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LS-4-O-2305 Revealing drug-induced conformational states of macromolecular complexes by high-resolution cryo-electron tomography

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Drug-induced conformational changes of macromolecular complexes provide indispensable insight into the function of cellular nanomachines and aid, among other things, to rationally design drugs targeting them. Unfortunately, only a few techniques exist, that allow observation of those changes in close-to-native environment.

Cryo-electron tomography (CET) is emerging technique that allows determining three-dimensional structure of macromolecular complexes in quasi-native environment by studying frozen-hydrated samples. However, despite providing tremendous insights into structure and organization of cellular organelles, flagella, microtubules and other large and/or symmetric objects, its ability to observe smaller asymmetric macromolecular complexes in resolution sufficient to reveal drug-induced conformational states has been disputed so far.

We present a high-resolution cryo-electron tomography pipeline (HRCET), which builds on open software (IMOD and PyTom) and combines state-of-the-art computational and imaging techniques like cryo-electron tomography (CET) with direct electron detector (DED), sub-volume averaging (SVA), robust contrast transfer function (CTF) correction, template-bias prevention or X-ray fitting to allow for unambiguous detection of moderate-sized conformational changes on even asymmetric molecular nanomachines.

Using the proposed pipeline, we detect antibiotic-induced change of 70S E. Coli ribosome. We show that drug-stabilized, drug-free and partially-assembled states can be computationally separated with sufficient resolution to allow for docking of high-resolution X-ray structures, facilitating biological interpretation of the observed changes. We also show that a semi-quantitative estimation of the drug affinity is possible using our technique.

The presented pipeline for high-resolution cryo-electron tomography (HRCET) represents another step towards user-friendly, highly reproducible workflow necessary for widespread application of cryo-electron tomography, ultimately allowing HRCET to become a reliable tool for answering some of the key questions that arise in practical drug design process.

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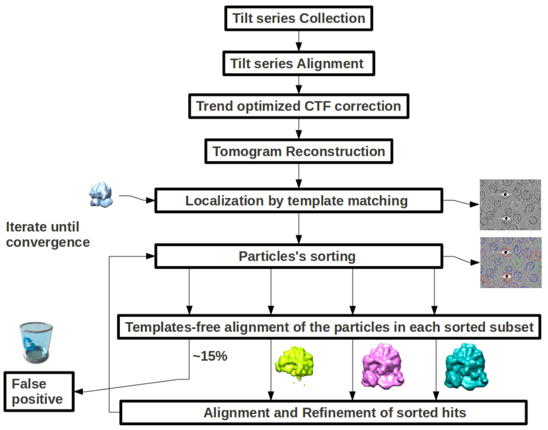

Fig. 1: Workflow for high-resolution cryo-electron tomography.Tilt series are collected using Titan KRIOS with Falcon I DED. IMOD (http://bio3d.colorado.edu/imod/) package combined with our own code is used to align and reconstruct the tomograms. Using PyTom (http://pytom.org/), particles are localized and averaged to arrive at final structures.

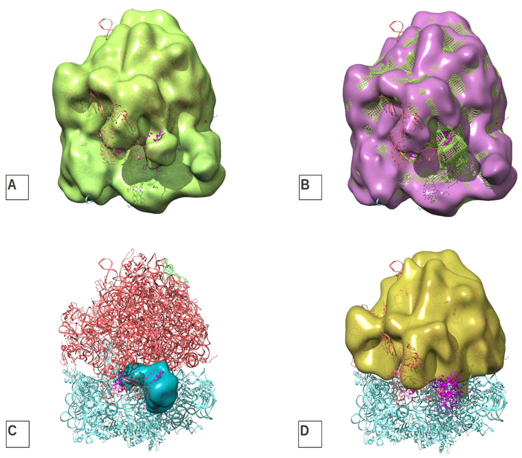

Fig. 2: Analysis of the found conformational states of 70S ribosome.A) 70S ribosome with EF-G. B) 70S only. Density relative to EF-G is overlapped as a green mesh. C) Density corresponding to the EF-G alone. D) Map of the 50S only, which was also identified in the datasets. Atomic ribbons are fitted X-ray structures (2WRI, 2WRJ).