Phasing in the Presence of Radiation Damage

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The brightest SR sources create havoc with macromolecular crystals when used at design specifications. The problem of radiation damage is not only severe in studies involving kinetics and mechanism where cryotechniques are not always viable, but is also significant for cryo-cooled (100K) crystals. In the course of the data collection, the diffraction power of the crystal is reduced, the mosaicity and overall B-factor go up, and eventually one will loose all higher order reflections. In addition to these general effects, some highly specific changes might occur, such as breakage of disulphide bonds and loss of definition of carboxyl groups [1,2,3]. Both the non-specific and the specific effects result in loss of perfect isomorphism throughout the data collection.

The multiple-wavelength anomalous dispersion (MAD) method has become the standard technique for structure determination. However, its main advantage, perfect isomorphism, can easily be swamped by radiation damage. At the ESRF MAD Undulator Beamlines ID29 and ID14-4, a large percentage of failed MAD experiments may be attributed to the occurance of radiation damage. In practice, this problem is often overcome by attenuating the beam or by the exclusive use of single-wavelength anomalous dispersion (SAD) experiments.

In this talk we will report on systematic experiments aim at understanding the consequences of radiation damage for MAD and SAD data collections. We will report on the consequences of specific and non-specific changes for the structure determination process. It will be shown that at current date, not only radiation damage itself is detorial for the phasing process, but more importantly our handling of its consequences. Examples will be shown on how to improve on this last issue.

[1] Raimond B.G. Ravelli and Sean M. McSweeney. The 'fingerprint' that X-rays can leave on structures. Structure, Feb 29, 2000, Vol 8, 315-328.

[2] Martin Weik, Raimond B.G. Ravelli, Gitay Kryger, Sean McSweeney, Maria L. Raves, Michal Harel, Piet Gros, Israel Silman, Jan Kroon, and Joel L. Sussman. Specific chemical and structural damage to proteins produced by synchrotron radiation. PNAS, Jan 18, 2000, Vol 97, no. 2, 623-628

[3] Wilhelm P. Burmeister. Structural changes in a cryo-cooled protein crystal owing to radiation damage. Acta Cryst., March 2000, Vol D56, 328-341.