



Supporting Information

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Dynamically Self-Assembling Metalloenzyme Models Based on Calixarenes

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Experimental Section

Calix[4]arene-4-sulfonic acid (CX4), metal chlorides, and acetylcholine chloride were purchased from Fluka. Azoalkanes **1-3** were synthesized as reported.^[1-3] UV spectra were recorded on a Varian Cary 4000, ¹H NMR spectra on a Jeol JNM-ECX400, and fluorescence spectra on a Varian Cary Eclipse instrument.

The pH (pD) of all solutions, which is important to control due to its pronounced effect on the binding constants of guest molecules,^[1] was adjusted by addition of NaOH (NaOD); the use of buffer was avoided because excess amounts of cations were recently shown to adversely affect the binding constants with CX4.^[4] In the case of titrations, two solutions containing identical amounts of CX4 and guests **1-3** were prepared with and without transition metal ion and the pH values were independently adjusted with NaOH before successive addition during titration. Binding constants and titrations were analyzed as described before.^[1-6]

Single crystals were successfully grown by slow ambient temperature evaporation from aqueous solutions containing 5 mM CX4 (as acid, affording strongly acidic solutions), 2 mM **1**, and 20 mM metal chloride; other conditions, e.g.,

after adjustment of neutral pH with NaOH, did not afford single crystals under our conditions. Single crystals were measured on a Bruker X8 APEX II CCD diffractometer with kappa geometry using MoK_α radiation ($\lambda = 0.71073 \text{ \AA}$, sealed tube) at $T = 173(2) \text{ K}$. Data integration and reduction were performed with the SAINT Bruker software suite.^[7] A semi-empirical absorption correction (SADABS)^[8] was applied. Structure solution and refinement were performed with the SHELX-97 software package.^[9] Structures were solved by direct methods and completed by successive interpretation of synthesis Fourier maps, followed by full-matrix least-squares refinement against F^2 . Hydrogen atoms were included in fixed, calculated positions and assigned a thermal parameter based on the bonded atom. The CIF files of the zinc and cobalt complexes are deposited under the codes CCDC 615736 and 615735, respectively, these data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Formula C₃₄H₅₅N₂O_{28.5}S₄Zn₂, $M_r = 1206.78$, colorless plate with dimensions $0.27 \times 0.20 \times 0.03 \text{ mm}^3$, triclinic, space group P $\bar{1}$, $a = 11.7817(17)$, $b = 14.189(3)$, $c = 14.969(3) \text{ \AA}$, $\alpha = 68.570(7)$, $\beta = 84.914(8)$, $\gamma = 84.269(7)^\circ$, $V = 2314.1(7) \text{ \AA}^3$, $Z = 2$, $\rho_{\text{cal}} = 1.732 \text{ Mg m}^{-3}$, $\mu = 1.317 \text{ mm}^{-1}$. $2\theta_{\text{max}} = 45.92^\circ$, measured reflections 47380, independent reflections 6260 ($R_{\text{int}} = 0.1355$). Carbon atoms, disordered oxygens and lattice waters were refined isotropically, other non-hydrogen atoms were refined anisotropically. The final anisotropic full matrix least-squares refinement on F^2 for 442 variables converged at $R_1 = 0.1500$ and $wR_2 = 0.3566$ with a goodness-of-fit of 1.051. In the final difference map the largest peak was 2.373 e \AA^{-3} and deepest hole – 1.231 e \AA^{-3} .

Formula C₃₄H₅₅N₂O_{28.5}S₄Co₂, $M_r = 1193.90$, pink plate with dimensions $0.28 \times 0.17 \times 0.02 \text{ mm}^3$, triclinic, space group P $\bar{1}$, $a = 11.8179(9)$, $b = 14.1354(13)$, $c = 14.9814(14) \text{ \AA}$, $\alpha = 68.717(6)$, $\beta = 84.570(6)$, $\gamma = 83.875(55)^\circ$, $V = 2314.4(4) \text{ \AA}^3$, $Z = 2$, $\rho_{\text{calcd}} = 1.713 \text{ Mg m}^{-3}$, $\mu = 0.997 \text{ mm}^{-1}$. $2\theta_{\text{max}} = 55.14^\circ$, measured reflections 78771, independent reflections 10646 ($R_{\text{int}} = 0.1615$). Disordered oxygens and lattice waters were refined isotropically, other non-hydrogen atoms were refined anisotropically. The final anisotropic full matrix least-squares refinement on F^2 for 614 variables converged at $R_1 = 0.1118$ and $wR_2 = 0.2991$ with a goodness-of-fit of 1.038. In the final difference map the largest peak was 2.548 e \AA^{-3} and the deepest hole – 0.926 e \AA^{-3} .

Additional Evidence for the Formation of the Ternary Complex at pH 2

The formation of the ternary complex ($\text{CX4}\bullet\mathbf{1}\bullet\text{Zn}^{2+}$) in strongly acidic solution (pH 2) can not only be followed by UV (Figure 5b of main text), but also by ^1H NMR (Figure S1) and fluorescence (Figure S2). Addition of Zn^{2+} causes an upfield ^1H NMR shift (as was observed at pH 7, see Figure 2 of main text), except that the shift was in this case more pronounced for the *endo* protons than for the *exo* ones; this is presumably related to the slightly different complexation geometries of **1** (at pH 7) and **1H⁺** (dominant at pH 2).^[11] In the fluorescence titration, an intensity increase was observed at pH 2, which signals the conversion of the virtually nonfluorescent protonated form (**1H⁺**)^[5] into the more strongly fluorescent ternary complex with zinc.

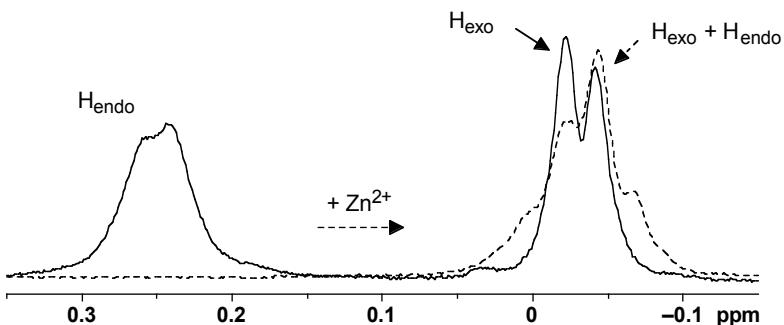


Figure S1. Shift in the ^1H NMR spectrum of **1** (2 mM) in the presence of CX4 (4 mM) in D_2O at pD 2.4 upon addition of 20 mM Zn^{2+} .

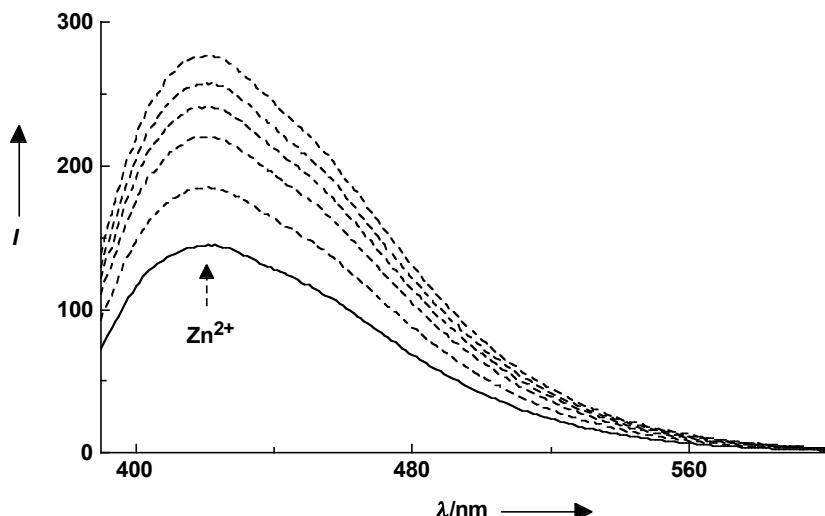


Figure S2. Changes in fluorescence spectra of **1** (1 mM) with CX4 (1.6 mM) at pH 2.0 upon addition of Zn^{2+} (up to 10 mM).

The interplay between cooperative and competitive binding (Scheme 2 of main text) applied at pH 2 as well. For example, when the CX4 complex of azoalkane **2** was formed at pH 2 (solid line in Figure S3, note again that **2** is protonated in its

CX4 complex at this pH)^[1] and Zn²⁺ was subsequently added, the UV titration led to a recovery of the UV absorption of the uncomplexed azoalkane **2** (characteristic absorption maximum at 373 nm in water)^[1] and not to the hypsochromically shifted absorption expected for the formation of a ternary complex, as was the case for azoalkane **1** (Figure 5b of main text).

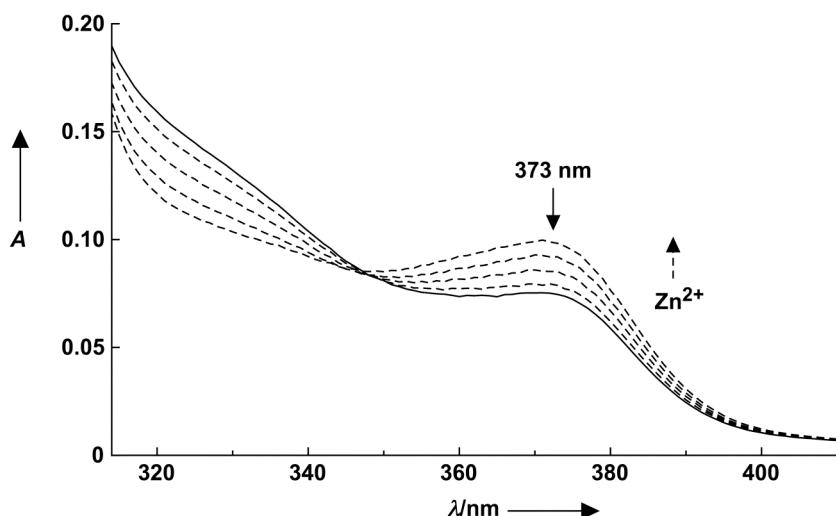


Figure S3. Changes in the UV spectra of **2** (2 mM) with CX4 (4 mM) in water at pH 2.0 upon addition of Zn²⁺ (up to 10 mM).

Additional Details on Binding Constants

By projecting a value of 4 M⁻¹ for K_2 (Scheme 3 of main text), it is possible to estimate the binding constant (K_5) of the (insignificantly populated) **1**•Zn²⁺ complex with CX4 through a thermodynamic cycle; this value is very high ($K_5 \approx 2 \times 10^6$ M⁻¹), even higher than that of protonated **1** ($K = 1 \times 10^5$ M⁻¹);^[1] the latter is only stabilized by a single positive charge. Interestingly, this 20 times larger binding constant for the “doubly charged **1**” (**1**--Zn²⁺) compared to the “singly charged **1**” (**1H⁺**) is exactly in the range one expects for a divalent compared to a monovalent cation.^[4] In other words, the affinity of Zn²⁺ to the complex CX4•**1** is 20 times larger than the affinity of H⁺, which accounts also for the observed displacement reaction in Equation (1), see main text. For comparison, in the absence of host, the binding constant of Zn²⁺ (ca. 4 M⁻¹) and H⁺ (ca. 3 M⁻¹, derived from its pK_a of 0.5)^[1] to the free guest **1** is about the same. The increased binding of Zn²⁺ to **1** in its CX4 complex provides another manifestation of the phenomenon of host-assisted metal-ligand bond formation due to the additional Coulombic interactions, which in this case favor the binding of the divalent cation (Zn²⁺) over the binding of the monovalent one (H⁺).

Description of Crystal Structures

The solid-state structure consists of CX4, metal ions, the organic guest (**1**), and lattice water. As expected from the acidic crystal growth conditions (see above), CX4 exists as a tetraanion, which is charge-balanced by two divalent transition metal ions. In the tetraanion, none of the four phenolic hydroxyl groups of CX4 is deprotonated,^[11] which allows the formation of a cyclic hydrogen-bonded network at the lower rim (O--O distance 2.70-2.74 Å) in a cone conformation. The organic guest **1** is immersed inside the CX4 cone, driven by hydrophobic interactions,^[2] and at the same time coordinated with one of its nitrogens to a transition metal ion. The bond lengths are 2.142(16) Å for Zn---N and 2.119(8) Å for Co---N, which is in the typical range of transition metal complexes incorporating nitrogen ligands.^[10,11]

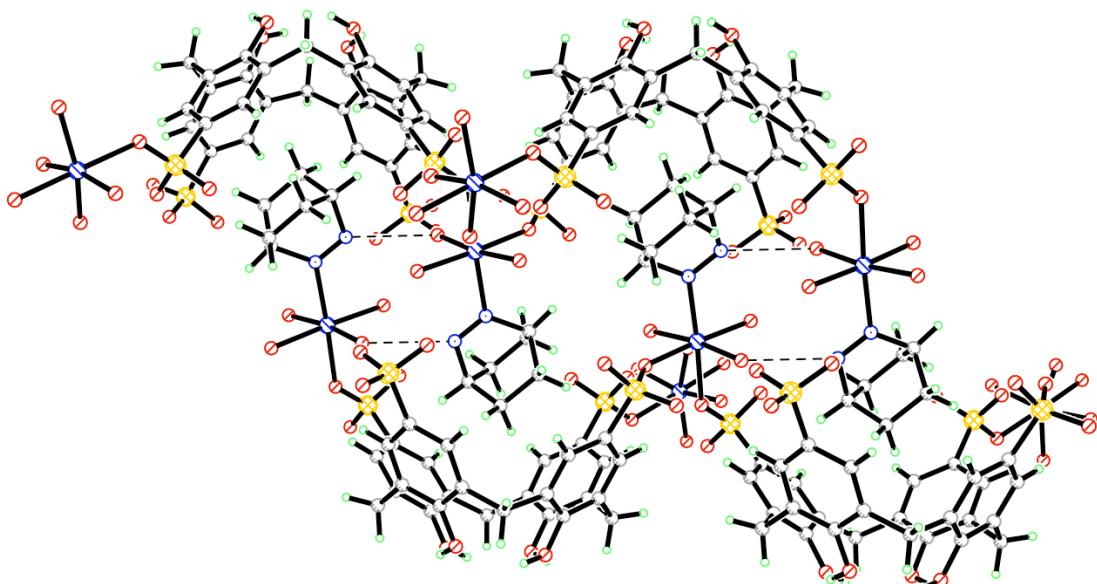


Figure S4. Bridging of two dimeric units (apparent hydrogen bonds are dashed); C atoms are grey, H green, N blue-dot, O red, S yellow, and M (Co or Zn) blue-stripe.

Important to note, the metal ion which ligates the guest immersed into a particular CX4 does not involve the sulfonato groups of the same CX4 within its first coordination sphere. Instead, it has three water molecules as ligands and it coordinates with a sulfonato group of a second CX4 to form a centrosymmetric dimer (see Figure 4a of main text). The formation of this dimer is assisted by apparent hydrogen bonds (dashed lines in the tetramer structure in Figure S4, N---O distance 2.87-2.91 Å) between the second azo nitrogen (the one not involved in the metal-ligand bond) and a water ligand of the metal ion coordinated with the organic guest immersed in the second CX4. The metal ions forming the dimeric units have a second sulfonato group

from an adjacent dimer as ligand, thereby bridging the dimers into chains (Figure S4), which arrange to layers of sheets in the *ab* plane to ultimately form an interesting metal-organic framework (Figure S5). Note that there is a second type of metal ions, which coordinate to a sulfonato group of each CX4 and five water molecules.

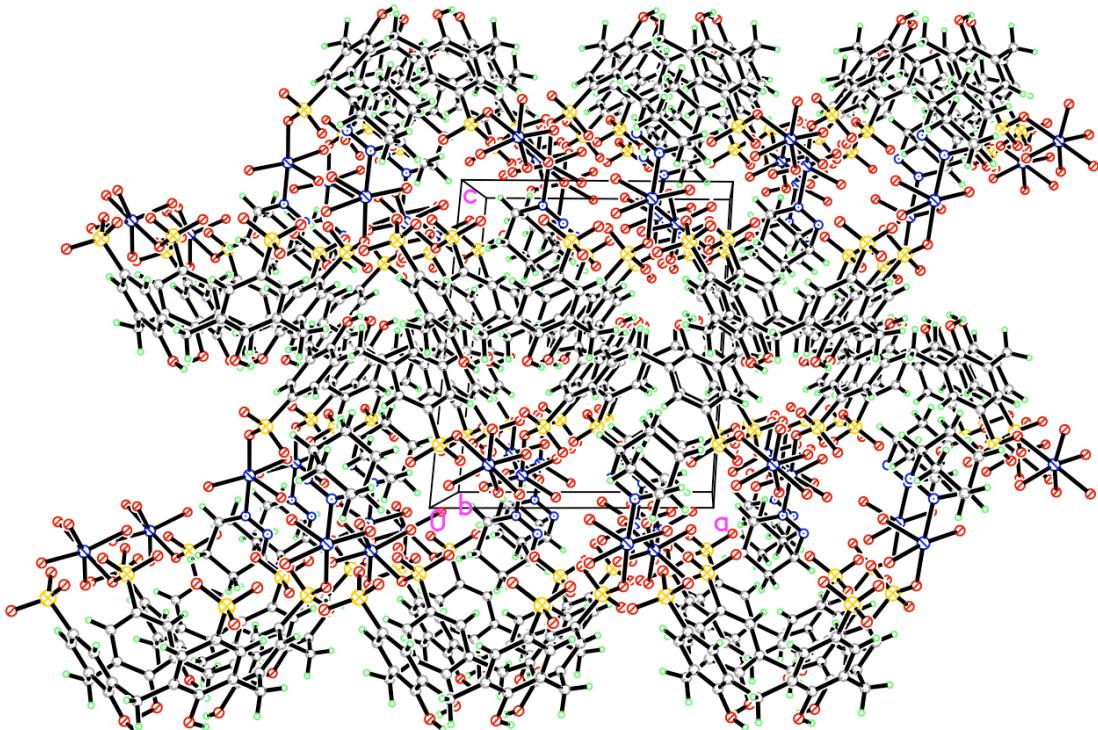


Figure S5. Packing diagram for the crystal structures, viewed down the *b* axis; C atoms are grey, H green, N blue-dot, O red, S yellow, and M (Co or Zn) blue-stripe.

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