Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

PEG-Copolymer-coated Iron Oxide Nanoparticles that Avoid the Reticuloendothelial System and Act as Kidney MRI Contrast Agents

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1. XRD patterns of mixed Fe/In oxide precipitates

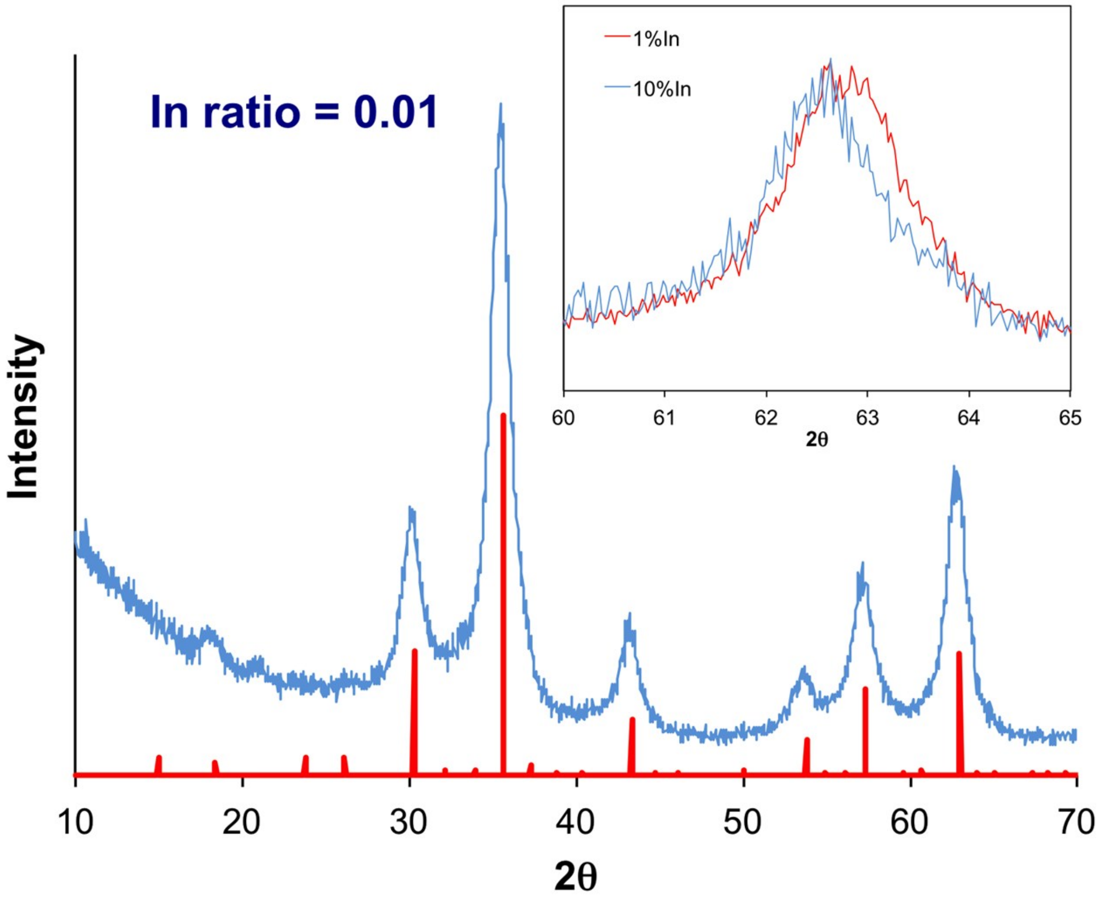


Figure 1S. DRX pattern of In/Fe oxide nanoparticles precipitated from In/Fe mixtures with a 0.01 In molar ratio. The vertical lines correspond to the spinel structure pattern of □-Fe$_2$O$_3$. In the inset, detail of the 62.983^o peaks for samples with 10% and 1% In ratios, respectively. A shift to smaller angles in the 10%In sample with respect to the 1%In sample is noticeable associated to a reduction of cell parameter due to the substitution of a part of the Fe^{3+} ions by In^{3+} ions with a larger ionic radius.

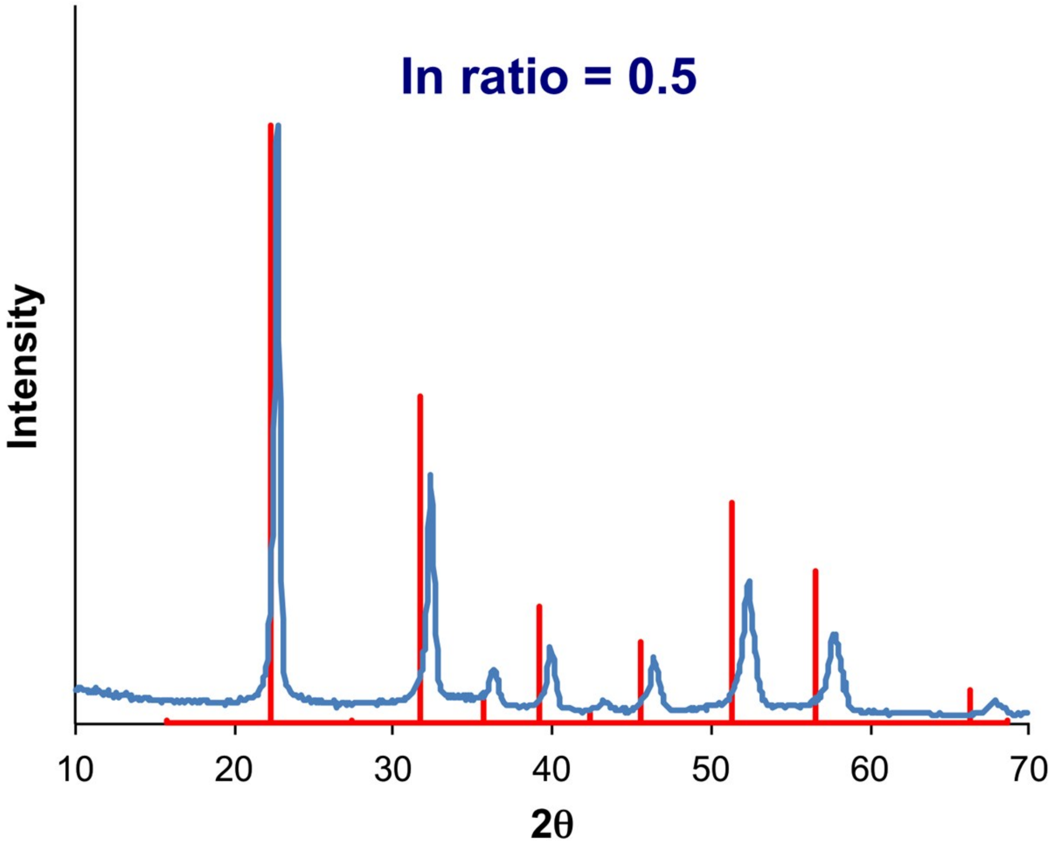


Figure 2S. DRX pattern of In/Fe oxide powders precipitated from solutions with a 0.5 In molar ratio. The vertical lines correspond to the pattern of $In(OH)_3$. The pattern of the precipitated powders is very similar in position and relative intensity to that of $In(OH)_3$ crystal structure only that the peaks of the precipitate are shifted to higher angles meaning that the cell parameter is shorter, as it should be expected when substituting half of the In^{3+} ions in the structure for Fe^{3+} ions with a smaller ionic radius.

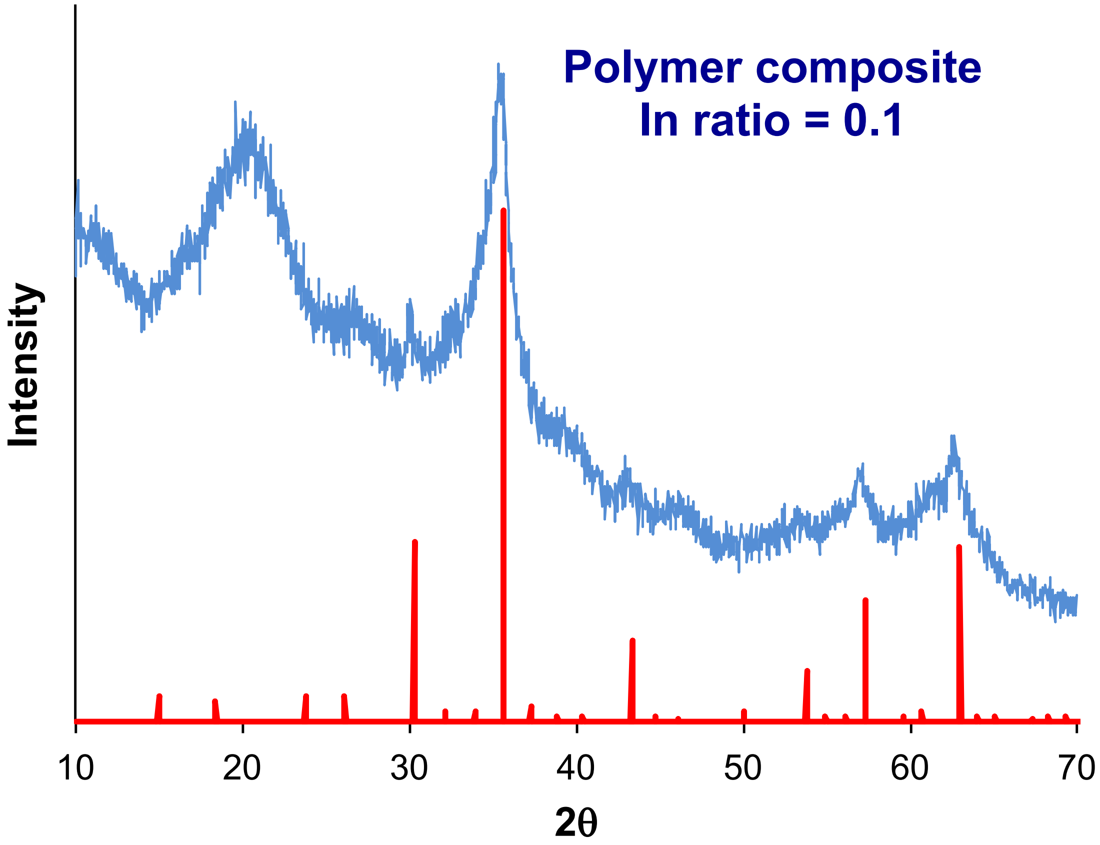


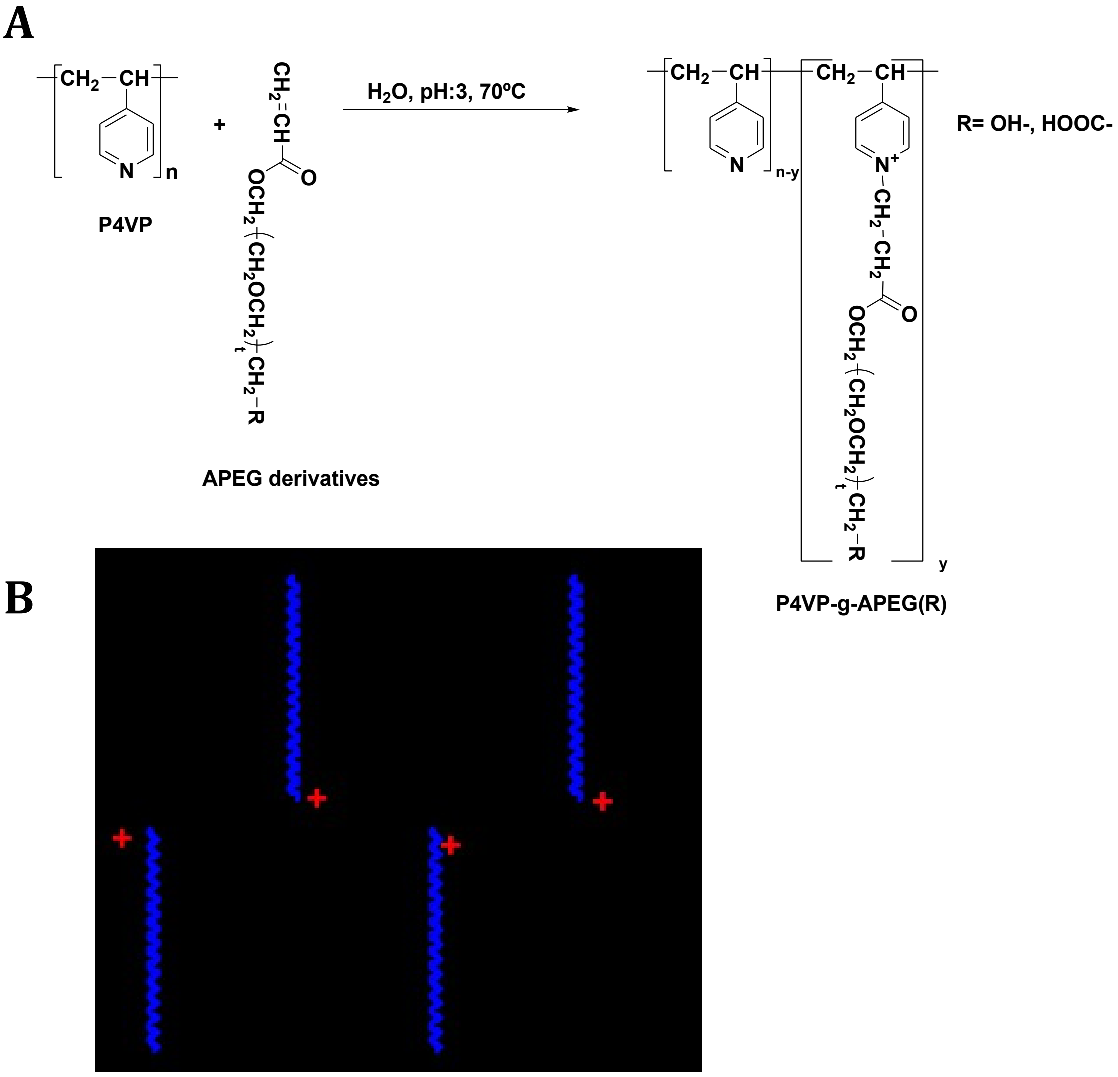
Figure 3S. DRX pattern of In/Fe oxide polymer composite nanoparticles precipitated from In/Fe mixtures with a 0.1 In molar ratio. The vertical lines correspond to the pattern of γ-Fe_2O_3. The pattern of the composite contains all the peaks of γ-Fe_2O_3 spinel structure and a wide peak at 20° corresponding to the polymer. The peaks are wider than for pure powders due to the smaller size of the nanoparticles.

2. P4VP-APEG copolymer synthesis and structure

The structure of the polymer used in the nanoparticle coating is shown in Figure 4S. The polymer shell is formed by a graft copolymer (P4VP-g-APEG) with a comb like structure that is obtained by grafting polyethylenglycol residues to the P4VP (Figure 4S). The coating process involves the carbon-nitrogen bond formation by a Michael addition reaction that proceeds in water at acidic pH, without further catalyst. This reaction involves the addition of the nucleophilic nitrogen of the pyridine, that becomes quaternized and therefore positively charged in this way, to the α,β-unsaturated carbonyl of the polyethylenglycol acrylates. Thus the backbone is a polyethylene chain with pyridine side groups. The APEG chains are linked to some of the pyridine groups by N-C bonds between the nitrogen of the pyridine groups and the β-carbon of the acrylate PEG ester, thus hanging perpendicular to the backbone.

NMR analysis shows a 100% reaction rate between the pyridine residues (Figure 4SC).

Thermogravimetry (TGA) and differential thermogravimetry (DTA) analysis on samples prepared with similar method but a different iron oxide content are shown in Figure 5S. Measurements were performed using a simultaneous DTA-TGA unit SDT-2960 (TA Instruments) in the range of 25ºC to 700ºC at a heating rate of 10ºC/min under air atmosphere. Thermogram shows a 6.6% weight loss up to 200ºC. Above this temperature the polymer is starting too degrade. Two different slopes are observed between 200ºC and 400ºC that may correspond to PEG and P4VP degradation processes, respectively. The dequaternization process, due to the cleavage of the C-N$^+$ bonds, has been reported to be observed around 300ºC. Thus the third weight loss step, between 450ºC and 550ºC can be attributed to pyridine moieties strongly bonded on iron oxide surface. The copolymer coating degraded completely at 600ºC leaving an inorganic content about 25%.



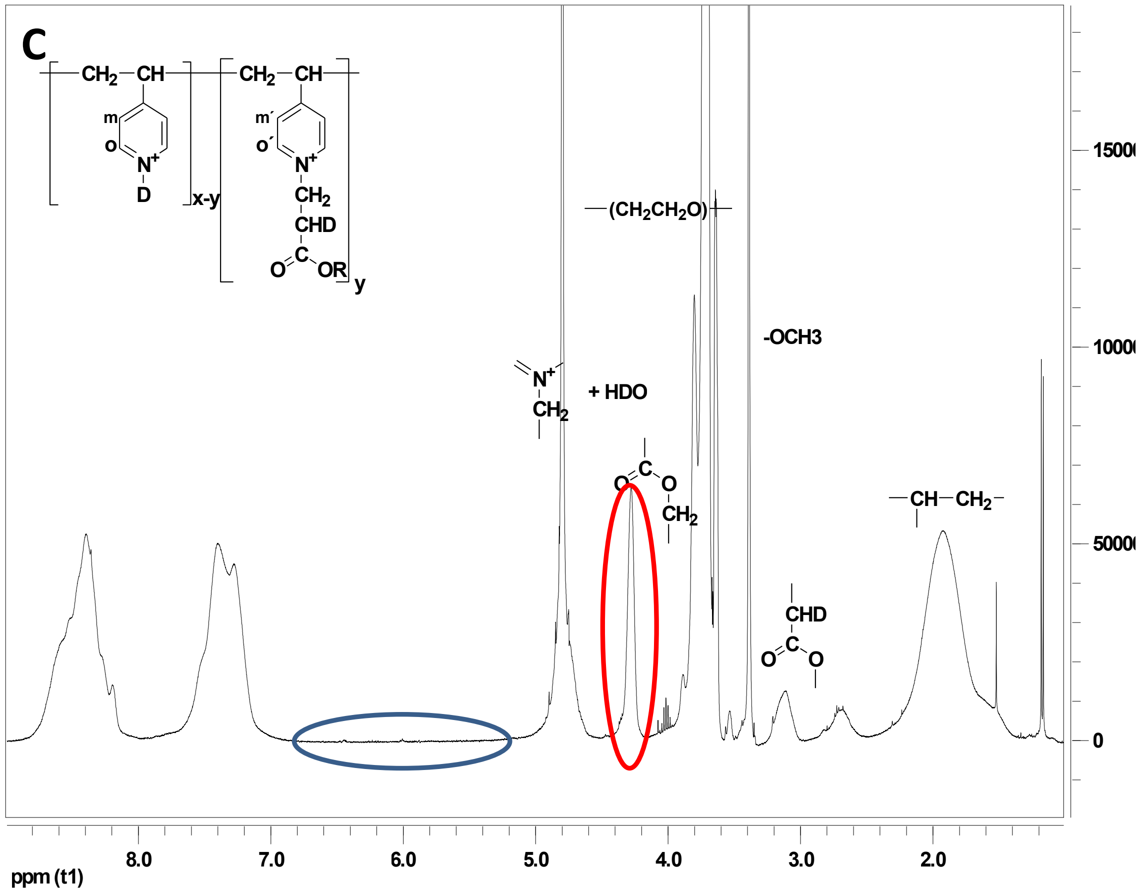


Figure 4S. A) Scheme of the Michael addition reaction between pyridine moieties of the P4VP nanoparticle polymer matrix and PEG acrylate chains. B) Drawing of the resulting brush grafted polymer structure. C) ^{1}H-NMR spectrum of the product of APEG grafting reaction to the P4VP chain by Michael addition of the acrylate group to the pyridine group depicted in A. The blue circle marks the region of appearance of vinyl protons of unreacted acrylate groups and the absence of signal in this region indicates a nearly 100% reaction yield. The red circle indicates the peak of protons of the –O–CH2– group in the PEG chain bonded to the acrylate group. The displacement of chemical the chemical shift to lower values and the appearance as a singlet instead of a triplet is also indicative of a 100% conversion of the acrylate double bond.

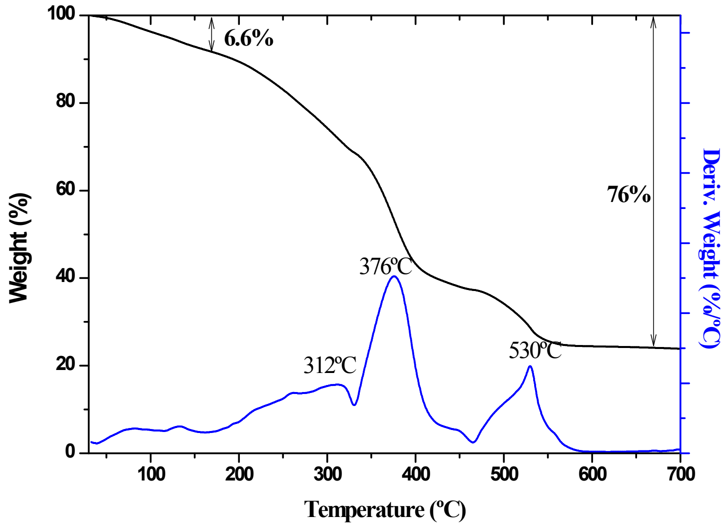


Figure 5S. TGA (black line) and DTA (blue line) curves for a nanoparticle sample similar to the one used in SPECT and MRI experiments, but with a higher iron oxide content (about 3 times higher).

2. TEM analysis of iron oxide nanoparticle size

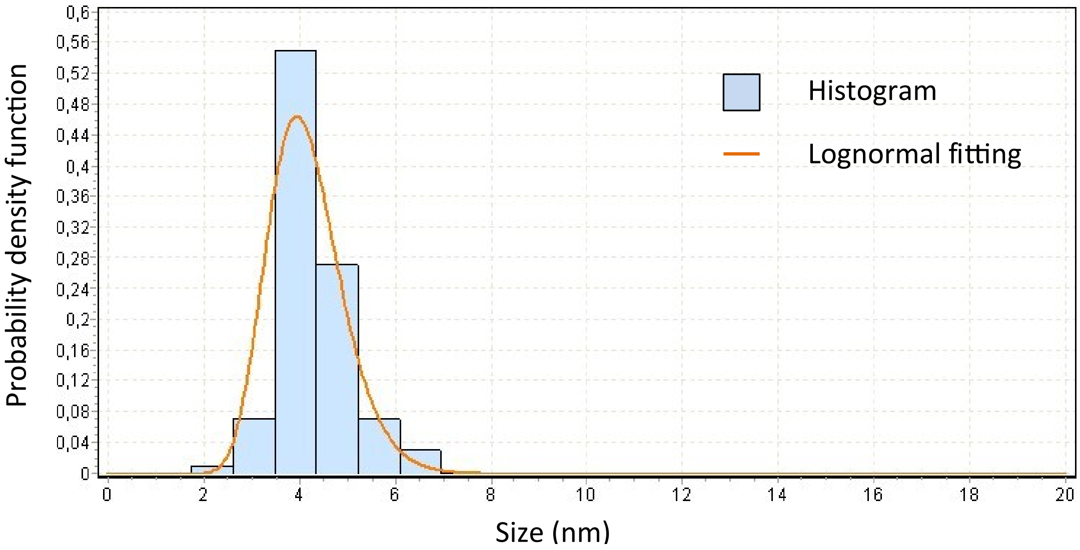


Figure 6S. Fitting of the TEM size distribution of iron oxide nanoparticles to a lognormal function. The obtained fitting parameters were σ=0.186, μ=1.408.

2. DLS analysis of iron oxide-P4VP nanoparticles suspended in water

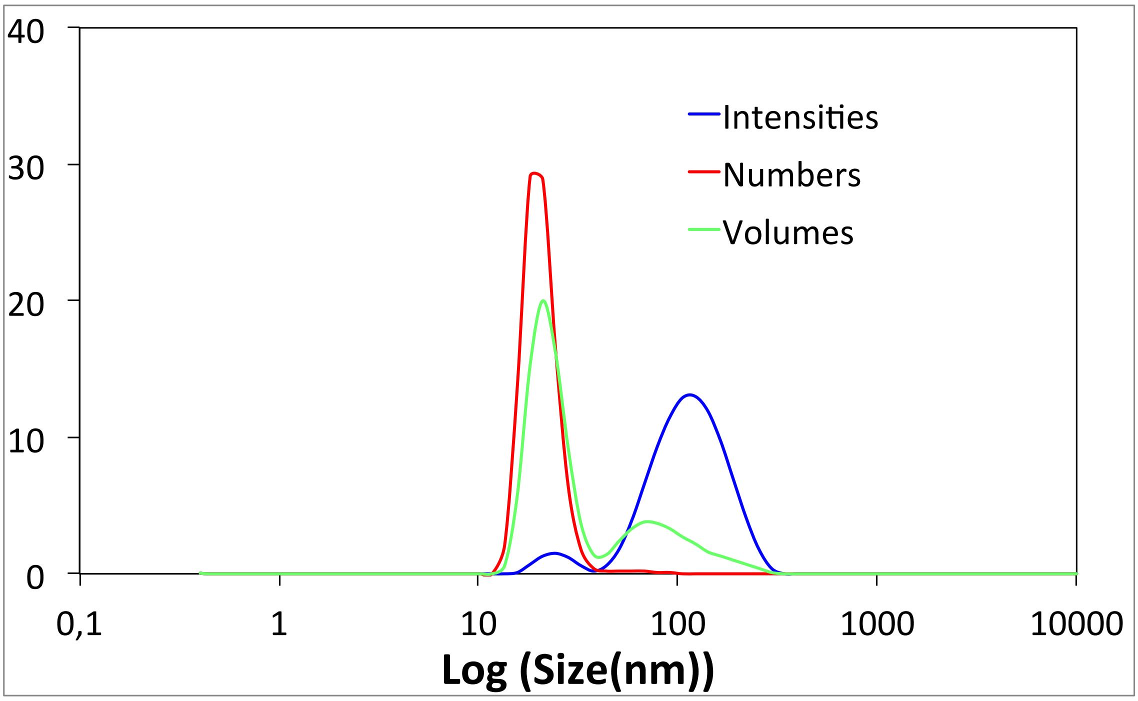


Figure 7S. Comparison of size distributions by numbers, volumes and intensities of iron oxide@P4VP@APEG composite nanoparticle suspension. The differences are due to the higher scattering intensity of large nanoparticles. The corresponding averages sizes are 18.4 nm, 49.3 nm and 127 nm, respectively. The polydispersity index was PDI=0.209.

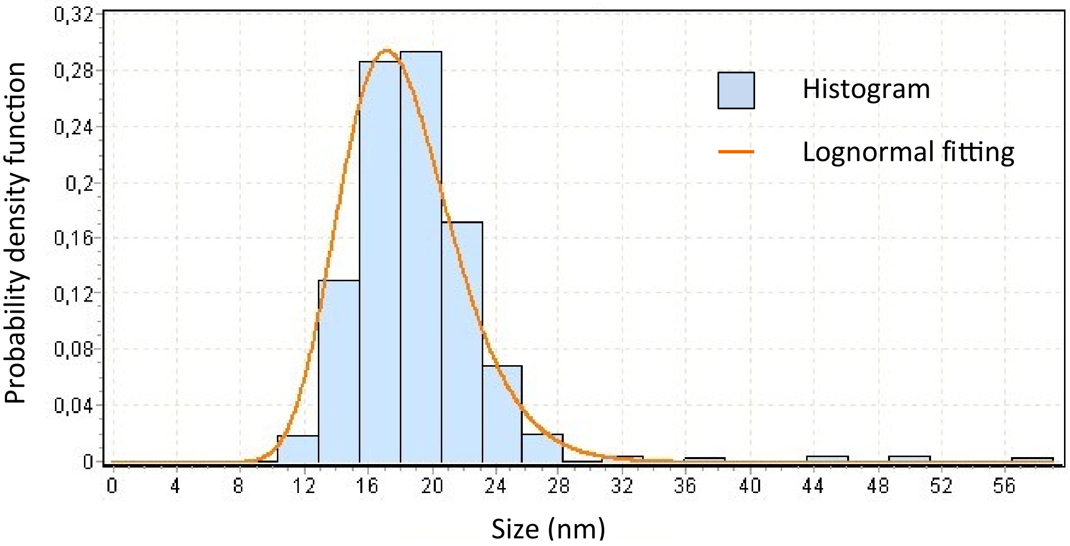
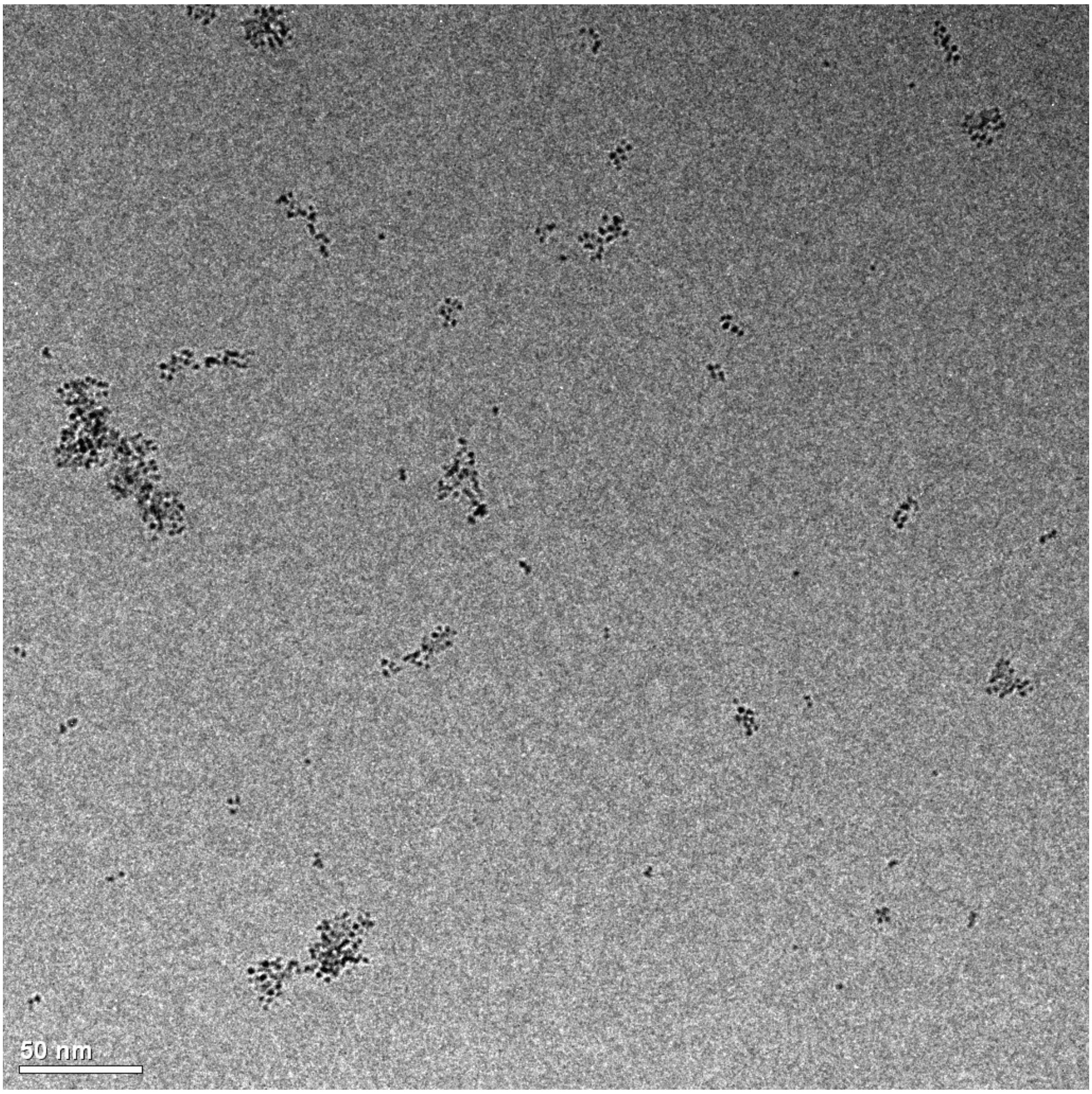
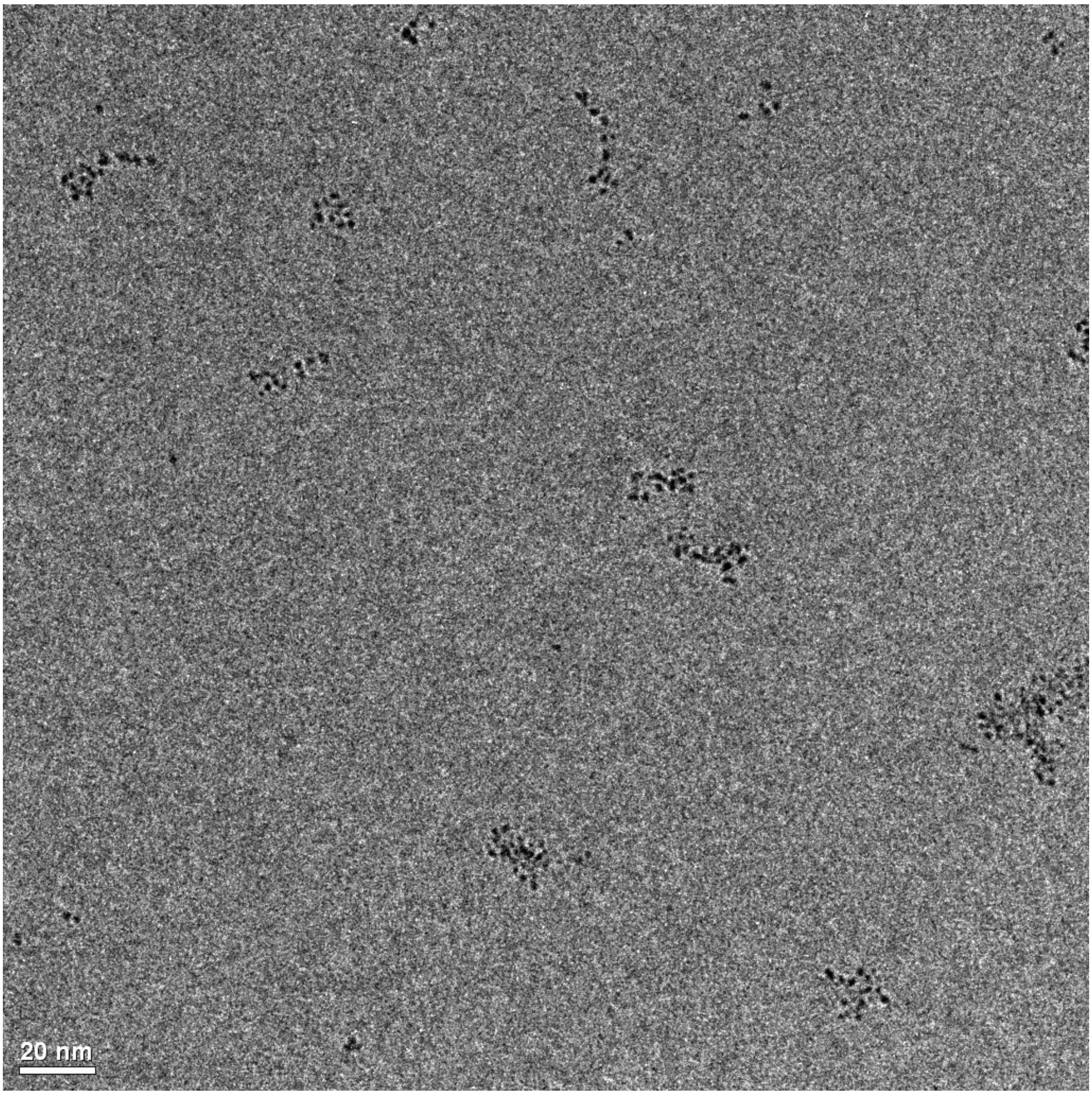


Figure 8S. Fitting of the DLS size distribution by numbers of the iron oxide@P4VP@APEG composite nanoparticles to a log normal function. The obtained fitting parameters were σ=0.199, μ=2.884.

3. cryoTEM observations




20 nm

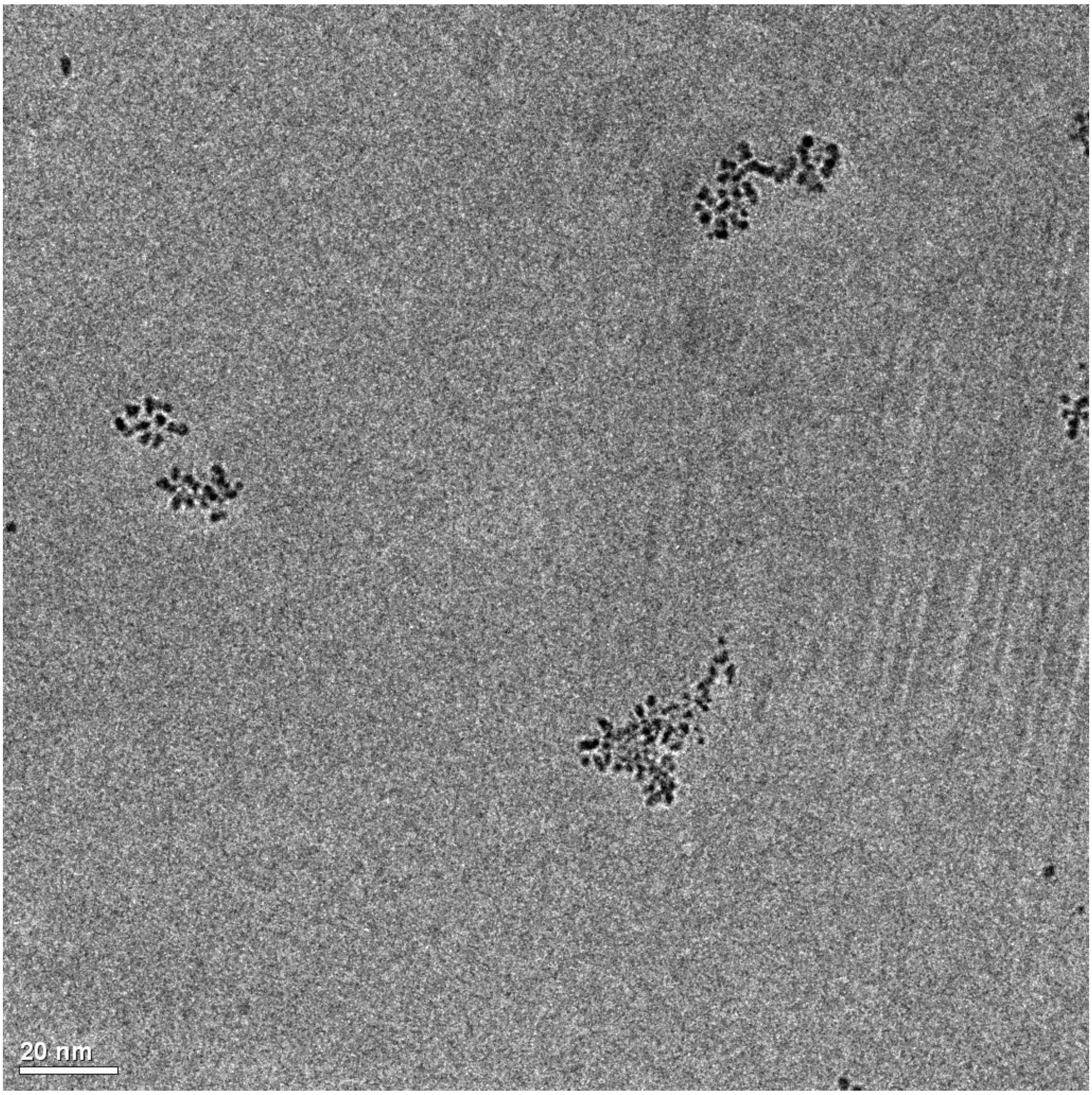


Figure 9S. Cryo TEM images of iron oxide@P4VP@APEG composite nanoparticle suspensions in water at pH = 7.4.

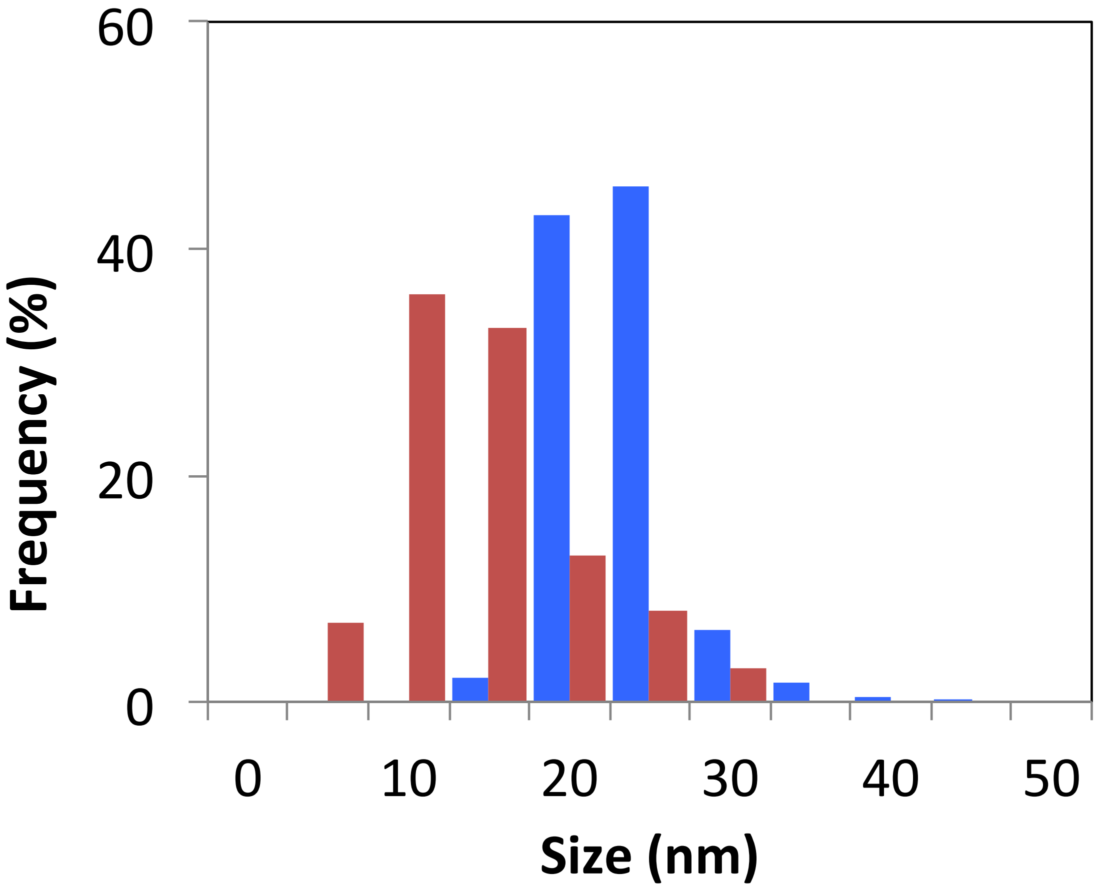


Figure 10S. Hystogram comparing the size distribution of iron oxide multicore nucleus from cryoTEM images, and that of hydrodynamic diameters of iron oxide@P4VP@APEG composite nanoparticles in water suspension from DLS mesurements. The averages sizes over 100 NPs were 18.4 nm and 11.5 nm respectively.

3. TEM histological inspection of kidney and liver tissue slices

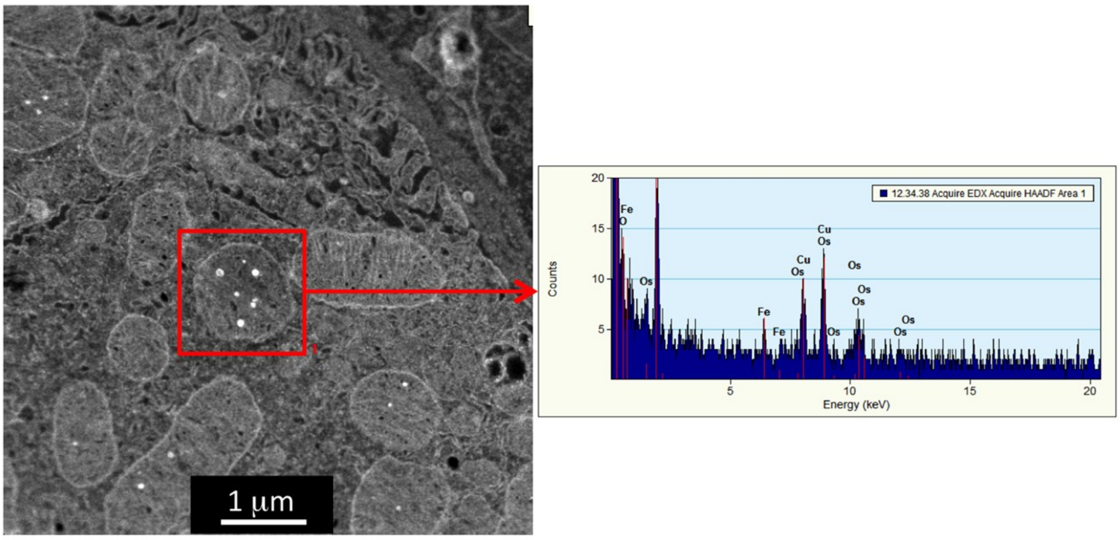


Figure 11S. TEM images of an iron oxide-P4VP-PEGA nanoparticle in a proximal tubule cell of a mouse kidney slice sacrificed 2h after injection, and EDX analysis of the area marked within the red square.

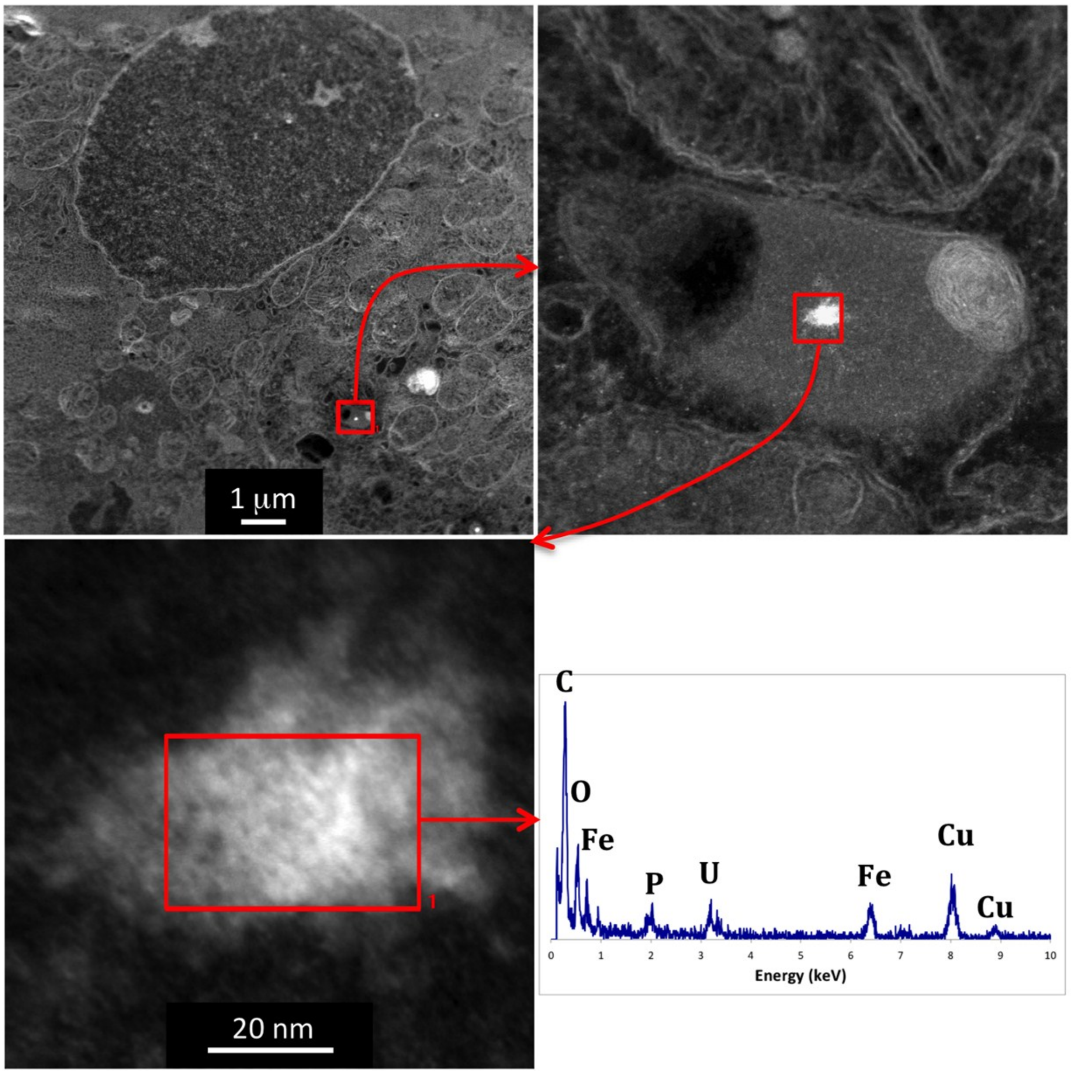


Figure 12S. TEM images at different magnification of an iron oxide-P4VP-PEGA nanoparticle in a proximal tubule cell of a kidney slice of a mouse sacrificed 24h after injection and EDX analysis of the area 1 marked with a red square.

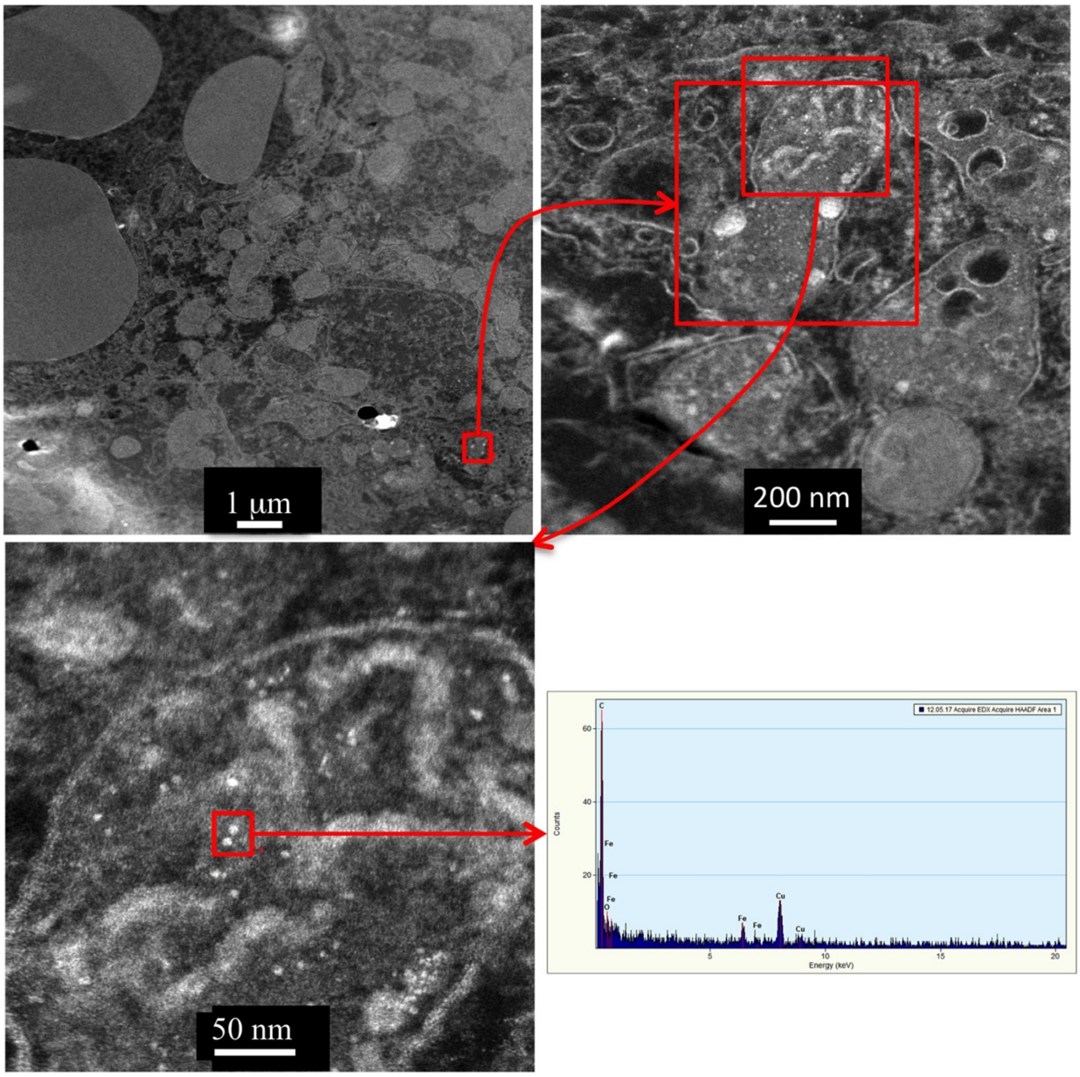


Figure 13S. TEM images at different magnification of an iron oxide-P4VP-PEGA nanoparticle in a liver slice of a mouse sacrificed 2h after injection, and EDX analysis of the area 1 marked with a red square.

4. Relaxometry measurements of IONPs aqueous suspensions

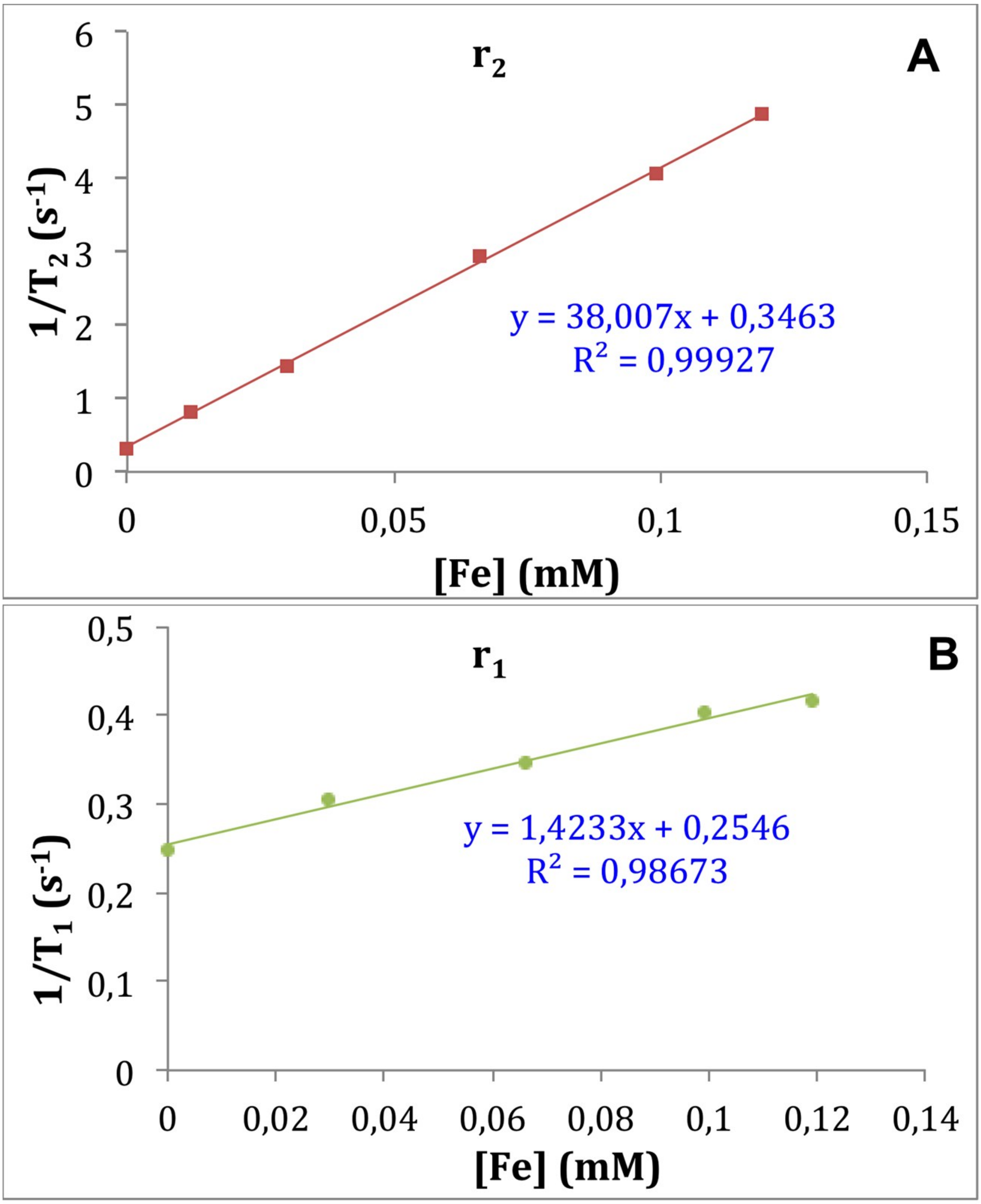


Figure 14S. Variation of the transverse (r_2), A, and longitudinal (r_1), B, relaxivities of the ferrofluid with the concentration of iron.