Protein-like proton exchange in a synthetic host cavity

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The mechanism of proton exchange in a metal–ligand enzyme active site mimic (compound 1) is described through amide hydrogen–deuterium exchange kinetics. The type and ratio of cationic guest to host in solution affect the rate of isotope exchange, suggesting that the rate of exchange is driven by a host whose cavity is occupied by water. Rate constants for acid-, base-, and water-mediated proton exchange vary by orders of magnitude depending on the guest, and differ by up to 200 million-fold relative to an alanine polypeptide. These results suggest that the unusual microenvironment of the cavity of 1 can dramatically alter the reactivity of associated water by magnitudes comparable to that of enzymes.

supramolecular | noncovalent | catalysis | proton | hydrogen–deuterium exchange

Water-mediated proton transfer in molecular cavities plays an essential role in the function of nature's remarkable metabolic machinery (1, 2). Of the broad diversity of enzyme-catalyzed reactions, those involving transfer of hydrogen are ubiquitous and of high fundamental importance (3). For instance, ATP synthase employs an internal chain of water molecules to mediate proton transfer across an electrochemical gradient (4, 5). This process drives ATP synthesis, which allows organisms to broadly meet their basic energy needs. Recent studies of tunneling in enzymatic hydron transfer reactions have other far-reaching and broad implications, namely the importance of the dynamic enzyme motions in driving catalysis (6, 7).

Of the methods used to study hydron transfer, kinetic studies of the mechanisms of amide hydrogen–deuterium exchange (HDX) have helped to elucidate the dynamic interactions between water and protein surfaces that are central to the unique capabilities of enzymes (8–13). Noncovalent interactions, such as electrostatic effects and hydrogen bonding, have also been shown to exert a significant influence on amide HDX rates. These properties are manifest in amide HDX rate constants that can vary by a factor of a billion for residues on the same protein (8). How these variations arise, and how such a property could be harnessed to drive enzyme-like reactivity, remain challenging questions.

In a growing effort to emulate the efficacy of biological receptors and enzymes, studies of the preparative, guest-binding, and catalytic properties of synthetic assemblies have been pursued (14–21). Whereas guest encapsulation has been reported in a variety of media, association processes of organic guests are generally more thermodynamically favorable in water (22, 23). Central to these studies is the notion that the displacement of a high-energy water cluster from a receptor drives guest association, and in some cases, subsequent catalysis. However, despite the abundance of structural data documenting diverse water clusters encapsulated in various host cavities, mechanistic descriptions of the dynamic processes between water and host are rare (24–32).

As part of the broader field of synthetic hosts previously mentioned, our group has developed a class of biologically inspired, anionic $K_{12}Ga_4L_6$ assemblies that exhibit catalytic properties similar to those of enzymes (**1**; Figs. 1 and 2*A*) (33, 34). Assembly **1** exhibits 12 intramolecular amide hydrogen bonds which, in analogy to structurally important peptide bonds found in polypeptides, preferentially stabilize the desired tetrahedral supramolecular structure over other conformers (35). Compound **1** and related hosts have been shown to catalyze several important chemical reactions with sizable rate accelerations (up to 10^6) and unusual selectivity reminiscent of enzyme catalysis (34, 36–41). Unlike most enzymes, the reactions catalyzed by **1** are functionally and mechanistically very diverse. Despite these differences, those reactions proceeding with the largest rate enhancements in **1** all involve proton transfer steps. Spurred by this insight, we have investigated the fundamental mechanism of proton transfer in **1** through amide HDX kinetics. The purpose of these studies is twofold: first, to probe the fundamental mechanisms of acid-, base-, and water-mediated proton transfer in a host that is known to promote enzyme-like rate accelerations in acid-mediated processes; second, to explore the effect of guest binding on proton exchange, which provides insight into the interplay of solvent and guest exchange for a synthetic active site. The results of these studies are broadly relevant to the ligand–receptor binding events involving biomacromolecules and the development of synthetic enzyme mimics that emulate the utility of their biological counterparts.

Results and Discussion

Initial studies revealed a puzzling relationship between the observed rate of amide HDX and the identity of the encapsulated guest. In the absence of guest, ^{1}H NMR analysis revealed that **1** undergoes first-order amide HDX relatively rapidly, as evidenced by the loss of a single protio amide resonance at 13.1 parts per million (ppm) (100 mM phosphate buffer, pD 8.40, 25 °C). In the presence of a cationic guest, this rate decreases by a magnitude that is commensurate with the binding affinity of the

Significance

Water drives molecular interactions central to the chemistry of life. Critical to life's metabolic machinery, enzymes catalyze reactions with remarkable selectivities and efficiencies resulting from noncovalent interactions between water, substrate, and the enzyme active site. Here, amide hydrogen–deuterium exchange kinetics provide quantification of the electronic and steric factors that influence exchange between water and a highly charged host molecule whose protective outer shell and hydrophobic cavity allow it to function like an enzyme. Investigations suggest that the host's interior microenvironment reacts with encapsulated water in a manner that is correlated to the host's ability to mediate acid catalysis, thereby providing a tool for developing enzyme-like microenvironments to mediate acid- or base catalysis.

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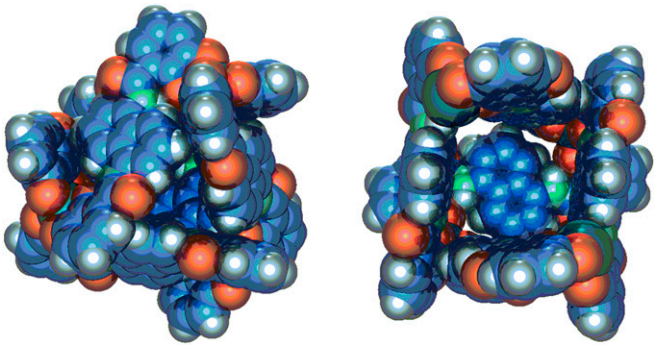


Fig. 1. (*Left*) Space-filling model of $K_{12}Ga_4L_6$ tetrahedron **1**. (*Right*) Rotated image of **1**, where a ligand has been removed to reveal the tetrahedron's interior.

host–guest pair. This relationship is represented in Fig. 3, where with excess guest, the log(k_{obs}) of amide HDX correlates linearly with log(K_a) (42) of the encapsulated guest. Thus, although it was clear that the presence of a strongly bound cationic guest inhibits host amide HDX, further investigations were necessary to explain this relationship.

To probe the influence of guest encapsulation on the mechanism of amide HDX, **1** was treated with increasing concentrations

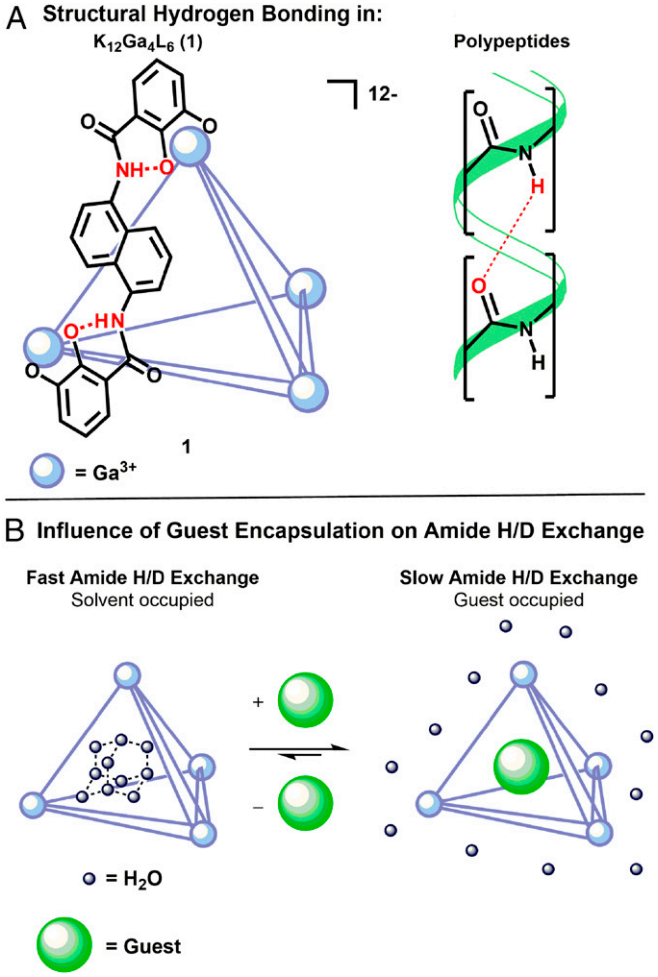


Fig. 2. (*A*) Structurally important hydrogen bonding in tetrahedron **1** (lines represent ligands and spheres represent Ga^{3+} centers), and polypeptides. (*B*) General scheme of solvent displacement from **1** by a guest molecule.

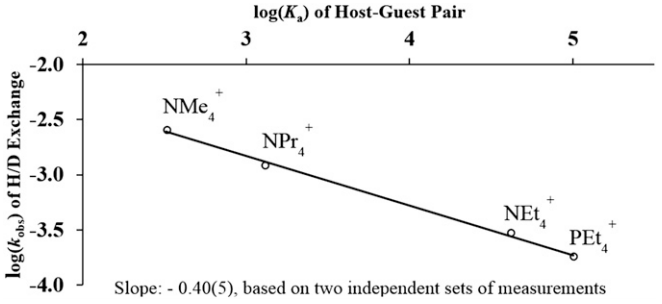


Fig. 3. Linear free-energy relationship between log(K_a) and log(k_{obs}) of amide HDX. All measurements were obtained using two equivalents of guest.

of guest, and proton exchange rates were measured (pD 8.40). In all cases, the rate of amide HDX decreased with increasing guest concentration until one equivalent of guest had been added (Fig. 4). Increasing the concentration of added guest past one equivalent did not cause as large a decrease in k_{obs}, suggesting that a saturation limit in guest had been obtained, which binds the interior of **1** and strongly inhibits proton exchange. In this manner, rate constants for HDX could be fit to $k_{obs} = k_3 f_A + k_2 f_B$, where f_A and f_B are the mole fractions of species **A** and **B**, which may be characterized by the absence or presence of encapsulated water (Fig. 5). This fitting enabled k_3 to be evaluated for G = NMe_4^+, NPr_4^+, and PEt_4^+ (see the *Supporting Information*). Based on this analysis, HDX proceeds up to 30× faster in **B** than **A**. We propose that encapsulated solvent provides a contribution to this difference (8–10 solvent molecules are expected to be encapsulated, based on thermogravimetric analysis) (42). It is also possible that HDX in the presence of excess **G** occurs simultaneously with guest exchange, as relevant guest self-exchange processes likely involve ligand distortion and occur 10–1,000× faster than the observed rates of HDX (43). Eyring analysis of HDX (**G** = PEt_4^+) revealed a large negative entropy of activation that is similar to the negative standard entropy of guest exchange and consistent with either hypothesis. The mechanism in Fig. 5 also provides a conceptual link between host–guest-influenced HDX and the Linderstrøm–Lang model of structurally hindered proton exchange by proteins (12, 13). Taken collectively, these data support a proton exchange mechanism involving encapsulated water, which can be modified by the presence of a competing guest.

To assess the extent to which acid- and base-mediated proton exchange processes are accelerated or retarded in **1**, the effects of bulk solution pD on HDX rates were investigated. Mechanistic studies have revealed that amide HDX proceeds by acid-,

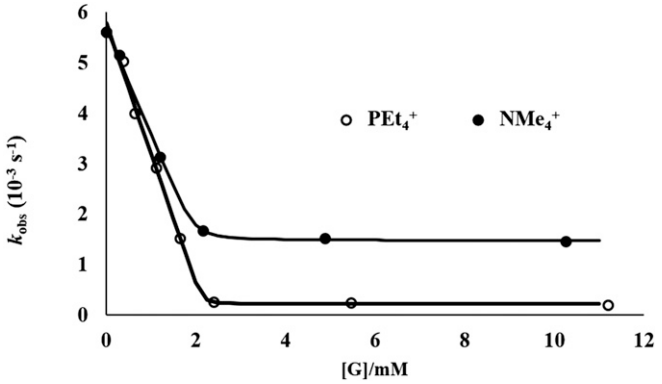
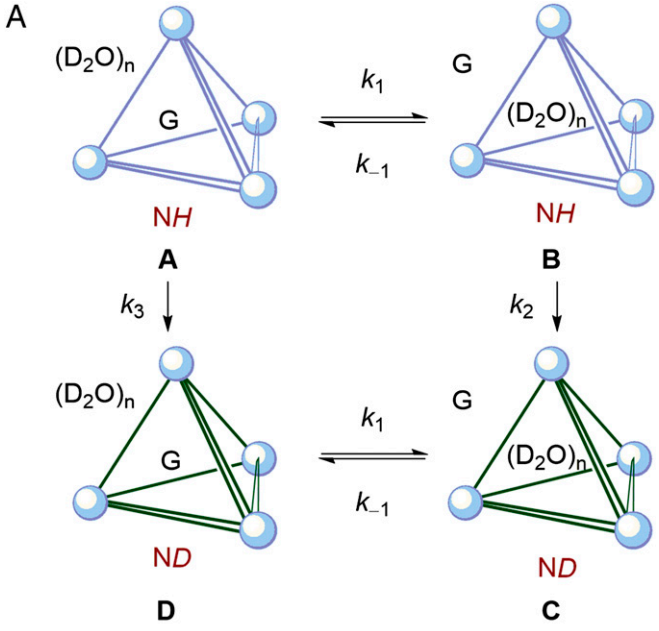


Fig. 4. Effect of increasing guest equivalents on k_{obs} of amide HDX with **G** = PEt_4^+, NMe_4^+ (pD 8.40; lines represent fits of experimental points to Eq. **1**. Error bars are omitted for clarity; see the *Supporting Information*).

base-, and to a lesser extent, water-mediated mechanisms, of which the dominant mechanism depends on bulk solution pH or pD. Experimental rate constants obtained at various pD values were fit to the logarithmic form of Eq. **2** (in Fig. 7*D*), from which rate constants for exchange catalyzed by acid (k_D), base (k_{OD}), and water (k_W) were obtained. These rate constants for host containing NMe_4^+, PEt_4^+, and solvent are tabulated in Table 1. The influences of the host are seen in the 10^5-fold acceleration of k_D in solvent-filled host, and in the deceleration of k_{OD} by 10^8-fold in PEt_4^+-filled host. For comparison, rate constants for a representative alanine polypeptide are included (44). The contributions of the various mechanisms are evident in the parabola-like relationship observed between log(k_{obs}) of amide HDX and pD (Fig. 6).

It is apparent that the rate minimum for HDX in **1** occurs at pD ~9, considerably higher than the minimum at pH ~3 for polypeptides in aqueous solution. This minimum is where the dominant mechanism of amide HDX switches from acid to base catalysis. The shift of the minimum for **1** corresponds to a 200,000-fold increase in k_D and decreases in k_{OD} [compare $(D_2O)_n$⊂**1**; Table 1] relative to a representative polypeptide. These dramatic differences can be understood in terms of the mechanisms for acid and base catalysis, shown in Fig. 7 (45). Regardless of whether the acid-catalyzed exchange proceeds via *N* protonation (more likely in a polyanionic host) or via the imidic acid, the transition state bears a positive charge. In contrast, the transition state for the base-catalyzed exchange bears a negative charge. The positive charge is stabilized and the negative charge is destabilized by electrostatics, resulting from the polyanionic nature of **1**. Similar behavior was seen in amide HDX in anionic (and cationic) micelles (9).

In the presence of a guest (NMe_4^+ or PEt_4^+), the pD-dependent curves in Fig. 5 are lowered and flattened. The data in Table 1 show that this is because both k_D and k_{OD} are decreased 40- to 100-fold for NMe_4^+ and 400- to 1,000-fold for PEt_4^+. This effect is not simply electrostatic, as it operates on both k_D and k_{OD}. Instead, when the interior of **1** is blocked by **G**, both acid- and base-mediated exchanges are retarded. We attribute this difference to a steric effect, whereby the guest interferes with the HDX, but we cannot be more specific as to how this operates.



B $$k_{obs} = k_3 f_A + k_2 f_B \quad (1)$$

Fig. 5. (*A*) General guest-dependent mechanism for amide H/D exchange at constant pH and (*B*) rate law describing this process, where f_A and f_B are the fractions of host existing as A and B, respectively, and guest self-exchange is faster than k_2, k_3 (42).

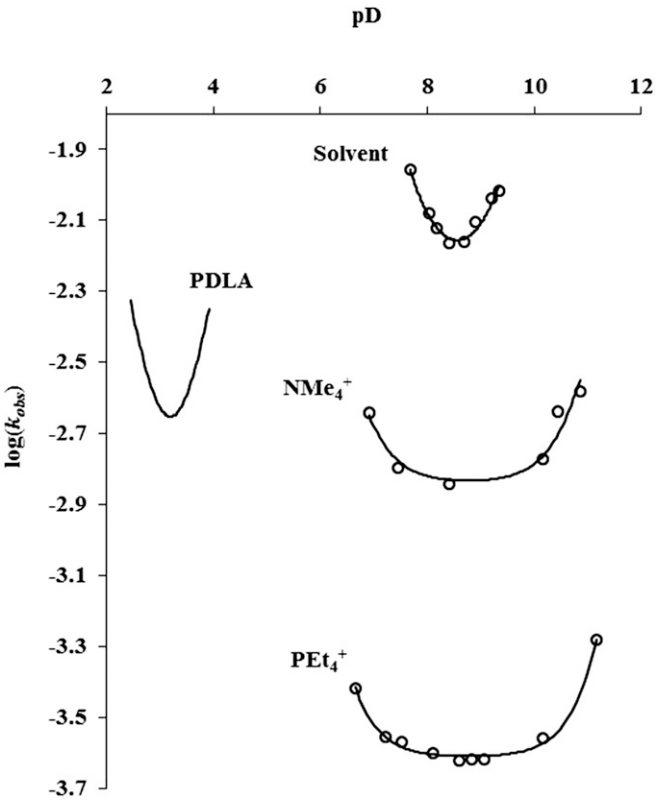


Fig. 6. Influence of bulk solution pD on k_{obs} of amide HDX. Varying the presence and type of guest affords order-of-magnitude changes in k_{obs}, whose minimum pD values are shifted up to 6 pD units relative to poly-DL-alanine (PDLA). Lines represent fits of experimental k_{obs} values to Eq. **2** (Fig. 7*D*; see Table 1 for rate constants).

It is surprising that k_W is hardly affected by the presence of **G**. The range of variation is only about 10-fold, and k_W is nearly the same as for a representative polypeptide. It is unusual for k_W to be detectable above the errors in measuring the contributions of k_D and k_{OD}. What makes detection possible in the presence of **G** is that both of those contributions are retarded relative to the water-mediated pathway, leading to curves that are flattened near the pD minimum, as can be seen in Fig. 6.

Evidence for the water-mediated pathway has historically been tenuous, which has limited the establishment of a firm mechanism. Water-mediated HDX is highly unlikely to proceed simply through N*H* removal by D_2O, as this process would have a maximum rate constant of 10^{-5} s^{-1}, based on an amide pK_a

Table 1. Rates of amide H/D exchange in 1

Rate constant	PEt_4^+⊂1	NMe_4^+⊂1	$(D_2O)_n$⊂1	PDLA*
logk_D $M^{-1}{\cdot}s^{-1}$	2.80 ± 0.03	3.81 ± 0.14	5.41 ± 0.04	0.0171
logk_{OD} $M^{-1}{\cdot}s^{-1}$	0.16 ± 0.02	1.15 ± 0.09	3.16 ± 0.04	8.48
logk_W s^{-1}	−3.62 ± 0.01	−2.84 ± 0.03	−2.25 ± 0.02	−3.04

*Rate constants (298 K) for PDLA were calculated using Arrhenius parameters from ref. 44.

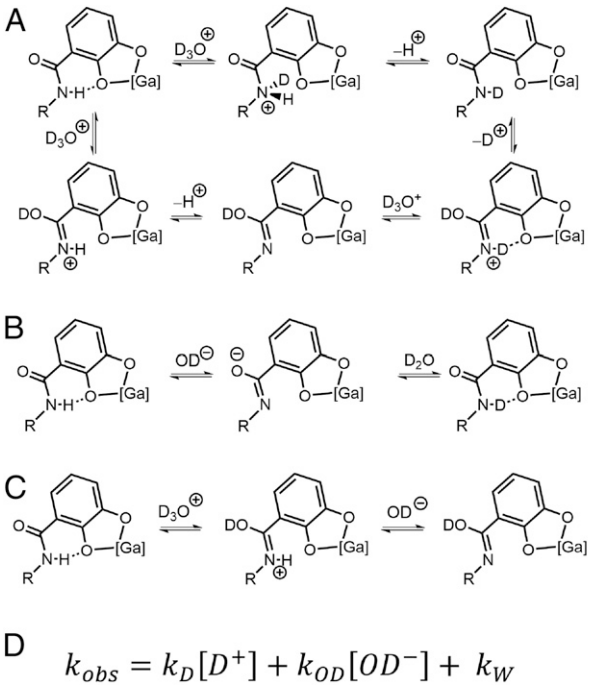


Fig. 7. Acid- (*A*), base- (*B*), and water- (*C*) mediated amide H/D exchange mechanisms, which can be described by (*D*) Eq. **2**.

of ~15 and an encounter-controlled reverse rate constant of 10^{10} $M^{-1}{\cdot}s^{-1}$. We instead propose the mechanism shown in Fig. 7*C*. Ordinarily this path too is unlikely, with a rate constant of 10^{-6} s^{-1}, based on a pK_a of about −1 for the *O*-protonated amide, a pK_W of ~15 in D_2O, and an encounter-controlled rate constant of 10^{10} $M^{-1}{\cdot}s^{-1}$ for deprotonation by OD^-. However, here the negative charge on the host can raise the pK_a of the *O*-protonated amide up to 4 units (46), and retard encounter only slightly, leading to an estimate consistent with the observed k_W. However, we cannot explain why the steric effect that retards k_D and k_{OD} is not also operative on k_W.

In summary, the observed rates of amide HDX in biologically inspired, anionic $K_{12}Ga_4L_6$ supramolecular assemblies **1** were correlated to the affinities of alkylammonium and phosphonium cations for the host interior. Guest titration experiments and modeling suggest that amide HDX proceeds through two different pathways contingent on the presence or absence of a guest, justifying this correlation. The rates of HDX catalyzed by acid and base are shifted by a factor of up to 200 million relative to those of an alanine polypeptide, an effect that we attribute to the polyanionic nature of **1**. Collectively, these experiments suggest that amide HDX occurs through encapsulation of water and that the unique electrostatics of the host dramatically alters reactivity of this transient hydrate. These results improve both our understanding of water in molecular recognition, and contributions of proton transfer to multistep acid catalysis in supramolecular cavities. Knowledge of these properties may enable the more judicious development of both synthetic and biomolecular microenvironments. More broadly, these insights are envisioned to link the fundamental processes of hydron transfer and enzyme-mimic structure, thereby providing a significant step toward the development of catalysts that rival the utility of biological enzymes.

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1. Buch-Pedersen MJ, Pedersen BP, Veierskov B, Nissen P, Palmgren MG (2009) Protons and how they are transported by proton pumps. *Pflugers Arch* 457(3):573–579.
2. Nagle JF, Tristram-Nagle S (1983) Hydrogen bonded chain mechanisms for proton conduction and proton pumping. *J Membr Biol* 74(1):1–14.
3. Liang Z-X, Klinman JP (2004) Structural bases of hydrogen tunneling in enzymes: Progress and puzzles. *Curr Opin Struct Biol* 14(6):648–655.
4. Fillingame RH (1992) H^+ transport and coupling by the F_0 sector of the ATP synthase: Insights into the molecular mechanism of function. *J Bioenerg Biomembr* 24(5):485–491.
5. Akeson M, Deamer DW (1991) Proton conductance by the gramicidin water wire. Model for proton conductance in the F_1F_0 ATPases? *Biophys J* 60(1):101–109.
6. Klinman JP, Kohen A (2013) Hydrogen tunneling links protein dynamics to enzyme catalysis. *Annu Rev Biochem* 82:471–496.
7. Knapp MJ, Klinman JP (2002) Environmentally coupled hydrogen tunneling. Linking catalysis to dynamics. *Eur J Biochem* 269(13):3113–3121.
8. Anderson JS, Hernández G, Lemaster DM (2008) A billion-fold range in acidity for the solvent-exposed amides of Pyrococcus furiosus rubredoxin. *Biochemistry* 47(23): 6178–6188.
9. Perrin CL, Chen J-H, Ohta BK (1999) Amide proton exchange in micelles. *J Am Chem Soc* 121(11):2448–2455.
10. Leichtling BH, Klotz IM (1966) Catalysis of hydrogen-deuterium exchange in polypeptides. *Biochemistry (Mosc)* 5(12):4026–4037.
11. Zhang Z, Smith DL (1993) Determination of amide hydrogen exchange by mass spectrometry: A new tool for protein structure elucidation. *Protein Sci* 2(4):522–531.
12. Englander SW, Mayne L, Bai Y, Sosnick TR (1997) Hydrogen exchange: The modern legacy of Linderstrøm-Lang. *Protein Sci* 6(5):1101–1109.
13. Skinner JJ, Lim WK, Bédard S, Black BE, Englander SW (2012) Protein dynamics viewed by hydrogen exchange. *Protein Sci* 21(7):996–1005.
14. Ariga K, et al. (2008) Challenges and breakthroughs in recent research on self-assembly. *Sci Technol Adv Mater* 9(1):014109.
15. Kahsar KR, Schwartz DK, Medlin JW (2014) Control of metal catalyst selectivity through specific noncovalent molecular interactions. *J Am Chem Soc* 136(1):520–526.
16. Soberats B, Sanna E, Martorell G, Rotger C, Costa A (2014) Programmed enzyme-mimic hydrolysis of a choline carbonate by a metal-free 2-aminobenzimidazole-based cavitand. *Org Lett* 16(3):840–843.
17. Oshovsky GV, Reinhoudt DN, Verboom W (2007) Supramolecular chemistry in water. *Angew Chem Int Ed Engl* 46(14):2366–2393.
18. Breslow R (1982) Artificial enzymes. *Science* 218(4572):532–537.
19. Breslow R (1995) Biomimetic chemistry and artificial enzymes: Catalysis by design. *Acc Chem Res* 28(3):146–153.
20. Murase T, Nishijima Y, Fujita M (2012) Cage-catalyzed Knoevenagel condensation under neutral conditions in water. *J Am Chem Soc* 134(1):162–164.
21. Habicher T, Diederich F, Gramlich V (1999) Catalytic dendrophanes as enzyme mimics: Synthesis, binding properties, micropolarity effect, and catalytic activity of dendritic thiazolio-cyclophanes. *Helv Chim Acta* 82(7):1066–1095.
22. Houk KN, Leach AG, Kim SP, Zhang X (2003) Binding affinities of host-guest, protein-ligand, and protein-transition-state complexes. *Angew Chem Int Ed Engl* 42(40):4872–4897.
23. Leung DH, Bergman RG, Raymond KN (2008) Enthalpy-entropy compensation reveals solvent reorganization as a driving force for supramolecular encapsulation in water. *J Am Chem Soc* 130(9):2798–2805.
24. Wang L-Y, Yang Y, Liu K, Li B-L, Zhang Y (2008) A new "opened-cube" $(H_2O)_{10}$ cluster and undulated water chain in porous metal–organic frameworks. *Cryst Growth Des* 8(11):3902–3904.
25. Lakshminarayanan PS, Suresh E, Ghosh P (2006) A hybrid water-chloride structure with discrete undecameric water moieties self-assembled in a heptaprotonated octaamino cryptand. *Angew Chem Int Ed Engl* 45(23):3807–3811.
26. Hazra A, Kanoo P, Mohapatra S, Mostafa G, Maji TK (2010) A flexible supramolecular host with a crowned chair octameric water cluster and highly selective adsorption properties. *CrystEngComm* 12(10):2775.
27. Li Y, Jiang L, Feng X-L, Lu T-B (2008) Anion dependent water clusters encapsulated inside a cryptand cavity. *Cryst Growth Des* 8(10):3689–3694.
28. Mandal S, et al. (2010) An unusual $(H_{(2)}O)_{(20)}$ discrete water cluster in the supramolecular host of a charge transfer platinum(ii) complex: Cytotoxicity and DNA cleavage activities. *Dalton Trans* 39(40):9514–9522.
29. Kurotobi K, Murata Y (2011) A single molecule of water encapsulated in fullerene C_{60}. *Science* 333(6042):613–616.
30. Wang X, Lin H, Mu B, Tian A, Liu G (2010) Encapsulation of discrete $(H_{(2)}O)_{(12)}$ clusters in a 3D three-fold interpenetrating metal-organic framework host with (3,4)-connected topology. *Dalton Trans* 39(27):6187–6189.
31. Yoshizawa M, et al. (2005) Endohedral clusterization of ten water molecules into a "molecular ice"within the hydrophobic pocket of a self-assembled cage. *J Am Chem Soc* 127(9):2798–2799.
32. Massera C, Melegari M, Ugozzoli F, Dalcanale E (2010) Formation of tetrameric water clusters driven by a cavitand template. *Chem Commun (Camb)* 46(1):88–90.
33. Caulder DL, Powers RE, Parac TN, Raymond KN (1998) The self-assembly of a predesigned tetrahedral M_4L_6 supramolecular cluster. *Angew Chem Int Ed* 37(13-14): 1840–1843.

34. Zhao C, et al. (2013) Chiral amide directed assembly of a diastereo- and enantiopure supramolecular host and its application to enantioselective catalysis of neutral substrates. *J Am Chem Soc* 135(50):18802–18805.
35. Janser I, Albrecht M, Hunger K, Burk S, Rissanen K (2006) Formation of triple-stranded dinuclear helicates with dicatecholimine ligands: The influence of steric hindrance at the spacer. *Eur J Inorg Chem* 2006(1):244–251.
36. Zhao C, Toste FD, Raymond KN, Bergman RG (2014) Nucleophilic substitution catalyzed by a supramolecular cavity proceeds with retention of absolute stereochemistry. *J Am Chem Soc* 136(41):14409–14412.
37. Hart-Cooper WM, et al. (2015) The effect of host structure on the selectivity and mechanism of supramolecular catalysis of Prins cyclizations. *Chem Sci (Camb)* 6(2):1383–1393.
38. Hart-Cooper WM, Clary KN, Toste FD, Bergman RG, Raymond KN (2012) Selective monoterpene-like cyclization reactions achieved by water exclusion from reactive intermediates in a supramolecular catalyst. *J Am Chem Soc* 134(43):17873–17876.
39. Pluth MD, Bergman RG, Raymond KN (2009) Proton-mediated chemistry and catalysis in a self-assembled supramolecular host. *Acc Chem Res* 42(10):1650–1659.
40. Fiedler D, van Halbeek H, Bergman RG, Raymond KN (2006) Supramolecular catalysis of unimolecular rearrangements: Substrate scope and mechanistic insights. *J Am Chem Soc* 128(31):10240–10252.
41. Hastings CJ, Pluth MD, Bergman RG, Raymond KN (2010) Enzymelike catalysis of the Nazarov cyclization by supramolecular encapsulation. *J Am Chem Soc* 132(20):6938–6940.
42. Sgarlata C, et al. (2010) External and internal guest binding of a highly charged supramolecular host in water: Deconvoluting the very different thermodynamics. *J Am Chem Soc* 132(3):1005–1009.
43. Davis AV, et al. (2006) Guest exchange dynamics in an M_4L_6 tetrahedral host. *J Am Chem Soc* 128(4):1324–1333.
44. Bai Y, Milne JS, Mayne L, Englander SW (1993) Primary structure effects on peptide group hydrogen exchange. *Proteins* 17(1):75–86.
45. Perrin CL (1989) Proton exchange in amides: Surprises from simple systems. *Acc Chem Res* 22(8):268–275.
46. Pluth MD, Bergman RG, Raymond KN (2007) Making amines strong bases: Thermodynamic stabilization of protonated guests in a highly-charged supramolecular host1. *J Am Chem Soc* 129(37):11459–11467.
47. Glasoe PK, Long FA (1960) Use of glass electrodes to measure acidities in deuterium oxide. *J Phys Chem* 64(1):188–190.
48. Covington AK, Robinson RA, Bates RG (1966) The ionization constant of deuterium oxide from 5 to 50°. *J Phys Chem* 70(12):3820–3824.