

## Supplementary Information

### FRET based pH probe with broad working range applicable to referenced ratiometric dual wavelength and luminescence lifetime read out

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**Note added after first publication:** This Supplementary Information file replaces that originally published on 26<sup>th</sup> February 2015, in which the mass calculations were incorrect.

### Materials and Methods

#### Preparation of the pH sensitive probe (4)

The amine reactive Eu-chelate Eu7D (**1**) was synthesized as previously reported.<sup>[1]</sup> Diamino dodecylamin (**2**) was obtained from Sigma Aldrich ([www.sigma-aldrich.com](http://www.sigma-aldrich.com)) and 5-(and-6)-Carboxynaphthofluorescein succinimidyl ester (**3**) was purchased from Molecular Probes ([www.lifetechnologies.com](http://www.lifetechnologies.com)).

25 µmol of compound (**2**) were dissolved in 2.5 mL of a sodium bicarbonate buffer (pH 9.0; 10 mM) and 5 µmol of compound (**1**) in 2.5 mL of the same bicarbonate buffer were added quickly. The mixture was stirred for 24 hours at room temperature. The reactants were removed by size exclusion chromatography with Sephadex G-10 (3 g, with ~ 50 ml bicarbonate buffer). Subsequently, 5 µmol of compound (**3**) were added to the eluate and stirred for further 12 hours at room temperature. The product (**4**) was purified again by size exclusion chromatography (Sephadex G-10) with Millipore water.

## MS (MALDI-TOF)

calcd. for  $[M]^- = [C_{65}H_{60}N_6O_{14}SEu]^-$ :  $m = 1333.23$ ;

$[M+H+Na]^+ = [C_{65}H_{61}N_6O_{14}SEuNa]^+$ :  $m = 1357.23$ ;

found  $m/z = 1356.3; 1357.2; 1358.2; 1359.2; 1360.2; 1361.2 [M+H+Na]^+$ .

## Buffers

50 mM Britton-Robinson buffer (BRB) solutions of the respective pH values were used throughout the measurements.

## Spectroscopy

All measurements were carried out in quartz cuvettes. Absorption spectra were recorded with a Varian Cary 300 Bio UV-Visible. Uncorrected luminescence spectra and decay profiles were recorded with a Varian Cary Eclipse Fluorescence Spectrophotometer at an excitation wavelength of 345 nm and emission slits of 1.5 or 2.5 nm. The luminescence decay profiles were recorded by integrating 100 excitation cycles with a delay time of 100  $\mu$ s and a gate time of 20  $\mu$ s.

## Mass spectrometry

MS was performed with an Autoflex III Smartbeam Laser 355 nm MALDI-TOF mass spectrometer from Bruker Daltonik (Bremen) with dithranol (trihydroxyanthracen) as matrix by accumulation of 2000 spectra. The relative intensities are reported as percentages relative to the base peak ( $I = 100\%$ ).

## Luminescence lifetime imaging according to the RLD method

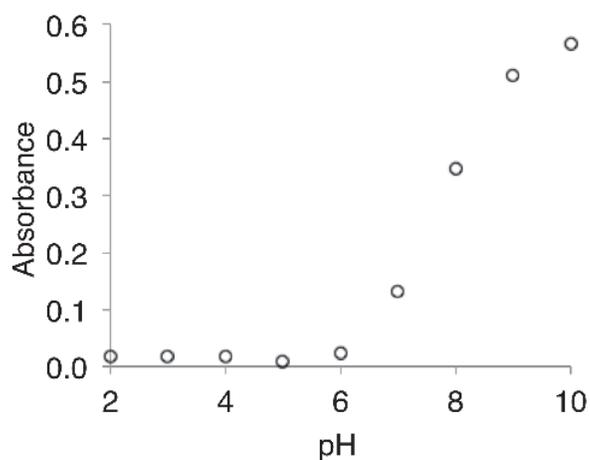
The calibration of the luminescence lifetime responses was performed by means of a custom-build imaging set up and samples (150  $\mu$ L of a 0.1 mM solution of the pH-probe (4) in 50 mM BRB solution of the respective pH values) prepared in microwell plates. The imaging system consisted of a PCO SensiCam 12 bit b/w CCD camera (PCO, Kelheim; [www.pco.de](http://www.pco.de)) equipped with a Schneider-Kreuznach Xenon 0.95/17 lens (Schneider Optische Werke; [www.schneiderkreuznach.com](http://www.schneiderkreuznach.com)), and a pulsable UVLUX335-HL-3 LED from Roithner Lasertechnik (Vienna; [www.roithner-laser.com](http://www.roithner-laser.com)). The excitation light was filtered with a UG 11 filter (Schott, Mainz, Germany, [www.schott.com](http://www.schott.com)) with a thickness of 2 mm. Emission light was detected using a D610/60 band pass filter (Chroma, Rockingham, VT, USA, [www.chroma.com](http://www.chroma.com)). The data acquisition in the CCD-based system for time-resolved imaging was controlled by the software module Look@MOLLI.<sup>[2]</sup> Luminescence lifetime measurements were carried out by means of time-resolved (gated) fluorescence detection according to the Rapid Lifetime Determination (RLD) method.<sup>[3,4]</sup> Each measurement was recorded with  $N=3$  and the parameters of the imaging system were set as follows:

pH	Binning	Gate [ $\mu$ s]	Delay 1 [ $\mu$ s]	Delay 2 [ $\mu$ s]	Lamp [ $\mu$ s]
3	2*2	120	3	63	700
4	2*2	120	3	63	600
4.5	2*2	120	3	63	500
5	2*2	120	3	63	400
5.5	4*4	80	3	43	350
6	4*8	50	3	28	250
6.5	4*8	50	3	28	200
7	4*8	35	3	20.5	150
7.5	8*8	20	3	13	70
8	8*16	10	1	11	40

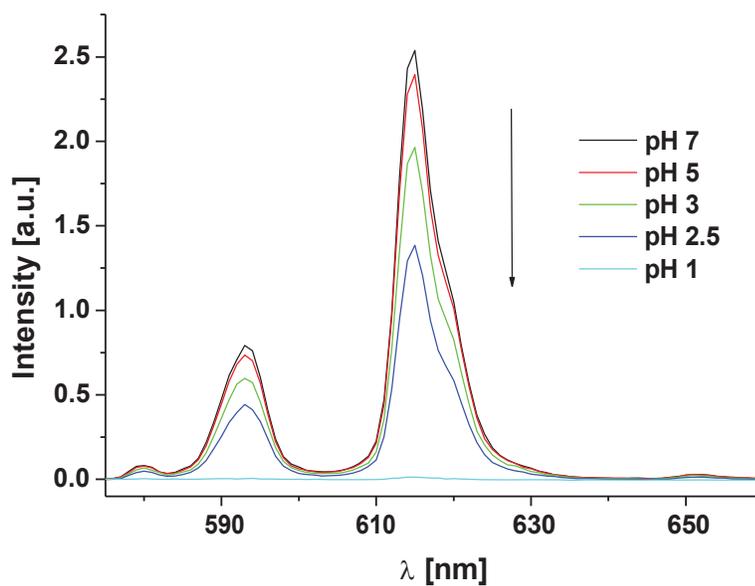
### Imaging of urine samples

Two different human urine samples (male) were measured. One sample was taken before and one sample 12 h after ingestion of 5 mg of vinegar. 150  $\mu$ L of the pH-probe (4) was added to 1 ml of each urine sample and stirred. The pH of both solutions was determined by two methods, the pH-glass electrode (Handylab 12 pH meter along with a Blue Line 27 flat membrane pH electrode; both from Schott; [www.schott.de](http://www.schott.de)) as reference standard and the luminescence lifetime imaging method (RLD) using the pH indicator probe (4) as described in this paper.

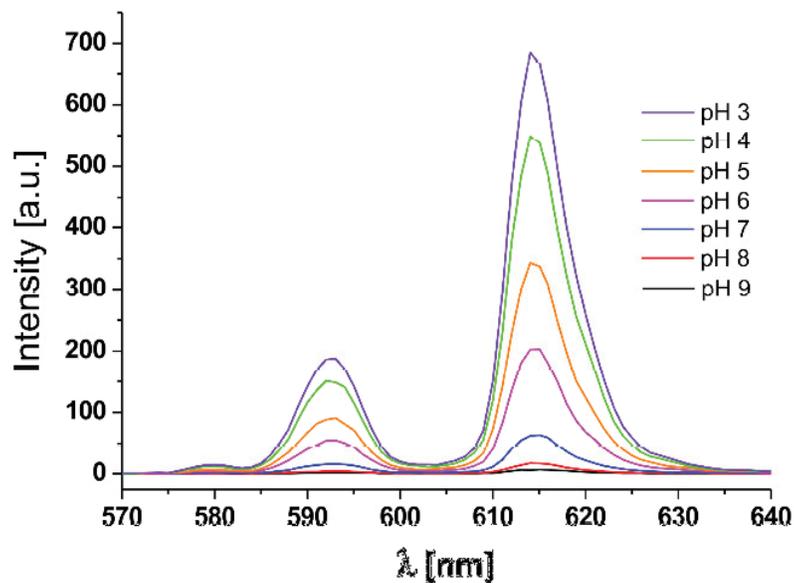
## Supplementary Figures



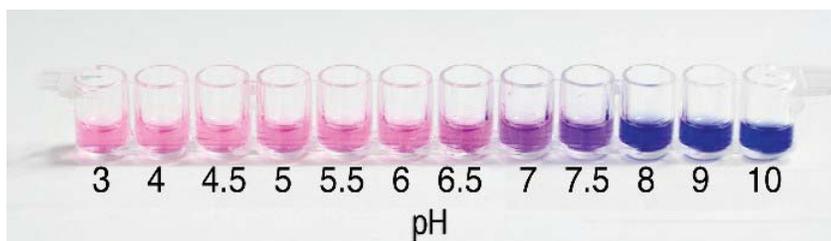
**Figure S1.** Change of the relative absorption of CNF (**3**) at 615 nm depending on pH, leading to a strong decrease of the red emission of the europium complex with increasing pH.



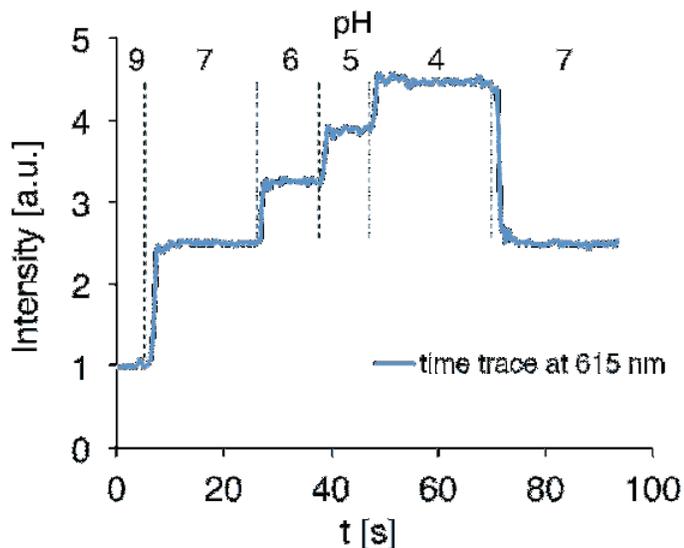
**Figure S2.** Luminescence of europium chelate Eu7D (**1**) depending on pH. At pH < 3 decomposition of the complex sets in, accompanied by a strong decrease of luminescence intensities.



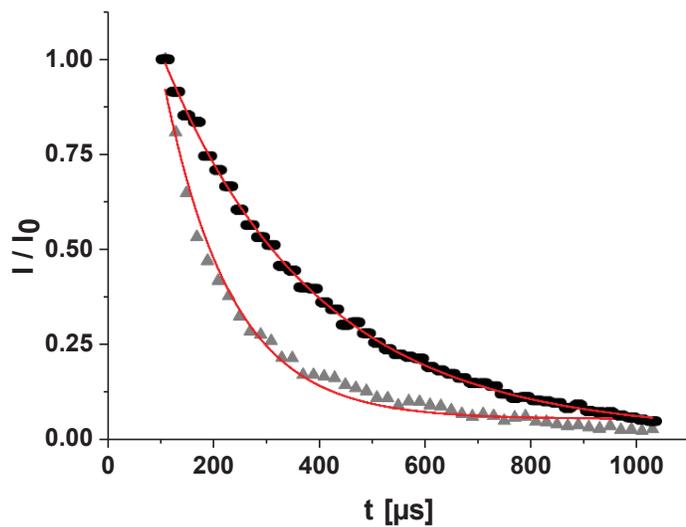
**Figure S3.** Emission spectra of (4) in response to pH recorded in time gated mode (400  $\mu$ s delay, 400  $\mu$ s integration time). The main emission peak of  $\text{Eu}^{3+}$  (614 nm) and the smaller satellite peak at 593 nm are quenched with increasing pH due to a higher absorption of the CNF unit.



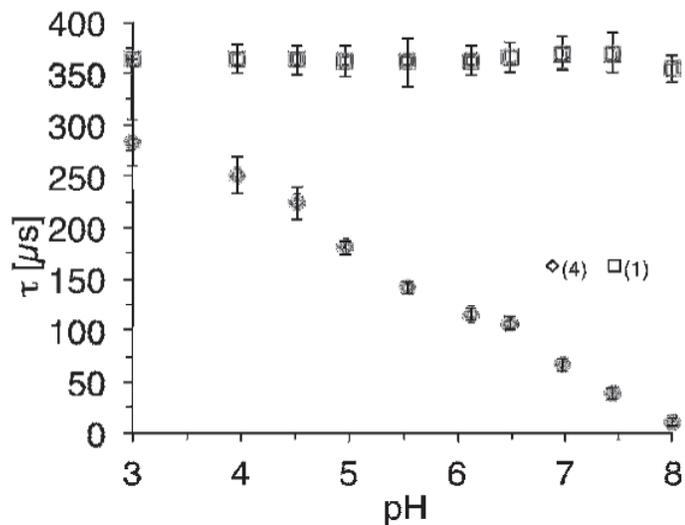
**Figure S4.** Visible color change of (4) in aqueous buffer in response to pH.



**Figure S5.** Time trace of the luminescent signal of (4) at 615 nm in response to varying pH. The changes in the signal appear immediately within less than 1 seconds ( $T_{90}$ ) or less than 2 seconds ( $T_{99}$ ). The signal change is completely reversible.



**Figure S6.** Normalized luminescence decay profiles of the 615 nm emission of (4) at pH 3 (■) and pH 6 (▲) with monoexponential decay fits (red lines) according to:  $y = A_1 \exp(-x/\tau) + y_0$ . These result in lifetimes  $\tau$  of 288  $\mu\text{s}$  for pH 3 and 127  $\mu\text{s}$  for pH 6, which is in good accordance with the values obtained with time-gated imaging (see Figure 7).  $I_0$  = Intensity after 100  $\mu\text{s}$  delay. Intensities  $I$  were acquired with 20  $\mu\text{s}$  integration time.



**Figure S7.** pH dependent luminescence lifetimes of the 615 nm emission of the europium chelate (1) (pure Eu-complex) and the pH probe (4) including spatial error bars within a round ROI of 0.5 cm diameter obtained by time-gated lifetime imaging. The data can be linearly fitted similarly to Figure 3b.

1. H. Takalo, V.-M. Mikkala, H. Mikola, P. Liitti, I. Hemmilä, *Bioconjugate Chem.* 1994, **5**, 278.
2. G. Holst, B. Grunwald, *Sens. Actuators, B* 2001, **74**, 78.
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