Supplementary Material (ESI) for Chemical Communications

A Liposome-based Nanostructure for Aptamer Directed Delivery

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Supporting Information (SI)

Materials. Hydrogenated soy phosphatidyl choline (HSPC), cholesterol (Chol), methoxypoly(ethylene glycol)- distearoyl-phosphatidyl-ethanolamine (MPEG-DSPE) and maleimide-terminated PEG-DSPE (MalPEG) were purchased from Avanti Polar Lipids (Alabaster, AL). Liposome extruder and extrusion membranes were also purchased from Avanti Polar Lipids. FITC-Dextran (4000), Sephadex G-50 gel, sodium bisulfite, sodium sulfite, 1-amino-2 napthol-4sulfonic acid, ammonium molybdate, perchloric acid, sulfuric acid, ammonium molybdate sln, and other solvents were purchased from Sigma-Aldrich Chemical. DNA synthesis reagents and TMR modifier were purchased from Glen Research (Sterling, VA). All the solutions were made using nanopure water obtained from a Millipore system (18.2 MΩ cm resistivity).

Reagent preparation. Fiske-Subbarow reagent: 29.9 g sodium bisulfite, 1 g sodium sulfite, 0.5 g 1-amino-2 napthol-4 sulfonic acid and 200 mL nanopure water were mixed by stirring. The mixture was filtered with a Büchner funnel to remove insoluble solid and stored in an amber bottle.

Ammonium molybdate sln: 2.2g ammonium molybdate, 20mL sulfuric acid and 1000 mL nanopure water were mixed and filtered to obtain a clear solution.

Sephadex Columns: 1g Sephadex G-50 gel beads powder was expended into 10 mL of HEPES buffer by stirring and allowed to soak overnight at 4ºC. The column was packed by slowly pouring beads down a glass rod and then spinning momentarily at 1500g for 5 minutes. The column was sealed and kept moist.

Instruments. An ABI3400 DNA/RNA synthesizer (Applied Biosystems) was used for sgc8 aptamer synthesis. The purifications were carried out on a ProStar HPLC system equipped with gradient unit (Varian) with C-18 column (Econosil, 5U, 250 × 4.6 mm) (Alltech Associates). The characterizations of all DNAs on concentration were performed with a Cary Bio-300UV spectrometer (Varian) by calculating the absorbance of DNA at 260 nm. Fluorescence of FITC-Dextran and TMR-sgc8 was monitored with plate reader (Safire, TECAN). Dynamic light scattering of liposomes was performed by Coulter LS Particle Analyzer. Vortexing of solutions was carried out using a Vortex Minimixer (Fisher Scientific); Flow cytometry (FACScan cytometer, Becton Dickinson Immunocytometry Systems); Confocal microscopy (FluoView 500, Olympus).

Aptamer preparation. Sgc8 aptamer was prepared based on the sequence from Shangguan et al.[9] A DNA sequence, 5′-Thiol-ATC TAA CTG CTG CGC CGCCGGGAAAATACTGTACGGTTAGA-TMR-3′, was synthesized by DNA synthesizer and purified by HPLC. All oligonucleotides were synthesized by solid-state phosphoramidite chemistry at a 1 μmol scale. The completed sequences were then deprotected in AMA (ammonium hydroxide/40% aqueous methylamine 1:1) at 65 °C for 20 min and further purified with reverse phase HPLC on a C-18 column.

Sgc8 aptamer was synthesized on a DNA synthesizer using standard bases with the following modifications: a thiol (S-S)-modified 5’ end to react and conjugate with the MalPEG on the liposome surface and a tetramethylrhodamine (TMR) fluorophore-labeled 3’ end to quantitatively characterize the

amount of aptamers linked on the liposome surface and monitor the liposome-cell interaction (5′-Thiol-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-TMR-3′) (Figure S1. B, C, D).

Preparation and characterization of sgc8 aptamer-conjugated liposome. Hydrogenated soy Phosphatidyl choline (HSPC), cholesterol (Chol), methoxypoly(ethylene glycol)- distearoyl-phosphatidyl-ethanolamine (MPEG-DSPE) and maleimide-terminated PEG-DSPE (MalPEG) (molar ratio 2:1:0.08:0.02, respectively) were mixed together inside a clean 100 mL round bottom flask under N_2 protection. The flask was sealed and vortexed for 30 minutes at room temperature. The mixture solution was applied for a slow rotating evaporation at 40 RPM, 55 ºC and 600 mg Hg until an evenly spread layer of thin lipid film was obtained on the bottom of the flask. The flask was placed under a high vacuum with N_2 for at least 2 hours or overnight to remove all the chloroform.

FITC-Dextran was dissolved in 20mM HEPES buffer (150 mM NaCl, pH 7.4) at 30 mg/mL. 3 mL FITC-Dextran solution was used to rehydrate the lipid film on the flask bottom by swirling in a 55 ºC water bath. After the entire lipid was removed from the flask bottom, the solution was transferred to a Cry-Freezing tube. The tube was subjected to freeze/thaw for 8 cycles in liquid nitrogen and 55 ºC water bath in turn. The solution was allowed to set overnight at 4 ºC.

The solution was extruded with the extruder (on a 50 ºC heating plate) 10 times through 0.4 μm, 0.2 μm and 0.1 μm polycarbonate membranes twice each. DLS was performed to examine the size distribution of liposome with the size distribution of 100 ± 20 nm (Figure S1.A). A small portion of such liposome solution was saved as pre-column sample for later calculation of lipid concentration. The rest of the liposome solution was transferred to the prepared Sephadex G-50 column to eliminate free

lipid, unincorporated FITC-Dextran and other impurities. The collected fractions (0.5 mL each) were applied for phosphate assaying and dye assaying:

Assaying Phosphate: Standard solutions of 0, 25, 50, 100, 150, 200, 500 nmol of PO_4^{3-} were prepared from a 1mM NaH_2PO_4 solution in replicates of 3. Approximately 100 nmol (about 7 μL) of each fraction from Sephadex column was added to a test tube. 0.7 mL perchloric acid was added to each tube, vortexed and placed in a 180 ºC-220 ºC oil bath for 90 min. The test tubes were cooled down. 0.7 mL Fiske-Subbarow reagent and 7mL ammonium molybnate sln were added to the tubes and applied for 15 min in boiled water bath. Absorption was measured at 815nm on the plate reader. The lipid concentrations were calculated by comparing with standard solutions.

Assaying Dye Loading: Standard solutions of both FITC and TMR dyes were obtained using the same method as phosphate standard solutions. The liposomes were burst with triton100 in 1:1 and added to a 383 plate with 3 replicates of each. The plate was measured with both FITC fluorescence (λ_{ex} = 488 nm, λ_{em} = 520 nm) and TMR fluorescence (λ_{ex} = 545 nm, λ_{em} = 580 nm). The FITC-Dextran amount was determined by using the following formula: (Dye/Lipid of sample x 100)/ (Dye/Lipid pre-column). The calculated data were plotted by comparing with standard FITC-Dextran solution. Liposomes were ready for aptamer conjugation.

The conjugation of sgc8 aptamer to liposomes was performed by adding 5-fold aptamer (compared to amount of MalPEG estimated from phosphate assay) to the liposome solution. The 3' thiol-modified aptamer was first activated by 100 mM TCEP solution at 4 ºC for 30 minutes. Then 5-fold of such activated aptamer was mixed with liposome and incubated overnight at 4 ºC. Following this, 2mM betamercaptoethanol (BME) was added to quench the unreacted maleimide group. The aptamer-liposome solution was re-run with Sephadex column to remove free aptamer, and the

lipid/FITC/TMR assays were repeated to characterize liposome composition. The distributions of lipid, FITC and TMR are depicted in Figure S1. B, C, D. Fraction #6 was identified as 100 nm liposomes linked with sgc8 aptamer and loaded with FITC-Dextran.

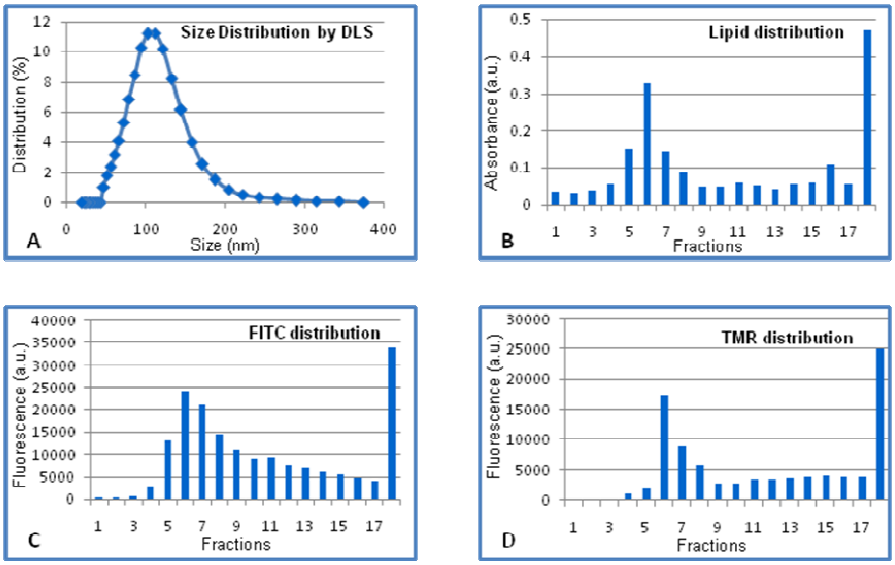

Figure S1. Characterization of liposomes. A. DLS of liposomes. Assaying of B, lipid; C, FITC; and D, TMR of prepared liposomes.

Dialysis of liposomes. The liposome solution was added to cut dialysis tubing (MW 4000) and set inside 500 mL HEPES buffer solution at 4 ºC (buffer was changed after each measurement). The fluorescence was measured every 24 hours (FITC: λ_{ex} = 488 nm, λ_{em} = 520 nm; TMR: λ_{ex} = 545 nm, λ_{em} = 580 nm).

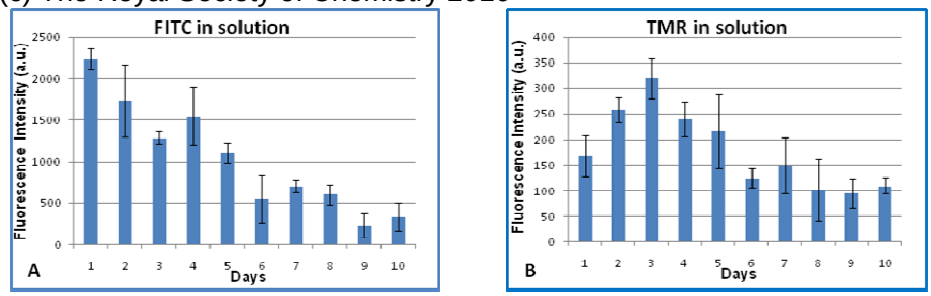

Figure S2. Dialysis anaylsis of liposomes on A, FITC and B, TMR. Three parallel experiments were performed each time.

Cell lines and buffers. CEM-CCRF cells (CCL-119 T-cell, human acute lymphoblastic leukemia) were obtained from ATCC. NB4 cells (acute promyelocytic leukemia) were obtained from the Department of Pathology at the University of Florida. The cells were cultured in RPMI medium supplemented with 10% fetal bovine serum and 100 IU/mL penicillin-streptomycin. The cell density was determined using a hemocytometer. Approximately 1×10^6 cells were dispersed in buffer for each flow cytometry test. The cells were kept in an ice bath at 4 °C during all experiments.