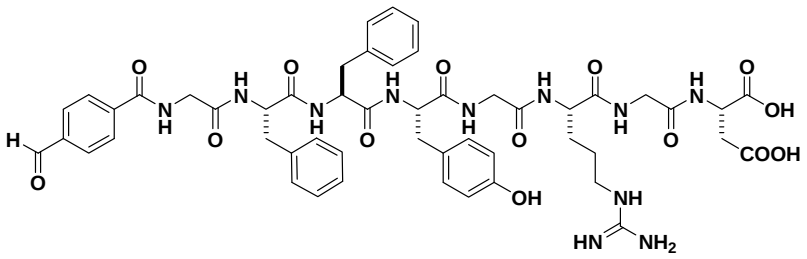
Supporting Information

Materials and methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai). 4-Formylbenzoic acid (FBA) and gemitabine hydrochloride were purchased from J&K Scientific. Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 400). LC-MS spectrometric analyses were performed at the LCMS-20AD (Shimadzu) system. 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. MTT data was recorded on a BioTek Synergy™ 4 Hybrid Microplate Reader. TEM images were done on a Tecnai G2 F20 system, operating at 200 kV.

Peptide systhesis: The peptide derivative was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin, the corresponding N-Fmoc protected amino acids with side chains properly protected by different group. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 1.2 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. At the final step, 4-Formylbenzoic acid (FBA) was used to attach on the peptide. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 times , followed by five steps of washing using DCM. The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TIS and 2.5% of H_2O for 30 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was filtrated and washed by ice-cold diethylether. The crude product was purified by HPLC and dried by lyophilizer.



Schem-S1*:** chemical structure of ***FBA-GFFYGRGD

Compound FBA-GFFYGRGD: ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H), 8.97 (t, *J* = 5.8 Hz, 1H), 8.26 (d, *J* = 23.1 Hz, 4H), 8.10 (d, *J* = 50.5 Hz, 8H), 7.63 (s, 1H), 7.22 (s, 4H), 7.15 (s, 5H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 4.49 (t, *J* = 28.2 Hz, 6H), 4.33 – 4.28 (m, 1H), 3.91 (d, *J* = 5.8 Hz, 1H), 3.75 (d, *J* = 3.6 Hz, 6H), 3.08 (s, 2H), 2.94 (d, *J* = 17.7 Hz, 4H), 2.84 – 2.65 (m, 5H), 2.61 (s, 1H), 1.72 (t, *J* = 15.2 Hz, 1H), 1.51 (t, *J* = 23.1 Hz, 4H). calc. M = 1049.42, obsvd. $(M+H)^{+}$ = 1050.70

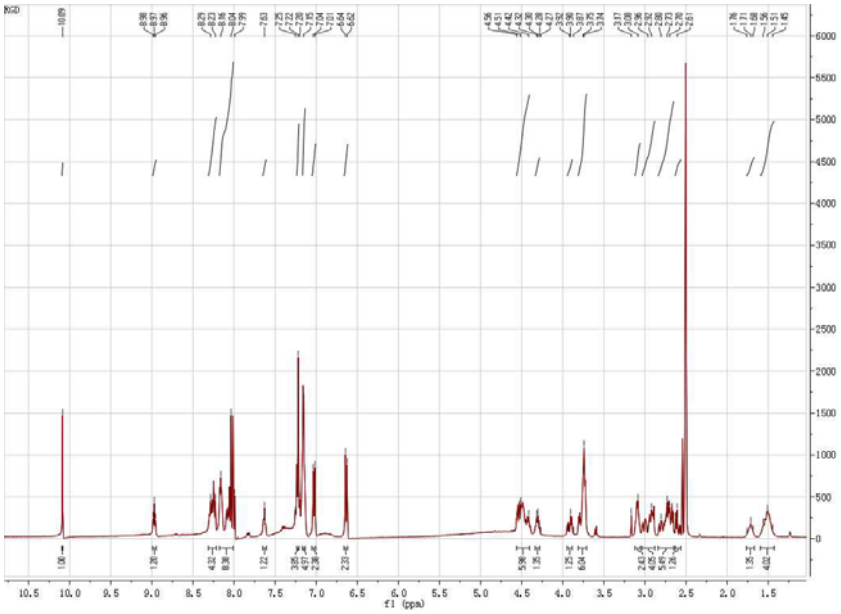


Fig.S-1. ^{1}H NMR of Compound ***FBA-GFFYGRGD***

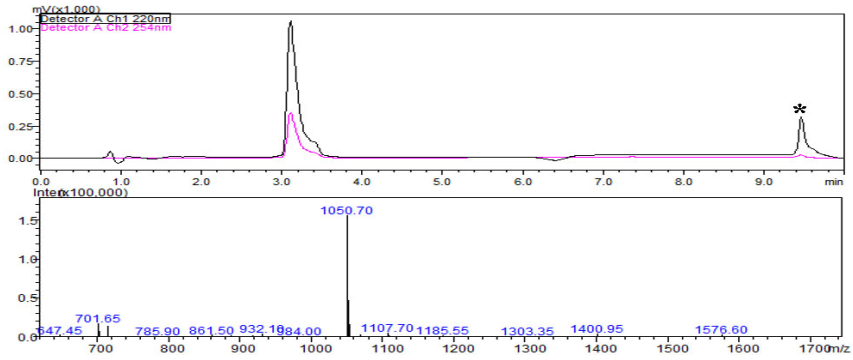
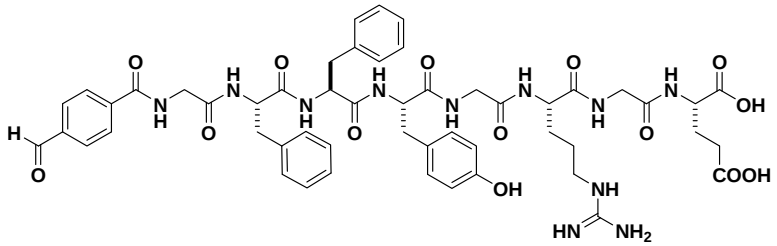


Fig.S-2. LC-MS of Compound ***FBA-GFFYGRGD*** (peak marked with * is system peak)



Schem-S2: chemical structure of ***FBA-GFFYGRGE***

Compound FBA-GFFYGRGE: ^{1}H NMR (400 MHz, DMSO) δ 10.09 (s, 1H), 8.96 (t, *J* = 5.8 Hz, 1H), 8.30 (d, *J* = 5.7 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.16 – 7.99 (m, 10H), 7.63 (s, 1H), 7.21 (d, *J* = 6.7 Hz, 4H), 7.16 – 7.13 (m, 6H), 7.01 (s, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 4.51 – 4.38 (m, 4H), 4.24 (ddd, *J* = 13.3, 11.0, 5.7 Hz, 3H), 3.94 – 3.88 (m, 1H), 3.80 – 3.69 (m, 6H), 3.12 – 2.98 (m, 3H), 2.95 – 2.85 (m, 3H), 2.82 – 2.67 (m, 4H), 2.27 (t, *J* = 7.5 Hz, 3H), 2.01 – 1.91 (m, 2H), 1.80 – 1.70 (m, 2H), 1.57 – 1.43 (m, 4H). calc. M = 1063.44, obsvd. $(M+H)^+$ = 1064.40

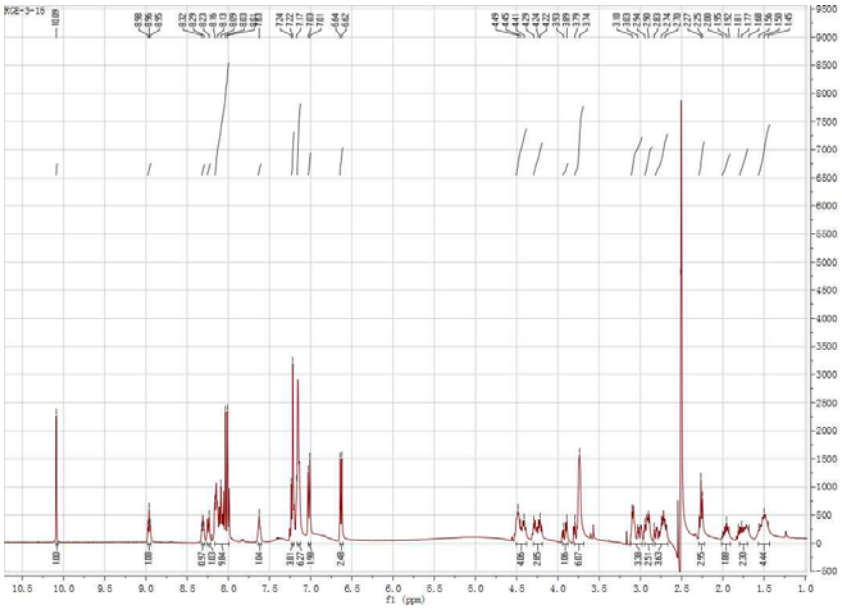


Fig.S-3. ^{1}H NMR of Compound ***FBA-GFFYGRGE***

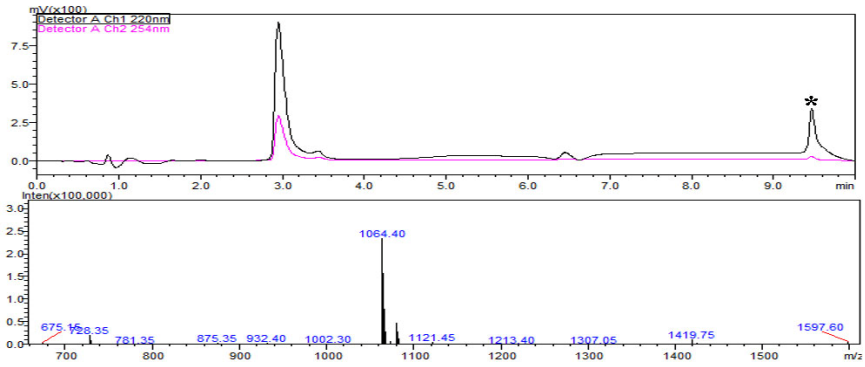


Fig.S-4. LC-MS of Compound ***FBA-GFFYGRGE***

Formation of gels： compound of ***FBA-GFFYGRGD*** **or** ***FBA-GFFYGRGE*** was firstly dissolved in 1×PBS with 2 equiv. Na_2CO_3 to adjust the pH value to 7.4. 1 equiv. of Gemcitabine was then added to the above solution, resulting in a homogeneous mixed solution by sonication and hydrogels would form within 30 minutes (the final peptide concentration = 0.5 wt%, about 4.76 mM).

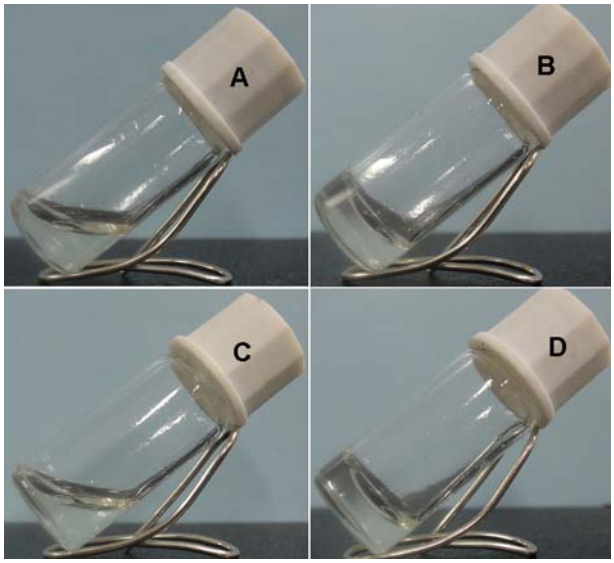


Fig.S-5. Optical photos of A) solution of ***FBA-GFFYGRGD*** and B) hydrogel of gemcitabine-RGD conjugates; C) solution of ***FBA-GFFYGRGE*** and D) hydrogel of gemcitabine-RGE conjugates

Confirmtion of Schiff base formation: To confirmtion the Schiff base formation between ***FBA-GFFYGRGD/ FBA-GFFYGRGE*** and gemcitabine, the hydrogels formed by above conjugates were lyophilized and the resulted powers were dissolved in DMSO-d_6 to obtain the ^{1}H NMR spectrums. As shown in Fig. S-6 and Fig. S-7, the characterized H of aldehyde on ***FBA-GFFYGRGD/ FBA-GFFYGRGE*** at 10.09 ppm was obviously disappeared in the presence of 1 equiv. of gemcitabine.

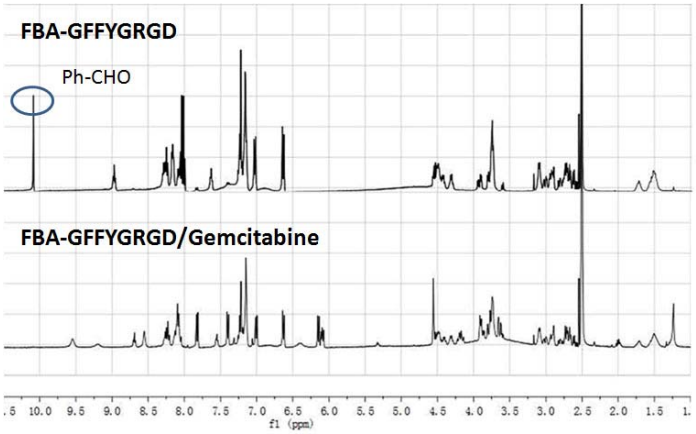


Fig.S-6. ^{1}H NMR spectrum of ***FBA-GFFYGRGD*** and ***FBA-GFFYGRGD/gemcitabine*** mixture

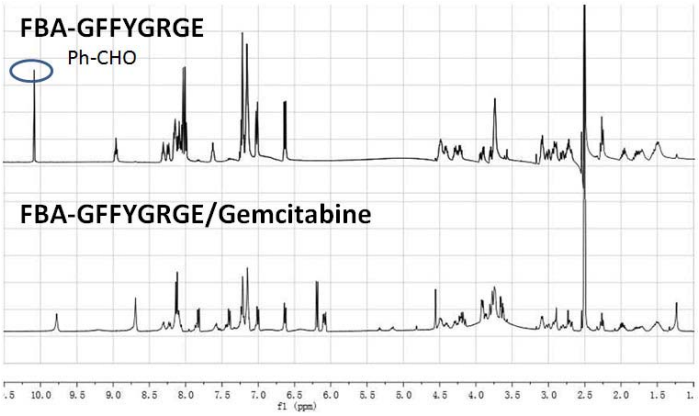


Fig.S-7. ^{1}H NMR spectrum of ***FBA-GFFYGRGE*** and ***FBA-GFFYGRGE/gemcitabine*** mixture

Rheology. The 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. The gels were firstly characterized by the mode of time sweep, followed by a dynamic frequency sweep in the region of 0.1–100 rad s^{-1} at the strain of 0.5% and a dynamic strain sweep at the region of 0.1%-10%.

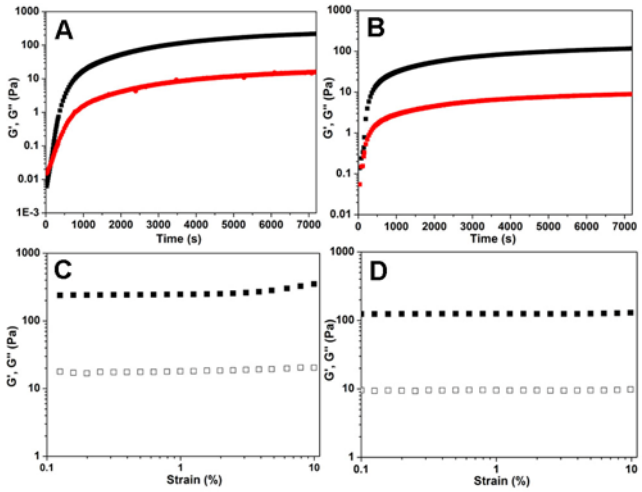


Fig.S-8. Time sweep at the strain of 0.5% of A) gel I formed by adding 1 equiv. of gemcitabine to PBS solution of ***FBA-GFFYGRGD*** and B) gel II formed by adding 1 equiv. of gemcitabine to PBS solution of ***FBA-GFFYGRGE*** and strain sweep of C) gel I and D) gel II

Preparation of TEM samples of compounds: a carbon-coated copper grid was vertically dipped into the hydrogel or solution for 5 seconds, washed by water two times and then placed in a desicator overnight before the TEM measurement

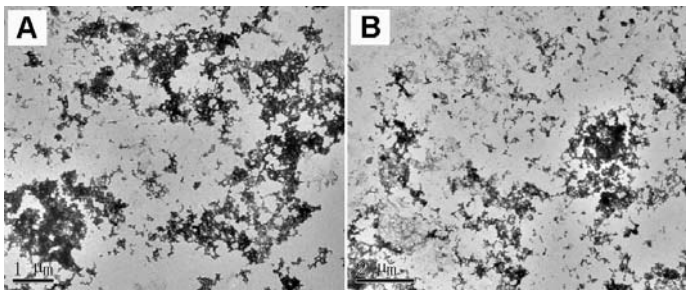


Fig.S-9. TEM images of A) peptide ***FBA-GFFYGRGD*** and B) peptide ***FBA-GFFYGRGE*** at a concentration of 0.5 wt% (5 mg/mL)

Determination of IC_{50} values on pancreatic cancer cells: The PAC-2, CFPAC-1 and BxPC-3 cells were seeded in a 96-well plate with the density of 10,000 cells per-well (total medium volume of 100 μL). 24 hours post seeding, the solutions with a serial of concentrations of peptide-gemcitabine conjugates or free gemcitabine in 100 μL of medium were added to each well (five wells for each concentration). Cells without the treatment of the compounds were used as the control. The MTT assays were performed after an extra culture time of 48 hours. All compounds were removed and 90 μL fresh medium was added for each well, 10 μL of MTT solution (5 mg/mL) was added and incubated for 4 hours in 37℃. Pipette out the spent media, formazon crystals at the bottom of each well were dissolved in 100 μL DMSO. After 15 minutes shaking at room temperature, absorbance at wavelength of 490 nm was tested using a BioTek Synergy™ 4 Hybrid Microplate Reader. The experiment was repeated for 3 times. IC_{50} values for the inhibition of cell viability were calculated from pharmacological inhibitory response curves using software Prism 5.0.

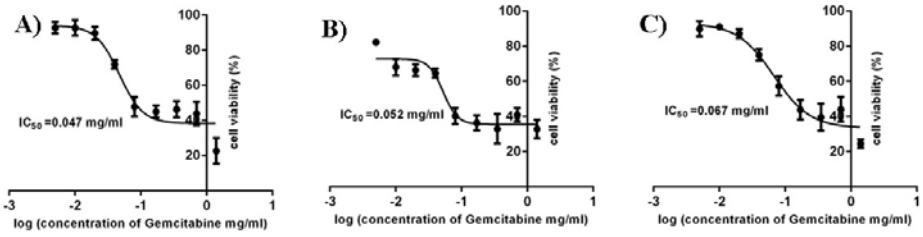


Fig. S-10 . Inhibition curve of A) Gemcitabine-RGD peptide , B) Gemcitabine-RGE peptide and C) Gemcitabine for PAC-2

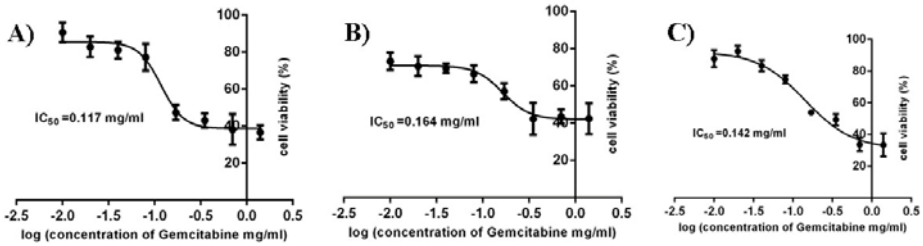


Fig. S-11 . Inhibition curve of A) Gemcitabine-RGD peptide , B) Gemcitabine-RGE peptide and C) Gemcitabine for CFPAC-1

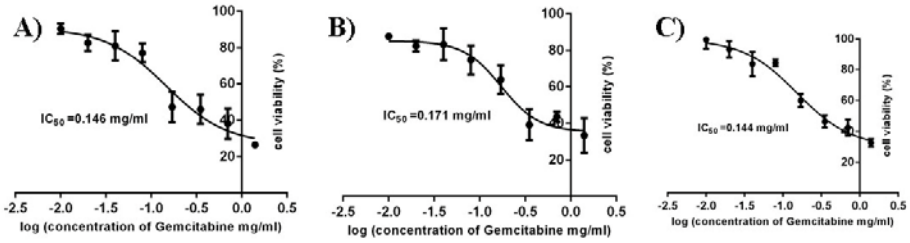


Fig. S-12 . Inhibition curve of A) Gemcitabine-RGD peptide , B) Gemcitabine-RGE peptide and C) Gemcitabine for BxPC-3

Drug release: Hydrogels in PBS solution containing 0.5 wt% of compound were formed in an Eppendorf tube at 25 °C. After 24 h, we added 0.25 mL of PBS on the surface of the hydrogels, 0.2 mL solution was taken out at the desired time point and 0.2 mL PBS was added back. For the following time points, 0.2 mL of PBS was taken out and 0.2 mL of PBS was added back at each point. We then monitored and calculated the release profile from the gel formed by a LCMS-20AD (Shimadzu) system. The experiment was performed at 37 °C in 3 parallel. The release profile in the presence of glycine or cysteine was obtained using similar procedure.

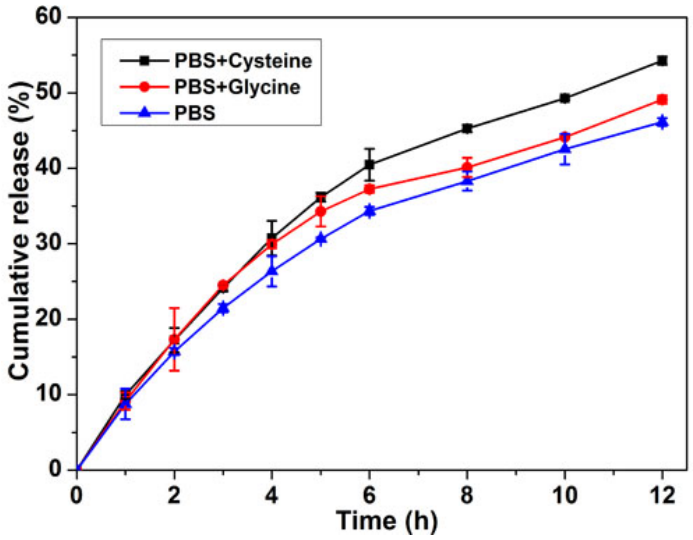


Fig. S-13. Drug release profile in the presence of 4.76 mM (equal molar concentration to gemcitabine in original gel) of glycine or cysteine in PBS buffer solution (pH = 7.4)