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
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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
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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
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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
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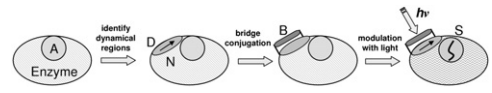
Conformational flexibility has been proposed to be a contributing factor to the catalytic efficiency of enzymes. It has been hypothesized that enzyme catalysis involves the use of internal motions to control the structural environment in the active-site to facilitate the targeted chemistry. A methodology 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
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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