Microsymposium

MS77.O02

Femtosecond Nanocrystallography and Characterization of Photosystem II

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Membrane proteins are extremely difficult to crystallize, however they are highly important proteins for cellular function. Photosystem I, one of the most complex membrane proteins solved to date took more than a decade to have a structure solved to molecular resolution. Large, well-ordered crystal growth is one of the major bottlenecks in structural determination by x-ray crystallography, due to the difficulty of making the “perfect” crystal. The development of femtosecond nanocrystallography, which uses a stream of fully hydrated nanocrystals to collect diffraction snapshots, effectively reduces this bottleneck\cite{Chapman2011} Photosystem II changed our biosphere via splitting water and evolving oxygen 2.5 billion years ago. Using femtosecond nanocrystallography we are developing a time-resolved femtosecond crystallography method \cite{Aquila2012} to unravel the mechanism of water splitting by determining the conformational changes that take place during the oxygen evolution process. Multiple crystallization techniques were originally developed in order to make the nanocrystals necessary for femtosecond nanocrystallography. For Photosystem II nano/microcrystals a free interface diffusion method, is used to increase yield over traditional methods. These crystals are then characterized by three different methods before being used for collecting diffraction data. The three methods currently used are optical microscopy, dynamic light scattering (DLS), and Second Order Nonlinear Imaging of Chiral Crystals (SONICC).


Keywords: Photosystem II, Femtosecond, Nanocrystal