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Surface Ligands in the Imprinting and Binding of Molecularly Imprinted Cross-Linked Micelles

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Abstract

Molecular recognition in water is challenging but water-soluble molecularly imprinted nanoparticle (MINP) receptors were produced readily by double cross-linking of surfactant micelles in the presence of suitable template molecules. When the micellar surface was decorated with different polyhydroxylated ligands, significant interactions could be introduced between the surface ligands and the template. Flexible surface ligands worked better than rigid ones to interact with the polar moiety of the template, especially for those template molecules whose water-exposed surface is not properly solvated by water. The importance of these hydrophilic interactions was examined in the context of different substrates, density of the surface ligands, and surface-cross-linking density of the MINP. Together with the hydrophobic interactions in the core, the surface hydrophilic interactions can be used to enhance the binding of guest molecules in water.

Keywords

molecular imprinting; binding; nanoparticle; micelle; cross-linking

Introduction

In molecular recognition, molecules interact with one another by noncovalent forces to form specific supramolecular complexes. The process can occur between small and large molecules, and is essential to many biological processes including ligand–receptor binding, enzymatic catalysis, and selective transport of nutrients across membranes. For molecular recognition to occur, the host molecule needs to possess a guest-complementary binding surface, ideally poorly solvated prior to binding. Traditionally, a concave structure is synthesized, preorganized by the covalent framework to engage multiple noncovalent forces with the guest molecule. This approach has led to countless small-molecule hosts (1–3) including macrocycles such as cyclodextrin, crown ether, and calixarene, as well as more flexible hosts (4–10) that utilize guest-triggered conformational changes to amplify guest binding (11–12). As the guest molecule becomes larger in size and more complex in structure, however, design and synthesis of its molecular host become increasingly

challenging, because the guest-complementary binding surface increases exponentially not only in size but also in complexity of the binding functionalities.

Molecular imprinting (13–14) takes a different approach to creating guest-complementary hosts (15–26). In this technique, the guest molecule (or surrogate) is used as the template. Functional monomers (FMs) interact with the template through noncovalent or cleavable covalent bonds. The template–FM complex is copolymerized and cross-linked with a large amount of a cross-linker to yield a polymer matrix with embedded template molecules. Removal of the templates leaves behind binding sites formed through the template polymerization, complementary to the template. The method is conceptually simple and highly attractive, because complementarity between the imprinted host and the guest does not require elaborate synthesis of complex organic molecules but, instead, relies on facile template–FM complexation and covalent capture by polymerization and cross-linking. Molecularly imprinted polymers (MIPs), indeed, have found broad applications in molecular recognition, separation, enzyme-mimetic catalysis, and chemical sensing (13–26). In addition to traditional macroporous polymers, imprinting could occur on surface and unimolecularly within dendrimers (27–28). Traditional MIPs are insoluble macroporous materials but soluble materials could be prepared by imprinting on polymeric nanoparticles (29–36) and within micro/nanogels (37–42).

Our group has been interested in the creation of functional receptors through biomimetic strategies (43–45). Recently, we reported a method to perform templated polymerization within cross-linked surfactant micelles to create molecularly imprinted nanoparticles (MINPs) (46). The method allowed us to prepare protein-sized nanoparticles with a controllable number of binding sites, thus bridging the gap between molecular receptors and macroscopic MIPs. The water-solubility of MINPs enabled us to study their binding by techniques typically used for molecular receptors, including fluorescence titration, isothermal titration calorimetry (ITC), and chemical derivatization (47).

In our previous studies, the binding functionalities were installed exclusively in the hydrophobic binding pocket created in the core of the MINP. Although the method worked successfully for many guests including nonsteroidal anti-inflammatory drugs (NSAIDs) (48), carbohydrates (49–50), and peptides (51–53), further improvement in binding affinity and selectivity is needed, particularly for molecules whose hydrophobic interactions are insufficient. In this paper, we report that the surface ligands on the MINP could contribute significantly to its binding but the interactions varied largely depending on the nature of the substrate and the structure of the surface ligands. The study thus revealed a previously neglected aspect of molecular recognition in our imprinted materials. Because the hydrophilic interactions provide additional driving force to the binding, they could be used rationally to enhance the binding of substrates with strong hydrophilicity.

Results and Discussion

Design and Synthesis of Surface-Functionalized MINPs

Synthesis of MINPs is shown in Scheme 1. Surfactant **1** has two sets of orthogonal cross-linkable groups: the tripropargylammonium headgroup can be cross-linked with diazide **2**

through the click reaction (54–55), and the methacrylate at the end of the hydrophobic tail can be co-polymerized with divinylbenzene (DVB) by free radical polymerization. The cross-linked micelle is typically functionalized by an azide-containing surface ligand (**3**) to enhance its water-solubility. The double cross-linking covalently fixes the micelle around the solubilized template molecule, converting the micelle into the molecularly imprinted nanoparticle (MINP). We often use a surfactant/template ratio of 50:1 in the preparation, as dynamic light scattering (DLS) estimated that each MINP contained approximately 50 (cross-linked) surfactant molecules. The 50:1 surfactant/template ratio would then afford one binding site per nanoparticle on average. As shown in a previous work of ours, the number of binding site could be tuned using different surfactant/template ratios in the preparation (46).

Surface ligand **3**, prepared from δ -gluconolactone and 2-azidoethylamine, is used in all our previous MINP syntheses. It is installed for two primary purposes. First, it creates a hydrophilic layer on the surface of the MINP, enhancing its solubility in water. Second, due to its poor solubility in typical organic solvents, we can recover the MINP conveniently by pouring the aqueous reaction mixture into acetone and wash off the template and other impurities using acetone/water and methanol (46).

In principle, we can decorate the surface of MINP with any azide-containing ligands (54). Because we had used different surface ligands on the cross-linked micelles to modulate their interactions with lipid bilayer membranes (56), we thought the surface ligands might be made to interact with the hydrophilic moiety of the template molecule to further strengthen the binding. In this study, we synthesized azido sugars **4–6** from glucose, cellobiose, and maltose, respectively, and employed them as surface ligands for the MINPs. The sugars vary in length and have different curvatures for **5** and **6**, due to the β and α glycosidic linkage between the two monosaccharides, respectively. We reasoned that, together with the linear and flexible **3**, ligands **4–6** would provide enough diversity for us to identify the important factors in the hydrophilic interactions on the micelle surface.

For our investigation, we used three templates (**7–9**). Compound **8** and **9** are commercially available and **7** was reported by us previously (46). These templates have different size, shape, and, importantly, distribution of hydrophobic/hydrophilic groups. Compound **7** is a facially amphiphilic derivative of bile salt; its hydrophilic and hydrophobic groups are on two opposite faces (57). Similar to **7**, compound **8** has an anionic carboxylate but contains an aromatic hydrophobe. Compound **9** is similar to **8** in its aromatic hydrophobic group but

has a nonionic hydrophilic sugar moiety. Our idea was that the different hydrophilic groups of these templates would interact with the surface ligands of the MINP differently and help us understand the key factors influencing the surface interactions. Previously, our study typically focused on the binding interactions in the hydrophobic core of the micelles (46).

The preparation of MINPs followed Scheme 1 and the detailed procedures are reported in the Experimental Section. In general, we monitored the surface-cross-linking and core-polymerization/cross-linking by ^1H NMR spectroscopy and DLS (Supplementary Material) (46). ^1H NMR spectroscopy showed disappearance of alkenic protons as the surfactant and DVB (core-cross-linker) underwent free radical polymerization. DLS showed an increase in size of the nanoparticles as surface ligands were attached and a slight decrease in size when core-polymerization shrank the cross-linked micelles. DLS also allowed us to estimate the molecular weight of the MINP and the number of (cross-linked) surfactants within the MINP. The surface-cross-linking has been confirmed by mass spectrometry (after cleaving the surface-cross-linkages) (58) and the DLS size by transmission electron microscopy (TEM) (51).

Effects of Surface Ligands on the Imprinting and Binding of MINPs.

Because the templates are fluorescent, we could determine the binding properties of the MINPs by fluorescence titration. Figure 1 shows the emission spectra of compound **7** upon titration with the MINP prepared with **7** as the template and **3** as the surface ligand, i.e., MINP₃(**7**). Addition of the MINP shifted the emission maximum of dansyl from ~550 to ~490 nm. Meanwhile, the emission intensity increased significantly. This was expected behavior for dansyl when it migrated from a polar to a nonpolar environment (59) and supports the binding of **7** by its MINP. The fluorescence data fit well to a 1:1 binding model and gave a binding constant (K_a) of $(230 \pm 40) \times 10^4 \text{ M}^{-1}$ in water (Figure 1b).

ITC is another commonly used technique to study binding (60). In addition to the binding constant, it affords a number of other important parameters including binding enthalpy (H) and the number of binding sites per particle (N). The binding free energy (G) can be calculated from K_a using equation - $G = RT\ln(K_a)$, and S from G and H . Our ITC titration of MINP₃(**7**) by template **7** showed a negative/favorable enthalpy. The data afforded a K_a value of $(230 \pm 31) \times 10^4 \text{ M}^{-1}$ (Table 1, entry 1), in excellent agreement with the value from the fluorescence titration.

Table 1 summarizes the binding data obtained for all three templates by the different MINPs. Entries 1–3 show the selectivity of our imprinted materials. Among the three template/guests studied, MINP₃(**7**) bound the template most strongly, with **8** and **9** showing 1/9 and 1/35 of the K_a value of **7**. The selectivity was expected, as our previous work has shown that the hydrophobic pocket generated through imprinting tends to be highly discriminating (46).

To our delight, the surface ligands indeed had a very pronounced effect on the MINPs prepared from template **7**. The binding for the template was the strongest with surface ligand **3** ($K_a = 230 \times 10^4 \text{ M}^{-1}$, entry 1) and the weakest with ligand **6** ($K_a = 22.6 \times 10^4 \text{ M}^{-1}$, entry 6). The difference was thus 10-fold even though the binding pocket inside the core stayed the same. For templates **8** and **9**, however, although ligand **3** remained the best, the strongest/

weakest ratio was much smaller, only 2.7 and 1.2, respectively. Therefore, the surface ligand of MINP could play quite a significant role in the molecular recognition, but the magnitude of the effect strongly depended on the template used.

The difference between the three templates is in the nature and size of their hydrophobe and the distribution of functional groups. Templates **8** and **9** both have a relatively small aromatic naphthyl group but possess an anionic and a nonionic hydrophilic group, respectively. The charge character was reflected in the overall stronger binding of **8** over **9**, presumably due to the electrostatic interactions between the negatively charged **8** and the positively charged MINP. These two templates is similar to a head/tail surfactant in their hydrophilic/hydrophobic arrangement, locating on the opposite ends of the molecule. As the hydrophobic group resides in the hydrophobic core of the cross-linked micelle, the hydrophilic groups are expected to point into the water and be solvated by water molecules.

For template **7**, the situation is different. In addition to the anionic carboxylate at the end of the molecule, the hydrophilic groups (hydroxyl and sulfonamide) are located on the opposite side of a very large hydrophobic steroidal surface. The large hydrophobic surface (including that from the dansyl group) gives a strong driving force for the template to interact with the nonpolar core of the micelle. The anionic group, with its strong tendency to stay in water, is expected to anchor the template near the surface of the micelle (46). Given the strong effect of the hydrophilic surface ligands on the imprinting/binding of MINP(**7**), the hydrophilic groups of **7** most likely faced the bulk water, interacting with the hydrophilic ligand through hydrogen-bonds (directly or mediated by water molecules), shown schematically in Figure 3. Although the “hydrophilic” face of **7** contains three hydrophilic groups, the rest of the structure on this face remains hydrophobic, being made of hydrocarbon. The arrangement proposed in Figure 3 allows the hydroxyl groups of the attached **3** to interact with the hydrophilic face of **7**, thus avoiding a complete exposure of partially hydrophobic surface to water—this likely was the reason for the enhanced binding of MINP₃(**7**) in comparison to the MINPs prepared with other surface ligands. Being cyclic, ligands **4–6** have significantly higher rigidity than **3**, and are expected to have difficulty adopting the “folded” conformation shown in Figure 3 to interact with the bound template **7**.

Bile salts are well-known to form mixed micelles with head/tail surfactants and lipids (62). In the most accepted model, the bile salt has its hydrophilic groups facing water, with its steroidal backbone facing the hydrocarbon core of the micelle (63), as we have proposed in Figure 3. In the same model, cholates can insert their steroidal backbone into the hydrophobic core of the mixed micelle, usually as dimers with their hydroxyl groups hydrogen-bonded to one another to avoid unfavorable hydroxyl–hydrocarbon contact. The latter scenario is not supported by our binding data, since the surface ligands exerted a strong influence on the binding of **7**, indicating significant interactions between the two—dimers with buried hydroxyl groups should not be able to do so. Furthermore, our ITC titrations revealed the number of binding site on average was close to 1 (Table 1), a result from the surfactant/template ratio of 50. The dimer model of the bile salt would predict at least two binding sites per nanoparticle with cooperative binding behavior. In a previous work of ours, we have shown that, even when two binding sites were generated for template

7 (with a surfactant/template ratio of 25), they are independent, possessing identical binding affinities within experimental error (46).

The binding model in Figure 3 is also consistent with the weak effect of surface ligands on templates **8** and **9**. These templates have their hydrophilic groups opposite to the aromatic hydrophobe, immersed in water. Being well-solvated, they have a much smaller need to interact with the hydroxyl groups of **3**.

Effects of “Double Surface-Functionalization”

In this study, the ratio of **1** to **2** was 1:1.2, leaving on average 0.8 alkyne per surfactant if the surface cross-linking happened quantitatively. The surface ligand was attached to the cross-linked surfactant by the click reaction to the quaternary ammonium headgroup. A previous study of ours indicated that the click functionalization was extremely efficient. Even for a polymeric azide (i.e., PEG 2000), 70–80% of the residual alkynes on the micelle surface could be successfully functionalized (54). For smaller surface ligands used in this study, we assumed the surface functionalization was quantitative.

The usage of cross-linker **2** translates to a significant distance between neighboring surface ligands, due to the many bonds in between the two ammonium headgroups (Figure 4a). We hypothesized that an increase in the density of the hydrophilic ligands might strengthen their interactions with the guest, particularly **9**, which has an abundance of hydroxyl groups. We thought the carboxylate of template **8** might be too small and thus might not benefit as much from such a change.

To increase the density of hydrophilic ligands, we designed and synthesized a new diazide cross-linker (**10**). As shown in Scheme 2, the compound was prepared in three simple steps from diazidoamine **11** and Boc-protected glycine *N*-hydroxysuccinimide ester **12**. Similar to surface ligand **3**, compound **10** carries a hydroxylated moiety derived from δ -gluconolactone (**15**).

We then prepared MINP using cross-linker **10** instead of diazide **2**. As a result, the MINP would have a layer of hydrophilic groups from the cross-linker itself. At the end of the surface cross-linking, we decorated the surface-cross-linked micelles with ligand **3** as usual, using the click reaction. Overall, we nearly doubled the surface hydrophilic groups because both the cross-linker (**10**) and the surface ligand (**3**) installed a δ -gluconolactone-derived hydrophilic ligand (Figure 4b). Since the linear and flexible ligand (**3**) was the best in our studies above, we did not use the cyclic azides (**4**–**6**) in these doubly surface-functionalized MINPs.

Table 2 summarizes the binding data for the MINPs prepared in this approach. Template **9**, indeed, benefited significantly from the double surface-functionalization. The binding constant for the template went from $8.30 \times 10^4 \text{ M}^{-1}$ (Table 1, entry 9) to $65.8 \times 10^4 \text{ M}^{-1}$ (Table 2, entry 3), an increase of 8-fold. However, the K_a value for both **7** and **8** by their own MINPs decreased upon double surface-functionalization (Table 2, entries 1 and 2). In fact, the binding for **8** was so weak that it could not even be measured in our hands.

Thus, as expected, a higher density of hydrophilic surface ligands enhanced the binding of MINP for template **9**, possibly due to multiple hydrogen bonds formed between the sugar moiety of the template and the amide/hydroxyl groups of **3** and **10**. Even though these groups are exposed to water, crowding them on the surface of the micelle should facilitate their noncovalent interactions.

Why did the other two templates suffer under the same condition? One possibility is the reduced surface cross-linking density. Cross-linker **2** has 4 carbons in between the two azido groups but **10** has 4 carbons and 1 nitrogen in between. Thus, at the same stoichiometry (surfactant/cross-linker = 1:1.2), the effective surface-cross-linking density should be lower in the MINPs prepared with **10** than with **2** as the surface cross-linker. A lower surface-cross-linking density is expected to decrease the rigidity of the binding pocket, detrimental to the binding.

If the lower surface cross-linking density is indeed the reason for the weaker binding for **7** and **8** (64), our results also imply that this parameter was more critical to the binding of guests with a smaller hydrophobe (i.e., **8**) than those with larger ones (i.e., **7**). The substitution of **2** by **10**, for example, lowered the K_a value of **7** by 9-fold (compare entry 1 of Table 1 and 2) but reduced that of **8** to undetectable. The result does make sense. Without the proposed hydrophilic interactions—due to its small-sized carboxylate—compound **8** needs to rely heavily on the hydrophobic interactions between the imprinted binding site and its naphthyl group. A poorly formed, narrow hydrophobic binding pocket for the naphthyl group (due to the low surface cross-linking density) could easily collapse in water, preventing the binding of the guest. For a template (i.e., **7**) with a much larger hydrophobic group, complete collapse of a correspondingly large binding pocket would cause too much stress to the cross-linked network—this could be the reason why the bile salt retained a reasonable binding affinity despite a lower surface-cross-linking density.

Conclusions

Molecular recognition in water is a very challenging topic in supramolecular chemistry because directional noncovalent interactions are often weakened significantly by competition from the solvent (65–66). MINPs, on the other hand, can bind various guests strongly and selectively in water (48–52, 67). Our previous studies focused exclusively on the molecular recognition between the template and the binding site in the hydrophobic core of the cross-linked micelle. Although the strategy worked quite successfully for a number of different templates, this work suggested there is an important, overlooked opportunity for additional improvement. Since the surface ligands could interact with the hydrophilic groups of the templates significantly, we could install different surface ligands—not limited to the ones studied in this work—to interact with the templates by design. We have reported a strategy to double the density of the surface ligands in this work. We expect similar approaches could increase the density further and magnify the hydrophilic interactions.

Another important learning from this study comes from the realization that the contribution of different intermolecular forces could be tuned quite rationally in our system. For example, Table 1 shows that binding of **8** by its MINPs was generally higher than that of **9**, most

likely due to the electrostatic interactions present in the former that are absent in the latter. However, with the double surface-functionalization, binding of **9** was enhanced while that of **8** disappeared. Since the lower surface-cross-linking density deteriorated the bindings of **7** and **8**, the enhancement of binding in **9** was even more significant, as the lower surface cross-linking density probably decreased the hydrophobic contribution to the binding of **9** just as it did to **7** and **8**.

Finally, this work shows that the surface-cross-linking density in MINP played a significant role in the imprinting and can be very sensitive to minute changes in structure of the surface cross-linker, especially for templates with relatively small/narrow hydrophobes. The learning will be useful in the design of future MINPs with improved binding properties.

Experimental Section

Synthesis

Syntheses of compounds **1**, **2**, **3**, and **7** were previously reported (46). Compounds **4–6** (68), **11** (69), and **12** (70) were synthesized according to literature procedures.

Compound 13.—A mixture of **11** (6.21g, 40.0 mmol) and **12** (5.425g, 20.0 mmol) in 100 mL dry CH₃CN was stirred at 70°C for 24 h. The solvent was removed by rotary evaporator and the residue was purified by column chromatography over silica gel using 20:1 methylene chloride/methanol as the eluent to afford an oily product (5.43 g, 87%). ¹H NMR (400 MHz, CDCl₃, δ): 4.03 (d, *J* = 4.6 Hz, 2H), 3.59–3.53 (m, 4H), 3.53 – 3.45 (m, 4H), 1.45 (s, 9H). ¹³C NMR (100 MHz, CDCl₃, δ): 169.6, 156.1, 80.1, 50.0, 49.6, 47.5, 46.7, 42.7, 28.7. ESI-HRMS (*m/z*): [M+H]⁺ calcd for C₁₁H₂₁N₈O₃, 313.1732; found 313.1728.

Compound 10.—A mixture of δ-gluconolactone (0.30g, 1.68 mmol) and **13** (1.07g 5.04 mmol) in pyridine (15 mL) was stirred at 65 °C for 16 h. The mixture was poured into water and washed with chloroform (3 × 20 mL) and ethyl ether (3 × 20 mL). The aqueous phase was concentrated to afford a white powder (0.64 g, 98%). ¹H NMR (400 MHz, D₂O, δ) : 4.40 (d, *J* = 4.0 Hz, 1H), 4.31 (d, *J* = 3.6 Hz, 1H), 4.12 (dt, *J* = 3.9, 2.4 Hz, 1H), 3.98–3.73 (m, 3H), 3.71–3.49 (m, 10H). ¹³C NMR (100 MHz, D₂O, δ): 174.6, 170.9, 73.3, 71.9, 71.0, 70.3, 62.6, 48.9, 48.3, 46.7, 45.3, 40.8. ESI-HRMS (*m/z*): [M+H]⁺ calcd for C₁₂H₂₃N₈O₇, 391.1684; found 391.1681.

Preparation of Molecularly Imprinted Nanoparticles (MINPs)

A typical procedure is as follows (46). To a micellar solution of compound **1** (9.3 mg, 0.02 mmol) in H₂O (2.0 mL), divinylbenzene (DVB, 2.8 μL, 0.02 mmol), compound **7** in H₂O (10 μL of a solution of 26.5 mg/mL, 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), CuCl₂ (10 μL of a 6.7 mg/mL solution in H₂O, 0.0005 mmol), and sodium ascorbate (10 μL of a 99 mg/mL solution in H₂O, 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), CuCl₂ (10 μL of a 6.7 mg/mL solution in H₂O, 0.0005 mmol), and sodium

ascorbate (10 μ L of a 99 mg/mL solution in H₂O, 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. ¹H NMR spectroscopy was used to monitor the progress of reaction. 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 (16 mg, 80%).

Determination of Binding Constants by Fluorescence Titration

A typical procedure is as follows. A stock solution containing MINP₃(**7**) (150 μ M) was prepared in Millipore water. Aliquots (2.0 μ L) of the MINP stock solution were added to 2.00 mL of the solution of **7** in Millipore water (0.2 μ M). After each addition, the sample was allowed to sit for 1 min at room temperature before the fluorescence spectrum was collected. The excitation wavelength (λ_{ex}) was 340 nm. The excitation slit width was 10 nm, and the emission slit width was 10 nm. The binding constant was obtained by nonlinear least squares curve fitting of the fluorescence intensity at 491 nm to a 1:1 binding isotherm.

Determination of Binding Constants by ITC

The determination of binding constants by ITC followed standard procedures (71–73). 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.

Preparation of MINP by surface-cross-linking of the micelle of **1**, surface decoration of the alkynyl-surface-cross-linked micelle (alkynyl-SCM) by ligand **3**, and core-cross-linking of the resulting material.

Figure 1.

(a) Emission spectra of compound **7** in the presence of 0–1.7 μM of MINP₃(**7**) in Millipore water. [**7**] = 0.5 μM . λ_{ex} = 340 nm. (b) Nonlinear least squares curve fitting of the fluorescence intensity at 491 nm to a 1:1 binding isotherm.

Figure 2.
ITC curve obtained at 298 K from titration of MINP₃(**7**) with **7** in Millipore water.
MINP₃(**7**) = 10 μM in the cell. The concentration of **7** in the syringe was 0.20 mM.

Figure 3.

Proposed binding of template 7 by MINP₃(7), with possible hydrogen bonds between the surface ligand and the hydrophilic groups of the template, possibly also mediated by water molecules.

Figure 4. Schematic representation of the cross-linking and surface ligands in MINP_{3,2} (a) and MINP_{3,10} (b).

Scheme 2. Synthesis of cross-linker **10** containing a sugar-derived group.

Table 1. Binding data for the MINPs prepared with different surface ligands.^a

Entry	MINPs	Guest	$K_a (\times 10^4 \text{ M}^{-1})$	N	G (kcal/mol)	H (kcal/mol)	$T S$ (kcal/mol)
1	MINP ₃ (7)	7	230 ± 31.1 (230 ± 40)	0.71 ± 0.05	-8.7	-0.28 ± 0.04	8.4
2	MINP ₃ (7)	8	(25.06 ± 3.47)		-7.4		
3	MINP ₃ (7)	9	(6.54 ± 1.42)		-6.6		
4	MINP ₄ (7)	7	42.1 ± 1.12 (44.3 ± 0.9)	0.83 ± 0.04	-7.7	-1.02 ± 0.11	6.6
5	MINP ₅ (7)	7	30.1 ± 1.04 (31.5 ± 1.9)	0.68 ± 0.09	-7.5	-9.23 ± 0.17	-1.8
6	MINP ₆ (7)	7	22.6 ± 1.05 (21.6 ± 1.1)	1.14 ± 0.27	-7.8	-4.15 ± 0.14	3.1
7	MINP ₃ (8)	8	50.9 ± 4.70	0.98 ± 0.03	-7.8	-1.63 ± 0.14	6.2
8	MINP ₄ (8)	8	21.8 ± 1.05	0.80 ± 0.03	-7.3	-1.74 ± 0.33	5.5
9	MINP ₅ (8)	8	27.4 ± 1.4	0.98 ± 0.02	-7.4	-6.94 ± 0.25	0.5
10	MINP ₆ (8)	8	18.7 ± 1.66	0.72 ± 0.07	-7.2	-7.66 ± 0.14	-0.3
11	MINP ₃ (9)	9	8.27 ± 1.60	1.20 ± 0.05	-6.7	-1.46 ± 0.09	5.3
12	MINP ₄ (9)	9	5.26 ± 0.14	1.21 ± 0.10	-6.4	-0.60 ± 0.05	5.9
13	MINP ₅ (9)	9	3.46 ± 0.57	1.19 ± 0.06	-6.2	-0.71 ± 0.05	5.7
14	MINP ₆ (9)	9	6.60 ± 0.13	1.22 ± 0.07	-6.6	-1.31 ± 0.1	5.3

^aThe titrations were performed in duplicates in Millipore water and the errors between the runs were <20%. The binding constants in parentheses were from fluorescence titration.

^bThe subscript denotes the surface-ligand (3–6) used in the MINP synthesis and the number in parentheses (7–9) the template molecule.

Table 2.

Binding data for the MINPs prepared with surface ligand **3** and diazide cross-linker **10**.^a

Entry	MINPs	Guest	$K_a (\times 10^4 \text{ M}^{-1})$	N	G (kcal/mol)	H (kcal/mol)	$T S$ (kcal/mol)
1	MINP _{3,10} (7)	7	26.2 ± 1.79 (26.9 ± 1.5)	0.95 ± 0.24	-7.4	-2.77 ± 0.15	4.6
2	MINP _{3,10} (8)	8	^b	-	-	-	-
3	MINP _{3,10} (9)	9	61.3 ± 1.98 (68.4 ± 2.6)	0.95 ± 0.24	-7.9	-5.76 ± 0.17	2.1

^aThe titrations were performed in duplicates in Millipore water and the errors between the runs were <20%. The binding constants in parentheses were from fluorescence titration. The subscript denotes the diazide cross-linker (**10**) and the surface-ligand (**3**) used in the MINP synthesis and the number in parentheses the template molecule.

^bThe binding was too weak to be measured.