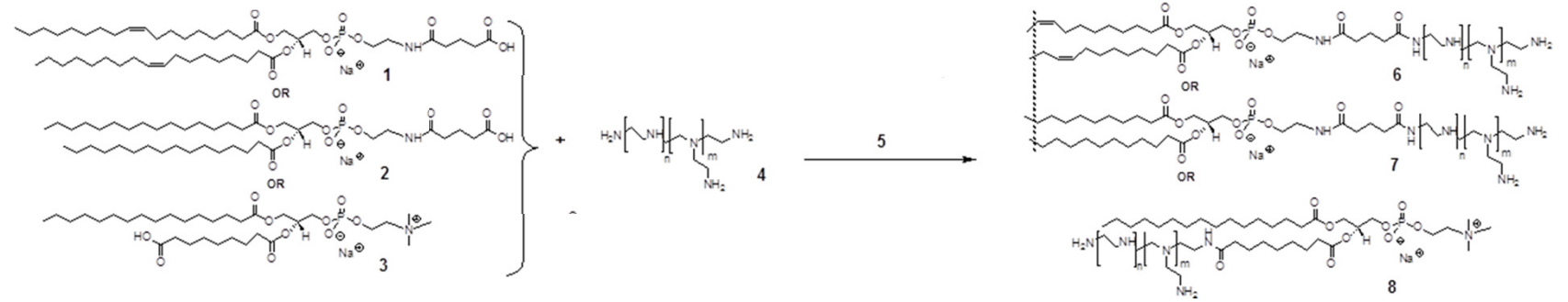
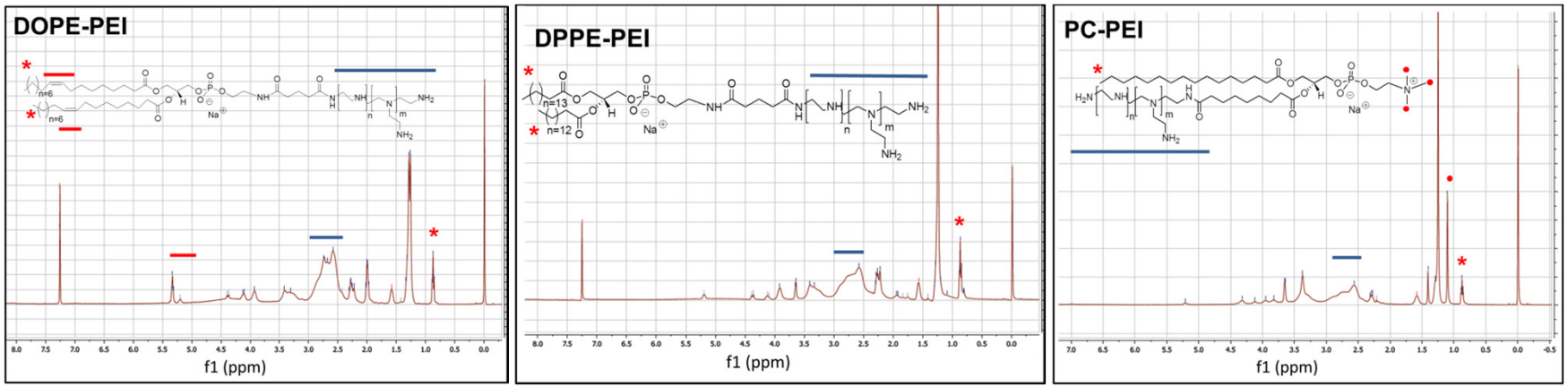
SUPPLEMENTARY MATERIAL

Synthesis of phospholipid-polyethylenimine conjugates

A

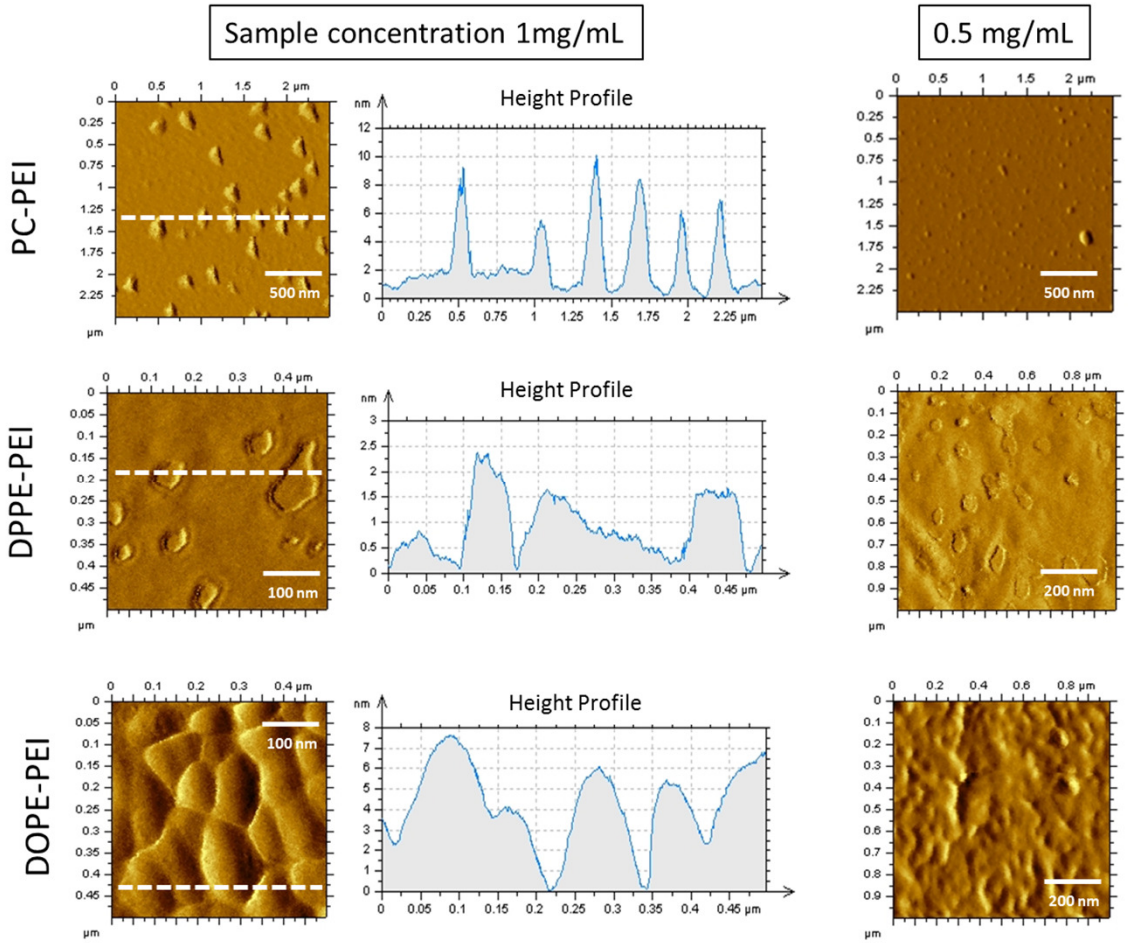


B

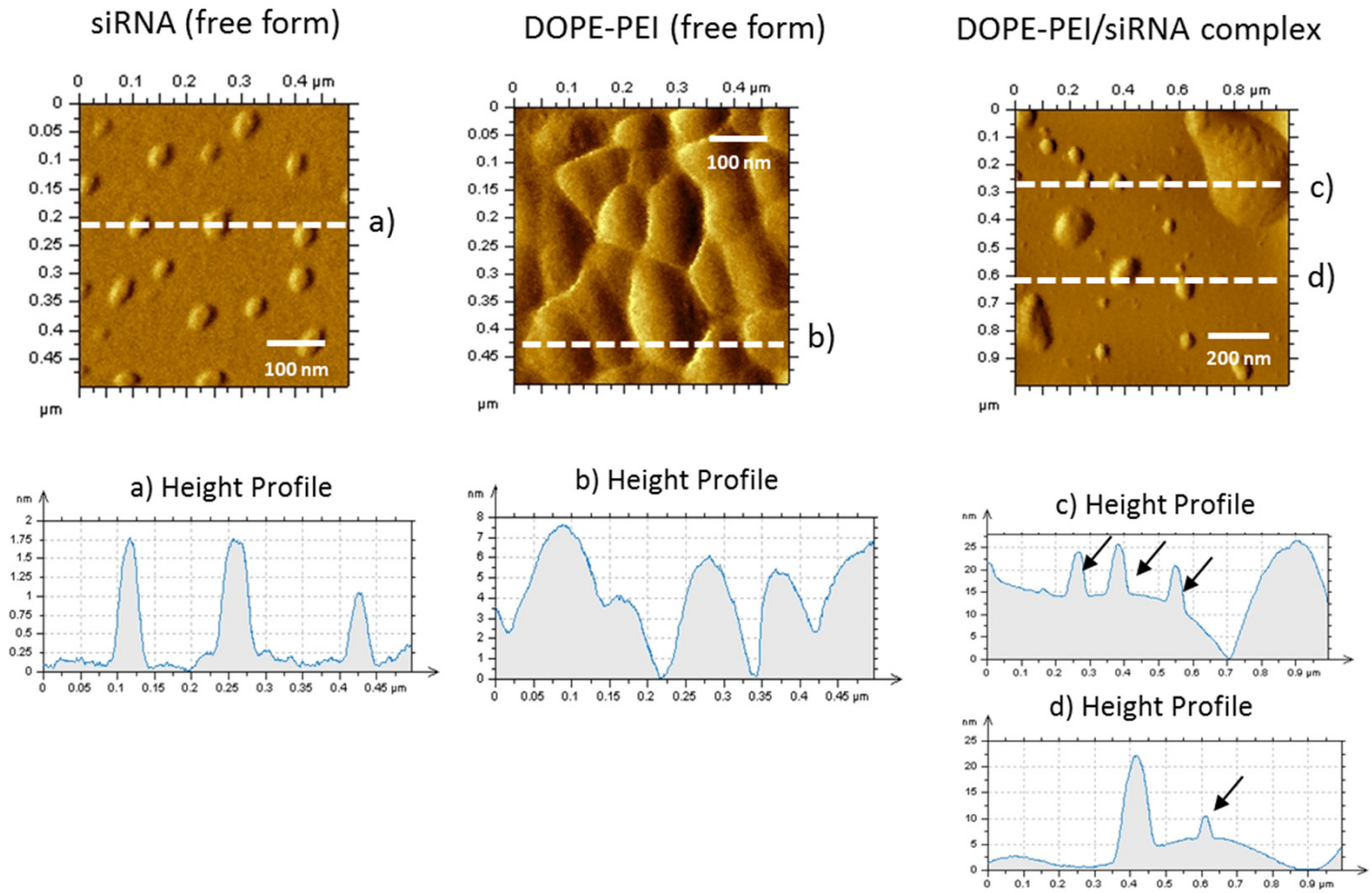


Supplementary Figure 1. (A) Synthesis scheme of phospholipid conjugation of Polyethylenimine (PEI). **1:** 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(glutaryl) (DOPE-NG) **2:** 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(glutaryl) (DPPE-NG) , **3:** 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine, (AzPC), **4:** Branched polyethylenimine (PEI, 1.8 kDa), **5:** N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide HCl (EDCI) and N-hydroxysuccinimide (NHS), **6:** DOPE-PEI, 7: DPPE-PEI, 8: PC-PEI. **(B)** NMR spectra of the conjugates with detectable characteristic peaks for PEI (blue) and phospholipids (red) used for conjugation. PPM values are as follows (where singlet, doublet, triplets, multiplet, broad singlets are noted as s, d, t, m, bs respectively): For PEI-DOPE, 1H-NMR in CDCl3, δ ppm 0.87 (t, 6H), 1.26-1.33 (d), 1.99-2.02 (m) and 2.22-2.30 (m), 2.40-3.00 (m), 3.31-3.41 (m), 3.93 (bs), 4.10-4.12 (m), 4.37-4.40 (d), 5.20 (bs) and 5.32-5.35 (t). For PEI-DPPE, 1H-NMR in CDCl3, δ ppm 0.87 (t, 6H), 1.26-1.28 (d), 1.75-1.94 (m), 2.22-2.28 (m), 2.25-2.30 (m), 3.33-3.41 (m), 3.91 (bs), 4.10-4.12 (m), 4.36-4.39 (d), 5.19 (bs). For PC-PEI, 1H-NMR in CDCl3, δ ppm 0.87 (t), 1.10 (s), 1.25-1.29 (m), 1.41 (s), 2.21-2.31 (m), 2.35-3.10 (m), 3.25-3.50 (m), 3.65-3.66 (d), 3.82-4.32 (m), 5.21 (bs).

<u>Phospholipid-Modified PEI free form or as a complex with siRNA</u> (Atomic Force Microscopy)



Supplementary Figure 2. Detailed structure of the conjugate assemblies. AFM images of phospholipid-PEI conjugates from solutions prepared at concentration of 1mg/mL and 0.5 mg/mL in BHG. For clarity, only the phase images are compared and presented. Height profiles i.e., a plot of height across a line (dashed line) on the XY plane from topography image are also shown. In case of PC-PEI, large irregular shaped aggregates were observed (notice the 500nm scale bar). Dilution to 0.5 mg/mL induced disaggregation to smaller structures. The DPPE-PEI samples formed spherical structures with a size of 50 to 100 nm with a few structures slightly larger. The DOPE-PEI structures displayed a continuous film-like structure with a bumpy texture. The separation between these bumps was slightly larger than 100 nm. DOPE-PEI and DPPE-PEI assemblies were stable even upon a two-fold dilution



Supplementary Figure 3. Analysis of micellar complex morphology by AFM. Topography DOPE-PEI/siRNA complexes is shown. DOPE-PEI, height profiles (across the dashed line from topography) of complexes, free siRNA and free DOPE-PEI are shown. When mixed with siRNA, DOPE-PEI complexes appeared as well-developed individualized rounded particles with size of 100 nm in diameter (average). The black bar is 100 nm. Height profile of the particles confirmed that siRNA was completely condensed and incorporated into the complexes (arrows in figure).

Methodology AFM images were obtained on an Agilent 5500 AFM/SPM microscope in acoustic AC mode using Si probes operating at a resonant frequency of 154 kHz. All measurements were carried out at room temperature and acquired images had a resolution of 512 x 512 pixels collected at a speed of 1 line/minute. A freshly cleaved mica surface was used as the substrate for imaging. Samples were prepared from siRNA and polymer solutions (1mg/mL in BHG) and diluted when necessary. To acquire images, 10-50 µl of the prepared sample was pipetted on to the mica surface and allowed to interact with the surface for 1-5 minutes. Excess solution was than dried with a gentle stream of air. Post-image processing of AFM images was done using Pico Image software provided with the instrument. The images were subjected to standard image processing techniques that included line correction, form removal, leveling and threshold adjusting. In all the AFM measurements, topography, phase and amplitude images were obtained. For clarity, only the phase images are compared and presented. Additionally, line profile images, i.e., a plot of height across a line on the XY plane from topography images were also plotted.