Nanoscribe 3D Printing of Versatile Microfluidic Mixers for Experiments with Biomolecules

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Primary CNF Tools Used: Nanoscribe 3D Printer

Abstract:

We report the fabrication and implementation of a 3D printed microfluidic mixer for studying biological macromolecules. The device mixes fluids by chaotic advection, enabling time-resolved structural measurements of large molecules and offers advantages over turbulent mixers in terms of sample consumption. Previous versions of the mixer have been used for time-resolved measurements for multiple biological systems and techniques, and there are many avenues for future work.

Summary of Research:

Microfluidic mixers have enabled time-resolved structural studies of biological macromolecules [1,2]. Snapshots of the reaction can be captured by different structural techniques, such as X-ray crystallography [3], Small Angle X-ray Scattering (SAXS) [4], and various spectroscopies [5]. Many time-resolved experiments have utilized flow-focused diffusive mixers [2], which rely on an outer sheath flow to thin the central sample stream so small molecules can rapidly diffuse into the stream for reaction initiation. Timescales ranging from single milliseconds to seconds are reachable. One reactant, however, must be relatively small and highly soluble for efficient diffusion. Thus, diffusive mixers are suited for reactions involving one large biological macromolecule, such as a protein or nucleic acid, and one small ligand.

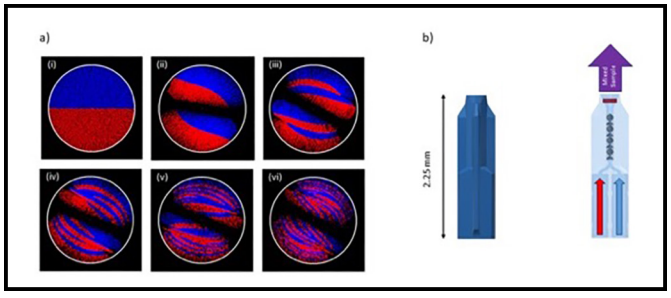

Figure 1: (a) Cross section of simulated flows after each mixing element inside the Kenics (b) CAD rendering of the exterior and cross section of the Kenics.

Reactions between two biological macromolecules, such as two proteins or a protein and a nucleic acid, are essential so there is interest in studying these interactions via time-resolved measurements. Turbulent mixers can achieve this, but high sample consumption is often prohibitive for lab-purified biological molecules. Mixing by chaotic advection is a strong alternative to diffusive and turbulent mixers, as the mixing is efficient enough for reactions between large macromolecules, and sample consumption is lower than turbulent mixers. The Kenics is a type of chaotic advection mixer that consists of a channel containing several helical mixing elements (Chemineer, Dayton, Ohio). As two fluids flow through, these mixing elements cause the fluids to undergo baker's transformations (Figure 1a); the two fluids are repeatedly stretched, split, and stacked on top of one another [6], and increasingly thin layers are produced, facilitating the diffusion of molecules between the two fluids. This design has efficient mixing for large molecules and can reach timepoints from 10 ms and beyond.

Kenics mixers can be fabricated using the Nanoscribe 3D printer at CNF. The Nanoscribe uses two-photon polymerization to print with submicron resolution, which is the level of precision required for our design (Figure 1b). The mixer was designed using Autodesk Inventor and the print settings were programmed in Describe. The inserts were printed with IP-S resin and 70% laser power. After printing, the inserts were placed in propylene glycol monomethyl ether acetate (PGMEA) for development for at least four days. Next, the inserts were submerged in IPA and sonicated for five minutes before UV curing for 60 minutes.

Previous versions of this mixer have been used for time-resolved SAXS (TR-SAXS) measurements [7]. Mixers of this design can also be coupled to other measurements. Absorbance spectroscopy was used to track the binding of myoglobin and azide, which caused a shift in the absorbance that was captured over time (Figure 2). The k value of the binding was accurately extracted from this data, demonstrating that kinetics experiments are possible [7]. Recent, CNF fabricated versions of this device are being used for x-ray spectroscopy measurements, with encouraging preliminary results.

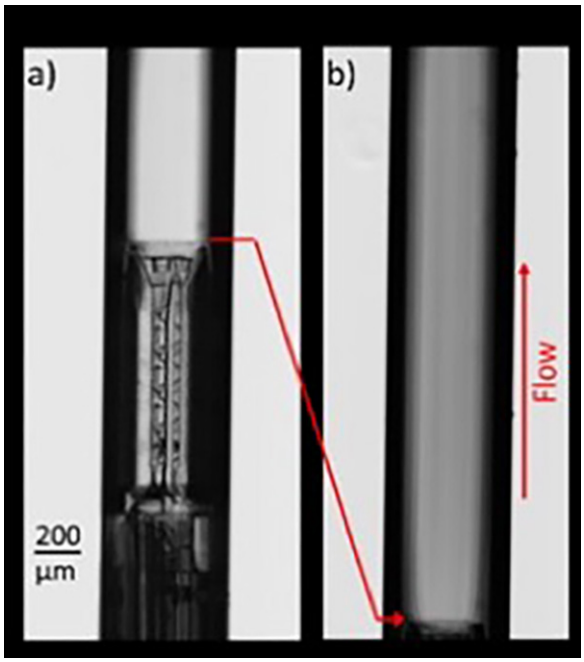

Figure 2: (a) Kenics mixer inside glass capillary absorbance spectroscopy measurements. (b) Fully mixed myoglobin and azide exiting the Kenics (tip of the mixer moved to the bottom of the field of view).

Conclusion and Future Steps:

Due to the high precision of the NanoScribe 3D Printer, we can fabricate a Kenics mixer for time-resolved experiments of a wide range of biological systems and a variety of techniques. Past work demonstrates the utility of this design for probing protein-protein, protein-nucleic acid, protein-ligand, and nucleic-acid ligand reactions with SAXS and absorbance spectroscopy. Future experiments will explore additional biological systems and use other techniques to capture the dynamics of biological reactions.

References:

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