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Molecularly Imprinted Materials: Towards the Next Generation

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ABSTRACT

This brief overview summarizes some recent developments from our Center for Molecular Imprinting related to the topic of this symposium. After a short presentation of the principle of molecular imprinting and recognition, the use of different materials including hybrids for the formation of the host will be discussed, followed by examples given of different formats used such as small polymer beads. In closing, potential directions for the next generation of molecular imprinting technology will be discussed.

INTRODUCTION

In the materials science area, defined structures in both organic and inorganic materials can be created using various structural templates to direct the fabrication process. As an example, polystyrene latex spheres have been used as template for the synthesis of highly ordered macroporous inorganic oxides [1]. Similarly, colloidal silica spheres were used to template a cross-linked polymer with ordered mesopores [2]. Following template removal, well-organized pore networks are formed in these materials, which have great potential uses in catalysis, separation technology, and biomaterials sciences. Not surprisingly, the same concept of template-assisted fabrication of defined structures has long been utilized in the research of molecular imprinting, aiming to generate molecular footprints in synthetic materials.

The molecular imprinting technique has attracted enormous research interests over the past decade. Commercialization of molecularly imprinted materials is now one of the major goals among the imprinting community, and indeed we have seen several startup companies utilizing molecular imprinting as a platform technology to be established. Compared to the supramolecular chemical approach [3], molecular imprinting is straightforward in producing tailor-made recognition materials, which display binding characteristics similar to biological antibodies, but with much more pronounced physical and chemical robustness. Uses of molecularly imprinted materials for separation, catalysis, polymer-assisted synthesis and biomimetic sensors have been demonstrated over the past years.

In general, molecular imprinting can be defined as a process of target directed synthesis of molecular hosts. For historical reasons, it has been largely related to the preparation of cross-linked synthetic polymers using a target molecule as a template. The complex formed between a template and appropriate functional monomers is fixed by co-polymerization with excess of a cross-linking monomer. After polymerization, the template is removed from the polymer matrix, which leaves binding sites specific for the original template, as well as its closely related structural analogues. Binding specificity of these sites is conferred by their well-defined shape and functionalities, which are complementary to those of the original template (Figure 1).

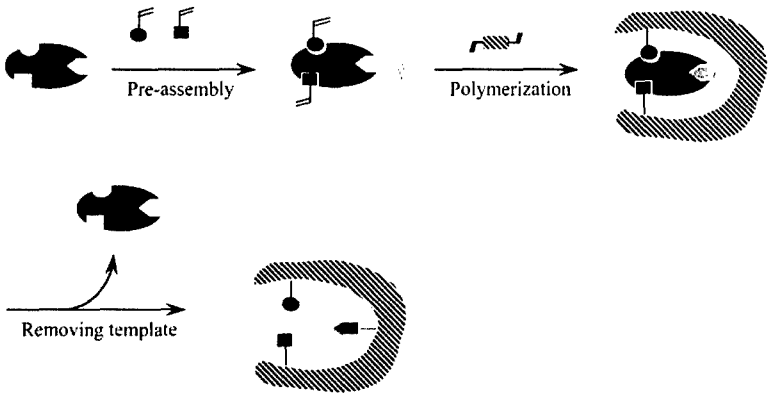


Figure 1. Schematic representation of a molecular imprinting process. Pre-assembly of functional monomers is driven by their complementary interactions with the template (in red). Co-polymerization with a cross-linker "freezes" binding groups to form a template-defined "cavity". Removal of the template by solvent extraction or chemical cleavage affords binding sites specific to the original template.

MOLECULAR IMPRINTING APPROACHES

Depending on the interactions between a template and functional monomers involved in the imprinting and rebinding steps, two distinct approaches have been followed to assemble molecularly imprinted polymers (MIPs). In the covalent approach pioneered by Wulff and co-workers, reversible chemical bonds are maintained between the template and the functional monomers during the polymerization and rebinding. In principle, this approach should lead to homogeneous binding sites, given the fact that the template-functional monomer complex is kept intact during the polymerization reaction. However, removal of the chemically bonded template from highly cross-linked polymer matrix is not a trivial task, and the re-binding process is normally much slower due to the necessary formation of the covalent bonds between the target compound and the MIP. Finally, this method requires prior derivatization of the template, and is often difficult to carry out except for those who are experienced in organic synthesis.

In the non-covalent approach invented by Mosbach and co-workers, various non-covalent molecular interactions such as hydrogen bond, ionic interactions and hydrophobic interactions are utilized. Due to the relatively weak interactions involved, often excess of functional monomers are added to stabilize the template-functional monomer complex during the polymerization, which often results in a heterogeneous distribution of binding sites. However, the large varieties of readily available functional monomers and the ease of preparation of MIPs have attracted the widely accepted use following this approach. Furthermore, the recent development of more potent functional monomers, e.g. use of metal coordinating interactions for specific amino acid sequences [4], should generate more homogeneous binding sites. In addition to the above-mentioned covalent and non-covalent approaches, attempts have been made to combine the advantages of both the covalent and non-covalent methods, whereby imprinting is carried out using polymerization of functional monomer being covalently coupled to a template, and selective rebinding by carefully designed non-covalent interactions [5].

MOLECULARLY IMPRINTED POLYMER BEADS

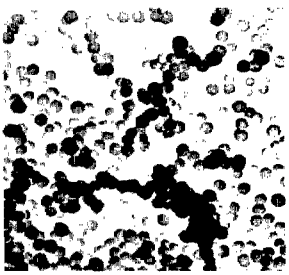
The most common format of molecularly imprinted materials is the cross-linked macroporous monolith, which is often ground and sieved to result in mostly irregularly shaped particles of different sizes. The grinding and sieving processes are labor intensive and waste useful polymers. When used as chromatographic stationary phases, the irregular particle shape also reduces column efficiency. For these reasons imprinted polymer beads are preferable. Although the well-established suspension and dispersion polymerization methods can be used to produce polymer beads, they are however not compatible with most non-covalent imprinting systems, since the aqueous continuous phase used interferes with the molecular interactions between template and functional monomers. In addition, hydrophilic functional monomers, such as the commonly used methacrylic acid, tend to partition into the water phase during the imprinting polymerization, which prevents their incorporation into the binding cavities. To solve all these problems, we have developed a suspension polymerization technique suitable for molecular imprinting in general [6]. This was achieved by using a liquid perfluorocarbon as the dispersing phase. The liquid perfluorocarbon is largely immiscible with most organic compounds and hence form an appropriate inert dispersing phase. A specially designed perfluorinated polymer surfactant was used to maintain stable emulsion droplets containing functional monomers, cross-linker, template and porogenic solvent during the polymerization. Using this technique we could obtain imprinted polymer beads ranging from 5 to 50 μm by varying the amount of the polymer surfactant. When used as a stationary phase in column chromatography, our imprinted polymer beads displayed a chiral resolution of amino acid derivatives similar to those achieved with the traditional ground and sieved MIP particles, however with low back pressure and at high flow rates.

To further simplify MIP preparation, we have recently introduced a precipitation polymerization method for the preparation of imprinted polymer microspheres [7,8]. In comparison with previous imprinting compositions, we started imprinting polymerization from a highly diluted solution of template, functional monomer, cross-linker and initiator. Uniform polymer microspheres ranging form 100 nm to 5 μm can be readily obtained, with favorable binding characteristics for different target compounds. Our polymerization condition is compatible with both covalent and non-covalent imprinting, because there is no interfering surfactant or stabilizer present in the reaction mixture. The small MIP microspheres can be easily suspended in assay solvents and dispensed, and yet readily collected by simple centrifugation. These characteristics are ideal for binding assays using MIPs instead of antibodies. Figure 2 shows the molecularly imprinted microspheres specific for the steroid hormone 17β-estradiol. As demonstrated by a competitive binding analysis, the MIP microspheres had very low cross-reactivity towards a compound that is only slightly different from the template, 17α-estradiol. The small particle size of imprinted microspheres also allows them to be used in microanalysis systems where fluidic apparatus has highly limited dimensions. In a later demonstration, similar molecularly imprinted microspheres were used as chiral selectors in capillary electrophoresis for enantiomer separations [9].

An attractive feature of imprinted microspheres is the fact that their binding sites are more accessible to bulky molecules such as a template labeled with an enzyme probe. In this way, the MIP bound not only the template itself, but also the template-enzyme conjugate in the same specific manner [10]. Thus we have successfully used imprinted microspheres in an enzyme-linked immunosorbent assay (ELISA) for determination of the herbicide, 2,4-

dichlorophenoxyacetic acid. The enzyme label tobacco peroxidase was used to catalyze both colorimetric and chemiluminescent reactions. The same assay was later improved to give much lower detection limit [11] along with potential high sample throughput [12].

It should be mentioned that similar cross-linking polymerization in dilute monomer solution was used to prepare molecularly imprinted microgels [13]. By adjusting monomer concentration and solvent composition, the cross-linking polymerization produced mainly intramolecularly cross-linked microgels. The model system utilized covalent bonds between a sugar derivative and a boronic acid monomer in the imprinting and rebinding reactions. Although binding selectivity of the obtained polymers was only modest, the obtainable nanometer-sized microgels were attractive for many potential applications.



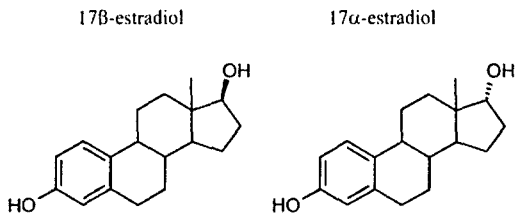


Figure 2. Molecularly imprinted microspheres against 17β-estradiol.

MOLECULARLY IMPRINTED HYBRID MATERIALS

Composite materials can combine desirable features of individual components. In many cases one component provides a stable structural framework to support other functional materials. In other cases it gives the final composite favorable physicochemical characteristics, for example magnetic susceptibility, that can be used to facilitate isolation of the hybrid materials.

As to the first aspect, molecularly imprinted composite polymers were prepared using preformed poly(trimethylolpropane trimethacrylate) beads as the supporting material [14]. The residual double bonds in the supporting beads were used to graft up to 64 mol% of molecularly imprinted cross-linked polymethacrylate. The resulting composite polymer beads were used as chiral stationary phase, which gave an enantioselectivity for the template molecule, Boc-L-Phe equivalent to the purely imprinted polymer, but with improved column efficiency. Instead of a polymer support, silica particles were also used as support and derivatized with vinyl monomers, followed by an imprinting polymerization with a metal coordinating monomer, N-(4-vinyl)-benzyliminodiacetic acid, using the enzyme ribonuclease A as template [15]. The imprinted composite material, when used as a stationary phase, demonstrated specific retention of the template protein due to the metal coordinated interactions between the adsorbent and the target protein. This protein-imprinted composite material represented one of the few early successes in imprinting against biomacromolecules.

By incorporating magnetic iron oxide, we have prepared superparamagnetic molecularly imprinted polymer beads using a suspension polymerisation methodology with a perfluorocarbon liquid as the dispersing phase. The imprinted polymers can be easily withdrawn from solution by

application of a magnetic field, which greatly simplified the separation and washing steps that are routinely used in binding assays [16].

IMPRINTING USING IMMOBILIZED TEMPLATE

For imprinting against small target molecules, the templates are commonly allowed to form complexes with functional monomers free in solution. An alternative route demonstrated by our group and others was to use a template immobilized on a solid support [17]. Following imprinting polymerization, the template and the carrier support were removed by chemical dissolution, which leaves surface imprinted sites on the obtained MIP. In this manner we could control not only the orientation of the binding cavities, but also the pore size distribution of the resulting MIPs, using for example silica beads with different porous structures as the carrier. Compared with imprinting against the free standing template, the surface imprinted binding sites resulting from immobilized template are much more accessible to large analyte-protein conjugates, or to analytes labeled with other bulky reporter entities such as gold colloids. It should be noted that our approach of using an analyte-carrier as template is analogous to the generation of biological antibodies using hapten-protein conjugates. In both cases specific binding sites for small target molecules are generated.

To demonstrate the concept of imprinting against immobilized template, we have chosen in one case theophylline as a model template compound. The template was immobilized by coupling 8-carboxypropyltheophylline to amino-functionalized porous silica beads. The pore volume was filled with a mixture containing the functional monomer, trifluoromethacrylic acid, divinylbenzene and a polymerization initiator. After polymerization, the silica gel was removed by treatment with aqueous HF, which resulted in macroporous organic polymer beads having surface-exposed binding sites (Figure 3). In addition to generating a surface imprinted polymer, the use of the silica framework as a pore-forming template gave well-controlled pore size distribution for the resulting organic polymer beads. When used in radioligand binding analysis using tritium-labeled theophylline as a probe, the imprinted organic polymer beads displayed binding characteristics similar to that obtained with the traditionally imprinted bulk polymers.

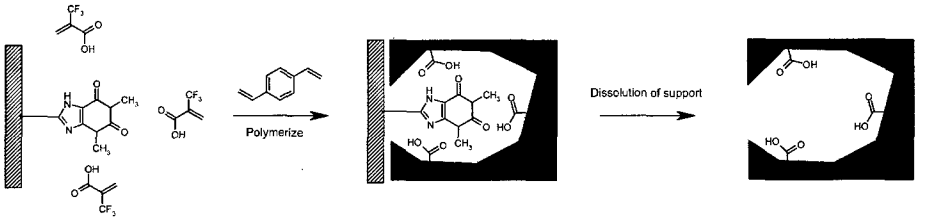


Figure 3. Molecular imprinting using immobilized template.

In a recent report, methacrylate-based mesoporous beads were imprinted against adenine and triaminopyrimidine analogues pre-immobilized on porous silica particles. Dissolution of the silica also resulted in surface confined binding sites specific for adenine and triaminopyrimidine [18].

IMPRINTED SCINTILLATION POLYMERS: A NEW SENSOR CONCEPT

Although molecularly imprinted polymers (MIPs) often display high binding affinity and specificity mimicking natural antibodies [19], there are only few examples of imprinted polymers capable of effective signal generation. For certain types of template molecules, it is possible to design special fluorescent functional monomers that are responsive to template binding, these monomers however can rarely be used when a different analyte is targeted. To circumvent the synthetic difficulties, we have proposed a concept of combining molecular imprinting with proximity resonance energy transfer for chemical sensing [20], where a universal reporter molecule is incorporated into close proximity of the imprinted binding sites. As a proof of principle, we developed a polymerizable organic scintillator and incorporated it into molecularly imprinted microspheres. The imprinted microspheres were prepared by the same precipitation polymerization method as discussed above. Due to the small size of the polymer beads, the organic scintillator was confined in close proximity to the imprinted binding sites that were created by the template, (S)-propranolol (Figure 4). When a tritium-labeled template binds to the MIP, the radioactive decay triggers the scintillator to generate a fluorescent light. The radioactive template free in solution is simply too far away from the scintillator to provide efficient energy transfer, therefore no fluorescence light can be observed. In this way our imprinted polymer can be looked at as a true biomimetic sensor - it directly reports the event of target binding with high selectivity. Following this concept, we have developed a competitive scintillation proximity assay using a radiolabeled template as the probe. The imprinted scintillation polymers could be used in both organic and aqueous solvents [21].

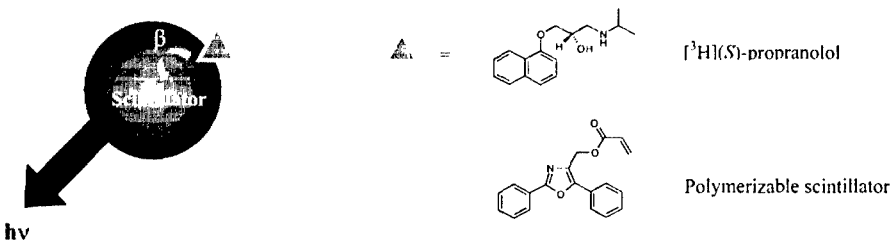


Figure 4. Molecularly imprinted scintillation polymer specific for (S)-propranolol.

"ANTI-IDIOTYPIC IMPRINTING" AND "DIRECT MOLDING": THE NEXT GENERATION

Until now, most research activities have focused on generating imprinted materials recognizing different target entities ranging from small molecules to proteins [22,23], and, even to whole cells [24]. Although in a limited number of examples, imprinted sites have been used to control chemical transformations [25,26], few attempts have been made to explore the utility of molecularly imprinted sites per se as a template to generate new chemical entities.

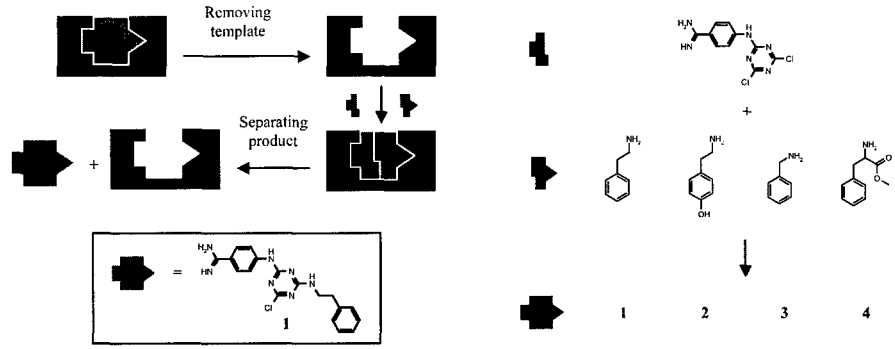


Figure 5. Anti-idiotypic imprinting for generating bioactive molecules.

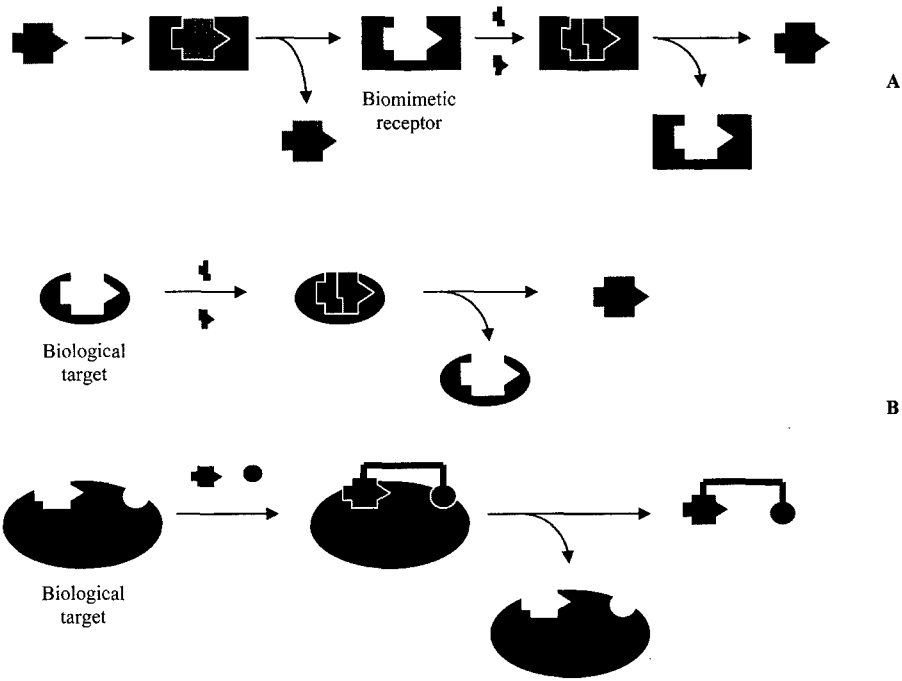


Figure 6. Schematic representation of the "anti-idiotypic" and "direct molding" methodologies. (A) The "anti-idiotypic imprinting" leads to new compounds mimicking the original template. (B) "Direct molding" assembles ligands by polymerization within or between sites of a biological target.

To address this issue, we have recently used pre-imprinted binding sites to generate bioactive compounds mimicking the original template, which was chosen from a known enzyme inhibitor [27]. This "double imprinting" is analogous to the formation of anti-idiotypic antibodies in the immune response system, where the combining site of the secondary antibody is an "internal image" of the original antigen. We envision that our synthetic anti-idiotypic imprinting approach should be useful for finding new drug candidates, especially when the three-dimensional structure of a biological target, a prerequisite for the rational drug design, is unresolved.

In our model anti-idiotypic imprinting we have chosen the medicinally interesting proteinase kallikrein as a model system. In the first step, a previously identified inhibitor (**1**) was used as the original template to prepare an imprinted polymer. The polymer contains specific binding "cavities" mimicking the enzyme's active site. These "cavities" were then used in a second round of imprinting to synthesize the original template, as well as that of new inhibitors using a small library of building blocks (Figure 5). In addition to the original template, we have identified a new enzyme inhibitor using this approach. In principle, our concept of using MIPs to screen building blocks instead of product libraries should greatly simplify the drug discovery process by saving enormous synthetic operations, since only the hit reactions need to be scaled up for further investigation.

The above approach can also be more schematically depicted as shown in Figure 6. Alternatively, a biological target can be used directly as a template to direct polymerization reactions [28] to give synthetic molecules affecting biological recognition process.

CONCLUDING REMARKS

Starting from relatively simple building blocks, molecular imprinting can be used to prepare synthetic materials mimicking biological antibodies and enzymes. Due to their physicochemical robustness, MIPs are favorable in many practical applications to replace biologically generated binding materials. It can be envisioned that MIPs will find potential applications in almost all aspects where natural antibodies have been utilized to provide selective target binding. Recent achievements in molecular imprinting on micro- and nano-spheres, molecularly imprinted "hierarchical structures" generated by using immobilized templates and simultaneous use of multiple templates at different dimensional scales are among the new breakthroughs. We foresee that the merger with novel preparation techniques in materials science will certainly open up new opportunities for molecular imprinting. As examples, microfabricated molecularly imprinted materials may be used in micro fluidic devices [29], and arrays of MIPs addressing different sample markers may lead to more intelligent artificial noses and tongues. In closing this discussion, we would like to point out that the borderline between regular molecular imprinting and the next generation leading to new bioactive molecules, using either the "anti-idiotypic imprinting" or the "direct molding" methodologies, is now being erased.

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