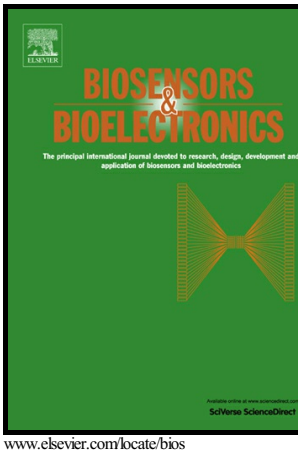

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Molecularly-imprinted polymer sensors: realising their potential

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Abstract

In parallel with recent developments in communications, nanotechnology and materials sciences, there has been extraordinary growth in the area of biosensors, with almost half of the total number of papers ever published (1962-2015) appearing in the last five-years (2010-2015). Molecular imprinting offers a route to the creation of specific and selective cavities in a 3D-polymeric network, which are complementary not only to the size and shape of a target species, but also provide interaction points and a coordination sphere around the template molecule. Given the challenges facing biosensor technologists, it is natural that this approach to create potentially highly stable synthetic ligands as an alternative to, or to compliment natural receptors, should emerge as a key line of interdisciplinary research. Despite the profuse amount of recent literature on molecularly-imprinted polymers (MIPs) and some limited commercial activity, these promising materials still need to overcome some limitations before taking their place in analytical market. In this review, we have focused on the most promising advances in MIP-based biosensors to illustrate how close to market they really are. We present our material under five main sections covering computational design, polymerisation strategies, material combinations, recent sensor designs and manufacturing issues. Each section provides technical details and evaluates the effect on sensor performance.

Keywords: MIP biosensors, computational polymer design, polymerisation conditions, monomer combinations, synthetic receptors, manufacturing MIPs.

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Introduction

The remarkable growth in both academic publications on biosensors and their commercial sales has been previously documented (Turner, 2013) along with their multifarious applications. Increasing interest in the replacement of biological receptors with semi-synthetic ligands such as engineered proteins and aptamers, and wholly synthetic analogues such as molecularly-imprinted polymers, has made a substantial contribution to the recent expansion of the field (Web of Science™, Thomson Reuters, 2015) (Figure 1). The materials component is at the heart of an effective sensor, because it defines the recognition selectivity and specificity, limits of detection and quantification, and overall quality and robustness of the results obtained. Polymer-based receptors attract attention due to possibilities for mass production, simplicity, improved shelf-life and cost, and because they potentially minimise batch-to-batch variation in performance. Also, automated production with predetermined features for both single and multi-analytical detection is another attractive opportunity created by polymer-based biosensors, with considerable potential for improved personalised healthcare (Turner et al., 2014). A plethora of polymerisation processes are now available to the sensor technologist and molecular

imprinting has emerged as one of the most promising approaches for producing excellent functional materials on sensor surfaces.

<<< Figure 1 here >>>

Molecular imprinting is a type of template-assisted synthesis that results in selective cavities in a 3D-polymeric network (Piletsky and Turner, 2006). Removal of templates from the polymer exposes cavities that are complementary not only in size and shape, but also with respect to interaction points and the coordination sphere of template molecules (Figure 2). Because of these interesting features, molecularly imprinted polymers (MIPs) have attracted increasing attention, as the numbers of the publications each year illustrate (Figure 1). In common with biosensors, the amount of literature related to MIPs has risen substantially over the past five years and now almost half of all papers ever written about the area were published between 2010-2015 (SciVerse SCOPUS, 2015). In addition, an interdisciplinary research area has spontaneously emerged combining biosensors and MIPs. MIPs have been considered as promising alternatives to their biological counterparts to develop novel matrices in diverse research fields such as chromatography, biotechnology, environmental science, food safety, biomedical sciences, and of course, biosensors. Although the fundamentals and conditions for polymerisation have been proposed and discussed in considerable detail, MIP-based biosensors still need to overcome some problems before taking their place in the biosensor market. First of all, heterogeneity of binding sites can cause non-specific interactions and so decrease the quality of the signal. In order to overcome this problem, workers have started to use combinatorial and computational methods to select the most appropriate functional monomer and

template:monomer ratio for polymerisation. Secondly, the random nature of polymerisation might also effect on the heterogeneity in distribution of imprinted cavities through polymeric backbone, causing unwanted variation in diffusional behaviour. Herein, controllable polymerisation strategies have been adopted including RAFT, ATRP, iCVD, and click-chemistry (for grafting) to improve MIPs. Also, new production methods such as core-shell, composites having multi-organic/inorganic components, ultrathin films and soft lithography for patterning have been developed. In order to enhance the signal quality and to widen applicability, various MIP-based sensor platforms have been developed including single analytes on multiple substrates, multi-analyte on single substrate arrays, microfluidic biochips and lab-on-valve systems. However, MIP-based biosensors still need to deliver satisfactory solutions as to how they can be economically manufactured and integrated into current devices. Notwithstanding this, there are some promising advances in these areas including the manufacture of MIP nanoparticles, and the development of screen-printable materials for disposable sensors and fully integrated biosensing instruments.

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This review is intended as an update to our earlier overviews of MIP-based optical (Piletsky and Turner, 2008) and electrochemical (Piletsky and Turner, 2002) sensors, which document the emergence of the field. In this review, we focus on promising recent advances in MIP-based biosensors to illustrate whether they are getting closer to the market or not. We present our material under five main sections covering computational design, polymerisation strategies, material combinations, recent sensor

designs and manufacturing issues. Each section provides technical details and evaluates the effect of sensor performance.

Computational Design

By using theoretical calculations to assess conformational and chemical complementarity between the template and functional monomers in different ratios and in different combinations, it is possible to improve sensor design and performance as well as confirming reproducibility and robustness. In addition, theoretical calculations give information about the type and combinations of dominant interactions between the template and functional monomers. In general, density functional theory (DFT) and Ab initio in respect of different restriction algorithms can be applied computationally after geometry optimisation studies. Nezhadali et al. used computational calculations to develop electrochemical biosensors for analyses and multivariate optimisation for biological samples (Nezhadali et al., 2013; Nezdahali and Shadmehri, 2013; Nezdahali and Mojarrab, 2014). Nezhadali et al. (2013) theoretically compared complex formation abilities of thiophene, 3-methylthiophene, pyrrole and furan with template molecules in the gas phase by applying a restricted Hartree-Fock method to detect 2-aminobenzimidazole in blood samples. On the basis of their electronic stabilisation energy calculations, they selected pyrrole as a functional electropolymerisable monomer. Nezhadali and Shadmehri (2013) applied Moller-Plesset perturbation theory to evaluate a complex with proper electronic stabilisation energy, i.e. higher binding energy following the most stable, lowest energy conformations. Nezhadali and Mojarrab (2014) used density functional theory using hybrid functional calculations during geometry optimisation to select the monomer with the higher recognition capability against hydrochlorothiazide in human

serum and pharmaceutical samples. Meanwhile, they applied multivariate statistical analysis to reduce the number of effective parameters to provide a cost-effective and more manageable factor set for analytical optimisation purposes. Azimi and Javanbakht (2014) also used density functional theory in accordance with a semi-empirical method in the gas phase with chloroform to localise the molecules in the maximally stable conformations. Following geometry optimisation, they calculated interaction energies depending on the Mullikan atomic charges and hydrogen bonding to evaluate the template/monomer molar ratio. These authors also considered the location of the crosslinker around the template molecules and the theoretical interaction ability of the imprinted cavities against template, by applying calculations for the polymeric chain with and without template molecules. They reported that not only functional groups, but also other atoms in both the template and cavity's backbone and charge distribution are involved in the imprinting process and controlled the recognition efficiency. Gholivand et al. (2012) also reported binding energy levels of template molecules with different monomers in the gas phase by applying density functional theory. In addition, when they took into account the effect of solvent in the energy calculation in accordance with the polarisable continuum model, in another calculation term in solution, they determined that solvent significantly influenced the stability and binding energy order of complexes. They successfully developed a sensitive biosensor for cyanazine detection in food samples by combining functional monomers with the appropriate solvent, in the light of their theoretical calculations. Hawari et al. (2013) described an interdigitated electrode platform for detection of mango ripeness. By selecting volatile organic compounds from different mango ripeness stages, they developed a selective biosensor through computational modelling of the optimum composition ratio of cross linker, functional

monomer and target molecules. For this purpose, they applied a semi-empirical method together with the Austin method and Polak-Ribiere algorithms, while taking into special consideration hydrogen bonds and Van der Waals interactions between the target molecules and the functional monomers. They found that hydrogen bonds naturally play a significant role in facilitating complex formation and in achieving high stability. Huynh et al. (2015) reported two functional monomers newly synthesised for biomimetic recognition of nicotine. The complex between these specific monomers and nicotine were characterised not only by high negative Gibbs free energy theoretically calculated using density functional theory, but also by a high stability constant determined by the titration curve. Using X-ray photoelectron spectroscopy, they also demonstrated that nicotine molecules had selectively adsorbed onto the imprinted cavities by means of a synergetic effect of coordination around zinc atoms and hydrogen bonding (Figure 3). As a conclusion, their theoretical calculations supply helpful data about functional monomer efficiency, proper monomer:template ratio and crosslinker composition. In addition, it is possible to determine dominant interactions between counter parts, not only in gas phase under theoretical conditions, but also in solution phase while considering polarisation factors.

<<< Figure 3 here >>>

Polymerisation Strategies

Polymerisation is concisely defined as attachment of small functional molecules to create a long 3D-network that could be produced by applying different external stimuli, including heat, light, charge and chemicals (Karimian et al., 2013a; Tran et

al., 2014; Holthoff et al., 2011; Nguyen et al., 2012). In general, polymerisation methods applied for producing regular polymers can be modified to create MIPs under the physicochemical conditions required for the imprinting process. Since the available space does not allow us to present all polymerisation approaches, only the most prominent examples will be discussed. As mentioned before, MIP-based biosensors may sometimes deliver poor signal quality due to variation in the distance of imprinted cavities from the sensor surface. In order to avoid this problem and to enhance the signal, researchers have focused on developing ultrathin polymeric films on the sensor surface. By reducing the film thickness, variation of imprinted cavity distance in a 3D-network can be limited. One alternative solution to this problem is grafting the initiator on the sensor surface by click chemistry and starting the polymerisation from the surface upward (Wang and Shannon, 2011; Gam-Derouich et al., 2012; Wei et al., 2011; Tan et al., 2013). Wang and Shannon (2011) reported MIP-based gold electrodes using click chemistry for electrochemical detection of hydroquinone. They immobilised the crosslinker following self-assembled monolayer formation and then produced hydroquinone-imprinted thin films on the gold surface. They also developed imprinted films by a drop-coating method for comparison purposes. They determined that MIP sensors based on click chemistry had a four times lower detection limit and three times higher sensitivity than drop coated devices. Gam-Derouich et al. (2012) applied aryl diazonium salt surface chemistry to immobilise the photoinitiator onto the gold surface. They reported a detection limit of 0.9 nM, using square wave voltammetry, and quite high selectivity against dopamine molecules (template) even in presence of L-3,4-dihydroxyphenylalanine (L-DOPA) as a competitor molecule. Wei et al. (2011) described an ultrasensitive surface plasmon resonance sensor including ultrathin MIP films having an impressive selectivity

against acephate (the template in this study) with detection limits of 1.14 x 10^{-13} M for apple and 4.29 x 10^{-14} M for cole (Brassica oleracea) samples, respectively. Tan et al. (2013) grafted functional monomer onto quantum dots to start polymerisation from the surface, while using the quantum dots as antenna for selective room-temperature phosphorescence sensing of haemoglobin. They made these non-toxic composites with a small particle size that facilitated good dispensability and stability in aqueous media. They reported that the sensors had good linearity over the range 1.0 x 10^{-7} to 5.0 x 10^{-6} M with high recoveries of 96.7-94.2% and 92.6-94.2% for urine and serum samples, respectively.

Another issue affecting biosensor performance with MIPs is the solution compatibility of the material's surface. Non-compatible surfaces may cause diffusion limitations; therefore sensor response time and signal quality problems can occur. Liu et al. (2014) inverted the hydrophobicity of CdSe quantum dots to a hydrophilic surface through a one-pot room-temperature reverse microemulsion, with the objective of specific opto-recognition of ractopamine. They reported a very low detection limit of 7.57 x 10^{-10} M and a precision, for five replicate detections of 1.51 x 10^{-8} M, of 2.09% relative standard deviation. Measurements of real samples were achieved, with good recovery ratios in the range of 82.79%-97.23%. El-Sharif et al. (2015) evaluated the effects of surface hydrophobicity (or wettability) on the protein recognition capability of imprinted sensor. They also compared bulk hydrogels with nanofilms on a sensor substrate by using acrylamide, N-hydroxymethylacrylamide, and N-isopropylacrylamide as functional monomers with differing hydrophobic characters. Interestingly, although the adsorption capabilities of the hydrogels had an inverse order with hydrophobicity as acrylamide > N-hydroxymethylacrylamide > N-

isopropylacrylamide; they showed that N-hydroxymethylacrylamide had the best recognition ability against the target protein with the order, in this case, being N-isopropylacrylamide < acrylamide < N-hydroxymethylacrylamide.

Reversible addition-fragmentation chain transfer (RAFT) and atom transfer radical polymerisation (ATRP), otherwise known as living polymerisation, are spectacular techniques to synthesise imprinted polymers with the same chain length and molecular weight (Pan et al., 2010; Peeters et al., 2014; Zhao et al., 2012). These techniques allow the sensor properties, such as surface homogeneity, thickness, response and water compatibility to be improved. Pan et al. (2010) reported RAFT polymerisation for facile surface grafting of stimuli responsive MIPs to obtain water compatible polymers for a wide range of templates. They suggested that this technique is efficient in improving the surface hydrophilicity because it allows easy grafting of any hydrophilic monomer. In addition, complicated and time consuming optimisation of the imprinting formulation could be eliminated because of the absence of any limitation in selecting the appropriate functional monomer, which makes the synthesis step more flexible and more tuneable. Peeters et al. (2014) described a RAFT polymerisation method to synthesise MIP platforms for thermal detection of histamine, in combination with graphene oxide to improve adsorption capability and enhance the sensor sensitivity. After a two-step initiator linking process that was confirmed with UV-vis spectroscopy, they achieved a surface grafting density of around 150 μmol/g, resulting in a thickness of about 2.4 nm and a length of about 34 nm. Wan et al. (2013) synthesised fluorescent particles via a multistep RAFT polymerisation procedure. They integrated the signalling element into a polymeric network, allowing the binding event to be monitored in real time by using sensitive

techniques such as fluorescent spectroscopy. Zhao et al. (2012) used ATRP to develop a label-free surface plasmon resonance spectroscopy biosensor for ametryn detection from soybean and white rice samples. They reported that the biosensor had a high linearity (R^2) of 0.9985 and low detection limits of 3.51 x 10^{-8} M for soybean and 6.19 x 10^{-8} M for white rice samples.

Initiated chemical vapour deposition (iCVD) is an interesting polymerisation techniques to synthesise polymeric nanotubes starting from traditional functional monomers, which also allows to researchers to design functional polymers for diverse applications such as biosensors, microfluidics, coatings and bioaffinity applications (Figure 4). Ozaydin Ince et al. (2013) applied this technique to create a simple but versatile concept to generate one-dimensional, surface-imprinted polymeric nanotubes for biorecognition of immunoglobulin G that exhibited high binding capacity and excellent specificity. They also mentioned that the iCVD method is valuable to this field because it offers new functional monomer combinations in addition to its simplicity and universality. In conclusion, it is clear that new polymerisation techniques continue to attract intense research interest in continuing attempts to improving MIP-based biosensor properties.

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Material combinations: from homogeneous to composite

In order to improve properties of MIP-based biosensors, attention has focused on combining functional materials to achieve a synergistic effect. For this purpose, various materials could be fused with MIPs in singular or multiple combinations

including, stimuli-responsive polymers (Pan et al., 2010), sol-gel derived xerogels (Holthoff et al., 2011), lanthanide ion complexes (Uzun et al., 2013), metal (Chang et al., 2013; Cennamo et al., 2015), silica (Wan et al., 2013; Feng et al., 2014) and carbon-based nanoparticles (Chen et al., 2010; Prasad et al., 2010; Peeters et al., 2014; Qui et al., 2012a, 2012b), quantum dots (Liu et al., 2014; Tan et al., 2013; Tan et al., 2014; Liu et al., 2013) and metallic oxides (Tran.T et al., 2014). In this way, the most powerful features of the materials could be exploited in superior combinations with enhanced properties. Pan et al. (2010) grafted poly(N-isopropylacrylamide) brushes onto imprinted particles to control the accessibility of imprinted cavities by adjusting the temperature around the lower critical solution temperature (LCST). They demonstrated that the specific template binding capability of MIPs was significantly decreased at an elevated temperature of 45^{o}C. Holthoff et al. (2011) used sol-gel derived, micron-sized xerogels thin films as a substrate for synthesis of TNT imprinted layers deposited onto a surface-enhanced Raman scattering (SERS) active surface. Using this combination of materials, they achieved a unique SERS band, which allowed detection and identification of template molecules with an apparent dissociation constant of 23 μM and a detection limit of 3 μM. They also recorded that the biosensor surfaces remained stable and retained their reversible recognition ability for at least 6 months. Uzun et al. (2013) described a combination of polymerisable lanthanide [terbium(III)] ion complex to synthesise L-histidine imprinted nanoparticles. In this study, the authors achieved well-distributed lanthanide ion complexes through the polymeric chain, which improved the fluorescent character of the nanoparticles. The L-histidine imprinted nanoparticles showed selectivity against not only L-histidine molecules, but also protein molecules having surface L-histidine residues.

Metallic silver and gold particles are generally chosen as core materials due to their electronic configuration and scaterring/plasmonic behaviour in respect of the light emitted. Chang et al. (2013) used silver microspheres as core materials to develop a 4-mercaptobenzoic acid imprinted substrate for SERS applications. This hybrid system had extremely high SERS activity and a detection limit of 10^{-15} M, with good reproducibility and selectivity depending on the gate effect of the imprinted cavities. Cennamo et al. (2015) combined five-branched gold nanostars with optical fibres and MIPs for the tri-dimensional and sensitive detection of 2,4,6-trinitrotoluene molecules. The intensity of the localised surface plasmon resonance (LSPR) of the gold nanostars in the optic fibres changed on interaction between the template and the imprinted cavities in the polymeric layer. These authors also compared the efficiencies of tapered and non-tapered optic fibres, and determined that both the penetration depth of the evanescent field and the number of interaction sites generated in the MIPs controlled the sensitivity and performance of the biosensor.

The use of silica-based materials as a core also attracts attention due to the ease with which they can be synthesised and modified, their cheapness and transparency. Wan et al. (2013) synthesised Z-L-phenylalanine MIP silica core-shell fluorescent microparticles through RAFT polymerisation. They reported a new nitrobenzo-xadiazole-based polymerisable monomer as a fluorescent probe, which emits light around 520 nm when interacting with L-phenylalanine molecules. Using this fluorescent property, they demonstrated that the system could be employed both in aqueous solution and directly in organic solvent for pharmaceutical process control. Feng et al. (2014) developed silica-based multiplexed composite nanoparticles

consisting of a ruthenium complex, silica core, poly-L-lysine (as linker) and gold colloids for near simultaneous tumour marker detection. They dually imprinted carcinoembryonic antigen (CEA) and carbohydrate antigen-199 (CA199) onto screen-printed electrodes. The sensor system was compared with a traditional double antibody sandwich-type immunoassay to show that the MIP-based multiplexed immunoassay had excellent potential for protein detection in clinical tests, due to its simplicity, cheapness, speed and sensitivity.

Carbon-based materials, such as carbon nanotubes, graphene and graphene oxide provide some of the most powerful additives because of their excellent conductivities, high surface area and ease of modification. Prasad et al. (2010) and Chen et al. (2010) used multi-walled carbon nanotubes (MWCNTs) as additive reinforcement materials to gain larger specific surface area and better electron transfer reaction. In this context, following direct acid treatment of the MWCNTs, they introduced vinyl functionalities by allylamine modification. Prasad et al. (2010) described detection of insulin in aqueous solution, blood serum and pharmaceutical samples, with a very low detection limit of 18.6 pM obtained by pulse anodic stripping voltammetry. Chen et al. (2010), on the other hand, reported detection of uric acid from aqueous solution with a detection limit of 22 μM using linear sweep voltammetry. Peeters et al. (2014) chose graphene oxide as an additive material to improve the binding capacity and sensitivity of MIPs, because of its small dimensions and high surface-to-volume ratio. They developed a graphene oxide-MIP hybrid biosensor for histamine detection using a heat transfer method, a sensing technique requiring just two thermocouples and an adjustable heat source. They achieved a low detection limit of 25 nM in buffer solution and concluded that the miniaturised system showed promise for analytical

applications due to the enhancement in binding and recognition properties of the MIP. Qiu et al. (2012a and 2012b) designed triplex systems, including graphene (oxide) / magnetite / MIPs, for chemiluminescence sensors for benzenediol isomers and epinephrine, respectively. They reported detection limits in the order of nM levels with very low relative standard deviation values of around 3.9%.

Due to their unique optical features, such as broad excitation, narrow tuneable emission, and excellent photostability, quantum dots, as a class of photoluminescent semiconductors, have also provided an attractive substrate for the synthesis of MIP-based composites for sensors. Tan et al. (2013) used 3-mercaptopropylsilane capped zinc sulfide quantum dots as core materials for selective room temperature phosphorescence biorecognition of haemoglobin. Following vinyl modification of the surface of the quantum dots, functionality was achieved via redox-initiated polymerisation of methacrylic acid, acrylamide and methylene bisacrylamide in the presence of bovine haemoglobin. This MIP-based system detected target protein in urine and serum samples, while the long lifetime of phosphorescence diminished interferences, such as autofluorescence and scattering, due to the biomatrix. Quantum dots have also been modified with amino groups to produce organic-inorganic surface imprinted composites as thinner shell materials (Tan et al., 2014). These authors emphasised that surface graft imprinting on the quantum dots has potential for quantification of target biomolecules both in aqueous solution and in complex biological fluids (Figure 5). The main problem with quantum dots is their surface instability in aqueous media (Liu et al., 2014). Liu et al. (2013) developed a stabilisation method for quantum dots by using ionic liquid to provide efficient binding between the composite ingredients and to improve fluorescence stability by

virtue of its high thermal and chemical stability. They reported a detection limit, precision for five replicates and relative standard deviation of 3.5 nM, 92 μM and 1.67%, respectively. Liu et al. (2014) also applied reverse microemulsion polymerisation to convert the hydropohobic surface into a hydrophilic one, as discussed above. Another inorganic substrate used to develop MIP-based biosensors is titanium oxide nanotubes (TiO_2 NT) (Tran et al., 2014). This team developed a highly ordered and vertically aligned TiO_2 NT arrays, starting from titanium foils. The molecularly-imprinted sensor exhibited high sensitivity against the template perfluorooctane sulfonate molecules, with a linear range from 0.5 to 10 μM, detection limit of 86 ng/mL and high selectivity in photoelectrochemical measurements.

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Recent sensor designs using MIPs

In general, MIPs have been separately synthesised through bulk polymerisation and then attached on the sensor surface. This approach causes problems including poor repeatability, high interference, cross-reactivity and batch-to-batch variation, which all stem from imprinting into bulky structures. Various alternatives to this have been proposed to improve sensor features by using difference polymerisation strategies and material/instrument combinations. In addition, mass production of more consistent MIP-based materials that could potentially be incorporated into sensors has been addressed (Poma et al., 2013) and has been separately reviewed elsewhere (Poma et al., 2010). In this section, we have sought to compile the recent designs for MIP-based biosensors in terms of: (i) polymerisation strategies; (ii) MIPs as controller/mediator or amplifier; (iii) MIPs as specific probes; (iv) MIPs as quenchers

in SERS; (v) MIPs on optical fibres; (vi) multianalyte-on-single substrate vs. array structures; (vii) nano-patterning: MIPs on-chip/microfluidics.

Polymerisation Strategies: In order to improve the performance of the sensors an obvious possibility is modification of the polymerisation strategy. We have already summarised some recent polymerisation approaches in respect of the polymerisation workflow, detailing the possible roots through nanofilm, nanoparticle, microcontact imprinting and epitope imprinting. A troponin T-imprinted electrochemical biosensor has been synthesised through cyclic vollammetric deposition of o-phenylenediamine in presence of the target analyte (Karimian et al., 2014). The formation of an ultrathin polymeric film on the substrate surface improved the sensor sensitivity and the thickness of polymeric film was easily adjusted by adjusting the scan rate and number of cycles during electropolymerisation. Sener et al. (2010 and 2011) used MIP nanoparticles as biorecognition element on quartz crystal microbalance and surface plasmon resonance systems. They easily attached the nanoparticles to the sensor surfaces via a temperature-controlled solvent evaporation technique (Sener et al., 2010). The use of MIP nanoparticles helped to achieve a more homogeneous and accessible distribution of imprinted cavities through the sensor surface that enhanced regular, rapid and homogeneous adsorption dynamics, while diminishing interference and improving signal quality. Yaqub et al. (2011) also used MIP nanoparticles (plastic antibodies) as artificial receptors for atrazine detection. Unlike in the previous studies, they had a two-step polymerisation approach: nanoparticle fixation via UV-photopolymerisation (second) after spin coating of MIP nanoparticles prepolymerised via thermal polymerisation. The imprinted nanoparticles showed linear calibration characteristics over a wide analyte range and no saturation effect in comparison to

designs using bulk polymers. Epitope and microcontact imprinting approaches are also attractive polymerisation strategies to improve the properties of MIPs by shortening diffusion paths and increasing the number of specific cavities by means of aligning template molecules on the surface of the polymeric phase (Diltemiz et al., 2013; Osman et al., 2013).

MIPs as controller/mediator or amplifier: Beyond the use of MIPs as biorecognition elements on the sensor surface, their applicability as smart guardians, controllers and mediators during biosensing process have been explored. Karimian et al. (2013b) combined smart polymers based on N-isopropylacrylamide and N,N'-methylenebisacrylamide with electropolymerised MIP films. They reported that variation in temperature effected on the sensor response, with the highest sensitivity achieved at 22^{o}C. The strategy developed in this study offered considerable potential for designing highly selective and controllable smart systems. Zhang et al. (2013) used molecularly-imprinted microgels as mediators for electrochemical recognition of 2,4-dichlorophenol. They introduced chlorohemin groups into imprinted cavities through the polymeric network to impart a reversible redox character to the microgel. The sensor showed good stability, acceptable repeatability, high recovery rates and an excellent linear response over the range 5.0-100 μM, with a detection limit of 1.6 μM. A highly sensitive electrochemical biosensor for oxytetracycline detection was developed by Li et al. (2012). In order to improve the biosensor properties, they used Prussian blue (PB) as an "artificial peroxidase" with high selectivity and catalytic activity as a functional monomer to synthesise the imprinted layer together with glucose oxidase-labelled oxytetracycline as a secondary amplifier. By this double-amplifying approach, they observed that the PB-assisted MIP biosensor showed

excellent analytical performance e.g. high sensitivity, good selectivity and reproducibility, and had potential for a wide range of applications.

MIPs as specific probes: Although MIPs have been reported in the literature for the fluorescence detection of a variety of analytes for a long time; the recent application of fluorescence polarisation techniques has made it possible to distinguish analyte molecules in situ without a separation step (Ton et al., 2012). Ton et al. (2012) developed MIP-based biosensors for rapid on-site detection of fluoroquinolone antibiotics in water and milk samples via fluorescence polarisation measurements. They reported very promising results with a low detection limit of 0.1 nM for enrofloxacin, which is below the permitted limit in ground water of 0.28 nM. They concluded that the assay was easy to apply without time-consuming centrifugation and separation steps. Reddy et al. (2013) used MIPs to create bioconjugation into sensing channels of a dual polarisation interferometer. They observed that the MIP adsorptive layer showed selectivity against target proteins even after interacting with real biological serum samples diluted to 1:100. They concluded that their thin-film MIP materials played a significant role in selective recognition and had potential for the detection of drugs, viruses, pesticides, toxins and bacteria, or for use in therapeutics, using simple adsorption, stripping and dual polarisation interferometry measurements.

MIPs as quenchers in SERS: Surface enhanced Raman scattering (SERS) has attracted considerable attention because of its high sensitivity and non-destructive nature. Unfortunately, the use and application of SERS are limited by problems such as the high reactivity and instability under ambient conditions associated with SERS-

active surfaces. MIPs inspired researchers to improve the properties of SERS-active surfaces by introducing recognition ability and also insulating the substrate surface. Chang et al. (2013) proposed a simple synthesis route for a core-shell silver-MIP hybrid SERS substrate for selective detection of 4-mercaptobenzoic acid. They observed the gate effect of MIPs and concluded that silver-MIP hybrids, as SERS platforms, could deliver ultrasensitive analytical platforms with detection limits at the femtomolar level. Site-controlled arrays of microdots approximately 6-12 μm in diameter were developed by Kantarovich et al. (2010) on SERS substrates for examining the uptake and release of S-propranolol via micro-Raman spectroscopy. They monitored individual dots as well as well-distributed multiple dots in arrays and suggested that the combination of nanolithography and SERS with MIPs might open up the possibility to synthesise miniaturised arrays for label-free, specific and quantitative detection. Holthoff et al. (2011) combined SERS substrates with a MIP xerogel synthesised via a sol-gel technique for 2,4,6-trinitrotoulene detection (Figure 6). They observed that the hybrid system showed reversible response and was effective, robust and stable in variety of environments. They emphasised that a successful MIP-SERS hybrid format would reduce size and cost while maintaining the high sensitivity, selectivity and portability required for military applications.

<<< Figure 6 here >>>

MIPs on optical fibres: Optical fibres are of interest because they offer many advantages such as ease of miniaturisation, handling and integration together with limited loss of light even over long distances, fast response time, high selectivity and low cost (Marazuela and Moreno-Bondi, 2002). Recently, surface plasmon resonant

coatings, especially silver and gold, have been combined with optical fibres through easy metal coating over the unclad portion of fibres. To introduce specific absorptivity, MIPs can play a key role to create 3D-memory on the metallic layer. An optical fibre sensor for the detection of tetracycline was developed by combining a silver coating on silica optical fibres with MIPs (Verma and Gupta, 2013). These authors reported that the optical fibre probes gave a linear response over a concentration range of 0.02 μM-0.96 μM, by recording surface plasmon resonance spectra in which a significantly large shift was observed in presence of target molecules. Cennamo et al. (2013, 2014, and 2015) reported a series of studies including combinations of optical fibres with surface plasmon resonance and MIPs. Compared to glass alternatives, polymer-based optical fibres have extraordinary advantages such as excellent flexibility, easy manipulation, great numerical aperture, large diameter and the ability to withstand smaller bending radii than glass (Cennamo et al., 2014). MIP/surface plasmon resonance biosensors for trinitrotoluene and L-nicotine were built using both tapered and non-tapered optical fibres and then their sensing performances were compared (Cennamo et al., 2013 and 2014). In addition, this group investigated the effect of gold nanoparticles on sensor performance by using five-branched gold nanostars added into the MIP layer (Cennamo et al., 2015). They emphasised that the sensing probes were easy to implement, cost-effective, rapid, did not need any particular user skills and were entirely free from electrical connections in the vicinity of the sample under examination (Figure 7). By using a fluorescent monomer, it is also possible to develop fluorescent optical-fibre sensors in association with MIPs acting as artificial receptors. Nguyen et al. (2012) described an intrinsic fluorescent optical fibre sensor for real-time detection of cocaine. The sensor exhibited a linear increase in fluorescence intensity in response to cocaine over the

concentration range 0-500 μM, with good reproducibility over one month and high selectivity over other drugs including ketamine, codeine, amphetamine sulphate, ecgonine methyl ester and buprenorphine hydrochloride. Ton et al. (2015) developed a disposable evanescent wave fibre-optic sensor for fluorescence detection of the herbicide, 2,4-dichlorophenoyacetic acid using a MIP layer coated over the fibres. They compared the efficiencies of ex-situ (dip-coating MIP particles synthesised beforehand) and in-situ (evanescent-wave photopolymerisation) deposition over the optical fibres. They reported that these disposable sensors detected targets in the low nM range and possessed high specificity and selectivity. These devices could also be useful for inexpensive and rapid on-site monitoring of environmental, food and biomedical analytes, especially given their portability.

<<< Figure 7 here >>>

MIPs on piezo-crystals: Quartz crystals have been widely used as transducers to develop MIP-based biosensors, not only for single molecules, but also for multi-channel and multi-analyte detection. This label-free technique is particularly well suited to larger analytes such as whole cells. As in the case of surface plasmon resonance, ready-to use analytical equipment is widely available and this has facilitated the plethora of research papers using this technique. Herein, we focus on some of the recent and most interesting developments and combinations. Croux et al. (2012) used reactive ion etching (RIE) technique to create mesa-like structures in order to diminish crosstalk as well as applying finite element analysis to simulate cross-channel frequency coupling capabilities. They developed a 4-channel MIP/NIP coated quartz crystal microbalance (QCM) biosensor array as a biosensing platform

for L-nicotine. They emphasised that, in addition to the relatively easy and inexpensive fabrication of the 4-channel biosensing platform, it could be enlarged to 8-, 16-, 32- and 64- etc channels with introduction of mesa-like structures (Croux et al., 2012). El-Sharif et al. (2015) combined protein-based MIPs with spectroscopy and QCM for characterisation of their molecular recognition efficiencies while varying hydrophobicity of the polymers. They achieved better selectivity when using hydrophilic polymeric matrix and claimed that the QCM sensor indicated MIP surface activity and provided physical interpretation in terms of hydrophilicity of polymers forming the MIP and protein recognition (El-Sharif et al., 2015). Kotova et al. (2013) focused on binding mechanisms between MIP and analyte, selectivity and ruggedness, in order to build a bridge between fundamental research and application, and thereby enhance the incorporation of MIPs into real products. They produced acrylate-based MIPs in two different shapes, as nanoparticles and nanofilms, on a QCM substrate for three different pharmaceuticals, ephedrine, leucovorin and anhydroleucovorin. They compared the binding efficiencies of template molecules with not only closely related compounds, but also some substructures in order to assess the most probable binding site in the template and MIPs (Kotova et al., 2013). Schirhagl et al. (2012) developed a sensitive MIP coating on QCM microfluidic chip by transferring antigenic determinants of antibody into polymeric structures through a two-step (also called double-imprinting) molecular imprinting approach. They created MIPs for the desired antigen after synthesising sacrificial antibody imprinted polymers. They also showed that it is possible to detect antigen molecules extracted into organic solvent with improved detection limits as well as in complex matrices, such as plasma or bread extract (Schirhagl et al., 2012). Suriyanarayanan et al. (2013) compared the efficiency of biotinyl moiety-selective polymer films created by

applying an interesting strategy using imprinting with two size regimes, the template (~1 nm) and sacrificial polystyrene beads (100-800 nm), to tune the porosity of MIP film and accessibility of the template to selective binding sites. They reported that the presence of an interconnected porous network through the MIP nanofilm improved recognition ability and substantially enhanced the sensitivity of the chemosensor (Suriyanarayanan et al., 2013). Latif et al. (2014) reported a biomimetic QCM coating produced via two different imprinting strategies, for label-free detection of bioanalytes varying from endocrine disrupting chemicals to bacteria. They applied bulk imprinting to develop a highly sensitive, selective and robust QCM sensor for real-time estradiol monitoring, while investigating the effects of porogen and cross-linker for creation of an imprinted polyurethane nanolayer. Separately, they developed a surface-imprinted polyurethane nanolayer through the double imprinting approach, to generate selective interaction sites for bacterial recognition, even in complex matrices such as growth media. They reaffirmed that molecular imprinting is a versatile method for designing artificial receptors not only for small molecules, but also for larger analytes like bacteria cells (Latif et al., 2014). Buchegger et al. (2014) combined ready-to-use resist with surface imprinting and UV- and thermo-nano-imprinting techniques to create a specific bacterial recognition layer by imprinting lipopolysaccharide (LPS) and lipoteichoic acid (LTA), instead of the whole bacteria cell. They also reported the effect of resist and stamp materials on the sensor properties, besides comparing the surface imprinting technique they developed with conventional techniques using acrylic-based monomer cocktails. They emphasised that the usability of the system for QCM measurements is limited due to some experimental restrictions, such as harsh alkaline conditions to remove bound micelles and extensive incubation and washing steps. Despite these limitations, they showed

that thermo-imprinted nano-films could be successfully implemented in a QCM assay as the recognition element (Buchegger et al., 2014). Yilmaz et al. (2015) developed a rapid and selective detection method via micro-contact imprinting of whole E. coli cells on the two classic, label-free sensing platforms, optical (SPR) and mass-sensitive (QCM), and compared their performances. They used a polymerisable form of amino acid (L-histidine) to create recognition layers similar to natural antibodies while gaining chemical and physical stability to enhance reusability and reduce production cost (Yilmaz et al., 2015). Such comparative studies have the potential to resolve important practical issue such as the choice of the best instrument for a particular application. However, a more fundamental comparison of SPR versus QCM was hampered in this case, since different fluidic conditions were used in the two devices.

Multianalyte-on-single substrate vs. array structures: Simultaneous multianalyte imprinting has been proposed in order to improve sensor performance while decreasing cost and cross-reactivity. In addition, competitive binding of multianalytes imprinted into same polymeric network offers high affinity and selectivity toward a group of corresponding templates, which facilitates cost-effective separation, short analysis time and reduces the effort needed to prepare different MIPs for different analytes separately (Zaidi, 2013; Feng et al., 2014). Zaidi (2013) developed a capillary electrochromatography system for detection of two amine-containing neurotransmitters, serotonin and histamine through dual-imprinting of them into a monolithic column. After optimisation of the polymerisation conditions with methylenesuccinic acid and methacrylic acid as monomers, and of the separation conditions by varying pH, organic modifier composition and buffer concentration in the eluent, they found that the monomer type significantly affected the polymer

performance. Finally, they observed highly reproducible results with good resolution, high separation efficiency and high selectivity. Feng et al. (2014) developed an electrochemiluminescence immunosensor for near simultaneous detection of tumour markers. They proposed the use of dual-imprinted film as a capturing probe with electrochemical detection of the target molecules. They compared their sensor performance with traditional double-antibody sandwich-type immunoassays. They produced their imprinted layer via in situ polymerisation of dopamine in the presence of different target antigens on antibody-decorated luminescence nanoparticles. They achieved detection limits in the pg/mL level and asserted that this strategy would provide a simple, cost-friendly, fast and sensitive approach for multiplexed immunoassay at the clinical level.

Array formats are one of the most promising alternatives to decrease costly and time-consuming steps. With this approach, it is possible to detect some analytes simultaneously or to improve signal quality by applying differentiation of responses from array spots (Linares et al., 2011). Wackers et al. (2014) designed a flow-through sensor cell segmented into four quadrants with a volume of 2.5 μL each, which allowed four simultaneous measurements on a single substrate. They monitored the heat changes during interaction between the MIP layer and analyte molecules via a heat-transfer method. The device was characterised with respect to quadrant separation, detection limit, specificity and cross selectivity. They concluded that the heat transfer method they used, in combination with the miniaturised, array format flow cell and MIPs layers, allowed simultaneous measurement of different targets down to the nM range without significant cross selectivity. Tran et al. (2014) described a titanium oxide-based nanotube array in combination with imprinted

nanolayers for sensitive and selective photoelectrochemical detection of perflurooctane sulfonate. They commented in the light of the results observed, that the array format could be used for the practical determination of perfluorooctane sulfonate in water samples even in presence of structurally similar competitors. Wang et al. (2013) developed a paper-based, multidisk plate grafted with MIPs for chemiluminescence detection of pesticides. They focused on the challenges i.e. low-cost, portable, fast and easy-to-setup detection for public use, and proposed grafting MIPs onto cellulose fibres in paper disks. By this approach, they achieved the detection of target pesticide at the femtomolar level under optimised conditions. Qiu et al. (2012a and 2012b) reported an array setup, which depended on multiplexed materials. In the first approach (Qui et al., 2012a), they detected multianalytes with a single array format by combining MIPs synthesised for different compounds. In the second approach (Qui et al., 2012b), they proposed an array format in double switching mode, by using imprinted and non-imprinted materials, to compare their performances. In both formats, they achieved rapid, sensitive, reusable and selective results because of the high adsorptive capability and excellent recognition ability of the MIP layers.

Nano-patterning: MIPs on-chip/microfluidics: A further philosophy is that selectivity should be built into a device at the fabrication stage, with the surfaces patterned through different lithographic techniques being used as substrates for synthesis of MIPs as well. Forchheimer et al. (2011) proposed a nano-imprint lithography technique to create molecularly selective nano-patterns for label-free sensor architectures. They subsequently formulated bottom-up (MIP) and top-down (lithography) procedures to fabricate the desired patterns and features over large areas

and at low cost. Because the results are comparable with those obtained with conventionally imprinted layers, they emphasised that the technique has the potential to mass produce inexpensive sensing platforms. Wang et al. (2011) combined MIPs with a lab-on-valve electrode system to develop sensitive and selective biosensors. The MIP layers were created in valve channels via in situ electropolymerisation of o-phenylediamine in the presence of the template molecule. A range of 0.01-0.2 μM with a detection limit of 3 nM was obtained as along with excellent selectivity, good sensitivity and low sample/reagent consumption. Hong et al. (2010 and 2012) developed a disposable cyclic olefin copolymer-based biochip by modifying a microchannel with MIPs. They optimised the shape and size of the imprinted nano-cavities in the size range of 10-10 nm and with a roughness of 2.5 nm, while adjusting the polymer composition and polymerisation conditions. They suggested that the MIP films proposed could be used to construct disposable biosensors for anesthetics, especially small molecules such as propofol, with relatively favourable sensitivity. MIPs were introduced into complementary metal oxide layer biological micro-electromechanical systems (CMOS BioMEMS) to fabricate integrated multi-type biosensors and readout circuits (Tsai et al. 2010). MIP-based and ion-sensitive field-effect transistor (ISFET) systems were fabricated to detect creatinine. Moreover, these devices had an integrated a ring-oscillator based readout circuit for reporting creatinine concentration. According to the results observed, both cases demonstrated the possibility of integrating multitype biosensors and readout circuits in CMOS BioMEMS based micro/nanosystems.

In conclusion, recent attempts to fabricate next generation and fully integrated devices have illustrated the possibility of improving the properties of MIP-based biosensors to

deliver rapid, selective, sensitive and highly reproducible responses at low cost. The excellent compatibility between disposable microfluidic chips and MIPs should also be noted.

<<< Figure 8 here >>>

Production/Operation Cost

Cost is the most important criteria for consumer products incorporating biosensors. The production cost determines both the product demand by the market and the benefits realised from the product by suppliers. Therefore, significant effort is expended to decrease the production and operational cost for developing biosensors at an industrial scale while conserving the desired features such as simplicity, rapidness, robustness, sensitivity, accuracy, precision and reproducibility. These challenges can be considered in two different ways: the need to decrease production cost and opportunities to decrease operational costs.

Production cost may be tackled by using cheaper starting materials such as silica, screen-printed electrodes, disposable plastic fibre optics, disposable biochips and paper etc (Wan et al., 2013; Moreira et al., 2013; Bakas et al., 2014; Feng et al., 2014; Ton et al., 2015; Hong et al., 2010; Wang et al. 2013). Wan et al. (2013) used cheaper materials as the core and then imprinted the target into shell in order to limit the production cost. Another case is the use of inexpensive screen-printed carbon/gold electrodes to design MIP-based biosensor architectures through surface imprinting (Moreira et al., 2013), sol-gel (Bakas et al., 2014) and dual-template imprinting (Feng et al., 2014). In addition, other disposable materials including evanescent-wave plastic

fibre optics, olefin-based microfluidics and especially paper have recently attracted much more attention as a substrate material to develop MIP-based biosensors (Figure 9).

<<< Figure 9 here >>>

In the second approach, multi-analytes on a single MIP or array formats might be developed to overcome the problems stemming from operational cost, as mentioned before (Iqbal et al., 2010; Shimizu and Clifton, 2010; Qui et al., 2012b; Wackers et al., 2014; Zaidi, 2013). In addition to some of the work discussed above, Iqbal et al. (2010) described a multichannel mass-sensitive biosensor as an electronic nose (e-nose) for simultaneous terpene detection from both fresh and dried herbs. They reported that the array proved its sensitivity, selectivity, repeatability, reproducibility and linearity with reversible responses over the concentration range <20-250 ppm, and was able to discriminate between molecules of similar molar mass, even for isomers, according to data validated with gas chromatography-mass spectrometry. Also, Shimizu and Clifton (2010) compared four selected MIP arrays in sensing applications in respect of efficiency, versatility and cost. Finally, Broeders et al. (2011) reported a miniaturised eight-channel sensor platform for biosensor applications. They chose histamine as model target to construct an integrated impedance analyser (Figure 10). They concluded that quasi-simultaneous time-resolved impedance measurements of the channels allowed extraordinary accuracy in a broad impedance range and that the system would be appropriate for performing stable measurements over intervals of several days for bioanalytical applications.

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Conclusion

As we concluded thirty-years ago (Turner et al., 1986) "It is uncertain exactly which direction will predominate in the next decade, but there is little doubt that the biosensor will impinge on the lives of an increasing number and wide range of scientists." The enticing possibility of new robust sensors that combine the exquisite sensitivity and specificity of biological receptors with the convenience and processability of polymers remains a hugely attractive goal. The sheer number and diversity of publications now addressing the key issues associated with MIP-based sensors surely suggests that such critical mass must shortly break onto the market with a novel and exciting range of analytical devices. Predicting exactly when this will happen, however, is a more difficult task. One indicator is the patent literature, which is indicating considerable activity with respect to MIPs (www.mipdatabase.com, 2015). Recent patents have included not only the synthesis of new MIP-based materials, but also device development, method validation and associated analysis (e.g. Knop, 2012; Nguyen et al., 2012; Lavold et al., 2014; Belbruno and Tanski, 2015). However, the process of transferring scientific advances into useful products can be painfully slow and requires multi-dimensional progress. Production cost and reproducibility of manufacture are important points, particularly since traditional MIP synthesis would have difficulty in supplying a reliable analytical reagent (as opposed to a crude chromatographic material). However, new methods offering MIP nanoparticles show great promise in this respect since they are consistent, readily amenable to purification and offer comparable performance to biological materials while delivering greater stability (Wan et al., 2013). Perhaps the

biggest challenge, however, is to find the "killer application" i.e. a MIP sensor that out performs all its rivals in a market niche that is big enough to support the R&D and commercial costs associated with introducing an entirely new technique to the market. The traditional paradigm dictates that clinical sensors are the most profitable and we have seen drives in this direction, for example, the sensor for the anaesthetic gas propafol, where a stable, online monitor would have life-saving advantages. However, the entry barrier in terms of regulatory barriers, is much lower for food and environmental applications, and the recent literature reviewed above illustrates a determine push towards devices to enhance food safety. Since a principal advantage of MIP sensors is robustness, environmental applications may be a good target, but profitability here is often very low and needs to be driven by legislation. Other possibilities are sensors where a long shelf life is necessary, such as devices for emergency situations e.g. triage, disaster management etc. As a final thought, it is absolutely clear that growth in demand for wearable, implantable or distributed sensors for applications in medicine, environmental monitoring, food safety analysis and security continue to grow apace and an imprinted polymeric device with sufficient specificity and selectivity is surely an attractive route to deliver much needed, real-time, chemical analysis.

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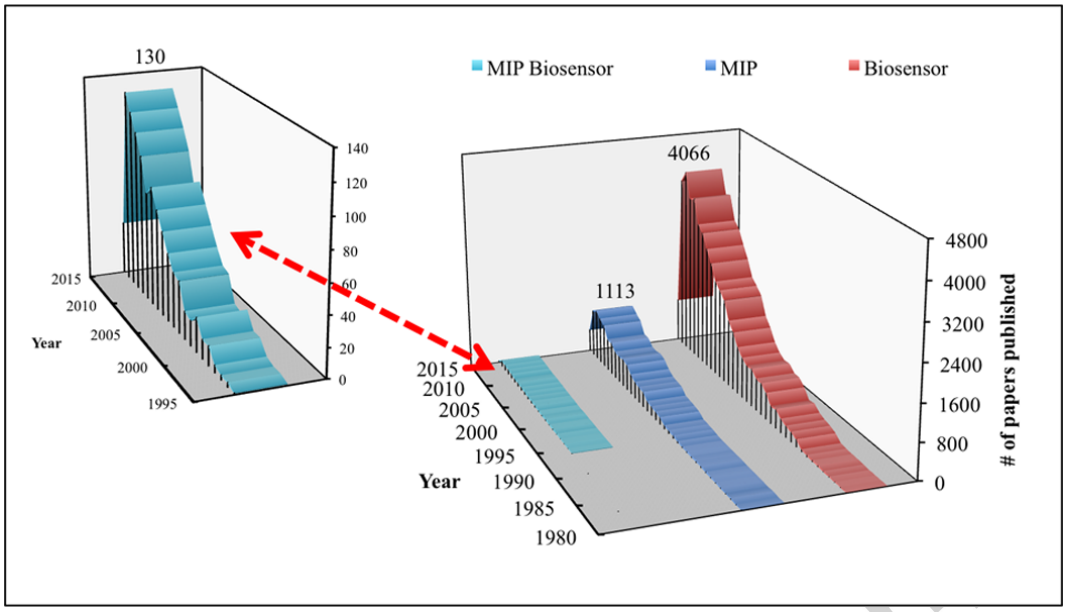

Figure 1. Numbers of publications with respect to time covering MIP biosensors, MIPs and Biosensors, respectively.

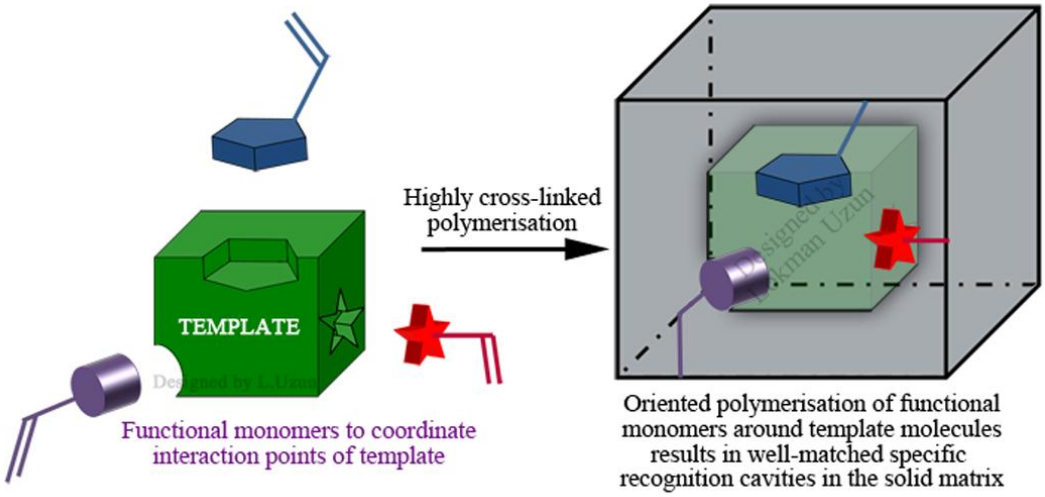

Figure 2. Schematic representation of molecular imprinting.

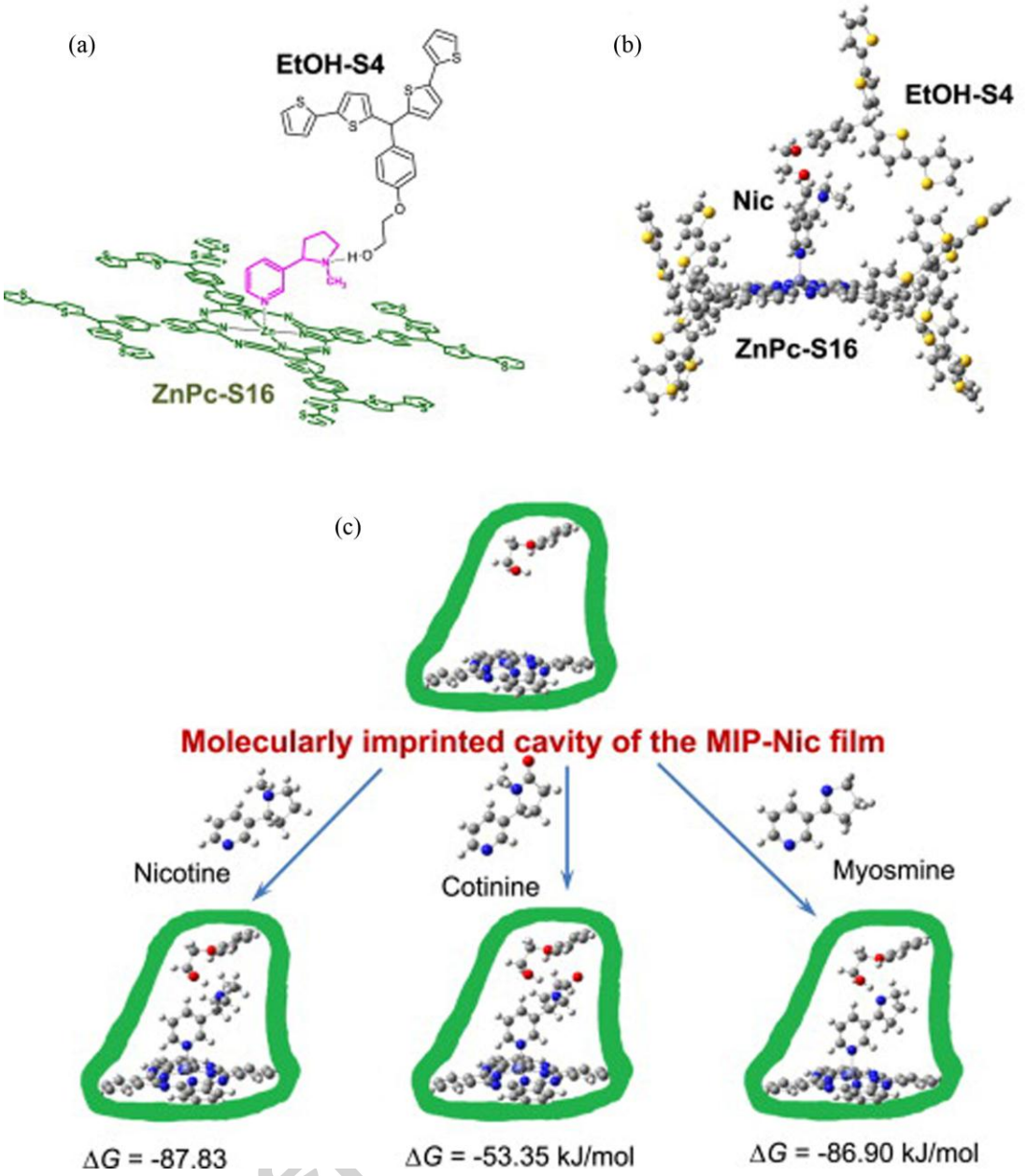

Figure 3. (a) 2D-structures of functional monomers (ZnPc-S16 and EtOH-S4) and template molecule (nicotine) in complexed form; (b) 3D-structure of complex optimised; (c) 3D-modelling of imprinted cavities with not only template molecule, but also with structural competitors (cotinine and myosmine). The negative Gibbs free energy values calculated for adsorption of nicotine, cotinine, and myosmine onto imprinted cavities reflects the spontaneous nature of interactions. Reprinted with permission from ref (Huynh et al., 2015).

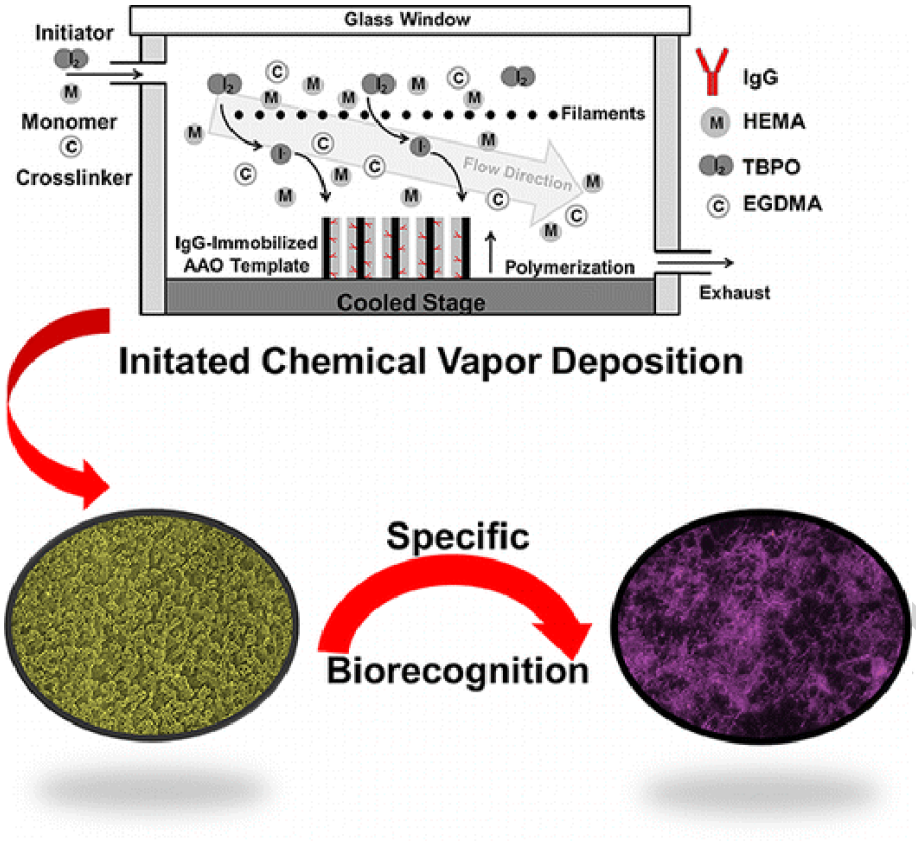

Figure 4. Schematic representation of the fabrication procedure for surface-imprinted and non-imprinted polymeric nanotubes on a bare AAO template using iCVD. Reprinted with permission from ref (Ozaydin Ince et al., 2013). Copyright 2013 American Chemical Society.

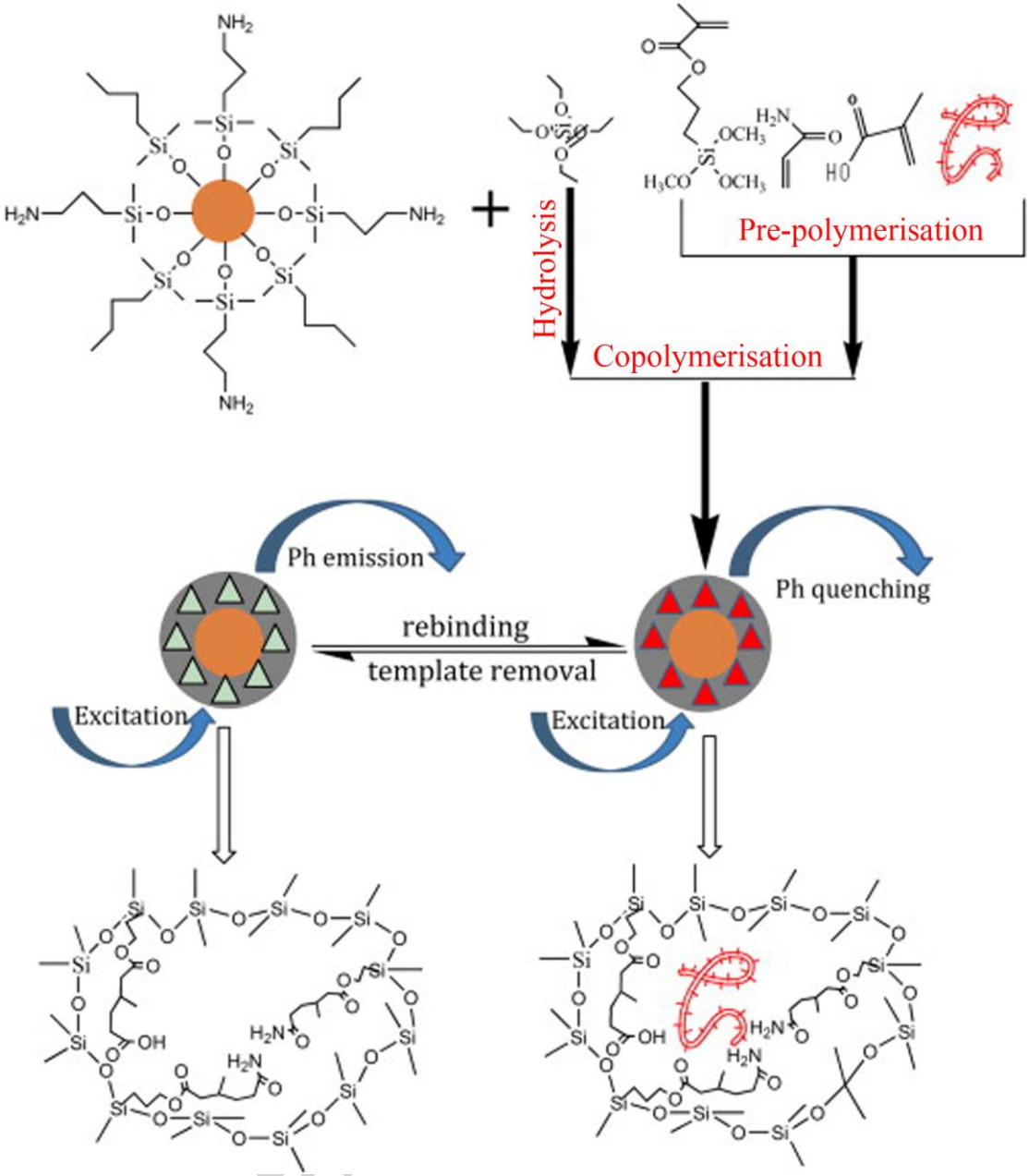

Figure 5. Schematic illustration of hybrid organic–inorganic molecular imprinting on the Mn-doped ZnS QDs. Reprinted with permission from ref (Tan et al., 2014).

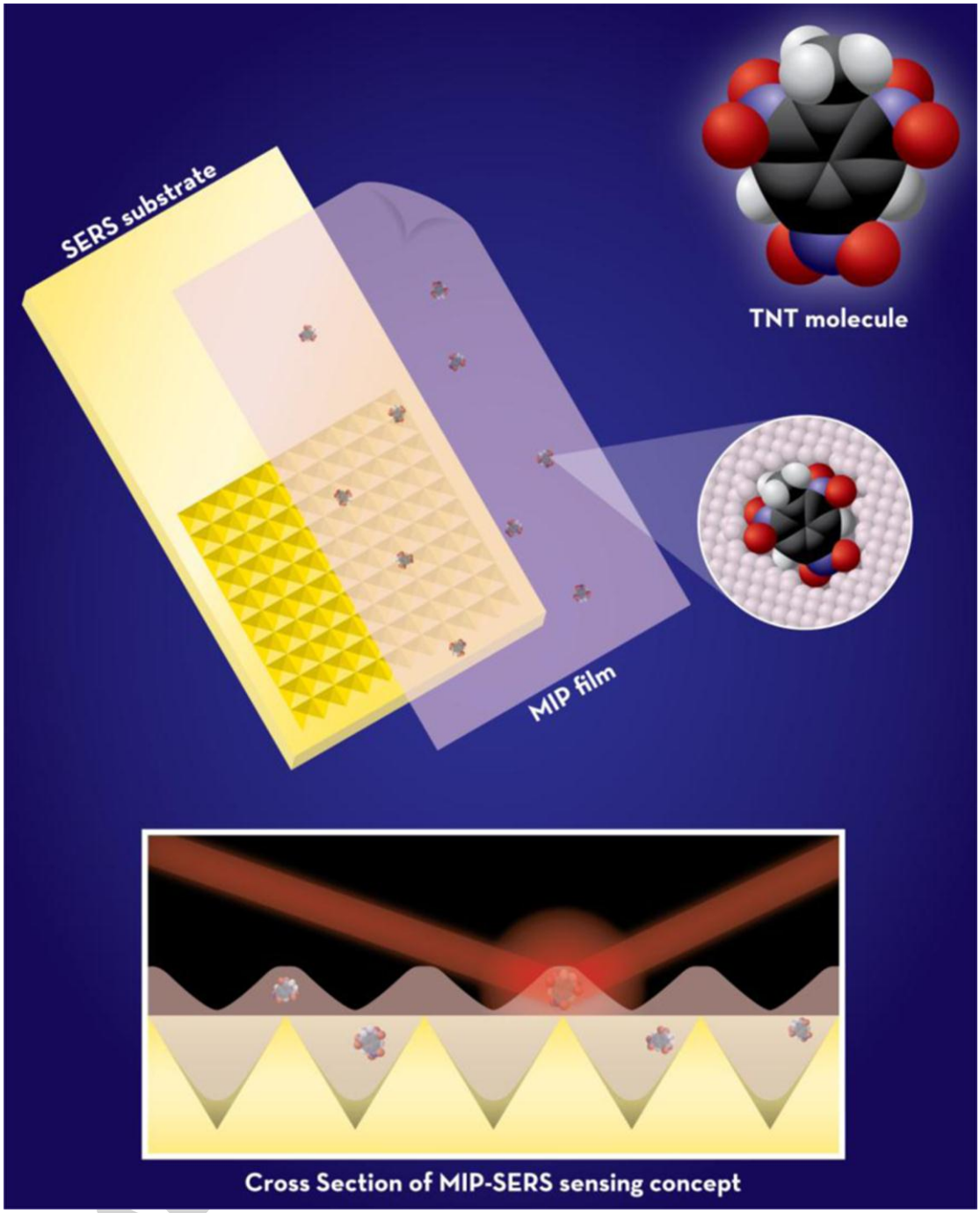

Figure 6. MIP-SERS detection concept. Reprinted with permission from ref (Holthoff et al., 2011).

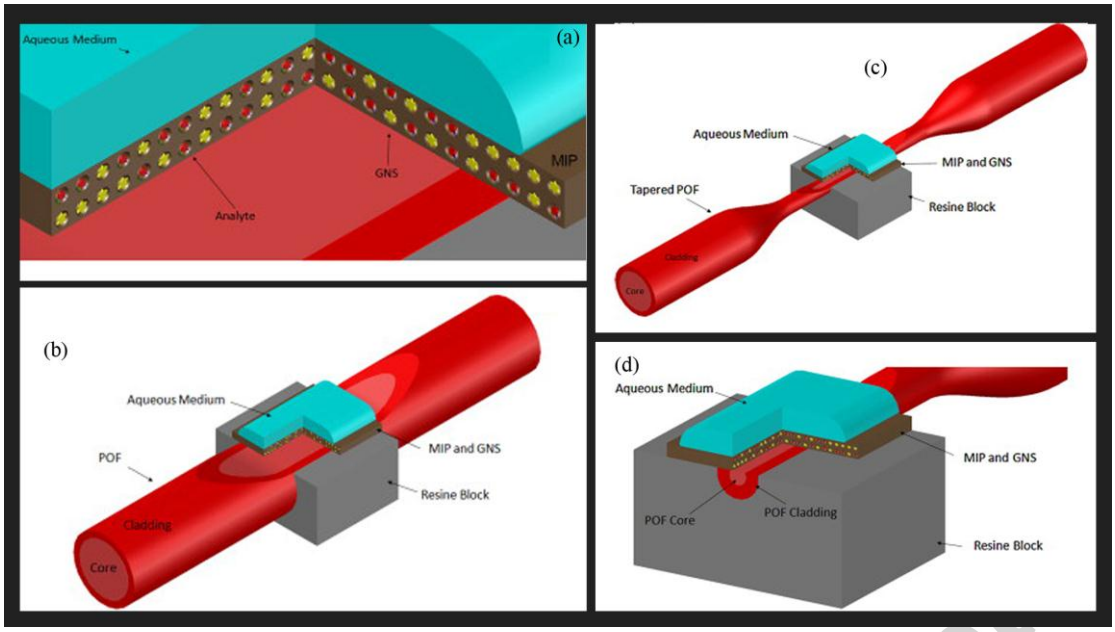

Figure 7. (a) Schematic representation of a GNS-MIP sensing layer after the interaction of analyte (TNT); (b) Untapered POF platform with GNS-MIP sensing layer; (c) Tapered POF platform with GNS-MIP sensing layer; (d) Cross-section view of sensing region. Adapted with permission from ref (Cennamo et al., 2015).

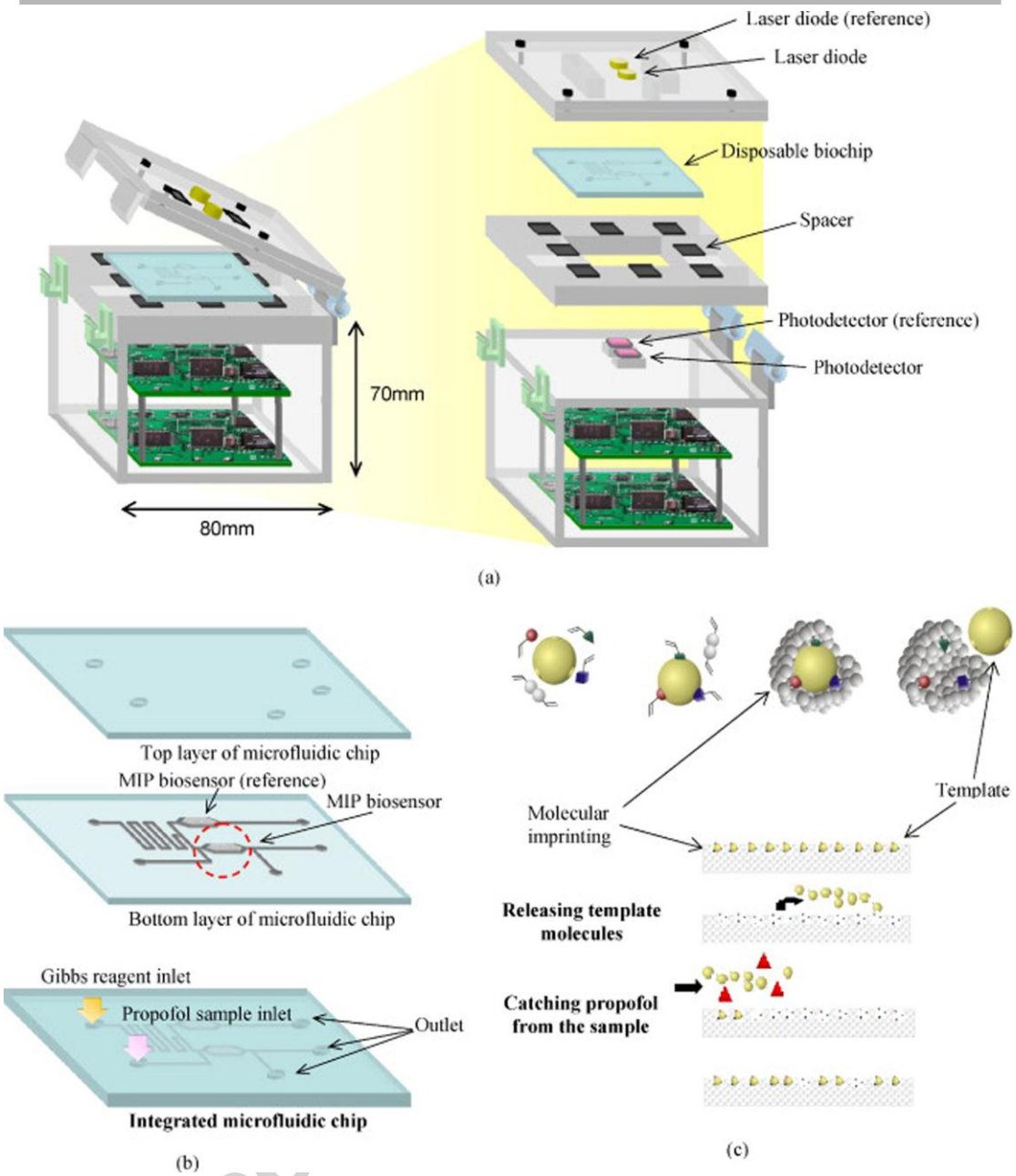

Figure 8. Schematic view of the fabrication of fully-integrated disposable microfluidic biochips with on-chip molecularly-imprinted biosensors: (a) the whole microfluidic system; (b) the microfluidic biochip; and (c) the working principle of molecular imprinting. Reprinted with permission from ref (Hong et al., 2010).

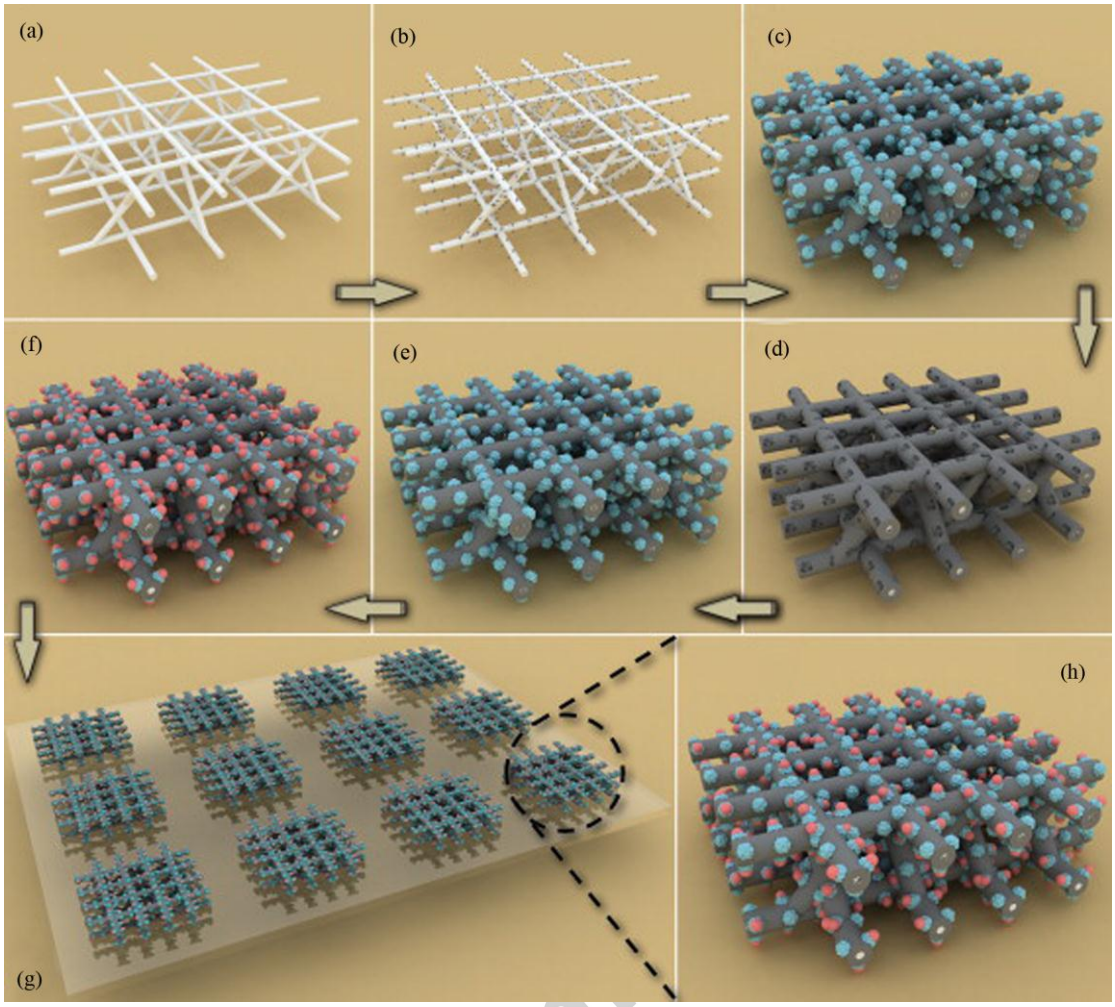

Figure 9. Schematic representations of the fabrication process of a paper based assay: (a) Cellulose based plain paper disk; (b) activation through silanisation of cellulose fibres; (c) formation of MIP layer by in situ polymerisation; (d) template removal from cavities; (e) adsorption of targets; (f) labelling of target with reagent; (g) assay in general view; (h) after competition. Reprinted with permission from ref (Wang et al., 2013).

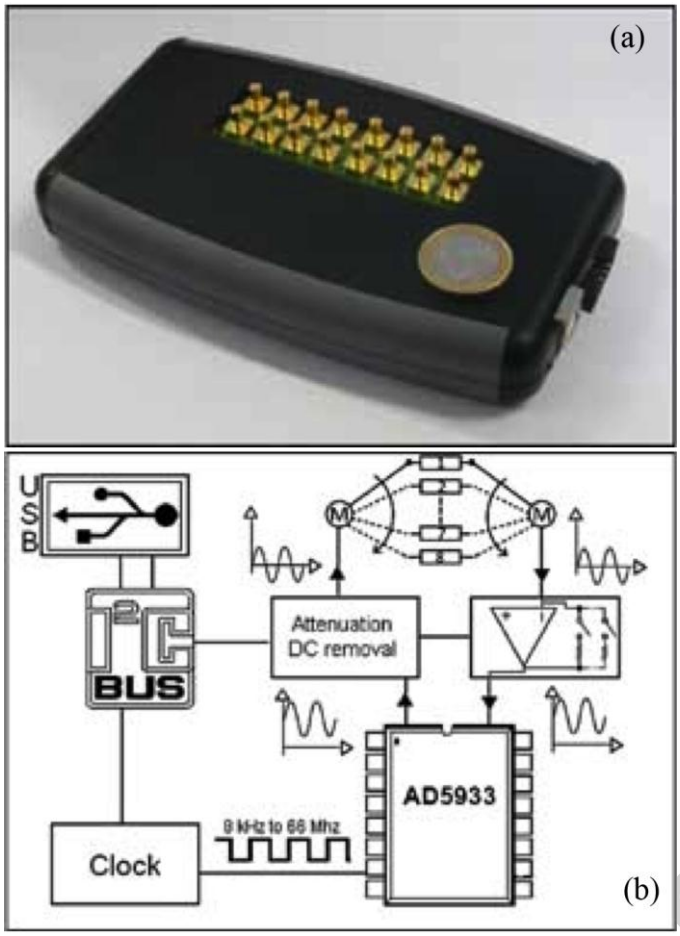

Figure 10. (a) Eight-channel impedance analyser with USB-port and SMB connectors visible and (b) simplified diagram of the measurement circuit. Adapted with permission from ref (Broeders et al., 2011).

Highlights

Extraordinary growth in the area over the past five-years (2010-2015).

MIPs emerge as a key line of interdisciplinary research

Focusing on the most promising advances in MIP-based biosensors

Advances in computational design and polymerisation strategies

Advances in material combinations, sensor designs and manufacturing issues

Opportunities for and barriers to commercialisation