Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Self-assembling dual component nanoparticles with endosomal escape capability

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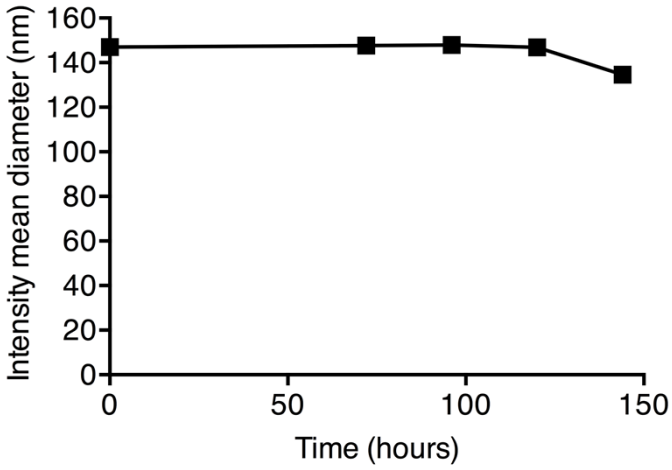

Figure S1. Dynamic light scattering (DLS) size analysis of the PDEAEMA dual component nanoparticles to assess particle stability. Error bars representing standard deviation are present but are smaller than the symbols on the graph.

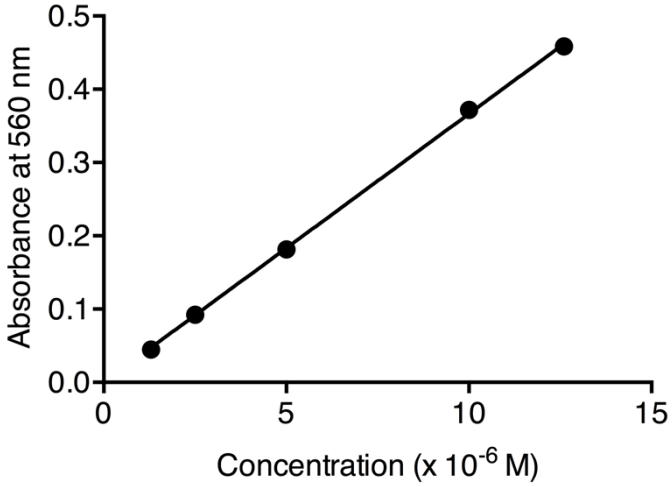

Figure S2. UV-vis absorbance standard curve of Rhodamine B octadecyl ester perchlorate measured at 560 nm in a 1:1 ethanol:water solvent mixture. The extinction coefficient at 560 nm of Rhodamine B octadecyl ester perchlorate was determined from this curve to be 36600 cm^{-1} M^{-1}. We determined the loading efficiency of Rhodamine B to be ~ 30% (see experimental section below).

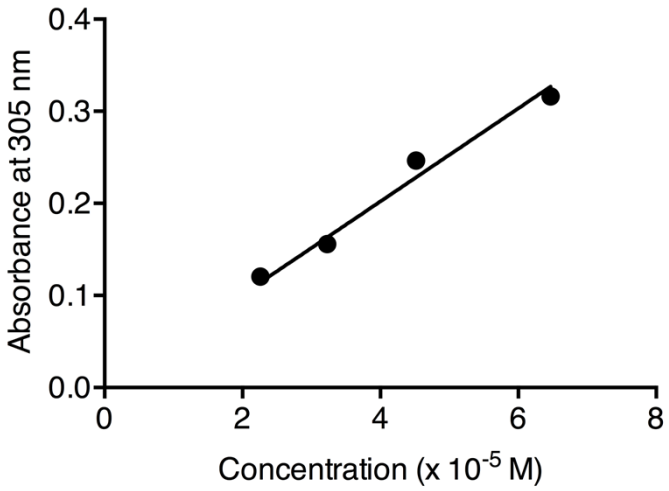

Figure S3. UV-vis absorbance standard curve of the nanoparticle polymeric formulation (1:5 mass ratio of PDEAEMA-b-PEG:PDEAEMA) measured at 305 nm in a 1:1 ethanol:water solvent mixture. The extinction coefficient at 305 nm of the 1:5 PDEAEMA-b-PEG:PDEAEMA nanoparticle formulation was determined to be 5048 cm^{-1} M^{-1}. We determined the concentration of nanoparticles after dialysis and filtering to be 0.23 mg mL^{-1} (see experimental section below).

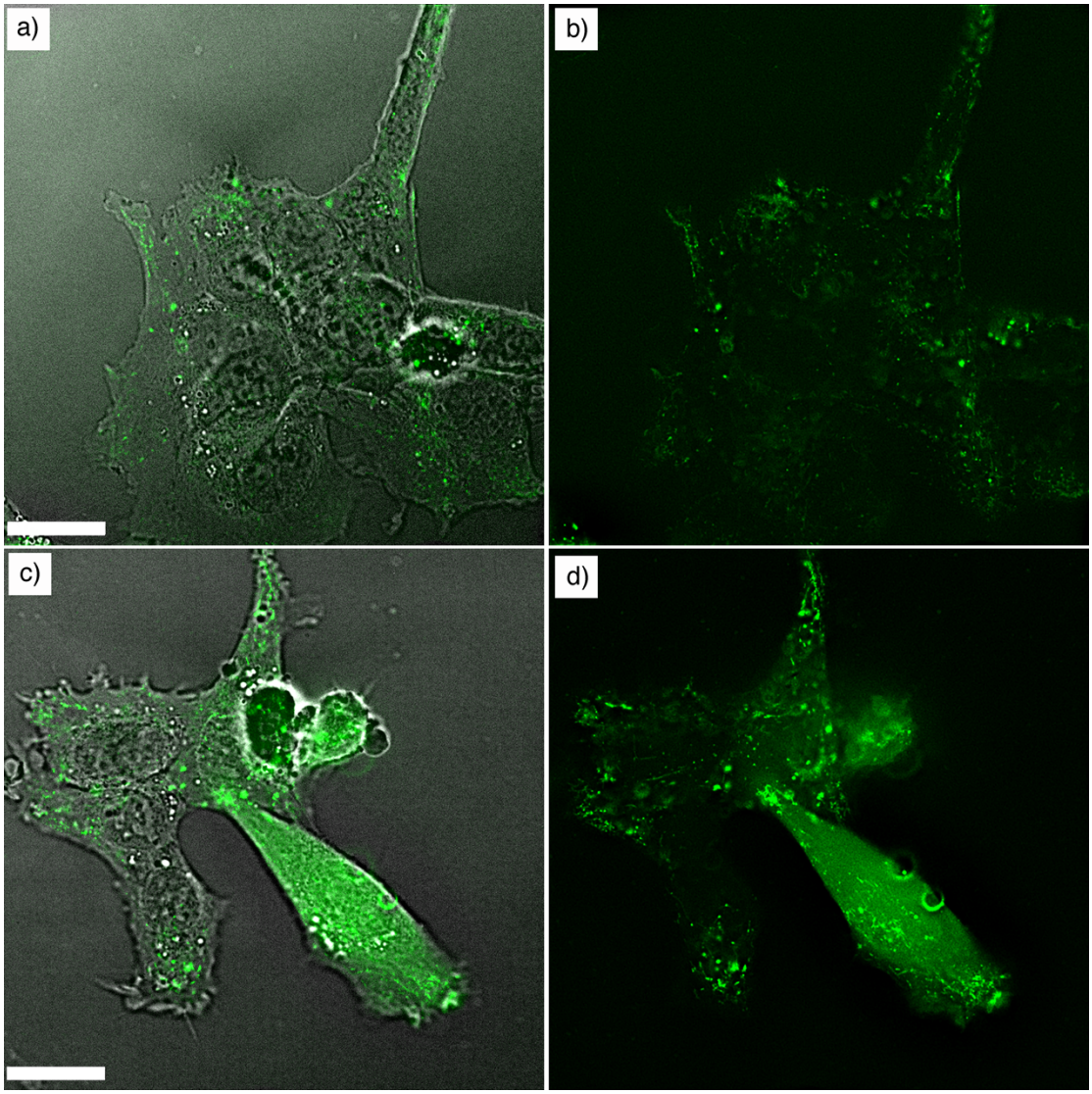

Figure S4 High-resolution images of 3T3 cells after the addition of calcein green, taken using a 60x objective. Figures a) and b) are images from the control experiment (calcein added without nanoparticles). Figure a) shows an overlay of brightfield and calcein (green) staining and b) shows calcein staining alone without brightfield for easier observation of the punctate calcein fluorescence. Figures c) and d) are representative images showing diffused calcein fluorescence when the nanoparticles are added with calcein where c) is an overlay of brightfield and calcein staining and d) is calcein staining alone without brightfield for easier observation of the calcein distribution. Scale bars represent 20 μm.

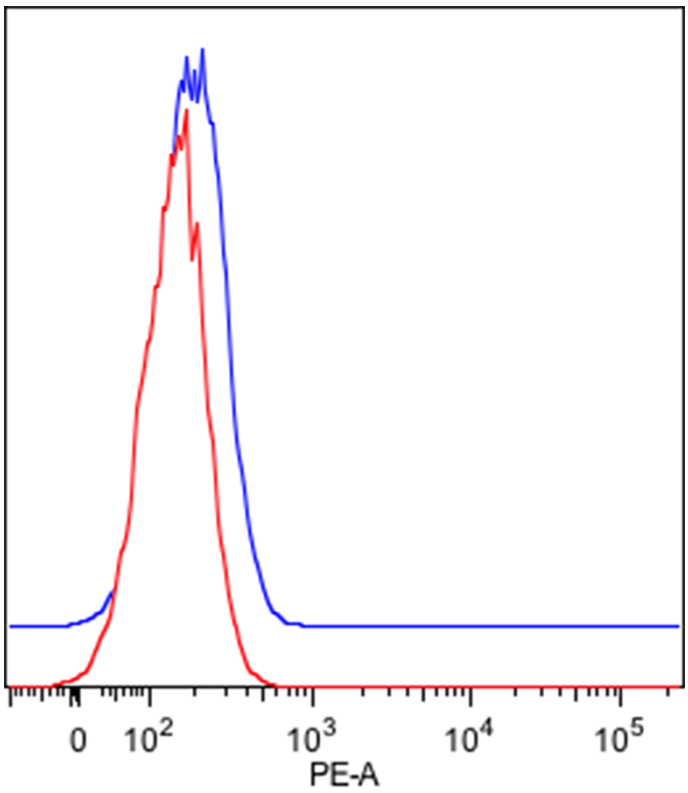

Figure S5. a) Flow cytometry histogram showing the fluorescence intensity of Rhodamine B loaded nanoparticles in 3T3 cells. Cells without nanoparticles (red line) and cells incubated with PDEAEMA dual component nanoparticles added at 2.2 μg mL^{-1} for 2 hours (blue line), show a uniform increase in fluorescence intensity of the cells.

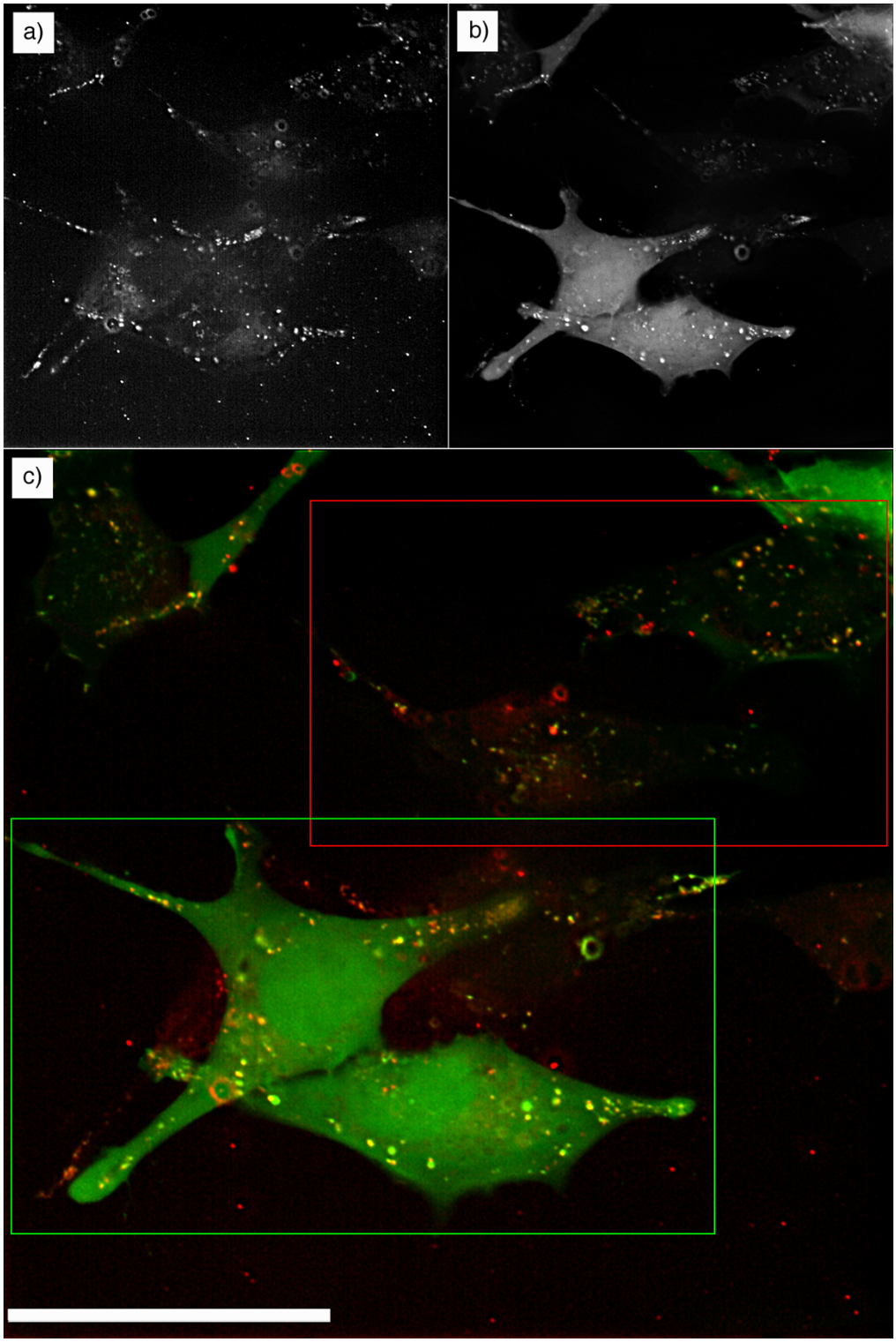

Figure S6. Representative multichannel cellular image showing a) Rhodamine B loaded PDEAEMA nanoparticle fluorescence b) calcein fluorescence and c) an overlay of nanoparticle (red) and calcein (green) fluorescence. This image demonstrates the presence of cells with internalized nanoparticles exhibiting either endosomal escape (boxed in green) or non-endosomal escape (boxed in red) behaviour. Scale bar represents 40 μm.

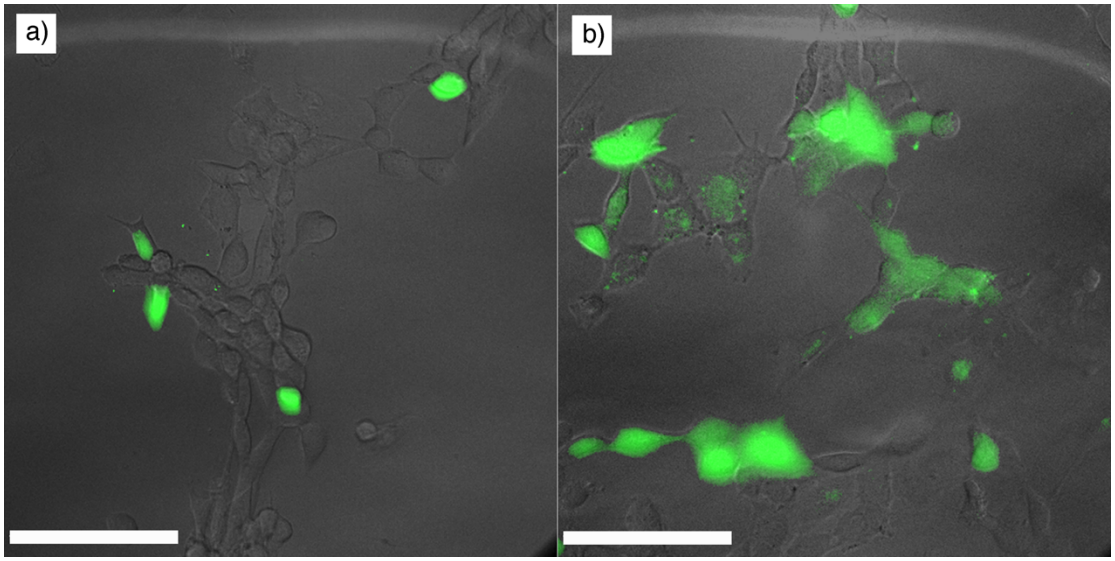

Figure S7. Images of 3T3 cells after the addition of calcein green, taken using a 40x objective. a) Cells treated with Bafilomycin A1 and PDEAEMA nanoparticles and b) cells treated only with PDEAEMA nanoparticles without Bafilomycin A1. The images are shown as an overlay of brightfield and calcein (green) staining. The gamma of the calcein channel was set to 0.5 to enhance dimly fluorescent areas while avoiding overexposure in highly fluorescent areas. Scale bars represent 100 µm.

Molecular weight calculation of PDEAEMA by NMR analysis

The 16-hydrogen peak from the RAFT agent at δ of 1.25-1.2 ppm (CH_3-C_8H_{16}-CH_2-), integration = 16H was compared with the 2-hydrogen polymer peak at δ of 2.7-2.6 (-CH_2-CH_2-N-), integration = 414H to obtain the number of monomers per RAFT agent.

$$Ratio\ of\ monomer\ to\ RAFT\ agent = \frac{414/2}{16/16} = 207$$

$$\therefore each\ polymer\ contains\ 207\ monomer\ units$$

$$Molecular\ weight\ of\ monomer = 185.26 g/mol$$

$$\therefore molecular\ weight\ of\ polymer$$
$$= 207\ x\ 185.26\ g/mol$$
$$= 38348.82\ g/mol$$
$$\approx 38000\ g/mol$$

Molecular weight calculation of PDEAEMA-b-PEG by NMR analysis

The 5-hydrogen aromatic ring peak from the RAFT agent at δ of 7.9-7.3 ppm (C_5H_5-C-), integration = 5H was compared with the 2-hydrogen polymer peak at δ of 4.4-4.0 (-COO-CH_2-CH_2-), integration = 150H to obtain the number of monomers per RAFT agent

$$Ratio\ of\ monomer\ to\ RAFT\ agent = \frac{150/2}{5/5} = 75$$

$$\therefore each\ polymer\ contains\ 75\ monomer\ units$$

$$Molecular\ weight\ of\ monomer = 185.26 g/mol$$

$$\therefore molecular\ weight\ of\ polymer$$
$$= (75\ x\ 185.26 g/mol) + 2000 g/mol\ PEG\ from\ RAFT$$
$$= 15894.50\ g/mol$$
$$\approx 16000\ g/mol$$

Nanoparticle disassembly analysis by dynamic light scattering

pH-dependent nanoparticle disassembly was studied in detail using dynamic light scattering. The region of instability was characterized based on the appearance of a secondary decay gradient on the autocorrelation function at larger delay times (characteristic of partial swelling or aggregation of particles). In contrast, the point of disassembly was determined based on a combination of low count rate that was indistinguishable from noise at 100% intensity of light,

as well as a significantly compromised autocorrelation function yielding unacceptable size data.

Determining the loading efficiency of Rhodamine B octadecyl ester perchlorate

A Rhodamine B octadecyl ester perchlorate UV-vis absorbance standard curve (Figure S2) was generated by dissolving various concentrations of the octadecyl Rhodamine B in a 1:1 ethanol:water solvent mixture and measuring its absorbance at 560 nm. The extinction coefficient of the octadecyl Rhodamine B was determined to be 36600 cm^{-1} M^{-1} at 560 nm based on the gradient of the standard curve. The synthesized nanoparticles were filtered using a 0.45 μm filter. A known volume of the nanoparticle solution was lyophilized then resuspended in a known volume of a 1:1 ethanol:water solvent mixture, and its UV-vis absorbance at 560 nm was measured. The final concentration of the Rhodamine B octadecyl perchlorate in the particles was calculated using the determined extinction coefficient, and the efficiency of loading determined as a percentage of the original mass of octadecyl Rhodamine B added during the synthesis of the nanoparticles. We determined this loading efficiency to be 30%. UV-vis absorbance measurements were conducted on a UV 3600 UV-vis NIR Spectrophotometer (Shimadzu, Japan).

Determining the concentration of nanoparticles after dialysis and filtration

A polymeric UV-vis absorbance standard curve was generated for the 1:5 mixture of PDEAEMA-b-PEG:PDEAEMA polymers by measuring the absorbance at 305 nm of the mixture of polymers at various dilutions in a 1:1 ethanol:water solvent mixture. The extinction coefficient of the 1:5 PDEAEMA-b-PEG:PDEAEMA polymer mixture was determined to be 5048 cm^{-1} M^{-1} at 305 nm based on the gradient of the standard curve. The synthesized nanoparticles were filtered using a 0.45 μm filter. A known volume of the nanoparticle solution was lyophilized then resuspended in a known volume of 1:1 ethanol:water solvent mixture, and its UV-vis absorbance at 305 nm was measured. The final concentration of nanoparticles was calculated using the determined extinction coefficient to be 0.23 mg mL^{-1}. UV-vis absorbance measurements were conducted on a UV 3600 UV-vis NIR Spectrophotometer (Shimadzu, Japan).

Calcein assay supplementary information

For cells treated with 4.4 x 10^{-11} g PDEAEMA particles per cell, 182 cells out of a total of 584 cells (~30%) exhibited diffused calcein fluorescence indicative of endosomal escape. In contrast, no cells exhibited diffused calcein fluorescence out of 574 cells in the case of calcein added without nanoparticles. For the cells treated with Bafilomycin A1 and 4.4 x 10^{-11} g PDEAEMA particles per cell, only 82 cells out of a total of 896 cells (~9%) exhibited diffused calcein fluorescence. In each analysis, more than 500 cells were counted from over 25 images captured at various locations in the well.