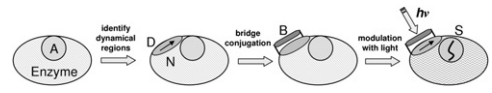
lateral surface pressure and the concentration, by using dilauroylphosphatidylcholine (DLPC) as a substrate. The Lag time as a function of DLPC-monolayer surface pressure and $GmPLA_2$-I concentration were determined.

1383-Pos Board B153
Antlion Strategy for Enzyme Specificity
Serdal Kirmizialtin, Ron Elber, Kenneth A. Johnson.
The University of Texas Austin, Austin, TX, USA.
The role of protein conformational dynamics in enzyme specificity and efficiency has held the fascination of enzymologists since the original debates over ''lock and key'' versus ''induced-fit'' mechanisms. Recent data has shown that the large, substrate-induced conformational changes are an important governor of enzyme specificity. Molecular dynamics simulations hold the promise for atomically detailed analysis of induced-fit mechanisms, but the large spatial and temporal scales are a challenge. Therefore, conformational transitions and induced fits on the millisecond scale have been studied infrequently and represent a major frontier in computer simulation. Here we investigated induced-fit mechanisms using Directional Milestoning applied to HIV reverse transcriptase for both correct and mismatched base pairs. The predicted rate and free energy profiles agree with available experimental data, including new single molecule kinetic measurements. The substrate-induced conformational change proceeds through a transition-state with motions of up to 25 Å in approximately 250 μs. The induced-fit mechanism affords specificity based upon a kinetic rather than a thermodynamic selection, which we liken to the manner in which an antlion captures its prey by digging a hole in the sand and waiting. Ants fall into the hole and are slow to escape and so they are eaten, whereas larger insects, which might see the antlion as prey, rapidly climb out of the hole. The substrate-induced conformational change is rapid and affords fast sampling of the bound nucleotide. A correct nucleotide leads to tight binding and alignment of catalytic residues to promote catalysis while a mismatched base precludes the formation of the tight binding state and the rapid opening of the specificity loop affords release of the mismatch.

1384-Pos Board B154
Construction of Peptidase-Resistant Substrates for Kinases
Angela Proctor[1], Qunzhao Wang[1], David S. Lawrence[1], Nancy L. Allbritton[1,2].
[1]University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [2]North Carolina State University, Raleigh, NC, USA.
Peptides composed entirely of native amino acids are used extensively in the biomedical community as substrates in assays for kinase activity. However, the usefulness of peptides is limited when kinase activity is measured in cell lysates or intact cells due to hydrolysis of the peptide bonds by peptidases. Replacement of native amino acids has been of high utility in the development of peptidase-resistant peptides for use as enzyme inhibitors as well as for therapeutic applications. Past efforts at creating peptidase-resistant substrates for kinases using a similar strategy have not generally met with success. The primary reason is that peptides with non-native residues are often very poor substrates for kinases. This work focuses on the development of degradation-resistant, peptide-based substrates for BCR-ABL and Akt (PKB). To understand which peptide bonds were most susceptible to hydrolysis, fluorescently tagged peptides were incubated in cell lysates and capillary electrophoresis was utilized to monitor peptide cleavage over time. All possible peptide fragments were synthesized and used as standards to identify the peptide bonds most vulnerable to cleavage. The residues adjacent to these fragmentation sites were replaced with non-native residues in an iterative fashion to stabilize the peptide. In a cell lysate, a modified BCR-ABL reporter possessed a half-life of 19 minutes, compared to 2 minutes for the unmodified reporter. Furthermore, this modified peptide retained activity as a substrate for BCR-ABL. Similar iterative replacement of non-native amino acids into an Akt substrate yielded a peptide with an extended lifetime in cell lysates which remained suitable for phosphorylation by Akt. Future work includes characterization of the native and modified reporters in single cells utilizing capillary electrophoresis.

1385-Pos Board B155
Direct Measurement of the Protein Response to an Electrostatic Perturbation that Mimics the Catalytic Cycle in Ketosteroid Isomerase
Santosh Kumar Jha, Minbiao Ji, Kelly J. Gaffney, Steven G. Boxer.
Stanford University, Stanford, CA, USA.
Understanding the role of electric fields and their fluctuations in the active site of enzymes in actuating efficient catalysis is a long-standing and fundamental problem in biochemistry. In this study, we have directly measured the dynamics of the electric field in the active site of a highly proficient enzyme, Δ^5-3-ketosteroid isomerase (KSI), in response to a sudden electrostatic perturbation which simulates the charge displacement that occurs along its catalytic reaction coordinate. Photoexcitation of a fluorescent analog (coumarin 183) of the reaction intermediate, which binds tightly in the enzyme's active site, mimics the change in electrostatic environment which occurs during the enzyme-catalyzed reaction. We measured the electrostatic response and angular dynamics of four probe dipoles in the enzyme active site by monitoring the time-resolved changes in the vibrational absorbance (IR) spectrum of a spectator thiocyanate moiety (a quantitative sensor of changes in electric field) placed at four different locations in and around the active site, using polarization-dependent transient vibrational Stark spectroscopy. The four different dipoles in the active site remain immobile and do not change their orientation during the simulated catalytic reaction. These results indicate that the active site of KSI is preorganized with respect to functionally-relevant electric fields.

1386-Pos Board B156
Engineering Hyper-Catalytic Enzyme by Photo-Activated Conformation Modulation
Pratul K. Agarwal[1], Christopher Schultz[2], Aris Kalivretenos[3], Brahma Ghosh[4], Sheldon Broedel, Jr.[2].
[1]Oak Ridge National Laboratory, Oak Ridge, TN, USA, [2]AthenaES, Baltimore, MD, USA, [3]Aurora Analytics, Baltimore, MD, USA, [4]Pfizer Inc., Andover, MA, USA.
Conformational flexibility has been proposed to be a contributing factor to the catalytic efficiency of enzymes. It has been hypothesized that enzyme catalysis involves the use of internal motions to control the structural environment in the active-site to facilitate the targeted chemistry. A methodology to modulate long time-scale enzyme dynamics was designed to test if controlled modulation of enzyme conformation leads to an increase in enzyme activity. Here we describe a chemical modification of Candida antarctica lipase B (CALB) that allows modulation of enzyme conformation to promote catalysis. Computational modeling was used to identify dynamical regions of CALB that impact the catalytic mechanism. Surface loop showing dynamical coupling to reaction were connected by a chemical bridge between Lys136 and Pro192, containing a derivative of azobenzene. The conformational modulation of the enzyme was achieved using two sources of light that alternated the azobenzene moiety in cis to trans conformations. Computational model predicted that mechanical energy from the conformational fluctuations facilitate the reaction in the active-site. The results were consistent with the hypothesis as enzyme activity was enhanced with photo-activation. Preliminary estimations indicate that the engineered enzyme achieved 8 to 52 fold better reaction rate.



1387-Pos Board B157
Protein Gels Degradation by Proteases
Noémie Ein-Eli[1], Zeineb Backey Backey[1], Marie-France Breton[1], Benjamin Cressiot[1], Didier Lairez[2], Gilbert Zalczer[3], Bénédicte Thiebot[1], Juan Pelta[1].
[1]LAMBE, CNRS - Evry and Cergy-Pontoise, France, [2]Laboratoire Léon Brillouin, CEA-Saclay, France, [3]Service de Physique de l'Etat Condensé, CEA-Saclay, France.
Tissues and organs are formed by specialized cells and by extracellular matrix (ECM) organized in 3-dimensional (3D) structure. ECM is a mix of various components, mainly of proteins. ECM remodeling is a physiological and pathological phenomenon (Raeber et al 2005). During tumor dissemination, invasive cells must liquefy the matrix to invade other tissues and establish distant metastases. Many groups study cancer cell behavior; however, up to now, few experimental data are focused on understanding the physical aspect of ECM degradation by enzymes.
The aim of this work is to find general behavior able to describe the physical mechanism of ECM enzymatic degradation.
Previous experimental studies have shown that the gelatin-gel degradation kinetics by thermolysin, a metalloproteinase, is diffusion limited (Lairez et al, 2007). A power law dependence on degradation time as a function of enzyme concentration was found and associated to a self-attracting enzyme random walk, leading to a continuum percolation model for proteolysis. Recently, we have demonstrated the same behavior with 2 serine proteases, trypsin and proteinase K (Breton et al, in preparation).
Here we study protein gel degradation by papain (a cystein protease) and chymotrypsin (a serine protease) by varying solvent viscosity, gelatin and enzyme concentrations. We obtain either a linear dependency or a power law of the degradation time (tc) as a function of enzyme concentrations: $tc \propto 1/[papain]^{1.1+/-0.1}$ and $tc \propto 1/[chymotrypsin]^{1.9+/-0.15}$. In the presence of glycerol, gel degradation kinetics with papain is reaction limited and enzyme diffusion is Brownian. With chymotrypsin, tc increase and can be superimposed to those

obtained without glycerol by taking into account both, the enzyme activity decrease and the solvent viscosity increase. Gel proteolysis kinetics is therefore diffusion limited and the diffusion is anomalous. We discuss possible explanation for these different mechanisms.

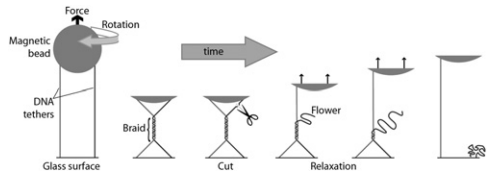
1388-Pos Board B158
Strain Tunes Proteolytic Degradation and Diffusive Transport in Fibrin Networks
Arjun Adhikari, Armen Mekhdjian, Alexander Dunn.
Stanford University, Stanford, CA, USA.
Proteolytic degradation of fibrin, the major structural component in blood clots, is critical both during normal wound healing and in the treatment of ischemic stroke and myocardial infarction. Fibrin-containing clots experience substantial strain due to platelet contraction, fluid shear, and mechanical stress at the wound site. However, little is understood about how mechanical forces may influence fibrin dissolution. We used video microscopy to image strained fibrin clots as they were degraded by plasmin, a major fibrinolytic enzyme. Applied strain causes up to 10-fold reduction in the rate of fibrin degradation. Analysis of our data supports a quantitative model in which the decrease in fibrin proteolysis rates with strain stems from slower transport of plasmin into the clot. We performed fluorescence recovery after photobleaching (FRAP) measurements to further probe the effect of strain on diffusive transport. We find that diffusivity perpendicular to the strain axis decreases exponentially with increasing strain, while diffusivity along the strain axis remains unchanged. Our results suggest that the properties of the fibrin network have evolved to protect mechanically loaded fibrin from degradation, consistent with its function in wound healing. The pronounced effect of strain upon diffusivity within fibrin networks offers a means of tuning the transport of proteins and other soluble factors within fibrin-based biomaterials, potentially addressing a key challenge in engineering complex tissues *in vitro*.

DNA, RNA Structure & Conformation I

1389-Pos Board B159
High-Bandwidth Magnetic Tweezers for Applying Torsion to Single DNA Molecules
Chang Jiang[1], Troy Albert Lionberger[2], Edgar Meyhofer[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of California, Berkeley, Berkeley, CA, USA.
The mechanical and functional properties of DNA arise from its double helical structure. It is now widely accepted that the torsional properties of DNA and DNA supercoiling play an important role in the kinetics of many DNA-binding proteins, but the mechanism underlying this relationship remains unclear. To address this gap in our understanding, we need an instrument that can accurately measure and control torsional stress applied to DNA. We have developed a high-bandwidth electromagnetic trapping system that can generate a uniform magnetic field in the sample region and apply constant torque above 10^2 pN·nm on the samples under study. The octupole magnetic trap is integrated into a microscope-based particle tracking system and can rotate superparamagnetic particles with three degrees of rotational freedom. The large signal bandwidth of the current in the coils can reach above 3kHz at 800uH inductive load and the heat generated by the current is dissipated by an active PID-controlled cooling system to prevent heating biological samples. The magnetic trap is being designed to independently control force and torque, allowing us to confine superparamagnetic particles in a trap with low torsional stiffness that is suitable for torque application and measurement at biologically relevant scales. To directly measure the torsional strain in DNA, we are planning to use superparamagnetic beads coated with metal on one hemisphere. Our magnetic torque tweezers are intended to quantitatively measure the changes of torsional stress in DNA and overcome the complexity and heating problems shared by previous optical and magnetic tweezers studies.

1390-Pos Board B160
Enhanced Flower Relaxation of a Braided DNA Complex
Johanna C. Palmstrom, Jun Lin, Omar A. Saleh.
University of California, Santa Barbara, CA, USA.
Single-polymer relaxation dynamics are key to understanding bulk-solution and intracellular rheology. Here, we investigate the effect of entanglements on polymer relaxation: we present a novel experimental system that permits entanglement of two DNA molecules by attaching them to a magnetic bead and braiding them together in a magnetic tweezer. We then actuate a relaxation event by photo-cleaving a bond that immobilizes one of the DNAs. The time course of relaxation is followed by tracking the magnetic bead position. We find that simple quasi-static models, that assume bead drag to be the dominant dissipative effect, fail to explain the data. Instead, the data require the inclusion of a polymer dissipation term: we introduce an enhanced 'flower' model [1] that posits that relaxation is dominated by the force-induced coiling-up of the free DNA end. Our analysis places limits on the the friction coefficient for the relative sliding of two interwound DNA molecules, a parameter which was somewhat inconclusively investigated in a recent study[2].

[1] Brochard-Wyart, F. Europhysics Letters, 1995. 30(7): pp. 387-392.
[2] Noom, M.C., et al. Nature Methods, 2007. 4(12): pp.1031-1036.



1391-Pos Board B161
Sequence-Dependent Mechanics of DNA
Krishnan Raghunathan[1], Alan Kandinov[1], Justin Blaty[1], **Joshua Milstein**[2], Jens-Christian Meiners[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]University of Toronto, Toronto, ON, Canada.
Double-stranded DNA is a semiflexible polymer that can naturally bend on length scales comparable to the size of large DNA-protein complexes like nucleosomes or protein-mediated DNA loops. The sequence of the substrate DNA does not only provide biochemical binding sites for the proteins, but also affects the local mechanical properties of the DNA. Notably, sequence can affect the intrinsic curvature of the DNA, as well as its bendability, or elasticity. While intrinsic bends in DNA and their role in protein-DNA complex formation are well studied, sequence-dependent elasticity still remains only vaguely explored. In order to separate sequence effects on elasticity from those on intrinsic curvature, we have designed sequences of DNA which have nearly identical curvatures but varied AT content and directly measured their mechanical elasticity using constant force axial optical tweezers. We found the persistence length to be highly dependent on the AT content of the DNA, differing almost thirty percent between sequences with nearly identical curvature but different sequence composition. This is a departure from conventional dinucleotide and trinucleotide models, which predict a much smaller difference between the two sequences, but consistent with estimates obtained from the crystallographic structures of protein-DNA complexes.

1392-Pos Board B162
Single Molecule Studies of the Effect of Spermidine on DNA Mechanics and Viral DNA Packaging
Nicholas A. Keller, Douglas E. Smith.
UCSD, San Diego, CA, USA.
Polyamine ions such as spermidine3+, along with monovalent and divalent salt ions, screen the negatively charged backbone of dsDNA and thereby facilitate processes in which DNA is confined in small spaces, such as viral DNA packaging. We use single-molecule optical tweezers assays to study Bacteriophage phi29 DNA packaging and the effect of spermidine, Mg2+, and Na+ on DNA condensation and elasticity. We determine the concentration of spermidine at which dsDNA condenses and we report a monotonic increase in stretch modulus and a monotonic decrease in persistence length at incremental spermidine concentrations up to the concentration at which dsDNA condenses. We also discuss the kinetics of spermidine binding onto dsDNA and the forces required to unravel condensed DNA.

1393-Pos Board B163
Triple Hydrogen Bonds in DNA Modify the Transition from Right- to Left-Handed Forms
Qing Shao, Laura Finzi, David Dunlap.
Emory University, Atlanta, GA, USA.
Double-stranded DNA usually adopts a right-handed B-form in aqueous solution, but alternative DNA conformations can also exist and play important roles in a wide range of cellular processes. For example, DNA melting (strand separation) is required to initiate DNA replication as well as transcription. Moreover, the over-production of left-handed Z-form DNA in cells is thought to be the trigger for auto-immunity in lupus. Therefore, understanding the underlying mechanics of right- to left-handed DNA transitions is very important to begin to understand how cellular processes depend on alternative DNA conformations. Two of the most influential factors related to this transition are the tension on DNA and the degree of hydrogen bonding. Magnetic Tweezers enable us to unwind single DNA molecules to investigate the dynamics of right- to left-handed DNA transitions at different tensions. Moreover, by substituting diaminopurine (DAP) deoxyribonucleotides for dATP in PCR reactions, completely triply hydrogen-bonded DNA fragments have been produced. These and normal DNA fragments