Watching The Release of a Photopharmacological Drug from Its Target Using Time-Resolved Serial Crystallography

Dr. Tobias Weinert[1], Dr. Maximilian Wranik[1], Prof. Dr. Michel O Steinmetz[1], Dr. Jörg Standfuss[1]
[1]*Paul Scherrer Institute*
tobias.weinert@psi.ch

Photopharmacology provides a powerful means to modulate the affinity and biological activity of small molecule drugs using light as a trigger. However, traditional structural biology has faced limitations in deciphering the molecular mechanisms behind these processes due to its inability to resolve critical transitions. In this presentation, I will detail our pioneering approach that employs time-resolved serial crystallography at the Swiss Light Source (SLS) and the Swiss X-ray Free Electron Laser (SwissFEL) to investigate the light-activated release of azo-combretastatin A4, a promising anti-cancer agent, and the ensuing conformational changes in tubulin. We successfully acquired an extensive series of structural snapshots, with logarithmic spacing in time ranging from 100 fs to 100 ms. These snapshots, in conjunction with quantum mechanics/molecular mechanics (QM/MM) simulations and time-resolved spectroscopy, provide direct molecular insights into the process. Our results elucidate how the cis-to-trans isomerization of the azobenzene bond instigates a change in ligand affinity, leading to the formation of an exit channel and the subsequent collapse of the binding pocket upon ligand release. Moreover, we establish that the ensuing global backbone rearrangements are connected to the mode of action of microtubule-destabilizing drugs.
This study emphasizes the value of time-resolved serial crystallography and computational simulations in advancing our comprehension of photopharmacology and facilitating the design of innovative, light-responsive therapeutic agents.
Figure 1: Sequential snapshots of light-induced azo-CA4 release from tubulin. The panels from left to right display isomorphous difference maps (negative (red) and positive (green) densities at 3σ) captured at key time points: 100 ns featuring ligand-centric changes, 100 µs highlighting alterations in the binding pocket, and 100 ms illustrating conformational changes propagating throughout the tubulin alpha subunit. The structures within the specified time ranges (depicted in orange) are compared to those from the preceding time intervals (shown in gray).

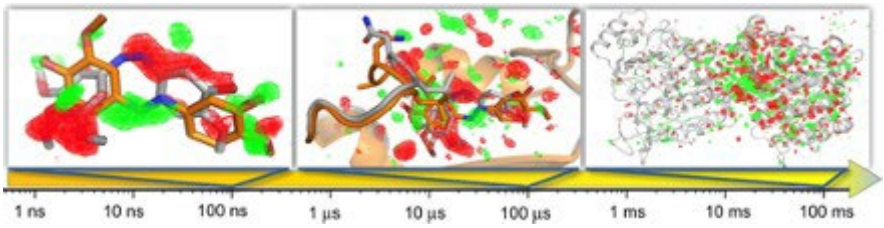

Figure 1