Analysis of molecular dynamics by 3D cryo-EM: the examples of HSP90 and Ribosomal complexes

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Single particle cryo-electron microscopy (cryo-EM) has achieved remarkable results in analysis and three-dimensional reconstruction of macromolecular complexes that can be captured in their natural state. Like most structural methods, this approach relies on averaging techniques. However structural and functional heterogeneity of a sample has usually been considered as a major obstacle for 3D structure determination. Finding solutions for separating images of molecules in different states has become a cornerstone of EM structural analysis. Finally when heterogeneity can be handled through image processing and particle sorting, it turns into an advantage by allowing the study of multiple states in equilibrium with each other, providing a 4D view of dynamics and macromolecular transitions related to a precise biological function. Our studies on Hsp90 (a 90 kDa heat-shock protein) [1] and on a tmRNA-ribosome complex [2] illustrate this approach.

Hsp90 plays a key role in the folding and activation of many client proteins involved in signal transduction and cell cycle control. The cycle of Hsp90 has been intimately associated with large conformational rearrangements, which are nucleotide-binding-dependent. However, up to now, our understanding of Hsp90 conformational changes derives from structural information, referring to the crystal states of either recombinant Hsp90 constructs or the prokaryotic homologue HtpG. By combining small-angle X-ray scattering and single-particle cryo-EM, we have obtained the first nucleotide-free structures of the whole eukaryotic Hsp90 (apo- Hsp90). In solution, apo-Hsp90 is in a conformational equilibrium between two open states. The structural changes involved in switching between the two Hsp90 apo-forms require large movements of the N-terminal domain and middle domain around two flexible hinge regions. Although large structural rearrangements, leading to partial closure of the Hsp90 dimer, were previously attributed to the binding of nucleotides, our results reveal that they are in fact mainly due to the intrinsic flexibility of the Hsp90 dimer.

In eubacteria, translation of defective messenger RNAs (mRNAs) produces truncated polypeptides that stall on the ribosomes. A quality control mechanism referred to as trans-translation is performed by transfer-messenger RNA (tmRNA), a specialized RNA acting as both a tRNA and an mRNA, associated with Small protein B (SmpB). So far, a precise description of the structural movements necessary to perform accommodation is still lacking. By using a construct containing the tRNA-like domain as well as the extended helix H2 of tmRNA we have studied the process of accommodation. The 3D structure shows how tmRNA and SmpB move into the ribosome decoding site after the release of EF-Tu•GDP.

The SmpB protein interacting with the small subunit decoding site stays in place while the one interacting with the large subunit moves away. Relative to canonical translation, an additional rotation of H2 is observed, suggesting that the large movement required to resume translation on tmRNA internal open reading frame starts during accommodation.

1 BRON P. et al., *Biol Cell*., **100**, (2008) 413-425.
2. WEIS F. et al. *submitted for publication*.

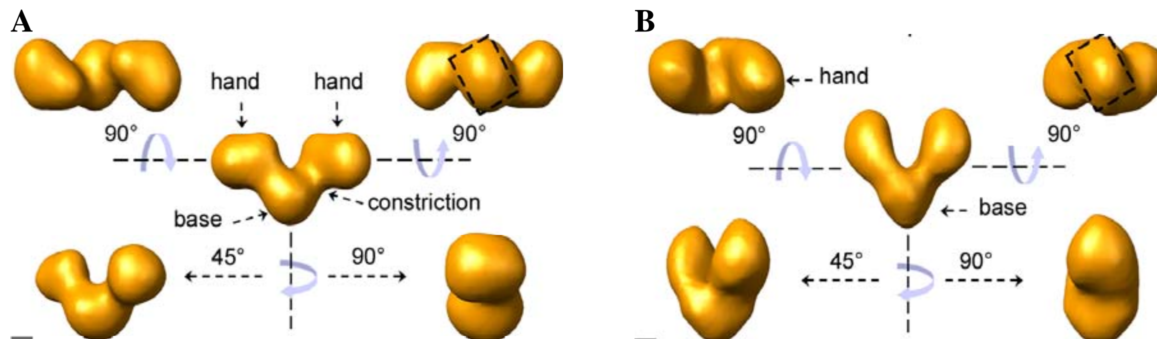

Figure 1. Three-dimensional reconstructions of the native eukaryotic apo-Hsp90 obtained by single-particle cryo-EM analysis (**A**) Different views of the predominant state. A total of 5587 images were included in the three-dimensional map. The model was built with C2 symmetry. The rectangular shape at the base of the three-dimensional volume is shown (dotted rectangle). (**B**) Same as (**A**), but showing the minority state of apo-Hsp90. A total of 598 images were included in the EM map.

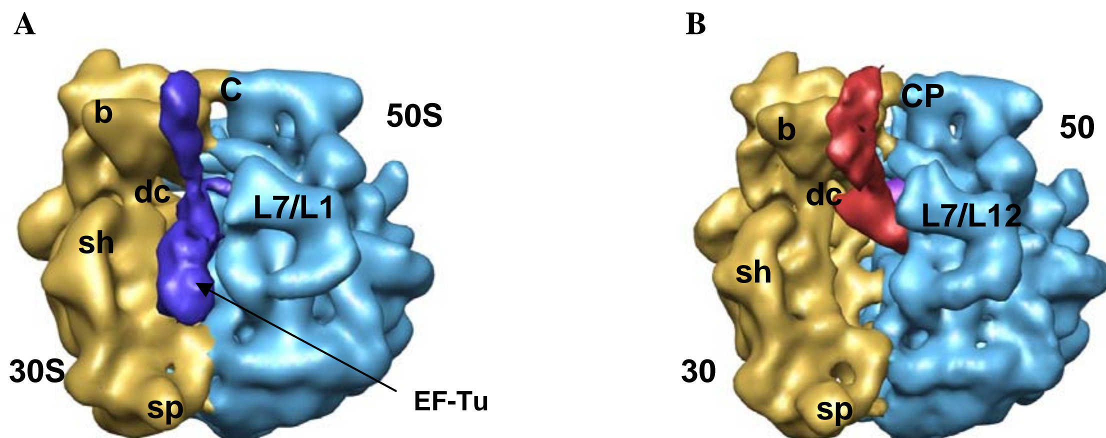

Figure 2. **Reconstruction of PKF-SmpB complex bound to the stalled ribosome**. (**A**) Cryo-EM map generated for one subset sorted by the local MSA method, corresponding to a pre-accommodated state before the release of EF-Tu. Density for EF-Tu-PKF-SmpB is in blue. (**B**) Cryo-EM map generated for the third subset and corresponding to the accommodated state after the release of EF-Tu·GDP. Density for PKF-SmpB is in red, the P-site tRNA is in purple.

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