matrix metalloprotease MMP1 on Type I collagen fiber from rat tail. Collagen is the most abundant protein in humans. Fibril forming Type I collagen is the main component of the extracellular matrix (ECM) that supports and defines most tissues. Degradation of collagen in the ECM by matrix metalloproteases (MMPs) is an important process in tissue remodeling. By tracking single fluorescently labeled MMPs moving on a collagen substrate, we were able to characterize the diffusive motion with high temporal resolution. These measurements suggest that proteolytic cleavage of the collagen substrate by Wild Type MMP1 (WT MMP1) both biases and hinders the diffusive motion of MMP1 on the collagen fiber. Both bias and hindrance are temperature dependent for WT MMP1. The diffusion was neither hindered nor biased for a point-mutant MMP1 and for MMP9, both of which are incapable of cleaving native Type I collagen from rat tail. To separate the effects of hindrance and bias, we specifically created hindrance by incubating collagen with WT MMP1 prior to measuring the motion of a catalytically inactive mutant MMP1. The resulting nonlinear diffusion of mutant MMP1 was characteristic of diffusion in hindered space. These results provide insight into the process of collagen degradation.

3914-Pos

High Frequency Asynchronous Rotation of Magnetic Microspheres and Biophysical Applications - Higher Sensitivity Regime for Magnetic Bead Biosensors

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Asynchronous rotation is an emerging platform technique with applications ranging from micro mixing to femtoliter viscometry and biophysical sensors (Applied Physics Letters 91, 224105 (2007)). Asynchronous rotation occurs when a driving magnetic field exceeds a critical frequency, above which the driving field is rotating faster than the driven body. The critical frequency depends on the viscosity of the fluid, size of the driven system, and the strength and quality of the driving field. The dynamics of a magnetic bead rotating at high frequencies were studied using a simple setup, consisting of a bright field microscope that was used to focus a 5 mW laser onto the particle of interest. The rotational frequency of the particle was measured by analyzing the intensity modulation of the laser beam, focused through the particle. Previously reported asynchronous rotation frequencies of magnetic beads range from 100 mHz to 12 Hz. Here, we report a system with an order of magnitude higher asynchronous rotation frequency, in water. The mixing efficiency and sensor sensitivity depend on the rotational frequency of the microsphere, among other factors. Higher rotational frequencies enable increased sensitivity of drag-based sensors, and also open up new capabilities for the method, such as novel force spectroscopy studies.

3915-Pos

Measurement of the Elastic Modulus of Individual Type I Collagen Fibrils

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The primary focus of this work was to measure the mechanical properties of hydrated individual type I collagen fibrils using atomic force microscopy (AFM) techniques. In particular, we are investigating anisotropy in the elastic modulus of these fibrils. Radial measurements were performed by compressing the fibril between the tip of an AFM cantilever and the flat substrate on which the fibril was supported. The elastic modulus was extracted from this data using a Hertzian analysis. To investigate the axial properties, a three-point bending technique was used. The fibril was suspended between adhesive supports and deflected centrally by the AFM tip. Here, the bending modulus was found by fitting the data to the equation describing the bending of an elastic beam affixed and supported at each end. Both the radial and the axial experiments were performed on fibrils assembled from pepsin digested collagen monomers, and the results were compared with similar measurements perform on intact, native fibrils. The outcomes are of interest to those investigating extracellular matrix mechanics, especially with application to cell differentiation and tissue engineering.

3916-Pos

How Do Osmolytes affect the Stability of Polycystin-1 PKD Domains?

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Autosomal dominant polycystic kidney disease is one of the most common life-threatening genetic diseases, and is a leading cause of renal failure. The majority of cases are caused by mutations in the PKD1 gene, which encodes for polycystin-1 (PC1). PC1 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. PC1's extracellular domain may function as a mechanical antenna that senses mechanical cues such as shear flow converting them into signaling processes that control cell differentiation and growth. PC1 is expressed along the renal tubule, where it is exposed to a wide range of concentrations of urea (from 5mM in the proximal tubule to up to ~800mM in the collecting duct). Urea is known to destabilize proteins. Other osmolytes found in the kidney such as sarcosine, betaine and trimethylamine N-oxide (TMAO) are known to counteract urea's negative effects on proteins. Here we used nano-mechanical techniques to study the effects of osmolytes on the biophysical properties of PC1's PKD wild-type and mutant domains. Upon increasing the concentration of urea we observe a systematic decrease in the mechanical stability. We also found that the refolding rate constant is slow down by urea (as much as ~5-fold at 2M). Moreover, we found that stabilizing osmolytes can effectively counteract the effect of urea at a ratio of 1:1 (urea/sarcosine) or 1:0.5 (urea/TMAO). We recently reported that some pathogenic missense mutations can significantly destabilize PKD domains. Interestingly, we found that stabilizing osmolytes increase the mechanical strength of a mutant domain. Our studies have the potential to provide new therapeutic approaches (*e.g.* through the use of osmolytes or chemical chaperones) for rescuing destabilized and misfolded mutant PKD domains.

3917-Pos

Real Time Detection of Mechanical Stress in Specific Cytoskeletal Proteins

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A molecular force sensor cassette (stFRET) was incorporated into actinin, filamin, and spectrin in endothelial cells and into collagen-19 in C. elegans. In vitro double strand DNA stretching essay confirmed the force sensitivity of stFRET. stFRET detected constitutive stress in all the proteins. In endothelial cells, the stress in actinin, filamin and spectrin could be eliminated by releasing focal attachments from the substrate. Our data also indicated the highest resting strain in spectrin in three cytoskeleton protein tested. When the intact C. elegans worm was reversibly stretched using micromanipulators, stFRET reversibly sensed the force in collagen-19. stFRET is a general purpose dynamic sensor of mechanical stress in filamentous proteins that can be expressed in cells in vitro and in whole animals.

We have developed another force sensor, named sstFRET, in which we substituted the alpha helix linker with a spectrin repeat domain. Our in vitro DNA stretching assay showed that 20 pN force is sufficient to deform the spectrin repeat linker, leading to a dramatic change of FRET. By inserting sstFRET into alpha-actinin, we succeeded in monitoring the change in actinin constitutive stress before and after cells are osmotically challenged. In addition, we observe substantially lower force loads on actinin in bovine aortic endothelial cells (BAEC) than that in human embryo kidney (HEK) cells.

3918-Pos

Unfolding and Refolding Dynamics of Filamin a Protein under Constant Forces

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Filamin A is an important cytoskelton cross-linking protein containing 24 immunogloble domains. Force spectroscopy of Filamin A has been studied by AFM. We use a new technology to study the response of Fimalin A to stretching force. When we apply a constant force ~50 pN to unfold a construct that is composed of 1-8 domains, distinct and nearly equal unfolding steps are observed (Fig. 1). When decreasing force to ~5 pN, refolding events of several previously unfolded domains is observed (Fig. 2). At the same time, from the force extension curve of unfolded proteins, the persistence length of polypeptide is estimated to be ~0.6 nm.