The rotor bead technique introduced here allows tracking of the complete three-dimensional trajectory of a dsDNA translocase in action. It also permits the application of torque in a laser tweezers apparatus using commercially-available microspheres.

1147-Plat
Reeling in DNA One Base at A Time: pcrA Translocation Coupled to DNA Looping Dismantles RecA Filaments
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The mechanism of helicase translocation on DNA remains controversial and the translocase activity driving their non-canonical functions such as protein displacement is poorly understood. Here, we used single molecule fluorescence assays to study a prototypical superfamily 1 helicase, *Bacillus stearothermophilus* PcrA, and discovered a progressive looping of ssDNA that is tightly coupled to PcrA translocation on DNA. Variance analysis of hundreds of looping events by a single protein demonstrated that PcrA translocates on ssDNA in uniform steps of 1 nt, reconciling discrepancies in previous structural and biochemical studies. On the forked DNA, rather than acting on the leading strand to unwind the duplex, PcrA anchored itself to the duplex junction and reeled in the lagging strand using its 3'-5' translocation activity. PcrA maintained the open conformation, not the closed conformation observed in crystallographic analysis, during looping-coupled translcation. This activity could rapidly dismantle a preformed RecA filament even at 1nM PcrA, suggesting that the translocation activity and structure-specific DNA binding are responsible for removal of potentially deleterious recombination intermediates.

1148-Plat
Protein-Mediated DNA Loops are Resistant to Competitive Binding
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The lac Repressor protein (LacI) is a canonical genetic regulatory protein. It represses transcription of the lac operon in E. Coli by simultaneously binding to two distant operator sites on the bacterial DNA and bending the intervening DNA into a loop. A set of substrate DNA constructs with intrinsic A-tract bends have been engineered by Mehta and Kahn, which were optimized to form hyperstable loops. We present single-molecule measurements of LacI-mediated loop formation and breakdown rates on these optimized DNA constructs and demonstrate that repeated formation and breakdown of the loops does not cease in the presence of 100 nM of free competitor DNA. While this observation dovetails with bulk competition assays in which the presence of competitor DNA disrupts the looped complexes only very slowly, our measured loop lifetimes of minutes disagree with an inferred lifetime of days from the bulk assays. We conclude that the LacI-DNA complex can exist in some non-looped conformation, which can re-loop, but is unexpectedly resistant to competition. We discuss possible scenarios for such a conformation in light of the data.

1149-Plat
Single Molecule Analysis of Substeps in the Mechanochemical Cycle of DNA Gyrase
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DNA gyrase is a molecular motor that harnesses the free energy of ATP hydrolysis to introduce negative supercoils into DNA. We have characterized the structural dynamics of processive supercoiling using a real-time single molecule assay in which DNA gyrase activity drives the directional, stepwise rotation of a submicron rotor bead attached to the side of a stretched DNA molecule. We are able to directly observe rotational pauses corresponding to rate-limiting kinetic steps under varying [ATP], and have used simultaneous measurements of DNA twist and extension in order to characterize transient supercoil trapping and DNA compaction during the reaction cycle. We have mapped out structural intermediates of the DNA:gyrase complex on a twist-extension plane, and have characterized transitions between these states driven by chemical events such as the cooperative binding of ATP. These measurements motivate several revisions to previous models based on lower resolution assays [1], and we will present our results in the context of a new branched kinetic model for the mechanochemical cycle. We are now using theoretical calculations together with measurements of force-dependent changes in extension in order to test specific geometric models for structural intermediates, and we have begun to analyze structure-function relationships using single-molecule analysis of gyrase fragments.
[1] Jeff Gore, Zev Bryant, Michael D. Stone, Marcelo Nollmann, Nicholas R. Cozzarelli, Carlos Bustamante, "Mechanochemical analysis of DNA gyrase using rotor bead tracking", Nature, 439 (2006)

1150-Plat
Two Structurally Different Families of DNA Base Excision Repair (BER) Proteins Diffuse Along DNA to Find Intrahelical Lesions
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Base excision repair (BER) proteins, endonuclease III (Nth) and VIII (Nei) from E. *coli* represent two distinct glycosylase families, which recognize and remove damaged DNA bases. One mechanism by which these glycosylases scan for DNA lesions is through a simple, one-dimensional diffusive search. To characterize this search mechanism, we have developed a single molecule assay in near TIRF to image Qdot-labeled, His-tagged Nth and Nei proteins interacting with YOYO-1 stained λ-DNA molecules elongated by hydrodynamic flow between 5μm silica beads. With an *in vitro* glycosylase activity assay, we confirmed that neither YOYO-1 stained DNA nor Qdot labeling significantly affects glycosylase activity. By imaging individual DNA "tightropes", we observed Qdot-labeled glycosylases interacting with DNA by either binding to or diffusing on DNA. With increasing ionic strength (50-500mM Kglutamate), although fewer glycosylases interacted per unit length of DNA, a greater fraction diffused along the DNA. At physiological ionic strength, (150mM KGlu) both Nth and Nei scan DNA for as much as 10 sec with a diffusion constant of ~1.5 x 10^5 bp^2 sec^{-1}, approaching the theoretical limit of rotational diffusion about the DNA helix. At these rates, the activation barrier for rotational diffusion of 0.7 K_bT is slightly below the maximum of ~2 K_bT for efficient target location. We observe no significant difference between Nth and Nei in the rate or mode of their DNA lesion search mechanism. Interestingly, at elevated ionic strengths, both families of glycosylases scan above the theoretical limit for free rotational diffusion (>5 x 10^5 bp^2 sec^{-1}). Therefore, the DNA/glycosylase interface may be optimized for physiological ionic strength, above which the glycosylase search mechanism shifts from rotational diffusion to a one-dimensional diffusion without rotation.

1151-Plat
Target-Site Search of DNA-Binding Proteins
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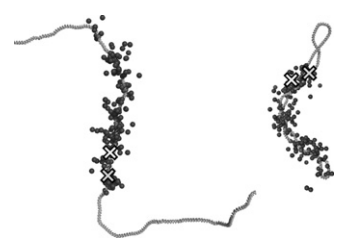
Gene regulatory proteins find their target sites on DNA remarkably fast; the experimental binding constant for *lac* repressor is three orders of magnitude higher than predicted by free diffusion alone. It has been proposed that nonspecific binding aids the search by allowing proteins to slide and hop along DNA. We develop a reaction-diffusion theory of protein translocation that accounts for transport both on and off the strand and incorporates the physical conformation of DNA. For linear DNA modeled as a wormlike chain, the distribution of hops available to a protein exhibits long, power-law tails. As a result, the long-time displacement along the strand is superdiffusive. Our analysis predicts effective superdiffusion coefficients for given nonspecific binding and unbinding rate parameters. Translocation rates experience a maximum with salt concentration (i.e., binding rate constant), which has been verified experimentally. Simulated protein trajectories on DNA (see figure) agree with our theoretical predictions of superdiffusive transport. Our analytical theory allows us to predict the binding and unbinding rate parameters that optimize the protein translocation rate and the efficiency of the search. Finally, we use our theory to predict rates of target site localization under various experimental conditions.



1152-Plat
Illuminating the DNA Binding Behavior of Mitochondrial Transcription Factor A
Géraldine Farge[1], **Onno D. Broekmans**[1], Niels Laurens[1], Linda Dekker[1], Maria Falkenberg[2], Erwin J.G. Peterman[1], Gijs J.L. Wuite[1].
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Mitochondria are the energy producing organelles of eukaryotic cells. Owing to their endosymbiotic evolutionary history, they contain their own genome (mtDNA) that encodes for thirteen proteins essential for ATP production.
In mammalian cells, multiple mtDNAs are compacted into protein-DNA complexes called "nucleoids". A major component of these nucleoids is the mitochondrial transcription factor A (TFAM), a member of the high mobility group (HMG) family of proteins. This abundant protein binds DNA with little sequence specificity, and is able to coat the entire mtDNA molecule. It not only serves a role in mtDNA packaging, but is also required for mitochondrial transcription. At this point, dynamics of the TFAM-DNA interaction remain unclear.
Experiments on single DNA molecules offer a very direct way to study TFAM dynamics. Tethered Particle Motion (TPM) experiments show that the system quickly equilibrates with the buffer, and that the end-to-end distance of the DNA decreases upon TFAM binding. Manipulations of single DNA molecules with two optical traps offer an explanation: TFAM decreases the DNA's stiffness (persistence length). A possible molecular mechanism for this decrease is that TFAM introduces bends in the DNA.
Adding single-molecule fluorescence to the dual optical trap illuminates TFAM's binding behavior. Literally seeing TFAM on the DNA, we can derive on- and off-rates, and determine that TFAM does not bind cooperatively. Interestingly and seemingly unrelated to its role in DNA organization, we also observe that TFAM can rapidly bind single-stranded DNA (ssDNA), but not when the ssDNA is under tension. The physiological function of TFAM binding to ssDNA could be related to its regulation of transcription.

Symposium 9: Biophysics of the Failing Heart

1153-Symp
What is the Effect of A Familial Hypertrophic Cardiomyopathy Mutation on Cardiac Myosin Function?
Susan Lowey.
University of Vermont, Burlington, VT, USA.
Familial hypertrophic cardiomyopathy (FHC) is a clinically and genetically heterogeneous disease which is a major cause of heart failure. The landmark discovery that a point mutation at residue 403 (R403Q) in the β-myosin heavy chain (MHC) can cause a lethal form of FHC was made in 1990, but the effect of this mutation on the functional properties of human cardiac myosin remains poorly understood. One problem has been that the prevalent mouse model for FHC expresses predominantly α-MHC. The β-MHC, however, is the predominant isoform in the ventricles of all larger mammals. Even though the α- and β-MHC share $>$ 90 % sequence identity, they differ ~ 2-fold in enzymatic and mechanical properties, raising the possibility that the effect of a disease mutation may depend on the isoform backbone. To address this question we used a transgenic mouse model in which the endogenous α-MHC was replaced with transgenically encoded β-MHC. A His-tag was cloned at the N-terminus of α- and β-MHC, along with the R403Q mutation, to facilitate isolation of myosin or its head subfragment-1 (S1).We find that the steady-state ATPase activity and *in vitro* motility of mouse α-MHC is enhanced by the R403Q mutation, as reported previously, but the R403Q mutation in a β-MHC background shows a slight reduction in activity. A more in-depth analysis of the R403Q phenotype is being undertaken by stopped-flow kinetics to measure the nucleotide turnover in these mutant S1 isoforms. In order to determine the extent of species-dependent differences, we are comparing the functional properties of β-cardiac myosin in the mouse with those in the rabbit, a model system which more closely resembles humans in protein composition and disease phenotypes.

1154-Symp
Reduced Responsiveness to β-Adrenergic Agonists in Murine cMyBP-C Cardiomyopathy
Richard L. Moss, Carl Tong.
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Myosin binding protein C (MyBP-C) is a thick filament accessory protein that has both structural and regulatory roles in striated muscle contraction. We are studying the roles of the cardiac isoform of MyBP-C in mouse models in which the cMyBP-C gene has been disrupted, resulting in ablation of the protein, and in mice expressing mutant protein in which residues that are phosphorylated *in vivo* by PKA have been replaced with ala or asp. Ablation of cMyBP-C results in a cardiac phenotype similar to many inherited cardiomyopathies in humans, i.e., septal hypertrophy, increased arrhythmic activity, and systolic and diastolic dysfunction. Studies of isolated myocytes from wild-type and null mice suggest that cMyBP-C regulates the kinetics of cross-bridge interaction with actin, a mechanism that is lost in the null mouse. Studies of myocytes from mouse lines expressing phosphorylation mutants of cMyBP-C indicate that PKA stimulation of contraction kinetics in myocardium is in large part due to phosphorylation of cMyBP-C, which appears to relieve a structural constraint on myosin and increases the likelihood of myosin binding to actin. Living myocardium expressing non-phosphorylatable cMyBP-C was found to exhibit depressed twitch force-frequency relationships and reductions in both frequency-dependent and β-agonist- induced acceleration of relaxation. These results can be explained by a model in which cMyBP-C phosphorylation accelerates cross-bridge interaction kinetics in wild-type myocardium, a regulatory mechanism that is lost in myocardium expressing the non-phosphorylatable mutant cMyBP-C. Supported by NIH R01 HL082900 and P01 HL094291.

1155-Symp
The Giant Elastic Protein Titin Role in Muscle Function and Disease
Henk Granzier.
University of Arizona, Tucson, AZ, USA.
No Abstract.

1156-Symp
Rescue of Familial Cardiomyopathies by Modifications at the Sarcomere Level and Ca^{2+} Fluxes
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University of Illinois, Chicago, Chicago, IL, USA.
Familial cardiomyopathies are commonly linked to missense mutations, deletions or truncations in sarcomeric, cytoskeletal, or intermediate filament proteins and give rise to hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) or restrictive cardiomyopathy. Although in the last two decades much information about the pathophysiology of genetically linked HCM and DCM has been provided by studies using transgenic animal models, there is still no therapy to prevent the development of the disease and increase survival in patients with HCM or DCM. Our emphasis here is on development of new therapies for treatment of HCM and DCM linked to mutations in thin filament proteins that are associated with increased and decreased myofilament sensitivity to Ca^{2+} respectively. We hypothesize that direct modifications of myofilament Ca^{2+} sensitivity and/or alteration in Ca^{2+} fluxes can serve as new therapeutic targets. Therefore if 1) HCM is associated with increased myofilament sensitivity to Ca^{2+}, interventions that desensitize the myofilament to Ca^{2+} may serve as potential new therapeutic targets and 2) DCM is associated with decreased myofilament sensitivity to Ca^{2+}, interventions that sensitize the myofilament to Ca^{2+} may serve as potential new therapeutic targets. There are several possible targets within myofilament proteins for altering myofilament Ca^{2+} sensitivity, in particular troponin I. Alterations in Ca^{2+} regulation by modification of sarcoplasmic reticulum Ca-ATPase (Serca2) or phospholamban levels are additional potential targets for HCM and DCM.

Minisymposium 2: Nanomedicine: Biophysical Approaches to Clinical Problems at the Nanoscale

1157-MiniSymp
4.0 Å Cryo-EM Structure of the Mammalian Chaperonin: TRiC/CCT
Yao Cong[1], Matthew Baker[1], Joanita Jakana[1], David Woolford[1], Stefanie Reissmann[2], Steven J. Ludtke[1], Judith Frydman[2], Wah Chiu[1].
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TRiC is a eukaryotic chaperonin essential for *de novo* folding of ~10% newly synthesized cytosolic proteins, many of which cannot be folded by other cellular chaperones. Unlike prokaryotic and archael chaperonins, each of its two rings consists of eight unique, but similar subunits. Using single particle cryo-EM, we determined the mammalian TRiC structure without any symmetry imposition at 4.7 Å resolution, which is the highest resolution asymmetric cryo-EM reconstruction to date. An analysis of this map allowed us to elucidate the relative orientation of the two rings and the two-fold symmetry axis location between them. A subsequent two-fold symmetrized map yielded a 4.0 Å structure, in which a large fraction of side chains and structural elements including loops and insertions appear as visible densities. These features permitted unambiguous identification of all eight individual subunits, despite their similarity. A Cα backbone model of the entire TRiC complex was subsequently refined from initial homology models against the cryo-EM density based on our subunit identification. A refined all-atom model for a single subunit