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REVIEW ARTICLE

Customizable Molecular Recognition: Advancements in Design, Synthesis, and Application of Molecularly Imprinted Polymers

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Molecularly imprinted polymers (MIPs) are where the complexity of receptor proteins meets the tunability of synthetic research. Receptor proteins, such as enzymes or antibodies, have functional cavities that act as docking platforms by recognizing and binding to complementary ligands. Once bound, a receptor-ligand complex may generate any multitude of cellular responses, including the regulation, uptake, and/or release of certain hormones, neurotransmitters, inorganic minerals, antigens, enzymes, and other molecules within an organism. Just like receptor proteins, MIPs are polymers with carefully selected functional groups that are spacially arranged to recognize target molecules. MIPs are generated by templating a functionalized polymer with a molecule, leaving a cavity that is complementary to the molecule upon removal. That cavity then has an affinity for the molecule that was templeted for later rebinding. The aim of MIP research is to recognize a desired target molecule with the precision of receptor proteins, and to maintain specificity and sensitivity towards the target molecule while tailoring functional properties for advanced applications. Resarchers are far from perfecting the delicate intracasy of mimicking such elegant biological processes, and improvements in all areas of MIP synthesis remains a vibrant and active topic. Various methods explored to synthesize MIPs with impressive recognition capabilities towards target molecules and the recent applications of MIPs are found herein. This review aims to dissect the synthetic steps required to generate MIPs, with emphasis on the more recent routes utilized and overall application advances.

Introduction

Creating polymers that harness reliable molecular recognition capabilities remains an incredibly vibrant research area for a variety of advanced applications. Specifically, the fundamental chemistry behind molecular recognition is being further refined for its use in biomedical technology,[1,2] biosensor diagnostics,[3,4] and biomarker testing.[5,6] Molecularly imprinted polymers (MIPs) provide a process for molecular recognition that closely resembles that of naturally occurring binding sites found on receptor proteins.

MIPs act as synthetic polymer receptor, where the polymerization step partially engulfs a desired molecule. Upon removal of the chosen molecule, the remaining polymer matrix is left with the imprint of the molecule within a pore-like structure. The imprint is then specifically selective for a rebinding moment, where reintroduction of the molecule often leads to an observable degree of rebinding. Mimicry of binding site interactions found in naturally occurring binding site interactions have allowed for favorable synthetic alterations, resulting in customizable MIPs with adequate recognition capabilities.[7–9] Based heavily on the complimentary sterics and functionality between such binding sites, current MIP research has focused on the highly selective, specific, and rapid recognition capability of a desired target molecule.[10,11] Although molecular imprinting has been around since the late 70s to early 80s,[12,13] the library of target molecules utilized for MIPs expanded more notably in the early 90s.[14] As this research has progressed, target molecules have included more complex structures such as proteins[15,16] in addition to small molecules.

As the implementation of this chemistry for complex molecular detection increases, so do the number of areas requiring elaborate improvement. The synthesis of MIPs with sophisticated recognition capabilities (primarily affinity and sensitivity for target molecules) remains a difficult challenge. Additionally, with society aspiring toward a more renewable future, the need for materials that are reusable and recyclable is dire in all areas of synthetic research. MIPs have the potential to address this issue by retaining function after multiple rebinding cycles, making them reusable in certain applications, and new research has been observing more “green” approaches in solvent selection and overall preparation.

This review is a guide to understanding the primary processes utilized while synthesizing MIPs, focusing on the most current routes available in the “toolbox” of MIP synthesis. Overall, this review serves to provide information on (I) the chemical approach to synthesizing MIPs, as detailed in current literature, with a final glance at (II) the recent application advancements of MIP chemistry.

I. General design and synthesis framework

Considering the few elements required to generate MIPs, preparation is generally swift and therefore cost- and time-effective.

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Synthesis includes the combinatory use of a template molecule, crosslinking agent, functionalized monomer, occasional co-monomer, polymerization initiator and a suitable solvent.

Selection of the template molecule is based on the desired application for MIP use. The template molecule is often the molecule of interest within the specific application. Sometimes, the desired molecule is too large, and smaller portions of the molecule will be utilized instead. The greater majority of target molecules utilized in standard MIP synthesis are small molecules that are relatively easy to handle, where larger and more complex compounds tend to lack in selective templating. Usually, this is due to issues in solubility during imprinting, along with the size of the template compound being difficult to rebind within a narrow MIP pore.[17] As will be discussed later in this review, different types of imprinting have provided avenues to examining larger molecules. Where appropriate, the selection of a template that closely resembles that of a desired molecule for recognition (i.e. dummy template molecule) is also an option.[11,18] The dummy template molecule is a great choice if the desired detectable molecule is unstable under certain reaction conditions or is overall dangerous to handle.

As an example to this concept, *He* and coworkers recently reviewed MIPs that may detect and separate the highly toxic and expensive metabolite, aflatoxin (AF).[19] The detection of AF is highly desirable in the food industry, where AF contamination on crops remains a complex issue. Due to the hazardous nature of handling AF, structural analogues have been reported in the literature to bypass such preparation. Common dummy molecules to AF include 5,7-dimethoxycoumarin, quercetin, 7-acetoxy-4-methylcoumarin, and 6-phenyl-4-methyl-2-chromanone.[20–23]

Assembly of functional monomer around the template molecule forms an ideal bonding pattern along the template molecule (Figure 1a). Added crosslinker then allows for polymerization to selectively surround the template-monomer complex, forming the MIP (Figure 1b).

After polymerization, the template molecule is removed, exposing functional cavities located within the MIPs (Figure 1c). The shape, size, and overall orientation of functional cavities are complementary to that of the absent template molecule, effectively leaving a physical imprint of the template molecule within the polymer.[16,24] The functional cavities, or recognition sites, are therefore sterically and chemically arranged to facilitate specific rebinding of a target molecule (Figure 1d).

Additionally, generated MIPs are often co-created with non-imprinted polymer (NIP). The NIP is synthesized exactly like MIP but lacks the imprinted cavities that are formed by the inclusion of a template molecule. Without the inclusion of NIP, there is no reasonable way to consider the efficiency of the imprinted cavities. A comparison of binding efficiency between the MIP and NIP is regarded as the imprinting factor. Imprinting factor is a way to measure the properties of the imprinted portion of the MIP, independent of the polymer backbone. Due to the many ways that imprinted cavity rebinding with template can be observed, there is no single way to present imprinting factor data. However, the comparison is typically given as a ratiometric comparison of MIP data versus the complementary NIP. Observation of a higher imprinting factor (usually a value that is >1) correlate to MIP having greater selectivity for template molecules than NIP.[25]

i. Molecular Design

The decided use of MIPs ultimately determines the choice of the template molecule. MIPs have shown successful rebinding and recognition capability to an array of targets, including antibiotics,[26,27] bacteria,[28] herbicides,[29] pesticides,[30] pollutants,[31] proteins,[32] and even viruses.[33,34] MIPs have been extensively researched for use as drug delivery systems for this exact reason, and many notable reviews are available within current literature.[35–37] Due to the breadth of applications for MIPs, functional monomer(s) and crosslinker(s) for use in MIP preparation are chosen with properties (charge, size, chemical identity) of the template in mind. Commonly used functional monomers and crosslinkers utilized in MIP synthesis can be found in Figure 2 and Figure 3, respectively.[38–54]

Choice of solvent also plays a significant role in the overall success of MIPs synthesis. Chosen solvent must dissolve all the necessary reactants, while not interfering with the template-monomer complex. Additionally, a solvent that aids in a porous structure (porogen) is especially helpful in MIP synthesis. Solvents that act as porogens are preferred, as the polymeric material generated will be porous and therefore offer greater recognition properties. This is because the recognition site of the MIPs will be more accessible than a material that is nonporous. Not only are porogens important due to the physical attributes they impose on the resultant MIPs, but it is also imperative to the stability of the pre-polymerization step. Aside from the ability to dissolve all the necessary components, porogen selection must also negligibly interact with chosen monomers, functional groups, crosslinkers, and template molecules. The most popular solvents selected for MIPs synthesis include toluene, acetonitrile, dichloromethane, 1,4-dioxane, acetone, tetrahydrofuran, and chloroform. It is typical to choose non- to moderately polar aprotic solvents, as these solvent choices decrease hydrogen bonding interactions that are associated with common MIP bonding types. Efforts to adapt MIP synthesis towards sustainable solvents have been observed in more recent years with green reagents such as room temperature ionic liquids and deep eutectic solvents.[55] Additionally, some techniques presented within this review aim to adjust the solubility of MIP matrices so that solvents such as water may be more easily employed.

Template-monomer complex

The mechanism of template-monomer complex assembly relies on the type of bonding between the two entities. Covalent, semi-covalent, and non-covalent bonding strategies have been used to integrate the templating molecule into the polymeric MIP. This area of research has been reviewed extensively[56,57] in the available literature, but will be briefly summarized here.

Covalent reactions

Covalent bonding between template molecules and their functional monomers offers an effective approach towards imprinting MIPs with homogenous recognition sites.[56,58]A functional monomer is first chemically bound to the template by a labile covalent bond, followed by copolymerization with a crosslinker in the selected porogen. Removal of the template then leaves the recognition sites within the moiety available for a rebinding moment.[58]

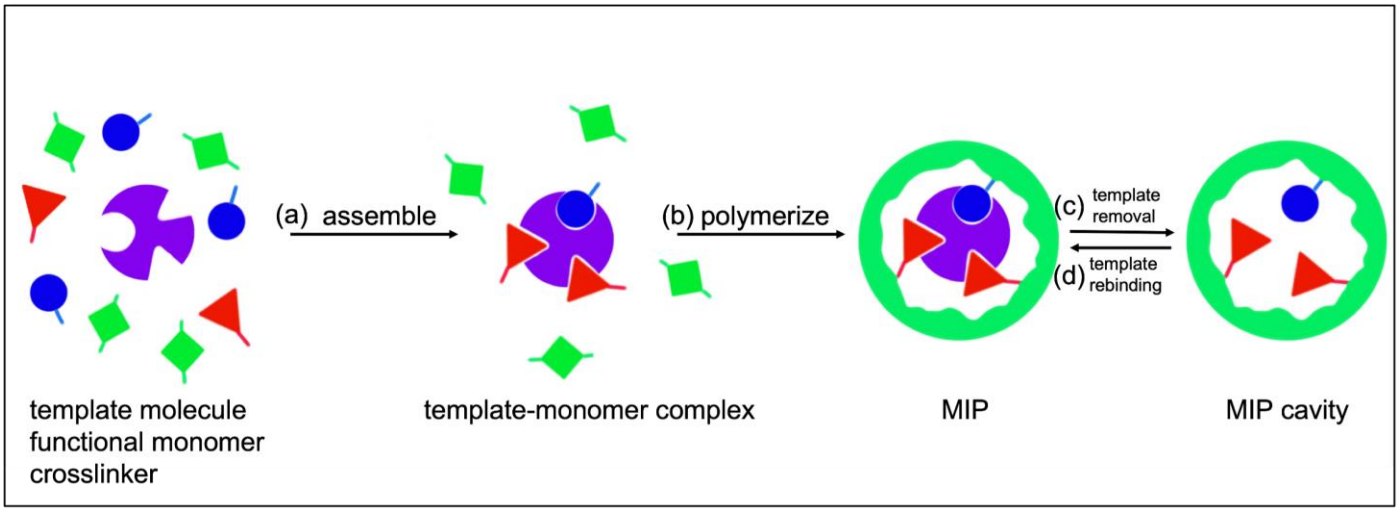

Figure 1. A depiction of the steps required for MIP synthesis. (a) Assembly of free functional monomer(s) and crosslinker around the template molecule form a complex with preferential bonding patterns. (b) Polymerization occurs around the template molecule, which remains within the generated polymeric cavity. (c) The template molecule is removed from the MIP, exposing the functionalities within the cavity. (d) Rebinding of the template molecule into the MIP to observe selectivity and sensitivity of the MIP.

The Wulff group developed the covalent imprinting approach where template molecules and functional monomers are joined via reversible covalent bonds prior to polymerization.[59] Historically these bonds are through boronate esters, ketal and acetals, and Schiff bases. Removal of the target analyte, once bound, requires cleavage of the bonds through chemical intervention. The resulting binding pocket is well-defined in that it is complementary to the sterics and functional arrangement of the target analyte. As the analyte rebinds to the imprinted cavity, the covalent bonds are then reformed during the rebinding process.[60]

Although this approach is straightforward, a limited number of examples utilizing covalent bonding for MIP formation are available in current literature. This is likely because multiple heteroatoms within the template molecule are required for this approach to be effective for imprinting, which is not always possible depending since the chosen template molecule is almost always application-specific.[57] Additionally, separation and subsequent removal of the target molecule from the polymer is more difficult when using this method given the nature of covalent bond strength.[56]

Non-covalent interactions

Non-covalent interactions have been the most popular imprinting technique utilized thus far among research of MIPs. Non-covalent bonding for MIP synthesis allows for a greater variety of potential applications due to reduced restriction in template molecule selection. Many variations of non-covalent interactions are observed in the literature for MIP formation, with most relying on hydrogen bonding,[61–65] pi-pi interactions,[66] or electrostatic interactions.[67–69]

The resulting molecularly imprinted cavities formed in the pre-polymerization step are decorated with non-covalent functionalities that are available for rebinding with a desired molecule or its analogues after polymerization and template removal. However, due to the nature of non-covalent interactions, MIPs prepared in this manner are not highly selective.[56] Uniformity within the formed cavities is difficult to control as it relies on the complicated ratio between monomer and template molecules. The equilibrium between the two must first be disturbed by adding supplementary monomer to generate template-monomer complexes. Increasing the functional monomer concentration causes an excess of functional groups to be present that do not form a complex with the template molecule. This leaves free-functional groups stationed on the MIP after polymerization, hindering specific recognition as they also have the ability to interact with target compounds.[56,57] Overall, noncovalent interactions within MIPs will decrease the selectivity and specificity but will increase the sensitivity of the cavity.

Semi-covalent reactions

Covalent imprinting significantly reduces the amount of non-specific recognition sites but lacks the breadth of viable template-monomer complexes.[70] Comparatively, non-covalent imprinting is a simple technique with a wide range of available template molecules yet lacks in specific binding due to the need for excess monomer. Combining the benefits of both covalent and non-covalent interactions, semi-covalent (dynamic covalent) bonding for the formation of template-monomer complexes is an effective approach. The first binding interaction occurs by reversible covalent bonding measures. These reversible bonds formed during template binding still allow tailored control of homogenous binding cavities. Generally, removal of the template molecule then occurs through hydrolysis, freeing the reversible covalent bonds and exposing the imprinted cavity.[71] Additionally, due to the use of covalent bonding in the pre-polymerization step, a random distribution of excess functionalized monomer is not present as in the non-covalent approach because the issues with template-monomer equilibrium are non-existent. After polymerization, functional groups imparting non-covalent interactions that decorate the cavity are utilized during the rebinding process.[70] This method offers a practical approach towards MIPs that contain greater specificity within the recognition sites without placing restrictions on template molecule structure.[57]

While semi-covalent imprinting may seem like the obvious approach to MIPs generation, there are certain drawbacks to consider. Mainly, rebinding can be difficult to achieve due to steric hindrance, and template removal by hydrolysis is not always favorable or achievable.[57]

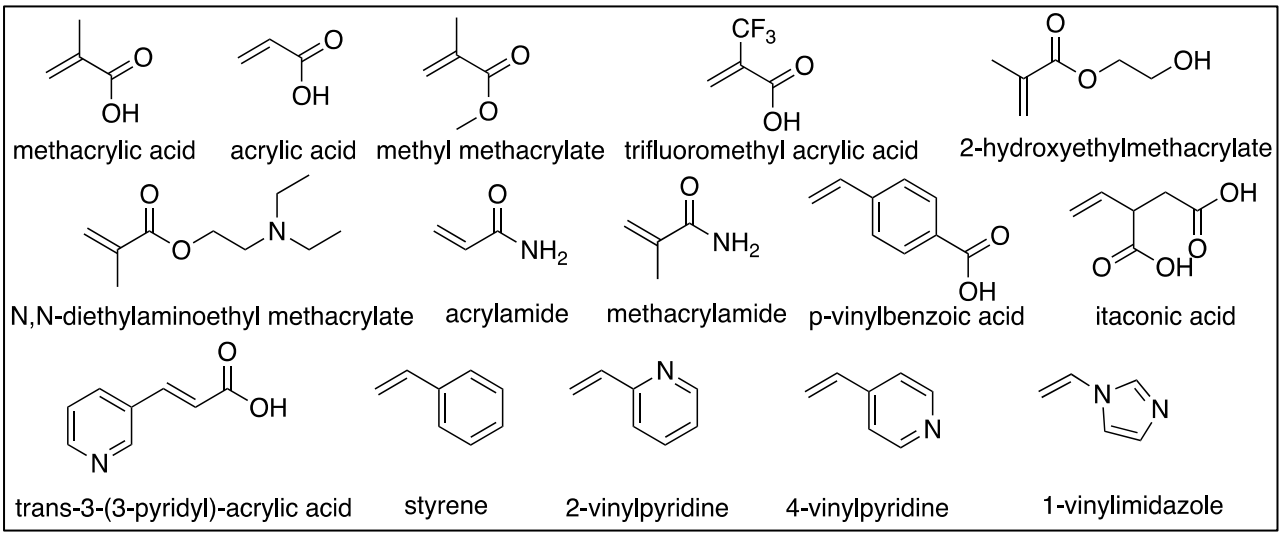

Figure 2. Common functional monomers utilized in the synthesis of MIPs.

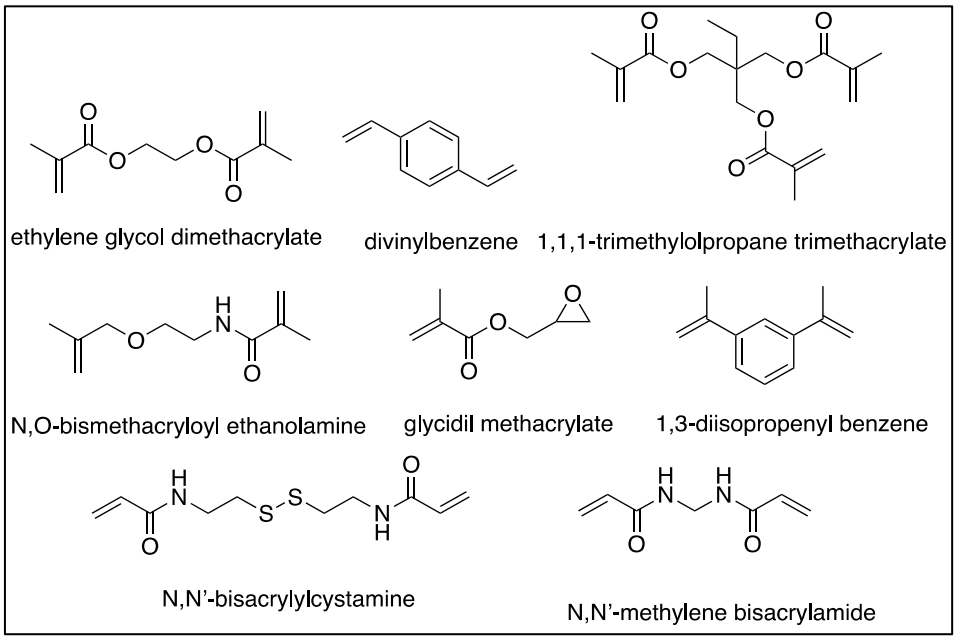

Figure 3. Common crosslinkers utilized in the synthesis of MIPs.

ii. Synthetic Techniques

Though MIPs have been prepared by several different polymerization pathways, given the current direction of MIP research, this review will focus on free radical and controlled radical polymerization techniques.

Traditional free radical polymerization

Free radical polymerization (FRP) has historically dominated the field of MIP preparation. This is because the FRP mechanism tolerates many reaction conditions and is compatible with a wide range of monomers. Additionally, FRP is a relatively straightforward process and can be conducted either neat or in solution. The method proceeds through three main steps; (1) initiation of radicals through use of a radical initiator, (2) propagation of monomer to form polymer chains, and (3) termination of active chain radicals.

Hu et al. demonstrated the use of FRP for the creation of MIP-coated quantum dots (QDs) to be used in the fluorescent detection of the sulfonamide antibiotic, sulfapyridine (SPD).[72]

Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), SPD, and Mn-doped ZnS QDs were combined for self-assembly in acetonitrile. The polymerization proceeded at an increased temperature with 2,2'-azobisisobutyronitrile (AIBN) as the radical initiator. Vigorous washing with a combination of methanol and acetic acid removed SPD from the fluorescent MIPs (FMIPs). The optimization of these particles for accurate fluorescent detection was investigated by adjusting the amount of Mn^{2+} with respect to the fluorescence quenching efficiency of their samples. FRP provided a wider range of viable functional monomers which extended the application potential for FMIPs with greater selectivity.

However, the researchers experienced issues with the sensitivity of these FMIPs in tap water, requiring an artificial increase in the concentration of SPD within the samples for measurable detection.

Daoud Attieh et al. synthesized water-compatible molecularly imprinted polymer nanogels by enzyme-mediated FRP.[73] The researchers sought to create a novel strategy for MIP nanogel synthesis in aqueous media that would be more environmentally friendly than existing MIP syntheses due imparting retrievability of an initiator enzyme. The environmental impact of this method was mitigated due to the use of an enzyme as an initiator instead of more commonly used synthetic initiators. Immobilized horseradish peroxidase (HRP) was used as a biocatalyst for initiation since it is a more sustainable and natural material than most initiators used in FRP. The immobilized form of HRP is used to prevent the initiator enzyme from getting entrapped in the cross-linked polymers, which allows the enzyme to be more readily reused.

For the synthesis of the MIP nanogels, 2,4-dichlorophenoxyacetic acid (2,4-D), a well-known and utilized herbicide, was used as a model template in aqueous media. By adding soluble HRP, 2,4-D, functional monomer 4-vinyl pyridine (4-VP), and water-soluble 1,4-bis(acryloyl)piperazine (PDA) as a crosslinker, self-assembly between all components was completed. For enzyme initiation to occur, HRP dosed with hydrogen peroxide (H_2O_2) enticed different oxidative states of enzymatic active sites (HRP-I and HRP-II). The combination of these oxidative catalytic states with H_2O_2 produced acetylacetone radicals, which then proceeded to initiate FRP as a polymerization mediator.

The enzyme-initiated MIPs were slightly more selective towards 2,4-D than similar analogues. To prevent entrapment of the enzyme so that it could be used in multiple cycles, HRP was covalently attached to 0.1 mm glass beads. Reuse up to 6 cycles was observed in the generated MIPs before nanogel yield began to decrease. This approach was successful at imparting an environmentally friendly approach at MIPs synthesis, initiating FRP by a reusable enzymatic unit.

The main issue that is typically observed with the use of FRP for MIP formation is the generation of products that are lacking in selective sensitivity and overall affinity for target analytes during the rebinding process. This stems from the inherent uncontrollability of free radical polymerization causing phenomena such as high polydispersity, buried template sites or difficulty in removing templating materials post-polymerization. As observed in this review, movement towards different varieties of polymerization techniques have therefore been expanding beyond a standard FRP approach.

Reversible-deactivation radical polymerization

When compared to conventional methods, reversible-deactivation radical polymerization (RDRP) generated MIPs involve a thermodynamically controlled process that affects how fast the growing polymer chains can propagate. By having a greater degree of control over the rate of chain growth in addition to negligible chain termination, MIPs generated by RDRP techniques have a narrower distribution of polymer chain length and therefore contain more homogenous polymeric moieties.

Of the RDRP techniques, the most prominent for synthesis of MIPs are reversible addition-fragmentation chain-transfer polymerization and atom transfer radical polymerization. However, alternative RDRP techniques are also present in the literature of which there are adequate reviews available.[74] For example, iniferter-induced radical polymerization has been explored for several years.[75] Additionally, it is important to note that nitroxide-mediated polymerization (NMP), also a RDRP approach, has been utilized to generate MIPs in the past,[76] but current research in this area is sparse. This could be attributed to most NMP initiators requiring relatively high activation temperatures, which negatively impacts biocompatibility with potential template molecules (i.e. proteins).

While these living radical polymerization techniques offer some promising results for bioapplication, especially biomedicine,[74,77] there are difficulties left to consider. Radical initiation often requires the utilization of toxic reagents, and sometimes the most optimal reactions produce toxic by-products that can incorporate themselves within MIP matrices. However, molecular recognition properties—namely selectivity and sensitivity during the rebinding process, have increased dramatically through these methods when compared to FRP approaches.

Reversible addition-fragmentation chain-transfer polymerization

One of the most popular types of RDRPs used in polymer synthesis today is through reversible addition-fragmentation chain transfer (RAFT) polymerization.[78,79]

RAFT polymerization processes seen recently aim to address a few major challenges in MIPs application. Mainly, addressing biological compatibility and hydrophilicity of MIPs while still maintaining competitive selectivity and sensitivity during utilization in aqueous media.

Ma et al. have been synthesizing fluorescent polymeric microspheres capable of selectively quantifying the concentration of small organic analytes in biological samples *via* RAFT approaches.[80] The obtained MIP microspheres proved an efficient approach towards the detection of specific analytes within complex biological mixtures by attaching well-defined thiol-terminated hydrophilic polymers to the MIP microspheres through RAFT precipitation polymerization (RAFTPP). RAFTPP is the addition of a RAFT agent into an otherwise general precipitation polymerization.[81] This technique has been utilized more recently in MIP preparation.[82]

RAFT coupling chemistry offered a versatile avenue towards preparing biologically compatible MIP microspheres while improving chemical recognition functionalities, molecular weight dispersity, and overall compatibility with aqueous media.

After template-monomer self-assembly, EGDMA, cumyl dithiobenzoate (CDB), and AIBN, were added to complete RAFTPP. Upon removal of the template molecule, another copolymerization of EGDMA and glycidyl methacrylate (GMA) *via* surface-initiated RAFT (SI-RAFT) yielded MIPs with surface exposed epoxy groups. Hydrophilic poly(hydroxyethylmethacrylate) (PHEMA) was then attached *via* thiol-epoxy "click" coupling, resulting in MIP particles functionalized with PHEMA brushes. The addition of PHEMA onto the MIPs significantly enhanced the surface hydrophilicity, which allowed for impressive specific rebinding moments in various aqueous media. Through SEM images, the diameter of each MIP microsphere showed an increase in nanoparticle size between the MIP and non-functionalized counterparts (Figure 4). The MIP microspheres were thus successfully functionalized with hydrophilic polymer, increasing MIP compatibility within aqueous media.

Hou et al. have more recently brought this chemistry a few steps

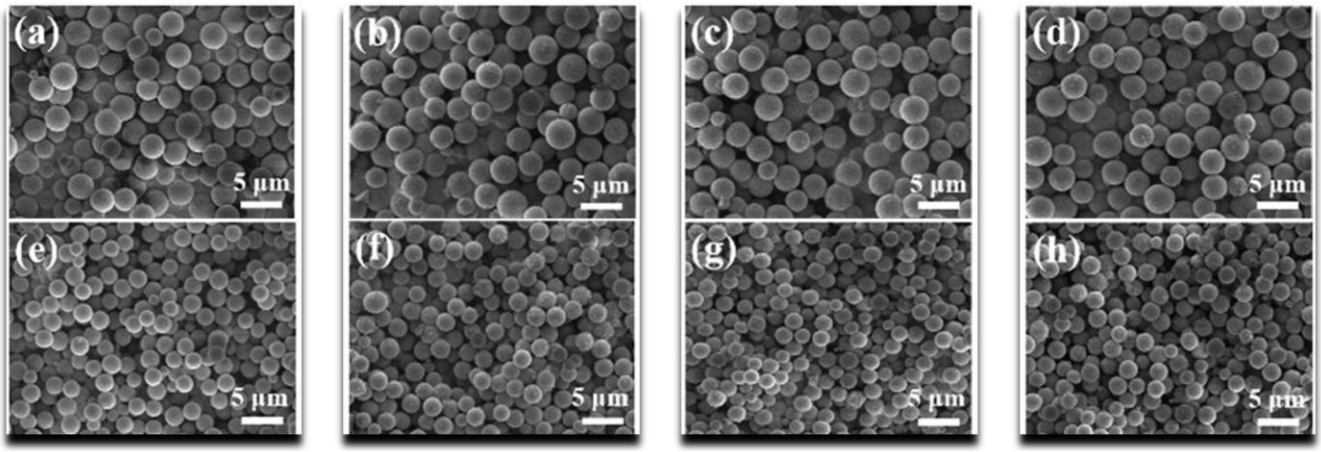

Figure 4. SEM images of unmodified MIP microspheres (a) compared to unmodified control polymer (e), epoxy-modified MIP microspheres (b) compared to epoxy modified control polymer (f), hydroxy-modified MIP microspheres (c) compared to hydroxy-modified control polymer (g), and PHEMA brush-modified MIP microspheres compared to PHEMA-brush modified control polymer (h). Reprinted and adapted with permission from Ma, Y.; Gao, J.; Zheng, C.; Zhang, H. Well-Defined Biological Sample-Compatible Molecularly Imprinted Polymer Microspheres by Combining RAFT Polymerization and Thiol-Epoxy Coupling Chemistry. *J. Mater. Chem. B* 2019, *7* (15), 2474–2483. Copyright 2019 Royal Society of Chemistry.

further, offering ratiometric fluorescent MIP microspheres by RAFT polymerization techniques with highly promising optosensing abilities.[83] The efficiency of ratiometric fluorescent detection of small organic analytes in real undiluted complex biological samples was investigated. 2,4-D was chosen as the template molecule in this study, while pure undiluted milk was selected to test the function of MIP microspheres in undiluted aqueous media upon successful synthesis.

First, "living" polymer microspheres were synthesized *via* RAFTPP of EGDMA and GMA, followed by a concentration-controlled SI-RAFT polymerization of red cadmium telluride quantum dot (CdTe QD) fluorophores. With EGDMA as the crosslinker, 4-vinyl phenol (4VP) as the functional comonomer, and methacrylate—urea—4-fluoro-7-nitrobenzofurazan (MA-Urea-NBD) as the fluorescent functional comonomer, surface imprinting with 2,4-D *via* a subsequent SI-RAFT polymerization was completed. By grafting a final hydrophilic polymer, poly(N-isopropyl acrylamide) (PNIPAAm), to the available vinyl groups of the "living" MIP microsphere structure, the polymer brushes addressed issues with solubility in the undiluted milk samples. The resulting MIP microspheres exhibited hydrogen bonding between the template molecule and MA-Urea-NBD units in acetonitrile when reintroduced to the template herbicide.

Not only were the MIP microspheres able to detect 2,4-D in the undiluted milk samples, but they demonstrated a distinct change in fluorescence intensity at 528 nm when the concentration of 2,4-D was increased (0-100µM) in the 2,4-D-MIP versus the corresponding 2,4-D-control polymer (CP), specifically with the NBD fluorophore included in the MIP production step. Negligible change was observed for the red CdTe QDs at 723 nm, which led to the observed color change from red to cyan with increasing 2,4-D (Figure 5). The 2,4-D-MIP had a lower limit of detection (0.13µM) than the current maximum contaminant level for 2,4-D in drinking water (0.14µM), and an overall imprinting factor of 3 when compared to the 2,4-D-CP. These MIP microspheres were also selectively sensitive to the specific template, such that accurate detection occurred in undiluted pure milk samples even with several template analogues present within the sample mixture.

Ultimately, the researchers were able to generate two different types of MIP microspheres *via* RAFT procedures that are capable of specific analyte detection within biological mixtures.

While RAFT polymerization techniques and modifications are certainly a promising avenue to increasing the homogeneity of MIP cavities, there are still other polymerization techniques that are adequate for generating MIPs with complex applications.

Atom transfer radical polymerization

Another popular RDRP approach towards MIP formation is through atom transfer radical polymerization (ATRP). ATRP produces complex polymers with ideal polydispersities and easily tunable molecular weights.[84] Compared to conventional polymerization techniques, ATRP offers a highly efficient and controlled technique towards the synthesis of MIPs.

ATRP tends to be rather sensitive to polar and acidic monomers, which makes the process for producing MIPs through ATRP challenging.[85] Certain monomer-template complexes are therefore not always feasible with ATRP. Use of ultra-low concentrations of transition-metal catalysts addresses this issue, and some research in this area shows low-toxicity and virtually no secondary pollution through this method.[86] Yet, as discussed previously, the use of any toxic substituents is non-ideal for MIPs with *in vivo* applications. Additionally, low-cost transition metal catalysts are air-sensitive and as a result can lead to premature polymerization. Therefore, metal-free ATRP approaches have been more recently explored for this area of synthesis.

A study conducted by *Ramakers* et al. demonstrates the use of photo-mediated ATRP (photo-ATRP) by use of 10-phenylphenothiazine (PTH) as a photoredox organocatalyst.[87] Through surface-initiated grafting of MIP films, the researchers were able to specifically rebind histamine, a natural biomolecule relevant in biomedical work and food safety regulation.[88,89]

MAA and EGDMA in the presence of histamine, PTH and dimethylacetamide (DMAc) were copolymerized by use of a pulse UV excimer laser to initiate photoATRP. The substrates were then rinsed with tetrahydrofuran (THF), ethanol (EtOH), and distilled water to remove histamine. While the growth of MIP film grafting

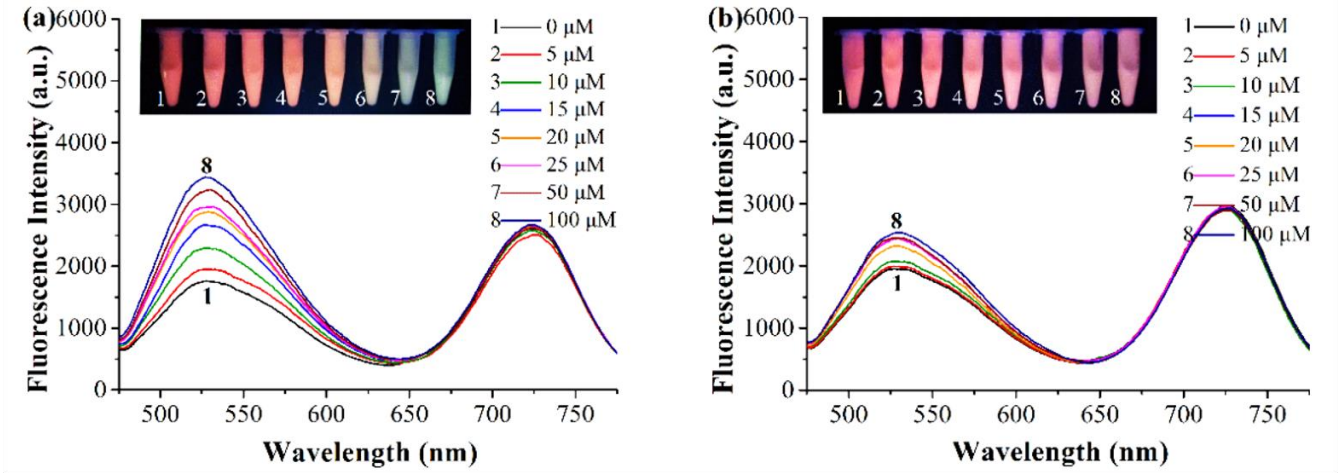

Figure 5. Fluorescence response of 2,4-D-MIPs (a) and the NIPs counterpart (b) with increasing concentration of 2,4-D from 0 to 100μM. Reprinted and adapted with permission from Hou, Y.; Zou, Y.; Zhou, Y.; Zhang, H. Biological Sample-Compatible Ratiometric Fluorescent Molecularly Imprinted Polymer Microspheres by RAFT Coupling Chemistry. *Langmuir* 2020, *36* (41), 12403–12413. Copyright 2020 American Chemical Society.

requires further investigation, the response of the MIPs through attachment to the created sensor was very strong. This is suggestive of photoATRP generating MIPs with high sensitivity and an abundance of suitable receptor sites specifically for histamine. This was especially prevalent when comparing the response of photoATRP generated MIPs to that of complementary photoATRP NIPs.

Researchers have also recently synthesized MIPs *via* a metal-free ATRP technique.[90] Here, *Bai* et al. successfully imprinted immunoglobin G (IgG) onto a modified electrode surface by use of metal-free visible-light-induced ATRP (MVL ATRP).

IgG was chosen as the template molecule due to its significance in work with viruses and bacteria within mammals. With regards to point-of-care, accurate and rapid detection of IgG is essential to treating an array of ailments. It would also be beneficial to detect IgG at lower concentrations so that illness may be determined at an earlier stage. MIP research for IgG detection not only addresses these requirements for IgG detection, but also offers greater production stability and cost-effectiveness in comparison to commonly available methods.

The imprinted polymers were generated on the surface of a modified Au electrode which could later be utilized as a biosensor for IgG. The Au electrodes were first modified with nano Au/nano Ni (AuNCs/NiNCs/Au) to increase the surface area and electron transfer ability at the electrode surface. High magnification SEM images were observed of the AuNCs/NiNCs/Au electrode surfaces before polymerization (Figure 6A). A complex of mouse IgG with fluorescein isothicyanate (FITC) provided both the template molecule and the photocatalyst (FITC-IgG). Thiol-initiator was immobilized onto the surface of the modified electrode, where acrylamide monomer, methylene bis-acrylamide as crosslinker, and the template-photocatalyst complex FITC-IgG, were dissolved in phosphate buffered solution and illuminated under visible light to induce photopolymerizaiton.

The template molecule IgG was removed by soaking the electrode in 10% (v/v) acetic acid solution with 10 g/L of sodium dodecylsulfate for 1 hour, and assisted with an electric potential of -0.6 V. The attached FITC photocatalyst no longer remained within the MIPs once IgG was removed, and therefore did not alter the electrode-measured intensity. The polymer/AuNCs/NiNCs/Au electrode surface (Figure 6B) and IgG-imprinted polymer/AuNCs/NiNCs/Au electrode surface (Figure 6C) were both compared through high magnification SEM images to the pre-polymerized electrode surfaces. Based on the SEM images, it was clear that the electrodes not only had been modified by introduction of the polymer, but the surface of the IgG imprinted polymer had noticeable porous topology once the template was removed. The researchers attributed this to the binding cavities that were left over following the removal of the template complex.

Based on the signal response of the electrode, an increase of IgG presented to the system (1.0×10^{-6} to 10 mg L^{-1}) showed an obvious decrease in the differential potential voltammetry of the MIP electrodes. The authors attributed this decrease to the rebinding moment of the IgG protein with formed cavities of the MIP, which incrementally blocked the probe from reaching the electrode surface.

The selectivity of the IgG imprinted electrodes was tested by using various proteins and analogues as interferents to the differential potential voltammetry probe. The data indicated that the cavities made on the IgG imprinted electrode were selective for IgG (29.3 +/- 0.7 μA) over the analogues (human serum albumin = 1.4 +/- 0.3 μA, bovine hemoglobin = 2.1 +/- 1.2 μA, myoglobin = 1.7+/- 1.2 μA, human immunoglobin M = 1.3 +/- 0.3 μA, human immunoglobin A = 1.3 +/- 0.3 μA) at the same concentration (10^{-4} mg L^{-1}) The imprinting factor of the IgGIPs/AuNCs/NiNCs/Au compared to the non-imprinted counterparts were also observed through signal response. The values of IgG detection were 10 times more selective in the IgG imprinted polymers versus the NIPs, and negligible for all other proteins and analogues.

It was determined that the MVL ATRP generated IgG imprinted polymers had comparable selectivity and higher sensitivity for IgG (limit of detection = 2.0×10^{-8} mg L^{-1}) than similar IgG sensors.

iii. Template Removal

Once polymerization is complete, the template molecule used to tailor the recognition site of the MIPs must be removed. Efficient removal of the template molecule is not always straightforward, and

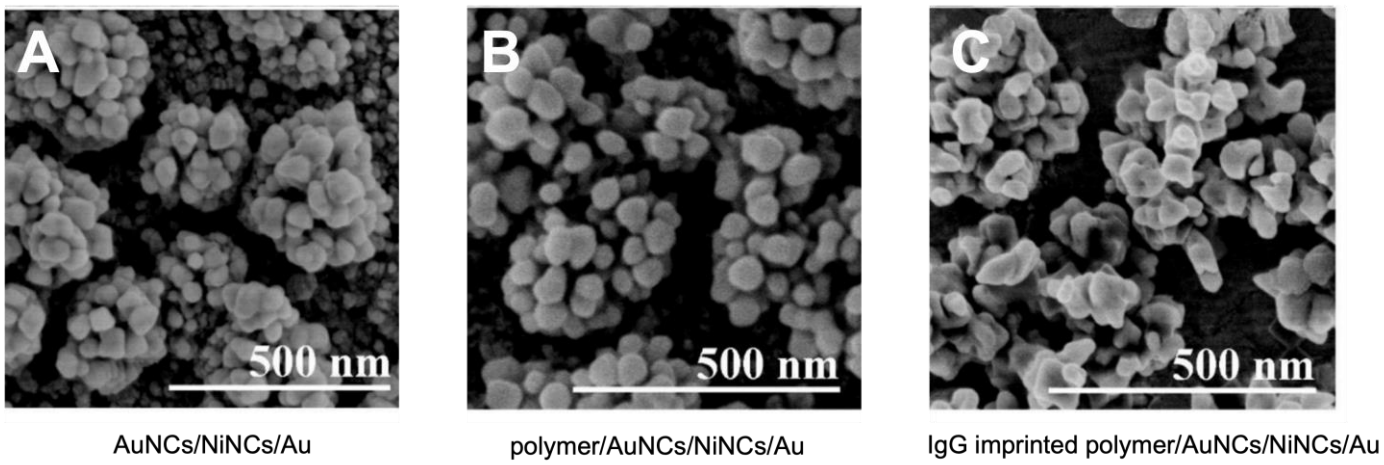

Figure 6. SEM images of: (A) the electrode AuNCs/NiNCs/Au surface before polymerization, (B) the modified electrode AuNCs/NiNCs/Au surface after polymerization had occurred but the template molecule IgG had not yet been removed, (C) the modified electrode AuNCs/NiNCs/Au after removal of the template molecule, leaving behind IgG imprinted cavities. Reprinted and adapted with permission from Bai, R.; Sun, Y.; Zhao, M.; Han, Z.; Zhang, J.; Sun, Y.; Dong, W.; Li, S. Preparation of IgG Imprinted Polymers by Metal-Free Visible-Light-Induced ATRP and Its Application in Biosensor. *Talanta* 2021, *226*. Copyright Elsevier 2021.

therefore this area of MIPs synthesis continues to expand. Depending on the strategy, removal of the template molecule can often leave the MIPs with undesirable properties. Vigorous and repetitive rinsing of the MIPs after polymerization with an appropriate solvent can be an effective way to remove the target molecule. However, if all the target molecules are not removed efficiently, leakage of the initial template molecule into the polymer during the rebinding step can result in undesirable and artificially skewed detection. A handful of techniques provide solutions to these issues, which mainly include surface imprinting, epitope/substrate imprinting, and solid-phase imprinting.

Surface Imprinting

Surface imprinting is a method of forming MIPs that came to fruition to eliminate the retention of template molecules within the interior of prepared microspheres after steps are taken intended for their removal. This leads to undesirable properties such as template elution difficulties, slow mass transfer, and low adsorption capacity of the target molecules. To tackle this issue, the surface imprinting technique grafts a functional polymer to the surface of a solid substrate, eliminating the interior of a MIP where template molecules have been shown to be retained. Additionally, this morphology aids in the ability of target molecules to bind with template sites because these sites are situated on the surface of the MIP instead of within.

The widely used nanomaterials as solid substrates for surface imprinting mostly fall into two categories: silicon dioxide (SiO_2) and iron (II,III) oxide (Fe_3O_4) nanoparticles. SiO_2 nanoparticles are the most used substrate for preparing nano-NIPs as they provide a porous structure which aids in the removal of template material. To ensure imprinting of proteins and small molecules to this substrate, some functionalization steps are necessary for molecular immobilization prior to MIP synthesis. For example, *Zhou* et al. investigated the utilization of modified silica gel solid substrates for the selective extraction of teicoplanin from water samples using surface molecularly imprinted polymers (Figure 7).[91] To generate the surface MIPs, they first functionalized the silica gel with 3-aminopropyltriethoxysilane to obtain an amino-functionalized silica gel. These particles were then introduced to teicoplanin template molecules which bind to the amino groups on the silica surface followed by functional monomer HPMA for pre-polymerization. The system was subsequently cross-linked by employing trimethylolpropane trimethacrylate to generate the MIPs. The template molecules were removed via extraction with an acrylic acid/methanol solution until teicoplanin was no longer detected by HPLC. These particles were subsequently dispersed in prepared lake, spring, and Pearl River water samples with known teicoplanin concentrations and characterized by HPLC for teicoplanin extraction. This system exhibited strong adsorption capacity (152.6 mg/g at TEC concentration of 1mg/mL) and selectivity for teicoplanin (compared against peptide antibiotics: VGM, BAT, CS and macrolide antibiotics: SPM and TIM). Additionally, the generated surface MIPs were able to extract between 81.4-94.6% of the total teicoplanin content from real water samples. This therefore serves as an example of the successful removal of template material after surface MIP synthesis and selective capture of target molecules utilizing the surface imprinting method with SiO_2.

Compared to SiO_2 nanoparticles, core-shell MIPs based on Fe_3O_4 nanoparticles have gained a wider attraction due to the high convenience of separation of Fe_3O_4 from the surrounding media by applying a simple magnet. Therefore, these magnetic MIPs are widely used in protein purification and proteomic research. *Islam* et al. took advantage of this technology to develop an environmentally friendly method for the detection of Ni(II) in food and water samples.[92] To do so, the group generated Fe_3O_4 graphene oxide (GO) by co-precipitating Fe_3O_4 nanoparticles on GO sheets which were then modified to introduce vinyl groups on the surface (Fe_3O_4@GO) (Figure 8). The functionalized particles were subsequently reacted with allyl-modified triethylenetetramine that had been coordinated to Ni(II) as the template molecule. A cross-linker, ethylene glycol dimethacrylate (EGDMA), was then introduced to the system to complete the formation of the surface MIP. The Ni(II) template was

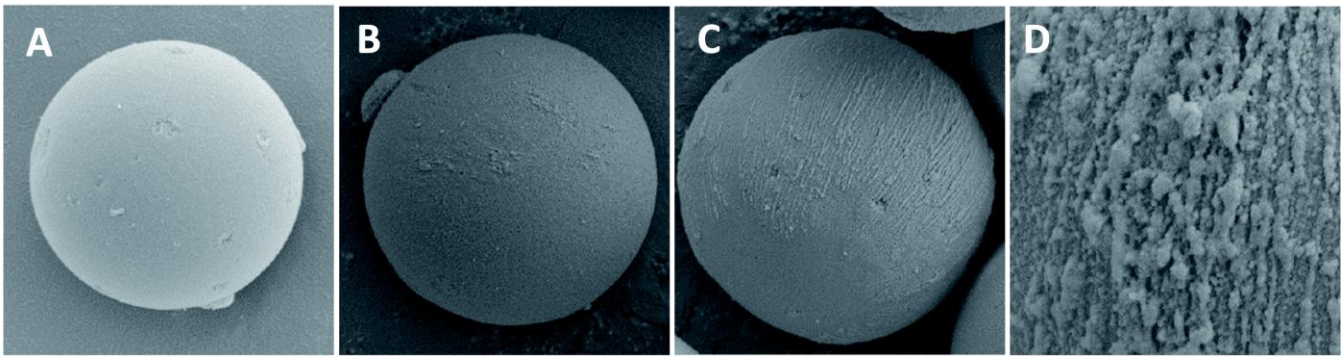

Figure 7. SEM images depicting the stages of MIP synthesis. (A) a modified silica gel particle, (B) a non-imprinted particle, (C) 10K magnification of a surface modified imprinted particle and (D) 30K magnification of a surface modified imprinted particle. Reprinted and adapted with permission from Zhou, H.; Peng, K.; Su, Y.; Song, X.; Qiu, J.; Xiong, R.; He, L. Preparation of Surface Molecularly Imprinted Polymer and Its Application for the Selective Extraction of Teicoplanin from Water. *RSC Adv.* 2021, *11* (22), 13615–13623. Copyright 2021 Royal Society of Chemistry.

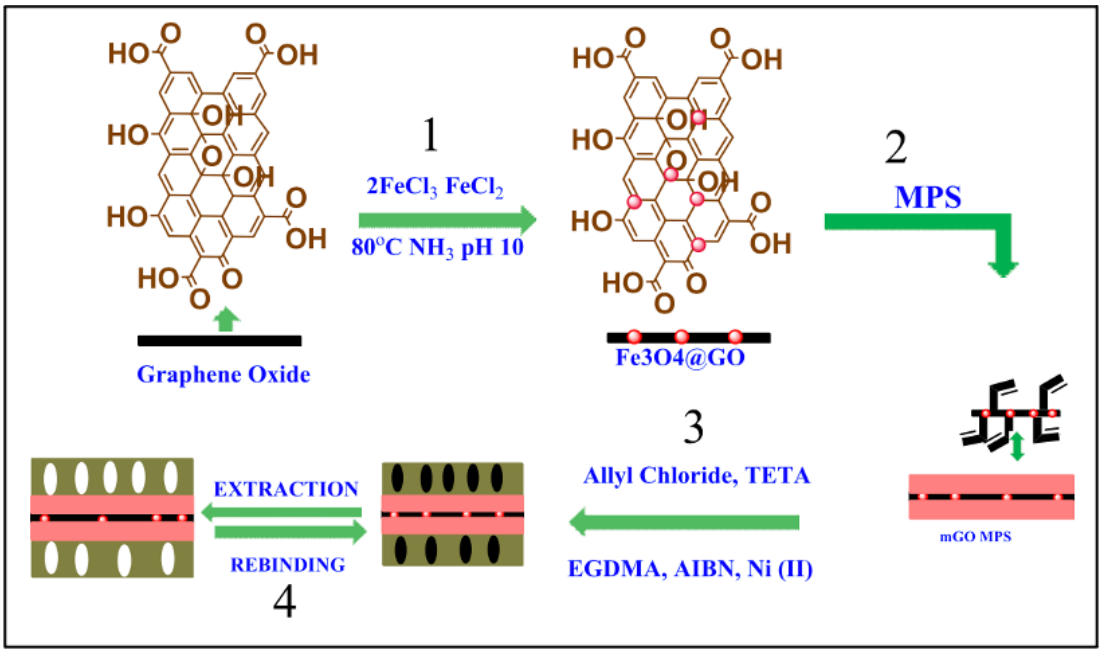

Figure 8. A schematic representation of the synthetic route to the synthesis of Fe_3O_4 based MIPs on graphene oxide. Step one shows the functionalization of graphene oxide with Fe_3O_4 via co-precipitation. Step two corresponds to the addition of methacrylate groups to the surface of the particles via coupling with 3-methacyloyl propyl trimethoxy silane (MPS). The MIP was then generated in step three via the introduction of allyl chloride and subsequent polymerization in the presence of Ni(II). Reprinted with permission from Islam, A.; Javed, H.; Chauhan, A.; Ahmad, I.; Rais, S. Triethylenetetramine-Grafted Magnetite Graphene Oxide-Based Surface-Imprinted Polymer for the Adsorption of Ni(II) in Food Samples. *J. Chem. Eng. Data* **2021**, *66* (1), 456–465. Copyright 2021 American Chemical Society.

removed by extraction with acid, followed by neutralization of the MIP. This system was reported to have a high adsorption capacity for Ni(II) of 48.4 mg/g. When evaluated against Cu(II), Zn(II), Cd(II) and Pd(II), it was found that the surface MIP's capacity was reduced but did still show a level of selectivity towards Ni(II). The surface MIPs did perform significantly better than non-imprinted polymers which showed almost the same adsorption values for the alternate ions as Ni(II).

Surface imprinting has been proven to be a viable technique for addressing the issue of template retention on the interior of MIPs, which has shown to induce negative effects on the behavior of the MIPs and their binding capabilities. Several substrate materials have been evaluated for this purpose and have shown to produce MIPs with exceptional binding properties while achieving complete template elimination.

Epitope/Substrate Imprinting

Although surface imprinting addresses the problems that arise with macromolecular mass transfer, whole proteins are still difficult to use efficiently for imprinting due to their complex structure, numerous functionalities, and risk of denaturation. Therefore, decreasing the complexity of protein structure and preparing MIPs with templates containing protein epitopes is a promising strategy. As natural antibodies against a protein recognize their target *via* certain sequential or conformational epitopes, MIPs can also be synthesized using an epitope-approach. Epitope-imprinted polymers can not only distinguish the epitope peptide but also recognize the entire protein in real samples. If the right epitope is selected, epitope imprinting has various advantages, such as an abundant choice of templates, improved applicability in various reaction conditions, and defined orientation to bind the target proteins.

Recently, *Zhang* et al. investigated the implementation of epitope imprinting for the detection of human hemoglobin (HbA).[93] Their work involved preparing two HbA imprinted polymers by different approaches: a hierarchical approach where chemisorption of the template (N-terminal pentapeptide VHLTP-amide of HbA) is performed before electropolymerization (scopoletin monomer) and a single-step method of template and functional polymer mixtures. The MIPS were then evaluated against the binding properties of entire proteins and specific peptide molecules. The group utilized squarewave voltammetry to evaluate the binding of these molecules to the imprinted sites within the MIPs finding the hierarchically prepared particles had greater binding ability than particles prepared *via* a one-step synthesis (K_d [dissociation constant] of 7.13 vs 68.6, respectively). However, when further evaluating the systems utilizing surface enhanced infrared absorption (SEIRA) Spectroscopy and atomic force microscopy (AFM) , they found that non-site-specific binding was also taking place within the MIPs. This was because HbA would also strongly bind to a non-imprinted polymer film produced in the same way as the MIPs but without a template for cavity generation. It was determined that this was due to non-covalent, out-of-cavity hydrophobic interactions between the target molecules and the polymer. It is therefore obvious that while epitope imprinting is a promising technique for the specific detection/adsorption of entire protein structures, there is still research required to achieve a perfected technique that is understood and fully behaves in the desired manner.

Solid-Phase Imprinting

While surface imprinting and epitope/substrate imprinting have made improvements for adsorption efficiency and protein targeting, room for enhancement of MIPs remain.

Solid-phase imprinting addresses issues such as limitations in solvent compatibility, template removal, and material recyclability. This imprinting technique begins by functionalizing a solid support, such as glass beads, with molecules that immobilize the template molecules. These materials are then transferred to a vessel where the template is introduced and bound to the substrate surface. Functional monomers are then added, along with initiator to initiate polymerization around the template molecules in the interstitial spaces between the solid supports. The excess material or polymer particles formed at a distance from the solid supports are then washed away with solvent, leaving behind polymers that were strongly bound to the template molecules. These can then be swollen with an alternate solvent to be removed from the solid supports and template molecules and collected for evaluation and use.

Chiarello et al. evaluated the effect of polymerization time on the binding properties of nanoMIPs prepared using solid-phase imprinting (Figure 9).[94] This system was targeted at the adsorption of ciprofloxacin and did so using functionalized glass beads as the solid substrate and acrylic acid, N,N'-methylene-bis-acrylamide, N-isopropylacrylamide (NIPAAm), and N-tertbutylacrylamide as the polymerization medium. They investigated a polymerization time range from 15 minutes to 5 hours and tracked the adsorption and selectivity properties along with particle size and the polymerization polydispersity index. They determined that because polymerization time influences the size of the MIP along with the rigidity, the selectivity and binding capabilities of the system were also affected. This was also attributed to the resultant steric hinderance of the MIP cavity with ciprofloxacin. It was seen that short polymerization times, below 2 hours, generated nanoparticles of low selectivity and high binding affinity; intermediate polymerization times resulted in both high selectivity and binding affinity; and long polymerization times found low binding affinity and selectivity values.

To further investigate the impact of various synthetic conditions, *Cavalera* et al. evaluated the influence of bound template morphology, solvent, and the constituents of the polymerization mixture on the ability to bind ciprofloxacin.[95] In each case, the MIPs were synthesized on modified glass beads and were fully soluble in water, which is a difficult property to achieve when utilizing other methods of MIP synthesis. They found that in all evaluated cases, the synthesized nanoMIPs were able to bind ciprofloxacin in buffered water with binding constants on the order of those for naturally occurring antibodies (10^5-10^7L mol^{-1}) . They attributed these values to the retention of only higher affinity MIPs from the solid-phase imprinting process, which are then utilized in the binding affinity characterization. The group then evaluated the specific polymerization parameters for their effect on the behavior of the synthesized MIPs. It was seen that pH had a significant influence on the binding capabilities of the particles based on the solvent they were generated in. Particles prepared in water showed a decreasing binding constant with increasing pH while those prepared in acetonitrile showed an increasing value. This is due to the state of protonation of the polymers involved in each of these systems which directly influences the number of available binding sites and therefore the equilibrium binding constant for ciprofloxacin. As all of these modifications to the polymerization system still showed significant binding characteristics for ciprofloxacin, this study is an example of the flexibility of the solid-phase imprinting method while noting that the selected system still influences these properties and the modes by which these MIPs are formed.

Additionally, solid-phase imprinting allows for the formation of MIPs in a variety of solvents, including water. For example, *Mourão* et al. investigated utilizing this technique for the detection of nucleotides (which are hydrophilic molecules) in water.[96] They found that MIPs synthesized by first anchoring Fe^{3+} on glass beads followed by the introduction of adenosine 5'-monophosphate (AMP) as the template molecule. The polymerization medium was then added, which was comprised of NIPAAm, 1-(vinylbenzyl)thymine, and N,N-ethylenebis(acrylamide) to generate the MIPs. The beads were then washed with 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer solution to obtain particles for adsorption characterization. These nanoMIPs showed high selectivity for AMP in MES buffer solution when evaluated against a range of other nucleotides with similar morphologies and a maximum binding capacity of 1350 nm/mg of MIP (Figure 10). This experiment is representative of the ability of solid-phase imprinting to broaden the range of solvent systems that can successfully be used with MIPs.

iv. Post-Imprint Modifications

Conclusion of MIP synthesis is usually reached after removal of

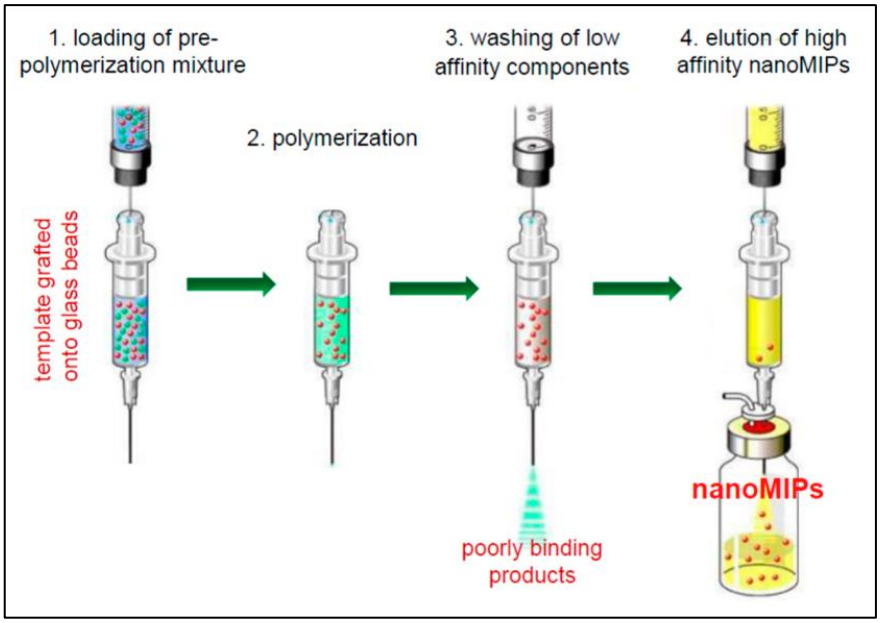

Figure 9. A representation of the solid-phase imprinting process for nanoMIP generation, where (1) the pre-polymerization mixture is loaded onto glass beads, (2) polymerized, and (3) the poorly bound products are washed out before (4) nanoMIPs are eluted for collection. Reprinted under the Creative Commons Attribution License from reference Charello, M.; Anfossi, L.; Cavalera, S.; Di Nardo, F.; Artusio, F.; Pisano, R.; Baggiani, C. Effect of Polymerization Time on the Binding Properties of Ciprofloxacin-Imprinted Nanomips Prepared by Solid-Phase Synthesis. *Polymers* 2021, *13* (16).

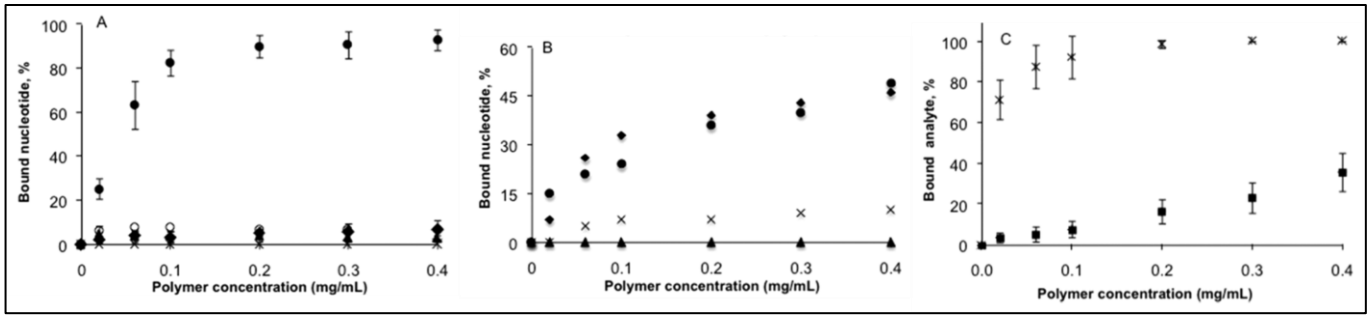

Figure 10. Plots of the equilibrium binding isotherms of AMP versus other nucleotides where AMP is represented by circles, GMP by diamonds, CMP by triangles and UMP by crosses on imprinted (filled) and non-imprinted (unfilled) particles. (A) represents polymers obtained in the presence of VBT and (B) shows polymers obtained in the absence of VBT. (C) depicts the binding properties of polymers synthesized in the presence of adenosine (star) and ATP (square). GMP = guanosine 5′-monophosphate disodium salt; CMP = cytidine 5′- monophosphate disodium salt; UMP = uridine 5′-monophosphate disodium salt; ATP = adenosine 5′-triphosphate disodium salt hydrate. Reprinted and adapted with permission from Mourão, C. A.; Bokeloh, F.; Xu, J.; Prost, E.; Duma, L.; Merlier, F.; Bueno, S. M. A.; Haupt, K.; Tse Sum Bui, B. Dual-Oriented Solid-Phase Molecular Imprinting: Toward Selective Artificial Receptors for Recognition of Nucleotides in Water. *Macromolecules* 2017, *50* (19), 7484–7490. Copyright 2017 American Chemical Society.

the template molecule. However, more recently there have been efforts to impart adaptations to MIPs after the initial polymerization and imprinting steps.[97–101]

Just as post-translational modifications found in the biosynthesis of proteins and complex molecules allow for an increase in proteomic diversity, post-imprint modifications (PIMs) impart multi-functionality into the binding cavity of previously prepared MIPs. This permits for an increase in molecularly recognizable substances and therefore additional efforts towards greater affinity and selectivity within synthetic recognition sites. By including modular and adaptable functionalities into the binding cavities, PIMs of MIPs (PIM-MIPs) have opened another avenue for discovery in this field.[102]

Saeki et al. have produced PIM-MIPs through usage of a multi-functionalized PIM reagent (PIR).[103] By use of the PIR, a PIM-MIP that possesses multiple functionalities and additional reaction sites within a single binding cavity was generated.

Through surface initiated ATRP, a MIP film was synthesized. A prostate specific antigen (PSA) was used during polymerization as a template for specific targeting of glycoproteins that are present in many prostate-related diseases. In a typical PIM-MIP synthetic route, imparting multiple functionalities within the binding cavities can be challenging due to necessary template modification needed to provide additional functional groups post-template removal. By using a new PIR (PIR-C), the template molecule did not require alterations for sequential PIMs to occur.

First, the selection of a fluoro-functional monomer that contained boronic acid groups was used to facilitate template binding through the interaction between cis-diol groups to form boronate ester linkages. Following template removal, thiol groups were left

exposed within the binding cavity, where the PIR-C was attached through disulphide bonds. The added PIR-C was functionalized with both secondary amino acid groups and a carboxyphenyl group to allot for another PIM to occur, while retaining template-rebinding selectivity for PSA compared to IgG. When treated with 1 mg mL^{-1} of human serum albumin (HSA), the less selective binding cavities were masked and affinity was far greater for PSA than IgG (K_a = 1.18 x 10^9 M^{-1}) (Figure 11). The secondary amino acid groups attached by the PIR-C were then utilized to introduce fluorescent Alexa Fluor 647 onto the MIP, where an increasing uptake of the desired PSA resulted in a corresponding decrease of fluorescent intensity. The addition of the final PIM with Alexa Fluor 647 demonstrated comparable selectivity to the optimized MIPs pre-modification (K_a = 2.82 x 10^9 M^{-1}).

The researchers concluded that an increase in specific rebinding for the template PSA was only plausible when the orthogonal dual interaction sites were both present within the polymer matrix. Specific rebinding of the target was found to be highly selective and sensitive through their synthetic approach by incorporating a PIR with optimized crosslinking density and blocking treatments. This ultimately provided evidence that multi-functionality is important for the specific recognition capabilities of PIM-MIPs for some selected templates, especially if those templates cannot withstand intricate modifications. Thus, the use of PIRs that contain a variety of functional group capabilities for specific analyte detection is an area of PIM-MIPs research that deserves further acknowledgement.

Additionally, *Tsutsumi* et al. continued to investigate PIM-MIPs for the specific fluorescent detection of proteins through nanogels instead of bulk- and film-based MIPs.[104]

This time, utilization of a bivalent functional monomer enabled specific target protein binding alongside the addition of secondary amino acid groups. HSA was used as the template protein for imprinting of the nanogels.

Following template removal, the secondary amino acid groups were reacted with fluorescent dye, ATTO 647 N, to form a PIM-MIP nanogel. As template rebinding with HSA occurred, an increase in fluorescence was observed. Alongside this finding, the researchers determined through use of reference proteins that the synthesized binding cavities were fluorescently selective and responsive only towards HSA.

Fluorescent reporter molecules incorporated into MIPs are more efficiently achievable through current PIM work. However, fluorescent signalling is not the only important substituent to include in MIPs application. MIPs with interesting morphologies and chemistries incorporated as PIMs are of considerable value in this field as well.[105] As is observed in this review, exploring the efficiency of multi-step PIMs is of great importance to the future of PIM-MIPs synthesis.

Research reported in Polymer Chemistry by *Montagna* et al. utilized a general approach to synthesizing PIM-MIP nanoparticles containing hydrophilic polymer brushes on the nanoparticle surface through RAFT coupling chemistry.[106] Free radical polymerization was first conducted to form MIP nanoparticles with moderate binding properties. By imprinting propranolol as a model template molecule through non-covalent bonding with MAA, and copolymerizing with 1,1,1,-trimethylolpropane trimethacrylate (TRIM) as a crosslinker, spherical nanoparticles were obtained. Impressive selectivity and affinity for propranolol was observed by comparing the MIPs to their NIP counterparts (Figure 12). In contrast to that of atenolol, an analogue to propranolol, the MIPs showed greater affinity for propranolol.

Through residual double bonds remaining mostly on the surface of the resultant MIP nanoparticles, a dithioester and trithiocarbonate were both attached to the surface of the nanoparticles through RAFT coupling chemistry. The resulting PIM-MIPs were decorated with living polymer and retained impressive rebinding properties.

The researchers then extended the living polymer chains with NIPAAm to provide the MIP with hydrophilic properties. This modification allowed for specific binding in water compared to the non-specific binding observed in both the free radical and RAFT-coupled MIP nanoparticles in aqueous media. The specific binding itself did not increase by the addition of NIPAAm, but rather remained unaffected (0.07 to 0.08 mg mL^{-1} at K_{50}) This finding highlights the importance of the first PIM step for increasing binding efficiency on MIPs, while also demonstrating the versatility of PIMs with added desirable traits through a multi-step approach.

II. Recent Application Advancements

While many parameters have been explored in the synthetic approach to MIPs, there are a few notable application advancements that are more prevalent in the literature today. Such application areas include a variety of environmental and food related efforts, while others spread more deeply into biomedical and point-of-care procedures. There are many suitable reviews on these topics,[107–109] and therefore, the most recent techniques to address an array of applications will be discussed herein.

MIPs generated with fluorescence in mind, electrochemical MIP-based sensors, and MIPs that incorporate magnetic material are all of considerable interest recently.

i. Fluorescent Imaging

The ability to detect specific molecules, such as proteins, is highly sought-after in many areas of chemistry and biomedical research. MIPs that harness fluorescence for this purpose offer a unique approach towards fast and accurate detection of target molecules, with an emphasis on specific selectivity of those materials.

In a recent study, *Li* et al. developed molecularly imprinted polymers with carbon-dot (MIP@CD) fluorescent sensors to improve the detection of the over-the-counter drug, naproxen.[110] When released into the environment from mediums such as sewage, naproxen negatively impacts natural bodies of water by promoting water pollution.

MIP@CDs were synthesized by adding the template naproxen to 3-aminopropyl triethoxysilane (APTES), tetraethyl orthosilicate (TEOS) and previously synthesized CDs. The product was then washed with methanol until there was no naproxen detected by UV-Vis spectroscopy.

UV-vis absorption peaks at 360 nm and 448 nm in both MIP@CDs and non-imprinted polymers with carbon-dots **(**NIP@CDs**)** confirmed that the MIP layer did not alter the optical properties of the CDs. They further studied the optimization of the detection conditions for naproxen to determine the highest fluorescent quenching ratio. This was used to test naproxen

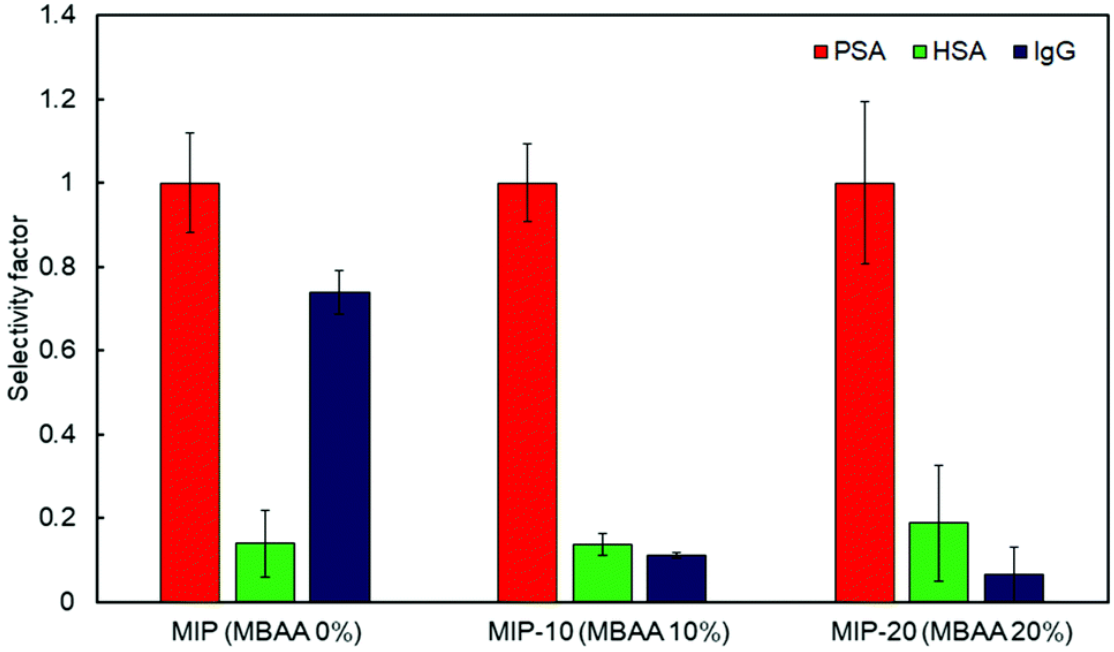

Figure 11. The determined selectivity factors of non-crosslinked (MIP), 10% crosslinked (MIP-10) and 20% crosslinked (MIP-20) MIPs with dual orthogonal binding with respect to PSA (red), HSA (green), and IgG (blue). The MIPs were pre-treated with 1mg mL^{-1} of HSA prior to the determined selectivity to mask any undesirable, non-uniform binding cavities. Reprinted with permission from Saeki, T.; Takano, E.; Sunayama, H.; Kamon, Y.; Horikawa, R.; Kitayama, Y.; Takeuchi, T. Signalling Molecular Recognition Nanocavities with Multiple Functional Groups Prepared by Molecular Imprinting and Sequential Post-Imprinting Modifications for Prostate Cancer Biomarker Glycoprotein Detection. *J. Mater. Chem. B* 2020, *8* (35), 7987–7993. Copyright 2020 Royal Society of Chemistry.

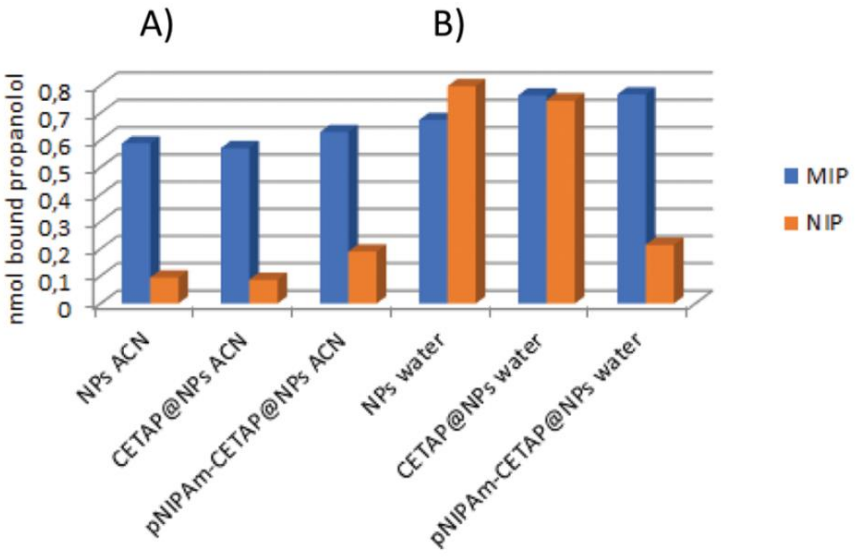

Figure 12. A comparison in the amount (nmol) of bound propranolol between imprinted MIPs (blue) and non-imprinted NIPs (orange) in (A) acetonitrile and (B) water, at varying stages of synthesis. The final MIPs (pNIPAm-CETAP@NPs) far outperform the binding of propranolol than that of their NIP counterparts in both acetonitrile and water. Reprinted and adapted with permission from Montagna, V.; Haupt, K.; Gonzato, C. RAFT Coupling Chemistry: A General Approach for Post-Functionalizing Molecularly Imprinted Polymers Synthesized by Radical Polymerization. *Polym. Chem.* 2020, *11* (5), 1055–1061. Copyright 2020 Royal Society of Chemistry.

containing samples and determine the selectivity of the MIP@CDs. Results showed strong anti-interference when in the presence of structural analogues ibuprofen, ketoprofen, and fenoprofen. The MIP@CDs had higher fluorescence quenching efficiency for naproxen due to the exact complementary shape and size of the imprinted cavities for the naproxen over the analogous compounds. Comparatively, the NIP@CDs were inefficient in the observed quenching behavior for naproxen and all of the analogues.

Competitive binding for naproxen versus the structural analogues was tested by increasing the concentration ratior of ibuprofen to naproxen from 0 to 4. The MIP@CD fluorescence quenching ratios for naproxen were unaffected by the increased concentration of competitor analogue, demonstrating high affinity of naproxen to the MIP@CDs. Additionally, the detection limit of naproxen for MIP@CDs (0.03 μM; range of 0.05-4 μM) was less than and comparable to existing naproxen sensors. Naproxen recovery studies

were then performed in real samples to demonstrate MIP@CDs for naproxen sensing in practical scenarios.

Additionally, *Wang* et al. focused on creating a new catalyst-free approach to the synthesis of fluorescent MIP (FMIP) nanoparticles using naproxen as a template molecule.[111]

In this method, APTES was coupled with FITC prior to polymerization as a fluorescent silane monomer (FITC-APTES). Varying amounts of FITC-APTES were used to synthesize FMIPs that would allow an observable distribution of FITC fluorophores. The resulting polymers, FMIP1 and FMIP2 varied in color; however, IR spectroscopy displayed negligible differences in polymer composition.

Upon testing the fluorescent properties of the FMIPs and the no-imprinted FNIPs, the imprinting factor between FMIP1 And FNIP1, and FMIP2 and FNIP2 were compared. FMIP2 had a greater imprinting factor than FMI1 (3.7 to 2.3, respectively), and was therefore selected to further test the fluorescence response of the FMIP for naproxen against structural analogues (Figure 13a). A much higher fluorescence response to naproxen was observed compared to all of the analogues, proving that the FMIP had impressive specific recognition capabilities (Figure 13b). Additional examination of time-dependent fluorescent response showed that FMIP2 had a shortened fluorescent response time (≤1 minute) to naproxen, widening opportunities for the development of real-time detection techniques if the FMIPs were to be immobilized on a solid substrate. Increased concentration of naproxen within the FMIP2 system was then tested to identify the detection limit. While the detection limit (2 μM with a range of 10-80 μM) was comparable to the reported fluorescence spectrometry method utilized (0.5 μM with a range of 2-87 μM), efforts to increase the detection sensitivity are required for future FMIPs.

Fluorescent detection of proteins was investigated when researchers *Kubo* et al. detected bovine serum albumin (BSA) using protein imprinted hydrogels (PI gels) composed of poly(ethylene glycol)(PEG).[112] The fluorescent dye, 8-anilino-1-napthalenesulfonic acid (ANS), was incorporated into a PEG-containing monomer prior to polymerization to assist in observing the efficiency of the PI gels. The ANS moiety was found to selectively interact with the hydrophobic part in BSA due to the imprint effect. AAm and NIPAAm were then added as additional functional monomers to aid in the adsorption of BSA by the PI gels, mainly for hydrogen bonding interactions, along with 4-vinylbenzyl trimethylammonium chloride (VBTMAC) for increased ionic interaction. It was found that the ionic interactions of VBTMAC did aid in the effectiveness of BSA adsorption, but the hydrogen bonding of AAm and NIPAAm did not contribute to increased rebinding. However, excessive amount of VBTMAC randomly interacted with BSA which decreased specific binding interactions. Concentration of VBTMAC was optimized to improve adsorption selectivity and alter the fluorescence intensity. The new PEG-ANS monomer improved selectivity to BSA while still incorporating the fluorescent dye into the PI gels.

Researchers *Yang* et al. had a different approach: they investigated the development of a triple emission MIP sensor that would allow detection of folic acid solely by the emission of color.[113] The researchers successfully developed a MIP sensor that showed impressive selectivity and sensitivity in detecting folic acid within real samples while maintaining their integrity. The group first synthesized two separate MIPs templated with folic acid by encapsulating green- and red-fluorescent quantum dots (g-QD and r-QD, respectively) on the surface of SiO_2 nanoparticles and imprinting through a sol-gel reaction. The MIPs were centrifuged and washed with MeOH or ACN (dependent on g-MIP or r-MIP) to remove the templated folic acid. The green- and red-emission non-imprinted polymers (g-NIPs and r-NIPs, respectively) were prepared in the same manner as both g-MIPs and r-MIPs but without the addition of folic acid. Optimized formulations were then synthesized through a post-imprinting method of mixing varied ratios of g-QD and r-QD MIPs to make triple-emission MIP. The same was done for the NIPs.

Detection results were evaluated using a portable UV lamp to observe a blue color change corresponding to the folic acid content within the complex samples. The corresponding fluorescence between the triple-emission MIPs and NIP counterparts were also investigated. As the concentration of folic acid was increased (0 – 50 ppm), a decrease in green fluorescence (≥1 ppm) followed by a decrease in red fluorescence (≥5 ppm) was observed in the MIP. As a result of the red and green fluorescence being hindered through the uptake of folic acid in the MIP, expressed blue fluorescence intensified (Figure 14A). The ratiometric intensity chanve of red-, green-, and blue-emission peaks was found to obey a logistic function ($r^2 = 0.9988$) (Figure 14A inset) and an extremely low limit of detection (0.0052 ppm). The NIP sensor had unaffected red fluorescence with increased addition of folic acid (Figure 14B), however, green fluorescence was raised. The researchers then eliminated the effect of spectral overlap from the blue-emission peak (Figure 14B inset) and determined that the green- and red-emission peaks were weakly quenched by increasing folic acid. The NIP also did not change from yellow to a pink-purple color until the concentration of folic acid was higher than 30 ppm (Figure 14B). The researchers concluded that the MIPs contained numerous folic acid specific cavities within the imprinting layer, and that the small amount of intensity found within the NIP sensors was attributed to nonspecific adsoption.

The use of MIPs in fluorescent imaging has also recently been applied to biological applications such as bioimaging.

Wang et al. synthesized MIPs using a peptide chain (WKFKGRDIY) template, fluorescent calcium peroxide copper acrylate comonomer, NIPAAm, N,N‘-bisacrylylcystamine crosslinker, AIBN initiator, and 4-vinylpyridine.[114] The peptide chain selected is that of the CD47 extracellular domain of a cancer cell, utilizing the epitope imprinting technique discussed previously. CD47 is part of the superfamily of immunoglobin and is often overexpressed on the surface of cancer cells, making it a suitable target for the detection of cancer. After being incubated for only 4 hours, the cells displayed a notable blue fluorescence when bound with the fluorescent MIP. The MIP was able to target CD47-positive cells and perform accurate fluorescence diagnosis analysis. Increased fluorescence response was observed when CD47-positive cells were in higher concentration when combined with both the MIP and NIP, however, the MIP demonstrated much stronger fluorescence response in comparison to the NIP. Additionally, specific binding to CD47-positive cells was tested by introducing 293T (normal) cells. Although fluorescence was detected for these cells, the response was

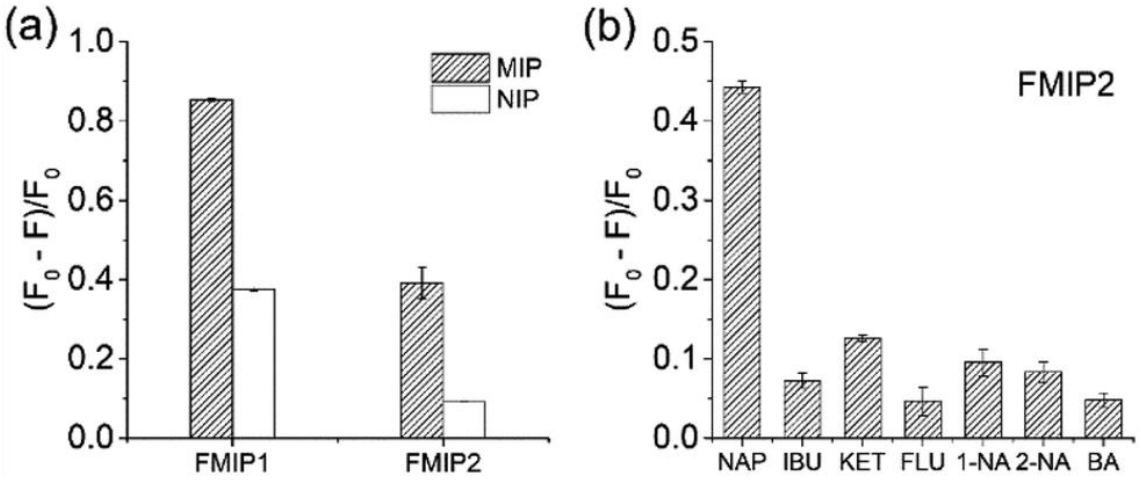

Figure 13. (a) Fluorescent response between FMIP1 and FMIP2 and the corresponding NIPs specifically for naproxen, and (b) the fluorescent response of FMIP2 to (*S*)-naproxen (NAP) and the following analogues (*S*)-ibuprofen (IBU), (*R, S*)-ketoprofen (KET), (*R, S*)-flurbiprofen (FLU), 1-napthylacetic acid (1-NA), 2-napthrylacetic acid (2-NA) and benzoic acid (BA). Reprinted and adapted with permission from Wang, F.; Wang, D.; Wang, T.; Jin, Y.; Ling, B.; Li, Q.; Li, J. A Simple Approach to Prepare Fluorescent Molecularly Imprinted Nanoparticles. *RSC Adv.* 2021, *11* (13), 7732–7737. Copyright 2021 Royal Society of Chemistry.

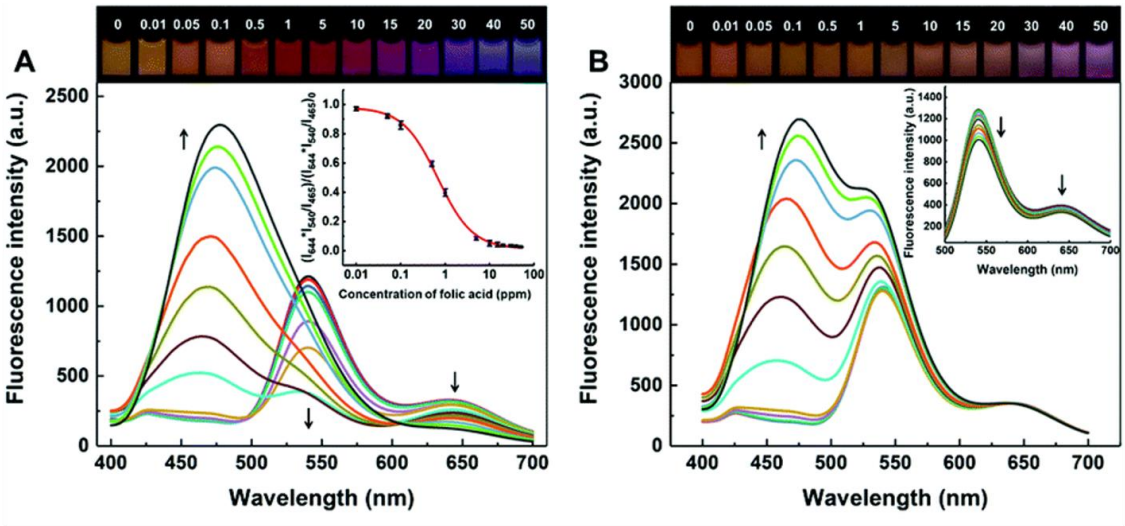

Figure 14. Fluorescence response of: (A) the triple-emission MIP with increasing concentration of folic acid from 0 to 50 ppm and the effects on red (620-750 nm), green (495-570 nm), and blue fluorescence (475-495 nm). Inset chart is the logarithmic function of the ratiometric change of intensity for green-, red-, and blue-emission peaks. (B) The triple-emission NIP counterpart, with increased concentration of folic acid from 0 to 50 ppm and the corresponding red, green, and blue fluorescence. Inset is the fluorescence response of green- and red-emission peaks with blue-emission spectral overlap removed for clarity. Reprinted and adapted with permission from Yang, Q.; Li, C.; Li, J.; Wang, X.; Arabi, M.; Peng, H.; Xiong, H.; Chen, L. Rational Construction of a Triple Emission Molecular Imprinting Sensor for Accurate Naked-Eye Detection of Folic Acid. 2020, *12*, 6529. Copyright 2020 Royal Society of Chemistry.

considerably weak. Interestingly, the MIP and NIP response to the 293T cells was indistinguishable, suggesting that the imprinted cavity had no role in the fluorescence observed with the normal cells. This means that the MIP they synthesized was not only able to recognize peptides, but that it was binding specifically to the CD47 protein located on the cancer cell membrane.

ii. Electrochemical Sensors

An avenue of MIP exploration that has great potential is that of electrochemical sensors. Aside from the preparation of MIPs being rather simple, electroanalytical chemical sensors that utilize imprinting techniques have a multitude of advantages. Most notably, modification of an electrode surface with MIP or MIP film greatly increases the selectivity of the sensor. Multi-walled carbon nanotubes (MWCNT) and metal organic frameworks (MOFs), made of metal ions and organic ligands, have been used to improve the performance and selectivity of electrochemical sensors by preventing the assembly of undesirable guest nanoparticles. *Han* et al. detected chloro-1,2-propanediol (3-MCPD) with a hybrid MOF/MWCNT MIP system.[115] 3-MCPD is a common additive in food that may be linked to lower sperm count and certain cancers. The molecularly imprinted electrochemical sensor (MIECS) developed to improve the selectivity of 3-MCPD.

The MOF/MWCNT hybrid (ZIF-65@CNTs) was developed on carboxylated MWCNT (cMWCNT). cMWCNTT/MOF-199 composites were added to the surface of glassy carbon electrodes to improve sensitivity and increase the overall surface area of the

electrode. With 3-MCPD as the template molecule and *o*-phenylenediamene (*o*-pd) as the functional monomer, the two were first joined through hydrogen bonding to form a pre-polymerization solution. Then, an electrochemical polymerization on the cMWCNTT/MOF-199 modified electrode was performed with the 3-MCPD/*o*-pd solution. Removal of the template molecule required the electrodes to soak in a methanol/acetic acid (v/v 9:1) eluent before rinsing with distilled water and drying at room temperature. With potassium gerricyanide as the redox probe, the electrochemical performance of the modified electrodes were tested. The MIP-modified electrodes had a lower observed current when compared to the electrodes that lacked MIP. This was attributed to the electron transfer on the surface of the electrode being hindered because of the non-conductive membranes leftover within the imprinted cavities. Then, during the rebinding of 3-MCPD, a lower current was observed when compared to the emptied imprinted cavities, due to the lessened conductive area of the electrode.

Cheng et al. developed molecularly imprinted chemiluminscence (MIECLS) sensors for the detection of bisphenol S (BPS) in drinking water.[116] BPS is often used as a substitute for bisphenol A (BPA) so plastics can be advertised as "BPA-free", but BPS has been found to be an environmental toxin that has disruptive effects to the endocrine system and is released into drinking water in commercial water bottles.

The researchers used a cobalt nitride (CoN) nanoarray as a metal nitride, grown on carbon cloth (CC) as a bifunctional electrocatalyst. Metal nitrides have emerged in more recent sensing applications as electrode enhancers. CoN specifically can increase electronic conductivity, provide a large specific surface area, is chemically stable, resistant to corrosion and possesses favorable catalytic activity. BPS was used as the template molecule, and the MIP was synthesized with a mixture of MMA and EGDMA. The MIECL sensor was made by immersion of the electrode surface into the MIP solution before removing the template molecule in a 9:1 rinse of methanol and acetic acid.

The morphology of the MIECL sensor was characterized by SEM (Figure 15A-D), where the CoN precursor was observed to have a needle-like nanoarray morphology (Figure 15A). The morphology was unchanged after the modification of the electrode by an ammonia nitride transformation (Figure 15B). Then, the modified electrode was evenly covered with a MIP film (Fig. 15C), and a similarly uniform distribution of the NIP membrane along the modified electrode surface was observed (Figure 15D). There was no distinguishable difference between the MIP and NIP before elution. Transmission electron microscopy (TEM) analysis confirmed the porous structure of the CoN/CC at low- and high-magnification (Figure 15E and F, respectively).

Observation of the electrochemiluminescence response showed that the MEICL sensor was sensitive to BPS in solution by a notable decrease in the electrochemiluminescence response. The MEICL sensors had a BPS-sensitive limit of detection (8.01×10^{-10} mol L^{-1}) that was comparable to current BPS detection methods, and was unaffected by the introduction of analogous molecules.

Grothe et al. investigated the detection of the molecule 4-amino benzoate (BZC).[117] BZC is known as a local anaesthetic that is commonly used in dentistry, but has recently been traced as a common additive circulating in drug misuse. Using electropolymerization, an electrochemical MIP (e-MIP) using BZC as a template molecule and 3-amino-4-hydroxybenzoic acid (3,4-AHBA) as a functional monomer was synthesized. In addition to functioning as the functional monomer, 3,4-AHBA was necessary for preventing oxidation of BZC. After optimization of the e-MIP cyclic voltammetry cycles, applied scan rate of electropolymerization, and incubation time, theoretical studies were performed to reduce experimental time. A realistic simulation of the e-MIP synthesis was developed by a combined Monte Carlo-Quantum Chemistry protocol. Results of the e-MIP confirmed the presence of BZC in human urine, but more data will be necessary to apply the e-MIP to *in situ* rapid identification of various drugs within mixed samples. The group worked to optimize the sensors performance and hoped to create a long-term stable e-MIP that is available to the public.

iii. Magnetic Response

Fe_3O_2 nanoparticles are an emerging platform to produce surface imprinted MIPs. Along with the inherent benefits of being surface imprinted, MIPs with superparamagnetic Fe_3O_2 cores offer enhanced retrievability *via* an external magnet. This enhances the template removal process by removing time consuming processes such as centrifugation or filtration and eliminates the need for organic solvents (application dependent). The combination of magnetic retrievability from the nanoparticle core with imprinted surfaces that are highly specific to sought after targets allow for magnetic MIPs to be highly useful for applications in extraction and separation fields. Sought after targets include toxic and carcinogenic hexavalent chromium, microbial biomarkers and flavonoids that would otherwise be discarded with agricultural waste.

Kumar et al. investigated targeting one of these toxic compounds *via* the selective adsorption of Cr(VI) from wastewater using a dichromate ion-imprinted amine functionalized silica-capped iron oxide nanoparticle.[118]

The MIPs utilized a dichromate ion template and implemented a coprecipitation polymerization method. The reaction mixture consisted of 4-vinylpyridine as a ligand, methyl methacrylate (MMA) as a functional monomer, EGDMA as a crosslinking agent and AIBN as an initiator. Selectivity of Cr(VI) MIPs was investigated through batch sorption experiments in a binary phase solution system with various competitor ions of comparable atomic radii. A greater distribution coefficient and selectivity coefficient for Cr(VI) was observed when compared to all other ions under study. The high selectivity of Cr(VI) was attributed to complexation with the binding site. The Cr(VI) imprinted MIP effectively removed over 96% of spiked Cr(VI) ions from real water samples from a local canal.

Another example of magnetic MIPs comes from *Rajpal* et al. who developed MIPs for the detection the *Pseudomonas aeruginosa* biomarker pyocyanin in various biological media, with applications in the culture free detection of *P. aeruginosa.*[28] To produce such MIPs, they first prepared silica modified Fe_3O_2 nanoparticles with tetraethoxysilane. The modified nanoparticles were then functionalized with 3-methacrylooxypropyltrimethoxysilane to display vinyl end groups for later polymerization of various monomers and crosslinkers in the presence of pyocyanin. The imprinting factor was measured for four combinations of monomers

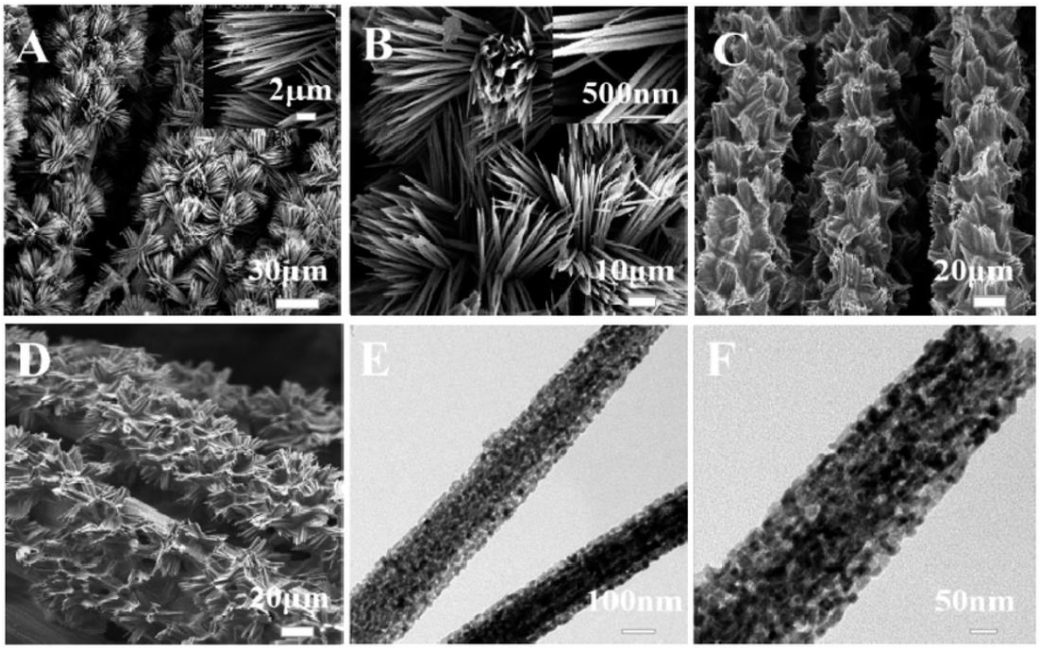

Figure 15. SEM images of (A) the CoN precursor at 30μm with inset of 2μm magnification, (B) the CoN/CC at 10μm with inset of 500nm magnification, (C) MIP with probe(Ru(bpy)$_3^{2+}$/Nafion) /CoN/CC prior to elution, (D) NIP with probe (Ru(bpy)$_3^{2+}$/Nafion)/CoN/CC prior to elution, (E) low-magnification TEM image of CoN/CC, (F) high-magnification TEM image of CoN/CC. Reprinted with permission from Cheng, R.; Ding, Y.; Wang, Y.; Wang, H.; Zhang, Y.; Wei, Q. A Novel Molecularly Imprinted Electrochemiluminescence Sensor Based on Cobalt Nitride Nanoarray Electrode for the Sensitive Detection of Bisphenol S. *RSC Adv.* 2021, *11* (18), 11011–11019. Copyright 2021 Royal Society of Chemistry.

and crosslinker. MIPs prepared with a methacrylic acid(MAA) monomer and divynyl benzene(DVB) crosslinker had an imprinting factor of 5.0, which is impressive considering the next highest imprinting factor was found to be 1.301 for an MIP prepared with acrylamide(ACR) monomer and DVB crosslinker. The DVB-MAA-MIPs were able to detect pyocyanin in bacterial cell culture as well as various buffers. Batch sorption experiments with pyocyanin and other structurally similar molecules revealed specificity of the MIPs for pyocyanin.

As agricultural industries produce waste often containing useful compounds, efforts to extract these compounds are also in development. For example, *Tian* et al. developed a hierarchically assembled magnetic MIP to sequester leutolin (LTL), a flavonoid with various pharmacological applications.[119]

In a layer-by-layer assembly, polyethylenimine (PEI) was first attached to amino modified Fe_3O_2 nanoparticles. The template, LTL, was then immobilized on the surface of the generated Fe_3O_4-PEI nanoparticles before attaching a polydopamine imprinting layer. Subsequent elution of the LTL templates left behind the layer-by-layer assembled MIP whose imprinting layer resembles a thin sandwich. This layer-by-layer assembly offers more orderly and evenly distributed imprinted sites as well as improved adsorption capacity for LTL when compared with a copolymerized MIP. Additionally, the layer-by-layer assembled MIP showed much shorter adsorption equilibrium times compared to related LTL-imprinted polymers, higher maximum LTL adsorption compared to precursor Fe_3O_2-NH_2 nanoparticles as well as selectivity for LTL compared to four small molecules with structural similarities. The adsorption selectivity of Fe_3O_4-LTL-MIPs for LTL and the four analogous compounds was compared to that of the Fe_3O_4-NIPs (Figure 16). The MIPs showed a higher adsorption capacity for LTL (66.03 μmol g^{-1}). The MIPs also demonstrated a high imprinting factor (3.94) which suggested great selectivity of the MIPs towards LTL versus the NIPs. Importantly, the MIPs successfully sequestered LTL from a more chemically complex sample; honeysuckle leaf extract. The superparamagnetic Fe_3O_4-NH_2 core allowed for a magnetic separation compared to other LTL-MIP detection methods, which commonly use centrifugation or filtration in combination with longer preparation times and higher temperatures to retrieve LTL.

MIPs with magnetic functionality are becoming increasingly useful with applications of sensing as well as retrieval of compounds from complex media for both bioremediation and resource preservation from otherwise discarded products.

Summary and Outlook

The “toolbox” for MIP chemistry is rapidly expanding, with notable advancements in the preparation of MIPs with high affinity and sensitivity towards target molecules. Recent literature in the MIP field has improved the overall understanding of fundamental receptor-target interactions and broadened application potentials of MIPs into fields such as biomedical sciences and environmental remediation.

Recent advancements in the preparation of MIPs has moved from traditional radical polymerization towards controlled radical polymerization techniques. Synthetic choices such as the use of RDRPs allow for the preparation of more homogenous MIP samples. The use of RDRPs as well as employing PIMs enhances specificity towards target molecules.

Furthermore, the inclusion of core-shell architecture in MIPs has allowed for major improvements in the field. These include surface

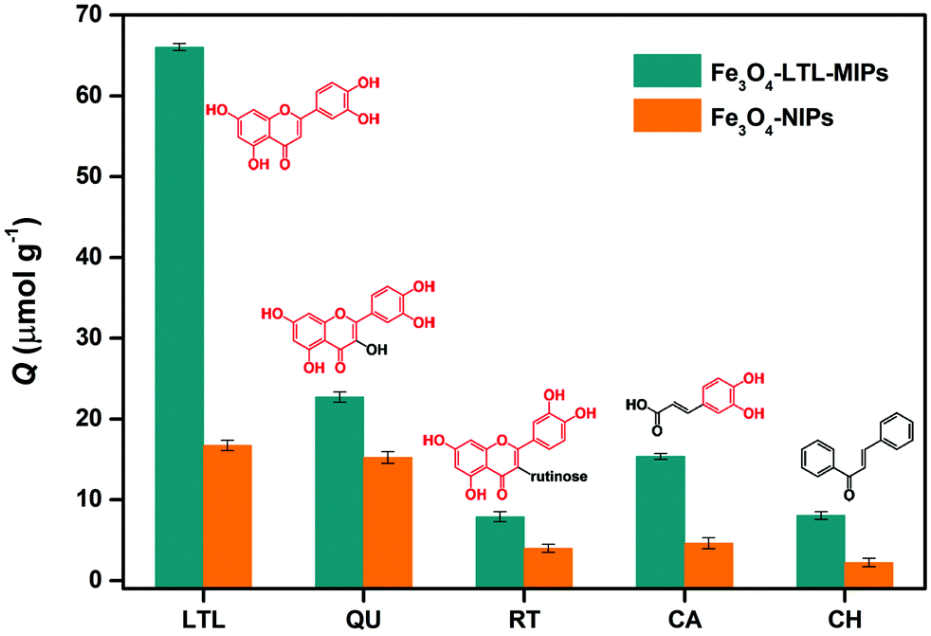

Figure 16. Adsorption selectivity of Fe_3O_4-LTL-MIPs and Fe_3O_4-NIPs for the template molecule luteolin (LTL), analogous compounds to the template quercetin (QU) and rutin (RT), and coexisting competitors of the template caffeic acid (CA) and chalcone (CH). Reprinted with permission from ian, X.; Gao, R.; Wang, Y.; He, Y.; Hussain, S.; Heinlein, J.; Tian, J.; Pfefferle, L. D.; Tang, X.; Tang, Y. Layer-by-Layer Assembled Magnetic Molecularly Imprinted Nanoparticles for the Highly Specific Recovery of Luteolin from Honeysuckle Leaves. *Green Chem.* 2021, *23* (10), 3623–3632. Copyright 2021 Royal Society of Chemistry.

imprinting which enhances removability of target molecules after MIP preparation through enhanced mass transfer, solid-phase imprinting which broadens the pool of compatible solvents, and magnetic retrievability of the MIPs whose cores are superparamagnetic. Additionally, solid phase imprinting and magnetic retrievability both improve material recovery. Through enhancements in recovery of MIPs and reversible bonding of bound target molecules, the lifetime of MIPs is being extended.

The overall application of MIPs fit into an array of research areas but follow similar patterns in the targeted detection and removal of substances within complex mixtures. Drugs like naproxen have been imprinted and detected in real water samples by MIPs with fluorescent capabilities. In an epitope-type imprinting approach, peptide chains found on cancer cells have been imprinted into MIP cavities for later fluorescent imaging and were distinguishably selective towards the entirety of the cancer cells compared to the normal cells. Through surface imprinting onto the sensor, MIPs have been used to improve electrochemical sensor sensitivity by aiding in the selectivity directly at the electrode. The improved sensors have comparable, if not lower limits of detection for targeted molecules extracted from real samples. MIPs that facilitate a magnetic response are of increased interest in physically separating toxic substances from real samples and have also been reported to recover valuable substances from waste production.

There are still considerable milestones that require attention before such synthetic materials replace their biological equivalents for complex molecular recognition.

Expansion is necessary in the overall selection of functional monomers and crosslinkers that are compatible with MIP preparation techniques. By widening the list of monomers and crosslinkers available for this process, future imprinting will be able to handle more sensitive and biocompatible template molecules. As a result, the library of template molecules imprinted into MIPs following an epitope imprinting approach may see a tremendous increase in the next decade.

The future of MIP adaptation will need to continue moving towards more post-imprinting techniques, where functionality is added after the imprinting process to not only increase selectivity, but to adjust the properties of the polymers. Solubility, sensitivity, selectivity, and other adjustments can then be made onto the MIP to increase application. On a similar note, the recyclability of MIPs is an important feature allowing for a future with more sustainable synthetic practices. Future work in this field will likely see a greater emphasis on material recyclability where a multitude of rebinding moments may be observed before the MIP or template molecule is no longer retrievable.

With these areas in mind, MIPs have gained considerable traction in the last decade, where the generation of more intricately detailed and fine-tuned materials is surely underway.

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