significant variations in their dielectric characterization in the radiofrequency range. Measurements can be made using a probe and an automatic network analyzer. This work proposes the use of dielectric data for glucose level monitoring. The results will enhance the development of instrumentation and methodology for effective glucose monitoring.

1704-Pos Light-controlled Manipulation Of Membrane Potential In Cells Using Qdot® Nanocrystals

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Board B680

Long-term, real-time monitoring of cellular activity is crucial for cell biology. Because of their noninvasive nature, optical approaches are best suited for this task. However, the ability to activate cells by changing their membrane potential without pharmacological or chemical intervention is a huge challenge for optical cell-based assays.

To address this problem, we are developing a light-controlled stimulation platform with semiconductor nanoparticles (quantum dots or Qdot® nanocrystals) utilizing their unique physical and photochemical properties. This platform is based on a photovoltaic mechanism generating free charge carriers (e.g. electrons) in nanoparticles upon light illumination. When photo-excited nanoparticles are placed in close proximity to a cell, free charge carriers create the cumulative electromagnetic field, thus modulating the cell membrane potential.

In our experiments, cells were cultured on top of multilayers of nanoparticles deposited on a glass substrate. Nanoparticles did not produce any adverse effects on cellular morphology and physiological responses. We were able to repeatedly depolarize the membrane in non-excitable cells and generate a light-triggered action potential in excitable cells upon light illumination.

Since the absorption spectrum of semiconductor nanoparticles is broad, they can be excited by any wavelength shorter than their emission maxima, thus making them compatible with various optical recording methods. We have designed an integrated optical assay that combines optical stimulation (via a nanoparticle-coated substrate) and optical recording (via Fluo-4). We performed these experiments on both hippocampal neurons and NG108 cells and demonstrated the increase of intracellular calcium concentration as a result of light-controlled depolarization.

In summary, in contrast to other methods of cellular stimulation, our nanoparticle-based optical activation platform allows to stimulate cells physiologically and repeatedly in a manner compatible with optical methods of registration thus providing a complete solution for cell-based studies.

1704.1-Pos Long Term Culturing Of Primary Adult Human Liver Cells Reveals An Existence Of A New Type Of Stem Cell In The Adult Human Liver

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Board B681

Growth patterns and interactions of adult primary human liver cells (PHLC) in long term culture condition have never been investigated, since there was no suitable culture medium to do such experiments. After preparing an original special medium for this purpose, growth patterns, interactions and protein and gene expression in PHLC had been observed in 4 months. The results of this experiment show that Epithelial to Mesenchymal Transition (EMT) in Hepatocytes occurred in a small amount. But there were mesenchymal type of cells, which are negative to CD34, CD45, CD14 and Albumin and had enormous ability to multiply under growth factors and cytokines. These cells go to quiescent condition and organize Embryonic Body (EB) like Liver Body (LB) during serum deficiency and again multiply in increased serum content of culture medium. They also answer to Embryonic signal peptides BMP-2,-4 stimulation by expressing Hepatocyte specific genes (Albumin, Transthyretin, alfa-1-Antitrypsin). A part of these cells positively immunostain to human Albumin in cell culture and in "Liver condition" in SCID mice liver. All these results bring to suggestion an existence of embryonic Mesoendodermal origin of stem cells in the adult human liver, which under special circumstances could differentiate to Hepatocytes and other tissue specific cells of Mesoendodermal origin. Excellent growth of these new Stem Cells in the special cell culture medium makes it possible to use these cells for bioengineering of tissues in vitro condition.

Molecular Mechanics & Force Spectroscopy

1705-Pos A Holographic Optical Tweezers Setup For Force Measurements On Biomaterials

Astrid van der Horst, Nancy R. Forde

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Board B682

In recent years, optical tweezers have proven their suitability in the field of single-molecule experiments. Micrometer-sized particles can be trapped and used as handles to manipulate the even smaller molecules, and the picoNewton forces that can be exerted and measured with this non-invasive technique lie in the force range of many biomolecular properties and events, including for example the mechanical forces exerted by molecular motors. When probing more complex systems, however, such as red blood cells or protein networks, the 3D character of such materials calls for more flexibility in manipulating trapped particles. With holographic optical

tweezers, multiple optical traps can be independently manipulated in three dimensions, adding the necessary flexibility to the interactive control over multiple trapped particles.

Here, we present a setup where the versatility of holographic tweezers is combined with high-speed (>kHz) camera imaging, to perform quantitative force measurements on biomaterials. The force calibration of holographic tweezers is less straightforward than for single-beam laser tweezers. Our initial experiments therefore include investigating the position-dependence of the trap stiffness for a single holographic trap. In addition, the stiffness dependence of a trap on the position of other traps is investigated. After full characterization of the trap stiffness, our setup can be used to probe the elastomeric properties of structural protein networks.

1706-Pos In Situ Force Calibration and Applications of a High-Bandwidth Magnetic Manipulator

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Board B683

Existing methods for calibrating magnetic manipulators rely on the use of Stokes' Law to convert observations on the drive current and the position and motion of magnetic particles into forces. A good description of the range of forces available and the general behavior of the system can be obtained by this technique, but significant error can be introduced by variations in bead size, shape, and content as well as magnetic field and field gradient. We present a force calibration method that is bead and location specific, eliminating the above sources of error. The Three Dimensional Force Microscope (3DFM) is capable of applying forces at bandwidths above 3 kHz. Driving a bead with a wide-bandwidth white noise force such as the 3DFM can produce has the same effect on the PSD of the bead's motion as an increase in thermal force. We demonstrate how a comparison of the undriven and white-noise-driven motion of a bead in a measured potential can be used to determine a calibration coefficient relating the drive current to force.

1707-Pos Versatile Horizontal Force Microscope to Probe Micropipette-held Cells and Particles

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University of California Davis, Davis, CA, USA.

Board B684

We have turned the core of the atomic force microscope on the side and combined it with pipette nanomanipulation. The resulting cantilever-based force microscope allows us to apply and measure compression, stretching, adhesion, and dissociation forces in the horizontal direction. The integrated micropipette setup facilitates the easy manipulation and mechanical interrogation of a broad variety of test objects like individual cells or functionalized microparticles. Test objects can be brought into feedback-controlled contact with the AFM cantilever anywhere along its length, considerably enlarging the range of forces that can be applied with a single cantilever. In addition to this greatly enhanced versatility, the horizontal configuration also enables a "side view" of the cantilever during experiments and allows for optical inspection of the instantaneous deformation of the test object. At the same time, the translation of test objects to/from the cantilever by micropipette manipulation substantially reduces unwanted hydrodynamic coupling effects. We present a variety of example experiments that illustrate the functionality and versatility of the instrument, e.g., compression tests on red blood cells and neutrophils, and adhesion measurements on biomolecule-coated microspheres.

1708-Pos Viscosity Effect On The Afm Force Measurements

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Board B685

Atomic force microscopy (AFM) based techniques have been used to study protein folding/unfolding and to measure the mechanical properties of macromolecules. In these experiments, the forces are measured from the deflection of the AFM cantilever, which is submerged in the buffer solution. When the cantilever moves relative to the surrounding liquid, the viscous drag force cause errors in the measurements, especially when the cantilever moves at a high speed and/or the buffer solution has a high viscosity. The small dimensions and irregular shapes of the cantilever and the AFM liquid chamber make it difficult to calculate or measure the viscosity effects. Using our AFM that was modified for single molecule manipulation experiments, we determined the viscous drag force on the AFM cantilever by measuring deflections of the cantilever under controlled displacement of the sample surface. Drag forces were measured in liquids with various viscosities and on cantilevers of several sizes. These measurements are essential for the interpretation of our experimental data of the macromolecular crowding effect on the mechanical stability of protein molecules, where the measurements were performed in solutions with high viscosities.

1709-Pos Optical Trapping Electrophoresis: Direct Measurement of Electrical and Drag Forces

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[2] *Univ of the Pacific, San Francisco, CA, USA.*

Board B686

We use an optical trap to measure

1. the electrical force on a charged particle in aqueous electrolyte in an external electric field and
2. velocity-dependent hydrodynamic drag forces in the absence of a field.

The particle's free electrophoretic velocity is also measured with the trap turned off. The velocity at which the drag force is equal in magnitude (but opposite in direction) to the electrical force is calculated and found to agree well with the measured electrophoretic velocity. These experiments directly verify the superposition assumption of O'Brien and White [1]: the total force on the particle is a simple sum of the isolated electrical and drag forces.

Measurements were done in the Debye-Hückel and Smoluchowski regimes. The effective particle charge $Q_{eff} = F_E/E$, where F_E is the measured electrical force, is much smaller than the charge of the hydrodynamic particle Q_H calculated from the electrophoretic velocity via the Smoluchowski equation and Debye-Hückel electrostatic-potential profile. Although the trapped particle and bulk solvent are stationary, solvent flows near the particle in a direction opposite to F_E due to E-driven counterions. This local electroosmotic counterflow exerts a drag force that reduces F_E by a factor $(2\kappa a/3)^{-1}$, where κ^{-1} is the Debye screening length and a is the particle radius. Calculations indicate a possibility of opposing the local counterflow by an externally-imposed velocity field.

Direct force measurements provide an improved understanding of electrokinetic phenomena near the particle/electrolyte interface.

References

[1]. R.W. O'Brien and L.R. White, J. Chem. Soc. Faraday II, *74*:1607–1626 (1978)

1710-Pos Hydrophobic Interactions at a Single Molecule Level Studied by Dynamic Force Spectroscopy

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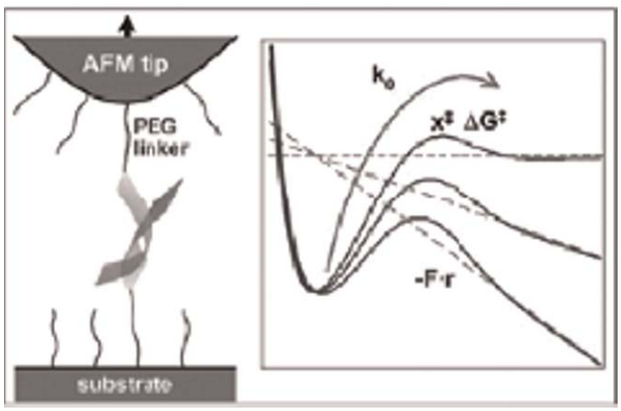
Duke University, Durham, NC, USA.

Board B687

Hydrophobic interactions are among the most important interactions between biological molecules in aqueous environment. These interactions are critical for many biological processes, including folding and assembly of biological macromolecules and structures. Recent theories indicate that the nature of hydrophobic interactions changes for objects with ~1 nm dimensions. Experimental measurements of interactions between nanoscale hydrophobic molecules are scarce because of the low solubility and aggregation phenomena. The novel single molecule force spectroscopy approach measures parameters that characterize energy landscape of intermolecular forces and can be applied to study interactions between hydrophobic molecules with sizes on a nanometer length scale.

Methodological aspects that facilitate force spectroscopy measurements of hydrophobic interactions will be described. Results for interactions between single alkane, fullerene and alpha-synuclein fragments in water will be presented.

Hydrophobic molecules are tethered by hydrophilic PEG linkers to AFM probes and substrates removing the uncertainty of the aggregation state of solution-based approaches and spurious surface effects. The statistical analysis of measured rupture forces considers different sources of systematic errors and reveals the activation energy and distance to the activation barrier characterizing the potential of mean force between single molecules in water.



1711-Pos Salt-Bridges as Molecular Velcro in Elastic Intrinsically Disordered Proteins

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Board B688

Most key players in the contractile machineries possess intrinsically disordered domains that perform essential force and signal transduction functions. These disordered domains exist as ensembles of rapidly equilibrating conformations that manifest as unique elasticity when subjected to external force. We are performing systematic structural and nanomechanical analysis of intrinsically disordered domains in the force generating and bearing molecules; including myosin S2 region (hing regions in coiled-coil α-helix), titin PEVK segment (polyproline II helix-coil), nebulin repeats (transient α-helix) and calmodulin (disordered connector). The nanomechanical properties are being measured by atomic force microscopy of single molecules and the force-strain curves analyzed by Steered Molecular Dynamics (SMD) simulations to identify force-relevant structural transitions. SMD on a titin PEVK structure demonstrated the dynamic breaking and rejoining of salt bridges identified by NMR are major force generating events that appear to determine PEVK elasticity as revealed by AFM. SMD simulations of the mechanical unfolding of a Rosetta structure of actin binding nebulin repeats indicated the importance of unfolding and reorientation of α-helix and the dynamics of salt bridges in the major force events during stretch. SMD of a calmodulin x-ray structure showed the stabilizing effect of calcium binding to acidic groups against mechanical unfolding. In all of these systems, salt bridges play a key role in how intrinsically disordered proteins respond to mechanical stress. We propose that the long-range, non-stereospecific nature of electrostatic interactions provide a facile mechanism to tether and untether the flexible chains, which in turn affect elasticity as well as control the accessibility of protein-protein interaction to these nanogel like proteins in the signaling transduction. (Musc. Res. Cell Motil. (2005), *26*, 291–301)

1712-Pos The Effect of Divalent Cations (Mg2+, Zn2+, Mn2+, and Ca2+) On The Hybridization Efficiency Of DNA

Jennifer Nichols, Joseph Buthker, Kumar Sinniah
Calvin College, Grand Rapids, MI, USA.

Board B689

Divalent cations are ubiquitous in biological systems and play a very important role in DNA structure and function. Divalent cations can affect biological processes such as replication, recombination, and gene expression when their concentrations are varied, but the predominant effects of divalent cations are observed at the single molecule level as these cations can induce significant structural changes in DNA conformations. For example, recent studies involving Atomic Force Microscopy (AFM) have found that concentrations of zinc and magnesium that are significantly higher than those of physiological levels cause DNA to kink. These kinks are characterized as sharp, abrupt bends found in ssDNA. In this poster we present a force spectroscopy study to determine the effects of four divalent cations (Mg^{2+}, Zn^{2+}, Mn^{2+}, and Ca^{2+}) on short DNA duplexes. The extent to which the divalent cations interact with the DNA was monitored through hybridization efficiency. A slight increase in hybridization efficiency was observed at cationic concentrations near physiological levels, while a significant decrease was found at higher concentrations. A variation in the rate of decrease in the hybridization efficiencies was detected amongst the different cations, thereby indicating a difference in binding which is attributed to slightly different modes of interaction, as well as varying affinities of these divalent cations to DNA. In addition to examining the hybridization efficiencies, helical rupture forces were also investigated. A nominal rupture force of ~ 50 pN for the single molecule DNA interaction seemed unchanged with varying concentrations of the divalent cations.

1713-Pos Measurement of The Binding Affinity of Intercalated DNA Complex by Stretching Single DNA Molecules with Optical Tweezers

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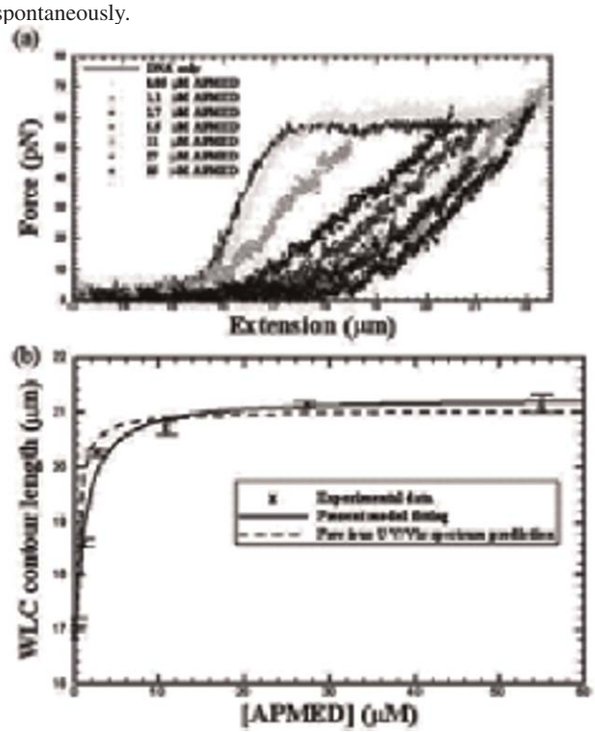
Board B690

Thermodynamic profiles for the interaction of small molecule with nucleic acid are crucial to rational drug design. To determine the binding energetics of the drug-DNA interactions, accurate prediction of binding affinity K_A is essential. We presented here a single molecule approach, together with a dual-beam optical tweezers system, to explore K_A and binding free energy ΔG_{obs} at the zero-force condition. We have synthesized a monointercalating agent, N-(2-aminoethyl)-N'-pyrene-1-ylmethylethane-1,2- diamine (APMED), whose pyrene structure can be inserted between Watson-Crick basepairs. APMED-DNA complex's zero-force contour length was obtained by fitting the force extension data in the low force regime with the inextensible wormlike chain model. Then, K_A can be determined from the changes in zero-force contour length of drug-DNA complex as function of drug concentration. The results are $K_A = 8.8\ (\pm 1.7) \times 10^5$ (1/M) (where the error bar represents the 95 % confidence interval), and the exclusion number n=3.8±0.2, which indicates an average of one quarter of the intercalation sites are occupied by APMED molecules. Furthermore, the binding free energy of APMED-DNA interaction at 20±0.5 °C is −7.94±0.13 kcal/mol, indicating that the interaction tends to occur spontaneously.



1714-Pos The Effect of Tether Length in Single Molecule Force Spectroscopy

Sarah G. Kamper, Laura Porter-Peden, Ronald Blankespoor, Mark Vander Wal, Kumar Sinniah
Calvin College, Grand Rapids, MI, USA.

Board B691

Single molecule force spectroscopy provides unique insights into the biomolecular interaction landscape. Using an external force to rupture the non-covalent interactions present in biomolecular systems, kinetic and thermodynamic parameters can be estimated from single molecule studies. However, the extent to which these parameters are affected by the tethers and linkers which attach the biomolecules to probes and surfaces is unclear. In this poster, we present a single molecule study of the non-covalent interactions between carbonic anhydrase enzyme and a tethered sulfonamide

inhibitor using an atomic force microscope. Using linkers and tethers of varying lengths, we estimate the kinetic and thermodynamic parameters for the enzyme-inhibitor complex based on loading rate dependence studies. We demonstrate that the inhibitor tethers contribute to the measured kinetic and thermodynamic parameters and should be considered in the development of theoretical models.

1715-Pos Revisiting the Folding Energy Landscape of E. coli RNase H in the Optical Tweezers

Jesse Dill, Carlos Bustamante, Susan Marqusee

UC Berkeley, Berkeley, CA, USA.

Board B692

We revisit the force-dependent folding and unfolding kinetics of the protein E. coli Ribonuclease H (RNase H). This protein's equilibrium energetics and folding pathway have been heavily studied in bulk. In 2005, a folding intermediate was observed in single-molecule mechanical denaturation experiments that had strikingly similar characteristics to the folding intermediate state observed via bulk spectroscopy.

With a new optical tweezers instrument that has better time resolution than the one used previously, we have characterized a short-lived intermediate in the unfolding trajectory of E. coli RNase H and compared its dynamics and mechanical properties to the folding intermediate. The structure of this unfolding intermediate and its relevance to bulk folding studies of RNase H will also be discussed.

1716-Pos Molecular Insights into the Mechanical Properties of Titin I-Band Domains

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Board B693

The mechanical properties of structural proteins are conserved and refined just as are other biologically important functions. This is true of the giant, rope-like muscle protein titin which spans half the length of a sarcomere and has structural, organizational, and mechanical roles. The region of titin found in the I-band of a sarcomere is composed of many tens of immunoglobulin-like (Ig) domains and is exposed to force under normal physiological conditions connecting the free-hanging ends of the myosin filaments to the Z-disc. The atomic force microscope provides an ideal tool to expose single molecules to tensile forces and measure their mechanical properties. Recent data show an apparent mechanical hierarchy in the I-band domains. Domains near the C-terminus in this region unfold at forces 2–3 times greater than domains near the beginning of the I-band. Though all these domains are thought to share a common fold and topology, the sequences of neighboring domains vary greatly with sequence identities in the range of 20% – 30%. We found that these sequences, however, are highly conserved across species as widely separated as humans, chickens, and zebrafish. This implies that the mechanical properties of each domain are well conserved functions. We seek to determine the molecular bases that determine the unique mechanical stabilities of each I-band Ig domain. We used sequence analysis techniques to search for properties common to weak domains vs strong ones and have found several interesting results such as tendencies for larger amino acid side-chains in mechanically stronger domains and several other more specific trends. We now aim to use mutagenesis techniques to test the true roles played by differentially conserved properties in weak and strong domains. This approach further illuminates the complex subtleties in the mechanical design of these domains.

1717-Pos Influence of Divalent Cations on the Integrin a7b1-Invasin Interaction Studied by the Biomembrane Force Probe Technique

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Board B694

Divalent manganese, magnesium and calcium cations play a complex and not yet fully understood regulatory role in cell surface receptors of the integrin family. In this study, the Biomembrane Force Probe technique was applied to investigate the influence of Mn^{2+} and Mg^{2+} on the specific receptor-ligand interaction between $\alpha7\beta1$, an integrin found in skeletal myoblasts and myofibers, and invasin, an outer membrane protein of enteropathogenic Yersinia bacteria. Invasin is known to bind with high affinity to multiple members of the integrin family - an important step in the internalization of the Yersinia bacteria into the eukaryotic cell. Genetically modified variants of both $\alpha7\beta1$ and invasin were immobilized on dextran coated melamine beads after which the Biomembrane Force Probe was applied to measure force induced dissociation of formed receptor-ligand pairs. Rupture force spectra were obtained under different ionic concentrations of Mn^{2+} and Mg^{2+}. This allowed us to investigate the influence of ionic surroundings on the $\alpha7\beta1$-invasin interaction under non-equilibrium conditions. In accordance with previously published models on integrin regulation, our results show

that both Mn^{2+} and Mg^{2+} support ligand binding through what seems to be a synergetic enhancement of ligand affinity.

1718-Pos Forced dissociation of the Neural Cell Adhesion Molecule complex

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Board B695

In this work, we use all-atom steered molecular dynamics simulations to dissociate the complex formed between the outermost two Ig domains (Ig12) of the Neural Cell Adhesion Molecule (NCAM). NCAM is a member of the Ig super family of cell adhesion molecules (IgSF CAMs). It is abundant in the brain and plays an important role in the development of the nervous system. Force measurements and binding assays show that Ig12 is involved in NCAM homophilic binding. We therefore consider the Ig12-Ig12 complex present in the crystal structures of both the Ig12 and the Ig123 NCAM fragments. We explicitly solvated the complex (total of 168000 atoms) and subjected it to either constant velocity or constant force pulling. We find that the force response consists of two distinct stages:

(i) An initial stretching phase, wherein the end-to-end length of the complex (5 nm) steadily increases by as much as 100% due only to a change in the relative orientation of adjacent domains on the same molecule.
(ii) Dissociation of the complex, which is preceded by partial unfolding at high (7 pN/ps), but not low (0.7 pN/ps) loading rates.

We find that the initial stretching phase involves the rupture of only one of the two intra-molecule, inter-domain H-bond linkers - E16-K98 and not E11-R177. We also show that a low constant force of 50 pN is sufficient to stretch the complex by 60% of its end-to-end length in 6 ns. Analysis of the unbinding trajectories shows that, among the hydrophobic residues identified from the crystal structures as important, F19 sustains a greater tensile force than Y65. In addition to the atomistic details of the NCAM Ig12 complex rupture, the simulations also reveal how a multi-domain IgSF CAM complex responds to near-physiological tensile forces.

1719-Pos Direct Observation Of Equilibrium Folding/Unfolding Transitions Of Single Calmodulin Proteins By AFM

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Board B696

Single-molecule force spectroscopy by AFM allows investigating the unfolding and refolding kinetics of proteins. For the proteins studied so far with this technique, unfolding usually occurs far from thermodynamic equilibrium, i.e. the probability for refolding is negligible in the force regime necessary for unfolding. In equilibrium systems, such as overstretched DNA, polysaccharides or coiled-coil motifs, both unfolding and refolding takes place at rates generally too fast to observe individual transitions. Here we present data on the mechanical unfolding of Calmodulin which occurs close to equilibrium. Numerous folding/unfolding transitions can be observed in the traces while stretching the protein at extremely low pulling velocity (1 nm/s). This data yields direct access to the unfolding and refolding rates under force and allows a detailed mapping of the potential energy surface for the mechanical unfolding and refolding of the protein. The two globular domains of Calmodulin unfold independently at forces $<$20 pN and refold at ~10 pN. Calmodulin binds 4 calcium ions and accomplishes a large conformational transition upon binding of a target peptide (e.g. the wasp venom peptide Mastoparan or fragments from Myosin light chain kinase). Our experimental data shows that binding of calcium ions clearly increases the folding rate, whereas binding of target peptides significantly decreases the unfolding rate of the protein. With this assay, the energetics and stoichiometry of ligand binding becomes directly observable in great detail. In particular, these experiments reveal the existence of different binding modes for target peptides from Myosin light chain kinase and Mastoparan.

1720-Pos Kinetics and Thermodynamics of Enzyme-Inhibitor Interactions

Laura Porter-Peden[1], Sarah G. Kamper[1], Kumar Sinniah[1], Ronald Blankespoor[1], Dejian Zhou[2], Chris Abell[2], Trevor Rayment[3]

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Board B697

A single molecule force spectroscopy study was used to examine the non-covalent interactions between a carbonic anhydrase (CA) enzyme and its inhibitors. Since covalent immobilization of the CA enzyme leads to random orientations, the enzyme was immobilized electrostatically on a charged pyridinium surface with its active site pointing upwards for easy access. The active site was probed with a sulfonamide inhibitor attached to an Atomic Force Microscope (AFM) cantilever. The single molecule rupture force data was obtained from a force distribution plot for the CA enzyme-sulfonamide complex. Rupture force data obtained at various loading rates was fit as a single energy barrier for the carbonic anhydrase-inhibitor complex and was used to obtain kinetic and thermodynamic parameters. The parameters were estimated based on two microscopic models and the Bell-Evans model. The disso-

ciation rate for the enzyme-inhibitor complex was found to be significantly faster than the natural spontaneous dissociation rate, as the activation energy barrier is lowered in the pulling experiments.

1721-Pos Stabilization of Cardiac Titin's N2b-unique Sequence by Disulphide Bridges Alters Molecular Spring Properties Depending on Redox State

Anika Gruetzner, Wolfgang A. Linke

University of Muenster, Muenster, Germany.

Board B698

The N2B-unique sequence (N2Bus) is an intrinsically disordered protein segment of 572 amino-acid residues specific to the cardiac isoform of titin, the giant elastic muscle filament. Previously we reported that the measured contour length (Lc) of the N2B-unique sequence, which extends in cardiac sarcomeres during physiological stretching, sometimes reaches much less than ~210nm, the expected value if the N2Bus were a permanently unfolded molecular spring. Here we used single molecule AFM force spectroscopy to investigate the mechanical characteristics of the N2Bus directly. We generated a recombinant construct containing the human N2Bus flanked on either side by two immunoglobulin-like domains. AFM force-extension curves recorded in 200mM PBS showed a mean Lc=120nm for the N2Bus, but there was a bimodal distribution centered at ~95nm and ~180nm. We speculated that the different Lc-values might be due to the presence of disulphide-bridges in the N2Bus. Various disulphide-connectivity prediction algorithms indeed suggested a few S-S bridges may form between the six cysteine residues contained within the N2Bus. Using the biochemical Ellman's Test on a recombinant N2Bus construct we demonstrate the presence of three S-S bonds under oxidizing, but not reducing, conditions. Single-molecule AFM stretch experiments performed in the presence of 10mM DTT revealed that under reducing conditions the N2Bus has a single mean Lc of ~210nm, consistent with full polypeptide extension. We conclude that S-S-bridges forming in the N2Bus under oxidizing conditions mechanically stabilize this titin region. Thus, titin extensibility and spring-force generation differ depending on the redox state, presumably also in the intact cardiac cell.

1722-Pos Fibrin Gel Porosity, Transport, and Elastic Modulus: Microbead Measurements

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University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Board B699

We report on measurements of fibrin structure and mechanics using thermally diffusing and magnetically driven microbeads in fibrin gels. Microbead experiments reveal local information about gel formation, network pore size, and storage modulus. This information is helpful in understanding the formation of blood clots, the propagation of proteins and drugs through clots, and the mechanics of stemming blood flow.

Our experiments reveal heterogeneity in the fibrin gel network, including probe diffusion among pores in the fibrin network, and non-linear motion of driven beads. We also report on efforts to extrapolate local microbead measurements to measure the material's macroscopic rheology. We measure fibrin polymerization by observing probe diffusion over the course of gel formation. The parameters we vary in our experiments include: bead size, bead surface chemistry, polymerization chemistry (pH, Ca, and protein concentrations), and fibrinogen genotype. Experiments employ a novel Multiforce High Throughput System for magnetic force microscopy.

1723-Pos Long Tandem Repeat Region of Fibrin's alphaC Region Correlates With Large Fiber Extensibility

Michael R. Falvo, Daniel Millard, E. Timothy O'Brien, Lifang Ping, Richard Superfine, Susan T. Lord

University of North Carolina, Chapel Hill, NC, USA.

Board B700

Fibrin fiber networks are the major structural component of blood clots and knowledge of their mechanical properties is crucial to an understanding of hemostasis, thrombosis and embolism. Though clot mechanics has been studied for several decades at the macroscopic level, there is little detailed understanding of the molecular scale response of fibrin to applied stress. Our work focuses on illuminating how the individual fibers,and ultimately the protein monomer, deform and accommodate strain. We have reported previously on the high extensibility (exceeding 300%) of individual fibrin fibers. Extensibilities of this magnitude are unexpected considering the highly ordered structure of the fibrin fiber revealed by crystallography and electron microscopy. We hypothesize that the majority of the strain is accommodated by a region of tandem amino acid repeats within flexible αC region. This region is intriguingly reminiscent of repeat sequences in elastomeric proteins such as elastin, resilin, and spider-silk. The striking similarity of the primary structure of the tandem repeat region of the αC with other elastic proteins suggests it exhibits similar mechanical properties, namely flexibility, extensibility and elasticity. Our study focuses on this portion of the protein by mechanically evaluating fibrins with varying lengths of the tandem repeat region. Unlike the rest of the protein which is very well conserved, the length of this region varies dramatically across species. Using a combined Atomic Force Microscopy / Fluorescence Optical Microscope, we have stretched freely suspended fibers made of human, mouse and chicken fibrinogen which have long, intermediate and zero length tandem repeat regions respectively. We focus on the extensibility, or strain at breaking of these fibers. Our result show correlation between extensibility and the length of the tandem repeat.

This work is supported by the NSF (DMR Biomaterials Program).

1724-Pos Study of Bimolecular Integrin-Fibrinogen Interactions Using a Laser Tweezers-Based Model System With an Electronic Force Clamp

Rustem I. Litvinov, Andrew Fisher, Andrew J. Schissler, Joel S. Bennett, John W. Weisel, Henry Shuman

University of Pennsylvania, Philadelphia, PA, USA.

Board B701

A laser tweezers-based force clamp system has been developed to measure the bond lifetimes of single-molecule protein-protein interactions at constant tensile forces. A LabVIEW programmable command signal to an inverting integrating analog amplifier positions the laser so that a trapped ligand-coated latex bead periodically contacts and separates from a receptor-coated silica pedestal. The program controls the duration of contact between bead and pedestal surfaces, the magnitude of compressive force during contact, the duration of bead separation, and the magnitude of the tensile force when bonding occurs. The electrical signal corresponding to the lateral force applied by the laser tweezers on the trapped bead is measured with a back focal plane detector, and is fed back to the input of a high gain amplifier. When a bond forms between the protein-coated surfaces, the feedback loop is effectively closed and the amplitudes of both the compressive and tensile forces closely match the command signal. The maximum tensile force that can be applied to separate the interacting surfaces is limited by the maximum laser power to ~100 pN. The force clamp system was used to quantify the forced unbinding of purified individual integrin αIIbβ3 and fibrinogen molecules. The integrin αIIbβ3 is abundant on platelets, and fibrinogen is a blood plasma protein. The regulated ability of αIIbβ3 to bind fibrinogen plays a crucial role in platelet aggregation and hemostasis and is largely determined by shear. Therefore, the lifetime distributions of the αIIbβ3-fibrinogen complex were obtained under a constant tensile force of ~70 pN, mimicking the effect of hydrodynamic blood flow on an adherent platelet. The data clearly demonstrate that the bond lifetimes of the surface-bound αIIbβ3 and fibrinogen are heterogeneous, indicating complex and multiple pathways for αIIbβ3-fibrinogen unbinding.

1725-Pos Effect Of The Pulling Position On The Dynamic Strength Of Integrin/ligand Interactions

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Integrins are the main mediators of leukocyte firm adhesion to the vascular endothelium. Lymphocyte function-associated antigen-1 (LFA-1) is a leukocytic integrin that binds via its alphaL I domain to the intercellular adhesion molecule-1 (ICAM-1) of the endothelium. The I domain of LFA-1 has been shown to change its structural conformation to reach a high affinity, open state [1,2]. During leukocyte recruitment, the affinity of integrin/ligand complexes may be affected by hydrodynamic drag forces caused by the bloodstream. The zero force affinity to ICAM-1 of the open I domain has been shown to be ~500-fold higher than that of the wild type. However, little is known about how the affinity of the interaction may be affected by dynamically pulling the complex from different positions. The aim of this work was to characterize the dynamic strength of wild type and open I domain/ICAM-1 complexes by pulling from different positions (N- and C- termini) using the atomic force microscope (AFM). Wild type and open I domains (100 μg/ml) were immobilized via a histidine Tag linker to the AFM tip, while human ICAM-1 (50 μg/ml) was immobilized on the dish. Force curves were acquired bringing the AFM tip into contact with the sample surface and pulling at different loading rates (retraction speeds: 0.37–37 μm/s). Rupture forces were determined from retraction curves with adhesion events. The most probable rupture forces were plotted versus loading rates obtaining the dynamic force spectra of the interaction. These were used to determine the dissociation rate constants and the widths of the interaction energy barriers.

References

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1726-Pos AFM Visualization and Nanomechanics of Single Clathrin-Mediated Endocytotic Complexes

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We continue to develop and apply new schemes of atomic force microscopy (AFM) and related analyses to characterize clathrin triskelia and their macromolecular complexes in receptor-mediated endocytosis. The intricacies of these clathrin-containing complexes and their significance for subcellular trafficking via interactions with adaptor proteins, membrane lipids, and other cofactors, have inspired numerous structural and functional studies. Here we resolve variable profiles of triskelia on mica surfaces for the first time by AFM at a resolution comparable to electron microscopy. Classical three-leg, filamentous pin-wheel shapes, as well as non-planar triskelion conformations and dimers, are readily observed. Pentagonal and hexagonal lattice structures are well visualized in a variety of clathrin assemblies with or without AP180 adaptors, resembling those of native CCVs purified from bovine brains. Single-organelle measurements by AFM force-volume and force spectroscopy reveal considerable detail and variability in nanoscale mechanics and energetics of clathrin lattices. Native CCVs can withstand several hundred pN of AFM compressive force before crumbling in physiological buffers, while reconstituted clathrin baskets, which lack an underlying membrane, are significantly softer. AFM force spectros-

copy reveals internal energetic barriers that may characterize CCV lattice elongation and triskelion extraction or unfolding. These results once again show AFM to be a powerful tool for biomedical imaging and nanometric characterization.

1727-Pos Bacterial Extracellular Matrix as a Responsive Hydrogel

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Biofilms are heterogeneous bacterial colonies that consist of cells and extracellular polymeric substance (EPS). This complex polymer matrix is made of polysaccharides, proteins, nucleic acid and other biomolecules. It plays a significant role in transport of nutrients and signaling molecules, and acts as a protective barrier between the cells and their environment. The physical properties of EPS determine the morphology and stability of the biofilm colonies, affecting, e.g., mass transfer and cells' phenotype. Until now, information on the mechanic properties of intact biofilms is inadequate, in that data acquired using conventional macroscopic testing techniques does not reflect the spatial heterogeneity of the colonies. Thus, we have developed a method involving atomic force microscopy (AFM) to measure EPS rigidity. Measurement of EPS elastic modulus as a function of environmental conditions was obtained by this method and demonstrated on a model Streptococcus mutans biofilm. This species is responsible for dental plaque, and grows in the steep pH gradients that develop as a response to sugar nutrients. We show that the modulus increases almost three times as pH is lowered from pH 7 to pH 5, and that it varies with spatial position. These results show that the soft, hydrated EPS gel contains labile charges that cause the hydrogel to soften and stiffen according to the proton concentration in the surrounding environment. The change is reversible, and we hypothesize that this responsive behavior of EPS protects the cells against their own acidic metabolic products and prevents cell damage by external agents. We anticipate that increased knowledge of the physical properties of the EPS will contribute to biological insight of biofilms and facilitate the development of strategies to counteract their undesirable involvement in engineering applications and drug-resistant infections.

1728-Pos Single -Molecule Force Measurement of IL-6 and IL6-Receptor Interaction in Living Cells

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Board B705

Interleukin-6(IL-6) plays important role in immune system and induces growth, differentiation or activation of several lines of human cells. It was proved that IL-6 exerted its biological function by binding a specific membrane receptor subunit(IL-6R) , then the dimeric complex dimerize and bind to two signal-transducing molecules, gp-130, to form a functional hexameric complex. Here we report a single-molecule level probe of the interaction between IL-6 and IL-6R on the surface of individual living U937 cell. Our experiment were conducted using homemade optical tweezers with subpiconewton force resolution and millisecond time resolution. We measured the interaction force spectroscopy between the bead enveloped IL-6 and IL-6R located the surface of living cells. By differential analysis we obtain the rupture force of single-molecule bond of IL-6 and IL-6R on living cell surface, and find it increases as loading rate, for example, the rupture force is 42.17 pN at loading rate of 3100 pN/s. we also verified that the interaction of IL-6 coated on 5μm bead with the cell were specific to IL-6R by negative contrast sets, saturating the IL-6 receptor on cell surface, and statistic analysis. This single-molecule force spectroscopy assay provided a wealth of biochemical and biophysical information about IL-6 mediated bonds, such as the rupture force, the dissociation rate, reactive compliance educed by Bell's model. This analysis present here may serves as a general framework to study mechanism of receptor mediated interaction between ligand and receptor on living cellls.

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1729-Pos In Vivo Detection of Protein Strain in Cells and Animals

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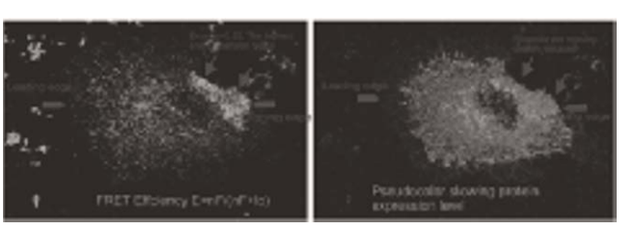
Mechanical stress is a universal stimulus transduced into biochemical signals that affect ion channel activity, gene expression, cell migration and differentiation. The distribution of stress within and about cells is distributed into many different chemical components that presumably can change dynamically. To examine the time and space variation of stress we developed a molecular force sensor named pFRET (protein fluorescence energy transfer) with GFP pair of Venus and Cerulean connected by a 50 angstrom alpha-helix. MD modeling suggests that pN forces will result in significant strain and changes in FRET efficiency E where strain=$((1/E)-1)^{1/6}$. We integrated pFRET into multiple fibrous cell components including collagen-19, filamin A, alpha-actinin, alpha-spectrin and myosin II A and B and obtained in acutely transfected 3T3 and HEK293 cells and *C. elegans*. We measured FRET with real time simultaneous frame wide field imaging of moving worms and confocal imaging of cells. The efficiency images showed spatial and time varying strains that were different for the different host proteins. This promises to be a powerful method to study the role of fibrous proteins in mechano-cell biology.



Tuesday, February 5, 2008

Symposium 13: Voltage-dependent Proton Channels Come of Age

1730-Symp Properties and Functions of Voltage-Gated Proton Channels

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The elusive gene for the voltage-gated proton channel was identified in 2006 (Ramsey et al, 2006, *Nature 440*:1213; Sasaki et al, 2006, *Science 312*:589). Some predictions based on the electrophysiological behavior of native proton currents have been borne out, but there have been surprises. The protein has four transmembrane doamins that resemble S1–S4 of ordinary voltage-gated ion channels, but lacks S5–S6 that contain the aqueous pore. Thus, the channel evidently lacks a water-filled pore, consistent with the predictions of studies showing that the channel is perfectly selective for protons and the conductance has a large deuterium isotope effect and profound temperature sensitivity. Based on competition between H^+ and Zn^{2+} (a potent inhibitor), the external binding site for Zn^{2+} was predicted to comprise 2–3 His residues. Remarkably, the human proton channel protein does have two His residues facing the external solution, and mutating them to Ala greatly attenuates inhibition by Zn^{2+} (Ramsey et al, 2006). However, most major features of proton channels await structural explanation. An archetypal characteristic of proton channels is the sensitivity of gating to pH, which suggests the existence of titratable sites. These groups have not been identified. The mechanism of proton selectivity is unknown. We are uncertain whether the channel is a monomer or multimer. "Activation" of the proton conductance in phagocytes involves phosphorylation by PKC of the channel or a distinct regulatory protein. The putative phosphorylation site remains hypothetical. Finally, the mechanism of voltage sensing has eluded structural identification. Despite the similarity of the proton channel to the voltage sensor of other channels, it is not clear that the mechanisms are identical. In summary, many key properties of the voltage-gated proton channel remain unexplained.

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1731-Symp Proton Channels In Phagocytes: From Postulate To Reality

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Phagocytic white blood cells kill microbes with superoxide radicals produced by a membrane enzyme, the phagocytic NADPH oxidase (phox). This process is essential for innate immunity, and patients with defective phox suffer from severe recurring bacterial and fungal infections. Voltage-gated proton channels provide a compensating charge for the electrons currents generated by the phox, and were postulated long ago to be required for bacterial killing by phagocytes. Electrophysiological recordings further established that, in phagocytes, proton and electron transport are closely associated: phox activation induces a −60 mV shift in the threshold of voltage-activation of proton currents, and the two activities co-segregate in excised patches. This close interaction led us to propose that the phox itself functions as proton channel. Consistent with this hypothesis, we showed that the expression of phox isoforms in HEK-293 cells is sufficient to generate proton currents that recapitulate the properties of native proton channels. However, proton currents persist in phagocytes lacking phox and are absent in COS-phox cells that express a functional oxidase, suggesting instead that the phox is not a proton channel but modulates the activity of a separate channel molecule. The cloning of the voltage-gated proton channel Hv1 will reveal whether Hv1 is the only channel that sustains the activity of the NADPH oxidase, and whether proton currents carried by Hv1 are also modulated by the activity of the phox. This will establish the physiological role of voltage-gated proton channels and open new avenues for the treatment of diseases linked to altered innate immunity.

1732-Symp Voltage-gated proton channels

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Voltage-gated proton channels (Hv channels) were described in snail neurons, mammalian phagocytes, alveolar cells and microglia. They show high proton selectivity of ion permeation and voltage dependence that is pH-sensitive. We have identified a membrane protein, VSOP (also called Hv1) that consists of four transmembrane helices with homology to the voltage sensor of voltage-gated ion channels (Sasaki et al, 2006) and reported that this protein recapitulates most properties of Hv channels. The presence of VSOP/Hv1 protein on phagosome membranes supports the idea that VSOP/Hv1 regulates phagosomal pH and membrane potential. It has been proposed that Hv channels play roles in regulating NADPH oxidase activities through their involvement in membrane potentials and pH. Hv channels also potentially provide protons as substrate for conversion from superoxide anions to hydrogen peroxide, and further conversion by myeloperoxidase to hydrochlorite. Since pathogen or apoptic cells are often engulfed in closed membrane compartments, that are called phagosomes, it will be important to address whether voltage-gated proton channels exist on phagosome membranes. For this purpose, we purified phagosomes from mouse activated neutrophils following treatment with magnetic beads conjugated with IgG. Phagosomes were collected using the magnetic system and western blot was performed using anti-mouse-VSOP polyclonal antibodies. These antibodies were generated against the whole C-terminal cytoplasmic polypeptide of mVSOP. The phagosome membrane fraction was verified by the detection of p47phox, a subunit of NADPH oxidase complex, which is known to be exclusively present in phagosomes, but not on plasma membranes, using anti-p47phox antibody. Band of VSOP/Hv1 (31kd) was clearly detected in the membrane preparation positive for p47phox signal. To gain insights into physiological functions of VSOP/Hv1, we established a mouse line with null expression of VSOP/Hv1 protein, and are studying how phagocytosis function is impaired.