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.

This work was supported by National Institutes of Health Grant GM62414 and by the U.S. Department of Energy, Office of Biological and Environmental Research, under contract W-31-109-Eng-38.

Keywords: high-throughput, structural genomics, structure determination

P.04.23.1

Acta Cryst. (2005). A**61**, C260

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

P.04.23.2

Acta Cryst. (2005). A**61**, C260

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.

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Keywords: modelling of proteins, statistical methods, high resolution

P.04.23.3

Acta Cryst. (2005). A**61**, C260

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

P.04.23.4

Acta Cryst. (2005). A**61**, C260

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