

## ESI

### **Core-Shell Polymer Nanoparticles for Prevention of GSH Drug Detoxification and Cisplatin Delivery to Breast Cancer Cells**

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### Synthetic procedures for PEG<sub>x</sub>-*b*-BPCL di block polymers:

Synthetic procedure for PEG<sub>5000</sub>-*b*-BPCL diblock copolymer is discussed in detail in the main manuscript and the remaining series of polymers were prepared in a similar manner.

**Synthesis of BPCL:** CCL (1 g, 3.87 mmol), TEG (6.35 mg, 0.038 mmol) and Sn(Oct)<sub>2</sub> (7.7 mg, 0.019 mmol). **Yield:** 690 mg (69 %). **<sup>1</sup>H NMR** (400MHz, CDCl<sub>3</sub>) δ ppm: 4.15 (s, 2H), 3.64 (m, 2.1 H), 3.44 (m, 1H), 2.44 (t, 2H), 2.34 (t, 2H), 1.94 – 1.84 (m, 4H), 1.45 (s, 9H). : **FT-IR (cm<sup>-1</sup>):** 2974, 2931, 1726, 1457, 1364, 1256, 1156, 1100, 957, 846, 726 and 647. M<sub>n</sub> (NMR) 23,400 g/mol; M<sub>n</sub> (GPC): 15,000 g/mol; M<sub>w</sub> (GPC) =21,100 g/mol. PDI (GPC) = 1.40

**Synthesis of PEG<sub>350</sub>-*b*-BPCL:** CCL (1 g, 3.87 mmol), mPEG<sub>350</sub> (13.5 mg, 0.038 mmol) and Sn(Oct)<sub>2</sub> (7.7 mg, 0.019 mmol). **Yield:** 760 mg (76 %). **<sup>1</sup>H NMR** (400MHz, CDCl<sub>3</sub>) δ ppm: 4.14 (s, 2H), 3.64 (m, 2.3H), 3.45 (m, 1H), 2.44 (t, 2H), 2.34 (t, 2H), 1.93 – 1.81 (m, 4H), 1.45 (s, 9H). : **FT-IR (cm<sup>-1</sup>):** 2973, 2931, 1726, 1457, 1364, 1251, 1156, 1099, 957, 846, 720 and 647. M<sub>n</sub> (NMR) 23,800 g/mol; M<sub>n</sub> (GPC): 16,300 g/mol; M<sub>w</sub> (GPC) =20,000 g/mol. PDI (GPC) = 1.22.

**Synthesis of PEG<sub>750</sub>-*b*-BPCL:** CCL (1 g, 3.87 mmol), mPEG<sub>750</sub> (28.5 mg, 0.038 mmol) and Sn(Oct)<sub>2</sub> (7.7 mg, 0.019 mmol). **Yield:** 740 mg (74 %). **<sup>1</sup>H NMR** (400MHz, CDCl<sub>3</sub>) δ ppm: 4.14 (s, 2H), 3.64 (m, 2.7 H), 3.45 (m, 1H), 2.44 (t, 2H), 2.34 (t, 2H), 1.93 – 1.81 (m, 4H), 1.45 (s, 9H). : **FT-IR (cm<sup>-1</sup>):** 2973, 2931, 1726, 1457, 1364, 1251, 1156, 1099, 957, 846, 720 and 647. M<sub>n</sub> (NMR) = 25,300 g/mol; M<sub>n</sub> (GPC) = 16,700 g/mol; M<sub>w</sub> (GPC) = 20,200 g/mol. PDI (GPC) = 1.20.

**Synthesis of PEG<sub>2000</sub>-*b*-BPCL:** CCL (1 g, 3.87 mmol), PEG<sub>2000</sub> (76 mg, 0.038 mmol) and Sn(Oct)<sub>2</sub> (7.7 mg, 0.019 mmol). **Yield:** 700 mg (70 %). **<sup>1</sup>H NMR** (400MHz, CDCl<sub>3</sub>) δ ppm: 4.14 (s, 2H), 3.64 (m, 3.8 H), 3.46 (m, 1H), 2.44 (t, 2H), 2.34 (t, 2H), 1.93 – 1.84 (m, 4H), 1.45 (s, 9H). : **FT-IR (cm<sup>-1</sup>):** 2973, 2931, 1726, 1457, 1364, 1251, 1156, 1099, 957, 846, 720 and 647. M<sub>n</sub> (NMR) 26,200 g/mol; M<sub>n</sub> (GPC): 22,400 g/mol; M<sub>w</sub> (GPC) =22,400 g/mol. PDI (GPC) = 1.31.

### Synthetic procedures for PEG-*b*-CPCL di block polymers:

Synthetic procedure for PEG<sub>5000</sub>-*b*-CPCL diblock copolymer is discussed in detail in the main manuscript and the remaining series of polymers were prepared in a similar manner.

**Synthesis of CPCL:**Trifluoroacetic acid (0.2 mL), BPCL (200 mg) and DCM (5.0 mL). <sup>1</sup>H NMR (400MHz, CDCl<sub>3</sub>) δ: 4.14 (t, 2H), 3.84- 3.64 (m 2.1 H), 3.57 (m, 1H), 2.54 (t, 2H), 2.36 (t, 2H), 1.99 – 1.67 (m, 4H). FT-IR (cm<sup>-1</sup>): 3450, 2880, 1720, 1352, 1252, 1180, 1092, 948, 917, 841 and 730. M<sub>n</sub> (NMR) 18,300 g/mol; M<sub>n</sub> (GPC): 9,000 g/mol; PDI (GPC) = 1.30.

**Synthesis of PEG<sub>350</sub>-*b*-CPCL:**Trifluoroacetic acid (0.2 mL), PEG<sub>350</sub>-*b*-BPCL (200 mg) and DCM (5.0 mL). <sup>1</sup>H-NMR (400MHz, CDCl<sub>3</sub>) δ: 4.14 (t, 2H), 3.64 (m 2.28H), 3.57 (m, 1H), 2.56 (t, 2H), 2.38 (t, 2H), 1.99 – 1.67 (m, 4H). FT-IR (cm<sup>-1</sup>): 3450, 2880, 1749, 1352, 1252, 1180, 1092, 948, 917, 841 and 730. M<sub>n</sub> (NMR) 18,700 g/mol; M<sub>n</sub> (GPC): 11,000 g/mol; PDI (GPC) = 1.24.

**Synthesis of PEG<sub>750</sub>-*b*-CPCL:**Trifluoroacetic acid (0.2 mL), PEG<sub>750</sub>-*b*-BPCL (200 mg) and DCM (5.0 mL). <sup>1</sup>H -NMR (400MHz, CDCl<sub>3</sub>) δ: 4.14 (t, 2H), 3.74- 3.64 (m 2.7 H), 3.57 (m, 1H), 2.56 (t, 2H), 2.38 (t, 2H), 1.99 – 1.67 (m, 4H). FT-IR (cm<sup>-1</sup>): 3450, 2880, 1749, 1352, 1252, 1180, 1092, 948, 917, 841 and 730. M<sub>n</sub> (NMR) 19,900 g/mol; M<sub>n</sub> (GPC): 12,200 g/mol; PDI (GPC) = 1.19.

**Synthesis of PEG<sub>2000</sub>-*b*-CPCL:**Trifluoroacetic acid (0.2 mL), PEG<sub>2000</sub>-*b*-BPCL (200 mg) and DCM (5.0 mL). <sup>1</sup>HNMR (400MHz, CDCl<sub>3</sub>) δ: 4.14 (t, 2H), 3.74- 3.64 (m 3.9 H), 3.57 (m, 1H), 2.56 (t, 2H), 2.38 (t, 2H), 1.99 – 1.67 (m, 4H). FT-IR (cm<sup>-1</sup>): 3450, 2880, 1749, 1352, 1252, 1180, 1092, 948, 917, 841 and 730. M<sub>n</sub> (NMR) 21,000 g/mol; M<sub>n</sub> (GPC): 12,700 g/mol; PDI (GPC) = 1.18.

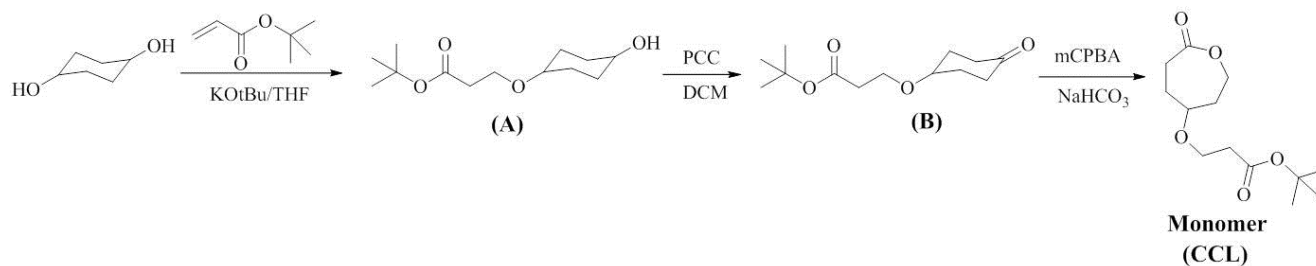
### **Synthesis of the Polymer-cisplatin Conjugate:**

**Synthesis of CPCL-CP:** CPCL (20 mg), NaOH (2 mL, 1 mg.mL<sup>-1</sup>) and aquated cisplatin (16.4 mg, 55 mmol). **FTIR** (cm<sup>-1</sup>): 3290, 2929, 2880, 1660, 1560, 1395, 1360, 1090, 1050, 930, 830 and 545.

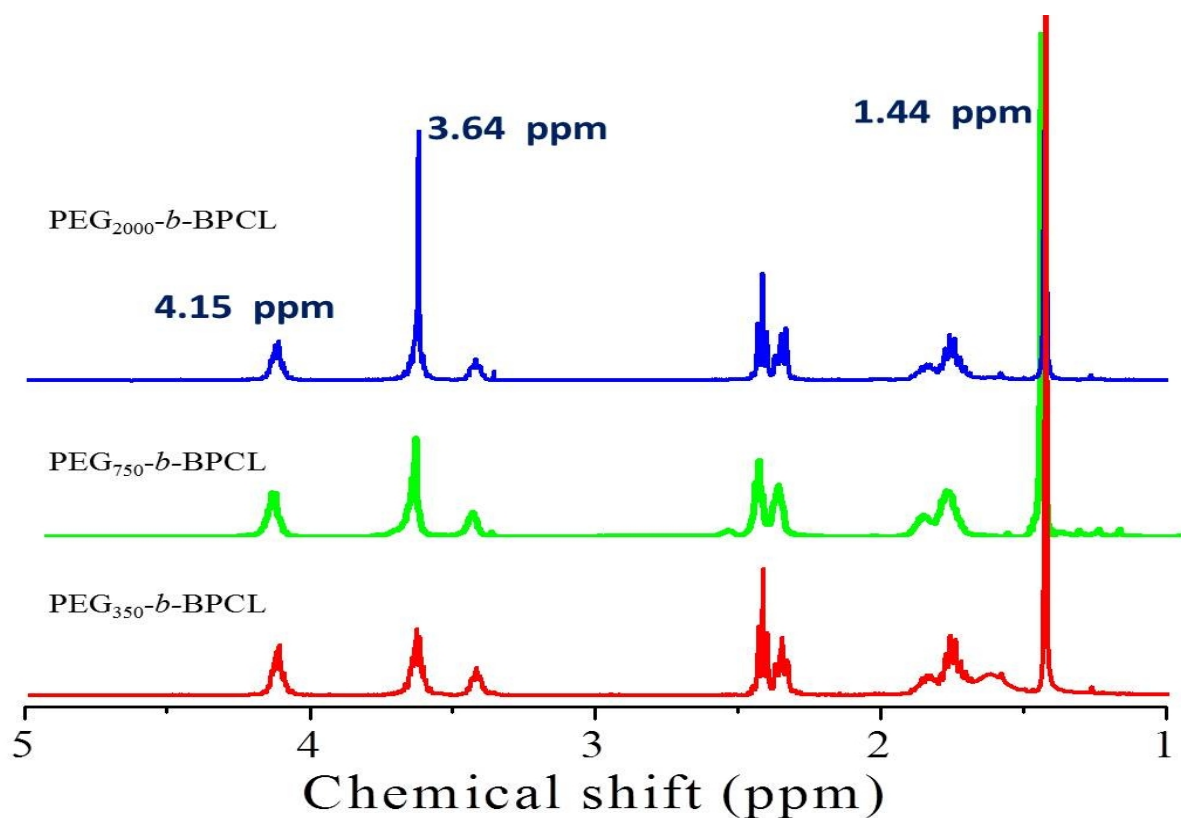
**Synthesis of PEG<sub>350</sub>-*b*-CPCL-CP:**PEG<sub>2000</sub>-*b*-CPCL (20 mg), NaOH (2 mL, 1 mg.mL<sup>-1</sup>) and aquated cisplatin (16.4 mg, 55 mmol). **FTIR** (cm<sup>-1</sup>): 3300, 2920, 2880, 1660, 1560, 1395, 1360, 1090, 1050, 930, 830 and 549.

**Synthesis of PEG<sub>750</sub>-*b*-CPCL-CP:** PEG<sub>2000</sub>-*b*-CPCL (20 mg), NaOH (2 mL, 1 mg.mL<sup>-1</sup>) and aquated cisplatin (16.4 mg, 55 mmol). **FTIR** (cm<sup>-1</sup>): 3300, 2950, 2880, 1660, 1565, 1395, 1350, 1090, 1050, 940, 830 and 548

**Synthesis of PEG<sub>2000</sub>-*b*-CPCL-CP:** PEG<sub>2000</sub>-*b*-CPCL (20 mg), NaOH (2 mL, 1 mg.mL<sup>-1</sup>) and aquated cisplatin (16.4 mg, 55 mmol). **FTIR** (cm<sup>-1</sup>): 3250, 2920, 2880, 1660, 1560, 1399, 1360, 1090, 1050, 935, 830 and 548.



**Figure SF 1:** Synthesis of monomer CCL.



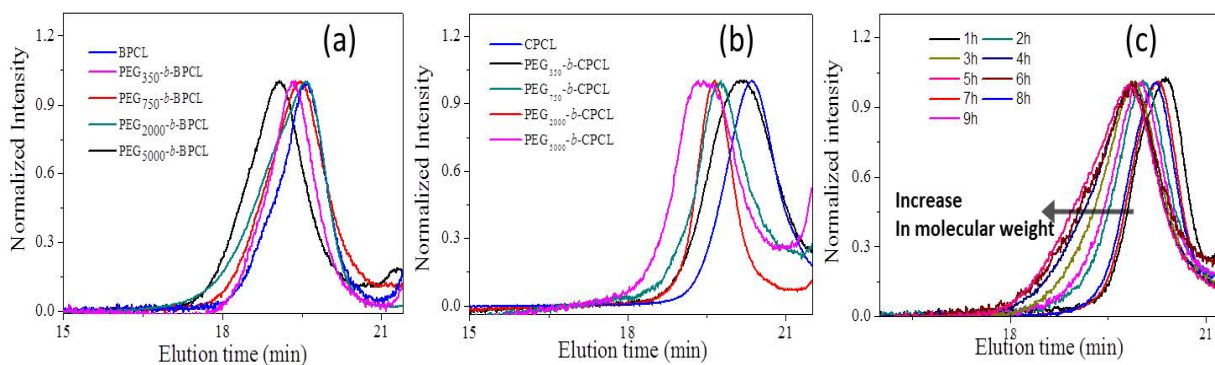
**Figure SF 2:** <sup>1</sup>H-NMR spectra of PEG<sub>x</sub>-*b*-BPCL diblock polymers in CDCl<sub>3</sub>.

**Note:** The quantification of caprolactone units (repeating units- $X_n$ ) in diblock polymers were determined by comparing the -OCH<sub>2</sub>CH<sub>2</sub>O- in the PEG part at 3.64 ppm for 28 protons (PEG<sub>350</sub>), 68 protons (PEG<sub>750</sub>), and 180 protons (PEG<sub>2000</sub>) with the methylene protons of PCL at 4.15 (OCH<sub>2</sub>) or 2.31 (COCH<sub>2</sub>). The peaks corresponding to the ether proton – OCH<sub>2</sub>CH<sub>2</sub>COO-Bu were appeared along with OCH<sub>2</sub>CH<sub>2</sub>O- in the PEG part at 3.64 ppm. It was also taken into consideration during the determination of n units in the PCL chains.

**Table ST 1.** Molecular weights and molecular weight distribution of PEG<sub>x</sub>-b-BPCL and PEG<sub>x</sub>-b-CPCL diblock polymers.

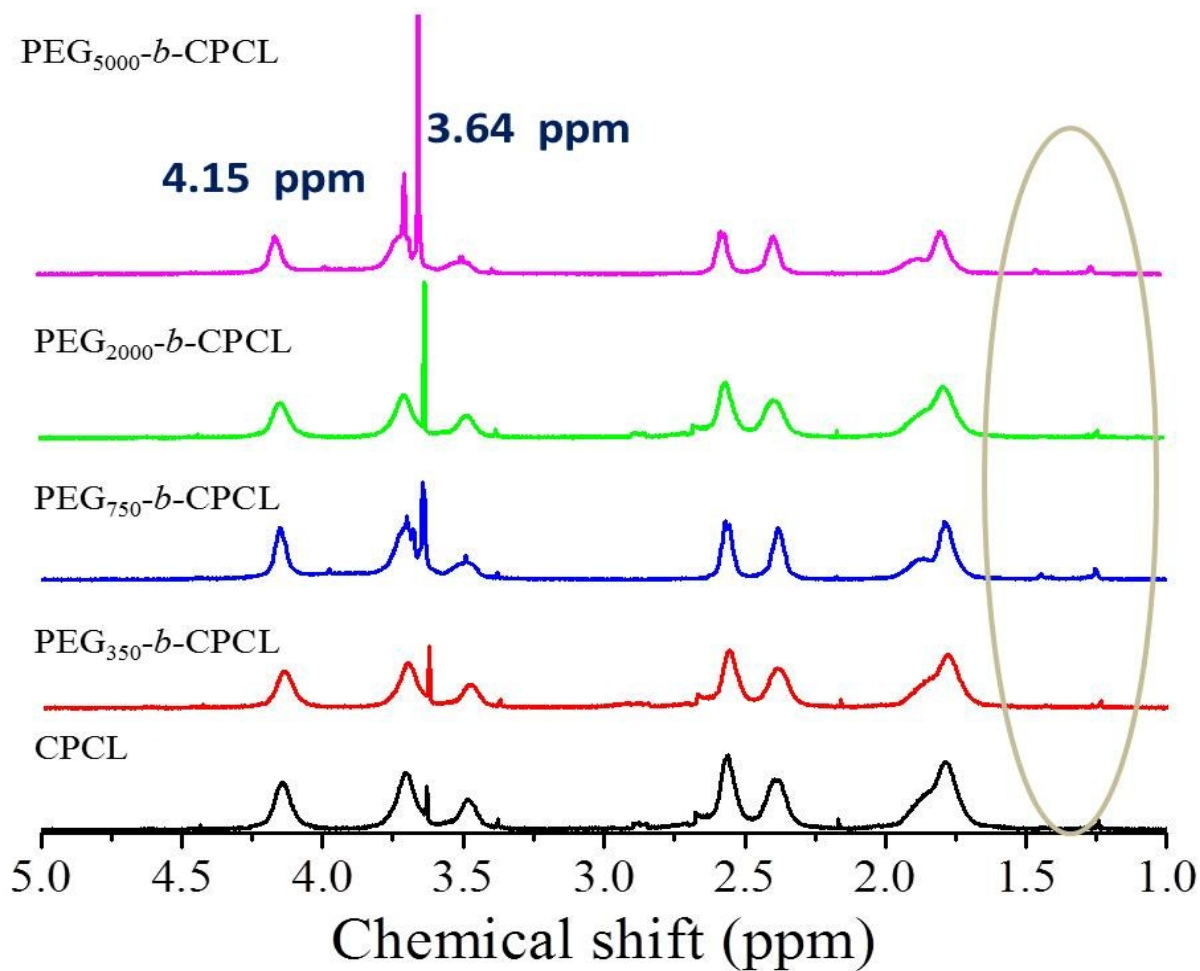
Polymer	n <sup>a</sup> (NMR)	M <sub>n</sub> <sup>b</sup> (g/mol, NMR)	M <sub>n</sub> <sup>c</sup> (g/mol, GPC)	M <sub>w</sub> <sup>c</sup> (g/mol, GPC)	M <sub>w</sub> /M <sub>n</sub> <sup>c</sup> (GPC)
BPCL	90	23,400	15,000	21,100	1.40
PEG <sub>350</sub> -b-BPCL	91	23,800	16,300	20,000	1.22
PEG <sub>750</sub> -b-BPCL	95	25,300	16,700	20,200	1.20
PEG <sub>2000</sub> -b-BPCL	94	26,200	17,000	22,400	1.31
PEG <sub>5000</sub> -b-BPCL	100	35,800	19,100	23,700	1.24
CPCL	90	18,300	9,000	11,700	1.30
PEG <sub>350</sub> -b-CPCL	91	18,700	11,000	13,700	1.24
PEG <sub>750</sub> -b-CPCL	95	19,900	12,200	14,600	1.19
PEG <sub>2000</sub> -b-CPCL	94	21,000	12,700	15,100	1.18
PEG <sub>5000</sub> -b-CPCL	89	23,000	13,100	22,200	1.69

<sup>a</sup> Number of repeating units are determined by <sup>1</sup>H-NMR. <sup>b</sup> M<sub>n</sub> was calculated based on M<sub>n</sub> = (repeating unit mass) x n. <sup>c</sup> Molecular weights and PDI determined by GPC using polystyrene as standard in THF



**Figure SF 3:** GPC chromatograms of PEG<sub>x</sub>-b-BPCL(a), PEG<sub>x</sub>-b-CPCL(b). The GPC chromatogram of polymerization kinetics of PEG<sub>2000</sub>-b-BPCL where the aliquots were taken at different time intervals.

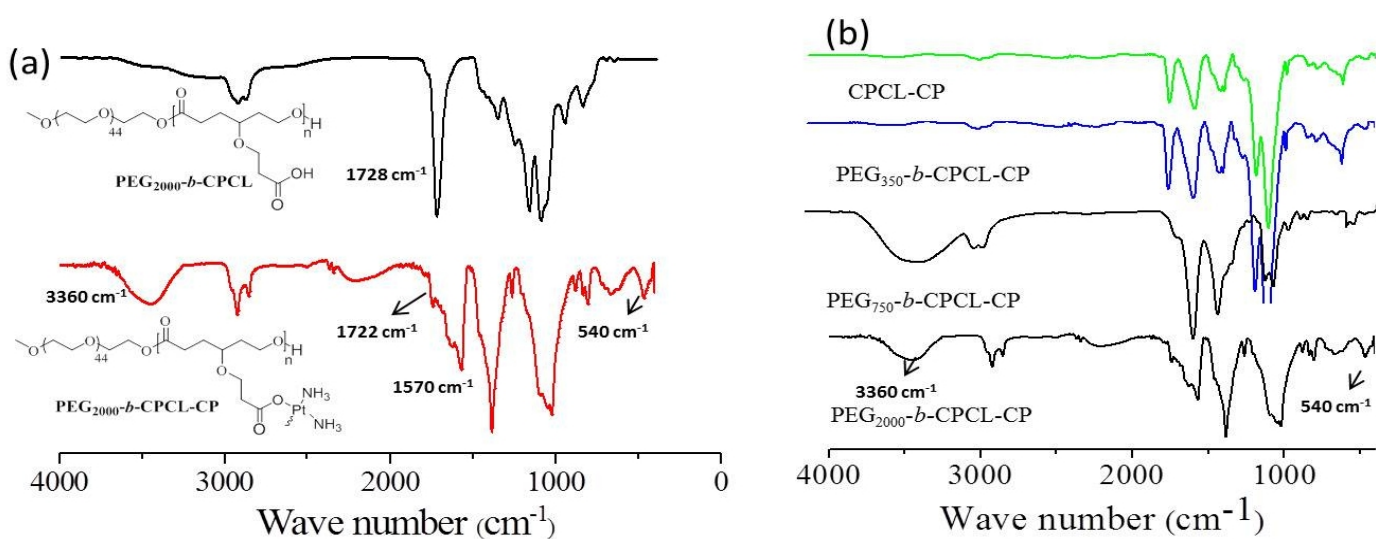
**Note:** From GPC chromatograms all block copolymer (PEG<sub>x</sub>-b-BPCL and PEG<sub>x</sub>-b-CPCL) showed monomodal distribution and confirmed the formation of high molecular weight polymers. As shown in SF 7b after deprotection of t-butyl group molecular weights decreased. Ring opening polymerization (ROP) of monomer CCL was carried out using PEG<sub>2000</sub> as an initiator and Sn(Oct)<sub>2</sub> as catalyst at 130 °C to study reaction kinetics in solvent free route. Molar ratios of monomer to initiator to catalyst kept as between  $[M_0]/[I_0] = 100$ . At different time intervals aliquots were collected and precipitated in cold methanol. GPC chromatograms of ROP kinetics showed increase in molecular weights with time.



**Figure SF 4:**  $^1\text{H-NMR}$  spectra of PEG<sub>x</sub>-*b*-CPCL diblock polymers.

Note: The comparison of figure SF-2 and SF-4 clearly showed that the *t*-Bu group peak vanished at 1.45 ppm in the deprotected diblock polymers.





**Figure SF 5:** FT-IR spectra of PEG<sub>x</sub>-b-CPCL-CP.

**Note:** The cisplatin conjugates with COOH groups of CPCL showed new peaks at 3000 cm<sup>-1</sup> (NH from cisplatin), 540 cm<sup>-1</sup> (Pt-O). The carbonyl peak shifted from 1720 cm<sup>-1</sup> to 1570 cm<sup>-1</sup>, upon cisplatin conjugation. This observation confirmed the formation of polymer-cisplatin conjugates.

**Calculation of Drug Loading Content (DLC) for PEG<sub>5000</sub>-*b*-CPCL-CP was described below following the report by Xu et al. (*Chem. Commun.* 2013, 49, 33–35):**

$$f = \frac{m_{Pt,exp}}{m_{Pt,th}} \times 100\% = \frac{W_{Pt}/M_{Pt}}{W_{acid}/M_{acid} \times 2} \times 100\%$$

$$= \frac{20.2/195}{79.8 - \{[(20.2 \times 34.6)/65.4] \times (20200/25200)\}}/404 \times 100\%$$

$$f = 75.7\%$$

**Calculation of Drug Loading Content (DLC):**

$$DLC = \frac{\text{initial feed} \times 75.7\%}{\text{polymer amount} + (\text{initial feed} \times 75.7\%)} \times 100\%$$

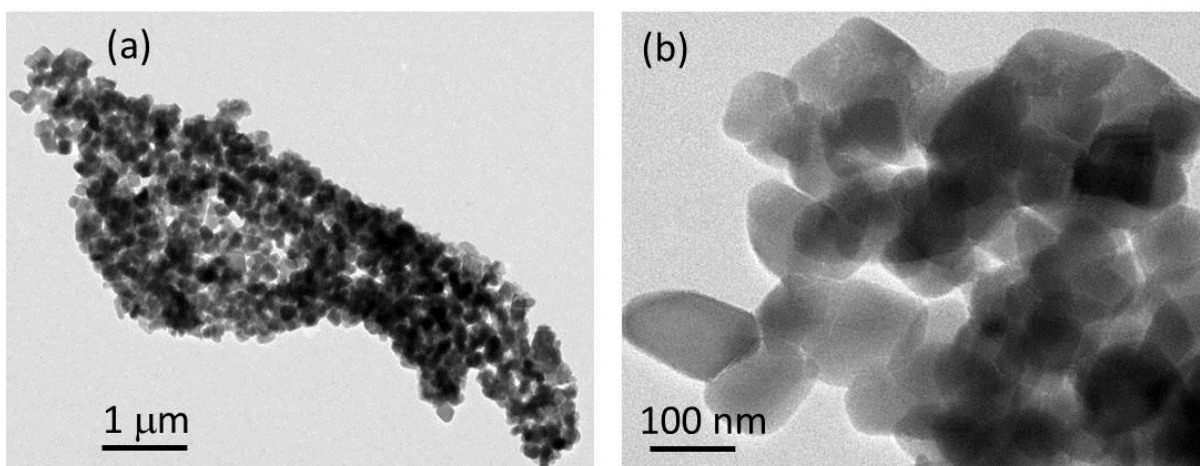
$$= \frac{15 \times 75.7\%}{20 + (15 \times 75.7\%)} \times 100$$

$$DLC = 36\%$$

Note: For remaining polymer-drug conjugates DLC was calculated in similar way and tabled below.

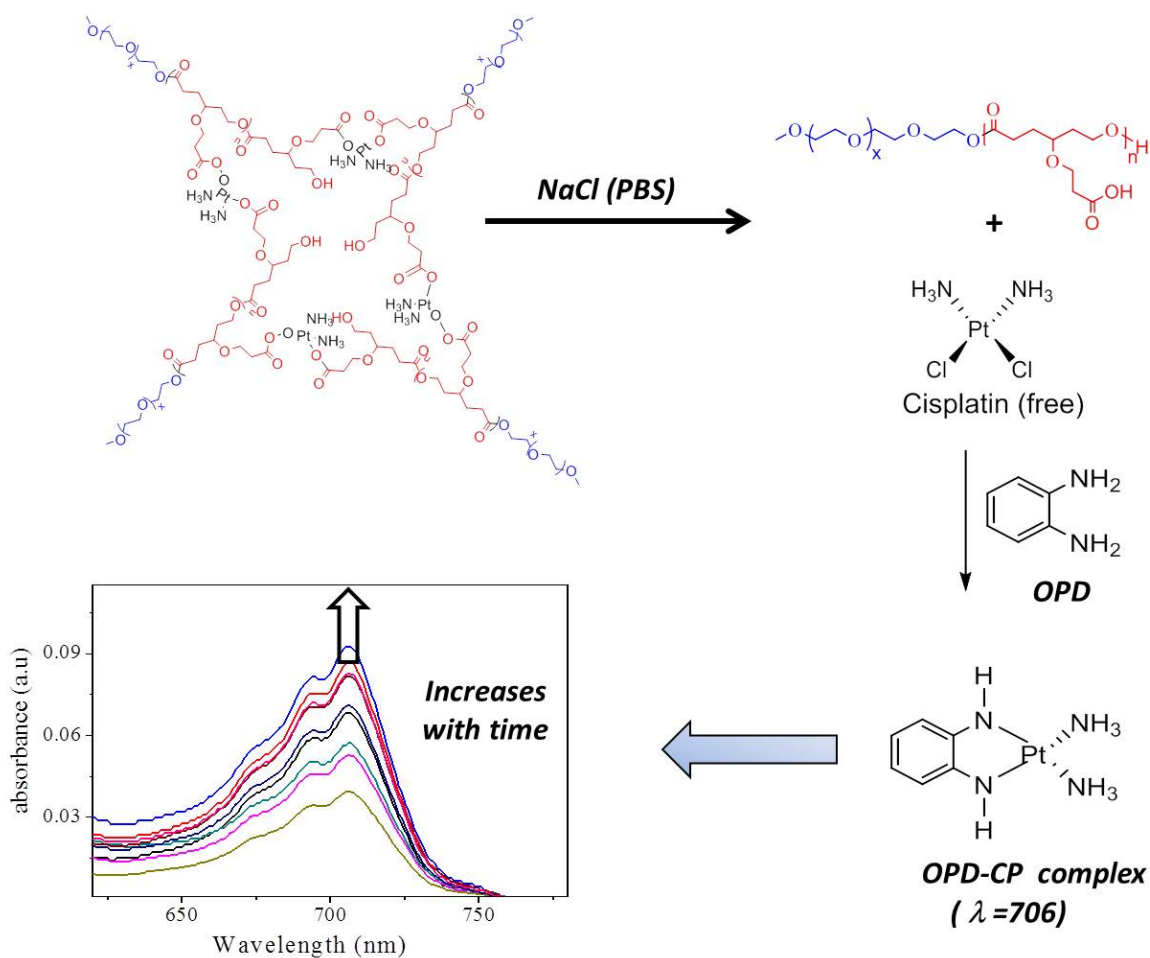
**Table ST 2:** *DLC determination using TGA and table showed the determined DLC using TGA and UV (using OPD assay) techniques.*

S.No	Polymer drug conjugates	DLC (TGA)	DLC (UV)
1	CPCL-CP	28 %	25 %
2	PEG <sub>350</sub> - <i>b</i> -CPCL-CP	30 %	28 %
3	PEG <sub>750</sub> - <i>b</i> -CPCL-CP	31 %	34 %
4	PEG <sub>2000</sub> - <i>b</i> -CPCL-CP	31 %	27 %
5	PEG <sub>5000</sub> - <i>b</i> -CPCL-CP	36 %	39 %



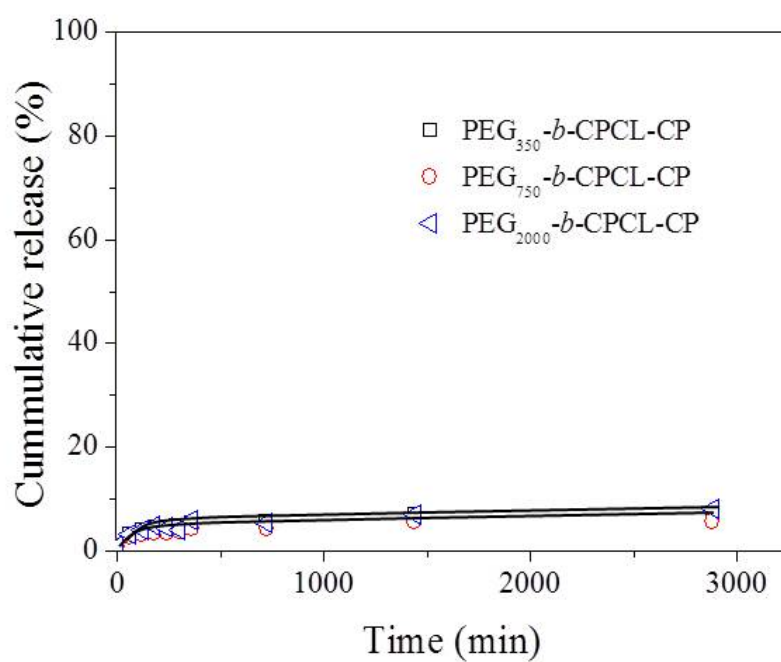
**Figure SF 6:** HR-TEM images of PEG<sub>2000</sub>-*b*-CPCL-CP nanoparticles.

**Note:** The additional HRTEM images of the PEG<sub>2000</sub>-*b*-CPCL-CP drug conjugates, confirmed the nanoparticles formation.



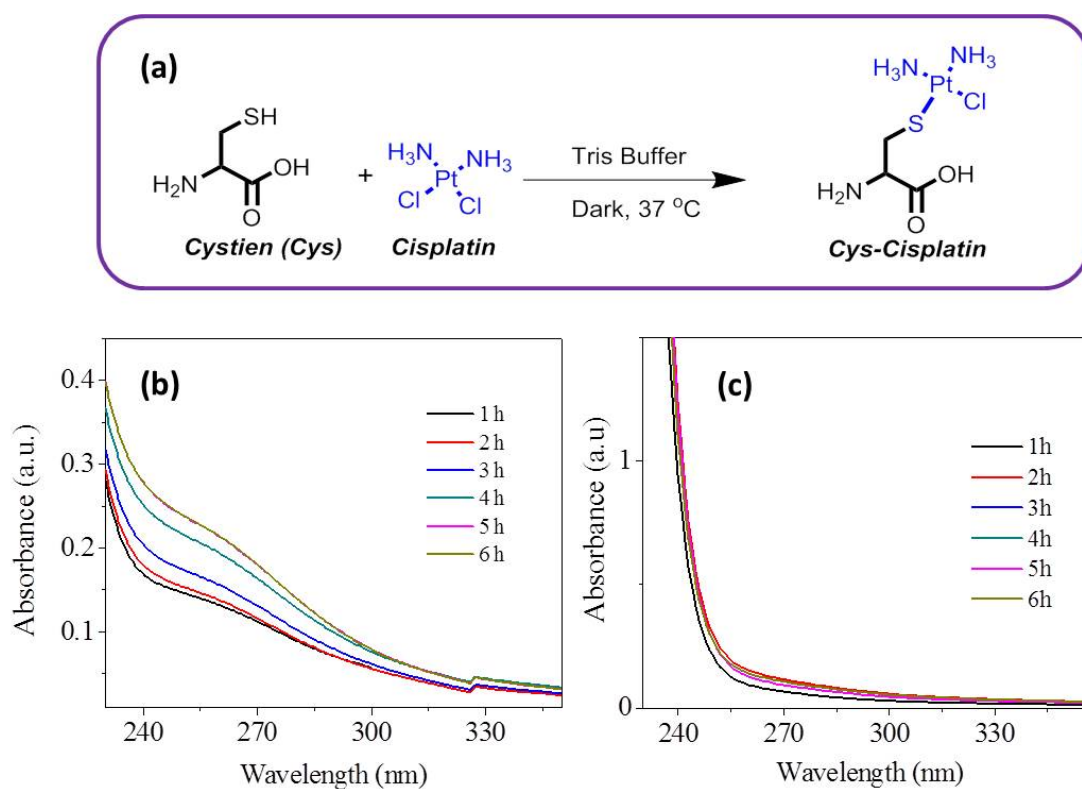
**Figure SF 7:** OPD assay for the determination of cisplatin content in dialysis method.

**Note:** The *in vitro* drug release of the drug conjugates were determined as shown above. The chloride and phosphate anions present in saline (pH = 7.2) and PBS (pH = 7.4) are known to participate in dechelation of the COO-Pt bond. The chloride anions attach on the Pt-OOC-polymer linkage to regenerate the cisplatin and the amount of cisplatin released in the media could be estimated with the help of *o*-phenylenediamine colorimetric assay. In this method, cisplatin released from the polymeric carrier were treated with OPD (*o*-phenylenediamine) for the estimation of the released drug. The amount of cisplatin drug released in this solution could be determined by measuring the absorbance at 706 nm (the absorbance of OPD-Pt complexes shown above).



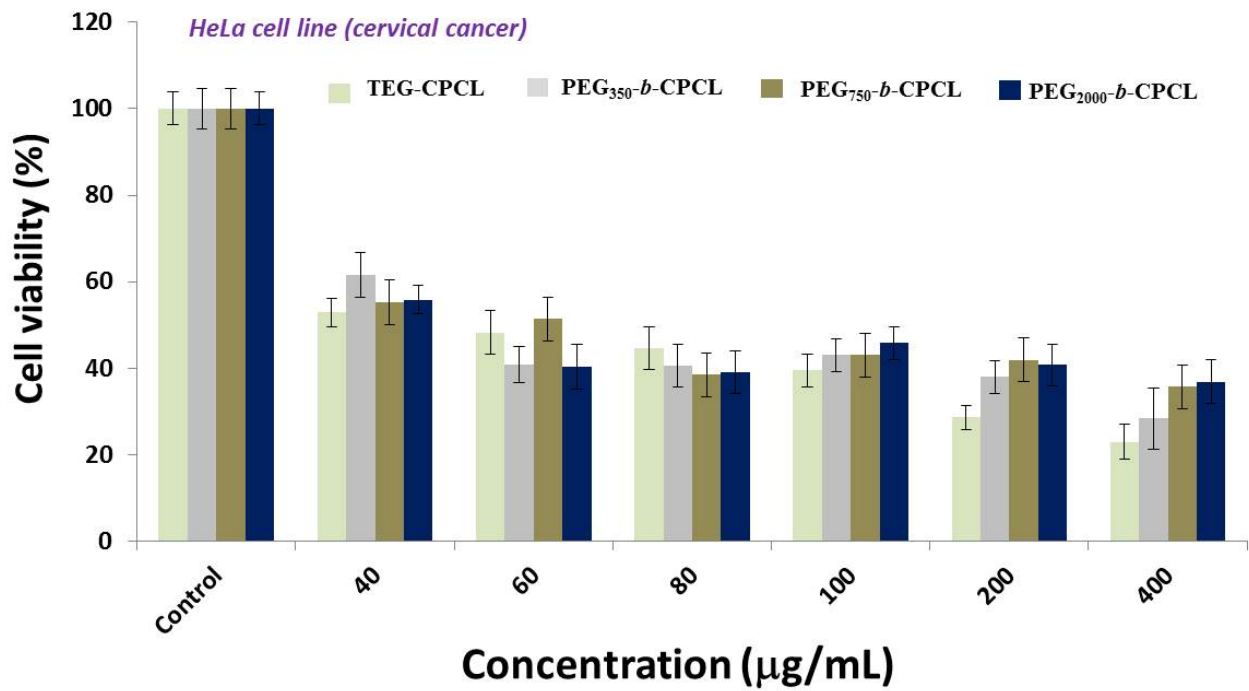
**Figure SF 8:** In vitro drug release profiles of polymer drug conjugates in milli Q water at 37 °C.

**Note:** The stability of the nanoparticles in water was found to be very good. (showed only 5 % release).

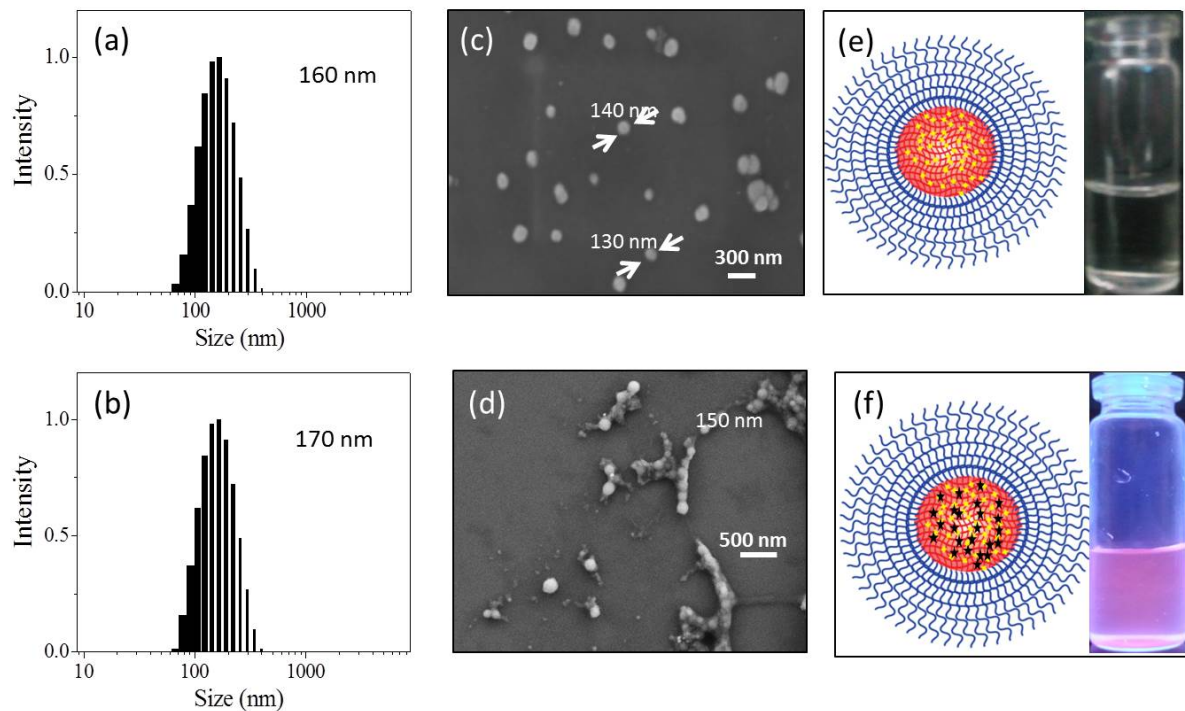


**Figure SF 9:** Determination of reaction kinetic for reaction between CP and Cysteine

**Note:** As mentioned in main manuscript S-containing compounds play a major role in cisplatin resistance. Amino acid like cysteine present in cytoplasm, which can bind to Pt. The reaction kinetics of Cysteine and cisplatin reaction ( in Tris buffer at 37 °C) was monitored using UV spectroscopy. Cisplatin reacted faster with Cysteine as shown in SF 10a, whereas the drug conjugate (PEG<sub>2000</sub>-*b*-CPCL-CP) did not show any reaction. Hence, it can be concluded that the drug conjugates stable against cysteine.



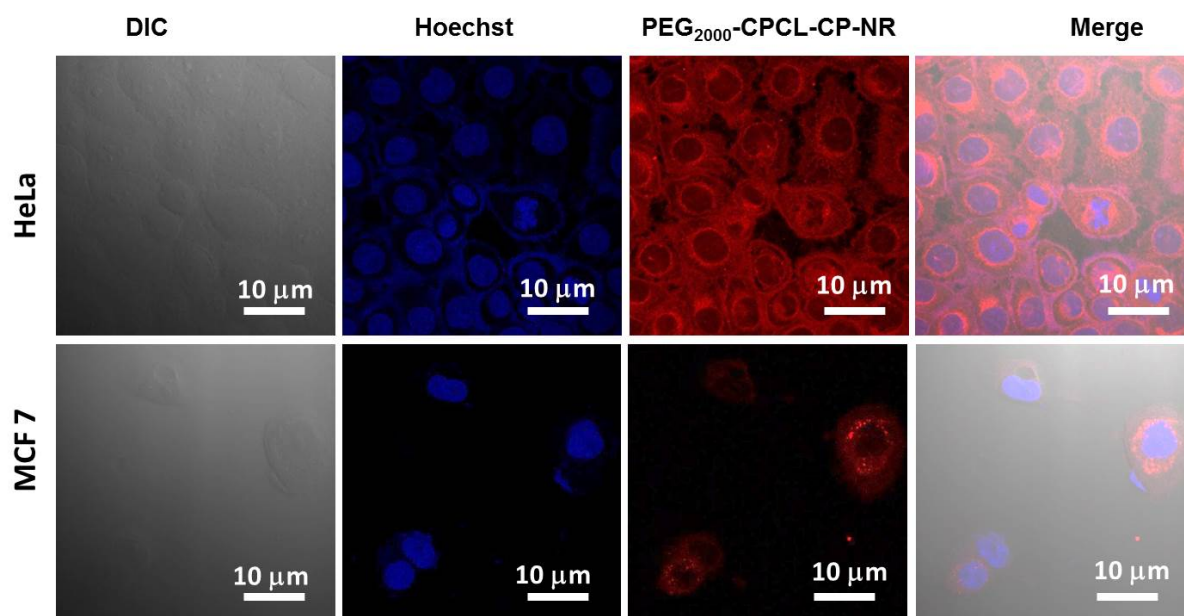
**Figure SF 10:** Cytotoxicity of diblock polymers.



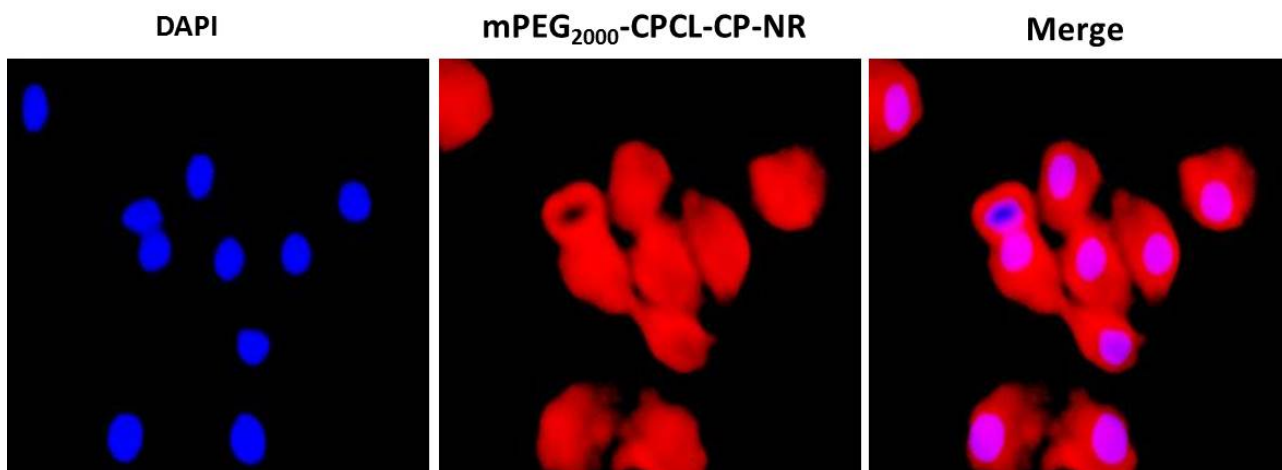
**Figure SF 11:** DLS histograms of PEG<sub>2000</sub>-b-CPCL-CP (a) PEG<sub>2000</sub>-b-CPCL-CP-NR (b) FESEM images of PEG<sub>2000</sub>-b-CPCL-CP (c), PEG<sub>2000</sub>-b-CPCL-CP –NR nanoparticles, and vial before (e) and after (f) NR encapsulation.

**Note:** DLS histograms of PEG<sub>2000</sub>-b-CPCL nanoparticles before (a) and after (b) NR loading are shown above. The samples exhibited monomodal distribution identical size. These results are further supported by FESEM studies, where spherical nanoparticles were observed for nascent and NR loaded drug conjugates.





**Figure SF 12:** CLSM images of PEG<sub>2000</sub>-*b*-CPCL-CP-NR nanoparticles. *In HeLa and MCF 7 cell line in second set of experiment. The data is incoherence with the data that showed in Figure 9. The nucleus was counterstained with Hoechst (blue). The cells were observed through red channel to locate NR fluorescence (red).*



**Figure SF 13:** Fluorescence microscopy images of PEG<sub>2000</sub>-*b*-CPCL-CP-NR nanoparticles.

**Note:** The cellular uptake of the NR encapsulated cisplatin-polymer conjugate was deliberate by fluorescent microscopy technique in HeLa cells. As shown in above images, strong NR fluorescence was observed in the cytoplasm of the cells. This confirms the accumulation of drug conjugates inside the cell.