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Tuning surface-cross-linking of molecularly imprinted cross-linked micelles for molecular recognition in water

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Abstract

Molecular recognition in water is an important challenge in supramolecular chemistry. Surface-core double cross-linking of template-containing surfactant micelles by the click reaction and free radical polymerization yields molecularly imprinted nanoparticles (MINPs) with guest-complementary binding sites. An important property of MINP-based receptors is the surface-cross-linking between the propargyl groups of the surfactants and a diazide cross-linker. Decreasing the number of carbons in between the two azides enhanced the binding affinity of the MINPs, possibly by keeping the imprinted binding site more open prior to the guest binding. The depth of the binding pocket can be controlled by the distribution of the hydrophilic/hydrophobic groups of the template and was found to influence the binding in addition to electrostatic interactions between oppositely charged MINPs and guests. Cross-linkers with an alkoxyamine group enabled two-stage double surface-cross-linking that strengthened the binding constants by an order of magnitude, possibly by expanding the binding pocket of the MINP into the polar region. The binding selectivity among very similar isomeric structures also improved.

Keywords

binding, cross-linking, hydrophobic interactions, micelle, molecular imprinting, nanoparticle, water soluble

Disciplines

Chemistry

Tuning Surface-Cross-Linking of Molecularly Imprinted Cross-Linked Micelles for Molecular Recognition in Water

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ABSTRACT. Molecular recognition in water is an important challenge in supramolecular chemistry. Surface–core double cross-linking of template-containing surfactant micelles by the click reaction and free radical polymerization yields molecularly imprinted nanoparticles (MINPs) with guest-complementary binding sites. An important property of MINP-based receptors is the surface-cross-linking between the propargyl groups of the surfactants and a diazide cross-linker. Decreasing the number of carbons in between the two azides enhanced the binding affinity of the MINPs, possibly by keeping the imprinted binding site more open prior to the guest binding. The depth of the binding pocket can be controlled by the distribution of the hydrophilic/hydrophobic groups of the template and was found to influence the binding in addition to electrostatic interactions between oppositely charged MINPs and guests. Cross-linkers with an alkoxyamine group enabled two-stage double surface-cross-linking that strengthened the binding constants by an order of magnitude, possibly by expanding the binding pocket of the MINP into the polar region. The binding selectivity among very similar isomeric structures also improved.

Key Words: molecular imprinting, binding, nanoparticle, micelle, cross-linking, hydrophobic interactions, water-soluble

Introduction

Molecular recognition in water is central to many biological processes but has been a difficult challenge in supramolecular chemistry.[1-2] Part of the challenge comes from the fact that hydrogen bonds, one of the best tools for selective intermolecular interactions, are compromised by competition from the solvent in aqueous solution. Hydrophobic and van der Walls interactions can be strong if a sufficient complementary area of interaction can be created. Their nondirectional nature, however, makes it difficult to utilize them for selective molecular recognition by design.[3] Even though macrocycles such as cyclodextrin, calixarene, and particularly cucurbituril[4-6] can be made into excellent supramolecular hosts in water, modifying their shape to match an arbitrary guest with a potentially complex shape is challenging due to the highly symmetrical nature of most macrocycles.[7-8]

Molecular imprinting is a technique to create complementary binding sites for molecules of all kinds of size[9-10] and molecularly imprinted polymers (MIPs) continue to find new applications in different areas of research.[11-19] The technique traditionally involves the formation of a complex between the template molecule (often the guest or its analogue) and functional monomers (FMs) that can interact with the template with either noncovalent or cleavable covalent bonds. The template–FM complex is typically formed in a mixture of a large amount of a cross-linker and an inert solvent as porogen. Free radical polymerization of the above mixture yields a polymer matrix with embedded template molecules. Removal of the templates leaves behind cavities complementary to the template molecule, with the FMs turning into specific binding groups in the binding site. In addition to traditional macroporous molecularly imprinted polymers (MIPs) obtained from the above procedures, imprinting could occur unimolecularly within dendrimers.[20-21] The heavy cross-linking in traditional MIPs makes it completely insoluble but soluble materials could be prepared by imprinting on polymeric nanoparticles[12, 22-28] and within less highly cross-linked micro/nanogels.[29-34]

Our group has been very interested in the creation of biomimetic receptors capable of functioning in aqueous environments.[35-37] Recently, we designed and synthesized doubly cross-linkable surfactants such as **1** (Scheme 1).[38] The compound has a tripropargylammonium headgroup that allows us to cross-

link its micelle on the surface by the well-known copper-catalyzed alkyne–azide click reaction.[39-40] Its tail is functionalized with a polymerizible methacrylate for the cross-linking of the micellar core through free radical polymerization with divinyl benzene (DVB) solubilized in the micelle. The cross-linked micelle is then functionalized by an azide-containing surface ligand (**3**) to enhance its water-solubility. The poor solubility of **3** in acetone allows us to recover the molecularly imprinted nanoparticles (MINPs) conveniently by precipitation of the MINP aqueous solution into acetone and washing the resulting precipitate with acetone/water and methanol.[38]

Scheme 1.

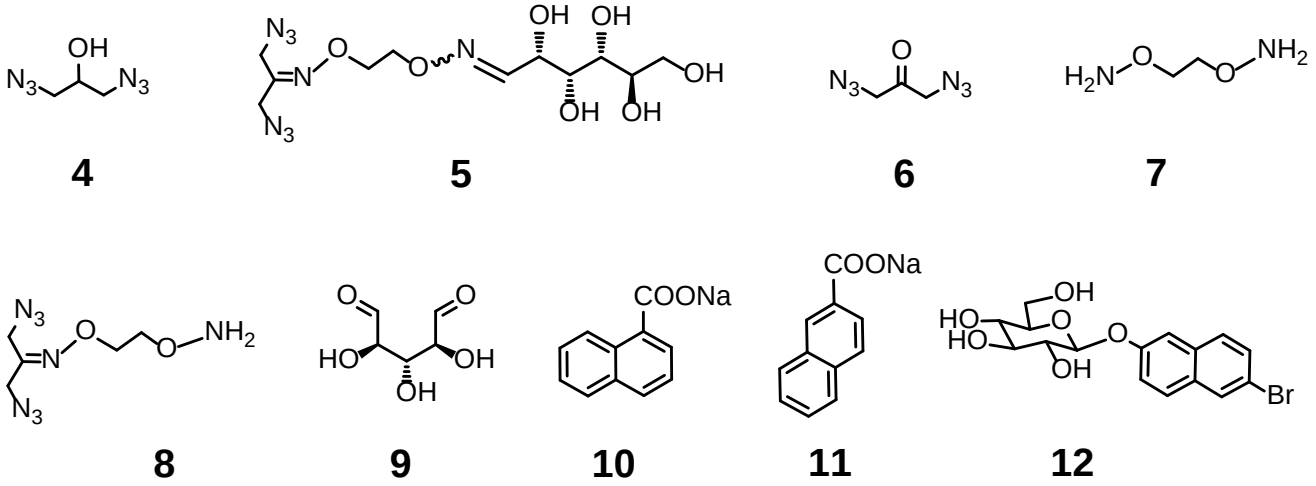
Importantly, either amphiphilic[38, 41] or hydrophilic molecules,[42-44] sometimes with suitable FMs, can be incorporated into the micelles as templates. The resulting MINPs displayed excellent molecular recognition in water for a wide range of biologically interesting molecules including small-molecule drugs,[45] carbohydrates,[42-43] and peptides.[41] The number of binding sites per nanoparticle can be controlled through tuning the surfactant/template ratio. MINPs are estimated to have ~50 cross-linked surfactants by dynamic light scattering (DLS). A 50:1 surfactant/template ratio afforded MINPs with an average of one binding site per nanoparticle and a 25:1 ratio afforded two binding sites, as revealed by isothermal titration calorimetry (ITC).[38] These properties make MINPs bridge the gap between water-soluble molecular receptors and macroscopic MIPs to achieve selective molecular recognition in water.

Because the integrity of the MINP binding sites is maintained by the surface–core double cross-linking, tuning the cross-linking density is expected to affect the molecular recognition properties of these nanoparticle receptors strongly. The core-cross-linking density is best tuned by the amount of DVB used.[38] In this work, we evaluated two different strategies to control the surface-cross-linking. An oxime-containing diazide cross-linker was synthesized, whose modular structure allows easy modification. We found that good water-solubility and rigidity of the surface-cross-linker were highly important parameters to the micellar imprinting. In addition, properly functionalized surface-cross-

linkers could be used to achieve two-stage double surface-cross-linking that improved the binding properties of MINPs strongly.

Results and Discussion

Design and Synthesis of Materials. The excellent functional-group compatibility of the click reaction gives us much flexibility in the design of surface cross-linkers and different ways of surface-cross-linking. Diazide **2** is our most commonly used surface-cross-linker (Scheme 1).[38, 41-45] Since flexibility of the surface-cross-linker and surface ligand is important to the binding of MINPs,[46] we designed two diazide cross-linkers **4** and **5** from ketone **6** that was prepared in one step from commercially available 1,3-dichloroacetone. Both compounds contained three carbons in between the two azides, one carbon shorter than diazide **2**. Compound **4** was prepared by simple reduction of the ketone of **6** with sodium borohydride. Compound **5** had the ketone of **6** linked to glucose through dialkoxyamine **7**. The glucose was included to enhance the surface hydrophilicity of the MINP. The design of **5** is modular, allowing us to decorate the surface of MINP potentially with other sugars and even different ligands. Previously, we have found the surface ligands of the cross-linked micelles could be engineered to interact with lipid bilayer membranes in specific manners.[39] Compound **8** was a diazide cross-linker terminated with an alkoxyamine. The alkoxyamine in principle should enable another round of surface-cross-linking after the click reaction, using externally added dialdehyde **9**, for example.



For our investigation, we used three templates (**10**–**12**). All three compounds share the common motif of having an aromatic hydrophobe connected to a hydrophilic moiety. Compound **10** and **11** are constitutional isomers differing only in the position of the carboxylate. Their small difference makes them particularly suitable for the study of binding selectivity of MINPs. Compound **12** is a commercially available nonionic sugar derivative. Its extensive hydroxyl substituents help us evaluate the importance of hydrogen bonds in the imprinting and binding of MINPs, especially when additional hydroxyl groups are introduced through **9**, for example.

The preparation of MINPs followed Scheme 1, with detailed procedures for the synthesis and characterization reported previously.[38, 41-45] Generally, the surface- and core-cross-linking of the micelles was monitored by ^{1}H NMR spectroscopy. The click cross-linking between **1** and **2** in the surface-cross-linked micelles (SCMs) has been verified by mass spectrometry after the 1,2-diol in the cross-linkage was cleaved by periodate.[47] Dynamic light scattering (DSL) was used to estimate the molecular weights of the nanoparticles and their size. The DLS size(~5 nm with ligand **3**) has been confirmed by transmission electron microscopy (TEM).[48-49]

Effects of Surface Cross-Linker on the Imprinting and Binding of MINPs. The binding properties of MINPs were studied by ITC, one of the most reliable methods to study intermolecular interactions.[50] The technique has the benefit of affording a number of important parameters including

binding enthalpy (ΔH) and the number of binding sites per particle (N), in addition to the binding constant (K_a). The binding free energy (ΔG) can be calculated from K_a using equation $-\Delta G = RT\ln(K_a)$, and ΔS from ΔG and ΔH. We also studied some of the bindings by fluorescence titration. Because the emission of compound **10** and **12** was quite weak in 50 mM Tris buffer (pH 7.3), for the purpose of comparisons, we used ITC binding data exclusively in this study. We have in multiple cases confirmed that binding constants and stoichiometry obtained from these two techniques agree with each other.[38, 41, 44-45]

Our ITC titrations showed that the MINP prepared with **10** as the template, i.e., MINP(**10**), bound its template with a K_a value of 6.46×10^5 M^{-1} when the conventional C4-diazide was used (Table 1, entry 1). The binding constant decreased by 2.8 times with the C3 diazide **4** (entry 2) but increased by 3.2-fold to $K_a = 20.7 \times 10^5$ M^{-1} when the more hydrophilic C3 diazide **5** was used (entry 3). We noticed that **4** was fairly insoluble in water and some of it remained even at the end of MINP preparation. Most likely, the poor solubility of this compound caused insufficient surface-cross-linking of the MINP, which could be the reason for the weaker binding affinity observed. For these reasons, we removed compound **4** in our further investigation. When the cross-linker (i.e., **5**) was made water-soluble, the C3 linkage between the two azides clearly helped the binding of the MINP.

Table 1

MINP(**10**) displayed significant selectivity for the template in comparison to its isomer, regardless of the surface-cross-linker used. With **2** used in the preparation, it showed a smaller binding constant for guest **11**, with $K_a = 1.25 \times 10^5$ M^{-1} (entry 4). MINP(**10**) prepared with C3 diazide **5** also displayed a weaker binding for **11**, with $K_a = 4.04 \times 10^5$ M^{-1} (entry 5). As far as the difference in binding free energy between the template (**10**) and its isomer (**11**) is concerned, MINP prepared with **2** gave 0.9 kcal/mol and MINP prepared with **5** afforded 1.1 kcal/mol. The ratio between the binding constants for

the matched/mismatched guests was 5.2 with **2** and 5.1 with **5**. Thus, the binding selectivity stayed nearly constant regardless of the surface-cross-linker.

MINP(**11**) showed a similar trend in the binding affinity. For example, the replacement of the C4 cross-linker (**2**) with the C3 cross-linker (**5**) increased the K_a value for the template from 7.59 to 30.8 × 10^5 M^{-1}, by 4.1-fold (entries 6 and 7). The binding selectivity basically stayed the same. The difference in binding free energy between the template (**11**) and its isomer (**10**) was 1.1 kcal/mol with either surface-cross-linker.

What could be the possible reason for the improved binding affinity but similar binding selectivity when **5** replaced **2** as the surface-cross-linker? An important clue could be the fact that the shorter surface-cross-linker (**5**) strengthened the binding for both the matched and mismatched guest molecules, as shown in Table 1. In other words, the higher surface-cross-linking density from **5** did not improve the complementarity between the template and the imprinted binding site significantly, or the change would help the template more than its structural analogue. A possible explanation for the results is that the shorter cross-linker did a better job preventing the collapse of the binding pocket in the aqueous solution than the longer, more flexible one. Prior to the binding, the strong cohesive energy of water and the unfavorable exposure of the vacated hydrophobic imprinted site to water create a very unfavorable situation. Although this unfavorable situation is the exact driving force for the rebinding of the template afterwards, the system could also mitigate the situation by a partial or complete collapse of the binding site. On the other hand, because MINP was prepared through surface-core cross-linking of the micelles with the template trapped inside, the cross-linked network by its memory effect favors the binding site being open, in the non-collapsed state. Since the shorter cross-linker helped both the matched and mismatched guests, we suspect that the surface-cross-linking plays an important role in preventing the collapse of the binding pocket. Another possible reason is steric in origin: cross-linker **5** is significantly larger than **2**, with the dialkoxyamine and the glucose attached. These groups, together with the associated solvent molecules, might help the binding site stay open, due to their steric repulsion. Regardless of the exact reason for the nearly constant binding selectivity, a shift of the carboxylate in

one position (from **10** to **11** or vice versa) could be detected easily by our MINPs with either cross-linker, highlighting the success of the molecular imprinting.

Another interesting trend observed in our binding data is that the mismatched guest—i.e., **11** for MINP(**10**) and **10** for MINP(**11**)—gave quite similar binding constants, about 1.20–1.25 × 10^5 M^{-1} with **2** as the cross-linker and 4.0–4.3 × 10^5 M^{-1} with **5**. Most likely, these numbers simply reflect the general driving force for these isomeric guest molecules to enter a (mismatched) hydrophobic binding site. In contrast, a larger difference was observed in the binding constants for the MINPs and their own templates: 20.7 × 10^5 M^{-1} for **10** by MINP(**10**) and 30.8 × 10^5 M^{-1} for **11** by MINP(**11**) (Table 1, entries 3 and 7). Not only so, the binding between **11** and its own MINP was always stronger than that between **10** and its own, regardless of the surface-cross-linker. This is a very interesting trend because the two compounds are isomers and have identical hydrophobes and the same hydrophilic carboxylate. The only difference between the two is the location of the carboxylate.

The carboxylate of template **10** and **11** is ionic and highly hydrophilic. It is expected to stay on the surface of the micelle during imprinting and binding, most likely ion-paired with one of the cationic surfactant headgroups. Such an arrangement also ensures the solvation of the carboxylate by water molecules, which tends to be very strong for ionic groups in aqueous solution. Because of this "hydrophilic anchoring", we expect the imprinted binding site for **11** to be deeper into the hydrophobic core of the cross-linked micelle than that for **10**.

Once the above picture is made clear, it seems fairly reasonable that the mismatched guest has the same driving force to enter the binding pocket, determined by the size of the pocket and the exposed hydrophobic surface area of the guest, with the latter being constant for the two isomers having the same naphthyl hydrophobe. The difference between the two surface-cross-linkers themselves for the mismatched guests (i.e., 1.20–1.25 × 10^5 M^{-1} with **2** and 4.0–4.3 × 10^5 M^{-1} with **5**) was totally reasonable from viewpoint of collapsed versus non-collapsed binding sites: with the binding site kept more open by the shorter cross-linker, the overall driving force for any hydrophobic guest to enter the pocket should be higher.

What is the reason for the overall stronger binding for **11** and its own MINP? We propose that it is due to the polarity of the binding site itself. Generally speaking, the deeper the binding pocket reaches into the micellar core, the smaller is its polarity. Near the surface, MINP mostly consists quaternary ammonium groups, triazoles from the click reaction, and any carbons and other functional groups from the cross-linkers. For a shallow pocket created from **10**, the binding site near the surface is quite polar from these functional groups. Deeper into the core, the MINP consists of the hydrophobic chain of **11** and DVB; the polarity thus decreases significantly. A less polar binding pocket should be more poorly solvated than a more polar one in water and should give a larger hydrophobic driving force for the binding of **11** by its own MINP. It should also be pointed out that this difference in the polarity of the binding site should be most pronounced for the "matched" guest/MINP pairs. During such a binding, the binding site is expected to desolvate nearly completely, assuming the binding site is perfectly complementary to the guest. Under such a circumstance, removing water from a nonpolar binding site (i.e., desolvation) should be more favorable than from a polar site. On the other hand, when the "mismatched" guest and MINP bind, desolvation will be incomplete due to the poor fitting of the guest in the binding pocket. The effect of desolvation necessarily will be much weaker in such a case.

Table 1 also shows that the shorter surface-cross-linker helped the nonionic template **12**.[51] The binding constant going from the longer **2** to the shorter **5** increased the K_a value from 2.84×10^5 M^{-1} to 11.2×10^5 M^{-1}, by 3.7-fold in this case (entries 10 and 11). Thus the effect of the replacement was nearly constant in all three templates (i.e., a 3-4-fold increase in binding constant). This independency from the substrates does seem to be consistent with the notion that the change was mostly in the MINP itself, as suggested by our binding-site-collapse model. The binding between **12** and MINP(**12**) was somewhat weaker than those between **10** or **11** and their corresponding MINPs. The difference probably reflected the favorable electrostatic interactions between the anionic templates and their cationic MINPs. We have shown previously that electrostatic interactions did play a significant role in the MINP binding when the surfactant and the template carried opposite charges.[38]

Two-Stage Double Surface-Cross-Linking. Compound **8** is the intermediate in the synthesis of **5** (Scheme 2). The interesting feature of this compound is its multifunctionality: the two azides can be used to cross-link the micelles and the alkoxyamine may be used for another round of cross-linking using dialdehyde such as **9**. We reasoned that this type of double surface-cross-linking might not only increase the surface-cross-linking density but also expand the binding site into the polar region of the cross-linked micelle.

Scheme 2.

Table 2 compares the MINPs prepared via the traditional one-stage surface cross-linking using diazide **8** and those with the double surface-cross-linking. What we noticed was that by itself, **8** was worse than **5** and even worse than **2**, despite its C3 tether. For example, for templates **10**, **11**, and **12**, the MINPs prepared with **8** bound its own templates with a binding constant of K_a = 1.37, 2.97, and 2.31 × 10^5 M^{-1}, respectively (Table 2, entries 1, 4, and 7). These numbers were consistently lower than those for the corresponding MINPs prepared with the 4-carbon-based cross-linker **2** (Table 1, entries 1, 6, 10), let alone the 3-carbon-based **5** (Table 1, entries 3, 7, 12). We attributed the poor performance of **8** to its low water-solubility—overall, this compound is considerably more hydrophobic than the multihydroxylated **5**. As shown by the earlier data for the MINP prepared with **4**, aqueous solubility of the cross-linker is important to its reaction with the alkyne groups on the micelle and strongly affects the performance of the final MINPs.

Even though we started at a lower level for **8** as stated above, the two-stage double surface-cross-linking was very helpful. As shown in Table 2, addition of dialdehyde **9** increased the K_a values by an order of magnitude for all three templates. The changes correspond to 1.3–1.7 kcal/mol of binding free energy, suggesting that the second surface-cross-linking was quite significant to the formation of the binding pockets.

In Table 1, when **5** was used as the surface-cross-linker, the binding (between a MINP and its own template) followed the order of **11** > **10** > **12** (Table 1, entries 3, 7, 11). In the earlier discussion, we have attributed the order to the favorable electrostatic interactions between the anionic templates (**10** and **11**) and their cationic MINPs, as well as the deeper, more hydrophobic imprinted binding pocket in case of MINP(**11**). In Table 2, when the MINPs were constructed with the two-stage double surface-cross-linking, the binding followed the order of **11** > **12** > **10**.[52] Thus, although **11** remained superior in its imprinting and binding, the nonionic **12** overtook **10** in the doubly surface-cross-linked micelles. One likely reason is that, in the expanded imprinted pockets, the multiple hydroxyl groups from **9** might be engaged in hydrogen-bonding interactions with the hydroxylated portion of **12**. Although the expanded portion of the binding site is fairly hydrophilic being composed of functional groups from **8** and **9**, guest binding will partially desolvate the binding site, facilitating its hydrogen-bonding interactions with the template.

Table 2

We also tried glyoxal (HCO-CHO), another water-soluble dialdehyde for the second surface-cross-linking but saw no improvement at all in the binding properties. Glyoxal itself exists as a mixture of cyclic and acyclic oligomers of the hydrate, similar to other small aldehydes. ^{1}H NMR spectroscopy showed that the parent compound(s) disappeared after cross-linking (catalyzed by aniline). Although we did not see any aldehyde peaks for the cross-linked MINPs but that could simply mean that the residual aldehyde existed as a hydrate. It is possible that the two aldehyde groups in glyoxal were simply too close to allow the compound to bridge the alkoxyamine groups on the surface of the micelle for the second round of cross-linking.

Another improvement of the doubly surface-cross-linked MINPs was in their binding selectivity. Table 2 shows that the ratio of binding constants between **10** and **11** was 12.9/1.9 = 6.6 for MINP(**10**).

This number was higher than that for MINP(**10**) prepared with **5** as the surface cross-linker (Table 1, 20.7/4.04 = 5.1). The ratio of binding constants between **11** and **10** for MINP(**11**) was 33.7/2.9 = 11.6 (Table 2), also higher than the corresponding ratio for MINP(**11**) prepared with **5** as the surface cross-linker (Table 1, 30.8/4.25 = 7.2). Compounds **10** and **11** are isomeric structures with small differences; it is encouraging that the two-stage double surface-cross-linking consistently improved the binding selectivity among highly similar structural analogues.

Conclusions

Even though selective molecular recognition in water is considered highly challenging due to the compromise of hydrogen bonds by solvent competition,[1-2] the molecularly imprinted cross-linked micelle is a versatile platform for creating nanoparticle receptors to bind all kinds of molecules in water.[41-43, 45, 48-49] In this work, we have shown that the surface-cross-linker could be tuned rationally to enhance the binding properties of MINPs. Shortening the tethers between the two azides by even one carbon clearly helped the binding, most likely by keeping the binding pockets in the open state prior to binding. Two-stage double surface-cross-linking was another useful strategy, enabled by the multifunctionality of compound **8**. The two-stage cross-linking could not only increase the surface-cross-linking density of the MINP but also expand the imprinted binding site into the polar region of the cross-linked micelle. It is important that these strategies can help any guests, ionic or nonionic, in terms of binding affinity and selectivity. Favorable hydrogen-bonding interactions could also be introduced through this strategy between the hydrophilic portion of the template and the MINP. Finally, good water-solubility is key to the performance of the surface-cross-linker. Although micelles have certain capacity to solubilize hydrophobic molecules in water, our formulation normally includes 1 equivalent of DVB to the cross-linkable surfactant. Since this is the highest amount of DVB that could be solubilized by surfactant **1** in the micelle,[38] it is good not to "burden" the micelle with any additional nonpolar solutes such as a poorly water-soluble surface-cross-linker.

Supporting Material

ITC titration curves, additional figures, and NMR spectra of key compounds (PDF).

Acknowledgments

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Competing Interests

There are no conflict of interest to declare.

Authors' Contribution

S.Z. performed the research. Y.Z. and S.Z. designed the research study and analyzed the data. Y.Z. wrote the paper.

Materials and Methods

Syntheses of compounds **1**–**3**,[38], **4**,[53] **6**,[54] **7**,[55] and **9**[56] have been reported.

Compound 8. A solution of compound **6** (2.99 g, 21.4 mmol) and **7** (3.94 g, 42.8 mmol) in ethanol (30 mL) was stirred at 50 °C for 24 h. After the solvent was removed by rotary evaporation, the residue was purified by column chromatography over silica gel using 1: 2 ethyl acetate/hexane as the eluent to afford a colorless oil (1.72 g, 38 %). ^{1}H NMR (600 MHz, CCl_3D, δ): 5.50 (s, 2H), 4.34 (t, J □ 6.0 Hz, 2H), 4.27 (s, 2H), 4.00 (s, 2H), 3.92 (t, J □ 6.0 Hz, 2H) ppm. ^{13}C NMR (150MHz, CCl_3D, δ): 150.6, 73.6, 72.7, 51.1, 45.7 ppm. ESI - HRMS calcd for $C_5H_{10}N_8O_2$ (*m/z*): $[M + H]^+$, 215.0927; found, 215.0996.

Compound 5. A solution of compound **8** (437 mg, 2.1 mmol) and D-glucose (1875 mg, 10.5 mmol) in a 3:1 mixture of methanol and water (30 mL) was stirred at 50 °C for 24 h. After the solvents were removed by rotary evaporation, the residue was dissolved in a 3:1 mixture of acetone and methanol and allowed to sit at room temperature overnight. The precipitate was removed by filtration and the filtrate was concentrated by rotary evaporation to obtain a pale yellow oil, which was dissolved in 30 mL of methylene chloride. The solution was allowed to stand at -20 °C for 30 min and the solution was

carefully decanted to discard the precipitate formed. The process was removed two other times and the solution obtained was concentrated by rotary evaporation to give a colorless oil (710.6 mg, 90 %). The product was a 4:1 mixture of the *E*/*Z* isomers. ^{1}H NMR (400 MHz, D_2O, δ): 7.42 (d, *J* □ 8.0 Hz, 1H), 4.23 (m, 5H), 4.16 (s, 2H), 3.97 (s, 2H), 3.81 (d, *J* □ 8.0 Hz, 1H), 3.65 (m, 2H), 3.47 (m, 2H). ^{13}C NMR (100 MHz, D_2O, δ): 153.5, 152.6, 151.5, 72.8, 72.5, 72.3, 71.9, 71.0, 70.8, 70.7, 70.7, 70.3, 70.1, 69.9, 66.3, 62.8, 62.6, 50.7, 50.7, 45.9, 45.8 ppm. ESI - HRMS calcd for $C_{11}H_{20}N_8O_7$ (*m/z*): $[M + H]^+$, 377.1455; found, 377.1526.

Molecularly Imprinted Nanoparticles (MINPs). A typical procedure is as follows.[38] To a micellar solution of compound **1** (9.3 mg, 0.02 mmol) in H_2O (2.0 mL), divinylbenzene (DVB, 2.8 μL, 0.02 mmol), the appropriate template in H_2O (10 μL of a solution of 40 mM, 0.0004 mmol), and 2,2-dimethoxy-2-phenylacetophenone (DMPA,10 μL of a 12.8 mg/mL solution in DMSO, 0.0005 mmol) were added. The mixture was subjected to ultrasonication for 10 min before compound **2** (4.13 mg, 0.024 mmol), $CuCl_2$ (10 μL of a 6.7 mg/mL solution in H_2O, 0.0005 mmol), and sodium ascorbate (10 μL of a 99 mg/mL solution in H_2O, 0.005 mmol) were added. After the reaction mixture was stirred slowly at room temperature for 12 h, compound **3** (10.6 mg, 0.04 mmol), $CuCl_2$ (10 μL of a 6.7 mg/mL solution in H_2O, 0.0005 mmol l), and sodium ascorbate (10 μL of a 99 mg/mL solution in H_2O, 0.005 mmol) were added. After being stirred for another 6 h at room temperature, the reaction mixture was transferred to a glass vial, purged with nitrogen for 15 min, sealed with a rubber stopper, and irradiated in a Rayonet reactor for 12 h. The reaction mixture was poured into acetone (8 mL). The precipitate was collected by centrifugation and washed with a mixture of acetone/water (5 mL/1 mL) three times. The crude produce was washed by methanol/acetic acid (5 mL/0.1 mL) three times until the emission peak at 480 nm (for the dansyl) disappeared and then with excess methanol. The off white powder was dried in air to afford the final MINP (typically 70–80%). For the two-stage surface-cross-linking, compound **8** was used instead of **2** in the above procedure. After 0.012 mmol of **9** and aniline (2.19 μL, 0.024mmol) were added, the reaction mixture was stirred for another 12 h before the core-cross-linking took place.

Aniline is known to catalyze oxime formation in aqueous solution.[57] All other procedures were identical.

Determination of Binding Constants by ITC. The determination of binding constants by ITC followed standard procedures.[58-60] In general, a solution of an appropriate guest in Millipore water was injected in equal steps into 1.43 mL of the corresponding MINP in the same solution. 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 MINP to the guest. The smooth solid line is the best fit of the experimental data to the sequential binding of *N* binding site on the MINP. The heat of dilution for the guest, obtained by titration carried out beyond the saturation point, was subtracted from the heat released during the binding. Binding parameters were auto-generated after curve fitting using Microcal Origin 7.

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51. Because the compound differed significantly from 10 and 11, we did not perform additional selectivity studies.

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Table 1. Binding data for the MINPs prepared with different surface-cross-linkers.[a]

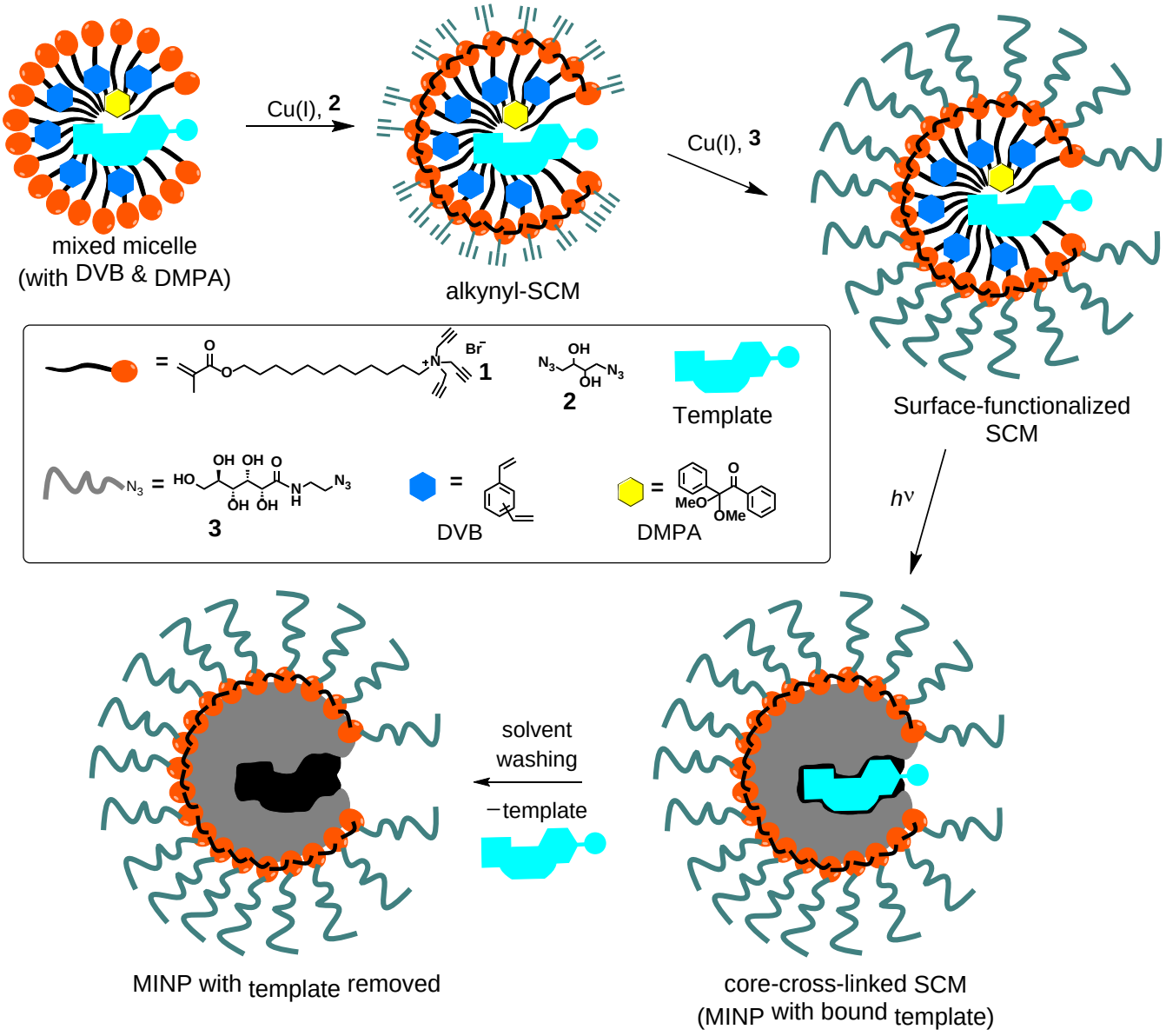
entry	template	cross-linker	guest	K_a ($\times 10^5$ M^{-1})	N	ΔG (kcal/mol)	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)
1	**10**	**2**	**10**	6.46 ± 2.05	0.71± 0.08	-7.9	-11.04 ± 1.8	-3.5
2	**10**	**4**	**10**	2.28 ± 0.71	1.07 ± 0.35	-7.3	-20.8 ± 8.2	-13.5
3	**10**	**5**	**10**	20.7 ± 3.0	0.86 ± 0.08	-8.7	-107.6 ± 11.7	-98.9
4	**10**	**2**	**11**	1.25 ± 0.14	0.74 ± 0.08	-7.0	-14.9 ± 2.0	-7.9
5	**10**	**5**	**11**	4.04 ± 1.15	1.12 ± 0.054	-7.6	-5.3 ± 0.4	2.3
6	**11**	**2**	**11**	7.59 ± 0.56	1.02 ± 0.01	-8.1	-20.6 ± 0.2	-12.5
7	**11**	**5**	**11**	30.8 ± 3.0	1.08 ± 0.01	-8.8	-103.3 ± 1.8	-94.5
8	**11**	**2**	**10**	1.20 ± 0.08	1.15 ± 0.03	-7.0	-3.8 ± 0.1	3.2
9	**11**	**5**	**10**	4.25 ± 1.45	0.89 ± 0.12	-7.7	-2.1 ± 0.4	5.6
10	**12**	**2**	**12**	2.84 ± 0.72	0.84 ± 0.14	-7.5	-41.8 ± 8.5	-34.3
11	**12**	**5**	**12**	11.2 ± 3.3	1.07 ± 0.06	-8.3	-22.7 ± 1.8	-14.4

[a] The titrations were performed in duplicates in 50 mM Tris buffer (pH=7.3) and the errors between the runs were <20%.

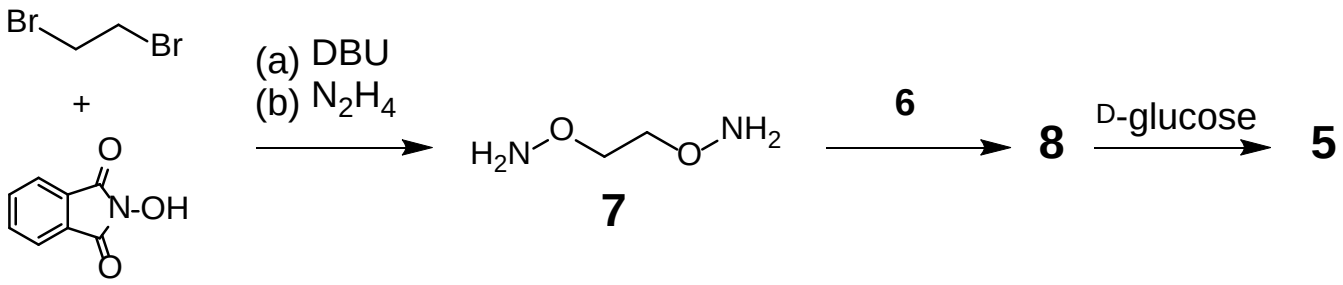
Table 2. Comparison of binding properties of MINPs prepared via one- and two-stage surface-cross-linking.[a]

entry	template	cross-linker	guest	K_a ($\times 10^5$ M^{-1})	N	ΔG (kcal/mol)	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)
1	**10**	**8**	**10**	1.37 ± 0.63	1.03 ± 0.85	-7.0	-16.4 ± 15.2	-9.4
2	**10**	**8** + **9**	**10**	12.5 ± 1.1	1.03 ± 0.01	-8.3	-75.6 ± 1.5	-67.3
3	**10**	**8** + **9**	**11**	1.9 ± 0.5	1.01 ± 0.07	-7.2	-27.1 ± 2.6	-19.9
4	**11**	**8**	**11**	2.97 ± 0.18	1.02 ± 0.01	-7.3	-38.6 ± 0.4	-31.3
5	**11**	**8** + **9**	**11**	33.7 ± 9.2	0.95 ± 0.03	-9.0	-15.0 ± 0.6	-6.0
6	**11**	**8** + **9**	**10**	2.9 ± 2.1	0.91 ± 0.03	-7.5	-75.4 ± 3.2	-67.9
7	**12**	**8**	**12**	2.31 ± 1.09	1.17 ± 0.07	-7.3	-11.5 ± 1.5	-4.2
8	**12**	**8** + **9**	**12**	24.7 ± 3.8	0.88 ± 0.02	-8.6	-132.6 ± 2.8	-124.0

[a] The titrations were performed in duplicates in 50 mM Tris buffer (pH=7.3) and the errors between the runs were <20%. [**1**]/[**8**] = 1:1.2 for the one-stage surface cross-linking. [**1**]/[**8**]/[**9**] = 1:1.2:0.6 for the two-stage surface cross-linking. Excess (3 equiv) of surface ligand **3** was used in all the MINPs to terminate all the surface alkyne groups.



Scheme 1. Preparation of MINP by surface-cross-linking of micelle of **1** with diazide **2**, surface decoration of the alkynyl-surface-cross-linked micelle (alkynyl-SCM) by ligand **3**, and core-cross-linking of the SCM to afford MINP with an internal binding site complementary to the template molecule.

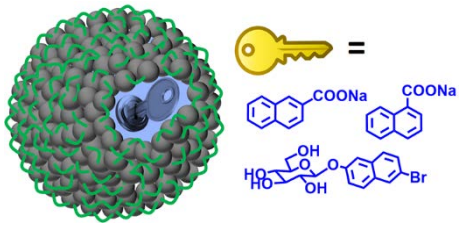


Scheme 2. Syntheses of cross-linkers **8** and **5**.

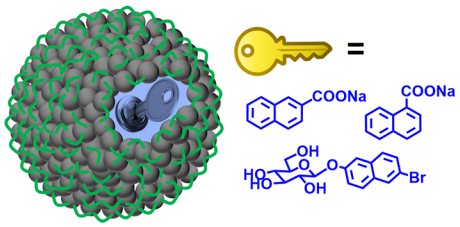
TOC graphic

Tuning Surface-Cross-Linking of Molecularly Imprinted Cross-Linked Micelles for Molecular Recognition in Water

*Shize Zhang and Yan Zhao**



Molecularly imprinted micelles are a versatile platform for creating water-soluble nanoparticle receptors for various guest molecules. The binding properties of these receptors could be controlled rationally through increased surface-cross-linking density and judicious two-stage surface-cross-linking that enhances the rigidity of the binding pocket and expands the pocket into the polar region of the cross-linked micelle.



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