

Article

Carbon dots/iron oxide nanoparticles with tuneable composition and properties

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Abstract: We present a simple strategy to generate a family of carbon dot/iron oxide nanoparticles (C/Fe-NPs) that relies on the thermal decomposition of iron (III) acetylacetonate in the presence of a highly fluorescent carbon-rich precursor, while polyethylene glycol serves as the passivation agent. By varying the molar ratio of the reactants, a series of C/Fe-NPs have been synthesized with tuneable elemental composition in terms of C, H, O, N, Fe. The quantum yield is enhanced from 6% to 9% as the carbon content increases from 27% to 36%, while the room temperature saturation magnetization is improved from 4.1 emu/g to 17.7 emu/g as the iron content is enriched from 17 to 31%. In addition, the C/Fe-NPs show excellent antimicrobial properties, minimal cytotoxicity and demonstrate promising bioimaging capabilities, thus showing great potential for the development of advanced diagnostic tools.

Keywords: magnetization, photoluminescence, carbon dots, magnetic nanoparticles, antimicrobial

1. Introduction

Superparamagnetic iron oxide nanoparticles (Fe-NPs) with sizes below 20 nm have been widely explored in a wide range of biomedical applications, including magnetically controlled gene and drug delivery [1, 2], hyperthermia treatment [3], bioseparation of cells [4], cell tracking and tissue repair [1], magnetic resonance imaging (MRI) [5], biosensors [6], diagnosis and treatment of bacterial infections [7]. Common strategies for the preparation of Fe-NPs rely on thermal decomposition [8], coprecipitation [9], sol-gel methods [10], hydrothermal and solvothermal treatment of iron-rich precursors [5]. Accurate control of the reaction conditions (nature of organometallic reactants, iron salts, surfactants, solvents, temperature, duration), purification protocols and passivation strategies can lead to tailor-made Fe-NPs with narrow size distribution [11].

Given that Fe-NPs are susceptible to oxidation and are particularly prone to agglomeration, surface protection and passivation strategies are essential to impart long-term structural and colloidal stability. To that end, surfactants and macromolecules are physically adsorbed or chemically attached to Fe-NPs, while passivating layers based on precious metals, silica and carbon can accommodate various functional groups, facilitating further conjugation with ligands, biomolecules and nanostructures. An expanding body of literature focuses on Fe-NPs combined with peptides [12], DNA [13], aptamers [14], lipids [15], drug molecules [16], biopolymers [17],

dendrimers [18], rare earth elements [19], metal organic frameworks [20], graphene oxide [21], carbon nanotubes [22] and fullerenes [23].

Particular emphasis is given to the development of hybrid materials based on Fe-NPs that exhibit fluorescent properties stemming from the presence of organic dyes [24], quantum dots [25], graphene quantum dots [26] and carbon dots (C-dots) [27]. In particular, hybrid nanoparticles composed of Fe-NPs and C-dots (C/Fe-NPs) combine the supreme magnetic properties of Fe-NPs with the excellent photoluminescent behaviour and non-toxic nature of C-dots [28, 29] and are ideal candidates for photothermal therapy [30], optical bioimaging [31], dual-modal bioimaging [32], bacteria sensing [33] and antimicrobial treatment [34]. Although, a number of interesting approaches have been proposed for the synthesis of C/Fe-NPs [14, 27, 33, 35-41], the development of environmentally benign, cost and time effective synthetic strategies yielding a versatile range of C/Fe-NPs remains an open challenge.

In this study we demonstrate a facile method to fine-tune the chemical composition and, thereby, the optical and magnetic properties of C/Fe-NPs, thus generating a novel family of multifunctional nanomaterials. We present a simple strategy to synthesise a series of C/Fe-NPs with varying chemical composition that exhibit tuneable magnetization coupled with superior wavelength-dependent optical properties, bioimaging capabilities, non-toxic nature and antimicrobial activity.

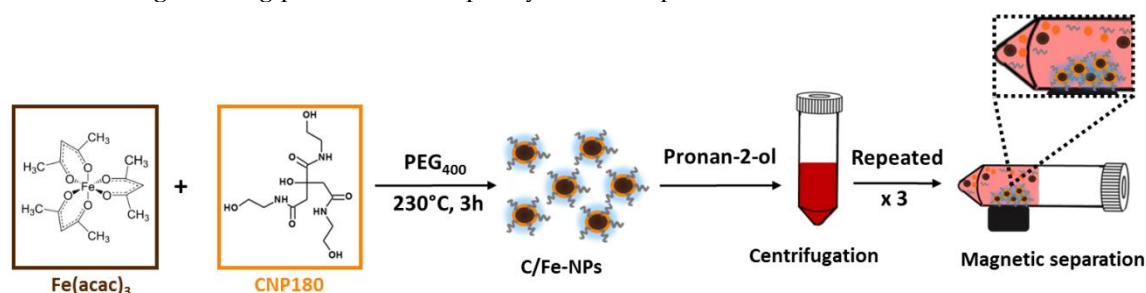
2. Materials and methods

2.1. Materials

Iron (III) acetylacetonate $\geq 99.9\%$ trace metal basis ($\text{Fe}(\text{acac})_3$) and ethanolamine $\geq 98\%$ (EA) were purchased from Sigma Aldrich. Polyethylene glycol 400 (PEG_{400}), citric acid monohydrate 99.5% (CA), propan-2-ol 99% and diphenyl ether 99.5% were obtained from Alfa Aesar.

2.2. Synthesis of magnetic C-dots

Magnetic C-dots were synthesized by thermal decomposition of iron and carbon precursors dispersed in the high boiling point solvent diphenyl ether, as presented in Scheme 1.



Scheme 1. Schematic representation of the process followed for the synthesis of C-Fe/NPs.

Highly photoluminescent C-dots precursor (CNP180) was synthesized via thermal treatment of CA and EA (molar ratio 3:1) at 180°C , in accordance with previously reported method [42]. Subsequently, various amounts of iron (III) acetylacetonate (2.1, 1.9, 1.7, 1.6, 1.5 and 1.1 mmol) were added into CNP180 along with 7.5 mmol of polyethylene glycol 400 and 20 mL of diphenyl ether. Subsequently, the mixture of precursors was heated under reflux with constant stirring for 3 hours at 230°C , to facilitate the formation of an amorphous coating of CNP230 on the iron oxide-based centres. The precipitate was purified by adding 40 mL of propan-2-ol to the mixture, followed by centrifugation at 8000 rpm for 10 min and this step was repeated three times. The C/Fe-NPs thus received were dispersed in distilled water, left for 15 min with a magnet attached to the vial's wall and the particles not attracted by the magnet were discarded. Finally, all samples were freeze-dried and afterwards stored under ambient conditions.

2.3. Characterization

Elemental analysis was conducted with a CHNS Elemental Analyzer (Flash 2000) equipped with a column for oxygen determination that was calibrated against 2,5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl)thiophene (Thermo Scientific, UK). The carbon, hydrogen, nitrogen analysis was carried out in aluminium pans, while oxygen analysis in silver pans (both types of pans were received from CE Instruments).

Magnetization curves of the solid samples were obtained at temperatures of 296 and 70 K by using a low temperature Faraday balance magnetometer with a continuous flow cryostat. More detailed magnetic measurements were carried out on selected samples at the VSM option of the Quantum Design PPMS apparatus with the oscillation frequency of 40 Hz in the temperature range from 4 to 300 K. The zero field cooled (ZFC) and field cooled (FC) magnetic susceptibility curves were measured in the magnetic field of 100 Oe. The FC data was collected on cooling the nanoparticles in the desired field, whereas the ZFC magnetic susceptibilities were measured after cooling the samples in zero applied field to 4 K, then applying the magnetic field of 100 Oe and recording the data while slowly warming the nanoparticles up to 300 K. The mass specific magnetization and magnetic susceptibility values are reported.

Transmission Electron Microscopy (TEM) images were recorded by the FEI T12 Spirit TEM operated at 120 kV. A droplet of 0.05 mg/mL aqueous suspension was deposited on a carbon coated copper grid (Agar Scientific, USA) and dried under air.

Fourier Transform Infrared (FTIR) spectra were obtained at Nicolet IS5 spectrometer (Thermo Fisher Scientific, USA) within the range of 4000-500 cm⁻¹. For each measurement, the samples were scanned 128 times at a resolution of 2 cm⁻¹.

X-ray diffraction (XRD) patterns were recorded by Bruker D2 Phaser diffractometer equipped with a Lynxeye 1-dimensional detector using Cu K α radiation. Similar volumes of C/Fe-NPs were scanned for 60 minutes in the 2 θ range of 5-80°.

Thermogravimetric analysis (TGA) measurements were carried out using TGAQ500 instrument (TA Instruments, UK). Samples were heated over the range of 25-500°C at a heating rate of 10°C/min, under continuous nitrogen flow.

Ultraviolet-visible (UV-Vis) spectra of aqueous dispersions of C/Fe-NPs placed in Hellma Analytics quartz cuvette with 1.0 cm pathlength were recorded at room temperature by means of a UV-3600 spectrophotometer (Shimadzu, USA).

Photoluminescence (PL) spectra of aqueous dispersions of C/Fe-NPs were recorded at room temperature by Horiba Fluoromax spectrofluorometer and the samples (also placed in Hellma Analytics quartz cuvette with 1.0 cm pathlength) were excited at wavelengths between 275-500 nm, with 25 nm increment.

The quantum yields (QY) of C/Fe-NPs dispersions were determined using anthracene (Sigma Aldrich, QY_R=0.27 at λ_{ex} =365 nm) as a standard reference dye and calculated from the Equation:

$$QY = QY_R \times \left(\frac{m}{m_R} \right) \times \left(\frac{\eta^2}{\eta_R^2} \right) \quad (1)$$

where QY_R is the quantum yield of the reference dye, m and m_R refer to the slope calculated from a linear regression of the tested material and the reference dye, A and A_R are the absorbance values of tested material and the reference dye, η and η_R are the refractive indexes of the solvents used to disperse the tested material and the reference dye, respectively. Anthracene was dissolved in ethanol (η =1.36) while the examined samples were dispersed in ultra-pure water (η_R =1.33). To minimize re-absorption effects, absorbance values were kept below 0.10.

Photoluminescence lifetime decays Photoluminescence lifetime decays were measured by Edinburgh Instruments LifeSpec-II equipped with two high-repetition rate picosecond pulsed diode lasers operating at 450 nm (EPL-450) and 375 nm (EPL-375). Time-correlated Single Photon Counting (TCSPC) data acquisition unit processed the detector signals. The PL lifetime decays were recorded with 10,000 peak counts for the aqueous solutions transferred into quartz cuvette (1 cm pathlength) at room temperature over 200 ns time range. The same parameters were kept for recording the instrument response function (IRF), while using dilute aqueous dispersion of

colloidal silica (Ludox HS-30, Sigma Aldrich). The average PL lifetimes (τ_{avg}) were calculated from the Equation:

$$\tau_{\text{avg}} = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} \quad (2)$$

where τ_i is the time component of multiexponential decay fitting and α_i is the fractional weight for each time component.

X-ray photoelectron spectroscopy (XPS) was performed using SPECS spectrometer equipped with Al K α X-ray radiation source (emitting photons 1486.64 eV) and a hemispherical analyzer (PHOIBOS 150) set to the pass energy of 20 eV, while the XPS system base pressure was 2×10^{-9} Pa. The XPS peaks were resolved by curve fitting (with the use of CASA XPS® software) with a sum of Gaussian (70%) and Lorentzian (30%) lines. The secondary electron background was subtracted following the Shirley procedure.

Cytotoxicity of C/Fe-NPs against epithelial HeLa cervix cancer cell line was evaluated by the standard MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay (Sigma Aldrich). The HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific) supplemented with 10% Fetal Bovine Serum (Thermo Fisher Scientific) and 1% Penicillin-Streptomycin (10,000 U/mL, Thermo Fisher Scientific). At first, 50 μ L of cell suspensions with the density 1×10^5 cells/mL were seeded in a 96-well plate and left overnight in the incubator (37°C, 5% CO₂), in order to facilitate the adhesion of the cells to the bottom of each well. After 24 h, the cells were incubated with C/Fe-NPs (10-200 μ g/mL in DMEM) for 24 h. Then, 20 μ L of 5 mg/mL MTT solution was transferred into each well and was further incubated for 2 h. Afterwards, 150 μ L of 0.7M lysis buffer was added into each well to liquefy the formazan crystals. Finally, the optical density (OD) of tested materials was measured by a microplate reader (Thermo Scientific, UK) with a set up wavelength at 595 nm. Cell viability was calculated via the Equation:

$$\text{Cell viability [\%]} = \frac{\text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100 \quad (3)$$

where OD_{control} and OD_{treated} were recorded in the absence and the presence of C/Fe-NPs, respectively. All assays were repeated at least in triplicates and an average value was used for calculations.

In vitro fluorescence imaging was done for the HeLa cells cultured in DMEM supplemented with 10% FBS (v/v) and 1% Penicillin-Streptomycin (v/v) at 37°C (5% CO₂, 95% air) for 24 h. Subsequently, liquid dispersions 50 μ g/mL C/Fe-NPs were added into each well with the HeLa cells and incubated for further 24 h. Afterwards, the media was aspirated and the cells were washed three times with PBS buffer (pH 7.2). The fluorescence imaging in live cells was performed with the use of Zeiss Axio Scope A1 microscope equipped with band-pass filters. The internalisation of fluorescent nanoparticles was investigated under a bright field as well as with the use of 366 nm, 488 nm and 512 nm excitation wavelengths.

Antimicrobial studies. The culturing method has been described elsewhere [43]. Briefly, an inoculated nutrient broth with a single loop of either Escherichia coli (*E.coli*) or Staphylococcus aureus (*S. aureus*) was incubated for 24 h at 37°C, while using SciQuip Incu-Shake MIDI orbital shaker (SciQuip Ltd, Newtown, Wem, Shropshire, UK) set as 200 rpm. Afterwards, the bacteria cultures were centrifuged twice, followed by the disposal of supernatant. Then, the cultures were resuspended and further diluted in nutrient broth, in order to get absorbance values equivalent to a 0.5 McFarlane standard, as measured by Biochrom WPA S800 visible spectrophotometer.

The method used for antimicrobial testing required to disperse 0.1g of C/Fe-NPs in 1mL nutrient broth, which had to be further autoclaved. Afterwards, 1 mL of tested bacterial strain and 8 mL sterile nutrient broth were added into each dispersion and the mixture was incubated for 24 h at 37°C under 200 rpm. Subsequently, 1 mL aliquot was taken from each suspension and further serially diluted by transferring 100 μ L of it into 900 μ L Ringer's solution with ¼ strength. The dilution process was repeated until the number of bacterial colonies fall between 30 to 300. Subsequently, 100 μ L of each one from the series of diluted suspension was spread onto agar-gelled nutrient plates, with the use of sterile plastic spreader. In the final step, the agar plates were

incubated at 37°C for 24 h and the number of bacterial colonies was estimated. The comparison with the control plate allowed to calculate the overall percentage of bacterial colonies decrease accordingly to the following Equation:

$$\text{Bacterial colony decrease [\%]} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100 \quad (4)$$

Experiments for each type of C/Fe-NPs were performed in triplicates and the average values are reported.

3. Results and discussion

It has been previously demonstrated that pyrolysis at 230°C of the fluorescent precursor CNP180 (derived via thermal treatment of EA and CA at 180°C for 30 min as discussed in the experimental section) leads to the formation of well-defined spherical NPs (referred hereafter as CNP230) with average size 19 nm [42]. In this study CNP180 was subjected to pyrolysis at 230°C, but in the presence of varying amounts of $\text{Fe}(\text{acac})_3$ and upon the addition of PEG_{400} in order to synthesise a series of well-defined C/Fe-NPs. Preliminary work indicated that both the PL intensity (Supplementary Information, Figure S1) and the crystallinity (Supplementary Information, Figure S2) of the C/Fe-NPs are optimised following 3 hours of pyrolytic treatment, thus this pyrolysis time was kept constant for all experiments described here. TGA analysis suggests that $\text{Fe}(\text{acac})_3$ abruptly decomposes at temperatures around 230°C (loss weight 91% at 236°C), while CNP180 and PEG_{400} are much less prone to degradation at this temperature region (Figure 1a).

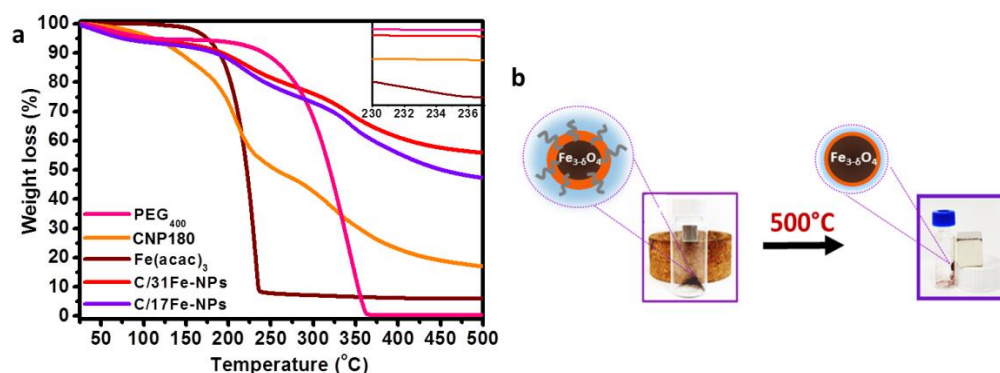
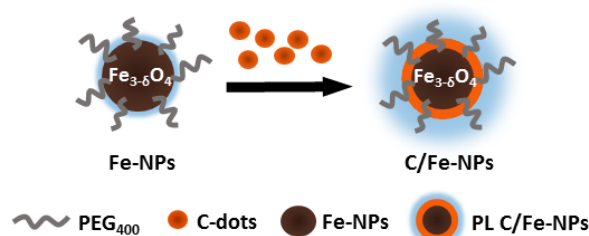


Figure 1. (a) TGA curves of C/17Fe NPs, C/31Fe NPs, PEG_{400} , CNP180 and $\text{Fe}(\text{acac})_3$; (b) Photos demonstrating the magnetic nature of C/17Fe NPs prior (left image) and after (right image) the TGA scan presented in a.

The TGA profiles point to the early formation of iron-rich nuclei and the subsequent formation of carbon-rich corona (Scheme 2). Interestingly, C/17Fe-NPs continue being attracted by the magnet even after the completion of the TGA scan (up to 500°C) (Figure 1b).



Scheme 2. Scheme presenting the build-up of C/Fe-NPs

On the basis of this approach and by varying the weight ratio between $\text{Fe}(\text{acac})_3$ and CNP180 a series of C/Fe-NPs was synthesised. Elemental analysis (Table 1) suggested that the iron content of the C/Fe-NPs varies between 17 and 31 wt%; hereafter the notation C/17Fe-NPs is used to indicate

17% iron content, C/31Fe-NPs to indicate 31% iron content, etc. At the same time, the carbon content monotonically decreases from 36.3% for C/17Fe-NPs to 26.9% for C/31Fe-NPs. Within this family of materials, the oxygen, nitrogen and hydrogen content is between 34.7-36.2%, 3.9-6.5% and 3.6-4.3%, respectively.

Table 1. Elemental analysis of C/Fe-NPs

	C	H	N	O	Fe
CNP230	43.30	5.39	9.03	42.28	----
C/17Fe-NPs	36.30	4.66	6.50	35.16	17.38
C/23Fe-NPs	31.45	4.29	5.38	36.11	22.77
C/24Fe-NPs	31.03	4.16	4.86	36.16	23.79
C/27Fe-NPs	28.90	3.97	4.33	35.87	26.93
C/31Fe-NPs	26.85	3.62	3.85	34.68	31.00

A major advantage of our synthetic strategy is a facile one-pot and single-step fabrication procedure, which have potential to be easily scale up and modified. In contrast, previous reports refer to tedious multistep synthetic procedures [14, 27, 33, 35-41].

The TEM image of C/17Fe-NPs (Figure 2a) reveals the presence of nanoparticles with centre-coating structure. In addition, C/17Fe-NPs demonstrate a relatively high level of homogeneity with an average particle size 6.4 nm (Figure 2b). The good separation between particles might be attributed to the presence of the carbogenic shell and the passivating role of PEG₄₀₀ layer.

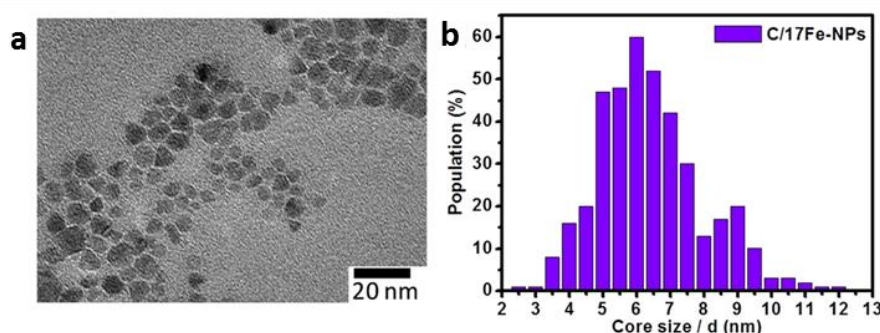


Figure 2. (a) TEM image with (b) size histogram of C/17Fe-NPs.

The XPS survey spectrum of C/31Fe-NPs (Figure 3a) indicates a very low iron content on the surface, thus confirming that iron is predominantly located on the centre. The high-resolution C1s spectra of C/31Fe-NPs (Figure 3b) can be deconvoluted into three peaks located at 284.1eV, 285.7eV and 287.5eV that correspond to C-C/ C=C, C-N/ C=N, C-O/ C=O, respectively [31, 35, 44]. The high-resolution Fe2p spectra of C/31Fe-NPs (Figure 3c) demonstrated the occurrence of Fe2p_{3/2} and Fe2p_{1/2} peaks centered around 722 eV and 711 eV, which point to Fe³⁺ and Fe²⁺ oxidation states, respectively [32, 35, 44, 45]. The high-resolution O1s spectra of C/31Fe-NPs (Figure 3d) can be deconvoluted into two peaks located around 531eV and 529 eV, which further confirm the presence of carboxyl groups and iron metal oxides, respectively. Similar results were obtained for other C/Fe-NPs.

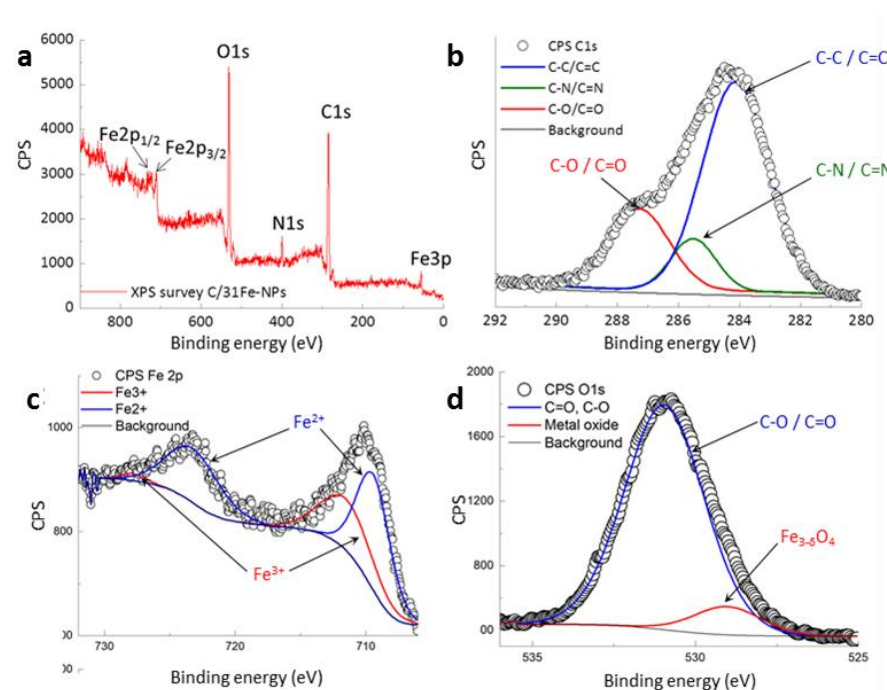


Figure 3. (a) The XPS survey together with deconvoluted (b) C1s, (c) Fe2p and (d) O1s XPS spectra of C/31Fe-NPs. Circular points refer to the data collected, while the solid lines refer to the fitted curves.

XRD patterns (Figure 4a) for C/31Fe-NPs, C/27Fe-NPs and C/24Fe-NPs show well-defined diffraction peaks at $2\theta = 30.02^\circ$, 35.14° , 42.96° , 53.50° , 56.93° and 62.63° attributed to the (220), (311), (400), (422), (511) and (440) crystal planes of spinel-type (magnetite/maghemite) phase, respectively. Those crystalline peaks appear much weaker for C/17Fe-NPs and C/23Fe-NPs, as expected due to their lower iron content. As shown in Figure 4b-f, only C/31Fe-NPs, C/27Fe-NPs and C/24Fe-NPs are strongly attracted by a magnet, while C/23Fe-NPs and C/17Fe-NPs fail to do so. The FTIR spectra of C/Fe-NPs (Figure 4g) show the presence of peaks centred around 588 cm^{-1} (Fe-O stretching vibration), 1054 cm^{-1} (C-OH stretching vibration), $1232\text{--}1352\text{ cm}^{-1}$ (C-O and C-N stretching vibrations), 1750 cm^{-1} (C=O stretching vibration), 2900 cm^{-1} (C-H symmetric and asymmetric stretching vibrations) and 3240 cm^{-1} (N-H and O-H stretching vibrations) [14, 31, 35, 36, 44, 46]. The peaks in the range of $1540\text{ to }1697\text{ cm}^{-1}$ are associated with the C=O stretching and N-H in-plane bending vibrations of amide I and II absorption band, respectively [32, 36, 37, 44, 47]. The FTIR spectroscopy confirms the presence of oxygen-rich groups on the C/Fe-NPs surface that render the particles dispersible in polar media.

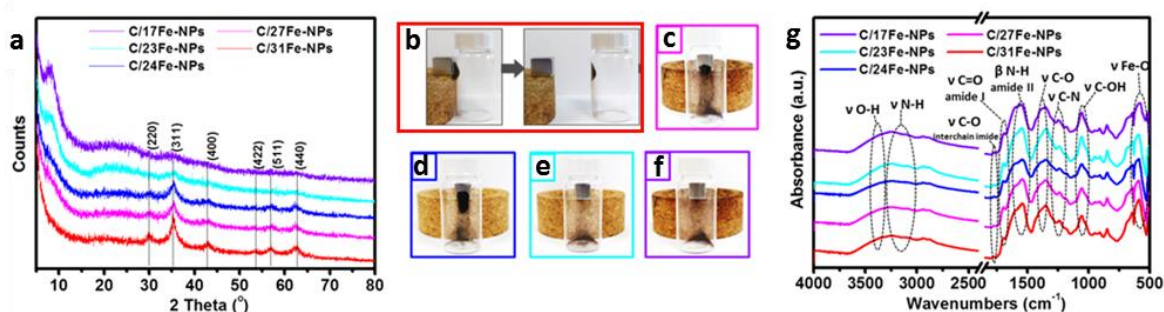


Figure 4. (a) XRD patterns of C/Fe-NPs. The photos of (b) C/31Fe-NPs, (c) C/27Fe-NPs, (d) C/24Fe-NPs, (e) C/23Fe-NPs, (f) C/11Fe-NPs in the presence of an external magnet. (g) FTIR spectra of C/Fe-NPs.

Figure 5 shows typical ZFC and FC magnetic susceptibilities recorded from selected C/Fe-NP samples in magnetic field of 100 Oe. The data obtained in the ZFC mode show broad peaks

signifying a transition from the low temperature blocked state to the superparamagnetic regime at high temperatures [48]. The blocking temperatures (T_B) corresponding to the maxima in the ZFC curves are higher for samples with larger Fe content, in agreement with the conjecture that those samples contain bigger Fe-NPs which are expected to become superparamagnetic at higher temperatures [49].

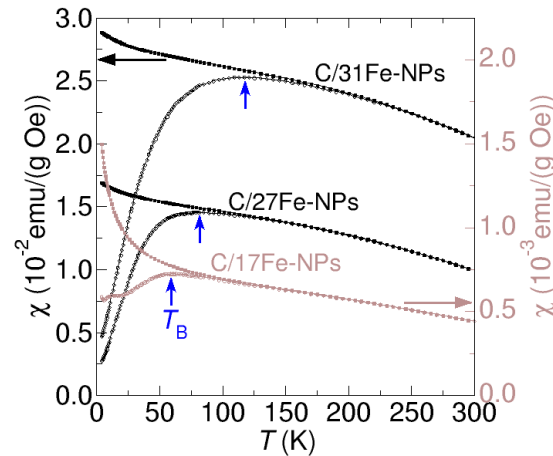
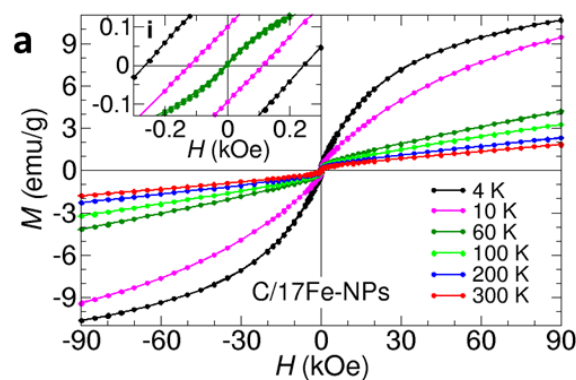


Figure 5. Temperature dependencies of magnetic susceptibilities for selected C/Fe-NPs measured in the ZFC (empty dots) and FC (filled dots) modes using the applied magnetic field of 100 Oe. Data shown in black was plotted on the left vertical axis, whereas brown data points are using the brown vertical axis located on the right-hand side. The blocking temperatures are indicated by vertical blue arrows.

The isothermal magnetization measurements shown in Figure 6 further indicate that C/Fe-NPs exhibit a predominant superparamagnetic behaviour close to the room temperature. Upon cooling, onsets of hysteresis loops are observed in the $M(H)$ curves at temperatures between 150 and 50 K indicating transitions to the blocked state. As shown in insets i of Figure 6, the coercivity increases with decreasing temperature, reaching values of 0.25-0.30 kOe at 4 K.

The magnetization of C/Fe-NPs does not reach saturation even at 90 kOe and 4 K. Therefore, the saturation magnetization (M_s) was estimated by extrapolating the magnetization versus $1/H$ to the limit when the inverse magnetic field approaches zero. The resulting M_s values for selected C/Fe-NPs are shown in the inset ii of Figure 6c. When calculated per gram of $\text{Fe}_{3-δ}\text{O}_4$ ($0 \leq \delta \leq 0.33$), the M_s values at 4 K fall within 50-56 $\text{emu/g-Fe}_{3-δ}\text{O}_4$ and are smaller than 90 and 84 emu/g expected for bulk magnetite and maghemite, respectively [50]. We note that substantially reduced M_s is characteristic for Fe-NPs with sizes in the range of few nanometres and was attributed to the presence of disordered spins on the surface of spinel cores [48, 51-58]. The saturation magnetization of C/Fe-NPs decreases upon heating, signifying thawing of the surface spins [59-61].



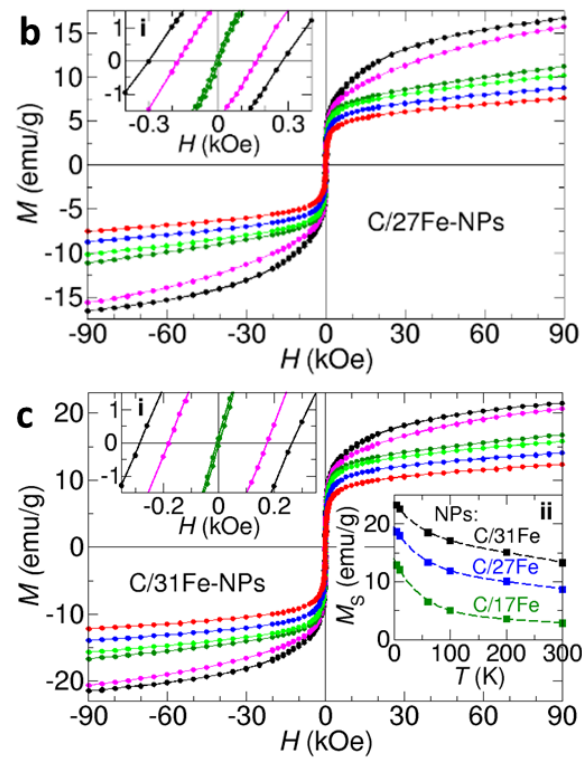


Figure 6. Magnetic hysteresis loops recorded at temperatures of 4 K (black dots), 10 K (pink dots), 60 K (dark green dots), 100 K (green dots), 200 K (blue dots) and 300 K (red dots) from C/17Fe-NPs (panel a), C/27Fe-NPs (panel b), and C/31Fe-NPs (panel c). Insets i show expanded M - H plots near the origin. Inset ii of panel C presents the saturation magnetization as a function of temperature for C/17Fe-NPs (black squares), C/27Fe-NPs (blue squares), and C/31Fe-NPs (dark green squares), together with fits using Equation 5 (dashed lines).

As illustrated in the inset ii of Figure 6c, the temperature dependencies of M_s can be well described by the phenomenological formula [61, 62].

$$M_s(T)/M_s(0) = (1 - B T^{3/2}) + A \exp(-T/T_f) \tag{5}$$

in which the first term accounts for a Bloch-like variation of magnetization expected for magnetically ordered core moments whereas the second term represents the effect of freezing of disordered surface spins in a spin-glass-like state at temperatures below T_f . Fitting Eq. (5) to the $M_s(T)$ data resulted in parameters collated in Table 2. The obtained values of the Bloch constant B are similar to those previously reported [62, 63] for NPs of Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ with comparable sizes. The A constant scales with the relative amount of surface spins compared to magnetically ordered core moments. Therefore, the observed systematic increase in the A values as the Fe content is reduced indicates a growing fraction of surface spins, in line with an increasing surface-to-volume ratio expected when spinel NPs are getting smaller. Simultaneously, the saturation magnetization ascribed to the magnetically ordered spinel cores dwindles, providing a further indication for a decrease in sizes of Fe-centres as the Fe content is reduced.

Table 2. Magnetic parameters derived from least-squares fits of the $M_s(T)$ data shown in the inset ii of Figure 6c with Equation 5

Sample	A (-)	B ($10^{-5} \text{ K}^{-3/2}$)	T_f (K)	$M_s(0)$ (emu/g)
C/31Fe-NPs	0.42 ± 0.05	4.0 ± 0.2	52 ± 2	17.7 ± 0.5
C/27Fe-NPs	0.75 ± 0.08	4.2 ± 0.2	53 ± 2	11.7 ± 0.4
C/17Fe-NPs	2.57 ± 0.15	5.2 ± 0.3	49 ± 2	4.1 ± 0.1

The UV-Vis spectra of magnetic C-dots (Supplementary Information, Figure S3a) reveals the presence of absorption peaks at 270 nm and a hump around 320 nm, which are attributed to π - π^* electron transitions of polyaromatic chromophores as well as n - π^* electron transitions of carbonyl (C=O) and amine (C-N) functional groups [32, 36] [30, 41]. Although the absorptivity of aqueous dispersions of C/Fe-NPs are rather low [36, 44, 64, 65], their aqueous dispersions display a strong blue luminescence under the UV light (Supplementary Information, Figure S3b). The fluorescence spectra of C/31Fe-NPs and C/17Fe-NPs (Figure 7) show an excitation wavelength independent contribution ($\lambda_{em}=455$ nm) that has been attributed to the presence of citrazinic acid and an excitation wavelength dependent emission at higher λ_{ex} that is characteristic for carbogenic NPs [42]. This fluorescent mode arises from surface emission states, edge defects, self-trapped excitons, crosslink enhanced emission, quantum confinement in the context of sp^2 islands within a sp^3 matrix [14, 31, 32, 36, 45]. Typically C-dots exhibit the strongest PL within the blue region [66], but red-shifted emission is achieved via extensive π -conjugated domains, high levels of surface oxidation and incorporation of heteroatoms such as N, S, P [67].

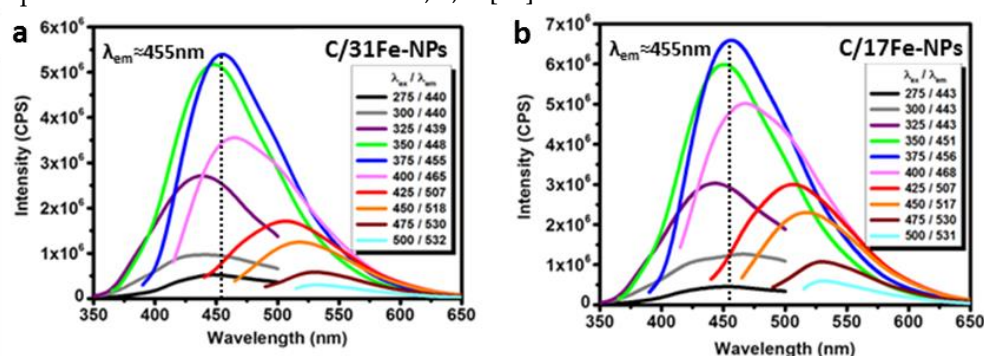


Figure 7. PL spectra of aqueous dispersions of (a) C/31Fe-NPs and (b) C/17Fe-NPs.

As shown in Supplementary Information Figure S4, the QY at $\lambda_{ex}=365$ nm increases with carbon content from 6% for C/31Fe-NPs, C/27Fe-NPs, C/24Fe-NPs to 7% for C/23Fe-NPs and 9% for C/17Fe-NPs. Those values are consistent with previous studies that reported QY ranging from 4.6 to 8% for related systems [30, 44, 45]. The time-resolved PL lifetime decays at $\lambda_{ex}=375$ nm (Supplementary Information, Figure S5) and 450 nm (Supplementary Information, Figure S6) of aqueous dispersions of C/Fe-NPs follow complex multi-exponential decay profiles, in agreement with previous studies [36]. The average PL lifetimes (τ_{avg}) of C/Fe-NPs are ranging from 12.43 ns up to 12.80 ns (Supplementary Information, Figure S5) compared to $\tau_{avg}=13.06$ ns for CNP230 (Supplementary Information, Figure S7), $\tau_{avg}=1.5$ -4 ns for the auto fluorescence of the cells, $\tau_{avg}=1$ -5 ns for organic dyes and $\tau_{avg}=6.4$ ns for citrazinic acid. Those data indicate a weak dependence on the iron content and a rather limited effect of the magnetic core on the microenvironment of the excited states within the carbogenic corona. The larger τ_{avg} observed for C/Fe-NPs carry promise for cellular labelling, tissue analysis and microfluidic devices [68].

The viability of HeLa cancer cells incubated with various concentrations of C/Fe-NPs (as estimated using standard MTT assays) is shown in Figure 8. At the highest concentration studied (200 μ g/mL) the viability of Hella cells was found to be 95% for C/17Fe-NPs, 91% for C/23Fe-NPs, 90% for C/24Fe-NPs, 87% for C/28Fe-NPs, and 86% C/31Fe-NPs. For comparison the viability of Hela cells incubated with 200 μ g/mL of CNP230 (Supplementary Information, Figure S8) and Fe-NPs is 97 and 61%, respectively. In principle, the cytotoxicity of NPs critically depends upon the nature of the surface functional groups [69]. Previous studies demonstrated the low toxicity of C-dots derived from CA and EA [36], in contrast to the toxic nature of heavy-metal based quantum [70] and the cell type-specific activity of the Fe-NPs [71, 72].

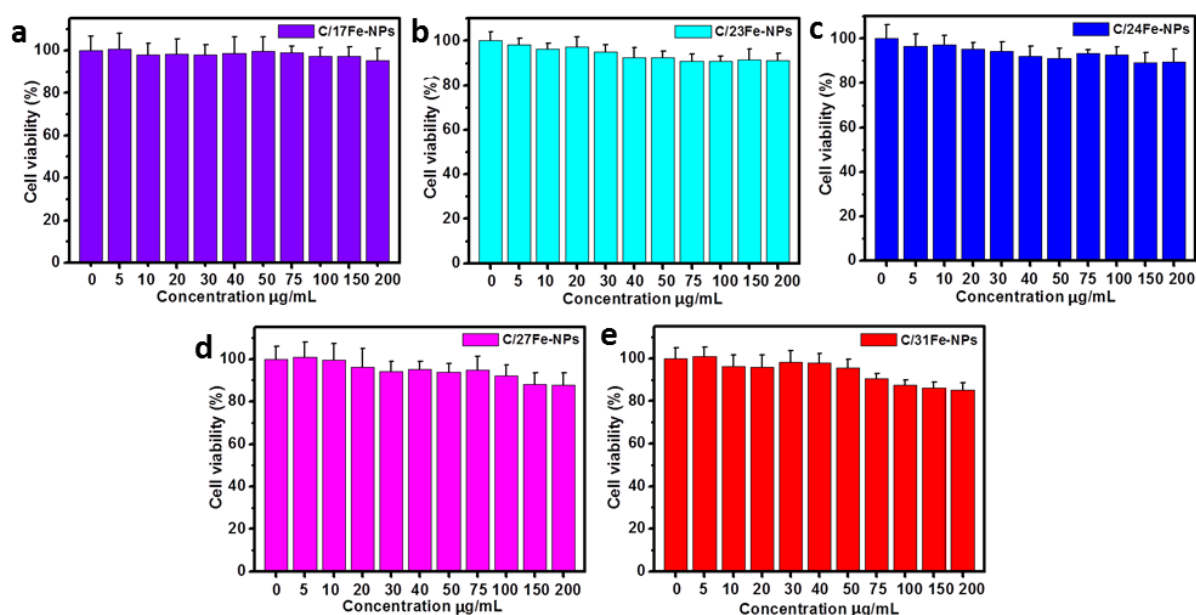


Figure 8. The viability of HeLa cell line calculated from MTT assay after their incubation for 24 hours with (a) C/17Fe-NPs, (b) C/23Fe-NPs, (c) C/24Fe-NPs, (d) C/27Fe-NPs, (e) C/31Fe-NPs.

Fluorescent microscope images of HeLa cell line incubated with 50 µg/mL of C/31Fe-NPs (Figure 9a) and C/17Fe-NPs (Figure 9b) for 24 h indicated that C/Fe-NPs penetrate the cells and are predominantly accumulated in the cytoplasm [37]. When illuminated with blue, green and red radiation, the internalised C/Fe-NPs can reveal morphological information regarding both the cytoplasm and the membranes.

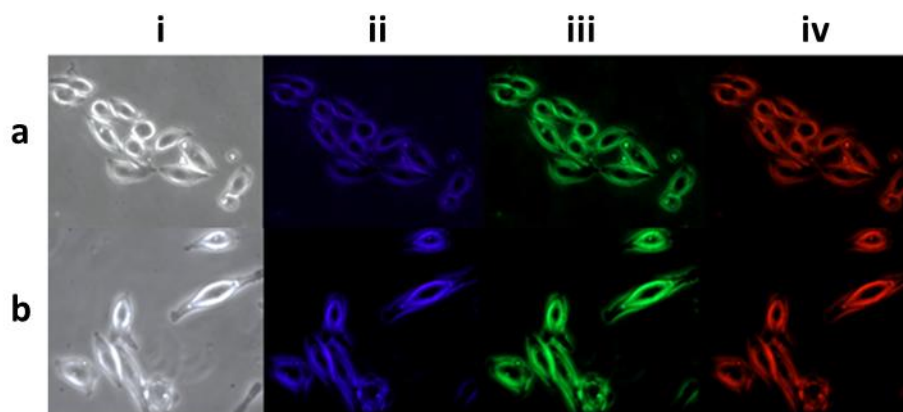


Figure 9. Fluorescent microscope images of HeLa cell line incubated with 100 µg/mL of (a) C/31Fe-NPs and (b) C/17Fe-NPs under the bright field (i) as well as the UV (ii), blue (iii) and green (iv) excitation wavelengths.

Overall C/Fe-NPs with low cytotoxicity and superior fluorescent properties act as efficient surveillance probes, while having the added advantage of being able to be directed via an external magnetic field to targeted destinations [32].

Finally, the antimicrobial activities of CNP230 and C/Fe-NPs were tested against *S. aureus* and *E. coli* (a representative Gram-positive and Gram-negative strain, respectively). As summarised in Table 3, C/Fe-NPs demonstrate excellent antimicrobial activity after incubation with bacterial species for 24 h at 37°C, causing 99.0-99.9% and 99.5-99.9% reduction in *E.coli* and *S.aureus* colonies, respectively. Very similar performance was observed for CNP230 as well as for a gallery of C-dots based materials [73], while Fe-NPs synthesized as a control sample via the same synthetic approach but without the carbogenic coating displayed 91.4% and 99.9% decrease in *E.coli* and *S.aureus* under

identical conditions, respectively [34, 74]. The data suggest that C/Fe-NPs could be viewed as highly reliable antimicrobial agents, able to combat bacterial contamination, preventing infectious diseases [75].

Table 3. Antimicrobial performance of CNP230 and C/Fe-NPs

Material	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
	% decrease	% decrease
	24h	24h
CNP230	99.9	99.9
C/Fe17-NPs	99.9	99.9
C/Fe23-NPs	99.9	99.9
C/Fe24-NPs	99.9	99.9
C/Fe27-NPs	99.0	99.5
C/Fe31-NPs	99.5	99.9

4. Conclusions

The study presents a facile strategy to fine-tune the structure and the chemical composition and, thereby, the optical and magnetic properties of C/Fe-NPs. A systematic investigation within this series of materials indicated that the C/17Fe-NPs with carbon content 36 wt% and iron content 17% show QY close to 9% and magnetization 4.1 emu/g, while C/31Fe-NPs with carbon content 27% and iron content 31% show QY 6 % and magnetization 17.7 emu/g (Figure 10). C/Fe-NPs are non-toxic materials that can be used as advanced diagnostic tools, while showing excellent antimicrobial activity against *E.coli* and *S.aureus*.

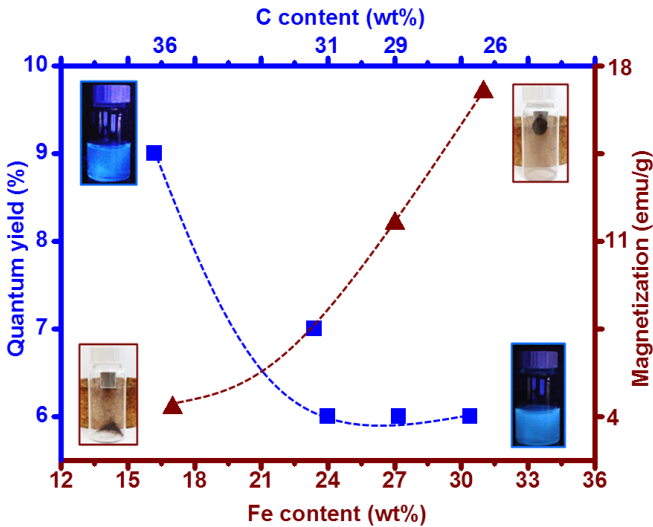


Figure 10. Quantum yield and magnetization of a series of C/Fe-NPs with varying C and Fe contents.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1 , Figure S1: (a) The PL spectra (λ_{ex} = 375 nm) of aqueous dispersions of C/Fe-NPs prepared from identical reactant mixtures with C/31Fe-NPs, but at various times of pyrolysis (b). Figure S2. (a) XRD patterns of C/Fe-NPs prepared from identical reactant mixtures with C/31Fe-NPs but at various times of pyrolysis; (b-g) Photos depicting various magnetic properties in the presence of external magnet. Figure S3. (a) Absorption spectra of aqueous dispersions of 0.01 mg/mL C/Fe-NPs; (b) Photos of C/31Fe-NPs (red), C/24Fe-NPs (blue) and C/17Fe-NPs (purple) under daylight and ultraviolet light. Figure S4. Integrated PL intensity of (a) C/17Fe-NPs, (b) C/23Fe-NPs, (c) C/24Fe-NPs, (d) C/27Fe-NPs and (e) C/31Fe-NPs in water as a function of optical absorbance at 365 nm. Figure S5. Time-resolved fluorescence decay profiles for aqueous solutions of (a) C/17Fe-NPs, (b) C/23Fe-NPs, (c) C/24Fe-NPs, (d) C/27Fe-NPs, (e) C/31Fe-NPs (E) at λ_{ex} = 375nm. Figure S6. Time-

resolved fluorescence decay profiles for aqueous solutions of (a) C/17Fe-NPs, (b) C/23Fe-NPs, (c) C/24Fe-NPs, (d) C/27Fe-NPs, (e) C/31Fe-NPs at λ_{ex} = 450 nm. Figure S7. Time-resolved fluorescence decay profiles for aqueous solutions of CNP230 recorded at λ_{ex} = 375 nm (grey colour) and λ_{ex} = 450 nm (dark yellow colour). Figure S8. (a) The MTT assay results for HeLa cells incubated with CNP230 for 24 h; (b) The fluorescence microscope images of HeLa cells with internalised CNP230.

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References

- Demirer, G.S.; Okur, A.C.; Kizilel, S. Synthesis and Design of Biologically Inspired Biocompatible Iron Oxide Nanoparticles for Biomedical Applications. *J. Mater. Chem. B* **2015**, *3*, 7831-7849.
- Shen, L.; Li, B.; Qiao, Y. Fe₃O₄ Nanoparticles in Targeted Drug/Gene Delivery Systems. *Materials* **2018**, *11*, 324-353.
- Xie, L.; Jin, W.; Zuo, X.; Ji, S.; Nan, W.; Chen, H.; Gao, S.; Zhang, Q. Construction of small-sized superparamagnetic Janus nanoparticles and their application in cancer combined chemotherapy and magnetic hyperthermia. *Biomater. Sci.* **2020**, *8*, 1431-1441.
- Lu, A.H.; Salabas, E.L.; Schuth, F. Magnetic Nanoparticles: Synthesis, Protection, Functionalization, and Application. *Angew. Chem. Int. Ed.* **2007**, *46*, 1222–1244.
- Lee, N.; Yoo, D.; Ling, D.; Cho, M.H.; Hyeon, T.; Cheon, J. Iron Oxide Based Nanoparticles for Multimodal Imaging and Magnetoresponse Therapy. *Chem. Rev.* **2015**, *115*, 10637–10689.
- Wu, W.; Jiang, C.Z.; Roy, V.A.L. Designed synthesis and surface engineering strategies of magnetic iron oxide nanoparticles for biomedical applications. *Nanoscale* **2016**, *8*, 19421-19474.
- Xu, C.; Sun, S. Applications of Iron Oxide-Based Magnetic Nanoparticles in the Diagnosis and Treatment of Bacterial Infections. *Front. Bioeng. Biotechnol.* **2019**, *7*, 141.
- Sun, S.; Zeng, H. Size-Controlled Synthesis of Magnetite Nanoparticles. *J. Am. Chem. Soc.* **2002**, *124*, 8204-8205.
- Lobato, N.C.C.; Mansur, M.B.; Ferreira, A.M. Characterization and Chemical Stability of Hydrophilic and Hydrophobic Magnetic Nanoparticles. *Mater. Res.* **2017**, *20*, 1-11.
- Itoh, H.; Sugimoto, T. Systematic control of size, shape, structure, and magnetic properties of uniform magnetite and maghemite particles. *J. Colloid Interface Sci.* **2003**, *265*, 283-295.
- Effenberger, F.B.; Couto, R.A.; Kiyohara, P.K.; Machado, G.; Masunaga, S.H.; Jardim, R.F.; Rossi, L.M. Economically attractive route for the preparation of high quality magnetic nanoparticles by the thermal decomposition of iron(III) acetylacetonate. *Nanotechnology* **2017**, *28*, 115603-115611.
- Shen, W.Z.; Cetinel, S.; Sharma, K.; Borujeny, E.R.; Montemagno, C. Peptide-functionalized iron oxide magnetic nanoparticle for gold mining. *J. Nanopart. Res.* **2017**, *19*, 1-12.
- Kumar, A.; Jena, P.K.; Behera, S.; lockey, R.F.; Mohapatra, S. Multifunctional magnetic nanoparticles for targeted delivery. *Nanomed.: Nanotechnol. Biol. Med.* **2010**, *6*, 64-69.
- Jin, H.; Gui, R.; Sun, J.; Wang, Y. Facile self-assembled magnetic nanoparticles/apptamer/carbon dots nanocomposites for highly sensitive up-conversion fluorescence turn-on detection of tetrodotoxin. *Talanta* **2018**, *176*, 277–283.
- Liang, J.; Zhang, X.; Miao, Y.; Li, J.; Gan, Y. Lipid-coated iron oxide nanoparticles for dual-modal imaging of hepatocellular carcinoma. *Int. J. Nanomedicine* **2017**, *12*, 2033–2044.
- Akbarzadeh, A.; Mikaeli, H.; Zanghami, N.; Mohammad, R.; Barkhordari, A.; Davaran, S. Preparation and *in vitro* evaluation of doxorubicinloaded Fe₃O₄ magnetic nanoparticles modified with biocompatible copolymers. *Int. J. Nanomedicine* **2012**, *7*, 511–526.
- Cai, L.; Dai, Y.; Cao, A.; Cao, M. The effects of CS@Fe₃O₄ nanoparticles combined with microwave or far infrared thawing on microbial diversity of red seabream (*Pagrus major*) fillets based on high-throughput sequencing. *Food Microbiol.* **2020**, *91*, 103511-103518.

18. Cai, H.; Li, K.; Li, J.; Wen, S.; Chen, Q.; Shen, M.; Zheng, L.; Zhang, G.; Shi, X. Dendrimer-Assisted Formation of Fe₃O₄/Au Nanocomposite Particles for Targeted Dual Mode CT/MR Imaging of Tumors. *small* **2015**, *11*, 4584–4593.
19. Lastovina, T.A.; Bugaev, A.L.; Kubrin, S.P.; Kudryavstev, E.A.; Soldatov, A.V. Structural studies of magnetic nanoparticles doped with rare-earth elements. *J. Struct. Chem.* **2016**, *57*, 1444–1449.
20. Wu, Y.; Ma, Y.; Xu, G.; Wei, F.; Ma, Y.; Song, Q.; Wang, X. Metal-organic framework coated Fe₃O₄ magnetic nanoparticles with peroxidase-like activity for colorimetric sensing of cholesterol. *Sens. Actuators B Chem.* **2017**, *249*, 195–202.
21. He, F.; Fan, J.; Ma, D.; Zhang, L.; Leung, C.; Chan, H.L. The attachment of Fe₃O₄ nanoparticles to graphene oxide by covalent bonding. *Carbon* **2010**, *48*, 3139–3144.
22. Zhao, T.; Ji, X.; Guo, X.; Jin, W.; Dang, A.; Li, H.; Li, T. Preparation and electrochemical property of Fe₃O₄/MWCNT nanocomposite. *Chem. Phys. Lett.* **2016**, *653*, 202–206.
23. Sepahvand, S.; Farhadi, S. Fullerene-modified magnetic silver phosphate (Ag₃PO₄/Fe₃O₄/C₆₀) nanocomposites: hydrothermal synthesis, characterization and study of photocatalytic, catalytic and antibacterial activities. *RSC Adv.* **2018**, *8*, 10124–10140.
24. Chekina, N.; Horak, D.; Jendelova, P.; Trchova, M.; Benes, M.J.; Hruby, M.; Herynek, V.; Turnovcova, K.; Sykova, E. Fluorescent magnetic nanoparticles for biomedical applications. *J. Mater. Chem.* **2011**, *21*, 7630–7639.
25. Zhang, R.C.; Liu, L.; Xu, X.L. Synthesis and characteristics of multifunctional Fe₃O₄-SiO₂-CdS magnetic-fluorescent nanocomposites. *Chin. Phys. B* **2011**, *20*, 1–4.
26. Wu, X.; Zhang, Y.; Han, T.; Wu, H.; Guo, S.; Zhang, J. Composite of graphene quantum dots and Fe₃O₄ nanoparticles: peroxidase activity and application in phenolic compound removal. *RSC Adv.* **2014**, *4*, 3299–3305.
27. Bhattacharya, K.; Deb, P. Hybrid nanostructured C-dot decorated Fe₃O₄ electrode materials for superior electrochemical energy storage performance. *Dalton Trans.* **2015**, *44*, 9221–9229.
28. Kelarakis, A. From highly graphitic to amorphous carbon dots: A critical review. *MRS Energy Sustain.* **2014**, *1*, 1–15.
29. Kelarakis, A. Graphene quantum dots: In the crossroad of graphene, quantum dots and carbogenic nanoparticles. *Curr. Opin. Colloid Interface Sci.* **2015**, *20*, 354–361.
30. Wang, H.; Shen, J.; Li, Y.; Wei, Z.; Cao, G.; Gai, Z.; Hong, K.; Banerjee, P.; Zhou, S. Magnetic iron oxide–fluorescent carbon dots integrated nanoparticles for dual-modal imaging, near-infrared light-responsive drug carrier and photothermal therapy. *Biomater. Sci.* **2014**, *2*, 915–923.
31. Zhuo, S.; Guan, Y.; Li, H.; Fang, J.; Zhang, P.; Du, J.; Zhu, C. Facile fabrication of fluorescent Fe-doped carbon quantum dots for dopamine sensing and bioimaging application. *Analyst* **2019**, *144*, 656–662.
32. Han, C.; Zhang, A.; Kong, Y.; Yu, N.; Xie, T.; Dou, B.; Li, K.; Wang, Y.; Li, J.; Xu, K. Multifunctional iron oxide-carbon hybrid nanoparticles for targeted fluorescent/MR dual-modal imaging and detection of breast cancer cells. *Anal. Chim. Acta* **2019**, *1067*, 115–128.
33. Ahmadian-Fard-Fini, S.; Salavati-Niasari, M.; Ghanbari, D. Hydrothermal green synthesis of magnetic Fe₃O₄-carbon dots by lemon and grape fruit extracts and as a photoluminescence sensor for detecting of *E. coli* bacteria. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2018**, *203*, 481–493.
34. Dong, X.; AlAwak, M.; Tomlinson, N.; Tang, Y.; Sun, Y.P.; Yang, L. Antibacterial effects of carbon dots in combination with other antimicrobial reagents. *PLoS ONE* **2017**, *12*, 1–16.
35. Guo, Y.; Tang, D.; Zhang, L.; Li, B.; Iqbal, A.; Liu, W.; Qin, W. Synthesis of ultrathin carbon dots-coated iron oxide nanocubes decorated with silver nanoparticles and their excellent catalytic properties. *Ceram. Int.* **2017**, *43*, 7311–7320.
36. Liu, X.; Jiang, H.; Ye, J.; Zhao, C.; Gao, S.; Wu, C.; Li, C.; Li, J.; Wang, X. Nitrogen-Doped Carbon Quantum Dot Stabilized Magnetic Iron Oxide Nanoprobe for Fluorescence, Magnetic Resonance and Computed Tomography Triple-Modal In Vivo Bioimaging. *Adv. Funct. Mater.* **2016**, *26*, 1–13.
37. Kumar, A.; Chowdhurni, A.R.; Laha, D.; Chandra, S.; Karmakar, P.; Sahu, S.K. One pot synthesis of carbon dots entrenched chitosan modified magnetic nanoparticles for fluorescence based Cu²⁺ ion sensing and cell imaging. *RSC Adv.* **2016**, *6*, 58979–58987.
38. Lou, L.; Yu, K.; Zhang, Z.; Li, B.; Zhu, J.; Wang, Y.; Huang, R. Functionalized magnetic-fluorescent hybrid nanoparticles for cell labelling. *Nanoscale* **2011**, *3*, 2315–2323.

39. Zhang, S.; Niu, H.; Hu, Z.; Cai, Y.; Shi, Y. Preparation of carbon coated Fe₃O₄ nanoparticles and their application for solid-phase extraction of polycyclic aromatic hydrocarbons from environmental water samples. *J. Chromatogr. A* **2010**, *1217*, 4757–4764.
40. Wang, M.; Fu, Q.; Zhang, K.; Wan, Y.; Wang, L.; Gao, M.; Xia, Z.; Gao, D. A magnetic and carbon dot based molecularly imprinted composite for fluorometric detection of 2,4,6-trinitrophenol. *Microchimica Acta* **2019**, *186*, 1–11.
41. Wang, Z.; Guo, H.; Yu, Y.; He, N. Synthesis and characterization of a novel magnetic carrier with its composition of Fe₃O₄/carbon using hydrothermal reaction. *J. Magn. Magn. Mater.* **2006**, *302*, 397–404.
42. Krysmann, M.J.; Kellarakis, A.; Dallas, P.; Giannelis, E.P. Formation mechanism of carbogenic nanoparticles with dual photoluminescence emission. *J. Am. Chem. Soc.* **2012**, *134*, 747–50.
43. Gibbons, E.N.; Winder, C.; Barron, E.; Fernandes, D.; Krysmann, M.J.; Kellarakis, A.; Parry, A.V.S.; Yeates, S.G. Layer by Layer Antimicrobial Coatings Based on Nafion, Lysozyme, and Chitosan. *Nanomaterials* **2019**, *9*, 1563.
44. Li, B.; Wang, X.; Guo, Y.; Iqbal, A.; Dong, Y.; Li, W.; Liu, W.; Qin, W.; Chen, S.; Zhou, X.; Yang, Y. One-pot synthesis of polyamines improved magnetism and fluorescence Fe₃O₄–carbon dots hybrid NPs for dual modal imaging. *Dalton Trans.* **2016**, *45*, 5484–5491.
45. Tiwari, A.; Verma, N.C.; Turrkan, S.; Debnath, A.; Singh, S.; Draeger, G.; Nandi, C.K.; Randhawa, J.K. Graphitic Carbon Coated Magnetite Nanoparticles for Dual-Mode Imaging and Hyperthermia. *ACS Appl. Nano Mater.* **2019**, *3*, 896–904.
46. Hu, F.; Wei, L.; Zhou, Z.; Ran, Y.; Li, Z.; Gao, M. Preparation of Biocompatible Magnetite Nanocrystals for *In Vivo* Magnetic Resonance Detection of Cancer. *Adv. Mater.* **2006**, *18*, 2553–2556.
47. Wang, J.; Zhang, B.; Wang, L.; Wang, M.; Gao, F. One-pot synthesis of water-soluble superparamagnetic iron oxide nanoparticles and their MRI contrast effects in the mouse brains. *Mater. Sci. Eng. C* **2015**, *48*, 416–423.
48. Kolhatkar, A.G.; Jamison, A.C.; Litvinov, D.; Willson, R.C.; Randall Lee, T. Tuning the Magnetic Properties of Nanoparticles. *Int. J. Mol. Sci.* **2013**, *14*, 15977–16009.
49. Knobel, M.; Nunes, W.C.; Socolovsky, L.M.; De Biasi, E.; Vargas, J.M.; Denardin, J.C. Superparamagnetism and Other Magnetic Features in Granular Materials: A Review on Ideal and Real Systems. *J. Nano. Nanotech.* **2008**, *8*, 2836–2857.
50. Cullity, B.D.; Graham, C.D. *Introduction to Magnetic Materials*, 2nd ed.; Wiley-IEEE Press: Hoboken, USA, 2009.
51. Goya, G.F.; Berquó, T.S.; Fonseca, F.C.; Morales, M.P. Static and dynamic magnetic properties of spherical magnetite nanoparticles. *J. Appl. Phys.* **2003**, *94*, 3520.
52. Martinez, B.; Obradors, X.; Balcells, L.; Rouanet, A.; Monty C. Low Temperature Surface Spin-Glass Transition in γ -Fe₂O₃ Nanoparticles. *Phys. Rev. Lett.* **1998**, *80*, 181–184.
53. Mercante, L.A.; Melo, W.W.M.; Granada, M.; Troiani, H.E.; Macedo, W.A.A.; Ardison, J.D.; Vaz, M.G.F.; Novak, M.A. Magnetic properties of nanoscale crystalline maghemite obtained by a new synthetic route. *J. Magn. Magn. Mater.* **2012**, *324*, 3029–3033.
54. Coey, J.M.D. Noncollinear Spin Arrangement in Ultrafine Ferrimagnetic Crystallites. *Phys. Rev. Lett.* **1971**, *27*, 1970–1972.
55. Iglesias, Ó.; Labarta, A. Finite-size and surface effects in maghemite nanoparticles: Monte Carlo simulations. *Phys. Rev. B* **2001**, *63*, 184416.
56. Dutta, P.; Pal, S.; Seehra M.S.; Shah, N.; Huffman, P.G. Size dependence of magnetic parameters and surface disorder in magnetite nanoparticle. *J. Appl. Phys.* **2009**, *105*, 07B501.
57. Phan, M.H.; Alonso, J.; Khurshid, H.; Lampen-Kelley, P.; Chandra, S.; Repa, K.S.; Nemati, Z.; Das, R.; Iglesias, Ó.; Srikanth, H. Exchange bias effects in iron oxide-based nanoparticle system. *Nanomaterials* **2016**, *6*, 221.
58. Demortière, A.; Panissod, P.; Pichon, B.P.; Pourroy, G.; Guillon, D.; Donnio, B.; Bégin-Colin, S. Size-dependent properties of magnetic iron oxidenanocrystals. *Nanoscale* **2011**, *3*, 225–232.
59. Kachkachi, H.; Ezzir, A.; Nogués, M.; Tronc, E. Surface effects in nanoparticles: application to maghemite γ -Fe₂O₃. *Eur. Phys. J. B* **2000**, *14*, 681.
60. Shendruk, T.N.; Desautels, R.S.; Southern, B.W.; van Lierop, J. The effect of surface spin disorder on the magnetism of γ -Fe₂O₃ nanoparticle dispersions. *Nanotechnology* **2007**, *18*, 455704.

61. Aquino, R.; Depeyrot, J.; Sousa, M.H.; Tourinho, F.A.; Dubois, E.; Perzynski, R. Magnetization temperature dependence and freezing of surface spins in magnetic fluids based on ferrite nanoparticles. *Phys. Rev. B* **2005**, *72*, 184435.
62. Desautels, R.D.; Skoropata, E.; Chen, Y.Y.; Ouyang, H.; Freeland, J.W.; van Lierop, J. Increased surface spin stability in γ -Fe₂O₃ nanoparticles with a Cu shell. *J. Phys.: Condens. Matter*. **2012**, *24*, 146001.
63. Rani, S.; Varma, G.D. Superparamagnetism and metamagnetic transition in Fe₃O₄ nanoparticles synthesized via co-precipitation method at different pH. *Physica B: Condensed Matter*. **2015**, *472*, 66.
64. Hu, Y.; Wang, P.; Bunker, C.E.; Teisl, L.R.; Reibold, M.; Yan, S.; Qian, H.; Hea, D.; Sun, Y.P. Preparation and optical properties of magnetic carbon/iron oxide hybrid dots. *RSC Adv.* **2017**, *7*, 41304-41310.
65. Fan, R.J.; Sun, Q.; Zhang, L.; Zhang, Y.; Lu, A.H. Photoluminescent carbon dots directly derived from polyethylene glycol and their application for cellular imaging. *Carbon* **2014**, *71*, 87-93.
66. Fernandes, D.; Heslop, K.A.; Kelarakis, A.; Krysmann, M.J.; Estevez, L. In situ generation of carbon dots within a polymer matrix. *Polymer* **2020**, 122159.
67. Ding, H.; Zhou, X.X.; Wei, J.S.; Li, X.B.; Qin, B.T.; Chen, X.B.; Xiong, H.M. Carbon dots with red/near-infrared emissions and their intrinsic merits for biomedical applications. *Carbon* **2020**, *167*, 322-344.
68. Berezin, M.Y.; Achilefu, S. Fluorescence Lifetime Measurements and Biological Imaging. *Chem. Rev.* **2010**, *110*, 2641-2684.
69. Magrez, A.; Kasas, S.; Salicio, V.; Pasquier, N.; Seo J.W.; Celio, M.; Catsicas, S.; Schwaller, B.; Forró, F. Cellular Toxicity of Carbon-Based Nanomaterials. *Nano Lett.* **2006**, *6*, 1121-1125.
70. Yang, S.T.; Cao, L.; Luo, P.G.; Lu, F.; Wang, X.; Wang, H.; Mezziani, M.J.; Liu, Y.; Qi, G.; Sun, Y.P. Carbon Dots for Optical Imaging in Vivo. *J. Am. Chem. Soc.* **2009**, *131*, 11308-11309.
71. Patil, U.S.; Adireddy, S.; Jaiswal, A.; Mandava, S.; Lee, B.R.; Chrisey, D.B. In Vitro/In Vivo Toxicity Evaluation and Quantification of Iron Oxide Nanoparticle. *Int. J. Mol. Sci.* **2015**, *16*, 24417-24450.
72. Li, L.; Mak, K.Y.; Shi, J.; Koon, H.K.; Leung, C.H.; Wong, C.M.; Leung, C.W.; Mak, C.S.K.; Chan, N.M.M.; Zhong, W.; Lin, K.W.; Wu, E.X.; Pong, P.W.T. Comparative In Vitro Cytotoxicity Study on Uncoated Magnetic Nanoparticles: Effects on Cell Viability, Cell Morphology, and Cellular Uptake. *J. Nanosci. Nanotechnol* **2012**, *12*, 1-8.
73. Stachowska, J.D.; Murphy, A.; Fernandes, D.; Gibbons, E.N.; Krysmann, M.J.; Kelarakis, A.; Burgaz, E.; Moore, J.; Yeates, S.G. A rich gallery of carbon dots based photoluminescent suspensions and powders derived by citric acid/urea. *Sci. Rep.* **2021**, *11*, 10554.
74. Azam, A.; Ahmed, A.S.; Oyes, M.; Khan, M.S.; Habib, S.S.; Memic, A. Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. *Int. J. Nanomedicine* **2012**, *7*, 6003-6009.
75. Huang, K.C.; Shieh, D.B.; Yeh, C.S.; Wu, P.C. Antimicrobial Applications of Water-Dispersible Magnetic Nanoparticles in Biomedicine. *Curr. Med. Chem.* **2014**, *21*(29).

