Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Supporting Information

Delivery of coumarin-containing all-trans retinoic acid derivative via targeted nanoparticles encapsulating indocyanine green for chemo/photothermal/photodynamic therapy of breast cancer

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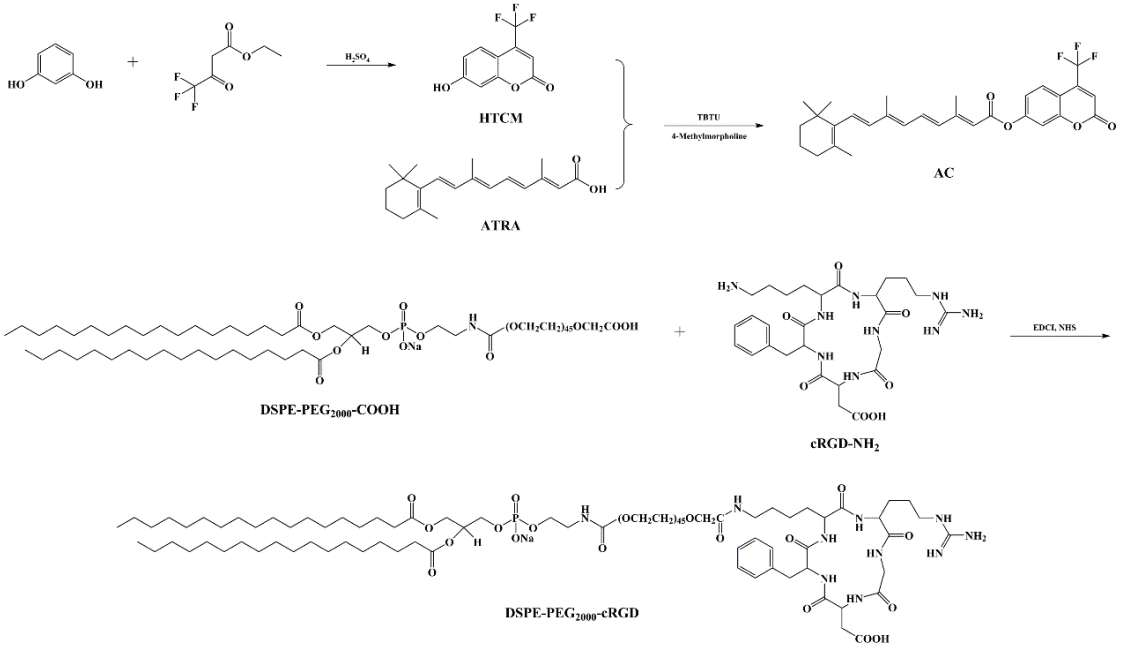

Figure S1. Synthetic route of AC and DSPE-PEG$_{2000}$-cRGD.

1. Synthesis and characterization

Synthesis of 7-hydroxy-4-trifluoromethyl coumarin (HTCM). As shown in **Figure S1**, HTCM was synthesized by the following procedures according to the previous literature.[1, 2] Ethyl 4,4,4-trifluoro-3-oxobutanoate (1.8 g, 10 mmol) and resorcinol (1.1 g, 10 mmol) were dissolved in 1,4-dioxane (25 mL). Then 5 drops of

concentrated sulfuric acid was added into the reaction mixture and refluxed for 5 h. After the reaction completed, the mixture was poured into ice water (100 mL) and stirred, followed by precipitation and filtration. Finally, the white solid was recrystallized twice from the mixture of ethyl alcohol and deionized water to obtain HTCM. ^{1}H-NMR (600 MHz, DMSO) δ 10.95 (s, 1H), 7.56 (dd, *J* = 8.8, 1.8 Hz, 1H), 6.91 (dd, *J* = 8.9, 2.4 Hz, 1H), 6.84 (d, *J* = 2.4 Hz, 1H), 6.76 (s, 1H). ^{13}C-NMR (101 MHz, DMSO) δ 162.63 (s), 159.33 (s), 156.39 (s), 140.18 (d, J = 31.9 Hz), 126.61 (s), 123.62 (s), 120.88 (s), 116.26 – 110.56 (m), 104.66 (d, J = 210.2 Hz), 40.00 (dp, J = 42.0, 21.0 Hz). ESI-MS (m/z): 229.2 $[M-H]^{+}$.

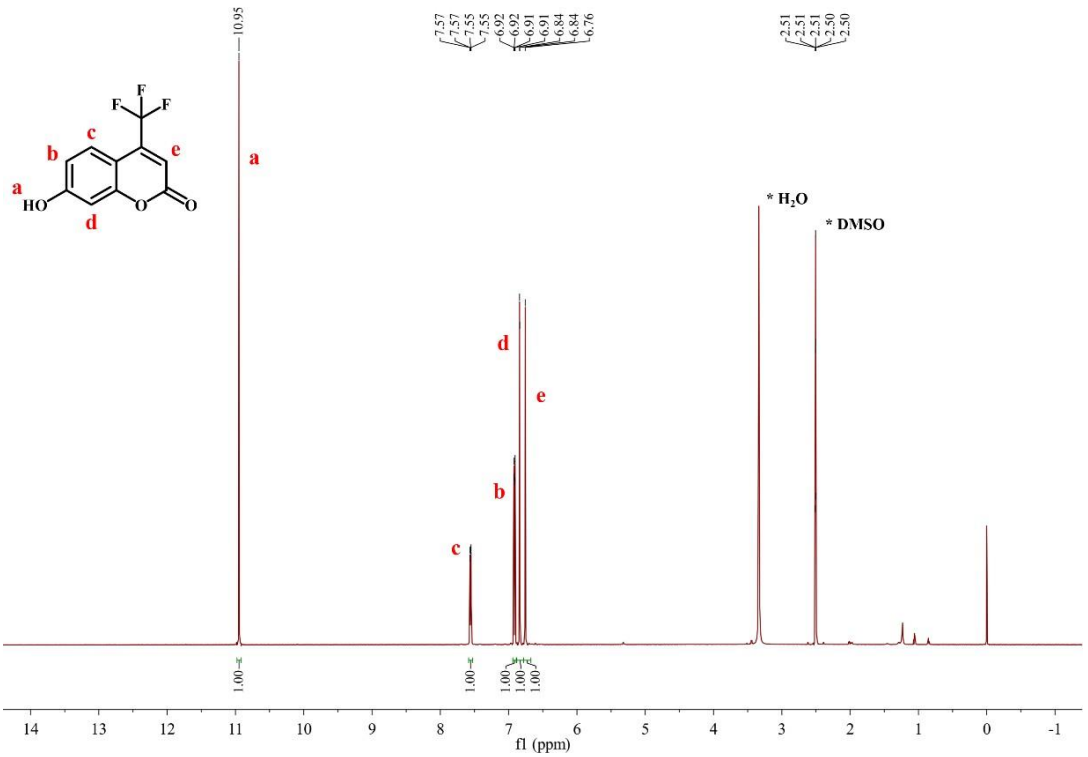

Figure S2. ^{1}H-NMR spectrum of HTCM.

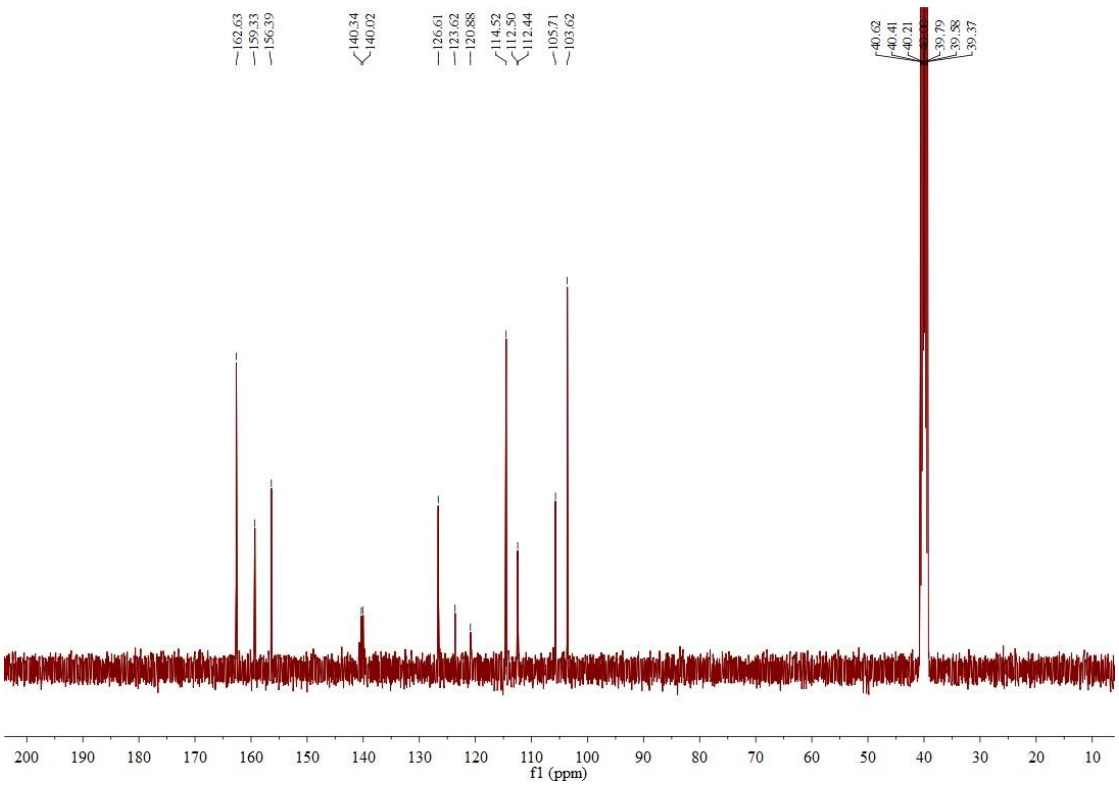

Figure S3. ^{13}C-NMR spectrum of HTCM.

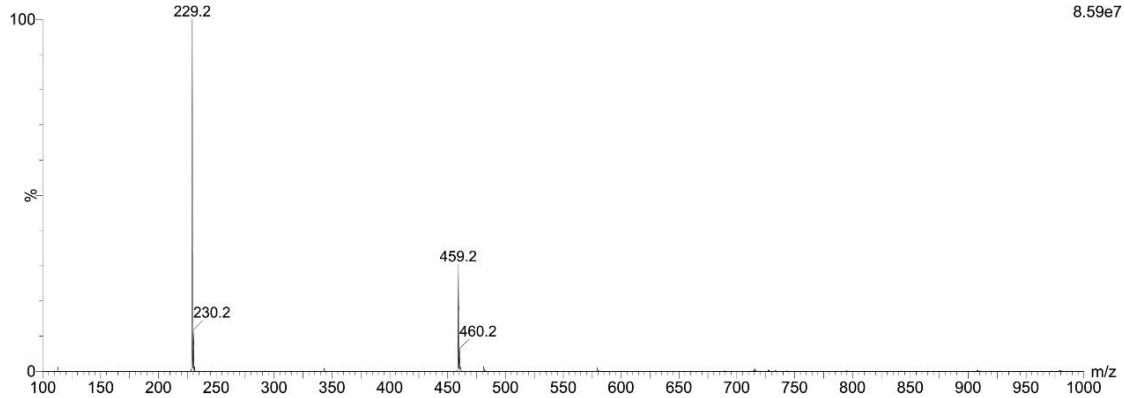

Figure S4. ESI-MS spectrum of HTCM.

Synthesis of coumarin-containing all-trans retinoic acid derivative (AC). AC was synthesized according to previous work from our laboratory.[3] Briefly, ATRA (1.3569 g, 5 mmol) was dissolved in 15 mL DMF with TBTU (1.9265 g, 6 mmol) and 4-Methylmorpholine (0.6069 g, 6 mmol) under ice-water bath stirring evenly for 0.5 h. HTCM (1.1507 g, 5 mmol) was then added into the mixture. Thereafter, the reaction was allowed to warm to room temperature and stirred overnight. Then the reaction mixture was diluted with ether and washed with water (3×25 mL). The organic layer was dried over by sodium sulfate and the solvent was removed under reduced pressure.

The crude product was subsequently purified by column chromatography on silica gel to obtain the targeted compound in the form of a yellow powder. ^{1}H-NMR (600 MHz, DMSO) δ 7.76 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 2.3 Hz, 1H), 7.31 (dd, J = 8.8, 2.3 Hz, 1H), 7.22 (dd, J = 15.1, 11.6 Hz, 1H), 7.06 (s, 1H), 6.56 (d, J = 15.0 Hz, 1H), 6.32 (dd, J = 19.0, 13.9 Hz, 2H), 6.22 (d, J = 16.1 Hz, 1H), 6.14 (s, 1H), 2.38 (s, 3H), 2.10-1.92 (m, 5H), 1.70 (s, 3H), 1.58 (dt, J = 8.8, 4.8 Hz, 2H), 1.49-1.38 (m, 2H), 1.03 (s, 5H). ^{13}C-NMR (151 MHz, DMSO) δ 164.35 (s), 158.02 (d, J = 208.6 Hz), 155.08 (s), 154.20 (s), 141.18 (s), 137.72 (s), 137.29 (s), 135.00 (s), 133.62 (s), 130.26 (d, J = 21.0 Hz), 129.18 (s), 126.11 (s), 123.02 (s), 119.98 (s), 117.03 (s), 116.40 (s), 111.46 (d, J = 58.1 Hz), 103.63 (s), 40.04 (dp, J = 42.0, 21.0 Hz), 33.74 (d, J = 183.6 Hz), 29.28 (s), 21.99 (s), 19.18 (s), 13.83 (d, J = 194.0 Hz). ESI-MS (m/z): 513.5 $[M+H]^{+}$.

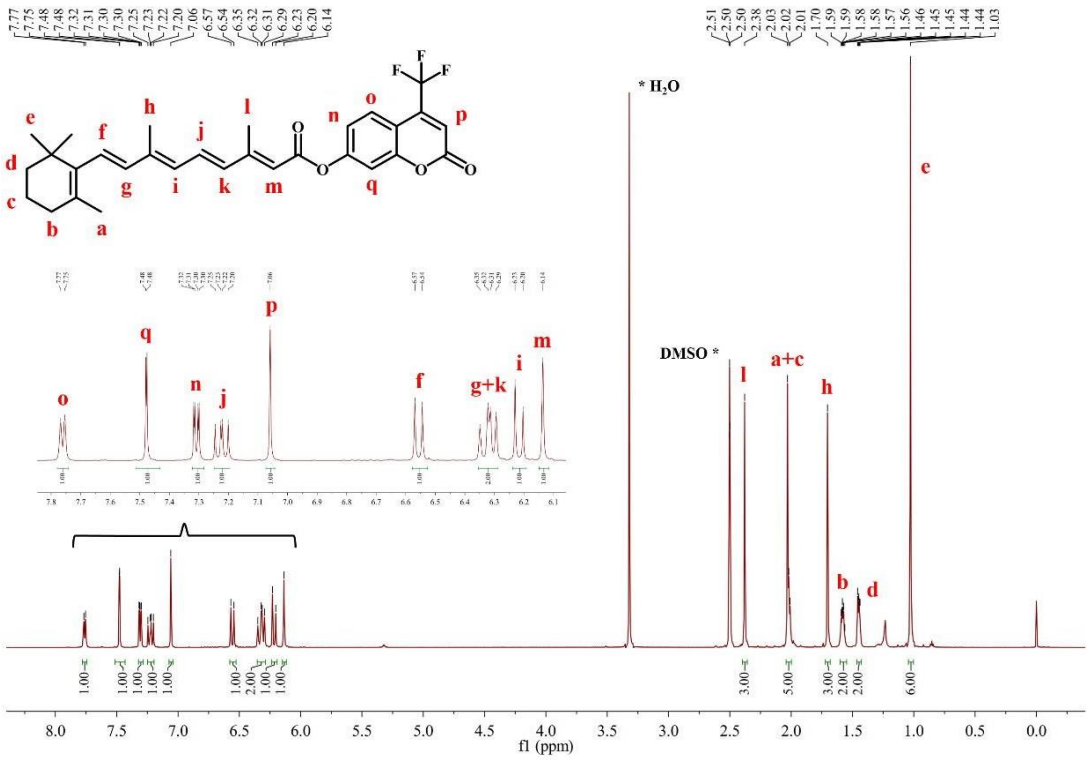

Figure S5. ^{1}H-NMR spectrum of AC.

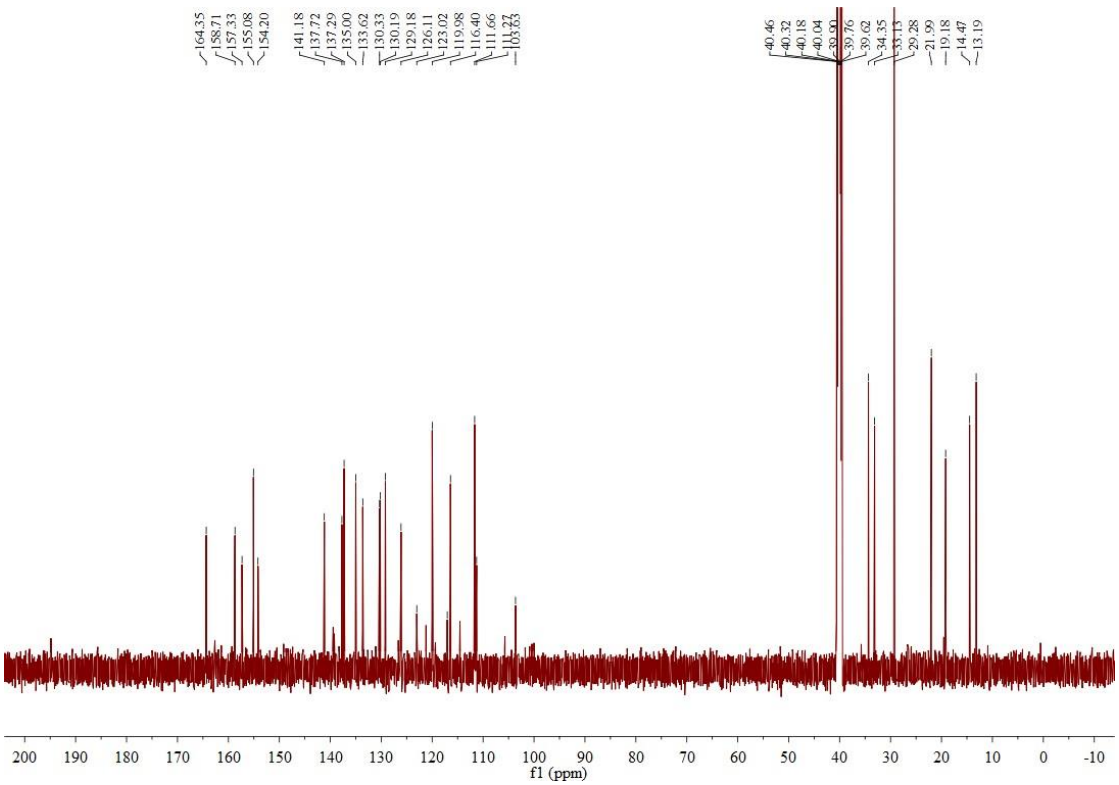

Figure S6. ^{13}C-NMR spectrum of AC.

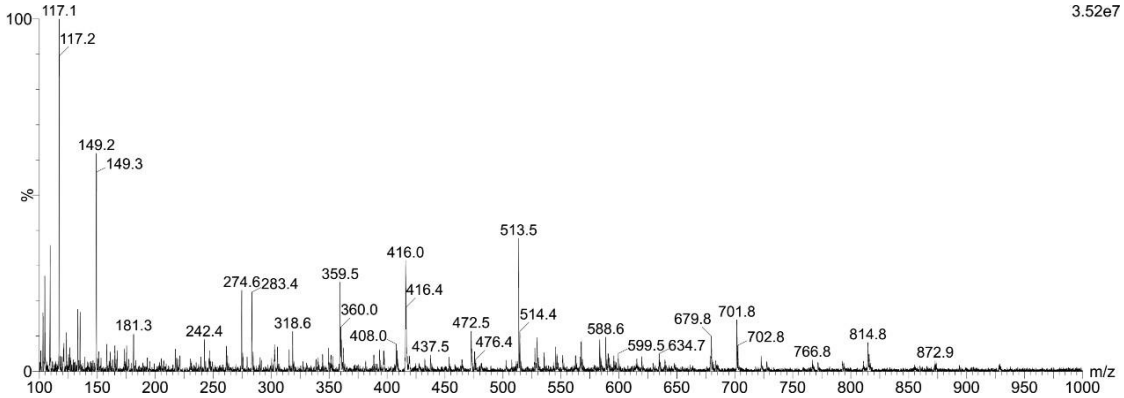

Figure S7. ESI-MS spectrum of AC.

Conjugation of cRGD with DSPE-PEG$_{2000}$-COOH. DSPE-PEG$_{2000}$-COOH were covalently functionalized with cRGD by amidation reaction between DSPE-PEG$_{2000}$-COOH and cRGD using EDCI and NHS as coupling reactants (**Figure S1**).[4, 5] The certain mass of DSPE-PEG$_{2000}$-COOH (100 mg, 0.05 mmol), EDCI (19.2 mg, 0.1 mmol) and NHS (11.5 mg, 0.1 mmol) were dissolved in DMF and stirred at 0 ℃for 0.5 h. Then cRGD (36.2 mg, 0.06 mmol) was added into the reaction solution and stirred for 24 h at room temperature. Finally, DSPE-PEG$_{2000}$-cRGD was collected as a white powder after dialysis and freeze-dried.

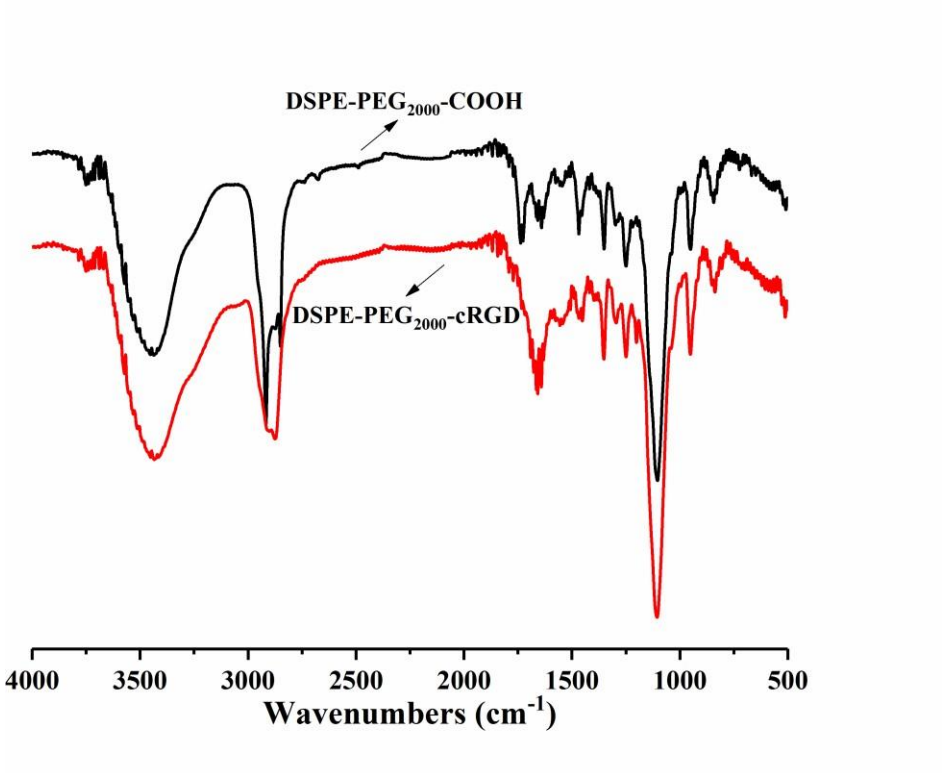

Figure S8. FT-IR spectra of DSPE-PEG_{2000}-COOH and DSPE-PEG_{2000}-cRGD.

As shown in **Figure S8**, DSPE-PEG_{2000}-COOH presented an absorption peak at 1725 cm^{-1}, which can be regarded as the carbonyl stretch of the carboxyl group.[6] New absorption peaks appeared at 1658 and 1560 cm^{-1}, which corresponded to the amide carbonyl vibration and the N-H bending vibration, respectively.

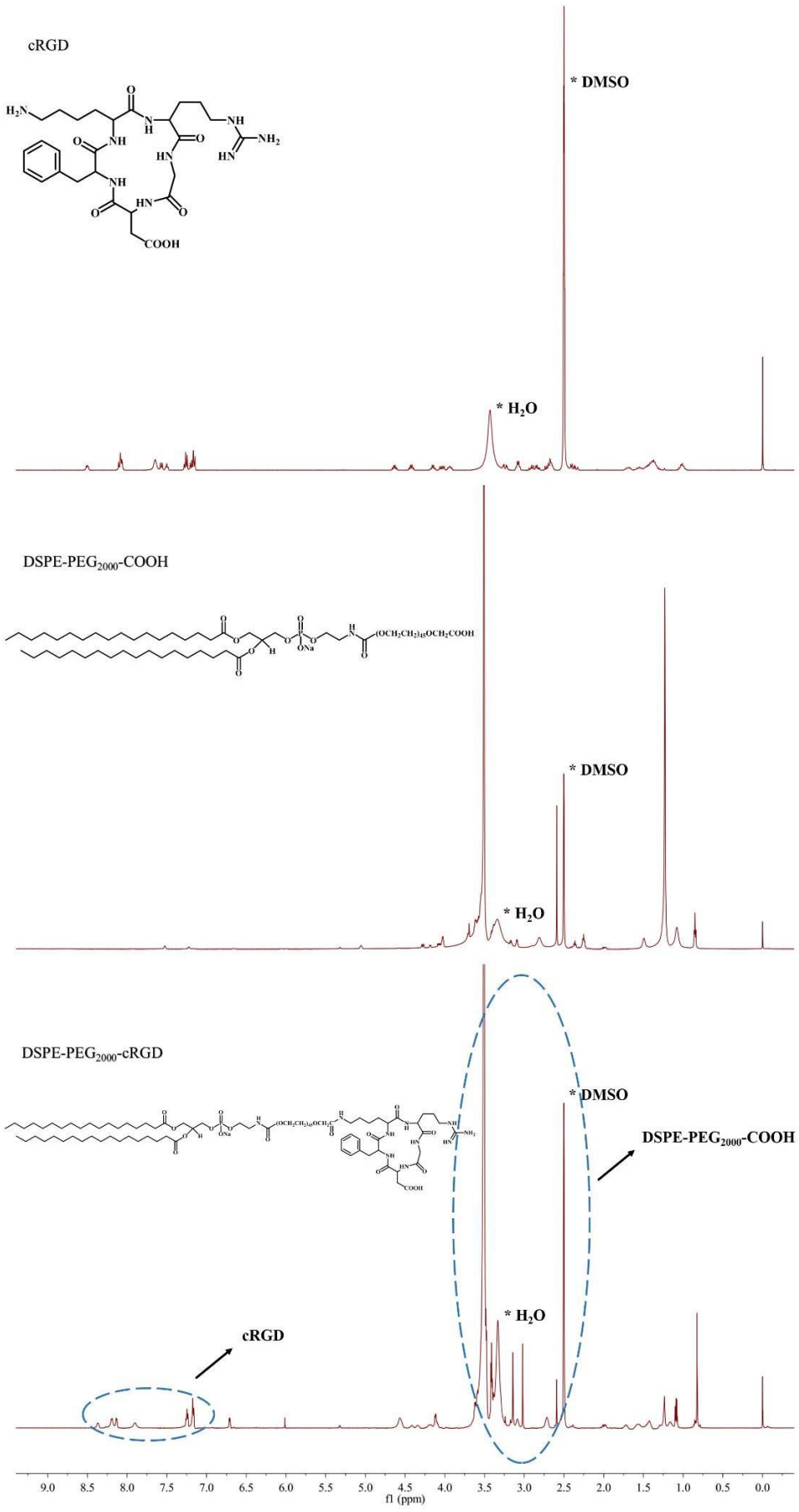
cRGD
* DMSO
* H2O
DSPE-PEG2000-COOH
* DMSO
* H2O
DSPE-PEG2000-cRGD
* DMSO
DSPE-PEG2000-COOH
* H2O
cRGD
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

Figure S9. ^{1}H-NMR spectrum of cRGD, DSPE-PEG$_{2000}$-COOH and DSPE-PEG$_{2000}$-cRGD.

The successful conjugation of cRGD to DSPE-PEG$_{2000}$-COOH was further confirmed by ^{1}H-NMR spectral in **Figure S9**. The ^{1}H-NMR spectrum of DSPE-PEG$_{2000}$-cRGD simultaneously possessed the same chemical shift as benzene ring and secondary amide (-CONH-) of cRGD at δ 6.82-8.41 ppm, which indicated the successful coupling of cRGD at the terminal of DSPE-PEG$_{2000}$-COOH.

2. Preparation and characteration

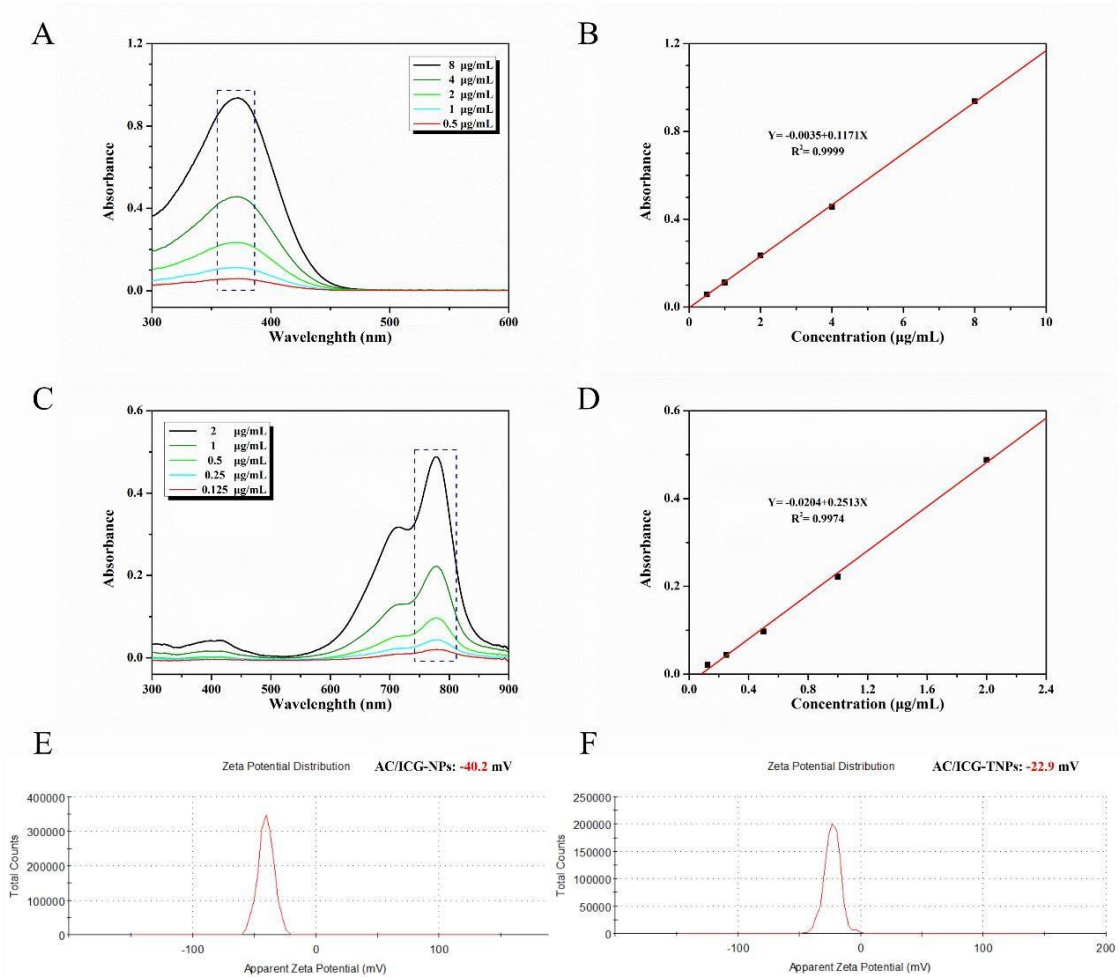

Figure S10. UV-vis spectroscopy of AC (A) and ICG (C) in methanol at different concentrations. Calibration curves of AC (B) and ICG (D) detected by UV-vis spectrometer. Zeta potential of AC/ICG-NPs (E) and AC/ICG-TNPs (F).

Reference:

1. Q. Sun, J. Li, W. N. Liu, Q. J. Dong, W. C. Yang and G. F. Yang, *Analytical chemistry*, 2013, **85**,

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3. C. You, H. Wu, M. Wang, Y. Zhang, J. Wang, Y. Luo, L. Zhai, B. Sun, X. Zhang and J. Zhu, *Chemistry*, 2017, **23**, 5352-5360.
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6. H. Wu, H. Shi, H. Zhang, X. Wang, Y. Yang, C. Yu, C. Hao, J. Du, H. Hu and S. Yang, *Biomaterials*, 2014, **35**, 5369-5380.