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Revealing Protein Binding Dynamics Using Time-Resolved Diffraction Experiments at SwissFEL

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The creation of movies of biomolecules "in action" based on time-resolved diffraction experiments has come within reach thanks to the availability of X-ray-Free-Electron Lasers (XFELs), such as SwissFEL, the new large scale facility at PSI. This project targets the development of XFEL-based methods for dynamic studies of protein-binding events, using photo-cleavable derivatives of proteins that specifically bind to streptavidin as a versatile model system.

The flexibility and adaptability of the structure of proteins is a key property with a strong influence on their functionality. The mobility of protein chains allows opening and closing of channels as well as the adaptation of binding pockets to regulate binding processes [1]. XFELs delivering ultra-bright and femtosecond short x-ray pulses provide attractive means to access such processes using X-ray diffracton methods with high temporal and spatial resolution. In so-called serial femtosecond crystallography (SFX) [2], each diffraction pattern is acquired from a fresh microcrystal. The serial approach also opened the paths to dynamic studies, in particular for proteins that react on light stimulation with a conformal change. In this project we rely on the so-called fixed target approach to SFX: the crystalline sample is deposited on a thin film support, which is mounted on a scanning stage and scanned through the beam, thus sequentially probing the individual micro-crystals with the tightly focused X-rays [3].

As protein crystallographic data collection critically depends on the sample preparation, we will first concentrate on the fabrication of sample holders to accommodate the crystalline samples, enclose them between ultrathin X-ray transparent films and provide access for the optical laser, which is used to trigger the binding of a caged cofactor to the protein of interest. For typical measurements, we will need a few hundreds of support structures, which means that they must be mass-fabricated using silicon or polymer technologies available in the micro- and nanotechnology lab at PSI. in collaboration with the team at Uni Basel, we will then synthesize photocaged derivatives, and perform their characterization, crystallization and co-crystallization experiments, and investigate the binding dynamics in XFEL measurement campaings. In the later phase of the project, we will design and produce new variants of the cofactor that allow addressing specific characteristics of streptavidin binding.

References

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[3] M.S. Hunter et al., Fixed-target protein serial microcystallography with an x-ray free electron laser, Sci. Rep. 2014, 4, 6026.