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Ab-initio* Structure Determination of SMU.440 Protein from *Streptococcus mutans
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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
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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
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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
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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

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SAD Phasing at the Presence of Pseudo-translational Symmetry
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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