SUPPORTING INFORMATION

LHRH-Targeted Cisplatin-Loaded Magnetite Nanoclusters for Simultaneous MR Imaging and Chemotherapy of Ovarian Cancer

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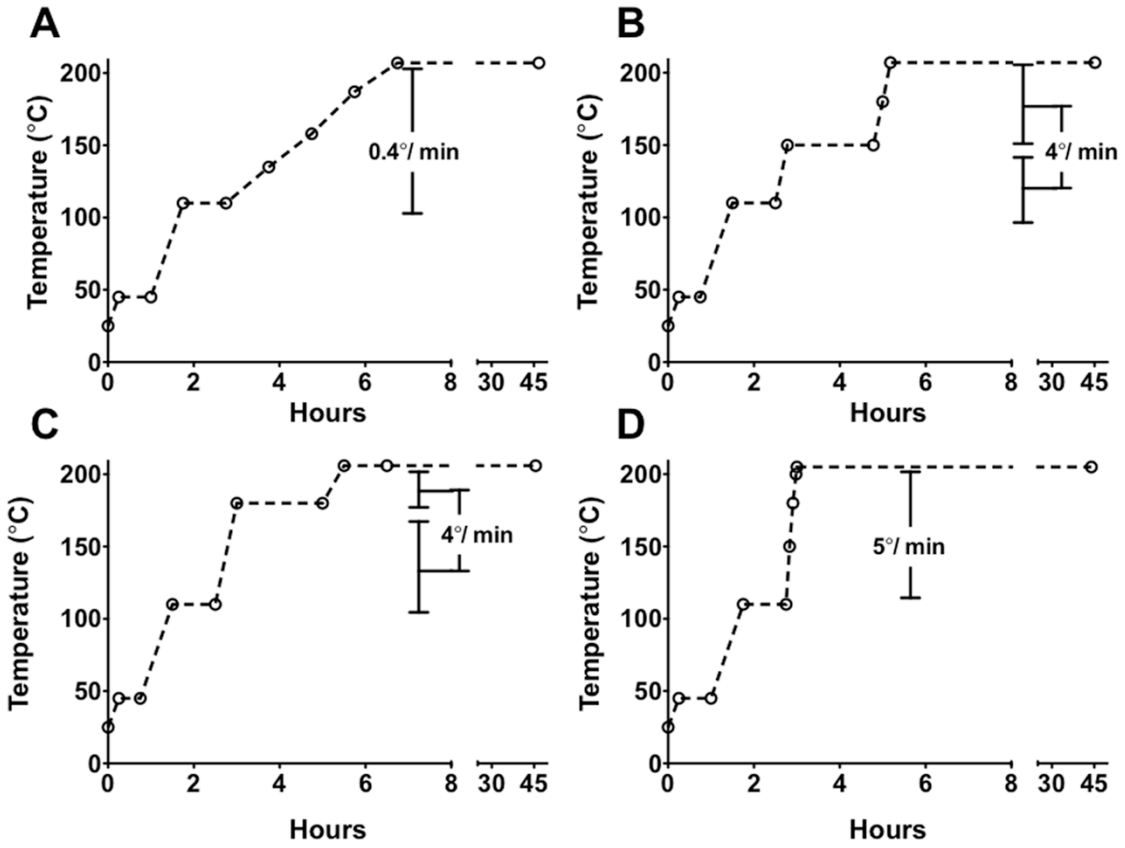


Figure S1. Various heating regimes during the synthesis of MNPs by thermal decomposition of $Fe(acac)_3$.

Table S1. Physicochemical characteristics of MNCs stabilized by homopolymer PLD, PLE, and PLD_{100}-b-PEG_{113}.

Formulation [a]	Abbreviation	DLS Characteristics [b]		
		D_{eff} (nm)	PDI	ζ -potential (mV)
PLE_{100}/MNP	hE_{100}-MNC	27.3	0.171	-34.6
PLD_{100}-*b*-PEG_{113}/MNP	D_{100}-MNC	94.8	0.189	-24.7
PLD_{100}/MNP	hD_{100}-MNC	67.2	0.168	-47.2

(a) The size of the MNPs in all formulations was 7.3 ± 4 nm as quantified by Image J software from TEM images.

(b) DLS characteristics were measured in de-ionized (DI) water at 1 mg/ml MNCs at 25°C.

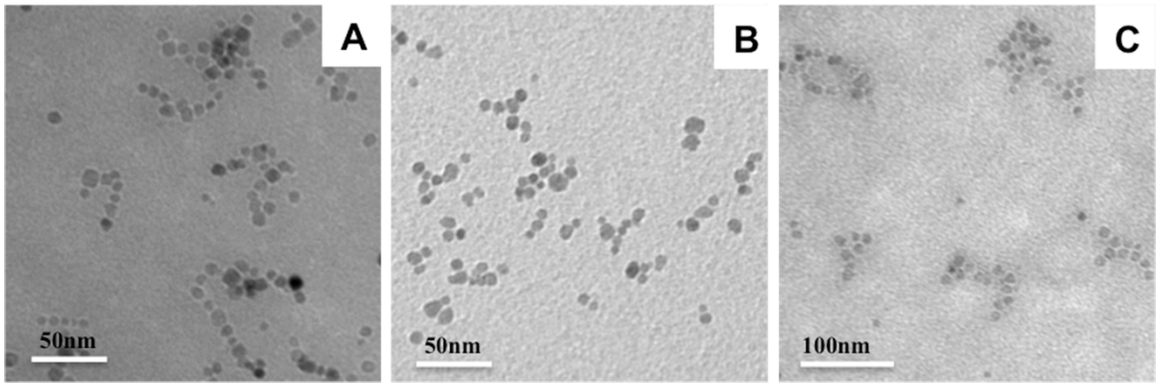
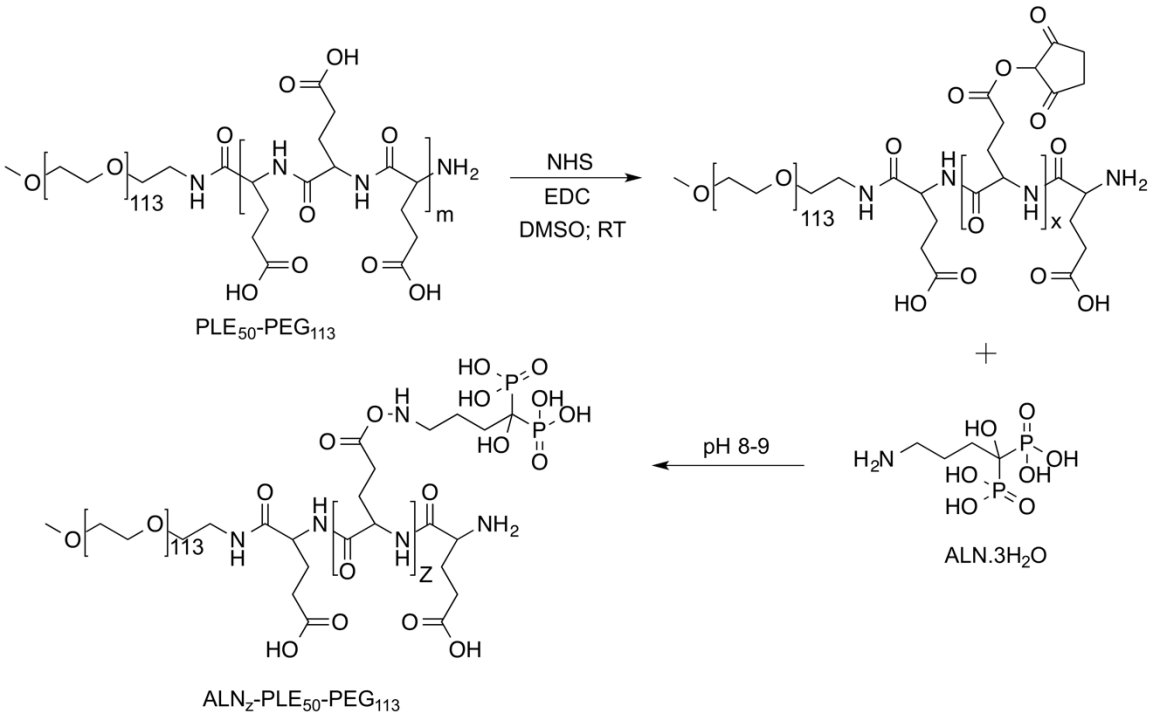


Figure S2: TEM images of **(A)** E_{10}-MNC**, (B)** E_{50}-MNC and **(C)** E_{100}-MNC formulations

Scheme S1. Schematic representation of ALN conjugation on PLE_{50}-b-PEG_{113} copolymer.



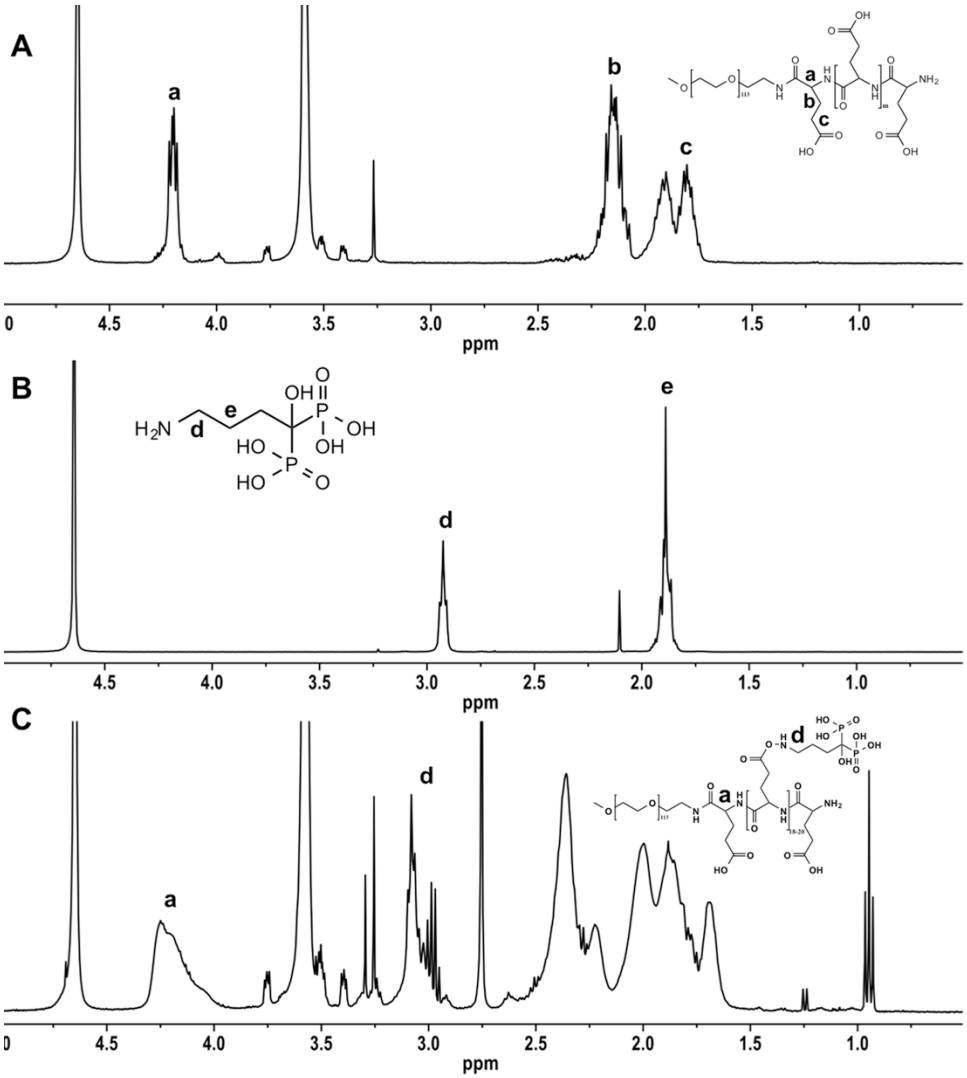


Figure S3. ^{1}H-NMR in D_2O of **(A)** PLE_{50}-*b*-PEG_{113}, **(B)** ALN and **(C)** ALN-modified PLE_{50}-*b*-PEG_{113}. Peak assignments are as follows: **(A)** δ ppm= 1.85 (2H, m, -CH_2-; c), 2.2 (2H, m, CH_2; b), 4.2 (H, t, -CH-; a); **(B)** δ ppm= 1.88 (2H, m, -CH_2-; e), 2.9 (2H, t, -CH_2; d); **(C)** δ ppm= 2.9 - 3.1 (2H, m, -CH_2-; d), 4.28 (H, d, -CH-; a).

Table S2. Quantitation of ALN conjugation by ^{1}H-NMR

Formulation	ALN units per chain (D_{conj})
A_6-MNC	5.8
A_{12}-MNC	10.7
A_{19}-MNC-1	19.3
A_{19}-MNC-2	18.5

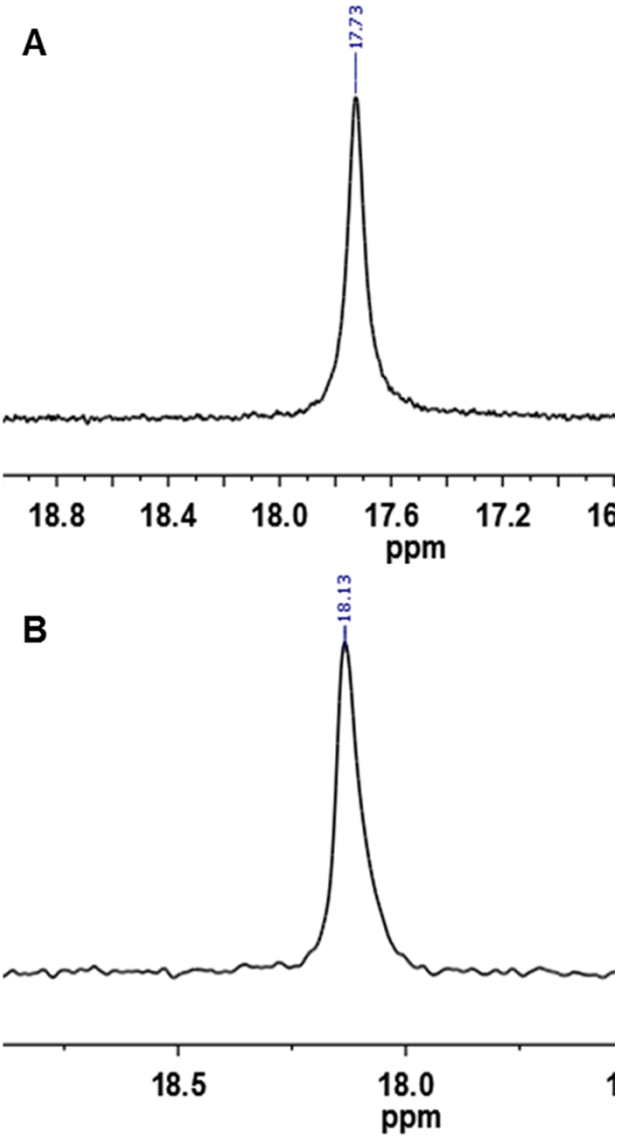


Figure S4. 31P-NMR in D2O of **(A)** unconjugated ALN (δ ppm: 17.73) and **(B)** ALN-modified PLE_{50}-PEG_{113} (δ ppm: 18.13)

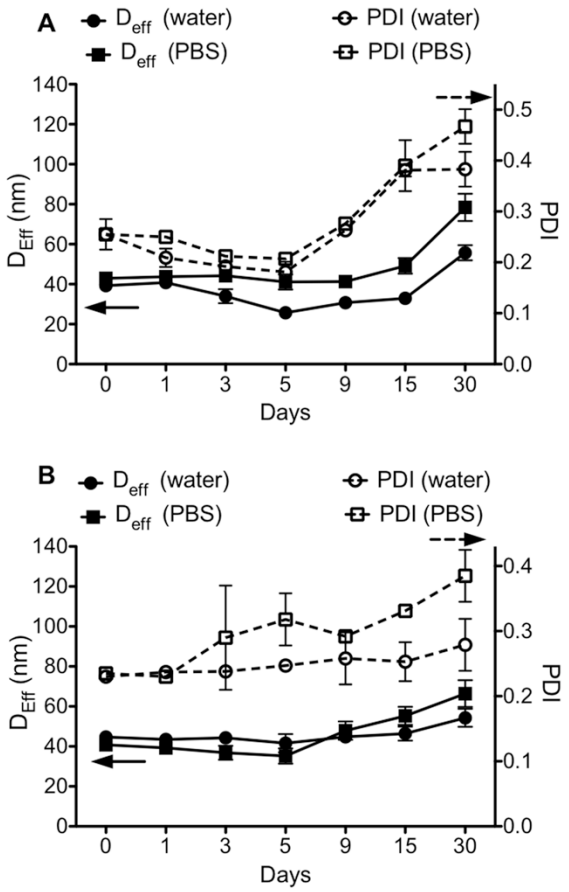


Figure S5. Colloidal stability of **(A)** A_6-MNC and **(B)** A_{12}-MNC formulations in DI water and PBS, pH 7.4 at RT.

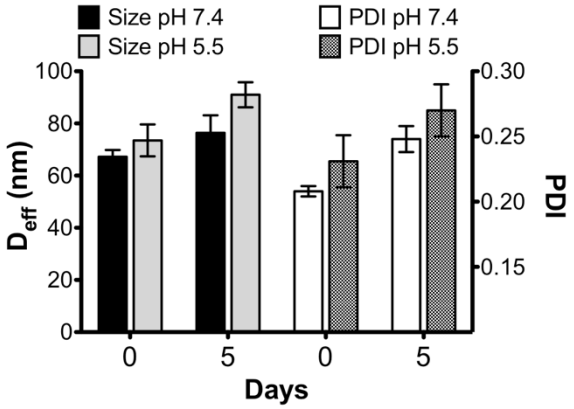


Figure S6: Colloidal stability of cisplatin loaded Pt-A_{19}-MNC formulation before and after cisplatin release from the formulation. The particle size (D_{eff}) of the samples used for release kinetics study (Figure 6) was determined by DLS before and at the end of the release study

Table S3. T_1 and T_2 relaxivity measurements of different formulations at 7T in DI water.

Formulation	r_1 ($s^{-1} \cdot mM^{-1}$)	r_2 ($s^{-1} \cdot mM^{-1}$)
A_6-MNC	1.00 ± 0.11	74.3 ± 4.2
Pt-A_6-MNC	na	na
A_{12}-MNC	1.6 ± 0.28	56 ± 1.6
Pt-A_{12}-MNC	2.1 ± 0.04	49.2 ± 3
A_{19}-MNC-1	1.1 ± 0.3	288.6 ± 5.8
Pt-A_{19}-MNC-1	1.3 ± 3.11	392.1 ± 2.04
A_{19}-MNC-2	1.04 ± 0.48	244.3 ± 3.8
Pt-A_{19}-MNC-2	1.3 ± 0.11	247 ± 3.7
Feridex	n.a.	269.4

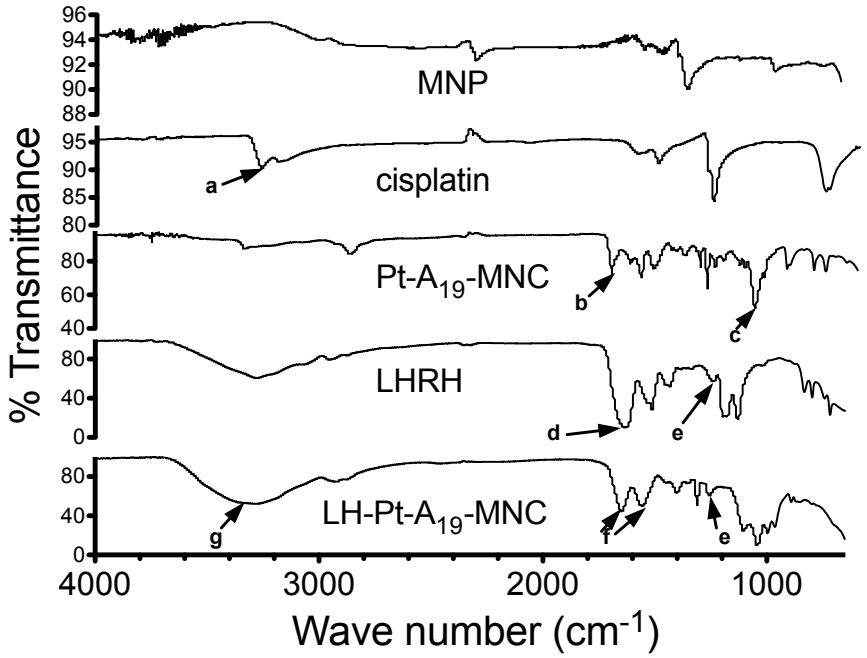


Figure S7. FTIR spectra of LHRH-conjugated Pt-A_{19}-MNCs, the drug and various intermediates. Labels: **a** - 3280 cm^{-1}: N-H stretch of the primary amine of cisplatin; **b** - 1730 cm^{-1}: carbonyl stretch of carboxylic acid on the copolymer; **c** - 1110 cm^{-1}: C-O stretch of PEG; **d** - 1650 cm^{-1}: N-H bend of primary amines; **e** - 1260 cm^{-1}: C-N stretch of aliphatic amines; **f** - 1660, 1580 cm^{-1}: Signals of secondary amide bonds following conjugation of LHRH to the MNC; **g** - 3400 cm^{-1}: N-H stretch of secondary amide bonds.

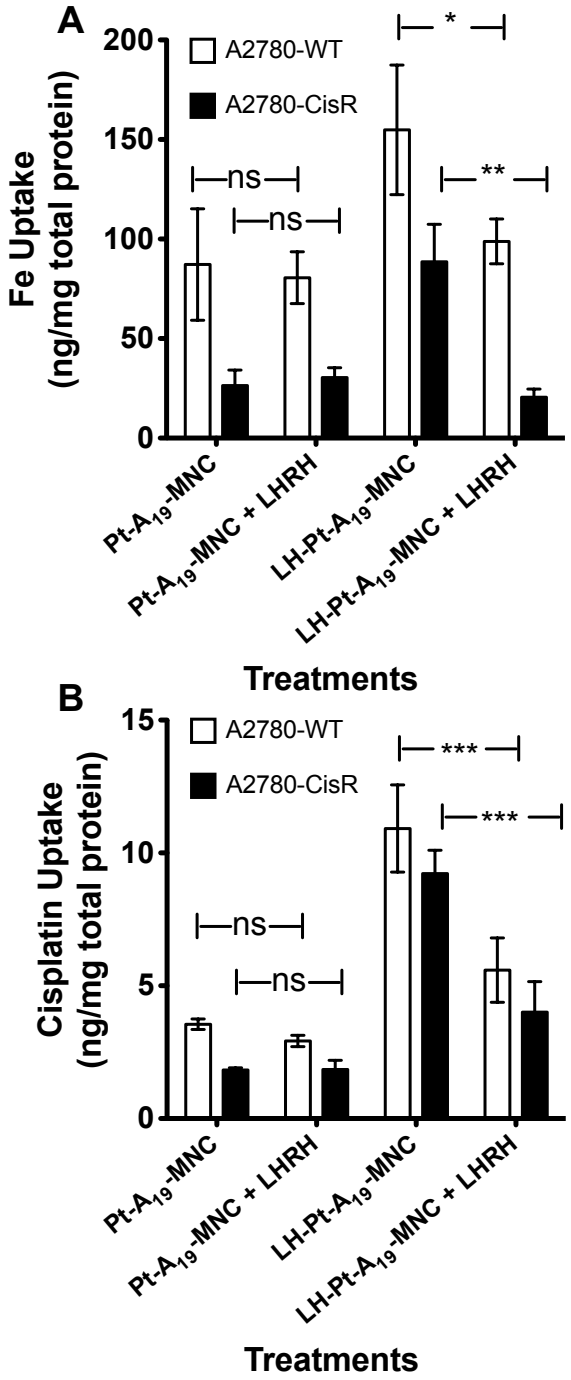


Figure S8. Inhibition of intracellular uptake of **(A)** Fe and **(B)** Cisplatin (Pt) in A2780-WT and A2780-CisR cells pre-incubated with D-Lys-6-LHRH peptide for 1h followed by 6h incubation with targeted and non-targeted PtMNC formulations. Statistical significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (n=3).

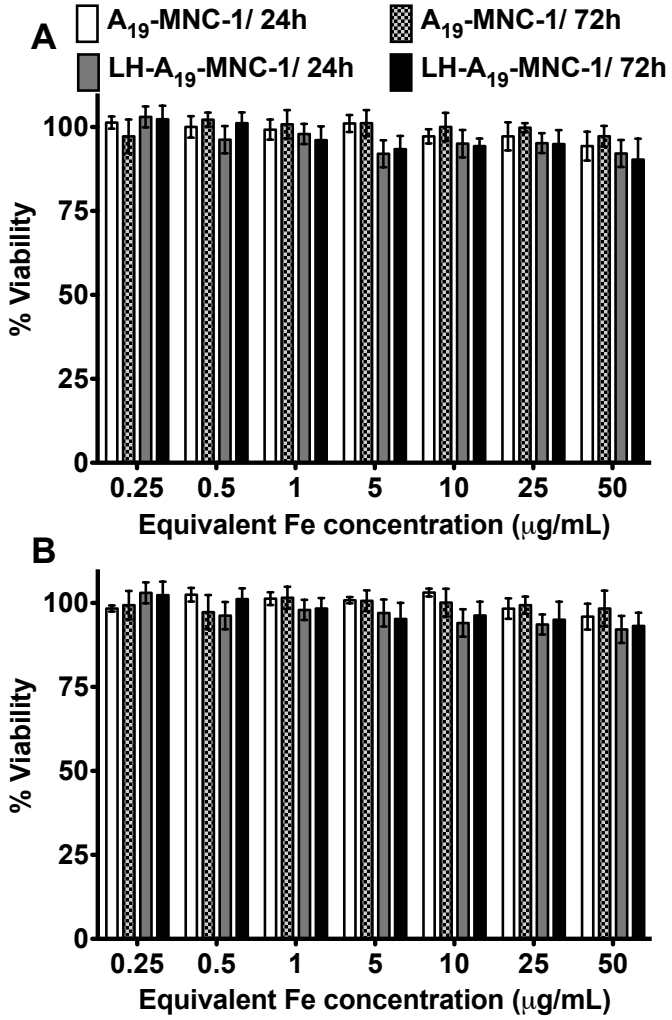


Figure S9. Formulation toxicity of unloaded non-targeted A_{19}-MNC-1 and targeted LH-A_{19}-MNC-1 formulations in **(A)** A2780-WT and **(B)** A2780-CisR cells. Cells were seeded in 96-well plates at a density of $3 \cdot 10^3$ cells/well and incubated at 37°C in 5% CO_2. After 24 h, they were treated with various concentrations of the said formulations and further incubated for 24h or 72h. At the end of each treatment period cell viability was measured by MTT assay. Mean ± SD (n = 12).

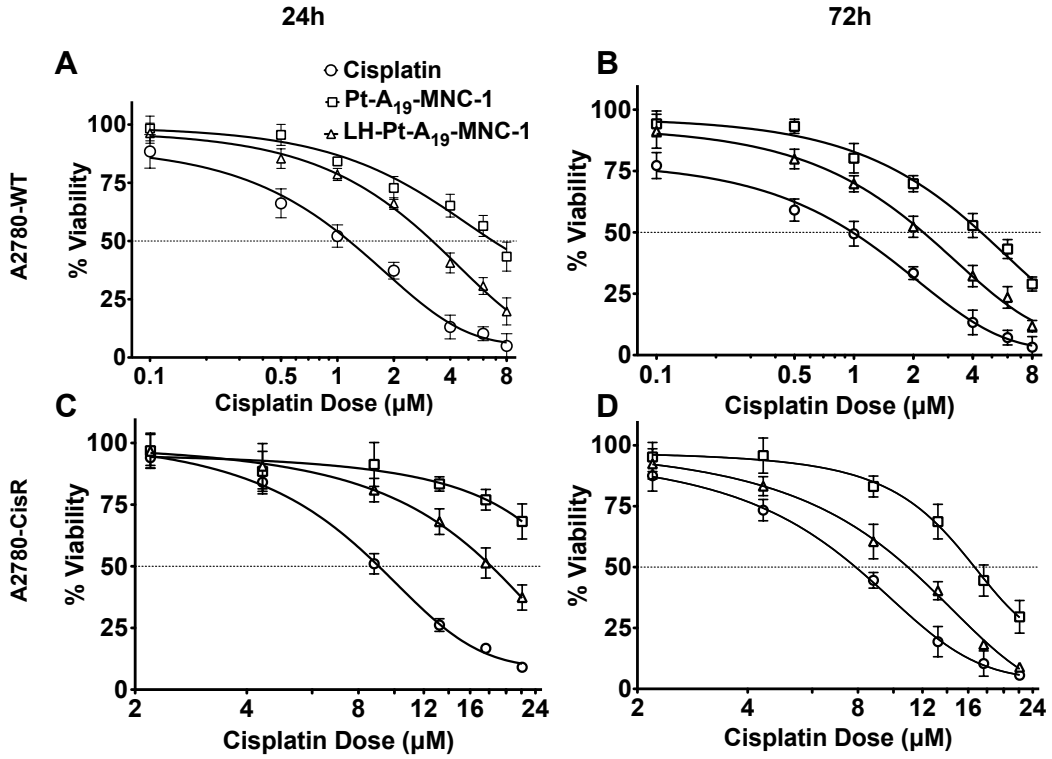


Figure S10. Cytotoxicity of cisplatin and LHRH-targeted and untargeted cisplatin-loaded MNCs in **(A, B)** A2780-WT and **(C, D)** A2780-CisR cells exposed for drugs for **(A, C)** 24 h and **(B, D)** 72 h. Data are mean ± SD (n = 6).