References

[1]. Wiita, A.P., Perez-Jimenez, R., Walther, K.A., Gräter, F., Berne, B.J., Holmgren, A., Sanchez-Ruiz, J.M., and Fernandez, J.M. (2007). "Probing the chemistry of thioredoxin catalysis with force" Nature, in press.

Single Molecule Biophysics - IV

2563-Pos Direct Imaging Of Human Rad51 Nucleoprotein Dynamics On Individual Double-Stranded DNA Molecules

Jovencio Hilario, Ichiro Amitani, Ronald J. Baskin, Stephen C. Kowalczykowski

University of California, Davis, Davis, CA, USA.

Board B677

Human Rad51 protein (Rad51) is central to recombinational repair of double-strand DNA breaks. It functions by polymerizing onto DNA and promoting the pairing and exchange of DNA strands between the damaged and intact homologous chromosomes. We visualized the real-time assembly and disassembly of Rad51 nucleoprotein filaments on individual double-stranded DNA molecules by single-molecule fluorescence microscopy. Rad51 assembly extends the DNA by 65%. Nucleoprotein filament formation occurs via rapid nucleation followed by growth from these nuclei. However, growth does not continue indefinitely, and nucleoprotein filaments terminate when 1–2 kb in length. The dependence of nucleation on of Rad51 concentration, suggests that ~3 Rad51 monomers are involved in the initial binding events. Rad51 nucleoprotein filaments are stable and remain extended when ATP hydrolysis is prevented; however, when ATP hydrolysis is permitted, filaments decrease in length as a result of protein dissociation and/or conversion to ADP-bound nucleoprotein complexes. Dissociation of Rad51 from dsDNA is slow and incomplete, demonstrating the necessity for a dsDNA translocating enzyme to accelerate disassembly.

2564-Pos Observing Prokaryotic Nucleotide Excision Repair: one molecule at a time

Neil M. Kad[1], Hong Wang[2], David M. Warshaw[1], Bennett Van Houten[2]

[1] *University of Vermont, Burlington, VT, USA,*
[2] *NIEHS National Institutes of Health, Research Triangle Park, NC, USA.*

Board B678

Nucleotide excision repair (NER) corrects DNA damage induced by a range of insults, from UV-induced thymine dimers to protein-DNA crosslinks. Here, we study the prokaryotic NER system from B. caldotenax which is comprised of three proteins: UvrA, UvrB, and UvrC. These proteins bind and operate sequentially to remove a 12–13nt oligonucleotide containing the DNA lesion. By using fluidics to suspend DNA strands between surface immobilized beads, we create tightropes upon which Qdot-labeled expressed Uvr proteins are seen to bind and slide. Using this assay we probe the interaction between DNA and the first two enzymes in the repair process: UvrA and UvrB. We have observed two modes of DNA interaction for UvrA: a transient static mode and a diffusive mode. The static interactions have an average lifetime of ~10s, and the diffusive interactions are slower than the predicted barrier-less diffusion constant on DNA. At 1M salt, the diffusion constant increases towards barrier-less diffusion suggesting electrostatic interactions are important for its physiological interaction with DNA. In agreement with solution studies we observe that UvrB requires UvrA to bind DNA. However, we find UvrB does not stably bind DNA, instead UvrB too shows two modes of interaction: transient static, and diffusion between defined regions on the DNA. Using two color differential labeling of UvrA and UvrB, we have observed rapid coincident diffusion under high salt conditions, mirroring UvrA alone. At physiological ionic strength, whether UvrA assists in UvrB's diffusive motion remains to be established. These experiments mark the beginning of a new single molecule approach to understanding the kinetics and order of processes that underlie prokaryotic NER. To our knowledge this is the first time a multi-protein complex has been visualized at the single complex level using differentially Q-Dot labeled constituent proteins.

2565-Pos Single Molecule Study Of Addab Helicase

Natali Fili[1], Christopher Batters[2], Mark I. Wallace[3], Mark S. Dillingham[4], Martin R. Webb[1], Justin E. Molloy[1]

[1] *National Institute for Medical Research, London, United Kingdom,*
[2] *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom,*
[3] *Chemical Research Laboratory, University of Oxford, Oxford, United Kingdom,*
[4] *Department of Biochemistry,University of Bristol, Bristol, United Kingdom.*

Board B679

DNA helicases are multi-functional motor proteins which translocate along DNA using the energy released from ATP hydrolysis. Translocation is coupled to diverse enzymatic activities, such as duplex unwinding, strand degradation and protein displacement. Resolving such complex biochemical properties requires a sensitivity and time resolution high enough for detecting rare and short-lived stochastic events. Unlike bulk measurements, single molecule approaches fulfil this requirement. We have developed a novel fluorescence-based assay, which allows us to monitor DNA unwinding by single helicases *in vitro*. Biotinylated double-stranded DNA (dsDNA) fragments are specifically immobilised on poly-ethylene glycol-coated surfaces through biotin-streptavidin interaction. Total internal reflection fluorescence (TIRF) microscopy is used to achieve high signal-to-noise ratio. The helicase activity is probed by a fluorescently labelled version of the *E.Coli* single-stranded DNA-binding protein (SSB), which preferentially binds single-stranded DNA (ssDNA). Single, helicase-mediated DNA unwind-

ing events are observed as fluorescent spots of increasing intensity, as increasing numbers of SSB molecules bind to the ssDNA product of the helicase. Using an objective-based TIRF microscope, these events are recorded at video rate. Increase in the fluorescence intensity directly correlates to the rate at which, single helicase molecules unwind DNA. Here, we use this assay to study the properties of the helicase/nuclease, AddAB, of *Bacillus subtilis*. Recombinant AddAB is allowed to bind the free dsDNA ends and its activity is stimulated by addition of ATP. The enzymatic properties of AddAB are studied as a function of DNA length, DNA sequence, ATP and Mg^{2+} concentration. A variety of AddAB mutants are used to dissect the mechanism by which AddAB activity is regulated by the recombination hotspot sequence (Chi). This assay adds to the single molecule toolbox available for studying DNA processing enzymes.

2566-Pos Single-molecule Studies of DNA Replication: Correlating Replisome Structure and Function

Joseph J. Loparo, Samir M. Hamdan, Charles C. Richardson, Antoine van Oijen

Harvard Medical School, Boston, MA, USA.

Board B680

DNA replication requires the coordinated activity of a large number of enzymes at the replication fork. Understanding the mechanisms controlling this organization requires a direct probing of the dynamics of fully functional replisomes during replication. Observations at the single-molecule level provide the most direct way to visualize the complex biochemistry of the replisome and to quantify the many transient intermediates essential to replication. We present results of single-molecule studies of functional phage T7 replisomes. We monitor the length of individual DNA molecules during replication and detect the formation and release of replication loops at the replication fork. Time-lapsed fluorescence microscopy of individual T7 replisomes demonstrate highly processive replication. Using these assays, we unraveled the molecular mechanisms that control the timing of replication-loop dynamics and ensure a timely reset of the replisome during the replication cycle.

Furthermore, we describe a novel assay that combines single-molecule fluorescence detection of labeled DNA polymerases with the mechanical manipulation of DNA. Changes in DNA length monitor the activity of enzymes at the fork, while changes in the fluorescence signal provide information on the stoichiometry and exchange of the components of the replisome. The combination of these two measurements allows us to correlate replisome function with its time-dependent structure. Studies to date have focused on the bacteriophage T7 replication machinery. The single-molecule methodologies described here, however, are directly applicable to other problems in genomic maintenance.

2567-Pos Single Molecule Fluorescence Studies of Transcriptional Regulation

Silvia Zorrilla[1], Denis Chaix[2], Carlos Alfonso[3], Germán Rivas[3], Dolores Peréz-Sala[3], Catherine A. Royer[2], Nathalie Declerck[2], M. Pilar Lillo[1]

[1] *Instituto de Química-Física Rocasolano. CSIC, Madrid, Spain,*
[2] *Centre de Biochimie Structurale, CNRS, INSERM, Univ. Montp. 1, Montpellier, France,*
[3] *Centro de Investigaciones Biológicas, CSIC, Madrid, Spain.*

Board B681

Transcriptional regulation is at the basis of bacterial cell proliferation under different growth conditions and is also crucial for the development of major human diseases such as cancer. Transcriptional regulation is achieved through a complex network of interactions including protein/DNA, protein/protein and protein/metabolite interactions. Therefore, detailed studies of the physical-chemical parameters governing these interactions are required for understanding the molecular basis of regulation processes and can be helpful in the quest for new antimicrobial agents or pharmacological targets. We are currently investigating on the control mechanism of a bacterial regulator involved in the regulation of carbon metabolic pathways (CcpN), as well as of nuclear receptors involved in the development of human diseases (PPAR). Our experimental approach is mainly based on fluorescence methodologies, including single molecule approaches such as fluorescence correlation and cross-correlation spectroscopy (FCS and FCCS), in combination with other biophysical techniques, in particular analytical ultracentrifugation. Combining these techniques allows precise and quantitative determination of the affinity, cooperative and stoichiometry of the complexes under true equilibrium conditions and to address challenging protein/DNA interactions. The most relevant results of these analyses will be presented.

This work was supported by the Marie Curie program (MERGT-CT-2006-046474) and by the Spanish Dirección General de Investigación (DGI) BFU2006-03905/BMC.

2568-Pos Visualizing Activator-dependent Transcription Initiation Start To Finish Using Multi-wavelength Single-molecule Fluorescence Microscopy

Larry J. Friedman, Jeff Gelles

Brandeis University Biochemistry Dept., Waltham, MA, USA.

Board B682

Regulated transcription initiation requires DNA binding of RNA polymerase and transcription factors, and the progression of the resulting complexes through multiple biochemical intermediates. In a molecular ensemble, this machinery can simultaneously exist in a large number of discrete biochemical states, making difficult the elucidation of full kinetic mechanisms for initiation. We here report the comprehensive kinetic mechanism of initiation at a prototypical

activator-dependent promoter, the σ^{54}-specific *glnALG* promoter of *Escherichia coli*. We circumvent the complexity of ensemble analysis by using multi-wavelength total internal reflection fluorescence microscopy to follow individual initiation reactions on surface-anchored DNA molecules that contain σ^{54} promoters. A Cy3 dye label on the σ^{54} subunit provides the signal for monitoring individual holoenzyme promoter bindings on DNA templates labeled with an Alexa-488 dye. Single transcription initiation events are identified by hybridizing the RNA transcript with an antisense oligonucleotide labeled with a third dye (Cy5). The observations reveal two distinct intermediates with σ^{54}RNA polymerase bound to a closed promoter. Moreover, open complex formation, escape into transcript elongation, and σ^{54} departures are detected and the interconversion kinetics for all states are measured. The experiments confirm that open complex formation or a closely associated process is the rate-limiting step during initiation, and demonstrate that open complex formation efficiently commits the polymerase to transcript synthesis. In contrast, both forms of closed complex are kinetically unstable so that multiple promoter binding and release events typically occur before a successful initiation. The results imply that closed complexes are kinetically responsive to changes in the σ pool even at this highly occupied σ^{54} promoter. Subsequent to open complex formation we nearly always observe σ^{54} release before detecting transcript production, indicating that the initiation subunit is efficiently ejected near the beginning of transcript synthesis.

2569-Pos Structure, Dynamics and Branch Migration of Holliday Junctions: A Single-Molecule Fluorescence Study

Mikhail A. Karymov[1], Mathivanan Chinnaraj[2], Aleksey Bogdanov[1], Swapnil Agarwal[1], Yuri L. Lyubchenko[1]

[1] *University of Nebraska Medical Center, Omaha, NE, USA,*
[2] *University of Alabama at Birmingham, Birmingham, AL, USA.*

Board 683

The Holliday junction (HJ) is a central intermediate of various genetic processes including homologous and site-specific DNA recombination and DNA replication. Elucidating the structure and dynamics of HJ's provides a basis for understanding the molecular mechanisms of these genetic processes. Our previous study (1) led to a model in which branch migration is a stepwise process consisting of consecutive migration and folding steps. These data revealed that a hop can be longer than one base pair; moreover we hypothesized that continuous runs over the entire sequence homology (10 bp) can occur. Direct measurements of the single-molecule FRET dependence on the donor-acceptor distance are required to justify this model. We performed FRET measurements of six immobile HJ constructs with varying numbers of base pairs between fluorescent dyes located on the opposite arms. The HJ designs were made in such a way that the distances between the donor and acceptor dyes would simulate one-base pair migration hops of a HJ having 10-bp homology. The FRET values determined for each acceptor-donor separation closely matched the values for the steps on the FRET time trajectories. These data showed that the junction is capable of migrating over several base pairs in a single hop confirming our model. We have also studied effect of GC-content on the lifetime of folded conformations during branch migration in mobile HJ. One GC pair placed at the border of the homology region almost blocks the migration over this site. On the other hand, a GC-pair placed in the middle of the migration region increases the lifetime of the folded state at this migration step approximately threefold.

Supported by the NSF grant PHY-0615590 (YLL).

References

1. Karymov et al (2005) *Proc Natl Acad Sci U S A* ***102***, 8186–91.

2570-Pos Modeling the Free Energy Cost of DNA Loop Formation

David P. Wilson[1], Todd D. Lillian[1], Sachin Goyal[2], Noel C. Perkins[1], Alexei V. Tkachenko[1], Jens-Christian Meiners[1]

[1] *University of Michigan, Ann Arbor, MI, USA,*
[2] *Cornell University, Ithaca, NY, USA.*

Board B684

We calculate the enthalpic and entropic contributions to the free energy cost of DNA loop formation common to many gene regulation systems. We describe the loop-forming, inter-operator DNA sequence using a dynamic, nonlinear elastic rod model capable of including sequence-dependent curvature and stiffness (Goyal et al). We use our model to determine the equilibrium (minimal energy) loop shape for each of the binding topologies allowed by the structure of the DNA-protein complex. The shape of the minimal energy loop determines the enthalpic contribution to the free energy. A sequence-dependent Hamiltonian is then constructed to describe linear perturbations about both the stress-free, intrinsically curved DNA, as well as the protein-bound looped DNA. The entropic contribution to the free energy is then calculated by comparing the normal(eigen) modes of free and looped states. The free energy allows us to determine loop stability as well as looping probability (Stockmayer J-factor) and reaction rates. Our computations reveal that entropic contributions can be as large as 10% of the free energy for DNA segments of the order of a persistence length (50 nm) for the LacI-DNA complex. Our work is also in good agreement with recent single molecule force dependent looping rate measurements for the LacI-DNA system (Chen et al).

2571-Pos Visualizing the Formation and Collapse of DNA Toroids

Bram van den Broek, Maarten C. Noom, Joost van Mameren, Gijs J.L. Wuite

VU University of Amsterdam, Amsterdam, The Netherlands.

Board B685

In eukaryotic and prokaryotic organisms as well as viruses, strongly positively charged polyamines play an important role in the organization of DNA. Interestingly, polyamines can cause DNA to undergo a sharp condensation phase transition in vitro. In this process, DNA molecules aggregate into highly ordered structures, generally with toroidal or rod-like shapes, consisting of many circumferen-

tially wound DNA strands. Toroidal DNA condensates are of particular interest because of their striking similarity with the morphology of compacted DNA found in viruses and sperm cells. Despite previous (single-molecule) experiments addressing the forces and energies involved in the process of DNA condensation and de-condensation, little is known about the dynamics of toroid formation and the stability of such a condensate. Here we show that the reversible condensation of single DNA molecules into toroidal structures under the influence of polyamines can be visualized with high spatial- and temporal resolution using a combination of optical trapping and fluorescence microscopy. We demonstrate that the minimal stable condensate consists of two DNA loops and we follow the unravelling of this structure into a non-condensed form when the DNA overlap is further reduced. In addition, we observe the nucleation dynamics of toroidal structures as well as its stepwise growth. Finally, we show that toroidal condensates can incorporate DNA from both sides at equal rates, providing a basis for diffusion-driven mobility of condensates along DNA. Moreover, this result suggests a mechanism that explains the formation of closely spaced toroids, as observed in sperm cells. An extensive, quantitative understanding of DNA toroid formation dynamics will be pertinent to the development and optimization of applications based on DNA-condensation, such as gene therapy.

2572-Pos Base Sequence Effects In Nanopore Unzipping Of DNA

Virgile Viasnoff, Ulrich Bockelmann

CNRS, ESPCI, Paris, France.

Board B686

Nucleic acids can be driven through an individual nanopore formed by a heptamer of α-hemolysin reconstituted in a lipid bilayer. Applying a voltage U across the membrane leads to an ion current flowing through the pore. The α-hemolysin pore exhibits an open diameter of 1.8 nm. Therefore, only single stranded DNA or RNA can be threaded through. The ion current through the pore transiently drops during the passage of a nucleic acid. Molecular constructs featuring a duplex part preceded by a single stranded overhang can be threaded into the pore, transiently held in the pore at low voltage U and subsequently unzipped at higher U.

Using α-hemolysin pores, we measure the statistical distribution of unzipping times for different base sequences and bias voltages. The results are compared to a theoretical study, which take the DNA base sequence explicitly into account. The unzipping process is described by a biased random walk in a one-dimensional energy landscape determined by the sequential basepair opening. Distributions of translocation times are numerically calculated as a function of duplex length, applied voltage und temperature. A rich dynamics is revealed for the coupled unzipping and translocation, bridging two different asymptotic regimes. One is a predominantly diffusive behaviour with a sequence specific effective diffusion constant. The other one is a dynamics dominated by pinning of the unzipping by rare events in the base sequence, namely the appearance of strong energy barriers to be thermally jumped over.

2573-Pos Tethered Particle Motion: Theory and Experiment for Looped and Unlooped DNA

Lin Han[1], Kevin Towles[2], John F. Beausang[2], Hernan Garcia[1], Rob Philips[1], Philip Nelson[2]

[1] *Caltech, Pasadena, CA, USA,*
[2] *Univ Pennsylvania, Philadelphia, PA, USA.*

Board B687

Tethered particle motion (TPM) is an experimental technique to monitor conformational changes in single molecules of DNA in real time, by observing the position fluctuations of a micrometer-size bead attached to the DNA. We present some recent work on theoretical problems inherent in the interpretation of TPM experiments, both in equilibrium and dynamical aspects. (1) The mean-square motion of the tethered bead can be used to report on the characteristics of its tether. We report on Monte Carlo simulation results, and compare them to experiments, relevant to bead diameters between 200–1000 nm and tether lengths between 100–1000 nm. (2) Relatively long camera shutter times are sometimes convenient experimentally, but complicate the theory. We report a new correction scheme that compensates for this, and improves our agreement with new experimental data. (3) TPM is an attractive method for studying DNA loop formation by protein complexes such as LacI. We report a new Gaussian sampling Monte Carlo simulation method that roughly concurs with new TPM experimental data for loop sizes around 300 basepairs. (4) Experimental determination of looping kinetics is complicated by the near coincidence of the loop formation/breakdown lifetimes with the diffusion time of the bead in typical experiments. We present a new modification of Hidden Markov modeling that disentangles these two processes and hence yields rate constants from noisy experimental data.

References

JF Beausang, C Zurla, C Manzo, D Dunlap, L Finzi, and PC Nelson. 2007. DNA looping kinetics analyzed using diffusive hidden Markov model, Biophys. J. *92* L64–L66.

JF Beausang and PC Nelson. 2007. Diffusive hidden Markov model characterization of DNA looping dynamics in tethered particle experiments, Physical Biology (in press).

2574-Pos The Dynamic behavior study of DNA probe by TIRF

Che C. Hsu, Yujia Cui, Shiue Hua Chen, Chien Ming Wu, Tzu Sen Yang, Ian C. Hsu*

National Tsing Hua University, hsinchu, Taiwan

Board B688

The dynamical behavior of DNA, which was immobilized on DNA microarray, have puzzled the gene chip researchers. In general, there are two types of immobilization methods, i.e., charge-charge interaction by poly-L-lysine coating and covalent binding to the glass surface at the one end of DNA. In this study, we aim to distinguish

that either DNA is like a seaweed wavering in the solution (wavering mode) or like a rope sticking on the surface (sticking mode) of glass. The two modes have quite different hybridization efficiency.

Because evanescent wave generated by TIRFM exhibits exponential decay with increasing distance z from the interface, the signal intensity excited by evanescent wave is thus sensitive to the change of the location of the signal emmitor along z direction. Therefore, the TIRFM is adequate to gauge the vertical location of the fluorescence dye. We measure the TIRF signal of Cy3 labeled 300-bp DNA molecules which was immobilized on the coverslip via a biotin-streptavidin. To determine which mode that the behavior DNA are belong to, we record the time trace of the single Cy3 fluorescence intensity to see if there is a changes in intensity corresponding to the vertical displacement. We also performed the same measurement for the othe type of immobilization methods, i. e., charge-charge interaction by poly-L-lysine coating.

In this presentaion, we will also report the theoriotical estimated size of fluorescence signal produced by the wavering of dye in the exponentially decayed evanescent excitation field. The amount and the time characteristic of the wavering is determined by Brownian motion. This wavering signal should be larger enough to stand out from the noise of a steaty single dye.

2575-Pos Probing The Assembly Of A 30S Ribosomal Subunit Fragment Using Multi-color Single-molecule FRET

Edward A. Lemke, Jean-Pierre Clamme, David Horning, Yann Gambin, James R. Williamson, Ashok A. Deniz

The Scripps Research Institute, La Jolla, CA, USA.

Board B689

The 30S ribosomal subunit can be assembled *in vitro* from a 16S rRNA and 21 ribosomal proteins. Previous ensemble studies were used to construct an assembly map for this complex process comprising multiple binding and folding events, but many of the molecular underpinnings of the assembly process remain unknown. Here, we report the use single molecule fluorescence techniques to study in greater detail the assembly of a ribonucleoprotein containing an rRNA fragment that forms the minimal binding-site for proteins S15, S6 and S18 in the central domain of the 30S ribosomal subunit. The rRNA fragment consists of 5 helices forming an upper and a lower three-way junction. To understand the cooperativity between upper and lower junction folding, three fluorescent dyes were introduced that can concurrently report on the inter-arm proximities of the two junctions. Furthermore, alternating laser excitation was used to remove extraneous peaks and to evaluate relative FRET efficiencies for this three-dye system, permitting the folding-state distributions of the RNA to be studied. The Mg^{2+}-dependent and protein-induced folding of the rRNA were studied to provide insights into the assembly of this fragment, one of the first steps on the assembly landscape of the 30S ribosomal subunit.

2576-Pos Controlling Single RNA Conformational Dynamics with Non-Equilibrium Steady State Methods

Xiaohui Qu, Glenna Smith, Kang Taek Lee, Ying Lee, Tobin Sosnick, Tao Pan, Aaron Dinner, Norbert F. Scherer

University of Chicago, Chicago, IL, USA.

Board B690

The evolution of RNA conformation with Mg^{2+} concentration ($[Mg^{2+}]$) is typically determined from equilibrium titration measurements or nonequilibrium measurements with a single $[Mg^{2+}]$-jump. Here, the folding of single RNA molecules is measured in response to a series of periodic changes of the Mg^{2+} concentration. The 260-residue catalytic domain of the RNase P RNA from Bacillus stearothermophilus is immobilized in a microfluidic flow chamber and the RNA conformational changes are probed by fluorescence resonance energy transfer (FRET). The kinetics of population redistribution after a $[Mg^{2+}]$-jump and the observed connectivity of FRET states reveal details of the RNA folding pathway. The FRET trajectories for jumps between $[Mg^{2+}]$=0.01 and 0.1mM always exhibit 2-state behavior, whereas jumps between 0.01mM and >=0.4mM exhibit two-state unfolding but multi-state folding. The RNA molecules in either conformation (low or high FRET states) prior to the $[Mg^{2+}]$ increase are observed to undergo dynamics in two distinct regions of the free energy landscape that are separated by a high barrier. We describe the RNA structural changes involved in crossing this barrier as a "hidden" degree of freedom because the changes do not alter the detected FRET value but alters the observed dynamics and kinetics. The FRET state populations do not achieve their equilibrium values at the end of the $[Mg^{2+}]$ intervals due to the long memory of the "hidden" degrees of freedom, thereby creating a non-equilibrium steady-state condition. Because the period of $[Mg^{2+}]$-jumps is adjustable, regions of the free energy landscape that are virtually inaccessible in standard equilibrium measurements or single-jump experiments can potentially be interrogated. We also discuss a theoretical analysis of the periodic perturbation experiments to extract memory kernels that describe the interaction of the observed degree of freedom with the bath of (slow) conformational changes.

Support provided by NIGMS/National Institutes of Health (RO1 GM073104) and the Alexander von Humboldt Foundation

2577-Pos Single-molecule Studies of DNA Polymerase Sliding Along DNA

Candice M. Etson[1], Samir M. Hamdan[2], Charles C. Richardson[2], Antoine M. van Oijen[2]

[1] *Harvard University, Boston, MA, USA,*
[2] *Harvard Medical School, Boston, MA, USA.*

Board B691

DNA polymerases are critical to all aspects of genomic maintenance, including repair, replication and recombination. They cata-

lyze the condensation of dNTPs to form a new strand of single-stranded DNA complimentary to a template strand. Synthesis proceeds in the 5′ to 3′ direction and must begin at the 3′ end of a primer. With the total number of polymerases present in the cell being limited, it seems unlikely that they would rely solely on 3-D diffusion to rapidly find their starting point when needed. To test if DNA polymerases may use 1-D diffusion along the DNA speed up the search process, we have directly observed individual, fluorescently labeled bacteriophage T7 DNA polymerases interacting with double-stranded DNA. We have tethered the DNA to a coverslip within a flow cell via a streptavidin-biotin linkage, and used buffer flow to stretch it by applying hydrodynamic force. At low concentrations, individual proteins can be seen diffusing along the length of the DNA. Individual protein positions can be determined with high precision by fitting their point-spread functions to a 2-D Gaussian, and their trajectories can be analyzed to determine a diffusion coefficient for each individual molecule. The average binding lifetime is seen to decrease as the salt concentration increases, as expected for binding by electrostatic interaction. However, a lack of statistically significant change in the average diffusion coefficient suggests that the protein moves along the DNA by sliding rather than by microscopically hopping.

2578-Pos Effect Of Force And Supercoiling On The Dynamics Of CI-mediated Dna Loop

Carlo Manzo[1], Chiara Zurla[1], Sankar Adhya[2], David Dunlap[1], Laura Finzi[1]

[1] *Emory University, Atlanta, GA, USA,*
[2] *NCI, NIH, Bethesda, MD, USA.*

Board B692

Loop formation induced by the bacteriophage lambda repressor, or CI protein, has been recently proposed as the mechanism that allows maintenance of the lysogenic phase after infection of E.coli, while guaranteeing an efficient and rapid switch to lysis in the presence of the proper inducing signals. A detailed characterization of the dynamics of CI-mediated loop formation and breakdown is lacking. Our particular interest is to study how force and supercoiling, similar to those experienced by the viral DNA in the host cell, can affect the rate of occurrence and the duration of the loop.

With this goal in mind, we carried out magnetic tweezers experiments on two DNA fragments, capable of forming either a 390 bp- or a 2300 bp-long loop. In the first case, the loop formation/breakdown process is observed in real time while varying both force and supercoiling. In the second case, the lifetime of the DNA loop is measured by means of the "force jump" method. Our preliminary measurements show that both force and supercoiling have considerable effects on the loop dynamics. This suggests that the mechanical properties of DNA and tensions exerted upon it can play a major role in looping and therefore in gene regulation.

2579-Pos Single-Molecule Studies of p53 Sliding Along DNA

Anahita Tafvizi[1], Jason Leith[1], Alan Fersht[2], Leonid Mirny[3], Antoine van Oijen[1]

[1] *Harvard University, Boston, MA, USA,*
[2] *University of Cambridge, Cambridge, United Kingdom,*
[3] *Massachusetts Institute of Technology, Boston, MA, USA.*

Board B693

The recognition of specific sites on DNA by transcription factors is central to the regulation of gene expression. To locate its target site on DNA, a transcription factor (TF) must recognize its site amongst millions to billions of alternative sites on DNA. This selection process happens on the timescale of a few seconds within the crowded environment of the cell. Studies suggested that TFs in order to facilitate their search process alternate between 3D diffusion in solution and 1D diffusion along DNA until they reach their target site (1,2). The duration of such a search depends on the rate at which a TF slides along DNA and the frequency with which it alternates between 1D and 3D diffusion.

We are interested in the 1D searching mechanism of p53, a transcription factor that functions as a tumor suppressor in human cells. We are using single-molecule techniques to observe diffusion of the fluorescently labeled p53 proteins along individual, stretched DNA molecules. The position of individual fluorescently labeled proteins relative to DNA can be tracked as a function of time. By analyzing these trajectories, we are able to determine the 1D diffusion coefficient and the frequency with which the protein alternates between 1D and 3D diffusion. During the 1D search process the p53 protein either slides along the DNA by keeping close contact with the duplex and tracking the helical pitch, or undergoes microscopic hopping on and off the DNA. By measuring the 1D diffusion of the p53 proteins as a function of ionic strength, we can differentiate between these two mechanisms.

References

1. Riggs, A. D., Bourgeois, S., & Cohn, M. (1970) J Mol Biol *53*, 401–417.
2. Slutsky, M. & Mirny, L. (2004) Biophys J.

2580-Pos Single-Molecule Observation of RecA-Mediated Pairing and Strand Exchange Processes

Hsiu-Fang Fan[1], Hung-Wen Li[1,2]

[1] *McGill University, Montreal, QC, Canada,*
[2] *National Taiwan University, Taipei, Taiwan.*

Board B694

RecA recombinases play a central role in homologous recombination pathway. Once they assemble on single-stranded (ss) DNA, the

RecA/ssDNA filament mediates the pairing of homologous DNA sequence and strand exchange processes. We used tethered particle motion (TPM) experiments to investigate the details of E. coli RecA mediated pairing and strand exchange steps at the single molecule level. Individual RecA-coated ssDNA substrates bound with sub-micron sized polystyrene beads, were introduced to a reaction chamber with homologous duplex DNAs tethered on surface. TPM experiments measure the DNA tether length change according to the bead Brownian motion. Therefore, the appearance and Brownian motion amplitude of the tethering beads permit the direct observation of RecA-mediated pairing and strand exchange processes in real-time. Firstly, we noted that pairing and strand exchange steps are more efficient under low pH condition. Secondly, the pairing process occurs in both ATP and its non-hydrolyzable analog, ATPgS state. Lastly, significant amount of ATP is required to efficiently carry out pairing/strand exchange steps. These single-molecule results provide more details on the complexity of RecA-mediated processes.

2581-Pos Single Molecule studies of Barrier-to-autointegration factor

Dunja Skoko

Michiyo Mizuuchi, Robert Craigie, Kiyoshi Mizuuchi. Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD, USA.

Board B695

Barrier-to-autointegration factor (BAF) is a cellular protein identified as a component of HIV and MoMLV preintegration complexes isolated form infected cells. MoMLV preintegration complexes containing BAF successfully avoid autointegration which would destroy the viral DNA before integration into the host genome. BAF has sequence non-specific DNA binding surfaces at the two ends of a stable symmetric dimer, and it condenses duplex DNA presumably by bridging two DNA segments. Viral DNA condensation is considered to be involved in the autointegration prevention.

To study the BAF mediated DNA condensation process in detail, we employed fluorescence-labeled BAF protein and total internal reflection fluorescent microscopy to visualize the condensation of single DNA molecules by BAF. BAF binds to the duplex DNA quickly and uniformly, and DNA condensation starts at the point of DNA that experiences least tensile force under the conditions of the experiment.

In addition, our single DNA molecule assay allows as to characterize the BAF-DNA affinity; the apparent K_d is around 8nM. While the association is rather quick, observed dissociation, takes place at two different time scales. Characteristic dissociation rate constants are $K_{off(slow)}$=0.01/s and $K_{off(\ fast)}$=1/s, suggesting two different modes of DNA binding. We currently investigate the reason behind the two dissociation rate constants.

2582-Pos Fluorescence Cross-Correlation Spectroscopy Measurements In Vivo Reveal The Asymmetric Incorporation Of SiRNAs Into RISC In Human Cells

Joerg Muetze, Thomas Ohrt, Wolfgang Staroske, Karin Crell, Petra Schwille

TU Dresden, Dresden, Germany.

Board B696

Short double stranded RNA molecules have emerged as key regulators of gene expression, controlling developmental programs as well as functioning as a defence mechanism against viruses and transposons. Small RNAs use Argonaute-containing complexes called RNA-Induced Silencing Complex (RISC) to identify cognate RNA transcripts whose expression is to be silenced. By combining laser scanning microscopy, fluorescence correlation and cross-correlation spectroscopy (FCS/FCCS) and biochemical methods, we have exploited the interaction of short interfering RNAs with RISC in vivo. We established a functional and stable EGFP-Ago2 expressing 293 cell line, with expression levels suitable for FCS/FCCS. Using this in vivo system combined with highly sensitive FCS and FCCS it is possible to gain vast information on relative binding, concentration and mobility. FCS measurements combined with different cellular treatments revealed an interconnection between P-body integrity and Ago2 mobility. Analysis of various micro-injected fluorescently labelled siRNAs with FCCS showed the asymmetry dependent incorporation of the antisense strand into RISC over time in human cells. Furthermore, modified silencing inactive siRNAs were investigated towards their incorporation into RISC.

2583-Pos Nanodosimetry Study by Single Molecule Approaches

Tzu Sen Yang, Yujia Cui, Chien Ming Wu, Jem Mau Lo, Wun Yi Shu, Chi Shiun Chiang, Kuo Ning Chiang, Chung Shan Yu, Su Tang Lo, Chun Yu Chuang, Ian C. Hsu*

National Tsing Hua University, Hsinchu, Taiwan.

Board B697

Cancer therapy with Auger electrons has recently become apparent. The radiotoxicity of Auger electron is caused primarily by the induction of DNA double-strand breaks (dsb). We present here a single molecule approach with a dual-beam optical traps, to directly probe the DNA dsb by Auger electrons. We synthesize the bifunctional compound of ^{99m}Tc-tricarbonyl complex with a pyrene derivative, in which the planar aromatic molecule can be inserted between Watson-Crick basepairs. From the experimental data of the DNA dsb at two applied forces, i.e., 10 pN and 20 pN, we found that with the intercalated ^{99m}Tc, an Auger electron emitter, DNA dsb rate was increased proportionally to the concentration of ^{99m}Tc. A model was proposed to correlate DNA dsb rate and radioactivity per DNA molecule to deduce DNA dsb yield per decay (n_d) by ^{99m}Tc. Our

results concluded that n_d=0.024±0.011 and 0.232±0.097 at fixed tension of 10 pN and 20 pN, respectively (Figure 1). We anticipate that this novel approaches for investigating DNA nanodosimetry will contribute to the progress in developing targeted cancer radiotherapy using Auger electron emitters.

Figure 1 Effect of radioactivity on the DNA double strand break rate at two applied forces. Also plotted are results fitted by a model that correlates DNA dsb rate and radioactivity per DNA molecule DNA dsb yield per decay (n_d) by ^{99m}Tc.

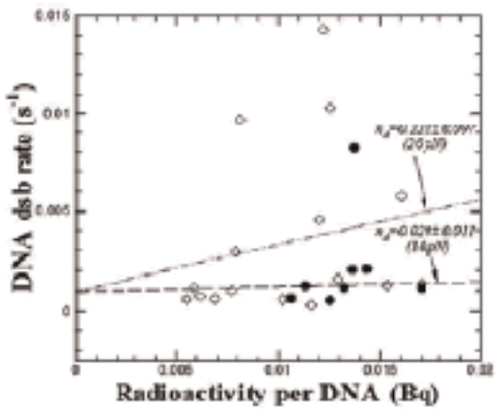

Figure 1 Effect of radioactivity on the DNA double strand break rate at two applied forces. Also plotted are results fitted by a model that correlates DNA dsb rate and radioactivity per DNA molecule to deduce DNA dsb yield per decay (n_d) by ^{99m}Tc.

2584-Pos Single Molecule Measurements of DNA Immobilized in a Biological Nanopore

Robert Purnell, Kunal K. Mehta, Jacob J. Schmidt

UCLA, Los Angeles, CA, USA.

Board B698

There is significant interest in the use of biological and synthetic nanopores to perform sensitive and rapid sensing of single molecules. Biological nanopores, in particular alpha-Hemolysin (aHL), offer the capability of sensing a large variety of single molecules at a rapid rate. This is particularly attractive for DNA because rapid, single-molecule sequencing would significantly reduce system costs and enable high-throughput analysis of extremely small samples. Previous work with single-stranded DNA (ssDNA) in aHL has used DNA hairpins, which cannot traverse the aHL pore, to immobilize single-stranded regions inside the pore and measure the resulting blockage currents in a low bandwidth. However, hairpins have been shown to produce current signals with multiple states, making it difficult to establish a reference conductance value for each base. In this study, strands are terminated with a streptavidin-biotin cap on the 5′ end of the strand. In addition to preventing complete translocation, streptavidin (d = 4 nm) cannot pass into the interior of the pore (d = 2.5 nm). Measurements of ssDNA strands immobilized in aHL in this way demonstrate highly consistent currents. Here, we report measurements of the currents of adenine, cytosine and thymine polyhomonucleotide strands immobilized inside the aHL pore. These results will serve as baseline values for future experiments to measure differences between the nucleotides dynamically as a heteronucleotide strand traverses the pore.

2585-Pos Fluctuation Theorem Analysis of DNA Repair Protein Motion on Flow-Extended DNA

Yihan Lin, Tong Zhao, Zishaan Farooqui, Xiaohui Qu, Chuan He, Aaron R. Dinner, Norbert F. Scherer

University of Chicago, Chicago, IL, USA.

Board B699

We analyze single-molecule tracking measurements of the C-terminal domain of the Escherichia coli DNA repair protein Ada sliding on DNA extended by flow with a drift-diffusion Langevin description of the system. We derive a fluctuation theorem for the influence of flow on the motion. Systematic variation of the flow enables us to extract microscopic friction parameters, suggesting that isolated Ada proteins undergoing unimpeded one-dimensional diffusion are unlikely to be effective in identifying DNA lesions for repair. Extensions of the present fluctuation theorem analysis are discussed.

2586-Pos Coliphage 186 genetic switch: a single molecule study

Chiara Zurla[1], Rachel Shubert[2], Ian B. Dodd[2], Keith Shearwin[2], Laura Finzi[1]

[1] *Emory University, Department of Physics, Atlanta, GA, USA,*
[2] *University of Adelaide, School of Molecular and Biomedical Science, Adelaide, Australia.*

Board B700

The 186 repressor (186CI) is a positive and negative transcriptional regulator and is the key protein involved in the maintainance of the lysogenic state of coliphage 186. The molecular mechanism through which this is accomplished is extremely complex, since it depends on the binding of 186 CI to four sets of binding sites: *pR*, *pL*, *FR* and *FL*. CI binds to the three *pR* operators in an all-or-none manner and represses the transcription from the lytic promoter *pR*. The weak *pL* operator regulates the transcription from the promoter for the CI gene and is located ~100 bp downstream of *pR* in face-to-face orientation. Repression of *pR* stimulates transcription from *pL*. The distal *FR* and *FL* sites assist repression of *pR* and are located ~300 bp upstream of *pL* and downstream of *pR*, respectively.

The crystal structure of the 186CI protein revealed a heptamer of dimers assembled in a weel-like arrangement and provided an intriguing model for understanding the structural basis of the 186 genetic switch. According to the proposed model, the cooperative binding of the protein to multiple operators would permit the formation of alternative DNA loops between the *pL* and *pR* region, as well as the distal binding sites, with the DNA wrapping around the outside of the large 14-mer structure.

We performed single molecule experiments to study the formation of alternative DNA loops in DNA fragments containing the wild-type 186 regulatory regions as well as mutations in the distal *FL* and/or *FR* sites. Here we present preliminary results obtained using the magnetic tweezer setup and the tethered particle motion technique.

Our results will help elucidating lysogenic regulation in coliphage 186. Comparison with similar results obstained on bacteriophage lambda will provide insights into alternate ways to assemble functional genetic switches.

2587-Pos Mapping Nucleosome-DNA Interactions on Single Molecules of Chromatin Isolated from Living Cells

Diego F. Ramallo Pardo[1], Kelly M. Trujillo[2], Cory Hillyer[2], Mary Ann Osley[2], Steven J. Koch[1]

[1] *University of New Mexico, Albuquerque, NM, USA,*
[2] *University of New Mexico School of Medicine, Albuquerque, NM, USA.*

Board B701

We are pursuing the development of methods for single-molecule analyses of chromatin isolated from living yeast cells. Chromatin Immunoprecipitation (ChIP) techniques are common and powerful methods for the study of protein-DNA interactions. However, ChIP has resolution limitations and presents normalization difficulties, and more importantly, only measures averages from a population of molecules. Thus, we are seeking to develop a single-molecule method capable of nucleosome mapping with near base pair resolution on individual chromatin fragments. The main hurdle is the isolation and tethering of intact, soluble and specific chromatin fragments from cells without disruption of the native chromatin structure. We are initiating our project by isolating chromatin from the *PHO5* gene, a well-studied yeast gene that contains four positioned nucleosomes at its promoter when the gene is repressed. We have placed two unique restriction enzyme sites surrounding the *PHO5* promoter present on a plasmid. After transformation of this plasmid into yeast, *PHO5* chromatin fragments will be isolated from cells grown under repressing conditions and digested with the unique restriction enzymes. We will also isolate *PHO5* chromatin from induced cells, where it has been shown by ChIP that the four positioned nucleosomes are lost from the promoter. These two populations of chromatin fragments will be ligated to complementary sticky ends on microspheres for purification and analysis. We will characterize nucleosome positions in the two *PHO5* chromatin populations by unzipping the chromatin with optical tweezers, as has recently been shown with a model nucleosome *in vitro* system[1]. Ultimately, we will use our method to study *in vivo* chromatin remodeling that is critical to fundamental processes such as gene transcription by RNA polymerase II and repair of DNA damage.

References

1. Shundrovsky et al. *Nature: Structural and Molecular Biology.* (2006) *13*: 549–554.

2588-Pos Chromatin Dynamics Study Using Magnetic Tweezers

Pooja Gupta, Miroslav Tomschik, Jordanka Zlatanova

University of Wyoming, Laramie, WY, USA.

Board B702

In living cells, DNA is wrapped around proteins called histones in the form of chromatin fibers which limits its accessibility to proteins and protein complexes involved in DNA transcription, replication, recombination and repair. These processes occurs throughout the life of a cell, and therefore chromatin structure must change to allow the genetic information of the DNA to be processed. In order to study the dynamics of chromatin fibers and how their mechanical properties may affect their biological function, we use a single-molecule technique, Magnetic Tweezers. In this instrumental set-up, a single DNA molecule is attached at one terminus to surface of the flow cell and at the other to a magnetic bead. The manipulation of the magnetic bead by external magnets introduces positive or negative supercoiling in the DNA molecule and stretches it with a defined force. We attempt to follow chromatin fiber assembly in real time and to measure nucleosome strength in individual chromatin fibers, at physiological ionic conditions. Such measurements will help us to understand the behavior of the chromatin fiber upon application of tension and/or torsion, to mimic similar conditions created by physiological processes *in vivo*.

2589-Pos Nucleosome Immobilization Strategies For Single-Pair FRET Microscopy

Wiepke Koopmans, Thomas Schmidt, John van Noort

Leiden Institute of Physics, Leiden, The Netherlands.

Board B703

Single pair Fluorescence Resonance Energy Transfer (spFRET) microscopy is a powerful technique for observing conformational dynamics in DNA-protein complexes. Sample immobilization in principle allows the extension of observation times, limited only by photobleaching. It is of crucial importance however, that immobilization itself does not introduce artifacts in the DNA-protein complex under study.

In previous work we applied spFRET microscopy to study the dynamics of mononucleosomes [Koopmans et al., J. Flu., 2007]. We found that the structural integrity and structural dynamics of nucleosomes are seriously affected by their immobilization to a poly(ethylene glycol) (PEG)-coated surface: over 80% of the reconstituted nucleosomes were disassembled upon immobilization and showed no FRET. We observed efficient FRET on the remaining nucleosomes, but only 3% of this population showed breathing dynamics.

Here we report on various nucleosome immobilization strategies, such as point attachment to PEG or BSA coated surfaces, and

confinement in porous agarose or poly-acrylamide gels. We compared their ability to maintain the structural integrity of immobilized nucleosomes. Control experiments on freely diffusing nucleosomes were carried out to verify the FRET efficiency and conformational dynamics observed on immobilized nucleosomes.

2590-Pos Use of Single-Molecule Imaging to Analyze the Distribution of Binding Ability in RNA Aptamer Populations

Mark P. Elenko[1], Jack W. Szostak[2], Antoine M. van Oijen[1]

[1] *Harvard Medical School, Boston, MA, USA,*
[2] *Harvard Medical School, Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA, USA.*

Board B704

The number of possible sequences for nucleic acid and protein biopolymers of functionally plausible lengths is literally beyond astronomical, exceeding the probable number of atoms in the universe. The nature of the functionality landscape across sequence space for such molecules is of great interest to the origins of life field, and, more pragmatically, to those interested in the possible design, screening, or in vitro evolution of functionally useful molecules. A key question concerns the likelihood of finding particular functional abilities (binding or catalysis) in a pool of sequences with a given length and/or other complexity determining attribute.

RNA is a particularly good candidate for exploration in this area, owing both to its centrality in the RNA world hypothesis and the current interest in developing RNA aptamer based drugs. Work analyzing a small number of RNA aptamers and ribozymes suggests a direct and quantifiable relationship between macromolecular complexity and ability for functional RNA (Carothers et. al., 2004). However, current estimates of the number of molecules (of a specified size) with a desired functionality are based solely on the results of possibly lengthy and probably biased in vitro selections.

This project uses a single-molecule fluorescence microscopy technique (Total Internal Reflection, or TIR) to analyze binding kinetics in populations of RNA aptamers. Initial work with known GTP aptamers confirms the viability of measuring both off and on times for binding. The goal is to enable quantification of the distribution of binding ability in heterogeneous high complexity pools. In addition to addressing important questions of probability in the RNA world, this is potentially useful for designing and understanding in vitro selection experiments, a key tool for the origins of life field and the expanding field of applied molecular evolution.

2591-Pos The Reaction of Glyoxal with Single DNA Molecules: Structural Evidence of Force-Induced DNA Melting

Leila Shokri[1], Chen Lu[1], Micah J. McCauley[1], Ioulia Rouzina[2], Mark C. Williams[1,3]

[1] *Department of Physics, Northeastern University, Boston, MA, USA,*
[2] *Department of Biochemistry Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA,*
[3] *Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston, MA, USA.*

Board B705

When a long polymeric double-stranded DNA (dsDNA) molecule is stretched beyond its B-form contour length, a transition occurs in which the DNA helix suddenly extends to around 1.7 times its original contour length as the force is increased by just a few piconewtons. A quantitative model has been proposed to describe this transition as a force-induced melting transition, analogous to thermal melting, where dsDNA is converted into single-stranded DNA (ssDNA). The force-induced melting model accurately describes the thermodynamics of DNA overstretching as a function of solution conditions and in the presence of DNA binding ligands. However, the overall structure of the melted DNA is not known. To determine the extent to which the DNA base pairs are exposed to solution during the transition, we have undertaken a study of lambda DNA overstretching in the presence of glyoxal, a chemical used previously to map DNA thermal melting. At physiological pH, the glyoxal reaction is expected to be essentially irreversible, but it occurs very slowly. In contrast, at alkaline pH, the glyoxal reaction rate is expected to be greater, but also reversible. Therefore, we examined DNA overstretching over a range of pH and salt concentrations, where we held the DNA molecules for a fixed amount of time at different extensions of the DNA overstretching plateau. Subsequent stretches show that in the presence of glyoxal, a significant fraction of the DNA melted by force becomes permanently melted under all of the conditions studied. Therefore, a significant fraction of the DNA base pairs are exposed to solution when the DNA is overstretched. This result provides quantitative evidence that DNA overstretching is accompanied by disruption of the DNA helical structure, including exposure of the DNA base pairs to solution.

2592-Pos Unusually Slow Unwinding Fluctuations Observed in Negatively Supercoiled B-DNA

Ramreddy Tippana

Institute of JACQUES MONOD, Paris, France.

Board B706

Here we have been used a magnetic tweezers setup to control both the stretching force and the relative linking number ΔLK of a DNA molecule containing a torsionally labile 40 base-pairs (bp). In the presence of 10 mM potassium phosphate ions, and in the absence of divalent ions, negatively supercoiling the DNA molecule while maintaining it under a ~0.34 pN extending force induces the formation of a cruciform DNA structure. The secondary structure

of the 40 bp in the cruciform state was estimated using Mfold. The predicted secondary structure indicates that the 40 bp in the cruciform form an imperfect hairpin. Deletion of these 40 base pairs from the 5kb DNA molecule in which they were embedded abolished the fluctuations. Once the cruciform DNA structure is formed, the rate of extrusion and resorption of the 40 bp cruciform structure is directly and reversibly controlled by varying ΔLK or the applied stretching force. The measured equilibrium lifetime of the cruciform structure is 16 ± 2 seconds, and in low salt lifetimes follow single-exponential distributions. In higher salt conditions cruciform extrusion is a two-step kinetic process, as addition of 150 mM Nacl or 5 mM Mg^{2+} led to the stabilization of intermediate states lying between the native and cruciform states of the DNA. Mutations in the loop did not affect the kinetics of cruciform formation/destruction. However, mutations in the stem dramatically reduced the lifetime of the cruciform structure or completely abolished the fluctuations. A mutation which destabilized the stem region while nevertheless preserving overall cruciform extrusion was shown to yield an equilibrium lifetime of 6 ± 1 seconds.

Wednesday, February 6, 2008

Symposium 18: Damaged Proteins-Structural and Biological Consequences

2593-Symp Proteome Dynamics - Parameterising Protein Turnover At A Global Level

Robert Beynon

University of Liverpool, Liverpool, United Kingdom.

An important, if overlooked, aspect of proteome characterization is the definition of the intracellular stability of individual members of the proteome, data that inform systems models and network dynamics, and which allow us to define mechanisms that regulate protein turnover. Global determination of protein stability in the cell is, coupled with absolute quantification of protein abundance defines one half of the protein turnover cycle, with protein synthesis (itself a product of mRNA abundance and translational activity) as the other. Indeed, the failure to fully define the parameters of this cycle may be the most compelling reason for the lack of a strict correlation between the abundance of a protein and its cognate transcript. Metabolic incorporation of stable isotope labeled amino acids, coupled with high resolution separation and mass spectrometry, permit accurate measurement of intracellular stability on a global scale, from which we can infer relationships between intracellular stability and function, and identify factors that dictate the rate at which proteins are sequestered into the degradative apparatus. In this presentation, I will discuss approaches to acquisition of large data sets of protein stability data, particularly in respect of isolated single cells but also in intact animals. I also will describe a novel approach to absolute quantification that is extendible to entire proteome. Finally, I will discuss some recent data that address structural determinants of protein stability.

This work was supported by grants from the BBSRC.

2594-Symp The Molecular Basis of Alzheimer's and other Protein Misfolding Diseases

Louise C. Serpell

University Sussex, Falmer, Brighton, United Kingdom.

Many unrelated proteins and peptides assemble to form amyloid fibrils that accumulate in the tissues in the misfolding diseases. These diseases include Alzheimerâ™s disease, Diabetes type 2 and the spongiform encepthalopathies. Although the precursor proteins share no similarities in native structure or primary sequence, the fibrils that are formed are rich in beta-sheet structure and share a common core conformation known as the cross-beta structure. Understanding the process of abnormal protein assembly is central to understanding pathology of the misfolding diseases. Small, oligomeric assemblies of amyloidogenic peptides may play a key role in cell toxicity and tissue degeneration. Recent advances in the structure determination of the amyloid core structure have lead to a clearer picture of the internal architecture of the amyloid fibril. Relating this structure of the mature fibril to the structure of the toxic oligomer is the next important step in understanding the disease process.

2595-Symp The Proteasomes Regulatory ATPases Stimulate Protein Degradation by Using A "Key-in-a-lock" Mechanism to Open the Gate in the 20S Particle

David Smith[1], Yifan Cheng[2], Julius Rabl[1], Shih-Chung Chang[1], Soyeon Park[1], Dan Finley[1], Alfred Goldberg[1]

[1] *Harvard Medical School, Boston, MA, USA,*
[2] *University of California-San Francisco, San Francisco, CA, USA.*

Protein degradation in all cells is an ATP-dependent process. In the eukaryotic 26S proteasome, the 20S core particle degrades proteins while associated with the six ATPases (RPT1-6) in the base of the 19S regulatory particle, and in archaea, the 20S functions with the homologous PAN complex, a homo-hexameric ATPase. These ATPases unfold protein substrates and catalyze the translocation of the unfolded polypeptides into the 20S particle. They are also members of the AAA family but contain a conserved C-terminal HbYX motif. We recently showed that upon binding of ATP to PAN this motif binds to intersubunit pockets in the 20S's outer alpha-ring, linking the ATPases to the 20S and trigger opening of a gated channel for substrate entry. This gate in the 20S's alpha-ring is formed by the N-termini of the alpha-subunits excludes substrates when closed. Eight-residue peptides corresponding to PAN's C-terminus (and containing the HbYX motif) can compete with PAN for binding to the 20S particle and by themselves cause gate opening. Therefore the proteasomal ATPases stimulate proteolysis by using their C-termini like a "key-in-a-lock" to catalyze substrate entry. To understand the mechanism of gate opening, we used Cryo-Electron Microscopy, to show that binding of this C-terminal ATPase domain to the 20S induces a rotation in the proteasomes' alpha-subunits, positioning a critical pro17 reverse-turn loop into a position that stabilizes the open-gate conformation. Mutations of these residues in yeast revealed that the more complex eukaryotic