

observed in the dry state, both of which are consistent with literature values for CaCO<sub>3</sub>.

## 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

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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  $\beta$  sheet, we showed that not only hydrophobic core packing plays important roles in determining the mechanical stability of TNfn3, backbone hydrogen bonds in  $\beta$  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  $\beta$  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

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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

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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 are of 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 indicate 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.

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### 3307-Pos Board B354

#### Effect Of Temperature On The Mechanical Properties Of Fibronectin

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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

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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*

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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