MS07 P01

Autodep 4.1 : A web-based deposition and archival system S. Sen, G.Van Ginkel, A.Kapopoulou, G. Sahni, G.J. Swaminathan, R.H.Newman, S.Velankar, K.Henrick
Macromolecular Structural Database, European Bioinformatics Institure, Hinxton, Cambridge CB10 1SD. United Kingdom. Email: ssen@ebi.ac.uk

Keywords: protein database, computer software, structural biology

The Macromolecular Structure Database (MSD) (http://www.ebi.ac.uk/msd/) [1] group is one of the four partners in the worldwide Protein Data Bank (wwPDB) [2], the consortium entrusted with the collation, maintenance and distribution of the global repository of macromolecular structure data. Structures can be deposited at the MSD using the AutoDep [3] deposition tool or with the other wwPDB partners using the ADIT system. The AutoDep 4.1 is an extension of the previously developed software AutoDep 4.0 [4], [5] which was a complete rewrite of the original AutoDep system. The AutoDep system allows value-added information to be returned in a safe and secure manner into the password-protected deposition session only accessible to the depositor, following annotation of the structure by curation staff, within 2 days of deposition. AutoDep 4.1 is also available for download and installation in-house, where a deposition can be completed and validated before uploading the whole deposition session to the MSD site, where submission to the wwPDB can be completed in minutes. The extended version of AutoDep (4.1) provides detailed information regarding the ligand binding sites, Uniprot sequence mapping and taxonomy information, applets for viewing the files, in addition to structure factor validation statistics, quaternary structure assessments, and new ligand dictionaries. With structures being determined at an ever increasing rate, it is imperative that deposition tools keep pace with this exponential growth of data. We believe that the latest release of AutoDep has significantly automated the deposition process, reduced the time taken to deposit a structure, and at the same time harnessed services offered by the MSD group in returning useful information to the depositor.

[1] H.Boutselakis et al., *Nucleic acid Res*., 2003, 31, D458.
[2] H.Berman et al., *Nature Struct. Biol*., 2003, 10, 980.
[3] D. Lin et al., *Acta Crystallogr*., 2000, D56, 828.
[4] M.Tagari et al., *Nuclei Acids Res*., 2006, 34, D287.
[5] G.J.Swaminathan et al., *Acta Crystallogr.* 2006, A62, S15.

MS07 P02

High resolution X-ray structure of Triclinic Hen Egg-White Lysozyme at 0.83Å Akifumi Higashiura[a], Hiroaki Tanaka[b], Koji Inaka[d], Masaru Sato[c], Shigeru Sugiyama[d], Sachiko Takahashi[b], Mari Yamanaka[b], Moritoshi Motohara[c], Satoshi Sano[c], Tomoyuki Kobayashi[c], Mamoru Suzuki[a], Tetsuo Tanaka[c] and Atsushi Nakagawa[a], [a]*Institute for Protein Research, Osaka University, Osaka, Japan,* [b]*Confocal Science Inc., Tokyo, Japan,* [c]*Japan Aerospace Exploration Agency, Ibaraki, Japan,* [d]*Maruwa Food and Biosciences, Inc., Nara, Japan.*
E-mail: hgsur-a@protein.osaka-u.ac.jp

Keywords: high resolution X-ray crystallography, data treatment, refinement method

Recent progress on using high brilliance and small divergence synchrotron beamlines, X-ray data collection at low temperature and technical advances in crystallographic analysis have significantly improved the resolution on X-ray crystallography. In this study hen egg-white lysozyme was used as a model protein for high resolution X-ray crystallography. High resolution crystals were obtained from highly purified hen egg-white Lysozyme. Data collections were performed using synchrotron radiation from SPring-8 beamline BL44XU and Photon Factory beamline BL5A. Two crystals were used to increase completeness and two data sets were collected for high and low resolution data to avoid the saturation of high intensity diffraction for each crystals. The high resolution diffraction data were observed up to 0.75Å. The data were integrated, scaled and merged using the *DENZO* and SCALEPACK programs. At the merging process low resolution dataset were carefully truncated. Hen egg-white lysozyme crystals belong to space group *P*1 with the cell dimensions a=26.7, b=31.0, c=33.7Å, α=89.2, β=72.5 and γ=67.7 degree. The overall R_{merge} based on intensities for all data was 5.4% with its completeness of 92.0% against data to 0.76Å. Refinement was carried out by *SHELX* programs. The refinement were proceeding against data to 0.83Å. An *R*-factor and *free-R*-factor was 11.0% and 12.6%, respectively. Hydrogen atoms were added to the model at predicted positions, lowering the *R*-factor and *free-R*-factor by approximately 1.0%. The final model consists of 1,305 atoms (without hydrogen atoms) and 163 water molecules. 24 side chains and 1 main chain were modeled in two conformations. *B* factors were 7.4, 10.1 and 19.6Å for main chain, side chain and water molecules. At the refinement process using loose restraints gave different distribution of bond length and angles. These results implied the necessity to improve refinement method of high resolution X-ray crystallography.

MS07 P03

The crystal structures of sphericase at 0.80Å resolution - insights into the catalysis mechanism of subtilisins Orna Almog[a], Marina de Leeuw[a] Ana González[b]
[a]*Department of Clinical Biochemistry, Faculty of Health Sciences, Ben-Gurion University, Beer Sheva, 84105 , Israel.* [b]*Stanford Synchrotron Radiation Laboratory, 2575 Sand Hill Road, MS 99, Menlo Park, CA 94025 , USA.* E-mail: almogo@bgu.ac.il

Keywords: serine protease, psychrophilic, calcium binding

We previously determined the crystal structure of Sph, a mesophilic subtilisin, at 0.93 Å resolution using the Eu/MAD method. The final model included all 310 amino acids, one disulfide bond, 679 water molecules and five calcium ions. None of the calcium binding sites correlated with the well-known high affinity calcium-binding site (site I; also known also as Site A) of the subtilisin family. In this study, we determine the crystal structure of Sph at a higher resolution and in a different crystal form using the molecular replacement method and the above-mentioned Sph structure as the starting model. Compared to the 0.93 Å model of Sph, there was further improvement in the details of the electron density map, which enabled us to locate a hydrogen atom in the catalytic site and a borate anion bound to the active site residue Ser250. Structural analysis indicated a structure identical to S41, a

psychrophilic (cold-adapted) subtilisin which shares high sequence identity (60%) with Sph, as well as the same calcium binding mode. We suggest that the five calcium ions found in these two subtilisins act as counter ions for the acidic residues found on their surface. Since Sph is a mesophilic subtilisin and S41 is a psychrophilic subtilisin and their structure is identical, we conclude that Sph and S41 most probably have the same origin and are evolutionally related.

MS07 P04

New insights for the catalytic and inhibition mechanisms of periplasmic Nitrate reductase (Nap) from *Desulfovibrio desulfuricans* from structural and spectroscopic análisis Shabir Najmudin[a], Pablo J. González[a], Catarina Coelho[a], José Trincão[a], Isabel Moura[a], José J. G. Moura[a], Carlos D. Brondino[b]* and Maria J. Romão[a] [a]*REQUIMTE/CQFB, Departamento de Química, FCT-UNL, 2829-516 Monte de Caparica, Portugal.* [b]*Departamento de Física, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, 3000 Santa Fe, Argentina.*
E-mail: shabir@dq.fct.unl.pt

Keywords: periplasmic nitrate reductase, Mo-sulfido ligand, novel catalytic mechanism

The periplasmic nitrate reductase from *Desulfovibrio desulfuricans* ATCC 27774 was isolated from cells grown in anaerobiosis and in the presence of nitrate. This enzyme is isolated as a soluble monomeric protein of 80 kDa having a Mo atom in the active site, coordinated to a *bis*-MGD moiety [1]. Previous EPR studies in both catalytic and inhibiting conditions showed that this Mo ion has a high flexibility of coordination when reacted with reducing agents, substrates or inhibitors. In the present work, samples both in as-purified conditions and reacted with substrates and inhibitors were crystallized and the corresponding structures were solved from 1.99 to 2.45 Å resolution. These results together with a single-wavelength anomalous dispersion experiment at above the iron edge (λ= 1.77 Å) of the as-purified (native) enzyme strongly suggests the presence of a sulfur atom instead of oxygen at the sixth position of coordination of the Mo ion. Analysis of the crystallographic data of the reacted samples of Nap indicates that neither azide nor cyanide binds to the Mo atom, and that perchlorate blocks the funnel-like cavity, hindering the substrate entrance and product release. In addition, EPR studies indicate that the turnover Mo(V) signal is not produced by a Mo-substrate. Based on these results, new hypotheses for the catalytic mechanism are proposed.

[1] Dias J.M., Than M. E., Humm A., Huber R., Bourenkov G. P., Bartunik H. D., Bursakov S., Moura J. J.G., Moura I.,Romão M. J. *Structure*,1999, **7**, 65-79..

MS07 P05

PURY: The database of geometric restraints of hetero compounds Miha Andrejašič[a], Dušan Turk[a] , [a]*Jožef Stefan Institute, Ljubljana, Slovenija.*
E-mail: miha.andrejasic@ijs.si http://pury.ijs.si/

Keywords: macromolecular structure refinement, structure determination using X-ray diffraction; topology

The paper of Engh and Huber (1991) [1] with description of accurate geometrical parameters of amino acid residues has set a new standard in macromolecular crystallography. A similar step forward in the area of nucleic acids was made by Parkinson et al. (1996) [2]. With the increasing number of macromolecular structures also complexes with "hetero" ligands and their variety is growing. Obtaining correct geometry of the hetero ligands is often crucial for understanding of biological relevance of a structure. The correctness of conclusions may however be hampered by the error contained in parameters describing geometry of a compound.
Therefore we have developed a "PURY" database, which contains lists of atom classes, bonds connecting them as well as angle and chirality, planarity and conformation parameters. The three last in the forms of improper and dihedral angles. Each entry in the list has associated target value and a force constant derived from the standard deviation of the list entry. The database is compiled from close to 162540 entries present in the latest release of small molecule crystal structures deposited in Cambridge Crystal Structure Database [3]. PURY database contains about 1946 atom classes, and lists of 28882 bonds, 223059 bonding angles and 235764 dihedral and 62437 improper angle terms.
Direct comparison with Engh Huber parameter set revealed that PURY parameters essentially correspond to EH target values in spite the fact that they have not been generated on a an expert selected list of entries with a much higher number of repetitions. Coordinative bonds with metal ions are included too. The database and the server allow generating parameters also for hydrogen atoms, although these parameters have much larger standard deviations due lower precision of their positions and lack of proper atom class assignment, which does not yet include hydrogen bond analysis.
The database can be used through web server "http://pury.ijs.si/", where from a deposited coordinates in a PDB format, topology and parameter files in forms for refinement programs MAIN, CNS and RefMac are generated. SHELEX output is in progress too. The server will in near future provide topology and geometry parameter files for all currently deposited hetero compounds in Protein Structure Database.
Basics of the server use as well as analysis of accuracy, reliability of the derived terms will be presented and demonstrated.

[1] Engh, R.A. and Huber, R. (1991): Accurate bond and angle parameters for X-ray protein structure refinement. Acta. Cryst. A47, 392-400.
[2] G. Parkinson, J. Vojtechovsky, L. Clowney, A.T. Brunger, H.M. Berman: New Parameters for the Refinement of Nucleic Acid Containing Structures, Acta Cryst. D, 52, 57-64 (1996).
[3] Allen, F.H., Bellard, S., Brice, M.D., Cartwright, B.A., Doubleday, A., Higgs, H., Hummelink, T., Hummelink-Peters, B.G., Kennard, O., Motherwell, W.D.S., et al (1979): The Cambridge Crystallographic Data Centre: computer-based search, retrival, analysis and display of information. Acta Cryst. **B35**, 2331-2339.