High-resolution 3D Structure Determination of Dynamic Macromolecular Complexes by Single Particle cryo-EM

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Using the latest developments in electron microscopic hardware combined with advanced computational image processing it is now possible to determine structures of large and dynamic macromolecular complexes at near atomic resolution. We have determined the structure of a 70S ribosome-SelB complex at 3.8 Å resolution which is sufficient to determine a de novo structure of SelB bound to the ribosome. SelB is the elongation factor specific for the delivery of the selenocysteine-tRNA to the ribosome. This also requires a stop codon in the mRNA being recoded into a signal for selenocysteine incorporation by a SECIS element in the pre-mRNA. Selenocystein incorporation is already a rather inefficient process in vivo making the structure determination of SelB bound to the ribosome an evasive target in structural biology for a long time. Successful structure determination in fact requires extensive optimization of the ribosome-SelB complex preparation and image sorting of a rather heterogeneous population of ribosome complexes. This strategy allows not only the structure determination at very high resolution but also the simultaneous structure determination of numerous functionally distinct states of the ribosome-SelB complex.
As a control we also have determined the structure of the ribosome-EFTu complex at 3.2 Å resolution. EF-Tu is the elongation factor responsible for transport of all canonical aminoacyl-tRNAs to the ribosome. Having both structures available at high resolution we obtained a detailed view of how the ribosome can be hijacked by SelB to allow the recoding of a stop signal into a signal for selenocysteine incorporation.