Supplemental material to JDT-2014-0267.

Synthesis and NMR analysis of the DSPE-PEG2000-folate construct.

Folate was conjugated to DSPE-PEG-amine as reported previously (Hartley, 2011). A molar excess of folic acid (16.7 mg) was added to a round bottom flask and dissolved 667 µL of anhydrous dimethyl sulfoxide (DMSO). Anhydrous DMSO was further dried by addition of molecular sieve. DSPE-PEG-amine (66.7 mg) was dissolved in 333 µL of pyridine and added to the round bottom flask. N,N'dicyclohexylcarbodiimide (DCC) (21.7 mg) was then added to the reaction mixture. The reaction ran for 4 hours under nitrogen at room temperature in the dark. The pyridine was removed by evaporation. Distilled deionized water (ddH<sub>2</sub>O) was added to make a mixture of 90% water by volume. The unreacted folic acid, DCC, byproducts, and DMSO were removed by dialysis with a 3500 molecular weight cut-off. Other larger byproducts were removed by centrifugation. After removal of byproducts and reactants, an equal volume of chloroform was added to extract the product (DSPE-PEG-folate). A drop of hydrochloric acid was added to the aqueous phase to protonate the product to make it more soluble in chloroform. The characteristic yellow of folate shifted from the aqueous phase to the chloroform phase. The chloroform phase was isolated and dried for storage. Some product was dissolved in deuterated DMSO and analyzed by proton NMR to confirm attachment of the folic acid to DSPE-PEG-amine. See Figure S1. The proton signals at 8.64, 7.64 and 6.64 are assigned to folic acid. The signals from 3.3 to 3.6 are assigned to the methylene hydrogens of PEG. The peak at 1.23 is assigned to methylene hydrogens on DSPE. The peaks around 2.5 are an artifact of hydrogenated DMSO.

