# Chaotic Advection Mixer for Capturing Transient States of Diverse Biological Macromolecular Systems with Time-Resolved Small Angle X-Ray Scattering

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Time-resolved mixing experiments, in which a microfluidic device is used to rapidly mix two species together to initiate a reaction, are a powerful tool to collect snapshots of the time-progression of macromolecular interactions. These mixing experiments are compatible with a variety of experimental techniques as structural probes, but Small Angle X-ray Scattering (SAXS) is particularly well suited to these studies as it can capture changes in the overall shape, size, and level of compactness of biological macromolecules with unconstrained motions in solution. Flow-focused diffusive mixers have been used successfully for time-resolved SAXS experiments, but they require that one of the reactants is small and highly soluble to achieve the rapid diffusion required to uniformly initiate a reaction. Although this requirement can be easily met for a broad range of biological macromolecule-ligand systems, many drug targets tend to be partially hydrophobic, not highly soluble, and not easily available in large quantities. Additionally, diffusive mixers preclude the study of the interaction of two large biological macromolecules, such as two proteins or a protein-nucleic acid system, as the diffusion times for these larger molecules can be longer than the reaction times of interest.

We present a novel coupling of the Kenics-style chaotic advection mixer with SAXS to study diverse classes of macromolecular interactions, including reactions between two large biological macromolecules or one large biological macromolecule and a ligand. The mixer is comprised of a series of eight helical elements with alternating left- and right-handedness. Rapid mixing is achieved by creating ultra-thin layers of each species via baker's transformations (stretching, splitting, and layering of the liquids), so even large proteins can be mixed in as fast as a few milliseconds. The mixer itself was fabricated with a NanoScribe 3D Printer and our sample cell design presents a sufficiently large observation region which permits a good signal to noise ratio. Timepoints from 10-2000 ms can be reached by changing flowrates or the position of the X-ray beam relative to end of the Kenics mixer. We used this mixer to study a variety of different biological questions, such as RNA folding, protein conformational changes, protein-protein associations, and protein-nucleic acid complex formation. With this mixer, we captured transient reaction states that evade observation by typical equilibrium measurements and visualization of these short-lived states can elucidate the mechanism of these reactions or reveal the initial stages of comple