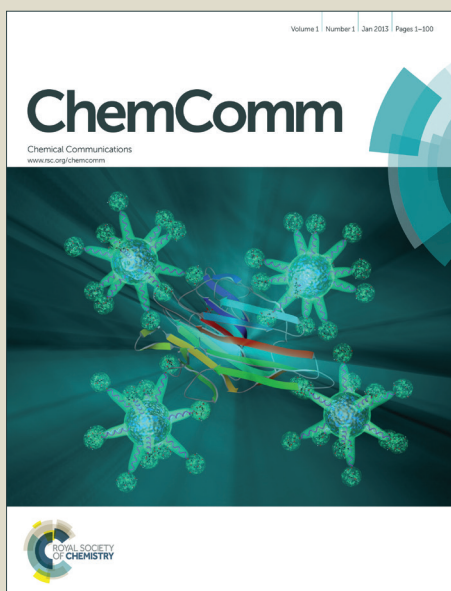This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

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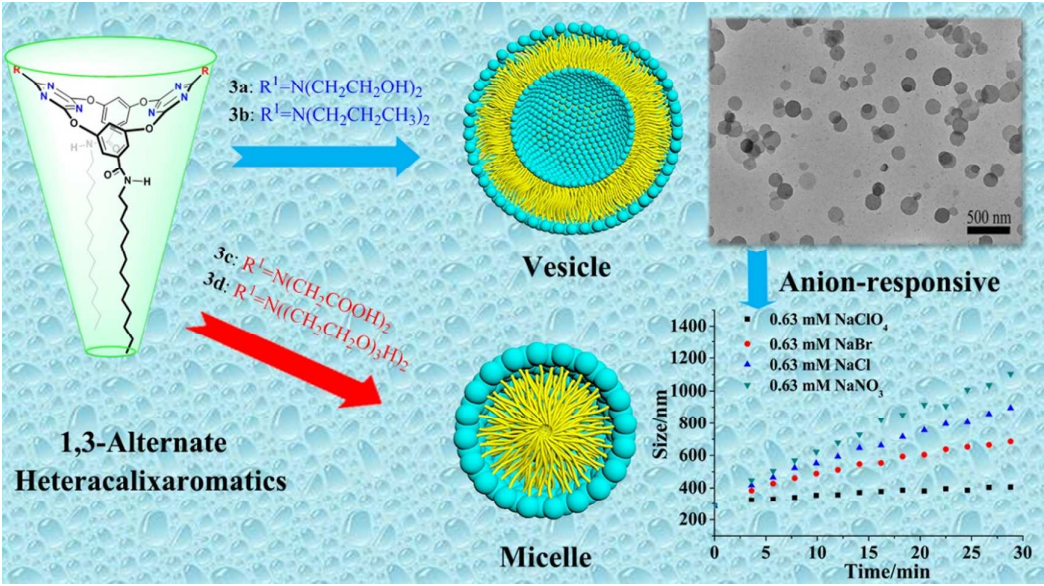
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ARTICLE TYPE

Regulated Assemblies and Anion Responsive Vesicles Based on 1,3-Alternate Oxacalix[2]arene[2]triazene Amphiphiles

Qing He, Zhi-Tang Huang, De-Xian Wang*

**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 followed the selectivity of $NO_3^- > Cl^- > Br^- > ClO_4^-$, driven**
10 **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
15 heteracalixaromatics the structural feature of self fine-tunability.[1a,1b] 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
20 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 with the two triazine rings, has been shown an unique anion acceptor based on anion-π
25 interactions.[4a,4b]

Anion-π interactions have attracted much attention in recent years.[6] Whereas 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
30 still limited.[9,10-13] In this context for example, Saha et al[10] reported anion sensing using naphthanediimides (NDI)[20] as π electron-deficient receptor. Matile's group designed NDI-based rods that that can transport anions across lipid bilayer membranes.[11] The same group also reported new catalytic reactions based on
35 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]triaizine derived
40 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
45 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 were reported herein.

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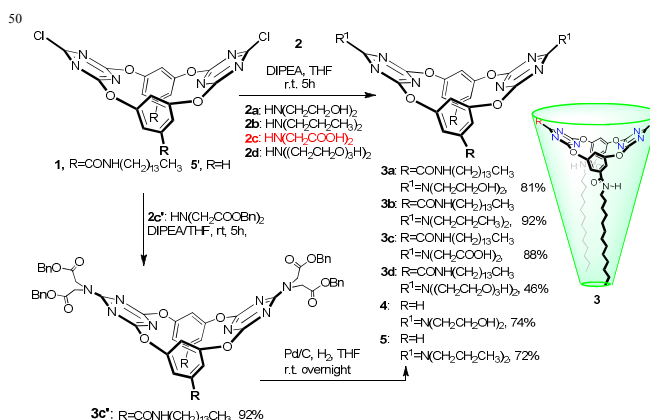
Scheme 1 Synthesis of amphiphiles **3**, **4** and **5**.

We designed the target molecules by introducing N-tetradecyl formacyl groups on the larger rim of benzene rings, whereas
55 groups of different polarity and hydrophilicity were incorporated into the larger rim of triazine rings, in order to investigate the regulation of substitutent effects on both self-assembly and responses of aggregates towards anions. Aromatic nucleophilic displacement reactions between starting compound **1**, which was
60 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, the reactions proceeded smoothly in THF at ambient
65 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
70 afforded **3c′** high efficiently (92%). Deprotection of **3c′** with Pd/C under hydrogen atmosphere produced the desired amphiphile **3c** in 88% yield. The structural characterizations of

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all the amphiphilic compounds were established by spectroscopic and elemental analysis (Figs. S26-37).

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 as opalescent, whereas **3c** and **3d** were clear dispersions, respectively. The assemblies of **3a** and **3b** were firstly assessed with TEM, SEM and LSCM experiments. As shown in Fig. 1 and Figs. S1-S6, 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 (Figs. 1A and B), and the spherical and partially collapsed particles demonstrated by SEM images (Figs. S1 and S2) indicate the vesicular structure of the aggregates. After a fluorescent probe lucigenin was encapsulated inside the aggregates of **3a** and **3b**, blue dots are visualized with LSCM experiments (Figs. S8 and S9), further supporting the feature of hollow vesicles. DLS measurements gave average hydrodynamic diameters of 248 nm for **3a** (Fig. S11) and 185 nm for **3b** (Fig. S12), 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 an average diameter of 3.5-4.5 nm (**3c**) and 4.5-5.5 nm (**3d**) (Figs. 1C and D, Figs. S5 and S6), respectively. Such diameters, when in comparison with the extended molecule lengths (2.8 nm for **3c** and 3.5 nm for **3d**, see Fig. S15), indicate 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 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 (Figs. S16-S19). The low CAC values less than 2.1 μM indicated the designed amphiphilic molecules showed strong self-assembling abilities in water. The dramatic different aggregation behaviours from **3a** and **3b** (vesicle), to **3c** and **3d** (micelle), and the low CACs obtained are very interesting and 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. Because 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,1b,5a] the conjugation of bridging oxygen atoms with triazine rings gave the amphiphilic molecules a cone-like rather than cylindrical molecular shape, which are obviously favored to form aggregates with low CAC values. Possessing similar shape and same hydrophobic moieties, the various morphologies from vesicles to micelles owe 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. Whereas the compounds of **3a** and **3b** bearing substituents of lower polarity and hydrophilicity than that of **3c** and **3d**, preferred to form lamella with 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 pKa 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 played the leading roles on regulating the morphologies of the aggregates.[18]
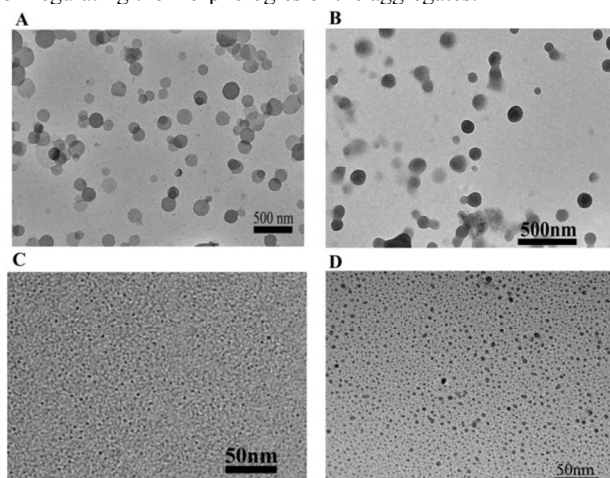

Fig. 1 TEM images of self-assemblies (5×10^{-5} M) of **3a** (A), **3b** (B), **3c** (C) and **3d** (D) in water. Vesicles were not stained.

Our interest to vesicular entities encouraged us to carry out XRD experiments to get further insight of the vesicular structure of **3a** and **3b**. From the diffraction peaks observed at 2θ=2.30° for **3a** (**Fig.** S20) and 2θ=2.74° for **3b** (Fig. S21), the thicknesses of vesicular membranes were estimated as 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), the measured thicknesses larger than 2.7 nm and 2.8 nm but less than the double of the extended molecular lengths, indicate the formation of a double-layer membrane in which the hydrophobic tails are directed inward and the macrocyclic cavities are coated on the surface. It worth addressing that the diffraction intensity of **3a** (Fig. S20) is stronger than that of **3b** (Fig. S21), suggesting highly ordered membrane was formed with **3a**, which is most probably due to the induced bis(2-hydroxylethyl)amino groups on the triazine rings. The polarity and intermolecular interaction of the substituents regulated the hydrophobic/hydrophilic balance and strengthened the 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 response of vesicular entities constructed with **3a** to 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 average 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, 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 indicated 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
5 salts (0.63 mM) were applied. As shown in Fig. 2B, anions affect the vesicle sizes in a different degree. The increments of the sizes caused by anions follow the order $NO_3^- > Cl^- > Br^- > ClO_4^-$. Such selectivity, and which cannot be fully explained by Hofmeister series, indicate that specific non-covalent interaction most
10 probably dominant 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
15 interactions and hydrogen bonding may contribute to the surface-anion interactions.
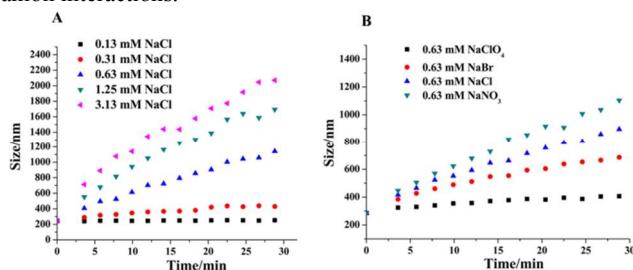

Fig. 2 Vesicular size of **3a** in response to (A) NaCl with different concentrations and (B) various anions.

20 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
25 concentration was increased to 6.3 mM that the increases of average hydrodynamic diameters are detected (Fig. S22). Dynamic effect of anions on the vesicle size is also observed during the measurement time interval of 30 minute. Interestingly, effect of various anions on the size of vesicles of **3b** follows the
30 same order, i.e. $NO_3^- > Cl^- > Br^- > ClO_4^-$ with 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 only be one of the main contributions.
35 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, all the protons of **5** keep intact after the addition of anions, which is in agreement
40 with our previous works that anion-π interactions alone could not cause the change of chemical shifts of oxacalix[2]arene[2]triazine.[4] The down-field shift of hydroxyl protons of **4**, however, indicates the formation of hydrogen bonding between hydroxyl groups and anions (Fig. S24). The
45 aforementioned outcomes suggested therefore the cooperative anion-π interactions and hydrogen bonding most probably dominated the selective vesicular responses of **3a** to anions.
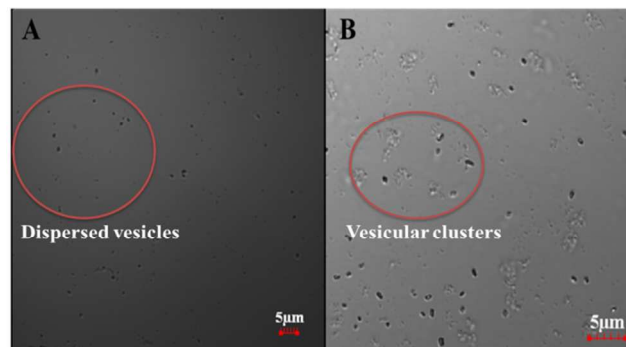

Fig.3 LSCM images (captured in the visible light channel) of vesicles of
50 **3a** before (A) and after (B) treatment with NaCl (6.3 mM) for 30 min.

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** (Figs. S13 and S14), respectively, were determined, indicating the association of anions on the
55 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 assumption was then exemplified with LSCM. As shown in Fig. 3A, well dispersed vesicles of **3a** are observed in the absence of salt. After
60 treatment with sodium chloride for 30 min, most vesicles exist as clusters with obviously increased size (Fig. 3B and Fig S10). The vesicle clustering was also illustrated with TEM images (Fig. S7), in which the vesicles form extensive areas of close contact, resulting larger aggregates.

65 ## Conclusions

In summary, taking oxacalix[2]arene[2]triazene as a molecular platform, novel functional amphiphilic molecules have been synthesized efficiently. Despite of their shape-persistent 1,3-alternate conformations, the heteracalixaromatic amphiphiles
70 showed low CAC values in the range of 10^{-6}-10^{-7} M. Regulation of the self assembled aggregates from vesicles to micelles were achieved through elaborately varying the polarity and hydrophilicity of the substitutents attached on the larger rim of triazine rings. The vesicular entities of **3a** were responsive to
75 anions, following the selectivity of $NO_3^- > Cl^- > Br^- > ClO_4^-$, as a result of cooperative anion-π interactions and hydrogen bonding between the surface engineered macrocyclic cavities and anions. This study convinced the future application of heteracalixaromatics on design of anion responsive materials[21] in
80 supramolecular chemistry.

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