Supporting Information

Engineering red-emitting multi-functional nanocapsules for magnetic tumour targeting and imaging

Julie T-W Wang[1]*, Umberto Martino[1], Rehan Khan[2], Paul Southern[3], Dönüş Tuncel[2]*and Khuloud T. Al-Jamal[1]*

[1] School of Cancer and Pharmaceutical Sciences, Faculty of Life Science & Medicine, King's College London, UK

[2] Department of Chemistry and UNAM−National Nanotechnology Research Centre, Bilkent University, Turkey

[3] UCL Healthcare and Biomagnetics Laboratories, Royal Institution of Great Britain, London, UK

* To whom correspondence should be addressed.

E-mail: khuloud.al-jamal@kcl.ac.uk; dtuncel@fen.bilkent.edu.tr; tzu-wen.wang@kcl.ac.uk

Supplementary Methods

UV-Vis and fluorescence measurements

The absorption, excitation and emission spectra of PLGA-Oligomer was analysed by a UV-Vis spectrometer (Perkin Elmer Lambda 2S UV) and a fluorimeter (Perkin Elmer Lambda LS 50B Luminescence) respectively. PLGA-Oligomer was dissolved in THF and underwent serial dilution. The absorption spectra from 200 nm to 800 nm was investigated in single scan mode with a data interval of 0.1 nm at a speed of 240 nm/min. Slit value was fixed at 2.0. Fluorescence excitation and emission spectra were obtained in single scan mode at a scan speed of 200 nm/min and a slits aperture of 5.0/5.0. A quartz cuvette with 1 cm optic path was used for the measurements.

Supplementary Figures

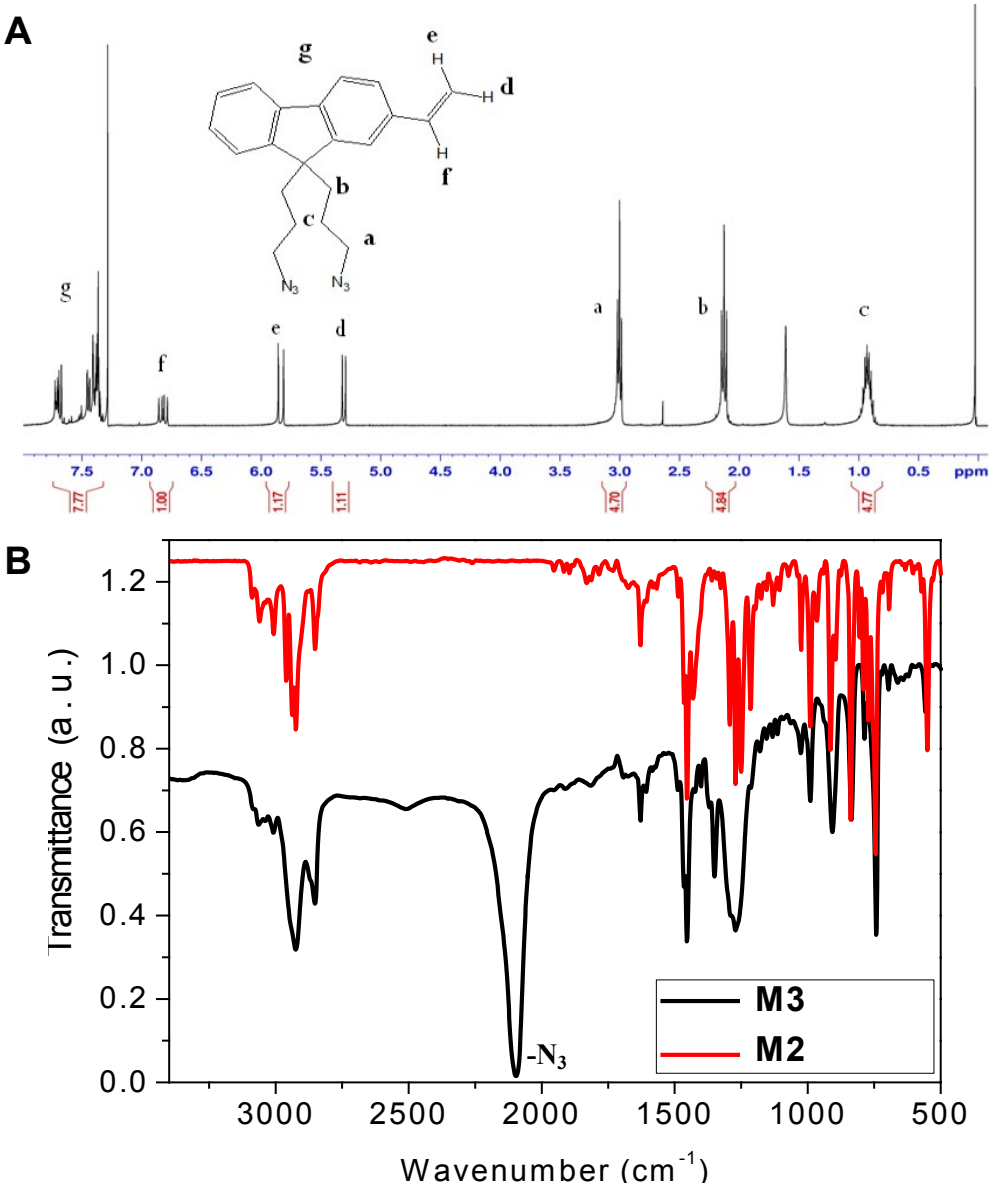

Figure S1: Characterisation of M2 and M3. (A) ^{1}H-NMR (400 MHz, CDCl3, 25°C) spectrum of M3. **(B)** FTIR spectra of M2 and M3.

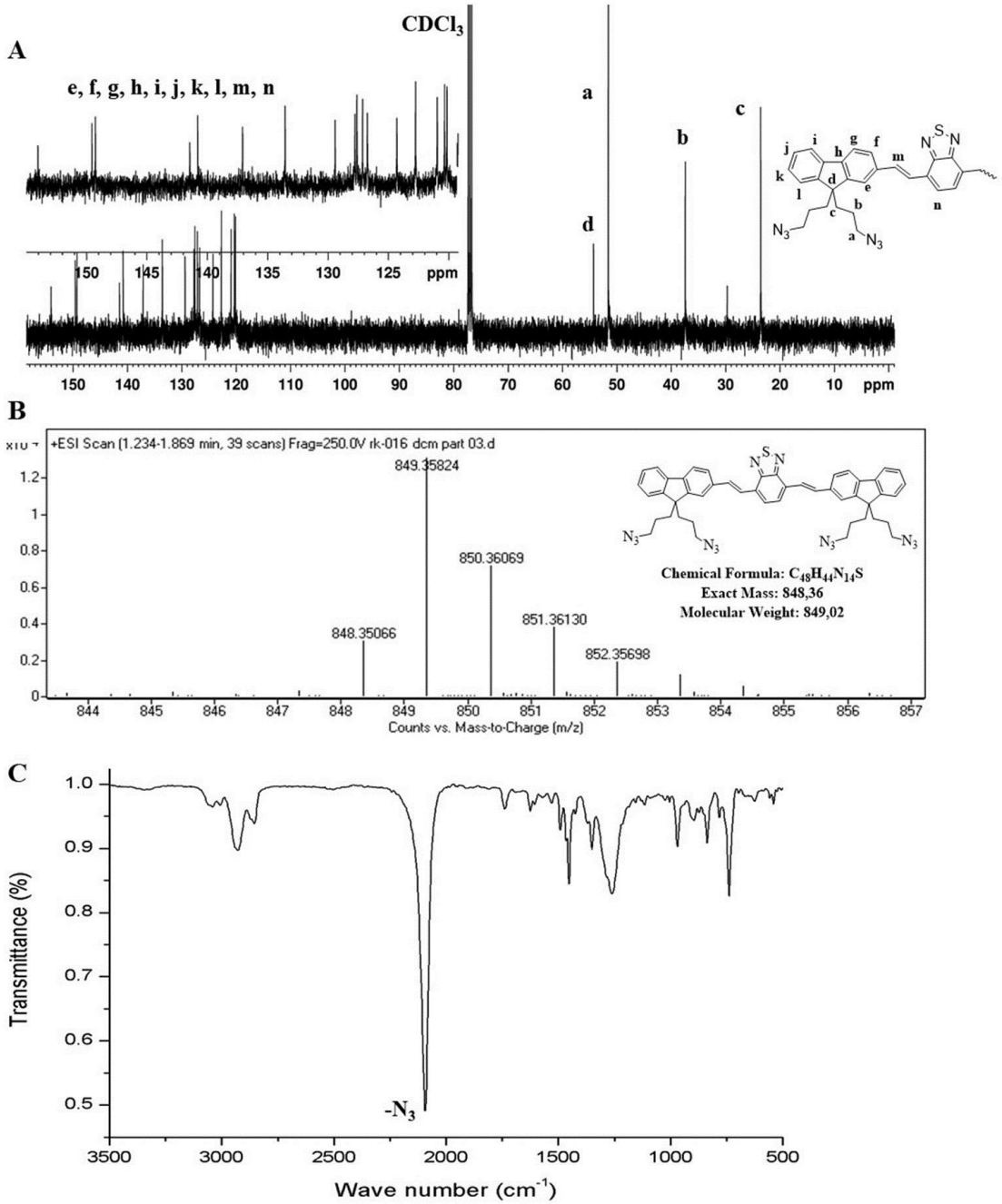

Figure S2: Characterisation of Oligomer 1. (A) ^{13}C-NMR (400 MHz, $CDCl_3$, 25^{o}C) spectrum **(B)** HRMS-TOF spectrum **(C)** FTIR spectrum**.**

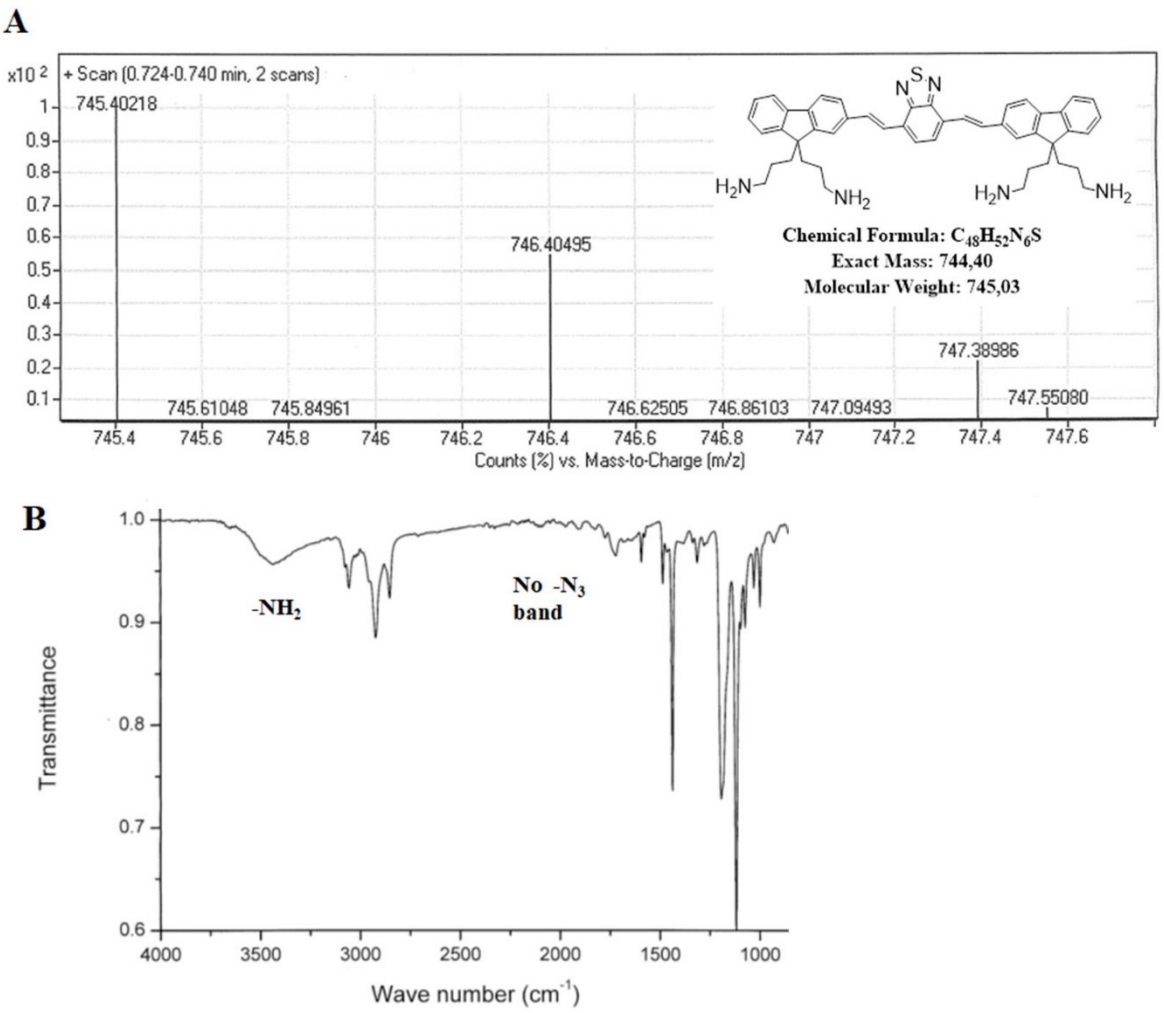

Figure S3: Characterisation of Oligomer 2. (A) HRMS-TOF spectrum **(B)** FTIR spectrum.

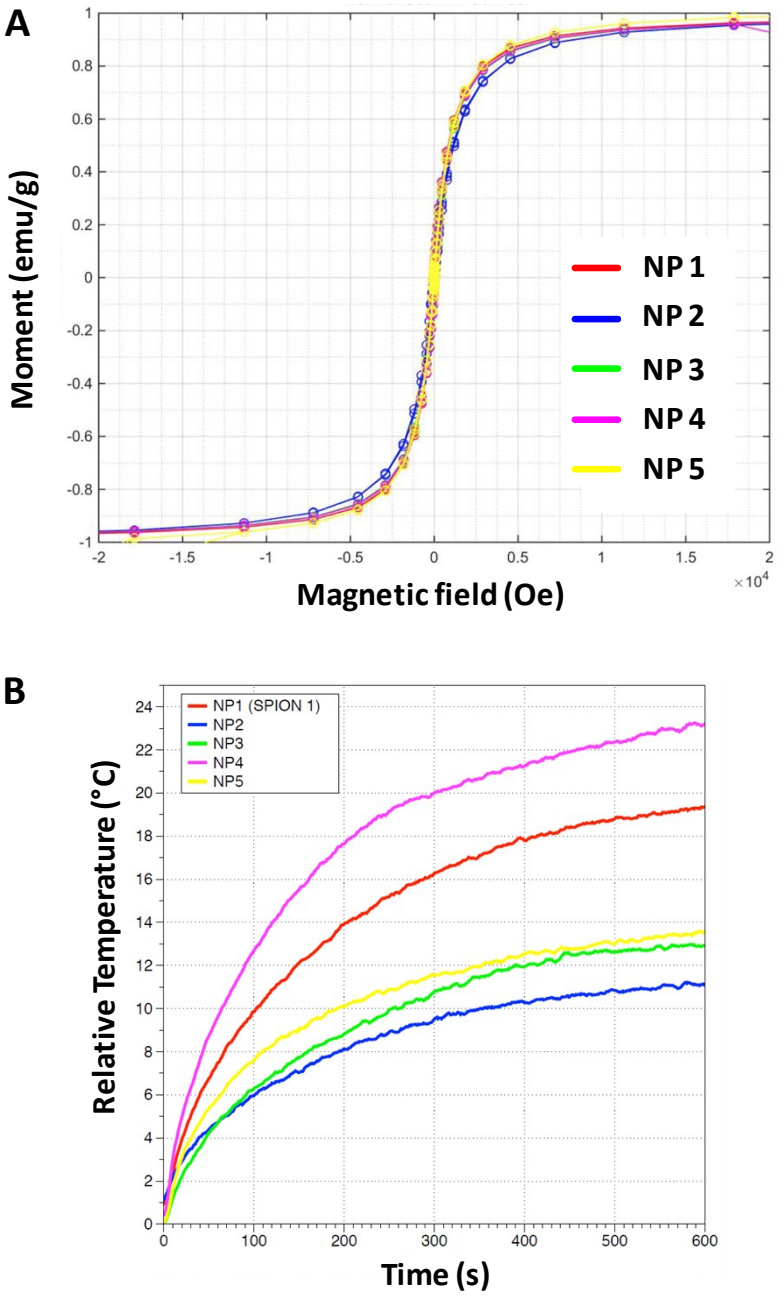

Figure S4: Characterisation of Fe_3O_4 nanoparticles (NPs) synthesised by the high-temperature decomposition method. (A) Magnetization hystresis curves measured by SQUID. The curves were normalised by the weight of the magnetic material quantified by ICP-MS **(B)** Comparison of Heating curves.

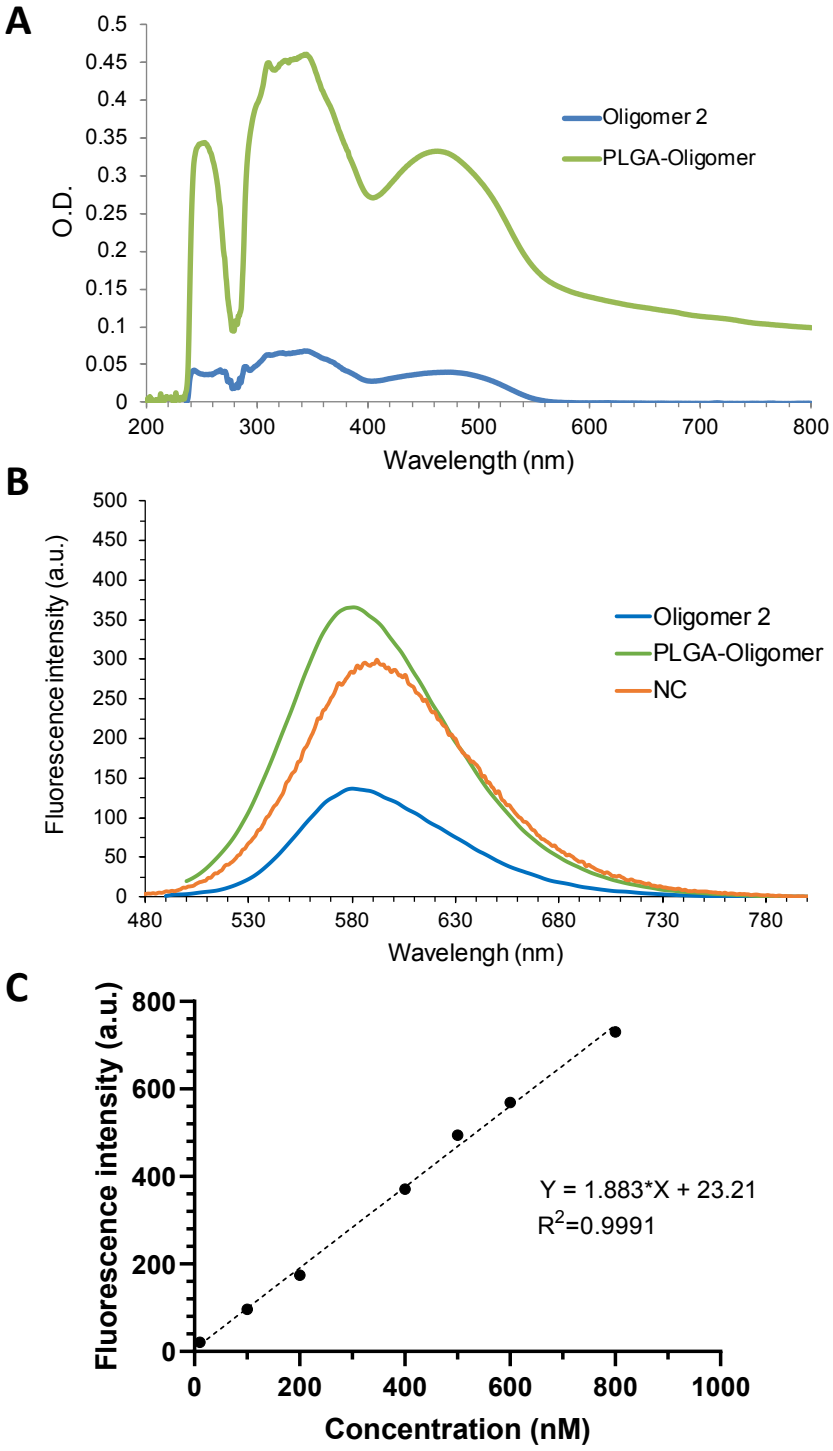

Figure S5: Optical properties assessments of Oligomer 2, PLGA-Oligomer and NC. (A) Absorption spectra of Oligomer 2 and PLGA-Oligomer. **(B)** Fluorescence spectra of Oligomer 2, PLGA-Oligomer and NC. **(C)** Calibration curve of PLGA-Oligomer. Oligomer 2 and PLGA-Oligomer were dissolved in THF while NC was dispersed in PBS after purification. For the fluorescence measurements in B, excitation was performed at 463 nm. For the calibration curve in C, the measurements were performed with excitation at 463 nm and the emission at 587 nm. Fluorescence measurements of PLGA-Oligomer by fluorimetry.

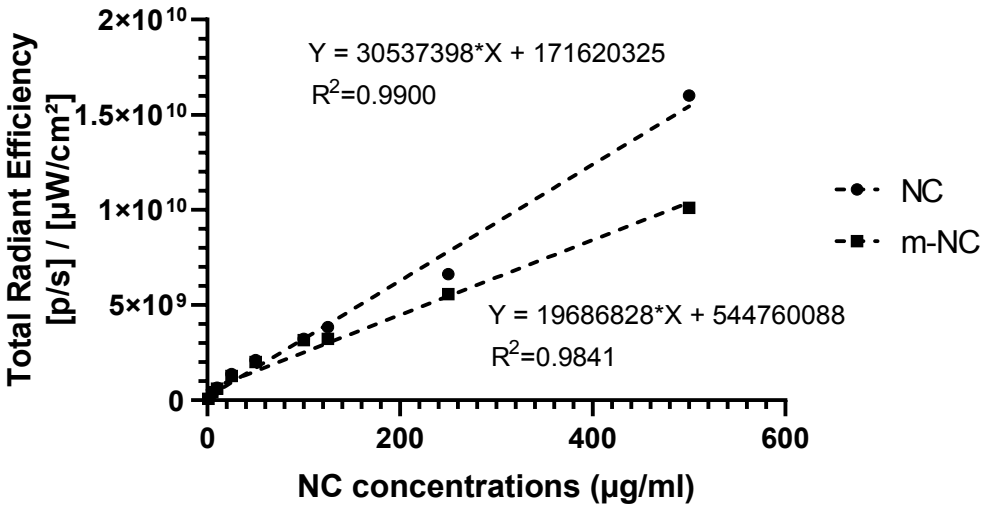

Figure S6: Fluorescence measurement of NC and m-NC at different concentrations by IVIS Lamina III optical imaging system. The concentrations of NC and *m*-NC were up to 500 µg/ml at polymer concentration. Fluorescent signals were measured at the excitation of 500 nm and emission at 620 nm.

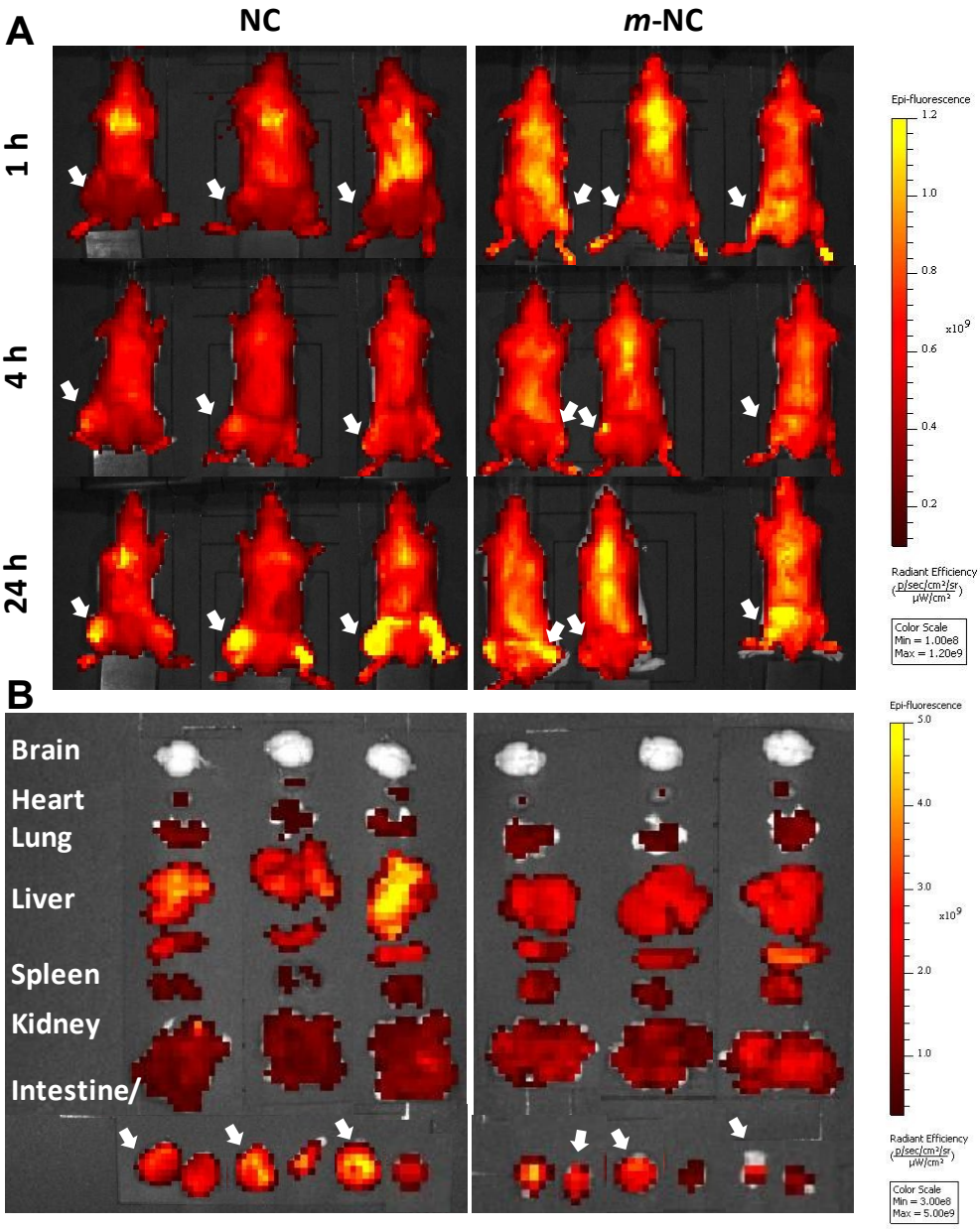

Figure S7. *In vivo* whole-body optical imaging and *ex vivo* biodistribution of NC and m-NC in CT26 tumour-bearing Balb/c mice after intravenous administration. (A) Whole-body images from dorsal views of mice at 1, 4 and 24 h post injection. **(B)** *Ex vivo* images of tissues excised at 24 h post injection. Balb/c mice were transplanted subcutaneously with CT26 tumours at the lower flanks. When tumours reached ~7-8 mm in diameter, mice were *i.v.* injected with NC or *m*-NC (250 mg polymer/kg and 50 mg SPION/kg). Magnetic field was applied by placing a permanent magnet (0.515 T) at one side of the tumours (most of them were the left tumour, except one tumour was on the right side) for 1 h (pointed by arrows). All images were obtained by IVIS Lumina III (λ_{ex}: 500; λ_{em}: 620 nm). Data were analyzed by Living Image®4.3.1 Service Pack 2 software.

Table S1: **Synthesis and characterisation of Fe_3O_4 nanoparticles under different nucleation and growth duration**

Fe_3O_4 nanoparticles (NPs)	Nucleation time (min)	Grown time (min)	Weight loss (%)[a]	Intrinsic loss power (ILP, nHm^2/kg) [b]
NP1	60	30	13.47	0.04
NP2	60	120	32.97	0.02
NP3	60	60	32.36	0.03
NP4	120	60	16.90	0.06
NP5	30	30	20.29	0.03

[a] The weight loss related to oleic acid coating was measured by thermogravimetric analysis (TGA).
[b] The calculation of ILP values were based on the iron content measured by ICP-Ms but did not account for the oleic acid coating. The values were therefore lower than the actual values.

Table S2: **Physicochemical characterisation of different formulations of NCs prepared by the emulsification/solvent evaporation method.**

PLGA (%)	PLGA-Oligomer (%)	Diameter (nm)*	PDI*	Zeta-potential (mV)*
90	10	222.9 ± 2.1	0.156	-47.5 ± 1.3
80	20	253.6 ± 3.5	0.200	-32.3 ± 1.2
50	50	362.6 ± 7.1	0.215	-29.4 ± 1.7

*Measurements were performed by dynamic light scattering in 10 mM NaCl (n=3).

Table S3: Physicochemical characterisation of PLGA-PEG NCs formulations used for the *in vivo* studies after 10X concentration.

10x NCs	Diameter (nm)*	PDI*	Zeta-potential (mV)*
NC	214.5 ± 0.8	0.242	-19.8 ± 1.0
***m*-NC**	242.0 ± 3.9	0.238	-10.5 ± 1.0

*Measurements were performed by dynamic light scattering in 10 mM NaCl (n=3)