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Recent efforts on phase determination by S-SAD

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Structure determination by the sulfur-SAD (single wavelength anomalous scattering) method is gaining considerable interest in the community. The technique offers the advantage of structure determination from data collected on native crystals using the weak sulfur anomalous scattering signal.

To increase the success rate of the S-SAD structure determination we have studied several approaches, in addition to using longer wavelength X-rays, for enhancing the anomalous scattering signal-to-noise ratio (S/N) contained in the S-SAD data set. These approaches include:

1 Data averaging from multiple crystals.
2. The collection of multiple (lower exposure) data sets (MDS) from the same crystal for a given total X-ray dose, which are processed together to give the phasing data set.
3. The use of highly "sensitive" X-ray area detectors.

We are also developing a database (the SSAD_DB) aimed at documenting all experimental information related to the successful sulfur-SAD phasing experiment for future data mining and archival. Examples and results from these studies and details of the SSAD_DB will be presented.

Keywords: sulfur-SAD, signal-to-noise, database

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Macromolecular OD-structures

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Properties of macromolecular order-disorder (OD) structures are discussed using several examples from literature. These OD-structures are composed of identical molecular layers and all pairs of adjacent OD-layers are geometrically equivalent, whereas non-equivalent triplets are possible and their presence means twinning or one-dimensional disorder. A special feature of an OD-twin is that the twin axis is parallel to a non-crystallographic symmetry axis. If the OD-twin is a twin by pseudomerohedry, this property reduces the contrast of twinning tests. Failure to recognise twinning can lead to further confusion during structure solution, as a contrast MR solution can be found in a higher symmetry space group. Contrary to this situation, the non-crystallographic symmetry of OD-layers can be helpful in the case of OD-twins by reticular merohedry, where it allows replacing detwinning by easier procedure of demodulation. The procedure also applies to allotwins containing individuals belonging to different space groups. Provided that an account was made for the interference terms between ordered domains, similar correction of the diffraction data can also be used in the case of partially disordered OD-structures and there is a report of successful experimental phasing using demodulated data from a partially disordered crystal. Correction of diffraction data from OD-twins, allotwins and partially disordered OD-structures improves overall quality of the atomic model and helps interpret weaker fragments of the electron density.

Keywords: macromolecular, OD, twinning

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Extending the limits in solving and rebuilding molecular replacement structures

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New developments are making it possible to solve and, importantly, rebuild structures with much less independent experimental information than formerly required.

Not long ago, molecular replacement was considered to be difficult if the sequence identity between the target and the template was below 40%. Developments in the algorithms, including the introduction of likelihood [1], have increased the reach of molecular replacement so that a sequence identity of 30% is usually sufficient. Nonetheless, crystallographers have frequently been in the frustrating position of having solved the molecular replacement problem without being able to see clearly how to rebuild the structure to complete the structure solution. In some cases, the correct molecular replacement solution is ambiguous, even though it is in a short list of possibilities.

By combining advanced algorithms in Rosetta (protein structure modelling), Phaser (molecular replacement) and AutoBuild (rebuilding and refinement), we have been able to extend the limits so that about half the structures in the twilight zone below 30% sequence identity can successfully be solved, rebuilt and refined to final models, without access to experimental phase information [2]. In the new procedure, accessible as phenix.mr_rosetta in the Phenix suite [3], electron density fit is added to the advanced potential functions that guide protein modelling in Rosetta, the best intermediate models are chosen by their likelihood scores in Phaser, and the completion of building and refinement is carried out in AutoBuild. Optionally, models can be improved in Rosetta prior to the initial molecular replacement calculation.

Other techniques allow us to exploit molecular replacement to kick-start multiple crystal averaging, thereby obtaining much better maps than would be obtained from a single crystal form. For example, a molecular replacement solution could be obtained for human angiotensinogen, using an ensemble of three templates with sequence identities of about 22%, but the structure could not be refined successfully. Four crystal forms of rat and mouse angiotensinogen resisted molecular replacement with the same ensemble model. However, by using as a model the electron density for human angiotensinogen, phased by molecular replacement, one of the other crystal forms could be solved. An iterative averaging approach eventually yielded excellent density, and all five structures could be solved [4]. In a similar fashion, density from experimental phasing in one crystal form can be used to solve the structure of another crystal form.

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Keywords: molecular replacement, modelling, multi-crystal averaging