Supporting Information

A biocompatible supramolecular hydrogel with multivalent galactose ligands inhibiting Pseudomonas aeruginosa virulence and growth

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1. Material and Method

2-chlorotrityl chloride resin, 2-(naphthalen-6-yl) acetic acid and all amino acids were purchased from Shanghai GL Biochem. Alkaline phosphatase was provided by Thermo Fisher Scientific. The plant lectin concanavalin A (Con A) and peanut agglutinin (PNA) were purchased from Sigma. Crystal violet and 3-3'-dipropylthiadicarbocyanine iodide (disC3(5)) were purchased from Macklin and APExBIO, respectively. Reagents for live/dead and CCK8 assays were provided by Dojindo Laboratories (Shanghai, China). *P. aeruginosa* strains (ATCC 27853) and *E. coli* strains (ATCC 25922) were obtained from the American Type Culture Collection (ATCC, MD, USA). All the other raw materials were obtained from Aladdin and without further purification unless mentioned. All the synthetic peptide were purified by Waters 600E Multisolvent HPLC system with CH_3CN (0.1% of TFA) and water (0.1% of TFA) as eluents. NMR spectra were obtained from a Unity Inova 400 by using DMSO-*d6* as a solvent. LC-MS spectra were obtained from an Agilent 6120 Quadrupole LC/MS system with an ESI resource. MALDI-TOF MS analyses were carried out on a Bruker Ultraflex-Treme mass spectrometer (Germany). Rheological studies were recorded on a Thermo Scientific HAAKE RheoStress 6000 rheometer (Germany). CD spectroscopy studies were carried out on a JASCO J-810 spectrometer. Fourier transform infrared spectroscopy (FTIR) characterizations were obtained on a PerkinElmer spectrophotometer (USA). Transmission electron micrograph (TEM) and Scanning electron micrograph (SEM) images were obtained from a Hitachi HT7700 TEM and Hitachi S-4700 SEM (Japan). Confocal microscopy images were recorded on a Leica TCS SP5 confocal fluorescence microscope.

2. Synthesis of Fmoc-Ser[Gal(OAc)$_4$]-OH

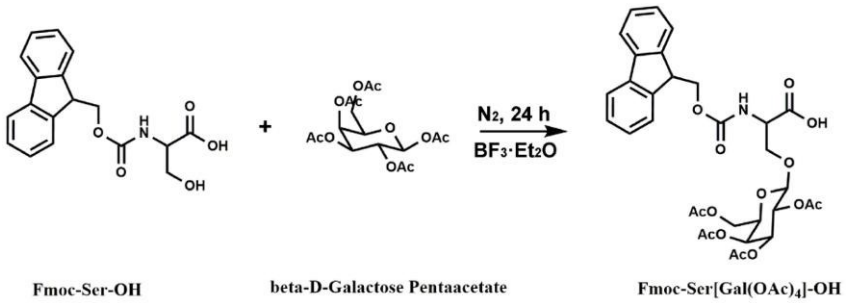

Fig. S1. The synthetic route for the preparation of Fmoc-Ser[Gal(OAc)$_4$]-OH from Fmoc-Ser-OH and β-D-galactose pentaacetate

Synthesis of Fmoc-Ser[Gal(OAc)$_4$]-OH: Fmoc-Ser-OH (2.096 g, 2.5 equiv.) and β-D-galactose

penta-acetate (1 g , 1 equiv.) were dissolved in dry CH_3CN (25 mL) and stirred at the atmosphere of N_2. After the solution was cooled to 0 °C in an ice-water bath, $BF_3 \cdot Et_2O$ (0.97 mL, 3 equiv.) was added slowly over 20 min. Then the reaction was allowed to react at room temperature for 24 h. The reaction was monitored by TLC (DCM/MeOH=10:1). Then CH_2Cl_2 was added and extracted with brine for three times. The organic layer was separated and dried by Na_2SO_4. After the solvent was removed by rotary evaporation, the crude product was purified by column chromatography using DCM/MeOH as eluents, and afford the final product of Fmoc-Ser[Gal(OAc)$_4$]-OH (yield: 63%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm) = 12.93 (s, 1H), 7.91-7.88 (d, 2H), 7.73-7.71 (d, 2H), 7.45-7.38 (m, 3H), 7.36-7.30 (m, 2H), 5.26-5.24 (d,1H）, 5.17-5.13 (dd, 1H), 4.94-4.88 (m, 1H), 4.78-4.75 (d, 1H), 4.33-4.27 (m, 2H), 4.26-4.24 (d, 1H), 4.23-4.18 (m, 2H), 4.06-4.03 (d, 2H), 3.93-3.87 (q, 1H), 3.81-3.77 (q, 1H), 2.11 (s, 3H), 1.99 (s, 3H), 1.93 (s, 3H), 1.91 (s, 3H). MS: calcd M = 657.2058, obsd $(M-H)^-$ = 656.2006.

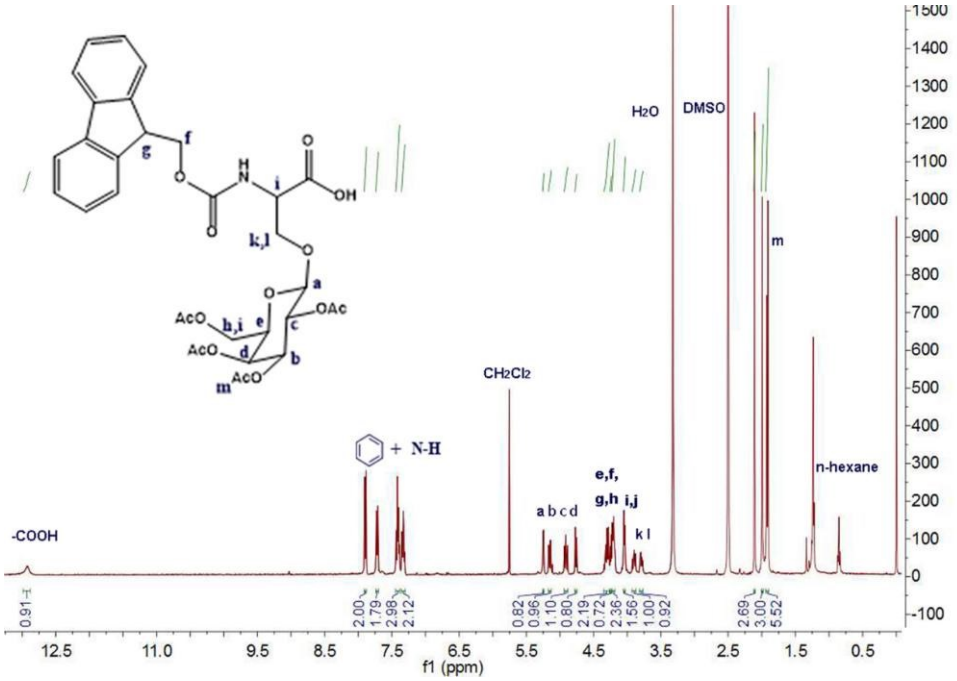

Fig. S2. ^{1}H NMR of Fmoc-Ser[Gal(OAc)$_4$]-OH in DMSO-*d6*.

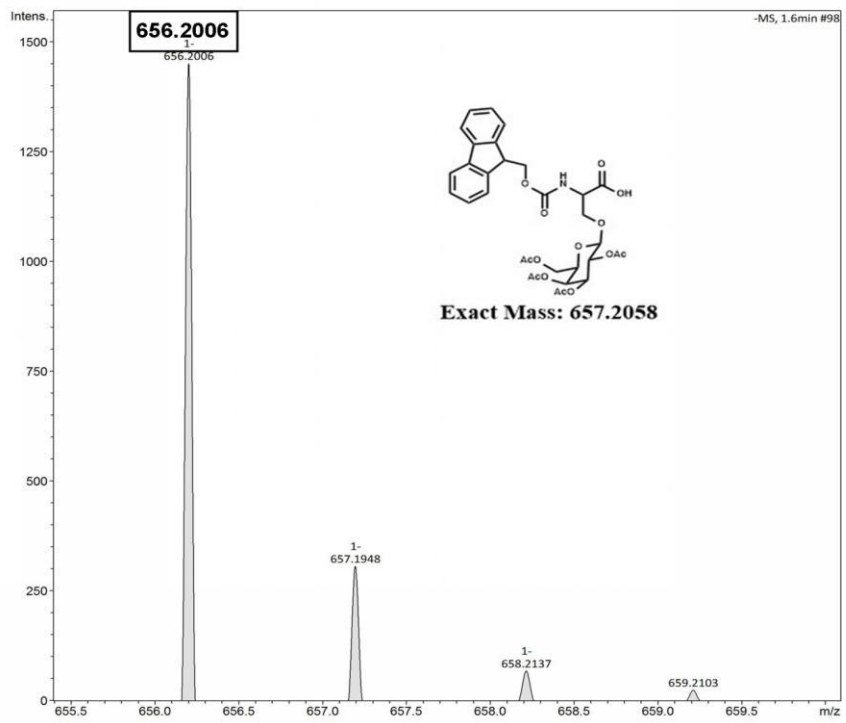

Fig. S3. LC-MS spectrum of Fmoc-Ser[Gal(OAc)$_4$]-OH.

3. Synthesis of SGYp

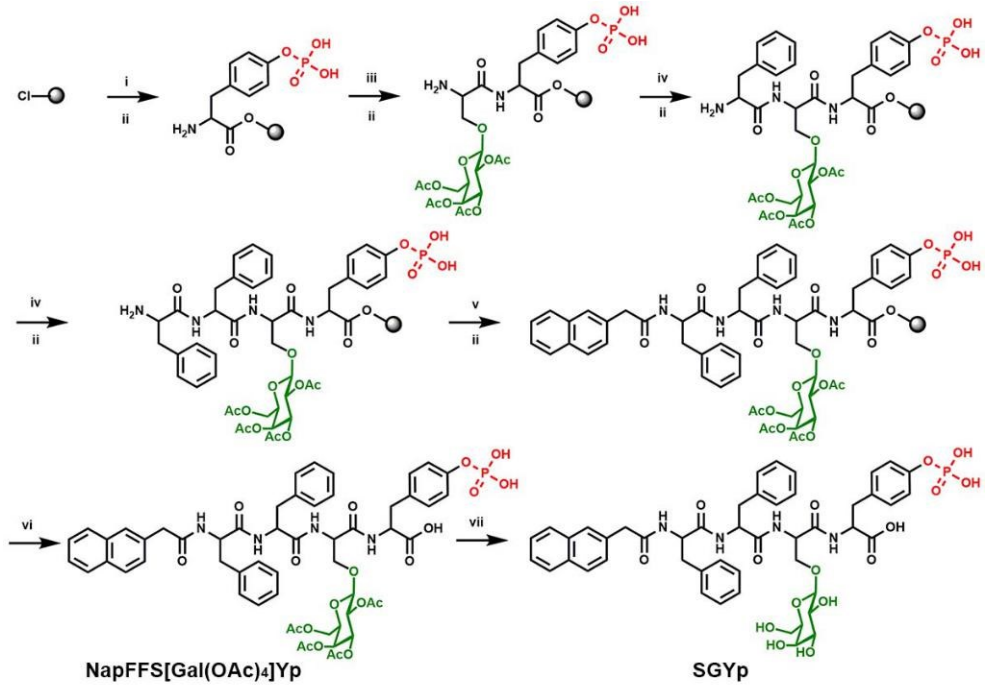

Fig. S4. The synthetic route of for the preparation of SGYp from corresponding amino acids.

Synthesis of NapFFS[Gal(OAc)$_4$]Yp: NapFFS[Gal(OAc)$_4$]Yp was synthesized on 2-chlorotritylchloride resin by following the standard solid-phase peptide synthesis protocols from

Fmoc-Tyr(H_2PO_3)-OH, Fmoc-Ser[Gal($OAc)_4$]-OH, Fmoc-Phe-OH and 2-(naphthalen-6-yl) acetic acid. First of all, 2-chlorotritylchloride resin (1 g) was suspended in dry dichloromethane (DCM), swelled under nitrogen atmosphere for 0.5 h, and then washed by dimethylformamide (DMF) for three times. Fmoc-Tyr(H_2PO_3)-OH and *N,N*-diisopropylethylamine (DIEA) were dissolved in DMF and added to the reactor. After reaction for 1 h, the resin was washed by DMF for three times, and quenched by the mixture solution (DCM:MeOH:DIEA=80:15:5). Then the resin was washed by DMF and treated by 20% piperidine in DMF to remove the Fmoc-protecting groups on Fmoc-Tyr(H_2PO_3)-OH. The peptide chain was extended by following the standard Fmoc solid phase peptide synthesis procedures with the application of corresponding Fmoc-protected amino acids and HBTU. Finally, the O-acetyl protected glycopeptide was cleaved from the resin with a mixture of TFA/H_2O=95:5, and purified by high performance liquid chromatography (HPLC) using H_2O−CH_3CN as eluents. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm) = 8.35-8.24 (m, 2H), 8.19(d, 1H), 8.12(d, 1H), 7.85(d, 1H), 7.77-7.73(dd, 2H), 7.57(s, 1H), 7.43(m, 2H), 7.26-7.05 (m, 15H), 5.25-5.24 (m, 1H), 5.16-5.09 (m, 1H), 4.96-4.90 (m, 1H), 4.81-4.77(d, 1H), 4.64-4.49(m, 4H), 4.45-4.40(m, 1H), 4.22-4.16(m, 1H), 4.07-4.03(d, 1H), 3.81-4.71(m, 3H), 3.07-2.99(m, 3H), 2.98-2.88(m, 3H), 2.81-2.75(m, 1H), 2.73-2.67(m, 2H), 2.08 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H). MS: calcd M = 1140.3617, obsd $(M + Na)^+$ = 1163.5010.

Synthesis of SGYp: SGYp was prepared from the deacetylation reaction of NapFFS[Gal($OAc)_4$]Yp (1 equiv.), which was treated with MeONa (1 M, 10 equiv.) in MeOH and stirred at room temperature for 4 h. Then the crude product of SGYp was purified by HPLC and the final yield was approximately 20%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm) = 8.30-8.24 (d, 1H), 8.22-8.16 (d, 1H), 7.86-7.83 (d, 1H), 7.8-7.22 (m, 2H), 7.60-7.57 (d, 1H), 7.49-7.42 (q, 2H), 7.28-7.0 (d, 17H), 4.64-4.46 (m, 3H), 4.40-4.38 (q, 1H), 4.16-4.13 (d, 1H), 4.03-3.97 (m,1H), 3.66-3.62 (dd, 1H), 3.59-3.47 (m, 6H), 3.46-3.38 (m, 4H), 3.08-3.02 (d, 2H), 3.02-2.97 (d, 1H), 2.96-2.89 (m, 2H), 2.87-2.74 (m,2H), 2.73-2.63 (m,1H). MS: calcd M = 972.3194, obsd $(M+Na)^+$ = 995.828.

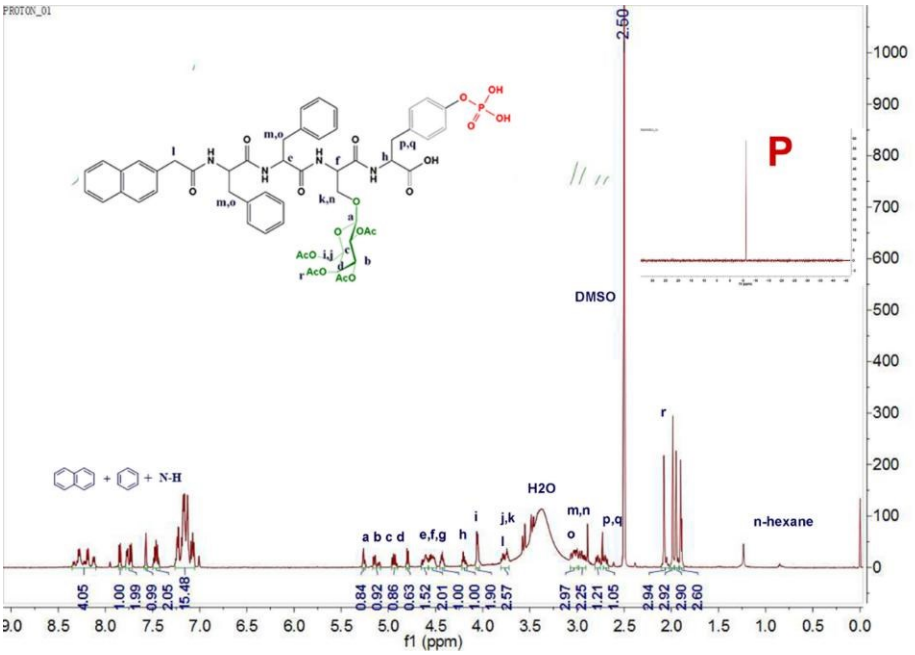

Fig. S5. ^{1}H and ^{31}P NMR of NapFFS[Gal(OAc)$_4$]Yp in DMSO-*d6*.

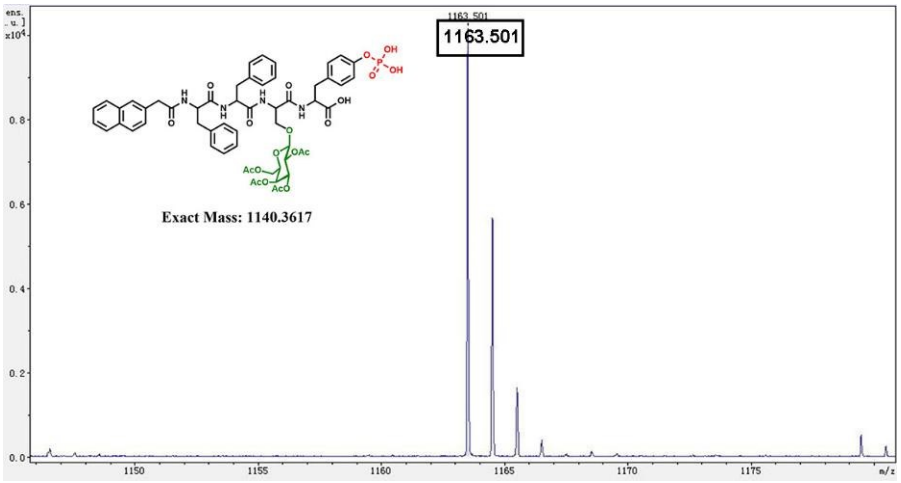

Fig. S6. TOF-MS spectrum of NapFFS[Gal(OAc)$_4$]Yp.

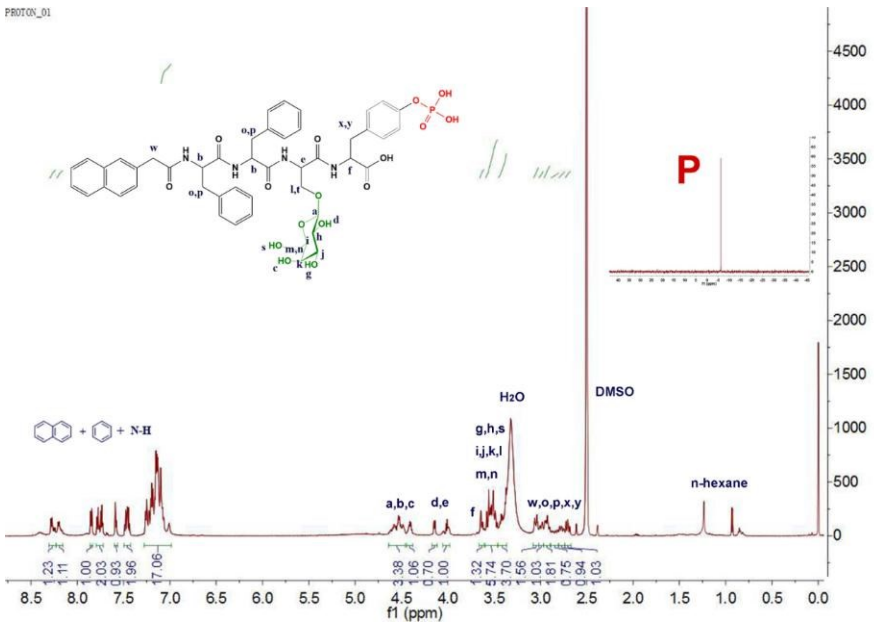

Fig. S7. ^{1}H and ^{31}P NMR of gelator precursor SGYp in DMSO-*d6*.

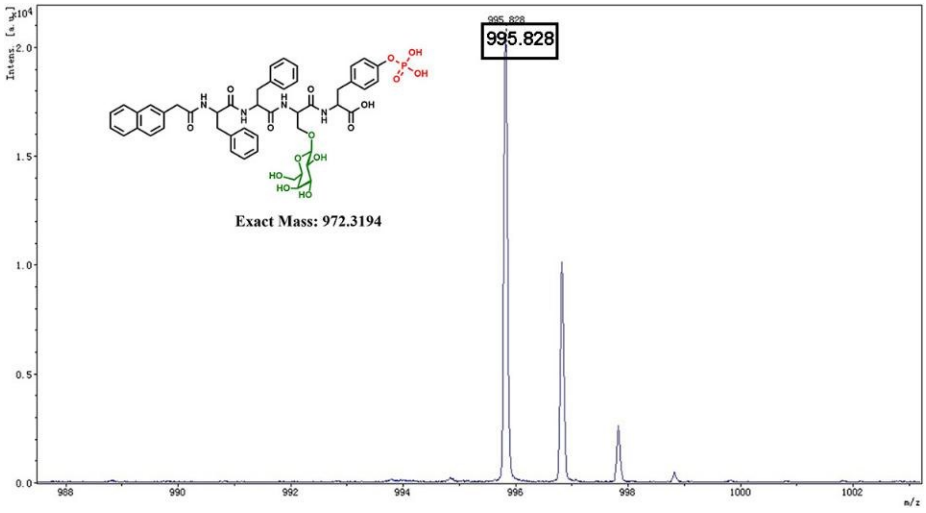

Figure S8. TOF-MS spectrum of gelator precursor SGYp.

4. Synthesis and characterization of SYp

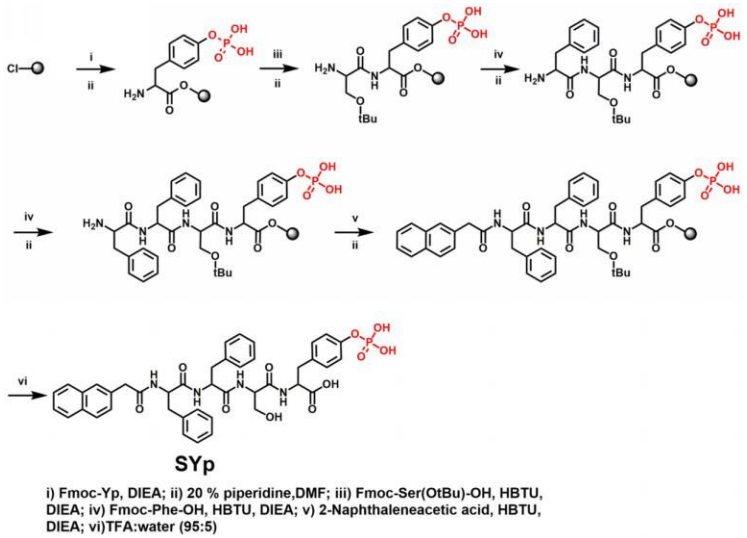

Fig. S9. The synthetic route of for the preparation of SYp via solid phase synthesis.

Synthesis of SYp: SYp was synthesized on 2-chlorotritylchloride resin by following the standard solid-phase peptide synthesis methods from Fmoc-Tyr(H_2PO_3)-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Phe-OH and 2-(naphthalen-6-yl) acetic acid. Then SYp was purified by HPLC with a yield of 45%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm) = 8.5-8.3 (d, 3H), 8.21-7.51 (d, 4H), 7.50-7.45 (d, 2H), 7.35-6.9 (d, 15H), 4.75-4.25 (t, 4H), 3.5-3.45 (d, 4H), 3.2-2.6 (d, 6H). MS: calcd M = 810.2666, obsd $(M+H)^+$ = 811.369, obsd $(M+Na)^+$ = 833.345.

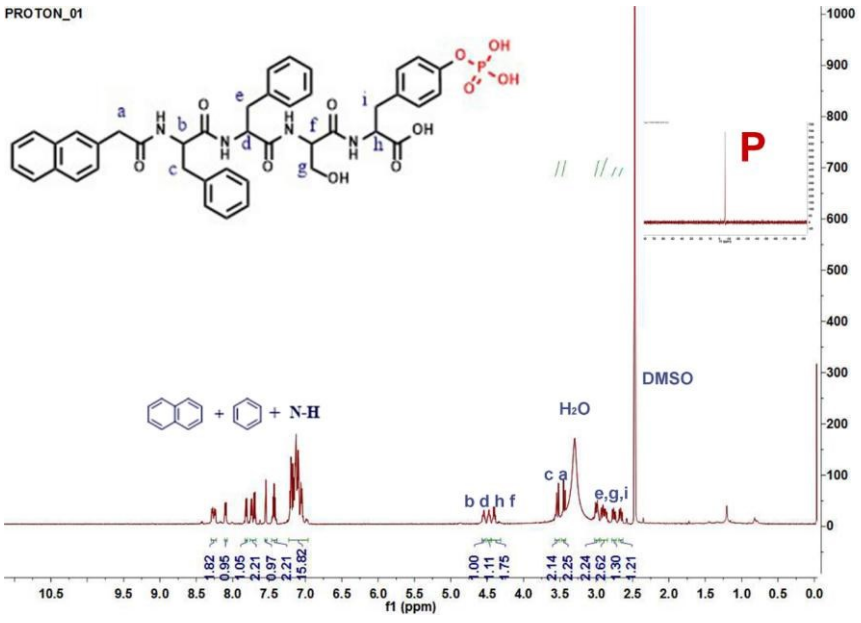

Fig. S10. ^{1}H and ^{31}P NMR of SYp in DMSO-*d6*.

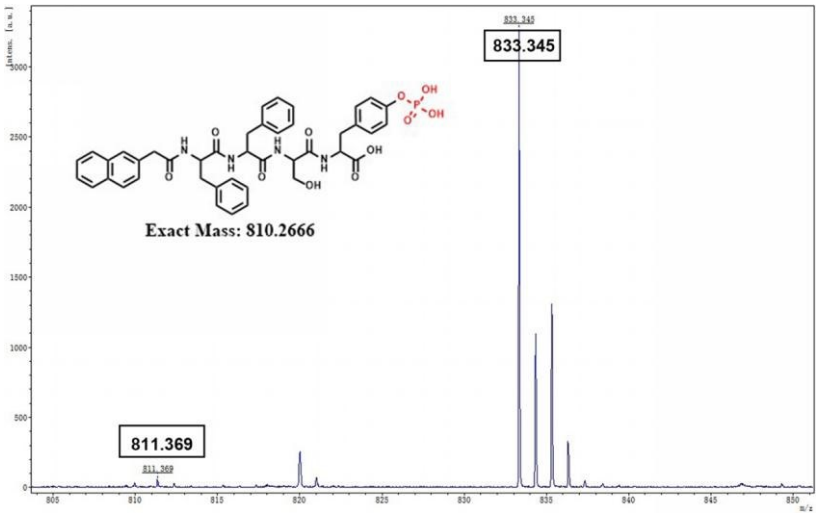

Fig. S11. TOF-MS spectrum of SYp.

5. Gelation tests of SYp

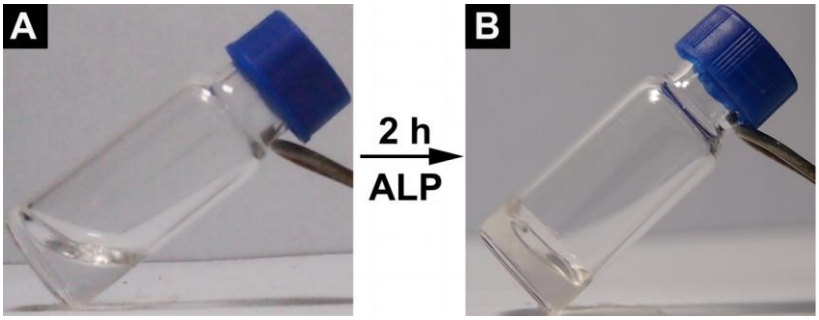

Fig. S12. (A) Optical images of the solution of SYp (0.6 wt%, pH=7.4); and (B) the SY hydrogel triggered by alkaline phosphatase (ALP 10 units/mL).

6. Rheological tests of SGY

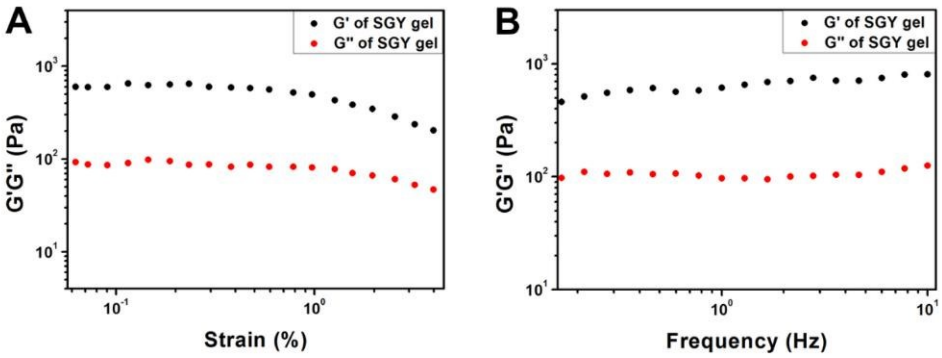

Fig. S13. (A) Strain dependence and (B) frequency dependence of the dynamic storage moduli (G') and the loss moduli (G'') of the SGY gel (0.6 wt%).

7. Determination of the critical concentration of SGY for supramolecular self-assembling

The critical concentration of SGY for supramolecular self-assembling was determined by fluorescence measurements using Nile red as a probe. A series of SGY solutions with increased concentrations of 25 μM to 1000 μM was prepared in PBS buffer. After incubating with 1 μL ethanol solution of Nile red (2.5 μM) for 24 h in dark, the fluorescence intensities of Nile red at different concentrations of SGY were examined (Ex = 543 nm, Em = 563 nm-750 nm). The critical concentration of SGY was determined by the dose-dependent curve plotted with fluorescence intensities of Nile red versus SGY concentrations.

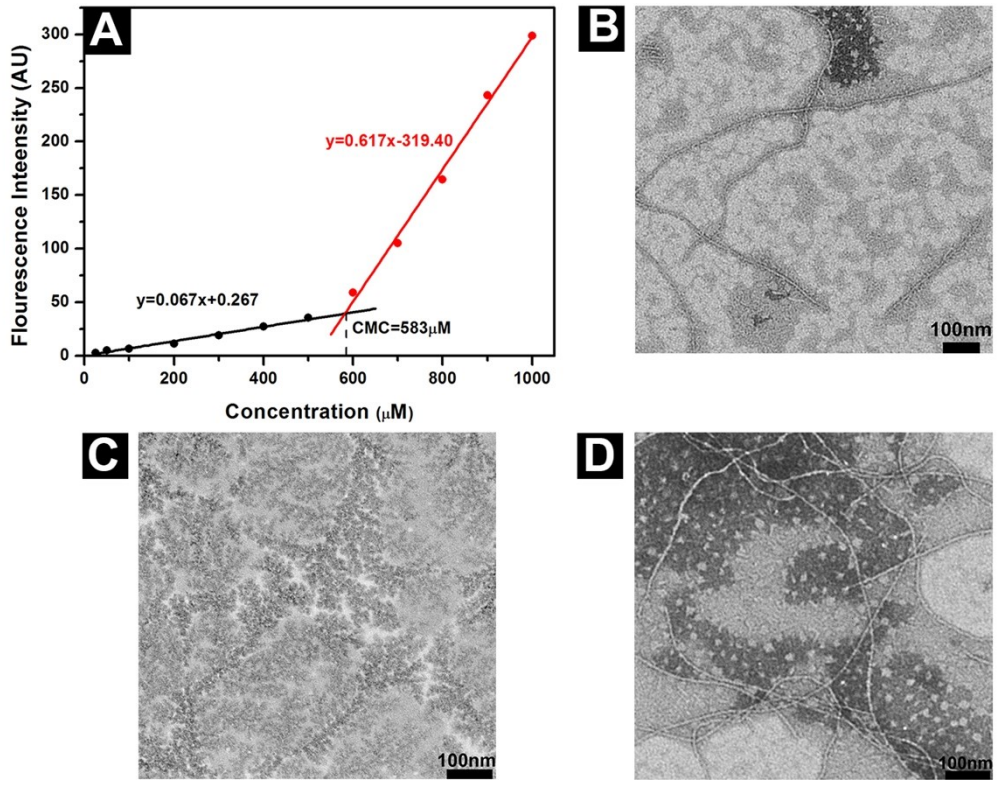

Fig. S14. (A) The dose-dependent curve plotted with fluorescence intensities of Nile red versus SGY concentrations. (B) TEM image of the nanostructures self-assembled from SGY at the critical self-assembling concentration (583 μM). (C) TEM image of the nanostructures self-assembled from SGY at 400 μM. (D) TEM image of the nanostructures self-assembled from SGY at 800 μM.

8. Cytocompatibility tests of SGYp and SGY

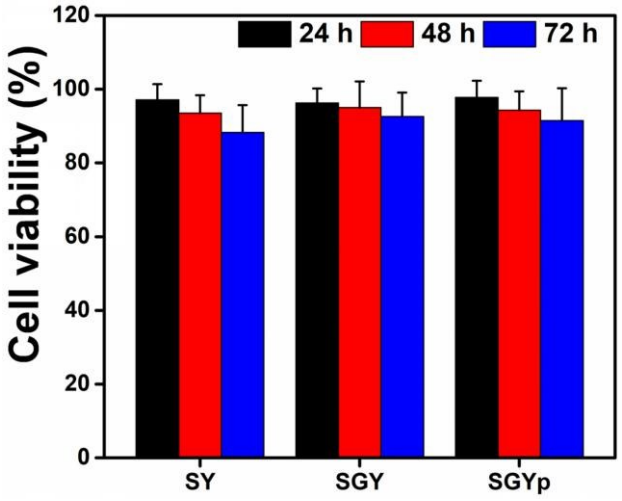

Fig. S15. Cytotoxicity tests of the molecule of SGYp (500μM), the SGY gel (1.0 wt%) and the SY gel (1.0 wt%) towards HUVEC cells over the course of 1, 2, 3 days.