

Supplementary Information

Ln[DO3A-*N*- α -(pyrenebutanamido)propionate] complexes: optimized relaxivity and NIR optical properties

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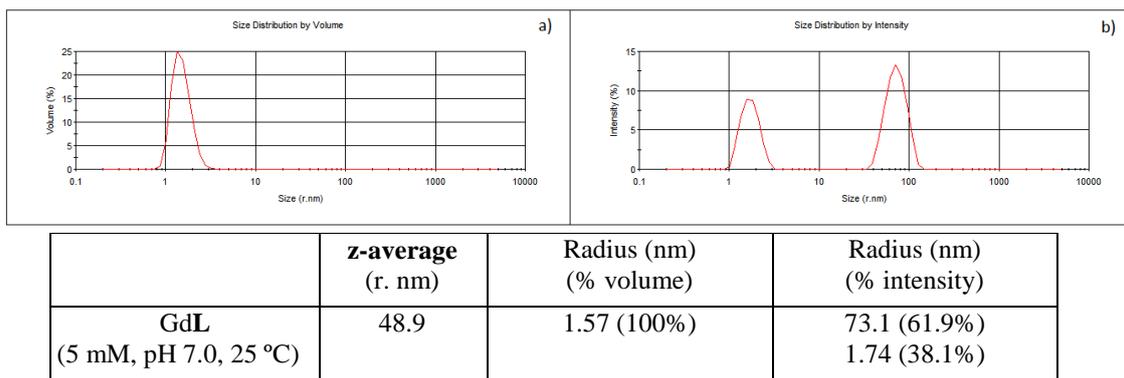


Figure S1. Size distribution in: a) volume (%); b) intensity (%) for a GdL solution (5.0 mM, pH 7.0, 25 °C) at a concentration well above the *cmc* (0.6 mM).

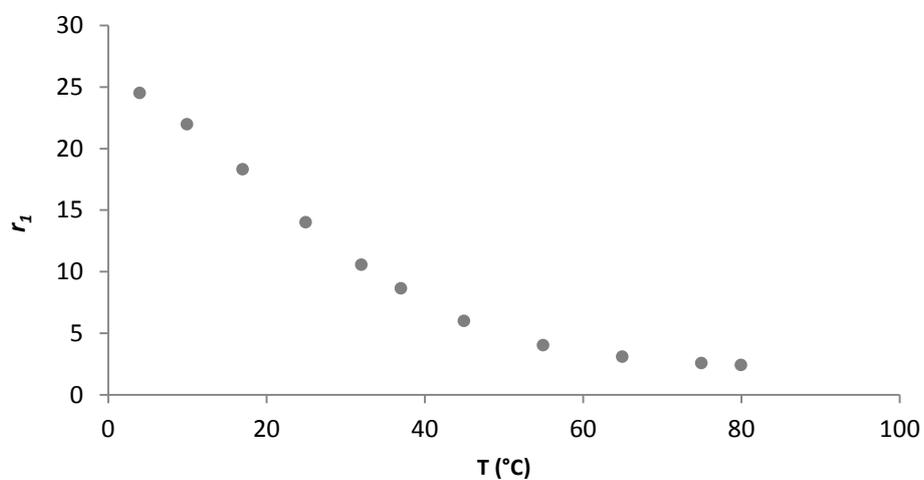


Figure S2. Temperature dependence of the water proton relaxivity for GdL (20MHz , 1mM, pH 6.0).

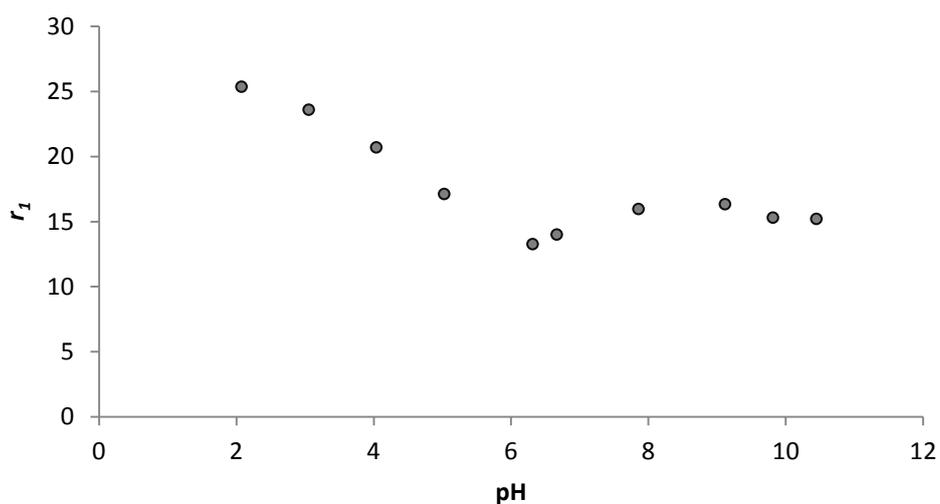


Figure S3. pH dependence of the water proton relaxivity for GdL (20 MHz, 1 mM, 25 °C).

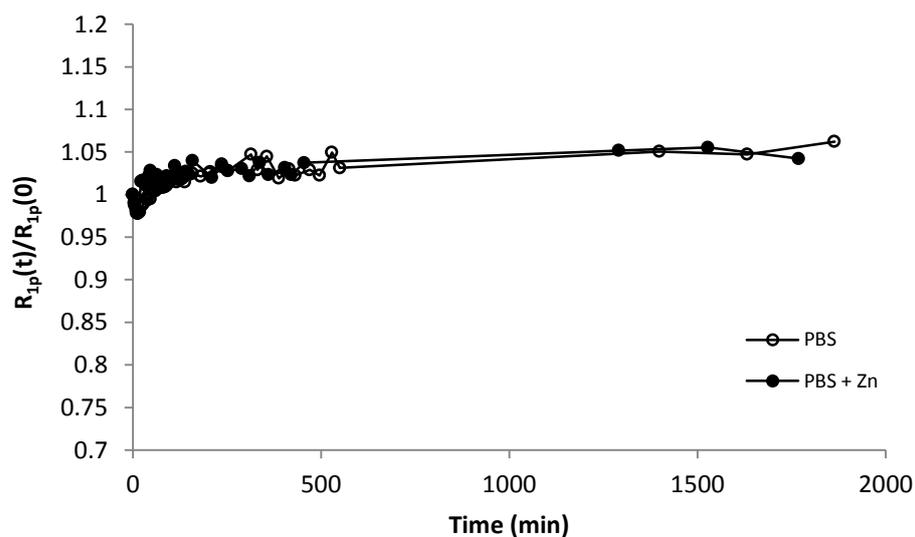


Figure S4. Evolution of the relative water proton paramagnetic longitudinal relaxation rate $R_{1p}(t)/R_{1p}(0)$ (20 MHz, pH 7.1, 25 °C) for a 1.5 mM solution of GdL in 10 mM phosphate buffer(○) and in phosphate buffer containing an equimolar amount of Zn^{2+} (●).

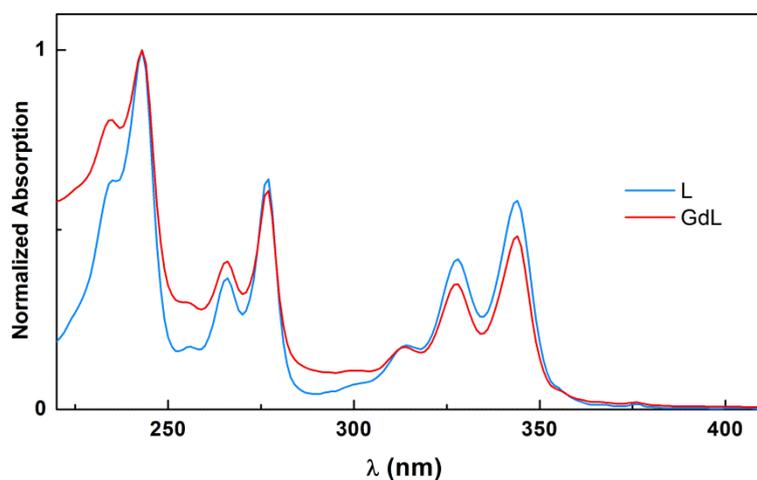


Figure S5. UV-Vis spectra for the free ligand **L** and for the Gd**L** complex in water (1.0×10^{-5} M, pH 7.0, 25 °C).

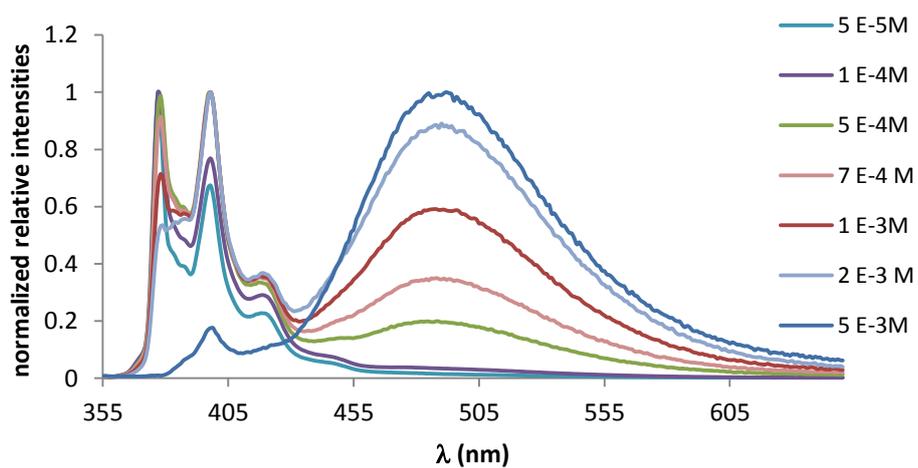


Figure S6. Fluorescence spectra for free ligand **L** in non-deoxygenated water (pH 7.0, 25 °C) over the concentration range 5×10^{-5} - 5×10^{-3} mol.dm⁻³ (λ_{exc} = 345 nm).

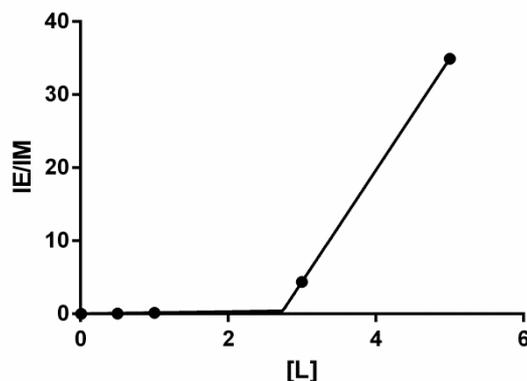


Figure S7. Changes in the emission properties of the free ligand **L** as the ratio of the fluorescence emission intensity for the *excimer* (490 nm) and for the monomer (377 nm) (I_E/I_M) as function of ligand concentration. Line fitted to **equation S1**.

The experimental data in **Figure 5** (manuscript) and **Figure S6** were fitted (Prima GraphPad) to a sigmoidal model (**equation S1**).[1,2]

$$\left(\frac{I_{Exc}}{I_{Mono}}\right) = \left(\frac{I_{Exc}}{I_{Mono}}\right)_0 + A_1 c + d(A_2 - A_1) \ln\left(\frac{1 + e^{(c-cmc)/d}}{e^{-cmc/d}}\right)$$

Equation S1

c - complex (GdL) or free ligand (**L**) concentration;

A_1 and A_2 - represent the limits of sigmoidal function that describes the behaviour of $d(I_{Exc}/I_{Mono})/dc$ (derivative of I_{Exc}/I_{Mono} in order to c);

d - is the time constant of the function and is related to the range of concentration over which the abrupt change in I_{Exc}/I_{Mono} occurs;

cmc - is the center of the sigmoidal function.

Table S1. Best fit values for the fitting (Prima GraphPad) of the experimental data of I_{Exc}/I_{Mono} vs [GdL] (**Figure 5** in manuscript and **Figure S6**) to **equation S1**.

Best-fit values	GdL
$(I_{Exc}/I_{Mono})_0$	-1,764
A_1	0,3660
d	0,007795
A_2	3,119
cmc	0,6454
Std. Error	
$(I_{Exc}/I_{Mono})_0$	0,007529
A_1	0,06346
d	71873
A_2	0,002088
cmc	0,01454

The E titration data (**Figure 7** in manuscript) were fitted to **equation S2** with n , number of equivalent binding sites, fixed to 1.

$$R_1^{pobs} = 10^3 \times \left\{ \left(r_1^f \cdot c_1 \right) + \frac{1}{2} \left(r_1^e - r_1^f \right) \times \left(n \cdot c_{HSA} + c_1 + K_A^{-1} - \sqrt{\left(n \cdot c_{HSA} + c_1 + K_A^{-1} \right)^2 - 4 \cdot n \cdot c_{HSA} \cdot c_1} \right) \right\}$$

Equation S2

n - number of equivalent binding sites, fixed to 1 in the fitting.
 r_1^f - relaxivity of the free GdL form, non-complexed with HSA
 r_1^e - relaxivity of the GdL form bound to HSA
 c_1 - analytical concentration of GdL
 c_{HSA} - analytical concentration of HSA
 K_A - association constant GdL/HSA

Table S2. Biodistribution, stated as percentage of injected dose per gram of organ (%ID/g \pm SD), of ^{153}Sm in Wistar rats at 1 and 24 hours after *i.v.* injection. Results are the mean of 4 animals.

Organ	1 hour %ID+SD	24 hours %ID+SD
Blood	0.3071 \pm 0.0290	0.0015 \pm 6.5 $\times 10^{-5}$
Liver	0.2095 \pm 0.0617	0.4701 \pm 0.1216
Spleen	0.1784 \pm 0.0581	0.3012 \pm 0.1646
Kidney	0.0629 \pm 0.0105	0.0242 \pm 0.0026
Heart	0.0385 \pm 0.0224	0.0044 \pm 0.0014
Lung	0.1084 \pm 0.0220	0.0274 \pm 0.0104
S. Intest.	0.0352 \pm 0.0190	0.0066 \pm 0.0011
L. Intest.	0.0125 \pm 0.0015	0.0035 \pm 0.0029
Bone	0.0365 \pm 0.0078	0.0411 \pm 0.0057
Muscle	0.0095 \pm 0.0048	0.011 \pm 0.00010
Brain + Cerebellum	0.0213 \pm 0.0134	0.0007 \pm 8.15 $\times 10^{-5}$

APPENDIX 1: Analysis of NMRD and ^{17}O NMR data

NMRD and ^{17}O NMR data have been analysed within the framework of Solomon-Bloembergen-Morgan theory.

^{17}O NMR spectroscopy

From the measured ^{17}O NMR relaxation rates and angular frequencies of the paramagnetic solutions, $1/T_1$, $1/T_2$ and ω , and of the acidified water reference, $1/T_{1A}$, $1/T_{2A}$ and ω_A , one can calculate the reduced relaxation rates and chemical shifts, $1/T_{2r}$ and $\Delta\omega_r$, which may be written as in Equations (A1)-(A3), where, $1/T_{1m}$, $1/T_{2m}$ is the relaxation rate of the bound water and $\Delta\omega_m$ is the chemical shift difference between bound and bulk water, τ_m is the mean residence time or the inverse of the water exchange rate k_{ex} and P_m is the mole fraction of the bound water. [3, 4]

$$\frac{1}{T_{1r}} = \frac{1}{P_m} \left[\frac{1}{T_1} - \frac{1}{T_{1A}} \right] = \frac{1}{T_{1m} + \tau_m} + \frac{1}{T_{1os}} \quad (\text{A1})$$

$$\frac{1}{T_{2r}} = \frac{1}{P_m} \left[\frac{1}{T_2} - \frac{1}{T_{2A}} \right] = \frac{1}{\tau_m} \frac{T_{2m}^{-2} + \tau_m^{-1} T_{2m}^{-1} + \Delta\omega_m^2}{(\tau_m^{-1} + T_{2m}^{-1})^2 + \Delta\omega_m^2} + \frac{1}{T_{2os}} \quad (\text{A2})$$

$$\Delta\omega_r = \frac{1}{P_m} (\omega - \omega_A) = \frac{\Delta\omega_m}{(1 + \tau_m T_{2m}^{-1})^2 + \tau_m^2 \Delta\omega_m^2} + \Delta\omega_{os} \quad (\text{A3})$$

The outer sphere contributions to the ^{17}O relaxation rates $1/T_{1os}$ and $1/T_{2os}$ can be neglected according to previous studies.[5] Therefore, Equations (A1-A2) can be further

simplified into Equations (A4) and (A5):

$$\frac{1}{T_{1r}} = \frac{1}{T_{1m} + \tau_m} \quad (\text{A4})$$

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m} \quad (\text{A5})$$

The exchange rate is supposed to obey the Eyring equation. In equation (A6) ΔS^\ddagger and ΔH^\ddagger are the entropy and enthalpy of activation for the water exchange process, and k_{ex}^{298} is the exchange rate at 298.15 K.

$$\frac{1}{\tau_m} = k_{\text{ex}} = \frac{k_B T}{h} \exp\left\{\frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT}\right\} = \frac{k_{\text{ex}}^{298} T}{298.15} \exp\left\{\frac{\Delta H^\ddagger}{R} \left(\frac{1}{298.15} - \frac{1}{T}\right)\right\} \quad (\text{A6})$$

In the transverse relaxation, the scalar contribution, $1/T_{2\text{sc}}$, is the most important [Equation (A7)]. $1/\tau_{s1}$ is the sum of the exchange rate constant and the electron spin relaxation rate [Equation (A8)].

$$\frac{1}{T_{2m}} \cong \frac{1}{T_{2\text{sc}}} = \frac{S(S+1)}{3} \left(\frac{A}{\hbar}\right)^2 \left(\tau_{s1} + \frac{\tau_{s2}}{1 + \omega_S^2 \tau_{s2}^2}\right) \quad (\text{A7})$$

$$\frac{1}{\tau_{s1}} = \frac{1}{\tau_m} + \frac{1}{T_{1e}} \quad (\text{A8})$$

The ^{17}O longitudinal relaxation rates in Gd^{3+} solutions are the sum of the contributions of the dipole-dipole (dd) and quadrupolar (q) mechanisms as expressed by Equations

(A11-A13) for non-extreme narrowing conditions, where γ_S is the electron and γ_I is the nuclear gyromagnetic ratio ($\gamma_S = 1.76 \times 10^{11} \text{ rad s}^{-1} \text{ T}^{-1}$, $\gamma_I = -3.626 \times 10^7 \text{ rad s}^{-1} \text{ T}^{-1}$), r_{GdO} is the effective distance between the electron charge and the ^{17}O nucleus, I is the nuclear spin (5/2 for ^{17}O), χ is the quadrupolar coupling constant and η is an asymmetry parameter :

$$\frac{1}{T_{1m}} = \frac{1}{T_{1dd}} + \frac{1}{T_{1q}} \quad (\text{A9})$$

with:

$$\frac{1}{T_{1dd}} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{GdO}^6} S(S+1) \times [3J(\omega_I; \tau_{d1}) + 7J(\omega_S; \tau_{d2})] \quad (\text{A10})$$

$$\frac{1}{T_{1q}} = \frac{3\pi^2}{10} \frac{2I+3}{I^2(2I-1)} \chi^2 (1 + \eta^2/3) \times [0.2J_1(\omega_I) + 0.8J_2(\omega_I)] \quad (\text{A11})$$

In Equation (A3) the chemical shift of the bound water molecule, $\Delta\omega_m$, depends on the hyperfine interaction between the Gd^{3+} electron spin and the ^{17}O nucleus and is directly proportional to the scalar coupling constant, $\frac{A}{\hbar}$, as expressed in Equation (A12).[6]

$$\Delta\omega_m = \frac{g_L \mu_B S(S+1) B}{3k_B T} \frac{A}{\hbar} \quad (\text{A12})$$

The isotopic Landé g factor is equal to 2.0 for the Gd^{3+} , B represents the magnetic field, and k_B is the Boltzmann constant.

The outer-sphere contribution to the chemical shift is assumed to be linearly related to $\Delta\omega_m$ by a constant C_{os} [Equation (A13)]. [7]

$$\Delta\omega_{os} = C_{os} \Delta\omega_m \quad (\text{A13})$$

NMRD

The measured longitudinal proton relaxation rate, $R_1^{obs} = 1/T_1^{obs}$, is the sum of a paramagnetic and a diamagnetic contribution as expressed in Equation (A14), where r_1 is the proton relaxivity:

$$R_1^{obs} = R_1^d + R_1^p = R_1^d + r_1 [\text{Gd}^{3+}] \quad (\text{A14})$$

The relaxivity can be divided into an inner and an outer sphere term as follows:

$$r_1 = r_{1is} + r_{1os} \quad (\text{A15})$$

The inner sphere term is given in Equation (A16), where q is the number of inner sphere

water molecules. [8]

$$r_{is} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{1m}^H + \tau_m} \quad (\text{A16})$$

The longitudinal relaxation rate of inner sphere protons, $1/T_{1m}^H$ is expressed by Equation (A11), where r_{GdH} is the effective distance between the electron charge and the ^1H nucleus, ω_I is the proton resonance frequency and ω_S is the Larmor frequency of the Gd^{3+} electron spin.

$$\frac{1}{T_{1m}^H} = \frac{2}{15} \left(\frac{\mu_0}{4\pi} \right)^2 \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{r_{GdH}^6} S(S+1) \times [3J(\omega_I; \tau_{d1}) + 7J(\omega_S; \tau_{d2})] \quad (\text{A17})$$

$$\frac{1}{\tau_{di}} = \frac{1}{\tau_m} + \frac{1}{\tau_{RH}} + \frac{1}{T_{ie}} \quad \text{for } i = 1, 2 \quad (\text{A18})$$

where τ_{RH} is the rotational correlation time of the $\text{Gd-H}_{\text{water}}$ vector.

For small molecular weight chelates (fast rotation), the spectral density function is expressed as in Equation (A19).

$$J(\omega; \tau) = \left(\frac{\tau}{1 + \omega^2 \tau^2} \right) \quad (\text{A19})$$

For slowly rotating species, the spectral density functions are described the Lipari-Szabo approach.[9] In this model we distinguish two statistically independent motions;

a rapid local motion with a correlation time τ_l and a slower global motion with a correlation time τ_g . Supposing the global molecular reorientation is isotropic, the relevant spectral density functions are expressed as in Equations (A20-A24), where the general order parameter S^2 describes the degree of spatial restriction of the local motion. If the local motion is isotropic, $S^2 = 0$; if the rotational dynamics is only governed by the global motion, $S^2 = 1$.

$$J(\omega_I; \tau_{d1}) = \left(\frac{S^2 \tau_{d1g}}{1 + \omega_I^2 \tau_{d1g}^2} + \frac{(1 - S^2) \tau_{d1}}{1 + \omega_I^2 \tau_{d1}^2} \right) \quad (\text{A20})$$

$$J(\omega_S; \tau_{d2}) = \left(\frac{S^2 \tau_{d2g}}{1 + \omega_S^2 \tau_{d2g}^2} + \frac{(1 - S^2) \tau_{d2}}{1 + \omega_S^2 \tau_{d2}^2} \right) \quad (\text{A21})$$

$$\frac{1}{\tau_{dig}} = \frac{1}{\tau_m} + \frac{1}{\tau_g} + \frac{1}{T_{ie}} \quad i = 1, 2 \quad (\text{A22})$$

$$\frac{1}{\tau} = \frac{1}{\tau_g} + \frac{1}{\tau_l} \quad (\text{A23})$$

$$J_i(\omega_I) = \left(\frac{S^2 \tau_g}{1 + i^2 \omega_I^2 \tau_g^2} + \frac{(1 - S^2) \tau}{1 + i^2 \omega_I^2 \tau^2} \right) \quad i = 1, 2 \quad (\text{A24})$$

The rotational correlation time, τ_{RH} is assumed to have simple exponential temperature dependence with an E_R activation energy as given in Equation (A25).

$$\tau_{RO} = \tau_{RO}^{298} \exp \left[\frac{E_R}{R} \left(\frac{1}{T} - \frac{1}{298.15} \right) \right] \quad (\text{A25})$$

The outer-sphere contribution can be described by Equations (A26 and A27) where N_A is the Avogadro constant, and J_{os} is its associated spectral density function as given by Equation (A15). [10, 11]

$$r_{ios} = \frac{32 N_A \pi \left(\frac{\mu_0}{4 \pi} \right)^2 \hbar^2 \gamma_S^2 \gamma_I^2}{405 a_{GdH} D_{GdH}} S(S+1) [3 J_{os}(\omega_1, T_{1e}) + 7 J_{os}(\omega_S, T_{2e})] \quad (\text{A26})$$

$$J_{os}(\omega, T_{je}) = \text{Re} \left[\frac{1 + 14 \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2}}{1 + \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{1/2} + 49 \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) + 19 \left(i\omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right)^{3/2}} \right] \quad (\text{A27})$$

$j=1,2$

The longitudinal and transverse electronic relaxation rates, $1/T_{1e}$ and $1/T_{2e}$ are expressed by Equation (A28 and A29), where τ_v is the electronic correlation time for the modulation of the zero-field-splitting interaction, E_v the corresponding activation energy and Δ^2 is the mean square zero-field-splitting energy. We assumed a simple exponential dependence of τ_v versus $1/T$ as written in Equation (A30).

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25} \Delta^2 \tau_v \{4S(S+1) - 3\} \left(\frac{1}{1 + \omega_S^2 \tau_v^2} + \frac{4}{1 + 4\omega_S^2 \tau_v^2} \right) \quad (\text{A28})$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS} = \Delta^2 \tau_v \left(\frac{5.26}{1 + 0.372\omega_S^2 \tau_v^2} + \frac{7.18}{1 + 1.24\omega_S^2 \tau_v^2} \right) \quad (\text{A29})$$

$$\tau_v = \tau_v^{298} \exp \left[\frac{E_v}{R} \left(\frac{1}{T} - \frac{1}{298.15} \right) \right] \quad (\text{A30})$$

The diffusion coefficient for the diffusion of a water proton away from a Gd^{3+} complex, D_{GdH} , is assumed to obey an exponential law versus the inverse of the temperature, with activation energy E_{DGdH} , as given in Equation (A31). D_{GdH}^{298} is the diffusion coefficient at 298.15K.

$$D_{GdH} = D_{GdH}^{298} \exp \left\{ \frac{E_{GdH}}{R} \left(\frac{1}{298.15} - \frac{1}{T} \right) \right\} \quad (\text{A31})$$

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