SUPPORTING INFORMATION

Confinement of a tris-aqua Gd(III) complex in silica nanoparticles leads to high stability, high relaxivity and supresses anion binding

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I. Materials and Instrumentation

Solvents and starting materials were purchased from Aldrich, Acros, Fluka and used without further purification. Tetraethoxysilane (TEOS, 99.999 %), TritonTM X-100, Hexanol (> 99 %), IgepalTM CO-520, Cyclohexane (> 99.5 %), Ammonium Hydroxide (28 - 30 %), ultrapure Nitric Acid (65%) and tert-Butan(ol-d) were purchased from Sigma-Aldrich and used without further purification. D_2O (99.9 % atom D) was obtained from Eurisotop. All water solutions were prepared from ultrapure laboratory grade water that has been filtered and purified by reverse osmosis using Millipore MilliQ reverse-osmosis cartridge system (resistivity 18 MΩcm). Lanthanide chloride salts were purchased from Aldrich. The precise metal ion content was determined by colorimetric titration in acetate buffer (pH = 4.5) using standardized H_2Na_2edta solution (Merck) and Xylenol orange indicator. 8-(benzyloxy)quinoline-2-carbaldehyde was prepared according to literature procedure.[1]

- Nuclear Magnetic Resonance (NMR)

^1H NMR spectra were recorded on Bruker Avance III 400 and 500 spectrometers. Chemical shifts are reported in ppm with the solvent as the internal reference.
The low frequency R_1 measurements between 5 kHz and 35 MHz were performed on a Stelar Spinmaster FFC-2000 NMR relaxometer (Stelar s.r.l., Mede PV, Italy).[2] The R_1 values at higher frequencies were measured on spectrometers at 20 (Bruker minispec), 50 (Bruker Minispec "mqvar" ND2318), 100 (Bruker Avance), 200 (Bruker A200) and 400 MHz (Bruker A400).

- Mass Spectrometry (ES-MS)

Mass spectra were acquired with a Finigan LXQ-linear ion trap (THERMO Scientific, San Jose, USA) equipped with an electrospray source.

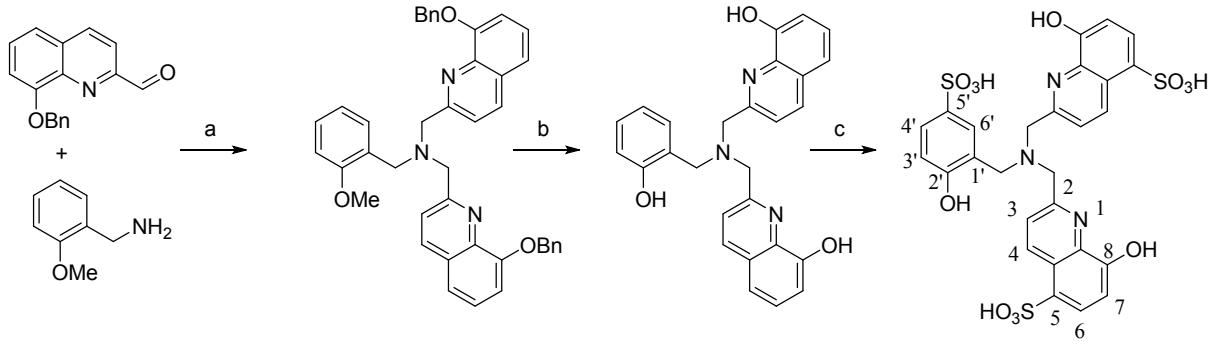
- Scanning Electron Microscopy (SEM)

The size and morphology of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$-doped silica nanoparticles ($[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@SiNPs) were measured by scanning electron microscopy (SEM Zeiss Ultra plus 55). A mother suspension of prepared nanoparticles (see synthesis hereafter) was diluted in 10 mL of ethanol and a drop of the resulting solution was deposited on an Ultra-thin carbon film supported by a lacey carbon film on a 400 mesh copper grid from Eloise. After evaporation of the

solvent, the particles were observed at an operating voltage of 15 kV with Inlens and STEM detectors.

II. Synthesis

- Synthesis of the ligand dhqN-SO_3



Scheme S1. Synthesis of dhqN-SO_3 – reagents and conditions: a) $NaBH(OAc)_3$, DCE, 71 % ; b) BBr_3, DCM, 35 % ; c) H_2SO_4/SO_3, 50 %

1-(8-(benzyloxy)quinolin-2-yl)-N-((8-(benzyloxy)quinolin-2-yl)methyl)-N-(2-methoxybenzyl)methanamine : To a solution of (2-methoxyphenyl)methanamine (479 mg, 3.49 mmol) and 8-(benzyloxy)quinoline-2-carbaldehyde (2.76 g, 10.5 mmol) in extra dry 1,2-dichloroethane (30mL) under argon, $NaBH(OAc)_3$ (6.33g, 21 mmol) was added. After stirring for 24 h at room temperature, the reaction was quenched by adding 1M $NaHCO_3$ aqueous solution (30mL), extracted with dichloromethane (2x50mL) and washed with brine (2x30mL). The organic layer was dried on Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by column chromatography on aluminium oxide 90 (Activity III, 0.063-0.200 mm, dichloromethane) to provide the desired product (1.56 g, 71 %) as a white solid.
^1H NMR ($CDCl_3$) δ (ppm): 7.95 (d, $J_{4,3}$ = 8.5 Hz, 2H, H4), 7.84 (d, $J_{3,4}$ = 8.5 Hz, 2H, H3), 7.61 (d, $J_{6'-5'}$ = 7.6 Hz, 1H, H6'), 7.44 (d, J = 6.6Hz, 4H, HBn), 7.29-7.16 (m, 10H, HBn, H5 and H6), 7.12 (m, 1H, H4'), 6.93 (dd, $J_{7,6}$ = 6.9Hz, $J_{7,5}$ =1.5 Hz, 2H, H7), 6.83 (m, 1H, H5'), 6.74 (d, $J_{3'-4'}$ = 8.2 Hz, 1H, H3'), 5.35 (s, 4H, CH_2-quinoline), 4.08 (s, 4H, CH_2-Bn), 3.80 (s, 2H, CH_2-phenol), 3.67 (s, 3H, OCH_3)
ES-MS**:** m/z (%): 632.4 $[M + H]^+$

2,2'-(2-hydroxybenzylazanediyl)bis(methylene)diquinolin-8-ol: To a solution of 1-(8-(benzyloxy)quinolin-2-yl)-N-((8-(benzyloxy)quinolin-2-yl)methyl)-N-(2-methoxybenzyl)methanamine (680 mg, 1.08 mmol) in extra dry dichloromethane (20mL) under argon at 0°C, 1M solution of BBr_3 in dichloromethane (20mL, 19.4 mmol) was added dropwise. After stirring overnight at room temperature, MeOH (20mL) was added dropwise and the solution was concentrated under vacuum. This operation was repeated four times until bromobenzyl is totally removed. Then the resulting residue was dissolved in a minimum amount of methanol (2mL) and precipitated by addition of diethyl ether (50mL). The yellow powder

formed was collected by filtration and washed with diethyl ether (2x10mL). The crude product was then purified by column chromatography on RP18 silica gel (m, Water/Acetonitrile/TFA 70:30:0,01) to provide the desired product (471 mg, 35 %) as a yellow solid.
^{1}H NMR (CD_3OD) δ (ppm): 8.85 (d, $J_{3,4}$ = 8.6 Hz, 2H, H4), 8.13 (d, $J_{4,3}$ = 8.6 Hz, 2H, H3), 7.74 (dd, $J_{6,5}$ = 8.2, $J_{6,7}$ =7.2 Hz, 2H, H6), 7.66 (dd, $J_{5,6}$ = 8.2, $J_{5,7}$=1.5 Hz, 2H, H5), 7.44 (dd, $J_{7,6}$ = 7.2, $J_{7,5}$=1.5 Hz, 2H, H7), 7.29 (dd, $J_{6',5'}$ = 7.5 Hz, $J_{6',4'}$ =1.5 Hz, 1H, H6'), 6.90 (m, 1H, H4'), 6.67 (m, 1H, H5'), 6.50 (dd, $J_{3',4'}$ = 8.0, $J_{3',5'}$ =0.8 Hz, 1H, H3'), 4.82 (s, 4H, CH_2-quinoline), 4.23 (s, 2H, CH_2-phenol).
ES-MS**:** m/z (%): 438.4 $[M + H]^+$

dhqN-SO_3: 2,2'-(2-hydroxy-5-sulfonatobenzylazanediyl)bis(methylene)bis(8-hydroxy-quinoline-5-sulfonate): 2,2'-(2-hydroxybenzylazanediyl)bis(methylene)diquinolin-8-ol (80 mg, 0.183 mmol) was dissolved in the minimum amount of oleum (2mL) and stirred for 30 minutes at room temperature. The reaction was quenched by adding ice and concentrated under reduced pressure. The resulting residue was dissolved in ethanol (2mL) and precipitated by addition of diethyl ether (50mL). The yellow powder formed was collected by filtration and washed with diethyl ether (2x10mL) to give the desired compound (62 mg, 33 %) as a yellow solid.
^{1}H NMR (D_2O) δ (ppm): 8.89 (d, $J_{4,3}$ = 8.8 Hz, 2H, H4), 7.76 (d, $J_{6,7}$ = 8.2 Hz, 2H, H6), 7.54 (d, $J_{3,4}$ = 8.8 Hz, 2H, H3), 7.39 (s, 1H, H6'), 6.98 (d, $J_{3',4'}$ = 8.4 Hz, 1H, H3'), 6.86 (d, $J_{7,6}$ = 8.2 Hz, 2H, H7), 6.00 (d, $J_{4',3'}$ = 8.4 Hz, 1H, H4') 4.85 (s, 4H, CH_2-quinoline), 4.52 (s, 2H, CH_2-phenol)
Elemental Anal. (%) Calcd. for dhqN$SO_3 \cdot 3H_2O$, $3H_2SO_4 \cdot$(MW = 1025,96) : C 31.61, H 3.44, N 4.10, found C 31.54, H 3.62, N 4.01

Melting point : 284°C

- Synthesis of the complexes

Solutions (5-10·10^{-3} M) of lanthanides complexes $[Ln(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ (Ln = La, Lu) for NMR measurements were prepared in situ by dissolving equimolar amounts of the dhqNSO_3 ligand and of the corresponding lanthanide chloride salt $LnCl_3$ into D_2O followed by adjustment of the pD with solutions of KOD in D_2O (1 M and 0.1 M).
2 to 5·10^{-3} M solutions of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ were prepared in situ by mixing the appropriate amounts of dhqNSO_3 ligand and $GdCl_3.6H_2O$ (99.99%; Aldrich) in mQ water followed by adjustment of the pH with NaOH solution in water (1 M and 0.1 M). Gd(III) stock solutions were prepared by dissolving the appropriate amounts of $GdCl_3.6H_2O$ in water.
All solutions were checked with the xylenol orange test to verify the absence of free gadolinium. Similar conditions of concentration and pH have been used for the relaxivity studies. 10·10^{-3} M solutions of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ at pH=7.4 have been used for SiNPs synthesis.

- Synthesis of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs

A ternary microemulsion procedure was used, adapted from a previously reported method.[3, 4] 1.3 mL of Igepal CO-520 was stirred with 10 mL of cyclohexane during 10 min to produce a homogeneous system. Then, 480 µL of the doping aqueous solution (320 µL of water and 160 µL of a 10 mM aqueous solution of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$) were added, followed by 100 µL of NH_4OH (28 - 30 % by weight). The resulting mixture was stirred for 30 min and finally 100 µL

of TEOS were added. The reactive medium was left at room temperature for 24 h under gentle magnetic stirring. The resulting microemulsion was disrupted by adding 30 mL of acetone and the formed silica NP washed three times with ethanol and three times with water. After the addition of acetone and each washing, the suspension of NPs was centrifuged at 10,000 r/min during 15 min and the supernatant was carefully removed. Finally, the wet residue of NPs (about 150 mg) was dispersed in 200 µL of H_2O to yield about 320 µL of a metastable (that tend to aggregate in water after a week) mother suspension of NPs suitable for relaxivity measurements. Spontaneous aggregation does not increase in serum.

III. Characterization Methods

- NMR Samples

Capillary NMR tubes. 55 µL of a suspension of NPs in H_2O were put in a sealed Wilmad capillary tube (1.7 mm OD, 1.5 mm ID, 110 mm in length). Then, the capillary was placed in a 5 mm NMR tube as holder. To lock the external magnetic field, a second capillary filled with an auxiliary D_2O solution (3mM) of the $[Tb(ttha)]^{3-}$ complex (ttha = triethylene tetraamine hexaacetate) was juxtaposed in the holder tube, parallel to the first capillary containing the investigated solution. The $[Tb(ttha)]^{3-}$ complex was used to induce a significant paramagnetic susceptibility shift of the resonance frequencies of the deuterium nuclei and residual HOD protons.[5] Then, the spectrometer field could be locked to the deuterium frequency of the auxiliary solution, the residual HOD protons giving a signal well separated from that of the H_2O protons in the mother suspension of NPs. The relaxation times T_1, T_2 and $T_{1\rho}$ of the H_2O protons in the presence of NPs could then be measured easily.[6]

5 mm NMR tube. A volume of 100 µL of the mother suspension of freshly prepared NPs in H_2O was diluted with an equal volume of with H_2O (100 µL) in order to get a large proton NMR signal at the resonance frequencies $\nu_I \leq 50$ MHz and then transferred into a 5 mm NMR tube.

- NMR Sequences

On the conventional spectrometers, the relaxation times T_1 of the H_2O protons in the capillaries were determined with the sequences of inversion recovery.[6] The longitudinal relaxation times T_1 were measured in the 5 mm NMR tubes from 5 kHz to 35 MHz on a commercial Stelar relaxometer by using the prepolarized (PP) and non-polarized (NP) sequences below and above about $\cong$ 12 MHz, respectively.[2]

- Concentration of Complex in Solution

From bulk NMR susceptibility shift. The paramagnetic Ln(III) ions in the mother suspension induce a susceptibility shift of the H_2O proton resonance frequency which is proportional to the concentration of the ions.[7-9] This shift was readily obtained as the difference of the measured frequencies in capillaries containing $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs and $[Y(dhqNSO_3))(H_2O)_3]^{3-}$@Si25NPs.

The rotational correlation time of which can be safely estimated from the standard Stokes-Einstein diffusion law

- Number of Complexes per Nanoparticle

The mean number of complexes per particle (n_{cplx}) in a suspension was derived from its volume V, the mass of silica of the suspended NPs, and the Gd(III) concentration. The mass of silica was obtained by lyophilizing the suspension for 24 h to afford dry silica of density 1.95 $g \cdot cm^{-3}$. 15 mg of dry NPs were obtained by lyophilizing 0.5mL of a NP solution with Gd(III) concentration of 0.18 mM, leading to $n_{cplx} \cong 60$.

IV. NMR of Lanthanide Complexes

The ^1H NMR spectra at 298 K of the Lu(III) and La(III) complexes show the presence of broad overlapping signals that coalesce at 343 K to afford a single set of 9 signals. This behavior can be interpreted in terms of a fast exchange at room temperature between the two possible helical conformations (Λ and Δ) of the tripodal complexes. The presence of such exchange is confirmed by the ^1H NMR spectra at 278 K that shows the 17 signals, expected for a rigid helical structure, despite some overlap.

$[Lu(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$

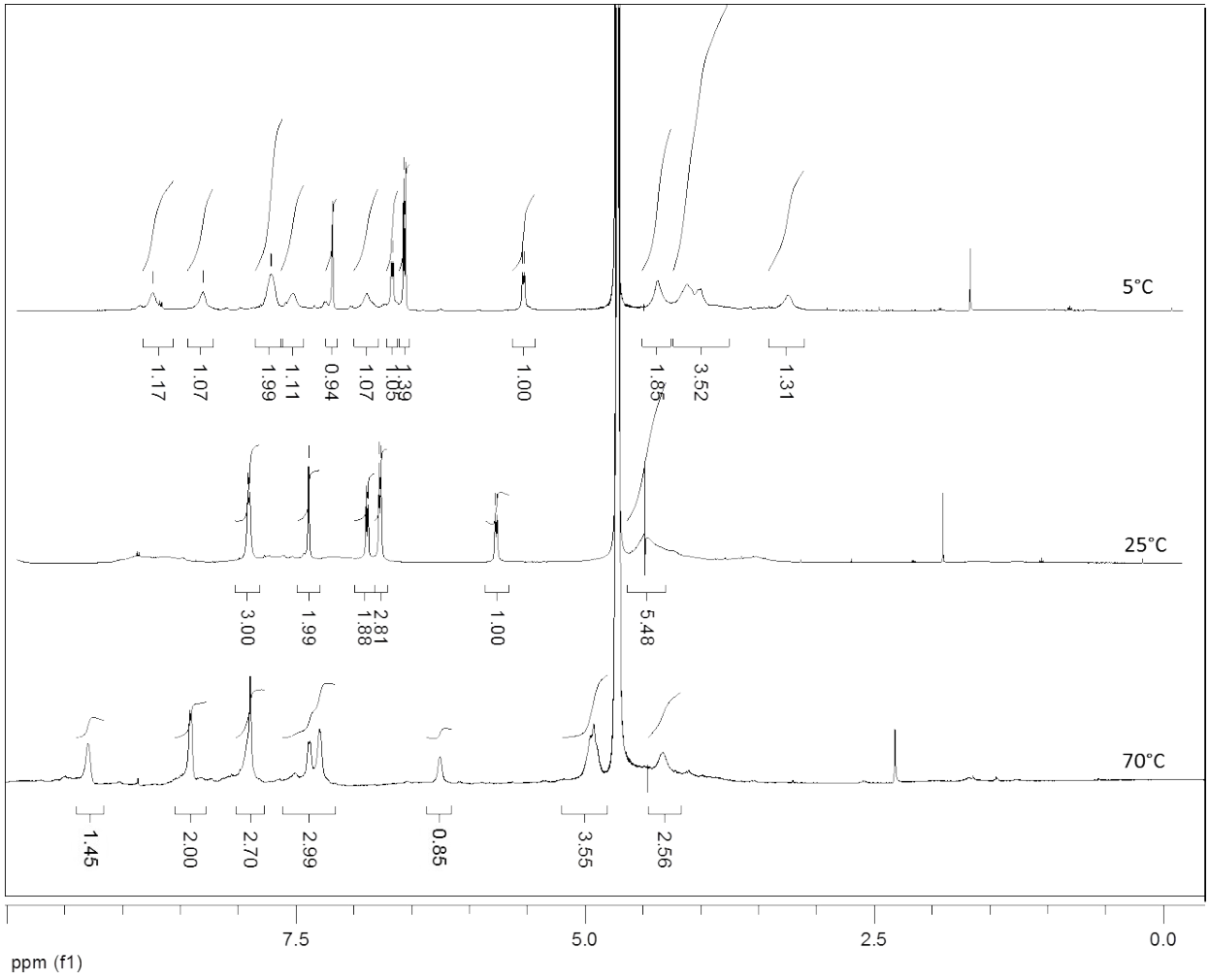


Figure S2. ^{1}H 500 MHz NMR spectra of $[Lu(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ at various temperature in D_2O at pD = 7.4

$[Lu(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ ^{1}H NMR (D_2O) δ (ppm) 70°C : 9.31(2H, H4), 8.43 (2H, H6), 7.93 (1H, H6'), 7.89 (2H, H3), 7.40 (1H, H3'), 7.29 (2H, H7), 6.27 (1H, H4'), 4.96 (4H, CH_2-N quinoline), 4.30 (2H, CH_2-N phénol)

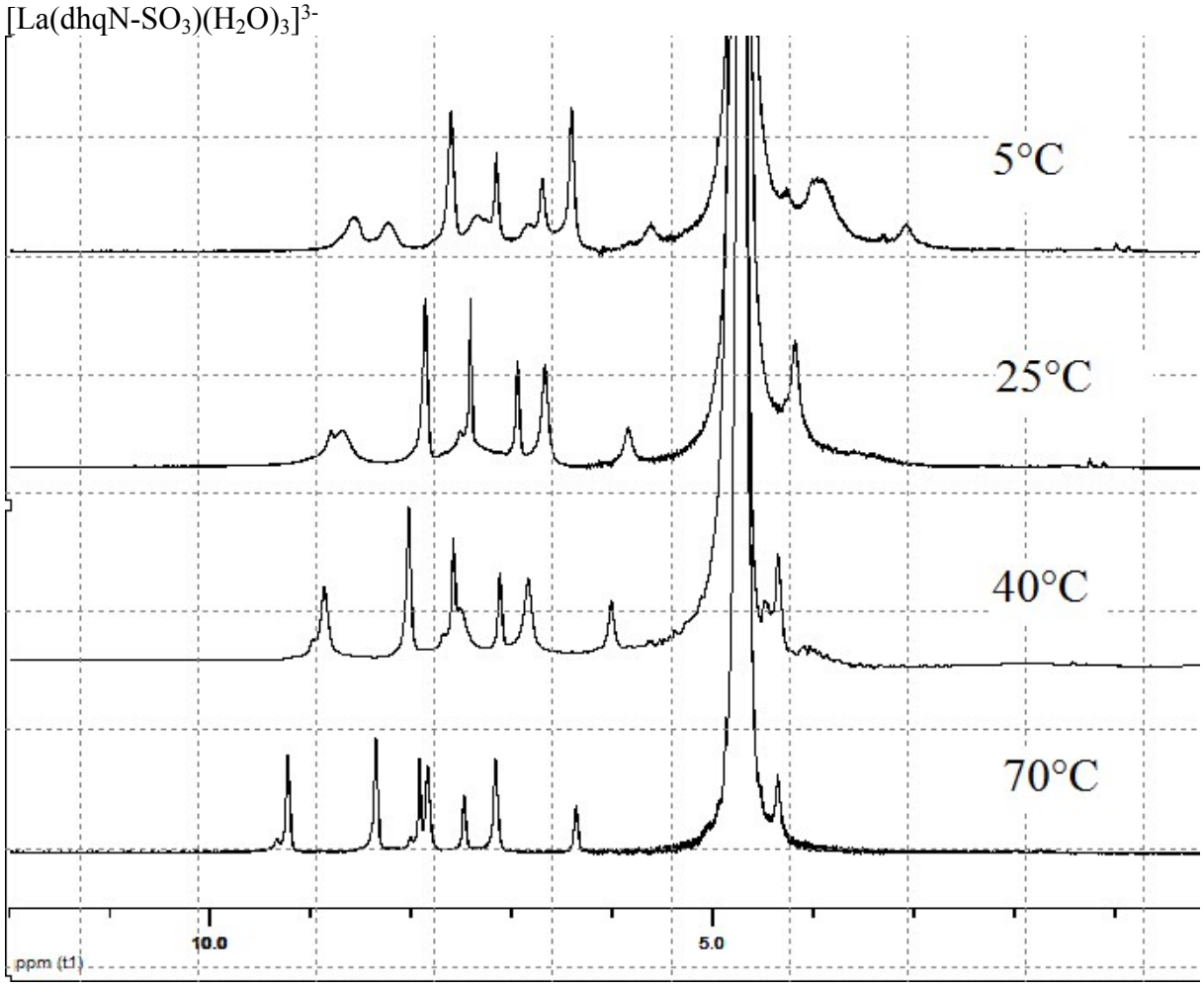


Figure S3. ^{1}H 500 MHz NMR spectra of $[La(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ at various temperature in D_2O at pD = 7.4

$[La(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ ^{1}H NMR (D_2O) δ (ppm) 70°C : 9.23(2H, H4), 8.35 (2H, H6), 7.92 (1H, H6'), 7.84(2H, H3), 7.48 (1H, H3'), 7.16(2H, H7), 6.36 (1H, H4'), 4.36 (2H, CH_2-N phénol)

V. Determination of pGd and pZn via spectrophotometric competition batch

- $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$

Gd(III) displacement from $[Ln(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ by the benchmark ligand DTPA (diethylenetriaminepentaacetate pGd = 19.6) was monitored by spectrophotometry. Batch titration techniques were employed. Various aliquots of DTPA stock solution (a range of molar ratios of 1: 0.01 up to 1:100 for dhqN-SO_3:DTPA was used) were added to $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ solution. The pH was adjusted to pH=7.4 by using TRIS buffer (0.1M). This method allows a precise measurement of the pGd ($pM = -\log[M]_{free}$ with $[M]_t=10^{-6}$ M $[L]_t=10^{-5}$ M at pH=7.4)[10]

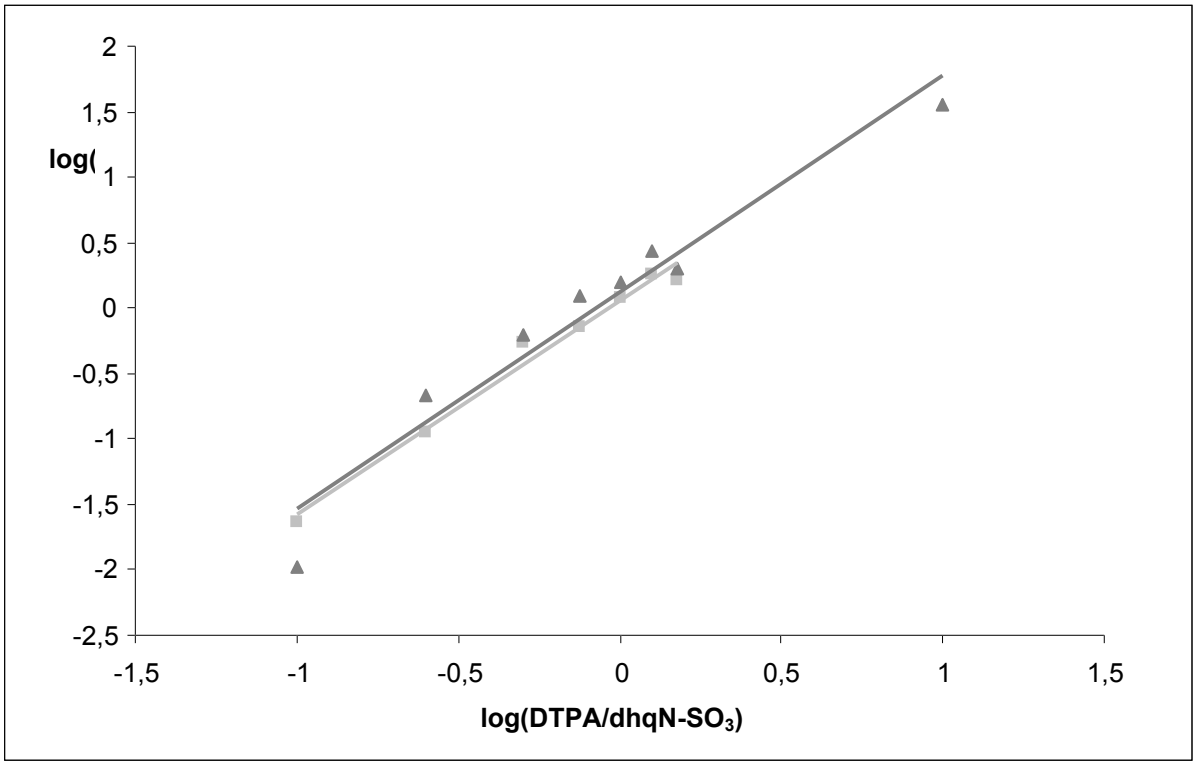


Figure S4. Competition titration log/log plots against DTPA

The plot directly gives the difference in pM between dhqN-SO_3 and DTPA (log([DTPA]/[L] when log([Gd(DTPA)]/[GdL]=0.)

$\Delta pM = -0.2$
pGd= 19.1-0.2 = 18.9

The stability of the complex is very high for a q=3 compound and is comparable to the stability of the mono aquo complex $[Gd(DTPA)(H_2O)]^{2-}$ (pGd= 19.1)[8].

- $[Zn(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$

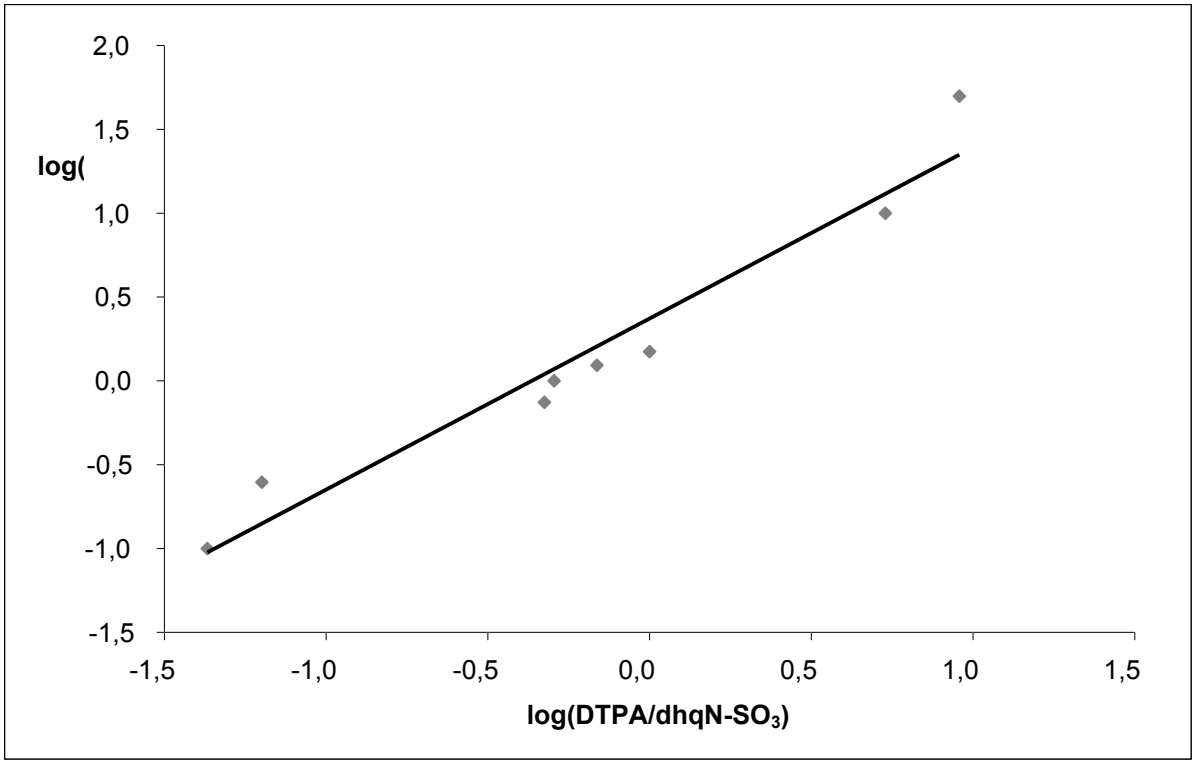


Figure S5. Competition titration log/log plots against DTPA

This method allows a precise measurement of the pZn The plot directly give the difference in pM between the dhqN-SO_3 ligand and DTPA (log([DTPA]/[L]) when log([Gd(DTPA)]/[GdL]=0.)
at pH 7.4
ΔpM = -0.4
pGd= 15.4-0.4 = 15.0

VI. Relaxivity measurements

For the measurements of the relaxivity of the complex $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ in the presence of BSA, a solution was prepared by mixing a 2mM solution of the complex at pH~7.4 in water, with a stock solution of BSA 10% m/v in H_2O at pH~7.4 (BSA= 4.5% m/v so $0.68 \cdot 10^{-3}$ M).

For the measurement in the serum, a solution has been prepared by mixing appropriate volumes of a 2mM solution of the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex or a 0.5mM solution of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs, at pH~7.4 in water in fetal bovine serum (ligand~$0.2 \cdot 10^{-3}$ M).

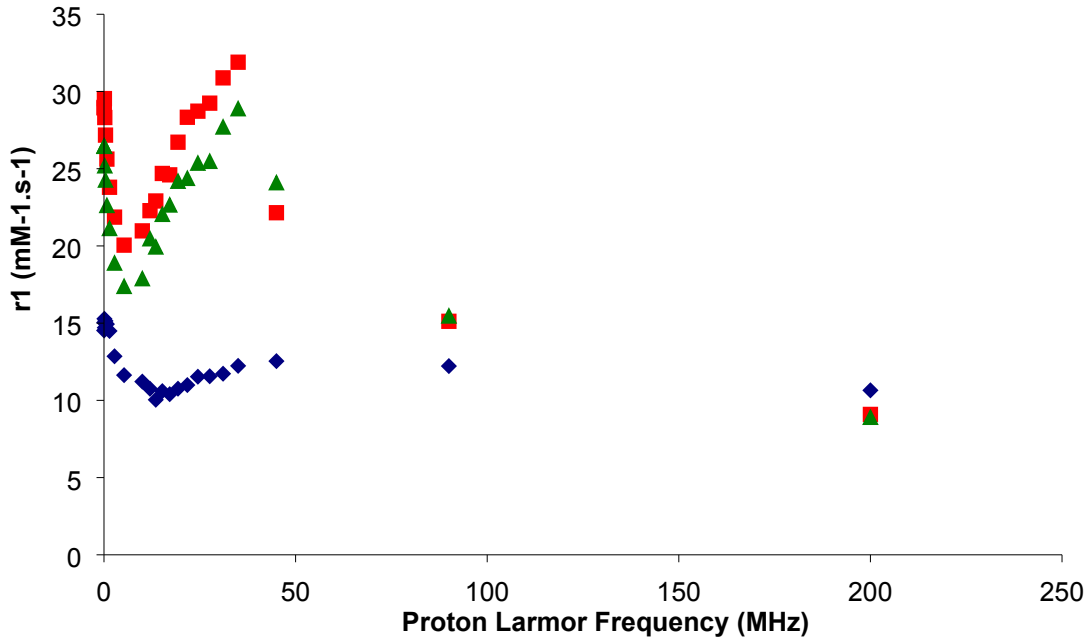


Figure S6. NMRD profiles of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ at 25°C in water at pH=7.4 (♦), BSA (■), Serum (▲)

The NMRD profile of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ in water shows that the r_1 of the complex at 20MHz and 200MHz is 10.65 $mM^{-1}.s^{-1}$. This value is in agreement with a q=3 complex.

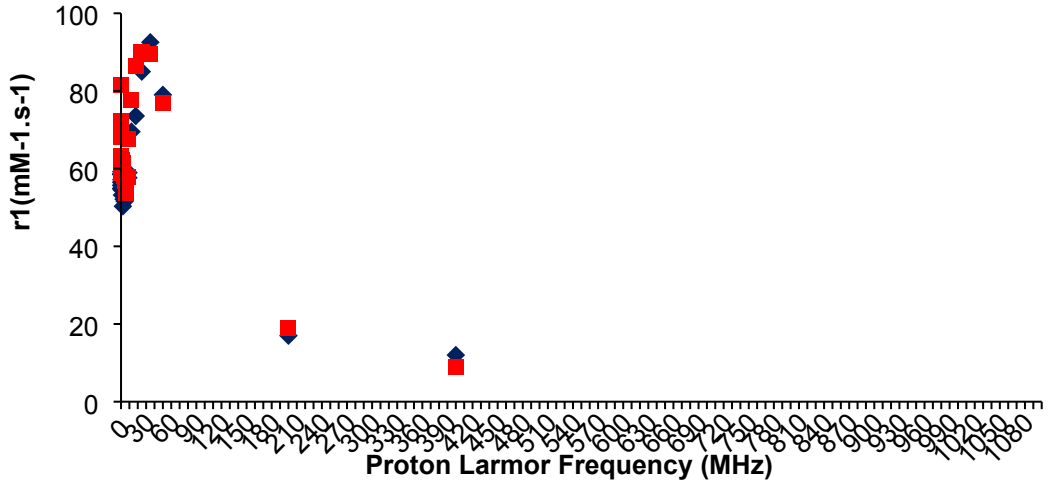


Figure S7. NMRD profiles of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs at 25°C(♦) and 37°C(■) ; C=0.4mM pH =7.6.

In order to study the replacement of coordinated water by anions, solutions have been prepared by mixing appropriate volumes of a stock solution containing the complex, either a 2mM solution of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ or a 0.5mM solution of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs, at pH~7.4 and of a 50mM stock solution of the anions.

r_1 ($mM^{-1}.s^{-1}$) 25°C	0.8 T	1.2 T	2.3T	4.7 T	9.3T
$[Gd(DTPA)(H_2O)]^{2-}$	4.39±0.06	4.26±0.06	4.16±0.06	4.06±0.06	3.62 ±0.06
$[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$	12.23±0.08	12.54±0.08	12.22±0.08	10.65±0.08	8.54±0.07
Si25NP	90±5	78±5	41±4	18±2	9±1

Table S8. r_1 ($mM^{-1}s^{-1}$) values of the $[Gd(DTPA)(H_2O)]^{2-}$ and $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complexes, free or embedded in 25 nm NPs in water at pH = 7.4 and 25°C

	$[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$	$[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs
H_2O	10.65±0.08	18±2
Serum	8.9±0.07	15±2
Carbonates (1eq)	9.53±0.07	
Carbonates (50 eq)	7.02±0.07	17±2

Table S9. r_1 ($mM^{-1}s^{-1}$) values of $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ and $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ @Si25NPs at pH=7.4, 25°C and 200MHz with various anions

VII. Potentiometric Titrations

Ligand protonation constants of dhqN-SO_3 ligand were determined by potentiometric titrations. For that purpose, 10 mL solutions of dhqNSO_3 (10^{-3} M) acidified to pH~2.5 were titrated in a thermostated cell (25.0°C +/- 0.1°C) under a stream of argon with the help of a 0.1 M KOH solution added by means of a 5 mL piston burette (Metrohm). The ionic strength μ = 0.1 M was fixed with KCl. Titrations were carried out with a Metrohm 751 GPD Titrino potentiometer equipped with a combined pH glass electrode (Metrohm). Calibration of the electrode system was performed prior to each measurement. The electromotrive force is given by $E = E° + s$ pH and both $E°$ and s were determined by titrating a known amount of HCl by 0.1 M KOH at μ = 0.1 M (KCl), using the acid range of the titration. The value used for the ion product of water is log K_w = 13.77.[11] More than 150 data points were collected for each experiment.
The data were mathematically treated by the program HYPERQUAD2000.[12] All values and errors represent the average of at least two independent experiments.

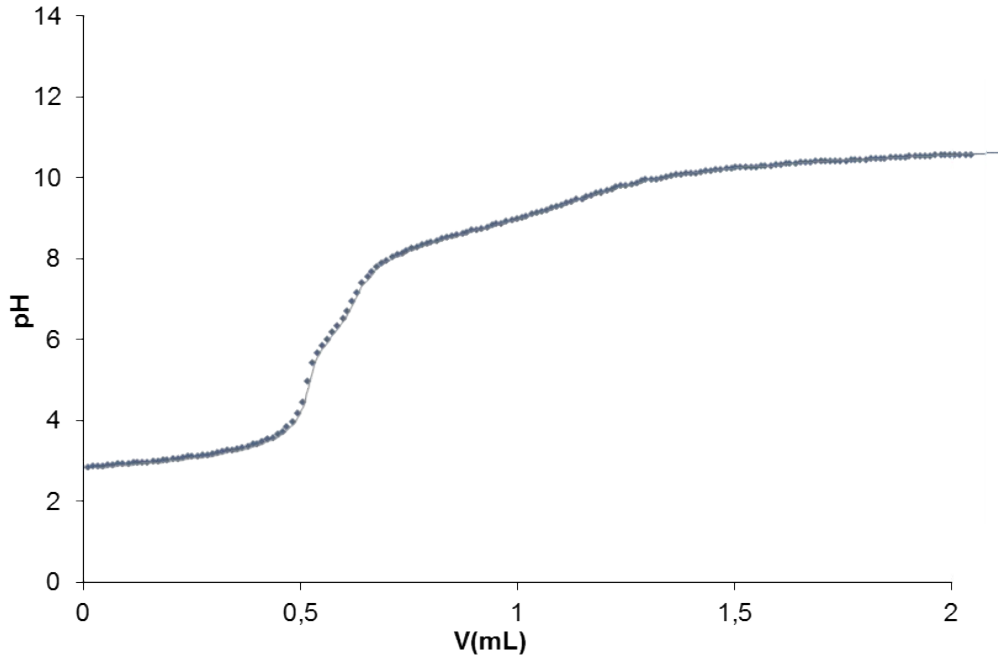


Figure S10 Potentiometric titration curve for dhqN-SO_3, NaOH 1mM added. T = 25 °C, I =0.1 M (KCl).

The pKa values of the six protonation sites have been determined by potentiometric measurements. They are respectively 3.77 (pKa1+2) for the pyridine nitrogens, 6.17 for the central nitrogen, 8.57 and 9.43 for the hydroxyl groups of the quinoline and 9.70 for the phenol.

VIII. Transmetallation

The transmetallation process in the presence of Zn^{2+} was monitored for both [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$ and [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$@Si25NPs in phosphate buffer by relaxometry [13]. The [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$ was reacted with one equivalent of $ZnCl_2$ in phosphate buffer. Relaxation times were measured at 50 MHz and 25°C at different times. The figure S11 shows the evolution of the ratio $R_1(t)/R_1(t=0)$.

The kinetic index defined as the time required to reach $R_1(t)/R_1(t=0)=0.80$ is 10 min for our compound. We conclude that the [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$ complex is not resistent to transmetallation.

The [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$@Si25NPs was also reacted with one equivalent of $ZnCl_2$ in phosphate buffer. Relaxation times were measured at 50 MHz and 25°C at different times. The following figure shows the evolution of the ratio $R_1(t)/R_1(t=0)$ for the [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$@Si25NPs and for the the [Gd(dhqN-SO_3)$(H_2O)_3]^{3-}$@Si25NPs reacted with $ZnCl_2$.

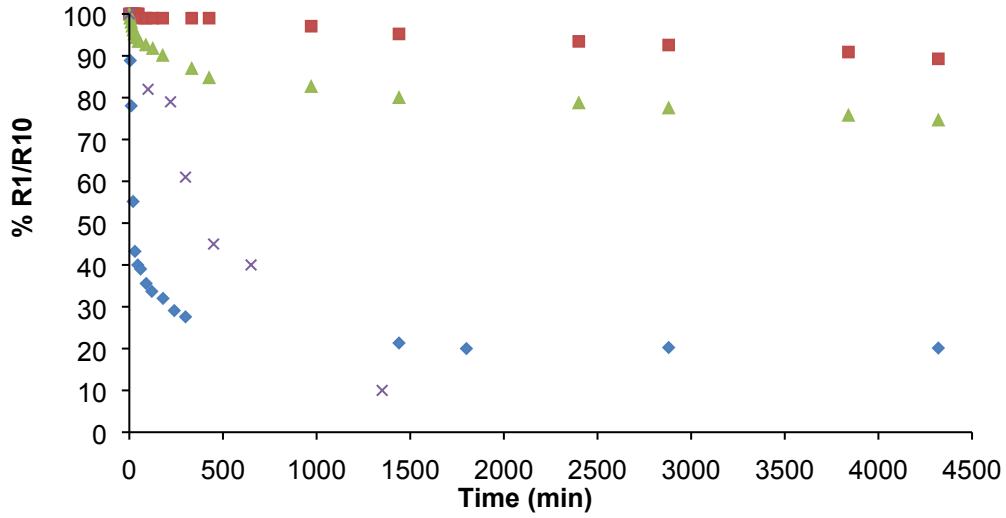


Figure S11. Time evolution of the ratio R_1/R_{10} of the relaxation rates $R_1 = 1/T_1$ at time t and $R_{10} = 1/T_{10}$ at time 0 for the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ (♦) and [Gd(DTPA-BMA)] (x) complexes in the presence of one equivalent of $ZnCl_2$ and of the embedded complexes $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs in the absence (■) and presence (▲) of one equivalent of $ZnCl_2$. This transmetallation test is carried out in phosphate buffer (pH=7.4) at 50 MHz, 25°C.

We observe that the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex is more stable in regard to transmetallation when incorporated in the silica nanoparticles. The kinetic index defined as the time required to reach $R_1(t)/R_1(t=0)=0.80$ is only 10 min for the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$ complex, but exceeds 2,000 min for the embedded complexes $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs. The decrease of relaxation rate observed after one day could be due to the aggregation of particles since the same decrease is observed for the $[Gd(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs in the absence of zinc.

IX. Lifetime measurement

Phosphorescence lifetimes were measured in time-resolved mode on a Fluorolog FL 3-2iHr spectrometer from Horiba-Jobin Yvon-Spex equipped with a double grating excitation monochromator, a iHR320 imaging spectrometer and a Hamamatsu R928P photomultiplier.
Quartz cells with an optical path of 1 cm were used in a FL-1057 housing including an elliptical mirror and a focusing lens.

Selective excitation of $Eu(^5D_0)$ level was accomplished by a SpectraLED source S-390 (FWHM 15 nm) from *Horiba Scientific* and coupled to a *Jobin Yvon* NL-C2 Pulse Diode controller and a DH-HT TCSPC controller including a SpectraLED output. The output signal of the photomultiplier was fed to a PC and controlled and analyzed with the Data Station (v2.7) and Decay Analysis (v6.8) softwares from *Horiba Scientific*. Lifetimes are averages of 3 independent determinations with a calculated Chi-square < 2. The emission lifetimes of the $Eu(^5D_0)$ excited level have been measured in D_2O and H_2O and were used to calculate the number of co-ordinated water molecules q by using the equation $q = 1.05(\Delta k_{obs})$ established[14] using crystalline complexes where $k_{obs} = 1/\tau_{obs}$, $\Delta k_{obs} = k_{obs}(H_2O) - k_{obs}(D_2O)$.

By using this equation, we obtain q = 3.1 and 3.0 for the free and embedded complexes, respectively, with an estimated uncertainty in q of ± 0.5.

Table 1. Lifetimes (ms) measured at 298K with λ_{ex} = 390 nm in H_2O and D_2O solutions.

		τ_{obs}	q
$[Eu(dhqN\text{-}SO_3)(H_2O)_3]^3$	H_2O	1	3.1(5)
	D_2O	1.34(2)	
$[Eu(dhqN\text{-}SO_3)(H_2O)_3]^{3-}$@Si25NPs	H_2O	0.30(1)	3.0(5)
	D_2O	2.14(8)	

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