

result in improved performance across multiple AFM modalities, including single molecule force spectroscopy. However, instrumental drift in AFM remains a critical issue that limits the precision and duration of experiments. Previously, we developed an active optical stabilization technique to improve tip-sample stability at ambient conditions. However, force drift also occurs via uncontrolled deflection of the zero-force position of the cantilever. We found that the primary source of force drift in liquid for a popular class of soft cantilevers is their gold coating, even though they are coated on both sides to minimize drift. While removing the gold led to ~10-fold reduction in reflected light, we nonetheless achieved a 10-fold improvement in force stability of bioAFM, with a sub-pN force precision over a broad bandwidth (0.01-20 Hz) just 30 minutes after loading. We subsequently extended AFM's sub-pN bandwidth by a factor of ~50 to span five decades of bandwidth ($\Delta f \approx 0.01$ -1,000 Hz) by developing an efficient process to modify a short ($L = 40 \mu\text{m}$) commercially available cantilever (BioLever Mini) with a focused ion beam (FIB). Measurements of mechanically stretching individual proteins showed improved force precision coupled with state-of-the-art force stability and no significant loss in temporal resolution compared to stiffer, unmodified cantilevers. Ongoing work in our lab extends this concept down to ultrashort cantilevers ($L = 10 \mu\text{m}$) along with instrumental development to detect these cantilevers in a commercial AFM. Such cantilevers enable sensitive detection of protein unfolding and refolding with ~1 μs time resolution. Importantly, these cantilevers were robust and were reused for SFMS over multiple days. Hence, we expect these responsive, yet stable, cantilevers to broadly benefit diverse AFM-based studies.

34-Subg

Revealing Structure and Dynamics of Telomere Maintenance Proteins on DNA: One Molecule at a Time

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Telomeres play important roles in maintaining the stability of linear chromosomes. A specialized protein complex, called shelterin or telosome, binds to and protects telomeres at chromosome ends. Telomere maintenance involves dynamic actions of multiple proteins interacting with long repetitive sequences and complex dynamic DNA structures, such as T-loops. Furthermore, it was shown recently that in contrast to cohesion along chromosome arms, sister telomere association is a specialized process requiring a tighter association provided by the cohesin subunit SA1 in conjunction with specific proteins from the shelterin complex. To better understand the telomere maintenance pathways, we established complementary single-molecule imaging platforms: a newly developed Dual-Resonance-frequency-Enhanced Electrostatic force Microscopy (DREEM) technique capable of revealing DNA paths in protein-DNA complexes, fluorescence imaging of quantum dot-labeled proteins for tracking dynamics of proteins on DNA tightropes, and a nanochannel based imaging platform for studying protein-mediated DNA-DNA pairing/looping in real time. I will highlight our recent results on: 1) Revealing DNA paths inside TRF2 complexes during DNA compaction through DREEM. 2) Cohesin SA1 and shelterin protein TRF1 mediated sister telomere cohesion. 3) Dynamics of SA1 and TRF1 mediated DNA-DNA pairing inside nanochannels.

35-Subg

Revealing the Mechanical Regulation of Hemostasis with Novel Approaches in Single-Molecule Manipulation

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¹Biological Chemistry and Molecular Pharmacology, Pediatrics, Wyss Institute, Harvard University, Cambridge, MA, USA, ²Program in Cellular and Molecular Medicine, Boston Children's Hospital, Boston, MA, USA. Adhesion molecules in the circulatory system operate in a dynamic environment of forces and flows. In previous work, we have shown that such forces can regulate hemostatic activity by acting on von Willebrand Factor (VWF), a multimeric glycoprotein capable of binding to platelets to form a platelet plug in primary hemostasis. In particular, using optical tweezers we have shown that the VWF A2 domain can act as a single-molecule mechanical switch, unfolding to enable enzymatic cleavage by the enzyme ADAMTS13 [1]. To further investigate this system, we are developing two novel single-molecule assays. First, using massively parallel single-molecule manipulation using centrifugal force [2], we are studying how mutations related to von Wil-

lebrand disease can change the kinetics of force-mediated enzymatic cleavage of the A2 domain. To ensure reliable measurements, our molecular constructs are based on self-assembled DNA nanoswitches that we have developed, which provide a specific molecular fingerprint for enzymatic cleavage [3]. Second, we are studying how changes in flow profile related to injury can change the structure and function of full-length VWF by using a high-speed hydrodynamic trap designed to elongate large polymeric proteins about a stagnation point.

References:

- [1] X. Zhang*, K. Halvorsen*, C.-Z. Zhang, W.P. Wong[^], and T.A. Springer[^], "Mechanoenzymatic cleavage of the ultralarge vascular protein, von Willebrand Factor," *Science* 324 (5932), 1330-1334 (2009). (*equal contributions, [^]co-corresponding/senior authors)
- [2] K. Halvorsen, W.P. Wong, "Massively parallel single-molecule manipulation using centrifugal force," *Biophysical Journal* 98 (11), L53-L55 (2010).
- [3] K. Halvorsen, D. Schaak, and W.P. Wong, (2011), Nanoengineering a single-molecule mechanical switch using DNA self-assembly, *Nanotechnology* 22 (49), 494005 (2011).

Subgroup: Biological Fluorescence

36-Subg

Engineering of Bacterial Phytochromes for in vivo Imaging

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Visualization of biological processes non-invasively in live animals is an invaluable approach in basic and translational biology. The progress in optically-based *in vivo* imaging requires genetically-encoded near-infrared probes [1]. In a near-infrared region of the optical spectrum (650-900 nm) mammalian tissue is more transparent to light because the combined absorption by hemoglobin and water is minimal. On the basis of bacterial phytochromes we have engineered three types of near-infrared fluorescence probes, which utilize present in mammalian tissues heme-derived biliverdin as a chromophore. These probes include several spectrally distinct permanently fluorescent proteins (iRFP670, iRFP682, iRFP702, iRFP713 and iRFP720) [2], fluorescent proteins that are photoactivatable from low to high brightness (PAiRFP1 and PAiRFP2) [3] and bimolecular fluorescence complementation probe that reports on protein-protein interactions (iSplit) [4]. The designed near-infrared proteins were imaged in tumor models in living animals. The multicolor deep-tissue and whole-body fluorescence imaging [2-4] and multicontrast photoacoustic imaging [5] techniques aided by the developed probes should become common approaches in cell and developmental biology, in studies of cancer and pathogen invasion, and in biomedicine.

- [1] Piatkevich K.D., Subach F.V. and Verkhusha V.V. *Chem. Soc. Rev.*, 2013, **42**, 3441-3452.
- [2] Shcherbakova D. M. and Verkhusha V. V. *Nat. Methods*, 2013, **10**, 751-754.
- [3] Piatkevich K. D., Subach F. V. and Verkhusha V. V. *Nat. Commun.*, 2013, **4**, 2153.
- [4] Filonov G. S. and Verkhusha V.V. *Chem. Biol.*, 2013, **20**, 1078-1086.
- [5] Krumholz A., Shcherbakova D. M., Xia J., Wang L. V. and Verkhusha V. V. *Sci. Reports*, 2014, **4**, 3939.

37-Subg

Fluorescence Nanoscopy by Polarization Modulation (SPoD) and Polarization Angle Narrowing (ExPAN)

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Recently, we presented a new fluorescence super-resolution wide-field approach (Hafi et al., *Nature Methods*, (2014))¹ that explores the possibility of disentangling different sets of fluorescence markers by their transition dipole moment orientation. We will present the principle ideas behind the approach and discuss the possibilities as well as current limitations. We first demonstrate that the angle range for exciting single molecules of