

Ribosome Subunit Joining Frozen in Time

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In this issue of *Structure*, Chen et al. (2015) report the use of a mixing-spraying method of time-resolved cryogenic electron microscopy, which allowed the progression of ribosomal subunit association to be visualized on the millisecond timescale.

Conformational changes of macromolecules and macromolecular complexes that occur on millisecond timescales underlie many cellular processes. Elucidation of these structural rearrangements is particularly important for the investigation of complex and dynamic molecular machines such as DNA and RNA polymerases, the spliceosome, and the ribosome. The functional cycles of these molecular machines consist of many steps and involve sequential interactions with multiple ligands. However, X-ray crystallography and cryogenic electron microscopy (cryo-EM) provide only static snapshots of the most stable conformations of macromolecular complexes, while many transient states and the order of structural transitions escape visualization by the conventional applications of these methods. Hence, time-resolved determination of macromolecular structure is one of the frontiers of modern structural biology.

A number of spectroscopic methods can be used to measure the kinetics of conformational changes on second to millisecond timescales; however, they do not provide a high-resolution depiction of structural transformations. Cryo-EM can, in principle, be used for time-resolved experiments. However, combining the efficient mixing of macromolecules and ligands with quick-embedding in vitreous ice for imaging remains technically difficult. The article by Chen et al., published in this issue (Chen et al., 2015), makes impressive strides toward addressing these challenges by visualizing the progression of ribosomal subunit association via structural snapshots at 9–12 Å resolution acquired on the millisecond timescale.

In bacteria, the formation of 70S ribosomes occurs during the initiation phase

of protein synthesis and involves the binding of mRNA, initiator tRNA, and three initiation factors (IF1, IF2, and IF3) to the small (30S) subunit, followed by the joining of the large (50S) subunit. In vitro, however, the 30S and 50S subunits can associate with each other in the absence of initiation factors, mRNA and tRNA forming the vacant 70S ribosome, thus providing a simple way to study the structural aspects of 70S formation. In particular, it remains unclear whether the intersubunit bridges (i.e., the contacts between the small and large subunits) that stabilize the 70S structure are formed simultaneously or in a stepwise, hierarchical manner. Chen et al. followed the spontaneous association of ribosomal subunits from the bacterium *E. coli* using a recently developed method for time-resolved cryo-EM (Lu et al., 2009). The 30S and 50S subunits were rapidly mixed in a nano-fabricated microfluidic channel and were then sprayed as a plume of droplets, which were deposited over the EM grid and plunge-frozen in liquid ethane (Figure 1). This process took place in a humidity- and temperature-controlled environmental chamber. Changing the length of the microfluidic channel allowed the authors to vary the time between mixing and embedding the sample in the vitreous ice for imaging. 3D reconstructions of 70S ribosomes were obtained at 60 ms, 140 ms, and 15 min after mixing. The number of 70S particles increased over time, whereas the number of the 50S particles decreased, indicating that at 60 ms and 140 ms the sample was under pre-equilibrium conditions.

3D reconstructions of 70S ribosomes were determined at 9–12 Å resolution, thus allowing an unambiguous detection of intersubunit bridges. This is a signifi-

cant improvement over a recently published study of the association of ribosomal subunits (Shaikh et al., 2014) that was conducted using a similar mixing-spraying method of time-resolved cryo-EM, but produced low (23–33 Å)-resolution structures. No stepwise formation of intersubunit bridges was detected by Chen et al., as all of the intersubunit bridges were visualized in newly associated 70S ribosomes even at the earliest time point, i.e., at 60 ms after mixing of ribosomal subunits. This observation is consistent with an early time-resolved hydroxyl-radical RNA probing study of ribosomal subunit joining (Nguyenle et al., 2006) that showed a concurrent formation of intersubunit bridges. Acquiring data at time points earlier than 60 ms after mixing will be required to further validate this conclusion. The bacterial initiation factor IF2, as well as its eukaryotic ortholog, eIF5B, is known to facilitate subunit joining *in vivo* (Melnikov et al., 2012). Hence, future time-resolved cryo-EM studies of ribosome subunit association in the presence of IF2 will be essential for the understanding of the molecular mechanism of subunit joining during the initiation phase of protein synthesis.

Chen et al. also made a number of interesting observations regarding the conformational dynamics of newly assembled 70S ribosomes, which were found to be structurally heterogeneous and were classified into three distinct conformational classes. In 10% of the vacant 70S ribosomes, the small ribosomal subunit was rotated by ~8° relative to the large subunit when compared to the majority (90%) of 70S ribosomes that were observed in the non-rotated conformation. The rotated vacant 70S ribosome reconstructed by Chen et al. is very similar

in conformation to rotated ribosomes containing tRNAs bound in the hybrid (A/P and P/E) states that were previously visualized by cryo-EM and X-ray crystallography (Agirreabala et al., 2008; Dunkle et al., 2011; Julián et al., 2008). Chen et al. also found that the non-rotated vacant 70S ribosomes were composed of two distinct structural classes, which differed in the degree of rotation of the head domain of the 30S ribosomal subunit relative to the rest of the 30S subunit. This rotation of the 30S subunit head, which occurs in the plane orthogonal to the plane of intersubunit rotation, was previously observed in a number of cryo-EM and X-ray structures of the ribosome and was termed “head swivel” (Schuwirth et al., 2005). The ratio of rotated ribosomes, non-rotated ribosomes, and non-rotated ribosomes with head swivel did not change between 60 ms and 140 ms after mixing ribosomal subunits, indicating that the equilibrium between these conformations in the 70S population was established on a timescale faster than the time resolution of this time-resolved cryo-EM study.

Taken together, the time-resolved cryo-EM experiments performed by Chen et al. provide important insights into the structural dynamics of the ribosome

and suggest cooperativity in the formation of intersubunit bridges during subunit joining. Furthermore, this key advance

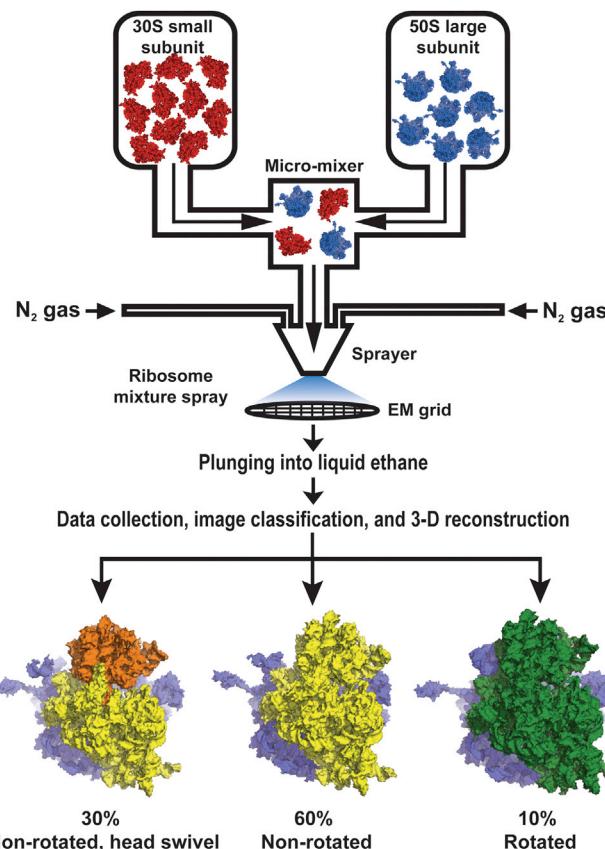


Figure 1. Visualizing the Progression of Ribosomal Subunit Association using a Mixing-Spraying Method of Cryo-EM

Large, 50S (blue), and small, 30S (red), ribosomal subunits are rapidly combined in a micro-mixing chamber before being sprayed onto a cryo-EM grid by humidified nitrogen gas. The grid is rapidly plunged into liquid ethane to trap the ribosomes and ribosomal subunits in vitreous ice for imaging. Three distinct populations of ribosomes were observed: non-rotated ribosomes (~60%), non-rotated ribosomes in which the head of the small subunit was swiveled (~30%), and ribosomal complexes in which the 30S subunit was rotated relative to the large subunit (~10%). The three classes of ribosomes observed by Chen et al. are represented in the figure by equivalent high-resolution crystal structures of *E. coli* ribosomes (Dunkle et al., 2011; Schuwirth et al., 2005). In the non-rotated, head-swiveled ribosome, the body of the small subunit is shown in yellow and the head domain of the small subunit is shown in orange (PDB: 2AWB, 2AW7 [Schuwirth et al., 2005]). The entire small subunit is shown in yellow in the non-rotated ribosome (PDB: 2AVY, 2AW4 [Schuwirth et al., 2005]). The small subunit is shown in green in the rotated ribosome (PDB: 3R8S, 4GD1 [Dunkle et al., 2011]).

in the time-resolved determination of macromolecular structure paves the way for future kinetic studies of the structural

dynamics of the ribosome and other large macromolecular complexes.

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