Innovations in Electric Field-Stimulated X-Ray Crystallography

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Though time-resolved biophysical and crystallographic methods have advanced dramatically in recent decades, most contemporary approaches still cannot yield the necessary temporal and structural resolution to address problems such as understanding the concerted motions underlying allostery and catalysis. First presented in 2016, electric field-stimulated X-ray crystallography (EF-X) offers a unique tool to map both molecular motions and the underlying energy landscapes. Application of a strong (~1 million V/cm) electric field to a macromolecular crystal results in a precise pattern of forces localized to the presence of charges on each molecule; the resulting motions can be observed in time-resolved X-ray diffraction data. Despite an effective proof-of-concept, the initial hardware implementation of EF-X imposed stringent requirements on crystal robustness, limiting the widespread applicability of the method. Here, we report a number of significant technical advances that remove many of those prior constraints. In particular, these advances both physical and osmotic stress on the crystals while increasing flexibility in experimental design. Perhaps most crucially, remote EF-X data collection is now possible via a permanent community accessible setup at APS BioCARS. Together, these advances transform EF-X from a promising experimental approach to a fully-realized biophysical technology.