A Designed Scaffold for Near-Atomic Resolution Cryo-EM Imaging of Small Proteins

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Project Goals: Research in the UCLA-DOE Institute for Genomics and Proteomics includes major efforts in imaging technology development. The specific goal of this work is to make it possible to determine high resolution structures of proteins smaller than 50 kDa by cryo-EM, so that this powerful technique can be applied to cellular proteins and enzymes within the research scope of the DOE.

Abstract:

Recent technical advances in cryo-electron microscopy (cryo-EM) have made it possible to determine the three-dimensional structures of large protein assemblies and macromolecular complexes with atomic level detail. However, proteins smaller than about 50 kDa are currently too small to be imaged at high resolution by cryo-EM, leaving most protein molecules in the cell beyond the reach of this powerful structural technique. In recently published work (1), we designed a modular, symmetric scaffolding system to rigidly bind and display smaller proteins to make them amenable to visualization by cryo-EM. Our novel protein scaffold solves two key problems – rigidity and modularity – that have limited the utility of previous scaffolding methods. A designed protein cage with cubic symmetry serves as the core. A small 17-kDa protein (DARPin) serves as a modular adaptor, which can be edited (e.g. based on phage display experiments) to bind a wide range of target proteins. The DARPin adaptor (which is itself alpha-helical) is genetically fused to the self-assembling cage subunit in a semi-rigid fashion by a continuous alpha helical connection. Imaging the scaffold by itself (without a bound cargo protein) showed that the DARPin was held rigidly enough to visualize it at near-atomic resolution (1). In new work (2), this protein scaffold is used for the first time to bind and display 12 copies of a small 26 kDa protein, sfGFP. We show that the bound cargo protein is held rigidly enough on the exterior of the scaffold to visualize it at a resolution of 3.8 Å. Structural details of the cargo protein are visible, making it the first demonstration of near-atomic resolution for a protein smaller than 50 kDa by cryo-EM. The designed scaffold is modular and can be modified through modest changes in its amino acid sequence to bind and display diverse proteins for imaging, thus providing a general method to break through the lower size limitation in cryo-EM. Future aims for optimization and broader application are noted.

Publications

1. Liu Y, Gonen S, Gonen T, Yeates TO. (2018). Near-atomic cryo-EM imaging of a small protein displayed on a designed scaffolding system. *Proc Natl Acad Sci USA* **115**, 3362-3367.
2. Liu, Y., Huyn, D., and Yeates, T.O. (2018) A 3.8 Å Resolution Cryo-EM Structure of a Small Protein Bound to a Modular Imaging Scaffold, https://www.biorxiv.org/content/10.1101/505792v1

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