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Investigation of the supramolecular assembly of luminescent lanthanide nanoparticles surface functionalized by *p*-Sulfonato-Calix[4]arenes with charged aromatic compounds

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In the memory of Pr Guy Solladié, Pr of organic chemistry and former director of the ECPM.

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Abstract: The surface coordination of *p*-sulfonato- (C[4]), *p*-sulfonatothia- (SC[4]) and *p*-sulfonato-sulfoxocalix[4]arene (SO₂C[4]) on La_{0.9}Tb_{0.1}F₃ nanoparticles (TbNP) was investigated by UV-Vis absorption and luminescence spectroscopies. SO₂C[4] displayed the strongest affinity towards the NPs and a significant antenna effect leading to observation of the characteristic Tb emission bands upon calixarene excitation. NPs decorated by a shell of SO₂C[4] were prepared and the interaction with rhodamine 6G (R6G) was studied. The progressive addition of R6G resulted in a strong Tb to R6G energy transfer process, as revealed by a long lived emission of R6G upon SO₂C[4] excitation. The FRET efficiency was almost quantitative (87-90 %), pointing to an average distance of 28 to 32 Å between the R6G and the Tb emitting centres of the NP. An association of one R6G per SO₂C[4] was deduced with a very strong apparent affinity ($K = 2.5 \times 10^7 \text{ M}^{-1}$) in water. Similar interactions were observed with paraquat, resulting in TbNP luminescence quenching. In both cases, an unexpected increase of the association constant of the cation with SO₂C[4] was observed when SO₂C[4] is anchored at the surface of the NP.

Introduction

Lanthanide complexes are a very important class of luminescent probes and sensors,^[1] that can be employed in numerous applications such as imaging,^[2] DNA sensing,^[3,4] determination of enzyme activity and fluoroimmunoassays^[5] after an appropriate biolabeling step.^[6]

The *f-f* optical properties related to electronic transition of Ln(III) ions (a "line-like" spectral emission signature and very long excited-state lifetimes, which allows for time-gated detection) are very appealing for bioanalysis that typically requires low background noise detection. However, the associated extremely weak absorptivity^[5] makes direct excitation inefficient. This issue can be overcome with the use of light-harvesting chromophores that will photosensitize Ln ions *via* the antenna effect.^[7]

Among the numerous and various antennas reported in the literature, the family of calixarenes^[8] is widely studied as Ln chelator and antenna for more than 30 years.^[9] Exhibiting

convergent O-donor atoms at the lower rim for Ln coordination and comprising a tetraaryl binding pocket, the *para* position can be modified with a sulfonate group which drastically increases its water solubility,^[10] allowing the study of their interaction with Ln cations in water, an important prerequisite for bioanalysis.^[11] Water soluble Tb complexes of *p*-sulfonatocalixarene derivatives have been reported to be more luminescent than their Pr, Sm, Eu and Dy counterparts,^[12] the very high affinity of the ligand towards Tb ions allowing the detection of this cation at the sub-ppb level in aqueous solution.^[13] Encapsulation of Tb complexes of *p*-sulfonatothiacalix[4]arene in silica nanoparticles (NPs) led to an enhanced luminescence emission, illustrating the significant input of nanoscience in the search of designing brighter probes.^[14] Calixarenes are also widely used as supramolecular host modules for molecular recognition, as their hydrophobic cavities can host charged and neutral guests such as solvent molecules,^[15] pesticides,^[16] and biorelevant compounds (dopamine, testosterone...)^[17] Luminescent nanodevices taking advantage of calixarene supramolecular recognition have been developed for host-guest sensing by surface decoration of Au-, Ag-, and Si-NPs or quantum dots by calixarenes.^[18]

In recent years, our research team has reported the design of water-soluble inorganic Tb doped LaF₃ NPs decorated with capping antennas, exhibiting exceptional brightness (up to $2.2 \times 10^6 \text{ M}^{-1}\text{cm}^{-1}$).^[19,20] This work (realized with hydroxyisophthalic derivatives, a good Tb photosensitizer^[21]) paves the way to the design of novel bright NPs capped with various efficient Tb antennas, such as water-soluble *p*-sulfonatocalixarene derivatives. To the best of our knowledge, no study is reported on the design of luminescent inorganic Ln NPs decorated with *p*-sulfonatocalixarenes that takes advantage of the dual role of Tb sensitizer and supramolecular host for molecular recognition. Herein, we aim at investigating (i) the ability of *p*-sulfonatocalix[4]arene derivatives to photosensitize Tb ions from the Tb doped LaF₃ NPs through surface coordination (**Figure 1**

Figure 1. Representation of the different steps leading to the proposed supramolecular assembly. (1) NP photosensitization by surface capping ligands and (2) host-guest interactions between *p*-sulfonatocalix[4]arenes capped on the TbNPs and aromatic charged compound (orange oval). The three *p*-sulfonatocalix[4]arene derivatives employed in this study with their corresponding acronyms and X functionalities. Structures of the two guest compounds (R6G and PQ).

step 1) and (ii) the supramolecular assembly originating from interactions of the most promising calixarene capped NPs with two different charged aromatic compounds, namely rhodamine 6G (R6G) and paraquat (PQ) (Figure 1 step 2).

Results and Discussion

Surface Photosensitization of TbNPs by calix[4]arenes

La_{0.9}Tb_{0.1}F₃ NPs (TbNPs) were synthesized in aqueous solution under microwave irradiation according to previously published procedures,^[19,20] and their shape and composition (doping level) were characterized by surface potential measurement, DLS and ICP-AES analysis, respectively (Figure S1 and Table S1). The concentration of the NPs was determined using the typical average size of these NPs previously determined by TEM measurement, the density of La_{0.9}Tb_{0.1}F₃ calculated as the percentage weighted density of LaF₃ and TbF₃, and the total amount of La (or Tb) amount in the solution, as determined by ICP-AES analysis.^[19,20] The surface potential of free nanoparticles in water was determined by DLS to be $\xi = +30$ mV, allowing for potential electrostatic interactions with negatively charged molecules at the surface. The three *p*-sulfonatocalix[4]arene derivatives employed in this study are presented in Figure 1. They were synthesized starting from *p*-tert-butylphenol according to protocols described in the literature.^[8,12,22–28] ¹H and ¹³C NMR spectra of C[4], SC[4] and SO₂C[4] are shown in Figures S2 to S7.

The spectroscopic properties of the three *p*-sulfonatocalix[4]arene derivatives were first studied. As evidenced in Figure 2, all C[4] exhibit a strong absorption band in the UV domain, associated to $\pi \rightarrow \pi^*$ transitions centered on the phenol moieties.^[29] The maxima of absorption display both strong hyperchromic and bathochromic shifts from 283 to 303 and 345 nm respectively for C[4], SC[4] and SO₂C[4]. The fluorescence emission and excitation spectra of the calixarene derivatives were also recorded (Figure S8).

Figure 2. UV/Vis absorption spectra of C[4] (black), SC[4] (blue) and SO₂C[4] (green).

The ability of the three *p*-sulfonatocalix[4]arene derivatives to photosensitize Tb contained in the La_{0.9}Tb_{0.1}F₃ NPs was then investigated, by titration experiments consisting in the addition of increasing amounts of each of the calixarenes to a solution containing TbNPs (HEPES 50 mM, pH 7.0). The titrations were monitored by recording of the time-gated emission spectra of the solutions upon excitation into the maximum of the excitation bands of the calixarenes. In such conditions (delay = 60 μ s), the short-lived autofluorescence of calixarenes is negligible. Figure 3a represents the evolution of the emission spectra in the case of SO₂C[4]. As evidenced by Figure 3a, the introduction of the calixarenes in the solution resulted in the observation of four main emission bands at 485, 545, 590 and 620 nm, corresponding to the typical ⁵D₄ $\rightarrow$ ⁷F_J (*J* = 6 to 3 resp.) electronic transitions of Tb.

Figure 3. (a) Evolution of the photoluminescence spectra of a 5.5 $\times 10^{-10}$ mol.L⁻¹ solution of La_{0.9}Tb_{0.1}F₃ (HEPES 50 mmol.L⁻¹, pH 7.0) upon addition of SO₂C[4] (λ_{exc} =335 nm) and (b) evolution of the Tb centered emission intensity at 545 nm (⁵D₄ $\rightarrow$ ⁷F₅ transition area of emission spectra from Figure S9) as a function of the number of SO₂C[4] (black, λ_{exc} =335 nm), SC[4] (blue, λ_{exc} =315 nm) or C[4] (red, λ_{exc} =290 nm) per NP.

The coordination of the calixarenes at the surface of the NPs (documented to happen through both the phenolate and sulfoxy oxygen atoms of $\text{SO}_2\text{C}[4]$)^[12] allows for a calixarene to Tb energy transfer leading to Tb emission. As it can be seen in **Figure 3b** and Figure S9, the photosensitization was particularly efficient in the case of $\text{SO}_2\text{C}[4]$, whereas C[4] and SC[4] only led to weak photosensitization, confirming the results observed for the photosensitization of free Tb^{3+} cations in aqueous solutions.^[12,13] It is surmised that the CH_2 bridges partially hinders the coordination of the phenolate groups, while the coordination with the soft S atoms is better, but far less efficient than the hard coordination of the hard O atoms of the SO_2 groups associated to the phenolates.^[30] In the case of $\text{SO}_2\text{C}[4]$, the surface photosensitization first strongly increased and finally led to a plateau region, corresponding to a monolayer coverage of calixarene (*vide infra*), with a final amplification of the Tb emission by a factor of 1200 compared to naked NPs at $\lambda_{\text{exc}} = 335$ nm. The effect of the pH on NP sensitization by $\text{SO}_2\text{C}[4]$ was also investigated and the antenna effect was shown to be more efficient at pH 7.0 (Figure S10). In these conditions, using the intercept between the line obtained by linear regression on the first eight points and the horizontal line formed at the plateau region, an estimation of the full coverage of the NPs by the calixarene led to ca. 1000 $\text{SO}_2\text{C}[4]$ per NP (**Figure 3b**). By calculation of the surface of the NP (with a diameter of 21 nm determined by TEM^[19,20]) and the surface occupied by $\text{SO}_2\text{C}[4]$ (calculated as an ellipse with diameters of 13 and 13.5 nm as shown in Figure S11, in accordance with the literature^[31,32]), a number of 1005 $\text{SO}_2\text{C}[4]$ per NP is obtained (see Section IV of Supporting Information), in line with the experimental value. In the following assays, a ratio of 900 ligands per NP (90% of total surface capping) will be preferred to avoid the presence of free ligand in solution.

Figure 4: Absorption (orange) and normalized emission spectra of R6G (black) and of $\text{SO}_2\text{C}[4]$ capped TbNP (green) in HEPES (50 mM, pH 7.0).

As shown in Table S1, the capping of the positively surface charged NP (+ 30 mV) by $\text{SO}_2\text{C}[4]$ in water leads to a negative zeta potential of -27 mV that can be explained by the presence of negative groups on $\text{SO}_2\text{C}[4]$, and the surface charge remains negative even in the buffered conditions of photoluminescence experiments (-15 mV in HEPES 50 mM pH 7.0)

Interaction of Rhodamine 6G with $\text{SO}_2\text{C}[4]$ capped TbNPs

Rhodamine 6G (R6G, **Figure 1**) is a prototypical fluorescent dye exhibiting fluorescence at a maximum of 555 nm in aqueous solution.

A particular feature is that both absorption and emission spectra overlap the emission spectrum of Tb (**Figure 4**). Noteworthy, evidences of R6G interaction with *p*-sulfonatocalixarenes and *p*-

Figure 5. Time-resolved emission spectra of TbNP capped with $\text{SO}_2\text{C}[4]$ (HEPES 50 mM, pH=7) upon addition of different equivalents of R6G (number of equivalents per $\text{SO}_2\text{C}[4]$ are indicated by different colors ($[\text{TbNP}] = 5.5 \cdot 10^{-10} \text{ mol.L}^{-1}$, 900 $\text{SO}_2\text{C}[4]$ per NP, $\lambda_{\text{exc}} = 335 \pm 10$ nm, delay time of 60 μs , integration time of 2 ms). Inset: Evolution of the time-resolved emission of R6G at 570 nm (●) and 605 nm (●) upon titration of $\text{SO}_2\text{C}[4]$ by R6G. Fits of the data (see text) are indicated as solid lines..

sulfonato-thiacalixarenes have already been reported in the literature,^[33,34] and its inclusion *via* encapsulation of the benzoate moiety of the R6G guest within the calixarene cavity was investigated.^[33] However, to the best of our knowledge, no study on the interaction between R6G and *p*-sulfonato-sulfoxocalix[4]arene, SO₂C[4], has been reported up to date. We thus aimed to investigate the possible interaction of R6G with SO₂C[4] capped at the surface of the NPs.

To question such an interaction, we planned to follow the time-resolved emission spectra of a solution of SO₂C[4] capped TbNPs (HEPES 50 mM, pH 7.0) upon addition of increasing amounts of R6G with excitation of the calixarene antenna (**Figure 5**). A delay of 60 μ s was applied between the light pulse and the beginning of the emission measurement in order to measure only the long-lived emission signals, while avoiding the R6G fluorescence, whose excited state lifetime is in the nanosecond range.^[35] A typical example of Tb-centered emission spectrum is illustrated in **Figure 5** (black line) after SO₂C[4] excitation.

After addition of R6G, two phenomena were observed: (a) a decrease of Tb related emission intensity and (b) the apparition of a long lived broad band with maximum around 560 nm. These phenomena are typical of the occurrence of a Förster resonance energy transfer (FRET) process occurring between Tb and R6G. This can only happen when the acceptor and donor are in close proximity, a condition satisfied in the case of the interaction of R6G guest with the SO₂C[4] host (**Scheme 1**). Thus, the Tb emission decreased as a part of its energy is transferred to R6G instead of being directly radiatively emitted, and the R6G emission band appears. Interestingly, the intensity of this new emission band increases upon initial additions of R6G (see inset of **Figure 5**), before reaching a plateau after an addition of R6G corresponding to nearly one R6G per SO₂C[4]. The time-resolved FRET observed here highlights a close proximity (so by extension an interaction) between the calixarenes capped at the surface of the NPs and the R6G, and the maximal intensity obtained for a R6G / SO₂C[4] ratio of one implies a possible inclusion of R6G in the host cavity. Such an energy cascade from the calixarene to the Tb ions and finally to an organic dye has already been observed in complex nanometric architectures.^[36]

In further spectroscopic measurements, the temporal emission decays were recorded for five different quantities of R6G added. The Tb emission decay was recorded at 490 nm (**Figure 6a**), while that of R6G was monitored at 570 nm (**Figure 6b**), a wavelength at which only minute Tb emission can be observed.

Scheme 1. Schematic representation of FRET between photosensitized Tb-NPs (donor) and R6G dye (acceptor) through the interaction of R6G in *p*-sulfonato-sulfoxocalix[4]arenes (SO₂C[4]).

Figure 6. PL intensity decays of (a) TbNP donors ($\lambda_{em} = 490 \pm 1$ nm) and (b) R6G acceptors ($\lambda_{em} = 570 \pm 1$ nm) for different ratios of R6G per SO₂C[4] upon pulsed excitation ($\lambda_{exc} = 335 \pm 1.5$ nm) in HEPES 50 mM, pH=7.0.

Without R6G acceptor, the long lived Tb-centered emission is mono-exponential, with a lifetime of 725 μ s. Considering that it is not possible in our case to determine the radiative lifetime of Tb, we used different values of radiative lifetime reported in the literature, e.g. 4.75 ms^[37] and 9.02 ms^[38] for Tb in aqueous solution at ~290 K, Tb centered luminescence quantum yields of $\Phi_{Tb} = 0.726 / 4.75 = 0.15$ or $0.726 / 9.02 = 0.08$ were respectively calculated. At 570 nm (**Figure 6b**, black line), a low signal attributed to traces of Tb emission is observed.

Upon addition of R6G, the Tb donor curves decrease in intensity and in decay time (**Figure 6a**) while the R6G acceptor curves (**Figure 6b**) increase in intensity and lifetime with a maximum obtained for ca. R6G / SO₂C[4] = 1:1, in line with the time-resolved emission spectra observations (**Figure 5**). PL lifetime of the Tb emission is now composed of two main components (PL intensity decay times are summarized in Table S2), the first one (fixed at 726 μ s) related to Tb not involved in FRET, the second one (between 100 and 300 μ s) corresponds to the lifetime of Tb atoms contained in the NPs which are involved in FRET to R6G.

At 570 nm, a mono-exponential lifetime in the hundred microsecond range is measured, related to R6G long lived photosensitization. This is clear evidence of FRET originating from TbNP sensitization as the intrinsic fluorescence lifetime of R6G is in the nanosecond range.^[35] **Figure 7** summarizes the emission intensity decay times of donor in the presence of acceptor (τ_{DA} , striped bars) and acceptor in the presence of donor

Figure 7. PL intensity decay times of donor in the presence of acceptor (striped bar, $\lambda_{em} = 490$ nm) and acceptor in the presence of donor (solid bar, $\lambda_{em} = 570$ nm) for different ratios of R6G per SO₂C[4] upon pulsed excitation ($\lambda_{exc} = 335 \pm 1.5$ nm) in HEPES 50 mM at pH 7.0. A 10% error is indicated for lifetime measurements. Data in Table S2.

(τ_{AD} , black bars). For a given number of R6G per SO₂C[4], both lifetimes are comparable, in agreement with the theory of FRET,^[39] which expects similar values of τ_{DA} and τ_{AD} when the donor lifetime in absence of acceptor is far longer than the acceptor lifetime, respectively 726 μ s and few ns in our case. From these experiments, the Förster radius R_0 , corresponding to the distance between the acceptor and the donor at which the energy transfer efficiency is equal to 50%, can be determined.

$$R_0 = 0.02108 (\kappa^2 \Phi_D n^{-4} \int I_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda)^{\frac{1}{6}} \text{ nm} \quad (1)$$

Using equation (1),^[40] with a refractive index $n = 1.33$ (aqueous solution), the orientation factor $\kappa^2 = 2/3$ (dynamic averaging as justified by the isotropic emission and long lifetime of Tb), the wavelength λ (in nm), the normalized (to unity over the integral) TbNP emission spectrum with intensity $I_D(\lambda)$, and the R6G absorption spectrum with molar extinction coefficient $\epsilon_A(\lambda)$ (in $\text{M}^{-1} \cdot \text{cm}^{-1}$), Förster distances R_0 of 4.4 nm ($\tau_{rad} = 4.75$ ms) and 3.9 nm ($\tau_{rad} = 9.02$ ms) were obtained. Moreover, donor (Tb) to acceptor (R6G) distances (r) and FRET efficiency (E_{FRET}) can be quantified with Tb emission intensities and decay times with (subscript DA) and without acceptor (subscript D and A), by using Equations (2) and (3) respectively.

$$E_{FRET} = 1 - \frac{I_{DA}}{I_D} = 1 - \frac{\tau_{DA}}{\tau_D} \quad (2)$$

$$r = R_0 \left(\frac{I_{DA}}{I_D - I_{DA}} \right)^{1/6} = R_0 \left(\frac{\tau_{DA}}{\tau_D - \tau_{DA}} \right)^{1/6} \quad (3)$$

The data recorded for 1.5 R6G / SO₂C[4] was chosen for the calculations in order to ensure the conditions of at least one R6G per calixarene, and the resulting FRET efficiency and acceptor-donor distances are presented in

Table 1. The results based on time decays and intensities are very similar. Both methods led to comparable results pointing to an efficient energy transfer and an average distance of 31-32 Å ($\tau_{rad} = 4.75$ ms) and 27-29 Å ($\tau_{rad} = 9.02$ ms) were obtained between the Tb donors in the NP matrix and the R6G embedded in the calixarene cavity.

Table 1. FRET efficiency (E_{FRET}) and donor-acceptor distances (r) (right panel) as calculated from donor decay times (τ) or PL intensities (I) from experiment with 1.5 R6G/ SO₂C[4] (from Figure S12). ^a calculated with $\tau_{rad} = 4.75$ ms. ^b calculated with $\tau_{rad} = 9.02$ ms.

	Based on τ_D	Based on I_D
E_{FRET}	$87 \pm 9 \%$	$90 \pm 9 \%$
r (nm) ^a	3.2 ± 0.3	3.1 ± 0.3
r (nm) ^b	2.9 ± 0.3	2.7 ± 0.3

Finally, the spectroscopic data of **Figure 5** could be used to obtain an estimation of the host guest interaction between calixarene and R6G, as modeled by the following equilibrium:

From the fitting of the data of **Figure 5**, a very strong association constant of $2.5(8) \times 10^7 \text{ M}^{-1}$ was calculated. Noteworthy, the inclusion of R6G into C[4] and SC[4] (not bound to a NP) has already been studied in *d*₆-DMSO solutions by ¹H NMR spectroscopy,^[33] but the reported values of the association constants are far smaller in that case, 2.5 and 1.32 M^{-1} , respectively. Unfortunately, the very weak solubility of R6G in D₂O precluded the study of the inclusion monitored by ¹H NMR.

In order to get further insight into the interaction of R6G with calixarene, we turned our attention towards the possibility to investigate the system by molecular modeling. In order to probe the conformation and donor-acceptor distances of the R6G-SO₂C[4] inclusion complex at the surface of NPs, molecular docking experiments were undertaken on a model TbF₅ complex. Host-guest species were generated using Autodock Vina^[41] (See experimental section for more details), the lowest energy conformer (**Figure 8**) involves the pendant benzoate moiety of the R6G bound within the SO₂C[4] cavity via π - π interactions, in line

Figure 8. Molecular model of the inclusion complex between R6G and SO₂C[4]-TbF₅ (H atoms omitted for clarity; centroid depicted as a red sphere; host carbon atoms in purple, guest carbon atoms in orange).

with previous studies,^[33] and is also borne out for the other five lowest energy conformations (Figure S13). The SO₂C[4] ⊃ R6G complex is augmented by weak CH-hydrogen bonds between the R6G arylmethyl groups to the calixarene sulfonate (SO---HC_{mean} = 3.5 Å), with an overall distance between the R6G_{centroid} and the Tb ion of 9.4 Å (Figure S14 for centroid definition) and a net stabilization of -16 kJ.mol⁻¹ upon formation of the host-guest species.

These data suggest that observation of FRET distances at ca. 32 Å (*vide supra*) indicate that energy transfer occurs beyond directly coordinated Tb (9.4 Å) and indeed from adjacent and subsurface Tb sites to the R6G. To investigate this and determine the average internuclear distances on the doped NP surface, a hexagonal phase La_{0.89}Tb_{0.11}F₃ surface was modelled using VESTA, assuming a homogeneous doping distribution within the NP (Figure S15).^[42] The internuclear distances were calculated for the closest 13 groups of symmetry equivalent Tb atoms within the lattice (Figure S16) – constituting surface and subsurface Tb sites, arriving at a mean internuclear distance of 18.7 Å. This value of 18.7 Å, added to the calculated Tb-R6G_{centroid} distance of ca. 9.5 Å for the six lowest energy conformations, correlates well with the experimental value of the FRET donor-acceptor distance ($r \approx 32$ or 28 Å depending on τ_{rad}) determined by PL experiments.

Interaction of paraquat with SO₂C[4] capped TbNPs

Considering the strong interaction of R6G into SO₂C[4], the doubly charged 1,1'-dimethyl-4,4'-bipyridyl cation (paraquat) was envisaged for its inclusion. **Figure 9** displays the evolution of the time gated emission spectra of the TbNPs covered by 900 equivalents SO₂C[4] upon addition of increasing amounts of

paraquat. A reduction of the Tb emission is observed, as previously observed on Tb(III) complexes of SO₂C[4] with pyridinium derivatives^[43] and we attribute this luminescent quenching to an electron transfer between the calixarene excited state and the paraquat (PQ)^[44] moiety in its cavity.

The electron transfer quenching of the NPs emission by PQ²⁺ has been analysed by using the Stern-Volmer relationship (Equation 4).^[45]

$$\frac{I_0}{I} = 1 + K_{SV}[PQ^{2+}] \quad (4)$$

where I_0 and I denotes the emission intensities in absence and presence of PQ²⁺ and K_{SV} is the Stern-Volmer quenching constant.

A linear behaviour has been observed, with a quenching constant $K_{SV} = 8.7 \times 10^5 \text{ M}^{-1}$. In addition, the apparent binding constant (K_A) and number of binding sites (n) were determined by Equation 5.

$$\log \frac{I_0 - I}{I} = \log K_A + n \log [PQ^{2+}] \quad (5)$$

As n is very close to the unity (0.93), it is assumed that the interaction is directed by the formation of complexes with the calixarenes and that the unspecific interaction at the surface of NPs is negligible. With this formalism, the apparent affinity constant was found equal to $3.46 \times 10^5 \text{ M}^{-1}$. As a figure of comparison, values ranging from $7.3 \times 10^4 \text{ M}^{-1}$ (in rat plasma)^[16,46] to $9.3 \times 10^5 \text{ M}^{-1}$ (D₂O, pD = 7)^[47] have been reported in the literature for unbound C[4] and paraquat. However, the sulfoxo derivative SO₂C[4] has been far less studied. To further investigate the interaction of PQ with SO₂C[4], a titration was performed, in which the ¹H-NMR spectra of free SO₂C[4] (without

Figure 9. a) Time-resolved emission spectra of Tb-NP ($5.5 \times 10^{-10} \text{ mol.L}^{-1}$) capped with SO₂C[4] (900 per NP) upon addition of different equivalents of paraquat (number of equivalents per SO₂C[4] are indicated by different colors, $\lambda_{exc} = 335 \pm 10 \text{ nm}$, delay time of 60 μs , integration time of 2 ms). b) Stern-Volmer plot for the quenching of NPs emission by PQ (●) and fit according to the equation $\log(I_0/I) = 873369 [PQ] + 1.0208$ ($R^2 = 0.9986$). c) Representation of $\log(I_0 - I)/I$ as a function of $\log[PQ]$ (●) and fit according to the equation $\log(I_0 - I)/I = 0.9304 \log[PQ] + 5.5389$ ($R^2 = 0.9973$) in dotted line.

Figure 10. a) Evolution of the ¹H NMR spectra of SO₂C[4] upon addition of increasing amount of PQ (400 MHz, D₂O, pD = 7.4). b) Variation of the chemical shift of the PQ1 (♦), PQ2 (▲) and SO₂C[4] (●) protons as a function of added equivalents of PQ in solution and fit (—).

NPs) were monitored in the presence of increasing amounts of PQ in D₂O (Figure 10). Titration of PQ into the solution of SO₂C[4] induces upfield shifts (8.24 ppm to 7.94 ppm) of the aromatic protons of the calixarene. Similarly, while free PQ displays two well resolved doublets corresponding to H atoms in *meta* (PQ1) and *ortho* (PQ2) positions of the pyridyl rings, the presence of SO₂C[4] resulted in the upfield shift of these doublets and in a broadening of the signals.

It is surmised that the inclusion of PQ into the calixarene cavity leads to strong π - π stacking interactions resulting in the positioning of the different aromatic protons in the shielding regions of the neighboring aromatic rings, with a concomitant upfield shift. As a result of a strong overlap with the peak of the residual water, the influence of inclusion on the peak of the methyl groups of paraquat could not be investigated. The broadening of the signals of PQ may also be attributed to the onset of desymmetrization of the PQ molecule, one of the pyridinium moiety likely being more affected by its inclusion into the cavity. The interaction was further confirmed by a 2D-NOESY experiment at 1 equivalent of PQ (Figure S17), revealing a dipolar interaction between the aromatic protons of the calixarene and the high field peak of PQ.

Using the chemical shifts of the aromatic protons of the calixarene, it was possible to determine the association constant. A very weak value of 86(9) M⁻¹ was obtained in these conditions, pointing again to a large enhancement of the association of the charged aromatic compounds with the calixarene when this latter is anchored at the surface of the NPs. The origin of this drastic effect is not yet clear and is probably a combination of solvation effects and of the polarization of the electronic cloud of the calixarene upon coordination to the positively charged surface of the NP.

Conclusion

C[4], SC[4] and SO₂C[4] were shown to be able to coordinate to the surface of Tb doped LaF₃ NPs. In the case of SO₂C[4], the coordination is accompanied by a strong photosensitizing effect

leading to Tb emission upon calixarene excitation. Considering previous work on the interaction of Ln cations with SO₂C[4], the interaction is expected to occur through the bonding of the oxygen atoms from phenol functions of the lower rim, leaving the cavity exposed to the solvent and to the potential inclusion of aromatic compounds. Such an interaction was investigated with R6G and paraquat and this was modeled in the case of R6G. The interaction was also evidenced by FRET experiments that revealed a strong association and a close proximity of R6G with the surface of the TbNP. In both cases, the association was observed to be strong and interestingly stronger than for the parent systems lacking the coordination of the calixarene to the surface of the NPs. A similar increase of affinity constant has been reported for calix[4]arene derivatives grafted on surface of gold NPs in interaction with quaternary ammonium cations.^[48] Although the origin of this enhanced association is not yet fully clear, the phenomenon might be very appealing within the frame of time-gated luminescence detection, taking into consideration that calixarenes are widely used for the detection of various compounds, among which herbicides^[49] or fungicides.^[50]

Experimental Section

Synthesis and characterisation of NP. TbNPs were synthesized according to previously published procedures and their shape and composition were characterized by TEM, DLS (Figure S1), zeta potential measurement (Table S1) and ICP-AES analysis.^[19] The concentration of the NPs was determined (following the previously published method)^[19] using the average size of the NPs as determined by TEM measurement, the density of La_{0.9}Tb_{0.1}F₃ calculated as the percentage weighted density of LaF₃ and TbF₃, and the total amount of La (or Tb) amount in the solution, as determined by ICP-AES analysis. ICP-AES analysis of samples in water were performed on a Varian 720 spectrometer equipped with a quartz Meinhard nebulizer and a cyclone spray chamber. DLS measurements (giving access to hydrodynamic diameter) and zeta potential measurements were performed on an Amerigo Particle Size & Zeta Potential Analyzer (Cordouan Technologies).

Synthesis of sulfonatocalix[4]arenes. *p*-sulfonatocalix[4]arene, C[4],^[22,23] *p*-sulfonatothiacalix[4]arene, SC[4],^[24,25] and *p*-sulfonato-sulfoxocalix[4]arene (SO₂C[4])^[8,26,27] were obtained according to literature procedures and characterized by ¹H and ¹³C NMR (Figures S2 to S7)

Spectroscopy. UV-Vis spectroscopic measurements were performed with 10×10 mm² quartz suprasil certified cells (Helma Analytics). UV/vis absorption spectra were recorded on a Specord spectrometer from Jena Analytics. Time-gated emission spectra were recorded on an TECAN Spark multimode microplate reader with monochromators, working with a Xe flash lamp in low-binding black 96-wells microplates from Greiner bio-one. Photoluminescence kinetics (lifetimes) were recorded on an Edinburgh Instrument FLP920 spectrometer, working in the Multi-Channel-Scaling (MCS) mode, using a Xenon flash lamp as the excitation source. The errors on the lifetimes are estimated to 10%. For conventional luminescence measurements, a continuous 450W Xe Lamp and a red sensitive R928 photomultiplier from Hamamatsu in Pelletier housing for visible detection (230 to 900 nm) were used.

Spectrofluorimetric titrations of TbNPs. Various quantities of ligand (C[4], SC[4] or SO₂C[4]) in HEPES (pH 7.0, 0.05 M) were added to HEPES buffered solution (0.05 M, pH 7.0) of La_{0.9}Tb_{0.1}F₃ NPs (5.5 10⁻¹⁰ mol.L⁻¹) in wells of a 96-well microplate. Time-gated emission spectra were measured for each well. Excitation at 335 nm with a bandwidth of 20 nm, emission measured between 520 and 570 nm with a 1 nm step, an emission bandwidth of 5 nm, a lagtime of 60 μs and an integration time of 2 ms.

FRET assays between SO₂C[4] capped TbNPs and R6G. Various quantities of R6G in HEPES (pH 7.0, 0.05 M) were added to HEPES buffered solution (0.05 M, pH 7.0) of La_{0.9}Tb_{0.1}F₃ NPs (5.5 10⁻¹⁰ mol.L⁻¹) with 900 SO₂C[4] per NP, in wells of a 96-well microplate. Time-gated emission spectra were measured for each well. The samples were excited at 335 nm with a bandwidth of 20 nm, and the emission measured between 450 and 650 nm with a 1 nm step, an emission bandwidth of 5 nm and an integration time of 2 ms. Lagtime was first optimized with a solution of R6G in order to suppress R6G fluorescence due to direct excitation and was settled at 60 μs.

Interaction assays of paraquat with SO₂C[4] capped TbNPs. Various quantities of paraquat, in HEPES (pH 7.0, 0.05 M) were added to a HEPES buffered solution (0.05 M, pH 7.0) containing La_{0.9}Tb_{0.1}F₃ NPs (5.5 10⁻¹⁰ mol.L⁻¹) and 900 SO₂C[4] per NP in wells of a 96-well microplate. Time-gated emission spectra were measured for each well. Excitation was performed at 335 nm with a bandwidth of 20 nm, emission measured between 450 and 650 nm with a 1 nm step, an emission bandwidth of 5 nm, a lagtime of 60 μs and an integration time of 2 ms.

NMR experiments. ¹H, ¹³C NMR APT and ¹H-¹H NOESY spectra were recorded on a BRUKER 500 MHz spectrometer. Paraquat and/or SO₂C[4] solutions were prepared in D₂O and acetone was used as an internal reference (peak at 2.22 ppm). ¹H NMR spectra of SO₂C[4] (4.62.10⁻³M in D₂O) with various equivalents (0, 0.25, 0.5, 0.75, 1, 2, 4 or 6 molar equivalent) of paraquat, and ¹H-¹H NOESY spectrum of SO₂C[4] (4.62.10⁻³M in D₂O) with 1 eq. of paraquat were recorded. pD of the solutions was maintained at 7.4 with DCl and NaOD solutions in D₂O.

Molecular docking experiments. Computational calculations were performed on the open-source molecular builder Avogadro^[51] using the Universal Force Field (UFF)^[52] to obtain structures suitable for docking. In the case of the SO₂C[4], the model was built by adaptation of a previously obtain solid-state structure.^[12] Docking simulations to determine the lowest energy host-guest species were performed using Autodock Vina^[42] to produce six low energy conformers (Figure S13). The host-guest species were processed and visualized using PyMOL^[53] and CCDC Mercury.^[54] The modelling of the surface internuclear distances was performed using VESTA^[42] by doping a hexagonal phase LaF₃ (5 × 5 × 2 or 10 × 10 × 4) unit cell with 11% terbium (La_{0.89}Tb_{0.11}F₃), approximating the NP as a planar surface with a homogenous distribution of Tb ions.

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Entry for the Table of Contents

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p-sulfonato-sulfoxo-calix[4]arenes were shown to strongly bind to the surface of Tb doped LaF_3 nanoparticles, providing an excellent antenna effect for Tb emission. In the presence of some aromatic charged compounds, a strong interaction of the aromatic compounds into the cavity of the surface anchored calixarenes is observed, with a surprising enhancement of the association as compared to the free calixarene in solution.

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