their tail domains under a total internal reflection fluorescence microscope. Although dynein-f occasionally showed one-dimensional diffusion, imaging of the quantum dot with nanometer precision indicated processive stepping behavior of individual dynein-f molecules mainly with 8 nm step and without large steps (>24nm). In an axoneme, this slow and processive dynein-f could impede microtubule sliding driven by other faster dyneins. To obtain further insights into the *in vivo* roles of dynein-f, we measured the sliding velocity of microtubules driven by a mixture of dyneins -c and -f at various mixing ratios. The velocity was modulated as a function of the ratio of dynein-c in the mixture. This modulation suggests that dynein-f acts as a load in the axoneme, but forces pushing dynein-f molecules forward seem to accelerate their dissociation from microtubules.

Platform AQ: Molecular Mechanics & Force Spectroscopy

1825-Plat Probing Equilibrium Binding Energies With Chemical Force Microscopy

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Force spectroscopy experiments reveal a wealth of kinetic behaviors exhibited by individual intermolecular bonds under external loading. While the conventional description of force spectroscopy has focused on probing kinetics the bond rupture under non-equilibrium fast loading conditions, using bond rupture measurements as a way to probe equilibrium potential energy surfaces remains virtually unexplored. We use an analytical approach and numerical simulations to show that measuring rupture forces as a function of the probe stiffness in the near-equilibrium regime could provide an estimate of the free energy of bond dissociation. We have tested this prediction in the experiment using chemical force microscopy measurements of the interactions between a small number of well-defined functional groups, and show that these measurements can estimate the free energy of an individual hydrogen bond. In another application we have used chemical force microscopy to measure the strength of the interactions of single chemical functional groups with the sidewalls of vapor-grown individual single-wall carbon nanotubes. We show that it is possible to combine the results of ab initio calculations and the rigorous kinetic description of the force spectroscopy experiment to predict binding force distributions for a single molecular contact that match the experimental results. Interestingly, our analysis reveals the important role of molecular linkage dynamics in determining interaction strength at the single functional group level.

1826-Plat Effects of Multiple Bonds Rupture in Force Spectroscopy Measurements of Interactions between Biotin and Streptavidin

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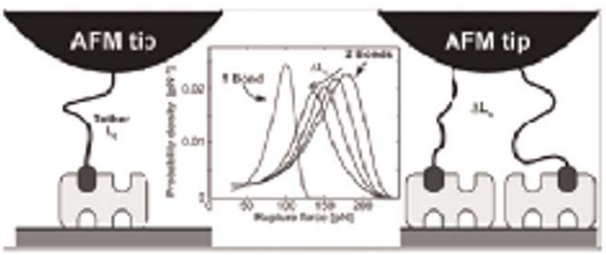
Force spectroscopy is becoming a widespread experimental tool in biophysical research. Often it has been used to uncover molecular details of protein folding and receptor-ligand interactions. Kinetic parameters that include dissociation rate and distance to the activation barrier are commonly extracted from experiments. Accuracy of these parameters depends on the single-molecular nature of the measured rupture forces. In the atomic force microscopy (AFM) studies of receptor-ligand pairs it is often difficult to ensure that the measured rupture forces correspond to interactions between single molecules. Moreover, the distribution of rupture forces in AFM measurements frequently does not follow the distribution predicted based on the single-molecule models and complicated kinetic theories have been proposed to explain this discrepancy. Here we propose that the deviation from the single-molecule distribution of rupture forces comes from occasional ruptures of double-bonds that occur during one rupture event. An analytical theory that takes into account difference between lengths of the tethers that attach ligands to AFM probes is developed and applied to successfully explain the distribution of the measured rupture forces between biotin and streptavidin molecules.



1827-Plat Direct Detection of Double Stranded poly(A) Conformations by Single Molecule Atomic Force Spectroscopy

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Eukaryotic mRNA is modified at its 3′ end by a stretch of ~200 adenines. Because of the biological significance of this adenine tail, the structure of polyadenylic acid, (polyA) has been extensively studied. In bulk measurements poly(A) was found to form single-stranded helices under neutral or alkaline pH, but double-stranded (ds) helices under acidic pH. To the best of our knowledge such ds-poly(A) structures have never been examined at a single-molecule

level, which is of biological relevance. Here, we use AFM to investigate the mechanical stability and molecular elasticity of poly(A) under acidic pH conditions. We find that force-extension curves of poly(A) in citrate buffer (pH~5.5, 150 mM NaCl) display a plateau feature at 54±2 pN that is very similar to the B-S overstretching transition of native double stranded DNA. Thus, this mechanical fingerprint is indicative of double-stranded poly(A) structures formed under acidic pH, albeit these structures are mechanically weaker as compared to native dsDNA, for which the overstretching transition occurs at a higher force of 65 pN. In some AFM recordings, the force abruptly dropped to zero during the plateau transition indicating that the two strands separated during the measurement. This observation further supports our conjecture that the mechanical stability of ds-poly(A) with a non-canonical base pairing is lesser than that of native dsDNA with the Watson-Creek type of base pairing.

This study is supported by NIH and NSF.

1828-Plat Nanoscale Mechanics and Assembly of Hierarchal Forms of Collagen Fibrils

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Connective tissues and extracellular matrix predominantly consist of a support framework of fibrous biopolymers formed from members of the collagen superfamily of proteins, various elastic fibrils, and proteoglycans. Together these molecules provide a scaffold for cellular housing and tissue support. The primary structural proteins are the fibril forming collagens, which lend rigidity to the matrix. Efforts to characterize the mechanobiology of these systems have been impeded by the fact that, while both the macroscopic mechanics of the composite system and the nanoscopic mechanics of the collagen molecule have been studied extensively, the native mechanical properties of the fundamental fibril from which the composite system is built remain largely unknown. It is precisely the native form of collagen that needs to be understood for rational design of biomimetic systems for tendon or corneal replacements or for development of scaffolds for use in engineered soft tissue constructs.

We are able to extract collagen fibrils from *Cucumaria Frondosa*, a species of echinoderm whose dermis exhibits a neurally controllable rigidity. This source of collagen allows for the study of the mechanics of individual native, intact fibrils. We have used atomic force microscopy to image and manipulate these fibrils and the proteoglycans of extracellular matrix. Through the use of nanoscale indentation and three-point bending, we have demonstrated that the elastic modulus of the collagen fibril is anisotropic, having an axial modulus that is ~150 fold stiffer than the radial. The presence of the fibril associated proteoglycans in the system under study has the added benefit that the interactions of these molecules can be investigated. We have made use of these interactions to induce the assembly of orthogonally stacked sheets of aligned collagen fibrils - an artificial corneal stroma.

1829-Plat Molecular Basis of Fibrin Clot Elasticity

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Blood clots must be mechanically stable to stop hemorrhage, yet elastic enough to buffer blood's shear forces. Upsetting this balance can result in clot rupture, leading to life-threatening thromboembolic disease including stroke, pulmonary embolism and heart attacks. With the global rise in obesity (a condition associated with cardiovascular disease) and atrial fibrillation (a disorder of heart rhythm characterized by irregularity) in world populations, cardiothrom-boembolic disease has reached epidemic proportions, creating an urgent need to understand and control the mechanisms that govern blood clot elasticity. Fibrin, the main component of a haemostatic plug, or blood clot, is formed from molecules of fibrinogen activated by thrombin. Although it is well known that fibrin possesses considerable elasticity, the molecular basis of this elasticity is unknown. Here we use atomic force microscopy (AFM) to probe the mechanical properties of single molecules of fibrinogen and fibrin protofibrils. The results show that the mechanical unfolding of the coiled α-helices (commonly known as 'coiled-coils') of single fibrinogen molecules and also fibrin protofibrils is characterized by a distinctive force plateau (an intermediate transition state which has been related to elasticity) in the force-extension curve. Steered molecular dynamics (SMD) simulations of single fibrinogen molecule stretching agree closely with the AFM results and relate the plateau force to a detailed stepwise unfolding of fibrinogen's coiled α-helices and also its central domain. AFM data also show that varying pH and calcium ion concentrations alters the mechanical resilience of fibrinogen. Employing a combination of experimental and computational techniques we have uncovered the first direct evidence that the coiled α-helices of fibrinogen are a major source of fibrin elasticity.

1830-Plat Mapping Mouse Gametes Interaction Forces Reveals Several Oocyte Membrane Regions with Different Mechanical and Adhesive Properties

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We studied the interaction involved in the adhesion of mouse gametes and on the mechanical properties of the oocyte membrane. The oocyte has an asymmetric shape and its membrane is composed of two distinct areas. One is rich in microvilli, the other is smoother and without microvilli. With a Biomembrane Force Probe (BFP) adapted to cell-cell measurements, we have quantified the separation forces between a spermatozoon and an oocyte. Microvillar and amicrovillar areas of the oocyte surface have systematically been probed and compared. In addition to a substantial difference in the

elastic stiffness of these two regions, the experiments have revealed the presence of two types of membrane domains, with different mechanical and adhesive properties, both distributed all over the oocyte surface, i.e. in both microvillar and amicrovillar regions. If the gamete contact occurs on the first type of domains, the oocyte membrane deforms only elastically under traction. The pulloff forces in these domains are higher in the amicrovillar region. For a contact of the spermatozoon on the other type of domains, there can be a transition from elastic to viscoelastic regime and then tethers are extruded from the oocyte membrane.

1831-Plat Nonlinear Response Probed by Laser-Trapping Microrheology

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Microrheology can be used to probe the viscoelastic properties of materials on micrometer scales and is thus applicable in cells. Cytoskeletal polymers are in general semiflexible and have strong non-linear elastic responses. Nonlinear response can not be probed by "passive" microrheology which relies on thermal fluctuations. We have here developed a high-bandwidth technique for active 2-particle microrheology with which we can probe linear and nonlinear responses of soft materials. Micron-sized colloidal probe particles are driven by an oscillating optical trap, and the resulting correlated motions of neighboring particles are detected by laser interferometry. Lock-in detection at the driving frequency and at its second harmonic makes it possible to measure the linear and the non-linear response of the embedding medium at the same time. We demonstrate the sensitivity of the method by detecting a second-harmonic response in water which is of purely geometric origin and which can be fully understood within linear hydrodynamics.

1832-Plat Monitoring the Drug-Induced Rheological Response of a Live Cell through Bio-Microrheology

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Bio-microrheology, the study of deformation and flow of biological material at the microscale, is applicable to a range of biomaterials from biopolymers to live cells. We use bio-microrheology techniques, primarily live-cell particle tracking, to monitor the time-dependent rheological response of various adherent cell lines to specific drugs. In addition to developing appropriate experimental protocols and data analysis methods, we seek a deeper understanding of the link between biochemical drug pathways and cell rheology.

Symposium 17: From Protein Crystals to Amyloid Fibrils: Condensed Colloidal Phases in Biology (APS/BPS)

1833-Symp Phase Transitions in Protein Solutions

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Globular proteins in aqueous solutions show transitions between dilute, concentrated and crystalline phases, similar to the transitions between the gas, liquid and solid phases of atoms and small molecules. However, the transitions in protein solutions also show many distinct features. Most notably, coexisting dilute and concentrated liquid phases in protein solutions are metastable with respect to crystallization. Furthermore, phenomena such as amorphous aggregation or regular self-assembly, which are common in protein solutions, rarely are exhibited by small molecules. We argue that all these unique features of protein phase behavior stem from the short-range and highly anisotropic nature of the protein interactions and formulate a simple model that provides a correct description of phase diagram of globular protein solutions.

We present experimental results for liquid-liquid phase separation, crystallization and aggregation of several gamma crystallins, globular proteins from eye lens involved in cataract disease. In particular, we compare the phase diagrams of several mutant human gamma-D crystallins that differ by a single point mutation. Remarkably, the solubility of these mutants decreases with increasing temperature in sharp contrast to the behavior of the native protein. At the same time liquid-liquid coexistence curve remains unaffected by mutations. We show how this apparent discrepancy is easily explained in a framework of our "aeolotopic" model which takes into account the short range and anisotropy of the protein interaction. We argue that this type of models could provide adequate theoretical basis for the analysis of protein crystallization and other protein condensation phenomena.

1834-Symp Morphology of Dense Colloidal Phases

David Weitz

Harvard University, Cambridge, MA, USA.

Colloidal particles exhibit some behavior that is similar to that of proteins in terms of their phase and crystallization behavior. While colloids are not completely like proteins, they can serve as a model with which to understand some of the underlying phase behavior. This talk will review the phase behavior of colloids, both crystals and gels. This can serve as a benchmark to which the much more complex behavior of proteins can be compared.

1835-Symp Coupled Computer Simulations and Experiments of Relevance to Alzheimer Disease

H. Eugene Stanley

Boston University, Boston, MA, USA.