Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Metal induced self-assembly of V-shape protein into 2D wavy supramolecular nanostructure

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1. Construction expression vector of SM5S and SM4S protein:

Expression vectors of SM5S and SM4S are constructed by gene engineer, PCR was employed to complete 5 site mutations. The designed primers are shown blow. (Table S1)

		SM4S (5'-3')		SM5S (5'-3')
Primer1	**D75H**	GAGGAACATGAAGTGTGGCATGTG CACACTTCATGTTCCTCCTCTGAATT	**E76H**	GAGGAAGATCATGTGTGGCAGG TGCCACACATGATCTTCCTCCTCT
Primer2	**Q79H**	TGTGTGGCATGTGATCATAGGAG GATCACATGCCACACATGATCTTC	**Q79H**	TGTGTGGCATGTGATCATAGGAG GATCACATGCCACACATGATCTTC
Primer3	**L137H**	TGGTGAAACACCAGGTGGAAGAGG CACCTGGTGTTTCACCAGCTGAATG	**L137H**	TGGTGAAACACCAGGTGGAAGAGG CACCTGGTGTTTCACCAGCTGAATG
Primer4	**E141H**	ACACGTGCACCAGCTCTCCC GGTGCACGTGTTCCACCTGG	**E141H**	ACACGTGCACCAGCTCTCCC GGTGCACGTGTTCCACCTGG
Primer5	**stop**	ACCTGCGTGAGTGACTCGAGCAC CTCGAGTCACTCACGCAGGTAGGC	**stop**	ACCTGCGTGAGTGACTCGAGCAC CTCGAGTCACTCACGCAGGTAGGC

2. Expression and purification:

Three kinds of protein are expressed in *Escherichia Coli* strain BL21(ED3), incubating in 5ml LB liquid medium including Kanamycin (100μg/mL) overnight, cells are inoculated into 500ml LB liquid medium containing Kanamycin (100μg/mL) until OD600 is about 0.1 and incubated till OD600 is about 0.6-0.8. Then IPTG was added to the final concentration of 1mM and the cells were incubated for 4hours. Collected by centrifugation, cells were ultrasonicated and remove sediment by centrifugation, the soluble fraction is purified over anion-exchange chromatography (DEAE) and further purification was performed on G75 and G25 to remove nucleinic acid and salt. Final protein is verified by page-gel electrophoresis (Fig. S1).

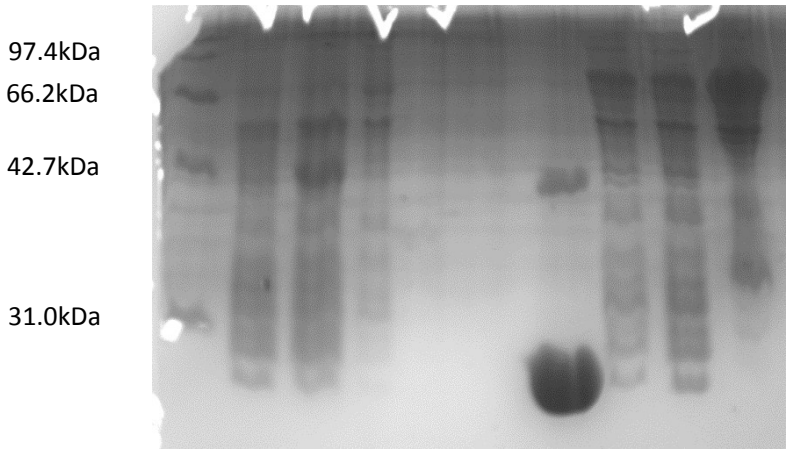

Fig. S1 Page-gel electrophoresis of SM5S, revealing 21kDa protein we purified, similar to the SMAC Wild Type: 20429Da.

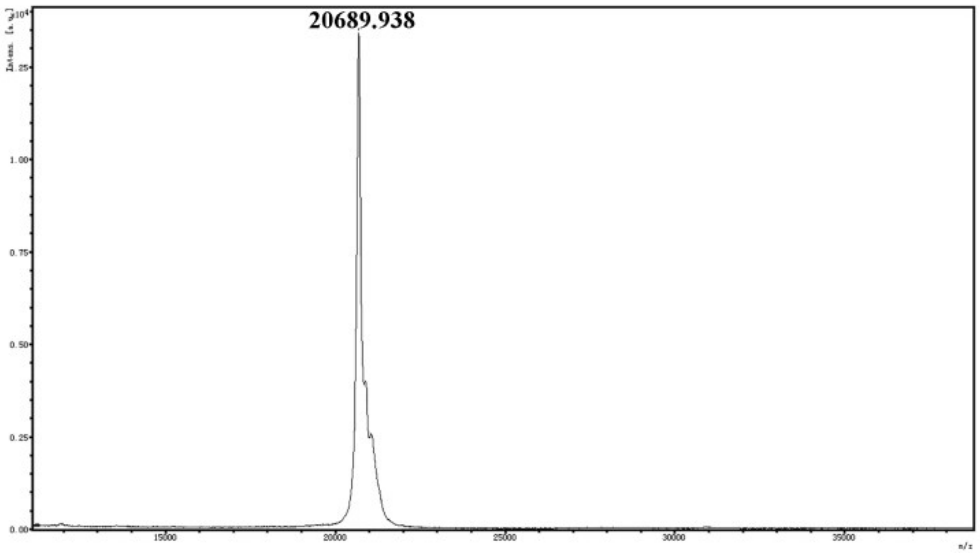

Fig. S2 MALDI-TOF result also demonstrates we have successfully obtained target protein SM5S.

Circular dichroism spectrum (CD) characterization:

All three kinds of proteins were carried out on CD, 500μl of each sample was used for CD measurement in TBS buffer (10mM Tris-HCl, pH7.0, 1mM EDTA) and the final concentration is about 1μM. Background spectra in the absence of protein were measured and subtracted and all CD signals were recorded from 190nm to 700nmwith scan rate 0.5 per second. (Fig. S3)

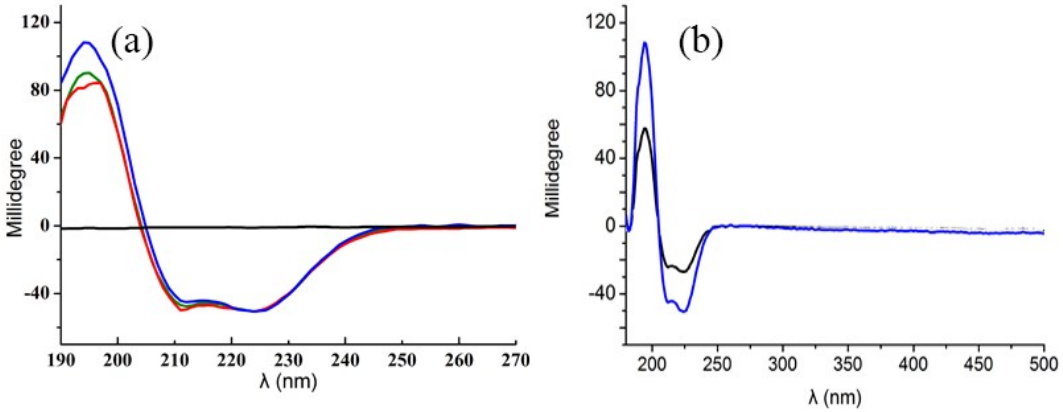

Fig. S3 (a) CD signals of SMAC WT, SM4S and SM5S normalized at 222 nm, the data reveals that the three kinds of protein are similar in second structure. (b) CD spectra of SM5S (black), SM5S &20eq.Zn^{2+} (blue) are recorded from 190nm to 500nm after incubating overnight. No excess peak was generated, indicating that we do not obtain the huge helix assembly through BH1-Zn-BH2 bond.

Non-specific Protein-Protein Interactions (PPI) by Computational Calculation:

The initial model of SMAC monomer was derived from the crystal structure (pdb ID: 1FEW) and its homodimeric structure was determined by a rigid-body protein-protein docking procedure using ZDOCK[1]. This program uses the Fast Fourier Transform algorithm to search exhaustively the entire rotational and translational space of one SMAC monomer with respect to the fixed another SMAC monomer for predicting the structure of their complex with the best shape complementarity, desolvation, and electrostatics. The rotational sampling interval around each Cartesian angles was set at 15 degree. A total of 3600 docked poses were sampled and then submitted to energy minimization using CHARMM force field to improve the energies and eliminate clashes. The top 10 ranking complexes with the low ZDOCK energy-based scores were selected for analysis of the contact interface according to the previously reported structural information[2]. Finally, the resulting SMAC dimer model was used to perform site-directed mutagenesis at position 75, 79, 137, and 141 to histidine by PyMol tool. The homomultimeric complexes were constructed by exploring only the part of search space that contains the chelating bis-histidine residues and conforms to the C2 symmetry.

3. Characterization of protein self-assembly:

DLS was performed to study the system. SM5S was dissolved in TBS buffer (10mM Tris, pH7.0). the final concentration is about 1μM, samples were made with addition of different Zn^{2+} concentration and incubated overnight prior to DLS measurement. (Fig. S4a) The influence of assembling time on the size of protein self-assemblies was also studied by DLS. Dissolved in TBS and 20eq. Zn^{2+} was added in system, the DLS signal of sample was recorded for 4h (Fig. S4b)

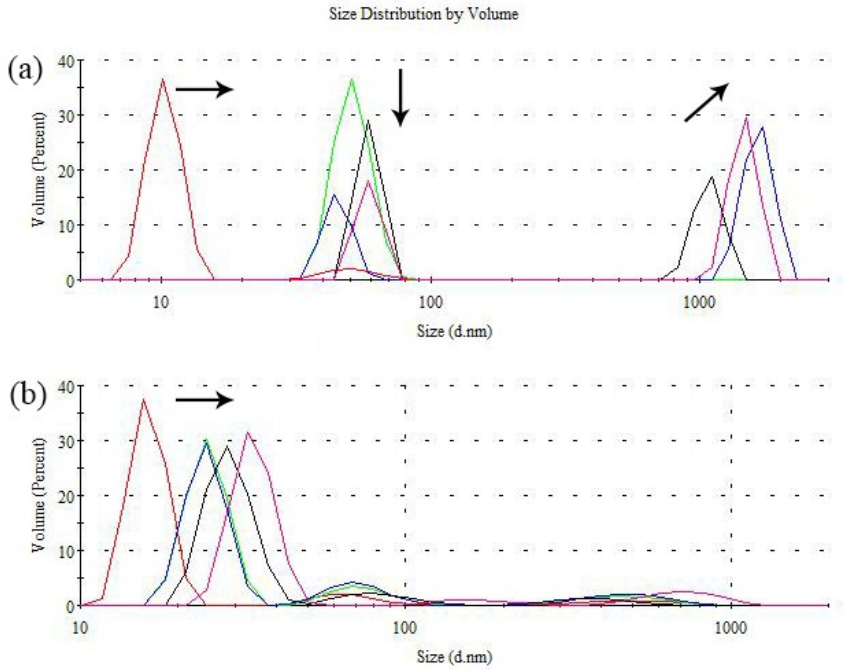

Fig. S4 (a) 1μM samples were treated with different ratio of Zn^{2+}, 0μM (red), 20μM (green), 40μM (black), 50μM (magenta), 100μM (blue), and incubated overnight before DLS test. Size of sample without Zn^{2+} is about 10 nm, when the Zn/protein ratio is 20, main peak moves to 50nm, higher Zn^{2+} concentration results in the drop of peak and generating new large peaks, accompany of forming larger self-assembly. (b) 20eq. Zn^{2+} is added in SM5S, DLS of the same sample was recorded at 0h (red), 1h (green), 2h (blue), 3h (black), and 4h (magenta). Size of sample became bigger as time went by.

TEM imaging was used to study morphology of protein self-assemblies. The sample was prepared by placing a drop of the samples on a 300-mesh, carbon-coated copper grid for 10 min, after negative stained by 4% Sodium phosphotungstate for 40s, samples were dried in air before measurement. The observation was performed with a JEOL1011 transmission electron microscope operating with an acceleration voltage of 200 kV. (Fig. S5)

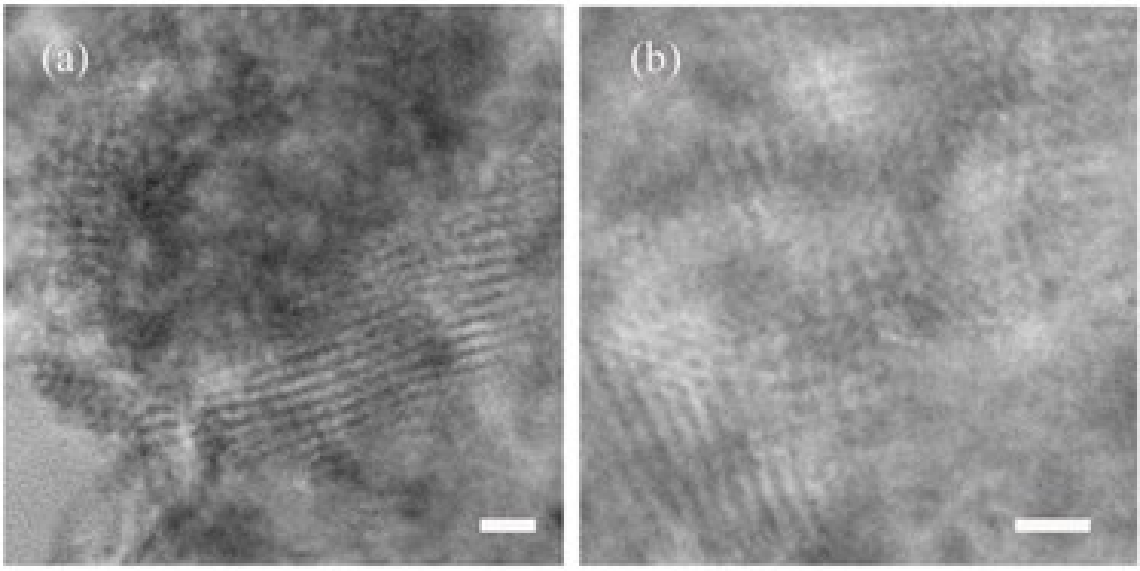

Fig. S5 TEM images (a-b) show the width of single nanowire is about 3.5±0.6 nm. Further prove our hypothesis: protein fist form nanowire with the help of BH1-Zn-BH1 bond.

AFM is the main tool we study about our protein assembly, all the sample were prepared by dropping 10μl solution on silicon slice which have been hydroxylated, and adsorbed for 10 min. Then wash the silicon slice three times using water, air dried before employing AFM. (Fig. S6)

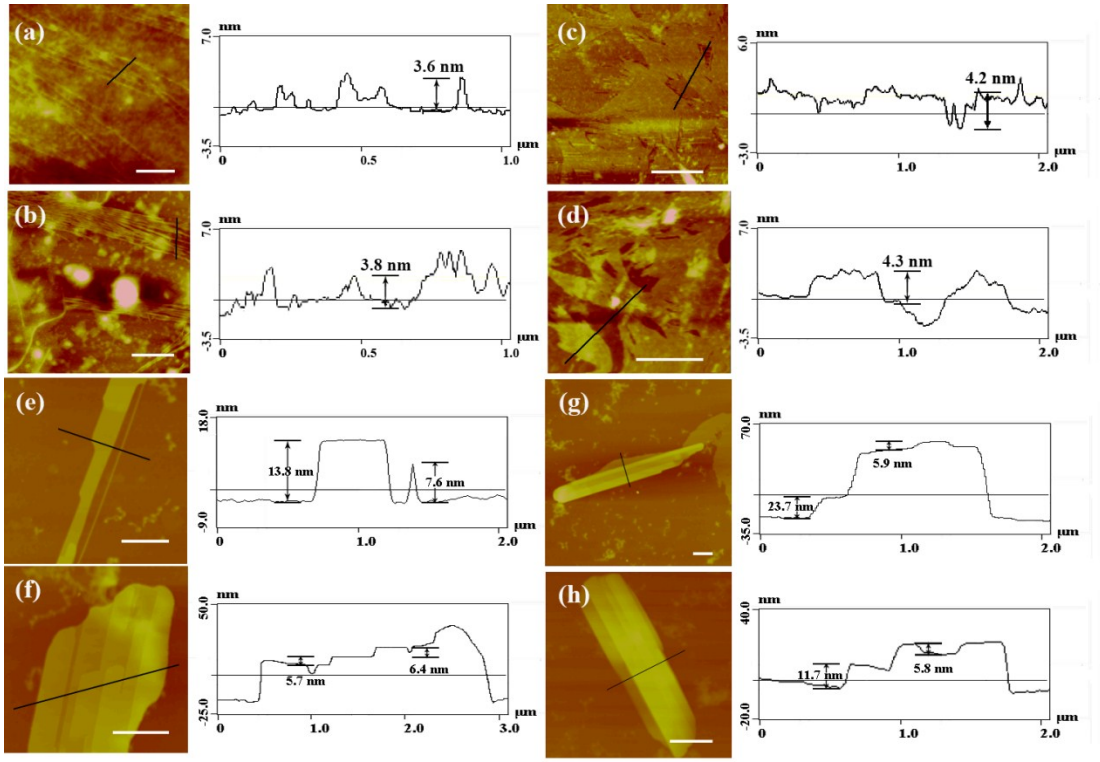

Fig. S6 AFM images with different concentration of Zn^{2+}, (a-b) metal/protein ratio is 10 and 20, dissociative nanowires were obtained. When the ratio raises to 40 or 50, bundling assemblies were observed (c-d). With high protein concentration and high metal/protein ratio, single and multiple 2D-layer supermolecular polymers were detected from AFM (e-h).

4. Characterization of protein self-assembly with FRET:

MALDI-TOF MS characterization of FITC and rhodamine B modified SM5S protein (SF and SRB). The MALDI-TOF MS is carried on Bruker MALDI-TOF, the concentration of modified protein is 20μM, and the matrix we used is sinapic acid, 10eq. of sample. After standardizing with protein Ⅱ standards, mass spectrum is record using LP5000-20000 mode. Fig. S7 show the FITC modified SM5S mass spectrum, the rhodamine B MS is shown in Fig. S8.

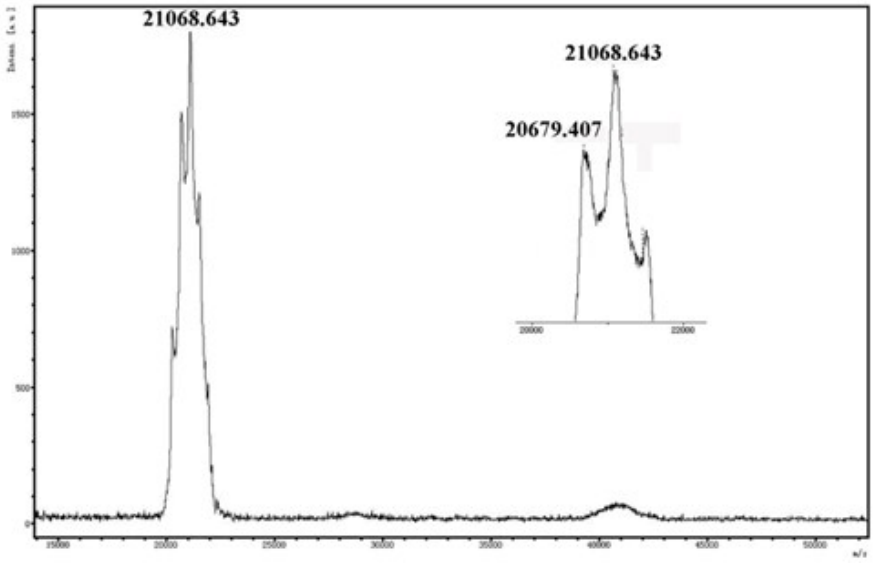

Fig. S7 MALDI-TOF MS of FITC modified SM5S protein, the zoomed image of peak shows that D-value between two peaks is about 389, which is the molecular weight of FITC (Mr=389.38g/mol). Because there are three amino on surface of monomer, 1-3 FITC molecules could be modified on the surface of SM5S monomer.

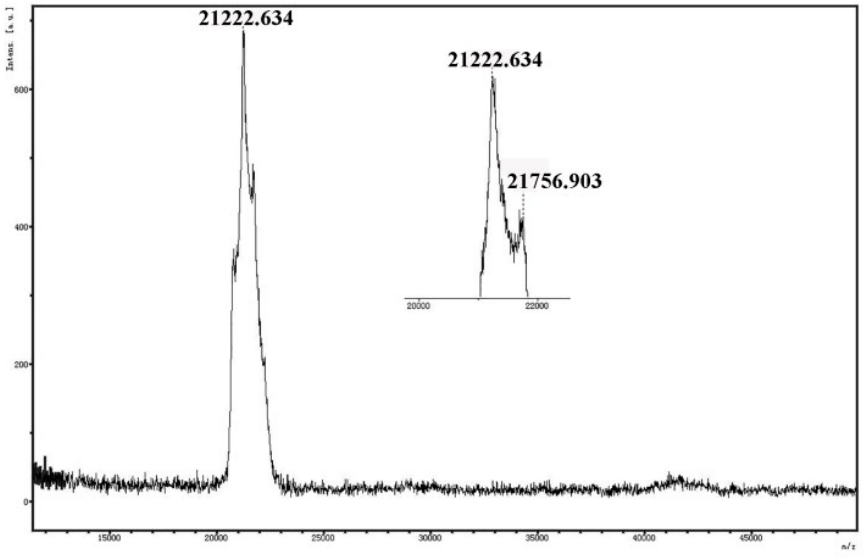

Fig. S8 MALDI-TOF MS of rhodamine B modified SM5S protein, the zoomed image of peak shows that D-value between two peaks is about 534.26, which is about the molecular weight of rhodamine B (Mr=536.09g/mol). Because there are three amino on surface of monomer, more than one rhodamine B molecules could be modified on the surface of SM5S monomer.

All the fluorescence measurements are carried on the 5301PC fluorescence spectrophotometer. Modified proteins (SF and SRB) were dissolved in TBS buffer and the concentration of protein samples is about 1μM.

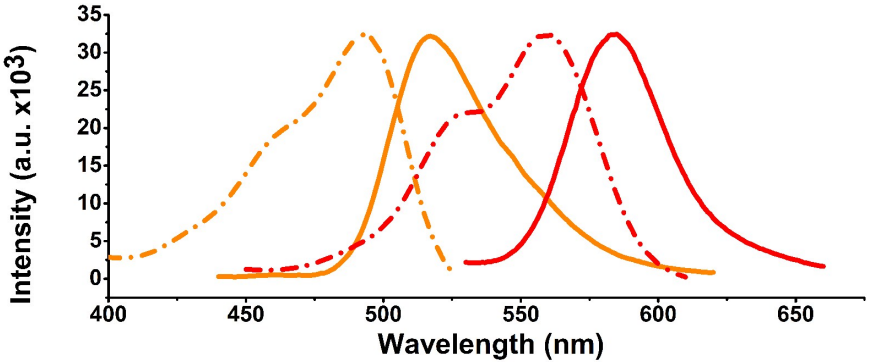

Fig. S9 The excitation and emission spectra of SF and SRB proteins, solid lines are the emission spectra of SF (orange excited at 460 nm) and SRB (red excited at 520 nm). Dash lines are the excitation spectra of SF (orange) and SRB B (red). There is a great overlap between the emission of SF and excitation of SRB, which reveals the two chromophores are suit as a pair of donor and acceptor.

Calculation of the quantum yields of SF and SRB:

The quantum yields of SM5S-FITC (SF) and SM5S-rhodamine B (SRB) were determined by the method reported by Williams et al and estimated using the fluorescein standards as follows:[3]

$$\Phi_x = \Phi_{st}\left(\frac{m_x}{m_{st}}\right)\left(\frac{n^2{}_x}{n^2{}_{st}}\right) \quad \text{(equation 1)}$$

where Φ_{st} represents the quantum yield of the standard, Φ_x represents the quantum yield of sample, m_{st} represents the slope of the linear fit for the standard, m_x represents the slope of linear fit for sample, and n is the refractive index of the buffer. Since both buffers were aqueous, the refractive index ratio was approximated to be unity.

Due to there is great difference between the wavelength of maxima absorbance of SM5S-FITC and SM5S-rhodamine B, we respectively use FITC (Φ=0.93) and rhodamine (Φ=0.89) as standards for test. The final results are shown as follow (Fig. S10)

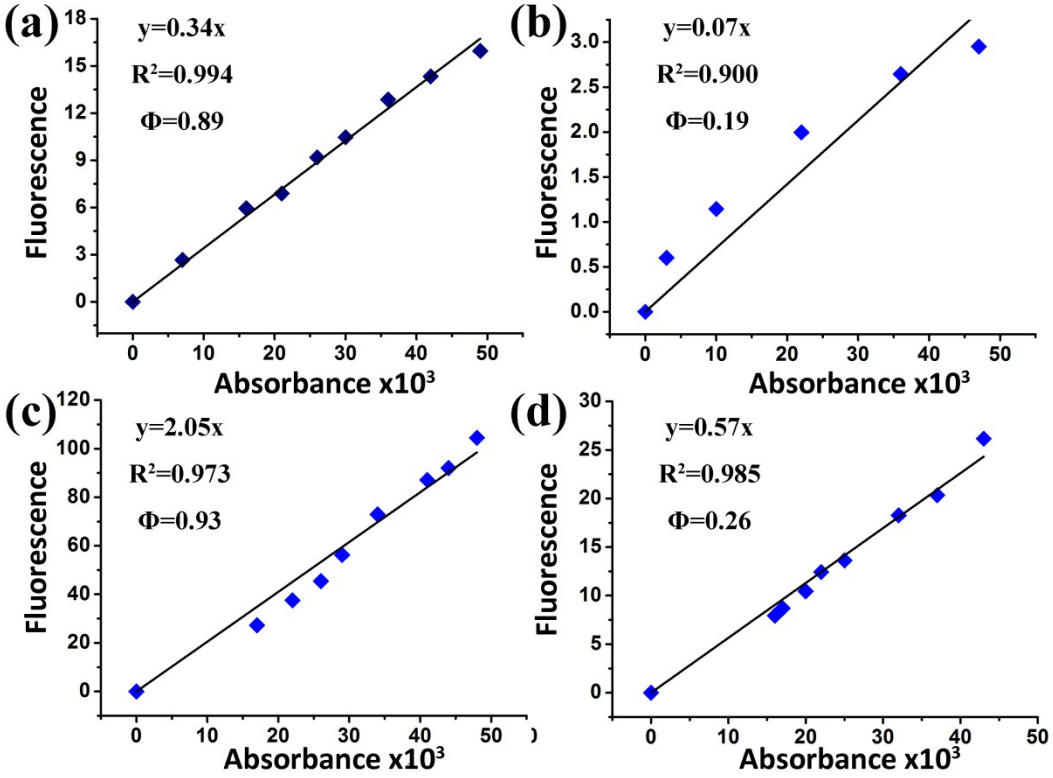

Fig. S10 Fluorescence spectrum-absorbance plots of (a) rhodamine B standard (c) FITC standard and (b) SM5S-rhodamine B (d) SM5S-FITC. The calculated quantum yields of SF and SRB are 0.26 and 0.19.

Fluorescence test of FRET event occurred in this system:

Emission at 650nm, the excitation spectrum is recorded from 400nm to 620 nm, slit widths are 10 nm (excitation) and 20 nm (emission). The emission spectrum is excited at 400nm and detected from 420nm to 650nm. The final excitation spectra are normalized at 497nm (Fig. S11a) and 554nm (Fig. S11b). The emission spectrum is excitation normalized (shown Fig. 8).

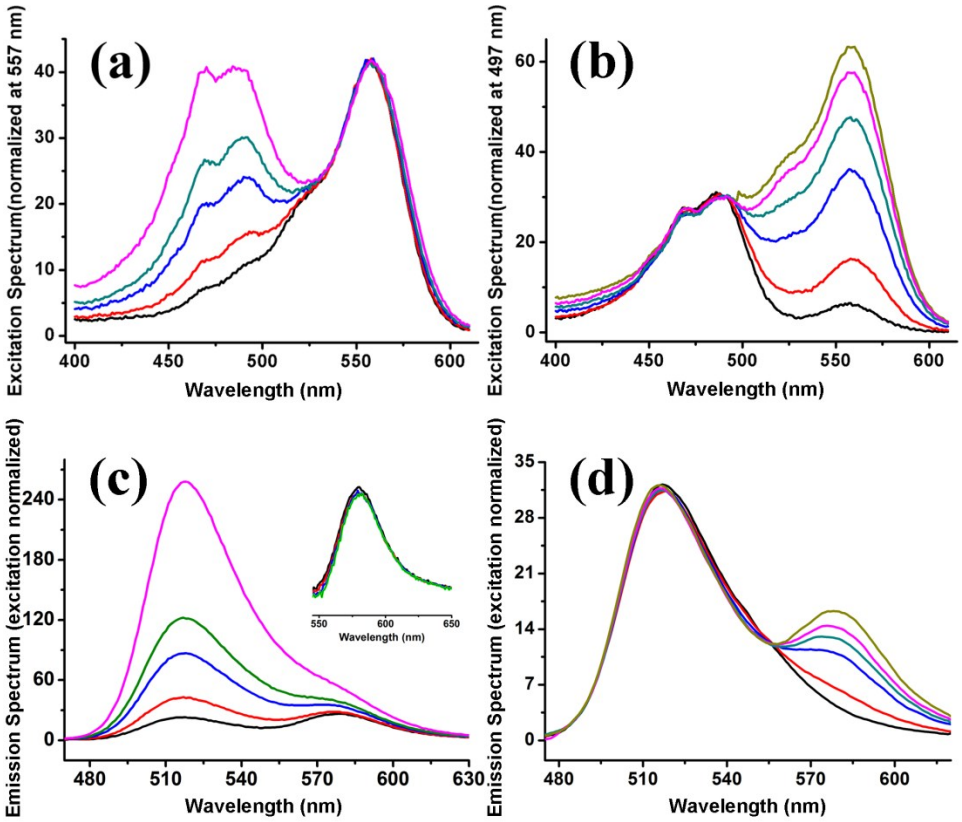

Fig. S11 Excitation spectra of SF-SRB system. (a) FRET system tested with increasing FITC and constant rhodamine B. The ratio could be calculated from the spectrum: FICT: rhodamine B=0.1(black) 0.2(red) 0.5(blue) 0.6(green) and 1(purple) (b) FRET system tested with increasing rhodamine B and constant FITC. The ratio could be calculated from the spectrum: FICT: rhodamine B=5(black) 2(red) 5/6(blue) 5/7(green) and 5/8(purple). Emission spectra of system with increasing amounts of the donor and invariant acceptor (c) and increasing amounts of the acceptor and invariant donor (d) before assembling through metal coordination.

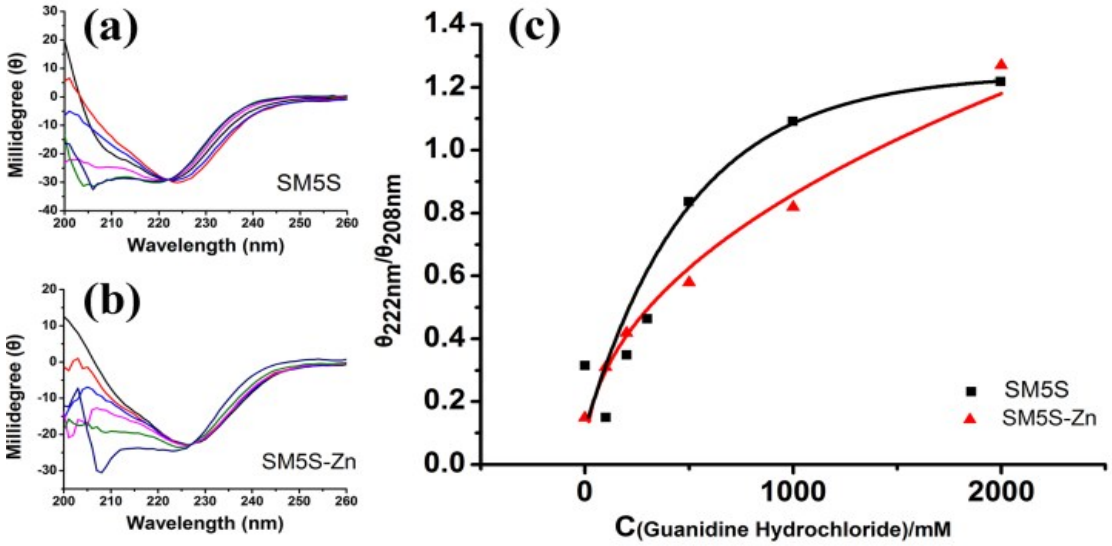

Fig. S12 (a-b) CD signals of SM5S protein and SM5S with 50equivalent Zn^{2+} normalized at 225nm, both systems were treated with 0mM (black line), 100mM (red line), 200mM (light blue), 500mM (magenta), 1M (green) and 2M (blue) guanidine hydrochloride before CD measurements. (c) Fitting lines of $\theta_{222nm}/\theta_{208nm}$ of SM5S signal protein (black) and SM5S with Zn (red).

Thermal stability Analysis

Temprature-dependent denaturation curves were analyzed using a two-state unfolding model reported by Pace et al and determining the fraction folded as follow:[4]

$$F_F=([\theta]-[\theta]_D)/([\theta]_F-[\theta]_D) \quad \text{(equation 2)}$$

where [θ] is the observed ellipticity and $[\theta]_F$ and $[\theta]_D$ are respective the ellipticities of the SM5S folded and denatured states.

The samples of SM5S and SM5S-Zn assembly are tested in neutral pH (TBS 50mM, pH=7.0) and the concentration of SM5S is 1μM. CD signals are recorded from 190 nm to 260 nm each 5 degree from 30℃ to 95℃. The minimum value at 225 nm of each CD spectrum is used for calculation of folded fraction; Because of the excess Zn^{2+}, when the concentration of protein self-assembly is higher (performed at 1μM), the metal induced protein self-assemblies are easy to aggregate along the increasing temprature, resulting the decrease of protein concentration and CD signal. Therefore, the assembly solution is diluted 5 times (final concentration is 200nM). The final CD signal will be multiplied by 5 when we use the data for folded fraction calculation.

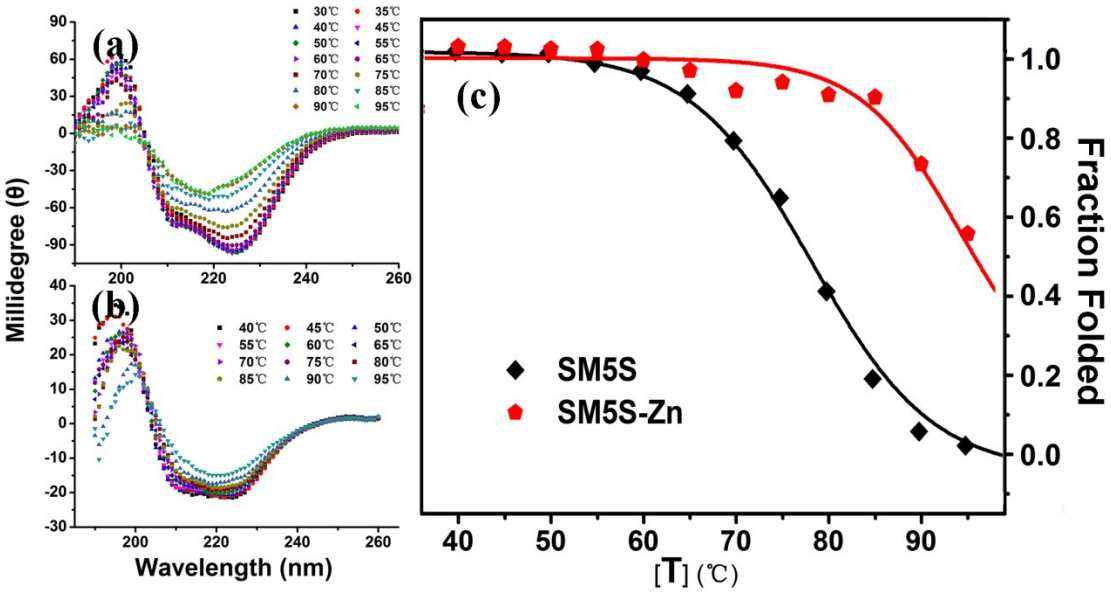

Fig. S13 CD spectra of SM5S (a) and SM5S-Zn (b) which are recorded each 5 degree from 40 to 95 degree. The folded fraction is calculated by using the equation 2 and shown in (c), the thermal stability of protein increase remarkably after self-assembling, the Tm have raised from 65 degree to 85 degree.

AFM images of SM5S incubated with Ca^{2+}, Mg^{2+}, Cu^{2+}, Ni^{2+}:

Other metal ions Mg^{2+}, Cu^{2+}, Ni^{2+}, and Ca^{2+} have been tried to obtain protein assembly. We did not detect regular protein assembly coordination with Cu^{2+}, Ca^{2+}, some small protein assemblies have been found after incubating with Mg^{2+} and Ni^{2+}, the size of assemblies is about 500nm, but no larger assembly nor 2D complex nanostructures are found, we speculate that Ni/Mg^{2+}-histidine$_4$ complex may show plane quadrangle rather than Zn-histidine$_4$ quadrangular pyramid, which may prevent the two proteins bond through BH1-metal-BH1 because of steric hindrance. What is more, protein assembly is formed through Low efficiency BH2-metal-BH2 site. Thus, the size of protein self-assembly always shows small.

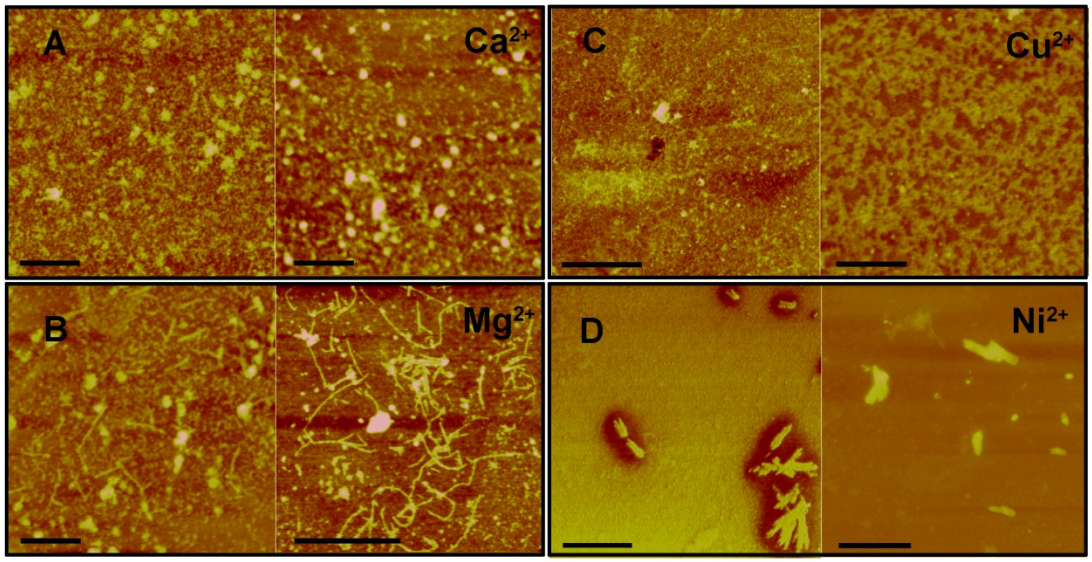

Fig. S14 AFM images of SM5S incubated with Ca^{2+}, Mg^{2+}, Cu^{2+}, Ni^{2+} with different metal/protein ratios, the better results of protein self-assemblies were shown in A (Ca^{2+}), B (Mg^{2+}), C (Cu^{2+}), D (Ni^{2+}). No regular protein assembly was detected after coordination with Cu^{2+}, Ca^{2+}. Some small protein assemblies have been found after incubating with Mg^{2+} and Ni^{2+}. The scale bars in AFM images are 1μm.

References for Supporting Information Section

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4 a) Pace, C. N. *Methods Enzymol*. **1986**, 131, 266–280; b) Jennifer R. L.; Robert S. H. *J. Biol. Chem.*, **2002**, 40, 37272-37279.