SUPPLEMENTAL INFORMATION FOR:

Anti-HER2 immunoliposomes for selective delivery of electron paramagnetic resonance imaging probes to HER2-overexpressing breast tumor cells

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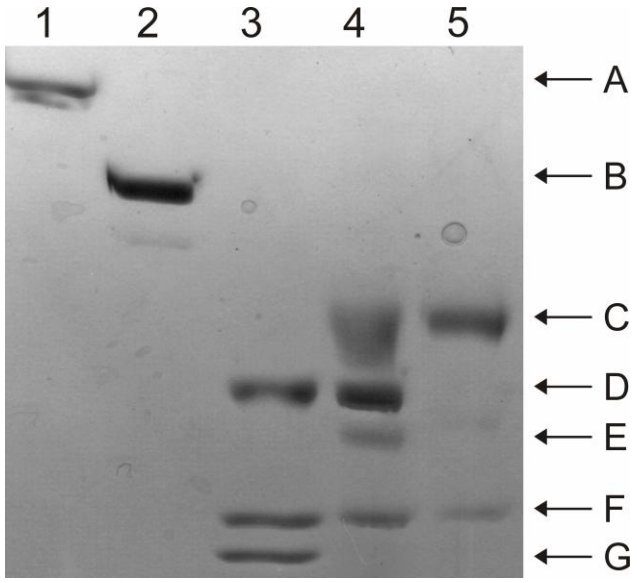
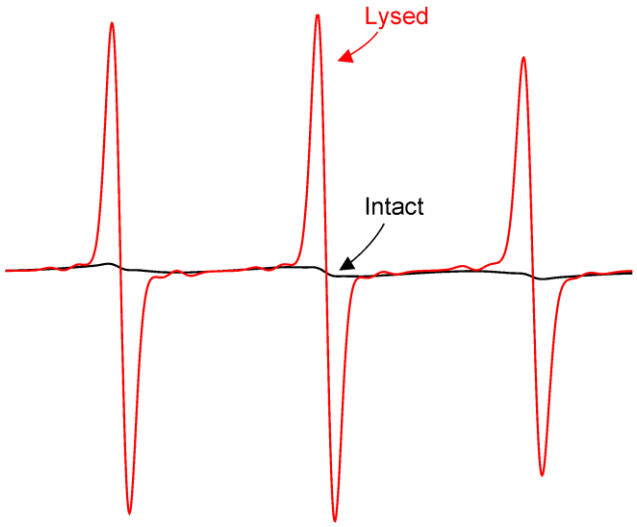
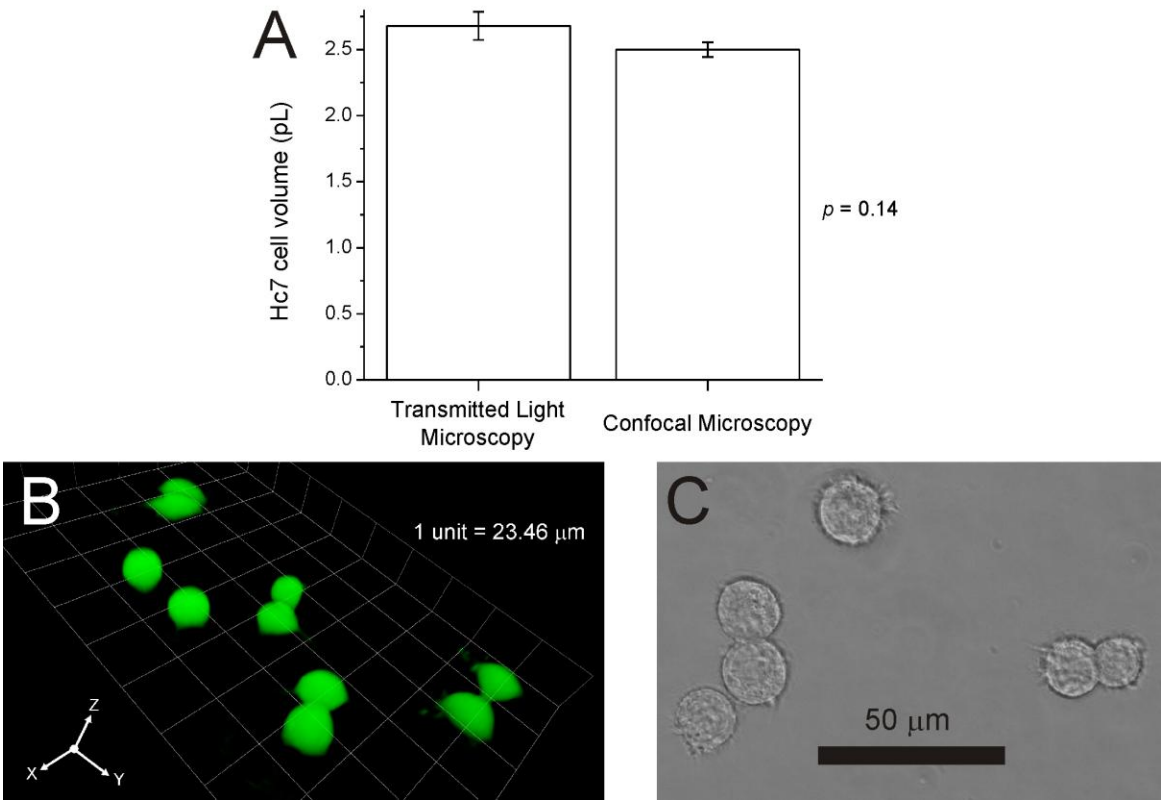


Supplemental Figure S1. Preparation of trastuzumab Fab' fragments and coupling to liposomes. Samples containing 20 µg total protein were resolved by SDS-PAGE under non-reducing conditions and stained with Coomassie Brilliant Blue according to standard protocols. Lane 1 is the whole antibody (band A), which was digested with pepsin to form $F(ab')_2$ fragments (lane 2, band B). Reduction of $F(ab')_2$ with cysteamine yielded Fab' fragments (lane 3, band D), with some reduction to non-immunoreactive heavy and light chains (lane 3, bands F, G). Lane 4 shows an unpurified immunoliposome preparation. Antibody fragments are attached to lipids bearing a nominally 2-kDa poly(ethyleneglycol) head group with a terminal maleimide. Linkage to the PEG-maleimide lipid increases the total molecular weight and causes a shift in fragment band positions on the gel. PEG-maleimide-coupled Fab' fragments (lane 4, band C) are distinct from those that were uncoupled (lane 4, band D). Additionally some coupling of individual heavy and light chains occurs (lane 4, bands E, F). Purification of the preparation by gel filtration results in pure immunoliposomes that are predominantly labeled with trastuzumab Fab' fragments (lane 5).



Supplemental Figure S2. Immunoliposomes retain high concentrations of encapsulated nitroxides. Purified immunoliposomes encapsulating 150 mM nitroxide **1** were measured by EPR spectroscopy before and after lysis. After the antibody coupling procedure, intact liposomes still retain nitroxide at concentrations that are sufficiently high to quench the spectral signal (black trace). Lysis of the liposomes dequenches the nitroxide molecules to generate a spectral signal that is ~60-fold greater (red trace). Lysis was achieved with the addition of 1% v/v Triton X-100, and sonication for 1 min.



Supplemental Figure S3. Hc7 cell volume determination. Average Hc7 cell volume was determined independently by confocal and transmitted light microscopy (A, error bars represent S.E.M.). Volumetric determination from 3-D reconstructions of serial optical sections acquired on a laser-scanning confocal microscope yielded an average volume of 2.50 pL per cell (n = 95, representative reconstruction shown in panel B). Alternatively, measuring the diameter of spherical, trypsinized Hc7 cells on a wide-field light microscope gave an average volume of 2.68 pL per cell (n = 68, representative image shown in panel C).

For confocal microscopic measurements, Hc7 cells were cultured on No. 1 glass coverslips and stained with Calcein-AM for 30 minutes at room temperature. Plates were washed with PBS and imaged on a laser-scanning confocal microscope (LSM510, Carl Zeiss, Oberkochen, Germany). Excitation used 488-nm emission from an argon ion laser, and emission was passed through a 505-nm long-pass filter prior to photometric quantitation. Images were captured on an array-photomultiplier tube. Z-axis slices were obtained at 1 µm intervals. Volumetric measurements were made using built-in algorithms in Volocity 5.1 (Improvision/Perkin Elmer, Waltham, MA).

For transmitted light microscopy, Hc7 cells were trypsinized and suspended in PBS. The suspensions were placed on No. 1 glass coverslips and immediately imaged with transmitted light on an inverted epifluorescence microscope (described in the Methods section). Cell diameters were measured in Adobe Photoshop CS3 and referenced to a fiducial length standard imaged at the same magnification.