3059-Pos
Application of Shotgun DNA Mapping to Yeast Genomic DNA Shotgun Clones
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Shotgun DNA mapping (SDM) is the ability to identify the genomic location of a random DNA fragment based on its naked DNA unzipping forces compared with simulated unzipping forces of a published genome. We have previously demonstrated proof of principle for shotgun DNA mapping by using plasmid pBR322 unzipping data amongst yeast genome background[cite preprint]. Currently we are validating the technique using unzipping data from yeast genomic DNA. Genomic DNA from yeast (S. cerevisiae) has been digested with restriction endonucleases to produce a library of random fragments, which we used to create a limited library of shotgun clones. Single-molecule unzipping constructs derived from these clones will be unzipping with optical tweezers (OT). In parallel, we have created a library of simulated possible unzipping force profiles, based on the known yeast genome sequence. The OT data and the library will be used in our existing SDM algorithms to identify each shotgun clone, and success rate will be determined via DNA sequencing of the clones. A major application of SDM we are working towards is mapping of nucleosomes and RNA Polymerase II molecules on native chromatin. We will report our progress towards this goal and also discuss other applications of SDM, including splice variant and telomere analysis.

3060-Pos
Separating Static and Dynamic Heterogeneity in Single-Molecule FRET Experiments with Burst Variance Analysis (BVA)
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Histograms of single-molecule FRET efficiency (E) are frequently employed to characterize macromolecular dynamics, and have been been used to better understand the structure and function of proteins such as E.coli RNA Polymerase, Calmodulin and LacY. While such plots are useful for characterizing simple distance changes represented by shifts in mean E value, substantial static heterogeneity, dynamic heterogeneity or both may exist within a single sample, making interpretation of the resulting E histogram difficult. To address this problem we introduce Burst Variance Analysis (BVA), in which we generate a 2D histogram of the mean FRET of a given molecule (E) versus its standard deviation (SD). We use simple theoretical considerations to predict the expected SD, and produce confidence intervals rigorously defining the boundaries beyond which the SD is consistent with dynamics. To validate the method, we performed both numerical simulations and experiments on well-characterized dynamic DNA hairpins. We found that BVA can detect dynamics near the diffusion timescale and over several orders of magnitude; additionally, we used it to distinguish between static and dynamic subpopulations, and separate them for analysis. Using BVA, we analyzed conformational dynamics in the Klenow fragment of E.coli DNA polymerase I, and found evidence for both static and dynamic subpopulations indistinguishable from one another in a simple E histogram. We expect this method to be broadly applicable to single-molecule FRET analyses of macromolecules, and to aid in identifying hidden static or dynamic heterogeneities in their behaviour.

3061-Pos
Visualizing the 1D Diffusion of Eukaryotic DNA Repair Factors Along a Chromatin Lattice
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The ability of DNA binding proteins to efficiently locate a target sequence or structure amongst a vast excess of nonspecific genomic DNA is a critical function affecting a variety of processes such as DNA repair, gene expression and DNA replication. This genomic search is further complicated in eukaryotes where DNA is organized into chromatin and it remains unclear whether nucleosomes act as obstructions which disrupt the scanning process, or whether eukaryotic DNA-binding factors are capable of bypassing these obstacles. Through the use of a single-molecule optical microscopy assay that aligns arrays of DNA molecules in an extended configuration we are able to visualize the facilitated diffusion of the mismatch repair factors Msh2-Msh6 and Mlh1-Pms1 along naked DNA as well as chromatin. Under physiological salt conditions Mlh1-Pms1 moved along DNA by a one-dimensional random walk and exhibited characteristics consistent with a model where the protein moved via a hopping mechanism while wrapped around DNA in a ring-like configuration. Notably, the average diffusion coefficient obtained for Mlh1-Pms1 is approximately an order of magnitude greater than that of its mismatch repair partner Msh2-Msh6 suggesting that these proteins may travel along DNA by distinct mechanisms. Additionally, application of hydrodynamic flow strongly biased the motion of Mlh1-Pms1, highlighting the importance of our technique that maintains DNA in an extended conformation in the absence of perturbing forces. Mlh1-Pms1 was able to freely bypass nucleosomes as it diffused along DNA whereas Msh2-Msh6 was partially confined by nucleosome barriers, passing much less frequently. This work demonstrates that Mlh1-Pms1 is capable of rapidly traveling along naked DNA as well as chromatin whereas the movement of Msh2-Msh6 is significantly hindered by the presence nucleosomes and suggests possible roles that facilitated diffusion may play in mismatch repair.

3062-Pos
Antiviral Signaling Mediated By RIG-I Translocation Activity
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RIG-I is a cytosolic multi-domain protein that detects viral RNA and elicits an antiviral immune response. Two N-terminal caspase activation and recruitment domains (CARDs) transmit the signal and the regulatory domain prevents signaling in the absence of viral RNA. 5'-triphosphate and double stranded (ds) RNA are two well known viral PAMPs (pathogen associated molecular patterns) that enable RIG-I to discriminate pathogenic from self-RNA. However, the function of the ATPase domain that is also required for activity is less clear. Using PIFE (protein induced fluorescence enhancement), a newly developed single-molecule fluorescence assay we discovered a robust, ATP-powered dsRNA translocation activity of RIG-I. The CARDs dramatically suppress translocation in the absence of 5'-triphosphate and the activation by 5'-triphosphate triggers RIG-I to translocate preferentially on dsRNA *in cis*. This functional integration of two RNA molecular patterns may provide a means to specifically sense and counteract replicating viruses.

3063-Pos
Following the Motions of a DNA Helicase on DNA in Real Time
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Using several experimental approaches, we are investigating the dynamics of individual PcrA DNA helicase interactions on DNA templates. Bulk biophysical and biochemical measurements are done in parallel to ascertain the viability and integrity of the protein and DNA substrates. For "Pacman", we are investigating the relationship between the hydrolysis of ATP and the mechanical motion of the enzyme along a DNA substrate. We have designed a mutant PcrA with two cysteines for attachment of a pair of dyes to follow internal protein motions using single-pair fluorescence resonance energy transfer (spFRET). For "Ring-a-bell" we are following the interaction of PcrA with replication protein RepC. Alone, PcrA is typically capable of translocating only ~80 bp before dissociating from a DNA template. With RepC, PcrA can achieve rolling circle replication of thousands of bp on a plasmid. Should the two proteins be bound together, they should create specific low and high spFRET signals as they translocate along a specifically labeled DNA substrate. And finally we are studying the interaction of PcrA and Holiday Junction DNA. Does PcrA separate the DNA from the center or from the ends of the DNA junction? We have upgraded our evanescent field fluorescence microscope to use alternating red (648 nm) and green (532 nm) lasers to illuminate the sample. We have added multiple syringes operating under computer control to follow experiments in real-time whereupon ATP is introduced into the liquid flow chamber. We have also upgraded our scanning confocal microscope to improve the alignment of the laser and the ease of use of the instrument.

3064-Pos
Quantum Dot Probes for Single-Molecule Rotation of Cell Surface Proteins
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Rotation of membrane proteins is a sensitive measure of their aggregation state and interactions. We have investigated the use of asymmetric quantum dots (QD) as non-bleaching imaging probes providing orientation-dependent optical signals from individual cell surface proteins. A commercial QD emitting at ~605 nm measures 10.9 x 5.3 nm and exhibits an initial fluorescence anisotropy of 0.11. Calculated rotational correlation times (RCT) for rotations about the particle short and long axes, 0.18 μs and 0.12 μs respectively, suggest that this nanoparticle can probe μs timescale molecular rotation. These QDs, and

the related 655nm QDs, conjugated using streptavidin to anti-insulin receptor antibody, are easily visualized bound to 2H3 cell insulin receptors (IR). Blinking of spots demonstrates imaging of individual QDs. We excite fluorescence from cell-bound QDs with non-polarized illumination and record orthogonally-polarized fluorescence images using an image splitter and an EMCCD camera. Image pairs are separated and one polarization is corrected for the optical path g-factor and for displacement, rotation and dilation relative to the other polarization. Time-dependent fluorescence from regions containing individual QDs in image pairs are extracted and the time-autocorrelation function for polarization fluctuations calculated either from actual polarization or from a combination of auto- and cross-correlations of polarized fluorescence intensities. Individual 655 nm QDs exhibit peak polarization fluctuations with an RMS amplitude of ~0.06. These fluctuations decay over 30-50 ms. Whether this slow decay represents hindered rotation of individual IR or results from crosslinking of multiple receptors by single QDs remains to be determined. Current work involves exploration of more highly-asymmetric QDs, use of faster detection methods and examination of the 2H3 cell Type I Fcϵ receptor where rotational dynamics on faster timescales have previously been explored in detail. Supported by NIH grant RR023156 and NSF grant CHE-0628260.

Molecular Mechanics & Force Spectroscopy I

3065-Pos

Identification of Infrared Spectrum from Human Bio-Energetic Campus

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The impact of living mater with unionized radiations is a theme of frequent researches for treatments in complementary medicine.

In presenting work we make the measurements of bio-energetic field applied to experimental fluids and we have given the explanations of the bio-energetic effect by quantum theory.

The technology of measurement was based on electro-mechanic principle, using a Laboratory pH Meter WPA-CD 7400, converting mV in units of intensity of bio-magnetic fields.

In bases of quantum theory (E= υ h) was calculated the Intensity of levels bio-energy, in mean value of 0.04 m eV, which correspond to 10^2 f T of bio-magnetic field, the Frequency of bio-magnetic wave, in value of $0.9*10^{12}$ Hz, the Bio-energetic wavelength=$2.5* 10^{-3}$ mm and corpuscular mass of bio-magnetic waves in value of ~ $2*10^{-32}$ Kg or 1/10 from electron mass, (M) when ''m''=$2/Ec^2$, in conformity with the parameter of mathematical counting or to prolong span of life cells.

Bio-energetic fluids can be used in technology of preparation of drugs, from homeopath medicine and in laboratory medicine for the changes of pH in liquid medium with cultivated stem cells for to prolong the span life of cells.

SPECIFICATIONS – pH Meter

Ranges	pH MV C	0.00 to 14.00 0 to ± 1999 -50 to +50	0.00 to 14.00 - -
Resolution		0.01pH/1 mV/0.1C	0.01 pH
Accuracy (electrical)		±0.01pH/1 mV/0.1C	±0.01 pH
Imput resistance		>10^{12} ohms	>10^{12}ohms
Auto Buffer Recognition		4.00 , 7.00 , 10.00 pH	-
Temperature Probe		TPA 100 platinum Resistance. User Correction up to ±2.00 C	-

Table 1

3066-Pos

Solvent Effect on the Unfolding Force of a Single Hydrophobic Polymer

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The hydrophobic collapse of a homopolymer is a much simplified model for studying the hydrophobic collapse of a protein. It is widely believed and theorized that the driving force for the hydrophobic collapse is the interfacial free energy between the polymer and the solvent. Therefore, changes in interfacial free energy should be directly proportional to the force to unfold the polymer in bad solvents. To test this hypothesis, we used single molecule force spectroscopy to unfold a single polystyrene chain in water-ethanol mixtures. Different percentage of binary mixture is used to create solvents with different interfacial energy with polystyrene. However, we do not see a linear correlation between the interfacial tensions with the unfolding forces. This is an indication that macroscopic properties such as the interfacial free energy cannot be directly applied to study certain systems in microscopic scale. In this study we also hypothesized a mechanism for the cause of this inconsistency.

3067-Pos

Active Stochastic Microrheology using Optical Tweezers

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Cells are dynamic structures capable of generating and reacting to physical cues in their environment. Measuring mechanical properties is thus essential for elucidating cell or other material structure-function in particular during dynamic rearrangement of the cytoskeleton. Although a variety of rheological techniques have been developed using video microscopy, AFM, and magnetic traps, the measurable frequency range is limited by the time to obtain the measurement, and forcing conditions such as amplitude, direction, contact geometry, and probe location. Here, we developed active stochastic microrheology using optical tweezers to enhance the temporal resolution and precision of detection. A stochastic force is generated by moving the trap relative to the sample. Both bead displacement and trap position are monitored simultaneously by separate position sensitive devices. With this method, both storage and loss shear moduli of the extracellular matrices can be extracted over a wide frequency range of $10^{-2} – 10^3$ Hz within a few minutes. Also, this method was used to probe the local mechanical environment of B-cell receptor using antigen specific interaction. We showed that the local mechanical properties are strengthened in response to antigen binding and repeated external excitation in a physiological range of 1–100pN. The mechanical responses can also be measured with respect to direction such as force applied normal and perpendicular to the cell membrane. This technique is useful in characterizing the mechanical properties at a user-defined location and magnitude, over a wide frequency spectrum, in a short time, and with a small deformation < 100nm. With these advantages, the method can also be applied to other cell processes, studies of complex fluids, fibril growth, and polymer solutions. Support from the NIGMS (GM-076689), an NSF Career Award (0643745), and the Singapore-MIT Alliance for Research and Technology (SMART-BioSyM) are gratefully acknowledged.

3068-Pos

A Single Molecule Force Spectroscopy Study of the Binding Interaction Between Insulin and G-Quadruplex DNA

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The formation of guanine (G)-quadruplex in the guanine-rich tandem repeats of the insulin-linked polymorphic region (ILPR) is linked to transcriptional effects on the insulin gene. Recent studies demonstrate that these G-quadruplexes can bind insulin, and while this may impact the transcription of insulin, little is known about the binding mechanism. A single molecule force spectroscopy study was performed to examine the selective binding of insulin to the ILPR G-quadruplex DNA. In this study, the insulin was covalently attached to a flat gold surface while the quadruplex DNA was attached to an AFM probe. The rupture force between insulin and quadruplex DNA was measured at various force loading rates. To confirm the specificity of the binding, control studies were performed by blocking the tethered G-quadruplex with ''free insulin'' in solution. Additional control studies were performed with a shorter DNA sequence incapable of forming a G-quadruplex on the AFM tip and a scrambled DNA sequence of the ILPR sequence. Results from the dynamic force-pulling studies are described based on the Bell-Evans and Dudko-Hummer models.

3069-Pos

A Single Molecule Study of Guanine Quadruplex DNA

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We present an atomic force microscopy (AFM) based single molecule force spectroscopy study of guanine (G) DNA quadruplexes. A bimolecular G-quadruplex was formed between a pair of single-stranded DNA molecules, each with two G-rich domains, that were immobilized on an atomic force microscope probe and an ultra-flat gold surface. G-quadruplex stability was examined as a function of the potassium ion concentration and loading rate (dynamic force spectroscopy). Kinetic and thermodynamic parameters for these single molecule G-quadruplexes were estimated with theoretical models, and the effect of the number of guanines in each G-rich domain was assessed. This study demonstrates that AFM-based single molecule force spectroscopy is a powerful tool for characterizing the structure, kinetics, mechanical and thermodynamic properties of G-quadruplexes.

3070-Pos

Mechanics of Lipid Bilayers of High Curvature

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Lysosomes, enveloped viruses, synaptic and secretory vesicles are all examples of natural nano-containers (diameter ~100 nm) which specifically rely on their