Electronic Supplementary Material (ESI) for ChemComm.


# Electronic Supplementary Information

## Controllably mixed-charged co-assembly of dendritic lipopeptides into invisible capsid-like nanoparticles as potential drug carriers

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# 1 Experimental Section

## 1.1 Materials and Methods

***Materials***

H-Lys-OMe·2HCl, Boc-Lys(Boc)-OH, Fmoc-Glu-OH, H-Glu(OtBu)-OtBu, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), benzotriazole-1-yl-oxytripyrrolidino phosphonium hexafluorophosphate (PyBop) and 1-hydroxybenzotriazole hydrate (HOBT) were purchased from GL Biochem Ltd. (Shanghai, China). Oleylamine, Oleic acid, N, N-diisopropylethylamine (DIEA) and trifluoroacetic acid (TFA) were obtained from Asta Tech Pharmaceutical Co., Ltd. (Chengdu, China). Dichloromethane (DCM), N, N-dimethylformamide (DMF), anhydrous diethyl ether, dimethyl sulfoxide (DMSO), chloroform-d ($CDCl_3$), and dimethyl sulfoxide-d6 (DMSO-$d_6$) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). Hoechst 33342 and cell counter kit-8 (CCK-8) were purchased from Dojindo Laboratories (Kumamoto, Japan). Dulbecco′s modified Eagle′s medium (DMEM), fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific (USA). Bovine serum albumin (BSA) and histone were obtained from Aladdin (Shanghai, China). Coenzyme Q10 (CoQ10) was purchased from Ark Pharm. NIH 3T3 fibroblasts cell line, mouse aortic vascular smooth muscle cells (MOVAS) and human glioblastoma (U87MG) Cells were purchased from Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China).

***Methods***

$^1H$ NMR spectra were recorded on a Bruker Avance II NMR spectrometer spectrometer at 600 MHz. The molecular weight of each compound was tested by matrixassisted laser desorption ionization time of flight mass spectroscopy (MALDI-TOF MS, Bruker Autoflex III) or electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS, Waters Q-TOF Premier). Dynamic light scattering (DLS) experiments were performed on a Malvern Zetasizer Nano ZS. UV-Vis absorbance spectra were measured

by a Perkin Elmer Lambda 650. Fluorescence was detected by fluorescence spectrophotometer (Hitachi F-7000). Transmission electron microscope (TEM, Tecnai GF20S-TWIN, FEI, USA) were used to observe the size and morphology of nanoparticles. Cellular uptake was observed by Confocal Laser Scanning Microscopy (CLSM, Leica TCP SP5).

## 1.2 Synthesis of Amphiphilic Dendrons

### *1.2.1 Synthesis of cationic amphiphilic dendritic lipopeptides (CDLs)*

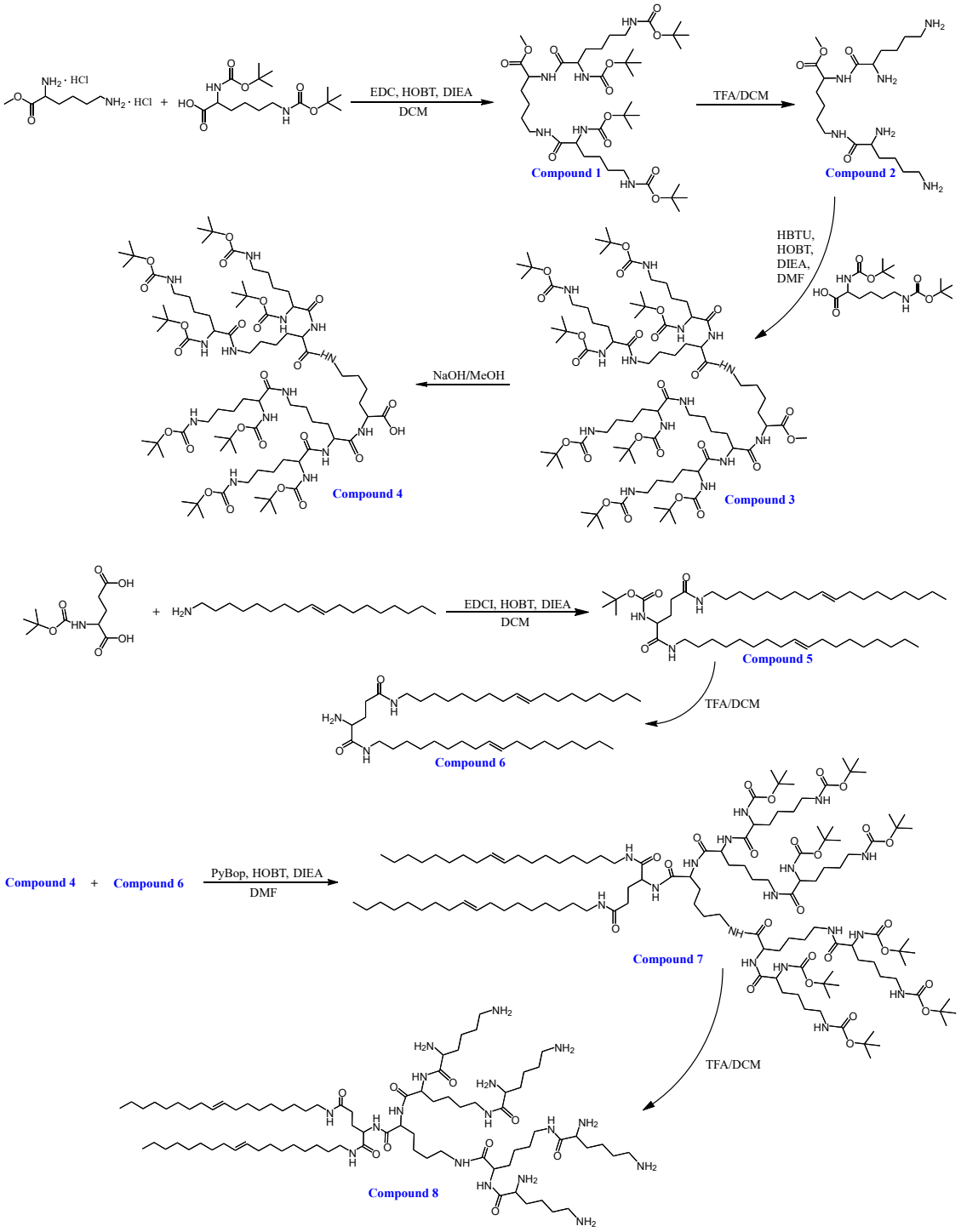


**Figure S1.** Synthetic route of CDLs.

***Synthesis of compound 4***

H-Lys-OMe·2HCl (4.0 g, 17.2 mmol), Boc-Lys(Boc)-OH (14.5 g, 41.9 mmol), EDC·HCl (10.5 g, 54.8 mmol) and HOBT (7.0 g, 51.9 mmol) were dissolved in anhydrous dichloromethane (DCM) under nitrogen atmosphere. Place Boc-Lys(Boc)-OH in the refrigerator at -20℃ before weighing. Subsequently, DIEA (29.0 mL, 17.6 mmol) was added with stirring in an ice bath. The solution was stirred at room temperature for 24 h. The solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 1** (yield: 91.69%). Then **Compound 1** (4.0 g, 4.9 mmol) were dissolved in anhydrous dichloromethane (DCM, 15 mL) under nitrogen atmosphere. Subsequently, TFA (14.5 mL, 188.0 mmol) was added dropwise with stirring in an ice bath. The solution was stirred at room temperature for 6 h. The solvent and TFA were removed under vacuum. The products were precipitated under stirring with ice anhydrous diethyl ether to obtain white powder **Compound 2**. Then **Compound 2** (1.8 g, 4.3 mmol), Boc-Lys(Boc)-OH (9.0 g, 26.0 mmol), HBTU (9.9 g, 26.0 mmol) and HOBT (3.5 g, 26.0 mmol) were dissolved in DMF under nitrogen atmosphere. Subsequently, DIEA (14.3 mL, 8.7 mmol) was added dropwise with stirring in an ice bath. Then the solution was stirred at room temperature for 48 h. The solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 3** (yield: 83.91%). Then **Compound 3** (2.9 g, 1.6 mmol) was dissolved in NaOH/MeOH (1 mol/L, 17.0 mL) in an ice bath. The solution was stirred at room temperature for 6 h to deprotect the methoxy group. MeOH in the mixture was removed by rotary evaporation and $CHCl_3$ was added to dissolve the residues. Adjust pH to 2~3 with HCl (1 mol/L) and the organic phase was dried with anhydrous $NaSO_4$ for

12 h. The filtrate was concentrated by rotary evaporation to obtain white powder **Compound 4**.

***Synthesis of compound 6***

Boc-Glu-OH (5.0 g, 20.2 mmol), Oleylamine (16.0 ml, 48.6 mmol), EDC·HCl (11.6 g, 60.7 mmol) and HOBT (8.2 g, 60.6 mmol) were dissolved in anhydrous DCM under nitrogen atmosphere. Subsequently, DIEA (34.0 mL, 20.6 mmol) was added dropwise with stirring in an ice bath. Then the solution was stirred at room temperature for 24 h. After reaction, the solution was removed by rotary evaporation and replaced with chloroform ($CHCl_3$). Then, the solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography and the pure **Compound 5** was obtained (yield: 79.52%). Then **Compound 5** (3.1 g, 4.2 mmol) was dissolved in anhydrous DCM (3 mL). Subsequently, TFA (3.1 mL, 40.6 mmol) was added dropwise with stirring in an ice bath under nitrogen atmosphere. The solution was then stirred at room temperature for 6 h. The DCM and TFA were removed under vacuum. The products were precipitated under stirring with ice anhydrous diethyl ether. **Compound 6** was obtained as white solid after removal of diethyl ether completely.

***Synthesis of CDLs (Compound 8)***

**Compound 4** (2.8 g, 1.6 mmol), **Compound 6** (1.4 g, 2.1 mmol), PyBop (1.3 g, 2.5 mmol) and HOBT (0.4 g, 2.5 mmol) were dissolved in DMF under nitrogen atmosphere. Subsequently, DIEA (1.5 mL, 9.0 mmol) was injected with stirring in an ice bath and then the solution was stirred at room temperature for 48 h. The solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 7** (yield: 85.73%). **Compound 7** (5.2 g, 2.2 mmol) was dissolved in anhydrous

DCM (14 mL) under nitrogen atmosphere. Subsequently, TFA (14.5 mL, 188.0 mmol) was added dropwise with stirring in an ice bath. The solution was stirred at room temperature for 6 h. The DCM and TFA was removed by rotary evaporation. The residues were dissolved in deionized water and dialyzed in a dialysis bag (Spectra/PorMWCO = 1000) for two days. The outer phase was replaced with fresh deionized water every 4 h. The solution in the bag was freeze-dried to receive the white solid of **Compound 8** (yield: 75.81%).

### *1.2.2 Synthesis of anionic amphiphilic dendritic lipopeptides (ADLs)*

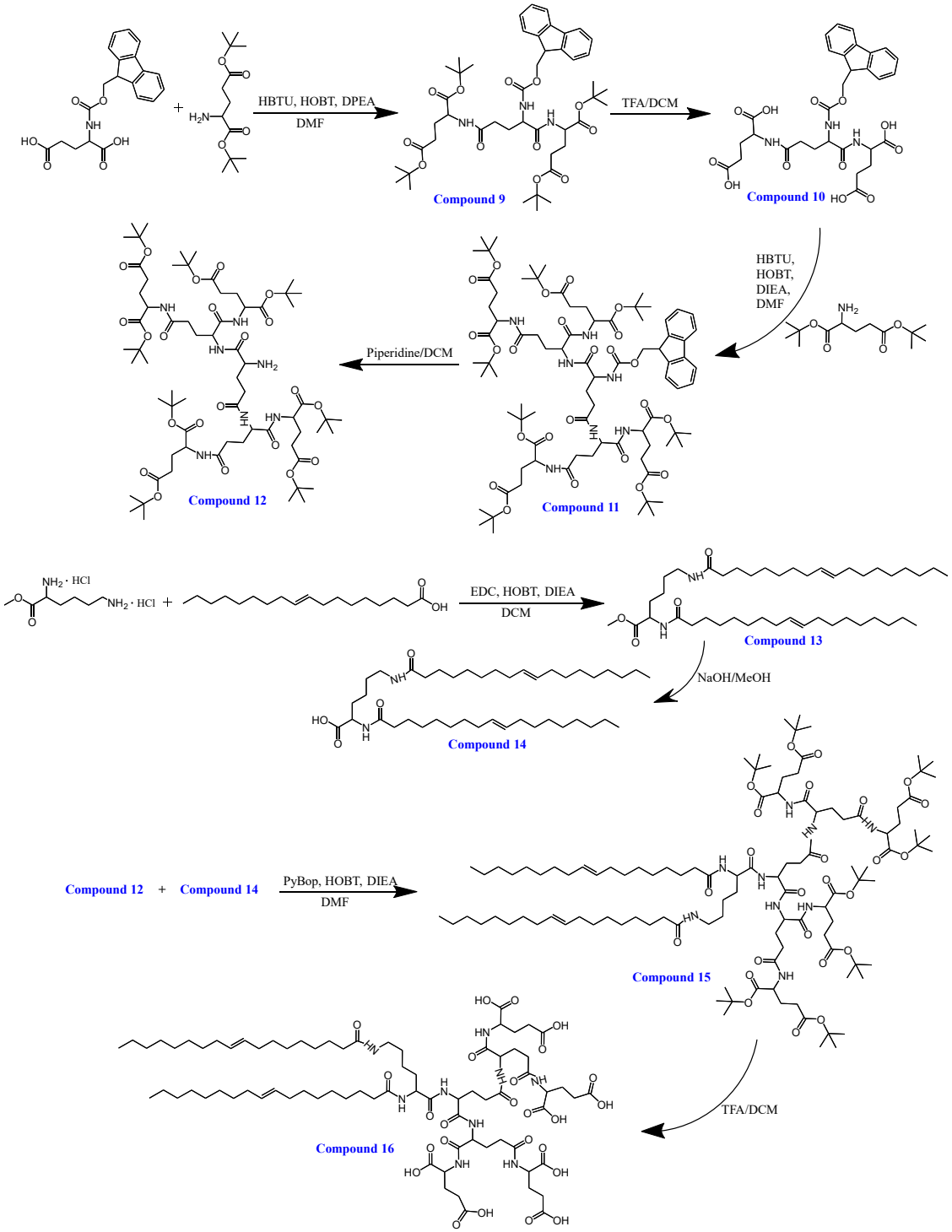


**Figure S2.** Synthetic route of ADLs.

***Synthesis of compound 12***

Fmoc-Glu-OH (5.0 g, 13.5 mmol), H-Glu (OtBu)-OtBu (9.6 g, 32.6 mmol), HBTU (15.5 g, 40.9 mmol) and HOBT (5.5 g, 40.8 mmol) were dissolved in DMF under nitrogen atmosphere. Subsequently, DIEA (23.0 mL, 139.0 mmol) was injected with stirring in an ice bath. Reaction in ice bath conditions for half an hour, then the solution was stirred at room temperature for 24 h. The DMF was removed by rotary evaporation and the solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 9** (yield: 92.38%). **Compound 9** (3.8 g, 4.5 mmol) were dissolved in anhydrous DCM (14 mL) under nitrogen atmosphere. Subsequently, TFA (14.5 mL, 188.0 mmol) was added dropwise with stirring in an ice bath. The solution was stirred at room temperature for 6 h. The DCM and TFA were removed under vacuum. The products were precipitated under stirring with ice anhydrous diethyl ether. **Compound 10** was obtained as white solid after removal of diethyl ether completely. **Compound 10** (2.6 g, 4.2 mmol), H-Glu(OtBu)-OtBu (7.34 g, 24.8 mmol), HBTU (9.42 g, 24.9 mmol) and HOBT (3.36 g, 24.8 mmol) were dissolved in DMF under nitrogen atmosphere. Subsequently, DIEA (14.0 mL, 84.8 mmol) was injected with stirring in an ice bath and then the solution was stirred at room temperature for 48 h. Then, the solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h. The filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 11** (yield: 80.02%). Then **Compound 11** (3.5 g, 2.2 mmol) was dissolved in DCM (23.0mL), and then piperidine was added in an ice bath. The solution was stirred at room temperature for 3h to deprotect Fmoc group. After reaction, the piperidine and DCM were removed by rotary evaporation and $CHCl_3$ was added to dissolve the residues. Then, the solution was washed with saturated sodium chloride

solution for several times to remove residual piperidine. The organic phase was dried with anhydrous $NaSO_4$ for 12 h. The filtrate was concentrated by rotary evaporation to obtain **Compound 12**.

***Synthesis of compound 14***

H-Lys-OMe·2HCl (5.0 g, 21.4 mmol), Oleic acid (14.0 ml, 44.3 mmol), EDC·HCl (12.4 g, 64.4 mmol) and HOBT (8.7 g, 64.3 mmol) were dissolved in anhydrous DCM under nitrogen atmosphere. Subsequently, DIEA (35.0 mL, 214.0 mmol) was added dropwise with stirring in an ice bath and the solution was stirred at room temperature for 24 h. The DCM was removed by rotary evaporation and replaced with $CHCl_3$. Then, the solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for 12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 13** (yield: 83.65%). Then **Compound 13** (3.0 g, 4.3 mmol) was dissolved in NaOH/MeOH (1 mol/L, 43.5 mL) in an ice bath. The solution was stirred at room temperature for 6 h to deprotect the methoxy group. The MeOH was removed by rotary evaporation and $CHCl_3$ was added to dissolve the residues. Adjust pH to 3~5 with HCl (1 mol/L) and the organic phase was dried with anhydrous $NaSO_4$ for 12 h. The filtrate was concentrated by rotary evaporation to obtain **Compound 14**.

***Synthesis of ADLs (Compound 16)***

**Compound 12** (3.4 g, 2.4 mmol), **Compound 14** (2.1 g, 3.2 mmol), PyBop (1.7 g, 3.3 mmol) and HOBT (0.5 g, 3.4 mmol) were dissolved in DMF under nitrogen atmosphere. Subsequently, DIEA (2.0 mL, 1.2 mmol) was injected with stirring in an ice bath and the solution was stirred at room temperature for 48 h. After reaction, the DMF was removed by rotary evaporation. Then, the crude product was redissolved with chloroform and the solution was respectively washed with saturated NaCl, saturated $NaHCO_3$ and HCl (1 mol/L) solutions for several times. The organic phase was dried with anhydrous $NaSO_4$ for

12 h and the filtrate was concentrated by rotary evaporation. The crude product was purified by column chromatography to obtain **Compound 15** (yield: 88.36%). **Compound 15** (4.0 g, 1.8 mmol) were dissolved in anhydrous dichloromethane (DCM, 14.0 mL) under nitrogen atmosphere. Subsequently, TFA (14.5 mL, 188.0 mmol) was added dropwise with stirring in an ice bath. The solution was stirred at room temperature for 6 h. The DCM and TFA was removed by rotary evaporation. The residues were dissolved in deionized water and dialyzed in a dialysis bag (Spectra/PorMWCO = 1000) for two days. The outer phase was replaced with fresh deionized water every 4 h. The solution in the bag was freeze-dried to receive the white solid of **Compound 16** (yield: 73.6%).

## 1.3 Characterizations of Amphiphilic Dendrons

### *1.3.1 $^{1}$H NMR analysis*

The $^{1}$H NMR spectra of the samples were recorded on a Bruker Avance II NMR spectrometer at 600 MHz. All products and intermediates are dissolved with a suitable deuterated reagent.

### *1.3.2 Mass spectrum analysis*

The samples were tested by matrix-assisted laser desorption-time-of-flight mass spectrometry MALDI-TOF MS or electrospray ionization-mass spectrometry (ESI-MS).

## 1.4 Preparation and characterizations of micelle systems

### *1.4.1 Cooperative self-assembly of CDLs and ADLs*

First, ADLs and CDLs were accurately weighed and dissolved adequately in DMSO. ADLs and CDLs were mixed with molar ratios of 4:1, 2:1, 1:1, 1:2, 1:4, respectively. Subsequently, the mixture was dropwise added into millipore water under ultrasonic conditions (15 min). After stirring for 1 h, the solution was dialyzed against millipore water for 24 h (Spectra/Por MWCO = 500) to obtain mixed-charged NPs (MNPs), which named MNPs (4:1), MNPs (2:1), ICNPs, MNPs (1:2) and MNPs (1:4) respectively. Anionic micelle systems (ANPs) was prepared as the same method just using ADLs. Cationic micelle systems (CNPs) was prepared as the same method just using CDLs.

### *1.4.2 Determination of critical assembly concentration (CAC)*

Critical assembly concentrations of CDLs and ADLs were determined by fluorescence spectrum using pyrene as a probe. The pyrene was dissolved in aqueous medium and the concentration was fixed at $6.00 \times 10^{-7}$ mol/L. The CDLs and ADLs were dissolved in the prepared solution of pyrene and the concentration ranged from $1.0 \times 10^{-7}$ to 1.0 mg/mL. Excitation pyrene spectra were recorded using fluorescence spectrophotometer from 300 nm to 380 nm with an emission wavelength at 395 nm. The fluorescence values of $I_{338}$ and $I_{334}$ were tested and used to calculate the values of $I_{338}/I_{334}$.

### *1.4.3 Size and zeta potential*

The size distribution and zeta potential of all micellar systems (includes MNPs (4:1), MNPs (2:1), ICNPs, MNPs (1:2) and MNPs (1:4) respectively were determined by a dynamic light scattering.

### *1.4.4 Transmission Electron Microscopy (TEM)*

Copper grid was dipped into the fresh ANPs, CNPs and ICNPs solution to produce the TEM sample and stained with 2wt% phosphotungstic acid. Until the solvent was evaporated completely by infrared lamp, the sample was observed using transmission electron microscopy.

### *1.4.5 Circular Dichroism (CD) Spectra*

ANPs, CNPs and ICNPs were dissolved in millipore water at the concentration of 100 μg/ml. 2 mL of the sample was added into a circular dichroism (CD) cuvette. Measurements were performed on a CD spectrometer (JASCO Corp J-1500, Japan) from 190 nm to 250 nm. Secondary structure of ANPs, CNPs and ICNPs were analyzed by CD Spectroscopy Deconvolution Software.

### *1.4.6 Stability analysis*

ANPs, CNPs and ICNPs (100 μg/mL) were prepared in pH 7.4 PBS. At designated time, aliquot of the solution was taken and monitored by DLS. ANPs, CNPs and ICNPs were prepared with different pH solution and the particle size changes were detected by DLS.

### *1.4.7 Protein adsorption assay*

BSA and histones were used as model protein to detect the protein adsorption, respectively. AMS, CMS and ICNPs were incubated with BSA solution in PBS at pH 8.5, 7.4 and 5.6 under mild shake (37 °C). The concentrations of the samples and BSA were 0.05 mg/mL. At given times (2 h and 4 h), aliquots of the mixture were taken and centrifuged at 8000 g for 15 min to precipitate the BSA adsorbed aggregate. The BSA concentration of supernatant was determined using an UV−vis spectrometer by measuring the maximal absorbance at 280 nm wavelength. Then, the adsorbed BSA on the aggregate was calculated against a standard calibration curve of BSA. The same method was used for anti-histones detection. The histones concentration of supernatant was determined using an UV-vis spectrometer by measuring the maximal absorbance at 275 nm wavelength.

### *1.4.8 CoQ10-loaded micellar systems*

CDLs and CoQ10 were dissolved adequately in DMSO under ultrasonic conditions. The mixture was added to pH 7.4 PBS under ultrasonic conditions. During the ultrasound process, the CDLs will self-assemble into micelles and the drug will be loaded into hydrophobic cavity (CoQ10-CNPs). The CoQ10-CNPs was dialyzed in a dialysis bag (Spectra/Por MWCO = 1000) with deionized water to remove the free CoQ10 and residual organic solvent at 4 ℃ for 24 h. Finally, the CoQ10-CNPs was obtained after freeze-drying. The whole process is performed in the dark and at room temperature. CoQ10-ANPs and CoQ10-(ICNPs) were prepared using the same method. The particle size of CoQ10-loaded micellar systems were detected by DLS. The drug loading (DL) of CoQ10-CNPs = (weight of CoQ10 in CoQ10-CNPs/weight of CDLs) × 100%; encapsulation efficiency (EE) of CoQ10-CNPs = (weight of CoQ10 in CoQ10-CNPs/weight of CoQ10 feeding) × 100%.

### *1.4.9 Light stability of CoQ10-loaded micellar systems*

Free CoQ10, CoQ10-ANPs, CoQ10-CNPs, and CoQ10-(ICNPs) containing the same concentration of CoQ10 were placed in colorless and transparent sealed vials (n = 3). The photodegradation test was performed at 25℃ by UV irradiation (λ = 254 nm, 4500 lx

illumination). Samples were taken at 1, 2, 4, 8, 12, 24, and 48 h to detect CoQ10 content by HPLC.

***1.4.10 In vitro drug release profile***

The CoQ10-(ICNPs) were dissolved in 1 mL of pH 7.4 PBS (ionic strength = 0.015 M). The samples were added into the dialysis bags (Spectra/Por MWCO = 1000). The dialysis bags were placed into centrifuge tubes at 37 ℃ under mild shake. The release medium is PBS/ethanol mixtures (4/1, v/v). At given times, 1 mL of dialyzate was taken for analysis and the same volume of fresh release medium was replenished immediately. The concentration of released CoQ10 in the dialysate was detected by UV-vis spectrophotometer at the absorbance at 275 nm. The CoQ10 release of CoQ10-CNPs and CoQ10-ANPs were measured by the same method.

***1.4.11 Hydroxyl radical scavenging ability***

Free CoQ10, CoQ10-ANPs, CoQ10-CNPs, and CoQ10-(ICNPs) containing the same concentration of CoQ10 were placed in a mixture solution (0.5% phenanthroline solution, 0.5% $FeSO_4$ solution and 0.01% $H_2O_2$, 1/1/1, v/v/v) and co-incubated at 37 ° C. At given times (1 h, 6 h, 12 h, 24 h and 48 h), samples were taken for analysis by UV-visible spectrophotometer at absorbance at 508 nm (As). $H_2O_2$ was replaced by 1ml ultrapure water and measured the absorbance as AB; CoQ-loaded formulations were replaced by 1 ml ultrapure water and measure the absorbance as Ap. The formula for the hydroxyl radical scavenging rate D (%) is as follows:

$$D\ (\%)=\frac{\text{As-Ap}}{\text{AB-Ap}}\times 100\%$$

***1.4.12 Cytotoxicity Testing***

NIH/3T3 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) FBS and 1% (v/v) penicillin-streptomycin solution at 37℃ in a humidified atmosphere containing 5% $CO_2$. The cytotoxicity of drug-loaded formulations was evaluated with a CCK-8 assay against NIH/3T3 fibroblasts cell. Cells were seeded onto 96-well plates ($1\times10^4$ cells/well). After culture for 24 h, cells were

exposed to fresh medium with various samples. Cells treated with blank culture media were negative controls. After 24 h of incubation, CCK-8 solution was added into each well and further incubated for 2 h. Absorbance at 450 nm was measured by a microplate reader (Thermo Scientific, USA). Cell viability was calculated as the ratio between sample absorbance and that of negative controls. The process of cytotoxicity testing of MOVAS cells and U87MG cells were same as NIH/3T3 fibroblast cells.

***1.4.13 In vitro cellular uptake***

RAW 264.7 macrophages were cultured in DMEM medium, which was changed every two days until 80% coverage was reached. Subsequently, the cells were digestedand $1 \times 10^5$ cells were seeded into a 6-well plate chambering cover-glass and incubated for overnight. Then 200 μL Dio-loaded nanopartilces were added into and incubate for another 4 h at 37 ºC, followed by washing to wipe off any free Dio-loaded nanopartilces. Afterwards, the cells were fixed in 4% paraformaldehyde for 8-10 min, washed, dyed with Hoechst 33342 and washed again. The coverslips were observed with a fluorescence microscope.

# 2 Characterizations of Amphiphilic Dendrons

## *2.1 Characterizations of CDLs*

### *2.1.1 Characterizations of Compound 1*

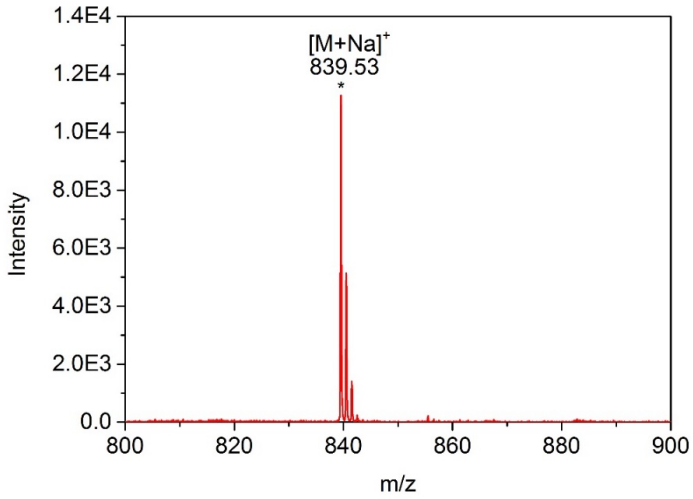


**Figure S3.** MALDI-TOF mass spectrum of Compound 1 (m/z, $[M+Na]^+$): 840.04 (calculated), 839.53 (observed).

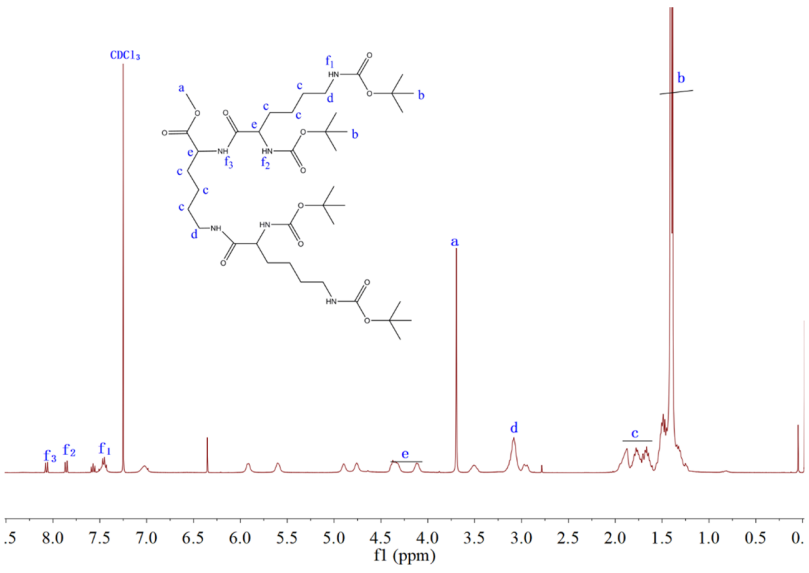


**Figure S4.** $^1$H-NMR spectrum of Compound 1 in $CDCl_3$.

### *2.1.2 Characterizations of Compound 3*

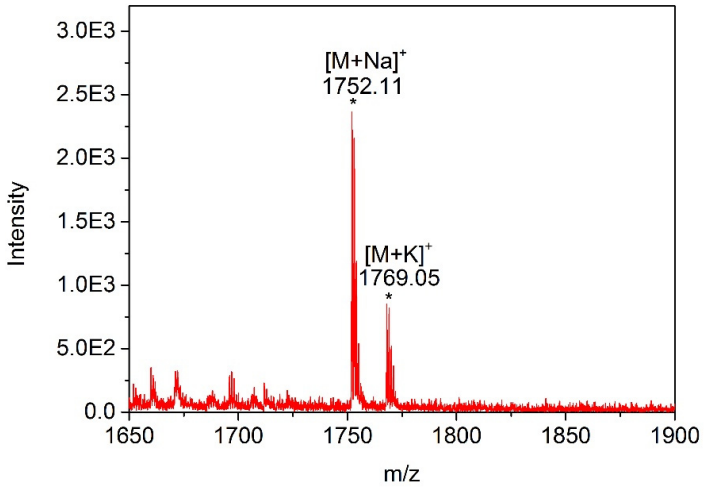


**Figure S5.** MALDI-TOF mass spectrum of Compound 3 MS (m/z, $[M+Na]^+$): 1753.20 (calculated), 1752.11 (observed). MS (m/z, $[M+K]^+$): 1769.20 (calculated), 1769.05 (observed).

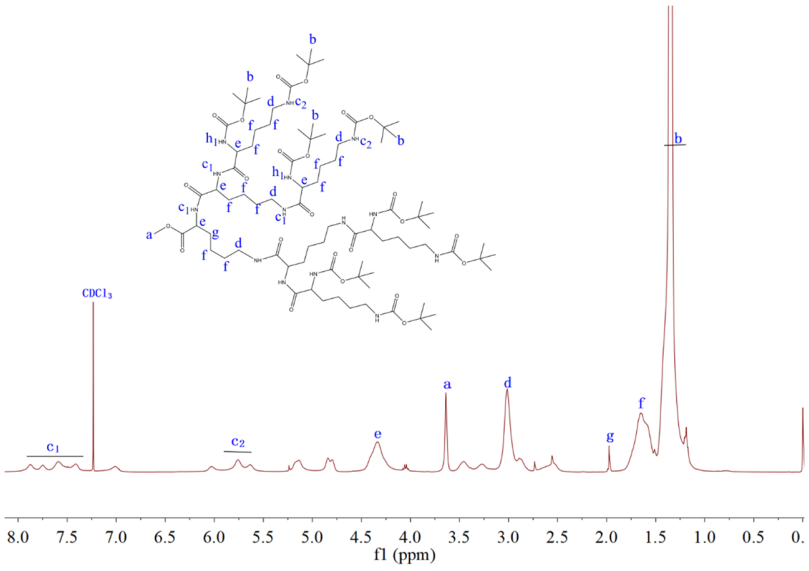


**Figure S6.** $^1$H-NMR spectrum of Compound 3 in $CDCl_3$.

### *2.1.3 Characterizations of Compound 5*

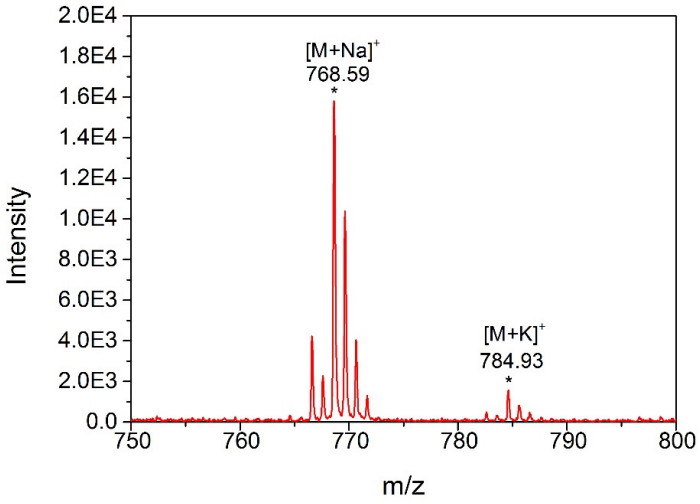


**Figure S7.** MALDI-TOF mass spectrum of Compound 5 MS (m/z, $[M + Na]^+$): 769.22 (calculated), 768.59 (observed). MS (m/z, $[M +K]^+$): 785.22 (calculated), 784.93 (observed).

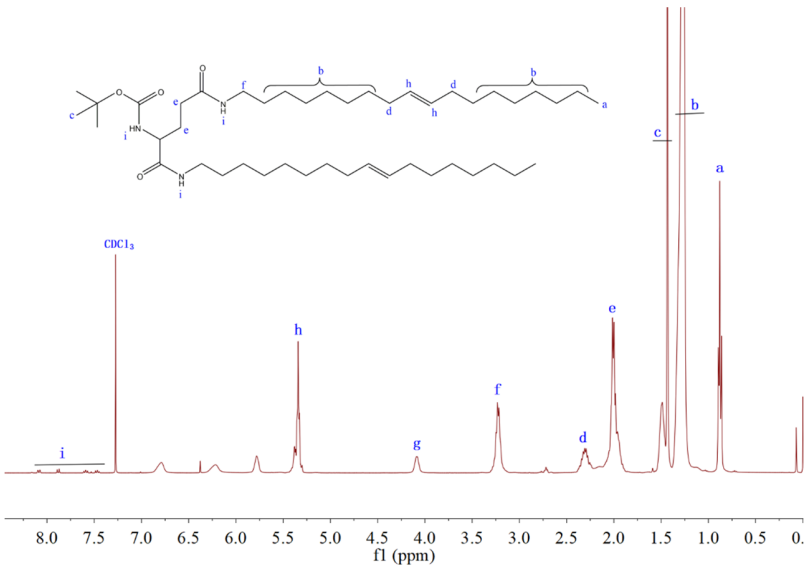


**Figure S8.** $^1$H-NMR spectrum of Compound 5 in $CDCl_3$.

### *2.1.4 Characterizations of Compound 7*

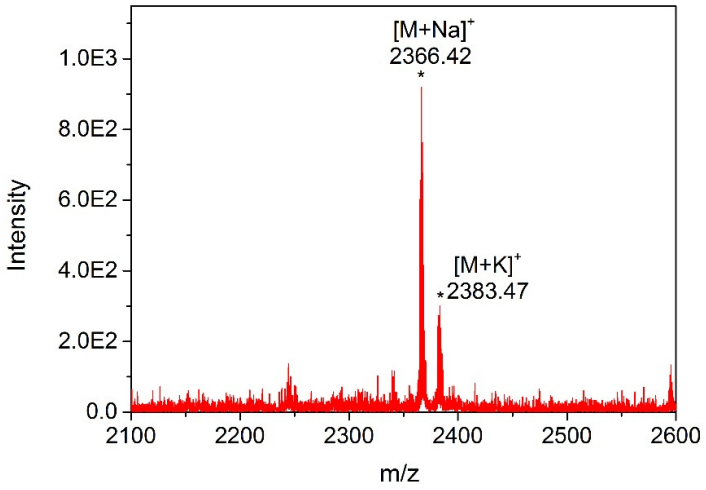


**Figure S9.** MALDI-TOF mass spectrum of Compound 7 MS (m/z, $[M+Na]^+$): 2367.26 (calculated), 2366.42 (observed). MS (m/z, $[M+K]^+$): 2383.26 (calculated), 2383.47 (observed).

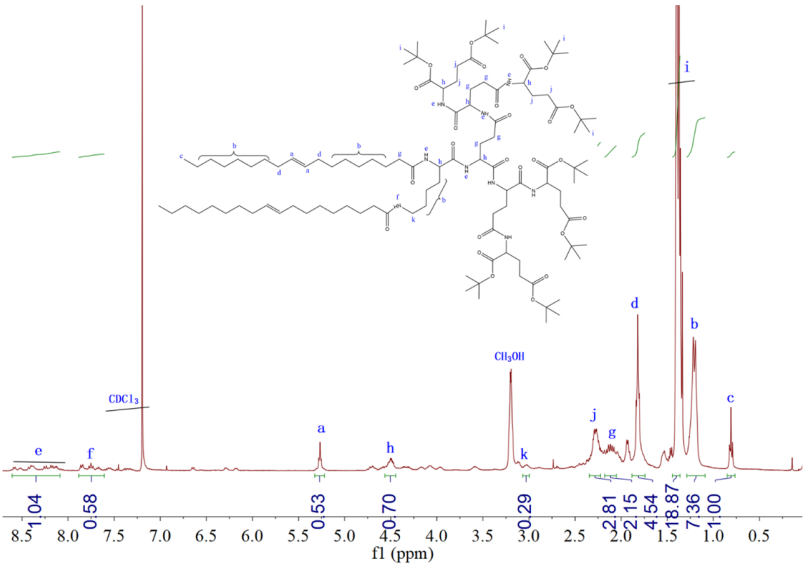


**Figure S10.** $^1$H-NMR spectrum of Compound 7 in DMSO-$d_6$.

### *2.1.5 Characterizations of Compound 8*

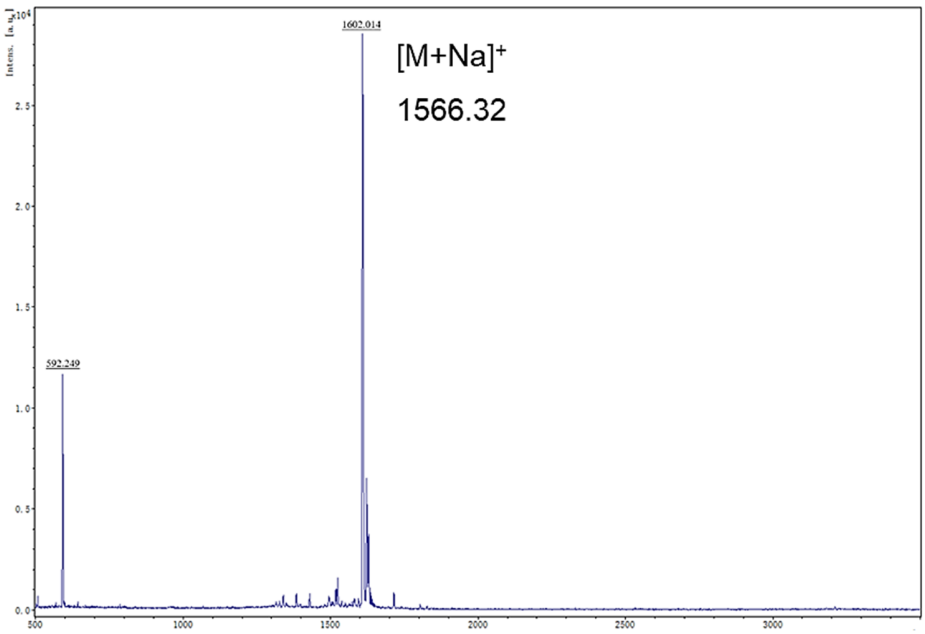


**Figure S11.** MALDI-TOF mass spectrum of Compound 8 MS (m/z, $[M+Na]^+$): 1566.33 (calculated), 1566.32 (observed).

### *2.2 Characterizations of ADLs*

### *2.2.1 Characterizations of Compound 9*

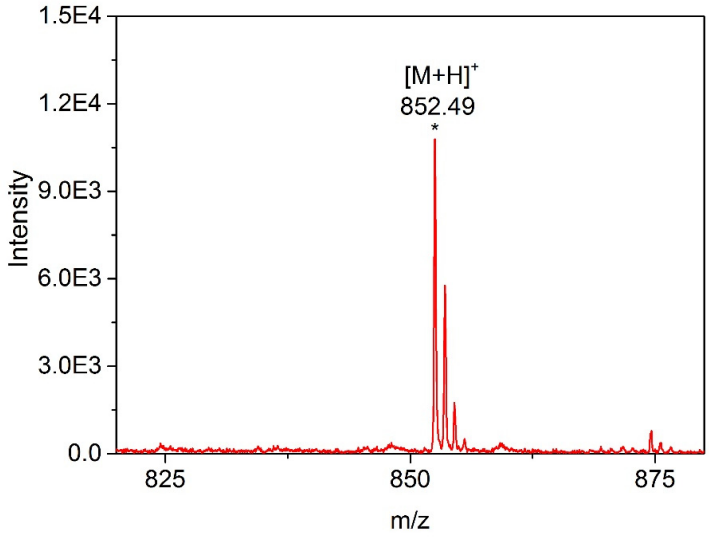


**Figure S12.** MALDI-TOF mass spectrum of Compound 9 MS (m/z, $[M+H]^+$): 853.04 (calculated), 852.49 (observed).

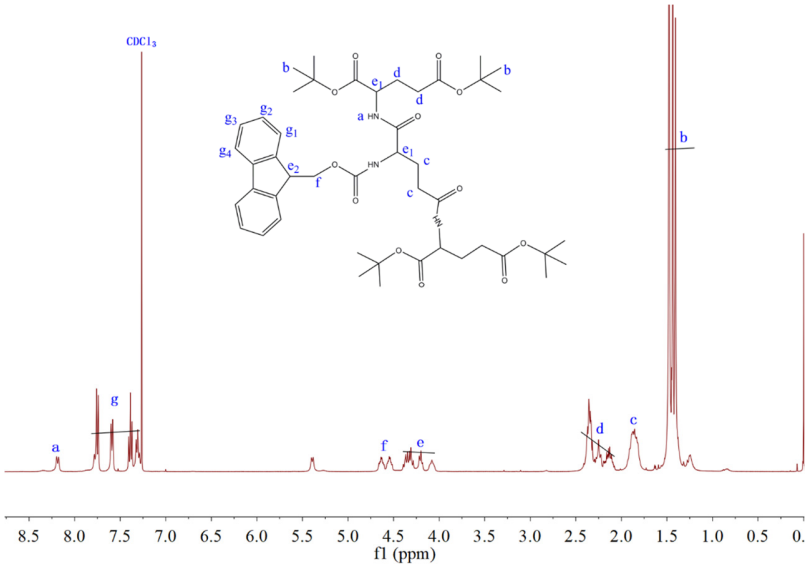


**Figure S13.** $^{1}$H-NMR spectrum of Compound 9 in $CDCl_3$.

### *2.2.2 Characterizations of Compound 11*

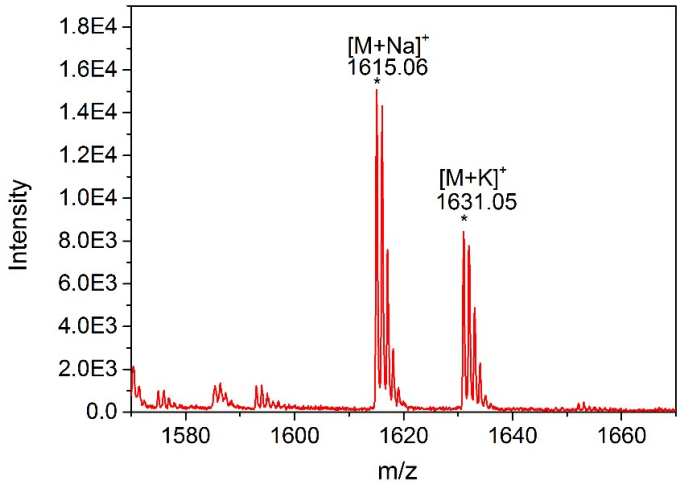


**Figure S14.** MALDI-TOF mass spectrum of Compound 11 MS (m/z, $[M+Na]^+$): 1615.93 (calculated), 1615.06 (observed). MS (m/z, $[M+K]^+$): 1631.93 (calculated), 1631.05 (observed).

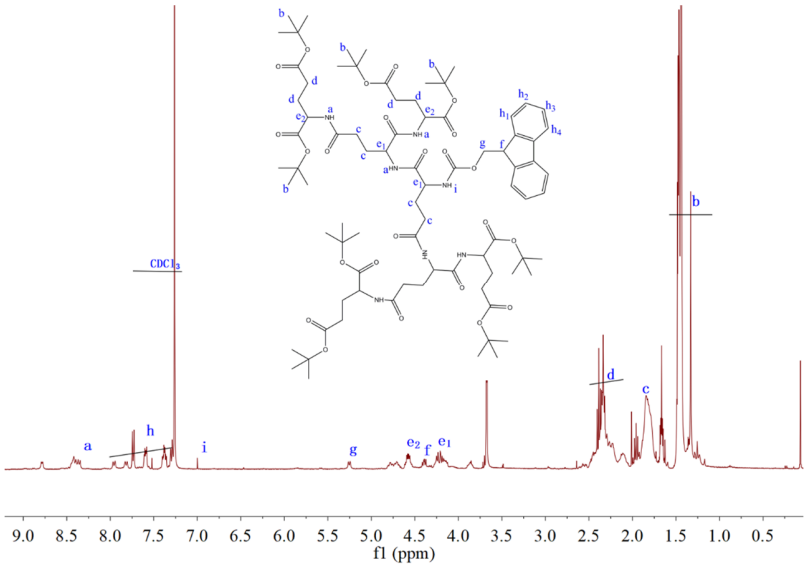


**Figure S15.** $^{1}$H-NMR spectrum of Compound 11 in $CDCl_3$.

### *2.2.3 Characterizations of Compound 13*

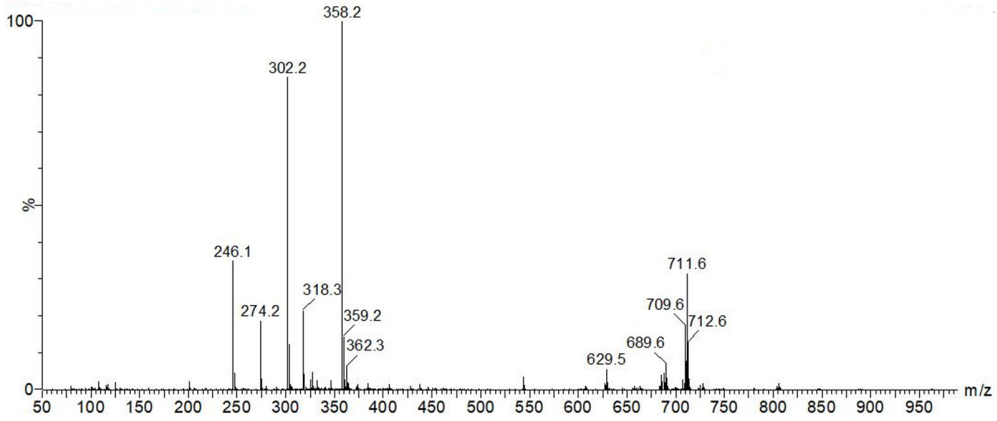


**Figure S16.** ESI-TOF mass spectrum of Compound 13 (m/z, $[M+Na]^+$): 712.1 (calculated), 711.60 (observed).

### *2.2.4 Characterizations of Compound 15*

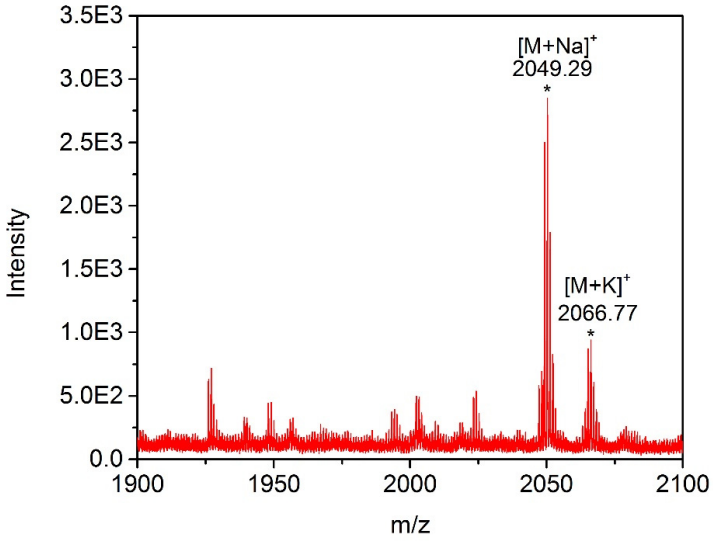


**Figure S17.** MALDI-TOF mass spectrum of Compound 15 MS (m/z, $[M+Na]^+$): 2050.77 (calculated), 2049.29 (observed). MS (m/z, $[M+K]^+$): 2066.77 (calculated), 2066.77 (observed).

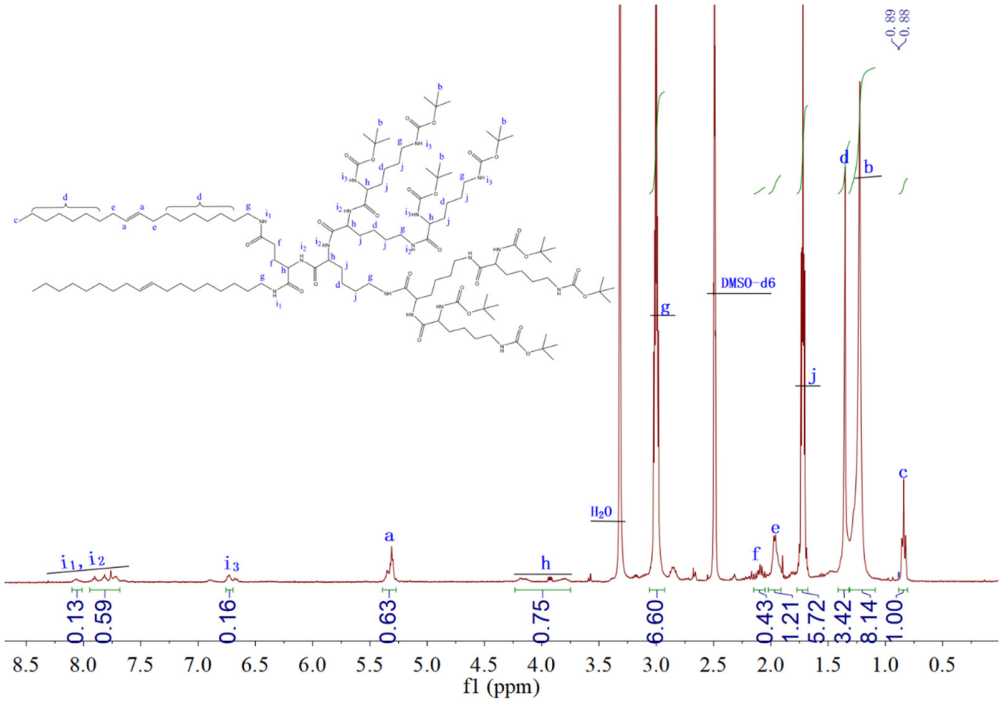


**Figure S18.** $^1$H-NMR spectrum of Compound 15 in $CDCl_3$.

### *2.2.5 Characterizations of Compound 16*

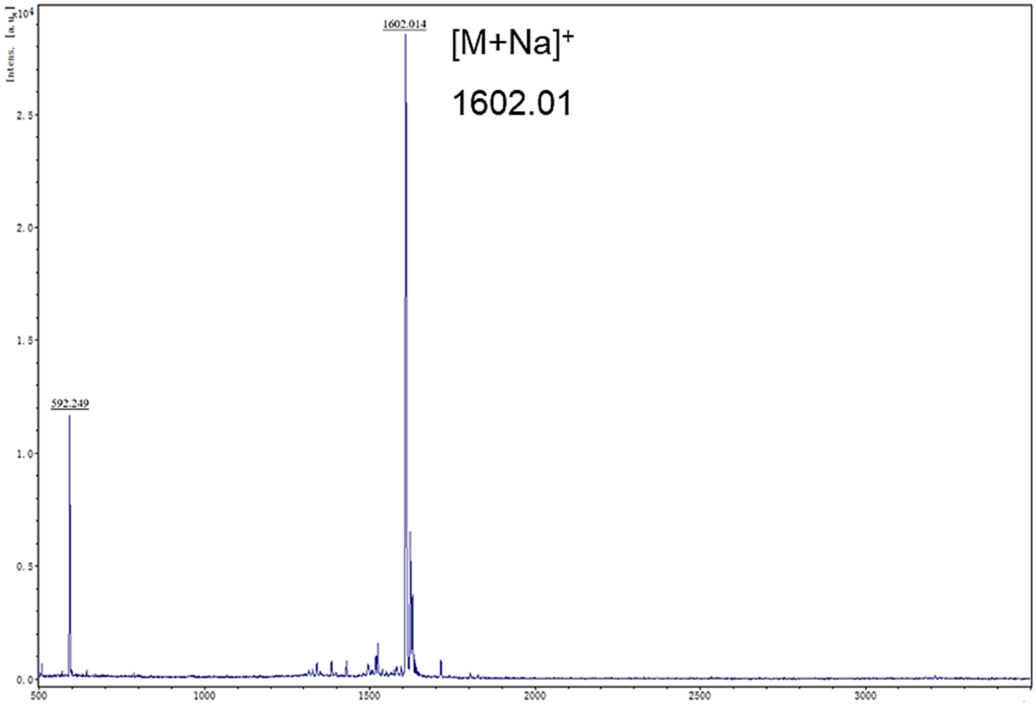


**Figure S19.** MALDI-TOF mass spectrum of Compound 16 MS (m/z, $[M+Na]^+$): 1601.90 (calculated), 1602.01 (observed).

# 3 Supporting Results

## *3.1 Particle size, Zeta potential, DL and EE*

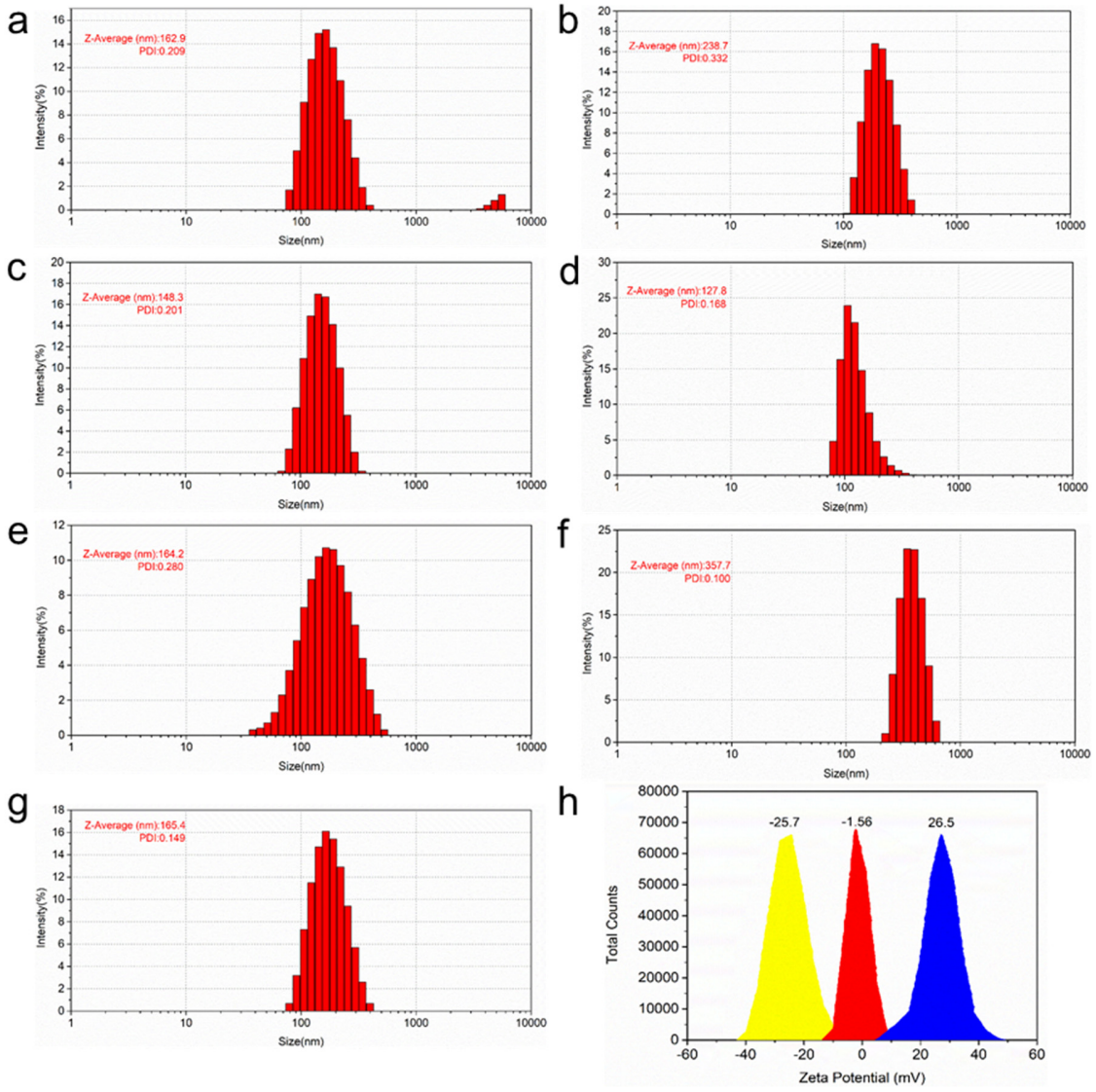


**Figure S20.** Particle size of (a) ANPs, (b) MNPs (4:1), (c) MNPs (2:1), (d) ICNPs, (e) MNPs (1:2), (f) MNPs (1:4) and (g) CNPs. (h) Zeta potential of ANPs (yellow), ICNPs (red), CNPs (blue).

The DL of CoQ10-CNPs was 14.41% ± 0.39 (n = 3), and EE of CoQ10-CNPs was 85.20% ± 0.51 (n = 3). The DL of CoQ10-ANPs was 14.18% ± 0.22 (n = 3), the EE of CoQ10-ANPs was 70.4% ± 0.34 (n = 3). The DL of CoQ10-(ICNPs) was 13.87% ± 0.28 (n = 3), the EE of CoQ10-(ICNPs) was 72.6% ± 0.44 (n = 3).

### *3.2 Stability Analysis*

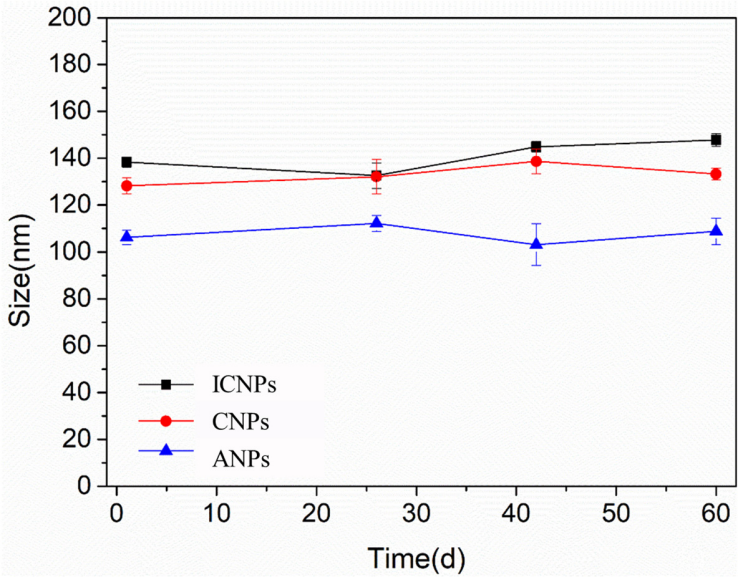


**Figure S21.** Stability analysis for the size changes of ANPs, ICNPs and CNPs in PBS (pH 7.4) determined by DLS (means ± SD, n=3).

### *3.3 Charge-tunable Behavior Study*

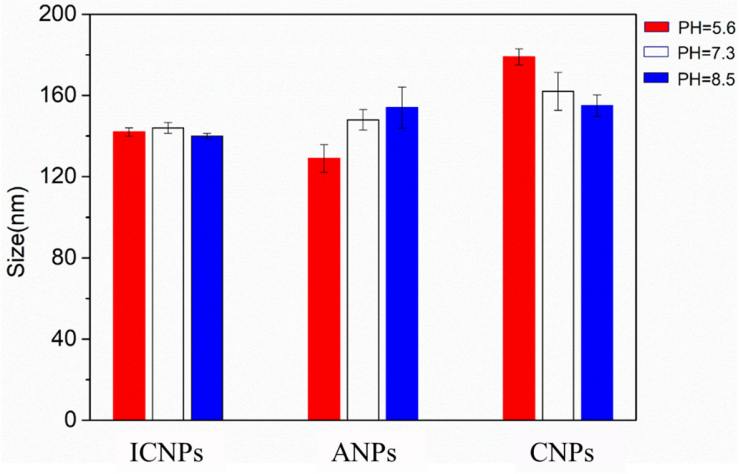


**Figure S22.** Size changes of ANPs, ICNPs and CNPs at different pH values (pH 8.5, 7.3 and 5.6) in PBS determined by DLS (means ± SD, n=3).

### *3.4 The cell cytotoxicity against MOVAS cells, U87MG cells and NIH/3T3 fibroblast cells*

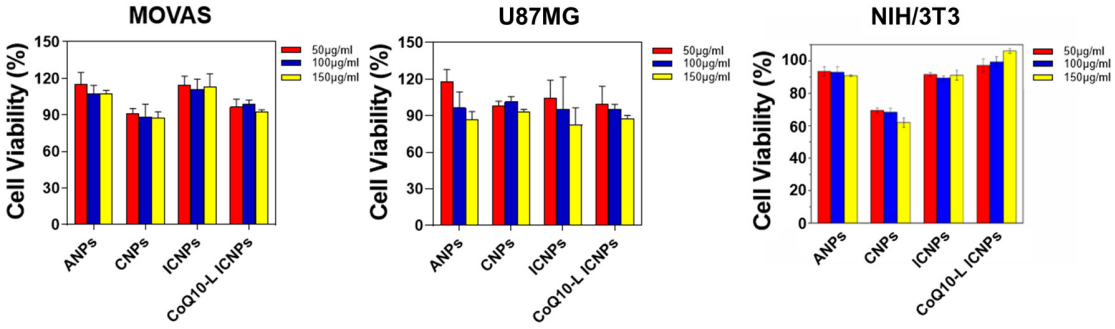


**Figure S23.** The cell cytotoxicity against MOVAS cells, U87MG cells and NIH/3T3 fibroblast cells of ANPs, CNPs and ICNPs.

### *3.5 Protein adsorption*

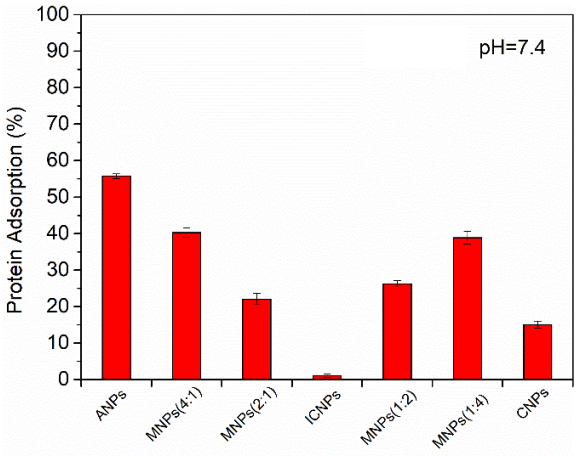


**Figure S24.** Protein adsorption of ANPs, ICNPs, CNPs and MNPs with different ratios of ADLs and CDLs in the presence of FBS in an aqueous solution (n = 3).

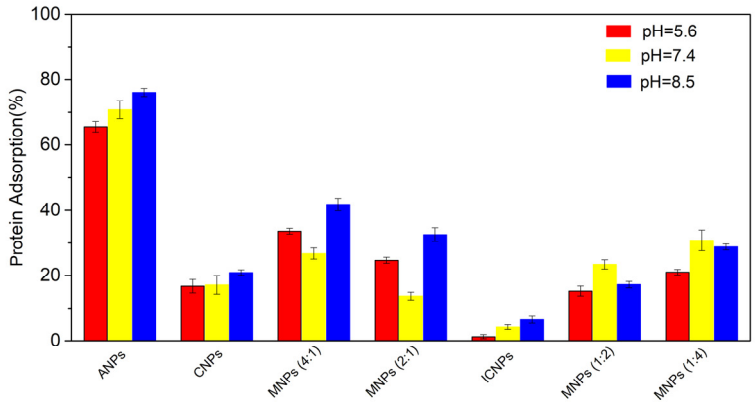


**Figure S25.** Protein adsorption of ANPs, ICNPs, CNPs and MNPs with different ratios at different pH values (pH 8.5, 7.4 and 5.6) in PBS.

### *3.6 In vitro release*

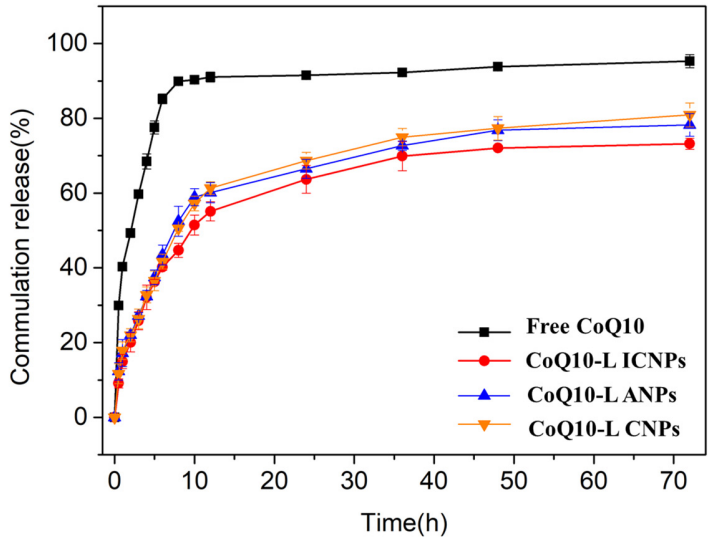


**Figure S26**. COQ10-loaded nanosystems in vitro release profile.

### *3.7 In vitro cellular uptake*

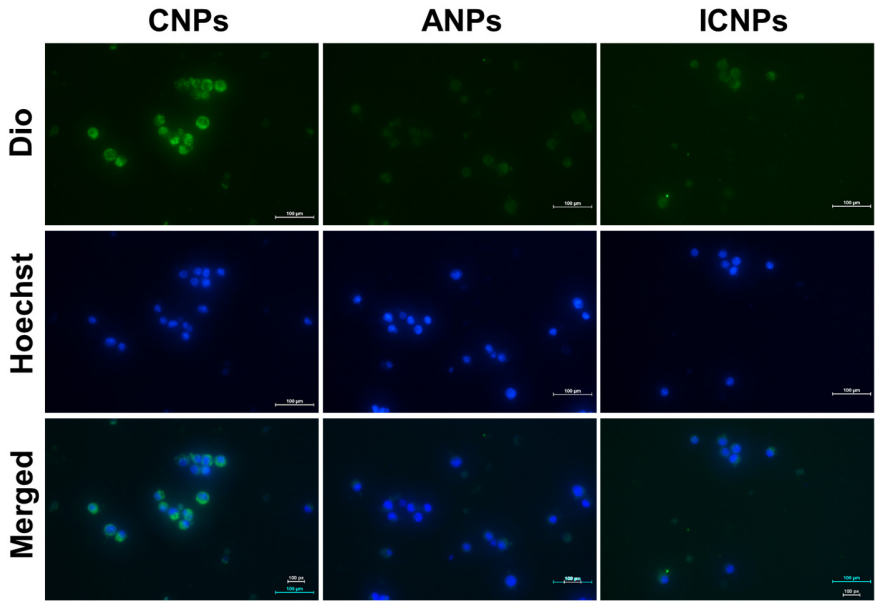


**Figure S27**. Fluorescent image of RAW 264.7 macrophage incubated with Dio-loaded nanosystems.