autophosphorylation by identifying co-evolving interdomain amino acid pairs in agreement with biochemical mutagenesis data [3]. We can now simulate the conformational transition between active and inactive conformations, quantify its free-energy barrier and its change as reaction to transmembrane forces exercised by the sensor domain. (unpublished data)

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1091-Plat
MD Simulations of KirBac1.1 Mutants Reveal Gating Changes at the Bundle Crossing Region
Anna Weinzinger.
University of Vienna, Vienna, Austria.
Inwardly rectifying potassium (Kir) channels play important physiological roles in a variety of cells including heart rate, insulin secretion or fluid balance. Their importance is further illustrated by the fact that inherited mutations are linked to diseases including Andersen Tawil Syndrome, Bartter syndrome or neonatal diabetes. Their activity is controlled by dynamical conformational changes that regulate ion flow through the central pore of Kir channels. Understanding the dynamical rearrangements of Kir channels during activation gating requires not only high-resolution structure information from channels crystallized in different conformations but insight into the transition steps. Guided by mutations that are known to increase channel activity (1), molecular dynamics simulations of the WT KirBac1.1 crystal structure and the G143E mutant have been performed. Full atomistic MD simulations revealed that introducing a glutamate in position 143 causes significant widening of the pore. In all our simulations the bundle crossing opened to 17 Å compared to the WT. Comparison of the mutant KirBac1.1 with the open structure of KirBac3.1 reveals that the pore radius reaches identical values. Furthermore the global rearrangements including a rotation and a bending of the lower part of TM2 are identical in both structures, suggesting that the final structure of the G143E mutant after 60 ns represents an open conformation. Simulations further revealed that deprotonation is essential for channel opening. This is further supported by investigations with non-charged amino acids in this position, which do not lead to channel opening on the nanosecond time scale.
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1092-Plat
Nanosecond-Timescale Dynamics of the Viral RNA-Dependent RNA Polymerase as a Determinant of Incorporation Fidelity
Ibrahim M. Moustafa[1], Victoria S. Korneeva[1], Jamie J. Arnold[1], Eric D. Smidansky[1], Laura L. Marcotte[2], Xiaorong Yang[1], Maria A. Sanchez Farran[1], Janna K. Maranas[1], David D. Boehr[1], James M. Hogle[2], Coray M. Colina[1], Craig E. Cameron[1].
[1]The Pennsylvania State University, State College, PA, USA, [2]Harvard Medical School, Boston, MA, USA.
The viral RNA-dependent RNA polymerase (RdRp) is required for replication of the genomes of RNA viruses. While many organisms may have evolved to make few mistakes during genome replication, RNA viruses appear to need genetic diversity for maximal fitness. This genetic diversity is created by the nucleotide misincorporation frequency of the RdRp. Perturbations in RdRp error rate therefore exhibit an antiviral effect. We have discovered a mutant poliovirus with a mutator phenotype caused by the change of His-273 of its RdRp to Arg. Kinetic experiments reveal an increase in the equilibrium constant for a conformational-change step that has been shown to be a major checkpoint for RdRp fidelity. The crystal structure of this derivative was unable to explain the biochemical observations. However, all-atom molecular dynamics (MD) simulations on the nanosecond timescale showed altered dynamics of the H273R derivative relative to the WT enzyme. By analyzing the conformational space sampled by the dihedrals of RdRp residues, we have identified RdRp residues whose dynamics correlate directly with fidelity. These residues lead to enhanced conformational flexibility of the active site of the low-fidelity H273R enzyme and diminished flexibility of the high-fidelity G64S enzyme studied previously. In general, the findings from MD simulations are supported by solution-state NMR experiments. Collectively, these experiments provide additional support for the existence of a network of RdRp residues whose dynamics control conformational changes at the active site required for incorporation fidelity. We suggest that small molecules which interfere with network dynamics should exhibit antiviral activity.

Platform: Cell Mechanics & Motility I

1093-Plat
Self-Propelled Particle Motion of Cells in Tissues
Sriram Manivannan, Jason P. Gleghorn, Jacquelyn M. Nestor, Yannis G. Kevrekidis, Celeste M. Nelson.
Princeton University, Princeton, NJ, USA.
Collective migration of cells has been identified in a number of biological processes including organ morphogenesis, tumor invasion, and wound healing. The mechanisms underlying initiation and maintenance of a collectively moving population of cells in the absence of external stimuli are unclear. Here we show that epithelial cells confined within monolayers can spontaneously organize and undergo rotational collective motion. We created artificial tissues using microlithography-based techniques and, in combination with time-lapse imaging and pseudo-automated cell tracking algorithms, we visualized the movements of cells within these tissues. We found that the coherence of group rotation is affected by the size and shape of the tissue. In addition, we found that disturbances within tissues, such as those resulting from cytokinesis, are able to switch the direction of rotation of cells. These collective motions emerged even in tissues that were treated with pharmacological agents to disrupt cell-cell connections. Using analytical agent based computer simulations, we found that cells within these tissues behave not as pure random walk particles, but instead as Vicsek-Czirok type self propelled, interacting particles; bearing some analogy to coherent motion of birds within a flock or fish within a school. These results indicate that basic principles of collective motion that govern animal behavior may be relevant in cellular motion. An improved understanding of these underlying principles will benefit future studies of collective cell migration.

1094-Plat
Cellular Membrane Tether (Nanotube) Retraction, Mobility, and Coalescence
Jin-Yu Shao, Baoyu Liu.
Washington University in St. Louis, Saint Louis, MO, USA.
During leukocyte rolling on the endothelium, membrane tethers (nanotubes)are extracted simultaneously from both leukocytes and endothelial cells because of the force imposed by the blood flow. Tether extraction has been shown to stabilize leukocyte rolling by increasing the lifetime of the adhesive selectin-ligand bonds that mediate leukocyte rolling. Over the past two decades, tether extraction has been studied extensively, both experimentally and theoretically. In contrast, much less is known about tether retraction. Tether retraction may occur in several occasions. For example, upon the breakage of the selectin-ligand bonds during leukocyte rolling, extracted tethers may retract back to the cells. In addition, during simultaneous tether extraction (two tethers, one from a leukocyte and the other from an endothelial cell, linked in series by receptor-ligand bonds), one tether will retract when the pulling force falls below the larger threshold force. In this work, with the micro-cantilever technique where latex beads affixed on silicon cantilevers were used as the force transducer, we extracted tethers either perpendicular or tangential to the neutrophil surface. Little movement of the tether-cell junction was observed during tangential tether extraction and no coalescence was observed during multiple tether extraction. Following adhesion rupture, spontaneous tether retraction was visualized by membrane staining, which revealed two phases: one was fast and exponential, whereas the other was slow and linear. Both phases can be reproduced with a mechanical model, showing that the first phase was dominated by elastic deformation recovery and the latter slow phase was driven by the far-field membrane tension on the cell body and the membrane-cytoskeleton adhesion. These results show for the first time how neutrophil tethers shorten upon instantaneous force removal and illustrate further how membrane tethers contribute to neutrophil rolling stability during the inflammatory response.

1095-Plat
Three-Dimensional Dynamics of a Eukaryotic Flagellum Revealed by High-Speed Holographic Microscopy
Laurence G. Wilson[1], Lucy M. Carter[2], Wilson C.K. Poon[2], Sarah E. Reece[2].
[1]The Rowland Institute at Harvard, Cambridge, MA, USA, [2]The University of Edinburgh, Edinburgh, United Kingdom.
Understanding the mechanics of the eukaryotic flagellum is a key challenge in biophysics. As well as being of scientific interest, there are clear therapeutic applications, not least in reproductive medicine. The physics of swimming sperm