

**PS04.17.06 CONFORMATIONAL ANALYSIS OF TRYPSIN AT ATOMIC RESOLUTION REVEALS EFFECTS OF CRYSTALLINE ENVIRONMENT.** Hans D. Bartunik, Alexandre N. Popov, Max-Planck Research Unit for Structural Molecular Biology, Protein Dynamics Group, MPG-ASMB c/o DESY, Notkestraße 85, 22603 Hamburg, Germany

The crystalline environment affects the relative orientation of the two  $\beta$ -barrel domains of bovine pancreatic  $\beta$ -trypsin (BPT) and induces conformational strain. The comparison is based on anisotropic refinement using SHELXL-93 [1] of BPT at 5°C in two different orthorhombic crystal forms [2, 3] at 1.0-1.1 Å resolution ( $R=8.3\%$ ;  $9.8\%$ ). The accuracy in the atomic positions is on average 0.01-0.02 Å for each structural model. When aligned on the active site region and one domain (150 residues; rmsd 0.09 Å, max. deviation 0.19 Å for the main chain atoms), the other domain exhibits relative coordinate shifts (rmsd 0.22 Å, max. 0.52 Å) as well as substantial changes in the conformational angles. The positional and conformational differences are particularly large for the  $\beta$ -strands 81-90 (near the surface) and 104-108. Molecular packing interactions further induce flexibility in a number of residues (35 in the one structure, 24 in the other) that are present in discrete alternate conformations. Correlations between alternate side chain locations are observed which extend over distances up to 20 Å; in several cases, water or sulphate molecules with partial occupancies are involved. Most of the ordered solvent (ca. 2.2 waters per accessible residue - nearly all in the first coordination shells) and the degree of anisotropy in the individual atomic temperature factors are essentially not affected by the crystalline environment. In both structures, only one residue (Gln 192) is not located in well-defined electron density; the flexibility may reflect its functional role in orienting substrates. The diffraction data were measured on the synchrotron beamline BW6/DORIS.

[1] Sheldrick, G. W. (1993). SHELXL-93, Univ. Göttingen.

[2] Marquart, M. et al. (1983). Acta Crystallogr. sect. B, 39, 480-490.

[3] Bartunik, H. D. et al. (1989). J. Mol. Biol. 210, 813-828.

**PS04.17.07 QUANTITATING CHANGES IN THE THREE DIMENSIONAL STRUCTURE OF VARIANT ENZYMES.** Richard Bott and Mark Knapp, Genencor International, 180 Kimball Way, So. San Francisco, CA

Site-specific substitutions made to change performance of an enzyme toward a desired purpose often result in subtle conformational adjustments. Often there is more than one structural consequence associated with any single substitution. In order to associate these structural consequences with altered function, it would be helpful to devise means of quantitating these changes. These changes could then be correlated with altered functionality in a series of variants.

*Bacillus lentus* subtilisin has been modified for improved proteolytic activity. The three-dimensional structures of several variants have been determined that display increased and decreased performance. It is necessary to obtain the highest resolution data in order to detect and quantitate these subtle changes that contribute to altered performance. Currently the resolution limit of the data can exceed 1.4 Å.

Results of our efforts to employ difference distance plots and other techniques will be presented. It is important to differentiate crystal lattice effects from mutational consequences when variants crystallize in different forms.

**PS04.17.08 CRYSTAL STRUCTURE ANALYSIS OF HUMAN TRANSTHYRETIN COMPLEXES WITH FLUORESCENT PROBES.** Vivian Cody, Joseph R. Luft, Walter Pangborn, Hauptman-Woodward Medical Research Institute, Inc., Buffalo, NY 14203

Fluorescent probes of the N-arylamino-naphthalene sulfonate type are used to assess the hydrophobicity of protein binding sites and as a means of monitoring conformational changes in biological macromolecules. Structure-activity data show that they can also act as competitive inhibitors for thyroxine ( $T_4$ ) binding to transthyretin (TTR). Fluorescence quenching studies of 8-anilino-1-naphthalene sulfonic acid (ANS) by competitive displacement of  $T_4$  from TTR was used to determine the binding affinity of  $T_4$  and to describe negative cooperativity in binding the hormone to the two equivalent sites on the TTR tetramer. These data supported two theoretical models for ANS quenching - one showing independent actions of the two hormone binding sites, and the other requiring interaction between the two sites. Similarly, the fluorescent probe N-(iodoacetyl)-N'-(5-sulfo-1-naphthyl)ethylenediamine (1,5-AEDANS) was shown to form a covalent bond with Cys-10 of TTR. In order to better understand negative cooperativity in hormone binding to TTR, we have carried out the X-ray crystal structure determination of human TTR co-crystallized with various fluorescent probes and report structural results for TTR complexed with ANS and 1,8-AEDANS. Both crystals diffract to 1.9 Å resolution and crystallize in the orthorhombic space group  $P2_12_12$  with two independent monomers in the asymmetric unit. Cell dimensions are isomorphous to previously reported lattices. Refinement of each structure was carried out to 1.9 Å resolution without inhibitor contributions using the program PROLSQ. Difference ( $F_o - F_c$ ) electron density maps based on these refinements reveal electron density in the center of the hormone binding domain in both data sets. In the case of 1,8-AEDANS, there is no density near Cys-10, but there is indication of a covalent link of 1,8-AEDANS to the  $\epsilon$ -amine of Lys-15, as was obtained in the crystal structures of N-bromoacetyl-hormone derivatives. Since the reactive acetyl group is the same for 1,5-AEDANS and 1,8-AEDANS, it is not clear why there is no involvement with Cys-10. Data for the ANS complex show density in the hormone binding site which is similar to that of 1,8-AEDANS. Higher resolution data for these complexes are needed to interpret changes in TTR conformation which may explain the mechanism of negative cooperativity. Supported in part by DK-41009.

**PS04.17.09 NEW CRYSTAL FORMS OF *ESCHERICHIA COLI*  $P_{II}$  COMPLEXED WITH VARIOUS LIGANDS AND STRUCTURE SOLUTION OF  $P_{II}$ /ATP/2-KETOGLUTARATE.** Karen Edwards, Peter Suffolk, Paul Carr, Eong Cheah and David Ollis. Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia

$P_{II}$  is a signal transduction protein involved in bacterial nitrogen regulation and plays a key role in regulating both the activity and level of expression of the enzyme glutamine synthetase (GS). Regulation of GS is achieved via a number of protein-protein interactions involving  $P_{II}$ , adenylyl transferase, nitrogen regulatory proteins I and II and uridylyl transferase (UTase/UR). The function of the regulatory enzymes is reversed upon uridylation of residue Tyr51 in  $P_{II}$  which occurs in response to a drop in cellular nitrogen levels.

The structure of unliganded *E. coli*  $P_{II}$  has been solved and refined to 1.9 Å resolution (Carr *et al.*, 1996). Recent biochemical evidence suggests an important role for ligands in effecting allosteric changes in  $P_{II}$  (Kamberov *et al.*, 1995). ATP, 2-ketoglutarate and glutamate have all been shown to bind to  $P_{II}$  and