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Conformational Heterogeneity of Macromolecules Analyzed by Cryo-Electron Microscopy

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In cryo-electron microscopy (EM), thanks to rapid freezing of the specimen, macromolecular assemblies and machines can be captured in their native, aqueous environment, and their native structure is preserved. In addition to the expected conformational heterogeneity of the assemblies that is due to fluctuations of the structure around the ground state, one can expect to capture molecules in different functional states, especially if the binding of a ligand induces a conformational change in the macromolecular assembly. Therefore, data set of images from an EM experiment must be interpreted as a mixture of projections from similar but not identical structures.

In order to separate images of molecules that are ligand-bound from those that are unbound and to reveal the conformational variability of the complex, we have developed two dedicated tools: (i) real-space analysis of 3-D variance/covariance in macromolecules reconstructed from a set of their projections [1] and (ii) a 3-D classification technique that enforces consistency among subsets of projections [2]. The variance in the 3-D mass distribution of the structure is calculated using a statistical bootstrap resampling technique, in which a new set of projections is selected with replacements from the available whole set of projections. In the new set, some of the original projections will appear more than once, while others will be omitted. This selection process is repeated a number of times and for each new set of projections the corresponding 3-D volume is calculated. Next, the voxel-by-voxel variance of the resulting set of volumes is calculated. The target variance is obtained using a relationship between the variance of arithmetic means for sampling with replacements and the sample variance. We applied this technique to data from a ribosomal complex containing EF-G and tRNAs (the 3-D volume is shown in Fig.1a) and calculated the 3-D variance map (Fig.1b). The variance map revealed high variability of the structure in the region of the intersubunit space of the ribosome, at the expected locations of tRNAs binding sites, at the location of the EF-G binding site, and around the L1 protein. These results indicated that the data set collected contained a mixture of various ribosome populations distinguished by ligand occupancy and movement of L1. Thus, we proceeded with the classification of the projections.

In the initial step of the classification scheme developed, for all directions of a coarse angular grid, experimental projections that fall in the same projection direction were sorted into two groups. In order to enhance the contribution of the ligand, clustering was performed using a circular mask in the expected position of the ligand (as known from other experiments and confirmed by the analysis of variance). Initial 3-D reconstructions were calculated from all those classes that had higher than average density within the area of the mask. The separation was further improved by performing a refinement with the projection matching procedure modified to work with two reference

volumes, starting with the two initial reconstructions and allowing the particles to move between the two sub-sets of experimental projections. This analysis has permitted us to separate a population in which EF-G exists with high occupancy in the intersubunit space (Fig.1c) from a population with low EF-G occupancy (Fig.1d).

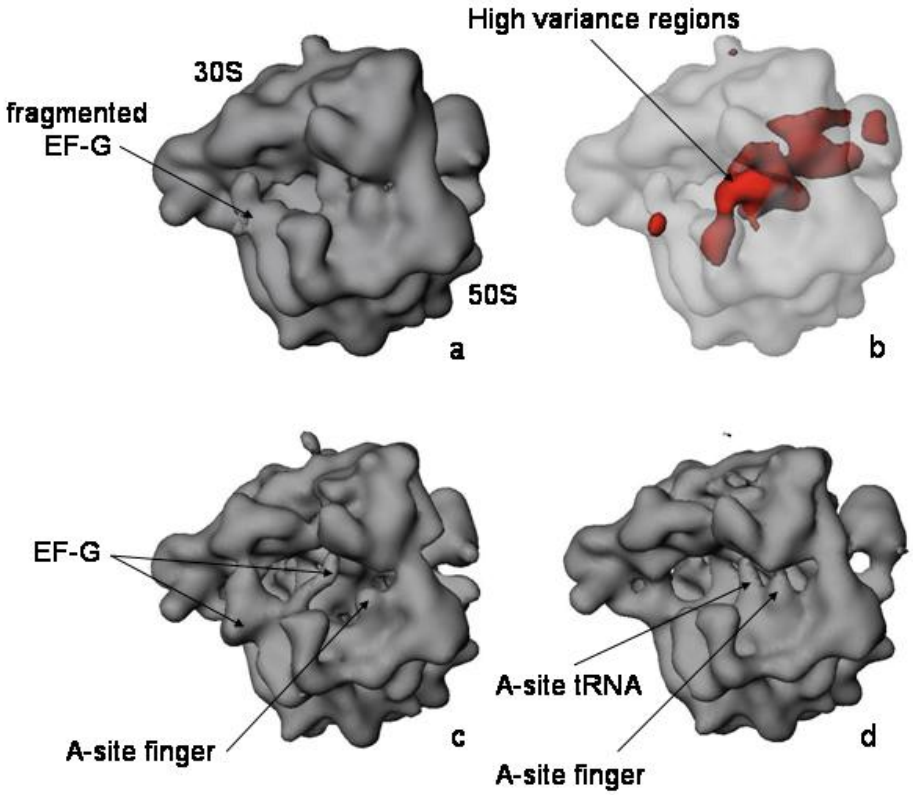

Fig.1 Analysis of variance and classification of a cryo-EM 3-D reconstruction of the 70S *E. coli* ribosome complexed with EF-G and tRNAs. (a) 3-D reconstruction of the 70S ribosome using the whole set of projections with two subunits (30S and 50S) marked. (b) Regions of high variance (shown in dark) within the complex shown in (a). 70S ribosome reconstructions, calculated from subsets of projections obtained using the developed classification scheme, showing (c) high EFG occupancy and (d) low EFG occupancy.

[1] P.A. Penczek *et al.*, Estimation of variance in single particle reconstruction using the bootstrap technique. *J. Struct. Biol.*, in press.
[2] P.A. Penczek *et al.*, A method of focused classification, based on the bootstrap 3-D variance analysis, and its application to EF-G-dependent translocation. *J. Struct. Biol.*, in press.

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