FEATURE ARTICLE

ChemComm
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Imprinted polymers—Tailor-made mimics of antibodies and receptors

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Received (in Cambridge, UK) 6th August 2002, Accepted 12th September 2002
First published as an Advance Article on the web 10th October 2002

The technique of molecular imprinting allows the formation of specific recognition sites in synthetic polymers through the use of templates or imprint molecules. These recognition sites mimic the binding sites of antibodies and other biological receptor molecules. Molecularly imprinted polymers can therefore be used in applications relying on specific molecular binding events. The stability, ease of preparation and low cost of these materials make them particularly attractive. This review focuses on recent developments and advances in the field of molecularly imprinted materials, with special emphasis on applications in immunoassays and sensors recently developed by our group and by others.

Molecularly imprinted polymers

Antibodies are routinely utilised as analytical reagents in clinical and research laboratories. Common applications are immunoassays and immunoaffinity separation,[1] but interest is also increasing in their use in biosensors.[2] These techniques have in common as a first step the binding of an analyte to an antibody. The binding utilises the exquisite recognition properties of an antibody for the antigen, in which the antigen fits exactly into the antibody's binding site, whereas other, even structurally related, compounds are excluded from the site.

The design and synthesis of biomimetic receptor systems capable of binding a target molecule with similar affinity and specificity to antibodies has been a long-term goal of bioorganic chemistry. One technique that is being increasingly adopted for the generation of artificial macromolecular receptors is molecular imprinting of synthetic polymers. This is a process where functional and crosslinking monomers are co-polymerised in the presence of a target analyte (the imprint molecule), which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerisation, their functional groups are held in position by the highly crosslinked polymeric structure. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the analyte. In that way, a molecular memory is introduced into the polymer, which is now capable of selectively rebinding the analyte (Fig. 1). The association between the imprint molecule and the monomers can be based on non-covalent interactions, such as hydrogen bonds, ionic bonds, hydrophobic interactions, van der Waals forces *etc*. or on reversible covalent bonds. For covalent imprinting, a polymerisable derivative of the imprint molecule has to be synthesised, and after synthesis of the polymer, the imprint molecule has to be removed by chemical cleavage. Non-covalent protocols are often experimentally more straightforward, but normally result in a certain heterogeneity of the binding sites, as different associated and dissociated states of the monomer–template complex are in equilibrium during imprinting. Protocols have also been suggested that combine the advantages of both covalent and non-covalent imprinting, that is, the target molecule is imprinted as a stable complex with the functional monomers formed *via* covalent interactions, whereas upon later use of the molecularly imprinted polymer (MIP), only non-covalent interactions come to play. As an example, Whitcombe and co-workers have reported the imprinting of a tripeptide (Lys-Trp-Asp) using a sacrificial spacer (*o*-hydroxybenzamide) between imprint molecule and monomer. After polymerisation, the covalent bonds between the imprint molecule and the monomers are hydrolysed leaving precisely positioned carboxy groups. During rebinding the peptide interacts with the polymer only via non-covalent interactions.[3]

Imprinting matrices and target molecules

At the time being, the majority of reports on molecularly imprinted polymers describe organic polymers synthesised from vinyl or acrylic monomers by radical polymerisation, and using non-covalent interactions. This can be attributed to the rather straightforward synthesis of these materials, and to the vast choice of available monomers with different functional groups. These can be basic (*e.g.* vinylpyridine) or acid (*e.g.* methacrylic acid), permanently charged (*e.g.* 3-acrylamidopropyltrimethylammonium chloride), hydrogen bonding (*e.g.* acrylamide), hydrophobic (*e.g.* styrene), metal coordinating, *etc.* These functional monomers are sometimes considered analogous to the 20 amino acids that constitute the building blocks of proteins. These simple monomers have association constants with the template that are too low for the formation of a stable complex (although in the final polymer, the formation of several simultaneous interactions and a favourable entropy term normally assure tight binding of the target molecule). During non-covalent imprinting, functional monomers have to be used in excess to shift the equilibrium towards complex formation,

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DOI: 10.1039/b207596b

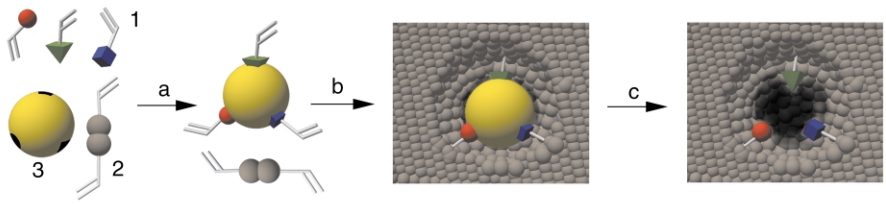

Fig. 1 Schematic representation of the molecular imprinting principle. 1: Functional monomers, 2: cross-linker, 3: template molecule; a: assembly of the pre-polymerisation complex, b: polymerisation, c: extraction of the template liberating the binding site.

resulting in some functional groups being randomly distributed throughout the polymer, which in turn is one of the reasons for non-specific binding. Compared to proteins that nature has selected for the required recognition and binding properties through evolution or, in the case of antibodies, clonal selection, this is a considerable drawback. Therefore, somewhat more sophisticated monomers are being designed that form more stable interactions with the template molecule or substructures thereof, and that can be used in a stoichiometric ratio.[4–7]

Other organic polymers are sometimes used for imprinting that are either better suited for a specific application or easier to synthesise in the desired form, for example poly(phenylene diamine),[8] overoxidised polypyrrole,[9] or polyurethanes.[10] Imprinting is also possible in inorganic matrices, in particular sol–gels of silica[11–14] or titanium dioxide.[15]

The molecular imprinting technique can be applied to different kinds of target molecules, ranging from small, organic molecules (*e.g.* pharmaceuticals, pesticides, amino acids and peptides, nucleotide bases, steroids and sugars) to peptides[16,17] and proteins.[18,19] One has to admit, however, that the imprinting of larger molecules such as, polypeptides and proteins is still a challenge, necessitating specially adapted protocols. There have even been a few reports on imprinting using whole bacteria or yeast cells as templates.[20,21]

Physical forms of MIPs

Traditionally MIPs have been prepared as bulk polymer monoliths followed by mechanical grinding to obtain small micrometer-sized particles. Whereas the materials obtained through this somewhat inelegant, but straightforward method still seem to be useful for many applications, others require MIPs in defined physical forms for which specially adapted synthesis methods are needed. For binding assays, small, spherical particles of below μm size are particularly well suited, whereas in sensors the MIPs are often used in the form of thin layers or membranes. Another aspect is the synthesis of binding sites close to the polymer surface, to avoid long response times and improve the steric accessibility of the sites. A method for the synthesis of MIP nanobeads by precipitation polymerisation has recently been developed in our laboratory.[22] Precipitation polymerisation can be performed with similar monomer mixtures as for bulk polymers, except that the relative amount of solvent present in the mixture is much higher. When polymerisation progresses, imprinted nanospheres or microspheres precipitate instead of polymerising together to form a polymer monolith. The method has the drawback that because of the dilution factor, higher amounts of imprint molecule are needed, although this may be compensated by the typically higher yields. In these materials, statistically a higher percentage of the binding sites should be accessible at the polymer surface.

Wulff's group has used an approach somewhat similar to the precipitation polymerisation mentioned above.[23] However, instead of precipitated particles, soluble polymer microgels were produced. These had a molecular weight in the range of 10^6 g mol^{-1}, that is, in the same order of magnitude as proteins, having only a small number of binding sites per molecule. Although microgels were readily obtained with optimised protocols, to obtain selective, imprinted materials proved to be more difficult with this technique, even though a covalent imprinting complex was used. Very recently, the group of Zimmermann published a report on molecular imprinting inside dendrimers (Fig. 2).[24] Their method involved covalent attachment of dendrons to a porphyrin (the template) core, cross-linking of the end-groups of the dendrons, and removal of the porphyrin template by hydrolysis. This approach seems to make several dreams of traditional 'imprinters' come through, as it ensures nearly homogeneous binding sites, quantitative template removal, the presence of only one binding site per molecule, and solubility in common organic solvents. Whether it is broadly applicable to different target molecules remains, however, to be shown.

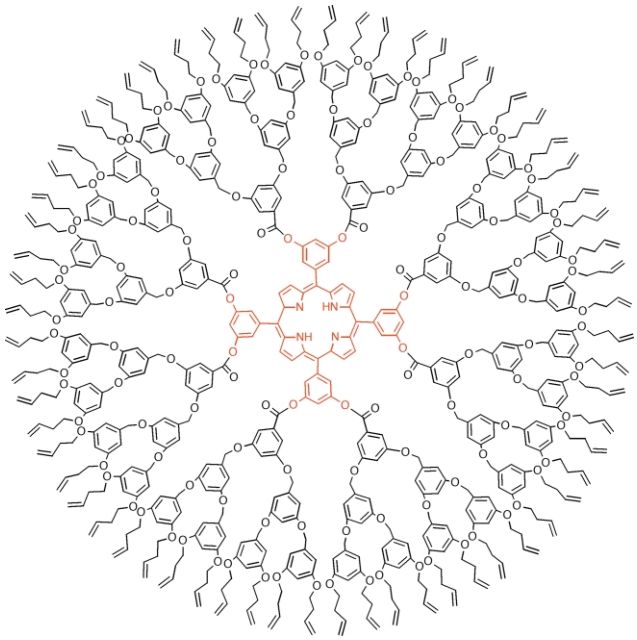

Fig. 2 A dendrimer with cross-linkable double bonds at the outer shell, and the covalently attached porphyrin template (in red) in the core.[24]

Whitcombe and co-workers have developed a technique for the creation of small imprinted beads based on emulsion polymerisation, *i.e.*, small beads are created from an oil-in-water biphasic system stabilised by a surfactant. The particularity of their protocol is that the template molecule (cholesterol) is part of the surfactant (pyridinium 12-(cholesteryloxycarbonyloxy)dodecane sulfate).[25] This results in all binding sites being situated at the particle surface, which was demonstrated by flocculation experiments using PEG-bis-cholesterol.

Another protocol for the creation of surface binding sites has recently been reported by our group. The imprint molecule is immobilised onto a solid support such as porous silica beads, prior to polymerisation.[26] Following imprinting polymerisation in the pores, the silica is removed by chemical dissolution, which leaves behind a porous polymeric structure. The binding

sites are now all situated at the surface of the polymer and should be uniformly oriented (Fig. 3).

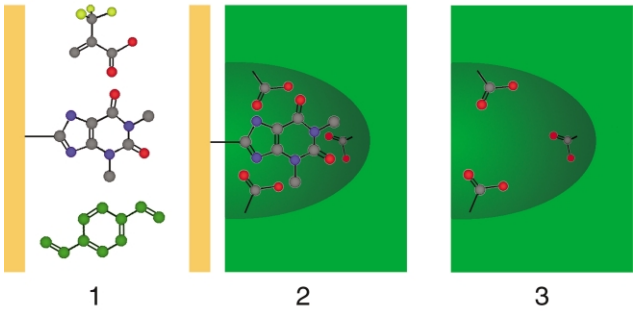

Fig. 3 Molecular imprinting of theophylline immobilized onto a solid support: immobilized template with monomers (1), composite material after polymerization (2), imprinted polymer after dissolution of the support (3).[26]

Thin imprinted polymer films have been used by many authors, in particular for sensors. For example, they can be *in situ* synthesised at an electrode surface by electropolymerisation,[8] or at a non-conducting surface by chemical grafting.[27] Another possibility is to use the 'sandwich' technique, that is, the synthesis of a thin (down to about one micron) MIP membrane between two flat surfaces.[28] Although not the most elegant, it often yields satisfactory results.

Imprinted polymers as antibody binding site mimics

Molecularly imprinted sorbent assays

The wide variety of techniques developed for the determination of analytes based on their specific recognition by an antibody, includes various configurations of immunoassays.[1,29] Molecularly imprinted polymers are certainly very different from antibodies; they are large, rigid and insoluble, whereas antibodies are small, flexible and soluble. However, MIPs share with antibodies one of their most important features, the ability to selectively bind a target molecule. Therefore, they could conceivably be employed in immunoassay-type binding assays in place of antibodies.

This was first demonstrated by Mosbach′s group with a MIP-based assay for the bronchodilator theophylline and the tranquilliser diazepam.[30] The format that was used was analogous to the first solid-phase immunoassay, a competitive radioassay for human growth hormone.[31] In short, to the sample (after extraction of the analyte into an organic solvent if necessary), typically in a 1.5 ml test tube, is added a fixed amount of radiolabelled analyte and a fixed amount of MIP. The MIP amount is chosen in order to bind 50% of the radioligand in absence of cold analyte under the conditions of the assay. The tubes are incubated under shaking until the equilibrium is reached, typically for a few hours, and then centrifuged to sediment the MIP. Unbound radioligand is quantified in the supernatant by liquid scintillation counting. With a series of known concentrations of cold analyte, a calibration curve can be recorded. The theophylline assay, for example, not only showed a very good correlation with an antibody-based enzyme immunoassay currently used in analytical laboratories in hospitals, but, surprisingly, even yielded a cross reactivity profile very similar to that of the natural monoclonal antibodies. From a selection of closely related substances, only 3-methylxanthine, which has one methyl group less than theophylline, was bound to the polymer to some extent (7% binding as compared to theophylline), whereas caffeine, which has one additional methyl group, showed virtually no binding.

This molecularly imprinted sorbent assay format has later been used by us and others to develop assay systems for several other compounds such as drugs,[17,32] herbicides[33,34] and corticosteroids.[35] Andersson and co-workers have shown that MIP-assays can even be performed directly with diluted blood plasma.[36] Table 1 shows as an example the cross-reactivity profile obtained with a MIP specific for the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D).[37] The values are compared to that of biological antibodies. Whereas the antibodies showed to be more selective when 2,4-dichlorophenoxybutyric acid and 4-chlorophenoxyacetic acid were tested, the 2,4-D-methylester is a particular case. It is bound equally well as, or better than, 2,4-D by the antibodies, which illustrates one of the differences between MIPs and antibodies: For raising antibodies against small molecules, prior to immunisation, the hapten has to be coupled to a carrier to obtain an antigenic conjugate. For 2,4-D this was done *via* its carboxy group, therefore the antibody does not recognise that group. This is normally not necessary with imprinted polymers; thus, in our case the MIP binds the methyl ester to only a few percent as compared to free 2,4-D. The results in Table 1 also show that the cross-reactivity profile is different depending on whether the assay was performed in aqueous buffer or in acetonitrile. This indicates that the relative contribution of the different types of non-covalent interactions to the binding is different in polar and non-polar solvents.

Non-radioactive labels

Imprinted-polymer-based assays are conveniently performed using radiolabels, because the labelled analyte has the same structure as the original template. However, this involves the handling of radioactive materials and produces radioactive waste, which is sometimes undesirable. Interest is therefore increasing in the development of alternative assay formats based on other detection methods that could use, just like immunoassays, an enzyme reaction or fluorescence for detec-

Table 1 Cross-reactivities of a 2,4-D-imprinted polymer for different related compounds with respect to the template and target analyte 2,4-D

	Binding compared to that of 2,4-D (%)			
Compound	Radioligand displacement assay in buffer[a]	Fluorescence assay in buffer[b]	Fluorescence assay in acetonitrile[b]	Monoclonal ELISA[d]
2,4-D	100	100	100	100
2,4-Dichlorophenoxybutyric acid	95	100	2	1.5–20[c]
2,4-D-methyl ester	7	4	1	30–160[c]
4-Chlorophenoxyacetic acid	24	42	50	0.5–2.8[c]
Phenoxyacetic acid	2	9	14	—
2,4-Dichlorophenylacetic acid	15	20	2	—
4-Chlorophenylacetic acid	10	15	1	—
Phenoxyethanol	< 0.1	< 0.1	< 0.1	—

[a] Data from reference 34; [b] Data from reference 37; [c] Data from reference 71; [d] Depending on antibody used.

tion. Several years ago we proposed competitive immunoassays that use a fluorescent probe[37] or an electroactive probe[38] for detection. These assays were based on a polymer imprinted with the herbicide 2,4-D, and the probes were not related to the analyte but had some structural similarity with it. It was shown that although binding of the probes to the polymer was only a few percent as compared to the analyte, specificity and selectivity of the assay were on a par with a competitive radioligand binding assay using the same polymer and the radiolabelled analyte (Table 1). The fluorescent assay could be performed in aqueous buffer as well as in organic solvents such as, acetonitrile.

The real challenge, however, has for us always been to use enzyme labels. Although most common with immunoassays, enzymes seemed to be less practical in MIPs assays for two reasons: First, they often only work in aqueous buffers, whereas the use of many imprinted polymers used to be restricted to organic solvents. Second, the rather hydrophobic nature and highly cross-linked structure of the polymer limits the access of the imprinted binding sites by the large protein molecules. However, during the last few years MIPs that perform well in aqueous solvents have been developed,[17,32,34,36] and we have shown that the problem of binding site accessibility might be circumvented by using, instead of large porous MIP particles, imprinted microspheres that have binding sites at or close to their surface. We have developed ELISA-type assays where the analyte was labelled with the enzyme peroxidase. Thus, colorimetry or chemiluminescence[39] could be used for detection. A colorimetric assay has also been reported by Piletsky and colleagues.[40] They have developed a method where the polymer is *in situ* synthesised in the wells of a polystyrene microtiter plate. Aminophenylboronic acid was polymerised in the presence of epinephrine (the target analyte) using oxidation of the monomer by ammonium persulfate. This process resulted in the grafting of a thin polymer layer onto the polystyrene surface. The polymer was then used in a competitive enzyme-linked assay with a conjugate of horseradish peroxidase and norepinephrine.

High-throughput systems

There is an ever-increasing demand for automated, high-throughput assaying and screening of natural products, as well as of biological and chemical combinatorial libraries. MIPs, owing to their specificity, ease of preparation, low price and high chemical and physical stability, could provide a useful complement or alternative to biological receptors for use as recognition elements in such assays. This is especially true in cases where a natural receptor does not exist or is difficult to obtain in large quantities. Our group has recently developed a high-performance MIP-based assay using a chemiluminescence imaging format.[39] Microtiter plates (96 or 384 wells) were coated with MIP microspheres using polyvinyl alcohol as a glue. The analyte is added together with a small amount of enzyme (tobacco peroxidase)-labelled analyte and incubated until the equilibrium is reached. After washing, the amount of polymer-bound 2,4-D-peroxidase conjugate is quantified using luminol as the chemiluminescent substrate. Light emission is quantified with a CCD camera-based imaging system (Fig. 4). This format allows for the simultaneous measurement of a large number of samples.

Another aspect in assay development is their possible use in automated systems for unattended monitoring. For such applications, flow systems are well suited. Their combination with chemically and physically stable, regenerable MIP materials seemed to us particularly promising. In a recent paper we described the design of a flow-injection ELISA-type MIP

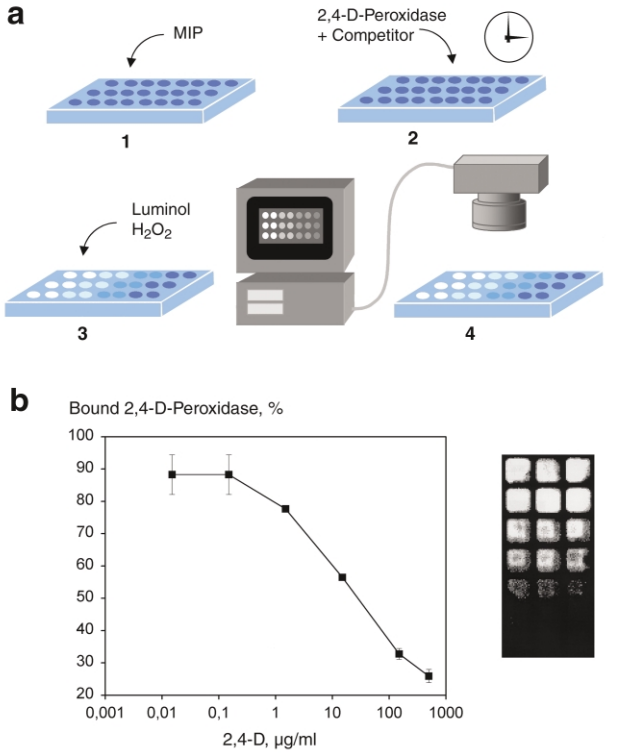

Fig. 4 MIP-based competitive chemiluminescence imaging ELISA. (a) Protocol: 1. The wells of a microtiter plate are coated with MIP microspheres, 2. Samples are added to the wells, containing the analyte 2,4-D and the 2,4-D–peroxidase conjugate, and incubated for 1 hour, followed by two washing steps, 3. The chemiluminescent substrate luminol and H_2O_2 are added, 4. The plate is imaged with a CCD camera. (b) CCD camera image of part of a 384-well plate obtained with a concentration series of 2,4-D in triplicate, and the resulting calibration curve for 2,4-D. Data from reference 39.

assay[41] using the same polymer and detection mode as described above for the imaging assay. A glass capillary was coated with the imprinted polymer and mounted in a flow system. A photomultiplier tube (PMT) was used for detection (Fig. 5). Calibration curves corresponding to analyte concentrations ranging from 0.5 ng mL^{-1}–50 µg mL^{-1} (2.25 nM–225 µM) were obtained, thus making the system one of the most sensitive MIP-based assays reported so far. A further increase in sensitivity by two orders of magnitude was obtained when detection was done in discontinuous mode and the chemiluminescence light was conducted inside the photomultiplier tube by an optical fibre bundle, thus yielding a dynamic range of 5 pg mL^{-1}–100 ng mL^{-1} (22.5 pM–450 nM).

A different format of a flow-injection chemiluminescence assay using MIPs has been developed by Lin and Yamada.[42] A polymer selective for 1,10-phenanthroline was prepared based on a ternary metal complex of the analyte, 4-vinylpyridine–Cu(II)–1,10-phenanthroline, in combination with styrene and divinylbenzene, and packed into a glass tube. When the analyte was injected into a buffer stream containing H_2O_2, it was complexed by the pyridine–Cu(II) binding sites and encountered H_2O_2 molecules. The Cu(II)–1,10-phenanthroline complex was able to catalyse the decomposition of hydrogen peroxide, the analyte 1,10-phenanthroline acting at the same time as the chemiluminescent substrate. In fact, during the reaction, a superoxide radical ion is formed, which reacts with 1,10-phenanthroline and gives a chemiluminescent emission. The 1,10-phenanthroline is destroyed during the chemiluminescent reaction, thus liberating the binding site for another analyte molecule. Although technically elegant, this detection system unfortunately appears to be limited in terms of possible analyte molecules.

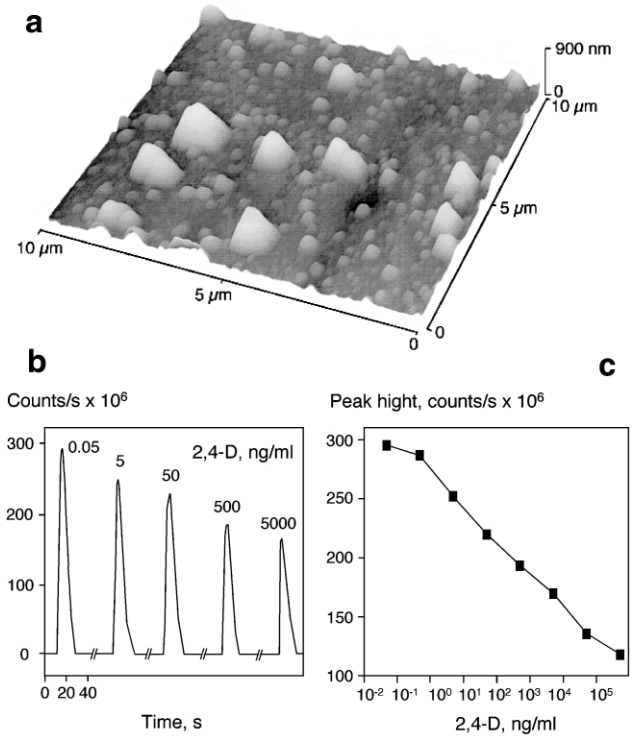

Fig. 5 MIP-based flow-injection capillary chemiluminescence ELISA. (a) Atomic force microscopy image of the polymer-coated inner capillary wall, (b) typical readout of the PMT obtained from the competitive assay in FIA mode at different 2,4-D concentrations, (c) calibration curve for 2,4-D. Data from reference 41.

Homogeneous assays

The competitive assays described above are heterogeneous formats. That implies that the bound fraction of the labelled analyte has to be separated from the unbound fraction before quantification of either of them. Although a majority of the common immunoassays use this format, homogeneous assays are gaining in interest, where the bound fraction of the labelled analyte can be quantified without separation from the free fraction. Such non-separation assays (although not truly homogenous since the polymer is insoluble) have also been developed with MIPs. One approach has been proposed where a fluorescent reporter group is incorporated into the MIP's binding sites. A fluorescent functional monomer, *trans*-4-[*p*-(*N*,*N*-dimethylamino)styryl]-*N*-vinylbenzylpyridinium chloride, has been used together with a conventional functional monomer to prepare a polymer imprinted with cyclic adenosine monophosphate.[43] Upon binding to the imprinted sites, the analyte interacts with the fluorescent groups, and their fluorescence is quenched, thus allowing the analyte to be quantified. Since the fluorophore acts at the same time as a functional monomer that recognises the analyte, it has to be specifically designed for each analyte. A way to introduce a universal reporter group into the polymer is by the use of proximity scintillation as the detection principle, a technique that we have combined with MIPs.[44,45] A scintillation fluor is randomly covalently incorporated into a MIP by copolymerisation. When the scintillation fluor is irradiated with β-rays, it emits fluorescent light that can easily be quantified. Small MIP microspheres were used that formed a stable suspension during the time required for the measurement. Since the scintillation fluors are located in close proximity to the imprinted sites, binding of a radiolabelled analyte results in excitation of the fluor and emission of fluorescent light. In the presence of cold analyte, some of the radiolabel is displaced from the MIP resulting in reduced fluorescence, as the distance is now too long to excite the fluors (Fig. 6). This competitive homogeneous assay, although it requires the use of radiolabels, has some considerable advantages. Since the scintillation fluor does not need to form a covalent or non-covalent bond with the template, the same fluor can be used for many different analytes. The assay is easier to automate as it does not rely on a separation step, and if a PMT array or an imaging method is used, high throughput can be obtained. Another advantage is that the binding can be followed in real-time and binding kinetics can be studied. A possible drawback of such assays is that the requirements in terms of selectivity of the polymer are higher than with heterogeneous assays due to the lack of a washing step.

Applications of MIP-based assays for screening of compound libraries

As outlined above, a possible use of MIPs is as artificial receptors for the screening of combinatorial libraries. Even though there have until now only been a few preliminary reports that demonstrated the feasibility of the approach,[46–50] we believe that MIPs will find applications in drug screening and development, in particular for the initial screening of large libraries. MIPs can be synthesised for molecules for which biological receptors cannot easily be obtained, and they seem to be well adapted to automated high-throughput screening methods. The first attempt to demonstrate the feasibility of this concept was made by Mosbach's group.[46] They established a library of 12 closely related steroid structures. Two of the library compounds were selected as targets and used to prepare two MIPs. It was then shown chromatographically that, as expected, the template steroids were specifically recognised by their respective MIPs out of the entire library. Of course, chromatographic methods, even if automated, can probably not provide the high sample throughput required to screen a large library. For this application, the above described imaging and homogeneous assay formats will be especially useful.

Sensors

In biosensors, a chemical or physical signal is generated upon the binding of the analyte to a biological recognition element such as, an antibody, a receptor or an enzyme. A transducer then translates this signal into a quantifiable output signal. The same general principle applies if a MIP is used as the recognition element instead of a biomolecule. Table 2 depicts the three

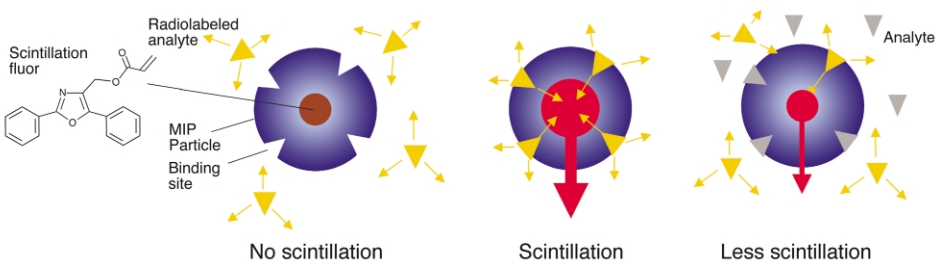

Fig. 6 Schematic representation of a competitive binding assay format based on proximity scintillation.[44]

different possibilities of transducing the binding event. In the simplest case, a certain change in one or more physicochemical parameters of the system (such as, mass accumulation) upon analyte binding, is used for detection. This principle is widely applicable and more or less independent of the nature of the analyte. In order to increase sensitivity and the signal-to-noise ratio, reporter groups may be incorporated into the polymer that generate or enhance the sensor response. If the analyte possesses a specific property (such as, fluorescence or electrochemical activity) this can also be used for detection.

Early attempts to utilise the recognition properties of MIPs for chemical sensing were for example ellipsometric measurements on thin Vitamin K_1-imprinted polymer layers,[51] the measurement of changes in the electrical streaming potential over an HPLC column packed with a MIP,[52] or permeability studies of imprinted polymer membranes.[53] Mosbach's group reported the first integrated sensor based on a MIP, a capacitance sensor consisting of a field-effect capacitor covered with a thin phenylalanine anilide-imprinted polymer membrane.[54] More recently, capacitive detection was employed by others in conjunction with imprinted electropolymerised polyphenol layers on gold electrodes.[55]

During the past few years, mass-sensitive acoustic transducers, in particular the quartz crystal microbalance (QCM), have become very popular in combination with imprinted polymers. These sensors are based on the first group of transducers (Table 2). They consist of a thin quartz disk with electrode layers on both sides, which can be put into oscillation using the piezoelectric effect. A thin imprinted layer is deposited on one side of the disk. Analyte accumulation in the MIP results in a mass change, which in turn causes a decrease in oscillation frequency that can easily be quantified by frequency counting. Possible reasons for the success of this transducer type are its relatively low price, its robustness and ease of use. In addition, it is relatively easy to interface the MIP with the sensor. A few years ago we reported, in collaboration with Kutner's group in Warsaw, the first enantioselective MIP-based QCM sensor (Fig. 7). For this sensor we relied on common acrylic polymers as the imprinting matrix.[28] The reason for that was the abundance of know-how available on such polymers, and their adaptability to many different template molecules due to the plethora of available functional monomers. The sensor, coated with a polymer imprinted with *S*-propranolol (a β-blocker), was able to discriminate between the *R* and *S*-enantiomers of the drug with a selectivity coefficient of $\alpha = 5$. Others have used different materials as the imprinting matrix. For example, a QCM has been used to construct an imprinted polymer-based sensor for glucose.[8] The polymer, poly(*o*-phenylene diamine), was electrosynthesised directly at the sensor surface in the presence of 20 mM glucose. In that way, a very thin (10 nm) polymer layer was obtained that could rebind glucose with certain selectivity over other compounds such as ascorbic acid, paracetamol, cysteine and to some extent fructose. Thin TiO_2 sol–gels have been used for imprinting of azobenzene carboxylic acid.[15] Very nice work in this area has recently been reported by Dickert's group.[21] They have

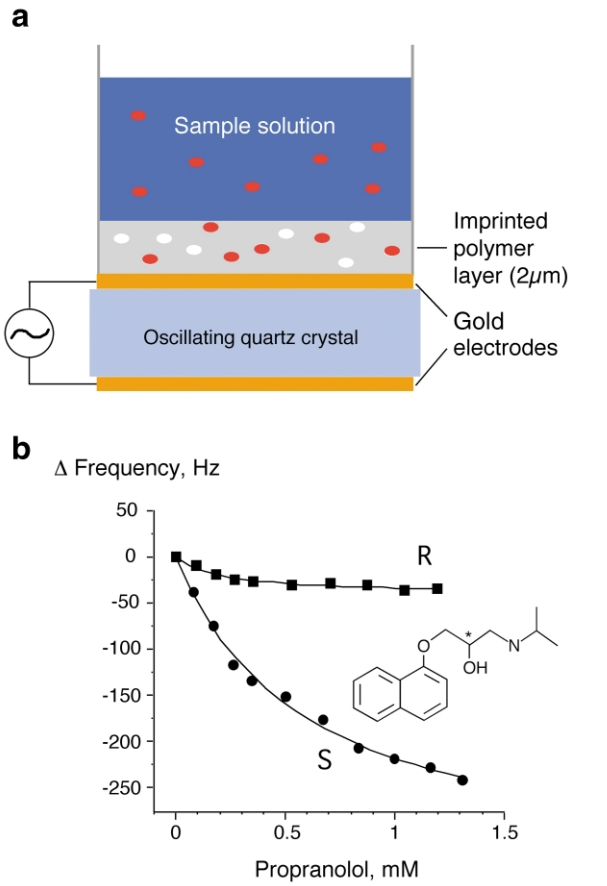

Fig. 7 (a) Schematic representation of a piezoelectric microgravimetry sensing device containing an imprinted polymer film as the recognition element. (b) Dependence of the resonance frequency change of the QCM resonator on the concentration of *R*-propranolol and *S*-propranolol. Adapted from reference 28, with permission.

produced imprints of whole yeast cells in polyurethane layers and in sol–gel layers at the surface of a QCM crystal using a stamping method. The sensor could be used to quantify yeast cells in suspension at concentrations between 1×10^4 and 1×10^9 cells per ml under flow conditions.

Other sensors belonging to the first group (Table 2) have been designed based on conductometric transducers.[56–58] Here, two electrodes are separated by an imprinted polymer membrane. Binding of the analyte to the polymer changes its conductivity, which is translated into an electrical signal. A sensing device for the herbicide atrazine which is based on a free-standing atrazine-imprinted acrylic polymer membrane and conductometric measurements has been constructed by Piletsky and coworkers.[59] According to the authors, the kind and molar ratio of crosslinking monomers used, and the relative amount of porogenic solvent in the imprinting mixture, were important factors not only for the flexibility and stability of the MIP membranes, but also because the conductometric response

Table 2 Different approaches to the transduction of the binding signal in MIP-sensors

Signal generated:	directly through the binding event	by the analyte	by the polymer
What is measured	Change in general physiochemical properties of the system	Specific property of the analyte	Change in the signal emitted by reporter groups incorporated into the polymer
Examples	Mass change (QCM) Capacitance change	Fluorescence Electrochemical activity IR Spectrum	Fluorescence Scintillation Spectral shift Proton release (pH)

seemed to depend on the ability of the MIP to change its conformation upon analyte binding.

If the target analyte exhibits a special property such as fluorescence[10,60] or electrochemical activity,[61] this can be exploited for the design of MIP-based sensors (Table 2, second group). If the analyte lacks such property, a competitive or displacement sensor format may be used. In collaboration with Turner and coworkers, we have developed a voltammetric sensor for the herbicide 2,4-D[38] where the electroactive compound 2,5-dihydroxyphenylacetic acid was used as a probe. MIP particles were coated as a thin layer onto a screen-printed carbon electrode (Fig. 8). The electrode was then incubated with

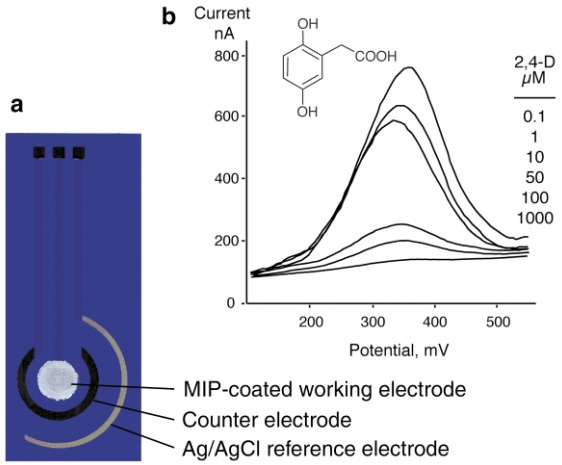

Fig. 8 (a) Screen-printed electrode coated with MIP-microparticles. The particles are covered with a thin agarose membrane. (b) Structure of the electroactive probe 2,5-dihydroxyphenylacetic acid, and differential pulse voltammetric scans obtained in the presence of different concentrations of the analyte 2,4-D.[38]

the sample to which the probe was added. In the presence of the analyte, some of the probe was displaced from the imprinted sites, whereas the remaining probe was directly quantified by differential pulse voltammetric measurements. We believe that, because of the potential low production costs, the combination of screen-printed electrodes and MIPs is particularly well suited for the design of disposable sensing elements.

An elegant way of designing the MIP/transducer couple is to have the signal generated by the polymer itself (Table 2, third group). This approach appears promising since it does not depend on a special property of the analyte, and moreover, should facilitate the integration and production of the sensing device. One example for such a format is a polymer containing a fluorescent metalloporphyrin as the reporter group, which acts at the same time as one of the functional monomers (Fig. 9).[62]

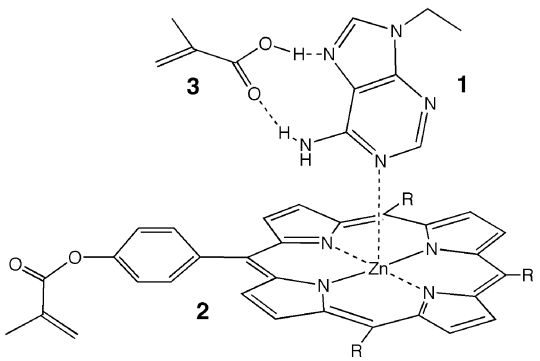

Fig. 9 Complex of the template 9-ethyladenine (**1**), a Zn-porphyrin signalling monomer (**2**) and methacrylic acid (**3**) as a co-monomer.[62]

Binding of the analyte 9-ethyladenine then results in quenching of the fluorescence of the polymer. The above-mentioned proximity scintillation-polymers belong at the same time to both the second and the third groups (Table 2). They have the advantage that the reporter group (scintillation fluor) does not necessarily need to be placed in the binding site.[44]

The signals generated by most transducer types are two-dimensional and provide only limited information about the composition of the sample. Although this is normally compensated by the high selectivity of MIPs, a different strategy is the use of transducer mechanisms that generate signals with a higher inherent information content. One way to achieve this is to exploit the high molecular specificity of absorption spectra in the mid-infrared spectral region (3500–500 cm^{-1}). The combination of MIPs and FTIR spectrometry might allow analytical problems to be addressed where the selectivity of the MIP alone is not sufficient, *e.g.*, when samples with complex matrices are to be investigated, or when structurally very similar analytes are present in the sample. Together with Mizaikoff's group, we have tried to combine imprinted polymers and infrared evanescent-wave spectroscopy in a chemical sensing device.[63] A MIP film selective for 2,4-D was deposited on a ZnSe attenuated total reflection element, which was mounted in a flow cell. Accumulation of 2,4-D in the MIP layer could be followed on-line and in real time by FTIR spectrophotometric measurements. Analyte binding was concentration dependent and could be quantified by integrating characteristic analyte bands.

Outlook

Even though molecularly imprinted materials have already found one niche application, namely, solid-phase extraction, for which they are commercialised, more work needs to be done to make them a real alternative or complement to biomolecules. In particular, what one hopes to achieve is the development of MIPs that contain a more homogeneous binding site population, have a higher affinity for the target analyte, and that can be routinely prepared and used in aqueous solvents. For some applications, in particular for trace analysis, a serious problem seems also to be the fact that complete removal of the template from a MIP is difficult if not impossible,[64,65] although sometimes this problem can be addressed by the use of a structural analogue of the target analyte as the template.[64,66] If at least some of these problems can be solved (a considerable part of the current research efforts on MIPs deals with their optimisation), the outstanding stability of MIPs, their low price and easy integration in standard industrial production processes are among the properties that should make them an attractive alternative to biomolecules in analytical chemistry and other applications. It is also clear that using a more systematic approach to design a MIP, rather than the good old trial-and-error method, would be desirable. In consequence, combinatorial[67–69] or computational[70] methods have been proposed to obtain an optimised polymer for a given target analyte. It appears that the development of imprinted polymer-based analytical methods is just about to leave the proof-of-principle stage, and researchers are starting to address specific analytical problems and to measure real samples. Fortunately, industry, as well as national and international funding agencies such as the European Commission seem to have recognised the great potential of MIPs in analytical chemistry and other areas.

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