with a high and low affinity. In addition, the ITC experiments of L-PGDS mutant proteins suggested that the active Cys65 residue is located at the high affinity-binding site. The results of these experiments will be discussed in this paper.

3423-Pos Board B151
The Evolutionary Constraints Imposed on Tyrosine Hydroxylation by its Beginnings as a Phenylalanine Hydroxylase; Studies of the Polypeptide Loop Determining the Substrate Specificity
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Tyrosine hydroxylase (TyrH), phenylalanine hydroxylase (PheH) and tryptophan hydroxylase (TrpH) belong to the family of aromatic amino acid hydroxylases (AAAH) and are genetically related. An ancient PheH probably was the ancestor of the modern enzymes. Their active sites are very similar, however their substrate specificities vary. They all hydroxylate phenylalanine; TyrH is the only one that can make DOPA from tyrosine. Previous work identified the aspartate at position 425 in TyrH, which lies in a very flexible and dynamic polypeptide loop, to be critical for the enzyme's achievement of this new activity. We started this project to examine whether alterations in the shape of this polypeptide loop could make a stronger TyrH. The crystal structure of TyrH shows D425 is at the end of this loop. We also knew that substituting it with a glutamate (D425E) decreased its DOPA forming ability 10-fold. We sought to find out if making the loop shorter but keeping the glu would make a strong TyrH. Therefore, we deleted separately and together the glutamines (Q424 and Q426) that are adjacent to D425. Of these three mutants, only D425EDQ426 has comparable activity to WT. Previously reported Vmax values are: WT, 150; D425ETyrH, 13.4. The value for D425EDQ426 is 118. We determined the specific activity for the enzyme by finding the ratio of Vmax/KM for tyrosine to Vmax/KM for phenylalanine and comparing this to WT's and previous mutants' values. The value for D425EDQ426 was 3.3, and for WT, 3.75, and for D425ETyrH, 0.34.

3424-Pos Board B152
Structural and Functional Basis for Substrate Specificity and Catalysis of Levan Fructotransferase
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Levan is β-2,6-linked fructan and serves as reserve carbohydrate in some plants and microorganisms. This fructan is usually utilized by enzymes such as glycoside hydrolase (GH) for producing a fructose. Levan fructotransferase (LFTase) of the GH32 family catalyzes an intramolecular fructosyl transfer reaction and results in production of cyclic difructose dianhydride, thus exhibiting a novel substrate-specificity. The mechanism by which LFTase carries out these functions via the structural fold conserved in the GH32 family is unknown. Here, we report the crystal structure of LFTase from *Arthrobacter ureafaciens* in apo form and complex with sucrose and levanbiose. Despite the structural similarity with members of the GH32 family, LFTase contains the −1 and −2 subsites for a difructosaccharide in an active site. This feature is unique among GH32 proteins and is facilitated by small side-chain residues in the loop region of a catalytic domain, which is conserved in the LFTase family. Together with functional analysis, our data provide a molecular basis for the catalytic mechanism of LFTase and suggest functional variations from other GH32 family proteins, notwithstanding the conserved structural elements.

3425-Pos Board B153
Co Recombination in Human IDO and TDO - A Comparison
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The heme enzymes tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) catalyze the oxidative cleavage of the L-tryptophan (L-Trp) pyrrole ring and the insertion of molecular oxygen to produce N-formylkynurenine, the first and rate-limiting step in the kynurenine pathway. The catalytic reaction involves the formation of a ternary complex including the protein, the ligand (O_2), which binds to the ferrous heme iron, and the substrate (L-Trp). For optimal turnover of the enzyme, the protein scaffold has to provide easy access for both O_2 and L-Trp to the heme active site. Efficient O_2 association may require transient docking sites to be accessible. As both oxygen atoms are inserted into the C2 - C3 bond of the L-Trp pyrrole ring during the reaction, efficient ligand escape is (most likely) not required. L-Trp binding calls for a rather large 'access gate'. In addition, the reaction product *N*-formylkynurenine must be expelled from the active site to allow the uptake of new reagents.

The molecular details of the catalytic mechanisms employed by TDO and IDO have, as yet, not been elucidated. Therefore, we have investigated ligand rebinding in ferrous IDO and TDO using flash photolysis at ambient temperatures, replacing the physiological ligand O_2 by CO, to disclose both similarities of and differences between the enzymes. CO allows us to exploit its excellent properties as a spectroscopic probe. Moreover, CO is not reactive towards L-Trp, so that we can focus on ligand and substrate dynamics in the absence of the ensuing enzymatic reaction.

3426-Pos Board B154
Molecular Dynamics Simulations of the Proton Transfer Reaction Between the Catalytic Residues in HTLV-I Protease
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In order to study the catalytic mechanism of Human T-cell leukemia virus type I (HTLV-I) protease, it is important to determine the protonation states of the two aspartic acid residues at its active site. Free energy simulations have been conducted to study the proton transfer reaction between the catalytic residues of HTLV-I protease using a combined quantum mechanical and molecular mechanical (QM/MM) molecular dynamic simulation. Free energy profiles for the reaction in apo-enzyme and in an enzyme-substrate complex will be presented. In addition, the structural features of HTLV-I protease will be compared and discussed for different protonation states of the active site residues.

3427-Pos Board B155
Evidence for Functional Role of C-H···S Hydrogen Bond in Enzyme Catalysis and Substrate Specificity: Type 1 Methionine Aminopeptidase
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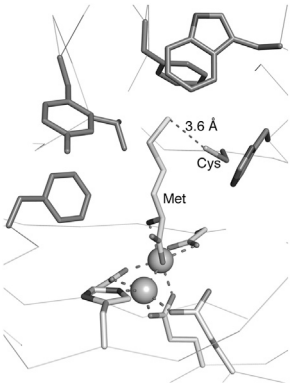
This study is about the functional role of a weak C-H···S hydrogen bond between conserved cysteine in the S1 pocket of methionine aminopeptidase (MetAP) and the substrate methionine during recognition and catalysis. Mutation of this conserved cysteine leads to relaxed substrate specificity, reduced stability and catalytic efficiency. Unexpectedly short distance (3.6 Å) is noticed between the cysteine S-atom from the enzyme and the carbon atom of the substrate. pKa of this cysteine was found to be much lower compared cysteines in proteins. Crystal structures of the mutant and the inhibitor complexes explain the polarizability and reactivity of the cysteine. Since the pKa is lower than the physiological pH, cysteine exists as a thiolate anion and interacts with substrate methionine by forming C-H···S hydrogen bond. The importance of this interaction is underscored from the fact that MetAPs process only peptides with methionine on the amino terminus. This is the first example of such a weak interaction playing critical role in the conserved function of a family of essential enzymes.



3428-Pos Board B156
Monitoring Conformational Changes that Occur to Blood Coagulant Prothrombin as it is Activated to Thrombin
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The blood coagulation protein thrombin is originally expressed as the zymogen prothrombin (ProT). Prothrombin contains the F1.2 (Gla + Kringle 1 + Kringle 2) domain and the inactive serine protease prethrombin-2 (PT2). Kringle 2 of F1.2 protects an immature anion binding exosite on ProT called pro-ABE II. The prothrombinase complex converts ProT to thrombin. Cleavage of ProT after R271 releases F1.2 from PT2. Cleavage of PT2 at R320 helps to generate the active, two-chain species thrombin that contains an A and B chain linked by a disulfide. Hydrogen-Deuterium Exchange coupled with MALDI-TOF mass spectrometry was used to monitor the conformational dynamics of ProT, F1.2 + PT2, PT2, and thrombin. During the activation process, the A-chain and the autolysis loop undergo changes in solvent accessibility that respond to the individual R271 and R320 cleavages. The resultant conformational properties aid in creating a protease that can accept substrates at the active site. Importantly, this coagulation protein system also uses anion binding exosites to control its functions. F1.2 protects pro-ABE II on both ProT and PT2 from solvent exposure. Moreover, this binding event exhibits a long-range