MS02-O5

Optimized diffraction data collection for native SAD phasing

Naohiro Matsugaki[1], Yusuke Yamada[1], Masahiko Hiraki[1], Ayaka Harada [2], Miki Senda[2], Toshiya Senda[2],

1. Photon Facroty, Structural Biology Research Center, Tsukuba, Japan
2. Structural Biology Research Center, Tsukuba, Japan

email: naohiro.matsugaki@kek.jp

Phasing macromolecular crystals by native SAD (single-wavelength anomalous dispersion) is attractive for de-novo structure determination because no derivative crystals are required. However, the method has been uncommon for 30 years due to experimental difficulties in using long wavelength X-ray required to enhance weak anomalous signals from naturally-presented light atoms in macromolecules. In diffraction measurement, long wavelength X-ray beam is severely absorbed by the sample or by the air on the beam path, which deteriorates the quality of diffraction data and results in burying small anomalous differences.

Diffraction data collection environment for native SAD phasing should be considered taking the above problem seriously into account. Photon Factory beamline BL-1A is routinely operated for long wavelength experiment where the diffractometer is equipped with a standing chamber enclosing both the goniometer and the X-ray detector, a helium cold stream recycling system, and a specially designed sample changer. X-ray absorption on the beam path does not matter at the beamlin as it is completely covered by helium. Under such optimized experimental environment we showed using the wavelength of 2.7 Å was advantageous in native SAD phasing against 1.9 Å, at around which most native SAD data collection has been performed so far [1].

Another aspect is the absorption by the sample itself. The wavelength used should be optimized for the sample size to most enhance the weak anomalous signal. However anisotropic amount of absorption due to the sample shape would be more problematic, which introuduces systematic errors into the diffraction data. Many trials have been made to correct the absorption effect in both empirical and analytical way, but it's much simpler if the sample is shaped in sphere. Shaping macromolecular crystal with deep UV laser [2] will solve the problem in a routine manner. We will present how the sample shaping is effective in native SAD phasing, as well as the discussion on the relationship between the sample size and the wavelength to be used.

References:

[1] Liebschner D. et al. (2016). Acta Cryst., D72, 728–741.
[2] Hasegawa et al., (2009). Journal of Crystal Growth, 312, 73-78.

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MS03 Combining methods in macromolecular structure determination, including special conditions MX

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MS03-O1

All is fair in phasing: the combined artillery in ARCIMBOLDO

Isabel Usón[1], Claudia Millán Massimo Sammito[2], Rafael J. Borges[2], Nicolas Soler[2], Iracema Caballero[2], Ana Medina[2]

1. ICREA at SBU, IBMB-CSIC, Barcelona, Spain
2. SBU, IBMB-CSIC, Barcelona, Spain

email: uson@ibmb.csic.es

Solving the phase problem is often a bottleneck in the determination of macromolecular crystal structures. Our approach to phasing, implemented in the software ARCIMBOLDO (http://chango.ibmb.csic.es/ARCIMBOLDO) relies on the combination of locating partial model fragments such as polyalanine alpha-helices with the program PHASER [1] and iterative density modification and main chain tracing with the program SHELXE [2]. In difficult cases, identifying correctly positioned fragments is not obvious and then many putative groups of fragments have to be tested in parallel. As the starting substructure constitutes a small fraction of the total and needs to be expanded, phasing will be self-validating in most ARCIMBOLDO use cases: once a solution can be identified as correct, it may contain errors but should be fundamentally right. Therefore all available information can be used to constrain the starting hypotheses. Typically used fragments encompass ubiquitous models, such as polyalanine helices, libraries of small local folds such as beta sheets extracted from the PDB and fragments of distant homologs. Restricting the purely *ab initio* search by predictions derived from bioinformatics can be enhanced with experimental data such as circular dichroism to decide which libraries should be used in ARCIMBOLDO_BORGES Alternatively, partial solutions can be filtered with the complementary information to discard incompatible solutions. The placed main chain models can be extended with probable side chains to generate multiple alternatives prior to expansion and we have used mass spectrometry in the context of natural products such as snake venoms, where composition is uncertain, to establish the structure. The combination with experimental phase information, too weak to render a solution on its own, can be performed in a number of ways: anomalous fragments can be searched for in PHASER along with normal fragments or independently determined and referred to the same origin. In either case, they can be combined in SHELXE. Alternatively the anomalous substructure can be determined from the map calculated from the partial fragments. A poor low resolution experimental map may be used in ARCIMBOLDO_SHREDDER [3] to phase a higher resolution crystal form.

Keywords: phasing, ARCIMBOLDO, SHELX