

Supplementary information for

Europium(III) based Nanooptode for Bicarbonate Sensing- a Multicomponent Approach to Sensor Materials

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Experimental Section

Reagents.

4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-ium bromide (**1**) was synthesized in house.¹ Europium (III) chloride hexahydrate (EuCl₃·6H₂O, 99.9%) was purchased from abcr GmbH. 1-bromooctadecane, tridodecylmethylammonium chloride (TDMACl), thioxanthen-9-one, trifluoroacetic acid (TFA), potassium carbonate (K₂CO₃), disodium phosphate (Na₂HPO₄), sodium L-lactate, sodium chloride (NaCl), sodium bicarbonate (NaHCO₃), sodium hydroxide (NaOH), hydrochloric acid (HCl), 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethane-1-sulfonic acid (HEPES), polystyrene (PS; Mw, 35,000), dichloromethane (CH₂Cl₂), methanol (MeOH), diethyl ether, toluene, triton X-100 and dialysis tube (cellulose membrane, avg. flat width 23 mm, MWCO 12400) were obtained from Sigma-Aldrich. All salts and solvents used were analytical grade or higher. All aqueous solutions were prepared by dissolving appropriate salts into Milli-Q water.

Preparation of EuL NPs. Four kinds of EuL PS NPs were prepared according to the previous report.² Surfactant was not used here because Eu.D03AC18 itself is an amphiphilic compound and it forms foam when its water solution is shaken. The amount of each component is listed in Table S1. Corresponding components were added to 0.5 ml of 1 % (w/w) polystyrene in toluene. Drops of CH₂Cl₂ were added to help dissolve. 3.5 mL of Milli-Q water was added to each sample. The two solutions were mixed and the two-phase system was sonicated with the probe (8 mm Ø, sonicating power: 60 W) with 1 second on and 1 second off timing for a total duration of 60 seconds while stirring at 300 rpm. This process was repeated 5 times with a waiting time of 60 minutes between each sonication during which the vial is left stirring at 300 rpm with a closed cap. After the fifth sonication, the samples were left open and stirred at 300 rpm for 48 hours to evaporate all the toluene. NPs were purified by dialysis (cellulose membrane, MWCO 12400).

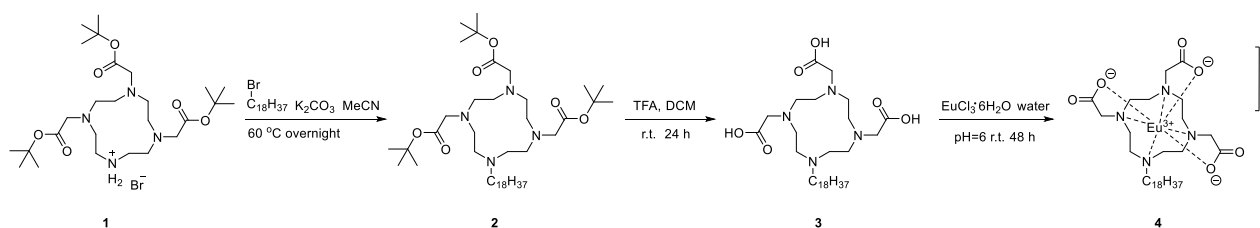
Table S1 The amount of each component for four nanooptode

	EuL / mg	TDMACl / mg	Thioxanthen-9-one / mg
NP1	7.5	0	0
NP2	7.5	5.7 (0.01 mmol)	0
NP3	7.5	0	2.1 (0.01 mmol)
NP4	7.5	5.7 (0.01 mmol)	2.1 (0.01 mmol)

Instrumentation and measurements. Emulsification was done with the sonicator (XS-sonic, FS-300N). Scanning electron microscopic (SEM) images were obtained under Jeol 7800F-prime scanning electron microscope (10 kV). Phosphorescence spectra and lifetimes were measured with a fluorescence spectrometer (Cary Eclipse) using disposable cuvettes (3 mL). 100 µl of the sample NP1 and NP2 stock were added to 3 ml of HEPES (20 mM, pH 7.40, 0.001% Triton-X100) in the cuvettes. For NP3 and NP4, only 10 µl of the stocks were used. All the stock solutions were well shaken before withdrawing. The testing solutions were uniform and transparent before and after HCO₃⁻ measurements. Fresh prepared NaHCO₃ (0.1 M and 1 M) were then added to the cuvettes and phosphorescence and lifetime were measured five times for each HCO₃⁻ concentration. The overall concentrations of EuL in the measurement system were calculated as 9.53×10⁻⁵ M for NP1 and NP2 and 9.53×10⁻⁶ M for NP3 and NP4 at most, without considering the residual salt in EuL and unbound molecules lost during the dialysis purification. The emission phosphorescence spectra were obtained by exciting at 380 nm while the excitation spectra were obtained with emission set at 620 nm. Related parameters, total decay time: 0.02 s; number of flashes: 1; decay time: 0.2 ms and gate time: 0.2 ms. The deviation from the five measurements mainly comes from the signal/noise ratio of the machine. For dynamic light

scattering (DLS), an ALV/LSE-3 & ALV-5000/60XO Correlator by ALV was used to measure the correlation function of the scattered laser intensity at 90° from the beam path of a 630nm Laser. The correlation function was then fitted using a regularized fit and then linear number weighted to yield the mean peak position. This was repeated for 5 runs per sample. Wolfram Mathematica 11 software was used for data analysis, lifetime fitting, and plotting.

General procedure for the synthesis of Eu.D03AC18 (EuL, **4**)



Scheme S1. Synthesis of Eu.D03AC18

tri-*tert*-butyl 2,2',2''-(10-octadecyl-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (**2**)

4,7,10-tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-ium bromide **1** (514 mg, 1 mmol), 1-bromooctadecane (350 mg, 1.05 mmol) and K_2CO_3 (815 mg, 5.9 mmol) were stirred in acetonitrile at 60 °C overnight. After cooling to room temperature, the suspension was filtered, and the solvent was removed by evaporation. The residue was purified by flash silica gel column chromatography with $CH_2Cl_2/MeOH = 10:1$ as the eluent. Dragendorff stain was used for the TLC. **2** was obtained as a yellow solid (591 mg, 77% yield). 1H NMR (500 MHz, $CDCl_3$): δ 3.51 – 3.39 (m, 3H), 3.28 (s, 2H), 3.23 – 3.13 (m, 2H), 3.13 – 2.93 (m, 6H), 2.90 – 2.65 (m, 6H), 2.41– 2.21 (m, 5H), 1.69 – 1.63 (m, 2H), 1.48 – 1.40 (m, 26H), 1.29 – 1.11 (m, 29H), 0.87 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.89, 170.53, 170.05, 82.31, 81.98, 81.80, 57.10, 55.98, 54.68, 53.13, 52.96, 50.46, 47.93, 32.06, 29.85, 29.83, 29.81, 29.80, 29.77, 29.75, 29.67, 29.65, 29.50, 29.41, 28.33, 28.27, 28.10, 28.10, 27.97, 27.77, 27.09, 26.89, 23.42, 22.83, 14.26. HRMS (ESI⁺) m/z calcd for $C_{44}H_{86}N_4O_6$: 766.6547, found 767.67609 [M+H]⁺.

2,2',2''-(10-octadecyl-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**3**)

2 (519 mg, 0.77 mmol) and 2 ml of trifluoroacetic acid (TFA) were added to 20 ml of CH_2Cl_2 . The resultant was stirred at room temperature for 24 h. Maldi-TOP-MS showed the deprotection was completed. TFA and CH_2Cl_2 were removed by rotovap and then the resultant was redissolved with 1 ml of CH_2Cl_2 . It was added dropwise to 30 ml of diethyl ether and white flocculent sediment was formed. White solid was obtained after centrifugation (343 mg, 74% yield). 1H NMR (500 MHz, CD_3OD): δ 4.15 (s, 2H), 3.70 – 3.35 (m, 13H), 3.28 – 2.89 (m, 11H), 1.83 (s, 2H), 1.51 – 1.19 (m, 31H), 0.90 (t, $J = 6.8$ Hz, 3H). ^{13}C NMR (126 MHz, MeOD): δ 174.78, 168.85, 162.49, 119.07, 116.75, 56.19, 56.06, 53.46, 52.96, 51.13, 49.62, 49.45, 49.29, 33.07, 30.79, 30.75, 30.72, 30.64, 30.47, 30.37, 27.78, 24.69, 23.73, 14.43. HRMS (ESI⁺) m/z calcd for $C_{32}H_{62}N_4O_6$: 598.4669, found 599.47964 [M+H]⁺. RP-HPLC $t_R = 6.1$ min (11 min method 5% → 100% $CH_3CN + 0.1\%$ FA) found 599.4687 [M+H]⁺.

Europium(III) 2,2',2''-(10-octadecyl-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (EuL, **4**)

3 (100 mg, 0.17 mmol) and $EuCl_3 \cdot 6H_2O$ (80 mg, 0.22 mmol) were added to 5 ml of water. pH was adjusted to 6 with NaOH (1M). The resultant was stirred at room temperature for 48 h. pH was adjusted twice during the reaction. Water was removed with a freeze dryer. The residual $EuCl_3$ is water soluble and it will be removed during the dialysis procedure of the nanoparticle preparation. HRMS (ESI⁺) m/z calcd for $C_{32}H_{59}N_4O_6Eu$: 748.3647, found 749.37593 [M+H]⁺. RP-HPLC $t_R = 7.7$ min (11 min method 5% → 100% $CH_3CN + 0.1\%$ FA) found 749.3767 [M+H]⁺.

Supplementary Results

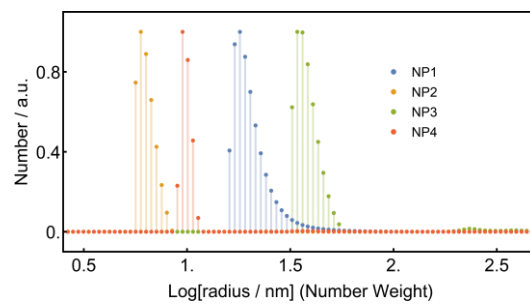


Fig. S1 DLS results for the size distribution for the EuL NPs.

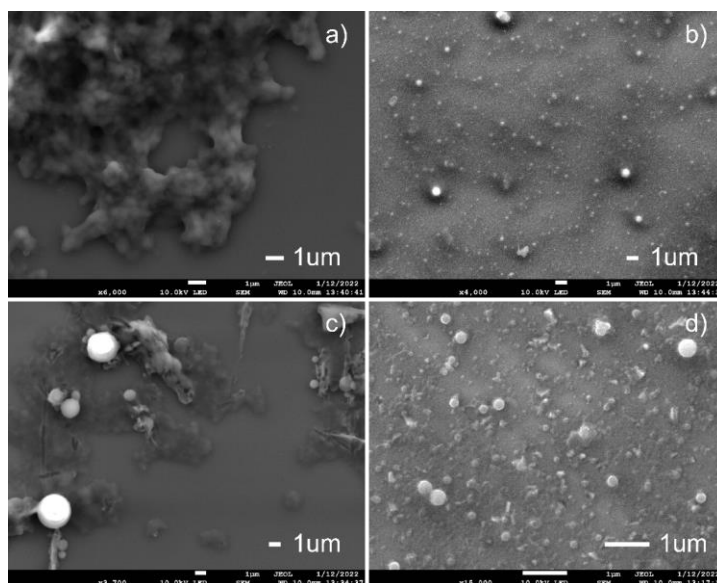


Fig. S2 SEM images of EuL a) NP1, b) NP2, c) NP3, d) NP4. The samples were deposited on cleaned polished side of the silicon wafers and left them dry before the measurements.

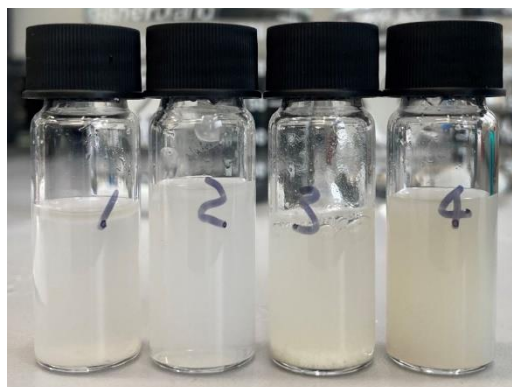


Fig. S3 Photo of EuL PS NP stock solutions in Milli-Q water 1) NP1, 2) NP2, 3) NP3, 4) NP4.

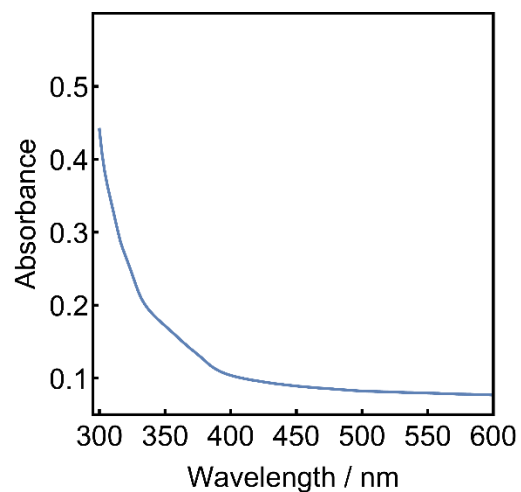


Fig. S4 UV-vis of NP4 (HEPES 20 mM, pH 7.40, 0.001% Triton-X100, 10 μ l of stock and 3 ml buffer).

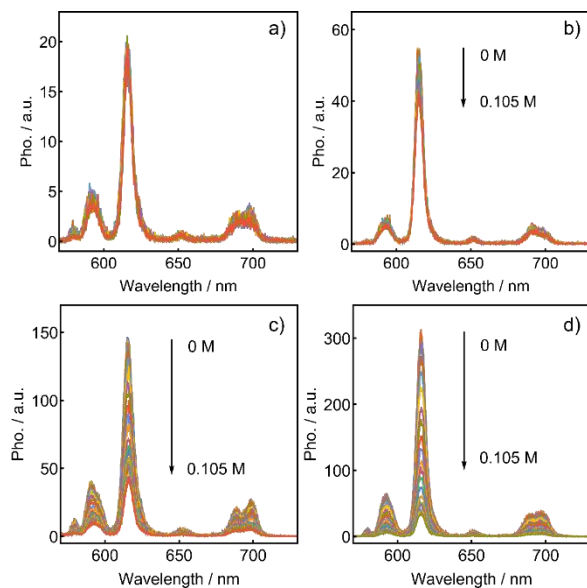


Fig. S5 Phosphorescence HCO_3^- response to different batches of NPs a) **NP1**, b) **NP2**, c) **NP3** and d) **NP4** (HEPES 20 mM, pH 7.40, 0.001% Triton-X100).

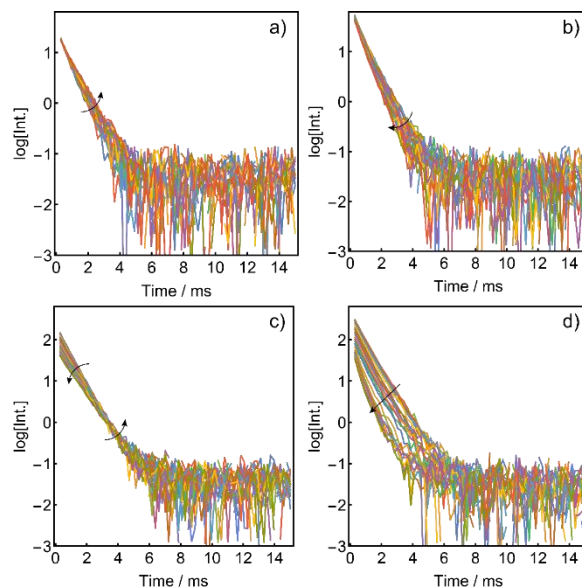


Fig. S6 Lifetime decay profiles of a) NP1, b) NP2, c) NP3, d) NP4 under different HCO_3^- concentrations.

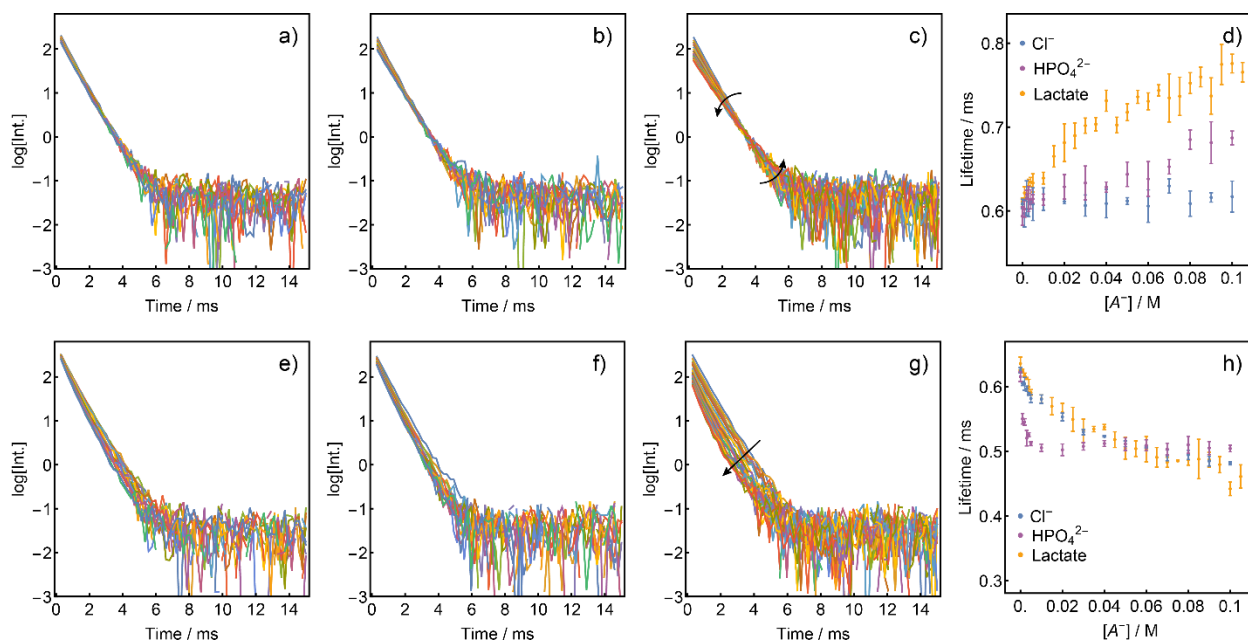


Fig. S7 Lifetime decay profiles of abcd) NP3, efg) NP4 with different interfering ions ae) Cl^- , bf) HPO_4^{2-} and cg) lactate under various concentrations. Titrations were conducted in HEPES buffer (20 mM, pH 7.40, 0.001% Triton-X100). Each data point in dh) is the average value of single exponential fitted results of three measurements for each concentration. Error bar: standard deviations from the triplicates.

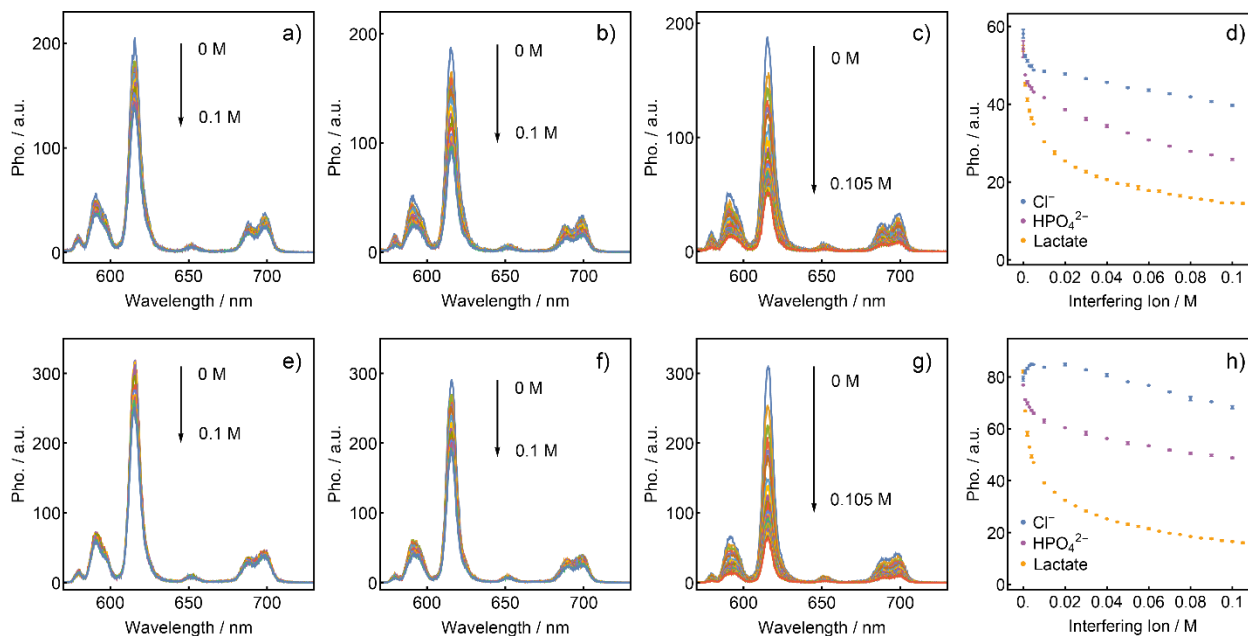
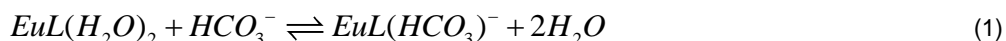


Fig. S8 Phosphorescence intensity response of abcd) **NP3**, efg) **NP4** to different interfering ions ae) Cl⁻, bf) HPO₄²⁻ and cg) lactate. Titrations were conducted in HEPES buffer (20 mM, pH 7.40, 0.001% Triton-X100). Each data point in dh) is the average value of three measurements and the intensity is calculated as the mean value of phosphorescence intensity from λ 605 to 638 nm. Error bar: standard deviations from the triplicates.

Theoretical Fitting

The bicarbonate association equilibrium is expressed with eq. 1



The binding constant β is expressed in eq. 2. Items in square brackets are the concentrations of species.

$$\beta = \frac{[EuL(HCO_3)^-]}{[EuL(H_2O)_2] \cdot [HCO_3^-]} \quad (2)$$

We assume the phosphorescence intensity I has a linear function to $[EuL(H_2O)_2]$.

$$I = a \cdot [EuL(H_2O)_2] + b \quad (3)$$

In eq. 4, EuL_{total} is the total concentration of the complex.

$$EuL_{total} = [EuL(H_2O)_2] + [EuL(HCO_3)^-] \quad (4)$$

The following eq.5 is derived from eq. 2, 3 and 4:

$$I = \frac{a \cdot EuL_{total}}{10^{(\log[HCO_3^-] + \log \beta)} + 1} + b \quad (5)$$

In the fitting progress, we induced one more factor k as a correction factor, which compensates for the deviation between experimental data and the ideal theory.

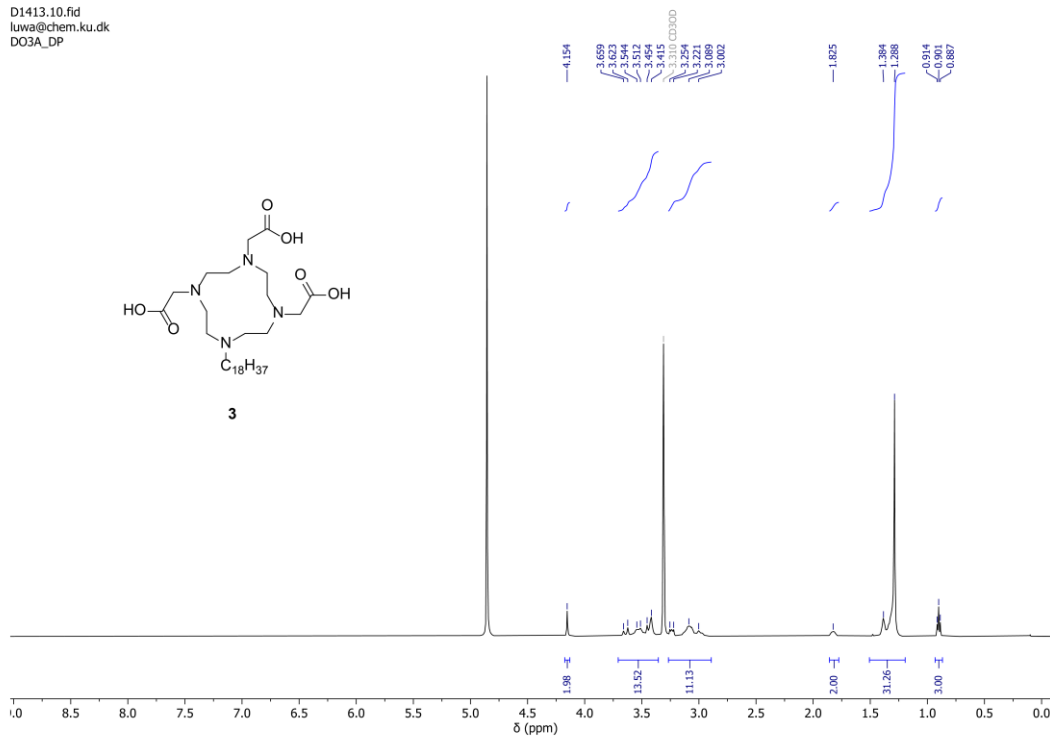
$$I = \frac{a \cdot EuL_{total}}{10^{k(\log[HCO_3^-] + \log \beta)} + 1} + b \quad (6)$$

HCO₃⁻ response (**Fig. 1cd**) was fitted with the eq. 6 and got logβ = 1.44 for NP3, and 1.46 for NP4. Corresponding results are summarized in Table S2.

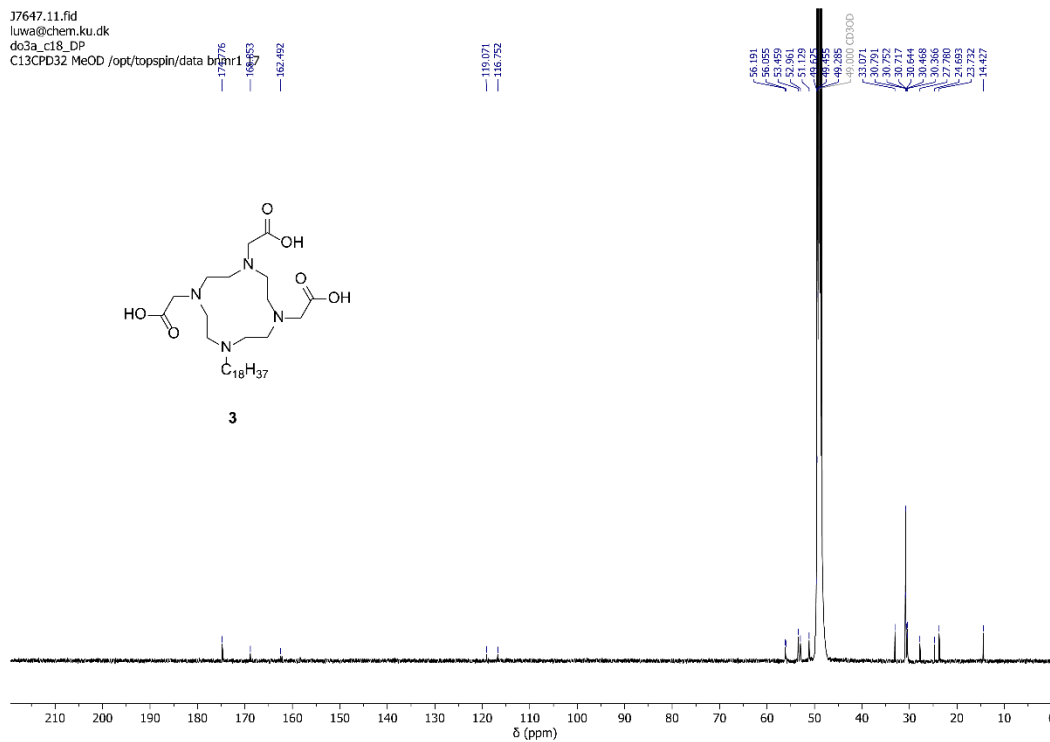
Table S2 Fitting results for **NP3** and **NP4** based on intensity signals and all four nanooptodes based on lifetime signals.

Intensity fitting	Function	R ²
NP3	$y = 6.3524 + \frac{34.1728}{1 + 10^{1.8607(1.4388+x)}}$	0.9997
NP4	$y = -3.0030 + \frac{81.2587}{1 + 10^{1.7772(1.4571+x)}}$	0.9995
Lifetime fitting		
NP1	$y = 0.5603 + \frac{0.1548}{1 + 10^{-3.7075(1.4205+x)}}$	0.9996
NP2	$y = 0.4773 + \frac{0.1156}{1 + 10^{2.2339(1.3985+x)}}$	0.9996
NP3	$y = 0.6035 + \frac{0.2313}{1 + 10^{-1.7322(1.2306+x)}}$	0.99992
NP4	$y = 0.3366 + \frac{0.2663}{1 + 10^{6.4464(1.2117+x)}}$	0.9985

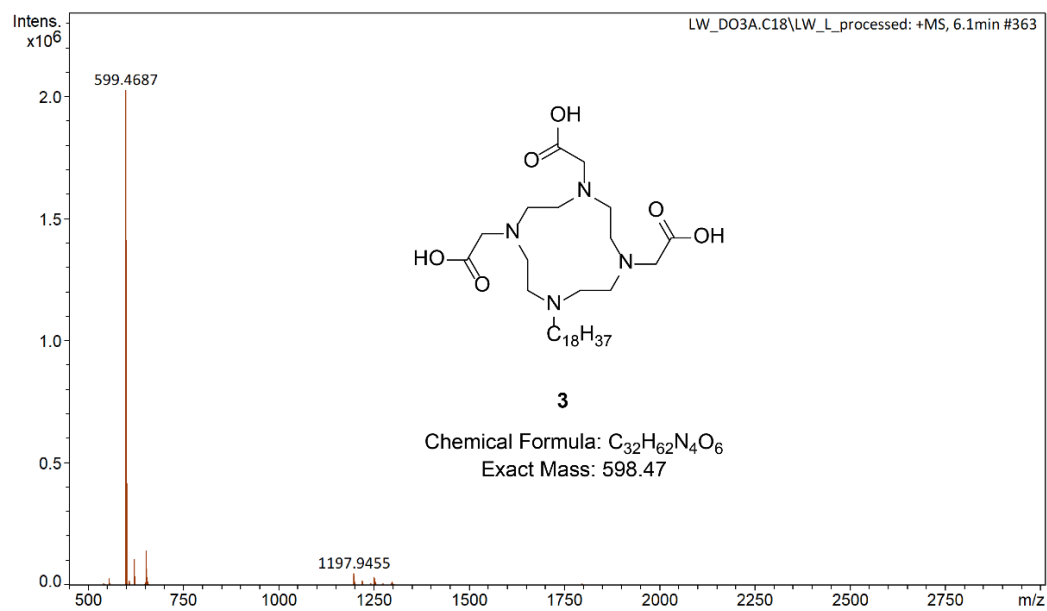
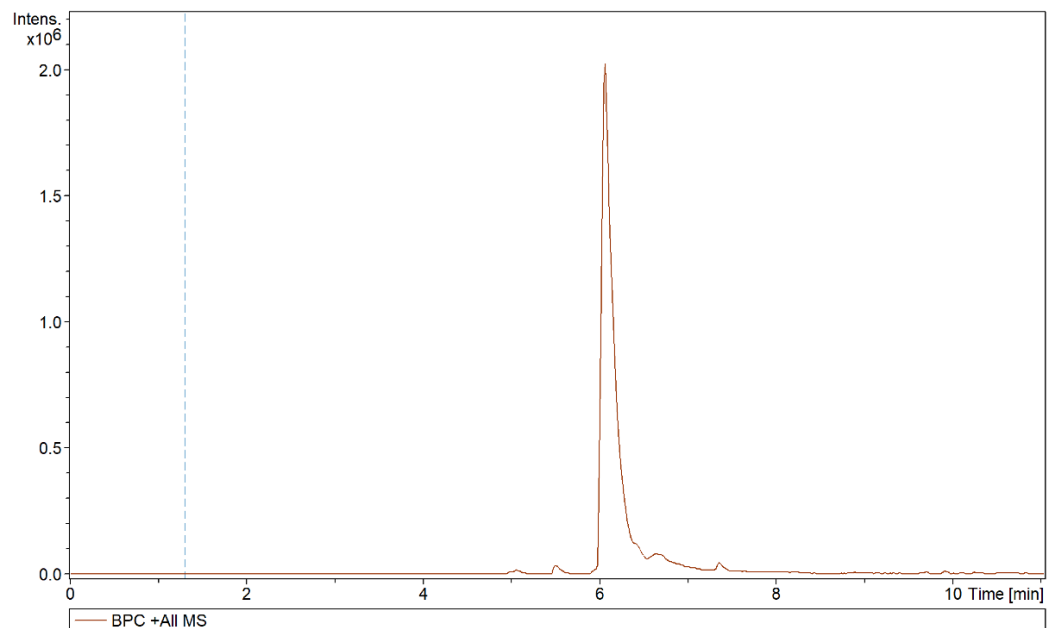
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luwa@chem.ku.dk
D03A_DP

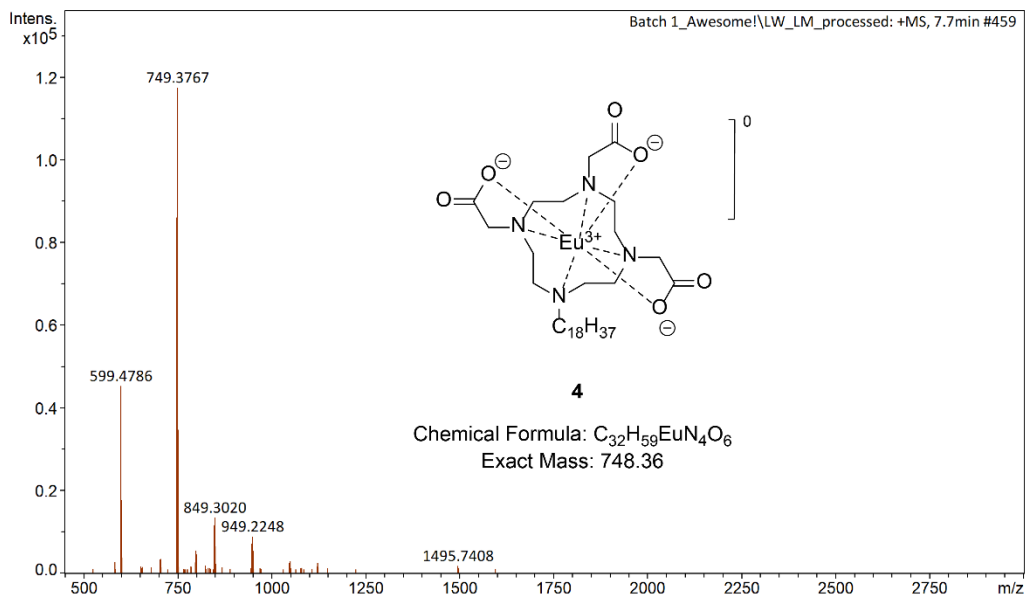
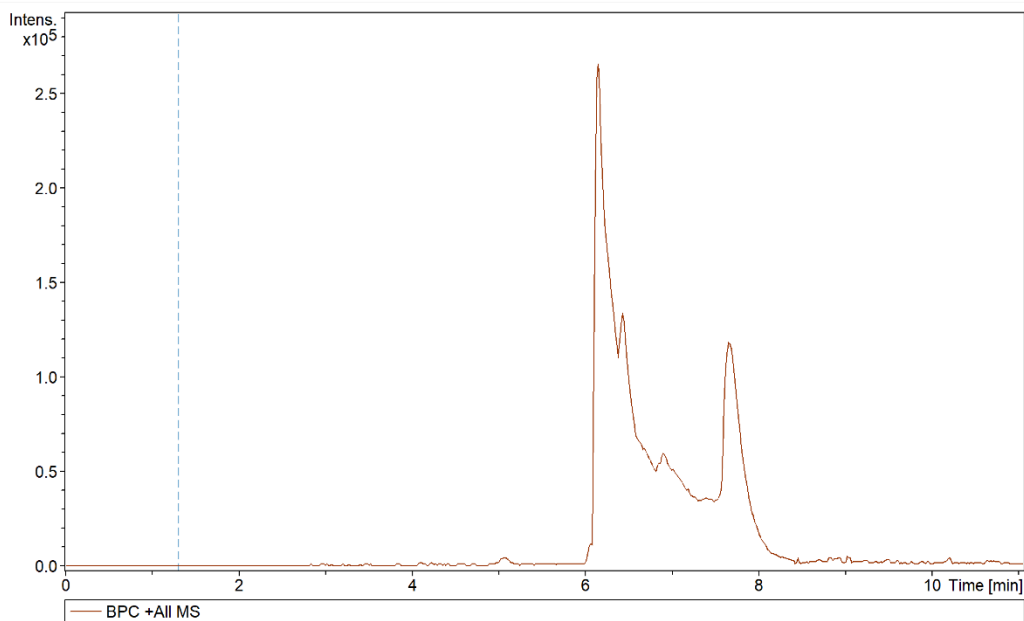


J7647.11.fid
luwa@chem.ku.dk
do3a_c18_DP
C13CPD32 MeOD /opt/topspin/data/bmr1



LC-MS





References

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