

## Supporting Information for

### **Lipid-Coated Nanoscale Coordination Polymers for Targeted Delivery of Antifolates to Cancer Cells**

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#### **MATERIALS AND METHODS**

**Reagents.** All chemicals, unless otherwise noted, were purchased from Fisher or Sigma Aldrich and used without further purification. Methotrexate was purchased from TCI America. 1,2-dioleoyl-3-trimethylammonium propane, chloride salt (DOTAP) and 1,2-stearoyl-3-trimethylammonium propane, chloride salt (DSTAP) were purchased from Avanti Polar Lipids. L- $\alpha$ -phosphatidylethanolamine, dioleoyl (DOPE) was a gift from Sigma Aldrich. Cell culture supplies, fetal bovine serum (Sigma), RPMI-1640 growth medium (Gibco), penicillin-streptomycin (Sigma), and phosphate buffered saline (Gibco) were purchased from the Tissue Culture Facility in the Lineberger Cancer Center at UNC-Chapel Hill. Annexin V FITC conjugate was purchased from Invitrogen.

**Instrumentation.** Microwave reactions were carried out in a CEM Discovery microwave or a CEM MARS 5 microwave. Scanning electron microscopy (SEM) was performed with a Hitachi 4700 Field Emission Scanning Electron Microscope, and transmission electron microscopy (TEM) was performed with a JEM 100CX-II Transmission Electron Microscope. A Cressington 108 Auto Sputter Coater equipped with a Au/Pd (80/20) target and MTM-10 thickness monitor was used to coat samples before SEM imaging. SEM micrographs were obtained on glass slides, and TEM micrographs were obtained on carbon-coated copper grids. Thermogravimetric analysis (TGA) was done on a Shimadzu TGA-50 equipped with a platinum pan, and samples were heated at a rate of 3°C/min under air. Powder x-ray

diffraction (PXRD) data was gathered on a Bruker SMART APEX II diffractometer using Cu radiation, and powder patterns were analyzed with the APEX II package using the phase ID plugin. UV-Vis absorption spectra were obtained using a Shimadzu UV-2401 PC UV-Vis recording spectrophotometer. Size and zeta potential information was obtained on a Malvern ZetaSizer dynamic light scattering instrument. Confocal microscopic images were obtained on glass slides and imaged on the Olympus FlowView confocal microscope at the UNC-CH Microscope and Imaging Facility. Images were analyzed using ImageJ (with the UCSD plugin) and PhotoShop.

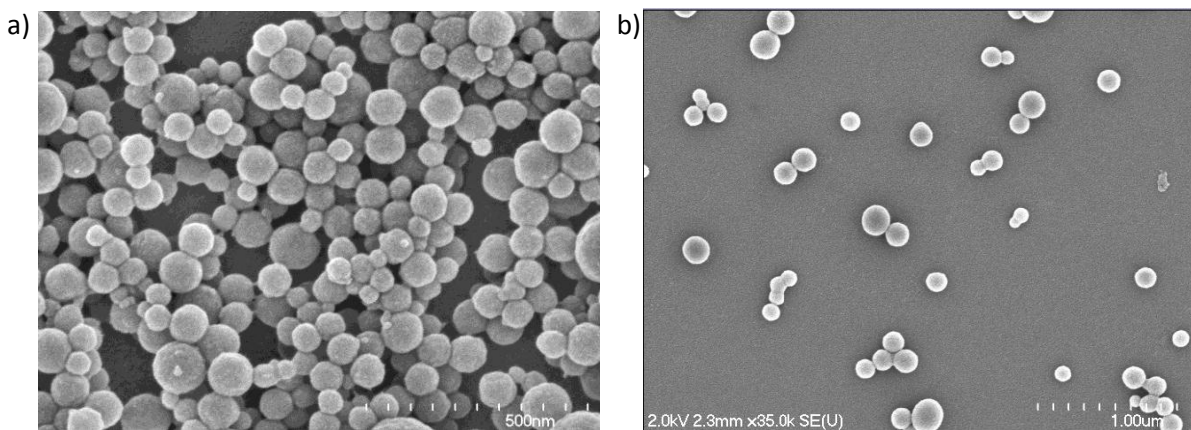
**Synthesis of [Ru(5,5'-CO<sub>2</sub>H-bpy)(bpy)<sub>2</sub>](PF<sub>6</sub>)<sub>2</sub> (carboxylated Ru(bpy)<sub>3</sub><sup>2+</sup> derivative).** 5,5'-CO<sub>2</sub>(Et-bpy) and Ru(bpy)<sub>2</sub>Cl<sub>2</sub> were prepared according to a literature method.<sup>34</sup> Ru(bpy)<sub>2</sub>Cl<sub>2</sub> (1.16g, 2.39 mmol) and 1.45 equivalents of 5,5'-CO<sub>2</sub>(Et-bpy) (1.04g, 3.46 mmol) were refluxed under argon in 160 mL of H<sub>2</sub>O:EtOH (1:1 v/v) for 8 h. After the removal of ethanol by rotary evaporation, the complex was precipitated by the addition of 5 mL of saturated aqueous ammonium hexafluorophosphate. This crude product was filtered and washed with ether, then hydrolyzed by reflux under argon in 100 mL of 4M HCl overnight. Precipitation was achieved by the addition of 5 mL of saturated aqueous ammonium hexafluorophosphate. The product was filtered and washed with water to remove excess HCl and washed with ether. Yield: 1.79 g (80%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>): 8.99 (d, 2H), 8.89 (m, 4H), 8.53 (d, 2H), 8.20 (m, 4H), 7.99 (s, 2H), 7.84 (d, 2H), 7.78 (d, 2H), 7.59 (t, 2H), 7.49 (t, 2H).

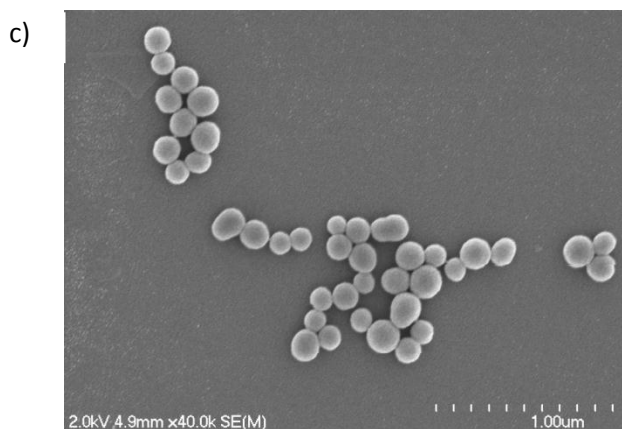
**Synthesis of DOPE-AA.** DOPE (50 mg, 0.067 mmol) was reacted with 4-methoxybenzoic acid (103.5 mg, 0.672 mmol) in dichloromethane (10 mL, anhydrous), in the presence of N,N'-dicyclohexylcarbodiimide (27.2 mg, 0.134 mmol) and 4-dimethylaminopyridine (16.42 mg, 0.134 mmol) under nitrogen. The reaction was stirred at room temperature for 24 h in the dark. After the removal of dichloromethane by rotary evaporation, the crude product was dissolved in chloroform, and the solution was washed with 4% Na<sub>2</sub>CO<sub>3</sub>, 0.2M HCl, water, and then dried over MgSO<sub>4</sub>. The resulting crude product was purified by column chromatography using silica gel and 5:1 chloroform/methanol (v/v) as the eluent. The product was obtained after removal of the solvents. Yield: 30 mg (51%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.82 (d, J = 7.6 Hz,

2H); 6.78 (s, 2H); 5.31 (m, 4H); 3.76 (s, 3H); 2.15 (s, 4); 1.97 (s, 6H); 1.62 (s, 10H); 1.46 (s, 4H); 1.21 (t, J= 14 Hz, 30H); 0.85 (t, J = 6 Hz, 6H).

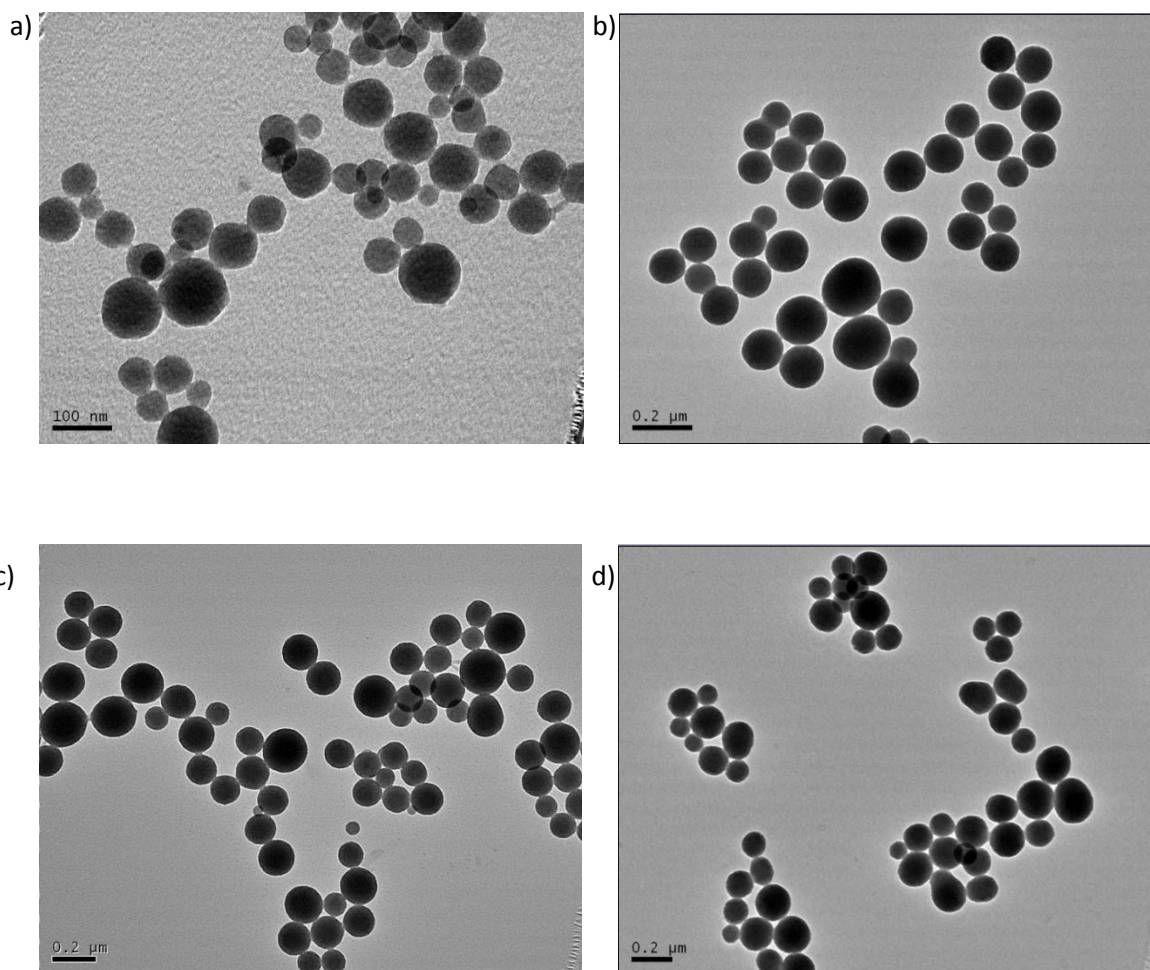
**Synthesis of liposomes.** 1:1 (by mol) DSTAP/DOPE liposomes were prepared by combining DSTAP (5 mg, 0.007117 mmol) and DOPE (5.295 mg, 0.07117 mmol) in chloroform. The solvent was removed under vacuum by rotary evaporation. The resulting lipid film was further dried under vacuum then hydrated with 1 mM aq. KCl. The dispersion was extruded three times through a 0.6  $\mu$ m polycarbonate filter membrane and ten times through a 0.1  $\mu$ m polycarbonate filter membrane. The resulting liposome formulation can be stored up to 1 week at 4 °C. Liposomes were made to contain DOPE-AA by incorporating 10 mol% DOPE-AA in the initial chloroform solution.

**Cell Lines.** CCRF-CEM acute lymphoblastic leukemia cells (ATCC# CCL-119) and Jurkat acute lymphoblastic leukemia cells (ATCC# TIB-152) were purchased from the Tissue Culture Facility of Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. Both cell lines were maintained as a suspension in RPMI-1640 growth medium (Cellgro) supplemented with 10% fetal bovine serum (Sigma) and 2% penicillin-streptomycin (Sigma).

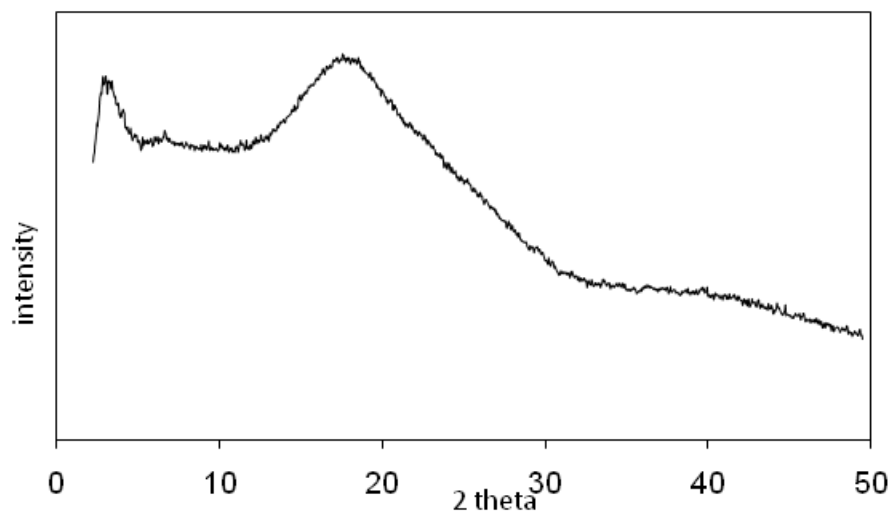




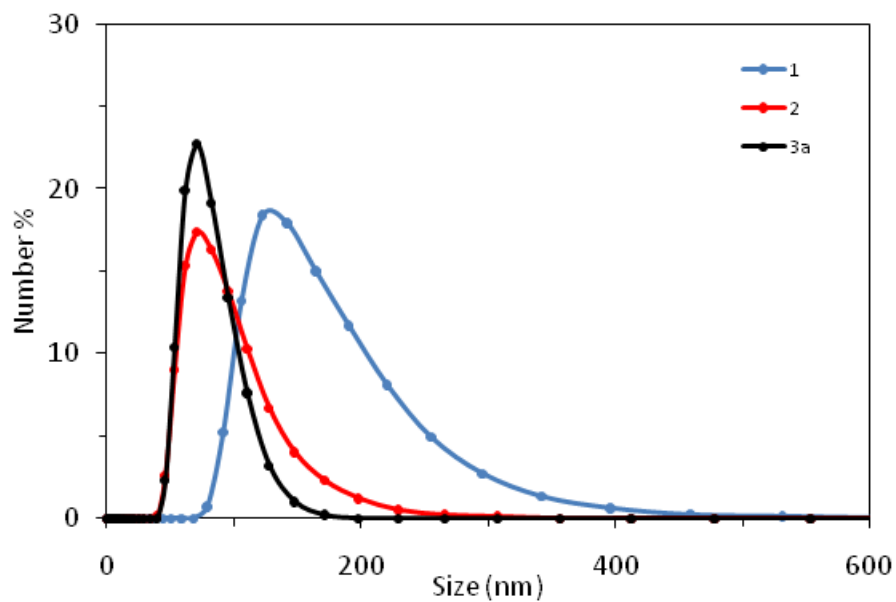
**Figure S1.** SEM images of (a) **1**, (b) **2**, and (c) **3a**.



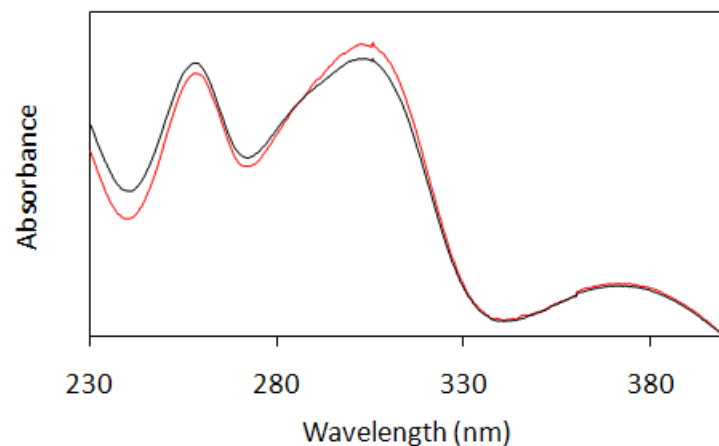
**Figure S2.** TEM images of (a) **1**, (b) **2**, (c) **3a**, and (d) **3b**.



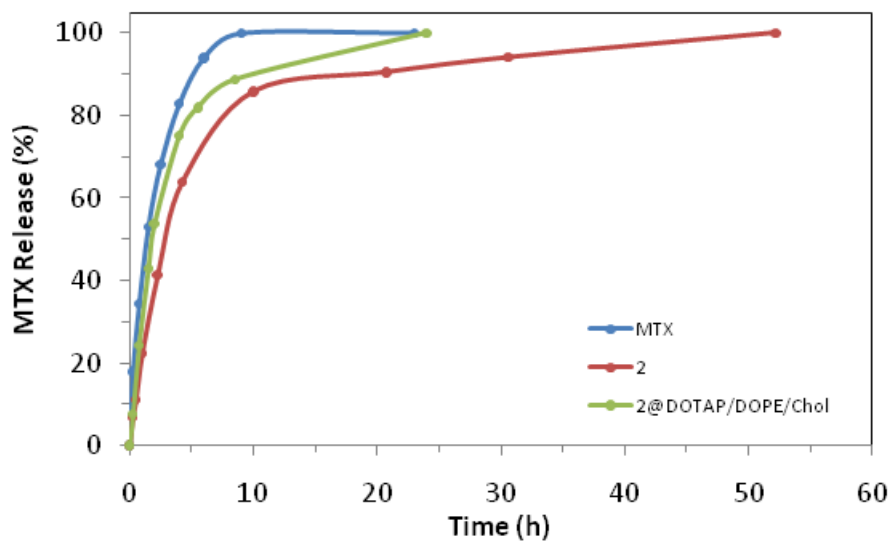
**Figure S3.** Powder X-ray diffraction pattern of **1** which shows that these particles are amorphous.



**Figure S4.** Number distribution obtained from DLS for **1**, **2**, and **3a**. Data for **1** was obtained in ethanol and data for both **2** and **3a** were obtained in 1 mM KCl (aq). **1** had a number average of 161 nm and a PDI of 0.204. **2** had a number average of 88.4 nm, PDI of 0.18, and a zeta potential of -27.2 mV. **3a** had a number average of 77.7 nm, PDI of 0.037, and a zeta potential of -10.0 mV.

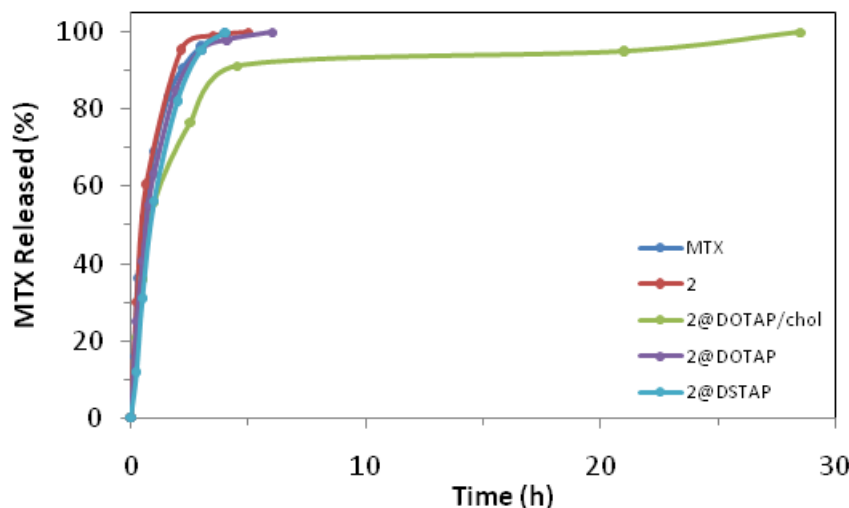


**Figure S5.** An absorption spectrum for MTX (black), compared to an absorption spectrum for a release profile aliquot from dialysis of **1** (red). The absorption profile does not change significantly, indicating that MTX remains unchanged under the synthetic conditions used.



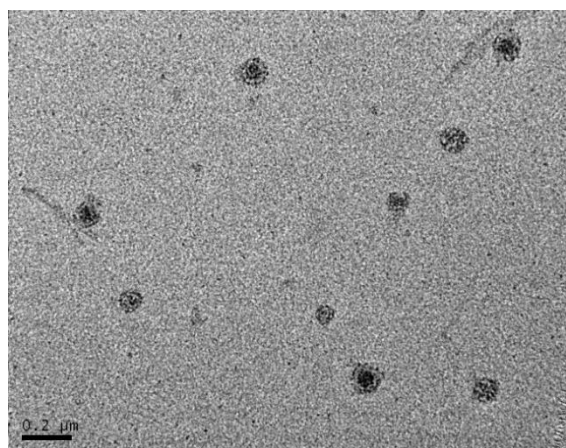
**Figure S6.** Release profiles of free MTX, **2**, and **2** coated with 4:48:48 (by mol) DOTAP/DOPE/cholesterol liposomes, showing no extra stabilization from this lipid coating. The release profile was performed in 8 mM PBS solution at 37 °C.



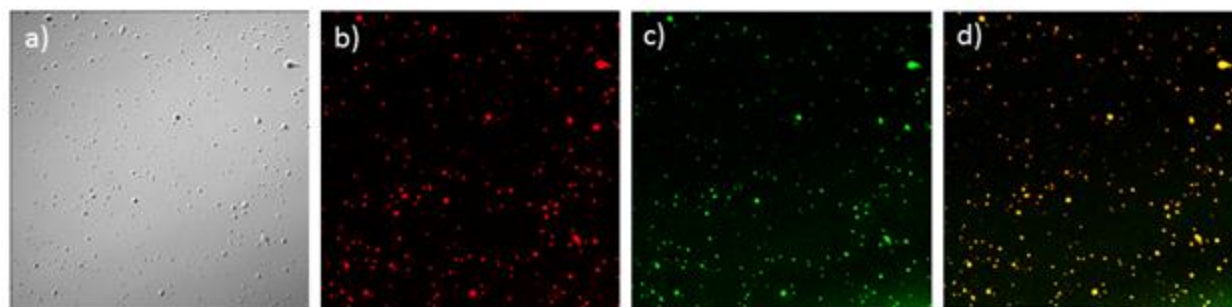


**Figure S7.** Release profiles for free MTX, **2**, **2** coated 1:1 (by mol) DOTAP/chol, **2** coated with DOTAP, and **2** coated with DSTAP, showing no extra stabilization upon lipid coating. Release profiles were performed in simulated body fluid (SBF) at 37 °C.

**Formulation of Simulated Body Fluid (SBF).** SBF was prepared by following a literature procedure.<sup>[1]</sup> To make 1 L of SBF, 800 mL of H<sub>2</sub>O was heated to 37 °C, and the following reagents were added sequentially in the order listed: NaCl (5.585 g), NaHCO<sub>3</sub> (0.965 g), Na<sub>2</sub>CO<sub>3</sub> (1.765 g), KCl (0.225 g), K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O (0.230 g), MgCl<sub>2</sub>·6H<sub>2</sub>O (0.217 g), HEPES (2-(4-(2-hydroxyethyl)-1-piperazinyl)ethanesulfonic acid, 11.928 g, dissolved first in 100 mL H<sub>2</sub>O), CaCl<sub>2</sub> (0.191 g), Na<sub>2</sub>SO<sub>4</sub> (0.072 g). The pH was raised to 7.40 by adding 0.75 mL of 1.0 M NaOH, and the solution was cooled to room temperature. More H<sub>2</sub>O was added to raise the volume to 1 L.

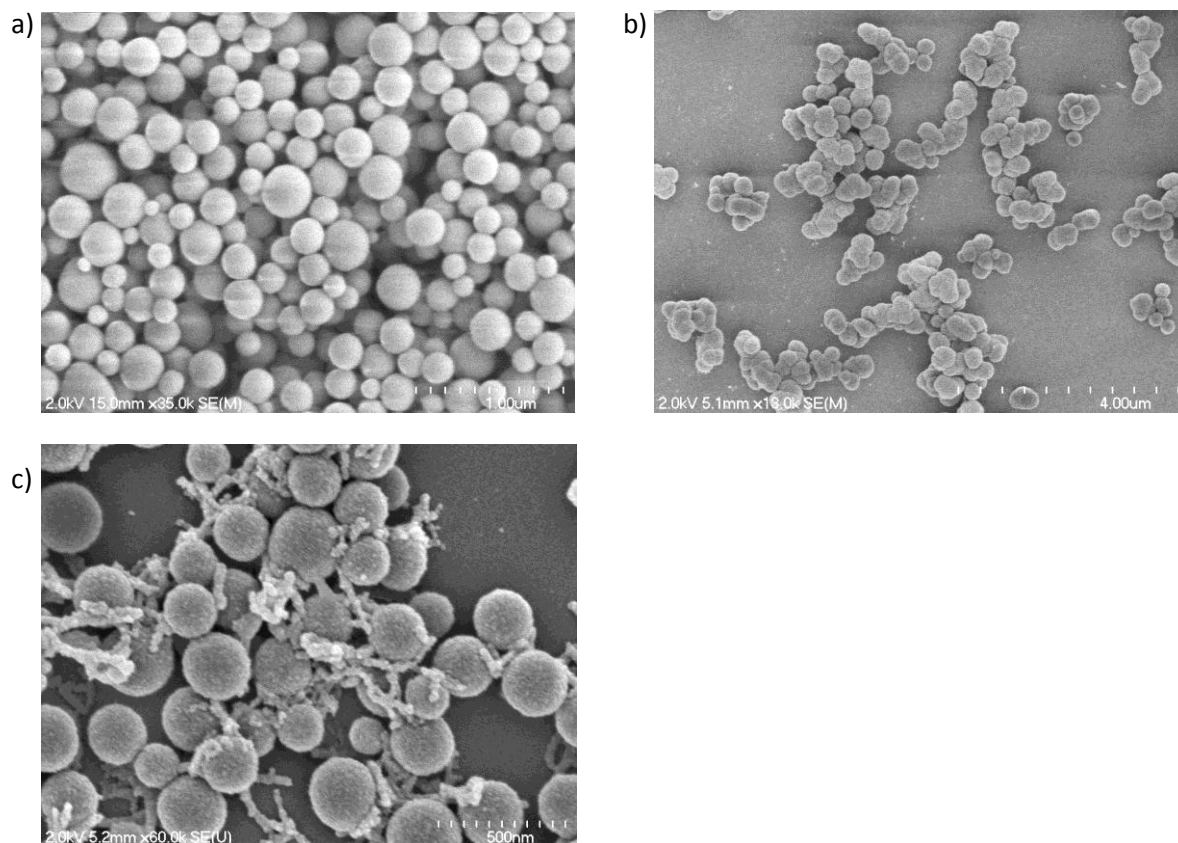


**Figure S8.** TEM image of DOTAP liposomes stained with 2% aq. K<sub>2</sub>PtCl<sub>4</sub>.



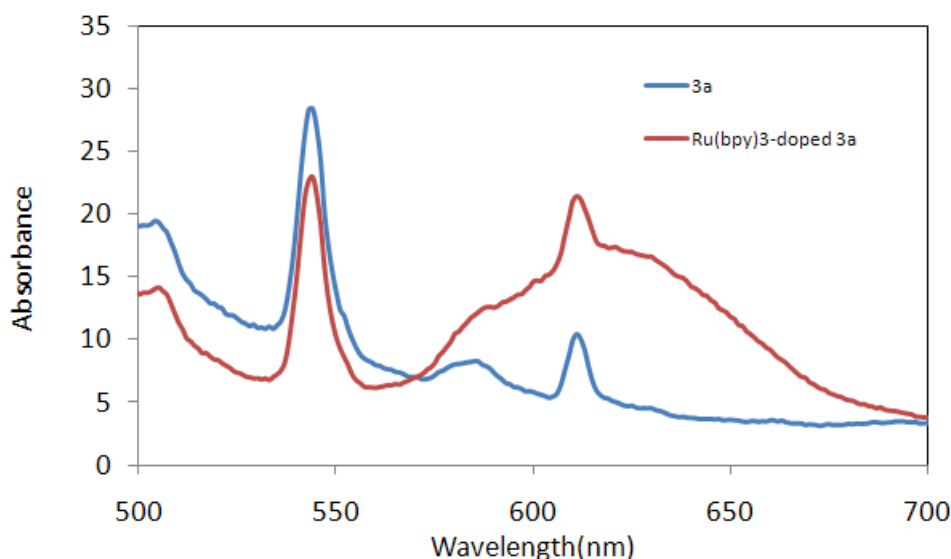
**Figure S9.** Confocal microscopy images of Ru(bpy)<sub>3</sub>-doped **3b** in which the lipid bilayer has been doped with 10 mol% DOPE-FITC. This experiment was conducted to show co-localization of particle fluorescence with the fluorescence of the lipid bilayer. a) DIC image, b) red fluorescence due to particles, c) green fluorescence due to FITC-doped lipid bilayer, and d) overlay of particle and lipid bilayer fluorescences showing co-localization.

**Synthesis of DOPE-FITC.** Fluorescein isothiocyanate (FITC, 27.3 mg, 0.07 mmol) was added to a small round-bottom flask and dried under high vacuum for 1 h. Anhydrous methanol (10 mL) and triethylamine (6.2  $\mu$ L) were added to the round-bottom flask, followed by 0.5 mL DOPE (100 mg/mL, 0.067 mmol in anhydrous dichloromethane). The reaction was magnetically stirred under nitrogen gas at room temperature for 5 h in the dark. After the removal of solvent by rotary evaporation, the product was redissolved in methanol (77 mg, 25 mg/mL, 100% yield).

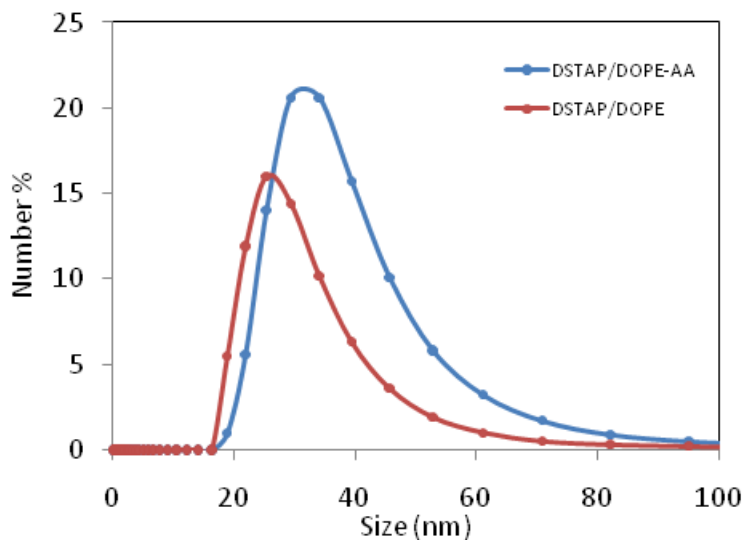




**Figure S10.** SEM images of (a) Ru(bpy)<sub>3</sub>-doped **3a**, (b) **4a**, and (c) Ru(bpy)<sub>3</sub>-doped **4a**. DLS data for Ru(bpy)<sub>3</sub>-doped **3a** shows a number average of 119.5 nm, PDI of 0.154, and zeta potential of -20.6 nm in 1 mM aq. KCl. DLS data for **4a** shows a number average of 82.4 nm, PDI of 0.345, and zeta potential of -11.8 mV in 1 mM aq. KCl. DLS data for Ru(bpy)<sub>3</sub>-doped **4a** gives a number average of 160.6 nm, PDI of 0.365, and zeta potential of -16.1 mV in 1 mM aq. KCl.



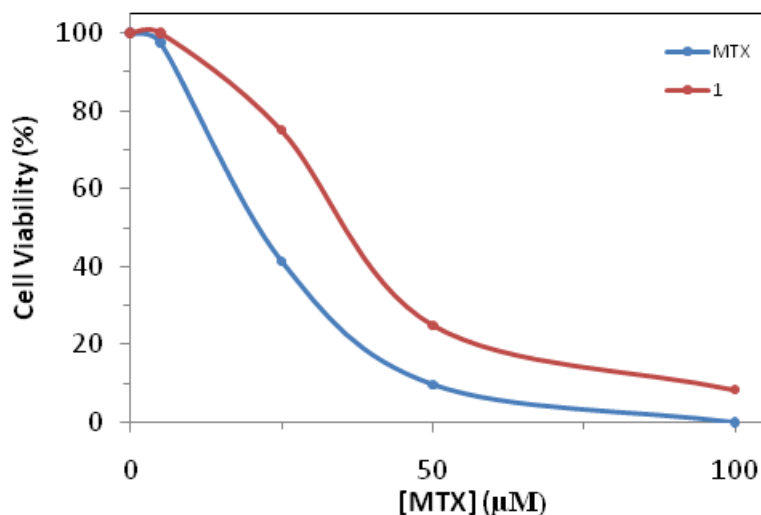
**Figure S11.** UV-Vis spectra of **3a** and 10 mol% Ru(bpy)<sub>3</sub>-doped **3a** showing absorbance attributed to the Ru(bpy)<sub>3</sub><sup>2+</sup> dye at ~630 nm. The sharp peaks at ~540 and ~620 nm are from the particles themselves.



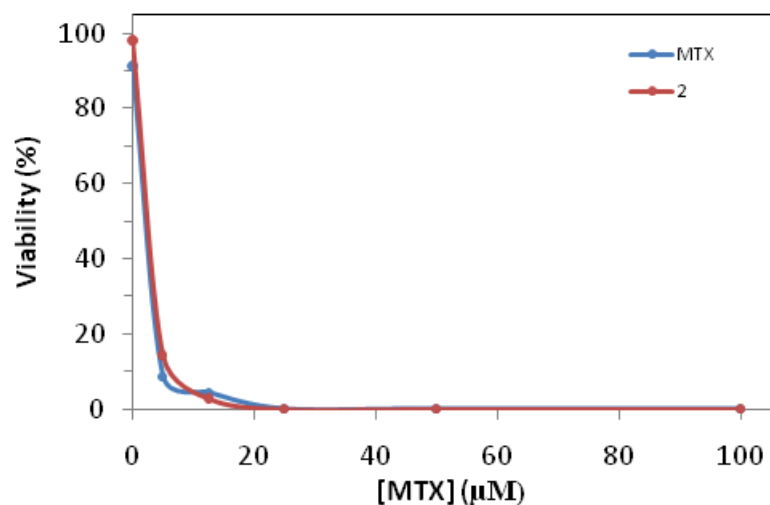
**Figure S12.** Number distribution obtained from DLS for 1:1 (by mol) DSTAP/DOPE liposomes and 10 mol% DOPE-AA 1:1 (by mol) DSTAP/DOPE liposomes. DLS data for the non-targeted

liposomes gave a number average of 25.7 nm, PDI of 0.318, and zeta potential of 59.5 mV in 1 mM KCl (aq). DLS data for the AA-targeted liposomes gave a number average 36.7 nm, PDI of 0.175, and zeta potential of 59.2 mV in 1mM aq. KCl.

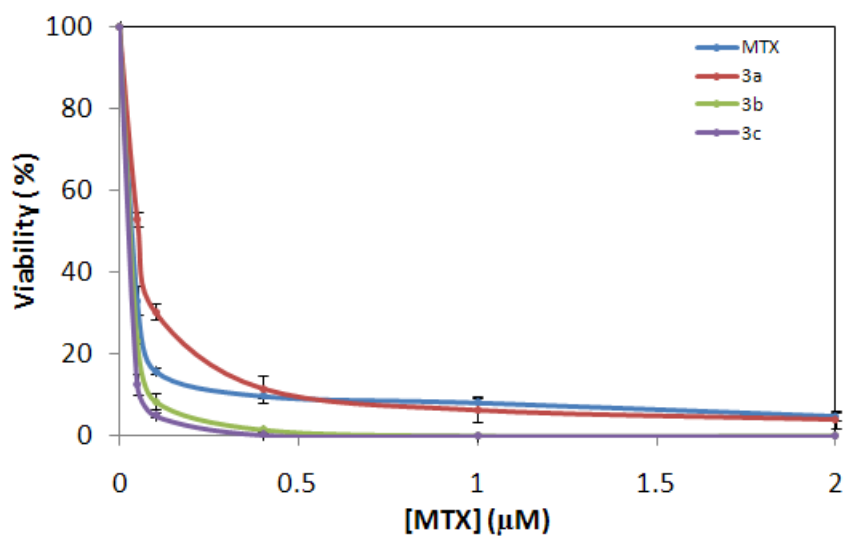
**Cytotoxicity assay of 1 and 2 against CCRF-CEM cells.** Confluent CCRF-CEM cells were centrifuged from suspension and a cell density was obtained from a hemocytometer. A 6-well plate was seeded with  $1.0 \times 10^6$  cells/well and a total of 1.5 mL media. The plate was incubated at 37 °C and 5% CO<sub>2</sub> overnight. Amounts of **1** or **2** as a particle suspension in RPMI-1640 medium were added to wells resulting in MTX concentrations of 0, 5, 12.5, 25, 50, and 100 µM. The plate was incubated at 37 °C and 5% CO<sub>2</sub> for 3 h and viability was determined via the trypan blue exclusion assay.



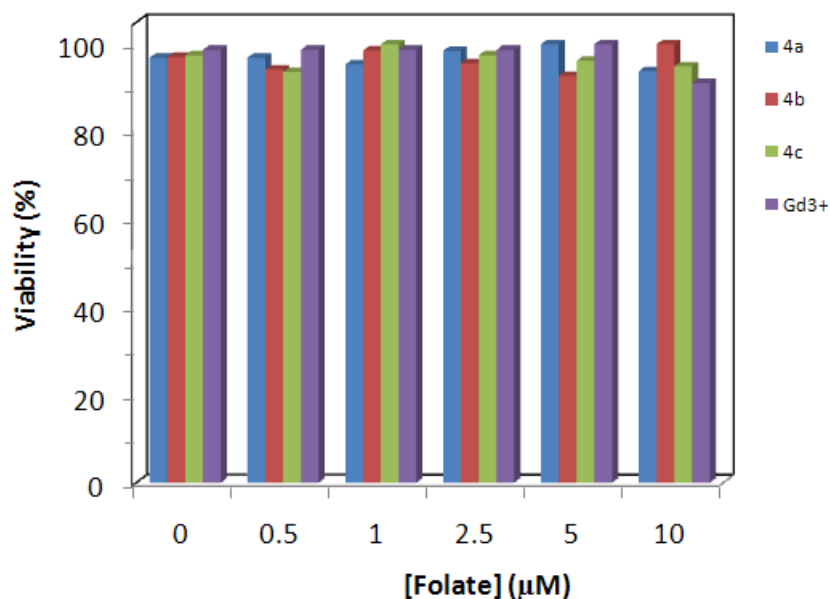
**Figure S13.** *In vitro* cytotoxicity curves for free MTX and **1**, displaying that there is no improvement in efficacy from **1**. IC<sub>50</sub> values for free MTX and **1** were 20 µM and 35 µM, respectively.



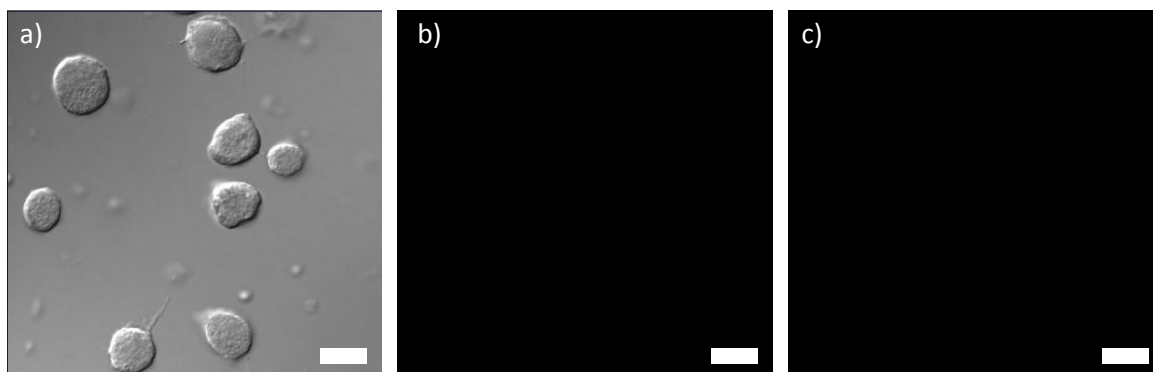
**Figure S14.** *In vitro* cytotoxicity curves for free MTX and **2**, displaying that there is no improvement in efficacy from **2**. The IC<sub>50</sub> value for free MTX and **2** was 1 μM.



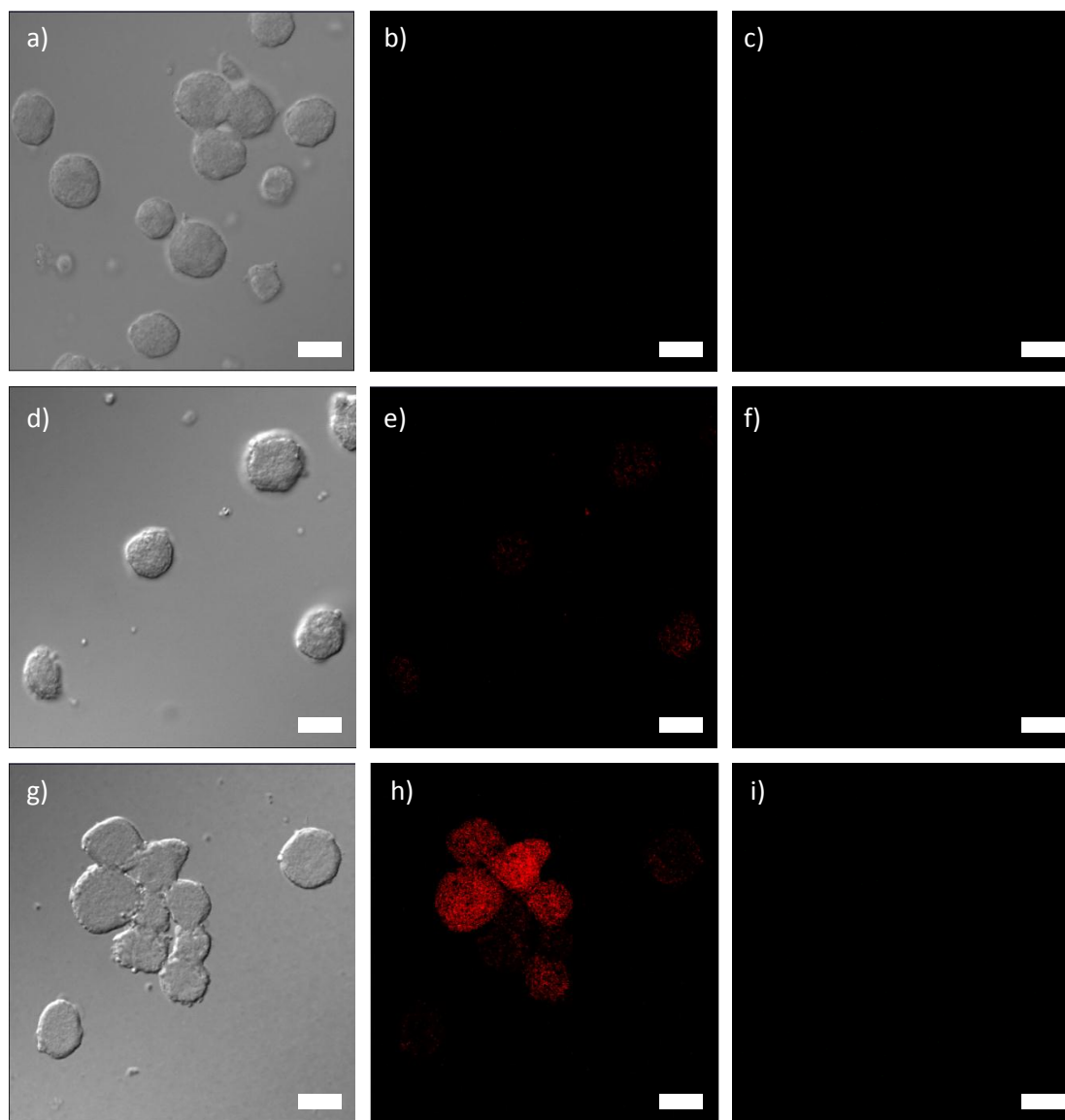
**Figure S15.** *In vitro* cytotoxicity curves for free MTX, **3a**, **3b**, and **3c**, displaying enhanced efficacy from lipid-coating and targeting.



**Figure S16.** *In vitro* viability assay testing free  $Gd^{3+}$ , **4a**, **4b**, and **4c**, displaying that no cytotoxic effect was observed due to free  $Gd^{3+}$  or the control vehicles **4a-c**. This experiment confirms that the cytotoxicity observed for **3a**, **3b**, and **3c** is due solely to MTX and no other cytotoxic agent.



**Figure S17.** Confocal microscopic image of Jurkat cells that were not incubated with particles. No fluorescence in any channel is observed. a) DIC, b) red channel (for particle fluorescence), c) green channel (for Annexin V FITC fluorescence). Scale bars are 25 μm.



**Figure S18.** Confocal images of Jurkat cells incubated with **4a** (a-c), **4b** (d-f), and **4c** (g-i). Cells incubated with **4c** shows enhanced uptake, but no apoptosis is apparent. Channels are: a,d,g) DIC, b,e,h) red from particles, c,f,i) green from Annexin V FITC apoptotic stain. Scale bars are 25 μm.

#### Reference:

[1] Oyane, A.; Kim, H.-M.; Furuya, T.; Kokubo, T.; Miyazaki, T.; Nakamura, T. Preparation and Assessment of Revised Simulated Body Fluids. *J. Biomed. Mater. Res.* **2003**, *65A*, 188.