occurs at these ss-dsDNA junctions and the physiological consequences of this 'breathing' is not well understood, in part because it has been difficult to measure in a sequence-specific manner in biological systems. We have used single and dimer pairs of 2-aminopurine (2-AP) residues, site-specifically placed at various positions on both sides of the ss-dsDNA junctions of DNA constructs, as spectroscopic probes of this breathing. Replacing adenosine with 2-AP in DNA has minimal biological or physical consequences, and these moieties absorb, fluoresce and display CD spectra at wavelengths >300 nm where other nucleic acid and protein components are transparent. The optical properties of these constructs have been used to measure the position-specific extent and nature of the fluctuations of forked and P/T DNA junctions. We find that spectroscopically measurable melting penetrates ~2 bps into the interior of the duplex region of these junctions under physiological conditions. In addition 2-AP bases in ssDNA loci directly adjacent to these junctions display significantly more unstacked character than do 2-AP probes located within long ssDNA sequences. Quenching of 2-AP fluorescence with acrylamide has independently confirmed these results. These local and transient DNA conformations have possible biological significance as interaction targets for DNA-manipulating enzymes, and we are using these breathing properties of DNA in isolation as a platform to study helicase activity on duplex DNA and ultimately the coupling of these helicases to the other components of the 'macromolecular machines of gene expression'.

1768-Pos Board B612

Conformational Dynamics between B- and Z-DNA probed via single-molecule FRET

Sangsu Bae[1], Doyoun Kim[2], Kyeongkyu Kim[2], Yanggyun Kim[3], Sungchul Hohng[1].
[1]Seoul National University, Seoul, Republic of Korea, [2]Sungkyunkwan University School of medicine, Suwon, Republic of Korea, [3]Sungkyunkwan University, Suwon, Republic of Korea.

Since the first discovery of the left-handed DNA structure, **Z-DNA**, in 1979, its biological role has been under constant debate, partly because of the unclear understanding of the Z-DNA formation mechanism in the cell.

Here, we report the first single-molecule FRET experiments on Z-DNA formation. A series of DNA duplexes containing varying CG repeats and different junction numbers were tagged with Cy3 (donor) and Cy5 (acceptor) for FRET measurements, and biotinylated at the end for surface immobilization. Prism-type single-molecule FRET setup was used to monitor the conformational dynamics of hundreds of single molecules simultaneously at varying salt concentrations and with or without Z-DNA inducing protein factors.

The salt-induced B-to-Z transition occurred fast with transition time of ~ 10 seconds at 5 M of $NaClO_4$, and was reversible because B-form DNA was readily recovered when the salt concentration was reduced back. The transition due to the Z-DNA binding domain ($hZ\alpha_{ADAR1}$) from the human editing enzyme, double-stranded RNA adenosine deaminase, however, was relatively slow with ~3 minutes of transition time. Contrary to the salt-induced cases, B-form DNA was not readily recovered after protein removal, which implies the tight binding between Z-DNA and $hZ\alpha_{ADAR1}$. We determined the free energy change of protein-induced B-Z transition by measuring the equilibrium constant of duplex DNAs with varying CG repeats. The junction free energy was also determined by comparing duplex DNAs with one B-Z junction or two B-Z junctions.

1769-Pos Board B613

DNA Conformation and Biomolecular Motors: New Nanomedicine Research Targets

Sonia Trigueros, Sonia Contera, John Ryan.
Oxford University, Oxford, United Kingdom.

DNA supercoiling is a feature of almost all DNA molecules. It is a powerful thermodynamic force that drives and directs many DNA associated processes in vivo. The level of supercoiling or DNA spatial conformation is constantly changing due to the activities of proteins and the environmental conditions of the cell. Local and temporal changes in DNA supercoiling affect many cellular processes such as replication, transcription recombination and chromosome organization.

DNA biomolecular motors such as DNA topoisomerases and DNA translocases are responsible for maintaining the steady state of supercoiling essential for cell viability. In prokaryotes, DNA supercoiling is expected to play an important role in site-specific recombination, a fundamental process to achieve resolution of dimeric chromosomes, allowing plasmids and chromosome segregation and consequently cell division. During this process, DNA undergoes multiple conformational changes due to the activity of Tyrosine recombinases and a DNA translocase known as FtsK.

I use cell biology, biochemical and biophysical techniques to study the role of DNA biomolecular motors and DNA topology in different cellular processes. In vitro, we demonstrate the topology dependence of the different steps in site-specific recombination events using DNA substrates with different superhelical density. By TIRFM, I characterize at the level of single molecule the activity of DNA molecular motors. Using high-resolution amplitude modulation atomic force microscopy (AM-AFM) in physiological buffer we characterize the nature of the forces that drive relevant DNA conformational changes by itself or after protein interaction during site-specific recombination events. Additionally, we observe for the first time the dynamics of DNA and the conformational changes of DNA during site-specific recombination events imaged by high-speed AFM at time resolutions up to 20 ms and sub-nm spatial resolution. Our current research is focus on DNA biomolecular motors as new nanomedice targets.

1770-Pos Board B614

Defect Excitation In Sharply Bent Dna And Its Micromechanical Consequences

Jie Yan.
National University of SIngapore, Singapore, Singapore.

Kinkable defect excitation in sharply bent DNA molecules have drawn attentions in recent years. Such excitations were recently observed in molecular dynamics simulations, and were experimentally observed in circular DNAs of 64 - 65 base pairs. In addition, it was shown that rare excitation of flexible defect provided an explanation to the unusually large cyclization probability of 94 bp DNA reported recently. In this presentation, we show that defect excitation also explains a few other experiments where unusual DNA mechanical responses were observed, and we present our predictions of other measurable DNA mechanical responses for future experiments. In addition, we provide a new experimental evidence demonstrating the breakdown of the traditional WLC model when DNA is sharply bent. Finally, we discuss the molecular details of the possible defects based on our molecular dynamics simulations.

1771-Pos Board B615

Manipulating Single dsDNA Molecules To Study Force Induced Phase Transitions

Claudia Danilowicz, Kristi Hatch, Charles Limouse, **Ruwan Gunaratne**, Julea Vlassakis, Jeremy Williams, Vincent Coljee, Mara Prentiss.
Harvard University, Cambridge, MA, USA.

Double stranded DNA (dsDNA) exists in a variety of different conformations *in vivo*, even in the absence of force. We have used magnetic tweezers to study the overstretching transition that takes place when dsDNA is pulled at constant force. We show that during this transition there is no significant single stranded DNA formation and the relaxation to the original B-DNA form exhibits variable hysteresis depending on the pulling ends. The extent of this hysteresis is also dependent on the type of salt used decreasing with increased screening efficiency, suggesting that the overstretched state is not melted DNA. We studied the effect of ion concentration for several monovalent cations and anions. Our results are consistent with existing theory that predicted that the overstretched states are forms of dsDNA that depend on the pulling technique, where 5'5' stretching produces a narrow fiber and 3'3' stretching produces a more widely spaced ladder with each case presenting a different characteristic charge spacing. In addition we studied the shear force required to denature long dsDNA when a constant force is applied to the 3'3' ends or the 5'5' ends. For lambda phage dsDNA, the critical forces for shearing by pulling from the 5'5' and 3'3' ends are 124.4 pN and 141.3 pN respectively, whereas the overstretching force is 65 pN for both cases. Given that short dsDNA molecules that shear before they completely overstretch have the same shear force for both pulling techniques, these results further support the theoretical proposal that overstretching dsDNA by pulling from the 3'3' ends produces a different structure than pulling from the 5'5' ends.

1772-Pos Board B616

Study Of Sequence Dependent Homolog Pairing With A Single Molecule Assay

Claudia Danilowicz, Chiu H. Lee, Kristi Hatch, Vincent W. Coljee, Nancy Kleckner, Mara Prentiss.
Harvard University, Cambridge, MA, USA.

One of the most prominent forms of shuffling genetic material is homologous recombination. Sexual reproduction, meiosis, is a hallmark of eukaryotic life on earth and is accompanied by homologous recombination of chromosomes which maximizes diversification while minimizing DNA damage. Different mechanisms have been proposed to explain how double stranded DNA (dsDNA) homologues find each other and lock together with their sequences matched so that crossovers between chromosomes result in new chromosomes;

however, the processes underlying this important feature of meiosis are not yet understood. In this work, we show that even in the absence of proteins or additional polymers, individual dsDNA molecules of 5 kb or longer in solutions containing only monovalent ions, can self-assemble into sequence dependent pairs, where this homologous dependent pairing can occur at lower dsDNA concentrations than those typically found in vivo. We have used magnetic tweezers to probe the stability of these molecular pairs and demonstrate that at room temperature they remain bound for shear forces up to 10 pN. To elucidate the mechanisms that underlie the observed homologous association of pairs, we studied the effect of sequence, dsDNA concentration, non specific competitors, temperature, and salt.

1773-Pos Board B617

Conformational Equilibria Of Bulged Sites In Duplex Dna Studied By EPR Spectroscopy And Differential Scanning Calorimetry

Bruce Robinson[1], Alyssa L. Smith[1], Pavol Chekan[2], Greg Brewood[3], Tamara M. Okonogi[4], Saba Alemayehu[3], Eric J. Hustedt[5], Albert S. Benight[3], Snorri Th. Sigurdsson[2].
[1]Univ Washington, Seattle, WA, USA, [2]Uinversity of Iceland, Reykjavik, Iceland, [3]Portland Biosciences Inc., Portland, OR, USA, [4]Fleming Pharmaceuticals, Fenton, MO, USA, [5]Vanderbilt, Nashville, TN, USA.

Conformational flexibility in nucleic acids provides a basis for complex structures, binding, and signaling. One-base bulges directly neighboring single-base mismatches in nucleic acids can be present in a minimum of two distinct conformations, complicating the examination of the thermodynamics by calorimetry or UV-monitored melting techniques. To provide additional information about such structures, we demonstrate how electron paramagnetic resonance (EPR) active spin-labeled base analogues, base-specifically incorporated into the DNA, are monitors of the superposition of different bulge-mismatch conformations. EPR spectra provide information in terms of ''dynamic signatures'' that have an underlying basis in structural variations. By examining the changes in the equilibrium of the different states across a range of temperatures, the enthalpy and entropy of the interconversion among possible conformations can be determined. The DNA constructs with a single bulge neighboring a single-base mismatch (''bulge-mismatches'') may be approximately modeled as an equilibrium between two possible conformations. Experiments on the bulge-mismatches show that basepairing across the helix can be understood in terms of purine and pyrimidine interactions, rather than specific bases. Measurements of the enthalpy and entropy of formation for the bulge-mismatches by differential scanning calorimetry and UV-monitored melting confirm that the formation of bulge-mismatches is in fact more complicated than a simple two-state process, consistent with the base-specific spectral data that bulge-mismatches exist in multiple conformations in the pre-melting temperature region. We find that the predictions of the nearest-neighbor (NN) model, based on data from DNA denaturation experiments, do not correlate well with the structures inferred from the base-specific EPR dynamics probe. We report that the base-specific spin probes are able to identify a bi-stable, temperature dependent, switching between conformations for a particular complex bulged construct.

1774-Pos Board B618

Closing The Lid On Dna End-to-end Stacking Interactions

Li Li, Suzette Pabit, Jessica Lamb, Hye Yoon Park, Lois Pollack.
Cornell University, Ithaca, NY, USA.

Recent experiments suggest that short DNA strands associate by end-to-end stacking. The stacking of hydrophobic ends of double-strand DNA molecules may provide the favorable free energy required for association. Here, we report interactions between DNAs with modified ends. DNA duplexes, 20 bp long, were capped with short T_4 loops at 2, 1 or 0 ends, and were placed in solutions dialyzed against buffer containing 20mM Mg^{2+}. Association was not observed in constructs with both ends capped. DNA-DNA interactions were characterized by measuring variations in small angle X-ray scattering (SAXS) curves at the lowest scattering angles. Second virial coefficients were computed from the SAXS data to further confirm that end-to-end stacking plays an important role in short strand DNA-DNA interactions.

1775-Pos Board B619

A Designed 3D Self-Assembled Crystalline DNA Array

Nadrian C. Seeman[1], Jianping Zheng[1], Jens J. Birktoft[1], Ruojie Sha[1], Tong Wang[1], Pamela E. Constantinou[1], Yi Chen[2], Chengde Mao[2].
[1]New York University, New York, NY, USA, [2]Purdue University, West Lafayette, IN, USA.

The precise control of the structure of matter is a central concern of the natural sciences. To this end, numerous investigators have developed self-assembling systems to produce targets of interest. Taking its cue from biological systems, structural DNA nanotechnology has used branched DNA motifs combined with cohesive ends to produce objects, nanomechanical devices and designed two-dimensional lattices. The details of these 2D lattices have been studied primarily by atomic force microscopy, whose resolution is 3-10 nm in typical analyses. The criteria for three dimensional self-assemblies are more strict, because the primary technique for their analysis is x-ray crystallography, whose resolution is limited only by the wavelength of the source (about 1 A). Previous efforts to produce self-assembled three-dimensional lattices have produced lattices that conformed to the design, but whose resolution was no better than 10 A, not really capable of revealing molecular structure. Here, we report the crystal structure at 5 A resolution of a self-assembled designed three-dimensional lattice based on the tensegrity triangle. Each edge of the tensegrity triangle contains two turns of DNA. The structure and sticky ends have been designed to be 3-fold symmetric, and at this resolution it appears to be so. The data are of sufficient quality to demonstrate clearly that it is possible to design a 3D lattice using the techniques of self-assembly based on molecular recognition.

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1776-Pos Board B620

Helix-Coil Transitions of Unusual DNA Structures by Measuring the Fluorescence Changes of 2-Aminopurine When Incorporated into DNA

Hui-Ting Lee, Lela Waters, Chris M. Olsen, Irine Khutsishvili, Luis A. Marky.
U. of Nebraska Medical Center, Omaha, NE, USA.

One focus of our research is to investigate the helix-coil transition of unusual DNA structures by comparing optical and calorimetric melting curves with fluorescence melts obtained by observing the fluorescence changes of 2-aminopurine (*AP*) when incorporated into DNA. In this work, we used a combination of temperature-dependent UV, circular dichroism (CD) and fluorescence spectroscopies, and differential scanning calorimetric (DSC) techniques to investigate the unfolding of: a) an intramolecular pyrimidine triplex, $A_3APA_3C_5T_7C_5T_7$; b) a G-quadruplex, $G_2T_2G_2TAPTG_2T_2G_2$; and c) a dodecamer duplex, CGCGA*AP*TTCGCG. Our experimental observations are as follows.

a) All melting curves of the triplex show similar monophasic transitions with T_Ms of 31 °C, ΔH_{vH}s of 38 kcal/mol and ΔH_{DSC} of 70 kcal/mol. Thus, this triplex unfolds in a non-two state fashion similar to the unfolding of the unmodified triplex but with a lower endothermic heat (by 12 kcal/mol). This indicates the presence of *AP* contributes with lower stacking interactions.

b) The G-quadruplex also unfolds in similar monophasic transitions: T_Ms of 52°C, ΔH_{vH}s of 44 kcal/mol and ΔH_{DSC} of 36 kcal/mol. The unstacking of *AP* follows the overall cooperative unfolding of the whole molecule. This quadruplex unfolds in two-state fashion similar to the unfolding of the unmodified quadruplex but with a higher endothermic heat (by 14 kcal/mol). This indicates that the presence of *AP* induces higher stacking contributions.

c) All melting curves of the dodecamer duplex show biphasic transitions. The T_M of the first transition depends on strand concentration while the T_M of the second one is independent, consistent with the presence of a duplex ← → hairpin ← → random coil equilibria. The central placement of *AP* destabilizes the duplex state, while does not affect the hairpin state.

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1777-Pos Board B621

Understanding the Chemistry of Cytosine Methylation Effects

Sarah Primrose[1], Stephanie McCarty[1], Janista Lek[2], Kimberly Fong[2], Yi Tian[3], Leonard J. Mueller[3], **Mary E. Hatcher**[4].
[1]Claremont McKenna College, Claremont, CA, USA, [2]Scripps College, Claremont, CA, USA, [3]UC Riverside, Riverside, CA, USA, [4]The Claremont Colleges, Claremont, CA, USA.

Proteins bind to DNA to initiate several important biological processes including DNA replication and transcription of genes, these processes are also highly regulated. A common method of bioregulation is cytosine methylation. Recent research suggests that cytosine methylation quenches DNA dynamics, preventing the DNA deformation often necessary for efficient protein-DNA complex formation. Using ^{31}P NMR, we have shown that methylation induced rigidity may stem from conformational changes in the backbone from BI to BII conformation. To explore the mechanism of these conformational changes, we have turned to the study of cytosine fluorination.