We have measured the spin-polarized electron momentum density distributions (magnetic Compton profiles) of UGe_2 using the synchrotron-based magnetic Compton scattering technique. The spin moment of UGe_2 has been determined as -1.15 μ_B at 10 K with an applied magnetic field of 0.5 T. Compared with the saturated magnetization of +1.40 μ_B, we have determined the orbital moment at +2.55 μ_B.

The magnetic Compton profiles are decomposed into partial profiles by fitting with the U-5f atomic profiles with different magnetic quantum number *m*. From the fitted results, we estimated the orbital moment at +2.90 μ_B. It gives a slightly higher value since this estimation does not take account of the partial quenching of the orbital moment due to hybridization.

We also found that the shape of the magnetic Compton profiles depend on temperature, indicating the spin-polarized, ground-state wave-functions vary with temperature.

[1] Saxena S. S. et al., *Nature*, 2000, **406**, 587.

Keywords: spin moment, magnetic Compton scattering, U-5f orbitals

MS50 Enzymes and Allostery

Chairpersons: Alexander Wlodawer, Silvia Onesti

MS50.27.1

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Allostery and Heteroinhibition of Human Thymidylate Synthase

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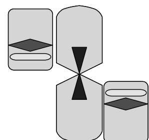
Thymidylate synthase (TS) is a homodimer which shows strong negative cooperativity between subunits. Unique property of human TS (hTS) among TS enzymes it that its active site loop (residues 181-197) can flip 180 degrees producing an inactive conformation [1]. Solution fluorescence studies have shown equilibrium between the active and inactive conformers [2]. We have developed bisphosphonate inhibitors that stabilize the inactive conformation and bind between dimers leading to the formation of hTS tetramers (but not higher oligomers) in solution. These inhibitors show positive cooperativity with antifolate inhibitors used in chemotherapy, which bind only to the active conformer. These data are consistent with a model in which hTS exists preferably as an asymmetric dimer with one subunit in the active conformation of loop 181-197 and the other in the inactive conformation.



Model of hTS homotetramer in which two subunits are connected by bisphosphonate inhibitor stabilizing the inactive conformation and two are inhibited by an antifolate with dUMP.

[1] Schiffer C. A., Clifton I. J., Davisson V. J., Santi D. V., Stroud, R. M., *Biochemistry*, 1995, **34**, 16279. [2] Phan J., Steadman D. J., Koli S., Ding W. C., Minor W., Dunlap R. B., Berger S. H., Lebioda L., *J. Biol. Chem*., 2001, **276**, 14170.

Keywords: chemotherapy, cooperative phenomena, inhibitor and drug design

MS50.27.2

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Structural Basis for Substrate Channelling of a Fatty Acid β-oxidation Multienzyme Complex

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Many enzymes are organized into multienzyme complex to catalyze sequential reactions termed the channelling mechanism. The purpose of our structural study is to elucidate this mechanism at the atomic level, focusing the fatty acid β-oxidation multienzyme complex from *Pseudomonas fragi*. We have determined two distinct crystal structures of the bacterial multienzyme complex that catalyzes the last three sequential reactions in the fatty acid β-oxidation cycle. The $\alpha_2\beta_2$ heterotetrameric structure shows the uneven ring architecture, where all the catalytic centers of 2-enoyl-CoA hydratase (ECH), L-3-hydroxyacyl-CoA dehydrogenase (HACD) and 3-ketoacyl-CoA thiolase (KACT) face a large inner solvent region. The substrate, anchored through the 3'-phosphate ADP moiety, allows the fatty acid tail to pivot from the ECH to HACD active sites, and finally to the KACT active site. Coupling with striking domain rearrangements, the incorporation of the tail into the KACT cavity and the relocation of 3'-phosphate ADP bring the reactive C2-C3 bond to the correct position for cleavage. The α-helical linker specific for the multienzyme contributes to the pivoting center formation and the substrate transfer through its deformation. This channelling mechanism could be applied to other β-oxidation multienzymes, as revealed from the homology model of the human mitochondrial trifunctional enzyme complex.

Keywords: beta-oxidation, multienzyme complex, three-dimensional protein structure

MS50.27.3

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Structural Biology of Cytochromes P450

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The mammalian cytochrome P450 enzymes are a family of membrane-associated haem-containing proteins which play a major role in the metabolism and subsequence clearance of numerous and diverse xenobiotics such as drug molecules. CYP3A4 is the most important member of P450 family, responsible for metabolising 50 % of drugs while CYP2C9 metabolises some 15 % of all marketed therapeutics. Both enzymes exhibit non-Michaelis-Menten kinetics, including homotropic and heterotropic cooperativity; to predict the *in vivo* clearance of drugs and drug-drug interactions, a better understanding of P450 allostery is required.

In the last few years, a number of mammalian P450 structures have been determined, including CYP2C9 [1] and CYP3A4 [2], both in unliganded forms and in complex with marketed drugs. These crystal structures provide insights into the principles of substrate binding for these promiscuous enzymes, and the structural basis of P450 allostery.

[1] Williams P.A., Cosme J., Ward A., Angove H.C., Vinkovic D.M., Jhoti H., *Nature*, 2003,**424(6947)**, 464-8. [2] Williams P.A., Cosme J., Vinkovic D.M., Ward A., Angove H.A., Day P.J., Vonrhein C., Tickle I.J., Jhoti, H., *Science*, 2004, **305(5684)**, 683-686.

Keywords: drug-protein interactions, drug metabolism, metalloproteins

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The Structure of Yeast Phosphofructokinase 1

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Phosphofructokinase 1 (PFK) catalyses the ATP-dependent phosphorylation of fructose 6-phosphate (Fru-6P) to fructose 1,6-bisphosphate, one of the principal regulatory steps in glycolysis.

The structure of 12S PFK from *S.cerevisiae*, a product of limited proteolysis of the native enzyme (known as 21S), has been solved at 2.9 Å resolution in complex with Fru-6P. This is the first crystal structure of eukaryotic PFK and one of the largest protein crystal structures known to date in atomic detail (approx. 600 kDa). We have determined the topology of the enzyme, the active site and the binding site of fructose-2,6-bisphosphate (Fru-2,6-P_2), the allosteric effector specific to eukaryotes. Still unknown is the effector binding site for ATP. A detailed interpretation has been carried out of the electron density map. The refined atomic model contains over 5,000 amino

acid residues and Fru-6P bound in the active site and in the Fru-2,6-P_2 effecttor site We have examined the consequesces on the enzyme structure and function of the two gene-duplication events that occured in the yeast gene compared to the prokatyotic gene.

Keywords: allostery, metabolism, regulation and reaction mechanisms of enzymes

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Molecular Basis for MSD and Catalytic Mechanism of the Human Formylglycine Generating Enzyme

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Sulfatases are enzymes essential for degradation and remodeling of sulfate esters. Formylglycine (FGly), the key catalytic residue in the active site, is unique to sulfatases. In higher eukaryotes, FGly is generated from a cysteine precursor by the FGly generating enzyme (FGE). Inactivity of FGE results in multiple sulfatase deficiency (MSD), a fatal autosomal recessive syndrome. We determined the FGE crystal structure by Ca^{2+}/Sulphur SAD phasing using in-house data collected at a wavelength of 1.54Å. Based on this structure, we report that FGE is a single-domain monomer with a surprising paucity of secondary structure and adopts a unique fold. The effect of all eighteen missense mutations found in MSD patients is explained by the FGE structure, providing a molecular basis of MSD. The catalytic mechanism of FGly generation was elucidated by six high-resolution structures of FGE in different redox environments. The structures allow formulation of a novel oxygenase mechanism whereby FGE utilizes molecular oxygen to generate FGly *via* a cysteine sulfenic acid intermediate [1].

[1] Dierks T., Dickmanns A., Preusser-Kunze A. Schmidt B., Mariappan M., von Figura K., Ficner R., Rudolph M.G., *Cell*, 2005, **121**, *in press.*

Keywords: enzyme catalytic mechanism, post-translational modification, genetic disease

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MS51 COMPLEMENTARY APPROACHES TO BIOLOGICAL STRUCTURE DETERMINATION

Chairpersons: Wah Chiu, Lucia Banci

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Estimating Protein Fold using Wide-angle Solution Scattering Data

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The secondary and tertiary structure motifs of protein folds have characteristic distributions of inter-atomic distances that produce features buried within the x-ray scattering pattern from a protein in solution. We have demonstrated that wide-angle x-ray solution (WAXS) scattering contains rich details of the secondary, tertiary and quaternary structure of multiple classes of proteins. Uses to date include the observation of ligand-induced structural changes and the monitoring of fold stages during chemical and radiation-induced protein denaturation.

WAXS scattering patterns obtained at high flux third generation synchrotron beam lines are not only sensitive to protein conformation states, but the scattering patterns generated can be quantitatively compared to data calculated from detailed structural models derived from crystallographic data. This method can be applied to almost any protein in solution including membrane proteins, large protein complexes and proteins with substantially disordered regions. As such, WAXS has the potential for being a sensitive, global method for detecting ligand-induced structural changes in proteins, narrowly categorizing proteins based on their scattering homology to known folds and elucidating the differences between crystal structures and aqueous conformations.

Keywords: protein structure analysis, WAXS, macromolecular structures

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Cryo-electron Microscopy of the Ribosome: Methods of Fitting, and Inference of Dynamics

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Cryo-electron microscopy yields “three-dimensional snapshots” of the ribosome and its interaction with ligands at time points determined by the use of antibiotics or GTP analogs. If several such snapshots are available for one of the functional processes, how can we obtain a seamless picture of its dynamics? We address the two aspects of this problem: the interpretation of medium-resolution cryo-EM density in terms of published coordinates of structural components, and the means of “interpolating” between subsequent structures inferred from the snapshots. This problem is exemplified by the investigation of the decoding process, for which four 3D snapshots are available [1,2].

[1] Valle M. et al., *Nat. Struct. Biol.*, 2003, **10**, 899. [2] Frank J., Sengupta J., Gao H., Li W., Valle M., Zavialov A., Ehrenberg M., *FEBS Lett.*, 2005, **579**, 959.

Keywords: real-space refinement, MD simulation, decoding

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Structure of the Acrosomal Bundle, a Biological Machine, at 9.5 Å Resolution

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In the unactivated Limulus sperm, a 60 μm-long bundle of actin filaments crosslinked by scruin is bent and twisted into a coil around the base of the nucleus. At fertilization the bundle uncoils and fully extends in five seconds to support a finger of membrane, the acrosomal process. This biological spring is powered by stored elastic energy and does not require the action of motor proteins or actin polymerization. Our 9.5 Å electron cryomicroscopic structure of the extended bundle [1] shows that twist, tilt, and rotation of actin-scruin subunits deviate widely from a "standard" F-actin filament. This deviation appears to be related to the packing requirements of the scruin cross-linkers. The structural organization allows filaments to pack into a highly ordered and rigid bundle in the extended state, but also suggests a mechanism for storing and releasing energy between the coiled and extended states.

[1] Schmid M.F., Sherman M.B., Matsudaira P., Chiu W., *Nature*, 2004, **431**, 104.

Keywords: actin, electron microscopy, macromolecular crystallography protein structures

MS51.27.4
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Beyond the Structure: how to Deal with Structural Disorder

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Structural disorder in proteins is probably as precious as a source of information as the structure itself. In fact, disorder implies mobility. If we are able to translate what is observed as disorder, both in X-ray and NMR structures, into dynamics, we are in a better position to understand function. NMR is a powerful tool to analyse mobility in terms of time scales of motions, from seconds down to picoseconds.

Novel approaches on how to deal with disorder by NMR will be shown, with particular reference to metalloproteins. Examples will range from the study of conformational flexibility at the active site of