Oligomeric self-assembly of a coiled-coil-based bipyramidal protein cage

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Coiled-coils (CC) are one of the most widespread type of protein super-secondary structure in nature and, due to their properties, have been widely used in protein engineering. Specific arrangements of CC dimers in a polypeptide chain can be used to design *de novo* polyhedral protein cages. However, the design of these modular nanostructures, named Coiled-Coil Protein Origami (CCPO), is intrinsically limited by the number of available orthogonal CC elements. This limitation can be surpassed by using an oligomeric bottom-up approach to cage self-assembly, thus facilitating the design of larger cages. Here, we describe the design and characterization of oligomeric CCPO assemblies with the case study of a bipyramidal CCPO cage, designed both as single-chain and as heterodimeric complex, showing a way to build oligomeric CC-based protein cages that undergo controlled self-assembly. Biophysical characterization and small angle X-ray scattering (SAXS) confirmed the proteins assumed the intended conformation in solution in all cases but one, indicating how the implementation of favorable topologies was crucial for the correct self-assembly of the protein cage. Additionally, we showed how, with the introduction of a protease recognition site in the heterodimeric CCPO bipyramid, we obtained a protein able to undergo an irreversible structural rearrangement and transition from an open conformation to a closed bipyramidal conformation, paving the way for designing dynamic and more complex protein cages.