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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^{obs} is the sum of a paramagnetic and a diamagnetic contribution as expressed in Eq. **[1]**, where r_1 is the proton relaxivity:

$$R_1^{obs} = R_1^d + R_1^p = R_1^d + r_1\left[Gd^{3+}\right] \qquad \textbf{[1]}$$

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

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

The inner sphere term is given in Eq. **[3]**, where q is the number of inner sphere water molecules.[1]

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

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

$$\frac{1}{T_{1m}^H} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g^2 \mu_B^2}{r_{GdH}^6} S(S+1)\left[\frac{3\tau_{d1}}{1+\omega_I^2\tau_{d1}^2} + \frac{7\tau_{d2}}{1+\omega_S^2\tau_{d2}^2}\right] \qquad \textbf{[4]}$$

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

$$\frac{1}{\tau_{\mathrm{d}i}} = \frac{1}{\tau_{\mathrm{m}}} + \frac{1}{\tau_{\mathrm{RH}}} + \frac{1}{T_{i\mathrm{e}}} \qquad i = 1\ ,2 \qquad \textbf{[5]}$$

The longitudinal and transverse electronic relaxation rates, $1/T_{1e}$ and $1/T_{2e}$ are expressed by Eq. **[7]-[8]**, 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 Eq. **[9]**.

$$\left(\frac{1}{T_{1e}}\right)^{ZFS} = \frac{1}{25}\Delta^2\tau_v\left\{4S(S+1)-3\right\}\left(\frac{1}{1+\omega_S^2\tau_v^2} + \frac{4}{1+4\omega_S^2\tau_v^2}\right) \qquad \textbf{[7]}$$

$$\left(\frac{1}{T_{2e}}\right)^{ZFS}=\Delta^2\tau_v\left(\frac{5.26}{1+0.372\omega_S^2\tau_v^2}+\frac{7.18}{1+1.24\omega_S\tau_v}\right) \qquad \textbf{[8]}$$

$$\tau_v=\tau_v^{298}\exp\left\{\frac{E_v}{R}\left(\frac{1}{T}-\frac{1}{298.15}\right)\right\} \qquad \textbf{[9]}$$

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.[2,3]

$$r_{1os}=\frac{32N_A\pi}{405}\left(\frac{\mu_0}{4\pi}\right)^2\frac{h^2\gamma_S^2\gamma_I^2}{a_{GdH}D_{GdH}}S\left(S+1\right)\left[3J_{os}\left(\omega_I;T_{1e}\right)+7J_{os}\left(\omega_S;T_{2e}\right)\right] \qquad \textbf{[10]}$$

$$J^{OS}\left(\omega,T_{je}\right)=\mathrm{Re}\left[\frac{1+\frac{1}{4}\left(i\omega\tau_{GdH}+\frac{\tau_{GdH}}{T_{je}}\right)^{1/2}}{1+\left(i\omega\tau_{GdH}+\frac{\tau_{GdH}}{T_{je}}\right)^{1/2}+\frac{4}{9}\left(i\omega\tau_{GdH}+\frac{\tau_{GdH}}{T_{je}}\right)+\frac{1}{9}\left(i\omega\tau_{GdH}+\frac{\tau_{GdH}}{T_{je}}\right)^{3/2}}\right] \qquad \textbf{[11]}$$

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}.[4,5]

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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 ω, and of the acidified water reference, $1/T_{1A}$, $1/T_{2A}$ and ω_A, one can calculate the reduced relaxation rates, $1/T_{1r}$, $1/T_{2r}$ and reduced chemical shifts (Eq. **[1]** - **[2]**), where $1/T_{2m}$ 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.[1,2]

$$\frac{1}{T_{2r}}=\frac{1}{P_m}\left[\frac{1}{T_2}-\frac{1}{T_{2A}}\right]=\frac{1}{\tau_m}\frac{T_{2m}^{-2}+\tau_m^{-1}T_{2m}^{-1}+\Delta\omega_m^2}{\left(\tau_m^{-1}+T_{2m}^{-1}\right)^2+\Delta\omega_m^2}+\frac{1}{T_{2OS}} \qquad \textbf{[1]}$$

$$\Delta\omega_\mathrm{r}=\frac{1}{P_\mathrm{m}}(\omega-\omega_\mathrm{A})=\frac{\Delta\omega_\mathrm{m}}{(1+\tau_\mathrm{m}T_\mathrm{2m}^{-1})^2+\tau_\mathrm{m}^2\Delta\omega_\mathrm{m}^2}+\Delta\omega_{os} \qquad \textbf{[2]}$$

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

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

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

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

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

$$\frac{1}{T_{2m}}\cong\frac{1}{T_{2SC}}=\frac{S(S+1)}{3}\left(\frac{A}{\mathrm{h}}\right)^2\tau_{S1} \qquad \textbf{[5]}$$

$$\frac{1}{\tau_{S1}}=\frac{1}{\tau_m}+\frac{1}{T_{1e}} \qquad \textbf{[6]}$$

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Binding Interaction of the two Gd(III) complexes

The binding parameters involved in the non-covalent interaction between two paramagnetic complexes, GdL^1 and GdL^2, 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:

$$R_1^{obs} = r_1^{GdL^1}\left[GdL^1\right] + r_1^{GdL^2}\left[GdL^2\right] + r_1^{Gd_2L^1L^2}\left[Gd_2L^1L^2\right] + R_1^{dia} \qquad [1]$$

where the r_i terms refer to the relaxivities, normalized to 1 mM, of the different paramagnetic species present in the solution. Note that $r_1^{Gd2L1L2}$ 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_1^{dia} 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:

$$R_1^{obs} = r_1^{GdL^1}\left(GdL_T^1 - \left[Gd_2L^1L^2\right]\right) + r_1^{GdL_2}\left(GdL_T^2 - \left[Gd_2L^1L^2\right]\right) + r_1^{Gd_2L^1L^2}\left[Gd_2L^1L^2\right] + R_1^{dia} \qquad [2]$$

$$R_1^{obs} - R_1^{dia} = r_1^{GdL^1}\left[Gd_2L^1L^2\right]\left(r_1^{Gd_2L^1L^2} - r_1^{GdL^1} - r_1^{GdL^2}\right) + r_1^{GdL^1}GdL_T^1 + r_1^{GdL^2}GdL_T^2 \qquad [3]$$

where GdL^1_T and GdL^2_T 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:

$$GdL^1 + GdL^2 \leftrightarrow Gd_2L^1L^2 \qquad [4]$$

$$K_A = \frac{\left[Gd_2L^1L^2\right]}{\left[GdL^1\right]\left[GdL^2\right]} = \frac{\left[Gd_2L^1L^2\right]}{\left[GdL^1_T - Gd_2L^1L^2\right]\left[GdL^2_T - Gd_2L^1L^2\right]} \qquad [5]$$

$$\left[Gd_2L^1L^2\right] = \frac{\left(K_A GdL^1_T + K_A GdL^2_T + 1\right) - \sqrt{\left(K_A GdL^1_T + K_A GdL^2_T + 1\right)^2 - 4K_A^2 GdL^1_T GdL^2_T}}{2K_A} \qquad [6]$$

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).

Figure S1. pH dependence of the longitudinal water proton relaxivity at 20 MHz and 25 °C of Gd**L**^{2}