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Early detection of SARS-CoV-2 with functionalized gold and molecularly imprinted polymeric nanoparticles: a mini review

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The novel coronavirus COVID-19 was first reported in Wuhan, China, in December 2019 and rapidly spread to the rest of the world, with the WHO declaring a global pandemic in March 2020. Rapid mutations of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have challenged its diagnosis and treatments. Reverse transcription-polymerase chain reaction (RT-PCR) tests are widely used for the diagnosis of COVID-19; however, they present several drawbacks including high cost, long turnaround time, and need for sophisticated lab infrastructure and trained technical personnel. Lateral flow tests based on antigen sensing are an interesting alternative since they offer rapid (15–30 min) and low-cost analysis, although their low sensitivity has led to several adopted tests being withdrawn from the market. Henceforth, the development of detection methods which are fast, robust, reliable, cost-effective, easy to use and portable is indispensable to prevent community transmission of COVID-19. We have reviewed two different emerging colloidal-based methodologies, (a) functionalized gold nanoparticles (functionalized AuNPs) and (b) molecularly imprinted polymers (MIPs), for fast, highly specific, and reliable identification of SARS-CoV-2. Different modifications of AuNPs with antibodies, antigens and nucleoproteins and their various assays including colorimetric, electrochemical, localized surface plasmon resonance (LSPR) and lateral flow immunoassays are discussed. In contrast, with MIP-based sensors, various antigen proteins and virus particles can be imprinted within the polymeric nanoplatform and hence can be detected with various readout techniques. The operating characteristics of these two emerging diagnostic platforms were critically reviewed and compared against each other.

Design, System, Application

Recent developments have highlighted that there remains an urgent need for accurate and low-cost point-of-care SARS-CoV-2 tests to break the chain of community virus transmission. Polymerase chain tests are not suitable for this purpose since the technique is costly and time-consuming. Furthermore, lateral flow tests lack sensitivity and are susceptible to environmental changes (*e.g.* pH, temperature) due to the use of antibodies as receptors. We will discuss two emerging technologies, including the use of gold and molecularly imprinted polymer nanoparticles (nanoMIPs) as highly selective synthetic recognition elements for SARS-CoV-2, combined with straightforward and rapid analysis methods. The review will focus on the design requirements for a portable test that uses nanoparticles as recognition elements and critically compare the systems (i) to traditional antibody-based assays and (ii) against each other. We will also discuss what prevents these systems from being commercialized given that lateral flow tests remain the preferred method of choice for tests in a home setting. The manuscript looks towards the future on how emerging technologies, based on versatile nanoparticles that can be modified towards other targets, can prevent future outbreaks of emerging pathogens.

1.0 Introduction

In December 2019 (Wuhan, China), a virus called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (COVID-19) was reported and shortly declared as a pandemic due to its high transmission rates.[1–3] There have been more than 545 million confirmed global cases as of June 2022, leading to a reported number of 6 million deaths with numbers still increasing.[4] Several vaccines have been developed globally and compulsory vaccination programs were implemented, which have been

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completed in most of the developed countries including some of the developing countries (12.13 billion vaccine doses have been administered globally). However, in most low-income countries, vaccination programs were run on a relatively smaller scale and could cover only a fraction of the population (~5%) due to lack of knowledge, insufficient medical campaigns, and poor medical facilities.[5,6] Furthermore, emerging variants due to the fast mutation rate of the virus remain a constant threat. Currently, reverse transcription-polymerase chain reaction (RT-PCR) is considered the gold standard technique for the detection of SARS-CoV-2. This process relies on the identification of viral RNA through nucleic acid reverse transcription and amplification.[7,8] However, this technique requires specialized equipment, reagents, facilities, and long turnaround time, during which community transmission occurs.[9,10] However, developing countries and deprived areas lack advanced facilities and, consequently, there is rapid spread of COVID-19. In addition to this, there are several inexpensive rapid antigen

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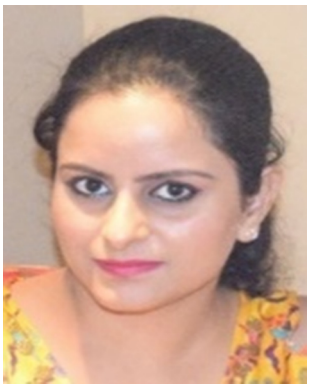

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lateral flow test kits on the market which are cheaper, easy to use and provide results in around 15–30 min. However, several rapid antigen test kits have been withdrawn from the market as they fail to meet the required sensitivity of the WHO norms (>80% for both symptomatic and asymptomatic cases). Consequently, the SARS-CoV-2 pandemic highlighted the need for more suitable and effective point-of-care tools for the detection and prevention of outbreaks.[11] SARS-CoV-2 is composed of four types of structural proteins, *viz.* spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins [Fig. 1].[12] The virus encompasses different protein spikes covering its outer membrane surface called S proteins. These spikes (SARS-CoV-2) have encountered mutations at multiple sites; moreover, more than one mutation type can be seen on the same mutation site. D614(7859), L5(109), L54(105), P1263(61), P681(51), S477(47), T859(30), S221(28), V483(28), and A845(24) are the top 10 mutation sites based on total number of occurrences.[13] It was found from the literature that D614G is the only mutation site that all current mutations have in common.

Early work focused on these proteins because they enable the virus to enter host cells. There are two different approaches for the diagnosis of COVID-19: the first involves the detection of viral proteins, and the second serological method involves the measurement of elevated immunoglobulin M (IgM) and immunoglobulin G (IgG) levels produced by the body in response to SARS-CoV-2 infection. Nanotechnology paves the way to early, convenient, low set-up, cost-effective virus detection. The detection of COVID-19 or its antigens could be determined optically, electrochemically, and by employing mass spectrometer/hybrid transduction platforms and other different analytical techniques. Considering the advantages of optical and electrochemical sensing methods, scientists have developed several nanoscale integrated sensing platforms specific for the SARS-CoV-2 as well as its antigens/proteins and antibodies.[14] There has been a growing demand for portable sensors capable of exhibiting high specificity, sensitivity and accuracy. To develop portable sensors, specifically point-of-care sensors, for the detection of SARS-CoV-2, it is crucial to identify or design a suitable molecular probe, also known as a molecular recognition element. A molecular probe should possess high affinity and selectivity and specifically recognize and bind the components of SARS-CoV-2 virus such as proteins or RNA genome. For example, spike (S1 and S2 proteins), nucleocapsid (N) and membrane (M) proteins can be targeted with specific (monoclonal) antibodies/aptamers (oligonucleotides) or MIPs. The source of the antibody can be patients with SARS-CoV-2; antibodies against these proteins were detected or can be developed *via* recombinant DNA technology utilizing immunogen; for example, SARS-CoV-2 antibody can be generated by sequencing peripheral blood lymphocytes of a patient exposed to SARS-CoV-2.[15] Furthermore, the part of proteins (S1, S2, N and M proteins) or RNA of the virus can be recognized using antigens, peptides, antisense nucleotides, or aptamers.

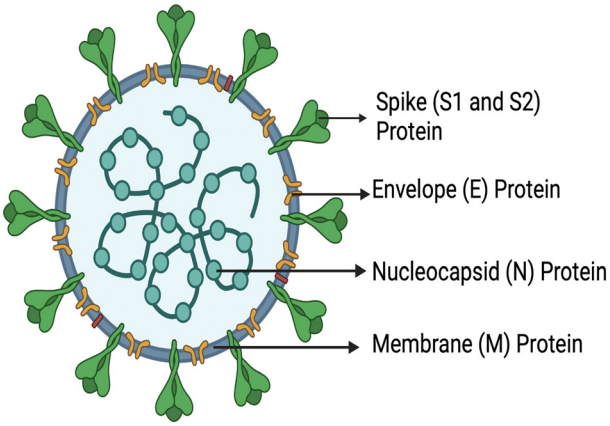

Fig. 1 Schematic representation of the structural proteins of SARS-CoV-2, created with **https://www.BioRender.com**.

Molecular recognition elements for SARS-CoV-2 can also be fabricated with molecularly imprinting technology/method; one can select the S, M and E protein or epitopes of the aforementioned proteins or the epitope of naming proteins using a range of functional monomers. There are thousands of monomers available that can be utilized for the fabrication of MIPs. Utilizing computational modeling and advanced techniques, suitable and optimal functional monomers can be selected. This approach aids in the identification of the monomers crucial for generating exceptionally selective and specific MIPs.[16] For example, computational software including SiteMap for predicting protein binding sites and MM-GBSA for calculating binding free energies of monomers at each site can be utilized.

In addition to that for signal transduction, different types of monomer such as fluorescent (such as fluorescein-*o*-methacrylate) and electroactive monomers (such as ferrocene-*o*-methacrylate) can be used for fluorescence and electrochemical detection, respectively.

Furthermore, an efficient sensor requires a signal transducer, which facilitates the transmission of signals during or after the binding event and nanomaterials can serve as effective signal transducers. Examples of such nanomaterials include magnetic nanoparticles (including

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iron oxide nanoparticles), metal nanoparticles such as AuNPs, quantum dots, and carbon nanostructures such as single-walled carbon nanotubes, graphene, cellulose-based materials, polystyrene microbeads[17–22] and many more are being used. These nanomaterials ranging in size from 1 to 100 nm possess unique properties such as conductance, thermal and chemical stability, distinctive optical characteristics, magnetic properties, increased strength, and high surface area to functionalize molecular probes.

These properties provide enhanced performance of the sensor in terms of response time, signal-to-noise (S/N) ratio, selectivity, and limits of detection. Electrochemical techniques are simple, inexpensive, and comparatively sensitive and are therefore routinely employed for the detection of bacteria, proteins, antibodies, and viruses.[23] In the context of SARS-CoV-2 electrochemical sensors, AuNPs have been effectively employed to enhance both electron transfer kinetics and signal amplification. The utilization of AuNPs in these sensors accelerates the electron transfer process, thereby improving the efficiency of electrochemical detection and enhancing the overall performance of the sensor. Moreover, AuNPs can be used in colorimetric assays and are compatible with most analytical techniques.

Of these materials, functionalized AuNPs and molecularly imprinted polymeric nanoparticles (nanoMIPs) have been extensively studied for the detection of COVID-19. The colorimetric detection method employing nanoparticles is an appealing method since it offers detection by the naked eye. On the other hand, nanoMIPs are also gaining attention because they are highly robust, inexpensive and thermally stable and there is no direct need for biological counterparts for the detection. There has been no review to date focused on the comparison of functionalized AuNPs and MIPs; however, there is a pressing need to address and compare these emerging technologies and research work based on functionalized AuNPs and MIPs.

Therefore, in this mini review, the use of AuNPs functionalized with antibodies, antigens and nucleotides, aptamers and MIPs/nanoMIPs for the detection of COVID-19 is systematically compared to the gold standard.

2.0 Materials used for SARS-CoV-2 detection

2.1 Functionalized gold nanoparticles (AuNPs)

AuNPs exhibit different chemical and physical properties that make them excellent materials for the development of biosensors.[24] Moreover, AuNPs can be synthesized *via* straightforward one-step reactions and display good biocompatibility, photostability, high electron transfer rate and catalytic activity.[25] Generally, AuNPs have been widely used in sensors, particularly since colorimetric assays based on AuNPs offer simplicity, cost-effectiveness, naked-eye response, high specificity and ease of use.[26] Henceforth, these have been employed for the development of efficient detection methodologies for nucleic acids, proteins and various chemicals and biomolecules. Sensing systems incorporated with AuNPs facilitate enhanced electronic transmission performance of sensors due to their electrically conductive properties.[27–30]

2.1.1 Antibody (Ab)-functionalized AuNPs. Several antibodies against spike S1 antigens have been used since these are the most significant markers of a previous infection with the virus. Since antibody tests can help identify a previously infected or recovered person but could not efficiently detect an active COVID-19 infection, these antibodies could be employed as a tool for the detection of antigens specific to

Table 1 Antibody-functionalized AuNPs for the detection of SARS-CoV-2

System	Detection or capture molecule/ligands	Target	Analytical method	Real sample testing/live/pseudo-virus	LOD	Ref.
Au–Pt NPs	Polyclonal	Spike S1 recombinant protein	Colorimetric	No	11 ng mL^{-1}	33
AuNPs	SARS-CoV-2 spike monoclonal antibody	Spike S1 recombinant protein	Colorimetric and electrochemical	No	48 ng mL^{-1} (colorimetric) and 1 pg mL^{-1} (electrochemical)	23
AuNPs on antibody-coated fluorine-doped tin oxide	SARS-CoV-2 spike S1Ab	Spike S1 protein	CV and DPV	—	6 pg mL^{-1}	34
AuNPs	Anti-spike antibody	COVID-19 viral antigen and virus	Colorimetry, surface-enhanced Raman spectroscopy	Yes	~1 pg mL^{-1} (colorimetry) ~4 pg mL^{-1} and ~18 virus particles/mL (SERS)	35
AuNPs	Polyclonal Abs against S1, membrane proteins and envelope proteins	Spike S1 proteins, membrane proteins and envelope proteins	Colorimetry	Yes	Not reported	36
AuNPs	Polyclonal Ab	Nucleocapsid (N) proteins	Localized surface plasmon resonance (LSPR)	No	150 ng mL^{-1}	37

SARS-CoV-2 within a few minutes. AuNPs when modified suitably could detect SARS-CoV-2 specifically within a few minutes employing various analytical methods. Different Ab-functionalized AuNPs and alloy nanoparticles used for the detection of SARS-COV-2 are summarized in Table 1.

Detection of viral antigens is more promising because antibody responses do not appear early when compared with a virus, thereby delaying the diagnosis and treatment.[31,32]

Fu *et al.* utilized polyclonal antibodies functionalized with Au–Pt alloy nanoparticles for the colorimetric detection of S1 spike proteins of SARS-CoV-2.[33] Karakuş *et al.* performed a rapid, selective, and dual-responsive electrochemical and colorimetric detection of SARS-CoV-2 with monoclonal functionalized AuNPs. Monoclonal antibodies were conjugated with 11-mercaptoundecanoic acid (MUA)-modified AuNPs by applying EDC-NHS (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride and *N*-hydroxysuccinimide) chemistry depicted in Fig. 2. The prepared system detected SARS-CoV-2 spike antigens in saliva samples with high specificity and did not show any cross-reactivity with other antigens such as *Streptococcus pneumoniae*, influenza A and MERS-CoV.[23] In the presence of the SARS-CoV-2 spike antigen, rapid and irreversible aggregation of AuNPs was observed which was attributed to antigen–antibody interactions. The interaction could also be inferred from the change in color of the solution from red to purple. This was validated spectroscopically using UV-visible spectrometry and red shift was observed with a detection

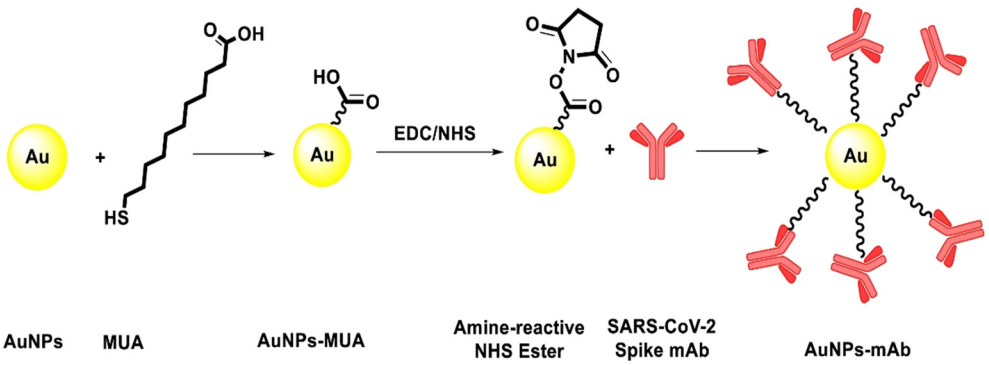

Fig. 2 Schematic presentation of the conjugation of the mAb on the surface of MUA-modified AuNPs. Reprinted from ref. 23. E. Karakuş *et al.*, *Anal. Chim. Acta*, 2021, 1182, 338939–338949. Copyright (2021) Elsevier.

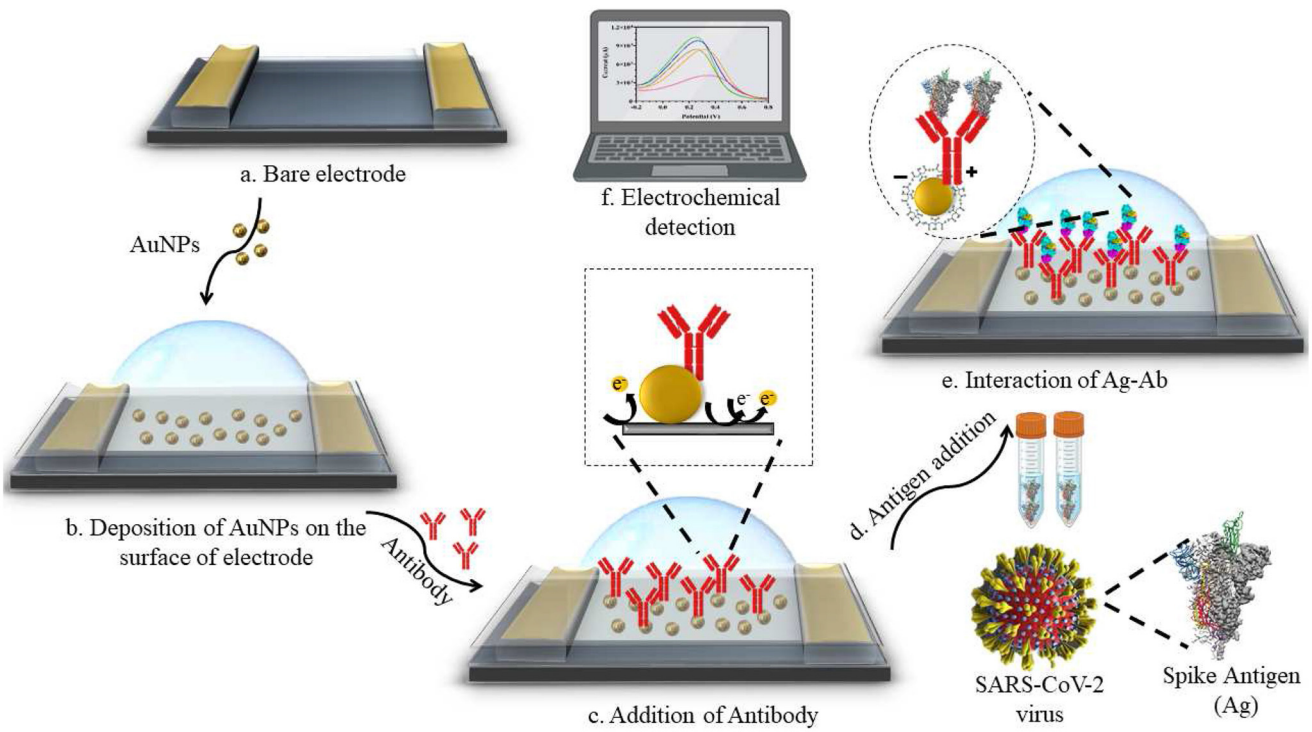

Fig. 3 Design of electrochemical sensing of SARS-CoV-2 spike S1 antigen using an electrode system consisting of Ag/AgCl as a reference electrode and platinum as a counter electrode. Reprinted from ref. 34. A. Robert *et al.*, *Anal. Chim. Acta*, 2021, 1188, 339207–339219. Copyright (2021) Elsevier.

limit of 48 ng mL^{-1}. Robert and colleagues developed an electrode consisting of a fluorine-doped tin oxide (FTO)/AuNP complex coupled with SARS-CoV-2 spike S1 antibody (SARS-CoV-2 Ab) as an immunosensor for SARS-CoV-2 spike S1 antigen as shown in Fig. 3.[34] The immunosensor was optimized for response time, temperature, pH and antibody concentration. Moreover, the platform displayed excellent sensitivity towards SARS-CoV-2 spike S1 antigen with a limit of detection down to 0.63 fM in standard buffer sample and down to 120 fM (6 pg mL^{-1}) in spiked saliva samples whilst displaying negligible cross-reactivity with other viral antigens.

Pramanik *et al.* reported an assay based on colorimetric and surface-enhanced Raman spectroscopy (SERS) for the determination of pseudo-SARS-CoV-2 (genetically modified virus which is not infectious) with anti-spike antibody-functionalized PEG-coated AuNPs.[35] In the presence of the antigen, AuNPs undergo aggregation due to the antigen–antibody interactions, which leads to a color change visible to the naked eye from pink to blue within 5 min. The response (LOD) for the developed sensor was 1 ng mL^{-1} for COVID-19 antigen and 1000 virus particles per mL for SARS-CoV-2 spike protein pseudotyped baculovirus. Ventura *et al.* reported highly sensitive COVID-19 detection in nasal and throat swabs by means of a colorimetric assay employing AuNPs to which three different antibodies for spike, envelope and membrane protein were attached.[36] A red shift was observed in the optical profile of the antibody-decorated AuNPs in the presence of viral particles. Additionally, the optical density was compared to the threshold cycle (C_t) of a real-time PCR, and it was observed that the proposed method could bind the virus with a detection limit comparable to that of the real-time PCR test. Another group led by Behrouzi *et al.* developed a sensitive and specific method for the detection of viral nucleocapsid protein using polyclonal antibody-coated AuNPs.[37] The method could sense the virus in 5 min with a LOD of 150 ng mL^{-1}.

2.1.2 Antigen-functionalized AuNPs. Antigens hold potential as ligands with high specificity and affinity towards SARS-CoV-2 antibodies and have therefore attracted significant attention to develop serological-based immunoassays.[38] SARS-CoV-2 proteins, specifically spike, envelope, membrane and nucleocapsid protein, are viral antigens and can be used for sensor development.[39] Of the four types of proteins, spike protein is the best early diagnostic marker of virus infection and could be detected before the appearance of clinical symptoms attributable to its high abundance on the surface. Antigen-functionalized AuNP-based immunoassays targeting IgG and IgM are summarized in Table 2. Funari and colleagues electrodeposited spike proteins on the surface of AuNPs and detected anti-SARS-CoV-2 spike protein antibodies.[40] Electrodeposition technique was used to fabricate the opto-microfluidic sensing platform with gold nanospikes to detect the target antibodies and the concentration of these can be obtained by correlating the localized SPR peak shift of the gold nanospikes. The limit of detection was obtained in the range of 0.08 ng mL^{-1}.

A lateral flow immunoassay capable of detecting IgM and IgG simultaneously has been developed by Li *et al.*[41] The methodology could be employed for the rapid screening of both symptomatic and asymptomatic SARS-CoV-2 carriers with appreciable specificity (90.63%) and sensitivity (88.66%).

Lew *et al.* developed another colorimetric serological assay to conduct large-scale testing of SARS-CoV-2 IgG in human plasma.[42] Four different epitopes located on the S and N proteins of the virus were conjugated to AuNPs to recognize target antibodies. The methodology was also employed to isolate and investigate the plasma samples of 35 patients and results showed 100% specificity and 83% sensitivity. Within 30 min of exposure of the sensor to the antibody, a specific optical response was observed, triggered by nanoparticle aggregation upon binding between the antibodies and AuNPs functionalized with epitopes. The limit of detection of this nanosensor was 3.2 nM with the conjugation of two antigenic epitopes (S14P5 and S21P2). Lorenzen *et al.* developed an electrochemical sensor for the fast and reliable detection of antibodies against COVID-19 in serum sample.[43] The research group modified the electrode with electro-synthesized PEDOT and AuNPs and immobilized truncated nucleoprotein (Naa160-406aa). The sensor developed was able

Table 2 Summary of antigenic protein-functionalized AuNPs for the detection of SARS-CoV-2 employing multiple techniques

System	Proteins	Antibody/analyte	Analytical method	Real sample testing	Ref.
AuNPs	SARS-CoV-2 spike protein	Spike protein antibody	Opto-microfluidic sensing	No	40
AuNPs	Recombinant antigen (MK201027) of SARS-CoV-2 spike protein	IgG and IgM antibodies	Lateral flow immunoassay	Yes	41
AuNPs	SARS-CoV-2 S and N epitope	IgG antibodies	Colorimetric serological assay	Yes	42
PEDOT–AuNP modified electrodes	Truncated nucleoprotein (Naa160-406aa)	IgG and IgM antibodies	Electrochemical detection	Yes	43
AuNPs	Nucleocapsid recombinant antigen ncov-ps-Ag8	IgG and IgM antibodies	Immuno-chromatography assay	Yes	44
AuNPs	Nucleoprotein	IgM antibodies	Lateral flow immunoassay	Yes	45
AuNPs	Membrane (M), spike (S) 1 and 2, nucleocapsid (N) and envelope (E)	IgA, IgM and IgG antibodies	RNA PCR	Yes	46

to detect the antibodies in about half an hour with high specificity. Liu *et al.* reported a colloidal gold immunochromatography assay to detect COVID-19 IgG and IgM antibodies using COVID-19 nucleocapsid recombinant antigen ncov-ps-Ag8 conjugated AuNPs within 15 min in human blood samples.[44] Moreover, the developed technology was able to detect COVID-19 antibodies with high sensitivity (95.85%) and specificity (97.47%). For the rapid, specific and inexpensive on-site diagnosis of COVID, Huang *et al.* developed a AuNP-based lateral flow assay to detect IgM antibodies.[45] The prepared AuNP-LF strips consisted of a coating of nucleoprotein on the analytical membrane and conjugation of IgM antibodies to the AuNPs. The efficacy of the assay was analyzed by testing serum samples of patients and the results were compared with that of RT-PCR, displaying a sensitivity of 100% and specificity of 93.3%. Shaw and coworkers developed another nanotechnology-based portable system for the detection of SARS-CoV-2 IgA, IgM and IgG with 88% sensitivity and 75% specificity.[46]

2.1.3 Nucleotide-functionalized AuNPs. AuNPs, when functionalized with suitably designed oligonucleotides specific for nucleoproteins of SARS-CoV-2, can diagnose SARS-CoV-2 in a matter of minutes.[47] The surface-modified AuNPs agglomerated in the presence of their target analyte, leading to a visual color change. Moitra *et al.* developed a colorimetric assay using AuNPs functionalized with thiol-modified antisense oligonucleotides (ASOs) specific to SARS-CoV-2 as displayed in Fig. 4.[47] The ASO-functionalized AuNPs agglomerated in the presence of an RNA sequence of SARS-CoV-2 evidenced from surface plasmon resonance. The cleavage of the RNA strand from the RNA–DNA complex of SARS-CoV-2 and ASO-functionalized AuNPs with addition of RNaseH led to the precipitation of AuNPs which could also be visualized with the naked eye as depicted in Fig. 4. The sensor system was highly selective and could sense viral RNA with a detection limit of 0.18 ng μL^{-1} of viral load.

Qiu *et al.* fabricated a dual-functional plasmonic biosensor for the clinical diagnosis of SARS-CoV-2.[48] The biosensor combines the plasmonic photothermal effect and localized SPR sensing transduction of 2D gold nanoislands functionalized with complementary DNA receptors. The biosensor displayed high sensitivity towards SARS-CoV-2 with a detection limit of 0.22 pM. Feng *et al.* developed another immunofluorescence assay for the detection of IgM and IgG.[49] The sensitivity and specificity of the immunochromatographic assay was observed to be 98.72% and 100% for IgG and 98.68% and 93.10% for IgM, respectively, when tested on serum samples from 28 clinical positive and 77 negative patients. Zhu *et al.* fabricated a nanoparticle-based biosensor assay coupled with RT loop-mediated isothermal amplification for COVID-19 diagnosis.[50] The system was found to be highly sensitive and displayed

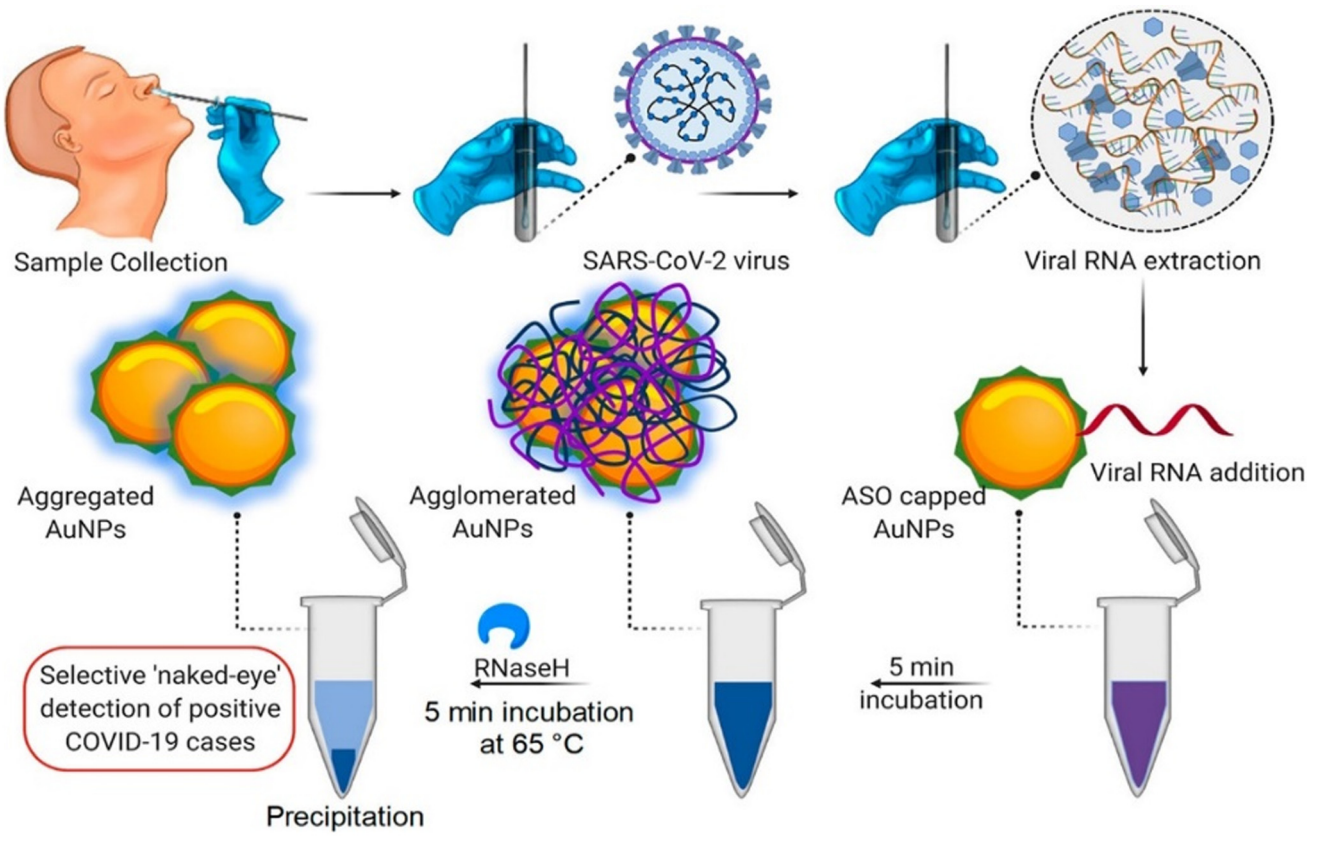

Fig. 4 Schematic representation for the naked-eye detection of SARS-CoV-2 RNA mediated by the suitably designed ASO-capped AuNPs. Reprinted from ref. 47. P. Moitra *et al.*, *ACS Nano*, 2020, 14, 7617–7627. Creative Commons public use license.

no cross-reactivity with non-SARS-CoV-2 templates (virus, fungi, synthetic nucleic acid sequences and bacteria). The efficiency of the assay was evaluated using oropharynx swab samples from COVID-19 positive (33/33) and negative (96/96) patients, demonstrating 100% specificity and sensitivity.

Díaza *et al.* developed a colorimetric method for the detection of various SARS-CoV-2 proteins employing AuNPs.[51] The sensor system employed different sizes of nanoparticles, sequences of oligonucleotides and buffer composition. In the presence of SARS-CoV-2, the oligonucleotides unfold, and their cholesterol moiety exposed to the aqueous medium gives a color change due to precipitation and aggregation of AuNPs [Fig. 5]. The system could detect the virus sequence in 15 min with high efficiency and selectivity.

CRISPR (clustered regularly interspaced short palindromic repeats) technology has been extensively used for the fabrication of highly specific and sensitive molecular assays including the recognition of viral RNA of SARS-CoV-2.[52] Based on this technology, Cao *et al.* reported the development of a colorimetric assay employing *trans*-cleavage activity of the CRISPR/Cas system to facilitate sequence-dependent aggregation of AuNPs as displayed in Fig. 6.[53] The assay provided a specific and sensitive detection of the viral RNA of SARS-CoV-2 which could also be observed with the naked eye with a response time of <1 min.

Another CRISPR–Cas-based biosensor equipped with a smartphone readout was developed for the ultrasensitive and selective determination of the virus by Ma *et al.*[54] The disaggregation of AuNPs induced by the degradation of single-stranded DNA led to observable color changes, which could be easily read on a smartphone device with a Color Picker app. Alafeef *et al.* developed a biosensor for the viral nucleocapsid phosphoprotein (N-gene) using highly specific antisense oligonucleotides (ssDNA) capped on AuNPs.[55] The authors optimized the conjugation of ssDNA concentration with AuNPs. Upon the addition of SARS-CoV-2 RNA to a graphene–AuNP-based platform, a red shift was observed through hyperspectral imaging [Fig. 7].

The same research group developed another colorimetric method for rapid and naked eye detection of the virus using a unique approach integrating nucleic acid amplification and plasmonic sensing of the virus with a response time of less than an hour.[56] The method employs plasmonic AuNPs capped with antisense oligonucleotides as a colorimetric

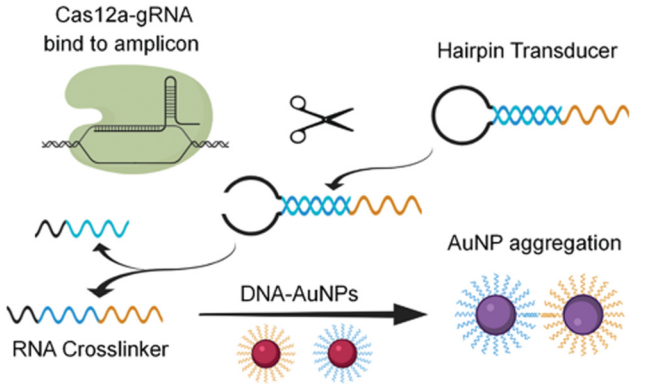

Fig. 6 Schematic representation of the principle of Cas-mediated AuNP aggregation and corresponding change in color from red to purple. Reproduced from ref. 53. Y. Cao *et al.*, *Chem. Comm.*, 2021, 57, 6871–6876. Copyright (2021) Royal Society of Chemistry.

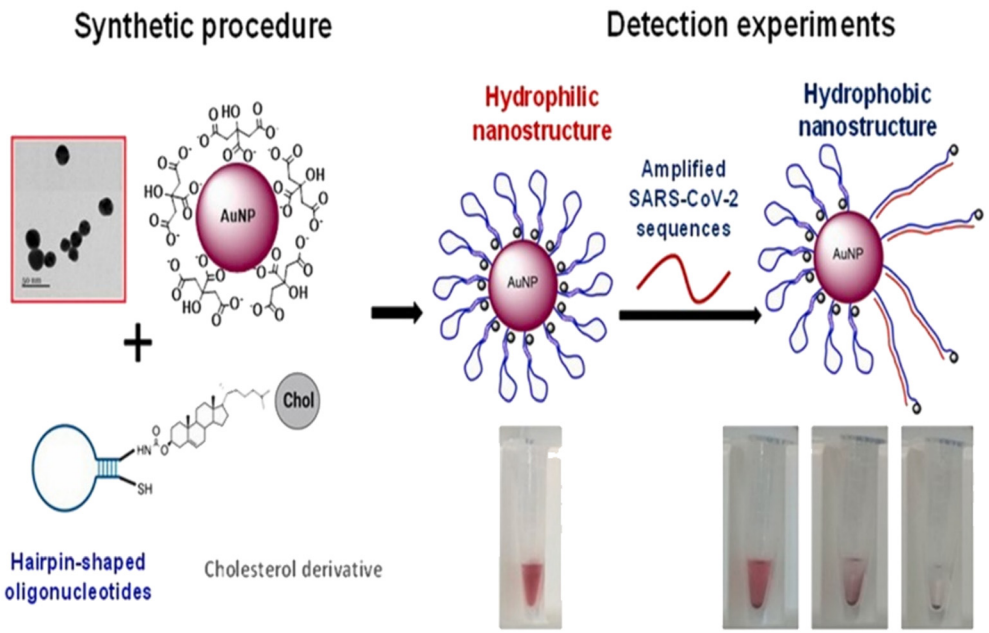

Fig. 5 Schematic representation of the sensor. AuNPs are modified with oligonucleotides containing a cholesterol moiety. The nanostructures are exposed to SARS-CoV-2 sequences, which induce the rearrangement of the hairpin structure of the oligonucleotides to an open structure. Thus, the cholesterol is exposed to the aqueous solution, causing the aggregation of the nanoparticles, detectable by the naked eye. Reprinted from ref. 51. C. R. Díaza *et al.*, *Talanta*, 2022, 243, 123393–123403. Creative Commons CC-BY license.

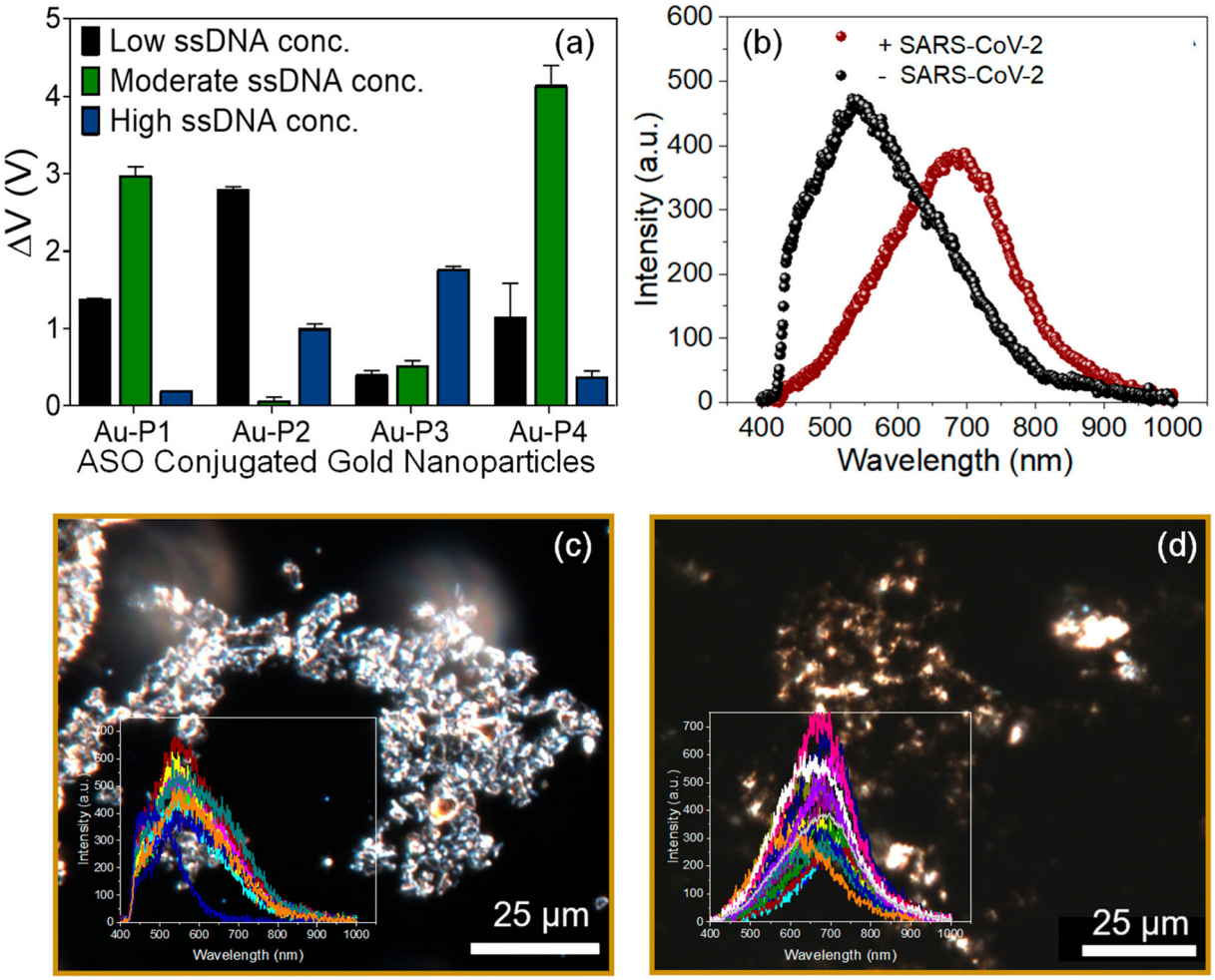

Fig. 7 (a) Optimization of the antisense ssDNA probes conjugated with AuNPs at different ratios to achieve maximum sensitivity and optimal signal output. (b) Average of the spectra obtained from the hyperspectral imaging from the graphene–Au–P_{mix} in the absence and presence of SARS-CoV-2 viral RNA. Enhanced dark-field microscopic-hyperspectral image (EDFM-HSI) of the graphene–Au–P_{mix} (c) in the absence of SARS-CoV-2 RNA and (d) in the presence of SARS-CoV-2 RNA with spectra collected from several pixels shown in the inset. Reprinted with permission from ref. 55. M. Alafeef *et al., ACS Nano,* 2020, 14, 17028–17045. Copyright (2021) American Chemical Society.

sensor to determine the nucleic acid specifically resulting in the aggregation of the AuNPs. Furthermore, the result can be monitored using a handheld optical reader to quantify the response. The dual detection of both Abs and antigens is highly of interest; therefore, Sadique *et al.* developed a novel category of electrochemical immunosensors for dual detection of SARS-CoV-2 antibodies and antigens by fabricating graphene oxide–gold nanocomposites.[57] DPV was employed to quantify the detection of SARS-CoV-2 antigens/antibodies [Fig. 8]. The LOD for the antigen immunosensor immunosensor was 3.99 ag mL^{-1}, while for the antibody this was 1 fg mL^{-1} and showed good consistency among patient serum and swab samples (Table 3).

2.1.4 Aptamer-functionalized AuNPs. Aptamers, single-stranded oligonucleotides, exhibit remarkable affinity and specificity towards their target molecules. These molecular entities can be synthesized using the systematic evolution of ligands by exponential enrichment (SELEX) methodology. Their versatile utility encompasses applications in areas such as bioimaging and therapeutics, serving as crucial recognition components within a multitude of detection platforms.[58] Aptamers rapidly found application in the field of sensors as innovative recognition elements. This is because they offer several advantages over antibodies, such as heightened stability, compact size, and strong affinity for small molecules. Moreover, they can be easily synthesized *in vitro*, straightforwardly modified, and can be flexibly designed within their sequences.[59] Therefore, researchers have used aptamers and functionalized them with AuNPs for the sensing applications of SARS-CoV-2.

Aithal and colleagues utilized aptamer-functionalized AuNPs for rapid detection test for SARS-CoV-2, which can detect 3540 genome copies per μL of inactivated SARS-CoV-2.[60] Researchers used two aptamers which have been developed previously with SELEX technology and can target the receptor-binding domain (RBD) of the SARS-CoV-2 spike proteins. The test uses plasmon absorbance spectra to detect 16 nM and higher concentrations of spike protein in

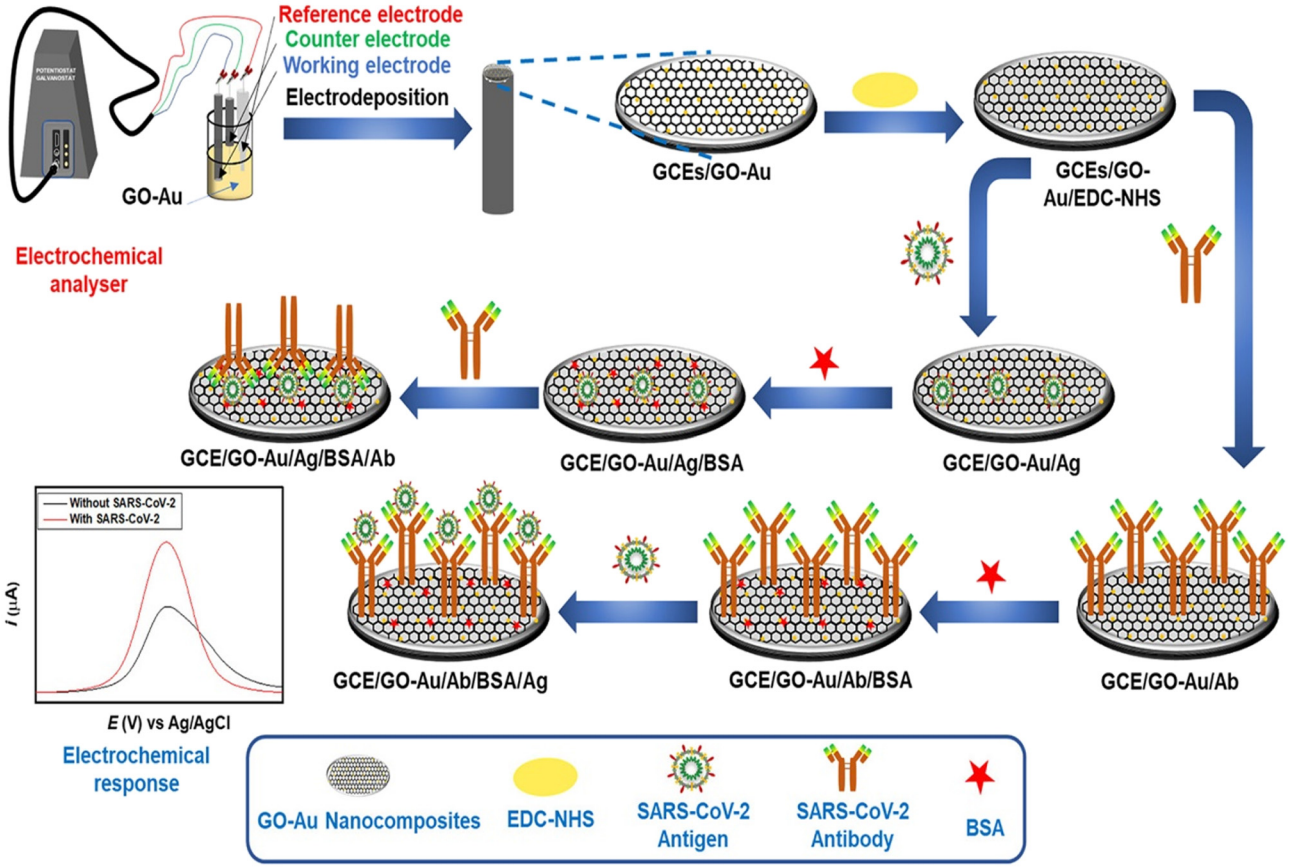

Fig. 8 Schematic of steps involved in the fabrication of SARS-CoV-2 antigen and SARS-CoV-2 antibody immunosensors. Reprinted from ref. 57. M. A. Sadique *et al.*, *ACS Appl. Bio Mater.*, 2022, 5, 2421–2430. Copyright (2021) Creative Commons public use license.

phosphate-buffered saline. The sensing signal is enhanced by adding a coagulant, $MgCl_2$ salt solution, to induce nanoparticle agglomeration, which depends on the amount of aptamer–protein binding. Adeel *et al.* developed a flexible aptamer-based electrochemical sensor for the rapid, label-free detection of SARS-CoV-2 S protein using a platform made of a porous and flexible carbon cloth coated with AuNPs.[61] The sensor displayed good selectivity, repeatability, and was tested in diluted human saliva spiked with different SARS-CoV-2 SP concentrations, providing lower limits of detection (LODs) of 0.11 ng mL^{-1} and 37.8 ng mL^{-1}, respectively. AuNPs were used to coat the flexible carbon cloth platform to increase its conductivity and electrochemical performance [Fig. 9]. The thiol-functionalized DNA aptamer was assembled with the AuNPs *via* thiol–Au bonds for the selective recognition of the SARS-CoV-2 spike protein.

Guan *et al.* fabricated a SERS nanoprobe by mixing AuNPs with Raman reporter Nile blue A (NBA).[63] These nanoprobes were then functionalized with spike protein aptamers and magnetic beads and redispersed in PBS-T buffer solution. The probes modified with aptamers cocktail, recognize the spike protein to form sandwich complexes, which are then detected by a handheld Raman spectrometer within 5 min with a LOD of 18 fM for the pseudovirus. Daniel *et al.* developed a low-cost, handheld device for rapid detection of SARS-CoV-2 RNA using aptamer-functionalized AuNPs synthesized by a novel method.[64] The device can detect SARS-CoV-2 RNA in less than 5 min by utilizing the selectivity of the aptamer to SARS-CoV-2 RNA.

Abrego-Martinez *et al.* demonstrated an aptamer-functionalized AuNP-based electrochemical biosensor for the detection of RBD in S protein of SARS-CoV-2.[62] The biosensor demonstrated excellent sensing performance, with a LOD of 1.30 pM (66 pg mL^{-1}) for SARS-CoV-2 S protein and was successfully applied for the detection of a SARS-CoV-2 pseudovirus. Gu *et al.* fabricated a new sensor based on spherical cocktail aptamer–AuNP (SCAP) for ultrasensitive detection of SARS-CoV-2 S protein based on surface-enhanced Raman scattering (SERS).[65] The aptamers are used to specifically bind to the SARS-CoV-2 S protein, while the AuNPs enhance the Raman signal through SERS. The developed detection platform can detect the SARS-CoV-2 S protein with a LOD as low as 0.7 fg mL^{-1}. Sun and co-workers reported the development of a new neutralizing agent called spherical neutralizing aptamer–AuNP (SNAP) to inhibit SARS-CoV-2 infection and suppress mutational escape.[66] SNAP exhibits exceptional binding affinity against the RBD of SARS-CoV-2 S protein and potent neutralization against authentic SARS-CoV-2 and mutant pseudoviruses.

Table 3 Oligonucleotide/DNA-functionalized AuNPs for SARS-CoV-2 detection platform

System	Detection or capture molecule/ligands	Target	Analytical method	Real sample testing/live/pseudo-virus	LOD	Ref.
AuNPs	Thiol-modified antisense oligonucleotides	N-gene (nucleocapsid phosphoprotein)	Colorimetric	Yes	0.18 ng μL^{-1}	47
Gold nanoislands (AuNIs)	DNA receptors	Spike proteins, membrane proteins and envelope proteins	Plasmonic photothermal (PPT) effect and localized surface plasmon resonance (LSPR)	Yes	0.22 pM	48
AuNPs	Nucleocapsid protein	IgM and IgG antibodies	Immunofluorescence assay	Yes	Not reported	49
AuNPs	F1ab (opening reading frame 1a/b) and np (nucleoprotein) genes	—	RT loop-mediated isothermal amplification	Yes	Not reported	50
AuNPs	Oligonucleotides	E and R (RNA-dependent RNA polymerase) genes, the insertion region of S protein	Colorimetric	Yes	10 nM (E), 20 nM (R), 500 pM each (E + R)	51
AuNPs	Cas12a-gRNA ribonucleoprotein	N and E gene	Colorimetric	Yes	Not reported	53
AuNPs	Single-stranded DNA	N gene	CRISPR-Cas12a powered visual biosensor	Yes	1 copy per µL for SARS-CoV-2 pseudoviruses 6.9 copies per µL	54
Plasmonic AuNPs	Antisense oligonucleotides	Nucleic acid	Colorimetric	Yes	10 copies per µL	55
Graphene oxide–gold nanocomposites	Antigen/antibody	Antigen/antibody	Electrochemical immunosensors	Yes	3.99 ag mL^{-1} (antigen), 1 fg mL^{-1} (antibody)	56

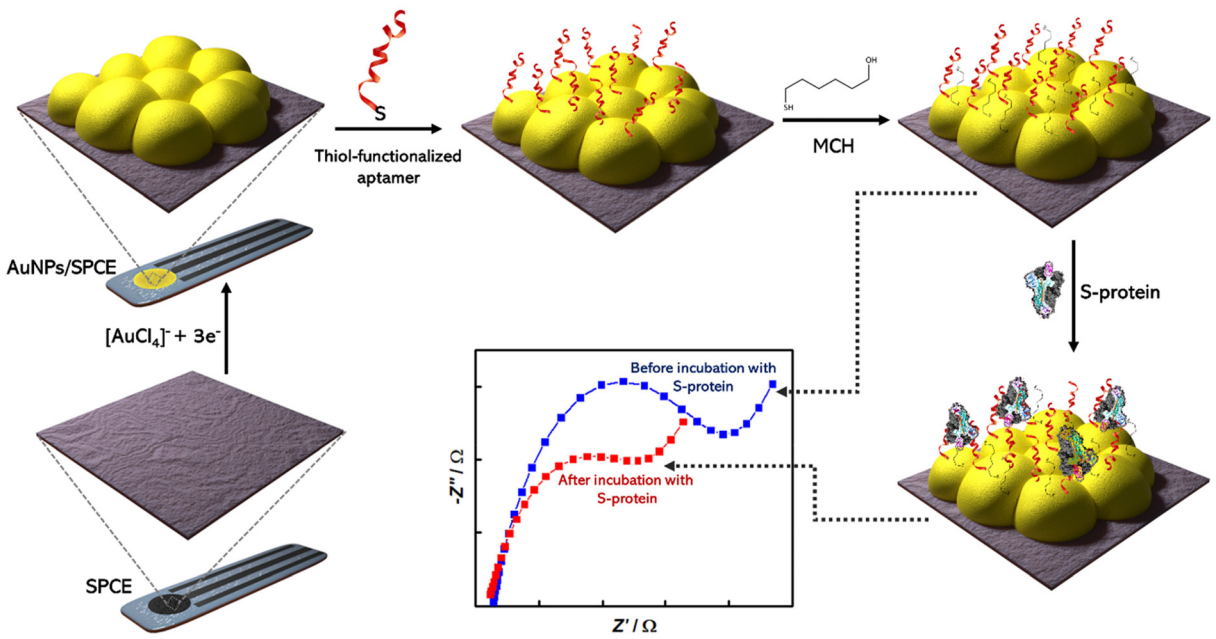

Fig. 9 Schematic representation of fabrication of aptasensor for SARS-CoV-2 S-protein detection using screen printed carbon electrode (SPCE); Reprinted from ref. 62; Abrego-Martinez *et al., Biosen. Bioelectron.,* 2022, 113595. Copyright (2022) Elsevier.

Incorporating aptamer-functionalized AuNPs for SARS-CoV-2 detection exemplifies a pioneering approach at the forefront of biosensing technology, harnessing the prowess of aptamers' precision, stability, and design versatility. This innovative strategy not only showcases enhanced sensitivity and specificity but also heralds a new era of rapid, reliable,

and adaptable diagnostic solutions for addressing the challenges posed by infectious diseases like COVID-19.

2.2 Molecularly imprinted polymeric nanoparticles

Molecularly imprinted polymeric nanoparticles (nanoMIPs) are artificial receptors, which possess tailor-made recognition sites that are complementary to the size, spatial orientation, and chemical functionality functional groups of the template molecule.[67,68] These artificial receptors display high selectivity and binding affinity towards a particular analyte (template) and can compete with antibodies in terms of affinity.[69] Moreover, these nanoparticles are highly robust and exhibit high chemical and thermal stability. Henceforth, these nanoMIPs have attracted significant attention in the development of biosensors for the detection of bacterial endotoxins and viruses.[70,71] Fabrication of nanoMIPs can be accomplished *via* various approaches, namely nanoprecipitation, mini-emulsion and solid-phase polymerization. In the nanoprecipitation method, a solution containing a mixture of monomers and template is polymerized with a suitable initiator and, after removal of the solvent (*via* evaporation or ultrasonication), nanoMIPs are obtained *via* precipitation.[72,73] Mini-emulsion polymerization is widely used to prepare nanoMIPs opposite to microemulsion where formation of emulsion is spontaneous. Moreover, high shear force needs to be employed to break up the emulsion into nanoscale particles to obtain mini-emulsions. In both nanoprecipitation synthesis and mini-emulsion polymerization, the template is removed using different methods including centrifugation, dialysis and extraction.[74] In solid-phase synthesis, the template is covalently bound to a solid phase such as glass beads which is used as an affinity medium to separate and collect high-affinity nanoMIPs.[75] In this approach, unreacted monomers and low-affinity nanoMIPs are removed using low-temperature elution, and then the high-affinity nanoMIPs are purified using elevated-temperature elution (60 °C) as shown in Fig. 10. The advantage of this approach lies in the covalent immobilization of the template molecule which ensures that the resulting high-affinity nanoMIPs are template-free. Furthermore, the covalent immobilization of the template molecule allows for a specific orientation, which results in the production of a more homogeneous binding site affinity, much like monoclonal antibodies.

Given their synthetic nature, MIPs possess excellent thermal and chemical stability. Moreover, MIP-based platforms can be deployed at different pH ranges, where most of the lateral flow rapid antigen tests fail to record accurate results.

For synthesizing MIPs against SARS-CoV-2, one can use a range of monomers. For instance, *N*-isopropylacrylamide and (hydroxyethyl)methacrylate can be used for hydrogen bonding and *N-tert*-butylacrylamide and 2-ethylhexylacrylate for hydrophobic interactions. Moreover, cationic monomers such as *N*-(3-aminopropyl)methacrylamide, 2-aminoethylmethacrylamide hydrochloride, 2-aminoethyl methacrylate, 3-guanidino-propylmethacrylate, methacrylic acid (MAA) and acrylic acid (AAc) are typically the monomers of choice to facilitate ionic interactions. It has been reported that a mix of monomers that enable hydrogen bond formation or ionic and hydrophobic interactions, particularly for protein targets, is crucial to produce MIPs with high affinity and selectivity. The most commonly used crosslinkers for organic polymerisation are ethylene glycol

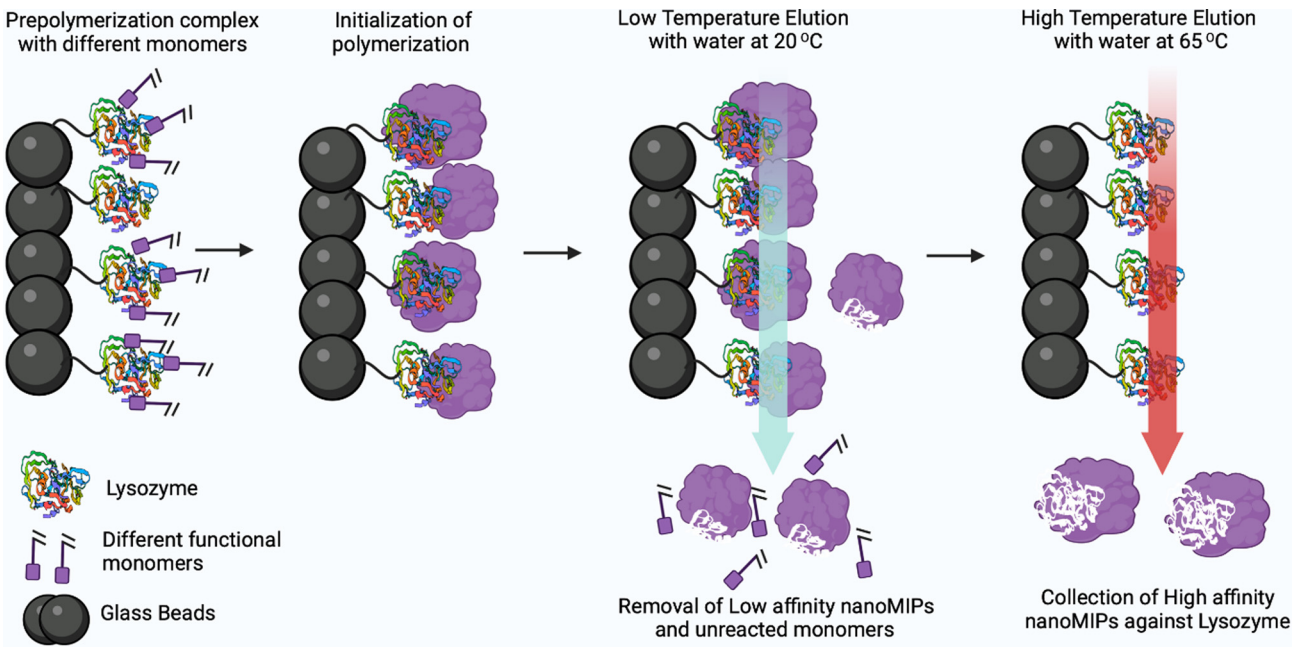

Fig. 10 Solid-phase synthesis to produce nanoMIPs against protein lysozyme (instead of lysozyme, other proteins or peptides can be imprinted). Reprinted from ref. 75. P. Singla *et al.*, *Anal. Bioanal. Chem.*, 2023, 415, 4467–4478; copyright (2023) Springer Nature.

dimethacrylate (EGDMA) and trimethylolpropane trimethacrylate (TRIM), whilst for aqueous polymerisation typically *N*,*N*-methylenebis(acrylamide) is used. To synthesize nanoMIPs, the entire protein can be imprinted; however, due to the fragile nature of proteins, their native confirmation might be altered during the polymerization process. Furthermore, proteins are often expensive and/or not available in sufficient amounts. To overcome these issues, short surface-exposed fragments of protein (*i.e.* antigenic determinants of a protein that binds to an antibody, known as epitopes) can be used as a template.[76]

Scientists at Rice University developed different methodologies for the detection of viruses; one for the detection of viral particles in the air and another consisting of a mobile phone reader with a chip capable of detecting virus in a blood sample.[77] A thin-film electronic device capable of detecting virus in air was developed under the RAPID (real-time amperometry platform using molecular imprinting for the selective detection of SARS-CoV-2) project, in addition to a plug-in tool to diagnose small amounts of virus in less than an hour. Another research group at Harvard and MIT are attempting to develop a face mask which lights up when it detects the presence of SARS-CoV-2 virus.[78,79] Using molecularly imprinted technology, scientists from Sixth Wave company were the first to develop MIPs for the rapid detection of SARS-CoV-2. A United States patent was filed on Accelerated Molecularly Imprinted Polymers (AMIPs™) technology and they incorporated this technology into a face mask. This mask will show a color change upon exposure to the virus exhaled in the breath.[36] Moreover, this company achieved the colorimetric detection of the delta variant of the SARS-CoV-2 virus causing COVID-19 infection using the same AMIPs™ technology.[80,81]

MIP Discovery (formerly MIP Diagnostics), a UK-based company, has developed a sensor for SARS-COV-2 using nanoMIPs synthesized *via* the proprietary solid phase approach. McClements and colleagues have used these nanoMIPs for the detection of the SARS-CoV-2 in clinical samples as well as the RBD (receptor binding domain) of spike protein using the heat transfer method.[82] The system was thermally stable and offered superior sensitivity towards antibodies with a LOD found to be in the range of fg mL^{-1}. In addition to this, Bhalla *et al.* developed a nanoplasmonic biosensor using the same nanoMIPs employed in the previous study for the detection of different variants of SARS-CoV-2.[83] The technology enabled the detection of spike proteins of the SARS-CoV-2 with a LOD of 9.71 fM (1.3 pg mL^{-1}), 7.32 fM (1.04 pg mL^{-1}) and 8.81 pM (1.2 ng mL^{-1}) for the alpha, beta and gamma variants of the proteins, respectively. Cennamo *et al.* developed a low-cost optical–chemical MIP sensor.[84] The sensor system displayed a change in the refractive index of the MIP nanofilm upon binding with the viral particles, which was investigated using a simple plasmonic D-shaped POF platform. The sensor employed a plasmonic plastic optical fiber sensor coupled with a synthetic MIP nanolayer for the detection of the spike protein of SARS-CoV-2. The research group firstly established the effectiveness of the MIP receptor to bind the spike protein and then measured clinical nasal swabs. The test results obtained using MIPs were compared with the RT-PCR results and were found to be more sensitive than RT-PCR with relatively fast response time. Raziq *et al.* reported on a MIP-based electrochemical sensor for the detection of SARS-CoV-2 antigen (ncovNP) as displayed in Fig. 11.[85] The ncovNP sensor displayed a detection and quantification limit of 15 fM and 50 fM (0.7–2.2 pg mL^{-1}), respectively, in lysis buffer.

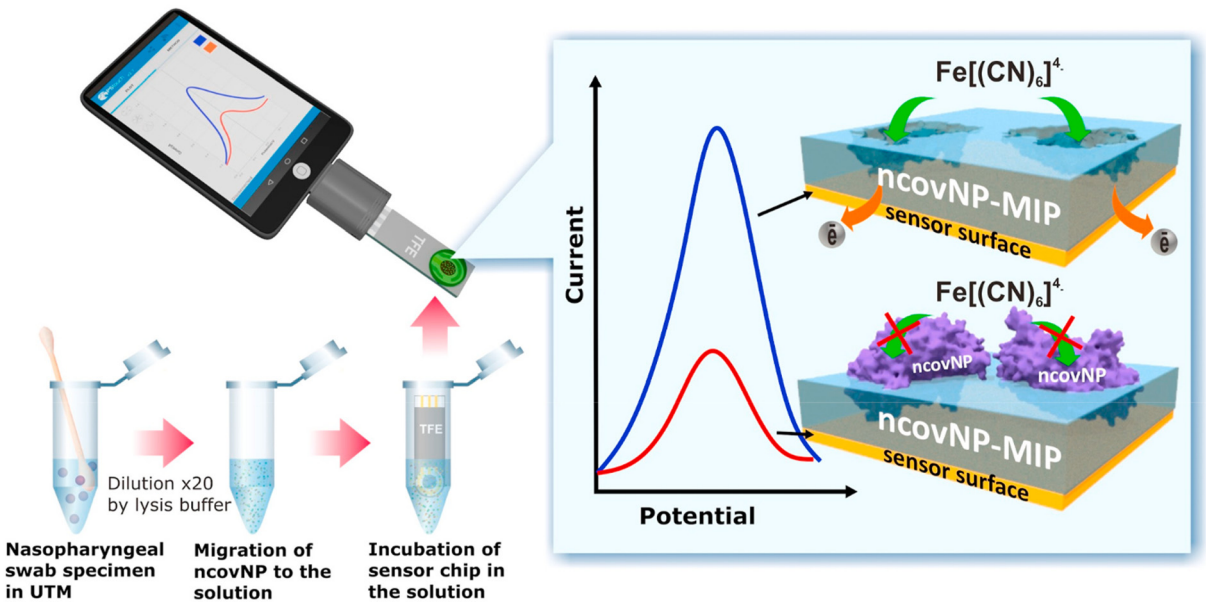

Fig. 11 COVID-19 diagnosis by the ncovNP electrochemical sensor analyzing the samples obtained from nasopharyngeal swabs of COVID patients. Reprinted from ref. 85. A. Raziq *et al.*, *Biosens. Bioelectron.*, 2021, 178, 113029–113036. Copyright (2021) Elsevier.

Table 4 Summary of molecularly imprinted based sensor platforms for the detection of SARS-CoV-2

System	Imprinted template	Target	Analytical method	Real sample testing/live/pseudovirus	LOD	Ref.
nanoMIPs	Spike protein epitope	Spike protein	Heat transfer method	Yes	~9.9 fg mL^{-1} (alpha) and ~6.1 fg mL^{-1} (delta) variant	81
nanoMIPs	Spike protein epitope	Spike protein	Nanoplasmonic biosensor	Yes	1.3 pg mL^{-1}, 1.04 pg mL^{-1} and 1.2 ng mL^{-1} for the alpha, beta and gamma variants	82
MIPs	Spike protein (S1 subunit, His-Tag)	S1 protein	Optical–chemical	Yes	3.8 mg mL^{-1}	83
MIPs	Spike protein	Spike protein S1 antigen	Electrochemical	Yes	0.7 pg mL^{-1}	84
MIPs	SARS-CoV-2 (2019-nCoV) spike protein (RBD)	Spike protein	Multiple techniques	Yes	1 ng	85
e-MIPs	SARS-CoV-2 pseudotyped particles (PPs)	SARS-CoV-2 PPs	Electrochemical	Yes	88.0 fg mL^{-1}	87

Additionally, the sensor could differentiate the target nucleoprotein from interfering proteins such as, S1, BSA, CD48 and E2 HCV.

Parisi *et al.* synthesized plastic antibodies based on MIPs using an inverse microemulsion method against the SARS-CoV-2 spike protein, employing the hydrophilic monomers acrylic acid and acrylamide. The synthesized nanoMIPs could recognize and bind to novel coronavirus SARS-CoV-2 RBD with high selectivity to inhibit the activity of spike protein. Moreover, these nanoMIPs inhibited the virus replication in Vero cell culture and thus are deemed suitable for the prevention and treatment of SARS-CoV-2 infection.[86] Sharif and colleagues electropolymerized water-soluble *N*-hydroxymethylacrylamide (NHMA) as a functional monomer and cross-linked this with *N*,*N*-methylenebisacrylamide using SARS-CoV-2 pseudoparticles (PPs) as a template to imprint. The obtained LOD was 88.0 fg mL^{-1}, suggesting its suitability for detection of SARS-CoV-2 with minimal sample preparation (Table 4).[87]

3.0 Comparison of functionalized AuNP- and nanoMIP-based platforms

For the development of highly sensitive, rapid, and reliable diagnostic tools for SARS-CoV-2 virus detection, both functionalized AuNPs and nanoMIPs are promising approaches. AuNPs functionalized with molecular recognition probes (antibodies, antigens, nucleotides, and aptamers) possess unique optical and physical properties, whilst MIPs exhibit high selectivity, sensitivity, robustness, reusability and batch-to-batch consistency. The major advantages of the nanoMIP platform are that there is no direct need to conjugate antibodies, proteins/antigens, nucleotides, and aptamers specific for SARS-COV-2. In contrast, AuNP-based sensor systems rely on the cross-linking of these nanoparticles with the aforementioned molecular recognition elements/biological counterparts. Immunosensors utilizing antibody-functionalized AuNPs and antigen-functionalized AuNPs are undoubtedly the most employed binders for detection of SARS-CoV-2 (and in diagnostics in general). They are relatively expensive and have limited shelf life and stability. Besides this, MIPs are cost-effective and can withstand high temperature and pH conditions without compromising the binding affinity and selectivity. Another crucial aspect to consider is the fabrication duration of individual sensors employing nanoparticles. Typically, immunosensors that utilize nanoparticles functionalized with antibodies involve more extensive procedures, demanding several hours to effectively immobilize biomolecules. The phase of obstructing nonspecific bindings using blocking solutions like BSA consumes a substantial period, approximately around 30 min. Aptamer functionalized AuNPs are also a viable alternative option to fabricate aptamer sensors for SARS-CoV-2 as it has excellent stability and cost-effectiveness as compared to antibodies, but the production time to design and fabricate aptamers can take weeks to months. Conversely, sensors based on MIPs might exhibit a shorter production time, particularly when employing the solid-phase synthesis method. The removal of the template through the dialysis technique or *via* immersing the electrode in extraction solutions (in the case of electropolymerization) might constitute one of the most time-intensive stages, lasting a few hours. Nonetheless, by utilizing solid-phase synthesis, the separation of high-affinity nanoMIPs can be achieved within a few minutes. There are varied functionalities of AuNPs modified for detecting antigens and antibodies. The nucleotide-functionalized AuNPs exhibit dual detection mode for both antigens and antibodies. The LOD with antibody-functionalized AuNPs is as low as 1 fg mL^{-1}, whilst with MIPs it is 6.1 fg mL^{-1}, both of which are suitable for lateral flow immunoassay in portable devices.

4.0 Conclusions

This review summarized and compared two of the most important and widely researched nanoparticle systems for

detection of SARS-CoV-2. The detection of SARS-CoV-2 is currently a global health concern and diagnostics play a crucial role in controlling the spread of infectious diseases. The diagnostic tools based on functionalized AuNPs as well as MIPs offer many advantages such as high selectivity, robustness, rapid response time, high specificity and in some cases, reusability. Furthermore, we have discussed the concept of molecular design for developing portable sensors capable of detecting the SARS-CoV-2 virus. This approach has the potential to be extended for detecting other pathogenic viruses, offering a valuable tool in preventing and addressing future pandemics. All these methodologies can identify the virus with high selectivity and sensitivity with a response time of less than an hour and, in a few cases, even minutes. However, functionalized AuNPs rely on other molecular recognition elements such as antibodies (especially mAbs), antigens, nucleotides, aptamers, and others. However, their very nature also brings some disadvantages: relatively high manufacturing costs together with difficult functionalization chemistry and relatively short shelf life at room temperature as well as time-consuming and sometimes complex production processes involving animals. Intriguing alternatives to these biological counterparts as molecular recognition are MIPs. Current imprinting techniques suffer from a heterogeneous "polyclonal" distribution of binding sites, poor performance in water, low capacity and slow mass transfer. The use of solid-phase approaches overcomes these issues, as the immobilized template enables an oriented immobilization, thus reducing the "polyclonality" of the imprinted sites. The synthetic nature of nanoMIPs allows them to withstand bacterial degradation. They therefore have a longer shelf life, even in the absence of refrigeration. This makes them viable for use in more remote geographical areas where healthcare infrastructure is more limited. Hence, nanoMIPs represent ideal candidates for replacing antibodies in lateral flow tests to be deployed in equatorial countries across the globe. However, they do encounter some challenges and involve ensuring reliable fabrication and reproducibility, achieving selective binding in complicated mixtures, and keeping their performance steady over longer time frames. Additionally, factors like making nanoMIPs work seamlessly with devices, efficiently scaling up production, and managing costs need to be considered. To overcome these challenges, future progress might involve fine-tuning the methods used to develop them to gain better control over their size and properties. Enhancing their design to make their binding strength and kinetics even better, and creating surfaces that fit perfectly with devices, are also potential avenues. Investigating new ways to cross-link these materials could also make them more durable. Using computer modelling could help predict and optimize their behaviour. It is very important to carefully check if these materials work well with living things. Finding good methods to deal with tricky samples and having experts from different areas like materials, chemistry, biology, and engineering collaborate is a crucial way to directly deal with these challenges. This comprehensive approach is absolutely essential to make sure that nanoMIPs become seamlessly integrated into a wide range of applications.

Conflicts of interest

The authors have no conflicts to declare.

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