Radiation damage in serial synchrotron crystallography at cryo- and room temperatures

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Radiation damage limits the accuracy of macromolecular structures in X-ray crystallography. Cooling to 100 K reduces the global radiation damage rate, so that cryo-crystallography became the method of choice over the past decades. The recent advent of serial crystallography, which spreads the absorbed dose over many crystals, thereby reducing damage, has rendered room-temperature (RT) data collection more practical, both enabling and requiring the study of specific and global radiation damage at RT. We performed sequential serial raster-scanning crystallography using the micro-focused synchrotron beam of ID13 at the ESRF and a fast single-photon-counting pixel-array detector. Two series of 40 and 90 full data sets of 2 Å and 1.9 Å resolution were collected at on Hen Egg-White Lysozyme (HEWL) crystals at RT and 100 K, respectively. Specific radiation damage at RT was observed at disulfide bonds but not at acidic residues, increasing and then fading away, a peculiar behavior that can be explained by differential diffraction intensity decay due to the non-uniform illumination by the X-ray beam. Specific damage to disulfide bonds is evident early on at RT and proceeds at a 5-fold higher rate than global damage. Our results suggest it is advisable not to exceed about 0.6 MGy in static and time-resolved serial and oscillation synchrotron crystallography experiments at RT, a rough yardstick that will change for proteins other than HEWL and at resolutions other than 2 Å.