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Regulated assemblies and anion responsive vesicles based on 1,3-alternate oxacalix[2]arene[2]triazene amphiphiles†

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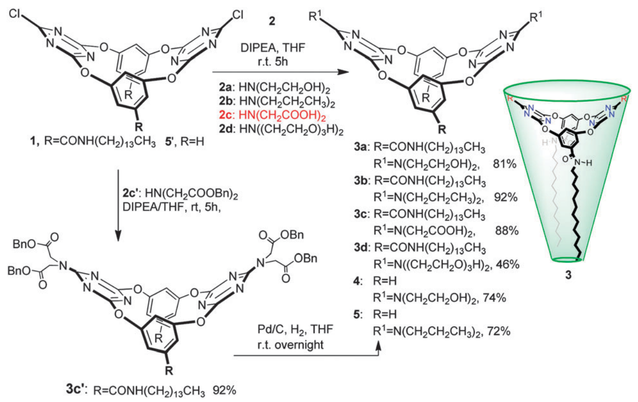
Regulated assemblies from vesicles to micelles based on 1,3-alternate oxacalix[2]arene[2]triazine amphiphilic molecules were reported. The vesicular entities were responsive to anions and followed the selectivity in the order $NO_3^- > Cl^- > Br^- > ClO_4^-$, driven by cooperative anion–π interactions and hydrogen bonding.

Heteracalixaromatics are a new generation of macrocyclic hosts in supramolecular chemistry.[1] Compared to conventional calixarenes, the introduction of bridging heteroatoms and their various conjugations with the adjacent aromatics endow heteracalixaromatics with the structural feature of self-fine-tunability.[1a,b] As a consequence, heteracalixaromatics exhibit versatile recognition abilities towards various guest species including metal ions and clusters,[2] neutral molecules,[3] and anions.[4] Heteracalixaromatics also provide a powerful platform to construct functional architectures.[5] As a representative example of heteracalixaromatics, oxacalix[2]arene[2]triazine, which adopts a shape persistent 1,3-alternate conformation and bears an electron-deficient V-shaped cleft formed by the two triazine rings, has been shown to be a unique anion acceptor based on anion–π interactions.[4a,b]

Anion–π interactions have attracted much attention in recent years.[6] Although the existence of anion–π interactions have been exemplified both theoretically[7] and experimentally,[4,8] the applications of such novel motifs in supramolecular chemistry are still limited.[9–13] In this context, for example, Saha *et al.*[10] reported anion sensing using naphthalenediimides (NDI)[20] as π electron-deficient receptor. Matile's group designed NDI-based rods that can transport anions across lipid bilayer membranes.[11] The same group also reported new catalytic reactions based on anion–π interactions.[12] It worth addressing that exploration of anion–π interactions in the functional molecular and supramolecular systems is one of the vital challenges.[9] Very recently, we reported size-regulable vesicles, the first examples of self-assembled vesicles from oxacalix[2]arene[2]triazine derived amphiphilic molecules in a THF–water system, based on anion–π interactions.[13] To explore the self-assemblies of oxacalix[2]arene[2]triazene based amphiphilic molecules in water, and to probe the responses of vesicles toward anions driven by cooperative non-covalent interactions, we designed new building blocks bearing deliberately incorporated substituent groups on the larger rim of the triazine rings. The effects of varying substituents on self-assembly, and the anion selectivities of the vesicles based on cooperative anion–π interactions and hydrogen bonding are reported herein.

We designed the target molecules by introducing *N*-tetradecyl formacyl groups into the larger rim of the benzene rings, whereas groups of different polarities and hydrophilicities were incorporated into the larger rim of the triazine rings, in order to investigate the regulation of substituent effects on both self-assembly and responses of aggregates towards anions. Aromatic nucleophilic displacement reactions between the starting compound **1**, which was prepared following our previous approach[13] and amines, bis-(2-hydroxy-ethyl)-amine (**2a**), *n*-dipropylamine (**2b**) and 3,6,12,15-tetraoxa-9-azaheptadecane-1,17-diol (**2d**), respectively, were performed (Scheme 1). In the presence of DIPEA as an acid scavenger,



Scheme 1 Synthesis of amphiphiles **3**, **4** and **5**.

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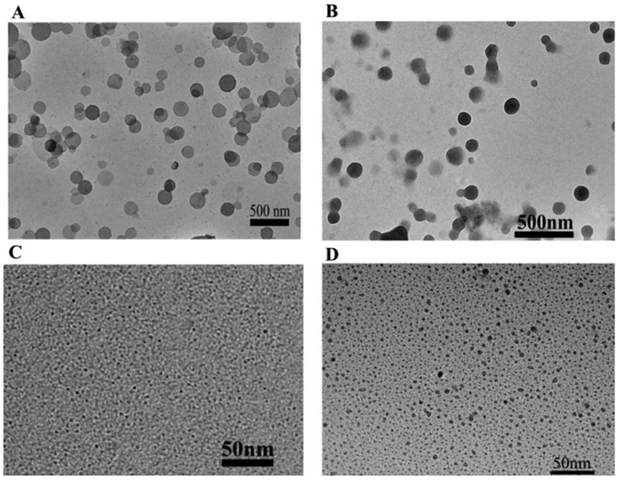

Fig. 1 TEM images of self-assemblies (5×10^{-5} M) of **3a** (A), **3b** (B), **3c** (C) and **3d** (D) in water. Vesicles were not stained.

the reactions proceeded smoothly in THF at ambient temperature to afford **3a**, **3b** and **3d** in 81%, 92% and 46% yields, respectively. However, the reaction of **1** and **2c** failed to give the desired compound **3c**. Then we changed our strategy by protecting the carboxyl group of **2c** with benzyl group, which gave **2c′**. Reaction of **1** and **2c′** under the above conditions afforded **3c′** with high efficiency (92%). Deprotection of **3c′** with Pd/C under a hydrogen atmosphere produced the desired amphiphile **3c** in 88% yield. The structural characterizations of all the amphiphilic compounds were established using spectroscopic and elemental analyses (Fig. S26–S37, ESI†).

With the amphiphilic compounds **3a–3d** in hand, we studied their aggregation behaviours systematically. Under a concentration of 5×10^{-5} M in water, **3a** and **3b** were opalescent, and **3c** and **3d** were clear dispersions, respectively. The assemblies of **3a** and **3b** were firstly assessed using TEM, SEM and LSCM experiments. As shown in Fig. 1 and Fig. S1–S6 (ESI†), spherical morphologies of **3a** and **3b** with average diameters of (250 ± 80) nm and (150 ± 100) nm, respectively, are observed from the TEM and SEM images. The sharp contrast between the periphery and centre of the spheres, as shown by TEM images (Fig. 1A and B), and the spherical and partially collapsed particles demonstrated by SEM images (Fig. S1 and S2, ESI†) indicate the vesicular structure of the aggregates. After a fluorescent probe lucigenin was encapsulated inside the aggregates of **3a** and **3b**, and blue dots are visualized with LSCM experiments (Fig. S8 and S9, ESI†), further supporting the feature of hollow vesicles. DLS measurements gave average hydrodynamic diameters of 248 nm for **3a** (Fig. S11, ESI†) and 185 nm for **3b** (Fig. S12, ESI†), respectively, which is in good agreement with the results of SEM and TEM. In contrast, the morphologies of **3c** and **3d** visualized by TEM images show spherical aggregates with average diameters of 3.5–4.5 nm (**3c**) and 4.5–5.5 nm (**3d**) (Fig. 1C and D; Fig. S5 and S6, ESI†), respectively. Such diameters, in comparison with the extended molecular lengths (2.8 nm for **3c** and 3.5 nm for **3d**, see Fig. S15, ESI†), indicate that both compounds tend to form micelles. We then took pyrene as a fluorescent probe to measure the critical aggregation concentrations (CAC) of **3a–3d**.[14] From the inflection points of the curves expressed as the emission ratio (I_1/I_3, I_1 = 373 nm and I_3 = 384 nm) *versus* concentrations, CACs of 1.8×10^{-6} M for **3a**, 4.0×10^{-7} M for **3b**, 2.1×10^{-6} M for **3c** and 4.2×10^{-7} M for **3d**, respectively, were estimated (Fig. S16–S19, ESI†). The low CAC values of less than 2.1 μM indicated that the designed amphiphilic molecules showed strong self-assembling abilities in water. The dramatically different aggregation behaviours from **3a** and **3b** (vesicle), to **3c** and **3d** (micelle), and the low CACs obtained are very interesting and are quite different from the traditional calix[4]arene based amphiphilic compounds.[15] In comparison with the cone conformation, 1,3-alternated calix[4]arene was thought to be more difficult to aggregate in water, with CACs usually two or more orders of magnitude higher than the former. The shape of 1,3-alternated calix[4]arene was postulated to be cylindrical and hence tended to form less-curved vesicles.[15a] Though oxacalix[2]arene[2]triazines have been demonstrated to adopt shape-persistent 1,3-alternate conformations,[1a,b,5a] the conjugation of bridging oxygen atoms with triazine rings gave the amphiphilic molecules a cone-like rather than a cylindrical molecular shape, which are obviously favored to form aggregates with low CAC values. Possessing a similar shape and the same hydrophobic moieties, the various morphologies from vesicles to micelles are owing to the subtle differences of the substituents on the larger rim of triazine rings (Scheme 1). The nature of the substituents attached on the triazine rings most probably affects the spontaneous curvature of the aggregates. The more polar and hydrophilic chains of **3c** and **3d** are expected to expand in water, which is in favor of the conical shape of the molecules. Therefore, **3c** and **3d** tended to form cone-like aggregates with larger curvature and resulted in the formation of the micelles. However the compounds of **3a** and **3b** bearing substituents of lower polarity and hydrophilicity than that of **3c** and **3d**, preferred to form lamellae with a smaller curvature, the latter then curved to vesicles. In addition to polarity and hydrophilicity, intermolecular hydrogen bonding between the substituents may also be contributable. For example, **3a**, with the pK_a value higher than that of **3c**,[16] is less able to form intermolecular hydrogen bonding than **3c**. The intermolecular hydrogen bonding between hydrophilic substituents would probably drive the ''side to side'' extensive stacking of the amphiphilic molecules of **3a** to strengthened ''head to head'' contacts of **3d**, with the latter leading to more curved arrays.[17] Polarity and hydrophilicity, and/or intermolecular hydrogen bonding of the substituent groups on the larger rim of triazine rings hence play leading roles in regulating the morphologies of the aggregates.[18]

Our interest in vesicular entities encouraged us to carry out XRD experiments to get further insight into the vesicular structures of **3a** and **3b**. From the diffraction peaks observed at $2\theta = 2.30°$ for **3a** (Fig. S20, ESI†) and $2\theta = 2.74°$ for **3b** (Fig. S21, ESI†), the thicknesses of vesicular membranes were estimated to be 3.84 nm (**3a**) and 3.36 nm (**3b**), respectively. In comparison to the extended molecular lengths of **3a** (2.7 nm) and **3b** (2.8 nm) (Fig. S15, ESI†), the measured thicknesses of larger than 2.7 nm and 2.8 nm but less than the double the extended molecular lengths, indicate the formation of a double-layered membrane in which the hydrophobic tails are directed inward and the macrocyclic cavities

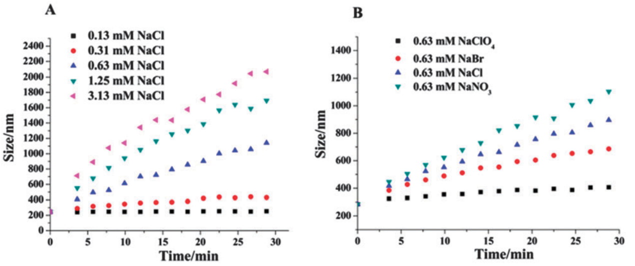

Fig. 2 Vesicular size of **3a** in response to (A) NaCl with different concentrations and (B) various anions.

are coated on the surface. It worth addressing that the diffraction intensity of **3a** (Fig. S20, ESI†) is stronger than that of **3b** (Fig. S21, ESI†), suggesting that a highly ordered membrane was formed with **3a**, which is most probably due to the bis-(2-hydroxylethyl)amino groups introduced into the triazine rings. The polarity and intermolecular interaction of the substituents regulated the hydrophobic–hydrophilic balance and strengthened intermolecular cohesion.

The surface-coated oxacalix[2]arene[2]triazines, which provide unique anion recognizable cavities constructed with the two electron-deficient triazine rings,[4] therefore led us to the study of the response of vesicular entities constructed with **3a** to the anions in water. When the concentration of sodium chloride was lower than 0.13 mM, DLS measurements demonstrated that the aggregate size of **3a** (expressed as the average of the hydrodynamic diameters) is hardly affected. When salt concentrations are in the range of 0.31–3.13 mM (Fig. 2A), the vesicles enlarge with the increasing concentrations. Interestingly, a dynamic change of the vesicle size corresponding to the salt concentrations is observed (Fig. 2A). At a certain concentration (0.63 mM, for example), the size of vesicles increases gradually from 245 nm recorded at 0 min to 1141 nm at 30 min with a 4-fold increment. Such outcomes indicate the formation of larger aggregations.[19] To explore the nature of the salt effect on the vesicle aggregation, various anions including chloride, nitrate, bromide and perchlorate in sodium salts (0.63 mM) were applied. As shown in Fig. 2B, anions affect the vesicle sizes to a different degree. The increments due to the size effects caused by anions follow the order $NO_3^- > Cl^- > Br^- > ClO_4^-$. Such selectivity, which cannot be fully explained by Hofmeister series, indicates that specific non-covalent interaction most probably dominates the interaction between anions and the surface of the vesicles.[13] Analysis of the structure of **3a**, which contains a V-shaped electron-deficient cavity formed by two triazine rings and bears bis(2-hydroxylethyl)amino groups on the larger rim of triazine rings as hydrogen bond donors, reveals that anion–π interactions and hydrogen bonding may contribute to the surface–anion interactions.

To certify this postulate, we chose **3b**, a ''pure'' electron-deficient receptor that excludes other non-covalent interactions as a control compound to study the anion effects on vesicles. When a sodium chloride concentration of 0.63 mM was applied, however, the size of the vesicles of **3b** did not change. Only when sodium chloride concentration was increased to 6.3 mM that the increases of average hydrodynamic diameters were detected (Fig. S22, ESI†). The dynamic effect of anions on the vesicle size is also observed during the measurement time interval of 30 minutes. Interestingly, the effect of various anions on the size of the vesicles of **3b** follows the same order, *i.e.* $NO_3^- > Cl^- > Br^- > ClO_4^-$ as that of **3a**. The above outcomes indicate that anion–π interaction should be attributable to the interactions between the surface of **3a** and anions. However, the larger anion concentrations needed implied that anion–π interactions might be only one of the main contributions.

To test the contribution from hydrogen bonding, we synthesized model compounds **4** and **5** (Scheme 1) and investigated their interactions with anions (as tetrabutylammonium salts) through ^{1}H NMR titrations. As shown in Fig. S25 (ESI†), all the protons of **5** remain intact after the addition of anions, which is in agreement with our previous studies that anion–π interactions alone cannot cause the change of chemical shifts of oxacalix[2]arene[2]triazine.[4] The down-field shift of the hydroxyl protons of **4**, however, indicates the formation of hydrogen bonding between hydroxyl groups and anions (Fig. S24, ESI†). The aforementioned outcomes suggested, therefore, that the cooperative anion–π interactions and hydrogen bonding most probably dominated the selective vesicular responses of **3a** to anions.

Moreover, upon the addition of anions, negative Zeta potentials of the vesicles being −8.0 ± 2.9 mV (0.63 mM) for **3a** and −11.3 ± 3.8 mV (6.3 mM) for **3b** (Fig. S13 and S14, ESI†), respectively, were determined, indicating the association of anions on the surface of vesicles. Despite the Coulomb repulsion between the vesicles, the formation of aggregates implied that anions probably acted as bridges to link the vesicles together. Such an assumption was then exemplified by LSCM. As shown in Fig. 3A, well dispersed vesicles of **3a** are observed in the absence of salt. After treatment with sodium chloride for 30 min, most vesicles exist as clusters with obviously increased size (Fig. 3B and Fig. S10, ESI†). The vesicle clustering was also illustrated using TEM images (Fig. S7, ESI†), in which the vesicles form extensive areas of close contact, resulting larger aggregates.

In summary, taking oxacalix[2]arene[2]triazene as a molecular platform, novel functional amphiphilic molecules have been synthesized efficiently. Despite their shape-persistent 1,3-alternate conformations, the heteracalixaromatic amphiphiles showed low CAC values in the range of 10^{-6} to 10^{-7} M. Regulation of

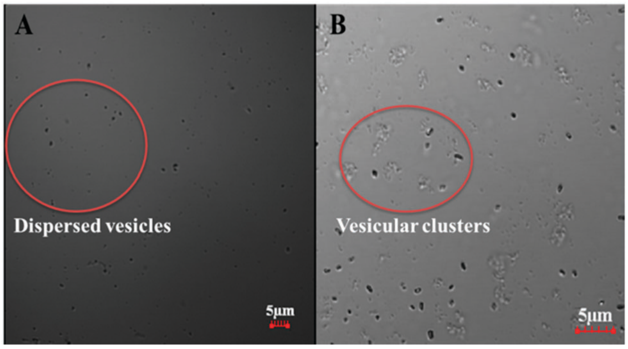

Fig. 3 LSCM images (captured in the visible light channel) of vesicles of **3a** before (A) and after (B) treatment with NaCl (6.3 mM) for 30 min.

the self-assembled aggregates from vesicles to micelles were achieved through elaborate variation of the polarity and hydrophilicity of the substituents attached on the larger rim of triazine rings. The vesicular entities of **3a** were responsive to anions, following a selectivity in the order $NO_3^- > Cl^- > Br^- > ClO_4^-$, as a result of cooperative anion–π interactions and hydrogen bonding between the surface engineered macrocyclic cavities and anions. This study has convincingly shown the potential of heteracalixaromatics for future applications in the design of anion responsive materials[21] in supramolecular chemistry.

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