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