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

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**Experiment section**

1. **Materials**

The diblock copolymer, poly(N-methyl-2-vinyl-pyridinium iodide)-b-poly(ethylene oxide) (P2MVP<sub>41</sub>-b-PEO<sub>205</sub>), was obtained by quaternization of poly(2-vinylpyridine)-b-poly(ethylene oxide) (P2VP<sub>41</sub>-b-PEO<sub>205</sub>) (Polymer Source, $M_w/M_n=1.03$, $M_w=13.3$ k) following a procedure described elsewhere. The degree of quaternization is about 90%. The bis-ligand compound 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L<sub>2EO<sub>4</sub></sub>) was prepared according to literature. Gadolinium chloride GdCl<sub>3</sub>·6H<sub>2</sub>O, europium nitrate Eu(NO<sub>3</sub>)<sub>3</sub>·5H<sub>2</sub>O 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:

$$f^+ = \frac{[+]}{[-]+[+]}$$  \hspace{1cm} (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 $R_\theta$ divided by the total polymer concentration. $R_\theta$ is obtained as

$$R_\theta = \frac{I_{\text{sample}} - I_{\text{solvent}}}{I_{\text{toluene}}} \times R_{\text{toluene}} \times \frac{n_{\text{solvent}}^2}{n_{\text{toluene}}^2}$$

(2)

where $I_{\text{sample}}$ is the scattering intensity of the micellar solution and $I_{\text{solvent}}$ is the intensity of the solvent. $I_{\text{toluene}}$ is the scattering intensity of toluene, and $R_{\text{toluene}}$ is the known Rayleigh ratio of toluene ($2.1 \cdot 10^{-2} \text{ m}^{-1}$). 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

$$R_h = \frac{kTq^2}{6\pi\eta\Gamma}$$

(3)

where $q$ is the scattering vector, $k$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the viscosity of the solvent, and $\Gamma$ 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:

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

(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:

$$\begin{align*}
K_R &= \frac{4\pi^2 n^2}{N_A\lambda_0^2} \left(\frac{dn}{dc}\right)^2
\end{align*}$$

where $n$ is the refractive index of solvent, $N_A$ is Avogadro’s number, $\lambda_0$ 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$^{-1}$ ($\theta=90^\circ$), 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

$$\begin{align*}
\frac{1}{S(q)} &= 1 + 2B_2 \frac{C}{M}
\end{align*}$$

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

$$\begin{align*}
\frac{K_R C}{R_\theta} &= \frac{1}{M} + 2B_2 \frac{C}{M^2}
\end{align*}$$

By plotting $K_R C/R_\theta$ 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/T₁ 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/T₁ 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.86

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 °C. 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.
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 $C$ ($C = C_t - C_{MC}$). (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$_2$EO$_4$) to the Ln-C3Ms solution.
Figure S5 Cryo-TEM images of Gd-C3Ms in PBS buffer (a) and PBS-EDTA solutions (b).

Reference:


