Controlled Mixing of Lanthanide(III) Ions in Coacervate Core Micelles

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This PDF file includes:

1. Experimental section;

2. Figures S1-S5;

3. References S1-S6.

Experiment section

1. Materials

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) ($P2VP_{41}$-b-PEO_{205}) (Polymer Source, M_w/M_n= 1.03, M_w= 13.3 k) following a procedure described elsewhere.[S1] The degree of quaternization is about 90%. The bis-ligand compound 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L_2EO_4) was prepared according to literature.[S2] Gadolinium chloride $GdCl_3 \cdot 6H_2O$, europium nitrate $Eu(NO_3)_3 \cdot 5H_2O$ and sodium chloride NaCl (analytical grade) were purchased from Aldrich and used without further purification. All stock solutions were made in acetate buffer at pH 5.

2. Method

Light scattering

Light scattering at an angle of 90 degrees was performed with an ALV light scattering-apparatus, equipped with a 400mW argon ion laser operating at a wavelength of 514.5 nm. All measurements were performed at room temperature. Titrations were carried out using a Schott-Geräte computer-controlled titration setup to control sequential addition of titrant and cell stirring. After every dosage, the laser light-scattering intensity (I) and the correlation function were recorded. The hydrodynamic radius and the scattered intensity are studied as a function of the mole fraction of positive charge, $f+$, which is defined as follows:

$$f+ = \frac{[+]}{[-]+[+]} \qquad (1)$$

where [-] and [+] are the molar charge concentrations of charged units on each polymer chain.

The light scattering intensity is expressed as the excess Rayleigh ratio R_θ divided by the total polymer concentration. R_θ is obtained as

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

where I_{sample} is the scattering intensity of the micellar solution and $I_{solvent}$ is the intensity of the solvent. $I_{toluene}$ is the scattering intensity of toluene, and $R_{toluene}$ is the known Rayleigh ratio of toluene ($2.1 \cdot 10^{-2}$ m^{-1}). The total polymer concentration is the sum of the concentrations of all components contributing to micelle formation. The CUMULANT method[S3] was used to analyze the mean apparent hydrodynamic radius (R_h) as

$$R_h = kTq^2 / 6\pi\eta\Gamma \quad (3)$$

where q is the scattering vector, k is the Boltzman constant, T is the absolute temperature, η is the viscosity of the solvent, and Γ is the measured average decay rate of the correlation function. The CONTIN method [S4] is used to analyze the distribution of particle (C3Ms) radii. The data was 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 Gamma, as suggested by Petr Stepanek for the "equal-area representation".[S5] The absolute values of $\Gamma_i W_i$ vary a lot from different samples, which makes it difficult to compare the results directly. Therefore, we normalized $\Gamma_i W_i$ with the highest value of $\Gamma_i W_i$ for each sample, and we call this probability in the CONTIN result.

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

$$\frac{K_R C}{R_\theta} = \frac{1}{M} \times \frac{1}{P(qR)} \times \frac{1}{S(q)} \quad (4)$$

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. For C3Ms, the R is closed to the core radius. $P(qR)$ and $S(q)$ are the form factor and the structure factor, respectively. K_R is an optical constant defined as:

$$K_R = \frac{4\pi^2 n^2}{N_{Av}\lambda_0^4}\left(\frac{dn}{dc}\right)^2 \quad (5)$$

where n is the refractive index of solvent, 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 Gd-C3Ms. We measured dn/dc of the micellar solutions using a differential refractive index detector (Shodex RI-71) and found a value of $1.58 \cdot 10^{-4}$ m^3/kg for Gd-C3Ms.

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 (which have a radius on the order of 20 nm). We therefore assume that $P(qR)$=1. At low concentrations, the structure factor can be approximated as

$$\frac{1}{S(q)} = 1 + 2B_2\frac{C}{M} \quad (6)$$

where B_2 is the second virial coefficient. Substitution into equation 4, we get

$$\frac{K_R C}{R_\theta} = \frac{1}{M} + 2B_2\frac{C}{M^2} \quad (7)$$

By plotting K_RC/R_θ versus C, we can obtain the molar mass of the micelles (M) from the intercept, from which we can obtain the aggregation number of the micelles.

Relaxometric measurements

Water proton relaxation measurements

The longitudinal water proton relaxation rate as a function of pH was measured at 25°C by using a Stelar Spinmaster (Stelar, Mede, Pavia, Italy) spectrometer operating at 20 MHz, by means of the standard inversion-recovery technique. The temperature was controlled with a Stelar VTC-91 air-flow heater equipped with a copper constantan thermocouple (uncertainty 0.1°C). The relaxometric characterization of the field-dependent relaxometry of the paramagnetic Gd(III)-probes solutions was carried out through the acquisition of the NMRD profiles. The proton $1/T_1$ NMRD profiles were measured at 25°C on a fast field-cycling Stelar relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.47 T (corresponding to 0.01-20 MHz proton Larmor frequencies). The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ± 1%. Additional data points in the range 20-70 MHz were obtained on the Stelar Spinmaster spectrometer. The concentration of the solutions used for the relaxometric characterization was determined according to a previously reported relaxometric method.[S6]

Cryogenic Transmission Electronic Microscopy (Cryo-TEM)

A few microliters of sample were placed on a bare copper TEM grid (Plano, 600 mesh) and the excess of liquid was removed with filter paper, followed by shooting the grid into liquid ethane cooled to -170 ºC. The sample vitrification procedure was carried out using a cryo-box (Carl Zeiss NTS GmbH, Oberkochen, Germany) equipped with humidity and temperature chamber. Samples were studied at an acceleration voltage of 120 kV. Images were recorded under low-dose conditions with a bottom-mounted CCD camera (UltraScan 1000, Gatan). For each sample, images were collected for a number of different regions in the sample.

Figures

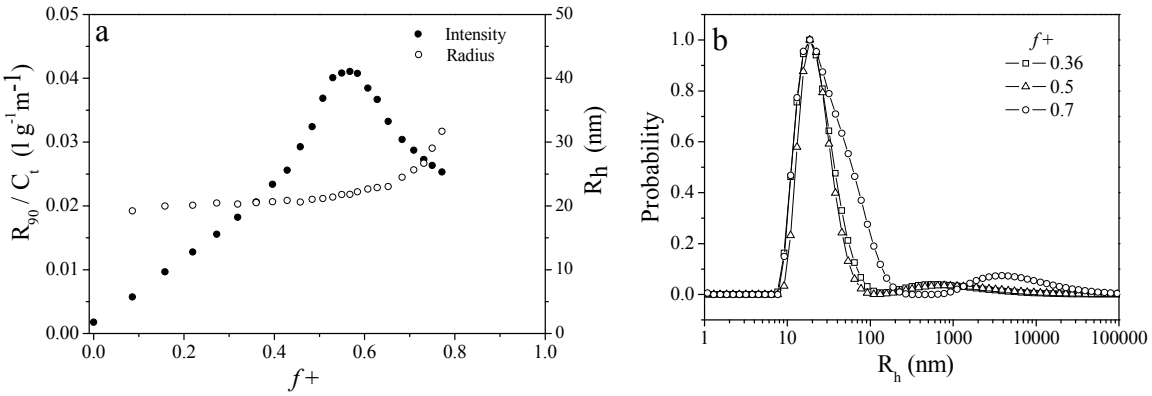

Figure S1 Light scattering titration of Gd-L_2EO_4 (Gd/L = 1/1.5) coordination complex with P2MVP$_{41}$-*b*-PEO$_{205}$. a: variations of light scattering intensity and hydrodynamic radius as a function of positive charge fraction. b: CONTIN analysis of size and size distribution of Gd-C3Ms at different f+.

The titration curve shows that the light scattering intensity increases immediately after the first addition of P2MVP$_{41}$-*b*-PEO$_{205}$ copolymers to the solution of the Gd-L_2EO_4 coordination complexes, indicating the formation of micelles. Upon adding the positively charged P2MVP$_{41}$-*b*-PEO$_{205}$ copolymers step by step, the intensity increases gradually and shows a maximum at $f+ \approx 0.5$. This maximum corresponds to the preferred micellar composition (PMC) where charge stoichiometry is satisfied.

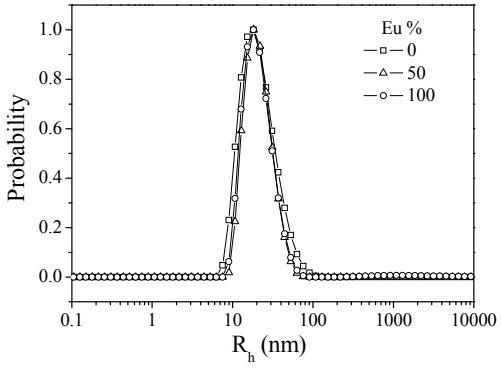

Figure S2 CONTIN analysis of size and size distribution of Eu/Gd-C3Ms at different Eu^{3+}/Gd^{3+} ratios (micelles are prepared in 20 mM acetate buffer, pH 5, total metal concentration is fixed at 0.5 mM).

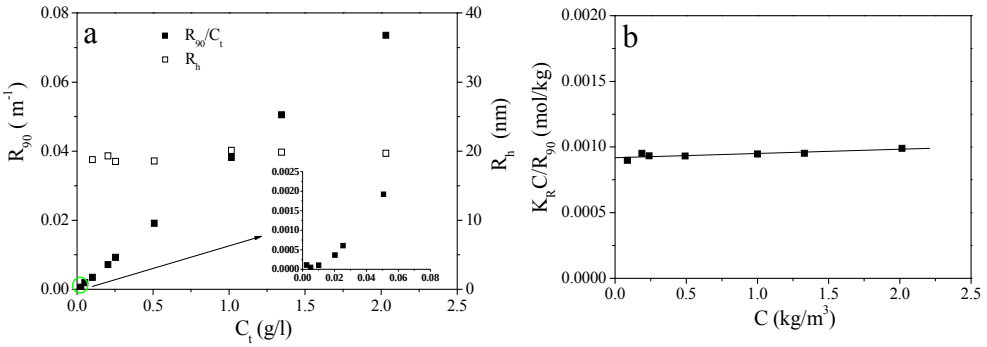

Figure S3 a: light scattering intensity and hydrodynamic radius versus total concentration of components. The CMC is estimated by extrapolating the intensity to the baseline. Inset shows a zoom in for low concentrations. b: K_RC/R_{90} is plotted as a function of C ($C = C_t - CMC$). (Gd-C3Ms are prepared in acetate 20 mM buffer, pH 5) The aggregation number of Gd-C3Ms is calculated from K_RC/R_{90} profile (See equation 7, experiment section). We find that around 40 $P2MVP_{41}$-*b*-PEO_{205} copolymers aggregate in one micelle, which means approximately 500 "LnL_3" coordination units are needed to compensate all the positive charges from these polymers. As a consequence, around 500 metal ions are contained in one micelle.

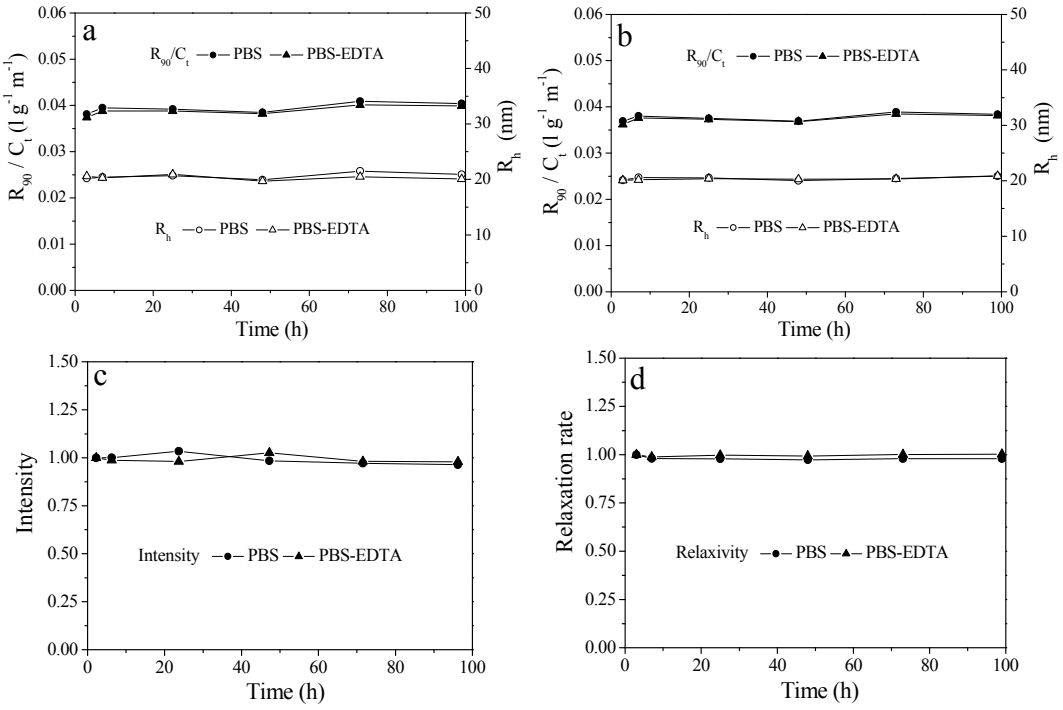

Figure S4 variations of light scattering intensity and micellar size of Eu-C3Ms (a) and Gd-C3Ms (b) in PBS buffers with and without EDTA over time. c: Variation of the luminescent intensity of Eu-C3Ms over time (normalized by the intensity of fresh Eu-C3Ms, Eu^{3+} concentration is 0.2 mM). d: Time dependence of longitudinal relaxation rate of Gd-C3Ms in PBS and PBS-EDTA solutions (normalized by the relaxation rate of fresh Gd-C3Ms, Gd^{3+} concentration is 0.5 mM). PBS: phosphate buffer saline, pH 7.4; PBS-EDTA: phosphate buffer saline, pH 7.4, with added EDTA (at the same concentration as that of L_2EO_4) to the Ln-C3Ms solution.

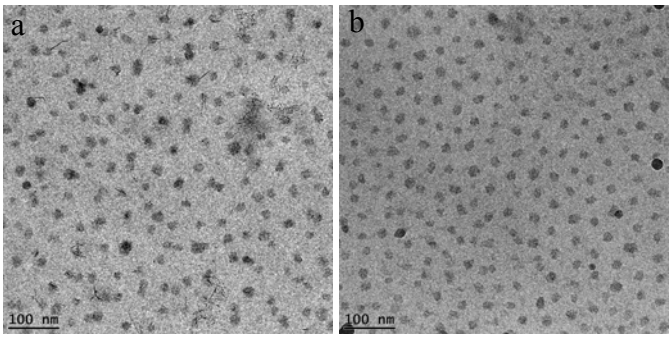

Figure S5 Cryo-TEM images of Gd-C3Ms in PBS buffer (a) and PBS-EDTA solutions (b).

Reference:

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