

Time-Resolved Cryo-Electron Microscopy of Ribosomal Subunit Reassociation using Microfluidic Mixing

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Single-particle reconstruction in electron microscopy combines, computationally, multiple orientations of multiple examples of a single structure presented on a TEM grid. In cryo-TEM, macromolecules are frozen in a thin aqueous film (<200 nm) on a thin carbon coated grid in liquid ethane at liquid nitrogen temperature. The single-particle reconstruction method typically captures static structures of macromolecular assemblies. The challenge of time-resolved cryo-EM is to rapidly mix reactants, then deposit them in a thin film in a short time (milliseconds) before plunging into liquid ethane. We have found that spraying the mixture with an air atomizer produces a thin enough aqueous film if the carbon film is freshly made and plasma-cleaned prior to plunging [1]. The mixing, reacting, and spraying steps were accomplished by a monolithic, microfabricated silicon device that incorporated a mixer, reaction channel, and pneumatic sprayer in a single chip. The device incorporates 2 T-mixers flowing into a single channel of 4 butterfly-shaped obstructions followed by a 100- μm wide, 40- μm deep, and 6000- μm long microfluidic reaction channel. This reaction channel is flanked by 2 ports connected to compressed humidified nitrogen gas at 50 psi to form the spray. This mixer sprayer is incorporated into a computer-controlled plunging apparatus [2]. The flow rate was 3 $\mu\text{L}/\text{sec}$ for each of the reactants: 30S and 50S.

This work reports the achievement of thorough mixing and time-resolved reassociation of 30S and 50S ribosomal subunits in the monolithic device. In the current configuration of the device, the mixed reactants spend 4 ms in the outlet channel, 0.4 ms in flight from the nozzle to the grid, and 5 ms on the grid being plunged into the cryogen, for a total reaction time of 9.4 ms. Analysis of the resulting micrographs show about 8% association of the 30S and 50S into the 70S form, consistent with the reaction kinetics of association determined biochemically [3]. Micromixers which allow for shorter and longer reaction times are now under development.

References.

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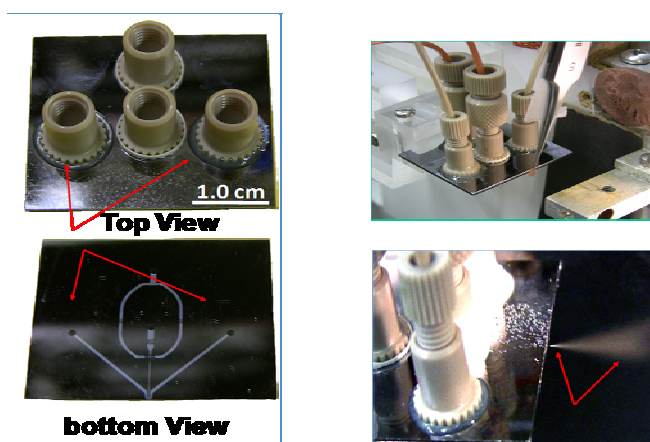


Figure 1. Micromixer. (*left*) Top and bottom views of the silicon micromixer. Arrows point to air inlets. The other two inlets are for the reactants. (*top right*) Micromixer mounted onto the plunging apparatus. (*bottom right*) During spray. Arrows point to spray plume.

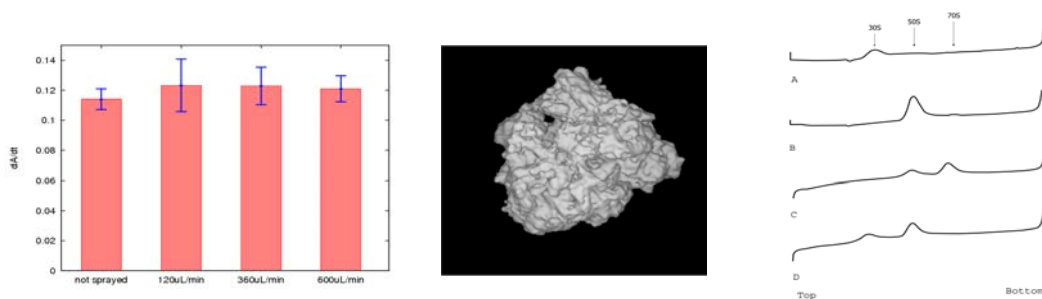


Figure 2. Preservation of specimen upon spraying. (*left*) Activity of malate dehydrogenase as a function of flow rate. (*middle*) Reconstruction of 7,705 70S ribosome images. Nominal resolution was 19Å. (*right*) Sucrose density profile of sprayed subunits: A, 30S subunit; B, 50S subunit; C, 30S+50S with 12 mM Mg²⁺; and D, 30S+50S with 0.1 mM Mg²⁺.