MS4-P5 Native SAD data collection environment at the Photon Factory BL-1A

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Native SAD (Single-wavelength anomalous dispersion) phasing, which utilizes naturally-included light atoms in macromolecules as anomalous scatterers, is an attractive crystallographic method for de-novo structural solution because no derivative crystals are required. The method appeared more than 30 years ago, however, it is still not ready to apply even at the latest synchrotron MX beamlines. Considering SAD phasing with heavy atoms is currently quite popular in MX, it is obvious the difficulty is in measuring accurately weak anomalous signal from light atoms. Using longer wavelength is required to enhance anomalous signals from light atoms, on the other hand causes experimental problems: severe beam absorption by the sample or air in the beam path, smaller angle of incident beam into the detector, etc. To overcome the absorption problem, a standing helium chamber was introduced on the long wavelength beamline BL-1A at the Photon Factory in order to perform diffraction experiment completely under helium environment. Helium cold stream is continuously supplied onto the sample positon and recycled to keep the gas consumption minimum. A dedicated sample changer was developed to minimize the contamination of air during the sample exchange. The whole beamline system allows performing native SAD experiments feasible at long wavelength even above 3 Å. The problem of 'small angle of incident beam into the detector' is mitigated by placing two area detectors in V-shape. Two Eiger X4M detectors were installed with the newly developed supporting system which allows to exchange the V-shape and the standard horizontal configurations. A mini-kappa goniometer was installed at the same time to increase the completeness of the data and to reduce the systematic error due to the beam absorption by the sample. The current status of the beamline as well as successful de-novo structural solutions using the wavelength ranging from 2.7 Å to 3.5 Å will be reported.

Keywords: native SAD phasing, macromolecular crystallography beamline

MS4-P6 ARCIMBOLDO_SHREDDER's contribution to MR: Phasing with fragments from distant homologs

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Most macromolecular structures are solved by molecular replacement, provided suitable search models are available. Distant homologs provide templates that may be too different to succeed, notwithstanding the overall correct fold or their featuring areas with close enough fragments. Successful ways to tackle this problem rely on the degree of conservation to select and improve search models, as implemented in Sculptor[1] or Ensembler[2].

ARCIMBOLDO[3] combines the search of small and accurate fragments with PHASER[4], with their expansion through density modification and autotracing with SHELXE[5]. ARCIMBOLDO_SHREDDER[6] uses fragments derived from distant homologs in a process driven by the experimental data. The following aspects will be illustrated in the solution of new and test structures:

- Model generation: contiguous polypeptide stretches, three dimensional volumes or structural unit
- Optimal fragment size to retrieve a minimum signal
- Model trimming against the rotation function
- Model optimization through gyre refinement against the rotation function
- Combination of partial fragment solutions with ALIXE (7)

ARCIMBOLDO_SHREDDER can be downloaded from http://chango.ibmb.csic.es/ARCIMBOLDO.

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Keywords: phasing, molecular replacement, small fragments, clustering, ARCIMBOLDO

MS4-P7 Fast iodide-SAD phasing for membrane protein structure determination

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Membrane proteins are hard to investigate. Each step of X-ray crystallographic pipeline from protein production to structure solution is non-trivial. In particular, experimental phasing techniques have shown their ineffectiveness in case of either soaking or co-crystallization of membrane protein crystals. A fast, easy and universal method is presented here for membrane protein structure determination. Four structures of target membrane proteins from four different largest classes have been solved via single-wavelength anomalous diffraction of iodide-soaked crystals. The method is highly efficient for various data collection strategies: standard and serial crystallography at synchrotron and XFEL sources.

Keywords: SAD, crystal soaking, iodide, membrane protein