



Supporting Information

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Reversible Cell-Specific Delivery of Chemotherapy Drugs Using Aptamer-Functionalized Liposomes

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General:

The chemicals and reagents used in this study were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA) and used as received unless otherwise specified. The aptamer with sequence 5'-GGT GGT GGT GGT TGT GGT GGT GGT GGT TTT TTT TTT TT-3', a random DNA control with sequence of 5'-GAG AAC CTG AGT CAG TAT TGC GGA GAT TTT TTT TTT TT-3', and the cDNA of the aptamer in 2'-O-Methyl RNA bases with sequence of 5'-CCA CCA CCA CCA CAA CCA CC-3' were obtained from Integrated DNA Technologies, Inc (Coralville, IA, USA). Fluorescence labeling and modifications on the oligonucleotides were achieved at the time of custom synthesis. HSPC, cholesterol, PEG2000-DSPE, and extruder were from Avanti Polar Lipids, Inc (Alabaster, AL, USA). The buffer for liposome preparation contained 25 mM HEPES at pH 7.6, 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 1 mM CaCl₂. The elution buffer for the column purification of liposomes was the same except that 35 mM, instead of 25 mM, HEPES at pH 7.6 was used. Sephadex G-100 medium was purchased from GE Healthcare (Chalfont St. Giles, UK)

Supplemental Figure 1. Analysis of Cells by FACS

The MCF-7 and LNCaP cells were plated in 24 well plates overnight with a density of 40,000/cm². On the experiment day, the medium was replaced by OptiMEM. The cells were subsequently incubated for 30 min before liposome solutions were added. After 5 h incubation, the cells were washed with PBS buffer (2× 500 μL/well) and subsequently

treated with 0.25% trypsin with EDTA for 20 min. The cells were then centrifuged at 1200 rpm for 5 min followed by removal of the trypsin solution by pipette. After the cells were washed with PBS buffer ($2 \times 500 \mu\text{L}/\text{well}$), they were fixed by a PBS solution containing 4% formaldehyde for 10 min at room temperature, washed again with PBS ($1 \times 500 \mu\text{L}$), and analyzed by FACS.

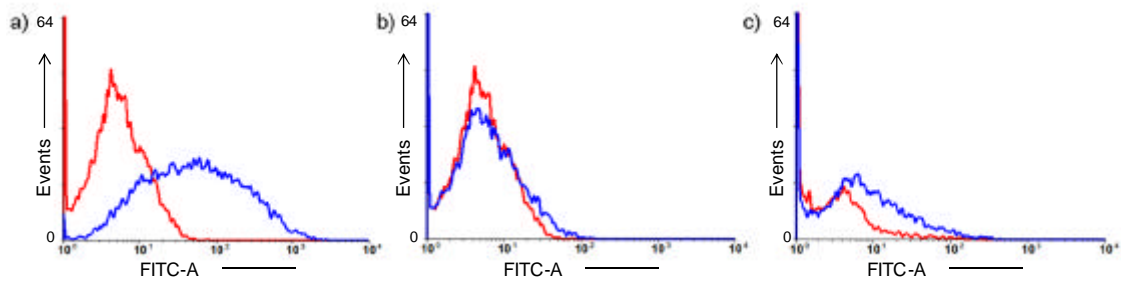


Figure S1. Flow cytometry analysis of untreated cells (red line) and cells treated with DNA-functionalized liposomes containing calcein (blue line). a) MCF-7 cells treated with aptamer-functionalized liposomes, b) MCF-7 cells treated with random DNA-functionalized liposomes, c) LNCaP cells treated with aptamer-functionalized liposomes.

To confirm that aptamer-liposome was indeed internalized and the fluorescence observed in Figure 1 (see text) was not from the surface bound calcein-containing liposomes, we carried out further uptake studies using fluorescence-assisted cell sorting (FACS). MCF-7 cells were incubated with NCL-aptamer-liposome similar to that for confocal studies except that trypsin (0.25%), a commonly used protease, was added to remove all NCLs on the extracellular membranes before the cells were fixed with formaldehyde. This trypsination step would remove cell membrane bound NCL-aptamer-liposomes that might otherwise give false FACS signals, so that the fluorescence detected by FACS would be exclusively from the liposomes internalized^[1,2]. As shown in Figure S1, the mean fluorescence intensity of the NCL-aptamer-liposome treated MCF-7 cells was 49.68, as compared to 4.38 of the untreated MCF-7 cells. In contrast, the MCF-7 cells treated with the liposomes coated with control DNA displayed a low mean fluorescence of 4.87, resembling that of untreated cells. LNCaP cells incubated with NCL-aptamer-liposome showed only slight increase in the mean fluorescence as

compared to the untreated LNCaP cells (2.89 versus 1.51), and the overall histogram remained nearly unchanged. These observations confirmed that the NCL-aptamer-functionalized liposomes could selectively bind to MCF-7 breast cancer cells and be readily internalized.

Supplemental Figure 2. Viability Study

LNCaP and MCF-7 cell lines were cultured in 96-well plates with ATCC recommended medium at concentrations so as to allow 70% confluence in 24 h. At the experiment day, cells were washed with PBS and incubated with Opti-MEM medium at 37 °C for 30 min. After the addition of various formulations of liposomes, cells was incubated for 5 h and then washed with PBS twice (100 µL/well). The cells were further incubated in pre-warmed fresh growth medium for 96 h. The cell viability was assessed colorimetrically with the MTT reagent (Sigma-Aldrich, St Louis, MO, USA) following the standard protocol. The absorbance was assessed with a microplate reader at 570 nm.

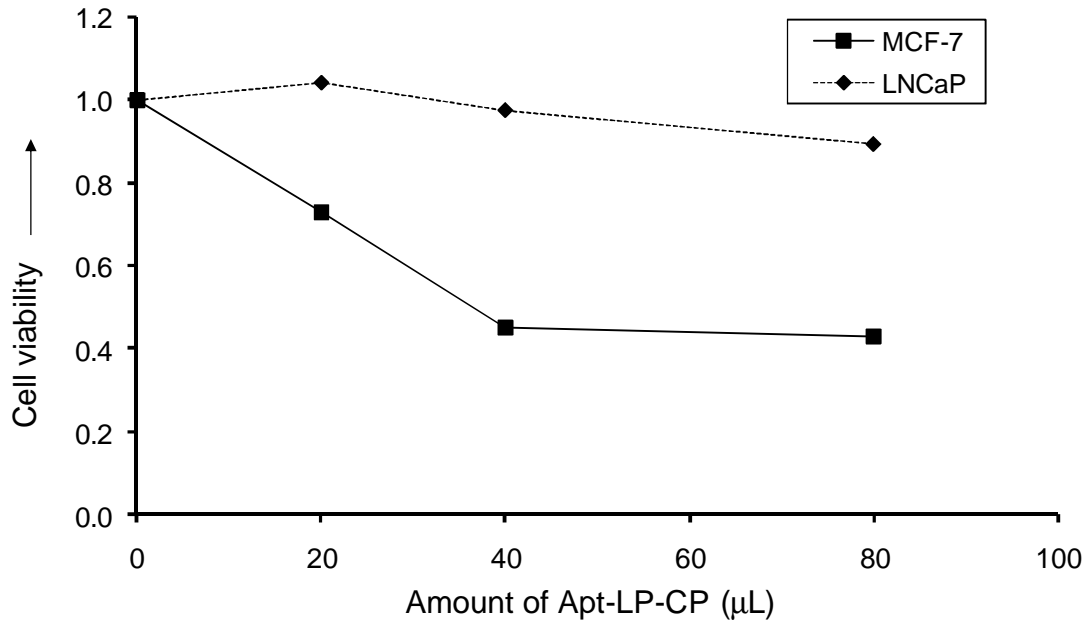


Figure S2. Cell viability assays of different liposome/cell combination: MCF-7 and LNCaP cells were treated with NCL aptamer-functionalized liposomes loaded with

cisplatin (Apt-LP-CP) at various volumes. Cell viability determined by MTT cytotoxicity assays after incubation for 96 h.

To optimize the condition for aptamer-liposome induced cytotoxicity to MCF-7 cells but not to LNCaP cells, we applied different volume of NCL-aptamer-liposome loaded with cisplatin (Apt-LP-CP) to these two cell lines. The original loading concentration of cisplatin was 4 mM. LNCaP and MCF-7 cells were first incubated with various volumes of Apt-LP-CP for 5 h. The medium was removed; the cells were washed with PBS (100 μ l). Fresh medium (100 μ L) was then applied. The plates were incubated for 4 days. The optimal dose of Apt-LP-CP was determined to be 40 μ L as largest viability difference between MCF-7 and LNCaP cells was obtained under this condition: Apt-LP-CP displayed noticeable toxicity towards MCF-7 cells (54.9% cells death at Day 4), while minimum cytotoxicity toward LNCaP cells (2.3% cells death at Day 4). The results represented the optimized condition for cell-specific targeting of Apt-LP-CP to MCF-7 cells (NCL positive cells).

Reference:

- [1] D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang, W. Tan, *Proceedings of the National Academy of Sciences of the United States of America* **2006**, *103*, 11838.
- [2] Z. Y. Xiao, D. H. Shangguan, Z. H. Cao, X. H. Fang, W. H. Tan, *Chemistry-a European Journal* **2008**, *14*, 1769.