

Figure S1

Fourier Transform Infrared (FTIR) spectra of bare (sample A), ex situ citrate coated (sample B) and in situ citrate-coated (sample C) Fe₃O₄ NPs in the region of the stretching vibration modes of carboxylate groups. The spectra were recorded in attenuated total reflection mode, with a Ge crystal. The spectra were acquired by using a Bio-Rad FTS6000 spectrometer with a resolution of 4 cm⁻¹. The components contributing to the bands were determined by a curve peak procedure (software XPSPEAK 4.1). The components at 1580 and 1390 cm⁻¹ are due to the asymmetric and symmetric, respectively, stretching vibrations of COO⁻ groups of citrate anions [1]. In this region the band of the bending vibration of H₂O molecules adsorbed on the surface of the NPs is present (ca. 1635 cm⁻¹). For the spectra of samples B and C, two additional components (cyan and magenta curves) were needed to fit the asymmetric shapes of the bands. In the case of bare NPs, the additional components (yellow, green and orange curves) might be due to features of the background.

[1] Mudunkotuwa IA, Grassian VH (2010) Citric acid adsorption on TiO₂ nanoparticles in aqueous suspensions at acidic and circumneutral pH: Surface coverage, surface speciation, and its impact on nanoparticle-nanoparticle interactions. *J Am Chem Soc* 132 (42):14986-14994.

<https://doi.org/10.1021/ja106091q>

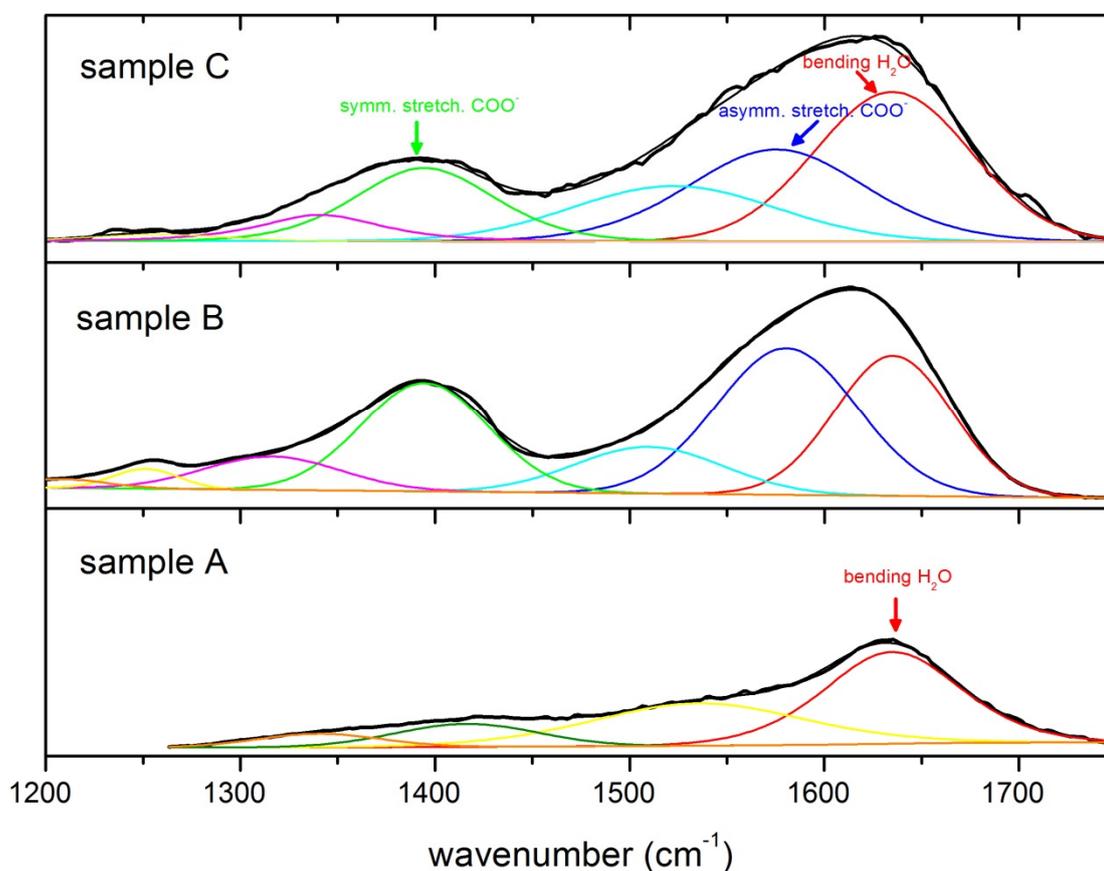


Figure S2

TEM images measured for sample B (magnetite NPs coated with citrate ex situ).

TEM images were recorded by using a High Resolution TEM JEOL 2010, operating at 200 kV, equipped with an energy dispersive system for x-ray analysis. Samples were prepared by depositing droplets of the diluted dispersions onto TEM grids covered with a polymer film. The samples were graphitized to make them conductive.

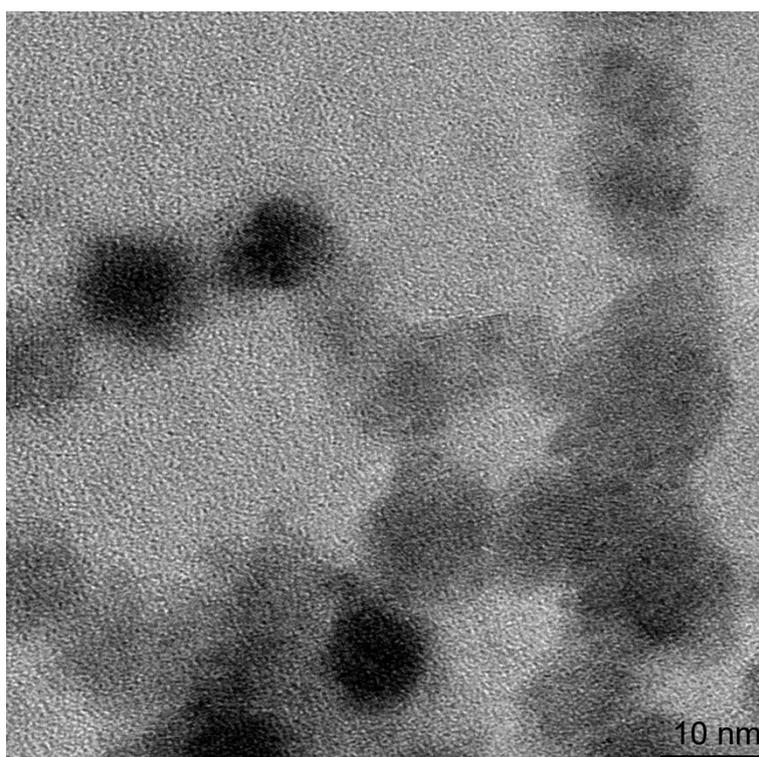
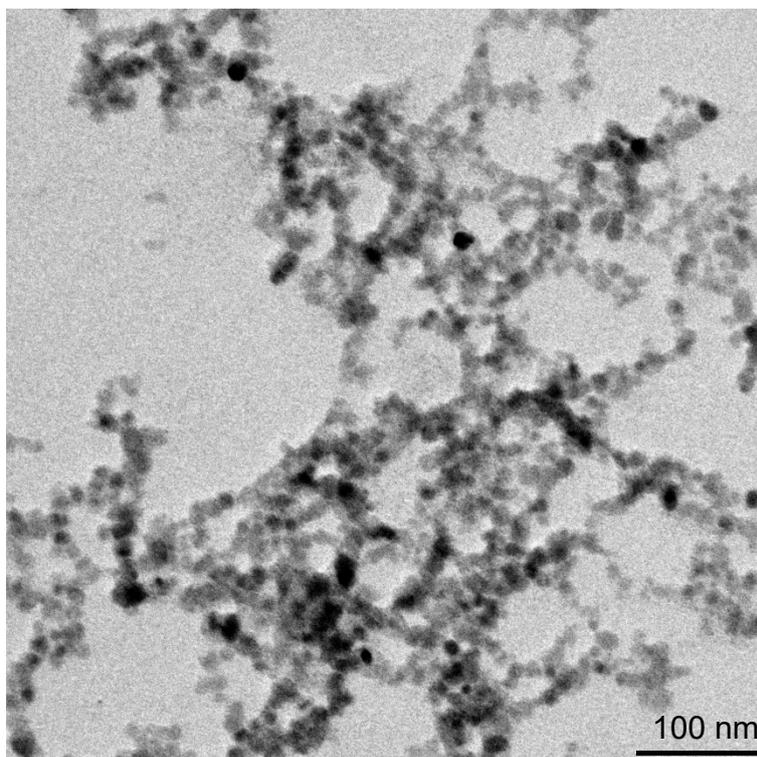


Figure S3.

Results of the Williamson-Hall analysis for the three samples. Filled squares represent the experimental data and the red line the least square fitting. The equation of the least square linear fitting and the linear correlation coefficient are reported for each data set. According to the Williamson-Hall analysis the peak width β (radian) has a contribution due to the size of the crystallite, D , and an additional contribution due to the elastic strain, 4ϵ .

$$\beta = \frac{K\lambda}{D \cos \theta} + 4\epsilon \frac{\sin \theta}{\cos \theta}$$

where K is the constant of the Scherrer equation, λ the x-ray wavelength and θ the scattering angle corresponding to the diffraction peak

By plotting $\beta \cos \theta$ vs. $\sin \theta$ for the diffraction peaks a straight line is obtained whose slope is 4ϵ and the intercept $\frac{K\lambda}{D}$

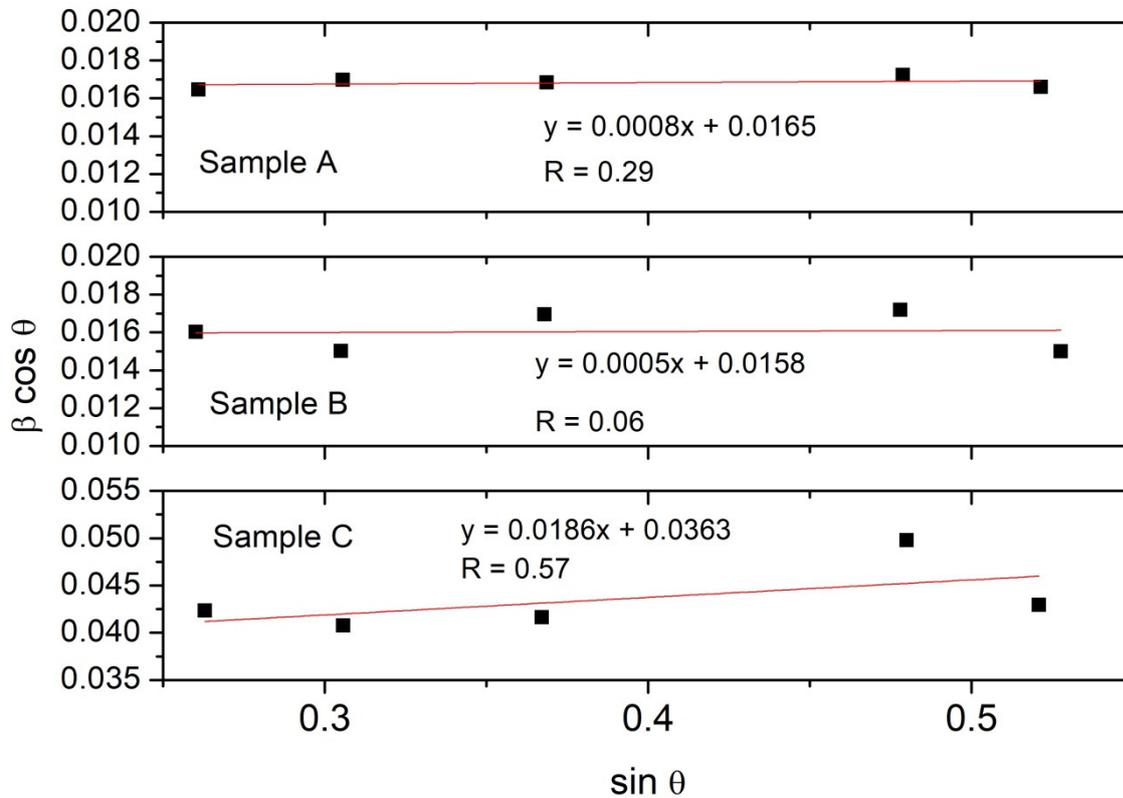


Figure S4.

Results of the analysis of the diffraction curves measured for sample A, B and C by the PM2K method. Black lines: experimental curves. Red lines: fitted curves. Grey lines: difference between experimental and fitted curves.

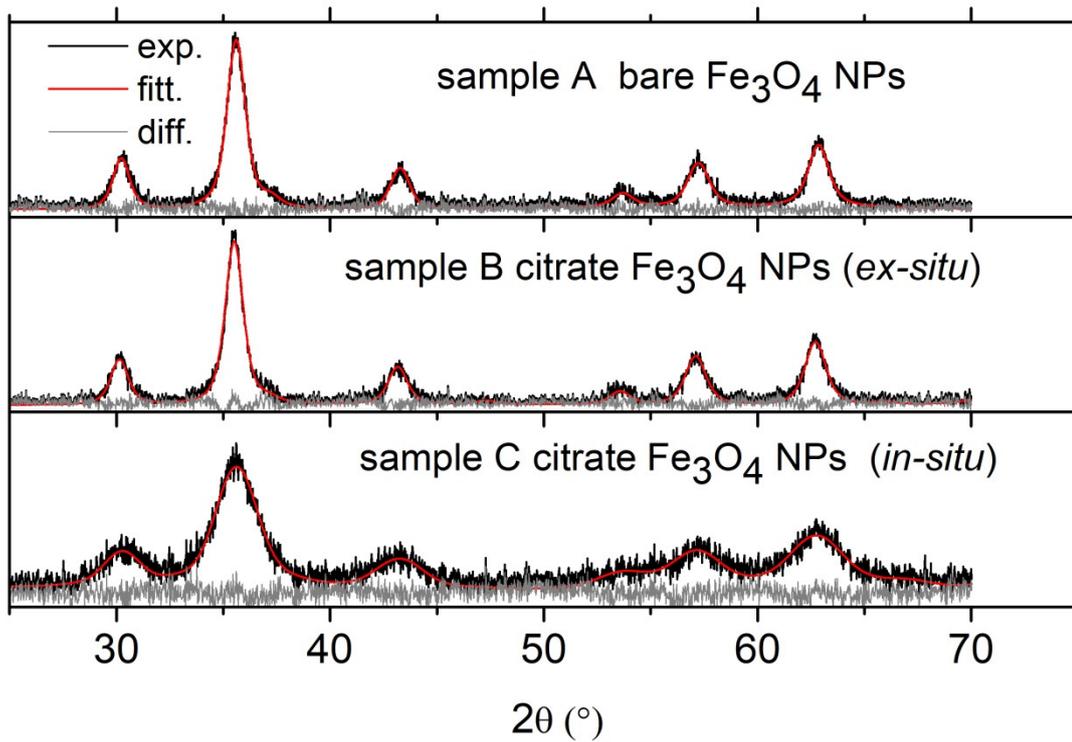


Figure S5.

DLS curves measured for sample C after one week of sedimentation. Solid line: Scattered intensity vs. hydrodynamic diameter. Dashed line: % of number of particles vs. hydrodynamic diameter.

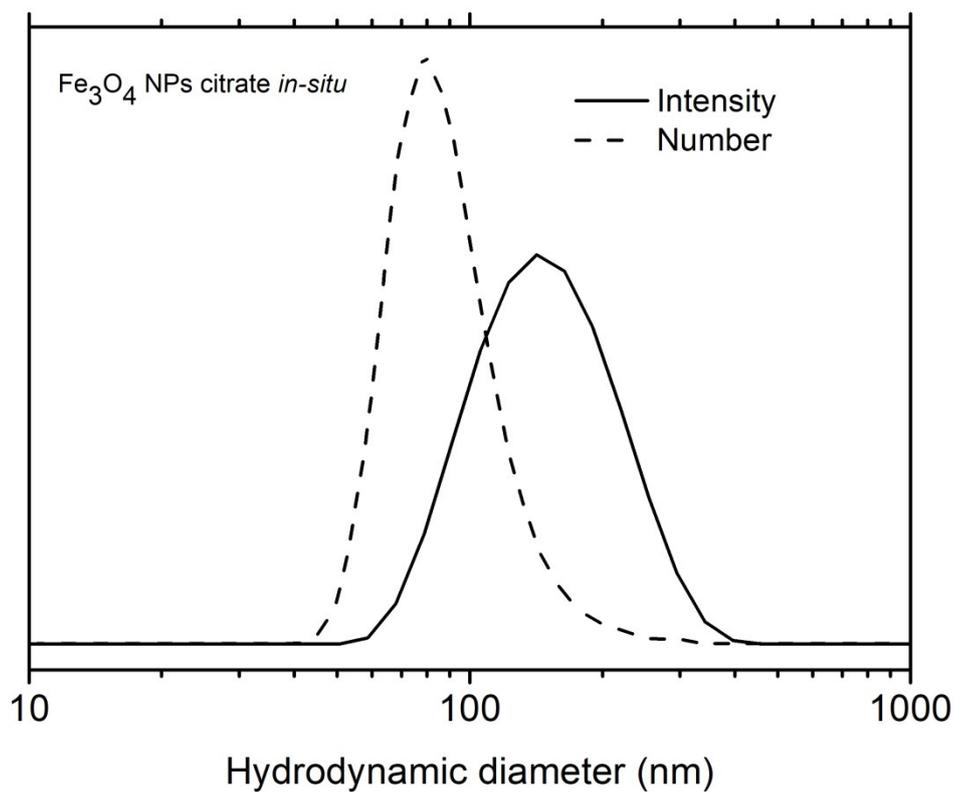
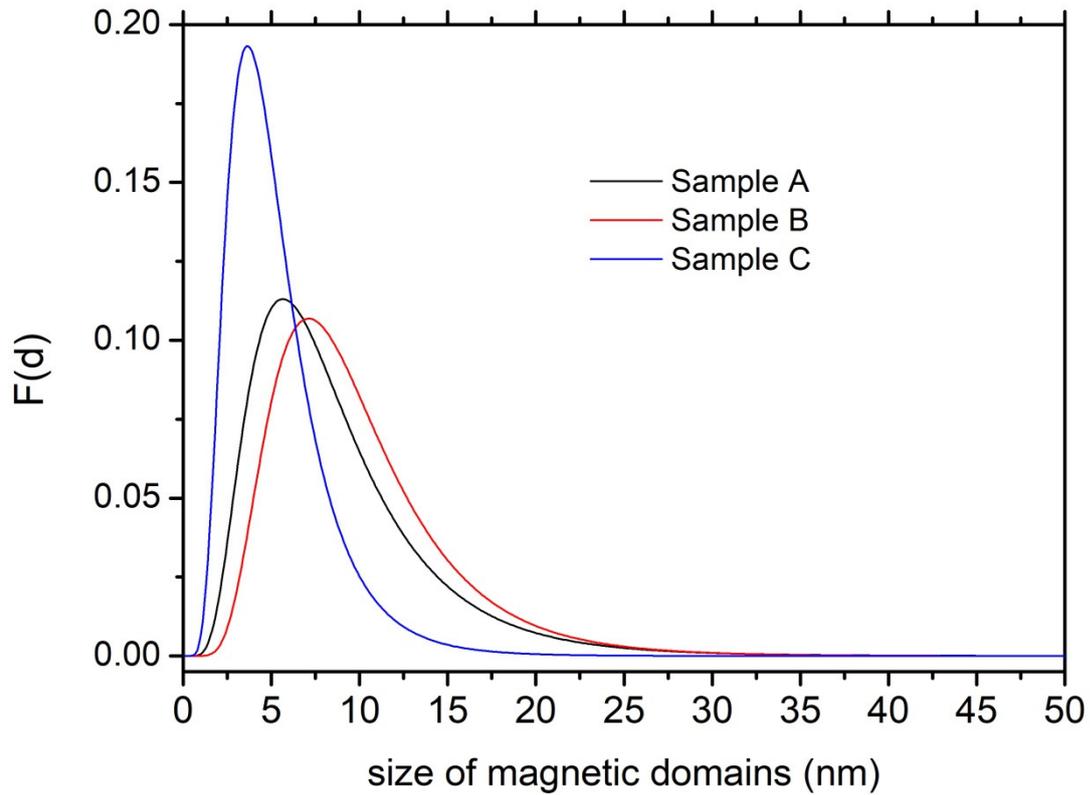


Figure S6.

Domain size (diameter) distribution curves obtained by fitting the magnetization vs. magnetic field curves for samples A, B and C. A lognormal distribution was used to fit the curves with the parameters reported in Table 5 of the text.



Analysis of magnetization curves vs. magnetic field intensity

The magnetization of superparamagnetic NPs as a function of the magnetic field intensity can be described by:

$$M(H) = M_s \int_0^{\infty} L(H, D) P_V(D) dD$$

where D is the diameter of the particles.

L(H,D) is the Langevin function which depends on the modulus of magnetic dipole and of the intensity of the magnetic field H:

$$L(H, D) = \coth(x) - \frac{1}{x}$$

$$x = \frac{\mu_0 \mu H}{k_B T}$$

where μ_0 is the vacuum permittivity ($4\pi \times 10^{-7}$ H/m, $4\pi \times 10^{-7}$ A/m²), μ the modulus of the magnetic dipole moment of the particles.

Assuming that the particles are spheres of diameter D the magnetic dipole moment is given by:

$$\mu = M_s V = M_s \frac{\pi}{6} D^3$$

where M_s is the saturation magnetization of the materials (A/m/Kg)

Since the particles are polydispersed we have to consider a particle size distribution (PSD) function.

For the PSD function the lognormal function was used:

$$P_V(V) = \frac{1}{\sqrt{2\pi} V w} \exp \left[-\frac{(\ln V - \ln m_V)^2}{2w^2} \right]$$

where m_V and w are the arithmetic mean of $\ln V$, and w the standard deviation of $\ln V$.

The $P_V(V)$ can expressed as a function of the particles' diameter:

$$P_V(D) = \frac{6}{\sqrt{2\pi} w \pi D^3} \exp \left[-\frac{(3 \ln D - 3 \ln m_D)^2}{2w^2} \right] = \frac{2}{\sqrt{2\pi} w' \pi D^3} \exp \left[-\frac{(\ln D - \ln m_D)^2}{2w'^2} \right]$$

$$M(H) = \int_0^{\infty} L(H, V) P_V(V) dV = \frac{\pi}{2} \int_0^{\infty} L(H, D) P_V(D) D^2 dD =$$

$$= \int_0^{\infty} L(H, D) \frac{1}{\sqrt{2\pi} w' D} \exp \left[-\frac{(\ln D - \ln m_D)^2}{2w'^2} \right] dD$$

With $w=3w'$

Taking into account that:

$$dV = \frac{\pi}{2} D^2 dD$$

The parameter m_D and w' are determined by fitting the experimental magnetization curves. The volume weighted mean diameter and the standard deviation can be calculated by the following formulas:

$$\langle D \rangle = \exp\left(\ln m_D + \frac{w'^2}{2}\right)$$
$$\sigma = \sqrt{\exp(2 \ln m_D + w'^2)(\exp(w'^2) - 1)}$$