Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Present state of MIP-based sensors for SARS-CoV-2

Aysu Yarmana,b, Sevinc Kurbanoglu,c and Frieder W. Schellera

a University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany, bMolecular Biotechnology, Faculty of Science, Turkish-German University, Sahinkaya Cad. 86, 34820 Beykoz, Istanbul, Turkey, cAnkara University, Faculty of Pharmacy, Department of Analytical Chemistry, Yenimahalle, Ankara, Turkey

1.1 Introduction

The outbreak of a COVID-19 was first reported in December in Wuhan, China, and since then, it has spread worldwide. WHO officially declared the pandemic on 12th March 2020. Therefore, its fast and accurate diagnosis of the virus is essential to prevent its spread. Diagnosis of COVID-19 uses three different approaches: i) viral gene detection, ii) detection of human antibodies, and iii) viral antigen assays, which have different fields of applications [1–3]. Up to now, detection of the viral genome by real-time reverse transcription-polymerase chain reaction (RT-PCR) is the most reliable procedure. Furthermore, it offers the detection of the uprising incidence by mutants of the virus. Antibody indication is the key in tracing the effect of vaccination and the immune response during infection. On the other hand, the indication of segments of the virus, so-called antigens, is the basis of the decentralized test (Point-of-Care) by dipsticks and lateral flow devices. Most immunoassays indicate the spike protein (S protein) or the nucleocapside protein (NP protein). The S protein is the binding place of the virus to the host receptor, the angiotensin-converting enzyme (ACE2), for entering the host cell (Fig. 1.1). This interaction has an affinity in the lower nanomolar range, and it was predicted from docking simulations.
that the peptide chain starting with Leu455, Phe486, to Tyr505 is involved in the binding of the receptor binding domain (RBD) of the S protein to ACE2 [4].

### 1.2 Molecularly imprinted polymers

The most important drivers in the field of molecular imprinting are Wulff and Mosbach ([58]; [59]). Nevertheless, the concept dates back to the 1930s, when Polyakov demonstrated specific adsorption properties of silica gel that recognized its target methyl orange ([60]).

MIPs are prepared by the copolymerization of functional monomer(s) and the target analyte (so-called template) in the presence or absence of cross-linkers, as demonstrated in Fig. 1.2 [5]. In the first step, a prepolymerization complex is formed. Template removal with an appropriate solvent leads to the formation of cavities, which mirror size, shape, and sometimes the functionality of the template molecules with a “molecular memory.” These cavities in the polymer network mimic the active sites of biomolecules like enzymes and antibodies. In nature, molecular recognition occurs via a whole spectrum of 20 amino acids, whereas MIPs are prepared by one up to six functional monomers.

Two main approaches have been used depending on the interaction between the template and functional monomers to form a prepolymerization complex. In the covalent approach, reversible covalent bonds are formed between the template molecule and functional monomer, which must be cleaved and reestablished during the removal and rebinding steps. The interaction is highly specific, and homogenous binding sites are obtained; nevertheless, it has been applied for only a limited number of analytes. By contrast, the noncovalent approach relies on weaker noncovalent
interactions such as hydrogen bonds, van der Waals forces, ionic bonds, and hydrophobic interactions. Since, in nature, most of the molecular recognition is based on noncovalent interactions, this approach is called the biochemist’s approach, whereas the covalent approach is known as the chemist’s approach. It serves a broader spectrum of analytes in comparison to the covalent approach. However, the yield of the binding sites is lower ([61]; [62]).

Various methods have been described for the MIP preparation (Table 1.1; [6]). Among them, bulk polymerization finds wide application. It results in monolithic structures, which must be subsequently grounded and sieved. Moreover, it is time-consuming, and slow binding kinetics are obtained. Alternatively, different MIP preparation methods, like suspension, precipitation, emulsion polymerization, surface imprinting techniques (microcontact-printing and electropolymerization), have been applied [7].

Due to structural complexity and fragility, the most successful results have been achieved for low-molecular weight templates compared to biomolecules such as proteins, nucleic acids, viruses, bacteria, and cells [8–21]. The common imprinting methods can disturb partially or completely their 3D structures. Furthermore, larger pores obtained after template removal can result in poor selectivity. To overcome these obstacles, only a small peptide portion or a fragment like a subunit has been described for larger molecules. This approach has been introduced for the
TABLE 1.1 General advantages and disadvantages of different types of polymerization types.

| Polymerization type | General advantages and disadvantages |
|---------------------|--------------------------------------|
| Bulk                | ✓ Simple and universal type of polymerization.  
                        ✓ No need for sophisticated instrumentation.  
                        ✓ Obtaining spherical materials.  
                        ✓ Providing reproducible results  
                        ✓ Allowing a large-scale examination of products.  
                        ✓ Requiring lengthy procedures.  
                        ✓ Resulting in irregularity in size and shape.  
                        ✓ Low performance. |
| Precipitation       | ✓ Providing uniform size and high yields of imprinted materials  
                        ✓ Creating homogeneous binding sites.  
                        ✓ One of the easiest and well-suited type with a high dilution factor.  
                        ✓ Requirement for a polymerization mixture in the presence of a much higher amount of porogen maker.  
                        ✓ The growing polymer chains are unable to occupy the entire volume. |
| Suspension          | ✓ An organic-based medium is mixed with an excess of water and the amount of suspension stabilizer.  
                        ✓ Two phases are mixed by stirring to form a suspension of organic droplets in the aqueous phase.  
                        ✓ The imprinted materials are scarce because water might disrupt noncovalent interactions between the template molecule and the monomers. |
| Multistep swelling  | ✓ Producing mono-disperse and outstanding materials with controlled diameter.  
                        ✓ The size of the imprinted materials might be controlled by changing the polymerization conditions.  
                        ✓ Requiring complex and long polymerization conditions.  
                        ✓ Requiring laborious procedure and aqueous emulsions. |
| Surface             | ✓ Producing mono-disperse materials and thin imprinted layers.  
                        ✓ Creating more accessible binding sites.  
                        ✓ Allowing rapid binding and high desorption rates.  
                        ✓ Providing more effective ability to recognize the template molecules.  
                        ✓ Providing a large specific surface area for the particles, hence leading to excellent a nity and selectivity.  
                        ✓ Requiring a complicated system and time-consuming procedure. |
| In situ             | ✓ Requiring a single-step preparation strategy.  
                        ✓ Being a cost-friendly fashion.  
                        ✓ Providing a, well-porous structure.  
                        ✓ Requiring a comprehensive and lengthy optimization procedure that needs to be optimized for every template molecules systems. |

Reprinted with permission [6]
first time by Rachkov and Minoura and called epitope imprinting because of the similarity to the biological determinants ([22–28]a).

Two main approaches have been described for the preparation of MIP-based sensors. In the first approach, the MIP is prepared in-situ direct on the transducer, whereas, in the second approach, MIPs have to be integrated on the surface of the transducer using several methods like spin-coating, drop-coating, layer-by-layer assembly, or grafting.

### 1.3 MIP sensors for SARS-CoV-2

In addition to the broad spectrum of MIPs for small molecules and proteins, the recognition of virus particles has been described in the literature [13]. Table 1.2 summarizes the analytical parameters of MIP-sensors for pathogenic viruses. The majority of these MIPs were prepared using the virus particle as the template. Among them, a multifunctional MIP allows the simultaneous visual indication of Hepatitis A and Hepatitis B viruses by evaluating the different color signals of two quantum dots [29]. In another work, subtypes of Influenza could be detected by individual MIP-QCM sensors with high specificity down to $10^{-5}$ particles per mil [30].

On the other hand, MIP-sensors for the dengue virus and HIV, respectively, used the epitope approach for the preparation of MIPs [31]. A mixture of a 35 amino acid peptide of the immunodominant region of the HIV-1 related glycoprotein 41 (gp41) and dopamine was “self” polymerized on a QCM-chip. The MIP bound both the peptide and the parent protein gp41 in the lower nanomolar concentration range with a LOD of 2 ng/mL for the protein. The authors claim satisfactory results for the detection of gp41 in human urine [31].

Parisi et al. realized the idea to develop “monoclonal-type” plastic antibodies based on MIPs to recognize the RBD of SARS-CoV-2 to block the binding of the spike protein to the ACEII-receptor [32]. They developed noncovalent imprinting MIP-nanoparticles with a diameter of around 80 nm and an imprinting factor for the RBD of 7. Specificity of the MIP was demonstrated by the neglectable binding of the RBD of SARS-CoV-2. The final aim is the intravenous application by blocking the binding of the virus spike to the ACE II-receptor, as shown in Fig. 1.3 [33].

The broad spectrum of transducers applied in biosensors has also been used in MIP-based sensors. Among them, the electrochemical and optical readout is the most frequently applied in comparison with quartz crystal microbalance [34] piezoelectric [35,36], thermal [37], and micromechanical [38] transducer. Herein we focus on these two most frequently used transducers since the MIP-sensors developed since the outbreak of pandemic are based on these techniques. In following sections we concentrated on
### TABLE 1.2 Molecular imprinting of some pathogenic viruses.

| Viruses                     | Functional monomers                                      | Cross-linkers | Templates                               | Method                          | Linear range                | LOD/maximum detected concentration | Application       | Ref.                                      |
|-----------------------------|----------------------------------------------------------|---------------|-----------------------------------------|--------------------------------|-----------------------------|-------------------------------------|-------------------|------------------------------------------|
| Tobacco mosaic viruses      | p,p' -diisocyanatodiphenylmethane containing 30% triisocyanate and bisphenol A | NS            | tobacco mosaic virus                    | Quartz crystal microbalance    | 100 ng mL\(^{-1}\) – 1 mg mL\(^{-1}\) | NS                    | Tobacco plant sap  | Dickert et al. [39]                      |
| Dengue virus                | Acrylamide and acrylic acid and N-benzylacrylamide       | NS            | The dengue virus NS1 protein            | Quartz crystal microbalance    | NS                          | NS                    | NS               | Tai, Lin, Wu, and Chen [40]              |
| Dengue virus (flavivirus)   | Acrylamide and acrylic acid and N-benzylacrylamide       | NS            | The dengue virus NS1 protein            | Quartz crystal microbalance    | 0.85 ng.Hz\(^{-1}\)          | Serum                 | Serum            | Tai, Lin, Wu, Huang, and Shu [41]        |
| Picornaviruses              | Polyurethane and bisphenol A                             | Phloroglucinol| Human rhinovirus serotype 2 (hrv2), Hrv14, Hrv1A | Quartz crystal microbalance    | 5.4 × 10(10) virions         | NS                    | NS               | Jenik et al. [42]                         |
| Hepatitis B virus           | N-Methacryloyl-L-tyrosine methyl ester and hydroxyethyl methacrylate | Ethylene glycol dimethacrylate | HBV antibody                           | Surface plasmon resonance      | NS                          | 21.4 mIU.mg\(^{-1}\)      | Serum            | Uzun, Say, Ünal, and Denizli [43]        |
| Human immunodeficiency virus| Polydopamine                                             | Polydopamine  | HIV-1 gp41                              | Quartz crystal microbalance    | 5–200 ng.mL\(^{-1}\) – 2 ng.mL\(^{-1}\) | NS                    | Human urine       | Lu et al. [31]                            |

(continued on next page)
### 1.3 MIP sensors for SARS-CoV-2

| Virus                          | Materials                                                                 | Antibody/Protein                        | Assay/Technique                             | Sensitivity | Ref.                                      |
|-------------------------------|---------------------------------------------------------------------------|-----------------------------------------|---------------------------------------------|-------------|-------------------------------------------|
| Influenza virus               | Acrylamide and methylimethacrylate and N-vinylpyrrolidone and methylacrylic acid | H1N3, H6N1, H5N1, H5N3 & H1N1          | Quartz crystal microbalance                 | NS          | Wangchareansak et al. [30]                |
| Human immunodeficiency virus  | 3-Aminobenzeneboronic acid                                               | 3-Aminobenzeneboronic acid             | Electrochemiluminescence                    | 1:20,000–1:50 anti-HIV-1 dilution ratios | Serum         | Zhou et al. [44]                          |
| Japanese encephalitis virus   | 3-Aminopropyltriethoxysilane                                             | Freeze-dried JEV vaccine               | Förster resonance energy transfer          | 2.5–45 nM   | Human serum                               | He et al. [45] |
| Japanese encephalitis virus   | 3-Aminopropyltriethoxysilane                                             | Tetraethoxysilicate                    | Fluorescence resonance energy transfer     | 24–960 pM   | Human serum                               | Liang et al. [46] |
| Hepatitis A virus             | Polydopamine                                                              | Polydopamine                            | Resonance light scattering                 | 0.04–6.0 nM | Human serum                               | Yang, Gong, Chen, Chen, and Cai [47] |
| Swine fever virus             | Acrylamide, methacrylic acid, methyl methacrylate and N-vinylpyrrolidone | Swine fever (Hog cholera) live vaccine | Quartz crystal microbalance                | 4–21 μg.mL⁻¹ | Klangprapan, Chokearpornchai, Lieberzeit, and Choowongkomon [48] |
| Dengue virus                  | Acrylamide, methacrylic acid methyl methacrylate, and N-vinylpyrrolidone | N,N′-(1,2-dihydroxyethylene) bisacrylamide | Dengue virus stamps                         | 10–10³ pfu mL⁻¹ | NS                                       | Navakul et al. [34] |

Reproduced with permission [49]
1. Present state of MIP-based sensors for SARS-CoV-2

FIGURE 1.3 Schematic representation of the interaction between Molecularly Imprinted Polymers (MIP)-based “monoclonal-type” plastic antibodies and SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Reprinted with permission [33].

FIGURE 1.4 Main approaches used in an electrochemical readout of MIP-based sensors. Reprinted with permission [5].

very first examples and more information about the recent studies can be seen from Table 1.3.

1.3.1 Electrochemical MIP-sensor for SARS-CoV-2 nucleoprotein

The electrochemical readout exploits three main transduction principles, which we recently reviewed (Fig. 1.4). Most research is based on the “gating effect” of a redox marker like ferri-/ferrocyanide, ferrocene, or ruthenium complexes [50,5]. After template removal, cavities are formed in the polymer layer, through which the redox marker permeates and reaches
### TABLE 1.3  MIP-based biomimetic sensors for SARS-CoV-2 detection.

| Template                           | Monomer                          | Transducer          | Detection Method | Linear range          | LOD          | Application                          | Ref.  |
|------------------------------------|----------------------------------|---------------------|------------------|-----------------------|--------------|-------------------------------------|-------|
| SARS-CoV-2 whole virus             | 3-AP                             | CNT/WO\textsubscript{3}-SPCE | EIS              | 7 to 320 pg/mL        | 57 pg/mL     | Nasopharyngeal swabs                | [63]  |
| SARS-CoV-2 whole virus             | NHMA MBAm (cross-linker)         | SPE                 | EIS              | 3 –7 log\textsubscript{10} pfu/mL | 4.9 log\textsubscript{10} pfu/mL | Saliva samples                     | [64]  |
| SARS-CoV-2 whole virus             | AAM, MAA, MMA, and NVP; DHEBA (cross-linker) | GO integrated Ag-SPE | CV               | 0.01 fM to 100 fM     | 0.1 fM       | Wastewater                          | [65]  |
| SARS-CoV-2 nucleoprotein           | m-PD                             | 4-ATP-modified Au-TFE  | DPV              | Up to 111 fM          | 15 fM (in lysis buffer) | Nasopharyngeal swab               | [2]   |
| SARS-CoV-2 nucleocapsid protein    | Arginine                         | Au/Gr modified SPCE | DPV              | 10.0-200.0 fM         | 3 fM         | Artificial nasal and saliva samples | [66]  |
| SARS-CoV-2 spike protein           | Pyrrole                          | Pt Electrode        | CA               | 0 μg/mL to 25 μg/mL   | NS           | NS                                  | [67]  |
| SARS-CoV-2 RBD                     | o-PD                             | MP-Au-SPE           | EIS              | 2.0 pg.mL\textsuperscript{-1} -40 pg.mL\textsuperscript{-1} | 0.7 pg.mL\textsuperscript{-1} | Saliva samples                     | [68]  |

*(continued on next page)*
| Template | Monomer | Transducer | Detection Method | Linear range | LOD | Application | Ref. |
|----------|---------|------------|------------------|---------------|-----|-------------|------|
| SARS-CoV-2 spike protein subunit S1 | APBA | 4-ATP-modified Au-TFME | SWV | 0-200 fM | 15 fM (in PBS) and 64 fM (patient’s nasopharyngeal samples) | Nasopharyngeal swab | [69] |
| SARS-CoV-2 spike protein subunit S1 | Aam, TBAm, and HEMA; BIS (cross-Linker) | POF-based SPR chip | SPR | NS | 0.058 μM | Nasopharyngeal swabs in universal transport medium and physiological solution (0.9% NaCl) | [57] |
| SARS-CoV-2 spike protein RBD epitope (GFNCYFPLQ) | Scopoletin | Au- SPRi chips | SPR | NS | | Artificial saliva samples | [70] |

3-AP: 3-aminophenol; AAM: Acrylamide; APBA: 3-aminophenyl-boronic acid; Au-TFME: Thin-film Au metal electrodes, BIS: N,N’-methylene bisacrylamide; CA: Chronoamperometry; DHEBA: N,N’-(1,2-dihydroxy-ethylene) bisacrylamide; HEMA: 2-hydroxyethyl methacrylate; MAA: Methacrylic acid; MBA: N,N’-methylethacrylamide; MMA: methyl methacrylate; m-PD: m-Phenylenediamine; NHMA: N-hydroxymethylacrylamide; NS: Not stated; NVP: N-vinylpyrrolidone; o-PD: o-Phenylenediamine; SPCE: screen-printed carbon electrode; TBAm: N-t-butylacrylamide
the electrode. This procedure is mainly suitable for redox-inactive analytes (from small molecules like drugs, toxins, pesticides to biomacromolecules like proteins, nucleic acids, and viruses) or even for the majority of redox proteins where direct electron transfer is not possible due to steric hindrance of the redox-active center. In contrast, the binding of electroactive analytes direct generates a faradaic current [5]. Hence the measurement is based on the direct electron transfer between the template and electrode. The third measurement principle relies on the detection of the signal generated by catalytically active analytes ([51b; [52,53]).

Among different electrochemical detection methods in MIP-based sensors, voltammetry is the most frequently used electrochemical readout method compared to potentiometric sensors like field-effect transistors or capacitors. In voltammetry, where the current is recorded as a function of potential, the effect of oxygen evolution and hydrogen generation should be considered. Moreover, false-positive results can be obtained in the presence of electroactive interfering substances, which can be suppressed by the application of more sensitive methods like differential pulse voltammetry (DPV) and square wave voltammetry (SWV) by the eliminating charging current [7,5].

The first biomimetic sensor described since the outbreak of Covid-19 utilized an electrochemical transducer, which targeted nucleoprotein (ncovNP) [2]. The sensor was prepared using electropolymerization. It can be prepared under mild conditions directly on the transducer, eliminating additional integration steps [54]. The use of cross-linkers is not needed. Further, the electrosynthesis is fast, and desired thickness can be achieved by controlling the charge passed through the electrode during polymerization. Controlling the thickness is one of the crucial parameters, especially for imprinting biomacromolecules, since the template molecules may be fully entrapped in the polymer matrix thus, hindering their removal and rebinding.

To obtain a more homogenous surface, the target analyte or their epitopes can be immobilized on the surface prior to electropolymerization. This method is called hierarchical imprinting and is more time-consuming as compared to random imprinting, which uses mixtures of the template and the functional monomer(s).

For the preparation of the ncovNP-MIP first, a self-assembled monolayer of 4-aminothiophenol (4-ATP) was formed on a gold thin film electrode (Au-TPE). Then it was incubated in DTSP solution for covalent immobilization of the template, ncovNP. As seen from the CVs, almost no current suppression was observed after incubation in 4-ATP, whereas significant signal suppression was obtained by subsequent immobilization of DTSP and ncovNP, respectively. According to molecular docking and quantum chemical calculations of the interactions between the ncovNP and the three candidate monomers m-phenylenediamine (mPD), dopamine, and EDOT, mPD was chosen as the optimal functional
monomer. The film was electrodeposited by polarizing the Au-ATP-DTSP-ncovNP electrode at 0.6 V with an optimized charge density. As seen from Fig. 1.5, after electropolymerization, further signal suppression was obtained, which is due to the formation of the nonconductive film. Subsequent removal of the template molecules with an ethanolic solution of 2-mercaptoethanol and 10% acetic solution led to an increase in the redox marker’s signal due to the formation of free cavities [2]. It was postulated that hydrogen bonds are formed between the accessible proton acceptor groups of the protein and the amino groups of mPD in the prepolymerization complex (Fig. 1.6; [2]).

Samples were prepared in lysis buffer (pH 7.2) containing 27.5 mM of Tris-HCl, 12.5 mM of EDTA, 1.5% (v/v) of TritonX-100, and 0.1% SDS diluted with MQ water to obtain the desired concentration. Rebinding of the template was measured in ferri/ferrocyanide solution by applying DPV (Fig. 1.7). The sensor showed a linear response up to 110 fM with a LOD and a LOQ of 15 fM and 50 fM, respectively, which are in the clinical range.

To illustrate the selectivity of the developed ncovNP-MIP sensor various proteins (of SARS-Cov-2 spike protein: S1, 75 kDa, pI: 6.0; Cluster of Differentiation 48: CD48, 22 kDa, pI:9.3; Hepatitis C virus surface viral antigen: E2 HCV, 47 kDa, pI: 8.2; bovine serum albumin: BSA, 66 kDa, pI: 4.7) with different size, molecular weight, and isoelectric point (pI) were studied. As seen from Fig. 1.7, the highest response was obtained for the target analyte ncovNP compared to the interfering proteins.
Moreover, the performance of the sensor was applied in nasopharyngeal swab specimens. The calibration graph was prepared first in spiked COVID-19 negative samples. The sensor had a pseudo linear response in the range of 0.22 fM and 333 fM. LOD and LOQ were calculated to be 27 fM and 90 fM, respectively. The sensor was further applied to COVID-19 positive patients. The performance of the MIP sensor was compared with RP-PCR, and a correlation was found. It was claimed that it could be applied in a portable sensing platform.

In a proof-of-concept study, we applied the epitope approach by utilizing a peptide from the RBD as the template for the electrosynthesis of MIPs for the S-protein [55]. After chemisorption of the heptapeptide from the receptor-binding domain of the spike protein of SARS-CoV-2 on the gold electrode, electrodeposition of the polymer, and template removal, both the target peptide and the RBD were bound in the lower nanomolar concentration range.

1.3.2 SPR-based MIP-sensor for SARS-CoV-2

Optical readout relies on the measurement of the optical changes like refractive index, fluorescent, and chemiluminescent upon the interaction of the (bio)recognition element with the target analyte.
Surface plasmon resonance (SPR) is an optical phenomenon, which is frequently used in optical MIP-sensors. It is based on the measurement of refractive index changes near the sensor surface ([9,56]; Fig. 1.8). It enables label-free and in-situ detection of the binding event, including the determination of the binding affinities and kinetics.

Cennamo et al. recently described the first prototype of a plastic optical fiber (POF)-based SPR sensor using a MIP for the specific recognition of the S1 subunit of SARS-CoV-2 spike protein [57]. To check and optimize the process, bovine serum albumin was first used as a template. The protocol
was then transferred for the preparation of a MIP for the S1 subunit of SARS-CoV-2 spike protein. The MIP was prepared as follows: acrylamide, N-t-butyl acrylamide, 2-hydroxyethyl methacrylate were mixed with a molar ratio of 1:0.5:0.6, in 15 mM phosphate buffer (pH 7.4.) The final concentration of N,N′-methylene bisacrylamide in the monomeric mixture was 0.19 M. After the mixture had been ultrasonicated and degassed with nitrogen, the template was added. Finally, APS (0.08% w/v) and TEMED (0.06% w/v) were added. 50 μL of the final solution was dropped on the previously allythiol modified D-shaped POF-covered gold SPR surface and polymerized. Polymerization was ended by washing the sensor with Milli-Q water. Template molecules were removed with trypsin and SDS. Fig. 1.9 illustrates the response of the bare electrode of the MIP before and after template removal. Fitting the data obtained upon the rebinding of the template to Hill equation, LOD and affinity constant were calculated to be 0.058 μM$^{-1}$ and 2.318 μM$^{-1}$, respectively.

The authors further investigated the specificity of the developed sensor in a universal transport medium using MERS-CoV Spike S1 protein, which results in almost no detectable signal. Moreover, the sensor was applied to detect SARS-CoV-2 virions obtained by nasopharyngeal (NP) swabs in UTM and physiological solution (0.9% NaCl).

### 1.3.3 First commercial MIP-sensor for SARS-CoV-2

MIP diagnostics developed MIP–nanoparticles for the recognition of the spike-protein using the receptor-binding domain (RBD) as the template ([https://www.mip-dx.com/covid19-nanomip](https://www.mip-dx.com/covid19-nanomip)). The RBD region of the spike glycoprotein is unique to SARS-CoV-2, and therefore the nanoMIP will not detect other human coronaviruses but should detect the current variants of SARS-CoV-2. The nanoMIPs are within the 40–80 nm range and allow for simple integration into sensor platforms because they can be readily functionalized with an amine group which enables attachment of a label of choice or on the sensor surface. The Nano-MIPs have been
1. Present state of MIP-based sensors for SARS-CoV-2

FIGURE 1.9 (A) Blue resonance peak: bare gold surface before the functionalization. Red resonance peak: red-shifted resonance due to the MIP layer before template extraction with
covalently coupled to an electrode surface and integrated into a thermal resistance sensor platform. Sensitivity has been demonstrated to be below 5 fg/mL for the RBD with an affinity constant (KD) ranging from 2 to 18 nM.

1.4 Conclusions

The general motivation to develop biomimetic recognition elements like aptamers and MIPs is the substitution of antibodies in diagnostics, therapy, and downstream processing. The recently published European Union Reference Laboratory for alternatives to animal testing: “Recommendation of Non-Animal-Derived Antibodies” underlines the importance of these biomimetic concepts. As reported in this chapter, a few MIP-based sensors for the determination of the viral load of SARS-COV-2 have been developed. However, it is questionable whether these sensors can displace or even partially substitute the “gold standard” of quantitative RT-PCR in SARS-CoV-2 diagnostics. On the other hand, the analytical performance of the presented MIPs should allow its application in lateral flow devices, which visually indicate the presence of the virus above a given threshold concentration.

Acknowledgments

This work was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy – EXC 2008/1 (UniSysCat) – 390540038 [Gefördert durch die Deutsche Forschungsgemeinschaft (DFG) im Rahmen der Exzellenzstrategie des Bundes und der Länder – EXC 2008/1 (UniSysCat) – 390540038].

References

[1] M. Drobysh, A. Ramanaviciene, R. Viter, A. Ramanavicius, Affinity Sensors for the Diagnosis of COVID-19, Micromachines 12 (2021) 1–19 390.

[2] A. Raziq, A. Kidakova, R. Boroznjak, J. Reut, A. Ópik, V. Syritski, Development of a portable MIP-based electrochemical sensor for detection of SARS-CoV-2 antigen, Biosensors and Bioelectronics 178 (2021) 1–7 113029.https://doi.org/10.1016/j.bios.2021.113029.
1. Present state of MIP-based sensors for SARS-CoV-2

[3] M. Yüce, E. Filiztekin, K.G. Özkaya, COVID-19 diagnosis —A review of current methods, Biosensors and Bioelectronics 172 (2021) 1–15 112752. https://doi.org/10.1016/j.bios.2020.112752.

[4] Z. Liu, X. Xiao, X. Wei, J. Li, J. Yang, H. Tan, et al., Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2, Journal of Medical Virology 92 (6) (2020) 595–601. https://doi.org/10.1002/jmv.25726.

[5] A. Yarman, F.W. Scheller, How reliable is the electrochemical readout of MIP sensors? Sensors (Switzerland) 20 (9) (2020) 1–23 2677. https://doi.org/10.3390/s20092677.

[6] Y. Saylan, Ö. Erdem, F. Inci, A. Denizli, Advances in biomimetic systems for molecular recognition and biosensing, Biomimetics 5 (2) (2020) 1–16 20. https://doi.org/10.3390/BIOMIMETICS5020020.

[7] A. Yarman, S. Kurbanoglu, I. Zebger, F.W. Scheller, Simple and robust: The claims of protein sensing by molecularly imprinted polymers, Sensors and Actuators B: Chemical 330 (2021) 1–12 129369. https://doi.org/10.1016/j.snb.2020.129369.

[8] O.S. Ahmad, T.S. Bedwell, C. Esen, A. Garcia-Cruz, S.A. Piletsky, Molecularly imprinted polymers in electrochemical and optical sensors, Trends in Biotechnology 37 (3) (2019) 294–309. https://doi.org/10.1016/j.tibtech.2018.08.009.

[9] N. Bereli, S. Akgönnüllü, S. Ashyüce, D. Cimen, İ. Gokturk, D. Turkmen, et al., Molecular imprinting technology for biomimetic assemblies, Hacettepe Journal of Biology and Chemistry 48 (5) (2020) 575–601. https://doi.org/10.15671/hjbc.801427.

[10] M. Feröz, P. Vadgama, Molecularly imprinted polymer modified electrochemical sensors for small drug analysis: Progress to practical application, Electroanalysis 32 (11) (2020) 2361–2386. https://doi.org/10.1002/elan.202060276.

[11] R.A. Hand, E. Piletska, T. Bassindale, G. Morgan, N. Turner, Application of molecularly imprinted polymers in the anti-doping field: sample purification and compound analysis, Analyst 145 (2020) 4716–4736. https://doi.org/10.1039/d0an00682c.

[12] O. Hayden, P.A. Lieberzeit, D. Blas, F.L. Dickert, Artificial antibodies for bioanalyte detection - Sensing viruses and proteins, Advanced Functional Materials 16 (10) (2006) 1269–1278. https://doi.org/10.1002/adfm.200500626.

[13] G. Jamalipour Soufi, S. Irvani, R.S. Varma, Molecularly imprinted polymers for the detection of viruses: Challenges and opportunities, Analyst 146 (10) (2021) 3087–3100. https://doi.org/10.1039/d1an00149c.

[14] J. Kalecki, Z. Iskierko, M. Cieplak, P.S. Sharma, Oriented immobilization of protein templates: A new trend in surface imprinting, ACS Sensors 5 (12) (2020) 3710–3720. https://doi.org/10.1021/acssensors.0c01634.

[15] M.A.R. Khan, T.C. Moreira, J. F., Riu, F. Sales, G M., Plastic antibody for the electrochemical detection of bacterial surface proteins, Sensors and Actuators B: Chemical 233 (2016) 697–704. https://doi.org/10.1016/j.snb.2016.04.075.

[16] C. Malitesta, E. Mazzotta, R.A. Picca, A. Poma, I. Chianella, S.A. Piletsky, MIP sensors - The electrochemical approach, Analytical and Bioanalytical Chemistry 402 (5) (2012) 1827–1846. https://doi.org/10.1007/s00216-011-5405-5.

[17] G. Moro, F. Bottari, N. Sleegers, A. Florea, T. Cowen, L.M. Moreno, et al., Conductive imprinted polymers for the direct electrochemical detection of β-lactam antibiotics: The case of cefquinome, Sensors and Actuators B: Chemical 297 (2019) 1–9 126786. https://doi.org/10.1016/j.snb.2019.126786.

[18] G. Ozcêlikay, S. Kurbanoglu, A. Yarman, F.W. Scheller, S.A. Ozkan, Au-Pt nanoparticles based molecularly imprinted nanosensor for electrochemical detection of the lipopeptide antibiotic drug Daptomycin, Sensors and Actuators B: Chemical 320 (2020) 1–7 128285. https://doi.org/10.1016/j.snb.2020.128285.

[19] J.G. Pacheco, P. Rebelo, M. Freitas, H.P.A. Nouws, C. Delerue-Matos, Breast cancer biomarker (HER2-ECD) detection using a molecularly imprinted electrochemical sensor, Sensors and Actuators B: Chemical 273 (2018) 1008–1014. https://doi.org/10.1016/j.snb.2018.06.113.
[20] F.W. Scheller, X. Zhang, A. Yarman, U. Wollenberger, R.E. Gyurcsányi, Molecularly imprinted polymer-based electrochemical sensors for biopolymers, Current Opinion in Electrochemistry 14 (2019) 53–59. https://doi.org/10.1016/j.joelec.2018.12.005.

[21] A. Yarman, F.W. Scheller, The first electrochemical MIP sensor for tamoxifen, Sensors (Switzerland) 14 (5) (2014) 7647–7654. https://doi.org/10.3390/s140507647.

[22] L. Cenci, R. Tatti, R. Tognato, E. Ambrosi, C. Piotto, A.M. Bossi, Synthesis and characterization of peptide-imprinted nanogels of controllable size and affinity, European Polymer Journal 109 (2018) 453–459. https://doi.org/10.1016/j.eurpolymj.2018.08.031.

[23] D. Dechtrirat, K.J. Jetzschmann, W.F.M. Stöcklein, F.W. Scheller, N. Gajovic-Eichelmann, Protein rebinding to a surface-confined imprint, Advanced Functional Materials 22 (24) (2012) 5231–5237. https://doi.org/10.1002/adfm.201201328.

[24] K.J. Jetzschmann, A. Yarman, L. Rustam, P. Kielb, V.B. Urlacher, A. Fischer, et al., Molecular LEGO by domain-imprinting of cytochrome P450 BM3, Colloids and Surfaces B: Biointerfaces 164 (2018) 240–246. https://doi.org/10.1016/j.colsurfb.2018.01.047.

[25] H. Nishino, C.S. Huang, K.J. Shea, Selective protein capture by epitope imprinting, Angewandte Chemie - International Edition 45 (15) (2006) 2393–2396. https://doi.org/10.1002/anie.200503760.

[26] L. Pasquardini, A.M. Bossi, Molecularly imprinted polymers by epitope imprinting: a journey from molecular interactions to the available bioinformatics resources to scout for epitope templates, Analytical and Bioanalytical Chemistry 413 (2021) 6101–6115. https://doi.org/10.1007/s00216-021-03409-1.

[27] A. Rachkov, N. Minoura, Recognition of oxytocin and oxytocin-related peptides in aqueous media using a molecularly imprinted polymer synthesized by the epitope approach, Journal of Chromatography A 889 (1–2) (2000) 111–118. https://doi.org/10.1016/S0021-9673(00)00568-9.

[28] K. Yang, S. Li, L. Liu, Y. Chen, W. Zhou, J. Pei, et al., Epitope imprinting technology: Progress, applications, and perspectives toward artificial antibodies, Advanced Materials 31 (50) (2019) 1–17. https://doi.org/10.1002/adma.201902048.

[29] L. Luo, F. Zhang, C. Chen, C. Cai, Visual simultaneous detection of hepatitis A and B viruses based on a multifunctional molecularly imprinted fluorescence sensor, Analytical Chemistry 91 (24) (2019) 15748–15756. https://doi.org/10.1021/acs.analchem.9b04001.

[30] T. Wangchareansak, A. Thitithanyanont, D. Chuakheaw, M.P. Gleeson, P.A. Lieberzeit, C. Sangma, Influenza A virus molecularly imprinted polymers and their application in virus sub-type classification, Journal of Materials Chemistry B 1 (16) (2013) 2190–2197. https://doi.org/10.1039/c3tb00027c.

[31] C.H. Lu, Y. Zhang, S.F. Tang, Z. Bin Fang, H.H. Yang, X. Chen, et al., Sensing HIV related protein using epitope imprinted hydrophilic polymer coated quartz crystal microbalance, Biosensors and Bioelectronics 31 (1) (2012) 439–444. https://doi.org/10.1016/j.bios.2011.11.008.

[32] O.I. Parisi, M. Dattilo, F. Patitucci, R. Malivindi, S. Delbue, P. Ferrante, et al., Design and development of plastic antibodies against SARS-CoV-2 RBD based on molecularly imprinted polymers that inhibit in vitro virus infection, Nanoscale 12 (2021) 16885–16899 2020.05.28.120709. https://doi.org/10.1039/c3tb00027c.

[33] F. Puoci, Monoclonal-type plastic antibodies for covid-19 treatment: What is the idea? Journal of Functional Biomaterials 11 (2) (2020) 9–12. https://doi.org/10.3390/jfb11020043.

[34] K. Navakul, C. Sangma, P. Yenchitsomanus, S. Chunta, P.A. Lieberzeit, Enhancing sensitivity of QCM for dengue type 1 virus detection using graphene-based polymer composites, Analytical and Bioanalytical Chemistry 413 (2021) 6191–6198. https://doi.org/10.1007/s00216-021-03410-8.
1. Present state of MIP-based sensors for SARS-CoV-2

[35] E.A. Beliaeva, B. Pluhar, T.N. Ermolaeva, B. Mizaikoff, N.A. Karaseva, Synthesis and application of molecularly imprinted polymers for trypsin piezoelectric sensors, Sensors and Actuators B: Chemical 280 (2018) 272–279. https://doi.org/10.1016/j.snb.2018.10.022.

[36] S. Chunta, R. Suedee, W. Boonsriwong, P.A. Lieberzeit, Biomimetic sensors targeting oxidized-low-density lipoprotein with molecularly imprinted polymers, Analytica Chimica Acta 1116 (2020) 27–35. https://doi.org/10.1016/j.aca.2020.04.017.

[37] R.D. Crapnell, F. Canfarotta, J. Czulak, R. Johnson, K. Betlem, F. Mecozzi, et al., Thermal detection of cardiac biomarkers heart-fatty acid binding protein and ST2 using a molecularly imprinted nanoparticle-based multiplex sensor platform, ACS Sensors 4 (10) (2019) 2838–2845. https://doi.org/10.1021/acssensors.9b01666.

[38] K. El Kirat, M. Bartkowski, K. Haupl, Probing the recognition specificity of a protein molecularly imprinted polymer using force spectroscopy, Biosensors and Bioelectronics 24 (8) (2009) 2618–2624. https://doi.org/10.1016/j.bios.2009.01.018.

[39] F.L. Dickert, O. Hayden, R. Bindeus, K.-J. Mann, D. Blaas, E. Waigmann, Bioimprinted QCM sensors for virus detection—screening of plant sap, Analytical and Bioanalytical Chemistry 378 (8) (2004) 1929–1934. https://doi.org/10.1007/S00216-004-2521-5.

[40] D.F. Tai, C.Y. Lin, T.Z. Wu, L.K. Chen, Recognition of dengue virus protein using epitope-mediated molecularly imprinted film, Analytical Chemistry 77 (16) (2005) 5140–5143. https://doi.org/10.1021/ac0504060.

[41] D.F. Tai, C.Y. Lin, T.Z. Wu, J.H. Huang, P.Y. Shu, Artificial receptors in serologic tests for the early diagnosis of dengue virus infection, Clinical Chemistry 52 (8) (2006) 1486–1491. https://doi.org/10.1373/clinchem.2005.064501.

[42] M. Jenik, R. Schirhagl, C. Schirk, O. Hayden, P. Lieberzeit, D. Blaas, et al., Sensing picornaviruses using molecular imprinting techniques on a quartz crystal microbalance, Analytical Chemistry 81 (13) (2009) 5320–5326. https://doi.org/10.1021/ac8019569.

[43] L. Uzun, R. Say, S. Ünal, A. Denizli, Hepatitis B surface antibody purification with hepatitis B surface antibody imprinted poly(hydroxyethyl methacrylate-N-methacryloyl-l-tyrosine methyl ester) particles, Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 877 (3) (2009) 181–188. https://doi.org/10.1016/j.jchromb.2008.12.004.

[44] J. Zhou, N. Gan, T. Li, F. Hu, X. Li, L. Wang, L. Zheng, A cost-effective sandwich electrochemiluminescence immunosensor for ultrasensitive detection of HIV-1 antibody using magnetic molecularly imprinted polymers as capture probes, Biosensors and Bioelectronics 54 (2014) 199–206. https://doi.org/10.1016/j.bios.2013.10.044.

[45] K. He, C. Chen, C. Liang, C. Liu, B. Yang, X. Chen, et al., Highly selective recognition and fluorescent detection of JEV via virus-imprinted magnetic silicon microspheres, Sensors and Actuators B: Chemical 233 (2016) 607–614. https://doi.org/10.1016/j.snb.2016.04.127.

[46] C. Liang, H. Wang, K. He, C. Chen, X. Chen, H. Gong, et al., A virus-MIPs fluorescent sensor based on FRET for highly sensitive detection of JEV, Talanta 160 (2016) 360–366. https://doi.org/10.1016/j.talanta.2016.06.010.

[47] B. Yang, H. Gong, C. Chen, X. Chen, C. Cai, A virus resonance light scattering sensor based on mussel-inspired molecularly imprinted polymers for high sensitive and high selective detection of Hepatitis A Virus, Biosensors and Bioelectronics 87 (2017) 679–685. https://doi.org/10.1016/j.bios.2016.08.087.

[48] S. Klangprapan, B. Choke-arprornchai, P.A. Lieberzeit, K. Choowongkomon, Sensing the classical swine fever virus with molecularly imprinted polymer on quartz crystal microbalance, Heliyon 6 (6) (2020) e04137. https://doi.org/10.1016/j.heliyon.2020.e04137.

[49] A.A. Malik, C. Nantasenamat, T. Piacham, Molecularly imprinted polymer for human viral pathogen detection, Materials Science and Engineering: C 77 (2017) 1341–1348. https://doi.org/10.1016/j.msec.2017.03.209.
[50] P. Lach, M. Cieplak, K.R. Noworyta, P. Pietra, W. Lisowski, J. Kalecki, et al., Self-reporting molecularly imprinted polymer with the covalently immobilized ferrocene redox probe for selective electrochemical sensing of p-synephrine, Sensors and Actuators: B. Chemical 344 (2021) 1–10 130276. https://doi.org/10.1016/j.snb.2021.130276.

[51] S. Yang, C. Bai, Y. Teng, J. Zhang, J. Peng, Z. Fang, et al., Study of horseradish peroxidase and hydrogen peroxide bi-analyte sensor with boronate affinity-based molecularly imprinted film, Canadian Journal of Chemistry 97 (12) (2019) 833–839. https://doi.org/10.1139/cjc-2019-0134.

[52] A. Yarman, Development of a molecularly imprinted polymer-based electrochemical sensor for tyrosinase, Turkish Journal of Chemistry 42 (2) (2018) 346–354.

[53] A. Yarman, Electrosynthesized molecularly imprinted polymer for laccase using the inactivated enzyme as the target, Bulletin of the Korean Chemical Society 39 (4) (2018) 483–488. https://doi.org/10.1002/bkcs.11413.

[54] P.S. Sharma, A. Pietrzyk-Le, F. D’Souza, W Kutner, Electrophysically synthesized polymers in molecular imprinting for chemical sensing, Analytical and Bioanalytical Chemistry 402 (10) (2012) 3177–3204. https://doi.org/10.1007/s00216-011-5696-6.

[55] A. Yarman, G. Caserta, X. Zhang, P. Borrero, S. Frielingsdorf, E. Supala, et al., Biosensors 2021, 31th Anniversary World Congress on Biosensors, Potential vs shortcomings of epitope MIPs for the recognition of natural and recombinant proteins, 2021.

[56] P. Singh, SPR biosensors: Historical perspectives and current challenges, Sensors and Actuators B: Chemical 229 (2016) 110–130. https://doi.org/10.1016/J.SNB.2016.01.118.

[57] N. Cennamo, G. D. Agostino, C. Perri, F. Arcadio, G. Chiaretti, E. M. Parisio, et al., Proof of concept for a quick and highly sensitive on-site detection of SARS-CoV-2 by plasmotic optical fibers and molecularly imprinted polymers, Sensors 21 (2021) 1–17.

[58] R. Arshady, & K. Mosbach. Synthesis of Substrate-selective Polymers by Host-Guest Polymerization. Makromolekulare Chemie, 692 (1981) 687–692.

[59] G. Wulff, A. Sarhan. Use of polymers with enzyme-analogous structures for the resolution of racemates. Angew. Chem. Int. Ed. Engl, 11 (1972) 341–344.

[60] M.V. Polyakov, Adsorption properties and structure of silica gel. Zhur Fiz Khim, 2 (1931) 709–805.

[61] C. Alexander, H.S. Andersson, L.I. Andersson, R.J. Ansell, N. Kirsch, I.A. Nicholls, J. O’Mahony, M.J. Whitcombe, et al., Molecular imprinting science and technology: A survey of the literature for the years up to and including 2003. Journal of Molecular Recognition, 19 (2) (2006) 106–180. https://doi.org/10.1002/jmr.760.

[62] B.T.S. Bui, K. Haupt, Molecularly imprinted polymers: synthetic receptors in bioanalysis. Analytical and Bioanalytical Chemistry, 398 (6) (2010), 2481–2492. https://doi.org/10.1007/s00216-010-4158-x.

[63] H.A. Hussein, A. Kandeil, M. Gomaa, R. Mohamed El Nashar, I.M. El-Sherbiny, R.Y.A Hassan, SARS-CoV-2-Impedimetric Biosensor: Virus-Imprinted Chips for Early and Rapid Diagnosis, ACS Sensors 6 (11) (2021) 4098–4107. https://doi.org/10.1021/acssensors.1c01614.

[64] H.F. El Sharif, S.R. Dennison, M. Tully, S. Crossley, W. Mwangi, D. Bailey, ..., S.M. Reddy, Evaluation of electropolymerized molecularly imprinted polymers (E-MIPs) on disposable electrodes for detection of SARS-CoV-2 in saliva, Anal. Chim. Acta (2022) 339777. https://doi.org/10.1016/j.aca.2022.339777.

[65] W. Sukjee, A. Thitithayanont, S. Manopwisedjaroen, S. Seetaha, C. Thepparit, C. Sangma, Virus MIP-composites for SARS-CoV-2 detection in the aquatic environment, Mater. Lett. 315 (September 2021) (2022) 131973. https://doi.org/10.1016/j.matlet.2022.131973.

[66] T. Zhang, L. Sun, Y. Zhang, Highly sensitive electrochemical determination of the SARS-COV-2 antigen based on a gold/graphene imprinted poly-arginine sensor, Anal. Methods 13 (47) (2021) 5772–5776. https://doi.org/10.1039/d1ay01478a.
1. Present state of MIP-based sensors for SARS-CoV-2

[67] V. Ratautaite, R. Boguzaite, E. Brazys, A. Ramanaviciene, E. Ciplys, M. Juozapaitis, …, A. Ramanavicius, Molecularly imprinted polypyrrole based sensor for the detection of SARS-CoV-2 spike glycoprotein, Electrochim. Acta 403 (2022) 139581. https://doi.org/10.1016/j.electacta.2021.139581.

[68] M. Amouzadeh Tabrizi, J.P. Fernández-Blázquez, D.M. Medina, P. Acedo, An ultrasensitive molecularly imprinted polymer-based electrochemical sensor for the determination of SARS-CoV-2-RBD by using macroporous gold screen-printed electrode, Biosens. Bioelectron. 196 (2021) (2022). https://doi.org/10.1016/j.bios.2021.113729.

[69] A.G. Ayankojo, R. Boroznjak, J. Reut, A. Ópik, V. Syritski, Molecularly imprinted polymer based electrochemical sensor for quantitative detection of SARS-CoV-2 spike protein, Sens. Actuators B 353 (2022). https://doi.org/10.1016/j.snb.2021.131160.

[70] Z. Bognár, E. Supala, A. Yarman, X. Zhang, F.F. Bier, F.W. Scheller, R.E. Gyurcsányi, Peptide epitope-imprinted polymer microarrays for selective protein recognition. Application for SARS-CoV-2 RBD protein, Chem. Sci. 13 (5) (2022) 1263–1269. https://doi.org/10.1039/d1sc04502d.
Non-Print Items

Abstract
The need for fast, selective, accurate, and cost-effective in situ analysis in various fields like medical diagnosis, environmental, food, or pharmaceutical analysis has pushed the development of biosensors. Due to their fragility, lower stability under harsh conditions such as high/low pH, organic solvents, high pressure, or temperature, the biological recognition elements have been substituted by fully synthetic counterparts like aptamers and molecularly imprinted polymers (MIPs). MIPs have recently found applications for the detection of SARS-CoV-2. Herein we describe the potential and challenge of application in this field.

Keywords
Molecularly imprinted polymers; Biomimetic sensors; SARS-CoV-2, Electrochemical readout; Optical readout