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Identification of SARS-CoV-2 by Gold Nanoparticles

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Abstract: The SARS-CoV-2 outbreaks highlighted the need for effective, reliable, fast, easy-to-do and cheap diagnostics procedures. We pragmatically experienced that an early positive-case detection, inevitably coupled with a mass vaccination campaign, is a milestone to control the COVID-19 pandemic. Gold nanoparticles (AuNPs) can indeed play a crucial role in this context, as their physicochemical, optics and electronics properties are being extensively used in photothermal therapy (PTT), radiation therapy (RT), drug delivery and diagnostic. AuNPs can be synthesized by several approaches to obtain different sizes and shapes that can be easily functionalized with many kinds of molecules such as antibodies, proteins, probes, and lipids. In addition, AuNPs showed high biocompatibility making them useful tool in medicine field. We thus reviewed here the most relevant evidence on AuNPs as effective way to detect the presence of SARS-CoV-2 antigens. We trust future diagnostic efforts must take this 'old-fashioned' nanotechnology tool into consideration for the development and commercialization of reliable and feasible detection kits.

Introduction

Focus on the SARS-CoV-2

The Coronaviruses, belonging to the *Coronaviridae* family, are characterized by an enveloped, non-segmented, positive-sense RNA (Yang *et al.*, 2020; Liu *et al.*, 2020b). In humans, they can induce severe symptoms at respiratory, hepatic, and enteric level. In December 2019, a novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) quickly spread from China to the whole world (Mi *et al.*, 2020), inducing the well-known Covid-19 disease. As of 4 April 2022, time of this article drafting, there were approximately 486 million confirmed infection cases worldwide (https//:covid19.who.it; Dong *et al.*, 2020). The easy human-to-human transmission is ascribed to the virus airborne nature, which makes it easy to spread through air droplets especially in the last two virus variants, namely Omicron and Omicron 2 (Rowe *et al.*, 2022; Dance, 2022). Also, the biomolecular interaction has been characterized straight after the beginning of the pandemic outbreak and explained by the selective binding of the virus spike protein to the angiotensin-converting enzyme 2 (ACE2) receptors, which are over-expressed by type 2 pneumocytes in human airways (Kumar *et al.*, 2021). The further virus uptake is due to the lysis of spike proteins induced by specific proteases of pneumocytes, which favors the entry by endocytosis or membrane fusion (Mirastschijski *et al.*, 2020). Once within cells, the SARS-CoV-2 releases its positive-sense RNA within the host cytoplasm, which triggers the production of pp1a and pp1ab polyproteins, and promotes the replication and transcription of viral RNA. The further transcription of viral mRNA induces the production of viral structural components like membrane, spike and nucleocapsid proteins (Zhang *et al.*, 2021). The viral particles are transported to the proximity of the host plasma membrane through the Golgi, and the new formed viruses escape by exocytosis, a specific mechanism promoting the infection of neighboring cells (Nakagawa *et al.*, 2016; Mason, 2020) (Fig. 1).

The massive inoculation of different kinds of vaccines worldwide has provided a reduction of Covid-19 outbreaks; in addition to the more conventional vaccines containing a weakened or killed virus, mRNA vaccines, such as the BNT162b2 vaccine, produced by BioNTech (in collaboration with Pfizer) delivers a small segment of mRNA loaded in lipidic nanovesicles into the cells (Kitchin *et al.*, 2020). This mRNA portion has the particularity to have the instructions to produce the spike proteins activating immune cells

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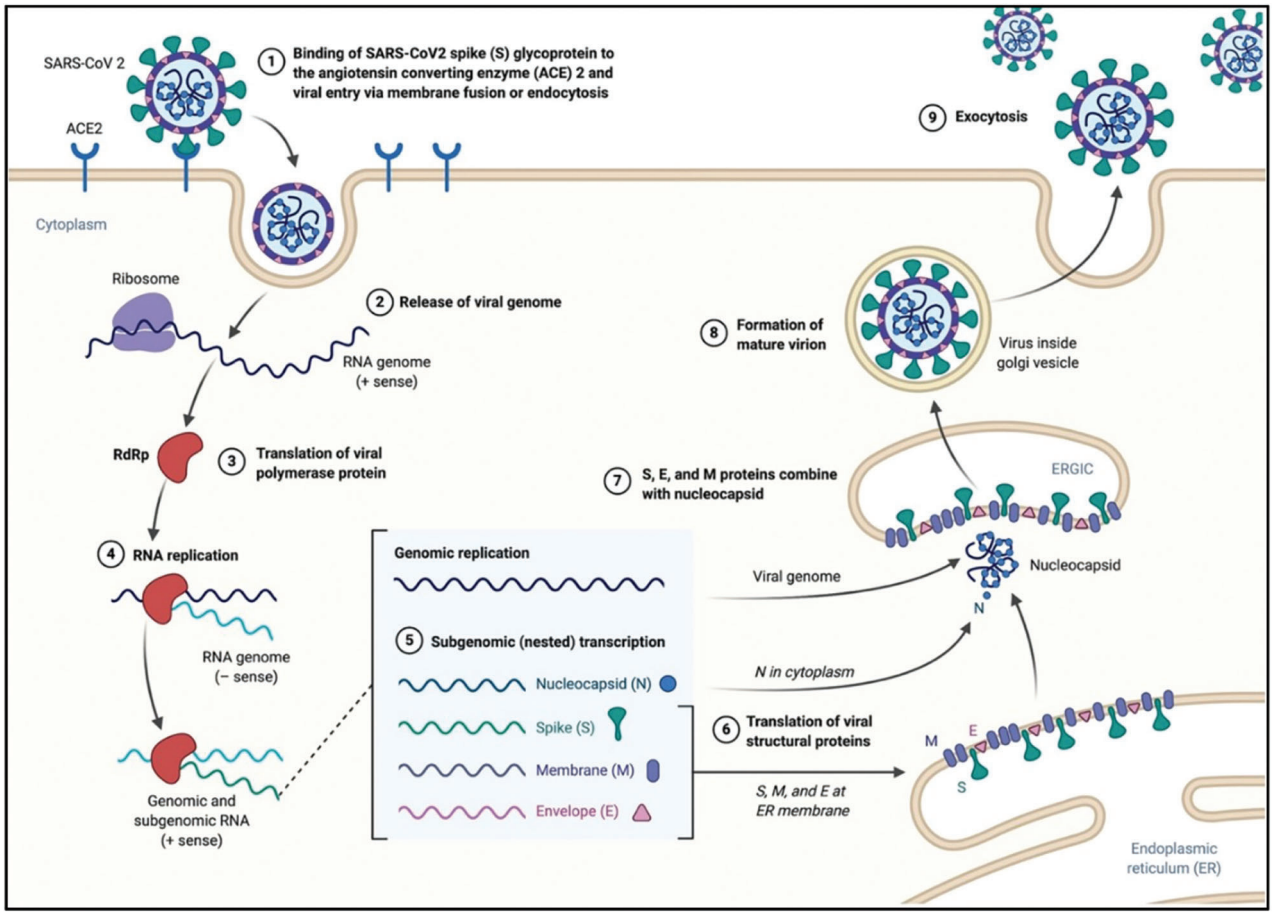

FIGURE 1. Mechanism of infection and life cycle of SARS-CoV-19 (Alanagreh *et al.*, 2020).

producing antibodies (Teijaro and Farber, 2021). The lipidic nanostructures are constituted by different types of lipidic molecules, but one of these is positive charged. Then, these molecules bind mRNA (negatively charged) but lose their positive charge when the basic conditions of the bloodstream occurred to reduce their adverse effects in human body (Dolgin, 2021). However, scientists were developing new strategies for enhancing the endosomal release of therapeutic nucleic acids, such as the use of new surfactants that enhance the delivery of mRNA into the cells (Røise *et al.*, 2022). Nevertheless, the scientific community also focused on the early diagnosis of the virus, to develop remedies that can support the active vaccination campaign. Indeed, the diagnostic assays can be a powerful tool to facilitate the surveillance after the vaccination as vaccinated people can also be infected by the virus (Fresco-Taboada *et al.*, 2022). In this scenario, nanotechnologies could be an optimal alternative for early SARS-CoV-2 diagnostic, especially using metallic NPs. Among these, gold (Au) based nanomaterials are probably the best choice for its unique physicochemical properties as it allows a combination of reliable detection (for diagnostic) together with a low toxicity (de Matteis and Rizzello, 2020; Silva Lopes *et al.*, 2014; de Matteis *et al.*, 2017).

Physicochemical properties of AuNPs

The physicochemical properties of AuNPs are several. However, some of them turn out to be suitable with a view to using these nano-objects as a diagnostic or therapeutic agent (Dykman and Khlebtsov, 2011; Yeh *et al.*, 2012). First, the Localized Surface Plasmon Resonance (LSPR) is by far the 'hallmark' among all the AuNPs physical properties. LSPR is an optical phenomenon occurring when polarized light triggers the oscillation of free conduction electrons on the AuNPs surface (de Matteis *et al.*, 2019). This specific feature of AuNPs makes possible a strong improvement of the optical extinction compared to the conventional organic molecules.

In general, the position of the LSPR peak is strongly dependent on the nanoparticle's physical properties such as such shape and size. In addition, the dielectric properties (Saison-Francioso *et al.*, 2015) as well as the local environment, namely surface-confined molecules substrate, solvent, and substrate, influence the LSPR phenomenon (Peixoto de Almeida *et al.*, 2014) (Fig. 2).

Moreover, the enhanced absorption of light by AuNPs has tremendous implications in different medicine sectors such as cancer treatment (Dykman and Khlebtsov, 2011) namely photothermal therapy applications (Bai *et al.*, 2020; De Puig *et al.*, 2015) and NP-based biosensors using colorimetric point-of-care devices (Iarossi *et al.*, 2018). In the latter case, the color of NPs solution changes due to plasmonic coupling between the NPs, as the color shift from red to purple color is due to aggregation in solution (Ghosh and Pal, 2007).

In addition to this, the use of Au in the body is tolerated due to its high reduction potential which makes it less ionizable and then stable in the body (Albanese and Chan, 2011). Several studies performed in living cells and animal

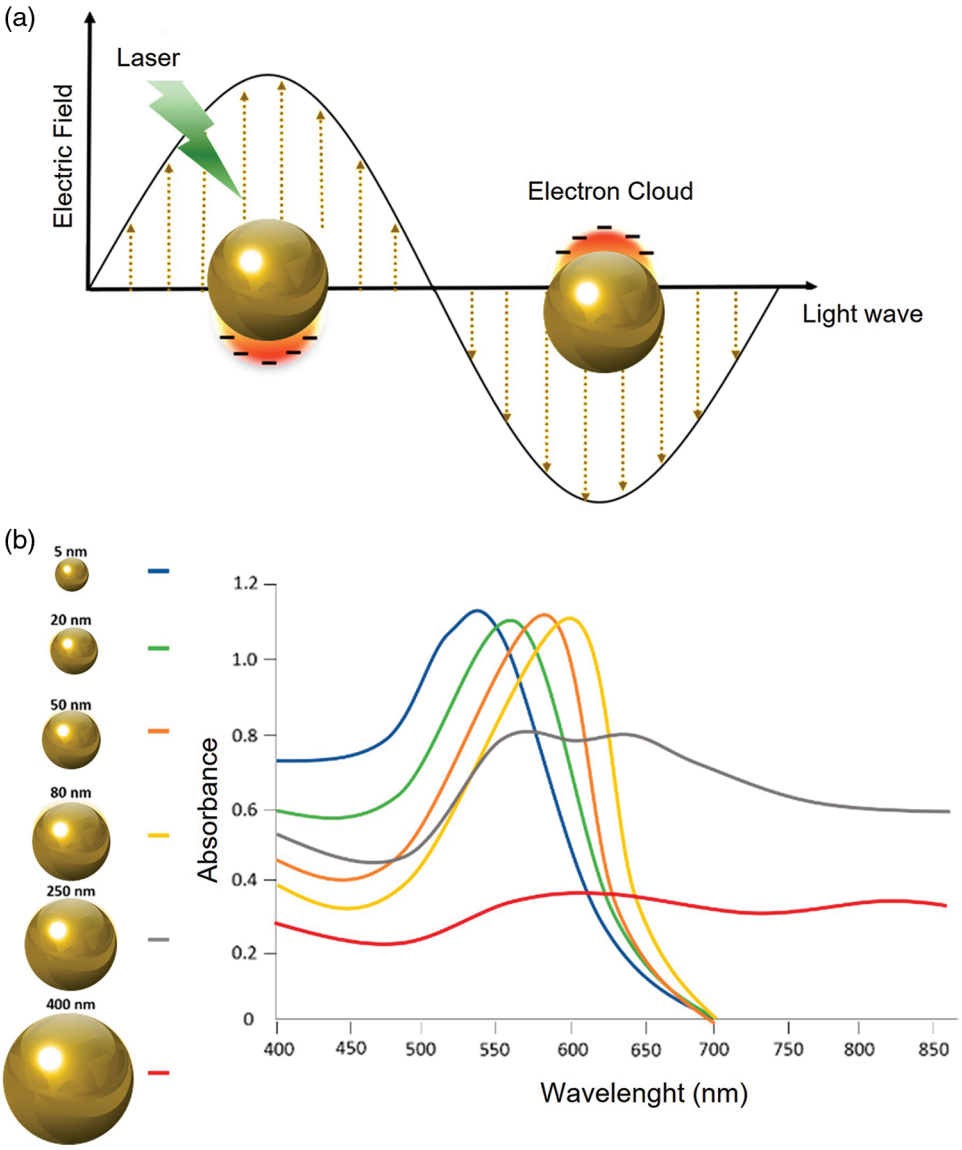

FIGURE 2. LSPR of AuNPs (a); Different UV-visible absorption spectra based on AuNPs size (b). Adapted from Kim and Lee (2018).

models demonstrated the biocompatibility of AuNPs (Zhang *et al.*, 2011; de Matteis *et al.*, 2020) which therefore is strongly dependent on NPs size and concentrations (Kang *et al.*, 2020) as well as on the cell types. In particular, the small and high doses correspond to the high toxicity (Vecchio *et al.*, 2012; Sani *et al.*, 2021).

The surface functionalization of AuNPs with several types of polymers, proteins, peptides, surfactant is a good approach to reduce the impact of NPs in cells (Mahato *et al.*, 2019).

In a typical photothermal treatment, the cancer cells are heated using temperatures between 41°C to 43°C by NIR stimulation after the uptake of Au nanomaterials (such as Au nanorods or Au nanoshells); as consequence, malignant cells can be destroyed (Zaho *et al.*, 2014; de Matteis *et al.*, 2021). As well as being a therapeutic agent, AuNPs can be used as diagnostic tools by exploiting other intrinsic characteristics of Au at the nanoscale.

For example, it is well known that the Raman scattering, representing the footprint of a certain molecule, has several applications in different fields (Cheng *et al.*, 2018). However, its use is limited by the small cross section associated to the Raman scattering that is 10–30 to 10–25 cm^2 per molecule (Nie and Emory, 1997). To improve the scattering, the molecules should be in the proximity of the AuNPs in order to exploit their local optical fields; this triggers a signal enhancement called Surface-Enhanced Raman Scattering (SERS) (Shuker and Gammon, 1970; Lee *et al.*, 2011). The latter is dependent on the AuNPs physicochemical properties such as shape, size, and aggregation. In particular, the best enhancement can be obtained on AuNPs aggregates or cluster having a size ranging from 20 nm to 60 nm (Kneipp *et al.*, 1997).

LSPR can also modulates the quantum yield of fluorescent dyes near their surface, thus inducing fluorescent enhancement or, alternatively, quenching, a process known as Metal Enhanced Fluorescence (MEF) (Geddes and Lakowicz, 2002; Shankar *et al.*, 2009). The two distinct effects (enhancement or quenching) are functions of the distance between the Au surface and the dye. This phenomenon is based on the fluorescence resonance energy transfer (FRET), responsible of the fluorescence quenching. FRET regards the energy transfer between fluorophores. It is widely used to study the potential interaction of biological molecules (such as proteins, lipids, or nucleic acids) located

in proximity. The mechanism exploits the presence of two fluorescent molecules, called donor and acceptor. The donor can be excited at a specific wavelength. This molecule emits energy which, in turn, can be transmitted to the acceptor, that consequently emits fluorescence at a different wavelength of emission compared to the donor. To this respect, AuNPs can improve the sensitivity and efficiency of FRET due to their large molar extinction coefficients (Chen *et al.*, 2012).

The latter is also extremely useful for enhancing the sensitivity of standard colorimetric assays (Brennan *et al.*, 2009). One advantage is the possibility to make a real-time monitoring with a good reproducibility and sensitivity, making AuNPs suitable for point of care (POC) diagnostics (Mariani and Minunni, 2014). For example, the plasmon-induced color switch has been exploited to detect altered levels of cytokines, proteins, DNA in cancer (Reddy *et al.*, 2012), to monitor the bacteria contamination in food (Waswa *et al.*, 2007) and in laboratory medicine (Helmerhorst *et al.*, 2012).

In the context of X-ray-based diagnostic, AuNPs are applied as alternative tools to the traditional contrast agents like iodine or barium sulfate (Künzel *et al.*, 2013). The need to replace the standard contrast agents is attributable to their high kidney toxicity (Kaller and An, 2022). AuNPs are particularly proper for this application due to the high atomic number (Z = 79) and density (=19.3 g/cm^3) (Cutler *et al.*, 2012). Furthermore, AuNPs show high X-ray attenuation coefficients providing sharper images enhancing the time of blood circulation (Hernandez-Rivera *et al.*, 2017; Leung *et al.*, 2011).

For biomedical, forensic, and environmental applications is crucial to detect chemical and biological molecules. Developing affordable, highly sensitive, miniature sensors is the only way to achieve this goal. As a rule, sensors comprise two parts: a recognition element for selectively binding the target analyte, and a transducer able to highlight the binding event (Naresh and Lee, 2021). The performance of a capable sensor is deeply dependent on these two elements based on selectivity, response time, and limits of detection (LOD) (Mathew *et al.*, 2021). It is well known that nanomaterials have remarkable physicochemical properties that can be of great assistance in creating new recognition and transduction processes for chemical and biological sensors miniaturizing sensor elements (Malik *et al.*, 2021). AuNPs are remarkably interesting for their several properties as described above and, in addition, high oxidative stability and low toxicity compared to others types of inorganic materials (Bakand *et al.*, 2012). They can use alone or in combination with other types of nanomaterials such as covalent organic frameworks-AuNPs composite (COFs-AuNPs) to monitor kidney injury (Boyacıoğlu *et al.*, 2022) Tumor Necrosis Factor-alpha (TNF-α) (Yola and Atar, 2021) or to detect toxins potentially dangerous for living organisms and environment (Karaman *et al.*, 2021).

Diagnosis of COVID-19 Using AuNPs

Common standard tests

It is indeed accepted that an early diagnosis represents the best method to prevent the uncontrolled virus transmission. Three broad groups of diagnostic tests are developed to monitor the spread of COVID-19 (Jarrom *et al.*, 2022):

(i) Tests able to detect the existence of coronavirus in the respiratory secretion consisting in the amplification of viral nucleic acid by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) (Kevadiya *et al.*, 2021);

(ii) Antigen tests (rapid diagnostic tests) to directly detect SARS-CoV-2 proteins produced by virus in respiratory secretions (Interim guidance WHO, 2020). Today, represents the golden standard for a rapid, yet effective way to monitor the positive cases.

(iii) Serum tests revealing the presence of antibodies against SARS-CoV-2 in blood.

Specialized laboratories process the samples from nasopharyngeal swabs but, as the spread of the virus progresses, self-screening tests have also been developed. Then, the antigenic as well as the serological approaches have been developed as laboratory-based tests. Serological tests can be useful to indirectly identify the virus, that is through the detection of antibodies produced in the serum following the interaction with the pathogen. Rapid *in vitro* diagnostic tests for the qualitative detection of IgG and IgM antibodies against SARS-CoV-2 in human serum, plasma, venous whole blood, and acupuncture were developed by some companies such as Abbott BinaxNow (https://www.abbott.com/), bioMérieux SA (https://www.biomerieux.it/), AESKU Diagnostics (https://www.aesku.com/), Roche (https://www.roche.com/) and other ones. However, the limitation about sensitivity and specificity as well as the LOD quantity highlights the need for different and more sensitive approach able to monitor the COVID-19 spread in the population.

In this framework, a lot of nanomaterials, thanks to the aforementioned properties seems to be the best choice (Hasanzadeh *et al.*, 2021).

AuNPs-based tests

The WHO guidelines reported the methodology of Point-Of-Care (POC) devices based on the detection of viral genes and proteins (Amer *et al.*, 2013) These “pieces” of virus are anchored on the NPs surface and the detection is based on electrical, optical, and electrochemical strategies (Lee *et al.*, 2018). The physicochemical properties of AuNPs have been thus investigated in terms of both detection and treatment of the SARS-CoV-2 (Bidram *et al.*, 2021). In particular, the AuNPs-based colorimetric detection is particularly suitable for the red-to-blue shift as consequence of LSPR coupling among NPs (Li *et al.*, 2015) permitting a rapid screening without the use of expensive instruments. Some works improved AuNPs using anti-spike (Pramanik *et al.*, 2021) or anti-Nucleocapsid (N) antibodies attached on the AuNPs surface by colorimetric reading. SERS was used for the virus identification, using 4-aminothiophenol as reporter molecule linked on AuNPs by Au-S bonds (Pramanik *et al.*, 2021). The addition of the virus with the AuNPs in the reaction mixture induced the aggregation of NPs, which was immediately distinguished even by naked eye thanks to the solution color change from pink to blue. The aggregated AuNPs created “hot-spots” showing a strong SERS signal

derived from 4-aminothiophenol and antibody attached on the NPs surface. This methodology allowed detecting up to 4 pg/mL of viral proteins in five minutes. Moreover, the effectiveness of the nano system on the virus spread was demonstrated using Cellosaurus HEK293T cells, known to express angiotensin-converting enzyme 2 (ACE2) receptor. Then, anti-spike antibodies functionalized AuNPs effectively blocked the SARS-CoV-2 infection by inhibiting its replication. In a recent strategy (Aithal *et al.*, 2022), AuNPs were decorated by a certain aptamer able to bind the SARS-CoV-2 spike proteins. The spikes were recognized upon addition of a coagulant. According to this approach, the AuNPs that did not recognize the spike underwent an aggregation process, whereas the binding event was detected by SPR. This system allowed detecting a concentration down to 16 nM of free spike proteins.

Femtomolar detection of spike proteins was carried out using plasmonic metasensor technology which can compress electromagnetic fields (Ahmadivand *et al.*, 2021). The advantages of these structures were the low-radiative losses and a strong electromagnetic fields confinement. A plasmonic label-free toroidal metasurfaces at terahertz frequencies were fabricated (~0.4 THz) coupled with colloidal AuNPs functionalized by specific monoclonal antibody to improve the binding features of the molecules. After excitation, the resonance shifts based on the different concentrations of spike proteins binding antibodies. The LOD of this device was about 4.2 fM. Engineered terahertz (THz) plasmonic metamaterials, in particular toroidal materials, are new technologies for diagnosis for their low impact *in vivo* and *in vitro* systems and in addition, they are non-destructive platforms (Ahmadivand *et al.*, 2018). As firstly described, the coupling with AuNPs is very useful for the detection of proteins due to the opportunity to detect several targets observing distinct resonance peaks (Xu *et al.*, 2016).

AuNPs-based serum tests

In this context, smart devices were developed for IgG detection, using AuNPs (Li *et al.*, 2015; Liu et al., 2020a; Pohanka, 2021; Ahmadi *et al.*, 2021). Among these, an interesting platform based on a lateral flow immunoassay strip (LFIAs) has been recently created (Wen *et al.*, 2020; Gupta *et al.*, 2020) (Fig. 3a). The nucleocapsid proteins of SARS-CoV-2 were immobilized on the strip to specifically bind IgG-functionalized AuNPs. The goodness and reproducibility of the assay was finally assessed using serum samples from clinically diagnosed cases of COVID-19. The presence of target proteins was evaluated following the color footprint of AuNPs, making this test particularly suitable to equip the conventional test and to use it in low-resource countries. In addition, LFIAs can be potentially applied in mass screening or home tests. A colorimetric assay was developed to detect IgGs in plasma using AuNPs functionalized by antigenic epitopes (Lew *et al.*, 2021). These immunodominant linear B-cell epitopes are present on spike and nucleocapsid proteins of SARS-CoV-2 showing great affinity with IgG. For this reason, the AuNPs surface was decorated with epitopes to bind specific antibodies. The binding event induced NPs aggregation triggering the plasmonic absorbance change, thus allowing detection of the virus with a LOD of 3.2 nM. The clinical tests, performed on human plasma medium, showed the identification of coronavirus with 100% of specificity, also in circulating COVID-19 variants (Lew *et al.*, 2021).

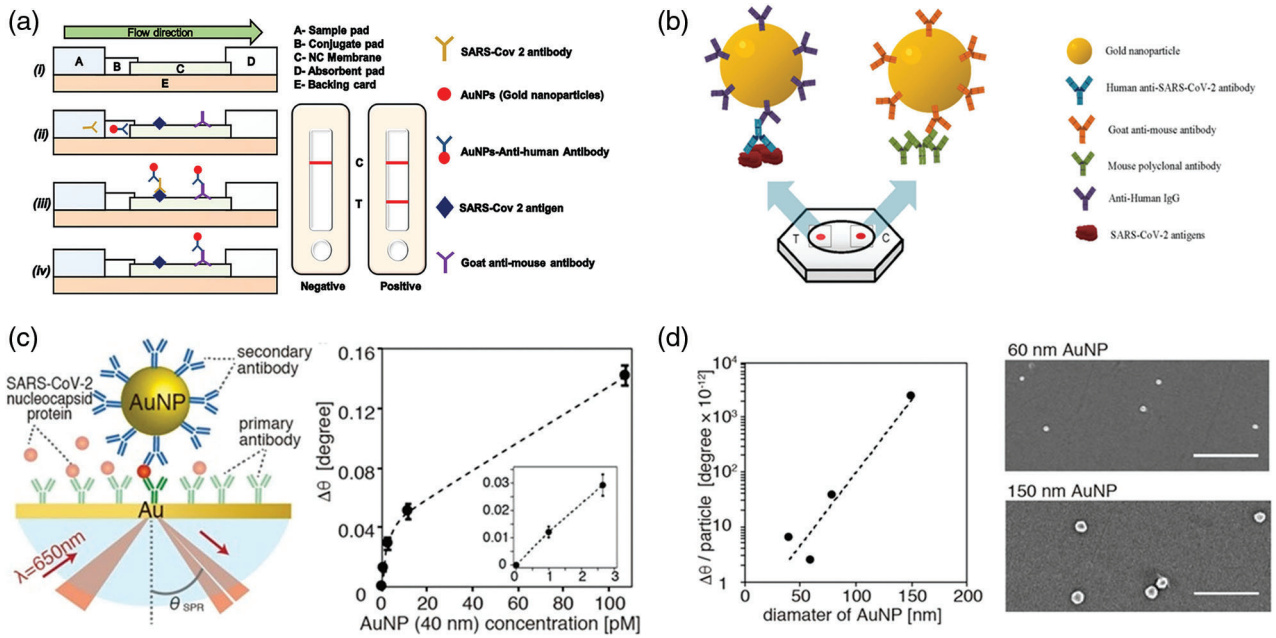

FIGURE 3. (a) Schematic representation of Lateral flow assay (LFA) using AuNPs. In the figure is reported the conjugation between the antibody and antigen as well as the relative positive results on the test (Gupta *et al.*, 2020); (b) Rapid flow-through dot-blot immunoassay (FT-DBA) using AuNPs functionalized by anti-human IgG. AuNPs with goat anti-mouse can bind to mouse polyclonal antibody (control) (Sil *et al.*, 2021); (c) identification of nucleocapsid protein of SARS-CoV-2 using AuNPs and SPR angular shift (Δθ) degree related to 40 nm AuNPs at different concentrations (pM); (d) Angular shift as function of different sizes of AuNPs and SEM acquisitions of AuNPs after SPR measurements (Yano *et al.*, 2022).

Another work focused on the recognition of SARS-CoV-2 using serological test implemented with dot-blot assay (FT-DBA) for the detection of IgG (Sil *et al.*, 2021). In this approach, AuNPs were functionalized with anti-human IgG (hIgG-AuNPs) able to bind the virus antigens immobilized on nitrocellulose membrane.

The selectivity and sensibility were higher compared to ELISA standard tests, obtaining results within two minutes, with sensitivity and specificity of about 98% (Fig. 3b).

Recently, Yano *et al.* (2022) created a platform in which the SPR of AuNPs, conveniently functionalized by antibodies, was used to find the nucleocapsid proteins of the SARS-CoV-2. The large size of the AuNPs (150 nm) enhanced the sensitivity detection with respect to the smaller AuNPs allowing to found protein virus at fM levels. The experimental data were both theoretical and experimental comparing size of 40 nm and 150 nm showing an increase of SPR angle of 0.94 degree for larger NPs; the smaller exhibited only a 0.06 degree (Figs. 3c and 3d).

AuNPs-based nasal swab tests

The nasal swab are the most popular tests. In a recent experimental work, an Au antigen–antibody nanoplatform was developed showing a sensitivity and specificity higher than 95% (Della Ventura *et al.*, 2020). The photochemical immobilization technique (PIT), a surface functionalization procedure, was employed to absorb antibodies on the AuNPs surface. Then, the nanoplatform was used to bind three surface viral proteins, namely the spike, the envelope, and the membrane proteins (S, E, and M, respectively). The AuNPs-protein interaction triggered the red shift of the absorption spectra. Alafeef *et al.* (2020) developed a paper-based colorimetric sensors to

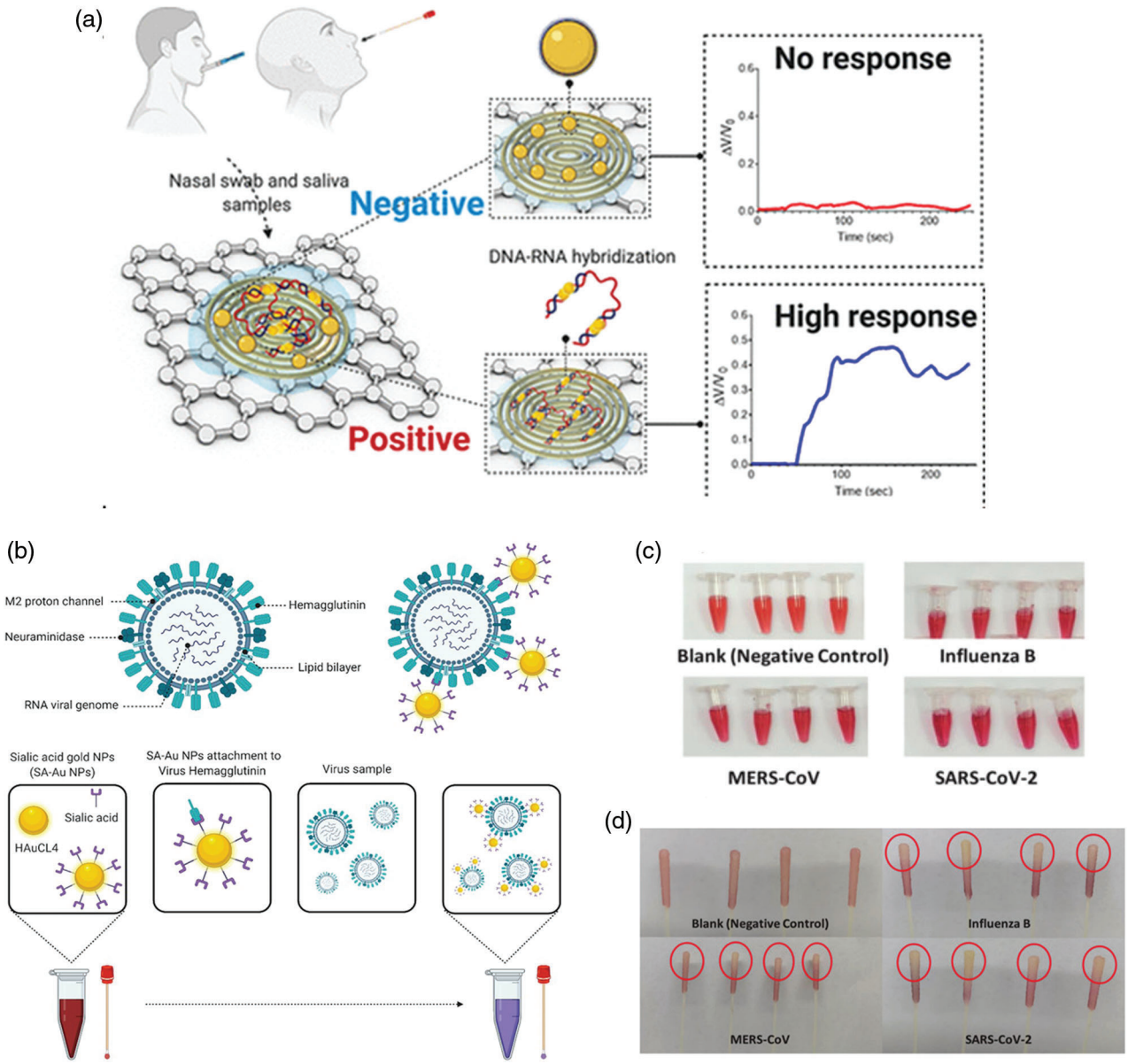

FIGURE 4. (a) Schematic principle of paper based colorimetric sensors with AuNPs-ssDNA. In the presence of SARS-CoV-2, the AuNPs-ssDNA bind viral RNA triggering a strong electrochemical signal. Reprinted by permission of American Chemical Society (Alafeef *et al.*, 2020); (b) Colorimetric detection of SARS-CoV-2 based on AuNPs conjugated with sialic acid, (c) positive swabs of different kind of viruses included SARS-CoV-2 after immersion in solution containing AuNPs and (d) colored tips of samples compared to the negative control (Alfassam *et al.*, 2021).

digitally detect SARS-CoV-2 by its viral RNA. The sensors were created using AuNPs functionalized by antisense oligonucleotides (ssDNA) able to bind nucleocapsid coronavirus protein, which generated electrochemical response by graphene-ssDNA-AuNP surface (Fig. 4a).

Hyperspectral microscopic data, together with the evidence on clinical samples of positive COVID-19 and healthy asymptomatic subjects, confirmed the effectiveness of this sensors with 100% of accuracy and a sensitivity of 231 copies μL^{-1}. The LOD was 6.9 copies/μL without other amplification cycles. A detailed protocol for a nano-amplified colorimetric test to relieve SARS-CoV-2 without the need of RNA extraction was recently reported (Alafeef *et al.*, 2021). This test was very effective due to the nano-amplification of plasmonic AuNPs previously functionalized by antisense oligonucleotides (ASOs), used as a colorimetric reporter to bind and amplify the viral genetic material. The use of ASOs was crucial for their ability to bind nucleocapsid proteins; the NPs aggregation induced a change in plasmonic response of the NPs upon interaction with the virus. The LOD was recorded to 10 copies/μL showing high specificity and sensitivity without using expensive equipment. In addition, this test can be used for a quantitative response using a handheld optical reader. Rodríguez-Díaz (Rodríguez-Díaz *et al.*, 2022) produced a colorimetric test based on AuNPs (15–38 nm) and PCR. In this specific conformation, the cholesterol is folded inside and the AuNPs solution takes on a red color. On the other hand, when the target is present, the molecules change their conformation anchoring the target, thus determining the aggregation of the nanostructures. The result was spectrophotometrically quantifiable using patient respiratory swab samples. Finally, the authors implemented PCR procedure to complete the detection in 1 h. Recently (Alfassam *et al.*, 2021), AuNPs were conjugated with sialic acid, a glycoprotein binding lung epithelial cell. This molecule represented a common target of respiratory virus, as it binds the viral surface protein hemagglutinin. Nasopharyngeal swabs containing SARS-CoV-2 were immersed in the solution containing the SA-AuNPs and, after 20 min, the authors observed a change in color detected by the UV-Vis spectrophotometry (Figs. 4b–4d).

AuNPs Combined Machine Learning to Detect SARS-CoV-2

The validation of the SARS-CoV-2 devices is a critical challenge to verify the optimal performance of diagnostic, especially the one carried out at home for self-monitoring (Vandenberg *et al.*, 2021). In this perspective, a recent work developed a Dense Neural Network (DNN) as a machine learning (ML) approach (Beduk *et al.*, 2022) to validate the accuracy of POC device in which AuNPs were associated with a graphene sensor. Firstly, the device can recognize the ACE2 enzyme (due to its affinity to bind spike proteins of coronavirus) and it was finally integrated in a potentiostat connected with a smartphone by wireless.

The nasopharyngeal swabs were collected by several patients affected by alpha (B.1.1.7), beta (B.1.351), delta (B.1.617.2) virus variants as well as negative patients. The SARS-CoV-2 was find using the machine learning model showing an accuracy of 99.37% (Figs. 5a and 5b). Similar approach was used by Liang *et al.* (2022) to overcome the limitation in the use of SPR based AuNPs for the detection of novel coronavirus. In particular, the authors focused on the disadvantages emerging in the analysis by SPR since the information of spatial and temporal distribution during the analysis were ignored reducing the accuracy and sensitivity. The authors proposed a test based on the analysis of images acquired by microscopy. These images were extracted following interaction between spike proteins adsorbed on

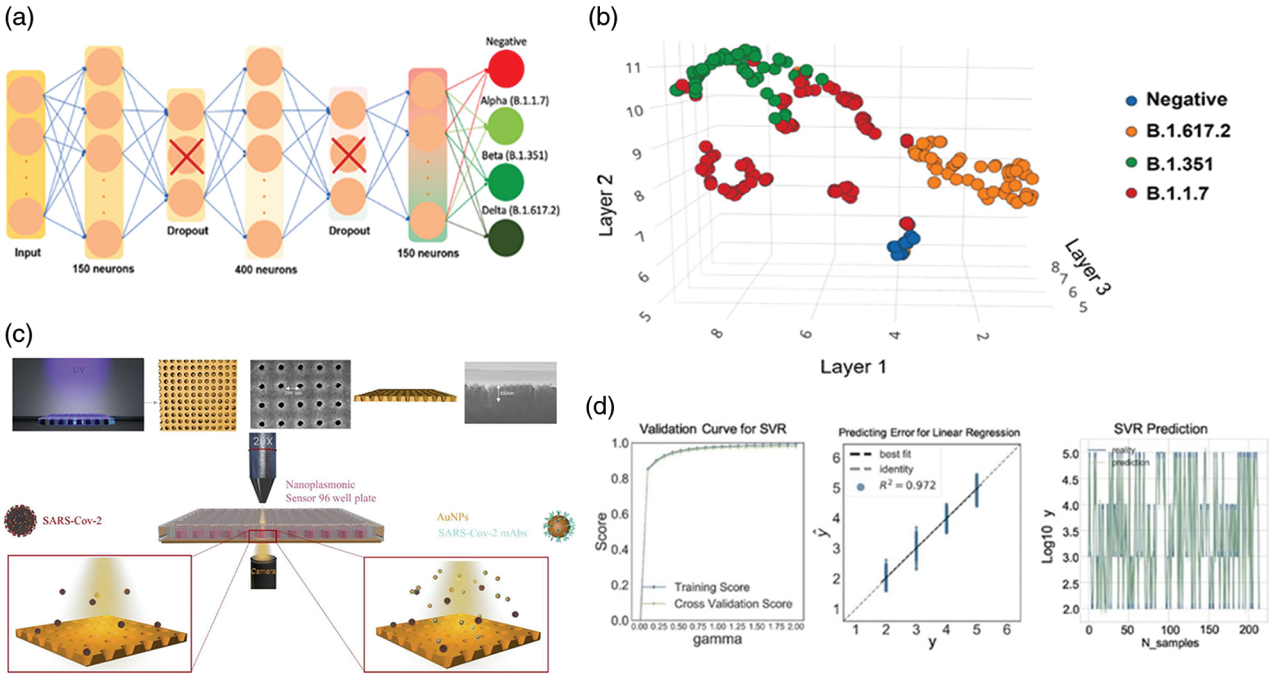

FIGURE 5. Graphical representations of The Dense Neural Network (DNN) (a) and records acquired from positive e negative tests performed using nasopharyngeal swabs (b), Adapted from Beduk *et al.* (2022); (c) summary scheme of the plasmonic device for SARS-CoV-2 detection using microscope. Machine learning SVM regression models to predict the virus concentration (R^2 > 95%), (d) Adapted from Liang *et al.* (2022).

TABLE 1

LOD, specificity, Sensitivity, time and sample volume used by different AuNPs-based SARS-CoV-2 tests

Reference	LOD	Specificity	Sensitivity	Time	Sample volume
Pramanik *et al.*, 2021	4 pg/mL^{-1}	–	–	5 min	–
Aithal *et al.*, 2022	16 nM	–	–	10 min	20 μL
Ahmadivand *et al.*, 2021	42 fM	–	–	20 min	–
Liu *et al.*, 2020a	–	97.47%	95.85%	15 min	10 μL
Ahmadi *et al.*, 2021	–	96.6%	90%	15–20 min	20 μL
Wen *et al.*, 2020	1.2 mg/mL^{-1}	100%	69.1%	15–20 min	10 μL
Lew *et al.*, 2021	3.2 nM	100%	83%	30 min	20 μL
Yano *et al.*, 2022	85 fM	–	–	1 min	70 μL
Della Ventura *et al.*, 2020	–	>95%	>95%	Few min	100 μL
Alafeef *et al.*, 2020	6.9 copies/μL	100%	231 copies/μL^{-1}	5 min	–
Alafeef *et al.*, 2021	10 copies/μL	100%	96.6%	<1 h	<10 μL
Rodríguez-Díaz *et al.*, 2022	>103–104 copies/μL	–	–	15 min	10–15 μL
Beduk *et al.*, 2022	5.14 ng/mL for S1 and 2.09 ng/mL for S2	–	–	1 min	–
Liang *et al.*, 2022	125.28–106 vp/mL	–	–	12 min	50 μL
Behrouzi *et al.*, 2021	5 ng/mL	–	–	–	4 μL

the sensors and AuNPs decorated with anti-SARS-CoV-2 antibodies. Binding results in transmission inhibition in the far field. Consequently, a reduction in gray values can be observed that is proportional with virus concentration that was detected in the range of 125.28 to 106 vp/mL with a LOD of 100 vp/mL. At this point, following further extractions of other images with different shades of colors corresponding to certain concentrations of pathogen, these were inserted into the machine learning program useful to detect SARS-CoV-2 quickly and safely (Figs. 5c and 5d).

Behrouzi and Lin (2021) developed a convolutional neural learning able to increase the efficiency of SARS-Cov-2 test by analysis of images. The method is based on the double-coffee ring phenomenon using LSPR by the presence of AuNPs functionalized using specific antibodies against the coronavirus permitting to detect virus with a LOD of 5 ng/mL. With this aim, as sensing system a hydrophilic membrane characterized by a porous pattern and hydrophobic barriers was used. When AuNPs bond to viral units, a specific image characterized by a double coffee ring can be displayed. In this way, the neural network will be able to detect this interaction. Indeed, a small ring was formed in the center due to the interaction between the NPs surrounded by a second ring produced instead by the hydrophobic barrier.

In the Table 1 are reported the most important characteristic of the sensors described in this review.

Conclusions

In the last year we witnessed a huge and common human effort towards the fight of the life-threatening COVID-19 infections. If on one side mass vaccination surely stopped the uncontrolled circulation of the virus, on the other a huge diagnostic campaign helped tracking positive cases, which can be then easily quarantined. With respect to the latter approach, we have witnessed a fast development of several different diagnostic kits, used both in the clinic as well as within common pharmacies. In this work, we described the most recent efforts to produce SARS-CoV-2 diagnostic tool based on AuNPs. Thanks to their unique physicochemical properties, AuNPs allowed indeed the detection of viral antigens even at picograms level, without the need of specialized equipment, in a cheap way. Since the pandemic situation is it is constantly evolving, we believe the development of next generation diagnostic kits should take into consideration the use of nanomaterials, to develop a nanoscale personalized platform able to detect viral RNA in specific manner with an optimal limit of detection and high sensitivity.

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References

Ahmadi A, Mirzaeizadeh Z, Omidfar K (2021). Simultaneous detection of SARS-CoV-2 IgG/IgM antibodies, using gold

nanoparticles-based lateral flow immunoassay. *Monoclonal Antibodies in Immunodiagnosis and Immunotherapy* **40**: 210–218. DOI 10.1089/mab.2021.0027.

Ahmadivand A, Gerislioglu B, Ramezani Z, Kaushik A, Manickam P, Ghoreishi SA (2021). Functionalized terahertz plasmonic metasensors: Femtomolar-level detection of SARS-CoV-2 spike proteins. *Biosensors and Bioelectronics* **177**: 112971. DOI 10.1016/j.bios.2021.112971.

Ahmadivand A, Gerislioglu B, Tomitaka A, Manickam P, Kaushik A et al. (2018). Extreme sensitive metasensor for targeted biomarkers identification using colloidal nanoparticles-integrated plasmonic unit cells. *Biomedical Optics Express* **9**: 373–386. DOI 10.1364/BOE.9.000373.

Aithal S, Mishriki S, Gupta R, Sahu RP, Botos G, Tanvir S, Hanson RW, Puri IK (2022). SARS-CoV-2 detection with aptamer-functionalized gold nanoparticles. *Talanta* **236**: 122841. DOI 10.1016/j.talanta.2021.122841.

Alafeef M, Moitra P, Dighe K, Pan D (2021). RNA-extraction-free nano-amplified colorimetric test for point-of-care clinical diagnosis of COVID-19. *Nature Protocols* **16**: 3141–3162. DOI 10.1038/s41596-021-00546-w.

Alafeef M, Dighe K, Moitra P, Pan D (2020). Rapid, ultrasensitive, and quantitative detection of SARS-CoV-2 using antisense oligonucleotides directed electrochemical biosensor chip. *ACS Nano* **14**: 17028–17045. DOI 10.1021/acsnano.0c06392.

Alanagreh L, Alzoughool F, Atoum M (2020). The human coronavirus disease COVID-19: Its origin, characteristics, and insights into potential drugs and its mechanisms. *Pathogens* **9**: 331. DOI 10.3390/pathogens9050331.

Albanese A, Chan W (2011). Effect of gold nanoparticle aggregation on cell uptake and toxicity. *ACS Nano* **5**: 5478–5489. DOI 10.1021/nn2007496.

Alfassam HA, Nassar MS, Almusaynid MM, Khalifah BA, Alshahrani AS et al. (2021). Development of a colorimetric tool for SARS-CoV-2 and other respiratory viruses detection using sialic acid fabricated gold nanoparticles. *Pharmaceutics* **13**: 502. DOI 10.3390/pharmaceutics13040502.

Amer HM, Abd El Wahed A, Shalaby MA, Almajhdi FN, Hufert FT, Weidmann M (2013). A new approach for diagnosis of bovine coronavirus using a reverse transcription recombinase polymerase amplification assay. *Journal of Virological Methods* **193**: 337–340. DOI 10.1016/j.jviromet.2013.06.027.

Bai X, Wang Y, Song Z, Feng Y, Chen Y et al. (2020). The basic properties of gold nanoparticles and their applications in tumor diagnosis and treatment. *International Journal of Molecular Sciences* **21**: 2480. DOI 10.3390/ijms21072480.

Bakand S, Hayes A, Dechsakulthorn F (2012). Nanoparticles: A review of particle toxicology following inhalation exposure. *Inhalation Toxicology* **24**: 125–135. DOI 10.3109/08958378.2010.642021.

Beduk D, Ilton de Oliveira Filho J, Beduk T, Harmanci D, Zihnioglu F et al. (2022). All in one SARS-CoV-2 variant recognition platform: Machine learning-enabled point of care diagnostics. *Biosensors and Bioelectronics: X* **10**: 1–7. DOI 10.1016/j.biosx.2022.100105.

Behrouzi K, Lin L (2021). Double-coffee ring nanoplasmonic effects with convolutional neural learning for Sars-Cov-2 detection. In: *2021 21st International Conference on Solid-State Sensors, Actuators and Microsystems (Transducers)*, pp. 381–384, Orlando, FL, USA. DOI 10.1109/Transducers50396.2021.9495602

Bidram E, Esmaeili Y, Amini A, Sartorius R, Tay FR, Shariati L, Makvandi P (2021). Nanobased platforms for diagnosis and treatment of COVID-19: From benchtop to bedside. *ACS Biomaterials Sciences and Engineering* **14**: 2150–2176. DOI 10.1021/acsbiomaterials.1c00318.

Boyacıoğlu H, Yola B, Karaman C, Karaman O, Atar N et al. (2022). A novel electrochemical kidney injury molecule-1 (KIM-1) immunosensor based covalent organic frameworks-gold nanoparticles composite and porous $NiCo_2S_4$@CeO_2 microspheres: The monitoring of acute kidney injury. *Applied Surface Science* **578**: 152093. DOI 10.1016/j.apsusc.2021.152093.

Brennan D, Justice J, Corbett B, McCarthy T, Galvin P (2009). Emerging optofluidic technologies for point-of-care genetic analysis systems: A review. *Analytical and Bioanalytical Chemistry* **3**: 621–636. DOI 10.1007/s00216-009-2826-5.

Chen NT, Cheng SH, Liu CP, Souris JS, Chen CT et al. (2012). Recent advances in nanoparticle-based förster resonance energy transfer for biosensing, molecular imaging and drug release profiling. *International Journal of Molecular Sciences* **13**: 16598. DOI 10.3390/ijms131216598.

Cheng C, Li J, Lei H, Baojun L (2018). Surface enhanced Raman scattering of gold nanoparticles aggregated by a gold-nanofilm-coated nanofiber. *Photonics Research* **6**: 357–362. DOI 10.1364/PRJ.6.000357.

Cutler CS, Hennkens HM, Sisay N, Huclier-Markai S, Jurisson S (2012). Radiometals for combined imaging and therapy. *Chemical Review* **113**: 858–883. DOI 10.1021/cr3003104.

Dance BA (2022). Omicron's lingering mysteries. *Nature 603.*

De Matteis V, Cascione M, Rizzello L, Manno DE, Diguglielmo C, Rinaldi R (2021). Synergistic effect induced by gold nanoparticles with polyphenols shell during thermal therapy: Macrophage inflammatory response and cancer cell death assessment. *Cancers* **13**: 3610. DOI 10.3390/cancers13143610.

de Matteis V, Cascione M, Toma CC, Rinaldi R (2019). Engineered gold nanoshells killing tumor cells: New perspectives. *Current Pharmaceutical Design* **25**: 1477–1489. DOI 10.2174/1381612825666190618155127.

de Matteis V, Rizzello L, Cascione M, Liatsi-Douvitsa E, Apriceno A et al. (2020). Green plasmonic nanoparticles and bio-inspired stimuli-responsive vesicles in cancer therapy application. *Nanomaterials (Basel)* **10**: 1083. DOI 10.3390/nano10061083.

de Matteis V, Rizzello L, Di Bello MP, Rinaldi R (2017). One-step synthesis, toxicity assessment and degradation in tumoral pH environment of SiO2@Ag core/shell nanoparticles. *Journal of Nanoparticle Research* **19**: 14. DOI 10.1007/s11051-017-3870-2.

de Matteis V, Rizzello L (2020). Noble metals and soft bio-inspired nanoparticles in retinal diseases treatment: A perspective. *Cells* **9**: 679. DOI 10.3390/cells9030679.

de Puig H, Tam JO, Yen CW, Gehrke L, Hamad-Schifferli K (2015). Extinction coefficient of gold nanostars. *The Journal of Physical Chemistry C* **119**: 17408–17415. DOI 10.1021/acs.jpcc.5b03624.

Della Ventura B, Cennamo M, Minopoli A, Campanile R, Bolletti Censi S, Terracciano D, Portella G, Velotta R (2020). Colorimetric test for fast detection of SARS-CoV-2 in nasal and throat swabs. *ACS Sensors* **5**: 3043–3048. DOI 10.1021/acssensors.0c01742.

Dolgin E (2021). The tangled history of mRNA vaccines. *Nature* **597**: 318–324. DOI 10.1038/d41586-021-02483-w.

Dong E, Du H, Gardner L (2020). An interactive web-based dashboard to track COVID-19 in real time. *The Lancet Infectious Diseases* **20**: 533–534. DOI 10.1016/S1473-3099(20)30120-1.

Dykman LA, Khlebtsov NG (2011). Gold nanoparticles in biology and medicine: Recent advances and prospects. *Acta Naturae* **3**: 34–55. DOI 10.32607/20758251-2011-3-2-34-55.

Fresco-Taboada A, García-Durán M, Aira C, López L, Sastre P et al. (2022). Diagnostic performance of two serological assays for the detection of SARS-CoV-2 specific antibodies: Surveillance after vaccination. *Diagnostic Microbiology and Infectious Disease* **102**: 115650. DOI 10.1016/j.diagmicrobio.2022.115650.

Geddes CD, Lakowicz JR (2002). Editorial: Metal-enhanced fluorescence. *Journal of Fluorescence* **12**: 121–129. DOI 10.1023/A:1016875709579.

Ghosh SK, Pal T (2007). Interparticle coupling effect on the surface plasmon resonance of gold nanoparticles: From theory to applications. *Chemical Reviews* **107**: 4797–4862. DOI 10.1021/cr0680282.

Gupta R, Sagar P, Priyadarshi N, Kaul S, Sandhir R et al. (2020). Nanotechnology-based approaches for the detection of SARS-CoV-2. *Frontiers in Nanotechnology* **2**: 143. DOI 10.3389/fnano.2020.589832.

Hasanzadeh A, Alamdaran M, Ahmadi S, Nourizadeh H, Bagherzadeh MA et al. (2021). Nanotechnology against COVID-19: Immunization, diagnostic and therapeutic studies. *Journal of Controlled Release* **336**: 354–374. DOI 10.1016/j.jconrel.2021.06.036.

Helmerhorst E, Chandler DJ, Nussio M, Mamotte CD (2012). Real-time and label-free bio-sensing of molecular interactions by surface plasmon resonance: A laboratory medicine perspective. *The Clinical Biochemist. Review* **33**: 161–173.

Hernandez-Rivera M, Kumar I, Cho SY, Cheong BY, Pulikkathara MX, Moghaddam SE, Whitmire KH, Wilson LJ (2017). High-performance hybrid bismuth-carbon nanotube based contrast agent for X-ray CT imaging. *ACS Applied Materials and Interfaces* **9**: 5709–5716. DOI 10.1021/acsami.6b12768.

https://www.abbott.com/.

https://www.aesku.com/.

https://www.biomerieux.it/.

https://www.roche.com/.

Iarossi M, Schiattarella C, Rea I, Stefano LD, Fittipaldi R et al. (2018). Colorimetric immunosensor by aggregation of photochemically functionalized gold nanoparticles. *ACS Omega* **3**: 3805–3812. DOI 10.1021/acsomega.8b00265.

World Health Organization (2020). Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: Interim guidance. WHO/2019-nCoV/Antigen Detection/2021.1.

Jarrom D, Elston L, Washington J, Prettyjohns M, Groves P (2022). Effectiveness of tests to detect the presence of SARS-CoV-2 virus, and antibodies to SARS-CoV-2, to inform COVID-19 diagnosis: A rapid systematic review. *BMJ Evidence-Based Medicine* **27**: 33–45. DOI 10.1136/bmjebm-2020-111511.

Kaller MO, An J (2022). Contrast agent toxicity. In: StatPearls [Internet]. Treasure Island (FL). StatPearls Publishing. 2022 Jan–PMID 30725844.

Kang MS, Lee SY, Kim KS, Han DW (2020). State of the art biocompatible gold nanoparticles for cancer theragnosis. *Pharmaceutics* **12**: 701. DOI 10.3390/pharmaceutics12080701.

Karaman C, Karaman O, Yola BB, Ülker İ, Atar N et al. (2021). A novel electrochemical aflatoxin B1 immunosensor based on gold nanoparticle-decorated porous graphene nanoribbon and Ag nanocube-incorporated MoS_2 nanosheets. *New Journal of Chemistry* **45**: 11222–11233. DOI 10.1039/D1NJ02293H.

Kevadiya BD, Machhi J, Herskovitz J, Oleynikov MD, Blomberg WR et al. (2021). Diagnostics for SARS-CoV-2 infections. *Nature Materials* **20**: 593–605. DOI 10.1038/s41563-020-00906-z.

Kim HS, Lee DY (2018). Near-infrared-responsive cancer photothermal and photodynamic therapy using gold nanoparticles. *Polymers* **10**: 961. DOI 10.3390/polym10090961.

Kitchin N, Absalon J, Gurtman A, Lockhart S, Neuzil K et al. (2020). Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *The New England Journal of Medicine* **383**: 2439–2450. DOI 10.1056/NEJMoa2027906.

Kneipp K, Wang Y, Kneipp H, Perelman LT, Itzkan I et al. (1997). Single molecule detection using surface-enhanced raman scattering (SERS). *Physical Review Letters* **78**: 1667–1670. DOI 10.1103/PhysRevLett.78.1667.

Kumar V, Doshi KU, Khan WH, Rathore AS (2021). COVID-19 pandemic: Mechanism, diagnosis, and treatment. *Chemical Technology and Biotechnology* **96**: 299–308. DOI 10.1002/jctb.6641.

Künzel R, Okuno E, Levenhagen RS, Umisedo NK (2013). Evaluation of the X-ray absorption by gold nanoparticles solutions. *International Scholarly Research Notices* **2013**: 1–5. DOI 10.1155/2013/865283 ID 865283.

Lee J, Morita M, Takemura K, Park ET (2018). A multi-functional gold/iron-oxide nanoparticle-CNT hybrid nanomaterial as virus DNA sensing platform. *Biosensors and Bioelectronics* **102**: 425–431. DOI 10.1016/j.bios.2017.11.052.

Lee M, Lee S, Lee JH, Lim HW, Seong GH et al. (2011). Highly reproducible immunoassay of cancer markers on a gold-patterned microarray chip usingsurface-enhanced Raman scattering imaging. *Biosensors and Bioelectronics* **26**: 2135–2141. DOI 10.1016/j.bios.2010.09.021.

Leung MKK, Chow JCL, Chithrani D, Lee MJG, Oms B et al. (2011). Irradiation of gold nanoparticles by X-rays: Monte Carlo simulation of dose enhancements and the spatial properties of the secondary electrons production. *Medical Physics* **38**: 624–631. DOI 10.1118/1.3539623.

Lew TTS, Aung KMM, Ow SY, Amrun SN, Sutarlie L, Ng LFP, Su X (2021). Epitope-functionalized gold nanoparticles for rapid and selective detection of SARS-CoV-2IgG antibodies. *ACS Nano* 15(7). DOI 10.1021/acsnano.1c04091.

Li M, Cushing SK, Wu N (2015). Plasmon-enhanced optical sensors: A review. *Analyst* **140**: 3. DOI 10.1039/C4AN01079E.

Li X, Zhang Q, Hou P, Chen M, Hui W et al. (2015). Gold magnetic nanoparticle conjugate-based lateral flow assay for the detection of IgM class antibodies related to TORCH infections. *International Journal of Molecular Medicine* **36**: 1319–1326. DOI 10.3892/ijmm.2015.2333.

Liang J, Zhang W, Qin Y, Li Y, Liu GL et al. (2022). Applying machine learning with localized surface plasmon resonance sensors to detect SARS-CoV-2 particles. *Biosensors* **12**: 173. DOI 10.3390/bios12030173.

Liu C, Mao B, Martinez V, Chen X, Li Y et al. (2020a). A facile assay for rapid detection of COVID-19 antibodies. *RSC Advances* **10**: 28041–28048. DOI 10.1039/D0RA04107F.

Liu C, Zhou Q, Li Y, Garner LV, Watkins SP et al. (2020b). Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. *ACS Central Sciences* **6**: 315–331. DOI 10.1021/acscentsci.0c00272.

Mahato K, Nagpal S, Shah MA, Srivastava A, Maurya PK et al. (2019). Gold nanoparticle surface engineering strategies and their applications in biomedicine and diagnostics. *3 Biotech* **9**: 2440. DOI 10.1007/s13205-019-1577-z.

Malik P, Gupta R, Malik V, Ameta RK (2021). Emerging nanomaterials for improved biosensing. *Measurement: Sensors* **16**: 100050. DOI 10.1016/j.measen.2021.100050.

Mariani S, Minunni M (2014). Surface plasmon resonance applications in clinical analysis. *Analytical and Bioanalytical Chemistry* **406**: 2303–2323. DOI 10.1007/s00216-014-7647-5.

Mason RJ (2020). Pathogenesis of COVID-19 from a cell biology perspective. *European Respiratory Journal* **55**: 2000607. DOI 10.1183/13993003.00607-2020.

Mathew M, Radhakrishnan S, Vaidyanathan A, Chakraborty B, Rout CS (2021). Flexible and wearable electrochemical biosensors based on two-dimensional materials: Recent developments. *Analytical and Bioanalytical Chemistry* **413**: 727–762. DOI 10.1007/s00216-020-03002-y.

Mi YN, Huang TT, Zhang JX, Qin Q, Gong YX, Liu SY, Xue HM, Ning CH, Cao L, Cao YX (2020). Estimating the instant case fatality rate of COVID-19 in China. *International Journal of Infectious Diseases* **97**: 1–6. DOI 10.1016/j.ijid.2020.04.055.

Mirastschijski U, Dembinski R, Maedler K (2020). Lung surfactant for pulmonary barrier restoration in patients with COVID-19 pneumonia. *Frontiers in Medicine* **7**: 254. DOI 10.3389/fmed.2020.00254.

Nakagawa K, Lokugamage KG, Makino S (2016). Viral and cellular mRNAtranslation in coronavirus-infected cells. *Advances in Virus Research* **96**: 165–192. DOI 10.1016/bs.aivir.2016.08.001.

Naresh V, Lee NA (2021). Review on biosensors and recent development of nanostructured materials-enabled biosensors. *Sensors* **21**: 1109. DOI 10.3390/s21041109.

Nie S, Emory SR (1997). Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *Science* **275**: 1102–1106. DOI 10.1126/science.275.5303.1102.

Peixoto de Almeida M, Pereira E, Baptista P, Gomes I, Figueiredo S et al. (2014). Chapter 13–Gold nanoparticles as (Bio) chemical sensors. *Comprehensive Analytical Chemistry* **66**: 529–567. DOI 10.1016/B978-0-444-63285-2.00013-4.

Pohanka M (2021). Point-of-care diagnoses and assays based on lateral flow test. *International Journal of Analytical Chemistry* **2021**: 1–9. DOI 10.1155/2021/6685619.

Pramanik A, Gao Y, Patibandla S, Mitra D, Mccandless MG et al. (2021). The rapid diagnosis and effective inhibition of coronavirus using spike antibody attached gold nanoparticles. *Nanoscale Advances* **3**: 1588–1596. DOI 10.1039/D0NA01007C.

Reddy PJ, Sadhu S, Ray S, Srivastava S (2012). Cancer biomarker detection by surface plasmon resonance biosensors. *Clinics in Laboratory Medicine* **32**: 47–72. DOI 10.1016/j.cll.2011.11.002.

Rodríguez-Díaz C, Lafuente-Gómez N, Coutinho C, Pardo D, Alarcón-Iniesta H et al. (2022). Development of colorimetric sensors based on gold nanoparticles for SARS-CoV-2 RdRp, E and S genes detection. *Talanta* **243**: 123393. DOI 10.1016/j.talanta.2022.123393.

Røise J, Han H, Li J, Kerr DL, Taing C et al. (2022). Acid-Sensitive surfactants enhance the delivery of nucleic acids. *Molecular Pharmaceutics* **19**: 67–79. DOI 10.1021/acs.molpharmaceut.1c00579.

Rowe BR, Canosa A, Meslem A, Rowe F (2022). Increased airborne transmission of COVID-19 with new variants implications for health policies. *MedRxiv* **133**: 915. DOI 10.1101/2022.01.13.22269234.

Saison-Francioso O, Lévêque G, Boukherroub R, Szunerits S, Akjouj A (2015). Dependence between the refractive-index sensitivity of metallic nanoparticles and the spectral position of their localized surface plasmon band: A numerical and analytical study. *The Journal of Physical Chemistry C* **119**: 28551–28559. DOI 10.1021/acs.jpcc.5b08357.

Sani A, Cao C, Cui D (2021). Toxicity of gold nanoparticles (AuNPs): A review. *Biochemistry and Biophysics Reports* **26**: 100991. DOI 10.1016/j.bbrep.2021.100991.

Shankar S, Rizzello L, Cingolani R, Rinaldi R, Pompa PP (2009). Micro/Nanoscale patterning of nanostructured metal substrates for plasmonic applications. *ACS Nano* **3**: 893–900. DOI 10.1021/nn900077s.

Shuker R, Gammon R (1970). Raman-scattering selection-rule breaking and the density of states in amorphous materials. *Physical Review Letters* **25**: 222–225. DOI 10.1103/PhysRevLett.25.222.

Sil BK, Jamiruddin MR, Haq MA, Khondoker MU, Jahan N et al. (2021). Aunp coupled rapid flow-through dot-blot immuno-assay for enhanced detection of SARS-COV-2 specific nucleocapsid and receptor binding domain igg. *International Journal of Nanomedicine* **16**: 4739–4753. DOI 10.2147/IJN.S313140.

Silva Lopes T, Gomes Alves G, Pereira R, Granjeiro JM, Correa Leite PA (2014). Advances and potential application of gold nanoparticles in nanomedicine. *Journal of Cellular Biochemistry* **120**: 16370–16378. DOI 10.1002/jcb.29044.

Teijaro JR, Farber DL (2021). COVID-19 vaccines: Modes of immune activation and future challenges. *Nature Review Immunology* **21**: 195–197. DOI 10.1038/s41577-021-00526-x.

Vandenberg O, Martiny D, Rochas O, Belkum AV, Kozlakidis Z (2021). Considerations for diagnostic COVID-19 tests. *Nature Review Microbiology* **19**: 171–183. DOI 10.1038/s41579-020-00461-z.

Vecchio G, Galeone A, Brunetti V, Maiorano G, Sabella S et al. (2012). Concentration-dependent, size-independent toxicity of citrate capped AuNPs in Drosophila melanogaster. *PLoS One* **7**: e29980. DOI 10.1371/journal.pone.0029980.

Waswa J, Irudayaraj J, DebRoyc C (2007). Direct detection of *E. coli* O157: H7 in selected food systems by a surface plasmon resonance biosensor. *LWT-Food Science and Technology* **40**: 187–192. DOI 10.1016/j.lwt.2005.11.001.

Wen T, Huang C, Shi FJ, Zeng XY, Lu T et al. (2020). Development of a lateral flow immunoassay strip for rapid detection of IgG antibody against SARS-CoV-2 virus. *Analyst* **145**: 5345–5352. DOI 10.1039/D0AN00629G.

WHO Health Emergency Dashboard. https://covid19.who.int/.

Xu W, Xie L, Zhu J, Xu X, Ye Z, Wang C, Ma Y, Ying Y (2016). Gold nanoparticle-based terahertz metamaterial sensors: Mechanisms and applications. *ACS Photonics* **3**: 2308–2314. DOI 10.1021/acsphotonics.6b00463.

Yang X, Yu Y, Xu J, Shu H, Liu H et al. (2020). Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. *The Lancet Respiratory Medicine* **8**: 475–481. DOI 10.1016/S2213-2600(20)30079-5.

Yano T, Kajisa T, Ono M, Miyasaka Y, Hasegawa Y (2022). Ultrasensitive detection of SARS-CoV-2 nucleocapsid protein using large gold nanoparticle-enhanced surface plasmon resonance. *Scientific Report*, **12**, 1060. DOI 10.1038/s41598-022-05036-x.

Yeh YC, Creran B, Rotello V (2012). Gold nanoparticles: Preparation, properties, and applications in bionanotechnology. *Nanoscale* **4**: 1871–1880. DOI 10.1039/C1NR11188D.

Yola ML, Atar N (2021). Novel voltammetric tumor necrosis factor-alpha (TNF-α) immunosensor based on gold nanoparticles involved in thiol-functionalized multi-walled carbon nanotubes and bimetallic Ni/Cu-MOFs. *Analytical and Bioanalytical Chemistry* **413**: 2481–2492. DOI 10.1007/s00216-021-03203-z.

Zaho J, Wallace M, Melancon MP (2014). Cancer theranostics with gold nanoshells. *Nanomedicine* **9**: 2041–2057. DOI 10.2217/nnm.14.136.

Zhang Q, Xiang R, Huo S, Zhou Y, Jiang S et al. (2021). Molecular mechanism of interaction between SARS-CoV-2 and host cells and interventional therapy. *Signal Transduction and Targeted Therapy* **6**: 233. DOI 10.1038/s41392-021-00653-w.

Zhang XD, di Wu XS, Liu PX, Yang N, Zhao B et al. (2011). Size-dependent *in vivo* toxicity of PEG-coated gold nanoparticles. *International Journal of Nanomedicine* **6**: 2071–2081. DOI 10.2147/IJN.S21657.