observed in the dry state, both of which are consistent with literature values for CaCO3.

Molecular Motors & Force Spectroscopy II

3304-Pos Board B351

Single Molecule Force Spectroscopy and Steered Molecular Dynamics Simulations Reveal the Mechanical Design of the Third FnIII Domain of Tenascin-C

Qing Peng, Shulin Zhuang, Meijia Wang, Yi Cao, Yuanai Khoo, Hongbin Li.
University of British Columbia, Vancouver, BC, Canada.

By combining single molecule atomic force microscopy, proline mutagenesis and steered molecular dynamics simulations, we investigate the mechanical unfolding dynamics and mechanical design of the third FnIII domain of tenascin-C (TNfn3) in detail. The mechanical stability of TNfn3 is found to be similar to that of other constituting FnIII domains of tenascin-C, and the unfolding process of TNfn3 is an apparent two-state process. The hydrophobic core packing of TNfn3 was previously reported as the key element of its mechanical stability. Here, employing proline mutagenesis to block the formation of backbone hydrogen bonds and introduce structural disruption in β sheet, we showed that not only hydrophobic core packing plays important roles in determining the mechanical stability of TNfn3, backbone hydrogen bonds in β hairpins are also responsible for the overall mechanical stability of TNfn3. Furthermore, proline mutagenesis revealed that the mechanical design of TNfn3 is very robust and proline substitution in β sheets only leads to mild reduction in mechanical stability. We also compare the AFM results with those of SMD simulations to understand the molecular details underlying the mechanical unfolding of TNfn3. We found that the mechanical unfolding and design of TNfn3 is significantly different from its structural homologue the tenth FnIII domain from fibronectin. These results serve as a starting point for systematically analyzing the mechanical architecture of other FnIII domains in tenascins-C and will help to gain a better understanding of some of the complex features observed for the stretching of native tenascin-C.

3305-Pos Board B352

Single-molecule Force Spectroscopy Reveals Engineered Metal Chelation Is A General Approach To Enhance Mechanical Stability Of Proteins

Hongbin Li, Yi Cao, Teri Yoo.
University of British Columbia, Vancouver, BC, Canada.

Significant mechanical stability is an essential feature shared by many elastomeric proteins, which function as molecular springs in a wide variety of biological machinery and biomaterials of superb mechanical properties. Despite the progress in understanding molecular determinants of mechanical stability, it remains challenging to rationally enhance the mechanical stability of proteins. Using single molecule force spectroscopy and protein engineering techniques, we demonstrate that engineered bi-histidine metal chelation can enhance the mechanical stability of proteins significantly and reversibly. Based on simple thermodynamic cycle analysis, we engineered a bi-histidine metal chelation site into various locations of the small protein, GB1, to achieve preferential stabilization of the native state over the mechanical unfolding transition state of GB1 through the binding of metal ions. Our results demonstrate that the metal chelation can enhance the mechanical stability of GB1 by as much as 100 pN. Since bi-histidine metal chelation sites can be easily implemented, engineered metal chelation provides a general methodology to enhance the mechanical stability of a wide variety of proteins. This general approach in protein mechanics will enable the rational tuning of the mechanical stability of proteins. It will not only open new avenues toward engineering proteins of tailored nanomechanical properties, but also provide new approaches to systematically map the mechanical unfolding pathway of proteins.

3306-Pos Board B353

Mechanical function and Biophysical Properties of the REJ region of Polycystin-1

Meixiang Xu, Liang Ma, Odutayo Odunuga, Edwardo Delgado, Andres Oberhauser.
UTMB, Galveston, TX, USA.

Mutations of Polycystin-1 (PC1) account for 85% Autosomal dominant polycystic kidney disease (ADPKD), which is the most common life-threatening inherited disease worldwide. PC1 has been implicated to be involved in renal tubule and kidney morphogenesis as a mechanosensor and transduce the signals into cellular response. Most domains of the long PC1's ectodomain of are mechanical stable Ig-like motifs and may function as effective force transmitters to regulate the multi-function properties of PC1. The REJ region is a major component of PC1s ectodomain (30% or ~1000 aa); however its structure and function remains unknown. Here we used protein engineering in combination with single-molecule AFM and circular dichroism (CD) techniques to elucidate the structure and mechanical properties of this region. Our studies indicates that the REJ region has complex mechanical properties. Stretching a protein construct which includes four PKD Ig-like domains and the complete REJ region, resulted in saw-tooth patterns with 3-10 force peaks with a wide range of unfolding forces of 50-250 pN, suggesting that the extra force peaks must originate from the REJ region. We also made several REJ constructs (I27)3-REJ FN4-(I27)2 and (I27)3-REJ FN3,4-(I27)2 and expressed them in bacteria and insect cells. Stretching these constructs generated peaks characteristic of the unfolding of titin I27 as well as other more complex unfolding events which we attribute to the unfolding of REJ domains. The complexity of the REJ domain unfolding force patterns suggests that these domains may have unfolding intermediates. These results support the hypothesis that PC1 is a mechano-transducer with a novel molecular architecture and elastic properties well-suited for sensing and transmitting distinct mechanical signals with a wide range of strengths.

This work is funded by the NIH (R01DK073394) and by the Polycystic Kidney Foundation (grant 116a2r).

3307-Pos Board B354

Effect Of Temperature On The Mechanical Properties Of Fibronectin

Isaac T.S. Li[1], Gilbert C. Walker[1,2].
[1]University of Toronto, Toronto, ON, Canada, [2]University of Pittsburgh, Pittsburgh, PA, USA.

Fibronectin (Fn) is a multi-domain protein in the extracellular matrix whose primary function is to provide mechanical strength for cell adhesion. In particular the fibronectin cell binding fragment containing exclusively fibronectin type III repeats (FnIII) are studied due to their similarity in structure, their mechanical strength and their direct involvement in cell binding. Previous experimental studies on the mechanical properties of FnIII using single molecule force spectroscopy have focused on the mechanical strength hierarchy of FnIIIs and the folding intermediate of FnIIIs under physiological conditions. Here, we want to explore the mechanical unfolding of FnIII under conditions that disrupt the folding of the protein. In particular, we report studies of the effect of temperature on the mechanical strengths of FnIII.

3308-Pos Board B355

Nanomechanical Manipulation Of Skeletal-muscle Titin With Force-ramp Optical Tweezers

Zsolt Martonfalvi.
University of Pecs, Pecs, Hungary.

Titin is a filamentous protein that spans the half sarcomere and functions as a molecular spring, a sarcomeric template, and possibly as a mechanosensor. The molecule has become a popular experimental model for exploring mechanically-driven protein folding because of its structure as a tandem array of similar beta-barrel domains. The force versus extension curve of titin, recorded in constant-displacement-rate experiments, is characterized by entropic-chain behavior onto which sudden, stepwise contour-length fluctuations caused by domain unfolding are superimposed. Recent force-clamp experiments revealed a complex, multi-stage force response during folding, suggesting that the unfolded chain collapses not solely by entropic mechanisms.

To explore the nanomechanical detail in titin's folding and unfolding, here we stretched single molecules of purified skeletal-muscle titin with force-ramp optical tweezers. Titin was extended in cycles of stretch and relaxation, during which the loading rate was kept constant by using a fast (500 Hz) feedback. Loading rates ranged between 1-10 pN/s, and minimal relaxation loads were 2-10 pN. We found that above 50 pN partially unfolded titin molecules often deviated significantly from the pure wormlike-chain behavior and displayed a variable stretch modulus of tens to hundreds of pN. When partially unfolded titin was relaxed to 2 pN and restretched immediately, we did not observe significant domain refolding. Thus, while enthalpic elasticity mechanisms may assist the collapse of the unfolded and extended protein chain, complete domain refolding requires longer periods of time spent in a highly contracted state at low (< 2 pN) forces.

3309-Pos Board B356

Mechanical Properties of Type IV Pili in Pseudomonas Aeruginosa

Shun Lu[1], Ahmed Touhami[1], Hanjeong Harvey[2], Edie Scheurwater[2], Lori Burrows[2], John R. Dutcher[1].
[1]Department of Physics, University of Guelph, Guelph, ON, Canada, [2]Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada.

Type IV pili (Tfp) are thin flexible protein filaments that extend from the cell membrane of bacteria such as *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*. The mechanical properties of Tfp are of great importance since they

allow bacteria to interact with and colonize various surfaces (1). In the present study, we have used atomic force microscopy (AFM) for both imaging and pulling on Tfp from *P. aeruginosa* (PAO1) and from its PilA, PilT, and PilB mutants. A single pilus filament was mechanically stretched and the resulting force-extension profiles were fitted using the worm-like-chain (WLC) model. The statistical distributions obtained for contour length, persistence length, and number of pili per bacteria pole, were used to evaluate the mechanical properties of a single pilus and the biogenesis functions of different proteins (PilA, PilT) involved in its assembly and disassembly. Importantly, the persistence length value of ~ 1 μm measured in the present study, which is consistent with the curvature of the pili observed in our AFM images, is significantly lower than the value of 5 μm reported earlier by Skerker *et al.* (2). Our results shed new light on the role of mechanical forces that mediate bacteria-surface interactions and biofilm formation.

References:
1- L. Craig, M.E. Pique and J.A. Tainer, Nat. Rev. Microbiol., 2, 363-378 (2004).
2- J.M. Skerker and H.C. Berg, Proc. Natl. Acad. Sci. USA, 98, 6901-6904 (2001).

3310-Pos Board B357

Pathogenic Mutations alter the Mechanical Stability of Polycystin-1 Immunoglobulin PKD Domains

Liang Ma, Mexiang Xu, Andres Oberhauser.
UTMB, Galveston, TX, USA.

Mutations in polycystin-1 can cause Autosomal Dominant Polycystic Kidney Disease (ADPKD), which is a leading cause of renal failure. The available evidence suggests that PC1 acts as a mechanosensor, receiving signals from the primary cilia, neighboring cells, and extracellular matrix. polycystin-1 is a large membrane protein that has a long N-terminal extracellular region (about 3000 aa) with a multimodular structure including sixteen Ig-like PKD domains, which are targeted by many pathogenic missense mutations. The mechanical properties of the wild-type PKD domains are known; however, nothing is known about the effects of pathogenic mutations on the biophysical properties of PKD domains. Here we investigated the mechanical properties of several pathogenic missense mutants on the first Ig-like domain (PKD-d1) by using a combination of protein engineering and single-molecule atomic force spectroscopy. We found that missense mutations alter the mechanical unfolding and refolding pathways of PKD-d1 resulting in distinct mechanical phenotypes. Using a simple 2-state energy diagram we find that point mutations can affect the free energy of mechanical unfolding/refolding and the position of the transition state. Depending on the pulling speed we find that a mutation can increase or decrease the unfolding forces. This indicates that the pathogenic mutants can affect the PKD domain's normal response to external mechanical forces and may help us understand the molecular mechanisms underlying ADPKD.

This work is funded by NIH (grant R01DK073394) and by the Polycystic Kidney Foundation (grant 116a2r).

3311-Pos Board B358

Calibration Of Optical Tweezers In Viscoelastic Media

Mario Fischer[1], Andrew C. Richardson[2], S. Nader S. Reihani[3], Lene B. Oddershede[2], **Kirstine Berg-Sorensen[1]**.
[1]Technical University of Denmark, Kgs Lyngby, Denmark, [2]University of Copenhagen, Copenhagen, Denmark, [3]Institute for Advanced Studies in Basic Sciences, Zanjan, Iran, Islamic Republic of.

The application of optical traps to manipulate or monitor intracellular processes in in vivo systems calls for precise procedures both to evaluate the characteristics of the trap and the viscoelastic properties of the intracellular medium. Here, we verify experimentally the authenticity of a calibration procedure, specifically developed for use in calibration of optical tweezers in complex, viscoelastic media (Fischer and Berg-Sørensen, J. Opt. A: Pure Appl. Opt. 9, S239 (2007)). We demonstrate the application of the procedure in i) water and in ii) entangled F-actin without cross-linkers. As the theoretical background of the procedure is essentially convenient formulations of the fluctuation-dissipation theorem, we name the procedure as the ''FDT-method''. The analysis of the measurements in water may be validated directly: We can compare the results for the spring constant to results obtained with well-established methods and those for the viscoelastic properties to theoretical predictions. In the actin solution, we may similarly compare our results with results of microrheology experiments in the literature.

3312-Pos Board B359

Understanding Receptor Kinetics And Mechanics In Phagocytosis Uptake Using Deformable Polyelectrolyte Microcapsules As Force Sensors

Vamsi Kodali[1,2], James Larsen[1,2], Stephan Schmidt[3], Andreas Fery[3], Jennifer E. Curtis[1,2].
[1]School of Physics, Georgia Institute of Technology, Atlanta, GA, USA, [2]Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, Atlanta, GA, USA, [3]Lehrstuhl für Physikalische Chemie II, Universitt Bayreuth, Bayreuth, Germany.

Macrophages play a dominant role in early host defenses against infection. Their defense consists of the ingestion of unwanted objects via an actin-driven membrane deforming process called phagocytosis. Understanding the receptor kinetics that trigger phagocytosis and the tightly controlled mechanics that drive this extremely expedient event is difficult. Here we present a new method of measuring phagocytotic kinetics and mechanics using deformable, mechanically calibrated polyelectrolyte microcapsules - a hollow shell approximately 4.5 μm in diameter. IgG biofunctionalized capsules are readily ingested by macrophages. The forces exerted by the cell during uptake are measured by visualization of the deformed capsules throughout uptake. We have established the lower limit of phagocytotic forces by identifying which capsules of which strength collapse during phagocytosis. We have found capsules that buckle at 130-150nN as measured by AFM, deform and then buckle during phagocytosis. Using this method, we can monitor subtle changes in the capsule shape throughout the event, including the classic squeezing deformation that arises from a contractile actin belt that travels up around the particle as it is consumed. This method can be extended to unravel the roles of the diverse molecular species involved in phagocytosis including several different myosin motors, actin binding proteins and other signaling molecules. Using drugs or molecular biology to interfere with certain molecules, the resultant change in the deformation sequence sheds light on the suppressed molecule's role. In our first series of experiments, PI3-Kinase inhibitor LY294002 has been applied to the macrophages. These preliminary experiments have shown that microcapsules that typically collapse become extremely deformed, no longer collapse. We interpret these results to indicate that PI3-Kinase is required for the closure of the phagocytotic cup.

3313-Pos Board B360

Direct Observation of Protein Complex Disassembly by Single Molecule Force Spectroscopy

Morten Bertz, Matthias Rief.
TU München, Garching, Germany.

In recent years, single molecule force spectroscopy has opened unique possibilities to investigate the mechanical properties of single protein molecules. So far, experiments have focused on the mechanical behavior of a protein's tertiary structure. For a large number of proteins, however, multiple folded protein molecules are arranged into a multi-subunit complex. How the quaternary structure of such a complex responds to force is not clear. Here, we present a toolkit to study the mechanical properties of dimeric and trimeric protein complexes by single molecule force spectroscopy. We apply these methods to two different model systems:

The dimeric α-crystallin domain is the building block of Hsp 16.5 from Methanococcus janashii. We can directly observe that this dimer dissociates at ~ 200 pN into two metastable monomeric subunits, which subsequently unfold independently.

The two most N-terminal domains of human titin are assembled into an antiparallel complex by telethonin in the Z-disk region of the sarcomere. We show that the Ig domains of titin are stabilized in the presence of telethonin, and that this stabilization is optimized to provide a high level of mechanical strength in the sarcomere. The dissociation force of the titin-telethonin complex exceeds 600 pN, making it one of the highest rupture forces known to date.

Single molecule force spectroscopy allows us not only to measure directly the dissociation forces of protein complexes, but also to observe the mechanical hierarchy of the involved building blocks. Building on these results, future experiments will attempt to observe the assembly of protein complexes using single molecule force spectroscopy.

3314-Pos Board B361

Calcium Dependent Dynamics and Forced Unbinding of Cadherins

Marcos Sotomayor, David P. Corey.
Harvard Medical School / HHMI, Boston, MA, USA.

Members of the cadherin superfamily of proteins have been implicated in selective cell adhesion, signaling, and more recently in mechanical processes such as sound transduction. While it is well known that the extracellular repeats of cadherin proteins mediate cell-cell adhesion in a calcium-dependent manner, the molecular mechanisms behind the influence of calcium in cadherin's adhesion dynamics and its mechanical response are not well understood. In addition, the architecture and strength of adhesion complexes formed by different members of the cadherin family remain debated. Here we report molecular dynamics simulations of classical cadherins type I (C-cadherin) and type II (Cadherin-8 and Cadherin-11) involving systems with up to 355,000 atoms. The simulations show how calcium ions control the structural integrity of cadherin's linker