P.02.01.1
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Ab-initio* Structure Determination of SMU.440 Protein from *Streptococcus mutans
Jie Nan, Xiao-Yan Zhang, Xiang-Yu Liu, Yu-He Liang, Xiao-Dong Su, *Department of Biochemistry and Molecular Biology, College of Life Sciences, Peking University, Beijing 100871, China*. E-mail: su-xd@pku.edu.cn

SMU.440 is a 138 residue hypothetical protein from *Streptococcus mutans*, a primary pathogen for human dental caries. It's a function unknown protein with few sequence homologues. In this work, SMU.440 protein was expressed, purified and crystallized. Two sets of diffraction data were collected, including a native dataset to 2.4 Å resolution with satisfying statistics (Rsym= 3.8%), and Hg-derivative dataset to 2.4 Å resolution but with a high mosaicity of 1.9 and low completeness. The crystals belong to spacegroup $P2_12_12$ and there are 2 molecules per asymmetric unit (AU). The structure was determined by SIRAS method. Self rotation function showed a 2-fold NCS in the AU, but only one heavy atom site could be found per molecule, RESOLVE could only trace a partial (about 40%) structure and gave a poor density map. An initial NCS matrix was found using the lsq_etc. function of the O program. The partial structure combined with NCS information was input to Arp/Warp and Resolve for iterative model building and manually adjustment. Finally, a 130 residue model for structure refinement was obtained.
Keywords: SMU.440, ab-initio structure determination, iterative model building

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X-ray Structure Determination of Hydroxyphenylpyruvate Reductase at 1.47 Å Resolution
Andreas Heine[a], Verena Janiak[b], Maike Petersen[b], Gerhard Klebe[a], [a]*Department of Pharmaceutical Chemistry and* [b]*Pharmaceutical Biology, Philipps-University Marburg, Marburg, Germany.* E-mail: heinea@mailer.uni-marburg.de

Hydroxyphenylpyruvate reductase (HPPR) is involved in the biosynthesis of rosmarinic acid in plants. HPPR was identified, purified and cloned from suspension cultures of *Coleus blumei* [1] and subsequently expressed from E. *coli* and purified for crystallization. HPPR belongs to the family of D-isomer specific 2-hydroxyacid dehydrogenases and catalyzes the NAD(P)H dependent reduction of hydroxyphenylpyruvates to the corresponding lactates. HPPR shows only low sequence identity of about 30 % compared to other proteins from this enzyme family.

Suitable crystals of HPPR for X-ray diffraction were obtained from 30% MPD, 0.2 M NaCl, pH 7.5 and diffracted to 1.47 Å resolution at the Bessy synchrotron. The structure was determined by exhaustive molecular replacement methods. A potential solution obtained with the program PHASER resulted in reasonable electron density for 30% of the molecule. Iterative cycles of automated model building with ARP/wARP resulted in a virtually complete model. The obtained protein structure shows a high structural similarity to other oxidoreductases.

[1] Kim K.H., Janiak V.; Petersen M. *Plant Mol. Biol.*, 2004, **54**, 311-323.
Keywords: hydroxyphenylpyruvate reductase, biosynthesis, protein structure determination

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SIR2004: New Features for *ab-initio* Crystal Structure Solution
Benedetta Carrozzini[a], Maria C. Burla[b], Rocco Caliandro[a], Mercedes Camalli[c], Giovanni L. Cascarano[a], Liberato De Caro[a], Carmelo Giacovazzo[a], Giampiero Polidori[b], Riccardo Spagna[c], [a]*IC-CNR, Bari, Italy.* [b]*Dipartimento di Scienze della Terra, Perugia University.* [c]*IC-CNR, Rome Section, Italy.* E-mail: benedetta.carrozzini@ic.cnr.it

SIR2004 [1], the evolution of the SIR2002 program [2], is devoted to the *ab initio* solution of crystal structures by direct methods. Several new features implemented in SIR2004 make this program more efficient: it is able to solve both small/medium size structures as well as macromolecules (up to 2000 atoms in the asymmetric unit at atomic resolution data). The new algorithms succeed also in solving several protein structures with data resolution up to 1.4-1.5 Å, providing interpretable electron density maps.

According to circumstances, the SIR2004 phasing process may apply tangent procedures and/or Patterson methods. The new phasing strategy is also based on: a) an optimal use of the figures of merit, one of which may be successfully applied in the early stages of the phasing process; b) the use of the protein envelope in the direct space refinement.

A powerful graphic interface makes friendly the user interaction with the program. SIR2004 can run on any PC or WorkStation (Operating systems: Windows 9x/2000/Me/NT/XP; Linux, Unix).

[1] Burla M.C., CaliandroR., Camalli M., Carrozzini B., Cascarano G.L., De Caro L., Giacovazzo C., Polidori G., Spagna R., *J. Appl. Cryst*., 2005, **38**. [2] Burla M.C., Camalli M., Carrozzini B., Cascarano G.L., Giacovazzo C., Polidori G., Spagna R., *J. Appl. Cryst*., 2003, **36**, 1103.
Keywords: computer programs, *ab-initio* structure determination, macromolecular crystallography

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High Throughput Technique in Structural Bioinformatics
S. Selvanayagam[a], D. Velmurugan[a], T. Yamane[b], [a]*Department of Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai – 600 025.* [b]*Department of Biotechnology and Biomaterial Science, Graduate School of Engineering, Nagoya University, Furo-Cho, Chikusa-Ku, Nagoya 464-8603, Japan.* E-mail: s_selvanayagam@rediffmail.com

As the available macromolecular sequences exceed far in number than the available three-dimensional structures, high throughput techniques are necessary to unravel the 3D-structures of selected macromolecular sequences in the area of Structural Genomics. ACORN program deposited in CCP4 is a comprehensive and efficient phasing procedure for the determination of protein structures when atomic resolution data are available. The structure solution program SHELXD is useful for locating the anomalous scatterers from SIR, SAS, SIRAS or MAD data. SHELXE estimates the native phases and the corresponding weights from SHELXD output. The phases obtained from ACORN and SHELXE are of superb quality to allow automated model building to be carried out in ARP/wARP. Minimal manual model building is required and the structure determination can be completed using maximum likelihood refinement program REFMAC. Attempts are here made in extending the applications to the structure elucidation of Catalase of approximately 57 kDa molecular weight using atomic resolution data (for *ab initio* phasing using ACORN) and Thermolysin of approximately 34 kDa molecular weight using 1.7 Å anomalous scattering data. Detailed presentation will be made on the various options in these in High Throughput structure determination of macromolecules.
Keywords: *ab-initio* structure determination, macromolecular crystallography, SAS

P.02.02.3
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SAD Phasing at the Presence of Pseudo-translational Symmetry
Yao He[a], Zhengjiong Lin[a,b], Yuanxin Gu[a], Haifu Fan[a], [a]*Institute of Physics, Chinese Academy of Sciences, Beijing 100080, China.* [b]*Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.* E-mail: heyao@cryst.iphy.ac.cn

Pseudo-translational symmetry results in some reflections group(s) having systematically weak intensities. In the presence of pseudo-translational symmetry, the heavy-atom (anomalous-scatterer) substructure determined by conventional methods from the Bijvoet differences will not be the actual substructure but rather the averaged or approximately, the basic substructure, which will have no or very weak contribution to the systematically weak reflections. SAD phasing based on such averaged heavy-atom substructures will cause

abnormally large errors in the phase of systematically weak reflections. To avoid this, special treatment is needed. Direct methods have been developed to solve the phase problem for small structures having pseudo-translational symmetry. The method can be used to obtain the actual heavy-atom substructures from the Bijvoet differences in the presence of pseudo-translational symmetry. Various phasing procedures have been tested and compared using a set of artificial protein SAD data.

Keywords: SAD phasing, pseudo symmetry, proteins

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Automated Web- and Grid-Based Protein Phasing with *BnP*

Charles M. Weeks[a], Naimesh Shah[a], Mark L. Green[b], Russ Miller[a,b], William Furey[c], *[a]Hauptman-Woodward Institute, Buffalo, NY. [b]Center for Comp. Res., SUNY at Buffalo. [c]VA Med. Center, Pittsburgh PA & Dept. Pharmacol., U. Pittsburgh*. E-mail: weeks@hwi.buffalo.edu

BnP is a protein structure-determination package that couples the direct-methods program *SnB*, used to locate heavy-atom substructures, with parts of the protein-phasing suite *PHASES* [1]. Thus, *BnP* provides an automated pathway from intensity data to an unambiguous protein electron-density map. In large or difficult cases, substructure determination can be a bottleneck. However, the *Shake-and-Bake* algorithm that is used to phase substructures can be readily adapted to a parallel computing environment and throughput increased in direct proportion to the number of available nodes.

Versions of *BnP* with a Java interface are currently available from http://www.hwi.buffalo.edu/BnP/. In addition, a new interface has been developed in PHP, a general-purpose scripting language that is especially suited for web development and allows users to run *BnP* from a browser displaying dynamically created web pages. It supports remote computation and has the capability of distributing multiple parallel jobs over a computational grid. The PHP version has been implemented on a stable prototype grid that was developed at SUNY Buffalo's Center for Computational Research and includes hardware at several different locations. An elegant backfill facility provides access to idle CPU time on many machines and makes it available for *BnP* calculations without disturbing other jobs. This work was supported by NIH grant EB002057 & NSF ACI-0204918.

[1] Weeks C. M., *et. al*., *Z. Kristallogr*., 2002, **217**, 686-693.

Keywords: shake-and-bake, parallel computing, automation

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A Deterministic Algorithm for Phasing Using Triplet and Quartet Invariants

Alexander B. Smith, Nick Sahinidis, *Chemical and Biomolecular Engineering, University of Illinois. Urbana, IL.* E-mail: absmith2@uiuc.edu

Traditional approaches to the crystallographic phase problem minimize merit functions of structural geometry to determine the missing phases [1]. To accurately model the diffraction physics, these merit functions are highly nonlinear and multimodal. As a result, phasing requires the solution of challenging global optimization problems. Trial-and-error, in combination with local search, has been used extensively to solve these optimization problems but is a tedious and difficult process, even for small molecules.

For centric structures, the phase problem has recently been approached via combinatorial optimization techniques that are guaranteed to find a global optimum of a minimal principle formulation of the phase problem [2]. This methodology leaves no ambiguity regarding the correctness of the phases thus derived.

We study how the addition of quartet invariants to the phasing model affects the resolution limits of the previous work [2], which only included triplet invariants. Phasing is accomplished with a polynomial-time binary Gaussian elimination algorithm. For a collection of structures, our methodology leads to considerably improved solutions at lower resolutions.

[1] Debaerdemaeker T., Woolfson M. M., *Acta Crystallographica A*, 1983, **39**,193-196. [2] Vaia A., Sahinidis N. V., *Acta Crystallographica A*, 2003, **59**(5), 452-458.

Keywords: direct methods, low-resolution phasing, optimization

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Neutron Structure Determination via Macromolecular H/D Derivatives

David A. Langs, Herbert A. Hauptman, Hongliang Xu, *Hauptman-Woodward Medical Research Institute, Inc., 700 Ellicott St., Buffalo, NY 14203 USA*. E-mail: langs@hwi.buffalo.edu

The principle of H_2O/D_2O solvent variation (Schoenborn, 1976) in neutron diffraction has long been used as a tool for structural phasing. The first crystal structure application of this procedure gave a 5Å map for the peptide antibiotic gramicidin A that was originally crystallized from ethanol (Koeppe & Schoenborn, 1984). A gramicidin derivative was synthesized for which the two methyl groups of Val^1 had been deuterated, to be contrasted with the native wild-type hydrogenated structure. Unfortunately crystals of sufficient size could not be obtained to help extend the initial 5Å model to the 2.5Å limit of the native data.

A problem arises when multiple H/D replacement sites are covalently bound to the same atom, in that these atoms will be only 1.7Å apart: the substructure can not be easily determined by conventional ΔE direct methods unless data are measured to better than 1.2Å. This is highly unlikely due to the weak flux rates at most neutron scattering facilities.

We have devised a new structure determination method for such H/D derivative applications which allows one to obtain the macromolecular phases directly without first having to solve the substructure, such that lower resolution neutron data sets can be successfully utilized. Support from NIH grant EB002057 is gratefully acknowledged.

[1] Schoenborn B. P., *Biochim. Biophys. Acta*, 1976, **457**, 41-55. [2] Koeppe R. E. , Schoenborn B. P., *Biophys. J*., 1984, **45**, 503-507.

Keywords: neutron diffraction, direct methods, macromolecular phase determination

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A Modified ACORN to Solve Protein Structures at Resolutions of 1.7 Å or Better

Jiaxing Yao[a], Michael Woolfson[b], Keith Wilson[a], Eleanor Dodson[a], *[a]Department of Chemistry, University of York, York,UK.* [b]*Department of Physics, University of York, York,UK.* E-mail: yao@ysbl.york.ac.uk

The first development of ACORN provided an efficient density modification procedure for the *ab initio* solution of protein structures with diffraction data to better than 1.3 Å starting with poor phases. An initial phase set could be obtained from a variety of sources such as the position of a heavy atom, a set of scatterers such as Sulphur atoms that had been positioned from anomalous dispersion measurements, a fragment or a very low homology model placed from a molecular replacement search. New procedures have been developed that yield good quality maps with data sets of resolution down to 1.7 Å. These new developments involve the artificial extension of data to atomic resolution and novel density-modification processes that develop density at atomic positions that was previously suppressed. The several known protein structures have been tested starting from a heavy atom, small α-helix and a model from molecular replacement search. The F-map from ACORN can be trace easily and the E-map can show most atom positions with the data extended to atomic resolution.

Keywords: data extension, density modification, Sayre equation refinement

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Electron Density of $ScRh_3B_x$: Relation of the Electron Density to the Hardness

Masahiko Tanaka[a], Toetsu Shishido[b], Masaoki Oku[b], *[a]National Institute for Materials Science, Tsukuba, Japan. [b]Institute for*