MS62 ADVANCES IN COMPUTATIONAL METHODS FOR PROTEIN CRYSTALLOGRAPHY

Chairpersons: Piet Gros, Kevin D. Cowtan

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New Likelihood-based Phasing Methods in *Phaser*

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We have been developing a new program, *Phaser*, to apply likelihood to solving macromolecular crystal structures by molecular replacement and experimental phasing methods.

Initial experiences with molecular replacement using brute-force likelihood targets in the program *Beast* [1] showed that likelihood had greater power to discriminate correct solutions, but the brute-force approach was prohibitively slow. To address this problem we have developed likelihood-based fast rotation [2] and fast translation [3] functions. The combination of these fast targets in *Phaser* with powerful automation strategies makes it possible to solve many difficult molecular replacement problems routinely.

More recent developments in *Phaser* focus on experimental phasing. The program includes new likelihood targets for phasing by SAD [4], as well as by MAD or MIRAS (unpublished). Completion of the heavy-atom substructure is accomplished through the automated interpretation of log-likelihood-gradient maps.

Applications of *Phaser* to difficult structure solutions will be discussed, along with plans for future development.

[1] Read R.J., *Acta Cryst.*, 2001, **D57**, 1373. [2] Storoni L.C., McCoy A.J., Read R.J., *Acta Cryst.*, 2004, **D60**, 432. [3] McCoy A.J., Storoni L.C., Read R.J., *Acta Cryst.*, 2005, **D61**, *in press*. [4] McCoy A.J., Storoni L.C., Read R.J., *Acta Cryst.*, 2004, **D60**, 1220.

Keywords: likelihood, molecular replacement, experimental phasing

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Broken symmetries in macromolecular crystallography

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The space-group symmetry of a crystal structure imposes a point-group symmetry on its diffraction spectrum, giving rise to so-called symmetry-equivalent reflections. This introduces a certain redundancy in diffraction data recorded with area detectors and is exploited in macromolecular X-ray crystallography to increase the accuracy of the data by averaging over the symmetry-equivalent measurements (data merging).

We will discuss two instances where the symmetry in reciprocal space is broken, *i.e.* where symmetry-related reflections are no longer equivalent. One such situation occurs in the presence of resonant (anomalous) scattering, when the resonant sites display anisotropy in their local atomic environment. The second example of a broken symmetry is when the sample suffers from site-specific radiation damage during the X-ray measurement. Both situations commonly occur in macromolecular crystallography. In such cases, the genuine intensity differences between symmetry-related reflections can actually be exploited to yield useful phase information in the structure solution process. In this approach, the usual separation of the data merging and phasing steps is abandoned. At the phasing stage, structural (*i.e.* real-space) models are refined which can account for the observed intensity differences between symmetry-related reflections, thus yielding phase information.

Keywords: resonant scattering, anomalous dispersion methods, MAD phasing

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Statistical Direct Methods of Phase Determination

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In the minimal principle method, one of the most successful direct methods of phase determination, the phase problem is formulated as a problem in constrained global minimization. The cosine minimal function, based on probabilistic estimates of the cosines of the structure invariants, serves as the foundation of an optimization procedure called *Shake-and-Bake* [1] that automatically and repetitively alternates reciprocal-space phase refinement with a complementary real-space density modification to impose the atomicity constraints.

A new statistical minimal function and its minimal principle, based on the statistical properties of the structure invariants themselves, have recently been formulated. Favorable applications of the corresponding statistical *Shake-and-Bake* algorithm have been made to the *ab initio* phase determination of small proteins as well as Se-atom substructures, and the results have shown an overall improvement in success rate relative to traditional *Shake-and-Bake*.

Statistical *Shake-and-Bake* is being incorporated as the default optimization procedure in newly distributed versions of the *SnB* and *BnP* computer programs. This research was supported by NIH grants EB002057 and GM072023.

[1] Weeks C.M., Miller R., *J. Appl. Cryst*., 1999, **32**, 120.

Keywords: phase problem, direct methods, shake-and-bake

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Direct Use of SAD Phase Information in Automated Model Building and Refinement

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The incorporation of prior phase information in a maximum likelihood formalism has been shown to strengthen model refinement. However, the currently available likelihood refinement target using prior phase information has shortcomings: the refinement target considers experimental phase information indirectly and statically in the form of Hendrickson-Lattman coefficients. Furthermore, the current refinement target implicitly assumes that the prior phase information is independent from the calculated model structure factor.

We have derived a multivariate likelihood function that overcomes these shortcomings and directly incorporates experimental phase information from a single-wavelength anomalous diffraction (SAD) experiment allowing for the simultaneous refinement of heavy atom and model parameters [1]. We have implemented this SAD function in the refinement program REFMAC5 [2]. The SAD function has been tested on many different real test cases yielding consistently better results than currently available functions. In some cases, the automated model building program ARP/wARP [3] can only successfully build a model when using the SAD function.

[1] Skubak P., Murshudov G.N., Pannu N.S., *Acta Cryst*.,2004, **D60**, 2196–2201. [2] Murshudov G.N., Vagin A.A., Dodson E.J., *Acta Cryst.*, 1997, **D53**, 240–255. [3] Perrakis A., Morris R.M., Lamzin V.S., *Nature Struct. Biol.*, 1999, **6**, 458-463.

Keywords: automated model building and refinement, macromolecular X-ray crystallography, prior phase information

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Hybrid Programming in Crystallography: Phenix.refine and Phenix.hyss

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The *Phenix* project [1] is an international collaboration aimed at the development of a comprehensive, highly automated software system for the solution and refinement of macromolecular crystal structures. At Lawrence Berkeley Laboratory we have developed