UNIVERSITÀ DEGLI STUDI DI PAVIA
DIPARTIMENTO DI FISICA

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This is my appraisal on the PhD thesis by Alberto Fringuello Mingo

Mingo presents a throughout experimental study of novel Gd and Mn-based Magnetic Resonance Imaging (MRI) contrast agents focusing on the ones yielding a positive contrast. A fruitful comparison with the properties of comercially available contrast agents is carried out at the aim of developing new agents characterized both by an improved longitudinal relaxivity (r_1) at the magnetic fields where clinical MRI scanners operate as well as by an enhanced chemical stability, in order to avoid undesirable toxicity which may eventually lead to pathologies in patients with reduced renal function.

In the first chapter a nice introduction to the very basic aspects of nuclear magnetic resonance and of MRI is presented along with the main properties of the MRI contrast agents. In particular, the main constraints which should be considered in the design of a contrast agent and the drawbacks which should be avoided are introduced: the need of a high r_1, a high stability, rapid clearance, low osmosity, possible artifacts caused by contrast agents in MRI acquisition protocols, etc… Mingo was able to summarize in this part the essential concepts needed to follow the subsequent parts without introducing a heavy formalism and making the reading enjoyable, even if in a few points a more detailed presentation would have been beneficial, as in the presentation of the RF pulse sequences, for example.

In the second chapter the theoretical models used to analyze the contrast agents relaxivity are presented. The contribution to the relaxivity from the inner sphere and outer sphere processes are described along with the main parameters (e.g. hydratation number) and relevant correlation times, as the one describing the rotational motions, the water exchange and the electron spin fluctuations. A series of simulations evidencing how the relaxivity changes upon varying the different parameters is shown. These are particularly useful to unravel which are the most important parameters that one should optimize in order to increase the relaxivity of a contrast agent. Furthermore, the role of the prototropic exchange in the relaxation mechanism is outlined.

The most original and important results are presented in the third chapter. In this chapter the NMR dispersion (NMRD) profiles of the main comercial Gd-based contrast agents are presented and analyzed in the light of the models presented in the second chapter. The experimental results show that there is a neat enhancement of the relaxivity when the contrast agents are dissolved in the human plasma, with respect to the relaxivity found in saline solutions or in viscous solutions of the body fluid. Studies as a function of the pH of the solution, of the viscosity and of the HSA protein concentration show that there is not a dominant factor which

determines the experimental relaxivity but that all three contribute. In fact, Mingo shows that the NMRD profiles of the commercial Gd-based contrast agents can be explained starting from the NMRD profile of the agent in saline solution with the addition of the viscosity, HSA and prototropic exchange effects. Then, the relaxivity of two novel Gd-based contrast agents with enhanced longitudinal relaxivity are presented. These agents show a relaxivity which is about twice the one of the commercially available contrast agents for magnetic fields relavant for MRI clinical use. Also here the NMRD profiles can be explained by taking into account the three factors described above and it is evidenced that the increased relaxivity has to be associated with an enhanced contribution from the inner sphere processes and, in particular, with a more significant prototropic exchange and a more effective binding of the contrast agents to the HSA proteins, yielding a slowing down of the rotational motions. Moreover, ^{17}O R_2 measurements have been performed to investigate the effect of the substitution of different chemical groups on the Gd-based contrast agents and how the water exchange time is affected by them. Finally, the chemical stability of these novel contrast agents has been assessed by studying the transmetallation of Gd with Zn as a function of time, under conditions which are more demanding than the ones found in vivo. This sort of stress test of the toxicity shows that one of the two novel contrast agents is quite stable against transmetallation, making it a potential candidate for future clinical applications.

The final chapter concerns the relaxometric properties of novel Mn-based contrast agents, both with a single Mn^{2+} ion per molecule or in the form of nanoparticle. The former ones are candidates for blood pool MRI contrast agents, showing a remarkable enhancement of the relaxivity thanks to the strong binding to HSA. The Mn nanoparticles are of potential interest in the MRI detection of tumours. In fact, owing to their size, they can hardly perfuse in healthy tissues but can leak when tumours enhance the local permeability. A MRI study of animal models with mammary tumours were used to test a particular Mn-nanoparticle-based agent. It is shown that the contrast to noise ratio (CNR) reached is similar to that obtained with a dose 5 times larger of a commercial Mn-based contrast agent. Nevertheless, the maximum CNR enhancement is obtained at acquisition times much longer than the ones of commercial agents and, hence, that further studies are needed to optimize this aspect.

Overall the thesis evidences a rather careful experimental activity performed using different techniques and experimental approaches (NMRD, MRI, ^{17}O R_2, etc…), combining measurements in different experimental conditions which allow both to better understand the physical mechanisms underlying the longitudinal relaxivity as well as to simulate the clinical conditions. The analysis of the results is appropriately performed and a well balanced discussion of the possible explanations outlined. Part of the presented research activty has already been published in two peer-reviewed international journals, described in four submitted European patents and it is likely that other publications will originate from the work presented in this thesis. This demonstrates not only the timeliness and originality of this work but also the technological impact that it may have.

Therefore, I am fully convinced that Alberto Fringuello Mingo deserves to defend his thesis.

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Finally, I have a few minor comments on the two first chapters that should be considered in the editing of the very final version of thesis:

- At p.16 one reads that "any element with an odd number of particles in the nucleus can be used for MRI". It would be more appropriate to replace "particles" with "nucleons". Moreover, one should consider that also nuclei with an even number of nucleons (e.g. ^{14}N, ^{2}H) could be used for MRI.
- In the same paragraph, while discussing the H atom, the analogy with a tiny bar magnet is presented but one should consider that the atom magnetic moment is basically determined by the electron not by the proton.
- In Eq.1.4 one should explain why M_x and M_y vanish (random phase approximation). Accordingly the last sentence of the first paragraph of p.19 is incorrect: while each nuclear spin precess around B_0 the total magnetization does not precess, namely the x and y components of the magnetization are averaged out.
- p.27. The fact that Gd metal orders ferromagnetically is totally irrelevant here. Ferromagnetism is a collective phenomenon while in the CA one has an isolated Gd moments which does not interact with the one of Gd in the other CAs.
- At p.49 and 50 the "Curie nuclear spin relaxation" is introduced and one reads that the relaxation rates are proportional to $\langle S_z \rangle^2$ and hence to the square of the magnetic field intensity. This is not correct, the nuclei probe the mean squared amplitude of the local electron spin fluctuations ($\langle S_{x,y,z}^2 \rangle$) which weakly depends on the magnetic field for $k_B T >> \mu_B H$, they do not probe the average component of $\langle S_z \rangle$, which is proportional to the total magnetization.

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Yours sincerely,

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