Supporting Information.

Multi-Functional Magnetic and Upconverting Nanobeads as Dual Modal Imaging Tools

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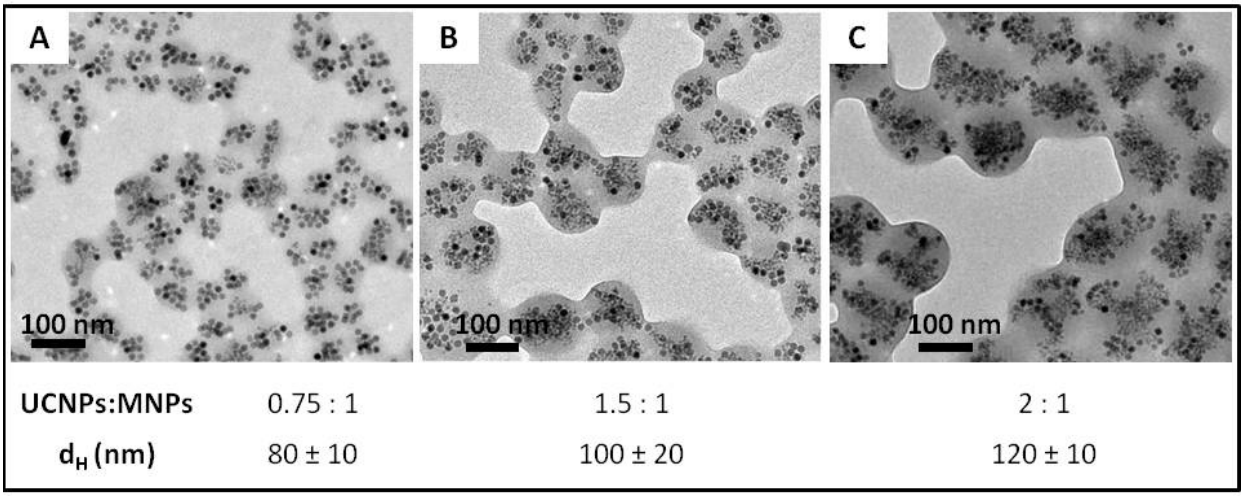


Fig. S1. TEM images and relative average hydrodynamic diameters of MUCNBs (6 nm UCNPs) obtained with different ratios UCNPs/MNPs: 0.75 (**A**), 1.5 (**B**) and 2 (**C**).

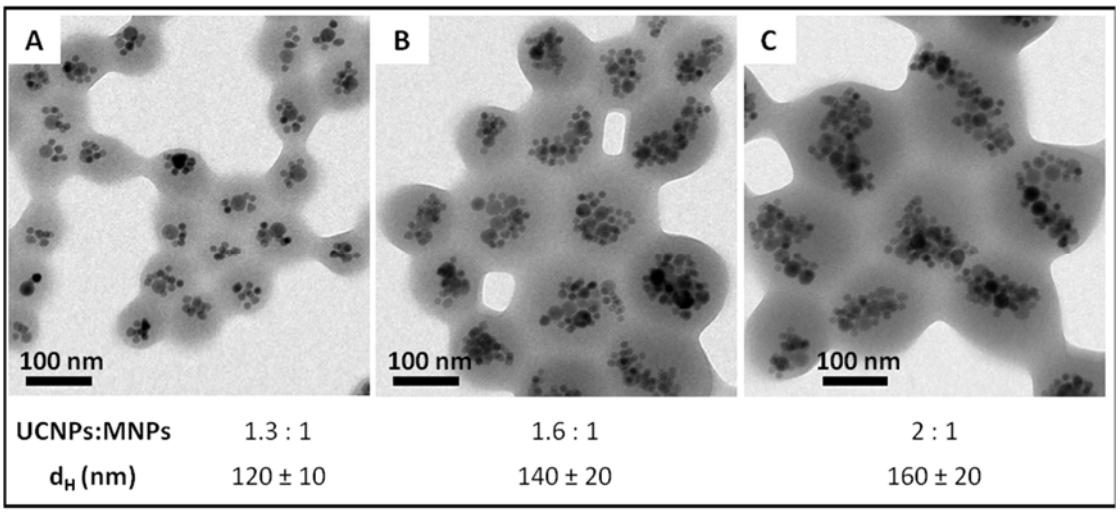


Fig. S2. TEM images and average hydrodynamic diameters of MUCNBs (23 nm UCNPs) obtained with different ratios UCNPs/MNPs: 1.3 (**A**), 1.6 (**B**) and 2 (**C**).

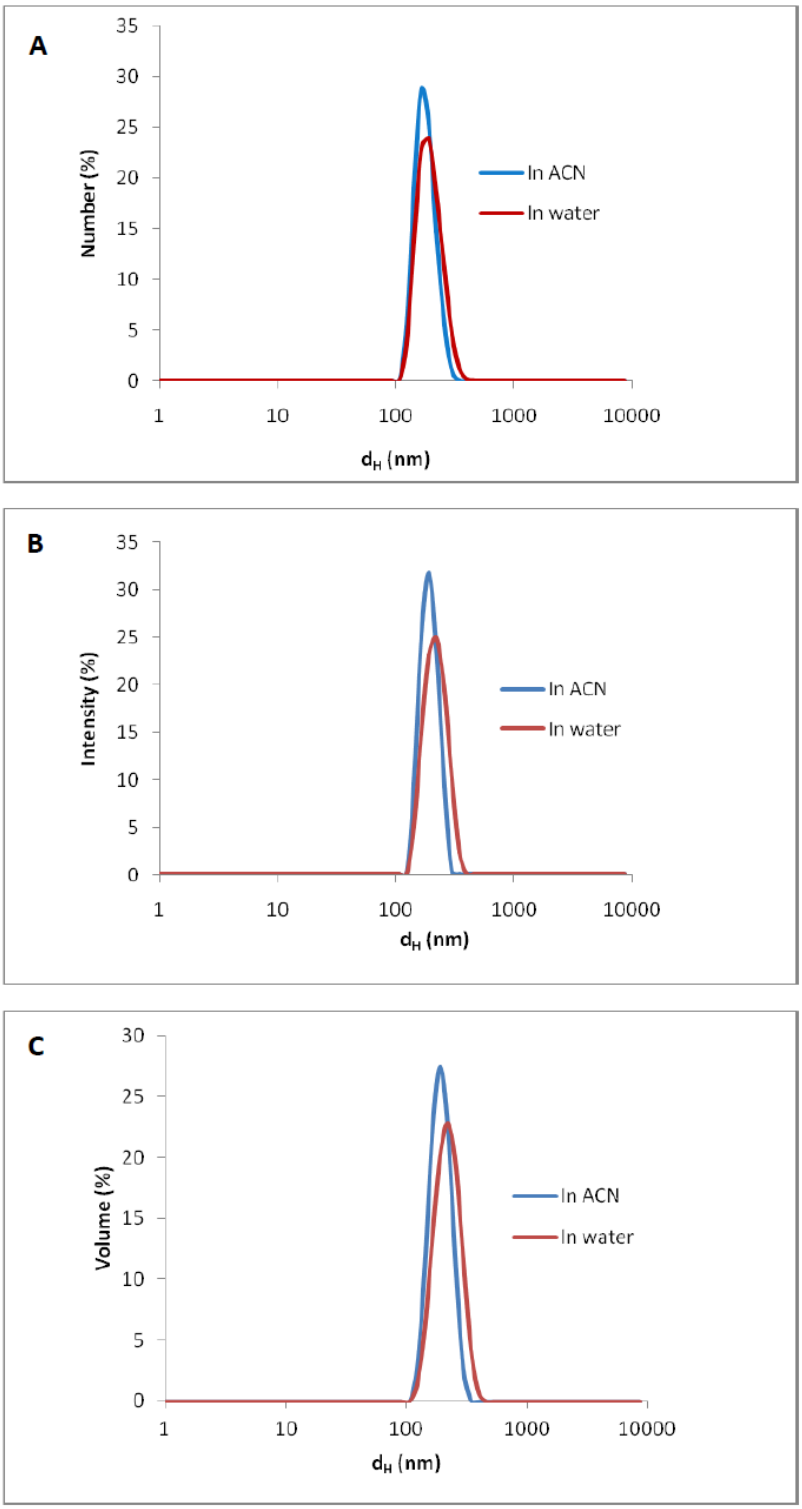


Fig. S3. Dynamic light scattering (DLS) graphs of MUCNBs (containing 23 nm UCNPs and 15 nm IONPs) measured by number (**A**), intensity (**B**) and volume (**C**). For each graph the measurements of the beads in acetonitrile (ACN) and soon after the transfer to water are reported. The peaks for the samle in ACN and water are narrow indicating well monodisperse beads and only a slightly increase of the hydrodynamic size occurs when the beads are transferred from ACN into water likely due to a more hydrated polymer shell in water.

Table S1: Average DLS hydrodynamic diameters measured by number, intensity and volume for MUCNBs as prepared in ACN and after water transfer (data refer to Figure S3).

	d_H by Number	**d_H by Intensity**	**d_H by Volume**
MUCNBs in ACN	150 ± 40	160 ± 30	160 ± 30
MUCNBs in water	160 ± 20	170 ± 30	170 ± 30

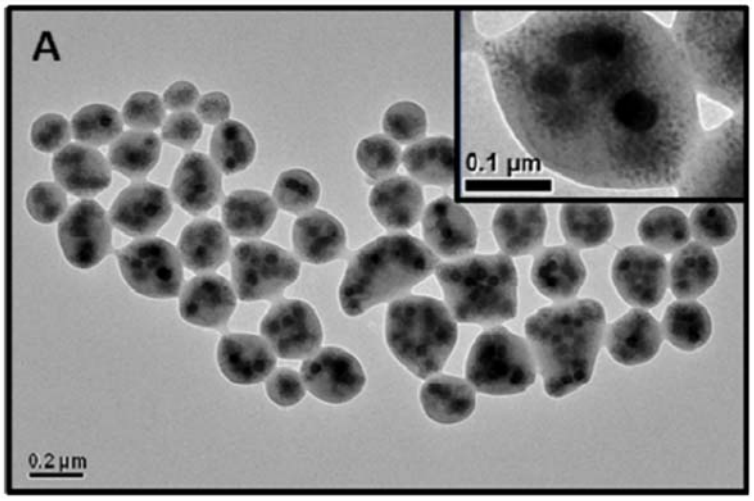


Fig. S4. TEM image of MUCNBs with a hydrodynamic size of 173 nm (PdI 0.07), prepared with UCNPs with a size of 30-40 nm and manganese iron oxide nanoparticles with a size of 8 nm. The inset shows one nanobead at high magnification; inside the bead it is possible to observe a big agglomerate of small manganese iron oxide nanoparticles and only four big UCNPs. On such sample no luminescence was recorded (data not shown).

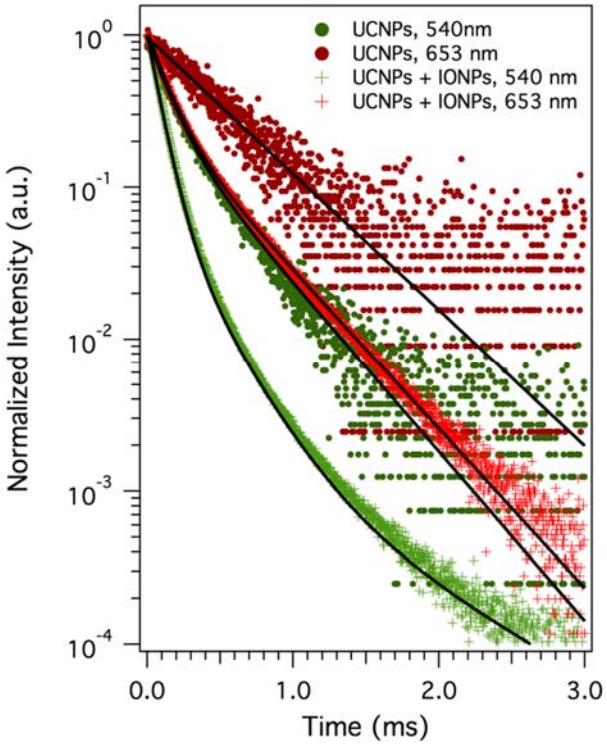


Fig. S5: Time dependent decay of the 540 nm (green) and 653 nm (red) luminescence of nanobeads loaded with UCNPs only (circles) and of MUCNBs loaded with both UCNPs and iron oxide nanoparticles (UCNPs + IONPs). Black lines are multi-exponential fits to the data, tabulated in Table S2 and described in the methods below.

Table S2: Upconversion luminescence lifetimes of nanobeads that contain UCNPs. Numbers in parentheses indicate the standard deviation in units of the last digit. A_i and τ_i are the amplitude and lifetime of component *i* of the multiexponential fits of the time dependent luminescence traces. τ_{eff} is the effective lifetime, calculated using the method described below.

Sample	**Effective lifetime**	**Lifetime parameters** *(i = 1, 2, 3)*	
Wavelength	τ_{eff} *(μs)*	A_i	τ_i *(μs)*
Nanobeads with UCNPs only			
540 nm	212(5)	0.690(9)	131(2)
		0.31(1)	392(5)
653 nm	479(5)	1.0	479(5)
MUCNBs (UCNPs + IONPs)			
540 nm	98.4(3)	0.906(1)	81.23(9)
		0.091(1)	247(1)
		0.0029(1)	776(12)
653 nm	214.2(9)	0.705(2)	129.5(5)
		0.295(2)	416.3(9)

Method for fitting lifetime components and calculating effective lifetimes.

Each time-dependent luminescence intensity curve, $I(t)$, was fit with Edinburgh FLS980 software using with one to three exponential functions according to the equation:

$$I(t) = \sum_i A_i \exp\left(-\frac{t}{\tau_i}\right) \qquad (S1)$$

Here, A_i and τ_i are the amplitude and lifetime of component *i* of the multiexponential fits. The amplitude values are normalized such that the sum of all amplitudes A_i equals 1. To faciltate comparison of lifetime traces with multiexponential decays, we calculated an *effective lifetime*, τ_{eff}, which aggregates all lifetime data into a single lifetime, using the following equation.

$$\tau_{eff} = \frac{1}{I_0}\int_0^{\infty} I(t)dt = \sum_i A_i\ \tau_i \qquad (S2)$$

In principle, τ_{eff} can be integrated from the raw $I(t)$ curve. Functionally, it can be shown, by substituting equation *S1* into equation *S2*, that τ_{eff} is also the average of the individual lifetimes τ_i weighted by their respective, normalized amplitudes, A_i.

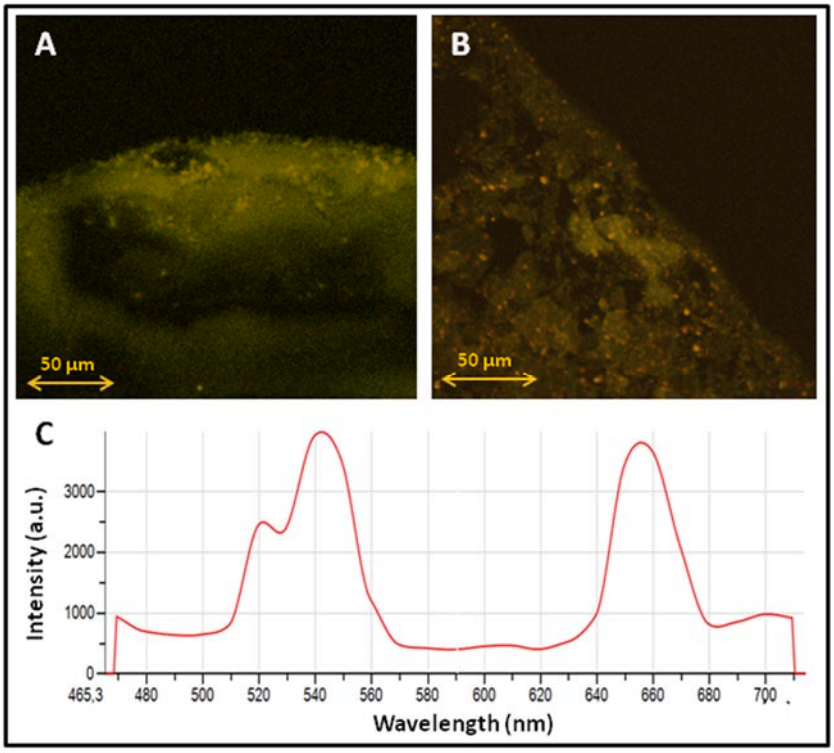


Fig. S6. Confocal spectral images of MUCNBs with UCNPs of 20 nm (**A**) and 6 nm (**B**) (λ_{exc} = 980 nm, λ_{em} = 500-680 nm). **(C)** Example of an emission spectrum recorded in correspondence with the yellow spots in figures A and B (λ_{exc} = 980 nm).

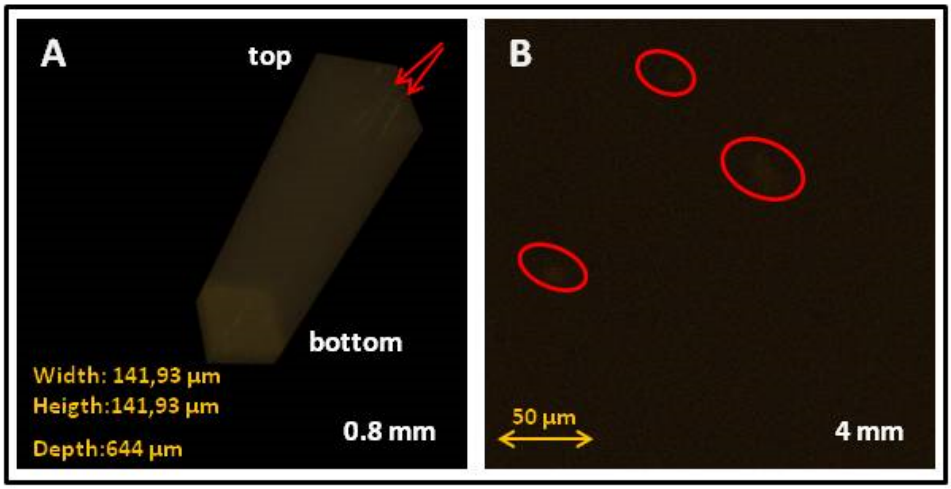


Fig. S7. 3D Confocal spectral image showing MUCNBs (UCNPs with a size of 20 nm) at the base and on the top of an agarose gel of 0.8 mm thickness (**A**). Confocal spectral images of MUCNBs (UCNPs with a 20 nm size) deposited on the top of an agarose gel of 4 mm thickness (**B**). For both the images λ_{exc} = 980 nm, λ_{em} = 500-700 nm.

Table S3: Relaxivity values (r_2) measured at 1.5 and 9.4 T for single IONPs of 15 nm size and MUCNBs.

	1,5 T r_2 ($mM^{-1}s^{-1}$)	9,4 T r_2 ($mM^{-1}s^{-1}$)
IONPs	112 ± 1	208 ± 8
MUCNBs	122 ± 2	206 ± 3

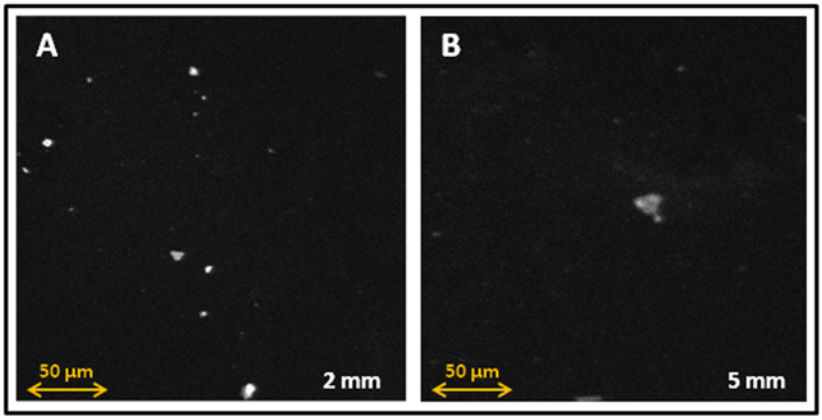


Fig. S8. Confocal images of MUCNBs (UCNPs with a size of 20 nm) on top of an agarose gel of 2 mm (**A**) and 5 mm (**B**) thickness, recorded with the two-photon source (λ_{exc} = 970 nm, λ_{em} = 500-700 nm).

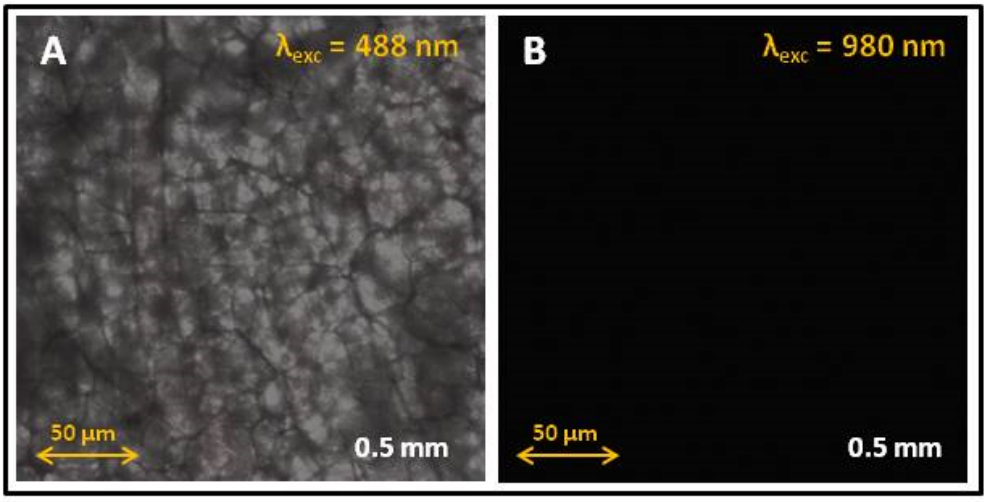
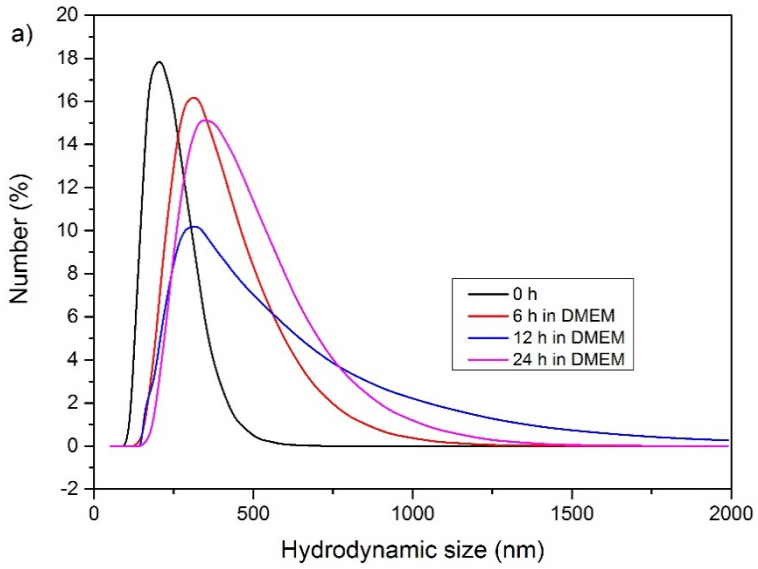


Fig. S9: Confocal image of the autofluorescence of a mouse skin slide of 0.5 mm thickness with λ_{em} = 525-700 nm at λ_{exc} = 488 nm (**A**) and λ_{exc} = 980 nm (**B**).

Stability of MUCMBs in cell culture media



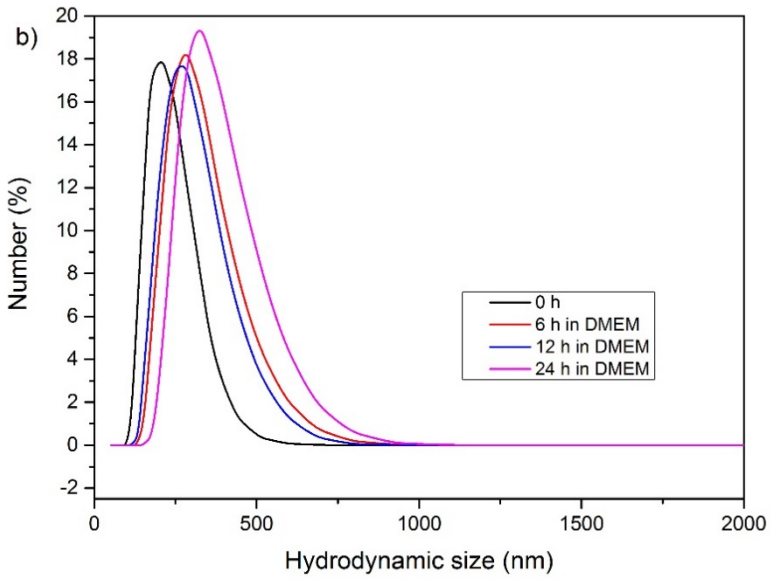


Fig. S10: DLS hydrodynamic size in number % of MUCNBs incubated in DMEM (6 µg Fe /mL) for 0, 6, 12 and 24 h after magnetically recovering the MUCNBs and re-dispersing them in water (**a**) or after having kept the MUCNBs in water undisturbed for 6 h (b) . The average hydrodynamic sizes of MUCNBs were gradually increasing with time when kept in DMEM media with also the increase of the PDI values. An increase in the polydispersive index (PDI) values is measured (Table S4). This trend indicates the partial adsorption of proteins on the charged bead surface. Interestingly, if the MUCNBs are kept in water for additional 6 h, their hydrodynamic size gradually decreases. This size decrease is likely due to the partial detachment of the serum proteins from the bead surface.

Table S4: Average hydrodynamic size of MUCNBs exposed to cell culture media and then re-dissolved in water, immediately after water re-dispersion (second column) and 6 hours after (third column).

	DLS avarage sizes refer to Figure 1aJust after media exposure and water redispersion			DLS avarage sizes refer to Figure 1b Re-measurment after 6 h in water		
Sample	Z-Ave	PdI	Number Mean	Z-Ave	PdI	Number Mean
	D (nm)		D (nm)	D (nm)		D (nm)
0 h	243±3	0.056	229±3	243±3	0.056	229±3
6 h in DMEM	413±5	0.148	366±9	338±8	0.109	312±5
12 h in DMEM	704±1	0.315	412±90	312±3	0.094	292±2
24 h in DMEM	480±3	0.163	429±4	395±12	0.125	366±13