

formation of a productive complex. Conversely, interference with these dynamics provides a possible mechanism by which nonnucleoside analogue inhibitors of NS5B block de novo initiation of RNA synthesis.

### 391-Pos

#### Simulating the Relaxation of DNA Supercoils By Topoisomerase I

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Many cellular processes involving DNA, including replication and transcription, result in significant superhelical stresses. During transcription, for example, RNA polymerase locally untwists about a helical turn of the DNA double helix. Then to elongate the RNA transcript, it proceeds along the template strand of the DNA and thereby induces supercoiling. DNA topoisomerases play an important role in relieving these stresses. Here we focus on understanding the action of human DNA topoisomerase I (Topo1) which operates in three basic steps: (i) cleaving a single strand of the DNA double helix, (ii) allowing the DNA superhelical stresses to relax, and (iii) religating the DNA. Recently, the Dekker lab, at Delft University of Technology, performed single molecule experiments to probe the relaxation of supercoils by topo1. A significant molecular dynamics (MD) effort (>100 cpu years) by the Andricioaei lab, at the University of California Irvine, characterized the energetics and topological changes of topo1 in complex with only a short fragment of DNA (~20 bp). Including a longer length of DNA to represent a biologically relevant length-scale (greater than a persistence length), is computationally prohibitive for MD and was necessarily neglected. Here we introduce an elasto-dynamic rod model as a first approximation to provide a dynamic description of the DNA as it relaxes. The rod model describes bending and torsion of the DNA helical axis, electrostatic and self-contact interactions, and approximates the hydrodynamic drag on the molecule. For our simulations, we provide as initial conditions, a plectonemic supercoil. The MD simulations serve to provide boundary conditions to the rod model by characterizing the torque applied to the DNA by topo1 as it rotates. Here we present preliminary results for the relaxation rates of supercoils.

### 392-Pos

#### Nucleoprotein Complex Formation By *Bacillus Subtilis* Spo0J/ParB

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Although prokaryotes lack the machinery utilized by eukaryotes to achieve well organized chromosome segregation, all cells must faithfully segregate their chromosomes in every cell division cycle. In many bacteria, this process is dependent upon a partitioning locus composed of an ATPase called ParA, a DNA binding protein called ParB, and centromere-like binding sites (*parS*) that are present adjacent to the origin of replication. ParB binds the *parS* sites and the ParA ATPase acts on the ParB-*parS* complex to facilitate segregation of replicated origins toward opposite cell poles. In *Bacillus subtilis*, the ParA protein is called Soj and the ParB protein is referred to as Spo0J. There are eight *parS* sites surrounding the origin of replication of the *B. subtilis* chromosome. Spo0J can bind to these sites and spread along the DNA up to 15 kilobases, forming a nucleoprotein complex. The Spo0J-*parS* complexes are not only a substrate for Soj/ParB, but they also serve to recruit the highly conserved structural maintenance of chromosomes (SMC) complex to the origin. The SMC condensin complex appears to function in both organizing the origin region and facilitating chromosome segregation. To investigate how Spo0J spreads along the DNA and ultimately how this complex recruits SMC, we have employed single molecule fluorescence imaging to directly observe the formation of the nucleoprotein complex of purified *B. subtilis* Spo0J and lambda DNA. We characterize the physical properties of this nucleoprotein complex and the kinetics of its formation.

### 393-Pos

#### Theoretical Analysis of the Molecular Mechanism of Stabilization of Nova-RNA Complex System: Fragment Molecular Orbital Method Based Quantum Chemical Calculation For the Effect of the Complex Formation on the Electronic State of Biomacromolecular System

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The RNA-binding proteins (RBPs) specifically recognize the shape and/or sequence of RNA molecules for binding the target. The relationship between

three-dimensional structure and RNA-binding mechanism of RBPs can be analyzed by performing computer simulations to provide deeper insight into this issue. In the present study, we performed Fragment Molecular Orbital (FMO) based quantum chemical calculations for neuro-oncological ventral antigen third KH domain (NOVA)-RNA complex system to study the molecular mechanism from the viewpoint of electronic state of biomacromolecules. We investigated the effect of the complex formation on an electronic state of NOVA. We found that the charge redistributes all over the structure and that the secondary structure of NOVA is remarkably associated with the change of electronic state in the complex formation. The results indicate that the whole protein structure participates in realization of the best energetic stabilization in the complex formation and we speculate that secondary structure could play an important role to obtain the optimum inter-molecular interaction energy by associating with charge redistribution. Further, we employ molecular dynamics simulation method to consider structures fluctuating around the equilibrium state. We perform FMO calculations for the obtained snapshots and examine the change of electronic state. The results will provide deeper insight into the relationship between electronic state and structural fluctuation. The details will be reported at the meeting.

### 394-Pos

#### Investigating Classic Lac Repressor-Dna Looping Experiments Using a Computational Rod Model

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Protein mediated DNA looping is a well known gene regulatory mechanism. A commonly studied system that controls gene expression is lactose repressor (LacI) induced DNA looping. In two in vitro studies, the Müller-Hill group investigates how the lac repressor protein in *E. coli* forms loops with linear and cyclized DNA. Their experiments analyzed LacI induced looping on linear DNA over a wide range of interoperator lengths (6-21 helical turns) and on supercoiled DNA minicircles of 452 base pairs. In these experiments, electron microscopy, non-denaturing polyacrylamide gel electrophoresis, and DNase I protection experiments were used to detect loop formation, estimate loop size, quantify loop stability, and for supercoiled DNA to detect loop topology ( $\Delta Lk$ ). In our study, we exercise our computational rod model to make side-by-side comparisons of our predictions with their experimental observations. By making comparisons, we look to understand the energetic cost of loop formation and the resulting topology of the looped complex.

### 395-Pos

#### High Throughput Screening of Aptamers For Human Thrombin and Factor IXa

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The traditional method for discovery of DNA/RNA aptamers is *in vitro* evolution (SELEX), where multiple cycles of partitioning and amplification enrich aptamer candidates from a pool containing randomized segments of length, *m*. Previous to our work the consensus sequence for alpha-thrombin aptamers (ThbA) was found via five rounds of SELEX in a DNA pool with *m*=60, while factor IXa aptamers (FIXaA) were discovered in an RNA library of *m*=40 after 8 rounds. We isolated the same ThbA, FIXaA and a novel carbohydrate aptamer (CA) to validate our new method, High Throughput Screening of Aptamers. HTSA uses a single partitioning step, PCR, and counts survivors by massively parallel sequencing. We found the minimal ThbA in a library of DNA hairpins loops (*m*=15) containing 56,000 copies of each of the 1.1 billion possible sequences. We distinguished two sequence motifs well above the background. The ThbA motif contains the consensus (counted 46,000 times) and dozens of related sequences. The leading candidate in the CA family (29,000 counts) is a novel aptamer that binds glucose (*K*<sub>d</sub>=1,400 nM) and alpha-methylmannoside (*K*<sub>d</sub>=500 nM). A known FIXaA was counted 52,000 times from a library of RNA hairpins (*m*=16 with 14,000 copies of each sequence). HTSA simplifies and shortens the discovery process, exhaustively searches the space of sequences within a library, simplifies characterization of the core binding domain, reduces the quantity of the target required, eliminates cycling artifacts, allows multiplexing of targets, and requires no complex automation.

### 396-Pos

#### Engineered Holliday Junctions As Single-Molecule Reporters For Protein-Dna Interactions

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Protein-DNA interactions are essential for gene replication and expression. Characterizing how proteins interact with and change the structure of DNA

upon binding is crucial in elucidating the mechanism of protein function. Here, we use engineered DNA Holliday junction (HJ) as a single-molecule FRET reporter to study how CueR, a Cu(I)-responsive MerR-family metalloregulator, interacts with its DNA substrate for transcriptional regulation. By analyzing the single-molecule structural dynamics of the engineered HJ in the presence of varying concentrations of both apo- and holo-CueR, we show how this metalloregulator interacts with and change the structures of the two HJ conformers, forming various protein-DNA complexes at different protein concentrations. We also show how apo- and holo-CueR differ in their interactions with DNA, as well as their similarities and differences with other members of the MerR-family of regulators, in particular in their mechanisms of switching off gene transcription after activation. This method of using engineered HJs to quantify changes in the structure and dynamics of DNA upon protein binding provides a new tool to elucidate the correlation of structure, dynamics, and function of DNA-binding proteins.

### 397-Pos

#### Complex Kinetics of the $\lambda$ Repressor-Mediated DNA Loop

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$\lambda$  repressor-mediated DNA loop formation and breakdown were monitored by Tethered Particle Microscopy (TPM) (1). The dwell times of the looped and unlooped DNA states, as revealed from TPM traces, were analyzed and revealed a complex kinetics for both loop formation and loop breakdown. A mechanism is proposed where  $\lambda$  repressor non-specific binding to DNA may play an important physiological role.

(1) C. Zurla, C. Manzo, D.D. Dunlap, D.E.A. Lewis, S. Adhya, L. Finzi, "Direct demonstration and quantification of long-range DNA looping by the  $\lambda$  bacteriophage repressor.", *NAR*, **37**, 2789-2795, 2009.

### 398-Pos

#### Free Energy Landscape of Nonspecific Protein-DNA Encounter

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Although structural, thermodynamic and kinetic studies of protein-DNA recognition have enhanced our understanding of both nonspecific and specific interaction mechanism, a few points are still in questioned, which are related to (i) how fast a protein can reach a given target on DNA and how long it will reside on DNA to perform its function, (ii) the energetic nature of protein-DNA interactions accompanied by conformational change, and (iii) the state of water in the DNA grooves and its role in the process of protein-DNA recognition. Here we have used the nuclease domain of colicin E7 (N-ColE7) from *E. coli* in complex with a 12-bp DNA as the model system to draw a picture of how a protein is encountering DNA. Brownian Dynamics (BD) coupled with Molecular Dynamics (MD) simulations are performed to provide the encountering process in multiple timescales. Several encounter complexes, which have different positions and orientations of protein around DNA in the initial structures, are extracted from MD trajectories. Then those encounters are simulated using BD to estimate the association rates at different protein binding sites on DNA, characterize the reaction pathway based on the free energy landscape and determine the spatial and orientational aspects required for the association. The results facilitate better understanding of sequence-independent protein-DNA binding landscapes and suggest the favorable encounter states.

### 399-Pos

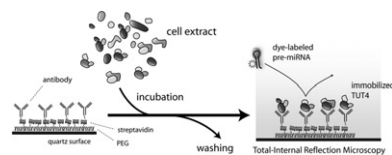
#### Single-Molecule Study on MicroRNA Machineries: MicroRNA processing With Immunoprecipitates At the Single-Molecule Level

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MicroRNAs (miRNAs) regulate gene expression via RNA silencing. Drosha initiates miRNA biogenesis by releasing a hairpin RNA (pre-miRNA) from a primary miRNA transcript. The pre-miRNA is processed into the mature miRNA by Dicer. It was also discovered that TUT4 (terminal uridylyl transferase 4) interferes with Dicer processing by uridylating pre-miRNAs. Discovery of these enzymes, however, was not accompanied with the study on the molecular mechanisms because of the lack of purified recombinant proteins. Here we report a novel method that combines single-molecule fluorescence

with immunoprecipitation, which is useful for studying proteins that are difficult to purify. On quartz surface in a microfluidic chamber, where single-molecule observation is going to be made, TUT4 proteins in crude cell extract



are immobilized with specific antibody. After effectively washing away unwanted other proteins from the chamber, the interaction between proteins and dye-labeled RNAs are observed in real time. The direct observation reveals the uridylation process at the molecular level and helps identify distinct modes of action. This newly developed method of immunoprecipitation in singulo may be applied in studying other proteins such as Drosha that cannot be obtained as purified.

## Membrane Physical Chemistry I

### 400-Pos

#### Nanomechanics of Lipid Bilayers: Heads or Tails?

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Understanding the effect of mechanical stress on biological membranes is of fundamental importance in biology since cells are known to perform their function under the effect of a complex combination of forces. The chemical composition and the lateral organization of such membranes are the ultimate responsible for determining their cellular scaffold and function. Micrometer-scale assays have revealed a wealth of information regarding the overall membrane mechanical resistance. Nonetheless, they are restricted to the use of giant bilayers, thus providing a mesoscopic outlook on the bilayer mechanical stability. Here we use force spectroscopy to quantitatively characterize the nanomechanical stability of supported lipid bilayers as a function of their chemical composition thanks to a molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve, hallmark of bilayer rupture. By systematically probing a set of bilayers exhibiting different chemical composition, we first show that both the headgroup and tail have a decisive effect on their mechanical properties. While the mechanical resistance dramatically changes for phospholipids composed of a 18:0 chain with varying headgroups within a wide range (3nN-66nN), the chain length increases the mechanical stability in ~ 6 nN for every extra pair of -CH<sub>2</sub> groups present in the chain along the series DMPC-DSPC. Furthermore, each unsaturation in the chain readily decreases the mechanical stability of the bilayer by ~1.5 nN. Finally, and contrary to previous belief, we demonstrate that upon introduction of cholesterol the mechanical stability of membranes not only increases in the liquid phase (DLPC) but also for phospholipids present in the gel phase (DPPC). This work highlights the compelling effects of subtle structural variations of the chemical structure of phospholipid molecules on the membrane behaviour when exposed to mechanical forces, a mechanism of common occurrence in nature.

### 401-Pos

#### The Phase Behavior of Supported Lipid Bilayer Mixtures and Cell Membranes Imaged By Secondary Ion Mass Spectrometry

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Supported lipid bilayers have been used as a tool to study the biophysical properties of model membranes with defined compositions. Special attention has been given to the role of cholesterol on the phase behavior of lipid membranes, in particular, to the formation of lipid rafts and complexes. Common techniques used to elucidate the phase behavior of binary and ternary lipid bilayers (i.e. fluorescence and atomic force microscopy) have been limited by their inability to provide direct information on the spatial composition of membranes. Secondary ion mass spectrometry (NanoSIMS) has proved to be a powerful tool for imaging the lateral organization of lipid bilayers with a spatial resolution in the order of tens of nanometers. In this study, a NanoSIMS is used to image lipid bilayers containing isotopically-labeled cholesterol. Additionally, human cell membranes are also imaged.

### 402-Pos

#### Direct Measurement of Time-Dependent Domain Coarsening in Giant Unilamellar Vesicles

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Liquid domains appear on giant unilamellar vesicles (GUVs) composed of a ternary mixture of saturated phospholipids, unsaturated phospholipids, and cholesterol when the temperature is quenched below the miscibility transition temperature. If the vesicle is taut, domains diffuse freely in the membrane and coalesce when they collide. This process is called coarsening. As the domains coarsen, the average radius of the domains increases with time as  $t^x$ , where  $x$  is the power law exponent of radius growth. The power law exponent has been