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Counterion-Controlled Self-Sorting in Amphiphilic Calixarene Micellar System

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ABSTRACT

Molecular recognition of small molecules and ions by artificial receptors in microheterogeneous media such as micelles and vesicles can, in principle, provide better models of biological systems in comparison with bulk solutions. In this work we have investigated the complexation of an organic fluorescent probe with amphiphilic calixarene receptor below and above the critical micelle concentration (CMC). For concentrations below the CMC, the probe forms a host-guest complex with the calixarene behaving like a traditional host-guest system operating in bulk solution. Above the CMC, multiple equilibrium processes are established and the probe can exchange between the recognition site of the calixarene in the monomeric state, micellized state and/or the micellar hydrophobic core. Careful analysis of the results obtained from NMR and fluorescence experiments allows us to propose a quantitative model to describe the system. The increment of the local concentration of Na^+ counterions at the Stern layer displace the dye to the micelle core through competitive binding of Na^+ in the cavity of the receptor and is decisive for the observed self-sorting behavior.

KEYWORDS

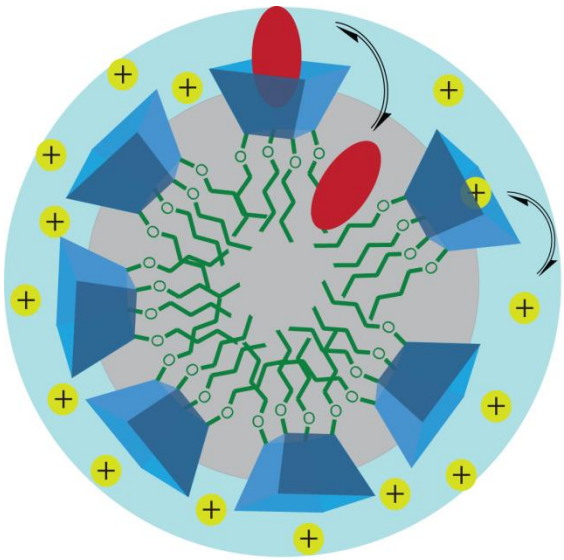
Supramolecular Chemistry; micelle; self-sorting; calixarene;

INTRODUCTION

p-Sulfonatocalix[*n*]arenes (SCn) are water soluble macrocyclic host molecules formed by *n* 4-hydroxybenzenesulfonate units linked by methylene bridges first described by Shinkai and co-workers in 1984. [1–11] After this pioneering work, SCn started to attract increasing attention and were shown to be prime building blocks for solid state supramolecular chemistry, as receptors for biological relevant molecules, as components of supramolecular polymers and supra-amphiphiles or to design and conceive dynamic sensors based on indicator displacement assays.[12–20] During their earlier studies on the chemistry of SCn, the Shinkai's group noted that these molecules can be readily *O*-alkylated at the lower phenolic rim to afford macrocyclic amphiphiles with "host-guest recognition sites".[4] This special class of amphiphiles was shown to aggregate into globular micelles with critical micelle concentrations (CMC) that decrease with increasing the length of the alkyl chains and are little affected by the ring size.[4],[21–24] Despite of not being extensively investigated, the potential of amphiphilic SCn to be applied in the formulation of hydrogels and multifunctional drug delivery vehicles is currently being explored.[25–28]

Some of the most important applications of micelles arise from their ability to solubilise and compartmentalize both apolar, polar and ionic compounds. The globular structure of these aggregates with the hydrophobic chains pointing into the interior and the polar or ionic head groups interfacing with the bulk aqueous solution provide them with three main solubilisation sites: the interfacial region or Stern layer (in the case of ionic surfactants) where counterions and other ionic and polar substances bind to, the palisade layer and the inner core where medium polarity and apolar molecules are preferentially located, respectively.[29–31] In the case of amphiphilic host molecules, like SCn, the macrocyclic cavity can also bind neutral and ionic species providing an additional

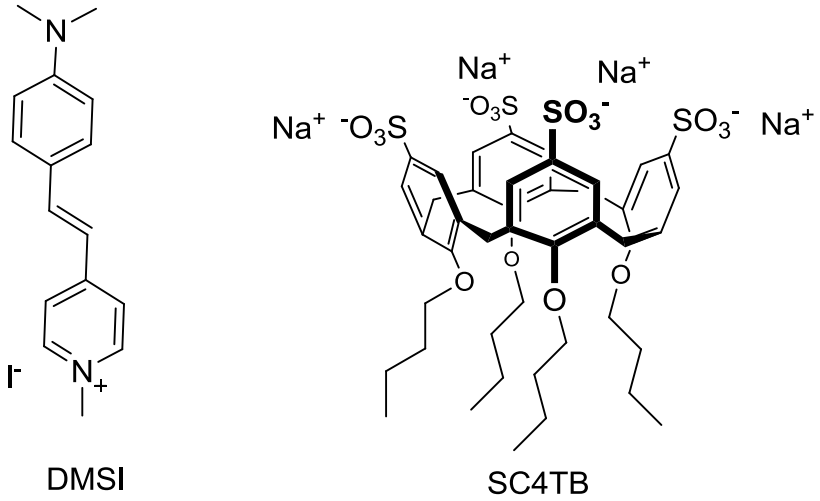
solubilisation site to these micelles. Moreover, while in conventional micelles exchange between species located within inner core/palisade layer and the Stern layer usually do not occur in the case of SCn based micelles the guest can, in principle, exchange between the cavity and the other solubilisation sites (Scheme 1). This particular property allow the study of molecular recognition and self-sorting events within the micellar aggregates where local concentrations can be exceptionally high and the active species are compartmentalized in specific locations.[32–35] This situation is more attractive, from the biomimetic point of view, as it has more resemblances with biological systems than traditional molecular recognition studies carried out in bulk solution. However, these studies are of increased complexity and the development of analytical models to carry quantitative analysis is challenging.



Scheme 1. Cartoon representation of a micelle assembled from amphiphilic macrocycles showing possible exchange of cations between the Stern layer and the host's cavity and organic molecules exchanging between the micellar core and macrocyclic recognition site.

Herein we aim to report the study of the complexation of a model guest molecule, *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide (DMSI), with SC4

tetrabutyl ether (SC4TB) at concentrations below and above the CMC (Scheme 2). With this work we aim to compare the binding ability of amphiphilic SC4 in their monomeric and micellized state and explore self-sorting phenomena in the system through evaluation of guest translocation from the host cavity to the micellar pseudophase. This question is pertinent to amphiphilic receptors and, to the best of our knowledge, was not addressed for amphiphilic SCn.



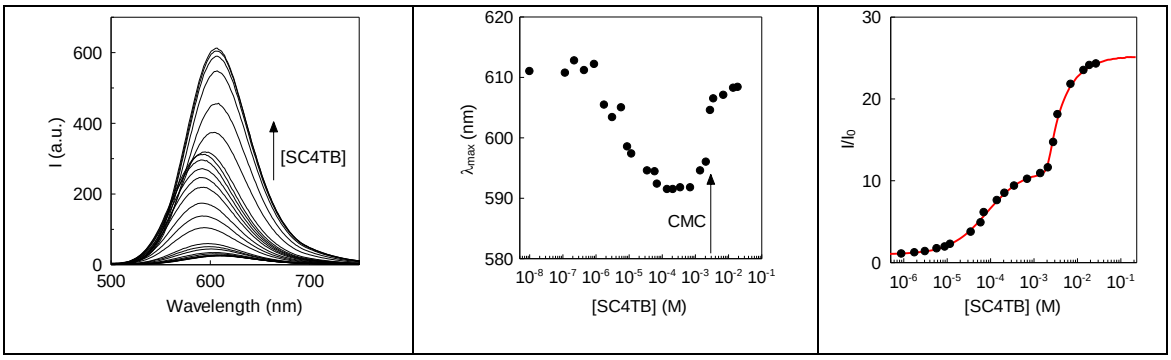
Scheme 2. Structures of guest and amphiphilic host molecules.

RESULTS and DISCUSSION

Figure 1(a) shows the spectral variations observed for the emission spectra of DMSI in the presence of increasing concentrations of SC4TB. As can be observed the emission intensity increases with the concentration of SC4TB and the emission maximum blue shifts from 610 nm in the absence of SC4TB to 592 nm in the presence of 2 mM of SC4TB. Note that DMSI is insensitive to the presence of both PBS buffers and Na^+ cations (see Supporting Information Section). Above this concentration the blue shifting tendency is inverted and a value of 608 nm is observed at 10 mM of SC4TB (Figure 1(b)). In the addition to the biphasic shifts observed in the emission maxima, Figure

1(c) shows that the fluorescence intensity increases with the concentration of SC4TB almost reaching a plateau for $I/I_0 \approx 11$ around 2 mM and above this concentration displays second increase in I/I_0 and approximates a new plateau ($I/I_0 \approx 25$) for concentrations above 10 mM. Taken together these results support the existence of the fluorescent probe in three distinct microenvironments: the bulk solution, the host cavity and the micellar pseudophase.

A similar behaviour to that observed for concentrations below 2 mM was previously reported for DMSI in the presence of SC4.[36] The observed spectral modifications were shown to be due to the formation of a 1:1 host:guest complex between DMSI and SC4 with an association constant of $K = 1x10^5$ M^{-1} in methanol. Similarly, we attribute the observed spectral modifications observed below 2 mM to the formation of an 1:1 host-guest complex with SC4TB. On the other hand, the spectral modifications observed above 2 mM are suggested to be due to the aggregation of SC4TB into micelles and the gradual translocation of the guest from the SC4TB cavity to the micellar pseudophase. These observations are supported by the coincidence of this concentration value with the reported CMC for SC4TB (3 mM).[23,24] It is worth noting that the CMC corresponds to a more or less narrower concentration range rather than a point and therefore more sensible properties (such as the emission of fluorescence probes fluorescence) can be affected by the presence o micellar aggregates at concentrations slightly below the CMC.[37] It should be remarked that critical micelle concentration for SC4TB is mainly unaffected by the presence of DMSI (see supporting information section).



(a) (b) (c)

Figure 1. (a) Fluorescence spectra of DMSI (10 μM) upon addition of increasing concentrations of SC4TB. (b) Plot of the emission maximum position against the SC4TB concentration.(c) Representation of the fluorescence intensity at 610 nm as a function of the SC4TB concentration.

Further evidences for the translocation of the guests from the SC4TB cavity to the micellar pseudophase can be obtained from ^{1}H NMR experiments. As can be observed in the presence of 1 equivalent of SC4TB (0.5 mM) the ^{1}H NMR signals of the guest appear considerable broadened and displaced upfield indicating the formation of the host-guest complex. Full assignment of all signals cannot be unequivocally carried out due to significant overlap and broadening upon complexation, but is evident that the signals corresponding to the pyridinium group display high upfield complexation induced chemical shifts. The *N*-methyl protons of the pyridinium group show a large upfield displacement of ca. -1.65 ppm while the signals of the dimethylamino group remain almost unchanged suggesting that the guest is partially included through the pyridinium group leaving the aminostyril group outside the cavity. Upon increasing the concentration of SC4TB above 1 mM the signals start to resolve and those of the pyridinium group are displaced downfield with respect to the host-guest complex while all other signals are slightly displaced upfield. Again, the signal of the pyridinium methyl protons is particularly elucidative since it is displaced from 2.6 ppm in the host-guest complex to ca. 4.05 ppm in the presence of 6 mM of SC4TB. Together, these

observations suggest that the DMSI is gradually translocated from the calixarene cavity to the micellar pseudophase upon micellar aggregation of the amphiphilic calixarene host.

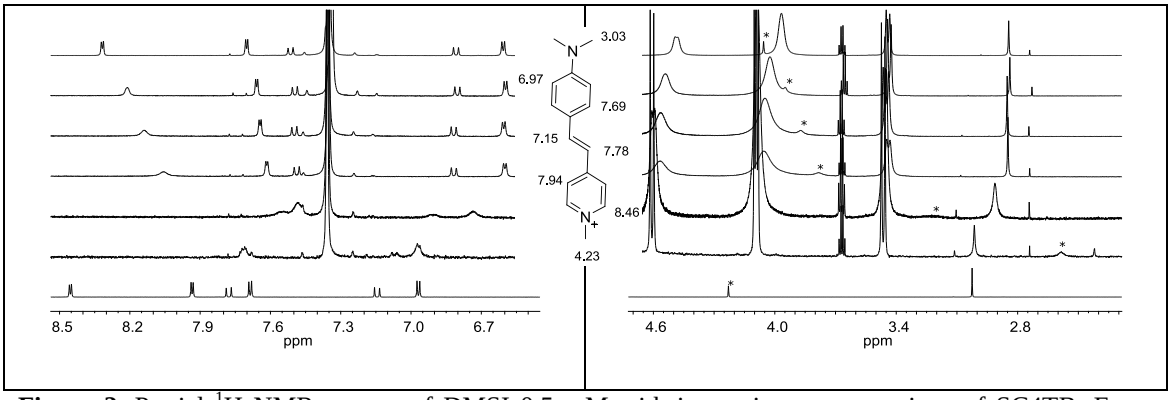


Figure 2. Partial ^{1}H NMR spectra of DMSI 0.5 mM with increasing concentrations of SC4TB. From bottom to the top: 0.0, 0.5, 1.0, 1.8, 2.3, 3.0 and 6.0 mM of SC4TB. All spectra were acquired in D_2O at 25 ºC. The signal marked with * corresponds to the pyridinium methyl protons of DMSI.

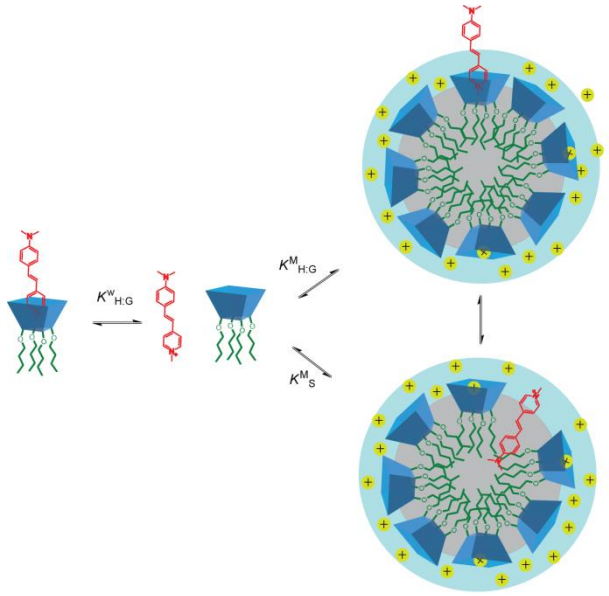
By considering the experimental observations described above it is possible to propose a scheme that to accounts for the different binding events taking place in solutions of amphiphilic calixarenes and guest molecules (Scheme 3). Above the CMC, the guest molecule can for 1:1 complex with monomeric SC4TB with an association constant $K^{w}_{H:G}$, it can form a 1:1 complex of the inclusion type with micellized SC4TB ($K^{M}_{H:G}$) or it can be solubilized in the micellar pseudophase with a partition constant K^{M}_{S}. Combining the pseudophase micellar model with a 1:1 binding model it is possible to obtain the expressions to calculate the concentrations of all species in solution (See SI). The model assumes, as approximation, that above the CMC the concentration of micellized SC4TB is given by $[SC4TB]_M = [SC4TB]_0$-CMC and therefore is only valid when the CMC is much higher than the concentration of guest molecule.[30]

By assuming this model, the experimental fluorescence data (Figure 1c) can be readily fitted to obtain the three binding constants. $K^{w}_{H:G}$ is obtained from the fluorescence variations below the CMC and therefore can be kept constant when the

complete concentration range is considered. In order to reduce the number of adjustable parameters the limiting value for the emission of DMSI in the cavity of SC4TB molecules in their micellized state is considered to be equal to that observed for monomeric host-guest complexes. By assuming these constraints, the experimental data was successful fitted (see figure 1c) and the following parameters were obtained: $K^{w}_{H:G}$ = (1.3±0.2)x10^4 M^{-1}; K^{M}_{S} = (1.4±0.5)x10^4 M^{-1} and $K^{M}_{H:G}$ = (1.1±0.5)x10^3 M^{-1}. It is worth noting that the value of $K^{w}_{H:G}$ compares with that reported for the *N*-methylpyridinium cation $K^{w}_{H:G}$ = (9.1±0.1)x10^4 M^{-1} but is important to stress that the binding mode of this guest is substantially different to than observed for DMSI. [38] In the former case it was proposed that the *N*-methyl group points towards the exterior of the host's cavity while in the present case the ^{1}H NMR data suggests the *N*-methyl group of the pyridinium group is included into the cavity. It is also important to compare the binding ability of SC4TB in the monomeric and micellized states ($K^{w}_{H:G}$ and $K^{M}_{H:G}$).

At first instance, one should expect these binding constants to be comparable values as the conformation of SC4TB remains the same as well as the size and properties of his recognition cavity.[23] However, it should be taken into account that the reported degree of counterion binding for SC4TB micelles is 69%.[24] This value suggests that the attractive coulombic interactions that may contribute for the stabilization of the complex in the monomeric state are partially neutralized upon aggregation resulting in lower stability of the complex and promoting the translocation from the receptors cavity to the micellar pseudophase. Further, the increase in the local concentration of Na^+ at the Stern layer may promote the formation of competitive Na^+ complexes with micellized SC4TB decreasing the apparent binding constant $K^{M}_{H:G}$.[39,40] Figure 3 compares the [SC4TB]-dependent mole fraction distribution of the DMSI

species calculated from the binding constants reported above with that calculated assuming the same value for $K^{w}_{H:G}$ and $K^{M}_{H:G}$ ($1.3x10^{4}$ M^{-1}). As can be observed in the first case the translocation of the guest from the receptors cavity to the micellar pseudophase is almost quantitative while in the second case the guest is predicted to be evenly distributed between the two sites.



Scheme 3. Cartoon representation of the possible binding events that take place between SC4TB and DMSI above the CMC.

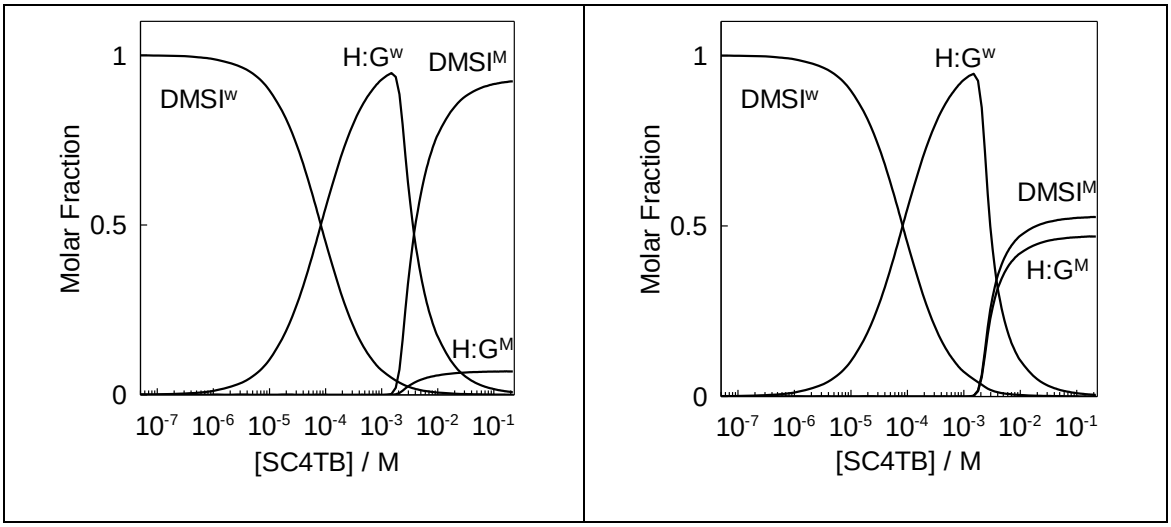


Figure 3. Mole fraction distribution for the DMSI species plotted against the total concentration of SC4TB calculated from (a) the experimentally observed values for the binding constants and (b) assuming that $K^{w}_{H:G} = K^{M}_{H:G} = 1.3x10^{4}$ M^{-1} and $K^{M}_{S} = 1.4x10^{4}$.

In order to further investigate the system and confirm the formation of host-guest complexes below the CMC a displacement assay was carried out using tetratethylammonium (TEA) chloride as competitor. As can be observed in figure 4, as the concentration of TEA increases the emission intensity decreases due to the formation of a host-guest complex between SC4TB and TEA. As a consequence DMSI is displaced from the host's cavity to the bulk solution. The data can be fitted using a competitive binding model to obtain a binding constant for TEA of K^{TEA} = $(4.4\pm1.0)x10^{2}$ M^{-1}. This value is comparable to that observed for the complexation of tetramethylammonium cation with SC4TB (K^{TMA} = $(2.8\pm0.1)x10^{2}$ M^{-1}).[41] This experiment supports the formation of the inclusion complex with DMSI at concentrations below the CMC.

Another interesting feature of the experiment reported in figure 4 is observation of minimum value around [TEA] = 40 mM and subsequent increase in the fluorescence intensity. This observation is attributed to a decrease of the CMC with the concentration of TEA. Electrical conductivity experiments showed that the CMC of SC4TB shifts from 3 mM in the absence of additives to 1.4 mM in the presence of 3 mM TEA (see SI). The magnitude of the CMC shift is expected to increase for higher concentrations of TEA but due to the higher conductivity of concentrated TEA solution is was not possible to obtain the CMC in these conditions. It is well established that the CMC of ionic surfactants decrease with the salt concentration and that the magnitude of this decrease if higher for more lipophilic ions.[42] Therefore, by using chemical stimuli, the organic probe can be displaced from the host's cavity to the bulk solution or to the

micellar pseudophase depending on the concentration and nature of the employed competitor. These observations are summarized in scheme 4.

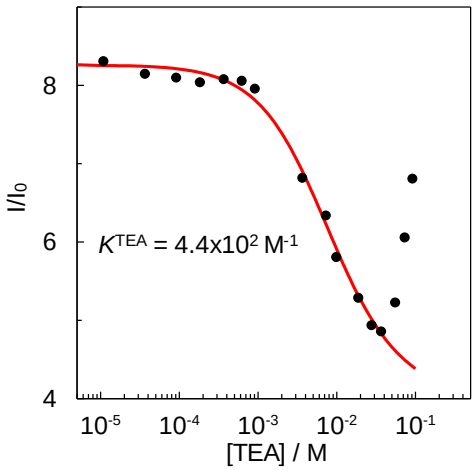
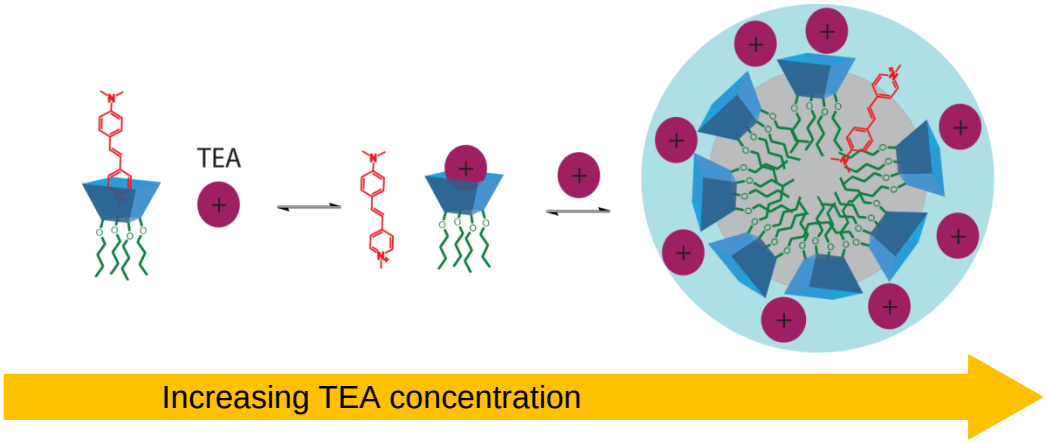


Figure 4. Fluorescence intensity (610 nm) variations observed for DMSI (12 μM) upon addition of increasing concentrations of TEA competitor in the presence of 0.2 mM of SC4TB.



Scheme 4. Cartoon representation of the recognition and aggregation behavior observed for SC4TB and DMSI in the presence of increasing concentrations of TEA.

In conclusion, we have observed that the binding affinity of SC4TB for a cationic model guest (DMSI) is substantially different in the monomeric and micellized state most probably due to the high concentration of Na^+ at the Stern layer leading to charge neutralization of the amphiphilic host in the micellar state and probably to competitive binding of sodium cations. This reduction in the apparent host-guest

binding constant results in a self-sorting process that is translated into the efficient translocation of the guest from the host cavity to the micellar pseudophase. In addition, a simple mathematical model has been developed to describe the distribution of the guest between all possible microscopic localization. This model can also be applied to fit experimental data and obtain the relevant binding (partition) constants.

Supporting Information

Critical micelle concentration determination in the presence of $DSMI^+$ and derivation of the distribution model is reported in the supporting information section.

Experimental Details

5,11,17,23-tetrasulfonato-25,26,27,28-tetrakis(n-butyl)calix[4]arene was available from previous studies.[23,24,43] All other compounds were commercial available and were used without further purification. The fluorescence spectra of *trans*-4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide were measured on a Cary Eclipse instrument with an excitation wavelength of 450 nm. The emission slit was 10 nm and the excitation slit was 5 nm. ^{1}H NMR spectra were recorded at 25 °C on a Varian Inova 750 spectrometer. Electrical conductivity was measured by using a Radiometer CDM3 conductivity meter with a cell constant of 0.968 cm^{-1}. The conductivity meter was calibrated with two KCl conductivity standard solutions (0.0100 M, with κ = 1413 $\mu S cm^{-1}$ at 25.0 °C; and 0.100 M, with κ = 11.28 mS cm^{-1} at 25.0 °C) supplied by Crison. The temperature was kept constant to within ±0.1 °C by passing thermostated water through a jacketed vessel holding the solution.

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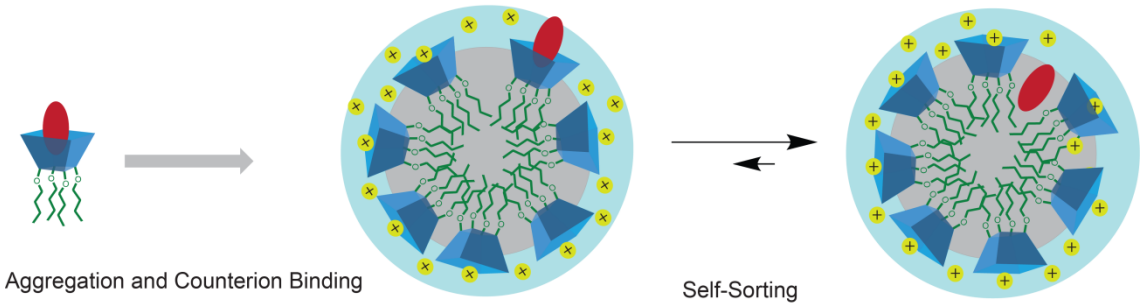
Graphical Abstract



Guest translocation from the host cavity to the micellar core is affected by competitive counterion binding.