Structure of Protein Assemblies by Comparative Modeling and Electron Microscopy

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We explore structural characterization of protein assemblies by a combination of electron cryo-microscopy (cryoEM) and comparative protein structure modeling (1). Specifically, our method finds an optimal atomic model of a given assembly subunit and its position within an assembly by fitting alternative comparative models into a cryoEM map. The alternative models are calculated by MODELLER (2) from different sequence alignments between the modeled protein and its template structures. The fitting of these models into a cryoEM density map is performed by a new density fitting module of MODELLER (Mod-EM). Identification of the most accurate model is based on the correlation between the model accuracy and the quality of fit into the cryoEM density map. To quantify this correlation, we created a benchmark consisting of eight proteins of different structural folds with corresponding density maps simulated at five resolutions from 5 to 15 Å, with three noise levels each. Each of the proteins in the set was modeled based on 300 different alignments to their remotely related templates (12-32% sequence identity), spanning the range from entirely inaccurate to essentially accurate alignments. The benchmark revealed that one of the most accurate models can usually be identified by the quality of its fit into the cryoEM density map, even for noisy maps at 15 Å resolution. Therefore, a cryoEM density map can be helpful in improving the accuracy of a comparative model. Moreover, a pseudo-atomic model of a component in an assembly may be built better with comparative models of the native subunit sequences than with experimentally determined structures of their homologs.

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