Self-Assembling Supramolecular Nanostructures Constructed from *de Novo* Extender Protein Nanobuilding Blocks

To construct chain-like polymeric nanostructures, we designed *de novo* extender protein nanobuilding blocks (ePN-Blocks) by tandemly fusing two *de novo* binary-patterned proteins with various linkers. The ePN-Blocks with long helical linkers or flexible linkers were constructed and analyzed by native PAGE, size exclusion chromatography-multiangle light scattering (SEC-MALS), small-angle X-ray scattering (SAXS), and transmission electron microscopy, suggesting the formation of various structural homooligomers. Subsequently, we reconstructed heterooligomeric complexes from extender and stopper PN-Blocks by denaturation and refolding. The SEC-MALS and SAXS analyses showed that extender and stopper PN-Block (esPN-Block) heterocomplexes formed different types of extended chain-like conformations depending on their linker types.

Living organisms are maintained by various self-assembling biomolecules including proteins, nucleic acids, sugars, and lipids. The structural and functional design of artificial proteins and protein complexes as desired is one of the ultimate goals of protein science and protein engineering. To achieve this goal, we consider that the design of artificial proteins that self-assemble into supramolecular complexes is an important step in the emerging field of "synthetic structural biology." Several years ago, we solved the three-dimensional structure of a *de novo* protein WA20 designed by binary patterning. The WA20 formed an intermolecularly folded dimeric 4-helix bundle structure [1]. Recently, we designed and constructed a protein nanobuilding block (PN-Block), WA20-foldon, by fusing an intermolecularly folded dimeric *de novo* protein WA20 and a trimeric foldon domain of T4 phage fibritin [2]. WA20-foldon formed several types of self-assembling nanoarchitectures in multiples of 6-mers, including a barrel-like hexamer and a tetrahedron-like dodecamer.

In this study, to construct chain-like polymeric nanostructures, we designed *de novo* extender protein nanobuilding blocks (ePN-Blocks) by tandemly fusing two *de novo* binary-patterned WA20 proteins with helical or flexible linkers (**Fig. 1**) [3]. The ePN-Blocks with long helical linkers or flexible linkers were expressed in soluble fractions of *Escherichia coli*, and the purified ePN-Blocks were analyzed by native PAGE, size exclusion chromatography-multiangle light scattering (SEC-MALS), small-angle X-ray scattering (SAXS), and transmission electron microscopy. These results suggest the formation of various structural homooligomers.

Subsequently, we reconstructed heterooligomeric complexes from extender and stopper PN-Blocks by denaturation and refolding. The SEC-MALS and SAXS analyses showed that extender and stopper PN-Block (esPN-Block) heterocomplexes formed different types of extended chain-like conformations depending on their linker types. **Figure 2** shows three-dimensional structural modeling of the esPN-Block (HL4) heterocomplex, e1s2 (HL4), based on SAXS analysis [3]. A rigid-body model structure of one extender and two stopper PN-Blocks [e1s2 (HL4)] was constructed based on the crystal structure of the WA20 dimer (PDB code 3VJF) [1] to explain the experimental $p(r)$ with consideration of the helical linker rigidity following linking of C and N terminals. The rigid-body model [**Fig. 2(A)**] shows an extended Z shape, and the simulated $I(q)$ and $p(r)$ from

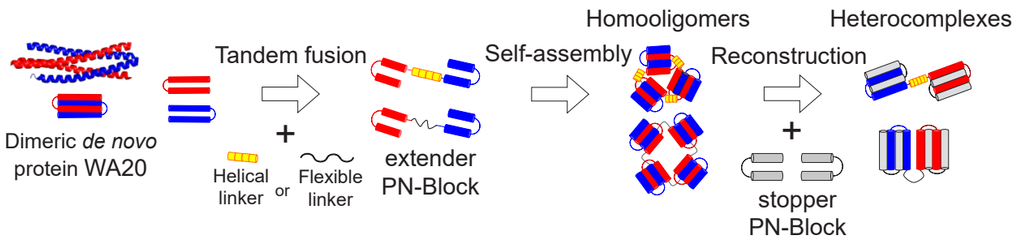

Figure 1: Schematic illustration of design and construction of extender protein nanobuilding blocks (ePN-blocks) and their complexes [3].

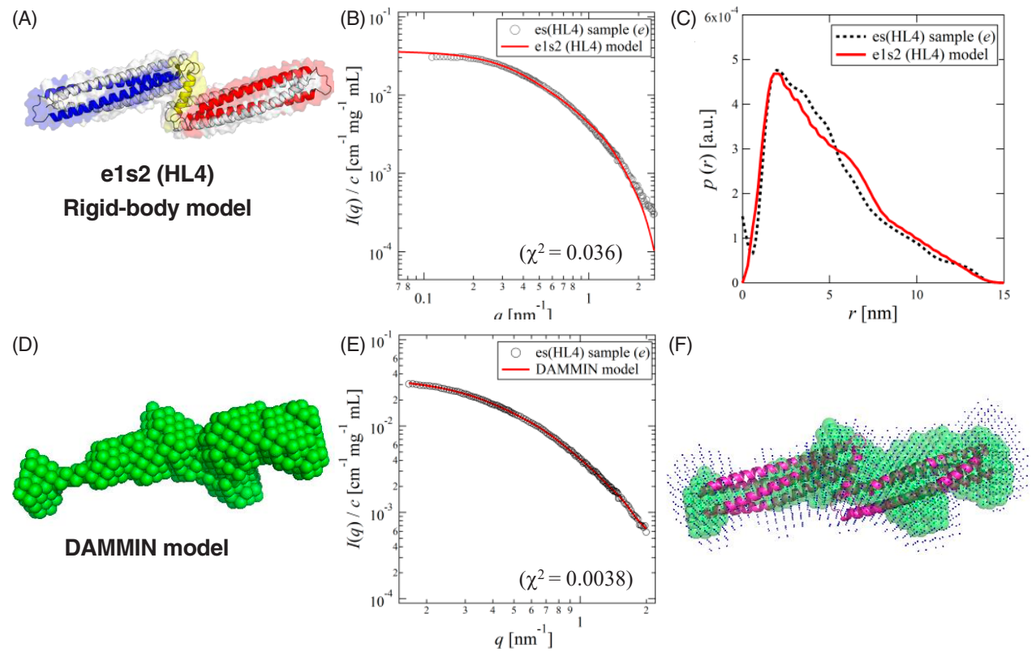

Figure 2: Three-dimensional structural modeling of the esPN-Block (HL4) heterocomplex [e1s2 (HL4)] based on SAXS analysis. **(A)** A rigid-body model structure of one extender and two stoppers (e1s2) of the esPN-Block (HL4) heterocomplex. **(B)** The concentration-normalized scattering intensity of the esPN-Block (HL4) heterocomplex [es (HL4)] sample obtained by the SAXS experiment (open circles) and fitting of $I(q)$ simulated from the rigid-body model of e1s2 (HL4) (red line). **(C)** The pair-distance distribution function $p(r)$ of the esPN-Block (HL4) heterocomplex sample (*e*) calculated from the SAXS data (black dash line) and $p(r)$ simulated from the rigid-body model of e1s2 (HL4) (red line). **(D)** A dummy atom model of e1s2 (HL4) reconstructed from the SAXS data using *ab initio* modeling programs DAMMIF and DAMMIN. **(E)** The concentration-normalized scattering intensity of the esPN-Block (HL4) heterocomplex sample (black open circles) and fitting of $I(q)$ simulated from the DAMMIN model of e1s2 (HL4) (red line). **(F)** Superimposition of the rigid-body model (magenta ribbon representation) and the dummy atom model (green) of e1s2 (HL4). Blue dots represent an averaged model from ten structural models calculated by DAMMIF. Reprinted from reference [3] with permission. Copyright © 2018, American Chemical Society.

the rigid-body model closely resemble those from the SAXS experiment [**Fig. 2(B)** and **2(C)**]. Moreover, the low-resolution dummy atom model was reconstructed from the SAXS data using *ab initio* shape modeling programs DAMMIF and DAMMIN [**Fig. 2(D)** and **2(E)**]. The DAMMIN model is superimposed on the rigid-body model as shown in **Fig. 2(F)**.

Moreover, atomic force microscopy imaging in liquid suggests that the esPN-Block heterocomplexes with metal ions further self-assembled into supramolecular nanostructures on mica surfaces. Taken together, the present study demonstrates that the design and construction of self-assembling PN-Blocks with *de novo* proteins is a useful strategy for building polymeric nanoarchitectures of supramolecular protein complexes.

REFERENCES

[1] R. Arai, N. Kobayashi, A. Kimura, T. Sato, K. Matsuo, A. F. Wang, J. M. Platt, L. H. Bradley and M. H. Hecht, *J. Phys. Chem. B* **116**, 6789 (2012).
[2] N. Kobayashi, K. Yanase, T. Sato, S. Unzai, M. H. Hecht and R. Arai, *J. Am. Chem. Soc.* **137**, 11285 (2015).
[3] N. Kobayashi, K. Inano, K. Sasahara, T. Sato, K. Miyazawa, T. Fukuma, M. H. Hecht, C. Song, K. Murata and R. Arai, *ACS Synth. Biol.* **7**, 1381 (2018).

BEAMLINE

BL-10C

N. Kobayashi[1, 2] and R. Arai[1] ([1]Shinshu Univ., [2]NINS-ExCELLS)