Characterizing protein conformational changes using resampled time-resolved serial X-ray crystallography data

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With the development of serial crystallography at both X-ray free electron laser (XFEL) and synchrotron radiation sources, time-resolved X-ray crystallography is increasingly being applied to study conformational changes in macromolecules. A successful time-resolved serial crystallography study requires the growth of microcrystals, a mechanism for synchronized and homogeneous excitation of the reaction of interest within microcrystals, and tools for structural interpretation. Here we utilize time-resolved serial femtosecond crystallography (TR-SFX) data collected from microcrystals of bacteriorhodopsin to benchmark different approaches to structural refinement and illustrate how resampling strategies may estimate coordinate uncertainty. We utilize singular value decomposition from resampled data to minimize phase bias in difference Fourier electron density maps, and we quantify residual densities for transient water molecules using a Polder omit analysis utilizing resampled data. We suggest that these tools may assist others in judging the confidence with which observed electron density differences may be interpreted as functionally important conformational changes.

[1] A. Vallejos et al., Characterizing protein conformational changes using resampled time-resolved serial X-ray crystallography data. In preparation (2024).
[2] D. Zoric et al., Structural changes in cytochrome c oxidase following the reduction of dioxygen to water. In preparation (2024).
[3] R. Dods et al., Ultrafast structural changes within a photosynthetic reaction centre. Nature 589, 310-314 (2021).