Characterization of the BLUF photoreceptor PAC using time-resolved crystallography

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Abstract

Reactions of biological macromolecules can be studied by time-resolved crystallography (TRX), as it provides high special and temporal resolution. TRX requires the ideally instantaneous initiation of the reaction of interest, to ensure that fine structural changes are not “blurred out” by the different molecules in the crystal reacting in a non-synchronized manner. Luckily, naturally light-sensitive signaling proteins such as photoreceptors are ideal target to study fast biochemical processes using pump-probe TRX.

The BLUF photoreceptor PAC is of interest in this study. PAC contains a Blue-Light sensor Using Flavin (BLUF sensor domain) sensor domain coupled to an adenylyl cyclase (AC) effector domain, involved in the conversion of ATP into cAMP. For this, PAC protein was produced, purified and characterized to verify stability and functionality. Complete X-ray crystallography datasets of PAC were collected in its dark state with and without ATP bound in the active site. Pump-probe TRX using synchrotron and XFEL sources in combination with FTIR spectroscopy was performed. Structural changes around the FMN chromophore for several time points could be elucidated.

The data shows the rotation of the highly conserved glutamine in the flavin chromophore pocket, which initiates a change in the hydrogen bonds network. Displacement of the neighboring methionine initiates signal transmission to the adenylate cyclase domain. Cryo-trapping experiment also enabled to capture a late reaction time-point, where PAC adopts the so called “Tryptophan-in” conformation (Trp90-in/Met92-out) in the light activated state.

The performed experiments help to better understand the signaling process of PAC photoreceptors, which can serve as a basis to design novel optogenetic tools.