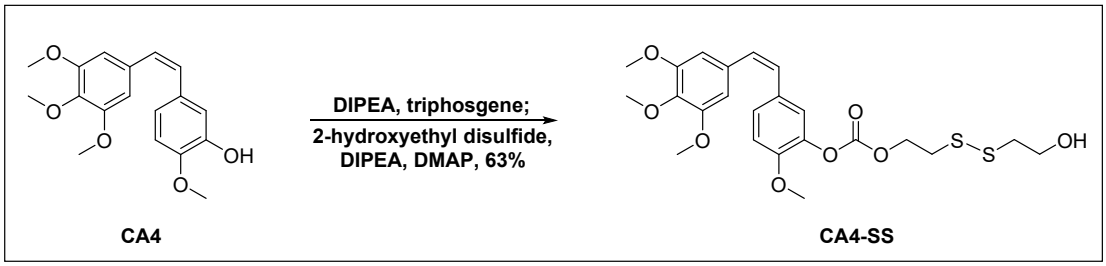
Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Electronic Supporting Information

for

An activatable, carrier-free, triple-combination nanomedicine for ALK/EGFR-mutant non-small cell lung cancer high-permeable targeted chemotherapy

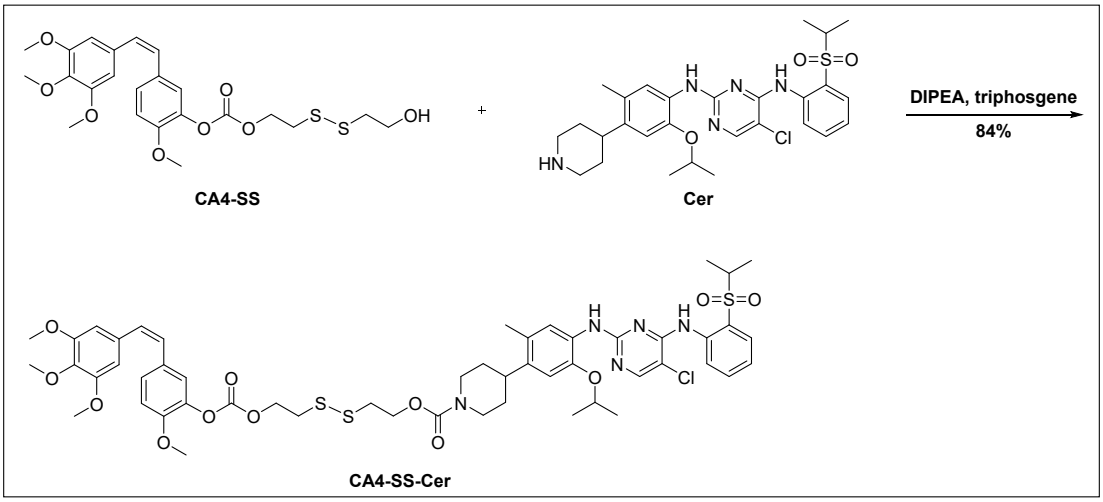
Synthesis of CA4-SS



To a stirred solution of CA4 (63.2 mg, 0.2 mmol, 1.0 equiv) in dry dichloromethane (DCM, 40 mL) was added tiphosgene (BTC, 1.188 g, 4.0 mmol, 20.0 equiv) at 0 °C. A solution of N,N-diisopropylethylamine (DIPEA, 0.99 mL, 6.0 mmol, 30.0 equiv) in dry DCM (6.0 mL) was dropwise added into the CA4 solution at 0 °C for 5 min and then allowed to warm up to room temperature. The reaction was stirred for 3.0 hours at room temperature under N_2 environment until the completion of the reaction. The mixture was concentrated under reduced pressure afforded the crude product (yellow solid). The crude product was directly used for next step.

The residue crude product was diluted with DCM (10 mL), and then dropped into an dry tetrahydrofuran (THF, 20mL) solution, containing triethylamine (0.82 mL, 0.78 mmol), 2-hydroxyethyl disulfide (154.0 mg, 1.0 mmol, 5.0 equiv), and 4-dimethylaminopyridine (DMAP, 14.7 mg, 0.12 mmol, 0.6 equiv) at -10 °C for 10 min and then allowed to warm up to room temperature. The mixture was stirred for another 20.0 hours at room temperature under N_2 environment, and then quenched with water. After evaporated under reduced pressure, and then re-dissolved with DCM, the combined extracts were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography (petroleum ether / ethyl acetate = 1:2) to give CA4 (62.6 mg, 63.1%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 7.15 (dd, J = 2.0, 8.0 Hz, 1H), 7.10 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.49 (s, 2H), 6.44 (s, 2H), 4.47 (t, J = 7.0 Hz, 2H), 3.86 (t, J = 8.0 Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H), 3.71 (s, 6H), 3.03 (t, J = 7.0 Hz, 2H), 2.88 (t, J = 6.0 Hz, 2H), 2.32 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 153.03, 153.00, 150.19, 137.18, 132.38, 130.10, 129.77, 128.36, 128.11, 122.69, 112.21, 105.86, 66.51, 60.92, 60.27, 56.04, 55.94, 41.53, 36.71.

Synthesis of CA4-SS-Cer



To a stirred solution of CA4-SS (52.0 mg, 0.105 mmol, 1.0 equiv) in dry DCM (25 mL) was added tiphosgene (BTC, 622 mg, 2.10 mmol, 20.0 equiv) at 0 °C. The DIPEA (0.52 mL, 3.15 mmol, 30.0 equiv) was dropwise added into the CA4-SS solution at -10 °C for 5 min and then allowed to warm up to room temperature. The reaction was stirred for 4.5 hours at room temperature under N_2 environment until the completion of the reaction. The mixture was concentrated under reduced pressure afforded the crude product (yellow solid).

The residue crude product was diluted with DCM (12 mL), and then dropped into an dry DCM (30mL) solution, containing DIPEA (0.020 mL), and Cer (58.5 mg, 0.105 mmol, 1.0 equiv) at -10 °C for 10 min and then allowed to warm up to room temperature. The mixture was stirred for another 20.0 hours at room temperature under N_2 environment, and then quenched with water. After evaporated under reduced pressure, and then re-dissolved with DCM, the combined extracts were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel column chromatography (petroleum ether / ethyl acetate = 1:1) to give CA4-SS-Cer (95.1 mg, 83.9 %) as white solid. R_f 0.55 (petroleum ether / ethyl acetate = 1:1); ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 9.61 (s, 1H), 8.56 (d, *J* = 8.0 Hz, 1H), 8.13 (s, 1H), 7.96 (s, 1H), 7.93 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.78 (s, 1H), 7.63-7.58 (m, 1H), 7.27 (t, *J* = 8.0 Hz, 1H), 7.14 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.10 (d, *J* = 2.0 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.71 (s, 1H), 6.49 (s, 2H), 6.46 (s, 2H), 4.58-4.50 (m, 1H), 4.47 (t, *J* = 7.0

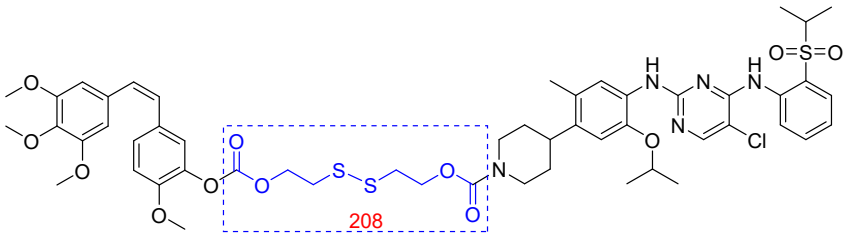
Hz, 1H), 4.44-4.20 (m, 4H), 3.84 (s, 3H), 3.83 (s, 3H), 3.70 (s, 6H), 3.30-3.20 (m, 1H), 3.04-2.97 (m, 4H), 2.95-2.78 (m, 3H), 2.17 (s, 3H), 1.82-1.50 (m, 4H), 1.36 (d, J = 7.0 Hz, 6H), 1.32 (d, J = 6.0 Hz, 6H). HRMS (ESI^+): m/z calcd for $[C_{52}H_{63}ClN_5O_{12}S_3]$ 1080.3324, found at 1080.3332.

Preparation of CA4-SS-Cer NPs and UA@CA4-SS-Cer NPs

The nanoparticles were prepared by the nanoprecipitation method. Briefly, CA4-SS-Cer (0.54 mg) was dissolved in EtOH (1.1 mL) and then added dropwise into deionized water (9.9 mL) under vigorous stirring at room temperature for 30 min. After that, the solution was dialyzed against distilled water to remove EtOH and un-self-assembled molecules.

As for UA@CA4-SS-Cer NPs, the mixture of CA4-SS-Cer (0.54 mg) and UA (0.23 mg) was dissolved in EtOH, and then the remaining procedure was similar to the preparation of UA@CA4-SS-Cer NPs.

Drug loading efficiency (DLE) was calculated using the following formula: DLE (%) = Weight of encapsulated drug/Weight of the whole nanoparticles × 100%.



CA4-SS-Cer Mol. Wt.: 1079

CA4-SS-Cer NPs: DLE (%) = (1079-208)/1079 = 80.7%

UA@CA4-SS-Cer NPs: DLE (%) > 80.7%

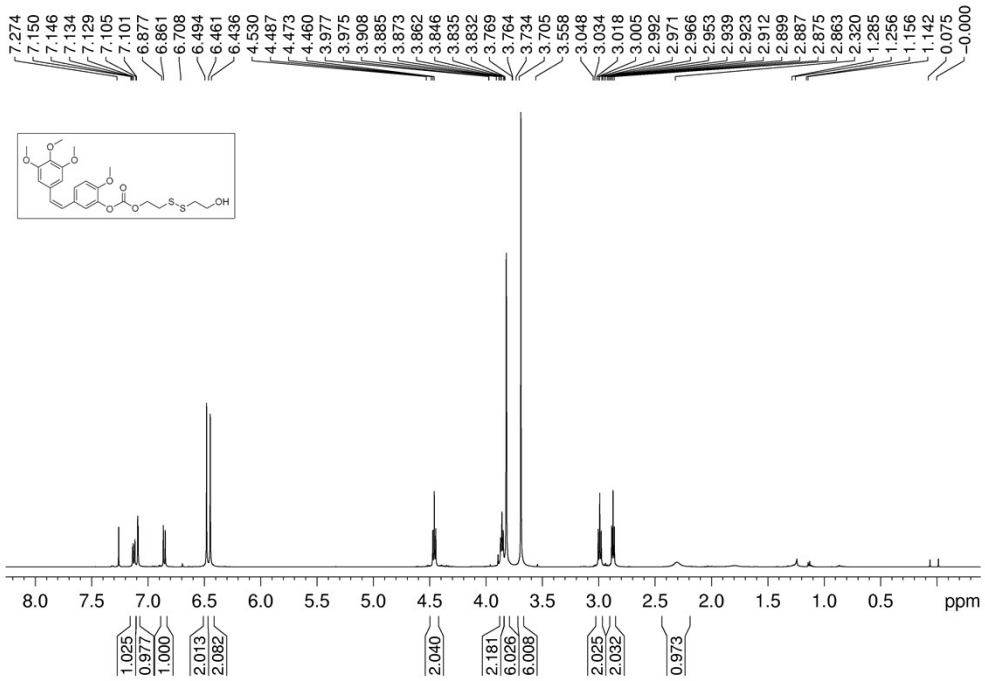


Figure S1 ^{1}H NMR of **CA4-SS** (500 MHz, $CDCl_3$)

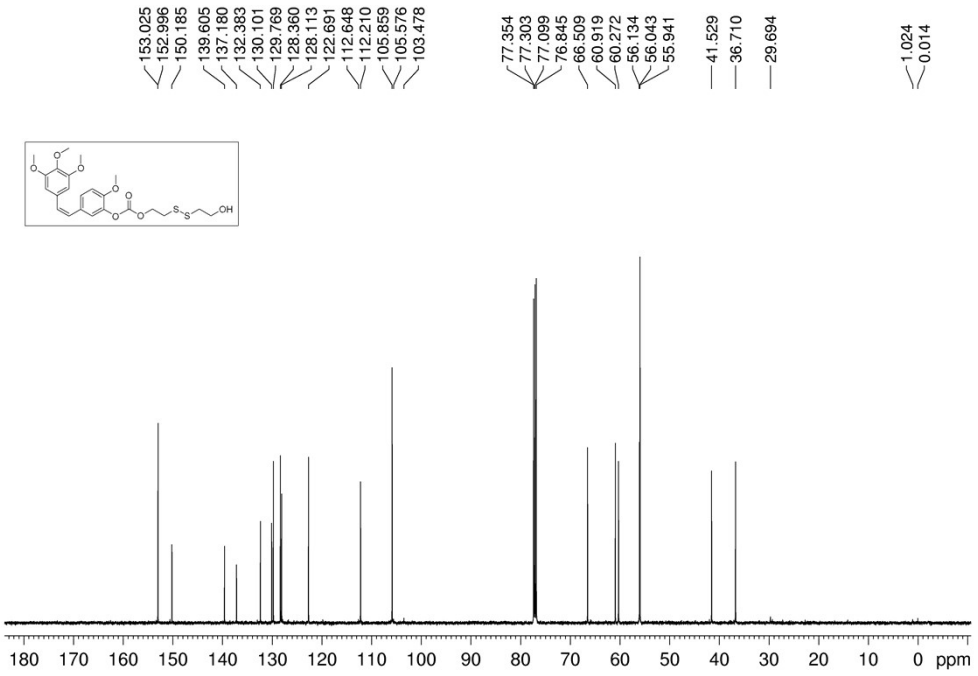


Figure S2 ^{13}C NMR of **CA4-SS** (125 MHz, $CDCl_3$)

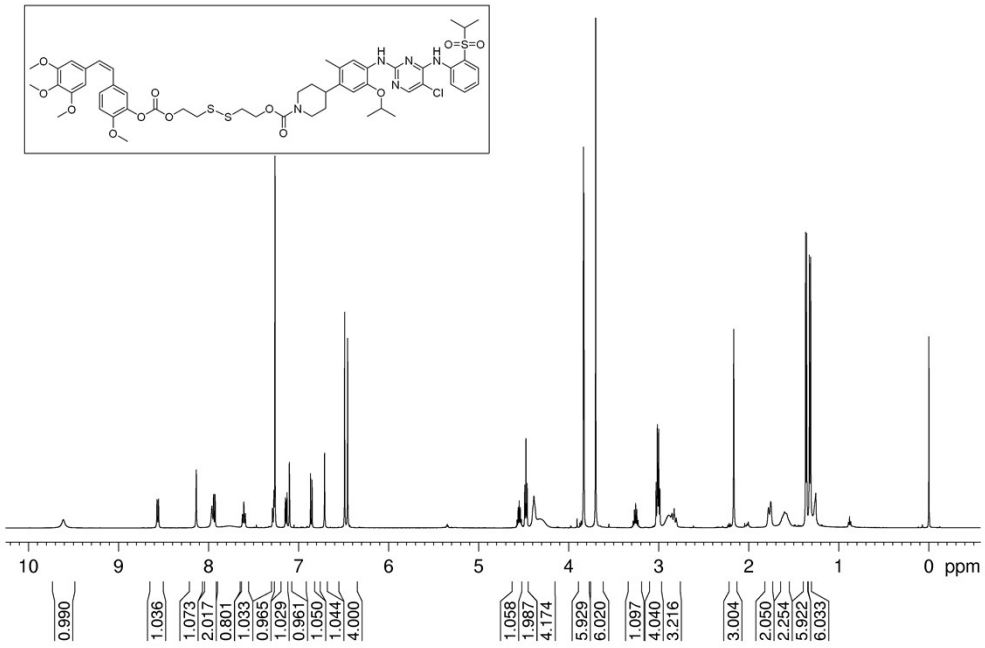


Figure S3 ^{1}H NMR of **CA4-SS-Cer** (500 MHz, $CDCl_3$)

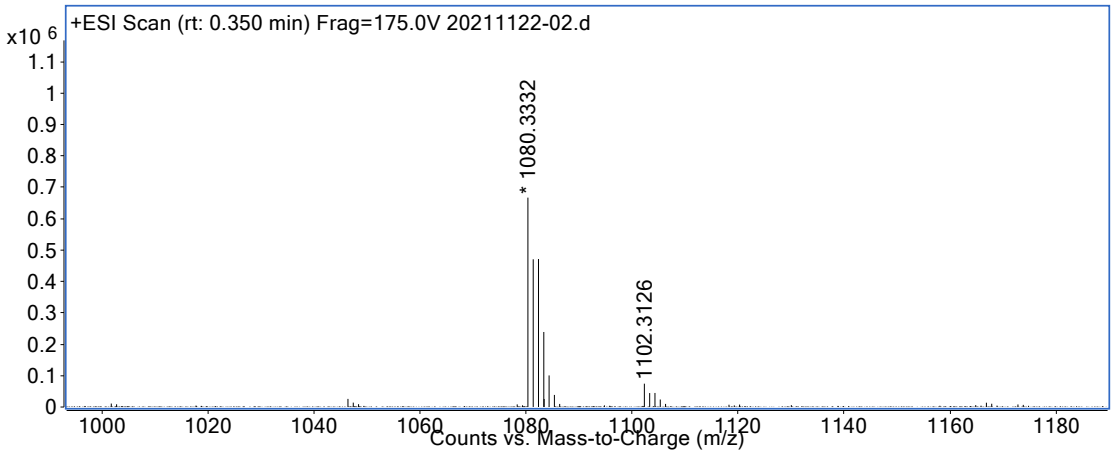


Figure S4 ESI-MS (m/z) $[M+H]^+$ of **CA4-SS-Cer**

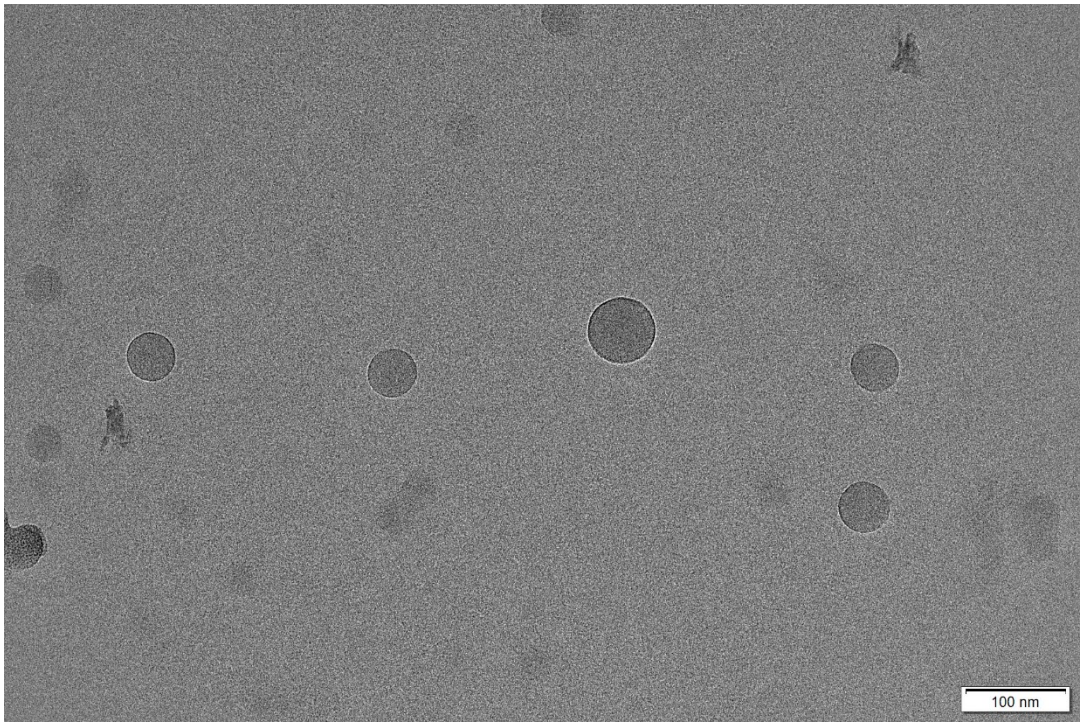


Figure S5 The TEM image of **CA4-SS-Cer NPs**

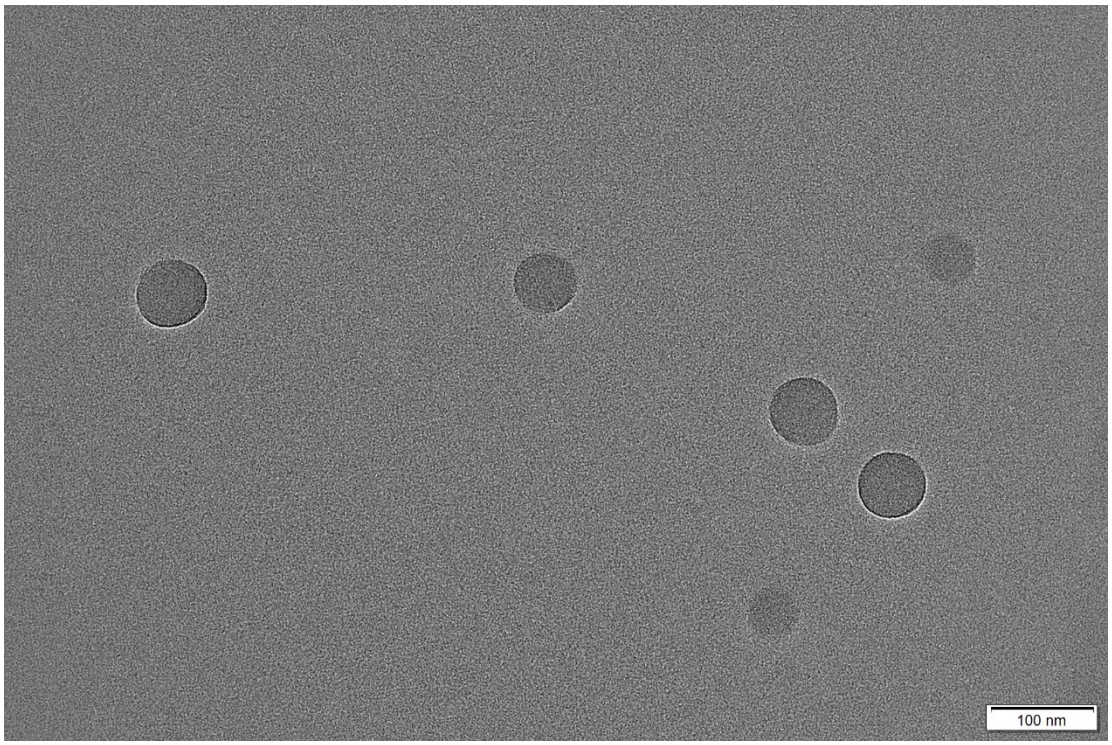


Figure S6 The TEM image of **UA@CA4-SS-Cer NPs**

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name:	1		
SOP Name:	mansettings.nano		
General Notes:			
File Name:	2021-05-12.dts	**Dispersant Name:**	Water
Record Number:	10	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	2021年10月26日 15:49:55

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	181.2	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	68.76	**Peak 1:**	78.13	100.0	28.73
PdI:	0.115	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.953	**Peak 3:**	0.000	0.0	0.000
Result quality	Good				

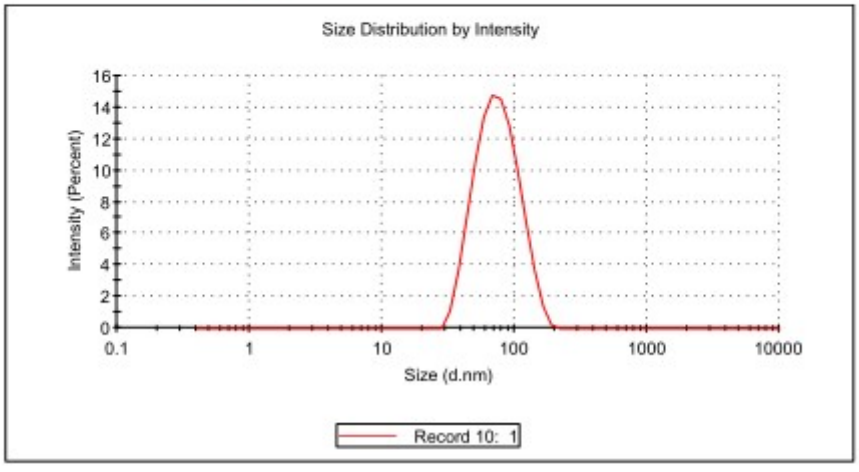


Figure S7.1 The diameter distribution of **CA4-SS-Cer NPs** in aqueous solution.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: 2

SOP Name: mansettings.nano

General Notes:

File Name:	2021-05-12.dts	**Dispersant Name:**	Water
Record Number:	11	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	2021年10月26日 15:52:28

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	182.6	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	68.97	**Peak 1:**	75.52	100.0	25.19
PdI:	0.119	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.951	**Peak 3:**	0.000	0.0	0.000

Result quality Good

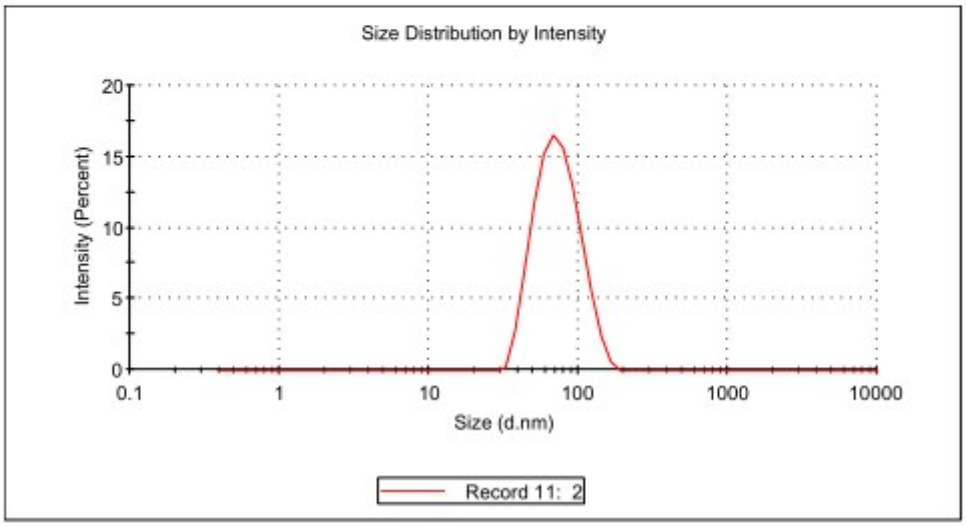


Figure S7.2 The diameter distribution of **CA4-SS-Cer NPs** in aqueous solution.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: 3

SOP Name: mansettings.nano

General Notes:

File Name:	2021-05-12.dts	**Dispersant Name:**	Water
Record Number:	12	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	2021年10月26日 15:55:01

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	182.2	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	9

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	68.30	**Peak 1:**	78.05	100.0	30.06
PdI:	0.127	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.951	**Peak 3:**	0.000	0.0	0.000

Result quality Good

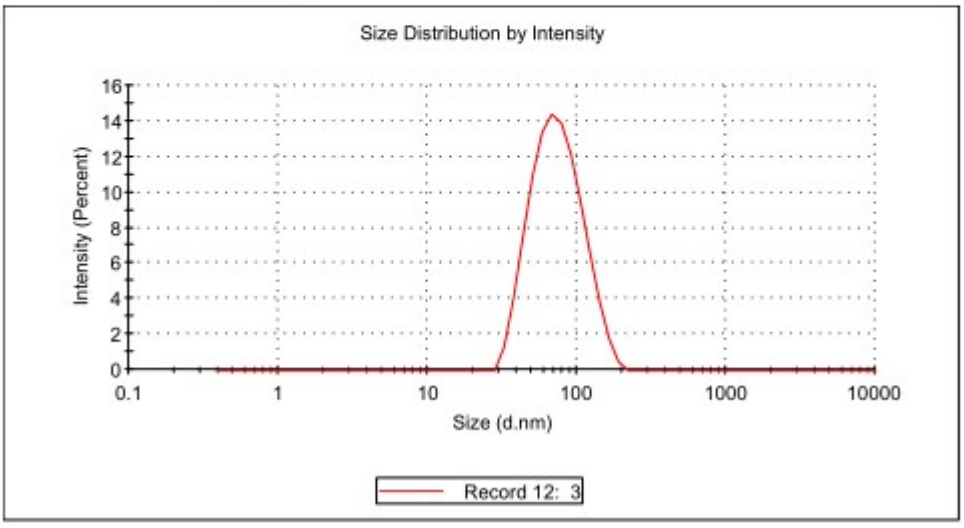


Figure S7.3 The diameter distribution of **CA4-SS-Cer NPs** in aqueous solution.

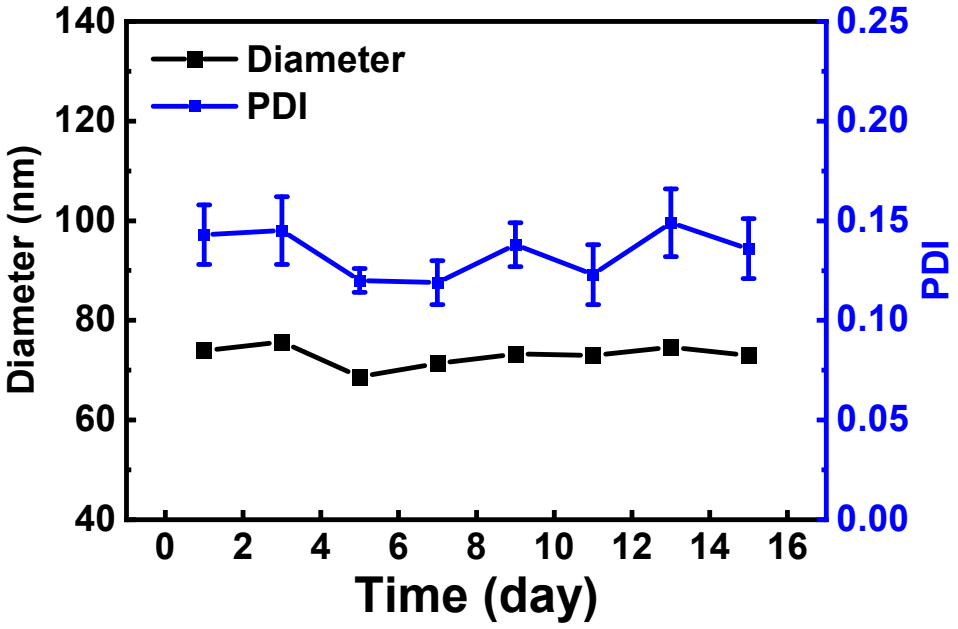


Figure S8 The change of average diameter and PDI of **CA4-SS-Cer NPs** over time.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: 2

SOP Name: mansettings.nano

General Notes:

File Name:	1023-0.05 乙醇-3.dts	**Dispersant Name:**	Water
Record Number:	10	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	2021年10月28日 11:57:18

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	222.2	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	91.93	**Peak 1:**	105.9	100.0	41.50
PdI:	0.122	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.954	**Peak 3:**	0.000	0.0	0.000

Result quality Good

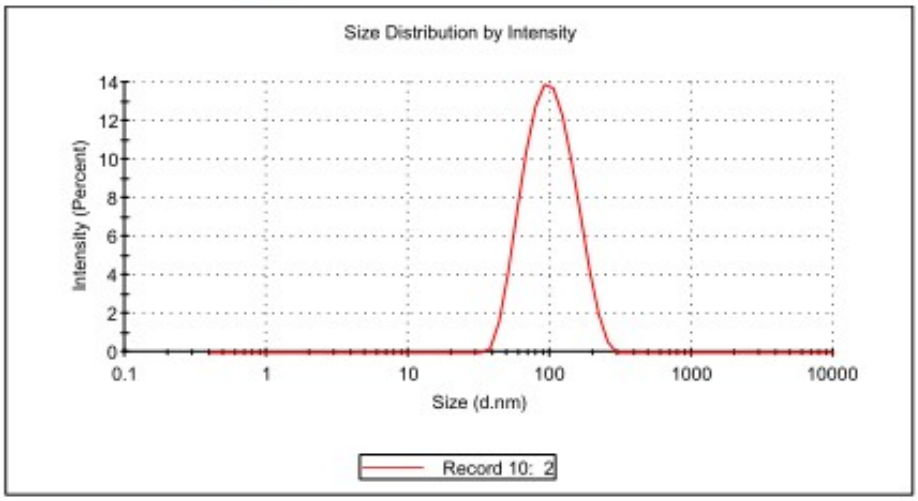


Figure S9.1 The diameter distribution of **UA@CA4-SS-Cer NPs** in aqueous solution.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: 2

SOP Name: mansettings.nano

General Notes:

File Name:	1023-0.05 乙醇-3.dts	**Dispersant Name:**	Water
Record Number:	10	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	2021年10月28日 11:57:18

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	222.2	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	91.93	**Peak 1:**	105.9	100.0	41.50
PdI:	0.122	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.954	**Peak 3:**	0.000	0.0	0.000

Result quality Good

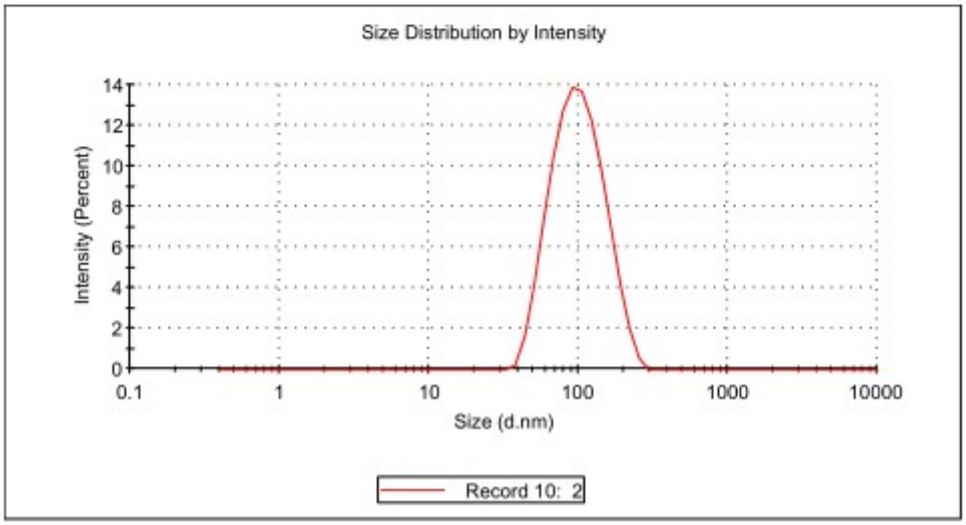


Figure S9.2 The diameter distribution of **UA@CA4-SS-Cer NPs** in aqueous solution.

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: 3

SOP Name: mansettings.nano

General Notes:

File Name:	1023-0.05 乙醇-3.dts	**Dispersant Name:**	Water
Record Number:	11	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	2021年10月28日 11:59:41

System

Temperature (°C):	25.0	**Duration Used (s):**	70
Count Rate (kcps):	221.2	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	8

Results

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	91.42	**Peak 1:**	103.3	100.0	39.28
PdI:	0.134	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.953	**Peak 3:**	0.000	0.0	0.000

Result quality Good

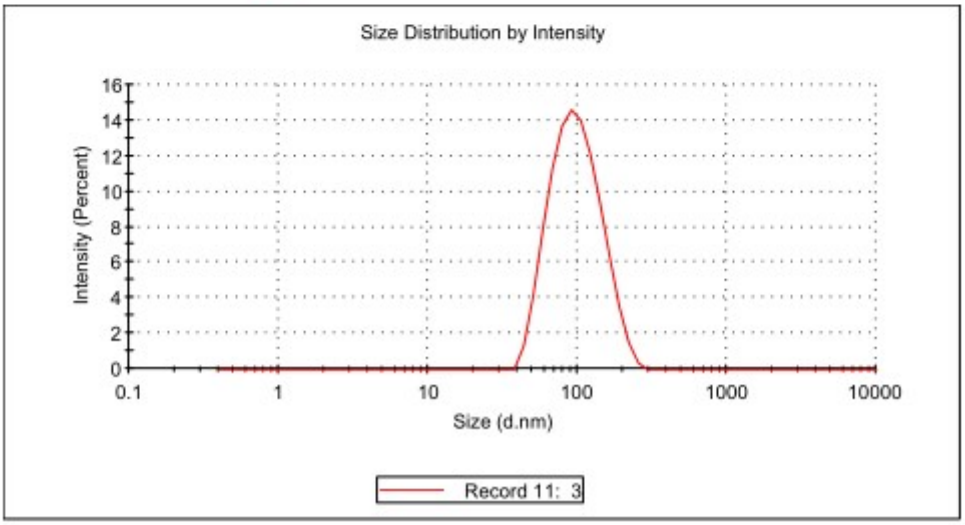


Figure S9.3 The diameter distribution of **UA@CA4-SS-Cer NPs** in aqueous solution.

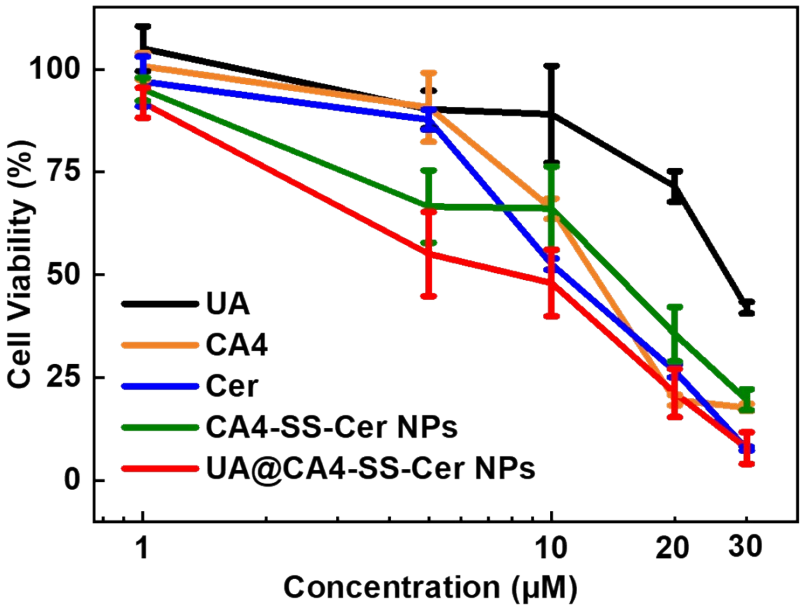


Figure S10 A549 cell viabilities assay (MTT test) .

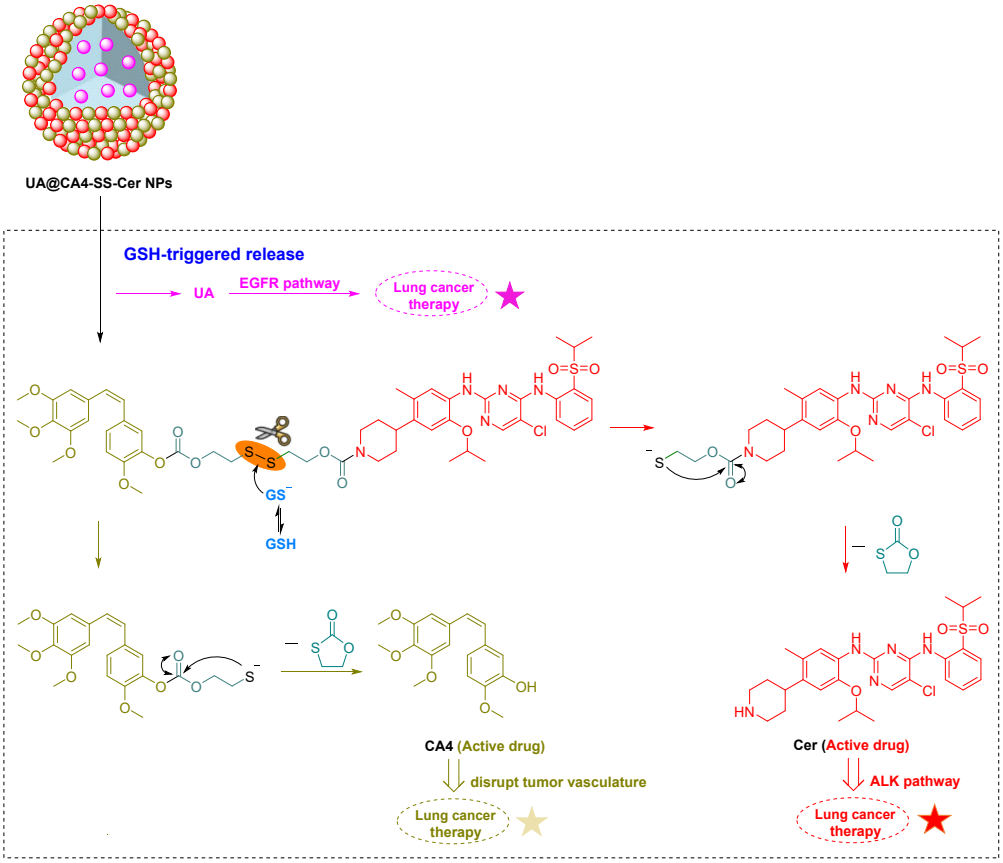


Figure S11 The proposed mechanism of **UA@CA4-SS-Cer NPs** for the triple-combination deep-penetrating therapy.