Electronic Supplementary Material (ESI) for ChemComm.

Experimental Supporting Information

Materials and general methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai, China). Taxol was purchased from Baoman Bio (Shanghai, China). All the other starting materials were obtained from Alfa (China). Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. The solvents were dried according to regular protocols. All other chemicals were reagent grade or better.

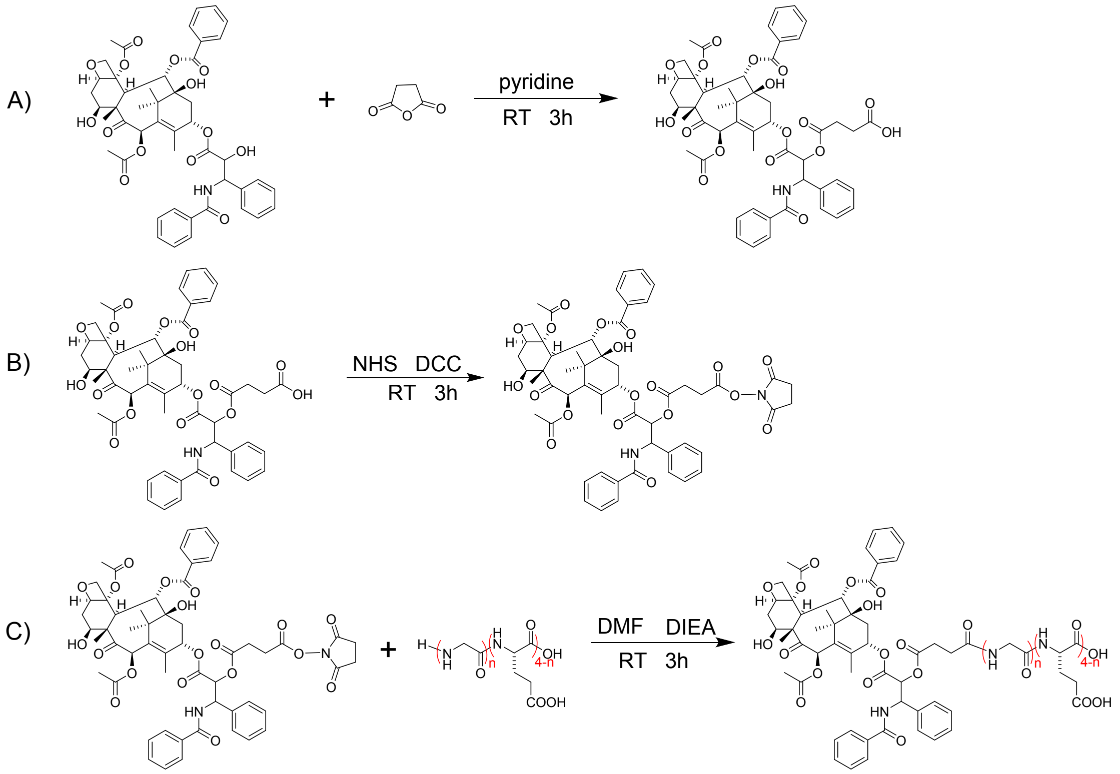
General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 400) using DMSO-d_6 as the solvent; HR-MS were received from VG ZAB-HS system (England). HPLC was conducted at LUMTECH HPLC (Germany) system using a C_{18} RP column with MeOH (0.05% of TFA) and water (0.05% of TFA) as the eluents; TEM images were done on a Tecnai G2 F20 system, operating at 200 kV; LC-MS was conducted at the LCMS-20AD (Shimadzu) system, and rheology was performed on a AR 1500ex (TA instrument) system using a parallel plate (40 mm) at the gap of 400 μm.

Synthesis and characterizations:

Peptide synthesis: The peptide derivative 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. The resin was firstly swelled in dry DCM for 5 minutes (5 mL of DCM per-gram of resin). And then the first amino acid in DCM (1 molar per gram of resin, concentration = 1 molar /5mL of DCM, with 2 equiv. of DIPEA) was loaded on the resin at the C-terminal with the loading efficiency about 1.0 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of

Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-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 DCM for 1 min (5 mL per gram of resin). The peptide derivatives were cleaved from the resin by a mixture of 95% trifluoroacetic acid, 2.5% triisopropylsilane, and 2.5% dd-H_2O for 30 minutes. Then dried via rotary evaporation, followed by ice-cold diethylether precipitation. The resulting precipitate was centrifuged for 10 min at 4 ^{0}C at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was ready for the next step.

Preparation of Succinated Taxol (Taxol-SA): The synthesis of succinated taxol was according to Scheme S-1A. 0.50 g (0.59 mmol) of paclitaxel was dissolved in 12 mL of pyridine and 0.90g (7.6 mmol) of succinic anhydride was then added. After being stirred at room temperature for 3 hours, the organic solvent was removed under reduced pressure. 20 mL of water was added, and the resulting mixture was stirred for 20 min. The precipitate separated was then dissolved in acetone, water was slowly added. 0.52 g of fine crystals were collected. (Yield = 92.0%)



Taxol-G_nE_{4-n} (Compound 1: n=3; Compound 2: n=2; Compound 3: n=1)

Scheme S-1. A) Synthetic route for Taxol-SA (Succinated Taxol); B) Synthetic route for N-hydroxyl succinimide (NHS) activated succinated taxol; C) solution phase organic synthetic route for designed compound **1**, **2**, and **3**.

Synthesis of Taxol-GGGE, Taxol-GGEE, and Taxol-GEEE: 100 mg of Taxol-SA (0.105 mmol) was dissolved in 10 mL of dichloromethane. 1.1 equiv. (13.3 mg, 0.116 mmol) of N-Hydroxysuccinimide and 26 mg (0.126 mmol) of N,N'-dicyclohexylcarbodiimide were added. After being stirred 3hrs at room temperature, the solution was filtered to remove precipitation, dried and evaporated under reduced pressure to yield a white power. The white powder was used for the next step without further purification. The white powder obtained was dissolved in 5 mL N,N-Dimethylformamide, 40 mg (0.126 mmol) of GGGE was then added with 120 μL of N-Ethyldiisopropylamine. After being stirred overnight, HPLC was used for purification to yield the title product in a yield of about 70%. Other compounds (Taxol-GGEE and Taxol-GEEE) were prepared by similar procedures.

Taxol-GGGE: ^{1}H NMR (400MHz, DMSO-d_6) δ 8.07-8.23 (m, 5H), 7.97-8.02 (d, 2H), 7.84-7.89 (d, 2H), 7.64-7.77 (m, 3H), 7.49-7.58 (m, 3H), 7.43-7.48 (t, 4H), 6.29 (s, 1H), 5.79-5.86

(t, 1H), 5.50-5.57 (t, 1H), 5.40-5.45 (d, 1H), 5.32-5.38 (d, 1H), 4.89-4.94 (d, 1H), 4.65 (s, 1H), 4.19-4.26 (m, 1H), 4.07-4.13 (m, 1H), 3.97-4.04 (m, 2H), 3.66-3.77 (m, 7H), 2.21-2.34 (m, 6H), 2.11 (s, 3H), 1.91-2.01 (m, 1H), 1.77 (s, 4H), 1.49 (s, 4H), 0.96-1.05 (d, 6H). HR-MS: calc. M^+= 1253.45, obsvd. $(M+1)^+$= 1254.4583.

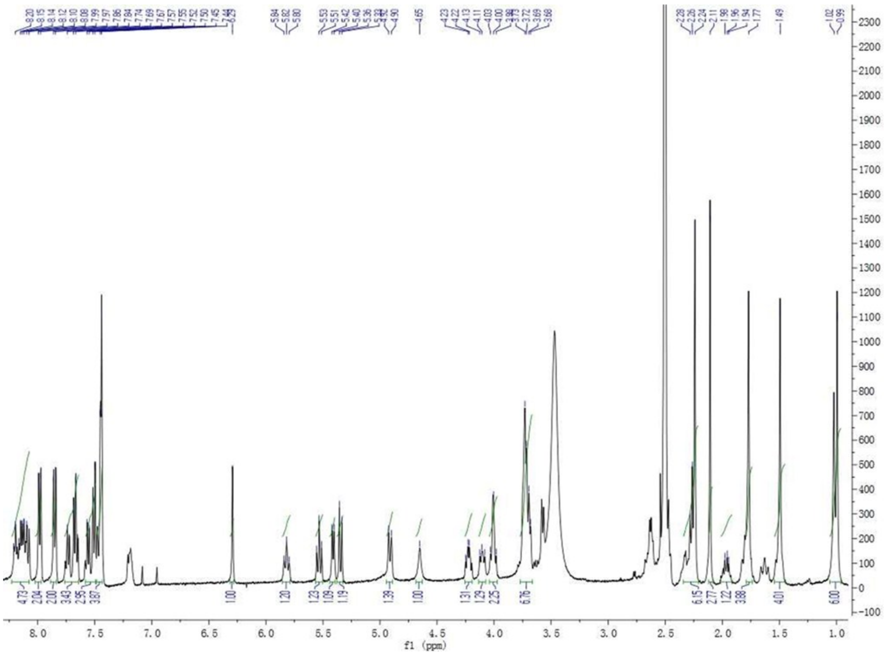


Figure S-1. ^{1}H NMR spectrum of compound **1** (Taxol-GGGE).

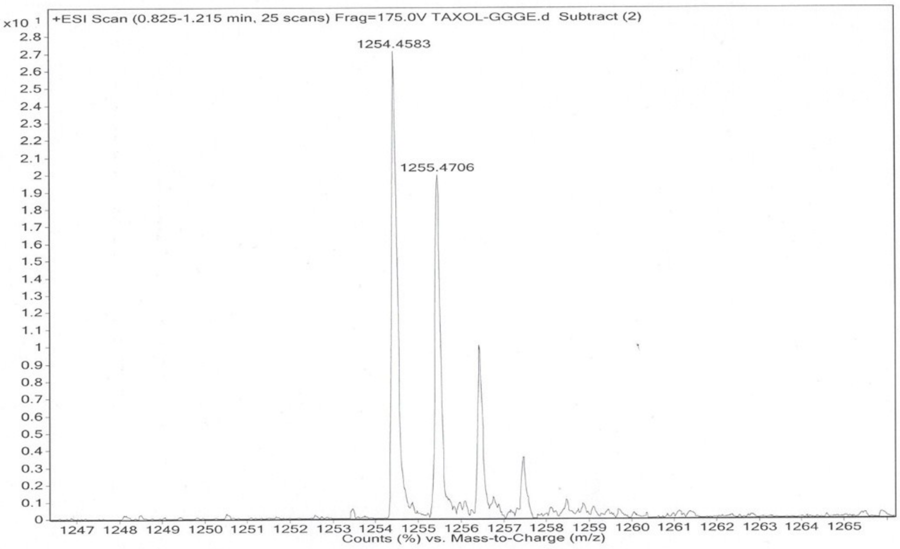


Figure S-2. HR-MS spectrum of compound **1** (Taxol-GGGE).

Taxol-GGEE: ^{1}H NMR (400MHz, DMSO-d_6) δ 9.27-9.21 (d, J=8.42Hz, 1H), 8.19-8.27 (m, 2H), 8.06-8.11 (t, 1H), 7.96-8.04 (t, 3H), 7.83-7.88 (d, J=7.54Hz, 2H), 7.72-7.77 (t, 1H), 7.64-

7.70 (t, 2H), 7.54-7.59 (t, 1H), 7.42-7.53 (m, 6H), 6.29 (s, 1H), 5.79-5.86 (t, 1H), 5.50-5.57 (t, 1H), 5.39-5.43 (d, J=5.27Hz, 1H), 5.32-5.37 (d, 1H), 4.89-4.94 (d, 1H), 4.65 (s, 1H), 4.29-4.35 (m, 1H), 4.08-4.19 (m, 2H), 3.97-4.04 (t, 2H), 3.72-3.76 (t, 2H), 3.66-3.70 (t, 2H), 2.57-2.71 (m, 3H), 2.54 (s, 2H), 2.21-2.35 (m, 8H), 2.11 (s, 3H), 1.87-2.00 (m, 2H), 1.71-1.84(m, 6H), 1.59-1.67 (m, 1H), 1.45-1.55 (m, 4H), 0.95-1.06 (d, 6H). HR-MS: calc. M^{+}= 1325.48, obsvd. $(M+1)^{+}$= 1326.4802.

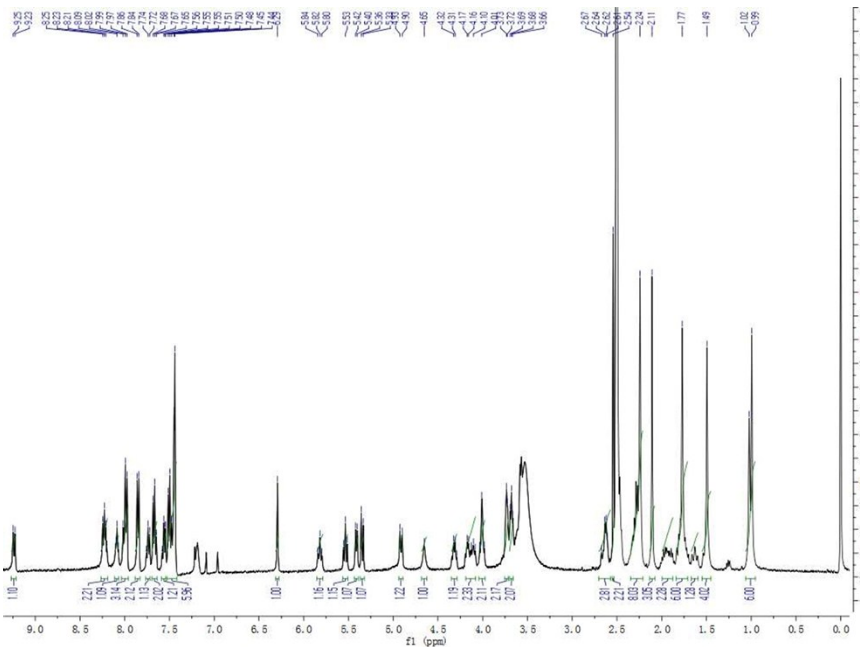


Figure S-3. ^{1}H NMR spectrum of compound **2** (Taxol-GGEE).

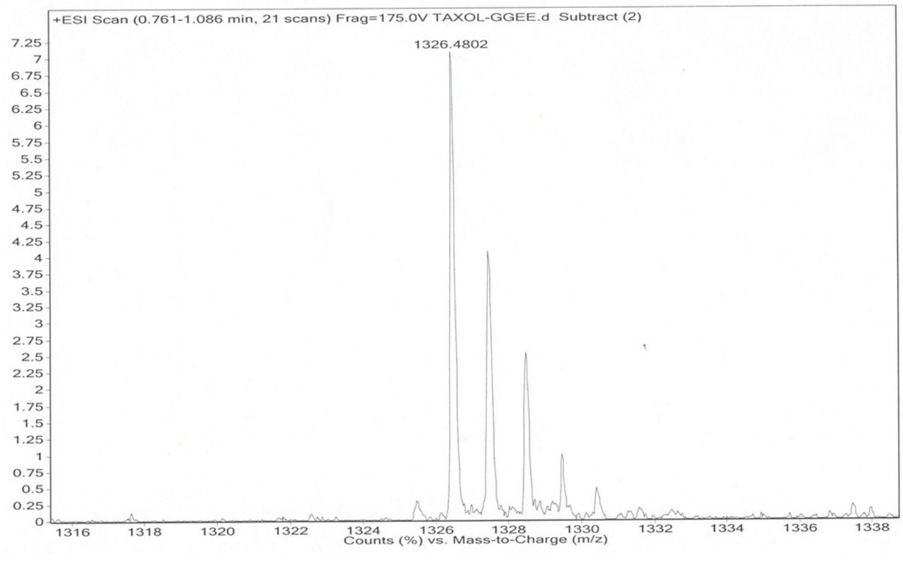


Figure S-4. HR-MS spectrum of compound **2** (Taxol-GGEE).

Taxol-GEEE: ^{1}H NMR (400MHz, DMSO-d_6) δ 9.21-9.26 (d, J=8.29Hz, 1H), 8.13-8.20 (m, 2H), 8.02-8.09 (m, 2H), 7.96-8.01 (d, J=7.38Hz, 2H), 7.83-7.87 (d, J=7.30Hz, 2H), 7.71-7.76 (t, 1H), 7.64-7.70 (t, 2H), 7.54-7.58 (t, 1H), 7.42-7.52 (m, 6H), 7.16-7.23 (m, 1H), 6.29 (s, 1H), 5.79-5.86 (t, 1H), 5.51-5.57 (t, 1H), 5.39-5.44 (d, J=7.17Hz, 1H), 5.32-5.37 (d, 1H), 4.89-4.94 (d, 1H), 4.66 (s, 1H), 4.22-4.31 (m, 2H), 4.15-4.19 (m, 1H), 3.98-4.04 (t, 2H), 3.71-3.75 (m, 1H), 3.65-3.69 (m, 1H), 2.57-2.71 (m, 3H), 2.54 (s, 2H), 2.21-2.34 (m, 10H), 2.11 (s, 3H), 1.84-2.01 (m, 4H), 1.72-1.81(m, 6H), 1.59-1.67 (m, 1H), 1.47-1.56 (m, 4H), 0.96-1.06 (d, 6H). HR-MS: calc. M^+= 1397.50, obsvd. $(M+1)^+$= 1398.5008.

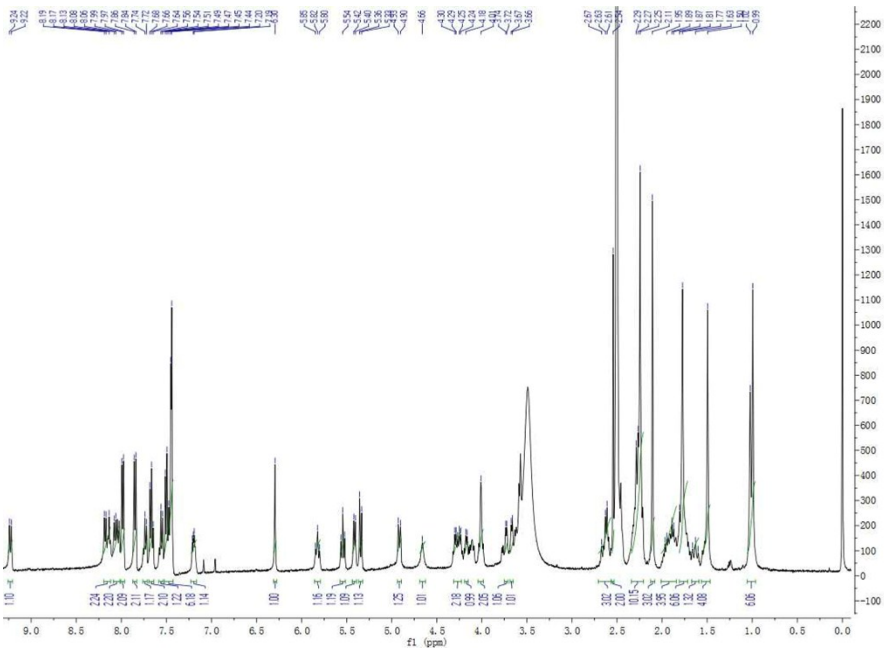


Figure S-5. ^{1}H NMR spectrum of compound compound **3** (Taxol-GEEE).

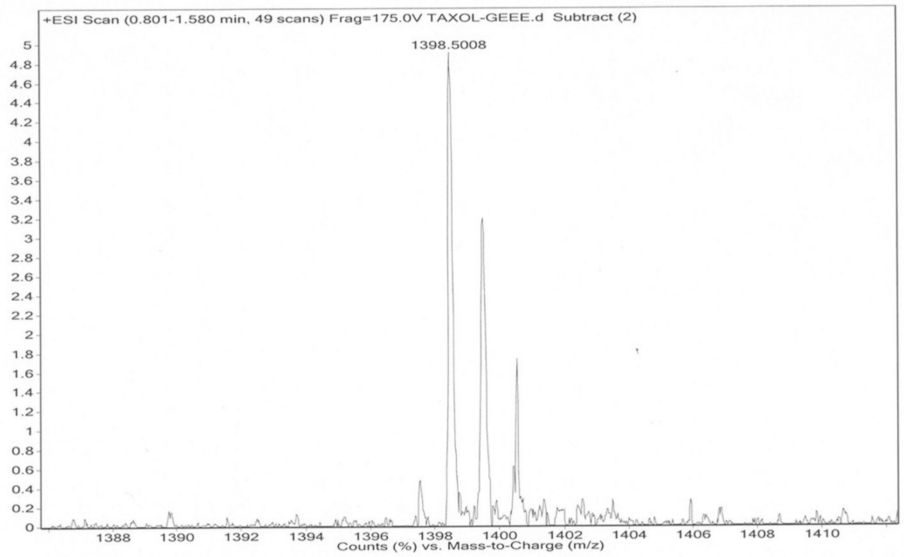


Figure S-6. HR-MS spectrum of compound compound **3** (Taxol-GEEE).

Critical Micelle Concentration: Solutions containing different concentrations of the compounds were used for the test and the light scattering intensity was recorded for each analysis. The CMC values of compounds in PBS solution were determined by dynamic light scattering (DLS). The intensity data and binding plot were analyzed using Origin software.

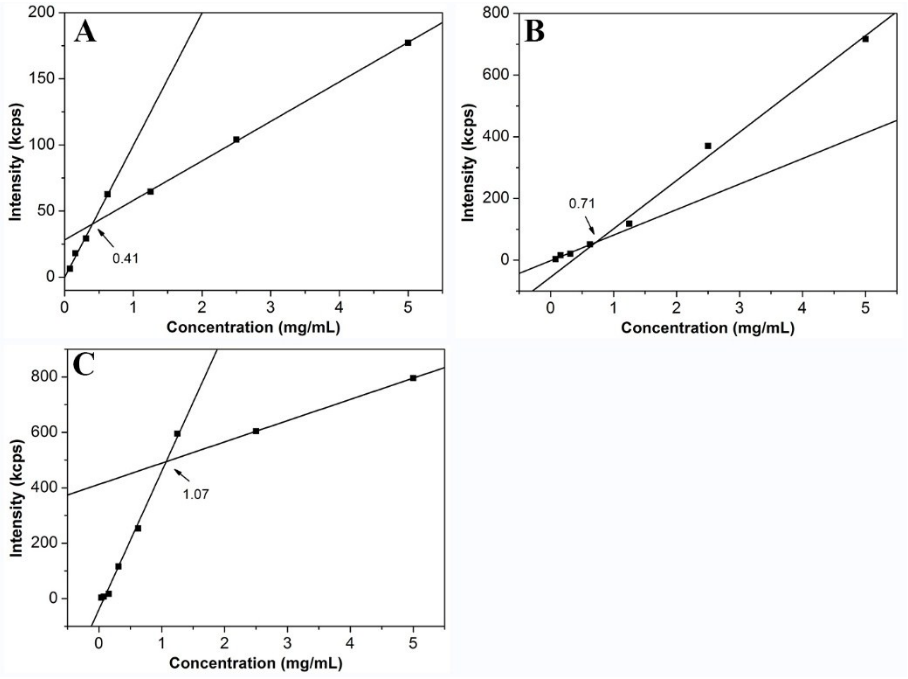


Figure S-7. Critical micelle concentration (CMC) value of compounds used in this study: A) compound **1**; B) compound **2**; C) compound **3**.

Preparation of hydrogel: 1 mg of Taxol-GGGE (compound 1, about 0.80 μmol) were dissolved in 0.2 mL of PBS buffer solution containing 0.17 mg (2 equiv. to compound 1) of Na_2CO_3 (2 equiv. of Na_2CO_3 were used to neutralize the carboxylic acids on Taxol-GGGE to make the final pH value to 7.4). The gel would form after being kept at room temperature (20-25 ℃) for about 5 minutes (the final concentration of hydrogelators was 4 mM). Other gels of 2 and 3 were prepared by similar procedures and the final concentration of hydrogelator was 4 mM.

Rheology: Rheology test was done on an AR 1500ex (TA instrument) system, 40 mm parallel plates were used during the experiment at the gap of 400 μm. The dynamic time sweep was conducted at the frequency of 0.5 rad/s and the strain of 0.5%. Dynamic strain sweep was performed and the strain values within the linear range were chosen for the following dynamic frequency sweep. The gels were characterized by the mode of dynamic frequency sweep in the region of 0.1-100 rad/s at the strain of 0.5%.

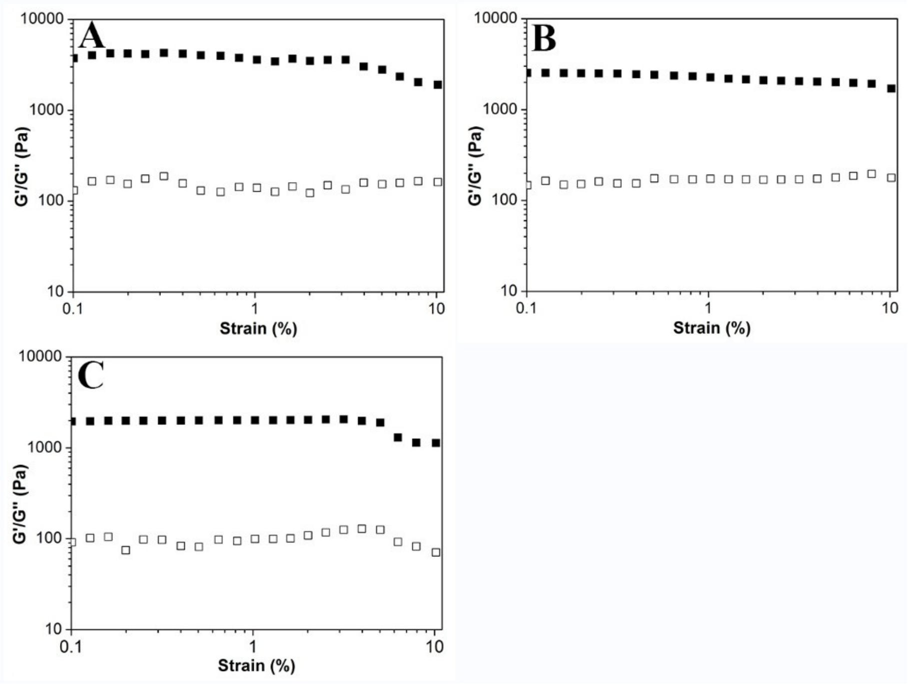


Figure S-8. Rheological measurements with the mode of dynamic strain sweep at the frequency of 0.5 % for PBS solutions containing: A) 0.5 wt % Taxol-GGGE (compound 1); B) 0.5 wt % Taxol-GGEE (compound 2); C) 0.5 wt % Taxol-GEEE (compound 3). (closed symbols: G' and open symbols: G'')

IC_{50} values:

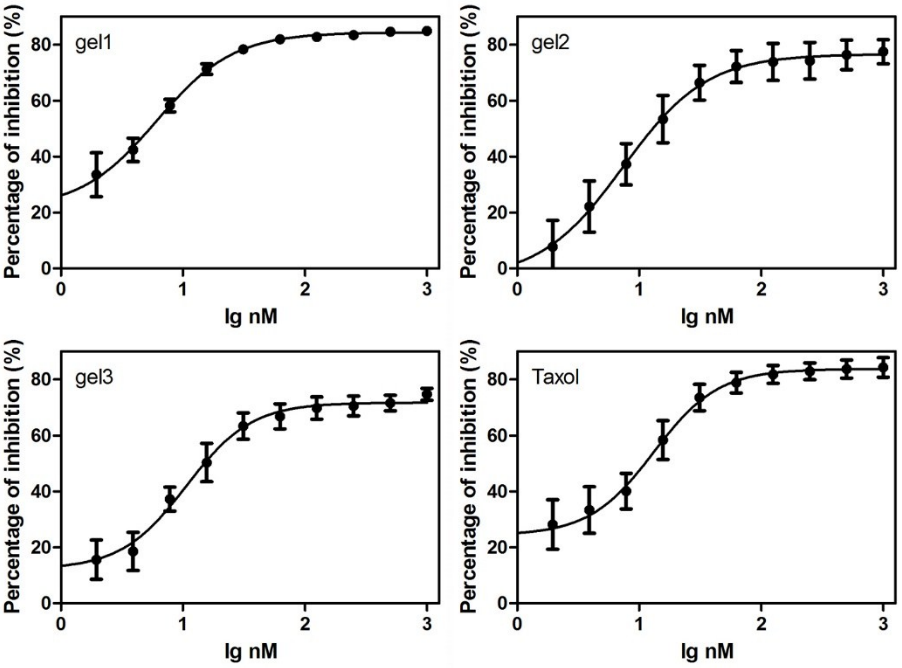


Figure S-9. Representative congress curve of inhibition of HepG2 cells to determine the IC_{50} value of different formulations of taxol.

Determination of zeta potential of self-assembled structures: The zeta potential of the self-assembled structures was measured by a zeta potential analyzer (Zeta Pals, Brookhaven Instruments, Huntsville, NY, USA). Hydrogels formed from 0.5 wt% of precursors in PBS were diluted for 5 times by PBS. The zeta potential was then measured with palladium electrodes at 25℃, and the mean of three readings was taken. Precursor solutions and PBS were filtered through a 0.22 μm membrane before the preparation of hydrogels.

Nanofibers in all of three hydrogels exhibited negative zeta potentials in PBS with the number of -44, -48 and -58 eV for the assembly formed by compound 1, 2 and 3, respectively.

Tumor inhibition assay: Animal study was performed in compliance with the relevant laws and institutional guidelines of Nankai University, and the committee have approved the experiments. Aiming to build breast tumor model, we inoculated Female Balb/c mice with 2×10^5 4T1-luciferase cells in the mammary fat pad. The tumor growth was monitored every other day. Tumor volume was recieved through calculating by the formula: length × width × (Length + Width)/2. When tumors size reached ~50 mm^3, mice were randomly divided into different treatment groups. The day of giving drugs was designated as day 0, all drugs were injected with an interval of 3 days, and the i.v. injection was stopped after 12 days. Mice weight was also monitored after receiving treatment.

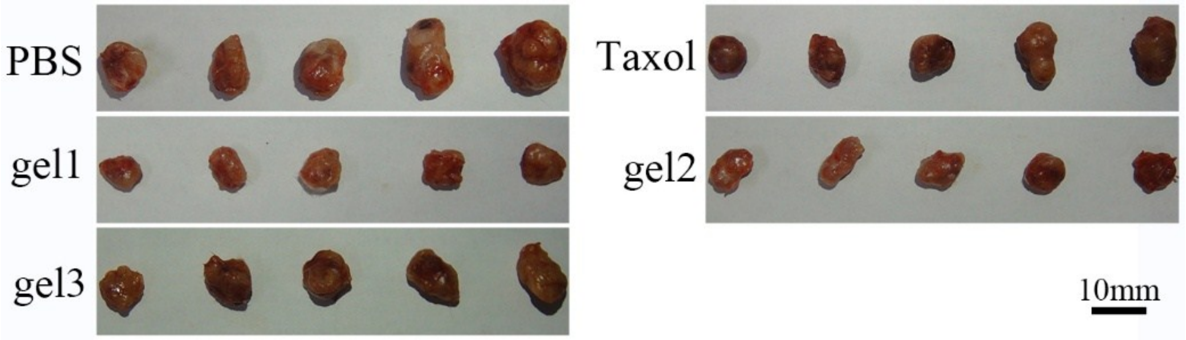


Figure S-10. Digital photograph of representative tumors from PBS, Taxol, gel1, gel2 and gel3 group.

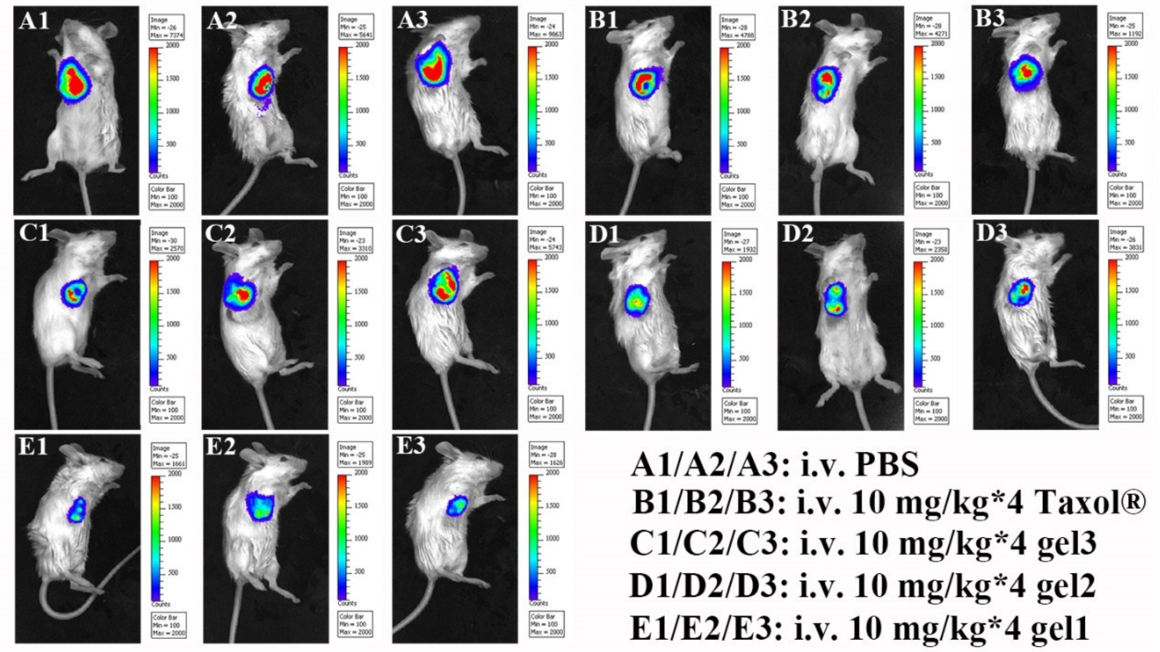


Figure S-11. Bioluminescent imaging on 4T1-luciferase tumor-bearing BALB/C mice 14 days after given indicated treatment. Tumor cells are displayed with a red blue color bar, red color indicating the highest bioluminescence intensity.

Determination of the hydrolysis percentage of different compounds: At different time points, the sample was dissolved in Methanol and the solution was used to run LC-MS to determine the hydrolysis percentage of different compounds. The areas of peaks at 220 nm were used to determine the hydrolysis percentage of different compounds containing taxol. The experiment was repeated 3 times.

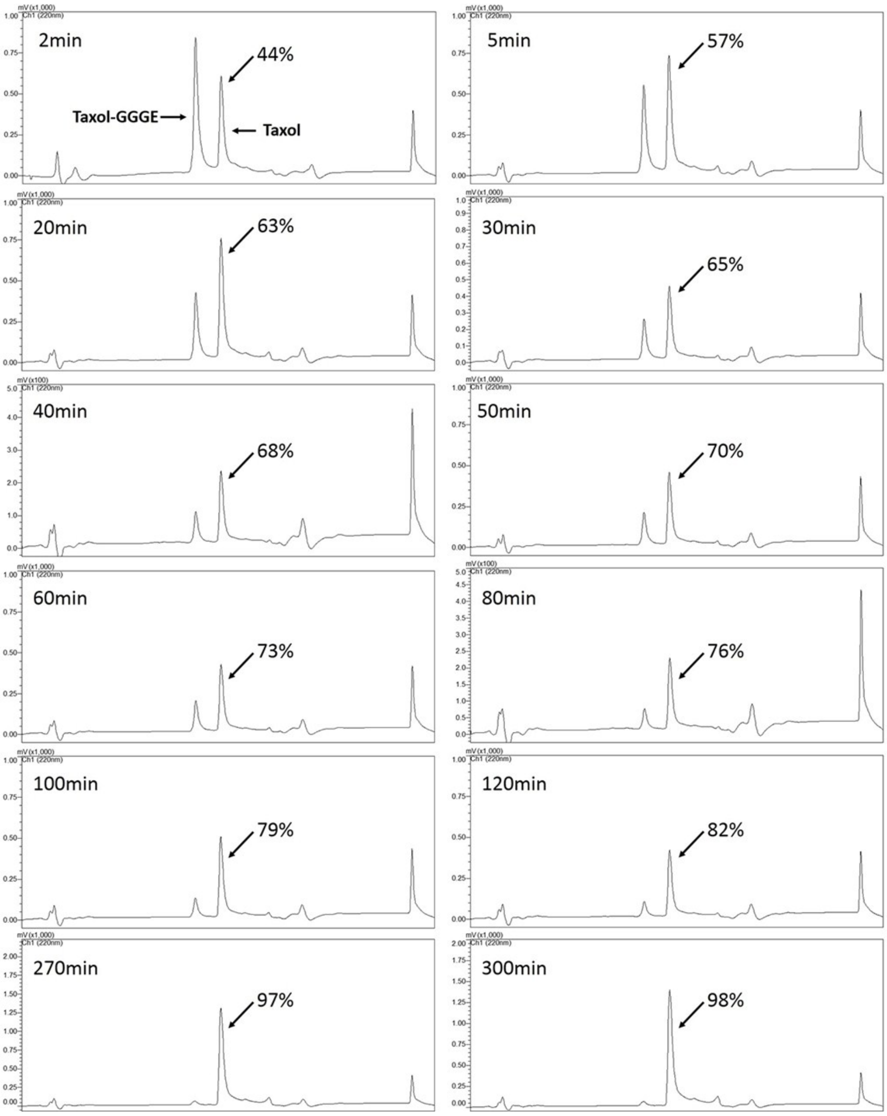


Figure S-12. The representative LC traces used to plot the hydrolysis percentage of compound **1** (taxol-GGGE).

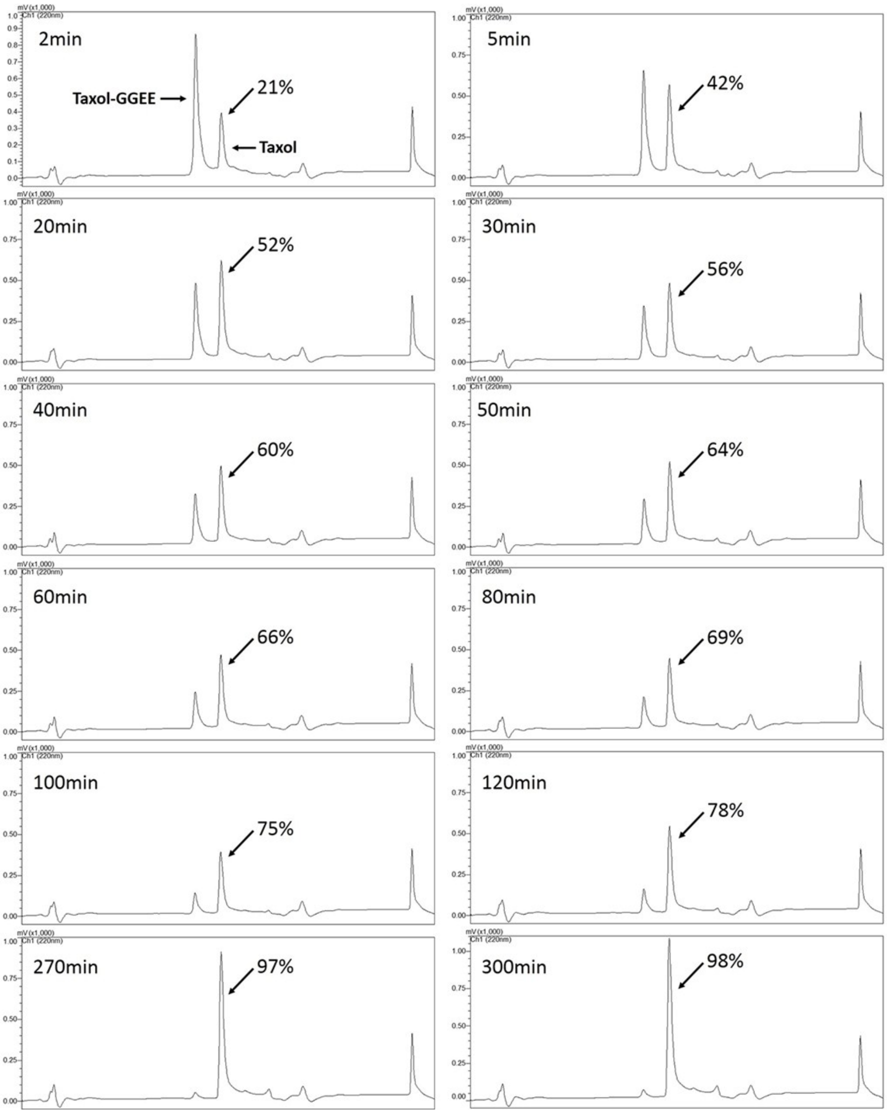


Figure S-13. The representative LC traces used to plot the hydrolysis percentage of compound **2** (taxol-GGEE).

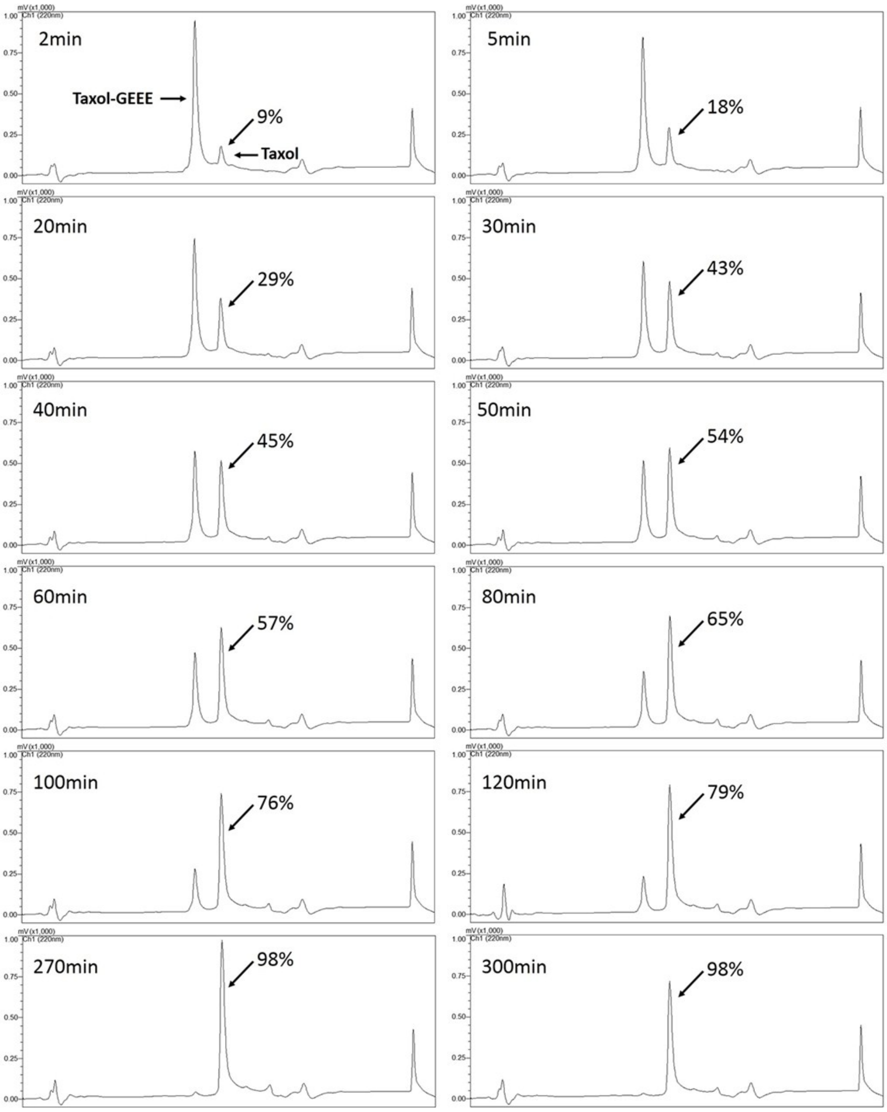


Figure S-14. The representative LC traces used to plot the hydrolysis percentage of compound **3** (taxol-GEEE).