Large longitudinal relaxivities were observed in MnII-containing Prussian blue analogue nanoparticles. At low concentrations and high field (7 T), a remarkable positive contrast enhancement was seen which exceeded that of clinical contrast agents and was attributed to the very large proportion of surface atoms of these coordination nanoparticles.

Magnetic resonance imaging (MRI) is a non-invasive technique that allows the diagnosis of diseased zones. In 30% of clinical tests, contrast agents (CAs) are used to enhance the image quality. Designing positive CAs denoted as T1-CAs with larger longitudinal relaxivities \( r_1 \) is one way to reduce the injected dose into the patient. The brightening conferred by such CAs originates from the fast proton relaxation of water molecules coordinated to paramagnetic ions with a large spin, a long electronic relaxation time and a fast water exchange rate such as in GdIII chelates commonly used as clinical CAs and in MnII complexes.\(^1\)\(^–\)\(^3\) One valuable strategy to improve their efficiency and delay their blood clearance is to use larger structures including these paramagnetic complexes or ions. A longer rotational correlation time leading to local reorientation of the complexes leads to low values of relaxivities. Hence, active paramagnetic species directly included into the core of NPs is a way to circumvent this problem, as described for some GdIII and MnII oxides,\(^7\)\(^,\)\(^8\)\(^,\)\(^12\)\(^–\)\(^17\) fluorides\(^18\) and metal organic frameworks.\(^19\)\(^–\)\(^25\) Among microporous networks, cyano-bridged coordination networks such as Prussian blue analogue (PBA) NPs have been extensively investigated for their magnetic/electronic properties,\(^26\)\(^–\)\(^29\) but less focus has been placed on MRI-CAs, despite the FDA approval of Prussian blue. Citrate coated Prussian blue NPs of 13 nm have been reported\(^30\)\(^,\)\(^31\) with a modest negative \( T_2 \) contrast enhancement, while GdIII-containing polycyanometallate NPs below 5 nm coated with polysaccharides (either in acidic\(^32\) or neutral\(^33,\)\(^34\) aqueous media) and PB NPs containing GdIII\(^35\) structures of around 100 nm revealed high longitudinal relaxivities. Recently, large MnII PBA particles have been reported (on exchange resin or with silica) with large transverse relaxivities \( (100 < r_2 < 205 \text{ mM}^{-1} \text{s}^{-1}) \) that are adapted to \( T_2 \)-weighted images.\(^36\)

In this communication, \( K_{\text{eq}} = 32 \text{ MnII}_{x} \text{InIII}_{1-x} \text{[FeII(CN)}_6\text{]} \) NPs with controlled contents of MnII have been obtained between 5 and 21 nm through coprecipitation by a one-step process in water without any additional reactant, followed by post-coating by dextran. These new compounds (abbreviated as MnInFe@Dextran NPs) exhibit large longitudinal relaxivities for MnII-based CAs (and \( r_2/r_1 \) close to 2) that exceed those of MnII oxide NPs. A remarkable positive T1 contrast enhancement was registered exceeding that of clinical chelate Gd-DTPA (Magnevist) under conditions usually unfavorable to T1 paramagnetic oxide NPs, i.e. at a low paramagnetic ion concentration \( (0.2 \text{ mM of MnII}) \) under high field (7 T) and with short TR MRI sequences.

Nanoparticles were produced without any additive by fast mixing of an aqueous solution of hexacyanoferrate(iii) with an aqueous solution containing MnII and InIII salts in variable ratios, \( x = [\text{MnII}]/[\text{InIII}] \) from \( x = 0.05 \) to \( x = 0.9 \) (see ESI†). Formation of stable colloidal solutions without aggregation was monitored by dynamic light scattering (DLS) and the results reveal hydrodynamic diameters that depend on the MnII ions content for \( x > 0.4 \) (Fig. S1, ESI†). Without the introduction...
of InIII ions \((x = 1)\), the colloidal solution was unstable. Introduction of only 10% of InIII ions enables the control of NPs with hydrodynamic diameters below 20 nm while 90% of InIII ions lead to a hydrodynamic diameter of 7 nm. This is related to the high insolubility of InFe PBA that controls the nucleation rate. Powders of NPs obtained with \(0.05 < x < 0.9\) were recovered by adding 25 equivalents of the dextran monomer per ferrocyanide and flocculation with acetone. MnInFe@Dextran NPs were subsequently dispersed in water up to 10 mM with stability for over a period of months without any change in the hydrodynamic diameter. Transmission electron microscopy (TEM) was carried out on these dispersions which revealed homogeneous nanoparticles isolated by dextran chains, with an average size ranging from 4 ± 1 nm to 20.7 ± 3.5 nm for \(x = 0.05\) and \(x = 0.9\), respectively (Fig. 1 inset and Fig. S2, ESIF). X-ray powder diffraction performed on the powders confirmed the face centered cubic structure of the NPs in all samples (Fig. S3, ESIF), with a decrease of the average cell parameter from 10.43 Å to 10.13 Å as the MnII ion content increased from \(x = 0.05\) to \(x = 0.9\), which is in good agreement with the smaller ionic radius of the MnII ion compared to that of the InIII ion. This indicates that MnII ions are not inserted in the coordination network. Fourier transform infra-red (FT-IR) spectra recorded for the different compounds revealed a broad band with two contributions at 2067 cm\(^{-1}\) and 2108 cm\(^{-1}\) that were attributed to the superimposed asymmetric vibrations of the bridged cyanides MnIII–NC–FeII\(^\dagger\) and InIII–NC–FeII, respectively, with the most intense contributions shifted to low frequencies for larger MnII proportions (Fig. S4, ESIF). The composition of metal ions and dextran was assessed by elemental analysis (Fig. S5, ESIF) that showed a decrease of the ferrocyanide vacancies from 20% to 10% upon decreasing \(x\). It is possible to estimate the number of dextran chains (~100) from the size and composition considering an average degree of polymerization of 247, leading to around 3 anchoring points between each chain and the surface of NPs. Magnetic measurements recorded at 5 K (Fig. S6, ESIF) confirmed the paramagnetic behaviour of all samples, with a coherent increase of the magnetization value at 5 T as the MnII contents were increased (the other ions InIII and low spin FeII are diamagnetic). Relaxometry measurements were registered at 37 °C (310 K) under a field of 1.5 T on the colloidal solutions obtained for samples with \(0.05 < x < 0.9\) (Fig. 1).

The longitudinal and transverse relaxivities \(r_1\) and \(r_2\) \((i = 1\) and 2 respectively\) expressed per mM of MnII ions (the other ions being diamagnetic) show a strong dependence on the MnII content indicating that all MnII ions are not equivalent depending on their location at the surface or in the core of the particles. Large longitudinal relaxivities between \(r_1 = 9\) mM\(^{-1}\) s\(^{-1}\) up to \(r_1 = 15\) mM\(^{-1}\) s\(^{-1}\) have been determined for samples with \(x = 0.3\) to \(x = 0.05\), respectively. A value of \(r_1 = 15\) mM\(^{-1}\) s\(^{-1}\) per MnII ion is 40 times that of 7 nm MnO nanoparticles\(^\dagger\) and about twice that reported for the best 2.5 nm MnO nanoparticles\(^7,8\) (also recorded at 1.5 T). Since in this range of the MnII content the size of particles is around 5 nm, the high proportion of MnII ions located at the surface of particles (calculated to be around 45%, Fig. S7, ESIF) may explain this efficiency, as these ions have a larger number of coordinated water molecules during efficient exchange with bulk water.† Upon increasing the MnII proportion from \(x = 0.4\) to \(x = 0.9\), the \(r_1\) values decreased from 5 mM\(^{-1}\) s\(^{-1}\) to 1.6 mM\(^{-1}\) s\(^{-1}\) (expressed per mM of MnII ions). In this range of the MnII content, the size of particles increases together with the MnII content resulting in (i) a decrease of the relative quantity of ions located at the surface of nanoparticles and (ii) an increase in the number of MnII ions located in the core of the particles and thus less activity upon proton relaxation. The transverse relaxivities \(r_2\) follow the same trend as that followed by \(r_1\) with values comprised between 30 mM\(^{-1}\) s\(^{-1}\) and 1.4 mM\(^{-1}\) s\(^{-1}\) (per mM of MnII ions), and \(r_2/r_1\) ratios between 1.1 and 1.8, which confirm that these particles are expected to behave as positive CAs (Fig. S8, ESIF). These measurements were reproduced for different batches of particles.

Among the various samples, nanoparticles with a proportion of 33% Mn (\(x = 0.33\) denoted as NP-33%) were selected as they display the best relaxivity for the minimum total amount of metallic ions of FeIII, InIII and MnII (see calculation in Fig. S9, ESIF). The nuclear magnetic relaxation dispersion (NMRD) profile of NP-33% at 37 °C shows a typical frequency dependence of \(r_1\) for slow tumbling CAs, which confirms that MnII ions are incorporated in the core of NPs (Fig. 2a). In addition, the low frequency profile discards any free MnII ions. The decrease of longitudinal relaxivity \(r_1\) at 300 MHz (7 T) is similar to that usually observed in nanoparticles.

![Fig. 1](image1.png) Longitudinal relaxivity \(r_1\) dependence of MnInFe@Dextran NPs on different MnII ion contents. Inset: the TEM image of NP-33%.

![Fig. 2](image2.png) (a) NMRD profile of NP-33% at 37 °C. (b) T1-weighted MR images of left: NP-33% at [MnII] = 0.2 mM, center: Gd-DTPA clinical CA at [GdIII] = 0.2 mM and right: water, from a 7 T clinical MRI system with a spin gradient sequence with TR = 22 ms and TE = 3 ms. (c) Cell internalization of NP-33% coated by Dextran-TRITC after 60 min.
for paramagnetic nanoparticles. Longitudinal relaxivity was also registered at 60 MHz (3 T) at 5 °C which increases from \( r_1 = 1.0 \text{ mM}^{-1} \text{ s}^{-1} \) at 37 °C to 12.9 \( \text{ mM}^{-1} \text{ s}^{-1} \) at 5 °C (Fig. S10, ESI†), suggesting that the water exchange is not the limiting parameter but rather the rotational correlation time is. In addition, the filtrate obtained after ultrafiltration of the colloidal solution was analyzed: its relaxation time is similar to that of water, and ICP measurement reveals negligible MnII and InIII addition, the filtrate obtained after ultrafiltration of the colloidal solution after 6 months and by DLS measurements recorded in serum at 5 mM (Fig. S11, ESI†).

Importantly, a large \( T_1 \)-weighted contrast enhancement was observed for NP-33% under a field of 7 T (300 MHz) at a concentration of 0.2 mM in MnII ions exceeding that of Gd-DTPA (Fig. 2b). The high contrast observed at high field and low concentrations may be further increased if recorded at 3 T (60 MHz). This highlights the remarkable activity of NP-33% as a \( T_1 \)-CA compared to MnII-based oxides (that are usually compared to pure water). This efficiency can be related to a moderate \( r_2 \) (and thus low \( r_2/r_1 \)) due to a weak number of paramagnetic sites contained in these 5 nm NPs (only 240 MnII atoms per particle, since InIII and FeII are diamagnetic) combined to the efficient exchange of water on the MnII atoms located at the periphery of NPs. Indeed, the presence of dextran does not impede the exchange of water molecules as the “bare” NP-33% have a smaller relaxation \( (r_1 = 7 \text{ mM}^{-1} \text{ s}^{-1} \text{ at } 37 \text{ °C and } 1.5 \text{ T}) \) compared to coated NP-33%, due to the decrease of the rotational correlation time. This again suggests that the former is the parameter limiting the relaxivity of these nanosystems.

Cytotoxicity of NP-33% was examined by the MTT test performed on two cell lines. The HEK293 cells were unaffected after 24 h below 5 mM concentrations of NP-33% and comparable to pure dextran (no toxicity at 8 mg mL\(^{-1}\), Fig. S12, ESI†). Murin mammalian cells revealed an IC\(_{50}\) of 3.8 mg mL\(^{-1}\) (532 \mu M of NP-33%) after 24 h, while it remained non-toxic for short times (1 h) up to 19 mg mL\(^{-1}\) (2 mM, Fig. S12, ESI†). In summary, NP-33% were observed to be non-toxic to weakly toxic up to fairly high concentrations for short and long times, respectively, and appears to be dependent on the cell line. In vitro studies were also performed to monitor cell internalization by confocal microscopy at \( \lambda = 500-700 \text{ nm} \) using NP-33% coated with dextran chains labeled with 0.1% of the fluorescent tetramethylrhodamine (TRITC) (Fig. S13, ESI†) and were fully characterized as the unlabelled ones (Fig. S14, ESI†). Fast internalization in less than 30 min was observed with the formation of vesicles (Fig. 2c and Fig. S15, ESI†) and no penetration was detected in the cell nucleus.

In summary, the high proportion of atoms located at the surface of these nanoparticles as compared to oxide or metal nanoparticles due to the low metallic density of microporous PBAs leads to remarkable longitudinal relaxivities per MnII atom among the largest reported for MnII with along that of Si quantum dot clusters doped with MnIII.\(^{37}\) As a consequence of a low \( r_2/r_1 \) ratio, a large \( T_1 \)-contrast enhancement is registered at low concentrations of MnII ions under high field conditions and short MRI sequences exceeding that of Gd-based clinical CAs. Their high stability, low toxicity and water-based preparation at room temperature make this family of MnII-based PBA contrast agents promising candidates for \textit{in vivo} MRI diagnosis. Incorporation of \(^{111}\)In and other active probes for single photon emission computed tomography (SPECT) will lead to multimodality on these new CAs.\(^{38}\)

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Notes and references

‡ Because of less than 10% ferrocyanide vacances in this range of the MnII content and albeit the microporosity of the network, internal MnII ions that bear a small number of water molecules are not expected to play a relevant role in these compounds.


