MS08-2-7 Identifying bacteriorhodopsin light-induced artefacts in time-resolved crystallography #MS08-2-7

Q. Bertrand [1], T. Weinert [1], J. Standfuss [1]
[1]PSI - Villigen (Switzerland)

Abstract
Time-resolve crystallography at X-ray free electron lasers (XFELs) allows the investigation of ultrafast protein reactions on the femtosecond time scale. However, getting insight on such rapid structural movements implies that the protein reactions of interest have to be triggered on the same time scale. This forces the use of photosensitive samples for which the reaction can be triggered in the femtosecond time range using lasers. However due to the crystal inherent properties, lasers are not able to trigger reaction for all proteins within one crystal. If the amount of molecules for which the reaction of interest has been triggered is too low, the structural signal from the reaction is unrecoverable. To counter this issue, the laser power applied to the sample might be increased, to simply deliver more photon to the crystal thus increasing the chances to trigger a reaction.
But any excessive laser power used during the experiment may lead to multi-photon excitation and may increase the probability of observing artefacts rather than native protein motion. Although structural effects of this multi-photon absorption are unknown, they are discussed in the X-ray laser field as a potential risk that could make data from femtosecond laser experiments unrelated to physiological protein motions.
Those questions were raised and the subject was largely discussed for Bacteriorhodopsin (bR), a model protein for which the photo-cycle has been extensively studied in the past years. To answer interrogations about the correct behaviour of bR's photo-cycle under various laser power, we performed a power-titration experiment. This latter was performed through a typical pump probe time-resolve serial crystallography (TR-SX) experiment using a single time delay of 10 ps, but registered with different laser power applied to the sample.
Our experiment shows that clear new retinal features appear in the difference maps and extrapolated maps when an extreme laser power is used. Those features are additional peaks in the difference maps around the retinal that could be modelled as a second retinal conformation in extrapolated maps and only appears using high laser power. This conformation closely resembles one only visible later on the photo-cycle and it seems that the use of extreme laser power accelerates the behaviour of the retinal through its photo-cycle. This delay of 10 ps provides overlaps with available datasets from several experiments already recorded with various laser powers and the comparison with those data will give insight about the impact of different laser setups on this light induced artefact.

References
Nango et al., 2016; Nogly et al., 2018; Weinert et al., 2019; Nass Kovacs et al., 2019; Grünbein et al., 2020.