Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Biotechnological Promises of Fe-filled CNTs for cell shepherding and magnetic fluid hyperthermia applications

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1. Characterizations and Techniques

Thermogravimetric analyses. Thermogravimetric analyses were performed using a TGA Q500 (TA Instruments), treating the samples placed in Pt pans with the following procedure: isotherm at 100°C for 20 min (to remove residual solvent, if any), ramp from 100 to 800 C at 10 °C·min^{-1}, under N_2 or air (flow rate on the sample of 90 mL·min^{-1}).

X-Ray photoelectron spectroscopy. X-ray photoemission spectroscopy (XPS) analyses were performed on a Thermo Scientific K-Alpha XPS system (Thermo Fisher Scientific, UK). For each analysis, about 0.5 mg of material were deposited on a copper thin film, which was then fixed on an XPS sample holder using a double side adhesive conductive carbon tap (SPI supplies). Spectra were collected using a monochromatized Al-K α radiation (1486.6 eV). The surface normal emitted photoelectron were analysed in a double-focusing hemispherical analyzer and recorded on a multi-channel detector. All the spectra were acquired in the constant analyzer energy mode. The Thermo Scientific Avantage software (Thermo Fisher Scientific) was used for digital acquisition and data processing.

X-ray diffraction. Powder X-ray diffractions patterns were recorded on a XPER-PRO diffractometer working with a Cu K-α radiation at 293 K.

Mössbauer Spectroscopy. After having sealed the sample in an aluminum foil, spectra were recorded in transmission geometry using a Wissel GmbH spectrometer equipped with a Cyclotron ^{57}Co(Rh) source operating at room temperature, and a Reuter Stokes proportional counter for γ-rays detection. Spectra were fitted to a sum of Lorentzians by least-squares refinement using Recoil 1.05 Mössbauer Analysis Software.[1]

Magnetic susceptibility. SQUID measurements were performed on a MPMS-5500 Quantum Design instrument in the temperature range 5–300 K. Experimental data were corrected for the sample holder and for the diamagnetic contribution of the sample using Pascal's constants.

UV-visible spectrometry. Absorbance of Fe@CNTs dispersions placed in plastic cuvettes were recorded with an Analytic Jena Specord 205 UV-visible spectrophotometer.

Transmission electron microscopy. Samples were prepared by drop-drying 0.1 mg·mL^{-1} dispersions of Fe@CNTs in CH_2Cl_2 on to carbon-coated Cu grids (200 mesh, Electron

Microscopy Sciences). Images were acquired with a Philips Tecnai 10 transmission electron microscope (TEM) operating at 80 KeV and in bright-field mode.

High-resolution transmission electron microscopy. Samples were prepared via drop-drying Fe@CNTs methanolic dispersions onto a copper grid mounted “lacey” carbon film. Transmission electron microscopy (TEM) was performed using a JEOL 2100F TEM (Field emission gun source, information limit <0.19 nm, 200 kV accelerating voltage) at room temperature. Analysis of nanoparticles was conducted using Image J software.

Optical Microscopy. Images of cells were acquired with a Leitz Labovert FS optical microscope equipped with a Leica DFC 290 camera.

2. Materials

Fe@CNTs synthesis and functionalization: Ferrocene, *N*-[2-(Trimethylsilyl)ethoxycarbonyloxy]succinimide (TEOC-OSu), isoamyl nitrite, morpholino-2-etanesulphonate (MES), ethyl-dimethylaminopropyl carbodiimmide hydrochloride (EDC·HCl) and N-hydroxy-sulpho-succinimide (NHSS) were obtained from Sigma-Aldrich, and used without additional purification. 4-aminobenzylamine was obtained from TCI and used without additional purification. Cetuximab was obtained from the Erbitux© formulation by discontinuous diafiltration in centrifugal concentrators (Vivaspin 6 10,000 g mol-1 molecular weight cut-off), and isolated through lyophylization. Dimethylformamide (DMF) and N-methylpyrrolidone (NMP) were obtained from Sigma-Aldrich and hydrochloridic acid (HCl) was obtained from Fisher Scientific. Methanol (MeOH) and toluene were purchased from Univar and distilled before use. Polytetrafluoroethylene and polycarbonate filters were obtained from Millipore (Fluoropore, Isopore, Omnipore or Durapore).

Biological experiments: Foetal calf serum were purchased from Invitrogen, while Dulbecco’s modified Eagle’s medium (DMEM), CO_2 independent medium and trypsin-EDTA were obtained from Gibco. T75 flasks were purchased from Corning. Trypan blue staining dye was obtained from Sigma-Aldrich as a 0.4% saline solution.

3. Methods

3.1 Fe@CNTs syntheses

1) Tol-Fe@CNTs synthesis

A 10 cm long silicon tubular substrate ("collector") was put inside a quartz tube, belonging to a horizontal furnace setup, in a temperature region of 850 °C. Then, under a constant Ar flow of 2 ml·min^{-1}, ferrocene (3.0 g) was introduced inside the quartz tube in a quartz recipient ("quartz boat") in a temperature region of about 550 °C. Toluene was then flown inside the furnace under the external argon purge of 2 ml·min^{-1} for 15 minutes. After that, the silicon substrate for the CNTs collection was moved in the cold zone of the furnace and cooled to room temperature under the inert Ar atmosphere. By this way about 500 mg of a black material were batchwise obtained.

2) Fc-Fe@CNTs synthesis

Two 10 cm long silicon tubular substrates ("collectors") were put inside a quartz tube, belonging to a horizontal furnace setup, in a temperature region of 850°C. Then, under a constant Ar flow of 2 ml·min^{-1}, ferrocene (3.0 g) was introduced inside the quartz tube in a in a quartz recipient ("quartz boat"), let 10 minutes in a temperature region of about 100°C and then pushed further in the oven in a temperature region of about 250°C. After that, the silicon substrates for the CNTs collection were moved in the cold zone at the end of the furnace and cooled to room temperature under the inert Ar atmosphere. By this way about 100 mg of a black material were batchwise obtained.

3) HCl washing of Fe@CNTs (HCl-Fe@CNTs)

In a 500 ml round bottom flask, Fe@CNTs (115 mg) were dispersed in 210 mL of methanol through 10' of sonication. To the resulting suspension was added 77 ml of a 4M HCl aqueous solution (19 ml of concentrated HCl 12 M and 58 ml of distilled water) and the mixture was sonicated for additional 10 min. The resulting mixture was stirred at room temperature for 16 hours. The reaction mixture was then poured in water (400 mL) and the material was filtered through a 0.45 μm Isopore filter, washed with 100 mL of water and resuspended in 100 ml of methanol via 10 minutes of sonication, poured again into 400 ml of water and filtered. These steps were repeated 3 times. For the last filtration, few drops of methanol were passed through the filter to help the drying process. Recovery of the black powder on the filter afforded 86 mg of HCl-Fe@CNTs.

4) Annealing of HCl-washed CNTs (Ann-Fe@CNTs)

Around 80 mg of HCl-Fe@CNTs were put inside a quartz boat and positioned in the center of the furnace, previously set at 580 °C and under an Ar/H_2 flow (ratio 9:1) of 1.8 ml·min^{-1}. The material was isothermally kept under such conditions for 15h, and then slowly cooled down through a stretch of time of nearly three hours to room temperature by switching the heating off.

3.2 Fe@CNTs functionalization

The typical procedures reported here were applied on Fc-Fe@CNTs, HCl-Fe@CNTs, Ann-Fe@CNTs and Tol-Fe@CNTs

1) Synthesis of Teoc-protected 4-aminobenzylamine **2**

In a 100 mL flask was introduced 4-aminobenzylamine (1 g, 8.19 mmol, 2.1 eq.) in a mixture of dioxane (10 mL) and water (10 mL). The solution was stirred and triethylamine (2.2 mL, 15.8 mmol, 4.1 eq.) and 1-[2-(Trimethylsilyl)ethoxycarbonyloxy]pyrrolidin-2,5-dione (1 g, 3.86 mmol, 1 eq.) were added successively. The reaction mixture was stirred overnight at room temperature and water (20 mL) was added. The solution was extracted three times with diethyl ether (3 x 40 mL). The organic phases were combined, washed with brine (30 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel chromatography (EtOAc) to yield 841 mg (82 %) of **2** as an orange oil.

^{1}H NMR (DMSO-D6, 400 MHz): δ 7.35 (t, 1H, *J* = 6.1 Hz), 6.88 (app d, 2H, *J* = 8.3 Hz), 6.48 (app d, 2H, *J* = 8.3 Hz), 4.92 (br s, 2H), 4.02 (m, 2H), 3.96 (d, 2H, *J* = 6.1 Hz), 0.90 (m, 2H), 0.01 (s, 9H); ^{13}C NMR (DMSO-D6, 100 MHz): δ 156.42, 147.44, 128.01, 126.90, 113.61, 61.41, 43.44, 17.41, -1.40; FTIR (solid HATR, cm^{-1}): 3355, 2952, 1694, 1624, 1516, 1471, 1436, 1358, 1247, 1177, 1126, 1059, 1041, 943, 857, 931, 767, 693, 662, 609, 587, 489; HRMS $C_{13}H_{13}N_2O_2Si$ [$M+H^+$] calcd 267.1523, exp 267.1525.

2) Synthesis of Teoc-protected benzylamine **3** (reference for TGA and XPS)

In a 100 mL flask was introduced benzylamine (420 mg, 3.92 mmol, 1 eq.) in a mixture of dioxane (10 mL) and water (10 mL). The solution was stirred and triethylamine (1.1 mL, 7.9 mmol, 2 eq.) and 1-[2-(Trimethylsilyl)ethoxycarbonyloxy]pyrrolidin-2,5-dione (1 g, 3.86 mmol, 1 eq.) were added successively. The reaction mixture was stirred overnight at room temperature and water (20 mL) was added. The solution was extracted three times with diethyl ether (3 x 40 mL). The organic phases were combined, washed with brine (30 mL), dried over $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel chromatography (EtOAc) to yield 680 mg (70 %) of **3** as an orange oil. The characterization data match those reported in the literature.[2]

3) Synthesis of Fe@CNTs-NHTeoc

70.6 mg of Fe@CNTs were placed in a 500 mL round bottomed flask and 100 mL of N-methyl-2-pyrrolidone (NMP) were added. The solution was sonicated for 20 min. 600 mg of Teoc-protected 4-aminobenzylamine **2** were introduced and the solution was sonicated for another 10 minutes. Isoamyl nitrite (1 mL) was added slowly and the reaction was stirred at 90 °C for 20 h. After cooling to room temperature, 200 mL of ethyl acetate were added and the solution was filtered on a durapore filter. The filter was placed in a beaker with 100 mL of ethyl acetate and sonicated for 2 minutes, the

filter was taken, put in another beaker with 50 mL of ethyl acetate and sonicated for 2 minutes. The filtered was then reused for the filtration and the two ethyl acetate solutions were filtered on the filter. This step was repeated twice and the solid was finally dried to obtain 70 mg of black powder.

4) Synthesis of Fe@CNTs-NH_2

In a 100 mL round-bottomed flask were introduced Fe@CNTs-NHTeoc (20 mg) and 10 mL of DMF. The solution was sonicated for 10 minutes, then 0.8 mL of a 1M solution of TBAF in THF was added and the reaction mixture was sonicated for 2 minutes. The reaction mixture was stirred 24 h at room temperature. 20 mL of ethyl acetate were added and the mixture was filtered on omnipore. The compound was dispersed in 20 mL of ethyl acetate through 10 minutes of sonication and filtered again on omnipore. This last operation was repeated twice. The solid was finally dried to obtain 17 mg of a black powder.

5) Synthesis of Fe@CNTs-Ab

The description of the synthetic strategy for Fe@CNTs bioconjugation is reported elsewhere.[3,4] This procedure was proportionally adapted on Tol-Fe@CNTs, Fc-Fe@CNTs, HCl-Fe@CNTs and Ann-Fe@CNTs amounts ranging from 25 to 30 mg in a per weight basis.

At the end of this procedure, each sample was further purified through discontinuous diafiltration against water, inside centrifugal concentrators of 300,000 $g \cdot mol^{-1}$ cut off, and isolated as powder by filtration through a hydrophilic polytetrafluoroethylene membrane with 0.45 µm porosity.

3.3 Determination of Fe@CNTs-Ab magnetic properties in solution

1) Determination of Fe@CNTs-Ab magnetic coagulation effectiveness (CE)

Stock solutions of Fe@CNTs-Ab were prepared by dispersing the different materials into CO_2 independent medium supplemented with 10% (v/v) foetal calf serum (complete CO_2 independent medium), at a constant Fe concentration of 30 $\mu g \cdot ml^{-1}$, by 60 seconds of sonication. 800 µl of the dispersion was then placed in a plastic cuvette of 1 cm of light path length. The dispersion was sonicated for further 60 sec inside the cuvette and immediately placed on the spectrophotometer dedicated support on which has been fixed a permanent parallelipedic Ne-B magnet (1X2X2 cm, Supermagnete), at a constant distance of 7 mm from the solution. The decrease in absorbance of the dispersion, due to the magnetic coagulation, was then recorded over an 8 minute time course.

2) Determination of Fe@CNTs specific absorption rates (SAR)

Solutions of Fe@CNTs-Ab were prepared by dispersing the different materials into CO_2 independent medium supplemented with 10% (v/v) foetal calf serum, at a constant Fe concentration of 120 $\mu g \cdot ml^{-1}$, by 60 seconds of sonication. 1.2 ml of this dispersion was placed inside a 1.5 ml polystyrene vial. This vial was then placed in the center of an 8 turns helical coil of 25 mm internal diameter, connected to an Ambrell Easy Heat LI induction heating equipment. A fiber optical temperature probe (Photon Control FTP-TF4-2M-SC) was introduced inside the solution through a small hole in the cap of the polystyrene vial. This fiber optical temperature probe was connected to a computer and the temperature evolution was recorded with the FluoSoft 2.9 software (Photon Control). Temperature evolution was followed during the application of an electromagnetic radiation of a frequency of 220 KHz at different magnetic field strengths (45 to 75 $KA \cdot m^{-1}$) for 15 minutes.

3.4 Determination of Fe@CNTs-Ab *in-vitro* magnetic performances

1) Cell culture

Human epithelial carcinoma cells (A431) cells were maintained in culture in 75cm² polystyrene flasks (Corning) with 20 mL of Dulbecco's modified Eagle's medium (DMEM). All media were supplemented with 10% of foetal calf serum and cells were grown under an atmosphere containing 5 % CO_2.

2) Cell preparation for magnetic filtration and MFH experiments

A431 cells at about 80% confluency were trypsinized for 3 minutes, centrifuged twice and resuspensed in complete CO_2 independent medium. Cells were counted and adjusted to the desired density with complete CO_2 independent medium.

3) Magnetic cell fishing

400 µL of a 430,000 A431 $cells \cdot ml^{-1}$ suspension in complete CO_2 independent medium was added to 400 µL of Fe@CNTs-Ab (dispersed at a constant Fe concentration of 60 $\mu g \cdot ml^{-1}$ by 60 seconds of sonication in complete CO_2 independent medium) and mixed for 10 minutes in a plastic vial. Then, a Ne-B permanent magnet (4X4X2 cm, Supermagnete) was placed against the vial and 100 µL supernatant aliquots were removed after 1, 2, 4 and 8 minutes of magnetic fishing. These aliquots were centrifuged in eppendorfs (3 minutes, 5000 rpm). The first 75 µL of the supernatant were discarded and the remaining culot (25 µL) was redispersed. Cells in this culot were counted (and imaged) in a Neubauer chamber.

4) Magnetic fluid hyperthermia

1000 µL of a 1,000,000 A431 $cells \cdot mL^{-1}$ suspension in complete CO_2 independent medium was added in 1.5 mL plastic vial to 200 µL of Fe@CNTs-Ab dispersion (at a

constant Fe concentration of 750 $\mu g \cdot ml^{-1}$, dispersed by 60 seconds of sonication in complete CO_2 independent medium), giving a final volume of 1.2 mL final cell density of 830,000 $cells \cdot ml^{-1}$ and Fe@CNTs-Ab concentrations of 125 $\mu g \cdot mL^{-1}$ in term of Fe. The vial was then placed in the center of an 8 turn helical coil of 25 mm internal diameter, connected to an Ambrell Easy Heat LI induction heating equipment. The vial was thermostated by a 37°C water bath. A fiber optical temperature probe (Photon Control FTP-TF4-2M-SC) was introduced inside the solution through a small hole in the cap of the polystyrene vial. This fiber optical temperature probe was connected to a computer and the temperature evolution was recorded with the FluoSoft 2.9 software (Photon Control). A pulsed (40 seconds on, 20 seconds off) electromagnetic radiation of a frequency of 220 KHz and magnetic field strength of 85 $KA \cdot m^{-1}$ was applied for 30 minutes. Control experiments consisted in the exposition during 30 minutes of cells to Fe@CNTs-Ab at the concentrations aforementioned but without electromagnetic radiation (negative MFH control). Also, positive and negative controls were done by exposition of 830,000 $cells \cdot mL^{-1}$ suspensions to respectively 70°C and 37°C for 30 minutes. After these treatments, the different suspensions were re-suspended by 10 seconds of vortex-mixing. 120 µL were removed, placed in eppendorfs and centrifugated at 1000 rpm for 5 minutes. The first 110 µL of supernatant were discarded and 10 µL of a 0.4% trypan blue saline solution was added to re-suspend the culot and stain death cells. 10 µL of this dispersion was placed on a microscope glass slide and covered with a glass cover slip. 10 images were taken per condition with an optical microscope to estimate the ratio between blue-stained (death) and non-stained (alive) cells.

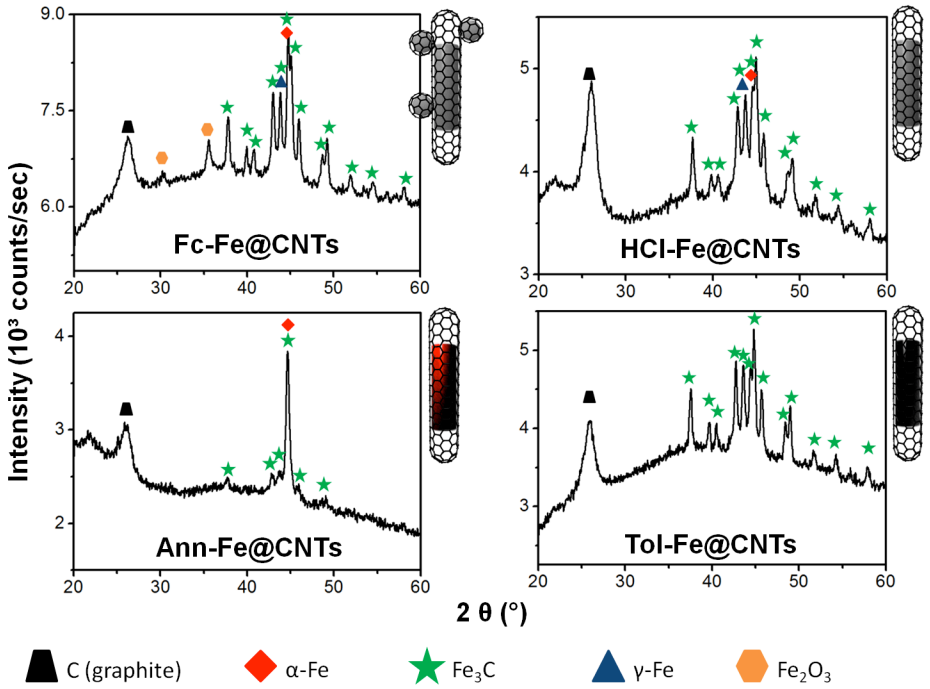
References

1 K. Lagarec and D. G. Rancourt, Department of Physics, University of Ottawa, Canada, 1998.

2 T. Y. Lam, Y.-P. Wang, R. L. Danheiser, *J. Org. Chem.*, 2013, **78**, 9396-9414.

3 R. Marega, L. Karmani, L. Flamant, P. G. Nageswaran, V. Valembois, B. Masereel, O. Feron, T. Vander Borght, S. Lucas, C. Michiels, B. Gallez and D. Bonifazi, *J. Mater. Chem.*, 2012, **22**, 21305–21312.

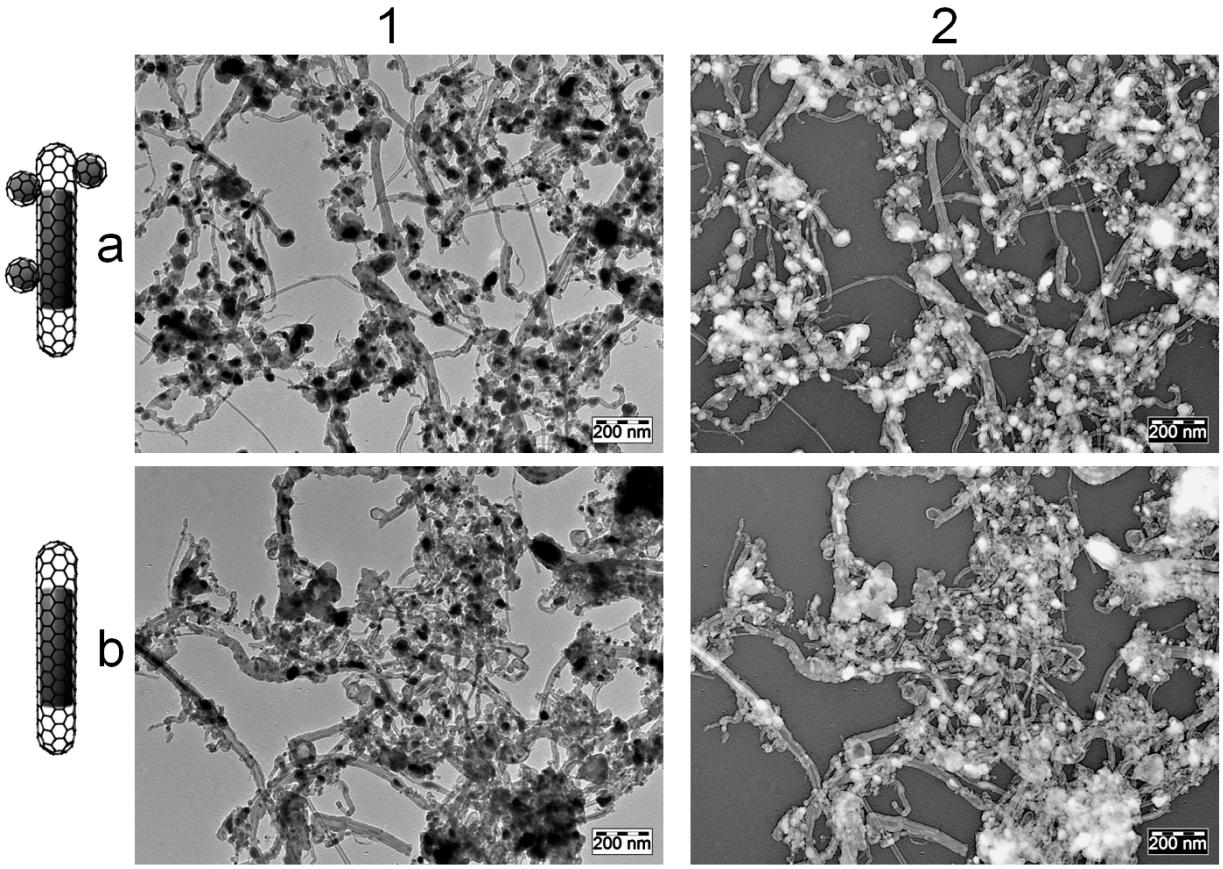
4 R. Marega, F. De Leo, F. Pineux, J. Sgrignani, A. Magistrato, A. D. Naik, Y. Garcia, L. Flamant, C. Michiels and D. Bonifazi, *Adv. Funct. Mater.*, 2013, **23**, 3173–3184.



Supporting Figure 1: Powder X-Ray Diffraction graphs of the four Fe@CNTs hybrids. One can observe in particular the disappearance of Fe_2O_3 after the HCl washings and the transformation of Fe_3C into α-Fe following the annealing.

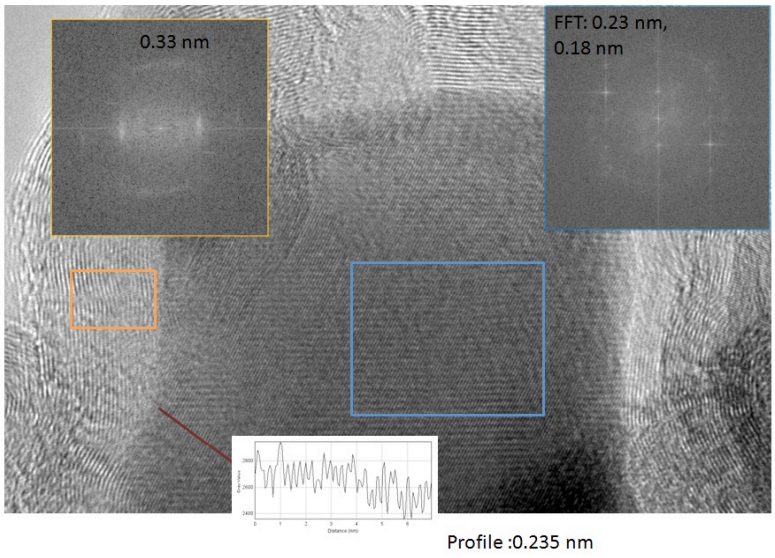
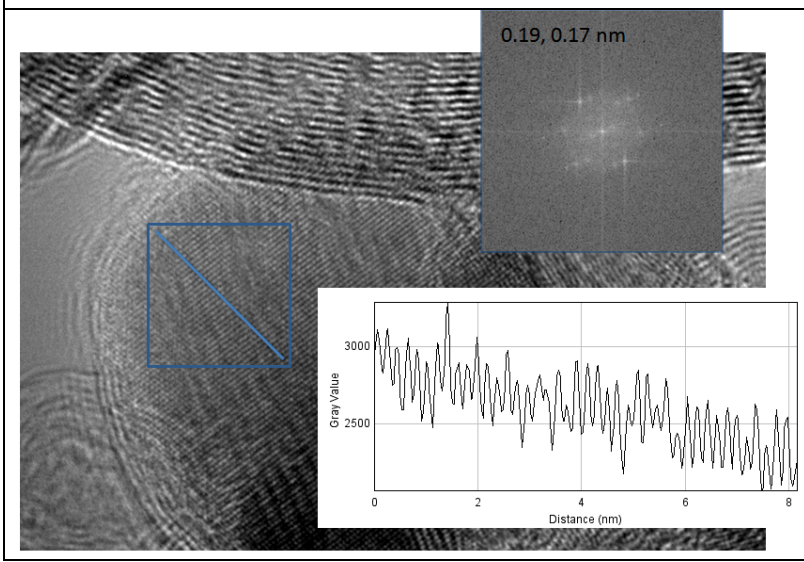
Fe@CNT hybrid	Species	Nature	δ (mm·s^{-1})	ε_Q (mm·s^{-1})	B_{Hf} (T)	$\Gamma/2$ (mm·s^{-1})	Abundance (%)
Fc-Fe@CNTs	γ-Fe	Singlet	-0.04(4)	-	-	0.26(6)	5
	Fe_3C	Sextet-1	0.18(1)	0.01(1)	20.6(3)	0.2*	76
	α-Fe	Sextet-2	0*	0	32.9(1)	0.1*	10
	γ-Fe_2O_3	Sextet-3	0.34(6)	0.05(6)	48.3(4)	0.2*	9
HCl-Fe@CNTs	γ-Fe	Singlet	-0.05(3)	-	-	0.21(5)	7
	Fe_3C	Sextet-1	0.19(4)	0.01(4)	20.6(3)	0.19(1)	88
	α-Fe	Sextet-2	0*	0*	33.6(5)	0.1(1)	5
Ann-Fe@CNTs	γ-Fe	Singlet	-0.1(1)	-	-	0.1(1)	2
	Fe_3C	Sextet-1	0.19(1)	0.01(1)	20.6(1)	0.34(4)	54
	α-Fe	Sextet-2	0.00	0.00	33.0(3)	0.11(2)	44
Tol-Fe@CNTs	γ-Fe	Singlet	-0.07(1)	-	-	0.1*	2
	Fe_3C	Sextet-1	0.18(1)	0.01(1)	20.6(1)	0.19(4)	98

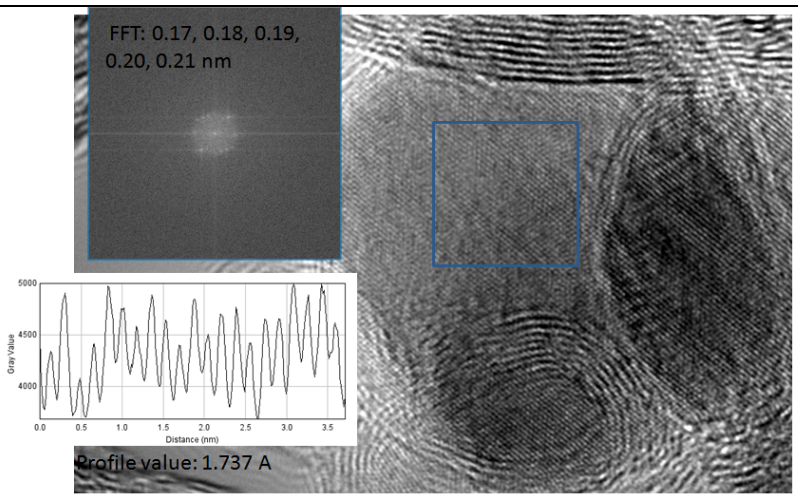
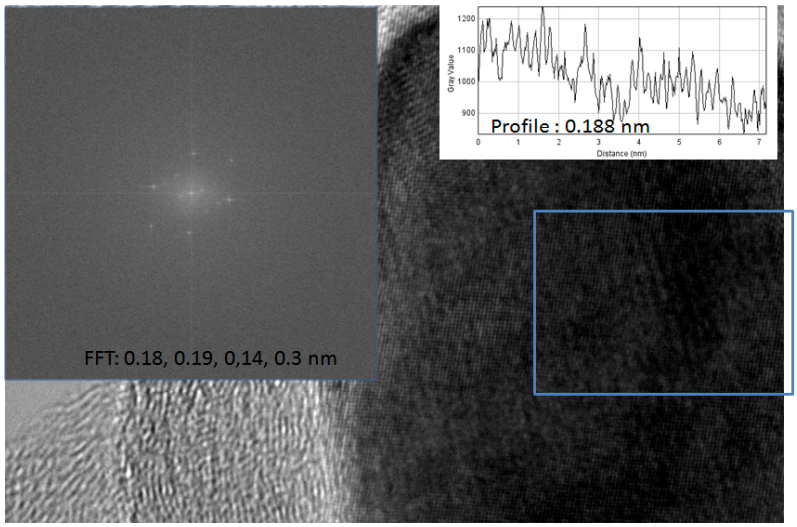
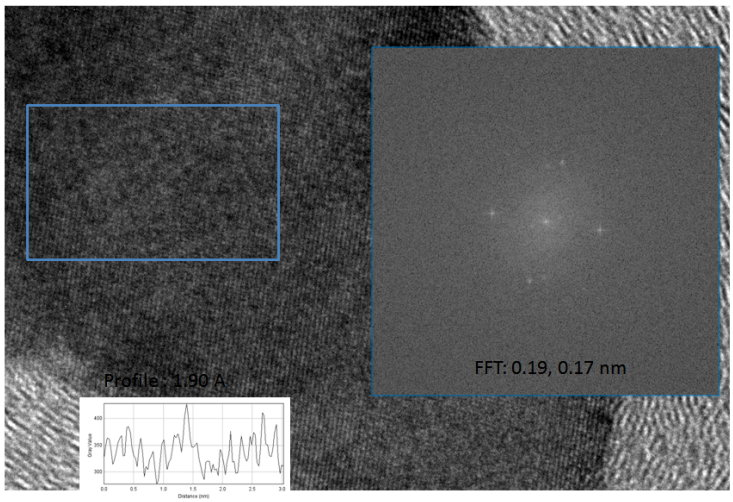
Supporting Table 1. ^{57}Fe Mossbauer parameters for the four Fe@CNTs hybrids at 298 K. δ, isomer shift (w.r.t α-Fe) ; ε_Q, quadrupole shift ; B_{Hf}, hyperfine magnetic field (in Tesla), *fixed parameter; $\Gamma/2$, half width at half maximum.

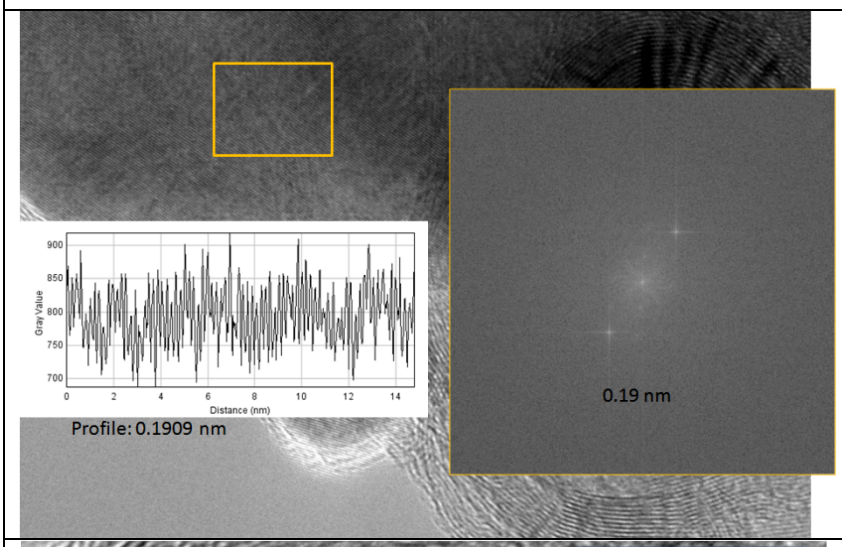
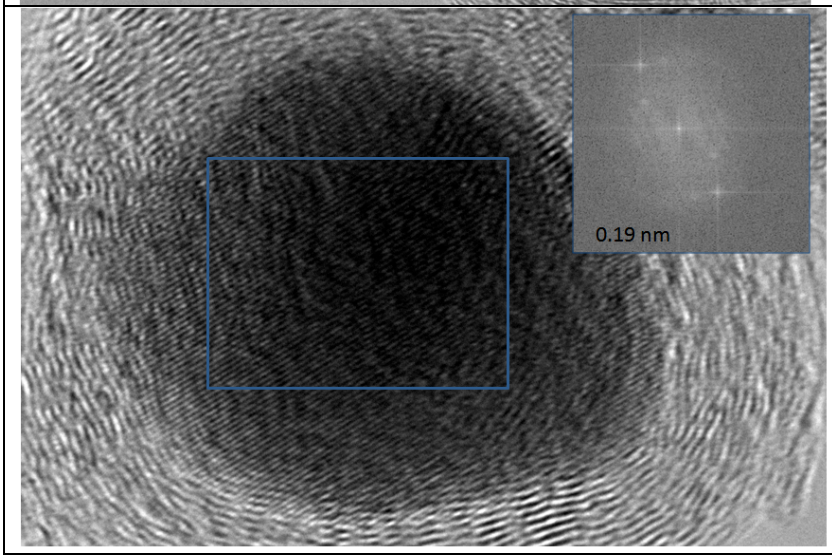


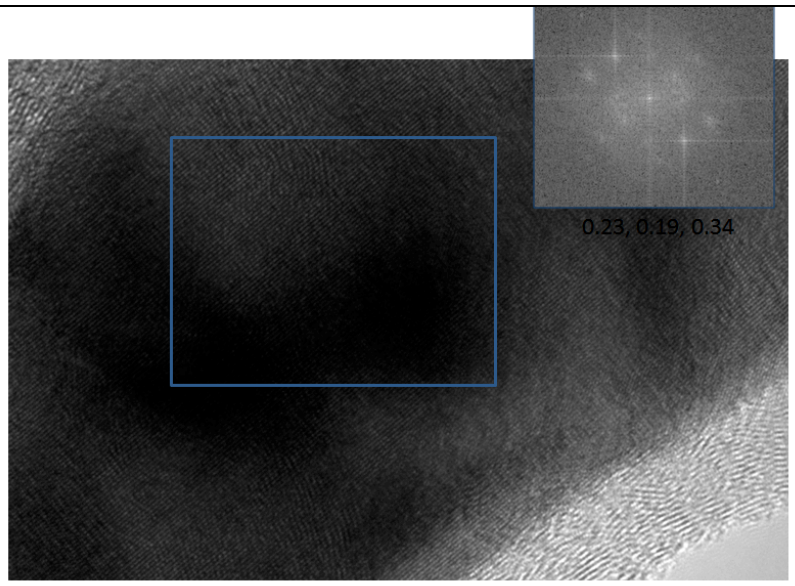
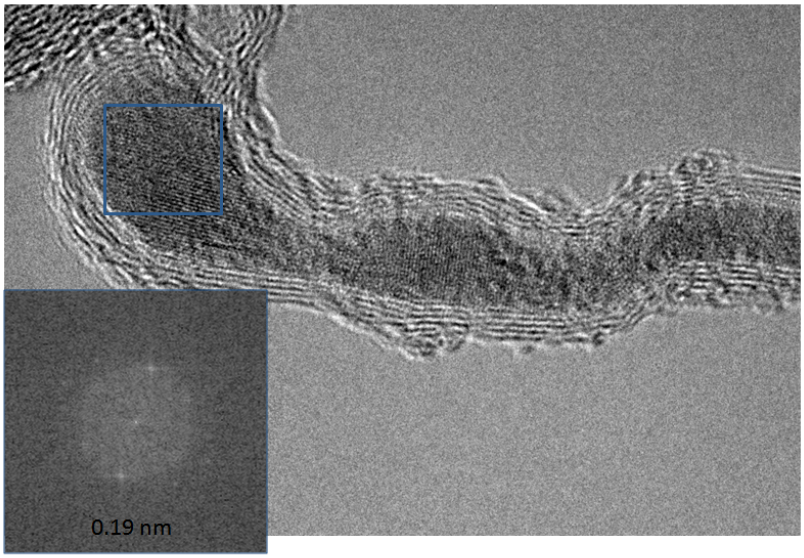
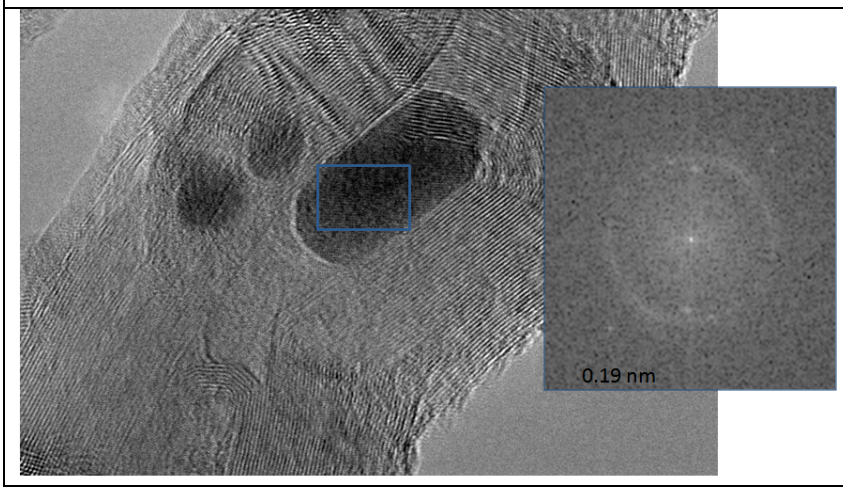
Supporting Figure 2: (1) TEM images of (a) **Fc-Fe@CNTs** and (b) **HCl-Fe@CNTs** at a 39kX magnification. (2) Same images after colour inversion.

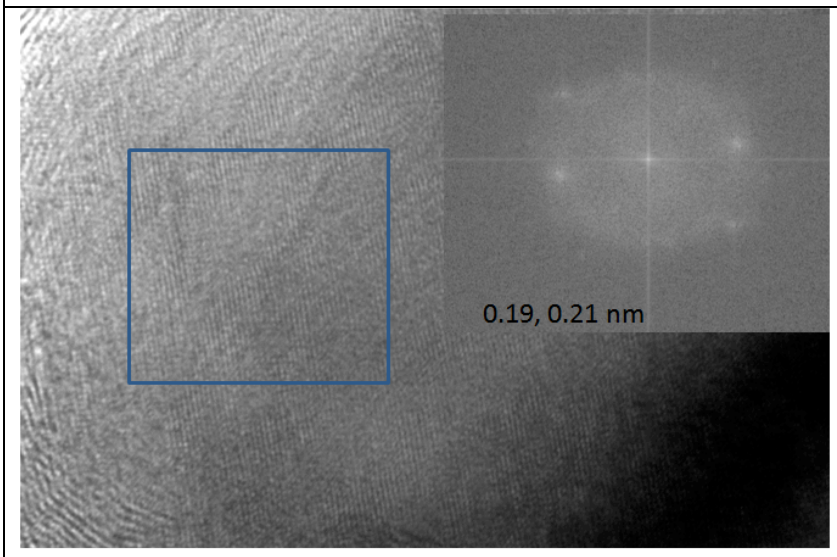
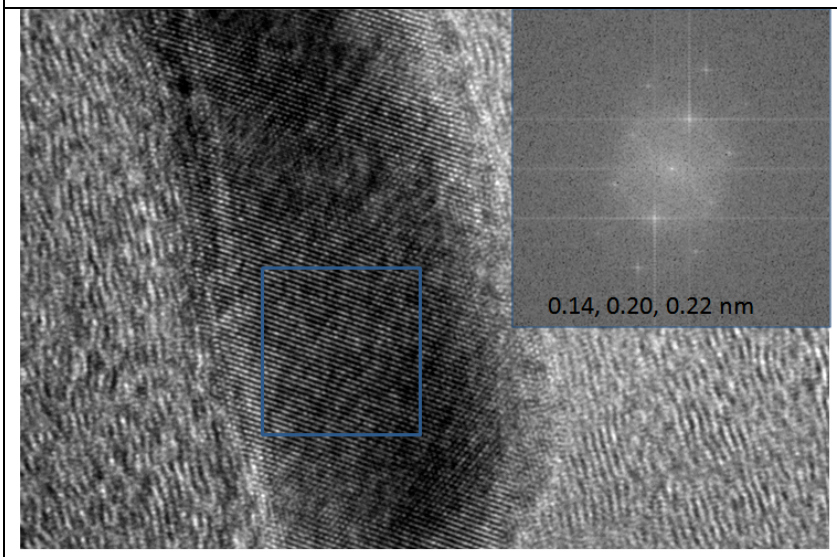
Supporting Table 2: HR-TEM images of **Fc-Fe@CNTs**, **HCl-Fe@CNTs** and **Ann-Fe@CNTs**, along with related d-values and their phase assignment. Insets represent FFT analyses of defined zones and profile height on selected lines.

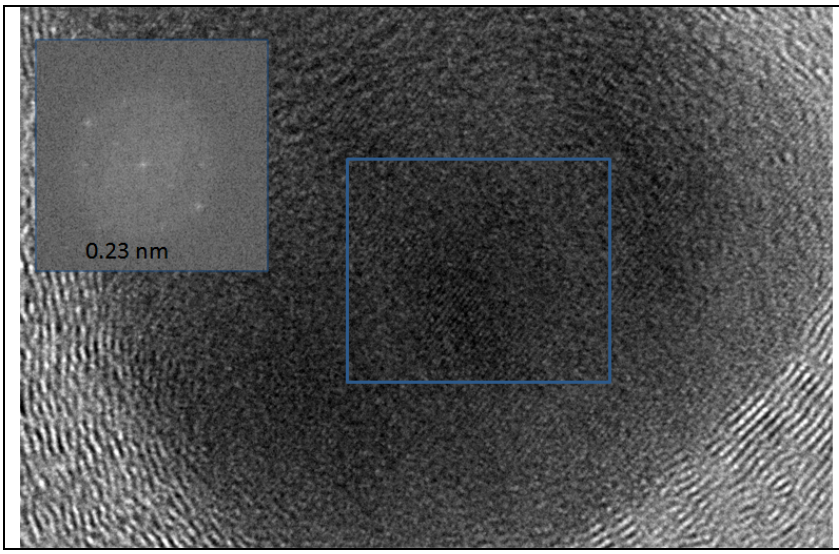
HR-TEM image (Fc-Fe@CNTs)	d-values (nm)	Phase assignment (lattice parameter)
	0.18 0.23(5) 0.33	Fe_3C (122) or γ-Fe (002) Fe_3C (121) Graphite (002)
	0.17 0.19	Fe_3C (230) Fe_3C (022) or (131)

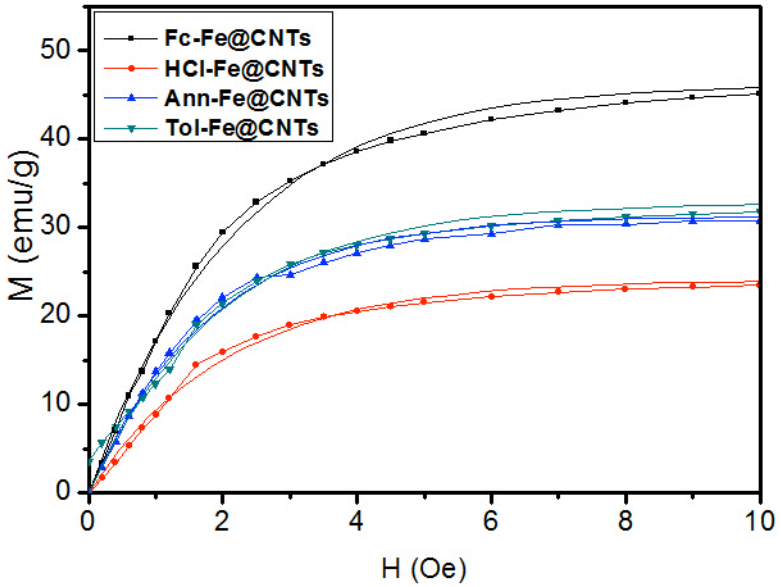
	0.17(4) 0.18 0.19 0.20 0.21	Fe_3C (230) Fe_3C (122) or γ-Fe (002) Fe_3C (022) or (131) Fe_3C (031) or γ-Fe (111) Fe_3C (211)
	0.14 0.18(8) 0.19 0.3	α-Fe (002) Fe_3C (122) Fe_3C (022) or (131) Graphite (002)
	0.17 0.19(0)	Fe_3C (230) Fe_3C (022) or (131)

HR-TEM image (HCl-Fe@CNTs)	d-values (nm)	Phase assignment (lattice parameter)
	0.19(1)	Fe_3C (022) or (131)
	0.19(1)	Fe_3C (022) or (131)

	0.19 0.23 0.34	Fe_3C (022) or (131) Fe_3C (121) or (210) Graphite (002)
	0.19	Fe_3C (022) or (131)
	0.19	Fe_3C (022) or (131)

HR-TEM image (Ann-Fe@CNTs)	d-values (nm)	Phase assignment (lattice parameter)
	0.19 0.21	Fe_3C (022) or (131) Fe_3C (211)
	0.14 0.20 0.22	α-Fe (002) α-Fe (011) Fe_3C (201)

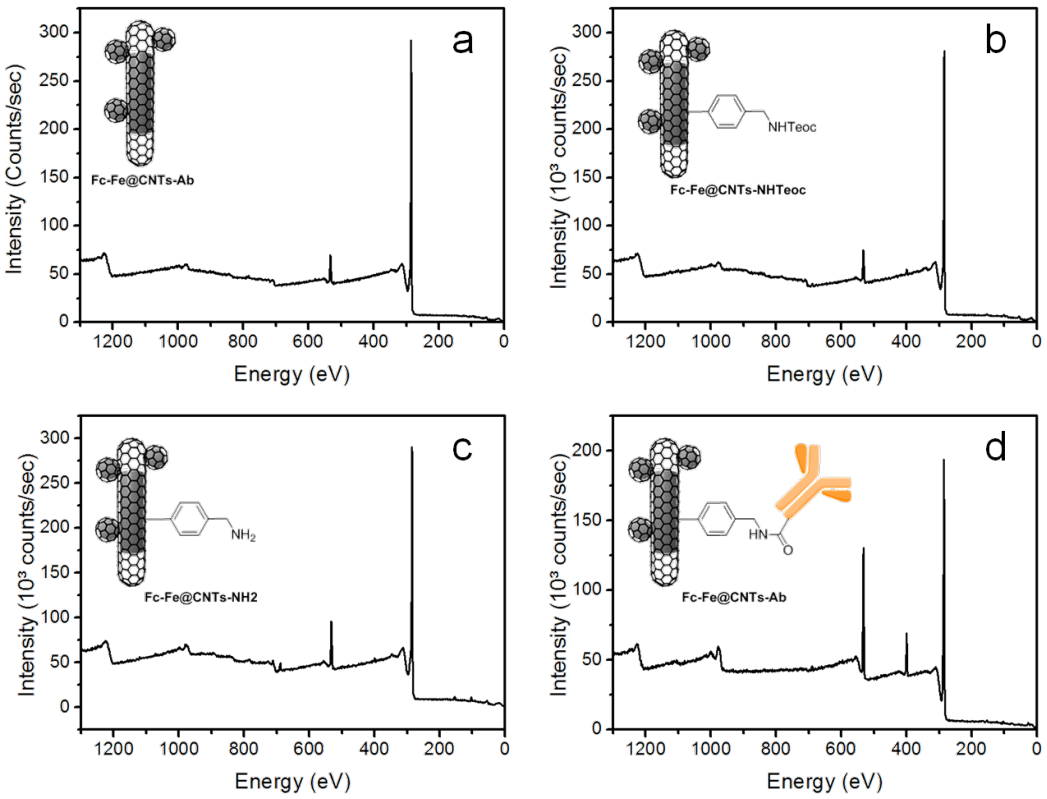
	0.23	Fe_3C (121) or (210)



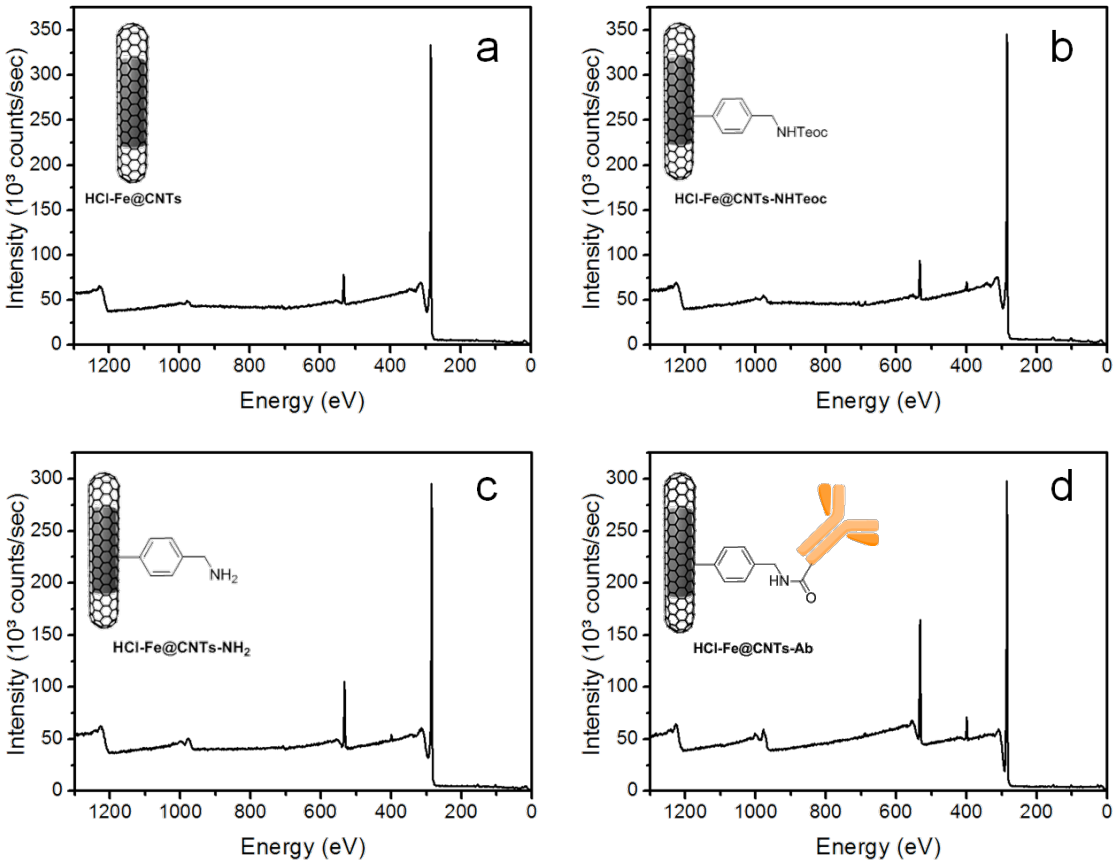
Supporting Figure 3 : Low-field magnetization curves of the four Fe@CNTs hybrids and Box-Lucas fitting.

Fitting Equation	y = a*(1 - exp(-b*x))			
Fe@CNTs Hybrid	Parameter	<>	σ	χ (a·b) emu·g^{-1}·KOe^{-1})
Fc-Fe@CNTs	a	46.27146	0.22489	
	b	0.46607	0.0092	21.56573936
HCl-Fe@CNTs	a	24.07248	0.15278	
	b	0.49083	0.01288	11.81549536
Ann-Fe@CNTs	a	31.30037	0.16704	
	b	0.55738	0.01285	17.44620023
Tol-Fe@CNTs	a	32.79823	0.30459	
	b	0.50424	0.01954	16.5381795

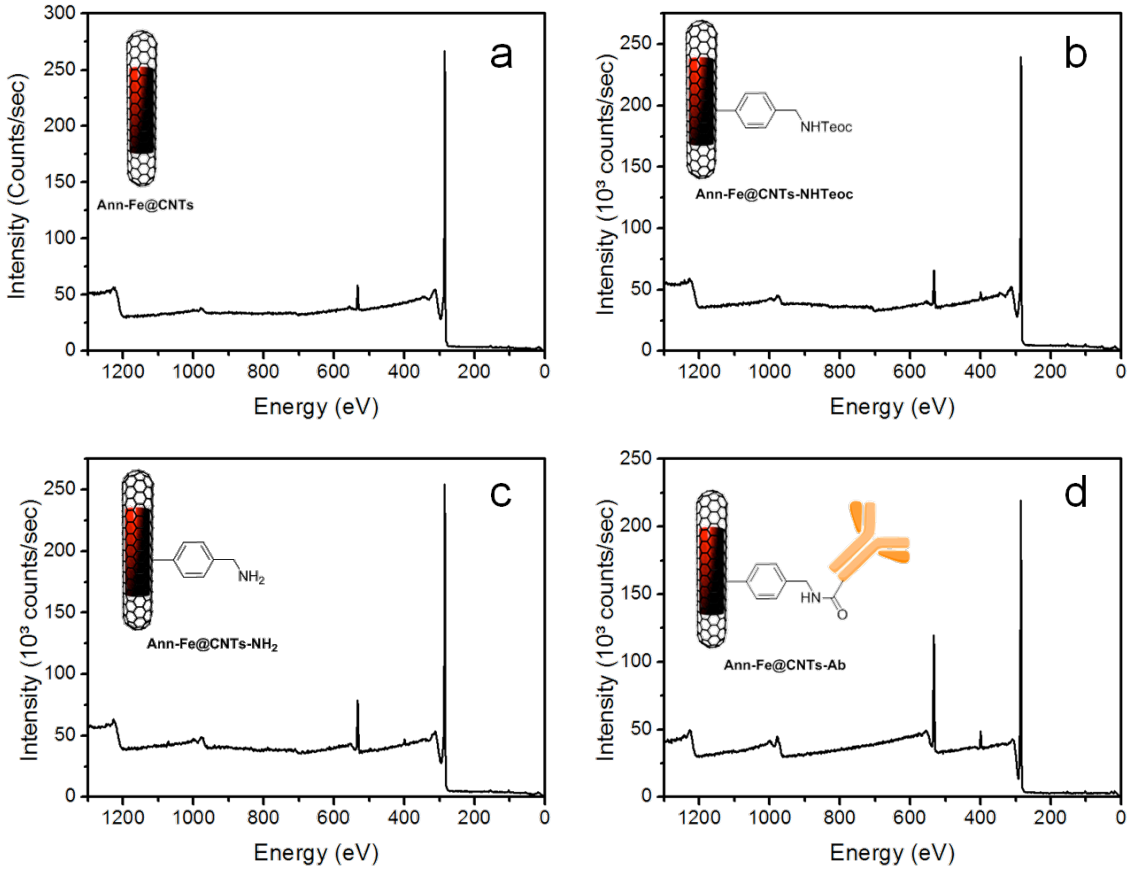
Supporting Table 3: Box-Lucas fitting equation values of the low-field magnetization curves of the four Fe@CNTs hybrids (cf. Figure S3) and calculated slope at origin (considered as low-field magnetic susceptibility χ) of the four Fe@CNTs hybrids.



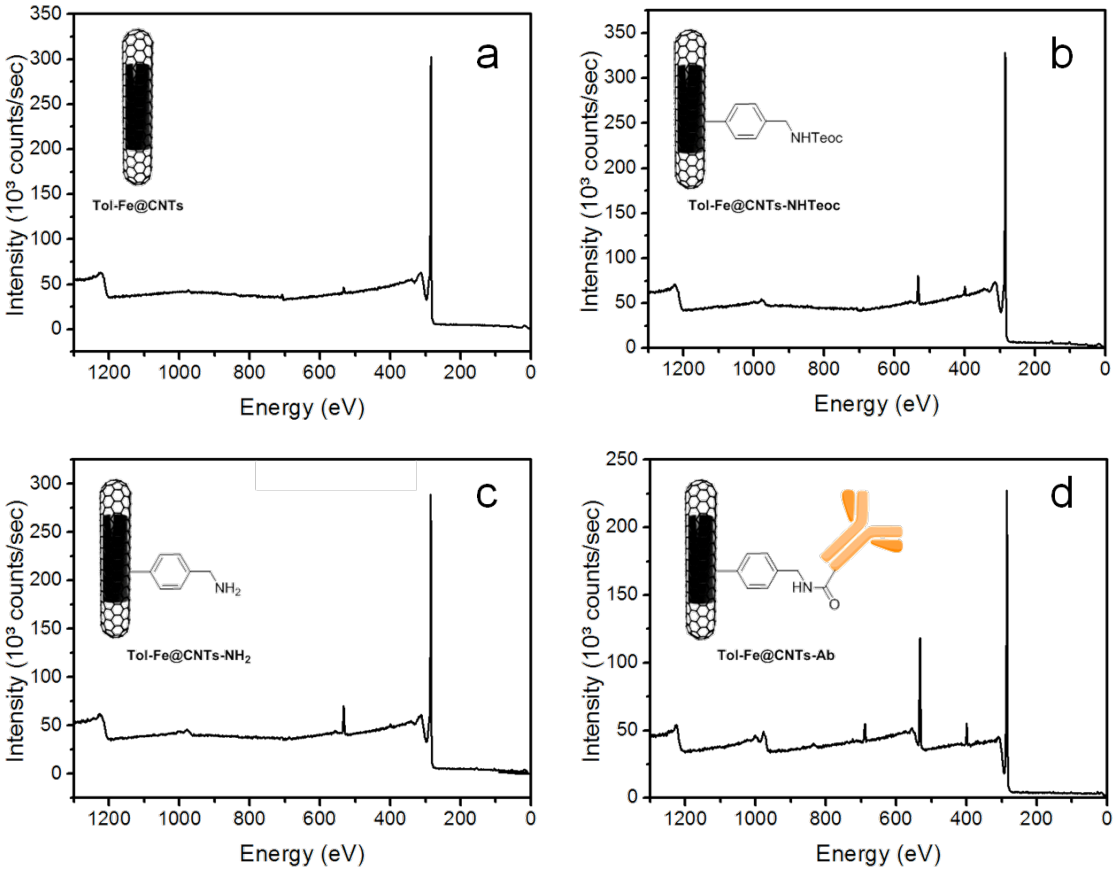
Supporting Figure 4: XPS characterization (survey scans) of **Fc-Fe@CNTs** series of derivatives ((a) **Fc-Fe@CNTs**, (b) **Fc-Fe@CNTs-NHTeoc**, (c) **Fc-Fe@CNTs-NH_2** and (d) **Fc-Fe@CNTs-Ab**).



Supporting Figure 5: XPS characterization (survey scans) of **HCl-Fe@CNTs** series of derivatives ((a) **HCl-Fe@CNTs**, (b) **HCl-Fe@CNTs-NHTeoc**, (c) **HCl-Fe@CNTs-NH_2** and (d) **HCl-Fe@CNTs-Ab**).



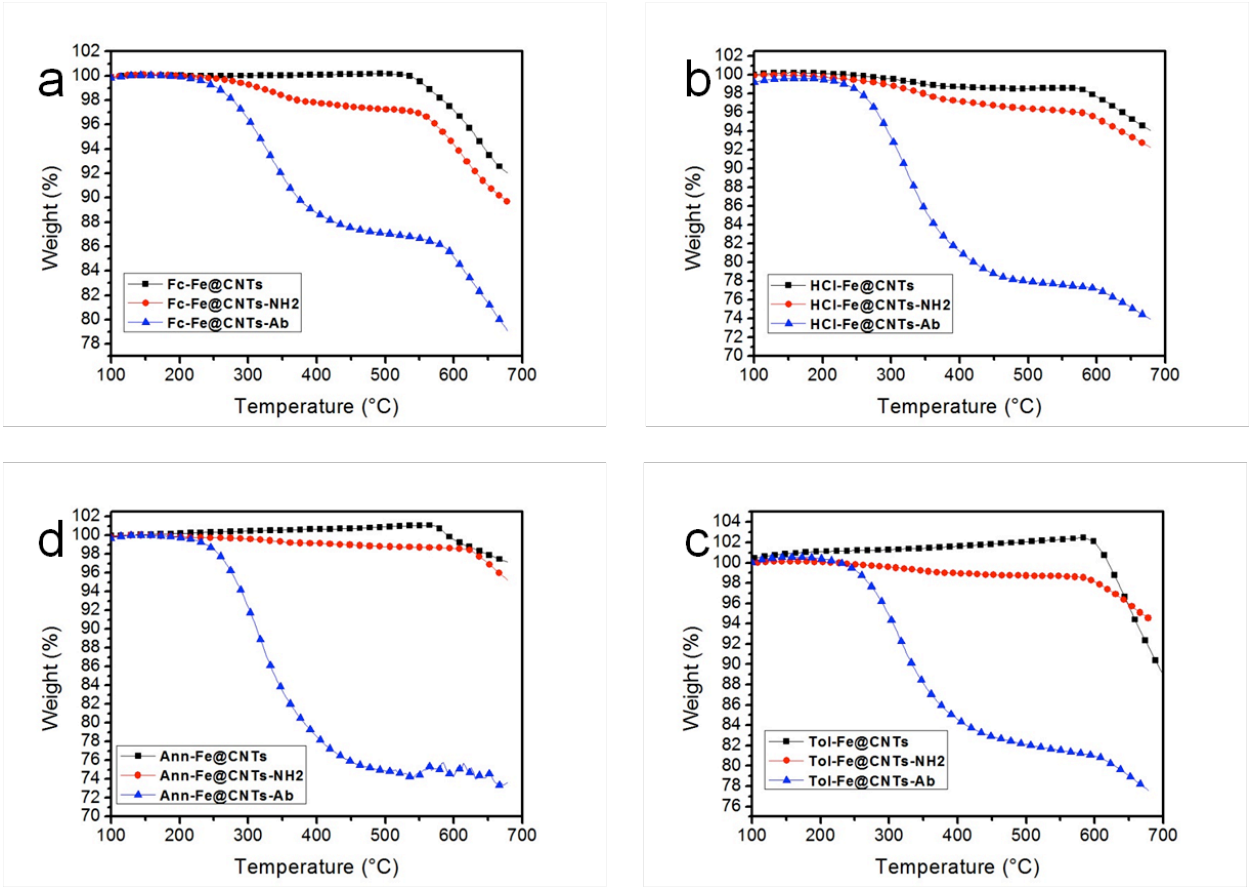
Supporting Figure 6: XPS characterization (survey scans) of **Ann-Fe@CNTs** series of derivatives ((a) **Ann-Fe@CNTs**, (b) **Ann-Fe@CNTs-NHTeoc**, (c) **Ann-Fe@CNTs-NH_2** and (d) **Ann-Fe@CNTs-Ab**).



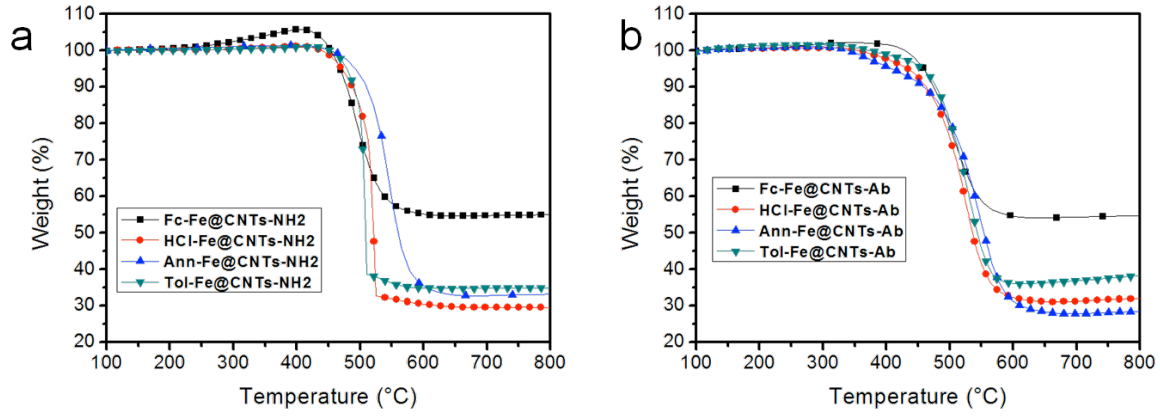
Supporting Figure 7: XPS characterization (survey scans) of **Tol-Fe@CNTs** series of derivatives ((a) **Tol-Fe@CNTs**, (b) **Tol-Fe@CNTs-NHTeoc**, (c) **Tol-Fe@CNTs-NH_2** and (d) **Tol-Fe@CNTs-Ab**).

Hybrid series	Element	Fe@CNTs		Fe@CNTs-NHTeoc		Fe@CNTs-NH2		Fe@CNTs-Ab	
		At %	Std dev.	At %	Std dev.	At %	Std dev.	At %	Std dev.
Fc-Fe@CNTs	C1s	95.77	2.11	91.08	0.33	88.71	1.37	77.23	2.24
	O1s	3.83	1.75	5.90	0.42	8.28	1.06	15.52	0.36
	N1s	n.d.	n.d.	1.90	0.33	1.46	0.19	5.69	1.67
	Si2p	n.d.	n.d.	1.12	0.42	1.56	0.46	1.21	0.16
	S2p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.36	0.10
HCl-Fe@CNTs	C1s	94.75	0.57	91.88	0.35	90.68	1.67	81.76	0.77
	O1s	4.44	0.48	4.97	0.19	6.63	1.65	14.43	0.30
	N1s	n.d.	n.d.	1.74	0.29	1.56	0.13	2.77	1.10
	Si2p	0.81	0.37	1.40	0.05	1.12	0.28	0.73	0.14
	S2p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.32	0.12
Ann-Fe@CNTs	C1s	94.79	0.51	91.07	0.15	90.28	0.16	81.94	0.15
	O1s	4.46	0.27	5.99	0.12	7.56	0.18	14.01	0.31
	N1s	n.d.	n.d.	1.62	0.16	1.49	0.19	2.99	0.42
	Si2p	0.74	0.31	1.32	0.11	0.67	0.14	0.85	0.17
	S2p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.21	0.07
Tol-Fe@CNTs	C1s	98.42	0.41	92.43	0.26	92.33	1.53	81.13	0.81
	O1s	1.58	0.41	4.89	0.14	5.47	1.56	14.12	0.17
	N1s	n.d.	n.d.	1.62	0.41	1.53	0.11	3.76	0.59
	Si2p	n.d.	n.d.	1.07	0.03	0.67	0.07	0.70	0.14
	S2p	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.30	0.05

Supporting Table 4: XPS atomic composition assessment for C1s, O1s, N1s, Si2p and S2p (expressed in atomic %) for **Fc-, HCl-, Ann-**, **Tol-Fe@CNTs** series of derivatives, resulting from a mean of three different survey scans. N.d. = non detected.



Supporting Figure 8: TGA analyses under N_2 plots: (a) **Fc-,** (b) **HCl-**, (c) **Ann-** and (d) **Tol-Fe@CNTs** series of derivatives.



Supporting Figure 9: TGA analyses under air plots of Fe@CNTs-NH_2 derivatives (a) and Fe@CNTs-Ab derivatives (b) (these TGA analyses on air were performed on the residue remaining after a first TGA under nitrogen, cf. Figure S8)

Fe@CNTs hybrid	Fe wt%	Organic fct. wt%	Ab wt%	Ratio Ab/Fe
Fc-Fe@CNTs-NH2	34.5	3.0	n.r.	n.r.
HCl-Fe@CNTs-NH2	19.1	2.2	n.r.	n.r.
Ann-Fe@CNTs-NH2	22.1	2.1	n.r.	n.r.
Tol-Fe@CNTs-NH2	23.1	3.3	n.r.	n.r.
Fc-Fe@CNTs-Ab	30.3	12.9	9.9	0.3
HCl-Fe@CNTs-Ab	16.5	22.0	19.8	1.2
Ann-Fe@CNTs-Ab	14.6	25.1	23.0	1.6
Tol-Fe@CNTs-Ab	20.6	17.9	14.6	0.7

Supporting Table 5: Fe loading (wt%) and degree of organic functionalization (wt%) for Fe@CNTs-NH_2 and Fe@CNTs-Ab. Fe loading was calculated from the residual weight after two consecutive TGA runs (one under N_2 and the following one under air) *via* the following equation:

$$Fe(wt\%) = \frac{(residual\ weight\ \%\ after\ N2\ run * residual\ weight\ \%\ after\ air\ run)}{MW_{(Fe2O3)}} * 2MW_{(Fe)}$$

Where $MW_{(Fe2O3)}$ is the molecular weight of Fe_2O_3 (159.694 g·mol^{-1}) and $MW_{(Fe)}$ is the molecular weight of Fe (55.847 g·mol^{-1})

The degree of organic functionalization was calculated from the first run under N_2 *via* the following equation:

$$Fct\ (wt\%) = residual\ weight\ \%\ at\ 500°C - residual\ weight\ at\ 500°C\ of\ corresponding\ raw\ Fe@CNTs$$

The degree of antibody functionalization was calculated *via* the following equation:

$$Ab\ (wt\%) = Fct\ of\ Fe@CNTs\text{-}Ab\ at\ 500°C\ (wt\%) - Fct\ of\ corresponding\ Fe@CNTs\text{-}NH_2\ at\ 500°C(wt\%)$$