ChemComm

COMMUNICATION
Aldrik H. Velders, Yun Yan *et al.*
Controlled mixing of lanthanide(III) ions in coacervate core micelles

ChemComm

Controlled mixing of lanthanide(III) ions in coacervate core micelles†

Junyou Wang,[a] Aldrik H. Velders,*[ab] Eliana Gianolio,[c] Silvio Aime,[c] Frank J. Vergeldt,[d] Henk Van As,[d] Yun Yan,*[e] Markus Drechsler,[f] Arie de Keizer,[a] Martien A. Cohen Stuart[a] and Jasper van der Gucht[a]

This article presents a facile strategy to combine Eu³⁺ and Gd³⁺ ions into coacervate core micelles in a controlled way with a statistical distribution of the ions. Consequently, the formed micelles show a high tunability between luminescence and relaxivity. These highly stable micelles present great potential for new materials, *e.g.* as bimodal imaging probes.

Complexes of lanthanide(III) ions are of considerable interest because of their unique optical and magnetic properties, arising from the electron configuration in the partially filled f-shell.[1] For example, gadolinium(III) complexes, with their seven unpaired electron spins, strongly decrease the spin–lattice relaxation time (T_1) of nearby water molecules in a magnetic field, which has led to their widespread application in contrast agents for magnetic resonance imaging (MRI) in medical diagnosis.[2] Other lanthanide complexes are known for their luminescent character and, in particular, complexes containing europium(III), terbium(III) and erbium(III) are used as sensors and optical imaging probes with long luminescent lifetimes, sharp emission bands and high resistance to photo-bleaching.[3] In biomedical diagnostics each imaging modality has its specific advantages and drawbacks,[4] and a recent trend is therefore to develop bi- or multimodal probe systems.[5] For combining the high resolution of MRI and the high sensitivity of optical imaging, incorporation of different lanthanide ions, such as gadolinium and europium, into one probe provides an excellent strategy.[6] In fact, several studies have focused on the design and synthesis of chelating ligands that can bind lanthanide (and other metal) ions for MRI and fluorescence imaging,[7] and others developed strategies for well-defined dinuclear and polynuclear complexes.[8] However, because of the large difference in sensitivity between, *e.g.* MRI and fluorescence, imaging modalities, multimodal probes would only be able to meet up to their potential and promise if more ions could be incorporated into one probe. Moreover, a good control of the mixing ratio between the different (lanthanide) ions is essential.[9] For solid particles, this control has proven to be a difficult task, because there is usually a preference for one lanthanide ion over another for specific crystal lattices, preventing a statistical distribution of a mixture of lanthanide ions in the particles.[10] Moreover, investigations of lanthanide-doped nanoparticles have shown that the doped ions in the core of the nanoparticle have properties different from the ones closer to the surface.[11] Here we present a simple and straightforward approach to incorporate different lanthanide ions, *i.e.* europium(III) and gadolinium(III), in a 20 nm (radius) coacervate micellar structure in a controlled and statistical manner. The luminescence and the effect on the magnetic relaxation rate of these bimodal probes containing several hundreds of lanthanide ions in the core have been studied and their stability against salt and competing ligands has been investigated.

The Eu/Gd micelles are formed in aqueous solution based on the electrostatic interaction between an anionic coordination polymer with both Eu³⁺ and Gd³⁺ ions, and a cationic-neutral diblock copolymer. The chemical structures of the ligand 1,11-bis(2,6-dicarboxypyridin-4-yloxy)-3,6,9-trioxaundecane (L_2EO_4)[12] and the diblock copolymer poly(*N*-methyl-2-vinyl-pyridinium iodide)-*b*-poly(ethylene oxide) (P2MVP₄₁-*b*-PEO₂₀₅)[13] are shown in Scheme 1. Eu³⁺ and Gd³⁺ ions are mixed with the L_2EO_4 ligand (L: dicarboxypyridine group) at a metal to ligand ratio M/L_2EO_4 of 1/1.5, where branched structures are formed with net three negative charges on the coordination "LnL₃" unit (Scheme 1c).[14]

[a] *Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands.*
  E-mail: junyou.wang@wur.nl; Fax: +31 317 483777; Tel: +31 317 482178
[b] *BioNanoTechnology Group, Wageningen University, Dreijenplein 6, 6703 HB Wageningen, The Netherlands. E-mail: aldrik.velders@wur.nl*
[c] *Department of Chemistry and Center for Molecular Imaging, University of Turin, Via Nizza 52, I-10125 Torino, Italy. E-mail: eliana.gianolio@unito.it*
[d] *Laboratory of Biophysics and Wageningen Nuclear Magnetic Resonance Centre, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands. E-mail: Henk.vanAs@wur.nl*
[e] *Beijing National Laboratory for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. E-mail: yunyan@pku.edu.cn*
[f] *Makromolekulare Chemie II, University of Bayreuth, 95440 Bayreuth, Germany. E-mail: Markus.Drechsler@uni-bayreuth.de*
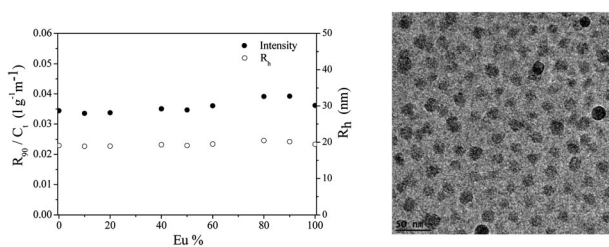† Electronic supplementary information (ESI) available: Experimental procedures, characterization and dynamic light scattering data. See DOI: 10.1039/c3cc39148e
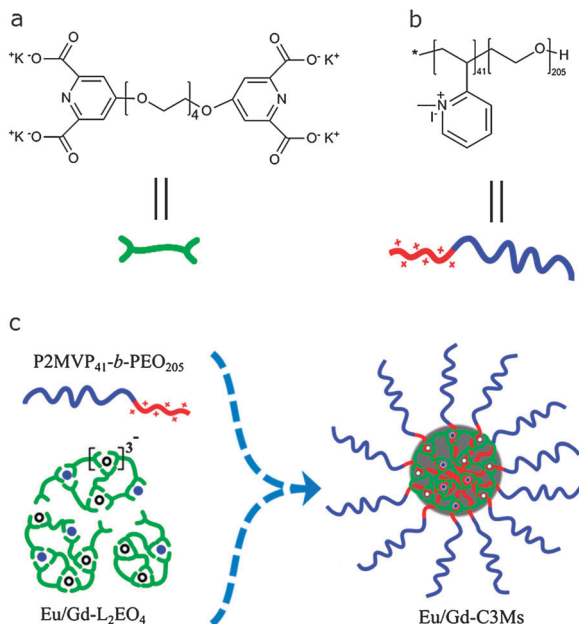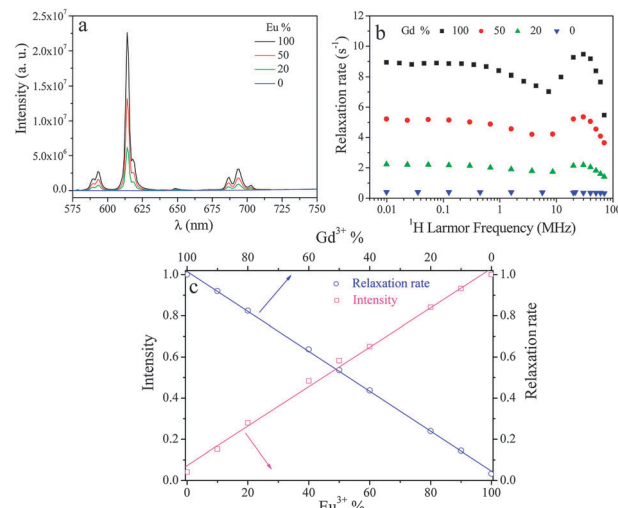
Scheme 1 (a) Structure of L_2EO_4; (b) structure of $P2MVP_{41}–PEO_{205}$; (c) formation of Eu/Gd-C3Ms (blue dots represent Eu^{3+} ions and white dots represent Gd^{3+} ions).

Fig. 2 Luminescent emission intensity (a) and NMRD profiles (b) of Eu/Gd-C3Ms at different Eu^{3+}/Gd^{3+} ratios. (c) Normalized luminescent intensity (red) and relaxation rate (blue) as a function of Eu^{3+}/Gd^{3+} ratio [Luminescent intensity and relaxation rate normalized by the values of Eu-C3Ms (100% Eu^{3+}) and Gd-C3Ms (100% Gd^{3+}), respectively. Eu/Gd-C3Ms were prepared in 20 mM acetate buffer, pH 5, and total metal concentration is 0.5 mM].

Upon mixing with the $P2MVP_{41}$-b-PEO_{205} copolymer, Eu/Gd–L_2EO_4 coordination structures quickly aggregate with the cationic block and the formed coacervate aggregates are stabilized by the neutral PEO chain, leading to the formation of micelles. We call this kind of micelle complex coacervate core micelles (C3Ms). The preferred micellar composition (PMC) is at the stoichiometric charge ratio where the opposite charges are neutralized completely (ESI,† Fig. S1). The hydrodynamic radius of the micelles is around 20 nm and both the intensity and radius are independent upon varying the Eu^{3+}/Gd^{3+} ratio (Fig. 1). The CONTIN results (ESI,† Fig. S2) indicate that there is only one dominant kind of particle in solution at all Eu^{3+}/Gd^{3+} mixing ratios. Cryo-TEM shows that the formed micelles have a core radius around 8 nm, so the thickness of the corona is about 12 nm, which fits with the values found for other micelles.[13,15] The critical micelle concentration (CMC) of Gd-C3Ms (100% Gd^{3+}) is found to be around 0.015 g L^{-1} (total concentration of polymer and Gd–L_2EO_4) and the aggregation number (the number of $P2MVP_{41}$-b-PEO_{205} per micelle) is around 40, which means that approximately 500 metal ions are contained in one micelle (ESI,† Fig. S3).

The Eu/Gd-C3Ms were further characterized by luminescence spectroscopy and magnetic relaxation measurements. For the

luminescence an excitation wavelength of 284 nm was used (Fig. 2a), with the L_2EO_4 ligand functioning as an antenna to transfer energy to a Eu^{3+} ion, which by itself has a negligible absorption coefficient at this wavelength.[16] The longitudinal relaxation rate of Eu/Gd-C3Ms was measured by recording the NMRD (Nuclear Magnetic Resonance Dispersion) curves (Fig. 2b). The relaxivity was found to be 20 mM_{Gd}^{-1} s^{-1} (30.9 MHz, 298 K), which is in the same order as observed with other micelle systems,[17] and corresponds to a relaxivity of $\sim 10^4$ $mM_{micelle}^{-1}$ s^{-1} for a 100% loaded Gd^{3+} micelle. In Fig. 2c, both the luminescence intensity at 614 nm and the relaxation rate of Eu/Gd-C3Ms are plotted as a function of the Eu^{3+}/Gd^{3+} ratio. We find linear dependences of both the luminescence and the relaxation rate on the amount of Eu^{3+} and Gd^{3+} ions, respectively, in the micelle. It is worth mentioning that Gd-C3Ms show negligible luminescence compared to Eu-C3Ms, while Eu-C3Ms do not contribute to the relaxation rate, indicating that the luminescence and relaxation are attributable to the Eu^{3+} and Gd^{3+} ions, respectively. These results further indicate that lanthanide complexes in the micelle do not interfere with each other and that the luminescence and relaxation rate of the micelle can be adjusted simply by changing the Eu^{3+}/Gd^{3+} composition ratio.

The high tunability of the physical properties of the Eu/Gd-C3Ms holds great promise from the application point of view, so we have further investigated the stability of the micellar structures at high salt concentrations and with competing ligand solutions. Complex coacervate core micelles typically strongly respond to ionic strength,[18] hence, we first studied the effect of salt on the micelles. Fig. 3 shows the light scattering intensity and hydrodynamic radius of Gd-C3Ms as a function of salt concentration. Remarkably, the intensity decreases only slightly and the radius does not change up to 500 mM NaCl; the critical salt concentration (CSC) where the Gd-C3Ms dissociate completely is around 800 mM. This is significantly higher than for previously reported

Fig. 1 Left: light scattering intensity and hydrodynamic radius of Eu/Gd-C3Ms at different Eu^{3+}/Gd^{3+} ratios. Right: Cryo-TEM images of Gd-C3Ms (micelles were prepared in 20 mM acetate buffer, pH 5, and total metal concentration is fixed at 0.5 mM L^{-1}).
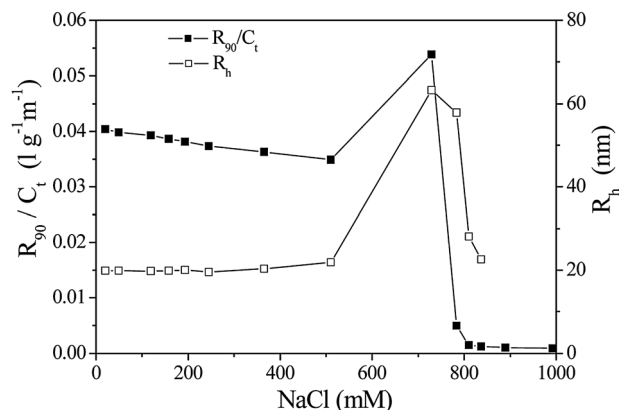
Fig. 3 The effect of salt concentration on scattering intensity and size of Gd-C3Ms.

systems (Zn-C3Ms, Fe-C3Ms), where micelles started to dissociate immediately upon increasing the salt concentration and fell apart completely around 200 mM salt concentration.[19] The iron(III) and zinc(II) micelles in these previous studies were formed from linear $M–L_2EO_4$ complexes ($M/L_2EO_4 = 1/1$) with only one or two negative charges for each coordination center, respectively. It is thus likely that the high stability of Gd-C3Ms is due to the branched structure and the high negative charge of $Gd–L_2EO_4$ complexes formed at a 1/1.5 ratio with three tridentate ligand moieties coordinating to a single lanthanide ion. Both the intensity and hydrodynamic radius of Gd-C3Ms show an increase followed by a sharp drop upon approaching the CSC point, which is due to the formation of worm-like structures near the CSC point.[20] In addition, the following stability tests were carried out by putting Eu-C3Ms and Gd-C3Ms in PBS buffer and PBS buffer with added EDTA as a strong competing ligand (at the same concentration as that of the L_2EO_4 ligand). The variations of the luminescence intensity of Eu-C3Ms, relaxation rate of Gd-C3Ms, light scattering intensity and hydrodynamic radii of both were recorded over a period of 100 hours. It turns out that the micelles in the different solutions do not change size, luminescent intensity (Eu-C3Ms) and relaxation rate (Gd-C3Ms) over time (ESI,† Fig. S4 and S5). The identical response to the PBS buffer with and without EDTA strongly corroborates the stability of the coordinated lanthanide ions in the micellar structures.

In conclusion, we reported a novel strategy to combine lanthanide(III) ions in a micelle system containing several hundreds of lanthanide ions in the coacervate core. The Eu^{3+} and Gd^{3+} amounts can be adjusted at will, leading to high tunability between luminescence and relaxivity of the micelles. Eu/Gd-C3Ms are very stable: the light scattering intensity and hydrodynamic radius, cryo-TEM images, the luminescent intensity and the relaxation rate remaining constant in PBS buffer and PBS–EDTA solution over a few days. The high tunability and stability provide great potential for use in new materials like bi- or multimodal imaging probes, for material or biomedical applications; also radioisotopes could be mixed in for additional diagnostic modality and/or for therapeutic purposes.[5b,21] The excellent response of the lanthanide ions and the stability of the micellar structures allow for further studies investigating the inner structure of coacervate micelles in detail, particularly the

dynamics of water and (buffer) ion exchange of the core with the bulk. On the other hand, further varying other lanthanide combinations as well as ligand and polymer variations open a wide field of investigation and applications.

Notes and references

1 J. C. G. Bunzli and C. Piguet, *Chem. Rev.*, 2002, **102**, 1897.
2 (*a*) D. D. Castelli, E. Gianolio and S. Aime, *Bioinorganic Medicinal Chemistry*, 2011, ch. 8, p. 223; (*b*) P. Hermann, J. Kotek, V. Kubicek and I. Lukes, *Dalton Trans.*, 2008, 3027.
3 (*a*) G. F. de Sa, O. L. Malta, C. D. Donega, A. M. Simas, R. L. Longo, P. A. Santa-Cruz and E. F. da Silva, *Coord. Chem. Rev.*, 2000, **196**, 165; (*b*) D. Parker, *Coord. Chem. Rev.*, 2000, **205**, 109; (*c*) C. M. G. dos Santos, A. J. Harte, S. J. Quinn and T. Gunnlaugsson, *Coord. Chem. Rev.*, 2008, **252**, 2512; (*d*) A. Ruggi, D. N. Reinhoudt and A. H. Velders, *Bioinorganic Medicinal Chemistry*, 2011, ch. 13, p. 383.
4 (*a*) Y. T. Lim, Y. W. Noh, J. H. Han, Q. Y. Cai, K. H. Yoon and B. H. Chung, *Small*, 2008, **4**, 1640; (*b*) W. J. M. Mulder, A. W. Griffioen, G. J. Strijkers, D. P. Cormode, K. Nicolay and Z. A. Fayad, *Nanomedicine*, 2007, **2**, 307.
5 (*a*) L. Frullano and T. J. Meade, *JBIC, J. Biol. Inorg. Chem.*, 2007, **12**, 939; (*b*) J. Kuil, A. H. Velders and F. W. B. van Leeuwen, *Bioconjugate Chem.*, 2010, **21**, 1709.
6 (*a*) J. C. G. Bunzli, *Acc. Chem. Res.*, 2006, **39**, 53; (*b*) B. K. Gupta, V. Rathee, T. N. Narayanan, P. Thanikaivelan, A. Saha, Govind, S. P. Singh, V. Shanker, A. A. Marti and P. M. Ajayan, *Small*, 2011, **7**, 1767.
7 J. M. Brysona, K. M. Fichterb, W. J. Chuc, J. H. Leec, J. Lia, L. A. Madsena, P. M. McLendona and T. M. Reinekea, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 16913.
8 D. J. Lewis, P. B. Glover, M. C. Solomons and Z. Pikramenou, *J. Am. Chem. Soc.*, 2011, **133**, 1033.
9 (*a*) C. S. Bonnet and E. Toth, *C. R. Chim.*, 2010, **13**, 700; (*b*) L. Pellegatti, J. Zhang, B. Drahos, S. Villette, F. Suzenet, G. Guillaumet, S. Petoud and E. Toth, *Chem. Commun.*, 2008, 6591.
10 (*a*) C. Dong, J. Pichaandi, T. Regier and F. van Veggel, *J. Phys. Chem. C*, 2011, **115**, 15950; (*b*) K. Riwotzki and M. Haase, *J. Phys. Chem. B*, 1998, **102**, 10129.
11 V. Sudarsan, F. van Veggel, R. A. Herring and M. Raudsepp, *J. Mater. Chem.*, 2005, **15**, 1332.
12 T. Vermonden, D. Branowska, A. T. M. Marcelis and E. J. R. Sudholter, *Tetrahedron*, 2003, **59**, 5039.
13 Y. Yan, N. A. M. Besseling, A. de Keizer, A. T. M. Marcelis, M. Drechsler and M. A. C. Stuart, *Angew. Chem., Int. Ed.*, 2007, **46**, 1807.
14 (*a*) T. Vermonden, W. M. de Vos, A. T. M. Marcelis and E. J. R. Sudholter, *Eur. J. Inorg. Chem.*, 2004, 2847; (*b*) Y. Yan, N. A. M. Besseling, A. de Keizer and M. A. C. Stuart, *J. Phys. Chem. B*, 2007, **111**, 5811.
15 J. Y. Wang, A. de Keizer, H. P. van Leeuwen, Y. Yan, F. Vergeldt, H. Van As, P. H. H. Bomans, N. Sommerdijk, M. A. Cohen Stuart and J. van der Gucht, *Langmuir*, 2011, **27**, 14776.
16 (*a*) J. P. Kirby, M. L. Cable, D. J. Levine, H. B. Gray and A. Ponce, *Anal. Chem.*, 2008, **80**, 5750; (*b*) M. D. Yilmaz, S. H. Hsu, D. N. Reinhoudt, A. H. Velders and J. Huskens, *Angew. Chem., Int. Ed.*, 2010, **49**, 5938.
17 D. D. Castelli, E. Gianolio, S. G. Crich, E. Terreno and S. Aime, *Coord. Chem. Rev.*, 2008, **252**, 2424.
18 I. K. Voets, A. de Keizer and M. A. Cohen Stuart, *Adv. Colloid Interface Sci.*, 2009, **147–148**, 300–318.
19 (*a*) J. Y. Wang, A. de Keizer, R. Fokkink, Y. Yan, M. A. Cohen Stuart and J. van der Gucht, *J. Phys. Chem. B*, 2010, **114**, 8313; (*b*) Y. Yan, A. de Keizer, M. A. Cohen Stuart, M. Drechsler and N. A. M. Besseling, *J. Phys. Chem. B*, 2008, **112**, 10908.
20 H. M. van der Kooij, E. Spruijt, I. K. Voets, R. Fokkink, M. A. Cohen Stuart and J. van der Gucht, *Langmuir*, 2012, **28**, 14180.
21 S. S. Kelkar and T. M. Reineke, *Bioconjugate Chem.*, 2011, **22**, 1879.