Paramagnetic Relaxation Enhancement in Hydrophilic Colloids Based on Gd(III) Complexes with Tetrathia- and Calix[4]arenes

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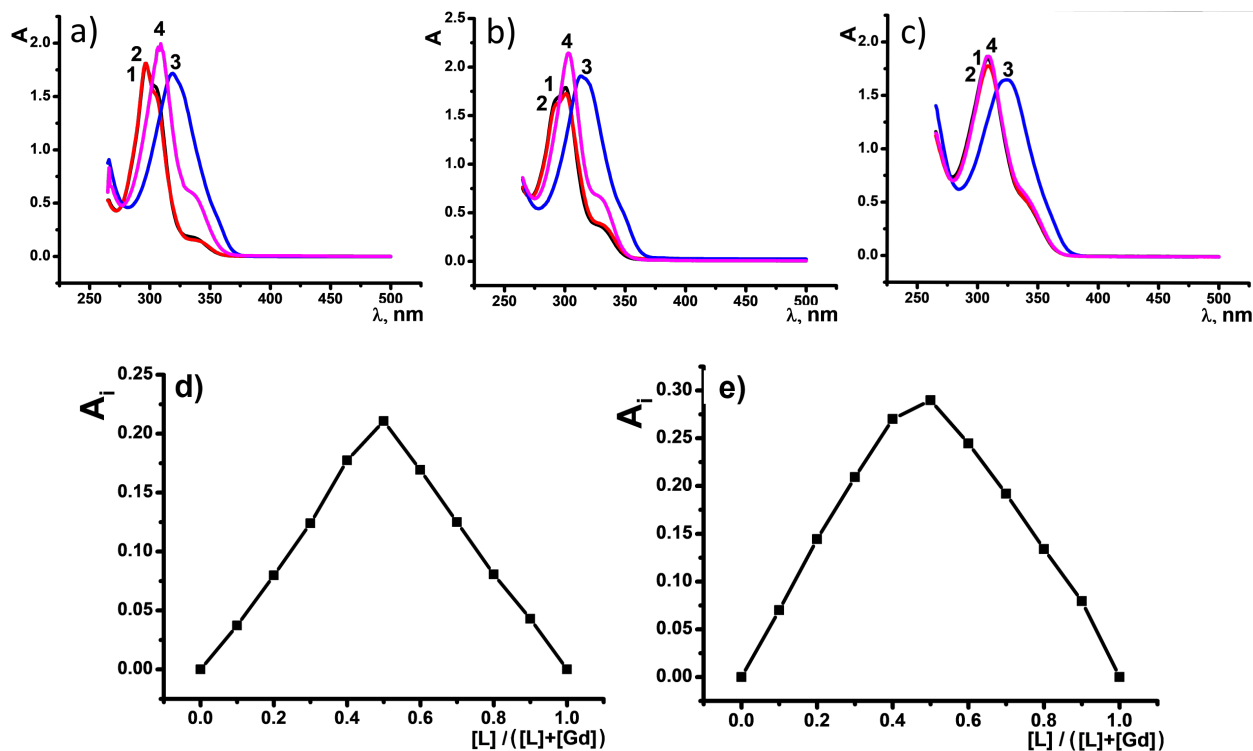
S1. Characterization of [Tb$_2$(TCAi)$_2$] complexes

Figure S1. UV-Vis spectra of TCAi and Tb - TCAi (1:1) systems in DMF (concentration 10^{-4} M): (1) TCAi (black); (2) Tb - TCAi (red); (3) [Tb$_2$(TCAi)$_2$] and TEA 1:8 (blue); (4) TCAi and TEA 1:8 (pink). (a) [Tb$_2$(TCA1)$_2$] and TCA1; (b) [Tb$_2$(TCA2)$_2$] and TCA2; (c) [Tb$_2$(TCA3)$_2$] and TCA3. Job plots for [Tb$_2$(TCA2)$_2$] (d) and [Tb$_2$(TCA3)$_2$] (e) where A$_i$= A$_\lambda$-A$_{\lambda,L}$([L] / ([L]+[Tb])) and λ=340 nm.

S2. Characterization of PSS-[Ln$_2$(TCAi)$_2$] and PSS-[LnCAi] colloids
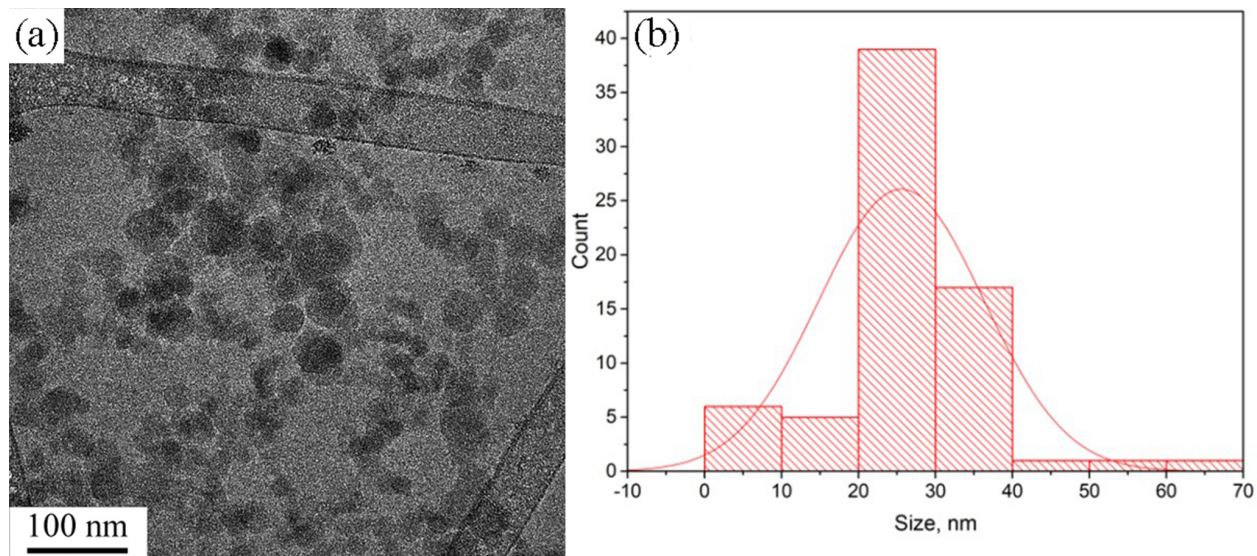

Figure S2. Cryo-TEM image of PSS-[Tb$_2$(TCA2)$_2$] (a) and corresponding size distribution (b).
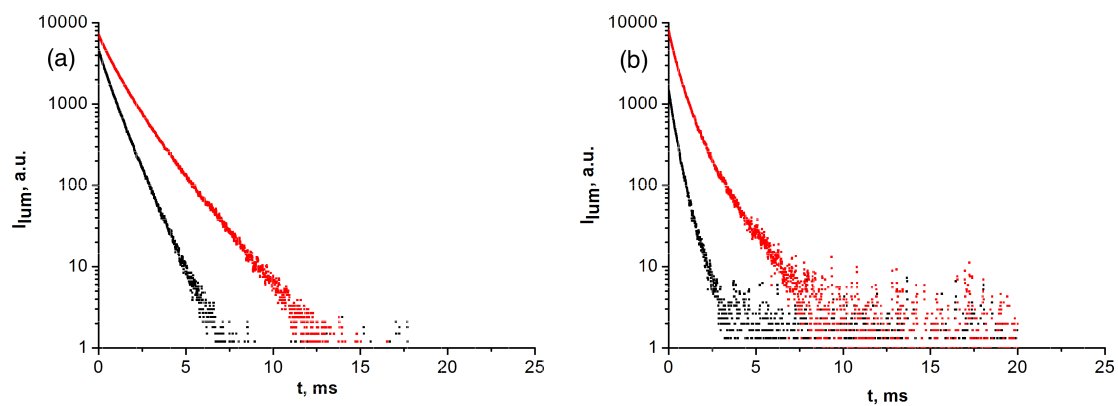

Figure S3. Luminescence decay curves of PSS-[Tb$_2$(TCA2)$_2$] (a) and PSS-[TbCA1] (b) in H$_2$O (black lines) and D$_2$O (red lines).

S3. Determination of Ln(III) losses during colloid preparation

In order to investigate in detail losses of Gd(III) ions in the preparation of PSS-[Gd$_2$(TCAi)$_2$] and PSS-[GdCAi] colloids, Gd concentration was measured by ICP-OES on the supernatants s$_1$ and s$_2$ collected after the first and second centrifugation, respectively, for samples PSS-[Gd$_2$(TCA2)$_2$] and PSS-[GdCA1], assumed as representative of the thiacalix[4]arene- and calix[4]arene-based colloids; the measured concentrations ($C_{M,ICP}$) are reported in Table S1. ^{1}H FFC NMR experiments were also performed on the supernatants with the aim of understanding in which form Gd(III) is lost; NMRD curves are shown in Figure S4.

In principle, the sum of the Gd concentration in s$_1$, s$_2$, and in the final colloids should be equal to $C_{M,NOM}$ (= 0.75 mM). The observed discrepancies (<15%) could be ascribed to the fact that the water volume used for the re-dispersion was only approximately equal to that of the withdrawn supernatant, as well as to errors in the analysis and possible losses in the working procedures. Notwithstanding these small differences, data in Table S1 strongly indicate that for PSS-[Gd$_2$(TCA2)$_2$] practically all the Gd(III) used in the preparation was incorporated in the colloids, with almost no Gd(III) losses in s$_1$; only a minor amount of Gd(III) was withdrawn in s$_2$ after re-dispersion. Moreover, NMRD curves in Figure S4 clearly show that the Gd(III) form in s$_2$ was the same observed in the colloids. On the contrary, for PSS-[GdCA1] the Gd(III) concentration in s$_1$ was quite high, corresponding to almost 2/3 of the nominal one, and the ^{1}H FFC NMR measurements (Figure S4) indicated that Gd(III) was present as aqua ion. In the supernatant s$_2$, the Gd(III) concentration was quite low and the NMRD curves could be associated to a mixture of Gd(III) aqua ions and Gd(III) complexes in the colloids. Considering that the Gd(III) concentration values measured for the colloids were similar within each class (i.e. PSS-[Gd$_2$(TCAi)$_2$] and PSS-[GdCAi]), from ICP-OES and FFC NMR data we can infer that for PSS-

[Gd$_2$(TCAi)$_2$] colloids Gd(III) is fully complexed by the ligands, with only minor losses due to supernatant withdrawal during washing. On the contrary, for PSS-[GdCAi] samples approximately one third of the initial Gd(III) seems to be complexed by the ligands, whereas a considerable amount is expelled as free aqua ion in the precipitation step.

Table S1. Gd(III) concentration determined by ICP-OES ($C_{M,ICP}$) and Tb(III) concentration determined by UV-Vis spectroscopy using xylenol orange as indicator ($C_{M,UV\text{-}Vis}$) on supernatants of PSS-[Ln$_2$(TCA2)$_2$] and PSS-[LnCA1] colloids.

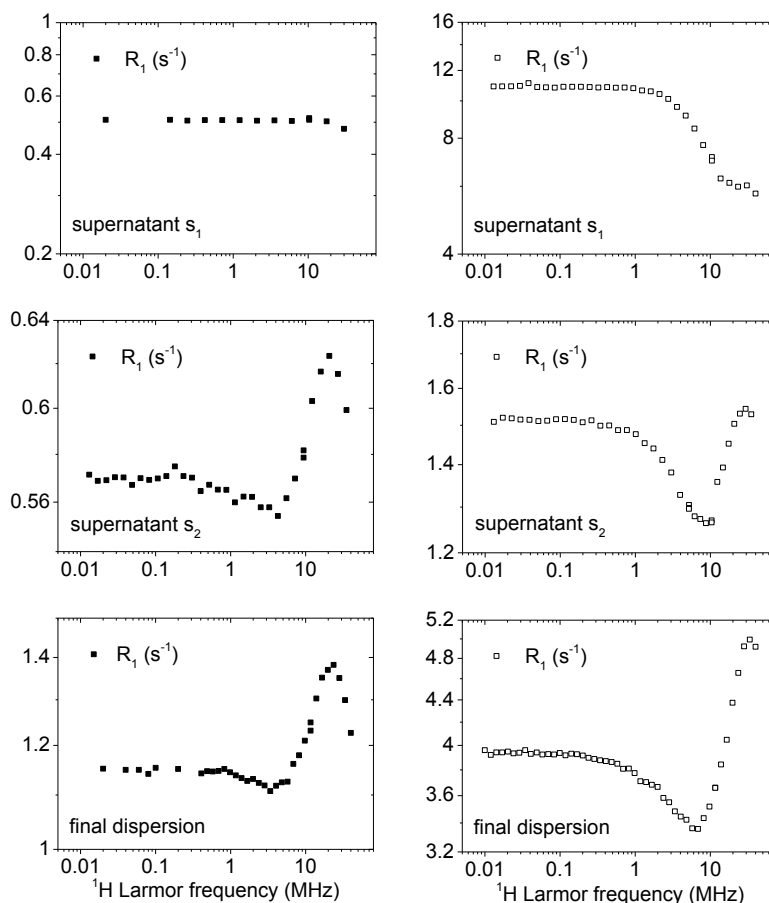|	s$_1$		s$_2$	
	$C_{M,ICP}$ (mM)	$C_{M,UV\text{-}Vis}$ (mM)	$C_{M,ICP}$ (mM)	$C_{M,UV\text{-}Vis}$ (mM)
PSS-[Ln$_2$(TCA2)$_2$]	0.02	0.05	0.12	0.02
PSS-[LnCA1]	0.40	0.29	0.06	0.11

Figure S4. R_1 NMRD curves for the supernatants s$_1$ and s$_2$ and for the final dispersion of PSS-[Gd$_2$(TCA2)$_2$] (full squares) and PSS-[GdCA1] (empty squares).

In order to check if the Tb-based systems show a behavior similar to that of the Gd counterparts, Tb(III) losses were estimated by UV-Vis spectrophotometric analysis using xylenol orange as indicator for free Tb(III) ions. The UV-Vis spectra of the supernatants recorded for the PSS-$[Tb_2(TCA2)_2]$ and PSS-[TbCA1] colloids are shown in Figure S5. The concentration of Tb(III) ions was determined from the absorbance of the complex with xylenol orange at 577 nm by comparison with a calibration curve; the obtained values are shown in Table S1. The concentration of free Tb(III) was quite high in the s_1 supernatant of PSS-[TbCA1], but very small in that of PSS-$[Tb_2(TCA2)_2]$, confirming the behavior observed for the corresponding Gd-based colloids by ICP-OES analysis and FFC NMR. Minor amounts of Tb(III) were found in the s_2 supernatants of both colloids. Differences between the concentrations of Gd(III) and Tb(III) for a given sample can be ascribed to variability in the sample preparation and to the fact that the UV-Vis method detects only free Tb(III) ions.
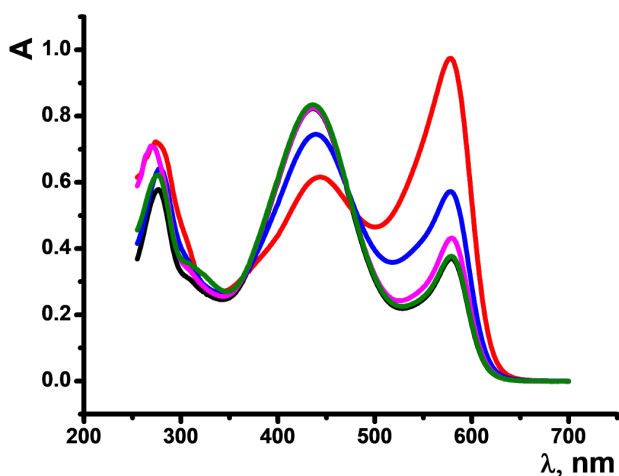

Figure S5. UV-Vis spectra of 1 mg/ml xylenol orange in water (black) and of s_1 and s_2 solutions of PSS-$[Tb_2(TCA2)_2]$ (s_1- pink, s_2- green) and PSS-[GdCA1] (s_1- blue, s_2- red) in the presence of xylenol orange.

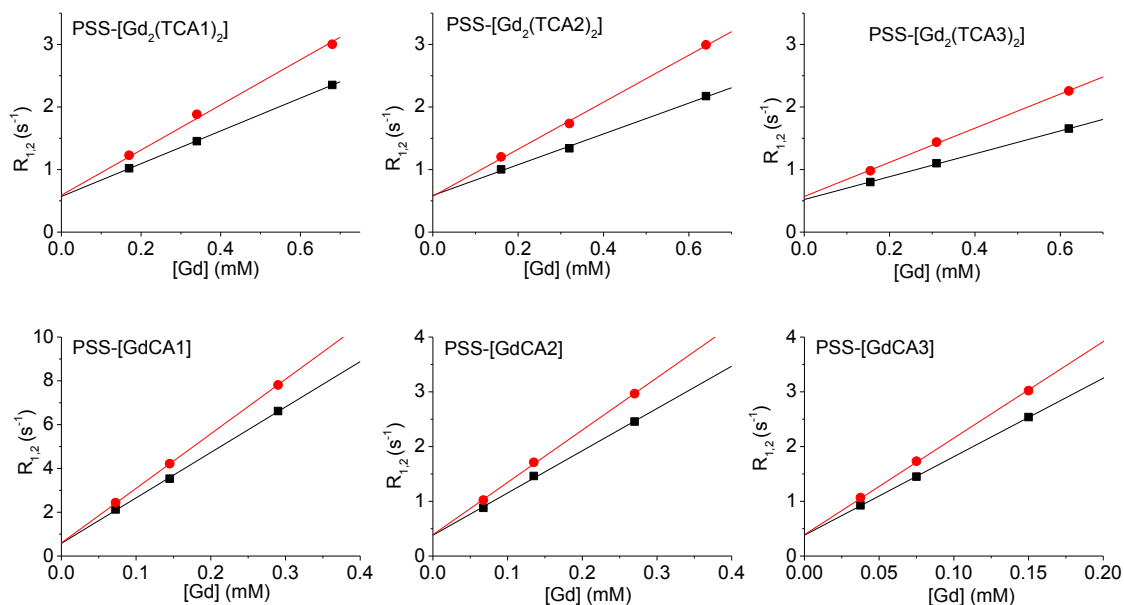## S4. Relaxivity properties of Gd(III)-based colloids

Figure S6. R_1 (black squares) and R_2 (red circles) *vs* Gd concentration measured at the ^{1}H Larmor frequency of 20.8 MHz for the indicated PSS-[Gd$_2$(TCAi)$_2$] and PSS-[GdCAi] colloids. The best linear fitting curves, obtained using Equation 1 in the article, are also shown as solid lines.