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