Novel Designed Rigidified Imaging Scaffolds For High-Resolution Structure Determination Of Small Proteins With Cryo-EM

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Numerous technical advances have made cryo-EM an attractive method for atomic structure determination. Cryo-EM is ideally suited for very large structures; symmetrical structures like viruses are especially amenable. However, problems of low-signal-to-noise in imaging small proteins makes it practically impossible to determine structures smaller than about 50 kDa, leaving a great many cellular proteins and enzymes (and nucleic acid molecules) outside the reach of this important structural technique. We have developed symmetric protein imaging scaffolds to display and solve the structure of small proteins. In earlier work (Liu Y, Huynh DT, Yeates TO. A 3.8 Å resolution cryo-EM structure of a small protein bound to an imaging scaffold. Nat Commun. 2019), we broke through this barrier by engineering novel scaffolds with sufficient rigidity and modularity to achieve resolution useful for interpreting atomic structure, reaching 3.8 Å resolution for a 26 kDa protein. To overcome the challenges of flexibility between the protein target and the imaging scaffold and further improve resolution, we have developed new computational tools to model (and then limit) range-of-motion by designing additional interfaces to rigidify our imaging scaffolds. With the new rigidified scaffolds, we can solve structures of small proteins at near-atomic resolution, reaching 3 Å resolution for sub-30 kDa protein targets. Current examples will be presented.