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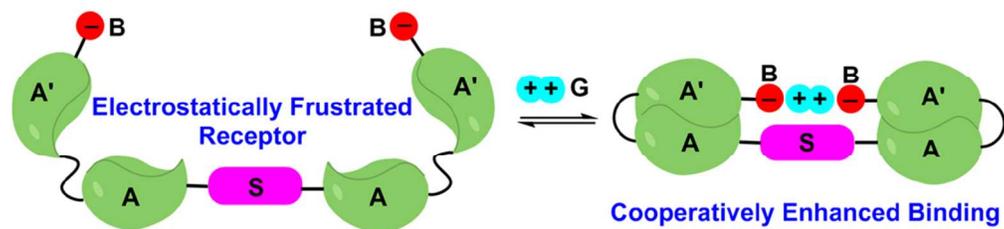
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# Rationally Designed Cooperatively Enhanced Receptors to Magnify Host–Guest Binding in Water

Roshan W. Gunasekara and Yan Zhao\*

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111

## Supporting Information Placeholder

**ABSTRACT:** When disengaged interactions within a receptor are turned on by its guest, these intrahost interactions will contribute to the overall binding energy. Although such receptors are common in biology, their synthetic mimics are rare and difficult to design. By engineering conflictory requirements between intrareceptor electrostatic and hydrophobic interactions, we enabled complementary guests to eliminate the “electrostatic frustration” within the host and turn on the intrahost interactions. The result was a binding constant of  $K_a > 10^5 \text{ M}^{-1}$  from ammonium–carboxylate salt bridges that typically function poorly in water. These cooperatively enhanced receptors displayed excellent selectivity in binding, despite a large degree of conformational flexibility in the structure.

## Introduction

Biological hosts have extraordinary abilities to recognize and bind guests in competitive aqueous environments, even well solvated hydrophilic small molecules whose binding is not expected to gain much binding enthalpy. A survey of biological and synthetic host–guest complexes by Houk et al. over a decade ago revealed a large gap between the two groups of receptors: whereas nanomolar or stronger affinities are frequently seen in the former, millimolar affinities represent the average for the latter.<sup>1</sup> Chemists indeed developed extremely tight binders in isolated cases;<sup>2–4</sup> such, nonetheless, remain as rare exceptions to the norm in synthetic supramolecular chemistry.

Interestingly, evident from the large number of tight-binding drugs developed for bioreceptors, there seems to be no fundamental deficiency in chemists’ ability to construct tight-binding guests for biological hosts. If this is indeed the case, the “deficiency” of synthetic host–guest complexes likely lies in the receptors that admittedly are less complex and smaller in size in comparison to common biological hosts.

The majority of synthetic receptors have been created using the concept of preorganization.<sup>5,6</sup> The concept played vital roles in the development of supramolecular chemistry in the last decades.<sup>7–22</sup> More recently, however, an increasing number of chemists began to wonder whether alternative strategies exist in constructing tight-binding receptors.<sup>23–28</sup> Since bioreceptors are often made of flexible peptide chains with rich conformational dynamics even in the folded state, it seems flexibility cannot be inherently detrimental to high binding affinity. Not only so, flexible bioreceptors must have

effective strategies to overcome the problem of negative conformational entropy when they tighten up in the presence of their guests.<sup>29</sup>

After studying protein and other naturally occurring receptors, Williams and co-workers proposed an interesting postulation that the driving force for guest-binding does not all have to come from direct host–guest interactions but may derive from cooperative strengthening of existing interactions within the host.<sup>23</sup> Essentially, *binding in bioreceptors can be delocalized over the entire structure, not confined at the host–guest interface.*

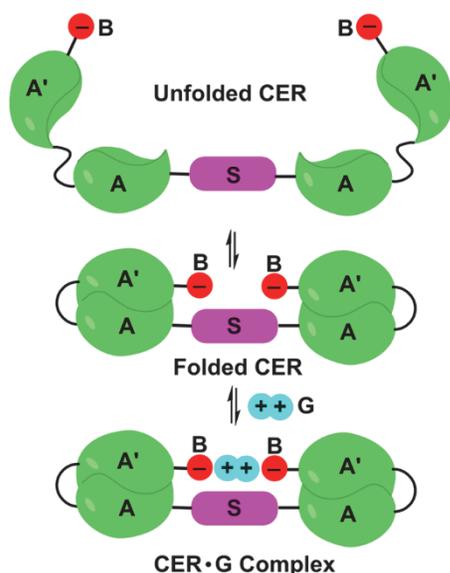
Delocalized binding in cooperatively enhanced receptors (CERs) has indeed been realized in several synthetic receptors. Kubik, Otto, and co-workers prepared a peptidic bismacrocyclic anion receptor whose hydrophobic interactions between the two macrocycles assisted the anion binding.<sup>30</sup> Carrillo et al. reported a crown ether-like macrocycle in which a remote intrahost hydrogen bond strengthened the binding of aromatic amino acid ester in an enantioselective fashion.<sup>27</sup> Our group reported an oligocholate foldamer host that exhibited strong cooperativity between the host conformation and guest binding, with the strongest binding occurring at the folding–unfolding transition.<sup>31</sup>

CERs essentially utilize the positive cooperativity between intrahost interactions and (direct) host–guest interactions to reinforce their guest-binding. An exciting implication of such receptors is that high binding affinity can be obtained even from weak (direct) binding forces, as long as sufficient intrahost interactions can be triggered by the guest. Unfortunately, despite the attractiveness and huge potential of such receptors, their rational design represents a formidable task. While preorganization gives chemists a clear path to follow in designing guest-complementary receptors, cooperative enhancement seems more of a rationale for existing phenomena as it stands. Even for the above mentioned synthetic CERs, their discovery appeared to be by accident rather than by design.

In this paper, we report a rational design of CERs that operate in aqueous solution. Weak ammonium–carboxylate salt bridges were enhanced by hydrophobic interactions within the receptor to afford strong binding in water. The key design of the system centers on the “electrostatically frustrated” intrahost interactions that could be strengthened by a suitable guest. Not only strong binding was obtained in water from relatively weak binding forces, excellent selectivity was also achieved for a highly flexible receptor.

## Results and Discussion

**Design of CERs.** As shown by Scheme 1, our CER consists of a central scaffold (**S**) to which two insulated “folding arms” are attached. Each arm can fold upon itself by the intrahost **A–A'** interactions. The two binding functionalities (**B**) are designed to be far apart in the unfolded CER but in proximity in the folded conformer. As a result, the electrostatic interactions between the two negatively charged **B**'s are in conflict with the **A–A'** interactions in the folded conformer and thus interfere with the folding. When a suitable, oppositely charged guest (**G**) binds, it engages direct electrostatic interactions and, more importantly, by neutralizing the electrostatically repelled **B**'s, strengthens the intrahost **A–A'** interactions. In this way, the formerly “frustrated” intrareceptor hydrophobic interactions are “turned on” by the guest and will contribute to the binding energetically. As will be shown by our study, the CER does not have to be fully unfolded prior to binding to be operative. As long as the intrahost **A–A'** interactions are not fully engaged before the CER binds the guest, they could contribute to the binding. Similar to biological CERs, the system has the “binding interactions” delocalized over much of the entire structure, with remote **A–A'** interactions being utilized to magnify the direct binding forces at the **B–G–B** interface.



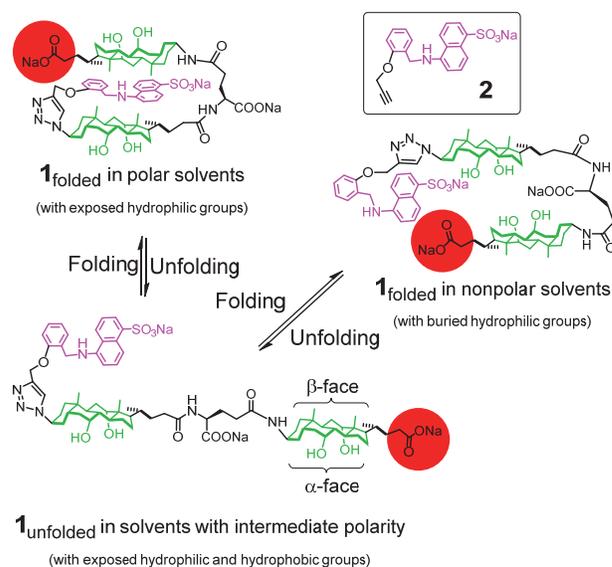
**Scheme 1.** Design of an electrostatically frustrated CER and its binding of an oppositely charged ligand to trigger intrahost **A–A'** interactions.

Notably, the CER is highly flexible by design. The guest-induced conformational change is strategically utilized instead of being avoided as in typical preorganized systems. Yet, because the optimal guest needs to match the **B–B** distance in the *folded* CER both electrostatically and geometrically, a strong binding selectivity may still be possible despite the flexibility.

**Synthesis and Conformational Study.** To realize the above design, we first synthesized bischolate **1** as the folding arm, with a fluorescent label to study its folding/unfolding (Scheme 2). Our group has a long interest in cholate foldamers except that the earlier examples had their monomers joined by amide groups on the hydrophilic  $\alpha$ -face of the cholate.<sup>32–34</sup> Because the two cholates in **1** need to interact through hydrophobic interactions of the  $\beta$ -faces in water, we connected the cholates by the  $\beta$ -amino group, with a flexible

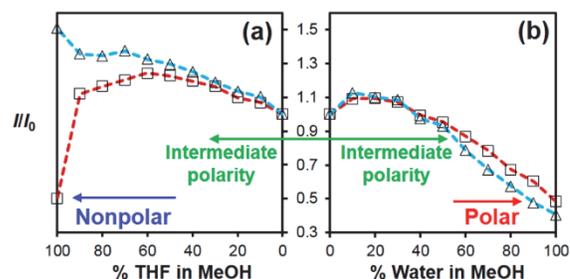
glutamic acid tether to facilitate the choate–choate interaction. Our previous work shows that a C4 tether in between two cholates allows the facial amphiphiles to interact with each other fairly easily.<sup>35</sup> In Scheme 2, the terminal carboxylate (highlighted by the red circle) corresponds to the binding functionality **B** in Scheme 1 and the two cholates are essentially **A** and **A'**, respectively.

The synthesis of **1** followed standard chemistry employed in other oligocholate synthesis<sup>32</sup> and can be found in the Supporting Information. Our synthesis left an azido group on the cholate, which made it convenient to label the arm with an environmentally sensitive fluorophore (**2**) using click chemistry.



**Scheme 2.** Idealized folding of bischolate foldamer **1** in polar and nonpolar solvents.

Figure 1 shows the maximum emission intensity of compounds **1** ( $\square$ ) and **2** ( $\triangle$ ) in two solvent mixtures. The intensity of each compound was normalized to the emission of the same compound in methanol so that the two compounds can be better compared. The x-axes are drawn such that the solvent polarity increases continuously from left to right all the way across Figure 1a,b.



**Figure 1.** Maximum emission intensity of bischolate **1** ( $\square$ ) and control compound **2** ( $\triangle$ ) normalized to the intensity of the same compound in methanol as a function of solvent composition in (a) THF/methanol and (b) water/methanol mixtures. The data points are connected by colored lines to guide the eye.  $\lambda_{\text{exc}} = 340$  nm. [Compound] = 2.0  $\mu\text{M}$ .

According to Figure 1, the two compounds responded to solvent polarity similarly at intermediate polarity, evident from the nearly overlapping  $I/I_0$  curves in between 30% THF/methanol and 50% methanol/water (indicated by the green arrow). However, the curves deviated from each other when the solvents became either more polar or less polar. Importantly, as the  $I/I_0$  curves moved apart, **1** ( $\square$ ) had stronger (normalized) emission than **2** ( $\triangle$ ) toward the polar end but weaker emission toward the nonpolar end.

The aminonaphthalene sulfonate in **1** and **2** is an analogue of the more common fluorophore dansyl, which emits strongly in nonpolar environments and weakly in polar ones.<sup>36</sup> Since a similar effect was operating in **2**, the stronger-than-usual emission of **1** in the most polar solvents suggests that its fluorophore has a higher environmental hydrophobicity than **2** in the most polar solvents, and vice versa in the most nonpolar solvents. This kind of crossing-over in solvent response was identical to what was observed in our cholate-based molecular baskets, which adopted a micelle-like conformation (with exposed hydrophilic faces) in polar solvents and reverse-micelle-like conformation (with buried hydrophilic faces) in nonpolar solvents.<sup>37,38</sup> Conceivably, as **1** folded in polar solvents via the hydrophobic cholate–cholate interactions (Scheme 2), the fluorophore was sensing the hydrophobic local environment and thus emitted more strongly than the control compound. When **1** folded in nonpolar solvents (in THF with low methanol), the hydrophilic faces turned inward, with the many polar groups toward the center of the molecule concentrating methanol near the fluorophore—this type of solvent-induced conformational change has been observed multiple times for both cholate foldamers<sup>32,38</sup> and nonfoldamers<sup>37,38</sup> under similar conditions.

Since the bischolate arm seemed to operate as intended, we prepared CER **3** by clicking three such arms (**5**) to 1,3,5-triethylbenzene. A control compound **4** was similarly prepared to help us understand the conformation of **3**. We chose the rigid trisubstituted benzene as the central scaffold so that the bischolate arms are separated or “insulated” from one another. Clearly, we did not want cholate–cholate interactions to occur across different arms.

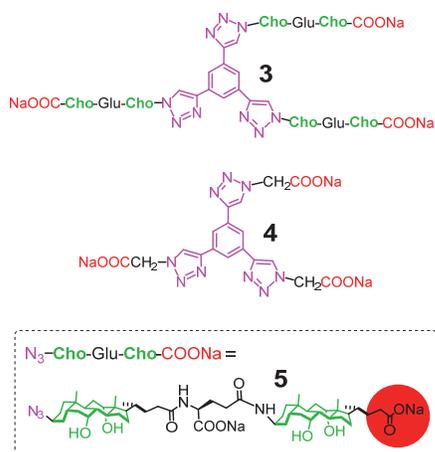
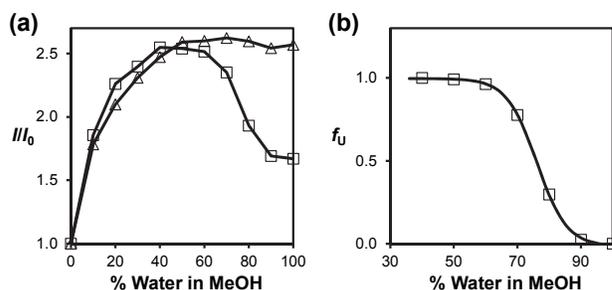


Figure 2a shows the  $I/I_0$  curves of **3** and **4**. We focused on the polar side of the solvent scale (i.e., methanol/water mixtures), as the receptor was designed to function in water through the hydrophobic interactions of the  $\beta$ -cholates. Remarkably, the curves for **3** and **4** once again nearly overlapped in <50% water/methanol but moved apart as the solvent became more polar, similar to what happened to **1** and

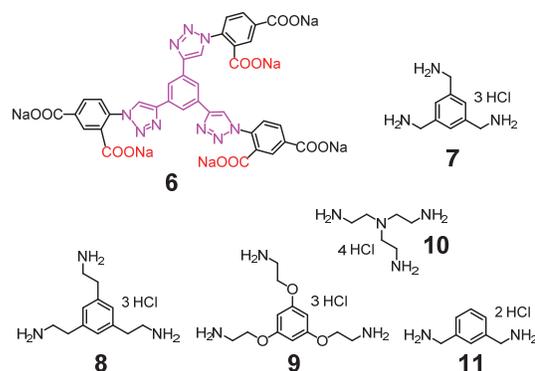
**2** in Figure 1b. Intermolecular aggregation was ruled out by dilution studies. More importantly, the fluorescence in >50% water/methanol displayed a sigmoidal transition, a hallmark of cooperative conformational change.<sup>39,40</sup> The data fit almost perfectly to a two-state unfolding–folding transition model (Figure 2b) that is characteristic of many proteins<sup>41</sup> and solvophobic foldamers,<sup>32,42</sup> suggesting that the proposed cooperative folding indeed was operating.



**Figure 2.** (a) Maximum emission intensity of “3-armed” **3** ( $\square$ ) and control compound **4** ( $\triangle$ ) normalized to the intensity of the same compound in methanol as a function of solvent composition.  $\lambda_{\text{ex}} = 240$  nm. [Compound] = 2.0  $\mu\text{M}$ . (b) Non-linear least squares curve fitting of the fluorescence data of **3** in  $\geq 40\%$  water/methanol to a two-state transition model, showing the fraction of unfolded conformer as a function of solvent composition.

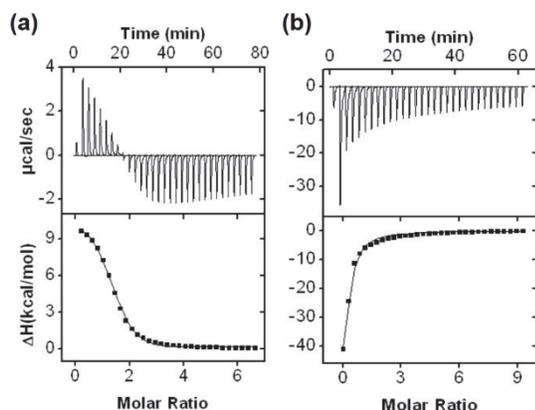
Taken together, it seems that the bischolate arms could fold hydrophobically in >50% water/methanol. The similar response of the 1-armed and 3-armed compounds toward solvent polarity suggests that these arms folded independently. The more important questions, however, were whether these arms indeed could enhance the binding of **3** as a receptor and which factors would control the cooperative enhancement.

**Guest-Binding of the CERs.** To evaluate the molecular recognition of **3**, we synthesized a hexacarboxylated analogue **6** as a control receptor, which lacks the key cooperative conformational change of **3**. Its three ortho carboxylates mimic the three terminal carboxylates from the cholates that are responsible for binding triammonium guests such as **7**. Its para carboxylates mimic the three glutamate carboxylates in the midsection of **3** to provide solubility in aqueous solution. Keeping the compounds charged is important to water-solubility of the host–guest complex, especially when the ammonium guest neutralizes the cholate or the ortho carboxylates in **3** and **6**, respectively.



The binding of the two receptors was studied by isothermal titration calorimetry (ITC). ITC is often the method of choice for binding studies. Not only could one determine binding constants ( $K_a$ ) in a broad range, other important parameters including the binding enthalpy, entropy, and the number of binding sites ( $N$ ) on the receptor could all be obtained simultaneously.

Both receptors (**3** and **6**) relied on the three introverted carboxylates for binding; the difference between the two was in how the carboxylates were folded back—by conformational changes and a rigid covalent framework, respectively—and whether cooperative conformational change was involved in the binding. As shown by Figure 3, the titration data for both compounds fit nicely to a 1:1 binding model but the two bindings had completely opposite heat of reaction, with **3** showing a positive/unfavorable enthalpy and **6** a large negative/favorable enthalpy.



**Figure 3.** ITC titration curves obtained at 298 K for the binding of **7** by (a) **3** and (b) **6**. The data correspond to entries 1 and 9 in Table 1. In a typical experiment, a 2–6 mM aqueous solution of the guest in Millipore water was injected in equal steps of 10.0  $\mu\text{L}$  into 1.42 mL of 0.05–0.2 mM solution of the host in Millipore water. The top panel shows the raw calorimetric data. The area under each peak represents the amount of heat generated at each ejection and is plotted against the molar ratio of the guest to the host. The smooth solid line is the best fit of the experimental data to the sequential binding of  $N$  equal and independent binding sites on the MINP. The heat of dilution for the guest, obtained by adding the guest to Millipore water, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

The thermodynamic parameters for the bindings are summarized in Table 1. Entries 1 and 9 show that the flexible CER (**3**) was able to bind triammonium **7** in water with a  $K_a$  of  $138 \times 10^3 \text{ M}^{-1}$ , ca. 6 times stronger than that of the more rigid control receptor (**6**). The difference corresponds to 1 kcal/mol binding free energy ( $\Delta G$ ). Formation of **3**·**7** was entropically driven, with a positive/favorable binding entropy ( $T\Delta S = 17.5 \text{ kcal/mol}$ ) that more than compensated the unfavorable binding enthalpy of  $\Delta H = 10.5 \text{ kcal/mol}$ . In contrast, the rigid receptor **6** has a large favorable enthalpy ( $\Delta H = -35.6 \text{ kcal/mol}$ ) that was offset by an also large entropic term ( $T\Delta S = -29.6 \text{ kcal/mol}$ ). To our delight, the number of independent binding sites ( $N$ ) for all the receptors (**3**, **6**, and **12** to be dis-

cussed later) was  $1.1 \pm 0.2$  according to the ITC titrations, indicating that 1:1 binding stoichiometry was indeed in operation as designed.

The binding data so far are consistent with the designed cooperatively enhanced binding. Not only was the flexible CER able to bind more strongly than the more “preorganized” control receptor **6** with the same number of salt bridges,<sup>43</sup> the two bindings had opposite driving forces. The entropically driven binding of **3** also strongly supports our CER design: since the intrahost hydrophobic interactions were expected to contribute to the binding and a large number of water molecules will be released to the bulk solution during hydrophobic association of the cholates, a strong entropic driving force is anticipated. According to Figure 2b, **3** was fully folded in 100% water. Since the folding was hydrophobically driven, the cholate–cholate hydrophobic interactions must have been already engaged to a large degree prior to binding. The fact that additional hydrophobic driving force could be “transferred” to the guest-binding suggests that the cholates were not tightly packed in folded **3** prior to the binding, as expected from the proposed repulsion between the terminal carboxylates.

**Table 1.** Binding data obtained by ITC<sup>a</sup>

Entry	Complex	$K_a$ ( $10^3 \text{ M}^{-1}$ )	$\Delta G$ (kcal/mol)	$\Delta H$ (kcal/mol)	$T\Delta S$ (kcal/mol)
1	<b>3</b> · <b>7</b>	$138 \pm 2$	-7.0	10.5	17.5
2	<b>3</b> · <b>7</b> <sup>b</sup>	$49 \pm 9$	-6.4	71.0	77.4
3	<b>3</b> · <b>7</b> <sup>c</sup>	$6.8 \pm 0.2$	-5.2	114.0	119.2
4	<b>3</b> · <b>7</b> <sup>d</sup>	$19 \pm 1.6$	-5.8	-1.6	4.2
5	<b>3</b> · <b>8</b>	$11 \pm 6$	-5.5	35.4	40.9
6	<b>3</b> · <b>9</b>	$8.0 \pm 1.0$	-5.3	-1.7	3.6
7	<b>3</b> · <b>10</b> <sup>e</sup>	--	--	--	--
8	<b>3</b> · <b>11</b>	$23 \pm 1$	-5.9	2.8	8.7
9	<b>6</b> · <b>7</b>	$24 \pm 10$	-6.0	-35.6	-29.6
10	<b>6</b> · <b>8</b> <sup>e</sup>	--	--	--	--
11	<b>6</b> · <b>9</b> <sup>e</sup>	--	--	--	--
12	<b>12</b> · <b>13</b>	$2.2 \pm 0.5$	-4.6	9.9	14.5
13	<b>12</b> · <b>14</b>	$150 \pm 30$	-7.1	-8.3	-1.3

<sup>a</sup> The titrations were generally performed in duplicates in water and the errors between the runs were generally <10%. The number of independent binding sites ( $N$ ) was found to be  $1.1 \pm 0.2$ . <sup>b</sup> The binding was determined in a 80:20 water/methanol mixture. <sup>c</sup> The binding was determined in a 60:40 water/methanol mixture. <sup>d</sup> The binding was determined in PBS buffer (pH 7.4, 137 mM NaCl, 2.7 mM KCl). <sup>e</sup> The binding was too weak to be determined by ITC.

The formation of **6**·**7** was enthalpically driven (Table 1, entry 9). The binding affinity for triammonium **7** by **6** in water was ~6 times stronger than that by a triphosphonate receptor ( $K_a = 4 \times 10^3 \text{ M}^{-1}$  in  $\text{D}_2\text{O}$ ) in the literature for the same guest.<sup>44</sup> The stronger binding by **6** likely comes from the secondary electrostatic interactions between the ammoniums on the guest and the para carboxylates of **6**. The enthalpic driving force seems reasonable. Although ionic interactions have been reported to afford positive entropy in some cases,<sup>45–48</sup> it is also well known that strong ionic interactions have favorable enthalpic contribution.<sup>47,49</sup> In the case of **6**, any favorable entropy obtained through release of water molecules during desolvation was probably overcome by increased order of the complex. One source for the higher order could come from the loss of conformational freedom in the receptor during binding. The free receptor is unlikely to have all the carboxylates on the same side of the molecule, due to electrostatic repulsion of the ortho carboxylates and multiple rotatable

bonds in the 1,3,5-tris(triazolyl)benzene scaffold. Binding between **6** and **7** would undoubtedly freeze the conformation of the host, leading to a reduction of entropy.

The intrahost hydrophobic contribution to the formation of **3**·**7** was additionally confirmed by the addition of methanol to the aqueous solution. As shown by entries 2 and 3 of Table 1, the binding affinity continued to decline with increasing amounts of methanol. Additionally, in PBS buffer, which contained significant amounts of electrolytes (NaCl, KCl, and sodium phosphate), the binding was also weakened significantly (entry 4). The result is consistent with our proposed binding mechanism. As the electrolytes lowered the repulsion among the negatively charged carboxylates in the folded CER, the intrahost cholate–cholate hydrophobic interactions become more fully engaged *prior to* the guest binding, destroying the very basis of the cooperative enhancement. These results are also in agreement with our earlier conclusion that, even though **3** was fully folded (Figure 2b), the cholates were not tightly packed due to the repulsion among the cholate carboxylates.

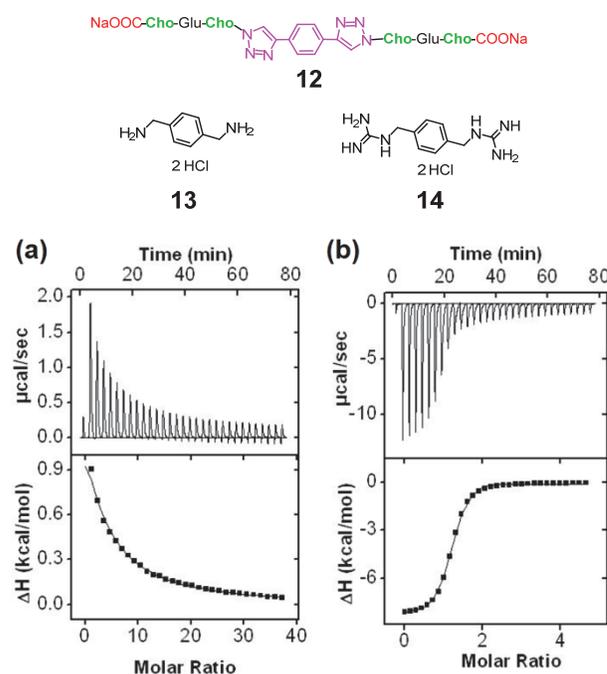
Our CER model in Scheme 1 predicts selectivity in the binding, as the optimal guest needs to fit in between the binding groups in the folded CER. The prediction was confirmed in the bindings of guests **8**–**11**. The addition of a single methylene spacer (**8** vs. **7**) lowered the binding affinity (of **8**) by an order of magnitude. Compound **9** differs from **8** by another oxygen spacer; its binding by **3** was similarly weak. Thus, despite the tremendous flexibility of the conformationally mobile CER, not only could it bind its guest tightly in water, it also did so with quite impressive selectivity.

Somewhat surprisingly, **3** had no detectable binding for the ammonium salt of TREN (**10**). It is unclear to us why this compound could not bind, given its similarity to **7** in size and the terminal amine groups. On the other hand, it is interesting to note that diammonium salt **11** was bound with quite a remarkable affinity in water. Even though its binding constant was weaker than that for **7** (as expected), a  $K_a$  of  $23 \times 10^3 \text{ M}^{-1}$  was 2–3 times higher than the “slightly-mismatched” triammonium **8** and **9**. We believe this result actually derived from our CER binding mechanism. Although three ammoniums are optimal for binding CER **3**, two such groups are sufficient to “disarm” the electrostatically frustrated bischolates. This is because when two salt bridges are formed between **3** and **11**, the third cholate carboxylate would not face significant repulsion in the guest-binding folded state. As a result, even when the third salt bridge was absent, all the other intrahost hydrophobic interactions among the cholates could be turned on by **11** to enhance its binding.

If the folding arms are essential to the CER, reducing its number should weaken the binding dramatically. To verify this hypothesis, we synthesized 2-armed CER **12** and studied its binding of diammonium **13** and diguanidinium **14**. As predicted, the 2-armed receptor displayed weaker binding for diammonium **13**, with a  $K_a$  of  $2.2 \times 10^3 \text{ M}^{-1}$  (Table 1, entry 12) or about 60 times weaker than that of **3**·**7**. It is worth noting that, although two salt bridges are formed in both **3**·**11** and **12**·**13**, the former complex was 10 times more stable than the latter. The result once again confirms that the intrahost cholate–cholate interactions were critical to the binding. Since three such interactions can be formed in **3**·**11** but only two in **12**·**13**, the higher stability of the former is anticipated, despite the same number of salt bridges formed in both complexes.

A stronger direct binding force between the carboxylate and guanidinium not surprisingly enhanced the binding even further, giving an impressive  $K_a$  of  $150 \times 10^3 \text{ M}^{-1}$  with  $\Delta G = -$

7.1 kcal/mol for **12**·**14** in water (Table 1, entry 13). As shown by Figure 4, the ITC curves for **13** and **14** once again displayed different types of driving forces, with the binding of diammonium **13** endothermic and the binding of diguanidinium **14** exothermic. If we assume the intrahost cholate–cholate interactions are hydrophobic and entropic in origin, the switching from entropy- to enthalpy-driven binding from **13** to **14** could suggest that cooperative enhancement by the intrahost interactions is more important to a receptor whose direct host–guest binding forces are weaker. Stated differently, the stronger the direct binding forces, the less the binding needs to rely on intrahost interactions to afford high binding affinity. Many bis- and tris-guanidinium–carboxylate host–guest complexes have been reported in the literature,<sup>46,50–52</sup> they often did not function in pure aqueous solution or displayed much weaker binding than what was observed for **12**·**14**. The enhanced binding in the CER suggests that cooperative hydrophobic intrahost interactions could indeed magnify polar interactions that are compromised by water.



**Figure 4.** ITC titration curves obtained at 298 K for the binding of (a) **13** and (b) **14** by **12**. The data correspond to entries 12 and 13 in Table 1.

## Conclusions

The significance of this work lies in the rational design of cooperatively enhanced receptors (CERs) that employ “hidden” intrahost interactions to magnify weak polar binding forces. Our strategy makes the binding delocalized over the entire structure of the receptor instead of being confined at the binding interface. This type of receptors essentially exploit the positive cooperativity between the guest-binding and intrahost interactions to augment each other.<sup>53</sup> Despite the flexibility of the receptor, high binding selectivity is still possible, even though the selection rule is quite different from what governs a preorganized receptor: instead of fitting snugly into a rigid pocket, the best guest needs to turn on the most number of non-engaged intrahost interactions prior to binding.

There is strong support for Williams's postulation of delocalized, cooperatively enhanced binding in biology. When streptavidin binds biotin, the melting point of the protein host increases by 37 °C and numerous backbone amide protons become resistant to H/D exchange.<sup>23</sup> In contrast to hundreds or thousands preorganized synthetic receptors already synthesized, very few CERs have been made by chemists. Hopefully, the rational design of CERs will accelerate the development of these biomimetic receptors and help chemists create ultrastable host-guest complexes even when strong direct host-guest interactions are unavailable—this could be one of many of nature's secrets in making the impossible possible. The electrostatic frustration illustrated in this work certainly is not the only strategy for CERs and additional designs will emerge for sure as more researchers join this pursuit.

Cooperative enhancement and preorganization are not mutually exclusive concepts in the design of supramolecular receptors. All the previous CERs<sup>27,28,30,31</sup> and the ones reported in this study all have a significant degree of preorganization, in the sense that some rigid scaffolds are used in the construction of the receptor to avoid total flexibility, which could be detrimental to both binding affinity and selectivity. A fine balance of the two strategies will most likely be needed for optimal complexes, as nature has amply demonstrated.

## ASSOCIATED CONTENT

### Supporting Information

Experimental details for the syntheses and additional figures. This information is available free of charge via the Internet at <http://pubs.acs.org>.

## AUTHOR INFORMATION

### Corresponding Author

zhaoy@iastate.edu

### Notes

The authors declare no competing financial interests.

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