the heat capacity associated with the formation of the complex. The position of the peak gives the energy of the barrier. The peak position and width depend on lipid chain length and saturation and physicochemical properties of the membrane. The energy barrier is crucial to the determination of the level of enzymatic activity and is hypothesized to be the microscopic origin of the "interface quality effects". Results of activity measurements showing precisely the role of the barrier are presented.

2322-Pos

Introducing Photox: A Novel Actin-Targeting Mono-ADP-Ribosyltransferase

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Photorhabdus luminescens is a pathogenic bacterium that produces many toxic proteins. Previously primarily known to target insects, *Photorhabdus* has been studied for its potential use in agriculture and the control of pests. However, *Photorhabdus* infections of humans are now beginning to be seen in the United States and Australia. The mono-ADP-ribosyltransferases (mARTs) are an enzyme class produced by numerous pathogenic bacteria and participate in diseases in plants and animals, including humans. We have discovered and characterized a novel mART from *P. luminescens*, which has been named Photox. This 46 kDa toxin shows high homology to other actin-targeting mARTs in key catalytic regions and a similar core catalytic fold. Furthermore, Photox shows *in vivo* cytotoxic activity against yeast, and growth recovery with the substitution of alanine for catalytic residues. *In vitro,* enzymatic activity is quite high (k_{cat}, 2235 $\pm$ 270 min^{-1}) and comparable with that of iota toxin from *Clostridium perfringens*. Substitutions of hallmark catalytic residues within Photox result in decreases in mART activity up to 20,000-fold. This toxin specifically ADP-ribosylates actin at Arg177, targeting each of alpha-, beta-, and gamma-actin isoforms, and inhibiting regular polymerization of actin filaments. By epifluorescent microscopy, Photox has been seen to associate with actin within yeast cells. After nearly a decade since the last addition to this enzyme family, Photox is the newest actin-targeting ADP-ribosyltransferase.

2323-Pos

Cellulase Enzyme Binding to Pre-Treated Biomass Particles Using Confocal Fluorescence Microscopy

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Mechanical and hydrothermal pretreatment of biomass produces cellulose-rich particles with high-surface area to volume ratio. In addition, pretreated particles with sizes on the order of 1000-micron^3 manifest diverse features, including sub-micrometer thin sheets and irregular yet voluminous, porous globules, with fibrous protrusions on the surface. Many of these features are observable on one single particle. These complex and porous particles are also observed to have numerous tunnels and crevices that can represent extensive pore volume for enzyme diffusion and provide additional surface area for cell-wall degrading enzymes to bind and react. To study the kinetics of cell-wall degrading enzymes binding to pre-treated biomass, we use confocal fluorescence microscopy, and take time-lapse, cross-sectional images of pretreated particles incubated in enzyme solution. By image reconstruction in both temporal and spatial domains, we attempt to elucidate cell-wall degrading enzymes binding kinetics.
Pretreated wood particles are first immobilized on glass surface by droplet drying method, and re-hydrated with sodium acetate buffer. At room temperature, fluorescently labeled *Thermobifida fusca* cellulases – Cel5A, Cel6B and Cel9A – of varying concentrations in sodium acetate buffer are added to the substrates. We use confocal microscope to record the fluorescence intensity of immobilized particles. Over a period of hours, enzyme binding is observable from increasing fluorescence intensity of the particles. Of the three enzymes, Cel6B has the highest affinity, while Cel9A the lowest. In addition, by comparing fluorescence and scanning electron microscope images, we note cellulase preferentially bind to the parts of particles with low auto-fluorescence, and with fibrous or sheet-like features, indicating presence of large number of accessible enzyme binding sites.

2324-Pos

Flexibility and Hydration of *Candida Antarctica* Lipase B in Organic Solvents

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We present a molecular dynamics (MD) study of Candida antarctica Lipase B (CALB) in organic media. This enzyme is used as catalyst in numerous industrial applications, often in organic solvents at rather dry conditions. It has been seen that the solvent e.g. impacts activity, selectivity and stability, and that careful selection of solvent can be very beneficial. It is therefore highly desirable to gain a better understanding of how enzymes behave in organic media. In this study, we focus on the flexibility and hydration level of the enzyme in different solvents, namely acetone, tert-butanol, methyl tert-butyl ether and hexane, under varying hydration conditions. While only minor structural differences are seen in the different media, we do observe that the flexibility, characterized by the root-mean square fluctuations, increases with increasing hydration level. The hydration level is in turn affected by the organic solvent properties. We observe that in polar solvents, more water is necessary to attain the same hydration levels as in non-polar solvents.
In order to investigate effects on flexibility purely originating from the organic solvent species, we compare results obtained from simulations carried out in different solvents, but where the hydration levels of the enzyme are similar. In experiments, one often accomplishes this by fixing the (thermodynamic) water activity. We will present a scheme for conducting MD simulations at fixed water activity, whose purpose is to make the calculations more compatible with this kind of experiments.
We have also extended our studies to include the effect of solvent on the stability of the Michaelis-Menten complex, formed by CALB and an ester substrate. Results for near-attack conformation populations for forming the tetrahedral intermediate will be discussed.

2325-Pos

The Role of P-loop in the Enzymatic Mechanism of Nucleotide Pyrophosphatases

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The phosphate-binding loop (P-loop) or Walker A sequence is a common feature of a large number of ATP and GTP binding proteins including kinases, cytoskeleton and DNA motors, membrane pumps and transporters. All known P-loop containing proteins are able to sense the difference between bound NTP and NDP via their P-loop which allows for hydrolyzing the beta-gamma phosphate bond of a nucleotide triphosphate. An exception to the rule is the enzyme dUTPase which specifically hydrolyses the alpha-beta pyrophosphate bond in dUTP into dUMP and PPi. Peculiarly, the target cleavage site and the catalytic water are in place in both dUDP and dUTP containing structures but only dUTP is hydrolyzed. We created mutations within the P-loop of human dUTPase to only perturb the gamma phosphate coordination of the bound nucleotide. Kinetic and thermodynamic data obtained with the mutants indicate that the P-loop only slightly affects nucleotide binding but accelerates cleavage of the alpha-beta pyrophosphate bond by several orders of magnitude. Unrelated bifunctional dUDP/dUTPase enzymes catalyze both dUDP and dUTP hydrolysis and do not contain a P-loop-like structure. Similarly, all other known nucleotide pyrophosphatases that couple hydrolysis to another reaction (e.g. DNA/RNA polymerases, ligases) lack the P-loop. Our investigations lead to the conclusion that uniquely, the P-loop provides negative discrimination against the hydrolysis of dUDP at the alpha-beta pyrophosphate bond by dUTPase. The physiological role of dUTPase is to keep cellular dUTP:dTTP ratios low in order to prevent uracil incorporation into newly synthesized DNA. In this respect, dUTP is the harmful species whereas dUDP is indifferent and its hydrolysis is probably wasteful. The P-loop was likely acquired by dUTPase to distinguish between the two potential NDP and NTP substrates whereas other pyrophosphatases do so via a coupled reaction or hydrolyze both.

2326-Pos

Mechanism of Disulfide Reduction by the Acidophilic Reductase Enzyme GILT

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Reduction of disulfide bonds is essential in lysosomal degradation of proteins. When delivered to the lysosomal lumen, proteins are denatured and subsequently proteolyzed. The acidic environment of the lysosome facilitates structural denaturation of the proteins; however, it also disfavors reduction of disulfide bonds by conventional means. Indeed, none of the thioredoxin or glutathione systems that confer reduction in the cytosol show reducing capacity at this low pH, thus necessitating the action of GILT, a newly discovered acidophilic reductase. GILT has optimal reducing capacity around pH 4 and has recently been implicated in a number of immunological processes, including bacterial infection and antigen processing by human immune cells. Still, very little is known today about the catalytic mechanism of this enzyme,

mainly due to a lack of relevant assays available at acidic pH. We have developed a single-molecule assay using force spectroscopy, which allows us to detect the reduction of single disulfides. The assay uses an Atomic Force Microscope to extend individual substrate proteins containing buried disulfides, thereby exposing the bonds to the solvent. When a disulfide bond is broken through reduction, this reaction is detected as a stepwise extension of the substrate polypeptide. This method enables measurement of reduction rates at a wide range of pH conditions. By altering the conformation of the disulfide through the applied strain on the substrate, we can dissect the mechanisms of enzymatic catalysis. Our results show that the enzymatic activity of GILT decreases as the strain on the substrate is increased. This feature is also seen in the enzyme thioredoxin but not in non-enzymatic reducing agents such as glutathione. These results shed light on the catalytic mechanism of GILT and establish single molecule force spectroscopy as a useful tool for characterizing enzymatic properties.

2327-Pos

Anisotropic Mechanical Response of the Enzyme Guanylate Kinase Perturbed by the DNA Molecular Spring

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Protein molecules are semi-rigid objects with organized but fluctuating conformation. For Guanylate Kinase, which catalyzes phosphoryl transfer between ATP and GMP, a large conformational change upon substrate binding occurs which is essential for enzymatic activity. With a DNA molecular spring stretching the molecule in distinct ways, we demonstrate that the enzymatic functions of substrate binding and phosphoryl transfer can be separately controlled mechanically.

Three different attachment points of the DNA spring on the surface of the protein were tested, corresponding to stretching the protein along three different directions. Using activity measurements with titration over substrate concentration, the kinetic parameters (i.e., binding affinity of substrates and catalytic rate constant) based on Michaelis-Menten kinetics were obtained in the presence and absence of the three different mechanical perturbations.

2328-Pos

Crystal Structure and Functional Analysis of Homocitrate Synthase, an Essential Enzyme in Lysine Biosynthesis

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Homocitrate synthase (HCS) catalyzes the first and committed step in the α-aminoadipate (AAA) pathway of lysine biosynthesis, which occurs in many fungi and certain archaea, and is a potential target for antifungal drugs. Here we report the crystal structure of the HCS apoenzyme from *Schizosaccharomyces pombe* and two distinct structures of the enzyme in complex with the substrate 2-oxoglutarate (2-OG). The structures reveal that HCS forms an intertwined homodimer stabilized by domain-swapping between the N- and C-terminal domains of each monomer. The N-terminal catalytic domain is comprised of a TIM barrel fold in which 2-OG binds via hydrogen bonds and coordination to the active site divalent metal ion, whereas the C-terminal domain is composed of mixed α/β-topology. In the structures of the HCS apoenzyme and one of the 2-OG binary complexes, a lid motif from the C-terminal domain covers the entrance to the active site of the neighboring monomer, whereas in the second 2-OG complex, the lid is disordered, suggesting that it regulates substrate access to the active site through its apparent flexibility. Steady state kinetic assays and *in vivo* yeast growth assays on wild-type enzyme and active site mutants allow us to elucidate its catalytic mechanism, including the residues implicated in catalysis. Together these results yield new insights into the mechanism and regulation of HCS, which provide a platform to identify small molecule inhibitors of HCS that may be optimized and used as anti-fungal agents.

2329-Pos

Kinetic Consequences of Mutations at an Allosteric Site in Arginase from the Thermophile *Bacillus caldovelox*

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Arginase is a binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. It is involved in ureagenesis and the control of arginine levels for production of proline, creatine, polyamines, and nitric oxide. The crystal structure of the enzyme from the extreme thermophile *Bacillus caldovelox* reveal a second arginine-binding site, located at the monomer-monomer interface. Binding of the guanidinum group of L-arginine by bidentate hydrogen bonds to Glu256 in one monomer and bifurcated hydrogen bond to Asp199 in the neighbor, was suggested to generate a catalytically competent conformation. Interestingly, in the rat and human liver arginases, an equivalent position is occupied by Arg-308, which is part of an S-shaped C-terminal motif, that is critical for oligomerization and cooperative response to the substrate. The bacterial arginase lacks this motif. To get some insight into the external site in *B. caldovelox* arginase, we examined the kinetic and structural consequences of mutations at Asp199 to asparagine and Glu256 to glutamine. Upon mutations, the hexameric subunit structure, affinity of the enzyme-manganese interaction, pH and temperature dependence of catalytic activity, thermal stability and tryptophan fluorescence properties of the enzyme were not altered. However, the hyperbolic kinetics exhibited by the wild-type enzyme (K_m= 3.5 mM) changed to cooperative for both variants (Hill coefficients of 1.5 ± 0.2). Results were not altered by agmatine (decarboxylated arginine) or low concentrations of guanidinium chloride. Our conclusion is that occupancy of the second site by L-arginine is not required for generation of a catalytically competent active site. Instead, by binding at the allosteric site, L-arginine acts as a typical allosteric activator. Thus, the intrinsic cooperative behavior is exhibited by the mutants because of their inabilities to bind the allosteric activator. Fondecyt 1070467.

2330-Pos

Catalysis Mechanism of Aminopeptidase from Streptomyces Griseus: A Quantum Mechanical/Molecular Mechanical Analysis

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Aminopeptidases are exopeptidases that catalyze the removal of N-terminal amino acids for peptides [1]. X-ray revealed that the streptomyces griesus aminopeptidase (SGAP) is a double zinc proteolytic enzyme [2,3] with strong preference for large hydrophobic amino acids. Two different schemes for the general catalytic pathway of SGAP are proposed [1,4]: OH- or H2O nucleophilic attack mechanism. We are investigating SGAP's catalytic mechanism by means of hybrid quantum mechanical/molecular mechanical calculations (AM1d/MM) and analogous small molecule module mechanism with both AM1/d and B3LYP/6-31++G(d,p) methods. A complex network of reaction pathways is generated so as to explore a variety of different putative reaction mechanisms. Our molecular dynamics simulations (SGAP binded with MET-ALA-ALA) for different protonation pattern in the active site indicate that the most probable scenarion is a nucleophilic attack by a Zn2+-bound hydroxide ion, with the GLU131 protonated. Small molecular model AM1 calculation with Gaussian 03 indicates that the Zn2+-bound hydroxide ion first attack the backbone C(O) of MET and then H of this hydroxide ion "migrate" to the adjacent N of ALA, then the C(O)-N peptide chain between MET and ALA is cleaved.

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2331-Pos

Substrate-Induced *Eisenia fetida* Protease Reactions Involve Both "Induced Fit" and "Lock and Key" Mechanisms

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The coupling between ligand binding and protein conformational change is the heart of biological network. "Lock and key" theory and "induced fit" theory were early contributions to our understanding for explaining how an enzyme binds to a substrate. It was accepted that the binding of a substrate to an enzyme is often accompanied by conformational changes of the enzyme. However, whether the substrate-induced complementary conformation is flexible or rigid after a catalytic reaction remains to be determined. By testing the enzyme activity and intrinsic fluorescence of a substrate non-specific *Eisenia fetida* protease-I with different substrates, we show that when this enzyme reacts with a first substrate, it utilises the "induced fit" mechanism. However, in its reaction with further substrates, either the "lock and key" or "induced fit" mechanisms will be used depending on the degree of conformational change required. In contrast to the high activity of the native protease, the chromozym-Th (or -Ch)-induced protease was unable to react with chromozym-U. Chromozym-U-induced enzyme, however, had high activity with chromozym-Th and chromozym-Ch. When low concentrations of GuHCl were used to disturb the conformation of the enzyme, only small changes in intrinsic fluorescence of the CTH-induced protease were