1142-Plat
Dynamic and Static Measurements of A Single and Double Phospholipid Bilayer System
Justin D. Berry[1], Curt M. DeCaro[1], Daniel A. Bricarello[2], Yicong Ma[3], Mrinmay Mukhopadyay[3], Gang Chen[3], Zhang Jiang[4], Alec Sandy[4], Suresh Narayanan[4], Atul N. Parikh[2], Sunil K. Sinha[3], Laurence B. Lurio[1].
[1]Northern Illinois University, DeKalb, IL, USA, [2]University of California, Davis, Davis, CA, USA, [3]University of California, San Diego, La Jolla, CA, USA, [4]Argonne National Laboratory, Argonne, IL, USA.
We investigate the surface height fluctuations of single and double bilayers of DPPE supported on silicon using x-ray photon correlation spectroscopy (XPCS). In this techique, x-rays are incident on the membrane in a grazing incidence geometry and diffusely scattered x-rays are measured using an area detector. Time fluctuations of the scattering pattern can then be analyzed to yield the relaxation rate of surface height fluctuations. Bilayer and double bilayer systems were prepared utilizing combination of Langmuir-Blodgett and Langmuir-Schaeffer depositions. Static structural measurements were also made on these systems as well as on more complicated systems consisting of triple and five-fold bilayers of DPPE. Relationships between structure and dynamics of these systems will be discussed.

1143-Plat
Coexistence of Immiscible Mixtures of Palmitoylsphingomyelin and Palmitoylceramide In Monolayers and Bilayers
Jon V. Busto[1], Maria Laura Fanani[2], Luisina De Tullio[2], Jesus Sot[1], Bruno Maggio[2], **Felix M Goni**[1], Alicia Alonso[1].
[1]Unidad de Biofisica (CSIC-UPV/EHU), Leioa, Spain,
[2]Universidad Nacional de Cordoba, Cordoba, Argentina.
A combination of lipid monolayer- and bilayer-based model systems have been applied to explore the interaction and organization of palmitoylsphingomyelin (pSM) and palmitoylceramide (pCer). Langmuir balance measurements reveal favourable interactions between the lipid molecules. A thermodynamically stable point is observed in the range 30-40 mol % pCer. The pSM monolayer undergoes hyperpolarization and condensation with small pCer concentrations, narrowing the liquid-expanded (LE) to liquid-condensed (LC) pSM main phase transition by inducing intermolecular interactions and chain ordering. Beyond this point, the phase diagram no longer reveals the presence of the pSM-enriched phase. Differential scanning calorimetry (DSC) of multilamellar vesicles reveals a widening of the pSM gel-fluid phase transition (41°C) upon pCer incorporation, with formation of a further endotherm at higher temperatures that can be deconvoluted into two components. DSC data reflect the presence of pCer-enriched domains coexisting, at different proportions, with a pSM-enriched phase that is no longer detected in mixtures containing >30 mol% pCer. Epifluorescence microscopy of mixed monolayers at low pCer content shows concentration-dependent, morphologically different pCer-enriched LC domain formation over a pSM-enriched LE phase, in which pCer contents close to 5 and 30 mol% can be determined for the LE and LC phases respectively. Fluorescence confocal microscopy of giant vesicles further confirms formation of pCer-enriched lipid domains. Vesicles cannot form beyond 40 mol% pCer contents. Altogether, the presence of at least two immiscible phase-segregated pSM-pCer mixtures of different compositions is proposed at high pSM content. A condensed phase (with domains segregated from the liquid-expanded phase) showing enhanced thermodynamic stability occurs near a compositional ratio of 2:1 (pSM:pCer). These observations become significant on the basis of the ceramide-induced microdomain aggregation and platform formation upon sphingomyelinase activity on cellular membranes.

1144-Plat
Diffusion of Nano-Meter-Sized Domains on A Vesicle
Yuka Sakuma[1], **Masayuki Imai**[1], Naohito Urakami[2], Michihiro Nagao[3,4], Shigeyuki Komura[5], Toshihiro Kawakatsu[6].
[1]Ochanomizu University, Tokyo, Japan, [2]Yamaguchi University, Yamaguchi, Japan, [3]National Institute of Standards and Technology, Gaithersburg, MD, USA, [4]Indiana University, Bloomington, IN, USA, [5]Tokyo Metropolitan University, Tokyo, Japan, [6]Tohoku University, Sendai, Japan.
The lateral diffusion of nano-meter-sized constituents (proteins, lipid rafts and so on) in lipid bilayers is of biological interest because the biochemical functions may be diffusion controlled. Saffman and Delbrück (SD) dealt with this issue using a hydrodynamic theory and derived an expression of the diffusion coefficient D forsmall domain size limit as $D(r)=k_BT[\ln(\eta_m h/\eta_m r)-0.0773]/4\pi\eta_m h$, where η_m and η_w are the viscosities of the membrane and the aqueous phase, respectively, h is the membrane thickness, r is the radius of the diffusing object. In this study we addressed the SD model by direct measurement of the intermediate scattering function of the nano-meter-sized liquid ordered domains in the fluid membrane using a contrast matching technique of neutron spin echo. We prepared ternary small unilamellar vesicles (SUVs) composed of deuterated DPPC, hydrogenated DOPC, hydrogenated cholesterol. The obtained intermediate scattering functions for the phase separated SUVs were fitted by a double exponential function, $S(q,t)/S(q,0)=A\exp(-D_0q^2t)+(1-A)\exp(-(D_0+D_d)q^2t)$, where A is the numerical constant, $D_0$and D_d are the diffusion coefficients of whole SUV and nano-meter sized domain, respectively. The fitting of $S(q,t)/S(q,0)$ gave $D_d = 2.3 \times 10^{-12}$ m^2/s, where the domains have the mean radius of 7.5 nm. The obtained domain diffusion coefficient agrees well with the SD prediction of $D= 2.35 \times 10^{-12}$ m^2/s using Ns/m^2 and Ns/m^2. Furthermore, by combining present data with the diffusion coefficient of a DPPC single molecule in L_d phase of $D_{DPPC} \approx 3.5 \times 10^{-12}$ m^2/s, we clearly demonstrates that the SD model well describes the observed diffusion coefficients in nano-meter length scale.

Platform W: Protein-Nucleic Acid Interactions II

1145-Plat
Single Molecule Studies of the Recognition Sequence Finding Mechanism of Protelomerase Telk
Markita P. Landry[1], Wai Mun Huang[2], Toshio Yanagida[3], Yann Robert Chemla[1].
[1]University of Illinois at Urbana Champaign, Urbana, IL, USA,
[2]University of Utah Health Sciences Center, Salt Lake City, UT, USA,
[3]Osaka University, Osaka, Japan.
Protelomerase TelK is an enzyme responsible for forming DNA hairpins in linear prokaryotic DNA. The mechanism by which this protein recognizes its target sequence and both quickly and accurately catalyzes DNA hairpin formation is poorly understood. To investigate the target recognition process, we used TIRF microscopy to visualize quantum dot-labeled TelK interacting with both nonspecific DNA and DNA containing the TelK target sequence. While many sequence-specific DNA-binding proteins (SSDBP) have been shown to scan DNA in 1D as their primary method for locating their recognition sequence[1], we surprisingly find that TelK does not move laterally on either aforementioned DNA substrate and therefore does not search by 1D scanning. Measurements of a c-terminally truncated TelK mutant reveal the same behavior. Interestingly, this mutant forms DNA hairpins 50 times slower than wild type, and dissociates from nonspecific DNA at a comparably lower rate than full-length TelK. These results suggest that dissociation from nonspecific DNA is an essential step in the recognition sequence search. Complementary studies with high-resolution optical tweezers reveal that TelK binding to DNA is a highly tension dependent process and condenses the molecule by several nanometers, consistent with crystal structures of the protein-DNA complex[2]. Remarkably, this condensation is observed on nonspecific DNA as well, despite the fact that these DNA distortions are energetically expensive. These findings suggest that the TelK target sequence search may involve 3D hopping and intersegmental transfer in lieu of 1D scanning. This may represent a novel SSDBP recognition sequence search mechanism.
1. Halford, S. et al. Nucl. Ac. Res. 32 (2004)
2. Aihara, H. et al. Mol. Cell. 27, 901 (2007)

1146-Plat
Bacteriophage phi29 Translocates DNA Along A Left-Handed Helical Path During Packaging
Craig L. Hetherington[1], Aathavan Karunakaran[1], Paul Jardine[2], Shelley Grimes[2], Dwight Anderson[2], Carlos Bustamante[1].
[1]UC Berkeley, Berkeley, CA, USA, [2]University of Minnesota, Minneapolis, MN, USA.
Bacteriophage phi29 employs a homomeric ring of RecA-like ATPases in order to package its dsDNA genome into the capsid at near-crystalline density. Previous single-molecule measurements of packaging have revealed the coordination of motor subunits, the step size of the motor, and the sensitivity of the motor to substrate modifications, thereby suggesting structural and kinetic models for the mechanism of translocation. However, traditional single-molecule experiments measure only the projection of the motor's motion onto the DNA longitudinal axis.
We directly observe that phi29 translocates DNA along a left-handed helical path by monitoring rotation of a bead attached to the side of the substrate DNA in a laser tweezers. Simultaneously, the response to applied torque is measured. This novel experiment probes the details of force and torque generation by the packaging motor. Combining these measurements with angstrom-scale laser tweezers observations of motor stepping suggests specific geometric models for the interaction of the motor and DNA during translocation.

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.

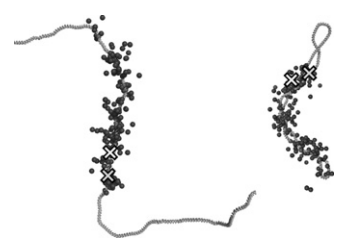
1147-Plat
Reeling in DNA One Base at A Time: pcrA Translocation Coupled to DNA Looping Dismantles RecA Filaments
Jeehae Park[1], Sua Myong[1], Anita Niedziela-Majka[2], Jin Yu[3], Timothy M Lohman[4], Taekjip Ha[1,5].
[1]Univeristy of Illinois at Urbana Champaign, Urbana, IL, USA, [2]Washington University School of Medicine, St. Louis, IL, USA, [3]University of California at Berkeley, Berkeley, CA, USA, [4]Washington University School of Medicine, St. Louis, MO, USA, [5]5Howard Hughes Medical Institute, Urbana, IL, USA.
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
Joel D. Revalee, Jens-Christian Meiners.
University of Michigan - Ann Arbor, Ann Arbor, MI, USA.
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
Aakash Basu[1], Lena Koslover[1], Allyn Schoeffler[2], Elsa Tretter[2], James M. Berger[2], Andrew J. Spakowitz[1], Zev D. Bryant[1].
[1]Stanford University, Stanford, CA, USA, [2]University of California Berkeley, Berkeley, CA, USA.
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
Andrew R. Dunn[1], Jeffrey P. Bond[1], David M. Warshaw[1], Susan S. Wallace[1], Neil M. Kad[2].
[1]The University of Vermont, Burlington, VT, USA, [2]The University of Essex, Colchester, United Kingdom.
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
Mario A. Diaz de la Rosa, Elena F. Koslover, Peter J. Mulligan, Andrew J. Spakowitz.
Stanford University, Stanford, CA, USA.
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].
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]University of Gothenburg, Gothenburg, Sweden.