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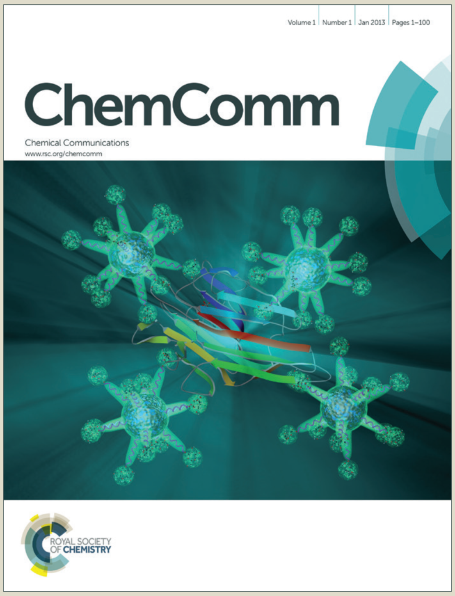

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AIE-Induced Fluorescent Vesicles Containing Amphiphilic Binding Pockets and the FRET Triggered by Host-Guest Chemistry

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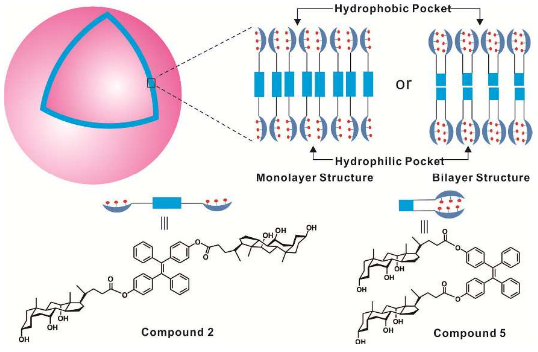
A series of tetraphenylethylene (TPE)-bile acid conjugates was described. It is found that the synergetic combination of the distinct properties of both TPE and bile acid units could directly afford uniform fluorescent vesicles with amphiphilic binding pockets in membrane. This structural feature of such vesicles provides a unique opportunity for facile construction of functional chemical systems through host-guest chemistry.

Self-assembled vesicles are three-dimensional hollow structure and have found numerous applications in chemistry, biology and materials science.[1] Among various self-assembled vesicles, the fluorescent ones, particularly with membrane receptors, have drawn increasing attentions due to their great applications in sensors, drug delivery, nanomaterials, and as important model systems of biological membranes to understand and mimic the molecular interactions and intercellular communication.[2] Up to now several strategies have been developed to produce fluorescent vesicles with attached receptors including embedding of fluorescent dyes and receptors in well-established liposome skeletons.[2a] However, the addition of guest molecules, such as fluorescent dyes, may induce morphological transitions in vesicular nanostructures, and also the fabrication is troublesome. As an alternative strategy, great efforts have been devoted to synthesis dye-labeled or pi-conjugated amphiphilic molecules for the formation of emitting vesicular assembles. Unfortunately, the emission of the vesicular structures would often be weakened significantly owing to the formation of detrimental excimer and exciplexe when the conventional luminophores are condensed and aggregated (aggregation-caused quenching, ACQ).[3] Thereby, the development of simple and straightforward method to produce vesicles with simultaneously strong fluorescence and membrane receptors is highly desirable.



Scheme 1. Schematic illustration of the vesicles derived from compound **2** and **5**, which show strong fluorescence and contain amphilphilic binding pockets.

Different from the aggregation-caused quenching, an unusual photophysical phenomenon of “aggregation-induced emission”(AIE), which is exactly opposite to the ACQ process, was observed in 2001.[4] It is found that a series of propeller-shaped molecules such as tetraphenylethene are non-luminescent in the diluted solutions but become highly emissive in the aggregate state. It is generally accepted that the aggregation effectively suppresses the intramolecular motions, blocks the nonradiative energy dissipation channels, and opens up the radiative decay pathway.[4] The novel and sensitive fluorescence turn-on nature of the AIE molecules makes them promising building blocks in the fabrications of organic light-emitting materials, bioprobes, and chemical sensors, etc. [4-5] Nevertheless, supramolecular self-assembly behavior of the AIE featured amphiphiles has been less investigated, especially in the formation of well-defined vesicular structures.[4-5] Thus, it will be extremely attractive to incorporate AIE attribute in supramolecular assemblies, since the rigid conjugated segment is able to be a part of the building blocks, and also AIE phenomenon can impart interesting photophysical properties to the formed assemblies.

Bile acids are a class of naturally occurring amphiphilic compounds, and play important roles in a wide variety of biological processes such as solubilization of fats in living organisms. Unlike conventional amphiphilic molecules, bile acids have a nonplanar steroidal skeleton with a convex

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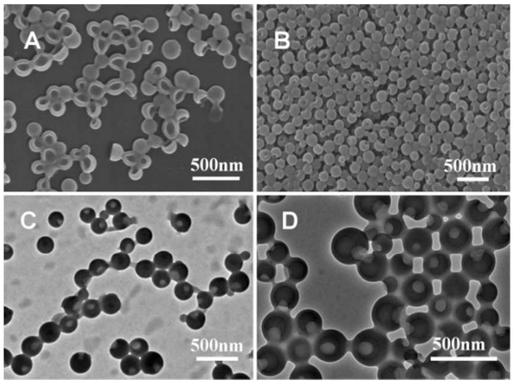

Figure 1. SEM images of the vesicles derived from compound **2** at fw = 40% (A) and compound **5** at fw = 50% (B) in water/acetone co-solvent system; TEM images of the vesicles derived from compound **2** (C) and **5**(D).

hydrophobic surface and a concave hydrophilic face.[6] Because of their rigid structure and unusual distribution of hydrophobic and hydrophilic regions, these molecules are able to form self-assembled aggregates with specific supramolecular arrangement, and have been utilized for construction of various supramolecular architectures.[6] Importantly, due to the rigid facial amphiphilicity, the unique hydrophobic and hydrophilic binding sites can be formed in the resultant assembles, which could serve as adaptable supramolecular host for carrying both hydrophobic and hydrophilic guest molecules of suitable size and shape.[6] Dependent on the guest molecule used, the binding affinities range from 4.9×10^4 to 7.7×10^{10} M^{-1}.[7] Many studies have indicated that the bile acids are powerful building blocks for creating functional assembles.

With the expectation of creating new functional supramolecular assembles, in this work, we synthesized a series of new TPE-bile acid conjugates and explored the synergetic self-assembly of both TPE and bile acid building blocks. Indeed, it is found that some of these new conjugates could facilely self-assemble into uniform and robust vesicles. Remarkably, the synergetic combination of the unique properties of both functional building blocks endowed the formed vesicles with not only strong fluorescence but also amphiphilic binding pockets in the inner and outer membrane surfaces (Scheme 1). The unique structural characteristic of such vesicles enables facile accessibility of functional chemical systems, specially the energy-transfer systems such as FRET process, through host-guest chemistry, indicative of promising applications of this new type of vesicles in various fields.

Scheme S1 in ESI shows the synthesis of the TPE-bile acid conjugates. As important intermediates the dimethoxylated and tetramethoxylated TPEs were synthesized through McMurry coupling reaction. In the case of dimethoxylated TPE, the McMurry coupling affords usually the mixture of E and Z stereoisomers, and their separation has been a difficult task by usual purification approaches.[5] In our work, the E and Z isomers were for the first time separated by automatic high-pressure purification system (HP Compact) as white and pale-yellow powders, and confirmed by NMR spectroscopy and single crystal diffraction (Figure S1-S2). Then, after the removal of the protection methyl group the resulting hydroxylated TPEs were further derivated to give six TPE-bile acid conjugates (compound **1**-**6**) through the esterification reaction with cholic acid, deoxycholicacid, and lithocholic acid, respectively.

As shown in Figure S3, all the synthesized conjugates are AIE-active, and categorized as blue-light emitters. Concretely, when these compounds dissolve in good solvent (acetone or methanol), they emit very faint lights with emission maximums at 375 nm under excitation of 325 nm. However, when water as a nonsolvent was added into the solutions of the TPE-bile acid conjugates, dramatic enhancement of luminescence was observed. All the six conjugates exhibit a similar emission evolution with increased volume fraction (fw) of water in solution (Figure S3), suggesting that the bile acid segments do not significantly affect the electronic structure of the TPE backbones. Generally, at a volume fraction lower than 40% the TPE-bile acids are virtually non-fluorescent (Figure S3-S4). When the fw is further increased, especially when fw > 60%, the emission is dramatically intensified, accompanying with a red-shift wavelength from 376 nm to 462 nm in the spectra. It is found that at about 90% water content the resultant emission reached a maximum intensity. This results are consistent with the observation in previously reported AIE systems, indicating that the self-assembly of the synthesized TPE derivatives bearing cholic acid, deoxycholic acid, and lithocholic acid units could open new opportunity to produce fluorenscent functional assembles with the conjugation of bile acid units.

Under SEM and TEM observations well-defined supramolecular aggregates were derived from the six TPE-bile acid conjugates in the co-solvent systems, especially with a higher fraction of water (Figure 1, Figure S5). The compound **1**, **2**, **3** and **5** self-assembled to vesicular structures with a diameter of ca.200 nm, respectively. They are uniform and the size distribution of the vesicles is narrow. Probably due to the lack of amphiphility or steric crowding, only spheres with different sizes were found for the case of compound **4** and **6**. Obviously, the degree of the hydrophobic–hydrophilic balance of the bile acid units introduced to the conjugates plays a critical influence on the morphology of the formed assembles. Additionally, we found that morphology of the formed aggregatesis strongly depedent on the volume fraction of water during self-assembly process. For example, the compound **5** formed irregular aggregate when the fraction of water was 10%, and then became spheres and bonded together when the fraction of water was increased to 30%. If the fraction of water increased to 50%, perfectly round vesicles were observed (Figure S6).

Considering the preliminary results and the structural feature of the studied conjugates, the compound **2** and **5**, which resemble the normal and bola type of amphiphilic molecules respectively, should provide good chance to realize the synergetic combination of the unique properties of both TPE and bile acid segments the formed aggregates. Careful TEM analysis of the resultant vesicles indicates that themembrane thickness is ca. 4.1 nm for the vesicle (**V2**) derived from compound **2** and ca. 4.9 nm for the vesicle (**V5**) derived from compound **5** (Figure S7), which are consistent with the length of one fully expended molecule **2** and the length of two molecule **5** (Figure S8), respectively. This result suggests the compound **2** self-assembled into monolayer vesicle, meanwhile compound **5** gave bilayer vesicular structure. To further reveal the molecule arrangement, wide-angle X-ray scattering (WAXS) and small-angle X-ray scattering (SAXS) experiments were performed to investigate the superstructure of the formed vesicles. A strong scattering signal (q= 17.2 nm^{-1}) corresponding to a d-spacing of 3.7 Å was detected (Figure S9A), which is approximated to the distance of the stacked adjacent TPE units.[8] In agreement with the TEM images, the lack of the scattering signals at 1.7 nm^{-1} and 2.5 nm^{-1} (Figure S9B), which respectively correspond to the length of compound **2** and compound **5**, indicates that the formed vesicles indeed have a monolayer or bilayer structure.

On the base of these results, we propose that the compound **2** and **5** self-assembled into monolayer or bilayer structured vesicles driven by the combination of multiple intermolecular interactions, including π-π stacking, hydrogen-bonding, and hydrophobic force (the model in Scheme 1). The hydrophobic tetraphenylethylene segments are tightly packed together through strong π-π stacking, while due to the facial amphiphility, the phase separation leads to the face-to-face and the back-to-back arrangement of cholic acid segments through the hydrogen-bonding and hydrophobic force. As a result, the formed vesicles exhibit strong turn-on florescence (Figure S9C-D), due to the blockage of intramolecular rotations of TPE (AIE effect). Also, thanks to the rigid skeleton of bile acid and TPE blocks, unlike lipid vesicles that easily lose their structural integrity when removed from solution, our vesicles are very robust and retain their spherical shape even on a solid substrate under high vacuum. Importantly, from this model it can be clearly seen that at molecular level the back-to-back arrangement of adjacent cholic acid segments could inevitably form hydrophobic pockets with their hydrophobic β-faces, and simultaneously hydrophilic pockets arises from the face-to-face hydrophilic α-faces.

The aggregation of bile acids has been widely studied in the last decade.[9] A widely adopted model argues that at lower number of monomer, bile acids aggregate to form single primary binding sites, whereas at high number of monomer further aggregation results in the formation of secondary binding sites. A general classification of these binding sites is marked by their encapsulation of both hydrophobic guest molecules (hydrophobic site) and hydrophilic molecules (hydrophilic site).[9] In this work, by using hydrophilic thioflavin T(ThT) as well as the hydrophobic fluorescent protein chromophore analog (GFP), which are widely used as probes to study hydrophilic and hydrophobic microenvironments or cavities,[10] we indeed confirmed the co-existence of the hydrophilic and hydrophobic pockets in our vesicles. Importantly, by synergetic exploration of the fluorescence property and host-guest chemistry of the vesicles described here the energy-transfer systems could be facilely established.

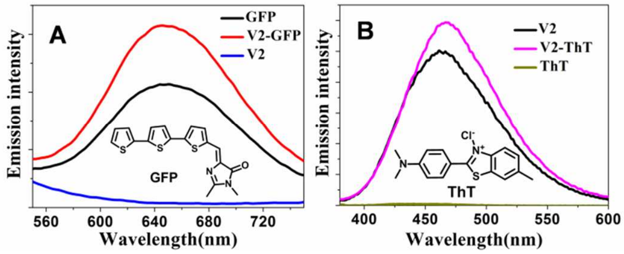

Figure 2. Comparison of the emission spectra of hydrophilic ThT probe (A) and hydrophobic GFP probe (B) in the absence and presence of the vesicle **V2**; λex = 335 nm for ThT and λex = 400 nm for GFP.

Figure 2A shows the fluorescence change of GFP in the absence and presence of the vesicle **V2**. Clearly, due to the nonradiative energy dissipation arising from the free rotations within molecule, the diluted GFP solution only shows diminished emission, but exhibits significant enhancement of fluorescent in the presence of the vesicles. The existence of hydrophobic microenvironments or pockets to accommodate GFP molecules and thus restrict the rotational freedom of the guest molecule should be responsible for the enhancement. Similarly, the experiments with hydrophilic ThT probe led to the same enhancement phenomenon (Figure 2B), and verified the existence of hydrophilic pockets in our vesicular system. In our system each conjugate molecule can form two binding sites. Based on the size of the formed vesicles, the geometry of the conjugate as well as the WAXS data, the density of the binding sites is estimated to be ca.3.5 per square nanometer.These results imply that these fluorescent vesicles with embedded amphiphilic binding sites should have high flexibility in the functionalization of the vesicles or in the creation of functional supramolecular systems.

Fluorescence resonance energy transfer (FRET) is a very fundamental phenomenon occurring in natural photosynthetic systems.[11] In this respect, the design and creation of non-covalent artificial FRET systems is specially favorable, as it helps to mimic light-harvesting assemblies in a supramolecular manner, which is closer to the strategy adopted in nature, and at the same time avoids tedious synthetic works. In this work, to demonstrate the facile accessibility of the vesicles described here to functional self-assembled systems, hydrophobic nile red (NR) and hydrophilic rhodamine B (RB) were chosen as guest molecules, and several binary composite FRET systems were first realized through simple host-guest chemistry of the embedded amphiphilic binding sites.

Figure 3A-B shows that the absorbance and emission spectra of RB and NR and the vesicle **V2**. The excellent overlap of donor fluorescence and acceptor absorption in the case of the vesicle and NR couple as well as in the case of the vesicle and RB couple provides a critical prerequisite for the induction of energy transfer. As shown in Figure 3C, upon the successive insertion of the guest dye NR in the hydrophobic binding site, the fluorescence intensity of the vesicle at 462 nm gradually decreased, and correspondingly, a new emission band at 605 nm appeared initially and shifted to 620 nm, probably due to increased amount of methanol. Clearly, an energy transfer from the stacked TPE units in the vesicles to the encapsulated NR occurred. Based on the experimental results, an energy transfer efficiency of 52.4% was achieved. Like the

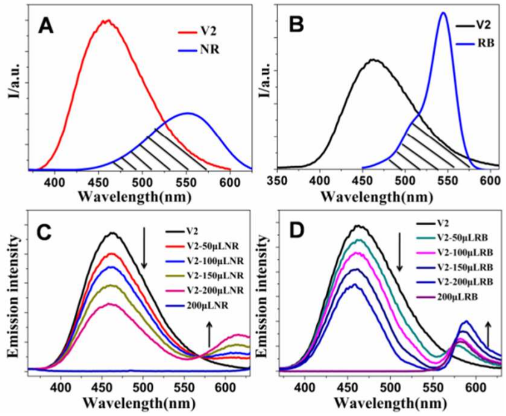

Figure 3. Absorption and emission spectra of (A) the vesicle **V2** and NR couple as well as (B) the vesicle **V2** and RB couple; Fluorescence evolution of the vesicle **V2** upon the encapsulation of NR (C) and RB (D); λex = 325 nm.

hydrophobic NR, an energy transfer system could also be constructed by the insertion of hydrophilic RB in the hydrophilic binding sites of the vesicles (Figure 3D). Additionally, the emission from direct excitation of hydrophobic NR at 325 nm is negligible due to ACQ effect (Figure 3C), and the RB emission with direct excitation have a relatively low intensity (Figure 3D), further confirming the occurrence of FRET process. It should be noted, however, that the energy transfer efficiency (41.8%) of the vesicle-RB system is much lower than that of the vesicle-NR system. The reason for this result is that the hydrophobic dye has stronger tendency to insert into hydrophobic pockets in aqueous media. Similar energy-transfer systems are also realizable through the host-guest chemistry of the vesicle **V5** (Figure S10). In the literature the polydiacetylene based vesicles with embedded binding sites were described,[12] which are related to our vesicular systems. The comparable FRET efficiency (from 33.3% to 61.5%) was also achieved.Thus, all these results indicate that the hydrophobic and hydrophilic binding sites (pockets) embedded in the TPE-based vesicles provide the required proximity and orientation of the inserted dye molecules for FRET process.

Encouraged by the success, we intend to further demonstrate the potentials of the vesicles for the creation of more complex functional supramolecular systems, such as the self-assemble cascade energy transfer system. Along with this consideration, NR and naphthalene (Nap) were chosen as guest dye molecules, and together with the fluorescent vesicle to form a FRET network. Figure S11A shows the absorption and fluorescence spectra of the individual dyes NR, Nap and the vesicle, indicative of a successive overlaps of donor fluorescence and acceptor absorption required for FRET network. To appropriately arrange these dye molecules in vesicular structure for the realization of cascade energy transfer, co-assembly of compound **2** and dye Nap was employed to form Nap-doped vesicles, and then the NR molecules were inserted into the hydrophobic pockets of the formed vesicles through host-guest chemistry. Fortunately, when the resultant assemble was excited at λ=273 nm, the fluorescence intensity of Nap at 336 nm and the TPE segment at 462 nm decreased, and meanwhile a new fluorescence band of the NR at 605 nm appeared (Figure S11B).These results confirmed the establishment of the cascade energy transfer in a particular order (Nap-**V2**-NR). It should be noted that since both **V2** and Nap can be excited at λ=273 nm (Figure S12), besides the cascade FRET process (Nap-**V2**-NR), the FRET process from **V2** to NR should simultaneously occur. In fact, in literature the cascade FRET process consists of multiple individual energy transfer events was also described.[13]

In summary, based on the synergetic combination of the unique properties of both TPE and bile acid units, a new strategy was developed to directly produce the self-assembled vesicles that not only show strong fluorescence but also contain hydrophobic and hydrophilic binding pockets embedded in the membrane surfaces. Compared to the vesicular structures reported so far, the distinct structural characteristic of the obtained vesicles provides a unique opportunity for facilely accessing to functional chemical systems through simple host-guest chemistry, as exemplified by the creation of FRET systems. Thus, we believe that this new type of fluorescent vesicles could hold great potential for the development of functional nanomaterials or serve as a new model system of biological membranes to understand the molecular interactions and intercellular communications.

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