

Integrating Nanofabricated Microfluidic Mixers and Flow Cells for Time-resolved Studies of RNA

CNF Project Number: 2158-12

Principal Investigator(s): Lois Pollack

User(s): Scout Fronhofer, Ilinca Hagi, Andrew Leibowitz

Affiliation(s): Cornell University, Applied and Engineering Physics

Primary Source(s) of Research Funding: National Institutes of Health

Contact: lp26@cornell.edu, shf56@cornell.edu

Research Group Website: <https://pollack.research.engineering.cornell.edu/>

Primary CNF Tools Used: Nanoscribe, Heidelberg DWL2000, Oxford PECVD, ABM mask aligner, Oxford RIE, Unaxis 770 Deep Silicon Etch, Suss SB8e Substrate Bonder, DISCO dicing Saw

Abstract:

RNA molecules serve many important biological functions, and they interact in interesting ways with other molecules, from ions to proteins to other nucleic acids. In order to get insight into RNA's biological roles, it is important to understand how RNA conformations change when they bind with other molecules. To investigate these structural changes we integrate 3D-printed microfluidic mixers with fabricated microfluidic channels, building flow cell devices that allow us to use the same type of mixer for both time-resolved fluorescence and time-resolved x-ray scattering measurements of RNA.

Summary of Research:

RNA interacts with RNA-binding proteins to form complexes that are involved in regulating various cellular processes [1-2]. Measuring how the structure of an RNA molecule changes when it interacts with a binding partner can help us understand the functional role of the RNA. Two useful techniques for characterizing these dynamic molecules are small angle x-ray scattering (SAXS) and fluorescence correlation spectroscopy (FCS). SAXS measures overall size and shape of the molecule, as well as the amount of association/aggregation within the sample, by detecting the x-rays scattered from molecules in solution. FCS provides information about the size and interactions of molecules in solution by measuring the fluctuations in emitted photons from fluorescently labeled molecules as they diffuse in and out of a laser confocal volume [3].

In order to capture the intermediate stages of an interaction between an RNA and its binding partner, we turn to time-resolved methods. Microfluidic mixers allow us to initiate reactions between biological

molecules and then probe the system at different positions along an observation region in order to measure a range of timepoints in the reaction. In past work we developed Nanoscribe 3D-printed microfluidic mixers which enable the rapid mixing of two input solutions. This type of chaotic advection mixer, called a Kenics, consists of a channel containing alternating helical elements[4]. A schematic of the Kenics mixer is shown in Figure 1. As the two solutions flow through the helical elements, they are repeatedly stretched, split, and stacked in a process known as a baker's transformation. This results in increasingly thin layers across which even relatively large biological molecules, like RNAs and proteins, can rapidly diffuse. The Kenics efficiently initiates reactions between RNAs their binding partners and has been used to conduct time-resolved SAXS (TR-SAXS) experiments [5]. In addition we have previously fabricated microfluidic channels that interface with our lab-built confocal microscope [6].



Figure 1: Schematic of a flow cell with a Kenics mixer.

Now, we have put these pieces together, integrating the 3D printed Kenics mixers with fabricated channels, enabling us to perform both TR-SAXS and TR-FCS using the same microfluidic mixer. Figure 2 shows flow cell devices for both techniques. With these microfluidic devices, TR-FCS and TR-SAXS can be used in complementary ways. For example, since FCS provides similar size information to SAXS through a different method, it can serve as a cross-validation. Also, TR-FCS can be performed in our lab to characterize a particular RNA system, allowing us to identify measurement conditions and time-scales of interest before taking the system to a synchrotron x-ray source. We have used these devices to collect data on RNA systems, using TR-FCS in our lab and TR-SAXS at the Advanced Photon Source (APS) at Argonne National Lab.

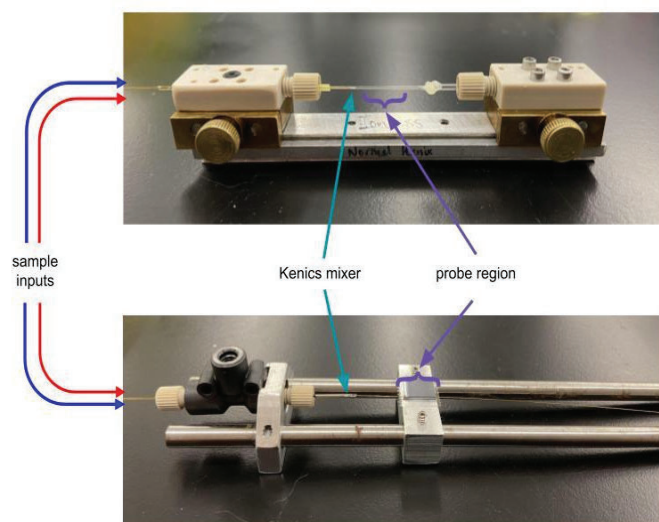


Figure 2: Top: Kenics flow cell device for TR-SAXS. Bottom: Kenics flow cell device for TR-FCS.

Conclusions and Future Steps:

We have built Kenics microfluidic devices for fluorescence applications and begun to use TR-FCS in combination with TR-SAXS to investigate RNA structure and dynamics. Further experiments will apply these methods to RNA-protein interactions to learn about how RNA structure changes in phase-separating systems. Future work may also include improving the time-point range of the TR-FCS device. Currently, the fastest accessible timepoint is limited to a few hundred milliseconds by the low flow speeds needed for TR-fluorescence measurements. However, timepoints in the tens of milliseconds or less could be reached by redesigning the channel and mixer geometry.

References:

- [1] Hentze, M. W., Castello, A., Schwarzl, T., & Preiss, T. (2018). A brave new world of RNA-binding proteins. *Nature reviews Molecular cell biology*, 19(5), 327-341.
- [2] Wadsworth, G. M., Srinivasan, S., Lai, L. B., Datta, M., Gopalan, V., & Banerjee, P. R. (2024). RNA-driven phase transitions in biomolecular condensates. *Molecular cell*, 84(19), 3692-3705.
- [3] Elson, E. L. (2011). Fluorescence correlation spectroscopy: past, present, future. *Biophysical journal*, 101(12), 2855-2870.
- [4] Saatdjian, E., Rodrigo, A. J. S., & Mota, J. P. B. (2012). On chaotic advection in a static mixer. *Chemical Engineering Journal*, 187, 289-298.
- [5] Zielinski, K. A., Katz, A. M., Calvey, G. D., Pabit, S. A., Milano, S. K., Aplin, C., & Pollack, L. (2023). Chaotic advection mixer for capturing transient states of diverse biological macromolecular systems with time-resolved small-angle X-ray scattering. *IUCrJ*, 10(3), 363-375.
- [6] Plumridge, A. (2020). Discovering design principles to re-engineer functional RNA elements using computational and microfluidic approaches. Cornell University.