Electronic Supporting Information

Following stability of amphiphilic nanoaggregates by using intermolecular energy transfer

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1 Experimental

1.1 Materials

Reagents and solvents were obtained from Sigma–Aldrich (Bornem, Belgium), Acros Organics (Geel, Belgium), ChemLab (Zedelgem, Belgium), Matrix Scientific (Columbia, USA) and BDH Prolabo (Leuven, Belgium), and were used without further purification. Lanthanide(III) chloride hexahydrates was obtained from Sigma–Aldrich (Bornem, Belgium).

1.2 Instrumentation

^{1}H and ^{13}C spectra were recorded by using a Bruker Avance 300 or 400 spectrometer (Bruker, Karlsruhe, Germany), operating at 300 or 400 MHz for ^{1}H and 75 or 100 MHz for ^{13}C.

IR spectra were measured by using a Bruker Vertex 70 FT-IR spectrometer (Bruker, Ettlingen, Germany).

Mass spectra were obtained by using a Thermo Finnigan LCQ Advantage mass spectrometer. Samples for the mass spectrometry were prepared by dissolving the product (2 mg) in DMSO (1 mL). The resulting solution was injected at a flow rate of 5 μL min^{-1}.

Accurate mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 µL/min and spectra were obtained in positive ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass.

TXRF measurements were performed on a Bruker S2 Picofox (Bruker, Berlin, Germany) with a molybdenum source. Lanthanide(III) solutions of approximately 1000 ppm in milli-Q water were prepared and 20 µL of this solution was mixed with 20 µL of a 1000 ppm Chem-Lab gallium standard solution (1000 µg/mL, 2-5% HNO_3). 2 µL of this mixture with similar Ln(III)-Ga(III) concentrations was put on a Bruker AXS quartz glass sample plate for measurement.

Solutions were dispersed in a 180 W Bandelin Sonorex RK 510 H sonicator equipped with a thermostatic heating bath.

Absorption spectra were measured on a Varian Cary 5000 spectrophotometer on freshly prepared aqua solutions in quartz Suprasil cells (115F-QS) with an optical pathlength of 1 cm.

Emission spectra and luminescence decays of Ln^{III} nanoaggregates were recorded on an Edinburgh Instruments FS920 steady state spectrofluorimeter. This instrument is equipped with a 450W xenon arc lamp, a high energy microsecond flashlamp mF900H and an extended red-sensitive photomultiplier (185–1010 nm, Hamamatsu R 2658P). All spectra are corrected for the instrumental functions. Luminescence decays were determined under ligand excitation (370 nm) monitoring emission of the $^5D_0 \rightarrow {}^7F_2$ transition of Eu^{III} complexes. Luminescence decays were analyzed using Edinburgh software; lifetimes are averages of at least three measurements. Quantum yields were determined by a comparative method with a standard reference; estimated experimental errors for quantum yield determination ±10%. Rhodamine 101 (Sigma) in ethanol (Q=100%) was used as a standard for the complexes. Solutions with a concentration of about 10^{-5} M were prepared to obtain an optical density lower than 0.05 at the excitation wavelength.

Two photon excitation measurements were conducted using an inverted optical microscope (TiU, Nikon) equipped with a piezoelectric stage (Physik Instrument (PI) GmbH & Co.). A femtosecond laser (800 nm, 80 MHz, 120 fs, Maitai-HP, Spectra Physics) was reflected by a dichroic mirror (T750 spxrxt, Chroma) and was focused on the sample by an objective lens (PlanApo, x60, N.A. 0.98, Nikon). 2PE emission from 550 nm to 720 nm was collected by a spectrograph (iHR320, Horiba) and 2PE spectra were recorded by a cooled charge-coupled device (CCD) camera (DU920P, Andor). A shortpass cutoff filter (ET750SP-2P, Chroma) was placed in front of the spectrograph in order to reject the excitation laser light.

Relaxometry: ^1H T_1 measurements were performed at 310 K, 1.41 T on a Bruker Minispec mq-60.

DLS measurements: Photon correlation spectroscopy was performed at 37 °C with a BIC multiangle laser lightscattering system with a 90° scattering angle (Brookhaven Instruments Corporation, Holtsville, USA). The intensity weighted diameter was measured on 0.1 wt% diluted suspensions in Milli-Q water, sonicated for 15 mins, passed through a 200 nm nylon filter before analysis and calculated by a non-negatively constrained least-squares (multiple pass) routine.

Confocal laser scanning microscopy was performed on an FV1000 (Olympus, Tokyo, Japan) with a UPlanSapo100xO (NA 1.40) objective lens. A 405-nm laser diode was guided to the sample with a DM 405/488/559/635 and emission was detected in two channels, 410-470 nm using a diffraction grating and 575-675 nm through a band pass filter for the donor and acceptor signals respectively. Image processing and analysis was performed with Fiji and home-developed software on MATLAB (version 7.11.0.584, MathWorks, Natick, MA).

Read out of the MTT assay was performed on a Safire^2 plate reader (Tecan, Männedorf, Switzerland).

1.3 Synthesis and characterization of ligands and complexes

DTPA-bisanhydride was synthesized as previously reported.[1]

6-nitro-2-oxo-2H-chromene-3-carboxylic acid[2, 3]

2-hydroxy-5-nitrobenzaldehyde (1 g, 1 eq.), Meldrum's acid (1.73 g, 2 eq.) and ytterbium triflate hydrate (0.192 g, 27 wt% Yb^{3+}, 5 mol%) were mixed intimately with a mortar and pestle. The resulting powder was stirred at 80 °C for 1 hour, dissolved in warm ethyl acetate, filtered through celite then recrystallised from ethyl acetate.

Yield: 1.41 g, 78%; ^1H NMR (400 MHz, D_2O, 25 °C, TMS) δ = 7.55 (d, 1 H) 8.48 (dd, 2 H) 8.67 ppm (s, 1 H). ESI-MS (-ve mode): *m/z*: calcd 234.1 $[M-H]^-$, found 233.9 $[M-H]^-$, 468.6 $[2M-H]^-$.

N-decyl-6-nitro-2-oxo-2H-chromene-3-carboxamide (nitro-coumarin amide)

6-nitro-2-oxo-2H-chromene-3-carboxylic acid (0.46 g, 1 eq.) and HATU (0.9 g, 1.2 eq.) were dissolved in dry DMF (10 mL) and DIPEA (0.31 g, 1.2 eq.) was added dropwise. Decylamine (0.37 g, 1.2 eq.) was dissolved with DIPEA (0.31 g, 1.2 eq.) in dry DMF (5 mL), added dropwise to the first solution and mixed overnight under argon atmosphere. The reaction mixture was then mixed with ethyl acetate and washed with brine solution 3 times. After drying the organic phase with magnesium sulfate and filtration, the filtrate was concentrated to allow recrystallization of the target material from ethyl acetate.

Yield: 0.48 g, 65 %; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 0.88 (t, 3 H), 1.26 (br, 14 H), 1.64 (m, 2 H), 3.48 (t, 2 H), 7.54, 7.57 (d, 1 H), 8.49, 8.50, 8.52, 8.53 (dd, 1 H), 8.62, 8.62 (d, 1 H), 8.98 ppm (s, 1 H). ESI-MS (+ve mode): *m/z*: calcd 374.4 $[M-H]^-$, found 373.1 $[M-H]^-$.

6-amino-N-decyl-2-oxo-2H-chromene-3-carboxamide (amino-coumarin amide)

N-decyl-6-nitro-2-oxo-2H-chromene-3-carboxamide (0.48 g, 1 eq.) was dissolved in THF/MeOH (9:1) with warming and reduced using H_2 and palladium/carbon (0.1 g, 5 % Pd, 20 wt%) by mixing overnight. After filtration through celite, the filtrate was concentrated to yield the pure target material.

Yield: quantitative; ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 0.88 (t, 3 H), 1.26 (br, 14 H), 1.64 (m, 2 H), 3.48 (t, 2 H), 6.88, 6.88 (d, 1 H), 6.97, 6.98, 7.00, 7.01 (dd, 1 H), 8.79 ppm (s, 1 H). ESI-MS (+ve mode): *m/z*: calcd 345.22 $[M+H]^+$, found 345.1 $[M+H]^+$, 688.8 $[2M+H]^+$.

DTPA-BC_{10}Coumarinamide and DTPA-BC_{12}

DTPA-bisanhydride (0.22 g or 2 g, 1 eq.) was dissolved in dry DMF and warmed to 50 °C. 6-amino-N-decyl-2-oxo-2H-chromene-3-carboxamide or decylamine (0.44 g or 2.179 g, 2.1 eq.) was dissolved in dry chloroform, added dropwise to the first solution and mixed for overnight.

DTPA-BC_{10}Coumarinamide: Yield: 0.38 g, 59%; ^{1}H NMR (300 MHz, DMSO, 25 °C, TMS) δ = 0.84 (t, 6 H), 1.24 (br, 28 H), 1.52 (m, 4 H), 2.29 (br, 8 H), 3.30 (m, 4 H), 3.48 (m, 10 H), 7.23, 7.26 (d, 2 H), 8.21, 8.22, 8.24, 8.25 (dd, 2 H), 8.64, 8.65 (d, 2 H), 9.12 (t, 2 H), 10.98 ppm (s, 2 H). ^{13}C NMR (100 MHz, pyridine d-5, 25 °C, TMS) δ = 14.41, 22.56, 26.91, 29.17, 29.21, 29.37, 29.44, 29.46, 31.77, 40.31 (under solvent), 50.50, 51.19, 52.19, 52.86, 55.33, 116.63, 118.52, 119.42, 119.54, 126.17, 136.1, 147.49, 150.06, 160.79, 161.25, 170.03, 173.03, 173.4 ppm. ESI-MS (+ve mode): *m/z*: calcd 1046.6 $[M+H]^+$, found 1046.5 $[M+H]^+$.

DTPA-BC_{12}: Yield: 2.8 g, 69%; ^{1}H NMR (300 MHz, DMSO, 25 °C, TMS) δ = 0.88 (t, 6 H), 1.20, 1.23 (br, 32 H), 1.35 (m, 4 H), 1.67 (m, 4H), 3.19 (br, 8 H), 3.52 (m, 4 H), 3.75, 3.80, 3.88 ppm (m, 10 H). ESI-MS (+ve mode): *m/z*: calcd 728.6 $[M+H]^+$, found 728.5 $[M+H]^+$.

(S)-2-(4-(((2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl)amino)benzamido)-N1,N5-didodecylpentanediamide (Folate-BC_{12})

Folic acid (0.5 g, 1 eq.) was dissolved in dry DMF (20 mL) with the assistance of sonication. HATU (0.95 g, 2.2 eq.) and DIPEA (0.32 g, 2.2 eq.) were added and the solution was mixed for 15 min. Dodecylamine (0.46 g, 2.2 eq.) and DIPEA (0.32 g, 2.2 eq.) were mixed in dry DMF (5 mL) and added dropwise to the first solution and mixed overnight under argon atmosphere. After evaporating most of the solvent, the crude product was mixed with ethyl acetate which resulted in a precipitate. The organic phase was washed with brine 3 times and the precipitate remained in the organic phase. The organic phase was dried with magnesium sulfate and filtered. The precipitate which remained in the magnesium sulfate was washed with ethyl acetate then extracted with warm DMF. Evaporation of the solvent resulted in the pure target compound.

Yield: 0.71 g, 81%; ^{1}H NMR (300 MHz, DMSO, 25 °C, TMS) δ = 0.85 (t, 6 H), 1.22 (br, 38 H), 1.87 (m, 2 H), 2.11 (m, 2H), 2.99 (m, 4 H), 4.29 (m, 1 H), 4.49 (d, 2 H), 6.62, 6.65 (d, 2 H), 6.97 (m, 1 H), 7.12 (m, 2 H), 7.65, 7.67 (d, 2 H), 7.82 (t, 2 H), 7.99, 8.01 (d, 1 H), 8.64 (s, 1 H), 11.71 ppm (br, 1 H). ^{13}C NMR (400 MHz, DMSO, 25 °C, TMS) δ = 14.42, 22.56, 26.76, 26.87, 29.17, 29.21, 29.47, 29.51, 31.76, 32.64, 38.89, 38.93, 46.41, 53.84, 60.29, 111.58, 121.92, 129.47, 132.55, 149.06, 151.21, 154.55, 154.58, 157.03, 166.49, 172.00, 172.07 ppm. ESI-MS (-ve mode): *m/z*: calcd 810.5 $[M+Cl]^-$, found 810.3 $[M+Cl]^-$.

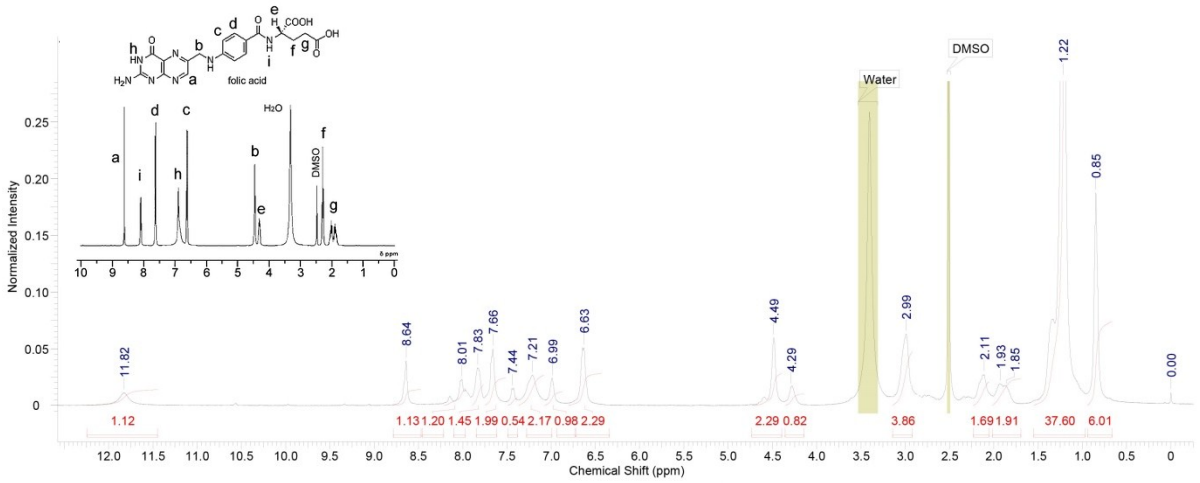

Fig. S1. ^{1}H NMR spectrum of folate-BC_{12}. Reference ^{1}H NMR spectrum of folic acid is provided (inset).

Lanthanide(III) complexes

The ligand (0.1 g, ±0.1 mmol, 1 eq.) was dissolved in dry pyridine (5 mL) and the $LnCl_3$ hexahydrate salt (0.11 mmol, 1.1 eq.) was added after grinding with a motar and pestle. The mixture was sonicated briefly and mixed for 15 mins before the pyridine was removed at 40 °C. The crude product was suspended in acetone and filtered over a Büchner. The solid was washed with an acetone/water 50:50 mixture (2 x 5 mL) to remove any free Ln(III) ions, rinsed again with acetone (2 x 10 mL) and dried in vacuo. The absence of free lanthanide ions was checked with an arsenazo indicator.

Gd(III)-DTPA-BC_{10}Coumarinamide: Yield: 74%; IR: $\tilde{\nu}_{max}$ = 1609 (COO^- asym. stretch), 1508 (amide II), 1396 cm^{-1} (COO^-sym. stretch); ESI-MS (-ve mode): *m/z*: calcd 1199.4 $[M-H]^-$, found 1199.4 $[M-H]^-$, 1277.0 $[M+Br]^-$. HRMS (+ve mode): *m/z*: calcd 1201.4457 $[M+H]^+$, found 1201.4464 $[M+H]^+$. The complex decomposes above 300 °C.

Eu(III)-DTPA-BC_{12}: Yield: 55%; IR: $\tilde{\nu}_{max}$ = 1608 (COO^- asym. stretch), 1508 (amide II), 1396 cm^{-1} (COO^-sym. stretch); ESI-MS (+ve mode): *m/z*: calcd 878.5 $[M+H]^+$, found 878.4 $[M+H]^+$. HRMS (+ve mode): *m/z*: calcd 878.4515 $[M+H]^+$, found 878.4502 $[M+H]^+$. The complex decomposes above 300 °C.

1.3 Preparation of NAs

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, 8.8 mg, 3 eq.) and the amphiphilic complex (Gd(III) 5 mg, ±0.04 mmol, 1 equiv. and Eu(III) 3.5 mg, ±0.04 mmol, 1 eq.) were dissolved in a 1:1 chloroform/methanol solution (2 mL). After evaporation of the solvents in from flask with septum and needle fitted in a vacuum oven at 50 °C, a thin film was obtained which was rehydrated with hot water (2 mL, 70 °C). To improve the solubility, the suspension was sonicated in a 180 W sonicator with a thermostatic bath at 65 °C for 15 min. Polyoxyethylene sorbitan monooleate or Tween 80® (34 mg, 6.5 eq.) was added as a surfactant followed by another 15 min of sonication to fulfil the process of nanoaggregate formation. Water was evaporated in a flask with septum and needle fitted in a vacuum oven overnight at 50 °C leaving a thin film. A small amount of sample was removed for DLS measurements. For preparation of samples for relaxometry measurements, the thin film was rehydrated with Milli-Q water (2 mL), sonicated for 15 mins and passed through a 200 nm nylon filter. The concentration of lanthanide(III) was analysed by TXRF before relaxometric measurements.

1.4 Cell culture stock solution

Preparation of the NAs was identical to section 1.3 with a stock concentration of 1.25 mM of both Gd(III) and Eu(III) complexes.

1.5 cell culture and sample preparation for imaging

HeLa cells were maintained in Dulbecco's modified Eagles medium (DMEM; Invitrogen, Carlsbad, California) without phenol red supplemented with 10% fetal bovine serum (FBS, Invitrogen) at 37°C under 5% CO2 atmosphere. For imaging, the cells were grown on 35-mm glass bottom dishes (MatTek, Ashland, Massachusetts). NAs in DMEM were added to the cells 24 hours before imaging with a final concentration of 50 µM. Before imaging, cells were washed with Hank's Balanced Salt Solution supplemented with both calcium and magnesium (HBSS; Invitrogen). Imaging was performed in HBSS.

1.6 MTT assay

The MTT assay (Life technologies) was performed according to manufactures instructions following the quick protocol. HeLa cells were used for the cell viability assay. For each condition, 8 measurements were performed.

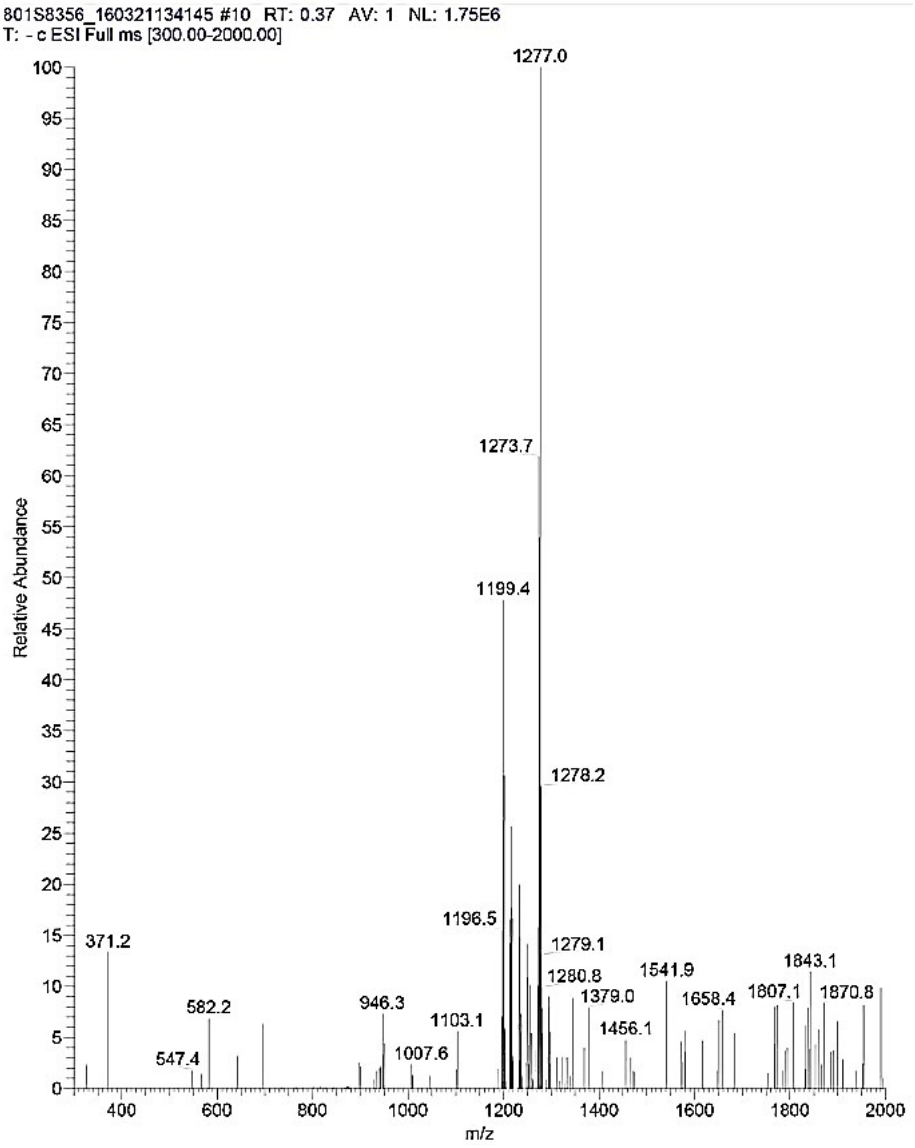

Fig. S2. ESI-MS Gd(III)-DTPA-BC_{10}Coumarin.

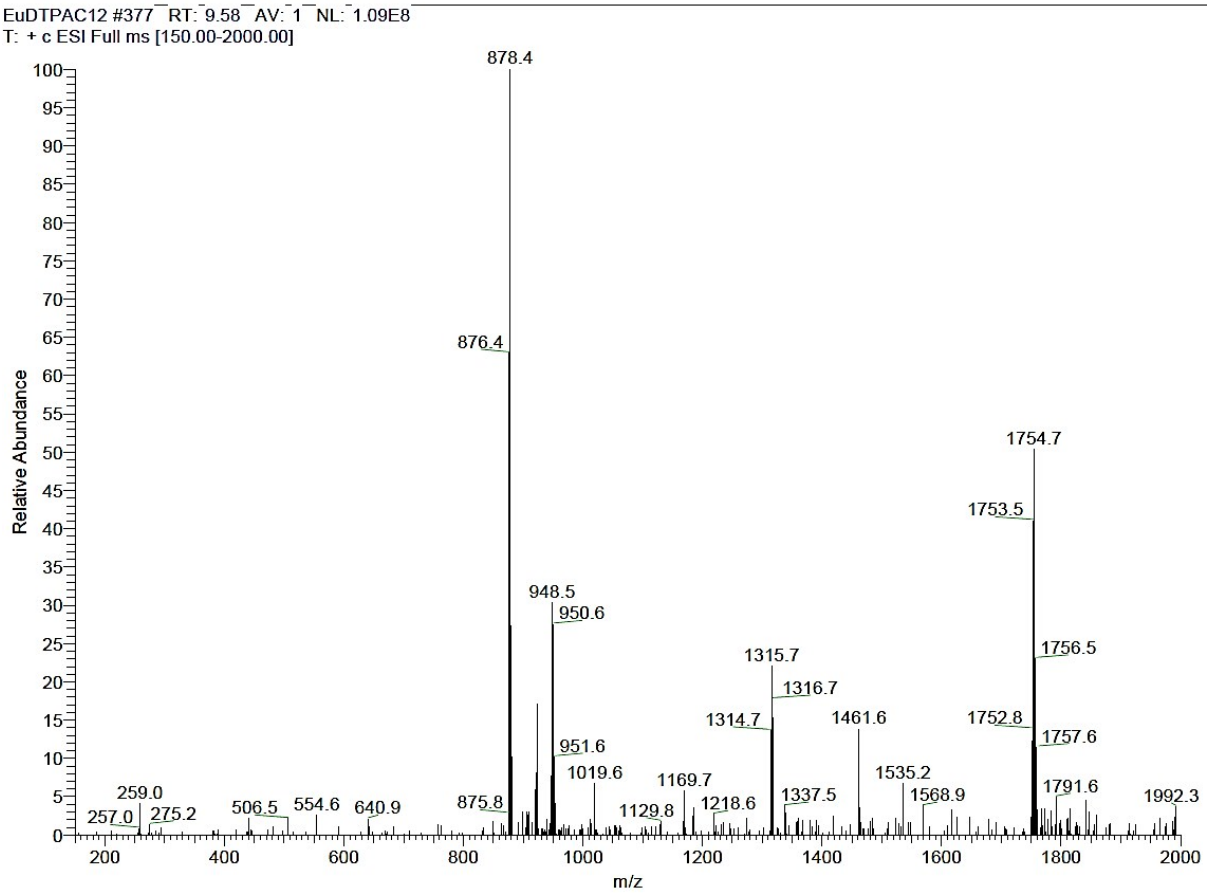

Fig. S3. ESI-MS Eu(III)-DTPA-BC_{12}.

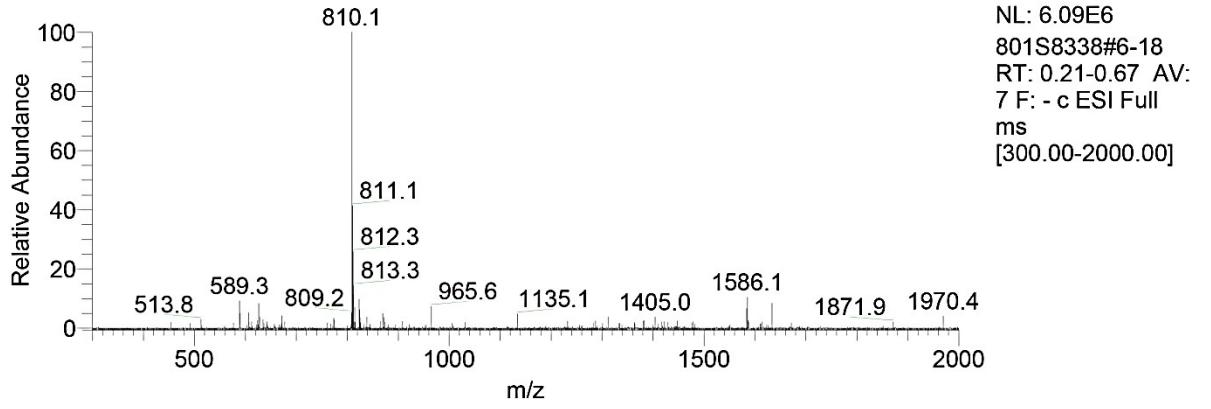

Fig. S4. ESI-MS Folate-BC_{12}.

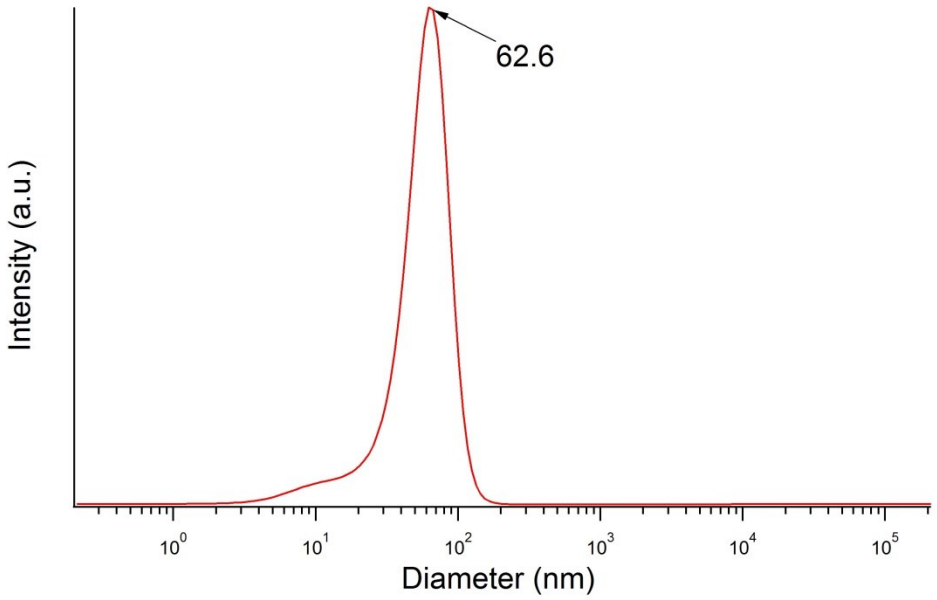

Fig. S5. DLS non targeted NAs (without folate).

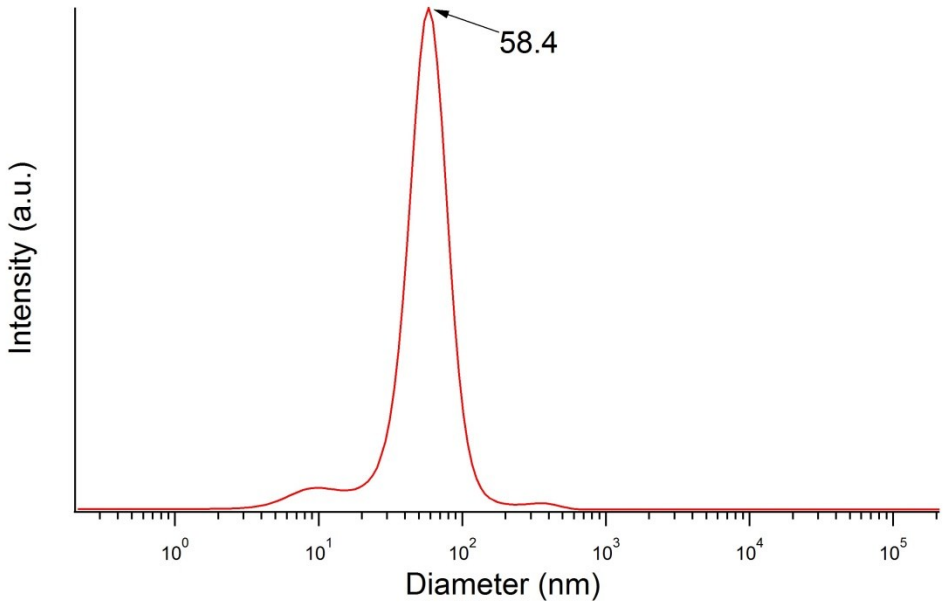

Fig. S6. DLS targeted NAs (with folate).

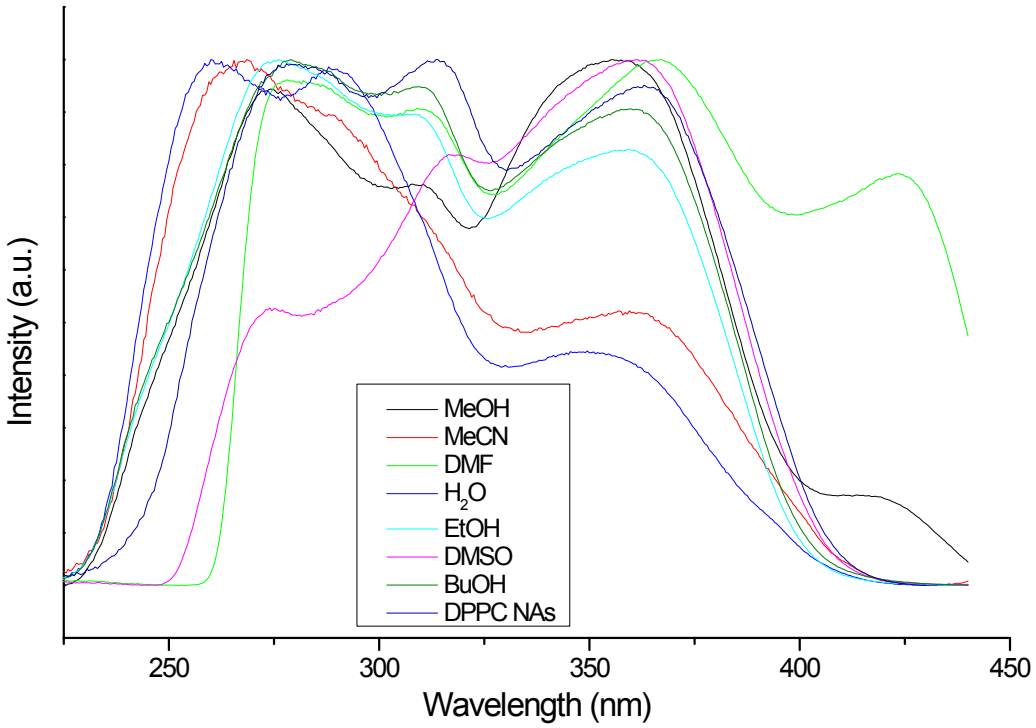

Fig. S7. Excitation and emission spectra Gd-DTPA-BC10coumarin in various solvents and dispersed in DPPC NAs (excitation spectrum taken while monitoring 450 nm, 10^{-4} M, 298 K).

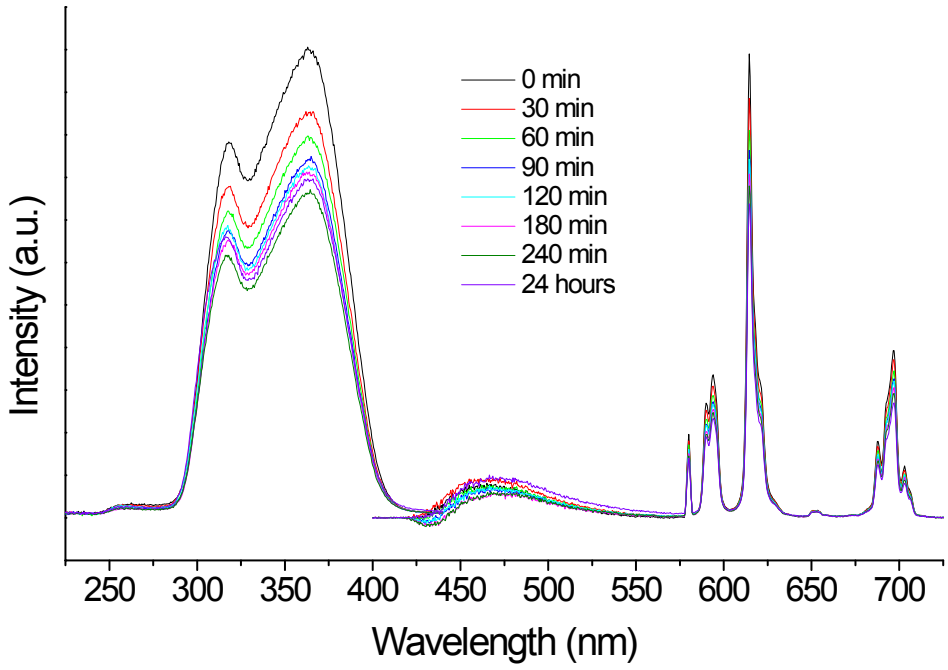

Fig. S8. Excitation and emission spectra of 1:1 FBS with Gd-DTPA-BC10coumarin/Eu-DTPA-BC12 DPPC NAs (FBS background subtracted, excitation at 370 nm and excitation spectrum taken while monitoring 615 nm, water, 10^{-4} M, 298 K).

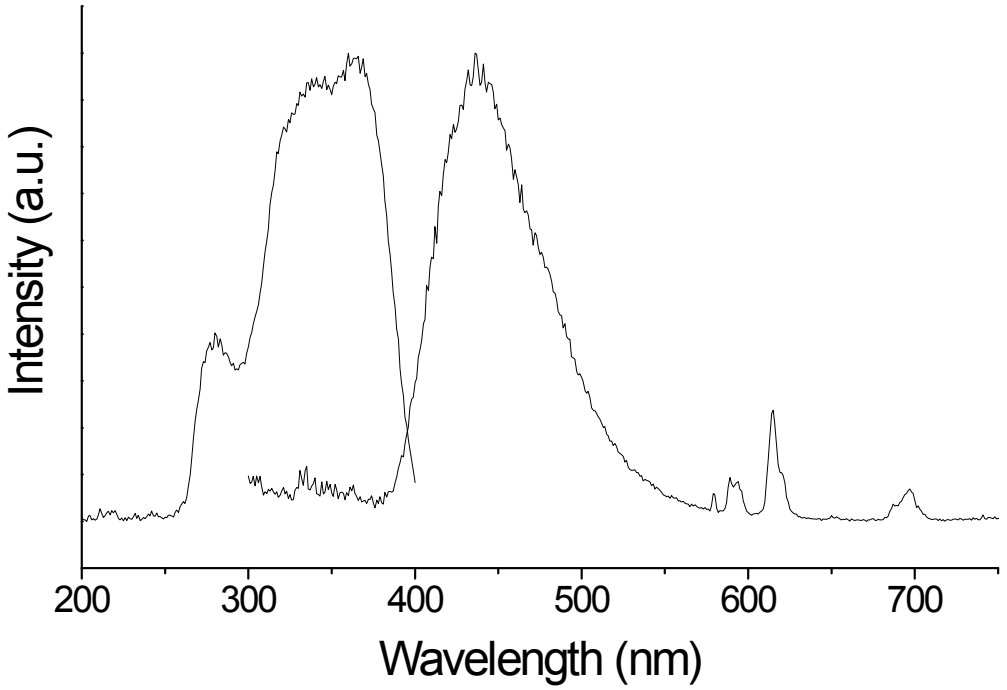

Fig. S9. Normalised and corrected excitation and emission spectrum Gd-DTPA-BC10coumarin and Eu-DTPA-BC12. Excitation wavelength is 370 nm and excitation spectrum is taken while monitoring 615 nm (MeOH:$CHCl_3$ 1:1, 10^{-4} M, 298 K).

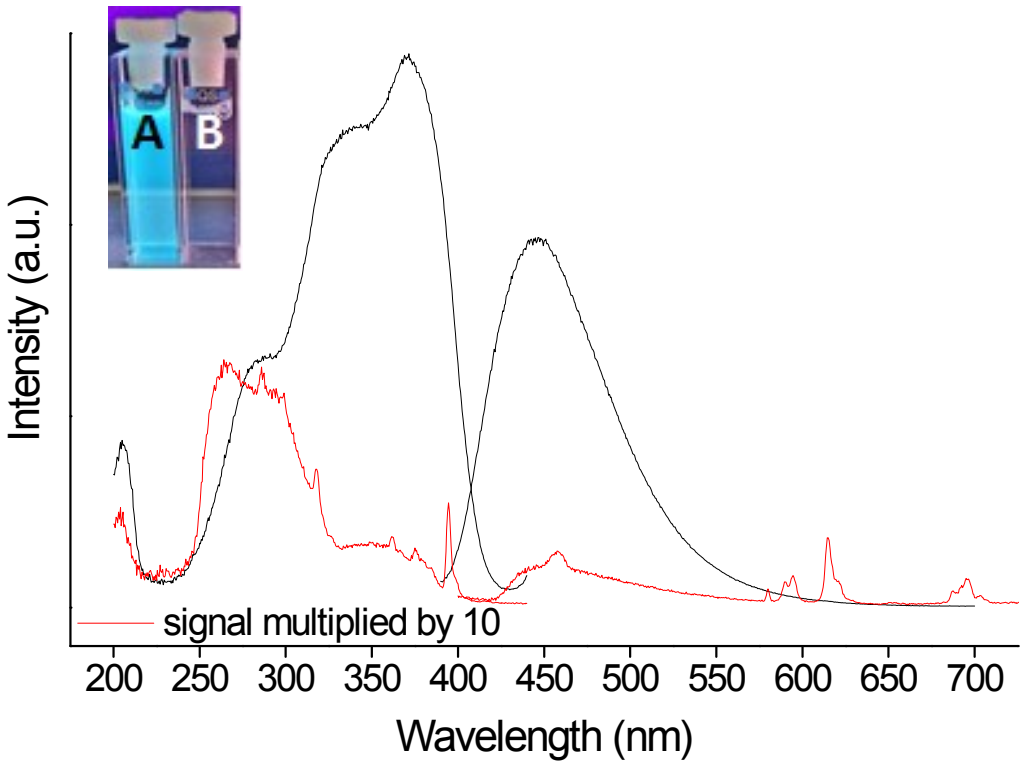

Fig. S9. Excitation and emission spectra Gd-DTPA-BC10coumarin DPPC NAs (black, cuvette A), Eu-DTPA-BC12 DPPC NAs (red, cuvette B) (water, 10^{-4} M, 298 K).

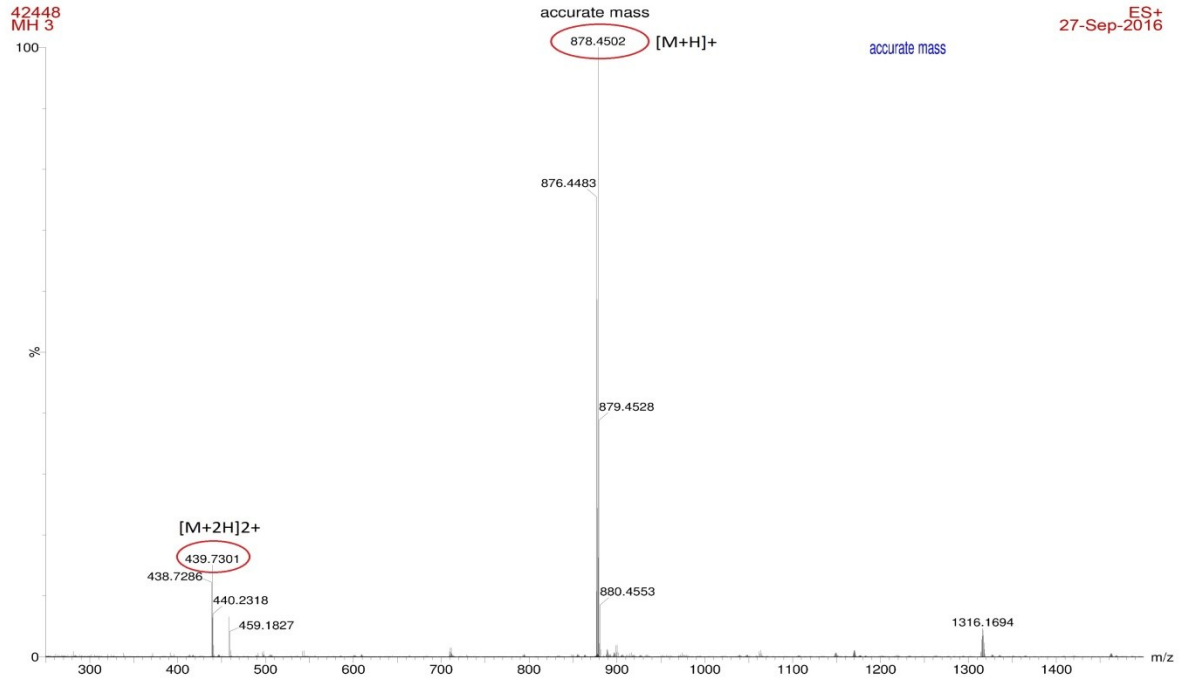

Fig. S10. HRMS Eu-DTPA-BC_{12}.

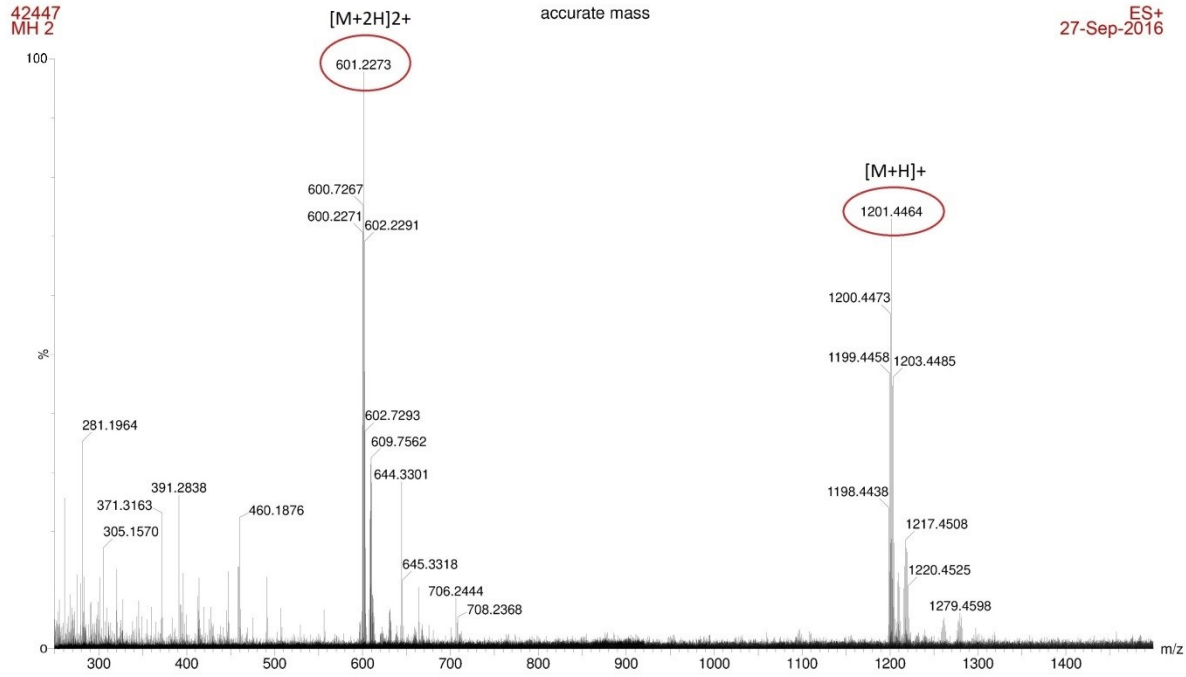

Fig. S11. HRMS Gd-DTPA-BC_{10}coumarinamide.

References

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2. B. Karami, M. Farahi and S. Khodabakhshi, *Helvetica Chimica Acta*, 2012, **95**, 455-460.
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