Supporting Information

Materials and general methods:

Materials: Fmoc-OSu, L-Tyr-OtBu and other Fmoc-amino acid were obtained from GL Biochem (Shanghai); Taxol was purchased from Tianfeng (Shenyang, P. R. China); Chemical reagents and solvents were obtained from Alfa and used as received. Alkali phosphatase (30 U/μL) was purchased from Takara Bio. (Dalian, P. R. China) (Catalog No. D2250); 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Invitrogen (Grand Island, NY); HepG2 cell line was kindly provided by Huaxi Medical College (Sichuan University, Chengdu, China).

General methods: $^1$H NMR and $^{31}$P NMR (Bruker ARX 400) were used to characterize the synthesized compounds. ESI-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System. Conversion of compound 1 was carried out by LCMS-20AD (Shimadzu). AFM was done on a Veeco multimode V system with the tip of Veeco RTESP. Rheology test was done on an AR 2000ex (TA instrument) system, 40 mm parallel plates was used during the experiment at the gap of 500 μm. Size distribution of nanospheres was obtained on a ZetaPALS+BI-90Plus system (Brookhaven Co.). Bio-RAD iMark™ Microplate Reader (Bio-Rad, America) was used in the MTT assay.

Peptide synthesis. Peptide of Fmoc-GYpK-OH was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected by a tert-butyl group. The first amino acid (Fmoc-Lys(Boc)-OH) was loaded on the resin at the C-terminal with the loading efficiency about 1.0-1.2 mmol/g. 20% piperidine in anhydrous N,N'-dimethylforma mide (DMF) was used during the deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotrizol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 ml per gram of resin), followed by five steps of washing using dichloromethane (DCM) for 2 min (5 ml per gram of resin). The peptide derivatives were cleaved from the resin by ice-cold reagent B and the mixture was stirred at room temperature for 30 minutes, filtered, and poured into ice-cold diethylether. The resulting precipitate was centrifuged for 10 min at 2 °C at 10,000 rpm. Afterward the supernatant was decanted and dissolved in double-distilled (dd) water and lyophilized.

The synthesis of Fmoc-L-Tyr(PO(OEt)$_2$)-OH was according the method we reported before.¹

Synthesis and characterizations of Fmoc-GYpK-OH:
Preparation of Fmoc-L-Tyr-OtBu (3): L-Tyr-OtBu (2.37 g, 10 mmol) and DIPEA...
(1.74 ml, 10 mmol) were dissolved in 75 mL of acetone with stirring; the solution of Fmoc-OSu (3.3 g, 9.8 mmol, dissolved in 75 mL acetone) was then added dropwise. After being stirred at room temperature (22-25 °C) overnight, the reaction mixture was concentrated, the residue was then purified by flash chromatography on silica gel (eluent: Hexane/Ethyl acetate = 3/1) to afford 4.4 g of title compound (95.8%). 1H NMR (300 MHz, DMSO-d6) δ 8.03-8.05 (d, 2H), 7.81-7.85 (t, 2H), 7.56-7.59 (t, 2H), 7.46-7.48 (m, 2H), 7.19-7.21 (d, 2H), 6.81-6.83 (d, 2H), 4.30-4.40 (m, 3H), 4.2-4.24 (m, 1H), 3.30-3.36 (m, 2H), 1.50 (s, 9H). MS: calc. M⁺ = 459.2, obsvd. (M+1)⁺ = 459.8.

**Scheme S-1.** Synthetic route for compound 5

**Preparation of Fmoc-L-Tyr(PO(OEt)₂)-OtBu (4):** I₂ (7.5 mmol, 1.5 equivalents) was added to a solution of the Triethyl phosphate (8 mmol, 1.6 equivalents) in 80 mL CH₂Cl₂ at 0 °C. 20 minutes after the addition, the clear, colorless solution was allowed to warm to room temperature (22-25°C). Then the solution was added dropwise, over a period of 30 minutes, to a flask containing Fmoc-Tyr-OtBu (5.0 mmol, 1.0 equivalent) and pyridine (20.0 mmol, 4.0 equivalents) in 50 mL CH₂Cl₂ at 0 °C. After an additional 2 hours, the reaction mixture was diluted with ethyl acetate (200 ml). The organic layer was washed with NaHSO₄ (5 wt%) (100 ml*3), brine (100 ml), dried over anhydrous MgSO₄, successively. The organic layer was concentrated by the rotary evaporator and the residue was then purified by flash chromatography on silica gel (eluent: Hexane/Ethyl acetate = 3/2) to afford 2.55 g of title compound (85.5%). 1H NMR (300 MHz, DMSO-d6) δ 8.03-8.05 (d, 2H), 7.82-7.84 (d, 2H), 7.57-7.59 (t, 2H), 7.42-7.48 (m, 4H), 7.25-7.28 (d, 2H), 4.10-4.40 (m, 8H), 3.11-3.13 (m, 1H), 3.03-3.07 (m, 1H), 1.49 (s, 9H), 1.37-1.41 (t, 6H). 31P NMR (δ -6.366 ppm). MS: calc. M⁺ = 595.2, obsvd. (M+1)⁺ = 596.0.

**Preparation of Fmoc-L-Tyr(PO(OEt)₂)-OH (5):** To a solution of 4 (600 mg, ~1.0 mmol) in 5 mL CH₂Cl₂, TFA (10 ml) was added at 0 °C. TLC was used to detect when the reaction was complete (usually the reaction took about 4 hours to finish), the solvent was then removed by a rotary evaporator. The residue obtained was
co-evaporated with toluene twice by a rotary evaporator. 530 mg of title compound was obtained after removing the solvent in vacuum (98.3%). $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 8.23-8.25 (d, 2H), 8.01-8.05 (t, 2H), 7.76-7.79 (t, 2H), 7.59-7.62 (m, 4H), 7.47-7.52 (d, 2H), 4.30-4.60 (m, 8H), 3.45-3.48 (m, 1H), 3.21-3.27 (m, 1H), 1.58-1.61 (t, 6H). 31P NMR ($\delta$ -6.348 ppm). MS: calc. M$^+$ = 539.2, obsvd. (M+H)$^+$ = 540.1.

Preparation of Fmoc-GpYK-OH (compound 7): the standard Fmoc solid phase peptide synthesis procedure was used to obtain compound 6 (Fmoc-Gly-Try(PO(OEt)$_2$)-Lys-OH) by using Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, and compound 5. After cleavage from the resin, precipitated from cold ether, and dried in the vacuum, successively, compound 6 was directly treated with 25 equiv. of TMSBr in 10 mL of dry DCM. The resulting solution was stirred at room temperature overnight. The solvent was removed by a rotary evaporator and 10 mL dd water was then added with stirring for 30 min at room temperature, the resulting solution was freeze-dried and purified by HPLC to afford compound 7 (Scheme S-2). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 7.875 (d, J=7.235, 2H), 7.640-7.715 (m, 2H), 7.375-7.428 (t, 2H), 7.290-7.340 (m, 2H), 6.935-7.075 (m, 2H), 4.205-4.285 (m, 3H), 3.630 (m, 2H), 3.065-3.155 (m, 5H), 2.635-2.725 (m, 1H), 1.145-1.198 (m, 6H). $^{31}$P NMR ($\delta$ -4.969 ppm). MS: calc. M$^+$ = 668.2, obsvd. (M+1)$^+$ = 669.5.

**i)** DMF, DIPEA; **ii)** 10% piperidine in DMF; **iii)** Folic acid (FA) NHS active ester (10), DIPEA, DMF
**Scheme S-2.** Synthetic route for compound 9 (Fmoc-GpYK-Taxol) and compound 1 (FA-GpYK-Taxol)

**Figure S-1.** $^1$H NMR of compound 7

**Figure S-2.** $^{31}$P NMR of compound 7

**Preparation of Fmoc-GpYK-Taxol (compound 9):** 158.3 mg of 7 (0.24 mmol) and 150 mg of Taxol-succinic acid NHS active ester (8) (0.16 mmol) were dissolved in 5 mL of DMF, 130 μL of DIPEA was then added. The resulting reaction mixture was stirred at room temperature overnight. Compound 9 was obtained by HPLC (156.4 mg, yield of 61.7%).
Preparation of Folate-GpYK-Taxol (compound 1): 150 mg of compound 9 was first dissolved in 4 mL of 10% piperidine in DMF, and the solution was stirred at room temperature for 2h. The compound of NH2-GYpK-Taxol was obtained by HPLC (90.2 mg, yield of 69.9%).

The Folic acid NHS active ester was synthesized according to the reference: 250 mg of the Folic acid (0.57 mmol), 65 mg of the N-hydroxyl succinimide (NHS, 0.57 mmol), and 78 mg (0.38 mmol) of dicyclohexylcarbodiimide (DCC) were dissolved in 8 mL of DMF. The reaction mixture was stirred at room temperature for 16 hours. 40 mL of the mixed solvent (acetone/ethyl ether = 30:70 v/v) was added with stirring. The resulting yellow solid was obtained by filtration and then washing with the mixed solvent twice. And it should be used for next reaction directly after synthesis.

After the Folic acid NHS active ester (93.6 mg, 0.174 mmol) being dissolved in 4 mL DMF, 80 mg of NH2-GYpK-Taxol (0.058 mmol) obtained above and 40 μL of DIPEA were added, successively. The resulting reaction mixture was stirred at room temperature overnight. Title product of 1 was obtained by HPLC (43.7 mg, yield of 42.02 %).

$^1$H NMR (400MHz, DMSO-d$_6$) $\delta$ 7.980 (d, J=7.198, 2H), 7.855-7.715 (m, J=7.223, 2H), 7.640-7.745 (m, 6H), 7.435-7.565 (m, 6H), 7.195-7.258 (m, 3H), 7.030-7.075 (m, 2H), 7.625-6.650 (m, 1H), 6.290 (s, 1H), 5.792-5.840 (t, 1H), 5.505-5.535 (m, 1H), 5.411 (d, J=7.259, 1H), 5.327-5.350 (m, 1H), 4.913 (d, J=10.382, 1H), 4.497-4.665 (m, 3H), 4.005-4.299 (m, 4H), 2.595-3.005 (m, 9H), 2.100-2.598 (m, 13H), 1.635-1.770 (m, 5H), 1.495-1.630 (m, 3H), 0.990-1.490 (m, 13H). $^{31}$P NMR ($\delta$ -6.110 ppm). MS: calc. M+ = 1804.6, obsvd. (M+1)+ = 1805.6.

Figure S-3. $^1$H NMR of compound 1
**Figure S-4.** $^{31}$P NMR of compound 1

![NMR Spectrum](image)

**Scheme S-3.** Chemical structure of the precursor of a SM hydrogelator and schematic hydrogelation catalyzed by phosphatase (Folic acid (FA) in blue and Taxol in red)
Formation of nanospheres and hydrogels: 2.0 mg compound 1 and 1 equiv. of Na₂CO₃ (to neutralize the phosphoric acid on 1) were dissolved in 1.0 mL of PBS buffer (pH = 7.4). The alkaline phosphatase was then added at a final enzyme concentration of 90 U/mL.

Size distribution of the nanospheres: The gels formed by the above procedure were incubated at room temperature overnight. 30 μL of gels were then diluted to 1.8 mL solutions by adding the PBS buffer solution or the DMEM medium with 10% of the FBS. Size distribution of the nanospheres in these two solutions was performed on a ZetaPALS+BI-90Plus system (Brookhaven Co.) immediately or after 24 hours.

Determination of the stability of the FA-GYK-Taxol in the nanospheres: The gels formed by the above procedure in eppendorf tubes (50 μL of gel in each tube) were firstly incubated at room temperature overnight. The gels were then incubated in a water bath at 37 °C. 150 μL of DMSO was added to the gels at different time points, and the resulting clear solution was used to determine the remained FA-GYK-Taxol in the gels by the LC-MS at different time points. Three samples at each time point were used.

Determination of IC₅₀ values of the compounds: The IC₅₀ values of Taxol, Taxol-succ, FA-GpYK-Taxol, and the nanospheres were evaluated by the MTT assay. The HepG2 cells were seeded in 96-well plates at a density of 2,000 cells per well with a total medium volume of 75 μL and incubated for 24 hours. Then 25 μL of the solutions containing a serial of concentrations of the above four compounds were added into the cells. 72 hours later, we replaced the medium with fresh medium supplemented with 15 μL MTT reagent (5 mg/mL). 4 hours later, the medium containing MTT was removed and DMSO (100 μL/well) was added to dissolve the formazan crystals. The optical density of the solution was measured at 492 nm, using a microplate reader (Bio-RAD iMark™, America). Cells without the treatment of the compounds were used as the control. The experiment was repeated for 3 times. The cell viability percent was calculated by the following formula:

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\text{The cell viability percent (\%) = OD}_{\text{sample}}/\text{OD}_{\text{control}} \times 100\%
\]

The concentrations of the compounds when 50% of cell viability was recorded represented the IC₅₀ values of the compounds.
**Table S-2.** Analysis of conversion from FA-GpYK-Taxol to FA-GYK-Taxol by LC-MS in the gel at different time points

<table>
<thead>
<tr>
<th>Time</th>
<th>Conversion (%)</th>
<th>gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelling point (~2.60 min)</td>
<td>66.37 ± 3.83</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td>84.96 ± 0.60</td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>86.56 ± 0.66</td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>86.39 ± 1.49</td>
<td></td>
</tr>
<tr>
<td>12 h</td>
<td>88.52 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>89.67 ± 0.12</td>
<td></td>
</tr>
</tbody>
</table>

*Note:* a PBS solution containing 0.2 wt% of FA-GpYK-Taxol was treated with the phosphatase (final enzyme concentration = 90 U/mL), the error bar represents the standard deviation, and the total experimental times = 3

**Figure S-5.** The light scattering data of the DMEM medium with 10% of the FBS
**Figure S-6.** The mol percentage of FA-GYK-Taxol remained in the diluted PBS buffer solutions upon being incubated at 37 °C at different time scales (determined by the areas of peak at 10.9 min. monitored by UV 220 nm detector)

**Figure S-7.** A typical LC trace for the gel in diluted PBS buffer solution upon being incubated at 37 °C at 12h

**Figure S-8.** A typical LC trace for Taxol
Figure S-9. The inhibition congress curves of Taxol, Taxol-succ, FA-GpYK-Taxol, and the nanospheres (IC$_{50}$ values were 24.8, 31.9, 19.6, and 27.3 nM, respectively)
