Supplementary Information

Exotic Nuclear Spin Behavior in Dendritic Macromolecules

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Materials and analytical NMR-measurements for evaluation of chemical syntheses

All the chemicals were purchased from commercial suppliers and used as received. If not otherwise stated, $^1$H NMR and $^{13}$C NMR spectra were acquired on a Bruker ultrashield 300 MHz spectrometer at 25 °C.

Synthesis of a bis-MPA-OH based dendrimers and investigations of singlet equilibration times

In further experiments another dendrimer based on the dendritic polyester bis-MPA-OH (MPA = 2,2-Bis(hydroxyl-methyl)propionic acid)) was designed. Modifications of bis-MPA-OH were performed to attach multiple GGA residues to the terminal functions of the bis-MPA-OH (Fig. S1). The mean number of derivatisation and $T_1$ and $T_2$ (Table 1) of protons of the core as well as the tripeptide residues have been determined as below.

Synthesis of bis-MPA-O-GGA-NH$_2$

![Figure S1](image_url)

Figure S1 Schematic Representation of HO-GGA-NH$_2$ and the bis-MPA-OH dendron (A) and modification of the bis-MPA-OH dendrimer by attaching HO-GGA-NH$_2$. Indicated in red and blue are the different proton pairs in which a singlet state can be populated.

To a round-bottom flask equipped with magnetic bar, Bis-MPA-OH dendrimer (31 mg, 0.0057 mmol), Boc-Ala-Gly-Gly-OH (310 mg, 1.01 mmol), EDCI (175 mg, 1.12 mmol) and DMAP (3.5 mg, 0.03 mmol) were dissolved in a mixture of dichloromethane (4.0 mL) and DMF (4.0 mL). The resulting mixture was stirred at room temperature for two days. After removal of the solvent under reduced pressure, the residue was dialyzed against water for three days and then lyophilized. bis-MPA-O-GGA-NH$_2$-Boc was then obtained and used for the next step. To the solution of bis-MPA-O-GGA-NH$_2$-Boc in dichloromethane (3.0 mL), TFA (3.0 mL) was added. The mixture was stirred for 2 hours and then concentrated under reduced pressure to give the crude product. After dialysis against water for 7 days and subsequent lyophilization, a solid was obtained.
**Figure S2** $^1$H NMR spectrum of 1.0 mM bis-MPA-O-GGA-NH$_2$ in D$_2$O recorded at $B_0 = 7$T (300 MHz proton frequency). The spectrum was averaged over 4 scans.

**Figure S3** $^{13}$C NMR spectrum of 1.0 mM bis-MPA-O-GGA-NH$_2$ in D$_2$O recorded at $B_0 = 7$T (75 MHz $^{13}$C frequency). The spectrum was averaged over 56039 scans.

**Estimation of the mean number of tripeptides per bis-MPA-O-GGA-NH$_2$ unit**

The mean derivatization number of bis-MPA-O-GGA-NH$_2$ molecule was estimated by using quantitative NMR experiments. The test sample contains bis-MPA-O-GGA-NH$_2$ at a concentration of 0.5 mM as well as pyrazine, which was employed as internal standard at the concentration of 50 mM. Firstly, it is assumed that bis-MPA-OH has been fully esterified with Ala-Gly-Gly-OH. Thus, the assumed molarity of tripeptide residues would be 24 mM. On the other hand, the actual molarity of tripeptide residues could be calculated by comparing the integral of the pyrazine signal with the integral of the methyl protons of the alanine. The actual derivatization
is 0.45. This results in a mean coverage of about 22 tripeptides per each bis-MPA-O-GGA-NH₂. These findings have been corroborated by ESI mass spectrometry, finding molecular masses of an m/z of 7954 (14 tripeptide residues +3 mu) as well as an m/z of 8139 (15 tripeptide residues +2 mu). Additionally, m/z of 7836 and 8023 have been found which might be attributed to losses of single peptides. The loss of tripeptides compared to the results of the estimation of the mean derivatization to cleavages during the ionization process. This mean derivatization is considerably lower than in the case of G5-PAMAM-GGA-NH₂ which is 0.69.[1]

Synthesis of G5-PAMAM-GGA-NH₂-Atto

To a solution of G5-PAMAM-GGA-NH₂(51) (36 mg, 0.7 µmol) in distilled water (3.0 mL), a solution of Atto488-NHS ester (1.0 mg) in DMF (3.0 mL) was added slowly. The resulting mixture was stirred at room temperature for 24 hours and then dialyzed against water for one week. The solvent was concentrated under reduced pressure to yield a viscous orange product. The average number of Atto488 per dendrimer has been determined to be 5 by comparing the integrals of the aromatic signals of Atto488 and the glycine signal from the tripeptide residue.

Figure S4 ¹H NMR spectrum of 0.1 mM G5-PAMAM-GGA-NH-ATTO in D₂O recorded at B₀ = 16.4T (700 MHz proton frequency). The spectrum was averaged over 16 scans.
Figure S5: $^{13}$C NMR spectrum of 0.1 mM G5-PAMAM-GGA-NH-ATTO in D$_2$O recorded at $B_0 = 16.4$T (175 MHz $^{13}$C frequency). The spectrum was averaged over 6144 scans.

NMR-Experiments

All NMR measurements have been performed at 37 °C. Longitudinal relaxation times ($T_1$) have been measured using an inversion recovery experiment.

Measurements on bis-MPA-OH dendron, bis-MPA-OH and bis-MPA-O-GGA-NH$_2$

Measurements of $T_s$ of the protons of the bis-MPA-OH core have been performed using the APSOC$^{[2]}$ sequence (APSOC pulse length 400 ms, APSOC pulse amplitude of 138 Hz). Integrations for $T_s$ and $T_1$ determination have been performed over the whole range of signals belonging to the CH$_2$ parts of the core. Pulse lengths and inter-scan delays have been optimized. For the $T_s$ measurements on the glycine signal of the GGA residue the SLIC sequence has been used (SLIC pulse length 90 ms, SLIC pulse amplitude of 16 Hz)$^{[3]}$.

Measurements on G5-PAMAM-GGA-NH$_2$-Atto

Measurements have been performed at 600 MHz using TopSpin4 for data processing. Determination of $T_1$ and $T_s$ has been performed with concentrations of 100 µM in D$_2$O and PBS respectively. The SLIC sequence$^{[53]}$ has been used for $T_s$ measurements and pulse lengths and inter-scan delays have been optimized (SLIC pulse length 410 ms, SLIC pulse amplitude of 11 Hz). For the measurements in the cytosol, B-cell cytosol with D$_2$O (5%) has been taken and G5-PAMAM-GGA-NH$_2$-Atto has been added to get a concentration of 100 µM of the dendrimer (figure S6). Measurements on the B-cells have been performed in the same way. A suspension of B-cells with 5 % D$_2$O has been placed in a Shigemi-NMR tube and the dendrimer has been added to make a concentration of 100 µM of G5-PAMAM-GGA-NH$_2$-Atto in the cell suspension. Afterwards $T_s$ has been determined. Upon lysis of the cells the dendrimers directly
bind to the membranes. After extensive washing of the lysed cells and taking them up in PBS (5% D\textsubscript{2}O) experiments can be repeated over several days and stored in between at 5 °C without change of the measured NMR parameters.

**Figure S6:** Thermal proton NMR spectrum of cell lysate with G5-PAMAM-GGA-Atto (red) and singlet filterer NMR spectrum of the same sample (blue).

**Determination of** $T\text{\textsubscript{1}}$ **and** $T\text{\textsubscript{s}}$ **in G5-PAMAM-GGA-NH\textsubscript{2}** **under the exclusion of oxygen**

For the exclusion of oxygen, a sample of G5-PAMAM-GGA-NH\textsubscript{2} in D\textsubscript{2}O (100 µM) has been prepared in a Young NMR tube. The sample has been frozen in liquid nitrogen and subsequently evacuated with a high vacuum pump and flushed with nitrogen. This process has been repeated 4 times and the sample was thawed under a constant flow of nitrogen. Determination of $T\text{\textsubscript{1}}$ and $T\text{\textsubscript{s}}$ has been done in the same way described for G5-PAMAM-GGA-NH\textsubscript{2}-Atto.

**Table S1** $T\text{\textsubscript{1}}$ and $T\text{\textsubscript{s}}$ data from measurements on the bis-MPA-OH derivatives. The colors indicate the proton pair on which the measurements have been performed according to the color coding in Fig. S19.

<table>
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<tr>
<th>Compound</th>
<th>Solvent</th>
<th>$T\text{\textsubscript{1}}$ [s]</th>
<th>$T\text{\textsubscript{s}}$ [s]</th>
</tr>
</thead>
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<tr>
<td>(4)bis-MPA-OH dendron (core)</td>
<td>D\textsubscript{2}O</td>
<td>0.38 ± 0.01</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>(5)bis-MPA-OH dendrimer</td>
<td>D\textsubscript{2}O</td>
<td>0.38 ± 0.01</td>
<td>1.7 ± 0.2</td>
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<tr>
<td>(6)bis-MPA-O-GGA-NH\textsubscript{2} (core)</td>
<td>D\textsubscript{2}O</td>
<td>0.40 ± 0.07</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>(6) bis-MPA-O-GGA-NH\textsubscript{2} (peptide)</td>
<td>D\textsubscript{2}O</td>
<td>0.72 ± 0.03</td>
<td>2.6 ± 0.1</td>
</tr>
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</table>
Determination of the ion influence

NMR measurements for determination of the singlet equilibration times ($T_s$) and longitudinal relaxation times ($T_1$) have been performed on a BRUKER 300 AV or 600 AV (Figure S7 and S8) using TopSpin3 for data processing. For each ion, two samples with G5-PAMAM-GGA-NH$_2$ (100 µM in D$_2$O) and HO-GGA-NH$_2$ (12.8 mM in D$_2$O) have been prepared respectively. Stock solutions of Gd(III)Cl$_3$, Mn(II)Cl$_2$, Fe(III)Cl$_3$, Zn(II)Cl$_2$ and Cu(II)Cl$_2$ with G5-PAMAM-GGA-NH$_2$ (100 µM in D$_2$O) have been prepared so that the addition of 2 µL of those solutions resulted in an increase of ion concentrations by 5 µM (Gd), 25 µM (Mn, Fe, Zn) or 50 µM (Cu). Afterwards 2 µL of those stock solutions have been added to the respective samples and $T_1$ and $T_s$ have been measured 3 times. The SLIC sequence has been used for $T_s$ measurements. Pulse lengths have been optimized after each titration step and inter-scan delays as well as the variable delay list have been adjusted to match approximately 5 times $T_1$ or $T_s$ respectively. The weighted average of those three measurements has been determined. Experiments for HO-GGA-NH$_2$ (12.8 mM) have been conducted in the same manner.
Figure S7. Relaxation experiments at 300 MHz. Singlet equilibration rate of GGA (1) (black) and G5-PAMAM-GGA-NH$_2$ (2) (red) (a) and longitudinal relaxation rates $T_{1}$ of GGA (1) (black) and G5-PAMAM-GGA-NH$_2$ (2) (red) (b-f) in D$_2$O depending on the concentration of different paramagnetic ions Gd(III), Mn(II), Fe(III), Zn(II) and Cu(II). Error margins have been determined by taking the standard deviation of 3 different measurements.

Measurements in HEPES buffered solutions have been conducted in the same manner in an aqueous solution of HEPES (10 mM) with 5% of D$_2$O. For each sample 6 datapoints in steps of 10 µM of Gd(III)Cl$_3$, 50 µM of Fe(III)Cl$_3$, Mn(II)Cl$_2$ and Zn(II)Cl$_2$ as well as 100 µM of Cu(II)Cl$_2$ have been recorded respectively. Measurements of those samples have been done at 600 MHz with a prodigy probe.
Figure S8: Relaxation experiments at 600 MHz. Longitudinal relaxation rates $T_1$ of GGA (1) (12.8 mM) (black) and G5-PAMAM-GGA-NH$_2$ (2) (100 µM) (red) in HEPES buffered aqueous solution (10 mM) in dependence of the concentration of different ions. Error margins are the standard deviation of 3 Experiments with 8 scans each.

Measurements in a HEPES buffered agarose gel

1 µL of a solution of G5-PAMAM-GGA-NH$_2$ in D$_2$O (2 mM) was placed in a 3 mm NMR tube which has been heated to 80 °C. Afterwards 199 µL of a solution of Agarose in H$_2$O (0.6 wt% with 5% D$_2$O) was added and the NMR tube was placed in a heating bath at 80 °C for 30 min to allow for the solutions to mix properly. Afterwards the sample was allowed to cool to room temperature allowing the agarose gel to form. The same procedure has been performed for the sample with 50 µM of Cu(II). In this case an additional 1 µL of a solution of Cu(II) in D$_2$O (10 mM) was added before the addition of the agarose solution. Measurements have been performed at 900 MHz.
Figure S9 The two samples in an agarose gel in 3 mm NMR tubes.

DOSY-NMR measurements

DOSY experiments were performed at 300 MHz using 16 points along the gradient. The diffusion coefficients that have been determined for G5-PAMAM-GGA and water at different concentrations of Cu(II) are shown in table S2.

Table S2: Diffusion coefficients (D) for D2O and G5-PAMAM-GGA at different Cu(II) concentrations.

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<tr>
<th>Cu(II) [µM]</th>
<th>G5-PAMAM-GGA [m²s⁻¹]</th>
<th>D2O [m²s⁻¹]</th>
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<td>1.92869x10⁻⁹</td>
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<td>100</td>
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<td>1.92538x10⁻⁹</td>
</tr>
<tr>
<td>150</td>
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<td>1.92414x10⁻⁹</td>
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<td>250</td>
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<td>1.91665x10⁻⁹</td>
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<tr>
<td>300</td>
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<td>350</td>
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<td>1.93186x10⁻⁹</td>
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<td>400</td>
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<td>1.93513x10⁻⁹</td>
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<tr>
<td>450</td>
<td>5.6393x10⁻¹¹</td>
<td>1.92382x10⁻⁹</td>
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<tr>
<td>500</td>
<td>5.72046x10⁻¹¹</td>
<td>1.91446x10⁻⁹</td>
</tr>
</tbody>
</table>

Cell cultures

Human Burkitt lymphoma B cell line Ramos and COS-7 cells were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Ramos and COS-7 cells were cultured at 37°C with 5% CO₂ in a humid atmosphere in RPMI 1640 and DMEM mediums respectively, (Gibco/Life Technologies, Carlsbad, USA). Both mediums were supplemented with 10% Fetal Bovine Serum (FBS, Biochrom, Berlin, Germany), 4 mM L-glutamine and 100 Units per ml of each penicillin and streptomycin (Lonza, Basel, Switzerland).
Primary hippocampal neurons were cultured as described before. Briefly, glia cells were first prepared from brain cortexes and seeded directly into 12-well plates in Minimum Essential Medium (MEM, Sigma-Aldrich) supplemented with 10% Horse Serum, 0.6% glucose, 1 mM L-glutamine and 100 Units per ml of each penicillin and streptomycin. Primary rat (*Rattus norvegicus*) hippocampal neurons were extracted from E18 pups and seeded on glass coverslips which have been previously coated with 1 mg/ml poly-L-lysine. These coverslips containing hippocampal neuron were then incubated on wells containing the 4-5 days *in vitro* old glia cells. Hippocampal neurons with 14-21 days *in vitro* (DIV) were finally used for the stainings.

**Cellular staining with fluorescent-dendrimer**

Ramos cells were incubated at 37°C with 5% CO₂ in a humid atmosphere with ~10 µg/ml of dendrimers coupled to atto488 in medium lacking FBS for ~16 hours (figure S9). Similarly, Ramos cells were incubated with 10 µg/ml of dendrimer-Atto488 and 100 µg/ml of Dextran-Alexa647 (Thermo-Fisher Scientific) for 30 minutes at 37°C in a humified cell incubator. Cells were pelleted by centrifugation at 300 x g and washed 3 times with 1 ml each of ice-cold 1x Dulbecco’s Phosphate Buffer Saline (DPBS) to remove excess of labeled dendrimer. Finally, cells were resuspended in DPBS to a concentration of ~100 million cells per ml and spotted on an 8-well imaging chamber (Sarstedt, Germany) or poly-L-lysine coated glass coverslips for microscopy or applied directly into the NMR sampler holder. COS-7 cells were first chemically fixed with 4% paraformaldehyde prepared in phosphate buffer saline (PBS) for 30 minutes at room temperature. After quenching excess of aldehyde with 100 mM glycine in PBS, cells were permeabilized using 0.1% Triton X-100 in PBS for 20 minutes followed by adding 10 µg/ml of labeled dendrimers in DPBS for 1 hour. Finally, cells were rinsed 2 times with DPBS for 5 minutes, one incubation with DAPI at 0.1 µg/ml in DPBS for 5 minutes, a final rinse in DPBS and mounted on a glass slide using Mowiol (6 g glycerol, 6 ml deionized water, 12 ml 0.2 M Tris buffer pH 8.5, 2.4 g Mowiol® 4–88, Merck Millipore). Living hippocampal neurons were incubated in their full medium with ~50 µg/ml of labeled dendrimer for 45 minutes at 37°C with 5% CO₂ in a humid atmosphere. After thorough washing with DPBS, cells were fixed, quenched and mounted as explained above for COS-7 cells.

**Microscopy**

The epifluorescence images of neurons were acquired using an Olympus IX71 microscope equipped with 1.4 NA/100x oil objective and an Olympus F-View II CCD camera. Ramos and COS-7 cells were imaged using a Nikon Eclipse Ti-E equipped with an HBO 100-W lamp and a Nikon DS-Q12 Camera. Ramos cells were imaged using a Plan Apo 100x/1.45 NA objective and COS-7 cells with a 1.4 NA/60X Plan apochromat oil immersion objective (both objectives from Nikon). Multicolor images obtained with a laser confocal scanning microscopy (Abberior Instruments GmbH, Göttingen, Germany) in figure 6d-f were acquired using a 405 nm, 489 nm and 561 nm lasers to image DAPI, Dendrimer-Atto488 and Dextran-Alexa647 respectively using a plan apochromat 100 x 1.4 NA oil-immersion objective (Olympus).
Selected epifluorescence images of living hippocampal neurons incubated with G5-PAMAM-GGA-Atto488 (3). Yellow arrowheads indicate example locations where (3) has been internalized within the cells.

Solid State NMR measurements

Solid state nuclear magnetic resonance (ssNMR) experiments have been conducted in a Bruker NMR spectrometer consisting of a wide-bore superconducting magnet at 20 T (~850MHz $^1$H Larmor frequency) equipped with a PH MAS DVT 850W6 EFREE BL3.2 C/N/H and an AVANCE IIIHD console. The cell lysate was washed in D$_2$O, centrifuged at 14,000 rpm for 3 minutes at 20 °C and any supernatant removed. The procedure was repeated 5 times and the sample was transferred into a 3.2mm zirconia rotor. All presented ssNMR experiments have been performed spinning the rotor at 18kHz at a temperature of 295K. The ssNMR spectrum is shown in Fig.S6.
**Figure S11** $^1$H MAS ssNMR (spinning speed 18kHz) spectrum of the cell lysate at $B_0=20$T (850MHz) and $T=295$K. The spectrum represents the sum of 4096x3 transients, recorded using an interscan delay of 10s. The asterisks in the inset denote the glycine signal from the GGA moiety in the G5-PAMAM-GGA-Atto488. The truncated peak close to 0 ppm originates from the silicon plug used to seal the sample inside the rotor.

**UV/VIS-Measurements**

UV-/VIS-measurements have been performed on an Eppendorf BioSpectrometer® kinetic. For those experiments, solutions of Cu(II)Cl$_2$ (500 µM), Mn(II)Cl$_2$ (250 µM), Fe(III)Cl$_3$ (250 µM) and Gd(III)Cl$_3$ (50 µM) in D$_2$O, D$_2$O with G5-PAMAM-GGA-NH$_2$ (100 µM) and D$_2$O with HO-GGA-NH$_2$ (12.8 µM) have been prepared and afterwards scanned in the range from 200 nm to 850 nm. The same measurements have been conducted with solutions of HO-GGA-NH$_2$ in D$_2$O (12.8 mM) and G5-PAMAM-GGA-NH$_2$ in D$_2$O (100 µM).

The results of the UV/VIS-measurements are shown in figures S12 to S23.
**Figure S12** UV/VIS spectrum of Gd(III)Cl$_3$ (50 µM in D$_2$O).

**Figure S13** UV/VIS spectrum of HO-GGA-NH$_2$ (12.8 mM in D$_2$O) and UV/VIS spectrum of HO-GGA-NH$_2$ (12.8 mM in D$_2$O) with 50 µM of Gd(III)Cl$_3$. 
Figure S14 UV/VIS spectrum of G5-PAMAM-GGA-NH$_2$ (100 µM in D$_2$O) and UV/VIS spectrum of G5-PAMAM-GGA-NH$_2$ (100 µM in D$_2$O) with 50 µM of Gd(III)Cl$_3$.

Figure S15 UV/VIS spectrum of Mn(II)Cl$_2$ (250 µM in D$_2$O).
Figure S16 UV/Vis spectrum of HO-GGA-NH$_2$ (12.8 mM in D$_2$O) and UV/Vis spectrum of HO-GGA-NH$_2$ (12.8 mM in D$_2$O) with 250 µM of Mn(II)Cl$_2$. 
Figure S17 UV/VIS spectrum of G5-PAMAM-GGA-NH₂ (100 µM in D₂O) and UV/VIS spectrum of G5-PAMAM-GGA-NH₂ (100 µM in D₂O) with 250 µM of Mn(II)Cl₂.

Figure S18 UV/VIS spectrum of Fe(III)Cl₃ (250 µM in D₂O).
Figure S19 UV/VIS spectrum of HO-GGA-NH₂ (12.8 mM in D₂O) and UV/VIS spectrum of HO-GGA-NH₂ (12.8 mM in D₂O) with 250 µM of Fe(III)Cl₃.
Figure S20 UV/VIS spectrum of G5-PAMAM-GGA-NH₂ (100 µM in D₂O) and UV/VIS spectrum of G5-PAMAM-GGA-NH₂ (100 µM in D₂O) with 250 µM of Fe(III)Cl₃.

Figure S21 UV/VIS spectrum of Cu(II)Cl₂ (500 µM in D₂O).
Figure S22 UV/VIS spectrum of HO-GGA-NH$_2$ (12.8 mM in D$_2$O) and UV/VIS spectrum of HO-GGA-NH$_2$ (12.8 mM in D$_2$O) with 500 µM of Cu(II)Cl$_2$. 
In case of the addition of Cu(II) to the solution of G5-PAMAM-GGA-NH₂ shows a new band clearly appearing at 300 nm. This indicates an interaction of the tripeptides with the Cu(II) ions which explains the influence of Cu(II) on $T_1$ and $T_s$ which is not observed in the cases of the other ions.

This change can also be seen by a change in color upon addition of Cu(II)Cl₂ to a solution of G5-PAMAM-GGA-NH₂ (100 µM) (Figure S19).

**Figure S23** UV/VIS spectrum of G5-PAMAM-GGA-NH₂ (100 µM in D₂O) and UV/VIS spectrum of G5-PAMAM-GGA-NH₂ (100 µM in D₂O) with 250 µM of Cu(II)Cl₂.

**Figure S24** A solution of Cu(II)Cl₂ in D₂O (12.5 mM) left compared to solution of Cu(II)Cl₂ in D₂O (12.5 mM) with G5-PAMAM-GGA-NH₂ (100 µM).
EPR measurements

All EPR measurements have been conducted at an X-band (ca. 9.5 GHz) CW EPR spectrometer (Bruker Elexsys E500 with a Bruker super-high Q resonator ER4122SHQE) at 30 K in a mixture of glycerol and D$_2$O (1:2). The temperature has been kept constant with a EPR900 cryostat from Oxford instruments with helium cooling. The modulation amplitude was 5 Gs at a microwave power of 6.325e-6 W. Besides the measurements mentioned in the main text, measurements of Cu(II) in the presence of G5-PAMAM-GGA-NH$_2$ have been conducted at different Cu(II) concentrations (100 µM, 200 µM, 300 µM, 400 µM and 500 µM). Those measurements showed no concentration dependency (figure S25).

![Figure S25](image)

**Figure S25** EPR spectra of different concentrations of Cu(II) in the presence G5-PAMAM-GGA-NH$_2$

Simulations of the EPR spectra have been conducted using the *pepper*-routine of EasySpin$^4$ (version 5.2.30) with ‘perturb2’ keyword in the software Matlab. The parameters for G5-PAMAM and G5-PAMAM-NH$_2$ are listed in Tables S3 and S4, respectively.

**Table S3.** EPR parameters used for simulation of the spectrum of G5-PAMAM. Peak-to-peak linewidths were set to 1.25 and 0.1 mT for the Gaussian and Lorentzian contribution, respectively.

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<td>60</td>
<td>572</td>
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<td>A tensor (2 x $^{14}$N) [MHz]</td>
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<td>32</td>
<td>32</td>
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<tr>
<td>A tensor (2 x $^{14}$N) [MHz]</td>
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Table S4. EPR parameters used for simulation of the spectrum of G5-PAMAM-NH$_2$. Peak-to-peak linewidths were set to 1.25 and 0.1 mT for the Gaussian and Lorentzian contribution, respectively.

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<td>A tensor (2 x $^{14}$N) [MHz]</td>
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</table>

Dynamic light scattering experiments

For an estimation of the hydrodynamic radius of G5-PAMAM-GGA in the presence of different Cu(II) concentrations, dynamic light scattering measurements have been performed. For the experiments, a solution of G5-PAMAM-GGA (10 µM) has been measured at Cu(II) concentrations of 0 µM, 10 µM, 20 µM, 30 µM, 40 µM and 50 µM respectively. Before measurements, the solutions have been sonicated in order to minimize aggregation. The measurements were performed at 37 °C and the particle size distribution was determined using a multimodal peak analysis with individual peaks analyzed by number as well as by intensity and mass. Per Cu(II) concentration 5 measurements have been performed with 100 acquisitions per measurement. The obtained particle size was shown to be in the range of the reported particle size of G5-PAMAM dendrimers.$^5$ However, no significant change in particle size could be observed by either analysis (figure S26).

Figure S26: Hydrodynamic radius of G5-PAMAM-GGA at different Cu(II) concentrations as determined by dynamic light scattering.


