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Supporting information for article:

Fixed target combined with spectral mapping: approaching 100% hit rates for serial crystallography

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S1. Single crystal spectroscopy to determine "safe" dose limit

S1.1. Method

Larger single crystals of carboxy-myoglobin, from the same crystallization batches that were used for the LCLS experiment, were mounted in thin-walled quartz capillaries (Hampton Research), flushed with CO gas before sealing with bees wax, on beamline ID30A-3 at the ESRF (Grenoble, France). Spectra were recorded at room temperature during X-ray exposure using the on-line microspectrophotometer (von Stetten *et al.*, 2015). In brief, the on-axis microspectrophotometer consists of two mirror objectives mounted at a 90° angle to the X-ray beam. White light (Ocean Optics DH2000-BAL) was delivered via an optical fibre (100 µm diameter) to give a 25 µm spot-size at the sample. Transmitted light was collected from the opposite objective via a 400 µm optical fibre and passed to a spectrometer (Ocean Optics QEPro). Data acquisition was controlled using Spectrasuite (Ocean Optics). The capillaries were mounted at a 45° angle to the goniometer rotation axis, so that the face of the capillary was perpendicular to the optical beam path. Spectra were recorded with an exposure time of 300 ms per spectrum during X-ray exposure. The X-ray energy was 12.8 keV, the X-ray beam size was 15 µm in diameter and the X-ray beam was attenuated by 90% to give a flux of 1×10^{12} ph/s. The average absorbed X-ray dose per 300 ms exposure was calculated using RADDOSE3D (Zeldin *et al.*, 2013). For a $50 \times 50 \times 50$ µm crystal this is 80 kGy.

S1.2. Result

A broadly accepted rule of thumb in macromolecular crystallography is that a structural change of less than 20% does not contribute significantly to the final electron density (Pearson *et al.*, 2007). More than 20% change in the myoglobin spectrum (assessed by taking time-slices through the spectra series at various points) was already observed by the second spectrum, recorded at 600 ms, suggesting that a conservative "safe" dose limit is 80 kGy.

Pearson, A. R., Pahl, R., Kovaleva, E. G., Davidson, L. & Wilmot, C. M. (2007). *J. Synchrotron Rad.* **14**, 92–98.

von Stetten, D. *et al.* (2015). *Acta Cryst.* D**71**, 15–26.

Zeldin, O. B., Gerstel, M. & Garman, E. F. (2013). *J. Appl. Cryst.* **46**, 1225–1230.