Association of Ribosomal Subunits as Studied by Time-Resolved Cryo-Electron Microscopy

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Abstract: The 30S and 50S subunits of the bacterial 70S ribosome are held together by at least 12 intersubunit contacts. These contacts include RNA-RNA, RNA-protein, and protein-protein interactions.
We have developed and implemented a microfluidic device that mixes two components to completion in 0.4 milliseconds (ms) and sprays the mixture in the form of microdroplets onto an EM grid that is being plunged into cryogen. The total reaction time before cryo-fixation can be as little as 9.4 ms. We have used this device to study association between the two E. coli ribosomal subunits. We have been able to trap association intermediates that form within 9.4 ms, and have determined three-dimensional cryo-EM reconstructions of these association intermediates.
According to our image analysis, about 25% of ribosomal subunits are already engaged in the formation of 70S ribosome particles within 9.4 ms. In the 3D reconstruction of 70S-like particles, bridges such as B2a, B2b, B3, and B7a have already formed, whereas bridges B2c, B4, and B6 are absent. Classification of the 70S-like images reveals a subclass of mature 70S, where the intersubunit bridges appear to have formed, in addition to a subclass corresponding to an immature 70S, where some bridges are missing.
We have recently collected cryo-EM data of subunit association after allowing subunit association for a longer reaction time by using a microfluidic device that allows 43 ms for reaction. At this reaction time, association has proceeded to about 49% completion. The 3D reconstruction of 70S-like particles at 43 ms also shows bridges missing, the same as those found in the reconstruction from the 9.4 ms device. Furthermore, preliminary classification of the 70S-like particles from 43 ms data set again show classes corresponding to mature 70S, with the final bridges formed, and an immature 70S, with several bridges missing. Our analysis of these two data sets suggests that formation of the initial bridges forms on the timescale determined by light scattering, but the final bridges form more slowly.

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