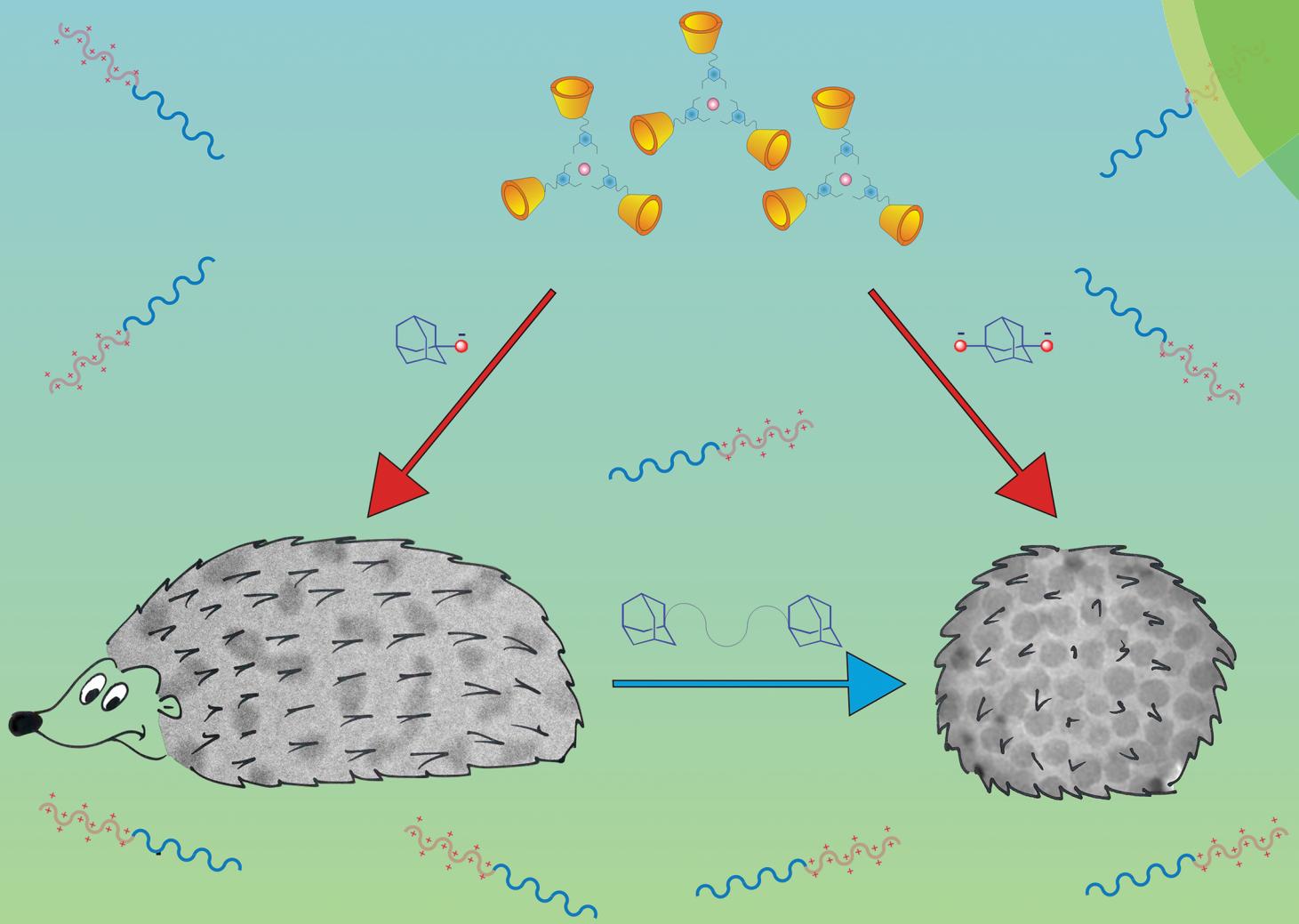


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## Cyclodextrin-based complex coacervate core–micelles with tuneable supramolecular host–guest, metal-to-ligand and charge interactions†

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Micelles have been recognized as versatile platforms for different biomedical applications, from bioimaging to drug delivery. Complex coacervate core micelles present great advantages compared to traditional micelles, however controlling the number of charges per core-unit and the stability is still a challenge. We here present cyclodextrin-based complex coacervate core micelles where the charge per core-unit can be straightforwardly tuned by cyclodextrin host–guest interactions. By varying the ratio between two adamantane guest molecules, 1-adamantanecarboxylic acid and 1,3-adamantanediacetic acid, the charge of the monomeric core-units can be finely tuned from 6– to 9–. By adding an adamantane bislinker, monomeric core-units can be combined together in dimeric and polymeric structures, increasing the micelles' stability. The orthogonal supramolecular host–guest and coordination-chemistry allows for well-controlled cyclodextrin-based complex coacervate core micelles that offer a versatile platform for designing future, e.g., responsive systems.

## 1. Introduction

Micelles provide versatile platforms in adaptive and responsive materials, e.g., for drug delivery due to the core capable of solubilizing and incorporating drugs.<sup>1</sup> In the last two decades, there has been growing interest in Complex Coacervate Core Micelles (C3Ms), which are formed by the electrostatically driven self-assembly of oppositely charged (block co)polymers.<sup>2,3</sup> Fundamental advantages of C3Ms, compared to, e.g., surfactant micelles, are (1) the high stability, (2) the relatively hydrophilic core, (3) the possibility to assemble with almost any charged molecules and (4) the prolonged circulation in the bloodstream.<sup>4–10</sup> Despite all the advantages and applications of C3Ms, controlling the number of charges of the building blocks and the stability is still a challenge. The first C3Ms, described by Harada and Kataoka, were formed by a rough assembly of oppositely charged polymers, without precise control over the charge.<sup>11</sup> In further studies, Cohen Stuart *et al.* achieved a higher charge control of micelle formation, compared to polyion micelles, by using coordination chemistry. The coordination between

dipicolinic acid molecules and di-cationic transition metal ions formed negatively-charged coordination polymers in the core,<sup>12</sup> neutralized by a positively-charged and neutral block copolymer (PMVP-PEO), forming metal-to-ligand based C3Ms.<sup>13,14</sup> By substituting first-row transition metal ions with lanthanide(III) ions, the core coordination polymers became branched and more stable. Changing the ratio between different lanthanides in the coordination core, for example Eu/Gd, different properties, such as luminescence and magnetic properties, could be simply adjusted.<sup>15</sup>

In recent studies, a better understanding of the formation and stability of C3Ms was achieved by using different generations of PAMAM dendrimers, which bear a well-defined number of charges. By adding the positively charged-neutral block copolymer (PMVP-PEO) several negatively-charged PAMAM dendrimers were encapsulated, forming the so-called dendrimicelles. Each negatively charged PAMAM dendrimer was considered as a monomeric core-unit, and below a critical number of charges per monomeric core-unit (*i.e.*, 8), no dendrimicelle could be formed. This suggested that (dendri)micelle formation depends on the number of charges per monomeric-unit in the core and that micelle stability increases with the increase in the number of charge-per-core units.<sup>16</sup>

A further understanding of the formation and stability of C3Ms was achieved by including nanoparticles (NP) inside dendrimers, inside micelles, with a so-called box-in-a-box structure.<sup>17,18</sup>

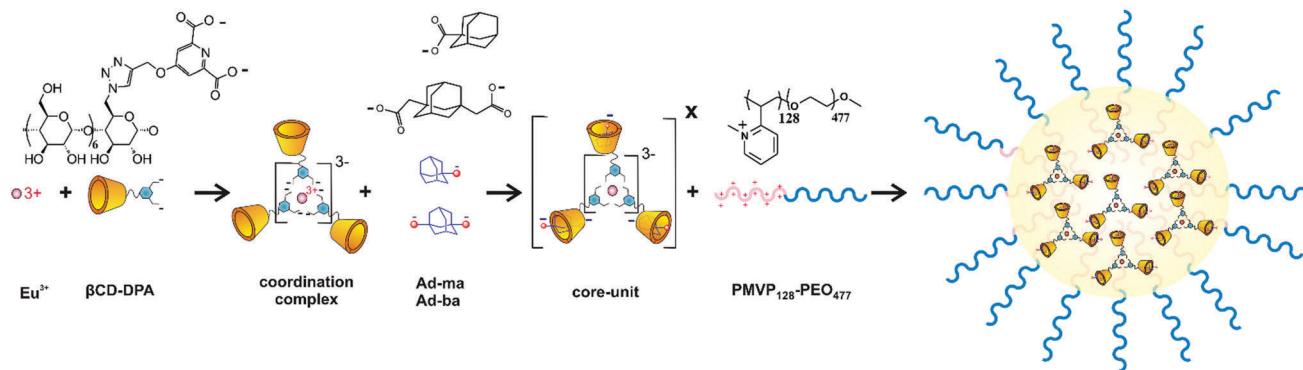
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**Scheme 1** Schematic illustration and molecular structures of core-units used for the formation of Cyclodextrin-based Complex Coacervate Core Micelles (C4Ms). One europium ion is coordinated by three  $\beta$ CD-DPA molecules, forming the coordination complex. By adding three adamantane molecules to the coordination structure, the core-unit is formed. By varying the ratio between the two adamantane guest molecules, 1-adamantanecarboxylic acid (mono-acid, Ad-ma) and 1,3-adamantanediacidic acid (bis-acid, Ad-ba), the charge can be tuned from 6 $-$  to 9 $-$  ( $X = 6, 7, 7.5, 8, \text{ and } 9$ ). By adding the block polymer, PMVP<sub>128</sub>-PEO<sub>477</sub> C4Ms are formed.

In this paper, we present a new class of coacervate-core micelles (see Scheme 1), called Cyclodextrin-based Complex Coacervate Core Micelles (C4Ms), in which the cyclodextrin is covalently tethered to a dipicolinic acid (CD-DPA) unit that can coordinate to metal ions. C4Ms offer the possibility of easy and fine tuning of the charge of the core-unit and the stability of the micelles *via* orthogonal supramolecular interactions gaining insight and control over the lower limit of charge-per-core-unit required for coacervation.

The cyclodextrin/adamantane host-guest pair was selected for its well-known high association constant in water.<sup>19–21</sup> By varying the ratio between the two adamantane guest molecules, 1-adamantanecarboxylic acid (mono-acid, Ad-ma) and 1,3-adamantanediacidic acid (bis-acid, Ad-ba), the charge of a Eu-CD-DPA complex can be tuned from 6 $-$  to 9 $-$ . By adding Ad-ma to the coordination structure, the monomeric unit<sup>6 $-$</sup>  was formed with the lowest charge per core-unit. By adding Ad-ba to the coordination structure, the monomeric unit<sup>9 $-$</sup>  was formed with the highest charge per core-unit. The intermediate charges per core-unit, such as monomeric unit<sup>7 $-$</sup> , monomeric unit<sup>7.5 $-$</sup> , and monomeric unit<sup>8 $-$</sup> , were formed by adding Ad-ma and Ad-ba in 2:1, 1:1, and 1:2 Ad-ma/Ad-ba ratios (Scheme 1). The charge per core-unit is a statistic average value, based on the stoichiometric addition of different ratios between Ad-ma and Ad-ba.

Cyclodextrin-based Complex Coacervate Core Micelles, C4Ms, based on monomeric Eu(III)-complex core-units with charged adamantane guest molecules can be formed by adding a positive-neutral block copolymer P2MVP<sub>128</sub>-PEO<sub>477</sub>. The stability of the C4Ms can be further adjusted by adding a double-guest bridging linker molecule. By adding this bislinker, monomeric core-units can be combined together in dimeric and polymeric structures, which results in an overall higher charge-per-core-unit. By increasing the bislinker concentration, the micelle stability increases. In this paper, we present a new class of versatile micelles, C4Ms, in which the orthogonal supramolecular interactions allow for a systematic investigation of the tuneable charge and micelle stability.

## 2. Experimental

Materials and methods, synthesis and micelle preparations are described in the following sections.

### 2.1 Materials and methods

Europium nitrate ( $\text{Eu}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ ), 1,3-adamantanediacidic acid, 1-adamantanecarboxylic acid, di-isopropylethylamine (DIPEA), amantadine HCl, propargyl bromide, and tris[(1-benzyl-1,2,3-triazol-4-yl)methyl]amine (TBTA) were purchased from Sigma Aldrich and used without further purification. Boc-Glu-OH and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) were purchased from TCI and used without further purification.  $\beta$ -Cyclodextrin monoazide was purchased from Cyclodextrin-Shop and used without further purification. All stock solutions were kept at pH 7. The block copolymer, poly(*N*-methyl-2-vinyl-pyridinium iodide)-*b*-poly(ethyleneoxide) (P2MVP<sub>128</sub>-*b*-PEO<sub>477</sub>), was obtained by quaternization of poly(2-vinylpyridine)-*b*-poly(ethylene oxide) (PVP<sub>128</sub>-*b*-PEO<sub>477</sub>) (Polymer Source,  $M_w/M_n = 1.03$ ,  $M_w = 13.3$  k) following a procedure described elsewhere. The degree of quaternization was 90%, as determined by DLS titration.<sup>22</sup>

All NMR measurements were carried out at 298 K on a Bruker Avance III 400 MHz, 500 MHz, or 600 MHz NMR spectrometer. The assignments were aided by COSY, HSQC, NOESY, ROESY and HMBC experiments. All mass spectra were acquired using ES ionization on a Thermo Finnigan LXQ Exactive Mass Spectrometer. Light scattering measurements (dynamic) and zeta potential measurements were carried out on a Malvern NanoSizer ZS, at an angle of 173 degrees, equipped with an argon-helium laser, operating at 632.8 nm at 25 °C. Multiangle measurements were performed using an ALV light scattering setup equipped with a 300 mW Cobolt Flamenco DPSS laser at 660 nm operated at 100 mW. Titrations were carried out using the Malvern NanoSizer ZS DLS and adding stock solutions stepwise to a 0.5 mL sample volume. All samples were measured at 25 °C. UV-vis measurements were carried out using a Hitachi U-2010 spectrophotometer using quartz



cuvettes of 1 cm path length. Fluorescence spectroscopy measurements were carried out on an Agilent Cary Eclipse Fluorescence Spectrophotometer using quartz cuvettes of 1 cm path length, and excitation and emission slits were set at 5 nm. Sonication was carried out in the sonication bath (Fisherbrand FB15053). Silica columns were prepared with silica of 40–63 µm, 60 Å, purchased from Screening Devices BV. Reversed silica columns were prepared with C18 functionalized irregular silica of 40–63 µm, 60 Å. For Cryo-TEM, the samples were cast on copper grids (400 mesh – 150 µm average hole size, Holey carbon) purchased from Electron Microscopy Sciences (EMS, Hatfield, PA, USA). After blotting, the samples were plunged into liquid ethane by using a Vitrobot Mark IV instrument. The grids were then transferred to a JEOL 1400 PLUS TEM operating at 120 kV.

## 2.2 Synthesis

The synthesis of diethyl 4-(prop-2-yn-1-yloxy)pyridine-2,6-dicarboxylate (2) and 1,5-bis(((3s,5s,7s)-adamantan-1-yl)amino)-1,5-dioxopentan-2-aminium (9) are described in the ESI.†

**2.2.1 Synthesis of pyridine-2,6-dicarboxylate modified  $\beta$ -cyclodextrin (5) (Scheme 2).** THF and water were purged with nitrogen for two hours. Compound 3 (200 mg, 0.16 mmol) and water (20 mL) were mixed under stirring. In the same flask, 2 (80 mg, 0.28 mmol), THF (10 mL), TBTA (6 mg, 0.01 mmol) and CuI (38 mg, 0.20 mmol) were added. The reaction was left stirring under a  $N_2$ -atmosphere at RT for 24 h, and the reaction was monitored using TLC. The volume of the reaction mixture was reduced to 5 mL by concentrating it *in vacuo*. The crude

reaction mixture was added directly to a reversed phase silica column C18 column (water:methanol from 2:1 to 1:1). This resulted in 167 mg of compound 4 (Scheme 2-i and Scheme S2, ESI†). The deprotection of compound 4 was performed by dissolving it (167 mg, 0.12 mmol) and  $K_2CO_3$  (83.5 mg, 1.2 mmol) in water (20 mL) (Scheme 2-ii). The reaction was heated to 70 °C and left stirring overnight. After concentration *in vacuo*, the product (5) was purified by dialysis (MW cut-off of 500–1000 kDa) over three days, replacing the water twice a day. The total yield was 153 mg (55%).

$^1H$  NMR spectrum (400 MHz,  $D_2O$ ):  $\delta$  8.21 (s, 1H, CH triaz.), 7.61 (s, 2H, CH pyr), 5.41 (s, 2H, pyr O-CH<sub>2</sub>), 5.13 (d,  $J$  = 3.6 Hz, 1H, H<sub>1''</sub>), 5.05 (m, 5H, H<sub>1</sub>), 5.03 (s, 1H, 1H<sub>6'f</sub>), 4.98–4.94 (m, 1H, H<sub>1'</sub>), 4.64 (m, 1H, H<sub>6'g</sub>), 4.21 (t,  $J$  = 10 Hz, 1H, H<sub>5'</sub>), 4.04–3.89 (m, 7H, H<sub>3</sub>), 3.89–3.78 (m, 18H, H<sub>6</sub> H<sub>5</sub>), 3.65–3.41 (m, 14H, H<sub>2</sub> H<sub>4</sub>), 3.14 (d,  $J$  = 12 Hz, 1H, H<sub>6'f</sub>), 2.74 (d,  $J$  = 12 Hz, 1H, H<sub>6'g</sub>).

$^{13}C$  NMR spectrum (150 MHz,  $D_2O$ ): ppm 172.46 (C=O), 165.98 (C-O pyr.), 155.04 (C C=O), 142.37 (C triaz.), 127.21 (C-H triaz.), 111.6 (CH pyr.), 101.88 (1C'', 1C), 101.34 (1C'), 83.07 (4C'), 80.79–81.28 (4C), 80.62 (4C''), 73.06–71.48 (2C, 3C, 5C), 70.40 (5C) 60.81 (6C) 51.29 (6C'). Calculated monoisotopic mass ( $[M - 2H]^{2-}$ ) for 5, M =  $C_{52}H_{76}N_4O_{39}$ , is 689.20. The experimental mass is 689.17.

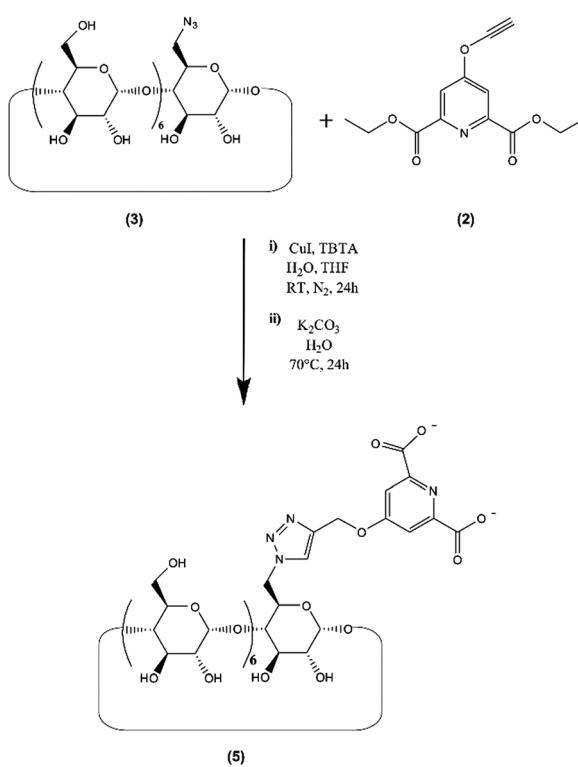
## 2.3 Micelles' preparation

Stock solutions of europium nitrate (5 mM), P2MVP<sub>128</sub>-*b*-PEO<sub>477</sub> (10 mM) (+),  $\beta$ CD-DPA (5 mM), adamantanes (1 mM) and bis-adamantane (1 mM) were prepared by dissolving the powders into MQ water and adjusting the solutions at pH 7. Micelles were prepared under sonication at room temperature to a final volume of 0.5 mL.

Micelle preparation consists of three steps (Scheme 1). First, the coordination complex between Eu<sup>3+</sup> and DPA was formed by adding europium ions at a final concentration of 0.2 mM.  $\beta$ CD-DPA was added in a 3:1 ratio of  $\beta$ CD-DPA/Eu. Second, the core-unit was formed by adding adamantane guest molecules to the coordination structure in a 1:1 ratio of Ad/ $\beta$ CD-DPA. The core-unit<sup>6-</sup> was formed by adding Ad-ma to the coordination structure at a molar ratio of 3:1 Ad-ma/Eu. The monomeric unit<sup>9-</sup> was formed by adding Ad-ba to the coordination structure at a molar ratio of 3:1 Ad-ba/Eu. The intermediately-charged core-unit<sup>7-</sup>, core-unit<sup>7.5-</sup>, and core-unit<sup>8-</sup> were formed by adding Ad-ma and Ad-ba to 1 in 2:1, 1:1, and 1:2 Ad-ma/Ad-ba ratios (the total adamantane concentration was kept at 1:1 Ad/ $\beta$ CD-DPA). Third, the block copolymer P2MVP<sub>128</sub>-PEO<sub>477</sub> was first sonicated for 10 minutes and then added in the correct amount to neutralize the negative core charges. After the addition of all compounds, the samples were left to equilibrate for 10 min.

C4Ms based on a mix of monomeric core-unit<sup>6-</sup> and dimeric units, called (C4Ms<sup>6-\*</sup>), were formed by adding 90% Ad-ma and 5% of NH<sub>2</sub>-Glu(Ad)-Ad compound 9, ESI†, with respect to the  $\beta$ CD-DPA concentration, to the europium complex and the block copolymer.

C4Ms based on polymeric core-units were formed by adding bislinker 9 to the coordination structure at a molar ratio of 3:1 of Ad/Eu and the corresponding amount of



Scheme 2 Reaction scheme of the synthesis of pyridine-2,6-dicarboxylate modified  $\beta$ -cyclodextrin.



the block copolymer. During all experiments, the concentration of the components was fixed above the CMC to prevent the equilibrium from shifting from micelles towards monomers (Fig. S2, ESI<sup>†</sup>).

### 3. Results and discussion

First, the formation of micelles based on monomeric metal complexes is described, followed by a discussion of the samples that contain bridging ligands connecting core-units.

#### 3.1 Tuning the size and stability of monomeric core-units

Scheme 1 shows the formation of Cyclodextrin-based Complex Coacervate Core Micelles (C4Ms) in three steps. The starting point for the C4Ms' formation was the coordination complex between Eu<sup>3+</sup> and DPA. Formation of the coordination complex was confirmed by fluorescence spectroscopy, exciting DPA, and observing Eu<sup>3+</sup> emission from <sup>5</sup>D<sub>0</sub> to <sup>7</sup>F<sub>j</sub>, confirming the antenna phenomenon and therefore the complex formation (Fig. S1, ESI<sup>†</sup>).<sup>23</sup> The sample presented a Eu<sup>3+</sup> lifetime of 1.5 ms, typical for a fully coordinated structure (data not shown).<sup>24</sup>

The following step for the micelle formation was to form the core-unit by adding adamantane guest molecules to the coordination complex. The charge of the core-unit was adjusted by varying the ratio between the two adamantane species, 1-adamantanecarboxylic acid (mono-acid, Ad-ma) and 1,3-adamantanediacidic acid (bis-acid, Ad-ba). By adding Ad-ma to the coordination structure, the core-unit<sup>6-</sup> was formed with the lowest charge per core-unit. By adding Ad-ba to the coordination structure, the core-unit<sup>9-</sup> was formed with the highest charge per core-unit. Intermediate charges per core-unit, such as core-unit<sup>7-</sup>, core-unit<sup>7.5-</sup>, core-unit<sup>8-</sup>, were formed by adding Ad-ma and Ad-ba in 2:1, 1:1, and 1:2 Ad-ma/Ad-ba ratios. The host-guest interaction between adamantanes and  $\beta$  cyclodextrin is already well reported in the literature.<sup>19,25</sup> Notwithstanding, the core-unit formation between cyclodextrin and adamantane molecules was confirmed by ROESY experiments (Fig. S16, ESI<sup>†</sup>).

The final step for micelle formation is the addition of the positive-neutral block copolymer P2MVP<sub>128</sub>-PEO<sub>477</sub>. The P2MVP<sub>128</sub>-PEO<sub>477</sub> block copolymer was selected due to its well-reported properties as the micelle counter polyanion.<sup>16,26</sup> Cyclodextrin-based complex coacervate core micelles' formation was confirmed by using zeta-potential and Dynamic Laser Scattering (DLS). Upon the addition of block copolymers, all the samples presented a zeta-potential of  $-0.4 (\pm 0.2)$  mV, confirming the neutralization of the core-unit charges.

DLS results confirmed the formation of micelles for C4Ms based on different core-units. Fig. 1 shows that the hydrodynamic diameter was 45 ( $\pm 6$ ) nm for C4Ms based on the core-unit charge above 6. The core-unit<sup>6-</sup> sample showed micellar aggregates with an apparent diameter of 100 nm, higher than the spherical micelles formed with the core-units<sup>7-,7.5-,8-,9-</sup>.

More investigations were performed by Cryo-TEM and multi-angle DLS with a polarizer. In Fig. 2a, Cryo-TEM results reveal that core-units<sup>9-</sup> are monodisperse and spherical, as expected.

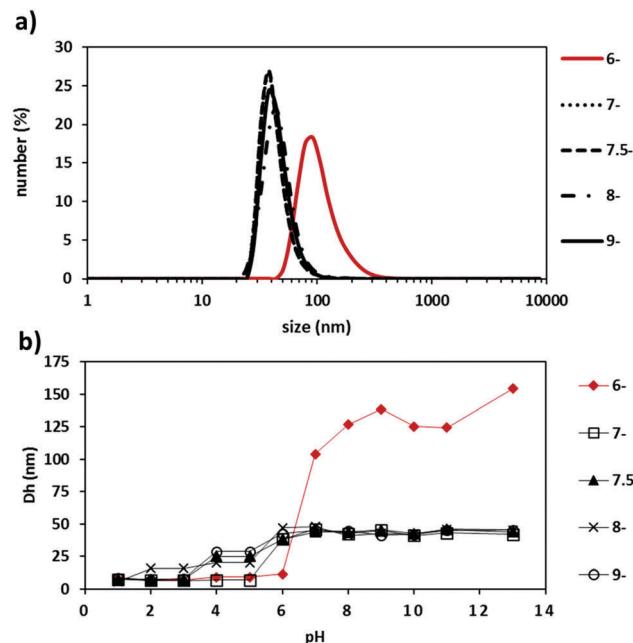


Fig. 1 (a) Hydrodynamic diameter distribution of C4Ms, based on different core-unit charges. By adding the block copolymer to the core-units<sup>7-,7.5-,8-,9-</sup>, stable micelles with a hydrodynamic diameter of 45 nm were formed. However, by adding the block copolymer to the core-unit<sup>6-</sup>, bigger micellar aggregates were formed. (b) Effect of pH on the hydrodynamic diameter, at different C4Ms' core-unit charges. C4Ms based on the core-unit<sup>6-</sup> showed lower stability at high pH, compared to higher charged core-units. (Samples were prepared at pH 7, and the final europium concentration was fixed at 0.2 mM.)

On the other hand, Fig. 2b shows that core-units<sup>6-</sup> micelles are not monodisperse and present a mixture of spherical and more elongated shape aggregates compared to the core-unit<sup>9-</sup> micelles. By introducing a 90 degree polarizer between the sample and the DLS detector, the vertical oriented light was polarized by micelles based on core-units<sup>6-</sup>. The polarization ratio of core-unit<sup>6-</sup> micelles was calculated to be around 0.21, confirming their stretched shape, whereas core-units<sup>9-</sup> have the typical depolarization ratio for spherical particles, around 0.01.<sup>28</sup>

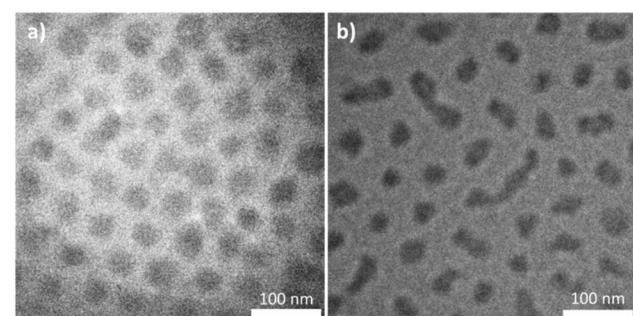


Fig. 2 Size and shape characterization at Cryo-TEM of C4Ms based on the core-unit charge<sup>9-</sup> (a) and C4Ms based on the core-unit charge<sup>6-</sup> (b). The highest core-unit charge C4Ms revealed homogeneously distributed spherical micelles, while the lowest core-unit charge C4Ms showed elongated micelles.



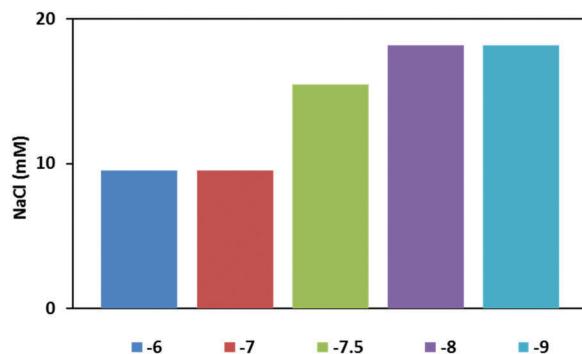


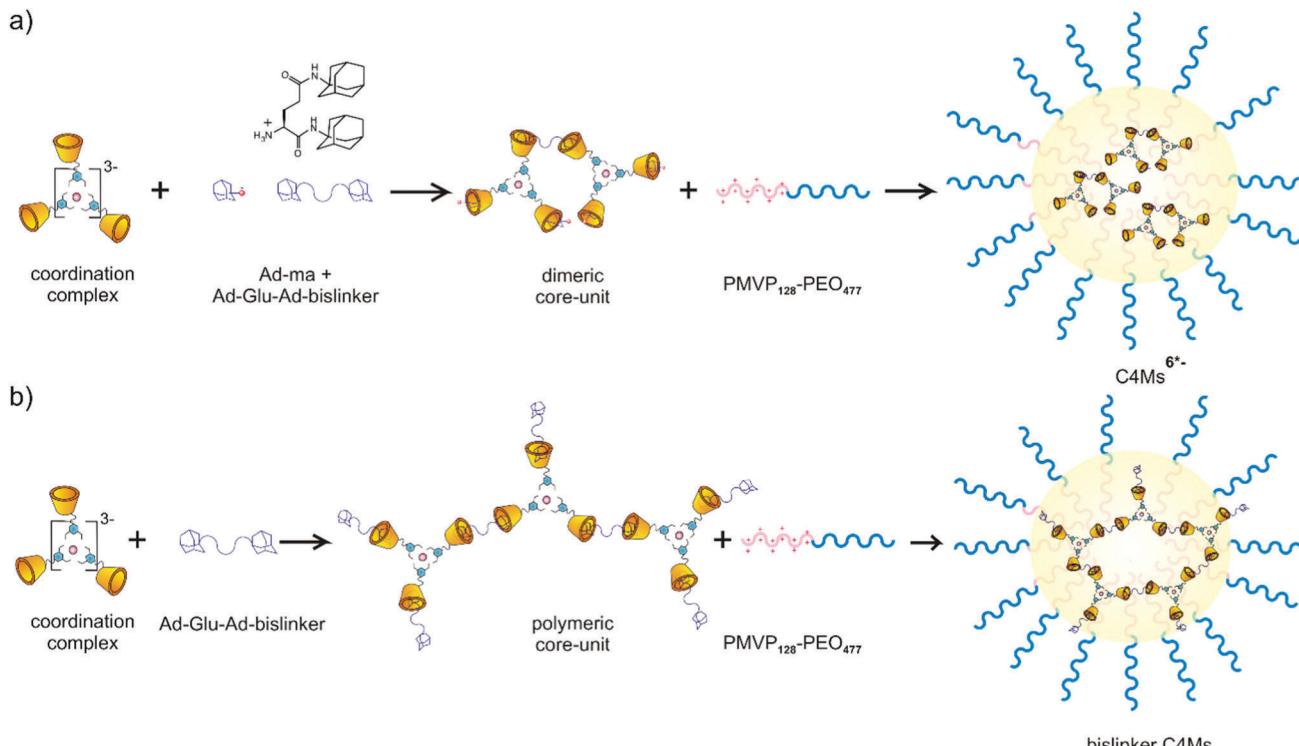
Fig. 3 Salt stability at different core-unit charges. The salt stability was calculated as the maximum salt concentration that C4Ms can tolerate, before the DLS intensity and the size decrease, as in Fig. S5 (ESI<sup>†</sup>). (Micelles were prepared at pH 7, and the final europium concentration was fixed at 0.2 mM.)

The Critical Micelle Concentration (CMC) was determined by titrating water to the core-units<sup>7–7.5–8–9–</sup> C4Ms, using DLS. The CMC was found to be around 1.5  $\mu$ M Eu<sup>3+</sup> ions (Fig. S2, ESI<sup>†</sup>).

A complete study on C4Ms' stability was performed by investigating them at different times, pHs, and salt and competitor concentrations. The size and the intensity of C4Ms were monitored over 36 h by using DLS. The size and intensity of C4Ms based on the core-units<sup>7–7.5–8–9–</sup> were stable up to 36 h (Fig. S3 and S4, ESI<sup>†</sup>). On the other hand, Fig. S3 and S4 (ESI<sup>†</sup>) show an increase in size and intensity of the core-unit<sup>6–</sup>. The C4Ms' stability was investigated at different pH values.

As shown in Fig. 1b, C4Ms based on the core-units<sup>7–7.5–8–9–</sup> were stable at basic pH, but they started to dissociate below pH 6. This effect can be related to the protonation of carboxylic acid groups on the adamantanes, as it would result in monomeric europium(III)-DPA coordination complexes with a net charge of 3–, too low to allow micelle formation.<sup>14</sup> The core-unit<sup>6–</sup> was not following the same stable trend and exhibited high scattered intensity and high size above pH 6.

The C4Ms' stability was also investigated against salt and competing free  $\beta$ CD. By adding salt to the C4Ms, we expect to screen the charges and, therefore, destabilize the complex. Fig. 3 shows a positive trend between salt stability and the core-unit charges. By increasing the core-unit charge, the charge stability against the salt increased. The Critical Salt Concentration (CSC), the point at which micelles start to dissociate, was determined by adding NaCl to micelles based on the core-units<sup>6–7–7.5–8–9–</sup>. C4Ms dissociated completely at 40 mM of NaCl (Fig. S5, ESI<sup>†</sup>). The intensity and hydrodynamic diameter of core-unit<sup>9–</sup> C4Ms showed a high peak at around 30 mM NaCl, representing the micellar shape transition from sphere to rod like.<sup>26,27</sup> Salt addition screens the charge interactions between core-units and block copolymers, thus weakening the driving force for micelle formation. Salt addition might also enhance the hydrophobic association between the adamantane guest and the cyclodextrin host;<sup>29</sup> the latter, however, does not negatively affect the micelle formation. We expect to destabilize C4Ms not only by changing the ionic strength but also by adding a



Scheme 3 Schematic illustration of C4Ms' formation, based on the Ad-Glu-Ad bislinker. (a) By adding 90% of Ad-ma and 10% of the Ad-Glu-Ad bislinker to the coordination complex, dimers can form inside the core. By adding the block polymer to the core dimers, C4Ms<sup>6+</sup> can form. (b) By adding the Ad-Glu-Ad bislinker to the core-unit, a polymeric network can form inside the core. By adding the block polymer to the core dimers, Ad-Glu-Ad bislinker based C4Ms can form.



supramolecular host competitor. By titrating free  $\beta$ CD to C4Ms,  $\beta$ CD competes with  $\beta$ CD-DPA in the core for hosting adamantane molecules, destabilizing the core of the micelle. Fig. S6 (ESI $\dagger$ ) presents the stability of C4Ms against free competing  $\beta$ CD. The same figure shows a positive trend between stability against  $\beta$ CD and core-unit charges. Core-unit $^{9-}$  C4Ms remained stable upon the addition of seven equivalents (5 mM) of  $\beta$ CD compared to the  $\beta$ CD-DPA ligand. On the other hand, the core-unit $^{6-}$  showed the lowest stability against the salt and competing  $\beta$ CD (9 mM and 2 mM respectively).

Moreover, the results showed that 6 negative charges per core-unit were not enough to form spherical, monodisperse and stable C4Ms. Therefore, we consider seven negative charges, the lowest limit of the core-unit charge required for proper coacervation of well-defined micelles. Based on these findings, we further exploited the supramolecular interactions to alter the size and the stability of core-unit $^{6-}$  samples.

### 3.2 Tuning the size and stability of core-units with bridging ligands

To increase the stability of C4Ms, a supramolecular linker was designed to create crosslinks between monomeric-units, increasing the net charge number per core-unit. A new guest adamantane bislinker (Ad-Glu-Ad) was synthesized and characterized. We investigated the size and the stability of C4Ms with the Ad-Glu-Ad bislinker, by using two different sample preparation methods. The first method, called “titration”, consisted of preparing one sample of C4Ms and titrating the increasing amount of the Ad-Glu-Ad bislinker into the just prepared C4Ms. The second method, called “premixing”, consists of the preparation of several samples with increasing stoichiometric ratios between Ad-Glu-Ad bislinker/Ad-ma, prepared before the addition of the block copolymer. A substantial difference between the two methods is that in the titration method, we add an excess of adamantanes to the already supramolecularly saturated micelles. However in the pre-mix, a stoichiometric addition of Ad-ma and the Ad-Glu-Ad bislinker with respect to the cyclodextrin concentration is added before block copolymer addition and micelle formation. A change in the titration experiment would therefore moreover confirm the dynamic exchange behavior of C4Ms’ building blocks between the core-unit and the environment.

By titrating the Ad-Glu-Ad bislinker to the core-unit $^{6-}$  micellar aggregates, a decrease in the size and intensity became visible already at the very first titration steps (0.03 mM Ad-Glu-Ad over 0.73 mM Ad-ma, thus 4% of Ad-Glu-Ad with respect to Ad-ma, Fig. S7, ESI $\dagger$ ). By titrating 0.06 mM of Ad-Glu-Ad (8% of Ad-Glu-Ad with respect to Ad-ma) to the core-unit $^{6-}$ , the formation of 59 ( $\pm 5$ ) nm micelles was triggered. The size of those micelles remained constant with the addition of 0.35 mM Ad-Glu-Ad (100% of Ad-Glu-Ad with respect to Ad-ma).

Fig. S8 (ESI $\dagger$ ) shows the effect of pre-mixing Ad-Glu-Ad and Ad-ma on the scattered intensity and size. The results indicate that above 0.04 mM of Ad-Glu-Ad (5% of Ad-Glu-Ad with respect to 90% Ad-ma), the intensity and size were in the same range of core-unit $^{9-}$  C4Ms. Above that value, the core-unit $^{6-}$  forms

the micelles similar in size to core-unit $^{9-}$  C4Ms. Based on the results in Fig. S7 and S8 (ESI $\dagger$ ), 5% Ad-Glu-Ad with respect to Ad-ma was chosen as the value to improve the stability of the core-unit $^{6-}$ , expecting statistically a mixture of the core-unit $^{6-}$  with new core-unit dimers, the whole being defined here as the core-unit $^{6-*}$  (Scheme 3a). Also, by taking 100% of Ad-Glu-Ad and no monomeric adamantane, a new core-unit based on polymeric structures was formed, core-unit $^{bislinker}$  (see Scheme 3b).

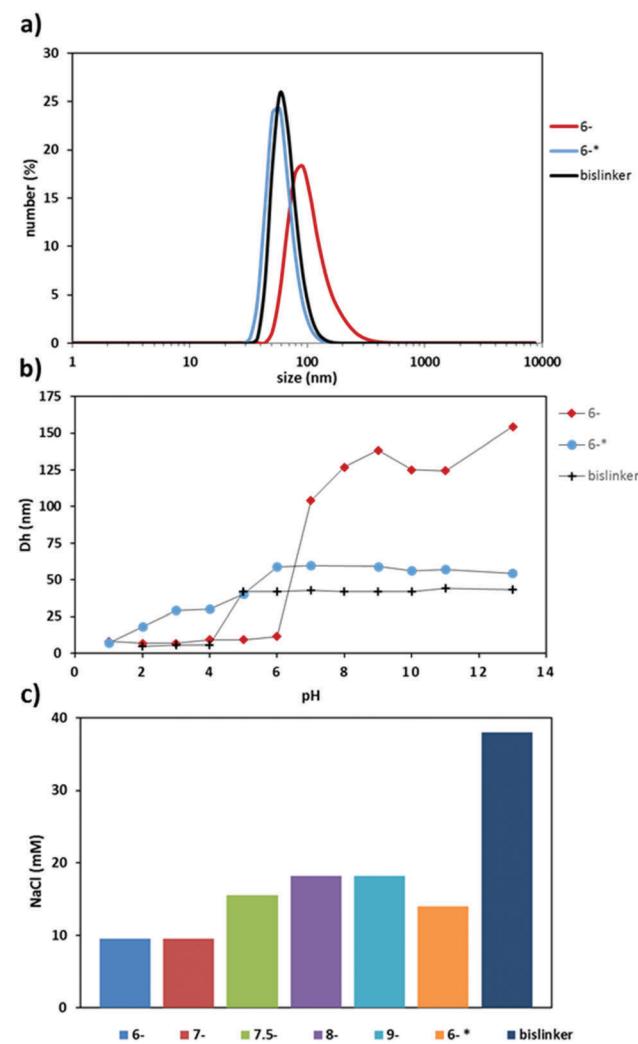


Fig. 4 (a) Hydrodynamic diameter distribution of monomeric-unit $^{6-}$  C4Ms, dimeric unit $^{6-*}$  C4Ms and polymeric unit C4Ms based on the bislinker. Dimeric unit $^{6-*}$  C4Ms and a polymeric unit form C4Ms of smaller size compared to the monomeric-unit $^{6-}$  C4Ms. (Micelles are prepared at pH 7, and the final europium concentration is fixed at 0.2 mM). (b) Effect of pH on the hydrodynamic diameter, at different C4Ms’ core-unit charges, C4Ms $^{6-*}$ , and Ad-Glu\_Ad C4Ms. C4Ms $^{6-*}$ , Ad-Glu\_Ad C4Ms shows stronger stability at high pH compared to monomeric-unit $^{6-}$  C4Ms. (c) Salt stability at different C4Ms’ monomeric core-unit charges, dimeric unit $^{6-*}$  C4Ms and polymeric unit C4Ms based on the bislinker. The salt stability experiment was performed by adding NaCl to different charged C4Ms. The salt stability was calculated as the maximum salt concentration that C4Ms can tolerate, before the DLS intensity and the size decrease, as in Fig. S5 (ESI $\dagger$ ). (Micelles are prepared at pH 7, and the final europium concentration is fixed at 0.2 mM).



The core-unit<sup>6-</sup>\* was able to form C4Ms of 59 ( $\pm 7$ ) nm, with a low polydispersity index (PDI), which were stable with time (Fig. 4). The bislinker C4Ms showed a size similar to the core-unit<sup>6-</sup>\*, around 60 ( $\pm 7$ ) nm in hydrodynamic diameter, which were stable with time. Micelles based on 100% Ad-Glu-Ad can form even if the residual charge per coordination complex unit is low (1.5<sup>-</sup>). The reason is due to the fact that the bislinker merges core-units together in polymeric and network structures that crosslinks with each other through the block copolymer, similarly to the bis-DPA lanthanide coordination polymers' core micelles reported before.<sup>15</sup> The preparation of C4Ms with 5% of bislinker C4Ms resulted immediately in more stable micelles against pH changes, compared to micelles based on the core-unit<sup>6-</sup>. The stability of core-unit<sup>6-</sup>\* based C4Ms against salt was comparable with the stability of core-unit<sup>7.5-</sup> C4Ms. These improvements in the stability of the C4Ms, achieved by adding Ad-Glu-Ad, corroborate the formation of core-unit dimers in the micelles. Interestingly, not all the 6<sup>-</sup> core-units need to be crosslinked to form micelles. The addition of a small amount of bislinkers (5% with respect to  $\beta$ CD-DPA concentration), which forms dimeric structures with an overall charge of 9<sup>-</sup>, suffices.

By preparing micelles based on 100% of bislinker, the core-unit<sup>bislinker</sup> based C4Ms resulted as stable against pH as core-unit<sup>9-</sup> based C4Ms (Fig. 4 and Fig. S9, ESI†). The stability of the core-unit<sup>bislinker</sup> C4Ms against salt and free  $\beta$ CD was at least double, respectively, four times higher in stability with respect to the other C4Ms prepared with the mono-adamantanes (Fig. 3 and Fig. S10, ESI†).

Combination of orthogonal supramolecular interactions such as cyclodextrin-adamantane host-guest chemistry and lanthanide coordination chemistry has proven to be a successful strategy to gain insight information in the complex surface-solute interface chemistry in which multivalency is of importance.<sup>30,31</sup> The same set of interaction motifs here provides a toolbox to investigate the difficulty in studying the inside of complex coacervate core micelles, in which the multivalent charge interactions between the block copolymer and oppositely-charged supramolecular metal complexes play a crucial role.

## 4. Conclusions

In conclusion, we successfully formed a new class of C3Ms, C4Ms, Cyclodextrin-based Complex Coacervate Core Micelles, based on supramolecular host-guest and metal-to-ligand interactions. We exploited the cyclodextrin/adamantane interaction to finely adjust the core-unit charge of the C4Ms to consecutively investigate the size and the stability of the micelles. The lowest number of charges per core-unit, core-unit<sup>6-</sup>, formed elongated, non-spherical micellar aggregates, around 100 nm in hydrodynamic diameter. These core-unit<sup>6-</sup> based C4Ms' aggregates were less stable against salt and free CD, compared to the highest charged core-unit<sup>9-</sup> based C4Ms. The results on intermediate core-unit charges confirmed that stability increases by increasing the core-unit charge. Moreover, by adding an Ad-Glu-Ad bislinker, the size and stability of core-unit<sup>6-</sup> C4Ms were adjusted,

by forming dimeric and polymeric core-units. We showed how to apply supramolecular host-guest chemistry to finely tune charges, size and stability of the important class of coacervate core micelles. Well-controlled cyclodextrin-based complex coacervate core micelles could offer a versatile platform for future responsive systems.<sup>32,33</sup>

## Conflicts of interest

There are no conflicts to declare.

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