visible in the 12- fold averaged structure. Due to selective binding of ADP within the dodecamer, the GS molecule deviates from exact 62 symmetry. This explains the preference of the C2 crystal form over hexagonal crystal forms which are observed for higher and lower ADP concentrations.

The use of R-free to monitor data overfitting when releasing strict non-crystallographic symmetry will be discussed and data for the choice of the reflections for the TEST-set (not well established for structures with non-crystallographic symmetry) will be presented.

Binding of two ADP molecules in an up-down fashion in the dodecamer sandwich, as seen in the new model, supports the biochemical finding of homotropic cooperative binding of substrate, for which kinetic data will be presented along with the crystallographic data.

PS04.17.33 THE BINDING SITES OF KRYPTON AND XENON IN PROTEINS : A SURVEY OF TEN COMPLEXES.
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It is now well established that the noble gases xenon and krypton bind to numerous proteins through weak Van der Waals interactions. In about fifty percent of the examined cases, the sites are sufficiently well defined (in terms of occupancy factors and phasing power) to be used as highly isomorphous heavy atom derivatives in MIR or SIRAS techniques (several examples will be presented during this meeting). The binding site environments of a set of ten proteins including elastase, cutinase, subtilisin, collagenase, lysozyme, urate oxidase, etc.. are analysed in terms of polar/hydrophobic interactions and close contacts to feature out a general prediction for the binding under moderate gas pressure (4 to 40 bar).

PS04.17.34 X-RAY STRUCTURE ANALYSIS OF APOFLAVODOXIN FROM ANABAENA PCC 7119.
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Many biological reactions are catalysed by flavoproteins, a large group of proteins carrying a flavin cofactor (either FMN or FAD). The redox properties of flavoproteins arise from the interaction between the apoprotein and the redox cofactor. Although the structures of many holoflavoproteins are known, there is no single apoflavoprotein of known three-dimensional structure.

We report here the X-ray structure of apoflavodoxin from Anabaena PCC 7119 at 2.0 Å resolution. Apoflavodoxin is a compact, well-folded protein with the same overall fold as holoflavodoxin (1). The transient cavity formed on removal of the FMN cofactor is filled by the indole ring of Trp57 that penetrates into the depression previously occupied by the pyrimidine portion of the isoalloxazine and by the ribityl. The closure of this aromatic gate disorganises the isoalloxazine and ribityl binding sites but leave the phosphate binding site intact. This suggest that the interaction between the FMN and the apoprotein could start at this place.

(1) Rao., S.T., Shaffie, F., Yu, C., Satyshur, K.A., Stockman, B.J., Markey, J.L. & Sundaralingam, M. Protein Science, 1, 1413-1427 (1992)

PS04.17.35 SHORT-CIRCUITING A WATER-MEDIATED ENZYME REACTION.
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A water-mediated hydrogen bond network coordinated by glutamate 58 has been proposed to play an important role in the thymidylate synthase (TS) reaction mechanism. We have addressed the role of glutamate 58 in coordinating the watermediated hydrogen bond network in the TS reaction, by cocrystalizing the E. coli TS mutant E58(60)Q with dUMP and the cofactor analog CB3717, and solving the X-Ray crystal structure to 2.5 Å resolution with a final R-factor of 15.3% (Rfree=23.7%). Using a difference Fourier analysis we were able to analyze directly the changes that occur between the wild-type and mutant structures. In the structure of the mutant enzyme the coordinated hydrogen bond network has been "short- circuited," providing an atomic resolution explanation for the disruption of the TS reaction by the E58Q mutant as well as confirming the proposal that the role of E58 in the TS reaction is to coordinate this conserved hydrogen bond network. The structure also shows an previously unobserved conformation for the cofactor analog, CB3717, which has implications for structure-based drug design and sheds light on the controversy surrounding the previously observed enzymatic nonidentity between the monomers of the TS dimer.

PS04.17.36 STRUCTURAL BASIS OF CHEMOTHERAPY RESISTANCE MEDIATED BY THYMIDYLATE SYNTHASE
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The structure of a mutant thymidylate synthase (TS) representative of a chemotherapy-resistant enzyme reveals distinct atomic shifts away from the active site of absolutely conserved residues critical for ligand binding.

TS inhibition is an important mechanism for the chemotherapy agent 5-fluorouracil (5-FU), which prolongs life in patients with stage III colon cancer. Cells containing a well-characterized mutant form of the human enzyme (Tyr 33 -> His) exhibit relative resistance to the 5-FU metabolite 5-fluoro-2'-deoxyuridine. To investigate the structural correlates of this chemotherapy resistance, we took advantage of the extensive collection of wild type and mutant structures of *E.coli* TS built so far in this laboratory and used site-directed mutagenesis to generate the corresponding mutation in *E. coli* TS (Tyr 4 -> His). The Tyr 4 -> His mutant *E. coli* TS was expressed in bacteria, purified by ion exchange chromatography and crystallized alone or in the presence of substrate and cofactor ligands. The structure of the mutant molecule was refined to a crystallographic R factor of 22% (R_{free} = 26%). Differences between mutant and wild type were also studied by examination of F_o - F_o difference maps.

Estimates of coordinate error yielded expected differences from true atomic positions of 0.35 - 0.4 Å. F_o - F_o difference maps allowed smaller changes between mutant and wild type structures to be discerned. These differences are concentrated in the region between the N-terminal A helix and the large central active site. The difference maps clearly reveal the loss of electron density around the position of the tyrosine hydroxyl group absent in the mutant enzyme. The hydrogen-bonding network linking the N-terminal A helix to the J helix that intervenes between the A helix and active site cavity is disrupted, since the Tyr 4 hydroxyl is no longer available to interact with side chain carbonyl of Val 170. A concerted movement of atoms away from the active site toward the mutant histidine residue propagates across