resistance is associated with inadequate target inhibition. Here we report our most recent efforts to create the analytical and chemical tools needed to directly measure the enzymatic activities of therapeutic targets including protein kinases, lipid-modifying enzymes and the proteasome. Fluorescent reagents are under development that report the activity of these various enzymes with the goal of performing biochemical assays in primary cells. The basic design incorporates enzyme substrates that are modified to create compounds which can be loaded into cells where they are modified by the enzyme of interest. Work has included modification of peptides to confer membrane permeability and to achieve long intracellular lifetimes. Microelectrophoretic separations combined with low-level fluorescence detection enable the quantitative analysis of these compounds from single mammalian cells. This capability addresses three major issues currently faced in the biochemical analysis of clinical samples: the need for direct measurement of the enzymatic activity of target proteins; sample size requirements that are feasible for clinical implementation; and sample heterogeneity that can mask pertinent aspects related to therapeutic response.

1190-Pos Board B100

CDP-Chase, a CDP-Choline Pyrophosphatase, is a Member of a Novel Nudix Family in Gram-Positive Bacteria

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A Nudix enzyme from *Bacillus cereus*, CDP-Chase, acts as a CDP-choline pyrophosphatase, hydrolyzing the phosphoanhydride bond of CDP-choline to produce CMP and phosphocholine. The structure of the free enzyme, determined to 1.8 Å resolution, shows that the enzyme is an asymmetric dimer. Each monomer consists of two domains, an N-terminal helical domain and a C-terminal Nudix domain. The N-terminal domain is placed relative to the C-terminal domain in such a way that produces an overall asymmetry. Residues that may be important for determining the asymmetry are conserved among a group of uncharacterized Nudix enzymes from Gram-positive bacteria. In addition to its Nudix activity, the enzyme has a 3' to 5' RNA exonuclease activity. This alternative activity appears to be facilitated by the asymmetry in the protein as the position of the N-terminal domain results in differences in the exposure of the two enzyme active sites. Two single-site mutations, E112A and E163A, were characterized to further investigate the mechanism of the enzyme. E112 is involved in the coordination of catalytic metals in both active sites, and E163 is only in close proximity in one of the active sites. Both mutations abolish CDP-choline pyrophosphatase activity but E112A has a much more profound effect on RNase activity, supporting a model where CDP-choline hydrolysis is catalyzed by one active site of the dimer and RNA exonuclease activity is catalyzed by the other. These data suggest that CDP-Chase is a member of a novel Nudix family in which structural asymmetry has a profound effect on the recognition of substrates by the Nudix enzymatic machinery.

1191-Pos Board B101

Active Sites of Enzymes are Crowded with Charge

David Jimenez-Morales, Jie Liang, Bob Eisenberg.

The chemistry of enzymes occurs at active sites that concentrate biological function into functional pockets. Functional pockets mix catalytic amino acids and substrate in tiny volumes. Here, we look for biological properties of that small space. We imagine that electric charge plays important roles, because even one charge in a small space produces large electric fields. To estimate densities of fixed charge, we measure the volume of functional pockets and count 'charged residues' in it. We collect locations of functional pockets from enzymes of known structure that catalyze the main six enzymatic reactions. Functional amino acids are identified by their participation in catalysis. We measure the volume of pockets using both solvent-accessible and molecular-surface models. 'Charged residues' are R, K and H (positive); E and D (negative). Charge density is extraordinarily large (~20 Molar on average, often larger). Mobile counterions for the fixed charge are presumably nearby in high density. Active sites do not resemble the infinitely dilute ideal solutions of classical enzyme kinetics. Their enormous charge density is comparable to the charge density of solid NaCl. Different types of enzymes have different charge densities. Hydrolases show the largest values of charge density. Some enzymes have extraordinarily large charge density -phosphoglycerate mutase (PDB = 1o98, density of charge 104 Molar, Molecular Surface), or sulfurtransferase (PDB = 1e0c, 109 Molar, Molecular Surface). Crowding of charged side-chains and ions produces enormous steric and electrostatic forces in these tiny active sites. The balance of these forces seems likely to be of great importance to enzyme function. Many charged pockets are also found away from active sites. Charged pockets are likely to be involved in many surface interactions. They may be reservoirs of electromechanical energy that can drive conformational changes.

1192-Pos Board B102

Strong Coulomb Interactions Between Internal and Surface Charges in Proteins

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Internal ionizable groups in proteins are essential for many biological processes. The molecular determinants of their pK_a values are poorly understood. To examine this problem, we measured previously the pK_a values of Lys, Arg, Asp and Glu at 25 internal positions in staphylococcal nuclease. The pK_a values are usually shifted substantially (as many as 6 pK_a units) in the direction that favors the neutral state because dehydration experienced by the ionizable groups in their buried positions is not compensated by interactions with polar or charged groups. A subset of variants with internal Lys residues showed evidence of interaction of the internal Lys with surface carboxylic groups. Using NMR spectroscopy and crystallography we have shown that internal Lys residues can have significant Coulomb interactions with surface Asp and Glu. Upon ionization the internal Lys residue remains internal and the proteins remain fully folded. The interactions between the internal Lys and surface carboxylic groups are long range (6.3 Å or more between charged atoms) and are mostly through protein. Long-range pairwise interactions as high as 2 kcal/mol have been measured. In some cases strong effects are governed by the sum of many weaker long-range interactions that cannot be decomposed experimentally into pairwise contributions. Overall, there is strong evidence that surface carboxylic groups stabilize the charged form of many internal Lys residues. The experimentally measured Coulomb interaction energies between internal and surface charges constitute invaluable constraints for benchmarking of structure-based electrostatics calculations. They also suggest a strategy for modifying the pK_a value of active site residues by the engineering of surface charges.

1193-Pos Board B103

Enzyme-Triggered Anticancer Lipid Prodrugs

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The use of liposomal drug delivery systems for cancer treatment is primarily hampered by the complications associated with making liposomal encapsulations that are: 1) stable outside the target area and 2) provide efficient drug release at the target. The escape of encapsulated drugs from the liposomes by passive diffusion often leads to suboptimal drug concentrations in the cancer tissues. Hence, we have constructed lipid prodrugs that can form liposomes and therefore overcome the necessity for liposome drug loading. The prodrugs were prepared using ether phospholipids esterified in the *sn*-2 position to anticancer drugs like chlorambucil, retinoic acids or prostaglandin, where the active drug can be released at the target tissue by means of secretory phospholipase A_2 ($sPLA_2$), an enzyme that is upregulated in various cancer tissues. In this work, the biophysical characterization, cytotoxicity and enzymatic hydrolysis of the prodrugs are presented. It is also shown how the mixing of the prodrugs with $sPLA_2$-cleavable phospholipids was found to enhance the properties of the liposomal formulation and to strengthen its targeting capacity.

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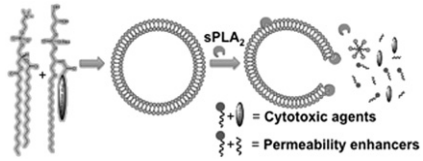

1194-Pos Board B104

Inhibitors of Leishmania Major Farnesyl Diphosphate Synthase: Crystallographic and Calorimetric Studies

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Leishmaniasis is a parasitic disease predominantly seen in tropical and subtropical regions. Different Leishmania species are responsible for different forms of the disease: Viscereal Leishmaniasis, Cutaneous Leishmaniasis and Mucutaneous Leishmaniasis. Cutaneous Leishmaniasis, which is caused, by either L. Tropica or L. major affects around 1.5 million people every year. It is transmitted by the bite of the sandfly, an intermediate host. Rodriguez et. al showed that the bisphosphonates, such as pamidronate inhibit the growth of lesions in mice when administered intraperitoneally. BPs target the Farnesyl Diphosphate synthase (FPPS), an enzyme of the mevalonate pathway in both parasite and humans, . Kinetic studies on LmFPPS showed that the Km for DMAPP is 53 μM. Inhibition cell based studies against intracellular L. donovani amastigotes show that the IC50 values for 1-(2-Hydroxy-2,2-bis-phosphono-ethyl) −3-phenyl-pyridinium