# Supramolecular Chemistry of *p*-Sulfonatocalix[*n*]arenes and Its **Biological Applications**

Dong-Sheng Guo and Yu Liu\*

Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, P.R. China

**CONSPECTUS:** Developments in macrocyclic chemistry have led to supramolecular chemistry, a field that has attracted increasing attention among researchers in various disciplines. Notably, the discoveries of new types of macrocyclic hosts have served as important milestones in the field. Researchers have explored the supramolecular chemistry of several classical macrocyclic hosts, including crown ethers, cyclodextrins, calixarenes, and cucurbiturils. Calixarenes represent a third generation of supramolecular hosts after cyclodextrins and crown ethers. Easily modified, these macrocycles show great potential as simple scaffolds to build podand-like receptors. However, the inclusion properties of the cavities of unmodified calixarenes are not as good as those of other common macrocycles. Calixarenes require extensive chemical modifications to achieve efficient endo-complexation.



*p*-Sulfonatocalix[*n*] arenes (SC*n*As, n = 4-8) are a family of water-soluble

calizarene derivatives that in aqueous media bind to guest molecules in their cavities. Their cavities are three-dimensional and  $\pi$ electron-rich with multiple sulfonate groups, which endow them with fascinating affinities and selectivities, especially toward organic cations. They also can serve as scaffolds for functional, responsive host-guest systems. Moreover, SCnAs are biocompatible, which makes them potentially useful for diverse life sciences and pharmaceutical applications.

In this Account, we summarize recent work on the recognition and assembly properties unique to SCnAs and their potential biological applications, by our group and by other laboratories. Initially examining simple host-guest systems, we describe the development of a series of functional host-guest pairs based on the molecular recognition between SCnAs and guest molecules. Such pairs can be used for fluorescent sensing systems, enzymatic activity assays, and pesticide detoxification. Although most macrocyclic hosts prevent self-aggregation of guest molecules, SCnAs can induce self-aggregation. Researchers have exploited calixarene-induced aggregation to construct supramolecular binary vesicles. These vesicles respond to internal and external stimuli, including temperature changes, redox reactions, additives, and enzymatic reactions. Such structures could be used as drug delivery vehicles.

Although several biological applications of SCnAs have been reported, this field is still in its infancy. Continued exploration of the supramolecular chemistry of SCnAs will not only improve the existing biological functions but also open new avenues for the use of SCnAs in the fields of biology, biotechnology, and pharmaceutical research. In addition, we expect that other interdisciplinary research efforts will accelerate developments in the supramolecular chemistry of SCnAs.

## 1. INTRODUCTION

Calixarenes, macrocycles composed of phenolic units linked by methylene groups at the 2- and 6-positions, are among the most widely studied organic supramolecular hosts and have been described as having "(almost) unlimited possibilities"<sup>1</sup> because they can be easily modified. They generally serve as simple scaffolds to build podand-like receptors, and the calixarene cavity is exploited only rarely.<sup>2</sup> Cavity complexation is the iconic feature of macrocyclic hosts and is appealing for construction of functional supramolecular architectures. However, the inclusion capabilities of the cavities of unmodified calixarenes are not as good as those of other common macrocycles such as crown ethers, cyclodextrins, and cucurbiturils; extensive chemical modification of calixarenes is necessary to achieve efficient endo-complexation.<sup>3</sup>

In contrast, *p*-sulfonatocalix[*n*] arenes (SCnAs, n = 4-8; Scheme 1), first reported by Shinkai et al. in 1984,<sup>4</sup> are a prominent family of water-soluble calixarene derivatives with a robust ability to bind guests in their cavities in aqueous media.<sup>5</sup> SCnAs have several advantageous features. First, they can be prepared easily in satisfactory yields by direct sulfonation of the upper rim of calixarenes. Second, they are highly water-soluble, and the driving forces for guest inclusion in the cavity, such as hydrophobic and  $\pi$ -stacking interactions, are more effective in aqueous media than in organic media. Third, the upper-rim sulfonate groups provide anchoring points that supplement the

Special Issue: Responsive Host-Guest Systems

Received: January 8, 2014 Published: March 25, 2014 Scheme 1. Structure of SCnAs (n = 4-8)



binding interactions intrinsic to the  $\pi$ -electron-rich cavities; as a result, SCnAs display especially strong binding ability and high selectivity toward various organic cations.<sup>6–9</sup> Finally, SCnAs are biocompatible. For example, in vitro, SC4A shows no hemolytic toxicity at concentrations up to 5 mM and elicits no nonspecific immune responses.<sup>10</sup> In mice, a single injection of SC4A at doses equivalent to 2-5 g in humans shows no acute toxicity, and the compound is rapidly cleared via elimination in urine without accumulating in the liver.<sup>11</sup> This biocompatibility makes SCnAs useful for diverse biological and pharmaceutical applications.<sup>12,13</sup> Comparing with the other two classical watersoluble macrocycles, cyclodextrins and cucurbiturils, SCnAs possess different cavity structure, framework rigidity, and complexation driving force, and then afford distinguishable recognition and assembly features in building responsive hostguest systems.

Although SCnAs were discovered 30 years ago, their functional applications are still being actively studied. Owing to their preorganized conical structures and the binding properties of their cavities, SCnAs have been used as amphiphiles and enzyme mimics and for molecular recognition and sensing, crystal engineering, catalysis, enzyme assays, and biological/medicinal chemistry.<sup>5,12-14</sup> Numerous reviews of various aspects of SCnAs have been published. For example, solid-state inclusion and crystal engineering applications were reviewed by Atwood et al., Raston et al., and others.<sup>14–16</sup> We reviewed the selective binding behaviors of SCnAs and their supramolecular polymerization reactions in aqueous solution.<sup>5,17</sup> Coleman et al. published excellent feature articles on the biochemistry of SCnAs in Chemical Communications.<sup>12,13</sup> Here, we highlight the most recent advances in the recognition and assembly properties that are unique to SCnAs, as well as their potential biological applications, focusing on work undertaken in our group, along with representative work of others. This Account is divided into three major sections based on the functions of SCnAs: fluorescent sensing systems and their use for monitoring enzymatic reactions, pesticide detoxification by complexation with SCnAs, and drug delivery via supramolecular binary vesicles formed by calixarene-induced aggregation. It is witnessed that SCnAs have the potential to connect supramolecular chemistry with biology/medicine in the form of high-performance applications. We believe that of special interest in future is the fabrication of SCnA-based supramolecular systems suitable for investigating biological processes and for opening new avenues for diagnosis of and therapy for diseases.

#### 2. FLUORESCENT SENSING SYSTEMS AND THEIR USE FOR MONITORING ENZYMATIC REACTIONS

Fluorescent sensing systems based on SCnAs and dyes have attracted considerable attention for detection of biological substances, such as the neurotransmitter acetylcholine and its precursor/metabolite choline.<sup>18–20</sup> These systems are based on the principle that addition of an analyte to a dye–host pair displaces the dye, which results in regeneration of its intrinsic emission (Scheme 2). The utility of such systems for measuring





absolute concentrations of analytes depends on the sensitivity and selectivity of the supramolecular host. To maximize sensitivity and minimize interference by nontarget species, as well as cost, the fluorescent dye should have a high quantum yield, and binding between the host and the dye should be strong and should result in a large fluorescence change (quenching or enhancement).

For chemosensing applications, we reported novel host–dye reporter pairs composed of SCnAs as hosts and lucigenin (LCG, N,N'-dimethyl-9,9'-biacridinium dinitrate) as the fluorescent guest (Scheme 3).<sup>21</sup> Free LCG is highly fluorescent in aqueous solution, but when it complexes with SCnAs (binding constant  $\approx 10^7 \text{ M}^{-1}$ ), it undergoes strong fluorescence quenching ( $I_{\text{free}}/I_{\text{bound}} = 140$ ). Owing to the high quantum yield of LCG and the high host–guest binding affinity, the dye, host, and analyte concentrations can be low, which makes the system economical and minimizes potential interference by nontarget species. The large fluorescence response makes this system highly sensitive to the addition of competitive analytes. Moreover, the SCnA·LCG reporter pairs are water-soluble, and the system can operate over a broad pH range.

However, like other host-dye sensing systems, this system lacks specificity for target analytes. SCnAs, which are negatively charged, can differentiate between cations and anions but show limited selectivity for structurally related cations. In addition, numerous biologically abundant cations, biopolymers containing positively charged residues, and even simple salts, which are ubiquitous in biological systems, also bind considerably to SCnAs. This nonspecificity makes determining absolute

1926

Scheme 3. (Enzyme-Coupled) STA for AChE and ChO Activities, with a Switch-Off Fluorescence Response



concentrations of analytes in biological media difficult if not impossible.

To circumvent this problem, we collaborated with Nau and co-workers, who developed supramolecular tandem assays (STAs),<sup>22-25</sup> to construct an SC4A·LCG-based STA for specific determination of the absolute concentrations of both acetylcholine and choline (Scheme 3).<sup>21</sup> This assay uses the indicator-displacement strategy for signaling in combination with acetylcholinesterase (AChE) and choline oxidase (ChO) to achieve high selectivity. In an STA, the only prerequisite is that the macrocycle have different affinities for the substrate and the product of the enzymatic reaction being monitored. In our system, SC4A differentiates well between choline (which is the ChO substrate and completes strongly with the dye) and betaine (which is the product of the ChO reaction and competes only weakly with the dye); the difference in binding constants is a factor of at least 200. Although a direct STA for AChE activity could not be achieved, because SC4A has similar binding affinities for acetylcholine and choline, an indirect STA was set up via an enzymatic reaction cascade involving in situ

conversion of the initial product, choline, to the final product, betaine. Because assays involving an enzymatic reaction sequence are generally referred to as enzyme-coupled assays, we refer to our assay as a substrate-selective enzyme-coupled STA.

In addition to allowing determination of the Michaelis constant  $(K_{\rm M})$  of an enzyme, STAs also offer the possibility of screening for enzyme inhibitors and, more importantly, the possibility of determining the absolute concentrations of substrates and products, acetylcholine, and choline in our case. Combining enzymatic reactions with conventional competitive sensing systems makes the determination of absolute concentrations of analytes feasible because an enzymatic reaction is the best example of a reaction in which the relative concentration of a target analyte (the substrate) changes dramatically (from the initial value to zero) while the concentrations of interfering metabolites and buffer ions remain constant. According to the Michaelis-Menten model, when an enzymatic reaction is occurring at a substrate concentration below the enzyme  $K_{M}$ , the initial reaction rate increases linearly with substrate concentration, and this relationship allows direct quantification of the substrate. Enzymatic conversion of substrates results in a fluorescence response, and calibration curves for the initial rates can be used to determine the concentrations of both acetylcholine and choline. Using this method with SC4A·LCG, we accurately (r = 0.998) quantified both analytes down to biologically relevant concentrations (low micromolar range) in a single sample. Although this integrated approach is far from being practically useful, it has clear-cut advantages in terms of specificity and sensitivity over previously described systems.

Two types of cholinesterases, AChE and butyrylcholinesterase (BChE), coexist throughout the bodies of vertebrates. The functions of AChE are well-known, but the importance of BChE has previously been underestimated. BChE has recently been shown to act both as a detoxification enzyme that scavenges anticholinesterase compounds and as an activator enzyme that converts prodrugs into their active forms. Moreover, in people with Alzheimer's disease, BChE activity





#### Accounts of Chemical Research

is higher than that in unaffected people, whereas AChE activity is lower. Owing to the increasing recognition of the biological and pharmacological significance of BChE, development of a sensitive, convenient method for assaying BChE activity is desirable. However, most current assays focus on AChE and, more importantly, cannot output qualitatively differentiated signals for AChE and BChE. To accomplish this end, we developed a robust, facile method for real-time, continuous monitoring of BChE activity using an STA with SC4A·LCG.<sup>26</sup> To achieve BChE specificity, we used succinvlcholine, which is degraded by BChE but not by AChE, as a substrate, and we used SC4A as the host because it exhibits a higher affinity for succinvlcholine than for its enzymatic-cleavage product, choline. With this system, we achieved direct, label-free monitoring of BChE activity through the switching off of the dye fluorescence. This assay readily discriminates between BChE and AChE, and monitoring of BChE activity is almost completely unaffected by the presence of AChE, even in excess (Scheme 4). This assay is potentially useful for the diagnosis of diseases in which BChE is an important marker, as well as for screening drugs to treat such diseases.

Taking advantage of the favorable characteristics of SCnA-LCG reporter pairs, Nau et al. used this type of enzyme assay to monitor enzymatic trimethylation of lysine residues in peptide substrates by histone lysine methyltransferases.<sup>27</sup> Hof et al. reported an array of SCnA-based indicator-displacement sensors that reliably distinguish between various analytes that are elements of the histone code, a set of post-translational modifications that control gene expression.<sup>28</sup> SCnA·LCG reporter pairs have been demonstrated to be best suited for this type of array because LCG has affinity for almost all SCnAs and the reporter pairs require no organic cosolvents. More recently, Nau et al. exploited SCnA·LCG reporter pairs for monitoring biomembrane transport in real time, named supramolecular tandem membrane assays.<sup>29</sup>

# 3. PESTICIDE DETOXIFICATION BY COMPLEXATION WITH SCnAs

The ability of SCnAs to form complexes with compounds of biological interest has been the subject of considerable research. For example, Hof et al.<sup>30</sup> reported that SCnAs bind selectively and with high affinity to histone trimethyllysine motifs involved in gene regulation and oncogenesis and that this binding disrupts the interaction between these motifs and their epigenetic reader proteins. Other researchers have reported that host-guest complexation can increase the bioavailability or decrease the systemic toxicity of various pharmacologically active compounds, such as anesthetics and antidiabetic and anticancer drugs.<sup>31-34</sup> In addition, the use of discrete hostguest complexation for drug delivery complements existing supramolecular drug formulation strategies based on liposomes and related systems.<sup>35</sup> However, macrocyclic complexation can also lower drug bioactivity, and although this result would be unfavorable for drug delivery, it might be useful for hazardous substance detoxification. In this section, we focus on the use of SCnA complexation for poison detoxification.

We have extensively studied the binding of viologens by SCnAs for the treatment of viologen poisoning (Scheme 5).<sup>36</sup> Viologens are used as herbicides (e.g., paraquat and diquat), as well as for an increasing number of scientific and technical applications. However, owing to their high toxicity, they pose considerable risks to human health and the environment. Viologen toxicity can be inhibited by host–guest complexation

Scheme 5. Biochemical Mechanism of Viologen Toxicity and Detoxification by Complexation with SC5A (HWR, Haber– Weiss reaction; HMP, hexose monophosphate pathway)



with SCnAs as a result of four factors related to the biochemical mechanism of toxicity: (1) SCnAs tightly bind viologens,<sup>37</sup> preventing their interaction with cellular reducing agents, (2) binding negatively shifts the reduction potentials of viologens, disfavoring generation of their radical cations, (3) radicals that do form are deactivated by hydrogen transfer from SCnAs, and (4) SCnAs can coordinate transition metal ions, which may be involved in catalyzing generation of the reactive oxygen species responsible for viologen toxicity. We validated the therapeutic effect of SCnAs by means of tests in mice. When viologen-poisoned mice ingested SC5A, even 2 h after poison exposure, the mortality rate decreased significantly; and SC5A ingestion also effectively prevented viologen-induced destruction of lung and liver tissue structures.

Subsequent to our work, Qi et al. reported paraquat detoxification by SC4A, as indicated by an in vivo pharmacokinetic study.<sup>38</sup> They used high-performance liquid chromatography to determine paraquat concentrations in rat plasma and showed that the peak plasma concentration and the area under the plasma concentration—time curve were markedly lower after SC4A treatment than in the untreated group. They also performed an in vitro intestinal absorption study to evaluate the effect of SC4A complexation on absorption pharmacokinetics, and they found that SC4A treatment efficiently impeded paraquat absorption, owing to formation of a stable host—guest complex.

The high binding affinities of SCnAs for viologens are essential for viologen detoxication, and binding alters their chemical and pharmacokinetic properties in vivo. SCnAs may find broader application for in vivo detoxification and removal of other hazardous substances, such as illegal food additives and environmental pollutants. The detoxication path is envisaged as follows: hazardous substances in vivo are encapsulated by the SCnAs, and then the host–guest complexes are rapidly eliminated in urine without being metabolized. The host– guest complexes can be expected to be cleared reasonably rapidly because of the rapidity with which free SCnAs are cleared and their lack of accumulation in the liver.<sup>12,36</sup> The only remaining requirement for supramolecular detoxication is the development of SCnAs with strong affinity for target hazardous substances. Considering the binding performance of SCnAs Scheme 6. Chemical Structures of Guest Molecules Used for CIA



they are potentially useful for treatment of poisoning by organic cation analogues.

### 4. DRUG DELIVERY BY SUPRAMOLECULAR BINARY VESICLES FORMED BY CALIXARENE-INDUCED AGGREGATION

Cyclodextrins, cucurbiturils, and SCnAs are widely studied water-soluble macrocycles. The former two tend to form threading structures with guest molecules, and this tendency has been exploited to fabricate various rotaxanes and catenanes. Inclusion of guest molecules in cyclodextrin and cucurbituril hosts has been found to prevent self-aggregation of the guests.<sup>39,40</sup> In contrast, SCnAs can promote the self-aggregation of aromatic or amphiphilic molecules by lowering the critical aggregation concentration (CAC), enhancing aggregate stability and compactness, and regulating the degree of order in the

aggregates.<sup>41</sup> We refer to this unique self-assembly strategy as calixarene-induced aggregation (CIA).

The prototype for CIA was reported in 2001 by Randaccio, Purrello, Sciotto, and colleagues,<sup>42</sup> who found that complexation between a SC4A derivative lower-rim modified with tetrakis-acetates and a cationic porphyrin led to the formation of aggregates with pH-tunable host/guest stoichiometries ranging from 4:1 to 4:7. These investigators subsequently performed a series of excellent studies on the synthesis of noncovalent multiporphyrin aggregates with programmable stoichiometry and sequence.<sup>43–45</sup> However, complexationinduced aggregation involving SCnAs did not receive much attention until 2009, possibly because porphyrins were the only guest species studied. Since 2009, the number of guest species has expanded considerably (Scheme 6): 22 guest molecules, divided into four categories (aromatic fluorescent dyes,<sup>41,42,46–52</sup> amphiphilic surfactants,<sup>53–61</sup> drugs,<sup>62</sup> and



Scheme 8. Construction of SC5A+PMA Supramolecular Binary Vesicles and Temperature-Responsive Drug Release from the Vesicles



proteins<sup>63</sup>), have been shown to undergo self-aggregation induced by complexation with SCnAs.<sup>64</sup>

When such aromatic and amphiphilic guests are used for CIA, two opposing forces are responsible for the formation of the resulting highly ordered assemblies: hydrophobic forces and repulsive electrostatic forces. Hydrophobic  $\pi$ -stacking interactions drive segregation of the alkyl chains and aromatic moieties in water, thus providing the impetus for selforganization. In contrast, electrostatic repulsion between the polar head groups, that is, the positively charged organic ammonium cations, prevents the formation of large threedimensional assemblies. Upon complexation with SCnAs, electrostatic repulsion between the polar head groups is replaced by electrostatic attraction between the head groups and the sulfonate groups of the SCnAs, and this attraction facilitates guest aggregation. We postulate that the CIA of guest molecules observed upon addition of SCnAs occurs in two steps (Scheme 7). First, the host and guest molecules instantaneously form a complex in which the two head groups of the guest are captured by the cavities of two host molecules, forming a 2:1 capsule-like complex driven by the host-guest interaction. Subsequently, additional guest molecules are readily

integrated into the complex, resulting in the formation of a 2:*n* complexes and then large three-dimensional aggregates. Although several noncovalent interactions, including host–guest, charge–charge,  $\pi$ -stacking, and hydrophobic interactions, stabilize the ultimate aggregates, strong host–guest interactions are a prerequisite for CIA. In summary, three key factors are generally required for high-performance CIA: (1) strong binding affinities between the SC*n*As and the polar head groups of the guests, (2) charge compensation between the hosts and guests, and (3) the preorganized cyclic scaffold of the SC*n*As. Because of the biocompatibility of SC*n*As, we have devoted a lot of our research effort to the use of CIA to fabricate supra-amphiphiles, which are of fundamental interest for drug-delivery applications.

For example, we used SC5A as the host and 1pyrenemethylaminium (PMA) as the guest to fabricate selfassembled binary supramolecular vesicles (Scheme 8).<sup>50</sup> Dynamic laser scattering indicated that the SC5A+PMA amphiphile self-assemblies have an average diameter of 99 nm and a narrow size distribution. Transmission electron microscopy showed that they have a hollow spherical morphology, convincingly indicating that they are vesicular.



Scheme 10. Enzymatic Responsiveness of Amphiphilic Assemblies of Myristoylcholine Fabricated in the Absence or Presence of SC4A



The bilayer membrane thickness is about 3 nm, which is on the same order of magnitude as the sum of one PMA length (7 Å) and two SC5A heights (14 Å), indicating that the vesicles are unilamellar. From these results, we deduced that the vesicles have the following structure: the hydrophobic pyrene segments are packed together in the interior of the vesicle walls, and the inner and outer surfaces of the walls consist of the hydrophilic phenolic hydroxyl groups of SC5A, which are exposed to water. SC5A and PMA are held together by host-guest and charge interactions. Notably, neither free SC5A nor PMA forms nanoscale aggregates itself. A control experiment revealed that the addition of excess 4-phenolsulfonic sodium (the subunit of SCnAs) does not cause any appreciable change in the absorption or emission spectrum of PMA. This result highlights one of the most fascinating aspects of supramolecular chemistry: two or more components can self-assemble into

higher-order structures that exhibit properties and functions that the individual components lack.

Many biomacromolecules, such as proteins and nucleic acids, change their behavior in response to a combination of environmental stimuli, rather than to a single stimulus. The construction of materials that could mimic this feature would be of great interest. Therefore, we constructed supramolecular binary vesicles based on host–guest complexation of SC4A with an asymmetric viologen (Scheme 9).<sup>57</sup> The CAC of the viologen decreases by a factor of ca. 1000 upon complexation with SC4A. The resulting vesicles respond to multiple stimuli—temperature, the addition of a cyclodextrin, and redox reactions—benefiting from the intrinsic reversibility of supramolecules. For example, reduction of the asymmetric viologen to its radical cation results in the formation of smaller vesicles, and reoxidation restores the original-size vesicles. The architecture of these vesicles containing entrapped doxorubicin

can be disrupted by reduction of the viologen to its neutral form, by an increase in temperature, or by the addition of cyclodextrins; and disruption triggers the efficient release of the entrapped doxorubicin from the vesicle interior. In vitro experiments showed that the loading of doxorubicin into the vesicles does not affect its toxicity to cancer cells, whereas encapsulation reduces the damage it causes to normal cells.

The construction of amphiphilic self-assemblies that respond to enzymatic reactions represents an increasingly important topic in biomaterials research, and applications of such assemblies for the controlled release of therapeutic agents at specific sites where a target enzyme is located are feasible. For example, we constructed cholinesterase-responsive supramolecular vesicles for use as a targeted drug-delivery system, using SC4A as the macrocyclic host and myristoylcholine as the guest (Scheme 10).<sup>58</sup> Although myristoylcholine is a natural substrate of cholinesterases, it cannot be used alone to fabricate an enzyme-responsive assembly, because the CACs of the substrate (myristoylcholine) and the product (myristic acid) are similar. Complexation of SC4A with myristoylcholine directs the formation of a supramolecular binary vesicle and decreases the CAC of myristoylcholine by a factor of ca. 100. Because the components are held together by noncovalent interactions, the assembled and unassembled states are in dynamic equilibrium, and enzymatic cleavage of free myristoylcholine results in disintegration of the self-assembled vesicles. Although the guest cleavage rate may be reduced by complexation with the host, noncovalent host-guest interactions are preferable to covalent modification of the substrates, which often results in loss of enzyme recognition. We found that binary vesicles consisting of SC4A and myristoylcholine respond specifically and efficiently to cholinesterase; cholinesterase-induced cleavage of myristoylcholine disrupts the hydrophilic-hydrophobic balance of the binary supra-amphiphiles, which results in vesicle disassembly. In addition, the release of a drug, such as the Alzheimer's drug tacrine, encapsulated in the vesicles can be triggered by this enzymatic cleavage. Cholinesterase is overexpressed in Alzheimer's disease, and therefore, this system has potential utility for the delivery of Alzheimer's drugs.65

A trypsin-responsive supramolecular vesicle was recently fabricated from SC4A as the macrocyclic host and protamine as the enzyme-cleavable guest.<sup>63</sup> Unlike the guests used previously for CIA, protamine is not a small molecule but rather a nonamphiphilic natural biological cationic protein, and its use represents a major expansion in the range of substrates that can be used for fabricating CIA assemblies. These SC4A+protamine vesicles have the potential to be useful for the controllable release of drugs at trypsin-overexpression sites. This principle can be expected to be adaptable for the construction of various enzyme-triggered self-assembled materials that can be used for site-specific responses.

#### 5. CONCLUDING REMARKS

In this Account, we summarized recent advances in the recognition and assembly properties of SCnAs, the biological functions of which have been extensively explored, owing to their biocompatibility. We highlighted various representative biological applications, including fluorescence-based sensing of biological substances, tandem assays for enzymatic activity, screening for enzyme inhibitors, detoxification of hazardous substances, and stimulus-responsive vesicles for drug delivery.

Taken together, these results demonstrate the feasibility of using SCnAs for disease diagnosis and therapy.

Some basic challenges remain to be resolved before the previously reported applications, as well as new applications, can be brought into practical use. One challenge is the chemical modification of SCnAs. Although calixarenes can readily be modified, the number of methods for SCnA modification is limited, and their derivatives are difficult to purify, mainly because of the strong polarity of SCnAs. Once SCnAs can be easily modified with various desired functional groups, the supramolecular chemistry of SCnAs can be expected to become increasingly fascinating, and the biological applications of these compounds should expand markedly. Finding methods to use both the preorganized scaffold and the cavity of SCnAs is another challenge, and such methods can be expected permit the establishment of novel strategies for molecular recognition, sensing, and assembly.<sup>66</sup> Such strategies may allow for multivalent binding, signal amplification, and integrating multiple functions into one self-assembling entity.

With covalent routes for generating SCnA derivatives and noncovalent strategies for fabricating functional host-guest systems in hand, our ultimate goal is to use the supramolecular chemistry of SCnAs to understand biological processes by establishing practical, smart host-guest systems for disease diagnosis and therapy. Although SCnAs are still far from being applied in the clinic, their supramolecular chemistry is actively being studied, and we are approaching our ultimate goal.

#### AUTHOR INFORMATION

**Corresponding Author** 

\*E-mail: yuliu@nankai.edu.cn.

Notes

The authors declare no competing financial interest.

#### **Biographies**

**Dong-Sheng Guo** obtained his Ph.D.in 2006 from Nankai University under the guidance of Professor Yu Liu and then joined Professor Liu's group as a faculty member. He was promoted to Associate Professor in 2008. His current research interest is calixarene-based supramolecular chemistry.

Yu Liu is a full professor in the Department of Chemistry of Nankai University. He obtained financial support from the National Outstanding Youth Fund in 1996 and is now supported by the Cheung Kong Scholars Programme of China. He won a National Natural Science Award, Second Prize, in 2010. His research focuses on molecular recognition and assembly of macrocyclic receptors.

#### ACKNOWLEDGMENTS

This research was financially supported by grants from the National Basic Research Program (also known as the 973 Program; No. 2011CB932502) and from the National Natural Science Foundation of China (Nos. 91227107, 21172119, and 21322207). We would like to express our sincere gratitude to group members Yi-Xuan Wang and Xin-Yue Hu for their assistance in the preparation of this Account.

#### REFERENCES

(1) Böhmer, V. Calixarenes, Macrocycles with (Almost) Unlimited Possibilities. *Angew. Chem., Int. Ed.* **1995**, *34*, 713–745.

(2) Gaeta, C.; Troisi, F.; Neri, P. *endo*-Cavity Complexation and Through-the-Annulus Threading of Large Calixarenes Induced by

Very Loose Alkylammonium Ion Pairs. Org. Lett. 2010, 12, 2092–2095.

(3) Coquière, D.; de la Lande, A.; Martì, S.; Parisel, O.; Prangé, T.; Reinaud, O. Multipoint Molecular Recognition within a Calix[6]arene Funnel Complex. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10449–10454 and references therein.

(4) Shinkai, S.; Mori, S.; Tsubaki, T.; Sone, T.; Manabe, O. New Water-soluble Host Molecules Derived from Calix[6]arene. *Tetrahedron Lett.* **1984**, *25*, 5315–5318.

(5) Guo, D.-S.; Wang, K.; Liu, Y. Selective Binding Behaviors of *p*-Sulfonatocalixarenes in Aqueous Solution. *J. Inclusion Phenom. Macrocyclic Chem.* **2008**, *62*, 1–21 and references therein.

(6) Shinkai, S.; Araki, K.; Matsuda, T.; Nishiyama, N.; Ikeda, H.; Takasu, I.; Iwamoto, M. NMR and Crystallographic Studies of a *p*-Sulfonatocalix[4]arene-Guest Complex. *J. Am. Chem. Soc.* **1990**, *112*, 9053–9058.

(7) Liu, Y.; Guo, D.-S.; Zhang, H.-Y.; Ma, Y.-H.; Yang, E.-C. The Structure and Thermodynamics of Calix[n] arene Complexes with Dipyridines and Phenanthroline in Aqueous Solution Studied by Microcalorimetry and NMR Spectroscopy. *J. Phys. Chem. B* **2006**, *110*, 3428–3434.

(8) Zhao, H.-X.; Guo, D.-S.; Liu, Y. Binding Behaviors of *p*-Sulfonatocalix[4]arene with Gemini Guests. *J. Phys. Chem. B* 2013, 117, 1978–1987.

(9) Cui, J.; Uzunova, V. D.; Guo, D.-S.; Wang, K.; Nau, W. M.; Liu, Y. Effect of Lower-Rim Alkylation of *p*-Sulfonatocalix[4]arene on the Thermodynamics of Host–Guest Complexation. *Eur. J. Org. Chem.* **2010**, 1704–1710.

(10) Paclet, M. H.; Rousseau, C. F.; Yannick, C.; Morel, F.; Coleman, A. W. An Absence of Non-specific Immune Response towards *para*-Sulphonato-calix[*n*]arenes. *J. Inclusion Phenom. Macrocyclic Chem.* **2006**, *55*, 353–358.

(11) Coleman, A. W.; Jebors, S.; Cecillon, S.; Perret, P.; Garin, D.; Marti-Battle, D.; Moulin, M. Toxicity and Biodistribution of *para*-Sulfonato-calix[4]arene in Mice. *New J. Chem.* **2008**, *32*, 780–782.

(12) Perret, F.; Lazar, A. N.; Coleman, A. W. Biochemistry of the *para*-Sulfonato-calix[n]arenes. *Chem. Commun.* **2006**, 2425–2438.

(13) Perret, F.; Coleman, A. W. Biochemistry of Anionic Calix[n]-arenes. *Chem. Commun.* **2011**, 47, 7303–7319.

(14) Atwood, J. L.; Barbour, L. J.; Hardie, M. J.; Raston, C. L. Metal Sulfonatocalix[4,5]arene Complexes: Bilayers, Capsules, Spheres, Tubular Arrays and Beyond. *Coord. Chem. Rev.* **2001**, *222*, 3–32.

(15) Dalgarno, S. J.; Atwood, J. L.; Raston, C. L. Sulfonatocalixarenes: Molecular Capsule and "Russian doll" Arrays to Structures Mimicking Viral Geometry. *Chem. Commun.* **2006**, 4567–4574.

(16) Danylyuk, O.; Suwinska, K. Solid-state Interactions of Calixarenes with Biorelevant Molecules. *Chem. Commun.* 2009, 5799–5813.

(17) Guo, D.-S.; Liu, Y. Calixarene-based Supramolecular Polymerization in Solution. *Chem. Soc. Rev.* **2012**, *41*, 5907–5921.

(18) Koh, K. N.; Araki, K.; Ikeda, A.; Otsuka, H.; Shinkai, S. Reinvestigation of Calixarene-Based Artificial-Signaling Acetylcholine Receptors Useful in Neutral Aqueous (Water/Methanol) Solution. *J. Am. Chem. Soc.* **1996**, *118*, 755–758.

(19) Bakirci, H.; Nau, W. M. Fluorescence Regeneration as a Signaling Principle for Choline and Carnitine Binding: A Refined Supramolecular Sensor System Based on a Fluorescent Azoalkane. *Adv. Funct. Mater.* **2006**, *16*, 237–242.

(20) Wen, L.; Sun, Z.; Han, B.; Imene, B.; Tian, D.; Li, H.; Jiang, L. Fabrication of Layer-by-Layer Assembled Biomimetic Nanochannels for Highly Sensitive Acetylcholine Sensing. *Chem.—Eur. J.* **2013**, *19*, 7686–7690.

(21) Guo, D.-S.; Uzunova, V. D.; Su, X.; Liu, Y.; Nau, W. M. Operational Calixarene-based Fluorescent Sensing Systems for Choline and Acetylcholine and Their Application to Enzymatic Reactions. *Chem. Sci.* **2011**, *2*, 1722–1734.

(22) Dsouza, R. N.; Hennig, A.; Nau, W. M. Supramolecular Tandem Enzyme Assays. *Chem.—Eur. J.* **2012**, *18*, 3444–3459.

(23) Hennig, A.; Bakirci, H.; Nau, W. M. Label-free Continuous Enzyme Assays with Macrocycle-fluorescent Dye Complexes. *Nat. Methods* **2007**, *4*, 629–632.

(24) Bailey, D. M.; Hennig, A.; Uzunova, V. D.; Nau, W. M. Supramolecular Tandem Enzyme Assays for Multiparameter Sensor Arrays and Enantiomeric Excess Determination of Amino Acids. *Chem.—Eur. J.* **2008**, *14*, 6069–6077.

(25) Nau, W. M.; Ghale, G.; Hennig, A.; Bakirci, H.; Bailey, D. M. Substrate-Selective Supramolecular Tandem Assays: Monitoring Enzyme Inhibition of Arginase and Diamine Oxidase by Fluorescent Dye Displacement from Calixarene and Cucurbituril Macrocycles. J. Am. Chem. Soc. 2009, 131, 11558–11570.

(26) Guo, D.-S.; Yang, J.; Liu, Y. Specifically Monitoring Butyrylcholinesterase by Supramolecular Tandem Assay. *Chem.*— *Eur. J.* **2013**, *19*, 8755–8759.

(27) Florea, M.; Kudithipudi, S.; Rei, A.; González-Álvarez, M. J.; Jeltsch, A.; Nau, W. M. A Fluorescence-Based Supramolecular Tandem Assay for Monitoring Lysine Methyltransferase Activity in Homogeneous Solution. *Chem.*—*Eur. J.* **2012**, *18*, 3521–3528.

(28) Minaker, S. A.; Daze, K. D.; Ma, M. C. F.; Hof, F. Antibody-Free Reading of the Histone Code Using a Simple Chemical Sensor Array. *J. Am. Chem. Soc.* **2012**, *134*, 11674–11680.

(29) Ghale, G.; Lanctôt, A. G.; Kreissl, H. T.; Jacob, M. H.; Weingart, H.; Winterhalter, M.; Nau, W. M. Chemosensing Ensembles for Monitoring Biomembrane Transport in Real Time. *Angew. Chem., Int. Ed.* **2014**, *53*, 2762–2765.

(30) Daze, K. D.; Pinter, T.; Beshara, C. S.; Ibraheem, A.; Minaker, S. A.; Ma, M. C. F.; Courtemanche, R. J. M.; Campbell, R. E.; Hof, F. Supramolecular Hosts that Recognize Methyllysines and Disrupt the Interaction Between a Modified Histone Tail and Its Epigenetic Reader Protein. *Chem. Sci.* **2012**, *3*, 2695–2699.

(31) Li, Q.; Guo, D.-S.; Qian, H.; Liu, Y. Complexation of *p*-Sulfonatocalixarenes with Local Anaesthetics Guests: Binding Structures, Stabilities, and Thermodynamic Origins. *Eur. J. Org. Chem.* **2012**, 3962–3971 and references therein.

(32) Guo, D.-S.; Zhang, H.-Q.; Ding, F.; Liu, Y. Thermodynamic Origins of Selective Binding Affinity between *p*-Sulfonatocalix[4,5]arenes with Biguanidiniums. *Org. Biomol. Chem.* **2012**, *10*, 1527–1536. (33) Wang, G.-S.; Zhang, H.-Y.; Li, D.; Wang, P.-Y.; Liu, Y. Characterisation and Antiproliferative Activity of Irinotecan and Sulphonatocalixarene Inclusion Complex. *Supramol. Chem.* **2011**, *23*, 441–446.

(34) Wang, G.-S.; Zhang, H.-Y.; Ding, F.; Liu, Y. Preparation and Characterization of Inclusion Complexes of Topotecan with Sulfonatocalixarene. *J. Inclusion Phenom. Macrocyclic Chem.* **2011**, *69*, 85–89.

(35) Ghosh, I.; Nau, W. M. The Strategic Use of Supramolecular pKa Shifts to Enhance the Bioavailability of Drugs. *Adv. Drug Delivery Rev.* **2012**, *64*, 764–783.

(36) Wang, K.; Guo, D.-S.; Zhang, H.-Q.; Li, D.; Zheng, X.-L.; Liu, Y. Highly Effective Binding of Viologens by *p*-Sulfonatocalixarenes for the Treatment of Viologen Poisoning. *J. Med. Chem.* **2009**, *52*, 6402–6412.

(37) Guo, D.-S.; Wang, L.-H.; Liu, Y. Highly Effective Binding of Methyl Viologen Dication and Its Radical Cation by *p*-Sulfonatocalix-[4,5]arenes. *J. Org. Chem.* **2007**, *72*, 7775–7778.

(38) Wang, G.-F.; Ren, X.-L.; Zhao, M.; Qiu, X.-L.; Qi, A.-D. Paraquat Detoxification with *p*-Sulfonatocalix-[4]arene by a Pharma-cokinetic Study. *J. Agric. Food Chem.* **2011**, *59*, 4294–4299.

(39) Biedermann, F.; Elmalem, E.; Ghosh, I.; Nau, W. M.; Scherman, O. A. Strongly Fluorescent, Switchable Perylene Bis(diimide) Host–Guest Complexes with Cucurbit[8]uril in Water. *Angew. Chem., Int. Ed.* **2012**, *51*, 7739–7743.

(40) Dsouza, R. N.; Pischel, U.; Nau, W. M. Fluorescent Dyes and Their Supramolecular Host/Guest Complexes with Macrocycles in Aqueous Solution. *Chem. Rev.* **2011**, *111*, 7941–7980.

(41) Guo, D.-S.; Jiang, B.-P.; Wang, X.; Liu, Y. Calixarene-Induced Aggregation of Perylene Bisimides. *Org. Biomol. Chem.* **2012**, *10*, 720–723.

(42) Costanzo, L. D.; Geremia, S.; Randaccio, L.; Purrello, R.; Lauceri, R.; Sciotto, D.; Gulino, F. G.; Pavone, V. Calixarene– Porphyrin Supramolecular Complexes: pH-Tuning of the Complex Stoichiometry. *Angew. Chem., Int. Ed.* **2001**, *40*, 4245–4247.

(43) Moschetto, G.; Lauceri, R.; Gulino, F. G.; Sciotto, D.; Purrello, R. Non-Covalent Synthesis in Aqueous Solution of Discrete Multi-Porphyrin Aggregates with Programmable Stoichiometry and Sequence. J. Am. Chem. Soc. 2002, 124, 14536–14537.

(44) Gulino, F. G.; Lauceri, R.; Frish, L.; Evan-Salem, T.; Cohen, Y.; Zorzi, R. D.; Geremia, S.; Costanzo, L. D.; Randaccio, L.; Sciotto, D.; Purrello, R. Noncovalent Synthesis in Aqueous Solution and Spectroscopic Characterization of Multi-Porphyrin Complexes. *Chem.—Eur. J.* 2006, *12*, 2722–2729.

(45) D'Urso, A.; Fragalà, M. E.; Purrello, R. From Self-assembly to Noncovalent Synthesis of Programmable Porphyrins' Arrays in Aqueous Solution. *Chem. Commun.* **2012**, *48*, 8165–8176.

(46) Guo, D.-S.; Chen, K.; Zhang, H.-Q.; Liu, Y. Nano-Supramolecular Assemblies Constructed from Water-Soluble Bis(calix[5]arenes) with Porphyrins and Their Photoinduced Electron Transfer Properties. *Chem.—Asian J.* **2009**, *4*, 436–445.

(47) Varga, O.; Kubinyi, M.; Vidóczy, T.; Baranyai, P. Bitter, I.; Kállay, M. Methylene Blue–Calixarenesulfonate Supramolecular Complexes and Aggregates in Aqueous Solutions. *J. Photochem. Photobiol.*, A **2009**, 207, 167–172.

(48) Lau, V.; Heyne, B. Calix[4]arene Sulfonate as a Template for Forming Fluorescent Thiazole Orange H-Aggregates. *Chem. Commun.* **2010**, *46*, 3595–3597.

(49) Megyesi, M.; Biczók, L. Considerable Change of Fluorescence Properties upon Multiple Binding of Coralyne to 4-Sulfonatocalixarenes. J. Phys. Chem. B **2010**, 114, 2814–2819.

(50) Wang, K.; Guo, D.-S.; Liu, Y. Temperature-Controlled Supramolecular Vesicles Modulated by *p*-Sulfonatocalix[5]arene with Pyrene. *Chem.—Eur. J.* **2010**, *16*, 8006–8011.

(51) Wang, K.; Guo, D.-S.; Liu, Y. Controlled Self-Assembly by Mono-*p*-sulfonatocalix[n] arenes and Bis-*p*-sulfonatocalix[n] arenes. *Chem.*—*Eur. J.* **2012**, *18*, 8758–8764.

(52) Basílio, N.; Piñeiro, Á.; Silva, J. P. D.; García-Río, L. Cooperative Assembly of Discrete Stacked Aggregates Driven by Supramolecular Host–Guest Complexation. J. Org. Chem. **2013**, 78, 9113–9119.

(53) Basilio, N.; García-Río, L. Sulfonated Calix[6]arene Host–Guest Complexes Induce Surfactant Self-Assembly. *Chem.—Eur. J.* 2009, 15, 9315–9319.

(54) Francisco, V.; Basilio, N.; García-Río, L.; Leis, J. R.; Maques, E. F.; Vázquez-Vázquez, C. Novel Catanionic Vesicles from Calixarene and Single-Chain Surfactant. *Chem. Commun.* **2010**, *46*, 6551–6553.

(55) Basilio, N.; Gómez, B.; García-Río, L.; Francisco, V. Using Calixarenes To Model Polyelectrolyte Surfactant Nucleation Sites. *Chem.—Eur. J.* 2013, 19, 4570–4576.

(56) Basilio, N.; Martín-Pastor, M.; García-Río, L. Insights into the Structure of the Supramolecular Amphiphile Formed by a Sulfonated Calix[6]arene and Alkyltrimethylammonium Surfactants. *Langmuir* **2012**, *28*, 6561–6568.

(57) Wang, K.; Guo, D.-S.; Wang, X.; Liu, Y. Multistimuli Responsive Supramolecular Vesicles Based on the Recognition of *p*-Sulfonatocalixarene and Its Controllable Release of Doxorubicin. *ACS Nano* **2011**, *5*, 2880–2894.

(58) Guo, D.-S.; Wang, K.; Wang, Y.-X.; Liu, Y. Cholinesterase-Responsive Supramolecular Vesicle. J. Am. Chem. Soc. 2012, 134, 10244–10250.

(59) Li, Z. Q.; Hu, C. X.; Chen, Y. Q.; Xu, H.; Cao, X. L.; Song, X. W.; Zhang, H. Y.; Liu, Y. Supramolecular Vesicles of Cationic Gemini Surfactants Modulated by *p*-Sulfonatocalix[4]arene. *Sci. China Chem.* **2012**, *55*, 2063–2068.

(60) Cao, Y.; Wang, Y.-X.; Guo, D.-S.; Liu, Y. *p*-Sulfonatocalix[4]arene-Induced Amphiphilic Aggregation of Fluorocarbon Surfactant. *Sci. China Chem.* **2013**, *56*, 1–8.

(61) Wintgens, V.; Coeur, C. L.; Amiel, C.; Guigner, J.-M.; Harangozó, J. G.; Miskolczy, Z.; Biczók, L. 4-Sulfonatocalix[6]arene-Induced Aggregation of Ionic Liquids. *Langmuir* **2013**, *29*, 7682–7688. (62) Qin, Z.; Guo, D.-S.; Gao, X.-N.; Liu, Y. Supra-amphiphilic Aggregates Formed by *p*-Sulfonatocalix[4]arenes and Antipsychotic Drug Chlorpromazine. *Soft Matter* **2014**, *10*, 2253–2263.

(63) Wang, K.; Guo, D.-S.; Zhao, M.-Y.; Liu, Y. Supramolecular Vesicle Based on the Complexation of *p*-Sulfonatocalixarene with Protamine and its Trypsin-Triggered Controllable Release Properties. *Chem.—Eur. J.* **2014**, DOI: 10.1002/chem.201303963.

(64) Basilio, N.; Francisco, V.; García-Río, L. Aggregation of *p*-Sulfonatocalixarene-Based Amphiphiles and Supra-Amphiphiles. *Int. J. Mol. Sci.* **2013**, *14*, 3140–3157.

(65) Guo, D.-S.; Zhang, T.-X.; Wang, Y.-X.; Liu, Y. Enzymeresponsive Supramolecular Polymers by Complexation of Bis(*p*sulfonatocalixarenes) with Suberyl Dicholine-based Pseudorotaxane. *Chem. Commun.* **2013**, *49*, 6779–6781.

(66) Wang, Y.-X.; Guo, D.-S.; Liu, Y. Phosphatase-responsive Amphiphilic Calixarene Assembly. *RSC Adv.* **2013**, *3*, 8058–8063.