Supporting Information

LHRH-targeted Nanogels as Delivery System for Cisplatin to Ovarian Cancer

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As mention in the main text two methods (in organic or aqueous media) were applied to synthesize PEGylated peptide using Fmoc-PEG-NHS (**Figure 1**). The organic route was the final procedure used for synthesis of PEG-LHRH. Initially PEGylated LHRH was synthesized in PBS buffer (pH 8, contains 10% DMF), followed by Fmoc-deprotection using 30% piperidine in PBS/DMF (v/v = 5/2). The advantage of Fmoc group (9H-fluoren-9-ylmethoxycarbonyl) is that it is cleaved under very mild basic conditions compare to Boc-protective group. During dialysis against PBS a small amount of white precipitate (presumably LHRH-PEG or/and PEG) was produced, which could be easily dissolved in DMF or after prolonged dialysis (1-2 days). When evaporation of solvents was used instead of dialysis, the similar precipitate was obtained probably due to the balance in the solubility of PEG or PEGylated LHRH in PBS/DMF mixture. All fractions (precipitates, supernatant) were collected and analyzed using H^1-NMR and UV spectrometry (**Figure S1**). To avoid the precipitation of PEGylated LHRH, the same synthetic scheme was carried out in organic solvent DMF in the presence of DIEA followed by Fmoc-deprotection using 30% piperidine/DMF (**Figure 1**).

Spectra show that NHS-PEG-Fmoc as well as reaction mixture LHRH-PEG-Fmoc have a notable signal at 270 nm, which corresponds to Fmoc group. As expected the LHRH (spectrum A2) possesses a peak at 230 nm and ~280 nm. The deprotected LHRH-PEG did not have any peak, which belongs to Fmoc group (spectra A12, B1). As mention in the main manuscript the synthesis carried out in PBS leaded to the formation of white precipitate, which is PEGylated LHRH (A12). Analysis of the reaction mixture revealed that the supernatant did not contain free LHRH or PEG. These results suggested that modification of LHRH by PEG was successful and product was fully recovered.

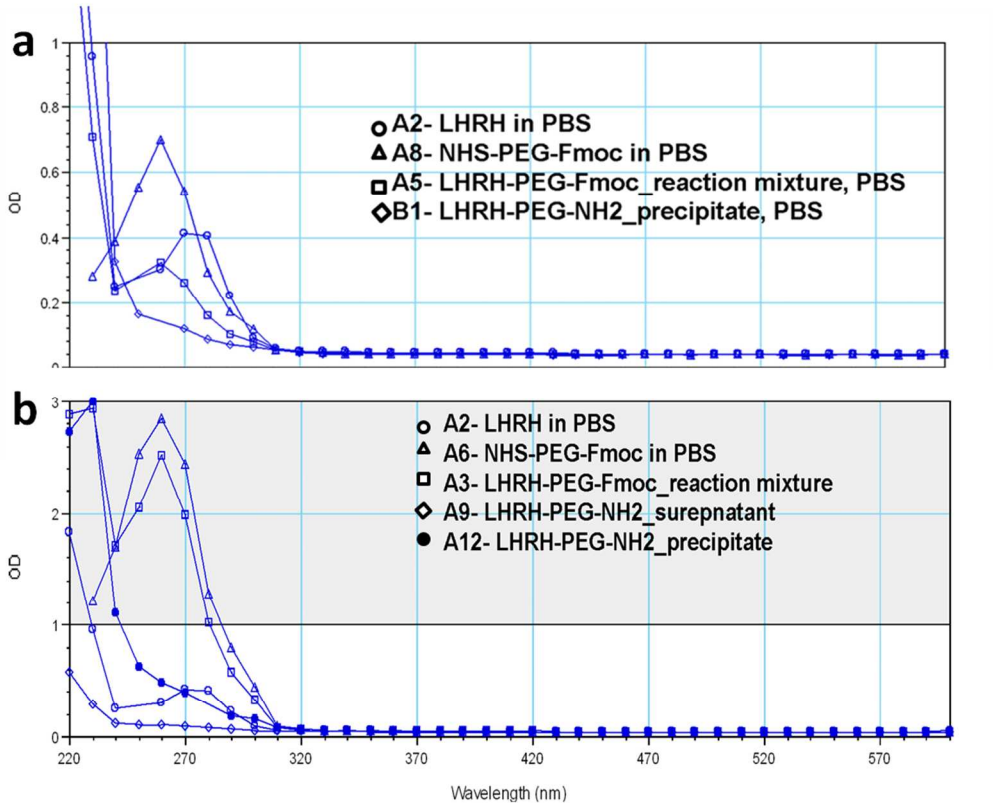

Figure S1. UV spectra for LHRH, intermediate compounds and final PEGylated LHRH in PBS buffer at concentration **a)** 0.5 mg polymer/ml and **b)** 2 mg polymer/ml.

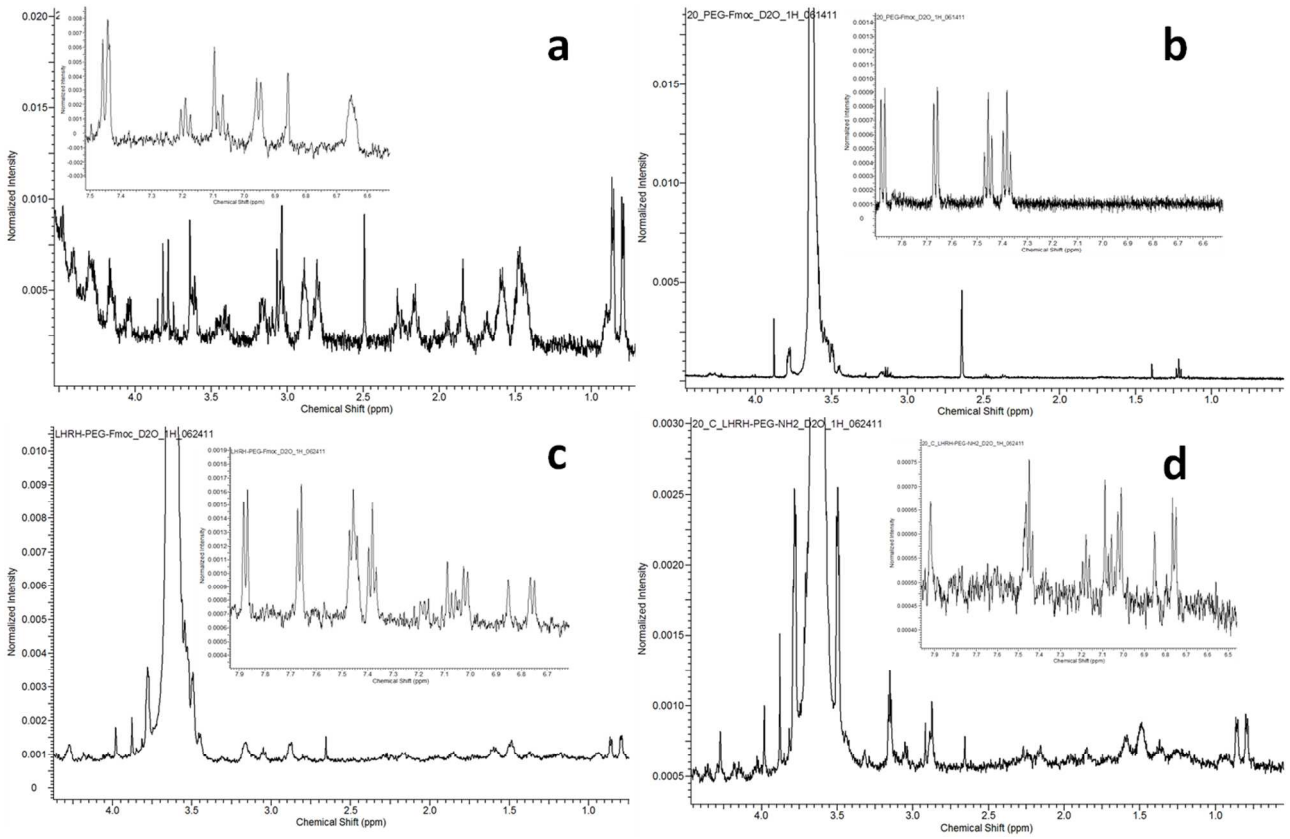

Figure S2. ^{1}H-NMR spectra of the intermediates and final PEGylated peptide. **a)** LHRH peptide, **b)** NHS-PEG-Fmoc, **c)** LHRH-PEG-Fmoc and **d)** LHRH-PEG-NH_2 in D_2O.

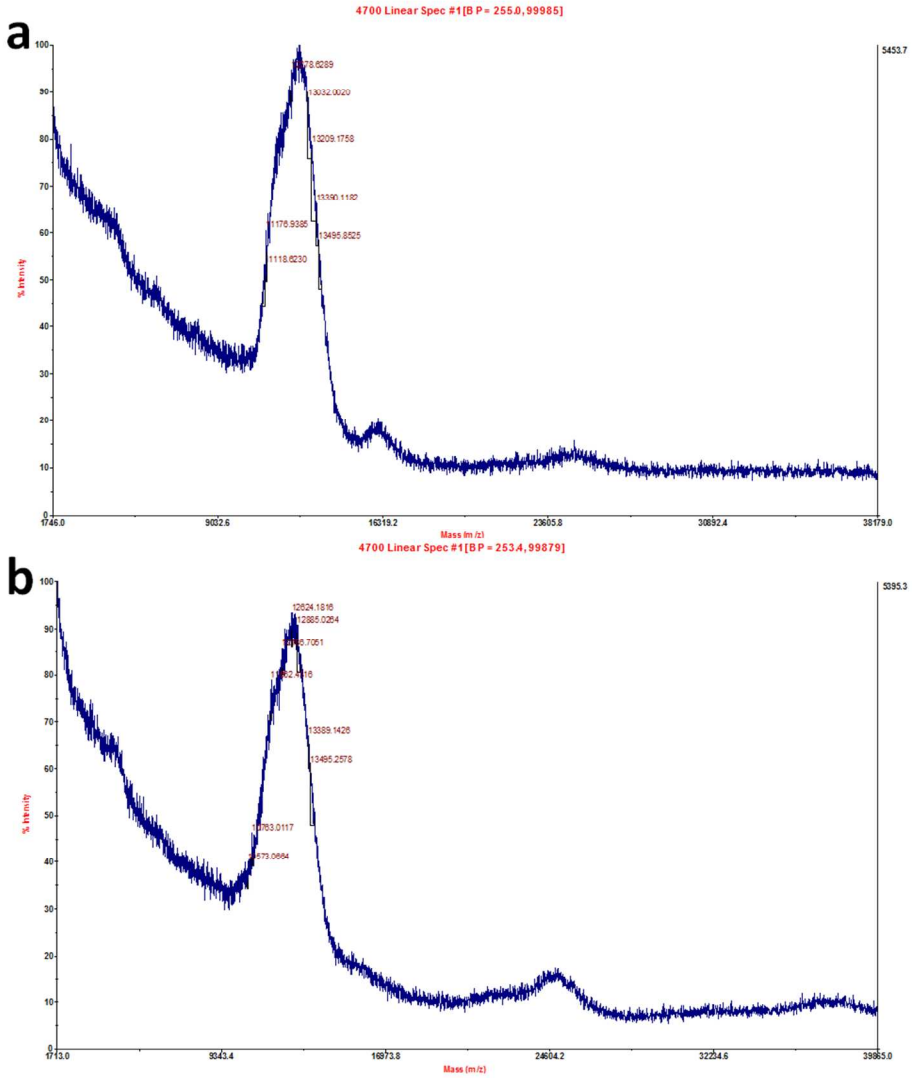

Figure S3. MALDI-TOF mass spectra of LHRH-PEG conjugates. **a)** LHRH-PEG conjugate in case of 1 eq. NHS-PEG-Fmoc during the reaction, **b)** LHRH-PEG conjugate with multiple PEG (5-fold excess of NHS-PEG-Fmoc).

PEGylated LHRH were dissolved in approximately 20-40 µL of acetonitrile and analyzed by matrix-assisted laser desorption/ionization tandem time-of-flight (MALDI-TOF) mass spectrometry. 1.0 µL of sample solution was mixed with 9.0 µL of matrix (10 mg/mL alpha cyano-4-hydroxycinnamic acid dissolved in 50% (v/v) aqueous acetonitrile containing 0.1% (v/v) TFA). All samples were desalted prior the measurements (same samples from ^{1}H-NMR study). Sample LHRH were prepared using Zip Tip columns, other samples using Zeba Desalt Spin Columns (0.5 ml).

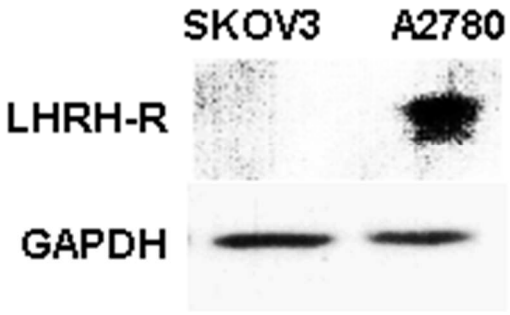

Figure S4. Expression of LHRH receptors in SKOV-3 and A2780 cell lines. For immunodetection of LHRH receptors, the cell lysates (40 µg of total protein per well) of A2780 and SKOV3 cells were subjected to 4-15% SDS-PAGE gel, followed by Western blot analysis. Goat polyclonal anti-LHRH receptor (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-GAPDH (1:5000, Cell Signaling Technology Inc., Beverly, MA) primary antibodies were used. GAPDH was used as a control for equal protein loading.

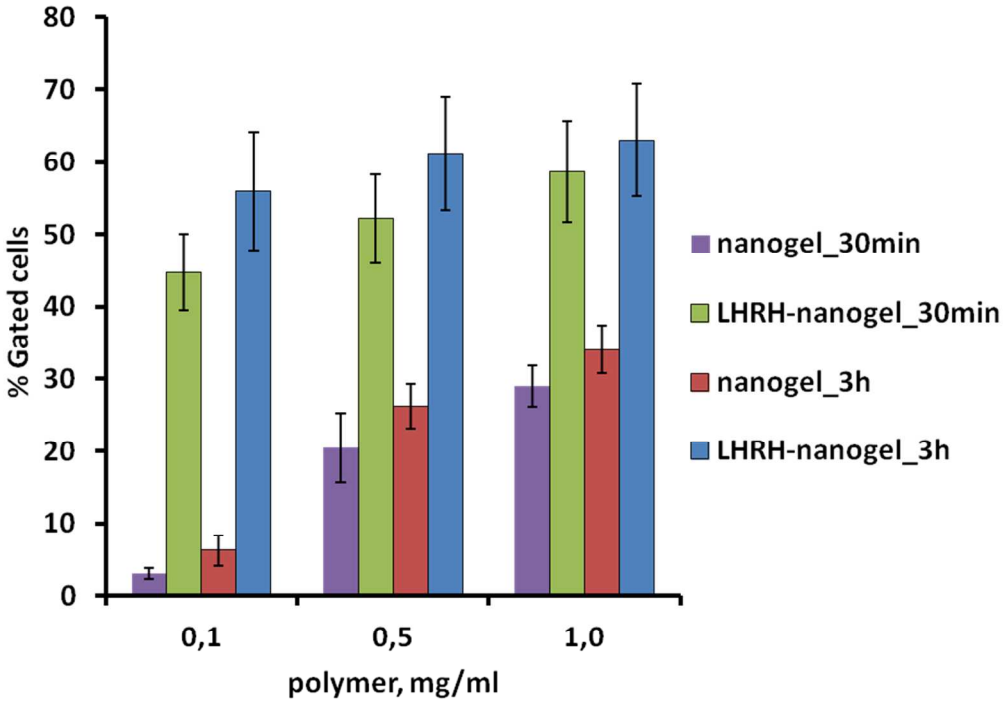

Figure S5. Uptake of FITC-labeled nanogel and LHRH-nanogel in A2780 cells as a function of exposure time (30 min, 3 h) and concentration of polymer (0.1-1.0 mg/ml).

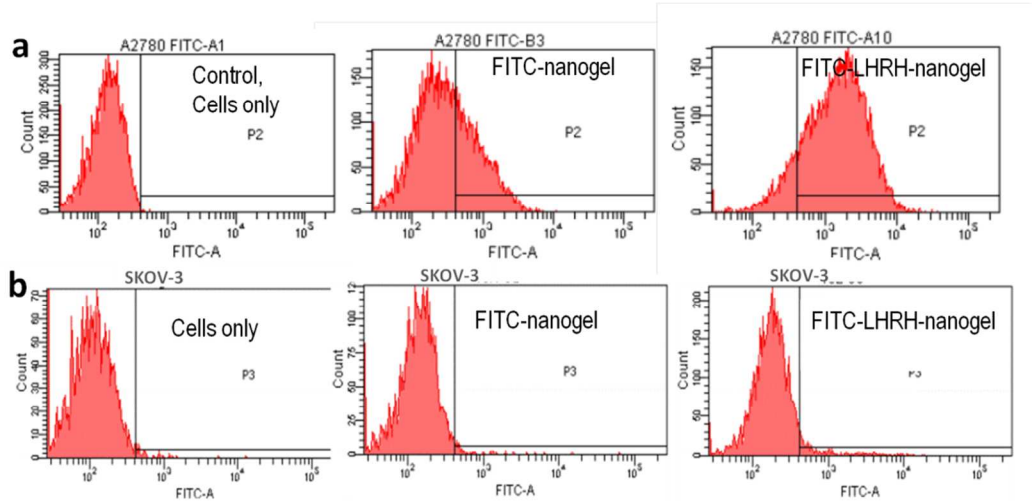

Figure S6. Histograms of fluorescence intensity in **a)** A2780 and **b)** SKOV-3 cell lines exposed to FITC-labeled nanogel and LHRH-nanogel (0.5 mg/ml, 3 h).

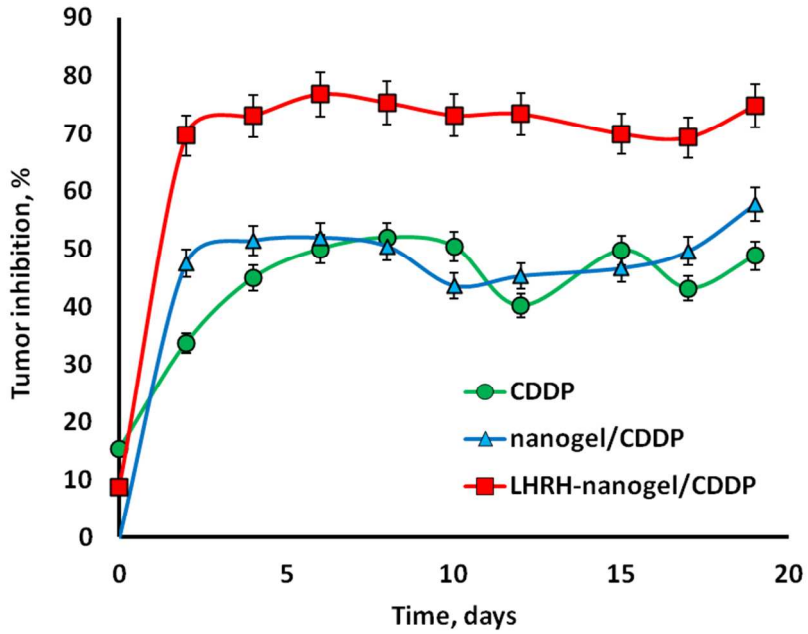

Figure S7. Tumor growth inhibition of ovarian xenografts in nude mice treated with CDDP formulations (CDDP, nanogels/CDDP and LHRH-nanogels/CDDP). Tumor inhibition (TI, %) = (1-Vt/Vc)*100, where Vt and Vc are mean tumor volumes in the treated and control (5% dextrose) groups, respectively.

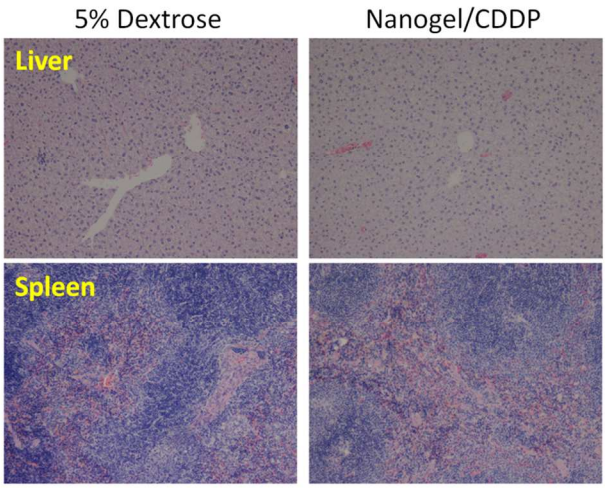

Figure S8. Light microscopy images (original magnification 100×) of hematoxylin and eosin-stained liver and spleen tissues from mice treated with various CDDP formats. The high accumulation of nanoparticles in the organs of reticuloendothelial system (such as liver and spleen) is well known and could be attributed to many factors such as size, shape or chemical composition of nanoparticles. Nevertheless, despite the high accumulation of Pt-loaded nanogels, no histopathological changes were observed in liver and spleen.