An Approach to Produce Uniform Artificial Protein Supramolecules

The design of artificial protein supramolecules has attracted great interest in developing novel medical nanomaterials. However, the production of uniform protein supramolecules remains a major challenge. We report a simple approach to produce uniform artificial protein supramolecules based on the geometric features of polyhedra. We demonstrated that the truncated icosahedral structure (soccer-ball shape) was uniformly constructed from the fusion protein composed of pentameric and dimeric protein domains. The supramolecule assembled from our designed fusion proteins was analyzed by small-angle X-ray scattering. Analysis showed that the molecular weight, diameter and overall shape were highly consistent with our designed structure.

Biocompatible protein-based nanomaterials have long been believed to be promising medical materials such as for vaccines and drug carriers [1]. Although various approaches to produce protein-based nanomaterials have been developed, most of them were based on random protein aggregations giving non-uniform materials, which is not desirable for applications. The key to producing uniform protein materials is the design of protein supramolecules produced by specific protein-protein interactions without random aggregation. One simple way to form protein supramolecules is to design a fusion protein composed of two different naturally oligomeric proteins. For example, Yeates' group successfully produced cubic protein supramolecules by using a fusion protein composed of the subunits from dimeric and trimeric proteins, respectively [2]. Although this is a very simple method of constructing protein supramolecules, three different shapes of protein supramolecule—cube, triangle prism, and triangle pillar—are produced at the same time owing to the geometric features of polyhedra.

In accordance with Euler's polyhedron formula (Eq. 1), we found that soccer-ball shape polyhedra (truncated icosahedron) could be produced as the sole structure if we designed a fusion protein composed of pentameric and dimeric proteins:

$$V + E - F = 2 \quad \text{(Eq. 1)}$$

where V, E and F are the number of vertices, edges and faces of polyhedra, respectively. The equation suggests that the number of pentagons is always 12 if a polyhedron is only composed of pentagons and hexagons such as a soccer-ball shape [3]. The hexagonal planes of the soccer-ball shape can be depicted by six line-segments provided from three different pentagonal planes (each pentagonal plane provides one line-segment) and three line-segments connecting the vertices of the pentagonal planes. The dimeric protein units in the fusion proteins connect the pentagonal protein units to form hexagonal planes, followed by forming a soccer-ball shape structure as shown in **Fig. 1**. We thus selected the template proteins, LSm protein as a pentameric protein unit and a coiled coil part of Myosin X (MyoX-coil), as a dimeric protein unit [4].

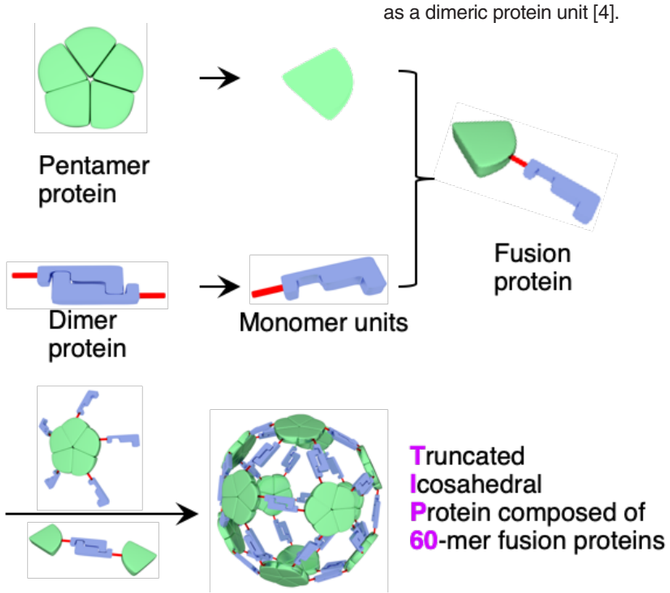

Figure 1: Schematic representation of our design approach to produce uniform protein supramolecules. Green colored pentamer and blue colored dimer proteins correspond to LSm and MyoX-coil, respectively. Copyright (2018) Wiley-VCH. Used with permission from ref. [4].

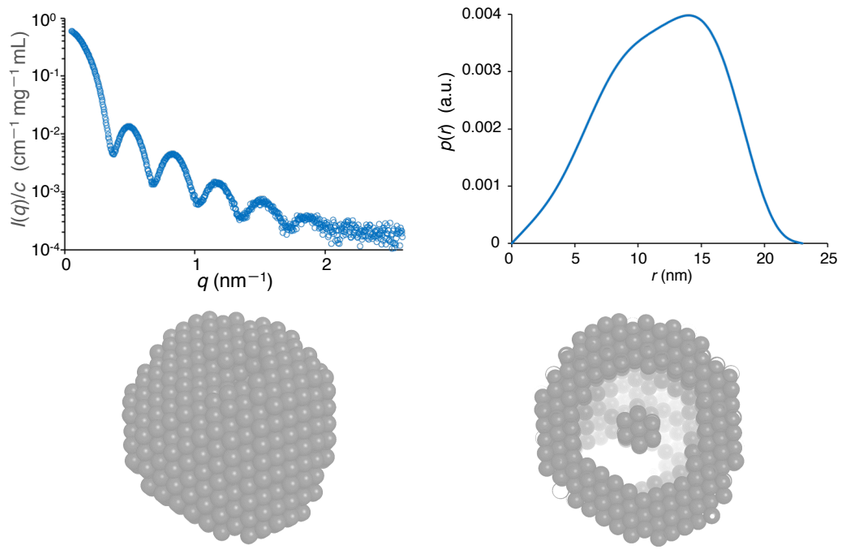

Figure 2: SAXS analysis data. The SAXS intensity of the protein supramolecule (upper left) and its pair-distribution function (upper right). A dummy atom model of the protein supramolecule: a full view (lower left) and cross-sectional view (lower right). (SASBDB accession code: SASDDZ8). Copyright (2018) Wiley-VCH. Used with permission from ref. [4].

The designed fusion protein was expressed in *E. coli* and purified by a Ni column. Complex formation of the purified protein was first analyzed by Blue-Native PAGE. The result clearly showed a single band at the molecular weight position corresponding to 960 kDa, suggesting that the molecular weight value was consistent with 1070 kDa calculated based on our designed 60-meric protein supramolecule. We therefore analyzed the structure by small-angle X-ray scattering (SAXS). For the SAXS analysis, the protein complex was further purified by an anion exchange column, followed by a size exclusion column. The SAXS profile of the purified protein complex showed the typical pattern of uniform spherical particles (**Fig. 2**). The radius of gyration and the molecular weight of the protein complex supramolecule were determined from the Guinier plots as 9.47 nm and 1063 kDa, respectively. The pair-distance distribution function $p(r)$ obtained from the SAXS data gave the maximum particle distance (D_{max}) as 23 nm. Interestingly, the shape of the distribution pattern was slightly left skewed, suggesting that the protein supramolecule has an inner space as reported in apo-ferritin, which is a natural hollow protein supramolecule [5]. The dummy atom model of the protein complex was reconstructed from the SAXS data using the *ab initio* modeling program DAMMIF [6]. The model structure also indicated that the protein supramolecule had a hollow spherical shape.

The shape, molecular weight and diameter of this protein supramolecule were also confirmed by transmission electron microscopy, dynamic light scattering analysis and SEC-MALS analysis [4]. All the analyses gave values that were consistent with those obtained from SAXS analysis. We thus designated the protein complex supramolecule as TIP60 (Truncated Icosahedral Protein composed of 60-mer fusion proteins). We also demonstrated that both the interior and exterior surfaces of TIP60 were functionalized by chemical modification [4]. Therefore, we believe that TIP60 can be used as a biomedical nanomaterial for various applications.

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