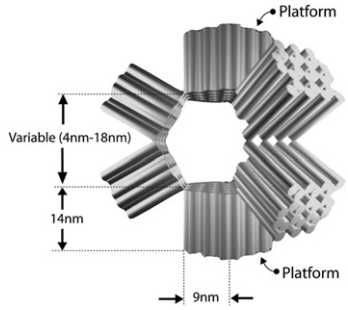
1473-Pos Board B317
A Novel Experimental Platform for DNA Mechanics Assays
Diane M. Wiener, Troy A. Lionberger, Steven A. Schrader, Katsuo Kurabayashi, Edgar Meyhöfer.
University of Michigan, Ann Arbor, MI, USA.
Single-molecule biophysics experiments have been widely employed to characterize the mechanics and dynamics of DNA and the activity of DNA-binding proteins. In typical experiments requiring DNA tethering, a DNA molecule is conjugated to a coverslip and observed with a light microscope parallel to the optical axis, limiting the resolution of optical and magnetic trapping measurements and direct observation of interactions with DNA-binding proteins. Another assay technique, where two beads tethering DNA are optically trapped, orients the DNA perpendicular to the optical axis, but this assay is limited to manipulating one DNA molecule at a time. We developed a device to overcome the limitations of previous assays. A microfabricated cantilever platform exposes the edge of a 50 nm thin gold surface that runs along the length of the sidewalls of patterned microfluidic channels. This narrow gold surface, oriented orthogonal to the optical axis, serves as a substrate for the covalent attachment of an end-thiolated DNA molecule through well established gold-thiol chemistry. The microfabricated cantilever device provides complete optical access to the DNA, and allows a DNA-tethered bead to be optically or magnetically trapped perpendicular to the optical axis, the experimentally preferred orientation. This experimental set-up allows for efficient, parallelized investigation of multiple DNA molecules in a single assay format. As a proof-of-concept demonstration, we have tethered λ-DNA and shorter DNA molecules between the gold edge and polystyrene beads and verify the conjugation by quantifying the Brownian motion of the tethered beads. This experimental platform has the potential for studying the mechanics of DNA and DNA-protein interactions, such as histone binding and RNA polymerase transcription, in conjunction with tethered particle motion, optical and magnetic trapping, and fluorescence imaging techniques all within a single focal plane.

1474-Pos Board B318
Feedback Control for Optical Tweezers Instruments: Position Clamping and Detection Laser Intensity Stabilization
Heikki Ojala, Anders Korsbäck, Anders E. Wallin, Edward Haeggström.
University of Helsinki, Helsinki, Finland.
Position clamping in optical tweezers allows us to use less laser power at a certain trapping stiffness which decreases the damage caused by the trapping laser. With intensity stabilized detection lasers smaller step sizes should be visible. High intensity laser light in optical tweezers can damage biomolecules under study. We employ digital feedback control of the trap position to increase the trap stiffness 13-fold[1]. We use predictive control to further increase, by ~30%, the effective lateral trap stiffness. This predictive approach provides higher control gain values than simple proportional control.
The trap is formed with a CW laser into an inverted microscope. The trap is moved with two orthogonal acousto-optical deflectors (AOD). A field-programmable gate array (FPGA) -board calculates the trap position and controls the AODs accordingly. Bead position is measured with two detection lasers using back-focal plane interferometry[2] with position sensitive detectors.
Molecular motors use step sizes as small as 0.34 nm[3]. To detect sub-nanometer movement we use our FPGA-controller to intensity stabilize the detection lasers[4]. The detection beams are coupled into polarization maintaining single-mode fibers. Part of the light emerging from the fiber is reflected onto a photodiode. The FPGA measures the intensity on the photodiode and controls the driving current of the detection laser diode. This setup pushes the low frequency region of the bright-noise spectrum (below 2 Hz a drop of ~1 dB for intensity signal) closer to the dark-noise limit of our detection electronics.
References:
[1] Wallin, A.E., et al., Applied Physics Letters, 2008. 92(22): p. 224104.
[2] Gittes, F. and C.F. Schmidt, Opt. Lett, 1998. 23(1): p. 7-9.
[3] Abbondanzieri, E.A., et al., Nature, 2005. 438(7067): p. 460-465.
[4] Carter, A.R., et al., Applied Optics, 2007. 46(3): p. 421-427.

1475-Pos Board B319
Is End-to-End Distance a Good Reaction Coordinate?
Nolan Harris[1], Eric Botello[1], Wei-Hung Chen[2], Kuan-Jiuh Lin[2], Ching-Hwa Kiang[1].
[1]Rice University, Houston, TX, USA, [2]National Chung Hsing University, Taichung, Taiwan.
Single-molecule force spectroscopy opens a new door for investigating detailed biomolecular interactions and their thermodynamic properties by pulling molecules apart while monitoring the force exerted on them. Recent advances in the nonequilibrium work theorem allows one to determine the free energy landscapes of these processes. However, the resulting free energy surfaces are expressed as a function of molecular end-to-end distance, which differs from the normal reaction coordinate, and raises the question whether molecular extension is a good order parameter. Using unfolding of the I27 domain of human cardiac titin, we show that the molecular end-to-end distance is a good reaction coordinate by comparing the free energies determined from force spectroscopy to traditional bulk chemical studies. The trends in protein unfolding free energies are consistent for force spectroscopy and bulk chemical studies, where the reaction coordinate is well-defined. The results demonstrate that the information from single-molecule pulling experiments are meaningful and useful for understanding the mechanism of protein folding.

1476-Pos Board B320
Rigid Linear Nano-Actuator Self-Assembled from DNA
Andrew M. Leifer[1], William M. Shih[1,2].
[1]Harvard Medical School, Boston, MA, USA, [2]Dana-Farber Cancer Institute, Boston, MA, USA.
A self assembling DNA nanostructure is proposed that will permit expansion and contraction in only one dimension. This actuator restricts two rigid platforms, each in the shape of a hexagonal disk of radius 9 nanometers and of depth 14 nanometers, to move apart from one another with separations between 4 and 18 nanometers. Designed to resist bending, shearing, or torsional stresses, the actuator is composed entirely of DNA and can be self-assembled from two single-stranded DNA plasmids and hundreds of single-stranded DNA oligonucleotides. The actuator is illustrated in the accompanying figure where DNA double helices are represented as cylinders. A detailed schematic is presented and preliminary findings are shown. The creation of nanometer-sized structures that can undergo constrained motion is a critical step towards building nano-machines.



1477-Pos Board B321
Position of the non-template DNA in the Polymerase II elongation complex revealed using single-molecule Fluorescence Resonance Energy Transfer
Joanna Andrecka[1], Barbara Treutlein[1], Maria Angeles Izquierdo Arcusa[1], Patrick Cramer[2,3], Jens Michaelis[1,3].
[1]Department of Chemistry and Biochemistry, Ludwig-Maximilians-Universität (LMU), München, Germany, [2]Gene Center, Ludwig-Maximilians-Universität (LMU), München, Germany, [3]Center for Integrated Protein Science, München, Germany.
Single Molecule Fluorescence Resonance Energy Transfer (FRET) was used as a quantitative tool to localize non-template DNA in the RNA Polymerase II (Pol II) elongation complex.
We measured the FRET efficiencies between 'antenna dye molecules' (ADMs) attached to the non-template DNA and several 'satellite dye molecules' (SDMs) attached to known positions within the Pol II elongation complex using the recently developed Nano Positioning System (NPS) (1). NPS combines x-ray crystallographic information, single-molecule FRET data and bayesian parameter estimation, a probability based analysis method, to compute the three dimensional probability density for the position of ADMs. Within the bayesian framework, we were able to account for errors in the determined Förster radii, errors in the measured FRET efficiencies and uncertainties in the SDM positions (due to the attachment of the SDMs via flexible linkers) as well as for geometric constraints. We determined positions of the non-template DNA at +1, -2, -4, -7 (within the transcription bubble) and -12, -15, -18 of the up-stream DNA. Together with the high resolution x-ray structure and our earlier studies (2) determining the position of the nascent RNA this study completes the picture of the Pol II elongation complex.
1. A. Muschielok, J. Andrecka, A. Jawhari, F. Brückner, P. Cramer and J. Michaelis, A nano-positioning system for macromolecular structural analysis. Nature Methods, accepted.
2. J. Andrecka, R. Lewis, F. Brueckner, E. Lehmann, P. Cramer and J. Michaelis, Tracking the position of mRNA in RNA polymerase II elongation complexes. PNAS 105, 135-140 (2008).