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FFC application notes: paramagnetic nanoparticles

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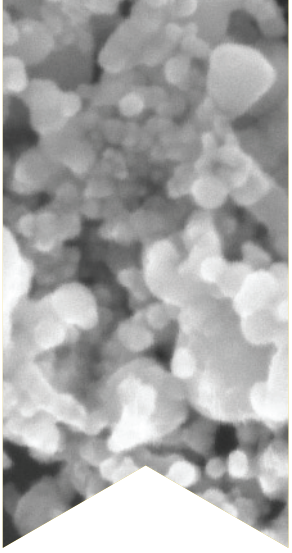

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PARAMAGNETIC NANOPARTICLES

Fast Field Cycling NMR application: paramagnetic nanoparticles as MRI contrast agents

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Introduction

The efficacy of a magnetic resonance imaging (MRI) contrast agent depends on its capability to increase the image contrast by increasing the relaxation rates of water protons selectively in specific tissues. This is achieved 1) by optimization of the paramagnetic relaxivity r1 of the agent and 2) by its functionalization towards specific biological targets **(FIG. 1)**. The relaxivity is the enhancement of the longitudinal relaxation rate of the water protons due to 1 mM paramagnetic ion concentration **[1]**. At the MRI magnetic fields, large relaxivity is achieved by slowing down the tumbling motion of the paramagnetic molecule used as contrast agent, and thus by increasing its size to the nanoscale. Functionalization of the agent is achieved in such a way that it becomes able to specifically react with a biological target. In turn, this often results in a slowly tumbling paramagnetic system composed of a Gd^{3+} complex covalently or non-covalently bound to a nano-size macromolecular substrate (to be selected depending on the clinical applications). $\mathbf{J}(\omega) \propto \mathbf{R}_1$. For these reasons, large efforts are devoted to the design and the characterization of paramagnetic nanoparticles as optimized MRI contrast agents. FFC relaxometry is the technique of choice for the characterization of the relaxation properties of these systems, because the field dependence of the paramagnetic relaxivity observed over a large magnetic field range can allow for the recovery of the many parameters on which it depends. This would not be possible if the measurements were performed at a single magnetic field or in a restricted magnetic field range.

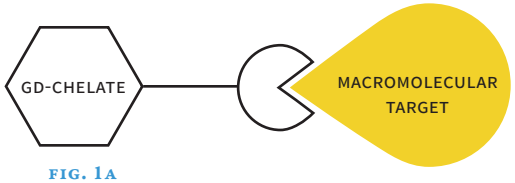

FIG. 1A

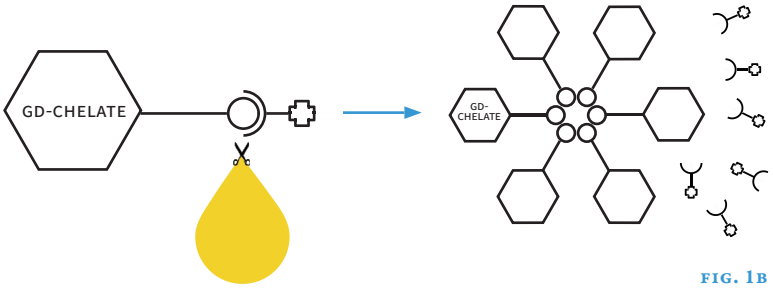

FIG. 1B

FIG. 1:
(a) Gd-based nanoparticles functionalized towards specific biological targets, and (b) Gd-based complexes forming paramagnetic nanostructures in the presence of specific targets.

Modelling of the relaxivity profile

The magnetic field dependence of paramagnetic relaxivity can be described by a number of structural, dynamic and electronic parameters. Whereas small paramagnetic complexes can be analyzed through the Solomon-Bloembergen-Morgan (SBM) model [2, 3], comprising

- the number ***(q)*** of the water molecules coordinated to each paramagnetic ion,
- the distance ***(r)*** of the corresponding water protons,
- the reorientation time of the molecule (τ_r),

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- the lifetime of the coordinated water protons (τ_M),
- the transient zero-field splitting (Δ_t) and
- the correlation time for electron relaxation (τ_V),

and through the translation diffusion model **[4]**, comprising:

- the distance of closest approach (d) between the paramagnetic ion and the water protons
- and the diffusion coefficients (D) of paramagnetic complexes and water molecules,

the analysis of the relaxivity profiles of gadolinium(III) or manganese(II) nanoparticles are complicated by the presence of static ZFS, which makes these models inaccurate. In fact, it is well known that static ZFS and/or hyperfine coupling with the metal nucleus affects dramatically the NMRD profiles with respect to the SBM model. A correct analysis of the paramagnetic relaxivity can be performed using the Florence NMRD model, which takes into account the effect of the static ZFS for molecular reorientation slower than the electron relaxation **[5–8]**, or the Grenoble **[9,10]** or Stockholm **[11–13]** models, which, besides taking into account the presence of static ZFS, can also be used outside the Redfield limit, i.e. for correlation times τ_V longer than the electron relaxation times.
The static ZFS can sizably affect the field dependence of the relaxivity profiles because it can change the ratio in the relaxivity values before and after the low field dispersion, and it can even cause the appearance of an additional dispersion in the profile, as shown in **FIG.2**. Contributions to relaxivity from water molecules bound to the paramagnetic nanoparticles outside the first coordination sphere of the paramagnetic metal, collectively indicated as second-sphere molecules, as well as contributions from internal reorientation motions (faster than the reorientation time of the whole molecule) should also be considered. Internal motions are usually modeled using the Lipari-Szabo model-free treatment **[14, 15]**.

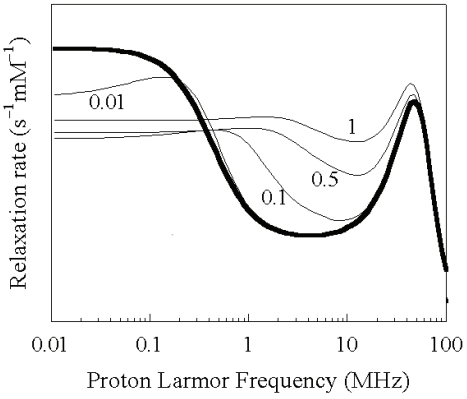

FIG. 2

FIG.2: *Relaxivity profiles calculated with the SBM model (in bold) and for different values of the static ZFS (0.01, 0.1, 0.5, 1 cm-1), all other parameters remaining unchanged.*

Examples of relaxivity profiles of paramagnetic nanoparticles

The relaxivity profile of a derivative of Gd-DTPA with a built-in sulfonamide (Gd-DTPA-SA), when free in solution, is similar to that expected for a small paramagnetic complex with one water molecule regularly coordinated to the gadolinium(III) ion, with a single dispersion at about 10 MHz **[16]**. However, sulfonamides are known to be specific inhibitors of carbonic anhydrase, being able to bind their catalytically active site (**FIG.1A**). Indeed, in the presence of carbonic anhydrase the profile shows a largely increased relaxivity, with a dispersion at about 1 MHz and a relaxivity peak in the high field region (**FIG. 3**). The presence of this relaxivity peak indicates that a complex is formed, and that the field dependent electron relaxation time is the correlation time for proton relaxation even at high fields, with the reorientation time being much longer. The profile cannot be well fitted with the SBM model, due to the presence of a small static ZFS that must be taken into account. The Florence NMRD model can be used to correctly analyze the relaxivity profile and obtain the best fit parameters (ZFS, Δ_t, τ_V and τ_M, assuming one water molecule regularly coordinated to the gadolinium(III) ion).

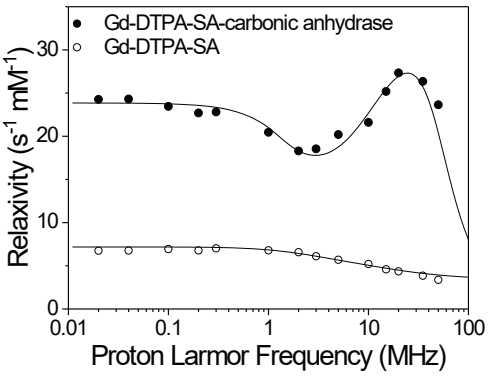

FIG. 3

FIG.3: *Water proton relaxivity profiles of a Gd-DTPA-SA in the absence and in the presence of carbonic anhydrase at 25 °C.*

MS-325 is a DTPA-derivative able to bind to serum albumin. Albumin binding increases the reorientation time of the complex in such a way that the relaxivity increases, with a relaxivity peak in the high field region of the profile. The relaxivity profiles could be fitted to determine the values of the parameters Δ_t, τ_V, τ_r and τ_M, by taking into account

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the presence of static ZFS and of fast local mobility (**FIG. 4A**) **[15].**
Gold nanoparticles covered with peptides functionalized with gadolinium complexes were shown to provide a large relaxivity at high fields; exceptionally high relaxivity was measured for gold nanostars, i.e. for nanoparticles with a star shape, instead of a spherical shape (**FIG. 4B**) [**17**]. From the analysis of the paramagnetic relaxivity profiles it was possible to show that this high efficiency is the result of optimized inner-sphere water exchange kinetics and particle surface-mediated elongation of second-sphere water lifetimes, and therefore of an enhanced outer-sphere and second-sphere effects.

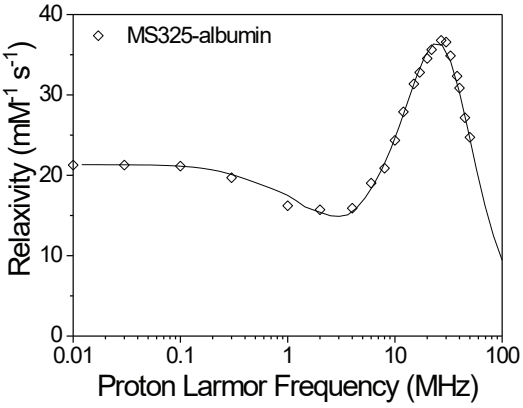

FIG. 4A

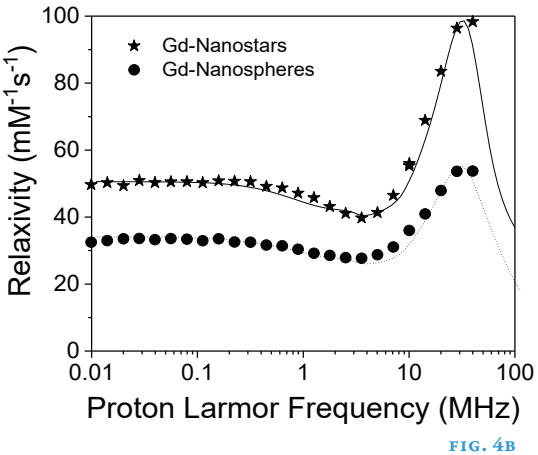

FIG. 4B

FIG.4A-B:
Water proton relaxivity profiles for (a) MS-325 bound to human serum albumin and for (b) spherical- and star-shape Gd-based gold nanoparticles, at 25 °C.

Carbon-based nanodiamond–gadolinium(III) aggregates with high relaxivity, independent of the MRI field strength, a finding unprecedented for gadolinium(III)–nanoparticle conjugates, have also been synthesized. The analysis of the paramagnetic relaxivity profiles suggests that the gadolinium(III) chelate retains rotational freedom even after attachment to nanodiamonds, and that a relatively large number of water molecules in the second coordination sphere must contribute to relaxivity [**18**].

Caspase probe 1 (CP1) is a gadolinium(III)-DOTA derivative with a DEVD peptide acting as a substrate for caspase-3/7 and tetraphenylethylene as aggregation agent. Caspases are activated during apoptosis and are unique biomarkers for this process. In the presence of caspase-3/7, the DEVD peptide is cleaved and the remaining gadolinium conjugate (Gad-AIE) aggregates (as shown in Fig. 1b). The aggregation leads to an increased relaxivity, with the peak in the high field region (**FIG. 5**) [**19**]. The relaxation profiles, analyzed with the inclusion of static ZFS and fast internal mobility contributions, confirm the occurrence of formation of large aggregates upon cleavage of the DEVD peptide. Therefore, CP1 is proposed as a MRI contrast agent (as well as a fluorescence probe) functionalized to monitor apoptosis in real-time, being thus able to provide precious information for evaluating cancer therapy response.

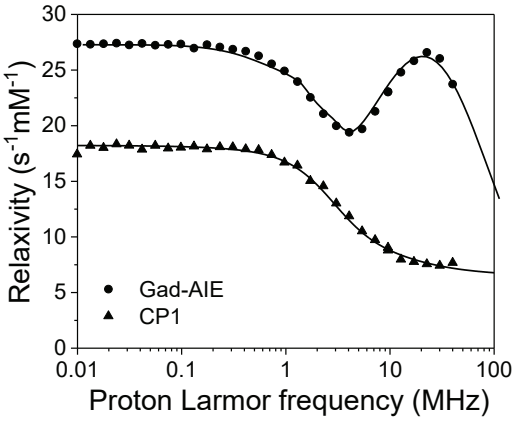

FIG. 5

FIG.5:
Water proton relaxivity profiles for Caspase probe 1 (CP1) and Gad-AIE at 25 °C. The appearance of the high field peak in the profile of Gad-AIE indicates the occurrence of aggregation due to cleavage of the DEVD peptide, present in CP1, by caspase-3/7.

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