

**Supporting information**  
**for**

**PAMAM dendrimeric conjugates with a Gd-DOTA  
phosphinate derivative and their adducts with  
polyaminoacids: the interplay of global motion, internal  
rotation and fast water exchange**

*Jakub Rudovský,<sup>†</sup> Mauro Botta,<sup>\*‡</sup> Petr Hermann,<sup>\*†</sup> Kenneth I. Hardcastle,<sup>§</sup> Ivan Lukeš<sup>†</sup> and Silvio  
Aime<sup>||</sup>*

<sup>†</sup>Department of Inorganic Chemistry, Charles University, Hlavova 2030, 128 40 Prague 2; <sup>‡</sup>Department of Environmental and Life Sciences, University of Eastern Piedmont, "A. Avogadro", Via Bellini 25/G, I-15100 Alessandria, Italy; <sup>§</sup>Department of Chemistry, Emory University, 1515 Dickey Drive, Atlanta, GA 30322, U. S. A.; <sup>||</sup>Department of Chemistry I. F. M., University of Turin, Via P. Giuria 7, I-10125 Torino, Italy.

**Table of contents:**

Figure S1. Relaxometric titration of the G2-(DO3A-PBnN{CS})<sub>16</sub> conjugate with GdCl<sub>3</sub> (page 3)

TEXT. NMR titration of G2-PAMAM dendrimer - <sup>1</sup>H NMR signal assignment (pages 4–5)

Figure S2. <sup>1</sup>H NMR (400 MHz) titration of the G2-PAMAM dendrimer (page 5)

Figure S3. VT-<sup>17</sup>O and <sup>1</sup>H NMRD profiles for all conjugated Gd(III) complexes (page 6)

Figure S4. Comparison of the <sup>1</sup>H NMRD profiles for the G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> conjugate at variable pH (page 7)

Figure S5. Comparison of <sup>1</sup>H NMRD profiles of G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> and G2-(Gd/Y-DO3AP<sup>BnN{CS}</sup>)<sub>16</sub> (page 8)

Figure S6. Relaxometric titration of G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> conjugate solution with poly(Lys)<sub>17</sub> (page 9)

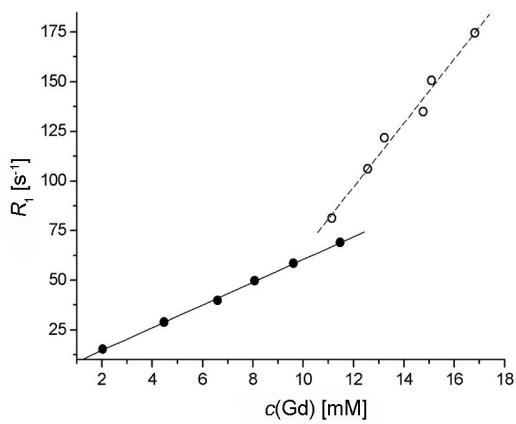
Figure S7. Dependence of proton relaxivity on pH for G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub>-poly(Arg)<sub>320</sub> system (page 10)

Table S1. Full relaxometric results for present PAMAM conjugates (page 11)

Table S2. Full relaxometric results for the G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub>-poly(Arg)<sub>56</sub> adduct (page 12)

Full SBM and LS equations in Scientist script (pages 13–17)

References (page 18)



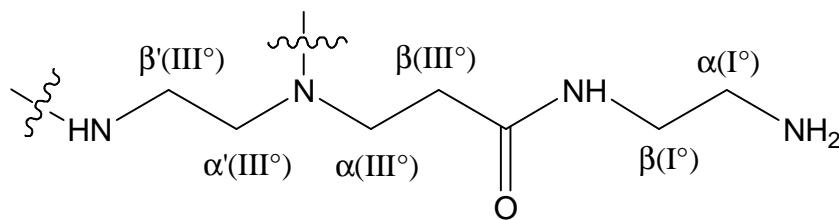
**Figure S1.**  $^1\text{H}$  NMR (400 MHz) relaxometric titration of a 1 mM solution of the G2-(DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> conjugate. The batch solution was divided into separate vials and  $\text{GdCl}_3$  was added up to 32 molar excess *per* conjugate. The lines are the linear fits to the experimental points. The slope of the solid line is  $5.7 \pm 0.1$  and indicates one coordinated water molecule. The slope of the dashed line is  $16 \pm 1$  and is the sum of the value for the conjugated complex and the “free” gadolinium aquaion  $[\text{Gd}(\text{H}_2\text{O})_8]^{3+}$ .

**The assignment of  $^1\text{H}$  NMR signals is spectra of pure G2-PAMAM dendrimer.** The assignment of tracked proton signals in  $^1\text{H}$  NMR PAMAM spectra followed the procedure of Sudmeier and Reilley.<sup>1</sup> This procedure assumes that the protons are deshielded with respect to their relative positions to the site of protonation. Also, if there are more sites that could be protonated, the overall effect is a linear combination of the local effects, expressed by Equation (S1):

$$\Delta d_i = \sum_{j=1}^N C_{ij} f_j \quad (\text{S1})$$

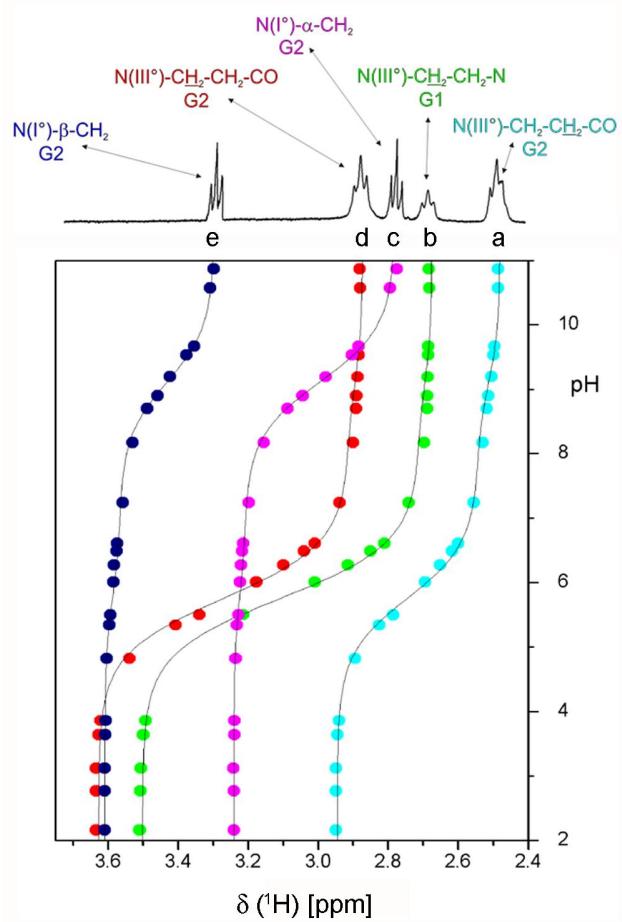
The overall change in chemical shift of the  $i$ th methylene group is given by the sum of protonated fractions  $f$  on the  $j$ th site adjacent to the group of interest multiplied by a respective protonation shift constant  $C$ . These constants were empirically determined over the large ensemble of related structures: e.g. for  $\text{N(I}^\circ\text{)}-\alpha\text{-CH}_2$  group  $C = 0.45$  for  $\text{N(I}^\circ\text{)}-\beta\text{-CH}_2$  it is 0.25 for  $\text{N(III}^\circ\text{)}-\alpha\text{-CH}_2$  it is 0.75 and for  $\text{N(III}^\circ\text{)}-\beta\text{-CH}_2$  it is 0.35. For this assignment it is assumed that there is one major protonation site for each methylene group in the PAMAM structure because both kinds of sites are well separated in the backbone (Chart S1).

**Chart S1.** Fragment of PAMAM structure with the assignment of  $^1\text{H}$  signals (for details see the text) with labeling used in the discussion.



The mutual intensities of the observed lines (downfield) is 2:1:2:2:2 (Figure S2). The well-resolved lines  $c$  and  $e$  show a chemical shift change of magnitude 0.35 and 0.25 ppm at pH 8.98, respectively. These

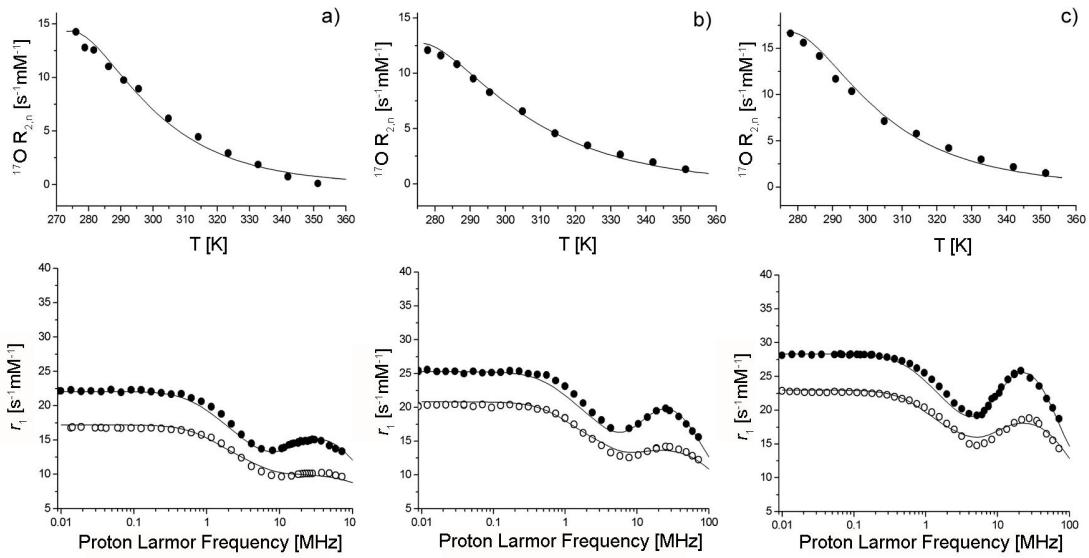
values correspond very well to  $\alpha$  and  $\beta$  CH<sub>2</sub> group adjacent to the terminal N(I°) amine, *i.e.* both signals include 32 protons.



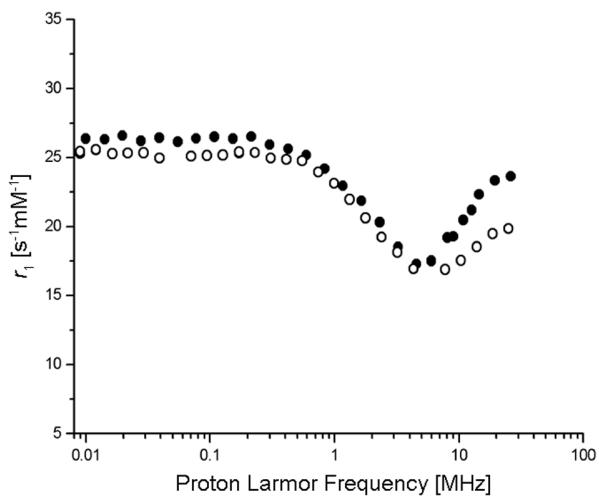
**Figure S2.** The <sup>1</sup>H NMR (400 MHz) titration of the G2-PAMAM dendrimer. The solid lines represent the best results from bi-sigmoidal simultaneous fitting.

The lines *b* and *d* show a major drop at pH 5.74 and the heights are about 0.9 ppm and 0.7 ppm, respectively, fitting to N(III°)- $\alpha$ -CH<sub>2</sub> protons. From their relative intensities in the spectra and the PAMAM architecture signal *b* corresponds to N(III°)-CH<sub>2</sub>-CH<sub>2</sub>-NH ( $\alpha'$ ) and signal *d* to N(III°)-CH<sub>2</sub>-CH<sub>2</sub>-CO ( $\alpha$ ). Finally, line *a* can be assigned to the N(III°)- $\beta$ -CH<sub>2</sub> protons as the main decrease is at pH

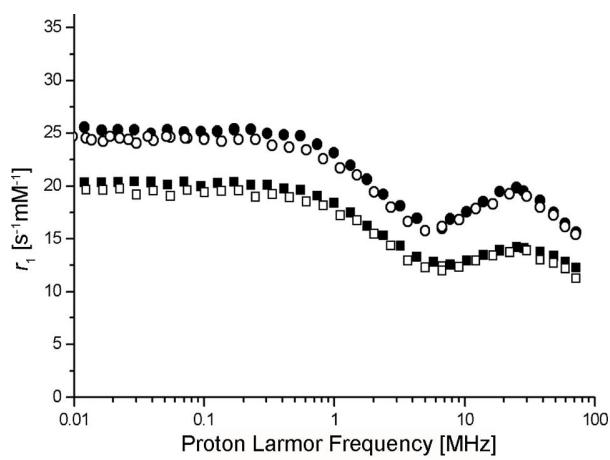
5.74 with height of 0.4 ppm. According to its somewhat lower chemical shift it was assigned to the N(III°)-CH<sub>2</sub>-CH<sub>2</sub>-CO group ( $\beta$ ).



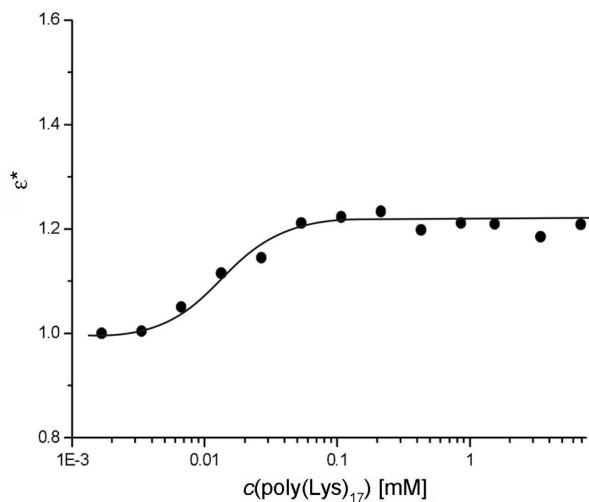
**Figure S3.**  $^{17}\text{O}$   $R_{2,n}$  (top) and  $^1\text{H}$  NMRD relaxation profiles (bottom; full circles 25 °C (●), open circles 37 °C (○)) for the G1-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>8</sub> (a), G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> (b) and G4-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>59</sub> (c) conjugates. Transverse relaxation rates ( $R_{2,n}$ ) were normalized to 1 mM concentration of gadolinium. The solid lines represent the best results of the simultaneous fitting to the experimental data (*vide infra*).



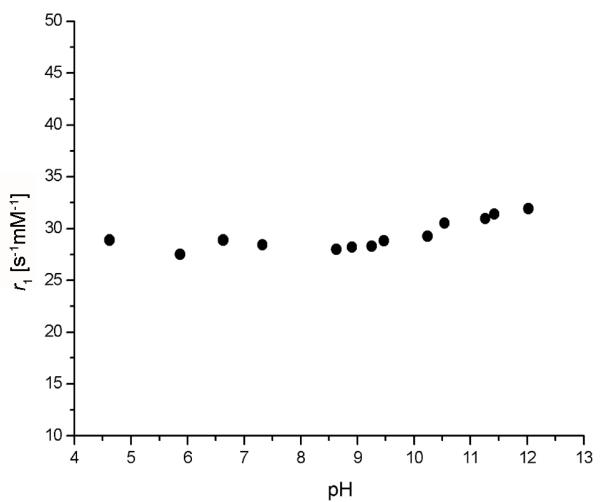
**Figure S4.** Comparison of the  $^1\text{H}$  NMRD profiles for the  $\text{G2-(Gd-DO3A-P}^{\text{BnN}\{\text{CS}\}}_{16}$  conjugate at pH 7.5 (open circles) and at pH 4.0 (full circles). Both profiles were measured at 25 °C.



**Figure S5.** Comparison of  $^1\text{H}$  NMRD profiles of G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> (full symbols) and G2-(Gd/Y-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> (open symbols) conjugates at pH 7 and 25 °C (circles) and 37 °C (squares).



**Figure S6.** Relaxometric titration of G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub> conjugate solution with poly(Lys)<sub>17</sub> (at 25 °C, pH 7.5 and 20 MHz). The solid line is just to guide the eyes. x-axis is in logarithmic scale.



**Figure S7.** The dependence of proton relaxivity on pH for G2-(Gd-DO3A-P<sup>BnN{CS}</sup>)<sub>16</sub>-poly(Arg)<sub>320</sub> system at 25 °C and 20 MHz.

**Table S1.** The full results of multi-parametrical simultaneous fitting of relaxation  $^1\text{H}$  and  $^{17}\text{O}$  NMR data at pH 7.5 for present PAMAM conjugates. The obtained values are shown in comparison with the values found for the parent  $\text{Gd}_2\text{-CS(DO3A-P}^{\text{BnN}\{\text{CS}\}})_2$  and  $\text{Gd-DO3A-P}^{\text{ABn}}$  complexes. The conjugates are labeled in a short form.

Parameter	Gadolinium(III) complex of				
	G1-8	G2-16	G4-59	$\text{CS(DO3A-P}^{\text{NBn}})_2$ <sup>a</sup>	$\text{DO3A-P}^{\text{ABnb}}$
$^{310}\text{r}_1 [\text{s}^{-1} \text{mM}^{-1}]$ <sup>c</sup>	10.1	14.1	18.6	6.1	4.2
$\Delta^2 [10^{20} \text{s}^{-2}]$	$0.1 \pm 0.03$	$0.07 \pm 0.04$	$0.06 \pm 0.04$	0.23	0.25
$^{298}\tau_{\text{M}}$ [ns]	$48 \pm 5$	$66 \pm 4$	$68 \pm 7$	53	16.2
$\Delta H_{\text{M}}^\# [\text{kJ mol}^{-1}]$	$55 \pm 5$	$50 \pm 4$	$51 \pm 5$	39	20.6
$^{298}\tau_{\text{Rg}}$ [ps]	$1560 \pm 280$	$2690 \pm 270$	$3140 \pm 210$	183	88
$E_{\text{rg}}$ [kJ]	$44 \pm 4$	$43 \pm 4$	$35 \pm 3$	37	29
$^{298}\tau_{\text{RI}}$ [ps]	$115 \pm 4$	$100 \pm 3$	$133 \pm 4$	—	—
$E_{\text{rl}}$ [kJ]	$27 \pm 2$	$20 \pm 1$	$28 \pm 1$	—	—
$^{298}\tau_{\text{v}}$ [ps]	$25 \pm 1$	$36 \pm 2$	$45 \pm 1$	15.9	11.2
$S^2$	$0.25 \pm 0.02$	$0.24 \pm 0.01$	$0.29 \pm 0.01$	—	—
$E_{\text{v}}$ [kJ]	<u>1</u>	<u>1</u>	<u>1</u>	1	1
$A/\hbar [10^6 \text{ rad s}^{-1}]$	<u>-2.89</u>	<u>-2.89</u>	<u>-2.89</u>	-2.89	-2.89
$R_{\text{GdO}}$ [ $\text{\AA}$ ]	<u>2.6</u>	<u>2.6</u>	<u>2.6</u>	2.6	2.6
$R_{\text{GdH}}$ [ $\text{\AA}$ ]	<u>3.1</u>	<u>3.1</u>	<u>3.1</u>	3.1	3.1
$A$ [ $\text{\AA}$ ]	<u>3.6</u>	<u>3.6</u>	<u>3.6</u>	3.6	3.6
$\Delta H_{\text{Mss}}^\# [\text{kJ}]$	<u>10</u>	<u>10</u>	<u>10</u>	10	15
$R_{\text{ss}}$ [ $\text{\AA}$ ]	<u>3.5</u>	<u>3.5</u>	<u>3.5</u>	3.5	3.6
$^{298}\tau_{\text{Mss}}$ [ns]	<u>1</u>	<u>1</u>	<u>1</u>	1	1
$q$	<u>1</u>	<u>1</u>	<u>1</u>	1	1
$q_{\text{ss}}$	<u>2</u>	<u>2</u>	<u>2</u>	1	1

<sup>a</sup> ref.<sup>2</sup>; <sup>b</sup> ref.<sup>3</sup>; <sup>c</sup> The relaxivity values were measured at 37 °C and 20 MHz; the underlined values were fixed during the fitting procedure.

**Table S2.** The full results of multi-parametrical simultaneous fitting of relaxation  $^1\text{H}$  and  $^{17}\text{O}$  NMR data at pH 7.5 for the G2-(Gd-DO3A-P $^{\text{BnN}\{\text{CS}\}}$ )<sub>16</sub>-poly(Arg)<sub>56</sub> adduct and the parent G2-(Gd-DO3A-P $^{\text{BnN}\{\text{CS}\}}$ )<sub>16</sub> conjugate.

Parameter	Gadolinium(III) complex of	
	G2-(DO3A-P $^{\text{BnN}\{\text{CS}\}}$ ) <sub>16</sub> -poly(Arg) <sub>56</sub>	G2-(DO3A-P $^{\text{BnN}\{\text{CS}\}}$ ) <sub>16</sub>
$^{298}r_1$ [s $^{-1}$ mM $^{-1}$ ] <sup>a</sup>	30.8	14.1
$\Delta^2$ [10 $^{20}$ s $^{-2}$ ]	0.04 $\pm$ 0.02	0.07 $\pm$ 0.04
$^{298}\tau_M$ [ns]	97 $\pm$ 9	66 $\pm$ 4
$\Delta H_M^\#$ [kJ mol $^{-1}$ ]	33 $\pm$ 2	50 $\pm$ 4
$^{298}\tau_{Rg}$ [ps]	3900 $\pm$ 100	2690 $\pm$ 270
$E_{rg}$ [kJ]	23 $\pm$ 6	43 $\pm$ 4
$^{298}\tau_{RI}$ [ps]	172 $\pm$ 14	100 $\pm$ 3
$E_{rl}$ [kJ]	38 $\pm$ 16	20 $\pm$ 1
$^{298}\tau_v$ [ps]	47 $\pm$ 2	36 $\pm$ 2
$S^2$	0.35 $\pm$ 0.03	0.24 $\pm$ 0.01
$E_v$ [kJ]	<u>1</u>	<u>1</u>
$A/\hbar$ [10 $^6$ rad s $^{-1}$ ]	<u>-2.89</u>	<u>-2.89</u>
$R_{GdO}$ [\mathring{A}]	<u>2.6</u>	<u>2.6</u>
$R_{GdH}$ [\mathring{A}]	<u>3.1</u>	<u>3.1</u>
$A$ [\mathring{A}]	<u>3.6</u>	<u>3.6</u>
$\Delta H_{Mss}^\#$ [kJ]	<u>10</u>	<u>10</u>
$R_{ss}$ [\mathring{A}]	<u>3.5</u>	<u>3.5</u>
$^{298}\tau_{Mss}$ [ns]	<u>1</u>	<u>1</u>
$q$	<u>1</u>	<u>1</u>
$q_{ss}$	<u>2</u>	<u>2</u>

<sup>a</sup>The relaxivity values were measured at 25 °C and 20 MHz; the underlined values were fixed during the fitting procedure.

## Full BSM (LS) equation set in Scientist form

IndVars: T,B

DepVars: R2o,R1h

Params: delta,Hm,tm0,Erl,Erg,tr0l,tr0g,tv0,acc,ro,rh,n,a,RLS,Hmss,tm0ss,rsf,n1

### **// Parameters and variables used**

// T - temperature at K

// B - used field at MHz

// R2o -  $^{17}\text{O}$  transversal relaxivity at ms-1 (unreduced)

// delta -  $\Delta_2$  trace of ZFS matrix

// tm0 - water residence time at 298 K

// Hm - water exchange free energy

// tr0l - rotational correlation time of local motion at 298 K

// tr0g - rotational correlation time of global motion at 298 K

// Er -rotation free energy

// tv0 - correlation time of electronic relaxation due to ZFS

// Ev - free energy of electronic relaxation

// acc - hyperfine coupling constant A/hbar of Gd-O interaction

// ro - Gd-O distance [m]

// rh - Gh-H distance [m]

// n number of inner sphere water molecules

// s - multiplicity of Gd - fixed to 3.5

// a - shortest distance form outer sphere to Gd(III) ion – needed for OS contribution

// D - diffusion cooeficient at 298 K - calculated from Hidman's semi-empirical equation

// RLS – Lipari-Szabo rigidity factor - should be between 1 (totally rigid) and 0

// n1 - number of water molecules in the second sphere

// rsf - Gd-SF distance

// Hmss - water exchange free energy for second sphere contribution

// tm0ss - water residence time at 298 K for second sphere

### // Physical constants implicitly used

// univ - universal gass constant R = 8.31441  
//  $\mu B$  - Bohr magneton  $\mu B$  = 9.274e-24  
//  $\mu_0$  - vaccum magnetic susceptibilty  $\mu_0$  =  $4\pi e^{-7}$   
// h - Planck constant h = 6.62617e-34  
// hbar = 1.0546e-34  
// NA - Avogadro constant NA = 6.023e23  
// k - Boltzman constant k = 1.38066e-23  
//  $\gamma_0$  - gyromagnetic ratio of 17O  $\gamma_0$  = -3.626e7  
//  $\gamma_h$  - gyromagnetic ration of 1H  $\gamma_h$  = 2.675e8  
// gs - elektrononic gyromagnetic ratio gs = 1.75977e11

### // Notes

// magnetic field is in Larmor frequencies - omegas (Wo a WS) are calculated as  $2\pi B / \text{ratio of gyros}$

### // Definitions of constants

s=3.5

univ=8.31441

sz=31.5

### // Definition of omegas

wh=6.28e6\*B

ws=658.21\*wh

wo=-0.1355514\*wh

### // Eyring equations

tm=((tm0^(-1)\*T/298.15)\*exp((Hm/univ)\*(0.003354-(1/T))))^(-1)

tmss=((tm0ss^(-1)\*T/298.15)\*exp((Hmss/univ)\*(0.003354-(1/T))))^(-1)

trl=((tr0l^(-1))\*exp((Erl/univ)\*(0.003354-(1/T))))^(-1)

trg=((tr0g^(-1))\*exp((Erg/univ)\*(0.003354-(1/T))))^(-1)

tv=((tv0^(-1))\*exp((Ev/univ)\*(0.003354-(1/T))))^(-1)

```

// Electronic relaxation-ZFS + SR

T1e=((1/25)*delta*tv*(4*s^2+4*s-3)*((1/(1+ws^2*tv^2))+(4/(1+4*ws^2*tv^2)) + dg/(9*trt))^(-1)
T2e=(delta*tv*((5.26/(1+(0.372*ws^2*tv^2)))+(7.18/(1+(1.24*ws^2*tv^2)))) + dg/(9*trt))^(-1)

// Spin rotation term

// TeSR=(dg/(9*trt))^(-1)

// Total electronic relaxation

// T1e=(T1eZFS^(-1)+TeSR^(-1))^(-1)
// T2e=(T2eZFS^(-1)+TeSR^(-1))^(-1)

// Total correlation times

trt=(trl^(-1)+trg^(-1))^(-1)
tc1l=(T1e^(-1)+trt^(-1)+tm^(-1))^(-1)
tc2l=(T2e^(-1)+trt^(-1)+tm^(-1))^(-1)
tc1g=(T1e^(-1)+trg^(-1)+tm^(-1))^(-1)
tc2g=(T2e^(-1)+trg^(-1)+tm^(-1))^(-1)
te1=(T1e^(-1)+tm^(-1))^(-1)
te2=(T2e^(-1)+tm^(-1))^(-1)

// R2 scalar - contact contrubution for 17O

cont=(1/3)*s*(s+1)*acc^2*(te1+(te2/(1+ws^2*te2^2)))
//cont=(1/3)*s*(s+1)*acc^2*(te1)

// R2 dipolar - pseudocontact contribution for 17O - Lipari-Szabo

dip1=((1/15)*4.535671e-45*s*(s+1))/(ro^6)
dip2O=(RLS*tc2g/(1+ws^2*tc2g^2))+((1-RLS)*tc2l/(1+ws^2*tc2l^2))
dip3O=(RLS*tc1g/(1+wo^2*tc1g^2))+((1-RLS)*tc1l/(1+wo^2*tc1l^2))
dip=dip1*(13*dip2O+7*dip3O)

// R2 dipolar Curie contribution for 17O

dip7=(1/5)*(wo^2*7.397e-107*7.94^4)/(9*1.9063324e-46*T^2*ro^6)
dip8=4*trg+(3*trt)/(1+wo^2*trt^2)
cur=dip7*dip8

// Total dipolar contribution to R2 of 17O

diptot=dip+cur

// Total R2 relaxation

T2m=(cont+diptot)^(-1)

```

```

// Radial frequency difference of bound and free water at rad/s
dwm=2*s*(s+1)*9.274e-24/(3*1.38066e-23*T)*acc*(B*1E6*6.28/2.675E8)

// Overall reduced T2 relaxation of 17O
num=(T2m^(-2))+(T2m*tm)^(-1)+dwm^2
denom=(tm^(-1)+T2m^(-1))^2+dwm^2
scam=num/denom
pm=n*1e-3/55.55
R2o=pm*scam/tm

// R1 dipolar - pseudocontact contribution for 1H - Lipari-Szabo
dip1H=5.16963e-43/rh^6
dip2H=(RLS*tc1g/(1+wh^2*tc1g^2))+((1-RLS)*tc1l/(1+wh^2*tc1l^2))
dip3H=(RLS*tc2g/(1+ws^2*tc2g^2))+((1-RLS)*tc2l/(1+ws^2*tc2l^2))
R1DIP=dip1H*(3*dip2H+7*dip3H)

// Total inner sphere milimolar contribution to 1H relaxivity
T1M=(R1DIP)^(-1)
R1IN=(N*1E-3/55.55)/(T1M+TM)

// Calculus for diffusion coefficient according to J.C.Hindman
B1=3.118150E-04
B2=5.062560E+03
B3=1.547920E+02
B4=1.629310E+03
D=(1E-4)/((B1*EXP(B2/T))+(B3*EXP(B4/T)))

// Freed's model of outer sphere contrubution to 1H relaxivity
TAU=A^2/D

// Spectral density function J(i) for proton spins
AI=TAU/T1e
CI=[0,1]*wh*tau
z2l=ai+ci
JI=RE((1+0.25*(z2l^0.5))/(1+z2l^0.5+(4/9)*z2l+(1/9)*(z2l^1.5)))

// Spectral density function J(s) for gadolinium electron states
AS=TAU/T2e
CS=[0,1]*ws*tau

```

```

z2S=aS+cS
JS=RE((1+0.25*(z2S^0.5))/(1+z2S^0.5+(4/9)*z2S+(1/9)*(z2S^1.5)))
// Calculation of outer sphere contribution to 1H relaxivity according to Freeds model
COST3=3.68e-20*S*(S+1)
R1OS=(COST3/(A*D))*((3*JI)+(7*JS))
// Second sphere contribution to 1H R1 relaxivity; dipolar R1 //contribution only
TC1SF=(T1E^(-1)+trt^(-1)+tmss^(-1))^(-1)
TC2SF=(T2E^(-1)+trt^(-1)+tmss^(-1))^(-1)
COST2SF=(5.16963e-43/RSF^6)
R1DIPSF=(COST2SF*((7*TC2SF/(1+WS^2*TC2SF^2))+(3*TC1SF/(1+Wh^2*TC1SF^2))))
T1MSF=(R1DIPSF)^(-1)
R1SF=(N1*1e-3/55.55)/(T1MSF+tmss)
// Overall R1 proton relaxivity - second sphere enabled
R1h=R1IN+R1OS+R1SF

```

## **References:**

- <sup>1</sup> Sudmeier, J. L.; Reilley, C. N. (1964) Nuclear Magnetic Resonance Studies of Protonation of Some Polyaminocarboxylate Compounds Containing Asymmetric Carbon Atoms. *Anal. Chem.* 9, 1698–1706.
- <sup>2</sup> Rudovský, J.; Botta, M.; Hermann, P.; Koridze, A.; Aime, S. (2006) Relaxometric and solution NMR structural studies on dimeric lanthanide(III) complexes of a phosphinate analogue of DOTA with a fast rate of water exchange. *Dalton Trans.* 2323–2333.
- <sup>3</sup> Rudovský, J.; Kotek, J.; Hermann, P.; Lukeš, I.; Mainero, V.; Aime, S. (2005) Synthesis of a bifunctional monophosphinic acid DOTA analogue ligand and its lanthanide(III) complexes. A gadolinium(III) complex endowed with an optimal water exchange rate for MRI applications. *Org. Biomol. Chem.* 3, 112–117.