Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

for

Self-assembly of engineered protein nanocages into reversible ordered 3D superlattices mediated by zinc ions

H. Chen, K. Zhou, Y. Wang, J. Zang, G. Zhao*

Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Food Science & Nutritional Engineering, China Agricultural University, Beijing Key Laboratory of Functional Food from Plant Resources, Beijing, 100083, China. E-mail: gzhao@cau.edu.cn

1. Protein preparation

cDNA encoding the full-length amino acid sequence of Dps (DNA-binding protein from starved *E. coli*) and its mutant Dps$^{-carboxyl}$ were cloned into the pET-3a vector (Novagen) and verified by DNA sequencing. (Fig. S2) Recombinant Dps protein was purified as previously described with some modification.[1] Briefly, the *E. coli* strain BL21 (DE3) which contained Dps expression plasmid was grown on LB medium supplemented with 50.0 mg/L of ampicillin sodium at 37 °C, and protein expression was induced with 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) when an optical density reached an A_{600} of 0.6. The cells were harvested after 8 h of induction and suspended in 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl. Next the cells were sonicated for 15 min on ice, and the lysate centrifuged for 10 min at 12 000 × g. The solution was then primarily purified by ammonium sulfate fractionation (60% saturated fraction). After centrifugation, the precipitate was dissolved in 50 mM Tris-HCl, pH 8.0, 0.5 M NaCl, followed by dialysis (14.4 kD cut-off) in 50 mM Tris-HCl, pH 8.0 three times. After centrifugation for 10 min at 12 000 × g, the precipitate was redissolved in 50 mM Tris-HCl, pH 8.0, 2 M NaCl, and was purified by Sephacryl S-300 gel filtration chromatography using equilibrated buffer (50 mM Tris-HCl, pH 8.0, 2 M NaCl), and then the protein was eluted with 50 mM to 1 M NaCl gradient by ion exchange chromatography after dialyzed against 50 mM Tris-HCl, pH 8.0, 50 mM NaCl.

Mutant Dps$^{-carboxyl}$ was purified as follows: after expression, the cells were harvested after 8 h of induction and suspended in 50 mM Tris-HCl, pH 8.0. Next the cells were sonicated for 15 min on ice, and the lysate centrifuged for 10 min at 12 000 g. The solution was then primarily purified by ammonium sulfate fractionation (60% saturated fraction). The precipitate was dissolved in 50 mM Tris-HCl, pH 8.0, followed by dialysis against 50 mM Tris-HCl, pH 8.0 three times. Then the protein was eluted with 0 mM to 1 M NaCl gradient by ion exchange chromatography, and further purified by Sephacryl S-300 gel filtration chromatography with equilibrated buffer (50 mM Tris-HCl, pH 8.0, 2 M NaCl). Protein purity was confirmed by SDS-PAGE (polyacrylamide gel electrophoresis). Protein concentrations were determined according to the Lowry method with bovine serum album as standard.

2. Transmission electron microscopy (TEM)

Samples were diluted with appropriate buffers to obtain the required concentration prior to being placed on carbon-coated copper grids (Beijing Zhongxingbairui Technology Co., Ltd) for 2 min. After excess solution was removed with filter paper, the samples were stained using 2% uranyl acetate (Beijing Zhongxingbairui Technology Co., Ltd) for 2 min.[2,3] TEM micrographs were imaged at 80 kV through a Hitachi H-7650 transmission electron microscope.

3. Dynamic Light Scattering (DLS) Analyses

Dynamic light scattering experiments were carried out at 25 °C using a Wyatt model WDPN-08 dynamic light scattering instrument.[4] The Dynamics software was used to calculate the size distribution of prepared proteins. Each measurement was averaged at least 15 runs. All samples were prepared in ddH_2O, and the added Zn^{2+} or NaCl solution did not exceed 5% of the total volume, therefore no corrections were made for sample dilution. After each addition, the samples were thoroughly mixed.

4. Zeta potential measurement

Zeta potential of wild-type Dps and its mutant $Dps^{-carboxyl}$ was measured by using a Delsa Nano C particle analyzer (Beckman Coulter, USA) at room temperature. Protein samples were buffered in 50 mM Tris, 20 mM NaCl, pH 8.0, with the concentration of 1.0 μM.

5. Small angle X-ray scattering (SAXS)

Small angle X-ray measurements were obtained as follows: the $Dps^{-carboxyl}$-Zn^{2+} complexes were centrifuged at 10, 000 × g for 5 min, and the samples were sealed in a metal ring using Kapton tape. Sample thickness was approximately 1.5 mm. Small angle X-ray scattering measurements were carried out at the BL16B beamline in the Shanghai Synchrotron Radiation Facility (SSRF). The wavelength was 0.1237 nm. 2D SAXS patterns were collected by a MAR CCD X-ray detector, the sample-to-detector distance was 2.835 m. One-dimensional SAXS data were obtained by azimuthally averaging the 2D scattering data. The magnitude of the scattering vector q is given by $q = 4\pi \sin\theta/\lambda$, where 2θ is the scattering angle. The simulated scattering patterns were obtained with *Scatter* (version 2.5)[5].

Supplementary Figures (Figs. S1-S6)

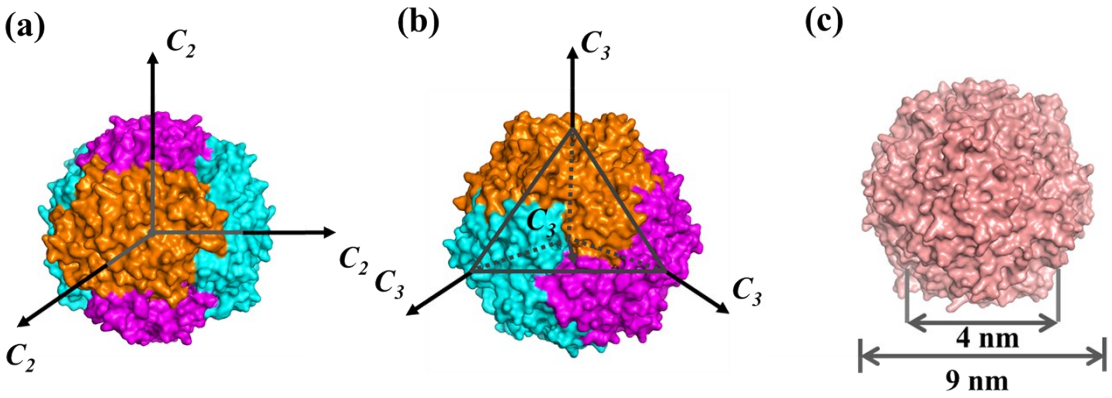

Figure S1. In the structure of Dps, four subunit trimers are placed at the vertices of a tetrahedron with a spherical-like structure, giving a 23-point-group symmetry. (a) The 2-fold symmetry axes of Dps; (b) The 3-fold symmetry axes of Dps; (c) The spherical dodecamers with inner diameter and outer diameter about 4 nm and 9 nm, respectively.

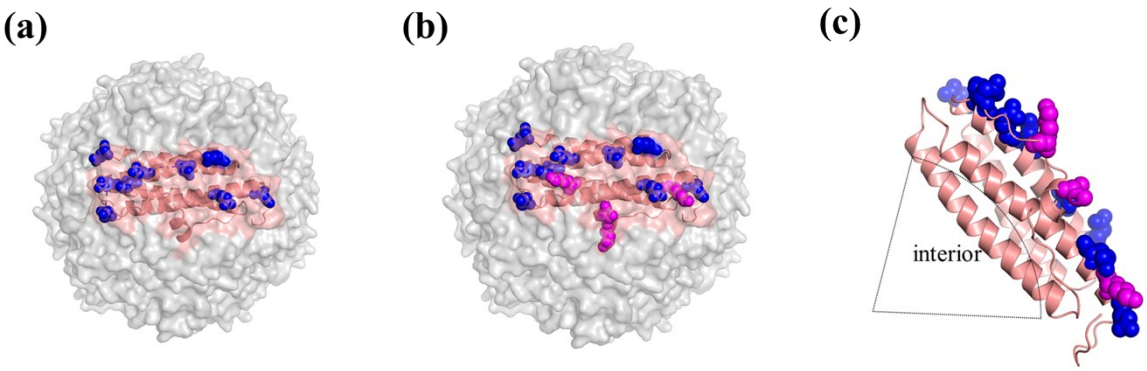

Figure S2. Redesign of the outer surface of Dps nanocages. (a) Distribution of nine intrinsic carboxyl groups on the exterior surface of each Dps subunit. (b,c) Four additional carboxyl groups were introduced on the outer surface of each subunit (pink).

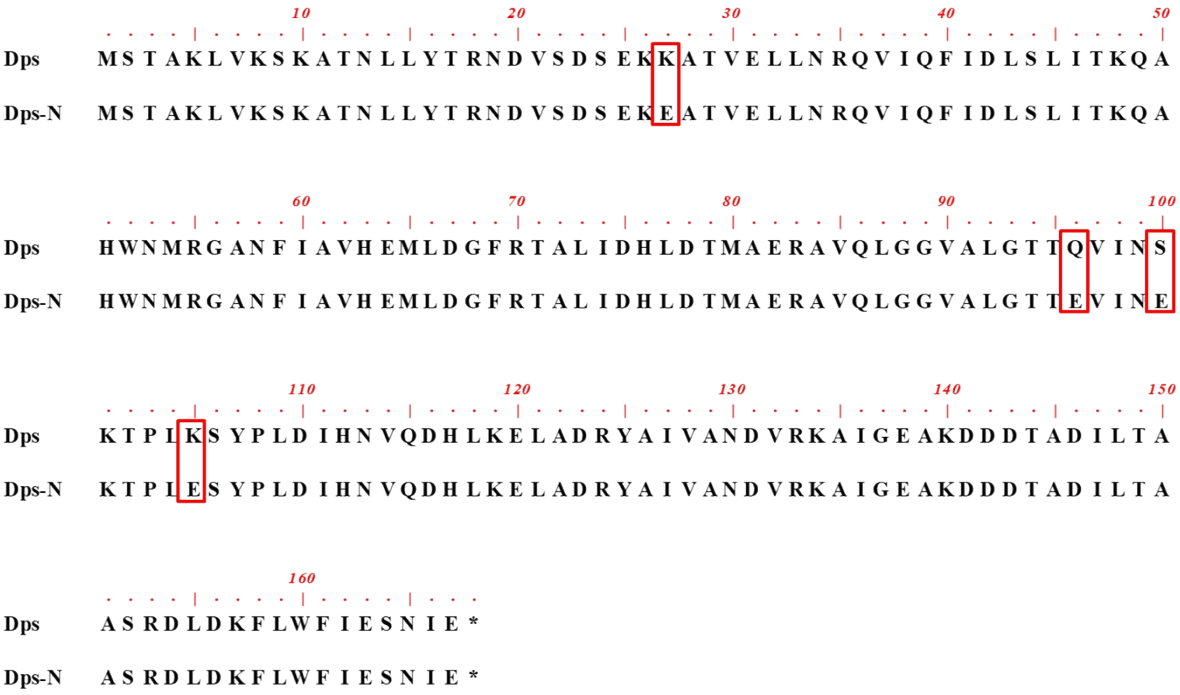

Figure S3. (a) Complete amino acid sequences of Dps and its mutant (Dps$^{-carboxyl}$). Mutation sites have been highlighted in red box (K27E, Q96E, S100E, K105E).

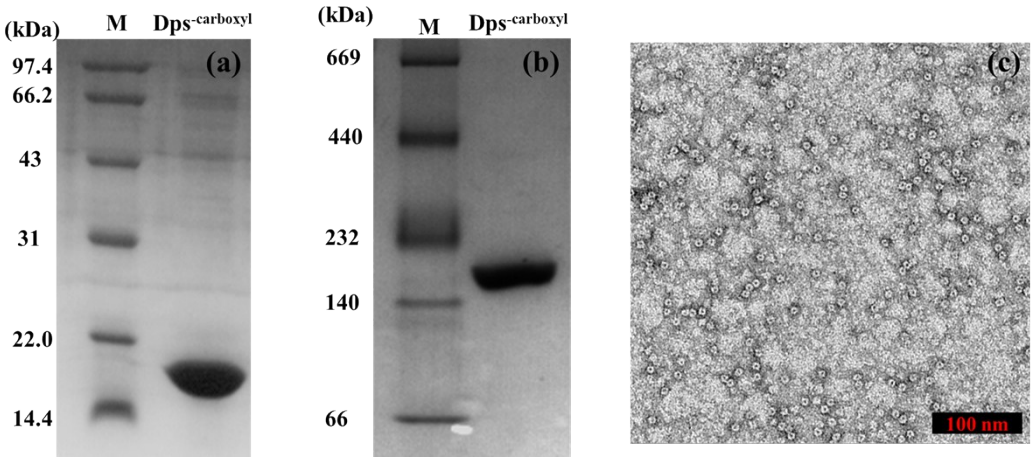

Figure S4. Characterization of Dps$^{-carboxyl}$ after purification. (a) SDS-PAGE and (b) native-PAGE analyses of purified Dps$^{-carboxyl}$. Land M, Protein markers and their corresponding molecular masses. (c) TEM images of Dps$^{-carboxyl}$. Scale bars represent 100 nm.

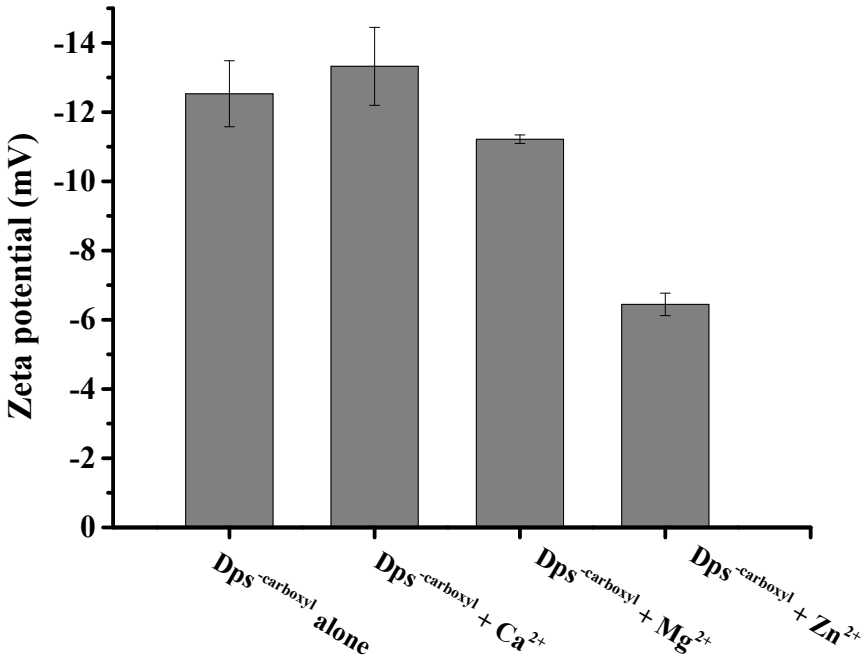

Figure S5. Zeta potential of Dps$^{-carboxyl}$ upon treatment with different metal ions. Condition: [Dps$^{-carboxyl}$] = 1.0 μM, the ratio of metal ions to protein is 200/1.

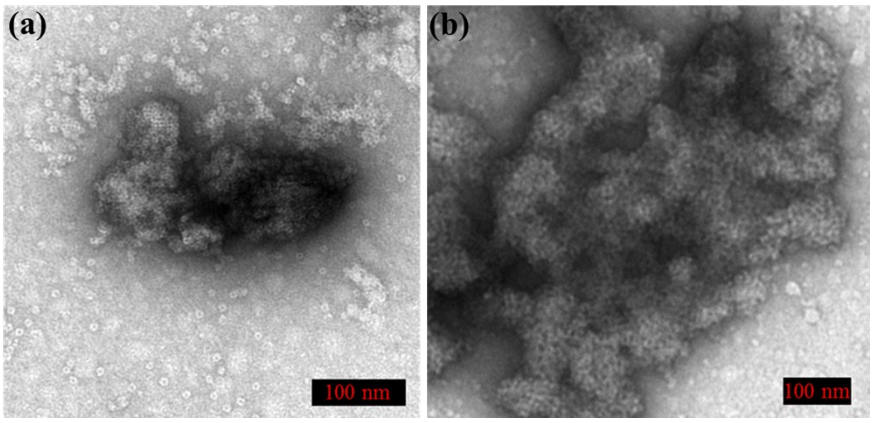

Figure S6. TEM images of (a) wild-type Dps, and (b) wild-type Dps mixed with Zn^{2+} (Dps : Zn^{2+} = 1 : 300). Condition: [Dps] = 1.0 μM, and protein are buffered in 50 mM Tris-HCl, pH 8.0.

References

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3. L. Chen, G. Bai, R. Yang, J. Zang, T. Zhou and G. Zhao, *Food Chem.*, 2014, **149**, 307-312.

4. H. Chen, S. Zhang, C. Xu and G. Zhao, *Chem. Commun.*, 2016, **52**, 7402-7405.

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