Supporting Information

Photon Upconversion Sensitized Nanoprobes for Sensing and Imaging of pH

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Quantification of pHrodo™ Red conjugation

The amount of pHrodo™ Red dye conjugated to the UCNP was calculated by measuring the emission spectrum of the conjugate upon 562 nm excitation with Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, Santa Clara, CA) and comparing the 585 nm emission intensity of the conjugated dye to a standard curve obtained from a dilution series of the pHrodo™ Red. Both the conjugate and the dye were diluted in 100 mM citrate, pH 3. The amount of pHrodo™ Red-dye conjugated to the UCNPs was calculated to be 56.9 nmol per mg of UCNP which resulted in a yield of 85 %.

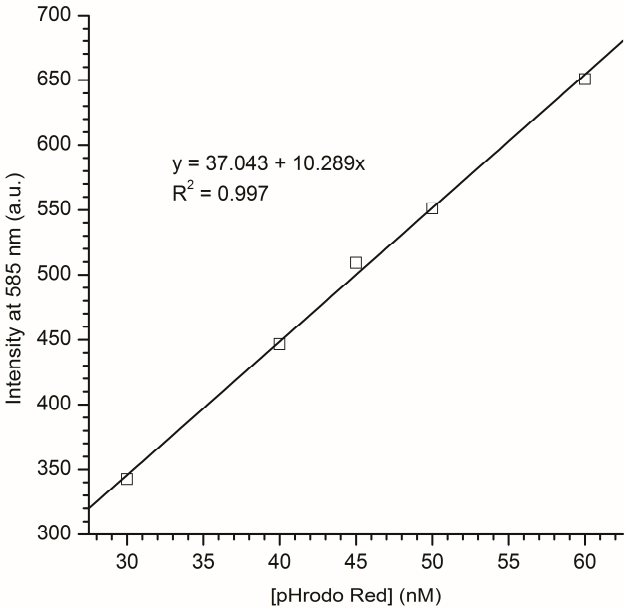

Figure S1. The standard curve of pHrodo™ Red-dye emission at 585 nm upon 563 nm excitation for calculation of the conjugation yield.

Determination of particle size and aggregation

The particle size of the UCNP-pHrodo™ Red-conjugate was measured in 0.01 mg/ml concentration in 25 mM MES-buffer (pH 3.75, 5.72 and 7), 150 mM NaCl with Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The particle size was measured by dynamic light

scattering after ~5 min, ~10 min and ~90 min from the. No further aggregation could be observed at pH 7.0.

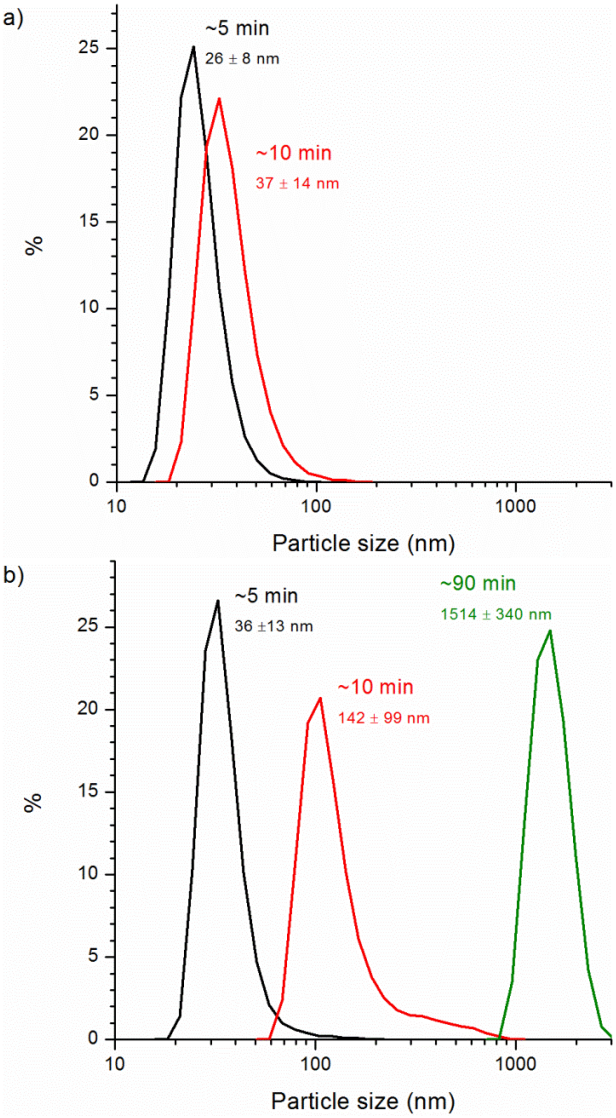

Figure S2. Dynamic light scattering (DLS) measurement of the UCNP-pHrodo™ Red -conjugate in pH a) 7.0, and b) 3.8 (25 mM MES buffer, 150 mM NaCl) after ~5 min and ~10 min (and ~90 min) from ultrasonication. Displayed are the size distributions by number.

Calibration of pH-nanosensor

The UCNP-pHrodo™ Red-conjugate was diluted to a concentration of 0.015 mg/ml in 200 mM phosphate-citrate buffer with pH 2.95, 3.26, 3.83, 4.04, 4.21, 4.47, 4.73, 4.93, 5.13, 5.44, 5.79, 6.55, 7.01 and 7.65 in three replicas. Both the upconversion photoluminescence of the UCNP at 550 nm a) and the UC-RET-sensitized emission of the pHrodo™ Red at 590 nm b) were measured with a microwell plate reader. 100-fold attenuation was used for the 550 nm signal by adding an optical density filter. The ratio of the luminescence intensity at 590 nm to 550 nm was calculated c). The 550 nm emission was not affected by the pH. A slightly increased luminescence was measured at low pH which is a result of aggregation of the nanoparticles. The UC-RET sensitized emission of the pHrodo™ Red at 590 nm showed linear correlation with pH. The 550 nm emission was used as the reference for the ratiometric response curve which was linear at the whole pH-range.

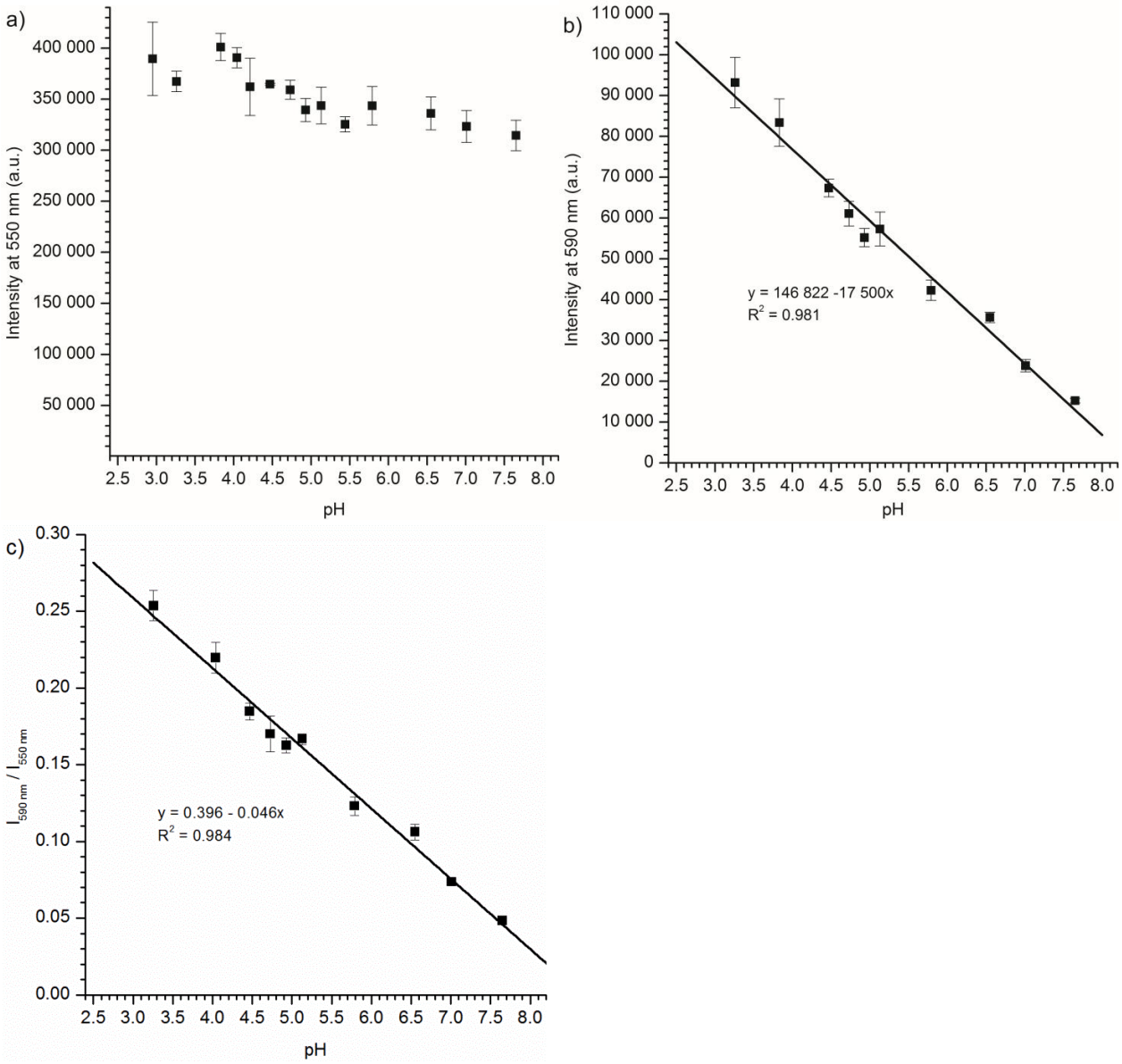

Figure S3. pH-titration curves of the pH-nanosensor in a buffer series with pH-range of 2.95–7.65. pH-response curves of a) upconversion photoluminescence emission of UCNP at 550 nm, b) UC-RET sensitized emission of pHrodo™ Red dye at 590 nm, and c) ratio of 590 nm and 550 nm measurements.

Fluorescence imaging

The UNCP-pHrodo™ Red-conjugate was imaged with Leica TCS SP5 scanning microscope. The UCNP-pHrodo™ Red-conjugate samples were dropped on a microscope slide with volumes of 50 µl in three different saline buffers of pH 7.1, 5.0 and 3.6. The emissions of the UCNPs and the pHrodo™ Red were scanned at 10 Hz rate with channels 525/50 nm (TRITC filter) with a confocal gain of 600 V of the PMT and 585/40 nm (FITC filter) with a confocal gain of 900 V of the PMT, respectively (Figure S4).

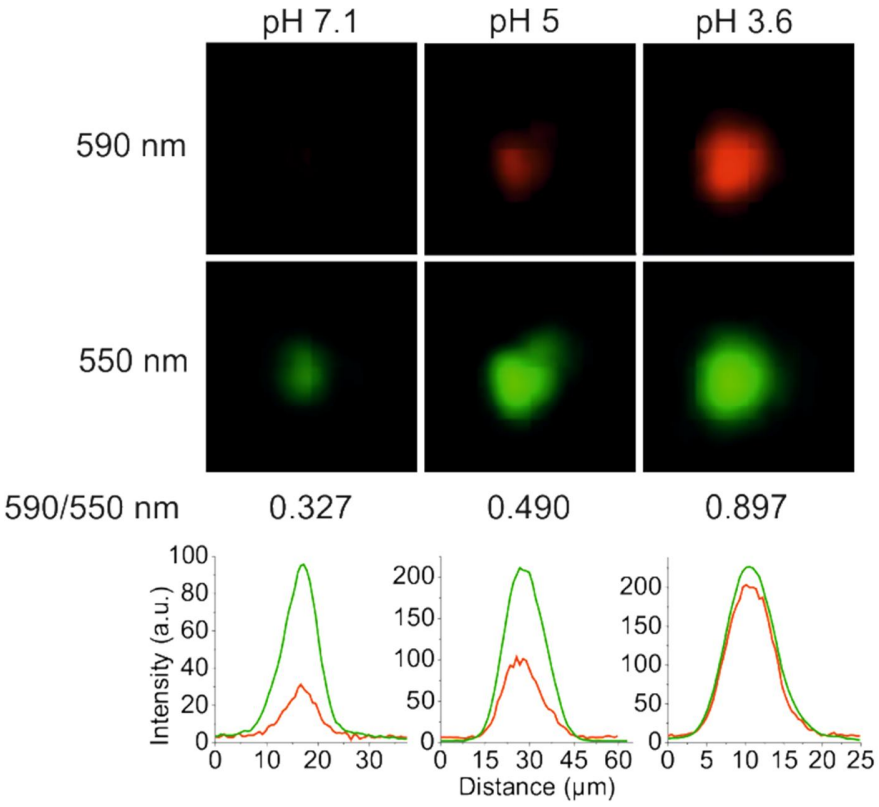

Figure S4. Fluorescence microscopy images of aggregates of UCNP-pHrodo™ Red -conjugate in three different pHs and two emission channels. The ratiometric response was calculated from the measured intensities of the red channel (pH indicator, PMT: 900 V) to the green channel (reference, PMT: 600 V) over the whole area of the aggregate spots. The histograms represent the intensity profiles of both red and green emissions measured from a line drew over the aggregate spots giving the diameters of approximately 15, 30 and 10 µm at pH 7.1, 5 and 3.6, respectively.

The cellular uptake of the particles into HeLa cells was also examined by confocal microscopy (Figure S5). The UCNPs were efficiently taken up by the cells as is seen from the signal obtained from direct excitation of pHrodo™ coupled to the UNCPs with a 561 nm diode laser. Direct excitation of the dye was used in the uptake studies as it could be visualized with a higher scanning speed. This fluorescence signals can only be recorded from living cells where the nanoprobes are expected to reside in endosomal compartments where the pH is below 6.

The pH response can be qualitatively visualized by examining the FRET signal outside of the cells as a reference, either in the culture medium or in an apoptotic cell, compared to a living cell (Figure S6). Outside of cells no FRET signal was detectable at pH 7.4; a faint signal was visible from a cell undergoing apoptosis and in a living cell two kinds of FRET signal are observable: Bright spots probably originating from late endosomes (pH 5 - 5.5) and less intense spots as would be expected from early endosomes (pH ~ 6).

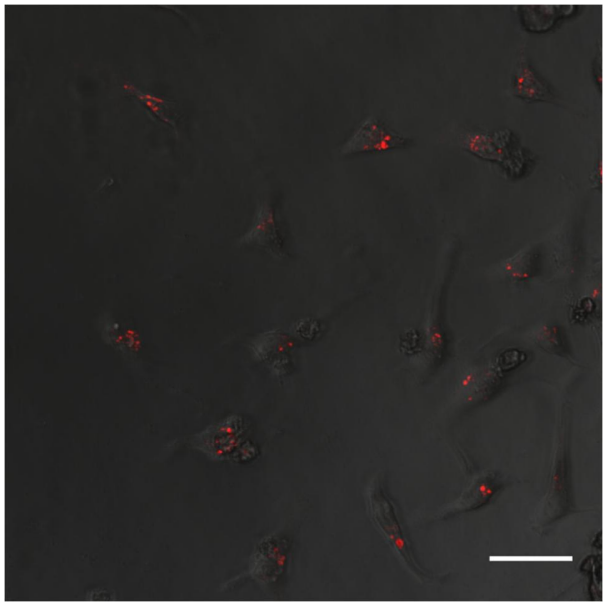

Figure S5. Non-ratiometric image of the UCNP-pHrodo™ - probes in cells, showing over 90% uptake and no or very low cytotoxicity. Added particle concentration was 50 µg/ml. The images were taken with a 561 nm diode laser by direct excitation of the pHrodo dye. The scale bar is 50 µm.

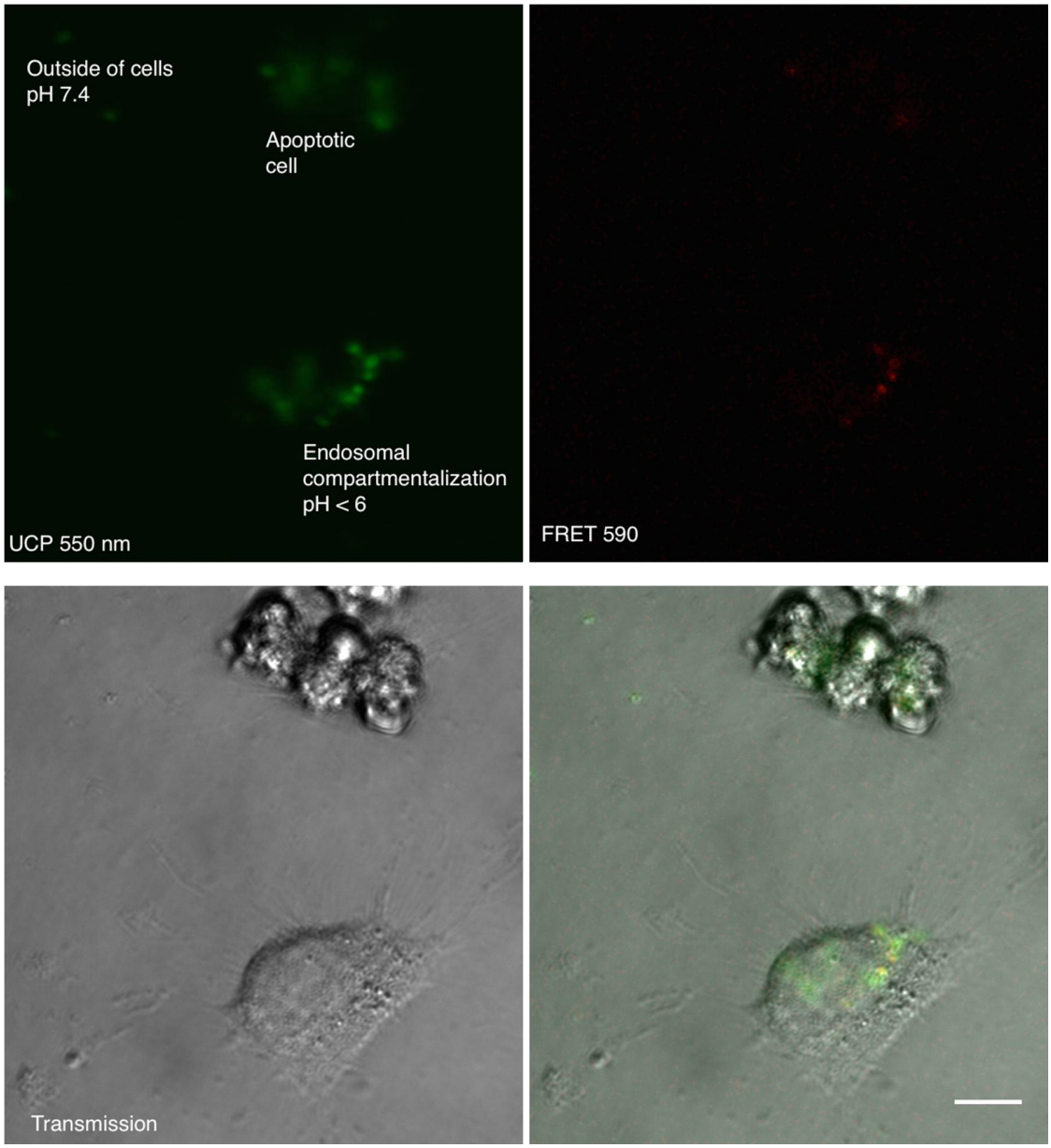

Figure S6. Ratiometric imaging of endosomal pH. Laser scanning confocal microscope image of HeLa cells loaded with UCNP-pHrodo™ probes. Up left green upconversion emission (510-560 nm), up right red emission (570-610 nm) of pH-sensitive FRET signal, on lower left a transmission micrograph and lower right a merged image with the transmission picture superimposed with the green and the red channel. A yellow color indicates a stronger red signal, and therefore, an acidic pH. Outside of the cells the pH of the culture medium is 7.4 and no FRET signal can be observed at positions where the green UCP emission can be recorded. The white scale bar is 10 µm.