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Supporting Information for

Constructing Functional Protein Nanotube by Small Molecule-Induced Self-assembly of Cricoid Proteins

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1. Overexpression and purification of wild type SP1 and SeSP1

The wild type SP1 domains were transformed into *E. coli* BL21 (DE3) and grew to 1 L LB culture containing 50 mg/ml ampicillin with shaking at 37°C. The protein expression was induced by adding 1 mM IPTG when OD_{600} reached 0.8. The cells were harvested 4 hr after induction. In purification, W. T. SP1 was first heated at 85 °C for 30 min and centrifuged at 15000 rpm for 30 min. The supernatant liquid was loaded onto a 20 mL DEAE ion-exchange column running in 20 mM Tris-HCl buffer, pH 6.3 with a salt gradient from 50 to 500 mM NaCl. The target protein was eluted at 500 mM NaCl. The sample was then dialyzed by 8 kDa cutoff dialysis membrane to milli-Q water and stored at -20 °C for the experiments. In SDS-PAGE sample preparation, 1 mL solution of 1L culture after IPTG induction of W. T. SP1 was obtained. After centrifugation at 10000 rpm for 10 min, the cell pellets were resuspended in 10 μL loading buffer. The mixtures were boiled for 20 min and loaded into the SDS-PAGE. Finally, the homogeneous and unique protein bands of ~12kD were obtained as shown in Figure S1a.

SP1-57Cys mutant expression vector pET-22b was obtained by quick change protocol PCR using upper primers 5'-GGCATGGAGTCTTGTGAGCTAAACC-3' and lower primer 5'-CTCGGTTTAGCTCACAAGACTCCAT-3'. The construction was confirmed by DNA sequencing using T7 sequencing primers. The plasmid with SP1-57Cys domain was transformed into cysteine auxotrophic *E. coli* strain BL21cysE51, which grew in 1 L M9 expression medium containing 50 pg/mL cysteine hydrochloride with shaking at 37 °C until OD_{600} reached 1.0. IPTG was added at a final concentration of 1 mM to induce expression for 30 min and then chloramphenicol (10 pg/mL) was mixed into the culture for another 10 min. The sediment cells were acquired by centrifugation at 8000 rpm and washed twice with saline (0.9% NaCl). The cleaned cells were resuspended in the M9 production medium with 100 pg/mL DL-selenocystine. Cells were harvested 3 hr after culturing at 28 °C. The purification of SeSP1 was in accordance with the purification of wild type SP1 as described above.

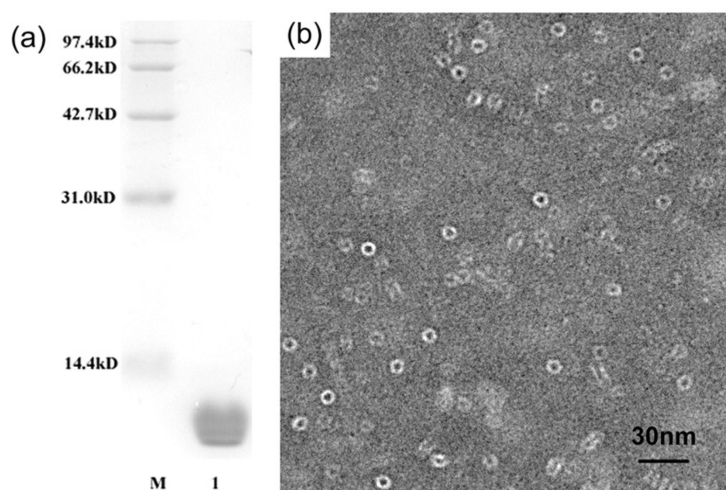

Fig. S1. (a) SDS-PAGE analysis of SP1 protein. Lane 1: the SP1 protein boiled in the presence of 2% SDS. (b) TEM image of ordered wild type SP1 dodecamer.

2. Electrostatic assembly of SP1 with ethylenediamine (EDA)

A 200 μL solution containing 25 mg/L SP1 was mixed with EDA in 10 mM PBS buffer, pH 7.0. The molar ratio between carboxyl groups of SP1(about 150 carboxyl groups on the surface of one SP1 nanoring) and the amino groups of EDA was 1:1. The final concentration of the EDA was 25 μM. The mixed solutions were stirred vigorously for 10 min and then left to stand for 1 hour.

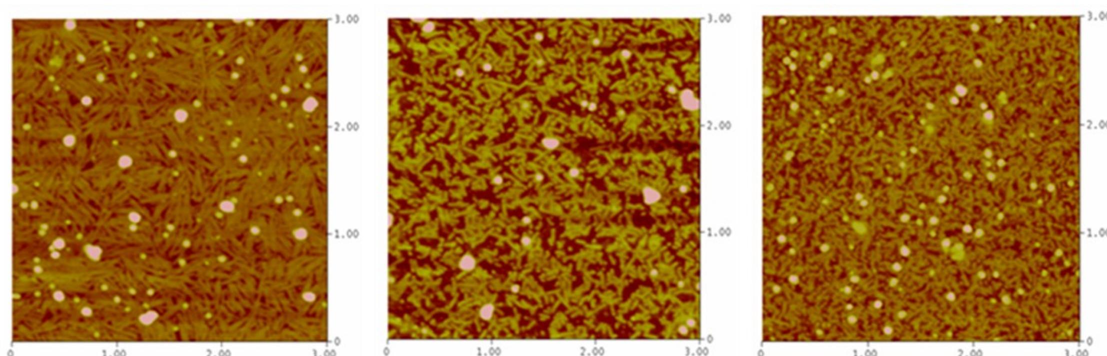

Fig. S2 The tapping-mode atomic force microscopy images of SP1-EDA electrostatic assembly at different ratios (the ratios of carboxyl groups of SP1and EDA were 1:1, 1:5, and 5:1 from left image to right, respectively).

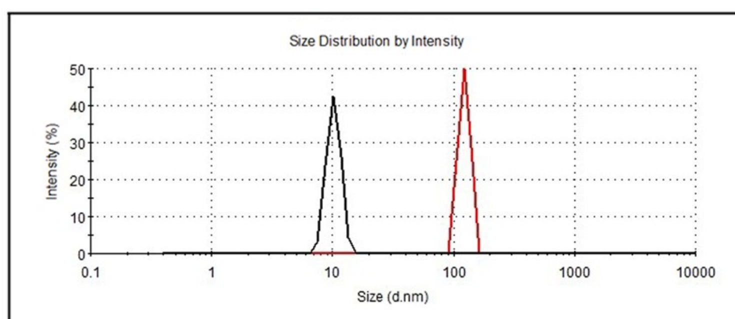

Fig. S3 Dynamic light scattering (DLS) analysis of free SP1 (black) and electrostatic assembly (red).

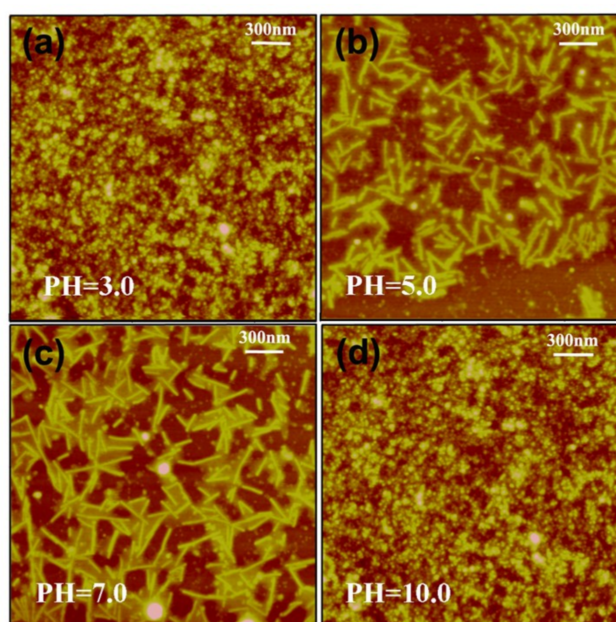

Fig. S4 The atomic force microscopy images of SP1-EDA electrostatic assembly at different pH.

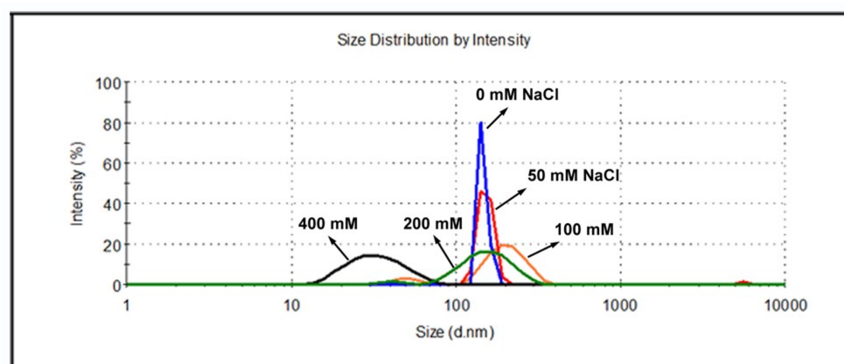

Fig. S5 DLS analysis of SP1-EDA electrostatic assembly at different concentration of NaCl (0 mM, 50 mM, 100 mM, 200 mM, and 400 mM).

3. Zero-length crosslinking of SP1/SeSP1 nanoring induced by EDA

A 1 mL solution containing 25 mg/L SP1/SeSP1 was mixed with EDC and Sulfo-NHS in 10 mM PBS buffer, pH 6.0. The molar ratio of the carboxyl groups of the SP1(about 150 carboxyl groups on the surface of one SP1 nanoring), EDC and Sulfo-NHS was 1:5:5. After 30 min of the stirring, 10 μL 2.5 mM EDA was added to the solution, stirring up for 2 hours at the room temperature. The mixed solution was then dialyzed by 8 kDa cutoff dialysis membrane to milli-Q water and stored at -20 °C for the experiments.

The MALDI-TOF mass spectrometry analysis determined the masses of both wild type SP1 and the SP1 protein covalently linked with excess EDA (10-fold). And most of the SP1 monomer can at least be combined with three of the EDA, meanwhile, there were a small amount of the SP1 monomer being linked to eight or thirteen of the EDA (Fig. S6).

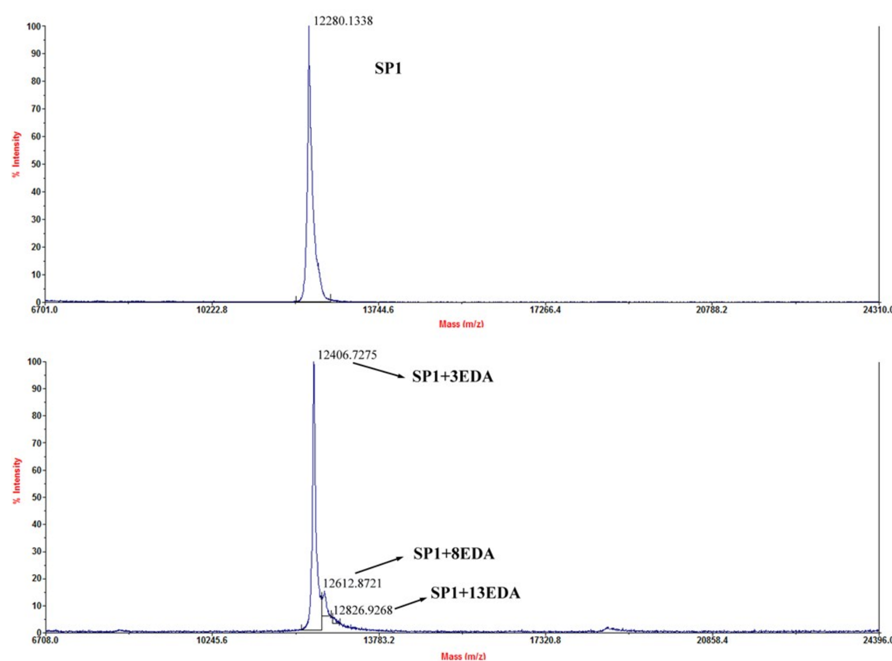

Fig. S6 MALDI-TOF mass spectrometry analysis. (a) wild type SP1. (b) SP1 protein covalently linked with 10-fold EDA.

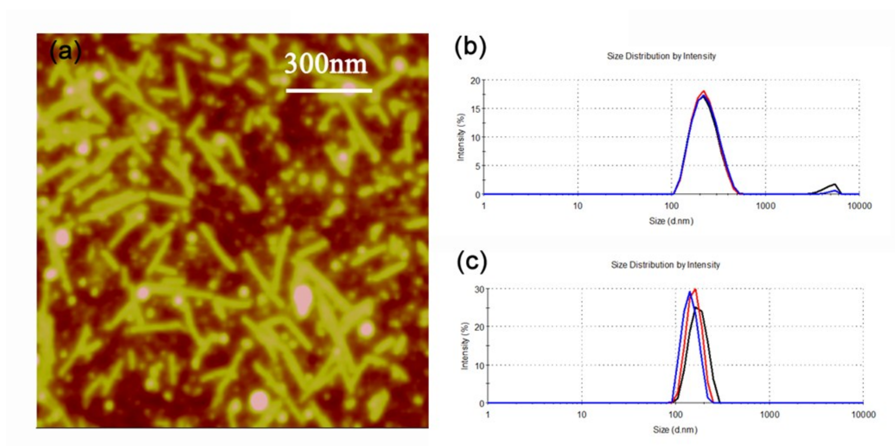

Fig. S7 The stability of the covalent assembly. (a) AFM image of SP1-EDAcc nanotubes heated at 100 °C for 30 min. (b) DLS analysis of SP1-EDAcc assembly at different concentration of NaCl, 0 mM (red), 100 mM (black), 400 mM (blue). (c) DLS analysis of SP1-EDAcc assembly at different pH. pH=3.0 (blue), pH=6.0 (red), pH=10.0 (black).

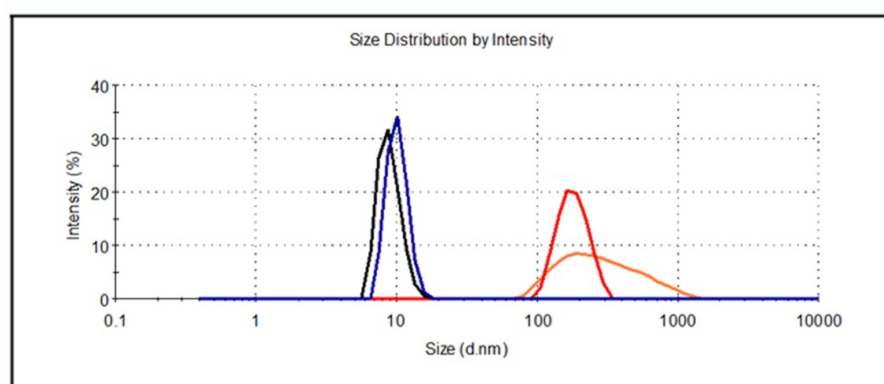

Fig. S8 DLS analysis of the mixed solution (SP1, EDC, and Sulfo-NHS, black) and the mixed solution with ethylamine (blue), EDA (red), and 1,6-hexanediamine (orange), respectively.

4. Determination of GPx activity of SeSP1-EDA covalent crosslinking

Determination of GPx activity used the coupled reductase assay system. The reaction system was a 400 μL solution containing 50 mM phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM GSH, 1 U of glutathione reductase, and appropriate SeSP1-EDA covalent crosslinking at 37 °C. The mixture was preincubated for 5 min, and then 50 μL of NADPH solution (2.5 mM) was added. After incubation for another 3 min at 37 °C, the reaction was initiated by the addition of 50 μL of 0.5 mM H_2O_2. The absorbance was recorded at 340 nm using a UV/vis spectrophotometer, SHIMADZU UV-2450. One unit of enzyme activity was defined as the amount of enzyme that catalyzes the turnover of 1 μmol of NADPH (ε = 6220 M^{-1} cm^{-1}, pH 7.0) per min. The specific activity was expressed in μmol·min^{-1}·$μmol^{-1}$ of enzyme.

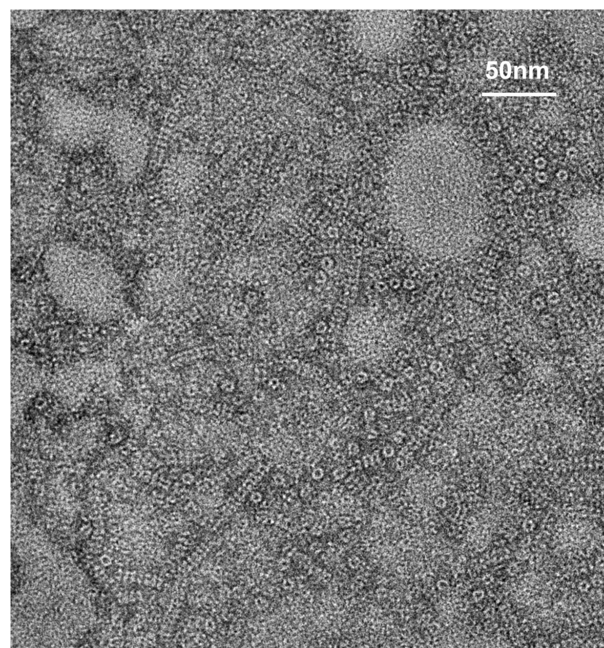

Fig. S9 Transmission electron microscope image of SeSP1-EDA covalent crosslinking nanotubes.

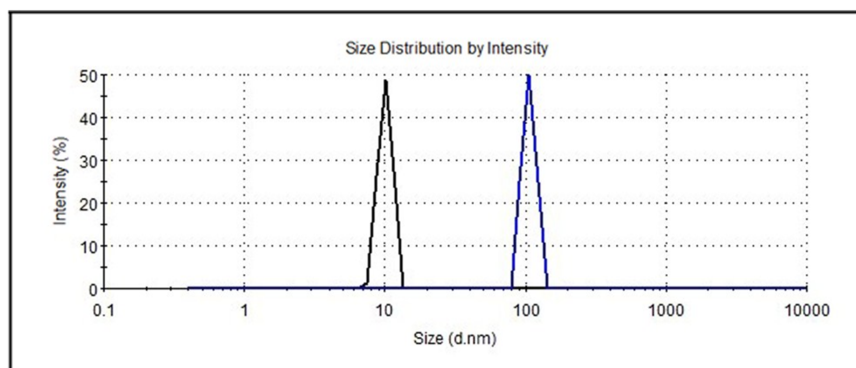

Fig. S10 DLS analysis of SeSP1 (black) and SeSP1-EDA covalent crosslinking nanostructure (blue).