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*

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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

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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

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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

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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

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$ScRh_3B_x$(x=0.0-1.0) has been investigated recently as an ultra-hard material. Crystal structure refinements and electron density analyses of this material were carried out by synchrotron X-ray powder diffraction. The powder diffraction data were collected using Multi-Detector System powder diffractometer at the BL-4B2 experimental station of the Photon Factory. The crystal structure refinements were performed using the Rietveld method and the electron density maps were calculated with the Maximum Entropy Method (MEM). The results of the refinements show that the crystal structure of $ScRh_3B_x$ is cubic with Pm3m space group, which has same atomic arrangement with perovskite structure. The lattice constant increases linearly according to the increase of B amount. In the electron density maps obtained by MEM analysis, electron density raises are obviously observed between B and Rh atoms. The rises of electron density show the existence of covalent bond between B and Rh atom. In spite of the liner increase of lattice constant according to the increase of B amount, the hardness of this series of compounds have a minimum between 0.4 and 0.7 of B contents. This change of hardness is supposed to be related to the amounts of the covalent bond in the crystal structure. The bond character of this series of compounds is also discussed based on the results of electron density analyses.

Keywords: borides, electron density, powder diffraction

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Structure Determination of a Novel Protein by Sulphur SAD using Novel Crystal mounting Method

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A crystal mounting technique was developed for the sulphur SAD method using longer wavelength X-rays. This technique is novel in that the a nylon loop is glued directly onto the tip of the micropipette and fixed as if the micropipette tip is located in the loop, so the solution caught in the loop can be aspirated through the micropipette just before flash freezing. Using this technique, the cryo-buffer and cryoloop can be removed easily before data collection to eliminate their X-ray absorption. The structures of novel proteins were solved using this technique in combination with chromium radiation. In the case of PH1109 from *P. horikoshii*, 90% of all residues were built automatically by *RESOLVE* using this technique, but only 76% were built for the dataset obtained using the standard loop. These results indicated that our crystal mounting technique was superior to the standard loop mounting method for the measurement of small anomalous differences, and yielded good results in sulphur substructure solution and phasing. We will demonstrate that the sulphur SAD method with a chromium source is more practical for macromolecular structure determination using our crystal mounting technique.

Keywords: sulphur, SAD, crystal mounting method

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IL MILIONE: A Complete Package for a Global Phasing, from Powders to Proteins

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IL MILIONE is a multipurpose compact, user friendly, efficient package for the global phasing of the crystal structures. The following tasks can be accomplished:

a) phasing and refining powder data. The program EXPO2004 [1] has been incorporated;

b) ab initio crystal structure solution of small, medium and macromolecules. The program SIR2004 [2] has been incorporated. Structures can be solved both by Patterson and Direct Methods (resolution up to 1.4-1.5Å, up to 2000 atoms in the asymmetric unit)

c) a new molecular replacement routine has been incorporated;

d) SAD-MAD, SIR-MIR, SIRAS-MIRAS cases can be faced. The new method provides quite simple and effective formulas both for locating heavy-atom/anomalous-scatterer substructures, and for phasing reflections ([3], [4]).

The program is highly automatic and suitable for high throughput crystallographic. Results of numerous applications will be shown.

[1] Altomare A., Caliandro R., Camalli, M., Cuocci C., Giacovazzo C., Moliterni A.G.G., Rizzi R., *J. Appl. Cyst*, 2004, **37**, 1025-1028. [2] Burla M. C., Caliandro R. Camalli M., Carrozzini B., Cascarano G.L., De Caro L., Giacovazzo C., Polidori G., Spagna R., *J. Appl. Cyst*, 2004, **38**, 000-000. [3] Giacovazzo C., Ladisa M., Siliqi D. (2002) *Acta Cryst.* A**58**, 598-604. [4] Giacovazzo C., Siliqi D., *Acta Cryst*., 2004, A**60**, 73-82.

Keywords: structure determination, crystallografic software, protein crystallography

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***OASIS-2004* and Difficult SAD Phasing**

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OASIS [1] is a direct-method program for resolving the phase ambiguity in single-wavelength anomalous diffraction (SAD) and in single isomorphous replacement (SIR) of proteins. The new version, OASIS-2004 includes algorithms for automatically tuning the scaling factor associated to the lack-of-closure error and for dynamically incorporating known structure fragment(s) in the iterative direct-method phasing. Details of the phasing strategy will be described. Application to SAD data from a series of known as well as originally unknown proteins will be given. The data sets were collected either with synchrotron radiation or with in-house sources (Cr-Kα and Cu-Kα) X-rays. Among the applications, an originally unknown protein with more than a thousand amino acids in the asymmetric unit has been solved with Cr-Kα sulfur-SAD data. Good quality phases have been successfully derived from sulfur-SAD data at the Bijvoet ratio $<|\Delta F|>/<F>$ lower than 0.6%. In all cases the combination of programs OASIS-2004, DM, RESOLVE-BUILD and ARP/wARP enabled automatic structure analysis from *ab initio* SAD phasing to model building. All resulted in a model containing more than 90% of the content of the asymmetric unit.

[1] Hao Q., Gu Y. X., Zheng C. D., Fan H. F., *J. Appl. Cryst.* , 2000, **33**, 980-981.

Keywords: SAD phasing, direct methods, proteins

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A Novel Method to Prepare Iodine Derivatives for In-house Phasing

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We developed novel procedures for efficient preparation of iodine derivatives of protein crystals that are most effectively employed for in-house phase determination. In this procedure, target native crystals are exposed by gaseous iodine. In the crystals, hypoiodous acids are