Structural models are auto-build and structures are refined, verified and analyzed using semi-automated computational tools. Functional analysis is being performed using a newly developed ProFunc server. 3D models of relevant members of the sequence family are generated and their quality is assessed. Majority of the steps in the MCSG pipeline are tracked in near real time by the database. All the structures and their analysis are made available to the public using the MCSG database and web tools. The MCSG structure determination pipeline when combined with data collection facilities at third generation synchrotrons, advanced software and computing resources resulted in significant acceleration of structure determination of novel proteins. Using this pipeline the MCSG has determined 112 novel structures in 2004.

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Keywords: high-throughput, structural genomics, structure determination

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Structural Insights into GnT-I Substrate Recognition & Specificity

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β-1,2-N-acetylglucosaminyltransferase I (GnT I; EC 2.4.1.101) is a key carbohydrate processing enzyme in the Golgi – it initiates the conversion of oligomannosyl to complex and hybrid N-linked glycans. GnT I, an inverting glycosyltransferase, catalyzes the addition of GlcNAc to the terminal 3-arm mannose of Man_5Gn_2 glycans found on glycoproteins in the secretory pathway.

We previously determined the x-ray crystal structure of the nucleotide-bound complex of GnT I in the presence of the native UDP-GlcNAc donor [1]. We now present a series of high-resolution donor analog complexes that provide insight into donor recognition and underscore the importance of the C2 position in catalysis.

We also report the structure of a ternary complex of both donor and acceptor substrates bound in the active site, which reveals how GnT I confers acceptor binding specificity by means of multiple subsites on the oligosaccharide.

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Keywords: glycosyltransferases, enzyme ligand complexes, active-site recognition

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On the Incompleteness of Atomic Models

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When working with incomplete models at a conventional resolution of about 2-3 Å, one may suppose for simplicity that unknown atoms may be found with the given probability at any point of the unit cell. Then the contribution of these atoms to structure factors can be taken into account statistically. This allows a proper modification of target values and a choice of the weighting scheme to be done [1].

When working at a resolution of 1 Å or higher, the model incompleteness changes its aspect. The conventional model of spherical atoms cannot describe the density deformation caused by interatomic interactions, and this discrepancy is significant for high-resolution structure factors. While all atoms are well defined at such a resolution, refinement without special precaution gives too large values for ADPs in order to 'cover' the deformation density missed in the model. At the same time, an introduction of multipolar models [2] may lead to overfitting the data. An intermediate model of dummy bond electrons was shown [3] to be capable to reduce the distortion of the ADPs even when the model is refined at the resolution of order of 0.9 Å that is not yet adequate for refinement of multipolar models.

[1] Lunin V.Y., Afonine P., Urzhumtsev A., *Acta Cryst*, 2002, **A58**, 270. [2] Hansen N., Coppens P., *Acta Cryst*, 1978, **A34**, 909. [3] Afonine P.V., Lunin V.Y., Muzet N., Urzhumtsev A., *Acta Cryst*, 2004, **D60**, 260.

Keywords: modelling of proteins, statistical methods, high resolution

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Ultra-high Resolution Measurement at BL41XU of SPring-8

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A proton and an electron play an important role to chemical reaction through biomacromolecules. In order to elucidate the reaction by X-ray crystallography, it is essential to make clear the structure in atomic resolution below 0.7 Å. The measurement in such resolution is achieved by not only improving the sample crystal but also optimizing the equipment of the beamline. BL41XU at SPring-8 with the high brilliance is the most suitable for such data collection.

(< 0.6 Å).

Example data collection was performed at 100 K, using the crystal of Endopolygalacturonase I. The X-ray was set at 0.6 Å, and R-AXIS V (Rigaku) was used as the detector. To acquire the complete data set, two data, in which the camera length was different, were collected. Consequently, diffraction spots were visible to a resolution of 0.62 Å. Intensity data were integrated and scaled, and $R_{\text{-sym}}$ of overall and outer shell (0.70-0.68 Å) were 3.2 and 30.0 %, respectively. As a result of structural refinement using SHELXL, R and R_{free} value of the obtained structure, in which 3,416 non-hydrogen atoms and 2,181 hydrogen atoms are included, is 9.72 and 10.78 %, respectively. Some other data are under analysis.

Keywords: very high resolution structure, crystallography instrumentation, synchrotron radiation

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A Challenge to Bonding Nature Study in Protein Crystallography

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Visualizing bonding electrons between atoms which construct a protein molecular, and precise positions of hydrogens in a protein leads us to the next stage of structural analysis of protein, such as precise chemistry and physics. The maximum entropy method is the most suitable tool to achieve this aim. This method enables us to visualize the least-biased electron distribution by refining the observed structure factors and phase by maximizing the information entropy of electron distribution which is not based on any chemical assumptions. As a result, the reliability factor of the obtained charge density becomes extremely low, for instance, R=2.4% for cytochrome *c*-553. Then, there are obvious differences between MEM charge density and the conventional Fourier map especially in the weak electron density region where the bonding electrons and the hydrogens exist. The obtained charge density clearly exhibits the characteristic anisotropy of the Fe-N coordinate bonds in the heme group and hydrogen charge density which can not be observed in the conventional method. Some other results for peroxidase, P450cam and P450nor will be also presented.

Keywords: protein crystallography, maximum entropy method, bonding electron

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Opening of the Safety-belt Loop of Human Aldose Reductase

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Aldose reductase (ALR2; EC 1.1.1.21) is a member of the aldo-keto reductase superfamily and it catalyzes the NADPH-dependent reduction of aldeydes to their corresponding alcohols. It is implicated in the polyol pathway and in diabetic complication.

The crystal structure of native aldose reductase has been determined to a resolution of 0.82 Å with a final R = 9.50 and R_{free} = 10.90 and a mean coordinate error for the fully occupied sites of the protein of 0.011 Å (from fully matrix inversion). The structure contains a large number of multiple conformations: 78 out of 316 residues were modeled in two conformations.

The overall structure folds into an eight-stranded α/β barrel with the active site located at the C-terminal end of the barrel and the $NADP^+$-binding site near the hydrophobic binding pocket [1]. The cofactor is held in place by the so-called 'safety-belt' (a loop between residue 216 and 227 of the canonical α/β barrel) [2].

The active site of the structure contains a citrate molecule in two conformations. One of the conformations stabilizes the closed position of the safety-belt, whereby the other permits the safety-belt to open. Due to the high resolution, the partially opened conformation of the safety-belt can be observed in the electron density.

[1] El-Kabbani O., Wilson D., Petrash J. M., Quiocho F. A., *Molecular Vision*, 1998, 4. [2] Wilson D.W., Bohren K.M., Gabbay K.H and Quiocho F.A., *Science*, 1992, **257**, 81.

Keywords: high-resolution refinement, active-site structure, loop modeling

P.04.23.6

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Crystal Structure of *Caenorhabditis elegans* Spermidine Synthase: in Preparation

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Caenorhabditis elegans (C.elegans) is a free living worm and a well established model organism to study general biological processes like development.

Consistent with reports on other organisms, growth of *C. Elegans* depends on polyamines. Polyamines such as putrescine, spermidine and spermineare aliphatic polycations, essential for regulation of cell proliferation and differentiation. Spermidine synthase isoneofthekey enzymes in the polyamine biosynthetic pathway.

This enzyme catalyzes the transfer of the aminopropyl group from decarboxylated S-adenosylmethionine to putrescine in the biosynthesis of spermidine.

Spermidine synthase from *C. Elegans* has been over-expressed in Escherichiacoli, purified by affinity chromatography and co-crystallized with putrescine, which is the substrate. The crystals diffract to 2.5Å and belong to the monoclinic P21 space group with unit cell dimensions, a=59.99, b=99.23, c=67.85Å and β=107.2°. The asymmetric unit contains two molecules. Model building and refinement are ongoing.

Keywords: *Caenorhabditis elegans*, polyamines, spermidine synthase

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Structure of *Ralstonia solanacearum* Fucose Binding Lectin at 0.94Å Resolution

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Ralstonia solanacearum is a soil-born bacterium belonging to the group of beta-proteobacteria. It is responsible for bacterial wilts in over 200 plants including potato, tomato and banana, and is capable of living for prolonged periods in soil, infecting hosts via the roots.

A 9.9 kDa fucose-binding lectin (RSL) has been found in *R. Solanacearum* extract. Ultra-high resolution diffraction data to 0.94Å data were collected from crystals of the recombinant RSL: α-methyl-fucose complex at ESRF, Grenoble. Superb phasing was obtained using the RSL:seleno-methyl fucoside complex, showing the crystals to contain three monomers, each of two 4-stranded β-sheets, with two sugar sites per monomer. The three monomers associate to form a 6-bladed β-propeller; the first time such an arrangement has been observed. ITC microcalorimetry and surface plasmon resonance studies are underway to define the fine specificity to fucosylated oligosaccharides present in plant cell walls, that may be the target for the lectin in soil.

Keywords: lectin crystallography, atomic resolution crystallography, synchrotron radiation crystallography

P.04.23.8

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Crystal Structures of Ribosomal Protein L10 in Complex with L7/12 N-Terminal Domains

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The L7/12 stalk of the large ribosomal subunit comprises protein L10 and multiple copies of L7/12. It is involved in binding of translation factors and stimulation of factor-dependent GTP hydrolysis. The stalk is disordered in available crystal structures of ribosomes or 50S subunits. We have determined crystal structures of *Thermotoga maritima* L10 in complex with three L7/12 N-terminal domain (NTD) dimers. The structures are in agreement with a multitude of biochemical data. A globular NTD of L10 encompasses the binding region for 23S rRNA. A long C-terminal helix (α8) of L10 shows a modular design with consecutive binding sites for L7/12 dimers. L10 helix α8 assumes different positions with respect to the NTD in different crystal forms and thus constitutes a mobile platform for the attached L7/12 molecules. The number of L7/12 dimers varies with the length of L10 helix α8 in different species. The structure of the L7/12 NTD dimers agrees with one mode of dimerization observed in isolated L7/12. The hinge region of L7/12 can bind in α-helical form to the NTD in isolation but is displaced by L10 upon complex formation and becomes disordered. The organization of the complex supports its function in factor recruitment and GTPase activation.

Keywords: L10-L7/12 complex, L7/12 stalk, ribosome structure

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Comparative Study of Thrombin Binding of Potassium vs. Sodium

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Thrombin, a critical serine protease responsible for blood coagulation, is an allosteric enzyme that binds an alkali metal cation near the substrate-binding site. We have obtained a crystal structure to a resolution of 1.9 Å for the potassium-bound form of thrombin and compared it to the existing structure of sodium-bound thrombin.[1]