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MIPs for Commercial Application in Low-cost Sensors and Assays – an Overview of the Current Status Quo

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Highlights

- The review aims to summarize recent commercially interesting innovations in MIP-based sensor design
- In addition to sensors, commercially viable assays based on MIPs are analysed and recent advances are summarized and discussed
- The review also analyses how commercial MIP companies could contribute to a next step in the commercialization of MIP-based detection systems
- The review contains a critical analysis and discussion on MIP-based sensors and assay commercialization as well as an outlook/recommendation for the future

Abstract

Molecularly imprinted polymers (MIPs) have emerged over the past few decades as interesting synthetic alternatives due to their long-term chemical and physical stability and low-cost synthesis procedure. They have been integrated into many sensing platforms and assay formats for the detection of various targets, ranging from small molecules to macromolecular entities such as pathogens and whole cells. Despite the advantages MIPs have over natural receptors in terms of commercialization, the striking success stories of biosensor applications such as the glucose meter or the self-test for pregnancy have not been matched by MIP-based sensor or detection kits yet. In this review, we zoom in on the commercial potential of MIP technology and aim to summarize the latest developments in their commercialization and integration into sensors and assays with high commercial potential. We will also analyze which bottlenecks are inflicting with commercialization and how recent advances in commercial MIP synthesis could overcome these obstacles in order for MIPs to truly achieve their commercial potential in the near future.

Keywords: Molecularly imprinted polymers, diagnostics, biosensing, lateral flow assays, commercialization

1. Introduction

1.1. Introduction to Molecularly Imprinted Polymers

The field of point-of-care (PoC) diagnostics has been rapidly developed over the past few decades by the introduction of novel sensing technologies and arrays such as the home-pregnancy test or the portable glucose sensors for diabetes patients [1, 2]. The core component of these novel technologies consists of bioreceptors that drive the selective recognition of the target-of-interest. Although bioreceptors are very powerful recognition elements due to the high affinity they inherently display for their target, their limited stability and relatively high-cost synthesis procedure have resulted in an increased research focus on the development of synthetic alternatives.

A kin to enzymes and antibodies, molecularly imprinted polymers (MIPs), might provide an answer. MIPs are artificial highly cross-linked polymeric receptors that are engineered towards the binding of specific analytes. This binding interaction is facilitated by nanocavities that are disturbed throughout the synthesized polymeric network, reflecting the conformation and chemical functionalities of the imprinted molecule or species [3-6]. The cross-linked nature of the polymer gives rise to distinct physical advantages over their biological counterparts. Enabling MIPs to resist harsh physical conditions (extreme pH and temperatures) that would render biological receptors useless [7-9]. This has led to MIPs being

deployed into numerous fields, including chemical separation, chromatography, affinity materials for sensors, binding assays, catalysis, and sorbents for solid phase extraction, to name just a few [10-17].

1.2. A brief history of MIPs

The concept of molecularly imprinting can be dated back as far as the 1930's, with the basic principal involving the formation of a polymeric network around a small extractable template molecule to leave a complementary nanocavity was noted [18-20]. Most commonly, the imprinting process is conducted by introducing the template molecule to functional monomer and functional crosslinking agent, before initiating polymerization by the addition of a photo/thermal initiator and the requisite conditions [21-24]. The template can then be extracted by means of mechanical grinding and solid phase extraction by prolonged washing with protic solvents [25-27]. (Figure 1)

Altering reagent ratios, monomer and crosslinker chemical compositions, and method of polymerization enables MIPs to be tailored towards a desired application. Particle sizes tend to be the driving force behind the selection of these parameters, with smaller particle sizes offering enhanced surface area and potentially greater binding properties. Over the years, polymerization techniques have become more sophisticated with methods such as bulk and suspension polymerization offering particle sizes ranging mid to low microns [28-30], whereas micro emulsions and precipitation polymerization offer low micron to sub-micron particle sizes [31-33]. These methods were developed throughout the late 20th century, with smaller particle sizes becoming more attractive towards the turn of the century. With smaller nanoparticle sizes offering a greater array of applications, advancing the synthesis of MIPs further. The development of solid phase synthesis ushered in the capability of reliably reproducing particle sizes in the nanometer range, offering the first reliable method of producing consistently performing MIPs [34-36].

Homogeneity in particle sizes are not the only benefits to gain from the advancements made in the field, with the imprinting process itself also being improved. Traditionally small molecules were the main target for MIPs, proving easy to imprint due to their relatively meager size. The imprinting of large molecule

such as proteins, fatty residues and even cells was deemed less efficient and complex. Therefore leaving MIPs developed for the sensing of these molecules relatively unexplored. This however changed with the introduction of solid phase synthesis, as the target molecule was now immobilized on a substrate rather than dissolved or dispersed in solution [37, 38]. This immobilization process therefore opened the door for the imprinting of larger molecules, with key epitopes of the aforementioned targets now able to be imprinted [39]. This revelation allowed the field to develop further, adding the capabilities of MIPs to sense complex biomarkers (*e.g.* cancer cells), which further strengthens their potential applicability in medical and clinical diagnostics [40, 41].

1.3. Incorporation of MIPs into chemical sensing platforms

The idea of integrating MIPs as selective recognition layers in sensing devices and assays emerged in the 90s, coupling MIPs to traditional biosensor readout technologies that act as transducers translating molecular binding into a measurable signal [42, 43]. Advancements in MIP synthesis procedures, micro-electronics and computation have accelerated this evolution over the past twenty years, with many new sensor and assay platforms emerging [44-46]. A variety of approaches ensued, with readout methods including but not limited to quartz crystal microbalances (QCM), electrochemical sensors (*e.g.* capacitance, resistance, conductivity, potential difference, etc.), and thermal methods (*e.g.* the so called “heat transfer method”) [47-53]. The combination of MIPs and their associated readout platforms has facilitated the analysis of many different mediums (*e.g.* urine, blood, wastewater, soil samples, etc.) for a whole host of analytes [54-59]. Device sensitivity and limit of detection (LoD) is therefore situationally dependent on the technique and MIP employed. This deviation in sensing capabilities has meant not one MIP readout combination has been elected superior, with certain techniques favored in specific situations [60-63]. Despite the fact that all MIP-based platforms offer the advantage of low-cost, straightforward receptor synthesis and increased physical and chemical stability, commercialization seems to remain tricky.

Part of the complexity of commercializing MIP-based sensors might lie in the combination of mass producing reproducible polymer batches and integrating them into handheld readout devices. However, assays can offer an alternative to the latter. Rather than coupling a MIP receptor layer to a transducer, a MIP can be further modified to exhibit effects that can be observed by the naked eye. A diverse array of systems have achieved this ability using fluorophores, dye molecules and nanoparticles to create visually identifiable assays for a host of molecules [64-66]. This version of MIP based analysis is aimed towards semi-quantitative / qualitative analysis, offering the capabilities of in the field-testing.

Although MIP-based assays have shown to be competitive in terms of specificity and sensitivity with state-of-the-art competitive assays such as ELISA and are compatible with *e.g.* lateral flow lab-on-a-chip technology, the commercial potential of these assays is yet to be fully exploited. In this review, we want to highlight the latest advances in MIP-based sensor and assay development and focus on commercially interesting platforms. We want to focus on the bottlenecks in translating commercially viable research outcomes to market-ready products and illustrate how the emergence of commercial enterprises that focus on mass-producing reproducible, highly selective MIP batches can accelerate the development of commercial MIP-based biosensors and assays in coming decades.

2. MIPs as receptors in sensing devices

As previously mentioned, MIPs can be combined with a wide variety of label-free readout techniques to create sensor devices for the detection of an essentially unlimited number of analytes-of-interest. This has led to nearly three decades of academic and applied research on incorporating MIPs into sensor prototypes. However, not all of these methods prove currently commercially viable due to several potential bottlenecks: the research is not advanced enough, the method lacks reproducibility, or the cost of commercialization is not feasible. In this article, we will summarize the most recent advances in MIP-based sensing and focus on technologies with a large potential commercial impact.

2.1. Electrochemical readout

The first fully integrated biosensor device was invented by Clark and Lyons in 1962 and has evolved into one of the most striking success stories in the history of biosensing. Glucose sensors have evolved from enzyme-modified Clark electrodes for amperometric glucose detection to wearable glucose meters combined with continuous insulin administration that have drastically changed the life of diabetes patients [67]. This evolution has led to a lot of research focus on integrating MIPs into electrochemical sensor platforms based on e.g. field-effect transistors, chemiresistors or amperometric, voltammetric, impedimetric, capacitive and conductometric readout principles [68]. Electrochemical MIP-sensors are extremely powerful and have shown to be capable of analyzing samples in all phases, though gaseous and liquid samples are preferable [69]. Carbon nano-tubes, conductive polymers, and metallic nanoparticles are among the accepted methods of incorporating this trait [70-72]. Among the conductive polymers, polypyrrole has especially shown to be commercially interesting for incorporation into electrochemical MIP sensors as it allows to directly create the receptors on the measurement substrate, opening up the possibility for mass-scale chip production. This has led to the development of sensors for various targets including hormone-disruptors, drugs, biomarkers and even DNA molecules [73-76].

2.1.1. Conductometric and impedimetric sensing

Conductometric measurements rely on the time dependent monitoring of the conductivity of the MIP receptor layer, with the binding of a target analyte changing the concentration of ionic species at the solid liquid-interface [77]. Conductance measurements are relatively straightforward to perform and require little instrumentation, making the technology interesting from a commercial point-of-view. However, to date only a limit number have of MIP-based conductometric sensors have been developed [78-80]. Exploiting the change in ionic concentration of solid-liquid interfaces is usually done by electrochemical impedance spectroscopy (EIS). EIS analyzes the impedance of a system in dependence of an AC, analyzing capacitive and inductive effects in addition to the purely resistive system, DC system used in conductometric sensing [81]. This makes EIS a powerful analysis tool, which has been extensively studied as a transducer mechanism in MIP-based sensing [82-84]. The compatibility with wire-based

sensing and miniaturization using microelectronics makes EIS useful for PoC *in vivo* diagnostics, a characteristic that was exploited by Wackers *et al.* for the monitoring of histamine concentration in intestinal fluids. They coated titanium electrode wires in a catheter with an adhesive polystyrene layer onto which they immobilized both MIP and non-imprinted particles by dip coating in order to obtain a differential measurement setup (see Figure 2) [85]. The resulting sensor was able to detect histamine in intestinal fluid in physiologically relevant concentrations, illustrating the potential of MIP-based sensing for *in vivo* monitoring gastro-intestinal disease markers.

MIPs have also been extensively studied as receptors for *in vitro* diagnostics with an interesting breakthrough being recently reported by Cai *et al.* They coated arrays of carbon-nanotube tips with MIP layers for the detection of papillomavirus-derived E7 protein [86]. Although the research is still somewhat on the fundamental side, the ultra-low detection limit in the subpicogram per liter range, makes the application commercially interesting in terms of diagnostics but also as a tool to monitor and study infectious diseases, a field that is highly relevant within a post-pandemic society.

2.1.2. Voltammetry and amperometry

Voltammetric approaches are highly interesting for bio(mimetic) sensing due to their versatility and low noise, enabling sensor engineers to develop devices with high sensitivities [87]. These methods typically make use of three electrodes (working, counter and reference) with an aqueous electrolyte inside a defined electrochemical cell. The voltage is swept within a defined range and the resulting current is monitored in time in response to increasing concentrations of the analyte of interest. An interesting characteristic of the method is that it enables the simultaneous detection of multiple analytes (if they give rise to current peaks at different voltages). Other advantages of voltammetric sensing are the compatibility with aqueous biological samples and the fact that the electrodes do not have to be noble metals with many applications relying on *e.g.* carbon-based electrodes [88-90].

Electrochemical interferences can be suppressed by methods such as differential pulse voltammetry (DPV) and square wave voltammetry (SWV), while also increasing the sensitivity towards the desired analyte. Zhang *et al.* illustrated the potential of DPV for *in vivo* monitoring of lactate levels in sweat, an application that can be useful for *e.g.* monitoring athletes during training and studying their performance [91]. They managed to immobilize MIP recognition layers on a mesh of silver nanowires in a wearable patch by electropolymerization (see Figure 3). The patch, consisting of the electrodes and a miniature PDMS flow cell were connected to a DPV analyser and allowed the researchers to analyse the lactate levels of a test person during exercise in clinically relevant concentrations (higher nM range). In this way, the sensor has demonstrated to be competitive with biosensor platforms and diagnostic techniques that require sampling and analysing lactate concentration *in vitro*. This work demonstrates that MIPs are compatible with the trend of wearable detectors for personalized medicine. Other voltammetric MIP sensors have been developed in recent years with high commercial potential for application in various fields including cancer diagnostics [92], soil contamination screening [93], water monitoring [94], and food safety [95].

Amperometric methods are a subset of voltammetric approaches that operate at a fixed, optimal potential. The analyte becomes trapped by a MIP and is reduced or oxidized at the operating potential, generating a current that is in proportion to the concentration of the analyte of interest [96]. Amperometric sensors have been the basis of most commercial glucose meters due to the combination of the advantages of voltammetry with the simplicity of working at a fixed potential, allowing miniaturization and straightforward signal processing. The incorporation of amperometric platforms have been studied almost immediately after the early start of the focus on MIP-based sensing in the mid-90s leading to many interesting detection platforms in various fields [97-99]. In a more recent study, Yang *et al.* imprinted poly(acrylonitrile-co-acrylic acid) layers with bisphenol A. They demonstrated the detection of the endocrine disruptor in seawater and paper cups with sensitivities comparable to LC/MS/MS [100]. Other

amperometric MIP sensors have demonstrated potential for the detection of prostate cancer markers [101], d-mannitol [102], and antibiotics [103].

2.1.3. Field-effect transistors

Field-effect biosensors typically consist of a semiconductor path separated by two conducting electrodes, the drain and the source. A third electrode, the gate, is used to apply a bios voltage over the sensor. The receptor layer is usually attached to an insulating layer (polymer, metal oxide...) covering the semiconductor path between the source and drain. Capture of the target by the recognition layer, will change the charge density on the solid-liquid interface, thereby directly influencing the conduction path between drain and source that can be electrically monitored [104]. The method offers a highly sensitive detection platform that was utilized by Dabrowski *et al.* for the detection of human serum albumin (HSA), a marker for liver and kidney disease [105]. Bithiophene MIPs were created on SiO₂ nanoparticles by electropolymerisation. The FET-chemosensor was able to detect HSA in sub-clinical femtomolar levels, providing a non-invasive alternative for the current gold standard test that requires the drawing of blood from patients. An albumin test is rarely done in isolation and usually part of a larger investigation of a patient blood study, making a test for albumin not commercially interesting on its own. However, the study does provide an excellent proof-of-principle for the detection of proteins in blood and urine and multiplexing the platform might provide a commercially interesting alternative for traditional lab tests. The same group has demonstrated similar results, using the sensor for the detection of human chorionic gonadotropin hormone, illustrating its potential application in home-pregnancy testing, and d-arabitol for fungal infection diagnosis [106, 107]. Other groups have used FET-based MIP sensors for the detection of prostate specific antigen in human plasma [108], glucose in buffer [109], and gluten in food products [110].

2.2. Acoustic wave devices

Acoustic wave sensors utilize piezoelectric materials, typically quartz, to generate an acoustic wave over the surface of a functionalized surface. An acoustic wave vibrating at a resonance frequency is created by making use of an appropriate electrical field over the sensor substrate. Depending on the cut of the piezoelectric material, the wave can travel along the surface of the sensor (surface acoustic wave or SAW devices) or away from the surface to the bulk of the substrate (bulk acoustic wave or BAW devices) [111].

Sensors based on SAW devices typically analyze the propagation of a wave over the surface of a piezoelectric film between two interdigitated transducers. SAW resonators for MIP-based sensing have been reported on in a limited amount of publications throughout the years [112-115]. In 2017, Mazouz *et al.* combined imprinted polypyrrole layers with SAW technology for the ultra-sensitive detection of glyphosate [116]. They managed to detect the herbicide in concentrations down to the picomolar concentration regime with limited cross-selectivity observed towards common competitors, illustrating the potential of the sensor to address a present-day hot topic in environmental monitoring. Earlier this year, Kidakova *et al.* reported on a similar device [117]. They immobilized protein markers for neurological diseases onto gold SAW sensor chips and electropolymerized a layer of *m*-phenylenediamine to create a mold of the protein on top of the sensor substrate. Shear acoustic waves were generated by a radio-frequency signal and the observed phase shift was observed to analyse mass loading on the sensor surface. Although the study was conducted in buffer solution, the results are highly promising from a diagnostic point-of-view, illustrating that the sensor is capable of distinguishing between cerebral dopamine neurotrophic factor and its homologue at concentrations in the sub-pg/mL range. This indicates that the sensor is competitive with state-of-the-art ELISA platforms and is able to detect protein markers within or even below the clinically relevant concentration regime.

The amount of BAW devices for MIP-based sensing that have been developed heavily outweighs the number of MIP sensors based on SAW technology. This finding can mainly be attributed to the popularity of quartz crystal microbalances (QCM) that have formed the basis of hundreds of publications on

chemical sensing in the past twenty years [118-120]. A QCM utilizes a slim piezoelectric quartz crystal that is sandwiched between two circular metal electrodes, which vibrates at a resonance frequency by applying an alternating current field is applied. As the mass on the surface increases (Δm) (ng), a proportional change in resonance frequency (Δf) (Hz) can be observed, as described by the Sauerbrey equation (mass sensitivity constant (C) ($\text{ng cm}^{-2} \text{Hz}^{-1}$)) [121];

$$\Delta m = -C\Delta f$$

MIPs are coated onto the surface of the crystal offering the possibility of real-time measurements in both the gaseous and liquid phases [122-125]. When a binding event occurs between the MIP and the target molecule, the change in mass at the surface causes a shift in the resonance frequency. This makes QCM-based sensors particularly interesting for detecting macromolecular targets such as proteins [126-128], viruses [129-131], bacteria [132-134], or whole cells [135, 136].

In 2018 Battal *et al.* developed a sensor for the detection of synthetic cannabinoids [137]. The QCM-based sensor displayed excellent selectivity for several commonly encountered cannabinoids and was able to detect them in spiked synthetic urine samples in concentrations down to the sub-pg/mL concentration regime (Figure 4). The sensor is commercially very interesting as it can find applications in various fields including clinical toxicology, forensic research and law enforcement, as these very addictive, potentially life-threatening designer drugs are flooding both the illicit and legal drug market [138]. Additionally, they are hard to detect using current drug tests, as drug producers tend to change their structure rapidly to avoid prosecution.

MIP sensors based on QCM technology also have potential in personalized medicine as evidenced by a publication of Chunta *et al.* in 2019 [139]. They constructed a MIP sensor capable of detecting high-density lipoprotein (HDL) cholesterol in the clinically relevant concentration regime. Their sensing platform showed little to no cross-selectivity towards other (lipo)proteins in blood and displayed an excellent correlation with the colorimetric enzymatic test that is considered to be the golden standard. In

addition, the sensor does not require sample pre-treatment and is therefore a lot faster than the latter. The authors then integrated this sensor into an array along with their previously developed sensor for low-density lipoproteins (LDL) to deliver a proof-of-concept for a multiple analyte self-test for metabolic syndrome [139, 140]. Other interesting sensor platforms have recently been developed for application in food safety [141-142], environmental screening [143-147], and pathogen detection [148, 149].

2.3. Thermal readout

The development of thermal methods that incorporate MIPs is a relatively untapped resource, and only over the last decade, the prospect of a thermal read-out has begun to be explored [150]. Initial publications exploring MIP-based thermal methods are sparse, with the first thermal MIP sensor with high application potential outlined by Lettau *et al.* [151]. This paper outlined how phenylacetate could be catalytically converted using a column packed with MIPs, leading to an increased temperature observed in the collected filtrate from a flow reactor. A follow up to this work introduced the first MIP-based thermistor in organic solvent, with binding events to the MIP observed as an exothermic peak signal that appears after the injection of an analyte. The paper then further elaborated on the differences that could be witnessed between the MIP and NIP, generating thermal readings in a similar manner seen to a traditional batch rebinding experiment. Similar platforms have been developed by the same group over the years [152-154].

A more direct thermal method emerged in 2012 when van Grinsven *et al.* reported on a novel thermal method for the identification of DNA point mutations using DNA melting curve analysis [155]. It was discovered as the temperature of DNA increases it undergoes a defined conformational change, increasing the surface area of itself by nearly 150%. This observation was known to increase the electrical resistance in the system, but the increase in thermal resistance was unreported. The readout technology was soon combined with MIPs for the detection of low-molecular weight compounds [156-159]. The combination of thermal sensing with surface imprinting techniques seems to work out particularly well for the detection of macromolecular targets including proteins [160], mammalian cells [161-163], and

microorganisms [164-166] in various matrices including wastewater, urine and blood. These results illustrate the importance of surface imprinting for creating synthetic receptors for macromolecular targets, which is expected to become of increasing importance in the near future for applications on the detection of *e.g.* immunoglobulines in assessing covid-19 immunity [161].

The method controls the input temperature underneath the sample in a stringent manner, while passively monitoring the temperature in the liquid measuring chamber above the sample passively in time. Binding of the target to the solid-liquid interface leads to an increase of the thermal resistance, which can be measured as either a decrease of the temperature inside the flow cell (constant input temperature) or a phase shift on the transmitted thermal wave [167]. The method offers a very low-cost readout platform, which requires little instrumentation, but the sensitivity of the system is restricted. The main problem associated with this technology is producing the receptor layers, with imprinting tricky to standardize in terms of mass-production and receptor immobilization.

In 2017, the technology was taken one step further towards commercialization by coating thermocouples with bulk imprinted MIP particles imprinted with cortisol and serotonin (see Figure 5) [168]. An adhesive layer was immobilized on the thermocouple and MIP particles were attached to the layer using dip-coating, enabling to directly detect neurotransmitters in liquid samples without the need for planar functionalized substrates. These findings open up the possibility of integrating thermocouples into *e.g.* catheters for the direct *in vivo* detection of small molecules. More recent research, focused on improving the sensitivity of the technology by directly grafting MIP receptor layers on planar electrodes for the detection of designer drug molecules [169]. Last year, Cornelis *et al.* reported on a new update from the readout technology side; they improved the detection limit of the methodology by two orders of magnitude by using a printed meander-structure as a heater and sensing element directly underneath the receptor layer [170]. The temperature inside the flow cell was monitored by a thermopile, enabling them to detect *E. coli* in apple juice in concentrations down to 100 CFU/mL.

2.4. Optical sensors

Optical sensing principles have been extensively used as transducers in a wide variety of MIP-based sensing devices and assays [171-173]. This group of detection platforms is quite heterogeneous and it is often hard to mark the border between sensors and assays. An example operating at the border of both would be a lateral flow assay connected to a surface plasmon resonance (SPR) analyzer. For the sake of simplicity, we will only discuss the direct detection of analytes on a sensor surface in this chapter, while all platforms that are based on luminescence, fluorescence or fluorescence quenching, dye or label displacement, enzymatic, pseudo-enzymatic or non-enzymatic color reactions and similar principles will be discussed in the next chapter as part of MIP-based assays.

SPR can be considered as one of the most powerful, and therefore popular, readout technologies and has been combined with MIPs for the detection of microorganisms [174, 175], amino acids, peptides and proteins [176-178], dangerous explosives [179-181], antibiotics [182-184] and other low-molecular weight compounds [185-189]. Detection upon rebinding of the target is based on changes in electron density at the surface of the sensor chip. Ertürk Bergdahl *et al.* reported on the development of a biosensor with high diagnostic potential last year [190]. They used microcontact printing to imprint tyramine layers on gold electrodes with secreted bacterial factor (RoxP). In this way, they managed to detect RoxP in sub-nanomolar concentrations with limited interference from other proteins. They used this sensor to detect RoxP in skin swaps (Figure 6) and validated the results using ELISA, thereby highlighting the potential applicability of the sensor in diagnostics. Other recently developed SPR-MIP sensors were used for the detection of anticancer drugs [191] and cardiac biomarkers [192].

Surface-enhanced Raman Spectroscopy (SERS) is another tool that has been used as an optical transducer for the direct detection of target-MIP rebinding at solid-liquid interfaces [193, 194]. SERS is particularly interesting because of its ultra-sensitive detection mechanism that allows for the detection of analytes in the femtomolar range [195]. Interesting applications using SERS-based sensors have been developed in recent years mainly focusing on food safety and environmental monitoring [196-199].

2.5. Bottlenecks commercialization MIP sensors

Despite tremendous advances in both imprinting technology and micro- and nano-electronics, commercially interesting MIP-based sensor platforms have only been reported on in a research setting. The valorization process, translating lab-based technology to the market is complex and in addition to commercial bottlenecks, which will not be studied in detail in this manuscript, many technical challenges need to be overcome to turn MIP sensors into true competitors for commercial biosensors, molecular diagnostics and traditional lab analysis.

From the device side, technical issues can lay either in creating handheld or user-friendly portable systems or in the creation of disposable, functionalized electrodes. The former is illustrated by the fact that handheld portable glucose meters are usually based on a straightforward conductometric readout platform that allows for easy miniaturization and relatively simple calibration. However, physiological glucose levels are typically in the millimolar range and creating sensors for other compounds might require higher sensitivities that require other readout principles that operate in the (sub-)nanomolar regime. With the field of micro- and nano-electronics rapidly expanding, commercial enterprises are coming up with simplified handheld/portable solutions: Palmsens offers portable alternatives for the high-end impedance analyzers by HP or Zürich Instruments for lab analysis. Similar trends can be observed for *e.g.* QCMs and SPRs with companies like Aspectus and OWLS building portable low-cost alternatives for high-end lab equipment offered by respectively QSense and Biacore (GE). As these devices will improve, the chance of companies not only offering platform technology but also integrated devices for specific biosensing applications will increase.

The authors of this paper are also working on founding a company, SENSIP BV, based on their thermal sensing technique. Both the possibility of creating a commercial readout platform and developing specific sensor applications are being explored. Therefore, apart from miniaturizing readout design and upscaling device production, a lot of R&D budget will be devoted to creating reproducible functionalized electrodes. In terms of particle immobilization onto wire-based or planar electrodes, research focuses on various angles including dip-coating, roll-coating, electro-polymerization, surface grafting and roll-to-roll

imprint lithography. An even more challenging third bottleneck that can be identified lies in the reproducible creating of large batches of MIP particles or layers. Achieving homogeneity in both morphological characteristics as binding affinity is a complex tasks and makes it hard to get rid of inter-batch variability, which is obviously detrimental in terms of creating commercial sensor applications.

3. Low-cost MIP assay formats

A possible alternative to integrated sensor devices that can address some of the proposed bottlenecks lays in the use of MIPs as recognition elements in assays. Although both terms are often used interchangeably in literature and there is some overlap between the concepts, making the difference feel somewhat arbitrary, we define MIP-based assays as sensing applications that indirectly detect target-rebinding leading to an optically obtainable signal, potentially amplified along the way. This facilitates both readout, as the result can often be seen with the naked eye or requires e.g. a smartphone camera, and receptor immobilization, as lab-on-a-chip technology allows for the creation of lateral flow assays and dipstick devices. An example from the biosensing industry can be found in home-pregnancy testing. Introduced in the 1970's, the home pregnancy test is a sandwich immunoassay that grants the colorimetric determination of the presence human chorionic gonadotropin (hCG) [200]. Comprising of a porous pad with a series of reactive components, a sample is introduced onto the surface of the pad and is carried along the device by capillary action alone. While traversing the pad the liquid sample (e.g. urine, blood, saliva) interacts with the reagents imbedded in the microstructure of the pad, in turn selectively chemically labelling the target analyte. Engineered antibodies comprise the backbone of this chemical labelling process, expressing desired physical traits (radioisotope, fluorescence, and chromophore) [201]. As with antibodies, MIPs can be manipulated and modified to exhibit specific traits upon the binding of a target analyte. MIPs therefore pose as a genuine synthetic alternative to previously biological sensing elements, with the earliest report of a "molecularly imprinted assay" being reported in 1993 by Vlatakis *et al.* [202]. This assay was analogous to competitive radiolabeled immunoassays, employing an imprinted ethylene dimethacrylate-co-methacrylic acid polymer to sense the presence of two unrelated compounds

(theophylline and diazepam). The results of this demonstration were impressive, with a linear range of 14-224 μM for theophylline and the results from 32 serum tests correlated with the same analysis from a corresponding enzyme-multiplied immunoassay technique. Cross-selectivity was proven to be on par with that of antibodies, though the major pitfall came at the cost of the developed MIP-based assay operating in organic solvent. Assays capable of operating in aqueous buffer solution and organic solvents were soon introduced, paving the way forwards for MIP-based assays to operate in biologically relevant environments [203-205].

Though the binding of target analytes and environments analysis could be conducted had drastically been improved, the use of radiolabeling was hindering the commercialization of the generated assays. The availability, handling, cost and coupling of isotopic labels was brought into question, deeming radiolabeled assays as unfeasible. Other methods were soon developed showing greater potential, with Piletsky *et al.* being one of the first to introduce fluorescently labelled analogues of triazine for the detection of unlabeled triazine [206]. Fluorescent characteristics would soon be incorporated into the physical properties of the imprinted polymers, utilizing fluorescent monomers, crosslinking agents and initiators [207]. This allowed fluorescent enhancement and quenching to be considered as potential mechanisms for the direct detection of analytes. Other methods were also considered, with MIPs being conjugated to enzymes offering enhanced sensitivities. Though with the integration of biological components the environments analysis can be conducted in once again becomes limited. However, Literature sources report the use on enzymes in conjugation with MIPs in colorimetric and chemiluminescence methodologies [208]. Direct coupling of modified analytes with powerful chromophores such as dye molecules has also been reported, enabling visual confirmation of binding events [209].

Since the inception of these concepts and methodologies, the field has however developed further; utilizing technologies not previously available to increase the commercial potential of these strategies. These advancements are guided by the needs of both commercial and industrial applications, increasing

the ease and speed of analysis. The innovation of the last decade has therefore brought these concepts closer to commercial fruition, bringing the need to re-examine the current state-of-the-art (Quantum dots, nanoparticles, displacement assays) and determine the current obstacles that must be overcome before these technologies will be translated to commercially available devices.

3.1. Quantum dot MIP assays

The use of conventional organic fluorophores in combination with imprinted systems has seen a decline over the last decade, with the use of quantum-dots (QDs) becoming ever more popular. QDs offer greater chemical stability, photostability and tunable spectral properties than traditional fluorophores, gifting distinct advantages to systems that utilize these fluorescent nanoparticles [210]. Thus QDs pose as a more capable, controlled, and reliable method of installing fluorescent characteristics into molecular imprinted systems. The first reported affiliation of QDs with MIPs was reported by Lin *et al.* in 2004 [211]. MIP layers were anchored onto CdSe/ZnS core-shell QDs permitting the selective detection of caffeine, uric acid and estriol in several solvents with the biggest effect size encountered in water, which opened up the possibility of using the system for biological analysis. This article however, only provided a first proof-of-principle and over the years, other groups were able to come up with more in-depth studies, demonstrating actual application of MIP-QD assays for *e.g.* environmental monitoring with LoDs reported in the relevant nM concentration regime [212-213].

In recent years, the amount of assays based on the combination of QDs and MIPs has rapidly increased with several commercially interesting technologies reported on by a wide range of groups. Vaneckova *et al.* formed thin imprinted polydopamine films with modified QD-antibody-antigen conjugates as the imprinted species for protein detection [214]. Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was then utilized in this proof of principle immunoassay, undertaking a dual approach that highlighted the sensitivity (from the MIPs) and selectivity (from the QDs-antibodies) of the method. The resulting LoD of the methodology was 4.2 µg and 1.6 µg respectively, with the analysis time

of each scan only taking 23 seconds. The main drawback of this technology is still the application of complex readout devices that increases the size and cost of the technology.

As most fluorescent systems utilize an optical readout method for interoperating the fluorescence signal, either the readout device must become compact (handheld) or the necessity of readout methods for validation must be overcome. Wang *et al.* overcome this necessity by introducing a facile QD-MIP based method for the direct detection of dopamine in tiny amounts of biofluid [215]. The system that is introduced is a paper-based assay that allows the detection of physiologically relevant amounts of dopamine in 10 μL of serum. Dual-emission (carbon and CdTe) QDs act as a core that is encapsulated inside a MIP shell, enabling spectroscopic and visual verification of the presence of dopamine in the presence of UV light. To facilitate ease of use, the nanoparticles were loaded onto small strips of filter paper by adsorption of the particles directly into the crevices of the substrate. The strips were then exposed directly to serum samples, and analyzed with a spectrometer and UV lamp (Figure 7). The dose-response curve for this system displayed a wide linear range ($0 - 1.2 \times 10^{-6}$ M) even in the presence of common ionic and molecular interfaces that can be found in biofluids, yielding an LoD of $100-150 \times 10^{-9}$ M. All results were validated with HPLC, which emphasizes that the assay is not only interesting because it is handheld and requires little instrumentation but also has enough diagnostic relevance. Over the past two years alone, many similar QD-based MIP assays have been developed for various purposes including medical diagnostics [216-218], food safety [219-220] and environmental screening [221-222].

3.2. Nanoparticle MIP assays

In addition to QDs, nanoparticles (NPs) have been used in conjunction with MIPs to create optical detection assays. Though some literature demonstrates that NPs can be incorporated into the bulk of MIPs [223], the majority of literature utilizes NPs as a separate sensing element. These assays are often combined with colorimetric detection, overcoming the need for an excitation source and fluorescent probes.

Wu *et al.* reported on the use of NPs with MIPs for the colorimetric detection of residues of the insecticide Cartap in tea [224]. Cartap was extracted from spiked and non-spiked samples of tea by means of magnetic molecular imprinted microspheres ($\text{Fe}_3\text{O}_4@\text{mSiO}_2@\text{MIPs}$) that utilized methacrylic acid as a functional monomer. Cartap was eluted from the MIPs and AgNPs were added to stimulate a color change turning the elution from yellow to grey dependent on the presence of the target. In this way, a semi-quantitative assay was developed allowing for the detection of Cartap in concentrations down to $\pm 5 \text{ mg L}^{-1}$. The reaction can be further quantified using a UV-VIS spectrophotometer, allowing for an LoD of 0.01 mg L^{-1} . Although this method is very interesting due to its simplicity, the multistep procedure makes it time-consuming and is not very sensitive. Zhao *et al.* introduced a method that can address the latter aspect, by using AuNPs for signal enhancement; they were able to construct a colorimetric sensor for the detection of atrazine with an LoD of $1.2 \mu\text{g L}^{-1}$ and an LoQ of $4.0 \mu\text{g L}^{-1}$ [225]. This makes the assay competitive with most sensor applications that are able to detect targets in the lower nanomolar concentration regime. However, sample pretreatment is still necessary for sample analysis, limiting the method's commercial potential. Other reports of nanoparticles being used in a similar fashion exist, though they all suffer the same drawbacks preventing commercial viability [226].

Kong *et al.*, highlighting the potential of a system that does not rely on sample pretreatment, developed a novel microfluidic paper-based colorimetric sensor based on MIPs and AgNPs [227]. A system was constructed that consisted of a MIP membrane that was imprinted with bisphenol A (BPA) (Outer layer), ZnFe_2O_4 NPs (second layer) and cellulose paper (Inner layer) (Figure 8). Upon the addition of 3,3',5,5'-tetramethylbenzidine (TMB) and peroxide to the structure, the NPs would oxidize the introduced peroxide generating OH species that would discolor the cellulose paper. The binding of BPA to the MIP layer would prevent this interaction, leaving the color of the cellulose paper unchanged.

This proved to be an extremely sensitive method with a LoD of 6.18 nM and a linear range of $10 - 1000 \text{ nM}$. Photoshop was used to quantify the color change, relating the change in greyscale to the concentration of BPA introduced. These results prove that it is possible to develop a low-cost MIP-NPs

assay that can be used for the detection of compounds in a relevant concentration regime using a simple camera and image processing software. The method does have some drawbacks such as pH deficiency and the need for environmentally unfriendly reagents. However, the combination of this type of assay in combination with a lateral flow device in the future could minimize the environmental impact of the method, as the required reagent volume would be drastically decreased.

3.3. Colorimetric and fluorometric MIP displacement assays

Displacement assays act as competitive assays between a labelled version of a target analyte and a non-labelled version. The modified analogue of the target species has specific functionalities facilitating the molecule to exhibit colorimetric or fluorometric properties. The first proof-of-principle of the concept was conducted in 1998 by McNiven *et al.* using a dye-labeled conjugate to detect chloramphenicol [228]. The colorimetric sensor proved immune to interferences and had a linear response to the over a 3 – 1000 $\mu\text{g mL}^{-1}$ range, being effective above, within and below the therapeutic range. Nicholls *et al.* detail a fluorescent variation of the approach in which they reported a displacement assay format for the detection of chlorophenolic contaminants in drinking water and packaging material [229]. Fluorescently labelled pentachlorophenol (PCP) was prepared as a “guest” molecule and used in a competitive binding assay format. Both labelled and non-labelled PCP was introduced in the presence of the MIP with less retention for the guest molecule observed. The guest molecule was also pre-bound to the MIP, with the reporter molecule being displaced in the presence of the higher affinity unmodified PCP analogues. The filtrates collected from the analysis were then tested with luminometers and compared to GC-ECD as a reference. A detection limit of 0.5 $\mu\text{g L}^{-1}$ was achieved, though in comparison to antibodies the method lacked sensitivity. These reports also focused on the spectral analysis of the method, whereas Li *et al.* highlighted in a more recent study how the method could be used for the visual verification of algal metabolites [230]. Though a greater sensitivity was not procured (48 $\mu\text{g L}^{-1}$), visualization of the displacement of fluorescently labelled analogue was made possible with a hand-held UV lamp (Figure 9). Thus removing the need for large and expensive pieces of analytical equipment.

One facet reducing commercial potential of MIP displacement assays is the need to include a chromophore/fluorophore. However, it has been shown that development of a reporting analogue is not required, and an unrelated (fluorescent) dye can be utilized instead. The first example of this was proposed in 1998 by Haupt *et al.* in which a fluorescent ligand displacement assay was outlined [231]. The assay uses a coumarin derivative as a nonrelated fluorescent probe for the detection of the herbicide 2,4-dichlorophenoxyacetic acid, producing a specific and selective probe that achieve a detection limit of 100 nM. Piletsky *et al.* demonstrated the same principle for the detection of L-phenylalaninamide by dye displacement [232]. Pre-loading the dye molecule onto the MIPs before loading the particles onto a column and eluting an aqueous solution containing the target molecule through. Drawing attention to the merits of high reproducibility yet maintaining a detection limit in the low μM . As chromophores are easy to incorporate into the method, Greene *et al.* showed that visual verification of binding events was possible [233]. Benzofurazan dyes were used in a competitive binding assay in the presence of six imprinted amine compounds that could be easily identified by the naked eye without require spectroscopic method.

Several MIP-based displacement assays have been used in recent years for the detection of a wide variety of compounds including biogenic amines, pesticides and narcotics [234-236]. The most apparent obstacles that need to be overcome to allow for commercialization lie in improving the sensitivity of these methods and the creation of homogenous batches of MIPs with high affinity. The use of dyes with high extinction coefficients could improve the sensitivity in the future, while integrating the assays in lateral flow devices can create dipstick devices suitable for market introduction.

3.4. MIPs as capture antibodies in immunoassays

In addition to being used as antibody-analogues in competitive assays as described in the previous chapter, MIPs have also been used as capture probes in traditional ELISA(-like) pseudo-immunoassays [237-239]. These type of assays profit from the popularity and associated technological advances that have been associated with ELISA-like assays. The ubiquity of immuosorbent assays in present day

diagnostics and analytical chemistry could overcome some of the thresholds associated with introducing new technologies into a well-established market. Furthermore, immunoassays are compatible with lab-on-a-chip technology, which opens up the possibility of integrating the assay into a lateral flow device. This characteristic was exploited by He *et al.* for the creation of a test strip for the pesticide triazophos (Figure 10) [240]. They immobilized an imprinted cellulose acetate layer on the test line of a lateral flow strip. Samples travelled by capillary transport from the sample pad to the absorbent pad via the MIP layer. The sample containing an unknown concentration was mixed with a solution containing a conjugate of triazophos with an IgG and a FITC label. When the sample reaches the MIP line, the conjugate will compete with any target molecule present in the sample, resulting in an inversely proportional correlation between target concentration and the signal. The results were benchmarked with LC-MS/MS with an LoD of $20 \mu\text{g mL}^{-1}$ in tap water. A similar assay was developed by Akbulut and Zengin deposited imprinted poly (N-acryloyl-L-phenylalanine) films on whatman paper strips for the detection of propranolol in human plasma [241]. These portable tests themselves are suitable for on-site analysis but all results have been assembled using a lab UV-VIS spectrophotometer. Additional tests in the future will have to prove that these tests can detect targets in relevant concentrations when combined with *e.g.* smartphone cameras, which have been used by other groups in the context of MIP-based sensing [242-243].

3.5. Bottlenecks MIP-based assay platforms

The overview presented in this chapter illustrates the commercial potential of MIP-based assays. In comparison to integrated sensor devices, receptor immobilization is often a lot easier with MIPs often simply being immersed in solution or deposited onto the bottom of a 96-well plate. In addition, assays profit from the popularity of immunoassays and can be easily integrated into lateral flow strips or dipstick, although they are also compatible with most forms of biosensors, especially those based on optical transducers. In addition, colorimetric assays can often be analyzed by the naked eye, as do some assays based on luminescence and fluorescence that do require some form of excitation source. Even the quantifiable assays that are now being analyzed using lab-based spectrophotometers could easily be

transferred to portable systems that are compatible with smartphone cameras or handheld spectrophotometers.

Up until now, the major bottleneck in developing MIP-based assays for commercial use has been, again, the production of homogenous receptors with high affinity on a large scale. Enzyme-based assays and immunoassays profit from the knowledge and knowhow the industry has in mass-producing them, especially with reactor technology enabling upscaling the production of these bioreceptors. However, MIP synthesis is profiting from the rapidly advancing field of polymer technology and the number of groups reporting on novel ways to create MIP particles, that are more homogenous in morphology and affinity towards their target, are increasing. With the development of these new techniques, new companies focusing on large-scale MIP synthesis are entering the commercial market, as we will discuss in the next chapter.

4. Commercial MIP synthesis

As described in previous sections, despite the huge commercial potential of imprinting technology (> 1000 patents on Scifinder), until date, the amount of companies that direct their resources towards MIPs is limited and efforts in this area have mostly been confined to laboratory research. Early commercialization of MIPs was aimed at application in purification and separation, pioneered by companies such as MIP Technologies, POLYINTELL (AFFINIMIPSPE products), and Supelco (previously Sigma Aldrich, now Merck). Acros (SupelMIP) for instance, offers MIPs cartridges for purchase for the solid phase extraction (SPE) of a number of environmental contaminants [2244]. Aspira Biosystems sells MIPs, made by epitope imprinting that are able to selectively capture microorganisms [245]. The advantages that MIP offers in this area include savings of time and costs, stability under extreme conditions and no influence of ions on extraction which can be an issue in other porous materials such as zeolites and clays [246], and superior selectivity over standard methods. The reason that early commercialization focused on purification and separation lies in the fact that polymer structure and homogenous binding affinity are not subjected to stringent requirements and bulk microparticles can be directly integrated into columns.

In terms of sensing applications, the main bottlenecks previously identified in previous chapters are the integration of MIPs into electrodes and assays and the large-scale production of homogenous, high affinity MIP particles. Especially the latter can be challenging with standard methods of producing nanoMIPs including electropolymerisation or lithographic techniques. Therefore, a few companies have been founded in recent years that focus on synthesizing MIPs on a large-scale for sensing purposes. Semorex synthesizes MIPs for the detection of proteins, with the potential of extending to therapeutics for cancer and treatment of bowel diseases. However, protein imprinted polymers are a challenge in itself since mass-production, if conventional approaches are followed, requires a large amount of the template which brings inherent costs.

MIP diagnostics has introduced an alternative approach that might change the field entirely. The company, based in Sharnbrook (UK), was founded in 2015 and focuses on MIP development using the solid-phase imprinting approach to produce high affinity nanoparticles as pioneered by the Piletsky group at the University of Leicester [36]. The advantages of this approach are that the template can be recycled to reduce costs while allowing for (semi) automated production by elution of the polymers instead of the template [37]. Their focus has been contract development work for other companies that have struggled with traditional disadvantages of antibodies, such as batch-to-batch variation or operation under broad temperature and pH conditions. Their produced nanoMIPs have been applied to remove micropollutants and microorganisms from drinking water, to direct replacement of antibodies in assays, and novel sensor formats. However, recent investment will enable scale-up of operations for development of an in-house portfolio of products, starting with commonly used biomarkers. This can speed up the development of sensing platforms based on these receptors in the future.

Canfarotta *et al.*, reported on the production of nanoMIPs for vancomycin, which were used as antibody-replacements in an ELISA-like format [247]. MIP Diagnostics' nanoMIPs were also integrated into gold screen-printed electrodes for the impedimetric detection of cocaine at trace levels [248]. A simpler and more universal approach to label-free detection of analytes, independent of their electrochemical

properties, might be the addition of monomers modified with a redox probe, such as done by Mazzotta *et al.* who used vinyl ferrocene and ferrocenyl methacrylate to detect vancomycin [249]. The nano-sized homogenous MIPs also meant a huge advantage in thermal MIP-based sensing, allowing to directly couple the nanoparticles to thermocouples for the detection of a wide variety of biomedical targets, ranging from small organic molecules to large proteins at physiologically relevant concentrations [250]. Crapnell *et al.* further extended this approach by functionalizing multiple thermocouples and integrating them into a single sensor setup for the multiplexed detection of cardiac biomarkers (Figure 11) [59]. The sensor demonstrated to be capable of simultaneously detecting the biomarkers in buffer and serum solutions. Betlem *et al.* recently introduced an alternative readout approach, using thermistors rather than thermocouples, which simplified analysis by measuring electrical resistance. Furthermore, the use of thermistors offers several advantages such as their low-cost, robustness, and highly sensitive response to small changes in temperature around a fixed base point [251].

In the future, companies like MIP Diagnostics hold the key for the commercialization of MIP-based sensing platforms as they focus on overcoming the main bottleneck standing in the way of commercialization. To do so, they will not only have to increase their focus on mass-producing their nanoMIPs but will also have to continue to work with research groups around the world to explore how these MIPs can be integrated into sensors. In doing so, they can profit from the knowhow of companies that are traditionally involved in the construction of biosensor and assay platforms. An exciting advancement recently announced by the company is the collaboration with Stream Bio, an enterprise that is specialized in bioimaging. The goal is to combine nanoMIPs and conjugated magnetic nanoparticles in a lateral flow assay for the development of a fast (10 minutes) diagnostic test for SARS-CoV-2. The test could improve the diagnostic capacity of healthcare systems worldwide by offering PoC diagnostics but can also be used to screen sewage water for SARS-COV-2. The latter is seen as a potential path of spreading the disease [252]. In the future, one would expect more assay formats that incorporate MIPs

and there is potential towards the area of oral therapeutics and drug delivery due to the nanoMIPs inherent biocompatibility [253].

5. Outlook

The literature analysis done in this review re-emphasizes the commercial potential of MIPs as alternatives for bioreceptors in sensor platforms and assay formats but also illustrates why commercialization of MIP-based sensors and assays lags somewhat behind in comparison to biosensors and assays based on natural receptors. This can be attributed in part to historical reasons. Biosensors emerged in an era when personalized medicine did not really exist yet while the need was there. Home-based self-testing for blood glucose levels was not possible using traditional, state-of-the-art detection platforms but it has changed the life of diabetics around the globe. Likewise, home pregnancy testing allowed women and couples in general, to assess their pregnancy status without needing to overcome the social and financial threshold of visiting a doctor every time. Biosensors filled a void that traditional laboratory analysis could not fill and acquired market relevance and momentum because of it. This momentum, and resulting financing, in combination with prior knowledge on coupling enzymes and antibodies to surfaces, has formed the basis of the success story of these platforms that have become ubiquitous in modern-day society.

Despite these success stories, traditional biosensors for personalized medicine have still not fulfilled their full potential, as lab-based analysis remains the golden standard due to the high sensitivity and high throughput associated with techniques such as PCR, LC- and GC-MS... However, gradually society is evolving and technological developments that are making their way into society such as smartphones and 3D printing have accelerated the development of e-health and biosensing platforms. MIP-based sensors and assays could also profit from this trend. Many of the examples highlighted in this review, illustrate how MIPs are compatible with smartphone readout, wearable sensors or lateral flow assays based on 3D-printed microfluidic platforms. This research has tackled one of the main bottlenecks traditionally hindering MIP-based sensing from being commercial: integration of MIP particles in electrodes and assay formats suitable for commercial production.

The main challenge remains to create MIPs in large batches that are homogenous in size and shape but also in their affinity towards their target. Recent advances in imprinting technology have led to MIP particles with affinities that are comparable to that of natural receptors, which is necessary to convince the market that MIPs have a benefit and create momentum in a manner similar to the first biosensors.

Companies such as MIP Diagnostics that come up with ways to not only improve the affinity of the target but also enable the large-scale production of homogenous batches of these particles, hold the key to commercial success. These new methodologies could compete with new advances in reactor synthesis of antibodies and enzyme. In this way, MIPs will no longer have a technological disadvantage over their natural counterparts and their benefits including low-cost price and superior mechanical, chemical and thermal stability will become more important in commercial diagnostics. In addition to the generic nature of the technology, which, in contrast to their natural counterparts, allows for the detection of nearly any target of nearly any size, these characteristics of MIPs still makes molecular imprinting a very interesting, commercially viable technology that can disrupt the diagnostic market for years to come.

Author Contributions Statement

J.L. and K.E. coordinated writing of the manuscript, wrote the introduction and assay sections, assembled and edited the article in close collaboration with T.J.C. P.S. and M.P. wrote the section on MIP companies and helped to study the industrial relevance of the field while H.D. and B.v.G. wrote the sections on thermal and electrochemical sensor platforms respectively.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Vitae

Mr. Joseph W. Lowdon MChem (Hons) obtained an integrated master's degree in chemistry from Manchester Metropolitan University in 2017. Over the course of his studies, he participated in multiple internships in the fields of synthesis and characterization of New Psychoactive Substances, synthesis of fluorescent monomers, and intelligent design of molecularly imprinted polymers. Since November 2017 he has begun to obtain his PhD in chemical and biological biosensing within the Smart Devices Research Unit of the Faculty of Science and Engineering at Maastricht University. His research interests include synthesis of biomimetic polymers for bio- and chemical sensing, biocompatible materials for drug delivery and the implementation of new technologies for healthcare management.

Dr. Hanne Diliën obtained a master's degree in chemistry (molecular design and synthesis) at KU Leuven in 2006. She started a PhD project in the development of low band gap polymers at Hasselt University in cooperation with IMEC and obtained her PhD in sciences in 2011. As a postdoctoral fellow she continued working at Hasselt University as a member of the Nanobiotechnology and Bioelectronics group. Since 2013, she is an assistant professor in the Maastricht Science Programme at Maastricht University. Furthermore, she is part of the Sensor Engineering department, where she leads the organic chemistry and polymer synthesis research lines. Dr. Diliën's research interests are focused on organic and polymer synthesis for applications in the (bio)medical field; such as synthesis of pharmaceuticals, hydrogels,...

Dr. Pangaj Singla has recently obtained his PhD from Guru Nanak Dev University, India. He studied the use of micelles and other drug delivery systems during his PhD. The past year, he has collaborated intensively with the group of Dr. Peeters at Newcastle University working on molecular imprinted polymers and thermal characterization of biosensor platforms and drug delivery systems.

Dr. Marloes Peeters obtained a master's degree in chemistry and chemical engineering from Eindhoven University of Technology. Subsequently, she moved to Hasselt University to complete a PhD on biosensing using Molecularly Imprinted Polymers in 2013. This was followed by postdoctoral

work at Hasselt University and at Queen Mary University of London. She started her independent research career at Manchester Metropolitan University in 2015, where she led the group that worked in the field of biosensors. In 2018, she was promoted to senior lecturer, after which she transferred to Newcastle University in 2019 where she holds the same position and leads her own research group, focused on biomimetic sensing. Her research interests include the development of novel biosensor platforms that can be used for sustainable healthcare and environmental applications.

Prof. Dr. Thomas Cleij obtained his Ph.D. in 1999 at Utrecht University in polymeric materials science. Subsequently, he was appointed as assistant professor at Louisiana State University and associate and full professor at Hasselt University. In 2009, he became involved with the efforts to start natural science education at Maastricht University. This resulted in the Maastricht Science Programme, which he started in 2011 as Dean and Programme Director. He has been appointed at Maastricht University as a full professor since 2012, Dean sciences since 2015 and Dean of the Faculty of Science and Engineering in 2019. He is the PI of the Sensor Engineering Department and his research interests are focused on the combination and interplay of novel functional polymeric materials and advanced applications in the life sciences. Examples of this research at the interface between chemistry, life science and physics include polymer-based biosensors, molecularly imprinted polymers (MIPs) for bio-analytical applications and polymeric hydrogels for tissue engineering.

Dr. Bart van Grinsven received a master's degree in bioelectronics and nanotechnology from Hasselt University in 2007. Subsequently, he was employed by TNO as a development engineer before returning to the BIOSensor group of Hasselt University in 2008. After obtaining his PhD in 2012 and working as a postdoctoral researcher in the same group, he moved to Maastricht University in 2014. Currently, he holds the independent position of associate professor and group leader within the Sensor Engineering Department at Maastricht University, leading the biosensor research line. His field of interest includes biosensor development, PoC testing and biomedical device engineering.

Dr. Kasper Eersels obtained a master's degree in bioelectronics and nanotechnology and a PhD in science at Hasselt University in 2009 and 2014 respectively. He continued working in the BIOSensor group at Hasselt University until 2015 as a postdoc after which he moved to KULeuven to work as a postdoctoral fellow at in the Soft Matter and Biophysics section of the department of Physics and Astronomy. In 2017, he transferred to Maastricht University, where he currently holds the independent position of assistant professor within the Sensor Engineering Department, leading the research on MIP-based sensing and their application in industrial and medical settings. His research interests include bio- and chemosensor development, biomedical application engineering and implementation of technology into healthcare.

Figures

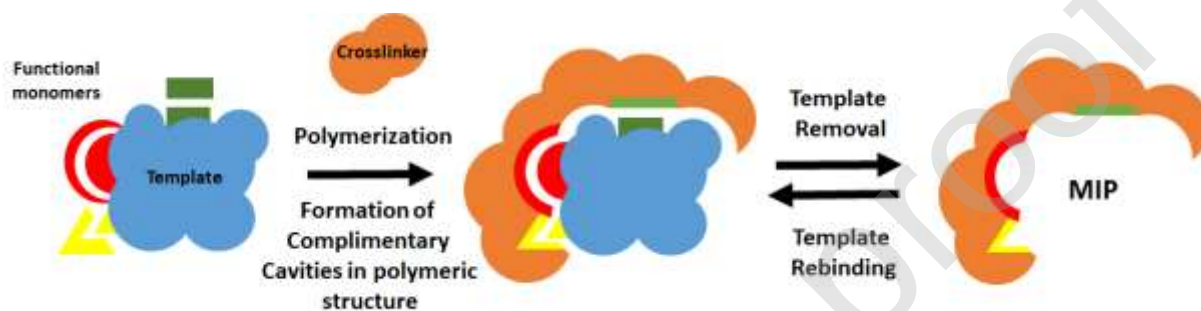


Figure 1: A generic schematic demonstrating the fundamental principles behind the synthesis and extraction of a MIP.

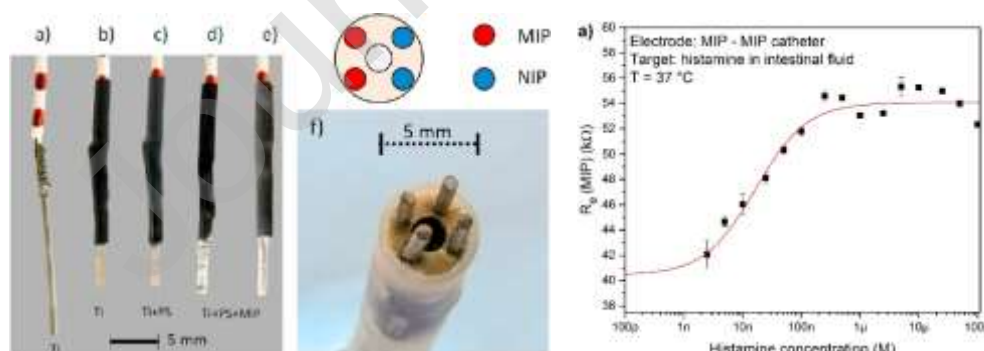


Figure 2: Thermocouples were coated with MIP particles by dip-coating a polystyrene layer onto a copper-welded titanium catheter wire (left). Two wires were coated with MIP particles, two with NIP

particles and integrated into one catheter (middle), allowing for the impedimetric detection of histamine in intestinal fluid (right). Figure reproduced from [79] with permission. Copyright Elsevier 2020.

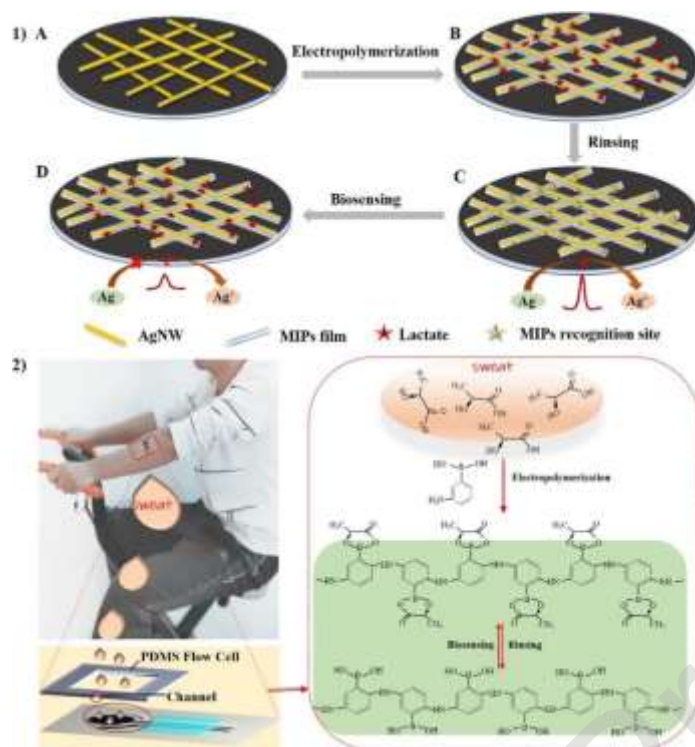


Figure 3: Lactate MIPs were made on top of a patch containing silver nanowires by electropolymerization (top). This patch, containing an integrated PDMS flow cell and a measuring chamber were attached to a test person's arm, allowing to monitor lactate levels in sweat. Reproduced with permission from [85]. Copyright Elsevier 2020.

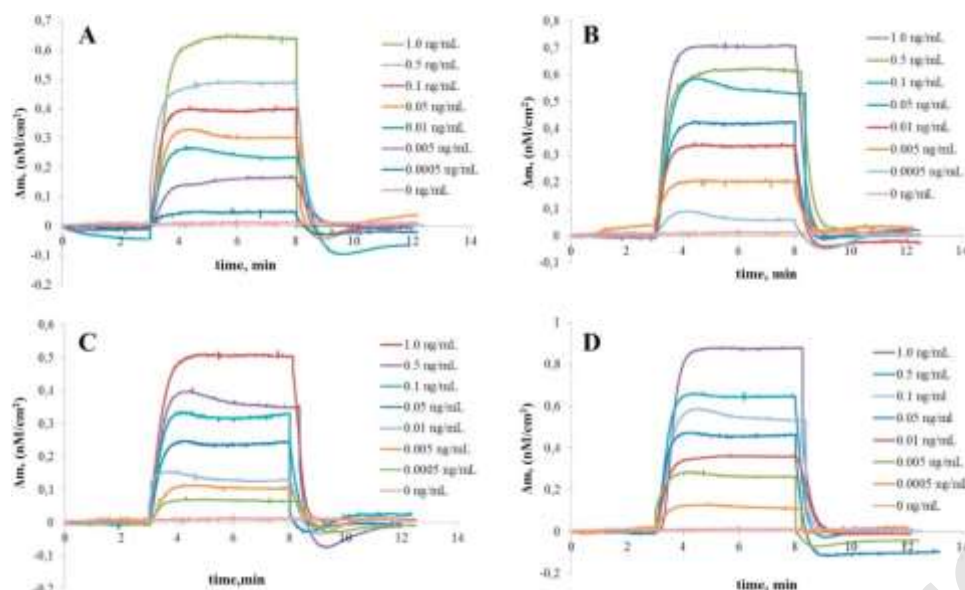


Figure 4 QCM-based detection of 4 different cannabinoids (JWH-018, JWH-073, JWH-018 pentanoic acid and JWH-073 butanoic acid respectively A-D). The data show that it is possible to quantify these compounds in synthetic urine down to concentrations in the pg/mL range. Re-used from [131] with permission. Copyright Elsevier 2018.

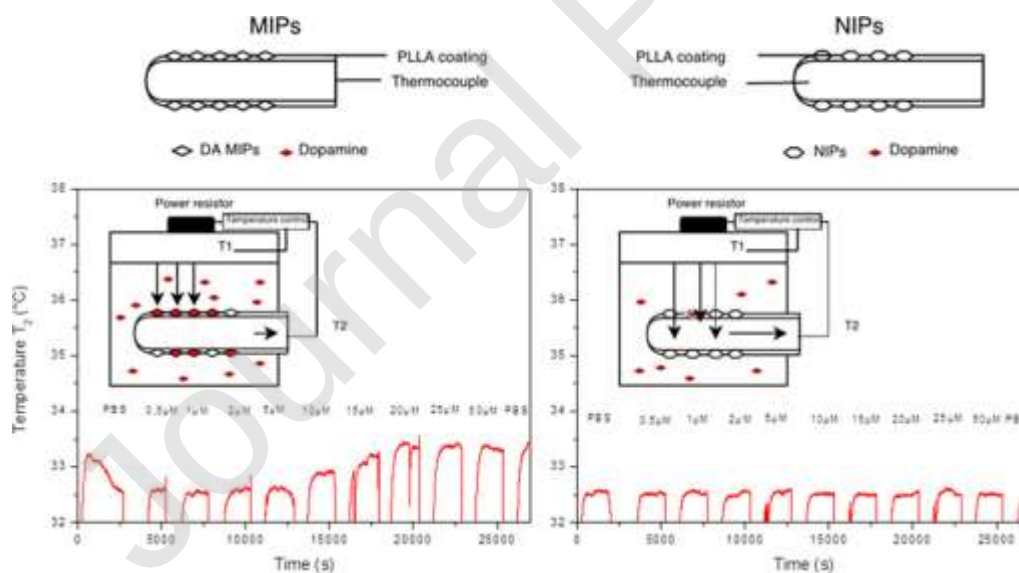


Figure 5 Thermocouples were dip-coated with a poly-lactic acid adhesive layer and roll-coated with MIP particles. These thermocouples were used to detect the presence of various neurotransmitter in buffer. Re-

used from [162] with permission on an open-access license agreement, Copyright American Chemical Society 2017.

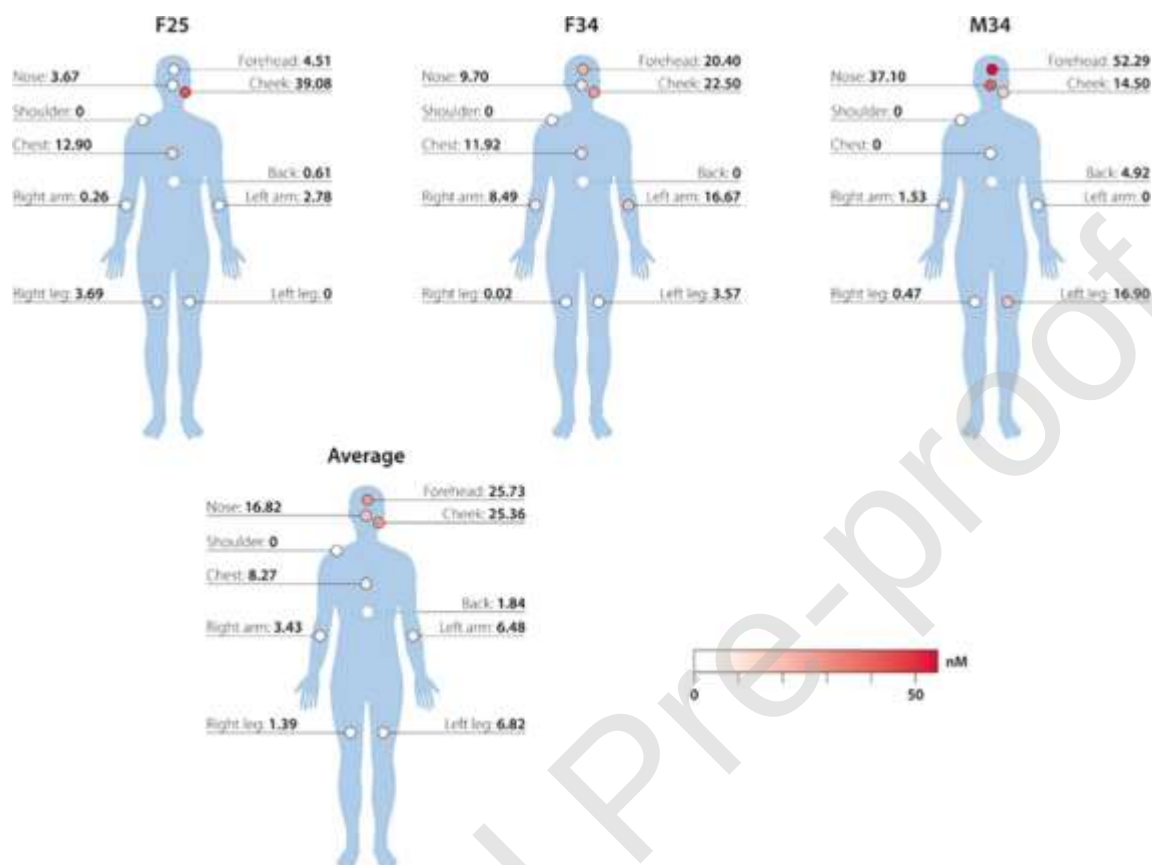


Figure 6: Gold SPR chips were coated with MIPs for the quantification of secreted bacterial factor (RoxP). Skin swaps were taken at various positions on the bodies of two female and one male volunteer and the MIP-based SPR sensor was able to detect RoxP on all positions with results being validated using a golden standard ELISA test. Adapted from [184] with permission. Copyright American Chemical Society 2019.

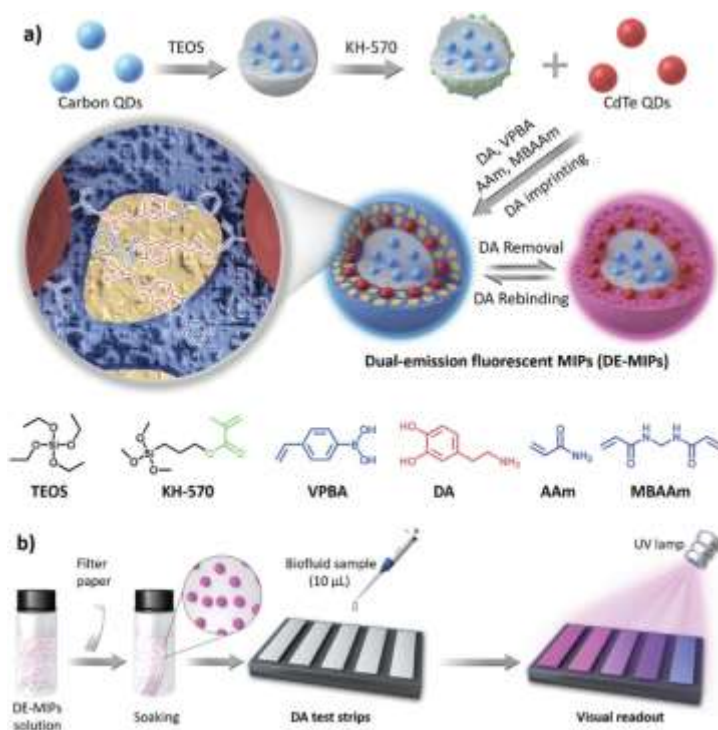


Figure 7: Fluorescent dual-emission MIP nanoparticles were made by integrating red and blue fluorescent quantum dots into the polymer shell (top). These particles were immobilized onto test strips by soaking them in MIP solution. The result is a pH-indicator-like test strip that allows the end-user to quantify the amount of dopamine in a biological sample. Adapted with permission from [209], Copyright John Wiley and Sons 2018.

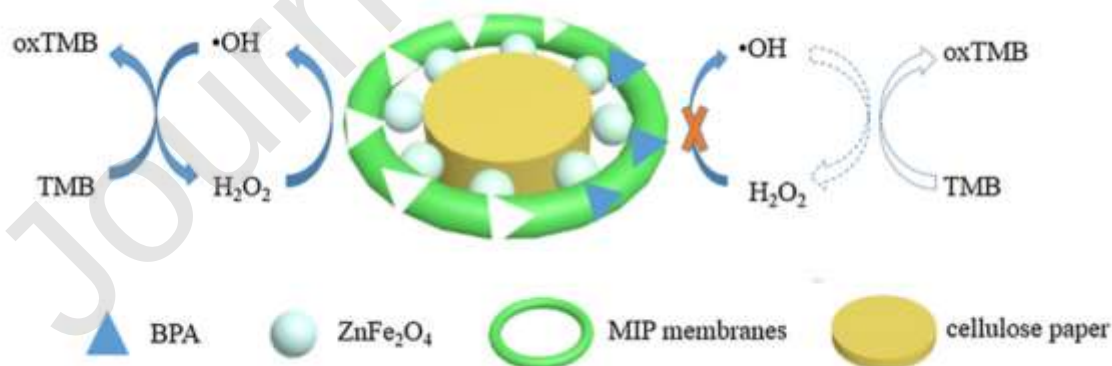


Figure 8: MIP membranes deposited round a cellulose paper, using nanoparticles as a spacer ring between the paper and the MIP layer. The MIP membranes selectively bind BPA, preventing the

oxidation of peroxidation, leaving the color of the cellulose paper unchanged. In absence of BPA, the generation of OH-species will discolor the paper. Re-used with permission from [221], Copyright Elsevier 2017.

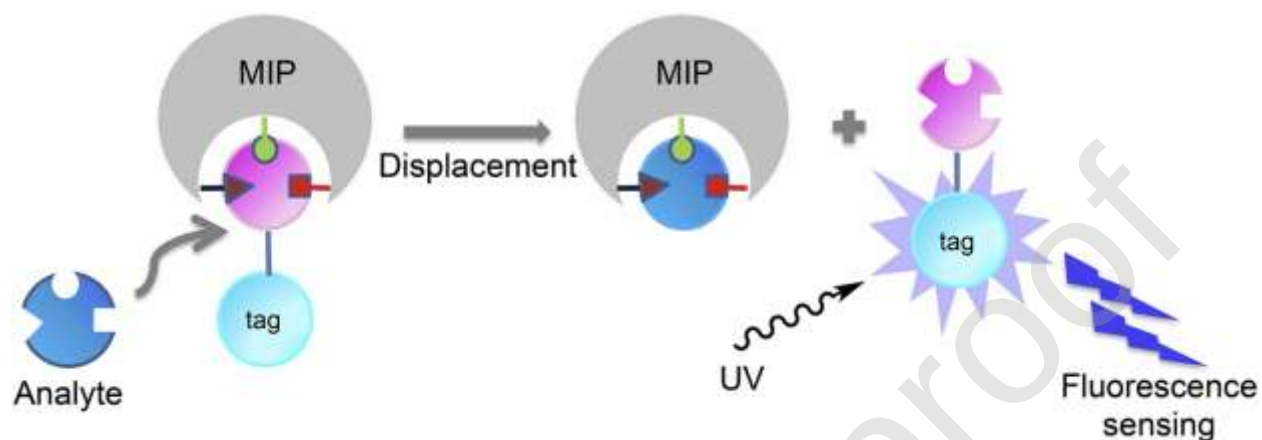


Figure 9: MIPs were imprinted with algal metabolites geosmin and 2-methylisoborneol, extracted and loaded with substrates coupled to fluorescent tag. In presence of the target, the fluorescently labeled substrate gets displaced due its lower affinity for the MIP. Illumination of the filtrate with a simple UV-lamp visually confirms the presence or absence of the target. Adapted with permission from [224]. Copyright Elsevier 2019.

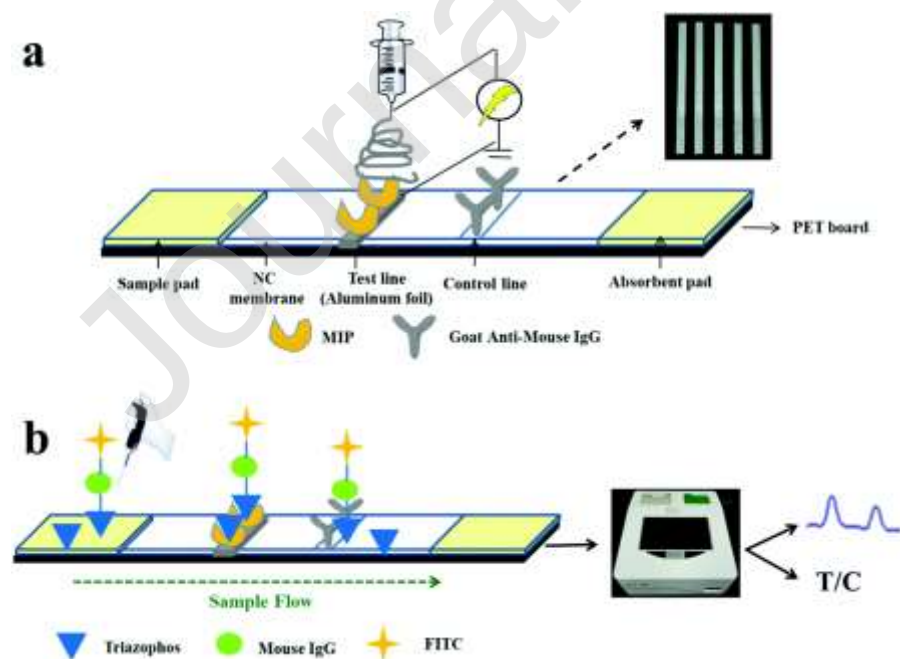


Figure 10: Triazophos MIPs can be used instead of antibodies in a lateral flow assay. MIPs were used as capture antibody in the sorbent test line path of the assay. Samples were mixed with FITC-labeled target molecules. Presence of the target prevents the labeled molecules to bind to the test line, leading to an inverse correlation between target concentration and fluorescence intensity on the test line. Adapted from [234] with permission. Copyright Centre National de la Recherche Scientifique (CNRS) and the Royal Society of Chemistry 2020.

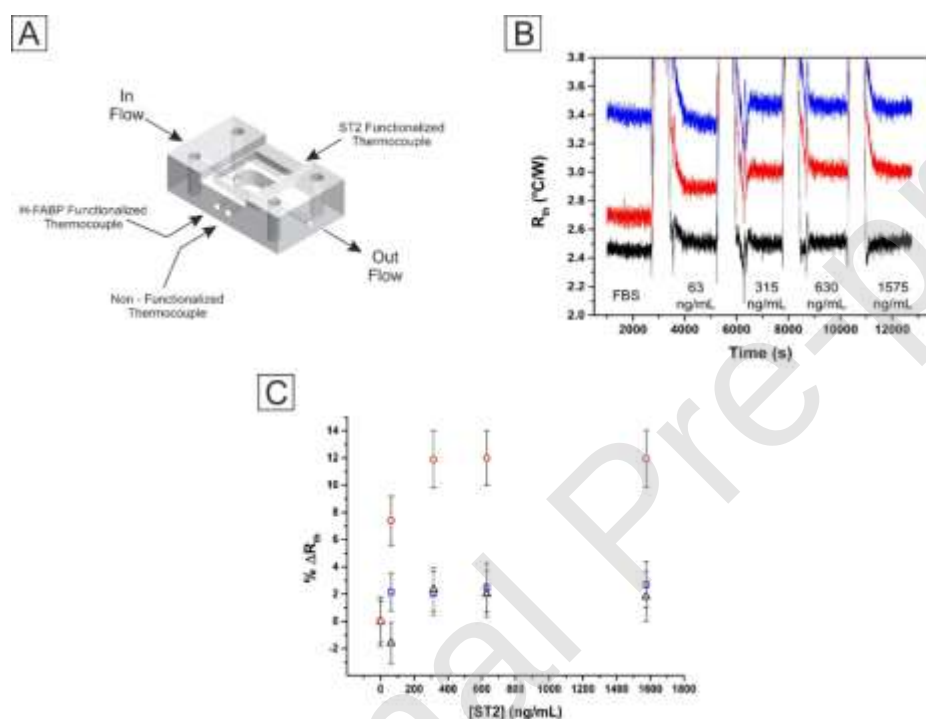


Figure 11. (A) Schematic of the triple thermocouple flow cell used. (B) Raw data HTM plot of the R_{th} over time for the addition of ST2 in FBS to a triple thermocouple set-up with one thermocouple functionalized with ST2 nanoMIPs (red), one functionalized with H-FABP nanoMIPs (blue), and one unfunctionalized thermocouple (black). (C) Plot of the R_{th} % change for the addition of ST2 in FBS to a triple thermocouple set-up with one thermocouple functionalized with ST2 nanoMIPs (red circles), one functionalized with H-FABP nanoMIPs (blue squares), and one unfunctionalized. Re-used with permission from [59], Copyright American Chemical Society 2019.

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