2041-Pos Board B11
Viscosity Effect On The AFM Force Measurement
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Atomic force microscopy based techniques have been used in the investigation of protein folding/unfolding in order to study the protein properties at the single molecule level. These experiments are performed in buffer solution, where the AFM cantilever is used to exert and measure the mechanical forces on the protein molecules under study. Due to the motion of the cantilever relative to the liquid, a viscous drag force, which can be significant when the liquid is viscous or the cantilever's speed is high, acts on the cantilever. The irregular shape of the liquid chamber and cantilever, and the special boundary conditions of the fluid make this problem complicated and difficult to solve analytically. We measured the viscous drag forces on different cantilevers for several different cantilever speeds in solutions of different viscosities. The results show that the viscous drag on a cantilever is determined by its geometry, its relative speed, the viscosity of the solution, and the separation between the cantilever and the sample surface. This method will be useful to make corrections to the unfolding force data of proteins as well as that of AFM based force measurements.

2042-Pos Board B12
AFM Visualization and Force Spectroscopy of Clathrin Triskelia
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We have applied atomic force microscopy (AFM) and single molecule force spectroscopy (SMFS) to characterize the structure and molecular mechanics of clathrin triskelia. The latter are the basic building blocks of the protein coat surrounding plasma-membrane-derived vesicles involved in receptor-mediated endocytosis. Here we resolve variable profiles of individual triskelia on mica surfaces for the first time by AFM, at a resolution comparable to that of electron microscopy. Classical three-leg, filamentous pin-wheel shapes, as well as dimers and non-planar triskelion conformations, are readily observed. Time sequences of AFM images clearly demonstrate conformation fluctuations in single triskelia when the latter are in physiological solutions. Additionally, AFM SMFS reveals, also for the first time, a series of internal energetic barriers that characterize triskelion heavy chain domain folding, corresponding to numerous alpha-helix hairpins of ca. 30 amino acid residues, and variable unfolding of larger, cooperative hairpin domains up to the size of the known repeating motif of ca. 145 amino acid residues. The dynamic domain rupture force ranges from a few 10s pN to over 500 pN, increasing continuously as the stretching loading rate changes over the range from 100 to 10^5 pN/s. The results once again show AFM to be a powerful tool for biomedical imaging and nanometric single molecule characterizations.

2043-Pos Board B13
Force Spectroscopy Of The Interaction Of Fibrinogen With Erythrocytes And Platelets
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Increased values of erythrocyte aggregation are associated with an augmented risk of cardiovascular and cerebrovascular conditions. The prevailing hypothesis for the mechanism of erythrocyte hyperaggregation is due to an increase in plasma adhesion proteins, particularly fibrinogen. Fibrinogen is a blood-borne glycoprotein comprised of three pairs of distinguishable polypeptide chains with three potential integrin-binding sites, but can also interact with cells through non-integrin receptors. Currently, fibrinogen-induced erythrocyte aggregation is thought to be caused by non-specific protein binding to erythrocytes membranes. In contrast, platelets are known to have integrin receptors expressed on the membrane surface (the $\alpha_{IIb}\beta_3$ glycoprotein complex). In the present work we evaluated the possible existence of a binding between human fibrinogen molecules and an unknown receptor on human erythrocytes. For the sake of comparison, an equivalent study was conducted with human platelets. The interactions were studied under physiological conditions by force spectroscopy, using an atomic force microscope (AFM) with fibrinogen-functionalized tips. Blood cells were deposited on poly-L-lysine treated glass slides. Single molecules were stretched by tip pulling after pressing the cantilevers on the samples. AFM images were carried out in tapping mode and force spectroscopy measurements in contact mode. The interaction forces between a fibrinogen-modified AFM probe and platelet/erythrocyte surface were determined from pN to nN levels. The main conclusion from our results is that there is a single molecule interaction between fibrinogen and an unknown receptor on erythrocyte membrane, with a lower affinity when compared with platelet binding. The existence of a membrane receptor involved on the fibrinogen-induced erythrocyte aggregation suggests the possibility of a drug therapy that could result in a significant decrease of erythrocyte aggregation in patients with different pathologies (e.g., hypertension and diabetes).

2044-Pos Board B14
Force Transduction In Smooth Muscle Cells
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Mechanical forces directly affect the form and function of tissues. Transmission of force from outside the cell through focal and junctional adhesions controls the maturation or disassembly of these adhesion sites and initiates intracellular signaling cascades that alter cellular behavior. To understand the mechanism by which living cells sense mechanical forces, and how they respond and adapt to their environment, a critical first step is to develop a new technology able to investigate cellular behavior at sub-cellular level that integrates an atomic force microscope (AFM) with total internal reflection fluorescence (TIRF) microscopy and fast-spinning disk (FSD) confocal microscopy, providing high spatial and temporal resolution. The integrated system is broadly applicable across a wide range of molecular dynamic studies in any adherent live cells, allowing direct optical imaging of cell responses to mechanical stimulation in real-time.
Thus, we are able to: (i) image with high spatial resolution or stimulate the apical cell surface using AFM, and (ii) quantitatively time-lapse image the cell-coverslip interface using TIRF, or FSD confocal image to study molecular dynamics and protein translocation between different sub-cellular structures. Significant rearrangement of the actin filaments and focal adhesions was shown due to local mechanical smooth muscle cell stimulation at the apical cell membrane that induced changes into the cellular structure throughout the cell body. By exploring innovative approaches like those used in these investigations, new information for understanding live cell restructuring and dynamics in response to mechanical force can be provided. Understanding how live cells adapt to mechanical force and how they are able to recognize and respond to mechanical stimuli represents an important biophysical problem.

2045-Pos Board B15
Using Atomic Force Microscopy to Measure Mechanical Strength of Nanometre Scale Protein Fibrils
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Mechanical properties are routinely measured on both biological and man-made fibres on the macro- and micro-scale to give valuable information on properties such as elasticity (Young's modulus), shear and rupture force. Here, methods will be described to progress such mechanical measurements to samples on the nanometer size scale, using atomic force microscopy (AFM). This technique is used to first localise and visualise a single fibril on a patterned surface with regularly repeating plateaus and troughs; controlled motion of the tip then is used to push down on a fibril hanging over a trough or pull it sideways while recording the force exerted on the fibril as it is being stretched. Performing such measurements over a range of forces allows the determination of the elastic Young's modulus as well as observation of non-elastic (irreversible) extension and ultimately the rupture force of protein fibrils with diameters down to a few nanometers.
Results will be presented for a range of protein fibrils. In particular, the properties of collagen fibrils with diameters on the order of 5 nm will be compared to those obtained for thicker collagen fibres.

2046-Pos Board B16
Mechanics of Spontaneously Beating Cardiac Myocytes Investigated by AFM
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Cardiac myocytes in vitro contract spontaneously as they are cultured under appropriate conditions. In order to clarify the contractile mechanism of cardiac myocytes, it is basically important to investigate the mechanical properties of cardiac myocytes. The atomic force microscope (AFM) is now a powerful tool for measuring the mechanics and the dynamics of cells at a single cell level. Previous studies revealed that the AFM allowed us to measure the amplitude and frequency of cells at their several positions, however the dynamic behavior has not been investigated as a function of cell position. In this study, we measured the spatial-dependent contractility of spontaneously beating