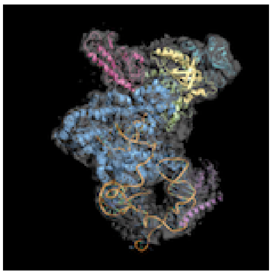

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Keynote 5 - Time-Resolved Macromolecular Structure Determination

Tuesday, 20 November 2018 10:00 (40)

Macromolecular crystallography changed substantially with the advent of the brightest X-ray sources the world has ever seen, the free electron lasers for hard X-rays (XFELs). The immense brilliance and the ultrashort pulses available at these machines triggered the development of serial crystallography, where a very large number of very small crystals are exposed to the X-ray pulses, one by one and in random orientation. We established time-resolved serial femtosecond crystallography (TR-SFX) at the Linac Coherent Light Source1. We followed the trans to cis isomerization of the central p-coumaric acid chromophore in the photoactive yellow protein in real time from 100 femtoseconds to 3 picoseconds2. With X-ray structures determined on the femtosecond time scale we structurally characterize protein structural changes on the excited state energy surface, as well as the isomerization reaction through a conical intersection, for the first time. Catalysis by bio-medically important enzymes is observed on different, slower time scales. The holy grail of time-resolved crystallography is a practical and general method to investigate enzyme catalysis. We developed such an approach called "mix-and-inject serial crystallography" (MISC) to trigger reactions by diffusion of substrate3, 4. We demonstrate that the approach is feasible using the M. tuberculosis β-lactamase (BlaC). BlaC is an enzyme that promotes broad-scale antibiotic resistance by chemically inactivating β-lactam antibiotics. We characterize the structure of the enzyme-substrate complex and that of a reaction intermediate along the catalytic pathway of the BlaC reaction with a third generation antibiotic (Ceftriaxone)5. This talk aims of addressing how MISC may be feasible at new-generation synchrotron X-ray sources.

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