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Supporting Information

Polymeric micelle-mediated delivery of DNA-targeting organometallic complexes for resistant ovarian cancer treatment

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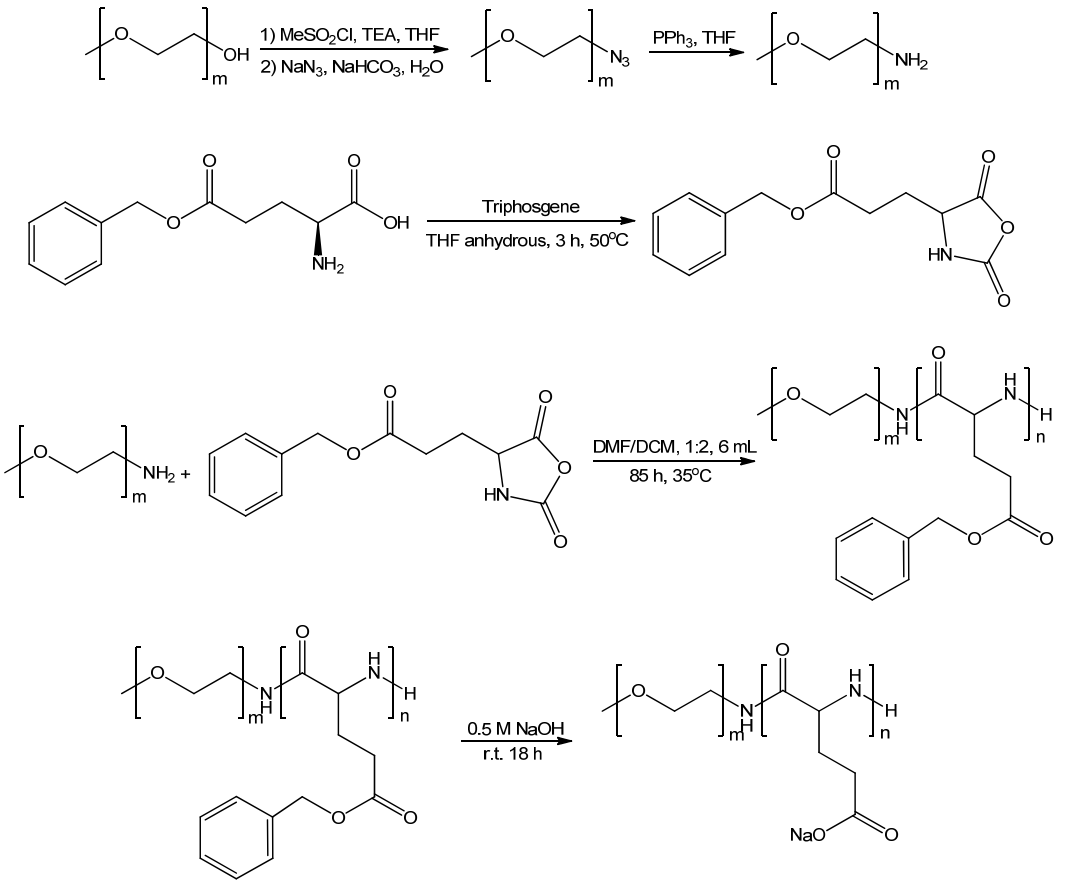
1. Synthesis of PEG-b-P(Glu) copolymer

CH_3O-PEG-NH_2 (MW_{PEG} = 10 kDa) was synthesized by modification of a previously reported method.[1] CH_3O-PEG-OH (5 g, 0.5 mmol) was co-evaporated with 30 mL of toluene to remove water, followed by addition of 50 mL tetrahydrofuran (THF) and methyl sulfonyl chloride (0.122 mL, 1.5 mmol). Triethylamine (0.229 mL, 1.65 mmol) diluted in 20 mL THF was added dropwise to the solution over 10 min at 0ºC and stirred overnight at 60ºC. The resultant precipitate was dissolved in 50 mL of water and the solution was cooled on an ice bath. To this solution was added 0.5 mL of 1 N $NaHCO_3$ (0.5 mL) and sodium azide (0.13 g, 2.0 mmol). THF was subsequently removed and the remaining aqueous solution was refluxed for 24 h before extraction with dichloromethane (DCM, 4 × 100 mL). The combined organic layers were dried over sodium sulfate and concentrated to yield CH_3O-PEG-azide. CH_3O-PEG-azide was then dissolved in 50 mL THF, and triphenylphosphine (0.314 g, 1.2 mmol) was added. The solution was stirred at 50^{o}C for 10 h. Upon cooling to room temperature, 3 mL water was added and the solution continued to stir overnight. THF was removed under vacuum, followed by addition of 100 mL water. The triphenylphosphinoxide precipitate was removed first by centrifugation at 13,000 rpm for 15 min, followed by filtration through a 0.22 μm filter. The water was removed in vacuo to yield CH_3O-PEG-NH_2 as a white solid. Yield: 4.5 g, 90%.

N-carboxyl anhydride of L-glutamic acid γ-benzyl ester was synthesized by the Fuchs-Farthing method using triphosgene.[2] L-glutamic acid γ-benzyl ester (1 g, 4.2 mmol) was dissolved in 20 mL of anhydrous THF and warmed to 50^{o}C. After an equivalent of triphosgene was added, the reaction mixture was allowed to stir at 50^{o}C for 3 h until a

completely homogeneous solution formed. The reaction mixture was then poured into 60 mL of hexane, and the resulting suspension was kept at -20°C overnight. N-carboxyl anhydride of L-glutamic acid γ-benzyl ester was obtained as white crystalline solid after filtration and washed with cold hexane. Yield: 868 mg, 78.2%.

The PEG-b-P(Glu) copolymer was synthesized by the previously reported procedure with slight modifications.[3] Briefly, N-carboxyl anhydride of L-glutamic acid γ-benzyl ester (300 mg, 1.14 mmol) in 2 mL of anhydrous N,N-dimethylformamide (DMF) was added to CH_3O-PEG-NH_2 (570 mg, 0.057 mmol) in 4 mL of anhydrous DCM and stirred for 85 h at 35°C under inert atmosphere. The product, PEG-b-poly(γ-benzyl-L-glutamate) (PEG-b-PBLG), was precipitated with diethyl ether. The degree of polymerization for PBLG was determined to be 20 by comparing the proton ratio of PEG (-OCH_2CH_2-: 3.688 ppm) and the phenyl group of PBLG (-$CH_2C_6H_5$: 7.350 ppm) by ^{1}H NMR ($CDCl_3$). PEG-b-PBLG (100 mg) was hydrolyzed with 40 mL of 0.5 M NaOH for 20 h at room temperature, and then dialyzed against DI water for 24 h. Completion of hydrolysis was confirmed by ^{1}H NMR in D_2O at room temperature.



Scheme S1. Synthesis of PEG-b-P(Glu) copolymer.

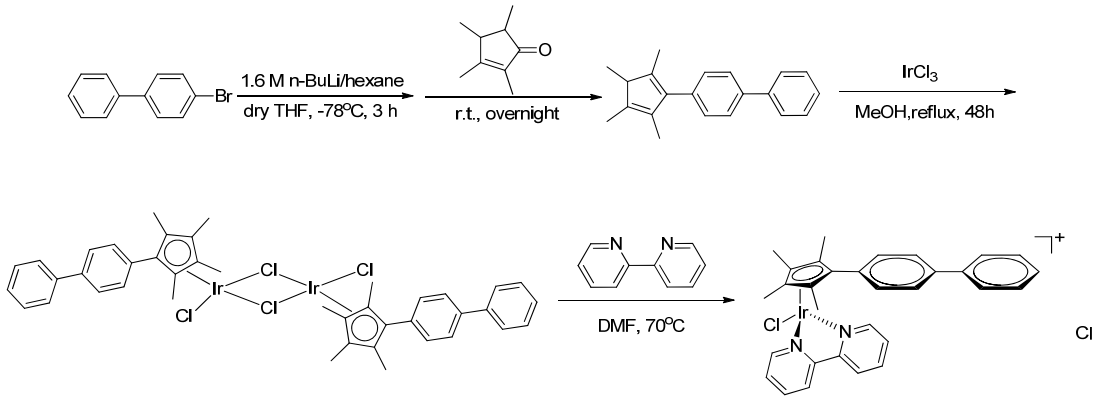
2. Synthesis of organometallic complexes

2.1. Synthesis of [(η^5-$C_5Me_4C_6H_4C_6H_5$)IrCl(bpy)]Cl (1)

[(η^5-$C_5Me_4C_6H_5$ C_6H_5)$IrCl_2$]$_2$ was synthesized by following the literature reported procedure.[4] 4-bromo-biphenyl (5.7 g, 24.5 mmol) was dissolved in dry THF (100 mL) and treated with 2.5 M n-BuLi/hexane solution (9.8 mL, 24.5 mmol) at -78^{o}C. After stirring at this temperature for 3 h, 2,3,4,5-tetramethyl-2-cyclopentenone (4.1 g, 29.4 mmol) was added. The reaction mixture was allowed to warm slowly to ambient temperature with stirring overnight. The resulting yellow solution was acidified with HCl (36%, 10 mL), stirred for another 30 min, then THF was removed by rotary evaporation. The water layer was extracted with DCM several times and washed with $NaHCO_3$ and water twice. The combined organic portions were dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness on a rotary evaporator to afford a light yellow powder. The product $Cp^{xbiph}H$ was recrystallized from chloroform and hexane (3:1). Yield: 4.25 g, 63%.

$Cp^{xbiph}H$ (0.2 g, 0.71 mmol) and $IrCl_3$•$3H_2O$ (0.25 g, 0.71 mmol) dissolved in methanol (20 mL) were heated under reflux in an N_2 atmosphere for 48 h. The reaction mixture was allowed to cool to ambient temperature and the dark green precipitate was filtered off. The volume of the dark red filtrate was reduced to 5 mL on a rotary evaporator. Upon cooling to ambient temperature, an orange precipitate appeared which was collected by filtration. The product was washed with methanol and diethyl ether, and dried in air. Yield: 60 mg, 11%.

[(η^5-$C_5Me_4C_6H_4C_6H_5$)IrCl(bpy)]Cl was synthesized according to the previously reported method.[5] [(η^5-$C_5Me_4C_6H_5$ C_6H_5)$IrCl_2$]$_2$ (87 mg, 0.08 mmol) and 2,2'-Bipyridine (25.6 mg, 0.16 mmol) in DMF (2 mL) was heated to 70^{o}C in an N_2 atmosphere overnight. The crude product was purified by silica gel column chromatography using a mixture of DCM and methanol (10:1, v/v). Yield: 11 mg, 10.5%. MS: *m/z* (%) = 657 $[M - Cl]^+$. NMR (400 MHz, $CDCl_3$, δ): 8.995 (d, 2H), 8.530 (d, 2H), 8.235 (t, 2H), 7.718 (d, 2H), 7.646 (m, 4H), 7.568 (d, 2H), 7.484 (t, 2H), 7.404 (t, 1H), 1.790 (s, 6H), 1.758 (s, 6H).

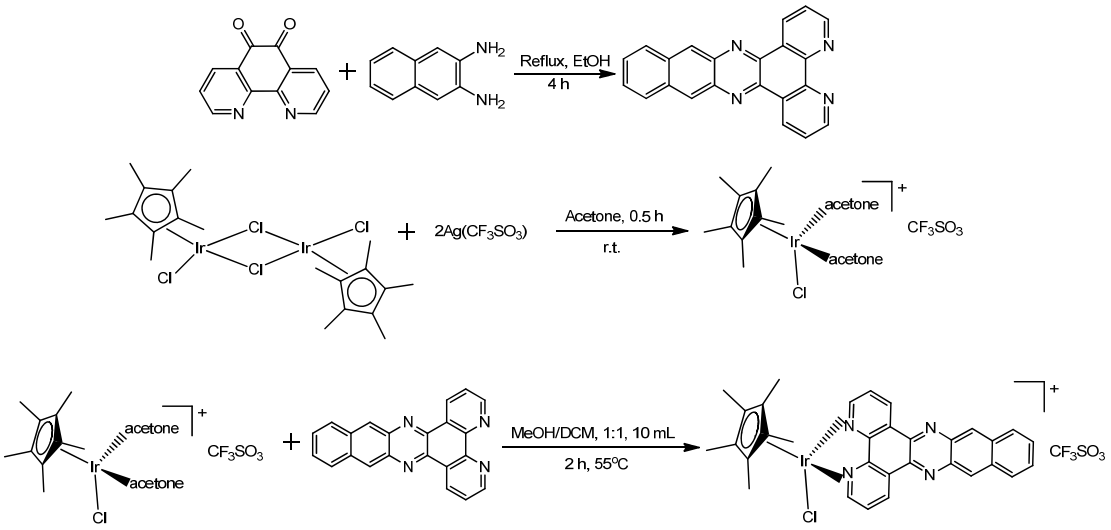


Scheme S2. Synthesis of $[(\eta^5\text{-}C_5Me_4C_6H_4C_6H_5)IrCl(bpy)]Cl$ (**1**).

2.2. Synthesis of $[(\eta^5\text{-}C_5Me_5)IrCl(dppn)](CF_3SO_3)$ (2)

The ligand dppn was synthesized by refluxing 1,10-phenanthroline-5,6-dione (130.6 mg, 0.62 mmol) and 2,3-diaminonaphthalene (110.6 mg, 0.7 mmol) in ethanol for 4 h. The yellow precipitates were collected, washed with cold ethanol, and dried under vacuum. Yield: 150 mg, 45%.

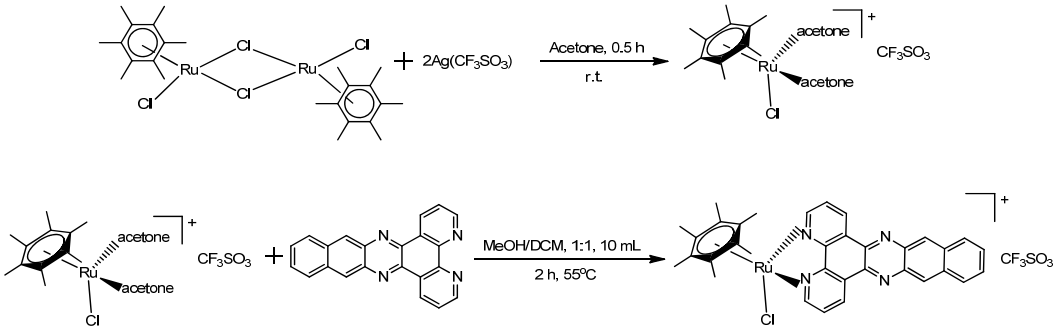
$[(\eta^5\text{-}C_5Me_5)IrCl(dppn)](CF_3SO_3)$ was synthesized by following the literature procedure.[6] Briefly, two equivalents of $Ag(CF_3SO_3)$ (25.7 mg, 0.1 mmol) were added to $[\{(\eta^5\text{-}C_5Me_5)IrCl_2\}_2]$ (40.0 mg, 0.05 mmol) in 10 mL acetone and stirred in the dark for 0.5 h. Filtration of the resulting AgCl precipitate and subsequent solvent removal under vacuum afforded $[(\eta^5\text{-}C_5Me_5)IrCl(acetone)_2](CF_3SO_3)$, which was stirred with the ligand dppn (33.2 mg, 0.1 mmol) in CH_3OH/CH_2Cl_2 (1:1, 10 mL) at 55°C for 2 h. Following volume reduction of the resulting clear solution to 2 mL and addition of CH_3OH (3 mL), the product was precipitated with diethyl ether, washed and dried in vacuo. Yield: 62 mg, 73%. MS: m/z = 695 $[M - CF_3SO_3]^+$. ^1H NMR (400 MHz, d_6-DMSO, δ): 1.755 (s, 15H, Cp*), 7.689 (m, 2H, dppn), 8.329 (m, 2H, dppn), 8.412 (m, 2H, dppn), 9.180 (s, 2H, dppn), 9.431 (dd, 2H, dppn), 9.732 (dd, 2H, dppn).



Scheme S3. Synthesis of $[(\eta^5\text{-}C_5Me_5)IrCl(dppn)](CF_3SO_3)$ (**2**).

2.3. Synthesis of $[(\eta^6\text{-}C_6Me_6)RuCl(dppn)](CF_3SO_3)$ (3)

$[(\eta^6\text{-}C_6Me_6)RuCl(dppn)](CF_3SO_3)$ was synthesized by following the literature procedure.[7] Briefly, two equivalents of $Ag(CF_3SO_3)$ (25.7 mg, 0.1 mmol) were added to $[\{(\eta^6\text{-}C_6Me_6)RuCl_2\}_2]$ (34.0 mg, 0.05 mmol) in 10 mL acetone and stirred in the dark for 0.5 h. Filtration of the resulting AgCl precipitate and subsequent solvent removal under vacuum afforded $[(\eta^6\text{-}C_6Me_6)RuCl(acetone)_2](CF_3SO_3)$, which was stirred with the ligand dppn (33.2 mg, 0.1 mmol) in CH_3OH/CH_2Cl_2(1:1, 10 mL) at 55°C for 2 h. Following volume reduction of the resulting clear solution to 2 mL and addition of CH_3OH (3 mL), the product was precipitated with diethyl ether, washed and dried in vacuo. Yield: 54 mg, 70%. MS: *m/z* (%) = 631 $[M - CF_3SO_3]^+$. ^{1}H NMR (400 MHz, d_6-DMSO, δ): 2.147 (s, 18 H, CH_3 C_6Me_6), 7.791 (m, 2 H, dppn), 8.30 (m, 2 H, dppn), 8.450 (m, 2 H, dppn), 9.224 (s, 2 H, dppn), 9.417 (dd, 2 H, dppn), 9.684 (dd, 2 H, dppn) ppm.



Scheme S4. Synthesis of $[(\eta^6\text{-}C_6Me_6)RuCl(dppn)](CF_3SO_3)$ (**3**).

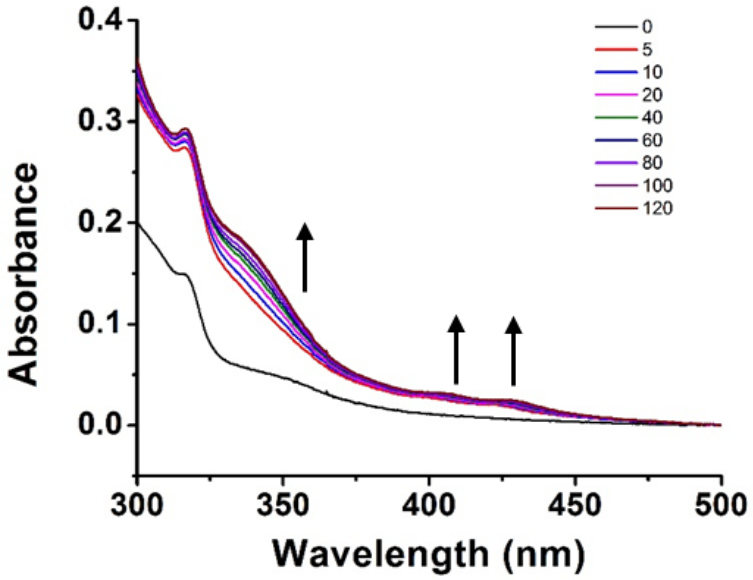


Figure S1. UV-vis spectra for the titrations of **1** (10 μM) in PBS (pH 7.2) with DNA (0-120 μM nucleotide).

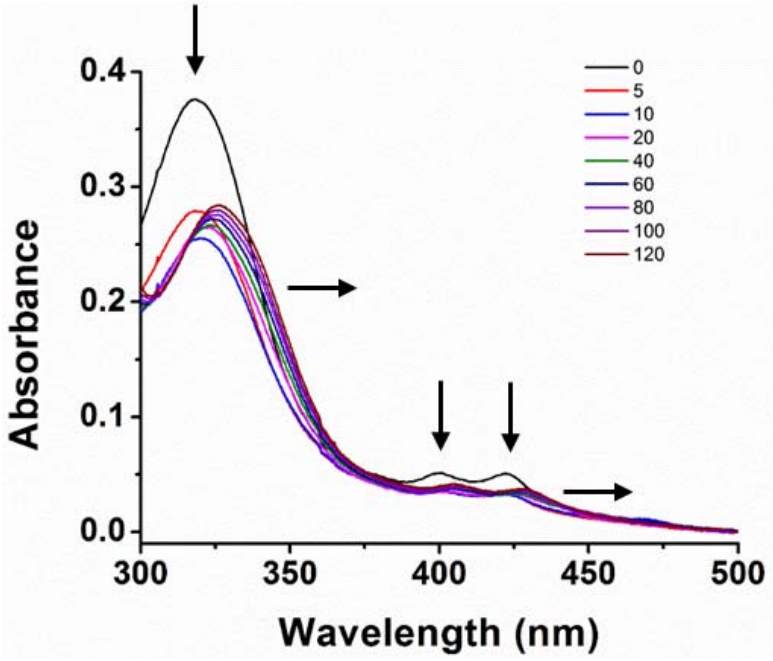


Figure S2. UV-vis spectra for the titrations of **2** (10 μM) in PBS (pH 7.2) with DNA (0-120 μM nucleotide).

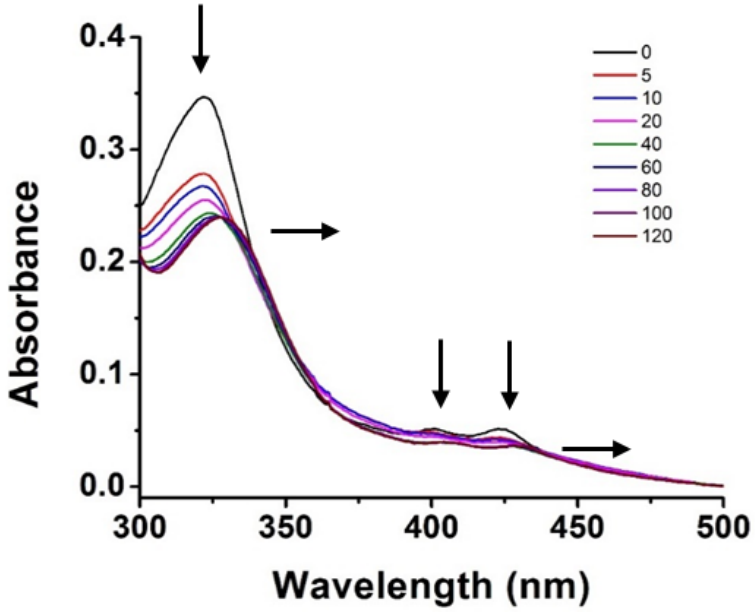


Figure S3. UV-vis spectra for the titrations of **3** (10 μM) in PBS (pH 7.2) with DNA (0-120 μM nucleotide).

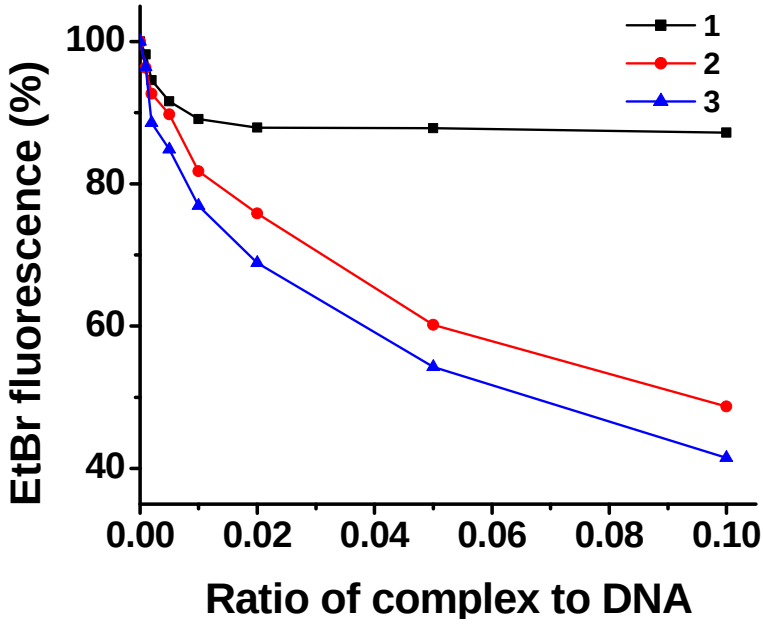


Figure S4. The decrease in the fluorescence of EtBr-bound DNA in the presence of increasing amounts of complexes **1**-**3**.

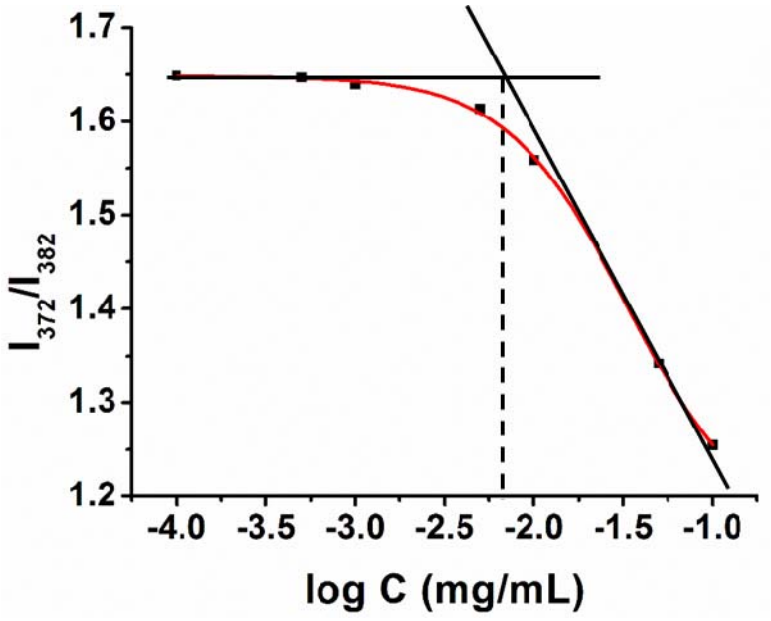


Figure S5. CMC determination of **m1** using pyrene as a probe. I_{372}/I_{382} refers to the ratio of the emission intensity of the first and third peaks in the fluorescence spectrum of pyrene. The CMC was obtained from the intersection of the two tangent lines as shown in the figure.

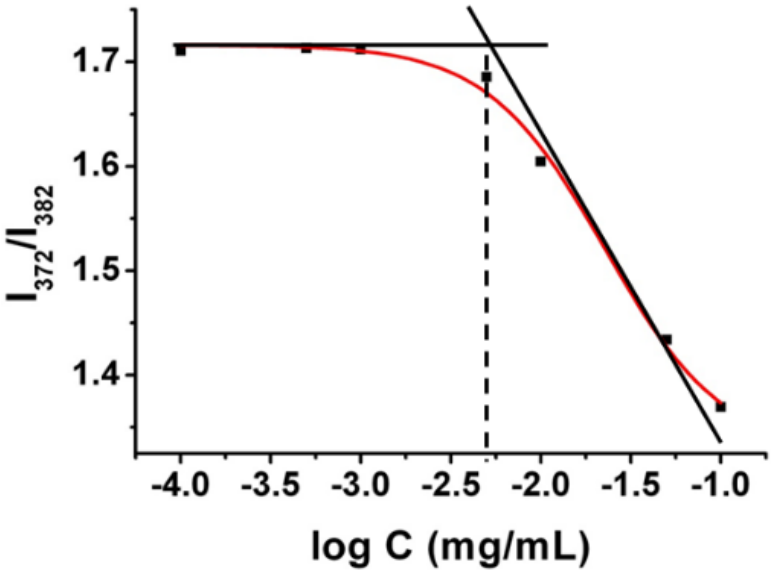


Figure S6. CMC determination of **m2** using pyrene as a probe I_{372}/I_{382} refers to the ratio of the emission intensity of the first and third peaks in the fluorescence spectrum of pyrene. The CMC was obtained from the intersection of the two tangent lines as shown in the figure.

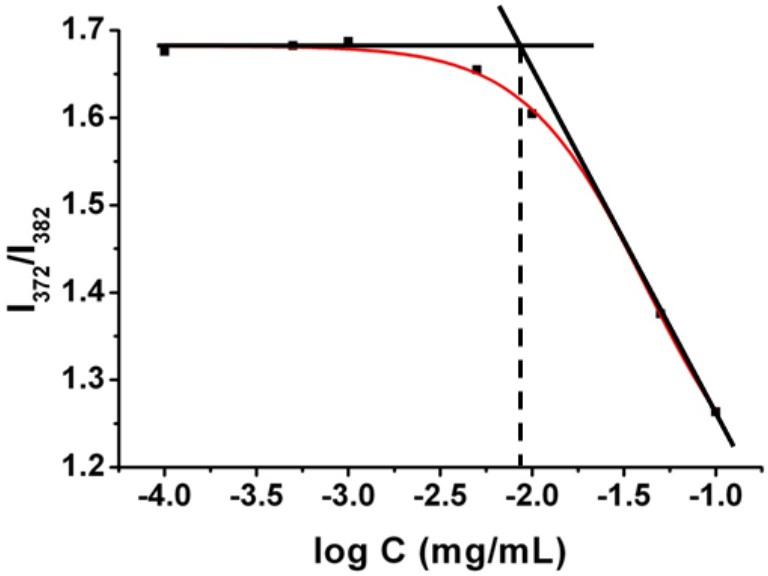


Figure S7. CMC determination of **m3** using pyrene as a probe I_{372}/I_{382} refers to the ratio of the emission intensity of the first and third peaks in the fluorescence spectrum of pyrene. The CMC was obtained from the intersection of the two tangent lines as shown in the figure.

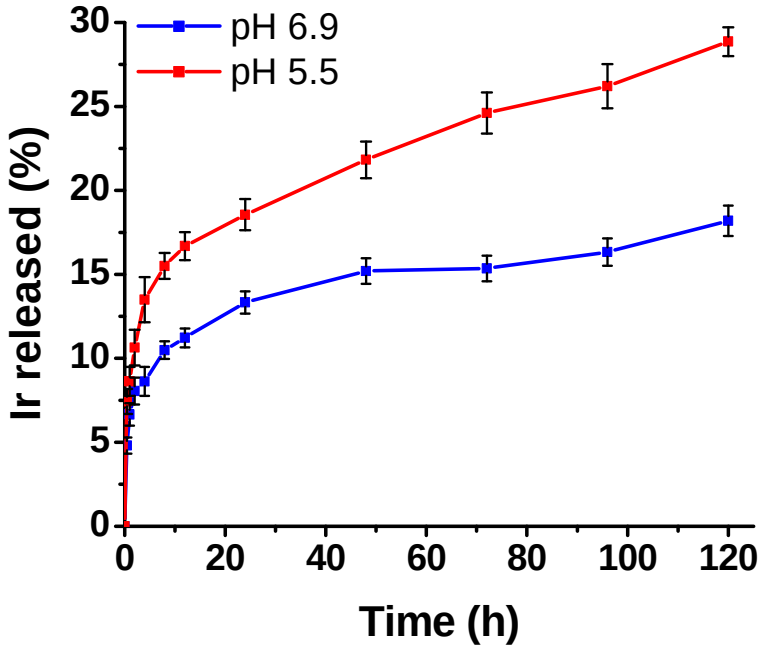


Figure S8. Cumulative release of **1** from **m1** under different conditions mimicking the early endosomes (10 mM PBS, pH 6.9, and 20 mM NaCl), and late endosomes and lysosomes (10 mM PBS, pH 5.5, and 70 mM NaCl) at 37°C.

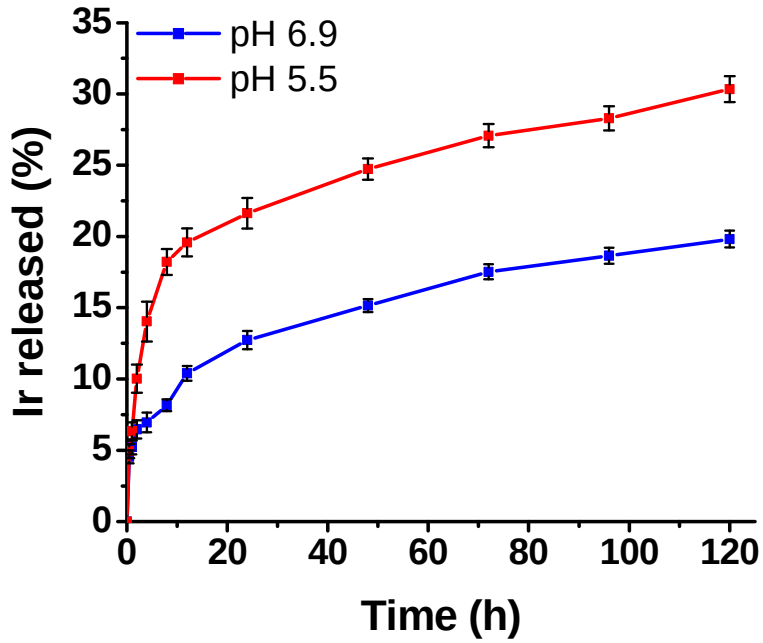


Figure S9. Cumulative release of **2** from **m2** under different conditions mimicking the early endosomes (10 mM PBS, pH 6.9, and 20 mM NaCl), and late endosomes and lysosomes (10 mM PBS, pH 5.5, and 70 mM NaCl) at 37°C.

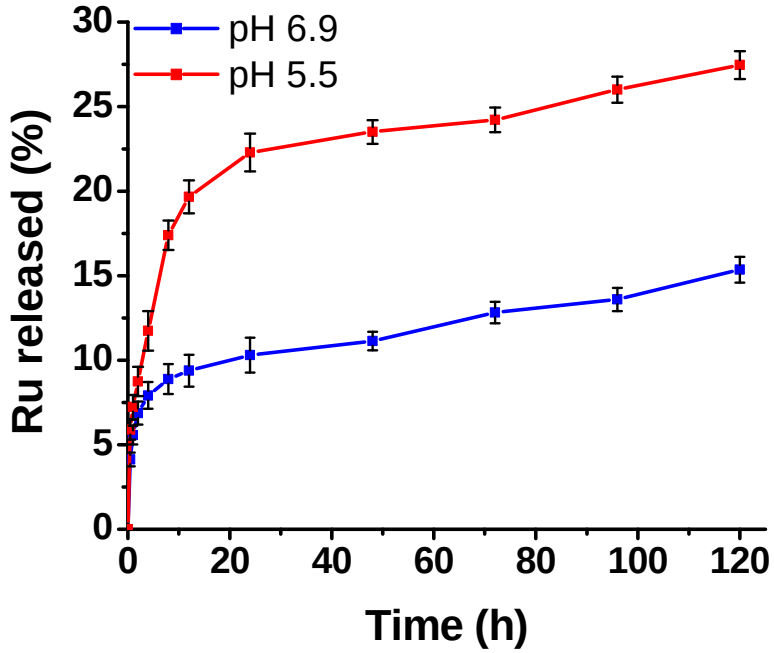


Figure S10. Cumulative release of **3** from **m3** under different conditions mimicking the early endosomes (10 mM PBS, pH 6.9, and 20 mM NaCl), and late endosomes and lysosomes (10 mM PBS, pH 5.5, and 70 mM NaCl) at 37°C.

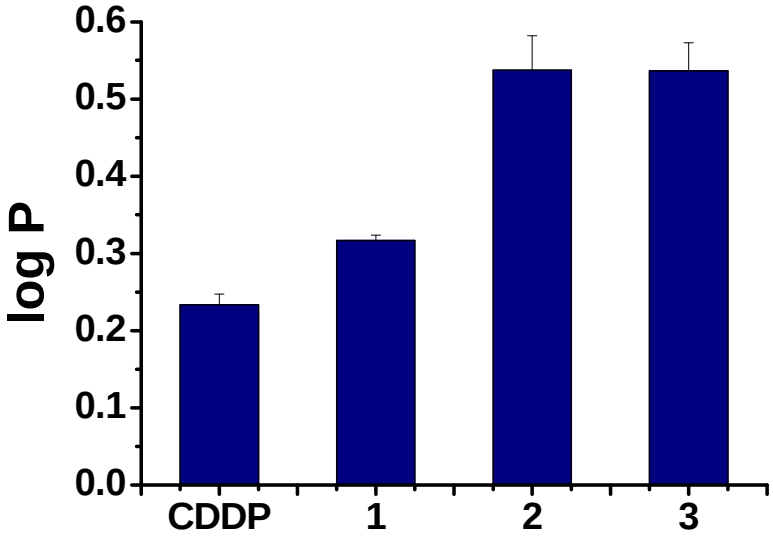


Figure S11. log P value of CDDP and complexes **1-3**.

Table S1. Characterization of RhB-micelles. Data are expressed as means ± S.D. (n = 3).

	TEM diameter [nm]	Number-Ave diameter [nm]	PDI[a)]	Zeta potential [mV]	Drug loading[b)]	[Complex]/[Glu][c)]
RhB-m1	44.6 ± 8.7	65.7 ± 3.42	0.190 ± 0.01	-9.86 ± 0.40	34.9 ± 0.22%	0.54 ± 0.01
RhB-m2	42.4 ± 7.6	56.3 ± 0.97	0.153 ± 0.01	-10.8 ± 0.26	35.1 ± 0.46%	0.46 ± 0.02
RhB-m3	43.2 ± 7.2	64.9 ± 3.34	0.129 ± 0.04	-13.5 ± 1.21	32.3 ± 0.58%	0.43 ± 0.02

[a)]Polydispersity index; [b)]Weight ratio of complex to micelle; [c)]Weight concentration of micelles.

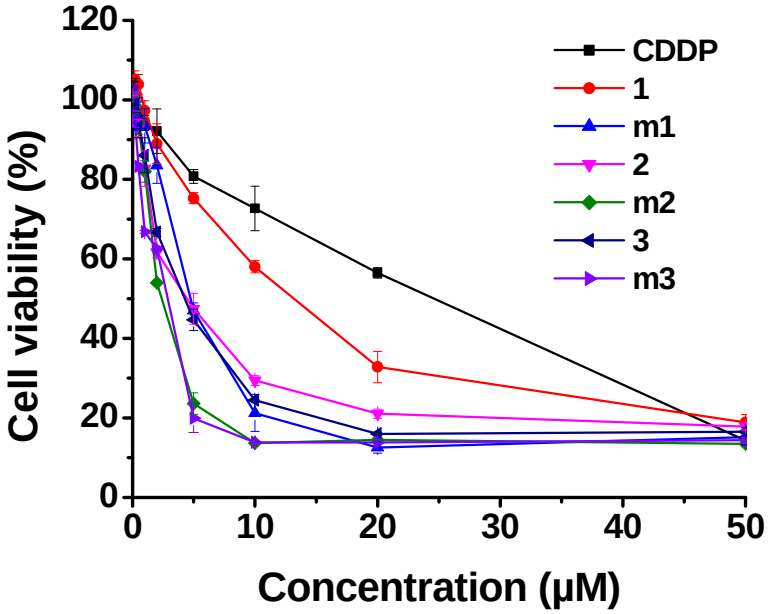


Figure S12. Cytotoxicity of CDDP, complexes **1-3** and **m1-m3** against OVCAR-3 cells. The cells were incubated with CDDP, complexes or micelles for 48 h followed by the MTS assay.

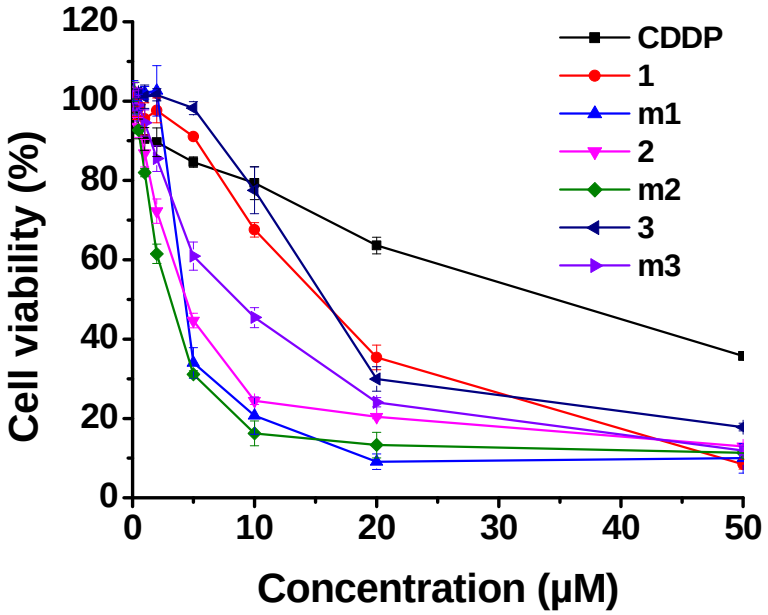


Figure S13. Cytotoxicity of CDDP, complexes **1-3** and **m1-m3** against SKOV-3 cells. The cells were incubated with CDDP, complexes or micelles for 48 h followed by the MTS assay.

Table S2. IC_{50} value (μM) of CDDP, complexes **1-3** and **m1-m3** against OVCAR-3 and SKOV-3 cells after 48 h incubation. Data are expressed as means ± S.D. (n = 3).

	CDDP	**1**	**m1**	**2**	**m2**	**3**	**m3**
OVCAR-3 cells	15.5 ± 0.14	4.14 ± 0.33	1.65 ± 0.03	1.92 ± 0.03	1.33 ± 0.11	5.19 ± 0.21	2.76 ± 0.36
SKOV-3 cells	37.07 ± 1.32	16.05 ± 0.75	4.76 ± 0.14	5.41 ± 0.22	3.61 ± 0.08	16.33 ± 0.53	10.61 ± 0.73

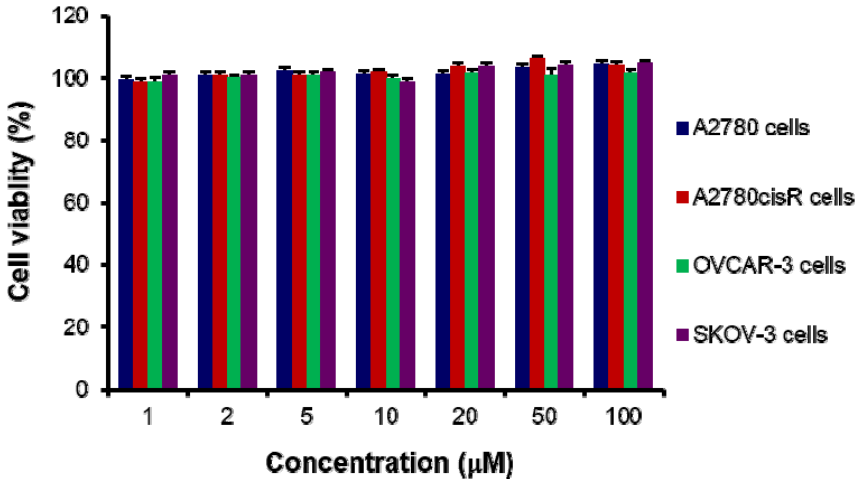


Figure S14. Cytotoxicity of the PEG-b-P(Glu) copolymer against A2780, A2780cisR, OVCAR-3 and SKOV-3 cells after incubation for 48 h followed by the MTS assay.

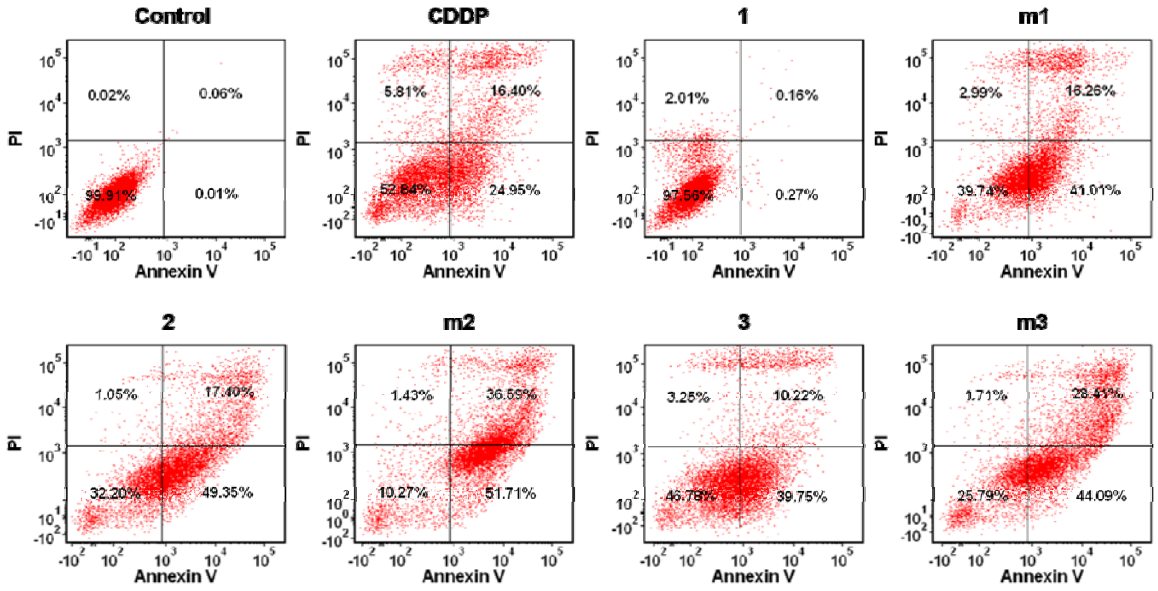


Figure S15. Annexin V/PI analysis of A2780 cells after the incubation with CDDP, complexes **1-3** and **m1-m3** for 48 h. The quadrants from lower left to upper left (counter clockwise) represent healthy, early apoptotic, late apoptotic, and necrotic cells, respectively. The percent of cells in each quadrant was shown on the graphs.

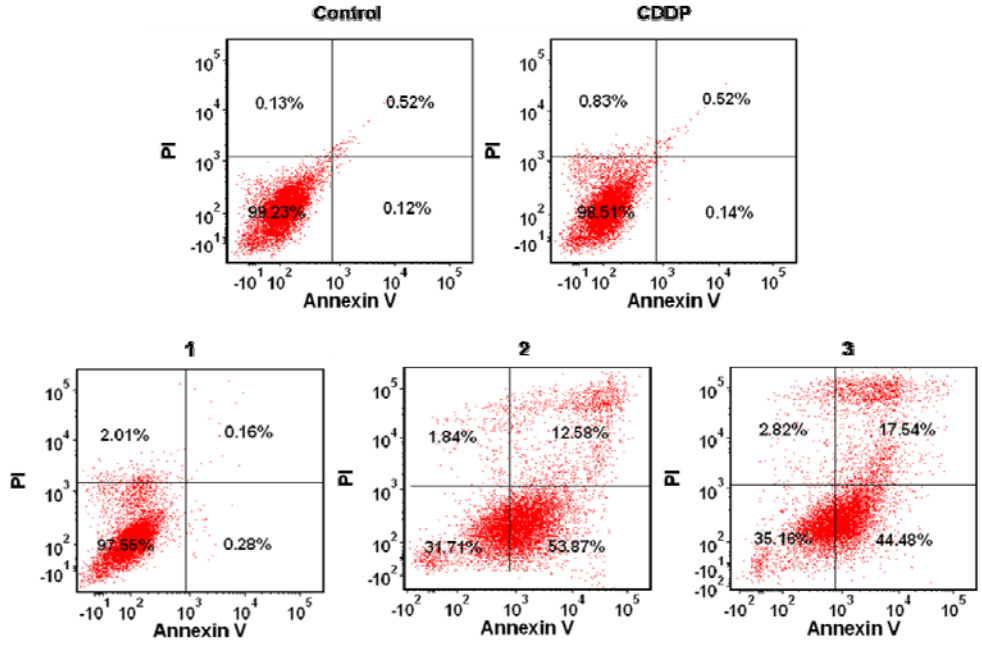


Figure S16. Annexin V/PI analysis of A2780cisR cells after the incubation with CDDP and complexes **1-3** for 48 h. The quadrants from lower left to upper left (counter clockwise) represent healthy, early apoptotic, late apoptotic, and necrotic cells, respectively. The percentage of cells in each quadrant was shown on the graphs.

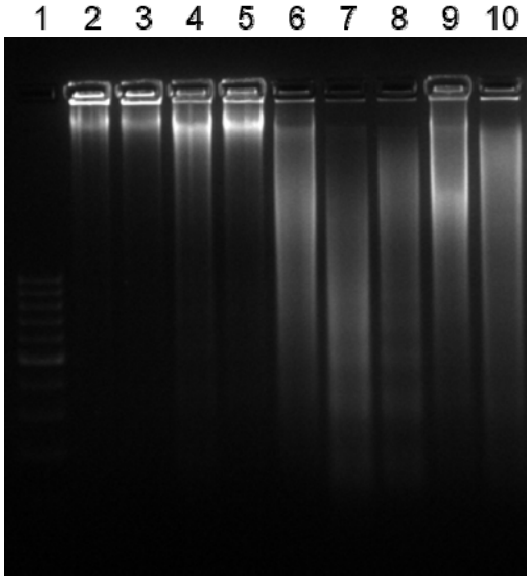


Figure S17. Analysis of DNA ladder on 2% (w/v) agarose gel at 35 V for 3 h after DNA extraction from the A2780 cells treated with CDDP, complexes and micelles. Lanes 1-10: DNA marker, control, PEG-b-p(Glu) copolymer, CDDP, **1**, **m1**, **2**, **m2**, **3**, and **m3**, respectively.

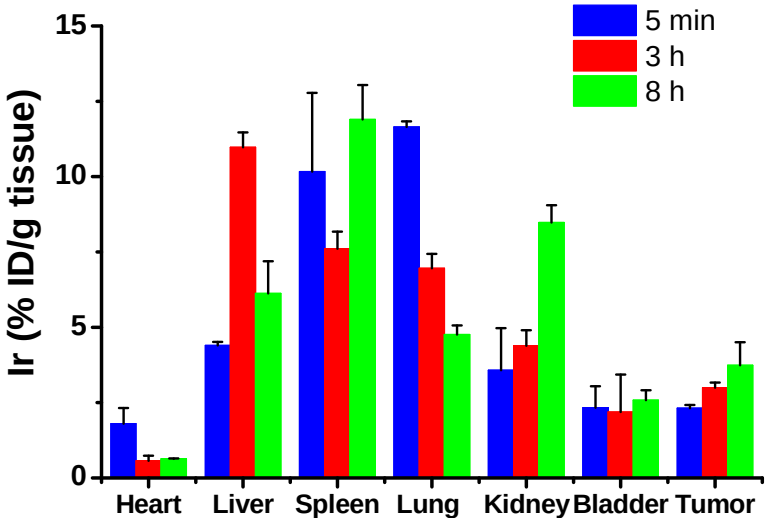


Figure S18. The biodistribution of **m2** in A2780cisR tumor bearing mice at different time after intravenous injection at a dose of 5 mg/kg based on iridium.

References

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