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X-ray Free-Electron Laser Based Methods for Structural and Ultrafast Dynamics Studies of a Photosynthetic Reaction Centre

DAVID ARNLUND
Institutionen för kemi och molekylärbiologi
Naturvetenskapliga fakulteten

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ABSTRACT
Life on earth is fuelled by the energy of sunlight, which must first be captured and converted into a chemical energy form useful to the cell. This process is known as photosynthesis and the major pathway of this energy conversion is *via* photosynthetic reaction centres. These enzymes convert the energy content of an absorbed photon into a transmembrane potential difference *via* the movements of electrons. Increasing our knowledge of the three-dimensional fold and structural changes that takes place within photosynthetic reaction centres is therefore of considerable importance for understanding biological photosynthesis.

The aim of this work has been to adapt methods for both crystallographic and solution phase structural studies of membrane proteins to the unique properties of X-ray free-electron laser (XFEL) radiation. To accomplish this, a new crystallization technique for the photosynthetic reaction centre from the purple bacterium *Blastochloris viridis* (RC_{vir}) was developed which was suitable for serial femtosecond crystallography (SFX) experiments at an XFEL. Our initial experiments at the Linac Coherent Light Source (LCLS), the world's first XFEL, yielded an SFX structure of RC_{vir} to 8.2 Å resolution. After the LCLS decreased the X-ray wavelength at which the facility could operate, and in combination with improved crystallization conditions, we later resolved the SFX structure of RCvir to 3.5 Å resolution.

Whether or not ultrafast structural changes in RC_{vir} occur in photosynthesis has been debated for two decades. We addressed this question by developing time-resolved wide-angle X-ray scattering (TR-WAXS) studies at the LCLS that could capture rapid structural changes in solubilized samples of RC_{vir}. Proof-of-principle experiments revealed a structural deformation that propagated through the RC_{vir} protein following multi-photon absorption by its cofactors, enabling a protein quake through a photosynthetic protein to be visualized. Further insight was provided by a second TR-WAXS experiment in which this structural signal was observed in the data as the pump laser fluence was decreased to less than one photon absorbed per RC_{vir} molecule. This result implies that, even under physiological conditions of normal sunlight, ultrafast protein structural rearrangements may influence the primary charge separation events of biological photosynthesis.