**Electronic Supporting Information** Production of a promising modular proteinaceous self-assembled delivery system for vaccination Chao Pan,<sup>‡a</sup> Jingqin Ye,<sup>‡a</sup> Sen Zhang,<sup>b</sup> Xiang Li,<sup>a</sup> Yixin Shi,<sup>a</sup> Yan Guo,<sup>a</sup> Kangfeng Wang, a,c Peng Sun, a,d Jun Wu, \*a Hengliang Wang \*a and Li Zhu \*a <sup>a</sup> State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Biotechnology, Beijing, 100071, PR China <sup>b</sup> State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Academy of Millitary Medical Sciences, Beijing, 100071, PR China <sup>c</sup> College of Life Science, Hebei University, Baoding, 071002, PR China <sup>d</sup> School of Medicine, Tsinghua University, Beijing, 100084, PR China \*Corresponding author: E-mail: jewly54@bmi.ac.cn, wanghl@bmi.ac.cn, junwu1969@163.com <sup>‡</sup>These authors contributed equally to this work. 

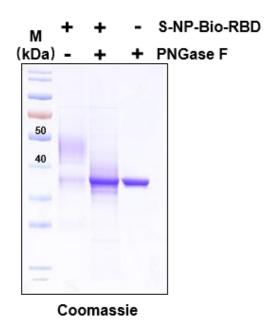


Fig. S1 SDS-PAGE analysis of S-NP that was incubated with or without PNGase F.

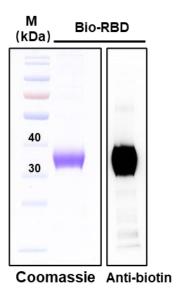
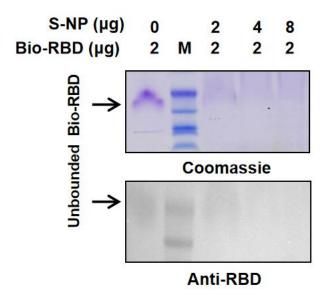
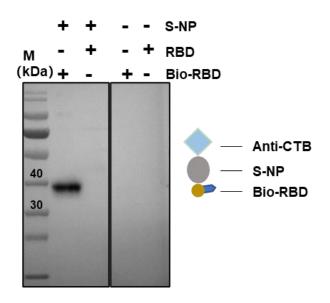


Fig. S2 Bio-RBD was analyzed by Coomassie blue staining and western blotting

using HRP-labeled streptavidin (SA) after SDS-PAGE separation.



**Fig. S3** Analysis of the binding between S-NP and Bio-RBD under different proportions after incubation at 37°C for 1 h by Coomassie blue staining and western blotting (M: marker).



**Fig. S4** RBD and Bio-RBD were separated by SDS-PAGE individually, and then transferred to PVDF membranes. After incubation with S-NP and buffer at 37 °C for one hour, the two membranes were incubated with anti-CTB antibody and HRP-labeled secondary antibody successively to analyze the connection of Biotin and mSA.

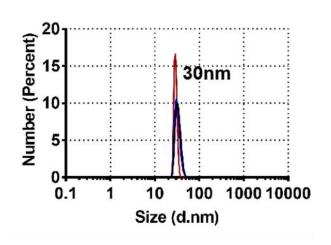
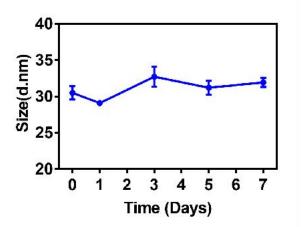
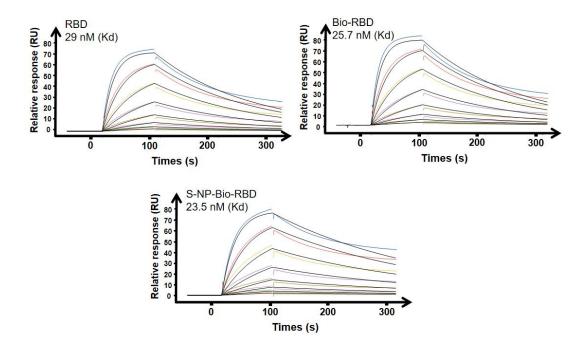


Fig. S5 S-NP-Bio-RBD was analyzed by DLS.



47 Fig. S6 Stability analysis of S-NP-Bio-RBD. S-NP-Bio-RBD solution was detected

by DLS when incubated at 37  $^{\circ}\mathrm{C}$  for different time periods.



**Fig. S7**. Measurement of affinity between ACE2 and RBD, Bio-RBD and S-NP-Bio-RBD by surface plasmon resonance. Different color lines indicate the different concentrations of RBD.

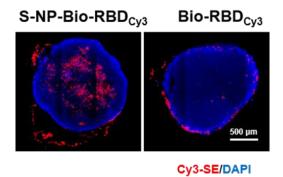


Fig. S8 Cy3-labeled Bio-RBD (Bio-RBD<sub>Cy3</sub>) was coupled with S-NP (S-NP-Bio-RBD<sub>Cy3</sub>). Balb/c mice were subcutaneously injected with S-NP-Bio-RBD<sub>Cy3</sub> or Bio-RBD<sub>Cy3</sub>, and immunofluorescence was performed to analyze the accumulation of antigen in dLNs 24 h after immunization.

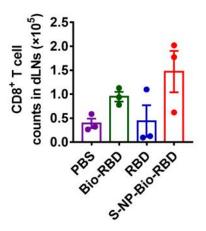
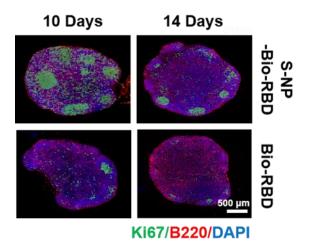


Fig. S9 CD8+ T cell counts in dLNs three days after vaccination (n = 3).



68 Fig. S10 Immunofluorescence was performed to analyze the formation of germinal

center in dLNs at different time points. Bar =  $500 \mu m$ .

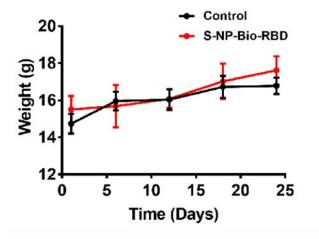


Fig. S11 Body weight of the mice was measured after immunization with S-NP-Bio-RBD (25  $\mu g$  RBD per mouse) for one month.

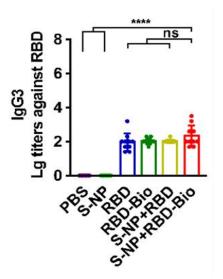


Fig. S12 IgG3 titers against RBD were measured in the serum of Balb/c mice immunized with S-NP, RBD, Bio-RBD, S-NP+RBD, and S-NP-Bio-RBD after the third immunization. \*\*\*\* p < 0.0001, ns: no significant.