Abstracts

Abdulquddus Noah	University of Warwick	Abdulquddus Noah, Maelle Lorvellec, Masanori Mishima	Centre for Mechanochemical Cell Biology, Warwick Medical School, University of Warwick	AFM measurement of cortical mechanics during cytokinesis. During animal cytokinesis, the mechanical force generated by the actomyosin network assembled at the equatorial cell cortex drives constriction of the cytoplasm into two daughter cells. However, cortical deformation results from a balance of active contractile forces, passive viscoelastic forces of the plasma membrane and cytoskeleton, and hydrodynamic forces. Theoretically, even uniform contractile forces can drive furrow ingression if the equatorial cortex is mechanically more deformable than the poles. This highlights the need for spatiotemporally resolved measurements of cortical viscoelasticity, which remain scarce, except for pioneering studies in large echinoderm embryos in the 1960s-1980s. Here, we aim to measure the viscoelastic properties of dividing cultured cells using atomic force microscopy, in correlation with cytoskeletal dynamics observed by confocal microscopy. Acute myosin inhibition will allow us to separate the contributions of active force generation and passive cortical mechanics. This approach will shed light on the mechanics underlying cytokinesis. It may also provide insight into the lack of cleavage furrow formation caused by a mutation in congenital dyserythropoietic anemia type III, which affects the Rho-family GAP activity of centralspindlin.	**Poster No. 1**
Alice Brinkworth	University College London	Alice Brinkworth, Aaron Guha, Pankti Vaishnav, Josie Ferreira	All University College London	In situ Structural Characterisation of the Plasmodium falciparum Microgamete Flagellum. Sexual reproduction in the malaria parasite Plasmodium falciparum relies on the rapid formation of the male microgamete, a cell consisting solely of a nucleus and flagellum. The defining event of this cellular transformation is the cytosolic assembly of eight axonemes in under 20 minutes. Despite the essential role of the motile microgamete flagellum in malaria transmission, their structural architecture and the mechanisms driving such accelerated assembly remain elusive. Using in situ cryo-electron tomography, we imaged Plasmodium falciparum microgametes to investigate the organisation of flagellar microtubules at high resolution. Analysis of over 250 tomograms has revealed striking deviations from classical eukaryotic flagella. Basal bodies composed of singlet microtubules, uncapped distal ends and a diversity of atypical axoneme morphologies suggest a distinct pathway tailored for rapid assembly. By employing sub-volume averaging, we have obtained structures of flagellar microtubules and associated complexes, a critical step towards identifying the proteins responsible for nucleating the flagellum. This approach has provided significant insight into the mechanisms underlying microgamete motility and assembly, advancing our understanding of male gamete formation to inform malaria transmission-blocking strategies.	
Amy Godfrey	Warwick Medical School, Centre for Mechanochemical Cell Biology	Rob Cross, Daniel Peet, Nicholas Carter	Warwick Medical School, Centre for Mechanochemical Cell Biology	Microfluidics can enable researchers from a wide range of disciplines to automate their protocols, manipulate existing systems in new ways, acquire high-throughput datasets and use precious resources efficiently. Substantial progress has been made towards making this technology accessible to a diverse audience. The existing methods nonetheless have their limitations; being costly in time or resources, requiring precarious or permanent assemblies, recycling used flow chambers, or they are incompatible with multiple light microscopy techniques. Here, we present a method to overcome such limitations, combining low-cost disposable flow chambers and repositionable ports, in combination with microtubule lattice studies and the Warwick Open Source Microscope (WOSM)-TIRF.	**Poster No. 2**
Andrew McMahon	University of Warwick	Andrew McMahon, Matthieu Blandenet, Christopher Tynan, Henrik Strahl, Seamus Holden	University of Warwick, Newcastle University, STFC Harwell Research Campus, Newcastle University, University of Warwick	The elongasome is protein motor complex in rod-shaped bacteria that inserts peptidoglycan into the cell wall circumferentially, allowing bacteria to grow via elongation. We previously found that MreB, an actin-like filament that orientates the elongasome, displays motion consistent with a molecular motor tug-of war mechanism between oppositely directed synthesis machineries1. Here, we present progress on understanding the molecular mechanisms and physiological role of elongasome tug-of-war. Western blot analysis demonstrated almost undetectably low RodA levels. To understand the potential role of stress sigma factors in the cell widening associated high RodA-levels, we analysed the induction of the SigM regulon, as well as strains carrying deletions of envelope stress sigma factors. High RodA levels did not activate a SigM response, thus ruling out associated regulation of other elongasome components. Using single-molecule imaging techniques, we have tracked and imaged various constituents of the Bacillus subtilis elongasome to further understand the roles of MreB and the cell wall synthesis proteins in cell growth. Further to this, we have used DNA-PAINT microscopy to study the distribution of elongasome components on MreB polymers and utilised MINFLUX3, a novel millisecond-resolution, nanometer-precision imaging technique, to understand how nanoscale changes in MreB motion result in the the macroscale changes in cell morphology. 1) Middlemiss, S., et al. Molecular motor tug-of-war regulates elongasome cell wall synthesis dynamics in Bacillus subtilis. Nat Commun 15, 5411 (2024). 2) Dion, M. F. et al. Bacillus subtilis cell diameter is determined by the opposing actions of two distinct cell wall synthetic systems. Nat. Microbiol. 4, 1294–1305 (2019). 3) Balzarotti, F. et al. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. Science 355, 606-612 (2017).	
Apolena Zounarova	University of Warwick / TU Dresden	Apolena Zounarova, Manas Chakraborty, Ferdos Abid Ali, Stefan Diez, and Anne Straube	University of Warwick, TU Dresden, University of Bristol, Max Planck Institute of Molecular Cell Biology and Genetics	Cytoplasmic dynein 1 is primarily known as the major retrograde microtubule-associated motor transporting cargo such as organelles and endocytic vesicles from the cell periphery to the cell centre. In addition to transport, dynein also organises the microtubule network by sliding and twisting microtubules relative to one another, contributing to mitotic spindle formation and alignment of polarised microtubule arrays in neurons and muscle cells. Dynein is typically activated upon binding to dynactin and a cargo adaptor, which aligns its motor domains parallel to each other, enabling processive movement. However, it remains unclear how dynein is activated when the cargo is another microtubule. Previous results have shown that when simultaneously contacting two microtubules, dynein becomes autoactivated, undertakes processive movement, and generates higher forces. We propose that dynein crossbridges microtubules using its two motor domains, and that their physical separation bypasses the requirement for binding to dynactin and cargo adaptor. To provide direct evidence for this mechanism, we introduce small tags into the microtubule-binding domain of human dynein to precisely track its stepping along the crossbridged microtubules. As a complementary approach, we employ cryo-electron microscopy to resolve dynein's crossbridging conformation at high resolution. Altogether, this work will advance our understanding of the distinct mechanisms by which dynein organises microtubules. Given that both microtubule bundling and sliding are physiologically relevant, it will also provide further insight into the molecular basis of dynein dysfunction in disease.	

Benjamin Friedrich	Physics of Life, TU Dresden	Maximilian Kotz, Abhimanyu Sharma, Veikko F. Geyer, Benjamin M. Friedrich	TU Dresden	Motile cilia are slender cell appendages, which bend rhythmically to propel ciliated cells or pump fluids in multicellular organisms. The highly conserved cytoskeletal core of cilia, the axoneme, contains regular spaced dynein motors, which bend the cilium. How oscillatory motor activity is controlled is debated for 50 years. As a new approach to this longstanding problem, we measure small fluctuations of the cilia beat [1]. We argue that these fluctuations reflect small-number fluctuations of motor activity, and can thus provide insight into mechanisms of motor control. We present the first theoretical model of the cilia beat that accounts for motor noise [2]. To rigorously test this model, we compare model predictions to experiments where dyneins have partially been extracted from cilia. The model reproduces key phenomena of experimental data, including "phase slips" in the beat of a single cilium. Our analysis of fluctuations suggests a new route to test models of the cilia beat and motor control. [1] Sharma et al. PNAS 2024 https://www.pnas.org/doi/10.1073/pnas.2406244121. [2] Kotz et al. arXiv 2025 https://arxiv.org/html/2512.10727v1.	
Bhagyashri Mahajan	CMCB/MCBB University of Warwick	Bhagyashri Mahajan (Presenter), Satyajit Mayor	NCBS, India and CMCB/MCBB University of Warwick, UK	The role of class I myosins in plasma membrane organization. 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. 1. Mayor, S., Bhat, A. & Kusumi, A. Cold Spring Harb. Perspect. Biol. 15, (2023). doi:10.1101/cshperspect.a041394. 2. Koester, D. V. et al. Proc National Acad Sci 113, (2016) doi: 10.1073/pnas.1514030113 . 3. Sil, P. et al. bioRxiv 2025.09.30.679578 (2025) doi:10.1101/2025.09.30.679578.	**Poster No. 3**
Erik Schäffer	University of Tübingen	Aleksandr Kostarev[1], Shu Yao Leong[1], Hiroshi Sugimoto[2]	1: University of Tübingen, Germany 2: Kobe University, Japan	Direct measurement of ultra-weak plant kinesin steps using silicon nanospheres as optical tweezers probes. Kinesin motor proteins are essential for both transport along cytoskeletal microtubules and cell division. In plants, cytokinesis relies on phragmoplast orienting kinesins (POKs). The kinesin-15/12 paralog KIN12D/POK2 can generate only about 300 fN of force. This low force is consistent with the idea that POK2 does not transport cargo, similar to weak kinesin-8 motors, the function of which is unknown in plants. To understand the mechanochemistry of these ultra-weak motors, it is necessary to detect their individual steps during ATP hydrolysis. However, the spatiotemporal resolution achieved with conventional probes in optical tweezers is insufficient for detecting fast steps at such low forces. To overcome this limitation, we used high–refractive-index silicon nanospheres for optical trapping. With these nanospheres, we achieved an unprecedented force resolution of 60 aN at room temperature in liquids with a force sensitivity of 2.7 fN $\mathrm{Hz}^{-1/2}$. We bound single POK2 or plant kinesin-8 motors to the nanospheres and were able to detect individual 8-nm steps with 4-nm substeps within a force range of 100-400 fN. These in vitro measurements establish a path toward identifying plant kinesin function based on their mechanochemistry and demonstrate the potential of silicon nanospheres for studying ultra-weak molecular machines.	
Eva Kreysing	University of Warwick, WMS	Eva Kreysing1, 2, 3, 4, Hélène Gautier1, Sudipta Mukherjee1, 2, 3, Katrin A. Mooslehner1, 2, 3, Leila Muresan1, 5, Daniel Haarhoff6, Xiaohui Zhao7, Alexander K. Winkel1, Tina Borić2, 3, Sebastián Vásquez-Sepúlveda2, 3, Niklas Gampl2, 3, Andrea Dimitracopoulos1, Eva K. Pillai1, Robert Humphrey1, 8, Ragnhildur Thóra Káradóttir8, and Kristian Franze1, 2, 3	1 Department of Physiology, Development and Neuroscience, University of Cambridge, UK 2 Institute of Medical Physics and Microtissue Engineering, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany 3 Max-Planck-Zentrum für Physik und Medizin, Germany 4Warwick Medical School, University of Warwick, UK 5 School of Computing and Information Science, Anglia Ruskin University, Cambridge, UK 6 Makespace Cambridge Ltd, Cambridge, UK 7 Department of Medicine, University of Cambridge, UK 8 Cambridge Stem Cell Institute, University of Cambridge, UK	During the development of the nervous system, neurons grow axons and dendrites to connect with other cells. As neurons become integrated into the neural network, they mature and develop electrical activity. While mechanical interactions between neurons and their environment are critical for axon growth and pathfinding, their role in the electrical maturation of neurons—and thus in the formation of circuits in the developing brain—remains poorly understood. Here, we identified environmental mechanics as a key regulator of neuronal maturation and discovered the pathway that links tissue mechanics to this process. Using electrophysiology, immunofluorescence, RNA sequencing, and CRISPR Cas9 knockdown in primary rat neurons, we showed that stiffer environments delay electrical maturation via Piezo1 activity, leading to downregulation of transthyretin and delaying synaptogenesis and electrical maturation. In Xenopus laevis embryos, we found that tissue stiffness was negatively correlated with synapse density, and that stiffening of the brain significantly delays synaptic activity in vivo. Our findings highlight the critical role of mechanical signals in neuronal maturation and suggest that local brain tissue stiffness is a key regulator of circuit formation in the developing brain.	

Farha Naaz	University of Warwick	Farha Naaz*, Alex Fellows#, Richard Wademan# & Anne Straube*	*University of Warwick, # LMB-MRC, Cambridge	Dissecting Directional and Lateral Dynamics in Intracellular Cargo Transport Using MINFLUX. Cellular cargoes, such as organelles and secretory vesicles, recruit molecular motors (kinesin and dynein) for transport along microtubules. Quantitative dissection of these behaviours is essential for understanding how motors coordinate long-range transport within crowded cellular environments. Bidirectional transport is crucial for neuronal function, and defects or mutations in motor-dependent transport have been linked to neurodegenerative diseases. These motors exhibit dynamic behaviours including processive runs, pauses, directional reversals, and potential switching between microtubule tracks or protofilaments. Quantitative resolution of these transitions is critical for understanding how motor coordination regulates cargo distribution. Using MINFLUX, a super high-resolution imaging technique, we investigate dynein and kinesin dynamics in neurons to understand their engagement with microtubules at high spatiotemporal resolution. We utilize inducible neurons expressing endogenously Halo-tagged motors (dynein, kif5a and kif5c) with low level labelling (2–100 pM) of Janelia Fluor Halo dyes to track individual fluorophores. By analysing XY trajectories of processively moving motors over time, we classify runs as diffusive, static, or actively moving based on velocity. To assess directional speed differences, we use EB3-mStayGold (EB3-mSG) as a plus-end microtubule marker, distinguishing actively moving motors from hitchhikers. Molecular motors frequently exhibit sideways stepping and reversals, making time resolution crucial for accurate trajectory analysis. To better capture true reversals and jumps, we applied smoothing techniques to the data. On axis displacements enables robust quantification of run lengths, dwell times, and reversal frequency. Simultaneously, off-axis displacement dynamics were analysed to detect abrupt lateral excursions consistent with microtubule switching or protofilament transitions. The precision we achieved ranged from 0.5–2.5 ms in time and 4–8 nm in the X and Y directions. By resolving fine-scale dynamic transitions within continuous trajectories, this approach advances our understanding of how molecular motors orchestrate regulated intracellular transport.	**Poster No. 4**
Girish Ram Mali	University of Oxford	Muyang Ren, Raman Dhaliwal, Alan Wainman, Kate Heesom, Charlotte Melia, Girish Ram Mali	1: Sir William Dunn School of Pathology, University of Oxford; 2: School of Biochemistry, University of Bristol	Ciliophagy promotes motile cilia recycling under stress. Motile cilia are microtubule-based cell surface extensions that play essential roles in fluid flow and cell signaling. Their fate under cellular stress conditions remains understudied. Here, we show that perturbed calcium signaling, a key modulator of cell stress responses, in the unicellular ciliate Tetrahymena thermophila leads to rapid resorption of intact surface cilia deep into the cytoplasm in ring-like structures. To track the fate of the internalised cilia, we used time-course proteomic analyses which revealed an upregulation of molecular chaperones, the autophagy machinery, and axonemal dynein assembly factors (DNAAFs) concomitant with ciliary regeneration. Transmission and volumetric electron microscopy (TEM and vEM) along with genetic experiments revealed that internalised ciliary fragments are encapsulated within autophago-lysosomes for processing to release free tubulin from ciliary doublets to allow cilia regeneration. We propose that the internalised ciliary rings undergo regulated “ciliophagy” for degradation and recycling to drive regeneration. As this process provides a robust model to track ciliary disassembly, we used ultrastructural expansion microscopy (U-ExM) to investigate the timed erasure of microtubule post-translational modifications which highlighted unexpected differences in the timing of tubulin post-translational modification removal during axoneme disassembly. Our work establishes a link between ciliary turnover and cellular homeostasis in response to stress.	
Glenn Carrington	University of Leeds	Glenn Carrington, Michelle Peckham	Faculty of Biological Sciences, Astbury Centre for Structural Biology and the School of Molecular and Cellular Biology, University of Leeds, Leeds, UK	Myosin under strain: Using Myosin 5 to find the final piece of the mechanochemical puzzle Processive myosin motors must coordinate their two heads to generate forward movement without premature detachment from actin. Myosin-5 is a high-duty ratio motor in which ADP release from the lead head is selectively gated by intramolecular strain, biasing detachment of the trailing head and ensuring processive stepping. Although the biochemical basis of this strain-dependent gating has been established for over two decades, its structural mechanism has remained unresolved. Here, we present high-resolution cryo-EM structures of actin-bound myosin-5 heavy meromyosin captured in a double-head-bound state during processive movement. The reconstruction resolves both motor domains simultaneously, revealing asymmetric conformations consistent with opposing intramolecular strain. While the trailing head adopts a canonical post-powerstroke architecture, the lead head is trapped in a distinct, mid-powerstroke conformation with ADP retained in the nucleotide pocket. Comparative structural analyses identify strain-associated rearrangements within key mechanochemical elements, including the relay helix, converter, and nucleotide-binding pocket. These observations suggest how mechanical distortion of the motor domain can stabilise ADP binding and delay its release under load. Together, the data define a structurally trapped intermediate that links mechanical strain to nucleotide state. By visualising how intramolecular tension modulates the active site, this work addresses a long-standing missing state in the myosin ATPase cycle. More broadly, it provides a framework for understanding strain-dependent mechanochemistry across diverse myosin classes, from unconventional transport motors to cardiac myosin.	

Henry Allmann	University of Bristol	Henry Allman, Liz Moruzzi, Dek Woolfson, Mark Dodding and Jessica Cross	University of Bristol	Controlling Kinesin-3 Activation with De Novo Protein Design. Kinesin motors convert ATP hydrolysis into mechanical work to drive intracellular transport. Members of the kinesin-3 family are regulated through a monomer–dimer equilibrium in which dimerisation activates processive motility. This intrinsic regulatory mechanism provides a powerful opportunity to engineer synthetic control over motor activity. Our goal is to use computational protein design to control kinesin-3 activation in cells by manipulating this equilibrium. Specifically, we aim to engineer de novo coiled-coil modules that can regulate motor dimerisation and therefore activity. Coiled coils provide a highly programmable design framework, with well-defined sequence–to-structure relationships, allowing precise control over oligomerisation. Looking forward, we are exploring the design of stimulus-responsive dimerisation systems that could enable externally controlled activation of kinesin-3. These will respond to triggers such as light, phosphorylation, or chemically induced dimerisation via cell-penetrating peptides or small molecules, providing temporal and spatial control over motor function in living cells.	**Poster No. 5**
Jakub Milczarek	University of Warwick	Jakub Milczarek, Ian Windham, Gregory Alushin, Richard J. McKenney, G. W. Gant Luxton, Kassandra M. Ori-McKenney	University of Warwick, The Rockefeller University, University of California Davis	Cryo-EM structure of nesprin-2G's calponin homology domain and F-actin binding interface. Introduction: The linker of nucleoskeleton and cytoskeleton (LINC) complexes connect the nucleus to cytoskeleton and are involved in mechanical crosstalk across between the organelle and cytoplasm. Nesprin-2 giant (G) is a LINC-associated protein able to bind actin filaments, which was recently shown to both: strongly associate with actin, and to bundle its filaments. No structure of nesprin-2G bound to actin was ever published. Method: Cryo-EM structure reconstruction was conducted in CryoSPARC, following a custom pipeline. Atomic model building and refinement were done with ISOLDE and Phenix, respectively. Result: We obtained a high-resolution model of nesprin-2G calponin homology (CH) domain in association with an actin filament. A detailed analysis and comparison with available structures of other CH domain-actin complexes revealed a deflection of the actin D-loop in our model that is not observed on any other deposited structure. Conclusion: A positive charge patch found on the nesprin-2G CH domain likely repelled actin's lysine 50, thus displacing the loop. No other conformational changes were seen.	**Poster No. 6**
Jingyi Luo	The university of Hong Kong	Jingyi Jingyi Luo, Chen Jing Khoo, Weixin Chen, Zheng liu, Boxuan Li, Wei Sin Lau, Xiang David Li, Shih-Chieh Ti	The University of Hong Kong	Therapeutic agents that target tubulin and disrupt microtubule dynamics have shown established clinical efficacy against diverse hematological malignancies and solid tumors for decades. However, the structure-activity relationship between therapeutic drugs and prognosis-associated variations in tubulin primary sequences remains unknown. Here, we reveal that tubulin protein families (i.e., isotypes) determine the intrinsic affinities for paclitaxel, one of the most successful tubulin-targeting chemotherapeutics. Biophysical characterizations and site-directed mutagenesis identify a point mutation, which is distal from the taxane-binding pocket, can invert the hypo-sensitivity of human β3-tubulin to paclitaxel. High-resolution (~2.3 Å) cryo-electron microscopy microtubule reconstructions reveal the allosteric effects of this single-residue substitution causing conformational changes that remodel the paclitaxel-binding pocket, and alter the lattice interactions for determining the microtubule intrinsic stability. Total internal reflection fluorescence (TIRF) microscopy-based assays demonstrates a decelerated GTP-to-GDP transition at the growing ends of mutant microtubules, evidenced by a prolonged GTP-cap lifetime, and consistent with structural data showing the catalytic αE254 residue adopting a less hydrolysis-competent conformation. Crucially, reverse genetics employing endogenous knock-in of this mutation sensitizes cancer cells to paclitaxel, validating its potent functional impact. Our findings illuminate how specific tubulin mutations sculpt unique conformational landscapes, providing a structural and mechanistic blueprint for understanding differential drug sensitivity. This work uncovers these tubulin allosteric effect can be exploited as druggable sites, paving the way for the strategic design of novel microtubule target agent based therapies to circumvent clinical resistance.	
Marcus Braun	Institute of Biotechnology CAS	Eva Lanska (1), Aniruddha Nagarajan (2), Tereza Humhalova (3), Valerie Siahaan (1), Jochen Krattenmacher (1), Lenka Libusova (3), Carsten Janke (4, 5), Zdenek Lansky (1), Marcus Braun (1,) Sandeep Choubey (2).	1: Institute of Biotechnology of the Czech Academy of Sciences. 2: The Institute of Mathematical Sciences, Chennai, Tamil Nadu, India. 3: Faculty of Science, Charles University, Prague, Czechia. 4: Institut Curie, PSL Research University, CNRS UMR3348, Orsay, France. 5: Université Paris-Saclay, CNRS UMR3348, Orsay, France.	Tau condensation generates a selective microenvironment around microtubules. 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 unknown. Here, we show that tau envelopes are multilayered condensates formed via prewetting-like transition. In this process, tau undergoes a concentration-dependent transition from a thin, cohesive, microtubule surface-adsorbed layer to thick, multilayered condensates. We demonstrate that this process is governed by inhomogenities in the microtubule lattice, such as patterns of tubulin post-translational modifications. We show 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-binding 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, like microtubules, F-actin, or DNA, as viable pathways to generate liquid-like condensates at physiological protein concentrations.	
Marija Zanic	Vanderbilt University	Laura B. Richardson(1), Elizabeth J. Lawrence(2,5), Abinaya Pinjakan(3), Marija Zanic(1,2,4*)	(1) Department of Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, USA; (2) Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, TN, USA; (3) School for Science and Math at Vanderbilt, Nashville, TN, USA; (4) Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA; (5) Current address: Department of Medicine & Health, University of Manchester, Manchester, United Kingdom	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. SSNA1 specifically localizes to sites of damage along the microtubule lattice, thus acting as a microtubule damage sensor. However, the effects 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 effects on microtubule mechanics and self-repair. We apply two distinct sources of force to investigate microtubule mechanics: kinesin-driven gliding assays and microfluidic flow. We find that SSNA1 binding increases microtubule rigidity and resistance to breakage under the physiological and controlled forces in our assays. Interestingly, 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, suggesting an alternative mechanism for restoring microtubule integrity in the absence of tubulin-mediated repair and providing new insights into SSNA1's mechanism of microtubule stabilization.	

Mark Dodding	University of Bristol	Laura O'Regan, Sarah Bristow, Zurine Anton, Anna Bodzeta, Jessica A Cross, Derek N Woolfson, Mark. P Dodding1*	University of Bristol	Kinase-gated coincidence detection controls kinesin-driven lysosome transport Kinesin motors drive long-range intracellular transport through coordinated cargo recognition and conformational autoregulation, yet the mechanisms that selectively control cargo engagement remain unclear. Here, we identify a phosphorylation-dependent gate on kinesin-1 activity mediated by the carboxy-terminal domain (CTD) of kinesin light chain 2 (KLC2). The KLC2 CTD is constitutively phosphorylated on multiple serine residues, suppressing membrane association via its amphipathic helix. We identify the NIMA-related kinase NEK10 as a KLC2-selective regulator of kinesin-1, restraining motor activation, cargo engagement, and lysosome transport; conversely, loss of NEK10 increases membrane association and, together with low-affinity adaptor interactions, promotes lysosome motility. These findings reveal a phosphorylation-regulated coincidence-detection mechanism—a kinesin-kinase code—that integrates adaptor binding and membrane cues to control kinesin-1–mediated transport and provide a mechanistic basis for understanding paralogue and isoform diversity in the kinesin-1 family.	
Masanori Mishima	University of Warwick	Andrejus Suchenko1, Clyde Savio Pinto1, Saskia E. Bakker1, Isabella M. Kolodny2, Hamdi Hussain1, Tomoyuki Hatano1, Karuna Sampath1, Krishna Chinthalapudi2, Sarah M. Heissler2, Mohan Balasubramanian1, Masanori Mishima1	1. University of Warwick; 2.The Ohio State University College of Medicine	Post-translational modifications of weak electrostatic tethering as a conserved strategy for regulating motor–track interactions. Molecular motors convert the chemical energy of ATP hydrolysis into mechanical work to drive diverse cellular processes. While stereospecific interactions between motors and cytoskeletal tracks underpin this chemomechanical cycle, weak electrostatic interactions mediated by flexible tails and surface loops play an essential role in supporting productive motor–track engagement during motility. In microtubule-based systems, post-translational modifications have been shown to regulate motor function in processes such as cargo trafficking (eg. the tubulin C-terminal tail, the "tubulin code") and mitotic spindle organization (eg. phosphorylation of tail and loop elements flanking the catalytic core of the kinesin KIF23/MKLP1). However, whether analogous regulatory mechanisms operate in actin-based motility has remained unclear. We recently discovered that arginylation of the acidic N-terminal tail of actin, which reduces its net negative charge, increases the detachment of actin filaments during myosin-II–driven surface gliding without affecting gliding velocity. Forced actin arginylation in Schizosaccharomyces pombe perturbs actin-based structures, including myosin-II–dependent actomyosin ring constriction. Together, these findings support a conserved strategy in which post-translational modification of weak electrostatic tethering modulates motor–track affinity and engagement without altering the intrinsic stepping kinetics of the motor.	
Matthieu Benoit	Institute Genetic & Development of Rennes	Charlotte Guyomar*, Clément Bousquet*, Siou Ku*, Claire Heichette, Laurence Duchesne, Romain Gibeaux, Matthieu P.M.H. Benoit†, Denis Chrétien† *Co-first authors. †Co-senior authors	Univ. Rennes, CNRS, Inserm, IGDR (Institut de Génétique et Développement de Rennes), UMR6290, ERL U1305, 35000 Rennes, France	Tubulin in the GTP state promotes incorporation of C- and D-type lateral interactions in microtubules. Tubulin polymerizes into microtubules with a delay between assembly and GTP hydrolysis, producing GTP/GDP-Pi caps at growing microtubule ends, which protect the unstable GDP-tubulin lattice from depolymerization. In prevailing models, cap shrinkage or loss would lead to catastrophes, stochastic switches from polymerization to depolymerization, whereas the reverse transition would correspond to rescues. However, both the structure of the GTP cap and the molecular mechanisms underlying these events remain poorly understood. Here, we performed a cryo-electron microscopy characterization of microtubules assembled in the presence of a range of GTP analogues, and found that a large proportion adopt previously uncharacterized types of lateral interactions between two protofilaments, which we termed C- and D-types. These interactions are shifted longitudinally by approximately 2 nm in opposite directions relative to the classical A- and B-types. The two protofilaments involved in these interactions rotate substantially towards each other, and engage distinct and weaker contacts than the A- and B-types. C- and D-types occur at a high frequency at the onset of microtubule self-assembly in the presence of GTP, when large GTP/GDP-Pi caps have been postulated. Altogether, our results suggest that the formation of C- and D-type lateral interactions is intrinsic to the GTP state of tubulin in microtubules, and that, in conjunction with GTP hydrolysis, they could destabilize the microtubule growing tip, leading to stochastic depolymerization events characteristic of their dynamic instability.	
Shivani Yadav	University of Warwick	Shivani Yadav and Anne Straube	Center for Mechanochemical Cell Biology, Warwick Medical School	Mechanical Response of KIF1C to Directional Load Bidirectional cargo transport within cells emerges from coordinated interactions between plus-end directed kinesins and minus-end directed dynein motors. How opposing motors avoid persistent tug-of-war states and instead achieve regulated directional switching remains an open question. Recent work has identified the FHF adaptor complex as a key regulatory scaffold that couples the kinesin-3 motor KIF1C to dynein in a codependent activation mechanism, suggesting that motor coordination may be controlled at the level of complex assembly. In such assemblies, the intrinsic mechanical properties of KIF1C are likely to play a central role in determining force balance and transport directionality. However, the single-molecule response of KIF1C to assisting and opposing loads remains to be understood, and it is unclear how association with adaptors such as FHF may modulate these properties. Here, we used optical trapping force-ramp assays to characterize the directional load response of KIF1C across a range of loading rates. Our preliminary results reveal a catch-bond behaviour of KIF1C under resistive loads. Further, we observe directional asymmetry in the loading-rate dependence of detachment, with resisting loads exhibiting saturation behavior not observed under assisting conditions. These findings suggest that KIF1C exhibits intrinsically directional mechanical responses that may bias outcomes during mechanical competition with dynein. Such asymmetry provides a potential mechanistic framework for understanding how FHF-mediated motor assemblies regulate bidirectional intracellular transport.	**Poster No. 7**

Varun Ramaswamy	Birkbeck College University of London	Varun Ramaswamy (1,2,3), Fabienne Beuron (1), Catarina Felisberto-Rodrigues (1,2,4), Stephen Hearnshaw (1), Craig McAndrew (2), Yann-Vaï Le Bihan (1,2), Edward Morris (2,5), Rob Van Montfort (1,2)	(1) Division of Structural Biology, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, United Kingdom. (2) Division of Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, London SM2 5NG, United Kingdom. (3) Present address: The Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, Malet Street, London WC1E 7HX, United Kingdom. (4) Present address: Discovery Sciences, AstraZeneca, Cambridge CB4 0WG, United Kingdom. (5) Present address: School of Molecular Biosciences, 351 Jarrett Building, Garscube Campus, University of Glasgow, Glasgow G61 1QH, United Kingdom	The kinesin-14 motor HSET (KIFC1) is a mechanical linchpin for the survival of cancer cells exhibiting centrosome amplification. While healthy diploid cells are largely independent of HSET for bipolar spindle assembly, cancer cells with supernumerary centrosomes are prone to lethal multipolar mitosis. To evade mitotic catastrophe, these cells rely on the minus-end directed force generated by HSET to cluster extra centrosomes into a pseudo-bipolar geometry. Despite its significance as a selective therapeutic target, the mechanochemical coupling between HSET's domains that facilitates microtubule cross-linking and sliding remains poorly understood. HSET is a member of the kinesin-14A family, characterised by a C-terminal catalytic motor domain, a long coiled-coil stalk, and an N-terminal microtubule-binding tail. While high-resolution crystal structures exist for the motor domain in its ADP-bound state, the inherent flexibility of the stalk and tail has historically hindered full-length structural determination. Consequently, little is known about the global conformational changes that mediate communication between the three domains of HSET. In this study, we use negative stain EM to present the first full-length 3D reconstructions of HSET at a resolution of 19 Å and investigate the global conformational changes that could govern force transduction along the length of the HSET molecule. To further resolve the mechanochemical state of the motor, we report a 3D reconstruction of ADP-bound HSET at 8 Å using cryo-EM with a Volta Phase Plate. By employing molecular dynamics flexible fitting (MDFF), we have integrated the global architecture from negative stain with the high-resolution local detail of cryo-EM. These data provide new visual insights into the interdomain communication and mechanical mode of action of a human kinesin-14A motor.	
Varun Ramaswamy	Birkbeck College University of London	Varun Ramaswamy (1), Szymon Manka (1), Darsh D. Sharma (2), Anna Akhmanova (3), Ankit Rai (2), Carolyn A. Moores (1)	(1) Institute of Structural and Molecular Biology, Birkbeck, University of London, UK. (2) Gujarat Biotechnology University, Gandhinagar, Gujarat 382355, India . (3) Cell Biology, Neurobiology and Biophysics, Faculty of Science, Utrecht University, The Netherlands	Full-length structures of kinesin-14 HSET (KIFC1) show a glimpse of inter-domain communication. Microtubules are components of the eukaryotic cytoskeleton involved in many cellular activities including cell division and motility. Microtubules are the targets for many small molecules that specifically stabilise these polymers and thereby perturb microtubule dynamics. Because of the ubiquity of microtubules, these small molecules are both experimentally useful and clinically valuable. However, the extent to which binding by these reagents modifies the molecular properties of microtubules – including the number of protofilaments from which microtubules are built - remains an area of active interest. Doublecortin (DCX) is a neuronal microtubule-associated protein that nucleates and stabilises microtubules and plays a central role in neuronal migration. DCX binds microtubules through two conserved DC domains, NDC and CDC, both of which bind to microtubules at the inter-dimer interface between protofilaments. This binding site has been hypothesised to support DCX's nucleation and stabilisation activities, and also renders it particularly sensitive to the number of protofilaments from which the microtubule is built. In vitro, nucleation in the presence of doublecortin typically produces 13-protofilament microtubules, which is the main architecture found in cells. Doublecortin binding has thus been used as a tool to differentiate different microtubule architectures. In this study, we used we first used TIRF microscopy to show that doublecortin binds to microtubules stabilised by the fluorescent taxol derivative, FChitax-3. We then used cryo-electron microscopy to determine the structures of FChitax-3-microtubules. The majority of microtubules in our data are built from 15- and 16-protofilaments. We find that the fluorescent moiety of FChitax-3 inserts itself at microtubule lateral contacts, thereby explaining this molecule's influence on microtubule architecture. DCX binds to these microtubules through NDC and our structure allows us to visualise the adaptation of the DC domain to the smaller inter-protofilament angle found in 15-protofilament microtubules. These data reveal the surprising adaptability of microtubule polymers and their cellular binding partners to modulation by small molecules.	**Poster No. 10**
Vishakha Karnawat	University of Warwick	Vishakha Karnawat, Algirdas Toleikis, Nicholas J. Carter, Justin E. Molloy & Robert A. Cross	University of Warwick	Kinesin-1 microtubule motors are ATP-fuelled, twin-headed cargo transporters that step processively along microtubules, with a load-dependent directional bias. Here we show using single molecule optical trapping that 1 mM ATPγS, a slowly-hydrolysed analogue, substantially defeats the biasing mechanism, whereas 1 μM ATPγS supports it. Our data argue that nucleotide binding puts kinesin into a previously unrecognised Await-Isomerisation (AI) state that is overpopulated by ATPγS and generates slow backsteps. In the working model we propose, exit from this AI state establishes hydrolytic competence and is potentiated by load-dependent neck-linker docking, which steers the tethered head towards its next on-axis binding site. By overpopulating the AI state, ATPγS reveals its pivotal role in the biasing mechanism, whose control logic maximises forward stepping under load in ATP by coupling steered diffusion-to-capture of the leading kinesin head to load-dependent neck linker docking and nucleotide hydrolysis on the trailing head.	**Poster No. 8**
Will Scott & Xuan Liu	University of Warwick	Will Scott, Xuan Liu, Mohan Balasubramanian, Masanori Mishima	Centre for Mechanochemical Cell Biology, Warwick Medical School, University of Warwick	Development of Highly Photostable Red Fluorescent Proteins by Design and Screening. Photobleaching remains a major limitation for live-cell imaging. Inspired by StayGold, an exceptionally photostable green fluorescent protein, we engineered a red fluorescent variant, StayRose, by substituting the conserved tyrosine at the chromocentre with 3-aminotyrosine via genetic code expansion. StayRose exhibits remarkably high photostability. Building on this, we are pursuing two complementary strategies to create equally photostable red fluorescent proteins without genetic code expansion: (1) transferring structural features from StayGold that contribute to photostability into a red fluorescent scaffold, and (2) creating a viability-based assay in bacteria to be used for screening for red mutants of StayGold. This will enable long-term, dual-color imaging at high spatiotemporal resolution, opening new possibilities for studying molecular motors and cytoskeletal filaments.	**Poster No. 9**