ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Molecularly imprinted nanoparticles as tailor-made sensors for small fluorescent molecules†

Cite this: *Chem. Commun.*, 2014, **50**, 5752

Received 27th February 2014,
Accepted 7th April 2014

DOI: 10.1039/c4cc01516a

www.rsc.org/chemcomm

Joseph K. Awino and Yan Zhao*

Water-soluble nanoparticles molecularly imprinted against naphthyl derivatives could bind the templates with high affinity and excellent selectivity among structural analogues in aqueous solution. Fluorescent dansyl groups installed during template polymerization allowed these nanoparticles to detect the presence of the target analytes by Förster resonance energy transfer.

Chemical sensors are important for a wide range of applications including clinical diagnostics, environmental remediation, drug analysis, and chemical detection. Sensors ideally should detect specific chemicals of interest with minimal interference from other chemicals present in the same sample. What is vital to the sensing specificity is typically a molecular-recognition unit in the sensor that binds the analyte with high affinity and selectivity.

Molecular imprinting is a technique to create guest-complementary binding sites, most often in a cross-linked polymer matrix.[1] It usually involves polymerization of a mixture of imprint molecules (*i.e.*, the templates), functional monomers, and cross-linkers into a highly cross-linked material. Template-complementary binding sites are created upon the removal of the templates from the polymer matrix. Because molecularly imprinted polymers (MIPs) potentially can be prepared for any molecule that can form a suitable template-functional monomer complex, molecular imprinting is a powerful technique for preparing synthetic receptors.

A key benefit of MIP is its predetermined binding selectivity (for the template or its mimics). This feature is enormously useful to molecular sensing in which the molecules of interest are typically known.[1c] Indeed, when coupled with optical,[2] mass,[3] refractive index,[4] or other signal-transducing mechanisms, MIPs have been used as sensors for a variety of analytes.[5]

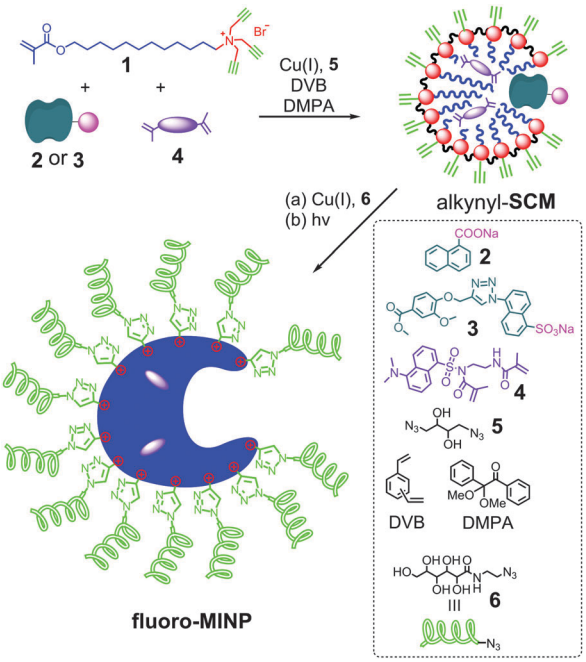
We recently reported a method to prepare molecularly imprinted nanoparticles (MINPs)[6] by surface-core cross-linking of surfactant micelles in water.[7] The nanoparticles imprinted against a bile salt derivative were found to bind the template among its structural analogues with excellent selectivity and affinity. Because the radius of the MINP (*ca.* 1.5 nm for the hydrophobic core and 2.5 nm including the surface ligands) is within the Förster distance (R_0) of many fluorophore pairs,[8] we reasoned that a MINP functionalized with an appropriate fluorophore should be able to detect analytes through Förster resonance energy transfer (FRET). As pointed out in a recent review, "ability to spectroscopically characterize binding sites" is a highly desirable feature for MIPs, especially if the materials can be made "either soluble or insoluble" and "readily processable".[9]

To demonstrate the concept, we first prepared an aqueous solution of cross-linkable surfactant **1**, template **2** (or **3**), and fluorescent dansyl derivative **4** that has two polymerizable methacrylate groups (Scheme 1). Surfactant **1** has a critical micelle concentration (CMC) of 0.55 mM and an aggregation number of 50 in water.[6] With [**1**] = 10 mM and [**1**]/[**2** or **3**] = 50/1, the resulting MINP was expected to contain on average one binding site per particle. To enable the resulting MINP to detect the template (the target analyte) by FRET, we chose to employ a naphthalene-containing template (**2** or **3**) that could serve as a FRET donor for the dansyl acceptor to be incorporated into the MINP through co-polymerization of **4**.

The details of the MINP synthesis and characterization are reported in the ESI† (Fig. S1–S6). As shown in Scheme 1, the micelles of **1** were first cross-linked *via* click chemistry on the surface by diazide **5** using Cu(I) catalysts. At this point, the organic additives including **4**, DVB (divinylbenzene), and DMPA (2,2-dimethoxy-2-phenylacetophenone, a photoinitiator) should simply be trapped within the SCM. Immediately after the surface-cross-linking, a sugar-derived azide (**6**) was added to the mixture to functionalize the surface of the alkynyl-SCM. The alkynyl-SCM had extra alkynes on the surface because the ratio of [**1**]/[**5**] was 1.2 in the reaction mixture while surfactant **1** had 3 alkynyl groups and cross-linker **5** only 2 azides. After surface-functionalization, UV irradiation triggered free radical polymerization of the methacrylate groups of **1** and **4**, as well as DVB solubilized within the SCM core. The micelles were able to solubilize one DVB per surfactant and this high level of DVB was found to enhance the rigidity of the core and the binding selectivity.[6]

Department of Chemistry, Iowa State University, Ames, Iowa 50011-3111, USA. E-mail: zhaoy@iastate.edu; Fax: +1-515-294-0105; Tel: +1-515-294-5845

† Electronic supplementary information (ESI) available: Syntheses and characterization of materials, experimental procedures for the binding studies, additional data and Figures. See DOI: 10.1039/c4cc01516a



Scheme 1 Preparation of fluoro-MINP.

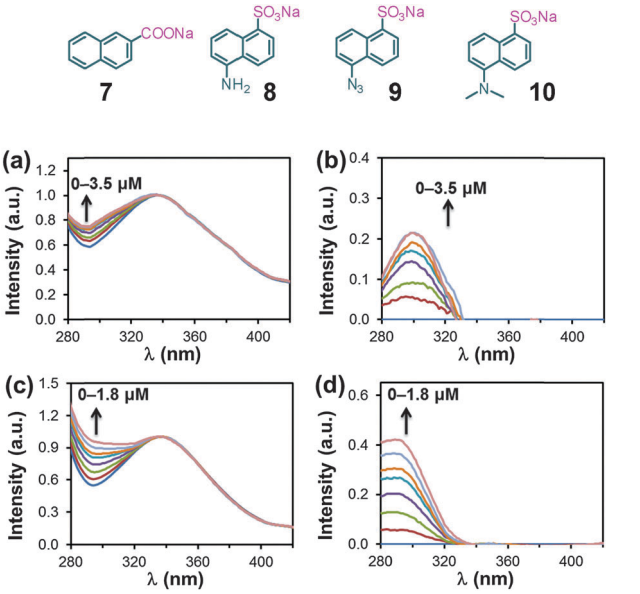


Fig. 1 (a) Normalized excitation spectra of fluoro-MINP (**2**) in the presence of different concentrations of **2** and (b) the excitation spectra with the contribution of fluoro-MINP (**2**) subtracted. (c) Normalized excitation spectra of fluoro-MINP (**3**) in the presence of different concentrations of **3** and (d) the excitation spectra with the contribution of fluoro-MINP (**3**) subtracted. The emission for the dansyl acceptor at 500 nm (λ_{em}) was monitored as the excitation wavelength (λ_{ex}) was scanned from 250 to 450 nm. [MINP] = 0.25 μM in 50 mM Tris buffer (pH 7.4).

At the end of the core-cross-linking, the fluoro-MINPs were recovered by precipitation from acetone, followed by methanol washing (to remove the imprint molecules).

The entire cross-linking process could be monitored easily by ^{1}H NMR spectroscopy. As shown in Fig. S1 and S2 (ESI†), upon surface-cross-linking, the sharp ^{1}H NMR signals of the surfactant were replaced by broad peaks and the protons near the ammonium headgroup (*e.g.*, propargylic protons) disappeared. The methacrylate and the DVB protons, although visible after surface-cross-linking, disappeared completely after core-cross-linking.

Fig. 1a and c shows normalized excitation spectra of fluoro-MINP (**2**) and fluoro-MINP (**3**) in the presence of different concentrations of **2** and **3** in Tris buffer (pH 7.4), when the dansyl emission at 500 nm was monitored. In the absence of binding, the donor fluorophore (**2** or **3**) would stay largely in solution, far from the dansyl acceptors embedded within the fluoro-MINPs. Titration of the fluoro-MINP with **2** or **3** would not affect the excitation spectrum (of the dansyl) since all the emission would be caused by direct excitation of the dansyl in this scenario. In the event of a binding, the donor molecule bound by the MINP would absorb light, undergo excitation, and transfer the excited energy to the dansyl acceptor in the same nanoparticle. In the latter case, the donor would contribute to the acceptor emission and thus peaks corresponding to the donor absorption would appear in the excitation spectrum of the dansyl acceptor.

We chose to have a 2 : 1 ratio of dansyl derivative **4** to the template (**2** or **3**) so that each MINP had 2 dansyl groups on average and a good chance existed for the naphthyl template to be within the Förster distance (R_0 = 2.2 nm)[10] of the dansyl acceptor during rebinding. Indeed, the characteristic contribution from the donor absorption (@ 290–310 nm) appeared when the template molecule was added to the "correct" fluoro-MINP (Fig. 1a and c). When the contribution of the acceptor (*i.e.*, the fluoro-MINP) was subtracted, a distinctive peak near 300–310 nm from the donor (**2** or **3**) appeared (Fig. 1b and d), indicative of increasing FRET with higher concentrations of the template added to the solution. When the "wrong" template was added, *e.g.*, **3** to fluoro-MINP (**2**) or **2** to fluoro-MINP (**3**), as shown in Fig. S7 and S8 (ESI†), the FRET signal was either absent or much weaker. Similar observations were made when the MINPs were titrated with other structural analogues (**7**–**10**), including **7** that only differed from **2** by the position of a carboxylate (Fig. S9–S16, ESI†).

The above results indicate that non-specific binding (from generic hydrophobic and electrostatic interactions) between the MINPs and the negatively charged template analogues could not trigger FRET, even those with very similar structures.[11] FRET was apparently a result of strong and specific binding, which was confirmed by isothermal titration calorimetry (ITC) shown in Fig. 2a and b. With ITC, we could obtain the binding data even for those structural analogues that caused no change in the fluorescence excitation spectra. Additionally, the technique allowed us to determine the number of binding sites (N) on the MINP.

The ITC binding data in Table 1 shows that the MINPs were highly selective in their binding. For MINP (**2**), the template itself gave a binding constant of $K_a = 0.43 \times 10^6$ M^{-1}, which translates to a binding free energy of $-\Delta G$ = 7.7 kcal mol^{-1}. The affinity was quite remarkable for a small molecule like **2** and should have resulted from the combination of hydrophobic interactions and electrostatic interactions between the oppositely charged MINP and the guest. None of the other anionic analogues, whether larger or smaller than **2**, showed any comparable binding; all the K_a values

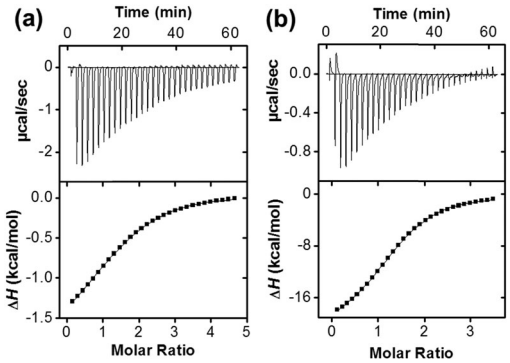


Fig. 2 ITC titration curve obtained at 298 K for the bindings between (a) fluoro-MINP (**2**) and **2** and (b) between fluoro-MINP (**3**) and **3** in 50 mM Tris buffer (pH 7.4). Additional ITC curves (Fig. S17–S18) are reported in the ESI.†

Table 1 Binding data for MINPs obtained by ITC[a]

Entry	MINP	Guest	K_a ($\times 10^6$ M^{-1})	$-\Delta G$ (kcal mol^{-1})	N
1	MINP (**2**)	**2**	0.43 ± 0.01	7.7	1.1
2	MINP (**2**)	**3**	—[b]	—[b]	—[b]
3	MINP (**2**)	**7**	0.0023 ± 0.0004	4.6	0.8
4	MINP (**2**)	**8**	0.0015 ± 0.0001	4.3	1.1
5	MINP (**2**)	**9**	0.0033 ± 0.0003	4.8	1.0
6	MINP (**2**)	**10**	0.0011 ± 0.0001	4.1	0.8
7	MINP (**3**)	**2**	0.0070 ± 0.0002	5.2	1.2
8	MINP (**3**)	**3**	1.00 ± 0.04	8.2	1.2
9	MINP (**3**)	**7**	0.0015 ± 0.0002	4.3	1.0
10	MINP (**3**)	**8**	0.0095 ± 0.0002	5.4	0.7
11	MINP (**3**)	**9**	0.0082 ± 0.0010	5.3	1.1
12	MINP (**3**)	**10**	0.0095 ± 0.0003	5.4	0.5

[a] The titrations were generally performed in duplicates and the errors in K_a between the runs were generally <20%. Binding was measured in 50 mM Tris buffer (pH = 7.4). [b] Binding was not detectable by ITC.

were at least two orders of magnitude lower than that for the template itself (Table 1, entries 2–6).

It is significant to note that stereoisomer **7** was bound by MINP (**2**) nearly 200 times weaker than **2**. The result highlighted the importance of hydrophilic anchoring during the imprinting: template **2** had to place its carboxylate group on the surface of the micelle; the ionic anchor must have oriented the hydrophobic group so that the resulting binding pocket could not accommodate the naphthyl and a misplaced carboxylate.

MINP (**3**) was also very selective. It bound its own template **3** with a K_a value of 1.00×10^6 M^{-1}, more than twice as that between MINP (**2**) and **2**. The higher affinity[12] was reasonable given the larger hydrophobic size of the guest, as hydrophobic interactions are known to be proportional in strength to the hydrophobic surface area buried upon binding.[13] None of the other guests, despite their similarities, was bound by MINP (**3**) in comparable affinity (Table 1, entries 9–12).

Convinced of the highly selective binding, we examined the FRET signals in the presence of potentially interfering structural analogues. Because of the stronger FRET of MINP (**3**) with its template, we examined the FRET detection of **3** in the presence of various structural analogues as potential interfering species. When 2 μM of **3** was added to a solution of 0.50 μM MINP (**3**), FRET from

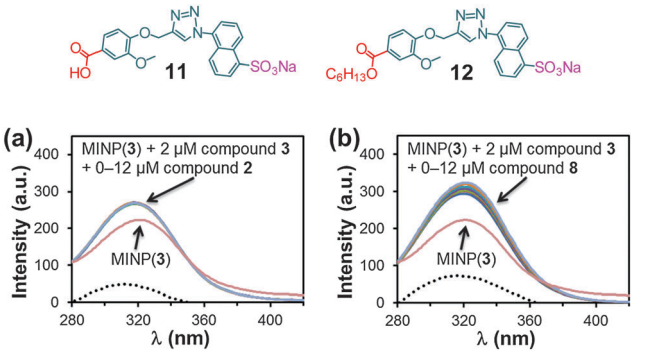


Fig. 3 (a) Excitation spectra of MINP (**3**) with 2 μM of compound **3**, titrated with 0–12 μM of compound **2**. (b) Excitation spectra of MINP (**3**) with 2 μM of compound **3**, titrated with 0–12 μM of compound **8**. The dotted spectrum in black was obtained by subtracting the MINP spectrum from that of the MINP plus compound **3**. The emission for the dansyl acceptor at 520 nm (λ_{em}) was monitored as the excitation wavelength (λ_{ex}) was scanned from 250 to 450 nm. [MINP] = 0.50 μM in 50 mM Tris buffer (pH 7.4).

the donor to the MINP acceptor was clearly visible in the excitation spectrum (Fig. 3a, compare the MINP spectra before and after the addition of compound **3**; the dotted spectrum in black was obtained by subtracting the MINP spectrum from that of MINP plus **3**, showing λ_{max} = 310 nm from the donor).[14] Significantly, when 2–12 μM of compound **2** (Fig. 3a), **7** (Fig. S19, ESI†), or **9** (Fig. S20, ESI†), was added,[15] the excitation spectra showed essentially no change. Compound **8** did show some interference (Fig. 3b). Since **8** and **9** were bound by MINP (**3**) similarly, the interference from **8** should derive from its spectroscopic instead of binding properties. We also examined the interference of two additional analogues of **3**, with the methyl ester hydrolyzed (in **11**) and replaced with a longer, hexyl group (in **12**), respectively. As shown in Fig. S21 and S22 (ESI†), these analogues did not affect the FRET signal at all, despite their similarity to **3**.

In summary, we have demonstrated that fluorescently-labelled MINPs can be generated against hydrophobic guests for highly specific binding among their structural analogues. The combination of predetermined binding properties from molecular imprinting and easy-to-perform FRET-based detection make these MINPs potentially very useful as sensors for small fluorescent molecules in water. Since the fluorophore was introduced independently from the molecular recognition-aspect of the imprinting, the FRET-detection and molecular imprinting in principle are orthogonal to each other.

We thank NSF for supporting the research.

Notes and references

1 (*a*) G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812; (*b*) G. Wulff, *Chem. Rev.*, 2001, **102**, 1; (*c*) K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495; (*d*) K. J. Shea, *Trends Polym. Sci.*, 1994, **2**, 166; (*e*) B. Sellergren, *Molecularly Imprinted Polymers: Man-Made Mimics of Antibodies and Their Applications in Analytical Chemistry*, Elsevier, Amsterdam, 2001; (*f*) B. Sellergren, *Angew. Chem., Int. Ed.*, 2000, **39**, 1031; (*g*) M. Komiyama, *Molecular Imprinting: From Fundamentals to Applications*, Wiley-VCH, Weinheim, 2003; (*h*) M. Yan and O. Ramström, *Molecularly Imprinted Materials: Science and Technology*, Marcel Dekker, New York, 2005; (*i*) C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony and M. J. Whitcombe, *J. Mol. Recognit.*, 2006, **19**, 106.

2 (*a*) A. L. Jenkins, O. Manuel Uy and G. M. Murray, *Anal. Commun.*, 1997, **34**, 221; (*b*) P. Turkewitsch, B. Wandelt, G. D. Darling and W. S. Powell, *Anal. Chem.*, 1998, **70**, 2025; (*c*) S. A. Piletsky, E. Terpetschnig, H. S. Andersson, I. A. Nicholls and O. S. Wolfbeis, *Fresenius' J. Anal. Chem.*, 1999, **364**, 512; (*d*) J. Matsui, M. Higashi and T. Takeuchi, *J. Am. Chem. Soc.*, 2000, **122**, 5218; (*e*) T. Takeuchi, T. Mukawa, J. Matsui, M. Higashi and K. D. Shimizu, *Anal. Chem.*, 2001, **73**, 3869; (*f*) A. Tong, H. Dong and L. Li, *Anal. Chim. Acta*, 2002, **466**, 31; (*g*) E. Mertz and S. C. Zimmerman, *J. Am. Chem. Soc.*, 2003, **125**, 3424; (*h*) N. T. Greene and K. D. Shimizu, *J. Am. Chem. Soc.*, 2005, **127**, 5695.

3 (*a*) F. L. Dickert, O. Hayden and K. P. Halikias, *Analyst*, 2001, **126**, 766; (*b*) M. Zayats, M. Lahav, A. B. Kharitonov and I. Willner, *Tetrahedron*, 2002, **58**, 815; (*c*) K. Das, J. Penelle and V. M. Rotello, *Langmuir*, 2003, **19**, 3921.

4 A. Kugimiya and T. Takeuchi, *Biosens. Bioelectron.*, 2001, **16**, 1059.

5 (*a*) *Molecularly Imprinted Materials–Sensors and Other Devices*, ed. K. J. Shea, M. Yan and M. J. Roberts, Materials Research Society, Warrendale, PA, 2002; (*b*) L. Ye and K. Haupt, *Anal. Bioanal. Chem.*, 2004, **378**, 1887; (*c*) M. J. Whitcombe, I. Chianella, L. Larcombe, S. A. Piletsky, J. Noble, R. Porter and A. Horgan, *Chem. Soc. Rev.*, 2011, **40**, 1547.

6 J. K. Awino and Y. Zhao, *J. Am. Chem. Soc.*, 2013, **135**, 12552.

7 (*a*) S. Zhang and Y. Zhao, *Macromolecules*, 2010, **43**, 4020; (*b*) S. Zhang and Y. Zhao, *J. Am. Chem. Soc.*, 2010, **132**, 10642; (*c*) X. Li and Y. Zhao, *Langmuir*, 2012, **28**, 4152; (*d*) S. Zhang and Y. Zhao, *Chem. Commun.*, 2012, **48**, 9998; (*e*) G. Chadha and Y. Zhao, *Chem. Commun.*, 2014, **50**, 2718.

8 B. W. v. d. Meer, G. Coker and S. Y. S. Chen, *Resonance Energy Transfer: Theory and Data*, VCH, New York, 1994.

9 S. C. Zimmerman and N. G. Lemcoff, *Chem. Commun.*, 2004, 5.

10 L. Stryer, *Annu. Rev. Biochem.*, 1978, **47**, 819.

11 We also tried 1-, and 2-naphthaleneamine as the guests and observed no FRET either. They were not studied further because their water-insolubility did not allow us to determine their binding by ITC.

12 Although compound **3** was significantly larger than **2**, the former had quite a number of oxygen and nitrogen atoms that might be hydrated when the molecule was in solution. The substantial desolvation during binding might be the reason why the binding for **3** was only marginally larger than that for **2** by their corresponding MINPs.

13 C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Krieger, Malabar, Fla, 2nd edn, 1991.

14 The λ_{max} of the donor (310 nm) was quite close to that of the acceptor (340 nm). An ideal FRET sensor should have very little overlap between the donor and the acceptor absorptions.

15 Compound **10** contained the same dansyl as the MINP (**3**) itself and thus was not compared in this study.