Supporting Information

Self-Assembly of Diphenylalanine Peptide into Microtubes with "Turn on" Fluorescence by an Aggregation-Induced Emission Molecule

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Experimental Section

Reagents. All chemicals were of analytical or higher grade. All solutions were prepared using ultrapure water from a Millipore Simplicity 185 water purification system (Millipore, Milford, MA, USA). The lyophilized diphenylalanine (NH_2-Phe-Phe-COOH, FF, ≥98%), 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) were purchased from Sigma-Aldrich Co. (USA). FF was characteristed by NMR: ^{1}H NMR (CD_3OD, 400 MHz): δ7.35~7.24 (m,10H), 4.52(dd, J_1 = 8.1 Hz, J_2 = 4.8 Hz, 1H), 3.99 (dd, J_1 = 9.0 Hz, J_2 = 4.8 Hz, 1H), 3.28~3.22 (m,2H), 3.02 (dd, J_1 = 13.9 Hz, J_2 = 8.1 Hz, 1H), 2.92 (dd, J_1 = 14.4 Hz, J_2 = 9.0 Hz, 1H). The electrospray mass spectra of FF is shown in Figure S1-A, which demonstrated the characteristic ions of FF in the positive mode including $[M+H]^+$ (*m/z* 313), $[M+Na]^+$ (*m/z* 335), $[2M+H]^+$ (*m/z* 625) and $[2M+Na]^+$ (*m/z* 647).

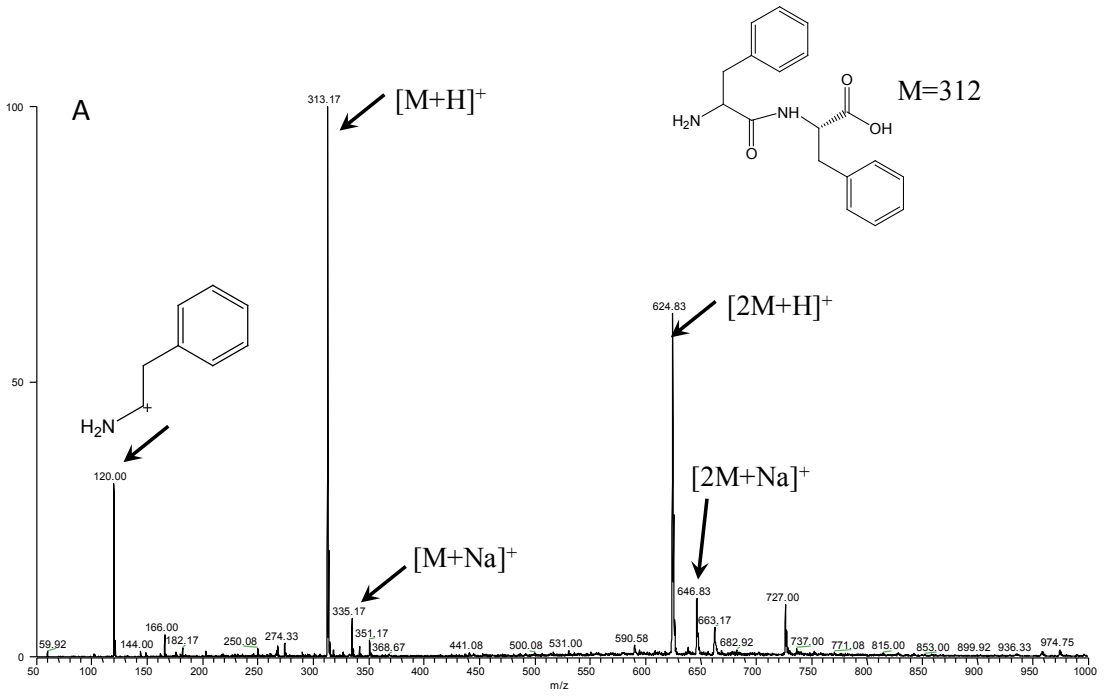
9, 10-Bis[4-(3-sulfonatopropoxyl)-styryl] anthracene (BSPSA) was synthesized according to our previous works.[15] Briefly, 9,10-Bis(4-methoxystyryl)anthracene was first synthesized by dissolving 9,10-dibromoanthracene, 4-methoxystryrene, K_3PO_4 and $Pd(OAc)_2$ in DMAC, and then stirred at 110 °C for 24 h. Then, the obtained 9,10-Bis(4-methoxystyryl)anthracene and dry CH_2Cl_2 were mixted and cooled to -78 °C, after which boron tribromide/CH_2Cl_2 (1.51 g/10 mL) was added. After stirring overnight, water was added to generate 9,10-Bis(4-hydroxystyryl)anthracene. At last, 9,10-Bis(4-hydroxystyryl)anthracene was added into anhydrous under nitrogen, after which NaOEt/anhydrous ethanol was slowly added. Then, with the addition of 1,3-propanesultone/ethanol, the mixture was vigorously stirred overnight

and a yellow product of BSPSA was precipitated. ^{1}H NMR (DMSO-d6, 400 MHz): δ 8.40 (d, J = 3.3 Hz, 2H), 8.38 (d, J = 3.3 Hz, 2H), 7.96 (d, J = 16.5 Hz, 2H), 7.74 (d, J = 8.7 Hz, 4H), 7.55 (d, J = 3.2 Hz, 2H), 7.53 (d, J = 3.2 Hz, 2H), 7.01 (d, J = 8.7 Hz, 4H), 6.86 (d, = 16.5 Hz, 2H), 4.13 (t, J = 6.5 Hz, 4H), 2.58 (t, J = 7.2 Hz, 4H), 2.03 (m, 4H). The electrospray mass spectra of BSPSA are shown in Figure S1-B, which shows the characteristic ion of $[M-2Na]^{2-}$ (*m/z* 328) in the negative mode.

Self-assembly of FF. For the self-assembly, 10 mg of lyophilized FF was dissolved in 100 μL HFP to disrupt any possible aggregation in the peptide sample. Then, 20 μL of FF/HFP (100 mg/mL) solution was transfered to a 1.5 ml centrifuge tube, and allowed to dry by evaporation under ambient conditions. After FF/HFP solution was dried completely by evaporation at ambient conditions, FF was re-solubilized in ultrapure water by addition of 1 mL water [20] or a certain concentration of BSPSA aqoeous solution and subsequent ultrasonic treated at 65 ° C for 30 min. The FF aqueous solution was then annealed at room temperature and allowed to age for one day. The obtained assemblage was utilized for further characterization.

Characterization Methods. Fluorescence microscope images were recorded on an Olympus IX71 fluorescence microscope (Olympus, Japan). 20 μL 0.5 mg/ml FF aqueous solution was pipetted onto a glass slide to dry by evaporation under ambient conditions. Pictures were then taken after the assembly procedures. Scanning electron microscope (SEM) photographs were taken on a Hitachi S-4800 scanning electron microscope (Hitachi, Japan) by dripping 30 μL 0.5 mg/ml FF aqueous solution on

glass slide to dry. Before SEM imaging, a thin layer of Pt was coated on the film surface by an Auto Fine Coater (JEOL) at a current of 20 mA for 150 s. Fluorescence spectra were recorded by a SHIMADZU RF-5301PC spectrophotometer (Shimadzu, Japan) at 25 °C (5-nm slit width, 1-cm path-length cuvettes,). For polarization experiments, the obtained microtubes after self-assembly of Phe-Phe in BSPSA solution were directly subjected to the test in aqueous solution. UV-Vis absorption spectra were obtained with a TU-1901 UV-Vis spectrophotometer (Beijing, China). Circular dichroism (CD) spectra were recorded on a Pistar π-180 spectrophotometer (Applied Photophysics Ltd., UK) at room temperature using 1 mm quartz cells. Scans were obtained in a range between 190 and 350 nm by taking points at 1 nm (time per point: 2 s, Bandwidth: 4 nm). Three spectra were averaged to improve the signal-to-noise ratio.



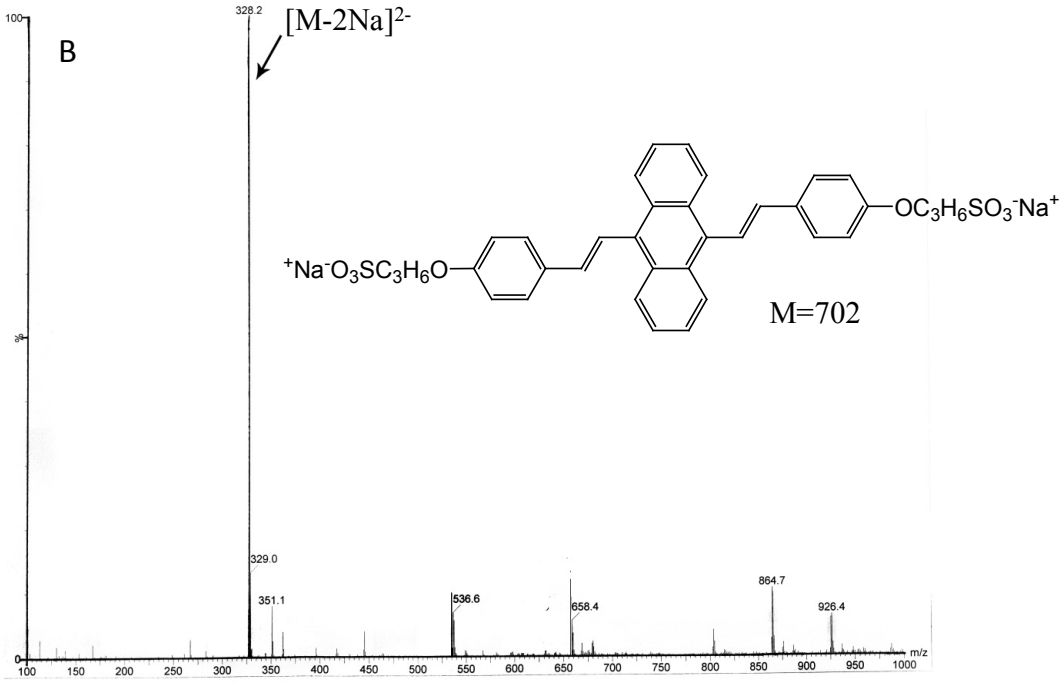


Figure S1. Mass spectra of FF (A) and BSPSA (B).

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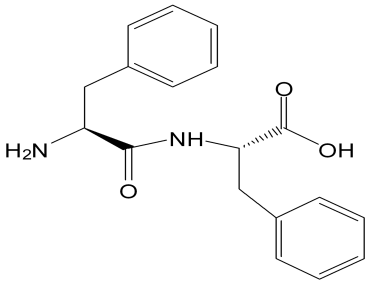
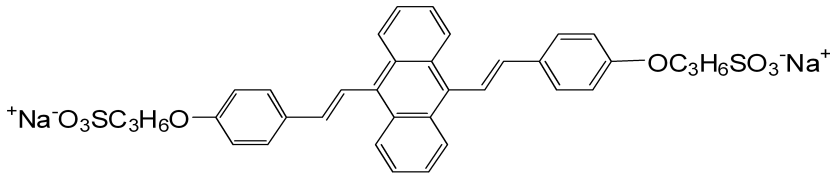


phenylalanine



9,10-bis[4-(3-sulfonatopropoxyl)-styryl] anthracene（BSPSA）sodium

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75 **Figure S2.** Molecular structure of phenylalanine and BSPSA.

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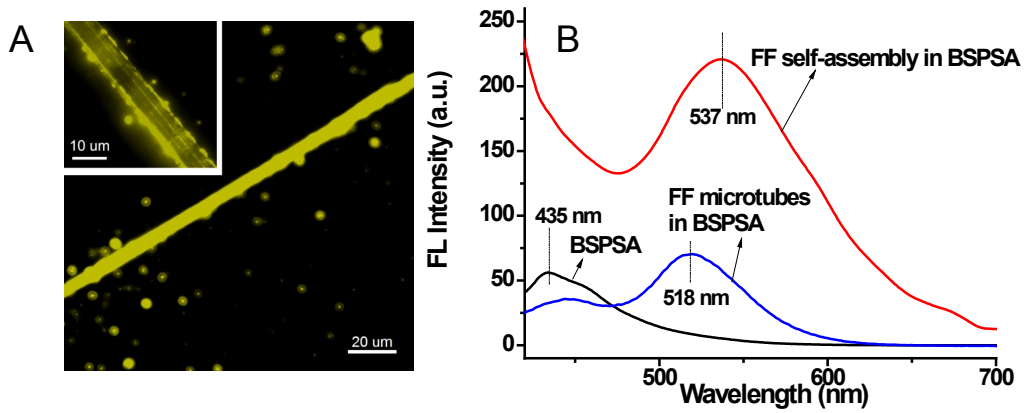
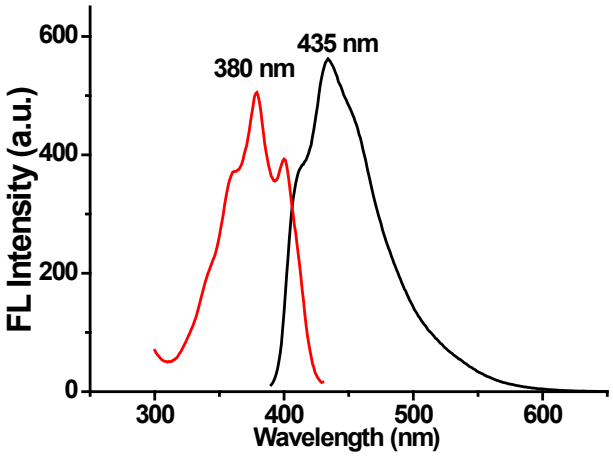


Figure S3. (A) Fluorescence microscope image of microtubes obtained by self-assembly of 2.0 mg/mL FF in water and then incubated with 0.01 mg/mL BSPSA solution. (B) Fluorescence spectra of BSPSA, microtubes of incubating formed non-fluorescence microtubes with BSPSA, and microtubes obtained by FF self-assembly in BSPSA solution. BSPSA concentration: 0.01 mg/mL.



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86 **Figure S4**. Fluorescence spectrum of BSPSA solution excited at 380 nm (black line)

87 and the emission spectrum (red line). BSPSA concentration: 0.01 mg/mL.

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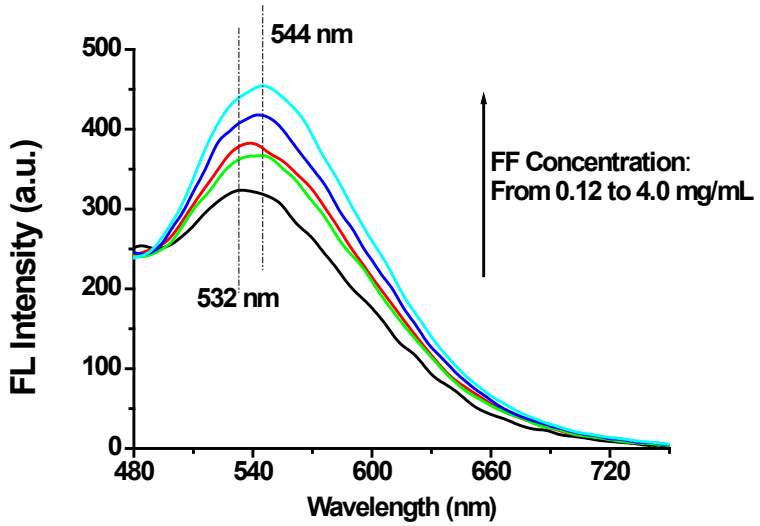

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91 **Figure S5.** Fluorescence spectra of different concentration of FF assemblage in

92 BSPSA solution. FF concentration: 0.12, 0.25, 0.5, 1.0, 2.0, 4.0 mg/mL, respectively.

93 BSPSA concentration: 0.01 mg/mL. Excitation wavelength: 404 nm.

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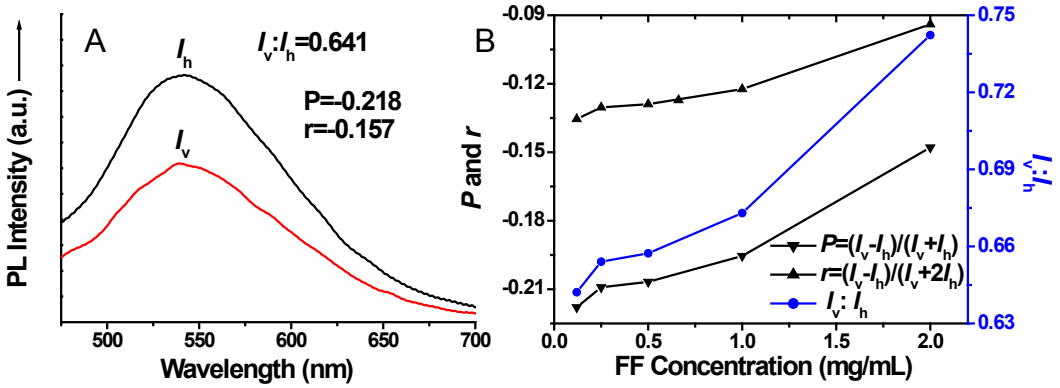

Figure S6. Polarized emissions from microtubes obtained by the self-assembly of different concentrations of FF in BSPSA solutions. FF concentration: 0.12, 0.25, 0.5, 1.0, 2.0 mg/mL, respectively. BSPSA concentration: 0.01 mg/mL. Excitation wavelength: 404 nm.

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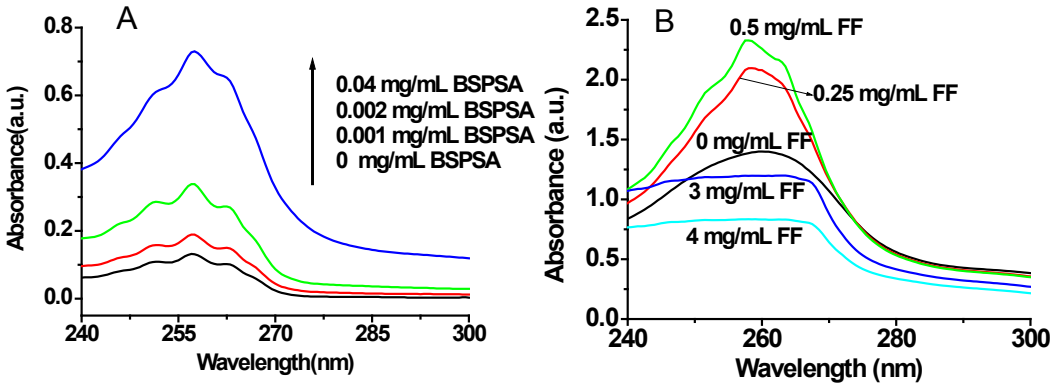

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105 **Figure S7**. UV-Vis absorption spectra of microtubes by self-assembly of FF in

106 BSPSA aqueous solution. (A) 2.0 mg/mL FF in different concentration of BSPSA

107 solution. (B) Different concentration of FF in 0.01 mg/mL BSPSA.

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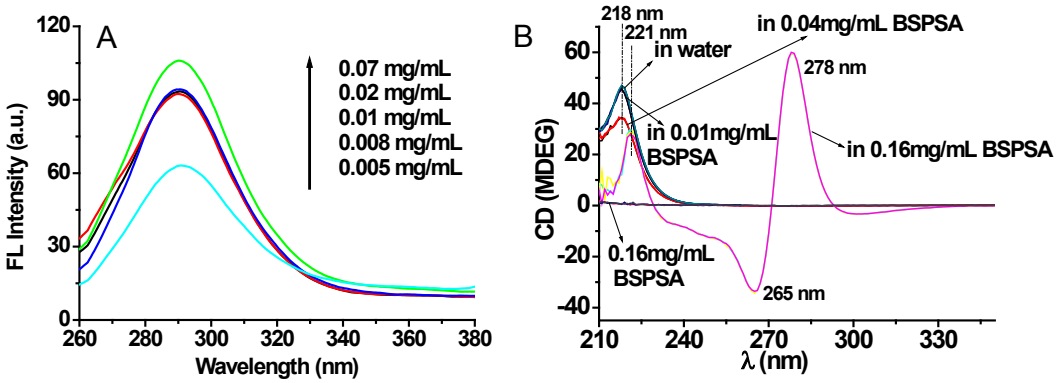

Figure S8. (A) FL spectra of 0.2 mg/mL FF solutions in different concentration of BSPSA aqueous solution (0.005 to 0.07 mg/mL). Excitation: 260 nm. (B) CD spectra of 0.16 mg/mL BSPSA solution, microtubes obtained by self-assembly of 2.0 mg/mL FF in water, 0.01, 0.04 and 0.16 mg/mL BSPSA aqueous solution. The samples were four times diluted for the test.