

Article

Selective Aqueous Anion Recognition in an Anionic Host

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THE BIGGER PICTURE Molecular recognition of anions in water is an important tool for catalysis, environmental remediation and biomedical applications. Selective recognition in aqueous solution is made more challenging by the need to overcome the hydration shell of anions: hydrophobic interactions are generally minimal, and dehydration of anions is energetically unfavorable. Self-assembled metal-ligand cage complexes are known to bind anions by exploiting charge matching: cationic cages can bind anions, and this charge matching is usually necessary to allow anion recognition in water. Here we show that *anionic* water-soluble $\text{Fe}_4\text{L}_4^{4-}$ cages can bind suitably sized non-coordinating anions in the host cavity. the selectivity is dependent on external function and cavity size, and the pendant anionic groups on the cage limit anion egress from the cavity.

Our long-term research focus is the application of functionalized metal-ligand cage complexes for selective molecular recognition in water: the pendant functional groups on the cage periphery can allow control of target recognition. The near-term application of this system will be selective triggered release of anions in water, which could be achieved by cage disassembly upon addition of an internal effector, or by modification of the external groups to allow selective control of guest release upon cage reaction. This paper sets the groundwork for future catch-and-release anion binding in water, which has immediate applications in environmental remediation of toxic chemicals.

SUMMARY

Water-soluble $\text{Fe}_4\text{L}_4^{4-}$ cages can be synthesized in a multicomponent self-assembly process exploiting functionalized trigonal ligands, Fe^{II} salts and water-soluble sulfonated formylpyridine components. The cages are soluble in purely aqueous solution and display an overall 4- charge, but are capable of binding suitably sized non-coordinating anions in the host cavity despite their anionic nature. Anions such as PF_6^- or AsF_6^- occupy the internal cavity, whereas anions that are too small (BF_4^-) or too large (NTf_2^-) are not encapsulated. The external anionic charge and sterically blocked ligand cores limit the exchange rate of bound anions, as no exchange is seen over a period of weeks with the anion-filled cages, and internalization of added PF_6^- by an empty cage takes multiple weeks, despite the strong affinity of the cavity for PF_6^- ions. In the future, this recognition mechanism could be used to control release of anions for environmental applications.

Supramolecular chemistry · Self-assembly · Host-guest systems · Anion binding · Molecular recognition

INTRODUCTION

Molecular recognition in water is vital for the application of synthetic receptors in biological environments and for environmental remediation.^{1,2} Different types of substrates require variability in receptor design: molecular recognition of neutral species in water is far more effective than in organic solvents, as one can exploit hydrophobic driving forces to favor binding.^{3,4} Recognition of soft, lipophilic

cations is also very well-explored, as CH- π and cation- π forces favor recognition.⁵ However, anion recognition in water is much more challenging, as hydrophobic interactions are generally minimal, and anions (unlike metal cations) are not receptive to interactions with properly oriented lone pairs. Most importantly, dehydration of anions is energetically unfavorable, which must be compensated by strong host:anion interactions, so affinities in water are often lower than in organic solvents.^{6,7} Examples of selective anion recognition in water can be seen with rigid

cavity-containing receptors,^{8,9} self-assembling macrocycles¹⁰⁻¹³ and hosts that exploit defined cavities with properly positioned hydrogen bond donors.¹⁴⁻²⁰ Alternate strategies such as coordination to rare earth centers are also effective.²¹

An alternative method to create defined binding cavities is to use self-assembly. Self-assembled metal-ligand cage complexes are highly versatile, and have myriad applications in molecular recognition, catalysis and cargo transport, among others.²²⁻²⁵ While many complexes are restricted to organic solvents, there are a number of examples of self-assembled cages that are soluble in, and stable to water.²⁶ Assembly in water confers greater target scope for molecular recognition, as the hosts can take advantage of hydrophobic effects to bind neutral species. Aqueous hosts have often been used to bind neutral guests and soft cations such as ferrocenium or tetraalkylammonium salts: the affinity is driven by either cation- π interactions between the guest and the aromatic host walls, by favorable charge matching between cationic guests and anionic hosts, or both.^{5,27-29}

Strategies to confer water-solubility on hosts fall into three general categories: take advantage of high charge in the assembly, either cationic or anionic, to favor dissolution (as seen with Raymond's Ga_4L_6 ¹²⁻ cages,³⁰⁻³² Fujita's M_xL_y ^{$n+$} Pd-pyridyl assemblies,³³⁻³⁶ Ward's cationic Copyrindylpyrazole cages,³⁷⁻³⁸ as well as others³⁹⁻⁴¹); incorporate charged or PEG groups to the periphery of normally organic-soluble cages;⁴²⁻⁴⁵ or exploit counterion effects to drive solubility of moderately charged cages.⁴⁶⁻⁴⁹ These latter two strategies have been used by Nitschke to assemble water-soluble metal-iminopyridine cages of a variety of sizes and shapes, as well as performing a detailed analysis of the effects of cage structure and metal ion on the stability of the cages in water.⁵⁰ Despite the presence of hydrolysable iminopyridine motifs coordinated to cationic metals, these cages can show resistance to hydrolysis for months, depending on coordinating metal.

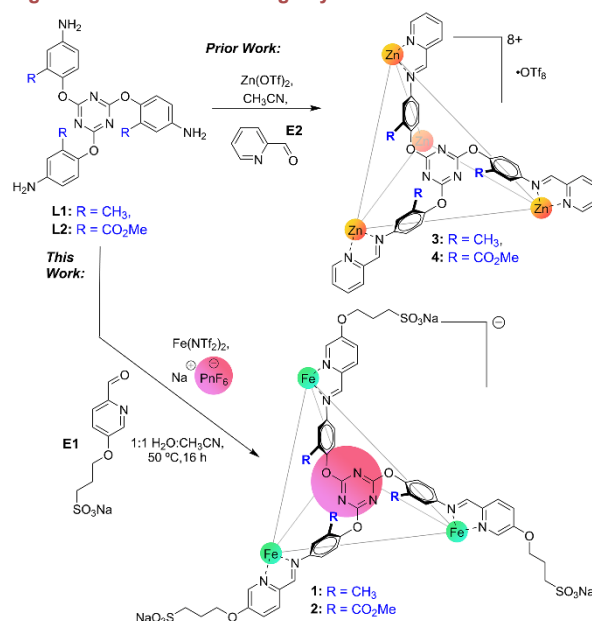
Cationic self-assembled cages are well-known to bind anions in organic solution,⁵¹ including challenging targets such as sulfate,⁵² as well as halides⁵³ and non-coordinating anions.^{54,55} There are also some examples that extend this recognition to purely aqueous solution, but they are far rarer,⁵⁵⁻⁵⁹ often requiring internally positioned H-bonding groups as well as overall cationic charge. The common anionic cages do not show affinity for anions, as might be expected.³⁰ Here, we show that an anionic receptor can strongly bind non-coordinating anions in aqueous solution, and this external negative charge acts as a barrier to guest exchange. Water-soluble Fe_4L_4 complexes can be assembled by multicomponent assembly of neutral tris-amine ligands, Fe^{2+} salts, and sulfonate-containing formylpyridines, and these overall anionic cages can bind non-coordinating anions strongly, in purely aqueous solution, with no observable guest exchange seen over weeks at ambient temperature.

RESULTS

Water-Soluble Cage Synthesis and Characterization

The first priority for aqueous anion recognition is to create hosts that are soluble in water. We have previously shown that the two ligands **L1** and **L2** (Figure 1) can be easily converted to M_4L_4 tetrahedra **3** and **4** upon multicomponent self-assembly with Zn salts and 2-formylpyridine (**E2**), and the complexes bind anions on the cage interior in CD_3CN .⁶⁰ Other work by the Kramer and Nitschke groups showed similar behavior for the unfunctionalized variants.^{61,62} Despite the 8+ charge of the Zn_4L_4 complexes, they are insoluble in water. To convert the organic-soluble complex to a water-soluble system, one could change the core ligand to incorporate solubilizing groups,⁴² modify the formylpyridine "endcap",⁶³ or exploit alternative counterions such as SO_4^{2-} .⁴⁶ In this system, two of these strategies were unsuccessful: formation of the carboxylate variant of ester **L2** proved challenging, and while self-assembly of **L1** with FeSO_4 in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ was possible, the complex proved quite sensitive, and the reaction was poorly repeatable. We therefore turned our attention to derivatizing the formylpyridine endcap. Aldehyde **E1** has been previously used to form water-soluble ML_3 fragments by Nitschke,⁶³ and was easily synthesized by combining 3-hydroxy-6-formylpyridine with propylenesultone.

Figure 1. Self-assembled Cage Synthesis

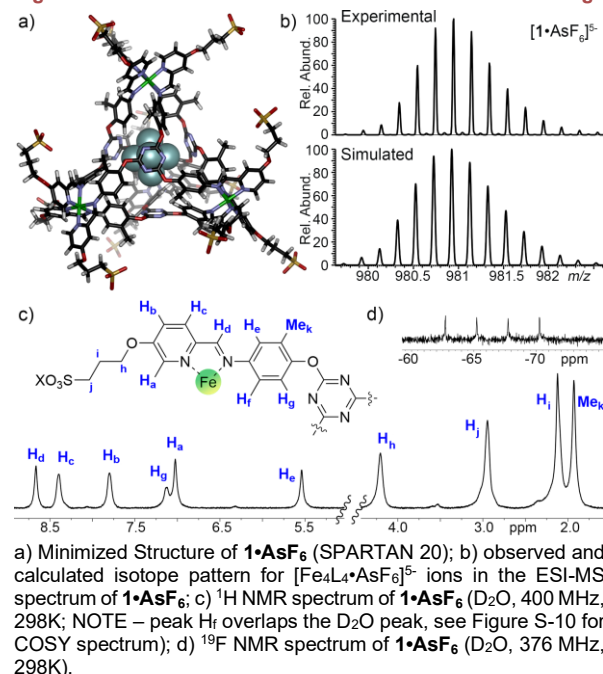


Self-assembly process for the formation of water-soluble cages **1** and **2**.

The organic components **E1** and **L1** were reacted with different Fe^{2+} salts in 1:1 $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ and heated for 50 °C for 16 h. When $\text{Fe}(\text{NTf}_2)_2$ was used, evidence of cage formation was seen, but the ^1H NMR spectrum showed multiple different products, although no unreacted components **E1** or **L1** were observed. When the process was repeated with ester ligand **E2**, there was no evidence of cage formation at all from the ^1H NMR spectrum.

However, when the syntheses were performed with $\text{Fe}(\text{NTf}_2)_2$ in the presence of AsF_6^- (10 mol.-eq of NaAsF_6 with respect to Fe^{2+}) in the reaction mixture, cage assembly was successful. When the components were reacted in a 3:1 aldehyde:ligand:metal ratio, sharp peaks for Fe_4L_4 cage **1** were seen in the product NMR, but a substantial amount of unreacted **E1** was present. The water-soluble aldehyde **E1** proved challenging to separate from the water-soluble cage **1**, so it was used as limiting reagent. When a component ratio **E1**: Fe^{2+} : **L1** = 1.5 : 1 : 1 was used, clean **1** was formed in high conversion. As can be seen in Figure 2b,c, the M_4L_4 complex **1** formed cleanly in the optimized conditions, and only one anionic species can be seen in the ^{19}F spectrum, that of bound AsF_6^- – no peaks for NTf_2^- are present (see Figures S6 – S13 for full characterization). This observation mirrored that seen with the partial formation of empty complex **1** with $\text{Fe}(\text{NTf}_2)_2$ alone – in that case, no signals for NTf_2^- were observed in the ^{19}F NMR spectrum at all. The reaction requires a mixture of 1:1 $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ to minimize decomposition of Fe^{2+} to iron oxide during the reaction: the assembly can be performed in pure water, but the mass recovery was much lower and no product was observed upon reaction in CH_3CN alone, as complex **1** is insoluble in CH_3CN .

Figure 2. Structure and Characterization of Anion-Bound Cage



As there are four Fe^{2+} cations in the cage architecture, the absence of the NTf_2^- counteranions was slightly unexpected, but the reason was quickly established by ESI-MS analysis. Both the impure sample of **1** and the pure $1\cdot\text{AsF}_6$ required negative mode to observe discrete peaks, and only negative ions were observed. Cage **1** is overall *anionic* in water – the observed charge state is 4-, indicating that all 12 sulfonate groups are anionic. The added NTf_2^- anions are evidently washed away during isolation. In the

presence of AsF_6^- , only a single peak for AsF_6^- is seen in the ^{19}F NMR, and only the mono- AsF_6 complex $1\cdot\text{AsF}_6^{5-}$ can be seen in the ESI-MS, along with some empty 1^{4-} . No evidence for any NTf_2^- or poly- AsF_6^- complexes could be seen. Acquisition of M^+ peaks from the empty **1** complex required lower spray voltage to obtain a clean spectrum, and this complex was far more prone to fragmentation (see Figures S-4 – S-5), but the only peaks for intact cage were the 1^{4-} ion, with no NTf_2^- species present.

This data suggests that suitably sized anions are bound inside the cavity of cages **1** and **2** in aqueous solution – the *anionic* host binds anions, which is certainly surprising. There are few hosts known with anionic pendant groups that are capable of binding anions in water, and they tend to be macrocycles that exploit directed H-bonds in the cavity, or show low binding affinities.^{8,64,65} We were unsuccessful in obtaining crystals that were suitable for scXRD, presumably due to the flexible arms at the periphery, but the minimized structure of $1\cdot\text{AsF}_6$ is shown in Figure 2a, illustrating the tight fit of the AsF_6^- anion in the cavity of 1^{4-} . While the binding of anions such as AsF_6^- in organic-soluble cages such as **3** or **4** in CH_3CN is known, those cages are *cationic*, and that positive charge is an important driving force for target binding: similarly sized neutral guests have a significantly lower K_a than anions.^{61,62} Encapsulating anions in aqueous solution requires overcoming the anion hydration energy, which is substantial (-71 kJ mol^{-1} for PF_6^- , -205 kJ mol^{-1} for ClO_4^- , -400 kJ mol^{-1} for SO_4^{2-}).⁶⁶⁻⁶⁸ In addition, the overall 4- charge of cage **1** provides a charge mismatch: while the localized environment of the cavity is cationic due to the Fe centers, the overall complex charge is anionic. Other examples of water-soluble cages with anionic peripheries and cationic metal centers do not bind anions in water, to our knowledge.^{30-32,55-59}

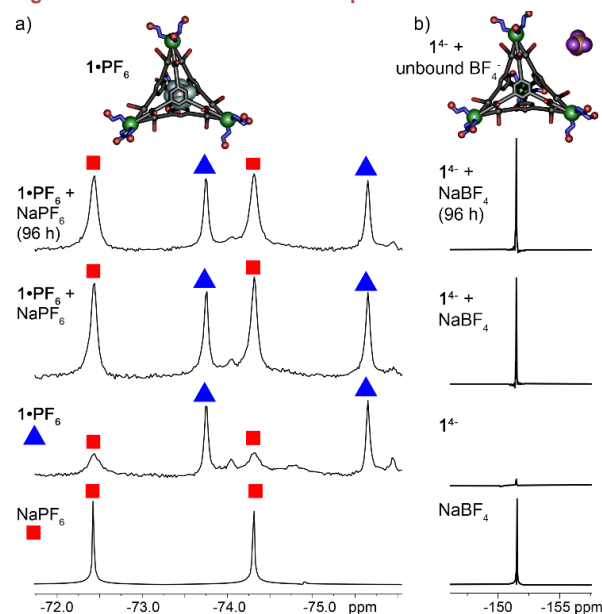
Anion-Binding Scope

The scope of the assembly process was then tested, varying the ligand (**L1** and **L2**) and added counterion, using the optimized component ratio with **E1** as limiting reagent. Ester ligand **L2** was slightly less amenable to assembly than **L1** – the empty cage **2** did not form with $\text{Fe}(\text{NTf}_2)_2$ alone, but the PF_6^- -bound complex $2\cdot\text{PF}_6$ was cleanly formed in the presence of NaPF_6 . Formation of cage **1** was successful in the presence of NaPF_6 , NaAsF_6 and NaSbF_6 in the reaction mixture and the ^1H NMR spectra of the $1\cdot\text{PnF}_6$ variants displayed identical numbers of proton peaks at very similar shifts (see Figure 2c and Figures S14 and S23, PnF_6 is used here as a collective label for PF_6^- , AsF_6^- or SbF_6^-). However, there were some noticeable differences in the broadness of the peaks, as well as in the ^{19}F spectra.

The clearest spectral evidence for internalized anions was with the PF_6^- and AsF_6^- complexes (see Figures 2, 3, S-16 and S-9). While the proton NMR signals varied only slightly, clear evidence for bound PF_6^- could be seen in the ^{19}F spectra. Two sets of ^{19}F doublets were seen, with the bound peaks 1.5 ppm upfield of the free PF_6^- (Figure 3a,

referenced to added hexafluoroisopropanol, HFIP). When NaPF_6 was added to the sample, the free PF_6^- signals were enhanced, with no change to the bound peaks. The signal for bound AsF_6^- were more challenging to determine due to the broader signals for AsF_6^- and the smaller changes in shift upon binding, but the As-coupled quartet for bound guest showed an upfield shift of 0.2 ppm. The ^{19}F spectra of SbF_6^- were unhelpful, due to the broadness and complex coupling pattern of the SbF_6^- anion, but the ^1H NMR spectra of **1**• SbF_6^- showed clear differences with the $\text{PF}_6^-/\text{AsF}_6^-$ spectra, indicating that the SbF_6^- anion is internally encapsulated – this is consistent with prior work, which indicated that SbF_6^- was the most strongly bound substrate for cages **3** and **4**.⁶⁰ In addition, the ESI-MS spectrum was very clean, showing only peaks for 1^{4-} and **1**• SbF_6^{5-} (see Figure S-25): all this data suggests that SbF_6^- is internalized in the same manner as PF_6^- or AsF_6^- .

Figure 3. Size-Selective Anion Encapsulation



a) ^{19}F NMR spectra of templated cage **1**• PF_6^- , along with spectra for cage + added NaPF_6 ; b) a) ^{19}F NMR spectra of unoccupied cage **1** with residual BF_4^- along with spectra for cage + added NaBF_4 showing no encapsulation of anion (D_2O , 298K, 376 MHz).

The assembly process was also tested with $\text{Fe}(\text{BF}_4)_2$ and $\text{Fe}(\text{PF}_6)_2$ (see Figure S63-S66). The ^1H and ^{19}F NMRs of the 1^{4-} and **1**• PF_6^- complexes formed this way showed peaks at identical shifts to the cages formed by reaction with $\text{Fe}(\text{NTf}_2)_2$ and $\text{NaBF}_4/\text{PF}_6$, although some additional line broadening was seen in the NMR spectra. This sheds light on the nature of the cations in the system – the majority of the cations upon isolation are Na^+ salts, as the added Fe^{2+} is mainly used in the assembly, although use of excess Fe^{2+} leads to residual Fe^{2+} in the system, and these paramagnetic ions broaden the NMR. Use of $\text{Fe}(\text{NTf}_2)_2/\text{NaPnF}_6$ minimizes this issue, favoring the sodium salt of the cages. There was no observed difference when KPnF_6 was substituted for NaPnF_6 .

Notably, PF_6^- , AsF_6^- and SbF_6^- are all highly similar in structure and properties, so would be expected to behave similarly. The scope of the anion binding was tested with other related anions, SO_4^{2-} , ClO_4^- and BF_4^- . These anions differ in size (slightly) from the successfully bound PnF_6^- ions, but more importantly have much higher dehydration energies. Reaction of **1** with FeSO_4 was unsuccessful, and no evidence for M_4L_4 assembly was seen in the NMR, only broad peaks for uncoordinated ligand. Successful formation of cage **1** was possible with both $\text{Fe}(\text{ClO}_4)_2$ and $\text{Fe}(\text{NTf}_2)_2/\text{NaBF}_4$. As ClO_4^- has no ^{19}F signature, obtaining unambiguous evidence for internalization was difficult without a scXRD structure, but the ESI-MS spectrum showed the same peak distribution as **1**• AsF_6^- , with only 1^{4-} and **1**• ClO_4^{5-} peaks present, indicating that ClO_4^- is likely bound in the cage. In contrast, while cage **1** could be formed in the presence of NaBF_4 , there was no evidence of the smaller BF_4^- being bound on the cage interior, as can be seen in Figure 3b. The ^1H spectrum is consistent with Fe_4L_4 cage formation, but no evidence for internalized BF_4^- was seen in the ^{19}F spectrum. A small amount of residual free BF_4^- is present, but no discrete peak for bound BF_4^- can be seen. When excess NaBF_4 was added, only one species is seen in solution, free BF_4^- . In addition, the ESI-MS analysis indicated a strong peak for the unoccupied $[\text{M}_4\text{L}_4]^{4-}$ ion, with only miniscule peaks for $[\text{M}_4\text{L}_4\cdot\text{BF}_4]^{4-}$ present (Figure S-33). This leads to a conclusion that BF_4^- is too small to bind effectively on the cage interior, whereas hydrated SO_4^{2-} is too large to effect templation: the “cutoff” for dehydration energy that can be overcome in cage templation likely lies around that of ClO_4^- (-205 kJ mol^{-1}).⁶⁶⁻⁶⁸

Anion Exchange

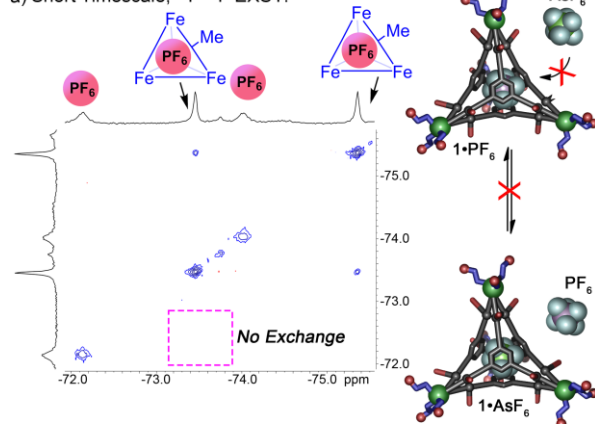
While the anionic self-assembled cages **1** and **2** can be synthesized with suitably sized anions bound on the interior, it was unclear what the effects of the peripheral anions on the cage exterior would have on the kinetics of guest exchange in solution. The organic-soluble cages **3** and **4** showed highly variable exchange properties, depending on the presence or absence of an anion on the cage interior.⁶⁰ Exchange times ranged from multiple weeks at 50°C (when one bound anion was displaced by another) to seconds at 23°C when adding anion to empty cage. The pendant functional groups on the ligands provide a blockage to guest egress and ingress, slowing exchange.

We therefore tested whether guest exchange was possible with various combinations of cages **1**•**X** and **2**•**X** in water (see Figures 4 and S-50 – S-61). As the possible exchange rates were highly variable, we performed two sets of experiments to access multiple different exchange regimes, both short (msec – sec) and long (hours – weeks). Cage **1**• PF_6^- (1 mM, D_2O), synthesized from **1**, **E1**, $\text{Fe}(\text{NTf}_2)_2$ and NaPF_6 , which contains small amounts of free PF_6^- as well as cage-bound PF_6^- , was subjected to a ^{19}F - ^{19}F EXSY experiment (Figure 4a, mixing time = 300 ms). Zero evidence of anion exchange was observed during this short-timespan experiment, indicating that if any exchange occurs, it is far slower than the EXSY timescale. Therefore, solutions of cages **1**• PF_6^- , **1**• AsF_6^- and **2**• PF_6^- (1 mM, D_2O)

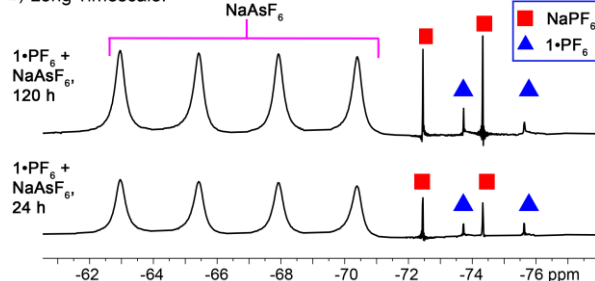
were treated with 10 mM anion (NaSbF_6 , NaAsF_6 , NaPF_6 and NaBF_4) and the systems monitored over time by both ^1H and ^{19}F NMR at ambient temperature. In all cases, no exchange was observed at all over a period of 2 weeks – no changes were seen in either the ^1H or ^{19}F spectra. The 1-PF_6 complex was also heated at 70°C for 16 h in the presence of 10 equivalents of NaSbF_6 , which did not cause any exchange. Some cage decomposition was observed at these elevated temperatures, but the intact cage retained the bound PF_6^- ion (see Figure S-57).

Figure 4. Restricted Anion Exchange in the Cage

a) Short Timescale, ^{19}F - ^{19}F EXSY:



b) Long Timescale:

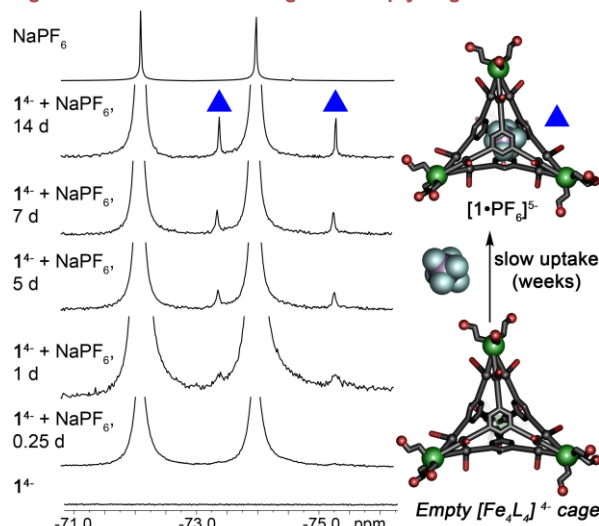


a) ^{19}F – ^{19}F EXSY spectrum of $1\text{-PF}_6 + \text{PF}_6^-$, indicating no exchange on the NMR timescale (2 mM, D_2O , 298K, 376 MHz, 300 ms mixing time); b) ^{19}F NMR spectra of 10 mM NaAsF_6 added to a solution of 1 mM $1\text{-PF}_6 + \text{PF}_6^-$ over time, indicating no exchange over a period of weeks (D_2O , 298K, 376 MHz, see Figure S-51 for full spectra).

The lack of exchange between PF_6^- and AsF_6^- is not likely to be due to one anion binding more strongly than the other, as no evidence of exchange was seen in either direction, i.e. adding PF_6^- to 1-AsF_6 , or AsF_6^- to 1-PF_6 . Evidently the egress of anion is highly restricted in this system, even more so than was observed in CD_3CN . Other guests were also tested, such as cyclohexane, which has been previously shown to bind in related M_4L_4 assemblies by Nitschke,⁵¹ but when excess cyclohexane was added to the empty 1^4 -complex in D_2O , no evidence of hydrocarbon encapsulation was seen. The ESI-MS spectra do offer some evidence of differential rates of anion release: the ratio of $[1]^4$ to $[1\text{-PnF}_6]^{5-}$ varies with anion size, with $[1\text{-PnF}_6]^{5-}$ peaks for the larger PnF_6^- ions being more prevalent (see Figures S-11, S-19 and S-25). This may suggest that the smaller

anions (e.g. PF_6^-) are more easily expelled upon Coulombic explosion in the ESI, which is somewhat consistent with the observation that small anions (e.g. BF_4^-) are not retained in aqueous solution. Even so, no expulsion of larger PnF_6^- anions was seen in solution by NMR.

Figure 5. Slow Anion Exchange into Empty Cage 1



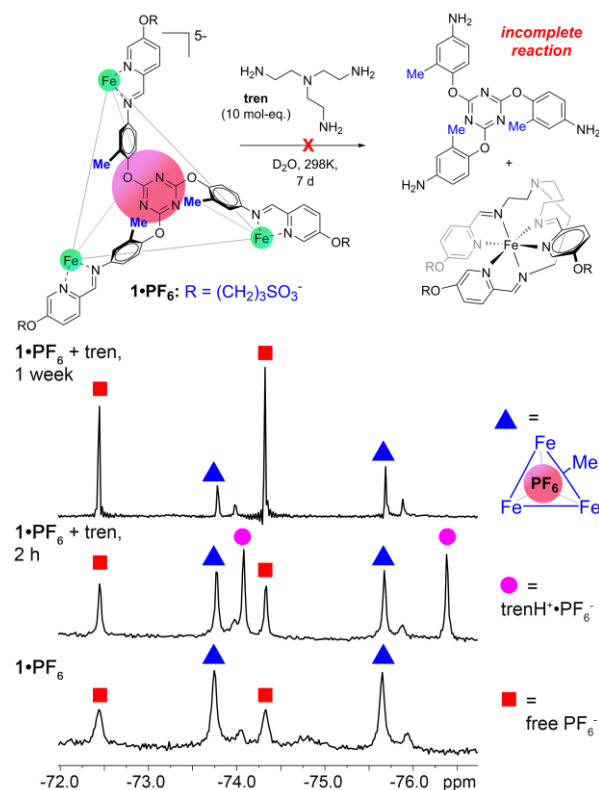
^{19}F NMR spectra of 10 mM NaPF_6 added to a solution of 1 mM 1^4 over time, indicating slow formation of 1-PF_6 over a period of weeks (D_2O , 298K, 376 MHz).

The next question was whether added anions could enter the cavity of previously synthesized cages at all, or whether the affinity was solely a templating effect in the self-assembly. The empty 1^4 -cage (1 mM) was treated with 10 mM NaPF_6 and the ^{19}F spectrum monitored over time. As can be seen in Figure 5, added PF_6^- could indeed bind in the empty 1^4 -cage, but very slowly – incomplete encapsulation was observed after 2 weeks at 23°C . This extremely slow exchange rate prevents determination of an accurate binding affinity, as equilibrium is not reached in a suitable amount of time. More forcing conditions (elevated temperature) lead to some cage decomposition, also preventing accurate analysis. However, it is clear that cage 1^4 -strongly restricts anion egress, as no loss of bound anion is seen in any of the samples tested.

Finally, we attempted to release the anions by disassembling the cage complex: excess tren (tris-(2-aminoethyl)amine, 10 mM) was added to a 1-PF_6 solution (1 mM, D_2O). The tren nucleophile is a well-precedented method of disassembling M-aminopyridine cages via transimination, allowing cargo release,⁶⁹ and the process usually occurs very rapidly. In this case, however, while some transimination occurred over a period of hours, ~50 % 1-PF_6 remained intact after 2 weeks reaction, indicating unusual stability of the anion-bound M_4L_4 cage in aqueous solution. Indeed, no solvolysis of the 1-X complexes was seen over a period of months at ambient temperature in D_2O – this is in contrast with other M_4L_6 and M_4L_4 M-aminopyridine complexes in water, which show decomposition over a period of minutes to days in aqueous

solution. The anion recognition properties of **1** are dependent on two facets: size- and shape complementarity, and anion dehydration energy. Suitably sized PF_6^- , AsF_6^- , SbF_6^- and ClO_4^- are strongly encapsulated in the cage. If the anion is too big, such as NTf_2^- , or too small, such as BF_4^- the empty cage can be formed with no encapsulated anion. Also, the strongly solvated sulfate ion $\text{SO}_4^{2-} \cdot 6\text{H}_2\text{O}$ is far too large to bind inside the cavity, despite it being of the correct size to fit on the interior after desolvation. The more weakly solvated ions can displace their waters in the assembly process, allowing recognition.

Figure 6. Cage Disassembly and Anion Release



^{19}F NMR spectra of 10 mM tris-(2-aminoethyl)amine (**tren**) added to a solution of 1mM [**1**· PF_6] $^{5-}$ over time, indicating incomplete transimination of the cage and PF_6^- release after a period of weeks (D_2O , 298K, 376 MHz).

DISCUSSION

This leads to the question of why the exchange is so slow with the fully formed assembly. Two possibilities present themselves: i) the aqueous solution could solvate the anions more strongly than in CD_3CN , thus requiring a larger desolvation penalty to pass through the portals of the host; ii) the external anionic environment could repel the entering anions, or iii) both factors are important. There is evidence for both factors: the templating anions are resistant to displacement by any other guest, be they anions of better size matching or neutral hydrophobic species. Egress of a

bound anion does not require desolvation, so this suggests a repelling effect by the anionic exterior. On the other hand, binding of PF_6^- is possible with the empty cage **1** $^{4-}$, albeit slowly, suggesting that the repulsive effect is not absolute, and anions can enter an empty cage, dependent on binding affinity. The very slow rate of this process compared to exchange in CD_3CN ⁶⁰ indicates that anion desolvation is an additional barrier to exchange in aqueous solution. It is possible that anion exchange requires decomplexation of the ligands to the Fe^{2+} centers, but this exchange mechanism is very uncommon for Fe-iminopyridine complexes⁵⁰ and the high stability of this complex in water makes it unlikely.

Figure 7. Anion Binding Mechanism

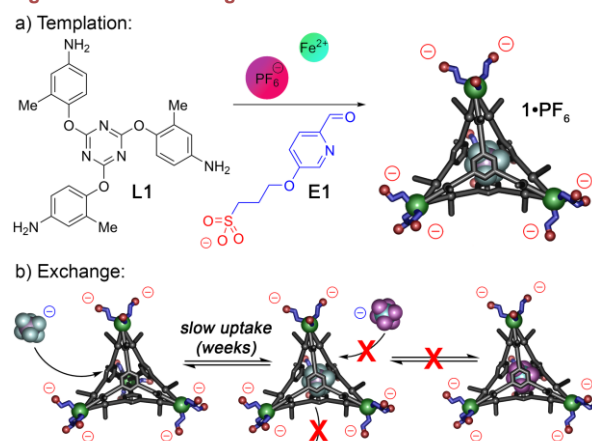


Illustration of the molecular recognition process: anions can template the formation of anionic cage **1**, but the external anionic slows guest entry and severely restricts guest egress.

Overall, the pendant functional groups on cages **1** and **2** both provide blockages to guest exchange: the ligand-centered groups act as doorstops to the revolving phenyl groups, slowing exchange when compared to the unfunctionalized variants, and the anionic groups at the periphery act as more of a “bouncer”, preventing entry except in limited circumstances. While the fully intact cages limit exchange, the templating effect occurs before assembly, so anions do not need to get past the bouncer to enter the cavity.

LIMITATIONS OF STUDY

The limitations observed in this system lie mainly in the fragility of the cages before complete self-assembly. Reaction must occur in a CH_3CN :water mix for solubility, and the free Fe^{2+} ions are prone to competitive reaction with water, depositing as iron oxide in the reaction mixture. While the cages are highly stable once formed, the accessible yield is relatively low due to this side reaction. In addition, the extremely slow exchange process makes determining binding affinities very challenging, as the system does not reach equilibrium over a period of weeks. Finally, using fluororous anions in water is challenging, as

small amounts of hydrolysis byproducts are often present and complicate NMR analysis.

Conclusion

In conclusion, we have shown that self-assembled water-soluble Fe_4L_4 cages can be synthesized by a multicomponent assembly process exploiting Fe^{2+} salts, anionic formylpyridine endcaps and trigonal functionalized tris-aniline ligands. Despite the overall 4- charge of the self-assembled cages, the lack of directed H-bonds in the interior, and the challenges of desolvating anions in aqueous solution, these anionic cages strongly bind suitably sized anions in water. Strongly solvated anions are not bound, but mildly solvated ClO_4^- are, as well as poorly solvated PF_6^- ions. The pendant anionic groups do not prevent anion binding, but they do add an additional layer of resistance to guest exchange, as no exchange can be seen between occupied cages and added anions over a period of weeks, and only very slow ingress of anions is seen with unoccupied cages: the anions act as a bouncer at the door, not letting other anions past. In addition, changing the ligand functional groups (from methyl groups in **1** to esters in **2**) significantly reduces the effectiveness of anion binding, suggesting future possibilities with these hosts for triggered, selective anion release in water.

Resource Availability

Lead Contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Prof. Richard Hooley (richard.hooley@ucr.edu).

Materials Availability

All unique/stable reagents generated in this study are available from the lead contact with a completed materials transfer agreement.

Data and Code Availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- GENERAL INFORMATION
- METHOD DETAILS
 - Synthesis of Cages
 - Mass Spectrometric Methods

SUPPLEMENTAL INFORMATION

Document S1. Figures S1–S74.

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AUTHOR CONTRIBUTIONS

N. B. Z., B. d. C. performed the synthesis and NMR characterization, C. C. performed the mass spectrometric analysis, R. R. J. and R. J. H. coordinated the project and designed the experiments. R. J. H. and N. B. Z. wrote the paper, and all authors contributed to the final draft.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Escobar, L., and Ballester, P. (2021). Molecular Recognition in Water Using Macrocyclic Synthetic Receptors. *Chem. Rev.* 121, 2445–2514. DOI: 10.1021/acs.chemrev.0c00522
2. Kubik S. (2022). When Molecules Meet in Water—Recent Contributions of Supramolecular Chemistry to the Understanding of Molecular Recognition Processes in Water. *ChemistryOpen*. 11, e202200028. DOI: 10.1002/open.202200028
3. Hof, F., Craig, S.L., Nuckolls, C., and Rebek, J. Jr. (2002). Molecular encapsulation. *Angew. Chem. Int. Ed.* 41, 1488–1508. DOI: 10.1021/ja034535e
4. Jordan, J.H., and Gibb, B.C. (2015). Molecular containers assembled through the hydrophobic effect. *Chem. Soc. Rev.* 44, 547–585. DOI: 10.1039/C4CS00191E
5. Beatty, M. A., and Hof, F. (2021). Host–guest binding in water, salty water, and biofluids: general lessons for synthetic, bio-targeted molecular recognition. *Chem. Soc. Rev.* 50, 4812–4832. DOI: 10.1039/D0CS00495B
6. Langton, M.J., Serpell, C.J., and Beer, P.D. (2016). Anion Recognition in Water: Recent Advances from a Supramolecular and Macromolecular Perspective. *Angew. Chem. Int. Ed.* 55, 1974–1987. DOI: 10.1002/anie.201506589
7. Evans, N.H., and Beer, P.D. (2014). Advances in Anion Supramolecular Chemistry: From Recognition to Chemical Applications. *Angew. Chem. Int. Ed.* 53, 11716–11754. DOI: 10.1002/anie.201309937
8. Jordan, J.H., Gibb, C.L.D., Wishard, A., Pham, T., and Gibb B. C., (2018). Ion–Hydrocarbon and/or Ion–Ion Interactions: Direct and Reverse Hofmeister Effects in a Synthetic Host. *J. Am. Chem. Soc.* 140, 4092–4099. DOI: 10.1021/jacs.8b00196
9. Sokkalingam, P., Shraberg, J., Rick, S.W., Gibb, B.C. (2016). Binding Hydrated Anions with Hydrophobic Pockets. *J. Am. Chem. Soc.* 138, 48–51. DOI: 10.1021/jacs.5b10937
10. Parks, F.C., Sheetz, E.G., Stutsman, S.R., Lutolli, A., Debnath, S., Raghavachari, K., and Flood, A.H. (2022). Revealing the Hidden Costs of Organization in Host–Guest Chemistry using Chloride-Binding Foldamers and their Solvent Dependence. *J. Am. Chem. Soc.* 144, 1274–1287. DOI: 10.1021/jacs.1c10758
11. Liu, Y., Parks, F.C., Sheetz, E.G., Chen, C.-H., and Flood, A. H. (2021). Polarity-Tolerant Chloride Binding in Foldamer Capsules by Programmed Solvent-Exclusion. *J. Am. Chem. Soc.* 143, 3191–3204. DOI: 10.1021/jacs.0c12562
12. Parks, F.C., Liu, Y., Stutsman, S.R., Debnath, S., Raghavachari, K., and Flood, A. H. (2018). Allosteric Control of Photofoldamers for Selecting between Anion Regulation and Double-to-Single Helix Switching. *J. Am. Chem. Soc.* 140, 17711–17723. DOI: 10.1021/jacs.8b10538
13. Liu, Y., Zhao, W., Chen, C.-H., and Flood, A. H. (2019). Chloride capture using a C–H hydrogen-bonding cage. *Science* 365, 159–161. DOI: 10.1126/science.aaw5145

14. Butler, S., and Jolliffe, K. (2021). Anion Receptors for the Discrimination of ATP and ADP in Biological Media. *ChemPlusChem* 86, 59–70. DOI: 10.1002/cplu.202000567
15. Berry, S.N., Qin, L., Lewis, W., and Jolliffe, K.A. (2020). Conformationally adaptable macrocyclic receptors for ditopic anions: analysis of chelate cooperativity in aqueous containing media. *Chem. Sci.* 11, 7015–7022. DOI: 10.1039/D0SC02533J
16. Borissov, A., Marques, I., Lim, J.Y.C., Félix, V., Smith, M.D., and Beer, P.D. (2019). Anion Recognition in Water by Charge-Neutral Halogen and Chalcogen Bonding Foldamer Receptors. *J. Am. Chem. Soc.* 141, 4119–4129. DOI: 10.1021/jacs.9b00148
17. Docker, A., Tse, Y.C., Tay, H.M., Taylor, A.J., Zhang, Z., and Beer, P.D. (2022). Anti-Hofmeister Anion Selectivity via a Mechanical Bond Effect in Neutral Halogen-Bonding [2]Rotaxanes. *Angew. Chem. Int. Ed.* 61, e2022145. DOI: 10.1002/anie.202214523
18. Docker, A., Marques, I., Kuhn, H., Zhang, Z., Félix, V., and Beer, P.D. (2022). Selective Potassium Chloride Recognition, Sensing, Extraction, and Transport Using a Chalcogen-Bonding Heteroditopic Receptor. *J. Am. Chem. Soc.* 144, 14778–14789. DOI: 10.1021/jacs.2c05333
19. Mosquera, J., Zarra, S., and Nitschke, J.R. (2014). Aqueous Anion Receptors through Reduction of Subcomponent Self-Assembled Structures. *Angew. Chem. Int. Ed.* 53, 1556–1559. DOI: 10.1002/anie.201308117
20. Jing, L., Deplazes, E., Clegg, J.K., and Wu, X. (2024). A charge-neutral organic cage selectively binds strongly hydrated sulfate anions in water. *Nat. Chem.* 16, 335–342. DOI: 10.1038/s41557-024-01457-5
21. Ramakrishnam Raju, M.V., Harris, S.M., and Pierre, V.C. (2020). Design and applications of metal-based molecular receptors and probes for inorganic phosphate. *Chem. Soc. Rev.* 49, 1090–1108. DOI: 10.1039/C9CS00543A
22. McTernan, C.T., Davies, J.A., and Nitschke, J.R. (2022). Beyond Platonic: How to Build Metal–Organic Polyhedra Capable of Binding Low-Symmetry, Information-Rich Molecular Cargoes. *Chem. Rev.* 122, 10393–10437. DOI: 10.1021/acs.chemrev.1c00763
23. Chakraborty, R., Mukherjee, P.S., and Stang, P.J. (2011). Supramolecular Coordination: Self-Assembly of Finite Two- and Three-Dimensional Ensembles. *Chem. Rev.* 111, 6810–6918. DOI: 10.1021/cr200077m
24. Rizzuto, F.J., von Krbek, L.K.S., and Nitschke, J. R. (2019). Strategies for binding multiple guests in metal–organic cages. *Nat. Rev. Chem.* 3, 204–222. DOI: 10.1038/s41570-019-0085-3
25. Ward, M.D., and Raithby, P.R. (2013). Functional behaviour from controlled self-assembly: challenges and prospects. *Chem. Soc. Rev.* 42, 1619–1636. DOI: 10.1039/c2cs35123d
26. Percástegui, E.G., Ronson, T.K., and Nitschke, J.R. (2020). Design and Applications of Water-Soluble Coordination Cages. *Chem. Rev.* 120, 13480–13544. DOI: 10.1021/acs.chemrev.0c00672
27. Dougherty, D. A. (2013). The Cation– π Interaction. *Acc. Chem. Res.* 46, 885–893. DOI: 10.1021/ar300265y
28. Pluth, M.D., Bergman, R.G., and Raymond, K.N. (2009). Proton-mediated chemistry and catalysis in a self-assembled supramolecular host. *Acc. Chem. Res.* 42, 1650–1659. DOI: 10.1021/ar900118t
29. Smithrud, D.B., Sanford, E.M., Chao, I., Ferguson, S.B., Carcanague, D.R., Evansek, J.D., Houk, K.N., and Diederich, F. (1990). Solvent effects in molecular recognition. *Pure Appl. Chem.* 62, 2227–2236. DOI: 10.1351/pac19906212227
30. Caulder, D.L., and Raymond, K.N. (1999). Supermolecules by Design. *Acc. Chem. Res.* 32, 975–982. DOI: 10.1021/ar970224v
31. Hong, C.M., Bergman, R.G., Raymond, K.N., and Toste, F.D. (2018). Self-Assembled Tetrahedral Hosts as Supramolecular Catalysts. *Acc. Chem. Res.* 51, 2447–2455. DOI: 10.1021/acs.accounts.8b00328
32. Bierschenk, S.M., Pan, J.Y., Settineri, N.S., Warzok, U., Bergman, R.G., Raymond, K.N., and Toste, F.D. (2022). Impact of Host Flexibility on Selectivity in a Supramolecular Host-Catalyzed Enantioselective aza-Darzens Reaction. *J. Am. Chem. Soc.* 144, 11425–11433. DOI: 10.1021/jacs.2c04182
33. Fujita, M., Tominaga, M., Hori, A., and Therrien, B. (2005). Coordination assemblies from a Pd(II)-cornered square complex. *Acc. Chem. Res.* 38, 371–380. DOI: 10.1021/ar040153h
34. Ueda, Y., Ito, H., Fujita, D., and Fujita, M. (2017). Permeable Self-Assembled Molecular Containers for Catalyst Isolation Enabling Two-Step Cascade Reactions. *J. Am. Chem. Soc.* 139, 6090–6093. DOI: 10.1021/jacs.7b02745
35. Fujita, D., Suzuki, K., Sato, S., Yagi-Utsumi, M., Yamaguchi, N., Mizuno, N., Kumasaka, T., Takata, M., Noda, S., Uchiyama, S., Kato, K., and Fujita, M. (2012). Protein encapsulation within synthetic molecular hosts. *Nat. Commun.* 3, 2093–2099. DOI: 10.1038/ncomms2093
36. Zaffaroni, R., Orth, N., Ivanović-Burmazović, I., and Reek, J.N.H. (2020). Hydrogenase Mimics in $M_{12}L_{24}$ Nanospheres to Control Overpotential and Activity in Proton-Reduction Catalysis. *Angew. Chem. Int. Ed.* 59, 18485–18489. DOI: 10.1002/anie.202008298
37. Tidmarsh, I.S., Faust, T.B., Adams, H., Harding, L.P., Russo, L., Clegg, W., and Ward, M.D. (2008). Octanuclear Cubic Coordination Cages. *J. Am. Chem. Soc.* 130, 15167–15175. DOI: 10.1021/ja805605y
38. Cullen, W., Metherell, A.J., Wrang, A.B., Taylor, C.G.P., Williams, N.H., and Ward, M.D. (2018). Catalysis in a Cationic Coordination Cage Using a Cavity-Bound Guest and Surface-Bound Anions: Inhibition, Activation, and Autocatalysis. *J. Am. Chem. Soc.* 140, 2821–2828. DOI: 10.1021/jacs.7b11334
39. He, Y. P., Yuan, L. B., Chen, G. H., Lin, Q. P., Wang, F., Zhang, L., Zhang, J. (2017). Water-Soluble and Ultrastable Ti_4L_6 Tetrahedron with Coordination Assembly Function. *J. Am. Chem. Soc.* 139, 16845–16851. DOI: 10.1021/jacs.7b09463
40. Roy, B., Zangrando, E., and Mukherjee, P.S. (2016). Self-assembly of a redox active water soluble Pd_6L_8 'molecular dice.' *Chem. Commun.* 52, 4489–4492. DOI: 10.1039/C6CC00042H
41. Liu, G., Yuan, Y.D., Wang, J., Cheng, Y., Peh, S.B., Wang, Y., Qian, Y., Dong, J., Yuan, D., and Zhao, D. (2018). Process-Tracing Study on the Post-assembly Modification of Highly Stable Zirconium Metal–Organic Cages. *J. Am. Chem. Soc.* 140, 6231–6234. DOI: 10.1021/jacs.8b03517
42. Mal, P., Schultz, D., Beyeh, K., Rissanen, K., and Nitschke, J.R. (2008). An unlockable-relockable iron cage by subcomponent self-assembly. *Angew. Chem. Int. Ed.* 47, 8297–8301. DOI: 10.1002/anie.200803066
43. Bolliger, J.L., Belenguer, A.M., and Nitschke, J.R. (2013). Enantiopure water-soluble $[Fe_4L_6]$ cages: host-guest chemistry and catalytic activity. *Angew. Chem. Int. Ed.* 52, 7958–7962. DOI: 10.1002/anie.201302136
44. Kishi, N., Li, Z., Yoza, K., Akita, M., and Yoshizawa, M. (2011). An M_2L_4 molecular capsule with an anthracene shell: encapsulation of large guests up to 1 nm. *J. Am. Chem. Soc.* 133, 11438–11441. DOI: 10.1021/ja2037029
45. Symmers, P.R., Burke, M.J., August, D.P., Thomson, P.I.T., Nichol, G.S., Warren, M.R., Campbell, C.J., and Lusby, P.J. (2015). Non-equilibrium cobalt(III) "click" capsules. *Chem. Sci.* 6, 756–760. DOI: 10.1039/C4SC03036B
46. Bolliger, J.L., Ronson, T.K., Ogawa, M., and Nitschke, J.R. (2014). Solvent Effects upon Guest Binding and Dynamics of a $Fe^II_4L_4$ Cage. *J. Am. Chem. Soc.* 136, 14545–14553. DOI: 10.1021/ja5077102
47. Percástegui, E.G., Mosquera, J., and Nitschke, J.R. (2017). Anion Exchange Renders Hydrophobic Capsules and Cargoes Water-Soluble. *Angew. Chem. Int. Ed.* 56, 9136–9140. DOI: 10.1002/anie.201705093

48. Grommet, A.B., Hoffman, J.B., Percástegui, E.G., Mosquera, J., Howe, D.J., Bolliger, J.L., and Nitschke, J.R. (2018). Anion Exchange Drives Reversible Phase Transfer of Coordination Cages and Their Cargoes. *J. Am. Chem. Soc.* **140**, 14770–14776. DOI: 10.1021/jacs.8b07900
49. Zhang, D., Ronson, T.K., Lavendomme, R., and Nitschke, J.R. (2019). Selective Separation of Polyaromatic Hydrocarbons by Phase Transfer of Coordination Cages. *J. Am. Chem. Soc.* **141**, 18949–18953. DOI: 10.1021/jacs.9b10741
50. Percástegui, E.G., Mosquera, J., Ronson, T. K., Plajer, A.J., Kieffer, M., and Nitschke, J.R. (2019). Waterproof architectures through subcomponent self-assembly. *Chem. Sci.* **10**, 2006–2018. DOI: 10.1039/C8SC05085F
51. Zhang, D., Ronson, T.K., and Nitschke, J.R. (2018). Functional Capsules via Subcomponent Self-Assembly. *Acc. Chem. Res.* **51**, 2423–2436. DOI: 10.1021/acs.accounts.8b00303
52. Custelcean, R., Bosano, J., Bonnesen, P.V., Kertesz, V., and Hay, B.P. (2009). Computer-Aided Design of a Sulfate-Encapsulating Receptor. *Angew. Chem. Int. Ed.* **48**, 4025–4029. DOI: 10.1002/anie.200900108
53. Riddell, I.A., Ronson, T.K., and Nitschke, J.R. (2015). Mutual stabilisation between M^4L_6 tetrahedra and $M^6X_4^{2-}$ metallate guests. *Chem. Sci.* **6**, 3533–3537. DOI: 10.1039/C5SC01083G
54. Lai, Y.-L., Xie, M., Zhou, X.-C., Wang, X.-Z., Zhu, X.-W., Luo, D., Zhou, X.-P., and Li, D. (2024). Precise Post-Synthetic Modification of Heterometal-Organic Capsules for Selectively Encapsulating Tetrahedral Anions. *Angew. Chem. Int. Ed.* **63**, e202402829. DOI: 10.1002/anie.202402829
55. Zhang, D., Ronson, T.K., Mosquera, J., Martinez, A., Guy, L., and Nitschke, J.R. (2017). Anion Binding in Water Drives Structural Adaptation in an Azaphosphatane-Functionalized $Fe^II_4L_4$ Tetrahedron. *J. Am. Chem. Soc.* **139**, 6574–6577. DOI: 10.1021/jacs.7b02950
56. Custelcean, R., Bonnesen, P.V., Duncan, N. C., Zhang, X., Watson, L.A., Van Berkel, G., Parson, W.B., and Hay, B.P. (2012). Urea-Functionalized M_4L_6 Cage Receptors: Anion-Templated Self-Assembly and Selective Guest Exchange in Aqueous Solutions. *J. Am. Chem. Soc.* **134**, 8525–8534. DOI: 10.1021/ja300677w
57. Sawada, T., and Fujita, M. (2010). A Single Watson–Crick G–C Base Pair in Water: Aqueous Hydrogen Bonds in Hydrophobic Cavities. *J. Am. Chem. Soc.* **132**, 7194–7201. DOI: 10.1021/ja101718c
58. Plajer, A.J., Percástegui, E.G., Santella, M., Rizzuto, F.J., Gan, Q., Laursen, B.W., and Nitschke, J.R. (2019). Fluorometric Recognition of Nucleotides within a Water-Soluble Tetrahedral Capsule. *Angew. Chem. Int. Ed.* **58**, 4200–4204. DOI: 10.1002/anie.201814149
59. Sudan, S., Chen, D.W., Berton, C., Fadaei-Tirani, F., and Severin, K. (2023). Synthetic Receptors with Micromolar Affinity for Chloride in Water. *Angew. Chem. Int. Ed.* **62**, e202218072. DOI: 10.1002/anie.202218072
60. da Camara, B., Bar Ziv, N., Carta, V., Mota Orozco, G.A., Wu, H.-T., Julian, R.R., and Hooley, R.J. (2023). Gated, Selective Anion Exchange in Functionalized Self-Assembled Cage Complexes. *Chem-Eur. J.* **e202203588**. DOI: 10.1002/chem.202203588
61. Ferguson, A., Staniland, R.W., Fitchett, C.M., Squire, M.A., Williamson, B.E., and Kruger, P.E. (2014). Variation of guest selectivity within $[Fe_4L_4]^{8+}$ tetrahedral cages through subtle modification of the face-capping ligand. *Dalton Trans.* **43**, 14550–14553. DOI: 10.1039/C4DT02337D
62. Xu, L., Zhang, D., Ronson, T.K., and Nitschke, J.R. (2020). Improved Acid Resistance of a Metal–Organic Cage Enables Cargo Release and Exchange between Hosts. *Angew. Chem. Int. Ed.* **59**, 7435–7438. DOI: 10.1002/anie.202001059
63. Ryan, H.P., Haynes, C.J.E., Smith, A., Grommet, A.B., and Nitschke, J.R. (2021). Guest Encapsulation within Surface-Adsorbed Self-Assembled Cages. *Adv. Mater.* **33**, 2004192. DOI: 10.1002/adma.202004192
64. Lisbjerg, M., Jessen, B.M., Rasmussen, B., Nielsen, B.E., Madsen, A.Ø., and Pittelkow, M. (2014). Discovery of a cyclic 6 + 6 hexamer of D-biotin and formaldehyde. *Chem. Sci.* **5**, 2647–2650. DOI: 10.1039/C4SC00990H
65. Yawer, M.A., Havel, V., and Sindelar, V. (2015). A Bambusuril Macrocyclic that Binds Anions in Water with High Affinity and Selectivity. *Angew. Chem. Int. Ed.* **54**, 276–279. DOI: 10.1002/anie.201409895
66. Liu, Y., Sengupta, A., Raghavachari, K., and Flood, A.H. (2017). Anion Binding in Solution: Beyond the Electrostatic Regime. *Chem* **3**, 411–427. DOI: 10.1016/j.chempr.2017.08.003
67. Smith, D.W. (1977). Ionic Hydration Enthalpies. *J. Chem. Educ.* **54**, 540. DOI: 10.1021/ed054p540
68. Collins, K.D. (2006). Ion hydration: Implications for cellular function, polyelectrolytes, and protein crystallization. *Biophys. Chem.* **119**, 271–281. DOI: 10.1016/j.bpc.2005.08.010
69. Castilla, A.M., Ronson, T.K., and Nitschke, J.R. (2016). Sequence-Dependent Guest Release Triggered by Orthogonal Chemical Signals. *J. Am. Chem. Soc.* **138**, 2342–2351. DOI: 10.1021/jacs.5b13016