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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,