glutamate dehydrogenase (GDH) ($t_{1/2}$ at 90°C = 12.5 h) from the thermo-philic sulfur-reducing archaeon *Thermococcus* ANI. GDH catalyses the oxidative deamination of L-glutamate to 2-oxyglutarate and ammonia, and provides an important link between carbon and nitrogen metabolism.

The *Thermococcus* GDH has been crystallized in two orthorhombic forms; form A, a primitive cell with dimensions 155.3 x 115.1 x 173.4Å, and form B, a C centred cell with dimensions 97.6 x 247.5 x 146.4Å. Two data sets have been collected from form A crystals using an in-house rotating anode (to 3.0Å) and a synchrotron source (to 2.4Å). The V_M was 2.75 Å^3/Da, assuming 6 monomers (M_r 47,000 Da) per asymmetric unit.

The structure was solved by automated molecular replacement using AMoRe, with a search model derived from the homohexameric *Pyrococcus furiosus* GDH. The four primitive orthorhombic spacegroups were searched for two independent occurences of one *P. furiosus* trimer, with the most significant solution observed in $P2_12_12_1$ (correlation 60.2 and R-factor 34.4%). The structure has been refined using molecular dynamics (XPLOR) and restrained least-squares methods (TNT), employing strict 6-fold non-crystallographic symmetry constraints.

The structure consists of two trimers arranged "back-to-back", the interface forming an extended β-sheet. The arrangement of each trimer is similar to that observed in the *P. furiosus* and *C. symbiosum* structures, and a structural comparison, along with the identification of potential structural determinants of enzyme thermostability will be discussed.

MS04.16.04 ONE SEQUENCE, TWO FOLDS: CRYSTAL STRUCTURE OF A MISFOLDED, METASTABLE FORM OF CD2. R. Leo Brady and Alison J. Murray, Department of Biochemistry, University of Bristol, Bristol BS8 1TD U.K.

We have recently determined the crystal structure of a misfolded, metastable form of the NH_2-terminal domain of the lymphocyte cell adhesion molecule CD2. When expressed as part of a glutathione-S-transferase fusion protein the polypeptide sequence for domain 1 of CD2 can fold in two different ways. The major (85%) monomeric component forms a familiar immunoglobulin superfamily fold as previously demonstrated by both X-ray crystallography and NMR spectroscopy. We now describe the structure of a second, dimeric form present in about 15% of recombinant CD2 molecules. After denaturation and refolding in the absence of the fusion partner dimeric CD2 is converted to monomer, illustrating the dimeric form represents a metastable folded state.

The crystal structure of this dimeric form, refined to 2.0 Å resolution, reveals two domains with overall similarity to the IgSF fold found in the monomer. However, in the dimer each domain is formed by the intercalation of two polypeptide chains. Hence each domain represents a distinct folding unit that can assemble in two different ways. In the dimer the two domains fold around a hydrophilic interface believed to mimic the cell adhesion interaction at the cell surface, and we demonstrate formation of dimer can be regulated by mutating single residues at this interface. This unusual misfolded form of the protein, which appears to result from inter- rather than intramolecular interactions being favoured by an intermediate structure formed during the folding process, illustrates that evolution of protein oligomers is possible from the sequence for a single protein domain. We suggest this exploitation of a finely-balanced folding intermediate capable of developing to two distinct folds may pre-date "domain swapping" as a mechanism for the evolution of protein oligomers. A biological role for the alternative fold cannot be ruled out, as in this state the adhesion interface is buried and effectively switched "off".

Analyses of a series of deletion and single-site mutations of CD2 support the proposed folding pathway for immunoglobulin superfamily domains implied by this structure. Identifying domain features important in directing folding pathways has important implications for protein design and engineering.

MS04.16.05 STRUCTURAL INSIGHT INTO THE FUNCTION OF GroEL/GroES/ADP COMPLEX. Zhaohui Xu, Arthur Horwich, Paul B. Sigler, Yale University and the Howard Hughes Medical Institute, 295 Congress Ave., New Haven, CT 06510

GroEL is a chaperonin, composed of 14 60-kD subunits arranged as two sevenfold rotationally symmetric rings stacked back-to-back with dyed symmetry. With the aid of Mg^{2+}, ATP and GroES [a 7-subunit (10kD each) co-chaperonin], GroEL enhances the protein-folding process by preventing the formation of irreversibly misfolded and/or aggregated non-native "off pathway" intermediates. Non-native polypeptides are bound avidly by GroEL primarily through hydrophobic contacts inside the opening of the central cavity. In an ATP-consuming cycle, the bound peptide is released to achieve a folded state or rebind to the chaperonin.

We have crystallized and solved the structure of a complex of GroEL/GroES/ADP to 3.0Å. While still under refinement, the structure has shown seven ADP molecules and one GroES 7-mer ring bind to the same GroEL ring where major structural rearrangement has occurred. The elucidation of the stereochemical details of this complex will give us insight into the mechanism by which GroEL-assisted folding is accomplished.

MS04.16.06 AN UNUSUAL ROUTE TO THERMOSTABILITY IN PYROPHOSPHATASES Adrian Goldman*, Tiina Salminen*, Alexei Teplyakov‡, Barry Cooperman† and Reijo Lahti*. *University of Turku, Turku, Finland; ‡European Molecular Biology Laboratory, Hamburg, Germany; †University of Pennsylvania, Philadelphia, USA.

Rigid-body motions of entire monomers to produce tighter oligomers may be yet another way in which proteins can be made thermophilic. By comparing the structures of *Thermus thermophilus* and *E. coli* PPases (T- and E-PPases) we showed that the chief determinant of the increased thermostability of T-PPase appears to be a change in the oligomerisation and oligomeric interfaces of these hexameric enzymes. Their sequences are 47% identical and the monomer structures superimpose extremely well (rmsd of 1 Å per Cα). However, the hexamers superimpose poorly (rmsd of 2.2 Å per Cα) because of a complex rigid-body motion: with respect to the E-PPase hexamer, each T-PPase monomer in the hexamer is skewed by about 1 Å in the xy plane, is 0.3 Å closer to the centre of the hexamer in the z-direction, and is rotated by about 7° about its centre of gravity. The rigid-body translation accounts for most of the difference between the hexamer and monomer superpositions. In T-PPase the hexamer is packed more tightly than in E-PPase: the amount of surface area buried upon oligomerisation increases by 16%, in particular at one of the trimer-trimer interfaces. Three small loops involved in oligomerisation adopt different conformations. Consequently, the number of hydrogen-bonding and ionic interactions almost doubles, which allows the tighter-packing to occur. The new interactions interlock across all the oligomeric interfaces in the hexamer.

Bacterial PPases, only 170 residues long, contain large active sites (≈15 active site residues; ≈12 Å across) and have little in the way of a hydrophobic core. Much of the protein, therefore, does multiple duty: for instance, one helix contributes to the hydrophobic core of the protein, provides active-site residues and is a key part of the trimer-trimer interfaces. (Thermo)stability and activity appear to be intimately linked. Although these enzymes are not allosteric, solution data on E-PPase variant proteins show that oligomerisation stabilises the conformation of the enzyme that binds substrate and *vice versa*. The D_3 bacterial inorganic pyrophosphatases seem to be a minimalist protein design.