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Molecular Recognition

Water-Soluble Molecularly Imprinted Nanoparticles (MINPs) with Tailored, Functionalized, Modifiable Binding Pockets

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Abstract: Construction of receptors with binding sites of specific size, shape, and functional groups is important to both chemistry and biology. Covalent imprinting of a photocleavable template within surface–core doubly cross-linked micelles yielded carboxylic acid-containing hydrophobic pockets within the water-soluble molecularly imprinted nanoparticles. The functionalized binding pockets were characterized by their binding of amine- and acid-functionalized guests under different pH values. The nanoparticles, on average, contained one binding site per particle and displayed highly selective binding among structural analogues. The binding sites could be modified further by covalent chemistry to modulate their binding properties.

Introduction

The active (binding, transport, or catalytic) sites of proteins are key to their intended functions. Researchers in the field of supramolecular chemistry have, over the last few decades, synthesized many synthetic receptors that mimic one or more aspects of these active sites, with the majority of them prepared through molecular synthesis.[1] Although molecular synthesis ensures synthetically pure and discrete functional molecules, the significant synthetic efforts required often become an impediment to scale-up and practical applications of the materials.

Molecular imprinting is a conceptually different approach to synthetic receptors.[2] Instead of building the receptors first and then trying to fit their guests into the structures, one simply co-polymerizes appropriate functional monomers (FMs) and cross-linkers around molecular templates. Removal of the templates generates guest-complementary binding pockets within the polymer matrix. Much progress has been made in molecularly imprinted polymers (MIPs) since Wulff[3] and Mosbach[4] respectively pioneered covalent and noncovalent imprinting (referring to the binding interactions between FMs and the template). The concept has also been extended beyond traditional macroporous polymers to imprinted surfaces[2] and even unimolecularly within dendrimers.[5]

Although molecular imprinting can create binding sites more efficiently than molecular synthesis, it is generally accepted that the binding sites obtained through imprinting are heterogeneous and less structurally defined.[2,6] According to a representative review,[7] MIPs ideally are "preparable in one (or few) high yielding synthetic step(s)", "able to be post-synthetically functionalized," and possess "homogeneous imprinted sites of high stability" with "high (binding) affinity with possibility to tune." Although highly desirable and important to the applications of MIPs, many of these features are yet to be realized with traditional imprinting techniques.

Herein, we report a method to construct tailor-made, hydrophobic binding pockets possessing specific binding functional groups in water-soluble nanoparticles. Our method has overcome some key challenges in conventional molecular imprinting including heterogeneous distribution of binding sites and difficulty in direct characterization of binding properties by spectroscopic methods. These molecularly imprinted nanoparticles (MINPs) were shown to distinguish guests based on their size, shape, and functional groups. Most interestingly, the binding pockets could be modified through standard chemistry to alter their molecular-recognition properties.[8] Different from traditional MIPs[2] or other reported imprinted nanoparticles,[9] our MINPs on average contained one binding pocket per particle, thus bridging the gap between the discrete receptors made through molecular synthesis and those less well-defined receptors made through traditional imprinting.

Results and Discussion

Materials design and synthesis

The method was a development from our recently reported molecular imprinting method using surface-cross-linked micelles (SCMs)[10] prepared from **1** (Scheme 1).[11] This surfactant (**1**) has a tripropargylammonium headgroup cross-linkable on the surface by the click reaction. The methacrylate group at the hydrophobic tail enables core cross-linking around a hydrophobic template solubilized by the micelle in water. In the pre-

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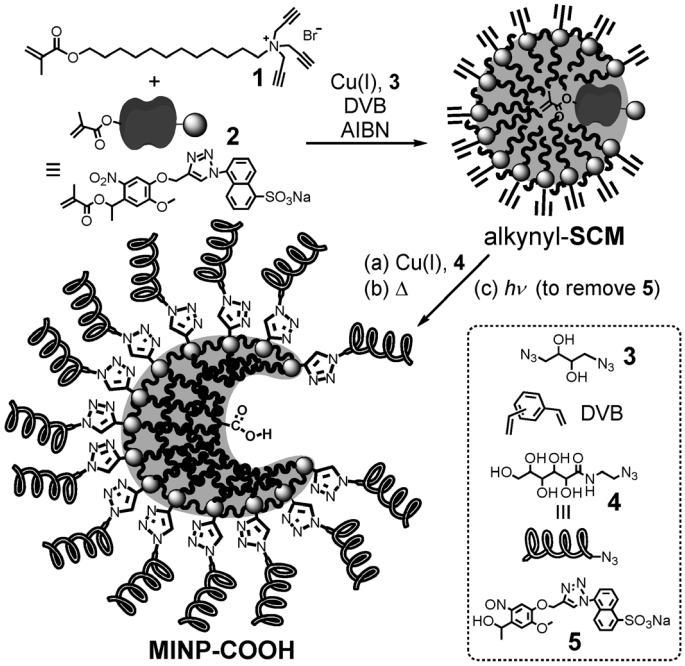


Scheme 1. Preparation of MINP–COOH.

vious work, we demonstrated that selective binding pockets could be created within the SCM for bile salts. The limitation of the previous method lies in the fact that only hydrophobic pockets with prescribed shapes could be created within the MINPs. Without specific binding groups, the pockets recognize guests primarily based on their size, shape, and hydrophobicity.

To install a functional group within a molecularly imprinted binding pocket, one normally has to employ FMs that bind the template through specific noncovalent interactions. Although such a method works well for conventional MIPs in organic media, it is completely unsuitable in our case because the entire imprinting takes place in aqueous solution. Not only do FMs with polar binding groups (e.g., methacrylic acid) tend to stay in water instead of within the hydrophobic core of the micelle, the intended noncovalent template–FM complex is also unlikely to be stable when a large amount of water is present.[12] Even if the template–FM complex is somehow made stable inside the micelle, the polar FM most likely would stay at the surfactant/water interface instead of in the hydrophobic core of the micelle. As a result, even if such imprinting is made to work, it will be difficult to have the polar functional group deep within the hydrophobic core of the resulting MINP. Needless to say, for molecular recognition in water, polar binding interactions typically are stronger in a more deeply imbedded hydrophobic microenvironment.

To overcome these challenging problems, we designed a photocleavable template (**2**) containing an *o*-nitrobenzyl linkage (Scheme 1). The overall hydrophobicity of **2** allowed it to be easily incorporated into the micelle of **1**. The sulfonate group of the template had a strategic purpose in our design: in addition to strengthening the binding with the cationic micelle of **1** through electrostatic interactions, it orients the template to make its methacrylate group point inward. Because the methacrylate group is nearly at the opposite end of molecule from the sulfate that is anchored at the micelle surface, the methacrylate (after polymerization and photo-deprotection) is expected to position the carboxylic acid deep inside the hydrophobic pocket of the final MINP–COOH (Scheme 1).

MINP–COOH synthesis was adapted from our earlier procedures.[11a] Click-cross-linking of the template-containing micelles by the water-soluble diazide **3** in the presence of a Cu^{I} catalyst created alkynyl–SCMs, which were surface-functionalized through another round of click reactions with sugar-derived **4**. The reactions were performed at 10 mM of **1** in water, above its critical micelle concentration (CMC) of 0.55 mM. Each SCM, according to our dynamic light scattering (DLS) study, contained approximately 50 surfactant molecules. Thus, a ratio of [**1**]/[**2**] = 1:0.02 in theory placed one template within each SCM, a feature verified in our previous bile salt-binding MINPs.[11a]

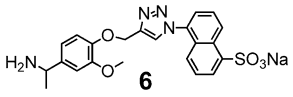
In the previous procedure, we employed photo-polymerization to cross-link the methacrylate of **1** with divinylbenzene (DVB) solubilized in the core. The method was clearly unsuitable with **2** having the photocleavable *o*-nitrobenzyl ether. We thus decided to solubilize a small amount of AIBN (azobisisobutyronitrile, a thermal initiator) at the beginning of the procedure and carried out thermal polymerization of the methacrylate and DVB at 75 °C for 16 h after the "surface-clicking". Fortunately, as hydrophobic interactions generally remain effective at high temperatures (and generally change from entropically driven to enthalpically driven with increasing temperatures),[13] template **2** was successfully cross-linked with the rest of the structure. Alkenic protons disappeared completely at the end of the thermal polymerization process as shown by ^{1}H NMR spectroscopy (Figure S1 in the Supporting Information).

Our last step in the materials synthesis was the photolytic cleavage of the *o*-nitrobenzyl linkage to remove the nitroso derivative **5** and vacate the binding site. The reaction progress could be monitored easily by fluorescence spectroscopy (Figure S2 in the Supporting Information) because of the fluorescent naphthalene group of **5**.[14]

According to DLS, the SCM, surface-functionalized SCM, and the final MINP-COOH averaged 3.5, 6.3, and 4.7 nm in diameter, respectively (Figure S3 in the Supporting Information). The size of MINP-COOH translated to approximately 50,000 Daltons in molecular weight (Figure S4 in the Supporting Information), comparable to many proteins in this regard. Overall, the MINP-COOH bears a resemblance to a water-soluble protein receptor with a hydrophilic exterior, a hydrophobic core, a specifically shaped hydrophobic binding site, and an internal functional group. It is worth mentioning that the sugar-derived surface ligand **4** was installed not just to make the MINP mimic a water-soluble protein in its surface hydrophilicity; its high crystallinity allowed the MINPs to be easily precipitated from solvents such as acetone while maintaining complete solubility in water and polar solvents such as DMF. As will be shown later, solubility in selected organic solvents was critical to the covalent modification of the MINPs.

Characterization of the carboxylic acid-containing binding pockets

To characterize the carboxyl-functionalized MINP receptors, we first studied the binding of a template analogue, **6**, which contained an amino group in the position of the methacrylate group in template **2**. As the carboxyl group in the MINP binding pocket was generated from the polymerized methacrylate of **2**, compound **6** upon binding should have its amino group in close proximity to the MINP carboxyl group. The host–guest binding thus should be driven by a combination of hydrophobic interactions (between the hydrophobic portion of **6** and the MINP) and an ammonium–carboxylate salt bridge. Being located in a hydrophobic microenvironment, the salt bridge should be particularly strong.[15] As in our bile salt-binding MINPs, electrostatic interactions between the positively charged cross-linked micelle and the sulfonate group should contribute as well.



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The water solubility of our MINPs allowed us to study their binding properties by using standard titration methods, a feature difficult to achieve with conventional imprinting that tends to yield insoluble macroporous polymers.[1c] As shown by Figure 1a, upon titration of **6** by MINP–COOH in Tris buffer

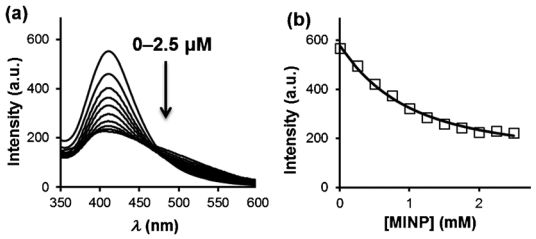
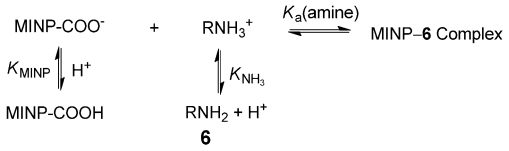


Figure 1. (a) Emission spectra of **6** ($\lambda_{ex}=300$ nm) upon addition of different concentrations of MINP–COOH in 50 mM Tris buffer (pH 7.4). [**6**] = 0.5 μM. (b) Nonlinear least squares fitting of the emission intensity of **6** at 415 nm to a 1:1 binding isotherm.

at pH 7.4, the emission at 410 nm decreased and a weaker peak at 470 nm appeared gradually. Although we could not be certain why the MINP-bound **6** emitted at a longer wavelength, it is possible that the binding slowed down the rotation around the σ-bond between the triazole and the naphthyl rings and enhanced the conjugation between the two aromatic groups. For the same reason, although environmentally sensitive dansyl fluorophores are often used to probe the local polarity of the binding pocket, we could not do so with **6**, as its emission depends on multiple factors. Nonetheless, the fluorescence data fit nicely to a 1:1 binding isotherm to afford a binding constant of $K_a=(1.5\pm0.3)\times10^6\ M^{-1}$ (Figure 1b).

To further understand the role of the MINP carboxyl in the binding, we performed similar fluorescence titrations of amine **6** at different pH values (2.2–9.5) in citrate phosphate and Tris buffers. The concentration (10–50 mM) of the buffers showed negligible effect on the obtained binding constants. Such results generally suggest that ionic strength does not play any significant role in the binding.[16] In the bile salt-binding MINPs, the negligible effect of ionic strength seemed to come from two opposing effects of the salts on the hydrophobic interactions and electrostatic forces involved in the binding, respectively.[11a]

Because both the host and the guests contain removable protons, we have to consider the acid/base properties of all the reactants in the binding.[17] Scheme 2 shows the acid–base



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Scheme 2. The acid–base equilibria and the binding of amine **6** by MINP–COOH.

equilibria involved and the binding of amine **6** by MINP–COOH. The acidity constant of (protonated) amine **6** (pK_{NH_3}) in solution is probably similar to that for 1-phentylethylamine ($pK_a=9.4$).[18] Assuming MINP–COOH deprotonates more easily than protonated **6**, we anticipate that the strongest binding between MINP–COOH and **6** would occur in between pK_{MINP} and pK_{NH_3}. Below pK_{MINP}, the dominant forms of the reactants are MINP–COOH and RNH_3^+ (i.e., protonated **6**). These two species cannot form the ammonium–carboxylate salt bridge directly. Instead, MINP–COOH needs to undergo an unfavorable deprotonation (in the acidic medium) in order for the binding to occur. The binding, thus, would be compromised by the deprotonation of the MINP carboxyl. Above pK_{NH_3}, $MINP–COO^-$ and RNH_2 (i.e., **6** itself) will dominate on the other hand. To form the ammonium–carboxylate salt bridge, **6** has to undergo an unfavorable protonation under the now basic conditions and, thus, would also weaken the binding.

Our titration confirmed the predictions. As shown by Figure 2, the binding was undetectable by fluorescence spectroscopy at ≤pH 5, became stronger with increasing pH, and

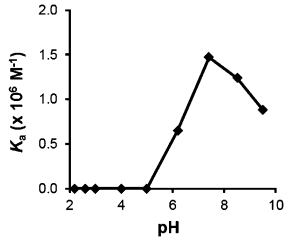
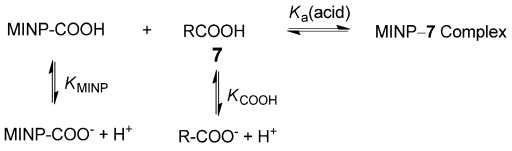


Figure 2. The apparent binding constants of MINP–COOH for **6** in citrate phosphate buffer (pH 2.2–6.2) and Tris buffer (pH 7.4–9.5) obtained by fluorescence titration.

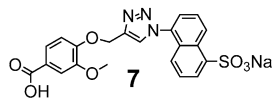
weakened again above pH 7.4. According to Scheme 2, the maximum K_a ($1.5\times10^6\,M^{-1}$) should reflect the binding when MINP–COO^- and RNH_3^+ predominate in the solution. If we take the midpoint between pH 5 (where the binding was still zero but began to rise) and pH 7.4 (where the binding was the strongest) as pK_{MINP}, the acidity constant of the MINP carboxyl is estimated to be 6.2. This value is significantly higher than acetic acid ($pK_a=4.76$) or benzoic acid ($pK_a=4.20$) in water. The larger pK_a for MINP–COOH is very reasonable and strongly supports the location of the acidic group in a hydrophobic microenvironment. It is well known from protein chemistry that a carboxyl group located in a hydrophobic pocket is more difficult to deprotonate than in aqueous solution, as the resulting carboxylate cannot be solvated properly in a hydrophobic microenvironment.[19]

The presence of the carboxylic acid in the hydrophobic binding pocket of MINP–COOH was verified additionally by its binding of the carboxylic acid guest **7**. Scheme 3 shows the



Scheme 3. The acid–base equilibria and the binding of acid **7** by MINP–COOH.

various acid–base equilibria involved in the binding. Because the carboxyl group of **7** is exposed to solvent, its pK_a is expected to be similar to that of 3,4-dimethoxybenzoic acid ($pK_a=4.43$).[20] As the binding between two carboxylic acids occurs through the hydrogen-bonded carboxylic acid dimer, both MINP–COOH and **7** need to be in the protonated form to achieve the strongest binding.



Indeed, as shown by Figure 3, the pH profile for the binding of **7** was nearly opposite to that of **6**: the strongest binding occurred at low pH values (2.2–2.6) and showed a sharp decrease as the solution became less acidic. The binding weakened significantly at pH 3 and became completely undetectable by fluorescence at $\geq$pH 6.2. If we take the midpoint between pH 2.6 (where the binding was the maximum) and pH 6.2 (where the binding first became zero) as the acidity constant of **7**, a value of 4.4 is obtained, exactly as predicted from the pK_a of 3,4-dimethoxybenzoic acid.

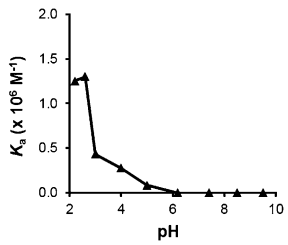


Figure 3. The apparent binding constants of MINP–COOH for **7** in citrate phosphate buffer (pH 2.2–6.2) and Tris buffer (pH 7.4–9.5) obtained by fluorescence titration.

Because the maximum binding constants for **6** and **7** were quite similar (1.5 and $1.3\times10^6\,M^{-1}$, respectively), it seems the ammonium–carboxylate salt bridge and the carboxylic acid dimer make similar contributions to the overall binding. This is a useful piece of information for molecular recognition in water. Recent work of ours shows that, although a (guanidinium–carboxylate) salt bridge could be strong, for molecular recognition in self-assembled hydrophobic entities such as micelles or lipid bilayers, a carboxylic acid dimer could be more useful. This is because the uncharged nature of a carboxylic acid dimer makes migration into a hydrophobic microenvironment easier.[21] Charged functional groups (e.g., ammonium, guanidinium, or carboxylate) often have a strong tendency to stay within or at least close to water to satisfy their solvation needs.[22]

Binding selectivity of MINP–COOH

An important property of molecularly imprinted materials is their binding selectivity. The above study demonstrated binding selectivity for acid- and base-functionalized template analogues in a pH-dependent manner. For example, at pH 7.4, the MINP receptor bound **6** with micromolar affinity but did not bind **7** at all. Under acidic conditions (pH 2.2–2.6), the exact opposite selectivity was achieved.

To understand the binding selectivity of the MINP receptor for other non-acidic/basic analogues (**8**–**11**), we switched to isothermal titration calorimetry (ITC) to determine the binding constants. One reason for the change was that fluorescence titration is unsuitable for bindings with lower binding affinities. Additionally, ITC could afford other useful information including binding enthalpy, entropy, and the number of binding sites per particle (*N*). Because these guests do not contain acid/base groups, we performed ITC titrations under neutral conditions in 50 mM Tris buffer.

ITC confirmed both the 1:1 binding stoichiometry and the binding affinity for **6** and yielded a K_a value very similar to that obtained by fluorescence titration (i.e., $1.5\times10^6\,M^{-1}$, see Table 1, entry 1). The titration curve and the fitting of the experimental data are shown in Figure 4. ITC was able to detect the binding of **7** (which could not be measured by fluorescence titra-

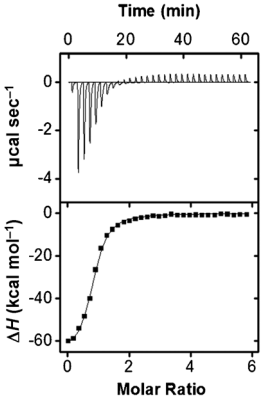


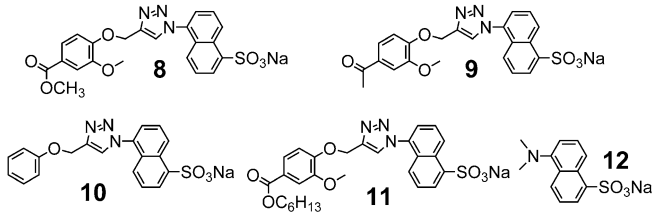
Figure 4. ITC titration curve obtained at 298 K for the binding of **6** in 50 mM Tris buffer (pH 7.4).

Table 1. Binding data for MINP–COOH obtained by ITC.[a]

Entry	Guest	K_a [10^4 M^{-1}]	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$-T\Delta S$ [kcal mol^{-1}]	N
1	**6**	152±6	−8.4	−70.2	61.8	1.0±0.1
2	**7**	8.8±0.5	−6.7	−15.3	8.6	0.6±0.1
3	**8**	76±3	−8.0	−29.8	21.8	0.7±0.1
4	**9**	73±6	−8.0	−32.1	24.1	0.6±0.1
5	**10**	0.6±0.5	−5.2	−7.2	2.0	0.4±0.1
6	**11**	5.2±0.7	−6.4	−8.2	2.8	0.4±0.1
7	**12**	0.27±0.06	−4.7	−3.5	−1.1	1.2±0.1
8	**6**[b]	3.1±0.5	−6.1	−5.0	−1.1	0.6±0.1
9	**12**[b]	25±2	−7.4	−9.6	2.2	0.7±0.1

[a] The titrations were generally performed in duplicate in 50 mM Tris buffer (pH 7.4) and the errors between the runs were <20% except in very weak bindings. [b] The host was MINP–CONHNaph.

tion) at pH 7.4 and, as expected, gave a much weaker K_a, approximately 1/17 of that for **6**. Ketone **8** and ester **9** were bound similarly as expected (entries 3 and 4) and were bound more strongly than acid **7**.[23] We were delighted to see the moderate selectivity for **6** over **8** or **9**. After all, these compounds were very similar guests in many regards.



It is interesting to consider the ionic state of the carboxyl group on MINP–COOH during binding. The binding study for amine **6** yields a pK_a of 6.2 for the MINP carboxyl. Upon "plugging" the binding pocket with a hydrophobic guest such as **8** or **9**, the immediate environment around the MINP carboxyl becomes more hydrophobic upon the expulsion of water from the binding pocket. We would not be surprised that the binding should further increase its pK_a so that the carboxyl stays "comfortably" protonated even when the bulk aqueous phase has a pH of 7.4. In other words, for an acidic (or basic) group relatively deep inside in a hydrophobic pocket in water, its acid or base property is not a fixed constant as in solution but is an intimate function of the guest present in the binding pocket. These properties apparently are critical to the binding and catalytic properties of proteins.[19]

Compound **10** is overall quite similar to **8** and **9** but lacks a methoxy group and a methyl ester or acetyl group. Its binding was two orders of magnitude weaker, testifying to the excellent shape/size selectivity of the MINP (Table 1, entry 5). Ester **11** has a hexyl group instead of the methyl group in **8**. Although its binding was stronger than that of **10**, the one order of magnitude reduction in K_a from methyl ester **8** indicated that the binding pocket was quite discriminating.

Table 1 shows that all the bindings studied by ITC were largely enthalpically driven, with generally unfavorable entropic terms. We do not believe the results imply that the contribution of hydrophobic interactions were insignificant. Although the classical hydrophobic effect is considered to be entropically driven,[24] the effect is multifaceted and the energetic characteristics may be different depending on the (aliphatic/aromatic) nature of the guests and the size/shape of the hydrophobic surfaces.[25]

Notably, MINP–COOH contained one binding pocket per nanoparticle, evident from the binding studies for those guests with strong bindings (in which the ITC curve fitting would be more reliable). This feature came directly from the surfactant aggregation number and the ratio of surfactant to template during the imprinting. As demonstrated in the bile salt-binding MINPs, if needed, the binding stoichiometry could be tuned by the surfactant/template ratio quite easily.[11a]

Covalent modification of the binding pockets

Similar to an active site of a protein, the binding pocket of MINP–COOH could be modified through covalent chemistry. This could be a great way to tune the binding properties of the MINP receptor. To demonstrate this feature, we dissolved the nanoparticles in DMF and activated the MINP carboxyl with 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (EDCI), a standard amide-coupling reagent. After treatment with 2-aminonaphthalene, the resulting MINP–CONHNaph displayed characteristic naphthalene emission at 410 nm (Figure S5 in the Supporting Information).

Most interestingly, MINP–COOH and MINP–CONHNaph displayed (anticipated) distinctly different molecular-recognition properties. As described earlier, amine **6** was the best guest for MINP–COOH at pH 7.4, as everything including size, shape, and functional groups matched perfectly between the host and the guest. Not surprisingly, dansyl sulfate **12**, which at most represented a "half-matched" guest, was too small to bind strongly to MINP–COOH (Table 1, entry 7, $K_a = 0.27\times10^4$ M^{-1} or 560 times weaker than that of **6**). Once naphthylated, however, the binding pocket was expected to bind dansyl sulfonate much better, as the 2-aminonaphthalene group was chosen to make up for what was missing in dansyl sulfate **12** from **6** (i.e., the phenyl–triazole spacer between the amino and the naphthyl group; compare the two structures in Figure 5). As shown by the data in Table 1 (entries 8 and 9), MINP–CONHNaph showed

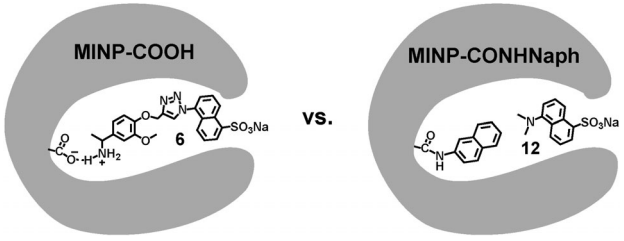


Figure 5. Binding of amine **6** by MINP–COOH versus binding of dansyl sulfonate **12** by MINP–CONHNaph.

a remarkable reversal of binding selectivity for **6** and **12**: whereas the bigger guest was preferred by MINP–COOH by 560 times, the smaller guest was bound more strongly by the naphthylated receptor than the larger one by eight times.

Conclusion

Molecular imprinting in surfactant micelles is a powerful method to create nanoparticle receptors that resemble water-soluble proteins. Their hydrophilic exterior,[10a,c,d,g] hydrophobic core,[26] and internal tailor-made binding sites[11] all could be tuned easily with the surface-cross-linked micelle platform. Previous noncovalent imprinting in the micelles only yielded hydrophobic pockets with predefined shape and size.[11] By combining covalent imprinting with a photoprotection strategy, we now can install specific functional groups within the binding pockets. Despite the many protein-like features, the MINP receptors are highly cross-linked materials with robust properties and long-term stability. Importantly, the entire preparation and purification of MINPs could be done in 2–3 days without special techniques. With their excellent molecular-recognition properties and facile preparation, we anticipate MINPs to become very useful in many applications where custom-made, specific binding sites are needed.

Experimental Section

General

Methanol, methylene chloride, and ethyl acetate were of HPLC grade and were purchased from Fisher Scientific. All other reagents and solvents were of ACS-certified grade or higher, and were used as received from commercial suppliers. Routine ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 or on a Varian VXR-400 spectrometer. ESI-MS was recorded on a Shimadzu LCMS-2010 mass spectrometer. UV/Vis spectra were recorded at ambient temperature on a Cary 100 Bio UV/Visible spectrophotometer. Fluorescence spectra were recorded at ambient temperature on a Varian Cary Eclipse Fluorescence spectrophotometer. ITC was performed by using a MicroCal VP-ITC Microcalorimeter with Origin 7 software and VPViewer2000 (GE Healthcare, Northampton, MA). Syntheses of the compounds are reported in the Supporting Information.

Synthesis of MINP–COOH

DVB (2.8 μL, 0.02 mmol), AIBN in DMSO (10 μL of 8.2 mg mL^{-1}, 0.0005 mmol), **2** in D_2O (10 μL, 0.0004 mmol) were added to a 2.0 mL micellar solution of surfactant **1** (9.3 mg, 0.02 mmol) in D_2O. (D_2O was used instead of H_2O to facilitate monitoring of the reaction progress by ^{1}H NMR spectroscopy.) The mixture was ultrasonicated for 10 min. Compound **3** (4.13 mg, 0.024 mmol), $CuCl_2$ in D_2O (10 μL of 6.7 mg mL^{-1}, 0.0005 mmol), and sodium ascorbate in D_2O (10 μL of 99 mg mL^{-1}, 0.005 mmol) were then added and the reaction mixture was stirred slowly at room temperature (25 °C). After 12 h, compound **4** (10.6 mg, 0.04 mmol), $CuCl_2$ in D_2O (10 μL of 6.7 mg mL^{-1}, 0.0005 mmol), and sodium ascorbate in D_2O (10 μL of 99 mg mL^{-1}, 0.005 mmol) were added and the mixture was stirred for another 6 h. The reaction vial was sealed with a rubber stopper and the reaction mixture was purged with nitrogen for 15 min before it was stirred at 75 °C for 16 h. The resultant solution (2.0 mL) was cooled to room temperature and poured into acetone (8.0 mL). The precipitate formed was washed five times with 1:4 water/acetone mixture and dried overnight in the dark to give an off-white powder. The power was dissolved in Millipore water (1 mL) and irradiated in a Rayonet reactor for 12 h. Water was removed under reduced pressure and the residual sample was washed five times with 1:4 water/acetone mixture in a centrifuge tube and dried to give the product as a white powder (15 mg, 75 %).

Synthesis of MINP–CONHNaph

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 10 μL of 61.0 mg mL^{-1} in dry DMF, 0.004 mmol) was added to a stirred solution of MINP–COOH (20.0 mg, 0.0004 mmol) in dry DMF (1 mL) at 0 °C under nitrogen. After 2 h, 2-aminonaphthalene (10 μL of 56.2 mg mL^{-1} in DMF, 0.004 mmol) was added and the mixture was stirred for 24 h at room temperature. The mixture was concentrated in vacuo and poured into 2 mL of acetone. The precipitate was collected by centrifugation and rinsed several times with 2 mL of acetone to afford the product as an off-white powder (16 mg, 80 %).

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[23] As discussed in the previous section, the weak binding for **7** under pH 7.4 was caused by the deprotonation of both MINP–COOH and **7** at this pH. In order to bind, both have to undergo an unfavorable protonation under this condition.

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FULL PAPER

Molecular Recognition

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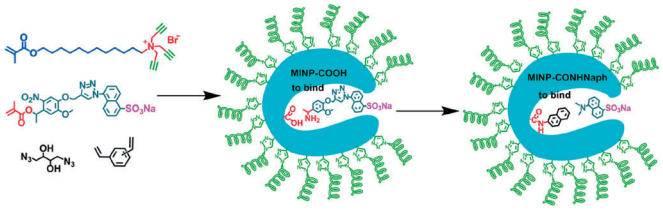
■■ – ■■

Water-Soluble Molecularly Imprinted Nanoparticles (MINPs) with Tailored, Functionalized, Modifiable Binding Pockets



Protein receptor mimics: Covalent imprinting within surface-cross-linked micelles in combination with a photodeprotection strategy yielded water-soluble molecularly imprinted nanoparticles with a single carboxylic acid group in the binding pocket. The acid-functionalized imprinted nanoparticles had excellent molecular recognition properties and can be modified covalently in the binding pocket.