Supporting Information

Highly Stable Near Infrared Dye Conjugated Cerasomes for Fluorescence Imaging-Guided Synergistic Chemo-Photothermal Therapy of Colorectal Cancer

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1. Synthesis Protocol

Protocol Synthetic route map will show on Figure S2B-C.

（1）synthesis of Z1

To a 50ml flask equipped with reflux condenser were added acetic acid(3ml),3-methyl-2-butanone(1.68ml,1.6mol), and phenylhydrazine (0.57g,0.519ml). The mixture was heated to reflux for 3h to get a dark yellow oil. Then washed with K_2CO_3, diluted with DCM, and concentrated for future use. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.99 – 7.91 (m, 1H), 7.48 – 7.40 (m, 1H), 7.33 – 7.24 (m, 2H), 2.32 (t, J = 7.1 Hz, 2H), 1.56 – 1.46 (m, 2H), 1.29 (d, J = 4.9 Hz, 12H), 0.90 (s, 3H)。MS （$C_{11}H_{13}N$）: 159.23, Actual result[M]$^+$: 159.2.

（2）synthesis of Z3

The 2,3,3-trimeth-ylindoleninium (637mg,0.004mol) and Iodoethane (779mg,0.005mol) were mixed in 1,2-dichlorobenzene(6ml) and heated at 110℃ for 12h under nitrogen. The mixture was cooled, and the solid was triturated with acetone to get a flesh-color powder without further purification. The powder was defined as **Z3**. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.99 – 7.91 (m, 1H), 7.48 – 7.40 (m, 1H), 7.33 – 7.24 (m, 2H), 1.40 (q, J = 8.0 Hz, 2H), 1.29 (s, 6H), 0.90 (s, 5H), 0.90 (d, J = 15.9 Hz, 2H)。MS （$C_{13}H_{18}IN$）: 315.20, Actual result[M]+: 314.09, [M-I]$^+$: 188.05

（3）synthesis of Z2

The 2,3,3-trimeth-ylindoleninium-5-sulfonate(637mg,0.004mol) and 6-bromohexanoic acid(0.98g) were mixed in 1,2-dichlorobenzene(6ml) and heated at 110℃ for 12h under nitrogen. Then the mixture was cooled, and moved away the upper liquid, diluted the solid with DCM, then triturated with Ethyl acetate to get a pink powder without further purification. The powder was defined as **Z2**. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.99 – 7.91 (m, 1H), 7.48 – 7.40 (m, 1H), 7.33 – 7.24 (m, 2H), 2.32 (t, J = 7.1 Hz, 2H), 1.56 – 1.46 (m, 2H), 1.29 (d, J = 4.9 Hz, 12H), 0.90 (s, 3H)。MS （$C_{17}H_{24}BrNO_2$）: 353.10, Actual result[M]+: 352.14, [M-Br]$^+$: 274.21

（4）synthesis of Z4

A mixture of acetic acid(5ml) and acetic anhydride(5ml) and N-[5-(phenylamino)-2,4-pentadienylidene] aniline monohydrochloride(1mmol) was stirred under

nitrogen, and slowly added Z2, then heated to reflux(120℃) for 4h,cooled down to 25℃, and agitated a mixture by addition of ether(15ml),removed the liquid, then dissolved by methanol and removed the solvent under reduced pressure to get the crude product, defined as **Z4**. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.99 – 7.91 (m, 1H), 7.59 – 7.52 (m, 3H), 7.50 – 7.40 (m, 3H), 7.39 – 7.24 (m, 3H), 6.16 (d, J = 17.9 Hz, 3H), 5.30 (d, J = 15.0 Hz, 1H), 5.16 (dd, J = 15.1, 1.0 Hz, 1H), 2.32 (t, J = 7.1 Hz, 2H), 2.00 (s, 3H), 1.51 (t, J = 7.0 Hz, 2H), 1.36 – 1.27 (m, 12H).MS（$C_{30}H_{35}BrN_2O_3$）: 551.52, Actual result[M-Br]$^{+}$: 471.30

（5）synthesis of Z5

The crude product of Z4 was dissolved in 10ml trimethylamine together with 10ml Ethyl, then slowly added Z3, waited until the smoke cleared, the mixture was stirred for 3h at room temperature, then the solvent was removed under pressure, and the crude product was added ether overnight, then purified by silica gel column chromatography with DCM/methanol=10:1 to afford the desired product, defined as **Z5**. The product's Rf was approximately 0.2, and mass spectrum result was M=523. ^{1}H-NMR (400 MHz, Chloroform-d) δ 8.04 (dd, J = 7.5, 1.5 Hz, 1H), 7.27 (tdd, J = 12.5, 7.4, 1.6 Hz, 3H), 7.11 (tdd, J = 7.5, 3.8, 1.6 Hz, 2H), 6.91 (td, J = 7.5, 1.5 Hz, 1H), 6.03 – 5.84 (m, 3H), 5.41 (dt, J = 15.2, 6.2 Hz, 1H), 5.10 (d, J = 15.0 Hz, 1H), 2.32 (t, J = 5.2 Hz, 2H), 2.03 – 1.97 (m, 2H), 1.51 (t, J = 5.2 Hz, 2H), 1.39 (td, J = 8.2, 1.8 Hz, 2H), 1.35 – 1.22 (m, 21H)。MS（$C_{35}H_{44}BrIN_2O_2$）: 731.56, Actual result[M-Br-I]$^{+}$: 523.33. After this step of purification, we can also obtained a product with similarity property of Z5, while the mass spectrum result was M=437, defined as 437.After analysis, we determined that the 437's structure was like what in **Figure S2A**.So we can purify these two compounds separately for future use.

（6）synthesis of Z6

Glycerol monostearate (1g, 0.0028mol) and 4,4'-Dimethoxytrityl chloride (1.417g, 0.0042mol) were mixed in anhydrous pyridine, then stirred and heated at 50℃ for 18h .Then the mixture was cooled, poured into the ice-water mixture, extracted with chloroform for three times, removed the insoluble residue, triple-time washed with DI-water, dryed with anhydrous sodium sulfate, after the filtration, the solvent was removed under pressure, then purified by silica gel column chromatography with EtOAc/Hex=2:1 to afford the desired product, defined as **Z6**. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.46 (dq, J = 5.7, 2.0 Hz, 2H), 7.31 (tt, J = 7.9, 2.0 Hz, 7H), 6.88 – 6.82 (m, 4H), 4.25 (dd, J = 12.3, 7.0 Hz, 1H), 4.15 (pd, J = 7.0, 4.9 Hz, 1H), 3.80 (s, 6H), 3.50 (dd, J = 12.3, 6.9 Hz, 1H), 3.42 (dd, J = 12.4, 6.9 Hz, 1H), 2.97 (d, J = 4.9 Hz, 1H), 2.35 (t, J = 5.5 Hz, 2H), 1.63 (tt, J = 7.8, 5.4 Hz, 2H), 1.36 – 1.21 (m, 10H), 1.26 (s, 22H), 0.89 (s, 1H), 0.93 – 0.85 (m, 2H)。MS（$C_{40}H_{62}O_6$）: 660.94, Actual result[M]$^{+}$: 655.33

（7）synthesis of Z7

A mixture with Z5, Z6, DMAP and EDC at the equivalent ratio of 1:1.1:0.4:2 was dissolved in 10ml chloroform, then stirred for 24h at room temperature, then purified by silica gel column chromatography with DCM/methanol=15:1 to afford the desired product, defined as **Z7**. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.49 – 7.43 (m, 2H), 7.41 (ddd, J = 6.6, 4.7, 1.4 Hz, 2H), 7.35 – 7.27 (m, 8H), 7.28 – 7.16 (m, 2H), 7.11 (dtd, J = 13.4, 7.5, 1.5 Hz, 2H), 6.92 (td, J = 7.5, 1.5 Hz, 1H), 6.88 – 6.82

(m, 4H), 6.00 (d, J = 15.0 Hz, 1H), 5.86 – 5.77 (m, 2H), 5.62 – 5.52 (m, 2H), 5.44 (d, J = 15.1 Hz, 1H), 4.75 – 4.62 (m, 2H), 4.29 (p, J = 7.1 Hz, 1H), 4.08 – 3.89 (m, 2H), 3.80 (s, 6H), 3.63 – 3.47 (m, 4H), 3.16 – 3.04 (m, 2H), 2.42 – 2.23 (m, 4H), 2.01 – 1.80 (m, 3H), 1.76 – 1.57 (m, 3H), 1.61 – 1.53 (m, 2H), 1.51 (dddd, J = 25.0, 13.2, 4.4, 2.3 Hz, 4H), 1.48 – 1.36 (m, 2H), 1.39 – 1.25 (m, 16H), 1.24 (s, 3H), 1.14 – 0.99 (m, 2H), 0.89 (t, J = 7.8 Hz, 3H)。MS（$C_{77}H_{102}BrIN_2O_7$）：1374.48，Actual result[M-Br-I]$^+$：1168.56

（8）synthesis of Z8

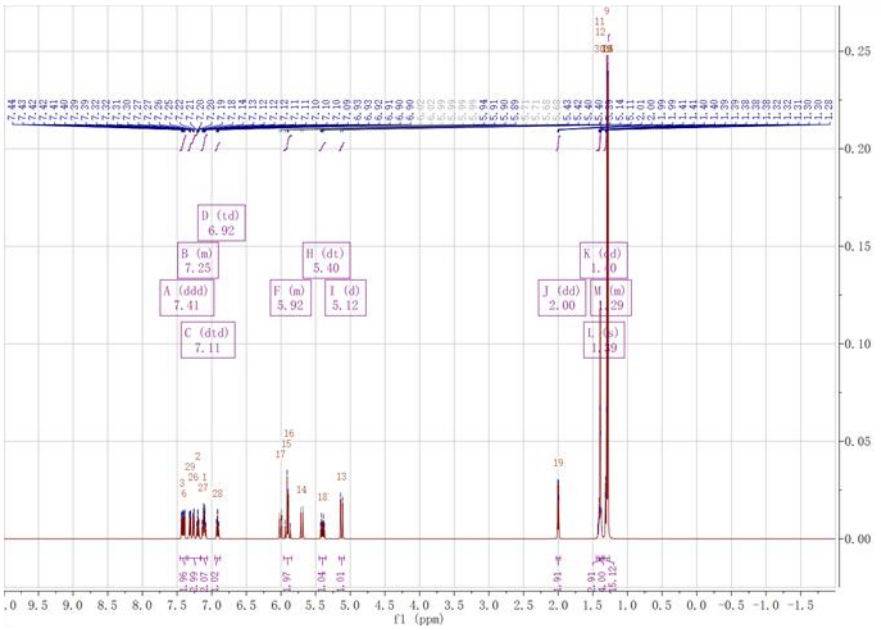
An equivalent Z7 was dissolved in 10ml DCM, then slowly added trifluoroacetic acid with 25 equivalent, then the solvent was turned to light yellow immediately. Stirred the solvent for 3h at room temperature, then removed DCM and TFA under pressure to get a dark blue solid, defined as **Z8**. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.81 (dd, J = 7.4, 1.5 Hz, 1H), 7.39 (dd, J = 7.4, 1.6 Hz, 1H), 7.28 (ddd, J = 15.7, 7.4, 1.5 Hz, 2H), 7.12 (dddd, J = 30.6, 15.0, 7.5, 1.6 Hz, 3H), 6.91 (td, J = 7.4, 1.6 Hz, 1H), 6.01 (dt, J = 15.3, 1.4 Hz, 1H), 5.90 (q, J = 15.1 Hz, 2H), 5.69 (dd, J = 15.0, 0.9 Hz, 1H), 5.39 (ddd, J = 15.2, 9.9, 2.9 Hz, 1H), 5.12 (d, J = 15.0 Hz, 1H), 4.44 (p, J = 7.0 Hz, 1H), 4.29 (tdd, J = 12.0, 6.1, 3.6 Hz, 3H), 4.04 (ddt, J = 12.5, 7.1, 2.7 Hz, 2H), 3.75 (dq, J = 12.3, 7.9 Hz, 1H), 3.63 (td, J = 12.4, 2.7 Hz, 1H), 3.48 (dq, J = 12.4, 8.0 Hz, 1H), 2.94 – 2.86 (m, 1H), 2.69 – 2.60 (m, 1H), 2.52 – 2.41 (m, 2H), 2.14 (dddd, J = 13.1, 10.6, 8.4, 3.4 Hz, 2H), 2.02 (qd, J = 12.5, 12.0, 1.8 Hz, 1H), 1.87 (tdd, J = 12.3, 9.4, 2.7 Hz, 1H), 1.74 – 1.55 (m, 4H), 1.56 – 1.38 (m, 4H), 1.42 – 1.30 (m, 1H), 1.33 (s, 1H), 1.32 (s, 3H), 1.34 – 1.26 (m, 4H), 1.25 (d, J = 29.5 Hz, 11H), 1.21 – 1.08 (m, 4H), 1.12 – 0.94 (m, 4H), 0.89 (t, J = 7.9 Hz, 3H), 0.76 (qdd, J = 12.6, 3.5, 1.8 Hz, 1H)。MS（$C_{56}H_{84}BrIN_2O_5$）：1072.11，Actual result [M-Br-I]$^+$：863.91

（9）synthesis of Z9

A mixture with Z8, succinic anhydride and DMAP at the equivalent ratio of 1:5:5 was added into a moderate amount of chloroform, stirred the solvent for 24h at RT, then washed with ammonium chloride aqueous solution, extracted by chloroform, after the filtration, the solvent was removed under pressure. Finally, the product was purified by silica gel column chromatography with DCM/methanol=15:1 to afford the desired product, defined as **Z9**, which was Glycerol @ heptamethine cyanine dyes, abbreviated as Gly@Cy7. ^{1}H-NMR (400 MHz, Chloroform-d) δ 7.40 (dd, J = 7.5, 1.5 Hz, 1H), 7.29 (ddd, J = 23.8, 7.5, 1.5 Hz, 3H), 7.14 (dtd, J = 28.0, 7.4, 1.5 Hz, 2H), 6.91 (td, J = 7.4, 1.5 Hz, 1H), 5.80 – 5.66 (m, 2H), 5.61 (dd, J = 15.0, 1.0 Hz, 1H), 5.21 (ddd, J = 15.0, 9.9, 1.1 Hz, 1H), 5.12 (d, J = 15.0 Hz, 1H), 4.68 (dd, J = 12.4, 6.9 Hz, 1H), 4.54 (dd, J = 12.4, 7.0 Hz, 1H), 4.40 (p, J = 7.0 Hz, 1H), 3.99 (dq, J = 12.4, 8.0 Hz, 1H), 3.69 (ddd, J = 37.7, 12.3, 6.9 Hz, 2H), 3.53 – 3.39 (m, 2H), 3.39 – 3.25 (m, 2H), 2.85 (ddd, J = 12.3, 2.3, 1.1 Hz, 1H), 2.75 (ddd, J = 12.2, 3.6, 2.0 Hz, 1H), 2.62 (ddd, J = 12.2, 4.0, 1.9 Hz, 1H), 2.52 – 2.37 (m, 3H), 2.35 – 2.06 (m, 4H), 2.05 – 1.92 (m, 1H), 1.67 – 1.59 (m, 2H), 1.51 – 1.26 (m, 6H), 1.31 (s, 4H), 1.29 – 1.15 (m, 15H), 1.18 – 0.96 (m, 2H), 1.08 (s, 3H), 0.94 – 0.84 (m, 2H), 0.89 (s, 2H)。MS（$C_{56}H_{84}BrIN_2O_5$）：1170.18，Actual result[M-Br-I]$^+$：963.25.

Figure S1

A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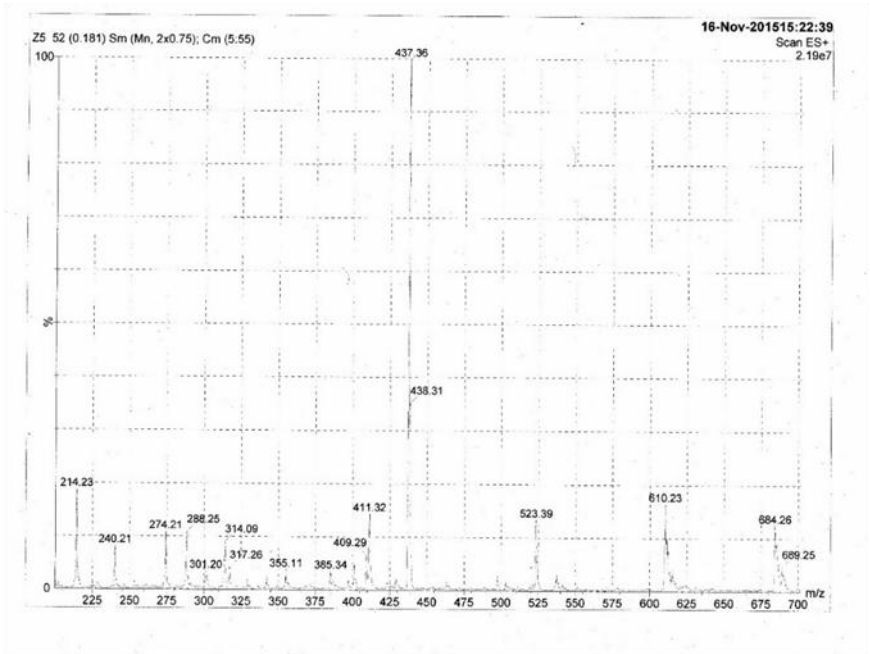
B (m)
7.25
A (ddd)
7.41
C (dtd)
7.11
H (dt)
5.40
F (m)
5.92
I (d)
5.12
J (dd)
2.00
M (m)
1.29
f1 (ppm)

B


Z5 52 (0.181) Sm (Mn, 2x0.75); Cm (5.55)
16-Nov-201515:22:39
Scan ES+
2.19e7
100
%
0
437.36
438.31
214.23
240.21
274.21
288.25
301.20
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411.32
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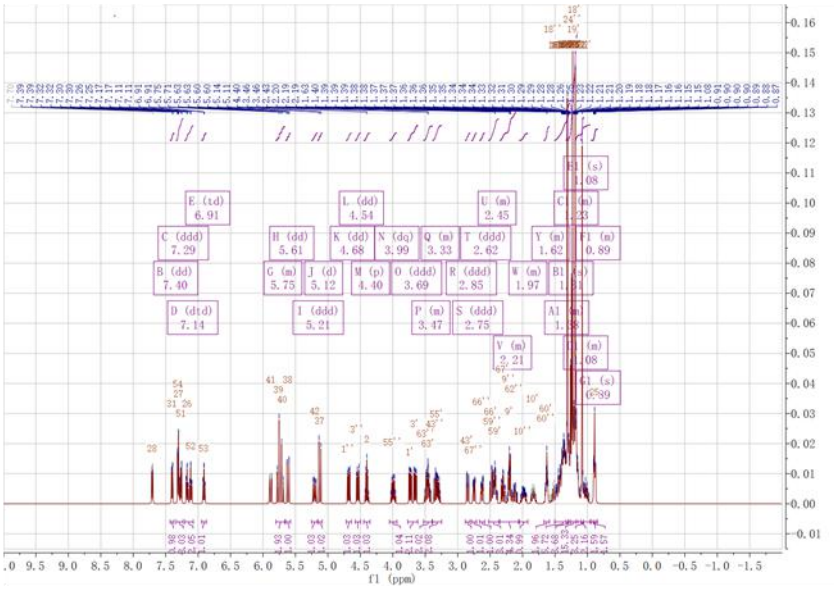
684.26
689.25
m/z

C



D

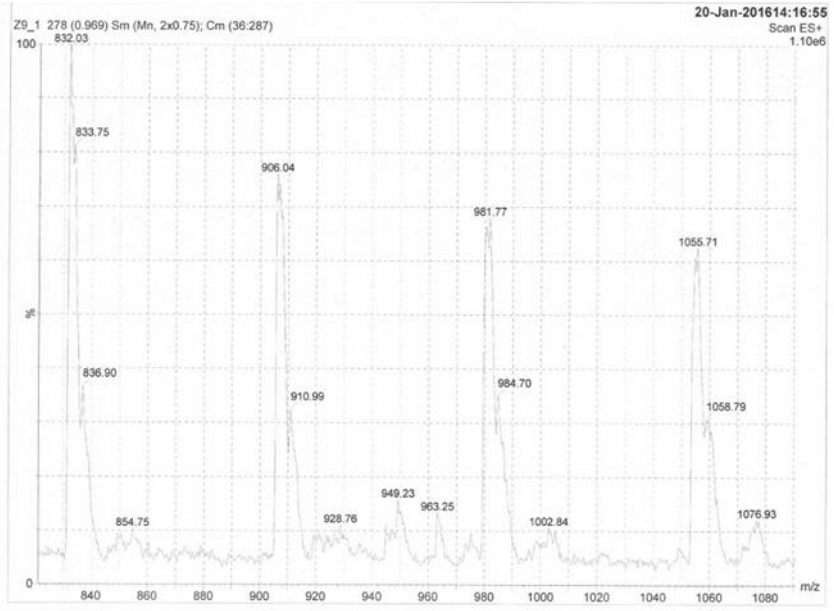


Figure S1: Mass spectrum and ^{1}H-NMR result of 437 and Gly@Cy7 (A) ^{1}H-NMR result of 437 (B) Mass spectrum result of 437 (C) ^{1}H-NMR result of Gly@Cy7 (D) Mass spectrum result of Gly@Cy7.

Figure S2

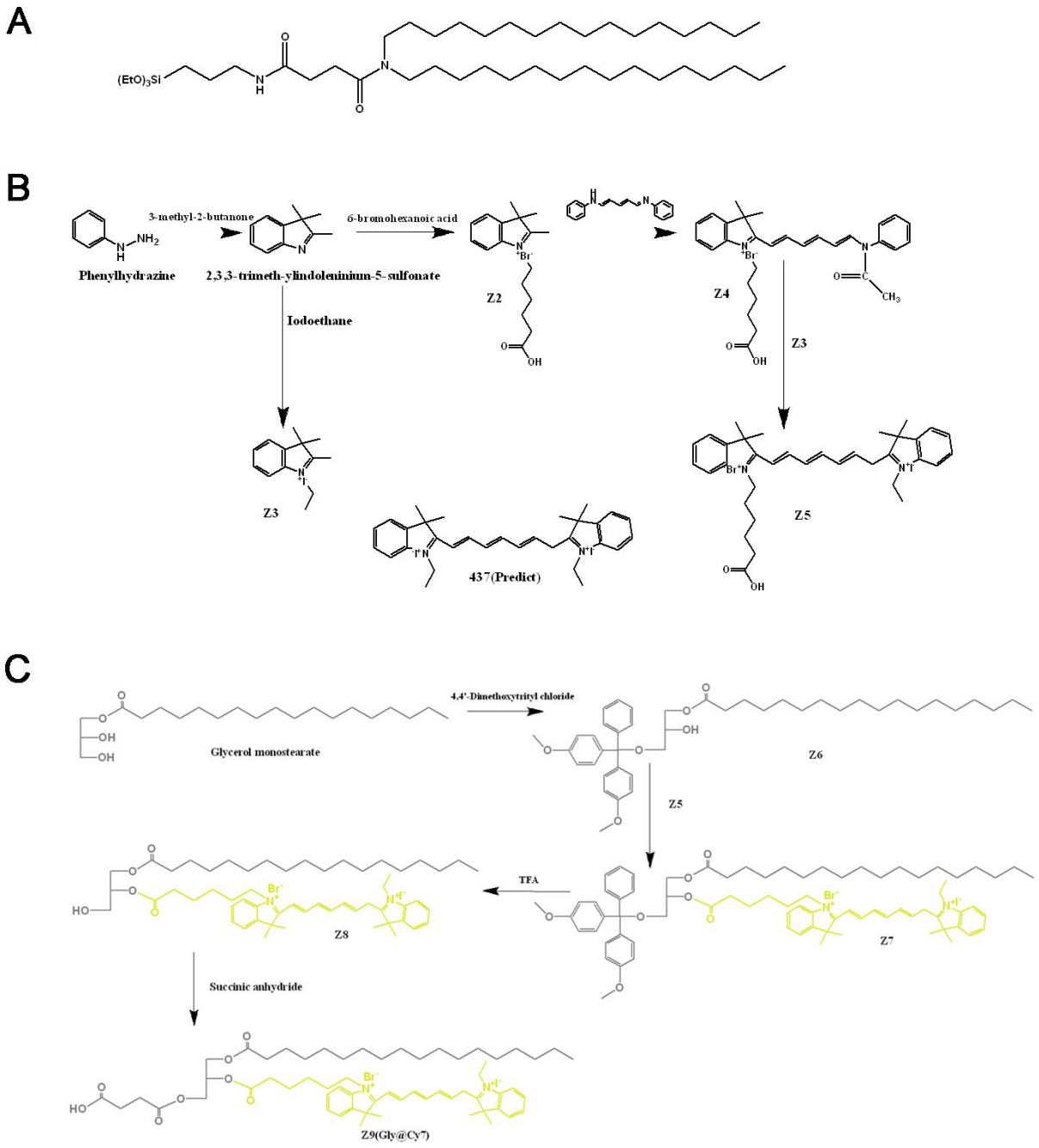


Figure S2: Synthetic scheme. (A) Structure of Cerasome forming lipid (B) Synthetic scheme of Cy7 and 437 (C) Synthetic scheme of Gly@Cy7.

Figure S3

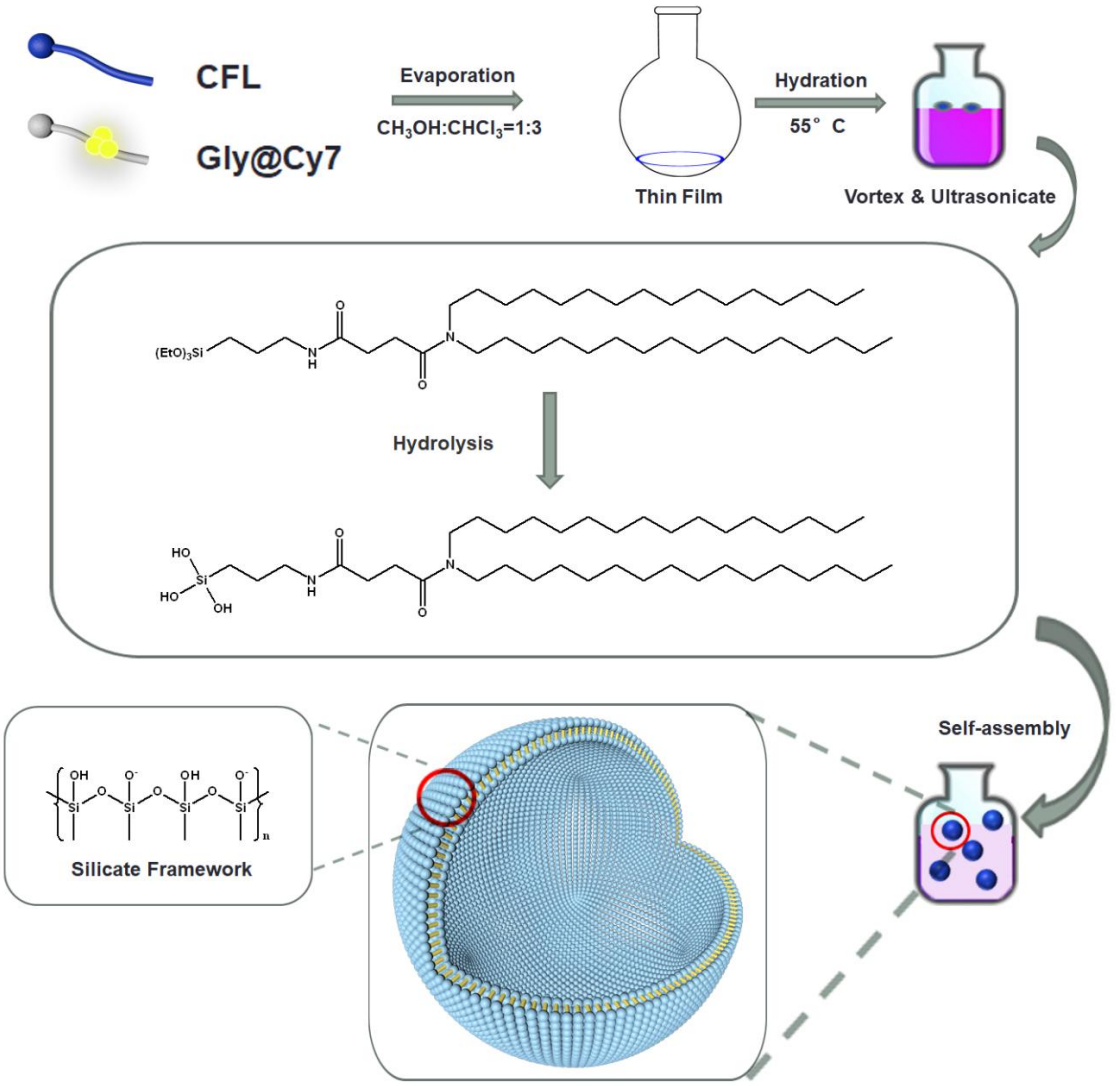


Figure S3: Schematic diagram of the synthesis of Gly@Cy7-Si nanoparticles.

Figure S4

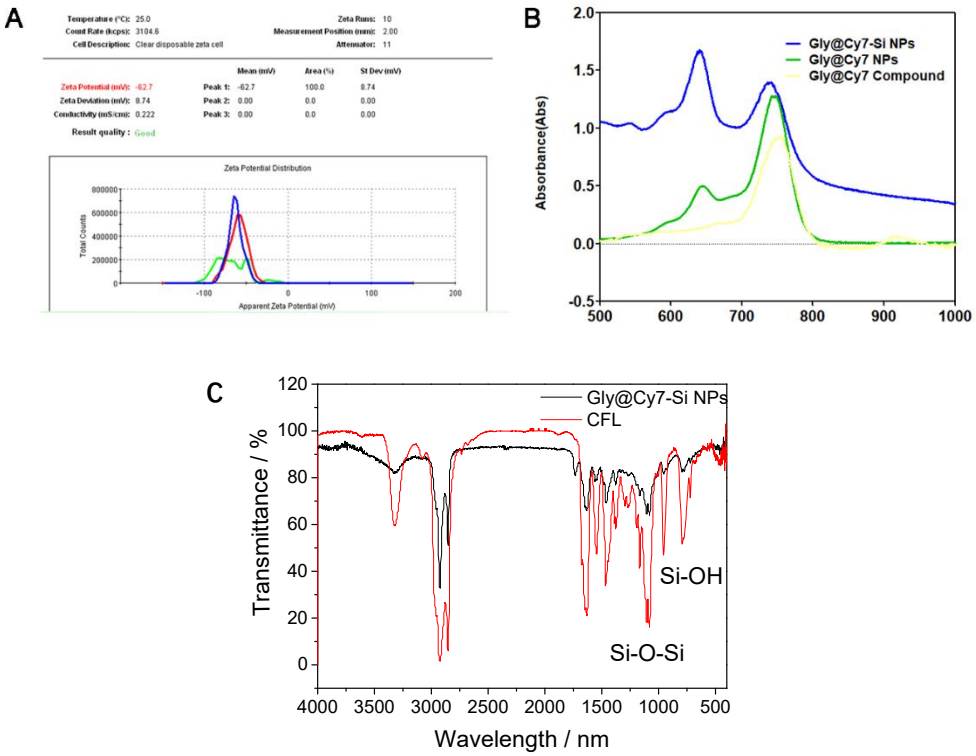


Figure S4: (A) Zeta-Potential of Gly@Cy7-Si NPs (B) UV-VIS spectra of Gly@Cy7 NPs, Gly@Cy7-Si NPs and Gly@Cy7 compound. (C) Fourier infrared spectroscopy of Gly@Cy7-Si NPs and CFL.

Figure S5

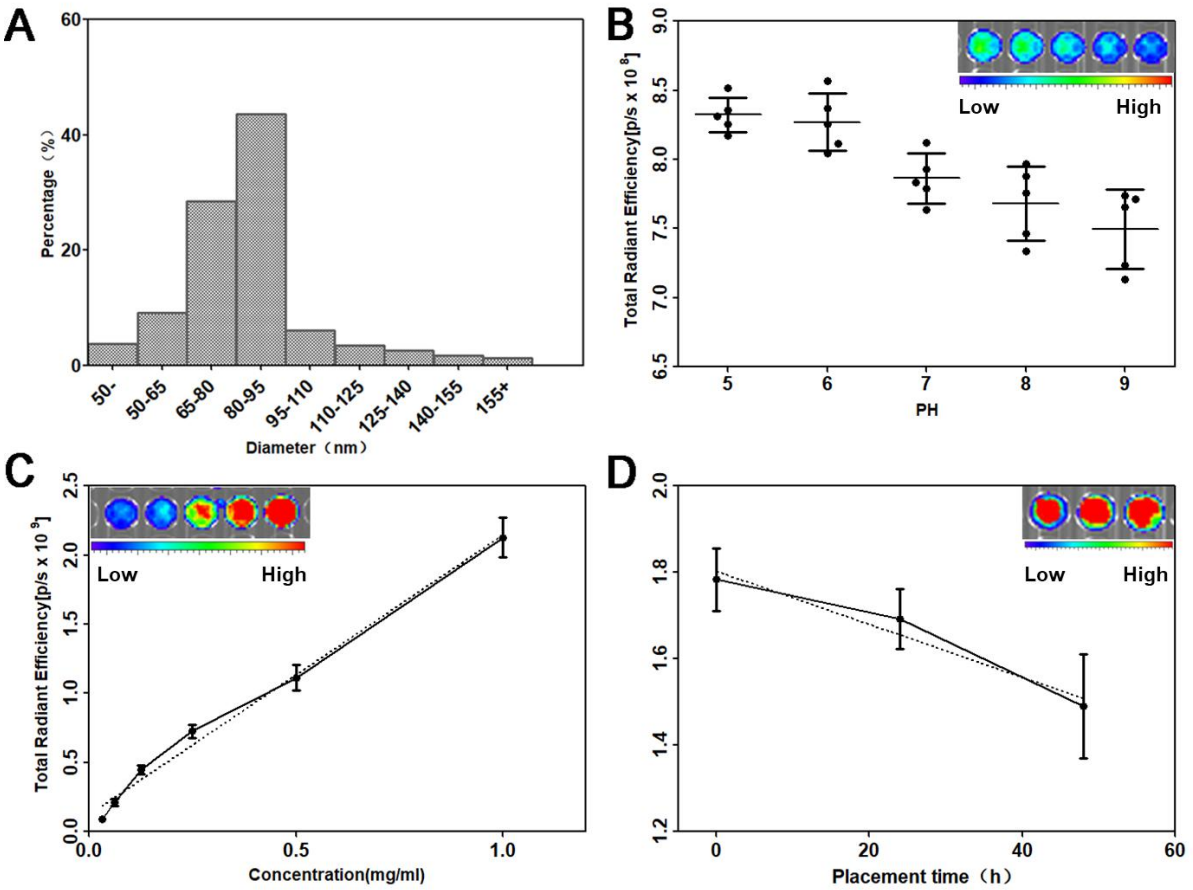


Figure S5: (A) Particle size distribution of Gly@Cy7-Si-DOX (B) Radiant efficiency under different PH (C) Radiant efficiency under different concentration (D) Radiant efficiency placed after different hours.

Figure S6

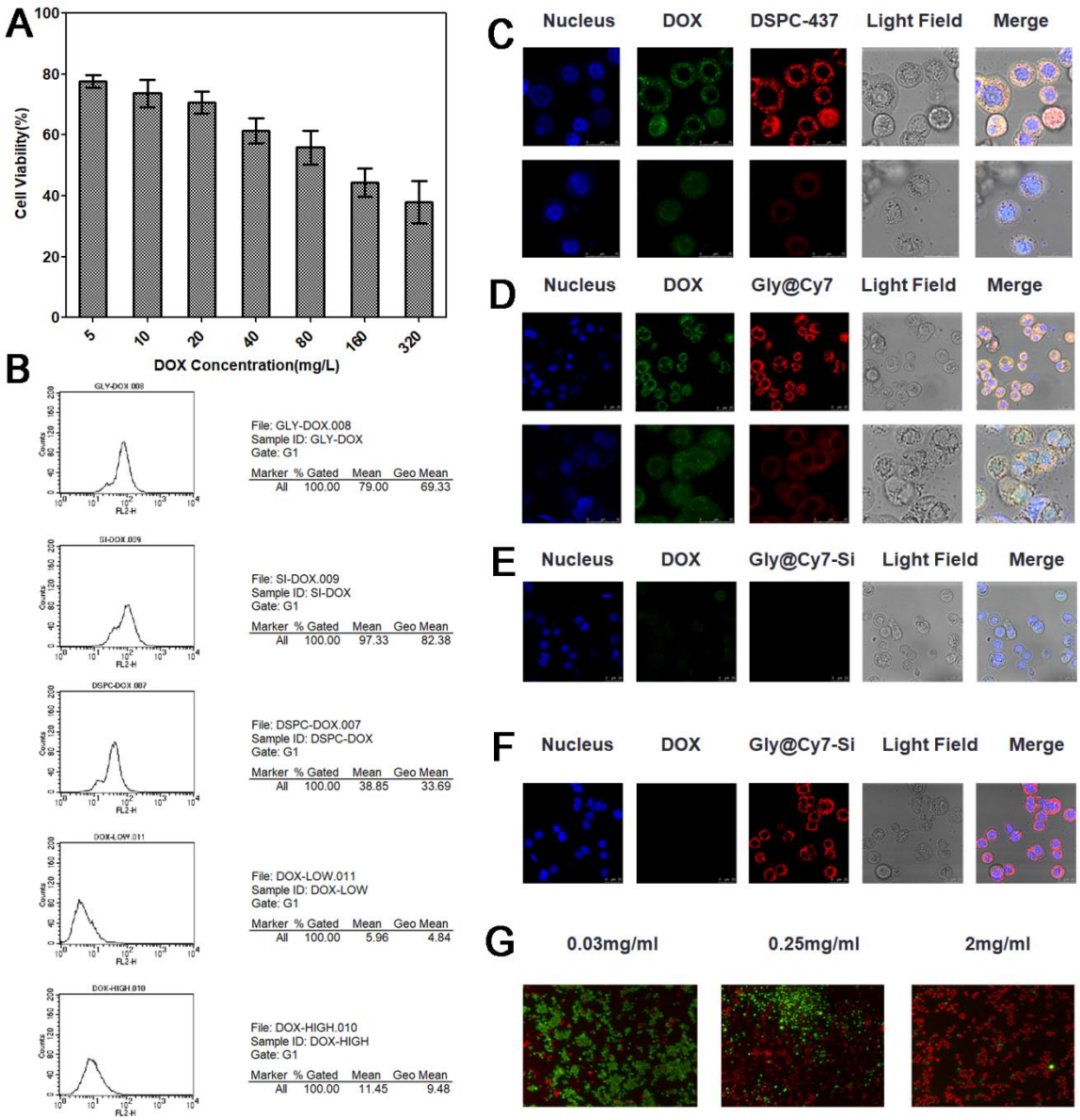


Figure S6:(A) In vitro cell therapy result via doxorubicin only (B) FACS analysis detailed data for the efficiency of HT-29 cell uptakes of various NPs(C)(D)(E)(F) In vitro cellular uptake before and after treatment by HT-29 cells of Gly@Cy7-DOX NPs, DSPC-437-DOX NPs, DOX only and Gly@Cy7-Si NPs only was effectively internalized into HT-29 cells. NPs showed red color (ex/em=640/700 nm), intracellular location of NPs could be clearly observed and the DAPI blue color was used for staining cell nuclei. (G) Gly@Cy7-Si NPs mediated photothermal killing effect to HT-29 cells in different concentration (Calcein-AM and PI staining): with laser irradiation for 10 min (NIR laser: 808 nm, 1.5 W cm^{-2}).

Figure S7

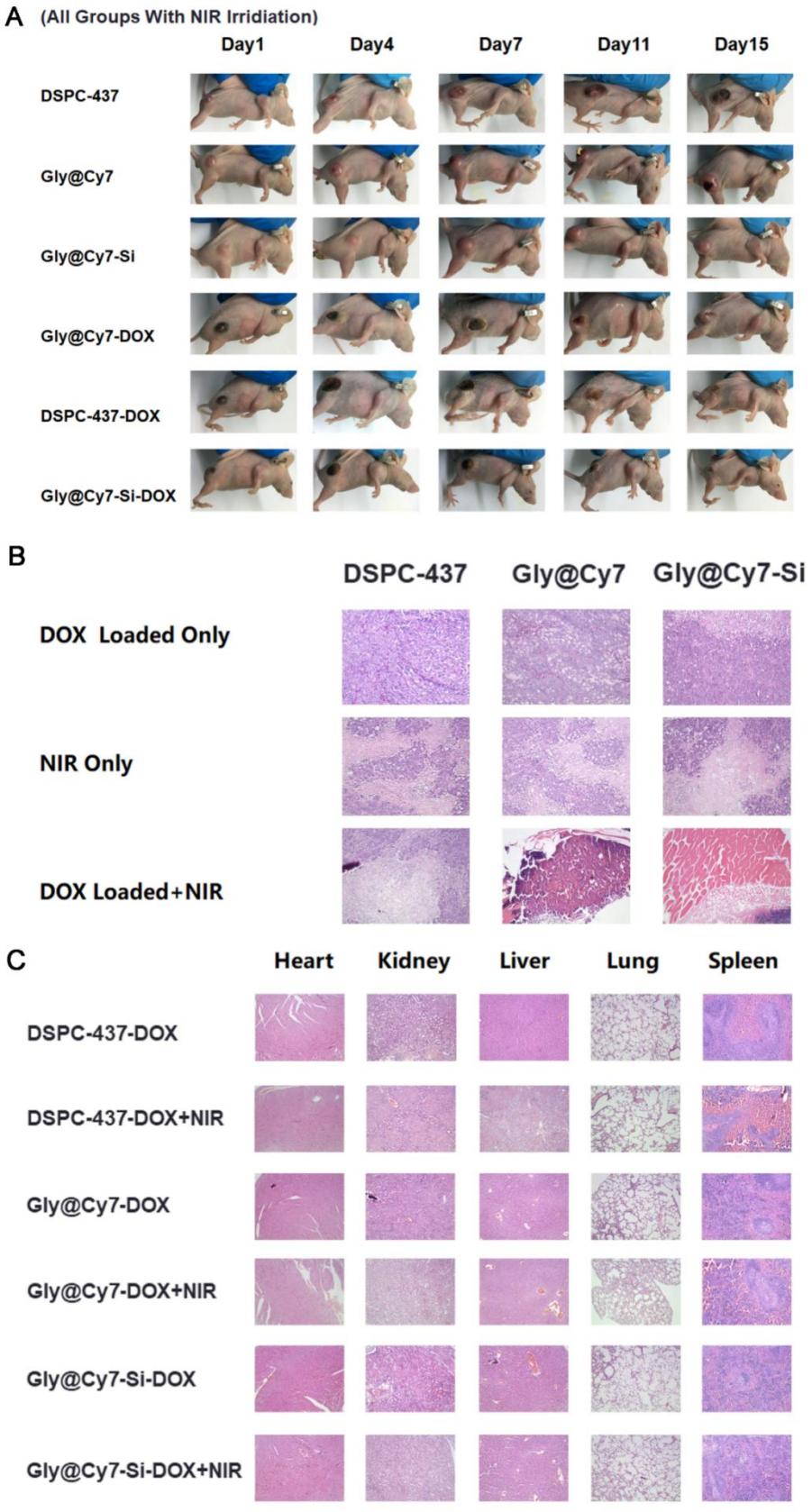


Figure S7: (A) Representative photographs of tumors bearing mice with time before and after

different treatments.(B)H&E stained slices of tumor with different treatment. (C)Histology staining of tissue slices (heart, liver, spleen, lung and kidney) collected from mice of different groups at day 15 after treatment.

Table S1

Day1

	Control	Gly@Cy7-Si	Gly@Cy7-Si-DOX
WBC(10^9/L)	6.28±0.73	4.58±0.63（↓）	4.54±1.24（↓）
RBC(10^12/L)	9.26±0.78	9.12±1.18	9.25±1.13
HGB(g/L)	152.0±13.4	148.8±21.6	152.6±15.2
HCT%	40.86±3.61	40.46±5.87	41.86±3.94
MCV(fL)	44.16±1.21	44.22±1.11	45.36±2.41
MCH(pg)	16.40±0.51	16.24±0.51	16.52±0.95
MCHC(g/L)	372.0±4.2	367.8±6.1	358.8±13.3
RDW%	20.20±3.68	19.06±2.81	18.02±3.08
PLT(10^9/L)	502.8±70.3	470.0±111.9	530.8±57.9
PCT%	0.184±0.026	0.184±0.049	0.204±0.025

	Control	Gly@Cy7-Si	Gly@Cy7-Si-DOX
MPV(fL)	3.76±0.15	4.00±0.12	3.92±0.23
PDW%	11.64±0.68	12.24±0.38	12.20±1.36
LYM(10^9/L)	4.16±0.36	2.94±0.63（↓）	2.96±0.87（↓）
LYM%	66.58±2.68	63.44±6.76	65.40±4.06
MID(10^9/L)	1.04±0.48	0.74±0.15（↓）	0.66±0.13（↓）
MID%	16.28±6.55	16.48±3.04	14.82±2.15
GRN(10^9/L)	1.08±0.42	0.90±0.12	0.92±0.30
GRN%	17.14±6.27	20.08±3.93	19.78±2.88
EOS(10^9/L)	<0.7	<0.7	<0.7

Day4

	Control	Gly@Cy7-Si	Gly@Cy7-Si-DOX
WBC(10^9/L)	6.20±1.19	6.83±0.94	4.12±0.90
RBC(10^12/L)	9.57±0.59	8.64±0.91	8.55±1.92
HGB(g/L)	158.0±11.8	146.3±16.6	142.4±31.4
HCT%	41.92±2.81	38.28±4.03	38.66±8.47
MCV(fL)	43.70±0.74	44.30±0.78	45.22±1.13
MCH(pg)	16.46±0.38	16.93±0.71	16.66±0.36
MCHC(g/L)	376.8±5.2	382.0±9.5	368.2±7.9
RDW%	18.68±1.19	19.50±1.47	18.46±1.02
PLT(10^9/L)	527.2±36.0	483.8±85.1	489.0±162.2
PCT%	0.194±0.015	0.170±0.033	0.190±0.067

	Control	Gly@Cy7-Si	Gly@Cy7-Si-DOX
MPV(fL)	3.76±0.17	3.58±0.21	4.00±0.19
PDW%	11.82±1.19	11.58±0.45	12.32±0.88
LYM(10^9/L)	4.58±1.18	4.85±0.75	2.92±0.64
LYM%	74.00±6.03	71.13±1.67	70.80±5.51
MID(10^9/L)	1.08±0.31	1.30±0.14	0.92±0.33
MID%	17.25±3.62	19.40±1.72	21.26±5.92
GRN(10^9/L)	0.53±0.17	0.68±0.10	0.28±0.08
GRN%	8.75±3.26	9.48±1.03	7.94±2.59
EOS(10^9/L)	<0.7	<0.7	<0.7

Day7

	Control	Gly@Cy7-Si	Gly@Cy7-Si-DOX
WBC(10^9/L)	8.18±2.09	6.18±1.88	6.26±2.19
RBC(10^12/L)	9.91±0.83	9.92±0.22	9.09±1.44
HGB(g/L)	158.8±14.8	160.6±6.2	142.6±23.8
HCT%	44.54±4.95	45.02±1.53	40.46±6.83
MCV(fL)	44.84±1.25	45.32±0.52	44.34±0.54
MCH(pg)	16.00±0.49	16.16±0.38	15.64±0.35
MCHC(g/L)	357.4±12.6	356.8±12.0	352.6±8.3
RDW%	18.42±1.68	17.50±0.81	18.28±1.82
PLT(10^9/L)	368.8±25.4	378.2±24.1	335.0±47.7
PCT%	0.123±0.010	0.128±0.008	0.110±0.016

	Control	Gly@Cy7-Si	Gly@Cy7-Si-DOX
MPV(fL)	3.50±0.23	3.52±0.11	3.42±0.08
PDW%	12.04±0.96	12.38±0.30	11.98±0.54
LYM(10^9/L)	6.02±1.73	4.24±1.53	4.52±1.50
LYM%	72.80±4.20	67.60±5.36	72.82±2.85
MID(10^9/L)	1.16±0.22	1.12±0.31	1.08±0.36
MID%	14.16±1.90	19.08±6.05	17.00±1.18
GRN(10^9/L)	1.00±0.25	0.82±0.25	0.66±0.39
GRN%	13.04±2.55	13.32±2.59	10.18±3.50
EOS(10^9/L)	<0.7	<0.7	<0.7

Table S1: Routine blood test result collected from mice of different groups at day 1,4,7 after treatment. All data were in the normal range.

Table S2

	Gly@Cy7-Si	DSPC-437	Gly@Cy7
AUC	1117.0±84.2%ID· h	992.6 ± 61.7%ID· h	906.7 ± 64.6%ID· h
T1/2	6.04±0.57h	4.45±0.66 h	4.78±0.95 h

Table S2: Pharmacokinetics profiles of the drug loaded cerasomes, which were monitored by determining the plasma level of DOX content. AUC and $T_{1/2}$ were calculated and mentioned in this table.

Table S3. Zeta potential and particle size of the nanoparticles.

	Gly@Cy7-Si-DOX NPs	Gly@Cy7-DOX NPs	DSPC-437-DOX NPs
Zeta Potential	**-30~-80 mV**	**-10~-20 mV**	**-10~-25 mV**
Particle Size	**101.2±25.8 nm**	**115.2±28.1 nm**	**135.2±39.6 nm**

Table S4. Survival number of different groups after treatment.

Day	PBS+Light	DOX	Gly@Cy7 +Light	DSPC-437+Light	Gly@Cy7-Si+Light	DSPC-437-DOX+Light	DSPC-437-DOX	Gly@Cy7-Si-DOX+Light	Gly@Cy7-Si-DOX	Gly@Cy7-DOX+Light	Gly@Cy7 -DOX
0	7	7	7	7	7	7	7	7	7	7	7
1	7	7	7	7	7	7	7	7	7	7	7
3	7	7	7	7	7	7	7	7	7	7	7
5	6	7	7	7	7	7	7	7	7	7	6
7	6	6	7	7	7	7	6	7	7	7	6
9	5	6	7	7	7	7	6	7	7	6	6
11	4	6	7	6	7	6	5	7	7	6	5
13	3	5	6	6	7	6	5	7	6	6	5
15	3	4	6	6	7	6	5	7	6	5	5