Supramolecular protein cage composite MR contrast agents with extremely efficient relaxivity properties

SUPPORTING INFORMATION

Relaxivity Equations:[1]

Analytical equation containing the decay constant, T_1, for the recovery of the net nuclear spin magnetization for a sample placed in a magnetic field which has be tilted out of equilibrium:

$$M_z(t) = M_{z\ Eqilibrium}\left(1 - e^{\frac{-t}{T_1}}\right)$$

- $M_z(t)$ nuclear spin magnetization in the z axis at time t in units of seconds
- $M_{z\ Equilibrium}$ equilibrium state of the nuclear spin magnetization in the z axis (maximum magnetization)
- T_1 decay constant for the recovery of spin in units of seconds

Observed T_1 of a specific sample type with a contrast agent present:

$$T_{1\ observed} = \left[\frac{1}{T_{1\ sample}} + (r_1)[Contrast\ Agent]\right]^{-1}$$

- r_1 relaxivity of a contrast agent in units of mM^{-1} $seconds^{-1}$
- [*Contrast Agent*] concentration of the contrast agent in units of mM

Solomon-Bloembergen-Morgan (SBM) model for PRE:

Relaxivity of contrast agent including the dipolar, scalar and Currie relaxation mechanisms:

$$r_1 = \frac{q \cdot [Constrast\ Agent]}{[Water]}\left[\frac{1}{T_{1M} + \tau_M}\right]$$

$$r_1 = \frac{q \cdot [Constrast\ Agent]}{[Water]} \left[\frac{1}{\left(\frac{1}{T_{1M}^{dipolar}} + \frac{1}{T_{1M}^{scalar}} + \frac{1}{T_{1M}^{currie}} \right)^{-1} + \tau_M} \right]$$

$[Water]$ = 55.6 Molar	concentration of water in units of (moles / liter), (fixed value)
q	number of inner sphere waters that bind to the Gd ion, (fitting parameter)
$T_{1M}^{dipolar}$	dipolar contribution to the relaxation time
T_{1M}^{scalar}	scalar contribution to the relaxation time
T_{1M}^{currie}	Currie contribution to the relaxation time
τ_M	residence time for the Gd bound water molecule, (fitting parameter)

Relaxivity of contrast agent considering only the dipolar relaxation mechanism (the dipolar mechanism was only considered in the fitting of the NMRD profiles in this work):

$$r_1 = \frac{q \cdot [Constrast\ Agent]}{[Water]} \left[\frac{1}{T_{1M}^{dipolar} + \tau_M} \right]$$

SBM analytical description of the dipolar relaxation time:

$$T_{1M}^{dipolar} = \frac{2C_{dd}}{15r_{IS}^6} [3J(\omega_I, \tau_{d1}) + 7J(\omega_s, \tau_{d2})]$$

Prefactor for relaxation:

$$\boldsymbol{C_{dd} = \gamma_I^2 \gamma_S^2 \hbar^2 S(S+1) \left(\frac{\mu_0}{4\pi}\right)^2}$$

$\gamma_I = 2.675 \cdot 10^8$	nuclear gyromagnetic ratio (second^{-1} Tesla^{-1})
γ_S = -1.760859778 $\cdot 10^{11}$	electronic gyromagnetic ratio (second^{-1} Tesla^{-1})
ħ = 1.054571628 $\cdot 10^{-34}$	Planck constant (Joules $\cdot$ seconds)
S = 7/2	spin quantum number for the Gd^{3+} ion
$\mu_0 = 4\pi \cdot 10^{-7}$	magnetic permeability of free space (Newton $\cdot$ Amps^{-2})

$r_{IS}^6 = 3 \cdot 10^{-10}$ distance between the nuclear and the electronic spin (meters),(fixed value)

Spectral density function:

$$J(\omega, \tau) = \frac{\tau}{1 + \omega^2 \tau^2}$$

ω Larmor frequency of nuclear or electric spin

τ correlation time where τ is either τ_{d1} or τ_{d2}

Correlation times in units of seconds:

$$\tau_{d1} = \left(\frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{T_{1e}}\right)^{-1}$$

and

$$\tau_{d2} = \left(\frac{1}{\tau_R} + \frac{1}{\tau_M} + \frac{1}{T_{2e}}\right)^{-1}$$

τ_R rotational correlation time for the Gd ion, (fitting parameter)

T_{1e} longitudinal electronic relaxation rate

T_{2e} transverse electronic relaxation rate

Electronic relaxation time (longitudinal and transverse) in units of seconds:

$$T_{1e} = \left[\frac{2\Delta^2}{50}(4S(S+1) - 3)\left(\frac{\tau_v}{1 + \omega_S^2 \tau_v^2} + \frac{4\tau_v}{1 + 4\omega_S^2 \tau_v^2}\right)\right]^{-1}$$

and

$$T_{2e} = \left[\frac{\Delta^2}{50}(4S(S+1) - 3)\left(3\tau_v + \frac{5\tau_v}{1 + \omega_S^2 \tau_v^2} + \frac{2\tau_v}{1 + 4\omega_S^2 \tau_v^2}\right)\right]^{-1}$$

$\tau_v = 1.4 \cdot 10^{-11}$ correlation time for instantaneous distortions of the metal complex polyhedron in units of seconds, (fixed value in the SBM fit)

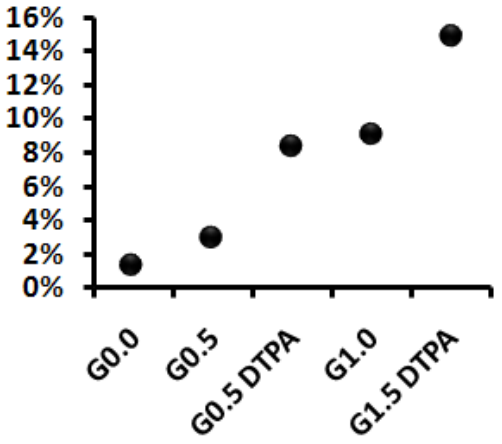
$\Delta^2 = 9 \cdot 10^{18}$ mean square fluctuation of the zero-field splitting in units of seconds^{-2}, (fixed value in the SBM fit)

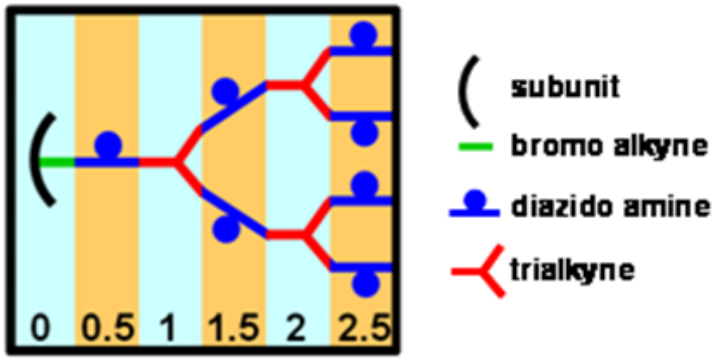
Fitting scheme:

1) Four fitting parameters were used (τ_R, τ_M, τ_V and Δ) to individually fit all twenty NMRD profiles while q was held at a value of 1 in these SBM fits. (20 profiles result from 2 preparations for both non-passivated and passivated batches for G0.0, G0.5, G1.5, G2.5 G3.5) Next average values for τ_V and Δ were calculated from the 20 individually fit profiles. This resulted in: $\Delta = 3.0 \cdot 10^9 \pm 0.3 \cdot 10^9$ and $\tau_V = 1.4 \cdot 10^{-11} \pm 0.2 \cdot 10^{-11}$.
2) The average values for τ_V and Δ were used to fit the averaged NMRD profile (all twenty data sets averaged) with q, τ_R and τ_M all set as fitting parameters. The individual NMRD profiles were also fit in this manner.

Details on the construction of the branched polymer modeled into the Hsp structure:

Generation	Volume addition at each gen (A^3)	Total volume added	% interior occupied	Added groups
G0.0	1332	2664	1.3%	24 bromo alkynes
G0.5	1568	5800	2.8%	24 diazido amines
G0.5 DTPA	5542	16884	8.3%	24 DTPA(Gd)s
G1.0	723	18330	9.0%	20 total (4 are in xlinks, 16 are not xlinked) trialkynes
G1.5 DTPA	5982	30294	14.9%	20 DTPA(Gd)s





Protein and Gd quantitation:

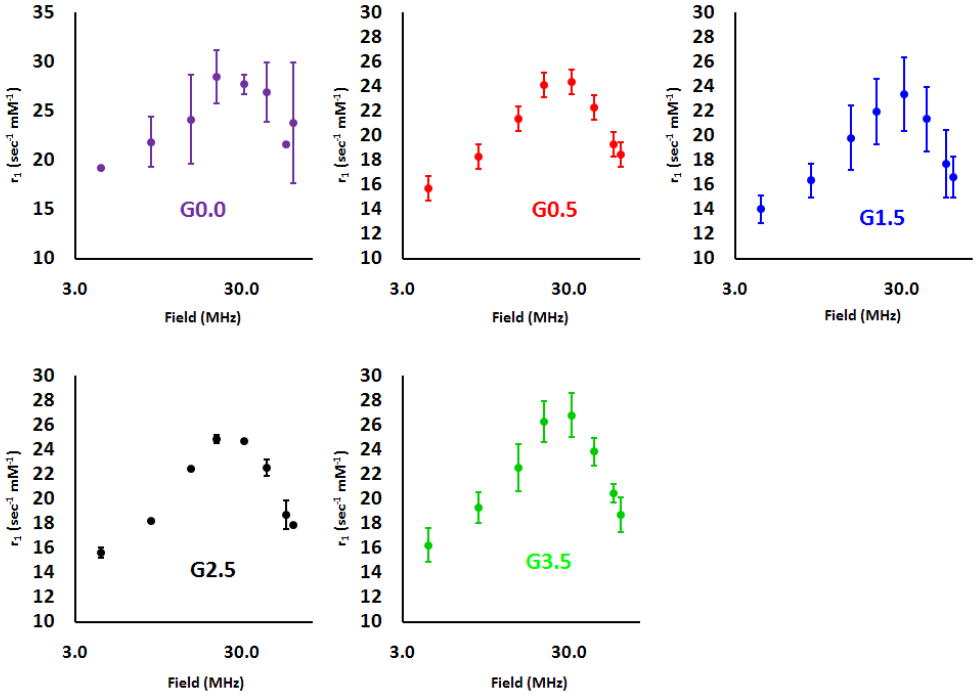
A BCA Protein Assay (bicinchoninic acid) from Pierce was used to quantitate the protein (HSP) concentration (www.piercenet.com) and the protocol provided by Pierce was used. Protein samples were analyzed in triplicate resulting in an average relative standard deviation of 2.1% for the twenty samples for the BCA assay. Energy Laboratories, Inc. performed the ICP-MS quantitation of the the Gd ions (www.energylab.com).

Comparison of protein cage – Gd based contrast agents:

Cage / Chelator	Ionic r_1 (MHz)	Particle r_1	Cage Diameter (nm)	Particle r_1 per volume ($sec^{-1} mM^{-1} nm^{-3}$)	Particle r_1 per mass[2] ($L g^{-1} sec^{-1}$)	Clinically relevant binding	Group Reference
CCMV / endogenous binding site	202 (62)	28,482[a]	28	2.5	7.8	No	Douglas / Young [3]
CCMV / metal binding peptide – genetic fusion	210 (62)	36,120[a]	28	3.1	9.9	No	Douglas / Young [4]
MS2 / DTPA-ITC	16.9 (64)	7,200	27	0.7	2.9	Yes	Kirshenbaum [5]
CPMV / DOTA-click	15.5 (64)	4,150	30	0.3	1.1	Yes	Finn [6]
CCMV / DOTA-NHS ester	46 (62)	2,806	28	0.2	0.8	Yes	Douglas / Young [4]
MS2 / bis(HOPO)-TAM	31 (60)	2,900	27	0.3	1.2	Yes	Francis [7, 8]
HSP-BP-DTPA-Gd	19 (62)	3,450	14	2.4	8.7	Yes	Douglas / Young this work
DTPA-Gd	4 (20)	4		12.2	7.3	Yes	[9]

NMRD profile and SBM fit of generations G0.0, G0.5, G1.5, G2.5 and G3.5:

Four experimental data points are averaged for all points in the plots below. These four points are comprised of two points from the passivated preparations and two from the non-passivated preparations. The error bars are plus and minus one standard deviation.



Einstein Stokes relation to estimate the rotational correlation time of HSP in water at 20 ^{0}C:

$$\tau_R = \frac{4\pi\eta r^3}{3kT}$$

τ_R = rotational correlation time (seconds)
η = Viscosity of water (water @ 20 ^{0}C = 0.001002 Pa sec = 0.001002 kg m^{-1} sec^{-1})
r = radius of the particle (Hsp = 6nm = 6 · 10^{-9} m)
k = boltzman constant (1.3806504 · 10^{-23} kg m^2 sec^{-2} K^{-1})
K = temperature in Kelvin

=(4*PI()*0.001002*(6*10^-9)^3)/(3*(1.38*10^-23)*296) = 2.2 *10^{-7}
=(kg m^{-1} sec^{-1}*(m)^3) / ((kg m^2 sec^{-2} K^{-1})*K) = seconds

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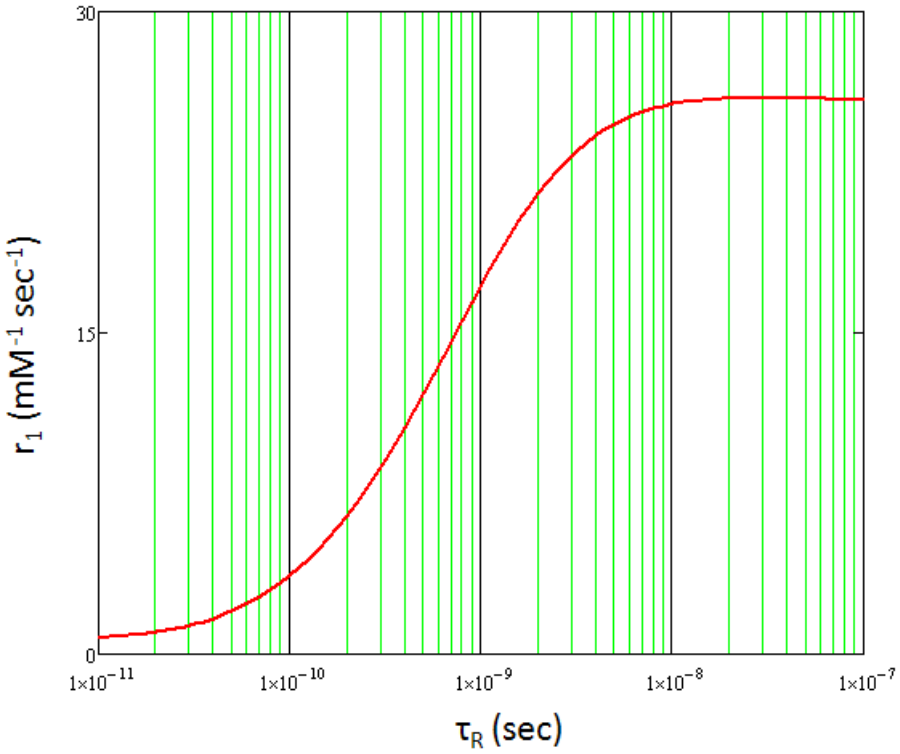
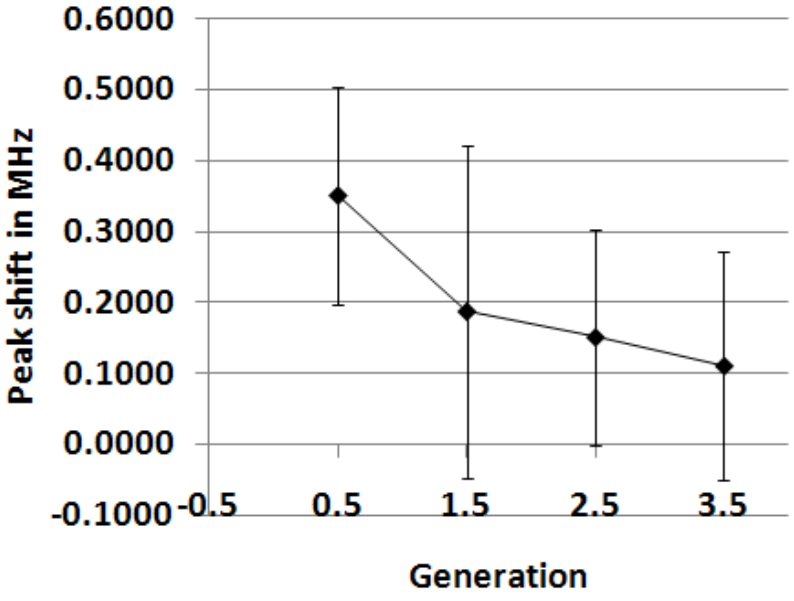
Relationship of r_1 and τ_R determined by the SBM model with q = 1.1, τ_M = 5.6*10^{-7} seconds and the magnetic field = 31MHz (0.73 Tesla):



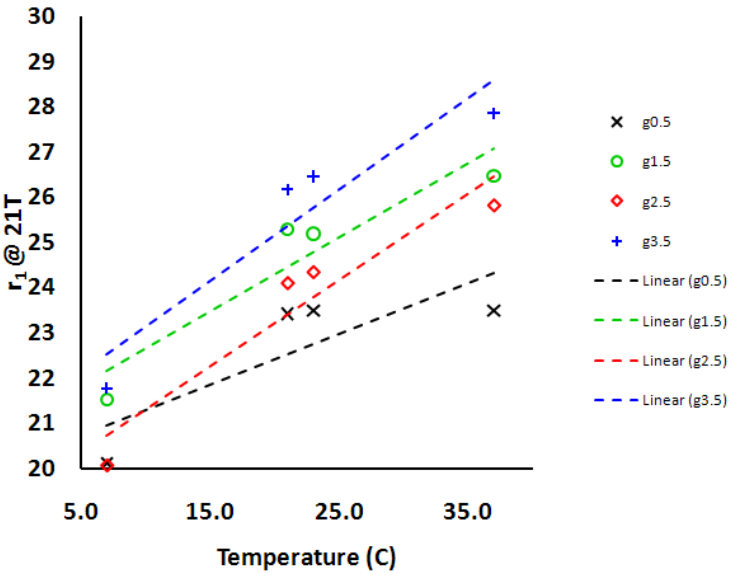
Peak shift in MHz for the peak located at approximately 31MHz:

Four experimental data points are averaged for the points below. These four points are comprised of two points from the passivated preparations and two from the non-passivated preparations. The error bars are plus and minus one standard deviation.



Plots of r_1 vs. temperature for G0.5, G1.5, G2.5 and G3.5:

Four experimental data points are averaged for the points below. These four points are comprised of two points from the passivated preparations and two from the non-passivated preparations.



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