Electronic Supplementary Material (ESI) for Nanoscale.

Biocompatible crosslinked β-cyclodextrin nanoparticles as multifunctional carriers for cellular delivery

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Supporting Information

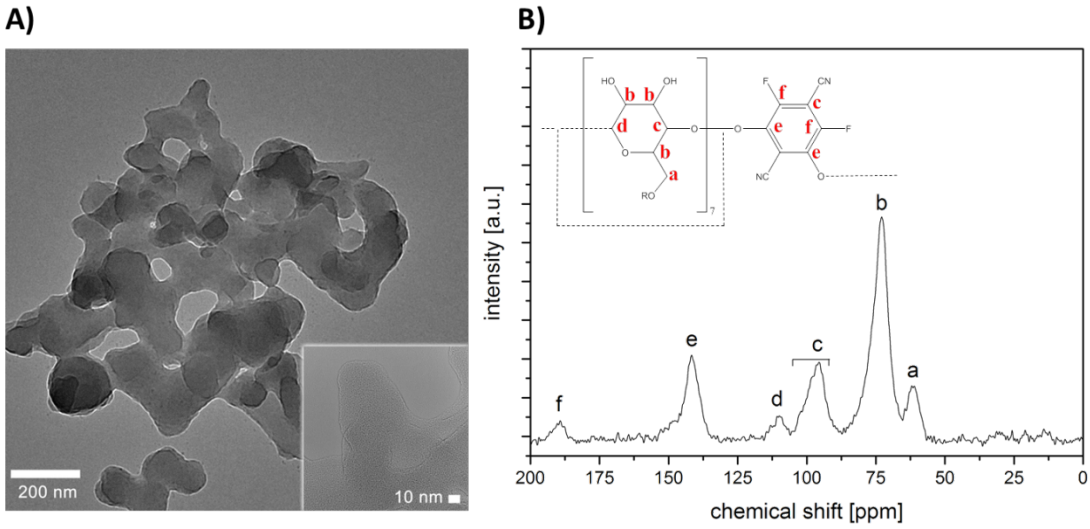

Figure S1: A) Transmission electron microscopy (TEM) image of β-CD NPs revealing a particle size distribution of around 100-200 nm. B) ^{13}C-MAS solid-state nuclear magnetic resonance (ssNMR) spectrum of β-CD NPs indicating the successful incorporation of the oligosaccharide compounds and the rigid organic linker into the crosslinked material.

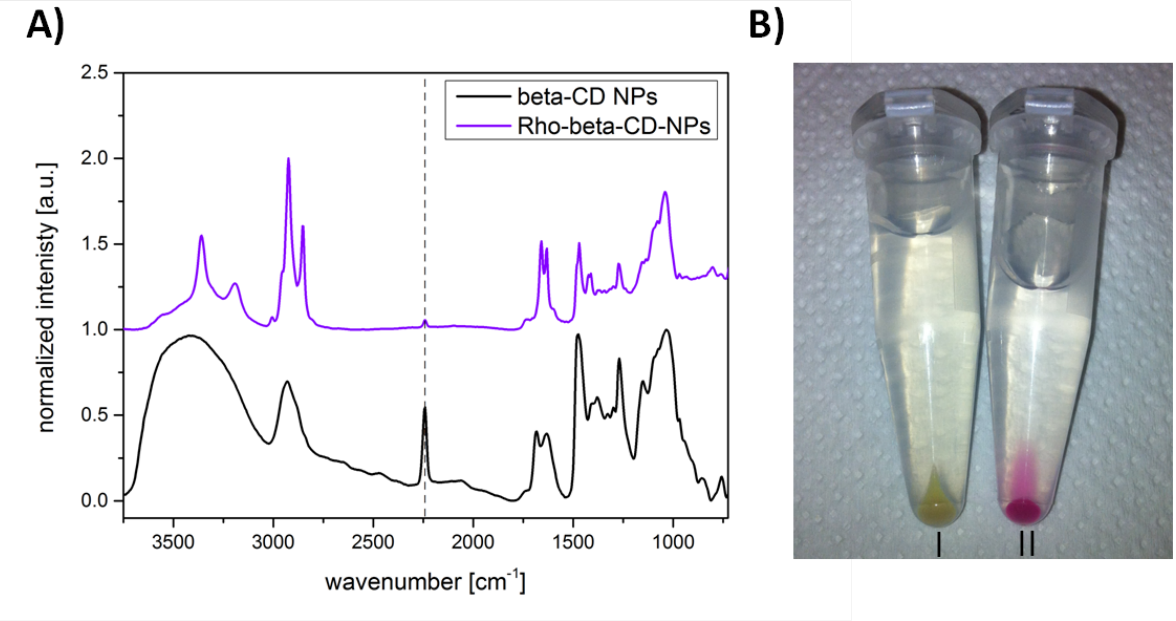

Figure S2: A) IR spectroscopy data of as-synthesized β-CD NPs (black) and rhodamine-labeled nanoparticles (violet). The successful attachment can be followed by the vanishing nitrile stretching vibration at 2245 cm^{-1} due to the covalent binding of the dye's azide groups to form tetrazole rings in a 1,3-dipolar cycloaddition. Spectra were normalized and shifted for clarity by 1.0 along the y-axis. B) Photograph of 1 mg of as-synthesized pale yellow β-CD NPs (I) and pink labeled Rho-β-CD NPs (II) in water after centrifugation, respectively.

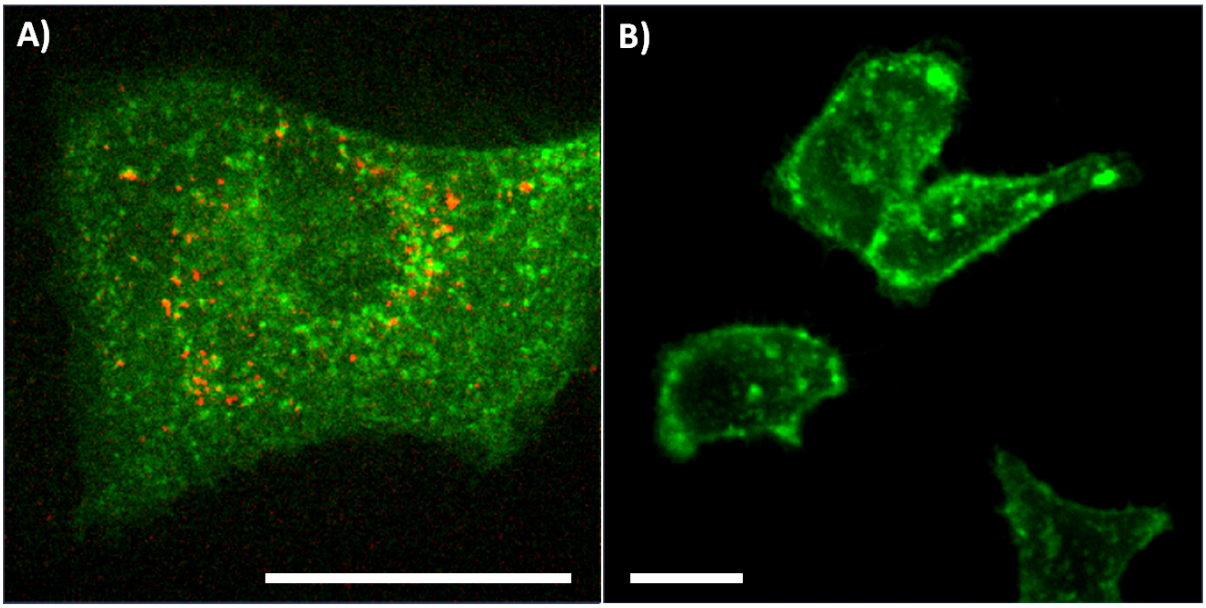

Figure S3. Fluorescence microscopy of HeLa cells incubated with rhodamine-labeled β-CD NPs (red) after 30 min (A), or pretreated with free inhibitors (e.g. β-cyclodextrin) for 30 min and incubated with rhodamine-labeled β-CD NPs for 30 min afterwards (B). Cell membranes are stained with WGA (green). Scale bars represent 10 µm each.

To test the targeting of sugar receptors with β-CD nanoparticles on HeLa cells, the receptors were either blocked or free. The functionality was evaluated in a receptor competition experiment. For this purpose, one part of the HeLa cells was pre-incubated with 5 µL of an inhibitor solution (10 mM aqueous solutions of D-glucose, D-L-arabinose, 2-deoxy-D-glucose or β-cyclodextrin, respectively), to block the receptors, for 30 min at 37 °C under a 5% CO_2 humidified atmosphere. Then the HeLa cells were incubated with 5 µg of rhodamine-labeled β-CD-NPs for 30 min at 37 °C under a 5% CO_2 humidified atmosphere. For staining the cell membrane, the cells were incubated with WGA for 2 min. The cells were washed three times with PBS, fresh medium was added and subsequently the cells were imaged. Clearly an enhanced sugar receptor-mediated β-CD NP uptake can be seen when the sugar receptors are available on the cell surface (A) compared to blocked receptors (B).

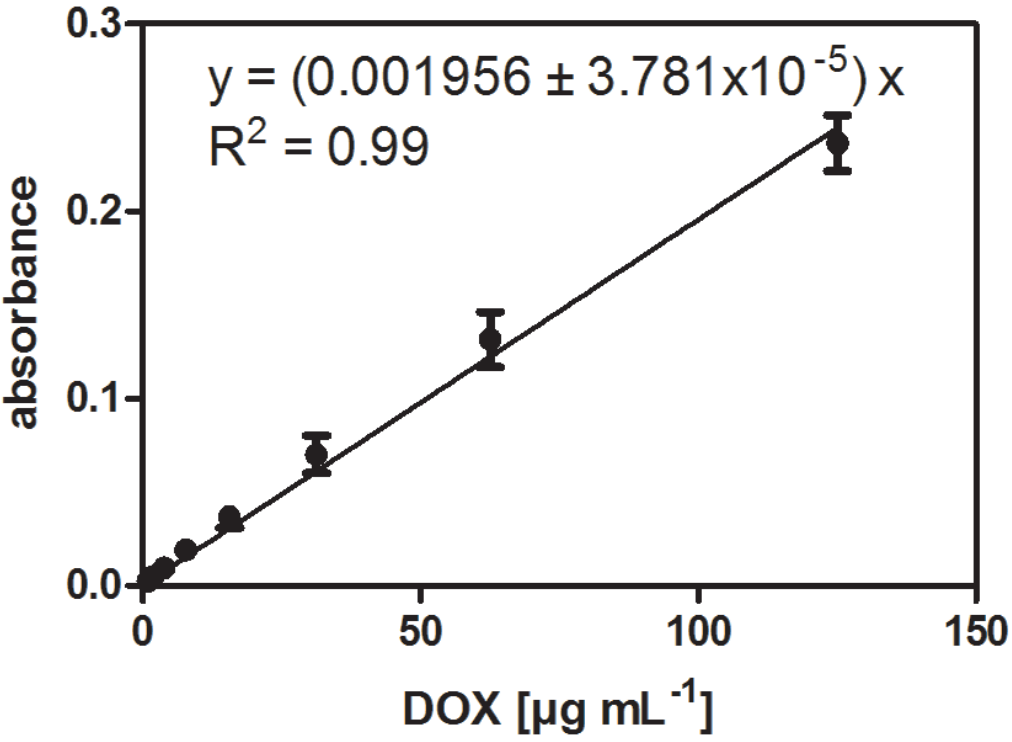

Figure S4: Calibration curve for doxorubicin (loading capacity) measured at 500 nm.

The loading capacity of the CD-NPs was determined by UV-Vis difference measurements of starting and supernatant DOX solution after centrifugation following the loading process. The

concentration of the DOX loading solution was 1 mg/mL and the concentration of nanoparticles 1 mg/mL. After several washing steps the supernatants were collected, revealing an absorbance of 0.750±0.008. By using the linear regression fit of the calibration curve this corresponds to a DOX content of 383 µg in the collected supernatants. Therefore, the total amount of loaded DOX was 117 µg per 0.5 mg nanoparticles leading to a loading capacity of 23 wt%.

To test the pH-responsive release of Hoechst from the nanoparticles, Hoechst-loaded particles were redispersed in 1 mL potassium phosphate buffer at pH 7.4, 6.0, 4.0 respectively. After a 20-minute incubation period, particle solutions were centrifuged to separate particles and supernatant and the UV-Vis spectrum of the supernatant containing the released Hoechst was measured. Release increased with decreasing pH as shown in Figure S5.

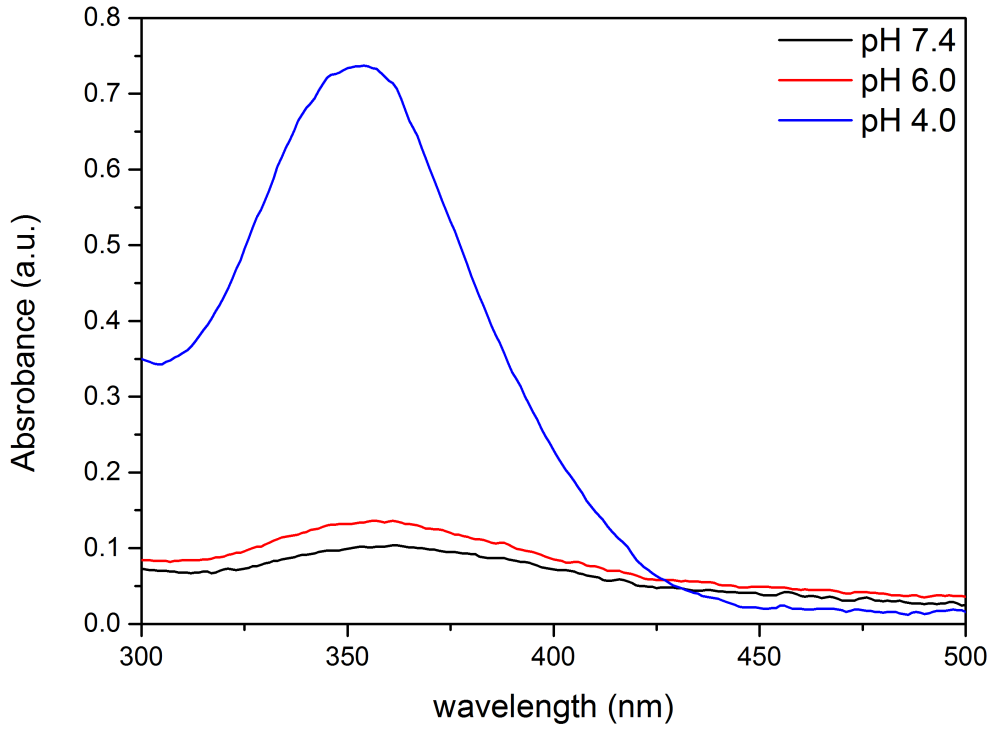

Figure S5: UV-Vis measurement of Hoechst released from nanoparticles at various pH values.

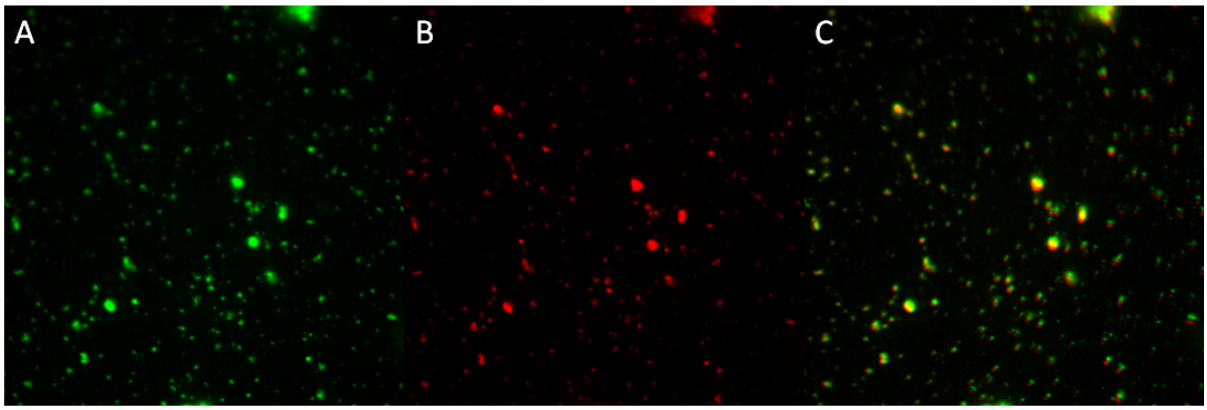

Figure S6: Fluorescence colocalization images of the liposome coated CD NPs. A) liposomes labeled with WGA 488, B) CD NPs labeled with TAMRA, C) merged image of both channels.

To investigate if the coating of the particles with liposomes was successful, fluorescence colocalization experiments were performed (Figure S6). For this purpose β-cyclodextrin particles were stained with TAMRA, a rhodamine dye, and the liposomes were marked with WGA 488. In the case of a successful coating the dyes should be colocalized, while an independent distribution indicates a failure of the particle coating. The images (A, B) show that both the liposomes and particles were stained successfully and the merged image (C) shows that both fluorescent stains are colocalized, proving that the particles were successfully coated with liposomes. The slight offset in the positions of the particles and the liposomes between both channels is caused by the Brownian motion of the particles, while switching to a different excitation wavelength to take the second image. Control experiments confirmed that there is no spectral overlap between both dyes, meaning the observed colocalization of the signals is caused by the successful coating.

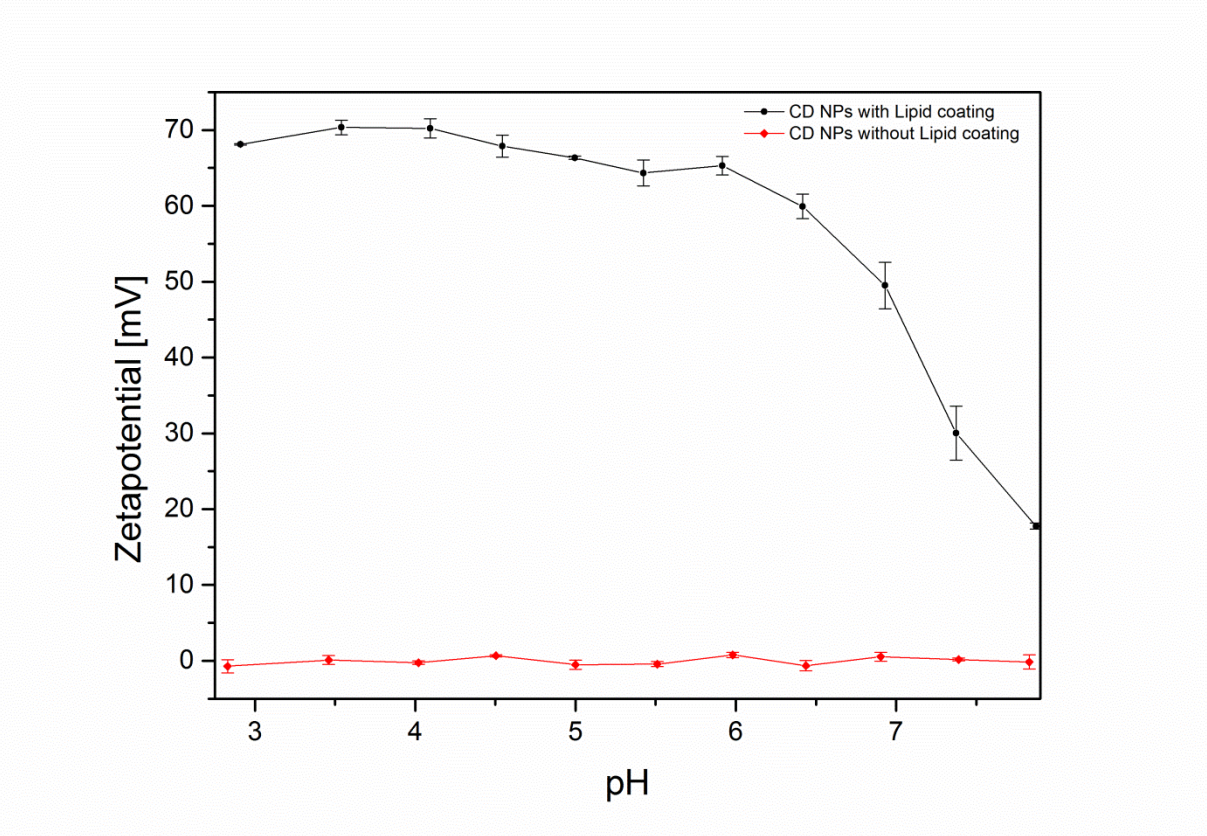

Figure S7: Titration curves of coated and non-coated CD NPs.

A further proof for successful lipid coating is a comparison of the zeta potentials of lipid-coated and non-coated particles. The β-CD-NPs without any coating show a more or less constant zeta potential over the whole pH-range from 3 to 8 of around 0 mV. After successful lipid coating the measured zeta potential is tremendously increased. This results from the positive charge of the DOTAP lipid and thus confirms a successful coating.