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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

P.02.04.2

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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

generated, and they form covalent bonds at the *ortho*-positions of accessible tyrosine residues by the aromatic electrophilic substitution. The resultant iodine derivatives are the most suitable for in-house phasing using longer wavelength X-rays, such as Cr-*Ka* X-rays, because anomalous signals of iodine are strengthen at the region of longer wavelength X-rays.

We applied this approach to native crystals of thaumatin. The crystals were successfully iodinated to generate enough phasing power by the Cr-*Ka* X-rays. As a result, the crystal structure of the iodinated thaumatin was solved by fully automated procedure. The present methods not only to contribute to the in-house *ab initio* structure determinations using Cr-*Ka* radiation, but also to promote the phasing at synchrotron facilities if their beamlines provide longer wavelengths than the conventional ones. In the poster session, the possibility of the present method to apply for other crystals will be discussed.

Keywords: iodinated-derivative, Cr-*Ka* X-rays, in-house phasing

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SAD Phasing at Bijvoet Ratio below 0.6%

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SAD method is getting more and more important in high-throughput determination of protein structures. An important factor affecting SAD phasing is the Bijvoet ratio $<|\Delta F|>/<F>$. This makes sulfur-SAD data a challenge in diffraction phasing. B.C. Wang [1] has demonstrated that the error-free sulfur-SAD data of Rhe with Bijvoet ratio at 0.6% can be successfully phased by the ISAS procedure. Ramagopal *et al.* [2] reported the test on phasing three sets of experimental sulfur-SAD data, glucose isomerase at λ=1.54Å, xylanase at λ=1.74Å and xylanase at λ=1.49Å. Bijvoet ratios are respectively 0.68%, 0.69% and 0.55%. In their test, SAD phasing was successful for the first two data sets but failed with the third one. Here we report the successful phasing of the xylanase SAD data at λ=1.49Å kindly supplied by Dr. Z. Dauter, Brookhaven National Laboratory, USA. *SHELXD* and *SOLVE* were used to locate and refine the sulfur atoms. *OASIS-2004* was used for the iterative SAD phasing, which incorporates dynamically known structure fragment(s). *DM* was used for density modification. *RESOLVE-BUILD* and *ARP/wARP* were used for automatic model building. The result is a structure model from *ARP/wARP* containing 300 of the total 303 residues.

*The first three authors contributed the same.

[1] Wang B.-C., *Methods Enzymol.* , 1985, **115**, 90-112. [2] Ramagopal U. A., Dauter M., Dauter Z., *Acta Cryst.*, 2003, D**59**, 1020-1027.

Keywords: sulfur-SAD phasing, direct methods, proteins

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SAD Phasing Using a Home Source, What is the Limit?

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With a diffraction system combining a high-brilliance rotating anode source, graded multilayer optics, a 3- or 4-circle goniostat and a sensitive CCD detector it is possible to solve protein structures from Cu-Kα native data only using SAD (single-wavelength anomalous diffraction) [1].

With highly redundant data this can be done with proteins that only contain Sulfur as the anomalous scatterer, despite the relatively low anomalous scattering contribution (f″) at Cu-Kα of only 0.56 electrons.

The possibility to solve a structure using SAD phasing depends on the number of anomalous scatterers and the strength of the anomalous signal. These are expressed in the Bijvoet factor $<\Delta F\pm>/<F>$.

In this study we will show a number of cases of structure determination using SAD phasing on native data collected on a home source and how the Bijvoet factor can be used to predict the success of SAD phasing.

[1] Debreczeni J.E., et al., *Acta Cryst.*, 2003, D**59**, 686-696

Keywords: SAD, accurate intensity data collection, anomalous scattering

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A New Lanthanide Complex for Solving Protein Structures Using Anomalous Scattering

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A complex of europium, $Na_3.[Eu(pyridine\text{-}2,6\ dicarboxylate)_3]$, was used to solve macromolecular structures by anomalous diffraction methods (MAD and SAD).

Crystals of thaumatin I from *Thaumatoccus daniellii* were soaked in 100 mM of the Eu complex. The structure was solved by the MAD method using data from synchrotron radiation measured to a resolution of 1.46 Å, at the L_{III} absorption edge of Eu.

Hen egg-white lyzosyme derivative crystals were obtained by cocrystallization in 100 mM of the Eu complex leading to a new crystal form belonging to space group C2. This new structure was solved by the SAD method using data collected with a rotating anode generator. Phases were extended to 1.3 Å resolution using data collected with synchrotron radiation at ë = 0.931 Å.

The symmetry of the free Eu complex is 23. In both structures, the complex is fixed on several sites, one of which is located on a crystallographic 2-fold axis. In the complex, the coordination of Eu is complete. Thus the complex is bound to the protein through the ligand only. In the experimental electron density maps of the two structures the electron density of the complex is well defined around the highly occupied Eu sites.

Keywords: macromolecular crystallography, heavy-atom derivative, anomalous diffraction

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Comparative Study of the Binding of Different Gd Complexes in Protein Crystals

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A series of Gd complexes was used for obtaining high-phasing power heavy-atom derivatives. Ease of use of the complexes and high success rate in obtaining good derivatives were demonstrated by a large number of tests [1]. Here we present a comparative study of the different complexes and data analysis of about 50 derivatives obtained with 8 different proteins.

For highly occupied binding sites a model of the corresponding complex was built and refined. This allowed identification of the binding mode of the complexes. For less occupied binding sites the binding site locations and occupancies were determined. Combining these different kinds of information may allow the identification of systematic patterns and thus to predict the behavior of the different complexes with proteins of unknown structure, depending on the nature of residues at the protein surface, and possibly on other factors such as the precipitating agent.

In addition to working on derivative crystals of several test proteins (urate oxidase from Aspergillus flavus, hypothetical protein Yggv from E. coli, glucose isomerase from Streptomyces rubiginosus, thaumatin from Thaumatococcus daniellii) several new protein structures were solved de novo using the complexes.

[1] Girard É., Stelter M., Vicat J., Kahn R., *Acta Cryst.*, 2003, **D59**, 1914-1922.

Keywords: macromolecular crystallography, heavy-atom derivative, anomalous diffraction