Electronic Supplementary Material (ESI) for Soft Matter.
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Revealing and tuning the core. structure, properties and function of polymer micelles with lanthanide-coordination complexes

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Experimental section

Materials

The bis-ligand compound 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L_2EO_4) was prepared according to literature.[1] The diblock copolymer, poly(N-methyl-2-vinyl-pyridinium iodide)-b-poly(ethylene oxide) ($P2MVP_{41}$-*b*-PEO_{205}), was obtained by quaternization of poly(2-vinylpyridine)-b-poly(ethylene oxide) ($P2MVP_{41}$-*b*-PEO_{205}) (Polymer Source, M_w/M_n = 1.10, M_w= 13.3 k) following a procedure described elsewhere.[2] The degree of quaternization is about 90%, estimated from the titration of anionic polyelectrolyte with known charge number. Chemical structures of the ligand and polymer are shown in Scheme 1. 3-(trimethylsilyl)propanoic-2,2,3,3-d4 acid sodium salt (TSP, 98% D), and deuterium oxide (99% D), lanthanide salts, $Eu(NO_3)_3{\cdot}6H_2O$ and $GdCl_3{\cdot}6H_2O$ were purchased from Sigma Aldrich and used without further purification. Micelles samples were prepared in MES (2-(N-morpholino) ethanesulfonic acid) buffer (20 mM, pH 6). For NMR measurements, micelles were prepared in D_2O with 20 mM NaCl. The pD was adjusted with either DCl (Sigma Aldrich, 35 wt% in D_2O) or NaOD (Sigma Aldrich, 40 wt% in D_2O) to 6.4 ± 0.1, corresponding to the pH 6 in MES buffer.[3]

Methods

Light scattering

Light scattering measurements were performed on an ALV instrument equipped with a 300 mW Cobolt Samba-300 DPSS laser operating at 532 nm. The detect angle was fixed at 90 degree and all samples were measured at room temperature. The light scattering intensity is expressed as the excess Rayleigh ratio R_θ:

$$R_\theta = \frac{I_{sample} - I_{solvent}}{I_{toluene}} \times R_{toluene} \times \frac{n^2_{solvent}}{n^2_{toluene}} \quad (1)$$

where I_{sample}, $I_{solvent}$ and $I_{toluene}$ are the scattering intensities of the micellar solution, solvent and toluene, respectively. $R_{toluene}$ is the known Rayleigh ratio of toluene (2.1 x10^{-2} m^{-1}) and n is the refractive of solvent (1.333) and toluene (1.497). The CONTIN method[4, 5] was used to analyze the size and size distribution of micelles. The data were analyzed with AfterALV program (AfterALV 1.0d, Dullware), which provides $\Gamma_i W_i$ as default output for each size fraction. Here, the intensity weighted contribution W_i is multiplied by average decay rate Γ, as suggested by Petr Stepanek for the "equal-area representation".[6] The absolute $\Gamma_i W_i$ was normalized with the highest value of $\Gamma_i W_i$ for each sample for clear comparisons.

The Rayleigh ratio can be linked to the concentration and mass of the scattering objects:

$$\frac{K_R C}{R_\theta} = \frac{1}{M} \times \frac{1}{P(qR)} \times \frac{1}{S(q)} \tag{2}$$

where C is the weight concentration of micelles, M is their molecular mass, and R is the radius of the object that contribute to scatter light. $P(qR)$ and $S(q)$ are the form factor and the structure factor, respectively. K_R is an optical constant defined as:

$$K_R = \frac{4\pi^2 n^2}{N_{Av}\lambda_0^4}\left(\frac{dn}{dc}\right)^2 \tag{3}$$

where N_{Av} is Avogadro's number, λ_0 is the wavelength of the incoming beam (532.0 nm), and dn/dc is the refractive index increment of the micelles, which is measured with a differential refractive index detector (Shodex RI-71), see below table:

DPA/Eu	2	3	6	10
dn/dc*10^4 m^3 kg^{-1}	1.17	1.32	1.46	1.46

In our experiments, the scattering vector $q = (4\pi n/\lambda_0)sin(\theta/2)$ is approximately 0.023 nm^{-1} (θ = 90°), so that qR is small for the micelles and we therefore assume that $P(qR)$=1. At low concentrations, the structure factor can be approximated as

$$\frac{1}{S(q)} = 1 + 2B_2 \frac{C}{M} \tag{4}$$

where B_2 is the second virial coefficient. Substitution into equation 2, we get

$$\frac{K_R C}{R_\theta} = \frac{1}{M} + 2B_2 \frac{C}{M^2} \tag{5}$$

By plotting $K_R C/R_\theta$ versus C, molar mass of micelle is obtained from the intercept, allowing further calculating the aggregation number of the micelles.

Fluorescence spectroscopy

Micelles solution in MES buffer was put in 1.0 cm quartz cells for steady state and lifetime measurements on a Cary-4000 spectrometer. The slit was set at 5 nm and the spectra were corrected for the instrumental function. The excitation is set at 395 nm, and the emission spectra was recorded in the range of 550–750 nm. The concentration of Eu(III) was fixed at 0.5 mM in all samples.

Cryo-TEM

Copper grids (300 mesh Quantifoil 2/2 or 400 mesh Lacey carbon) were exposed to a glow-discharge in air prior to being used. Four microliters of micellar solution was applied to each grid. Blotting and vitrification in liquid ethane were carried out with a Vitrobot Mark IV instrument (FEI Co.). Grids were then transferred to a Gatan CT 3500 cryo-stage and analyzed in a JEOL JEM2100 transmission electron microscope. Images were recorded at 200 kV under low-dose conditions with a 4K × 4K Gatan US4000 digital camera.

NMR

Samples were prepared in D_2O under the charge stoichiometry with a controlled pD at 6.4 ± 0.1 and TSP as an internal standard. All the ^{1}H-NMR measurements were carried out at 298 K on Bruker AMX-500 spectrometer (500 MHz). The spectrum were plotted and analyzed with MestReNova 9.0 software.

NMR relaxometry

The longitudinal relaxation rate of Gd-C3Ms was measured using a Maran Ultra 0.72 T NMR spectrometer controlled with RINMR software (Resonance Instruments Ltd., Witney, United Kingdom). T_1 and T_2 relaxation decay curves were recorded by means of an Inversion-Recovery-Carr-Purcell-Meiboom-Gill (IR-CPMG) pulse sequence. Each inversion step was applied before an echo train, 15 inversion steps were used of 1, 2, 4, 6.5, 9, 15, 25, 50, 140, 230, 320, 400, 500, 650 ms. CPMG echo train consisted of 2048 echoes of four data points each. Following settings were used: number of averages: 4; echo time: 0.225 ms; repetition time: 1000 ms; spectral width: 100kHz. T1-T2 measurements were analyzed with Levenberg–Marquardt non-linear least squares algorithm implemented in SPLMOD which fits a sum of exponential decays to the echo train resulting in a relative amplitude and T_1 and T_2 relaxation time constant. For all samples was used a single exponential fit.

Magnetic Resonance Imaging

T_1 map measurement was conducted in 3 T magnetic resonance imaging (MRI) scanner. The MRI scanner consists of an Avance console (Bruker, Karlsruhe, Germany) and a superconducting magnet with a 50 cm vertical free bore (Magnex, Oxford, UK). An openable 1 T/m gradient set (Bruker, Karlsruhe, Germany) was used. For induction and detection of the signal, bird cage RF coil 4cm in diameter was used.

An inversion recovery spin echo-turbo spin echo (IR-SE-TSE) sequence was used for the T_1 map measurement. The inversion recovery measurement was performed using the following setting: field of view: 28 mm x 28 mm; slice thickness: 3 mm; matrix size 128 x 128; number of averages: 4; echo 1 time: 4 ms; echo n time: 5ms; inversion steps: 10 (30, 40, 50, 60, 80, 100, 200, 400, 800, 1500ms); turbo factor: 8; repetition time: 2000 ms; spectral width: 50kHz. Data analysis was performed with IDL (Research Systems, USA) using home-made processing, fitting, and calculation routines. The data were fitted on a per pixel basis using a mono-exponential decay function yielding quantitative maps of an amplitude, relaxation rate R_1, and relaxation time T_1. In order to distinguish the samples a different number of glass capillaries were inserted inside of the test tubes.

Figure S1

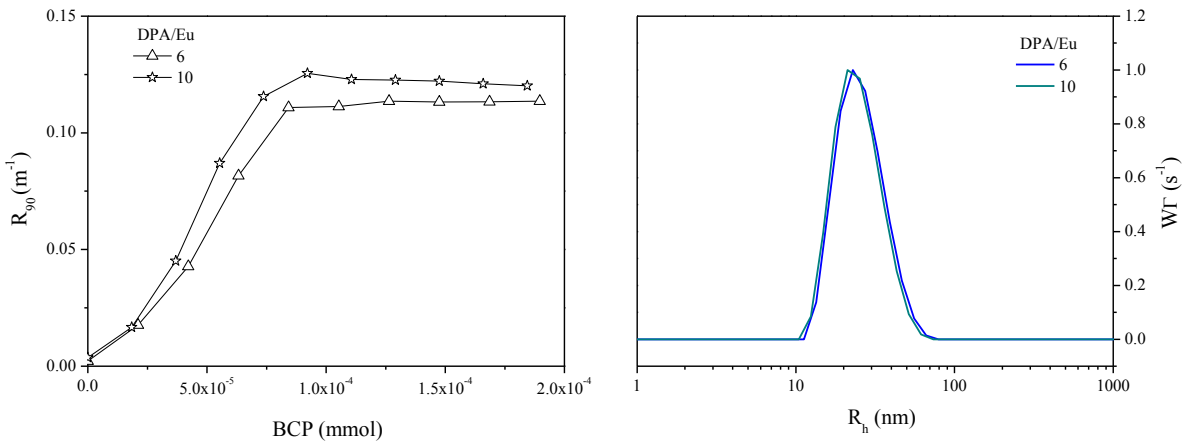


Figure S1 Left: titrate BCP into Eu-L_2EO_4 solutions under the DPA-Eu ratio of 6 and 10. Right: size and size distribution of Eu-C3Ms at different DPA/Eu ratios.

Figure S2

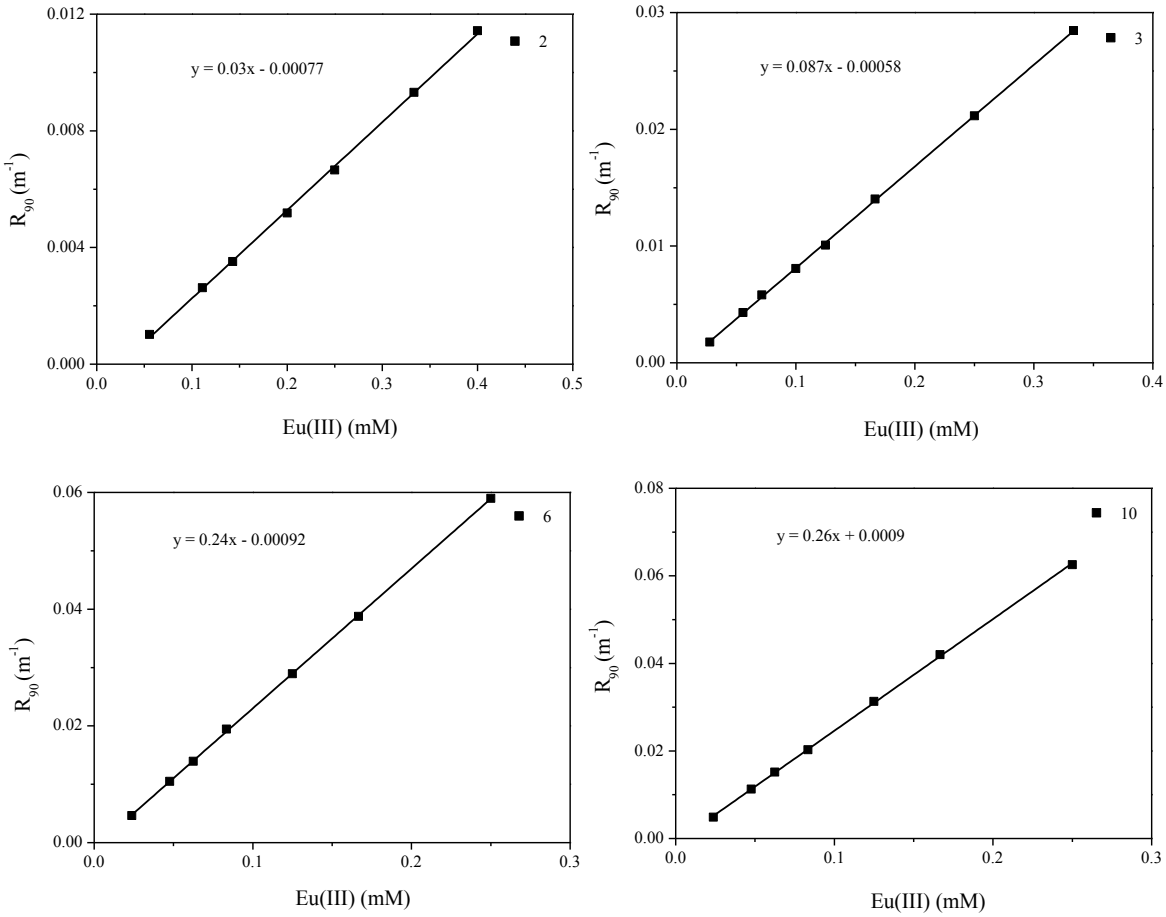


Figure S2 Intensity of Eu-C3Ms as a function Eu(III) concentration.

The intensity is represented as Rayleigh ratio R_θ, which is subtracted with the intensity from buffer solution and corrected by the intensity of toluene as a reference, see experimental section, method, equation 1. The CMC is determined by extrapolating the decay line to zero intensity, and calculated from the fitting formula, see table below:

DPA/Eu	2	3	6	10
CMC Eu(III) (mM)	0.026	0.0067	0.0038	0.0035

Figure S3

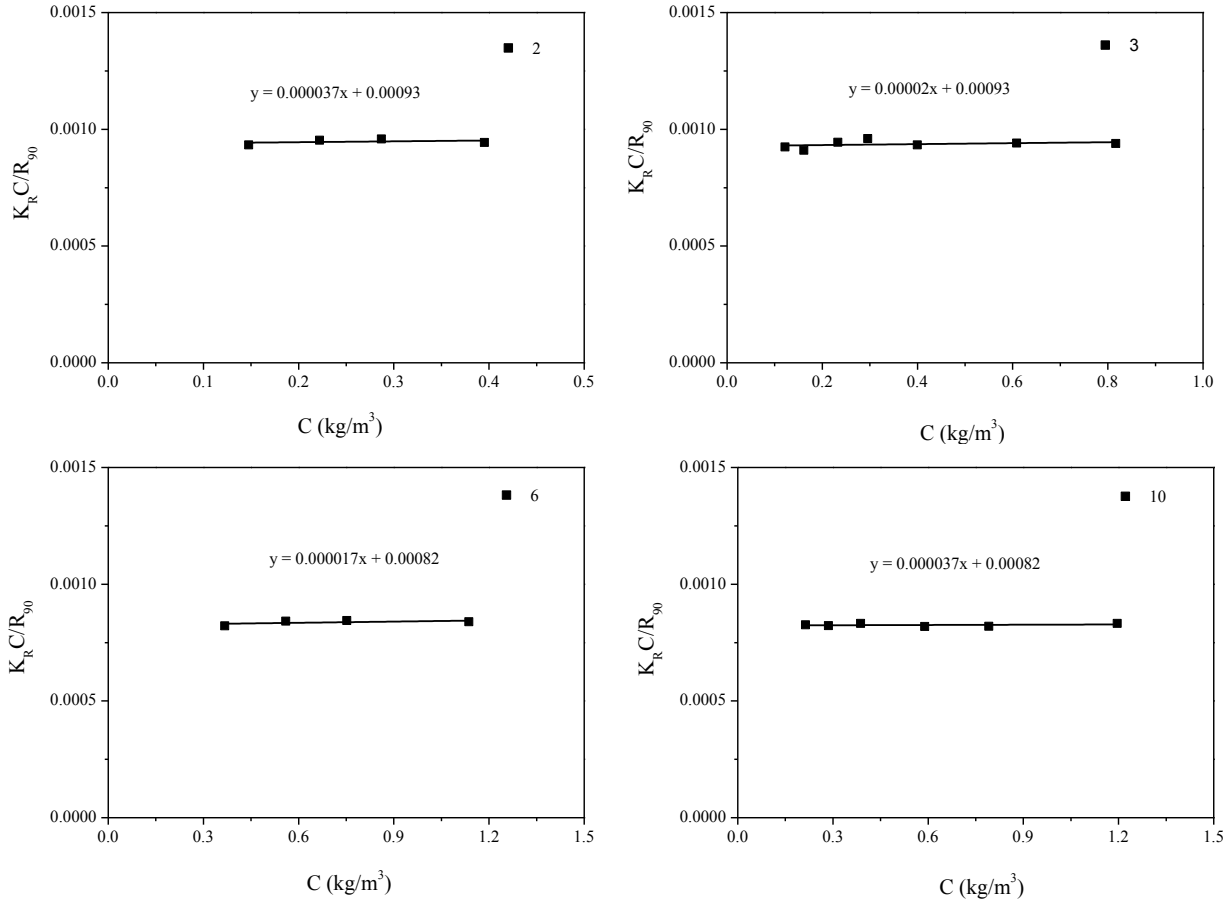


Figure S3 K_RC/R_{90} is plotted as a function of C (subtracted by CMC). The obtained aggregation numbers are summarized in the table.

The intercept of the line gives the micellar mass, $M_{micelle}$. Here we take DPA/Eu 2 as an example, to show how to calculate the aggregation number:

$$M_{micelle} = 1/0.00093 = 1.11 \text{ x } 10^3 \text{ kg/mol} = 1.11 \text{ x } 10^6 \text{ g/mol}$$

Under DPA/Eu ratio of 2, one Eu(III) binds one L_2EO_4 ligand, such a unit give 1 negative charge and a molecular weight of 828 g/mol. The molecular weight of the diblcok copolymer is 14500 g/mol, and one polymer contains 41 x 0.9 = 37 positive charges. (quaternization degree is around 90%)

The micelles are prepared at the charge stoichiometry, so we get

$$N_{Eu\text{-}L} \text{ x } 1 = N_{polymer} \text{ x } 37 \quad (6)$$

$$N_{Eu\text{-}L} \text{ x } 828 + N_{polymer} \text{ x } 14500 = 1.11 \text{ x } 10^6 \quad (7)$$

By solving equation 9 and 10, we find that the numbers of each components in one micelle. The calculations with other DPA/Eu ratios follow the same strategy, and the free ligand at ratio 6 and 10 is excluded in the calculation. The obtained aggregation numbers are included in the below table:

DPA/Eu	Eu (III)	L_2EO_4	$P2MVP_{41}$-*b*-PEO_{205}
2	910	910	25
3	434	651	39
6	286	601	53
10	262	558	54

Figure S4

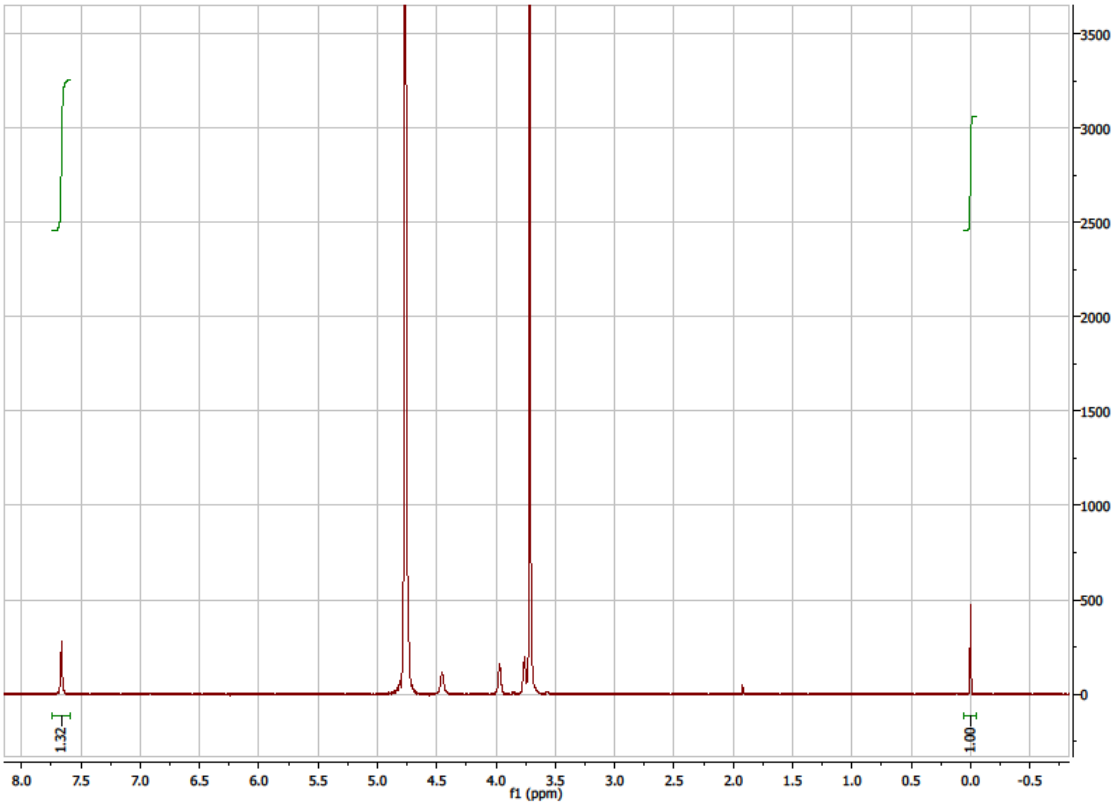


Figure S4 H-NMR spectrum of the Eu-C3Ms at DPA/Eu ratio of 10. Micelles at DPA/Eu ratio of 10 display an stronger peak at 7.7 compare to micelles formed at 6, because the original input already give the excess of ligand. Subtracted by this part, around 15% of the ligand are free estimated by an internal standard TSP (0 ppm).

Figure S5

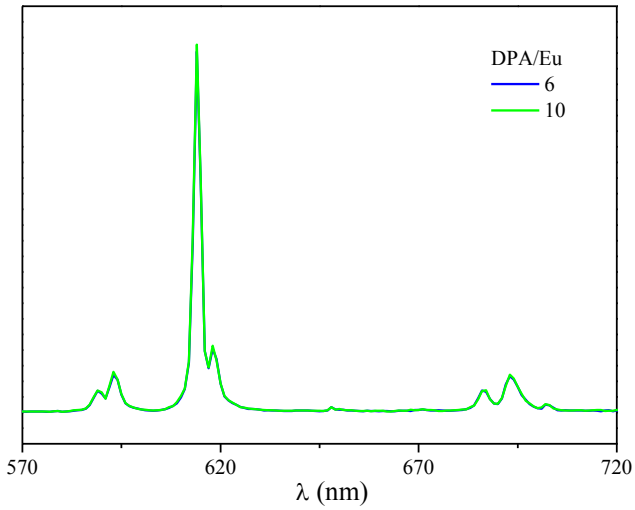
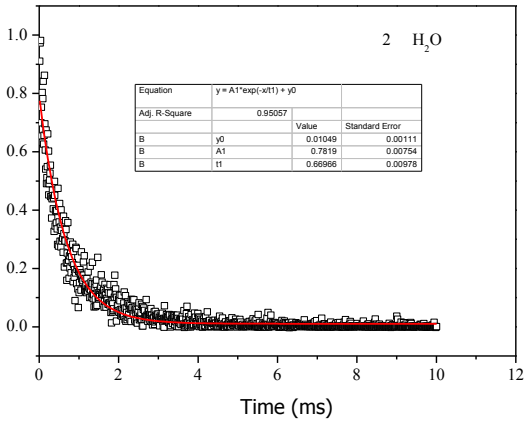
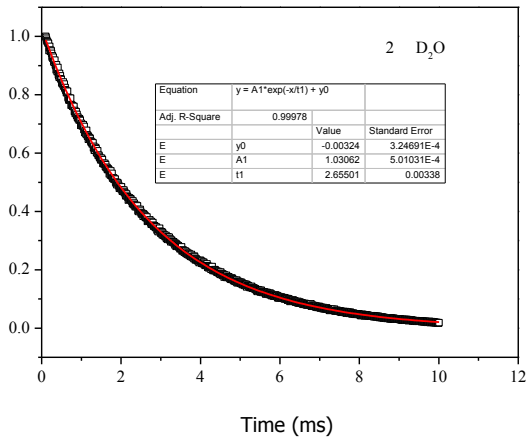
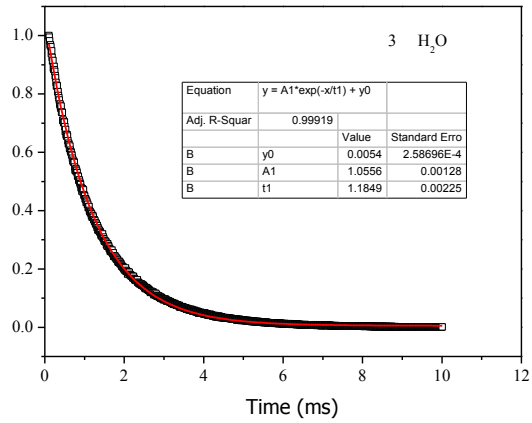


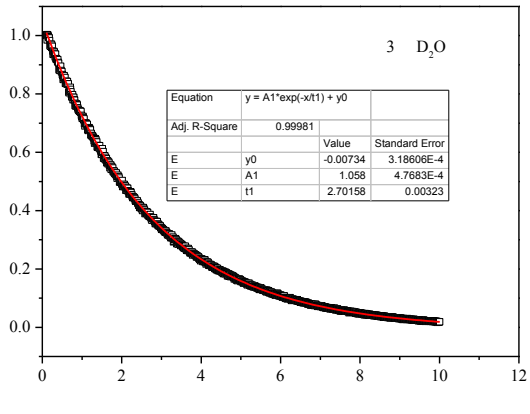
Figure S5 Luminescence emission of Eu-C3Ms prepare at DPA/Eu ratio of 6 and 10. (λ_{ex} =395 nm, [Eu] = 0.5 mM).

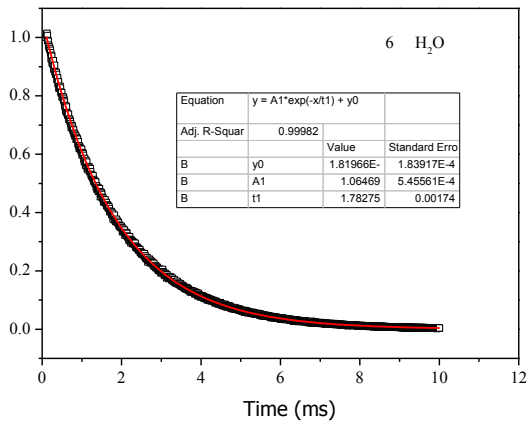
Figure S6

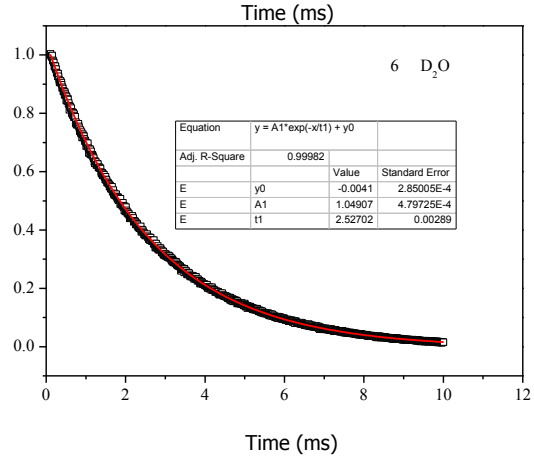

2 H_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Square 0.95057
Value Standard Error
B y0 0.01049 0.00111
B A1 0.7819 0.00754
B t1 0.66966 0.00978
Time (ms)


2 D_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Square 0.99978
Value Standard Error
E y0 -0.00324 3.24691E-4
E A1 1.03062 5.01031E-4
E t1 2.65501 0.00338
Time (ms)


3 H_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Squar 0.99919
Value Standard Erro
B y0 0.0054 2.58696E-4
B A1 1.0556 0.00128
B t1 1.1849 0.00225
Time (ms)


3 D_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Square 0.99981
Value Standard Error
E y0 -0.00734 3.18606E-4
E A1 1.058 4.7683E-4
E t1 2.70158 0.00323
Time (ms)


6 H_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Squar 0.99982
Value Standard Erro
B y0 1.81966E- 1.83917E-4
B A1 1.06469 5.45561E-4
B t1 1.78275 0.00174
Time (ms)


6 D_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Square 0.99982
Value Standard Error
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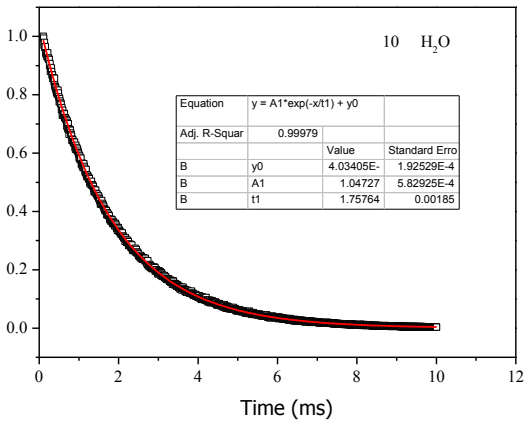
E A1 1.04907 4.79725E-4
E t1 2.52702 0.00289
Time (ms)


10 H_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Squar 0.99979
Value Standard Erro
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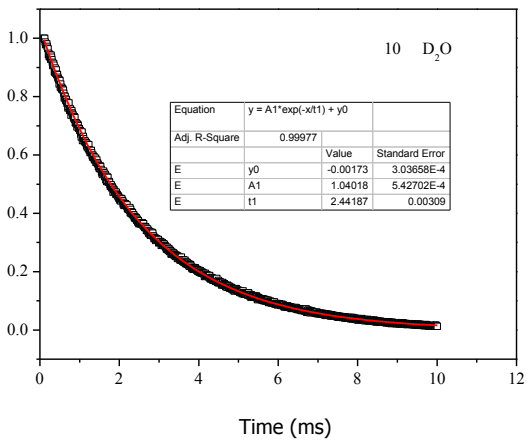
B A1 1.04727 5.82925E-4
B t1 1.75764 0.00185
Time (ms)


10 D_2O
Equation y = A1*exp(-x/t1) + y0
Adj. R-Square 0.99977
Value Standard Error
E y0 -0.00173 3.03658E-4
E A1 1.04018 5.42702E-4
E t1 2.44187 0.00309
Time (ms)

DPA/Eu	τ_{H2O} (ms)	τ_{D2O} (ms)	$q_{average}$
2	0.67	2.66	1.01
3	1.18	2.70	0.27
6	1.78	2.53	-0.1
10	1.79	2.44	-0.1

Figure S6 Life time measurements with λ_{ex} at 395 nm and λ_{em} fixed at 614 nm, the numbers are summarized in the table. τ: life time of the micelles; $q_{average}$: average number of inner sphere water molecules.

Figure S7

NMR relaxometry

DPA/Gd	R1 (s^{-1})	R2 (s^{-1})
2	16.8	17.6
3	12.5	14.0
6	5.3	7.7
10	4.4	7.1

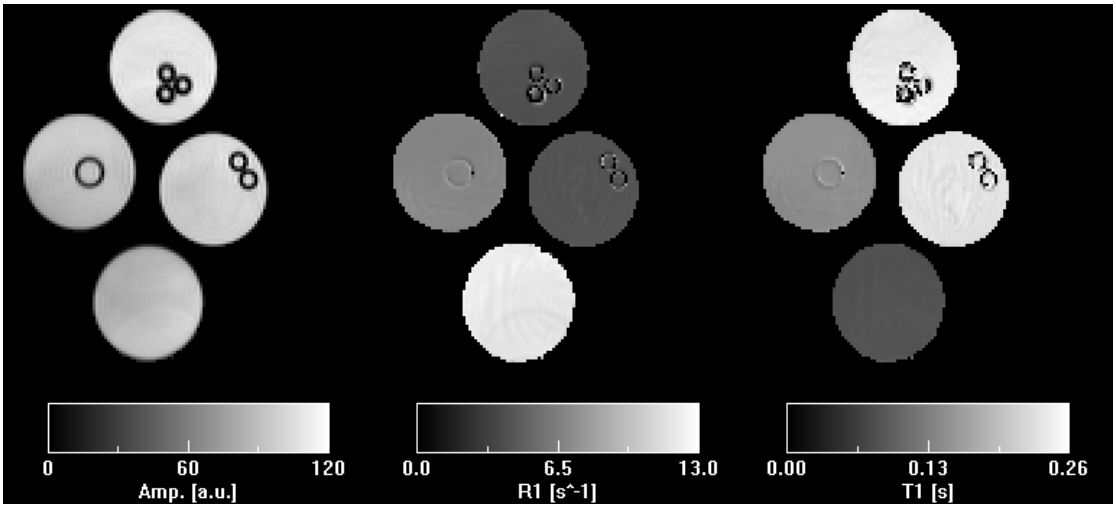


Figure S7 Longitudinal relaxation rate of Gd-C3Ms at different DPA/Gd ratio. (0.72T) MRI images (3T), from left to right: amplitude map (a.u.), R_1 relaxation rate map (s^{-1}), T_1 relaxation time map (s). Note, the relaxation rates in the Table deviate from the scale bars in the figure as they correspond to measurements performed at different magnetic field strengths. [Eu] = 0.5 mM.

Figure S8

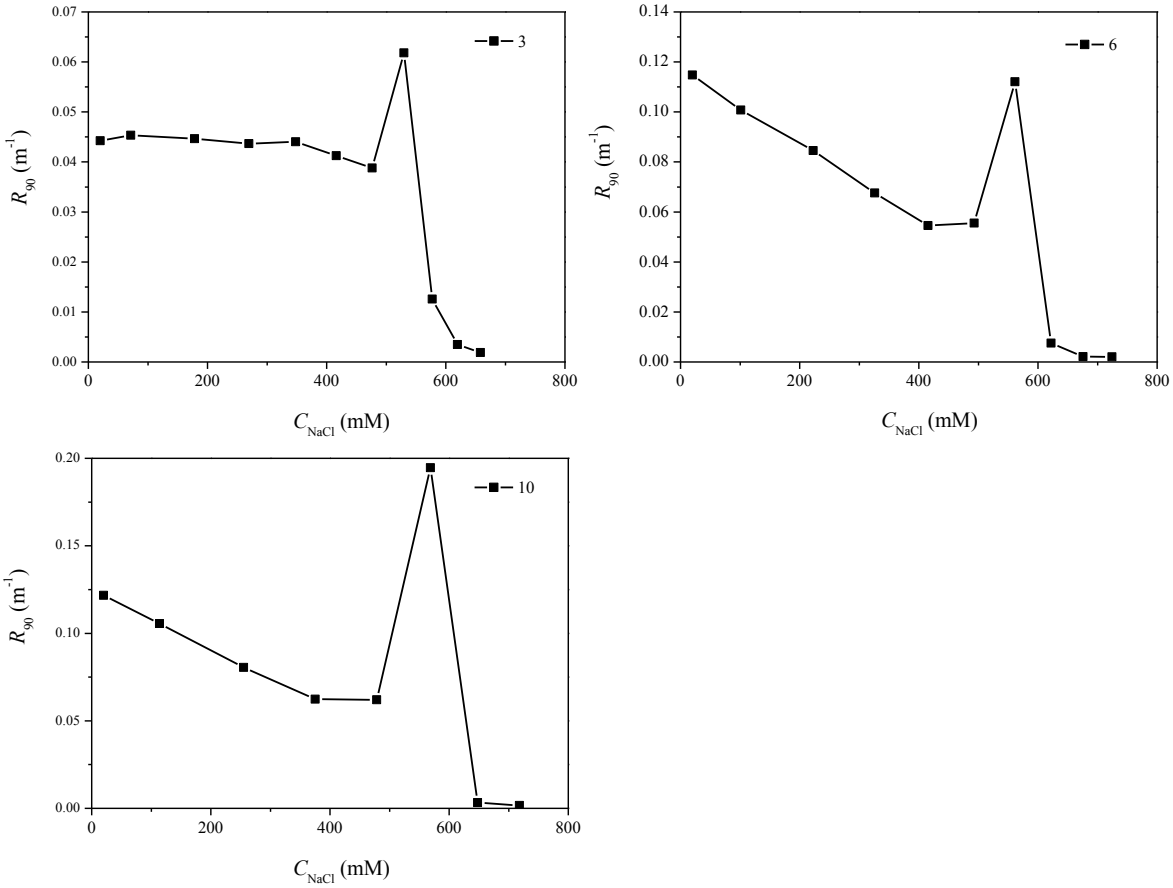


Figure S8 Salt titration curves of micelles formed at DPA/Eu ratio of 3, 6 and 10.

Reference:

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