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Molecularly Responsive Binding through Co-occupation of Binding Space: A Lock–Key Story

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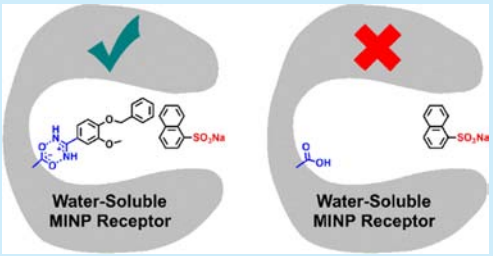
Supporting Information

ABSTRACT: When two guest molecules co-occupy a binding pocket of a water-soluble host, the first guest could be used as a signal molecule to turn on the binding of the second. This type of molecularly responsive binding strongly depends on the size of the two guests and the location of the signal molecule.



Biological systems have sophisticated mechanisms to detect and respond to a vast variety of signals present within themselves and in the environment. These processes occur over multiple length scales but, at the most fundamental level, often rely on biomacromolecules such as proteins to alter their molecular recognition, transport, or catalysis in response to specific chemical or physical stimuli.

One way to create molecularly responsive receptors (MRRs) is to mimic allosteric proteins through conformational communication between the allosteric and the main binding site.[1] Since conformation can determine the size, shape, and distribution of functional groups of a molecule, we can tune the binding rationally as long as we understand how to trigger a desired conformational change of a receptor using the signal molecule.

A different type of molecularly responsive binding may be obtained with receptors possessing a large enough binding pocket to fit two smaller guests. Cucurbit[8]uril,[2] for example, has a cavity that can accommodate two aromatic guests.[3] Binding of the first guest enhances the binding of the second, ideally if the two have opposite electronic properties so they can form a charge-transfer complex in the cavity.[4] In such examples, the first guest may be viewed as a signal molecule to turn on the binding of the second, particularly if the receptor binds the second guest negligibly in the absence of the first one.

The double-guest-binding of cucurbit[8]uril has been used to create numerous interesting materials since its discovery.[5] The macrocyclic receptor, however, has a cavity nearly fixed in size and shape, making it difficult to extract general rules governing the signaling process in this type of MRRs. When two guests co-occupy a binding pocket, what controls the interdependency of the bindings? Intuitively, a strong interdependency translates to a higher sensitivity in the signaling process. Does it matter which guest is used as the signal molecule and which guest is the one whose binding is being turned on, or all such naming is simply a matter of reference?

Herein, we report that specific rules indeed exist that govern the molecular signaling in water-soluble MRRs with two cohabitating guests. Not only are the relative sizes of the guests, but also is the location of the signal molecule critical to the performance of the MRRs. We expect that elucidation of these rules will expedite the development of MRRs and enable new designs of signal-controlled materials and devices.

The preparation of our MRR is based on our recently developed surface-cross-linked micelles (SCMs) from tripropargylammonium surfactants. The click-cross-linked nanoparticles can be functionalized in many ways for different applications.[6] Micelles of **1** can be similarly cross-linked on the surface using diazide **3** and Cu(I) catalysts and then decorated with hydrophilic ligand **4** (Scheme 1).

To create binding pockets within the SCMs, we added sulfonate **2**, DVB, and AIBN in the beginning of the reaction. The anionic template was readily incorporated into the cationic micelle but could not participate in the surface-cross-linking. It would, however, copolymerize with **1** and DVB via free radical polymerization during the thermally induced core-cross-linking. In the final step of the preparation, we cleaved the *ortho*-nitrobenzyl ester bond by photolysis to remove the template, creating a template-complementary binding pocket within the doubly cross-linked micelle (Scheme 1).[7]

Molecular imprinting is a powerful method to create binding sites in a polymer matrix.[8] Although imprinted nanoparticles have been reported in the literature,[9] our molecularly imprinted nanoparticles (MINPs) are characterized by complete water solubility, a tunable number of well-defined binding sites in the hydrophobic core, and protein-like nanosize (4–5 nm).[7,10]

When several structural analogues were examined as the guests for MINP–COOH, **5** was not surprisingly found to be the best, with a binding constant (K_a) of 1520×10^3 M^{-1} at pH 7.4 in 50 mM Tris buffer.[7] An important feature of the MINP receptors is the controllable number of binding sites on the nanoparticle. Since each SCM contains ca. 50 surfactants, a ratio of surfactant/

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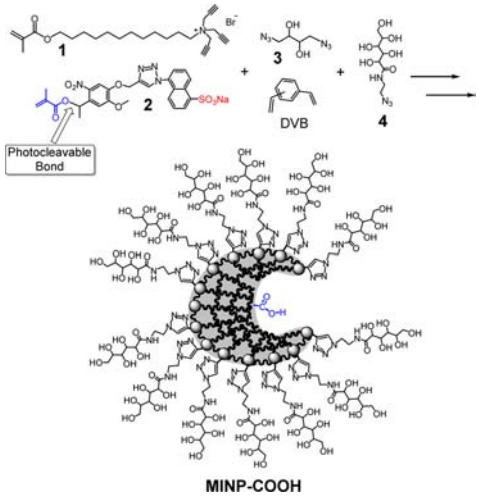
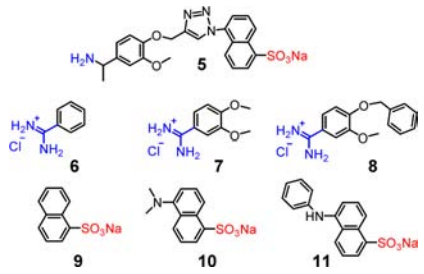
Scheme 1. Schematic Preparation of MINP−COOH from Cross-Linkable Surfactant 1 and Photocleavable Template 2[a]



[a]Diazide **3** and DVB (divinyl benzene) were surface and internal cross-linkers used in the synthesis, respectively.

template = 50/1 in the preparation afforded MINPs with an average of one binding site per nanoparticle.



The binding between MINP−COOH and **5** was driven by a combination of hydrophobic interactions, a carboxylate−ammonium salt bridge, and favorable electrostatic interactions between the negatively charged sulfonate of **5** and the positively charged MINP−COOH.[7] To create a pair of smaller guests that can fit into the MINP binding pocket, we split **5** into two parts: a salt bridge-forming aromatic amidinium salt (**6**, **7**, or **8**) and an aromatic sulfonate (**9**, **10**, or **11**). *Together*, they should occupy the binding site to fulfill all the main interactions between **5** and MINP−COOH. Amidinium salts were chosen because of the stronger amidinium−carboxylate salt bridge than ammonium−carboxylate, to facilitate experimental determination of the binding constants. (For the same reason, although the amino analogues were easier to synthesize than **6**−**8**, we did not perform the corresponding syntheses and binding studies.) The aromatic groups of **6**−**11** vary in size but in general mimic specific parts of guest **5**.

To understand the molecularly responsive binding, we first determined the binding constants of **6**−**11** individually by isothermal titration calorimetry (ITC).[11] The ITC-derived binding affinities were previously shown to agree well with those by other methods such as fluorescence titration.[7,10]

As summarized in Table 1, the binding of the amidinium salt increases with the size of the hydrophobic group, with K_a being 15, 32, and 1350 × 10^3 M^{-1} for guests **6**, **7**, and **8**, respectively (entries 1−3). This trend is understandable. Although the same carboxylate−amidinium salt bridge was involved in the binding, a larger aromatic hydrophobe can release more of the "high-energy" water molecules inside the hydrophobic binding pocket of MINP−COOH. Our previous study showed that the pK_a of the carboxyl group within the MINP pocket was 6.2, due to the hydrophobicity of the microenvironment.[7] At pH 7, it is largely in the deprotonated state. The acid−base equilibrium is thus not

Table 1. Binding Data for MINPs Obtained by ITC[a]

entry	host	guest	percent complexed[b]	K_a (10^3 M^{-1})	ΔG (kcal/mol)	Amp.[c]	N[d]
1	MINP	**6**	–	15 ± 4	−5.7	–[e]	0.7 ± 0.1
2	MINP	**7**	–	32 ± 4	−6.1	–[e]	0.5 ± 0.1
3	MINP	**8**	–	1350 ± 60	−8.4	–[e]	0.6 ± 0.1
4	MINP	**9**	–	1.3 ± 0.2	−4.2	–[e]	0.5 ± 0.1
5	MINP	**10**	–	2.0 ± 0.9	−4.5	–[e]	1.3 ± 0.3
6	MINP	**11**	–	39 ± 8	−6.3	–[e]	0.8 ± 0.1
7	MINP·**6**	**9**	12%	5.8 ± 0.4	−5.1	4.5	0.8 ± 0.1
8	MINP·**6**	**10**	20%	7.3 ± 0.5	−5.3	3.6	0.9 ± 0.1
9	MINP·**6**	**11**	12%	650 ± 30	−7.9	17	0.8 ± 0.1
10	MINP·**7**	**9**	21%	53 ± 7	−6.4	41	0.5 ± 0.1
11	MINP·**7**	**10**	21%	96 ± 4	−6.8	48	0.5 ± 0.1
12	MINP·**7**	**11**	21%	370 ± 50	−7.6	9.5	0.6 ± 0.1
13	MINP·**8**	**9**	68%	990 ± 70	−8.2	762	1.0 ± 0.1
14	MINP·**8**	**10**	89%	–[f]	–[f]	–[f]	–[f]
15	MINP·**8**	**11**	78%	–[f]	–[f]	–[f]	–[f]
16	MINP·**9**	**8**	6%	1940 ± 30	−8.6	1.4	1.0 ± 0.1
17	MINP·**10**	**7**	8%	36 ± 9	−6.2	1.1	1.7 ± 0.3
18	MINP·**11**	**6**	50%	19 ± 5	−5.8	1.3	0.8 ± 0.1

[a]The titrations were generally performed in duplicates in Millipore water and the errors between the runs were <10%. [b]The percentage of MINP−COOH being complexed with the first guest or signal molecule in the 1:1 mixture, calculated using the binding constants of the guests and the host/guest concentrations. [c]The amplification factor was defined as the binding constant of the titrating guest in the presence of the signal molecule divided by that of the same guest in the absence of the signal molecule. [d]The number of binding site per MINP determined by ITC. [e]Titration involved only MINP and the guest, thus no amplification being reported. [f]Binding was undetectable by ITC.

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expected to contribute significantly to the binding between MINP−COOH and the amidinium guests.

The same trend was observed in the sulfonates, with the highest K_a observed for **11**, which has the largest hydrophobe. The binding constants were generally 1−2 orders of magnitude lower than those of the amidinium salts (Table 1, entries 1−6), confirming again the significant contribution of amidinium−carboxylate salt bridge to the binding. For the fluorescent sulfonates, the 1:1 stoichiometry was confirmed by the Job plots for selected compounds, i.e., MINP−COOH with **10** and (MINP−COOH + **7**) with **10** (Figures S7 and S8, Supporting Information). The study also ruled out nonspecific binding between the positively charged MINP−COOH and the sulfonates.

To study the co-occupation of the MINP binding pocket, we titrated a 1:1 mixture of MINP−COOH and the amidinium salt (**6**, **7**, or **8**) with the sulfonate (**9**, **10**, or **11**). The 1:1 stoichiometry was maintained for better comparison among different pairs of signal/guest molecules. However, for different binding affinities to be determined accurately by ITC, the optimal MINP concentration (in the 1:1 mixture) was different, meaning that the percentage of the MINP receptor being bound by the first guest or signal molecule was also different. To take this parameter into consideration, we listed the value in each situation under the column, "percent complexed", in Table 1.

Another column, "Amp", summarizes the amplification factor of a signal molecule, defined as K_a for a particular guest in the presence of the signal molecule relative to that in the absence. The larger this number is, the more sensitive the binding is toward the signal molecule.

Our data revealed some very interesting trends. As shown in Table 1, for amidinium **6**, the largest amplification in binding was obtained for sulfonate **11**, whose K_a increased by 17-fold by the signal molecule (entry 9). For amidiniums **7** and **8**, the largest amplification was for sulfonates **10** and **9**, with the amplification factor being 48 and 762, respectively (entries 11 and 13). There is no correlation between the amplification factor and the percentage of the host complexed with the signal molecule, as the percentage varied from 12 to 89% for the pairs with small amplifications and 12 to 68% for the "optimal" pairs with large amplifications.

Shown more clearly in Figure 1, the strongest guest-triggered binding occurred always when the amidinium/sulfonate pair added together was equivalent to the "optimal" guest (i.e., **12**, the amidinium analogue of **5**).[12] This is quite reasonable because a

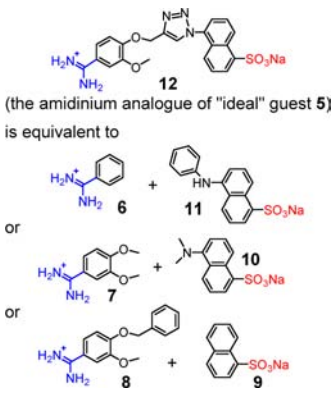


Figure 1. Comparison of "optimal" guest **12** with optimal pairs of guest in molecule-triggered binding.

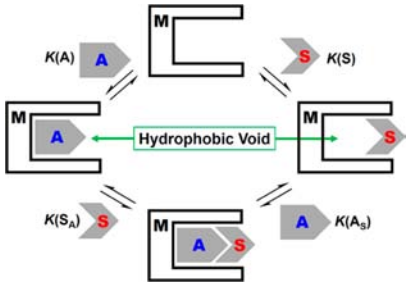
main driving force for the aqueous-based binding was hydrophobic interactions, which are maximized when the hydrophobic space created after template **2** is filled, whether with a single guest (i.e., **5**) or two cohabiting guests. Consistent with this postulation, the MINP receptor had a better tolerance for smaller guest pairs than for pairs too large to fit within the pocket. Sulfonate **10** or **11**, for example, bound to MINP−COOH with reasonable affinities (Table 1, entries 5 and 6) but showed no detectable binding in the presence of **8** (entries 14 and 15).

Among the three optimal pairs of guests (**6**/**11**, **7**/**10**, and **8**/**9**), the highest amplification in binding was for **8**/**9**, with the K_a of **9** increased by ∼760 times by the signal molecule, far larger than the 17 and 48 observed for **6**/**11** and **7**/**10**, respectively. Not sure what was responsible for the difference initially, we performed "reverse signaling" experiments, in which a 1:1 mixture of MINP−COOH and the sulfonates was titrated with the corresponding optimal amidinium identified earlier in the study.[13] To our surprise, the amplification was nearly negligible, ranging from 1.1 to 1.4 in all three cases, and once again exhibited no apparent correlation with the percentage of host complexed (Table 1, entries 16−18).

Thus, the signaling process in our MRR with cohabiting guests is not "symmetrical". It matters greatly which molecule is used as the signal molecule even when the pair of the guests together have the optimal size, shape, and functionalities for the binding pocket.

Scheme 2 shows our hypothesized co-occupation of the amidinium signal molecule (**A**) and the sulfonate guest (**S**) in the

Scheme 2. Schematic Representation of Co-occupation of the Binding Pocket of Host M by Two Guests (A and S) in a Sequential Binding



binding pocket of MINP−COOH (**M**). The amidinium salt was predetermined by its binding functionality to go deep inside the pocket (to form a salt-bridge with the MINP carboxyl group) and the sulfonate needs to stay near the surface (to allow the sulfonate to be solvated by water while maximizing hydrophobic contact with the MINP binding pocket). The binding geometry was also expected from the covalent imprinting process using template **2**.

For a good signaling process, we want K_a for the molecularly triggered binding to far exceed the value without the signal molecule. According to our binding experiments, the general trend for the optimal amidinium/sulfonate pairs was $K(S_A) \gg K(S)$ but $K(A_S) \approx K(A)$.

A close examination of Scheme 2 reveals the possible reason behind the "unsymmetry" in the molecular signaling. Amidinium **A** occupies the inner portion of the binding pocket. Its binding derives from the amidinium−carboxylate salt bridge and its hydrophobic contact with the MINP pocket. These two driving forces are largely the same, with or without **S** in the pocket. The

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only benefit one can envision for the binding with sulfonate **S** present is the additional hydrophobic contact between **A** and **S** in the **M·A·S** complex than in **M·A**; this probably accounts for the slightly stronger $K(A_S)$ than $K(A)$ observed in our titrations (Table 1, entries 16−18).

For sulfonate **S**, the situation is different. It occupies the outer part of the MINP binding pocket. In the formation of **M·A·S**, all the hydrophobic needs of the host and the guests are fulfilled. In the formation of **M·S**, which determines $K(S)$, however, a hydrophobic void is created within the **M·S** complex upon the binding of the guest. This hydrophobic space can only be filled by water molecules in the absence of the amidinium signal molecule and is highly unfavorable. Although a hydrophobic void also exists in the **M·A** complex, it is located near the surface of the MINP, connected to bulk water. As a result, the water molecules inside can easily exchange with those in the solution. The entropic cost of trapping water molecules can be very high, up to 2 kcal/mol for a single water molecule in some cases.[14] Thus, forming an internal hydrophobic void (as in **M·S**) is much more energetically costly than forming an external one (as in **M·A**). Of course, a perfect "blocking" of the binding site by **S** is not necessary in our model; the different size and nature of the hydrophobic void in **M·S** and **M·A** is what matters most.

With the above picture clarified, it is easy to understand that the larger the internal hydrophobic void, the stronger the need is to have it filled by a suitable molecule. This signal molecule, as shown by our experiments, essentially is the key to turn on the binding of the (second) guest. The guest whose binding is controlled by the signal molecule should stay near the surface of the water-soluble receptor to create the keyhole (i.e., the internal hydrophobic void) in our analogy. The most interesting discovery of our research is that, to have the strongest amplification in this type of molecularly responsive binding, one simply needs to make the key as large as possible, while making sure that the binding of the second guest is sufficiently strong so that the void space for the key can be created inside the lock, so to speak.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00527.

Experimental details, ITC titration curves, and additional figures (PDF)

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Notes

The authors declare no competing financial interest.

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(12) We did not synthesize compound **12**, as the current study focused on the co-occupation of guests in the binding pocket.

(13) Although an amidinium could also form a salt-bridge with a sulfonate, the binding is generally too weak in water (<100 M^{-1}) to compete with the other interactions in our study.

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