

## Cryo-EM of small proteins using designed assemblies as modular scaffolds

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Current advanced in cryo-electron microscopy have enabled the atomic level structure determination of extraordinary structures from viruses to large protein and nucleic acid complexes. However, signal-to-noise limitations make it extremely difficult to apply cryo-EM techniques to macromolecules smaller than about 50 kDa [1]. Breaking through this lower size limit is a key goal for the field, given that the average size for cellular proteins is in the 20 to 30 kDa range.

Recent developments in protein design have made it possible to create highly geometric protein assemblies, like cubic cages or clusters, with atomic level accuracy [2,3]. In new work we have used designed assemblies as scaffolds to which other small 'cargo' proteins can be attached for imaging by cryo-EM [4,5]. Two key challenges have been addressed in our scaffold designs. First, semi-rigid display of a cargo protein has been demonstrated using continuous alpha helical linkers between proteins. Second, modularity has been achieved by engineering a DARPin component as an adaptor module; DARPins can be selected for binding to diverse protein molecules. The scaffold cores are based on cubically symmetric (T) designed assemblies, providing additional advantages in symmetry for data processing and mitigation of the often recalcitrant problem of preferred orientation in cryo-EM. So far we have achieved a resolution of about 3.8Å for a 26 kDa cargo protein, GFP.

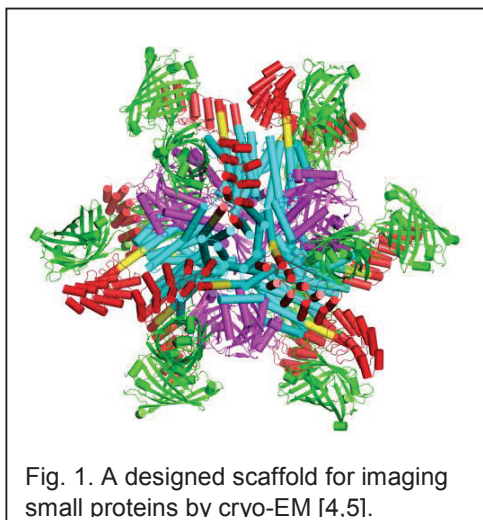


Fig. 1. A designed scaffold for imaging small proteins by cryo-EM [4,5].

### References:

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