

ELECTRONIC SUPPLEMENTARY INFORMATION

The Endocytic Pathway and Therapeutic Efficiency of Doxorubicin Conjugated Cholesterol-Derived Polymers

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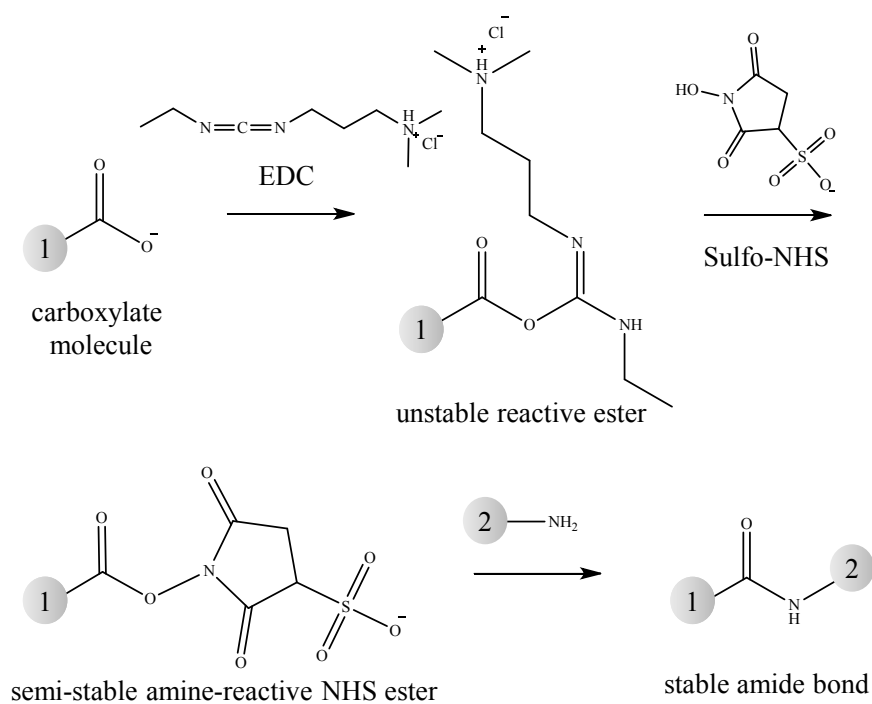
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RESULTS



Scheme S1: The formation of stable amide bonds via EDC/sulfo-NHS reactions. The scheme was adapted from Hermanson.

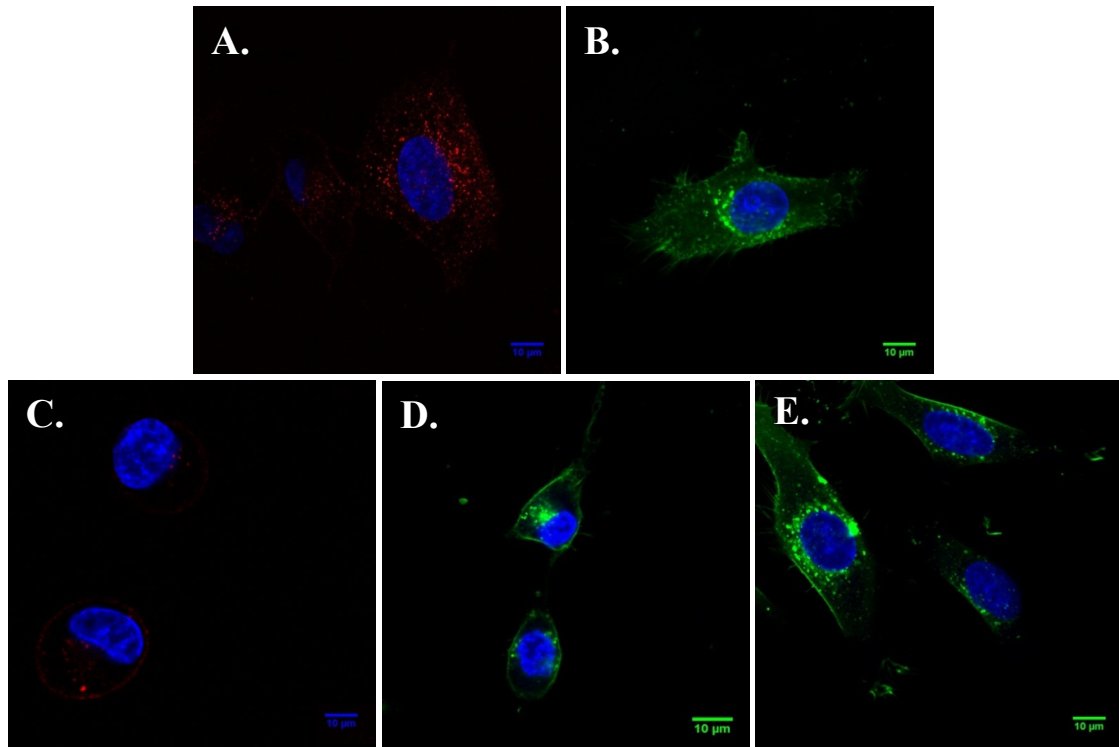


Figure S1: Intracellular distributions of endocytic markers in SHEP cells. Control samples consist of (A) human transferrin (16.7 $\mu\text{g/ml}$) and (B) lactosylceramide (0.81 μM) 1 hour treatments. (C) Human transferrin inhibition after 5 $\mu\text{g/ml}$ chlorpromazine treatment, lactosylceramide inhibition following a (D) 2.5 mM $\text{M}\beta\text{CD}$ and (E) 300 μM genistein treatments for 2 hours. Nuclei are visualized with Hoechst 33342 stain. Scale bars correspond to 10 μm .

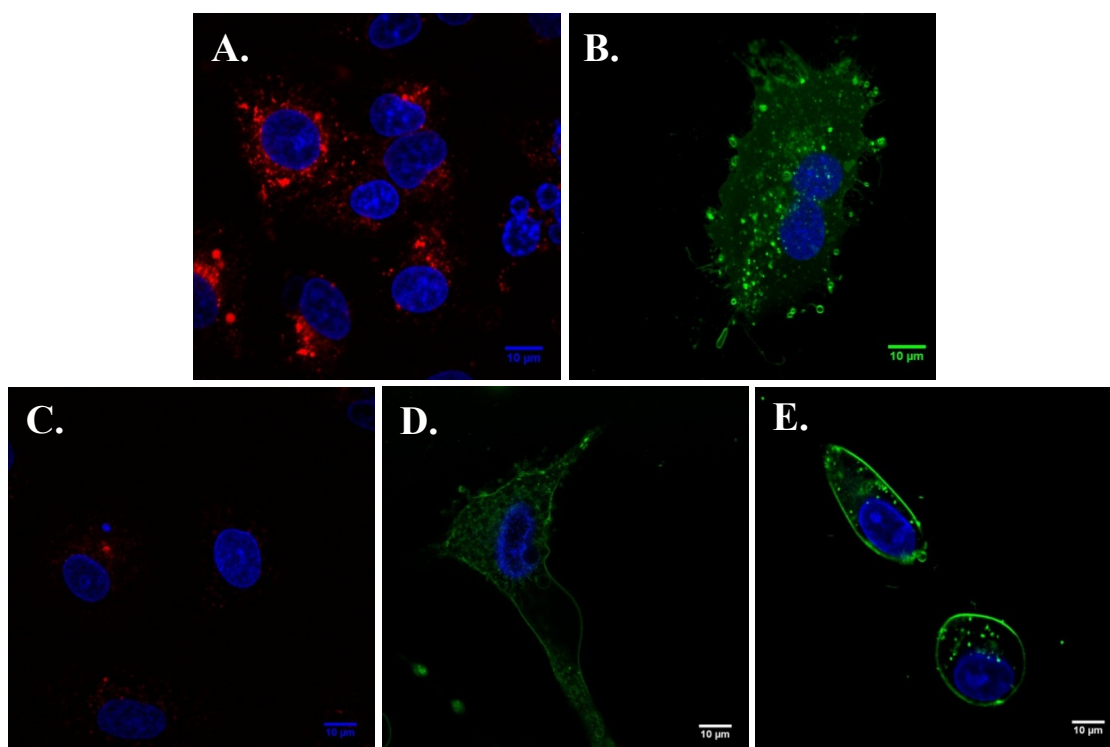
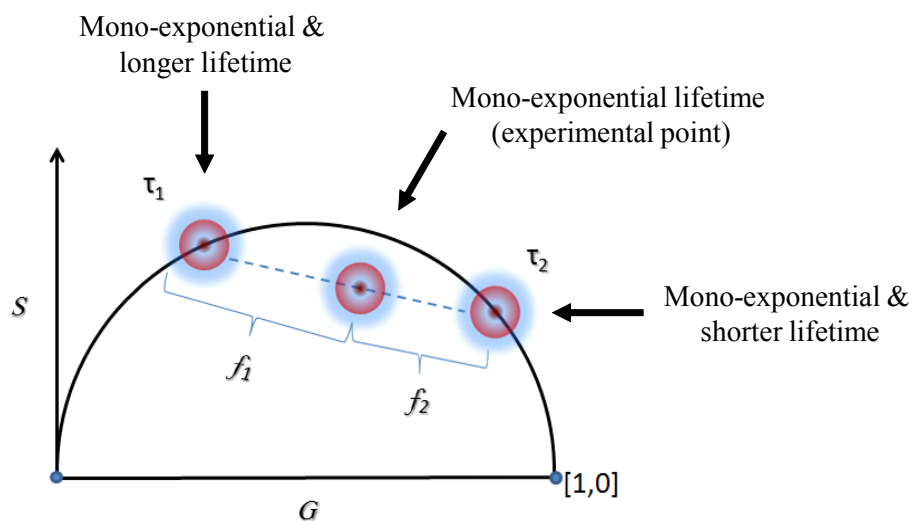


Figure S2: Intracellular distributions of endocytic markers in HepG2 cells. Control samples consist of (A) human transferrin (16.7 μg/ml) and (B) after 5 μg/ml chlorpromazine treatment, lactosylceramide inhibition following a (D) 2.5 mM MβCD and (E) 300 μM genistein treatments for 2 hours. Nuclei are visualized with Hoechst 33342 stain. Bars represent 10 μm.



Scheme S2: Schematic representation of a phasor plot. All single exponential lifetimes lie on the universal circle while multi-exponential lifetimes are a linear combination of their components.

Figure S3: Distribution of lifetimes for P(MAA-*co*-CMA)-DOX nanocomplexes ranging from 6.25:1 to 1250:1 (w/w) ratios with in (A) 1 hour and (B) 24 hours. 1.4 μ M free DOX was included as a control sample.

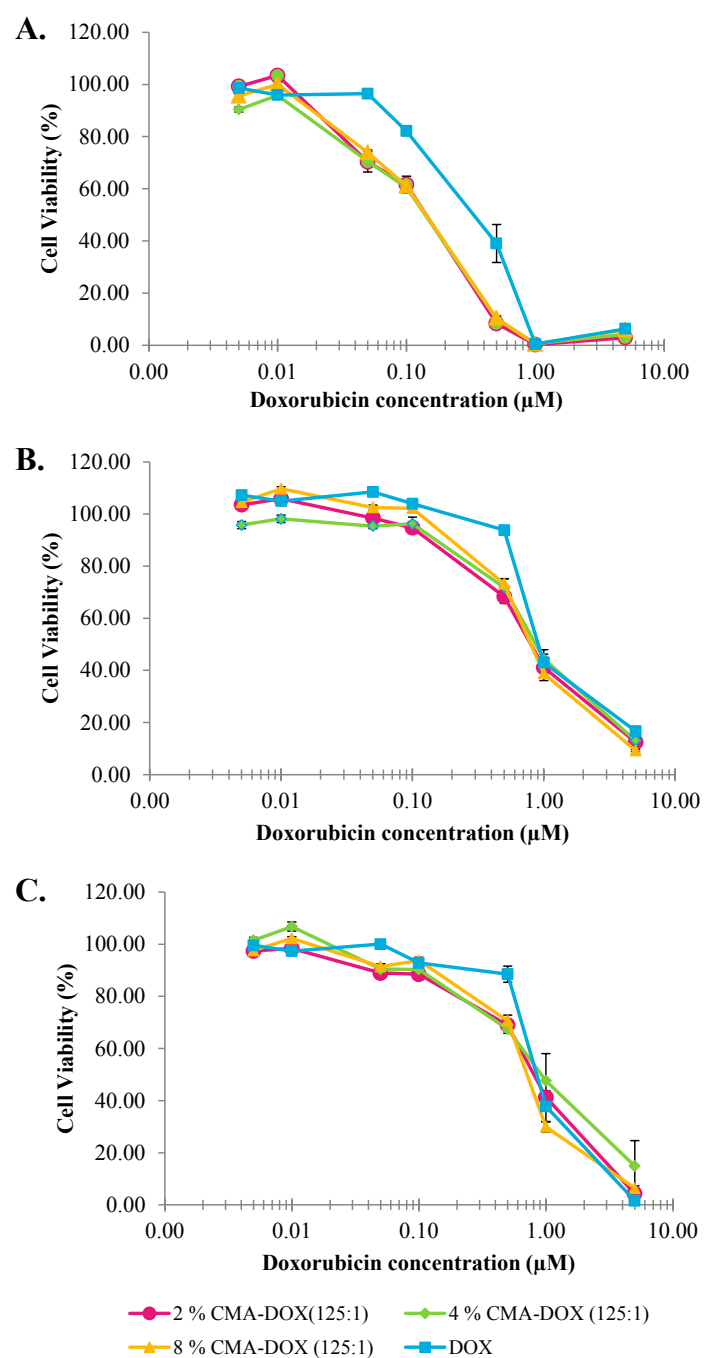


Figure S4: Toxicity of DOX and P(MAA-*co*-CMA)-DOX nanocomplexes prepared at 125:1 ratio in (A) SHEP (B) HepG2 (C) MRC5 cells for P(MAA-*co*-CMA) series. The assay was repeated three times in 4 replicates and the viability results were normalized according to the positive control (untreated cells). Error bars represent standard deviation.

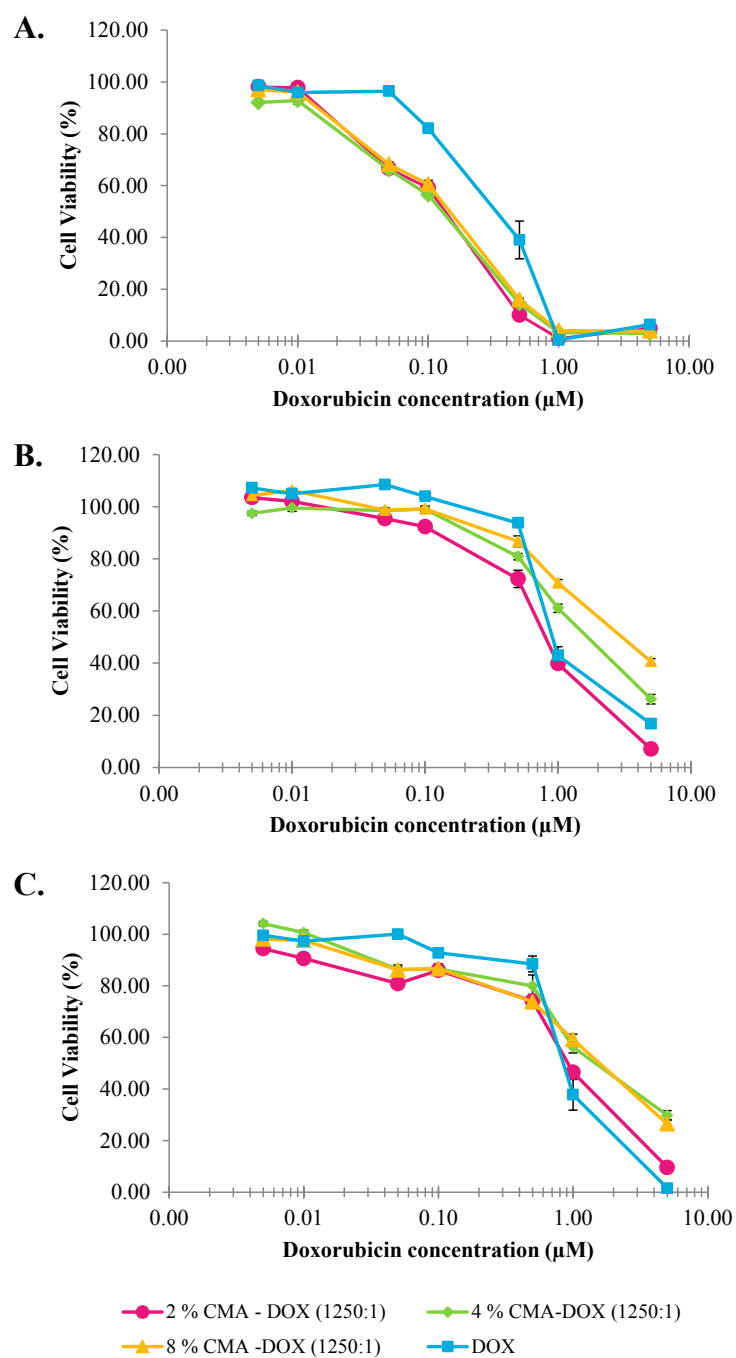


Figure S5: Toxicity of DOX and P(MAA-*co*-CMA)-DOX nanocomplex series at complexation ratios of 1250:1 in (A) SHEP (B) HepG2 (C) MRC5 cells, respectively. The assay was repeated three times in 4 replicates and the viability results were normalized according to the positive control (untreated cells). Error bars show standard deviation.

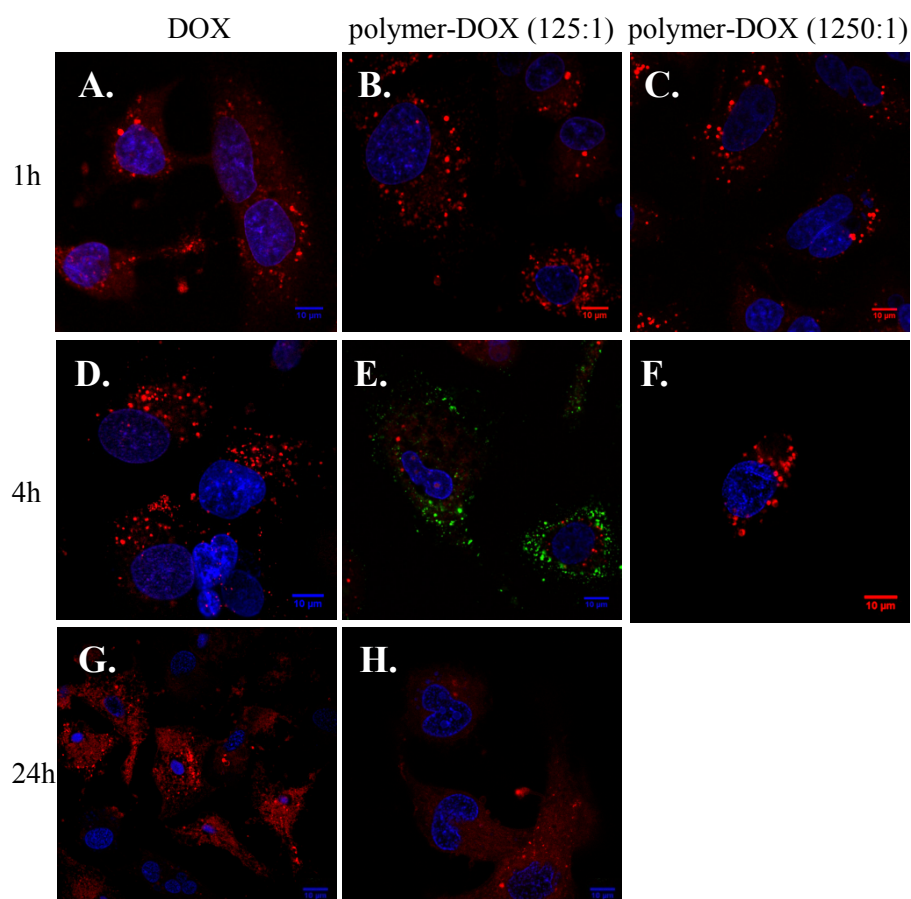


Figure S6: Confocal microscopy images of HepG2 cells treated with DOX (0.5 μ M) and P(MAA-*co*-CMA)-DOX nanocomplexes (125:1 and 1250:1) for (A-B-C) 1 hour, (D-E-F) 4 hours and (G-H) 24 hours. (E) Alexa Fluor 488 labelled 8 mol% CMA copolymers were utilized for preparing P(MAA-*co*-CMA)-DOX nanocomplexes at 125:1 and incubated in cells for 4 hours. Alexa Fluor 488 and DOX images were acquired at $\lambda^{\text{ex}} = 495 \text{ nm}$ - $\lambda^{\text{em}} = 591 \text{ nm}$ and $\lambda^{\text{ex}} = 485 \text{ nm}$ - $\lambda^{\text{em}} = 595 \text{ nm}$, respectively. Nuclei were stained with Hoechst 33342. Bars represent 10 μ m.

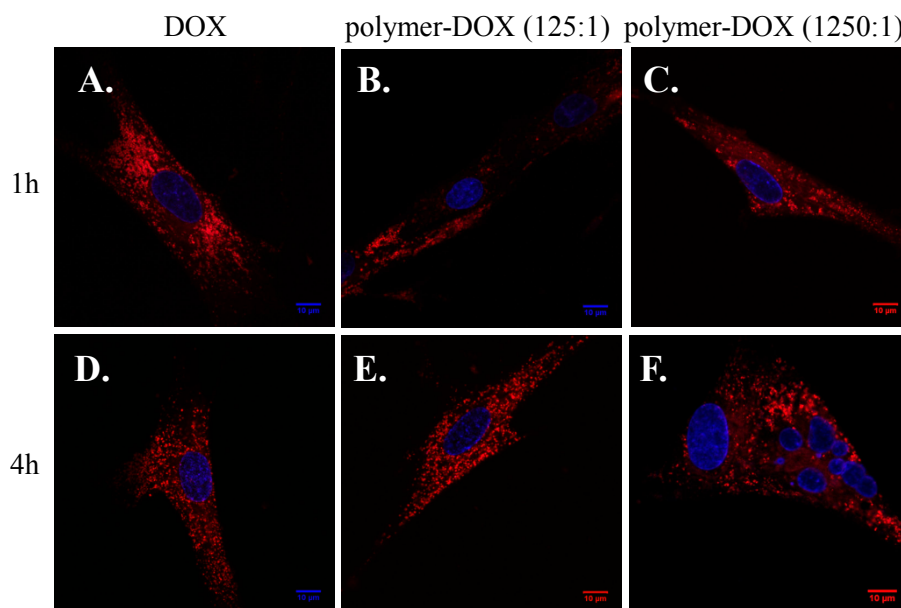


Figure S7: Confocal laser scanning microscopy exhibiting cellular internalization of 0.5 μM DOX and P(MAA-*co*-CMA)-DOX nanocomplexes at conjugation ratios of 125:1 and 1250:1 within (A-B-C) 1 hour and (D-E-F) 4 hours in MRC5 cells. Nuclei (represented in blue) were visualized with Hoechst 33342 stain. DOX fluorescent images were acquired at $\lambda^{\text{ex}} = 485 \text{ nm}$ and $\lambda^{\text{em}} = 595 \text{ nm}$. Scale bars indicate 10 μm .

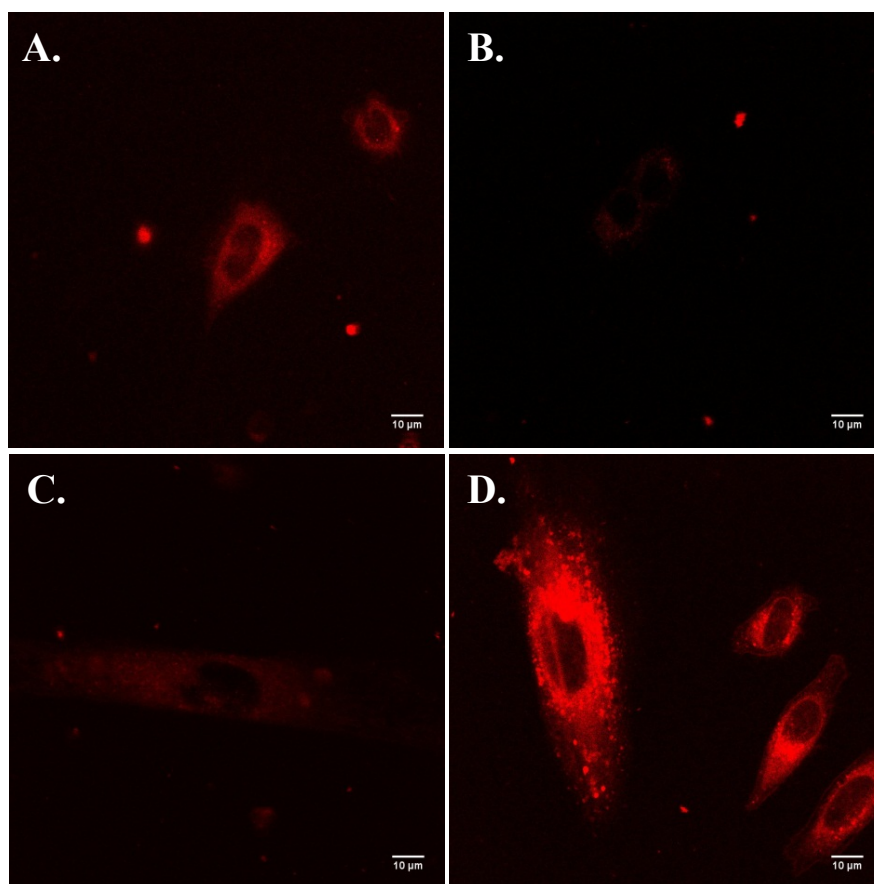


Figure S8: Inhibition of P(MAA-*co*-CMA)-DOX nanocomplexes after incubation with endocytic inhibitors (2 hours) in SHEP cells determined by confocal laser scanning microscopy. (A) No inhibitor, (B) genistein (300 μM), (C) chlorpromazine (5 μg/ml) and (D) MβCD (2.5 mM). Scale bars indicate 10 μm.