results in an increase in the length of the target DNA, and the rate of this increase depends exponentially upon the applied force. Surprisingly, a fast and a slow binding mode are revealed, indicating multi-step binding.The fast binding rate is weakly correlated with force and is responsible for most of the extension change, while the slow binding rate increases with force. This suggests that intercalation is rapid, and followed by a slower, force-dependent rearrangement of DNA-bound ruthenium complexes. These studies demonstrate the capability of optical tweezers to elucidate the mechanism of complex DNA-ligand interactions, which may facilitate the rational design of DNA binding ligands with specific DNA interaction properties.

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Single-Molecule Investigations of Von Willebrand Factor in Hydrodynamic Flow

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Von Willebrand factor (VWF) is a multimeric blood glycoprotein that plays a pivotal role in hemostasis - it serves as a ligand for platelet adhesion and aggregation forming a hemostatic platelet plug. We are studying how hydrodynamic forces that arise in the circulation can regulate hemostasis. For example, the binding of the VWF A1 domain to the platelet protein GPIbα is correlated to conditions of strong hydrodynamic flow. We are testing the hypothesis that quaternary unfolding of VWF under hydrodynamic stress activates the VWF adhesive function by exposing A1 domains for binding to the platelet glycoprotein GPIb, and are studying the dynamics of this process.

To accomplish this, we are developing powerful new techniques in single-molecule manipulation and detection to investigate how fluid forces generated in the bloodstream affect the structural conformation and function of VWF. We are creating various hydrodynamic flows to mimic a wide range of blood flow conditions, while directly visualizing the conformational dynamics of single VWF molecules in these environments. First, we have utilized a microfluidic device with a cross-slot geometry to study VWF under elongational flow. To expand these studies, we are also utilizing a microfluidic four-roll mill device that has a stagnation point that will allow prolonged observation of VWF under all flow conditions including elongational, shear, and rotational flow. In addition to the microfluidic devices, we are developing a custom ''shear wheel'' microscope to study fluorescently labeled VWF under shear flow. These novel techniques will enable us to understand how hydrodynamic forces in the bloodstream act on VWF to cause quaternary unfolding, and how this in turn regulates adhesive activity. These findings should also lead to a better understanding of bleeding disorders that result from abnormalities in flow conditions of the circulatory system.

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Real-Time Activation of Blood Platelets Studied with Magnetic Microparticles

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Under normal conditions, platelets are inactive and have very little interaction with each other and with other cells. Platelets become activated when they are exposed to biochemical or mechanical stimuli, for instance after vascular damage with exposure to collagen. An altered responsiveness of platelets to a stimulus can have major consequences, since it is known that platelets play a crucial role in hemostasis and are also involved in inflammatory as well as cardiovascular diseases. Therefore there is a need for novel technologies to quantify platelet activation as well as their activation sensitivity.

We study the real-time activation of platelets with the use of magnetic microparticles. The advantage of using magnetic particles over traditional fluorescent labeling is that the particles can capture and actuate the cells in a complex biological matrix. We will report data on two experimental configurations. In the first configuration we chemically stimulate the platelets and quantify the platelet response by measuring the association of antibody-coated magnetic microparticles to the platelets with the use of magnetic tweezers. In the second configuration we trigger the platelets by applied magnetic forces and quantify the platelet response by fluorescent imaging of the intracellular response processes. We study ensembles of platelets and record the temporal response as a function of the applied force. We interpret the data from the perspective of understanding the fundamental processes of platelet activation as well as for studying the feasibility of novel lab-on-a-chip diagnostic tests.

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Using DNA Handles in Optical Tweezers Studies of Protein Mechanics

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Optical tweezers, which utilize a highly focused laser beam to provide a trap for microscopic objects, have found broad application in mechanical studies of biomolecules. Mechanical response of proteins can be probed with optical tweezers by applying picoNewton-scale forces to microspheres tethered to a protein's ends, thereby manipulating its extension. However, the study of short molecules can be complicated by technical difficulties such as non-specific binding and unwanted optical interactions. To avoid these problems, biopolymers such as DNA can be used as handles attached to the molecule of interest, to make the lengths longer and reduce these undesired effects.

Elastin is an important structural protein, with mechanical properties of direct relevance to its vital physiological roles. With a fully extended contour length of < 200 nm, much smaller than the microspheres commonly used for optical manipulation, direct measurements of mechanical properties such as elasticity and flexibility are complicated by experimental challenges as mentioned above. DNA handles can be used to avoid many of these problems, but can present challenges for interpreting data, when trying to deconvolve the protein's response from measured data of the hybrid system.

In this work, we discuss how to take into account contributions from DNA handles. Through analysis of simulated force-extension curves of the hybrid elastin-DNA system, we develop an understanding of how parameters such as length of the DNA handles and of the protein affect output parameters such as persistence lengths. We then present experimental force-extension results of a hybrid system of elastin with DNA handles.

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Nanomechanics of Desmin Filaments Explored with Optical Tweezers

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Desmin forms the intermediate filament system of muscle cells where it plays important role in maintaining mechanical integrity and elasticity. Although the importance of intermediate-filament elasticity in cellular mechanics is being increasingly recognized, the molecular basis of desmin's elasticity is not fully understood. In the present work we explored the mechanical properties of purified and reconstituted desmin filaments by using optical tweezers.

Desmin, purified from chicken gizzard, was polymerized by the addition of MgCl2. Desmin assembly was monitored by using atomic force microscopy. For mechanical manipulation, desmin filaments were captured between two anti-desmin-coated latex beads, one of which was positioned in a force-measuring optical trap and the other one held and manipulated with a moveable micropipette. By moving the micropipette away from the trap, typically a few-hundred-nanometer section of the captured desmin filament was stretched. Non-linear portions of the elastic curves were fitted with the wormlike-chain model, yielding an average 1 nm apparent persistence length. In the force versus extension curves transitions appeared at 10 pN, which extended the filament by ~60 nm. This distance corresponds well to unit-length-filament (ULF) dimensions, suggesting that the ULF components of the desmin filament may be rearranged axially upon stretch.

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Versatile Optical Tweezers with Single Base-Pair Resolution

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The molecular dance by DNA and its accompanying proteins can be studied in great detail using optical tweezers. We have constructed a versatile optical trapping platform that operates as a highly stable dual or quadruple optical trap at the fundamental noise limit given by thermal fluctuations. This allows us to study important cellular processes such as DNA replication and DNA compaction in great detail. In a dual trapping assay we use differential detection to observe DNA polymerase activity at single base-pair resolution with a 100 Hz bandwidth. In quadruple trapping mode, two DNA molecules are manipulated simultaneously, which allows direct observation of the formation and rupture of single protein-induced DNA bridges.