**MS04.08.04 THE CELLULOSOME: A NOVEL MECHANISTIC CONCEPT IN MACROMOLECULAR AGGREGATES.** Pedro M. Alzari, Institut Pasteur, Paris, France.

The cellulosome, a cellulolytic multi-enzyme complex secreted by clostridia and other bacteria, is a specialized exocellular structure that enables cells to obtain energy from cellulose and hemicellulose, two abundant but intrinsically intractable substrates. Unlike other well-characterized macromolecular agregates such as viral capsids, the ribosome or the pyruvate dehydrogenase complex in which protein-protein interactions stabilize a highly ordered quaternary structure, the cellulosome makes use of a flexible mechanism to enhance the synergistic action of its various enzymatic components.

The cellulosome of the anaerobic thermophile *Clostridium thermocellum* contains numerous functional subunits, most of which are glycosidases conveying distinct carbohydrate specifities. Their catalytic domains display a variety of protein folding topologies including immunoglobulin-like and lectin-like folds, $(\beta/\alpha)_8$-barrels, and $(\alpha/\alpha)_6$-barrels. Genetic and crystallographic studies of these enzymatic components have revealed both a conserved active site architecture which has evolved to acquire different substrate specificities and dissimilar protein frameworks which have converged towards the same functional specificity. Most cellulosomal enzymes share a highly conserved duplicated domain - the dockerin domain - that serves to anchor the individual enzymes to a non-catalytic cellulosomal subunit. This scaffolding protein, called CipA (Cellulosome-Integrating Protein), harbors the cellulose-binding function of the complex, serves to attach the cellulosome to the cell surface, and is responsible of organizing the various enzymatic components into the complex. In addition to a cellulose-binding domain and a cell membrane-binding domain, CipA contains a linear tandem of homologous subunits - the cohesin domains - that specifically bind to the dockerin domains of glycosyl hydrolases, giving rise to the "rows of equidistantly spaced polypeptides" observed in electron micrographs. This modular mode of macromolecular assembly provides a simple mechanism to achieve a complex multi-functional structure, and may offer a wide range of applications for the design of new biochemical entities.

**MS04.08.05 DANCING WITH THE ELEPHANT: STRUCTURE AND ASSEMBLY OF THE FIBER-FORMING PROTEIN PILIN.** John A. Tainer, Hans E. Parge, Katrina T. Forest, and Elizabeth D. Getzoff. Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037

Attempting the three-dimensional description of protein fiber assemblies calls to mind the problem embodied in the Hindu fable about six erudite but blind men who came to "see" the elephant. Finding distinct elephant regions prompted their disparate comparisons to a wall, a spear, a snake, a tree, a fan, and a rope, so "...each was partly in the right and all were in the wrong." Sir Lawrence Bragg underscored the difficulty of applying crystallography to visualize fiber assemblies by dividing proteins into two broad classes: the globular proteins, which often exist as individual molecules and form excellent crystals, and the fiber-forming proteins that aggregate but do not crystallize due to the complex ways individual molecules aggregate (Bragg, 1975). Yet, without crystallographic structures, we risk the blind man's confusion in attempting the integration of the genetic, biochemical, and biological data for these critical and often multi-functional cellular assemblies.

Type IV pili are long, multi-functional fibers involved in the attachment, mobility, DNA transformation, and infectivity of many bacterial pathogens. Moreover, these intrinsically flexible pili bend, extend, and retract suggesting that the elephant we wish to visualize is not standing still but dancing. We isolated pilus fibers, disassembled them with high pH and *n*-octyl-β-D-glucopyranoside and obtained diffraction quality crystals by adding 1,2,3-heptanetriol. The 2.6 Å pilin structure reveals a novel ladle-shaped α-β roll fold with an extended α-helical spine. The pilin crystal structure combined with results from electron and force microscopy, fiber diffraction, and antibody binding suggests a testable assembly model and a means of dancing with the elephant, so that we approach a detailed understanding of pili function and assembly. Questions that can now be addressed include the structural basis for the high mechanical stability required for a fiber whose length (40,000 Å) is over 600 times its diameter (60 Å), and how these stealth fibers can undergo extreme sequence variation to escape the host immune response while maintaining their assembly and function.

Bragg, L. (1975) The development of x-ray analysis. Dover, NY.

**MS04.08.06 GroEL AND ITS LIGANDED STATES.** Sigler, Paul B., Department of Molecular Biophysics and Biochemistry, Yale University and the Howard Hughes Medical Institute, 295 Congress Ave., New Haven, CT 06510, USA

The bacterial chaperonin, GroEL, is a 14-subunit (60-kD each) double toroidal assembly that assists the folding of proteins in conjunction with a 7-subunit (10 kD each) complex, GroES, and the hydrolysis of $Mg^{2+}$-ATP. GroEL/GroES are essential components of *E. coli* and homologues are found in all eubacteria, archaea and eukaryotic cytoplasmic inclusions. Analogous proteins operate in the cytosol of eukaryotes. Chaperonins function by binding (and, perhaps, unfolding) nonnative proteins in the central cavity of the cylindrical double toroid preventing further misfolding and/or aggregation and releasing them in a form that permits refolding.

The structures of GroEL and its various liganded states will be presented in an effort to provide a structural context for what is known about their role in the folding process and as a basis for the design of genetic and biochemical experiments that further our understanding of the GroEL-assisted folding mechanism.

**PS04.08.07 STRUCTURE OF THE ALLOSTERIC TETRAMER THREONINE DEAMINASE.** T.Gallagher, E.Eisenstein, D.Chinchilla, J.Zondlo and G.Gilliland, Center for Advanced Research in Biotechnology of the Maryland Biotechnology Institute, University of Maryland, Shady Grove and the National Institute of Standards and Technology, 9600 Gudelsky Dr., Rockville MD 20850

The biosynthetic threonine deaminase from E. coli is a 220-Kdal tetramer whose activity is regulated by the allosteric ligands isoleucine and valine. SIRAS phases to 3.1 Å resolution based on a uranyl derivative, and native data to 2.3 Å, have led to a model of the enzyme and suggested a mechanism for the allosteric transition. Each 514-residue chain folds into 3 α/β domains, with the pyridoxal phosphate cofactor and the active site nestled between catalytic domains while the regulatory domain makes extensive quaternary interactions. Sigmoidal kinetics likely result from a structural transition in which the domains rearrange to modulate access to the active sites; biochemical evidence suggests that the transition involves a reduction in the symmetry of the tetramer from 222 to 2.

The unliganded structure, crystallized in space group I222 with lattice constants 84, 91, and 163 Å and one chain per asymmetric unit, is currently in refinement. Additional datasets from two other crystal forms, and from crystals grown in the presence of the allosteric ligands, have been collected. Thus a complete description of the tertiary and quaternary structure, and

discussion of the catalytic and allosteric mechanisms of this key regulatory enzyme, will be possible. Further interest to the science of crystal growth rises from the fact that two distinct crystal forms grow under identical conditions; this phenomenon will be explored in light of the molecular contacts.

**PS04.08.08 CUBIC, TETRAGONAL AND ORTHORHOMBIC CRYSTAL FORMS OF HORSE SPLEEN APOFERRITIN.** Geoffre, S., Gallois, B., Dautant, A., Granier, T., Langlois d'Estaintot, B., Michaux, M.A. & Précigoux, G., Unité de Biophysique Structurale, CNRS, Université de Bordeaux, 33405 Talence, France.

Ferritin is the universal iron storage protein utilized by most living cells to uptake and store iron, in a bio-available form , via redox mechanisms. X-ray structural studies show that haem is able to bind horse spleen apoferritin in a site similar to that observed in bacterioferritins with a stoichiometry 1:2.

In the crystal structures, and whatever the studied metalloporphyrin, the protoporphyrin IX is always observed free of metal. Beside that property, horse spleen apoferritin can be crystallized in three different space groups : cubic, F432 ; tetragonal, $P42_12$ and orthorhombic $P2_12_12$. The two later crystal forms were described some years ago (Harrison 1963, Hoy & al. 1974) but their crystal quality (6 Å resolution) could not allow a full structure investigation.

Thanks to a careful protein purification procedure, both horse spleen apoferritin crystals of tetragonal and orthorhombic forms, which diffract beyond 2.4 Å have been obtained. X-ray diffraction data were collected, on the three different crystal forms, with the LURE synchrotron radiation facilities.

We present a comparison of the structures of the three crystal forms : molecular packing and conformational differences will be discussed in relation with crystal symmetry differences.

**MS04.08.09 THE MONOCLINIC CRYSTAL STRUCTURE FORM OF BACTERIOFERRITIN FROM *E. COLI*.** Hospital, M.1, Dautant, A.1, Yariv, J.1, Précigoux, G.1, Kalb (Gilboa), A.J.2, Frolow, F.2 & Sweet, R.M.3 1Unité de Biophysique Structurale, CNRS, Université de Bordeaux, 33405 Talence, France. 2Departments of Structural Chemistry and Chemical Services, The Weizmann Institute of Science, Rehovot, Israel. 3Biology Department, Brookhaven National Laboratory, Upton, New York 11973-50000, USA.

The structure of a monoclinic, $P2_1$, crystal form of bacterioferritin from *E. coli* (cytochrome b1) was solved by molecular replacement and refined using as model the fundamental unit of this protein that consists of two protein subunits and a single haem. The haem is positioned in a special position on the two-fold axis of the dimer. The asymmetric unit of the monoclinic crystal consists of twelve such dimers and corresponds to the molecule of bacterioferritin (MW = 450 kD).

First, the orientation of the molecule has been successfully determined with a standard self-rotation followed by a locked self-rotation, then, the position in the unit cell, has been localized with the program AMoRe (Navaza, 1992). The model used was built from the coordinates of the tetragonal structure of cytochrome b1 (Frolow & al., 1994). Even at the 2.9 Å resolution, the following facts about this structure emerged. Thus it is confirmed that the haem is located at the interface of two subunits with as axial ligands the sulfur atoms of two symmetrically related Met52. Furthermore the presence of a di-metal center is observed in the inside of a four-helix bundle. The residues involved in the metal coordination spheres are four glutamates and two histidines. Both histidines ligands bind to the metals through their δ-nitrogen atoms.

The crystal packing corresponds to a situation halfway between the well known hexagonal closest packing and the orthorhombic all-face centred one.

**PS04.08.10 CRYSTALLOGRAPHIC STUDIES ON THE ICOSAHEDRAL CORE OF THE PYRUVATE DEHYDROGENASE MULTIENZYME COMPLEX FROM *BACILLUS STEAROTHERMOPHILUS*.** Tina Izard[a], Richard N. Perham[b], Arie de Kok[c], Wim G. J. Hol[a]. [a]Howard Hughes Medical Institute, Biomolecular Structure Center & Dept. Of Biological Structure, University of Washington, Box 357742, Seattle WA 98195-7742, USA, [b]Dept. of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK, [c]Dept. of Biochemistry, Agricultural University, Wageningen, The Netherlands

The bacterial and mammalian 2-oxoacid dehydrogenase multienzyme complex families catalyse the oxidative decarboxylation of 2-oxoacids (pyruvate, α–ketoglutarate and branched-chain 2-oxoacids) to produce the corresponding acyl-CoA and NADH. A well known member of the family is pyruvate dehydrogenase (PDH), occurring at the end of the glycolysis and providing the tricarboxylic acid cycle with acetyl-CoA. The architectural design of PDH is composed of a central core enzyme, dihydrolipoamide acetyltransferase (E2) with either octahedral (24-mer) or icosahedral (60-mer) symmetry, depending on the source of the enzyme. E2 binds the two peripheral enzymes, thiamin pyrophosphate (TPP) dependent decarboxylase (E1) and flavoenzyme lipoamide dehydrogenase (E3), leading to a molecular weight ($M_r$) of these systems of 5 to 10 million Da. In mammals and yeast, additional proteins are attached to the complex; the so-called protein X and a specific kinase and phosphatase. Deficiencies or malfunctioning of the complexes lead to severe pathological states such as numerous acidoses which are usually correlated with serious neurological dysfunctions.

The catalytic domain of E2 from *B. stearothermophilus* and *Enterococcus faecalis* PDH have been cloned, expressed in *E. coli* and purified. Of the former, crystals suitable for X-ray diffraction experiments grew within 10 days and diffract to about 4 Å resolution at cryo-temperatures. Here we describe the crystallisation of E2 from *B. stearothermophilus* and its preliminary analysis by X-ray crystallography.

**PS04.08.11 MOLECULAR AGGREGATION OF THE NEUROPHYSINS.** John P. Rose and Bi-Cheng Wang, Department of Biochemistry and Molecular Biology University of Georgia, Athens GA 30602, U.S.A.

Bovine neurophysin II has been crystallized in eight distinct crystal forms containing 1, 2, 3, 4, 6 and 12 molecules per asymmetric unit. The mode of molecular aggregation observed in the crystal structures may be paradigms of how the neurophysin-hormone complexes are packaged in the neurosecretory granules (NSG).

The neurophysins (NP) are a family of disulfide rich proteins responsible for the packaging and transport of the posterior pituitary hormones oxytocin (OT) and vasopressin (VP). Two closely related classes of neurophysins are known, one complexed with VP and the other with OT, this association reflecting the synthesis of each hormone and its associated NP via a common precursor.

During transport, the hormone is cleaved from its neurophysin carrier but remains associated with the protein as a non-covalent complex. The neurophysin-hormone complex is then stored in NSG until release into the blood stream. Within the NSG, the NP-hormone complex concentration can be as high as 1000 mg/ml. Although the mode of NP aggregation within the NSG is unknown, it has been postulated based on the high concentrations observed in the NSG that the complexes exist as dimers, higher aggregates, or even amorphous or crystalline precipitates thus the mode of NP association observed in the crystal structures may serve as a model for neurophysin packaging in the NSG's.

An analysis of the common modes of NP aggregation observed in the crystal structures will be presented.

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