Supramolecular Chemistry

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Dynamically Self-Assembling Metalloenzyme Models Based on Calixarenes**

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Macrocyclic host molecules have long been recognized as attractive models for enzymes.[1] When transition metals are coordinated in proximity to the hydrophobic cavities, the macrocycles resemble metalloenzymes in their structure and function. One approach, which has been pioneered by Breslow and co-workers for cyclodextrins,[2] and later extended to calixarenes,[3–6] involves the design of host

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molecules with covalently attached nitrogen ligands, which can be subsequently coordinated with transition metals to form sophisticated metalloenzyme mimics. The second principal approach, in which metalloenzyme models were studied from a structural point of view in the solid state, was explored by Atwood and co-workers[7,8] using *p*-sulfonatocalix[4]arene (CX4) as the host,[9] pyridine or pyridine-*N*-oxide as the guest, and the presence of metal ions. Herein, we present a simple supramolecular approach to metalloenzyme models in aqueous solution, which is based on the dynamic self-assembly between macrocyclic hosts with cation receptor properties, organic guests, and metal ions (Figure 1). In the resulting ternary complex, the guest is held in place by hydrophobic interactions with the host, while the metal ion experiences attractive Coulombic interactions with the negative charges positioned at the portal of the macrocycle.

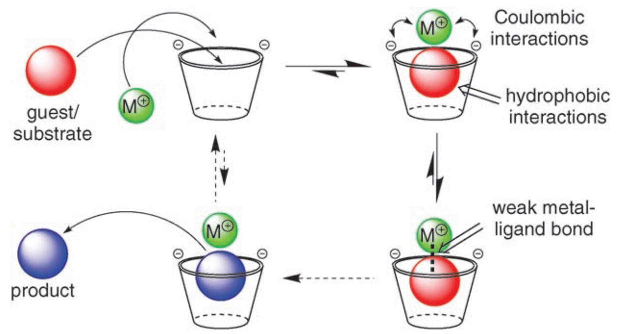
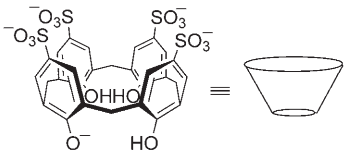
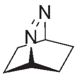


Figure 1. Dynamically self-assembling metalloenzyme model.

If the guest functions as a weak ligand, the host can assist the formation of a metal–ligand bond with the guest, which in turn reinforces the ternary complex and results in a positive cooperativity. Finally, if the guest possesses a functional group to allow a chemical reaction to occur, the catalytic activity of the coordinating metal may be probed. We now demonstrate the structural viability of the dynamic self-assembly approach (solid arrows in Figure 1) as well as thermodynamic and selectivity aspects by employing calixarenes (namely CX4) as hosts.



CX4 (at neutral pH)



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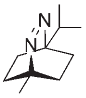

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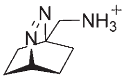

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Close inspection of Figure 1 reveals that the proper choice of the guest is quintessential. The guest should be sufficiently large to maximize hydrophobic interactions, but sufficiently small to allow the docking of cations to the upper rim of the host. It should possess functional groups to enable (weak) metal–ligand interactions and to allow chemical reactions and catalytic effects to be probed. We identified bicyclic azoalkanes, in particular 2,3-diazabicyclo[2.2.2]oct-2-ene (**1**), as ideal guest molecules. Among neutral guests, **1** has the highest binding constant with CX4 ($K \approx 1000\,\text{M}^{-1}$),[10] and its small spherical shape facilitates the concomitant binding of cations. Compared to common nitrogen ligands, bicyclic azoalkanes are weak ligands,[11] which is nicely reflected in their weak basicity ($pK_a \approx 0.5$ for **1**).[12] Most importantly, the complexation of **1** by the host and subsequent ligation of the metal can be conveniently monitored by optical spectroscopy, because **1** shows strong fluorescence in water[13] and has a weak but distinct near-UV absorption which displays characteristic shifts in different host environments[14] and upon complexation (protonation).[12]

Addition of CX4 to a solution of **1** at neutral pH[15] led to the formation of the 1:1 complex with a UV absorption maximum at 366 nm (solid lines in Figure 2).[10,16] Upon addition of transition metals (e.g., Zn^{2+}, Co^{2+}, Mn^{2+}), a hypsochromic shift was observed (Figure 2), which signals the formation of a ternary complex in which the azo group functions as a monodentate ligand.[16] Control experiments revealed that the addition of the same metal ions (up to 20 mM) to aqueous solutions of **1** did not give rise to a significant shift in the UV spectrum, which reveals that the azo–metal complex has a very low binding constant ($K < 5\,\text{M}^{-1}$) in solution.[11] Only in the presence of the macrocyclic host will the guest and metal form the desired complex, that is, the host assists or "templates" the formation of the metal–ligand bond. In essence, the host brings two species together, like an enzyme does with a substrate and a catalytic center, to form a metal–ligand complex, which in the absence of host is not present in significant amounts because of low bimolecular affinity.[17]

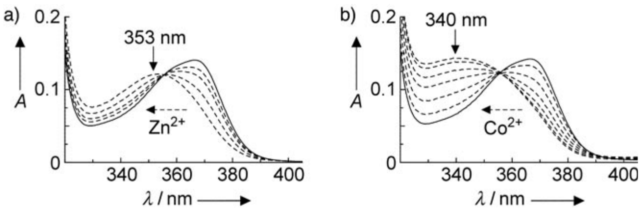


Figure 2. Changes in the UV spectra of **1** (2 mM) with CX4 (4 mM) in water at pH 7.0 (solid lines) upon addition (up to 10 mM) of a) Zn^{2+} and b) Co^{2+} ions (dotted lines).

Independent evidence for the formation of the ternary complex in the presence of Zn^{2+} ions comes from ^1H NMR studies. The formation of the inclusion complex between **1** (2 mM) and CX4 (4 mM) was established through intermolecular ROESY cross-peaks and the characteristic up-field shifts of the guest protons[10] which arise from the shielding effect of the CX4 aryl groups. Upon addition of Zn^{2+} ions (20 mM), the ROESY cross-peaks were retained and, more importantly, an extra up-field shift was observed (by ca. 0.3 ppm, Figure 3). This is consistent with the pictorial representation of the metal ion as a lid, which presses the guest somewhat more

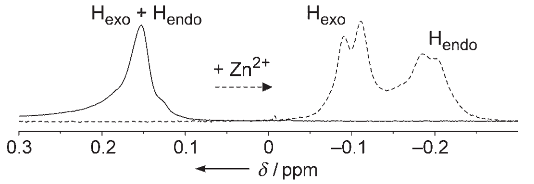


Figure 3. Shift in the ^{1}H NMR spectrum of **1** (2 mM) in the presence of CX4 (4 mM) in D$_2$O at pD 7.4 (solid line) upon addition of 20 mM Zn^{2+} ions (dotted line).

tightly into the cavity (Figure 4). Interestingly, when the bridgehead-alkylated derivative **2** (2 mM) was studied in the presence of CX4 (4 mM, $K = 480\,\text{M}^{-1}$),[10] the NMR shift upon addition of Zn^{2+} ions (20 mM) to the preformed complex was opposite (down-field by ca. 0.4 ppm), which signals a competitive binding, that is, the docking of the metal ion results in a steric interference with the bound guest, and the proper metal–ligand bond geometry can no longer be attained. Moreover, when Ca^{2+} ions (20 mM) were added to the CX4·**1** complex, a similar down-field shift (by ca. 0.3 ppm) was observed. This observation reveals that **1** is again released as a result of competitive binding,[18] because the ternary complex with the oxophilic Ca^{2+} ion is not stabilized by an additional metal–ligand bond as is formed with transition metals, and steric hindrance comes into play instead.

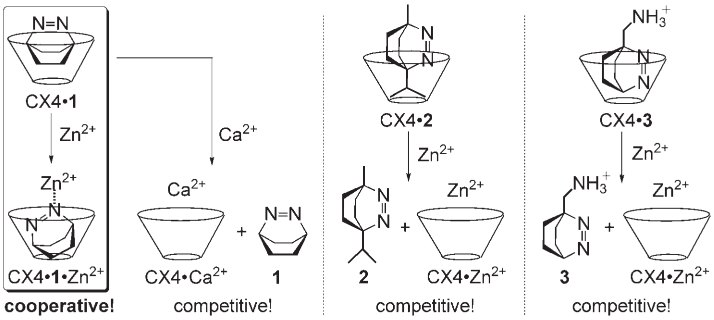


Figure 4. Interplay between cooperative and competitive binding.

The fluorescence of **1**, which is high in water, decreases upon complexation by CX4[16] as a result of exciplex-induced quenching by the electron-rich aryl groups of the surrounding host. When Zn^{2+} ions (which do not quench the fluorescence of free **1**) were added to the preformed host–guest complex, the fluorescence of **1** was further decreased, but reached a plateau at an intermediate metal-ion concentration (Figure 5 a). This observation signals the formation of the ternary complex, in which the fluorescence intensity is modulated due to altered photophysical properties of the complexed chromophore. For comparison, when zinc ions were added to the CX4 complex of the 1-ammoniomethyl derivative **3** (Figure 5 b), which is stabilized by the additional positive charge ($K = 60\,000\,\text{M}^{-1}$ at pH 7.0), the fluorescence increased, because the binding is again competitive and leads to release of the guest. For **3**, this is primarily due to charge repulsion between the ammonium group and the zinc ions. A similar fluorescence recovery arising from competitive binding was found for **2**.

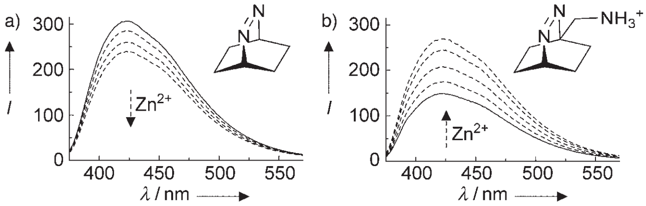


Figure 5. Changes in the fluorescence spectra at pH 7.0 (solid lines) upon addition of Zn^{2+} ions (up to 10 mM; dotted lines) to: a) azoalkane **1** (1 mM) with CX4 (1.6 mM) and b) azoalkane **3** (1 mM) with CX4 (0.5 mM).

As can be seen, a fascinating interplay between cooperative binding (formation of ternary complex) and competitive binding (release of guest) occurs. As depicted in Figure 4, the formation of the ternary complex (the metalloenzyme model) occurs only if 1) metal and guest binding do not clash because of steric or electrostatic factors, 2) a transition-metal ion is employed, and 3) the proper geometry for formation of the metal–ligand bond can be attained. A very high selectivity results, with only the parent **1** forming the desired ternary complex; even slight variations, such as the alkylation in **2** or the introduction of an ammonium group in **3**, suppress its formation. This result is in sharp contrast to the generally accepted very poor selectivity of CX4 towards guest binding, which has led Rebek and co-workers to correctly conclude that such simple hosts can recognize little more than a positive charge.[19] Our results show that an otherwise unselective host can be twisted into a highly selective one simply by exploiting additional supramolecular interactions.

The dramatic increase in the selectivity of the ternary complex is made possible by a “triple recognition”. In other words, three supramolecular interactions act in concert (Figure 1). This expands the concept of Tabushi et al., who emphasized the need for a double recognition of the guest in metalloenzymes (hydrophobic and metal–ligand interactions);[20] the present metalloenzyme models incorporate additionally weak Coulombic interactions between the metal and the host, which allows for a rapid exchange in a dynamic supramolecular assembly. For comparison, the metal in the previously designed metalloenzyme mimics[1–6] was tightly incorporated into the host through interaction with covalently attached nitrogen ligands.

Circumstantial evidence for the ternary complexes implicated in solution comes also from the crystal structures of the zinc and cobalt complexes, which are isostructural (Figure 6 and see the Supporting Information).[15] Note that **1** is immersed with its hydrophobic portion in the CX4 cone, while it serves at the same time as a monodentate σ-donor ligand in the first coordination sphere.

Besides the structural aspects, the binding constants are also very relevant because a rapid dynamic equilibrium applies in solution (Figure 7). The binding constant of Zn^{2+} ions with CX4 was determined as $2000 \pm 200\,\text{M}^{-1}$ at neutral pH (K_1) by analyzing the fluorescence recovery of **2** and **3** upon addition of zinc ions (see above). This value is comparable to that obtained with the similarly large

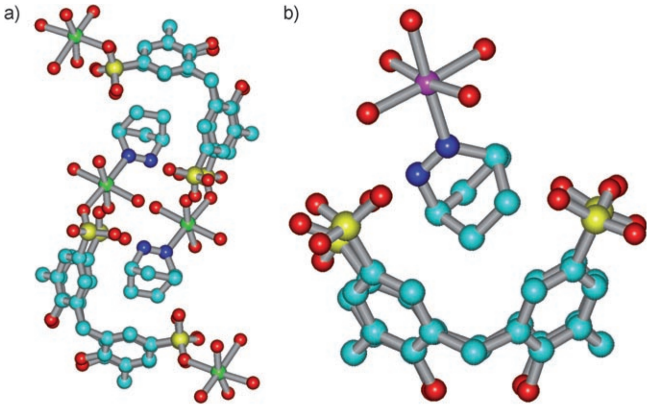


Figure 6. Crystal structures of the complexes formed between CX4, **1**, and transition-metal ions: a) the mutually encapsulating, binuclear repeat unit (with Zn^{2+} ions, green) and b) view of ternary complex (with Co^{2+} ions, magenta; C turquoise, N blue, O red, S yellow).

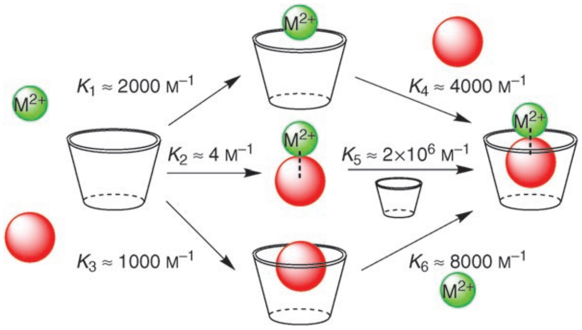


Figure 7. Binding constants in ternary complexes.

Mg^{2+} ions.[18] The binding constant of **1** in the absence of metal ions amounts to $1000\,\mathrm{M}^{-1}$ (K_3).[10,12,16] By titrating CX4 into a solution of **1** (2 mM) in the presence of an excess of Zn^{2+} ions (20 mM, this direct UV titration is possible because free **1** has no sizable affinity to Zn^{2+} ions, while CX4 is virtually quantitatively complexed at this concentration of Zn^{2+} ions), a binding constant of $4000 \pm 500\,\mathrm{M}^{-1}$ was determined, which corresponds to the apparent binding constant of **1** to the $CX4\cdot Zn^{2+}$ complex (K_4). From these three binding constants, the remaining ones can be calculated (K_6) or estimated (K_2, K_5). The increased binding in the presence of Zn^{2+} ions (factor of 4, K_4/K_3) reflects the positive cooperativity of the binding (synergistic effect). This value is consistent with the low binding constant between Zn^{2+} ions and uncomplexed **1** in aqueous solution (see above), although the values could also differ slightly because of different coordination spheres of zinc ions in water and when complexed to CX4.

The increase in the binding constants in the presence of the metal ion (K_4 versus K_3) as a result of the additional metal–ligand interaction may also be of interest for other areas where the modulation of guest binding constants is of interest, for example in sensor applications. A specific example is shown in Figure 8a for the fluorescence regeneration of **1** from its CX4 complex upon addition of acetylcholine as analyte.[16] As can be seen, the sensitivity of this fully water-soluble sensor system can be significantly enhanced by the addition of 10 mM zinc ions, thus expanding both the overall sensitivity (increase in fluorescence) as well as the accessible range for the sensing of this neurotransmitter.

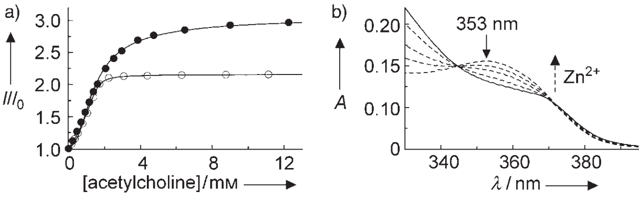
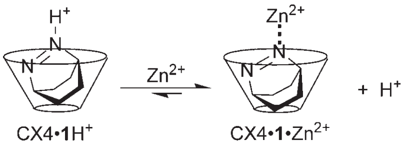


Figure 8. a) Fluorescence recovery of **1** (1 mM) with CX4 (1.6 mM) at pH 7.0 in the absence (open circles) and presence (filled circles) of 10 mM Zn^{2+} ions upon addition of acetylcholine (for the displacement assay principle, see Ref. [16]). b) Changes in the UV spectra of **1** (2 mM) with CX4 (4 mM) in water at pH 2.0 (solid line) upon addition of Zn^{2+} ions (up to 10 mM; dotted lines).

Let us finally bridge the gap to metalloenzymes. One of the prime assets of Zn^{2+} ions in hydrolytic metalloenyzmes is their ability to deprotonate ligands or water molecules in the course of metal–ligand bond formation.[21] This function could be nicely demonstrated in our self-assembling models as well. At lower pH values, for example, at pH 2, the guest is protonated in the CX4 complex ($CX4\cdot\mathbf{1}H^{+}$),[12,15] such that the UV absorption of **1** is shifted to the far UV region (Figure 8b, solid line).[16] Upon addition of Zn^{2+} ions, a new band emerges at 353 nm, which corresponds precisely to the absorption of the ternary complex ($CX4\cdot\mathbf{1}\cdot Zn^{2+}$, see Figure 2a). In this particular case, Zn^{2+} ions do not destabilize the complex of the cationic guest (as found for **3**), but rather deprotonate the guest by forming the metal–ligand bond [Equation (1)]. In other words, the docking of the zinc ion to the calixarene causes a shift in the pK_a value of the hydrophobically bound guest, thus showing a remote resemblance to its biological activity.



(1)

In summary, we have employed a dynamic self-assembly of a simple macrocycle, metal ions, and a rationally selected guest molecule to construct interesting structural metalloenzyme models in aqueous solution. Conceptually, the phenomenon of host-assisted metal–ligand bond formation stands out, as well as the high selectivity for the formation of a ternary complex. This strategy results in an interesting interplay between cooperative and competitive binding arising from a triple supramolecular recognition motif. With the valid proof of concept in hand, functional aspects (dashed arrows in Figure 1) need to be investigated next.

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