Supporting Information

Construction of supramolecular polymer by enzyme-triggered covalent condensation of CB[8]-FGG-based supramonomer

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Supporting Information Contents

Materials.

2-Chlorotrityl chloride resin, Fmoc-protected amino acids (Fmoc-Phe-OH, Fmoc-Gly-OH and Fmoc-Tyr(tBu)-OH), benzotriazole-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP), N-methylmorpholine (NMM) , N-hydroxybenzotriazole anhydrous (HOBt anhydrous), triisopropylsilane (TIS) and trifluoroacetic acid (TFA) were purchased from GL Biochem (Shanghai) Ltd. and used directly without further purification. Dimethyl formamide (DMF) and dichloromethane (DCM) were rigorously dried with sodium and then redistilled prior to use. Horseradish peroxidase (HRP) was purchased from Aladdin Industrial Corporation. All water used was prepared on a Millipore water purification apparatus with a minimum resistivity of 18.0 MΩ cm. H_2O_2 and other chemicals were purchased commercially with analytical grade purity. Cucurbit[8]uril (CB[8]) was synthesized according to the method reported by Kim.[1]

Instruments and measurements

HPLC experiment was carried out with a reversed-phased Shimadzu HPLC system equipped with a semi-preparative Varian Dynamax C18 column and a SPD-20A UV-Vis detector. Samples were eluted with CH_3CN/water (50/50 by volume, containing 0.1% TFA) over 15 min. ^1H NMR spectroscopy, ^{13}C NMR spectroscopy and Diffusion-ordered NMR spectroscopy were performed on a Bruker AVANCE III 500 MHz instrument using D_2O as the solvent. ESI-MS spectra were conducted on a Thermo Finnigan LCQ AD Mass Spectrometer with an electro-spray interface. UV-vis absorption spectra were recorded on a Shimadzu 3100 UV-vis-NIR spectrophotometer. Fluorescence emission spectra were acquired on a Perkin-Elmer LS-55 luminance spectrometer. The excitation wavelength was set at 275 nm. All the experiments for UV-vis absorption spectra and fluorescence emission spectra were carried out at room temperature. Dynamic light scattering (DLS) measurements were conducted with a Malvern Zetasizer Nano ZS instrument equipped with a He-Ne laser and an avalanche photodiode detector. Samples were centrifuged or filtrated with syringe filter unit before test. Atomic force microscopy (AFM) was conducted using the tapping mode AFM on a NanoScope Multi-mode-AFM (Veeco, USA). Scanning electron microscopy (SEM) measurements were performed on a JEOL JSM-6700F scanning electron microscope with a primary electron energy of 3 kV. All AFM and SEM samples were prepared by

placing a 5 μL drop of solution to the surface of the freshly prepared hydroxylated silicon wafer and followed drying in air. Transmission electron microscopy (TEM) observations were recorded on a JEM-2100F electron microscope at an acceleration voltage of 120 kV. Samples for TEM observations were prepared by placing a 5 μL drop of the supramolecular polymer solution on formvar carbon-coated copper grids for 2 min and subsequent negative staining with 5 μL of sodium phosphotungstate aqueous solution (2% by mass) for 1 min.

Peptide synthesis and purification

Peptide FG4Y (NH_2-Phe-Gly-Gly-Gly-Gly-Tyr-COOH) was synthesized on a preloaded 2-chlorotrityl chloride resin with a standard Fmoc (9-fluo-renylmethoxycarbonyl) solid phase peptide synthesis (SPPS) method. Typically, to a peptide synthesis vessel, 0.3 mmol of 2-chlorotrityl chloride resin was added. After swelling the resin in DCM for 30 min, the first Fmoc-protected amino acid were loaded onto the resin by using 2.5 equiv. of Fmoc-Tyr(tBu)-OH and 5 equiv. of DIEA in DCM solution for 1 hour. Then Fmoc groups were removed by 20% (by volume) piperidine in DMF for 30 min. Other coupling reactions were carried out with 3 equiv. of each amino acid, 3 equiv. of HOBt, 3 equiv. of PyBOP, and 3 equiv. of NMM in DMF. Repetitive coupling reactions and Fmoc removals according to the Fmoc SPPS procedure resulted in the successful growth of the peptide sequence. Peptide cleavage was conducted with a mixture of TFA-H_2O-TIS (95: 2.5: 2.5 by volume) for 3 h. After filtration, the solution was concentrated under reduced pressure to produce the crude product. Pure product was obtained upon purification on a reversed-phased C18 column with water-acetonitrile (1:1 by volume) as eluent. The peptide was characterized by a Bruker 500 MHz spectrometer and ESI-MS spectrometer. ^{1}H NMR (500 MHz, D_2O, 25 ℃) d (ppm): 7.37 (m, 3H), 7.27 (t, 2H), 7.09 (m, 2H), 6.81 (m, 2H), 4.56 (m, 1H), 4.27 (t, 1H), 3.92 (m, 8H), 3.18 (m, 2H), 3.06 (m, 1H), 2.90 (m, 1H); ^{13}C NMR (500 MHz, D_2O, 25 ℃) d (ppm): 35.97, 36.74, 39.09, 42.16, 42.33, 42.45, 54.46, 54.78, 115.37, 115.42, 128.02, 128.51, 129.16, 129.39, 130.59, 133.71, 154.37, 169.81, 170.71, 171.42, 171.71, 171.95, 175.51; $C_{26}H_{32}N_6O_8$, ESI-MS: calc. $(M+H)^+ = 557.23$, obsvd. $(M+H)^+ = 557.2$.

Preparation of supramonomer

To a FG4Y solution (1 mM), 0.5 mM of CB[8] was added. Drastic sonication of the mixture for 1 minute produced a transparent solution, which indicated the complexation of FG4Y and CB[8] was accomplished and the supramonomer was obtained. Supramonomer used at other concentration was prepared by directly diluting the stock solution in pure water.

Preparation of supramolecular polymer

To the supramonomer solution at different concentrations, HRP (1.0×10^{-4} mM-5.0×10^{-3} mM) and H_2O_2 (2 equiv.) were added. Supramolecular polymers were formed upon incubation of the solution for several minutes to several hours according to the dosage of HRP.

Characterization of FG4Y

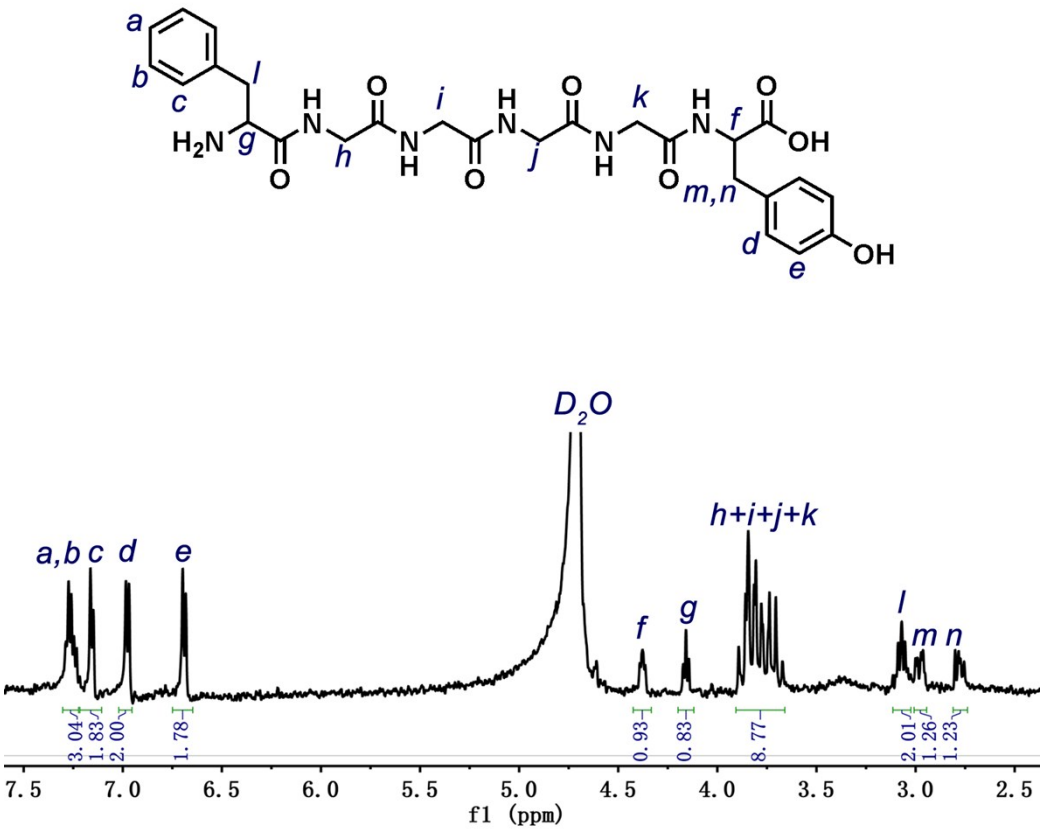

Figure S1. ^{1}H NMR spectrum recorded for FG4Y in D_2O.

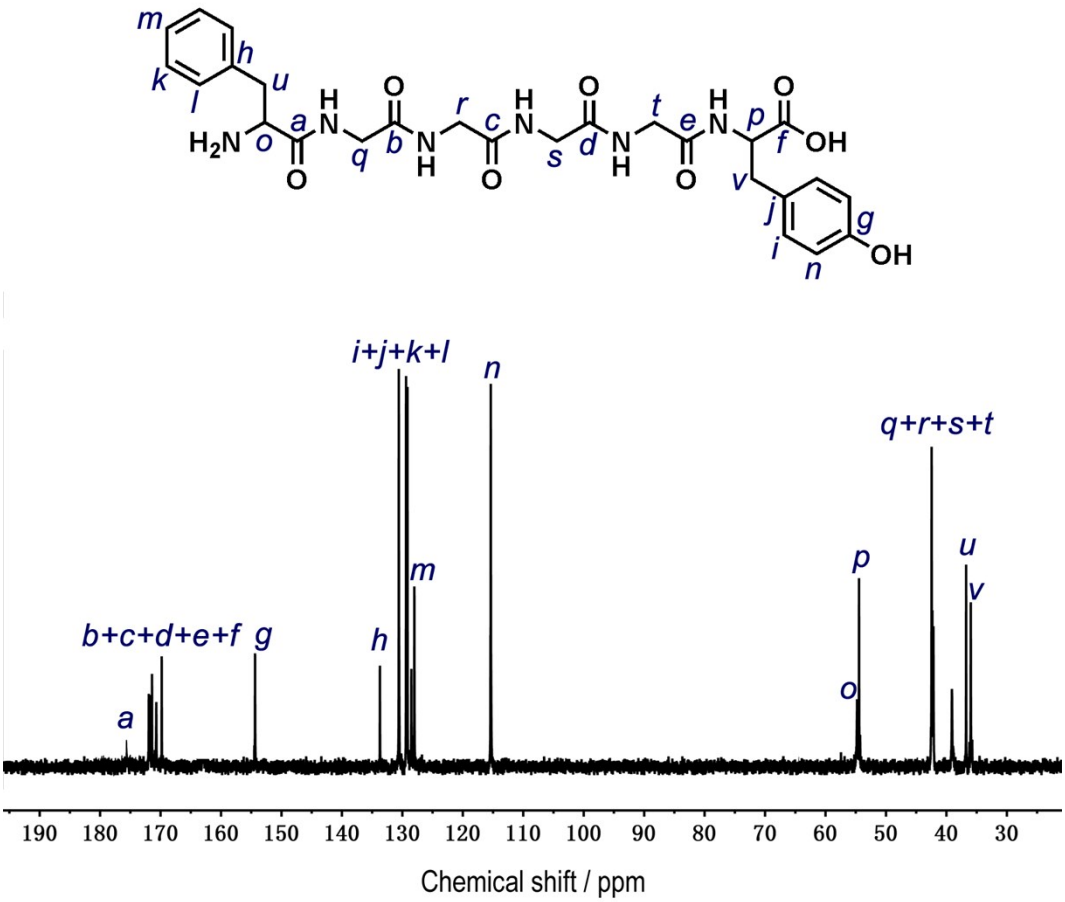

Figure S2. ^{13}C NMR spectrum recorded for FG4Y in D_2O.

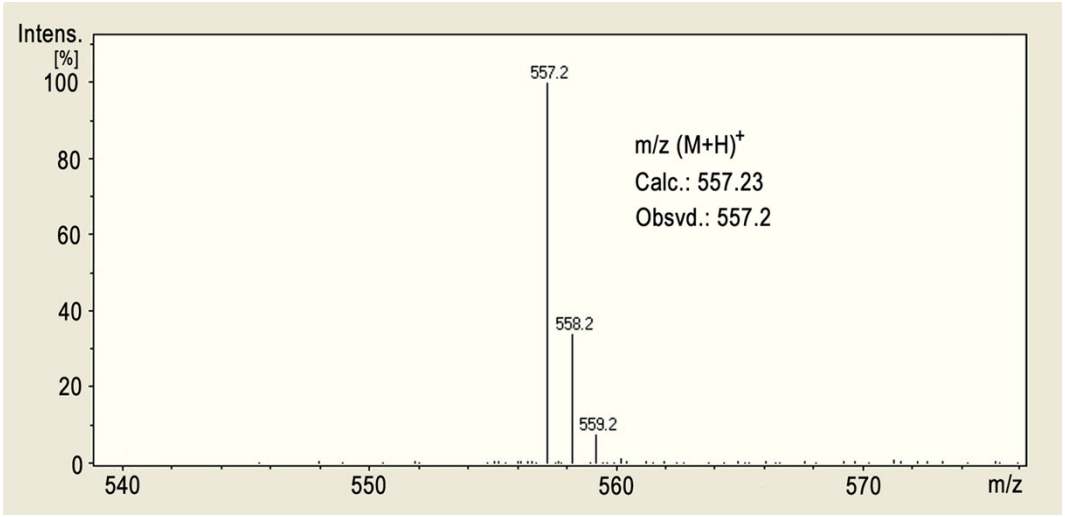

Figure S3. ESI-MS spectrum recorded for FG4Y.

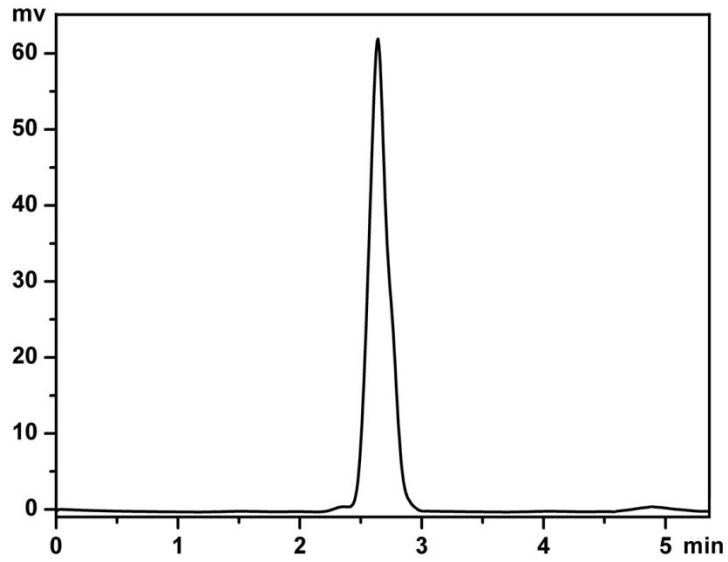

Figure S4. HPLC spetrum recorded for FG4Y.

Characterization of the supramonomer

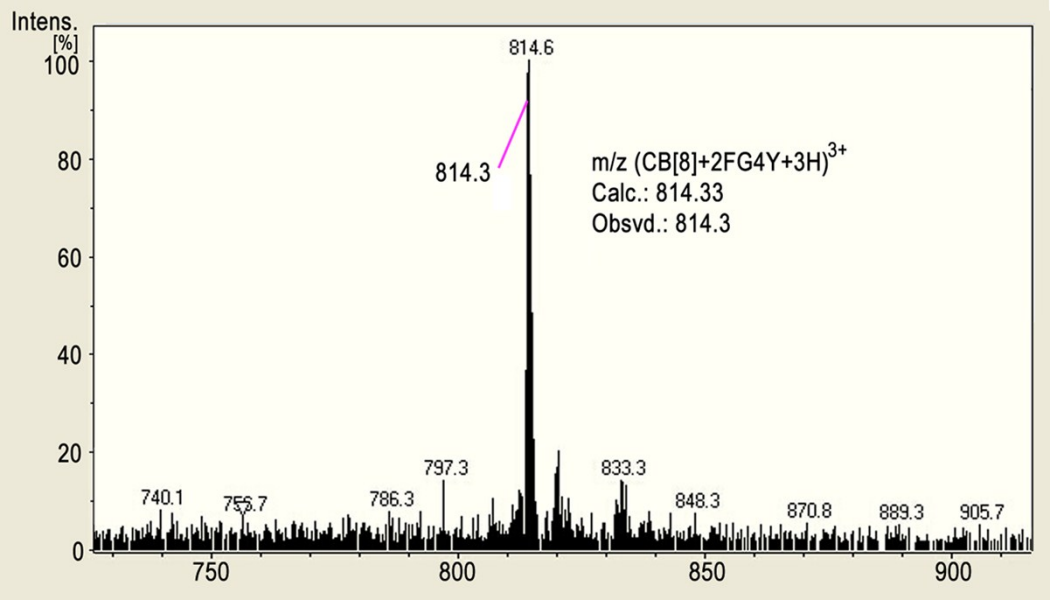

Figure S5. ESI-MS spectrum of the supramonomer prepared by the complexation of FG4Y and CB[8] in a molar ratio of 2:1 in aqueous solution.

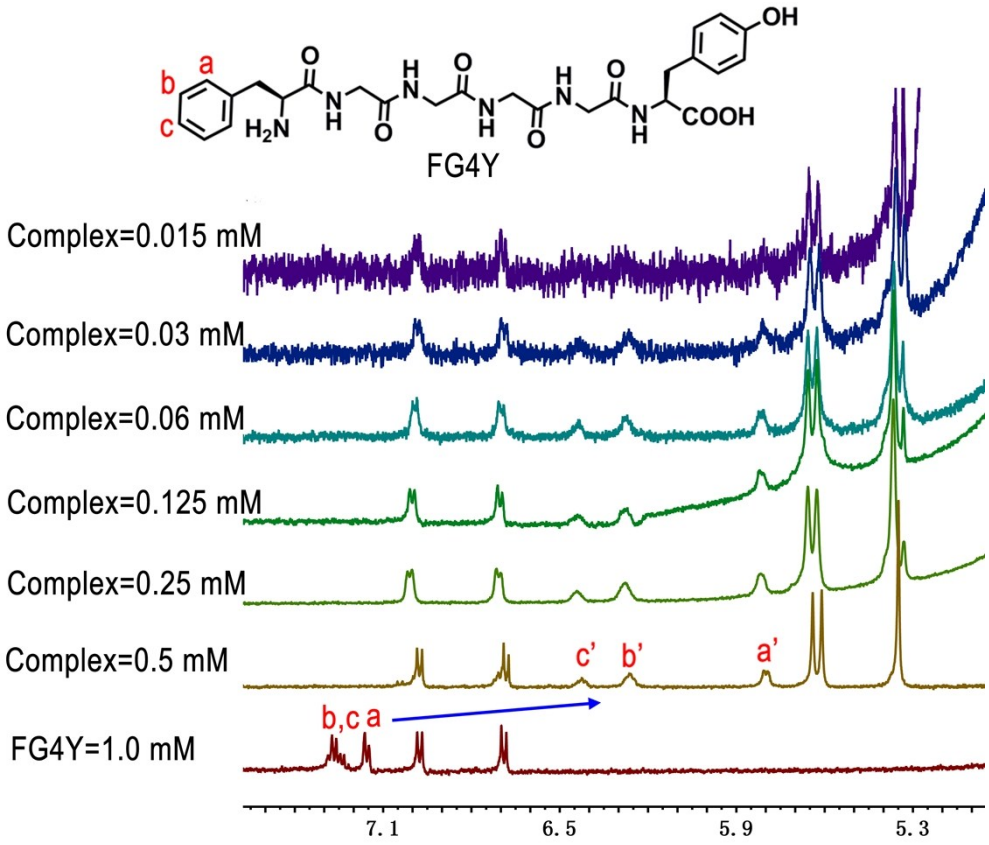

Figure S6. Partial ^{1}H NMR spectra of FG4Y at 1.0 mM and the ternary supramonomer prepared by the complexation of FG4Y and CB[8] in a molar ratio of 2:1 in different concentration.

Investigation of the catalytic conditions

It has been well-established that the phenol groups can be specifically coupled together through C-C bond or C-O bond under the catalysis of HRP in the presence of H_2O_2. Herein, to study the appropriate concentrations of HRP and H_2O_2 for FG4Y coupling, ^{1}H NMR experiments were performed.

Firstly, the HRP dosage was investigated by using a controlling variables method. The detailed procedure was carried out as follows: to a solution containing 1.0 mM of FG4Y and 5.0 mM of H_2O_2, different concentrations of HRP were added. After incubation overnight, the ^{1}H NMR spectra of the solutions were recorded and the phenol coupling yields were measured by calculating the diminished integral value of the phenol groups. As shown in Figure S7, from

2.5×10^{-5} mM to 2.5×10^{-4} mM, the coupling yield for phenol coupling was increased as increasing the HRP dosage. 2.5×10^{-4} mM, almost all the phenol groups were reacted, that is to say, 0.01 2.5×10^{-4} mM of HRP is an appropriate doze for 1 mM FG4Y coupling which consumes the minimum amount of HRP and achieves about 100% yield.

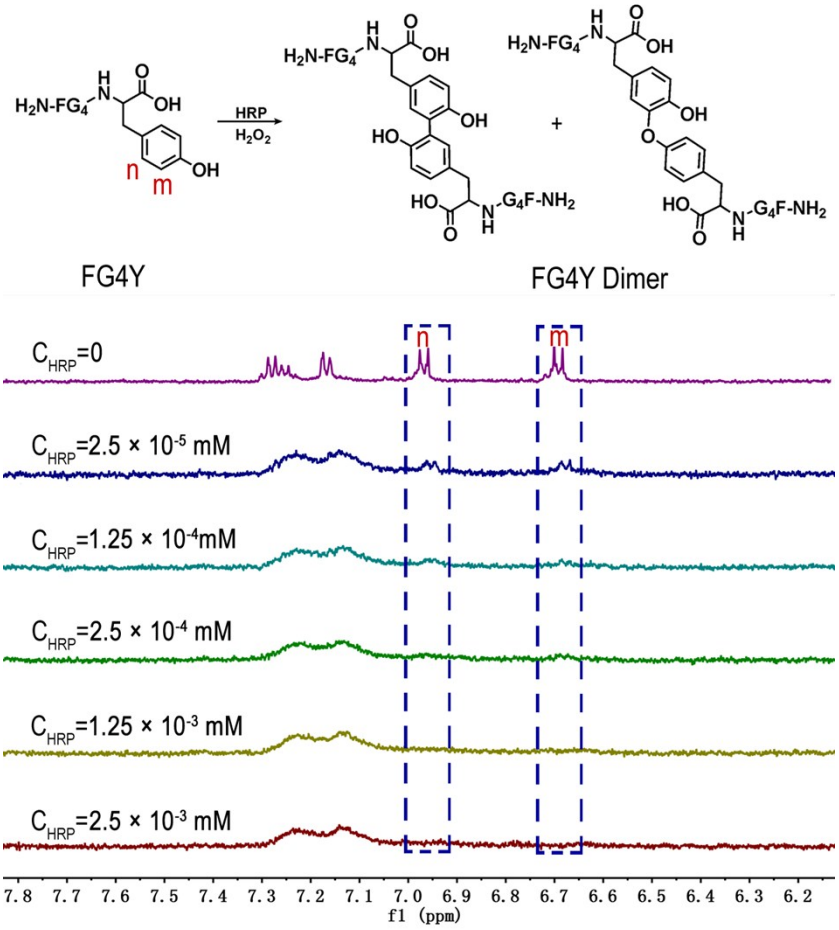

Figure S7. ^{1}H NMR spectra of FG4Y before and after the catalysis of different concentrations of HRP in the presence of H_2O_2. The concentrations of FG4Y and H_2O_2 were fixed at1.0 mM and 5.0 mM respectively in all cases while that of the HRP were varied from 0 to 2.5×10^{-3} mM.

Similarly, the appropriate H_2O_2 concentration was also studied. The concentrations of FG4Y and HRP were fixed at 1.0 mM and 2.5×10^{-4} mM respectively while the H_2O_2 concentration was varied. As shown in Figure S8, ^{1}H NMR results reveal that the minimum concentration of H_2O_2 is 1.0 mM for complete FG4Y coupling.

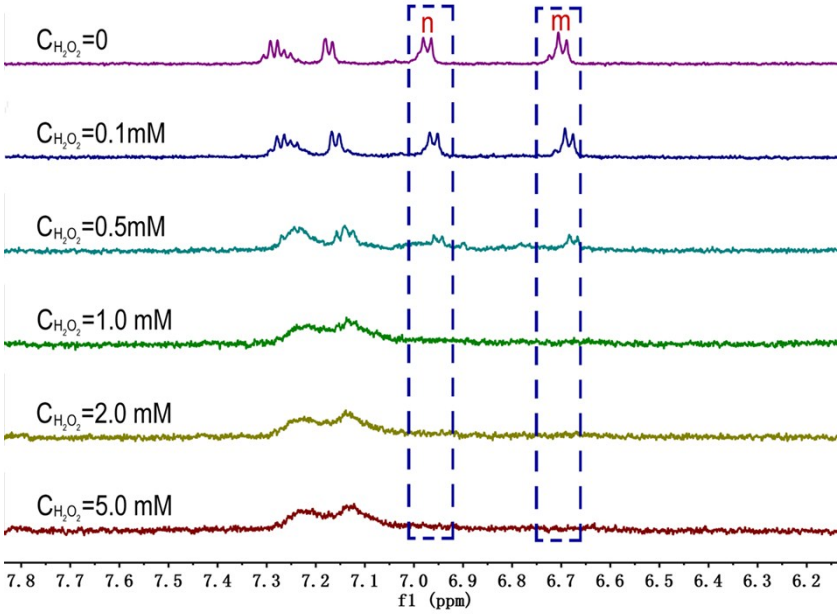

Figure S8. ^{1}H NMR spectra of FG4Y before and after the catalysis of HRP in the presence of different concentrations of H_2O_2. The concentrations of FG4Y and HRP were fixed at 1.0 mM and 2.5×10^{-4} mM in all cases while that of the H_2O_2 were varied from 0 to 5 mM.

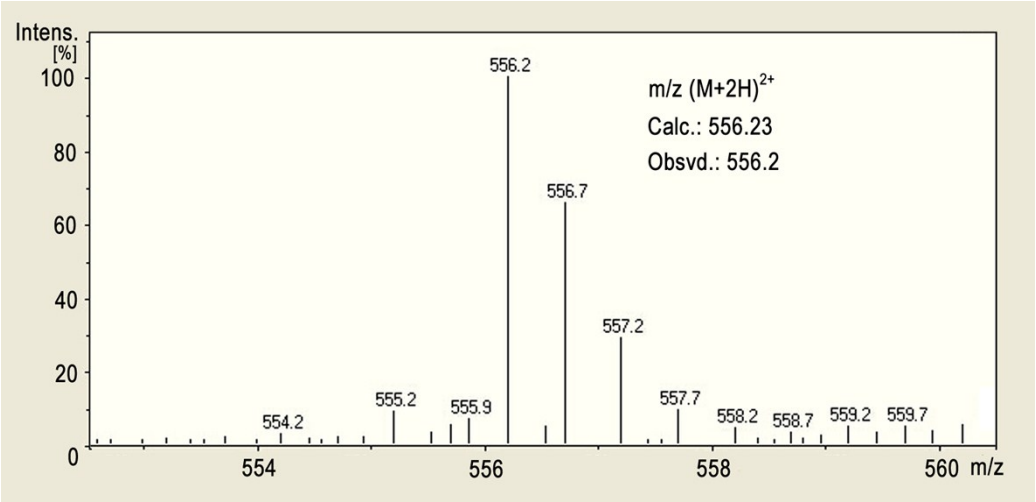

Figure S9. ESI-MS spectrum of the dimeric FG4Y formed by FG4Y coupling under the catalysis of HRP in the presence of H_2O_2. The concentrations of FG4Y, HRP and H_2O_2 are 1.0 mM, 2.5×10^{-4} mM and 1.0 mM, respectively. The calculated mass-to-charge ratio of the coupled FG4Y with 2 positive charges is 556.23 while the observed value is 556.2. This demonstrated that the FG4Y coupling was actually achieved upon the catalysis of HRP in the presence of H_2O_2.

Characterization of the supramolecular polymer

DLS measurements

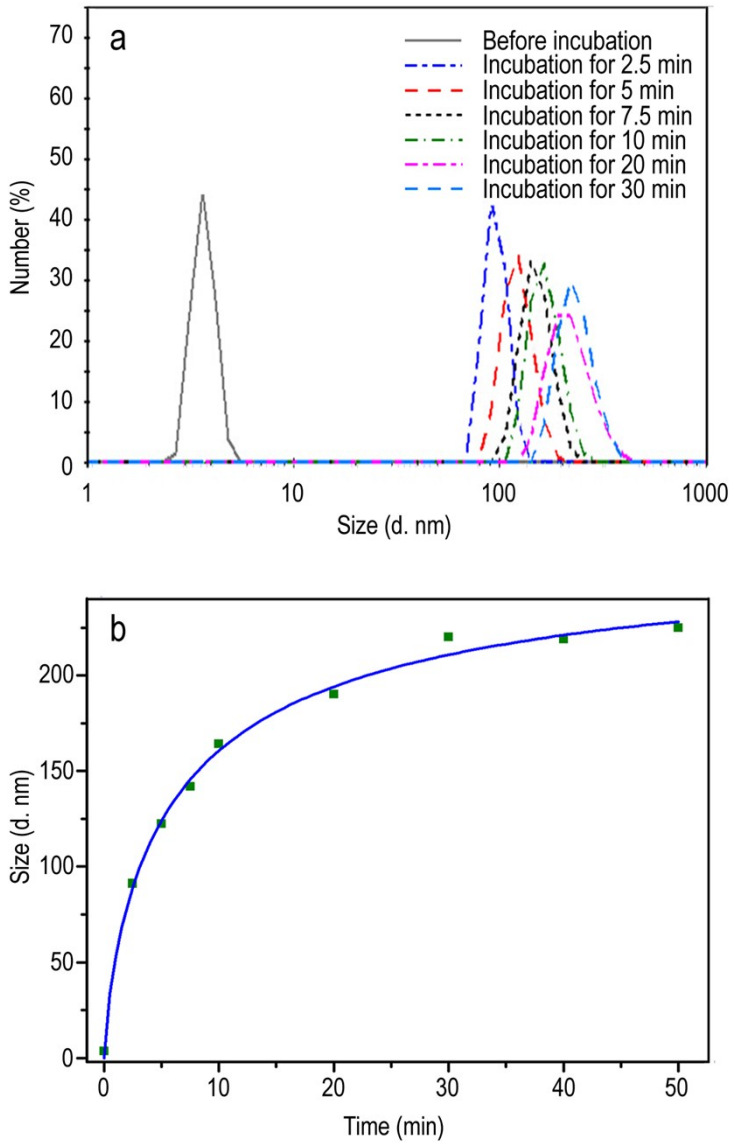

Figure S10. DLS analyses of the growth process of the supramolecular polymer. (a) Hydrodynamic sizes of the supramonomer-HRP mixed solution at different incubation times before and after the treatment with H_2O_2. The concentrations of supramonomer, HRP and H_2O_2 were 0.01 mM, 1.25×10^{-4} mM and 0.02 mM, respectively. (b) Plot of hydrodynamic size versus incubation time during the supramolecualr polymer growth process. The curve was drawn according to panel a.

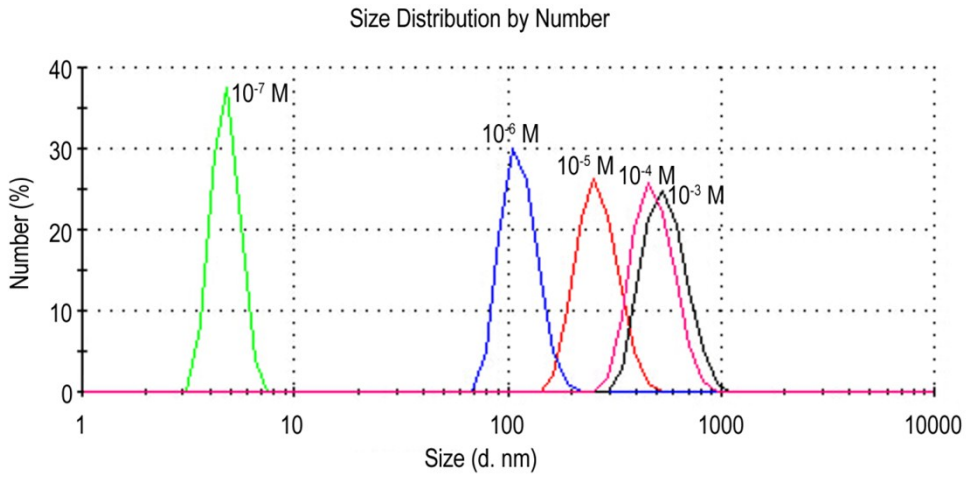

Figure S11. DLS measurements of the supramolecular polymer in different concentrations. The sizes of the supramolecuar polymer were 4.85 nm, 105.7 nm, 255.0 nm, 458.7 nm, 531.2 nm at 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M and 10^{-3} M, respectively. DLS results revealed that the size of the supramolecular polymer is concentration-dependent and high-molecular-weight supramolecular polymer cannot form until the concentration is above 10^{-6} M.

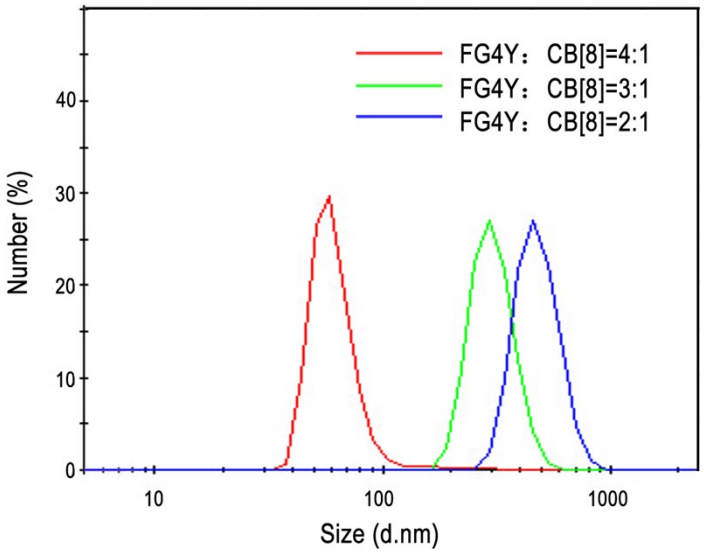

Figure S12. DLS analyses of the supramolecular polymer with different molar ratio of FG4Y and CB[8]. Samples were prepared by two steps. First, different molar ratio of FG4Y and CB[8] were mixed (the concentration of FG4Y is 0.2 mM), then HRP 2.5×10^{-4} mM and H_2O_2 (0.2 mM) were added to catalyze the polymerization. The hydrodynamic diameters of the samples were 58.77 nm, 295.3 nm, 458.7 nm at molar ratio of 4:1, 3:1 and 2:1, respectively. These results revealed that the accurate 2:1 molar ratio of FG4Y to CB[8] is favorable for the

Diffusion-ordered NMR spectroscopy (DOSY): Dosy was employed to further testify the formation of the supramolecular polymer. The higher diffusion coefficient (D) suggests the larger size of the compounds. For the supramonomer, the average diffusion coefficient was measured to be 2.51×10^{-10} m^2 s^{-1} (Figure S13). After enzymatic polymerization, a new diffusion coefficient of 1.95×10^{-10} m^2 s^{-1} was obtained (Figure S14), the decrease in the diffusion coefficient also implied that larger-sized supramolecular polymer was produced.

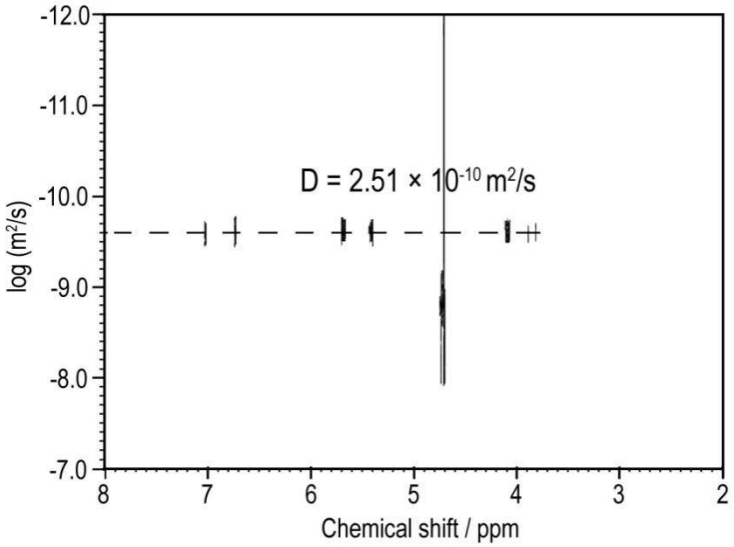

Figure S13. DOSY-NMR spectrum (600 MHz) of the solution of supramonomer (0.05 mM) in D_2O. The calculated D value is 2.51×10^{-10} m^2/s.

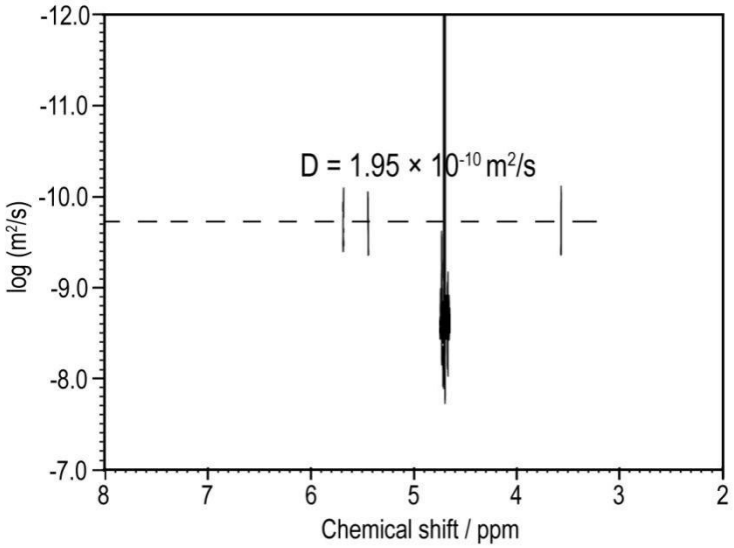

Figure S14. DOSY-NMR spectrum (600 MHz) of supramolecular polymer in D_2O. The calculated D value is 1.95×10^{-10} m^2/s.

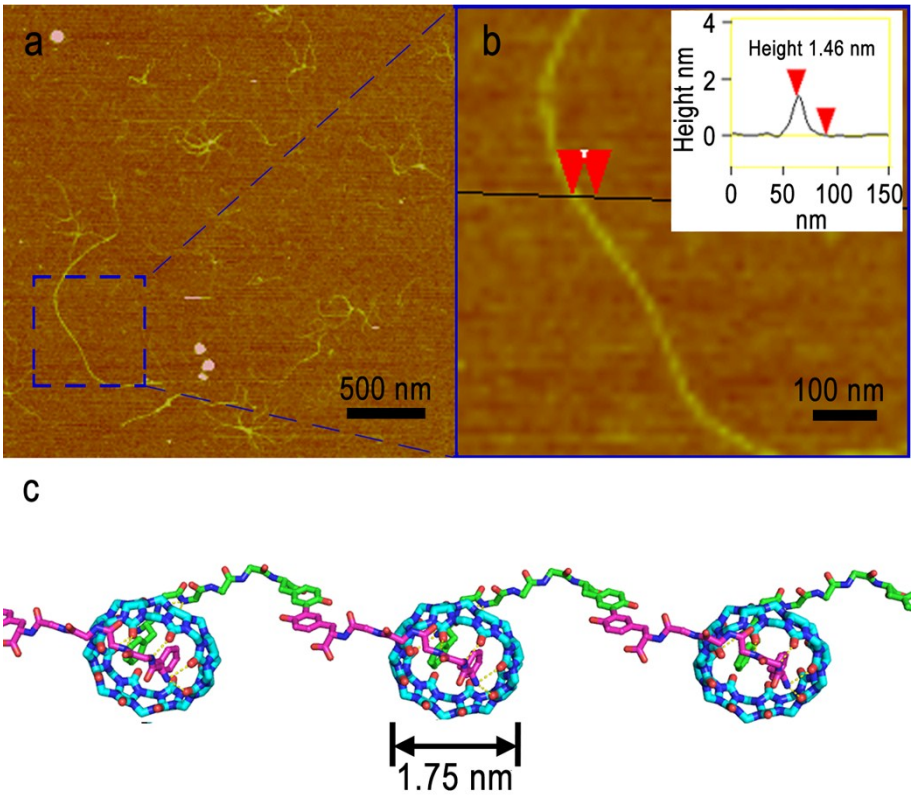

Figure S15. (a) AFM image of the supramolecular polymer at 0.05 mM; (b) partially amplificatory AFM image from panel a and the corresponding height curve of the supramolecular polymer. The supramolecular polymer appears to be fibril structure with height about 1.46 nm and length mainly around 400-500 nm. (c) Supramolecular polymer model made by software Pymol. The external diameter of the CB[8] molecule is 1.75 nm and the distance between two adjacent CB[8] of the supramolecular polymer is measured to be around 3.5-4.0 nm, which implied the fibrils are probably single-stranded supramolecular polymers and connected by hundreds of supramonomers.

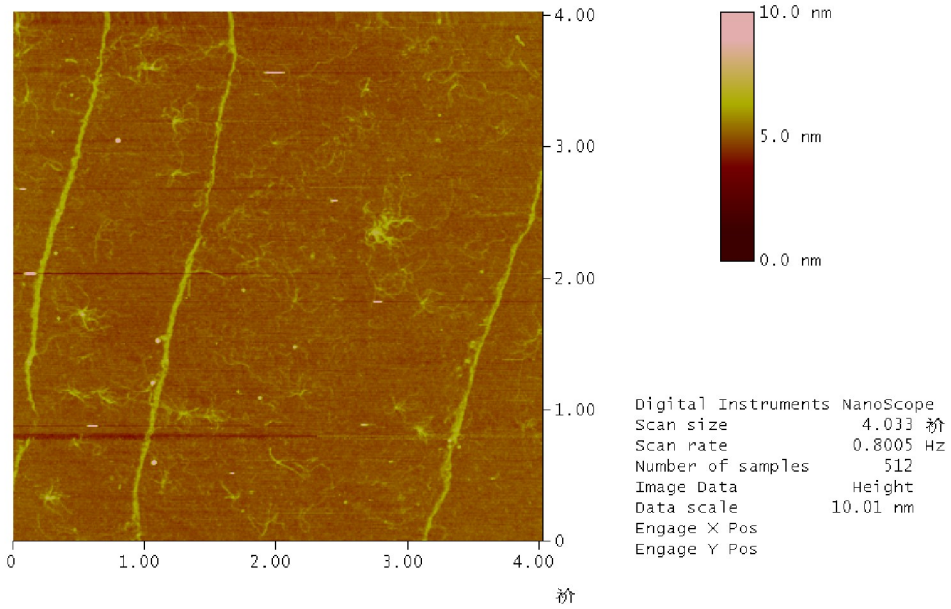

Figure S16. AFM image of the supramolecular polymer at 0.1 mM.

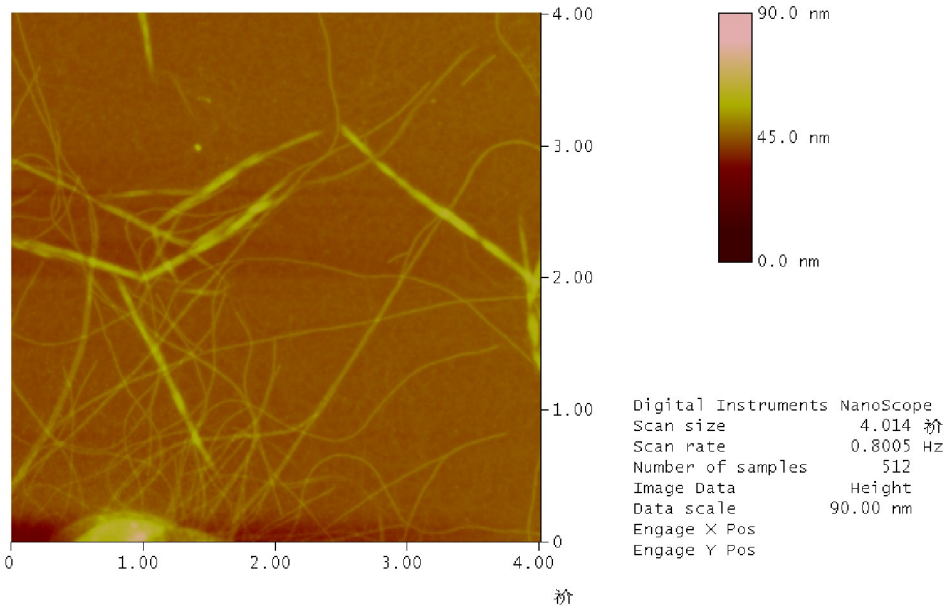

Figure S17. AFM image of the supramolecular polymer at 0.2 mM.

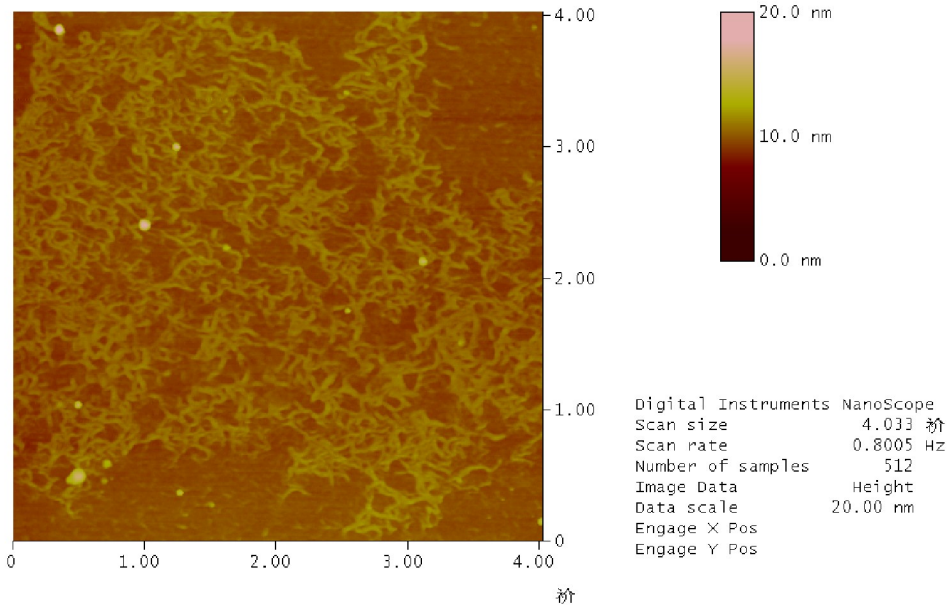

Figure S18. AFM image of the supramolecular polymer at 0.25 mM.

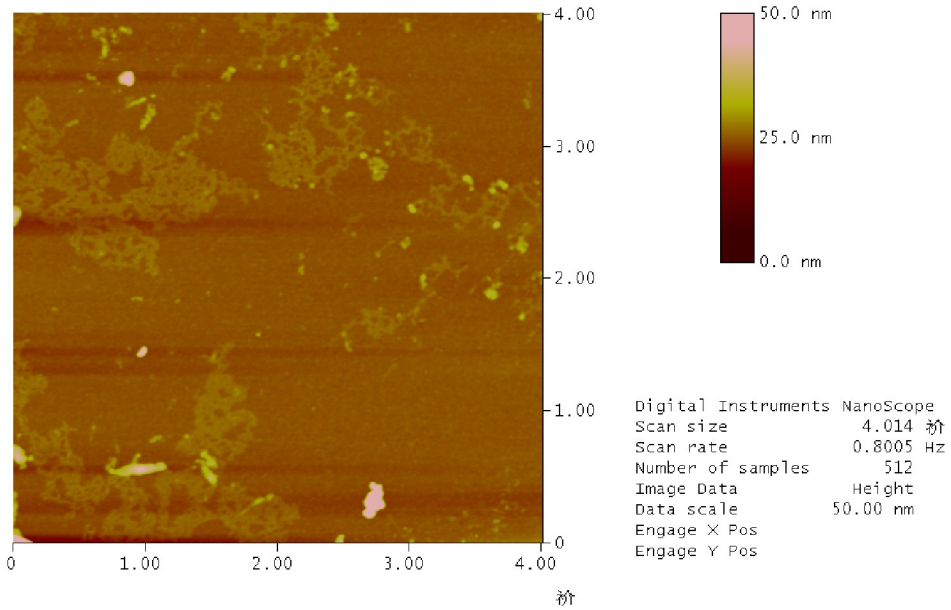

Figure S19. AFM image of the supramolecular polymer at 0.5 mM.

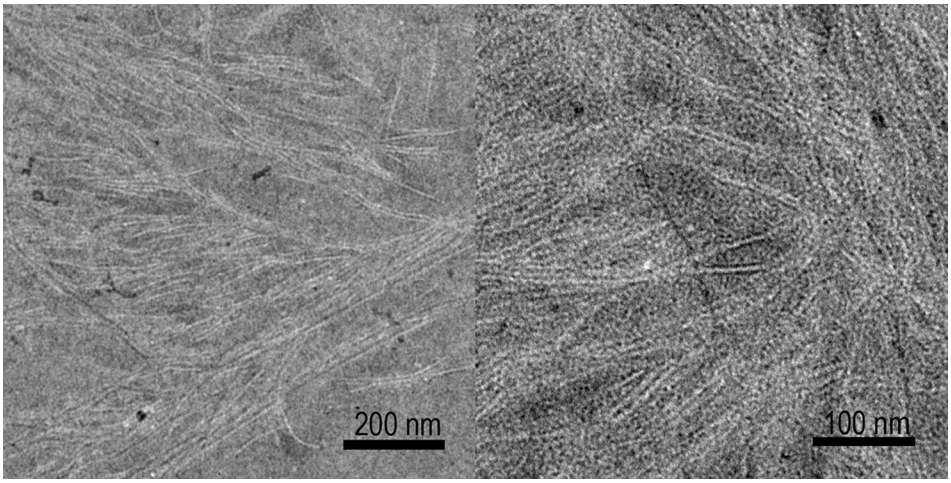

Figure S20. TEM images of the supramolecular polymer at 0.05 mM.

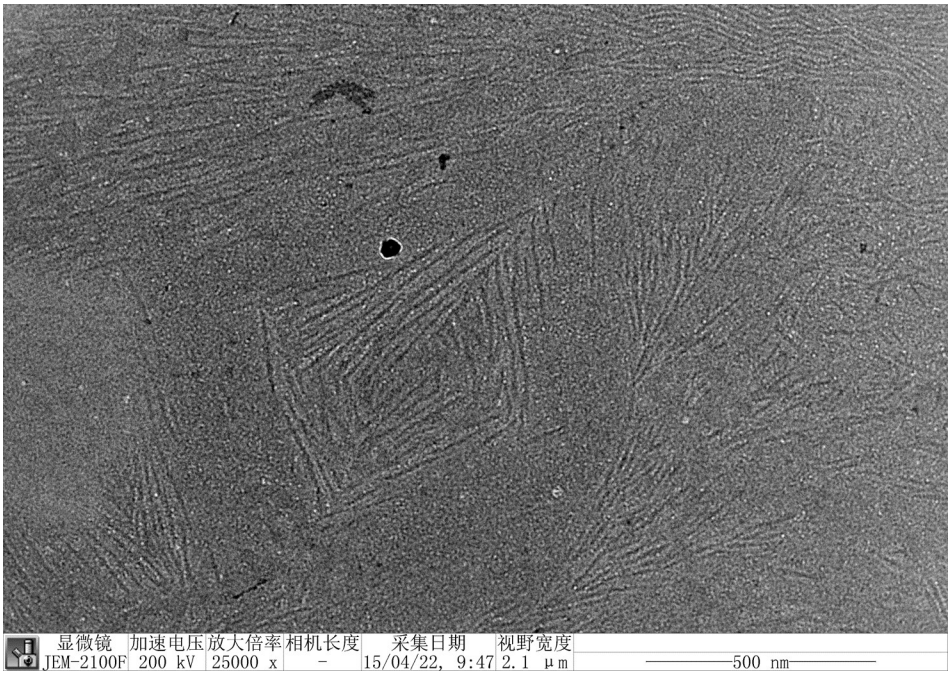

Figure S21. TEM image of the supramolecular polymer at 0.1 mM.

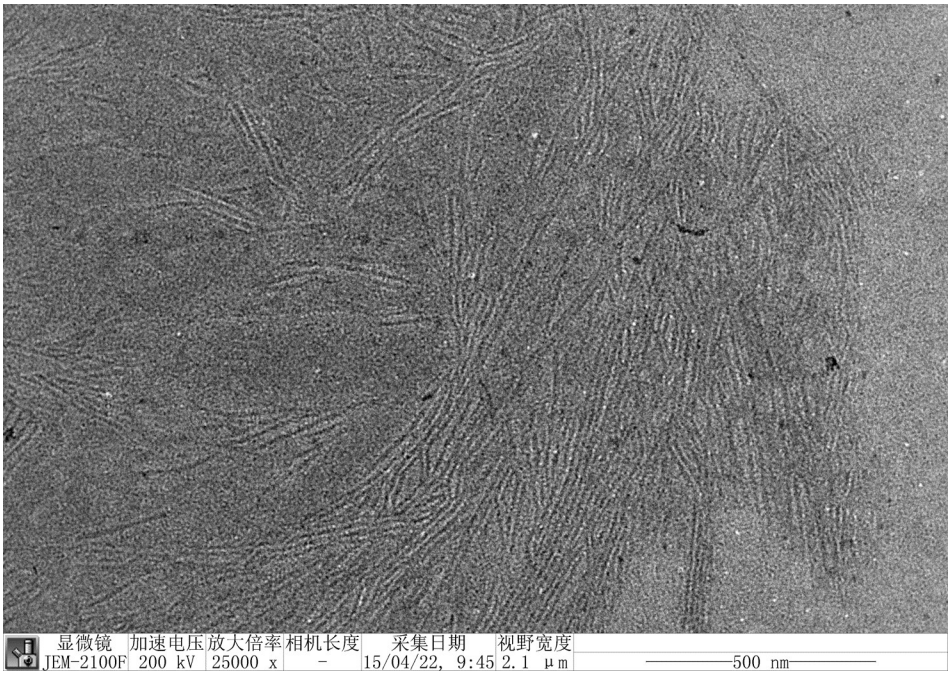

Figure S22. TEM image of the supramolecular polymer at 0.25 mM.

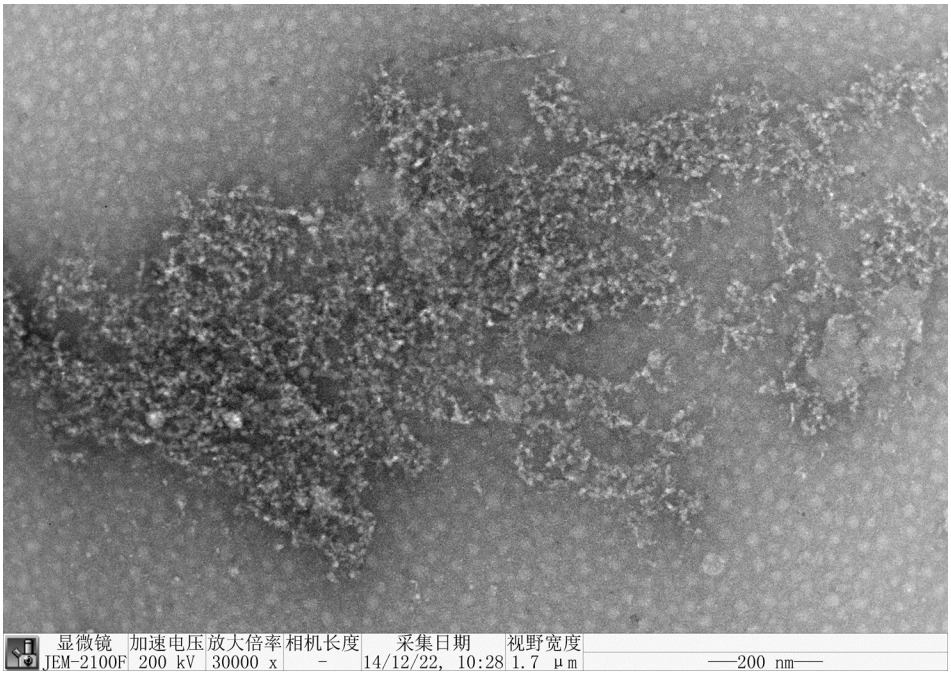

Figure S23. TEM image of the supramolecular polymer at 0.5 mM.

Control experiments

1. Attempt at preparation of supramolecular polymer by supramolecular complexation between enzymatically covalent FG4Y dimer and CB[8].

We have also carried out the approach to fabricate supramolecular polymer through supramolecular complexation of enzymatically covalent FG4Y dimer and CB[8]. However, such an approach was demonstrated to be unsuccessful. As can be seem in Figure S24, after enzymatic dimerization, the resulting FG4Y dimer could be hardly bound by CB[8], thus failing to generate supramolecular polymer. The steric hindrance effect caused by self-assembly of FG4Y dimer itself may be responsible for this phenomenon. The covalent bond is strong to overcome this barrier and make the covalent condensation of supramonomer successful, while the non-convalent supramolecular complexation is relatively weak and fail to achieve the polymerization of the FG4Y dimer. At this point, the advantage of covalent polymerization of supramonomer is obvious, which could transfer the difficulty of some supramolecular polymerization to easy covalent condensation, makes the fabrication of supramolecular polymer more smoothly.

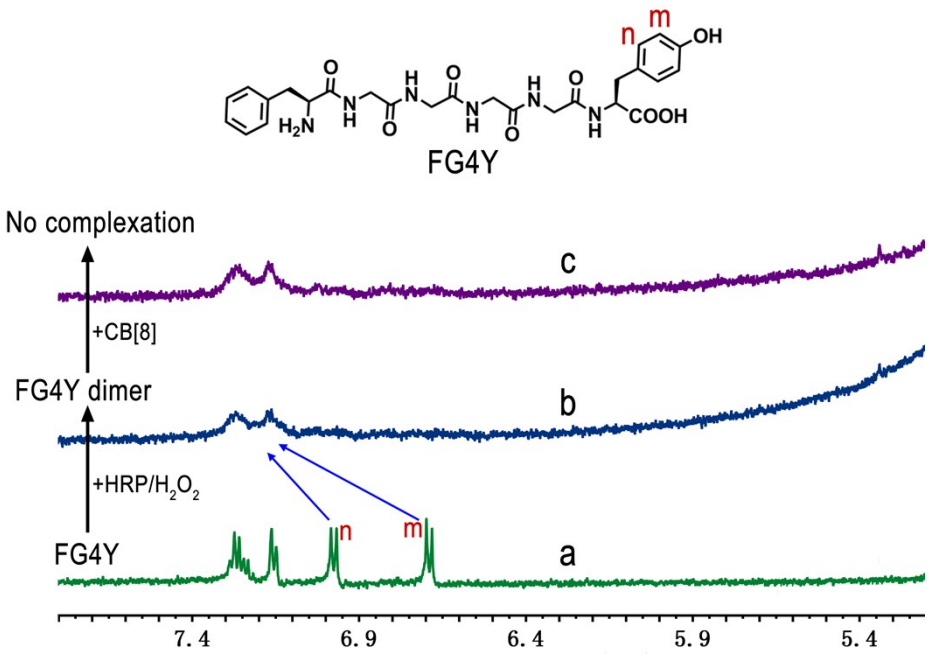

Figure S24. Partial ^{1}H NMR spectra of a) FG4Y (1.0 mM); b) dimeric FG4Y formed by addition of HRP (2.5×10^{-4} mM) and H_2O_2 (1.0 mM) to a; c) mixture after addition of CB[8] (0.5 mM) to b. There was no obvious difference on the chemical shift of benzene ring of phenylalanine between b and c, which meant the addition of CB[8] to b didn't induce complexation between the FG4Y dimer and CB[8].

2.AFM images of the control samples

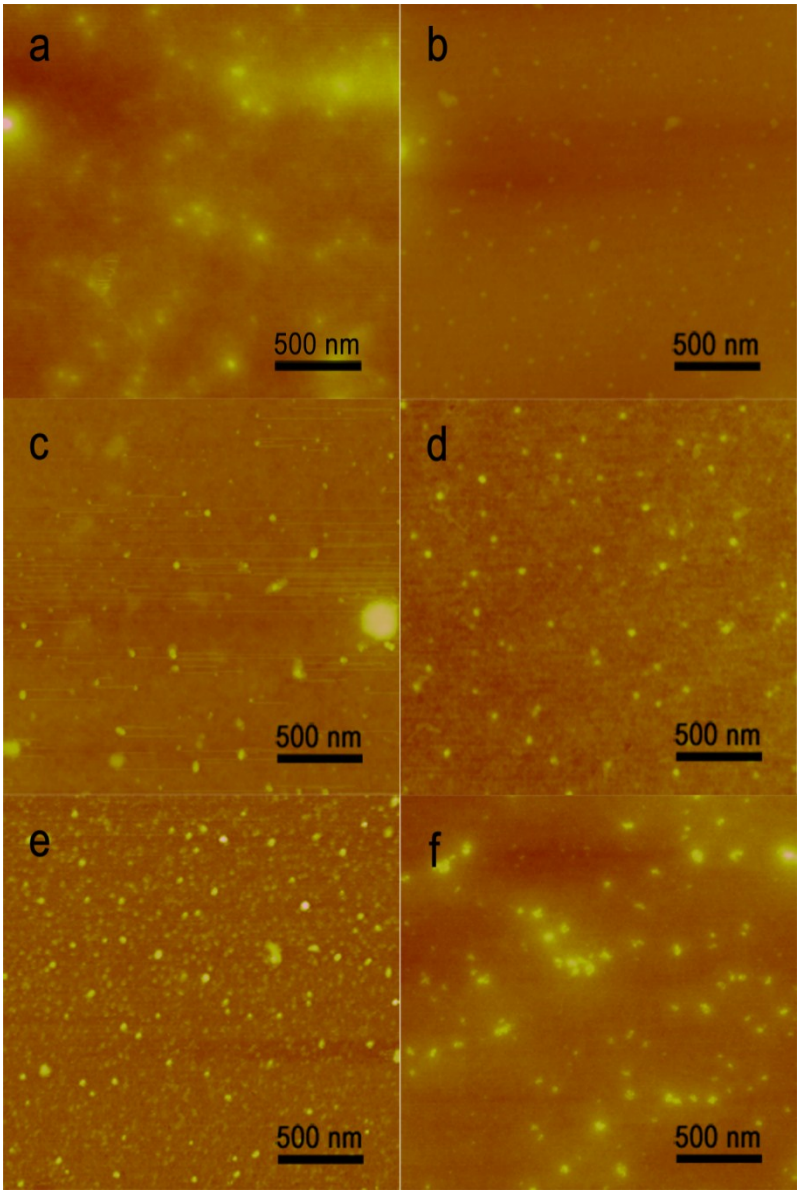

Figure S25. Height AFM images of the control samples. (a) FG4Y (0.1 mM), (b) HRP (2.5×10^{-4} mM), (c) HRP (2.5×10^{-4} mM) and H_2O_2 (0.1 mM), (d) supramonomer (0.05 mM), (e) supramonomer (0.05 mM) and H_2O_2 (0.1 mM), (e) supramonomer (0.05 mM) and HRP (2.5×10^{-4} mM).

References

[1] Kim, J.; Jung, I. S.; Kim, S. Y.; Lee, E.; Kang, J. K.; Sakamoto, S.; Yamaguchi, K.; Kim, K. *J. Am. Chem. Soc.* **2000**, *122,* 540-541.