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Stimuli-responsive molecularly imprinted materials: Fundamentals and applications

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Abstract

Stimuli-responsive molecularly imprinted polymers (MIPs) are exciting smart materials that are gaining substantial interest within the research community due to their versatility and possible widespread applications in biosensing, biomedicine and diagnostics, as well as chromatography and separation sciences. These materials offer significant advantages as recognition materials over their biological counterparts (antibodies) because of their ease and low cost of production along with their robustness and resistance to the extremes of temperature and pH. This much needed review aims to provide an updated summary of the various stimuli-responsive MIPs reported to date including those relying on thermo, pH, photo, biomolecule, ion, magnetic and electrical stimuli and includes their design and synthesis. The review also explores the potential applications of the stimuli-responsive MIPs, particularly in the fields of biosensors and diagnostics, along with biological imaging, drug delivery, disease treatments and interventions and the separation of targets from complex media. The advantages and disadvantages of the current stimuli-responsive MIPs set out in the review, allows for researchers to gather a concise understanding of these smart-materials and should pave the way for new methods of development and real-world applications. We believe the review is a helpful and necessary guide for the future evolution and application of stimuli-responsive MIPs.

Keywords

biomedicine, diagnostics, molecularly imprinted polymers, recognition materials, smart materials, stimuli responsive

1 | INTRODUCTION

Molecular recognition is ubiquitous in nature and crucial for the precision of the biological machinery. Commonly this refers to the interactions between a receptor and a ligand that display mutual molecular complementarity exemplified by antigen-antibody, DNA-protein, sugar-lectin, and RNA-ribosome interactions. Inspired by these biological systems chemists have developed new synthetic molecular recognition elements featuring a similar degree

of specificity and selectivity as their biological counterparts, but with the addition of robustness and fine tuneable properties. Molecularly imprinted polymers (MIPs) are a bio-mimetic class of polymers, that have gained particular attention due to the ease and low cost of manufacturing, a wide range of applications, as well as offering a wide freedom of design.^[1–9] MIPs are produced using a molecular imprinting technique that leaves cavities within a polymer matrix with an affinity for a particular template molecule (Figure 1).^[3]

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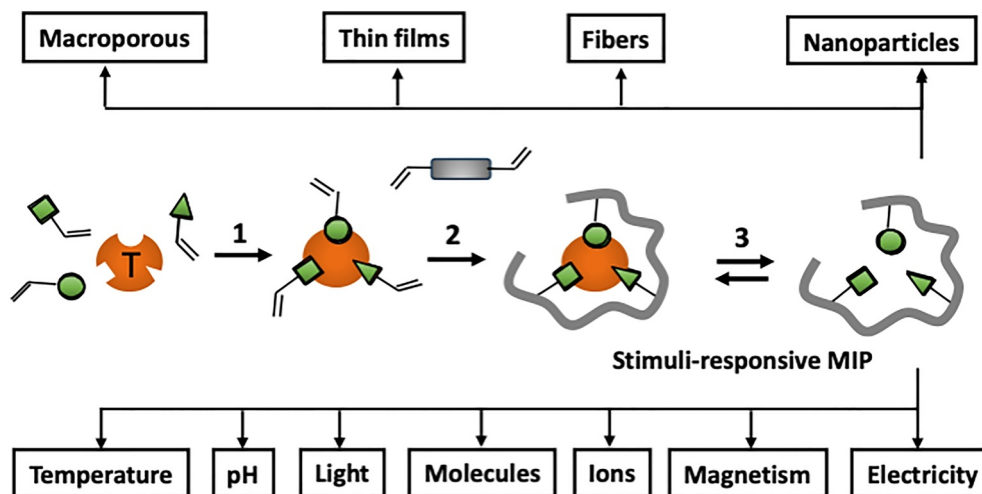


FIGURE 1 Schematic illustration of the molecular imprinting process to form a stimuli-responsive molecularly imprinted polymer (MIP) and stimuli used for response triggering. (1) Self-assembly of functional monomers and a template (T), (2) Polymerization (e.g. Free radical polymerization (FRP)) in presence of a crosslinking monomer followed by template removal (3) to form a stimuli-responsive MIP. Adapted under terms of the CC-BY license.^[3] 2012, from Hall et al., published by John Wiley&Sons Ltd.

The most widely used molecular imprinting process involves, as the first step, self-assembly of functional monomers around a template molecule by noncovalent interactions (e.g. hydrogen bonding, van der Waals forces, electrostatic forces, hydrophobic interactions).^[4–6] The MIP is then produced by free radical polymerization^[7] of these monomers in presence of a suitable crosslinker, forming a polymer with the template entrapped.^[8,9] The template is then extracted, leaving behind a cavity within the polymeric matrix that has a specific affinity towards the original template molecule. Due to the simplicity of this process, molecular imprinting is to date the most versatile technique for engineering synthetic molecular recognition elements mimicking biological receptors.^[10]

MIPs have traditionally found use as recognition materials in biosensors^[11–14] and as affinity based extraction and separation materials for demanding separations.^[15–17] More recently research has revolved around the design and advancement of MIPs mimicking other features of biological receptors.^[5,10] With natural receptors often being responsive towards external stimuli (change in temperature, pH, etc.) research has moved towards developing synthetic receptors mimicking these functions. Stimuli-responsive or “smart” MIPs are in this context gaining attraction.^[18–21] The simple techniques used to synthesise MIPs and the compatibility of the polymerization procedures with a wide range of conditions in terms of solvent and temperature allows for the incorporation of functional monomers that can alter the polymer properties when exposed to external stimuli.^[20,21] For instance, this can be manifested in a modulation of their recognition properties providing a switchable ability towards the binding or release of a target molecule or vice-versa the actual binding of the target can stimulate a secondary response, mainly optical or electrochemical.^[22] Presently there are an assortment of MIPs that have been adapted and

prepared to respond to specific external stimuli, particularly with changes in temperature, pH, incident light, ionic strength or the presence of specific molecules.^[18,20] As the field of stimuli-responsive MIP develops and expands (Figure 1), as do their applications and methodologies. Complementing previous excellent reviews of the field,^[18,20,21] we here provide an updated overview of responsive MIPs currently being developed. A particular emphasis has been made on receptor design strategies and new applications not covered in the previous reviews.

2 | MOLECULARLY IMPRINTED POLYMER FORMATS

2.1 | Macroporous polymers

The first-generation MIPs were produced by bulk imprinting where the template is entirely imprinted and entrapped within a highly crosslinked bulk polymer monolith. This was based on the extensive body of literature describing the synthesis of macroporous polymers and involves mixing all the components in solution (initiator, monomer, solvent, and template) followed by polymerization.^[8,23,24] This relied on the use of organic solvents, meaning only low molecular weight templates could be imprinted, as protein and biomolecular templates were at risk from denaturation.^[4,25] The polymers produced were typically hard and amorphous in nature and required a grinding and sieving process to achieve practical mass transfer rates which resulted in heterogeneous MIP particles in both shape and size.^[8,26] Despite these shortcomings, highly efficient imprinting of low molecular substructures or epitopes can be achieved with this approach making it useful for biological applications as well.^[27,28]

To accommodate the need for imprinting protein targets the bulk imprinting process evolved towards producing hydrogel-based polymers, by using water soluble monomers (e.g., acrylamides) and water as the polymerisation solvent.^[29] These hydrogel-based MIPs still require particle processing to enhance template access, but do allow for the imprinting of protein biomolecules without the fear of denaturation, while the flexibility and large mesh size of the hydrogel enables an easier release of the template.^[29] These types of MIPs are in generally low-cost and can be produced in large quantities in a short time period. However, post-processing and template removal is challenging and can potentially affect binding site integrity and in turn the affinity for the target molecule.^[30]

2.2 | Grafting techniques and thin film MIPs

Moving from 3D to 2D thin-film MIPs have become an alternative way of imprinting templates and allows for the integration of MIPs into sensor platforms.^[31] This method does not involve destructive process of grinding and sieving and bypasses thereby several cumbersome processing steps. With thin-film imprinting, the MIPs can be produced using various grafting techniques (grafting from/grafting to), drop coating, spin coating or electrocoating and the thin-film thicknesses ranging from nanometers using surface-initiated polymerizations into the lower micrometer scale with spin coating and electrocoating.^[32–35] Thin-film MIPs offer significant advantages over the traditional bulk MIPs as the MIP cavities are exposed at the surface of the film, they offer enhanced accessibility to the cavities and consequently an enhanced mass transfer rate, with most binding occurring at or near to surface of the MIP.^[36] MIPs in the thin-film format can be attached to solid surfaces and represent a “smart” coating that can be microstructured and integrated into microfabrication processes, allowing for improvements and advances in the performance of biosensors.^[37]

2.3 | MIP nanoparticles (nanoMIPs)

MIP-based nanoparticles are the latest adaptation of MIPs and offer the most versatility when incorporating the imprinting technology into devices and applications. Decreasing particle size increases the specific surface area allowing for much improved performance.^[38] In particular, the high surface area: volume ratio of the MIP nanoparticles means an increasing portion of templated sites being located at or near the particle surface resulting in faster binding/release kinetics, increased capacity and reduced non-specific binding compared to bulk and thin-film MIPs.^[39] As the MIP nanoparticles are directly

obtained in a one-pot reaction, post-polymerisation processing (grinding and sieving) is not needed. Moreover, as for antibodies, the solubility/dispersibility of nanosized MIPs make them amenable to the same in vitro assay formats as used in traditional immunoassays.^[40]

There are several ways to produce MIP nanoparticles, with the core-shell approach being a particularly popular method. Here, functional cores are first produced, followed by the grafting of an imprinted layer around the core.^[41,42] This approach allows for the use of cores with specific properties such as magnetic, antimicrobial, plasmonic, or signal enhancing properties.^[43,44] Particularly versatile in this regard is the use of nonporous silica nanospheres which are available in many sizes and are easy to functionalize.^[45] Attaching radical initiators or chain transfer agents on their surface allows straightforward grafting of thin polymer shells.^[42] The core shell method allows for the better control of the size and shell thickness and is suitable for the engineering of stimuli-responsive functions which can be based on the core and/or shell properties.

Precipitation polymerization is an alternative method used to produce MIP nanoparticles, whereby the components of the system are dissolved in the reaction solvent under high dilution and upon initiation, discrete polymer particles with low dispersity precipitate out.^[46] The particles are then collected, and the template is removed through washing. The formation of the MIP nanoparticles is a relatively quick and easy technique and typically produces good yields of nanoparticles, while also being a suitable method for imprinting a variety of compounds.^[47,48]

Mini-emulsion polymerisation is another method that is used to produce nanoparticle MIPs. Here an oil phase (containing template, monomers, cross-linker, initiator, and co-surfactant) is mixed with an aqueous phase (containing water and surfactant) and by shearing the mixture, a mini-emulsion, with stable droplets typically of a size between 50 and 500 nm, is created.^[49] Polymerisation is initiated and the MIP nanoparticles are recovered, followed by template removal, with the MIP nanoparticles ready for future use.^[50]

While these methods can produce very small high functioning particles, they are all associated with the difficulty in collecting the particles and lengthy protocols for removing the template. Inability to exhaustively remove the template commonly results in template bleeding which can potentially interfere in subsequent applications.^[29,38] This has led to the commonly used, solid-phase protocol. In this method the template is covalently immobilised on to the surface of a solid support (glass-beads, magnetic particles, etc.).^[51,52] The template containing support is brought in contact with the polymerisation solution and polymerisation is initiated resulting in the formation of nanoparticles around the template. After polymerisation the template-modified

support can be used for affinity-based enrichment of strongly binding MIP nanoparticles.^[53]

3 | FUNDAMENTALS AND APPLICATIONS OF STIMULI-RESPONSIVE MIPs

Stimuli-responsive MIPs are a rapidly developing class of materials, that offer immense potential and versatility in their design and responsive mechanisms. Here we explore the different types of stimuli-responsive MIPs and showcase their potential applications.

3.1 | Thermo-responsive MIPs

Nature is rich with examples of biopolymers responding to changes in temperature.^[54,55] The activity of most human enzymes is optimal at a physiological temperature of 37°C. Lowering the temperature slows down enzyme activity, while raising the temperature can cause enzymes to denature and stop functioning. Heat sensitive ion-channels changes their

ion permeability in response to temperature and can also be activated by a ligand binding event.^[56] Although mimicking these functions is beyond the scope of current research, MIPs featuring temperature-controlled molecular recognition are now well established.^[57] Thermo-responsive MIPs have been developed based on an extensive resource of literature describing thermo-responsive polymers, notably in the context of nanomedicine and drug delivery. The most widely studied polymer from this class is represented by poly(N-isopropylacrylamide)-based (pNIPAm) hydrogels as these can be engineered to exhibit a lower critical solution temperature (LCST) close to the human body temperature.^[58] Water is a good solvent below LCST, and the polymer is then swollen and deformable. In contrast, when the temperature rises above LCST, water is expelled leading to collapse and hardening of the gels.^[58] This has resulted in poly(NIPAm) being widely used as a major component within thermo-responsive MIPs with the binding capacity of such MIPs changing with variations in temperature.^[57,59,60] Hence, the expansion and contraction of thermo-responsive MIPs can be controlled by changing of the ambient temperature and this has been utilized within applications aiming at controlled drug release, catalysis, and in the field of separation science (Figure 2).^[61–63]

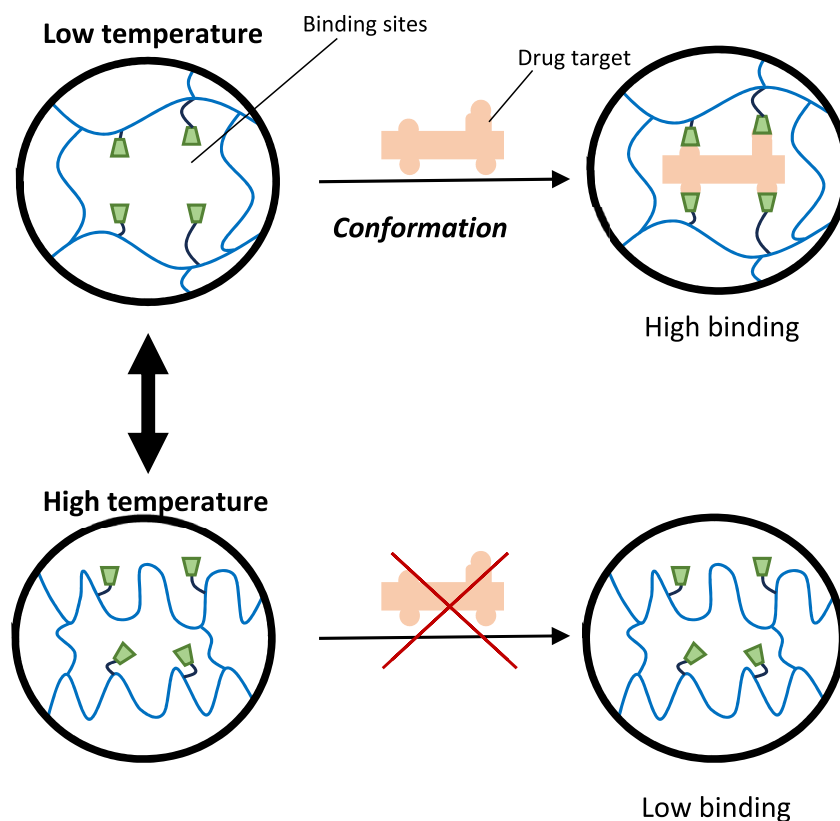


FIGURE 2 Schematic illustration of the drug-binding capacity regulation by conformational change of a thermo-responsive molecularly imprinted polymer (MIP) hydrogel with dynamic molecular binding sites at low and high temperatures.^[61] Reproduced under terms of the CC-BY license.^[61] 2022, Toyoshima et al., published by RSC.

One of the earliest mentions of temperature-sensitive and controllable MIPs was by Watanabe et al., by combining NIPAm with Acrylic Acid in the presence of a template molecule.^[64] This led to thermosensitive copolymer gels that could undergo large changes in swelling and conformation while retaining their molecular recognition ability in the shrunken states.^[64] Consequently, template uptake by the MIP was affected by temperature.

The thermo-responsive function has been extensively exploited to affinity enrich MIP nanoparticles produced using precipitation polymerisation, more recently in combination with solid phase synthesis.^[65] Early contributions by Hoshino et al. (built on their previous nanoparticulate work) exploited this principle using peptide-template modified agarose beads as stationary phase for the affinity enrichment of high affinity MIP nanoparticles.^[66] This was achieved by first allowing the “polyclonal” distribution of nanoMIPs to bind to the beads above LCST. Gradual lowering of the temperature down to $\approx 10^\circ\text{C}$ then led to release of the nanoMIPs in order of increasing affinity. The tightest binding nanoMIP isolated in this way had a size of approximately 30 nm and an exceptionally high affinity with a $K_d = 0.66\text{--}2.3\text{ nM}$.^[66,67] This solid phase methodology was subsequently adapted further, this time by using a glass-bead as the solid phase to immobilise the template. By conducting the polymerisation above the LCST and in presence of the template modified solid phase, the nanoMIPs adhered to the carrier could subsequently be released at lower temperatures.^[68]

Although the thermo-responsiveness in these examples is used as a means of enriching high affinity nanoparticles, these MIPs have found extensive use as reusable recognition material in sensors and diagnostic assays. This is due to an excellent recognition performance, combined with a cost effective and straightforward production. Small molecule targets like drugs of abuse and performance and image enhancing drugs are common targets for thermo-responsive

MIP nanoparticles. This is exemplified by the work of D'Aurelio et al., who developed such nanomaterials for the drug targets cocaine (CO) and morphine (MO) and incorporated them into electrochemical-based sensors (Figure 3). Low limits of detection (LOD 0.52 ng mL^{-1} for CO and 0.11 ng mL^{-1} for MO) and reusability distinguish these sensors over traditional single use immunoassays.^[69,70] Overall, the hydrogel nature and gentle conditions for producing them has enabled the successful imprinting of protein and biomolecule-based targets, leading to an increasing popularity of the solid-phase approach for producing nanoMIPs for protein targets.^[71] The quick adaptability of this approach to new protein targets is reflected in the rapid development of a SARS-CoV-2 nanoMIP and a corresponding sensor for the analysis of clinical samples in response to the COVID-19 pandemic.^[72]

Thermo-responsive MIP nanomaterials have also shown great promise as vehicles for targeted drug delivery.^[73,74] Most examples in the literature describe MIPs targeting surface proteins on cancerous cells. Embedding a drug into the MIP and utilising the thermo-responsiveness of the material, the drug can be released in response to temperature at the site of action. This is exemplified by the work of Yin et al., who created a sialic acid (SA)-imprinted mesoporous silica nanocarrier, which were loaded with the therapeutic drug doxorubicin (Figure 4).^[75] These imprinted mesoporous nanocarriers, were then tested against human hepatocellular carcinoma cells. Additionally, the work of Singla et al. used a double-imprinting technique to create cavities targeting the breast cancer cell receptor estrogen alpha (ER_α), and for the treatment drug (doxorubicin). This method allows for the drug molecule to be locked in place until needed, where it is released upon a thermal stimuli response.^[76] The forementioned examples are convincing demonstrations of new targeted drug delivery systems that have the potential for reducing the side-effects associated with current treatments.

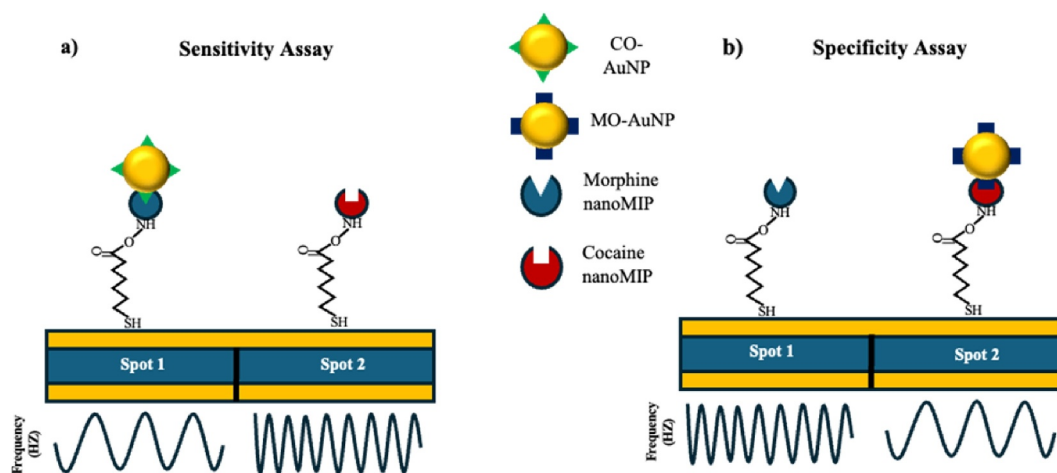


FIGURE 3 Schematic of sensitivity (a) and specificity (b) assays performed using multiplex nanoMIP QCM sensor.^[69,70] Reproduced under terms of the CC-BY license.^[69] 2021, D'Aurelio et al., published by MDPI.

3.2 | pH responsive MIPs

Polymers with Brønsted acidic or basic repeating groups (polyelectrolytes) contain ionizable groups with ionization states depending on the external pH.^[77] For loosely crosslinked gels, the polymer can respond to the concomitant increase in electrostatic repulsion by a conformational change, thereby translating a pH change into one or a combination of responses related to bulk properties for example, change in swelling, release of entrapped molecule, molecular recognition behaviour.^[78] Several examples of MIPs exhibiting these properties have been reported and we refer to a recent comprehensive review.^[18] A more recent example of a pH responsive drug delivery vehicle leveraged loosely crosslinked gels

featuring pH-degradable crosslinks.^[79] This could potentially be used for triggered release of entrapped cytostatic drugs at low pH tumorous microenvironments. For instance, Qin et al. used a zeolitic imidazolate (HmIm) metal organic framework (FZI-F8) loaded with doxorubicin (DOX) and polyvinylpyrrolidone coated carbon dots (CDs/PVP) as core and a degradable MIP shell as tumor sensitive biodegradable nanoparticles. The MIP shell was prepared from degradable monomers/crosslinkers, such as dimethylaminoethyl methacrylate, trifluoromethylacrylic acid (TFMA) and *N,N'*-diacryloylcystamine (BAC) as the functional monomers and crosslinker, respectively, with an epitope for a CD59 cell membrane glycoprotein target as template.^[80] In this work (Figure 5), hence, the MIP shell was designed to be degraded when exposed to Glutathione

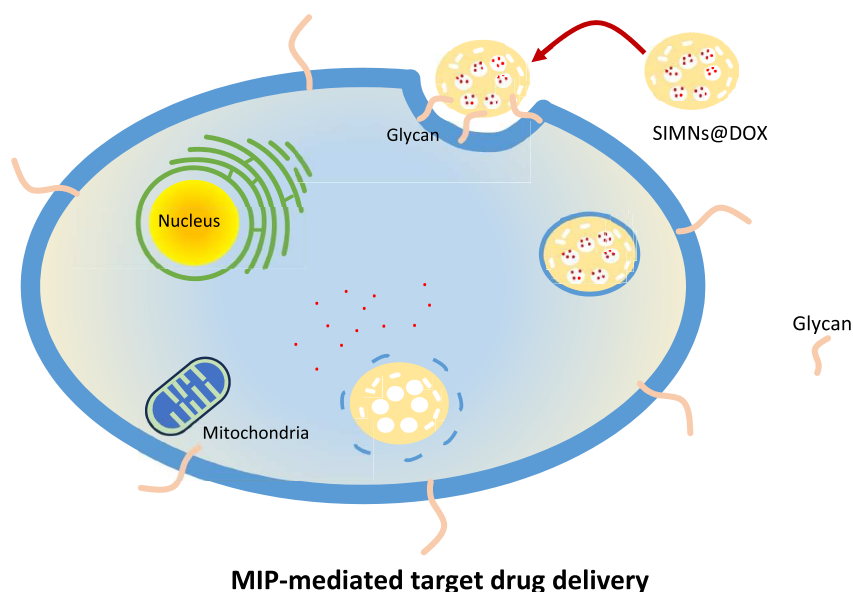
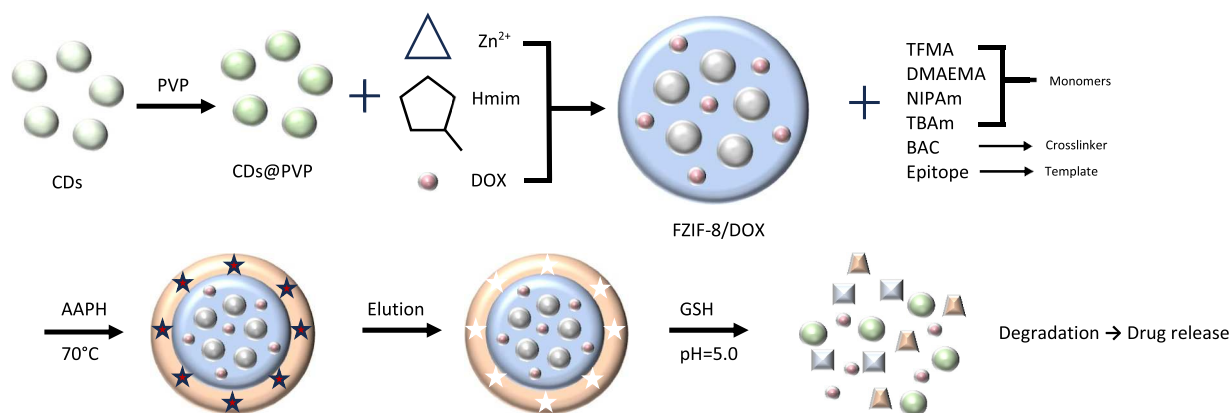


FIGURE 4 Thermo-responsive sialic acid (SA)-imprinted mesoporous silica nanocarrier for doxorubicin targeted drug delivery.^[75] Reproduced under terms of the CC-BY license.^[75] 2021, Yin et al., published by Elsevier.



and the lower pH of the tumour microenvironment thereby causing a pH triggered release of DOX at the tumour site.^[20,80] This release led to an inhibitory effect on the growth of tumors.^[80]

A more general pH responsive property of MIPs refers to template binding driven by electrostatic interactions, a consequence of the common use of Brønsted acidic/basic or hydrogen bonding functional monomers. This stimuli responsiveness is displayed by most MIPs, whereby acids, surfactants or organic solvents are used to elute template/targets from the MIP polymer matrix by breaking the non-covalent interactions between the template/target and the MIP.^[81] A common strategy used to ensure a high recovery of polymer-bound template has been to apply an acidic solvent extraction for template elution.^[82] Moreover, a combination of acids and anionic surfactants is commonly applied for the removal of protein-based templates from polyacrylamide-based hydrogels.^[83] This method not only breaks the non-covalent interactions between the protein template and MIP, but also denatures the protein molecule structure, making removal of the high molecular weight template easier. The resistance to such harsh treatments is an important feature that distinguishes MIPs from biopolymer derived affinity reagents.

Using pH responsive MIPs with changes in the pH environment affecting affinity has also been exploited within the field of biosensors. For instance, acidic buffers can be used to regenerate MIP-based SPR biosensors for reuse.^[53,84] MIP nanoparticles were covalently linked onto the surface of an SPR chip. Following rebinding performed in buffer at pH 7.4, a glycine at pH 2.0 wash is used to removal of the target molecules, allowing for reuse of the MIP immobilised SPR chip. This technique was explored further through the work of El-Sharif et al., who after, electropolymerizing a MIP film onto the gold surface of an SPR chip, used subsequent glycine at pH 2.0 washes to remove the template before the rebinding experiments, with the same glycine (at pH 2.0) wash used for MIP-SPR chip regeneration.^[35]

3.3 | Photoresponsive MIPs

Photo-responsive MIPs (PRMIPs) are synthetic recognition materials that under stimulus of a light of a specific wavelength, undergo chemical and/or physical changes in their structure. As such, photo-responsive materials have garnered

an interest in the scientific community, with photo-irradiation being seen as a 'clean' energy source that can be manipulated and directed relatively easily and if needed, remotely.

Traditionally, photo-responsive polymers are produced by simply incorporating light-sensitive monomers (chromophores) that respond to light in the desired fashion. Such monomers that can be considered for this use are: azobenzene,^[85,86] spirooxazine^[87,88] and spiropyran (Figure 6).^[89,90]

Depending on the chromophore incorporated there are numerous responses that can be imparted onto the material. For example, azobenzene is a widely studied chromophore due to its ability to undergo photoinduced isomerization switching from *cis* and *trans* conformations as a direct response to light stimuli.^[91,92] Other responses that can be observed from these 'smart' materials are shape memory effects (in which a temporary deformed material can recover to the original permanent shape).^[93,94]

In terms of the fabrication of PRMIPs, chromophores are included into the monomer mixture generally as the functional monomer. These materials can be fabricated through bulk^[95] or precipitation polymerisation,^[85] sol-gel methods,^[96] living polymerisation methods^[97] and surface modification methods.^[98] Due to the stipulation of their synthesis being only that a chromophore is added to the pre-polymerisation solution there is huge flexibility in the available methods for their production.

For example, Gong et al., produced a PRMIP for the detection of bisphenol A in water, utilizing an azo based chromophore. The target compound was then able to be selectively rebound or released from the PMIP via the use of either 365 nm or 440 nm light.^[85] In fact, azo based monomers are an excellent choice for the synthesis of PRMIPs as opposed to other photosensitive monomers as they generally can impart molecular recognition on the target itself. Other groups have also sought to utilise this photoinduced isomerisation characteristic of azo monomers such as Fang and colleagues who through living/controlled Atom Transfer Radical Polymerisation developed a dual responsive (Light and Temperature) MIP towards a target of 2,4-dichlorophenol in which tuneable rebinding properties can be seen.^[97]

When using azo based monomers, it is worth considering the molar ratio of cross-linker included into the polymerisation mixture. Cross-linkers, much like traditional MIPs are important in PRMIPs and can influence the properties of the

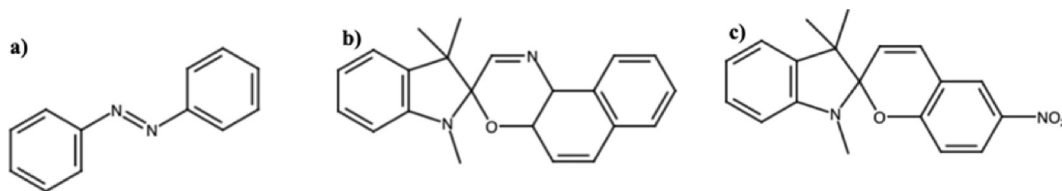


FIGURE 6 Chemical structures of potential light-sensitive monomers (chromophores) (a) Azobenzene, (b) Spirooxazine and (c) Spiropyran.

material, such as target selectivity and binding capacity. However, when considering the ability of azo compounds to switch between *cis* and *trans* conformations, a MIP that exhibits a structure that is confined and too rigid may be unsuitable for azo incorporation as photoisomerization may not be possible. This was observed by Gong and co-workers in their work developing PRMIP hydrogels.^[99]

Liu and co-workers developed a PRMIP sponge utilising spiropyran modified PVA, this is an intriguing example of a 'self-healing' or light-based regenerative material. The synthesised sponge that was capable of rebinding lead with distinct changes in the visual colour of the solution itself. This interesting response gives a rapid naked eye detection of Pb presence. Additionally, the sponge was able to be stripped and regenerated for at least 10 cycles, through a visual light water wash.^[100]

In addition to UV and visible light response, Liu and colleagues have developed a near infra-red (NIR) PRMIP for the controlled release of drugs modelled in porcine tissue. The synthesis of this material was achieved via the use of up-conversion nanoparticles (UCNPs) as core, with an azobenzene derivative as the functional monomer and paracetamol as the templated drug (Figure 7).^[98] This work is interesting as azobenzene derivatized monomers (MADPADS), which would normally require the use of UV light to induce their photoresponse, were crosslinked with triethanolamine trimethacrylate (TEAM) in the presence of paracetamol (APAP) template. This was achieved around a NaYF₄:Yb, Er@SiO₂-MPS (UCNPs) core and upon irradiating the UCNPs with NIR light (980 nm) caused the nanoparticles to emit green fluorescence (520–550 nm) thus inducing isomerisation in the azobenzene derivatives and as

such releasing paracetamol. This work provides an exciting advancement in the field and could have clinical significance as the patient would not be required to be exposed to a large dose of UV light to selectively release drugs around the area of interest.

Overall PRMIPs showcase an exciting future in the realm of responsive MIPs, with their ability to selectively rebind targets at the flick of switch making them an ideal candidate for selective removal of trace analytes or the controlled delivery of drugs in complex environments. Further work is however required in the field, especially in the field of azo derivatized monomers, as UV light is generally a requirement for photo-induced isomerization limiting their use in the clinical sector.^[85,86] With that in mind, due to light being considered a 'clean' energy source it is expected that further work in this realm will continue, and substantial advancements and developments will be made in this field.

3.4 | Biomolecule-responsive MIPs

MIPs are commonly referred to as synthetic replicas of natural receptors or antibodies (e.g. "plastic antibodies") due to their receptor like molecular recognition behaviour. However, this fails to consider secondary functions of biological receptors such as their allosteric behaviour or other recognition triggered effects. Thus, realistic replicas should also comprise such features. Biomolecule-sensitive MIPs were initially reported by Watanabe et al., whereby PolyNIPAm-based imprinted polymers were demonstrated to exhibit a temperature dependant change in volume,

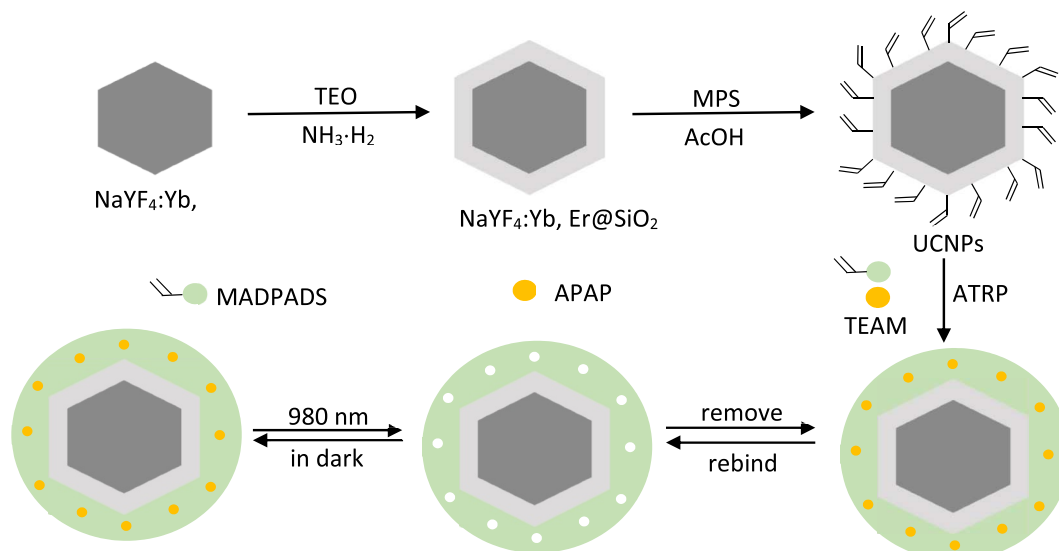


FIGURE 7 Schematic representation of NIR-light-responsive surface molecularly imprinted polymer (NSMIP) preparation.^[98] Reproduced under terms of the CC-BY license.^[98] 2020, Liu et al., published by Elsevier. MPS, methacryloxypropyltrimethoxysilane; TEO, tetraethoxysilane.

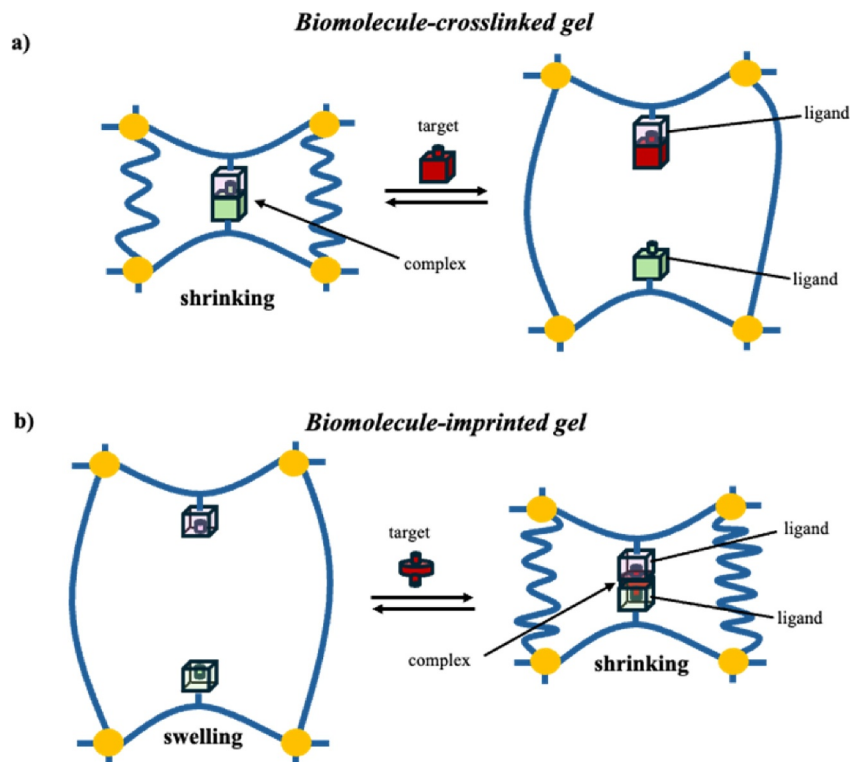


FIGURE 8 Schematic of swelling/shrinking behavior of biomolecule-responsive hydrogels (a) Biomolecule-crosslinked hydrogel (b) Biomolecule-imprinted hydrogel. Reproduced under terms of the CC-BY license.^[64] 1998, Watanabe, published by ACS.

affecting affinity, as a responsive change to variations in the concentration of a guest molecule.^[64] This study showed that the synthetic gels could undergo a large swelling change (conformational change), while still retaining the molecular recognition ability in the shrunken state. The gels exhibited a thermoresponsive swelling, consistent with conventional NIPAAm-based gels,^[101] and indicating the capability of conformational change depending on temperature. In addition to temperature, the swelling was also dependent on the concentration of template in the solution. This effect is schematically illustrated in Figure 8.^[64]

An important aspect to consider is the versatility of the polymerisation process that allows for the inclusion of signal markers, albeit electrochemical, fluorescent or colorimetric. This allows a stimulus response when the MIP binds to the target and forms the basis for the incorporation of MIPs in a variety of biosensors. Zhang et al., utilise this technique by using MIPs based on quantum dots-grated covalent organic frameworks (COFs) for the detection of quinoxaline-2-carboxylic acid (QCA). In this case the QCA target molecule quenches the fluorescent signal upon binding with the MIP, with the fluorescence quench due to the hydrogen bonding between the quantum dots-grated COF and the QCA target at the

imprinted binding sites.^[102] Additionally, Alanazi et al., included the electroactive monomer ferrocenyl-methylmethacrylate (FcMMA) into the polymer matrix of the MIP, which upon binding of the analyte presumably caused a polymer conformational change that in turn exposed the hindered FcMMA moieties contained within the polymer allowing the probe to signal the binding event.^[103]

Miyata et al., explored a unique concept by designing MIPs displaying a recognition dependent gel shrinkage behaviour in response to a tumour-specific glycoprotein biomarker.^[104] This concept was further explored using DNA-responsive MIP gels, with the shrinking caused by DNA duplex formation, here acting as reversible cross-linking points. Whilst not strictly biomolecule-responsive it is also worth including a note of creation of aptamer-MIP hybrids materials. These materials use an aptamer as a specific recognition element for chosen targets with the aptamer encapsulated within an imprinted hydrogel scaffold, the latter featuring thermal and pH responsive properties consistent with the nanomaterials produced by the solid-phase protocol (Figure 9). The combination of the aptamer with the molecularly imprinted gel produces a hybrid material that displays superior performance than its individual components. Due to the polymeric scaffold the aptamer is

locked in its optimal conformation, whilst also protecting it from degradation.^[105,106]

3.5 | Ion-responsive MIPs

Ion-responsive imprinted polymers are quickly becoming an area of interest within the molecular imprinting field, especially since anionic recognition drives a magnitude of process that are crucial for living cells. PolyNIPAm-based

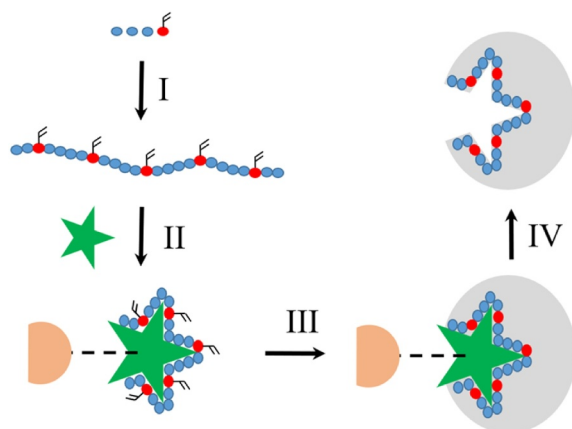


FIGURE 9 Schematic representation of the solid-phase synthesis of aptaMIP NPs. Red circle indicates the modified polymerizable base. Green Star representative of moxifloxacin template molecule. I: Synthesis of modified aptamer sequence. II: Complexation of aptamer with moxifloxacin target molecule attached to inert solid phase. III: Addition of polymer scaffold components, polymerisation and formation of polymer scaffold via TEMED initiated reaction; IV: Thermal (60°C) release of nanoparticle bearing aptamer sequence.^[105,106] Reproduced under terms of the CC-BY license.^[105] 2021, Sullivan et al., published by Wiley.

hydrogels have been proven to undergo volume changes in response to changes in ionic strength, thus leading to ion-responsive binding properties. Early incarnations of ion-responsive MIPs involve the molecular recognition microcapsules for ion-responsive controlled release. In the work of Chu et al., NIPAm based microcapsules containing Benzo-18-crown[6]-acrylamide crown ether receptors were produced for ion recognition.^[107] When specific cations such as Ba^{2+} are captured by the crown ether, the polymer swells closing its pores, while removal of the ions cause the polymer to shrink, thus opening the pores. These changes led to a Ba^{2+} triggered release of solutes contained within the prepared microcapsules.^[107]

More recently, highly oxanion-selective MIPs leveraging a hydrogen bonding based bioinspired design were reported.^[108,111–112] Recognition of isosteric oxanions (e.g. sulfate vs. phosphate) has so far posed a significant challenge in host-guest chemistry, and particularly the design of hosts featuring a switchable ion preference, for example, phosphate versus sulfate.^[110,112] Prototypes for these receptors are the sulfate and phosphate binding proteins which recognize their guests predominantly through multiple complementary hydrogen bonding (H-bond) interactions in a water-poor microenvironment.^[112] Notable, no or only few charged residues are involved in the oxanion binding site of the protein. Inspired by these receptors, Shinde et al. investigated whether molecular imprinting and charge neutral ureas such as FM1 as host monomers could offer a solution (Figure 10).^[110] MIPs were prepared using phenylphosphonic acid (PPA) as an organic soluble dianion as template in presence of FM1 in a two-fold molar excess. Due to strong monomer-template interactions this led to the formation of charge-neutral cleft like receptors featuring hydrogen bond donors in an optimal geometry for

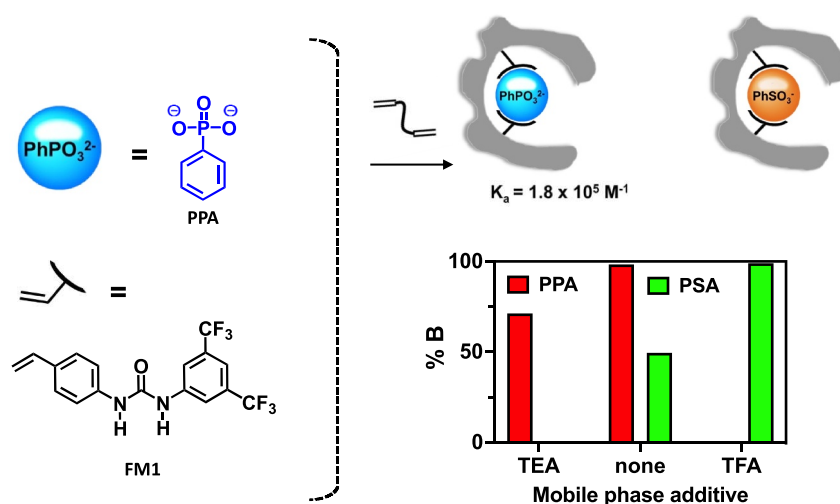


FIGURE 10 Schematic representation of an oxanion selective molecularly imprinted polymer (MIP) with phospho-sulfo switchable ion preference responsive to acidic or basic modifiers. 1,3-Diaryl Urea Monomer (FM1) was used as functional monomer and phenylphosphonic acid (PPA) as template. Ion selectivity was tested chromatographically on a MIP column by injecting PPA and phenylsulfonic acid (PSA) in an acetonitrile based mobile phase with triethylamine (TEA) and trifluoroacetic acid (TFA) as modifiers.^[110] Adapted under terms of the CC-BY license.^[110] 2022, from Shinde et al., published by ACS.

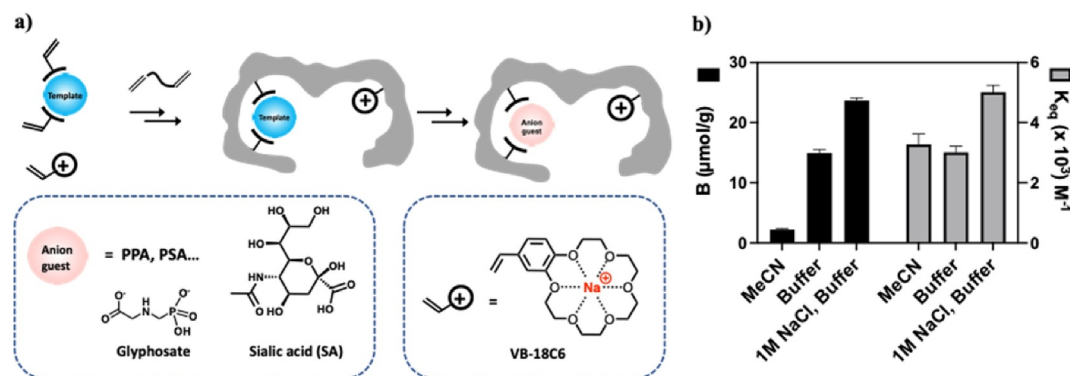


FIGURE 11 (a) Schematic representation of dual ion imprinting for the recognition of oxoanions in high salt media. The approach is based on a combination of cationic (VB-18C6) and urea (FM1) binding groups for simultaneous accommodation of the counteranion and oxoanion, here exemplified by glyfosate, sialic acid (SA), phenylphosphonic acid (PPA) and PSA (b) Binding and affinity of PPA on a dual ion imprinted polymer in response to high ionic strength.^[108,109] Adapted under terms of the CC-BY license.^[109] 2022, from Shinde et al., published by ACS.

complexation of isosteric oxyanions. The MIPs exhibited strong ion affinity ($K_{eq} = 4.5 \times 10^3 \text{ M}^{-1}$ for sulfate) in buffered media for both phosphate and the strongly hydrated sulfate anion. Interestingly, the oxyanion preference under anhydrous conditions was sensitive to the presence of basic or acidic modifiers in the test solution leading to a switchable phosphonate/sulfonate binding behavior. This performance challenges the best performing low molecular hosts and has a practical relevance in view of the strong need for robust anion binders.^[110]

The design of effective ion receptors is complicated by competition from their counterions a situation commonly leading to suppressed binding in high salt media. An approach to avoid this screening effect is to design dual ion receptors capable of accommodating both ions simultaneously.^[108,109] Imprinting offers in this context a highly versatile concept to design such receptors by combining anion and cation host monomers. This was demonstrated by imprinting PPA as its disodium salt in combination with FM1 and sodium ion selective 18-crown-6 monomers. The polymers displayed enhanced affinity for the template or inorganic phosphate or sulfate in competitive aqueous buffers, with affinity and selectivity increasing with increasing ionic strength (Figure 11).^[109] Hence, the presence of engineered sites for both ionic species dramatically increases the salt uptake in strongly competitive media such as brine.

By carefully tuning the composition of such dual ion receptors in terms of the combination of crosslinkers, positively charged and neutral hydrogen bonding monomers such MIPs can be further optimized for use in either organic or aqueous environments.^[108,109] This approach was subsequently used for imprinting more complex anions such as the pesticide glyfosate and the bioactive monosaccharide SA. In the latter case, aberrant SA expression is one of the key cancer indicators and hence, the development of specific capture tools for sialylated targets is an important step towards early cancer diagnosis.^[111] Based on the dual-ion imprinting approach, SA MIPs were developed capable of simultaneous complexation of SA's carboxylate group and

its counter-cation. The MIPs displayed SA recognition enhanced by the addition of the crown ether host. A striking demonstration of this effect was the dependency of binding on the SA counterion. In agreement with the 18C6 cation selectivity, SA binding was strongly enhanced for the K^+ , Na^+ and NH_4^+ salts of SA while binding of bulky lipophilic salts was suppressed, all in line with the documented affinity of these ions for the macrocycle (Figure 12).

Additional examples of ion-responsive MIPs are displayed in the work of Zhai et al., whereby MIP nanoparticles with synthetic binding pockets for Human apurinic/apyr-imidinic endonuclease/redox effector factor 1 (APE1) were produced in the form of polydopamine MIP shell coated magnetic nanoparticles.^[113] The nanoparticles displayed antibody-like binding and affinity towards the target with high specificity, displaying an efficient inhibition effect.^[113] The recognition and inhibition could be flexibly tuned by the addition of metal ions such as Mn^{2+} and Mg^{2+} in analogy with cofactor modulated enzyme action.

3.6 | Magnetic responsive MIPs

The stimuli responsive materials discussed above, fundamentally rely on stimuli responses that are derived from the polymeric network of the MIPs, like the swelling and shrinking of the polymer matrix or distortion of imprinted sites under stimulus. This ultimately leads to either an enhancement or reduction in binding affinity between the MIP and target. An additional response can be caused by the combined effect of composite materials interacting with MIPs. A common approach is to incorporate a magnetic (Fe_3O_4) nanoparticle with a MIP thus allowing the combined materials to exhibit a response under the stimulus of a magnetic field.^[114,115]

Magnetic MIPs have found use in a variety of applications, with a predominate area being the capture and removal of specific targets. Core-shell magnetic MIPs of approximate 45 nm were prepared using 4-vinylpyridine

as the functional monomer and trimethylolpropane trimethacrylate as the crosslinker, with a range of steroid and selective androgen receptor modulators as templates and targets. In this work the magnetic MIP nanoparticles, showed good selectivity and were capable of efficient binding and removal (under magnetic stimulus) of the target molecules from a complex matrix of river water. Additionally, Zhao et al., produced core-shell magnetic MIPs of the surface of magnetic carbon nanotubes for the detection of the antibiotic sulfamethoxazole, demonstrating a high imprinting effect, with fast adsorption kinetics and high adsorption capacity.

These materials served the purpose of using a magnetic stimulus to rapidly extracting sulfamethoxazole from milk and honey samples.^[116,117] This methodology is not limited to low molecular weight targets, with Liu et al., showing the successful development of magnetic MIP nanoparticles for the specific recognition of the protein target bovine haemoglobin (BHb). The resulting magnetic MIPs showed pronounced selectivity for BHb with recognition persisting in calf blood samples, thus demonstrating the practicality of the magnetic MIPs.^[118] Another potential exciting use of magnetic MIPs is as drug carriers. Ali et al., produced a biodegradable magnetic MIP as an anticancer drug carrier for the targeted delivery of Docetaxel. In this work (Figure 13), a magnetic MIP is created using a glucose-based biodegradable crosslinking agent, that degrades and releases the

anticancer drug (Docetaxel) at the active site.^[119] The magnetic properties of the MIP in this case are used to quickly concentrate the drug carrier at the site of the target. Through the application of an external magnetic field, this suggests a way to deliver drugs at target sites without affecting healthy cells.

3.7 | Electro-responsive MIPs

Electrochemical signal readouts are one of the preferred sensing strategies using MIPs^[120] as a biorecognition element for their high accuracy, specificity and sensitivity and fast time-to-result directly from complex samples.^[71,121] Adequate immobilisation is key to preserve the bioreceptor activity.^[122] Strategies include simple physisorption, biotinylated MIPs able to bind neutravidin-functionalised surfaces,^[123] electropolymerisation^[124] or covalent attachment, the latter yielding more stable and reproducible sensors.^[71] MIPs do not show any intrinsic redox activity^[3] and most templates do not exhibit electroactivity. The versatility in MIP synthesis implies the possibility of adjusting their monomer composition to integrate electroactive labelling and reach lower limits of detection.^[71]

The electrodeposition approach allows for enhanced control over deposit thickness,^[125] ion permeability, density, and porosity.^[124] Pores formed to bind the target analyte support fast kinetics and have demonstrated sensitivity, selectivity and stability as sensing layers.^[125] Signal enhancement strategies for improved electrical conductivity and higher surface area include conducting polymers but also graphene or carbon nanotubes.^[71,125] Conducting polymers such as polyaniline, polythiophene and polypyrrole are employed for their capacitance as well as ability to transfer charges from biological entities.^[124] Polyaniline fostered reproducible detection of perfluorooctanoic acid with a LOD at 1.08 ppb.^[126] Polyaniline was electropolymerized with poly-o-phenylenediamine for the detection of ciprofloxacin and quantifiable between 1 and 500 nM in tap and pond waters.^[127] The polymerisation of phytic acid-doped polyaniline enabled a label-free detection of SARS-CoV-2 antibodies down to 8 nM.^[128] Recently, the SARS-CoV-2 spike glycoprotein imprinted in polypyrrole was measurable between 0 and 25 µg/mL.^[129] This strategy was applied on a

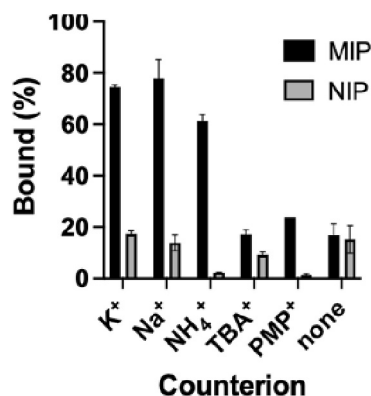


FIGURE 12 Influence of sialic acid (SA) counterions on the uptake of SA by a dual ion SA-imprinted polymer according to Figure 11.^[112] Reproduced under terms of the CC-BY license.^[111] 2021, Mavliutova, published by ACS.

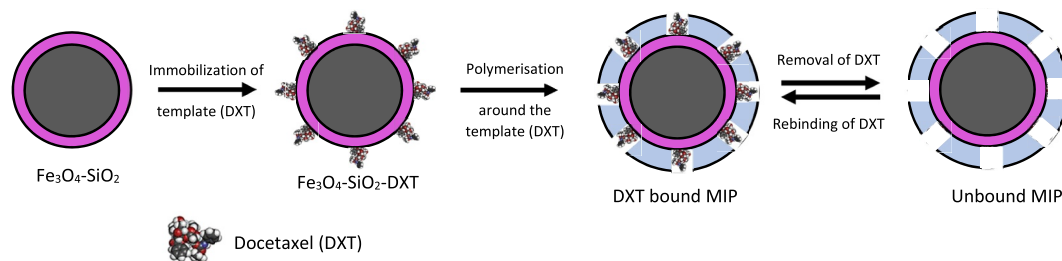


FIGURE 13 A schematical representation of a biodegradable magnetic molecularly imprinted polymer (MIP) as an anticancer drug carrier for the targeted delivery of docetaxel.^[119] Reproduced under terms of the CC-BY license.^[119] 2022, Ali, published by ACS.

microorganism scale with polypyrrole electropolymerized for the detection of *Listeria monocytogenes* at a limit of 70 CFU/mL.^[130] Electropolymerisation of poly(3,4-ethylenedioxythiophene) (PEDOT) yielded detection of melatonin between 0 and 100 μM , with limits of 0.171 μM .^[131] Electrosynthesis of a metallo-porphyrin-based MIP facilitated the recognition of the dipeptide carnosine between 0.1 and 1 mM, with a 55 μM LOD and performing in cell lysate.^[132]

Addition of polymerizable ferrocenes to the MIP pre-polymerization mixture have thus been demonstrated to strongly enhance detection sensitivity in potentiometric electroanalysis.^[133,134] Ferrocene methylmethacrylate supported the electrochemical detection of amphetamine^[135] and MDMA^[136] with respective LODs of 0.3 and 1.6 nM. The same ferrocenyl moiety allowed the selective recognition of glyphosate with a 3.7 pM LoD and perfluoroalkyl down to 0.84 ng mL⁻¹.^[137,138]

Adding a redox mediator directly into the MIP assembly has been undertaken more recently. A multi-layered sensor, involving polypyrrole and hexacyanoferrate above reduced graphene oxide functionalized with β -cyclodextrin, was able to quantify cortisol at a limit of 19.3 pM.^[139] Lee et al., electropolymerized β -cyclodextrin along with methylene blue and detected as low as 3.93×10^{-13} M of cortisol.^[140] Stephen et al. (2023) reported electropolymerized MIPs targeting bovine haemoglobin and bovine serum albumin (BSA) at minima of 50 pM. LoD.^[141] Methylene blue has also been utilised as the main monomer electropolymerized exhibiting a 29 nM limit of detection for melatonin.^[142]

Multi-analyte detection schemes are gradually emerging. Poly-o-phenylenediamine (PoPD) imprinted with creatinine and polymethylene blue on one electrode while another had albumin as template and ferrocene as redox mediator allowed to detect, in a single measurement, creatinine and albumin between 5.0 and 100 ng mL⁻¹.^[143] Another multiplexed scheme was published by Mugo et al., to detect epinephrine, lactate and cortisol in sweat with limits of detection respectively at 0.6 nM, 2.2 mM and 0.025 μM for each of the analyte stated previously.^[144] The dual detection of malathion and carbendazim was made possible, within linear ranges between 0.02–55 and 0.02–45 μM for each target respectively.^[145]

3.8 | Dual and multi-responsive MIPs

Dual/multi-responsive MIPs are systems that are produced to react to two or more external stimuli and even though these are currently less explored than single-responsive systems, dual/multi-responsive MIPs can offer increased versatility enabling the manipulation of material properties in multiple ways.^[146] Common examples of dual/multi-responsive MIPs often include

combinations such as magnetic/photo, magnetic/thermo, thermo/pH, thermo/photo and thermo/salt responsive, which are typical produced by replacing traditional functional monomers with those that respond to specific stimuli.^[147–150] Hua et al. developed a dual-responsive MIP with specific recognition for BSA, that combined thermo-responsive and salt-responsiveness.^[151] The hydrogel was formed by self-assembling the functional monomer N-[3-(dimethylamino)propyl]methacrylamide (DAPMA) with BSA and polymerizing in the presence of NIPAAm. This dual-responsive hydrogel demonstrated a clear memory of the template protein and could respond to temperature changes and variations in ionic strength.^[151] Salt ions played a key role in modulating the electrostatic interactions between the protein and the polymer chains, with increased salt concentrations reducing these interactions. The hydrogel's dual-responsiveness made it promising for applications like solid electrolyte membranes, electrode devices, protein delivery systems, and controlled-release sensors.^[146]

4 | CONCLUSIONS

The development of stimuli-responsive MIPs, has successfully gifted MIPs the capability of responding to external stimulus, mimicking natural antibodies further, while unlocking new opportunities for practical applications. With this, temperature, ion, biomolecule and pH-responsive MIPs have shown significant promise particularly with the production of nanoparticles using the solid-phase^[51] or magnetic template carrier^[52] protocols. These particles show excellent affinity, comparable to that of antibodies, and the stimuli-responsiveness allows for regeneration of the MIP, which is especially useful in biosensing and diagnostic applications. Moreover, these materials offer an exciting new approach to selective drug delivery. Other possibilities rely on the incorporation of additional functionality, such as photochemical, magnetic, or electrical, which allow the MIPs to respond to external stimulus. For example, the inclusion of a magnetic core within a MIP nanoparticle allows for an easy separation of the target molecule from a sample, whereas the inclusion of colorimetric or fluorescent tags within the polymer matrix allows for easy target detection.

The choice of monomers, crosslinkers and solvents during MIP preparation has a great impact on the stimuli-responsiveness, along with their performance. The functional monomers and template molecules should, when mixed in solution, form monomer-template complexes that persist throughout the polymerization step. Particularly rewarding is here to gain inspiration from the area of host-guest chemistry by engineering of designed host monomers for near stoichiometric imprinting of the template. More advanced combination of such host monomers allows the templating of binding sites for salts leading to dual ion

imprinted polymers where template binding can be ionically controlled and enhanced by increasing ionic strength of the medium. While many solvents used in stimuli-responsive MIPs, are organic solvents, these are mainly suitable for low-molecular weight templates that are stable in these solvents and hence, they are unsuitable for denaturation-susceptible biomacromolecules (proteins, enzymes, DNA), where instead low-reticulated hydrogel-based MIPs are preferred that can swell and shrink in response to external stimulus.

While stimuli-responsive MIPs represent a promising class of materials with the potential to positively impact areas such as biosensing, diagnostics, and drug delivery, significant challenges remain that must be addressed before their widespread adoption, especially with regards to in vivo applications. Research should focus on improving the biocompatibility, biodegradability, and stability of MIPs, with more work particularly needed to address biodegradation as most of the currently used scaffolds are not biodegradable. Production methods meanwhile need to be scalable and commercially viable. Moreover, deeper exploration into the safety and toxicity profiles in order to ensure that the polymers are safe towards healthy cells and tissues and can be cleared and eliminated after disease interventions or treatments, will be crucial for advancing into clinical use.

Despite these hurdles, the recent innovative approaches to SR-MIPs combined with the MIP's intrinsic ability to mimic antibodies in a customizable, tuneable way ensures that research in this field will continue to thrive.

AUTHOR CONTRIBUTIONS

Mark Sullivan: Conceptualization; writing—original draft preparation. **Perrine Lasserre:** Writing—original draft preparation. **Chester Blackburn:** Writing—original draft preparation. **Nicholas Turner:** Writing—original draft preparation; supervision; funding acquisition. **Börje Sellergren:** Conceptualization; writing—original draft preparation; supervision; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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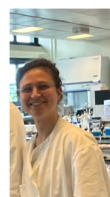
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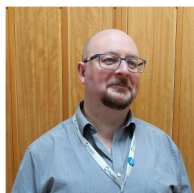
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