# Molecular Imprinting: Synthetic Materials As Substitutes for Biological Antibodies and Receptors[†]

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Molecular imprinting is a versatile technique providing functional materials able to recognize and in some cases respond to biological and chemical agents of interest. In contrast to biological antibodies, the best known receptors derived from biological combinatorial processes, molecularly imprinted polymers (MIPs) are obtained by template-directed synthesis. Thus, molecular imprinting can more properly be characterized as a "rational design" approach, allowing research and application problems to be solved. Using simple molecular building blocks, material chemists can now produce tailored synthetic materials of much improved stabilities able to replace or complement natural receptors.

## 1. Introduction

Studies of the underlying mechanism of enzyme catalysis and antibody formation began in the 1940s.[1] Discovery of the hypothetical "lock and key" relationship between enzyme and substrate and of the "induced folding" of a polypeptide chain around an antigen inspired researchers to pursue similar synthetic approaches with the aim of obtaining tailored binding materials by chemical means. Although the "instructive model" for antibody formation was not shown by later findings (which now support the "clonal selection theory") to be correct, at least it represented a conceptional beginning. Later studies demonstrated that use of an organic template allowed imprinted cavities in cross-linked polymers and inorganic matrices to be formed and has proven to be the most efficient way of making synthetic materials bearing selective molecular recognition sites.

The process of molecular imprinting involves the formation of recognition cavities through connecting of the different building blocks under the guidance of a molecular template (or print molecule) (Figure 1). Various driving forces can be utilized in the initial preassembly step in order to keep the building blocks associated with the template (or print molecule). Examples of such interactions include covalent bonding, hydrogen bond interaction, van der Waals forces, ionic interaction, metal coordination interaction, and hydrophobic effects. The fixation of binding groups can be achieved by various chemical reactions (e.g., different condensation and addition polymerizations), as long as these reactions do not disrupt the preformed template-building block complex. Although this can be achieved only under ideal conditions, simple free radical polymerization has turned out to be a feasible method compatible with a large variety of templates. Two distinct approaches have been followed for obtaining molecularly imprinted polymers, or MIPs as conveniently abbreviated,[2] those selected depending upon interactions between the template and the functional monomers involved in the imprinting and rebinding steps. In the covalent approach, pioneered by Wulff and coworkers, reversible chemical bonds are maintained between the template and the functional monomers during imprinting polymerization.[3] The same driving force is used for the MIPs obtained to subsequently bind the template. In principle, this approach can lead to homogeneous binding sites, since the template-functional monomer complex can be kept intact during the polymerization reaction. However, removal of the chemically bonded template from the highly cross-linked polymer matrix is often difficult to achieve, and the rebinding process is normally very slow because of the necessary formation of covalent bonds between the target compound and the MIP. Also, prior modification of the template is needed, which can require stringent synthetic conditions. In the noncovalent approach first reported by Mosbach and coworkers, noncovalent forces such as hydrogen bonds, van der Waals forces, ionic interactions and hydrophobic effects are utilized.[4,5] This mimics interactions prevailing in biomolecular recognition processes. Because of the relatively weak interactions involved, an excess of functional monomers is often added to stabilize the template-functional monomer complex during polymerization, which can result in heterogeneous binding sites requiring sometimes subsequent purification. However, the large number of functional monomers commercially available and the ease of preparation have attracted widespread use of this approach. The combinatorial synthesis[6,7] and in silico screening methodology[8] recently developed have also accelerated the process of obtaining optimal noncovalently imprinted polymers. Parallel to this, the rational design of more potent functional monomers, such as via metal coordination interactions to bind specific amino acid sequences, has led to binding sites becoming more homogeneous.[9] New potent functional monomers based on polymerizable amidines and ureas have been developed, allowing stoichiometric amounts of functional groups to be incorporated into imprinted polymers to

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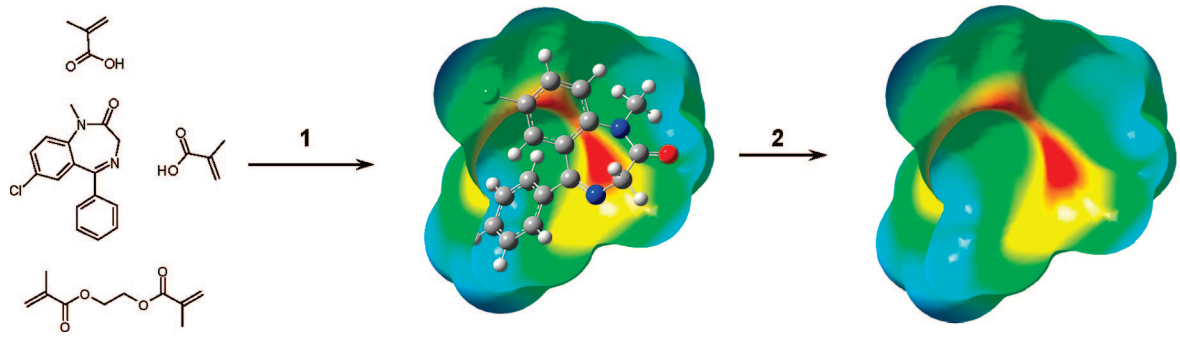


**Figure 1.** Schematic presentation of the molecular imprinting of diazepam (a drug used to treat anxiety). Specific binding sites are generated using methacrylic acid as a functional monomer. After polymerization (step 1) and removal of the template (step 2), binding sites containing carboxyl groups are left in the polymer matrix.

**Table 1. Comparison of MIPs with Antibodies and Aptamers**

| | MIPs | antibodies | aptamers |
|---|---|---|---|
| structural characteristics | synthetic polymers in which binding sites are stabilized by cross-linked 3D network structures | polypeptides folded into defined 3D structures | single-stranded DNA and RNA sequences folded into defined 3D structures |
| preparation | synthetic chemistry based on rational design; MIPs can be produced on a large scale; production costs are low and are often determined by the template molecules | use of an animal's immune response by means of a complicated combinatorial process; monoclonal antibodies can be produced on a large scale using the hybridoma technique | in vitro screening and amplification of combinatorial DNA or RNA sequences; rational design can be used to introduce new functions; large-scale production is limited because of the high costs |
| stability | high stability; can be used in both aqueous and organic solvents | low stability; used only under aqueous conditions | low stability; used only under aqueous conditions |
| binding site characteristics | noncovalently imprinted polymers often have heterogeneous binding sites showing broad affinity distributions | monoclonal antibodies have homogeneous binding sites | homogeneous binding sites |
| structural characterization | difficult for cross-linked amorphous materials | possible | possible |
| main target molecules | low-molecular-weight (<1000) compounds and metal ions | biomacromolecules and small immunogenic molecules that can be conjugated to protein carriers | small and large molecules |

reduce nonspecific adsorption.[10,11] In addition to the covalent and noncovalent approaches mentioned above, attempts have been made to combine the advantages of both the covalent and noncovalent methods, imprinting being carried out using polymerization of a functional monomer covalently coupled to a template, together with selective rebinding by carefully designed noncovalent interactions.[12]

In contrast to antibodies and single-stranded nucleic acid aptamers,[13,14] which are two of the best known receptors derived from combinatorial processes, MIPs are obtained from relatively simple molecular building blocks using template-directed synthesis, which is less time-consuming and more cost-effective. It is difficult to comment in a general way on the different receptors that are prepared, a basic comparison of these materials being provided in Table 1. MIPs, as purely synthetic materials, can in principle be designed from scratch to act not only as simple affinity adsorbents, but also as smart reporters responsive both to targeted molecules[15] and to different environmental stimuli.[16–19]

Potential applications of molecularly imprinted materials have been discussed in a wide variety of review papers, including those on the topics of affinity separation,[20] catalysis,[21] organic synthesis,[22] and chemical analysis.[23,24] Further, a more general article with emphasis on drug discovery can be referred to.[25] A comprehensive survey of scientific papers on the topic of molecular imprinting (until the year of 2003) has also been published, with its some 1500 references providing exhaustive information on prior work.[26] In the present review, we consider molecular imprinting primarily from the standpoint of materials science, examining the most recent developments in the area and discussing directions future developments are likely to take. The following part of this review will introduce recent developments of interesting physical formats of MIPs, including the control of porous structures of imprinted materials. This is followed by a discussion of representative new matrix materials that have been used in molecular imprinting. Micro- and nanofabricated MIPs are covered in a separate section, because these represent an area of increased research focus, where new functions of imprinted materials are being realized through building up more complex structures and reduced physical sizes. As an important step toward a new application area for discovery of new materials/molecules, in Section 5, the exploitation of imprinted nanocavities for directed synthesis (i.e., imprinting within imprinted sites) is subsequently discussed in more detail. Our perspectives for the prior area are given in the last conclusion section.

**Table 2. Different configurations of molecularly imprinted materials.**

| Configuration | Characteristics | Method of preparation |
|---|---|---|
| Monolith | The physical dimensions of monolithic MIPs are controlled by the accessible volume the reaction container provides. | In situ polymerization. |
| Bead | Particle size is controlled by additional surface-active reagents. | Suspension polymerization, emulsion polymerization, precipitation polymerization, and miniemulsion polymerization |
| Membrane/film | Thickness is controlled by the preparation methods. | In situ polymerization, surface-initiated polymerization, and electrochemical polymerization. |
| Molecular monolayer | A monolayer of self-assembled molecules. | Molecular self-assembly. |
| Microscale 3-D structure | A well-defined 3-D structure. | Soft lithography and microstereolithography. |
| Nanofiber, nanowire, nanotube | | Electrospinning and in situ polymerization inside nanochannels. |
| Dendrimer | Single binding site in well-defined globular macromolecules. | Multistep organic synthesis. |

## 2. Imprinted Materials of Controlled Physical Forms and Porous Structures

At the outset, we provide an overview of different configurations of molecularly imprinted materials developed recently (Table 2). Early demonstration of molecular imprinting was carried out using irregularly formed porous polymer particles obtained by grinding of imprinted polymer blocks followed by size fractionation by sieving. This procedure is simple and straightforward and leads to size-defined though irregularly shaped material. Most imprinting publications are still based on use of this method. However, the poor control over the exact physical form of the resulting MIPs and difficulties in scaling up MIP production are limiting factors here. In practical applications, it is preferable to generate imprinted binding sites in polymer matrices having defined physical shapes. Activities of this type have resulted in a variety of interesting MIP materials, as has been highlighted in recent reviews of novel MIP formats, including MIP beads and films,[27] membranes,[28] and in situ prepared monoliths.[29]

**2.1. MIP Beads.** Kempe and Kempe prepared spherical MIP beads recently using suspension polymerization carried out in mineral oil (liquid paraffin).[30] In acetonitrile, the binding of propranolol by the imprinted beads (the template being a drug used to treat hypertension) was much stronger than for a control polymer, although the MIP beads obtained showed a broad size distribution (Figure 2A). Using a polycarbonate-based spiral microflow reactor (Figure 2B), Zourob et al. showed that in mineral oil, this same imprinting reaction can produce monodisperse MIP beads (Figure 2C).[31] The new continuous phase (mineral oil) used in the study is less expensive than the perfluorocarbon liquid used earlier by Mosbach and Mayes[32] and affords clean polymer beads because of the reaction not requiring any stabilizer or surfactant. It remains to be seen whether suspension imprinting polymerization in mineral oil can be used to address a broader range of templates, because use of the polar porogen (acetonitrile) may have certain limitations.

Carter and Rimmer synthesized by means of a polymerizable amphiphilic binding monomer a series of molecularly surface imprinted core–shell particles.[33] The amphiphilic binding monomer was copolymerized together with ethylene glycol dimethacrylate on a cross-linked polystyrene core. The imprinted core–shell nanoparticles displayed surprisingly selective binding to the respective templates (caffeine, theophylline, propranolol, and atenolol) in aqueous solution. Pérez-Moral and Mayes also introduced a signaling component (fluorescent monomer) into the core of structured MIP nanoparticles.[34] The imprinted shell was synthesized using methacrylic acid as the functional monomer, providing selective binding to the original template (propranolol) of the core–shell particles. It was suggested that such imprinted core–shell nanoparticles be employed in nonseparation assays,[35,36] using fluorescent resonance energy transfer (FRET), for example, for signal transduction. Until now, this has not been demonstrated with use of imprinted core–shell nanoparticles, however.

Wang et al. prepared theophylline-imprinted, monodisperse polymer beads (Figure 2D) in one-step precipitation polymerization.[37] Beads (5 $\mu$m in diameter) larger than the earlier submicrometer spheres produced using the same method [38,39] were obtained through fine-tuning the reactant composition (of functional monomer, cross-linker, and solvent) and use of agitation. In recent work, we have shown it to be possible to gain precise control of the size of MIP beads from the nano to the micrometer range by changing the reaction conditions, obtaining uniformly imprinted nanoparticles or microbeads suitable for different applications.[40] Although for each new template an optimal set of conditions may need to be found experimentally, the precipitation polymerization method remains the most straightforward in providing regular polymer beads of excellent reproducibility.

**2.2. Imprinted Membranes.** Minoura et al. prepared photoresponsive imprinted membranes containing a polymerizable derivative of azobenzene, *p*-phenylazoacrylanilide.[41] They demonstrated that reversible isomerization of the photoresponsive compound (under both UV and visible light) serves to regulate the strength of the binding of the original template (dansylamide). For separation purposes, it would be of interest to obtain regulated permeation using stimuli responsive MIP membranes, which has not yet been achieved. In recent studies, instead of MIP membranes being fabricated directly, thin imprinted polymer layers have been grafted onto commercial microfiltration membranes for achieving favorable liquid flow characteristics. Yang et al. prepared molecularly imprinted nanotubes supported by a porous alumina membrane.[42] The imprinted nanotubes could be used directly for the separation of biomolecules, without the alumina support being removed. This may be an ideal system for studying mechanisms of template diffusion

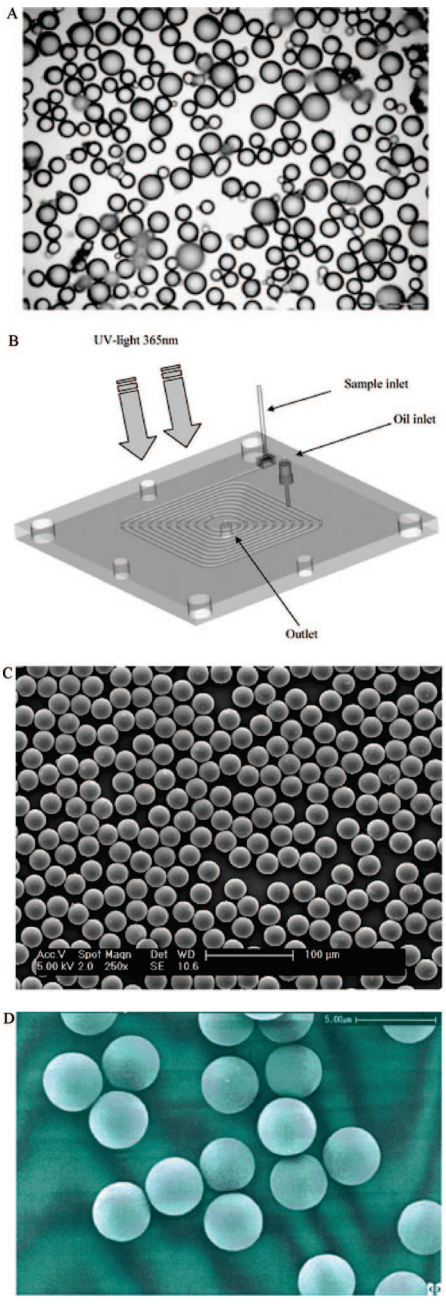


**Figure 2.** (A) Molecularly imprinted polymer beads selective for propranolol, prepared by suspension polymerization in liquid paraffin oil. Reproduced with permission from ref 30. Copyright 2004 Wiley-VCH. (B) Use of a spiral microflow-reactor channel allowing (C) monodisperse MIP beads to be obtained through suspension polymerization in mineral oil. Reproduced with permission from ref 31. Copyright 2006 The Royal Society of Chemistry. (D) Uniform MIP beads selective for theophylline, prepared by one-step precipitation polymerization. Reproduced with permission from ref 37. Copyright 2003 Wiley-VCH.

through narrow bore channels, because different imprinted binding sites can be grafted within the same type of alumina channel so as to provide an open tubular structure, in which the effects of passive diffusion of the template can be separated from any binding-facilitated or binding-delayed transport.

**2.3. Control of Pore Structure of MIPs.** Davis and co-workers used a sol–gel process to synthesize molecularly imprinted microporous silica. Using a carefully designed aromatic template, they attached functional organic groups (aminopropyl) to the walls of microcavities occupied by the template. Subsequent removal of the aromatic core resulted in an amino-functionalized micropore found to be a shape-selective base catalyst.[43] Precise control of the pore structure of imprinted silica matrices can also be achieved using scales of differing length. Dai and co-workers used surfactant micelles and metal ions to create cylindrical mesopores (diameter 2.5–4.0 nm) in silica material. The cylindrical pores were provided with imprinted cavities (1–3 Å) able to selectively bind the metal ion of the template.[44] These hierarchically imprinted materials exhibited not only fast binding kinetics and high capacity, but also favorable metal ion recognition capabilities. Schmidt et al. used different linear polymers of larger dimensions to adjust the morphology of spin-coated MIP films. Removal of the liner polymer by solvent extraction resulted in different porous structures in the propranolol-imprinted film.[45]

Large and well-defined pores of imprinted organic polymers were obtained when a sacrificial supporting material with defined structure, e.g., porous silica beads, and more recently, colloidal crystals, were present during the imprinting reaction.[46,47] After polymerization, the sacrificial support was removed by chemical dissolution, leaving large and regular pores defined by the skeleton of the sacrificial support. Alternatively, when the molecular template used for imprinting was immobilized on the sacrificial support, removal of the sacrificial silica resulted in surface-exposed molecular recognition sites in organic polymers.[48,49]

**2.4. Composites.** Composite materials containing imprinted polymers have been reported in several examples. By incorporating magnetic iron oxide, Ansell and Mosbach prepared superparamagnetic MIP beads using suspension polymerization in perfluorocarbon liquid. The composite beads can be easily recovered from solution by applying a magnetic field, enabling fast and simple MIP-based affinity separations.[50]

Sellergren and co-workers have studied different methods to graft MIP layer on preformed support materials, particularly on silica beads. These include the use of surface-immobilized radical initiator and iniferter,[51,52] the latter being more effective when combined with silica support compared to with cross-linked polystyrene beads. Although there is no evidence that such composite beads can outperform the traditional MIP particles as chromatographic stationary phases, the grafting methods developed can be applied in other systems, e.g., to coat preformed gold colloids or quantum dots with a thin MIP layer. The core of such composite materials, because of their interesting optical properties, would allow new MIP-based sensing materials to be developed.

## 3. New Supporting Matrices for Molecular Imprinting

In conventional molecular imprinting, a high level of cross-linking is used to ensure template-binding specificity. The stiff polymer network, however, prevents efficient removal of the template from the interior of the MIPs. The imprinted polymer also results in slow binding kinetics. Surface imprinting of large biological macromolecules that has been conceived[53] has been shown to overcome the limitation in

binding-site accessibility. Very recently, Shea and co-workers reported surface imprinting of proteins using specific epitopes (surface-exposed amino acid sequences) as templates. The templates were immobilized on solid supports during the imprinting reaction, allowing surface-exposed and well-defined protein-binding sites to be formed in the obtained MIP films.[54] Ratner and co-workers used a radio frequency glow-discharge (RFGD) plasma-deposition method to create imprinted sites on a diasaccharide matrix.[55] The imprinted material displayed selective recognition for a variety of template proteins, presumably via cooperative noncovalent interactions involving hydrogen bonds, van der Waals forces and hydrophobic interactions. Marty et al. investigated the use of liquid crystal networks as a basic material for molecular imprinting,[56,57] with the aim of simplifying template removal and improving site accessibility. Since the interactions that developed between the mesogenic moieties of the liquid crystal elastomers conferred additional stiffness on the network through noncovalent interactions, a low-level covalent cross-linking was found to suffice for ensuring binding-site integrity. Molecularly imprinted binding sites have also been successfully introduced into hydrogels.[58] Through use of a temperature-responsive moiety, Watanabe et al. were able to prepare imprinted polymer gels that underwent a large change in volume when exposed in a shrunken state to a template molecule.[59]

Imprinted microgels soluble in organic solvents were first reported by Biffis et al., using covalent interaction.[60]

The potential of MIPs has been studied for some time also as enzyme mimics.[61] Catalytic MIP microgels were prepared recently by Maddock et al. by use of noncovalent interaction.[62] The preparation method was similar to that employed in precipitation polymerization,[38,39] except that the reaction solvent was fine-tuned so as to yield discrete microgel particles. In a very recent paper, Wulff et al. described the preparation of imprinted nanogels (10–20 nm) that have catalytic activity comparable to more traditional bulky materials.[63] An unusual "postdilution method" was used in order to achieve effective molecular imprinting in the nanogels.

Imprinted polymers containing single binding sites (through monomolecular imprinting) have been achieved. Griebel and Maier synthesized hyperbranched polyesters containing single catalytic sites.[64] Imprinting inside of dendrimers was first reported by Zimmerman and co-workers (Figure 3).[65] There, template molecules were covalently attached to the focal groups of individual macromolecules prior to cross-linking of the periphery of the matrix molecules. They also introduced a chromogenic reporter into imprinted dendrimers, so that the selective binding of alkane diamines and tris(2-aminoethyl)amine could be monitored by following changes in UV absorbance.[66,67] Because every MIP contains a single binding site, ideally the same in each case, monomolecular imprinting is probably the method of choice for preparing MIPs that mimick real monoclonal antibodies. The downside of this approach is that it requires multistep organic synthesis and tedious purification, and can be applied only to templates of a few types possessing high molecular symmetry.

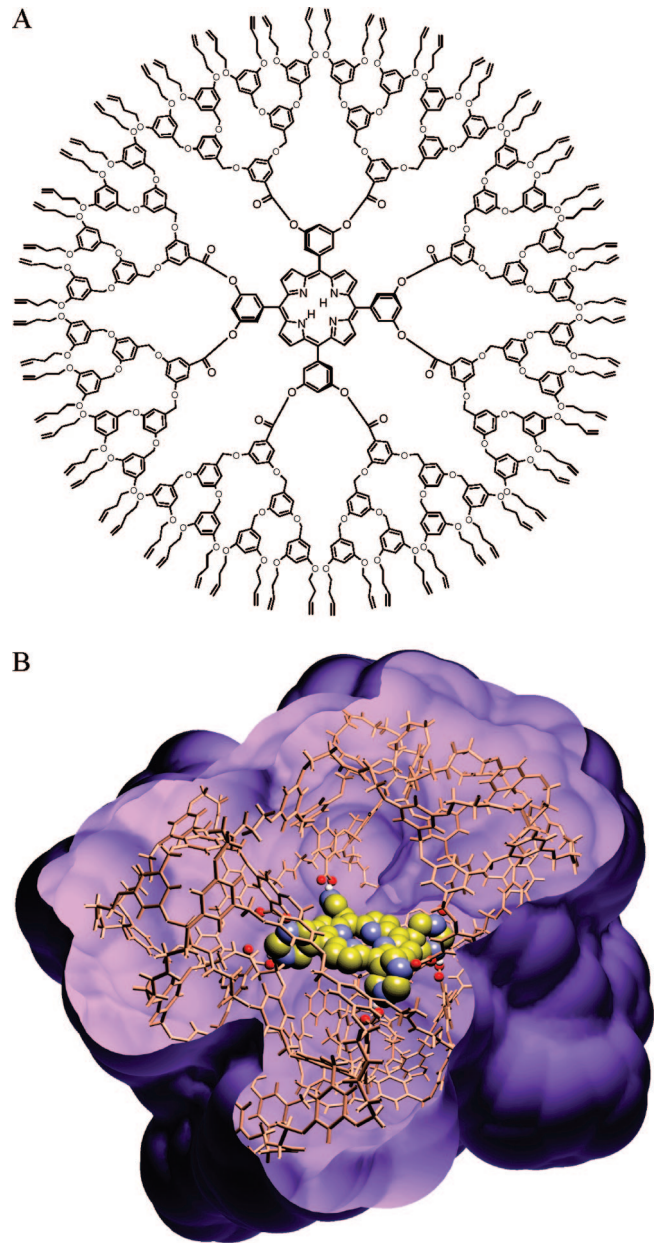


**Figure 3.** Monomolecular imprinting inside dendrimers. (A) Preassembled dendrimer prior to cross-linking of the peripheral vinyl groups. The porphyrin template is covalently attached to the core. (B) The finished dendrimer containing single binding sites for selected porphyrins. Reproduced with permission from ref.[65] Copyright 2002 Nature Publishing Group.

Molecularly imprinted monolayers have been studied in a number of cases. Piletsky et al. prepared a self-assembled monolayer (SAM) of hexadecyl mercaptan on a gold electrode in the presence of a cholesterol template and used the imprinted SAM to measure cholesterol concentration using potassium ferricyanide as an electrochemical tracer.[68] Lahav et al. used a photochemical imprinting method to create recognition sites for a naphthacenequinone derivative in a thiol monolayer on gold electrodes.[69] Following these proof-of-principle studies, the use of an organic SAM to create molecular recognition sites on gold electrodes for developing various chemical sensors was reported by several laboratories.[70–75] Boal and Rotello, using nanoparticles as scaffolds, assembled multivalent binding sites for flavin on gold colloids, using diaminopyridine and pyrene-function-

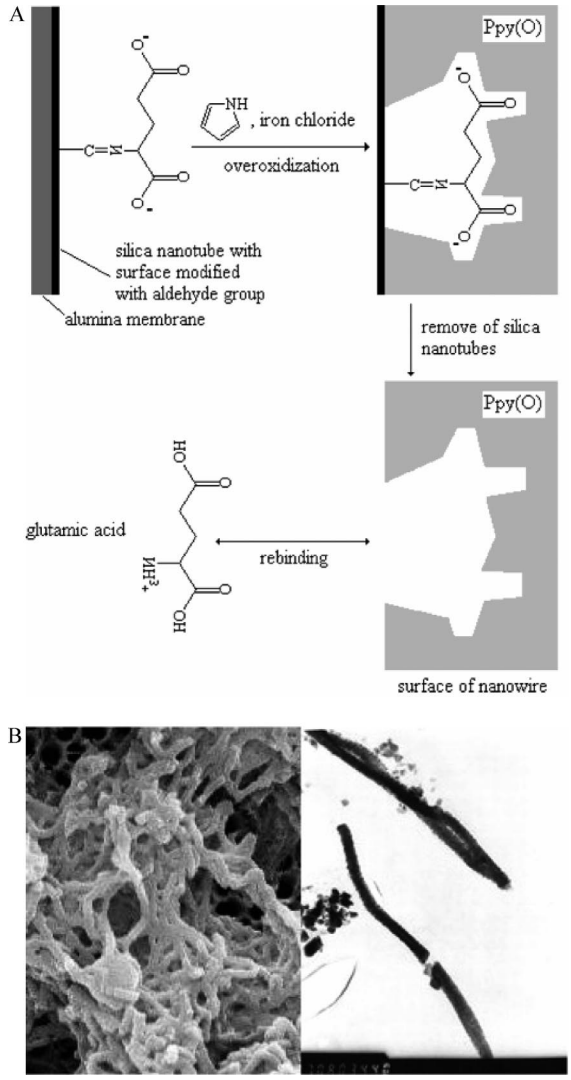


**Figure 4.** (A) Schematic representation of molecular imprinting on nanowires. Glutamic acid immobilized on the inner surface of a sacrificial nanopore membrane was used as the template. (B) SEM (left) and TEM image (right) of glutamic-acid-imprinted 100 nm diameter polypyrrole nanowires after removal of the alumina membrane. Reproduced with permission from ref 81. Copyright 2005 American Chemical Society.

alized thiol as building blocks. The limited mobility of thiols on gold colloids allowed optimal binding to occur between the recognition elements and the flavin template.[76] Similarly, the attachment of organic thiols to gold surface was used to capture specific high-order conformation of boronic-acid-appended poly(L-lysine). The special conformation of poly(L-lysine) was induced by its interaction through the pendant boronic acids with glucose templates. The fixed polymer conformation on the gold surface, after the glucose templates being removed, maintained their binding groups appropriately arranged, able to selectively rebind the sugar template.[77]

## 4. Micro- and Nanofabricated MIPs

The creation of microfabricated MIP filaments was first reported by Yan and co-workers, who made use of a soft lithography method, the imprinting polymerization reaction taking place within microchannels formed between a silicon wafer and a microfabricated poly(dimethyl siloxane) (PDMS) stamp.[78,79] The same method was also used to fabricate

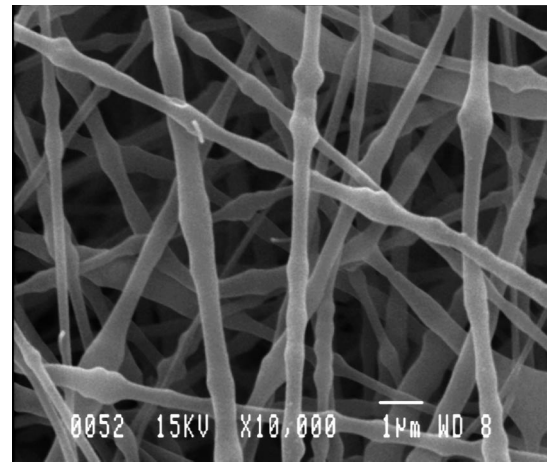


**Figure 5.** Scanning electron micrograph of electrospun composite nanofibers containing estradiol-imprinted nanoparticles. Reproduced with permission from ref 84. Copyright 2006 American Chemical Society.

imprinted optical waveguides for the fluorescent detection of target analytes.[80] One problem connected with PDMS microchannels is that the stamp material is not compatible with the most commonly employed imprinting systems, those containing acrylate, methacrylate, or styrene monomers. A more versatile method was introduced by Yang et al. for the preparation of surface-imprinted nanowires capable of selective recognition of not only small amino acids, but also large proteins (Figure 4).[81,82] In such cases, the templates were first immobilized on the inner wall of a nanopore alumina membrane. Following the imprinting reaction, which was confined within the nanopore channels, the supporting alumina was removed by chemical dissolution, similar to the use of a sacrificial scaffold reported previously,[48] leaving behind polymer nanowires having imprinted sites located only on the surface.

We developed two simple electrospinning methods recently for the preparation of molecularly imprinted nanofibers. In one case, we showed that imprinted nanofibers can be produced from a polymer solution directly, using a simple electrospinning technique.[83] After removal of the template, a pesticide 2,4-dichlorophenoxyacetic acid (2,4-D) by solvent extraction, imprinted binding sites were left in the nanofiber materials being able to selectively rebind the target molecule. Because this simple method requires that the conditions employed in forming the imprinted binding sites be compatible with the electrospinning process, it is applicable only to certain types of templates. A more general approach is to use premade MIP nanoparticles as a starting material to produce composite nanofibers by electrospinning.[84] As we have demonstrated, MIP nanoparticles can be easily encapsulated into electrospun nanofibers, the composite nanofibers obtained being very stable and maintaining favorable molecular recognition capabilities (Figure 5).

The controlled deposition of MIP microstructures for sensor applications has also been investigated. Huang et al. constructed an on-chip sensor device by photoirradiation of a cross-linkable polymer.[85] A cross-linkable polymer in solution can more easily be deposited on a plain surface than a simple monomer mixture can, through use of spin coating and other printing techniques. The final cross-linking reaction

can readily be supplied with short-wavelength photoirradiation. Combing sol–gel chemistry with spin-coating deposition, Marx and co-workers coated thin MIP film (70 nm) on ITO electrodes for fabrication of electrochemical sensors. The sensors displayed very high sensitivity and chiral selectivity for the model analytes investigated.[86] To achieve better control of the chemical composition of the cross-linkable polymers, Li et al. synthesized a series of linear copolymers of 2-methacryloylethyl methacrylate and methacrylic acid by use of atom-transfer radical polymerization (ATRP).[87] The linear copolymers were subsequently allowed to form stable complexes with the templates before being cross-linked. Because the overall fluid properties can be readily adjusted, prepolymers and macromonomers containing binding groups may possibly be adapted in the future to allow them to array MIP patterns on a micro and nanoscale. This has potential application in micro total analysis and in addressing samples that are difficult to analyze. The use of living radical polymerization (ATRP) to produce a surface-bound ultrathin (<10 nm) MIP layer was reported recently by Husson and co-workers, using fluorescent amino acid derivatives as templates.[88] It was shown that use of ATRP provided a microscopically smooth surface (rms roughness <2 nm) and a precise control of film thickness, two matters that are important in developing optical sensing devices. Unfortunately, direct use of ATRP for molecular imprinting in a noncovalent system may be complicated, because the catalytic complex formed by the Cu ion and acidic or basic ligands can easily be disrupted by various template molecules, resulting in the polymerization process not really being "living". In this regard the other controlled radical polymerization techniques using iniferters,[52] RAFT reagents,[89] and nitroxide mediated polymerization[90] seem to be more appropriate.

Conrad II et al. fabricated molecularly imprinted 3D microstructures, using a microstereolithography technique.[91] In doing so, they utilized a focused laser beam to polymerize a liquid monomer solution within a localized volume. The combination of laser-induced polymerization with an $x-y-z$ mobile sample stage enabled precisely controlled three-dimensional microstructures to be generated (Figure 6). These miniaturized, spatially resolved MIP structures can be used for advanced chemical sensing and diagnostic applications.

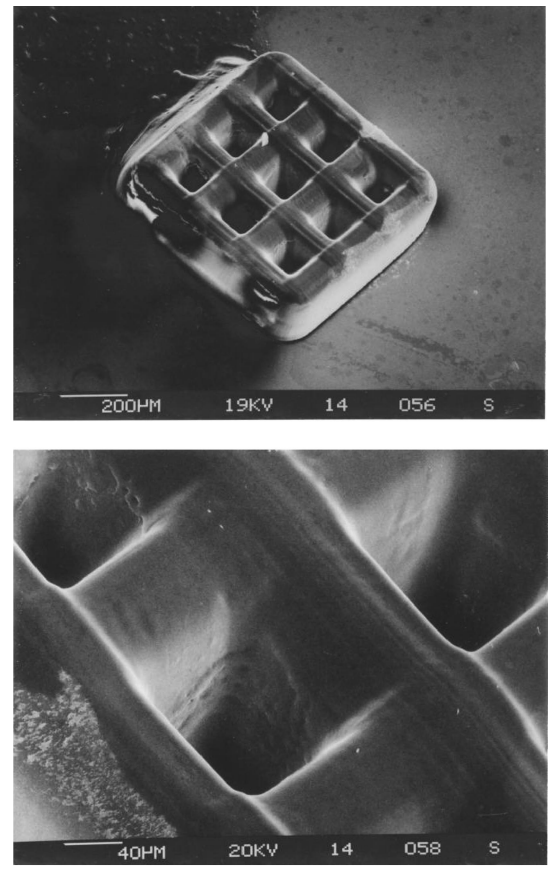


**Figure 6.** Scanning electron micrograph of a molecularly imprinted 3D microstructure (600 $\mu$m × 600 $\mu$m × 100 $\mu$m), using a microstereolithography technique. The magnified image (bottom) shows the wall to be approximately 20 $\mu$m thick. Reproduced with permission from ref 91. Copyright 2003 Wiley-VCH.

## 5. Exploitation of Imprinted Nanocavities

In the last paragraph we wish to draw the reader's attention to the potential of using cavities obtained by imprinting, apart from recognition per se, to other areas such as site-directed organic synthesis inside the nanovessels, similar to what is achieved by using active centers of biomolecules (enzymes).

Molecularly imprinted nanocavities reflect the stereostructure and functionality of the template molecules. The nanocavities can be utilized to control chemical reactions so as to yield new molecules possessing the functions desired. The possibility of using molecular imprinting to expedite the discovery of potential drug leads has been exploited in our laboratory recently.[92–96] Using the protease enzyme kallikrein as a model target, we first prepared an imprinted polymer with one of its known inhibitors, a triazine derivative.[94] We expected that the imprinted "pocket" would somehow mimic the active site of the enzyme, so that it could serve as a selective nanomold for assembling new inhibitors. To test our hypothesis, the imprinted polymer was challenged by various small building blocks composed of a common, positively charged dichloro-*s*-triazine derivative and a series of amine reactants (Figure 7). In addition to the original template, a high yield of a new kallikrein inhibitor was obtained. Those inhibitors that could not be effectively synthesized within the imprinted cavity turned out to be much less active in the enzyme assay.

A strategy of this sort should be especially useful when a ligand of low molecular weight is known to affect certain biological processes, because an MIP can be prepared using the known bioactive molecule as a template so as to afford binding sites mimicking those of the biological target. The MIP can be used to direct the synthesis of new ligands, a process that can be taken as a second imprinting (anti-idiotypic) step. Following the MIP-directed reaction, the various components can easily be separated from the MIP and characterized by use of standard analytical techniques for identifying the products (or the reaction routes) amplified by the MIP. In this way, considerable synthetic effort can be avoided because only the "hits" need to be scaled up and then subjected to both in-depth study in bioassays and further optimization.

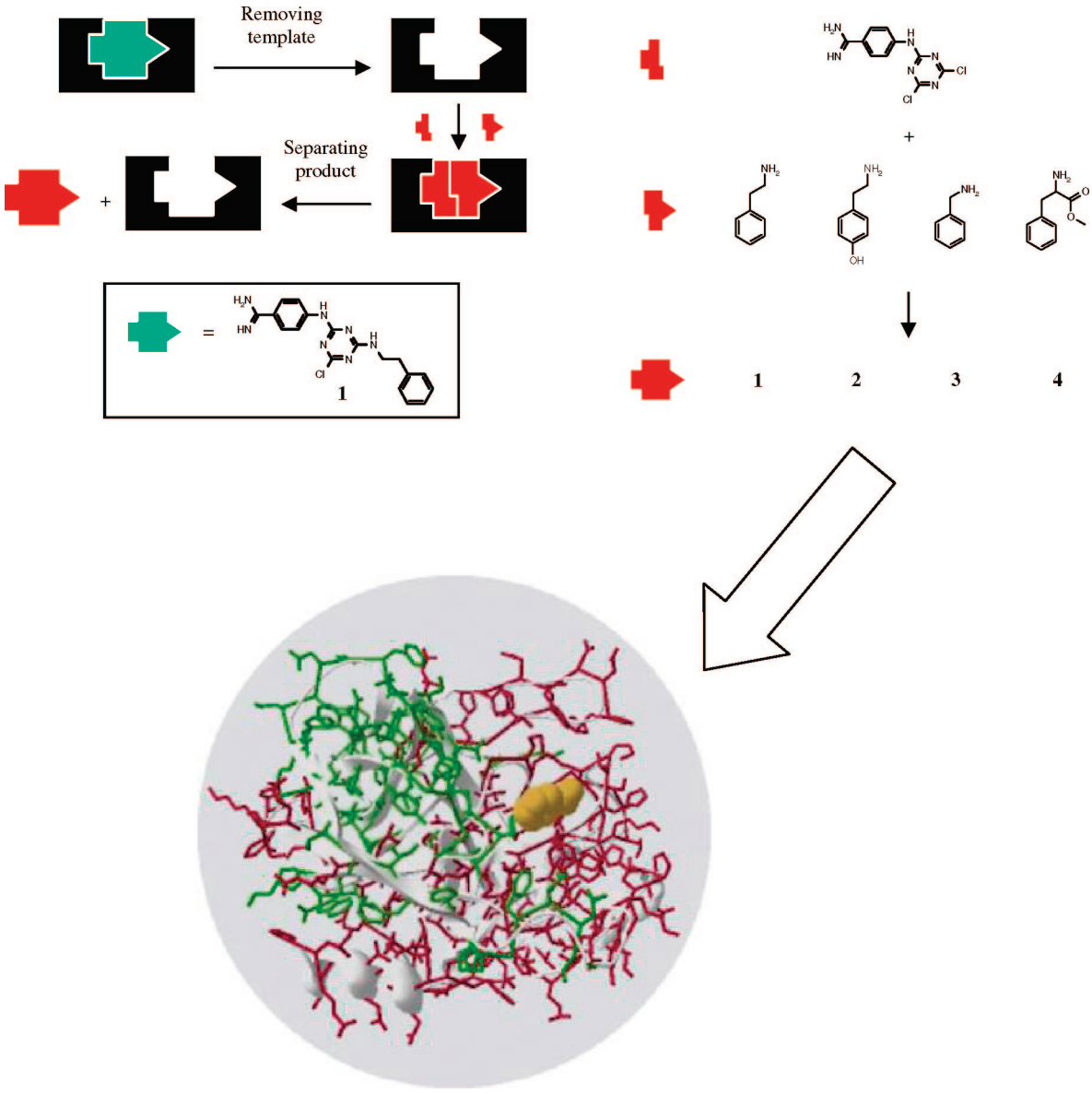


**Figure 7.** Generation of new enzyme inhibitors using imprinted binding sites. Top left: After removal of the template (in green) from the MIP (in black), the binding cavity was used to direct the assembly of reactants (in red). Top right: The chemical reactions investigated in the study. Bottom: A new inhibitor (**3**) showed kallikrein inhibition activity similar to that of the original template (**1**). Here, the enzyme's active site is blocked by an inhibitor, shown in yellow.

Further, one can, as we call it, apply "direct molding", for instance, of the active site of an enzyme. The same enzyme kallikrein was studied and a stereospecific inhibitor obtained allowing the constituents shown in Figure 7 to interconnect in the active site.[95] Although this approach is extremely appealing for finding, for example, new drugs, in particular if not much is known about the biomolecule (enzyme) in question, much more in depth studies are required.

Somewhat related is the preparation in polymers using molecular imprinting of nanovessels allowing a stereospecific reaction to take place as recently demonstrated for a Huisgen reaction.[96] In addition, the nice work on the use of surface-exposed binding sites to control the nucleation of inorganic crystals should be mentioned. Here, formation of the original template was facilitated by the complementary structure of the imprinted sites.[97] Vulfson and co-workers used calcite, a specific crystal phase of calcium carbonate, as a template for generating surface-exposed binding sites on a synthetic polymer. They found that the imprinted sites could selectively promote the growth of calcite instead of aragonite (another polymorph of calcium carbonate), which was normally formed under the experimental conditions selected.

## Concluding Remarks

Following the clear demonstration of the feasibility of molecular imprinting as such, new developments have emerged, including the creation of new and advanced functional materials, particularly with regard to their configuration and performance.

It should be emphasized in this context that it is extremely valuable to have an array of different strategies at hand, each tailored to a specific application. However, despite the exciting ongoing work we have reported, the commercial development of molecular imprinting is still in its infancy.

It is not clear at the moment to what extent MIPs can replace, or more appropriately complement, real biological receptors in terms of selectivity, binding strength, and homogeneity. In a recent study, Steinke and co-workers demonstrated that the chemical process of imprinting can be better controlled using ring-opening metathesis polymerization (ROMP) so as to provide a more homogeneous binding-site distribution.[98] We believe that new developments in synthetic polymer chemistry (such as new living polymerization reactions) will be introduced to the imprinting community rather quickly in the next period of time, the combination of new synthetic chemistry with micro- and nanofabrication techniques providing ever more exciting materials of unprecedented performance.

The introduction and development of new and basically different MIPs for dealing with complex samples is another challenge. The first examples of using MIP arrays as chemical sensors were reported by the Shimizu,[99,100] Dickert,[101] and Takeuchi groups.[102] As shown, the problem of the recognition provided by single imprinted polymer being relatively weak can be solved using an array of different MIPs simultaneously. The combination of MIP arrays with appropriate chemometrics should considerably simplify the analysis of more difficult samples. Much work is needed, however, in developing integrated sensors (an artificial nose and an artificial tongue) based on pattern recognition.

Molecular imprinting is multidisciplinary in nature and possesses a high potential for applications in particular through their capacity for serving as robust artificial receptors. We are confident that in the future there will be a large number of different systems and companies using this exciting new general platform technology, encompassing the analytical area including solid phase extraction, biosensor mimics, separation (e.g., the resolution of racemic mixtures), drug discovery, and beyond.

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