Electronic Supporting Information:

Experimental

Materials UV-visible and IR spectra were recorded on a Varian Cary 50 Scan UV-Visible spectrophotometer and on a Thermo Electron Corporation Nicolet 380 IR (KBr pellet) respectively. Transmission electron microscopy (TEM) measurements were carried out using a Philips CM10. ^{31}P -NMR spectra were obtained on a Varian Gemini spectrometer at 200 MHz with chemical shifts being reported as ppm from trimethylsilane as internal standard. Fluorescence study was performed on a Perkin-Elmer LS50B Luminescence spectrofluorometer. The size and the zeta potential of the nanocomplex was determined by dynamic laser light scattering (DLS) on a Nano-ZS (Red Badge) ZEN 3600 device (Malvern Instruments, Malvern, UK. The magnetic behavior at room temperature of the as-synthesized nanoparticles is characterized using both SQUID (superconducting quantum interference device) magnetometer and MIAplex® reader (Magnisense). MR imaging of the test tubes was performed using a 4.7 T MR scanner (Bruker). Cell suspensions were analyzed with a Beckton Dickinson FACSCalibur 3C (argon laser wavelength at 488 nm) flow cytometer. Ten thousand cells per sample were measured for forward-angle light scattering (FSC) and fluorescence. Fluorescence imaging on cells was carried out with a Nikon Optiphot-2 with addition of a coaxial-confocal module with Nipkow wheel (Technical Instruments, model K2 BIO). The optimal depth resolution was 0.5 mm. The excitement light source was a high-pressure mercury lamp. Appropriate fluorescence emission filters were used for Rhodamine B (rhodamine filter set: BP546, FT590, LP600). Images were collected using a cooled CCD camera (Micromax, Princeton Instruments, Evry, France). Display and analysis were performed with IPLab software (Scanalytics, Fairfax, VA).

Chemicals Iron dodecyl sulfate $Fe(DS)_2$ normal micelle (oil in water) are used to prepare γFe_2O_3 nanoparticles. The synthesis of $Fe(DS)_2$ was described previously [Y. Lalatonne, C. Paris, J. M. Serfaty, P. Weinmann, M. Lecouvey, L. Motte, *Chemical Communications* 2008, **22**, 2553]. The nanocrystal are surface passivated with (4-Amino-1-hydroxy-1-phosphonobutyl)phosphonic Acid (Alendronate) or with (6-Amino-1-hydroxy-1-phosphonohexyl)phosphonic Acid (Neridronate). The synthesis of Alendronate and Neridronate were described below. All others chemicals used were purchased from Sigma-Aldrich (St Louis, MO). Millipore H_2O was employed for the preparation of all aqueous solutions.

Synthesis of (4-Amino-1-hydroxy-1-phosphonobutyl)phosphonic Acid (Alendronate).
Alendronate was synthesized according to the general procedure [G. R. Kieczykowski, R. B. Jobson, D. G. Melillo, D. F. Reinhold, V. J. Grenda, I. Shinkai, *J. Org. Chem.* 1995**, 60***,* 8310] for linear aliphatic BPs and characterized by ^1H and ^{31}P NMR. BPs was prepared from the corresponding carboxylic acid precursor according to the following reaction carboxylic acid (150 mmol) and H_3PO_3 (150 mmol) were introduced in a three-necked round-bottom flask under inert atmosphere followed by 30 ml of methanesulfonic acid. After heating at 65°C for 1 h, PCl_3 (40mmol) was added slowly and the reaction allowed to proceed overnight at 65°C. The resulting yellow viscous reaction mixture was cooled to room temperature, quenched with 500 ml of ice-cold water (Neves et al., 2002). The pH was adjusted to 4.3 with a NaOH aqueous solution (0.5M) and the obtained white precipitate was collected by filtration. This solid was washed five times with a mixture of methanol/water (95:5), dialyzed for 3 days and freeze-dried to finally obtain linear aliphatic BPs (82%).
Carboxylic acid precursor: 4-aminobutyric acid. RMN ^{31}P $\{^1H\}$ (80.9 MHz, H_3PO_4/D_2O):

18.47. RMN ^{1}H (500 MHz, D_2O): 3.046 (m, 2H), 2.017 (m, 4H). I.R. (KBr): 1540, 1172, 1052 cm^{-1};

According to the solubility of BPs, the stock solution was prepared at 10 mM in water. Dilutions of the stock solution were conducted with Eagle's medium, and the highest tested concentration corresponded to 1 mM.

Synthesis of γFe_2O_3@HMBP nanocrystals: To prepare non-coated γFe_2O_3 particles, a solution of dimethylamine 40% in water ($(CH_3)_2NH$, 10.5 mL) is added to an aqueous micellar solution of ferrous dodecyl sulfate ($Fe(DS)_2$) (0.61 g, 10^{-3} mol). The solution is stirred vigorously for 2 h at 28.5 °C and the resulting precipitate of uncoated nanocrystals is isolated from the supernatant by centrifugation. This precipitate is washed with an acidic solution (HCl 10^{-1} $mol.L^{-1}$) and a solution of HMBP molecules (n = 10^{-4} mol in 30 mL of water) is added. The solution is stirred for two hours at room temperature. The precipitate that appears is washed with an acidic solution (HCl 10^{-1} $mol.L^{-1}$). Free HMBP are isolated from the coated particles thanks to a magnetic field and by centrifugation. The magnetic nanocrystals coated with HMBP molecules are dispersed in water. The initial pH is equal to 2 and then progressively increased to pH 7.4 by addition of sodium hydroxide NaOH (10^{-1} $mol.L^{-1}$). The iron concentration is deduced from ultraviolet-visible absorption.

Nanocrystal characterization: The average particle size was determined by transmission electron microscopy after deposition of a drop of the solution on a conventional TEM grid. Diluted ferrofluid ([Fe] = 5.10^{-4} $mol.L^{-1}$) at pH = 7.4 were used for the measurements of the hydrodynamic diameter and zeta potential, with Nano-ZS device. IR spectroscopy is used to confirm nanocrystal surface complexation via phosphonate groups. The average

number of molecules per nanocrystal is deduced with ^{31}P NMR spectroscopy. A range of concentrations of free Alendronate (NMR ^{31}P {1H} (80.9 MHz): 17.076 ppm) or Neridronate (NMR ^{31}P {1H} (80.9 MHz): 18.322 ppm) solutions added with NaH_2PO_4 (in capillary, 10^{-1} mol.L^{-1}; NMR ^{31}P {1H} (80.9 MHz): 0 ppm) was prepared for calibration. After chemical decomposition of the magnetic γFe_2O_3@HMBPs nanocrystals in acidic medium (nitric acid 65%), the ferrous ions are precipitated by addition of sodium hydroxide NaOH (10^{-1} mol.L^{-1}) in order to avoid shifting of the ^{31}P NMR signal. The supernatant is analyzed with ^{31}P NMR and the concentration (number of molecules per nanocrystal) of HMBP into the sample is deduced from this calibration plot. The magnetic properties were measured at room temperature by means of Squid magnetometer and using the MIAplex® reader (Magnisense). The physical bases of MIAplex® reader device, are reported elsewhere [22].

MR imaging of the test tubes was performed using a 4.7 T MR scanner. The iron concentration is between 0.031 to 1 mM. For measurements of T_2^* relaxation times, axial T_2^*-weighted SE images were obtained with TR values of 1500 ms and TE of 7 ms at 4.7T.

Conjugation of Rhodamine B to γFe_2O_3@Alendronate and characterization 10mL of ferrofluid γFe_2O_3@Alendronate in carbonate buffer ([Fe] = 4,1.10^{-3} mol.L^{-1}, n_{ald} = 2.3.10^{-6} mol) is added to an aqueous (pH=3) mixture containing Rhodamine B (0.239 g, 5.10^{-4} mol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (0.115 g, 10^{-3} mol) and *N*-Hydroxysuccinimide (NHS) (0.192 g, 10^{-3} mol). The mixture is stirred for 24 hours at room temperature. The resulting hybrid magneto-fluorescent nanoparticles formed a flocculate, which is dialysed for 4 days. No significant changes in the absorption spectrum of γFe_2O_3@Alendronate particles are noticed after Rhodamine B grafting. The efficiency of the conjugation is qualitatively and quantitativly evaluated using FTIR and

fluorescence spectroscopies.

Determination of the [Dye]/[Fe] Ratio: The molar ratio of the dye and iron of the magnetic particles was determined after chemical decomposition of the coupling between Rhodamine B and the particles in alkaline medium (pH 12, 24h). The magnetic particles were then separated from the fluorescent supernatant by magnetic decantation. After dilution in water, the dye concentration was deduced from ultraviolet-visible absorption and fluorescence (Rhodamine B, pH=7) and compared to the emission spectrum of an equal molarity of unadsorbed dye in water.

Cell viability: Human umbilical vein endothelial cell (HUVEC) from PromoCell (Heidelberg, Germany) and carcinoma cells line (MDA-MB-231; A435 and U87-MG) were obtained American Type Culture Collection respectively. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum.

Cell viability was evaluated using the MTT microculture tetrazolium assay. Cells were seeded at a density of $20X10^3$cells/well in 96-well flat-bottom plates (Falcon, Strasbourg, France) and incubated in complete culture medium for 24h. Then, medium was removed and replaced by 10% FCS-medium containing increasing concentrations of free Alendronate and γFe_2O_3@Alendronate nanocrystals with or without magnet. After 48 hours, cells were washed with phosphate buffered saline (PBS, Life Technologies) and incubated with 0.1 ml of MTT (2mg/ml, Sigma-Aldrich) for additional 4h at 37°C. The insoluble product was then dissolved by addition of DMSO (Sigma-Aldrich). Optical density was measured at 540 nm using a Labsystems Multiskan MS microplate reader. Each *in vitro* experiment was performed three times, with four wells per sample per experiment.

Flow cytometry: Cells were seeded on Petri dishes (Ø 30mm, density 50,000 cells per Petri dish), grown for 24 h and treated with γFe_2O_3@Alendronate Rhodamine for various time. Cells were washed twice in PBS. Treated and untreated cells were harvested by trypsinization (500l trypsin–EDTA). ([Fe]= 4.5 10^{-4} mol.L^{-1}, [Ald]= 25 10^{-6} mol.L^{-1}, [Rho]= 1.25 10^{-6} mol.L^{-1})

Cell labelling: The labeling of living cells is evaluated using Prussian blue staining for γFe_2O_3@HMBP nanocrystals and using fluorescence microscopy for γFe_2O_3@alendronate-Rhodamine nanocrystals.

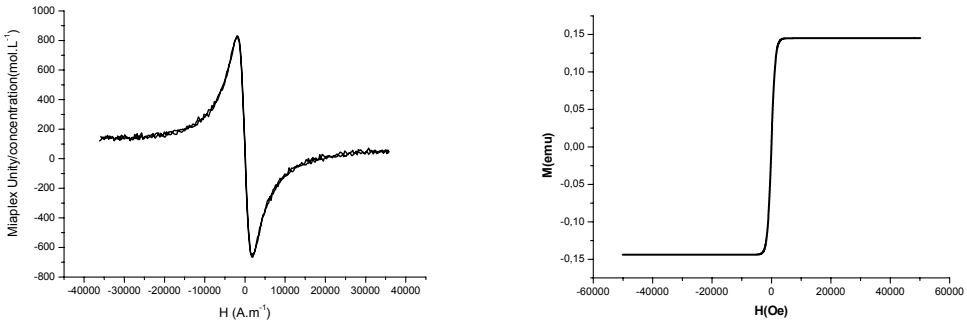
The principle of Prussian blue staining is that the ferric iron (Fe^{3+}) in the presence of ferrocyanide ion is precipitated as the highly colored and highly water-insoluble complex, potassium ferric ferrocyanide, Prussian blue. Cells were seeded on Petri dishes (Ø 30mm, density 50,000 cells per Petri dish), grown for 24 h and treated with γFe_2O_3@HMBP nanocrystals with or without magnet. The cells were then washed three times with PBS, fixed with paraformaldehyde (10 min) and dried at room temperature for 4 h. The attached cell monolayer was incubated with 5% potassium ferrocyanide (5 min), washed with PBS and then incubated again with solution containing 5% potassium ferrocyanide and 10% hydrochloric acid for 10 min and washed with distilled water three times. The iron particles in the cells were observed as blue dots using an optical microscope with phase contrast.

To determine the intracellular uptake of γFe_2O_3@Alendronate nanocrystals, fluorescence imaging was performed on human MDA-MB-231 breast carcinoma cells. For fluorescence microscopy, MDA-MB-231 cells were seeded on Petri dishes (Ø 30mm, density 50,000 cells per Petri dish) grown for 24 h and treated for various time (10 minutes, 1 hour and 6

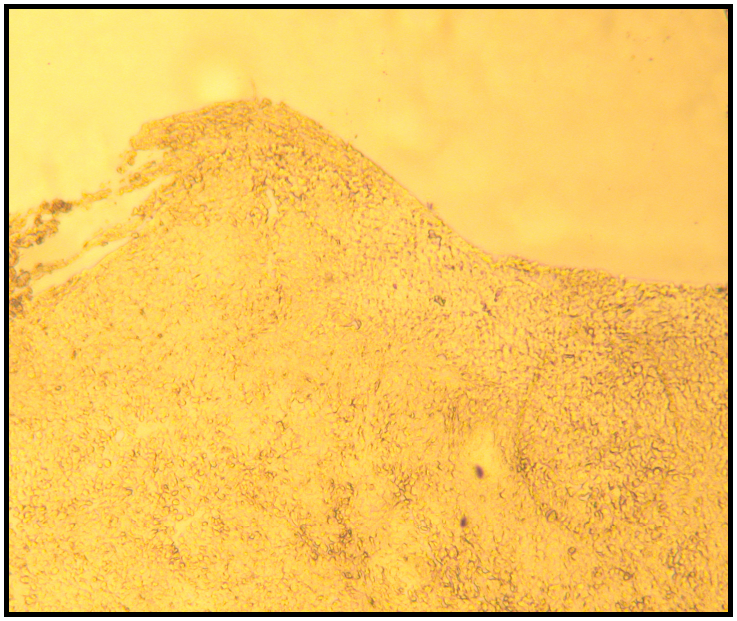
hours) with magneto-fluorescent nanoparticles ([Fe]= 10^{-4} mol.L^{-1}, [Ald]= 5 10^{-6} mol.L^{-1}, [Rho]= 0.25 10^{-6} mol.L^{-1}). Cells were washed twice in PBS and fixed with paraformaldehyde. For fluorescence microscopy, slides with coverslip bottom were used for imaging cells.

Xenografts in nude mice: All *in vivo* experiments were carried out with local ethical committee approval and accordingly to the UKCCCR guidelines. Animals were kept in a temperature-controlled room on a 12: 12 light-dark schedule with food and water ad libitum. MDA-MB-231 cells ($2x10^6$ cells) were inoculated subcutaneously (s.c) in 4-week-old athymic nude mice (nu/nu, Janvier, France, n= 24). Then, mice were arbitrarily placed in control (n= 6) and in each treated group (n= 6). The administration of Alendronate (7.5mg/kg) coupled or not with nanocrystals started 1 week after cell inoculation when tumors reached 30 mm^3. Control group received 0.1 mL of PBS 1X. Treatments or PBS solutions administration were injected near the tumor twice a week for 5 weeks. For nanocrystal treatments submitted to magnetic field, mice were anesthetized by intraperitoneal injection of 120 mg/kg ketamine and 6 mg/kg xylazine and a small magnet with a magnetic field of 0.3 T (Neodyme) were applied on the tumor for 30 min. Tumor volume was measured once a week along to major axes using callipers. Tumor volume (mm^3) was calculated as following: $V=(4/3)\pi R_1 x 2R_2$ where $R_1<R_2$. Each animal was weighted once a week during the treatment. Statistical significance was determined by the ANOVA using graph pad prism software. $P<0.05$ was considered significant.

Magnetic behavior



ESI 1: γFe_2O_3@Alendronate (a) second derivative of the magnetization (b) SQUID magnetization curves recorded at pH 7.4, in H_2O solutions.



ESI 2: Image of Perl's stained histological section of tumor treated with nanoparticles and with no applied magnetic field.