the related 655nm QDs, conjugated using streptavidin to anti-insulin receptor antibody, are easily visualized bound to 2H3 cell insulin receptors (IR). Blinking of spots demonstrates imaging of individual QDs. We excite fluorescence from cell-bound QDs with non-polarized illumination and record orthogonally-polarized fluorescence images using an image splitter and an EMCCD camera. Image pairs are separated and one polarization is corrected for the optical path g-factor and for displacement, rotation and dilation relative to the other polarization. Time-dependent fluorescence from regions containing individual QDs in image pairs are extracted and the time-autocorrelation function for polarization fluctuations calculated either from actual polarization or from a combination of auto- and cross-correlations of polarized fluorescence intensities. Individual 655 nm QDs exhibit peak polarization fluctuations with an RMS amplitude of ~0.06. These fluctuations decay over 30-50 ms. Whether this slow decay represents hindered rotation of individual IR or results from crosslinking of multiple receptors by single QDs remains to be determined. Current work involves exploration of more highly-asymmetric QDs, use of faster detection methods and examination of the 2H3 cell Type I Fcϵ receptor where rotational dynamics on faster timescales have previously been explored in detail. Supported by NIH grant RR023156 and NSF grant CHE-0628260.

Molecular Mechanics & Force Spectroscopy I

3065-Pos

Identification of Infrared Spectrum from Human Bio-Energetic Campus

Aurelian Udristioiu.
Emergency County Hospital Targu Jiu, Targu Jiu, Romania.

The impact of living mater with unionized radiations is a theme of frequent researches for treatments in complementary medicine.

In presenting work we make the measurements of bio-energetic field applied to experimental fluids and we have given the explanations of the bio-energetic effect by quantum theory.

The technology of measurement was based on electro-mechanic principle, using a Laboratory pH Meter WPA-CD 7400, converting mV in units of intensity of bio-magnetic fields.

In bases of quantum theory (E= υ h) was calculated the Intensity of levels bio-energy, in mean value of 0.04 m eV, which correspond to 10^2 f T of bio-magnetic field, the Frequency of bio-magnetic wave, in value of $0.9*10^{12}$ Hz, the Bio-energetic wavelength=$2.5* 10^{-3}$ mm and corpuscular mass of bio-magnetic waves in value of ~ $2*10^{-32}$ Kg or 1/10 from electron mass, (M) when ''m''=$2/Ec^2$, in conformity with the parameter of mathematical counting or to prolong span of life cells.

Bio-energetic fluids can be used in technology of preparation of drugs, from homeopath medicine and in laboratory medicine for the changes of pH in liquid medium with cultivated stem cells for to prolong the span life of cells.

SPECIFICATIONS – pH Meter

Ranges	pH MV C	0.00 to 14.00 0 to ± 1999 -50 to +50	0.00 to 14.00 - -
Resolution		0.01pH/1 mV/0.1C	0.01 pH
Accuracy (electrical)		±0.01pH/1 mV/0.1C	±0.01 pH
Imput resistance		>10^{12} ohms	>10^{12}ohms
Auto Buffer Recognition		4.00 , 7.00 , 10.00 pH	-
Temperature Probe		TPA 100 platinum Resistance. User Correction up to ±2.00 C	-

Table 1

3066-Pos

Solvent Effect on the Unfolding Force of a Single Hydrophobic Polymer

Isaac T.S. Li[1], Matthew Paige[2], Gilbert C. Walker[1].
[1]University of Toronto, Toronto, ON, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada.

The hydrophobic collapse of a homopolymer is a much simplified model for studying the hydrophobic collapse of a protein. It is widely believed and theorized that the driving force for the hydrophobic collapse is the interfacial free energy between the polymer and the solvent. Therefore, changes in interfacial free energy should be directly proportional to the force to unfold the polymer in bad solvents. To test this hypothesis, we used single molecule force spectroscopy to unfold a single polystyrene chain in water-ethanol mixtures. Different percentage of binary mixture is used to create solvents with different interfacial energy with polystyrene. However, we do not see a linear correlation between the interfacial tensions with the unfolding forces. This is an indication that macroscopic properties such as the interfacial free energy cannot be directly applied to study certain systems in microscopic scale. In this study we also hypothesized a mechanism for the cause of this inconsistency.

3067-Pos

Active Stochastic Microrheology using Optical Tweezers

Hyungsuk Lee, Carlos E. Castro, Yongdae Shin, Roger D. Kamm, Matthew J. Lang.
Massachusetts Institute of Technology, Cambridge, MA, USA.

Cells are dynamic structures capable of generating and reacting to physical cues in their environment. Measuring mechanical properties is thus essential for elucidating cell or other material structure-function in particular during dynamic rearrangement of the cytoskeleton. Although a variety of rheological techniques have been developed using video microscopy, AFM, and magnetic traps, the measurable frequency range is limited by the time to obtain the measurement, and forcing conditions such as amplitude, direction, contact geometry, and probe location. Here, we developed active stochastic microrheology using optical tweezers to enhance the temporal resolution and precision of detection. A stochastic force is generated by moving the trap relative to the sample. Both bead displacement and trap position are monitored simultaneously by separate position sensitive devices. With this method, both storage and loss shear moduli of the extracellular matrices can be extracted over a wide frequency range of $10^{-2} – 10^3$ Hz within a few minutes. Also, this method was used to probe the local mechanical environment of B-cell receptor using antigen specific interaction. We showed that the local mechanical properties are strengthened in response to antigen binding and repeated external excitation in a physiological range of 1–100pN. The mechanical responses can also be measured with respect to direction such as force applied normal and perpendicular to the cell membrane. This technique is useful in characterizing the mechanical properties at a user-defined location and magnitude, over a wide frequency spectrum, in a short time, and with a small deformation < 100nm. With these advantages, the method can also be applied to other cell processes, studies of complex fluids, fibril growth, and polymer solutions. Support from the NIGMS (GM-076689), an NSF Career Award (0643745), and the Singapore-MIT Alliance for Research and Technology (SMART-BioSyM) are gratefully acknowledged.

3068-Pos

A Single Molecule Force Spectroscopy Study of the Binding Interaction Between Insulin and G-Quadruplex DNA

Susanna Lynch[1], Rachel Battershell[1], Jordan Scott[1], Dejian Zhou[2], Kumar Sinniah[1].
[1]Calvin College, Grand Rapids, MI, USA, [2]University of Leeds, Leeds, United Kingdom.

The formation of guanine (G)-quadruplex in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) is linked to transcriptional effects on the insulin gene. Recent studies demonstrate that these G-quadruplexes can bind insulin, and while this may impact the transcription of insulin, little is known about the binding mechanism. A single molecule force spectroscopy study was performed to examine the selective binding of insulin to the ILPR G-quadruplex DNA. In this study, the insulin was covalently attached to a flat gold surface while the quadruplex DNA was attached to an AFM probe. The rupture force between insulin and quadruplex DNA was measured at various force loading rates. To confirm the specificity of the binding, control studies were performed by blocking the tethered G-quadruplex with ''free insulin'' in solution. Additional control studies were performed with a shorter DNA sequence incapable of forming a G-quadruplex on the AFM tip and a scrambled DNA sequence of the ILPR sequence. Results from the dynamic force-pulling studies are described based on the Bell-Evans and Dudko-Hummer models.

3069-Pos

A Single Molecule Study of Guanine Quadruplex DNA

Kumar Sinniah[1], Susanna Lynch[1], Heather Baker[1], Sarah G. Byker[1], Dejian Zhou[2].
[1]Calvin College, Grand Rapids, MI, USA, [2]University of Leeds, Leeds, United Kingdom.

We present an atomic force microscopy (AFM) based single molecule force spectroscopy study of guanine (G) DNA quadruplexes. A bimolecular G-quadruplex was formed between a pair of single-stranded DNA molecules, each with two G-rich domains, that were immobilized on an atomic force microscope probe and an ultra-flat gold surface. G-quadruplex stability was examined as a function of the potassium ion concentration and loading rate (dynamic force spectroscopy). Kinetic and thermodynamic parameters for these single molecule G-quadruplexes were estimated with theoretical models, and the effect of the number of guanines in each G-rich domain was assessed. This study demonstrates that AFM-based single molecule force spectroscopy is a powerful tool for characterizing the structure, kinetics, mechanical and thermodynamic properties of G-quadruplexes.

3070-Pos

Mechanics of Lipid Bilayers of High Curvature

Sai Li, Frederic Eghiaian, **Iwan A.T. Schaap**.
Third Institute of Physics, Georg August Universität, Gottingen, Germany.

Lysosomes, enveloped viruses, synaptic and secretory vesicles are all examples of natural nano-containers (diameter ~100 nm) which specifically rely on their

lipid bilayer to protect and exchange their contents with the cell. We have developed methods primarily based on atomic force microscopy that allows precise investigation of the mechanical properties of liposomes and that could be applied to study other related organelles/viruses. The mechanical properties of small, spherical vesicles were probed by applying very low forces (~ 0.1 nN), which led to a maximum 10 % deformation. The effects of lipid composition, temperature, osmotic pressure and the radius of curvature were studied for liposomes with diameters between 30 and 150 nm. The liposome deformation was modeled using finite element methods in order to extract the lipid bilayer elastic properties. For the larger liposomes we find a very good agreement with previously reported experiments on micrometer sized giant vesicles.

3071-Pos

Probing the Mechanical Properties of Single Scleroproteins with Optical Tweezers

Benjamin P.B. Downing[1], Astrid van der Horst[1], Ming Miao[2], Fred W. Keeley[2,3], Nancy R. Forde[1].
[1]Simon Fraser University, Burnaby, BC, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada.

Stretching and relaxing single proteins provides quantitative information on their elasticity and other mechanical properties. This can be done with optical tweezers, a technique in which the ends of the protein are chemically attached to micron-scale spheres, used to manipulate the protein and measure its response. We are working on the application of this technique to scleroproteins, nonglobular proteins whose mechanical properties are of direct relevance to their physiological roles. These proteins self-assemble into hierarchically organized load-bearing structures, often found in the extracellular matrix. The ability of optical tweezers to manipulate single molecules and higher-order structures suggests their application to probing the mechanical response at different hierarchies of assembly. Applying this technique to stretch these single proteins presents many challenges, including the production of constructs with appropriate labels for attachment to microspheres, relatively short contour lengths which can introduce experimental artifacts, and self-aggregation and binding interactions of these predominantly insoluble proteins, which make it difficult to isolate and manipulate single molecules. We discuss our work to overcome these challenges, with a specific focus on elastin.

3072-Pos

Elastic Behavior of ssDNA in Salty Solutions

Dustin McIntosh, Omar A. Saleh.
UC Santa Barbara, Santa Barbara, CA, USA.

The interaction between highly charged poly-ions, such as DNA, and the smaller ions in salty solutions is of fundamental importance to the basic processes of molecular biology (e.g., ion-mediated nucleic acid folding, collapse and stabilization of proteins). Despite its importance, this phenomenon is poorly understood, particularly for multivalent ions where mean-field theories (e.g. Debye-Huckel) break down. By stretching single denatured ssDNAs in monovalent salt solutions, we have established that force-extension measurements directly and quantitatively probe electrostatic effects on charged polymers in solution (O.A. Saleh *et al.*, PRL **102**, 068301 (2009)). We exploited access to the 'tensile blob' regime to show that, for a broad range of NaCl concentrations, ssDNA behaves as a real polymer in good solvent with a Kuhn length linearly proportional to the Debye length. Here, we present data on the effects of cations with different valences and chemistries on ssDNA structure. We find that the effects of divalent ions greatly exceed those predicted by simple Debye-Huckel calculations and discuss our data in the context of more realistic theories.

3073-Pos

Mechanical Unfolding of Cardiac Myosin Binding Protein-C by Atomic Force Microscopy

Árpád Karsai[1], Robert W. Kensler[2], Miklós Kellermayer[3], Samantha P. Harris[1].
[1]UC Davis, Davis, CA, USA, [2]University of Puerto Rico, San Juan, PR, USA, [3]Semmelweis University, Budapest, Hungary.

Cardiac myosin binding protein-C (cMyBP-C) is a member of the immunoglobulin (Ig) superfamily of proteins and consists of 8 Ig- and 3 fibronectin (Fn)-like domains along with a unique regulatory sequence referred to as the M-domain. Domains near the C-terminus bind tightly to myosin and mediate the association of MyBP-C with thick (myosin-containing) filaments, whereas N-terminal domains of MyBP-C, including the M-domain, bind reversibly to myosin S2 and/or actin. The ability of MyBP-C to bind to both myosin and actin raises the possibility that MyBP-C cross links thick and thin (actin-containing) filaments and thereby imposes a drag that regulates shortening velocity during contraction. To investigate the mechanical properties of the proposed thick-thin filament linkage, we used atomic force microscopy (AFM) and electron microscopy (EM) to assess the single molecule elasticity and mechanical stability of full-length mouse cardiac (c) MyBP-C expressed in sF9 cells. Force-extension curves showed that cMyBP-C is extensible via unfolding of individual domains evident as "saw tooth" peaks in force spectra. Spectra with up to 12 peaks were obtained. The force required to unfold the domains varied, with the least and most stable domains unfolding at forces <50 pN and >100 pN, respectively, suggesting that a mechanical hierarchy exists along cMyBP-C. EM images of purified, rotary shadowed cMyBP-C showed that molecules were frequently V- or U-shaped with lengths ~44 nm. These data indicate that cMyBP-C is extensible and contains regions with variable resistances that could slow sarcomere shortening or limit lattice expansion. Supported by NIH HL080367.

3074-Pos

Active Force Clamp Control of Optical Tweezers

Heikki Ojala[1], Kalle Hanhijärvi[1], Anders E. Wallin[1], Gabija Ziedaite[2], Dennis Bamford[2], Edward Hæggström[1].
[1]Department of Physics, University of Helsinki, Helsinki, Finland, [2]Institute of Biotechnology and Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland.

In a typical high-resolution optical tweezers (OT) experiment a molecular motor changes the contour length of a trapped dumbbell-construct. Unless the inter-trap distance is actively controlled the OT increases the load on the molecular motor as it steps along the template. To counter this phenomenon we implement a real-time controller for the OT to be used in constant force measurements.

We trap a dumbbell construct (bead-DNA-bead) in an inverted microscope by dividing a CW laser beam into a stationary trap and a steerable trap. Separate low power detection lasers and position sensitive detectors in the back-focal plane measure the position of both beads. The position of the bead in the stationary trap is used for constant-force feedback control. The feedback algorithm runs a Proportional-Integral-Derivative-controller on a field programmable gate array, and acousto-optical deflectors update the steerable trap position at a rate of 200 kHz.

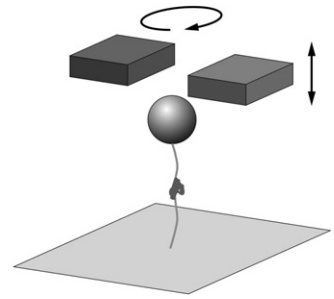
We test the force clamp control with a 10kb dsDNA molecule and present a theory explaining the power spectrum of the force clamped bead's position. We study the effect of controller bandwidth by digitally filtering the signal used for feedback control, and test the response time of our real-time controlled optical tweezers with a RNA hairpin opening/closing reaction.

3075-Pos

Protein-DNA Interactions Studies using Magnetic Tweezers

Matthew J. Wiggin, Nynke Dekker.
TU Delft, Delft, Netherlands.

Single molecule force spectroscopy techniques allow the forces, energy barriers, and mechanisms of biologically important structural transitions to be manipulated and observed at the single molecule scale. A major advantage of magnetic tweezers is the ability to manipulate not only the force in such systems, but also the torque, which is extremely important in processes involving the DNA double helix, since such processes frequently involve rotational motion. We will present recent results studying protein-DNA interactions using a newly constructed magnetic tweezer.



3076-Pos

Single-Molecule Atomic-Force Spectroscopy Captures a Novel Class of Molecular Nanosprings with Robust Stepwise Refolding Properties

Minkyu Kim[1], Khadar Abdi[1], Gwangrog Lee[2], Mahir Rabbi[1], Whasil Lee[1], Ming Yang[3], Christopher J. Schofield[3], Vann Bennett[1], Piotr E. Marszalek[1].
[1]Duke University, Durham, NC, USA, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]University of Oxford, Oxford, United Kingdom.

Biological systems are constantly under mechanical stress either during movement or when acted upon by external forces. The identification of proteins motifs that behave as biological springs will be important for understanding how cells respond to mechanical stimuli and can also propel the design of non-biological nanomaterials. We report here identification of a large class of alpha-helical spiral or solenoid-shaped proteins comprised of ANK-R, ARM, or HEAT repeats that rapidly and forcefully refold following stretching. Each of these repeats unfolds and refolds in equilibrium through discrete events involving