WK.03 Wise Use of Dose: Structure Solvability vs. Structure Integrity

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Workshop Fee: $60 for Students, $110 all others

Description

With the trend to use ever smaller crystals and the abundant flux available from 3rd generation synchrotron sources that permits acquisition of data sets from small protein crystals in a short time, the deleterious effect of radiation damage becomes an increasingly limiting barrier. Radiation damage is proportional to the absorbed X-ray dose which in turn is proportional to fluence on the sample (flux density times exposure time). Radiation damage starts with the first exposure. But by the time the general decay of diffraction peaks is noticeable in the diffraction pattern, structural changes have already occurred, in particular at highly sensitive sites. Therefore, care should be taken from the beginning to use the limited dose on a sample crystal carefully. Currently, the general prescription for exposure time per frame at 3rd generation beamlines is to not saturate the detector. This is certainly not a scientific approach to the question: How much exposure is enough to solve the structure, or, more precisely, the biological question that the crystallographic endeavor was aimed to answer.

How much exposure (=dose) is enough to solve a structure while maintaining sample integrity as much as possible is the central theme of the workshop.

While radiation damage is unavoidable with any X-ray crystallographic data acquisition, and while there is a one-to-one relation between the dose on a sample crystal and the number of photons in the diffraction peaks for a particular experimental condition, there are many ways to increase the efficiency of the applied dose with respect to solving the structure. The list of topics of the workshop covers a variety of techniques and approaches that can help to achieve the goal of minimum exposure for the different stages of the structure solving process, including phasing and refinement at low or high resolution. The goal of the workshop is to give the beamline scientists and project PIs guide lines and tools to consider for obtaining at the end of the process a data set and a structure least altered by radiation damage.

Topics of the Workshop:

0. Basic facts of radiation damage
1. How much exposure is enough for
   a. anomalous phasing
   b. refining a structure at different resolutions vs. structural effects of radiation damage
2. Strategy - Techniques to reduce overall dose for same final structure solving result
   a. orienting crystals (precisely) for Bijvoet pairs on same or adjacent frames
      (no loss of isomorphism due to radiation damage)
   b. reorienting sample vs. large total rotation angle at same orientation
      (better reduction of systematic errors)
      (better scaling, particularly for low symmetry crystals)
   c. the total dose distributed over several exposures vs. one exposure
      (effect of non-Poissonian uncertainties)
3. Mitigation of Radiation Damage
   a. scavengers
      (do they help?; role of cryoprotectant)
   b. low temperature
      (B-factor reduction, decrease of site specific damage)
   c. computational
      (zero-dose extrapolation; improved phasing; dose stamp for each reflection)
4. Reduction of background noise for better I/s
   a. optics and sample induced background vs. scattering angle
   b. detector distance
      (beam spot size and sample induced spot size vs. mosaicity and scattering angle)
   c. detector induced noise
      (analog (CCD) and counting detectors; compared to sample induced noise)
5. Optimal choice of wavelength
   (radiation damage for same statistical significance of diffraction peaks vs.
   dose for different wavelength)
6. Minibeams
   (do have smaller exposed volumes of a medium size crystal higher diffractive
   efficiency than the full crystal?)