behaving as an apparently linear sensor of the mechanical environment. This could serve as a built-in safety mechanism for protecting the sarcomere against structural disintegration.

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Glass: A Multi-Platform Specimen Supporting Substrate for Precision Single Molecule Studies of Membrane Proteins
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High resolution (≈1 nm lateral resolution) biological AFM imaging has been carried out almost exclusively using freshly cleaved mica as a specimen supporting surface, but mica suffers from a fundamental limitation that has hindered AFM's broader integration with many modern optical methods. Mica exhibits biaxial birefringence; indeed, this naturally occurring material is used commercially for constructing optical wave plates. In general, propagation through birefringent material alters the polarization state and bifurcates the propagation direction of light in a manner which varies with thickness. This makes it challenging to incorporate freshly cleaved mica substrates with modern optical methods, many of which employ highly focused and polarized laser beams passing through then specimen plane. Using bacteriorhodopsin from Halobacterium salinarum and the translocon SecYEG from Escherichia coli, we demonstrate that faithful images of 2D crystalline and non-crystalline membrane proteins in lipid bilayers can be obtained on common microscope cover glass following a straight-forward cleaning procedure. Direct comparison between data obtained on glass and on mica show no significant differences in AFM image fidelity. This work opens the door for combining high resolution biological AFM with powerful optical methods that require optically isotropic substrates such as ultra-stable1 and direct 3D AFM2. In turn, this capability should enable long timescale conformational dynamics measurements of membrane proteins in near-native conditions.
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Force Spectroscopy of DNA-Ctab Aggregates
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Aggregates of oligonucleotides and the surfactant, CTAB, have been studied with force spectroscopy using an atomic force microscope. Gold coated AFM tips were derivatized with long carbon chain thiols and thiols functionalized with carboxylic acid and amine head groups and used to measure the strength of interaction or adhesive force between the AFM tip and aggregate surface. Force curves were obtained at various locations on the aggregate surface showing consistently reproducible adhesion within a given spot and some variability in adhesion from one spot to another. Adhesive forces were also measured in the same way on a model system composed of self-assembled monolayers (SAMs) formed using the thiols mentioned above on gold coated silicon wafers for comparison. Forces of attraction between the derivatized tip and the model SAM surface were quite a bit lower compared to measurements made on aggregates. Some force curves obtained from aggregate surfaces showed broad retraction traces indicative of a strong adhesive force that may include a cascade of detachment steps. Currently, friction (lateral) force experiments are underway on both the aggregate surface and the model system to determine the surface free energy between the tip and surface and the extent of homogeneity on the aggregate.

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Fibrinogenesis and Fibrinolysis Followed with Nano-Thrombelastography
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Hemostasis is a complex process that relies on a sensitive balance between the formation and breakdown of the thrombus, a three-dimensional polymer network of the fibrous protein fibrin. Neither the details of the fibrinogen-fibrin transition, nor the exact mechanisms of thrombus degradation are fully understood at the molecular or supramolecular level. We investigated nanoscale changes in the viscoelasticity of the 3D-fibrin network during fibrinogenesis and streptokinase (STK)-induced fibrinolysis by using a novel, atomic-force-microscope (AFM)-based application of force spectroscopy, named nano-thrombelastography.
Clot formation was initiated by adding Ca^{2+} to fresh, anti-coagulated mixed human plasma droplet on a glass surface. In order to induce fibrinolysis, STK, at a final enzyme activity of up to 10,000 IU was applied in situ. For measuring the nanoscale elastic and viscous properties of the fibrin network, the tip of an AFM cantilever was immersed in the plasma droplet and oscillated vertically with a constant rate. The cantilever bending was correlated with fibrin-clot elasticity and viscosity in time. Morphological changes were followed by scanning AFM on polymerized fibrin deposited on mica surface.
Whereas the global features of the time-dependent change in cantilever deflection corresponded well to a macroscopic thrombelastogram, the underlying force spectra revealed large, sample-dependent oscillations in the range of 3-50 nN and allowed the separation of elastic and viscous components of fibrin behavior. Upon STK treatment the nano-thrombelastogram signal decayed gradually. The decay was driven by a decrease in thrombus elasticity, whereas thrombus viscosity decayed with a time delay. In scanning AFM images mature fibrin appeared as 17-nm-high and 12-196-nm-wide filaments. STK-treatment resulted in the decrease of filament height and the appearance of a surface roughness with 23.7 nm discrete steps that corresponds well to the length of a fibrinogen monomer.

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Direct Observation of Titin Immunoglobulin Domain Unfolding-Refolding in Muscle Sarcomeres
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Titin is the largest protein in the human body. Constituted by Immunoglobulin-like (Ig) domains and unstructured regions, titin determines the passive elasticity of muscle tissue. Current models of muscle elasticity propose a central role for the extension of its unstructured regions at low force, while relegating Ig domains unfolding reactions as a safety mechanism triggered only by unphysiologically high forces. Here we combine single-particle tracking and single-molecule force spectroscopy to observe the dynamics of Ig domains from I-band under physiological forces. Using quantum dots and centroid tracking techniques we show that in single myofibrils extended to an optimal sarcomere length of 3.1 μm, titin molecules labeled in situ undergo stepwise changes in length of 13 ± 3 and 22 ± 3 nm. Additionally, single-molecule experiments on a proximal native fragment of titin shows that the folding/unfolding step sizes of the proximal Ig domains of 10.4 ± 3.6 and 21.8 ± 4.7 nm, at 8 pN of force. These extensions closely match the distribution of step sizes observed in the intact single myofibril. Our studies demonstrate that Ig domain folding reactions in titin occur at physiological forces and sarcomere lengths and are thus likely to be a major component of muscle elasticity.

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DNA-Induced Viral Assembly Studied in Real-Time by Optical Tweezers, Acoustic Force Spectroscopy and Atomic Force Microscopy
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The simian vacuolating virus (SV40) can infect both human and apes and is of interest as capsule for drug delivery since it can efficiently infect a wide range of human cells. The icosahedral virus capsid is assembled from VP1 pentamers, and this assembly is stimulated by the presence of dsDNA. However, the real-time assembly process of SV40 and viruses in general is poorly understood limiting among other things our ability to manipulate capsid re-assembly in vitro for therapeutic purposes.
Here, we show the real-time assembly of capsid around dsDNA employing dual-trap optical tweezers (OT). Protein assembly around the DNA leads to a drastic change in the contour length, which is readily observed when dsDNA is incubated with VP1 pentamers, indicating both kinking and crosslinking. Additionally, a new technology, acoustic force spectroscopy (AFS) capable to apply very low constant forces, provides indications of substantial capsid-like-structure formation observable as real-time shortening of DNA in the presence of VP1 combined with major rupture events when stretching the DNA-protein structure afterwards. In order to obtain visual information about protein coverage and arrangement on DNA, we generated atomic force microscope (AFM) images. In these images we observe significant cluster formation on DNA providing a good indication that we observe partial assembly. These results reveal that we can indeed track the assembly of viral capsids and visualize intermediates. This in turn provides a window into the complex kinetics of viral capsid formation.