MS046.O05

Versatile and efficient rapid-mixing liquid jets

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The concept of rapid-mixing for initiation of protein function has been a corner stone of kinetic studies. As with stop-flow experiments, mixing can be used to accurately measure rates of reactions in solution using techniques such as UV-Vis spectroscopy. Although these experiments are rich in information regarding the timescales of protein function, the structural basis for this is still mainly derived main from coupling on static structural studies.
Fast time-resolved structural studies of proteins have now mainly relied on pump-probe experiments where protein function is triggered using laser pulses and the structure probed at a given time-delay. These techniques are only suitable for naturally photoactive proteins or those chemically modified by photo caging agents. Rapid diffusive mixing, on the other hand, allows for the initiation of a wide variety of processes and is not bound by these limitations.
During this work we have designed and fabricated versatile rapid-mixing nozzles capable of delivering ligands into micro-crystals in 100-200 microseconds, allowing for sub- and low-millisecond time-resolved experiments with proteins. The nozzles deliver the crystals as a free liquid jet suitable for XFEL experiments as well as rapid-mixing for time-resolved solution scattering and spectroscopy experiments.
Besides the high speed of mixing achievable with these devices, the soft-lithography approach to fabrication allows for the rapid design and prototyping as well as the reproducible fabrication of multiple nozzles simultaneously. Furthermore, the fabrication approach has been simplified to allow for the dissemination of the technique to the wider user community - an important step towards making TR-measurements more widely available.
In this work we present our devices as well as the extent of time-scales that can be achieved, their use in UV-Vis spectroscopy to define mixing times and their correlation to our 2-phase flow computational fluid dynamic (CFD) simulation of the liquid jet and mixing capabilities of the devices. These CFD simulations, backed by experimental data, allow for fast definition of the expected time-scales for specific experimental cases as well as for the iterative design of novel geometries prior to fabrication of the devices.
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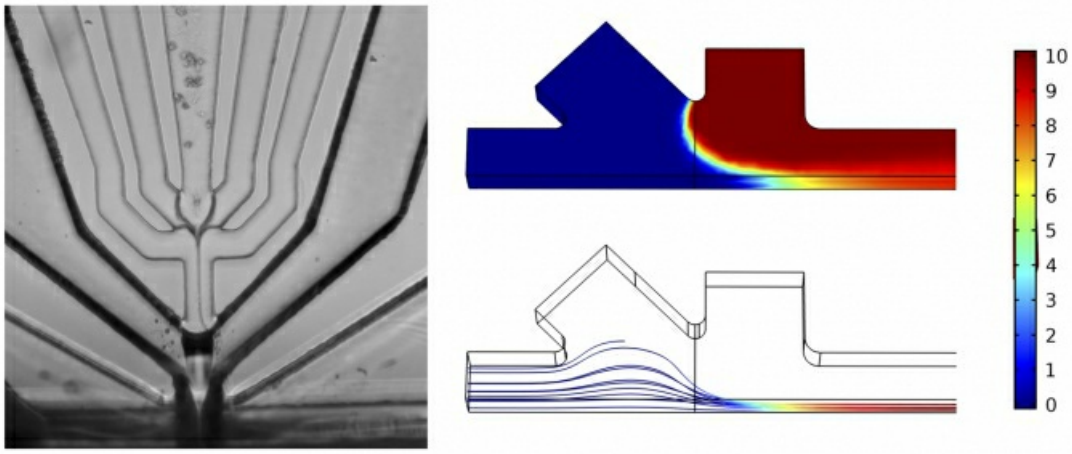

Left: Microfluidic mixing jet flowing 10 µm protein crystals in 40% PEG400 mixed with 20% PEG400 solution containing ligand. The liquid jet is gas flow-focused. Right: CFD simulation of triple-focused mixer. Colour gradient shows change in concentration of ligand. Lines show calculated particle trajectories along the mixer.

Keywords: rapid-mixing, liquid jet, time-resolved