Applications of Microfluidic Mixers for Time-Resolved SAXS and Crystallography Experiments

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Both SAXS and X-ray Crystallography (MX) have paved the way for rapid determination of macromolecular structures, including proteins, DNA, and RNA. However, structural information alone cannot fully explain the biological functions that these macromolecules perform. Relatively recent developments in microfluidic mixers allow for the rapid diffusion of a ligand, such as an ion or small molecule, into a co-flowing stream that contains a sample, like a protein or RNA. This triggers millisecond scale interactions that can be visualized by various structural techniques. The combination of these mixers with either SAXS or MX creates an exciting frontier for time-resolved experiments. The microfluidic mixers are constructed from concentric capillaries and are comprised of a flow-focused mixing region and a delay region (Calvey et al., 2016; Calvey et al., 2019). The mixing region creates a narrow stream of sample surrounded by ligand, which supports fast diffusion for uniform mixing of the ligand and sample. Next, there is a delay region, which simply controls how long the sample and ligand interact before eventually being probed by the X-ray beam. The mixers have a seemingly endless range of applications as they can be situated upstream of standard sample delivery hardware and are compatible with samples free in solution or in crystal form. A recent Time-Resolved SAXS experiment (Plumridge et al., 2018) incorporated these mixers with a standard SAXS sample cell to visualize the effect of added Mg2+ ions on a small segment of RNA, exploring reaction timescales from 10ms to 3000ms. This wide range of detection times was accessed using a combination of flow rates and mixers with the appropriately sized mixing and delay regions. An additional application for these mixers links them with Gas-Dynamic Virtual Nozzles (GDVNs) for Mix-and-Inject Femtosecond Serial Crystallography (Olmos et al., 2018). Key to this technique is the use of microcrystals and the rapid diffusion of small molecules to access proteins within them. Snapshots of the protein-ligand interactions can then be captured on the millisecond scale and beyond. These mixers are also currently being adapted for time-resolved spectroscopy. Overall, the mixers can be applied widely to probe different biological systems and designed for compatibility with multiple structural techniques. This technology has the potential to elucidate the details of many essential biological processes. References: Calvey G. D. et al. (2016) Struct. Dyn. 3, 054301 Calvey G. D. et al. (2019) Anal. Chem. 91(11), 7139-7144 Olmos J. L. et al. (2018) BMC Biol. 16(59) Plumridge A. et al. (2018) Nucleic Acids Res. 14(21), 7354-7365