the enzyme lead to the belief that this enzyme possesses some unique motifs. However, in the absence of atomic level structural information clear structure/function correlations are lacking. We are currently working towards obtaining a high-resolution structure of PaeLigD-PE using solution NMR methods.

Reference:
1) Zhu, H., and Shuman, S. (2006) J. Biol. Chem. 281, 13873-13881.
2) Zhu, H., and Shuman, S. (2008) J. Biol. Chem. 283, 8331-8339.

301-Pos Board B180
Zero-Mode Waveguides for Real-Time Observation of Single Nucleotide Incorporation
Zhuangxiong Huang, Serge Donkers, Jacob W.J. Kerssemakers, Nynke H. Dekker.
Delft University of Technology, Delft, Netherlands.
Single-molecule fluorescence studies on the incorporation of fluorescently labeled nucleotides by DNA polymerizing enzymes typically operate at nucleotide concentrations well below their Km values. While this is inevitable given the femtoliter observation volumes accessed via conventional fluorescence microscopy, the biological relevance of the insights gained into enzymatic kinetics may be compromised. Zero-mode waveguides (ZMWs), sub-wavelength holes in a thin metal film, provide an excellent solution to this problem by greatly reducing the observation volume [M. J. Levene, et al., 2003, Science 299, 682-686].
We have successfully designed and fabricated ZMWs of about 100 nm in width. In addition we have developed a surface treatment protocol based on PEG functionalization to make the ZMWs biocompatible, and to facilitate the controlled tethering of biomolecules [A. Crut, et al., Nanotechnology, in press (2008)]. In these structures, we have observed the real-time observation of single nucleotide incorporation at biologically-relevant concentrations in ZMWs, The latest scientific results will be presented.

302-Pos Board B181
Formation Of RecA Filament During The Mechanical Unzipping Of dsDNA To ssDNA: Competition With SSB Differentially Controls RecA Mediated SOS Response And Replication Repair
Carlo Zambonelli, Claudia Danilowicz, Nancy Kleckner, Mara Prentiss.
Harvard University, Cambridge, MA, USA.
RecA and its eukaryotic homologs Rad51 and Dmc1 carry out many DNA transactions: recombinational DNA damage repair, genome integration of incoming DNA, programmed recombination during meiosis, and stalled DNA replication forks processing, all reactions initiated forming a RecA ssDNA nucleoprotein filament. ssDNA/RecA interactions activate the ''SOS'' response through RecA co-protease activation, raising RecA concentration. RecA/ssDNA binding must be regulated such that it only occurs in specific situations: a feature involved in modulating specificity is competitive binding of SSB and RecA to ssDNA. We studied this competition using a magnetic tweezers assay system in which we follow the formation of RecA filaments, in the presence and absence of SSB, on a single ssDNA molecule obtained by mechanical unzipping of dsDNA. We examined various buffer conditions and the effects of several relevant nucleotides. When RecA and SSB tetramer are equimolar, SSB wins the competition and no stable RecA filament is observed; when RecA is in a 20-fold molar excess, stable RecA filament forms. At intermediate molar ratios mixed situations are observed. These results provide information on the competition dynamics between RecA and SSB at the single DNA molecule level. Our results confirm previous ensemble studies: RecA and SSB affinities for ssDNA provide an intrinsic differential control for RecA mediated DNA repair and recombination functions, independently by a RecA loading machinery. SOS response increases RecA concentration giving a molar ratio of RecA:SSB of ~30:1 outcompeting SSB without need for loading factors. These studies show that a ssDNA/RecA filament is stable for much longer than any ssDNA/SSB complex examined. This could imply that the free energy for bound RecA is much lower than that for SSB.

303-Pos Board B182
Control of DNA Replication by Anomalous Reaction-Diffusion Kinetics
Michel G. Gauthier, John Bechhoefer.
Simon Fraser University, Burnaby, BC, Canada.
DNA replication requires two distinct processes: the initiation of pre-licensed replication origins and the propagation of replication forks away from the fired origins. Experiments indicate that these origins are triggered over the whole genome at a rate I(t) (the number of initiations per unreplicated length per time) that increases throughout most of the synthesis (S) phase, before rapidly decreasing to zero at the end of the replication process. We propose a simple model for the control of DNA replication in which the rate of initiation of replication origins is controlled by the interaction with a population of rate-limiting proteins. We find the time set by reaction-diffusion kinetics for such proteins to find, bind to, and trigger a potential origin. The replication itself is modeled using a formalism resembling that used to study the kinetics of first-order phase transitions. Analyzing data from Xenopus frog embryos, we find that the initiation rate is reaction limited until nearly the end of replication, when it becomes diffusion limited. Initiation of origins and hence I(t) is suppressed when the diffusion-limited search time dominates. We find that, in order to fit the experimental data, the interaction between DNA and the rate-limiting protein must be subdiffusive. We also find that using a constant nuclear import of the limiting proteins leads to a more accurate description of the experimental data.

Protein-Nucleic Acid Interactions I

304-Pos Board B183
Single-molecule Study of Site-specific DNA Recombination by γδ Resolvase
Mingxuan Sun[1], Hua Bai[2], Nigel D. Grindley[3], John F. Marko[1].
[1]Northwestern University, Evanston, IL, USA, [2]University of Illinois at Chicago, Chicago, IL, USA, [3]Yale University, New Haven, CT, USA.
γδ resolvase is a serine recombinase coded by γδ transposon, which catalyzes DNA recombination between two *res* sites (114 bp) on a negatively supercoiled circular DNA, resulting in two catenated DNA circles. Each *res* site contains 3 different resolvase binding sites - site I, II and III, and each binds to a resolvase dimer. We have developed a single-DNA based system whereby synapsis and recombination should lead to torsional relaxation of a single supercoiled DNA. DNA relaxation catalyzed by γδ resolvase occurs at much higher rate on DNA substrate containing 2 res sites than those containing 1 or 0 res site. Furthermore, reactions on a 2-res-site substrate show a characteristic ~200 nm relaxation consistent with the +4 ΔLk observed to be associated with the recombination reaction in bulk experiments. We also have observed topoisomerase activity of γδ resolvase on the non-specific (0 res) DNA substrate.

305-Pos Board B184
Thermodynamics of Interactions between Histone-like Proteins from *Escherichia coli* (HU and IHF) and Intact Duplex DNA
Junseock Koh, Ruth M. Saecker, M. Thomas Record Jr.
Universiy of Wisconsin, Madison, WI, USA.
The structural homologs $HU_{\alpha\beta}$ and IHF are major nucleoid associated proteins (NAPs) of *Escherichia coli*, which organize chromosomal DNA and facilitate numerous DNA transactions. Using isothermal titration calorimetry (ITC), fluorescence resonance energy transfer (FRET) and a series of DNA duplex lengths (8, 15, 34, 38 and 160 base pairs) we establish three different nonspecific binding modes for both $HU_{\alpha\beta}$ (Koh et al., 2008) and IHF. Both the NAP:DNA mole ratio ([NAP]/[DNA]) and DNA length dictate the dominant NAP binding mode. On sufficiently long DNA, at low [NAP]/[DNA], both HU and IHF populate a noncooperative 34 bp binding mode with a binding constant of ~ 10^7 M^{-1} at 0.082 M Na^+. With increasing [NAP]/[DNA], both HU and IHF bound in the noncooperative 34 bp mode progressively convert to two moderately cooperative modes with site sizes of 10 bp and 6 bp and smaller binding constants. As DNA length increases at low [NAP]/[DNA], fractional population of the 34 bp mode increases. The 34 bp mode of IHF exhibits a large negative $\Delta C_{p,obs}^{\circ}$ and an exothermic ΔH_{obs}° (15 - 25°C), similar to previous observations for the specific complex of IHF and wrapped H'-DNA, whereas a small positive $\Delta C_{p,obs}^{\circ}$ and an endothermic ΔH_{obs}° were observed for the 34 bp mode of HU. From these and parallel studies at various salt concentrations we propose that DNA is wrapped on the body of IHF in the nonspecific 34 bp mode like the specific complex of IHF and H'-DNA, whereas DNA is bent but not wrapped in the 34 bp nonspecific HU-DNA complex. Other structural features of the binding modes of HU and IHF deduced from these studies are also discussed.
This work was supported by NIH grant GM 23467.

306-Pos Board B185
Novel Techniques for Study of the Nucleosome Core Particle Ionic Atmosphere and Its Role in Electrostatically-Driven DNA Packing
Kurt Andresen.
Colgate University, Hamilton, NY, USA.
The nucleosome core particle (NCP) is the primary mechanism for DNA compaction. While the wrapping of the DNA around the histone core is thought to be at least partially sequence dependent, the packing of the nucleosome core is believed to be almost entirely electrostatic in nature. Using novel techniques to probe the ionic atmosphere, we hope to elucidate details of this compaction and