Electronic Supplementary Information

Mn^{II}-containing coordination nanoparticles as highly efficient T_1 contrast agents for MRI

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Characterization techniques

The Dynamic Light Scattering (DLS) measurements have been performed on a Malvern Nanozetasizer Apparatus (equiped with a backscattering mode) on the colloidal solutions containing the particles. The number profile was used to estimate the size of the nanoparticles. This measurement was used as a qualitative measurement of the size of the particles or aggregates in solution, wich systematically includes a solvation shell. It provides an estimation of the particles' size directly after the synthesis.

The TEM mesurements have been done on a TEM JEOL 1400 with 120 keV incident electrons focused on the specimen.

X-Ray Powder Diffraction (XRPD) was performed on powders deposited on aluminium plate and recorded on a Philipps Panalytical X'Pert Pro MPD powder diffractor at CuKα radiation equipped with a fast detector.

Magnetic measurements were carried out with a Quantum Design MPMS-5S magnetometer working in the dc mode.

^{1}H NMRD profiles (nuclear magnetic resonance dispersion) were obtained at 37°C using a Stelar fast field cycling relaxometer (Mede, Italy) over a range of magnetic fields extending from 0.25 mT to 0.94 T (0.01–40 MHz). Measurements of T_1 and T_2 relaxation times were performed at 37°C on a Bruker Minispec systems mq20, mq60 and on a spectrometer Avance 300 (Karlsruhe, Germany) working at 20, 60 MHz and 300 MHz (0.47, 1.41 and 7T) respectively.

MR imaging was performed on a 7 T PharmaScan (70/16 US, Bruker, Ettlingen, Germany). One-millimeter thick image slice was collected using a RARE sequence. The parameters for T1 map were: TR/TE=5000,3000,1500,800,400,200,100,50ms/8.5 ms, RARE factor=2, MTX=256x256, FOV=35x27 (coronal) or 25x25 (coronal) mm, 2 averages, TA=35min21sec.
The T2 map sequence was a MSME of one millimeter thick with the following parameters: TR/TE=5000/10,89 ms, MTX=256x256, FOV=35x27 (coronal) or 25x25 (coronal) mm, 1 average, TA=16min.The number of echoes with a constant of 10,89ms was 64.
The sequence weighted T1 was a FLASH of one millimeter thick with the following parameters: TR/TE=21,6/3,5 ms, flip angle= 40°, MTX=256x256, FOV= 20x20 (coronal) mm, 128 averages, TA=9min

Absorption measurements have been carried out on a Cary 5000 Spectrophotometer on 3mL of the particles dispersed in aqueous solution.

Fluorescence mesurements were performed on a Fuoromax-4 Fluorospectrophotometer on 3mL of the particles dispersed in aqueous solution.

HEK293-cells, human embryonic kidney 293 cell line were cultured in DMEM (PAA, Linz, Austria), enriched with 4.5 $g.L^{-1}$ glucose, 1 % glutamine and supplemented with 10% FBS. TSA cells, mouse mammal carcinoma cells, were cultured in RPMI supplemented with 10% FBS. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cell survival was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cells (15 000 cells) were plated

in 96-wells plates 24 hours before the experiment. Cells were incubated with the indicated concentration of particles for either 1 or 24 hours, and placed at 37 °C, 5% CO_2. For MTT assay, 100 μL of 0.5 mg/mL MTT solution diluted in the phenol red free medium were added in each well and incubated for 4 h at 37°C in order that the surviving cells can form formazan crystals. These crystals were then dissolved by adding 100 μL per well of 10% Triton x100/0.1N HCl in anhydrous isopropanol solution. The plate was placed under gentle mixing and the absorbance was read at 570 and 650 nm. Results were calculated as the absorbance at 570 minus the absorbance at 650 nm and compared to the control condition. For the negative control condition, cells were incubated with 10uM of staurosporine.The experiment was performed in triplicate.

Confocal microscopy was performed on the Axiovert 200 LSM510 Meta microscope (Carl Zeiss, Jena, Germany) using a 40x oil immersion objective of 1.2 Numeric Aperture (N.A.). The 543 nm laser intensity was set up at 10 % of it maximum intensity and the emission was collected at 550-700 nm. For this experiment, cells were plated on Lab-Tek chamber I and grown in red phenol-free medium.

Synthetic procedure

Synthesis of $K_{4y-3+x}Mn^{II}_{x}In^{III}_{1-x}[Fe^{II}(CN)_6]_y$, nH_20 @ Dextran nanoparticles

Synthesis of $K_{4y-3+x}Mn^{II}_{x}In^{III}_{1-x}[Fe^{II}(CN)_6]_y$, nH_20 nanoparticles

The synthesis was carried out at 2°C. An aqueous solution (100 mL) of $MnCl_2$ and $InCl_3$ (0.5 mM,) was mixed rapidly with an equal volume of an aqueous solution containing $K_4Fe(CN)_6$ (0.5mM) under vigorous stirring. The solution was stirred for 30 minutes at 2°C and for 30 minutes at room temperature. An uncolored solution is obtained and nanoparticles' size is checked by Dynamic Light Scattering.

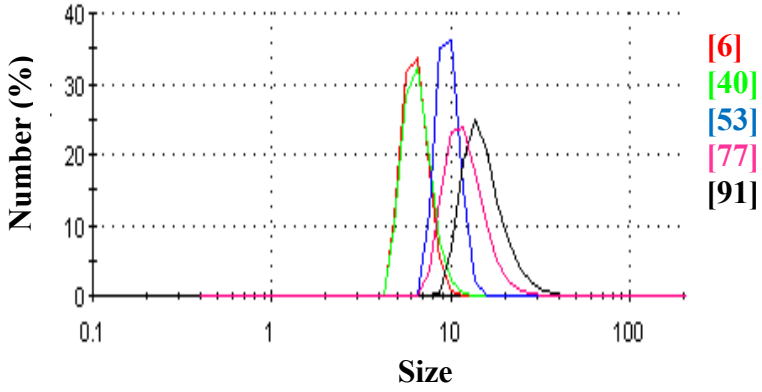
Particles coated with Dextran

To the freshly prepared aqueous solution of $K_{4y-3+x}Mn^{II}_{x}In^{III}_{1-x}[Fe^{II}(CN)_6]_y$, nH_20 nanoparticles, 25 equivalents of Dextran monomer (repeating unit MW = 162 $g.mol^{-1}$) per divalent metallic ion were added to the solution of nanoparticles. 0.8 volume of acetone with respect to the volume of the particles solution were added. The floculated particles were centrifugated at 8000 rpm during 15 minutes and the resulting solid was dried under vacum. The resulting powder of particles was characterized by Infra-red, TEM, XRPD, Elemental Analysis and SQUID mesurement.

Dispersion of $K_{4y-3+x}Mn^{II}_{x}In^{III}_{1-x}[Fe^{II}(CN)_6]_y$, nH_20 @ Dextran nanoparticles

The resulting powder is dispersed at 10mM of nanoparticles in 500 µL of water and sonicated during 5 minutes. The colloidal solution obtained was characterized by DLS. Longitudinal and transverse relaxation times (T1 and T2 respectively) mesurements as well as in vitro studies and MTT test have been done on the dispersion of particles.

Figure S1
Examples of Dynamic Light Scattering plots and hydrodynamic diameters extracted for the various samples depending on the Mn^{II} content



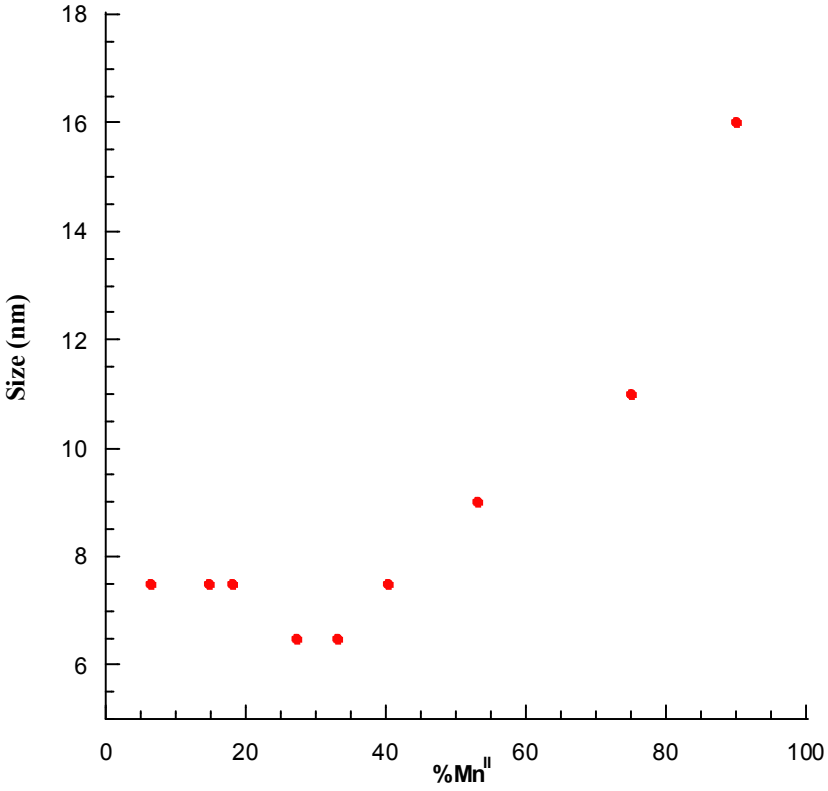


Figure S2
Transmission Electronic Microscopy images for Mn(II) contents of 6, 15, 25, 50, 77 and 91%

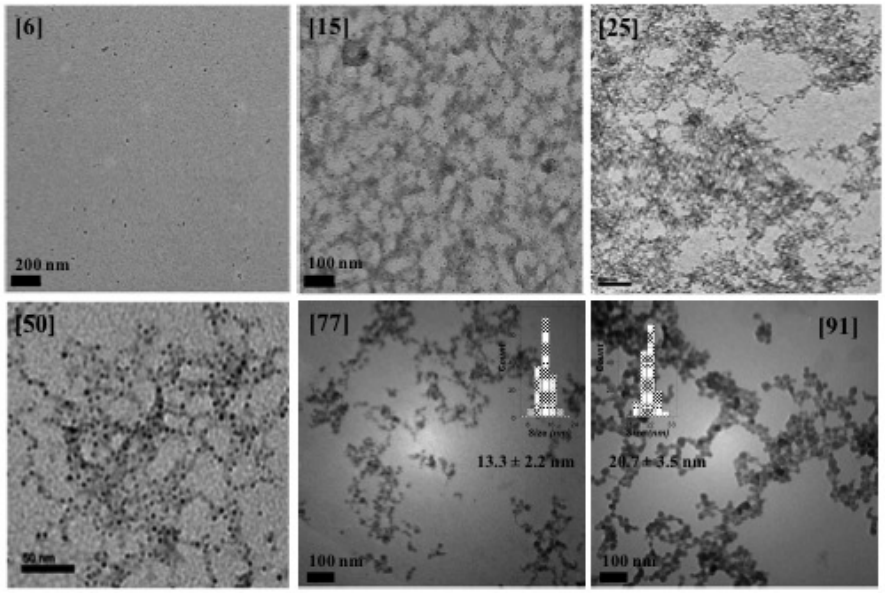
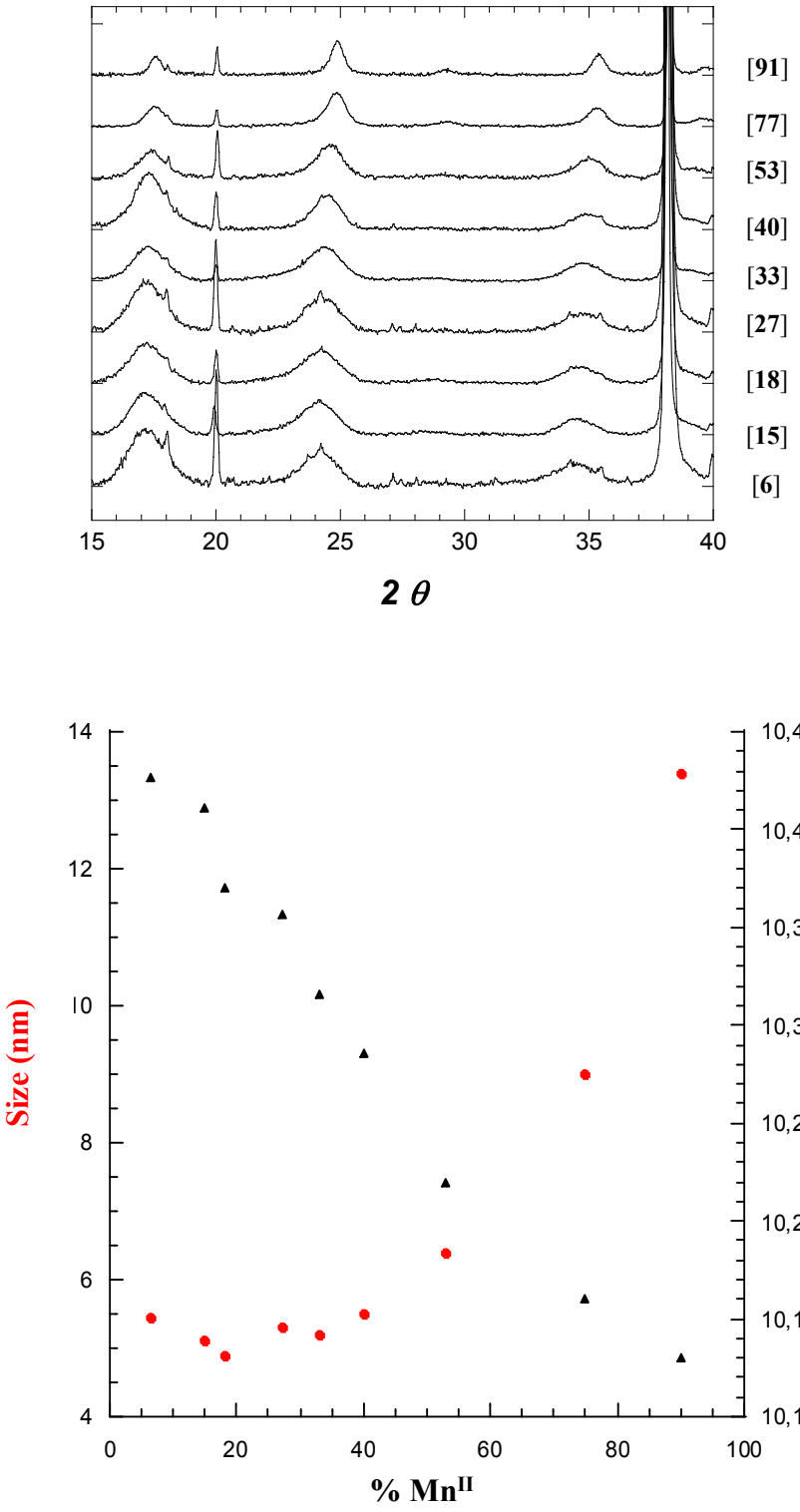


Figure S3
X-Ray powder diffractograms and cell parameter a (black triangles) and correlation length dependence (red cirles) over the Mn(II) content, extracted from X-Ray powder diffractograms



The nanoparticles' size and cell parameter for each sample were calculated from the (220) and (400) Bragg reflections using Sherrer's formula and Bragg law respectively.

Figure S4
Asymmetric vibration of the cyanide bond extracted from the Infra-Red spectra in the 2300-1900 cm^{-1} region for the different Mn^{II} contents

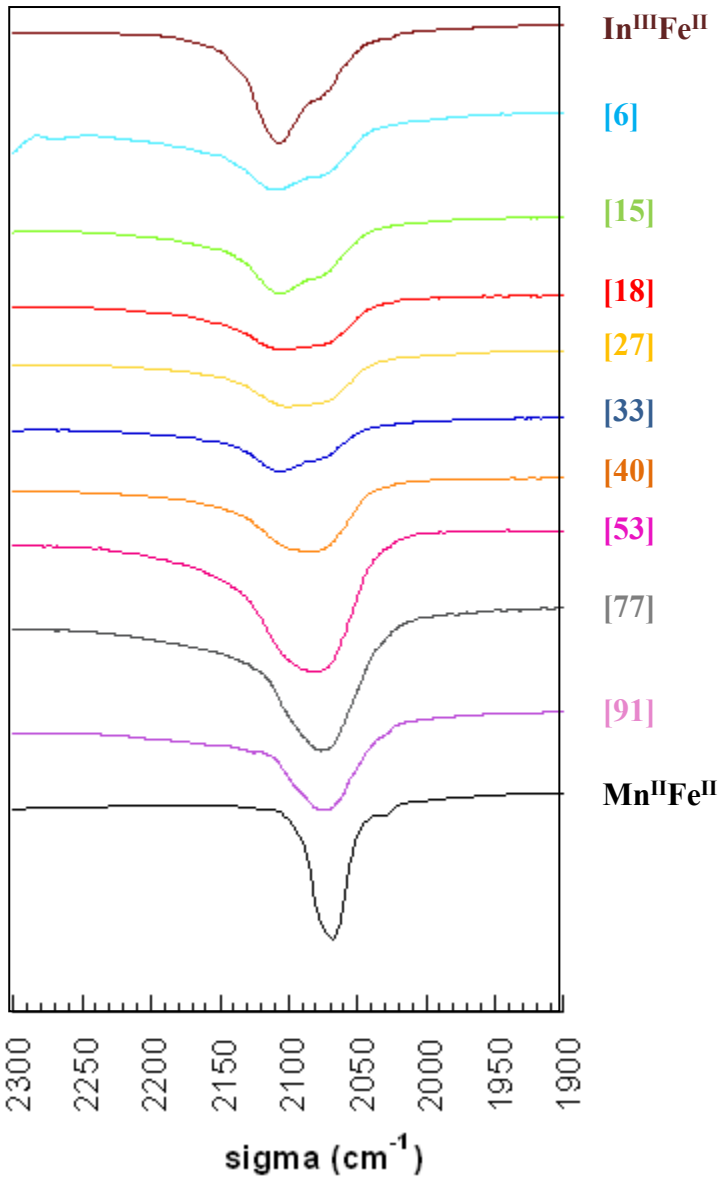


Figure S5
Composition determined by Elemental Analysis

%Mn^{II}	Formula units for $Mn^{II}_xIn^{III}_{1-x}[Fe^{II}(CN)_6]_y$ samples
[6]	$\mathbf{K_{0,70}Mn^{II}_{0,06}In^{III}_{0,94}[Fe^{II}(CN)_6]_{0,91}.49H_2O\ (dext)_{48}}$
[15]	$\mathbf{K_{0,75}Mn^{II}_{0,15}In^{III}_{0,85}[Fe^{II}(CN)_6]_{0,90}.55H_2O\ (dext)_{49}}$
[18]	$\mathbf{K_{0,86}Mn^{II}_{0,18}In^{III}_{0,82}[Fe^{II}(CN)_6]_{0,92}.31H_2O\ (dext)_{32}}$
[27]	$\mathbf{K_{0,83}Mn^{II}_{0,27}In^{III}_{0,73}[Fe^{II}(CN)_6]_{0,89}.65H_2O\ (dext)_{40}}$
[33]	$\mathbf{K_{0,81}Mn^{II}_{0,33}In^{III}_{0,67}[Fe^{II}(CN)_6]_{0,87}.36H_2O\ (dext)_{35}}$
[40]	$\mathbf{K_{1,00}Mn^{II}_{0,40}In^{III}_{0,60}[Fe^{II}(CN)_6]_{0,90}.33H_2O\ (dext)_{33}}$
[53]	$\mathbf{K_{1.01}Mn^{II}_{0,53}In^{III}_{0,47}[Fe^{II}(CN)_6]_{0,87}19H_2O\ (dext)_{26}}$
[77]	$\mathbf{K_{1,09}Mn^{II}_{0,77}In^{III}_{0,23}[Fe^{II}(CN)_6]_{0,83}22H_2O\ (dext)_{23}}$
[91]	$\mathbf{K_{1,11}Mn^{II}_{0,91}In^{III}_{0,09}[Fe^{II}(CN)_6]_{0,80}22H_2O\ (dext)_{23}}$

[**6**]: $K_{0.70}Mn^{II}_{0,06}In^{III}_{0,94}[Fe^{II}(CN)_6]_{0,91}.49H_2O\ (dext)_{48}$ (M= 9024 $gmol^{-1}$); calcd (%) for $C_{294.66}H_{579.38}N_{5.46}Mn_{0.06}In_{0.94}Fe_{0.91}K_{0.70}O_{289.69}$: C 39.22, H 6.47, N 0.85, Fe 0.56, K 0.30, Mn 0.04, In 1.20; found: C 38.4, H 6.33, N 0.84, Fe 0.58, K 0.29, Mn 0.04, In 1.21.

[**15**]: $K_{0.75}Mn^{II}_{0,15}In^{III}_{0,85}[Fe^{II}(CN)_6]_{0,90}.55H_2O\ (dext)_{49}$ (M= 9217 $gmol^{-1}$); calcd (%) for $C_{297.60}H_{597.41}N_{5.40}Mn_{0.15}In_{0.85}Fe_{0.90}K_{0.75}O_{298.70}$: C 38.78, H 6.53, N 0.82, Fe 0.55, K 0.32, Mn 0.09, In 1.06; found: C 38.3, H 6.45, N 0.82, Fe 0.52, K 0.29, Mn 0.08, In 1.00.

[**18**]: $K_{0.86}Mn^{II}_{0,18}In^{III}_{0,82}[Fe^{II}(CN)_6]_{0,92}.31H_2O\ (dext)_{32}$ (M= 6302 $gmol^{-1}$); calcd (%) for $C_{205.52}H_{396}N_{5.52}Mn_{0.18}In_{0.82}Fe_{0.92}K_{0.86}O_{198}$: C 39.17, H 6.33, N 1.23, Fe 0.82, K 0.53, Mn 0.16, In 1.49; found: C 38.1, H 6.16, N 1.2, Fe 0.83, K 0.52, Mn 0.16, In 1.51.

[**27**]: $K_{0.83}Mn^{II}_{0,27}In^{III}_{0,73}[Fe^{II}(CN)_6]_{0,89}.65H_2O\ (dext)_{40}$ (M= 8032 $gmol^{-1}$); calcd (%) for $C_{247.13}H_{533.80}N_{5.34}Mn_{0.27}In_{0.73}Fe_{0.89}K_{0.83}O_{266.90}$: C 36.96, H 6.70, N 0.93, Fe 0.62, K 0.40, Mn 0.18, In 1.04; found: C 36.6, H 6.63, N 0.95, Fe 0.75, K 0.44, Mn 0.22, In 1.23.

[**33**]: $K_{0.81}Mn^{II}_{0,33}In^{III}_{0,67}[Fe^{II}(CN)_6]_{0,87}.36H_2O\ (dext)_{35}$ (M= 6610 $gmol^{-1}$); calcd (%) for $C_{214.49}H_{420.23}N_{5.22}Mn_{0.33}In_{0.67}Fe_{0.87}K_{0.81}O_{210.11}$: C 38.98, H 6.41, N 1.11, Fe 0.74, K 0.48, Mn 0.27, In 1.16; found: C 38.00, H 6.25, N 1.11, Fe 0.77, K 0.48, Mn 0.28, In 1.19.

[**40**]: $K_{1,01}Mn^{II}_{0,40}In^{III}_{0,60}[Fe^{II}(CN)_6]_{0,90}.33H_2O\ (dext)_{33}$ (M= 6259 $gmol^{-1}$); calcd (%) for $C_{203.28}H_{395.42}N_{5.40}Mn_{0.40}In_{0.60}Fe_{0.90}K_{1.00}O_{197.71}$: C 39.01, H 6.37, N 1.21, Fe 0.80, K 0.62, Mn 0.35, In 1.10; found: C 38.3, H 6.23, N 1.29, Fe 0.87, K 0.57, Mn 0.35, In 1.09.

[**53**]: $K_{1,01}Mn^{II}_{0,53}In^{III}_{0,47}[Fe^{II}(CN)_6]_{0,87}19H_2O\ (dext)_{26}$ (M= 4865 $gmol^{-1}$); calcd (%) for $C_{161.2}H_{298}N_{5.22}Mn_{0.53}In_{0.47}Fe_{0.87}K_{1.01}O_{149}$: C 39.80, H 6.17, N 1.50, Fe 1.00, K 0.81, Mn 0.60, In 1.11; found: C 38.82, H 6.03, N 1.37, Fe 0.98, K 0.74, Mn 0.58, In 1.09.

[**77**]: $K_{1,09}Mn^{II}_{0,77}In^{III}_{0,23}[Fe^{II}(CN)_6]_{0,83}22H_2O\ (dext)_{23}$ (M= 4413 $gmol^{-1}$); calcd (%) for $C_{143}H_{274}N_{4.98}Mn_{0.77}In_{0.23}Fe_{0.83}K_{1.09}O_{137}$: C 38.92, H 6.26, N 1.58, Fe 1.05, K 0.97, Mn 0.96, In 0.60; found: C 37.87, H 6.10, N 1.45, Fe 1.02, K 0.92, Mn 0.92, In 0.59.

[**91**]: $K_{1,11}Mn^{II}_{0,91}In^{III}_{0,09}[Fe^{II}(CN)_6]_{0,80}22H_2O\ (dext)_{23}$ (M= 4399 $gmol^{-1}$); calcd (%) for $C_{142.8}H_{274}N_{4.8}Mn_{0.91}In_{0.09}Fe_{0.80}K_{1.11}O_{137}$: C 38.99, H 6.28, N 1.53, Fe 1.02, K 0.99, Mn 1.14, In 0.23; found: C 38.22, H 6.14, N 1.41, Fe 0.99, K 0.93, Mn 1.1, In 0.23.

Figure S6
Magnetization M=f(H) plots registered between 5T and -5T (after saturation under a field of 5T) at 5K

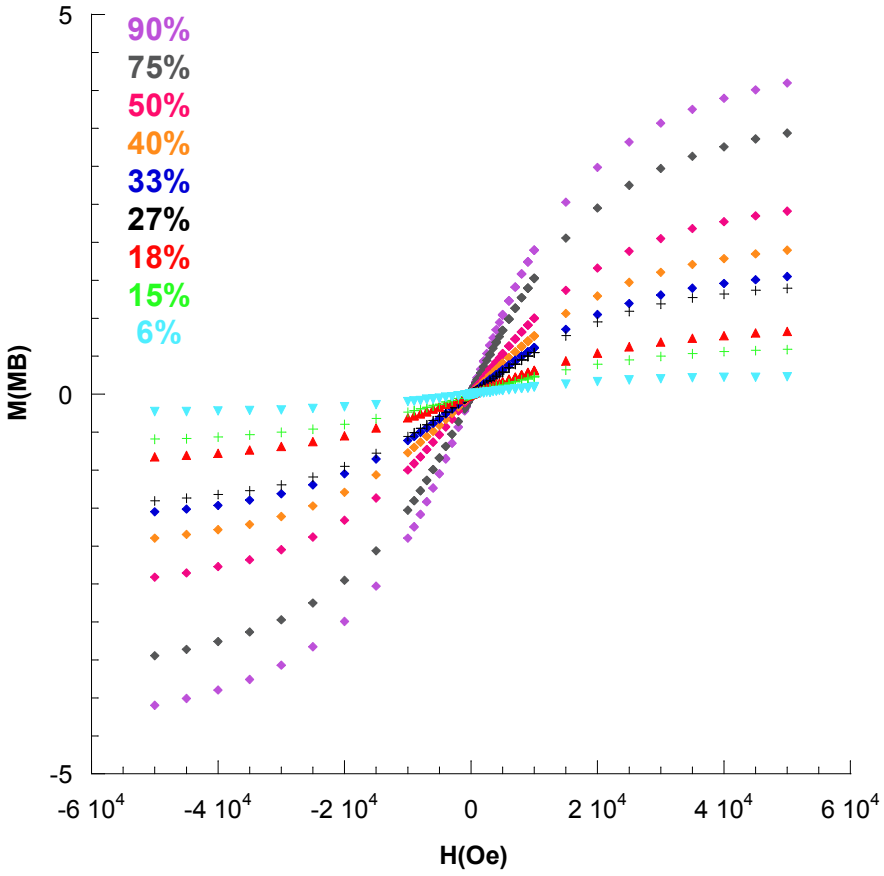


Figure S7
Determination of the surface proportion in a PBA nanoparticle

Calculation of the number of atoms contained in a cubic Prussian blue analogue nanoparticle with an edge of *a* nm

The total number of atoms N_T in a nanoparticle with a *fcc* structure possessing an edge of *a* nm and a unit cell parameter of c nm is composed of n^3 elementary cells where n=a/c:

$$N_T = 4n^3 + 6n^2 + 3n + 1$$

The number of atoms belonging to the surface N_S is given by:

$$N_S = 12n^2 + 2$$

Thus the percentage of atoms located at the surface *%S* is:

$$\%S = \frac{N_S}{N_T} = \frac{12n^2 + 2}{4n^3 + 6n^2 + 3n + 1}$$

In the case of Prussian blue, c=1 nm, so that the proportion of Fe (or Mn+In) atoms located at the surface is reported in the table for different sizes of particles

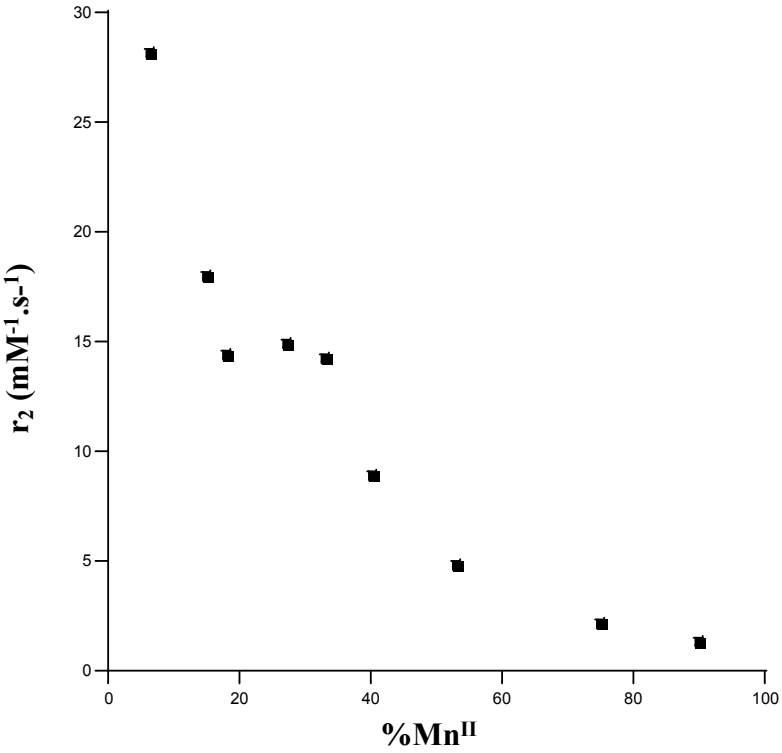
[Size] (nm)	***[5]***	***[8]***	***[10]***	***[12]***	***[16]***	***[18]***	***[22]***	***[30]***
N_T	666	2457	4631	7813	17969	21438	45563	113491
N_S	302	770	1202	1730	3074	3470	5810	10802
%S	45	31	26	22	17	16	13	10

This is a much larger proportion as compared to oxides or metals.

For example for a cubic particle of a compound with a fcc perovskite structure with a cell parameter that is half (0.5 nm) that of PBA, a 5 nm nanoparticle contains about 26% of atoms located at the surface.

Even though the surface area remains the same, the atoms proportion is not similar depending on the compound. The PBA show a larger proportion of atoms located at the surface as compared to other compounds like oxides or metals.

Figure S8
Evolution of the transverse relaxivity and r_2/r_1 at 60MHz and at 37°C depending on the Mn^{II} content



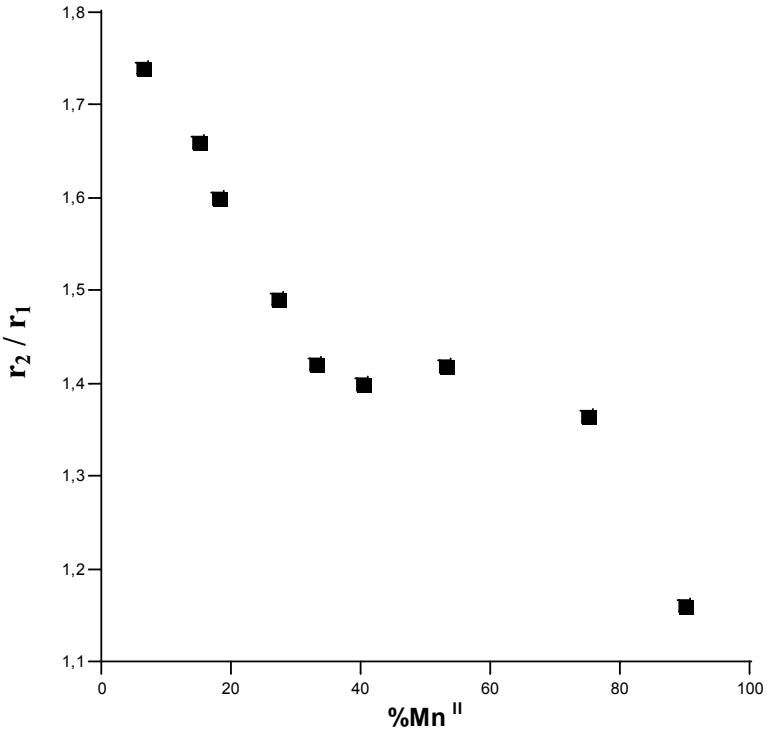
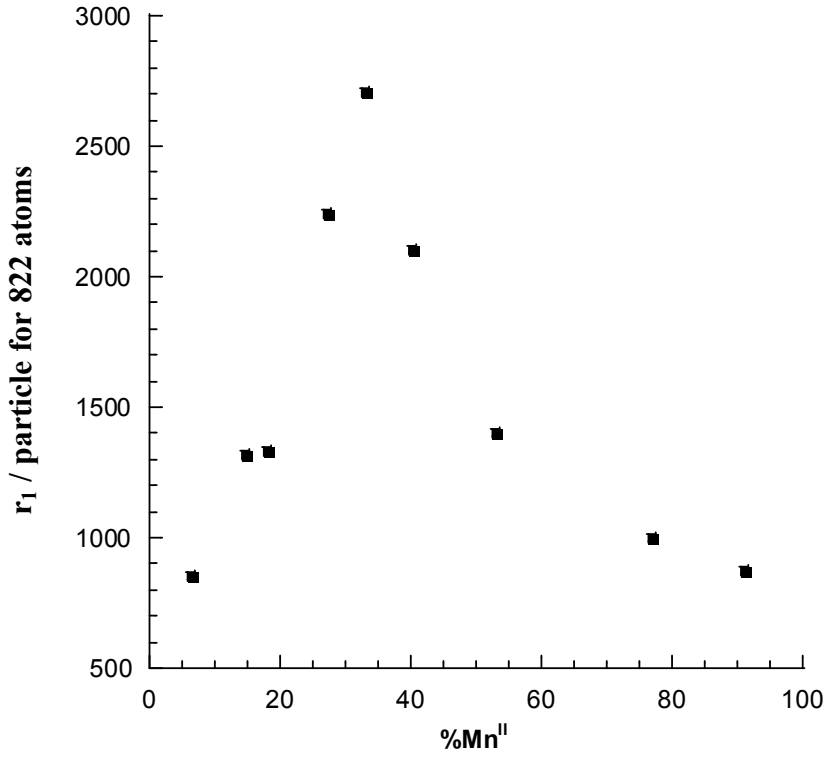


Figure S9
Optimal relaxivity per particle of 5 nm at 60MHz and at 37°C



Calculation of the optimal relaxivity:
The idea is to evaluate the relaxivity for a **constant number of metallic atoms** (Fe^{III}+In^{III}+Mn^{II}) in the solution, as the purpose is to obtain the maximum relaxivity for a minimum injected number of metallic atoms to minimize the toxicity.
We thus considered a number of 822 atoms that are included in a 5.4 nm cubic particle with a cell parameter of 1 nm (this size corresponds to particles' size for x=0.33). The calculation is based on the average Mn atomic relaxivity multiplied by the number of Mn atoms contained in the particle (=x*822 atoms).

Figure S10
Relaxivity measurement at 5°C and 37°C for NP-33% and NP-6%

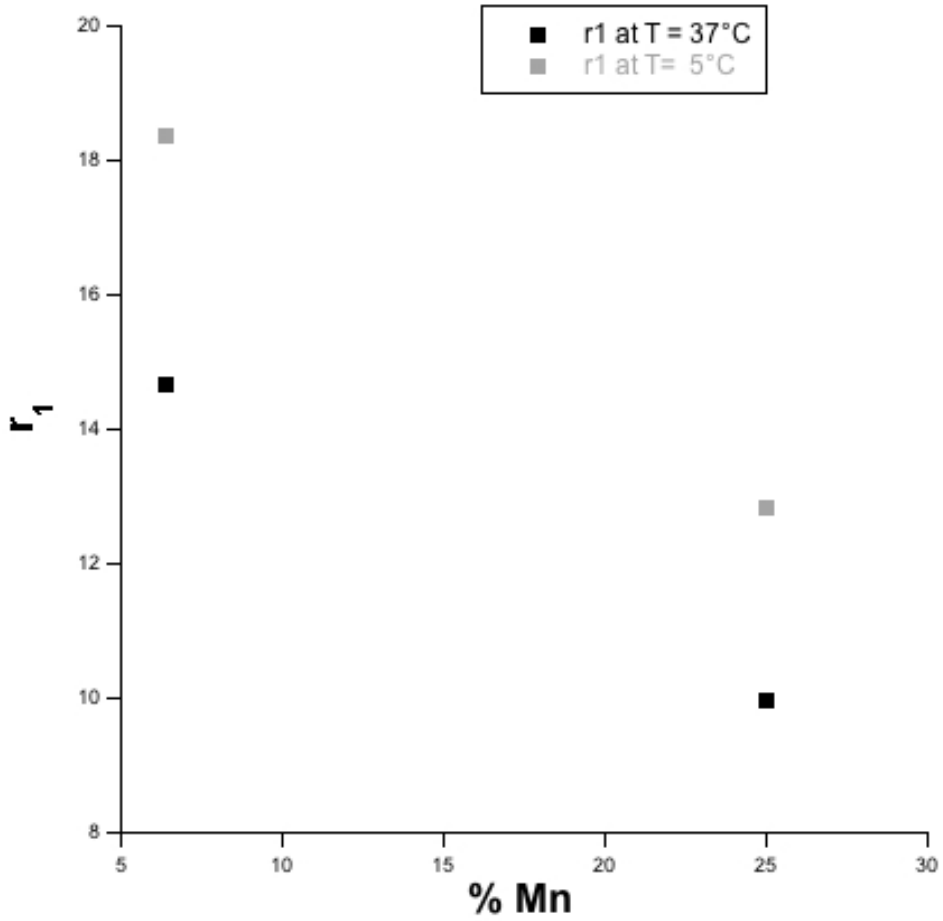


Figure S11
Dynamic Light Scattering performed in physiological serum for NP-33%

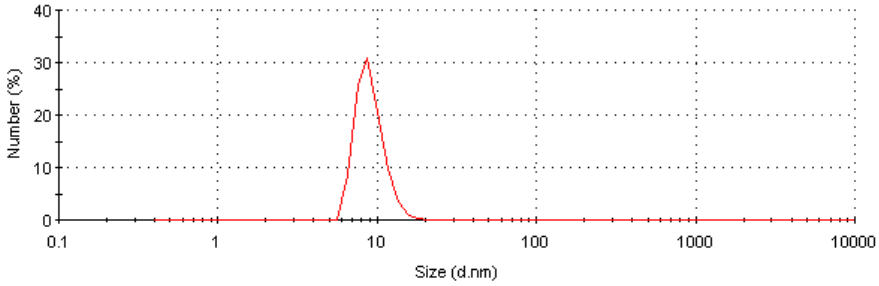
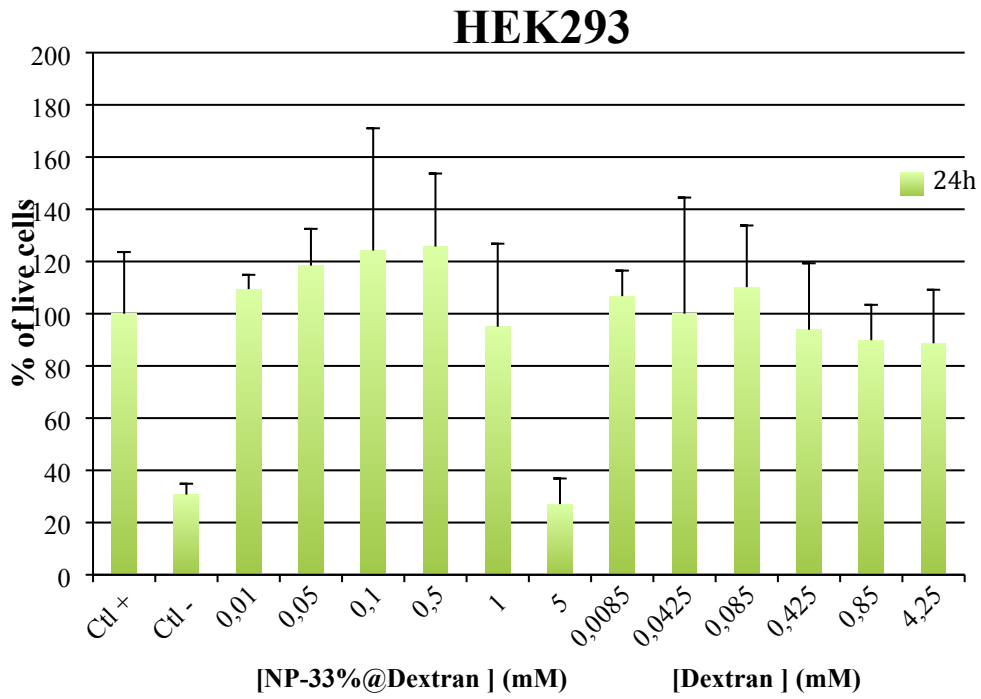
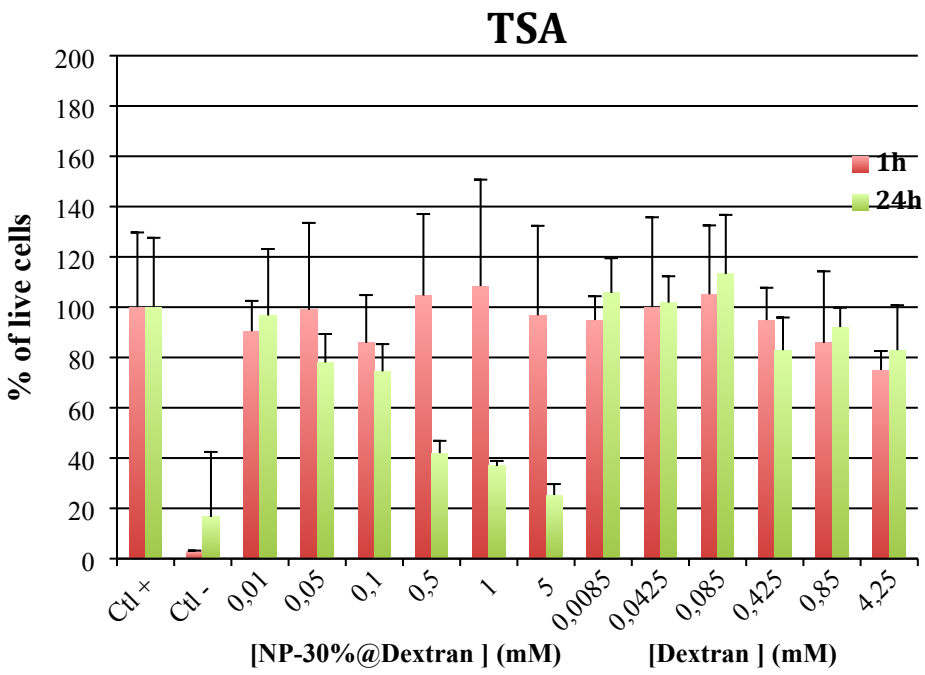


Figure S12

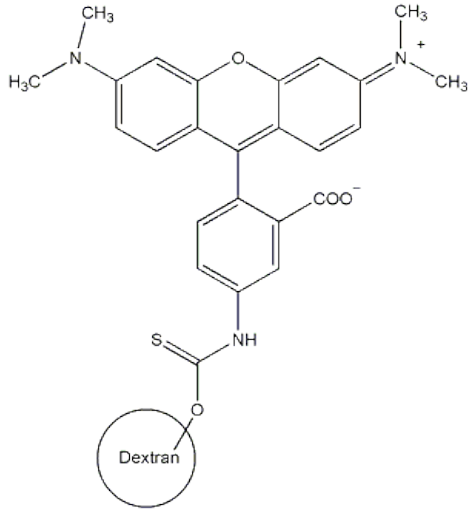
MTT test performed on two cell lines (HEK293 and TSA) and at different times after the injection of NP-33%@Dextran





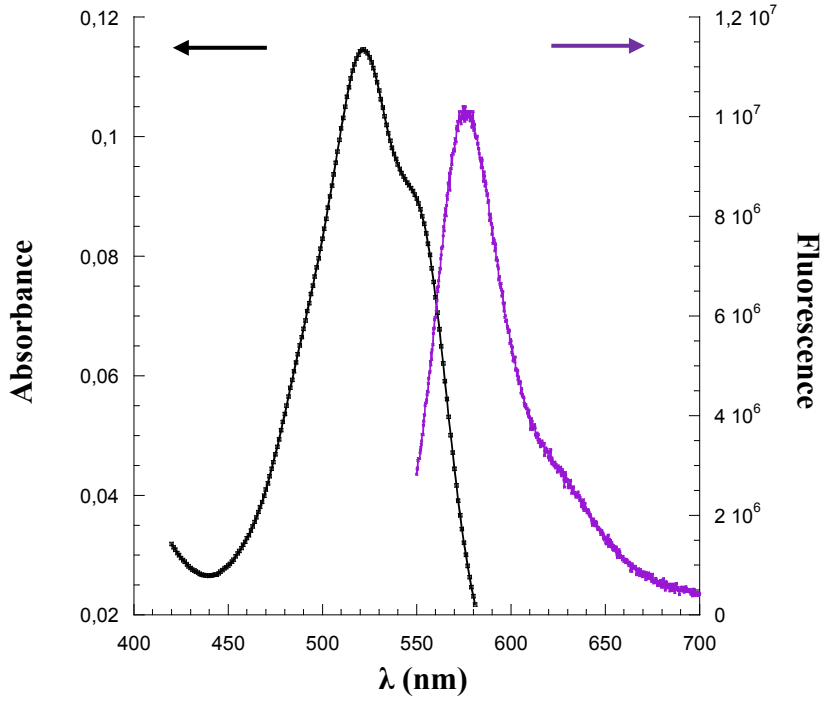
Ctl+: untreated cells, Ctl-: cells treated with 50 micromol of staurosporine (natural compound able to induce cell apoptosis)

Figure S13
Chemical structure of TRITC-Dextran (purchased from Sigma)



λexc = 543 nm
λem = 580 nm (maximum of emission)

Figure S14
Absorbance (black) and fluorescence (purple) of NP-33%@TRITC-Dextran ($K_{0,81}Mn^{II}_{0,33}In^{III}_{0,67}[Fe^{II}(CN)_6]_{0,87}.36H_2O$ (dext-TRITC)$_{53}$)



Absorption spectra of NP-33%@TRITC-Dextran has been recorded in the 200-800 nm range. Here only the absorption band centered on 543nm is presented. Concerning fluorescence spectra of NP-33%@TRITC-Dextran , the colloidal solution of particles was excited at 543nm and fluorescence was measured from 550 to 700 nm.

Figure S15
Cell internalization of NP-33%@TRITC-Dextran followed by confocal microscopy (left = transmission images ; right = fluorescence images ; scale bar = 20 µm)