# Protein synchronization methods and considerations: light activation and rapid mixing

**Diana Monteiro**[1]

*[1]Hauptman-Woodward Med. Res. Inst.*

*dmonteiro@hwi.buffalo.edu*

Dynamically-resolved, or better known time-resolved (TR), structural studies of proteins require efficient methods for synchronized activation followed by delivery of the sample to the X-ray beam. Deciding on the correct method of activation determines the time-resolution achievable during the experiment. Both the biological target and question, as well as the physical nature of the sample bring constraints that have to be taken into consideration, making the design of a successful time-resolved experiment is a multi-dimensional problem.

There are several current methods and tools that can be used to synchronize protein activity both in solution and in crystals. The most common approaches use either light activation or rapid mixing of substrates/ligands. Both methods have advantages and disadvantages as well as a large number of parameters that can be tuned and optimized: no technique is one-size-fits-all. As protein function synchronization is not trivial, it is unsurprising that most time-resolved structural biology experiments reported to date have focused on naturally photoactivatable proteins, as these systems can be directly activated using short laser pulses. Nevertheless, such proteins only account for roughly 0.5% of all known proteins. I have specialized in the development of both photochemical and microfluidic tools to address these challenges and will be presenting a roadmap to help guide researchers in designing their own time-resolved experiments. I will give examples of the work currently being carried out in the new synthetic chemistry laboratory at HWI, which focuses on the development of photocaged compounds. Photocaging protecting groups mask chemical moieties that are essential for function and can be removed with short pulses of light, triggering protein activity. I will also give examples of how microfluidics can be used to deliver crystal slurries to X-ray beams and how this technology can be used for efficient and fast mixing. All these tools are versatile and can be used in experiments both at synchrotron and XFELs sources.

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