Silk Nanoparticles: Proof of Lysosomotropic Anticancer Drug Delivery at Single Cell Resolution

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Supplementary Information

**Supplementary Figure 1.** Fluorescent standard used to normalise AF488-conjugated native and PEGylated silk nanoparticles. Fluorescence intensities of 50 – 1000 µg/ml native or PEGylated silk nanoparticles. Equations of lines used to normalise fluorescence values for cell-based studies.
**Supplementary Figure 2.** Verification that active gate contained of live singlets during flow cytometric analyses. (A) Location of all events on a classical FSC-A vs SSC-A flow cytometry dot plot. (B) Same data with a quadrant gate applied to separate calcein-AM positive live cells from propidium iodide (PI) positive apoptotic cells. (C) Location of all events located within the live gate. Two populations identified as: (i) live singlets, or (ii) doublets. Location of live gate used to record cells of interest during nanoparticle dosing studies.

**Supplementary Figure 3.** NH₄Cl substantially reduces lysosomal acidification in MCF-7 human breast cancer cells. Control MCF-7 cells (top) or MCF-7 cells pre-treated with NH₄Cl for 1 h and then dosed for 5 h with LysoTracker® Red in the absence or presence of NH₄Cl. Profile plot of 15 µm line on lysosomal signal (right) revealed substantial decrease in signal intensity. Scale bars = 10 µm.
**Supplementary Figure 4.** Selection of nuclei and analysis of nuclear-associated doxorubicin fluorescence. (A) Nuclear and doxorubicin channels of a multicolour image (left) were split and nuclei identified in the nuclear channel (centre). Regions of interest were applied to the doxorubicin channel and background fluorescence readings were taken. Scale bars = 10 µm. (B) Nuclear area and integrated density were determined and nuclear-associated fluorescence was calculated for each individual cell.