Supporting Information

Summary

- Synthesis scheme and ^{1}H NMR spectrum of HOOC-PEG-FA (**Figure S1**)
- Surface potential measurements of pristine CDs, CDs-bAPAE and CDs-bAPAE-PEG-FA as a function of the pH medium (**Table S1**)
- UV spectra of CDs-bAPAE-PEG-FA and CDs-bAPAE-PEG-FA/Dox (**Figure S2**)
- 3D emission spectra of CDs and CDs-bAPAE (**Figure S3**)
- Cytocompatibility of CDs-bAPAE-PEG-FA without or with NIR light stimulation, after 24 or 48h incubation with HDF, MCF-7 and MDA-MB-231 (**Figure S4**).
- Erythrolysis tests of CDs-bAPAE-PEG-FA and CDs-bAPAE-PEG-FA or free doxorubicin at increasing equivalent concentration of doxorubicin (**Figure S5**).
- 24 h uptake studies of drug free CDs-bAPAE-PEG-FA (**Figure S6**).
- 24 h uptake studies of doxorubicin loaded CDs-bAPAE-PEG-FA/Dox (**Figure S7**).

To attach PEG-FA chains to the CDs, the amine terminal of the NH2-PEG-FA derivative first needed to be modified. This was achieved by introducing a carboxylic functional group using succinic anhydride (SA), resulting in COOH-PEG-FA (Figure S1).

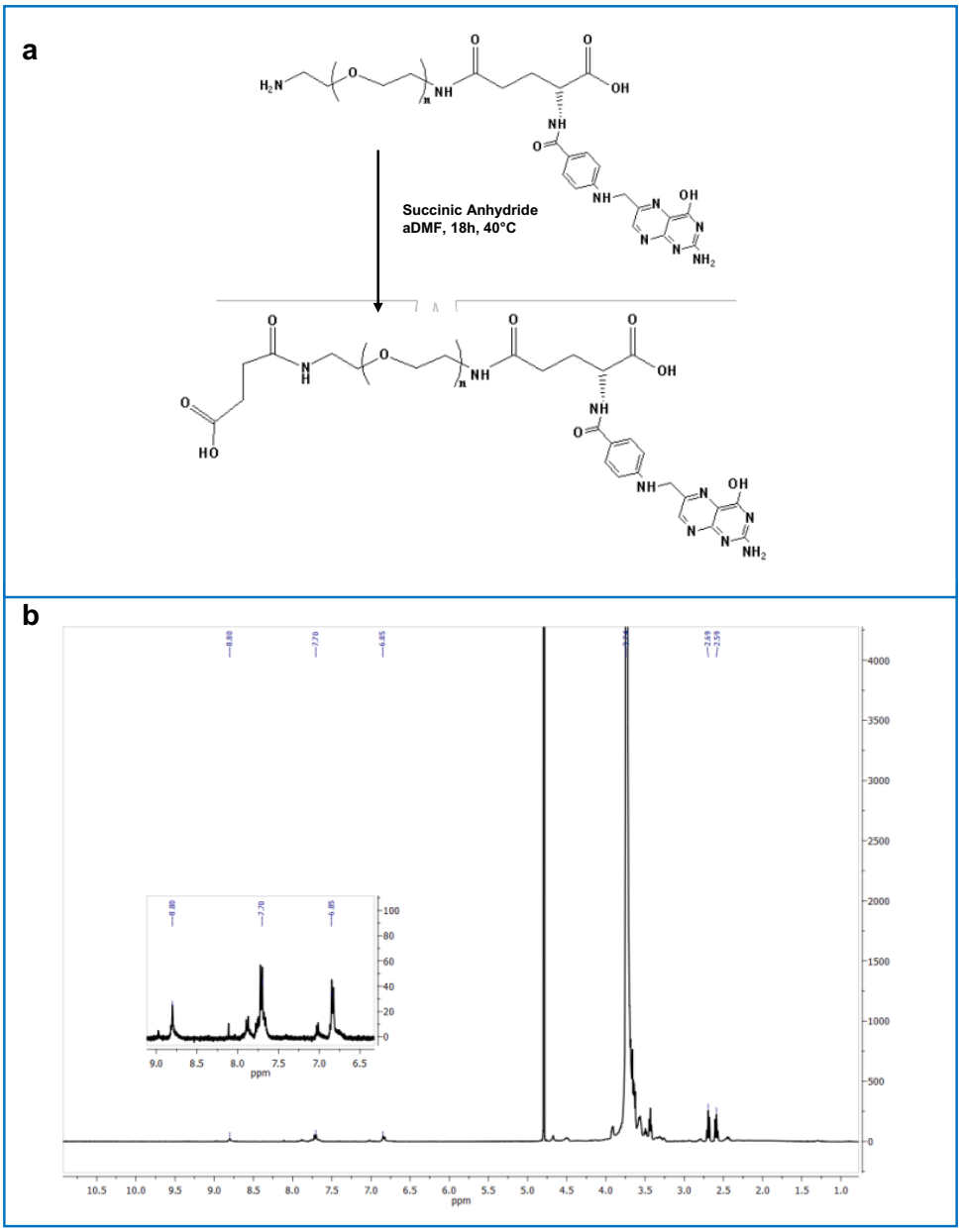

Figure S1. a) Synthesis scheme and b) ^{1}H NMR spectrum of HOOC-PEG-FA.

The functionalization of CDs was also confirmed by surface potential measurements (Table S1). The ζ-potential of CDs-bAPAE-PEG-FA was found to be close to neutrality. As expected, the analysis of the surface charge of CDs-bAPAE (TABLE 1.) shows that at pH 4, the ζ-potential is generally positive, consistent with the presence of polyamine (bAPAE). However, at pH 7 and pH 9, the charge of the system tends towards

neutrality. ζ-potential measurements of the final system CDs-bAPAE-PEG-FA confirmed values close to neutrality for all analyzed pH levels, with only a slight increase in potential measured at pH 4. This phenomenon might be attributed to the reduction of exposed primary amine groups on the surface of CDs-bAPAE after amide coupling, resulting in a slight decrease in surface potential at acidic pH.

Table S1. Surface potential measurements of pristine CDs, CDs-bAPAE and CDs-bAPAE-PEG-FA as a function of the pH medium.

pH	CDs ζ Potential (mV)	CDs-bAPAE ζ Potential (mV)	CDs-bAPAE-PEG-FA ζ Potential (mV)
pH 4	-12,2 (±5,75)	+12,73 (±5,53)	+0,323 (±3,86)
pH 7	-11,5 (±3,89)	-4,28 (±6,98)	-7,10 (±5,41)
pH 9	-9,41(±5,92)	-13,03 (±16,3)	-11,33 (±8,46)

For the bare CDs (Figure S2a). and CDs-bAPAE (Figure S2b)., in agreement with their UV spectrum, the maximum emission occurs at a wavelength of 440 nm (excitation: 343 nm).

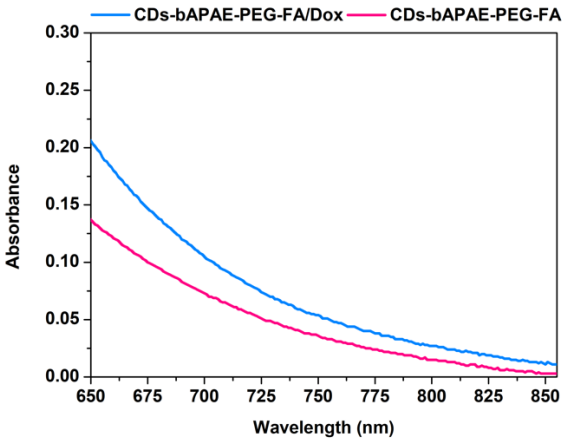

Figure S2. UV absorption of the CDs-bAPAE-PEG-FA and CDs-bAPAE-PEG-FA@Doxo conjugates in the NIR region.

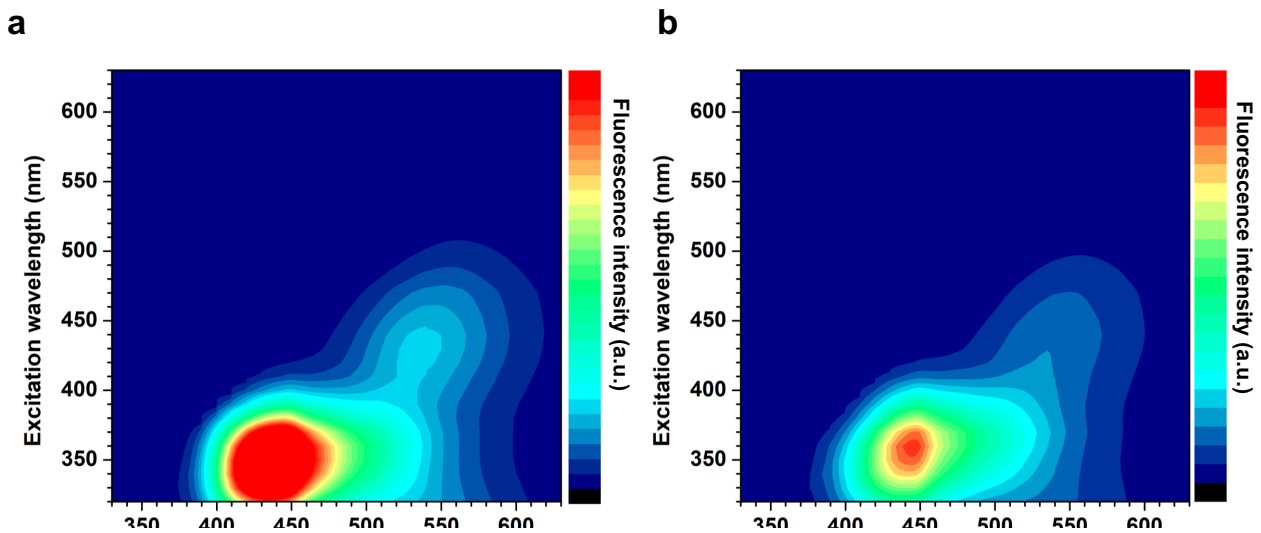

Figure S3. 3D emission spectra of a) CDs and b) CDs-bAPAE

To test the safety of passivated CDs, the cytocompatibility was evaluated on a healthy human dermal fibroblasts (HDF) and on two human breast cancer cell lines, MCF-7 and MDA-MB-231 using the MTS assay (Figure S3). Cell viability was also evaluated after photothermal treatment using concentrations of CDs-bAPAE-PEG-FA ranging from 0.1 to 3 mg mL^{-1}. (Figure S3 b, d) Results showed that the empty system is cytocompatible on all cell lines and time points analyzed (24 and 48 h).

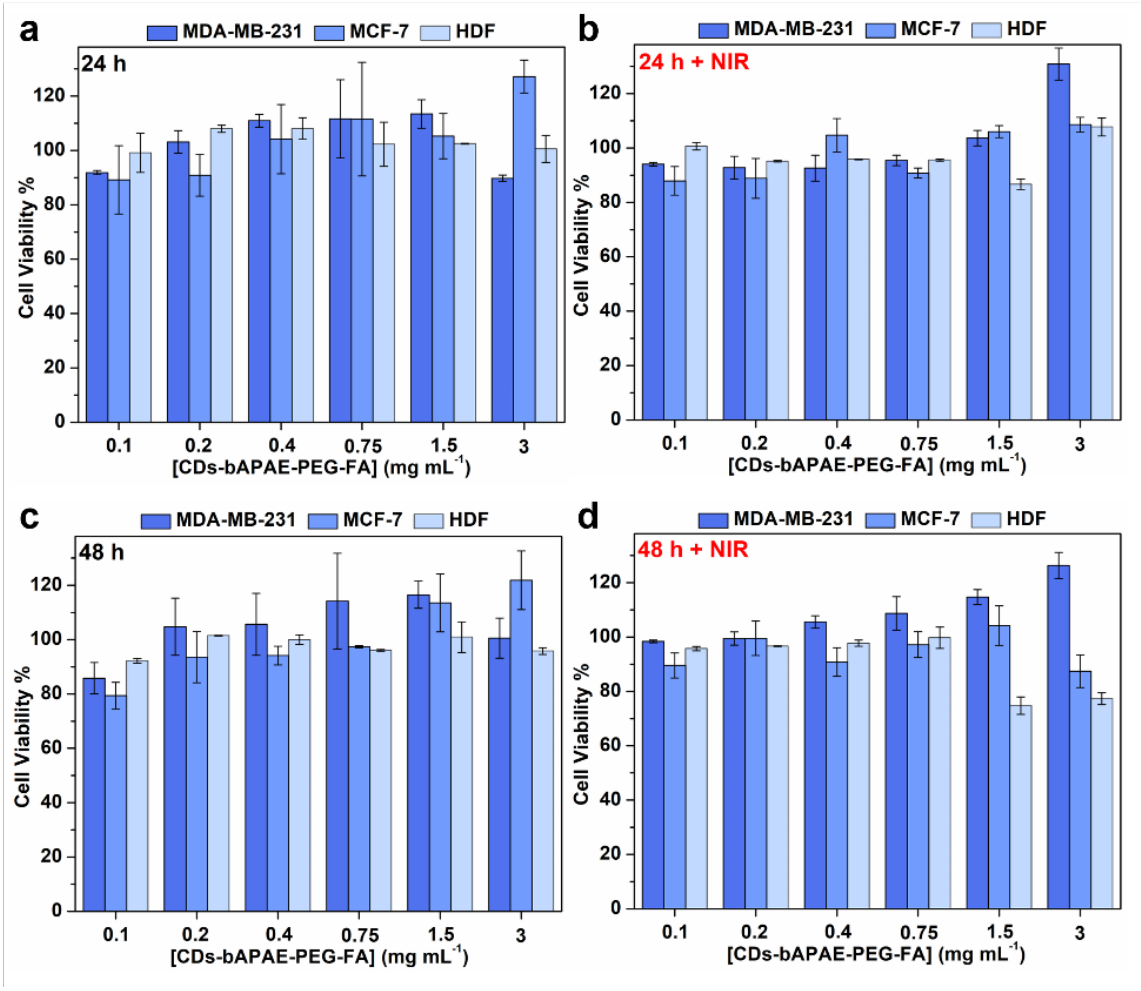

Figure S4. Cytocompatibility of CDs-bAPAE-PEG-FA without (a, c) or with (b, d) NIR light stimulation, after 24 (a, b) or 48h (c, d) incubation with HDF, MCF-7 and MDA-MB-231.

Further studies conducted on healthy red blood cells confirmed that the both the empty and the doxorubicin-loaded passivated CDs possessed high cytocompatibility on erythrocytes, indicating a possible safe use for parenteral administration (Figure S4).

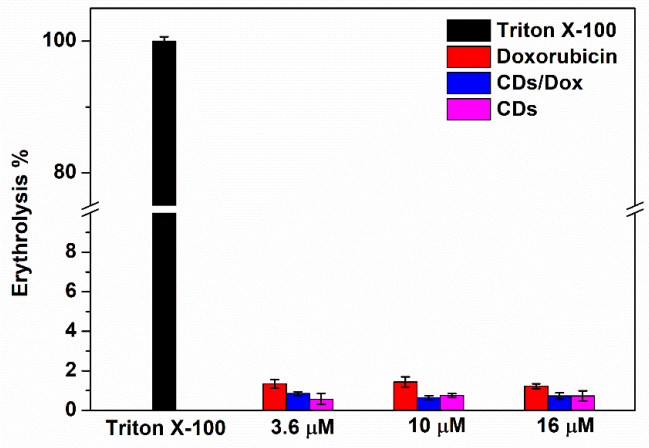

Figure S5. Erythrolysis tests of CDs-bAPAE-PEG-FA (magenta) and CDs-bAPAE-PEG-FA (blue) or free doxorubicin (red) at increasing equivalent concentration of doxorubicin.

The internalization process resulted time-dependent for each treated cell line (24h uptake micrographs - Figure S5).

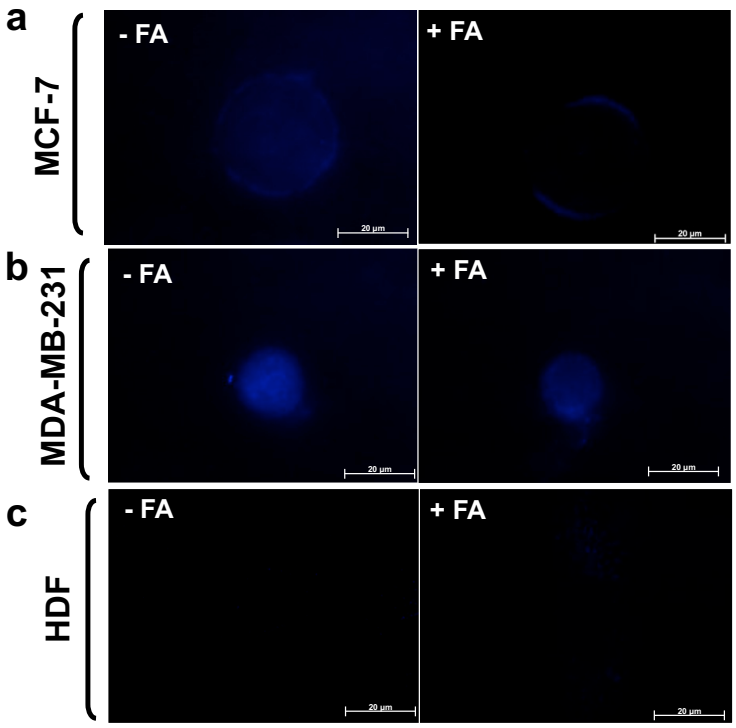

Figure S6. Uptake studies of drug free CDs-bAPAE-PEG-FA. Fluorescence micrographs acquired in DAPI channel (ex: 359 nm; em: 457 nm) of CDs-bAPAE-PEG-FA uptake after 24h incubation with a) MCF-7, b) MDA-MB-231 and c) HDF. Acquisitions of CDs-bAPAE-PEG-FA uptake in non-treated cells (-FA) and pre-treated with folic acid to obtain receptors saturation (+FA).

Doxorubicin uptake showed time-dependent internalization for all tested cell lines, consistent with the data obtained from qualitative investigation (Figure S6).

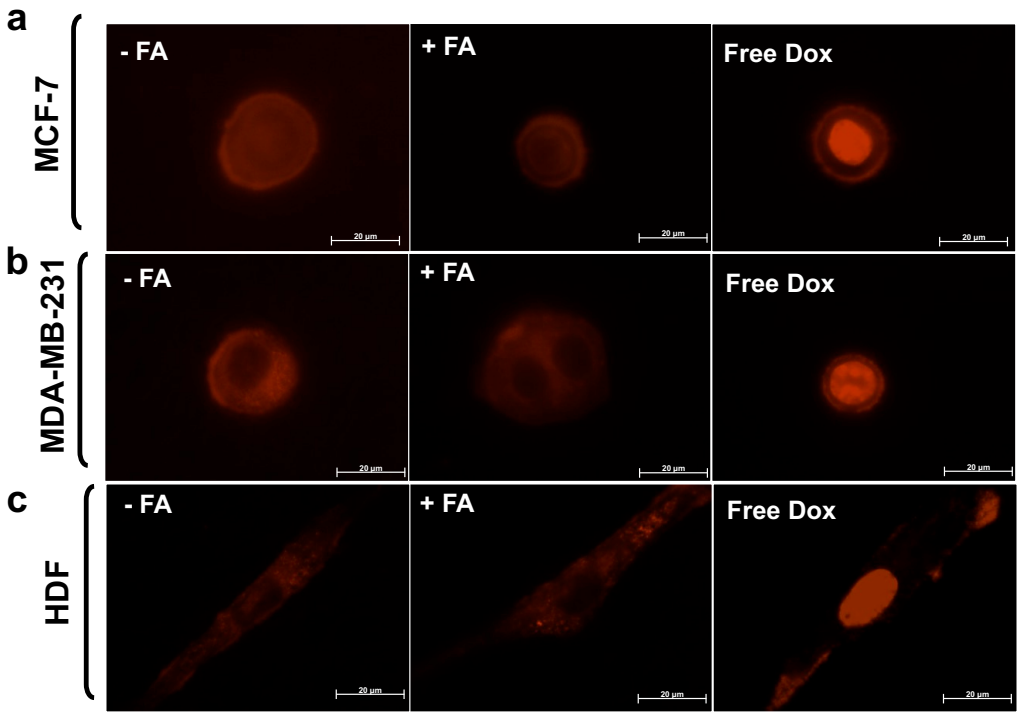

Figure S7. Uptake studies of doxorubicin loaded CDs-bAPAE-PEG-FA/Dox. Fluorescence micrographs acquired in Texas Red channel (ex: 561 nm, em: 594 nm) of CDs-bAPAE-PEG-FA/Dox uptake after 24h incubation with a) MCF-7, b) MDA-MB-231 and c) HDF. Acquisitions of CDs-bAPAE-PEG-FA/Dox uptake in non-treated cells (-FA), pre-treated with folic acid to obtain receptors saturation (+FA), and cells incubated with equivalent concentration of free doxorubicin (Free Dox).