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TRENDS

Molecularly imprinted polymers with multi-functionality

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Abstract Molecular imprinting is a very powerful synthetic method for preparation of robust materials with pre-designed molecular selectivity. Molecularly imprinted polymers (MIPs) are attractive substitutes for antibodies in many analytical and bioanalytical applications, e.g. for development of biosensors and for drug assays. In addition to selective molecular binding, new functions are being added to MIPs to make the synthetic materials responsive to different environmental conditions, making it possible to modulate the binding and release of different molecular targets and to simplify affinity separation. Introduction of signal-transduction functionality into MIPs also brings in new, more easily operated chemical sensors for detection and quantification of important analytical targets.

Keywords Molecular imprinting · Multi-functionality · Nanoparticle · Composite material · Controlled radical polymerization

Introduction

Molecularly imprinted polymers (MIPs) are now routinely synthesized in research laboratories to solve different technical problems. MIPs have pre-designed target selectivity and outstanding stability, and can be prepared in a large quantity (tens of grams) within a short period (2–3 days). These features make MIPs very attractive as affinity sorbents for analytical and preparative separations [1]. The high molecular selectivity of MIPs is attributed to the template-controlled generation of molecular binding sites in a cross-linked polymer network. During the process of molecular imprinting, a template molecule controls the sequential and directional placement of functional monomers, leading to the fixation of a template–functional monomer complex in a solidified polymer matrix. After removing the molecular template by solvent extraction, the sites previously occupied by the molecular templates become available to re-bind the template and its closely related chemical species [2]. Depending on the molecular interactions that maintain the template–functional monomer complex, the synthetic approaches for molecular imprinting can be divided into three categories: covalent molecular imprinting [3], sacrificial covalent molecular imprinting [4], and non-covalent molecular imprinting [5]. Among these three approaches, non-covalent molecular imprinting is now the method mostly used to prepare MIPs because of its simple synthetic process and the availability of numerous functional monomers. Because a high molecular binding selectivity is the main objective in developing MIPs, substantial efforts have been made in the past to improve the affinity and capacity of MIPs [5, 6]. Recent development also involves new MIPs that can maintain high molecular selectivity in an aqueous environment [7], because many biological samples must be treated in water-based solvent environments.

For many practical applications, it will be very beneficial if MIPs can have multiple functions rather than just target recognition alone. In this regard, multi-functional MIPs that have a tunable molecular binding property, stimuli response to environmental alterations, and a signal-transduction property are

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becoming increasingly interesting, because these new MIPs can be used to develop, e.g., controlled drug-delivery systems, more efficient unit-operation systems in affinity separation, and smart chemical sensors to enable direct detection and/or quantification of analytical targets. Important breakthroughs in multi-functional MIPs have been made during the past years, and more examples can be expected in the near future. In this text we will provide a short overview of multifunctional MIPs and discuss the trends in future development. Because of the limited space we will focus mainly on the synthetic aspects and use selected examples to illustrate the principles of designing multifunctional MIPs. For interested readers there are more comprehensive review articles in the literature that can provide more complete coverage of MIPs [8, 9].

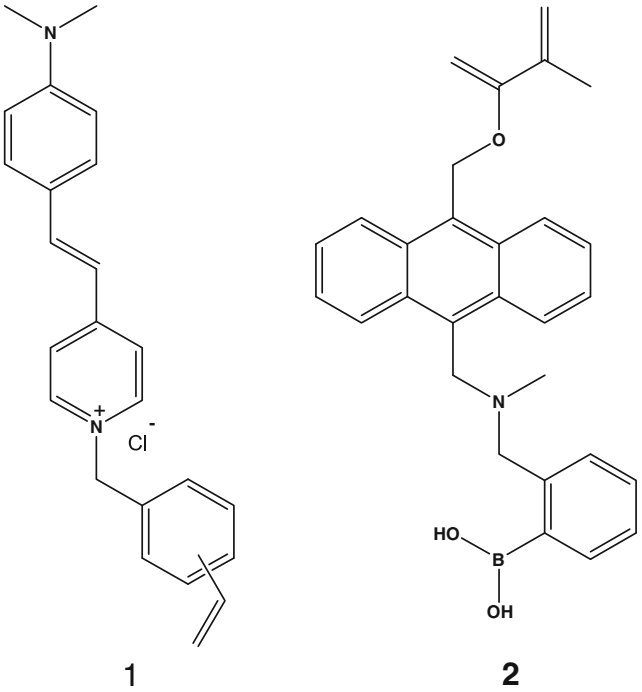
The types of functionality, in addition to selective molecular binding, can be numerous. Several additional functions can be introduced into MIPs without negatively affecting the very basic function of MIPs, i.e. selective molecular recognition. These additional functions include the materials' responses to magnetic field, electric field, photons, mechanical shear, and temperature, etc. The desired multi-functions can be achieved through different synthetic strategies. In the following we will summarize the most-adopted strategies that have offered multi-functional MIPs. These strategies are based on:

1. use of multi-functional monomers;
2. post-imprinting modification;
3. in-situ synthesis of MIP on functional nanoparticles; and
4. conjugation of nanoparticles with different functionality.

Use of multi-functional monomers

New functionality in addition to molecular binding can be added to MIPs using carefully designed multi-functional monomers. Unlike the more traditional functional monomers (e.g. methacrylic acid, 4-vinylpyridine) used in molecular imprinting, the multi-functional monomers possess not only a target-binding group, but also an additional moiety that can respond to the molecular binding event (Fig. 1). Consequently, the synthesized MIP can act as a smart chemical sensor (chemosensor) to signify the presence of a target analyte without requiring tedious sample-preparation steps.

As an early example, Turkewitsch et al. designed a fluorescent monomer, *trans*-4-[*p*-(*N*,*N*-dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride (1), that is able to bind cAMP (the molecular template) in the presence of another hydrophilic functional monomer, 2-hydroxylethyl methacrylate (HEMA) [10]. After polymerization and template removal, the fluorescent monomer was fixed within the imprinted sites and became sensitive to cAMP rebinding. The imprinted polymer underwent fluorescence quenching when exposed to aqueous cAMP. Thus the fluorescent monomer in this early work provided both template-binding and signal-transduction functions.



1 2

Fig. 1 Two representative monomers that contain both template-binding and fluorescence-reporting functionality

Another interesting fluorescent chemosensor was reported by Wang et al. for detection of fructose [11]. In this work the authors designed a polymerizable anthracene–boronic acid conjugate (2), a fluorogenic monomer that had significant enhancement of fluorescence emission after binding fructose. In the fluorogenic monomer 2, the boronic acid moiety was designed to bind the *cis*-diol molecule (fructose), which makes the boron accept the lone pair electrons on the nearby nitrogen. As a result, the lone pair electrons on the nitrogen can no longer quench the fluorescence of the anthracene moiety through photoelectron transfer. This type of "turn-on" fluorescence sensor has low background signal, and they are very attractive for analytical applications.

Using similar design principles, several bi-functional (template binding and signaling) monomers have been designed and used successfully to prepare MIP-based chemosensors [12–14]. When rationally designed multifunctional monomers are used to prepare nano-sized or "soluble" MIPs, fast binding kinetics can be achieved and the new MIPs can be kept as stable colloidal solutions to be handled more conveniently. This improvement can lead to more practical chemosensors that have faster response (within minutes) and simpler signal readout, e.g. using fluorescence detectors [15]. One technical challenge in this approach is that multi-step synthesis is often required to prepare the multifunctional monomers. It is also often difficult to predict the solubility of the specially designed

functional monomers in common imprinting solvents, which may limit the use of this synthetic approach for preparation of MIP-based sensors.

One alternative to designing new multi-functional monomers is the addition of environment-responsive monomers during the molecular imprinting process [16]. As recent examples, copolymerization of the traditional functional monomer and/or cross-linker with *N*-isopropylacrylamide (NIPAm) and azobenzene monomers has been used to introduce temperature and light-responsiveness into MIPs [17, 18]. The template binding to these MIPs can be controlled by temperature or UV or visible light, which can be useful for developing controlled delivery and release materials and systems.

Post-imprinting modification

Instead of using designed functional monomers, Takeuchi et al. developed an interesting alternative strategy to realize multifunctional MIPs. This method is based on controlled, site-specific modification of MIPs with a chemical reagent to bring in the additional function [19]. In a recent work, the authors first prepared a lysozyme-imprinted MIP grafted on a glass substrate. The imprinted cavities were designed to contain an exchangeable functional group (a carboxylic acid derivative) fixed to the MIP via a disulfide bridge (Fig. 2a). After removal of the protein template, the carboxylic acid derivative was replaced by an aminoethyl group through a disulfide exchange with aminoethylpyridyldisulfide. The terminal amine was then reacted with fluorescein isothiocyanate (FITC) to introduce the fluorophore [20]. The FITC-modified MIP had "turn-on" fluorescence emission when it bound the target protein, lysozyme, probably resulting from the dehydration of the cavity upon the protein binding. In this work, the in-cavity modification and fluorescent labelling was realized via the disulfide linkage, which required only mild reaction conditions and was convenient to perform.

Post-imprinting modification has also been performed on MIPs without affecting their imprinted cavities. Especially for imprinted microspheres and nanoparticles, modification of their surfaces does not compromise their interior binding sites but can improve the compatibility of the MIPs with aqueous solvent, which is often unavoidable in practical applications. Grafting hydrophilic polymer layer or polymer brushes on MIP beads has proved an effective method to reduce non-specific binding [21–23]. Of more interest is the addition of magnetic response to MIP microspheres, as reported by Zhao et al. using controlled radical precipitation polymerization (Fig. 2b). In this work the authors synthesized uniform MIP beads first, then continued to graft poly(glyceryl monomethacrylate) (poly(GMMA)) brushes on the MIP surface using reversible addition-fragmentation chain transfer (RAFT) polymerization [24]. The poly(GMMA) brushes were further used to capture the magnetic Fe_3O_4 nanoparticles formed by co-precipitation of Fe^{2+} and Fe^{3+} ions. Because all the chemical modifications were performed on the MIP surface, the imprinted cavities were kept intact and maintained a high molecular binding selectivity. The final composite magnetic beads not only possessed molecular binding selectivity for the template, 2,4-dichlorophenoxyacetic acid (2,4-D), but could also be easily separated from solution by applying an external magnetic field.

In-situ synthesis of MIP on functional nanoparticles

Using nanoparticles as solid supports, a thin layer of MIP can be synthesized in situ on the nanoparticle surface to furnish multifunctional composites. Both grafting-to and grafting-from radical polymerizations have been used to synthesize the composite MIP nanoparticles with a defined core–shell structure. Depending on the types of the core nanoparticles (e.g. quantum dots (QDs), magnetic nanoparticles), the in-situ synthesis will lead to core–shell MIPs that are fluorescent or have magnetic susceptibility (for a recent review, see Ref. [25]). In particular, the grafting-from synthesis of the MIP layer can be achieved using different living and/or controlled radical polymerization techniques (atom-transfer radical polymerization (ATRP) and RAFT polymerization), which enable more precise control of the MIP layer on the supporting nanoparticles [26, 27].

Lanthanide-doped upconverting nanoparticles (UCPs) have a special capability to turn low-energy near-infrared (NIR) or infrared light into shorter UV or visible emission. This unique property makes UCPs very attractive as optical probes for bioimaging applications, because excitation with NIR can have deeper tissue penetration and less photo damage for living cells. Using UCPs as the core particles, Beyazit et al. synthesized a thin layer of trypsin-imprinted polymer using NIR-induced photopolymerization (Fig. 3). In this system, when the UCPs were irradiated with a NIR source, they emitted visible light (λ_{em}=520–540 nm) that, in the presence of the photosensitizer eosin Y, initiated the photopolymerization [28]. Because the photopolymerization relied on the UCPs to provide the initiating visible light, it was only on the surface of the UCPs that the actual polymerization could take place. As a result, the localized photopolymerization enabled very efficient surface modification of UCPs with MIP and other functional polymers.

Conjugation of nanoparticles with different functionality

The fast progress in nanotechnology over the past twenty years has brought in numerous nanomaterials with

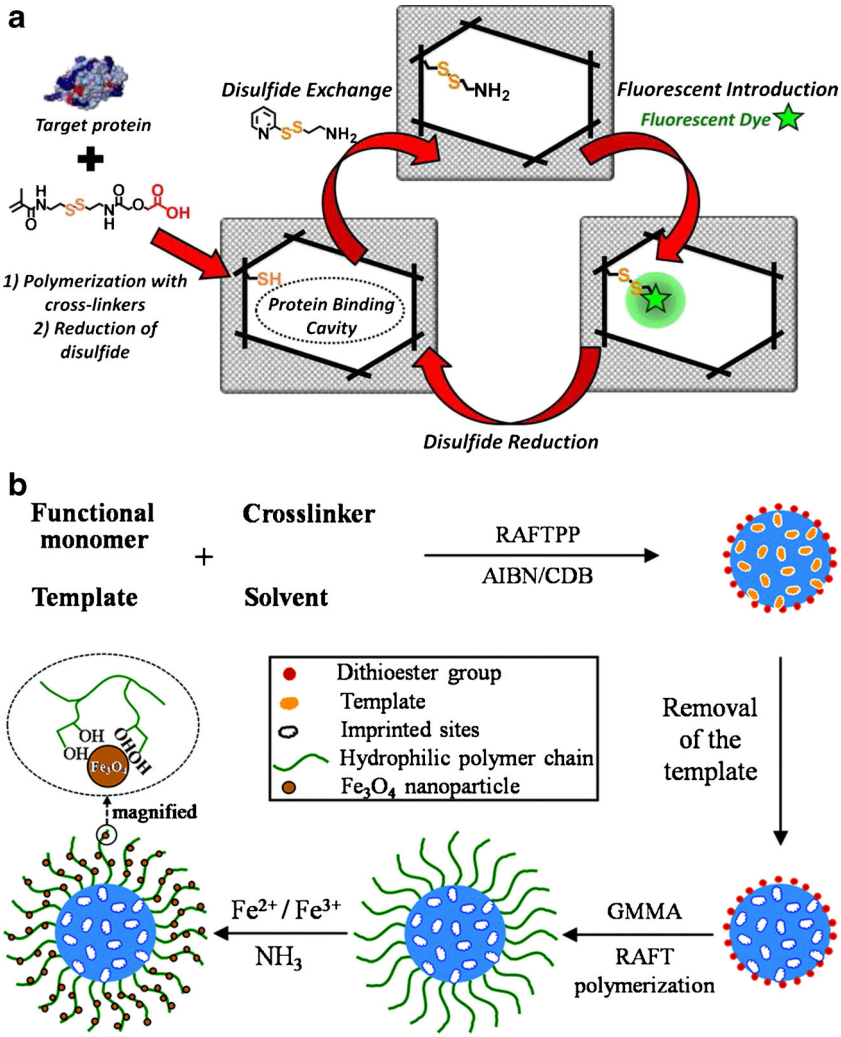


Fig. 2 (**a**)Preparation and post-imprinting modification of protein-imprinted polymer for fluorescent sensing of protein. Reproduced from Ref.[20] with permission from the American Chemical Society. (**b**)Preparation of magnetic MIP beads using post-imprinting modification. From Ref.[21] with permission from the Royal Society of Chemistry

unprecedented functionality. Because of their outstanding and unique electrochemical, magnetic, and optical properties, semiconductor QDs, magnetic nanoparticles, UCPs, nanodiamonds [29], and carbon nanodots (C-dots) [30] are among the most attractive nanoparticles being used in electronics, energy, and biomedical applications. As the number of commercially available functional nanoparticles continues to increase, it is interesting to combine these nanoparticle building blocks with existing MIPs to realize multi-functionality. Although multifunctional MIPs can be obtained by the in-situ synthesis of a MIP layer on nanoparticle support (as discussed above), this approach often requires multi-step reactions and the production is difficult to scale up. To address these problems, we proposed using chemical conjugation to combine pre-synthesized MIPs with other functional nanoparticles to realize new composites with the desired multi-functionality. Because the MIP component can be synthesized under optimized molecular imprinting conditions [31], it can be expected that the multifunctional composite obtained by the nanoparticle conjugation will maintain the best molecular recognition property.

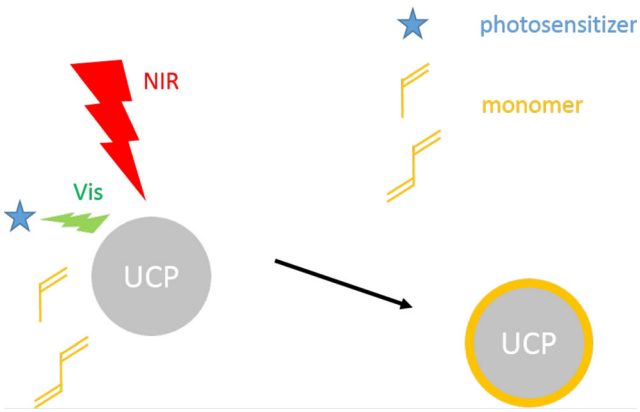


Fig. 3 In-situ synthesis of MIP layer on UCP surface using localized photopolymerization

Compared with intermolecular reaction between small organic molecules, chemical conjugation between larger solid particles is more difficult as a result of slower inter-particle collision and steric hindrance. Nevertheless, several high-

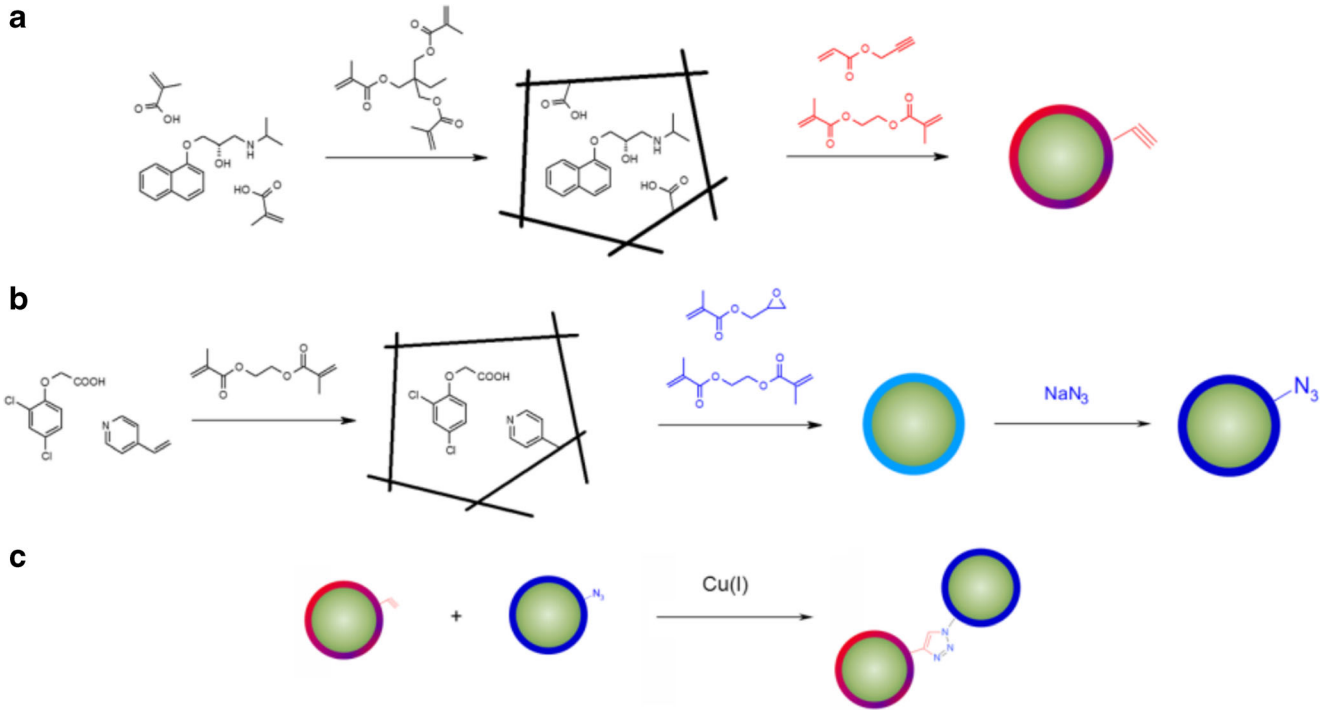


Fig. 4 Synthesis of clickable MIP nanoparticles using propranolol(**a**) and 2,4-D(**b**) as the templates, and conjugation of the two MIP particles using CuAAC click reaction(**c**)

efficiency coupling chemistries are available to enable fast and straightforward conjugation between MIPs and other functional nanoparticles. In this respect the click chemistry based on Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) [32] and the perfluorophenylazide (PFPA)-activated photoconjugation [33] are very suitable for preparing MIP–nanoparticle composites.

The use of CuAAC click chemistry to conjugate MIP nanoparticles was first performed using propranolol and 2,4-D imprinted particles as models. The imprinted nanoparticles were designed to have a core–shell structure and were synthesized by one-pot precipitation polymerization. In this design the imprinted binding sites were in the interior of the core–shell particles, and the particle shell was decorated with alkynyl and azide groups for the propranolol and the 2,4-D imprinted particles, respectively (Fig. 4). Mixing the two types of MIP particle with Cu(I) led to larger, covalently linked particle conjugates with dual molecular selectivity [34].

After achieving the efficient nanoparticle conjugation, we used the same click chemistry to conjugate azide-modified magnetic nanoparticles with alkyne-decorated, propranolol-imprinted nanoparticles. The obtained magnetic composite not only had high propranolol selectivity, but could also be quickly isolated from solution by simply using a permanent magnet [35]. In a further development, we revealed that the CuAAC-mediated nanoparticle conjugation can be confined at a well-defined liquid–liquid interface, leading to complex, colloidal particle-enveloped capsules (colloidosomes) that have multiple molecular selectivity for propranolol and *cis*-diols. Because of the boronic acid fluorophore immobilized in

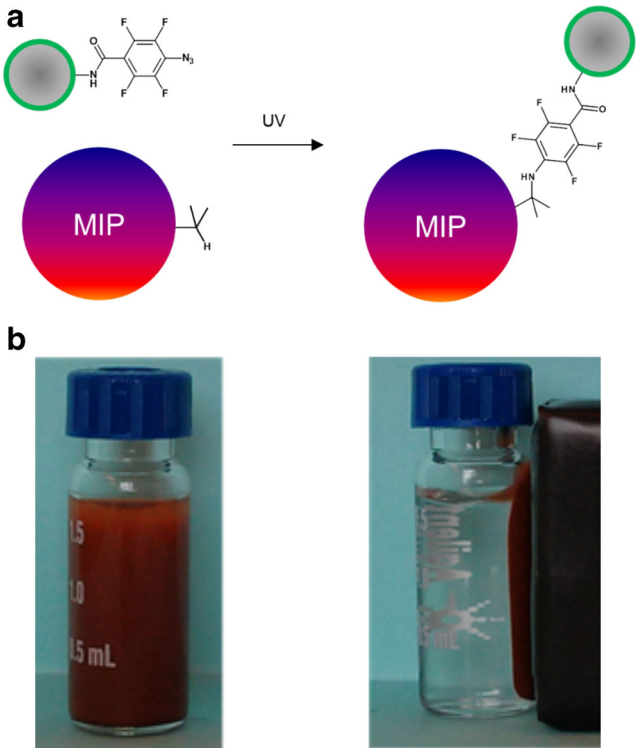


Fig. 5 (**a**)Preparation of magnetic MIP using PFPA-activated photoconjugation. (**b**)Separation of the composite particles using a simple permanent magnet

the interior, the colloidosomes could also act as a fluorogenic sensor for *cis*-diols [36]. The single colloidosome can therefore act as affinity adsorbent for multiple molecular targets and simultaneously as a fluorescent chemosensor.

By modifying the nanoparticle surface with PFPA reagents, it is also possible to use simple UV irradiation to directly link the PFPA-modified nanoparticles to ordinary MIPs. As one example, we achieved chemical ligation between PFPA-modified magnetic nanoparticles and unmodified MIP beads using a photoconjugation reaction (Fig. 5) [37]. Because a large variety of nanoparticles (e.g. silica, Au, QDs, magnetic nanoparticles, and C-dots) can be modified with the PFPA reagents [30], in principle there are unlimited possibilities for combining organic MIPs with other nanoparticles to realize a variety of multi-functionality. We expect that, starting from optimized MIPs and other nanoparticles as modular building blocks, high-efficiency chemical conjugation will lead to more interesting MIP-related multi-functional materials suitable for practical and new applications.

Outlook

Molecular imprinting has become an established synthetic method for preparation of organic polymers with pre-defined molecular recognition. One important direction for future developments is to integrate additional functions into MIPs, so that a single synthetic material has multiple useful functions. Such multifunctional materials with predefined molecular selectivity can have a broad range of applications, including fast magnetic separation, smart chemical sensors, controlled delivery systems, and switchable catalysts. Each of the different synthetic strategies leading to multifunctional MIPs has its own pros and cons; however, some general comments can be made regarding their synthetic accessibility. Compared with the approach of using multi-functional monomers, post-imprinting modification and nanoparticle conjugation are more straightforward and easier to implement in most research laboratories. Especially for MIPs designed to bind small molecules, post-imprinting modification on the MIP surface and conjugation between MIPs and functional nano objects are preferable, because these methods only affect the MIP surface and do not compromise the interior binding cavities in the MIP. For MIPs designed to bind large biomacromolecules, the in-situ synthesis of MIP on a functional-nanoparticle support will be a more suitable option, because the imprinted binding sites located on the surface are more critical for fast macromolecule recognition. It can be envisaged that intensified research into multi-functional MIPs will occur, and more systems will be based on post-imprinting modification of MIPs, in-situ synthesis of MIP on a functional-nanoparticle surface, and controlled conjugation of MIPs with functional nanoparticles. The advances in microfluidic reactors will provide very useful tools for preparing more uniform and multifunctional nanoparticle assemblies with outstanding stability and pre-defined molecular recognition capabilities.

References

1. Cheong WJ, Yang SH, Ali F (2013) Molecularly imprinted polymers for separation science: a review of reviews. J Sep Sci 36:609–628
2. Ye L, Mosbach K (2008) Molecular imprinting: synthetic materials as substitutes for biological antibodies and receptors. Chem Mater 20:859–868
3. Wulff G, Sarhan A (1972) The Use of polymers with enzyme-analogous structures for the resolution of racemates. Angew Chem Int Ed 11:341–341
4. Whitcombe MJ, Rodriguez ME, Villar P, Vulfson EN (1995) A new method for the introduction of recognition site functionality into polymers prepared by molecular imprinting – synthesis and characterization of polymeric receptors for cholesterol. J Am Chem Soc 117:7105–7111
5. Wulff G, Knorr K (2001) Stoichiometric noncovalent interaction in molecular imprinting. Bioseparation 10:257–276
6. Hall AJ, Manesiotis P, Emgenbroich M, Quaglia M, Lorenzi ED, Sellergren B (2005) Urea host monomers for stoichiometric molecular imprinting of oxyanions. J Org Chem 70:1732–1736
7. Zhang H (2013) Controlled/"living" radical precipitation polymerization: a versatile polymerization technique for advanced functional polymers. Eur Polym J 49:579–600
8. Alexander C, Andersson HS, Andersson LI, Ansell RJ, Kirsch N, Nicholls IA, O'Mahony J, Whitcombe MJ (2006) Molecular imprinting science and technology: a survey of the literature for the years up to and including 2003. J Mol Recogn 19:106–180
9. Whitcombe MJ, Kirsch N, Nicholls IA (2014) Molecular imprinting science and technology: a survey of the literature for the years 2004-2011. J Mol Recogn 19:106–180
10. Turkewitsch P, Wandelt B, Darling GD, Powell WS (1998) Fluorescent functional recognition sites through molecular imprinting. A polymer-based fluorescent chemosensor for aqueous cAMP. Anal Chem 70:2025–2030
11. Wang W, Gao S, Wang B (1999) Building fluorescent sensors by template polymerization: the preparation of a fluorescent sensor for D-fructose. Org Lett 1:1209–1212
12. Takeuchi T, Mukawa T, Shinmori H (2005) Signaling molecularly imprinted polymers: molecular recognition-based sensing materials. Chem Rec 5:263–275
13. Nguyen TH, Ansell RJ (2009) Fluorescent imprinter polymer sensors for chiral amines. Org Biomed Chem 7:1211–1220
14. Wagner R, Wan W, Biyikal M, Benito-Pen E, Moreno-Bondi MC, Lzraq I, Rurack K, Sellergren B (2013) Synthesis, spectroscopic, and analyte-responsive behavior of a polymerizable naphthalimide-based carboxylate probe and molecularly imprinted polymers prepared thereof. J Org Chem 78:1377–1389
15. Awino JK, Zhao Y (2014) Molecularly imprinted nanoparticles as tailor-made sensors for small fluorescent molecules. Chem Commun 50:5752–5755
16. Puoci F, Iemma F, Picci N (2008) Stimuli-responsive molecularly imprinted polymers for drug delivery: a review. Curr Drug Deliv 5: 85–96
17. Pan G, Guo Q, Cao C, Yang H, Li B (2013) Thermo-responsive molecularly imprinted nanogels for specific recognition and controlled release of proteins. Soft Mater:3840–3850

18. Fang L, Chen S, Guo X, Zhang Y, Zhang H (2012) Azobenzene-containing molecularly imprinted polymer microspheres with photo- and thermoresponsive template binding properties in pure aqueous media by atom transfer radical polymerization. Langmuir 28:9767–9777
19. Takeuchi T, Sunayama H, Takano E, Kitayama Y (2015) Post-imprinting and in-cavity functionalization. Adv Biochem Eng Biotechnol. doi:10.1007/10_2015_314
20. Sunayama H, Takeuchi T (2014) Molecularly imprinted protein recognition cavities bearing exchangeable binding sites for postimprinting site-directed introduction of reporter molecules for readout of binding events. ACS Appl Mater Interfaces 6:20003–20009
21. Haginaka J, Takehira H, Hosoya K, Tanaka N (2004) Uniform-sized molecularly imprinted polymer for (*S*)-naproxen selectively modified with hydrophilic external layer. J Chromatogr A 849:331–339
22. Yang K, Berg MM, Zhao C, Ye L (2009) One-pot synthesis of hydrophilic molecularly imprinted nanoparticles. Macromolecules 42:8739–8746
23. Moczko E, Poma A, Guerreiro A, de Vargas-Sansalvador IP, Caygill S, Canfarotta F, Whitcombe MJ, Piletsky S (2013) Surface-modified multifunctional MIP nanoparticles. Nanoscale 5:3733–3741
24. Zhao M, Zhang C, Zhang Y, Guo X, Yan H, Zhang H (2014) Efficient synthesis of narrowly dispersed hydrophilic and magnetic molecularly imprinted polymer microspheres with excellent molecular recognition ability in a real biological sample. Chem Commun 50:2208–2210
25. Wackerlig J, Lieberzeit PA (2015) Molecularly imprinted polymer nanoparticles in chemical sensing – synthesis, characterization and application. Sensors Actuators B 207:144–157
26. Xu L, Pan J, Dai J, Cao Z, Hang H, Li X, Yan Y (2012) Magnetic ZnO surface-imprinted polymers prepared by ARGET ATRP and the application for antibiotics selective recognition. RSC Adv 2:5571–5579
27. He Y, Huang Y, Jin Y, Liu X, Liu G, Zhao R (2014) Well-defined nanostructured polymers for highly selective magnetic separation of fluoroquinolones in human urine. ACS Appl Mater Interfaces 6:9634–9642
28. Beyazit S, Ambrosini S, Marchyk N, Palo E, Kale V, Soukka T, Bui BTS, Haupt K (2014) Versatile synthetic strategy for coating unconverting nanoparticles with polymer shells through localized photopolymerization by using the particles as internal light sources. Angew Chem Int Ed 53:8919–8923
29. Mochalin VN, Shenderova O, Ho D, Gogotsi Y (2012) The properties and applications of nanodiamonds. Nat Nanotechnol 7:11–23
30. Li H, Kang Z, Liu Y, Lee S-T (2012) Carbon nanodots: synthesis, properties and applications. J Mater Chem 22:24230–24253
31. Poma A, Turner APF, Piletsky SA (2010) Advances in the manufacture of MIP nanoparticles. Trends Biotechnol 28:629–637
32. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew Chem Int Ed 41:2596–2599
33. Liu L-H, Yan M (2010) Perfluorophenyl azides: new applications in surface functionalization and nanomaterial synthesis. Acc Chem Res 43:1434–1443
34. Xu C, Ye L (2011) Clickable molecularly imprinted nanoparticles. Chem Commun 47:6096–6098
35. Xu C, Shen X, Ye L (2012) Molecularly imprinted magnetic materials prepared from modular and clickable nanoparticles. J Mater Chem 22:7427–7433
36. Shen X, Xu C, Uddin KMA, Larsson P-O, Ye L (2013) Molecular recognition with colloidosomes enabled by imprinted polymer nanoparticles and fluorogenic boronic acid. J Mater Chem B 1:4612–4618
37. Xu C, Uddin KMA, She X, Jayawardena HSN, Yan M, Ye L (2013) Photoconjugation of molecularly imprinted polymer with magnetic nanoparticles. ACS Appl Mater Interfaces 5:5208–5213