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Keywords

molecular imprinting, folic acid, binding, nanoparticle, micelle, cross-linking, ion pairs

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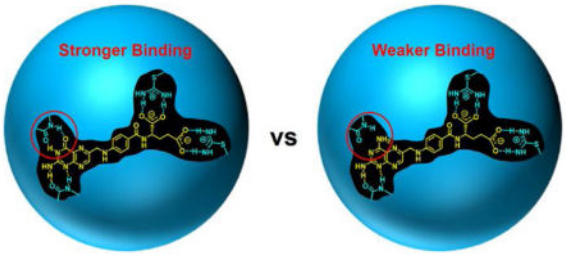
Abstract

Folate receptors are overexpressed on cancer cells and frequently used for targeted delivery. Creation of synthetic receptors to bind folic acid and its analogues in water, however, is challenging because of its complex hydrogen-bonding patterns and competition for hydrogen bonds from the solvent. Micellar imprinting within cross-linkable surfactants circumvented these problems because the nonpolar micellar environment strengthened the hydrogen bonds between the amide group in the surfactant and the template molecule. Incorporation of polymerizable thiouronium functional monomers further enhanced the binding through hydrogen-bond-reinforced ion pairs with the glutamate moiety of folate. The resulting imprinted micelles were able to bind folate and their analogues with submicromolar affinity and distinguish small changes in the hydrogen bonding patterns, as well as the number/position of carboxylic acids. The binding constant obtained was 2–3 orders of magnitude higher than those reported for small molecule synthetic receptors. Our binding study also revealed interesting details in the binding. For example, the relative contributions of different segments of the molecule to the binding followed the order of carboxylates > pyrimidine ring > pyrazine ring.

Graphical Abstract

Folic acids and analogues are important biomolecules but their distinction is hampered by the subtlety in their structural differences. Molecularly imprinted cross-linked micelles as protein-sized water-soluble receptors differentiate them with ease, using multiple hydrogen-bonding interactions and thiouronium binding groups.



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Supporting Material

Additional figures including fluorescence and ITC titration curves (PDF).

Keywords

molecular imprinting; folic acid; binding; nanoparticle; micelle; cross-linking; ion pairs

Introduction

Biological systems rely on molecular recognition for important processes such as binding, catalysis, and transport. Because individual noncovalent interactions are quite weak, they are used together to achieve sufficient strength. Chemists over the last several decades have used the same strategy to construct receptors for ions and small organic molecules, mainly in organic solvents.[1, 2]

To bind a guest molecule with high affinity and selectivity, its receptor needs to have a highly complementary binding interface for the guest. Macrocycles are a perfect platform for receptors, as long as their concave structure has the appropriate size and functionality for the guest molecule. A more recent platform is linear foldamers,[3–7] with a particular benefit of using guest-triggered conformational changes to amplify the guest binding.[8, 9]

Both macrocycles and foldamers represent the molecular approach to the construction of receptors. Herein, the receptor, typically larger than the guest molecule, is built step-by-step to encompass the guest. As the guest molecule becomes more complex, the receptors necessarily grow in size and complexity and their synthesis could become very difficult.

Molecular recognition in water takes the challenge to the next level,[10, 11] as hydrogen bonds, one of the best tools for directional molecular interaction, are compromised by competition from the solvent. Furthermore, when the receptor is built from a molecular scaffold, water-solubilizing groups need to be installed on the structure, in addition to the binding groups. As we move from simple organic guests to more complex biological molecules, design and synthesis of the corresponding molecular receptors could become an unsurmountable task.

Molecular imprinting is a very different approach to constructing receptors.[12–22] A noncovalent or covalent complex is first formed between the template/guest molecule and functional monomers (FMs) in a polymeric matrix and then trapped by heavy cross-linking. Removal of the templates leaves behind guest-complementary binding sites in the matrix, potentially with a high level of fidelity. In this method, the binding site is formed by facile covalent capture instead of total synthesis, making the synthesis much easier to perform. Not surprisingly, since their discovery, molecularly imprinted polymer (MIPs) have been used by researchers of diverse backgrounds for applications including separation, enzyme-mimetic catalysis, and chemical sensing.[12–22] Over the years, many different techniques have been developed to improve the properties of traditional MIPs, including imprinting unimolecularly within dendrimers,[23, 24] on polymeric nanoparticles,[25–32] and within micro/nanogels[33–38]

Folic acid is an important biological molecule. Because folate receptors are overexpressed on many cancerous cells,[39] folic acids are frequently used for targeted delivery of anticancer drugs.[40, 41] Creation of synthetic receptors for folic acid illustrates the typical challenges in

the molecular recognition of biomolecules in water.[42–46] The molecule is highly functionalized, with a glutamate coupled to a pteroate moiety. It is fully water-soluble with numerous hydrogen-bond donors and acceptors and two ionizable carboxylic acids. The natural folate receptor binds folic acid with an extremely highly affinity ($K_a \approx 10^{10}$ M^{-1}).[41] Most synthetic folate receptors could be used in water[42–44, 46] and those that functioned in water bound folic acid with relatively low affinities ($K_a = 10^2$–10^4 M^{-1}).[45]

Another challenge with biomolecules is their selective recognition among close relatives: molecules **2**–**4** differ subtly from folic acid, sometimes by only one or two hydrogen-bond donor/acceptor, other times by the number and location of carboxylic acids. Yet, they play very different roles in biology.

These challenges, together with our interest of designing biomimetic receptors,[47–49] prompted us to develop synthetic receptors for folic acid and its analogues. In this paper, we report that highly selective folate receptors could be prepared by molecular imprinting within doubly cross-likable micelles. The resulting molecularly imprinted nanoparticles (MINPs) use hydrogen bonds, salt bridges, and hydrophobic interactions to achieve strong and selective binding. The binding constant obtained by our receptor was 2–3 orders of magnitude higher than those reported in the literature by small-molecule receptors.[45] The materials bridge the gap between molecular receptors and macroscopic MIPs. Similar to traditional MIPs, they can be prepared conveniently in one-pot reactions without special techniques. Unlike macroscopic polymers, however, they behave like soluble molecular receptors with their nanodimension and a controllable number of binding sites.

Results and Discussion

Design and Synthesis of Functionalized MINPs

Preparation of MINP receptors is shown in Scheme 1. Cross-linkable surfactant **5** contains two sets of orthogonal cross-linkable groups and is dissolved in water above its CMC (0.41 mM).[50] We first cross-linked its micelle on the surface by the Cu(I)-catalyzed click reaction with diazide **6**.[51, 52] The surface-cross-linked micelle was further functionalized by the click reaction with monoazide **7**. The functionalization decorates the micelle with a layer of hydrophilic ligands to improve its water-solubility. The surface decoration also enabled us to recover the materials by precipitation from acetone and washing with organic solvents (see the Experimental Section for details). The micelle contained divinylbenzene (DVB) and DMPA (a photolytic radical initiator). Free radical polymerization was initiated normally after the surface-cross-linking and functionalization to cross-link the core between the methacrylate of **5** and DVB. A 1:1 ratio between the cross-linkable surfactant and DVB was normally used because this level of DVB in the core was found to be essential to the binding selectivity of MINP.[53]

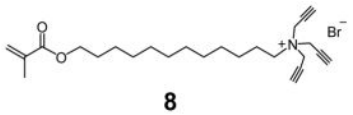
The template molecule typically had significant hydrophobicity and was incorporated into the micelle very easily. It served as a place holder inside the micelle as the surface–core double cross-linking “solidified” the micelle. Once the template was removed by repeated solvent washing, the resulting nanoparticle possessed a binding site complementary to the template molecule. The surfactant/template ratio was typically maintained at 50:1 in the

preparation because dynamic light scattering (DLS) showed that each MINP consisted of approximately 50 (cross-linked) surfactants. The 50:1 surfactant/template ratio translated to an average of one binding site per nanoparticle. We have shown that this number is fully tunable, simply by using different surfactant/template ratio in the preparation.[53]

The detailed procedures for the MINP preparation are reported in the Experimental Section. In general, the surface-cross-linking and core-polymerization/cross-linking was monitored by ^{1}H NMR spectroscopy and DLS (Supporting Information).[53] As the surfactant and DVB (core-cross-linker) underwent free radical polymerization, ^{1}H NMR spectroscopy showed disappearance of alkenic protons. DLS, on the other hand, revealed an increase in size for the nanoparticles as surface ligands were attached and a slight decrease in size when core-polymerization shrank the cross-linked micelles. DLS could also help us estimate the molecular weight of the MINP. The surface-cross-linking has been confirmed by mass spectrometry (after cleaving the surface-cross-linkages)[51] and the DLS size by transmission electron microscopy (TEM).[54]

Binding Properties of MINPs

Folic acid is rich in hydrogen-bonding functionalities and has two carboxylic acids. This was why we used amide-functionalized surfactant **5** in the majority of our MINP preparation. Folic acid is anionic under physiological conditions. The usage of a cationic cross-linkable surfactant is envisioned to help its inclusion in the micelle. Once incorporated into the micelle, folic acid is expected to stay close to the micellar surface to be solvated by water, due to its strong hydrophilicity. This location is also important to the removal of the template by the solvent washing (see the Experimental Section for details). The amide bond of **5** is only two carbons away from the ammonium headgroup and thus should be very close to the surface as well. We reasoned that the numerous surfactant molecules surrounding the template would hydrogen-bond with it using the amide. Although competition from solvent often makes hydrogen bonds ineffective for molecular recognition in water, the hydrophobic microenvironment of a micelle is known to strengthen hydrogen bonds.[55, 56] As a comparison, we prepared a MINP from compound **8**, another cross-linkable surfactant we frequently used.[57, 58] It has little hydrogen-bonding ability in the headgroup but the same cross-linkable functionalities (tripropargylammonium headgroup and methacrylate on the hydrophobic tail). As will be shown by the binding data (vide infra), surfactant **5** afforded a MINP with significantly stronger binding than **8**.



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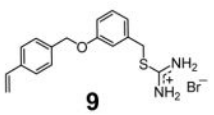

9

To recognize the glutamate moiety, we included FM **9** in the formulation. It is amphiphilic, just like the cross-linkable surfactant. The two hydrophobic aromatic rings help the molecule stay within the micelle. The thiouronium group, similar to guanidinium or amidinium,[59–62] is known to form strong hydrogen-bond-reinforced salt bridge with carboxylate.[63] Its vinyl group will copolymerize with the methacrylate and DVB to covalently fix the binding group on the MINP.

Folic acid is weakly fluorescent. As different concentrations of MINP$_{5}$(**1**)—i.e., MINP prepared with cross-linkable surfactant **5** and template **1**—were added to folic acid in 50 mM Tris buffer (pH 7.4), its fluorescence was slightly quenched (Figure 1a). Two isoemissive points were observed at 417 and 511 nm, indicating that the titration caused a continuous transition from the free to the bound guest. The emission intensity at 448 nm fit nicely to a 1:1 binding isotherm, yielding a binding constant (K_a) of (17.2 ± 2.7) × 10^5 M^{-1} in Tris buffer (Figure 1b). The submicromolar binding affinity was very impressive for a completely water-soluble guest. In comparison, molecularly constructed receptors in the literature were reported to bind folate with K_a = 10^2–10^4 $M^{-1.45}$ The 1:1 binding stoichiometry was confirmed in our case by the Job plot, which showed a clear maximum at a molar fraction of 0.5 (Figure 1c).

We also confirmed the binding constant obtained from the fluorescence titration by isothermal titration calorimetry (ITC). It is considered one of the most reliable methods for studying intermolecular interactions.[64] It has the benefit of giving a number of important parameters including binding enthalpy (ΔH) and the number of binding sites per particle (N), in addition to the binding constant. The binding free energy (ΔG) can be calculated from K_a using equation -ΔG = RTln(K_a), and ΔS from ΔG and ΔH. The ITC titration of **1** showed a negative/favorable enthalpy (Figure 2). The data afforded a K_a value of (17.0 ± 1.0) × 10^5 M^{-1} (Table 1, entry 1), in excellent agreement with the value obtained by fluorescence titration. The ITC titration gave the number of binding site of N = 1.2 ± 0.4, consistent with our surfactant/template ratio in the preparation and also the Job plot.

Table 1 summarizes the binding data obtained in the study. The binding data were generally obtained from ITC and selected examples were verified by fluorescence titration, with the K_a values obtained by the latter shown in parentheses.

Entries 1–4 of Table 1 show the effects of FM **9** on the MINP binding. As the amount of the FM increased in the formulation, the binding constant of the corresponding MINP for folic acid (**1**) peaked at 2 equiv **9**. Since folic acid has two carboxylates, the results indicate that a 1:1 stoichiometry is optimal for the imprinting and binding. MINP$_{5}$(**1**) prepared without FM **9** also gave a significant binding constant (K_a = 4.55 × 10^5 M^{-1}). Thus, even without the thiouronium FM, the template was readily included into the micelle. The discrete number of binding sites and clear trends in the binding constants ruled out any significant nonspecific binding during the titrations.

Entry 5 shows the binding of MINP$_{8}$(**1**) prepared with 2 equiv FM **9**. The binding constant (K_a = 2.47 10^5 M^{-1}) was 1/7 of that from MINP$_{5}$(**1**) prepared under the same conditions. The results support the importance of the amide groups in the imprinting, most likely from the proposed hydrogen-bonding interactions mentioned above.

Compounds **2**–**4** are close structural analogues of folic acid (**1**). Aminopterin **2** has an identical glutamate moiety and a 4-aminobenzoyl spacer as folic acid. The only difference is at the left end of the molecule, in the hydrogen-bonding pattern. Instead of D-D-A in the pyrimidine ring of **1**, compound **2** has a D-A-D motif, with everything else being the same (Chart 1). Yet, MINP$_{5}$(**1**) showed significant selectivity, with **2** only bound by MINP$_{5}$(**1**)

with K_a = 3.50 × 10^5 M^{-1} (Table 1, entry 6). This number is about 1/5 of that for **1**, corresponding to about 1 kcal/mol difference in the binding free energy.[65] Clearly, hydrogen bonds played an important role in the imprinting and binding of the folate derivatives, as supported by our earlier data. This is an important feature of our imprinting method. Molecular imprinting in water traditionally is considered a highly challenging topic.[66] In our case, the micellar environment circumvented the problem of solvent competition. Given the polarity of folic acid, it must stay near the surface of the micelle. It is very good news that hydrogen bonds between the amide of the surfactant and the template played noticeable roles in the imprinting and binding.

Pteroic acid **3** is missing the glutamate of folic acid. Its binding by $MINP_5$(**1**), thus, tests the importance of the carboxylic acids in the molecular imprinting. The binding constant was even weaker, with K_a = 0.217 × 10^5 M^{-1} (Table 1, entry 7) or nearly 1/80 of the value for **1**. Thus, the carboxylates contributed very strongly to the binding. In addition, fitting of the small-sized guest into the large pocket created for **1** should also be unfavorable.

The above result seems to contradict entries 1–4, showing different amounts of thiouronium FM **9** only changed the binding by 4-fold. However, the bindings in entries 1–4 were for several different $MINP_5$(**1**) always binding its own template (**1**). In these MINPs, regardless of the amounts of **9** present, the imprinting process was expected to form optimized binding pocket using monomers and cross-linkers available. Even in the absence of **9**, hydrogen bonds are anticipated to form with the rest of the MINP, whether with amide, triazole, or hydroxyl groups. Thus, different amounts of FM **9** only served to change the "perfectness" of the binding pocket but the binding was always for the "perfect" guest (i.e., folic acid as the template). In the case of binding of **3** by $MINP_5$(**1**), the binding pocket was created for **1** but pteroic acid **3** has a different number and position of the carboxylic acids from the template. In such a case, the guest was mismatched with the binding pocket and expected to be bound much more weakly.

Tetrahydrofolic acid **4** is similar to **2** in that the hydrogen-bonding motifs of the molecule is changed by the reduction of the pyrazine ring. Instead of two hydrogen-bond acceptors in **1**, the same place now has two hydrogen-bond donors instead. $MINP_5$(**1**) again was able to detect the change, with K_a = 9.01 × 10^5 M^{-1} (Table 1, entry 8). This binding constant is stronger than that for **2** (entry 6) by nearly 3 times, suggesting that the pyrazine part of the molecule played a less important role than pyrimidine in the molecular imprinting and binding of the receptor.

The benefit of creating receptors by molecular imprinting is that different receptors can be prepared with the same ease, without the need of any individual design, as formation of the binding pocket is taken care of by similar template–FM complexation and covalent capture. We thus prepared $MINP_5$(**2**) following similar procedures, with aminopterin as the template molecule. As shown by the binding data (Table 1, entries 9–12), this receptor showed as good binding properties as $MINP_5$(**1**): the receptor shows the strongest binding for its own template (**2**) among the structural analogues. This is another indication for the success of the imprinting. The guest that was bound most weakly was pteroic acid **3**, once again highlighting the key roles of the thiouronium–carboxylate salt bridge in the imprinting and

binding. If the change around the pyrazine ring between **1** and **4** were detected with a little difficulty by $MINP_5$(**1**) (relatively speaking), $MINP_5$(**2**) had no problem distinguishing **4**—its binding was nearly one order of magnitude lower than that for the template (**2**) itself. The results make sense. The only difference between **1** and **4** are in the pyrazine, which according to the earlier discussion contributed not as importantly as other parts of the molecule to the binding. The difference between **2** and **4** are in both the pyrazine and the pyrimidine ring in the hydrogen-bonding motifs. Since the latter seemed to be key contributors to the binding, differentiation between these two molecules is expected to be easier than between **1** and **2**.

Conclusions

Although molecular recognition in water is a very challenging topic in supramolecular chemistry,[10, 11] this work shows that molecular imprinting in cross-linked micelles is an effective and convenient solution to the problem. Despite the complexity of folate derivatives and their subtle structural differences, our MINP receptors easily distinguished them in the binding. The initial applications of MINPs focused on relatively hydrophobic molecules.[53, 67] Follow-up work indicated that MINPs could be generated for peptides with very similar side chains,[54, 63, 68] as well as mono- and oligosaccharides.[69, 70]. This work demonstrates that very hydrophilic molecules can be imprinted just as easily, as long as appropriate FMs are present. It also shows that MINP could be used to reveal the relative contributions of different functional groups to the binding, by comparing structural analogues in the binding. In the case of MINP receptors for folate, the relative contributions followed the order of carboxylates > pyrimidine > pyrazine. This is an extremely useful feature of our imprinted receptors, as it can help reveal the effectiveness of different functional groups in molecular recognition and allow researchers to focus on the most effective groups. Although it is difficult to measure the importance of hydrophobic interactions in folate recognition directly, the interactions are expected to be the "background", always present in molecular recognition in water. Even for the binding of carbohydrates (which typically lack any significantly hydrophobic groups) by lectins (their protein receptors in nature), release of water-molecules from the binding pockets is considered an important part of the binding free energy.[71] Prior to binding, the imprinted binding site of MINP is occupied by water molecules. Even though folic acid itself is not very hydrophobic, its binding releases these "high-energy" water molecules, similar to the binding of carbohydrates by lectins.

Experimental Section

Syntheses of compounds **5**,[50] **6**–**8**,[53] and **9**[63] were previously reported.

Preparation of Molecularly Imprinted Nanoparticles (MINPs)

A typical procedure is as follows.[53] To a micellar solution of compound **5** (10.2 mg, 0.02 mmol) in H_2O (2.0 mL), divinylbenzene (DVB, 2.8 μL, 0.02 mmol), folic acid (**1**) in H_2O (10 μL of a solution of 18.5 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 **6** (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 **7** (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. ^{1}H 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 448 nm (for folic acid) 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 $MINP_5$(**1**) (100 μM) was prepared in 50 mM Tris buffer (pH 7.4). Aliquots (1.0 μL) of the MINP stock solution were added to 2.00 mL of the solution of **1** in 50 mM Tris buffer (pH 7.4) (2.0 μM). 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 350 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 448 nm to a 1:1 binding isotherm.

Determination of Binding Constants by ITC

The determination of binding constants by ITC followed standard procedures.[72–74] In general, a solution of an appropriate guest in in 50 mM Tris buffer (pH 7.4) 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.

Acknowledgments

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65. The cross-linked micelles have numerous hydrogen-bonding functional groups including triazole, hydroxyl (from the surface cross-linker), and amide. In addition, water molecules are expected to be present to engage in and mediate hydrogen-bonding interactions with the guest and MINP. When a guest enters the binding pocket formed from the original template, if the hydrogen-bonding pattern between the original template and MINP cannot be formed by the new guest, the guest is expected to adjust itself in the binding pocket, e.g., by forming hydrogen bonds with other available groups or even solvent molecules nearby, to achieve the strongest binding possible to lower the overall free energy of the system. In addition, different number of water molecules will be involved in the desolvation of the host/guest and solvation of the final complex. Therefore, the

change in binding free energy from one guest to another is a composite term of many contributions. There is no attempt to assign the change to specific interactions in our discussion.

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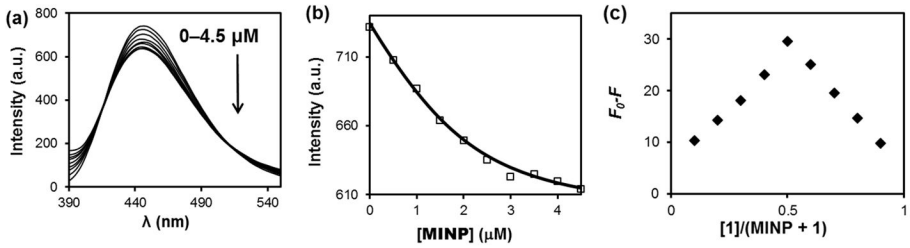


Figure 1.

(a) Emission spectra of folic acid **1** in the presence of 0–4.5 μM of $MINP_5$(**1**) in 50 mM Tris bugger (pH 7.4). The MINP was prepared with 2 equiv FM **9** to bind the glutamate. [**1**] = 2.0 μM. λ_{ex} = 350 nm. (b) Nonlinear least squares curve fitting of the fluorescence intensity at 448 nm to a 1:1 binding isotherm. (c) Job plot for the binding of **1** by $MINP_5$(**1**), in which the emission intensity at 448 nm was plotted against the molar fraction of **1**, at a constant total concentration of 10.0 μM in 50 mM Tris buffer (pH = 7.4).

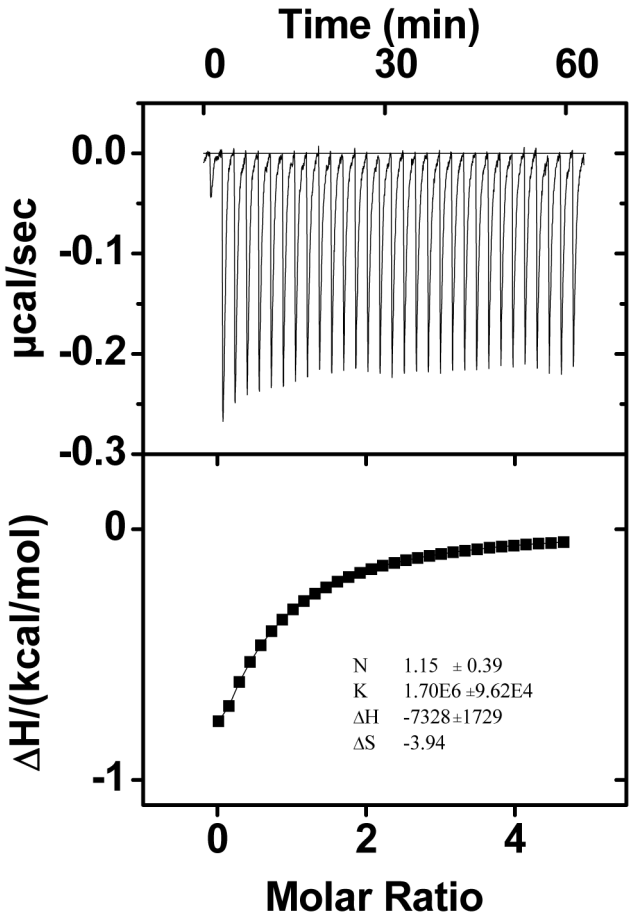
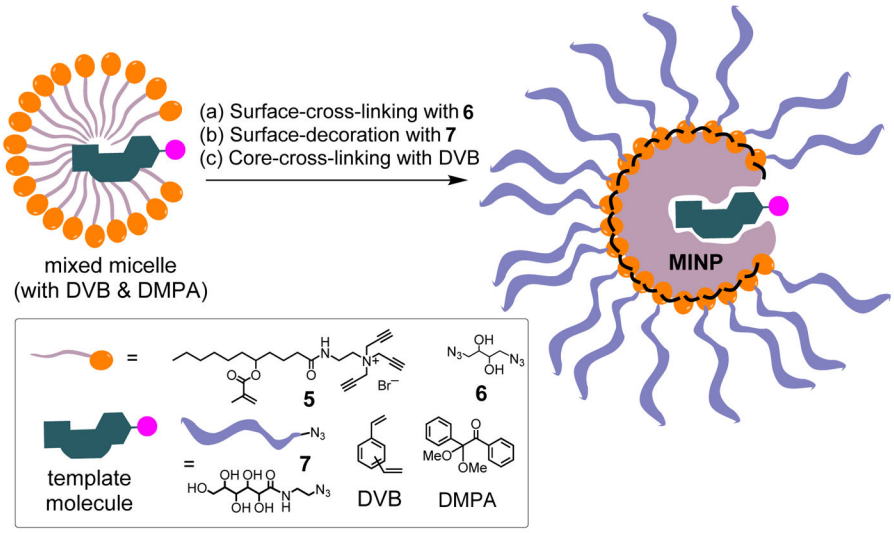


Figure 2.

ITC curve obtained at 298.15 K from titration of $MINP_5$(**1**) with **1** in 50 mM Tris buffer (pH = 7.4). The MINP was prepared with 2 equiv FM **9** to bind the glutamate. $MINP_5$(**1**) = 10 μM in the cell. The concentration of **1** in the syringe was 0.2 mM.



Scheme 1.

Preparation of MINP from templated polymerization of cross-linkable micelle.

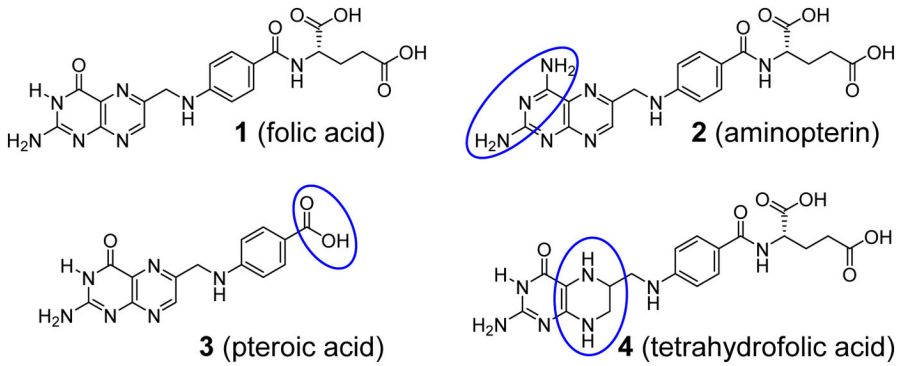


Chart 1.

Structures of folic acid and its analogues, with the differences from folic acid highlighted by blue circles.

Table 1

Binding data for MINPs (obtained by ITC unless indicated otherwise)[a]

Entry	MINP[b]	FM	Guest	−Δ*G* (kcal/mol)	*N*[c]	K_a (× 10^5 M^{-1})	−Δ*H* (kcal/mol)	*T*Δ*S* (kcal/mol)
1	$MINP_5$(**1**)	0 equiv **9**	**1**	7.7	0.9 ± 0.3	4.55 ± 1.30 (4.60 ± 1.40)	3.4 ± 1.1	4.3
2	$MINP_5$(**1**)	1 equiv **9**	**1**	8.2	0.9 ± 0.1	10.1 ± 2.5 (10.0 ± 2.2)	1.0 ± 0.1	7.2
3	$MINP_5$(**1**)	2 equiv **9**	**1**	8.5	1.2 ± 0.4	17.0 ± 1.0 (17.2 ± 2.7)	7.3 ± 1.7	1.2
4	$MINP_5$(**1**)	3 equiv **9**	**1**	8.4	0.8 ± 0.1	16.0 ± 1.0 (16.1 ± 2.3)	5.8 ± 0.8	2.6
5	$MINP_8$(**1**)	2 equiv **9**	**1**	7.4	0.7 ± 0.2	2.47 ± 0.14	3.8 ± 0.5	3.6
6	$MINP_5$(**1**)	2 equiv **9**	**2**	7.5	0.8 ± 0.2	3.50 ± 1.0 (3.70 ± 0.94)	9.7 ± 1.3	−2.2
7	$MINP_5$(**1**)	2 equiv **9**	**3**	5.9	0.9 ± 0.1	0.217 ± 0.052	19.5 ± 1.7	−13.6
8	$MINP_5$(**1**)	2 equiv **9**	**4**	8.1	1.3 ± 0.1	9.01 ± 0.22	15.2 ± 1.3	−7.1
9	$MINP_5$(**2**)	2 equiv **9**	**1**	7.4	0.8 ± 0.1	2.81 ± 0.31	3.2 ± 0.6	4.2
10	$MINP_5$(**2**)	2 equiv **9**	**2**	8.4	1.2 ± 0.3	13.6 ± 1.6	10.9 ± 3.0	−2.5
11	$MINP_5$(**2**)	2 equiv **9**	**3**	5.6	1.0 ± 0.2	0.127 ± 0.011	1.3 ± 0.2	4.3
12	$MINP_5$(**2**)	2 equiv **9**	**4**	7	1.0 ± 0.1	1.44 ± 0.16	29.0 ± 1.8	−22

[a]The titrations were generally performed in duplicates in 50 mM Tris buffer (pH = 7.4) and the errors between the runs were <20%. The binding constants in parentheses were from fluorescence titration and thus the number of binding sites and binding enthalpy/entropy were not available.

[b]The subscript denotes the cross-linkable surfactant used in the MINP synthesis and the number in parentheses the template molecule.

[c]Binding was too weak to be detected by ITC.

[c]*N* is the number of binding site per nanoparticle measured by ITC.