Tuning the relaxation rates of dual-mode T$_1$/T$_2$ nanoparticle contrast agents: a study into the ideal system

Natasha A. Keasberry$^1$, Manuel Bañobre-López$^2$, Christopher Wood$^1$, Graeme. J. Stasiuk$^{1,4}$, Juan Gallo$^{1,2,3,*}$, Nicholas. J. Long$^{1,3,*}$

$^1$Department of Chemistry, Imperial College London, South Kensington, London, SW7 2AZ, UK

$^2$International Iberian Nanotechnology Laboratory (INL), 4715-330 Braga, Portugal

$^3$Comprehensive Cancer Imaging Centre, Department of Surgery & Cancer, Hammersmith Campus, Imperial College London, Du Cane Road, London W12 0NN, UK

$^4$School of Biological, Biomedical and Environmental Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, UK
TEM characterisation

Figure S1  TEM micrographs of Fe$_3$O$_4$ nanoparticles with different protecting ligands and at different stages of functionalization. **A**, original oleic acid protected NPs. **B**, Aminoundecanoic acid protected NPs, NP1. **C**, Phosphate PEG protected NPs, NP2. **D**, Alenronic acid protected NPs, NP3. **E**, DOTA functionalised NPs, NP4. **F**, PEG functionalised NPs, NP10. Scale bar 50 nm in A, C, E and F, and 20 nm in B and D.
FT-IR characterisation

Figure S2  Infrared spectrum of the nanoparticles before and after the ligand exchange with sodium alendronate (neat, ATR plate). Top – sodium alendronate ligand; middle – oleic acid capped nanoparticles; bottom – alendronate capped nanoparticles.
Figure S3  Infrared spectrum of the PEGylated nanoparticle showing a more pronounced C=O amide stretch as a result of the PEG coupling reaction between a COOH on the PEG molecule and NH$_2$ on the alendronate.
Magnetic characterisation

![Graph showing magnetic characterisation for different samples.](image)

**Graph Key:**
- **NP1**
- **NP2**
- **NP3**

**Graph Legend:**
- **M (emu/gFe)**
- **H**

![Graph showing comparison between ALA, DOTA-Zn, and DOTAGd.](image)

**Graph Key:**
- **ALA**
- **DOTAZn**
- **DOTAGd**

**Graph Legend:**
- **M (emu/gFe)**
- **H**
Figure S4  Magnetic characterisation of the different samples. Results not corrected for diamagnetic component from the sample holder.
TGA characterisation

Calculation of density of ligands on nanoparticle surface
To determine the stoichiometry of the reaction, the density of ligands on the nanoparticles must be known. The density of AUA ligands on magnetite nanoparticles (6 nm) has been reported to be 11 ligands/nm$^2$.\textsuperscript{1} Prior literature measurements have determined that for bisphosphonate ligands, there are approximately 1.6 molecules per nm$^2$ of iron oxide nanoparticles for a nanoparticle core of approximately 6 nm diameter.\textsuperscript{2} This equates to about 180 bisphosphonate ligands on the surface. Calculations from thermogravimetric analyses gave an average of 115 ± 30 alendronate ligands coating the nanoparticle surface, which correlates well with the literature.

Figure S5  TGA curve showing the loss of mass from the sample of NP-alendronate (after solvent loss) against temperature.

From the TGA curve, the difference in mass after the loss of water when the sample is heated can then be used to calculate the number of ligands that coat the surface of one particle.\textsuperscript{3} The total mass of ligands lost can be found by subtracting the end mass (mass of NP core only) from the starting mass (mass of NP core + ligands).

Assuming the NP is a sphere, the volume of the NP, $V_{NP}$, can be calculated:

$$V_{NP} = \frac{4}{3}\pi r^3 = 1.13 \times 10^{-19} \text{cm}^3$$
(where \( r \) is the average radii of the nanoparticles as measured from TEM images).

To find the \( M_W \) of the nanoparticle core (no ligands), the mass of 1 particle can be calculated from the density, \( d_{NP} \) (magnetite density: 5.15 g/cm\(^3\)) and volume, \( V_{NP} \) (calculated) of the NP. The relationship between the mass of 1 particle to Avogadro’s number gives the \( M_W \) of the core.

\[
\text{Mass of 1 particle} = d_{NP} \times V_{NP} = 5.82 \times 10^{-19} g
\]

\[
M_W = \text{mass of 1 particle} \times \text{Avogadro’s number} = 350752.16 \text{ Da}
\]

The number of moles of the core can then be calculated, knowing the mass of the core (mass at end of run, from TGA curve):

\[
n(\text{core}) = \frac{m(\text{core})}{M_W(\text{core})} = 1.47 \times 10^{-9} \text{ mol}
\]

The number of moles of ligands per nanoparticle can also be calculated, knowing the total mass of ligands lost from the TGA curve, and the \( M_W \) of the ligand (271.08, known).

\[
n(\text{ligand}) = 1.79 \times 10^{-7} \text{ mol}
\]

From the number of moles of ligands per nanoparticle, and the number of moles of the core, the number of ligands per nanoparticle can be calculated from the relationship between the two.

\[
\text{no. of ligands per particle} = \frac{n(\text{ligand})}{n(\text{core})} = 121 \text{ ligands}
\]

TGA of each nanoparticle sample was run three times, and the average of the combined runs calculated to obtain the average number of ligands per sample (Table S1).

Table S1  Average number of ligands determined for each batch of NP-alendronate

<table>
<thead>
<tr>
<th>NP-alendronate sample</th>
<th>Average no. of ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1</td>
<td>123.02 ± 39.78</td>
</tr>
<tr>
<td>Batch 2</td>
<td>108.42 ± 33.14</td>
</tr>
<tr>
<td>Batch 3</td>
<td>115.59 ± 33.99</td>
</tr>
</tbody>
</table>
Quantification of DOTA coupling yield, and amount of Gd(III) after complexation

Washings from the coupling reaction were incubated with known amounts of Gd\(^{3+}\) overnight. These solutions were then mixed with 0.017 mM xylenol orange solutions in 0.2 M acetate buffer. The presence of free Gd\(^{3+}\) in the solution turned the colour of the xylenol orange from pink/orange to blue/purple. This change in colour can be measured by UV-Vis spectroscopy and using a calibration curve the amount of free and bound Gd\(^{3+}\) can be calculated. The amount of bound Gd\(^{3+}\) is then directly related to the amount of non-reacted DOTA from the reaction.

Concentration of DOTA 152±19 chelates per nanoparticle

**Calibration of Gd(III) standards**

Gd(III) standards (50 μL each) in the range 0 – 5 μg Gd(III) were made up from a solution of 6.06 mg/mL GdCl\(_3\) stock solution in acetate buffer at pH 6.0. To each 50 μL standard solution, 500 μL of 0.017 mM xylenol orange solution was added and the UV spectrum recorded from 350 – 650 nm. The ratio of the absorbances at 573 nm and 433 nm can be plotted against amount of Gd(III) to give a straight line calibration plot.

![UV-spectra of Gd(III) standards with xylenol orange solution added.](image)

**Figure S6** UV-spectra of Gd(III) standards with xylenol orange solution added.
Figure S7  Ratio of absorbance (573/433 nm) plotted against amount of Gd(III) (μg).
MRI phantoms

Figure S8   MR images of nanoparticle samples and reference (Dotarem®) at different concentrations. Top, $T_1$-weighted images. Bottom, $T_2$-weighted images.
Figure S9  MR image of nanoparticle samples NP5 and NP11 at different concentrations.
PEG functionalisation strategy

Scheme S1  Scheme showing the preparation PEGylated dual NPs
Table S2  Number of PEG molecules per nanoparticle as calculated from three independent TGA measurements.

<table>
<thead>
<tr>
<th>Sample</th>
<th>N PEG molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP10</td>
<td>71 ± 22</td>
</tr>
<tr>
<td>NP11</td>
<td>57 ± 16</td>
</tr>
</tbody>
</table>
Table S3  Characterisation of the different nanoparticles prepared for this study.

<table>
<thead>
<tr>
<th>Modification</th>
<th>NP</th>
<th>Zpot (mV)*</th>
<th>Hydrodynamic size (nm)</th>
<th>Coupling yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligands</td>
<td>NP1</td>
<td>-8</td>
<td>24.13</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>NP2</td>
<td>-9</td>
<td>15.22</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>NP3</td>
<td>-13</td>
<td>20.15</td>
<td>----</td>
</tr>
<tr>
<td>Initial functionalization</td>
<td>NP3</td>
<td>-13</td>
<td>20.15</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>NP4</td>
<td>-12</td>
<td>22.40</td>
<td>&gt;80% (DOTA)</td>
</tr>
<tr>
<td>Metal</td>
<td>NP4</td>
<td>-12</td>
<td>22.40</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>NP5</td>
<td>-16</td>
<td>31.87</td>
<td>&gt;95% (Gd)</td>
</tr>
<tr>
<td></td>
<td>NP6</td>
<td>-16</td>
<td>15.70</td>
<td>&gt;95% (Mn)</td>
</tr>
<tr>
<td></td>
<td>NP7</td>
<td>-17</td>
<td>23.90</td>
<td>&gt;95% (Zn)</td>
</tr>
<tr>
<td>Distance</td>
<td>NP5</td>
<td>-16</td>
<td>31.87</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>NP8</td>
<td>-10</td>
<td>37.70</td>
<td>75% (DOTA)</td>
</tr>
<tr>
<td></td>
<td>NP9</td>
<td>-15</td>
<td>40.70</td>
<td>61% (DOTA)</td>
</tr>
<tr>
<td>PEGylation</td>
<td>NP10</td>
<td>-14</td>
<td>35.54</td>
<td>62% (PEG₆)</td>
</tr>
<tr>
<td></td>
<td>NP11</td>
<td>-12</td>
<td>40.24</td>
<td>50% (PEG₉₆)</td>
</tr>
</tbody>
</table>

*All Zpot values and hydrodynamic sizes were obtained at 25°C in water.

All Zpot values measurements gave negative values. For nanoparticles bearing a terminal amino groups (NP1, 2, 3, 10 and 11) these values could initially seem unexpected. A closer look at the structure of alendronic acid shows the presence of a bisphosphonate group with pKa values of 2.72, 8.73, 10.5 and 11.6 that give rise to the overall negative values at the neutral pH used for the measurements.

Table S4  Measurement of the colloidal stability of the nanoparticles with time

<table>
<thead>
<tr>
<th></th>
<th>Hydrodynamic size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0</td>
</tr>
<tr>
<td>NP3</td>
<td>20.15</td>
</tr>
<tr>
<td>NP5</td>
<td>31.87</td>
</tr>
</tbody>
</table>
**PEG conformation**

To estimate the most probable conformation of PEG molecules on the surface of the nanoparticles the space occupied by each molecule (D) has to be compared to the Flory radius (Rf) of the PEG.

Rf for a mushroom conformation can be calculated according to following expression:

$$Rf = a \, n^{3/5}$$

where $a$ is the monomer length (0.35 nm for PEG), and $n$ is the number of repeats (6 or 96 for PEG600 and PEG5000 respectively)

D can be calculated knowing the number of PEG molecules per nanoparticles (see table S2), the surface of each particle (assuming they are perfectly spherical, $S = 4 \pi r^2$), and assuming that the projection of the space they occupy is a circle ($S = \pi r^2$).

**Table S5**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rf (nm)</th>
<th>D (nm)</th>
<th>Conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP8/10</td>
<td>1.55</td>
<td>0.71</td>
<td>Brush</td>
</tr>
<tr>
<td>NP9/11</td>
<td>5.41</td>
<td>0.79</td>
<td>Brush</td>
</tr>
</tbody>
</table>
References


