Electronic Supplementary Information to

Optimizing the high-field relaxivity by self-assembling of macrocyclic Gd(III) complexes

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Equations used for the analysis of NMRD data

The measured longitudinal proton relaxation rate, $R_{1}^{\text{obs}}$, is the sum of a paramagnetic and a diamagnetic contribution as expressed in Eq. [1], where $r_1$ is the proton relaxivity:

$$R_{1}^{\text{obs}} = R_{1}^{d} + R_{1}^{p} = R_{1}^{d} + r_1 \left( Gd^{3+} \right)$$  \[1\]

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

$$r_1 = r_{1is} + r_{1ss} + r_{1os}$$  \[2\]

The inner sphere term is given in Eq. [3], where $q$ is the number of inner sphere water molecules.\(^1\)

$$r_{1is} = \frac{1}{1000} \times \frac{q}{55.55} \times \frac{1}{T_{1m}^H + \tau_m}$$  \[3\]

The longitudinal relaxation rate of inner sphere protons, $1/T_{1m}^H$, is expressed by Eq. [4]:

$$\frac{1}{T_{1m}^H} = \frac{2}{15} \left( \frac{\mu_0}{4\pi} \right)^2 \gamma_i^2 g^2 \frac{\mu_B^2}{r_{GdH}} S(S+1) \left[ \frac{3\tau_{d1}}{1 + \omega_i^2 \tau_{d1}^2} + \frac{7\tau_{d2}}{1 + \omega_S^2 \tau_{d2}^2} \right]$$  \[4\]

where $r_{GdH}$ is the effective distance between the electron charge and the $^1H$ nucleus, $\omega_i$ is the proton resonance frequency and $\omega_S$ is the Larmor frequency of the Gd(III) electron spin.

$$\frac{1}{\tau_{di}} = \frac{1}{\tau_m} + \frac{1}{\tau_{RH}} + \frac{1}{T_{ie}} \quad i = 1 , 2$$  \[5\]

The longitudinal and transverse electronic relaxation rates, $1/T_{ie}$ and $1/T_{2e}$ are expressed by Eq. [7]-[8], where $\tau_V$ is the electronic correlation time for the modulation of the zero-field-splitting interaction, $E_V$ the corresponding activation energy and $\Delta^2$ is the mean square zero-field-splitting energy. We assumed a simple exponential dependence of $\tau_V$ versus $1/T$ as written in Eq. [9].

$$\left( \frac{1}{T_{ie}} \right)^{ZFS} = \frac{1}{25} \Delta^2 \tau_V \left[ 4S(S+1) - 3 \right] \left( \frac{1}{1 + \omega_S^2 \tau_\nu^2} + \frac{4}{1 + 4\omega_S^2 \tau_\nu^2} \right)$$  \[7\]
\[
\left( \frac{1}{T_{2e}} \right)^{ZFS} = \Delta^2 \tau_{v} \left( \frac{5.26}{1 + 0.372 \omega_{S}^{2} \tau_{v}} + \frac{7.18}{1 + 1.24 \omega_{S} \tau_{v}} \right) \quad [8]
\]

\[
\tau_{v} = \tau_{v}^{298} \exp \left\{ \frac{E_{v}}{R} \left( \frac{1}{T} - \frac{1}{298.15} \right) \right\} \quad [9]
\]

The outer-sphere contribution can be described by Eq. [10] where \( N_{A} \) is the Avogadro constant, and \( J_{os} \) is its associated spectral density function:\textsuperscript{2,3}

\[
r_{1os} = \frac{32 N_{A} \pi}{405} \left( \frac{\mu_{0}}{4\pi} \right)^{2} \frac{\hbar^{2} \gamma_{S}^{2} \gamma_{J}^{2}}{a_{GdH} D_{GdH}} S (S + 1) \left[ 3 J_{os} (\omega_{1}; T_{1e}) + 7 J_{os} (\omega_{S}; T_{2e}) \right] \quad [10]
\]

\[
J^{OS}(\omega, T_{je}) = \text{Re} \left[ \frac{1 + \frac{1}{4} \left( i \omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) \frac{1}{2}}{1 + \left( i \omega \tau_{GdH} + \frac{\tau_{GdH}}{T_{je}} \right) \frac{1}{2}} \right]^{\frac{1}{2}} \quad [11]
\]

where \( j = 1, 2 \), \( \tau_{GdH} = \frac{a_{GdH}^{2}}{D_{GdH}} \).

The same set of equations [3-5] can be used to evaluate the contribution to \( r_{1} \) of the water molecules in the second coordination sphere, \( r_{1ss} \textsuperscript{4,5} \).


\textbf{Equations used for the analysis of^{17}O NMR data}
From the measured $^{17}$O NMR transversal relaxation rates and angular frequencies of the paramagnetic solutions, $1/T_1$, $1/T_2$ and $\omega$, and of the acidified water reference, $1/T_{1A}$, $1/T_{2A}$ and $\omega_A$, one can calculate the reduced relaxation rates, $1/T_{1r}$, $1/T_{2r}$ and $\omega_r$, and of the acidified water reference, $1/T_{1m}$, $1/T_{2m}$ and $\omega_m$, one can calculate the reduced relaxation rates, $1/T_{1r}$, $1/T_{2r}$ and $\omega_r$, and of the acidified water reference, $1/T_{1m}$, $1/T_{2m}$ and $\omega_m$, one can calculate the reduced relaxation rates, $1/T_{1r}$, $1/T_{2r}$ and $\omega_r$, and of the acidified water reference, $1/T_{1m}$, $1/T_{2m}$ and $\omega_m$.

$$\frac{1}{T_{2r}} = \frac{1}{P_m \left[\frac{1}{T_2} - \frac{1}{T_{2A}}\right]} = \frac{1}{\tau_m \left[\frac{1}{T_2} - \frac{1}{T_{2A}}\right]} + \frac{1}{\tau_m \left[\frac{1}{T_{2m}} - \frac{1}{T_{2A}}\right]} + \frac{1}{\tau_m \left[\frac{1}{T_{2m}} - \frac{1}{T_{2A}}\right]} + \frac{1}{\tau_m \left[\frac{1}{T_{2m}} - \frac{1}{T_{2A}}\right]} [1]$$

$$\Delta \omega_r = \frac{1}{P_m} (\omega - \omega_A) = \frac{\Delta \omega_m}{(1 + \frac{\tau_m}{T_{2m}})^2 + \tau_m^2 \Delta \omega_m^2} + \Delta \omega_m [2]$$

Previous studies have shown that outer sphere contributions to the $^{17}$O relaxation rates are negligible.\(^3\) Equation [1] can be further simplified:

$$\frac{1}{T_{2r}} = \frac{1}{T_{2m} + \tau_m} [3]$$

The exchange rate is supposed to assume the Eyring equation. In Eq. [4] $\Delta S^\ddagger$ and $\Delta H^\ddagger$ are the entropy and enthalpy of activation for the water exchange process, and $k_{ex}^{298}$ is the exchange rate at 298.15 K.

$$\frac{1}{\tau_m} = k_{ex} = \frac{k_B T}{h} \exp \left\{ \frac{\Delta S^\ddagger}{R} - \frac{\Delta H^\ddagger}{RT} \right\} = \frac{k_{ex}^{298} T}{298.15} \exp \left\{ \frac{\Delta H^\ddagger}{R} \left( \frac{1}{298.15} - \frac{1}{T} \right) \right\} [4]$$

In the transverse relaxation the scalar contribution, $1/T_{2sc}$, is the most important, Eq. [5]. $1/\tau_{s1}$ is the sum of the exchange rate constant and the electron spin relaxation rate.

$$\frac{1}{T_{2m}} \approx \frac{1}{T_{2SC}} = \frac{S(S+1)}{3} \left( \frac{A}{h} \right)^2 \tau_{s1} [5]$$

$$\frac{1}{\tau_{s1}} = \frac{1}{\tau_m} + \frac{1}{T_{1e}} [6]$$

**Binding Interaction of the two Gd(III) complexes**

The binding parameters involved in the non-covalent interaction between two paramagnetic complexes, GdL₁ and GdL₂, can be determined by relaxometry using the well-consolidated Proton Relaxation Enhancement (PRE) method.

In a solution containing the two interacting systems, the observed water protons longitudinal relaxation rate is expressed by the following relationship:

$$R_{\text{obs}}^1 = r_1^{\text{GdL}_1} \left[ \text{GdL}_1 \right] + r_1^{\text{GdL}_2} \left[ \text{GdL}_2 \right] + r_1^{\text{GdL}_1\text{L}_2} \left[ \text{GdL}_1\text{L}_2 \right] + R_{\text{dia}}^1$$  \[1\]

where the $r_1$ terms refer to the relaxivities, normalized to 1 mM, of the different paramagnetic species present in the solution. Note that $r_1^{\text{GdL}_1\text{L}_2}$ represents the molecular relaxivity of the adduct, i.e., is two times higher than the average value normalized to 1 mM concentration of Gd(III). $R_{\text{dia}}^1$ is the diamagnetic contribution measured by replacing the Gd(III) complexes with a diamagnetic analogue.

The concentration of both the unbound complexes can be expressed in terms of the bound species:

$$R_{\text{obs}}^1 = r_1^{\text{GdL}_1} \left[ \text{GdL}_1 \right] + r_1^{\text{GdL}_2} \left[ \text{GdL}_2 \right] + r_1^{\text{GdL}_1\text{L}_2} \left[ \text{GdL}_1\text{L}_2 \right] + R_{\text{dia}}^1$$  \[2\]

$$R_{\text{obs}}^1 - R_{\text{dia}}^1 = r_1^{\text{GdL}_1} \left[ \text{GdL}_1 \right] \left( G_1 - r_1^{\text{GdL}_1} - r_1^{\text{GdL}_2} \right) + r_1^{\text{GdL}_2} \left[ \text{GdL}_2 \right] + r_1^{\text{GdL}_1\text{L}_2} \left[ \text{GdL}_1\text{L}_2 \right] + \left[ \text{GdL}_1 \right] + \left[ \text{GdL}_2 \right]$$  \[3\]

where GdL₁ₜ and GdL₂ₜ represent the total concentration of the two complexes in the aqueous solution.

The concentration of the adduct can be obtained by the expression of the chemical equilibrium:

$$\text{GdL}_1 + \text{GdL}_2 \rightleftharpoons \text{GdL}_1\text{L}_2$$  \[4\]
The combination between Eqs. 3 and 6 allows the analysis of the relaxation rate dependence on the total concentration of one of the two complexes chosen as independent variable in the titration (e.g. GdL$^2$ in the experiment reported in Fig. 7 left).

\[ K_A = \frac{[\text{Gd}L_1^2]}{[\text{Gd}L^1][\text{Gd}L^2]} = \frac{[\text{Gd}L_1^2]}{[\text{Gd}L^1_\gamma - \text{Gd}L_1^2][\text{Gd}L^2_\gamma - \text{Gd}L_1^2]} \quad [5] \]

\[ [\text{Gd}L_1^2] = \frac{(K_A \text{Gd}L^1_\gamma + K_A \text{Gd}L^2_\gamma + 1) - \sqrt{(K_A \text{Gd}L^1_\gamma + K_A \text{Gd}L^2_\gamma + 1)^2 - 4K_A^2 \text{Gd}L^1_\gamma \text{Gd}L^2_\gamma}}{2K_A} \quad [6] \]
**Figure S1.** pH dependence of the longitudinal water proton relaxivity at 20 MHz and 25 °C of GdL²