Hep3B cell line (ATCC HB-8064) was maintained in EMEM with 10% FBS at 37 C in a humidified atmosphere (air supplemented with 5% CO2) and passaged with 0.05% trypsin at a subcultivation ratio of 1:3. 1 x 10^6 Hep3B cells were exposed to various concentrations (particles/mL) of siRNA loaded SP94-targeted DOPC protocells and siRNA loaded DOTAP lipid particles for 48 h at 37 °C. Loaded siRNA silenced expression of cyclin A2. After incubation cells were harvested by gentle shaking in 5 mM EDTA for 30 min at 37 °C, centrifuged (1000 rpm, 1 min) to remove excess particles, fixed with 3.7% formaldehyde (15 min at room temperature), and permeabilized with 0.2% Triton X-100 (5 min at room temperature); cells were then exposed to a 1:500 dilution of anticyclin A2, labeled using an Alexa Fluor 488 antibody labeling kit, for 1 h at 37 °C. Cells were washed three times and resuspended in D-PBS for flow cytometry analysis performed by a FACSCalibur flow cytometer (Becton Dickinson; Franklin Lakes, NJ, USA) equipped with BD CellQuest software, version 5.2.1. Samples were acquired with the forward scatter channel in linear mode, and all other channels in log mode. Events were triggered on the basis of forward light scatter, and, a gate was placed on the forward scatter-side scatter plot that excluded cellular debris. Mean fluorescence intensity was determined using FlowJo software, version 6.4 (Tree Star, Inc.; Ashland, OR, USA). The initial protein concentration was taken to be the mean fluorescence intensity of antibody-labeled cells prior to treatment with siRNA-loaded particles. The number of particles necessary to reduce cyclin A2 expression by 90% was calculated from a plot of particle concentration versus cyclin A2 concentration.