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## Radiation damage in serial synchrotron crystallography at cryo- and room temperature

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X-ray crystallography is the most-prolific technique in structural biology but suffers from radiation damage, which limits the accuracy of the macromolecular structures. The introduction of cryo-cooling techniques greatly reduced the global radiation damage rate and was standardized on all X-ray crystallography beamlines at synchrotrons over the past decades. With the recent advent of serial crystallography, room temperature (RT) data collection was made more accessible as the absorbed energy is spread over a large number of crystals. So far, studies of specific and global radiation damage at RT still remain limited. Here, we used a sequential serial raster-scanning approach using the micro-focused beam of ESRF ID13 beamline in combination with a fast single-photon-counting pixel-array detector. Two series of 40 and 90 data sets were collected on Hen Egg-White Lysozyme (HEWL) crystals at RT and 100K, at resolutions of 2 and 1.9 Å, respectively. At RT, specific radiation damage was observed at disulfide bonds, but not on the carboxylic groups of acidic residues. The evolution of the specific damage at RT was monitored, and after an increase of damage with dose, its signal fades away. This peculiar behavior could be explained by differential diffraction intensity decay due to the non-uniform illumination by the X-ray beam and well modelled. Appearance of specific damage at RT is extremely fast and proceeds at a ~5-fold higher rate than global damage. Our results suggest it is advisable not to exceed about 0.4 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.

### References

[1] de la Mora E, Coquelle N, Bury CS, Rosenthal M, Holton JM, Carmichael I, Garman EF, Burghammer M, Colletier J-P, Weik M (2020) Radiation damage and dose limits in serial synchrotron crystallography at cryo- and room temperatures. *Proceedings of the National Academy of Sciences*: 201821522

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