Electronic Supplementary Information

Design and validation of a new ratiometric intracellular pH imaging probe using lanthanide-doped upconverting nanoparticles

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Figure S1. TEM images of NaGdF$_4$:Yb,Tm (left) and NaGdF$_4$:Yb,Tm@NaGdF$_4$:Nd,Yb (right) UCNPs and corresponding size distribution diagrams.
**Figure S2.** Overview TEM image of OA- (left) and PEI-coated (right) UCNPs.
Figure S3. Optical properties of UCNPs: 

A, UCL spectra of UCNPs (1.0 mg/mL), and B, intensity of the signals acquired under different pH environments under excitation at 980 nm and at a power of 1.0 W. 

C, UCL spectra of UCNPs, and D, intensity of the signals under different pH environments acquired under excitation at 808 nm and at a power of 1.0 W.
Scheme S1. Preparation of the pH sensitive ratiometric probe.
**Figure S4.** The upconversion emission (at 475 nm) lifetime decay curves of PEI coated UCNPs and UCNP-FL conjugates.

According to the theory,\(^1\) the fluorescence lifetime of the free donor of the FRET pair, \(\tau_D\), is

\[
\tau_D = \frac{1}{k_D + k_{Di}}
\]

And the lifetime of the donor in the presence of the FRET counterpart

\[
\tau_D = \frac{1}{k_D + k_{Di} + k_t}
\]

where \(k_D\) is the rate of the radiative decay of the donor, \(k_{Di}\) is the rate of the non-radiative decay of the donor, and \(k_t\) is the rate of the energy transfer from donor to acceptor.
Figure S5. Cytotoxicity evaluation of the UCNPs-FL ratiometric probe on HeLa cells.
Figure S6. Confocal laser scanning microscopy images of dried samples of UCNP-FL at pH 4.7 (a, b) and 7.3 (c, d). The images show the signal collected from the red channel in (a, c), and those from the green channel in (b, d). The scanning area is 102 μm × 102 μm.
Figure S7. Left, column graph representation of the average ratiometric signal (green channel over red channel) obtained from CLSM images of HeLa cells incubated with UCNPs-FL and subsequently washed and incubated at different pH values. Right, plot of the average ratiometric signal obtained from CLSM images of HeLa cells incubated with UCNPs-FL versus the incubation pH showing a linear relationship between both parameters.
Figure S8. Representative signal profiles across cells at different pH values obtained from ratiometric images. At pH values of 7.4 and 6.5 regions of lower signal intensity (thus lower pH) can be observed at the edges of the cells (red arrows). At pH values of 5.7 and 5.0 these regions are lost.
**Figure S9.** The bright field image and the fluorescence image (green channel) of HeLa cells incubated with the mixture of UCNPs (40 μg/mL) and FL (3 μg/mL).