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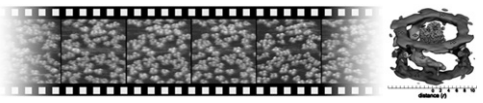
Membrane-mediated protein-protein and protein-lipid interactions, membrane protein localization, and related dynamics, modulate membrane protein function. So far membrane structure and dynamics could not be studied altogether lacking the technique that analyzes unlabelled proteins at submolecular lateral and high temporal resolution. Here we used high-speed atomic force microscopy (HS-AFM) to characterize the lateral and angular movements and interactions of unlabelled OmpF. OmpF distribution emerges from diffusion-limited aggregation, maximizing surface coverage and providing a multitude of local environments and contacts. Protein motion scales roughly with membrane crowding. However molecules display individuality of diffusion behavior ranging from fast moving (~400nm2/s) to immobile molecules trapped by favorable protein-protein associations. We derive the complete molecular interaction probability landscape that we compare with coarse-grained molecular dynamics simulation of the membrane protein interaction. HS-AFM may open a novel research avenue that bridges structure of individual membrane proteins and supramolecular membrane architecture.

HS-AFM movie frames (frame rate 477 ms) showing the motion of OmpF trimers in the membrane. Right: Experimental interaction potential map



2103-Plat

Probing Dielectric and Hydrogen Bonding Gradients in Biological Membranes

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Nitroxide spin-labeling in combination with EPR spectroscopy has found many applications in studying structure and dynamics of proteins and biological membranes. Recently, there has been a substantial interest in utilizing EPR to characterize local effects of polarity and hydrogen bonding in proteins and biological membrane systems. Here we report on employing an arsenal of advanced spin-labeling EPR methods to profile heterogeneous dielectric and hydrogen bonding environment along the α-helical chain of an alanine-rich WALP peptide that is anchored in a lipid bilayer in a transmembrane orientation. A series of WALP cysteine mutants was labeled with a pH-sensitive nitroxide IMSTL (S-(1-oxyl-2,2,3,5,5-pentamethylimidazolidin-4-ylmethyl) ester) that is similar in molecular volume to phenylalanine. The protonation state of this nitroxide could be directly observed by EPR allowing us to follow proton gradient across the membrane in the vicinity of the WALP α-helix, and, thus, to reconstruct the gradient in the effective dielectric constant. These experiments were complemented by assessing local polarity from characteristic changes in EPR spectra that were enhanced by the use of perdeuterated and ^{15}N-substituted nitroxides and high field EPR at 130 GHz (D-band). Formation of hydrogen bonds between the nitroxides and membrane-penetrating water molecules was observed directly in HYSCORE X-band experiments. Such measurements allowed us to derive experimental profiles of heterogeneous dielectric and hydrogen bonding environment along a typical transmembrane α-helix. Supported by: NSF-0843632 to TIS and NIH 1R01GM072897 to AIS.

2104-Plat

Pulmonary Surfactant Reduces Surface Tension to Low Values near Zero through a Modified Squeeze-Out Mechanism

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Pulmonary surfactant stabilizes the lung by reducing surface tension (ST) to low values near zero at end expiration. Atomic force microscopy studies revealed that, as surface pressure increases, spread surfactant films initially form solid micro- and nanodomains and then generate 3-5 multilayers which apparently remain associated with the surface monolayer. Time-of-flight Secondary Ion Mass Spectroscopy analyses revealed selective squeeze-out must occur because the multilayers are enriched in unsaturated fluid phospholipids (PL) while the remaining monolayer becomes enriched in disaturated PL. Taken together, these results are consistent with a modified squeeze-out model where surfactant vesicles interact with the air-water interface through surfactant proteins B- and C-containing adsorption/fusion pores. PL migration onto the surface results in vesicle instability generating a monolayer at equilibrium surface pressure, which remains functionally associated with excess bilayer material. Initially, the monolayer and associated reservoir have similar composition, but film compression causes fluid PL to migrate through the fusion pores into the multilayers. Gel phase PL are restricted because they are sequestered in micro- or nanodomains. This process results in monolayers highly enriched in disaturated PL which reduce ST to near zero. Film expansion allows fluid PL to regain the surface through the fusion pores. This mechanism explains both the rapid reincorporation of surfactant PL into the monolayer during adsorption and during film expansion and the progressive improvement in surface activity during repeated compression.

Platform: Cell & Bacterial Mechanics & Motility II

2105-Plat

Flexural Rigidity and Shear Stiffness of Flagella

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Cilia are thin subcellular organelles that line airways and other passages and bend actively to propel fluid and foreign materials. The ciliary cytoskeleton (the axoneme) consists of nine outer microtubule doublets surrounding a central pair of singlet microtubules. Large bending deformations of the axoneme involve relative sliding of the outer doublets driven by the motor protein dyneins. The genetics and cell biology of the ciliary structure and function have been studied extensively, but the mechanics of the axoneme remain unclear. In this study, we used the unicellular alga *Chlamydomonas reinhardtii* as the model system for their flagellum replicates the highly conserved molecular structure of the ciliary axoneme. Piconewton forces were applied perpendicularly on the tip of a single flagellum (length L) through a microsphere trapped in optical tweezers. Dividing the force (P) by the corresponding deflection of the flagellar tip (δ) yields the flexural stiffness of the flagellum ($K = P/\delta$), which was then used to calculate the apparent flexural rigidity ($EI = KL^3/3$). The contributions of major structural components to passive mechanical properties were quantified by testing on flagella of specific mutations. The average apparent flexural rigidity of wild-type, pf-3 (without nexin links), and pf-13 flagella (without outer dynein arms) was about 2700 ± 1100, 1300 ± 550, and 650 ± 140 pN·μm^2, respectively. In addition, the ratio of elastic shear stiffness (resistance to interdoublet sliding) to true flexural rigidity was estimated by the counterbend response in bent flagella manipulated with a glass microneedle. The quantitative understanding of axonemal mechanics will help illuminate the roles of certain genes and molecular structures in the normal and abnormal axoneme.

2106-Plat

Structure of Trypanosoma Brucei Flagellum Accounts for its Bihelical Motion

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Trypanosoma brucei is a parasitic protozoan that causes African sleeping sickness. It contains a flagellum required for locomotion and viability. In addition to a microtubular axoneme, the flagellum contains a crystalline paraflagellar rod (PFR) and connecting proteins. We show here, by cryoelectron tomography, the structure of the flagellum in three bending states. The PFR lattice in straight flagella repeats every 56 nm along the length of the axoneme, matching the spacing of the connecting proteins. During flagellar bending, the PFR crystallographic unit cell lengths remain constant while the interaxial angles vary, similar to a jackscrew. The axoneme drives the expansion and compression of the PFR lattice. We propose that the PFR modifies the in-plane axoneme motion to produce the characteristic trypanosome bihelical motility as captured by high-speed light microscope videography.

2107-Plat

Hydrodynamics of the Double-Wave Structure of Insect Spermatozoa

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