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filament with a pitch of ~1.3μm. If the MVC was forced to navigate through the intersection and the separation between actin filaments (<100nm) was less than the vesicle diameter, the MVC initially paused and then switched to the intersecting filament. For this to occur, motors must be free to diffuse on the fluid lipid (DOPC) vesicle surface and engage an actin filament anywhere that the vesicle surface contacts an actin filament. This 3D model system with added complexity will provide an experimental platform to understand how myosin Va motor ensembles maneuver their cargo through the complex actin cytoskeletal network.

124-Plat
Measurements and Simulations of the Fission Yeast Cytokinetic Ring Tension during Constriction
Harvey F. Chin[1,2,3], Erdem Karatekin[2,3], Thomas D. Pollard[4], Ben O'shaughnessy[1].
[1]Chemical Engineering, Columbia University, New York, NY, USA, [2]Cellular and Molecular Physiology, Yale University, New Haven, CT, USA, [3]Nanobiology Institute, Yale University West Campus, West Haven, CT, USA, [4]Molecular Cell and Developmental Biology, Yale University, New Haven, CT, USA.
Cytokinesis in animals and fungi requires the assembly and constriction of an actomyosin contractile ring at the site of cell division. However, the ring tension_the ring's principal mechanical property_has rarely been measured. Consequently, it has not been possible to relate the organization of the ring to its principal function. To address this, we recently developed a method to measure the cytokinetic ring tension in fission yeast for the first time (Stachowiak et al., Dev. Cell, 2014). We used micropipette aspiration on yeast protoplasts (whose cell walls have been enzymatically digested) to measure the membrane tension and imaged the geometry of the membrane furrow induced by the tense ring by fluorescence microscopy in order to deduce the ring tension from a force balance at the furrow. The availability of a method to measure cytokinetic ring tension has opened the door to observing the effect of mutations and drug treatments on ring tension that were previously invisible. Here, we develop the ring tension measurement to track ring tension in real time throughout the full course of constriction, and we measured tension in fission yeast mutants and wild type cells following drug treatments where the activity and dynamics of myosin or actin are compromised. We coordinated these measurements with a mathematical model of the constricting ring to test the mechanism of tension production and constriction. Insights into this mechanism that have resulted highlight the sensitivity to actin filament length and polymerization and the role of myosin activity in maintenance of steady state tension.

125-Plat
A Minimal System to Establish Microtubule-Based Cell Polarity *In Vitro*
Núria Taberner[1], Pierre Recouvreux[2], Sophie Roth[1], Marileen Dogterom[1].
[1]BionanoScience, TU Delft, Delft, Netherlands, [2]IBDM, Marseille, France.
From yeast to fibroblasts, many cell types have a defined polarity that allows them to directionally move, grow or divide. This polarity is typically defined by a polarized distribution of proteins at the cell cortex. We are interested in the emergence of polarity in systems where microtubules are directly involved in its establishment and maintenance by delivering polarity markers to the plasma membrane.
We developed two in vitro systems that allow for microtubule-based delivery of proteins to bio-mimetic cortices. These systems, consisting of elongated microfabricated chambers or emulsion droplets, allow for dynamic microtubules to self-assemble and organize in response to interactions with the chamber or droplet boundaries.
In micro-chambers, single events of microtubule-based delivery to a wall are imaged and quantified. Our experiments show that clustering of proteins at microtubule tips (fission yeast's mal3, kinesin tea2, and tip1) enhances prolonged docking of proteins to the wall receptors as opposed to non-clustering proteins (EB analog protein mal3). Moreover, pre-docked clusters at the wall can capture growing microtubule tips enhancing repeated deliveries at the same spot. These observations are very similar to the observation of clustering of polarity markers in living fission yeast cells.
With elongated emulsion droplets the global emergence of polarity in a closed system can be assessed under conditions where proteins can additionally diffuse within the lipid boundaries. With this minimal system we aim to establish the minimal mechanism by which microtubules can establish and maintain cell polarity in living cells. In parallel, we performed experiments in living yeast cells, which suggest that a simple artificial protein that combines membrane affinity with microtubule tip affinity is in principle enough to establish (but not maintain) polarity.

Platform: RNA Structure, Translation, and Splicing

126-Plat
Balanced Interactions between Ribosomal Subunits Allow Rapid Large-Scale Rotation
Lars V. Bock, Christian Blau, Andrea C. Vaiana, Helmut Grubmüller.
Theoretical and Computational Biophysics, MPI for biophysical chemistry, Goettingen, Germany.
The ribosome consists of two subunits which remain associated through intersubunit bridges during tRNA translocation, despite large-scale rotations of the small 30S relative to the large 50S subunit. Stable ribosomes undergoing rapid rotation require the subunit binding free energy to be sufficiently strong and constant for different rotation angles. Here we investigate how this is achieved considering the large shifts the intersubunit bridges experience in particular at the periphery of the rotational movement. Using molecular dynamics simulations of x-ray structures refined against cryo-EM maps of the ribosome in 13 intermediate states of spontaneous translocation, we study the dynamics and energetics of the intersubunit contact network. To that aim, residues were grouped into clusters based on their observed intersubunit contacts. In addition to the central contact clusters, strong continuous interactions were also found for peripheral clusters. This continuity is realized by changing contact partners in the course of rotation. The most peripheral B1 bridges are stabilized by a changing pattern of contacts between residues of opposite charge that adapt to the rotational state. Continuous contacts of the strong B4 bridge are ensured by the flexibility of H34 helix (50S subunit) which follows the rotational movement and contacts multiple positively charged arginines on the 30S protein S15. The tRNAs which span the two subunits add to an almost constant degree to the intersubunit binding enthalpy, despite their very different positions in the ribosome. These mechanisms keep the strength of intersubunit interaction similar for different rotation angles allowing rapid rotation.

127-Plat
Real-Time Observation of DNA Recognition by the RNA-Guided Endonuclease Cas9 using Single-Molecule FRET
Digvijay Singh[1], Samuel H. Sternberg[2], Jingyi Fei[3], Jennifer A. Doudna[2], Taekjip Ha[3].
[1]Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Department of Chemistry, University of California, Berkeley, Berkeley, CA, USA, [3]Center of the Physics of Living Cells, Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA.
The RNA-guided endonuclease Cas9 targets foreign DNA for degradation as part of an adaptive immune system in bacteria mediated by clustered regularly interspaced short palindromic repeats (CRISPR). Due in part to the ease of programmability, Cas9 is now being widely used in various organisms for site-specific genomic editing, genome-wide knockout screens, and transcriptional activation and repression. This system brings together three major classes of biopolymers (DNA, RNA and protein), but the specificity of different components for each other remains poorly understood. Perhaps most important, Cas9 has been shown to bind and cleave genomic DNA sequences with varying degrees of mismatches to the guide RNA, leading to potentially deleterious off-target effects. Previous methods to investigate the tolerability of mismatches have largely employed experimental methods that use Cas9-induced DNA cleavage as a readout of specificity and not direct binding. Recent ChIP-seq experiments suggest that off-target binding occurs at sites with complementarity in just a short 5-bp seed region, but the use of crosslinking obscures kinetic information and can potentially introduce artifacts. Here, we have used single-molecule FRET together with bulk biochemical assays to directly observe interactions between Cas9-RNA and DNA targets of varying sequence complementarity in real time. Using multiple distinct labeling geometries that report on both initial association and subsequent DNA unwinding, we develop a kinetic framework for the RNA-guided DNA interrogation process and provide insight into conformational changes that are required for target cleavage. Our findings resolve outstanding questions on the mechanism of DNA recognition by Cas9 and will facilitate improvements in genome engineering applications.