EMBO Workshop
European Cytoskeletal Forum

‘Structure and Function of the Cytoskeleton’

-

7 – 10 April 2026

Centre International Conference Sorbonne Université
CICSU - 4, place Jussieu, 75005 Paris, France

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Abstract Booklet
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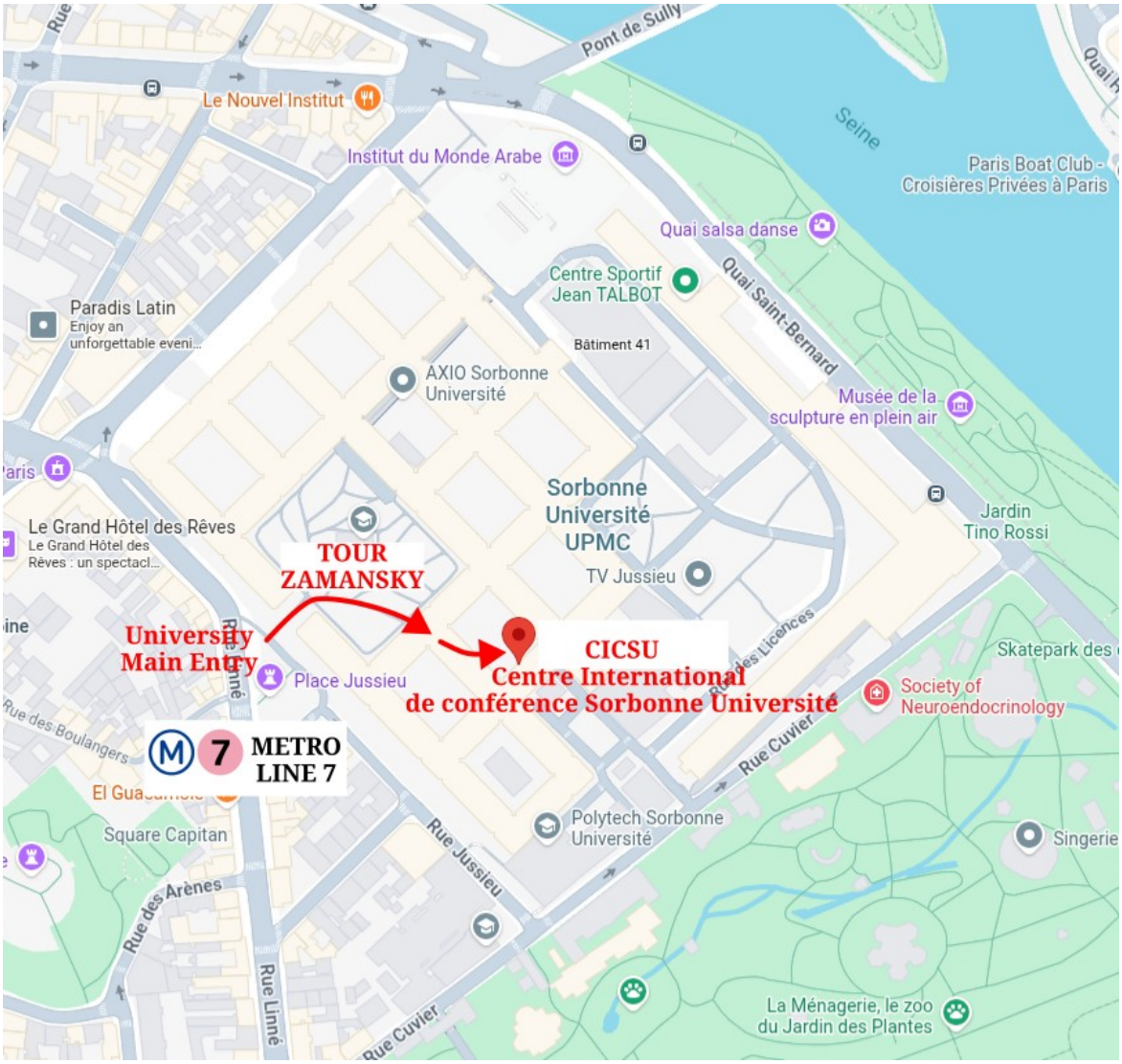

Schedule

Tuesday Arpil 7

1:00-2:00 PM	Registration	
2:00-2:15 PM	Welcome introduction	
2:15-4:05 PM	**SESSION 1.**	Chair: Guillaume Romet-Lemonne
2:15-2:40	Greg Alushin	Visualizing cellular force-sensing through the cytoskeleton
2:40-3:05	Dolores Perez-Sala	Fine tuning of intermediate filaments through cysteine modifications
3:05-3:20	Serapion Pyrpassopoulos	Mechanical tension extends the microtubule lattice and modulates kinesin-1 binding in an isoform-dependent manner
3:20-3:25	Thomas Germier	Gataca-Systems - Sponsor talk
3:25-3:50	Pekka Lappalainen	Regulation of actin filament assembly at focal adhesions ' or 'Biochemical, mechanosensitive and metabolic regulation of actin filament assembly at focal adhesions
3:50-4:05	Nivya Mendon	Characterization of the nuclear actin and its organization across species
4:05-4:35 PM	Coffee Break / Sponsors	
4:35-5:40 PM	**SESSION 2.**	Chair: Lu Yan Cao
4:30-4:35	Katie Pickup	Journal of Cell Science - The Company of Biologists
4:35-5:00	Florian Schur	A molecular atlas of the cell motility machinery
5:00-5:25	Hugo Wioland	How tropomyosins cover actin filaments
5:25-5:40	Helge Ewers	Multiscale imaging of cytokinesis
5:40-5:45	Oriol Nos Aguilà	Impetux - Sponsor talk
5:40-6:10 PM	**FLASH TALKS**	
6:10-7:30 PM	**POSTER SESION #1 (poster 1 to 50)**	
7:30-9:00 PM	COCKTAIL #1 - Tour Zamansky	

Wednesday April 8

9:00-10:35 AM	**SESSION 3.**	Chair: Christophe Le Clainche
9:00-9:25	Anne Straube	Cooperation of opposite polarity motors in cargo transport
9:25-9:50	Robert Grosse	An actin nucleoskleleton for nuclear integrity during confined migration
9:50-10:05	Philippe Dehio	Tuning of cell migration and stiffness by cellular energetics
10:05-10:30	Jana Helsen	Progressive coevolution of the yeast centromere and kinetochore
10:30-11:00 AM	Coffee Break / Sponsors	
11:00-12:30 PM	**SESSION 4.**	Chair: Arnaud Echard
11:0-11:25	Lukas Kapitein	Tuning the Tracks: Functional Diversity Encoded in Microtubule Lattice States
11:25-11:50	Johanna Ivaska	Cell adhesion dynamics and cancer cell motility
11:50-12:05	Helen Matthews	Oncogenic signalling primes the actin cortex for protrusion and motility
12:05-12:20	Juliette Mathieu	Cytoplasmic bridge maintenance in the choanoflagellate S. rosetta depends on Alix-dependent actin regulation.
12:20-12:25	Maya Ballet	Fluigent - Sponsor talk
12:25-2:00 PM	**LUNCH BREAK / POSTER SESSION #2 (Poster 51 to 100)**	
2:00-3:45 PM	**SESSION 5.**	Chair: Gijsje Koenderink
2:00-2:25	Sarah Köster	The physics of intermediate filaments: from molecular interactions to network mechanics
2:25-2:40	Makito Miyazaki	Spatiotemporal control of actin network assembly on lipid membranes in a reconstituted system
2:40-3:05	Thomas Surrey	Design principles for bipolar spindle organization by two motors.
3:05-3:30	Christophe Leterrier	The axonal cytoskeleton down to the nanoscale
3:30-3:45	Gilles Hickson	A RhoA Translocation Engine Couples Actomyosin Contractility to Membrane Throughput in Cytokinesis
3:45-4:15 PM	Coffee Break / Sponsors	
4:15-5:35 PM	**SESSION 6.**	Chair: François Robin
4:15-4:40	Lilian Fritz-Laylin	Cytoskeletal evolution and the origin of eukaryotic diversity
4:40-4:55	Pascal Martin	Self-organized chiral helical beating with actin-filament bundles and myosin-X motors in vitro.
4:55-5:20	Guillaume Charras	Keratins and desmosomes control the mechanical strength of epithelial tissues
5:20-5:35	Martina Boiardi	Cytoskeletal dynamics modulate cell cycle oscillations in frog egg extracts
5:35-7:00 PM	**POSTER SESSION #3 (Poster 101 to 150)**	
7:00-9:00 PM	COCKTAIL #2 - Tour Zamansky	

Thursday April 9

9:00-10:20 AM	**SESSION 7.**	Chair: Hugo Wioland
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9:25-9:50	Stefan Diez	Regulation of bidirectional transport and force on microtubules
9:50-10:05	Laura Haney	TDP-43 Regulates Microtubule Dynamics
10:05-10:20	Simona Buracco	Investigating the impact of cellular size on actin networks and their turnover
10:20-10:50 AM	Coffee Break / Sponsors	
10:50-12:25 AM	**SESSION 8.**	Chair: Anne Houdusse
10:50-11:15	Clara Nahmias	Microtubule associated proteins and molecular motors in breast cancer
11:15-11:30	Dhanya Cheerambathur	Kinetochores Link Rho GTPase Signaling to Actin Remodeling During Neuronal Dendritic Arborization
11:30-11:55	Carolyn Moores	Exploring cellular complexity through molecular mechanisms of cytoskeleton regulation
11:55-12:10	Michael Cianfrocco	Molecular mechanism of Taxol-induced microtubule stabilization
12:10-12:25	Sabine Windhorst	Discovery of a new evolutionarily conserved short linear F-actin binding motif
12:25-2:00 PM	**LUNCH BREAK / POSTER SESSION #4 (Poster 151 to 202)**	
2:00-3:35 PM	**SESSION 9.**	Chair: Manuel Théry
2:00-2:25	Virginie Hamel	Unveiling centriole architecture using expansion microscopy
2:25-2:40	Georgios Tsiavaliaris	Synthetic Arylindole Inhibitors Reveal Isoform-Specific Roles of Class I Myosins in Chirality and Brain Development
2:40-3:05	Ana-Maria Lennon-Dumenil	Shaping Immune Cell Behavior and Function through Changes in Cell Shape
3:05-3:20	Thomas Drexler	Resolving branch nucleation in space and time in macrophage podosomes using cryo-electron tomography
3:20-3:35	Valerie Siahaan	Tau phosphorylation impedes functionality of protective tau envelopes
3:35-4:05 PM	Coffee Break / Sponsors	
4:05-5:00 PM	**SESSION 10.**	Chair: Anne-Cécile Reymann
4:05-4:30	Verena Ruprecht	Cytoskeletal dynamics during phagocytic clearance in the early embryo
4:30-4:55	Martin Lenz	Transient contacts between filaments impart its elasticity to branched actin
4:55-5:10	Jayanti, Kumari	Tubulin-Like FtsZ Paralogs and the Evolution of Cytoskeletal Function in Asgard Archaea
7:30PM-0:00 AM	GALA DINER / LIVE MUSIC @ BUTTES CHAUMONT ORA Farmhouse / Pavillon du Lac - Parc des Buttes-Chaumont - 63 Rue Manin, 75019 Paris	

Friday April 10

9:00-9:40 AM	**SESSION 11.**	Chair: Olivia du Roure
9:00-9:25	Thibaut Brunet	Actin, myosins, and microvilli in the closest living relatives of animals
9:25-9:40	Waleed, Mirza	Active self-organization and maturation of mechanosensitive bio-molecular condensates
9:40-10:05	Kinneret Keren	Cytoskeletal dynamics in regenerating Hydra
10:05-10-20	Simli Dey	Asymmetric branched actin assembly driven by N-WASP around a membrane neck leads to neck breakage
10:20-10:50 AM	Coffee Break	
10:50-12:00 AM	**SESSION 12.**	Chair: Cécile Leduc
10:50-11:15	Marija Zanic	SSNA1 mechanically reinforces the damaged microtubule lattice
11:15-11:40	Jean-Leon Maitre	Mechanics of blastocyst morphogenesis
11:40-11:55	Bénédicte Sanson	Mechanisms at tricellular adherens junctions in epithelial morphogenesis
12:00	CLOSING REMARKS + DEPARTURE	

List of Participants

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List of Contributions (clickable)

Oral presentation Abstracts

(by alphabetic order of the name of the presenter)

Visualizing cellular force-sensing through the cytoskeleton

Gregory Alushin

Laboratory of Structural Biophysics and Mechanobiology, The Rockefeller University, New York, NY, USA

In addition to chemical cues, cells in the body must perceive physical forces at the molecular scale to orchestrate development and maintain homeostasis. Cells probe and respond to the physical properties of their local environments through the actin cytoskeleton, a network of protein polymers, motor proteins, and myriad associated partners. The Alushin lab uses structural, biophysical, and cell biological approaches to study how these factors collectively convert mechanical forces into biochemical signals which govern cell fate and behavior. The lab is developing new approaches for visualizing how biomechanical forces alter the structures of proteins to control their activities, revealing new mechanisms distinct from standard biochemical regulation. I will focus on our efforts to decipher how forces modulate the structures of actin filaments and force-sensitive actin binding proteins to regulate their binding interactions and functions. These insights provide a foundation for interpreting how mechanical signaling malfunctions in disease, which may provide new opportunities for therapeutic development.

Cytoskeletal Dynamics Modulate Cell Cycle Oscillations in Frog Egg Extracts

Martina Boiardi[1, 2], Nikita Frolov[1], Nicole Goede[1, 3, 4], Liliana Pineros Leyton[5], Lendert Gelens[1]

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[3] Laboratory of Animal Ecology, Global Change and Sustainable Development, Department of Biology, KU Leuven, Belgium

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The cell cycle is a tightly regulated biological oscillator, as demonstrated by the early embryonic divisions of the frog Xenopus laevis, where cells divide synchronously every 30 minutes without checkpoints or gap phases. This rapid cycling is driven by the periodic activity of cyclin B-Cdk1, the core regulator of cell cycle progression. Xenopus eggs cell-free extracts have demonstrated the ability of cellular structures to self-organize, and have enabled direct connections between this self-organization and the molecular network driving cell cycle oscillations. In particular, nuclei form when DNA material (e.g., demembranated sperm chromatin or commercial lambda-DNA) is added to extracts. The compartmentalization of the cyclin B-Cdk1 complex between the nucleus and the cytoplasm has been shown to play a crucial role in setting the periodicity of the cell division cycle, with slower oscillations associated with a higher nuclear-cytoplasmic volume ratio.

Extracts are capable of self-organization even in the absence of nuclei or centrosomes. The formation of cell-like compartments, mitotic spindles, and asters, requires microtubules, whereas other dynamic structures, such as contractile actomyosin networks, require F-actin filaments. How the cytoskeleton-guided self-organization of the cytoplasm influences the cell cycle period remains unclear. Here, we investigate how cytoskeletal networks contribute to setting the speed of cell cycle oscillations. Using cycling extracts, we observed periodic waves of actin contractions, microtubule aster (de)polymerization, and cell-like compartments formation, that persist over multiple cell cycles. Microtubules-guided self-organization significantly lengthens the cell cycle period, while periodic actomyosin contractions have minimal effect on the oscillation pace. A Cdk1-biosensor allowed us to investigate the period of the cell cycle oscillator in the absence of both cytoskeletal networks. Our findings suggest that cytoskeletal dynamics interact with the cell cycle regulatory network to modulate its tempo, and highlight the importance of considering their interplay to fully understand cell cycle regulation.

Investigating the Impact of Cellular Size on Actin Networks and Their Turnover

Simona Buracco[1], Alexandra Colin[1], Benoit Vianay[1], Louise Bonnemay[2], Laetitia Kurzawa[1], Manuel Thery[1, 2], Laurent Blanchoin[1, 2]

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[2] CytoMorpho Lab, Chimie Biologie Innovation, Institut Pierre-Gilles de Gennes, UMR 8132, CNRS, Ecole Supérieure de Physique et Chimie Industrielle de la Ville de Paris, Université Paris Sciences et Lettres, Paris 75005, France

Size scaling is a fundamental property of biological systems. Although cell type maintains a characteristic optimal size, cell size and morphology also vary with metabolic state, cell cycle phase, and aging. Across these changes, cells must adjust their intracellular structures to preserve functionality. Because cell size and shape depend on the actin cytoskeleton, understanding how actin architectures scale with cell size is essential. Yet the molecular mechanisms remain unclear. To address this, we quantified size-dependent changes in actin organization in mouse embryonic fibroblasts (MEFs).

We first identified a linear correlation between MEF volume and spreading area. Exploiting this, we used micropatterns of defined sizes to impose controlled changes in cell volume and achieved a tenfold size variation, pushing cells beyond their physiological range. Strikingly, mass spectrometry and dry-mass measurements revealed that total protein content—including actin—remained constant across all sizes, indicating that larger cells dilute their protein concentration. Despite unchanged total actin levels, F-actin content increased linearly with cell size. Larger cells assembled larger and more numerous structures, including focal adhesions, stress fibers, and lamellipodia, revealing proportional scaling of actin networks with cell size. This occurred without new actin synthesis, leading to a higher F/G-actin ratio in larger cells and depletion of the G-actin pool.

Functionally, reduced G-actin availability impaired actin dynamics. Photoactivation experiments showed a size-dependent decrease in actin turnover, accompanied by slower protrusion rates. Importantly, analogous trends were observed in the unpatterned cell population, confirming that these effects are not pattern-induced artifacts. Together, our results demonstrate that actin networks scale proportionally with cell size, but without the synthesis of additional actin, this scaling depletes the G-actin pool and limits actin dynamics. We propose a model of “G-actin pool tension,” in which competition for a finite monomer pool directly constrains actin network formation and remodeling in large cells.

Keratins and desmosomes control the mechanical strength of epithelial tissues

Guillaume Charras

University College London, UK

The ability of tissues to sustain mechanical stress and avoid rupture is a fundamental pillar of their function. Rupture in response to physiological levels of stress can be undesired, for example resulting from disease or genetic mutations, or be an integral part of developmental processes, such as during blastocoel formation in mouse or leg eversion in flies. Despite its importance, we know very little about rupture in cellularised tissues because it is a multi-scale phenomenon that necessitates comprehension of the interplay between mechanical forces and biological processes at the molecular and cellular scales. Using a combination of mechanical measurements, live imaging and computational modelling, we characterise rupture in epithelial monolayers. We show that, despite consisting of only a single layer of cells, monolayers can withstand surprisingly large deformations, often accommodating several-fold increases in length before rupture. At large deformation, epithelia increase their stiffness multiple-fold in a process controlled by a supracellular network of keratin filaments. Perturbing keratin organisation or desmosomes fragilises monolayers and prevents strain stiffening. Using computational and experimental approaches, we find that tissue stretch induces not only stiffening but also an increase in intercellular adhesion, perhaps due to catch-bond properties in desmosomes.

Kinetochores Link Rho GTPase Signaling to Actin Remodeling During Neuronal Dendritic Arborization

Dhanya Cheerambathur

University of Edinburgh

Dendrites, the signal receiving compartments of neurons, form complex tree like arbors essential for neural connectivity and function. Their development depends on the dynamic reorganization of the actin and microtubule cytoskeleton, coordinated by Rho GTPase signaling. However, how these molecular cues translate into precise neuronal architectures remains unclear.

We demonstrate that kinetochores, traditionally known for tethering DNA to spindle microtubules during chromosome segregation, surprisingly regulate actin dynamics to direct dendritic patterning in the C. elegans. Our works shows that the conserved kinetochore protein KNL 1 which serves as a scaffold linking microtubule and signaling components during mitosis also orchestrates actin organization post mitotically in neurons. Loss of KNL 1 in the neurons caused excessive dendritic branching and disrupted contact dependent repulsion required for normal dendrite arbor spacing. These alterations correlated with abnormal actin and microtubule organization. Pharmacological or genetic modulation of actin dynamics—via Latrunculin treatment or co depletion of formin mDia3—restored normal branching, indicating that KNL 1 regulates actin assembly pathways critical for dendrite morphogenesis.

Using an in-situ membrane tethering system, we found that KNL 1 intrinsically promotes actin assembly. When targeted to the plasma membrane, KNL 1 induced dynamic actin rich clusters enriched in active RhoA and actin assembly factors such as Arp2/3 and Ena/VASP, demonstrating its ability to locally stimulate F actin polymerization and Rho GTPase activity. Structure–function analyses revealed that this property resides in KNL 1’s N terminal domain, which hosts signaling modules rather than microtubule binding elements.

Our findings identify KNL 1 as a key regulator linking actin dynamics to dendritic architecture and reveal that kinetochore proteins, long studied for mitotic roles, also have critical post mitotic functions in shaping neuronal connectivity.

Molecular Mechanism of Taxol-Induced Microtubule Stabilization

Nicholas Vangos[1], Patrick DeLear[1], Marija Zanic[2], David Sept[1], Michael Cianfrocco[1]

[1] University of Michigan

[2] Vanderbilt University

Microtubules are polar polymers formed from tubulin heterodimers and are an essential component of the eukaryotic cytoskeleton. Most microtubules are dynamic and undergo repetitive cycles of polymerization and depolymerization. The nucleotide occupancy of tubulin controls microtubule dynamics, with GTP- and GDP-microtubules favoring polymerization and depolymerization, respectively. The dynamicity of microtubules is essential to their function. Indeed, the mechanism of action of many anticancer compounds is to interfere with microtubule polymerization or stability. Taxol (paclitaxel) is a front-line chemotherapeutic agent that stabilizes microtubules, ultimately leading to mitotic defects and cell death. Despite its prominent therapeutic role, the molecular basis for Taxol-mediated microtubule stabilization remains uncertain, with conflicting models existing in the field. To define the mechanism of Taxol, we implemented a new cryo-EM processing pipeline that allowed us to determine the structure of human microtubules bound to Taxol at 2.3 Å. Our structure is the highest-resolution microtubule structure to date, revealing 400+ water molecules throughout the structure. Comparison of this structure with 2.2 Å structures of GDP and GMPCPP microtubules showed that Taxol exploits allosteric switches within tubulin to 'flip' structural motifs in alpha and beta tubulin from GDP to a GTP-like state. Our structures also demonstrate that Taxol is a bona fide GMPCPP mimic, converting microtubules into a structural state nearly identical to that of GMPCPP. Moreover, inspection of the microtubule seam in these structures showed that both Taxol- and GMPCPP-mediated lattice expansion relieves strain in the GDP seam lattice. We propose that Taxol exploits several 'switch-like' motifs within tubulin to convert GDP-bound microtubules into a GTP-like state, where the increased lattice expansion strengthens lateral contacts at the microtubule seam. Not only does our work reveal the molecular mechanism of Taxol, but it also suggests that the tubulin nucleotide state controls microtubule

Tuning of Cell Migration and Stiffness by Cellular Energetics

Philippe Dehio[1], Nadine Stroehlein[2], Michael Sixt[1]

[1] Institute of Science and Technology Austria

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The actomyosin cytoskeleton is central to the regulation of cell shape, stiffness, and motility. Cellular force generation arises from the conversion of chemical energy, in the form of ATP, into mechanical work. A major force generator in the actin cortex is the myosin II ATP-fueled power stroke. In the absence of ATP—the rigor state—myosin II remains crosslinked to actin. Upon ATP binding, myosin detaches from actin, and ATP hydrolysis primes the myosin head for subsequent actin rebinding and force generation. However, it remains largely unexplored how low energy levels alter actomyosin cortex organization and, in turn, cellular mechanics.

To address this, glycolysis was inhibited in dendritic cells, causing a reduction in ATP levels and a concomitant decrease in migratory velocity. Under these low-energy conditions, pronounced actomyosin bundles formed. Pharmacological stabilization of myosin in its actin-unbound state abolished bundle formation. These findings implicate myosin in actin crosslinking during energy depletion—a mechanism reminiscent of rigor mortis, the ATP-dependent stiffening of post-mortem muscle. Similarly, energy-depleted dendritic cells exhibited increased cellular stiffness, suggesting that energy availability can directly tune cellular mechanics.

We propose that myosin II integrates cellular energy levels: under homeostatic metabolic conditions, ATP turnover permits normal contractility and force generation, whereas energy depletion enhances myosin II–mediated actin crosslinking, increasing cell stiffness. This cortical stiffening might protect the energy-starved cell from mechanical damage. Together, these results uncover a potential metabolic–mechanical axis linking cellular energy state to the biophysical properties of cells.

Asymmetric Branched Actin Assembly Driven by N-WASP Around a Membrane Neck Leads to Neck Breakage

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Actin assembly plays a critical role in various membrane remodelling processes, including intracellular trafficking. In this process, the membranes often form vesicular buds connected to the plasma membrane by saddle-shaped necks. However, the molecular mechanisms regulating actin assembly at these membrane necks remain poorly understood. Moreover, it is still unclear how this dense actin network produces force to carry out membrane fission. To address this fundamental question, in this work, we developed a reconstituted system featuring saddle-shaped membrane necks, generated from wrapping the lipid bilayers around beads grafted onto a glass substate. The formation of these curved membrane necks at the base of the beads was confirmed by the enrichment of the curvature sensing BAR protein SNX9. Our results show that upon introducing actin nucleation-promoting factor N-WASP, together with Arp2/3 complex and actin monomers, branched actin network assembles asymmetrically around the membrane necks. This asymmetric actin assembly exert force on the necks, leading to their displacement, as evidenced by the lateral and vertical displacement of the beads. This suggests that the neck structure was disrupted. In contrast, when using a truncated formed of N-WASP (pVCA), which still activates Arp2/3, the actin network grows symmetrically around the necks while no bead displacement was observed. These findings are consistent with recent reports showing that in mammalian cells, actin assembly at stalled clathrin-mediated endocytosis sites is asymmetric, acting like a “bottle opener” to pull off endocytic buds (Jin et al., 2022). Together, our findings provide new insights into how actin nucleation-promoting factors spatially regulate actin assembly at complex membrane geometries, advancing our understanding of processes such as endocytosis.

Regulation of bidirectional transport and force on microtubules

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Intracellular transport of vesicular cargo is a fundamental process mediated by teams of molecular motors that move along microtubules. Various cargos show complex motility pattern of bidirectional movement, including pauses and directional reversals owing to the simultaneous presence of opposite-polarity motors. However, the influence of various factors such as vesicle membrane composition and microtubule associated proteins (MAPs) on biasing this transport is unclear. Here, we addressed this question by reconstituting cargo motility along microtubules in vitro by attaching purified Dynein-Dynactin-BICD2 (DDB) and kinesin-3 (KIF16B) or kinesin-1 (KIF5B) to large unilamellar vesicles (LUVs). We investigated the influence of motor density and diffusivity (by varying the lipid composition of LUVs), in presence or absence of various MAPs such as tau, on the transport characteristics of vesicles. We found that this minimal system is sufficient to recapitulate fast unidirectional runs, pauses and reversals similar to in vivo cargo motility. In the presence of specific MAPs, the vesicle landing rates on microtubules as well as their reversal frequency and transport direction were impacted. Moreover, the lipid composition influenced the ability of the vesicles to circumnavigate dynamic roadblocks such as tau envelopes. We observed that gel-state vesicles were able to pass through, whereas most of the diffusive vesicles dissociated from the microtubules upon encountering tau envelopes. In summary, using in vitro liposome motility assays allows to delineate the influence of various factors impacting membrane-motor-microtubule interactions, eventually biasing the directionality of intracellular cargo transport.

Resolving Branch Nucleation in Space and Time in Macrophage Podosomes Using Cryo-Electron Tomography

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The actin cytoskeleton plays a key role in cellular morphology and motility by forming dynamic three-dimensional (3D) networks. Can we determine how these networks assemble over time by analysing their 3D architecture at a molecular level?

To investigate this, we employed cryo-electron tomography to visualize the molecular architecture of human macrophage podosomes, which are actin-rich structures that facilitate mechanosensing and membrane remodelling. Using subtomogram averaging, we assessed the polarity of the actin filaments and found that, in both the core and ring modules, two-thirds of the filaments had their barbed ends growing towards the ventral plasma membrane. This suggested a common nucleation mechanism. We then employed high-resolution template matching to identify Arp2/3 complex-mediated branch junctions. This enabled us to obtain a structure with a resolution of 9.4 Å. Quantitative analysis of branch orientation revealed that branching mainly occurs towards the ventral membrane. Additionally, branches at the periphery of the core were randomly oriented towards and away from the podosome centre. This suggests that the Arp2/3 complex drives nucleation of both core and lateral filaments. Given that podosomes emerge from cortical filaments along the ventral membrane and knowing the spatial orientation of both the mother and daughter filaments, we simulated successive generations of branches. Our model shows that, over time, the filaments become increasingly oblique to the ventral membrane and increasingly less parallel. Furthermore, a model with two generations of branches best fits our data on nascent podosomes, providing temporal insight into the growth of these actin networks.

By resolving Arp2/3 complex-mediated branch organization and linking it to higher-order architecture and temporal emergence, our work has advanced our mechanistic understanding of podosome assembly. Additionally, our mathematical framework provides a generalizable method for resolving sequential branching events in unperturbed environments, providing the first example of in situ 4D structural biology.

Multiscale Imaging of Cytokinesis

Helge Ewers

Freie Universität Berlin

After separation of duplicated genetic material, animal cells divide by cytokinesis. This process involves several simultaneous and successive steps of molecular reorganization at cell membrane, cortex and the spindle apparatus as the cleavage furrow ingresses and the spindle is transformed into the intercellular bridge. Dozens of proteins play a role in cytokinesis as the cytoskeleton and motor proteins dramatically reorganize the cell and finally organize abscission. However, a comprehensive view of the successive nanoscale reorganizations over cytokinesis remains untractable with current microscopy methods. Here, we developed a multimodal imaging and analysis framework to investigate the relative organization of key cytokinetic molecules over time, with a focus on septin reorganization during cytokinesis. We extract cytokinetic hallmarks from live-cell lattice light-sheet and expansion microscopy (ExM) to computationally define pseudotime and align nanoscale ExM images of cytokinesis. We discover a cytokinetic substep and create nanoscale maps of the intercellular bridge and the midbody in 6 stages for 20 involved molecules. We thus provide a framework for nanoscale analysis of cytokinesis, a process fundamental to life.

Unveiling centriole architecture using expansion microscopy

Virginie Hamel

university of Geneva

The centriole is an evolutionary conserved organelle coordinating fundamental biological processes including cell division, cellular signaling and cell motility. This organelle, of 500 nm long and 250 nm in diameter is composed of about 200 different proteins, some of which have been associated with human diseases. How these proteins are organized at the level of the centriole architecture and how does centriolar sub-structural elements assemble during centriole biogenesis is still poorly understood. Here, I will present the latest work from my laboratory, which tackle these structural cell biology questions using cryo-electron tomography and ultrastructure expansion microscopy (U-ExM), which enables a four-fold physical magnification of a biological specimen.

TDP-43 Regulates Microtubule Dynamics

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Microtubules are dynamic polymers essential for cellular organization and function, yet the details of their regulation via disease-associated proteins remains unclear. Trans Active Response DNA-Binding Protein 43 kDa (TDP-43) is best known for its roles in nuclear RNA metabolism and pathological aggregation in neurodegenerative disorders, like amyotrophic lateral sclerosis (ALS). TDP-43 notably shares features with microtubule-associated proteins including the ability to form liquid-liquid phase separated biomolecular condensates. Here we identify a direct role for TDP-43 in regulating microtubule dynamics. Using purified proteins, multi-wavelength TIRF microscopy, and biochemical assays, we show that TDP-43 binds both tubulin dimers (kD = 35 nM) and polymerized microtubules via a multifaceted mechanism that can promote microtubule bundling while also sequestering tubulin or microtubules within biomolecular condensates. Functional truncation mutants mapped the TDP-43-tubulin interaction to both the protein's N-terminal dimerization domain and canonical phase-separating domain. In Neuroblastoma-2A cells, TDP-43 depletion or overexpression altered microtubule organization and dynamics visualized through EB1 comet tracking. Upon Taxol treatment, cells displayed numerous significantly enlarged cytoplasmic TDP-43 condensates, linking cytoskeletal regulation to disease-relevant mislocalization. Together, these findings establish TDP-43 as a previously unrecognized regulator of microtubule dynamics and suggest that cytoskeletal disruption may be a key contributor to its role in neurodegenerative disease.

A RhoA Translocation Engine Couples Actomyosin Contractility to Membrane Throughput in Cytokinesis

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Cytokinesis is often portrayed as the progressive tightening of an actomyosin ring. Yet classic ultrastructure and modern live imaging agree on a deeper point: the ring is a laminated, membrane-coupled cortex that must continuously remodel as circumference decreases. This raises a central physical problem: how can constriction proceed without jamming membrane-associated cortical elements?

I will present a mechanochemical model in which cytokinesis operates as a RhoA translocation engine that couples a localized equatorial RhoA activation source to sustained cortical inflow, membrane compression and regulated membrane throughput. In this framework, actomyosin-driven inflow compresses heterogeneous, membrane-coupled domains at the equator, promoting F-actin turnover and biasing how active RhoA is partitioned among competing effector modules. A key prediction is that a membrane-proximal anillin–septin module, capable of assembling independently of F-actin, relieves congestion at the contractile ring by diverting membrane-coupled RhoA activity into the furrow flanks as closure proceeds, where it can be re-deployed to sustain further inflow. The resulting feedback architecture predicts regime behavior: depending on local geometry and load, closure can be smooth, pulsed or oscillatory.

I will discuss how this membrane-throughput view reinterprets diverse phenomena—including unilateral ingression, persistent “contractile units,” and context-dependent requirements for anillin and septins—and I will conclude with near-term, falsifiable predictions and a minimal agent-based modelling framework to formalize how local RhoA transport and membrane mechanics generate robust cell division.

Cell adhesion dynamics and cancer cell motility

Johanna Ivaska

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Integrins are the main cell adhesion receptors of extracellular matrix (ECM) ligands. They integrate ECM cues to the cellular cytoskeleton and regulate key cellular signaling pathways. By nucleating new adhesion sites and regulating the turnover of existing adhesions, integrins and components of the actin cytoskeleton control cell morphology, polarity and migration. I will describe our recent work on how cancer relevant signalling pathways balanced by kinases and phosphatases regulate integrin dynamics and cancer invasion. I will also discuss how phosphorylation controls the availability of adhesion components in invading cells.

Tuning the Tracks: Functional Diversity Encoded in Microtubule Lattice States

Lukas Kapitein

Utrecht University

Neurons depend on a highly organized microtubule cytoskeleton to support structural integrity and polarized transport. Within axons and dendrites, functionally distinct microtubule subsets coexist and selectively support the activity of specific motor proteins. Nonetheless, how these subsets are specified, and how motors distinguish between them, has remained an open question.

Here I will show that microtubule lattice conformation, specifically expanded and compacted lattice states, acts as a tunable structural parameter that generates functional diversity within microtubule networks. Using correlative fluorescence and cryo-electron microscopy, we find that the subset of stable microtubules is characterized by an expanded lattice conformation. Cell biological experiments furthermore reveal that lattice conformation underlies the selective motility of kinesin-1 along stable microtubules. Finally, in vitro reconstitution experiments demonstrate that lattice state directly governs how microtubule-associated proteins interact with microtubules, independently of tubulin post-translational modifications.

Together, these findings point to microtubule lattice plasticity as the key cell-biological mechanism to generate functional diversity within the cellular microtubule network.

The physics of intermediate filaments: from molecular interactions to network mechanics

Sarah Köster

University of Göttingen, Germany

When biological cells migrate or tissues develop, they experience high strains and stresses. Within the cytoskeleton, actin filaments and microtubules break easily under stain, as do their networks. By contrast, intermediate filaments have been shown to be remarkably energy dissipating and extensible, up to several times their original length. At the single filament level, this behavior can be precisely characterized by optical tweezer experiments and can be traced back to a very distinct hierarchical molecular architecture consisting of alpha-helical coils in a massively parallel arrangement. While these results suggest that intermediate filaments serve as "safety belts" and "shock absorbers" for the cell, it is not yet entirely clear whether the intriguing single filament behavior is relevant at the cellular level. Therefore, we complement our single filament measurements with active microrheology on filament networks and with cell stretching studies. Through this work we hope to elucidate the role of intermediate filaments within the composite cytoskeletal network and the complex environment of a biological cell.

Tubulin-like FtsZ Paralogs and the Evolution of Cytoskeletal Function in Asgard Archaea

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The evolution of the cytoskeleton was a key factor in cellular organization and division. Cytoskeletal proteins play central roles in membrane remodelling and cytokinesis across all domains of life, yet how these systems evolved remains incompletely understood. Asgard archaea, which are closely related to eukaryotes, possess a complex cytoskeletal repertoire, including actin, numerous actin binding proteins, and tubulin like proteins. To gain insight into ancestral cytoskeletal mechanisms, we investigated FtsZ, the evolutionary precursor of tubulin, in Candidatus Odinarchaeota.

Odinarchaeota encodes two divergent FtsZ paralogs, OdinFtsZ1 and OdinFtsZ2, which we characterized for their assembly and membrane interaction properties. OdinFtsZ1 forms curved protofilaments and directly associates with membranes through an N-terminal amphipathic helix, enabling membrane deformation in vitro. When expressed in E.coli, OdinFtsZ1 localizes to the membrane and disrupts normal cell division, resulting in elongated cells. Deletion of the amphipathic helix abolishes membrane association and restores normal cell morphology.

In contrast, OdinFtsZ2 assembles into stacked spiral filaments in vitro but lacks intrinsic membrane binding activity. Instead, it requires recruitment by the membrane anchor OdinSepF to induce membrane deformation. Consistently, OdinFtsZ2 is predominantly cytoplasmic when expressed alone in E. coli, but relocalizes to the membrane upon co-expression with OdinSepF.

Together, these findings demonstrate functional specialization among ancestral FtsZ paralogs and highlight how filament architecture and membrane tethering shaped early cytoskeletal roles in cell division, offering insight into the evolutionary origins of the eukaryotic cytoskeleton.

Shaping Immune Cell Behavior and Function Through Cell Deformation

Ana-Maria Lennon-Dumenil

Institut Curie, INSERM U932, France

Immune Cells circulate between tissues and lymphoid organs to achieve their surveillance function. As a consequence of this, they experience multiple events of cell deformation. During my talk, I will highlight how the actin cytoskeleton of immune cells enables them detecting these changes in cell shape and discuss the consequences of such mechansosensing events on their immune sentinel properties. I will further show how the mechanical responses of immune cells are regulated in vivo upon establishment of tissue fibrosis.

Transient Contacts Between Filaments Impart Its Elasticity to Branched Actin.

Martin Lenz

CNRS - U. Paris-Saclay - ESPCI

The biologically crucial elasticity of actin networks is usually understood as an interplay between the bending and stretching of its filaments. This point of view however fails when applied to the weakly coordinated branched actin networks found throughout the cell. Through experiments and theory, we show that their elasticity crucially involves reversible contacts between their filaments. These contacts can in turn be controlled through filament entanglement during network growth to regulate the final properties of the network. These properties could be key to understanding how moving cells dynamically adapt their cytoskeleton to their environment.

Self-Organized Chiral Helical Beating with Actin-Filament Bundles and Myosin-X Motors in Vitro.

Ludivine Chaix, Antony Lee, Pascal Martin

Physique des Cellules et Cancer, Institut Curie—PSL Research University, CNRS, Sorbonne Université

Biological systems have the ability to self-organize at large scales by coordinating the activity of small constituents that each consume energy from the environment. Wave-like beating of eukaryotic flagella constitutes a prototypical example of an active emergent behavior from the interaction of biofilaments and molecular motors. Our group has previously shown that, in the presence of myosin-II or myosin-V motors added in bulk, polymerizing actin filaments can self-assemble in vitro into polar bundles that exhibit symmetric flagellar-like beating in 2D. The assay is based on surface micropatterning of an actin-nucleation-promoting factor to control the geometry of actin polymerization. Here we report wave-like beating with myosin-X motors, confirming that the self-organization process is a robust property of motor-filament systems. Remarkably enough, beating with myosin X took the form of chiral helical waves that combine bending and torsion of the actin-filament bundles, which propagated from the anchored base toward the bundle's tip. Polymerization from the surface of microbeads allowed for the study of these 3D movements and how interactions between neighboring beads via their 'tentacles' resulted in effective attraction forces and bead clustering.

Cytoplasmic Bridge Maintenance in the Choanoflagellate S. Rosetta Depends on Alix-Dependent Actin Regulation.

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Cytoplasmic bridges are an evolutionary conserved feature of animal germ cells, arising from incomplete cytokinesis through the exclusion of abscission machinery from the intercellular bridge. To explore the evolutionary origin of this process, we investigated cytokinesis and abscission regulation in choanoflagellates, the closest living relatives of animals. The choanoflagellate Salpingoeca rosetta lives either as solitary cells or as multicellular chains in which neighboring cells remain connected by cytoplasmic bridges. We found that S. rosetta homolog of Alix, a major abscission component in animal cells, localizes to cytoplasmic bridges regardless of whether divisions are complete or incomplete. Surprisingly, Alix loss does not alter abscission timing in unicellular divisions but instead causes regression of cytoplasmic bridges within multicellular chains. Remarkably, this defect is rescued by reducing actin polymerization, suggesting that Alix helps counteract actin-dependent forces that otherwise destabilize the bridge. We will present our ongoing work aiming to investigate actin dynamic during incomplete cytokinesis in S. rosetta, and how Alix shapes the cytoskeletal environment to stabilize cytoplasmic bridges.

Oncogenic Signalling Primes the Actin Cortex for Protrusion and Motility

Helen Matthews, Joe Tyler, Rachel Lawson

University of Sheffield

Ras oncogenes drive cancer progression not only through driving proliferation but also by reorganising the cytoskeleton to promote motility and invasion. Using an inducible cell line where oncogenic Ras can be rapidly activated in normal epithelial cells, we characterised the molecular mechanisms underpinning Ras-dependent actin remodelling. We found that Ras activation triggers wholesale cytoskeletal reorganisation, inducing the disassembly of bundled actin structures such as stress fibres and the formation of dynamic protrusions. Atomic force microscopy revealed cell softening follow Ras activation, indicating a global change in cortex ultrastructure. These changes emerge over several hours and require a transcriptional response driven by ERK signalling and the transcription factor Fosl1. RNA sequencing identified a suite of actin-regulatory and motility-related genes that peak seven hours following Ras activation. Following this transcriptional response, TIRF microscopy revealed the emergence of periodic waves of Arp2/3-dependent actin polymerisation, which travel across the basal membrane resulting in protrusion formation upon reaching the cell edge. These waves are enriched for PIP3 and require local PI3K signalling. Importantly, ERK and Fosl1 are essential for the emergence of these waves suggesting that global actin remodelling is required to render the cortex permissive to local Ras/PI3K-dependent signalling. Finally, we examined the functional consequences of these changes: Ras-dependent actin remodelling increases cell motility and improves cell division fidelity by enhancing cell rounding during mitosis. Together, our results demonstrate that Ras modulates actin through two distinct, integrated mechanisms: a slow, ERK-dependent transcriptional response and a rapid, local PI3K-dependent stimulation of actin polymerisation. These pathways cooperate to drive the phenotypic plasticity required for cancer cell proliferation and invasion.

Structural diversity of intermediate filaments revealed by cryo-EM

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Intermediate filaments (IFs) are a diverse component of the cytoskeleton that exhibit remarkable cellular and tissue specificity. Their distinctive mechanical characteristics, including extraordinary elasticity, tensile strength, and distinctive stiffening behaviors under strain and compression, are pivotal for the mechanical resilience of cells and the integrity of tissues. The distinguishing characteristics of these elements are attributable to their intricate, hierarchical architecture, which differs fundamentally from the architectures of other cytoskeleton elements, such as actin and microtubules. The IFs family of proteins consists of over 70 distinct proteins, which are divided into six subtypes based on their sequence. Most of the members of the IF family of proteins assembled into ~10 nm think filaments . Why are there so many intermediate filaments in mammalians and are there structural difrencesses between the filaments ?

To answer these questions, we have determined the structures of assembled of two type III IFs, in cells. While our work unveils a novel construction principle for cytoskeletal filaments, it also indicates fundamental structural differences between different Ifs, expressed in different tissues.

Characterization of the Nuclear Actin and Its Organization Across Species

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Actin is a cytoskeletal element responsible for maintaining cell shape, transporting cargo, and responding to external stimuli. The role of cytoplasmic actin is well-defined, but its presence, organization, and function in the nucleus are still enigmatic. Nuclear actin is implicated in chromatin remodelling, transcription, DNA repair, and gene expression regulation. However, studies so far have not considered the actin isoforms, post-translational modifications, and cell-to-cell variations. Similarly, whether the nuclear actin dynamics are comparable to those of cytoplasmic actin remains unclear. To address this, our study combined biochemistry, cell biology, and fluorescence-based imaging to analyze nuclear actin content across cell lines. Biochemical fractionation reveals that the ratio of nuclear actin to cytoplasmic actin varies across the cell lines, and we further identified that the distribution of nuclear actin isoforms is also distinct depending on the cell lines. The nuclear actin filaments exist in various forms, ranging from monomers to short rods and long filaments, which might dictate the transcriptional activity. Based on these experiments, we also developed a serum response element reporter assay to dissect the mechanism of nuclear actin diversity in response to actin drugs as well as actin isoforms. Further, using a genomics-based approach, we have explored the role of beta and gamma actin in the regulation of global transcription. Overall, we have observed that the distribution and arrangement of nuclear actin can exhibit significant variations depending on the origin of the cell, and our study presents a framework for dissecting nuclear actin function.

Active Self-Organization and Maturation of Mechanosensitive Bio-Molecular Condensates

Waleed Mirza

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Mechanosensitive biomolecular condensates (such as focal adhesions and adherens junctions) form via liquid–liquid phase separation and are continually remodeled by mechanical forces and biochemical turnover. We present a mechanochemical framework based on a Cahn–Hilliard reaction–diffusion model that incorporates force-dependent binding and unbinding kinetics. Linear stability and eigenvalue analyses indicate that a spatially uniform state becomes unstable under certain force-influenced parameter regimes. This instability leads to the emergence of focal adhesion–like condensate patterns with a characteristic length scale, which can oscillate in time and produce recurring "load-fail" cycles. Nonlinear simulations further show that the reaction kinetics suppress Ostwald ripening, yielding a microphase-separated state with a condensate size that can be tuned by mechanical force and reaction rates. We find that the stiffness of the mechanical environment modulates the frequency and amplitude of the load-fail oscillations, with intermediate stiffness required to sustain persistent cycles. Moreover, simulations in micropatterned cell geometries (triangular, square, and elliptical) demonstrate that condensates preferentially nucleate in regions of high curvature, indicating that cell shape and boundary conditions strongly influence condensate organization. Our framework provides testable predictions for oscillation frequencies, condensate length scales, and spatial patterns as functions of mechanical parameters, biochemical reaction rates, and cell geometry. Importantly, we verify that these predictions are consistent with state-of-the-art experimental observations, validating the model and linking subcellular condensate mechanics to cell-wide mechanical behavior.

Spatiotemporal Control of Actin Network Assembly on Lipid Membranes in a Reconstituted System

Makito Miyazaki

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The actin cytoskeleton forms a dynamic mesh-like network that drives cellular deformations. The structural and functional properties of actin network are defined by its density and the activity of actin-binding proteins. However, how network density impacts the penetration ability and activities of actin-binding proteins remains unclear. Here, we developed a method to spatiotemporally control actin network assembly on a supported lipid bilayer using photolithography (Nano Lett. 2024) and optogenetics (Nat. Commun. 2025). This approach enables precise manipulation of the density, thickness, and shape of Arp2/3-mediated actin network assembly. Using this reconstituted system, we investigated how network density affects the interaction of two representative actin-binding proteins: myosin and ADF/cofilin. We found that the penetration of myosin filaments into the network was strictly inhibited by only a several-fold increase in network density due to the steric hindrance. Furthermore, penetrated myosin filaments induced directional actin flow when the network has a density gradient. On the other hand, ADF/cofilin penetrated into the network regardless of network density. However, network disassembly was dramatically inhibited by only a several-fold increase in network density. These findings reveal the network-density-dependent functions of actin-binding proteins, shedding light on the mechanical regulation of cytoskeletal dynamics by the actin network density.

Exploring cellular complexity through molecular mechanisms of cytoskeleton regulation

Carolyn Moores

ISMB, Birkbeck

The overall goal of our research is the elucidation of essential cytoskeleton regulatory mechanisms, applying reconstitution approaches and cryo-electron microscopy. There are significant challenges in understanding the specific contribution of the actin cytoskeleton to cellular processes because actin forms many different supramolecular arrays even within the same cell. We have investigated mechanisms by which turnover of the branched actin cytoskeleton via the Arp2/3 complex is regulated. This is essential during many cellular processes including cell migration and membrane remodelling. Cortactin is important for actin branch stabilization but the mechanism for this had been unclear. We determined the structure of cortactin-stabilized Arp2/3 actin branches and found that cortactin interacts with Arp2/3 at the branch junction and the new actin filament. Our structure shows how cortactin stabilizes the F-actin like interface of activated Arp3 with the first subunit of the new filament and binds and stabilizes inter-subunit contacts along it, explaining cortactin's contribution to regulation of branched actin network dynamics. We have also investigated the molecular mechanism by which metazoan Arp2/3 complexes are activated by SPIN90 dimers to nucleate bidirectional actin filaments. This unexpected finding has important implications for actin network organization and is contrary to previous ideas that SPIN90 monomers can activate Arp2/3. We showed that the SPIN90 dimerization domain is predicted to be present in multicellular animals, suggesting that this mechanism of bidirectional actin filament nucleation is conserved in metazoans.

Fine tuning of intermediate filaments through cysteine modifications

Dolores Perez-Sala

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Intermediate filaments are cytoskeletal elements that play key structural, mechanical and signaling roles. Moreover, they are important for cell and organelle homeostasis and integrate cellular responses, such as the response to oxidative stress. Cysteine residues are critical players in redox signaling. Several intermediate filament proteins possess redox sensitive cysteine residues. In particular, type III intermediate filaments possess a conserved cysteine residue that is a hotspot for posttranslational modifications of diverse nature. Curiously, structurally different modifications of this cysteine elicit distinct functional consequences, which can result in morphologically varied filament assemblies, and selectively impact cellular processes, in particular cytoskeletal crosstalk. The type III intermediate filament protein vimentin constitutes an example that illustrates how cysteine modifications can finely tune filament assembly and function in response to changes in the cellular context.

Mechanical Tension Extends the Microtubule Lattice and Modulates Kinesin-1 Binding in an Isoform-Dependent Manner

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Recent work has shown that the microtubule lattice possesses remarkable structural plasticity, with its conformation modulated by MAPs and motor binding. However, how this plasticity responds to mechanical forces remains poorly understood. We developed assays to measure the effect of tensile forces on single microtubules using optical tweezers and fluorescence microscopy. Decorating microtubules with quantum dots enabled us to measure, with nm-precision, mechanical distortions of ~0.4% under changes in average tensile force $\langle \Delta F \rangle$ = 10.4 pN, within the range of Fmin = 1.29 pN to Fmax = 20.4 pN, forces comparable to those generated by one to three kinesin-1 motors. Under forces in this range, the average binding rate of KIF5B decreased by ~20%, while its dissociation rate increased by ~10%, reducing its average run length. In extreme cases, run length dropped by up to 46% under tension. Interestingly, the effect was not uniform across microtubules subjected to similar tensile forces, and even for the same microtubule the response did not always repeat across successive force-cycling events, suggesting that the lattice can adopt multiple conformational states under tension. This behavior is consistent with a polymorphic lattice model in which distinct structural configurations can be differentially stabilized by mechanical stress, as confirmed by Brownian dynamics simulations and mechanical models. In contrast to KIF5B, no statistically significant effects were observed for KIF5C at the same range of forces. Together, these experiments and simulations provide new insights into how microtubules can act as sensors and transducers of mechanical and biochemical cues across the cell.

Mechanisms at Tricellular Adherens Junctions in Epithelial Morphogenesis

Bénédicte Sanson

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In embryos, epithelial sheets dramatically change their shapes, such as during gastrulation or organogenesis. Epithelial sheet plasticity requires individual cells to remodel their cell-cell contacts while they exchange positions, divide or leave the epithelium. Cell-cell contact remodelling requires the activity of the cytoskeleton and associated membrane proteins and is primarily studied at epithelial bicellular junctions, both in vivo and in cultured cells. But epithelial cells also form contacts with more than one cell, at tricellular junctions, where three neighbouring cells attach at their corners. Compared to bicellular junctions, however, how tricellular junctions remodel and by which mechanisms they contribute to morphogenesis, is poorly understood.

Specialised components at tricellular junctions have been identified but their functions in epithelial morphogenesis remain unclear. In Drosophila, we have discovered a new component of tricellular junctions, the transmembrane adhesion molecule Sidekick. Sidekick concentrates at tricellular adherens junctions in fly epithelia and is required for polarised cell rearrangements in developing tissues. We are using Sidekick as a molecular entry point to i) elucidate the organisation of cytoskeletal and membrane proteins at tricellular adherens junctions at the nanometre scale, using super-resolution microscopy (PAINT) in the intact embryo and ii) solve the mechanisms by which tricellular junctions control dynamics during cell rearrangements and other cell behaviours. We will present an update of our work.

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A molecular atlas of the cell motility machinery

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Actin filaments assemble into higher-order networks that generate the protrusive forces driving cell motility and shape changes. Yet the principles governing how these networks are organized and specialized remain unclear. How do actin filaments with distinct functional properties emerge within complex cytoskeletal architectures, and how do filament identity and network organization influence one another?

Our lab aims to uncover the biochemical and architectural principles governing actin cytoskeleton regulation and to build a molecular atlas of the motility machinery at the cell leading edge. By integrating molecular structure with higher-order organization, we seek to understand how cytoskeletal architecture enables cellular function. To achieve this, we combine high-resolution cryo-electron microscopy and tomography with biochemical and cell biological approaches to determine structures of actin-binding proteins and map their localization within native cellular environments.

Here, I present a workflow for large-scale 3D imaging of actin networks at single-filament resolution in lamellipodia of migrating cells. Tomographic volumes spanning up to 100 μm² enable tracing of individual filaments, describing the complete network architecture, and identifying branch junctions. Our data reveal pronounced filament heterogeneity, including micrometer-long filaments threading through the network, and identify filament subsets that define distinct topological entities within the lamellipodium.

Together, these advances reveal the detail and complexity of the actin cytoskeleton at the cell leading edge at a level not previously accessible, and establish a powerful framework for testing models of cytoskeletal organization.

Tau Phosphorylation Impedes Functionality of Protective Tau Envelopes

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Tau, an axonal microtubule-associated protein, is a critical regulator of microtubule function and stability. Tau interaction with microtubules is regulated by phosphorylation and hyperphosphorylation of tau is implicated in microtubule destabilization related to neurodegenerative disorders. How tau phosphorylation leads to microtubule destabilization is however unknown. Recently, it was shown that tau molecules can bind to microtubules in two distinct modes: either as (i) single tau molecules independently diffusing on the microtubule surface, or (ii) cooperatively-bound tau that form a cohesive tau "envelope" enclosing the microtubule lattice [1,2]. Tau envelopes locally compact the microtubule lattice [3] and form a selectively permissible barrier that inhibits kinesin-motors and protects microtubules against the activity of microtubule severing enzymes such as katanin [1]. Here we show that tau phosphorylation has deleterious effects on the microtubule-protective function of tau envelopes. Using in vitro reconstitution combined with TIRF microscopy and live-cell experiments, we found that tau phosphorylation destabilizes tau envelopes and decreases their integrity, leading to reduced microtubule protection against microtubule-severing enzymes [4]. Our data suggest that a perturbation of microtubule homeostasis linked to tau hyperphosphorylation in neurodegeneration, could be explained by the disassembly and impaired functionality of the tau envelopes.

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Cooperation of opposite polarity motors in cargo transport

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Bidirectional cargo transport in cells emerges from coordinated actions of plus-end directed kinesins and minus-end directed dynein motors. These opposite polarity motors show co-dependence, where one requires the activity of the other. However, when kinesin and dynein were linked together artificially, they undertook a tug-of-war with little net motility. We reconstituted complexes of dynein and the kinesin-3 KIF1C in the presence of dynactin and cargo adapters from purified recombinant human proteins and show that both motors can bind simultaneously to cargo adapters Hook3, BICD2 and BICDR1 to form co-motile complexes. Quaternary complexes of dynein, dynactin, hook3 and KIF1C (DDHK) form most efficiently and show directional, processive motility towards the plus and the minus end of microtubules in single molecule assays. KIF1C acts both as an activator and processivity factor for dynein. Activation requires only a fragment of the KIF1C non-motor stalk binding the cargo adaptor HOOK3. The interaction site is separate from the constitutive factors FTS and FHIP, which link HOOK3 to small G-proteins on cargos. We provide a structural model for the auto-inhibited FTS-HOOK3-FHIP1B (FHF) and explain how KIF1C relieves it. Collectively, we explain co-dependency by revealing how mutual activation of dynein and kinesin occurs through their shared adaptor. Many adaptors bind both dynein and kinesins, suggesting this mechanism could be generalised to other bidirectional complexes.

Properties of Nucleators and Motors Required for Bipolar Spindle Formation

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The microtubule cytoskeleton adopts distinct architectures at different cell cycle stages. Using microscopy-based biochemical in vitro reconstitutions and computer simulations, we explore how minimal microtubule/motor networks that mimic cellular architectures self-organize. We focus on the role of molecular motors and microtubule nucleators for bipolar spindle network organization during cell division. In doing so, we identify fundamental biophysical design principles of dynamic bipolar microtubule network organization.

Synthetic Arylindole Inhibitors Reveal Isoform-Specific Roles of Class I Myosins in Chirality and Brain Development

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Class I myosins are central regulators of actin cytoskeleton dynamics, membrane trafficking, and cell polarity, yet multiple short- and long-tailed isoforms coexist in cells with distinct and partially overlapping functions. Although genetic manipulations have been widely used to elucidate specific roles, their permanent effects can obscure stage- and isoform-specific functions. Small-molecule inhibitors complement these approaches by enabling acute, tunable, and reversible modulation of myosin behaviour; however, existing myosin-1 inhibitors (e.g. pentachloropseudilin) either lack selectivity or exhibit cytotoxicity, which preclude their use in vivo. Here, we report novel arylindol-based inhibitors, synthesized via metal-based coupling reactions, that selectively target class I myosins across species. The compounds bind with low micromolar affinity, potently suppress actomyosin ATPase and motor activity, while exhibiting improved specificity and reduced cytotoxicity. Computational modelling and molecular docking indicate an allosteric mechanism of inhibition that prevents the myosin motors from generating force, thereby blocking self-directed intracellular transport. Consequently, this interference can be used to effectively impair myosin-1–dependent processes, including endocytosis, secretion, and membrane tension regulation. In cell-based assays, the inhibitors effectively disturb myosin-1 localization, causing disturbances in trafficking and actin organization, which confirms target specificity. To evaluate efficacy in vivo, we employed Drosophila melanogaster, where these myosins function as single-headed motors with conserved, nonredundant roles. Myo1D (Myo31DF) and Myo1C (Myo61F) display antagonistic activities in left–right patterning, with Myo1D promoting dextral orientation via actin-based membrane trafficking and E-cadherin–mediated adhesion. Consistent with selective Myo1D inhibition, compound treatment induced mirror-image hindgut looping in ~20% of embryos, phenocopying Myo1D loss-of-function without affecting viability or growth. We also reveal a previously unrecognized role for Myo1D in brain morphogenesis, as both Myo1D-mutant and inhibitor-treated flies exhibit comparable abnormalities in the central learning centre. This work establishes arylindoles as selective pharmacological tools to probe class I myosin functions in cellular physiology, development, and disease.

Discovery of a New Evolutionarily Conserved Short Linear F-Actin Binding Motif

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Dynamic regulation of the actin cytoskeleton by actin-binding proteins (ABPs) is central to cellular organization and homeostasis, with binding mode critically shaping ABP function. Here, we identify a previously unrecognized short linear F-actin–binding motif (SFM) through cryo-electron microscopy of the ITPKA–actin filament complex. Using the structure-guided computational pipeline SLiMFold, we systematically identified 103 human proteins harboring SFMs and implicated in diverse cellular pathways. Phylogenetic analyses indicate that SFMs emerged de novo and are conserved across eukaryotes, displaying F-actin binding affinities in the low micromolar range (2–11 μM). Structure–function analysis defined key residues that mediate binding and tune affinity, while cryo-EM structures of two SFM–actin filament complexes reveal that SFM engagement reduces filament stiffness. Collectively, our results establish SFMs as a widespread class of actin-anchoring modules that modulate actin filament mechanics. These modules couple actin dynamics to a broad spectrum of cellular functions, providing a unifying framework for the actin-associated activities of numerous proteins.

How tropomyosins cover actin filaments

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Within a single cell, the actin cytoskeleton forms diverse networks of filaments, implicated in many fundamental functions. These actin networks are assembled from a common pool of actin and regulatory proteins, but how network differentiation is regulated has remained a very challenging question for decades. Recently, a family of actin binding proteins emerged as a key to elucidate this question: tropomyosins.

In every cell, multiple tropomyosin isoforms are coexpressed and have been shown to spatially segregate to specific networks. Tropomyosins has been proposed to give filaments an identity by regulating the recruitment and activity of the other cytoskeletal proteins. However, the mechanisms controlling tropomyosin localization are still unknown.

Our main hypothesis is that each tropomyosin isoform recognizes specific mechanical and biochemical signatures that determine to which actin network they get recruited. Such signatures include actin isoforms, mechanical constraints, other tropomyosins and actin binding proteins.

Experiments are performed with an in vitro reconstituted system, on purified and fluorescently labelled proteins. We take advantage of a microfluidic setup, designed in the lab, to quantify the assembly and disassembly of tropomyosin clusters on individual actin filaments, in tightly controlled biochemical conditions.

Our results reveal a new recruitment mechanism mediated by another actin binding protein, shedding light on previously misunderstood observations in cells.

SSNA1 Mechanically Reinforces the Damaged Microtubule Lattice

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SSNA1 (Sjögren's Syndrome Nuclear Autoantigen 1) is a microtubule-associated protein involved in key cellular processes, including cell division, intraflagellar transport, and axonal branching. Our previous work demonstrated that SSNA1 specifically localizes to sites of damage along the microtubule lattice, establishing it as a bona fide microtubule damage sensor. However, the e"ects of SSNA1 on microtubule mechanics or on the process of microtubule self-repair, which involves the incorporation of soluble tubulin dimers into lattice damage sites, are not known. Here, we use in vitro reconstitution with purified proteins and total internal reflection fluorescence (TIRF) microscopy to probe SSNA1's e"ects on microtubule mechanics and self-repair. We apply two distinct sources of force to investigate microtubule mechanics: microfluidic flow and kinesin-driven gliding assays. Our results show that SSNA1 binding increases microtubule rigidity and resistance to breakage under the physiological and controlled forces in our assays. Interestingly, we find that SSNA1's localization to microtubule damage sites prevents the incorporation of new tubulin dimers and thus inhibits lattice self-repair. Conversely, we find that SSNA1 does not recognize damage sites that have been repaired by tubulin incorporation. Together, our findings demonstrate that SSNA1 reinforces the mechanical strength of microtubules without promoting self-repair, providing new insights into SSNA1's mechanism of microtubule stabilization.

Poster Abstracts

Poster 1 - Cytoskeletal Dynamics at Synapses: Characterization of Novel Microtubular Regulators and Their Potential Role in Resilience to Neurodegeneration

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In neurons, the microtubule (MT) cytoskeleton composed of α- and β-tubulin, plays a key role in the development and maintenance of axons and dendrites. At the synaptic compartment, dynamic microtubules enter into dendritic spines, the tiny protrusions that support the post-synaptic side of most excitatory synapses in the brain, contributing to synaptic maintenance and plasticity. Furthermore, recent studies in our laboratory demonstrated that in Alzheimer's disease (AD) spines that were invaded by microtubules successfully resisted oAβ injury, whereas non-invaded neighbouring spines were frequently eliminated contributing to early decay of synaptic connections associated with memory failure.

In that context, we hypothesized that the entry of microtubules into the spines provides a direct route for the transport of selective synaptic components that may contribute to protect spines against amyloid toxicity. In order to identify the cargo molecules that are being trafficked, as well as proteins that promote microtubule entry, we employed a proximity labeling assay using a split version of the biotin ligase TurboID. One fragment was linked to a microtubular protein and the other to the F-actin-binding peptide LifeAct, allowing enzyme reconstitution and biotynilation of nearby synaptic effectors during microtubule entry into the spines. Target proteins will be then identified by mass spectrometry

This work will uncover key regulators and cargoes involved in microtubule entry into spines, shedding light on mechanisms of synaptic resilience and offering potential targets for early intervention in neurodegenerative diseases like AD

Poster 2 - How the Cytoskeleton, Via Septins, Regulates Thrombopoietin Receptor Trafficking

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The production of platelets from large multinucleated megakaryocytes requires complex series of events driven by the master regulator thrombopoietin receptor (TpoR) – JAK2-STAT 3/5 signalling. Being an indispensable component of platelet biology, understanding regulation of TpoR traffic and its cell surface localization becomes crucial. Recent reports indicated an effect of the actomyosin network on the traffic and signalling of TpoR. However, whether septins also affect TpoR traffic remained unknown. Septins were reported to be involved in the regulation of the surface expression of receptors like EGFR, ErBb2, etc. Previous reports linked septins to platelet biogenesis. But, the function of septins in TpoR signalling remained unknown. Our study showed that forchlorfenuron (FCF), a pan-septin dynamics inhibitor supressed the surface expression of TpoR. FCF treatment reduces TpoR vesicle speed while increasing the size of vesicles carrying TpoR. To further identify which septin was involved, we observed the traffic of TpoR in septin 9 knockdown cells. Surprisingly, knockdown lead to increased surface expression of TpoR without any effect on the TpoR vesicle speed unlike FCF. However, like FCF treatment, knockdown increased the TpoR vesicle size. Inhibition of septin dynamics with FCF also decrease the thrombopoietin induced TpoR-JAK2-STAT3/5 signalling. To elucidate the mechanism of septin mediated regulation, we checked the effect of FCF and septin 9 knockdown on microtubules. Both of them increased the filamentous tubulin levels in cells. Strikingly we observed a specific interaction of septin 9 with microtubule motor protein KIF1C. We further observed its colocalization with TpoR vesicles with KIF1C staining the cell periphery specifically in septin 9 knockdown cells. We therefore hypothesized that the traffic of TpoR vesicles may be hindered by KIF1C-Septin 9 interaction. Further experiments are in progress to verify this hypothesis. Our results thus establish septin 9 as a regulator of TpoR traffic, surface expression and signaling.

Poster 3 - The Ste20-Family Kinase GCK-4 Regulates Intestinal Lumen Formation Via Apical Actin Organization in Caenorhabditis Elegans

Mohamed Attaibi[1], Jorian Sepers[1], João Ramalho[1], Savvas Tzavellas[1], Jason Kroll[1], Ruben Schmidt[1], Ophelie Nicolle[2], Gregoire Michaux[2], Mike Boxem[1]

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Tubular structures are fundamental to multicellular life, enabling the transport of fluids, nutrients, and gases. Despite their structural diversity, from unicellular tubes to complex branched epithelia, a shared requirement of tubulogenesis is the formation of a continuous fluid-filled lumen. This involves establishment of an apical domain at the future lumen site, followed by lumen opening, expansion, and coalescence into a single lumen. Cytoskeletal organization and cell–cell junctions play central roles during these steps by providing mechanical resistance, facilitating lumen opening, and balancing outward forces generated by luminal secretion and osmotic pressure.

To uncover novel regulators that act downstream of polarity establishment to control patterning of the cytoskeleton and junctions during lumen opening and enlargement, we performed a screen targeting kinases with putative roles in C. elegans intestinal development. We identified GCK-4, an ortholog of the mammalian Ste20-family kinases LOK and SLK, as a regulator of continuous lumen formation that localizes to the apical domain during early intestinal development. Absence of GCK-4 resulted in loss of actin recruitment to the lumen formation site and impaired brush border development. Consequently, cell-cell junctions persisted at the intestinal midline, leading to fragmented and cystic lumen pockets. Mimicking phosphorylation of the sole Ezrin/Radixin/Moesin ortholog in C. elegans, the actin-membrane linker ERM-1, partially rescued the lethal cystic phenotype. In mammalian systems, ERM proteins are though to be regulated by LOK/SLK. However, despite partial rescue by phosphorylation-mimicking ERM-1 variants, we observed no defects in ERM-1 phosphorylation in animals lacking GCK-4. This indicates that the role of GCK-4 in C. elegans development is not mediated by direct regulation of ERM-1.

Our work highlights regulation of the cytoskeleton during an essential developmental process and suggests an essential role for apical actin enrichment during lumen formation. We are currently investigating the role of different actin conformations during this process.

Poster 4 - Mechanosensitivity of Actin Binding Proteins

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Living cells are constantly exposed to mechanical stimuli from their external environment, while also generating internal forces. The actin cytoskeleton plays a crucial role in both generating forces, and converting these mechanical stimuli into biochemical signals through a process known as mechanotransduction.

Although mechanotransduction by the cytoskeleton was previously attributed to force-induced conformational changes in associated proteins, evidence now shows that the filaments themselves can act as direct mechanosensors. Interestingly, actin filaments that are incorporated into diverse networks (e.g. the cortex, lamellipodia, stress fibers) experience various mechanical constraints, including tension, bending, torsion and compression. These forces can be generated by actin-binding proteins (ABPs), such as myosin motors, and can also arise from interactions with other cellular components.

In this context, my aim is to better characterize how the mechanical state of actin filaments regulates their affinity with ABPs. To carry out this study, I perform in vitro experiments on purified and fluorescently labelled proteins. Single actin filaments are subjected to controlled mechanical constraints (tension, bending and twisting) using microfluidics, optical tweezers or magnetic tweezers. We characterize the reversible binding of a recently identified mechanosensitive ABP, highlighting the coupling between the different mechanical constraints.

Poster 5 - HAX1 Couples Cytoskeletal Mechanics to Cell-Cycle Progression.

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Mechanical forces generated by the cytoskeleton must be coordinated with cell-cycle progression to ensure faithful cell division, yet the molecular integrators of these processes remain incompletely understood. HAX1 is identified as a potential regulator of mechanotransduction that couples actomyosin contractility, microtubule organization, and cell-cycle control, positioning it as a candidate mechanochemical integrator at mitosis and cytokinesis. Depletion of HAX1 in epithelial cancer cells reduces proliferative capacity and leads to accumulation at the G1/S boundary and in late mitosis, accompanied by increased numbers of cytokinetic bridges and mitotic stalling. Transcriptomic profiling demonstrates coordinated downregulation of MYC and E2F target genes, cyclins, and MCM replication-licensing components, indicating compromised DNA replication competence and a defective G1/S transition.

Mechanistically, HAX1 loss induces a hypercontractile state characterized by increased RhoA activity, elevated phosphorylation of myosin light chain MYL9, enhanced stress fiber formation, and disturbed focal adhesion architecture. HAX1 directly interacts with MYL9, suggesting a role in local control of actomyosin tension via modulation of myosin phosphorylation, and may influence YAP-related transcriptional responses and focal adhesion dynamics. In parallel, co-immunoprecipitation with SEPT2/SEPT7 supports a role for HAX1 in organizing septin–myosin assemblies within the contractile ring and along the cytokinetic bridge, suggesting that HAX1 participates in coordinating septin architecture with actomyosin contractility.

Beyond actomyosin regulation, HAX1 localizes to centrosomes and the cytokinetic bridge, and associates with microtubule and cytokinesis regulators including CKAP5, PRC1, septins, and membrane-trafficking factors. HAX1 depletion is associated with spindle defects, prolonged cytokinetic bridges, and increased sensitivity to microtubule-targeting agents and Aurora A kinase inhibition by alisertib, despite largely preserved Aurora A phosphorylation. Collectively, these findings support a model in which HAX1 functions as a mechanochemical integrator stabilizing actomyosin–septin–microtubule architecture at the spindle and cytokinetic bridge, thereby linking cytoskeletal mechanical homeostasis to cell-cycle progression, replication competence, and completion of abscission.

Poster 6 - Structural and Functional Insights into Lifeact and F-Tractin Binding to F-Actin

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Fluorescent peptides such as Lifeact and F-tractin are widely used to visualize filamentous actin (F-actin), yet both can perturb actin regulation. Using cryo-EM, we determined the Lifeact–F-actin and F-tractin–F-actin complexes, showing that both peptides insert an amphipathic helix into the same hydrophobic pocket between adjacent actin subunits. This binding site is also used by key actin-binding proteins, including cofilin and myosin, and our in vitro assays confirm that Lifeact and F-tractin compete with these regulators. Such competition provides a mechanistic explanation for the probe-induced alterations in actin dynamics and cell morphology observed in vivo. Finally, we show that the affinity of the probes for F-actin can be modulated by targeted mutations, offering a route toward optimized variants with reduced interference. Our findings provide critical insights into the molecular basis of Lifeact and F-tractin binding to actin and highlight the importance of carefully considering probe-induced artifacts when investigating actin dynamics in cell biology studies.

Poster 7 - A New CDC42 / Septin / BORG / Microtubule Interplay During Mitosis

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The laboratory has long been interested in cytoskeleton remodelling during cell adaptation to stress. In interphase, the interaction of septin filaments to actin fibers is under the control of a family of five CDC42 effectors, called the BORGs (Binder of Rho GTPases, also known as CDC42EPs). We found that in HeLa cells, Paclitaxel-induced CDC42 inactivation triggers the release of BORG2 (and BORG3) from actin stress fibers, with its subsequent proteasome-mediated degradation, resulting in a striking relocalization of septins from actin to microtubules (MTs) (Salameh et al., 2021). Such septin relocalization induces acquired resistance to the MT-stabilizing chemotherapy drug Paclitaxel (Targa et al., 2019; Salameh et al., 2021).

We now address the localization and function of BORGs during mitosis. We show that BORG2 and BORG4 coalign with MTs during mitosis, at the spindle, the midzone and the intracellular bridge, with partial septin (and possibly CDC42) overlapping. Cellular depletion of BORG2 by siRNA correlates with a delay in prometaphase, as observed in septin- or CDC42-depleted cells that are known actors of chromosome congression, and correlates also with a delay in metaphase. This highlights the role of the BORG cytoskeletal crosslinking protein as a novel player in mitosis, whose behavior might differs from that observed in the cytoplasm.

Poster 8 - Differential Effects of Thymosin Proteins on Actin Dynamics

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Thymosins are a group of small (5kDa) actin monomer-binding proteins that are thought to sequester actin monomers, inhibiting all polymerization. Almost all research has been carried out on one thymosin, thymosin beta 4 (TB4, TMSB4X gene). TB4 is believed to be the most abundant actin sequestering protein in many mammalian cells, acting as a 'buffer' to maintain a large pool of actin monomers for rapid polymerization. However, there are five thymosins in humans, and thymosin beta 10 (TB10, TMSB10 gene) is expressed at similar levels to TB4 in many cell types. Surprisingly, research on TB10 has been minimal, and little research on either TB4 or TB10 has been conducted in the last 20 years. Both TB4 and TB10 are 44 amino acid long proteins and share 75% identity (33 aas).

Here, we compare the biochemical properties of TB4 with TB10, to determine whether they are likely to be functionally redundant or might contribute to actin dynamics in different ways. Our current data suggest that TB10 is significantly different from TB4. In addition to having a higher affinity for ATP-bound actin monomers, TB10 displays a considerably greater ability to cause depolymerization of pre-polymerized actin filaments. Furthermore, profilin has less ability to shift actin's polymerization equilibrium when added to TB10 than when added to TB4. One possibility for the difference in depolymerization ability between TB4 and TB10 is that TB10 has a comparatively higher affinity for ADP-actin, inhibiting the ability of depolymerized monomers to re-charge actin with ATP. Our experiments using fluorescence polarization show that TB10 indeed has a significantly higher affinity than TB4 for ADP-actin.

Our results show that TB10 has a fundamentally different effect to TB4 on actin dynamics. Given the similar cellular abundances of TB4 and TB10, they are likely to have differential effects on cellular actin dynamics.

Poster 9 - Addressing the Role of Septins at the Membrane Through Cell-Free Assays

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The interplay of septins with membranes is essential to direct their function in the cell. We have been investigating how septins can interact with lipids, and alter their properties. To this end, we have designed bottom-up in vitro reconstituted assays to be able to decouple the function of septins from potential partners, in a cell-free environment. Distinct biomimetic lipid membranes (lipid bilayers, monolayers and liposomes (large or giant) were used to address the behavior and key roles of septins from yeasts to mammals in (i) their organization onto lipids, (ii) membrane remodeling and curvature sensitivity, (iii) building diffusion barriers and (iv) altering the mechanical properties of membranes. In addition, septins interact with multiple partners and we are thus interested, in the context of cell division, to assess a possible septin ESCRTs-interplay occurring during cytokinesis.

A set of complementary methods are undertaken from cryo-electron microscopy and tomography, scanning electron microscopy, fluorescence optical microscopy, microfluidics design and atomic force microscopy.

Poster 10 - Influence of Initial Cell Shape on Direction and Persistence of Migration

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Cells are able to actively migrate in 2D or 3D environments, individually or collectively.

This process is essential in morphogenesis, immune responses, wound healing but also cancer dissemination.

In the case of mesenchymal migration, cells reorganize their actin cytoskeleton to polarize and spatially segregate adhesion sites and forces, while the microtubule network act as a polarity compass and as tracks for intracellular trafficking. Both of these cytoskeletal networks are highly dynamic and have a fast turnover rate of a few minutes.

However, some cells can migrate directionally and persistently over several hours, maintaining a sustained polarity and architecture. This seems to imply the existence of a form of mechanical memory that maintains the directional bias in single cells, although the cytoskeleton is rapidly renewing. How is this memory supported in persistent cell migration ?

To investigate this question, we started by wondering how long a cell can be influenced by its initial shape during migration. Using a recent method of dynamic micropatterning, we are able to impose cell shape and then rapidly release the cells and study their migration. We can then play with different initial shapes and compare their influence on the direction and persistence of migration.

Poster 11 - Cdc42 And Rac2 Acts Through the Formin-like Frl/FMNL to Control Lamellocyte Shape and Encapsulation of Parasitoid Wasp Eggs in Drosophila

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Drosophila has been established as a powerful genetic model to study not only blood cell development but also innate cellular immunity. Lamellocytes are the most distinct blood cell type which are large key effector cells in the anti-parasitoid immune response in Drosophila. Findings on cell morphology and function of lamellocytes are based on pioneering studies conducted in the early 1980s. Here, we analyzed their remarkable cell cytoskeleton and cell morphology using high-resolution confocal imaging combined with an lamellocyte-specific candidate RNAi approach to identify key regulators of the lamellocyte morphology and functions. The distinct cytoskeleton determines the rigid disc-shaped morphology of giant lamellocytes, which enables passive circulation within the hemolyph. Once attached, lamellocytes reorganize their actin cytoskeleton and form lamellipodia-like protrusions to spread. Remarkably, cell spreading does not depend on WAVE-Arp2/3-branched actin filament nucleation but rather requires the formin-like protein (Frl/FMNL) downstream of Rac2 and Cdc42 signaling. Supporting this notion, RNAi-mediated depletion of either Frl/FMNL or Rac2 and Cdc42 but not Rac1 results in prominent changes in lamellocyte morphology and immune dysfunction. Our data further suggest a pathway in which Rac2/Cdc42 recruits Frl/FMNL to the cell cortex controling lamellocyte spreading and encapsulation of parasitoid wasps.

Poster 12 - Arp2/3 Regulates Endothelial Barrier Integrity

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A compromised endothelial barrier is associated with vascular leakage and tissue edema in various diseases such as sepsis; however, effective therapies that stabilize the vasculature are lacking. While much research has focused on junctional adhesion molecules, such as the adherens junction protein VE-cadherin, in regulating endothelial barrier permeability, the role of the underlying actin cytoskeleton in dynamic barrier regulation is less well understood. Here, we show that the Arp2/3 complex, which assembles branched actin networks implicated in junction assembly, regulates endothelial permeability. In cultured endothelial cells, vascular endothelial growth factor-A (VEGF-A) and thrombin compromised the endothelial cell monolayer in an isoform-specific Arp2/3-dependent manner. Inhibition of Arp2/3 using CK666 or gene silencing decreased VEGF- or thrombin-induced actin stress fiber formation and VE-cadherin internalization. Importantly, CK666 prevented VEGF-induced vascular leakage in vivo and decreased lipopolysaccharide-induced leakage in murine endotoxemia. Our results suggest a model where Arp2/3 balances endothelial barrier stability v/s permeability in an isoform-specific manner, along with an interplay with other actin-binding proteins, and that Arp2/3 inhibition may provide a means for vascular barrier stabilization.

Poster 13 - Structural Insights into Actin Filament Regulation

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Max Planck Institute for Molecular Physiology

The dynamic turnover of actin filaments (F-actin) plays a central role in controlling the shape and movement of eukaryotic cells. These processes depend on the precise regulation of actin polymerization and disassembly by a diverse set of actin binding proteins. Notably, the two polar ends of F-actin, being the only sites for the addition or loss of actin subunits, are especially important for controlling filament dynamics. Using cryo-electron microscopy (cryo-EM), we described some of the molecular mechanisms linked to the actin turnover cycle, including how different actin-binding molecules affect the stability of the F-actin ends at an atomic level. Our findings highlighting some of the mechanisms by which actin dynamics can be controlled in cells.

Poster 14 - Biochemical Communication and Self-Organization in Actin-Based Living Matter

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Understanding the basic principles of what is defining life is still an unresolved question, with broad applications from the field of fundamental sciences and origins of life to the applied field of synthetic cells design. Recent definition by Stuart Bartlett suggested that a living state is defined by four processes: dissipation, autocatalysis, homeostasis and learning (Bartlett & Wong, 2020). Cytoskeleton is of a primary importance for cell functions and has the property to generate large-scale assemblies from nanometer-scale proteins. It is therefore reasonable to think that cytoskeleton could behave as a “living material”(Banerjee et al, 2019).

In this work, we investigate how actin structures can communicate via biochemical signaling. We used an in vitro reconstituted system where actin structures are grown from micrometer-sized beads coated with a nucleation promoting factor for actin polymerization. Using this system, we study how biochemical interactions between actin networks give rise to collective behaviors. We show that these interactions induce collective motion and spatial organization that emerge when there is a sufficient density of structures. . Our results demonstrate that biochemical communication leads to emergent patterns at larger scales.

This study highlights cytoskeletal assemblies as active living matter systems, in which communication and information processing drive self-organization.

Poster 15 - The Microtubule plus-End Tracking Protein NAV1 Regulates Adhesion Dynamics During Cell Migration and Axon Pathfinding

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Axon guidance depends on coordinated interactions between microtubule (MT) and actin networks within the axonal growth cone. The MT plus-end tracking protein (+TIP) NAV1, the vertebrate ortholog of C. elegans axon guidance factor UNC-53, regulates growth cone motility and steering in vitro by coupling MT plus ends to peripheral actin filaments and promoting MT stabilisation. However, it remains unclear how NAV1-mediated MT capture within the growth cone influences axon pathfinding. Growth cone advance, similar to directed cell migration, relies on traction forces generated by actomyosin cables anchored to integrin-based adhesions known as point contacts, which are analogous to focal adhesions. In migrating cells, directional movement requires spatially regulated focal adhesion assembly and disassembly, processes that depend on coordinated MT and actin dynamics. To test whether NAV1 regulates point contact turnover during axonal growth cone steering, we first investigated its role in focal adhesion dynamics in non-neuronal cells. We show that NAV1 regulates directed migration in HeLa cells by controlling focal adhesion turnover and cytoskeletal polarisation. We demonstrate that NAV1 promotes MT-induced focal adhesion disassembly by stabilising MTs at the cell periphery, a process that depends on both its MT plus-end binding property and ATPase function. In addition, NAV1 facilitates focal adhesion disassembly by activating the small GTPase RAC1 through interaction with the RhoGEF TRIO. In neurons, NAV1 depletion in cultured retinal ganglion cells abolishes ephrin-A5-induced axonal retraction, a response driven by dynamic point contact turnover. Ongoing work aims to determine whether NAV1 deficiency disrupts point contact dynamics during Ephrin-A5-induced axonal retraction. Together, these findings identify NAV1 as a regulator of directed cell migration and focal adhesion turnover through F-actin-MT coupling and control of Rho GTPase signalling, and suggest that NAV1 may regulate adhesion dynamics in neurons during axon pathfinding.

Poster 16 - Actin Under Stress: Structural Integrity of Ezrin-Anchored Filaments Under Uniaxial Strain

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The actin cortex of eukaryotic cells forms a thin, dynamic network beneath the plasma membrane that is essential for maintaining cell shape, enabling motility, and transmitting mechanical forces. Actin filaments in this cortex are mechanically coupled to the membrane through linker proteins such as ezrin, allowing cells to sense and respond to external deformation. Despite its central role in cellular mechanics, the structural response of membrane-anchored actin filaments to lateral strain remains poorly understood.

Here, we introduce a reconstituted in vitro model system that enables controlled deformation of surface-bound actin filaments under uniaxial strain. Filamentous actin is specifically anchored to an elastic polydimethylsiloxane (PDMS) substrate via a silane- and PEG-based functionalization strategy, using the constitutively active ezrin mutant T567D as a defined molecular linker. This setup provides robust and reproducible mechanical coupling while maintaining accessibility for fluorescence-based analysis. Uniaxial stretching of the PDMS membrane imposes well-defined lateral strain, which is homogeneously transmitted within the elastic regime of the substrate. Fluorescence microscopy reveals that ezrin-anchored actin filaments undergo pronounced strain-induced reorientation along the stretch axis and display measurable changes in filament contour length. While a large fraction of filaments remains structurally intact under deformation, fragmentation events are observed in a subset of filaments, indicating heterogeneous mechanical stability at the level of individual filaments.

Together, these results establish a mechanically robust and well-defined platform to probe the structural response of ezrin-linked actin under lateral stress. By bridging elastic substrate mechanics with molecularly defined actin anchoring, this model system provides new opportunities to examine structure–function relationships within the actin cortex under physiologically relevant strain conditions.

Poster 17 - Kinetic and Structural Dissection of γTuRC Activation by Centrosomin Motif 1 (CM1)

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The γ-tubulin ring complex (γTuRC) is the main microtubule nucleator in cells. However, its native conformation deviates from the ideal 13-fold microtubule symmetry, limiting nucleation efficiency, a key aspect of spatiotemporal regulation of microtubule formation. We previously showed that γTuRC-mediated nucleation can be promoted by a slowly GTP-hydrolyzing tubulin mutant in vitro and that tubulin directly participates in ring closure. In cells, activation requires adaptors such as the centrosomal protein CDK5RAP2, which binds γTuRC via its Centrosomin Motif 1 (CM1). However, CM1 binding only promotes a partially closed conformation, raising the question of whether complete closure is required for activation.

Here, combining TIRF-microscopy-based in vitro reconstitutions with cryo-EM, we dissect γTuRC closure during nucleation. Using TIRF-based nucleation assays we show that CM1 is a potent γTuRC activator, increasing nucleation of wild-type microtubules by over 100-fold, with nearly all γTuRC complexes nucleating at high CM1 concentrations. Nucleation time after CM1 binding varies, reflecting the stochastic nature of the mechanism, even in the presence of an activator. Complementary cryo-EM reveals that CM1 potently accelerates human γTuRC-mediated nucleation by facilitating complete closure of γTuRC as the nascent microtubule assembles, which correlates with the increased activity in TIRF assays.

A 3.7 Å structure of the activated, nucleating complex identifies additional interactions that stabilize the closed state and notably, subunits unique to higher eukaryotic γTuRCs, which maintain the complex in an open, inactive conformation, also participate in its closure and activation.

Together, our results demonstrate that CM1 facilitates γTuRC activation by accelerating conformational closure, but that full activation depends on the nucleation process itself, with tubulin incorporation playing a critical role. This work provides a unified kinetic and structural framework for understanding γTuRC activation and the architectural logic underlying regulated microtubule nucleation in human cells.

Poster 18 - Actin Pulses Without Myosin II Support 3D Cell Intercalation

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Cell intercalation drives coordinated changes in cell position within developing tissues, contributing to organ shaping and ensuring proper physiological function. To change position, cells undergo major reorganisations of their cell–cell contacts, mediated in part by the Adherens Junction (AJ) complex. The mechanical forces required to remodel AJs depend on their association with the subcortical actin network. In 2D intercalation, which has been extensively studied, these forces largely arise from the contraction of the actomyosin network driven by Myosin II (MyoII).

In contrast, 3D cell intercalation, which shapes most organ structures, remains much less explored. To investigate this process, we study the morphogenesis of the Drosophila melanogaster tracheal system, focusing on the dorsal branch (DB), which forms exclusively through 3D intercalation without proliferation or apoptosis. This makes the DB an excellent 3D in vivo model. Notably, unlike 2D intercalation, DB intercalation proceeds independently of MyoII function.

To determine whether actin contributes to 3D tracheal cell intercalation, we developed an innovative in vivo tool enabling actin depolymerisation at single-cell resolution. Our results show that actin alone can sustain and transmit mechanical forces within intercalating cells. By imaging actin dynamics in vivo during 3D intercalation, we uncovered a progressive redistribution of actin from the cytoplasm toward the AJs, initiating at the tricellular AJs (tAJs). At tAJs, actin exhibits a distinctive pulsing behaviour that occurs independently of MyoII. By dissecting the subcellular origin and nature of this pulsatile actin network, we identified a new force-generating platform at tAJs, composed of the actin polymerase Enabled and the Arp2/3 actin nucleator complex. This platform localises to tAJs and varies dynamically with actin pulses. Together, these findings support a new model of 3D intercalation in which MyoII-independent actin pulses drive AJ remodelling across molecular, cellular, and tissue scales.

Poster 19 - Lmod2 Is a Processive Pointed-End Elongator of Actin Filaments

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The actin cytoskeleton drives essential processes like cell migration and muscle contraction. While multiple processive elongators of actin filaments in eukaryotes act at the barbed end, none have been identified at the pointed end. Here, we show that Lmod2 functions as a processive pointed-end polymerase in striated muscle sarcomeres. Single-molecule imaging reveals that Lmod2 stably associates with the pointed end, promoting elongation under conditions that usually cause depolymerization, including in the presence of profilin and tropomyosin. A dilated cardiomyopathy-associated mutation in Lmod2 disrupts its polymerase activity, linking this mechanism to cardiac dysfunction. Cryo-EM structures reveal a stepwise elongation process in which two Lmod2 molecules alternate to recruit monomers. This work establishes pointed-end polymerization as a novel actin assembly mechanism and links it to muscle diseases.

Poster 20 - Unraveling Actin Networks Dynamics and Size Regulation Using Reconstituted Environments.

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In cells, multiple actin architectures co-exist and are, notably, responsible for shape or motility. It remains unclear however how the size and the dynamics of these structures are set-up and maintained, individually and collectively. The ground rules maintaining the integrity and structure of actin networks have yet to be fully understood, especially in the context of network size scaling with varying cell size. In order to get free of the cell complexity, actin networks can be reconstituted from purified proteins in micro-fabricated environments: polystyrene beads are used to support the growth of the network, while flow chambers or microwells create a size-adjustable, defined, and controlled closed environment. Using this set-up, it is possible to decouple parameters of interest, such as environment volume, protein availability, and protein ratios, and investigate their respective influence on actin networks. These results represent a new step in the mechanistic understanding of how the available pool of components can determine actin networks sizes and dynamics. After establishing the ground rules for regulation of a single actin structure, it will be possible to incrementally complexify experiments and ask further questions such as the mechanisms for the co-existence of these various networks in environments of various sizes, or their reactive or adaptative capacities when faced with perturbation.

Poster 21 - SPIN90 Modulates the Architecture of Lamellipodial Actin in an ARPC5L Dependent Fashion.

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When stimulated by nucleation-promoting factors such as WAVE, the Arp2/3 complex generates branched actin networks. In contrast, when activated by SPIN90, the Arp2/3 complex generates linear actin filaments. The Arp2/3 complex in mammals, consists of 8 iso-complexes with different properties as there are two isoforms of Arp3, ArpC1 and ArpC5. Here, using recombinant Arp2/3 iso-complexes with defined composition, we show that SPIN90 selectively activates ArpC5L rather than ArpC5 containing complexes to generate linear actin filaments. Consistent with this, SPIN90 at the leading edge of migrating cells enhances the recruitment of ArpC5L but not ArpC5 containing Arp2/3 complexes to lamellipodia. These SPIN90-Arp2/3-ArpC5L complexes generate linear actin filaments that integrate into lamellipodia and impact protrusion efficiency. Moreover, loss of SPIN90 leads to an enrichment in actin filaments that are more perpendicular to the plasma membrane. Our results demonstrate that SPIN90 regulates the architecture and dynamics of lamellipodial actin in an Arp2/3 iso-complex dependent fashion.

Poster 22 - Cytoskeletal Alterations in Huntington’S Disease Affect Cortical Neuron Development.

Mariacristina Capizzi

Paris Brain Institute

Huntington’s disease (HD) affects brain development from the embryonic stage, beginning before the appearance of symptoms. The mutated huntingtin (HTT) protein plays a crucial role in axonal physiology, particularly through its interactions with microtubule-related proteins that regulate axonal transport and signalling. Our research revealed that the loss of HTT function and mutant HTT both disrupt axonal growth during development by affecting the cytoskeleton of growth cones via microtubule and actin disorganization. These findings prompt us to reconsider the role of HTT in the regulation of cytoskeletal architecture. Furthermore, this cytoskeletal disorganization extends to the axonal initial segment (AIS), affecting its structural integrity and axonal polarity. TRIM46, a microtubule bundler essential for AIS assembly and microtubule organization, is significantly downregulated in HD from the earliest stages of axonal maturation. This correlates with disrupted axonal compartmentalization, cargo transport, and neuronal polarity. These findings prompt further investigation into the role of cytoskeletal dysfunction in HD and the potential of cytoskeletal-targeting agents as therapeutic interventions for HD.

Poster 23 - Binding Features of Actin Binding Domains as a Primary Criterion for Protein Segregation on Different F-Actin Networks During C. Elegans Early Embryogenesis.

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Filamentous actin (F-actin) is one of the main constituents of cell cytoskeleton, being at the base of cellular architecture and orchestrating numerous biological processes involving cell shape remodeling and force generation. Notably, actin/myosin (actomyosin) contractility plays a crucial role during the early phases of C. elegans embryo development as it participates to the initial symmetry breaking and subsequent antero-posterior body axis establishment.

The actin cytoskeleton assembles into diverse structures, such as meshwork, parallel or antiparallel bundles, or under the shape of a branched network. Some Actin Binding Proteins (ABPs) can co-exist or cooperate on specific actin structures, while others segregate on different actin networks. Consequently, the complex interplay of the numerous ABPs could result in the formation of distinct protein network around each actin hyper-structure.

While several mechanisms may be at play, this project aims at deciphering the consequences of the specific binding modality of actin binding domains, such as competition for sterically exclusive F-actin binding sites as well as their residence time on F-actin. We use confocal spinning disc microscopy to explore the subcellular localization of actin binding domains (ABDs) and analyze their intrinsic binding dynamics by Fluorescence Recovery After Photobleaching (FRAP) experiments and Single-Molecule Total Internal Reflexion Fluorescence microscopy followed by single-particle tracking on actin filaments during the first division of the C. elegans embryos.

We hope that such work could provide the first basis for a "grammar" of interactions, indicating how ABD binding features contribute to protein location and function on specific actin network that may be key during C. Elegans early development.

Poster 24 - Confinement-Induced Formation of a Supracellular Peripheral

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Subcellular organization relies on a balance between positional stability and transport of components ranging over a large size scale. Cytoplasmic physical properties emerge as key regulators of this duality over space and time. Indeed, the cytoplasm due to its crowded and viscoelastic nature, can generate forces that oppose random or motor-driven motion of organelles, while also promoting agitation and transport through its active nature. Cytoskeleton networks are established as regulators of cytoplasm physical properties, but the mechanisms by which they may stiffen, fluidize or mix the cell interior remain mostly elusive. Here, using high-resolution imaging and magnetic tweezer-based rheology in sea urchin eggs, we provide an unprecedented study linking architecture, dynamics and mechanical function of cytoplasmic F-actin networks. These consist of dynamic and loosely connected filaments, forming a mesh with an average pore size of ~2µm. Through injection of actin stabilizers, we induced gain in density and connectivity of F-actin networks, with a near 1.5-fold decrease in pore size. Remarkably, we find that this stabilization leads to significant cytoplasm rigidification, with a 10-fold increase in both viscosity and elasticity, and consequent severe reduction in the diffusivity of components with sizes ranging from 200 nm to 10 µm. Interestingly, disassembly of F-actin networks also halts diffusive motion of components of various sizes, but only has a minor impact on cytoplasm viscoelasticity. This suggests that bulk F-actin networks can either promote cytoplasm agitation or act as mechanical stabilizers depending on their density or connectivity. Finally, we show that F-Actin network architecture varies over the cell cycle, with instances of dense and connected networks resembling those induced in eggs with actin stabilizers, and similar impacts on organelle diffusivity and cytoplasm viscoelasticity. Overall, our results propose bulk F-actin networks as tunable modules to regulate cytoplasm mixing and rheology, with important implications for cellular organization.

Poster 25 - Dynamic ERM Polarity Coordinates Speed and Directional Persistence During Cell Migration

Sebastien Carreno et al.

University of Montreal

Efficient cell migration depends on how fast a cell moves but also on how persistently it maintains its direction. While large-scale analyses established a robust coupling between speed and persistence, how cytoskeletal polarity is established, tuned, and dynamically remodeled to sustain this coupling remains unresolved.

Ezrin, Radixin, and Moesin (ERM) are cortical cytoskeletal linkers that integrate mechanical forces at the cell cortex by coupling actin filaments to the plasma membrane. During migration, ERMs were found enriched at the trailing edge, where they promote rear retraction. Here, we discover that ERMs form a dynamic front–back cortical polarity axis whose spatial asymmetry controls the coupling between migration speed and directional persistence.

Quantitative live-cell imaging reveals that ERM tunes the coupling between migration speed and persistence in a non-linear manner. An optimal regime of ERM polarity maximizes both parameters, whereas insufficient or excessive ERM activation uncouples them, leading to fast but erratic or slow but overly constrained migration. This functional regime is associated with a transient redistribution of ERMs to the leading edge during phases of acceleration, establishing a polarized actin cortex downstream of RhoA.

Fluorescence recovery after photobleaching demonstrates that ERM dynamics are spatially specialized: leading-edge ERMs are more dynamic and weakly associated with the plasma membrane, whereas trailing-edge ERMs are more stable, consistent with distinct cytoskeletal roles in protrusion and retraction. Using optogenetic manipulation of Moesin, we further show that imposing asymmetric ERM activation is sufficient to bias cell migration. Sustained ERM activation at one pole promotes retraction, while lower activation at the opposite pole permits protrusion and dictates directional persistence.

These findings identify ERM-mediated cortical polarity as a minimal cytoskeletal organizing module that couples migration speed to directional persistence, providing a general framework for how cells coordinate motion through spatially structured force transmission at the cortex.

Poster 26 - Elucidating the Molecular Mechanisms for the Emergence of Chirality in Actin Networks

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Chirality is a fundamental feature of biological organisation, evident from early embryonic development to large-scale anatomical asymmetries, such as snail pond shell coiling. While actin networks have been shown to spontaneously form chiral structures at the cellular level, the molecular mechanisms underlying the emergence of such asymmetry remain unclear. In this project, we investigate how chirality is transmitted from the scale of the actin filament to the actin network at the cellular scale.

Using microfluidics and open chamber method we reconstitute actin networks crosslinked by α-actinin and expose them to cofilin in order to induce rotation. We show that α-actinin binds stably but non-homogeneously to single actin filaments, protecting them from depolymerization and delaying initial cofilin binding and severing. Cofilin preferentially targets α-actinin–free regions, although colocalization of both proteins can occur at high cofilin concentrations.

Using polarized fluorescence microscopy, we will quantify how cofilin-induced filament rotation is transmitted to the whole bundle. In parallel, we will increase the complexity of the system by reconstituting actin networks grown from formins anchored to lipid surfaces, mimicking actin polymerization from focal adhesion sites. This combined approach will allow us to probe how growth dynamics, network formation and filament rotation together contribute to chiral actin organization at larger scale.

Poster 27 - Self-Organized Flagellar-like Beating in Vitro Driven by Myosin X

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Biological systems can self-organize at large scales through the coordinated activity of energy-consuming molecular motors. Our group has previously shown that myosin II or V, together with a parallel network of polymerizing actin filaments, can self-assemble in vitro into polar bundles that exhibit wave-like beating resembling that of eukaryotic flagella [1]. Here, we examine beating driven by myosin X, a “bundle specialist” involved in filopodia assembly in vivo. We found that myosin X also generates beating with waveforms similar to those produced by myosin II and V. The myosin signal within the bundles consisted of the superposition of two components: a stationary profile and a weaker propagating wave. The density waves were initiated at twice the beating frequency, in relation to curvature waves—like in myosin-V driven bundles [1]. The stationary signal corresponded to the processive motion of motors towards the bundle tip (“streaming”) and remained uniform except near the apex, where it decreased in proportion to the number of actin filaments. Remarkably, FRAP experiments revealed a localized, preferential myosin attachment at the dynamic tip of the bundles. These observations suggest a feedback mechanism between myosin affinity for actin and bending of the filament bundle.

[1] M. Pochitaloff, M. Miranda, M. Richard, et al.: Flagella-like beating of actin bundles driven by self-organized myosin waves. Nature Physics 18, 1240–1247 (2022).

Poster 28 - Mechanical Confinement and Calcium Signaling Orchestrate Germ Cell Activity

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Development of the female gamete requires the formation of a structure called ovarian follicle (or egg chamber in Drosophila), where the germ cells are surrounded by somatic cells. In Drosophila, egg chambers are produced during the morphogenetic process of encapsulation where a group of 16 interconnected germ cells (a germline cyst) is progressively enclosed by a layer of somatic cells. We showed previously that germ cells are not passive but actively generate cortical contraction waves during encapsulation. A defect in this contractility leads to defects in the formation of egg chambers. Thus, contractility in germ cells is essential for correct encapsulation; however, the developmental signals that trigger this cortical activity in germ cells are not known. Here we reveal that confinement of germ cells by the somatic cells which surround them at the time of encapsulation acts as a mechanical signal to trigger and/or maintain their cortical activity. We developed a minimal system allowing direct physical manipulation of intact germline cysts based on cell dissociation and confinement/ deconfinement methods. Combining physical perturbations, genetic tools, pharmacology, and live imaging allow us to dissect how mechanical cues regulate germ cells excitability. We found that isolated germline cysts display drastic reduction of cortical contractility. Restoring confinement immediately reactivates contractility in germ cells. We also show that Calcium Signaling is required during encapsulation and participates in this response to confinement. Using a calcium reporter, we showed that calcium is highly dynamic in germ cells during encapsulation. We demonstrated, unexpectedly, that artificially increasing basal calcium level in germ cells blocks contraction waves propagation and that confinement is required to maintain a moderate basal level of calcium to sustain germ cell activity. Our new confinement–deconfinement system opens the way to mechanistic studies that directly couple physical manipulation with in vivo genetics in an intact developmental context.

Poster 29 - Supracellular Acto-Myosin Cables Guide Cell Movement During Tissue Closure

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Tissue closure is a fundamental morphogenetic process required for normal embryonic development and tissue repair. Disruptions to these tissue closure mechanisms can lead to severe developmental abnormalities, such as the formation of cleft palate. However, the molecular and cellular processes underlying this essential function remain poorly understood, especially when fusing tissues are initially far apart. Moreover, the role of surrounding or substrate tissues in regulating tissue closure is largely unknown.

Adult epidermis in Drosophila forms from the fusion of two imaginal discs which have moved toward each other from opposite sides of the animal and merged to eventually differentiate. They use the underlying larval epidermis as a substrate for migration, which is subsequently eliminated to make space for the adult epidermis. We investigated the function of the larval epidermal tissue in thorax closure and we showed it actively participates to the movement of the disc, while it was initially considered as a passive migratory substrate for the colonization of new adult epidermal cells. Larval epidermal cells generate contractile supracellular cables bridging the two discs to move towards each other. Combining super-resolution microscopy and live imaging approaches, we revealed the dynamics of actin cytoskeleton remodeling to produce supracellular cables. Based on temporal transcriptomic profiles we generated, we are now identifying the molecular factors which regulate the contractility of these larval epidermal cells.

In conclusion, by using live imaging techniques, we uncovered a novel mode of tissue closure in which neighboring tissues produce supracellular acto-myosin cables to guide cell movement. This mechanism may also play a role in tissue closure processes in vertebrates.

Poster 30 - A DIAPH1-SOWAHC-CFL1 Axis Regulates Macropinocytosis and Chemoresistance in Cervical Cancer

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Formin mDia1 (DIAPH1) is a central regulator of actin dynamics underlying cell migration and plasma membrane remodeling, processes frequently dysregulated in cancer. Here, we uncover a previously unrecognized DIAPH1-dependent signaling axis controlling macropinocytosis and chemotherapeutic sensitivity in cervical cancer.

Using label-free quantitative mass spectrometry in CRISPR/Cas9-engineered DIAPH1 knockout (KO) HeLa cells, we mapped the DIAPH1 interactome and identified SOWAHC as a novel binding partner. The interaction was validated in reciprocal co-immunoprecipitation experiments, and SOWAHC protein levels were found to depend on DIAPH1.

Loss of either DIAPH1 or SOWAHC increased phosphorylation of the actin-severing protein cofilin (CFL1) under both basal and EGF-stimulated conditions, indicating reduced CFL1 activity. Functionally, DIAPH1, but not SOWAHC, was required for EGF-induced ruffling and optimal random cell migration. In contrast, macropinocytosis was impaired in both DIAPH1 and SOWAHC KO cells and was nearly abolished upon DIAPH1 loss. The persistence of ruffling in SOWAHC-deficient cells despite defective macropinocytosis uncouples ruffle formation from macropinosome closure and identifies SOWAHC as a selective regulator of macropinocytic uptake. CFL1-deficient cells exhibited constitutive membrane ruffling and preserved EGF-induced macropinocytosis, likely due to upregulation of the cofilin homolog Destrin.

Importantly, DIAPH1 loss markedly sensitized HeLa cells to cisplatin, the standard-of-care therapy for advanced cervical cancer, consistent with the macropinocytosis-dependent uptake of the cisplatin receptor.

Given the association of CFL1 with poor prognosis in cervical cancer, we examined the status of this axis in human tumors. Analysis of a tissue microarray comprising 322 cervical cancer cases revealed elevated DIAPH1 and SOWAHC expression in tumors relative to normal epithelium, stage-dependent expression patterns, and an inverse correlation with phospho-CFL1 levels.

Together, these findings identify a DIAPH1-SOWAHC-CFL1 signaling axis that coordinates actin remodeling, macropinocytosis, and chemotherapeutic responsiveness, uncovering an unappreciated vulnerability in cervical cancer.

Poster 31 - Influence of Structural Myosin Variants on Actin Network Architecture and Dynamics

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Beyond all the complexity of living cells, they can be described from physics perspective as spherical compartments filled with active matter. Active matter can be defined as systems in which energy is continuously consumed at the microscopic scale, leading to emergent behaviours in the macroscopic scale. In cells actin filaments and myosin motors are the core elements of the active matter. And one of the most fascinating features of active matter systems is the emergence of internal flow. In confined geometries, this flow can give rise to self-propulsion. This phenomenon demonstrates a fundamental aspect of cellular motility. While internal flows and active forces are central to cellular functions; the dense, complex and heterogeneous nature of the cytoplasm makes it challenging to investigate core physical principles behind. To overcome this challenge, we use minimal but functional model systems by preserving key ingredients. In this work, we investigated the effect of structural myosin variants on the dynamics, mechanics and architecture of the in vitro reconstituted actomyosin networks by using video particle tracking and imaging techniques. Our findings provide fundamental insights into understanding determinants of flow and contractility inside living cells.

Poster 32 - Dynamics of Competitive Actin Architectures

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In cells, different actin architectures coexist in a dynamic manner and are in principle in competition for the same pool of actin monomers and actin-binding proteins. In addition, cells constantly experience environmental changes requiring a fast adaptation of their structures in order to modify shape or type of movement for example. However, a comprehensive view of how these networks coexist in a dynamic manner in the cell, where resources are limited and how cells adjust the size of their dynamic actin architectures in relation to overall cell size is still lacking. Decoupling these parameters to assess their relative contributions in a cellular context is extremely challenging, so we have developed a bottom-up approach to identify the key molecular mechanisms involved in the coexistence of dynamic competitive actin architectures. We used a reconstituted system consisting of beads propelled by actin polymerization or micropatterns functionalized with lipids to mimic the polymerization close to a membrane in microwells, enabling us to work with a limited number of components. With this system, we reconstituted several dynamic actin architectures, competing for a limited pool of protein, over a period of multiple hours. We demonstrated that protein turnover and recycling ensure a fair distribution of the resources. Using a mathematical model, we demonstrated that competition strength is defined by turnover rate, amount of available proteins and number of competing structures. Finally, we established that when there are too many competing structures, turnover is no longer sufficient for all networks to coexist. By highlighting the importance of protein turnover and the limited pool of actin monomers, this work clearly illustrates possibilities and limitations for actin networks to coexist under resource constraints.

Poster 33 - Geodesic Actin Networks in p190RhoGAP-KO Endometrial Cancer Cells: How? Why? What For?

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The project focuses on p190RhoGAPs proteins, specifically the two isoforms, p190A and p190B, encoded by the ARHGAP35 and ARHGAP5 genes, respectively. P190RhoGAPs (p190) proteins are GTPase Activating Protein domains (GAP). Their role is to negatively regulate the activity of Rho GTPases, mainly RhoA. Rho GTPases regulate the organization of the actin cytoskeleton and, consequently, cell migration, invasion, and proliferation.

P190 proteins are altered in endometrial cancer. Indeed, the ARHGAP35 and ARHGAP5 genes have been identified as significantly mutated in 14% and 10% of endometrial tumors (Lawrence et al. 2014), (Pinault et al. in preparation), respectively. The majority of mutations are truncating mutations leading to a loss of function of p190 proteins.

To explore their role, we used CRISPR-mediated p190A and p190B KO HEC1A cells. We found that an atypical reorganization of actin, called “Cross-Linked Actin Networks” (CLANs), was observed in 25% of KO-p190A or KO-p190B cells. CLANs are described as geodesic actin structures composed of actin nodes connected by branches. CLANs are well-known and described in trabecular network cells, but nothing is known about the presence of CLANs in endometrial cancer.

The aim of the project is therefore to characterize this atypical actin organization by analyzing its composition, the signaling pathways that induce these CLANs, and their impact on cancer cells. We identified that alpha-actinin, MLC2 and proteins from the PDLIM family are present on CLANs. In addition, we identified pathways that are involved in their formation and maintenance in endometrial cells, both positive and negative regulation. Notably, partners of p190A and p190B identified by BioID are involved in a negative regulation of CLANs. Thanks to this knowledge we aim to better characterize the impact of CLANs on endometrial cells and tissues.

Poster 34 - Visualization of Actin Remodelling During Clathrin-Mediated Endocytosis in S. Cerevisiae Using Cryo-Electron Tomography

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Clathrin-mediated endocytosis is the primary cellular uptake pathway. Despite its importance, the actin architecture that drives membrane invagination remains elusive. Using cryo-electron tomography, we captured endocytic events at various stages in an unperturbed cellular environment.

A ribosomal exclusion zone was present at all endocytic sites. Within this zone, a dense actin network surrounded the membrane invagination. We only observed a clathrin coat at the tip of the invagination. To visualize the actin network, we traced the filaments using both the semi-automated Amira segmentation tool and our in-house AI-based tool. The network consists of filaments that are tangential to the side of the membrane invagination and that are oriented at a high angle to the plasma membrane. We observed bent actin filaments at the tip and side of the invagination, indicating that these filaments store elastic energy. Template matching of Arp2/3 complex-mediated branch junctions indicated that the actin filaments grow towards the plasma membrane, where actin monomers are incorporated. However, as they cannot push the plasma membrane due to its high turgor pressure, the filaments grow into the cytoplasm instead. As these filaments are anchored to the clathrin coat by myosin motors, this creates a pulling force on the coat. Together with the release of elastic energy, we propose that this can drive membrane invagination.

Building on our research into podosomes, and using the spatial distribution of branches around the invagination and the plasma membrane, we will now simulate successive generations of branches. This will enable us to resolve branch nucleation in space and over time during clathrin-mediated endocytosis in yeast.

Poster 35 - How Formin Uses Profilin to Nucleate Actin Filaments

Mark Zweifel, Naomi Courtemanche

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Formins assemble actin filaments for a wide range of cellular processes through dynamic interactions with the abundant, actin-binding protein profilin. Although the process of formin-mediated filament elongation is well characterized, the mechanism by which formins overcome energetic barriers to create new actin filaments is poorly understood. We coupled single-molecule and ensemble reconstitution assays with deterministic kinetic modeling to reveal how formins nucleate filaments. We found that formins assemble filament nuclei by sequentially binding three actin monomers in a process that takes place in parallel to spontaneous actin assembly. Whereas the formin FH2 domain preferentially binds actin monomers, the flexible FH1 domain facilitates de novo filament formation by binding and transferring profilin-actin complexes to a free FH2 dimer. This delivery mechanism stimulates filament nucleation in a striking, position-dependent manner that is tuned by profilin's differential affinity for multiple binding sites along the FH1 domain. The specialized architectures of FH1 domains dictate a balance in nucleation and elongation activities, thereby maximizing the efficiency of formin-mediated actin polymerization.

Poster 36 - Identification of Microproteins as Novel Actor of Secretion in Drosophila

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Secretory activity is essential for cell-cell communication and interorgan crosstalk and its dysfunction could disrupt tissue homeostasis, leading to developmental disease. While the machinery of secretion has been largely studied, the mechanisms underlying its regulation are not fully understood.

Recently, the family of microproteins, encoded by small ORFs and less than 100 aa in length, has been identified as new potential regulators of biological processes. Since a substantial proportion of microproteins encompass transmembrane domain, we hypothesize they might regulate secretory processes.

To identify novel regulators of secretion, we use the Drosophila spermatheca of the female reproductive system, which stores spermatozoa in its lumen. After mating, spermathecae undergoes cell remodeling to become highly secretory and release molecules essential for spermatozoa survival and female fecundity. Thus, we aim to identify novel transmembrane microproteins regulating the secretory machinery using spermatheca.

Since the cell arrangement of the spermatheca is largely unexplored, we first characterized its cell organization and secretory dynamics. We found that secretory and epithelial cells are tightly interconnected, a structural arrangement that likely facilitates communication within the organ also supporting their communication with surrounding tissues.

Moreover, using apical markers, we better defined the specialized microvilli-rich structure dedicated to secretion of the secretory cells. In particular, we found that after mating, secretory cells strongly remodel their actomyosin cytoskeleton surrounding this secretory region.

Secondly, to identify regulators of the secretory machinery, we performed comparative RNA-seq on virgin and mated spermathecae, specifically screening for transmembrane microproteins transcriptionally regulated upon mating. In parallel, we are investigating the role of one candidate microprotein which localizes to early endosomes, supporting its function in spermatheca remodeling and secretion.

Together, this work will enable the identification of microproteins involved in spermatheca remodeling supporting high secretory activity, and consequently spermatozoa survival and female fertility through their function in membrane biology.

Poster 37 - Role of βIII-tubulin (TUBB3) in the Progression and Chemoresistance of Ovarian Cancer

Baptiste D'Urso

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Ovarian cancer is one of the most aggressive gynecological cancers. It is often diagnosed at a late stage, contributing significantly to female mortality. This type of cancer is often treated, before and/or after surgical resection, with a combination of two chemotherapeutic agents: cisplatin and paclitaxel (or another Taxol derivative). Paclitaxel (PTX) binds to the β-subunit of microtubules and blocks their depolymerization, thereby preventing the formation of a mitotic spindle and entry into mitosis [Zhang et al. 2020]. In ovarian cancer, several clinical studies report the frequent development of resistance to taxane-based chemotherapies, including paclitaxel [DM. Roque et al. 2013].

βIII-tubulin, encoded by the TUBB3 gene, is a tubulin isotype initially associated with neuronal tissues, whose aberrant expression has been observed in several solid tumors, notably ovarian cancer.

In this study, we analyzed TUBB3 expression in taxane-sensitive and taxane-resistant ovarian cancer cell lines. Our results show significant overexpression of TUBB3 in resistant cell lines. Paclitaxel treatment induces TUBB3 expression. Functional analyses suggest that this overexpression contributes to the dynamic stability of microtubules, thereby reducing the efficacy of anti-microtubule agents. TUBB3 expression appears to alter the cellular phenotype, particularly in terms of adhesion, migration, and invasion, thereby promoting the metastatic process.

Taken together, our data suggest that TUBB3 may play a key role in the mechanisms of chemoresistance and may serve as a prognostic biomarker as well as a potential therapeutic target.

Poster 38 - Non-Muscle Actinopathy-Associated Loss of Function Variants of Actin Modulate the Reorganization Properties of the Actin Cytoskeleton

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Loss-of-function mutations in the gene of cytoplasmic β-actin represent a subgroup of diseases called non-muscle actinopathies. Among these mutations we investigated the cellular effects of a missense variant, G302A, and a four-amino-acid deletion, S338-I341del, in patient-derived fibroblast cells. We found that neither of the mutations affected the organization of actin or the width of the actin-filament bundles, while the mutation G302A reduced the stiffness of the cells as measured by using atomic force microscopy. The latter effect might be associated with the misorganization of tubulin in the mutant cells. When we challenged the cells by monolayer stretching and followed the mechanically-induced reorganization of the actin cytoskeleton, we found that the mutant cells behaved in a different way compared to wild type cells. G302A mutant cells showed more dense actin filament bundles within the cells. At the same time, the extent of cofilin reorganization from the cell periphery was increased upon stretch, and this correlated with an increased cofilin phosphorylation. In the case of the deletion, while the extent of cofilin phosphorylation increased, the extent of reorganization was unaltered; rather, the phosphorylation of myosin light chain, important in counteracting external force, was drastically reduced. We could partially rescue this fascinating effect by overexpressing the active form of the formin mDia. The latter observation may help devising an accessible treatment of patients with the S338-I341 deletion in the cytoplasmic β-actin sequence.

Poster 39 - Big Bad Bundlers: Mechanism of IQGAP1 Bundling

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Actin filament bundles with distinct dynamics, dimensions, and polarity form the structural basis of cellular protrusions such as filopodia, lamellipodia, invadopodia, and stress fibers, which are essential for cell motility, intracellular transport, and mechanical integrity. IQ-motif containing GTPase-activating protein 1 (IQGAP1) regulates actin filament growth and organizes filaments into higher-order bundled structures. Unlike classic actin-bundling protein that mediate side-binding interactions through well-defined actin-binding domains (I.e. fascin or alpha-actinin), IQGAP1 is proposed to bundle actin filaments via dimerization of its calponin homology (CH) domain. However, the specific residues and mechanisms underlying IQGAP1-mediated bundling, as well as the higher-order structures it generates, remain poorly defined. Here, we identify and characterize key residues within the CH domain of IQGAP1 that contribute to actin filament bundling. Using purified proteins and Total Internal Reflection Fluorescence (TIRF) microscopy, we assessed the effects of wild-type IQGAP1 and a bundling-deficient mutant (IQGAP1 DiB) on actin filament organization by quantifying the fluorescence intensity of labeled actin. To establish a quantitative benchmark for bundling, we generated a dose–response curve using the canonical bundling protein fascin, enabling discrimination between single filaments and bundles containing defined numbers of filaments. Using this framework, we show that IQGAP1 predominantly forms bundles composed of two to three filaments, whereas IQGAP1(DiB) but fails to bundle actin altogether. Notably, IQGAP1 favors antiparallel filament organization, while the DiB mutant exhibits a shift toward parallel bundling. Two-color TIRF assays reveal robust localization of IQGAP1 to filament sides at bundling sites, whereas the DiB mutant displays reduced side-binding. Together, these findings demonstrate that specific CH-domain residues are essential for IQGAP1-mediated higher-order actin bundling, with implications for the architecture, mechanics, and function of actin-rich cellular structures.

Poster 40 - Role of Septins in Maintaining Epithelial Mechanical Integrity During Morphogenesis

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Septins are ubiquitous, highly conserved, guanosine triphosphate (GTP)-binding proteins that organise into filaments and associate with membranes and other cytoskeletal elements, including actin and microtubules. Owing to these properties, they are referred to as the fourth cytoskeletal element. Discovered in yeast, septins are extensively studied for their role in cytokinesis; however, how septins contribute to the mechanical forces underlying tissue-scale morphogenesis in vivo remains unclear. Here, we investigate the pivotal role of septins in cell morphogenesis using Drosophila gastrulation as a robust, genetically tractable model system.

Septins are enriched at regions with high mechanical activity, including the apical cortex and yolk plugs at the bases of epithelial cells. Using the FLP-DFS genetic system, we deplete the maternal contribution to endogenous septins, and during gastrulation, as epithelial cells attempt to undergo stereotypical, coordinated shape changes, they progressively tear apart, ultimately leading to the collapse of the epithelial tissue. Basally, epithelial cells are connected to the yolk via yolk plugs, which progressively constrict and close as gastrulation proceeds. In septin-depleted embryos, yolk plugs reopen, and the basal membrane is fragmented.

Globally, loss of septins causes severe morphogenetic defects, including impaired ventral furrow formation and incomplete posterior midgut invagination, leading to gastrulation failure. Notably, these lethal defects emerge specifically during gastrulation, when mechanical tension builds during epithelial remodelling, rather than cellularization. These observations suggest that septins maintain epithelial mechanical integrity specifically under elevated mechanical stress generated during morphogenesis. Given their colocalization with actomyosin-rich regions, septins likely resist cell deformation by coordinating with contractile cortical networks during tissue-scale collective cell rearrangements.

Poster 41 - Novel mNeonGreen-Tropomyosin Fusions Reveal Functional Redundancy and Formin-Isoform Independent Localization of Yeast Tropomyosin Paralogs

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Actin cytoskeletal networks are composed of a wide range of regulatory proteins, each contributing to spatial and functional specificity. Tropomyosin is an actin-binding protein which protects actin filaments from cofilin-mediated disassembly. Distinct tropomyosin isoforms have long been hypothesized to differentially sort to subcellular actin networks and impart distinct functionalities. Nevertheless, a mechanistic understanding of their functional contributions to actin dynamics remains limited. In our study, we present functional mNeonGreen-Tpm fusion proteins that exhibit complete functionality as a sole copy, surpassing limitations of existing probes and enabling real-time dynamic tracking of Tpm-actin filaments across eukatyotic systems such as S. cerevisiae, S. pombe, and mammalian cells. Further, we utilize these fusion proteins to reveal that S. cerevisiae Tpm paralogs, Tpm1 and Tpm2, indiscriminately bind to actin filaments nucleated by either formin isoform - Bnr1 and Bni1 in vivo. These observations contrast to the paradigm of Tpm-formin pairing shown proviously in S. pombe and mammalian cells, suggesting species-specific evolutionary trajectories. We show that Tpm levels regulate balance between linear and branched actin networks in yeast cells and discover that increased Tpm2 levels can organize functional actin cables in absence of Tpm1 in vivo and restore normal actin network homeostasis. Overall, these findings support a concentration-dependent and formin-isoform independent spatial sorting mechanism for Tpm1 and Tpm2, and reveal unexpected functional redundancy between these Tpm paralogs despite their evolutionary divergence, providing insights into how duplicated tropomyosin genes evolve in a simple single cell eukaryote.

Poster 42 - Biorientation of Chromosomes in Cell Division -1 (BOD-1) in the Development of the Nervous System.

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Indian Institute of Science Education and Research Pune

In dividing cells, Biorientation of Chromosomes in Cell Division -1 (BOD-1) helps to align and biorient chromosomes at the metaphase plate by regulating kinetochore-microtubule attachments. BOD1 inhibits Protein Phosphatase 2A (PP2A) which helps to maintain phosphorylation, allowing kinetochore microtubule rearrangements. Loss of BOD1 causes chromosome misalignment, aneuploidy and subsequent apoptosis. Human patients with BOD1 mutations present with Intellectual Disability, learning and sensory deficits, high frequency hearing impairment and schizophrenia, while mice models also show ataxia and Autism Spectrum Disorder like symptoms, indicating a non-canonical role of BOD1 in terminally differentiated neurons. Proteins such as KMN (Knl1/Mis12/Ndc80) network proteins that regulate microtubule interactions during cell division, are known to regulate actin dynamics in neurons. Similarly, the secondary role of BOD1 in non-dividing cells may also be through the regulation of any of the cytoskeletal elements including actin and microtubules.

Using In Situ Hybridization we show that Bod1 mRNA is expressed in the developing brain and spinal cord of zebrafish embryos. We also confirm the protein expression of Bod1 in the nervous system in zebrafish embryos through a reporter line. Further, using a CRISPR mediated knockout model of Bod1 in zebrafish, we observe that there is no change in morphometric parameters and no microcephaly, indicating no change in the overall cell numbers and cell division is unaffected in the mutants. However, we see behavioural defects such as increased spontaneous tail coiling activity and optomotor defects in the mutant, indicating a role of Bod1 motor and optomotor neural circuits. Using a Dark Flash Habituation paradigm, we see a trend in the form of decreased habituation-learning, indicating cognitive deficits in the mutant. Thus, we conclude that Bod1 has a distinct function in neurons, and its function may be through the regulation of the actin and microtubule cytoskeletal elements.

Poster 43 - aPKC Tuning Regulates Epithelial Polarity and Cell Morphology in Drosophila

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Epithelial cell polarity is fundamental to morphogenesis, with polarity proteins defining membrane domain identity and size. While the antagonistic interactions among apical, basolateral, and junctional proteins are well characterised, how these dynamic changes couple to cytoskeletal remodelling and cell shape remains an open question.

Atypical Protein Kinase C (aPKC) is a key determinant of apical identity in Drosophila, phosphorylating lateral and junctional proteins to exclude them from the apical domain. Using a chemical-genetics approach to acutely inhibit aPKC activity, we uncovered a hierarchy of aPKC substrates within the follicular epithelium. Low inhibitor levels lead to apical localisation of Yurt and trigger rapid endocytosis of Crumbs, while other substrates remain unaffected. Since Crumbs functions as both an apical anchor and an aPKC complex activator, we expected its depletion to disrupt phosphorylation of all aPKC targets. Surprisingly, Par-6 and aPKC remained stably localised at the apical membrane despite Crumbs endocytosis, though they failed to relocalise after mitosis without Crumbs. This indicates that Crumbs is essential for initial recruitment of Par-6/aPKC but dispensable for short-term maintenance.

We also found that the apical localisation of Yurt induced by partial aPKC inhibition promotes apical constriction through recruitment and activation of Rho kinase and Myosin II, key effectors of actomyosin contractility. Loss of Yurt impaired epithelial resistance to mechanical stretching, suggesting that Yurt plays a role in a stretch-response mechanism. As cells are stretched, apical aPKC activity becomes diluted, enabling Yurt to accumulate apically and the recruitment of Myosin. We are currently testing whether this Yurt plays a similar role in cells' resistance to stretching during development.

Our work reveals how polarity cues dynamically interface with the actomyosin cytoskeleton to control epithelial architecture, highlighting a dual role for aPKC and its partners in polarity and force generation during morphogenesis.

Poster 44 - Loss of ARHGAP19 Function Causes Inherited Neuropathy, with Cytoskeletal and Axonal Defects Supported by in-Vivo and in-Vitro Evidence

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Charcot-Marie-Tooth (CMT) disease, also called hereditary motor and sensory neuropathy (HMSN), is the most prevalent Mendelian inherited neuropathy, varying in prevalence across populations but estimated on average to occur at a frequency of ~ 1 in 2,500 individuals. Patients with CMT can range from mildly affected to severely disabled, and the disease presents with progressive distal wasting, weakness and sensory loss, often accompanied by foot deformity.

By using next-generation-sequencing, we identified 16 recessive variants in the RhoGTPase activating protein 19 gene (ARHGAP19) causing motor-predominant neuropathy in 25 individuals from 20 unrelated families. The ARHGAP19 protein acts as a negative regulator of the RhoA GTPase and we used various approaches to model these variants.

In-vitro biochemical and cellular assays revealed that patient variants impair the GTPase-activating protein (GAP) activity of ARHGAP19 and reduce ARHGAP19 protein levels. Parallel in-vivo studies in Drosophila and zebrafish models demonstrate a conserved role for ARHGAP19 orthologs in regulating locomotor capacity. In zebrafish model, we observed more pronounced axonal bundles and a significantly increased number of axonal branches, suggesting that ARHGAP19 LOF may cause CMT in part by perturbing the cytoskeleton of motoneurons. Indeed, alterations in neuromuscular junction morphology of Drosophila model larvae are similar to those observed in mutants for presynaptic cytoskeletal proteins, and our transcriptomic analyses in patient-derived fibroblasts showed that pathways associated with motor proteins and the muscular cytoskeleton are downregulated by ARHGAP19 LOF.

Taken together, our findings establish ARHGAP19 variants as a cause of inherited neuropathy acting through a loss-of-function mechanism. ARHGAP19 is a GAP with activity towards RhoA, and the downstream effectors are exciting potential new targets for therapy. Efforts are ongoing to identify potential small molecule drugs by use of Drosophila melanogaster drug repurposing screen to modify the disease progression.

Poster 45 - A Structural Model for the Autoinhibition of the γ-TuRC-Activating CM1 Domain

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The spindle is composed of microtubules, which are primarily nucleated from centrosomes. Precise spatiotemporal regulation of microtubule nucleation is essential for accurate spindle assembly and to prevent cell division and developmental defects. The γ-Tubulin Ring Complex (γ-TuRC) stimulates microtubule nucleation. During mitosis, γ-TuRCs are activated specifically at centrosomes through binding of the highly conserved N-terminal centrosomin motif-1 (CM1) domain, found in γ-TuRC tethering proteins such as Drosophila centrosomin (Cnn). However, how CM1 binding is prevented in the cytosol remains unclear. In this work, we provide a structural explanation for CM1 autoinhibition, revealing that intramolecular interactions mediate Cnn N-terminal folding to block γ-TuRC binding. Using a combination of structural approaches in collaboration with the structural biology team of Françoise Ochsenbein (I2BC Paris-Saclay), we show that the CM1 forms a conserved coiled-coil hairpin that buries a key phenylalanine residue known to be essential for γ-TuRC binding. While this mechanism appears sufficient for autoinhibition in humans, in flies an additional interaction with an upstream CM1 autoinhibition (CAI) domain is required to cover the second phenylalanine residue of the CM1 dimer. Collectively, our data provide a structural model for CM1 autoinhibition and reveal important evolutionary differences in CM1 regulation.

Poster 46 - An Apical Supracellular Actin Network Transmits Forces over Long Distances in Squamous Cells

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Epithelial tissues form protective barriers while supporting critical functions such as absorption and secretion. Their structural and functional integrity relies on adherens junctions, which coordinate migration and transmit forces between adjacent cells by connecting their actin cytoskeleton. We identified the presence of an apical supracellular actin network in squamous epithelial cells. Using squamous carcinoma A431 cells as a model, we characterized this network composed of star-shaped actin structures interconnected by linear actin bundles that span multiple cells. We found that the network's formation and maintenance require actomyosin contractility and intact adherens junctions, while tight junctions seem dispensable. Furthermore, this network dynamically reorganizes as cells migrate and preferentially aligns with the direction of movement. This contractile structure generates mechanical tension that extends across the apical surface of multiple cells. Our findings suggest that this supracellular actin network functions as a long-range force transmission device in squamous cells, advancing our understanding of the biomechanical properties of epithelia.

Poster 47 - Modelling Microtubule Dynamics in Axonal Growth Cones

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Neurons establish connections during embryonic development by sending out long polarized cell protrusions called axons. At the tip of the axon, actin polymerization leads to extension of lamellipodia and filopodia and axon elongation. This process is strongly regulated by extracellular guidance cues that signal to locally affect cytoskeletal dynamics. While actin provides the driving force for axon elongation, the dynamic distribution of microtubules (MTs) in the growth cone has a decisive role in axon navigation.

We investigate the role of microtubule (MT) and MT-actin interaction in growth cone guidance. We use live imaging of MTs in primary neurons to extract parameters of dynamic instability, such as rates of polymerization and de-polymerization as well as the transitions between those two dynamic states. These parameters are then used to model MT dynamics and MT-actin interactions within realistic growth cone morphology. We use the software Cytosim (Nédélec et al., 2007), to model individual dynamic MTs within the growth cone, and include a simplified actin network to simulate actin-MT interactions and retrograde flow.

Extracting growth cone shapes from imaging data led us to realize that shape alone has a strong influence of MT distribution and length. To obtain the typical splayed array of MTs however, interaction with internal actin bundles is necessary.

Combining microscopy, image analysis and modelling, we established a steady-state model of growth cone MT dynamics: In simulations of growth cones with symmetric (straight growing) and asymmetric (turning) growth cone morphologies, MTs distribute similarly to what we could measured in cultured neurons. These results highlight that the known intrinsic properties of the microtubules together with the interaction of the actin network, are sufficient to explain the MT architecture.

Poster 48 - Mechanical Properties of the Actin Cortex During Cell Cycle

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The actin cytoskeleton is a key player for mammalian cells mechanics and control of their shape. A particularly striking example is the shape change occurring during mitosis, when the cell swells and rounds up. This process is regulated by the actin cortex, a dense and dynamic network of actin filaments, myosin motors and associated proteins, located right under the plasma membrane.

It is already known that the mitotic cortex is thinner and more tense than the interphase one [1]. We want to investigate the mechanical properties of the cortex during all stages of the cell cycle. To do so, we use the FUCCI system in HeLa cells that uses fluorescence to identify not only mitosis but also G1, S and G2 phases [2]. We combine this with the “Magnetic Pincher”, a tool that was recently developed by the team and that uses two magnetic microbeads to measure the thickness and elasticity of the actin cortex [3]. To analyze our mechanical data, we use a two-part contact mechanics model, combining linear Hookean theory with the non-linear van Wyk's model of wool fibers [4].

Thanks to these techniques, we were able to confirm that the mitotic cortex is thinner than the interphase cortex. We also compare the elasticity of the cortex during the whole cell cycle and find out that the mitotic cortex seems softer than the interphase cortex. We also look at the linearity of the cortex to understand the rearrangement of the cortex structure. Finally, we try to combine all our results to get a whole picture of the actin cortex mechanics during cell cycle.

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Poster 49 - The Survival of Motor Neuron Protein as a Novel Actin-Bundling Factor: Identification and Mechanistic Insights

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Spinal muscular atrophy (SMA) is a severe neurodegenerative disorder characterized by the selective loss of functional α-motoneurons in the spinal cord and brain stem, resulting in progressive skeletal muscle atrophy due to denervation. SMA is caused by reduced levels or impaired function of the ubiquitously expressed Survival of Motor Neuron (SMN) protein. While SMN is best known for its essential roles in mRNA metabolism and motor neuron maintenance, accumulating evidence implicates SMN in actin cytoskeleton regulation. However, the structural and mechanistic basis of SMN–actin interactions has remained largely unexplored, in part due to longstanding limitations in obtaining SMN in a pure and biochemically tractable form. Here, we report the successful recombinant purification of multiple SMN variants and their comprehensive characterization with respect to actin regulation and structural organization. Using biochemical and biophysical approaches, we show that wild-type SMN directly interacts with both monomeric (G-actin) and filamentous actin (F-actin), with binding primarily mediated by the N-terminal half of the protein. In contrast, the C-terminal YG-box drives SMN self-association, promoting oligomerization into tetrameric, octameric and higher assemblies, as demonstrated by in vitro pull-down assays and analytical ultracentrifugation. Total internal reflection fluorescence (TIRF) microscopy revealed that SMN inhibits both actin nucleation and filament elongation. Strikingly, SMN induces robust actin bundle formation when present during polymerization or upon addition to prepolymerized actin filaments. These bundles are decorated by SMN in a uniform, periodic pattern along their length, identifying SMN as a previously unrecognized, disease-relevant actin-bundling protein . Ongoing work aims to resolve high-resolution structures of the F-actin–SMN complex, dissect the impact of post-translational modifications on SMN function, and elucidate cytoskeletal roles of SMN beyond the nervous system.

Poster 50 - Targeting Cytoskeletal Dynamics to Impair Invasion in Diffuse Intrinsic Pontine Glioma

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Diffuse midline gliomas (DMGs) are among the most aggressive pediatric brain tumours, with Diffuse Intrinsic Pontine Glioma (DIPG) representing the deadliest. It is characterized by diffuse infiltration and resistance to therapy. Recurrence typically arises beyond the initial tumour site, implicating invasion as a major driver of treatment failure. Bone Morphogenetic Protein 7 (BMP7), a secreted growth factor elevated in invasive DIPG cells, contributes to invasion and therapeutic resistance by modulating microtubule dynamics and immune responses. Highly invasive DIPG cells display microtubule-enriched cytoskeletal remodelling, increased BMP7 expression, and reduced intermediate filament content, suggesting a microtubule-dominant migratory phenotype. We hypothesize that BMP7 promotes invasion and immune evasion by stabilizing microtubules and polarizing naive immune cells toward a tumour-supportive phenotype. We will manipulate BMP7 levels in DIPG cells to assess microtubule changes via live-cell imaging and quantify matrix degradation (MMP2 activity). Co-culture assays with human microglia will determine how BMP7 influences immune cell polarization. BMP7 inhibition will be tested in combination with microtubule-targeting agents and radiation to evaluate cooperative therapeutic effects. Finally, zebrafish xenografts will enable real-time in vivo visualization of DIPG invasion and immune interactions. This work will reveal BMP7's role as a regulator of DIPG motility and immune modulation, offering new therapeutic intervention in this incurable pediatric cancer.

Poster 51 - Dissecting the Molecular Mechanism Regulating the Subcellular Targeting of Ena/VASP Proteins

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Ena/VASP proteins constitute powerful actin polymerases that accumulate at sites of active actin assembly, including microspike and lamellipodia tips, and focal adhesions (FAs), where they drive processive elongation of filament-barbed ends. Vertebrates express three Ena/VASP members: VASP, Mena and Evl, which share an N-terminal EVH1 domain, followed by a proline-rich region and a C-terminal EVH2 domain harboring actin-binding sites and a tetramerization motif. Yet, the mechanisms regulating Ena/VASP recruitment remain elusive. The availability of knockout mutants lacking all three paralogues (EVM-KO), allowed us to define the minimal domains required for subcellular Ena/VASP targeting. Notably, the EVH1 domain alone can mediate all aspects of VASP targeting when combined with the tetramerization motif to increase its functional affinity through avidity. Subcellular targeting has been additionally assumed to be regulated by post-translation modifications, including phosphorylation of tyrosine and serine residues. However, in contrast to phosphorylation of residues Y39 and S157, which were proposed to either prevent or be required for Ena/VASP targeting, we identify phosphorylation of Y16 in vertebrates, or the corresponding residue Y17 in the Ena/VASP ortholog UNC-34 of C. elegans, as the decisive regulatory event negatively controlling Ena/VASP recruitment and function. Accordingly, expression of phosphomimetic mutant Y16E, but not Y39E, in EVM-KO cells failed to rescue microspike formation and abolished accumulation of Ena/VASP proteins at lamellipodia tips and FAs. Furthermore, a phosphomimetic UNC-34 (Y17E) mutant in C. elegans is as paralyzed as an unc-34 null due to neuronal migration defects. These findings were corroborated by multicolor-TIRF imaging of VASP-mediated actin-filament elongation on lamellipodin-derivatized beads in the presence of soluble wild-type VASP and phosphomimetic mutants Y16E and Y39E, and by analytical ultracentrifugation using the Ena/VASP ligand ActA from Listeria. The detailed investigation of other processes including 2D-cell migration, protrusion persistence, actin turnover and actin assembly at Listeria surfaces are currently in progress.

Poster 52 - Dual Control of Microtubule and Actin Dynamics by Fidgetin-like 1 Dictates Developing Axon Trajectories

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Accurate axon navigation is essential for assembling neuronal circuits. This process depends on the growth cone, a cytoskeleton-based machinery that interprets extracellular guidance signals and translates them into precise axon mechanical behaviors. However, the molecular mechanisms coupling MT and F-actin dynamics to guidance signals remain largely unknown.

We previously identified the ATPase Fidgetin-like 1 (Fignl1) as a microtubule regulator critical for zebrafish motor axon pathfinding at guidance choice points. We here investigated whether and how Fignl1 controls axon guidance at the midline in the mammalian developing brain using the visual system as a model. We showed that Fignl1 loss-of-function affects Retinal Ganglion Cell (RGC) axon midline crossing and causes an excess of ipsilateral projections, as defective non-canonical Wnt5a/β-catenin signaling does. Consistently, Fignl1 loss-of-function leads to premature desensitization of contralateral RGC axons to Wnt5a repellent cue. This phenotype is characterized by their inability to maintain the inhibition of growth cone protrusive activity (based on F-actin nucleation) induced by Wnt5a. Consistently, Fignl1 colocalizes with F-actin filopodia in RGC growth cones and inhibits F-actin nucleation and polymerization in reconstructed cell-free systems. We further showed that Wnt5a-dependent phosphorylation of Fignl1 at S329 by PKC (i) enhances its F-actin-inhibitory function while suppressing its MT-depolymerizing activity, and (ii) is required for contralateral RGC axon repulsion in vitro and (iii) midline crossing in vivo.

In conclusion, our work uncovers a previously unrecognized mechanism by which non-canonical Wnt signaling tunes MT and actin dynamics to control cell behaviors, with broad physiological and pathological impacts ranging from embryonic morphogenesis to neuronal circuit wiring and cancer biology.

Poster 53 - Hypoxia Causes Microtubule Reorganisation in Epithelial Cells

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A highly regulated and dynamic microtubular cytoskeleton is vital for normal cellular function and the maintenance of homeostasis. While much is known about the mechanisms by which microtubules are regulated under physiologic conditions, the effect of pathological stimuli and how this contributes to disease progression remains poorly understood. Hypoxia is a prominent microenvironmental feature of a range of pathological states including inflammation, cancer, and ischemia. The effect of hypoxia on microtubules remains inconclusive and understanding the physiological response of the microtubules may provide significant advancement in the treatment of diseases. When Caco-2 intestinal epithelial cells are exposed to hypoxia they present with a perturbed microtubule structure, despite no change in protein expression of -tubulin. This effect has also been shown to be independent of HIF-1alpha, a key driver of hypoxia-induced transcription mediating cellular adaptation. Despite the appearance of the microtubules, there is no change to the free and polymerised pools of tubulin and thus we propose that they are not simply depolymerised but instead alter their structure to adapt to this period of hypoxic stress. Hypoxic exposure also results in an altered microtubule structure in primary colonic and small intestinal epithelial cells but not colonic fibroblasts, indicating some cell-type specificity. Future work will aim to characterise the mechanism by which microtubules respond to hypoxia as well as the functional consequences.

Poster 54 - Exploring Nuclear Actin Dynamics with Atomic Force Microscopy in Living Cells

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Actin contributes to several cellular processes, such as maintaining and regulating cell shape, motility and cell division. Even though the presence of actin in the nucleus was confirmed decades ago, the many roles of monomeric and polymeric actin in the nucleus are just being uncovered. These include transcription, chromatin organization, DNA damage repair, oocyte development and nuclear architecture.1

To investigate the mechanical properties of nuclei, we utilize atomic force microscopy (AFM). The expression of different nuclear-targeted actin mutants allows us to specifically control actin dynamics within the nuclear compartment and to measure changes in nuclear stiffness. Inducing nuclear actin polymerization through overexpression of either actin wildtype or actin-S14C increased the nuclear Young's modulus significantly, whereas overexpression of non-polymerizable actin-R62D had no effect.2

Our previous work revealed that nuclear envelope rupture in migrating cancer cells induces the formation of a transient and dynamic nuclear F-actin network.2 Here, we manually puncture nuclei with AFM cantilever tips to further explore the role of nuclear actin polymerization after nuclear envelope rupture. Utilizing icGAS as a marker for nuclear envelope rupture, we observed that actin rapidly polymerizes within the nucleus at the site of rupture, potentially plugging the rupture site and preventing leakage of nuclear contents. These F-actin structure persist for several minutes post nuclear envelope rupture. Analysis of Force-Distance curves acquired during the nuclear puncture experiments revealed an active force generation towards the cantilever, whenever nuclear actin was polymerized. Pharmacological treatment with actin depolymerizing drugs or expression of NLS-actin-R62D prevented the formation of nuclear actin plugs and resulted in deeper indentation of the cantilever into the nuclei. These results point towards a novel role of nuclear actin polymerization as a nucleoskeletal component necessary to withstand and counteract forces that damage the nuclear compartment.

1:(Ulferts et al., 2024) doi.org/10.1242/jcs.261630

2:(Kamaras*, Frank* et al., 2025) doi.org/10.1038/s44318-025-00566-2

Poster 55 - Examining a Non-Centrosomal Microtubule-Organizing Center in Drosophila Fat Cells

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The centrosome is the best-known microtubule-organizing center (MTOC); however, many differentiated cell types rely on non-centrosomal MTOCs (ncMTOCs) to organize their microtubule cytoskeleton. In larval Drosophila fat body cells, an ncMTOC is assembled at the nuclear envelope. This structure depends on the spectraplakin Shot, an evolutionarily conserved multidomain protein with central roles in cytoskeletal organization and crosslinking of actin and microtubules. Loss of Shot results in displacement of the ncMTOC

from the nuclear envelope to an ectopic cytosolic focus. In a previous loss-of-function genetic screen targeting microtubule-dependent motility, we found that pre-fusion autophagosomes undergo dynein–dynactin-dependent transport toward the perinuclear ncMTOC. Consistently, Shot depletion redirected autophagosomes to the ectopic cytosolic ncMTOC, indicating that organelle positioning closely follows ncMTOC localization. Building on these findings, we investigated the molecular architecture and positioning of the fat body ncMTOC using targeted loss-of-function approaches against ncMTOC components, including Shot, Msps, and Msp300. Strikingly, loss of Msp300, a nesprin-family protein required for anchoring the ncMTOC to the nuclear envelope, caused the ncMTOC to relocate beneath the plasma membrane. Autophagic vesicles were concomitantly repositioned to this new site, demonstrating that ncMTOC positioning is plastic and can be reassigned under specific conditions.

Together, our results identify the fat body ncMTOC as a dynamic and relocatable microtubule-organizing structure whose positioning depends on defined anchoring factors and directly influences intracellular organization. These findings provide new insight into ncMTOC architecture and highlight an unexpected level of flexibility in microtubule organization in differentiated cells.

Poster 56 - NuMA1 Controls Myonuclear Motility in Striated Skeletal Muscle Through AMPK Activity and Is Impaired in Duchenne Muscular Dystrophy.

Vincent Gache

INMG-PGNM-MNCA (Inserm/CNRS/Ucbl1)

Skeletal muscle consists in a bundle of thousands post-mitotic multinucleated cells, called myofibers, in which myonuclei are evenly spaced and positioned at the periphery. This myonuclear positioning i) shapes myonuclear domain (MND) in myofibers, essential for the transcriptional integrity of myofibers, ii) is driven by cytoskeleton and associated proteins and iii) is required for proper myofiber functions. In numerous of muscle diseases (i.e. myopathies), alteration of myonuclei localization (internalized and/or mispositioned) contributes to myofiber misfunctioning, supporting the need to better understand the fundamental mechanisms that regulates myonuclei dynamics and to restore the establishment of myonuclear domains in these diseases. In this study, we show that in Duchenne muscular dystrophy (DMD) myofibers, myonuclei are more dynamic and contribute to the failure in MNDs settings. To identify new actors regulating this MND settings, we performed a mass spectrometry (MS)-based proteomic analysis to identify MAPs in myotubes/myofibers and performed a siRNA screening on selected candidates. This approach highlighted NuMA1 as a new factor controlling myoblast fusion and myonuclear positioning through the control of nuclear-microtubule-organizing-center (n-MTOC) integrity and microtubule network orientation. Strikingly, while NuMA1 is restrained to myonuclei in mononucleated myoblasts, it progressively accumulates in the cytoplasm during muscle cell differentiation, preferentially with microtubule (MT) nucleation spots at the vicinity of the nuclear membrane. We identified that AMP Kinase activity has an essential role in NuMA1 nuclear accumulation through the specific phosphorylation on serine-1853 and the ability of myonuclei to accumulate NuMA1 is correlated to their motility in myofibers. Finally, we show that nuclear NuMA1 content is increased in DMD patients and mdx mouse model, contributing to more dynamic myonuclei that can manipulated pharmacologically through the control of AMPK activity. Altogether, our data identify a novel mechanism by which nuclear sequestration of a MAP allows to couple nuclear positioning and motion to MT organization.

Poster 57 - Citron Kinase Balances Protrusion and Retraction and Controls Spreading and Anteroposterior Polarity in Interphase Migrating Cells

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Non-muscle myosin II (NMII) activity regulates cell morphology through its contractile and actin cross-linking functions. NMII-dependent contraction is controlled by phosphorylation of the regulatory light chain (RLC) downstream of specific kinases such as MLCK and is counteracted by the phosphatase MYPT1, which is inhibited by RhoA/ROCK signaling. Citron kinase (CITK) also phosphorylates NMII downstream of RhoA, in the context of cytokinesis. Here, we report that in interphase cells CITK mainly localizes to protrusive regions, specifically the lamellipodium. siRNA-mediated depletion of CITK in HeLa cells induced pronounced overspreading and loss of anteroposterior polarity. Unlike NMII-deficient cells, CITK-depleted cells exhibited highly bundled actin fibers together with enlarged protrusive regions. We found that CITK-deficient cells were strongly contracted, as indicated by elevated phospho-RLC levels, enlarged focal adhesions, and nuclear accumulation of YAP. Abnormal spreading and polarity loss were rescued by expression of siRNA-resistant CITK. In contrast, Arp2/3 inhibition prevented cell enlargement but failed to restore polarization, whereas inhibition of ROCK or MLCK dissolved actin bundles without recovering cell size or polarity. Overspreading appeared to be Rac1-dependent, supported by the aberrant redistribution of actin-regulatory proteins, including Arp2/3 and WAVE2, along the entire cell periphery. Using DIC imaging, we observed that CITK-depleted cells attached to the substrate approximately twice as fast as control cells and displayed altered membrane ruffling dynamics, characterized by an accelerated initial wave of actin ruffles followed by an abnormal quiescent phase as cells reached maximal spreading. Together, these findings support a dual role for CITK in regulating membrane ruffling, promoting Rac1-driven protrusion while restraining excessive spreading through RhoA/mDia signaling. Ongoing work focuses on defining the local contribution of CITK to the RhoA/Rac balance using photoactivation approaches, as well as mapping the role of distinct CITK domains and associated protein networks in cell spreading and polarization using single-molecule microscopy and mass spectrometry.

Poster 58 - Identification of PKN2 and MOB4 as Coordinators of Collective Cell Migration

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In animals, collective cell migration is critical during development and adult life for repairing organs. It remains, however, poorly understood compared with single cell migration. The polymerization of branched actin by the RAC1-WAVE-Arp2/3 pathway is established to power membrane protrusions at the front of migrating cells, but also to maintain cell junctions in epithelial monolayers. Here we have identified novel regulators of collective cell migration using a two-pronged approach: candidates were extracted from publicly available RAC1-WAVE-Arp2/3 dependency maps and screened in a second step using CRISPR/Cas9 genetic inactivation. In a wound healing assay, PKN2 knockout (KO) MCF10A cells display decreased collective migration due to destabilization of adherens junctions, whereas MOB4 KO cells display increased collective migration with a loss of migration orientation. Upon wound healing, PKN2 relocalizes to lateral junctions and maintains coordinated migration in the monolayer, whereas MOB4 relocalizes to the front edge of leader and follower cells collectively migrating towards the wound. The role of MOB4 in controlling collective migration requires YAP1, since MOB4 KO cells fail to activate YAP1 and their phenotype is rescued by constitutively active YAP1. Together, these findings reveal two complementary activities required for coordinating cells in collective migration.

Poster 59 - Expansion of the Membrane Associated Periodics Skeleton During Neurite Extension

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Neurons rely on a complex cytoskeleton to protect their long and thin processes lifelong. A central component of the neuronal cytoskeleton is the membrane associated periodic skeleton, a network composed of actin rings that are interspaced by spectrin tetramers that forms a periodic lattice along most of the length of a neurite. Suggestive of an essential role for neuronal stability and function, mutations in spectrin result in neurite breakage in C.elegans and are associated with ataxia in human patients. To ensure its neuroprotective role, the membrane associated periodic skeleton needs to continiously expand with the outgrowing neurite. Most of the length of a mature neurite occurs during neurite stretch growth, a growth phase following growth cone collapse when the neurite must scale its length to the growing skeleton of the organism. Although this growth phase accounts for the major length of many neurites and occurs from embryogenesis until adulthood, it remains poorly understood how the cytoskeleton continously extends with the growing neurite. To close this gap, we developed a temporally controllable fluorescent probe, which allows us to visualize the expansion of newly synthesized, endogenous spectrin into the extending membrane associated periodic skeleton during neurite stretch growth in living C. elegans. We can show that spectrin expands the outgrowing lattice from specific extension sites that are distributed along the length of the neurite at rates that scale with the neurites growth rate. Surprisingly, spectrin incorporation and turnover were especially enriched within the presynaptic area of our model motor neuron, despite the uniform expansion of the membrane associated periodic skeleton. Finally, genetic perturbation experiments to interfere with synapse function or positioning supports the central role of the synapse in regulating spectrin kinetics within this motor neuron.

Poster 60 - Dynamics of Supracellular Keratin Bundling in Stretched Epithelia

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There is broad consensus that intermediate filaments play a key role in protecting cells and tissues from large deformations. However, little is known about how they fulfil this function. Here we demonstrate that epithelial cells undergo a slow adaptation to stretch that couples a star- bundling transition of keratin filaments and the escape of the nucleus from its keratin cage. The bundling transition begins with a depletion of keratin filaments at tri-cellular junctions followed by a progressive accumulation in thick bundles that bisect cell-cell junctions. Bundling is a cooperative process that initiates in a few scattered cells and propagates to their neighbours through desmosomes, leading to the growth of multicellular clusters that contain a percolated network of thick keratin bundles. Bundling dynamics are slow and strongly influenced by the interaction between actin and keratin. Informed by a computational model, we provide evidence that keratin bundling generates a compressive stress on the nucleus, which is relaxed by nuclear escape from the keratin cage. The topological transitions identified here provide epithelia with a multiscale mechanism to adapt to sustained stretch.

Poster 61 - SH3BGRL Proteins Are Thioredoxin Fold–Containing Actin Filament Pointed End Capping Proteins (TPECs)

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Cellular actin dynamics are tightly regulated by actin-binding proteins with specialized domains. Here, we identify SH3BGRL (SH3-domain binding glutamic acid-rich like) proteins as modulators of actin dynamics, characterized by their thioredoxin (Trx) fold and the absence of the canonical enzymatic site. The Trx fold is generally associated with enzymatic activity; however, in this context, it functions non-enzymatically to enhance actin nucleation, inhibit depolymerization and cap the growing pointed end of the actin filament. Our results indicate that all SH3BGRL isoforms weakly promote actin nucleation in vitro by stabilizing energetically unstable actin dimers and trimers. Using all atoms molecular dynamics simulations and in vitro assays that directly probe the pointed end of the actin filament, we show that SH3BGRL proteins efficiently inhibit actin subunit addition at the pointed end by direct association with the terminal actin subunits. However, SH3BGRL proteins are less effective at preventing subunit loss from the pointed end, but they can cooperate with tropomodulin to enhance this activity in an isoform-specific manner, indicating that all isoforms are capable of forming a tripartite complex with actin and tropomodulin. Interestingly, Saccharomyces cerevisiae Aip5 (actin-interacting protein 5) appears to bind the pointed end via a similar mechanism, implying that a subset of thioredoxin fold proteins may be evolutionarily conserved actin-binding proteins across kingdoms.

Based on our results, we propose a new and more appropriate name that reflects the function of the SH3BGRL protein family: thioredoxin fold–containing pointed end capping proteins (TPECs).

Poster 62 - Molecular Principles Governing the Spatial Segregation of Actin Networks During Cell Migration: A Biochemical Study of the Roles of Arp2/3, α-Actinin-1, and Myosin II.

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Cell migration is a highly coordinated process driven by the dynamic remodeling of the actin cytoskeleton. Actin filaments are organized by actin-binding proteins (ABPs) into distinct networks with specific architectures, dynamics, and mechanical properties that collectively enable cell movement. At the leading edge, a dense, branched actin meshwork known as the lamellipodium, nucleated by the Arp2/3 complex, generates protrusive forces that push the membrane forward. In contrast, within the cell body, linear actin filaments are crosslinked by α-actinin-1 and assembled into antiparallel bundles, contracted by myosin II, to form stress fibers that generate tension between focal adhesions and pull the cell forward. Efficient migration relies on coordinated but spatially segregated actin networks, yet the mechanisms underlying this organization remain unclear. While actin nucleators are known to define network architecture at the molecular scale, we hypothesize that actin filament orientation, polarity, and density within these networks selectively recruit or exclude motors and crosslinkers. To test this hypothesis, we use biochemical assays with purified proteins. We combine kinetic analyses of actin assembly using fluorescence spectroscopy with single–actin-filament observations by TIRF microscopy, as well as in vitro reconstitution of lamellipodia- and stress-fiber-like networks. These approaches allow us to dissect the localization, cooperation, and competition between key actin regulators in cell migration, including Arp2/3, myosin II, and α-actinin-1. By linking actin network architecture to the selective recruitment of its regulators, this work uncovers fundamental principles that drive the spatial organization of the cytoskeleton during cell migration.

Poster 63 - Mitochondrial Homeostasis Mechanisms in Migrating and Differentiating Neurons During Cortical Development

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Mutations in the X-chromosomal Doublecortin (DCX) gene are a leading cause of malformations of cortical development (MCDs), resulting in lissencephaly in hemizygous males and subcortical band heterotopias (SBH) in heterozygous females. Patients of both sexes suffer from intellectual disability and severe epilepsy. Data from Dcx-knockout mouse models have revealed that defects in neuronal migration, morphology, and hyperexcitability are very likely to cause these symptoms. However, the precise molecular mechanisms remain unknown and effective treatment is not available. Recently, mitochondrial abnormalities have been described in hippocampal neurons of Dcx-knockout mice, which could possibly underlie the cellular defects. Preliminary data from our group suggest mitochondrial trafficking defects and disrupted mitochondria-ER interactions, indicating disturbed mitochondrial homeostasis and calcium regulation. Here, we will further characterise these mitochondrial impairments during hippocampal development to link them to neuronal migration, heterotopia formation, and epilepsy. We will use FAST-SIM microscopy to track mitochondrial movement and microtubule dynamics in real time, investigate potential Dcx interactions with dyneins and kinesins, and employ functional assays and Ca2+ imaging to assess mitochondrial function during neuronal migration and maturation. Finally, we will test whether pro-mitophagic drugs can rescue migratory defects and hyperexcitability. We will use both Dcx-knockout mice and human induced pluripotent stem cell-derived models to enhance the translatability of our findings, and potentially pave the way for future therapeutic approaches.

Poster 64 - Actin Cortex Mechanics Ruled by Density and Contact

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The cortex is a thin contractile meshwork of actin filaments that provides structural integrity to the cell and regulates its shape. We developed the cell pincher, a new method to directly probe the actin cortex of live cells by positioning large magnetic beads across the cell boundary. We are thus able to measure precisely and dynamically the thickness of the cortex, as well as the its mechanical properties with a direct measurement unbiased by whole cell deformation. We made the striking observation of a systematic inverse correlation between the cortex thickness and its elastic modulus. Thinner cortices are stiffer, while thicker cortices are softer, from ~20 kPa for 150nm cortices to ~2kPa for 400nm. This relationship is robust to drug treatments affecting contraction and nucleation and is observed in a host of fibroblasts, epithelial, immune and cancer cells. This inverse correlation is adequately explained by strong variations in actin filament density, and we verify by simultaneously measuring actin intensity and cortex thickness that the quantity of actin is practically unchanged in thick cortices compared to thin ones. This potential control of cortex density, through swelling of a dynamic network, is expected to have a major functional impact on the cell surface mechanics with distinct bending and area expansion modulus. Additionally, when some cortices are sufficiently compressed, we observe a strong stiffening that is well explained by fiber mechanics theory where the elasticity is governed by the density of contacts between filaments. This fiber-like behavior is nearly-absent in strongly contractile cell types, but becomes dominant when cells are treated with myosin inhibitors or in less contractile cell types. Rapid transitions between these states is observed, and can be triggered by opto-genetic activation of the RhoA pathway. These results show that cortex mechanics thus emerge from density-controlled actin architectures and contractility.

Poster 65 - Defining and Quantifying the Human Actinome

Henry Higgs

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As we approach the 100th anniversary of its discovery, actin continues to excite and perplex many of us. Many proteins that influence actin dynamics and organization have been discovered, but numerous aspects of their function remain to be defined. Even less defined is how these proteins work together to affect the assembly and disassembly of specific actin-based structures in a spatially- and temporally-defined manner to carry out a specific cellular function. Two examples that my lab studies are CIA (calcium-induced actin, PMID 41279891) and ADA (acute damage-induced actin, PMID 35290799). Both are transient (lasting <2 min) and extensive (CIA polymerizes 20-30% of a U2OS cell's actin in 30 sec). While we know something about the assembly factors for these processes (INF2 for CIA, Arp2/3 complex and FMNL formins for ADA), we do not know how the filaments are organized for their specific functions, nor how they are disassembled so quickly.

Spurred by this knowledge gap, my laboratory is taking a more holistic approach to actin dynamics, by defining 'the actinome', defined as 'the ensemble of proteins controlling the dynamics, organization, and force-generating capability of the actin cytoskeleton'. By our current tally, the human actinome contains ~300 proteins. One initial goal is to quantify actinome protein concentrations (in molecules per cell) in select human culture lines (HeLa, others) and primary cell types (neutrophils, platelets), using label-free whole-cell proteomics. Our current results suggest that publicly-available datasets are insufficient for robust quantification, particularly for small proteins like thymosins, and we are using alternate means for their quantification. In this presentation I will use these data to assess 1) how actin is 'buffered' by monomer-binding proteins, and 2) how this information can be used to make mechanistic insights on CIA, ADA and other actin-based processes.

Poster 66 - Mechanical Imprinting at ECM-Adhesions Via the Talin Interactome Leads to Lasting Effects on the Cardiomyocyte Cytoskeleton and Contractile Function

Thomas Iskratsch

Queen Mary University of London

Cells connect to the extracellular matrix (ECM) via integrins, through which they sense the mechanical properties of their environment and in response adapt their behaviour and function(1- 2). On their cytoplasmic face sits a complex mechanical signalling machinery with the mechanosensitive protein talin an integral component.

Here, we aimed to investigate how talin mechanosensing is regulated in cardiomyocytes (the contractile cells of the heart), and how this is further transduced downstream, influencing the cardiomyocyte phenotype and function.

Using a combination of nanopillar arrays, FRET, FRAP and optogenetic approaches we identify a specific mechanism for cardiomyocyte rigidity sensing. Single cardiomyocyte adhesions sense simultaneous (fast oscillating) cardiac and (slow) non-muscle myosin contractions. Together this modulates the tension on the mechanosensitive adaptor protein talin(1), with effects on the molecule confirmation and interactome. Immunoprecipitation and FRAP experiments indicate that the talin interaction proteins DLC1, RIAM and Paxillin each preferentially bind to adhesions and talin at specific extracellular matrix stiffness(3). Optogenetic experiments confirm direct competition between the individual proteins. The competition is further regulated through adhesion related kinase pathways, suggesting a mechanical imprinting mechanism. Loss of DLC1 binding at the adhesions results in phenotypic changes of the cardiomyocytes and myofibrillar disarray.

Together, these experiments surprisingly reveal a mechanical imprinting that is resulting in changes to the talin interactome, cytoskeletal organisation and cardiomyocyte mechanics and could be critically involved in the progression of cardiac disease and heart failure.

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Poster 67 - Role of Unconventional Myosin 1C on Actin Reorganization and Lysosome Membrane Dynamics

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Myosin 1C is an unconventional, actin-based motor protein that links membranes to the actin cytoskeleton and regulates intracellular trafficking and cytoskeletal remodeling. Although the molecular basis of Myo1C's regulation of actin dynamics at membranes is not entirely understood, mutations in this protein have been associated with diseases such as metabolic

dysfunction, hearing loss, and retinal degeneration. In this work, I combined biochemical reconstitution, live actin assays, and in vitro membrane systems to study the molecular activity of Myo1C. Myo1C was purified from HEK293 cells using affinity chromatography and tested for its effect on actin organization. In polymerization assays, Myo1C altered the rate of linear filament elongation. Using branched actin networks with the VCA domain of human WASP protein and the Arp2/3 complex, I observed that Myo1C increased the polymerization rate of branched actin formation and the number of junctions compared to controls. These findings could indicate that Myo1C enhances branched actin nucleation, although additional replicates are required for statistical confirmation. To explore the impact of Myo1C function on membranes, I employed Giant Unilamellar Vesicles (GUVs) with acidic luminal pH and PIP3-containing lipids to allow myosin recruitment. Incorporating the LysoFlipper probe, a fluorescent probe that targets the lysosome membrane and reports membrane tension changes through fluorescence lifetime changes, into the GUVs will enable measurement of membrane tension by fluorescence lifetime imaging microscopy. Myo1C was recruited to GUVs and promoted the assembly of actin cages, both linear and branched, around the vesicles. Primary results showed that actin remodeling and Myo1C recruitment could increase membrane tension, suggesting a mechanistic role in integrating actin dynamics with organelle mechanics. In conclusion, these results suggest Myo1C has an essential function in regulating actin cytoskeleton architecture and membrane properties. This will provide a framework for understanding how Myo1C affects hearing loss, retinal degeneration, and metabolic disorders.

Poster 68 - VASH1-Mediated Tubulin Detyrosination Is Regulated by Phosphorylation in DRG Neurons, Shaping Growth Cone Architecture.

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The establishment of functional neuronal circuits depends on precise axon navigation and branching, processes driven by tightly controlled cytoskeletal dynamics within the growth cone. Post-translational modifications of microtubules, particularly the α-tubulin detyrosination/tyrosination cycle, play essential roles in defining microtubule dynamics and stability. This cycle is characterized by the enzymatic removal and re-addition of a gene-encoded tyrosine residue at the C-terminus of α-tubulin. While tyrosination implies the tubulin tyrosine ligase (TTL), tubulin detyrosination involves the vasohibin cysteine proteases in combination with their peptide cofactor SVBP (VASH1-SVBP and VASH2-SVBP) and the atypical metalloprotease MATCAP1. How extracellular cues could influence this cycle is poorly understood.

Here, we uncover a direct link between C-type natriuretic peptide (CNP)-cGMP-PRKG1α signaling pathway and tubulin tyrosination status in dorsal root ganglion (DRG) neurons. CNP activation induces a marked DRG growth cone enlargement, together with increased tyrosinated tubulin in the growth cone and reduced detyrosinated tubulin along the axon. These changes were abolished by DT-3, a PRKG1α inhibitor. Pharmacological inhibition or genetic deletion of the tubulin detyrosinating enzyme VASH1 mimics the CNP-induced cytoskeletal reorganization. In vitro kinase assay identifies VASH1 as a direct substrate of PRKG1α, which selectively phosphorylates a serine residue within its C-terminal domain. This specific phosphorylation markedly suppresses VASH1-SVBP binding to microtubules and detyrosination activity of the complex. This phosphorylated form of VASH1 is revealed within DRGs by the use of a newly developed antibody and abolished in the presence of DT-3.

These findings reveal VASH1-SVBP detyrosination enzyme as a key effector downstream of CNP-cGMP-PRKG1α signaling, establishing a mechanistic link between an extracellular guidance pathway and the microtubule modification machinery that shapes neuron architecture.

Poster 69 - Mechano-Metabolic Regulation of Cell Motility and Invasion in Glioblastoma

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Glioblastoma (GBM), the most common type of primary brain cancer in adults, is a devastating and often fatal disease. Rapid invasion of the GBM cells into healthy brain parenchyma prevents curative surgery and limits the efficacy of adjuvant therapies. Previous work has shown that GBM cells are sensitive to the mechanical properties of the surrounding microenvironment, which influences the speed and directionality of their migration. However, the importance of such biomechanical cues during GBM invasion in vivo remains poorly understood.

Metabolic plasticity and adaptation represent another key feature of GBM. These cancers are typically characterized by altered lipid and amino acid metabolism, with multiple metabolic profiles coinciding with the different molecular/transcriptional GBM subtypes within a single tumor. Importantly, several recent studies have highlighted that the metabolism, mechanosensing, and migration of cancer cells are intrinsically connected. In GBM, invasive phenotypes have previously been associated with both stiff and compliant microenvironments, as well as with both mitochondrial respiration and aerobic glycolysis. A likely reason for these discrepancies is the extensive heterogeneity and plasticity of different GBM tumors and models—a trait which warrants the use of holistic and unbiased research techniques.

We are combining high-content CRISPRa screening, cutting-edge mechanobiology tools, and orthotopic GBM xenografts to investigate the mechano-metabolic regulation of cell motility in GBM. An unbiased screen targeting ~3,000 metabolic genes in GBM spheroids spreading on mechanically defined substrates will reveal the metabolic pathways that promote or inhibit GBM cell migration in soft vs. stiff microenvironments. Proof-of-concept experiments using co-cultures of human GBM cells demonstrate the suitability of the technique for separating slow-moving cells from highly migratory ones. After the CRISPRa screen has been concluded, top hits will be investigated in vitro and in vivo to provide mechanistic insights about their relationship with established mechanosensory pathways and cellular structures, including the cytoskeleton.

Poster 70 - Exploring α-Tubulin N-Acetyltransferase 1 (ATAT1)-Dependent Molecular Pathways in Lung Cancer Cells.

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The cytoskeleton of eucaryotic cells is formed by actin microfilaments, microtubules (MTs) and intermediate filaments (IFs) that cooperate in essential cytoskeletal functions such as cell adhesion, division and migration. MTs are composed of α/β-tubulin heterodimers and their diversity and functions are regulated by the presence of different post-translational modifications (PTMs) on α/β-tubulin.

α-tubulin acetyltransferase 1 (ATAT1) is the enzyme responsible for α-tubulin acetylation at lysine 40 (K40), a well-studied α-tubulin PTM. Although ATAT1 and tubulin acetylation have been implicated in cancer, little is known about the mechanisms and the relevance of ATAT1 in cytoskeleton-mediated functions of cancer cells. By using non-small cell lung cancer (NSCLC) ATAT1-silenced models we investigated whether ATAT1 directly modulates cell division and migration, both hallmarks of cancer when deregulated.

Herein, we demonstrate that ATAT1-silenced cells exhibit prolonged mitosis and increased mitotic defects, including polar and lagging chromosomes, as well as chromatin bridges. Moreover, ATAT1 silencing alters the balance between α-tubulin tyrosination and detyrosination during mitosis, suggesting an effect on MT dynamics. Proper regulation of MT dynamics is not only important for mitosis but also for cell migration. Indeed, we also show that ATAT1 silencing affects migration rate in wound healing assay and focal adhesions (FAs) signalling.

Finally, RNA sequencing experiments were employed to identify the molecular pathways modulated in ATAT1-silenced cells. The omics results support the in vitro evidence of ATAT1's involvement in cell migration, highlighting alterations in genes that regulate the actin cytoskeleton, IFs, FAs and cell motility. Differential proteomics analyses confirm the modulation of FA pathways.

In conclusion, in this work we demonstrated that ATAT1 and acetylated tubulin play a significant role in the build-up and function of the mitotic apparatus and influence the migration capacity and adhesion properties of cells, features that are often deregulated in cancer.

Poster 71 - Uncovering the Role of QPCTL in Microtubule Cytoskeleton Regulation Using CRISPR-Cas9 Screening

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Cellular division, a tightly regulated process, involves microtubules forming the mitotic spindle to ensure accurate chromosome segregation, governed by the spindle assembly checkpoint (SAC). As microtubule dynamics are essential for mitotic fidelity, they are employed as major pharmacological targets in cancer therapy. Microtubule-targeting agents (MTAs), like nocodazole, disrupt microtubule dynamics, causing mitotic arrest and apoptosis. Conversely, certain genes confer resistance to MTA therapy when downregulated.

To identify key regulators microtubules, we conducted a CRISPR-Cas9 positive screen using Nocodazole, a microtubule destabilizer, to select for genes involved in microtubule cytoskeleton regulation. Next-Generation Sequencing coupled with MAGeCK analysis identified few sgRNA' conferring survival advantage under Nocodazole treatment. Our study explores the role of QPCTL, a CRISPR-Cas9 screening gene, conferring nocodazole resistance.

We further observed that QPCTL knockout (QPCTL-KO) cells showed an increased survival, faster wound closure, and bypassed nocodazole-induced G2/M arrest, confirmed by fluorescence-activated cell sorting. Reintroducing QPCTL cDNA reversed these effects, thereby restoring G2/M arrest. Immunostaining with Nocodazole indicated that QPCTL KO cells had less prometaphase arrest, bypassing the cell cycle possibly due to mitotic slippage or improved spindle assembly. Cold-stable assay showed better-assembled microtubules in QPCTL KO cells compared to wild-type (WT) cells. Additionally, MAD2L2, a SAC component and reported QPCTL interactor, showed decreased levels in QPCTL KO cells in western blot analysis. This suggests QPCTL KO may enhance microtubule assembly through MAD2L2 modulation. We employed proximity labeling to identify QPCTL interacting partners, and further studies are required to explain how QPCTL-KO enhances Nocodazole resistance and Microtubule dynamics

Poster 72 - In-Plane Resistance of Tissue to External Strain

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Polar epithelial cells assemble into thin yet robust sheets that withstand mechanical in-plane stress through strong conformal contacts, maintained by specialized cell–cell junctions anchored to the viscoelastic cortex. Here, we examine how free-standing epithelial monolayers respond mechanically to central indentation, using a colloidal probe to probe orientation-dependent effects. Apart from this, we also employ cysts and hemicysts (domes) to explore the mechanical properties of lumen-forming tissue. Tissue tension is quantified by modeling the deformed monolayer as a minimal surface. Viscoelastic properties are obtained from force relaxation. Our analysis shows that tension in the basal cortex dictates the purely elastic response to in-plane extension, whereas the apical cortex is softer and dissipative, producing hysteresis at shallow indentation depths. At greater indentation depths, apico-basal polarity becomes irrelevant: cells undergo apical compression, and the response is dominated by the prestressed basal cortex driving in-plane mechanics. These insights are particularly important for lumen-forming epithelia, which are subject to pronounced apical compression due to elevated Laplace pressure.

Poster 73 - Molecular Atlas of Cellular Actin Networks at Single Filament Resolution Using Montage Cryo-ET.

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Cell motility depends on the protrusive force generated by the actin cytoskeleton at the leading edge of cells. In lamellipodia, thin protrusions at the front of migrating cells, actin forms higher-order networks with versatile physical properties. These properties are attributed to the reciprocal regulation between actin binding protein activity and the underlying actin network geometry.

Cryo-electron tomography (cryo-ET) has been used to characterise actin networks in cellular protrusions at leading cell edge, including lamellipodia, but these descriptions have been mostly limited to small areas, capturing only 1-2 percent of cellular protrusions. More specifically, such characterisations only provide snapshots of defined regions. This does not allow filament tracing in its entirety, in order to establish the global architecture and connectivity of actin filament networks. Hence, we lack an understanding of how actin network geometries orchestrate cell migration.

To overcome these limitations, we have established a novel large-scale, high-resolution montage cryo-ET workflow, to image entire leading edge of migrating cells. Seamless 3D tomogram stitching is achieved by combining neural network based denoising strategies with novel optical flow-based algorithms. This allows segmentation and vectorisation of the actin cytoskeleton at the complete leading edge of a moving cell at single filament resolution, spanning areas up to 150 µm2. Our computational analysis pipeline enabled us to vectorise all the filaments in such large areas with filament reaching length scales that were not observed in previous studies. The obtained data provides a ground-truth understanding of how the geometrical complexity of the actin cytoskeleton steers directional cell migration and defines lamellipodial shape. Further, this method allows quantitative analysis of entire cellular actin network rearrangements associated with emergent migratory behaviours.

Poster 74 - Structural Insights into Assembly and Stress Resistance of Lens Intermediate Filaments: Filensin and Phakinin

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Intermediate filaments (IFs) are fundamental components of the cytoskeleton, yet the molecular mechanisms governing the assembly of tissue-specific IF systems remain poorly understood. In the eye lens, the IF proteins filensin and phakinin are uniquely expressed, and mutations in these proteins are associated with cataract formation. Despite this physiological relevance, the assembly principles of filensin–phakinin filaments have remained poorly defined.

Here, we first examined the interaction between filensin and phakinin in cellular and tissue contexts, resolving previous uncertainty by demonstrating their co-localization within shared filamentous networks. Building on this observation, we purified recombinant human filensin and phakinin and reconstituted filament assembly in vitro, enabling controlled structural and biophysical analysis. Biochemical assays revealed equimolar co-assembly, while domain truncation experiments identified distinct contributions of individual regions to filament length and stability. Notably, our data indicate that the rod domains of filensin and phakinin form a stable hetero-octameric core, providing a structural framework for higher-order filament assembly. TEM revealed smooth, elongated filaments, providing direct structural evidence of organized assembly. Furthermore, analysis of disease-associated variants uncovered specific mutations that disrupt filament integrity and reduce resistance to mechanical and thermal stress, linking defined structural perturbations to functional failure and pathological phenotypes. Ongoing single-particle cryo-EM studies aim to further elucidate the architectural features of these assemblies.

Together, our results provide insight into the assembly principles of tissue-specific IF systems and highlight the importance of integrating biochemical, structural, and cellular approaches to understand cytoskeletal organization.

Poster 75 - Functional Characterization of Cdc42EP1/Borg5 in Rho-GTPase Signaling and Actin Remodeling-Based Motility

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Small GTPases of the Rho-family are crucial for fundamental cellular processes including cell shape maintenance and change, cell and tissue differentiation as well as intra- and intercellular transport. All these phenomena are mediated through the exertion of pushing and pulling forces mediated by actin filament polymerization and myosin II-driven contraction, respectively. The Rho-GTPase Cdc42 has been implicated in both processes through binding effectors promoting actin filament polymerization or more indirectly signaling to the organization of the actin cytoskeleton, such as through kinases (e.g. MRCK). We have previously studied the specific cellular activities associated with the Cdc42 effectors FMNL2 and -3, formin family actin polymerases and N-WASP, a ubiquitously-expressed activator of actin filament branching by Arp2/3 complex. Here we are exploring the potential impact of the Cdc42-interactor Cdc42EP1 (also known as Binder of Rho-GTPase 5, Borg5), the largest member in this family of five and prominently expressed in cell migration models such as B16-F1 melanoma cells. Interestingly, Borg family proteins have also been implicated in the regulation of the cytoskeleton through septin filaments, although a detailed mechanistic understanding of all these connections, in particular in the context of Rho-GTPase outputs is missing.

Stable, genetic disruption of Cdc42EP1/Borg5 consistently altered the morphology and migratory behavior of B16-F1. Changes included a counterintuitive, lamellipodial widening with dramatic suppression of their protrusion rates, translating into significantly suppressed migratory performance. Furthermore, cells lacking the Borg family member displayed various alterations in gene expression and phosphorylation patterns all consistent with an overall increase in myosin II-driven cell contractility. In addition, Borg5 disruption coincided with a marked increase in active levels of their partner GTPase Cdc42. Future experiments will disentangle to what extent observed phenotypes depend on potential changes in Cdc42 signaling to its remaining effectors versus perhaps on changes in septin-mediated actin cytoskeleton regulation or both.

Poster 76 - P-Glycoprotein Inhibitor Reverses Colchicine Resistance in Breast Cancer Cell Monolayer and Spheroid Models

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Colchicine, a mitotic poison targeting rapidly dividing cells, has shown promise in cancer therapy. However, its clinical use faces challenges, including possible cellular resistance caused by the overexpression of P-glycoprotein (P-gp). Thus, we evaluated the ability of P-gp inhibitor to resensitize resistant breast cancer cells to colchicine. First, the combined effect of colchicine and P-gp inhibitor was measured in a monolayer of triple-negative breast cancer cell line UFH-001 and its resistant variant UFH-001R. The combinations showed higher synergistic cytotoxicity in resistant cancer cells compared to the non-resistant variant. Next, to uncover the mechanism behind the synergic cytotoxicity, P-gp activity and expression were analysed using rhodamine 123 and immunoblot assays. Since colchicine disrupts microtubule dynamics, we further investigated microtubule morphology. Using fluorescence microscopy after immunostaining of alpha-tubulin, we observed changes in microtubule organization after treatment with the drug combinations in both cell lines. Because these microtubule alterations are linked to cytostatic effects, we also performed cell-cycle analysis of both UFH-001 and UFH-001R cells by flow cytometry after treatment with selected combinations. Finally, to better simulate the tumor microenvironment in vivo, cancer cell spheroids of the same cell lines were used to evaluate the cytotoxic effects of the drug combinations in 3D. Several combinations demonstrated strong synergistic cytotoxicity, which was comparable to that observed in a cell monolayer. To determine how the compound combinations affect different layers of the spheroids, we visualized immunolabeled phosphohistone H3, a marker of cell proliferation, using confocal fluorescence microscopy. Collectively, our results indicate that the combinations of colchicine with a P-gp inhibitor have considerable potential for the treatment of colchicine-resistant cancers.

Poster 77 - Decoding Katanin Activation: How Microtubule Engagement Overcomes Autoinhibition

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Microtubule-severing enzymes remodel the microtubule (MT) cytoskeleton to support essential cellular processes, yet the molecular mechanisms that couple microtubule engagement to catalytic activation remain insufficiently defined. Using the Caenorhabditis elegans Katanin complex—comprising the MEI-1 (p60) AAA+ ATPase and its regulatory partner MEI-2 (p80-like)—we delineate two interdependent regulatory layers that together ensure activation occurs only at the appropriate substrate interface.

We show that the N-terminal domain of MEI-1 functions as an autoinhibitory module that suppresses ATP turnover and prevents interactions with tubulin C-terminal tails in the absence of MEI-2. Removal of this domain produces a constitutively active ATPase, enabling identification of pore-loop residues critical for sensing the microtubule lattice and relaying this signal to the AAA+ core. In parallel, we identify two basic microtubule-binding domains within MEI-2 that are essential for recruiting Katanin to microtubules; their substitution compromises MT binding and disrupts meiotic spindle assembly. These domains are evolutionarily conserved, underscoring the broad relevance of this regulatory strategy.

Together, these results define a coordinated mechanism in which MEI-1 autoinhibition and MEI-2–mediated MT binding jointly govern the transition from substrate recognition to ATPase activation. In our presentation, we will discuss how this multilayered control prevents futile ATP consumption, ensures precise remodeling of meiotic microtubules, and provides a conceptual framework for understanding how microtubule-severing enzyme are regulated across species.

Poster 78 - Mitochondrial G-Actin Activates a Transcriptional and Metabolic Rewiring Program that Affects Macrophage Behaviour

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[1] Cytoskeletal Dynamics in Cell Migration and Cancer

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Actin networks display diverse architectures and turnover dynamics across all eukaryotic cells. How specific actin pools (monomeric, G-actin and filamentous, F-actin) control mitochondrial DNA transcription and metabolism in cancer cells remains poorly understood. In metastatic colorectal cancer (mCRC), including microsatellite stable (MSS) tumors, 90% of patients express a truncated Adenomatous polyposis coli (APC) protein that lacks its actin nucleation activity. Importantly, mCRC patients have been associated with poor response to immunotherapy. Here, we found that APC helps 'safeguard' the ratio G-/F-inside mitochondria. Next, we demonstrated that an increase towards mitochondrial G-actin promotes TFAM recruitment to the mtDNA promoter region and enhances mitochondrial gene expression, without altering mitochondrial dynamics or mass. As a result, OXPHOS complexes, oxygen consumption and ATP production increase, while generation of ROS species diminishes. These metabolic changes were associated with a metabolic shift from glucose oxidation toward fatty acid synthesis in cancer cells. This metabolic rewiring in cancer cells triggers macrophage polarization and migration. Collectively, our findings uncover a novel role of actin within mitochondria in shaping cancer cell metabolism and immune modulation, offering new therapeutic avenues in mCRC.

Poster 79 - Guidance Mechanisms of Dendritic Cells' Transmigration Through Lymphatic Endothelia

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Among cell biological phenomena, guided cell migration stands as one of the most intricate yet elusive ones. We utilize antigen-presenting dendritic cell (DC) entry into the lymphatic system as a model for guided cell migration. DCs cross lymphatic endothelial barriers to enter the lymphatic vessels, and later reach the lymph nodes, in a lymphatic endothelial chemokine CCL21-dependent manner.

We have shown (Liaqat et al. 2024, The EMBO J.) that RAB6- and ELKS-dependent targeted exocytosis of CCL21 at the multicellular junctions of lymphatic endothelial cells determines the preferential DC entry site. Such localized secretions require the proper transport of exocytic vesicles to LECs' multicellular junctions by the microtubular network. Along these lines, we have revealed that LECs possess an intrinsic property leading to microtubules targeting their multicellular junctions. Hence, in our current study, we employ an array of in vivo, explant and primary cell culture studies to investigate how microtubule growth and exocytic vesicles are directed towards the multicellular junctions, resulting in targeted exocytosis ,thus, determining the transmigration hotspots for DC entry into the lymphatics. Therefore, our ongoing studies help unveil the poorly defined mechanisms of guidance cue formation and their association with microtubule-driven cell polarity. The specificities in approaches and results will be presented at the meeting.

Poster 80 - Daam-, FMNL-, and mDia-Family Formins Synergize to Regulate Cortical Actin Dynamics in B16-F1 Mouse Melanoma Cells

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The actin-rich cell cortex is a viscoelastic structure tethered to the plasma membrane and essential for processes such as cell migration and division. Yet, the full set of its actin assembly factors driving its formation remains elusive. Unlike the Arp2/3 complex, which generates short, branched filaments limiting sustained myosin-driven force generation, formins assemble long, linear filaments ideally suited for actomyosin-mediated contraction. To identify the complete inventory of formins regulating cortex mechanics, we systematically screened Diaphanous-related formins for interactions with active Rho-family GTPases implicated in contractility using Y2H-assays. This screen identified Daam1/2 and FMNL2/3 and was further supported by the prominent rear cortical localization of constitutively-active EGFP-tagged formin variants as well as by immunostaining of their endogenous counterparts in polarized cells. Furthermore, for the first time we demonstrate release of autoinhibition of inactive Daam1 and FMNL2 sandwich complexes by respective GTPases using TIRF imaging on the single-filament level. Of note, CRISPR/Cas9-generated Daam1/2 double-knockout cells exhibited marked polarization defects evidenced by the formation of multiple protrusive fronts, impaired 2D-migration and altered focal adhesion dynamics, with a particularly pronounced defect in rear-end retraction. However, contrary to previous work, we found no evidence that Daam1/2 participate in the formation of finger-like protrusions at the leading edge. By contrast, FMNL2 appears to function as a dual role formin acting both at the cell front and the rear. Using analytical ultracentrifugation we reconcile previous controversies by demonstrating that the affinity of FMNL2 for active GTPases, particularly Rac1, depends on the GBD and presence of the complete FH3 domain. Finally, we show that loss of either Daam1/2, FMNL2/3 or mDia1/3 caused comparable defects in cell cortex stiffness, whereas combined loss of Daam1/2- and mDia1/3-family formins led to amplified cortical defects including rigidity, blebbing and duration of cytokinesis demonstrating their functional synergy to establish and maintain cortical integrity.

Poster 81 - Migrating in Confinement: Measuring the Pushing Forces Exerted by Cells.

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Cell migration is required for biological processes such as development, immune response, and wound healing unfortunately, it is also a key factor in cancer metastasis. Extensive work has been done on cells migrating on 2D substrates more recently this has been extended to 3D matrices where it has been seen that migrating cells push and pull on the environment. However, both approaches have drawbacks while 2D assays are convenient for imaging they are far from the physiological reality and 3D matrices provide a closer to native environment but are challenging to standardize and image. It has been shown that when immature dendritic cells (iDCs) are confined between 2 plates (so called 2.5D), they show a higher diffusion coefficient, since non adhesive cells do not migrate on a planer surface. This indicates that mechanical confinement of cells in vivo can influence migration. Is this also true for cancer cells since tumors are often dense networks of extracellular matrix presenting a complex 3D environment where cancer cells will experience confinement and exert forces.

For this reason, PDMS microchannels are used to physically confine the cell and to limit the migration to one dimension. We can then position optically trapped beads in the path of the migrating cell and study the interaction of the cell with this obstacle. Using the cancer cell line, MDA-MB 231, expressing LifeAct RFP and EB3 GFP we visualize the actin and microtubule behavior. We study the effect of chemo-attractants and surface passivation on the cell's migration in these channels and if this is influenced by the topography of the channels, as has been shown to be the case for immune cells. Finally, we aim to measure the pushing and pulling forces exerted by the leading edge of a cancer cell migrating in confinement on an optically trapped bead.

Poster 82 - Exploring the Dynamics of Actin Networks in Proprioception Process in Arabidopsis

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The ability of plant organs to sense their local curvature and driving the progressive establishment of organ straightening is known as proprioception. This is a key mechanism in achieving correct shape and regulating the growth dynamics in combination with gravisensing and light perception [1,2]. In this study, we aim to explore molecular players driving proprioception in Arabidopsis. Previous experiment using myosin-XI mutants suggested a role of actin in this process [3]. The proprioceptive insensitivity in this mutant could be either due to the disruption of their cargo function or due to the modification of tension in actin cables. To explore the role of actin filaments we analyzed actin network with confocal microscopy. In wild type, during the gravitropic-upward-curving-phase, these long actin bundles deviate from their straight-alignment and become wavy. During the de-curving-phase, these actin bundles regain their straight configuration. Then we produced RNAi-villin plants where actin configuration was specifically altered in fibre cells in stem. Using computational phenotypic tool, these lines appeared to be insensitive to proprioception confirming the importance of actin filament in controlling plant posture. We are exploring the actin configuration in both mutants and how it could modify the growth pattern and auxin action during plant movements.

Keywords: Proprioception, actin network, confocal microscopy, plant shape.

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2. Moulia et al., (2019). Posture control in land plants: growth, position sensing, proprioception, balance, and elasticity. Journal of Experimental Botany, 70(14), 3467-3494.

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Poster 83 - Selective Control of Microtubule Dynamics by Tubulin Isotypes and Posttranslational Modifications

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Microtubules (MTs) are key components of the eukaryotic cytoskeleton and are important for cellular functions and homeostasis. The tubulin code, encompassing different isotypes and post-translational modifications (PTMs) of tubulin, is suggested to control MT properties and functions by modulating their building blocks –the tubulin proteins. Perturbed tubulin PTMs or mutations in tubulin can lead to neurodevelopmental and bleeding disorders, neurodegeneration and male infertility.

The aim of our study is to determine how the tubulin PTMs and isotypes can affect these intrinsic properties - MT dynamics and mechanics. To perform reproducible measurements we need to obtain pure functional tubulin with controlled PTMs and isotypes. For this we established a 2-step purification method based on cycles of polymerization and depolymerization, followed by an ion exchange chromatography. Tubulin was purified from human cell lines, mouse brains and platelets without any detectable associated proteins; allowing us to perform reproducible dynamic, structural and mechanical measurements. Analysing dynamic MTs reconstituted in-vitro from different tubulin variants and analysed using TIRF-microscopy, we find that some tubulin PTMs as well as isotypes confer specific dynamic properties to MTs. In particular, polyglutamylation modulates MT growth and length. Using Cryo-Electron Tomography, we observed that both PTMs and isotypes impact the ultrastructure of the microtubule lattice.

Our data demonstrate that tubulin isotypes and PTMs affect both MT dynamics and lattice architecture. As our tubulin is purified from cells and tissues, the PTMs and isotype composition we studied reflects the physiological situation in the organism and can thus be directly translated into how the tubulin code affects microtubule behaviour in living cells. Disruptions in the tubulin code leading to perturbations in MT intrinsic properties could significantly influence cell functions and contribute to disease mechanisms.

Poster 84 - Microtubule dynamics in the presence of actin networks

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The eukaryotic cytoskeleton is a composite network of biopolymers, largely responsible for maintaining cellular integrity, cell division, motility and intracellular transport. These cellular functions rely on the precise coordination and interactions between dynamic actin filaments and microtubules. Inside the cell, actin filaments can organize themselves into various structural forms such as filamentous networks or tightly packed bundles. One interesting question is how the actin network architecture influence microtubule dynamics. We investigate this question by studying an in vitro reconstituted system, where we polymerize tubulin subunits inside pre-assembled filamentous and bundled actin networks. The two actin network architectures are achieved with the aid of crowding agent. Experiments using total internal reflection fluorescence microscopy reveal that microtubules are more stable in filamentous actin networks than when interacting with bundled actin networks. Moreover, microtubules exhibit pause events in filamentous actin networks whereas they bend in the presence of bundled actin networks. We further investigate how local actin network inhomogeneities, filamentous network coverage and bundle thickness influence microtubule dynamics. Thus, our study provides a thorough overview at how the surrounding actin network architecture affects microtubule dynamics and behavior.

Poster 85 - Regulation of Apical Cortex Mechanics

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The mechanical properties of epithelial tissues are governed by the actomyosin cortex underlying the apical plasma membrane. This cortex is tightly coupled to tight junction (TJ) assembly, yet how actin and non-muscle myosin II (NM2) isoforms selectively regulate apical cortex mechanics remains poorly understood. Here, we combine genetic and drug-based perturbations with atomic force microscopy (AFM)-based nanoindentation to analyze the molecular control of apical cortical mechanics in MDCK II cells.

Our mechanical model extends purely elastic descriptions by incorporating cortical relaxation, effectively enlarging the parameter space with a flow component and enabling separation of cortical tension and area compressibility. We show that loss of γ-actin leads to pronounced softening of the apical cortex and reduced cortical tension, despite compensatory upregulation of other cytoskeletal components following γ-actin depletion. In contrast, NM2B knockout cells maintain near-normal cortical tension but display a significant reduction in area compressibility, revealing a distinct role for NM2B in stabilizing the elastic response of the cortex. Disruption of TJ assembly through cingulin knockout similarly reduces apical stiffness and cortical tension, highlighting the mechanical coupling between TJ scaffolding and cortical force transmission.

To relate mechanics to cortical architecture, we complement AFM measurements with super-resolution imaging and quantitative analysis of actin organization. STED microscopy of isolated apical cortices reveals a dense actin meshwork, in contrast to the stress fiber-dominated basal architecture observed by single-molecule localization microscopy, highlighting the intrinsic mechanical polarity of epithelial cells.

Together, these results demonstrate that apical cortex mechanics emerge from a coordinated, isoform-specific interplay between actin, NM2 isoforms, and TJ assembly, and establish AFM-based mechanical decomposition as a powerful framework for resolving epithelial cortical tension and viscoelasticity.

Poster 86 - Autoregulatory Self-Oxidation Restricts MICAL1-Driven Actin Filament Disassembly

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One of the actin-binding proteins is the monooxygenase Molecule Interacting with CasL 1 (MICAL1). MICAL1 catalyzes the NADPH-dependent, stereospecific oxidation of filamentous actin by targeting methionine residues in the actin D-loop, leading to filament destabilization and disassembly. Consequently, MICAL1 contributes to cytoskeletal remodeling during processes such as cell migration, axonal guidance, synaptic plasticity, and changes in cell morphology. Its dysregulation leads to defects in cytoskeletal integrity and has been associated with neurological disorders, including epilepsy.

MICAL1 activity is regulated by an autoinhibited conformation; one of the binding partners that activates MICAL1 is the family of small GTPase Rab. During our studies of MICAL1, we observed time-dependent changes in its enzymatic activity in the presence of NADPH. This observation suggests that MICAL1 may possess more than one autoregulatory mechanism.

Here, we show that MICAL1 undergoes self-oxidation in the presence of NADPH, leading to structural damage and progressive enzyme inactivation. The data reveal a rapid and progressive loss of activity occurring within minutes of incubation at 37 °C, observed for both the isolated monooxygenase domain and the full-length protein. Our structural analysis indicates protein aggregation during self-oxidation. To identify residues affected by this process, liquid chromatography–mass spectrometry was employed, revealing oxidatively modified regions within the protein. We propose that self-oxidation constitutes an intrinsic autoregulatory mechanism that limits prolonged MICAL1 activity in cells, thereby preventing excessive and sustained destabilization of the actin cytoskeleton.

Poster 87 - NHSL3 Interacts with Ena/VASP Proteins and the Scar/WAVE Complex and Promotes Cell Migration.

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Tight control of mesenchymal cell migration is important for embryonic development and its deregulation causes disease. It is driven by lamellipodia protrusion at the leading edge of the migrating cell. This is controlled by Rac-Scar/WAVE-Arp2/3 complexes driving actin filament nucleation coupled to Ena/VASP proteins mediating actin filament elongation. These activities are coordinated by leading-edge proteins, such as Lamellipodin and NHSL1. Here, we discovered KIAA1522/NHSL3 as an additional regulator of these essential actin effectors.

We reveal that NHSL3 promotes cell migration. NHSL3 co-localises at the edge of lamellipodia with Ena/VASP proteins and the Scar/WAVE complex. We show that it binds to Ena/VASP proteins and the Scar/WAVE complex and functions to inhibit Scar/WAVE-Arp2/3 activity in cells. NHSL3 interacts with the Scar/WAVE complex subunit Abi and, in contrast to other known Scar/WAVE complex binders, additionally to the CYFIP1/2 subunit through 3 short linear motifs. Thus, control of actin filament nucleation and elongation at the leading edge of mesenchymal cells is more complex than anticipated. Our study provides insights into the intricate regulation of lamellipodial actin networks highly relevant for understanding control of mesenchymal cell migration during development and diseases.

Poster 88 - A Modular GCP Code Regulates γ-Tubulin Ring Complex Geometry in C. Elegans

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Microtubules are nucleated by γ-tubulin ring complexes (γ-TuRCs), which in most eukaryotes template canonical 13-protofilament microtubules. In contrast, C. elegans predominantly assembles 11-protofilament microtubules, but the composition, structure and organization of subunits in its γ-TuRCs are not understood. Using single particle cryo-electron microscopy, we determined the structure of the native C. elegans γ TuRC, revealing a cone-shaped assembly consistent with an 11-protofilament microtubule lattice. In addition to conserved γ-tubulin small complexes (γ-TuSCs), we identified a striking reorganization of GCP proteins within the region that corresponds to human GCP4–6. In this region, GCP2 interleaves with the nematode-specific subunits GTAP-1 and GTAP-2, which accommodate an unusual actin-containing bridge in the complex. In contrast to native γ-TuRCs, we found that recombinant subcomplexes containing C. elegans GCP2, GTAP-1 and GTAP-2 oligomerize into microtubule-nucleating assemblies compatible with a 13-protofilament lattice. Together, our work defines the structural basis of an 11-protofilament nucleation template, and demonstrates how differences in GCP composition can reshape γ-TuRC geometry to accommodate evolutionarily diverse microtubule architectures.

Poster 89 - Centrosomal Microtubules Control Dendritic Cell Integrity and Coordinated Motility in Complex Environments

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Immune surveillance depends on the continuous migration of dendritic cells (DCs) through densely packed and mechanically heterogeneous tissues. To reach lymph nodes and sites of inflammation, DCs must deform their cell body and nucleus while maintaining structural integrity and directional motility. How microtubules (MTs) contribute to this mechanically demanding mode of migration is not well understood. Here, by studying a key centrosome-associated factor AKNA, we identify centrosomal MT architecture as a key critical determinant of DCs motility and behavior in confined environments.

Using super-resolution imaging we show that in low-complexity setting of 2D confinement wild-type DCs establish a compact γ-tubulin–positive microtubule-organizing center (MTOC) that nucleates a radial MT array aligned with a polarized Golgi apparatus. In this configuration, cells migrate with coherent morphology and persistent trajectories. In the absence of AKNA, MTs become highly disordered along with strong reduction in γ-tubulin foci and altered centrosome and actin cytoskeleton organization. Moreover, AKNA-deficient DCs display reduced directional persistence, indicating impaired coordination.

When challenged in mechanically complex environments, by using microfabricated devices and 3D collagen matrix these defects become pronounced. In dense 3D collagen matrices, AKNA-deficient DCs exhibit striking shape instability, frequent cell fragmentation and death. Similarly, in microfabricated devices of different geometry that mimic steric tissue complexity, the AKNA deficient DCs tend to stall and remain trapped between obstacles. In contrast, bone marrow–derived macrophages lacking AKNA display normal centrosomal γ-tubulin, MT organization, and morphology, indicating a cell-type–specific requirement.

Together, these findings reveal that AKNA-dependent centrosomal MTs form a load-bearing and orienting scaffold that preserves dendritic cell integrity and coordinates their motility in complex three-dimensional environments.

Poster 90 - Investigating the Molecular Mechanisms of Cell Shape Changes During Forebrain Roof Plate Morphogenesis

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In vertebrates the forebrain roof plate (RP) invaginates to give rise to the two cerebral hemispheres, the failure of which leads to holoprosencephaly. Our laboratory investigates the mechanistic and molecular basis of forebrain RP invagination using chick embryos. We observed that the midline of the RP neuroepithelium is significantly thinner than the lateral regions suggesting that the differential thickness of the RP may play a role in its invagination. Indeed, constitutive activation of BMP signaling in the RP midline increases thickness by reducing pCofilin, a protein crucial for actin remodelling. This alters the midline morphology from the normal W-shape to a V-shape. Similarly, overexpressing non-phosphorylable Cofilin increases the thickness of the midline NE. Since Lim kinase (LIMK) phosphorylates Cofilin, this study indicates that BMP signaling through activation of LIMK phosphorylates Cofilin to regulate actin dynamics and cell shape for proper forebrain RP invagination.

To further elucidate the molecular mechanisms driving cell shape changes during forebrain RP invagination, I am investigating the roles of the cytoskeleton and associated signaling pathways. Immunostaining revealed abundant apical F-actin and phosphorylated myosin light chain (pMLC), hallmarks of strong actomyosin contractility. While microtubules showed apico-basal alignment. I observed that pharmacological disruption of actin polymerization using Latrunculin-B or Cytochalasin-D blocked RP invagination without altering the molecular identity of the tissue by affecting the morphogenesis alone. Further, I am exploring the role of the YAP/TAZ pathway, known to regulate cell shape and indirectly modulate Cofilin activity. Upon constitutive activation of YAP, the thickness of neuroepithelium increased with concurrent decrease in pCofilin, which altered actin organization and induced abnormal cell proliferation. Conversely, inhibiting YAP function produced U/V-shaped invaginations. These findings highlight the critical interplay between cytoskeletal dynamics and YAP signaling in forebrain RP morphogenesis. Further investigations are underway to uncover the downstream targets of YAP involved in this process.

Poster 91 - Targeting Class-IX Myosins with Adhibin: A Strategy to Interfere with RhoA-Driven Metastasis

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RhoA signaling is a central regulator of actin remodeling and actomyosin contractility, requiring precise spatiotemporal control to coordinate adhesion, migration, growth, and cytokinesis. Although this pathway represents an attractive therapeutic target to prevent cancer cell metastasis, most efforts have focused on downstream effectors. Pharmacological targeting of RhoGAPs, which restrain RhoA activity, remains largely unexplored. Modulating RhoGAP localization or function provides an alternative strategy to perturb cytoskeletal programs driving metastatic dissemination, circumventing the challenges of enhancing RhoGAP activity. We identified adhibin as a first-in-class allosteric inhibitor of class-IX myosins (Myo9a/Myo9b), monomeric motors that uniquely couple ATP-dependent force generation with localized RhoA inactivation through a C-terminal RhoGAP domain and actin-crosslinking activity. Adhibin binds to the Myo9 motor domain, suppressing ATPase activity and actin-based motility. Across various carcinoma and melanoma cell models, adhibin disrupts spatial RhoA signaling, leading to loss of front–rear polarity, focal adhesion disassembly, impaired actomyosin contractility, and global actin cytoskeletal disorganization that compromises cell–cell contacts. These defects impair single-cell and collective migration, as well as cytokinesis. Studies in organotypic cell models revealed minor interference with growth and proliferation. In entorhinal–hippocampal slice cultures, adhibin reduced the predominant Myo9b expression in astrocytes, while only slightly affecting neurofilament light chain fragmentation, consistent with preserved axonal integrity and absence of cytotoxicity. Along with its non-pathological profile, the results support adhibin as a safe and effective therapeutic candidate. To advance translational potential, we generated and screened a library of adhibin analogues, identifying compounds with enhanced potency, improved tolerability, and low-dose efficacy, while retaining robust inhibition of RhoA-driven metastatic behaviors. Together, these findings establish myosin-9 inhibitors as a powerful tool to interrogate RhoA-dependent processes in development and regeneration, and, importantly, as a strategy to limit metastatic progression that is currently under investigation in xenograft models, patient-derived samples, and brain tissue models.

Poster 92 - PDLIM4 Is a Scaffolding Protein for Stress Fiber Organization

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Actin stress fibers are higher-order contractile structures composed of actin filaments crosslinked by proteins such as α-actinin. Yet, the mechanisms that organize and stabilize bundled filaments into coherent stress fiber networks remain poorly understood. PDLIM4 is an actin-associated protein containing two known protein interaction domains, a PDZ domain and a LIM domain, connected by a large intrinsically disordered central region. Despite documented interactions with actin and α-actinin-1, the identity of the domains mediating these and possible other protein-protein interactions, as well as the role of PDLIM4 in stress fiber architecture have not been reported. To address these questions, we combined PDLIM4 knockout and rescue experiments using domain-specific mutants in U2OS cells with in vitro biochemical assays. Loss of PDLIM4 resulted in abnormally thin and poorly organized stress fibers. On the other hand, over-expression of full-length PDLIM4 induced formation of cytoplasmic aggregates, which we exploited to identify interaction partners using aggregate-based enrichment of different actin binding proteins. Further interaction partners were identified and confirmed using BioID proximity labeling, and pull-down experiments. Finally, a systematic domain-deletion analysis was applied to define regions required for PDLIM4 aggregation and stress fiber localization. Our results support a model in which PDLIM4 enables multivalent interactions that physically organize α-actinin–bundled filaments within stress fibers, possibly aided by PDLIM4 dimerization and selective recruitment of other actin binding proteins to ‘Z-disc like’ structures of stress fibers. Together, these findings identify PDLIM4 as a structural regulator of actin stress fibers and provide mechanistic insight into how modular adaptor proteins tune the actin cytoskeletal architecture.

Poster 93 - HIV-1 Utilizes Actin Disassembly Factors to Remodel Actin Meshwork for Viral Assembly and Release in T Cells

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HIV-1 assembly occurs at the T-cell plasma membrane and requires viral Gag multimerization and budding. These steps likely depend on the local disassembly of the filamentous actin network that present a barriere for virus egress (1). Thus actin disassembly cofactors may be involved in that process. Here, we investigated three such cofactors, coronin, cofilin and WDR1, in HIV-1 infected primary blood T cells. Using siRNA in HIV-infected T cells and in Gag viral particles–producing cells, combined with STED super-resolution microscopy imaging, we found that cofilin or WDR1 depletion increases cortical F-actin density, elevates intracellular Gag, reduces Gag assembly site formation and polarization at the cell membrane, and impairs particle release, with cofilin depletion having the strongest effect. Furthermore, WDR1 interacts with Gag at the cell membrane, whereas cofilin is enriched in Gag viral particles. These factors likely contribute to F-actin disassembly at local Gag assembly sites that is required for efficient HIV-1 assembly and release in T cells.

(1) HIV-1 diverts cortical actin for particle assembly and release. Dibsy R, Bremaud E, Mak J, Favard C, Muriaux D. Nat Commun. 2023 Oct 31;14(1):6945.

(2) HIV-1 budding requires cortical actin disassembly by the oxidoreductase MICAL1. Serrano T, Casartelli N, Ghasemi F, Wioland H, Cuvelier F, Salles A, Moya-Nilges M, Welker L, Bernacchi S, Ruff M, Jégou A, Romet-Lemonne G, Schwartz O, Frémont S, Echard A. Proc Natl Acad Sci U S A. 2024 Nov 26;121(48):e2407835121.

Poster 94 - Tau Condensation Generates a Selective Microenvironment Around Microtubules

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Tau is a neuron-specific microtubule-associated protein implicated in a range of neurodegenerative diseases known as tauopathies. Recent studies have shown that tau molecules on microtubules can organize into cohesive structures, known as tau envelopes, which can restrict the interaction of other microtubule-associated proteins, such as severing enzymes, with the microtubule lattice. The formation of tau envelopes presumably involves tau-tau interactions, however, the underlying mechanism is unknown. Here, we show that tau envelopes are multilayered condensates formed via prewetting-like transition. Increasing tau concentration results in transition from a thin, cohesive, microtubule surface-adsorbed layer to thick, multilayered condensates. We demonstrate that the condensed tau layers efficiently recruit tau interactors, such as soluble tubulin, to the microtubule surface, which results in enhanced microtubule polymerization. In addition, tau condensates act as potent microtubule bundlers. Our results show that surface-adsorbed, cooperatively-bound tau molecules constitute a protective sheath around microtubules, while tau condensates forming on top of this sheath form a distinct microenvironment of tau interactors, locally regulating microtubule-related processes. We thus propose a dual role of microtubule-bound tau, and suggest prewetting-like transitions on charged surfaces of polymeric filaments, as viable pathways to generate liquid-like condensates at physiological protein concentrations.

Poster 95 - Actin Bodies Assemble from Dynamic Actin Filaments to Safeguard Quiescent Cells from Ageing and Unveil an Unexpected Activity of Actin Nucleator upon Quiescence Exit.

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The majority of the cells spend most of their lives in quiescence, a temporary absence of proliferation. Upon quiescence establishment, S. cerevisiae, like many other organisms, reorganizes several cellular machineries. Among these, the actin cytoskeleton is undergoing one of the most drastic transformation and is remodeled into an original structure called Actin Bodies. In this study, we shed light on the molecular mechanism at work during this process. We found that actin cables rapidly vanish and actin patch formation ceases at the plasma membrane following Arp2/3 inactivation. Then, with time, the dynamic actin structures remaining in the cytoplasm gather to form Actin Bodies in a Svr2 dependent fashion. Actin continue to polymerize, then actin bodies embedded actin filaments are stabilized by the combined action of fimbrin and capping protein. Our results also indicate that actin bodies safeguard quiescent cells from stress and aging and are key structures for actin nucleator activation upon quiescent exit.

Poster 96 - CLASPs Stabilize the Pre-Catastrophe Intermediate State Between Microtubule Growth and Shrinkage

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Cytoplasmic linker-associated proteins (CLASPs) regulate microtubules in fundamental cellular processes including cell division, migration, and neuronal development. CLASPs stabilize dynamic microtubules by suppressing microtubule catastrophe and promoting rescue, the switch-like transitions between growth and shrinkage. Yet, how CLASPs specifically modulate microtubule transitions is not understood. Here, we investigate the effects of CLASPs on the pre-catastrophe intermediate state of microtubule dynamics, employing distinct microtubule substrates to mimic the intermediate state. Surprisingly, we find that CLASP1 promotes the depolymerization of stabilized microtubules in the presence of GTP, but not in the absence of nucleotide. This activity is also observed for CLASP2 family members and a minimal TOG2-domain construct. Conversely, we find that CLASP1 stabilizes unstable microtubules upon tubulin dilution in the presence of GTP. Strikingly, CLASP1 drives microtubule substrates with vastly different inherent stabilities into the same slowly depolymerizing state in a nucleotide-dependent manner. We interpret this state as the pre-catastrophe intermediate state. Therefore, we conclude that CLASPs suppress microtubule catastrophe by stabilizing the intermediate state between growth and shrinkage. This work provides critical mechanistic insights into how CLASPs stabilize microtubules, underlying their roles in diverse cellular contexts.

Poster 97 - Tau Protein as a Regulator of Cell Adhesion and Migration

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Tau is a microtubule-associated protein that stabilizes microtubules and promotes their bundling, while also binding actin to co-organize the actin and microtubule cytoskeletons. Tau is primarily expressed in neurons and its alterations are involved in neurodegenerative diseases. Over the last decade, tau has emerged as a prognostic factor in many cancers, although the underlying mechanisms remain unclear. Cell adhesion and migration are tightly regulated by microtubules and actin through focal adhesions and are often altered in neoplastic transformation, constituting a hallmark of cancer. Interestingly, recent genomic studies have linked tau to the focal adhesion pathway.

Our aim is to explore how tau impacts cell adhesion and migration through its effects on microtubules, actin and focal adhesions. To this end, we use ectopic expression of normal and pathological forms of tau in human epithelial RPE-1 cells. Our first results reveal that tau expression in epithelial cells alters their migration speed and directionality. Using crossbow-shaped patterns mimicking the structure of migrating cells, we show that pathological forms of tau increase the size of focal adhesions along with microtubule bundling at the leading edge, and also alter actin organization. We are currently using live microscopy to evaluate how focal adhesion turnover and the cytoskeleton dynamics in the vicinity of focal adhesions are impacted by tau proteins.

Overall, our results should highlight the role of tau proteins in mechanisms that are misregulated in tumorigenesis.

Poster 98 - Manipulating Mammalian Somatic Cells to Undergo Semi-Closed Mitosis

Monica Gobran, Henning Urlaub, Peter Lenart

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At mitotic entry, CDK1-CyclinB1 is activated abruptly and irreversibly in a switch-like manner. This triggers a highly ordered sequence of events in rapid succession: chromosomes condense, the microtubule and actin cytoskeleton reorganize, and the nuclear envelope breaks down. However, by disabling positive feedback loops of CDK1 activation, we were able to convert this all-or-none response to a gradual transition, enabling us to study intermediate CDK1 activation states.

We show that when CDK1 is partially inhibited, cells biochemically complete mitosis; they accumulate and then degrade Cyclin B1 with normal kinetics. However, while nuclear pores begin to disassemble, the overall structure of the nuclear envelope remains intact. We show that this results in the release of spindle assembly factors to the cytoplasm and formation of a cytoplasmic spindle, while the intact nuclear envelope prevents access to chromosomes.

We characterized this state in detail revealing intriguing similarities to semi-closed mitosis that is observed in fungal species and microbial eukaryotes, and that is considered to be the ancestral form of division. Interestingly, in the cytoplasm all events of mitosis, such as centrosome maturation, spindle formation, reorganization of the actin cortex take place and proceed in normal order and timing. However, microtubules do not assemble on or attach to chromosomes and therefore chromosome segregation does not occur.

Together our data suggest that by dialing down CDK1 activity, cells can be reverted to semi-closed mitosis, whereby they complete all cytoplasmic events of division, including spindle assembly. However, disassembly of the nuclear envelope appears to require higher CDK1 activity, which in turn is necessary for completing nuclear events of mitosis and importantly for chromosome segregation.

Poster 99 - Understanding Tissue Mechanics Through Tumour Organoids

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Tumours exhibit altered physical properties that manifest across spatial scales. Compared to healthy tissue, solid tumours are typically stiffer, which can partly be attributed to the extracellular matrix. However, the contributions of single cell properties and collective cell behaviour to the emergent tissue properties remain unclear.

Aiming to elucidate the role of single cells in multicellular assemblies found in tumours, we investigated patient-derived colorectal liver metastasis organoids. Organoid lines from different patients varied in morphology, consistently comprising either a single central lumen or a multitude of small lumina. In both cases, the lumen-facing sides of the cells are enriched in actin. We performed bulk organoid compression with AFM and found that single-luminal organoids are stiffer and more elastic than multi-luminal organoids. Concurrently, Brillouin microscopy in situ showed a higher Brillouin frequency shift for single-luminal organoids, indicating lower compressibility. The frequency shift was shown to increase with increasing organoid size, alongside an increase in nuclear elongation, which we attribute to an increase of stress acting in the tangential direction. Furthermore, the nuclei in multi-luminal organoids were larger, less spatially ordered and displayed a more irregular shape than their single-luminal counterparts, which we hypothesise is related to an altered keratin organisation. Overall, our data suggest that the mechanical properties of organoids are coupled to the physical properties of their constituent cells.

Poster 100 - Thrombospondin-5 Mediated ECM Remodeling Regulates Cell Migration in Breast Cancer Metastasis.

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Breast cancer (BC) metastasis is critically shaped by the tumor microenvironment, yet how specific stromal ligands remodel extracellular matrix (ECM) architecture and mechanotransduction to regulate invasion remains poorly understood. We identify thrombospondin-5 (THBS5/COMP) as a myofibroblastic cancer-associated fibroblast (myoCAF) marker highly enriched in invasive ductal carcinoma (IDC) and define its functional role in ECM remodeling and cancer cell invasion.

THBS5 upregulation in CAFs profoundly alters ECM organization in cell-derived matrices (CDMs) and collagen plugs, reducing fiber alignment and collagen contractility while increasing ECM pore size. Mechanical measurements reveal that THBS5-CAF CDMs exhibit increased viscoelasticity, accompanied by proteomic changes suggestive of altered collagen bundle architecture and ECM composition. Compared to THBS2, which primarily increases matrix stiffness, THBS5 uniquely imprints dynamic mechanical properties onto the CAF-derived ECM.

Functionally, THBS5-modified CDMs synergize with soluble THBS5 to promote migration of MDA-MB-231 breast cancer cells via syndecan-4 (SDC4) and integrin-β1–dependent signaling. This invasive behavior is actomyosin contractility-dependent but matrix metalloproteinase-independent, consistent with a mechanically permissive invasion mode. THBS5 acts as a ligand to reduce active membrane integrin-β1 through enhanced internalization and delayed recycling, leading to distinctive rounder and bifurcated cancer cell morphologies. At the nuclear level, cancer cells cultured on THBS5-rich matrices display robust YAP nuclear translocation independent of soluble ligand, reduced nuclear wrinkling, and induction of the heterochromatin mark H3K9me3, linking CAF-derived ECM cues to nuclear mechanics and epigenetic regulation. Similar nuclear phenotypes are observed in normal fibroblast CDMs and THBS5-expressing CAFs.

Together, our findings establish THBS5 as a spatially acting CAF-derived ECM ligand that integrates matrix viscoelasticity, integrin trafficking, and nuclear mechanotransduction to regulate breast cancer invasion, highlighting THBS5-driven stromal niches as potential therapeutic targets in IDC.

Poster 101 - CKAP5 Role in Neurite Growth Cone Navigation

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The molecular mechanisms underlying the protein interaction networks that govern neurite guidance in the human nervous system remain incompletely understood. Actin and microtubule dynamics are well established as central drivers of neurite protrusion and navigation: actin generates the propulsive force for forward extension, while selective microtubule polymerization directs the growth. Microtubules are thought to steer the growth cone by stabilizing actin structures within defined regions. Cytoskeleton-associated protein 5 (CKAP5) has emerged as a critical mediator of cross-talk between these networks. In vitro, CKAP5 bridges actin filaments and microtubules, while in cells it promotes microtubule polymerization along actin bundles, positioning CKAP5 as a potential orchestrator of neurite navigation.

Here, we report alterations in morphology and dynamics of neurite growth cone in the SH-SY5Y neuroblastoma cell line following CKAP5 knockdown. To facilitate high-resolution analysis of neurite outgrowth and steering, we developed PDMS silicone chambers for controlled microscopic assays. Under CKAP5 knockdown conditions, growth cones exhibited lowered responsiveness to gradients of both attractive and repulsive guidance cues. Collectively, these findings support a role for CKAP5 in organizing growth cone cytoskeletal architecture, thereby shaping its morphology and directional progression.

Poster 102 - Ultrasound-Induced Cytoskeletal Disassembly Drives Selective Apoptosis in Patient-Derived Oral Cancer Cells

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India accounts for more than a quarter of the total oral cancer burden globally. Despite advances in current treatment therapies, the survival rates over the last five decades have remained unchanged. The severe side effects associated with conventional radio- and chemotherapy are attributed to the nonselective targeting that damages both cancer cells and adjacent normal tissues. Here, we describe an alternative low-frequency (39kHz) pulsed ultrasound (US) approach that leverages the distinct mechanical vulnerability of cancer cells. We show that ultrasound applied at optimised parameters induces selective apoptosis of patient-derived oral cancer cells without significantly damaging healthy normal epithelial cells. Molecular studies reveal that US treatment enables the disruption of focal adhesions (FAs) and cytoskeletons (F-actin and myosin IIA fibers) in oral cancer cells, which correlates with the decrease in contractility. The mechanical vulnerability of oral cancer cells to US-mediated mechanical forces is due to reduced expression of Tpm2.1, a cytoskeletal mechanosensory protein. Furthermore, ultrasound treatment reduces proliferation in oral cancer cells. The findings of this study indicate that US treatment not only triggers selective apoptosis but also reduces the invasive and proliferative properties of patient-derived oral cancer cells. Overall, this work suggests that it will be possible to lay the foundation for developing potentially safer, personalized, and non-invasive ultrasound-based mechano-treatment against oral cancer.

Poster 103 - CAP1-Dependent F-Actin Disassembly Governs Epidermal Development

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Actin filament turnover is central to cell shape remodeling and adhesion, yet how F-actin disassembly contributes to epithelial tissue development in vivo is poorly understood. Cyclase-associated protein 1 (CAP1) has long been recognized as a multifaceted regulator of actin dynamics and signaling in cell-free and cultured systems. However, its function in mammalian tissues has remained poorly defined. Here, we bring CAP1 biology into the context of in vivo epidermal development, providing the first demonstration that its actin-disassembly activity is indispensable for skin morphogenesis.

Using in utero gene manipulation in mouse embryos, combined with live imaging and mutational dissection, we show that CAP1 is essential for maintaining adherens junction (AJ) integrity, apicobasal polarity, and proper cell shape. CAP1 loss did not alter cAMP levels but led to elevated F-actin content, reduced actin turnover, and severe disorganization of junctional actin networks. CAP1 was broadly cytoplasmic, consistent with a role in global actin dynamics. Notably, pharmacological stabilization of F-actin phenocopied CAP1 depletion, highlighting that dynamic actin disassembly, not polymerization, is the critical driver of morphogenesis in this system.

Structure–function rescue experiments in vitro and in vivo revealed that the helical folded domain (HFD), which enables CAP1 to cooperate with proteins such as cofilin and ADF (actin depolymerization factor/destrin) to sever and depolymerize filaments, is required for AJ organization and morphogenesis. In contrast, a domain supporting G-actin nucleotide exchange was dispensable. Thus, CAP1 promotes epidermal tissue architecture primarily through its F-actin disassembly activity in vivo.

Our findings position CAP1 as a central actin regulator in a developmental setting and provide direct evidence that regulated filament disassembly is a key organizing principle for epithelial tissues. By establishing CAP1 function in a mammalian morphogenetic context, this work bridges actin biochemistry with tissue-scale biology, underscoring the importance of actin turnover for development, homeostasis, and disease.

Poster 104 - Identification and Characterization of a Plasma Membrane-Associated Non-Centrosomal MTOC in Fruit Fly Nephrocytes

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The microtubule (MT) network supports key cellular processes, including intracellular transport and cell division, through its polarized architecture. While centrosomal MT-organizing centers (MTOCs) are typical in most cells, specialized cell types often form non-centrosomal (nc)MTOCs. In larval insect cells, polyploidy achieved through endoreplication renders the centrosome obsolete, and MTOCs are reassembled onto the nuclear membrane. This process is mediated by the Drosophila spectraplakin Shot, which, together with its partners, anchors to the nuclear membrane and functions in microtubule binding and organization.

However, polyploidy can also arise through cell fusion, as in Drosophila larval garland nephrocytes—large, binucleate, highly organized cells that filter blood. We found that nephrocytes exhibit ncMTOCs that differ markedly from those in other cells, possibly as a consequence of this distinct origin. MT minus ends predominantly localize beneath the plasma membrane (PM), organized by Shot, indicating the formation of a novel PM-anchored ncMTOC. We show that this process is mediated by the slit diaphragm, integrins, and their adaptor protein talin. Loss of slit diaphragm components, integrins, or talin causes Shot dissociation from the PM, ectopic MTOC formation, and disorganized cellular architecture. Although integrins are known to stabilize MT plus ends at the cortex, we demonstrate that endogenous talin interacts with Shot, providing the first evidence for integrin-mediated organization of MT minus ends.

Nephrocytes possess labyrinthine membrane invaginations forming filtration channels whose depth depends on endocytosis and recycling. We show that the PM-anchored MTOC directs recycling of lacunar components to these channels, as loss of MT minus-end motors abolishes their formation. Because garland nephrocytes are homologous to mammalian glomerular podocytes, and integrins and cytoskeletal dynamics are central to kidney pathologies, our findings provide new insight into disease-relevant mechanisms.

Poster 105 - The Role of Class I Myosins in Plasma Membrane Organization

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The plasma membrane of a living cell participates in a myriad of physiological processes such as ion and nutrient homeostasis, signal transduction, trafficking of molecules in/out of the cell, cell division, cell adhesion, cell migration and cell-cell communication. Orchestration of these functions requires compartmentalization of specific proteins and lipids into distinct functional domains and their spatio-temporal regulation. A wealth of experimental observations regarding the nature and dynamics of membrane component organization, suggest that the membrane bilayer is maintained as an active composite of the membrane in conjunction with a dynamic actin cytoskeleton, and the extracellular matrix at the outside of the cell (1). In all eukaryotic cells non-muscle class II myosin proteins serve as motors that power the contractility of the actin skeleton, either as stress fibers or at the actin cortex, providing the potential to drive the active-organization of molecules that associate with actin. Class I myosins on the other hand are directly associated with the lipid membrane and could also serve as key ingredients in active membrane lipid organization in the cell (3).

To understand this complex system, we have taken a bottom-up approach by reconstituting this active composite membrane system, utilizing actin filaments and motor proteins, to understand molecular organization of molecules that are associated with dynamic actin filaments (2). Here, I reconstitute a minimal system with purified class I myosin proteins, actin filaments on supported lipid bilayers in an attempt to understand the role of Myosin1c in active membrane organization.

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Poster 106 - Confinement-Induced Formation of a Supracellular Peripheral Star-Shaped Actomyosin Network Ensures Tumor Cluster Collective Amoeboid Migration

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Collective cell migration is essential for morphogenesis, tissue homeostasis or wound healing, and is exploited by cancer cells during metastatic progression. Beyond well-characterized traction-based migration, a recent mode of collective movement independent of substrate adhesion—collective amoeboid migration (CAM)—was identified in patient-derived advanced gastro-intestinal cancers (1). Biophysical modeling established that CAM relies on three minimal requirements: i) polarization of actomyosin contractility at the cluster rear, ii) cell "jiggling," i.e. oscillations around fixed positions, and iii) sufficiently strong jiggling-generated friction forces to propel clusters (2).

Tumor clusters are thought to disseminate in highly confined environment such as inter-fibrillar extracellular matrix space, micro-vessel lumens or peritoneum recesses. Whereas studies on single cell response to confinement have unveiled a key role of the nucleus in sensing the compression and triggering increased cellular contractility (3-4), little is known on the collective response to confinement by cells within a confined cluster.

Here, using live imaging and laser ablation techniques in microchannel-based microfluidics, we reveal that cells from the periphery of the cluster respond to confinement by generating a supracellular star-shaped cortical actomyosin network composed of pulsatile nodes and contractile cables anchored at vinculin-rich adherens junction. Vinculin depletion markedly impairs the network formation, reduces its contractility, and abolishes CAM. Nuclear tracking and biomechanical analysis further reveal that vinculin-KO clusters are softer and display defective jiggling. Strikingly, increasing actomyosin contractility or elevating compressive forces fully restores CAM in vinculin-deficient clusters. We propose a working model whereby vinculin and the supracellular cortical actomyosin cage regulate CAM by controlling global cluster stiffness and cell–cell cohesion.

Understanding how tumor cell groups adapt to confinement and migrate in low-adhesion environments may open new avenues to inhibit collective invasion in aggressive cancers.

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Poster 107 - Exploring the Structural and Molecular Mechanisms of γ-Tubulin Ring Complex Regulation in Drosophila Melanogaster

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Microtubule nucleation is templated by multi-protein γ-tubulin ring complexes (γ-TuRCs), which are recruited to centrosomes during mitosis by γ-TuRC tethering proteins, including those containing the conserved Centrosomin motif 1 (CM1) domain. Strong evidence indicates that CM1–γ-TuRC interactions stimulate microtubule nucleation.

Recent high-resolution cryo-electron microscopy studies revealed that cytosolic γ-TuRCs adopt a semi-open conformation due to the presence of the GCP4/5/6 core subcomplex, which splays outward relative to the geometry of a canonical 13-protofilament microtubule. This configuration likely represents a low-activity state that prevents ectopic microtubule nucleation in the cytosol. Consequently, γ-TuRCs are thought to undergo large-scale conformational rearrangements to become fully active upon recruitment to microtubule-organizing centers (MTOCs), such as centrosomes. However, the molecular basis of γ-TuRC activation remains unclear.

Although γ-TuRCs were long thought to have a uniform composition within a given species, we have demonstrated that Drosophila γ-TuRCs are compositionally heterogeneous. Notably, the small but essential γ-TuRC component Mozart1 (Mzt1) is expressed exclusively in sperm cells, despite Mzt1 being required for γ-TuRC assembly and mitosis in other systems, including yeast, plants, and human cells.

Additional heterogeneity arises from the presence of the GCP4/5/6 subcomplex. While this subcomplex is absent in budding yeast—where CM1 binding promotes γ-TuSC oligomerization into γ-TuRCs—even in Drosophila, the CM1 domain of Centrosomin (Cnn) can recruit γ-tubulin to centrosomes when the GCP4/5/6 subcomplex is depleted.

In this project, we aim to determine the first near-atomic-resolution structure of native Drosophila γ-TuRCs in the presence and absence of the CM1 domain, representing inactive and active states, respectively. This work will provide mechanistic insight into γ-TuRC activation and reveal how CM1-mediated binding occurs in the absence of Mozart proteins.

Poster 108 - Filament Severing Promotes Actin Ring Contractility Propelled by the Diffusible Crosslinker Anillin

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Contractile cytoskeletal structures are involved in diverse cellular processes like cell migration, cell polarization, and cell division. Diffusible crosslinkers can drive filament bundle contraction, propelled by Brownian motion channeled in the direction of increasing filament overlaps. How filament severing influences the process is unknown. Essential for cell division in metazoans are the diffusible actin-filament crosslinker anillin and the actin-filament severing factor cofilin, which both localise to the contractile ring of bundled actin filaments that drives the formation of the cleavage furrow during cytokinesis. Here, by using in vitro reconstitution and TIRF imaging, we show that severing of actin filament promotes the contraction of rings formed from bundled actin filaments that are diffusibly crosslinked by anillin proteins. To explain the observation, we employ the cytoskeleton simulation suite Cytosim and show that anillin-driven expansion of newly formed overlaps, after filament cutting, is sufficient to drive ring contraction. Higher mobility of shorter actin filaments, observed both in simulations and experimentally, suggests that filament cutting tips the balance between crosslinker-generated filament-filament sliding in direction of increasing overlap and protein-protein friction, which is known to exponentially scale with the number of crosslinkers, and thus strongly correlates with filament legths. Upon filament cutting new overlaps can form, while simultaneously the now shorter filaments can move more readily. Our results demonstrate that a minimal system of actin filaments, diffusive crosslinkers and actin severing factors is sufficient to generate robust actin ring contraction, revealing how filament severing, in tandem with diffusible crosslinkers, contribute to actin-filament network contractility.

Poster 109 - Exploring the Biochemical Impact of the β-Actin G74S Mutation: Insights into Histidine Methylation and Dynamic Effects on Actin

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Single-point mutations in the cytoskeletal actin isoforms are associated with various disease symptoms, including distinct facial abnormalities, intellectual disability, and developmental disorders, as observed in Baraitser-Winter Cerebrofrontofacial Syndrome (BWCFF). While these mutations disrupt actin's biochemical functions, the molecular mechanisms by which they do so remain largely unclear. Here we describe the effects of the BWCFF-causing β-actin G74S mutation which produces a strong BWCFF phenotype, using in silico and in vitro approaches. The mutation is located in actin's sensor loop (residues 70–78), a region involved in nucleotide binding and protein interaction. We first analyzed the effects of the mutation on N3-methylation of histidine-73, a post-translational modification that stabilizes F-actin by enhancing polymerization rates while reducing the ATPase activity. Analyses show that significant rearrangements are required for methylation to occur in the presence of the mutation. However, these adjustments lead to a slower reaction rate in vitro and reduced levels of Histidine-73 methylated mutant actin in cellulo. We then examined the effects of the mutation on protein dynamics and function. Using molecular dynamics simulations and biochemical assays, we analyzed the mutation's effects and the impact of methylation deficiency on dynamics and function of monomeric actin. Our simulations reveal destabilization of the β-sheet in subdomain 1, which impairs actin's folding and stability. This results in a thermally less stable protein, as demonstrated by differential scanning fluorimetry (ΔTM = -8.6°C and -7.1°C for G-actin and F-actin, respectively). Furthermore, the mutant protein exhibits a more frequent and wider opening of the nucleotide binding cleft at both the base and mouth, resulting in an increased nucleotide exchange rate (0.14 s-1) compared to wild-type actin (0.04 s-1). These findings provide insights into the combined impact of the G74S mutation and consequent methylation deficiency on disease pathology and enhance our understanding of actinopathies at the molecular level.

Poster 110 - Mechanical Properties of Filopodia like Structures

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A cell's ability to interact with its environment through membrane protrusions is fundamental to development, immune defense, tissue repair, enabling interpretation and navigation of complex 3D environments of extracellular matrices (ECM). Membrane protrusions can take various forms (filopodia, lamellipodia, blebs) with actin machinery engaging with the cell membrane, pushing it forward and retracting it when required. During migration, protrusions can interpret various signals, matrix stiffness, pore sizes or chemokine gradient, allowing the cell to travel the path of least resistance.

Assuming that these protrusions can exert not only pulling but pushing forces to probe/deform/displace the ECM, the global aim of the project is to measure these pushing forces and identify the mechanisms producing them.

Using beads trapped in an optical tweezers, membrane tethers are pulled from adherent cells, and the growth of the actin cytoskeleton can be studied. Additionally, the forces applied during this process can be measured and the presence of pushing forces can be identified.

Furthermore, we investigate if actin growth into the tether is dependent on a specific bead coating. Interestingly, our preliminary results indicate that, regardless of the bead coating, actin grows into the pulled tether. However, the type of growth, continuous from the base or patchy throughout the tube, and the forces exerted by the tube on the bead vary depending on the bead coating. Finally, we look at how microtubules behave and polymerize in the membrane tethers depending on the presence of actin or not.

By mimicking cells filopodia in this way, we begin to tackle the question of how a cell's cytoskeleton coordinates to form F-actin–rich protrusions such as filopodia, the dependency of this machinery on specific signaling pathways, while also quantifying the forces generated during the process.

Poster 111 - Chromosome-Associated Myosin Promotes Bivalent Separation in Mammalian Oocyte Meiosis

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Microtubule-based kinesin motors associate with chromosomes during cell division in order to facilitate chromosome segregation during anaphase. Actin-based myosin motor proteins, however, are generally thought to be dispensable for chromosome segregation. Here, we report that non-muscle myosin IIB associates with chromosomes and promotes bivalent separation in mammalian oocyte meiosis. During anaphase, non-muscle myosin IIB accumulates within the regions of the chromosome arms that mediate cohesion between the homologous chromosomes. Inhibition of non-muscle myosin IIB induces the formation of chromatin bridges as well as lagging chromosomes during anaphase. Thus, we demonstrate that rapid and accurate separation of bivalents in mammalian oocytes requires actin and a chromosome-associated myosin.

Poster 112 - Interaction Between Formin Fus1 and Ras GTPase Promotes Cell-Cell Fusion in Fission Yeast

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Formins form a well-conserved family characterized by formin-homology (FH) 1 and 2 domains, which catalyze the assembly of linear actin filaments. Regulation depends on more variable, often N-terminal, domains, which can contain a GBD-FH3 domain that binds small Rho-family GTPases. The formin Fus1, one of three formins in the fission yeast Schizosaccharomyces pombe, assembles the actin fusion focus, which is essential for cell fusion. This actin structure concentrates secretory vesicles carrying cell wall digestive enzymes at the site of cell fusion. Previous work showed that Fus1 N-terminus (Fus1N), which contains a putative GBD-FH3 domain, is sufficient for localization to the tip of the mating projection. Interestingly, expression of Fus1N in fus1Δ cells also induced cell lysis, suggesting that Fus1 has a second function in cell wall digestion, independent of F-actin assembly.

Using two-hybrid and co-immunoprecipitation (co-IP) assays, we found that Fus1N binds several Rho-family proteins, as well as Ras1, the only Ras GTPase in fission yeast. Based on Alphafold3 predictions, we designed a Fus1 mutant allele deficient in this interaction. Two-hybrid analysis and co-IP confirmed that the mutation specifically disrupted the interaction between Fus1N and Ras1, while interactions with other GTPases remained unaffected. Interestingly, a mutant Fus1N, while localized at the fusion site, did not induce cell lysis, contrary to wild-type Fus1N. The mutation of full-length Fus1 also partly compromised cell-cell fusion. Thus, a complex between the formin Fus1 and the Ras1 GTPase promotes cell-cell fusion. We are currently investigating how this pathway promotes cell wall digestion independently of actin assembly.

Poster 113 - High Leupaxin Expression Alters Durotaxis of Metastatic Breast Cancer Cells Via Inhibition of the Paxillin-Focal Adhesion Kinase Pathway

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As cells migrate through different tissues, they encounter varying stiffnesses, a mechanical cue that can guide their migration. This process, known as positive durotaxis, usually involves cells preferentially migrating toward stiffer regions. However, we have previously demonstrated that, in contrast to most cancer cell lines, some cells exhibit negative durotaxis, migrating from stiffer to softer environments. Moreover, it was previously shown that subpopulations of several cancer cells lines exhibit adurotaxis, migrating in any direction of a stiffness gradient.

The loss of positive durotaxis ability may allow tumor cells to migrate away from the stiff primary tumor microenvironment into softer healthy tissue, initiating metastasis. The key mechanisms underlying whether cells display positive, negative durotaxis or adurotaxis remain unknown.

Using in-house fabricated stiffness gradient gels, we discovered that metastatic variants of MDA-MB-231 breast cancer cells, which preferentially metastasize to bones or the brain in mice, exhibit impaired durotaxis and mechanosensing responses to increasing stiffness, such as cell spreading, and focal adhesions formation and signaling. We have identified by single-cell RNA sequencing several focal adhesion, signaling, and cytoskeleton-related genes that are differentially expressed in these variant cell lines compared to parental cells. We focused on leupaxin, a protein localizing in focal adhesions and known to inhibit paxillin phosphorylation and cell spreading, which is overexpressed in the metastatic variants. Silencing leupaxin in the brain metastatic variants restores their durotactic abilities, while overexpressing leupaxin in the parental cells induces impairments of durotaxis and mechanosensing phenotypes similar to the metastatic variants. Finally, we showed that leupaxin disrupts the paxillin-focal adhesion kinase (FAK) pathway, leading to durotaxis alteration.

This new mechanosensing regulation mechanism specific to metastatic cells might be key to allow tumor cells to escape the stiff tumor microenvironment and invade the softer healthy tissue.

Poster 114 - Actin in Action: Novel Tools for Visualizing Actin Organization in Living Cells and Tissues.

Manos Mavrakis, Carla Silva Martins, Sophie Brasselet

Institut Fresnel, CNRS

Essential physiological functions, including cell division, cell adhesion and motility, and tissue morphogenesis, rely on the capacity of animal cells to change and adapt their shape. To accomplish these force-dependent tasks, animal cells make use of actin cytoskeletal filaments. The precise way in which actin filaments organize, i.e. how actin filaments are physically oriented in space, and how filament organization is remodeled in time, is determinant for force generation. Being able to measure actin filament organization directly in living cells and tissues thus promises to advance our understanding of how proteins and signaling pathways individually and collectively control actin-driven cellular functions. We will present the development of novel genetically-encoded, green- and red-fluorescent-protein-based reporters that allow non-invasive, quantitative measurements of actin filament organization in living cells and tissues by using polarization-resolved fluorescence microscopy. We will show examples of actin organization measurements in living mammalian cells in culture, as well as in living fission yeast, Drosophila and C.elegans embryos.

Poster 115 - Molecular Mechanisms Actin(G) on Endosomal Sorting

Kerrie McNally[1], Akaash Kumar[1], Megan Clapperton[1], Franck Perez[2], David Baker[3], James Manton[1], Emmanuel Derivery[1]

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The abundance and localisation of transmembrane proteins on the cell surface enables cells to sense and respond to environmental signals, facilitate nutrient uptake and migrate. Endosomes are important protein sorting stations, playing a key role in sorting endocytosed integral proteins (cargos) for lysosomal degradation or, alternatively, for recycling back to the plasma membrane. Several multi-protein complexes are essential for cargo recycling including the WASH complex, an endosomal Arp2/3 nucleation promoting factor. WASH-dependent endosomal branched actin is required for endosomal sorting of many cargos. The correct sorting of integral proteins is critical for maintaining cellular homeostasis, with mutations in sorting complexes causative for various human diseases, such as Ritscher-Schinzel syndrome being caused by mutations in the WASH complex.

However, the molecular mechanisms by which WASH-dependent branched actin drives endosomal sorting of cargos remains poorly understood. Here I will present the development of new technologies to uncover the underlying molecular mechanisms of endosomal sorting, with a focus on the role of branched actin in this process.

Imaging endosomal sorting is difficult; endosomes move rapidly and require fast imaging but the need to image multiple proteins during cargo sorting (cargos, sorting complexes, actin) slows acquisition. We have developed a multispectral imaging approach, capable of imaging 8-fluorescent channels simultaneously, without a loss in spatiotemporal resolution. We coupled this with synthetic biology to image endosomal sorting events with multiple cargos, sorting complexes and actin simultaneously, affording us an integrated view of a sorting event. In addition, we have developed nanobodies that specifically block endosomal branched actin nucleation, to assess the mechanistic role of branched actin both in our in vitro reconstitutions of sorting and in cells.

This unique combination of multispectral microscopy, de novo protein design and in vitro reconstitution aims to establish the molecular mechanisms and physical principles underlying endosomal sorting.

Poster 116 - Traversing a Bio-Condensate: Structure and Transport Dynamics in the Fission Yeast Actin Fusion Focus

Zeno Messi, Valentine Thomas, Hikari Mase, Sophie Martin

University of Geneva

Intracellular transport and local secretion play crucial roles in various biological processes, from neurotransmitter release at synapses to fungal hyphal steering and yeast gamete fusion. In Schizosaccharomyces pombe, the actin fusion focus–a dense actin aster assembled by the formin Fus1–concentrates secretory vesicles carrying cell wall digestive enzymes to facilitate cell-cell fusion. Our recent work highlights the role of Fus1's intrinsically disordered region (IDR) in the condensation of this secretory focus.

The IDR mediates focus condensation in vivo, forming an assembly that excludes ribosomes while allowing passage of localization factors, Myosin V-driven vesicles, and G-actin/profilin. Notably, replacing the Fus1 IDR with a domain that forms stable oligomers restores focus formation but impairs cell fusion, suggesting that dynamic condensation is critical for function. These findings imply that IDR-mediated condensation may be selectively permeable for actin network organization and vesicle concentration.

Despite these insights, the spatial arrangement and transport dynamics of the focus components remain unclear. We aim to determine how Myosin V-transported secretory vesicles navigate the crowded focus to reach the plasma membrane. Preliminary data suggest focus components do not maintain fixed relative positions but instead undergo rearrangements during maturation. Interestingly, the bulk of Myosin V appears to detach from the pool of secretory vesicles and participate in actin focus organization, raising the question of how vesicles and digestive enzymes traverse the Fus1 condensate.

To address these questions, we employ single-particle tracking (SPT) and high-accuracy imaging of sparsely labeled focus components using self-labeling protein tags (HaloTag). This approach allows us to track component dynamics across different condensation stages and in fusion-defective Fus1 IDR mutants, shedding light on how condensate properties influence function.

Our study will provide fundamental insights into how cargo navigates the dense environment of biomolecular condensates, potentially revealing a broadly conserved mechanism of vesicle transport and secretion in cellular organization.

Poster 117 - How Cells Sense and Respond to Multi-Component Fibronectin /Vitronectin Matrices During Early Adhesion

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The extracellular matrix (ECM) regulates cell adhesion and force transmission through its molecular composition and structural organization. Fibronectin (FN) and vitronectin (VN) are major adhesive ECM proteins that present shared RGD motifs but differ in molecular context, raising the question of how cells sense mixed ECM environments and translate these cues into mechanical adhesion during the earliest stages of contact.

Here, we investigated how αv-class integrins regulate adhesion initiation and force development on two-dimensional FN/VN matrices with defined ligand ratios. Using engineered fibroblast cell lines selectively expressing αvβ3, αvβ5, or both integrins, we quantified adhesion initiation and strengthening using atomic force microscopy–based single-cell force spectroscopy. Adhesion forces were measured as a function of contact time, and binding probability was assessed from repeated short contact–retraction cycles, enabling direct probing of integrin-mediated force transmission at early timescales.

We found that adhesion forces increased progressively with rising VN content, forming a continuous VN-dependent gradient that was lowest on FN, intermediate on FN/VN mixtures, and highest on VN. This dependence was already evident at early contact times and was reflected in both adhesion forces and adhesion strengthening rates. Adhesion forces adjusted continuously with FN/VN ratio, increasing nearly linearly with VN concentration, indicating that force generation reflects the relative abundance of ECM ligands rather than dominance of a single component.

Analysis of integrin-specific contributions revealed matrix- and time-dependent modes of force regulation. On FN, αv-class integrins exhibited early competitive interactions, whereas VN-rich matrices supported additive, independent contributions during adhesion maturation. FN/VN mixtures accelerated early adhesion initiation, while VN-rich environments dominated force strengthening over time.

Together, these results demonstrate that ECM composition tunes early integrin-mediated force transmission and determines whether αv-class integrins engage competitively or additively during adhesion maturation. This work highlights how multicomponent ECMs regulate cytoskeleton-coupled adhesion mechanics at the single-cell level.

Poster 118 - Multi-Scale Mechanics of the Cell Cortex in the Transition to Multicellularity

Djibril Ruben Montaville[1, 2], Hugo Lachuer[2], Nicolas Borghi[2], Olivia du Roure[1], Julien Heuvingh[1]

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The cell cortex is a thin layer of cytoskeleton beneath the plasma membrane of eukaryotic cells. It is an active gel made up of actin filaments that polymerize and depolymerize continuously, and that form a physically continuous, contractile network thanks to crosslinkers and molecular motors. The cell cortex is crucial for essential cellular processes such as division and migration. In addition, multicellularity involves the cell cortex into additional functions such as tissue morphogenesis, homeostasis, and repair. This is made possible by the modulation of tensions at cell-cell interfaces supported by specific junctional complexes.

We recently found in model epithelial cells that the transition from a unicellular to an epithelial state leads to a drastic change in the mechanics of the cortex, which becomes denser, twice as thin and three times as rigid. This change may constitute a response to the new mechanical constraints imposed by multicellularity. The microscopic determinants of this transition, and the mechanisms by which it contributes to tissue functions are, however, unknown.

To address question, we aim to characterize how cell-cell interfacial tension emerges from cell cortex mechanics. To do so, we will measure cortical stiffness, thickness and cell-cell interfacial tension at the mesoscopic scale using a custom magnetic pincher with a pair of intracellular magnetic beads. We will then combine these measurements with force inference to map absolute interfacial tensions across the tissue. Finally, we will determine its relation to molecular mechanics of the cell-cell junctions using FRET tension sensors inserted in adhesion proteins.

Poster 119 - A Novel Mechanism of Regulation of MYPT1 Via Direct Interaction with PKA Regulatory Subunits and Its Relevance in Platelet Function

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Background: Platelet spreading, aggregation and secretion at the site of vascular damage requires morphological changes driven by cytoskeletal remodelling. These processes are catalysed by the phosphorylation and dephosphorylation of the myosin II regulatory light chains by myosin light chain kinase and myosin light chain phosphatase (MLCP), respectively. The key component of MLCP is the targeting subunit (MYPT1), that directs the catalytic subunit near its target protein. The activation status of MYPT1 is determined by the relative activities of the Rho associated coiled-coil kinase (ROCK) and protein kinase A (PKA). PKA is the foremost effector of cyclic AMP (cAMP) signalling and is tethered to its targets by A kinase anchoring proteins (AKAPs). Aims: We aim to elucidate the direct effect of cAMP signalling on MLCP activity and its relevance on platelet function affecting thrombus formation. Methods: Affinity pulldown, immunoprecipitation and in situ proximity ligation assay (PLA) were used to test for direct interaction between the regulatory subunits of PKA (PKA-R) and MYPT1 in platelets, endothelial cells and HEK293T cells. Peptide array epitope mapping and substitution analysis were used to identify hot spots where the substitution resulted in loss of binding. Aggregometry was used to test for functional relevance of the MYPT1-PKA interaction in platelets. Results: GST-tagged PKA-Rs were able to pull-down MYPT1 in platelet lysates and HEK293T cells. Transfection of HEK293T cells with myc-tagged MYPT1 constructs narrowed down the site of interaction to a central region of MYPT1. PLA corroborated these interactions in situ. Targeting the MYPT1-PKA interaction led to increased thrombin-mediated platelet activation. Summary/Conclusions: Our study identifies a novel mechanism of regulation of MYPT1 via direct interaction with PKA-Rs. We propose that MYPT1 could act as an AKAP that tethers PKA to the MLCP signalling node. Disruption of the MYPT1-PKA interface in platelets shows the functional relevance of the interaction.

Poster 120 - A Quality Control Pathway that Prevents Cin8 Aggregation and Ensures Proper Spindle Morphology

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The yeast kinesin-5/Cin8 is a bidirectional kinesin responsible for spindle formation, kinetochore congression and spindle elongation. To exert these functions, Cin8 forms clusters composed of multiple Cin8 motors. Cluster formation is indispensable for spindle formation and elongation as it modulates the directionality of Cin8, leading to movement of the Cin8 motor clusters towards the plus-ends of microtubules. Interestingly, clustering of Cin8 is regulated by Hsp70 chaperones and deletion of the Hsp70 nucleotide exchange factor Sse1/Hsp110 leads to Cin8 over-clustering and uncontrolled plus-end-directed motility of Cin8. Previous work in the lab has shown that Cin8 is an aggregation-prone protein that forms detergent-resistant aggregates upon overexpression, leading to the hypothesis that uncontrolled Cin8 clustering may have Cin8 aggregation as a side effect.

Here, we investigate the molecular mechanisms that lead to Cin8 aggregation, and the quality control mechanisms utilized by cells to dispose of Cin8 aggregates. Using Bimolecular Fluorescence Complementation (BiFC) experiments, we show that Cin8 interacts with the SUMO-dependent ubiquitin ligase Slx5, under overexpression of both proteins. Using a GFP-trap approach, we identified that Slx5-Cin8 BiFC adducts contain a prion-like protein as well as two chaperone proteins involved in the regulation of amyloid-type aggregates. We further show that some of the identified proteins co-localize with the BiFC signal, supporting the idea that Slx5 targets Cin8 aggregates that form upon Cin8 overexpression.

To test whether Slx5-dependent degradation of Cin8 acts as a quality control pathway that operates upon deregulation of Cin8 clustering, we are currently monitoring Cin8 localization and spindle morphology in cells deleted for the Sse1 or Slx5 proteins or in mutants that prevent Cin8 SUMOylation and degradation.

These efforts aim to uncover novel quality control mechanisms that protect the mitotic spindle from protein aggregation.

Poster 121 - Self-Organization of Septin Filaments at an Artificially Reconstituted Intercellular Bridge: Mimicking Cytokinesis

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Cell shape evolves over time and varies with cellular function, and the resulting membrane curvature distribution modulates the organization of cellular components, their self-organized structures, and associated functions. We have recently discovered that human septins, an eukaryotic cytoskeletal protein, interact directly with membranes and undergo curvature-dependent self-organization when bound to the membranes, with microscale curvature sensitivity. Septins are a crucial component of animal cytokinesis and localize to the intercellular bridges bridging dividing cells during cell division. However, their role in these bridges and self-organised structures are not well described. In the present study, we have developed a new strategy to mimic the shape of intercellular bridges of dividing cells. This methodology reconstitutes the geometry of intercellular bridges in vitro using a microfluidic chip, and investigates the organisation of septin filaments in this confined space. Upon septins addition to the microstructures, coated with supported lipid bilayers (SLBs), septin interaction with the SLBs was monitored. Self-organised septin filaments within our reconstituted intercellular bridges were visualised using stimulated emission depletion microscopy (STED). We will present the developed methodology, the characterization of the lipid bilayers and the self-organization of septins at the artificial intercellular bridge, as well as their imaging by super-resolution microscopy. This method can potentially be used to decipher the confinement- or geometry-dependent behaviours of cellular components in vitro.

Poster 122 - Phase Separation Enables Dynamic Treadmilling of Actin Bundles

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Self-organization and collective motion of cytoskeletal filaments is essential for living systems. In cells, these dynamics are facilitated by the actin cytoskeleton through assembly and disassembly of actin filaments. While concepts of self-balancing for treadmilling have been discussed in the absence of actin-binding proteins, it has been shown that the presence of actin-binding proteins is essential for efficient and localized dynamic turnover in cells. However, proteins influencing the treadmilling rates destabilize the balance arresting persistent turnover of ordered actin structures. Here, we introduce an in vitro system where actin filament bundles are self-organized through actin polymerization and disassembly. Phase-separated condensates of zyxin and VASP balance cofilin-driven disassembly to enable persistent actin treadmilling. The condensates crosslink filaments into dynamic bundles while promoting barbed-end polymerization. This localized stabilization based on condensate-mediated bundling competes against the activity of cofilin and CAP1, enabling selective disassembly at pointed ends and recycling of monomers. We complement our experiments with agent-based simulations that quantitatively recapitulate key experimental findings to elucidate the physical basis underlying this emergent behavior. The combined approach demonstrates that robust treadmilling requires an optimal phase separation strength to maintain high local concentration while preserving condensate fluidity. A weakened phase separation fails to stabilize the bundles, whereas overly cohesive condensates impede filament dynamics. Experimental reconstitution and theoretical modeling together reveal a physical mechanism by which the material properties of multivalent protein condensates govern the cytoskeletal turnover and provide a model on how coupling of higher-order organization with actin dynamics enables persistent collective cytoskeletal motility.

Poster 123 - Deciphering the (Ultra-)Structural Architecture of the Golgi Apparatus and Its Associated Cytoskeletal Filaments by Cryo-Electron Tomography

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The Golgi apparatus is a central hub for membrane trafficking and intracellular communication. In mammalian cells, individual Golgi cisternal stacks are connected laterally to form a ribbon, essential for cell polarity and directed migration. Cytoskeletal filaments are essential for maintaining Golgi architecture and positioning, allowing it to fulfill its functions. A distinct connection between Golgi membranes, actin filaments, and Golgi-derived microtubules coordinates Golgi ribbon formation, maintenance, and disassembly. However, less is known about how the Golgi associates with intermediate filaments (IFs); here, only a link between vimentin and a trans-Golgi network (TGN) Golgin has been shown. Previous studies, mostly by fluorescence microscopy, room temperature electron microscopy, and single particle cryo-electron microscopy, focused on individual or a small number of players within this highly complex and dynamic assembly. Thus, in this study, we aim to provide the basis for integrating previous studies into a more holistic representation of the Golgi ribbon by providing an in situ ultrastructural framework derived from quantitative analysis of cryo-electron tomography (cryo-ET) of focused ion beam (FIB)-milled epithelial (hRPE1) and fibroblast (BJeH) cells. To this end, to integrate findings concerning the roles of the cytoskeleton in Golgi organization, we established a filament tracing and polarity determination method based on template matching and deep learning that provides the basis for efficient classification, subtomogram averaging, and inference of filament origins as well as transport directions. We annotated individual subunits of cytoskeletal filaments surrounding the Golgi, identified membrane structures with TGN identity surrounded by filamentous actin, IFs, and microtubules. Supplementally, we revealed the presence of IFs at cis-Golgi for which no corresponding adapter proteins have yet been identified. In conclusion, our analyses laid the cornerstone for developing an integrated model of the Golgi apparatus at ultra-structural resolution.

Poster 124 - What Makes a Cell One? On the Impact of Cell Size on Cytoskeletal Self-Organization

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Cell size varies over several orders of magnitude, from micrometers for stem cells to centimeters for oocytes. Cytoskeletal networks play a prominent role in the maintenance of structural integrity in relationship to cell size and shape determination. Such maintenance of structural integrity upon changes in metabolic or environmental context requires a strong coupling between cytoskeletal organization and cell size. In this study, we aim at investigating the interplay between cytoskeletal self-organization and the maintenance of mechanical integrity for cells to produce an integrated response to environmental cues. Particularly, we focus on the relationship between cell size and cytoskeletal self-organization.

We adapted a method based on membrane fusion mediated by the viral fusion protein VSV G in order to induce rapid changes in cell size, and monitor the following reorganization of actin and microtubule networks architectures and dynamics. We fuse enucleated cells called cytoplasts with nucleated cells on dynamic micro-patterns in order to produce syncytial systems with varying size and/or varying nuclei-to-spreading area ratios and analyze their polarization and migration abilities. We hence explore the relative contributions of spreading area and DNA-to-spreading area ratios in the interplay between cell size regulation, cytoskeletal organization and cellular functional integrity.

Our preliminary results suggest that below a critical size increase, actin and microtubule networks organization, polarization and migration capacities of enlarged systems are similar to the ones of single cells. Above this critical size, we observed a reorganization of the microtubule network from a centrosome-driven organization towards the formation of parallel microtubule bundles that correlates with a loss of polarization and effective migration capacities in larger systems. We now aim at exploring the potential changes in cytoplasm density and microtubule stability with cell size and their contribution to the apparent loss of cytoskeletal integration at large cell sizes.

Poster 125 - Simple Chromosome Partitioning Mechanisms

Helen Saville, Claire Jacquerie, Francois Nedelec

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We used simulations to explore mechanisms of chromosome partitioning with the aim of understanding the design principles of mitotic spindle assembly. Using artificial evolution of simulated models in a computer we uncovered a truly simple combination of cytoskeletal elements that will self-organize into a bipolar structure to pull on a kinetochore pair symmetrically and reliably. I will demonstrate the mechanism using live simulations and discuss how such elementary mechanisms might reflect the early evolution of mitotic mechanism in eukaryotes.

Poster 126 - Passive Nematic Organization of Actin Filaments Is Sufficient to Drive Membrane Deformation

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The shaping of cellular membranes relies on the mechanical interplay between the lipid bilayer and the underlying cytoskeleton. Recent theoretical studies predict that the orientational order of actin filaments alone can mechanically deform membranes, as the elastic energy cost associated with topological defects can be reduced by the formation of conical protrusions.

Here, we investigate this physical mechanism using a minimal reconstituted system. We utilize cell-sized giant unilamellar vesicles (GUVs) having their outer surfaces decorated with a dense layer of short, rigid actin filaments anchored to the membrane via the actin-membrane linker Ezrin. On spherical GUVs, the actin film exhibits nematic alignment and characteristic +1/2 topological defects. Upon a critical Ezrin concentration, a denser actin network is recruited, which is sufficient to generate outward membrane tubules. We observed longer tubules when increasing ezrin concentration. On these tubules actin filaments exhibit nematic alignment along the tubule axis.

These findings are consistent with theoretical predictions where the minimization of Frank elastic energy compensates for the increase in membrane tension and bending energy to drive tubulation. Our results provide new insight into how nematic actin order can drive cell surface deformation.

Poster 127 - Semi-Automated Tracking of in Vitro Actin Parameters

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Actin filament dynamics underlie key cellular processes, yet quantifying these behaviors produced in biomimetic assays visualized with fluorescence microscopy remains a major bottleneck due to the time, bias, and variability inherent to manual analyses. We developed a semi-automated image analysis pipeline that integrates the TSOAX image segmentation software with a custom interface for rapid identification, verification, and measurement of actin filaments imaged by in vitro Total Internal Reflection Fluorescence (TIRF) microscopy assays. Once actin filaments are segmented and tracked in each TIRF movie through time by TSOAX, several parameters related to actin polymerization and higher-order structures can be extracted, including filament counts (e.g., nucleation), elongation rates, bundling, and sites of filament branching. Our TIRF experiments spanned a range of actin assembly conditions, including concentration-dependent polymerization, reactions that accelerate or halt elongation, and those altering filament architecture by initiating filament bundling or branching. The semi-automated pipeline produced quantitative measurements that closely match published and manual analyses but require less time and reduce user bias. This adaptable computational approach resolves a key quantitative barrier in cytoskeleton research, expanding the experimental scale, reproducibility, and scope of in vitro actin studies, additional actin regulatory proteins, and potentially other cytoskeletal polymers.

Poster 128 - Environmental Sensing and Contractility Dictate the Plasticity of Collective Cancer Cell Migration Plasticity

Clémence Nguyen-Vigouroux[1], Tatiana Cetenovic[1], Célia Galleri--Paris[1], Johanna Protin[1], Elise Carraz-Billat[1], Charlotte Canet-Jourdan[1], Michel Ducreux[1], Jacques Mathieu[1], Fanny Jaulin[1], Florent Peglion[1]

1 Gustave Roussy

The metastatic dissemination of individual cells or cell collectives is a decisive step in the progression of cancers. Migrating single cells dynamically switch between mesenchymal traction-based and amoeboid propulsion-based mode of migration in a mechanism named plasticity. Collective migration has mainly been described as a traction-dependent mode of locomotion. While a propulsive collective migration has recently been reported, we questioned whether cell clusters are also endowed with plasticity. Here, we report that patient-derived digestive cancer organoids exhibit a preferred mode of migration but transition to the alternate strategy to adapt to external stimuli or the manipulation of intrinsic determinants. We showed that the tumor cell cluster plasticity observed in engineered microchannel devices also ensures metastatic seeding in a murine model of peritoneal carcinomatosis. These findings reveal an adaptive mechanism at play during tumor invasion that must further be decrypted to enable the design of therapeutic strategies preventing metastatic progression.

Poster 129 - Probing Membrane and Cytoskeletal Mechanics with SENSOCELL Optical Tweezers: From Surface Tension to Intracellular Micro-Rheology.

Oriol Nos Aguilà

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The plasma membrane, cytoskeleton, and nucleus exhibit mechanical properties that are essential for cell signalling, migration, polarity, and morphogenesis. Optical tweezers provide a direct and minimally invasive way to quantify these properties in living systems.

Here, we present the SENSOCELL optical tweezers platform (IMPETUX) for multi-scale measurements of cellular mechanics. The system enables membrane tension, force propagation, stiffness, and receptor–ligand interactions to be quantified through tether pulling, bead–cell adhesion, and indentation assays. We also showcase TimSOM, an automated active microrheology workflow for probing intracellular viscoelasticity in the cytoplasm and nucleus of cells, embryos, and organisms such as C. elegans.

Poster 130 - Aurora a Kinase Regulates Material Properties of Spindle Poles to Organize 3-D Nuclear Architecture Post-Mitosis

VIGNESH OLAKKAL et al.

Indian Institute of Science, Bangalore

Université Paris-Saclay

The proper shape and organization of the nucleus is critical for ensuring error-free development. In cycling animal cells, the nuclear envelope (NE) disassembles at the onset of mitosis and reassembles upon mitotic exit. This process is tightly coordinated with changes in microtubule based spindle pole organization: spindle poles enlarge during mitotic entry and shrink during mitotic exit. However, the biological significance of this coordination has remained unclear. In this study, we uncover a key role for Aurora A kinase in regulating the dynamic behavior of the spindle-associated protein NuMA (Nuclear Mitotic Apparatus) at spindle poles during anaphase. By establishing an ingenious tool to rapidly degrade Aurora A kinase at anaphase onset, we demonstrate that its activity is essential to maintain NuMA in a dynamic state at the poles. Upon Aurora A inactivation, NuMA loses its dynamicity and abnormally accumulates at the poles. This aberrant accumulation leads to bending of the segregated chromosome ensemble around the NuMA-enriched poles, ultimately resulting in disrupted nuclear morphology and impaired re-establishment of interphase chromosome organization. We further show that NuMA localization to spindle poles is governed by microtubule-dependent minus-end-directed dynein/dynactin motor, its coiled-coil domain, and its C-terminal intrinsically disordered region (IDR). Through mutagenesis, we identify arginine and aromatic residues within NuMA IDR as key determinants of multivalent homotypic (cation-pi) interactions that drive pole localization, while glutamine residues promote solid-like transitions upon Aurora A loss. Notably, a proof-of-concept experiment demonstrates that artificially increasing these homotypic interactions can phenocopy Aurora A inactivation. Together, our results highlight the importance of tightly regulating the material properties of spindle poles to ensure accurate nuclear assembly and genome organization following mitosis.

Poster 131 - Impact of Vimentin Intermediate Filaments on Actin Assembly Dynamics

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Vimentin intermediate filaments play essential roles in maintaining cell integrity and regulating numerous cellular functions. In particular, vimentin cooperates with the actin cytoskeleton in key cellular processes that rely on actin dynamics, such as such as migration, division, and mechanosensing. While there is evidence that these two cytoskeletal components interact in cells, the underlying molecular mechanisms are only partially understood. Actin and vimentin can interact through biochemical signaling pathways or via cross-linkers, but whether they engage in a direct protein-protein interaction has remained controversial, in part because such interactions are difficult to isolate and characterize in cells. Using in vitro reconstitution and total internal reflection fluorescence microscopy to monitor the elongation of single actin filaments, we show that vimentin promotes actin elongation at the barbed end in a dose-dependent manner. Neither the vimentin tail nor head domains are required for this effect, and both filamentous and non-filamentous vimentin enhance actin elongation. We find that this acceleration results from a stabilization of actin subunits at the barbed end. Strikingly, this effect depends on the nucleotide state of actin, as the acceleration is only observed for the elongation from ATP-actin, and not ADP-actin monomers. This stabilization also enables vimentin to promote the nucleation of actin filaments. Consistently, magnetic pull-down assays demonstrate a direct, albeit weak, interaction between vimentin and actin monomers. Altogether, these findings identify vimentin as an unexpected new actor in the regulation of actin dynamics at the barbed end and bring new insights into the functional role of vimentin through cytoskeletal crosstalk.

Poster 132 - Exploring the Role of Microtubules and Molecular Motors in T Cell Polarity at the Immune Synapse

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Biological context

In T cells, the cytoskeletal polarization is essential for the immune synapse (IS) formation. This structure, discovered in the 1990s ensures the concentration of signaling effectors at the point of antigen recognition and permits a precise polarized delivery of immune factors within minutes following antigen recognition at the surface of a presenting cell.

Objective

Although studied for decades, the mechanisms involved in this rapid polarization of T cells are still ill defined. The most recent models propose that the local recruitment of dynein motors at the synapse anchors some microtubules (MTs) to the cortex and exert pulling forces causing the MTs to slide along the membrane while dragging the centrosome. Yet, experimental data on the intracellular distribution of motors and microtubules and of their detailed role in IS formation are lacking. The aim of my PhD is to decipher the role of MT and molecular motors in T cell polarity.

Methods

To observe new details in the organization of MTs and the distribution of motors during IS formation we developed super-resolution microscopy methods such as expansion microscopy, as well as spatially controlled live-microscopy set-ups. We are also studying the consequences of MTs and motors impairment by drugs or genetic modifications on polarization.

Results and Conclusion

Through the gain in spatial and temporal resolution provided by our methods, we reveal MT and motor organizations that challenge current polarization models. In particular, we identify previously undescribed interactions of microtubules with both the nucleus and the actin cortex, which could change the repartition of forces generated during IS formation. Moreover, contrary to the models proposed in the literature, we do not observe a concentration of molecular motors at the synapse, but rather a homogeneous distribution. I will present these results and discuss how they can change our view of T-cell polarity.

Poster 133 - Ezrin Mutations Influence Actin Cortex Formation

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The actin cell cortex is a thin layer of a filamentous-actin network located just beneath the plasma membrane, encasing the entire cell with a dense, cross-linked , undergoing constant polymerization and depolymerization, contributing to cellular stability and motility.

Ezrin links the plasma membrane to the actin cortex of the cytoskeleton. Ezrin has been shown to be overexpressed in virtually all types of cancer, where it promotes dynamic remodeling of the actin cortex, facilitating increased cell motility, promoting cancer metastasis.

The distinct amino acids involved in the interaction between ezrin and actin remain elusive, so this project aims to quantify the effect of introducing point mutations into the actin-binding domain of ezrin on binding affinity. By identifying essential residues, this study may provide insights into potential therapeutic targets for modulating ezrin-actin interactions.

This is realized by reconstituting minimal actin cortices on solid-supported lipid bilayers, using the physiological ezrin-PIP2 linkage. Confocal laser scanning microscopy (CLSM) in combination with a fluorescence micrograph analysis algorithm were used to assess the impact of ezrin mutations and anchor lipid concentration on actin cortex formation and drastic changes in actin-binding ability were found, when introducing just a single point mutation into the actin-binding domain of ezrin. Additionally, colloidal probe atomic force microscopy (CP-AFM) is employed to investigate the binding affinity of the different ezrin mutants by determining the dissociation rate constant of the ezrin-actin interaction.

Poster 134 - The Role of Integrin Traffic Regulator Swip1 in Hybrid Epithelial/ Mesenchymal Cell State Transition

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Breast cancer is a common cancer in women and treating the metastatic breast cancer remains a significant challenge. Epithelial-to-mesenchymal transition (EMT), and the more recently recognized ability of cells to reside in a plastic hybrid E/M state, are critical in breast cancer metastasis. During this process, epithelial cells gradually lose their apical-to-basal polarity and cell-cell adhesion while increasing their adhesion to the surrounding microenvironment and acquiring invasive abilities. These changes are due to the altered expression and function of integrins, and in part, E-cadherin, which are both constantly shuttled between the plasma membrane and the endosomal compartments through endocytosis and exocytosis. Endocytic trafficking plays an important role in regulating cancer cell migration, however its role in the regulation of cell state transitions and EMT is less well characterized. Integrin endocytosis is regulated by multiple mechanisms and recently Swip1 (Swiprosin-1, or EFHD2), an actin-binding protein that functions as an integrin adaptor in the CG endocytosis pathway, has been shown to promote breast cancer cell migration through this pathway. High level of Swip1 is also a negative prognostic factor in triple negative breast cancer. However, the relevance of Swip1 in controlling EMT remains to be investigated in breast cancer. Using mouse breast cancer cell lines from different stages of metastasis, we observed a gradual transition from epithelial to mesenchymal states from primary tumors to distant metastases. Interestingly, Swip1 possesses opposing regulatory roles on endosomal trafficking of E-cadherin and integrin β1 in these cell lines. While Swip1 depletion increased the level of internalized E-cadherin, it reduced internalized integrin β1. Furthermore, upon Swip1 depletion, the cells altered their polarity and showed a partial transition towards a more epithelial state. Overall, these data indicate Swip1 as a regulator of hybrid epithelial-mesenchymal cell states through the control of E-cadherin and integrin β1 endocytosis in breast cancer.

Poster 135 - Actin-Driven Mechanical Adaptation Modulates Phagocytic Capacity in a Postmitotic Epithelium

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Tissue homeostasis relies on mechanical feedback loops that balance cell loss and proliferation. In postmitotic tissues, compensatory proliferation is not available and alternative adaptive mechanisms must preserve structure and function. The retinal pigment epithelium (RPE) is a postmitotic epithelium that undergoes pronounced structural remodelling during ageing, yet how these changes relate to mechanical homeostasis and the vision supporting function of photoreceptor outer segment (POS) phagocytosis remains poorly understood.

To address this, we established a reductionist in vitro model of age-associated cell loss by inducing large-scale apoptosis in stem cell-derived RPE monolayers. This cell density reduction recapitulates key structural features of aged RPE, including reduced cell height, shortened apical microvilli and reorganisation of the actin cytoskeleton. The remodelled tissue acquires a new mechanical homeostasis, characterized by tissue stiffening, increased elasticity, reinforced cell-cell junctions and elevated contractility.

Functionally, these age-like monolayers display altered POS phagocytosis associated with reduced apical actin ruffling and diminished apicolateral deformation. Transcriptomic analysis reveals broad changes in actin-associated genes, including nucleators, bundlers and membrane linkers. Pharmacological modulation of actin nucleators shows that Arp2/3- and formin-dependent actin remodelling differentially control particle number and size during phagocytosis, linking actin cytoskeletal dynamics to functional output.

Together, our results* show that age-related structural remodelling in a postmitotic epithelium establishes a distinct actin-driven mechanical homeostasis that directly modulates phagocytic capacity. In this new range of mechanical homeostasis, actin dynamics are redirected toward tissue reinforcement and preservation at the expense of plasticity required for phagocytosis. These findings highlight how cytoskeletal adaptation to cell loss alone can compromise tissue function, independent of additional stressors.

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Poster 136 - Topography-Dependent Interaction of BAR Proteins and Actin Components in a Minimal System

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Single amoeboid cells are able to adapt their cell shape rapidly in order to efficiently migrate through complex 3D environments and do not rely on adhesions. A key process here is actin polymerization which helps to propel the cell forward as well as to push fibers of the ECM away to make space. This is achieved by locally coupling actin pushing forces to topographic changes in the cell's environment experienced by the plasma membrane. However, the mechanism of how membrane curvature can trigger local actin polymerization is not well understood. Especially the role of curvature-sensitive BAR-proteins is not clear. By using a reconstituted system which consists of a solid-supported lipid bilayer featuring ridges, BAR-proteins and branched actin components, we aim to study the interplay between membrane topography and actin polymerization. We observed different localization and signal distribution for different BAR, I-BAR and F-BAR proteins along the ridges. Interestingly, PWA, a truncated and active version of actin nucleation promoting factor N-WASP, forms clusters preferably along the ridges in the absence of BAR proteins. Next steps will include measurements with full-length N-WASP. Moreover, polymerizing actin will be added to study its localization and density dependent on different BAR proteins. Ultimately, this system will allow us to better understand the generation of local pushing forces in adhesion-independent cell migration.

Poster 137 - The Kinesin Kip2 Promotes Microtubule Growth Using a Bipartite Polymerase Module to Deliver Tubulin to Microtubule plus Ends

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Kinesin molecular motors are essential for processes such as chromosome segregation and vesicular transport. To fulfill their function, many kinesins promote microtubule growth, but the underlying molecular mechanism remains unclear. One of the motors with the strongest microtubule growth-promoting activity is Kip2, a kinesin that is required for astral microtubule integrity and spindle positioning in yeast. Here, we show that Kip2's ability to polymerize microtubules is coupled to binding and transport of free tubulin. The unstructured, basic N terminus of Kip2 is essential for microtubule elongation in vitro and in vivo, binding free tubulin and transporting it to microtubule plus ends. In addition to the N terminus, ATP hydrolysis and motor activity are required for polymerization. Notably, transferring the Kip2 N terminus to kinesin-1, which lacks polymerase activity, converts kinesin-1 into a tubulin-transporting polymerase. We propose that motor-driven tubulin delivery to microtubule plus ends is an efficient mechanism used by kinesins to promote microtubule polymerization.

Poster 138 - Roles of Fascin, Cofilin, and Myosin-10 in Filopodial Dynamics and Cancer Metastasis

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Metastases remain a key challenge to treating cancer, driving the majority of cancer-related deaths and remission. Unfortunately, avenues for reducing their frequency and severity are still limited, making the identification of possible interventions an important treatment frontier. Over the past two decades, evidence has shown that finger-like filopodial protrusions at the leading edge of cells are formed at greater frequency in and are associated with cancer metastasis. Additional findings have implicated three actin-binding proteins involved in filopodia formation in worse cancer outcomes: fascin, myosin-10 (MYO10), and cofilin. Each play different but complimentary roles in filopodia. Fascin bundles actin filaments; MYO10 is thought to bind to PI(3,4,5)P3 at the leading edge to facilitate initial filopodial protrusion or traffic along bundled actin filaments; and cofilin severs and depolymerizes actin filaments to enable monomer recycling. Interfering with either of these proteins decreases cancer migration and metastasis, and perturbing MYO10 or fascin decreases filopodia number or length. Additionally, Fascin is a promising target with NP-G2-044 in clinical trials for solid tumor malignancies. My project aims to elucidate the contributions of fascin, cofilin, and MYO10 to actin assembly and disassembly pathways in filopodia by combining filopodia imaging in cancer cells and using a reconstitution system of filopodia-like structures made from supported lipid bilayers and frog egg extracts. I am asking how these three proteins are temporally recruited relative to actin and each other and how these dynamics are affected by lipid composition. Better characterization of the underlying molecular mechanisms will provide insight into how targeting these cytoskeletal proteins ameliorates metastatic potential at a molecular level, with the possibility of identifying more relevant drug targets.

Poster 139 - Visualizing the Immunological Synapse at Molecular Resolution Using Cryo-Electron Tomography

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Cytotoxic T lymphocytes eliminate virus-infected and cancer cells by forming immunological synapses with them. Cortical actin plays a critical role in stabilizing this interface, supporting receptor signaling and organizing the synapse architecture. However, the three-dimensional organization of the actin filaments underlying the plasma membrane at the immunological synapse, and its effect on plasma membrane geometry, remain poorly understood. Using cryo-electron tomography (cryo-ET), we analyzed immune synapses formed between human cytotoxic T cells and mouse cancer cells. We visualized the molecular architecture of the T cell interior, providing high-resolution snapshots of lytic granules transported by microtubules and curved actin filaments, as well as mitochondria and nucleii in close proximity to the synapse. Our data revealed uneven plasma membrane-plasma membrane spacing, with regions of very close apposition associated with actin nanofoci in the cytotoxic T cells. These nanofoci display a tent-like structure of oblique, branched filaments that are directed towards the plasma membrane, which is consistent with Arp2/3-mediated nucleation. The nanofoci deform the membrane of the cytotoxic T cells locally, suggesting that they play a role in receptor clustering and exclusion in the plasma membrane. Cryo-ET is uniquely capable of visualizing these contacts at a molecular level, capturing both these nanoscale events and the overall synaptic landscape. Our work provides structural insights that confirm assumptions and uncover unexpected details.

Poster 140 - Molecular Determinants of Inborn Errors of Immunity (IEI) Caused by Mutations in Cytoskeletal β-Actin

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Cytoskeletal β- and γ-actin, encoded by ACTB and ACTG1, are ubiquitously produced across non-muscle cells and orchestrate important cellular processes like migration and adhesion. Recently, heterozygous de novo mutations in ACTB have been identified in patients exhibiting severe and recurrent viral infections, elevated IgE levels, and thrombocytopenia, suggesting an underlying immunodeficiency. These findings prompted us to assess the potential role of ACTB mutations as Inborn Errors of Immunity (IEI). Mutations in β-actin can disrupt cytoskeletal remodelling in lymphocytes, leading to impaired cell migration or defects in actin-dependent structures such as the T-cell immunological synapse. We selected a cluster of five β-actin mutations (p.K315Δ, p.K315E, p.G342D, p.E364K, and p.G366V), all linked to immunodeficiency, for a comprehensive characterization of the underlying molecular mechanisms. Initial biochemical studies on purified recombinant variant proteins showed that the mutants were able to polymerize, but displayed significantly reduced thermal stability compared to wild-type β-actin. Molecular dynamics simulations indicated that the p.K315E, p.G342D, and p.E364K mutation induce conformational alterations in the monomeric G-actin state. Cultured patient-derived fibroblasts exhibited mutation-specific defects, exemplified by significantly faster (p.K315E) or slower proliferation (p.G366V), as well as adhesion defects (p.G366V) compared to healthy fibroblasts. Immunostaining of patient-derived fibroblasts and patient-derived T-cells using actin isoform-specific antibodies are currently being conducted to assess alterations in cytoskeletal architecture. Our multidisciplinary approach aims to uncover how these mutations alter molecular interactions and, in turn, perturb cellular actin dynamics, thereby providing new insights into disease etiology and pathogenesis.

Poster 141 - VASH2-SVBP Detyrosinating Enzyme Regulates Microtubule Dynamics

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The cycle of α-tubulin detyrosination/tyrosination is critical for cell and tissue integrity. Its deregulation leads to severe human pathologies including cancer, heart failure, and brain dysfunctions. This cycle is characterized by the enzymatic removal and re-addition of a gene-encoded tyrosine residue at the C-terminus of α-tubulin. While tyrosination imply only the tubulin tyrosine ligase (TTL), tubulin detyrosination involves two evolutionary distinct enzyme families: the vasohibin cysteine proteases in combination with their chaperone and cofactor SVBP (VASH1-SVBP and VASH2-SVBP) and the atypical metalloprotease MATCAP. We have previously shown a divergent mode of detyrosination between the two VASH complexes, with VASH1-SVBP and VASH2-SVBP driving the global and local detyrosination of microtubules, respectively. These behaviors were correlated with the microtubule-binding properties of their disordered N- and C-terminal regions of the VASHs. Specifically, the N-terminal region is responsible for a significantly longer residence time of VASH2-SVBP on microtubules compared to VASH1–SVBP.

Here, we show in reconstituted systems and cells that VASH2-SVBP strongly binds to the microtubule lattice and promotes microtubule stability. It is a potent microtubule growth factor in vitro that modifies dynamic instability by limiting the rate of microtubule shrinkage, severely reducing catastrophes, and increasing rescues at both ends. In addition, VASH2 is able to assist spontaneous nucleation and polymerization of microtubules. As for its stabilizing effect, the nucleating effect of V2 does not rely on its catalytic, detyrosinating activity. The two flanking regions of the central, catalytic domain of VASH2 appear crucial to these stabilizing and nucleating properties, with the N-terminal region having a specifically important role.

Our data reveal for the first time nucleating and stabilizing properties of VASH2-SVBP complex, apart its detyrosinating activity. All these properties are not shared by VASH1-SVBP. We are currently investigating the importance of VASH2-SVBP in the brain where this protein is detected during development.

Poster 142 - Mechanisms Governing Cortical Development and Disease: Actin- Dependent Control of Mitotic Division Plane Orientation in Baraitser–Winter–Associated Microcephaly

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Baraitser–Winter cerebrofrontofacial syndrome (BWCFF) is a rare neurodevelopmental disorder caused by pathogenic variants in ACTB and ACTG1, which encode the cytoplasmic β- and γ-actin isoforms, respectively. The cortical malformation spectrum of BWCFF includes microcephaly and lissencephaly, which are documented in more than 50% of affected individuals.

In our previous work, we demonstrated an altered orientation of the mitotic division plane in apical progenitor cells (APs) within BWCFF cerebral organoids carrying the ACTB p.Thr120Ile and ACTG1 p.Thr203Met variants. These variants were consistently associated with severe microcephaly and lissencephaly in the corresponding patient cohort (PMID: [Indra et al.]). Alterations in the division plane are a well-established mechanism underlying microcephaly, as they lead to premature depletion of the progenitor pool.

In contrast to these previously characterized ACTB and ACTG1 variants, another BWCFF hotspot variant in ACTB at position Arg196 is associated with microcephaly in only a subset of patients. Specifically, microcephaly has been reported in 15 out of 21 individuals currently enrolled in the clinical registry.

Here, we investigated cerebral organoids derived from induced pluripotent stem cells of two unrelated patients carrying the ACTB p.Arg196His variant, of whom only one presented with microcephaly and cortical malformations. Organoids derived from the patient with microcephaly showed a pronounced shift from vertical to horizontal cleavage planes in dividing APs, indicative of reduced proliferative capacity. In contrast, mitotic APs of the patient without microcephaly divide like the ones of the control group.

Together, these findings support our hypothesis that actin-dependent defects in mitotic spindle alignment constitutes a major pathogenic mechanism contributing to microcephaly in BWCFF. The pronounced interindividual variability observed within the patient cohort warrants further investigation

Poster 143 - C. Elegans Models to Uncover Both Functional Complexity of Actin and Molecular Mechanisms Underlying Human Non-Muscle Actinopathies

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In human two ubiquitous actin cytoplasmic isoforms, ACTB and ACTG1, perform redundant roles while retaining some level of differences, notably in their nucleotide sequence and regulation of expression. An analogous situation occurs in C. elegans as nematodes possess 3 cytoplasmic actin proteins (ACT-1, ACT-2 and ACT-3). Due to similarity of sequences and compensation effects, it is highly challenging to reveal their individual properties and contribution to the numerous actin functions. Consequently, the question of how actin isoforms balance is regulated and how the control of redundancy ensuring genetic robustness occurs when one actin is affected, as in Non-Muscle Actinopathies (NMA), remains largely unexplored. My team aims at elucidating both the pathophysiological mechanisms underlying these rare diseases affecting cytoplasmic actins and the mechanisms at work which sustains viability when one actin isoform is absent.

Using CRISPR-designed NMA C. elegans models expressing actin variants with non-synonymous substitution, or different actin knockouts, we have analysed functional outcomes from molecular in vivo dynamics to cell-scale mechanical properties and phenotypes. We will present our multi-scale characterisation of NMA variants (Hecquet et al, 2025), which has led to their quantitative classification, and unpublished findings regarding the impact on cortical mechanics of some of these variants as well as a panel of different KO conditions. Additionally, our team has generated novel tools to visualize endogenously-tagged actin isoforms in C. elegans (Quintin et al, 2025), which open new perspective. Altogether, this work will help to elucidate the functional complexity of actin in vivo.

Poster 144 - Role of MYO6 in the Structural Organization of Apical Microvilli in the Mouse Epididymal Epithelium

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Myosin VI (MYO6) is a unique minus-end-directed actin motor protein that is essential for maintaining the structural integrity and function of specialized mammalian epithelia forming apical brush border actin structures. In epithelial cells, MYO6 fulfills a dual role by mediating vesicular membrane trafficking and anchoring the plasma membrane to the actin cytoskeleton at the base of microvilli. Loss of MYO6 function in Snell Waltzer mutant mice results in severe defects, including deafness caused by cochlear sensory epithelial dysfunction, impaired nutrient absorption in the intestinal epithelium, and defective renal reabsorption in proximal tubules. In Drosophila, mutations in the Myo6 gene cause male infertility, whereas Snell Waltzer males display multiple abnormalities during spermiogenesis accompanied by an approximately 25% reduction in fertility. We hypothesized that the reduced fertility in MYO6-deficient males may be partially attributable to impaired sperm maturation within the epididymis, a highly segmented tubular organ that establishes a specialized luminal microenvironment through tightly regulated endocytic and exocytic trafficking of the epididymal epithelial cells. Using stimulated emission depletion super-resolution microscopy, transmission and scanning electron microscopy, we performed a comprehensive ultrastructural analysis of the epididymal epithelium in Snell Waltzer mice. Our results reveal that MYO6 deficiency leads to pronounced microvillar abnormalities, including defective anchoring of microvilli to the apical membrane, partial microvillar loss, and disruption of the characteristic two-zone cytoplasmic organization in subsets of epithelial cells compared with control mice. These findings identify MYO6 as an important cytoskeletal component required for maintaining the structural organization and, consequently, the function of the mouse epididymal epithelium.

Poster 145 - Dimensional Scaling of Mitotic Spindle Assembly

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During eukaryotic cell division, the replicated genome is equally partitioned between daughter cells by the mitotic spindle, a dynamic structure mainly composed of microtubules. Mitotic spindle size scales with cell dimensions during early embryonic cleavages. Several mechanisms including regulation of microtubule length or number have been proposed to explain size adaptation of the mitotic structure, however, it remains unclear how the mitotic spindle can robustly assemble over a large range of cell sizes even within the same organism while satisfying temporal constraints imposed by cell cycle events.

Using live imaging of spindle assembly in embryos of different species and of various sizes we identified conserved mechanisms involved in scaling of mitotic spindle size and assembly. We developed a methodology based on labeled-protein injection to visualize and measure microtubule dynamics in different non-model organisms in which genetic tools are limited. We found that the microtubule growth rate scales with cell size across metazoans.

In addition, by observing epithelial cell divisions in C. elegans, we provide the first characterization of spindle scaling mechanisms in somatic tissues in intact animals. Our data provide evidence that microtubule growth rate scales with cell volume in somatic cells. Interestingly, our work demonstrates that the size of the mitotic spindle scales with cell size in somatic cells, but depending on the cell type, either the length or the width of the spindle stops scaling. This altered mechanism in small somatic cells appears to tend towards a lower limit of spindle size scaling.

Together our data provide an exhaustive comprehension of spindle shape, size and assembly across a wide range of size and shed light on the distinct mechanisms that operate to ensure faithful cell division in different tissue or developmental contexts.

Poster 146 - Consequences of Functional Perturbation of Actin Monomer Binding Proteins in Mammalian Cells

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Cell migration and proliferation as well as communication and trafficking all depend on the fine-tuned and continuous remodeling of actin filaments (F-actin), which are nucleated and elongated by addition of monomers (G-actin), regulated in turn by several monomer-binding factors. In this project, we are exploring the relative functions of the dominant monomer-binding proteins, profilin and thymosin-β4 (or –β10) in B16-F1 mouse melanoma cells. These factors are well-established to exert specific activities in vitro, i.e. inhibition of actin nucleation and promotion of filament elongation for profilin as well as sequestration of monomers in case of thymosins. However, a precise, comparative analysis of the relative, cellular roles of these factors as well as determination of the consequences of their respective depletion is currently missing.

We are describing detailed characterizations of genetic disruptions of either one of the two profilin genes expressed in B16-F1, profilin 1 or -2 and thymosin-β4. Combined removal of both profilins is cellular lethal, but suppression of profilin activity to less than 1 ‰ eliminates bundled, linear actin filament arrays such as filopodia and modifies the actin binding protein composition of branched actin networks such as lamellipodia. These phenotypes upon drastic suppression of profilin activity coincide with reduction of efficient cell migration. In contrast, stable elimination of thymosin-β4 has little effects on lamellipodia protrusion, but increases the efficiency of cell migration, which is remarkable given the additional observation that genetic disruption of both profilin1 and thymosin-β4 significantly reduces actin expression. Furthermore, chronic thymosin-β4 removal also increases profilin expression.

Future efforts will thus aim at establishing (i) the precise interdependencies and functional connections between these actin monomer binding proteins and (ii) the relative functions of distinct β-thymosins expressed in B16-F1 cells, in particular β4- versus β10.

Poster 147 - Towards a New Anti-Cancer Therapy Targeting Microtubules

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Microtubules are the main target of classical chemotherapies based on taxanes or alkaloids, because of their role in cell division. Despite their efficiency, these therapies cause side effects and resistance. Therefore, it is imperative to explore new therapeutic perspectives. LIMKs (LIMK1 and LIMK2) are deregulated in many cancers and appear as new therapeutic targets. LIMKs are serine/threonine and tyrosine kinases. They play a crucial role in cytoskeleton dynamics by independently regulating both actin filament and microtubule turnover. LIMKs promote the free tubulin form, but the molecular requirements of this process are still unknown. We want to uncover the signaling pathway by which LIMKs regulate microtubule dynamics by identifying novel partners of LIMKs and understanding how they regulate each other.

A mass spectrometry approach of LIMK2 interactome has allowed us to identify candidates potentially linking LIMK2 to microtubule dynamics, especially HDAC6, a protein known to deacetylate tubulin. We confirmed the interaction of LIMK2 with HDAC6 by co-immunoprecipitation in transfected HEK293T cells. We demonstrated the interaction between endogenous LIMK2 and endogenous HDAC6 by immunoprecipitation in HEK293T, HepG2 and SH-SY5Y cells. We also characterized the interaction between LIMK2 and HDAC6. Currently, we aim to understand the physiological role of this interaction. In particular, we want to evaluate whether HDAC6 is a substrate of LIMK2 and whether the activity of one of these proteins is regulated by the other protein. We also want to determine the impact of this interaction on microtubule dynamics. Understanding the molecular mechanisms that link LIMK2 to microtubule dynamics will pave the way for the development of new targeted anti-cancer therapies, more selective with probably limited side effects.

Poster 148 - ROR2 Regulates Microtubule–Adhesion–Junction Crosstalk to Maintain Endothelial Structural Integrity in PAH

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Pulmonary arterial hypertension (PAH) is characterized by the disruption of the endothelial barrier, which is due to cytoskeletal abnormalities. We earlier demonstrated that loss of the atypical Wnt receptor, ROR2 (Receptor Tyrosine Kinase-like Orphan Receptor 2) that enhances endothelial adhesion and permeability by sustaining integrin β1–dependent focal adhesion (FA) signaling. In this current research, we identified a cytoskeletal role of ROR2 in microtubules stabilization and linking adherens junctions to the cytoskeleton.

In pulmonary microvascular endothelial cells (PMVECs), ROR2 knocking down resulted in a significantly reduced expression of acetylated α-tubulin, suggesting that ROR2 depletion leads to a loss of stable, long-lived microtubules. Moreover, ROR2 deletion reduced VE-cadherin and a disrupted intercellular VE-cadherin connection, indicating a disrupted cell-to-cell adhesion, develops alongside a disrupted cytoskeletal structure.

Within the junctional interface, ROR2 binds to VE-cadherin and enhances its adhesion to actin filaments and microtubules via β-catenin. ROR2 deficiency disrupts the binding of VE-cadherin and β-catenin and reduces their association with the cytoskeleton in adherens junctions. The destabilized microtubules also prevent the transport of VE-cadherin to cell-cell junctions and sustain the disassembly of junctions.

These results reveal ROR2 as a previously uncharacterized regulator of microtubule acetylation and junctional cytoskeletal connectivity. The disruption of this ROR2-based structural motif impairs endothelial barrier mechanics and offers a potential pathologic mechanism for microvascular abnormalities in PAH.

Poster 149 - Rab GTPases Allosterically Activate MICAL1 to Drive Actin Filament Disassembly

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Actin oxidation at specific methionine residues is a unique mechanism that leads to actin disassembly. This process is tightly controlled by the cytoskeletal effector MICAL, which selectively binds to and oxidizes actin filaments, thereby driving actin network remodeling. MICAL-mediated actin oxidation plays a central role in cytoskeletal reorganization during axon guidance, angiogenesis, vesicle trafficking, and cytokinesis. Recent cryoEM structures from our group and others revealed that MICAL1 is maintained in an autoinhibited state through an intramolecular interaction between its N-terminal catalytic domain and C-terminal coiled-coil domain. Although Rab GTPases and Plexin receptors have been reported as MICAL activators, the molecular mechanism by which MICAL1 autoinhibition is relieved to enable actin filament oxidation has remained unclear.

Here, we show that Rab GTPases directly activate MICAL1-mediated actin disassembly. We determined two cryoEM structures of MICAL1 in complex with either one or two Rab8 molecules. Together with SEC–SAXS measurements and hydrogen–deuterium exchange mass spectrometry, these data reveal that Rab8 binding induces an allosteric rearrangement of the MICAL1 coiled-coil domain, partially disrupting its interaction with the catalytic domain and exposing the F-actin binding site. Kinetic analyses further demonstrate that although MICAL1 contains two Rab8 binding sites, binding of a single Rab8 molecule is sufficient to achieve full enzymatic activation. In contrast to previous reports, we did not observe detectable binding or activation of MICAL1 by PlexinA1 receptor in our assays.

Together, our findings reveal a molecular mechanism by which Rab GTPases unlock MICAL1 activity to drive actin filament oxidation and disassembly.

Poster 150 - Intermediate Filament Heterogeneity and Emergent Tissue Mechanics in Glioblastoma

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Cells within tissues are mechanically heterogeneous, yet we understand little about how this heterogeneity shapes tissue mechanics and collective cell behavior. In this project, we will explore how mixing cell populations with different mechanical properties introduces emergent, non-linear tissue behaviors, and how these depend not only on the degree of heterogeneity but also on its spatial organization.

Glioblastoma is an ideal model for this question. This aggressive brain tumor exhibits well-documented heterogeneity in intermediate filament expression, which is associated with both invasiveness and treatment resistance. We hypothesize that the resulting mechanical heterogeneity plays a central role in tumor spatial organization and impacts its invasive behavior.

To create controlled mechanical heterogeneity, we will combine multiple approaches: sorting cells based on rheological properties using real-time deformability cytometry, inducing mechanical drift through iterative selection based on traction force measurements, and using genetic perturbations such as vimentin knockout. Optogenetic RhoA activation will allow us to control both the degree and spatial pattern of contractility with light, decoupling mechanical state from cell identity.

We will investigate how mechanical heterogeneity affects tissue dynamics on micropatterned 2D substrates, quantifying cell shape, migration, and forces via traction force microscopy. We will then extend to 3D spheroids and matrix invasion assays to connect our mechanical characterization to pathophysiological outcomes. Throughout, computational modeling will build a unified framework linking cytoskeletal heterogeneity, spatial organization, and collective behavior.

Poster 151 - Macrophage–Epithelial Interactions: Mechanics and Collective Dynamics

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The interactions between macrophages and alveolar epithelial cells, including alveolar type I (ATI) and alveolar type II (ATII) cells, are essential for maintaining immune defense, tissue repair, and overall lung homeostasis. These heterotypic contacts depend on cytoskeletal dynamics to sustain barrier integrity, enabling epithelial cells to adapt their cortical tension, adhesion strength, and mechanical stability in response to macrophage-derived signals. However, it remains unclear how these signals influence epithelial mechanics and the collective behavior of alveolar cell layers.

In steady-state conditions, macrophages and epithelial cells cooperate to maintain balanced immune activity. This prevents excessive inflammation that can damage tissue and promote chronic diseases such as chronic obstructive pulmonary disease or pulmonary fibrosis. Through pathogen clearance and the release of signaling molecules, macrophages activate epithelial defense pathways and initiate local immune responses. During injury or infection, macrophages remove debris and support epithelial regeneration, while epithelial cells rebuild the structural and functional integrity of the lung. Disruption of this complex system impairs repair processes, weakens barrier stability, and contributes to the progression of chronic lung pathologies.

The aim of this project is to understand how mechanical forces are transmitted between macrophages, ATI and ATII cells, as well as how these forces depend on the activation and polarization state of the macrophages, affecting epithelial cell mechanics, cell-cell adhesion, and barrier function. For this purpose, high-resolution microscopy techniques such as MIET (metal-induced energy transfer) are combined with force spectroscopy (AFM), molecular tension probes (MTPs), and impedance-based measurements (ECIS) to analyze both single-cell and collective effects.

Poster 152 - Selective Killing of Cancer-Associated Fibroblasts by Ultrasound-Mediated Mechanical Forces

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Recent studies show that stretch/ultrasound (US)-generated mechanical forces cause selective apoptosis in several cancer cells, tumor organoids and animal models without damaging normal cells. Cancer-associated fibroblasts (CAFs) are an integral tumor microenvironment (TME) constituent. They display altered biomechanical properties similar to cancer cells, such as matrix secretion and its remodeling, chemokine secretion, and high contractile force generation. We thus test the effect of US-mediated mechanical forces on patient-derived CAF survival. Surprisingly, US treatment causes CAF apoptosis (mechanoptosis) through a calcium-activated apoptotic pathway but not in normal fibroblasts. MicroRNA-21 (miR-21) secreted by primary cancer cells suppresses the mechanosensory cytoskeletal protein tropomyosin 2.1 (Tpm2.1) in CAFs. This Tpm2.1 depletion in CAFs is responsible for mechanoptosis. Interestingly, normal fibroblasts behave similarly when Tpm2.1 was depleted. Further, 3D gel contractility and migration assay confirm that a prolonged US treatment disrupts myosin IIA-mediated contractility, which CAFs primarily use to support cancer invasion. Since US treatment causes mechanoptosis and reduces contractility, this approach could be used to develop ultrasound-based CAF targeting therapy to augment cancer treatment.

Poster 153 - Nanoscale Ligand Spacing Regulates Mechanical Force-Induced Cancer Cell Killing

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Cancer cells sense and respond to the extracellular environment, with differences in nanoscale ligand spacing affecting their behavior. Emerging reports show that stretch/ultrasound-mediated mechanical forces promote apoptosis (mechanoptosis) by increasing myosin contractility. Since myosin contractility is critical for nanoscale-ligand spacing-regulated cell behavior, we study the effect of ligand spacing on mechanoptosis. Gold nanoparticle arrays were created with 35, 50, and 70 nm spacings and functionalized with cyclic-RGD peptide. Interestingly, the highest level of apoptosis was observed on 50 and 70 nm ligand spacing, where increased myosin contractility and peripheral Piezo1 channel localization causing calcium influx were observed. Perturbing cell-matrix interactions by nanomolar doses of Cilengitide (cyclic RGD pentapeptide) increases mechanoptosis on 35 nm ligand spacing to similar levels observed on 50 and 70 nm. Thus, nanoscale-level changes in binding domains regulate mechanoptosis through cell-matrix mediated mechanotransduction, and the synergistic action of ultrasound and Cilengitide can ultimately be applied to enhance tumor treatment.

Poster 154 - Towards a Clinically Relevant Molecular Taxonomy of Triple-Negative Breast Cancer: Insights from an Immunohistochemical Biomarker Panel

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Breast cancer is the most common malignancy among women worldwide, including in Algeria. Its marked heterogeneity is reflected in multiple molecular subtypes, among which triple-negative breast cancer (TNBC) stands out for its lack of hormone receptors and HER2 expression, limiting targeted therapeutic options.

This study aimed to evaluate prognostic and predictive biomarkers associated with TNBC, validate its distinct molecular profile, and propose a histoprognostic classification that captures its underlying biological diversity.

A total of 573 breast cancer cases were reviewed, including 64 TNBC patients treated at the Cancer Control Center (CLCC) of Batna. Formalin-fixed, paraffin-embedded biopsy samples were analyzed by immunohistochemistry (IHC) using a biomarker panel encompassing cytokeratins (CKs), EGFR, androgen receptor (AR), p53, BRCA1, tumor-infiltrating lymphocytes (TILs), and epithelial–mesenchymal transition (EMT) markers.

Protein expression profiling enabled the classification of tumors into six TNBC molecular subtypes. Over the study period, TNBC accounted for 15.53% of all breast cancer cases. The mean patient age was 50.11 ± 12.13 years, with 76.56% older than 40. Histologically, 89.1% were invasive ductal carcinomas, most commonly grade II (64.1%) according to the Scarff–Bloom–Richardson (SBR) system.

IHC-based molecular subtyping revealed a predominance of the basal-like 1 (BL1) subtype (53.1%), followed by BL2 (6.3%), mesenchymal (17.2%), luminal androgen receptor (LAR, 9.4%), mixed (4.7%), and unclassified (9.4%) subtypes, the latter exhibiting a mutant p53 expression pattern. Significant variations across subtypes were observed regarding patient age, tumor grade, and lymph node status. BRCA1 loss was more frequent among patients with a family history (23.44%). Survival analysis demonstrated a significant difference in overall survival ($p = 0.007$) among the six subtypes, with the LAR subtype showing the most favorable prognosis.

Keywords: triple-negative breast cancer, immunohistochemistry, molecular classification, prognostic and predictive biomarkers

Poster 155 - Ultrasound-Generated Nanoscale Mechanical Stimulation to Regulate Stem Cell Differentiation

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Harnessing nanoscale mechanical cues to direct stem cell fate represents a transformative strategy in cell-engineered tissue regeneration. In this study, we demonstrate that low-frequency ultrasound (LFU) induces nanoscale vertical displacements (30−65 nm) at the cell−substrate interface, effectively promoting osteogenic differentiation of human mesenchymal stem cells (hMSCs). Remarkably, this ultrasound-driven mechanotransduction occurs on both soft and rigid matrices, highlighting the robustness of the approach. Daily 30 min LFU treatments over 7 days result in significant osteogenic commitment, as confirmed by immunofluorescence and gene expression analyses. Mechanistically, the response is mediated by RhoA-ROCK-dependent myosin IIA contractility, with pharmacological inhibition validating the pathway's role in LFU-induced osteogenesis. This noninvasive, scalable, and cost-effective LFU treatment offers an interesting alternative to traditional biochemical or scaffold-based methods, paving the way for ultrasound-based bioreactors in regenerative medicine and 3D bone tissue engineering.

Poster 156 - Myosin MYO1C Is a Novel Host Target of Chlamydia Trachomatis for Actin Cage Recruitment at the Bacterial Inclusion

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Chlamydia trachomatis (Ct), a Gram-negative obligate intracellular pathogen, manipulates host actin dynamics to facilitate its entry, development, and exit. It assembles a dynamic actin cage around its intracellular niche, known as the 'inclusion', which provides structural stability for bacterial growth, and is crucial for exit via the non-cell-lytic extrusion process. We found that Ct recruits Myosin 1 C (MYO1C), a ubiquitous actin dependent motor protein, to the inclusion throughout its life cycle. Consequently, loss of MYO1C activity reduced Ct infection and the production of bacterial progenies. Mechanistically, MYO1C functions as a dynamic tether that assembles an actin cage around the inclusion membrane, as depletion of MYO1C or its inhibition by pentachloropseudilin (PCIP) leads to the loss of the actin network surrounding the inclusion. In vitro reconstitution assays revealed that the presence of purified MYO1C was necessary and sufficient to build an actin cage around giant membranous vesicles. In summary, our findings identified MYO1C as a novel host target of Ct and provided mechanistic evidence for its role as a dynamic tether to recruit the essential actin cage around the bacterial inclusion.

Poster 157 - A Streamlined CRISPR/Cas9 Strategy to Assess Endogenous Localization of Diaphanous-Related Formins

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Formins are potent actin-assembly factors acting downstream of RhoGTPase signaling that drive the nucleation and processive elongation of filament-barbed ends. Notably, ectopic expression of constitutively-active variants of mDia2 has been shown to induce numerous filopodia-like structures at the cell periphery, with mDia2 enriched at their distal tips. However, this localization could not be confirmed by immunostaining, which instead revealed prominent accumulation of mDia2 at the cleavage furrow and midbody in dividing cells across distinct cell types, raising questions about the physiological localization of this powerful formin upon ectopic overexpression. To resolve this controversy, we employed a CRISPR/Cas9-based in-trans paired-nicking strategy to generate an indel-free, homology-directed fluorescent knockin into the DIAPH3 gene in B16-F1 mouse melanoma cells. To this end, we used the bright monomeric fluorescent protein mNeonGreen to enable detection of low abundance endogenous proteins and then enriched knockin cells by FACS. This approach allowed direct visualization of the activation, localization, and dynamics of endogenous full-length mDia2 in vivo. Strikingly, mDia2 was undetectable at actin-based structures during interphase, whereas from the onset of prometaphase the protein displayed moderate centrosomal enrichment and fluctuating accumulation at distinct cortical regions. Subsequently, its localization stabilized at the equatorial plane prior to furrow ingression. As furrow ingression progressed, mDia2 increasingly concentrated at the equatorial cortex and ultimately accumulated at the midbody, with the exception of the midbody dark zone. FRAP analysis further revealed distinct dynamic behaviors of mDia2 at the cleavage furrow and midbody, with markedly slower turnover at the latter. Confocal microscopy additionally revealed virtually complete colocalization of mDia2 with Septin2 at cortical regions throughout cell division. In contrast to previous studies, however, β- and γ-actin accumulated indistinguishably at the cell cortex of dividing cells, including the equatorial region. The same approach is being currently employed to resolve the endogenous localization of additional formins.

Poster 158 - Reconstituting Contractile Dynamic Steady States of Actin Networks

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Actin architectures undergo continuous self-renewal, with their individual components constantly dissociating, recycling and reassembling, while at the same time contributing to an overall stable structure. This allows different subcellular networks of the cell to be dynamically regulated, providing them with the flexibility to grow, shrink, appear and disappear as needed. Hence, actin architectures are in a “dynamic steady state” (DSS), characterized by the continuous nucleation and growth of filaments, balanced by their disassembly and recycling. Active contraction by myosin motors further contributes to maintaining stable yet dynamic architectures. Due to the interdependencies of all these mechanisms, investigating their properties in cells is challenging. In vitro reconstituted networks, based on a minimal set of purified proteins, offer a powerful alternative allowing precise, modular control over individual components to reveal principles underlying DSS formation and maintenance. A purely recycling-based actin DSS has been recently achieved. However, in the presence of myosin, reconstituted actomyosin networks typically undergo only brief, transient contractions.

Here, we present the reconstitution of contraction-based actin networks that instead self-organize into a dynamic steady state, ceaselessly flowing microscopically and yet maintaining a stable and steady macroscopic architecture. Using lipid-coated microdevices to confine and guide network assembly and taking advantage of both myosin-based force generation and cofilin-based recycling of actin monomers, we assemble minimal yet realistic actin architectures in DSS, both in the presence and in the absence of actin turnover. Reconstituted architectures, all based on a balance between actin transport, contraction and recycling, include networks that contract continuously, or exhibit pulsed and even spiral-like contraction, as observed in cells. Using the flexibility of bottom-up reconstitution, we identify conditions leading to such active steady states, dissect the microscopic dynamics giving rise to them and test their robustness. These results pave the way to a more mechanistic understanding of cellular actin architectures.

Poster 159 - Nonmuscle Myosin 2 Paralogs Have Differing Biochemical and Mechanical Properties

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The are three myosin-2 paralogs, termed nonmuscle (NM) 2A, 2B and 2C, found in cells. These myosins have distinct, but sometimes overlapping localizations in cells. Why are three paralogs needed and to what degree are they specialized for different functions. We use kinetic and biophysical assays to explore their differential properties. We show they are all very slow enzymatically, but NM2A moves actin filaments three times as fast as NM2B/C. NM2B has the highest duty ratio. Optical trapping data suggest that the kinetics of NM2A and NM2B are markedly force dependent with NM2B exhibiting actin attachment durations of more than 100 sec when resisting loads of ~5 pN are imposed. Both NM2A and NM2B exhibit catch-bond-like behavior under resisting loads. Using micropatterning of parallel stripes containing bound formins, antiparallel arrays of actin filaments were formed. Addition of NM2B filaments contracted these filaments to produce linear actin filaments arrays being pulled towards the center of the pattern by the bipolar NM2 filaments. The motility rate of the NM2 filaments was slowed and many filaments stalled under conditions where they are likely exerting isometric tension on the oppositely oriented actin filaments. In contrast, NM2A initially contracted the actin filaments, but then actively shredded them, destroying the pattern. We used a myosin with an imbedded FRET-based force sensor to measure the force the individual myosin were exerting on the actin filaments. These data suggest that NM2B which is strongly localized to stress fibers would be capable of exerting isometric tension on actin filaments attached to adhesion plaques with very little expenditure of energy. NM2A which has a strong localization just behind the leading edge of many migrating cells might be used to shear actin filaments to hasten their depolymerization in order to feed more G-actin into the Arp2/3 complex driving forward membrane propulsion.

Poster 160 - The Neuronal MAP6 Assembles Actin Filaments in the Microtubule Lumen

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In cells, the actin and microtubule cytoskeletons are constantly remodeled to achieve diverse functions including cell polarization, differentiation and migration. Microtubules and actin filaments are therefore finely organized and tightly regulated. Both actin and microtubules have been extensively studied individually, revealing their specific properties. However, these two cytoskeletons do not function as separate elements within cell; instead, they act in concert at different stages of the cell life cycle, involving specific associated proteins. Among these proteins, we focus on MAP6, a neuronal effector known for its strong microtubule-stabilizing properties and recently identified as a microtubule-inner protein. Using cell-free assays, TIRF-microscopy and cryo-electron tomography, we show that MAP6 is a potent actin nucleator that organizes actin filaments into bundles, promotes the co-nucleation of microtubules and actin filaments, and, strikingly, drives the assembly of actin filaments in the microtubule lumen.

Poster 161 - Tissue-Resident Memory CD8+ T Cells Use Mechanosensing for Local Immunesurveillance

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Tissue-resident memory CD8+ T cells (TRM) constitutively scan stromal cells in their tissue of residence, such as skin, gut or visceral organs, to intercept microbial spread. While chemokines and integrin ligands produced at epithelial barriers are critical mediators of this process, their elevated constitutive expression in non-barrier organs might lead to excessive influx of immune cells. We recently found that TRM lodged in exocrine glands, such as salivary glands (SG), perform tissue scanning in the absence of chemoattractant or adhesion receptor signaling, thus bypassing the requirement for canonical migration-promoting factors. Instead, SG TRM sense local physical confinement through nuclear deformation, which acts as a mechanosensor. A downstream arachidonic acid and Ca2+-signaling pathway increases cortical contractility and F-actin turnover as force-generating module for autonomous motility.

Here, we examined the development and prevalence of chemokine-independent, nuclear mechanosensing-driven tissue surveillance in virus-specific CD8+ T cells isolated from multiple internal and barrier organs during the course of an infection. Our data suggest that early effector T cells share the ability with SG TRM to migrate independently of chemokine signals. This ability is maintained in TRM lodged across most but not all internal organs. In sum, tissue confinement alone can trigger actomyosin network-driven autonomous T cell surveillance across multiple tissues, providing a widespread complementary strategy to chemosensing-dependent migration.

Poster 162 - Identifying Interfaces in the Septin Cytoskeleton for Pharmacological Targeting Against Cancer

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The mammalian septin cytoskeleton participates in a diverse range of cellular processes. We had previously demonstrated that genetic targeting of SEPT7 is effective in suppressing tumorigenesis in vivo. Interestingly, loss of SEPT7 is well-tolerated by the hematopoietic system. Therefore, targeting septin-dependent cytoskeleton in solid tumors provides a means for tumor suppression without myelosuppression and other hematopoiesis-related side effects of anti-cancer treatments. In our earlier studies, we identified that the GTPase domain-dependent homodimerization of SEPT7 is dispensable for septin-dependent cell division. In search of pharmacologically targetable interfaces in the septin cytoskeleton, we analyzed the SEPT7-SEPT9 G-G interface using the SEPT7-W268A mutant. Unlike wild-type GFP-tagged SEPT7, the W268A mutant was mainly present in triton-soluble fractions similar to the GTPase domain mutant-SEPT7-G59V characterized earlier. Moreover, confocal microscopy revealed a diffuse localization of both mutants, indicating disruption of homopolymerization. However, unlike the G-domain mutant, the W268A mutation disrupted the SEPT7-SEPT9 interaction in pulldown assays. Therefore, we provide evidence that targeting the SEPT7-SEPT9 dimerization interface is a potential strategy for disrupting septin-dependent cytokinesis. Additionally, we have performed an in silico inhibitor screen to identify novel small molecule inhibitors targeting the SEPT7-SEPT9 interface. These findings open opportunities for the targeted inhibition of septin-dependent cytokinesis in solid tumors.

Poster 163 - Excessive Cortical Contractility Triggers Early Oocyte Polarization and Aberrant Cytoplasmic Flows in Mammals

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Female meiosis produces oocytes. In mammals, this process ends with two successive asymmetric divisions in size (meiosis I and II), resulting in a large haploid oocyte containing the maternal stores required for early embryonic development after fertilization. The geometry of these divisions is controlled in part by cortical tension, which decreases in a spatiotemporal manner after meiosis I entry due to actomyosin cortex reorganization, allowing the spindle to migrate along its long axis towards the closest cortex via a Myosin-II dependent pulling mechanism, permitting an asymmetric division in size. Mechanical defects are frequent in mammalian oocytes, mirroring the variability in cortical tension and Myosin-II cortical enrichment. Particularly, mouse and human oocytes with a too high cortical tension produce embryos that stop developing after fertilization. To elucidate the consequences of excessive cortical tension on oocyte development, we engineered mouse oocytes with excessive cortical contractility by forcing active Myosin-II to their cortex through cortical targeting of the RhoA/ROCK pathway. We observe that these oocytes progress through meiosis I as successfully as control ones, with comparable timing and geometry of division. However, their actomyosin cortex spontaneously polarizes upon meiosis I entry, forming a flattened zone opposite as to where division will occur. Directional cytoplasmic flows originate from this polarized zone, pushing the spindle away, towards the opposite cortex, resulting in faster spindle migration and random spindle orientation. These directional flows disrupt organelle distribution, reducing mitochondrial and Golgi content by half after meiosis I, potentially depriving the future embryo of energy reserves and vital organelles. Furthermore, CD9 and Juno, essential for oocyte fertilization, accumulate precociously and in a restricted manner in the polarized zone, potentially altering fertilization efficiency, still to be tested. Finally, we have shown that the phenotypes observed in these engineered mouse oocytes exist in natural populations of mouse and human oocytes.

Poster 164 - Identification of the Mechanical Properties of Cells and Spores

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Institute of Physical Chemistry

Understanding how the mechanical properties of fungal cell walls integrate with intracellular activity and cytoskeletal function is essential for explaining cellular resilience, growth, and transitions into dormant states. Here, we present a comparative mechanobiological analysis of Schizosaccharomyces pombe (S. pombe) and Sordaria macrospora (S. macrospora), focusing on how cell-wall stiffness, turgor pressure, and intracellular dynamics reorganize across life stages.

Using atomic force microscopy (AFM), we quantified the stiffness of the cell wall in living S. pombe cells, capturing their elastic response under turgor load. In contrast, nano-indentation measurements on S. pombe spores revealed a substantially increased rigidity, consistent with the compact architecture of the dormant state. To broaden this comparative perspective, we examined spores of S. macrospora and its melanin-deficient mutants. These measurements demonstrate that melanin content is a key determinant of spore-wall stiffness, with wild type (WT) spores displaying significantly higher rigidity than fus mutant (tetrahydroxynaphthalene reductase knockout leading to a partly defective melanin biosynthesis pathway), and albino mutant (Polyketide synthases knockout leading to completely defective melanin synthesis pathway).

To connect extracellular mechanics with intracellular activity, we performed optical tweezer (OT) measurements to probe non-equilibrium micromechanical behavior in S. pombe cells and spores. Living cells show clear signatures of active, ATP-driven fluctuations, while spores exhibit strongly reduced activity, reflecting the mechanical and physiological reorganization during dormancy.

Together, our results reveal how cell-wall mechanics, turgor pressure, and cytoskeletal activity form a coupled mechanical system that is fundamentally restructured between active growth and dormant survival. This work highlights the importance of integrating mechanical and cytoskeletal perspectives to understand fungal adaptability across life stages.

Poster 165 - NMIIA/IIB Levels Influence Actomyosin Network Dynamics to Regulate Thrombopoietin-Receptor Traffic and Signaling.

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Actin remodeling at the cellular level necessitates a rapid turnover of filaments. While it has been suggested that myosin activity may restrict filament length, which of the non-muscle myosin II isoforms (NMIIA, NMIIB) directly influence actin dynamics remains unclear. We conducted linear actomyosin simulation that showed higher network tension and faster network ruptur with NMIIA motors compared to NMIIB. Live-cell imaging in COS7 cells demonstrated a higher frequency of NMIIA-associated actin bundle-severing events compared to NMIIB, which exhibited significantly fewer occurrences. NMIIA overexpression also enhanced the formation of peripheral actin arcs, which were not present in cells overexpressing NMIIB. NMIIA overexpression led to enhanced peripheral localization of cofilin which might further contribute to enhanced actin severing. FRAP analysis, optical trap-based cortical force measurements, and actin network imaging collectively indicated that NMIIA, in contrast to NMIIB, facilitated enhanced actin dynamics. We investigated the physiological importance of NMIIA-dependent remodeling by analyzing the trafficking of the thrombopoietin receptor (TpoR), which is a crucial regulator of platelet production. In platelet precursor cells, increased expression of NMIIA correlated with enhanced surface expression and downstream signaling of TpoR. ROCK inhibition altered the cortical actin network and reduced TpoR surface expression and signaling. However, Arp2/3 inhibition by CK666 lead to enhanced surface expression and signalling indicating crucial roles of actin cytoskeleton remodelling on traffic of TpoR. Finally differentiation of HEL and CMK cell lines towards platelet lineage showd increased NMIIA expression, reduced thickness of cortical actin, increased expression of NMIIA along with enhanced surface expression and signalling of TpoR. Our findings indicate that NMIIA expression modulates actin network dynamics leading to enhanced TpoR surface expression and signaling.

Poster 166 - Auto-Organisation of Microtubules by Molecular Motors in Cell

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In vitro reconstitution experiments have shown that molecular motors not only transport cargo but also actively organize microtubules, aligning and sorting them by clustering plus and minus ends. Building on these principles, this project investigates how molecular motors influence microtubule organization in living cells. To focus on motor-mediated mobility rather than microtubule turnover, we work with stabilized microtubules. Once stabilized, microtubules fragment and self-organize into bundles around the nucleus, suggesting the involvement of active forces. Using cell micropatterning to impose defined geometries, we assess how cellular shape constrains the formation and spatial arrangement of these bundles, bridging in vitro principles of polarity sorting with in cellulo mechanisms.

Poster 167 - Investigating the Molecular Mechanisms of Formins in T Cell Migration

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T cells migrate through confined three-dimensional (3D) environments using a rapid, integrin-independent amoeboid mode that relies on coordinated actin remodeling and force transmission. While this migration requires dynamic cytoskeletal reorganization, how distinct actin assembly factors organize contractile networks across different physical environments remains unclear. Formins are key regulators of actin filament elongation, microtubule stabilization, and cytoskeletal architecture, yet their environment-specific roles in T cell migration are poorly defined.

We previously demonstrated roles for the two highly expressed T cell formins, Formin-like 1 (FMNL1) and mammalian Diaphanous 1 (mDia1) in 3D migration. Here, using 3D traction force microscopy, we found that FMNL1-deficient T cells exert significantly less pulling force on collagen matrices, indicating impaired force transmission. To further dissect the cytoskeletal functions of mDia1 and FMNL1 in T cell migration, we combined genetic deletion with targeted reconstitution using full-length formins or FH2 domain mutants to distinguish actin polymerization-dependent and -independent roles. Migration assays revealed distinct requirements for mDia1 and FMNL1. mDia1-deficient T cells did not have significant transwell migration defects and in 3D collagen mDia1-deficient T cells were fully rescued even without FH2-mediated actin polymerization, suggesting mDia1 primarily has a structural function and stabilizes microtubules in this setting. In contrast, FMNL1-deficient T cells required FH2 activity for transwell chemotactic migration but did not appear to need FH2 activity in 3D collagen, implying environment-dependent roles for FMNL1 in actin remodeling. Furthermore, Stimulated Emission Depletion (STED) microscopy revealed a different degree of colocalization of mDia1 with actin and Myosin-IIA compared to FMNL1.

Together, our findings define environment-specific roles for FMNL1 and mDia1 in tuning actin and microtubule cytoskeletal architecture during T cell migration and provide mechanistic insight into how T cells adapt to physical environmental constraints.

Poster 168 - Supercellular Front-Rear Polarity of Metastatic Tumour Clusters Invading Via Collective Amoeboid Migration

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Tumour invasion into surrounding tissues and distant organs is a defining feature of aggressive cancers. Distinct cancer types employ preferred invasion strategies that are highly adaptable to the environmental changes encountered during dissemination. Tumour cells may migrate individually or collectively, with collective migration being associated with a markedly higher metastatic potential. Collective migration has traditionally been considered traction dependent. However, analysis of primary tumour explants from patients with metastatic colorectal cancer revealed that a subset of tumour spheres is capable of spontaneous migration within confined, non-adhesive microfluidic environments. This previously undescribed, traction-independent mode of migration has been termed collective amoeboid migration (CAM). CAM is driven by supracellular enrichment of actomyosin at the rear of migrating cell clusters.

The establishment of front-to-rear (F/R) polarity is a prerequisite for both traction-based migration and single-cell amoeboid movement. In these contexts, F/R polarity has been shown to depend on a defined set of molecular regulators, including phospholipids, Rho GTPases, membrane–cortex linkers, and components of the PAR polarity complex. This study aims to elucidate the molecular cascade responsible for establishing supracellular F/R polarity in tumour clusters undergoing CAM. Preliminary data indicate that depletion of LGL1/2 significantly reduces CAM speed, whereas depletion of PRKCζ or PRKCι has no detectable effect. Together, these findings suggest that actomyosin contractility and apicobasal polarity signalling contribute to the regulation of F/R polarity during CAM.

Poster 169 - Uncoupling microtubule lifetime, stability and post-translational modifications.

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Microtubules undergo constant growth and disassembly. Using microinjection of tubulin, we measured the turnover of the microtubule network in living cells. We found that the network's half-life is 3 minutes, 80% of the network is renewed within 10 minutes, and 10% of microtubules survive for over 30 minutes. These dynamics were similar in quiescent cells, but slowed down in senescent cells. Surprisingly, we found that the amount of post-translational modification was independent of microtubule age. Old microtubules were neither more modified nor more resistant to nocodazole. These results demonstrate that microtubule modification is not affected by age or stability. They suggest that stabilisation and modification are rather consequences of the constant local activity of enzymes that add and remove chemical signals from microtubules.

Poster 170 - Pipeline for Automated Tracing and Polarity Determination of Cytoskeletal Filament in Cryo-Electron Tomograms

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In eukaryotes, the cytoskeleton is essential for a wide range of processes, including intracellular transport, cell migration, and cell shape control. To fulfill these functions, the cytoskeleton is organized into precisely controlled higher-order structures. As such, it is highly relevant for our understanding of the individual functions of the cytoskeleton to determine how its filaments are oriented relative to each other and other entities within the cell to form a given type of assembly.

In the case of actin filaments and microtubules, revealing their polarity can provide even deeper insights, as their intrinsic polarity is essential for their roles in directing cargo transport, positioning organelles, and organizing cellular architecture.

Cryo-electron tomography (Cryo-ET) has the capability to visualize cytoskeletal assemblies at high resolutions within 3d volumes (tomograms) that allow us to observe single filaments and differentiate between individual filament types, enabling quantitative ultrastructural analysis. However, tracing the filaments in crowded tomograms and determining, where applicable, their polarity is a cumbersome process, as a pipeline capable of handling a variety of filaments in different cellular contexts is still missing. To assess individual filaments in crowded in-situ tomograms, we are developing a tracing method based on template matching, which limits false positive output using deep-learning segmentation to restrict the tracing to regions of interest. An integrated determination of filament polarity is implemented at the end of the pipeline, using the templates' preferred orientation along the central line of the filament to estimate its direction. The pipeline was tested on actin filaments in lamellipodia and stress fibers, on microtubules, giving promising results.

Poster 171 - Beyond Actomyosin — Actin-Binding Proteins as Architects of Artificial Actin Networks

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Modified by different actin-binding proteins, the actin cytoskeleton self-organizes into diverse architectures in the cell. When coupled with myosin motor activity, these actin structures undergo continuous remodelling, giving rise to contraction, directed motion, and intracellular flow. To dissect the individual contributions of selected actin-binding proteins, their concentrations, and buffer conditions to cytoskeletal dynamics, we employ reconstituted minimal systems. These biomimetic setups allow us to systematically vary specific parameters in a controlled environment, enabling insights into the mechanisms by which actin-binding proteins orchestrate actin organization and force generation. Passive microrheology experiments in bulk networks establish a fundamental understanding of actomyosin network mechanics and activity. To explore the impact of physical confinement on network behaviour, we complement this approach with experiments in giant unilamellar vesicles (GUVs). Using time-resolved imaging techniques, this allows us to explore how geometry and boundary interactions modulate cytoskeletal organization and dynamics. Together, these complementary approaches provide a deeper understanding of the complex interactions and emergent behaviours within modified actomyosin networks.

Poster 172 - From Morphogenesis to Space Partitioning by Microtubules and Molecular Motors

Bhagyanath SURESH et al.

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The orientation and architecture of microtubules largely guide cellular organization. Despite the critical role played by molecular motors in self-organizing cellular microtubule arrays, the current view remains primarily MTOC-driven. We have previously demonstrated that the concurrent transport of molecular motors and microtubules can segregate molecular motors along the microtubule based on their polarity, thereby partitioning an infinite space into alternating regions of plus-end and minus-end motors. But cells are compartmentalized, with a limited pool of motors and microtubules. We have tried to mimic this scenario by imposing boundary conditions via micropatterning. We found that this confined space partitioning can limit the number of domains and generate polarized motor domains upon decreasing the area of exploration. The localization of domain is also determined by the relative ratio of motors with the dominant one trying to occupy the central space and the weaker one becomes peripheral. The length of the microtubules can control the number of domains. An increase in MT length can reorganize the domains and within a confined space, it can also give rise to a bipolar array. Hence, we demonstrate that within confinement, two opposing diffusive motors are sufficient to reconstitute the polar, radial, and bipolar microtubule architectures.

Poster 173 - Dissecting the Mitotic Roles of DCLK1 Isoforms: Insights into Cancer Cell Division.

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DCLK1 (Doublecortin-like kinase 1) is a microtubule-associated protein (MAP) with kinase activity that serves as a biomarker in various cancers. Its overexpression correlates with poor prognosis and contributes to carcinogenesis, tumour initiation, relapse, and therapy resistance. One potential mechanism involves DCLK1 disrupting cell division, leading to aneuploidy and uncontrolled proliferation —key hallmarks of cancer.

DCLK1 exists in four isoforms: DCLK1-aS and DCLK1-aL (with both kinase and MAP domains), and DCLK1-bS and DCLK1-bL (containing only the kinase domain). Isoform deregulation varies across tumour types: renal tumours typically upregulate both DCLK1-α and DCLK1-β, while colorectal tumours overexpress DCLK1-β and downregulate DCLK1-α. Our aim is to uncover how DCLK1 isoforms differentially affect cell division, which could inform more precise, patient-tailored cancer therapies.

In the present work, we used CAKI2 renal cancer cells, which overexpress DCLK1-α and DCLK1-β compared to healthy HK2 cells, mirroring renal tumour profiles. Using siRNA, we depleted DCLK1-α alone or both DCLK1-α and DCLK1-β, then compared mitotic phenotypes to control cells. DCLK1-α depletion caused curved or distorted mitotic spindles, whereas co-depletion of DCLK1-α/β did not. Additionally, upon either DCLK1-α depletion or DCLK1-α/-β co-depletion, congression defects were more frequent than in control cells.

These preliminary findings suggest that DCLK1 isoforms may distinctly influence mitotic spindle organization and chromosome alignment. Further work will determine whether these effects depend on DCLK1’s kinase activity, MAP function, or both.

Poster 174 - Basal Cortex Organization and Mechanics in Epithelial Cysts

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Epithelial cysts are a powerful model for studying how the organisation of the cytoskeleton shapes the basal mechanical properties of three-dimensional tissues. To investigate basal cortex mechanics, we combined atomic force microscopy (AFM) indentation with fluorescence imaging of actin isoforms. Indentation measurements revealed that altering levels of either γ-actin or non-muscle myosin IIB changed prestress, cortical stiffness, and fluidity, indicating that actin composition contributes to the mechanical polarity of the basal cortex. In contrast, loss of the tight junction protein ZO-1 did not affect basal cortex dynamics. Notably, γ-actin, typically described as apical in epithelial monolayers, was also detected at the basal cortex of cysts, suggesting a functional contribution to basal cortex dynamics.

Importantly, γ-actin localisation was sensitive to the mechanical microenvironment. Within cysts, cells in contact with stiff substrates exhibited a loss of γ-actin at both the apical and basal cortices, whereas neighbouring cyst cells retained basal γ-actin enrichment. In contrast, cysts fully embedded within the matrix maintained a more uniform γ-actin distribution. To independently probe tissue-level mechanics, we additionally performed micropipette aspiration on intact cysts. These experiments revealed differences in apical and basal tension distribution, providing insight into the fracture mechanics of living epithelial tissues.

Taken together, our results suggest that the organisation and mechanics of the basal cortex in epithelial cysts are regulated by actin isoform distribution and substrate interactions, highlighting a multiscale coupling between cytoskeletal architecture and tissue mechanics.

Poster 175 - Autophagy Inhibition: A Key to Overcoming Lung Cancer Resistance

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The inherent drug resistance of lung adenocarcinomas, together with the additional resistance acquired during long-term treatment, remains a major obstacle in non-small cell lung carcinoma (NSCLC) therapy. Colchicine (Col), a mitotic poison that disrupts dynamic instability of microtubules, shows promising potential in lung cancer therapy due to its cytostatic effects. However, its clinical application is limited by its ability to induce cytoprotective autophagy in lung adenocarcinoma cells (A549), thereby contributing to their inherent chemoresistance. This cytoprotective mechanism was also found to drive the further increased resistance observed in A549-R cells, a variant developed in-house through prolonged exposure to Col. Beyond elevated autophagy levels, A549-R cells exhibit extensive morphological and phenotypic remodeling, including increased cell size, polyploidy, EMT transition, microfilament reorganization, microtubule architecture changes, and mitochondrial network alterations, as revealed by 2D and 3D stimulated emission depletion microscopy. To overcome both inherent and acquired resistance, we combined Col with autophagy inhibitor chloroquine (CQ). This combination led to synergistic cytotoxic effect in A549 and A549-R cells growing both in 2D and 3D (spheroid) arrangement. Mechanistically, this synergic cytotoxicity of Col/CQ resulted from suppression of Col-induced cytoprotective autophagy, combined with disruption of cytoskeleton, causing formation of actin bundles, and microtubule stabilization and condensation. These changes also affected cell-cycle progression in both parental and resistant cells, further amplifying the cytotoxic outcome. Together, these findings demonstrate that targeting cytoprotective autophagy, in combination with cytoskeletal remodeling, represents a potent strategy to overcome drug resistance, improving the clinical applicability of Col in treating lung adenocarcinomas.

Poster 176 - Spatial Organization of Intermediate Filaments in Astrocytes Driven by Other Cytoskeletal Fibers

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The cytoskeletal networks of intermediate filaments, actin microfilaments, and microtubules interact dynamically, with each component influencing the organization and behavior of the others. In particular we have previously described the role of both actin retrograde flow and microtubule-dependent transport in the dynamic rearrangement of intermediate filaments (Leduc et al. JCB 2017). In the present work, our goal is to determine the relative contribution of actin retrograde flow and microtubule-dependent transport in the stereotypic organization of the intermediate filament network controlled by cell adhesion to the substrate. To tackle this question, we use cells cultured on micropatterned substrates of circular or square geometry to precisely and reproducibly control the distribution of focal adhesion, cell shape and cell size. This allows us to investigate actin, microtubule and intermediate filament organization dynamics and at steady state and quantify the effects of specific cytoskeletal drugs -namely the use of small inhibitors to affect specific processes related to cytoskeleton assembly and dynamic- on intermediate filament organization. These data are integrated into mathematical models of intracellular flows (Park et al. PRE 2021) and spring-like molecules dynamics (Portet et al. J. Theor. Biol. 2022) to generate predictive simulations of cytoskeletal organization under specific perturbations.

Poster 177 - Efficient Filopodia Formation Promoted by a Filopodial Myosin-Talin Complex.

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MyTH-FERM myosins from evolutionarily distant organisms, Myo10 in mammalian cells and DdMyo7 in amoebae, are essential for filopodia formation. Interestingly, there are functional links between these myosins and the highly conserved mechanosensor talin. Myo10 is implicated in talin-mediated integrin activation at filopodia tips (Miihkinen et al, 2021) and Dictyostelium TalinA (TalA) binds DdMyo7 and regulates its cortical dynamics (Galdeen et al, 2007).

TalA is localized to both the rear of migrating cells, as shown by others, but is also at the leading edge and at filopodia tips. TalA co-localizes with DdMyo7 at filopodia initiation sites and the tip throughout filopodia extension. talA null cells exhibit a 50% reduction in cortical levels of DdMyo7, consistent with FRAP data showing that cortical DdMyo7 is more dynamic in talA nulls (Galdeen et al, 2007). This reduced association is accompanied by a significant decrease in filopodia number (~ 50%), establishing that TalA is needed to stabilize DdMyo7 at filopodia initiation sites.

Deletion mapping and AlphaFold (AF) prediction was used to identify DdMyo7 and TalA interaction sites. The predicted TalA structure is highly similar to that of HsTLN1 – an N-terminal FERM domain followed by a long C-terminal rod made up of 13 helical bundles (R1-R13), organized similarly to those in HsTLN1. AF Multimer predicted an interaction between R11 and cDD of DdMyo7 (aa 884-952), a highly conserved region in Dictyostelid Myo7s. Deletion of R11 from TalA or cDD from DdMyo7 abolished their interaction. TalA-ΔR11 no longer co-localizes with DdMyo7 to the leading edge and filopodia tips and is instead highly enriched at the rear of the cell.

These results reveal that the DdMyo7-TalA interaction is important for proper localization of TalA and cortical anchoring of DdMyo7 that is critical for efficient filopodia formation.

Poster 178 - Archaeal Origins of the Eukaryotic Cytoskeleton: Functional Insights from Asgard Tubulin and Gelsolins

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Investigating the potential origins of the eukaryotic cell and the emergence of its cellular functions is an important consideration for predicting the characteristics of organisms that participated in eukaryogenesis. To trace the evolution of cytoskeletal components, we studied tubulin and gelsolin proteins from Asgard archaea, the closest known relatives of eukaryotes. We elucidated the structure and dynamics of Odinarchaeota tubulin (OdinTubulin). X-ray structures in apo, GTP-, and GDP-bound states, combined with phylogenetics, confirm that OdinTubulin diverges significantly from prokaryotic homologs FtsZ and CetZ, and branches in the same clade as eukaryotic tubulins. OdinTubulin forms tubules at high temperatures, with short discontinuous curved protofilaments that spiral around the wall of the tubule, more similar to FtsZ, rather than running parallel to its length as in microtubules. This study proposes that tubulin originated in archaea and that the switch to microtubule filament morphology was an important adaptation during eukaryogenesis. We also investigated the evolution of regulated actin dynamics by characterization of two domain gelsolins (2DGels) from Heimdallarchaeota (Heim) and Lokiarchaeota (Loki). Sequence analysis revealed that Heim and Loki2DGels have C-terminal extensions which are distinct between Loki and Heim phyla and show significant variability within each phylum. In the structures, Loki2DGel sequesters two subunits of G-actin and Heim2DGel a single G-actin. Domain 1 of Heim2DGel binds in the classic site between actin subdomains 1 and 3, similar to Loki2DGel and human gelsolin. We find evidence that these Asgard 2DGels can regulate eukaryotic actin in vitro and when ectopically expressed in eukaryotic cell culture. These data indicate that the last Asgard archaea common ancestor likely possessed a calcium-regulated 2DGel actin-filament depolymerization system, and that the emergence of calcium regulation of actin dynamics predates eukaryogenesis. This work provides structural and functional insights into the archaeal ancestry of the eukaryotic cytoskeleton.

Poster 179 - Emergence of Polar Order at +1/2 Topological Defects Driven by Type I Myosin Motors

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Topological defects in nematic actin organizations are recognized as key mechanical regulators of tissue morphogenesis. However, how myosin motor activity modulates these defects in actin nematics is not fully understood. Here, we investigated how myosin I motors re-organize dense, nematically ordered actin filaments and influence the emergence of topological defects. Myosin I functions as an actin-membrane linker, exerting forces on actin filaments while anchoring them to membranes. Using reconstituted systems in which nematic actin networks are coupled to supported lipid membranes via myosin I motor Myo1b, we showed that Myo1b drives actin filament accumulation at +1/2 comet-like topological defects. This filament accumulation leads to the formation of protrusion-like actin structures. Fluorescence polarization microscopy reveals that actin filaments within these defects are highly aligned and densely packed. We proposed that the comet head of the +1/2 defects acts as a physical barrier that impedes filament transport toward the defect core, resulting in filament accumulation at the comet tail. To elucidate the underlying physical mechanism, we developed a microscopic model of Myo1b-driven actin filament transport and solve it by numerical simulations. The model quantitatively reproduces actin accumulation near the comet head of +1/2 defects, in good agreement with our experimental observations.

Poster 180 - The Great Transformation of the Actin Cortex

Joe Tyler, Jason King

The University of Sheffield

Macropinocytosis is the bulk uptake of extracellular fluid and allows cells to access nutrients and information from the environment. This requires major deformations of the plasma membrane which must be organised in time and space in the absence of an external template. These deformations are best characterised in the amoeba Dictyostelium discoideum where a domain of active Ras and PIP3 pattern a ring of the Arp2/3 complex to generate a stereotypical cup-shaped structure. How the Arp2/3 complex is localised to the edge of the Ras/ PIP3 domain and excluded from its centre however, is unclear as canonical activators of the Arp2/3 complex including Rac are active across the whole structure. Here we describe an unexpected role for the contractile actin cortex in determining the localisation of the Arp2/3 complex. We propose that the ring of Arp2/3 complex acts as a mechanical insulator facilitating the connection of two regions of the actin cortex with vastly different physical properties. We discuss the implication of this Ras/PIP3 driven cytoskeletal organisation as a general mechanism to generate micron-scale structures with evidence from Dictyostelium and several mammalian systems.

Poster 181 - Actin and Microtubules Cooperate in Chromosome Capture During Oocyte Meiosis

Yamini Vadapalli, Peter Lenart

Max Planck Institute for Multidisciplinary Sciences

Accurate chromosomes capture and alignment are essential for error-free cell division, preventing chromosome mis-segregation and aneuploidy. In somatic cells, dynamic astral microtubules “search and capture” chromosomes, whereby microtubule growth, shrinkage, and lateral kinetochore attachments facilitate capture. However, in oocytes with large nuclei of up to hundred micrometers in diameter, this mechanism is insufficient. Instead, actin-driven processes appear to play a crucial role in chromosome capture. In starfish oocytes, a contractile F-actin network transports chromosome to the ~30 µm capture range of astral microtubules to prevent chromosome loss. Similarly, in human and porcine oocytes actin-driven chromosome clustering facilitates spindle assembly.

Here, to address the conservation of such actin-driven mechanisms in animal oocytes, we investigated chromosome capture in the jellyfish Clytia hemisphaerica, a cnidarian species at the base of animal phylogeny. In Clytia oocytes, astral microtubules nucleate away from the cortex, around the nuclear envelope, rather than being anchored at the cortex. The nuclear region is filled with an extensive F-actin network. While the F-actin network contracts, microtubule asters coalesce and collect chromosomes. Following chromosome capture, the single large aster migrates to the cortex, where spindle assembly and polar body extrusion take place. Aster-chromosome complex transport to cortex appears to be driven by cytoplasmic flows.

Together, our findings evidence a conserved role of F-actin in chromosome congression, and at the same time reveal exciting species-specific adaptations. By comparing starfish and jellyfish oocytes, we hope uncover conserved mechanisms governing chromosome capture and meiotic spindle assembly, as well as species-specific adaptations, diverse solutions to the same cellular function.

Keywords: Oocyte meiosis, chromosome capture, F-actin, microtubules, spindle assembly, cytoplasmic flows, evolutionary cell biology.

Poster 182 - ZNF185 Regulates Adhesion Disassembly and Cell Migration

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The LIM domain is a double-zinc-finger motif, which is present in many proteins particularly those associated with the actin cytoskeleton. ZNF185 is a LIM domain protein that does not share many similarities with other LIM domain proteins. It is linked to prostate and lung cancer progression, but its cellular functions are largely unknown. We reveal that ZNF185 localizes prominently to focal adhesions and a lesser extent to actin stress fibers in cultured U2OS cells. Furthermore, our live-cell imaging experiments provided evidence that ZNF185 arrives to focal adhesions after zyxin does and is especially prominent during focal adhesion disassembly. To further investigate the cellular function of ZNF185, we generated a ZNF185 knockout in U2OS cells. Interestingly, loss of ZNF185 led to larger and more elongated focal adhesions, which were typically located closer to cell edges. The lifetimes of focal adhesions in the ZNF185 knockout cells were >2-fold longer as compared to the wild-type cells. Moreover, loss of ZNF185 resulted in diminished cell migration speed, most likely due to the presence of abnormally stable focal adhesions. Collectively, these results suggest that ZNF185 is an important regulator of focal adhesion disassembly and cell migration.

Poster 183 - A New Mechanism of Regulation of LIM Kinases, LIMK1 and LIMK2, Triggers Their Activity on Cofilin and Actin Filament Remodelling

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LIM kinases, LIMK1 and LIMK2, are serine/threonine and tyrosine kinases. They are playing a crucial role in the regulation of cytoskeleton dynamics mediating actin filament and microtubule turnover. Thus, they are involved in many physiological processes, such as cell cycle, cell differentiation and migration, and neuronal plasticity. Consequently, they are involved in several pathologies especially cancers, neuronal diseases and neurofibromatosis. LIM kinases appear as promising therapeutic targets, however, they so far remain undruggable, as none of the molecules developed to inhibit their kinase activity has successfully reached the clinical stage. A better understanding of their activity and regulation is then required to go further into the design of efficient targeted therapies. Here, we pointed out the impact of a single amino acid towards LIMK activity on cofilin, their main substrate in actin filament remodelling. We showed that Tyr630 for LIMK2 and Tyr632 for LIMK1 mediate LIMK dimerization resulting in their transphosphorylation, which seems a prerequisite for their canonical phosphorylation on their Thr505/508 within their activation loop. These Tyr do not appear to be phosphorylated, their aromatic nature is rather the essential determinant to ensure proper LIMK activity on cofilin. These results were confirmed in the yeast Saccharomyces cerevisiae orthologue system. Molecular modelling experiments further strengthened the crucial role of these Tyr630/632 as they adopt two discrete well-defined conformations directly correlated to the status of the alpha-C-helix of the kinase domain of LIMKs. Our results unravel an unexpected regulation of LIMK activation, and pave the way for a better understanding of the regulation of their activity on cofilin and thus on actin filament remodelling.

Poster 184 - SPEEDFix: Time-Resolved Cryo-ET of Migrating Cells

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The formation of dynamic protrusions at the cell leading edge, such as lamellipodia, is orchestrated by a complex interplay of actin and actin-binding proteins. Migration direction and speed hinge upon rapid changes in actin architecture controlling lamellipodial protrusion and retraction, yet the specific organisational changes that underlie these dynamics remain poorly understood. particularly, how the transitions between fast- and slow-moving regions in close proximity are reflected at the molecular level is not fully resolved.

Cryo-electron tomography (cryo-ET) has emerged as a powerful tool for visualising actin network organisation in-situ, providing high-resolution insights into supramolecular geometries and architecture. However, conventional cryo-ET sample preparation approaches lack the temporal context necessary to understand inherently dynamic systems. As a result, they cannot reveal migration direction or identify which regions of the lamella were actively protruding or retracting at the moment of vitrification. To our knowledge, no existing methodology enables controlled, robust, and reproducible on-demand fixation of migratory behaviour for downstream cryo-ET imaging.

To address this gap, we developed SPEEDFix (Structural Proteomics of Ephemeral Events by Direct Fixation), a rapid fixation method integrated with live-cell fluorescence microscopy. This system enables extraction and fixation of cells on cryo-EM grids within seconds of observing a specific migratory event, at the press of a button, preserving a near-native state of transient cellular structures at a given time point. By combining SPEEDFix with montage cryo-ET, subtomogram averaging, and large-scale network analysis, we can map the molecular identities, network architecture, and transitions within protrusions. This reveals cell-wide arrangements, offering a powerful framework to correlate emergent migratory behaviours with underlying ultrastructure. This approach provides new quantitative spatial insights into the cytoskeletal mechanisms that drive cell migration. More broadly, SPEEDFix is adaptable to other transient cellular processes where structural context is currently inaccessible, including phagocytosis, organelle reorganisation, and transitions during cell division.

Poster 185 - Stretching Vimentin Intermediate Filaments Using Microfluidics

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Vimentin, an intermediate filament protein predominantly expressed in mesenchymal cells, exhibit unique mechanical properties of extensibility and resistance to breaking —features that are critical for cellular resilience under mechanical stress1.

To investigate the mechanical behavior of vimentin filaments, previous studies have used optical traps to examine the response of individual filaments under strain2,3. Here, I present a microfluidic-based system in which single vimentin intermediate filaments (VIF) are stretched by viscous drag with the fluid flow. Coupled to fluorescent microscopy, this approach enables simultaneous observation of multiple filaments, allowing high throughput studies of VIF mechanical properties. Based on fluorescence measurements, this system also allows us to study local deformations in VIFs under strain and how these deformations may be influenced by factors such as filament composition or binding proteins. A deeper understanding of VIF mechanics will provide valuable insights into their role in the cellular response to mechanical stress.

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Poster 186 - The Cellular Effects of the Non-Muscle Actinopathy (NMA) Mutants G74S and R183W Are Associated with Distinct Molecular Mechanisms

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Mutations in cytoplasmic β- and γ-actin are associated with a wide spectrum of rare diseases, called non-muscle actinopathies. A subset of mutations in β-actin results in a severe phenotype characterized by craniofacial anomalies and intellectual disability, which is commonly referred to as Baraitser-Winter cerebrofrontofacial syndrome (BWCFF). The variant R183W in cytoplasmic β-actin causes a phenotype distinct from BWCFF, called dystonia-deafness syndrome (DD). Here, we compared the effects of the G74S (BWCFF) and R183W (DD) mutations on the cellular properties of actin in patient-derived fibroblasts. Although these mutations are part of the same allosteric route maintaining the closed conformation of actin, we hypothesized that mutation-specific effects on actin dynamics and interactions could give rise to distinct phenotypes in patients. None of the mutations changed the cellular organization of the actin filament bundles analyzed by confocal and super-resolution (STED) microscopy. However, the R183W mutation reduced cell stiffness by 50%, whereas the G74S mutation decreased it to 25% of that of wild-type cells. The observed changes might be related to the misorganization of tubulin in the G74S mutant. Analyzing the reorganization properties of actin upon stretch revealed that the relocalization of cofilin from the cell periphery is decreased in the R183W mutant, while it is increased in the G74S mutant. We also found that applied stress influences the kinetics of actin contraction in the G74S mutant. Complementary in vitro experiments with recombinant cytoplasmic β-actin carrying the R183W mutation revealed impaired cofilin binding, providing a possible explanation for the phenotype observed in patient cells.

Poster 187 - Mechanical Polarity of the Actomyosin Cortex and Single Cell Morphogenesis

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Cell shape and its dynamic changes are fundamental to development, tissue homeostasis, and cancer progression. A key feature of cell morphology is its organisation along polarity axes such as the front-rear axis in migrating cells or the apico-basal axis in epithelial cells, critical for cellular function. The actomyosin cortex, a thin contractile network beneath the plasma membrane, plays a central role in shaping cells by generating forces on the cell membrane. While the cortex's role in 2D morphogenesis (e.g., mitotic rounding, cell spreading) is well-documented, its function in 3D remains poorly understood.

In this project we investigate how the actomyosin cortex mechanically shapes single polarized cells in 3D. We use a minimalist model: 3T3 fibroblasts adhered to fibronectin-coated circular micropatterns. This allows us to control cell spreading and characterize 3D cell morphology. Cells on 15–20 µm patterns (approximating suspended fibroblast diameter) adopt an elongated apico-basal morphology, while those on smaller or wider patterns adopt a truncated spherical shape. To assess whether this elongation reflects cortical actin gradients, we developed vertical patterns in collaboration with Raphaelle Renou (supervised by Sylvie Coscoy and Vincent Semetey), enabling direct observation of cells on their side and therefore the quantitative comparison of fluorescence along the apico-basal axis. Preliminary results show no apico-basal actin gradient and we plan on examining non-muscle myosin 2 distribution next. We also explore the role of cortex contractility, of the underlying vimentin cage and of polysomes in maintaining elongated morphology.

Future work will quantify cortex thickness and stiffness along the polarity axis and across morphologies using the magnetic pincher technique, which employs intracellular and extracellular magnetic beads to "pinch" the cortex and measure its mechanical properties.

Poster 188 - IntAct-U-ExM Enables Isoform-Specific Super-Resolution Imaging of Actin Networks Across Species

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Expansion microscopy (ExM) has revolutionized super-resolution imaging in cell biology due to its simple and inexpensive workflow. The use of ExM has revealed several novel insights into the nanoscale architectures of cellular protein complexes, especially the microtubule cytoskeleton in model and non-model systems. Despite tremendous progress in expansion microscopy protocols that preserve cellular ultrastructure (U-ExM), compatible probes for imaging actin isoforms with U-ExM are still lacking and have hindered the study of diverse actin isoforms and networks across model systems. Here, we use IntAct, an internally tagged actin that incorporates into cellular actin networks, to develop and optimize U-ExM of diverse actin network types in both yeast, cultured mammalian cells, and primary mouse neurons. Using expression of ALFA-tagged IntAct variants, we show robust visualization of actin patches, cables, and rings in yeasts, diverse actin networks such as actin cortex, stress fibers, filopodia, lamellipodium in mammalian cells, and the sub-diffraction limited actin rings in primary mouse hippocampal neurons at improved resolution. We also detect transient nuclear actin filaments using IntAct-U-ExM underscoring the advantages offered by our approach to image understudied actin structures. Overall, we demonstrate the effectiveness of IntAct-U-ExM for performing super-resolution imaging of various actin structures in an isoform-specific manner and highlight the potential of IntAct to study the nanoscale organization of diverse actin cytoskeletal networks across species.

Poster 189 - Dissecting the Roles of Cyclase-Associated Protein (CAP) in Lamellipodia Architecture and Dynamics by Using CAP1/CAP2 Double Knockout Cells

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Cyclase-associated protein (CAP) is a multidomain protein involved in several vital cellular functions, including morphogenesis, migration and endocytosis. In mammals, there are two isoforms: CAP1 that is widely expressed in non-muscle cells and CAP2 that is most strongly expressed in muscle cells. Biochemically, CAP accelerates nucleotide exchange in ADP-actin monomers and promotes rapid depolymerization of cofilin-actin filament pointed ends. Recent biochemical studies provided evidence that CAP can also associate with filament barbed ends to accelerate their depolymerization and induces actin filament bundling. To uncover the precise mechanisms by which CAP regulates the actin cytoskeleton, we generated CAP1 knockout and CAP1/CAP2 double knockout B16 cells. Loss of CAP resulted in reduced cell migration, larger cell area, thick stress fibers, and appearance of aberrant cytoplasmic actin aggregates. Besides actin, these aggregates contain cofilin and alpha-actinin, but not myosin II. Expression of CAP1-EGFP in the knockout cells partially rescued the actin aggregate and stress fiber phenotypes, but not the increased cell area. Interestingly, CAP1-EGFP displayed partially different distribution within the leading edge as compared to actin, being more enriched towards the back of the lamellipodium. Further knockout-rescue studies with specific CAP mutants are ongoing to uncover the precise roles of different biochemical activities of CAP in lamellipodia dynamics and other actin-dependent cellular processes.

Poster 190 - Investigating the Isotype-Specific Impact of Tubulin-Beta-8 on Microtubule Behaviour and Structure.

Jeraldine Weber

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As major contributors to cell division, microtubules are key drug targets in proliferative diseases such as cancer. Many chemotherapeutic agents disrupt microtubule dynamics by targeting the tubulin-beta subunit of tubulin. While at least ten tubulin-beta isotypes have been characterised in humans, these isotypes share a high level of sequence identity and similarity; it is also unclear whether all tubulin-beta isotypes share the same sensitivity to tubulin-targeting drugs.

Here we have demonstrated that tubulin-beta-8, the main tubulin-beta isotype in human oocytes, displays reduced sensitivity against the microtubule destabilising drug Nocodazole. We observed that Nocodazole does not inhibit the microtubule assembly of tubulin-beta-8 in vitro. In addition, the microtubules of human oocytes do not depolymerise in the presence of Nocodazole. Meanwhile, microtubules of mouse and bovine oocytes, which do not express tubulin-beta-8, depolymerise in the presence of Nocodazole. Multiple sequence alignment of human tubulin isotypes revealed an amino acid substitution within the Nocodazole binding site of tubulin-beta-8 that is not conserved in other isotypes. By mutating this residue, we will investigate whether this restores any sensitivity to Nocodazole. Finally, we are currently using cryo-EM to determine the structure of microtubules assembled from tubulin-alpha-1A/beta-8 and will investigate the binding affinity of tubulin-beta-8 to other tubulin-targeting drugs. This will improve our understanding of how microtubules are adapted in germ cells.

Overall, these results demonstrate how tubulin-beta sensitivity against tubulin-targeting agents can vary on an isotype-specific basis. Understanding this may allow for the application of precision medicine approaches to the prescription of the most appropriate chemotherapeutic agent, depending on the tubulin-beta isotype enrichment within a patient’s cancer cell profile.

Poster 191 - Septins Are Nuclear Proteins

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Actin, intermediate filaments and microtubules are the three major cytoskeleton components that are described in every cell biology text book. However, this traditional view is incomplete, as in recent years the septin protein family has been recognized as the fourth central element, due to their filamentous appearance and interactions with actin and microtubules. Septins are essential for various cellular processes such as cytokinesis, cell motility and compartmentalization. Dysfunctions are associated with several diseases, including Alzheimer's disease and cancer. Identified initially as purely cytoplasmic proteins, our data provide strong evidence that septins are also present in the nucleus. Using immunofluorescence imaging and subcellular fractionation, we show that septins are localized in the nucleus not only in cancer cell lines but also in primary cells. We focused on one septin member from each septin subgroup (SEPTIN2, SEPTIN7, SEPTIN9, SEPTIN10) and identified the region of the nuclear localization signal (NLS) by using sequential domain deletion constructs. A biotinylation-dependent screen for interaction partners (BioID) identified Importin-7 as a potential transport protein for septins. Co-Immunoprecipitation and proximity-ligation assays confirmed the binding of all four analyzed septins to Importin-7. Inhibition experiments validated Importin-7 as one of the main import factors responsible for nuclear translocation of septins. Using NLS-tagged SEPTIN2, we identified key nuclear processes with involvement of septins, e.g., splicing and mRNA processing. Additionally, nuclear SEPTIN2 was associated with several transcription factors implicating a general role in gene transcription regulation. This novel aspect of septin biology represents a new research avenue with potential implications for our comprehension of cellular homeostasis, nuclear processes and potentially disease formation.

Poster 192 - Structure of the a-C Linker Connecting Microtubule Triplets in Basal Body Centrioles

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Centrioles are massive protein assemblies at the cores of centrosomes and basal bodies. A hallmark of centriolar architecture is the A-C linker, which connects the A- and C-tubules of neighbouring microtubule triplets. Despite its identification over 50 years ago, the composition and structural details of the A-C linker were not previously known. Here, we report cryo-electron microscopy (cryo-EM) reconstructions of the native A-C linker bound to centriolar microtubule triplets in T. thermophila basal bodies. We discovered that the A-C linker adopts two distinct conformations: an extended form in the proximal centriolar microtubule triplet region, and a narrow form in the distal portion. These conformations are distinguished by unique A- and C-tubule-binding proteins and an alpha-helical bundle that protrudes from the proximal A-C linker into the cell body. We identified core A-C linker components, which repeat every 8 nm along the centriole axis via a fishbone-like alpha-helical lattice. The structure of adjacent A and C-tubules shows that the A-C linker forms a bridge with novel triplet-specific B-C junction proteins, allowing us to build the first molecular model of a centriolar microtubule triplet pair. Our results uncover surprising structural and biochemical heterogeneity in centriole-associated complexes and suggest how the A-C linker may coordinate its assembly with other centriolar components to generate functional basal bodies.

Poster 193 - Identification of Novel Microvillus Components Involved in Epithelial Cell Morphogenesis

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Brush border establishment is crucial for intestinal function. The microvillus tip protein EPS-8 localises to the apical membrane after establishment of polarity in epithelial intestinal cells in Caenorhabditis elegans. Knock-down studies have revealed that EPS-8 regulates intestinal morphology and microvilli length, processes that depend on actin filament organisation. Its apical localisation coincides with the formation of the intestinal lumen and microvilli. EPS-8 is essential for embryonic development, yet its function in regulating intestinal actin dynamics during brush border formation remains unclear. In order to elucidate its molecular role, we performed a TurboID-based proximity labelling assay to identify candidate interaction partners. We identified a number of promising candidates, such as the myosin HUM-4, as well as the C. elegans ortholog of the I-BAR protein BAIAP2L1, F49E2.2, and several other novel proteins. Through fluorescent protein fusions we confirmed their localisation in the microvillus tip. Interestingly, gene knock-outs revealed functional redundancy between many of the newly identified microvillus proteins, and we show that they contribute to actin filament organisation and microvillus stability. We generated domain knock-outs of HUM-4 and F49E2.2 to study their molecular function in maintaining brush border stability. Our in vivo work in the C. elegans intestinal brush border provides new insights into conserved mechanisms of microvillus morphogenesis and maintenance.

Poster 194 - Transgelin in Regenerating Cardiomyocytes: Potential Mediator of Mechanotransduction and Intercellular Crosstalk?

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Unlike adult mammals, zebrafish (Danio rerio) harbor the ability to regenerate their heart following injury. Following dedifferentiation and proliferation, cardiomyocytes (CMs) at border-zone extend F-actin-filled protrusions and invade to ultimately replenish the fibrotic scar tissue with new myocardium. We have previously shown that AP-1 transcription factors are important regulators of CM protrusion and invasion and activate gene regulatory networks to remodel the actin cytoskeletal network. One potential AP-1 target gene, tagln, has previously been shown to be upregulated in several cardiac injury models in zebrafish. Transgelin (Tagln), is an actin bundling/gelating protein, and its mRNA and protein levels have been shown to respond to changes in mechanical tension and matrix stiffness. The role of Tagln in CM regeneration, however, remains unknown.

Here, using conditional dominant-negative approaches, we verified that tagln is an AP-1 target enriched in border-zone CMs. In order to understand the function of Tagln in vivo, we generated CRISPR-based loss-of-function models and transgenic gain-of-function models. Using super-resolution confocal microscopy, we find that CM-specific overexpression of tagln leads to higher density of F-actin within CM protrusions at the wound border and in non-CMs directly adjacent to the wound border CMs. Strikingly, CM-specific tagln overexpression also induces early CollagenXII accumulation, endocardial activation in the injured area, and inhibition of collagen degradation/remodeling. These observations suggest that stabilization of the actin cytoskeleton within CMs affects the phenotypes of surrounding cells in the injured area. Currently, loss-of-function and additional gain-of-function studies are ongoing to investigate how Transgelin regulates the actin cytoskeleton of border-zone CMs to promote invasion and the mechanisms by which tagln overexpression in CMs affects surrounding cells at border-zone. Our studies will provide important mechanistic insight into the role of actin cytoskeletal remodeling in CM regeneration, and the potential role of tissue stiffness in triggering the regenerative niche at border-zone.

Poster 195 - Tying up Loose Ends: The Actin Cable Organizing Center Regulates Actin Cable Polarity, Function and Quality Control in Budding Yeast

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Actin cables are bundles of F-actin that are essential for their role as tracks for transport of cellular constitutions from mother to daughter cell during asymmetric cell division in the budding yeast, Saccharomyces cerevisiae. Here, we describe the Actin Cable Organizing Center (ACOC), a structure that modulates actin cable orientation, length and quality control. The ACOC localizes to the tip of the mother cell distal to the daughter cell (bud), a polarity site in yeast that is not well understood. It serves as a site for capture of the pointed end of actin cables emanating from the bud, which results in alignment of actin cables along the mother-bud axis. The ACOC is also a site for disassembly of ACOC-anchored actin cables, which is critical for actin cable length control, and is associated with actin disassembly proteins including coronin and Aip1p. In addition, the ACOC functions in actin cable quality control: mechanical stress associated with actin cable capture by the ACOC can result in breakage of captured cables at points of weakness along the cable and rapid elimination of ACOC-associated actin cable fragments. Finally, the ACOC is the site for anchorage and accumulation of mitochondria at the mother cell tip by the tethering protein, Mfb1p. Inhibition of ACOC function results in defects in anchorage of actin cables and mitochondria at the mother cell tip, misalignment and depolarization of actin cables, and defects in localization of mitochondria and Mfb1p. Conversely, deletion of MFB1, and the associated defects in anchorage of mitochondria at the mother cell tip, result in defects in ACOC function in actin cable control.

Poster 196 - Cardiomyopathy-Associated LMNA R89/E203 Mutations Impair Lamin Filament Assembly and Nuclear Mechanical Stability

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Laminopathies, a group of debilitating disorders including Dilated Cardiomyopathy (DCM), are caused by mutations in LMNA gene, which encodes the intermediate filament proteins Lamin A/C. These nuclear structural components are crucial regulators of nuclear mechanics and chromatin organization. Structural data from the LMNA rod domain reveal the close spatial association of the highly conserved E203 and R89, providing a strong structural basis for their potential role in Lamin A/C polymerization. However, despite this crucial insight into assembly, the specific molecular and cellular consequences of single-point mutations at these sites on nuclear mechanical stability and the pathogenesis of DCM remain largely undefined.

We hypothesize cardiomyopathy-associated LMNA 1B-domain mutations (E203G/K/Del and R89L) disrupt lamin filament assembly, leading to compromised nuclear mechanical integrity. We first investigated whether human recombinant lamin A, wild-type and mutants, were capable of reconstituting filament assembly in vitro. Using immunofluorescence assays to monitor nuclear architecture and atomic force microscopy (AFM) to measure biomechanics at the single-cell level. We identified structural and morphological destabilization in the nucleus of cells expressing Lamin A/C mutants compared to wild-type. Furthermore, quantitative nanoindentation analysis using AFM reveals the significant alterations in the biomechanical properties of cells expressing LMNA mutants, indicating the critical biomechanical phenotypes potentially linked to the pathogenesis of LMNA-related cardiomyopathies. Collectively, this work demonstrates that single-point mutations in the rod domain of LMNA are sufficient to interfere the filament assembly and globally weaken nuclear mechanical properties, leading to cardiomyopathies. Future studies extending these observations to in vivo models will be crucial for a comprehensive understanding of their impact on cardiac tissue mechanics.

Keywords: Laminopathies, Protein assembly, Mechanical stability

Poster 197 - Regulation of Cytoskeleton Acetylation in Mitochondrial Transport and Breast Cancer Metastasis

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Breast cancer metastasis is a major cause of death in women worldwide. Metastatic cancer cells reprogram mitochondrial trafficking to sustain their migratory and invasive behavior. Mitochondria positioning to sites of high energy demand is governed by a balance between opposing dynein and kinesin-1 (KIF5B) molecular motors whose regulation remains incompletely understood. Here, we identify the SYBU gene as a candidate prognostic marker downregulated in breast cancer metastasis. SYBU encodes syntabulin, a mitochondria outer membrane protein that interacts with dynein to counterbalance KIF5B-dependent anterograde transport along microtubules to the cell cortex. Loss of SYBU disrupts the balance, causing excessive KIF5B-driven mitochondria movement, microtubule damage and deacetylation. In turn, microtubule deacetylation reinforces KIF5B-mediated transport, creating a positive feedback loop that drives mitochondria distribution close to the cell periphery and enhances cancer cell migration. Pharmacological inhibition of tubulin deacetylase HDAC6 restores mitochondrial positioning and reduces cell migration in SYBU-deficient cells. Our findings identify SYBU as a key regulator of mitochondrial trafficking and microtubule acetylation. They raise the possibility that SYBU also regulates actin-based mitochondrial transport. These results pave the way to personalized therapeutic approaches targeting cytoskeleton remodeling for metastatic breast tumors with low SYBU expression.

Poster 198 - Elastic Resilience of Vimentin Networks Under Repetitive Mechanical Stress

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The cytoskeleton is crucial in maintaining cell shape and structural integrity. It consists of three types of filaments: actin filaments, microtubules, and intermediate filaments (IFs). Among them, actin filaments and IFs exhibit distinctly different force-strain behavior: while IFs show nonlinear behavior with exceptional extensibility and remarkable resistance against rupture at high strains, actin filaments break at low strains. Here we ask whether the unique mechanical resilience of vimentin IFs extends to the network scale under repetitive mechanical stress. We apply cyclic strains to in vitro reconstituted networks using optical tweezers and observe contrasting behavior between the two cytoskeletal networks: vimentin networks reach a steady state after only a few cycles and indicate an elasticity-dominated response. Moreover, the energy dissipation in vimentin networks shows weak rate-dependence at fixed strain. By contrast, actin networks fluidize and dissipate more energy compared to vimentin networks. Our work highlights the complementary nature of the components of the cytoskeleton, which - in the cell - are partly co-localized and believed to constitute a composite biological material.

Poster 199 - How Endolysosomal Super-Organelles Maintain Proteostasis in the Acentrosomal Mouse Oocytes

Gabriele Zaffagnini

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Oocytes are the long-lived female germ cells, which need to preserve their intracellular integrity to allow proper embryogenesis. Protein homeostasis (proteostasis) is essential in all cell types to maintain intracellular integrity. In somatic cells, endolysosomal trafficking and positioning are central to proteostasis maintenance and are tightly regulated. I have recently discovered that in mice, oocytes sequester and degrade aggregated proteins, a hallmark of impaired proteostasis, in novel endolysosomal "super-organelles", which I have named ELVAs. ELVAs harbour endosomes, lysosomes, and autophagosomes in a liquid-like matrix, and show emergent properties such as an autonomous regulation respect to free lysosomes. Largely inactive in immature oocytes, ELVAs relocate to the cell cortex shortly before fertilisation and activate protein degradation, thereby clearing the sequestered aggregates. Unlike endolysosomal trafficking in somatic cells, which mainly relies on microtubules, ELVAs relocation in acentrosomal oocytes entirely depends on the actin cytoskeleton. Impairment of ELVAs activation, or disruption of the ELVA matrix protein RUFY1, result in compromised embryonic development and premature female infertility, respectively. Overall, ELVAs represent a novel paradigm to maintain proteostasis in the long-lived mouse oocyte.

Poster 200 - Anillin Mediates Unilateral Furrowing During Cytokinesis by Limiting RhoA Binding to Its Effectors

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During cell division an actin-myosin ring assembles at the cell equator and its constriction generates the two daughter cells. During unilateral ring constriction one side of the cytokinetic ring, called the leading edge, ingresses before the opposite lagging edge. Anillin is a multidomain protein that binds to F-actin and myosin via its N-terminus and to active RhoA and the plasma membrane via its C-terminus. Previous work demonstrated that anillin mediates unilateral ring constriction in the one-cell C. elegans zygote by limiting myosin accumulation in the ring. The molecular mechanisms, however, by which anillin inhibits myosin accumulation in the ring were unknown. Here we address the role of anillin in this process and show that anillin inhibits not only the accumulation of myosin but also of other RhoA effectors such as formin and Rho kinase. Mutational analysis of anillin in the zygote suggests that anillin binding to active RhoA blocks the RhoA effector site and thereby dampens RhoA signaling. Adjacent to anillin's RhoA-binding domain (RBD) lays a disordered linker region and biochemistry and in vivo studies indicate that the linker region enhances binding of the RBD to active RhoA. We propose that linker function is influenced by the different strength of cortical flows at the leading and lagging edge. Strong flows at the leading edge prevent binding of anillin to RhoA resulting in high RhoA signaling and ring constriction. Weak flows at the lagging edge enhance linker-stimulated RhoA binding of anillin and thereby reduce RhoA signaling and myosin levels. Together this results in asymmetric RhoA signaling and unilateral ring constriction. In summary, we discover a RhoA GEF- and GAP-independent mechanism, where RhoA signaling is limited by anillin binding to the RhoA effector site. Spatial fine-tuning of anillin's inhibitory role on RhoA signaling enables unilateral furrowing and contributes to animal development.

Poster 201 - The Schizophrenia Associated Protein DISC1 Is a Multivalent Tetrameric Hub of Conserved Ancient Fold

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Disrupted in Schizophrenia 1 (DISC1) is a pleiotropic protein that plays essential roles in cell migration, intracellular signalling and cargo transport. In these contexts, DISC1 associates with a wide range of partner molecules. Prominent among those is NDE1/NDEL1, a centrosomal protein that forms part of the dynein motor complex.

Consistent with its involvement in cytoskeleton regulation, inhibition of DISC1 activity leads to neuronal migration defects during early embryogenesis. Moreover, mutations at the DISC1 locus are strongly linked to a spectrum of mental illnesses, including schizophrenia and depression. Despite its clinical relevance, the molecular architecture and function of DISC1 have remained poorly understood for decades.

Here, we present an unpublished cryo-electron microscopy structure of the entire conserved core region of DISC1. The structure reveals an intricate homotetrameric assembly centered on four copies of bacteria-derived UVR domains. Our mutational analysis confirms the critical contribution of UVR mediated dimerization towards complex stability in metazoan DISC1. Notably, this oligomerization capacity is strongly predicted to be preserved among DISC1 homologues across main eukaryotic phyla. In the disease context, we show that one of the DISC1 mutations most strongly linked to mental illnesses abrogates the UVR dimer interface and completely disrupts tetramer assembly.

We further demonstrate that the tetrameric architecture of DISC1 underpins its ability to simultaneously coordinate multiple copies of client proteins such as NDE1. Importantly, tetramerization and partner binding represent structurally independent functions of DISC1.

Together, our findings provide key structural evidence supporting an ancient evolutionary origin and a conserved scaffolding function for this mammalian protein implicated in schizoaffective disorders.

Poster 202 - Redundant Microtubule Abscission Mechanisms Buffer Mechanical Heterogeneities

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In the final step of cell division, cytokinesis, the cytoplasm is partitioned into two, forming a cytoplasmic bridge between the daughter cells. Typically, cytokinesis is followed by abscission, the physical severing of the bridge. The severing of the membrane during abscission is mediated by the ESCRT-III machinery, which also recruits microtubule severing proteins to remove the microtubules of the bridge. Pulling forces on the bridge have been proposed to act as regulators of abscission by influencing the localization of the ESCRT-III complex. However, how forces are integrated by cells during abscission in a developmental context remains elusive. Here, we use pluripotent stem cells, where abscission is slow, keeping the two daughter cells connected for hours, to show that the cytoskeleton buffers mechanical heterogeneities to ensure abscission. We show that cells can cut their microtubules in two distinct ways. Slow abscission is associated with an asymmetric cut in the microtubule lattice, while during fast abscission, the microtubules are gradually depolymerized on both sides of the bridge. We show that the contractility of the actin cortex mediates this choice between the two abscission modes through modulation of the master kinase Aurora B. Finally, the choice between the abscission mode depends on and impacts fate choice dynamics.