Supporting information:

1. Experimental details

Ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), ammonium hydroxide (NH_4OH), poly(allylamine hydrochloride) (PAH) and poly(sodium 4-styrenesulfonate) (PSSS) with an average molecular weight of 70,000 and other chemicals were obtained from Sigma-Aldrich. The materials were of analytical grade and used without purification.

Preparation of magnetite-PSSS nanoparticles

Magnetite-PSSS nanostructures were prepared using the modified co-precipitation method. Briefly, $FeCl_3 \cdot 6H_2O$ (1.1 g; 4 mmol) and $FeCl_2 \cdot 4H_2O$ (0.4 g; 2 mmol) were dissolved in deoxygenated Millipore water (100mL).The required volume of Fe^{2+}/Fe^{3+} solution (Ratio 4 : 2 mmol) was added into a 100 mL three-necked round-bottom flask under Argon. The required volume of PSSS (0.25 g in 10 mL) solution was added to the mixture and mixed for 15 minutes. Ammonia (NH_4OH (35%) was added by syringe until a pH 9.0 was reached. The resulting black precipitate was stirred for 1 hour and subsequently washed with Millipore water (6 washes, 20 ml) until pH neutral. The obtained precipitate was dried, under a vacuum at room temperature (Yield: 0.129 g, 34 %), and analysed using FTIR and Raman spectroscopy, TEM, and SQUID. The same preparation of magnetite-PSSS was used to provide samples for analysis.

Preparation of Fe_3O_4-PSSS-PAH/PE_{10}–RhB nanostructures

0.0058 g of Fe_3O_4-PSSS was dispersed in 10 ml of Millipore water. Fe_3O_4-PSSS nanoparticles were negatively charged, with driving of the electrostatic interactions, positively charged PAH (0.6 ml, 2.85×10^{-7} M) was added to the suspension, sample was sonicated for 10 minutes at 20ºC. Then the resulting PAH layer was deposited, negatively charged PSSS (1.2 ml, 2.85×10^{-7} M) was added into solution and sonicated again. After second layer of polyelectrolyte was deposited, the process was carried out in a cyclic method, until ten layers of polyelectrolytes producing multilayered magnetic nanostructures. After deposition multilayered magnetic nanostructures were magnetically separated, decanted and re-dispersed in 10 ml of degassed Millipore

water. 2 ml of magnetic suspension of multilayered magnetic nanostructures was mixed with Rhodamine B (RhB) solution (3 ml, $2.1x10^{-4}$ M) overnight (approximately 18 hours) in darkness at the room temperature. The unreacted Rhodamine B in the resulting mixture was washed with Millipore water several times (4x5 ml), until the washings turned colourless. Freshly prepared luminescent magnetic nanostructures were then removed by magnetic separation. The collected luminescent magnetic nanostructures were dispersed in 5 ml Millipore water and kept in darkness.

The final suspension was analysed using FTIR, PCS, Uv-vis, PL, TEM, and Zeta potential measurements. The same preparation of Fe_3O_4-PSSS-PAH/PE_{10} was used to provide samples for analysis. A schematic illustration of the fabrication magnetic luminescent nanostructures is presented in Fig. S1.

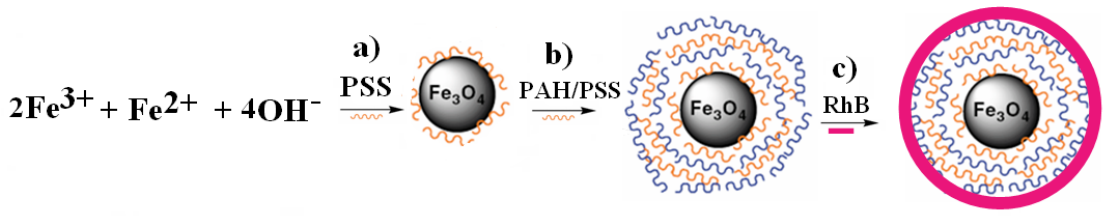

Figure S1 Schematic illustration of fabrication magnetic luminescent nanostructures using the Layer by Layer (LbL) technique. [1]

Preparation of primary rat cortical mixed glia

Primary cortical glial cells were prepared from 1-day old Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland). The rats were decapitated, the cerebral cortices were dissected and the meninges were removed. Tissue was bi-directionally chopped using a sterile scalpel and placed in 15ml falcon tubes (Sarstedt, Ireland) containing warm filter sterilized Dulbecco's Modified Eagle Media (2ml; DMEM; GIBCO, UK) supplemented with 10% heat inactivated foetal calf serum (Sigma, UK), streptomycin (100U/ml; GIBCO, UK) and penicillin (100U/ml; GIBCO, UK). The tissue was incubated for 20 min (37ºC; 5% CO_2:95% air; Nuaire Flow CO_2 incubator; Jencons, UK), triturated, filtered through a sterile mesh filter (40 µm; Becton Dickinson Labware, France), centrifuged at 2000 rpm for 3 min at 20°C and the pellet resuspended in warmed DMEM.

Equal numbers of resuspended cells were plated in 24 well plates at a density of 1.0 x 10^6 cells and incubated for 1 hour before addition of warmed DMEM. For analysis by confocal and light microscopy, cells were plated onto poly-L-lysine-coated (60 μg/ml) coverslips and grown at 37°C in a humidified 5% CO_2, 95% air environment for 12-14 days (with 3 intermediate changes of medium) until cells reached confluency. Previous work completed in our laboratory using this protocol have shown that this mixed glial culture comprises 30% microglia and 70% astrocytes.

2. Characterisation techniques:

Transmission electron microscopy (TEM) measurements

Samples were prepared for TEM by ultrasonically dispersing powder samples in Millipore water. One drop of the solution (5 μL) was placed onto a formvar-coated copper TEM grid. The grid was then dried in air. The size, shape and arrangements of the particles were analyzed in TEM images, obtained on a Jeol 2100 Transmission Electron Microscope. Particle sizes were determined by manually counting over 100 particles, in addition *x* and *y* measurements were determined for each particle.

Fourier Transformed Infrared spectroscopy (FTIR) measurements

Potassium bromide (KBr, for IR spectroscopy, Fluka) was used to prepare disks of the samples by pressing at a pressure of 8 MPa for 2-5 min. IR spectra were recorded between 4000 – 500 cm^{-1} by diffuse reflectance using a Perkin Elmer Spectrum One FT-IR.

Raman spectroscopy (RS) measurements

Raman spectra were recorded between 1000 and 4 cm^{-1} at room temperature. Raman spectra were fitted using GRAM/386 software. The excitation wavelength was 457 nm from an Ar^+ ion laser (Laser Physics Reliant 150 Select Multi-Line) with a typical laser power of ~ 17 W cm^2 in order to avoid excessive heating. The 100x-magnifying objective of the Leica microscope was capable of focusing the beam into a spot of approximately 1 μm diameter. To ensure a high signal–noise ratio, each RS spectrum is the average of 3 successive scans.

Superconducting quantum interference device magnetometer (SQUID) measurements

Magnetisation measurements were carried out on a SQUID magnetometer with a 5 T magnet (MPMS XL 5, Quantum Design) and a magnetic field of 10^{-8} amps/m with temperature being variable from 1 to 300 K. The magnetisation curves M (H_c) were obtained after cooling the sample to the measurement temperature in zero fields and then increasing the field from 0 to 50 kOe. In all cases, the powders were moderately compacted into the sampling tubes. The device operates typically with samples of mass 1.8 - 2 mg.

Nuclear Magnetic Resonance Dispersion (NMRD) measurements

T_1 and T_2 measurements by NMRD were obtained using a Spinmaster Fast Field Cycling Relaxometer operating at the frequency of 9.25 MHz.

Photon Correlation Spectroscopy (PCS)

PCS measurements were carried out in air at 25 °C at a backscatter angle of 173 º for all samples dispersed in water using a Malvern Zetasizer Nano ZS instrument.

Ultraviolet-visible Spectroscopy (UV-vis)

Samples were diluted to a known concentration in a quartz cuvette (2 ml working volume, 10 mm path length). Measurements were performed at room temperature using a Varian Cary 50 UV-vis spectrophotometer.

Photoluminescence Spectroscopy (PL)

Samples preparation was identical to UV-vis sample preparation. Measurements were performed at the room temperature using a Varian Cary Eclipse Fluorescence Spectrophotometer.

Zeta potential (ζ- potential)

Sample (~1 ml working volume) was injected into a disposable capillary cuvette and zeta potenial measurements were carried out in air at 25 °C for using a Malvern Zetasizer Nano ZS.

Thermogravimetric analysis (TGA) measurements

Thermogravimetric analysis (TGA) measurements were carried out in air for all the samples using a Perkin Elmer Pyris 1 TGA with a temperature scan rate of 10 °C min^{-1}. The analysis was performed in air on a sample of approximately 2 mg.

Nanostructure incubation

Cells were incubated in the presence of nanostructures (5μM) for 2 hours and prepared for analysis as detailed below in the following sections.

Analysis of nanostructure internalization by confocal microscopy

Cells were were washed twice with ice-cold PBS buffer (137mM NaCl, 2.7mM KCI, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.3) and then fixed with ice-cold methanol. Cells were washed three times with PHEM buffer (60mM PIPES, 25mM HEPES, 10mM EGTA, 2mM $MgCl_2$, pH 6.9). The coverslips were mounted onto glass slides for confocal microscopy (Zeiss, Axioplan 2).

Analysis of nanocomposite internalization by Prussian blue staining

Cells were were washed twice with ice-cold PBS buffer (137mM NaCl, 2.7mM KCI, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.3) and then fixed in ice-cold methanol. Cells were washed three times with PHEM buffer (60mM PIPES, 25mM HEPES, 10mM EGTA, 2mM $MgCl_2$, pH 6.9), incubated in the presence of Prussian blue reagent (4% HCI mixed with 4% hydrochloric acid (1.2 mmol/l) 1:1, freshly prepared) for 20 minutes, washed in deionized water, dehydrated through alcohol, cleared in xylene and mounted using DPX.

Flow Cytometry Analysis

Cells were washed twice with ice-cold PBS following treatment and incubated at 37ºC for 3-5 minutes in fresh PBS containing trypsin (30 μl). Media (200μl) was added and samples were centrifuged at 2000 rpm for 3 min. The pellet was resuspended in FACS buffer (0.1% sodium azide in PBS) and transferred to FACS

tubes. Measurements of the cell-associated fluorescence of non-treated and nanostructure-treated cells was completed on a DAKO $CyAN_{ADP}$ flow cytometer calibrated using Flow-Check Fluorospheres (Beckman Coulter, Ireland).

Cytotoxicity

The cytotoxicity of the nanostructures was evaluated by measuring the inhibition of cell proliferation using the MTT assay (Cell Proliferation Assay, Promega). Cells were incubated for 2, 18, 24 and 48 hours in the presence of nanostructures (1, 5, 10 or 20 μM). Cell viability was assessed by calculating the ratio of the number of viable cells in the nanostructure-treated culture with the non-treated culture.

Magnetic Resonance Imaging measurements

A nanocompostite-treated cell population was washed, pelleted and re-suspended in DMEM. Six cell phantoms were prepared in 2 ml cylindrical Eppendorf tubes, each containing 1 ml of 0.5 % warm agarose gel was and 1 ml of cell suspension containing either 2.0×10^6, 1×10^6, 0.5×10^6. 0.25×10^6, 0.05×10^6 or 0.01×10^6 cells/ml. Homogenous distribution of the cells was ensured by 30 seconds of trituration.

MRI was performed in a 7.0T Bruker Spectrometer with a small volume resonator used in transceiver mode. T2 times were calculated from a Multi-Spin-Multi-Echo (MSME) sequence (TR = 2000 ms, TE = 10 ms, 30 echoes with 10 ms echo spacing). T2* times were calculated from a Multi-Gradient-Echo (MGE) sequence (TR = 1500 ms, TE = 2.52 ms, 30 echoes with 5 ms echo spacing). Both sequences shared the following parameters: field of view = 2.56 cm, matrix = 156*156, slice thickness = 2 mm, number of averages = 1. The overall scanning time was between 5 and 6 minutes.

T2 and T2* times were calculated using the Image Sequence Analysis (ISA) software in the Bruker software, Topspin. The centre of the phantom was selected using the Region of Interest (ROI) tool. The same ROI was used to generate values for T2 and T2* time calculation. T2 and T2* times were fitted to a mono-exponential decay function. The field inhomogeneity, ΔB, due to the presence of the nanostructures was

calculated using $\Delta B\gamma = (1/T2^*) - (1/T2)$, where γ is the gyromagnetic ratio for hydrogen (42.58 MHz/T). ΔB was calculated for each of the phantoms.

3. Characterization of Fe_3O_4-PSSS nanostructures

FTIR measurements of Fe_3O_4-PSSS nanoparticles

Fourier Transform Infrared Spectroscopy confirmed the presence of PSSS on the surface of the particles. Peaks from 1180-1005 cm^{-1} represent PSSS. OH^- groups on the particle surface at 3440-3460 cm^{-1} are due to water molecules associated with the surface, and Fe-O stretch at 589 cm^{-1} respectively.

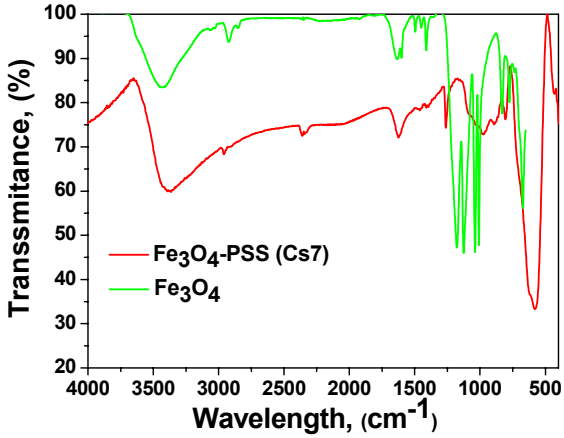

Figure S2 FTIR spectra of magnetite nanoparticles, with and without PSSS coating. Key; (green) pure PSSS (red) Fe_3O_4-PSSS.

Raman measurements of Fe_3O_4-PSSS nanoparticles

Raman spectra of the PSSS coated magnetite nanoparticles is presented in Fig. S3. Presence of PSSS is confirmed with a main peak found at 670 cm^{-1} [3]

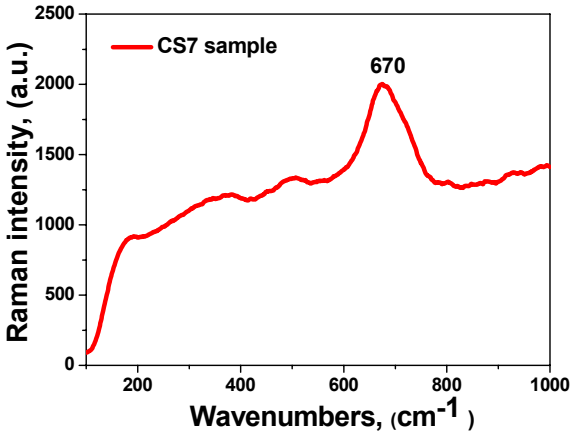

Figure S3 Raman spectra of PSSS-coated magnetite nanoparticles.

TEM and PCS measurements of Fe_3O_4-PSSS nanoparticles

TEM analysis of the magnetite-PSSS nanoparticles, Fig. S4, determined an average size distribution of 7.9 ±1.5 nm. TEM reveals cross-linking of nanoparticles due to PSSS being present. These particles are the starting point for deposition of PE layers.

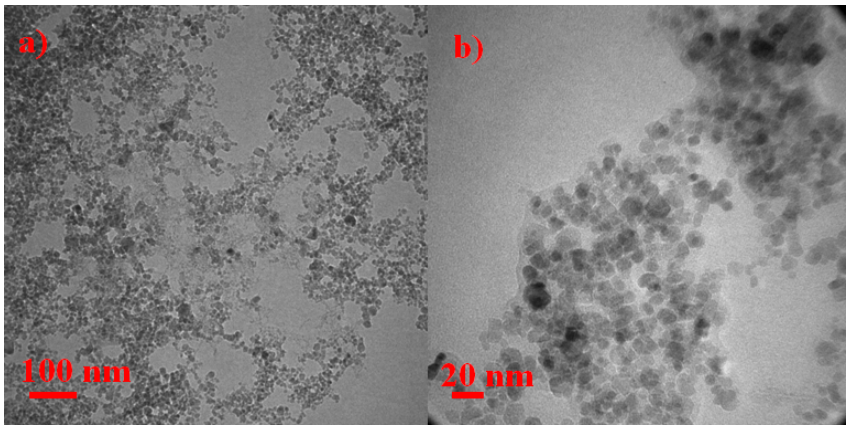

Figure S4 TEM images of PSSS-coated magnetite nanoparticles.

The stable magnetic suspension (last filtrate) was analysed by PCS. PCS measurements confirmed TEM data and showed presence of cross-linking between particles in an aqueous solution. The average of the hydrodynamic diameter of magnetite particles was 141.4 nm (PDI 0.141) and is presented in Fig. S5.

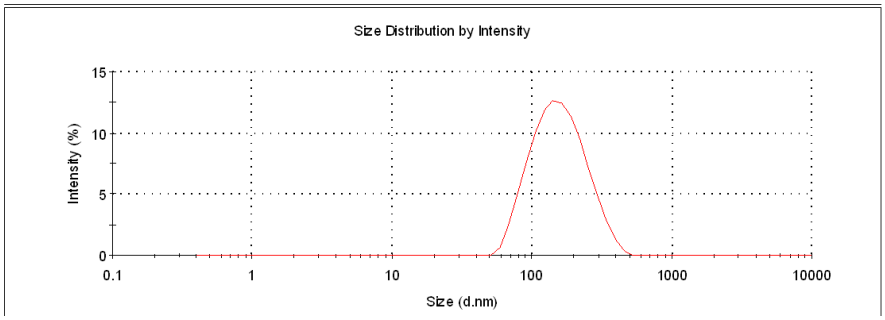

Figure S5 PCS data (3 averages) of PSSS-coated magnetite nanoparticles in Millipore water.

Magnetic measurements (SQUID) of Fe_3O_4-PSSS nanoparticles

PSSS functionalised magnetite demonstrates no hysteresis at 300K, while superparamagnetic behaviour is clearly evident at 300 K (Fig. S6). Magnetization (M_S) was 67.79 Am^2kg^{-1} K.

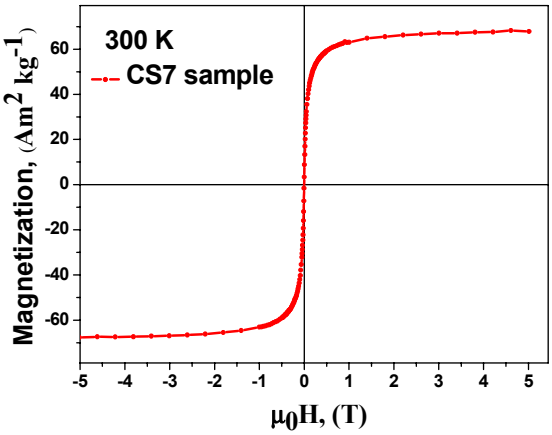

Figure S6. Magnetization curve versus magnetic field for PSSS-coated magnetite nanoparticles. Measurements were carried out at 27 °C.

NMRD measurements of Fe_3O_4-PSSS nanoparticles

Fig. S7 shows typical NMRD curves of superparamagnetic magnetite nanoparticles in Millipore water.

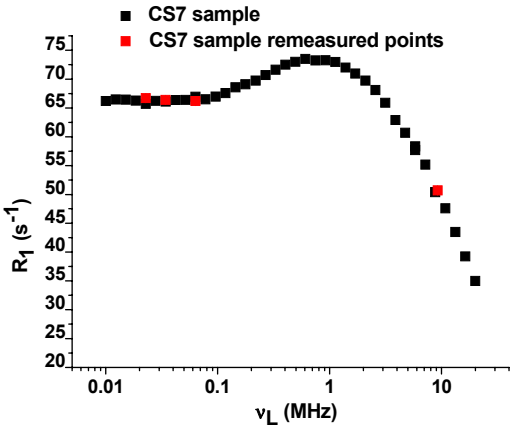

Figure S7. NMRD relaxation curve recorded at a measuring frequency of 20 MHz for PSS coated magnetite nanoparticles in Millipore water. Key: (black) PSSS-coated nanoparticles (red) data points correspond to the re-measured NMR of sample 3 days after initial measurements. Measurements were carried out at 24.8 ±1°C.

These findings are in agreement with TEM measurements, where nanoparticles are approximately 10 nm in diameter or lower. The sample has shown the highest relaxivity of ~ 73.43 $mM^{-1}s^{-1}$ at lower field 0.62 MHz. To confirm the suspension stability and repeatability, NMRD measurements were performed 3 days after initial measurements. No decrease in suspension stability was observed. Fig. S7 (red data points on the black curve), shows no change of the NMRD of curve at the selected frequencies (0.023, 0.034, 0.06, 9.28 MHz) 3 days after initial measurements.

$T_1 = 1.97x10^{-2}$ (error 0.0398%) [s^{-1}]
$T_2 = 3.25x10^{-3}$ (error 0.21%) [s^{-1}]

The PCS data obtained for the PSSS-coated magnetite nanoparticles do not show evidence of large amounts of aggregation after NMR field. Fig. S8 shows decrease in average of the hydrodynamic diameter of magnetite particles size (Table 1).

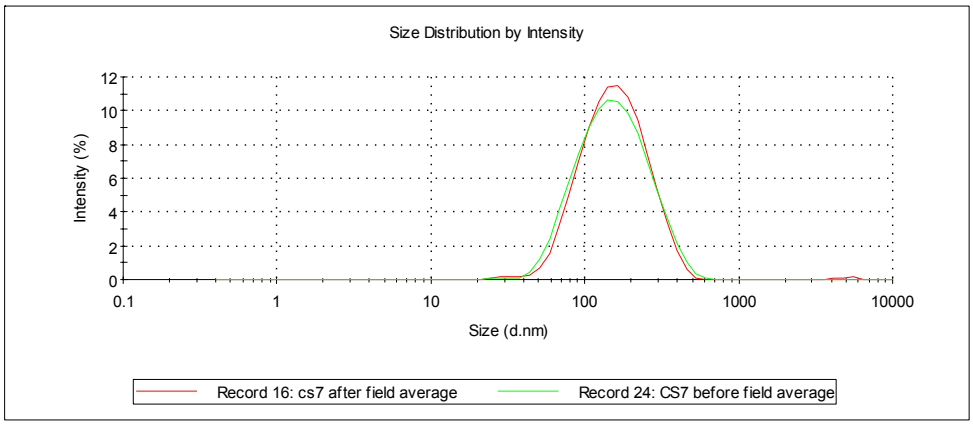

Figure S8. PCS data (3 averages) of PSSS-coated magnetite nanoparticles in Millipore water. Key: (green) before the NMR, (red) after NMR measurements.

Table S1. Summarised PCS and TEM data of PSS coated magnetite nanoparticles before and after NMR measurements.

Name	**Before NMR field**		**After NMR field**	
	TEM (nm)	**PCS (nm)**	**TEM (nm)**	**PCS (nm)**
CS7	8.8±0.6	171.6, PDI=0.203	9±1	138.2, PDI=0.203

The LbL deposition technique was used to coat the Fe_3O_4-PSS nanoparticles with 10 layers of PE. Fig. S9 shows ζ-potential as a function of deposited number PE layers for negatively charged Fe_3O_4-PSSS nanoparticles.

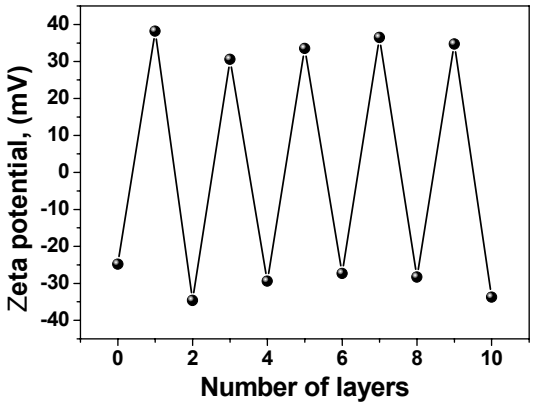

Figure S9 Zeta potential of the negatively charged Fe_3O_4-PSS coated PAH/PE_{10} nanoparticles as a function of PE layers numbers for PAH/PSS coatings.

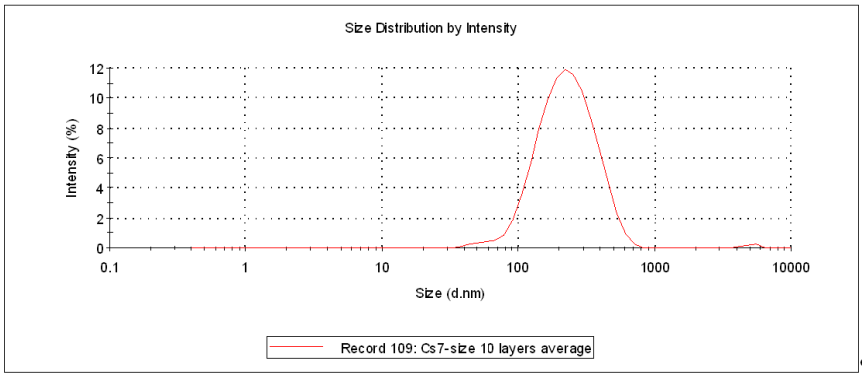

Figure S10. PCS data (3 averages) of Fe_3O_4-PSSS-PAH/PE_{10} nanostructure in Millipore water.

ζ-potential of multilayered Fe_3O_4-PSSS-PAH/PE_{10} nanoparticles was -33.1 mV. After sample was mixed with RhB (3 ml, $2.1 \cdot 10^{-4}$ M), the ζ-potential became more positively charged: -26.8 mV. The difference in the ζ-potential was -6.3 mV for the final product, indicating that RhB molecules were successfully deposited on the surface of the nanoparticles.

UV-vis and PL spectroscopic characterisation of bimodal fluorescent-magnetic Fe_3O_4-PSSS-PAH/PE_{10}-RhB nanostructures

Fig. S11 (a) presents the absorbance luminescence spectra of the Fe_3O_4-PSSS-PAH/PE_{10}-RhB samples. A peak at ~555 nm was identified, this is similar to pure RhB indicating that the origin of luminescent is RhB on the surface of the multilayered magnetic nanostructures. Nanostructures in solution retained a characteristic magenta colour in solution, indicating the generation of Rhodamine B in the final product.

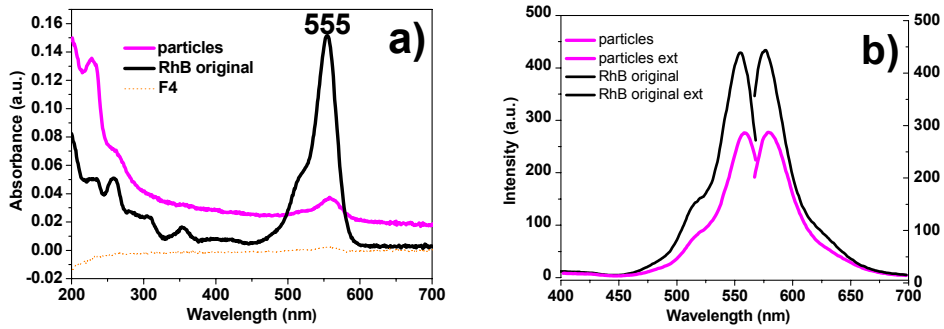

Figure S11. Room temperature absorbance, luminescence and excitation spectra of Fe_3O_4-PSSS-PAH/PE_{10}-RhB nanostructure. (λ_{ex}= 555 nm, λ_{em}= 578 nm). **a)** absorbance spectra **b)** luminescence and excitation spectra. Key: (black) Original RhB

solution (magenta) particles-RhB in water. Dotted orange line shows last washing (F4).

FTIR studies Fe_3O_4-PSSS coated PAH/PE_{10} nanoparticles

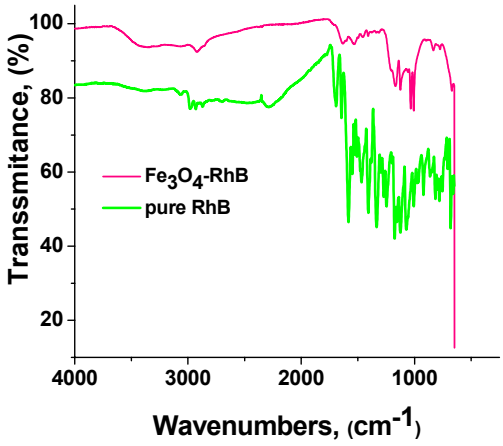

Figure S12. FTIR spectra of Fe_3O_4-PSSS-PAH/PE_{10}-RhB nanostructure. Key: (magenta) Fe_3O_4-PSS-PAH/PE_{10}-RhB, (green) pure Rhodamine B.

TGA results

The TGA curves for luminescent magnetic Fe_3O_4-PSS-PAH/PE_{10}-RhB nanostructures are shown in Figure S13.

It was noticed that there was a similar initial 1-2 % weight loss around 70 °C for primary PSSS coated Fe_3O_4 nanoparticles and 4 % for Fe_3O_4-PSS-PAH/PE_{10}-RhB nanostructures. This might be due to the decomposition of absorbed water on the surface of nanoparticles and nanostructures. For primary PSSS coated magnetite nanoparticles, the weight loss around before 260 °C (around 6 %) was observed due to the decomposition of PSSS from the surface of the nanoparticles.

The significant one step weight loss for magnetic-fluorescent Fe_3O_4-PSS-PAH/PE_{10}-RhB nanostructures could be seen around 260-500 °C could be mainly attributed to following decomposition of multilayered structure with RhB.

In addition, the TGA curves indicated the weight loss for the coated nanostructures was approximately 30 % higher than that of PSSS coated magnetite nanoparticles, suggesting the presence multilayered structure on the surface of primary PSSS coated magnetite nanoparticles.

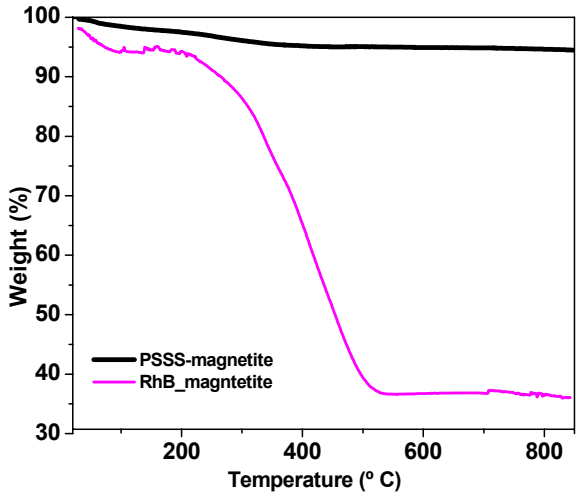

Figure S13. TGA spectra of primary Fe_3O_4-PSSS nanostructures and magnetic-fluorescent magnetic Fe_3O_4-PSS-PAH/PE_{10}-RhB nanostructures**.** Key: (black) Fe_3O_4-PSSS (CS7 sample), (magneta) Fe_3O_4-PSS-PAH/PE_{10}-RhB nanostructures.

Prussian Blue Staining

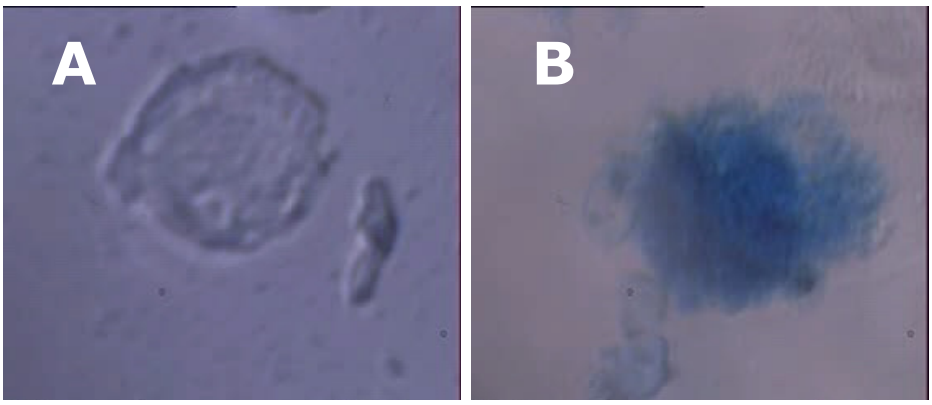

Figure S14 Prussian Blue staining of control-treated sample (A) and nanostructure-treated sample(B).

References:

[1] X. Hong, J. Li, M. Wang, J. Xu, W. Guo, J. Li, Y. Bai, T. Li. *Chemistry of Materials*, 16, 21, **2004**, 4022-4027.

[2] S. A. Corr, Y. P. Rakovich, Y. K. Gun'ko. *Nanoscale Research Letters*, 3, **2008**, 87-104.

[3] S.W. da Silva, T.F. O. Melo, M.A. G. Soler, E.C. D. Lima, M.F. da Silva, and P.C. Morais. *IEEE Transactions of Magnetics*. 39. 5. **2003** 2045-2047.