



SERS-based methods for the detection of genomic biomarkers of cancer

Aizhan Issatayeva^{a,*}, Edoardo Farnesi^{b,c}, Dana Cialla-May^{b,c}, Michael Schmitt^b,
Federica Maria Angel Rizzi^d, Daniel Milanese^a, Stefano Selleri^a, Annamaria Cucinotta^a

^a Department of Engineering and Architecture, University of Parma, Parco Area delle Scienze 181/a, 43124, Parma, Italy

^b Institute of Physical Chemistry (IPC) and Abbe Center of Photonics (ACP), Friedrich Schiller University Jena, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Helmholtzweg 4, 07743, Jena, Germany

^c Leibniz Institute of Photonic Technology, Member of Leibniz Health Technologies, Member of the Leibniz Centre for Photonics in Infection Research (LPI), Albert-Einstein-Straße 9, 07745, Jena, Germany

^d Department of Medicine and Surgery, University of Parma, Via Volturno 39/a, 43125, Parma, Italy

ARTICLE INFO

Keywords:

Cancer
DNA methylation
Genomic biomarkers
miRNA
Point mutation
Surface enhanced Raman spectroscopy

ABSTRACT

Genomic biomarkers of cancer are based on changes in nucleic acids, which include abnormal expression levels of some miRNAs, point mutations in DNA sequences, and altered levels of DNA methylation. The presence of tumor-related nucleic acids in body fluids (blood, saliva, or urine) makes it possible to achieve a non-invasive early-stage cancer diagnosis. Currently existing techniques for the discovery of nucleic acids require complex, time-consuming, costly assays and have limited multiplexing abilities. Surface-enhanced Raman spectroscopy (SERS) is a vibrational spectroscopy technique that is able to provide molecular specificity combined with trace sensitivity. SERS has gained research attention as a tool for the detection of nucleic acids because of its promising potential: label-free SERS can decrease the complexity of assays currently used with fluorescence-based detection due to the absence of the label, while labeled SERS may outperform the gold standard in terms of the multiplexing ability. The first papers about SERS-based methods for the measurement of genomic biomarkers were written in 2008, and since then, more than 150 papers have been published. The aim of this paper is to review and evaluate the proposed SERS-based methods in terms of their level of development and their potential for liquid biopsy application, as well as to contribute to their further evolution by attracting research attention to the field. This goal will be reached by grouping, on the basis of their experimental protocol, all the published manuscripts on the topic and evaluating each group in terms of its limit of detection and applicability to real body fluids. Thus, the methods are classified according to their working principles into five main groups, including capture-based, displacement-based, sandwich-based, enzyme-assisted, and specialized protocols.

1. Introduction

Cancer is one of the leading causes of mortality worldwide accounting for more than 10 million deaths annually [1]. An early-stage cancer diagnosis can significantly improve the survival chances of patients but is complicated by an absence or a weak manifestation of symptoms [2]. One promising solution to achieve an early-stage cancer diagnosis is the regular screening of the population [3]. Currently existing diagnostic techniques have limitations: tissue biopsy requires invasive and risky procedures, imaging-based techniques expose patients to ionizing radiation, and stool- and blood-based tests have limited accuracy and sensitivity [2–4]. Recently, liquid biopsy has gained research attention as a promising non-invasive and accurate screening

tool. Liquid biopsy is based on the analysis of body fluids for the presence of circulating tumor cells, circulating tumor DNAs/RNAs, exosomes, and other tumor-related substances that may serve as cancer biomarkers [2]. Circulating tumor nucleic acids are continuously ejected from tumors into body fluids and have a short half-life time (up to 2.5 h) which makes them ideal for real-time monitoring of tumor changes and therapy response [5]. Changes in nucleic acids may serve as genomic biomarkers for an early-stage cancer diagnosis due to a significant contribution of genetic alterations to cancer etiology [6].

Genomic biomarkers include but are not limited to an abnormal expression of nucleic acids, a mutation in their sequences, and epigenetic changes [2,7]. Alteration of the expression levels of microRNAs (miRNAs) has recently gained significant research attention. MiRNAs

* Corresponding author.

E-mail address: aizhan.issatayeva@unipr.it (A. Issatayeva).

<https://doi.org/10.1016/j.talanta.2023.125198>

Received 24 April 2023; Received in revised form 5 September 2023; Accepted 10 September 2023

Available online 15 September 2023

0039-9140/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

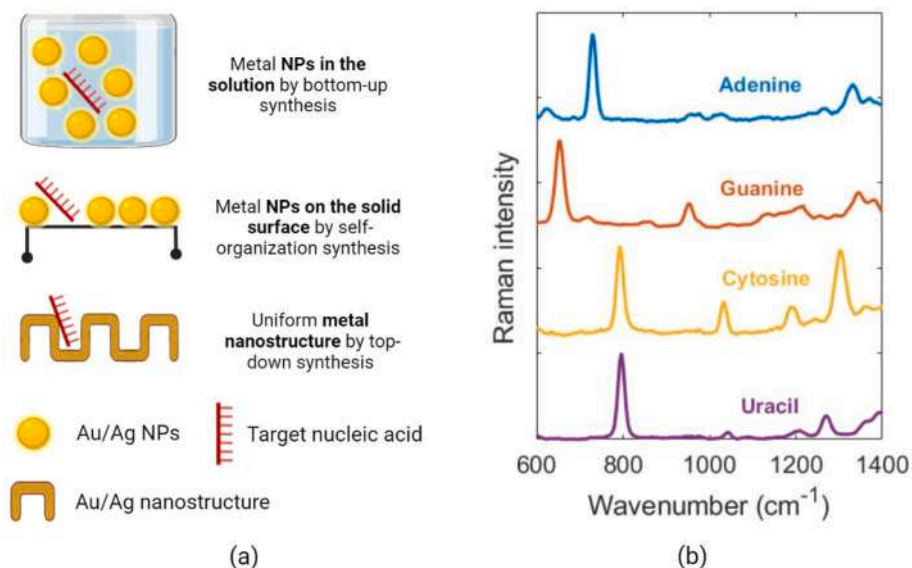


Fig. 1. Schematics of the SERS substrates and SERS spectra of the nucleic acids.

are short non-coding RNAs that typically consist of 22–25 nucleotides (nt) and are present in body fluids as free RNAs or inside exosomes [8]. They play a significant role in cellular processes by binding to mRNA and inhibiting gene expression. Therefore, abnormal levels of some miRNAs can lead to the silencing of tumor-suppressor genes or the modulation of oncogenes, a condition that favors cancer formation and spread, which makes miRNAs a promising cancer marker [8]. For example, the concentrations of miRNA-21, miRNA-155, miRNA-126, or miRNA-191 are about 10^{-14} – 10^{-13} M in the serum of healthy people, while their levels are upregulated in breast cancer patients (about 10^{-12} – 10^{-11} M) [9]. Circulating tumor DNAs are about 150 nt long fragments of DNA circulating in body fluids at a concentration of approximately 7–18 ng/mL (about 10^{-10} M) [2,10]. Those DNAs contain information about tumor-specific changes, such as single-nucleotide point mutations, copy number variations, chromosomal rearrangement, and DNA methylation levels [11]. Point mutation is defined as a change in a single nucleotide in a sequence. Single-point mutations in the KRAS, BRCA2, BRAF, and TP53 genes have been found to be positively correlated with cancer development and are an object of many studies [12,13]. DNA methylation, a process that involves an addition of a methyl group ($-CH_3$) to cytosines, has been also found to be altered during tumorigenesis. It was estimated that around 12% of cancers are associated with regional hypermethylation (at a particular gene) and 70% with global hypomethylation (through a whole genome) [14]. Some DNA methylation-based diagnostic kits are already available for the detection of cancer-related biomarkers in urine, plasma, and blood [15,16].

The most common technique for the miRNAs and DNA point mutation measurements is based on a target amplification by the polymerase chain reaction (PCR) coupled to the fluorescence-based detection of the amplified product [17]. DNA methylation is usually measured by converting the unmethylated cytosines to uracil with bisulfite treatment and further DNA point mutation detection, again with PCR and fluorescence [18]. This fluorescence-based technique is commercially available and widely applied, but it requires a multi-step assay and the use of a fluorescent dye (labeling) due to originally weak auto-fluorescence from biological samples [19]. One of the emerging attractive alternatives that have a good potential for liquid biopsy application is surface-enhanced Raman spectroscopy (SERS). SERS is one of the most sensitive analytical techniques that can provide molecular-specific and trace-sensitive information of a biological sample by analyzing the inelastic scattering by the molecules absorbed onto the metallic nanoparticles (NPs) [20,21].

SERS has been studied for the label-free direct detection of nucleic acids without any biological treatment, potentially providing more straightforward assays than the labeled fluorescence-based gold standard [22]. On the other hand, labeled SERS can achieve increased sensitivity by relying on Raman reporters, which are the molecules with strong absorption at the laser wavelength and high SERS response due to the additional resonance Raman enhancement [23]. Moreover, biological assays can be combined with SERS to reach target-specific detection. Labeled SERS with biological protocols may lose the advantage of simplicity but can still outperform the fluorescence-based gold standard in terms of multiplexing capabilities. This is due to the narrow Raman bands of the reporters, which allow several of them to be used simultaneously for the detection of multiple biomarkers [21,22].

This paper aims to analyze the SERS-based approaches presented in more than 150 papers for the detection of three types of genomic biomarkers, namely miRNA expression level changes, DNA point mutations, and abnormal DNA methylation. All methods will be arranged in groups according to their working principle. The future potential of the SERS-based methods to reach the clinical standard and replace or be combined with the current gold standard will be evaluated according to the detection abilities of the SERS-based protocols and their applicability for liquid biopsy.

2. SERS working principle

The working principle of Raman spectroscopy is based on the vibrational motion of a molecule caused by the incident light and subsequent scattering of the light with a changed frequency (inelastic scattering) [20]. Since the frequency shift depends on the vibrational modes of the analyzed molecule, the Raman information is specific to each molecule. Thus, the Raman spectrum, which represents the intensity profile of the scattered light as a function of the wavenumber, can provide a qualitative and potentially quantitative composition of an illuminated biological sample [24].

Inelastic scattering is a very rare event, so amplification techniques can be used for applications that require the detection of trace concentration ranges [20]. SERS exploits metal NPs to obtain chemical and electromagnetic (EM) enhancement. Chemical enhancement can typically achieve $10 - 10^2$ signal amplification due to the created charge-transfer state between the metal NPs and the molecule [25]. EM amplification is obtained when the light incident on the metal NPs induces a displacement of the electrons toward the positively charged

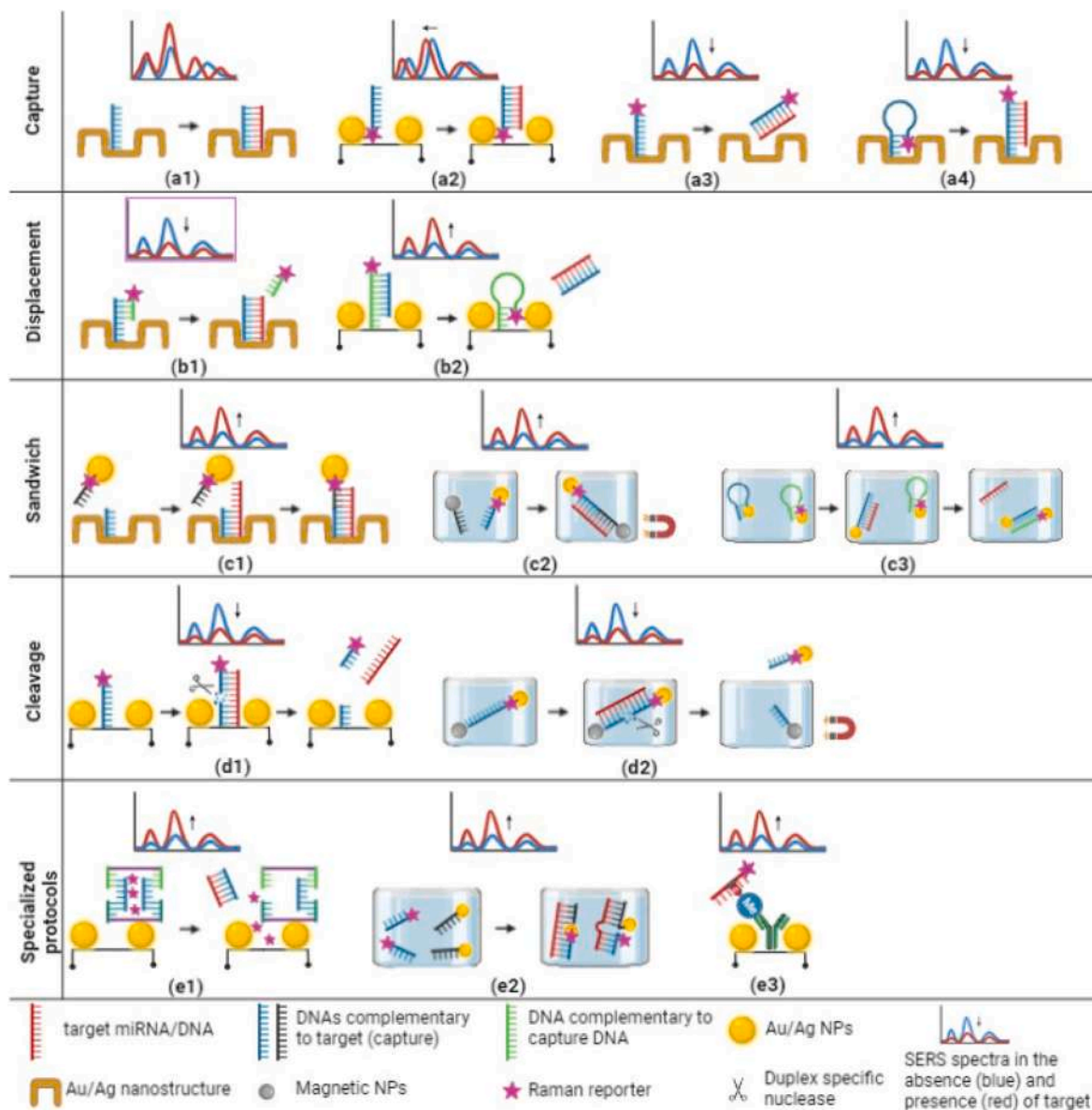


Fig. 2. Summary of the SERS-based protocols for the detection of genomic biomarkers.

atomic cores, which makes the NPs perform as dipole antennas [26]. This leads to an excitation of the surface plasmon polaritons at the interface between the metal and the dielectric and a subsequent EM field enhancement at the surface of the NPs [26]. Raman signal increases at the fourth power of the EM field resulting in significant amplification of the Raman fingerprint information from about 10^4 times [25] up to $10^{10} - 10^{11}$ times reported in some papers [27].

Since the EM field is created in close proximity to NPs, the SERS enhancement drops significantly with an increase in the distance from the NPs (D^{-10}) [28]. A considerable amplification of the SERS effect is observed in a region between two or more NPs, called a hot spot, due to the plasmonic coupling effect. The hot spots can be created by controlling the interparticle distance and varying the arrangement of the NPs. Different arrangements of NPs are achieved by three main types of fabrication techniques, such as a bottom-up approach, a self-organization process, and a top-down technique (see Fig. 1, a) [26]. The bottom-up method, which is mostly used for the fabrication of NP colloidal solutions, involves a reduction of silver or gold ions with the reducing agents and control of the NPs aggregation with the capping agents [29]. The self-organization process for the fabrication of an array

of NPs on the solid surface is usually achieved by attaching NPs to a silanized surface [29]. The top-down technique is usually applied to the production of ordered nanostructures to provide a regular and uniform arrangement of the hot spots which makes the SERS signal more reproducible. The top-down production includes lithography-based methods for the formation of the nanostructures followed by a vapor or electrochemical deposition of a metal layer [29]. The ordered nanostructures require a more complex fabrication procedure than the NPs in the colloidal solutions or on the solid substrates but provide better reproducibility and comparable enhancement [19].

3. SERS-based methods for the detection of potential genomic biomarkers

To the best of our knowledge, the SERS-based detection of nucleic acids was first presented by Vo-Dinh and his colleagues in 1994 [30]. Nucleic acids are made of chemical building blocks called nucleotides, which consist of a phosphate group, a sugar group (deoxyribose/ribose for DNA/RNA, respectively), and one of five nitrogen bases, namely adenine (A), thymine (T), guanine (G), cytosine (C), and uracil (U).

Double-stranded DNAs and heteroduplex DNA/RNAs are formed by binding of the complementary bases: G and C, and A and T/U (for DNA/RNA, respectively) [31]. The principle of the direct detection of nucleic acids is based on a difference in the Raman spectra of the five bases and the corresponding nucleotides. Fig. 1 (b) shows the normalized SERS spectra of the four bases of RNAs. As can be seen, each base can be discriminated from others by one or several characteristic peaks. For example, a peak at 732 cm^{-1} is associated only with A, while a peak around 790 cm^{-1} is found for C and U, so these two bases need to be discriminated by additional SERS information [32]. The SERS spectra of nucleotides are similar to the respective bases, but also contain the peaks of the sugar group and the phosphate group [33]. In the case of a mixture of several nucleotides (such as in the DNA/RNA sequence), the spectrum is constituted by the peaks of all present nucleotides. The spectra can be convoluted to determine the concentration of each nucleotide in the analyzed DNA or RNA and thereby differentiate the sequences [34].

Thus, to perform the direct SERS measurement of miRNA or DNA of interest, the sequences are absorbed on the Ag or Au nanoparticles. SERS spectra of different miRNAs and DNAs have different intensities of the peaks associated with the five nucleotides depending on the sequences and structures [35–48]. These differences can be found by manual analysis or an application of statistical tools, such as the partial least squares discriminant analysis (PLS-DA) and the principal component analysis (PCA). Morla-Folch et al. have shown that this technique can be used to discriminate single-stranded, double-stranded, hairpin RNAs with the same sequences and RNAs/DNAs with a point mutation [40, 48]. As for the DNA methylation-based biomarkers, the methylated cytosine can be distinguished from the non-methylated one because its characteristic SERS peaks ($790, 1028, 1303, 1636\text{ cm}^{-1}$) experience some frequency shift and intensity change due to methylation [14, 49–60].

The aforementioned works have measured each target sequence in water or buffer solution separately and in the absence of any other interfering substances. For liquid biopsy application, a biomarker of interest should be specifically captured from a pool of DNAs, RNAs, proteins, and other substances. Thus, the experiment should include a biological protocol that ensures target-specific detection. In most cases, the protocols rely on labeled SERS, which is based on the modification of the target or supporting sequences with a Raman reporter molecule (e. g., rhodamine X, methylene blue, 4-methoxy benzoic acid, and others). The biological protocol is designed in such a way that an introduction of the target sequence induces a change in the distance between the reporter and the SERS substrate. For example, the reporter originally located far enough from the NPs (producing no or weak SERS signal) can be brought into the SERS active region. As a result, the presence of the target sequence is detected not by a change in its own SERS response, but indirectly by an increase in the reporter's SERS signal because it is much stronger [31]. The amplitude of the signal variation depends on the concentration of the target (more targets induce more changes in the system), making this method suitable for quantitative application.

A literature review has shown that similar biological protocols are presented for miRNA, DNA point mutation, and DNA methylation detection. The reviewed protocols have been classified according to their working principle into five common groups for all biomarkers: capture-, displacement-, sandwich-, and cleavage-based methods, and a specialized group combining some distinctive protocols (see Fig. 2).

3.1. Capture of a target

The methods illustrated in Fig. 2 (a1-a4) are based on the immobilization of a single-stranded DNA (ssDNA), called capture DNA, on the surface of the SERS substrate. Capture DNA, being complementary to the target miRNA or DNA, is able to hybridize with it, while all other sequences and interfering substances are blocked from adsorption to the surface.

Fig. 2 (a1) shows the simplest version of the capture protocol in

which the hybridization of the capture DNA with the target causes the change in the SERS signal. In the label-free version of this protocol (presented in Fig. 2, a1), the SERS response is caused by the appearance of the miRNA in the SERS active region and the formation of new bonds [61,62]. Another way of applying label-free capture protocol is to use reverse transcription-recombinase polymerase amplification in order to specifically capture the target and amplify it. Then, the formed dsDNA amplicons can be mixed with NPs and directly analyzed. Koo et al. and Wang et al. have tested this protocol for the diagnosis of prostate cancer by detecting two RNA-based biomarkers in urine [63,64]. A more sensitive detection can be achieved by labeling the target. In this case, the reporter molecule attached to the target enters the SERS active region and induces a more significant SERS signal variation than in the label-free version [65–68]. This and other described protocols can be applied for all three types of biomarkers but with some modifications. For example, in the case of DNA point mutation, a mismatched DNA being only one nucleotide different from a matched DNA creates a partial hybridization with the capture DNA. However, due to the weaker stability of the partial hybridization a wash step, or the application of heat or electric potential can dehybridize a mismatched double-stranded DNA (dsDNA) while keeping a perfectly matched dsDNA stable [65–70]. As for DNA methylation, which does not involve a sequence change, the detection requires a preliminary bisulfite treatment. It converts all unmethylated cytosines to uracil while leaving the methylated cytosines unchanged. When the sequence is changed, the problem is traced back to the detection of point mutations in DNA [71,72].

The labeled version of the previously described protocol requires labeling of the target inside the body fluid, which is not convenient in real settings. Fig. 2 (a2) illustrates another capture-based approach, which does not require labeling of the target. The Raman reporter is immobilized on the surface of AgNPs through Ag–S binding and then capture DNA is attached to the reporter. The formation of a double-stranded complex after target capturing leads to mechanical stretching of the reporter's bonds and a frequency shift of its SERS peak by about 0.5 cm^{-1} for 10^{-17} M of the target. The authors have not specified the grating used to detect such a small spectral shift but claimed that the limiting factor is not the instrument's spectral resolution but the reproducibility of the measurements [73–75]. According to Zhang et al., the maximum root-mean-square variation in the peak position is about 0.022 cm^{-1} , so a shift greater than 0.15 cm^{-1} is considered significant and distinguishable [75].

Fig. 2 (a3) describes the following principle: thiolated ssDNA in comparison with thiolated dsDNA has a higher binding affinity towards the metal and graphene surfaces [76]. Therefore, under optimized conditions, the dsDNA formed after the hybridization of the capture DNA and the target sequence can be detached from the SERS surface. This leads to a decrease in the SERS response of the dsDNA or the reporter in the label-free and labeled versions, respectively [76–79].

Fig. 2 (a4) illustrates an application of a DNA hairpin as a labeled capture sequence [80–92]. Complementary sequences on the two ends of the capture DNA force it to form a hairpin structure. The target miRNA or DNA has a longer complementary sequence with the hairpin and a preferable intermolecular hybridization, which causes the hairpin to open up. The hairpin DNA allows good control over the distance between the reporter and the metal surface: in its closed position, the reporter is in proximity to the SERS surface, while in an open state, the reporter is moved at a distance equal to the length of the hairpin sequence, which reduces the output signal.

3.2. Displacement by the target

The methods illustrated in Fig. 2 (b1 and b2) are based on two partially hybridized DNAs (capture and placeholder) and the displacement of one of them by the hybridization with the target sequence. Fig. 2 (b1) shows the case when the target hybridizes with the capture DNA and the labeled placeholder strand is detached from the SERS surface,

resulting in a signal drop [93–105]. In the protocol shown in Fig. 2 (b2), the capture DNA is designed as a hairpin and labeled. The placeholder DNA initially hybridized with the hairpin opens it up, keeping the distance between the reporter and the SERS substrate. The target sequence hybridizes with the placeholder DNA, which leads to a detachment of the latter one, the closing of the hairpin, and movement of the reporter molecule toward the substrate [106–109]. Some papers have presented displacement-based methods combined with pre- [110] or post-amplification [111].

3.3. Formation of a sandwich

One of the most exploited techniques is a molecular sandwich approach that involves two capture DNAs complementary to the target from its two ends. Fig. 2 (c1) illustrates that one of the capture DNAs is attached to the SERS substrate, while another one is labeled and modified with the NP. The target sequence hybridizes with both captures and attaches them to each other. As a result, the NP is brought close to the SERS substrate, and the hot spot is created between the two SERS active agents. The reporter molecule, originally, is close to the NP, producing a moderate SERS signal, and after the target-induced hot spot creation, its signal increases by about three times [112–124]. The same method is also applied in the colloidal solution, where two or more metal NPs play the role of SERS-active agents [125–134].

Another version of this protocol applies a magnetic particle instead or together with one of the metal NPs (by covering the magnetic particle with an Au or Ag shell). As depicted in Fig. 2 (c2), the target sequence hybridizes with both DNAs and attaches the SERS-active labeled NP to the magnetic particle. An application of the magnet to the solution leaves only the magnetic particles with their attachments and removes all unattached labeled NPs and other interfering substances. In the absence of the target sequence, no labeled NP is attached to the magnetic particles and no SERS signal is observed [90,135–143]. The sandwich-based protocols shown in Fig. 2 (c1 and c2) can be used with the pre-amplification strategies, such as rolling circle amplification [144], strand displacement amplification [145], and hairpin-chain reaction [146–148].

The sandwich-based method can also involve hairpin DNAs. Fig. 2 (c3) presents two hairpin DNAs complementary to each other and attached to the NPs (one of them is labeled). In the presence of the target, the first hairpin opens, and its free end can open the second hairpin. The second hairpin replaces the target sequence due to a more preferable hybridization and releases it to a new cycle. The two hybridized hairpins fasten the NPs to each other, and the reporter in the hot spot produces a strong SERS signal [149–163].

The hairpin-based methods (capture, displacement, and sandwich) can be used for dual signal sensing: one target sequence is detected with the help of two reporters for better accuracy. In that case, one labeled hairpin DNA is immobilized on the SERS substrate and is opened up by the hybridization with another labeled hairpin. The second hairpin's reporter is close to the substrate producing the signal, while the first reporter is inactive. The target sequence hybridizes with the second hairpin, forcing it to leave the SERS substrate, and the first hairpin closes. The signal from the first reporter is increased, while the second reporter detaches from the metal NPs producing no SERS response [90, 164,165].

Some protocols (mainly hairpin-based) can be considered in combination with fluorescence-based detection in order to compensate for the limitations of each technique. Thus, a single molecule with appropriate properties can be used both as a fluorophore and a reporter, while the Ag/Au NPs serve as a fluorescence quencher and a SERS enhancer. The hairpins move the reporter/fluorophore to and from the NPs and produce opposite changes in the fluorescence and SERS signals [82,90, 149,156,159]. In that case, SERS can provide approximately as sensitive detection of the target nucleic acids as the standard fluorescence technique [159].

3.4. Cleavage by enzymes

The cleavage-based protocols depicted in Fig. 2 (d1, d2) involve enzymes that can be activated only in the presence of dsDNA, such as duplex-specific nuclease (DSN), Exo III, T7 exonuclease, S1 nuclease, and CRISPR-Cas12 enzyme. Fig. 2 (d1) shows the labeled capture DNA initially attached to the SERS substrate. Its hybridization with the target miRNA or DNA activates the enzyme, which cleaves the capture strand, so the reporter is detached from the substrate, and its SERS response is decreased. The advantage of this method is its ability to simultaneously provide a signal amplification because the target sequence is released to participate in a new cycle [164,166–172].

As shown in Fig. 2 (d2), the enzyme-assisted method can also be integrated with the magnetic separation technique in the NPs colloidal solution [165,173–176]. In this case, the presence of the target sequence also activates the enzyme, which leads to the detachment of the labeled NPs from the magnetic particles. The labeled SERS-active NPs leave the solution after magnetic separation, which results in the SERS signal drop.

3.5. Specialized protocols

Fig. 2 (e1-e3) illustrates the protocols with distinctive working principles, which require a complex design and an application of polymerases, ligases, or methylation-specific antibodies. Thus, the protocol shown in Fig. 2 (e1) demonstrates a closed capsule that has the reporter molecules trapped inside and can be opened by the target miRNA. The capsule can be a DNA hydrogel consisting of two acrylamide strands, two pairs of ssDNAs, and two capture DNAs [177–179]. In the presence of the target miRNAs, the capture DNAs hybridize with the targets, which leads to the opening of the capsule and the release of the reporters on the SERS substrate. Another type of capsule called a DNA microcapsule is formed by putting the reporters on the CaCO_3 particle, coating the particle with a layer of positively charged poly(allylamine hydrochloride), and covering it with a layer of negatively charged ssDNAs. The ssDNAs hybridize with the target miRNAs, which activates the DSN to cleave the formed duplex and open up the capsule. The reporters are released to the SERS substrate, and the signal is obtained [180].

Fig. 2 (e2) shows a protocol suitable for the discrimination of the sequences at a specific point, which makes it particularly useful for DNA point mutation detection. The technique is based on the application of a ligase and two DNA strands, one labeled and one modified with the SERS active NP. The strand hybridizes with the target DNA in such a way that the NP and the reporter are placed exactly at the point of potential mutation. In the case of an absence of the point mutation, the ligase is able to join the two DNA strands and bring the reporter into close contact with the NP, so a strong SERS increase is produced. No ligation occurs in the case of a mismatch, so there is no contact between the NP and the reporter and no SERS response [181–184]. Another way to achieve a specific point mutation detection is to apply a polymerase with the displacement-based method. Initially, the capture DNA is hybridized with the labeled DNA, and then both the target and the mismatched DNA hybridize with the capture at its opposite end. The polymerase extends the target DNA, and the labeled placeholder DNA is displaced. However, the mutated nucleotide in the mismatched DNA blocks the polymerase's activity, so the reporter remains attached to the SERS substrate, and no significant change in the SERS intensity is observed [185]. Additionally, the polymerase and the ligase can be used to selectively amplify the target DNA using PCR. The produced trigger DNAs can be further used in the sandwich-based protocol [186,187].

The protocol depicted in Fig. 2 (e3) is based on the application of methylation-specific antibodies, such as methyl-CpG-binding 2 protein and methyl-binding domain proteins, and is mostly suitable for DNA methylation detection [6]. The antibodies can be immobilized directly onto the SERS active material and grab only the methylated DNA sequences. The reporter on this methylated DNA generates a SERS signal,

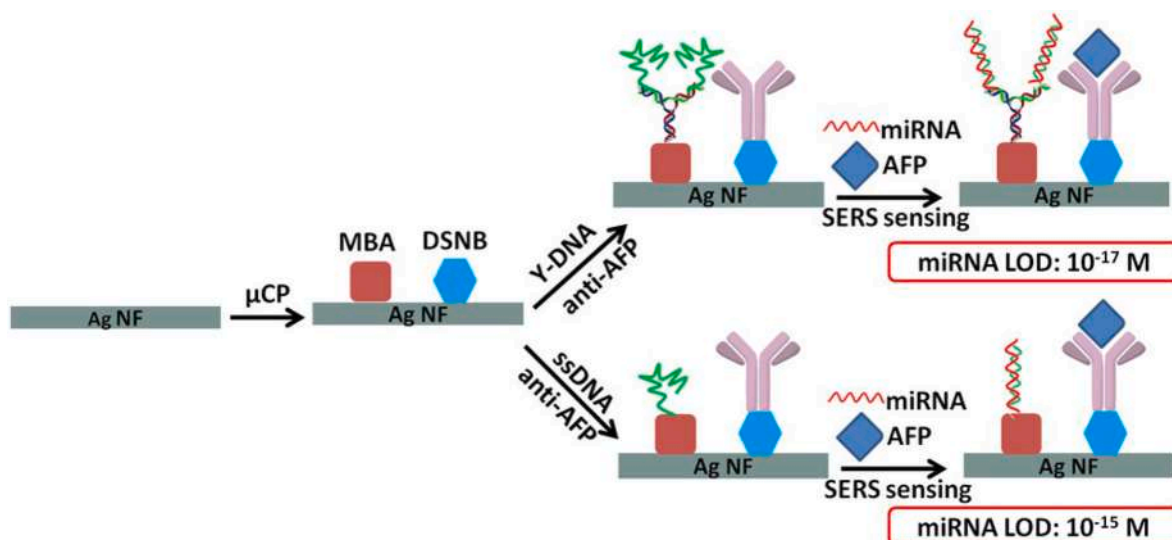


Fig. 3. Frequency shift-based protocol for the detection of miRNAs. Reprinted with permission from Cheng et al. [74]. Copyright 2018 American Chemical Society.

while the reporter on the unmethylated and unbound to the SERS surface DNA produces no or a weak SERS response [188–190]. Bisulfite treatment combined with DNA sequence change detection is more suitable for the regional methylation level measurement because of the specific capturing of the target DNA. The methods assisted by the methylation-specific antibodies can be applied for a global methylation evaluation.

The schemes presented in Fig. 2 can be used to review all the available SERS-based techniques for the detection of genomic markers and conduct a further evaluation of their performance. It can be noticed that the groups are arranged according to the increasing complexity of

the protocols: the capture-based method applies only one capture DNA, the displacement-based technique needs two DNAs, the sandwich approach uses additional NP, the enzyme-assisted method applies enzymes, and the last group requires the complex structures, polymerase, ligase, and antibodies. Since the most optimal protocols are those that can provide adequate performance with relatively simple assays, this arrangement may make an assessment of their complexity more convenient.

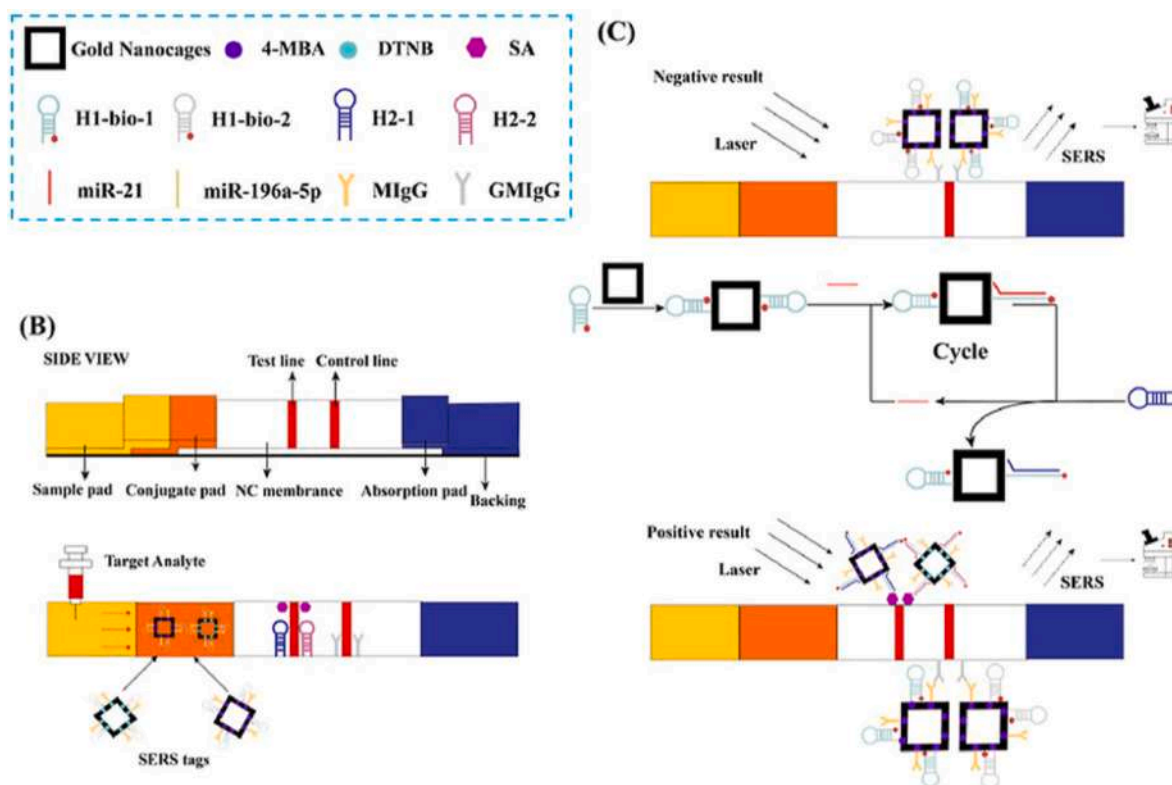


Fig. 4. Hairpin-assisted capture protocol for the detection of miRNAs. Reprinted with permission from Mao et al. [89]. Copyright 2021 Elsevier. Figure has been rearranged for better visibility.

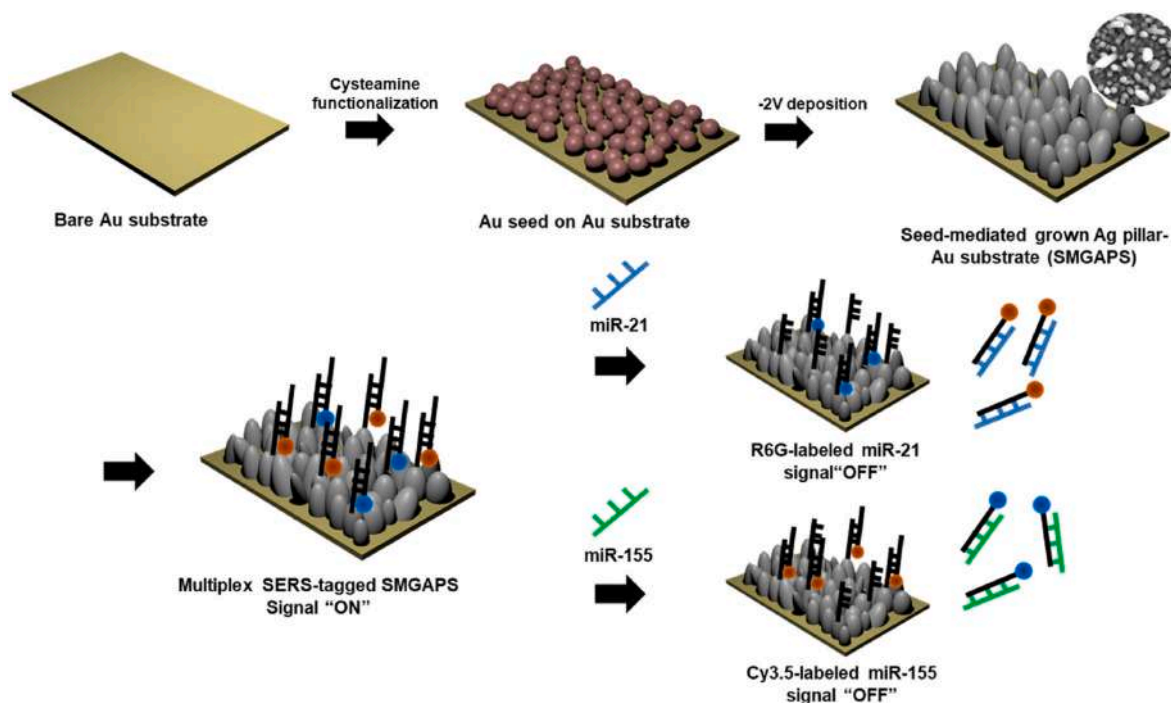


Fig. 5. Displacement-based protocol for the detection of miRNAs. Reprinted with permission from Kim et al. [97]. Copyright 2022 Elsevier.

4. Examples of the protocols with promising potential for liquid biopsy application

In this section, some original illustrations from the selected papers, which have shown promising detection abilities and interesting applications, are presented.

4.1. Detection of miRNA

Fig. 3 shows the frequency shift-based capture protocol presented by Cheng et al. [74]. Two Raman reporters, 4-methoxybenzoic acid (MBA)

and 5,5'-Dithiobis(succinimidyl-2-nitrobenzoate) (DSNB), are immobilized on the AgNPs film to achieve multiplex detection of two biomarkers, miRNA and alpha-fetoprotein (AFP). The capturing agents for two markers, DNAs complementary to the target miRNA and AFP antibodies, are covalently bound to the MBA and DSNB, respectively. In the case of capturing one of the targets, the respective reporter experiences mechanical stretching due to an increased load. The SERS spectrum of both reporters is shifted by about $0.4\text{--}0.5\text{ cm}^{-1}$ in response to 10^{-12} M of AFP and 10^{-17} M of miRNA. The authors have tested two different capturing approaches for miRNA: a simple ssDNA with one active sticky end and a Y-shaped structure with dsDNA as a scaffold and two active

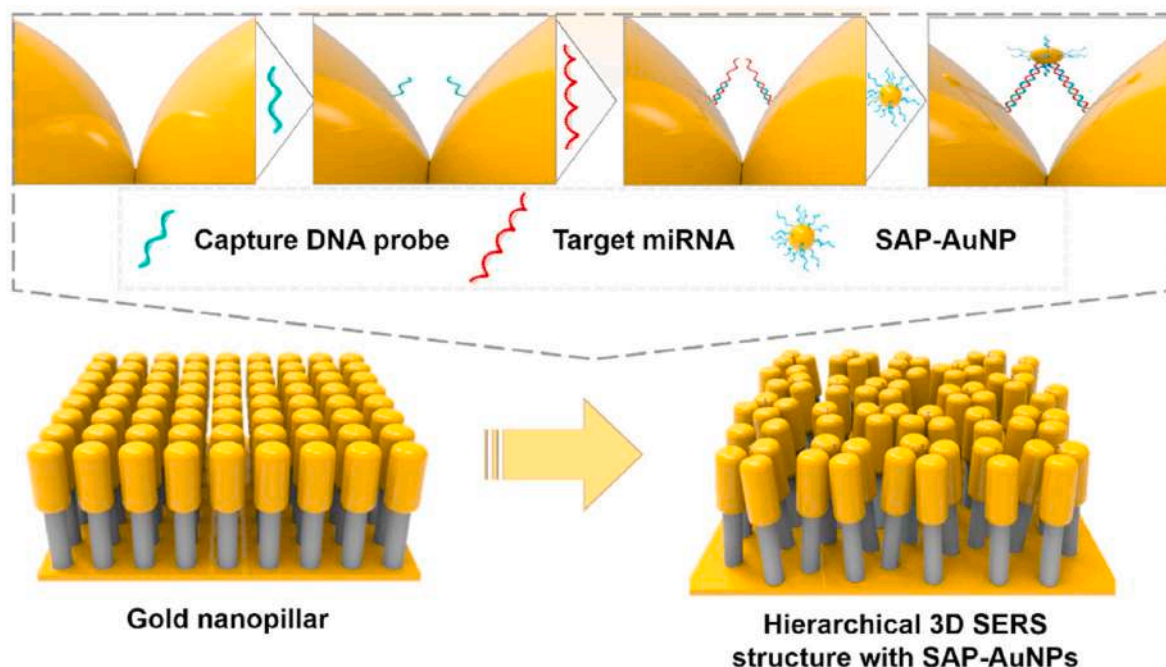


Fig. 6. Sandwich-based protocol for the detection of miRNAs. Reprinted with permission from Kim et al. [118]. Copyright 2022 Elsevier.

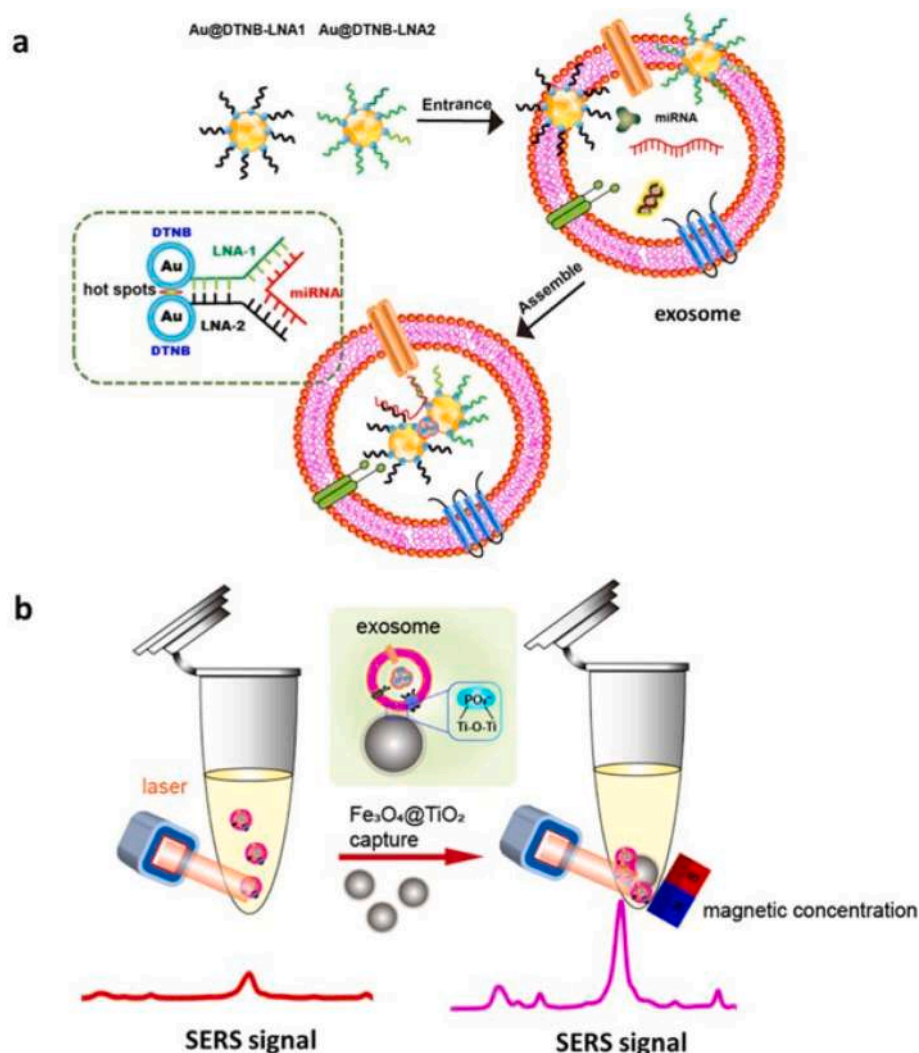


Fig. 7. Sandwich-based protocol for the detection of miRNAs. Reprinted with permission from Jiang et al. [131]. Copyright 2015 American Chemical Society.

sticky ends. Applying the Y-shaped DNA improved the detection sensitivity due to a more stable configuration and twice more capturing strands. Thus, a limit of detection (LOD), which is the smallest detectable concentration of the target, is $10^{-15}M$ and $10^{-17}M$ for single-stranded and Y-shaped configurations, respectively. The developed protocol has been successfully implemented to compare the miRNA-223 and AFP concentrations in the serum of hepatocellular carcinoma patients and in healthy serum.

SERS protocols, for example, the hairpin-assisted capture method, can be integrated with the lateral flow assays (LFAs), which is a platform used in point-of-care testing for fast analysis of biological samples [191]. Fig. 4 demonstrates the LFA, which consists of several main regions, such as a sample pad, a conjugate pad with the labeled Au nanocages modified with hairpin DNAs, a membrane with test and control lines that are covered with hairpin capture DNAs and mouse antibodies, respectively, and an absorption pad [89]. A sample of body fluid is dropped on the sample pad, and then it travels to the conjugate pad where the target miRNAs open up the hairpin DNAs on the nanocages. The open end of those hairpins can be captured by the hairpin DNAs on the test line. However, if the target sequence is absent and the nanocages' hairpins are left closed, the nanocages travel further to the control line and are captured by the anti-mouse antibody. As a result, the SERS signal measured on the test line is related to the concentration of the target miRNAs, while the signal from the control line signifies the number of unhybridized hairpins. The protocol has reached a LOD of

about $2-3 \times 10^{-12}M$ for the measurement of miRNA-21 and miRNA-196a-5p in the urine of lung cancer patients and healthy people.

The displacement-based protocol shown in Fig. 5 was presented by Kim et al. for multiplex sensing of two miRNAs [97]. As the SERS substrate, the authors used Ag nanopillars grown on the surface of the Au seeds at the Au substrate. Initially, two types of DNAs are immobilized on the surface of the substrate and then hybridized with the labeled DNAs complementary to the targets. In the presence of the target miRNAs, they hybridize with the respective captures and detach them together with the reporters from the substrate. As a result, initially strong SERS signals from the reporters decrease in response to the target miRNAs. The protocol has been tested in mouse urine and reached a LOD of about $4.51 \times 10^{-14}M$ and $1.65 \times 10^{-13}M$ for miRNA-21 and miRNA-155, respectively.

Many authors have proposed sandwich-based protocols for miRNA detection with or without magnetic separation. The optimal sensitivity and applicability in body fluids have been shown by Kim et al. and Jiang et al. [118,131]. Kim et al. have reached highly sensitive detection with the label-free sandwich protocol through the application of Au nanorods and AuNPs as SERS active materials [118]. Fig. 6 illustrates that the AuNP modified with the capture DNAs is brought in between the two adjacent nanorods using the hybridization of its capture DNAs with two target miRNAs that at the opposite ends are hybridized with the capture DNAs of each nanorod. This configuration allowed the positioning of the miRNA-DNA pairs in the hot spot created by three SERS-active

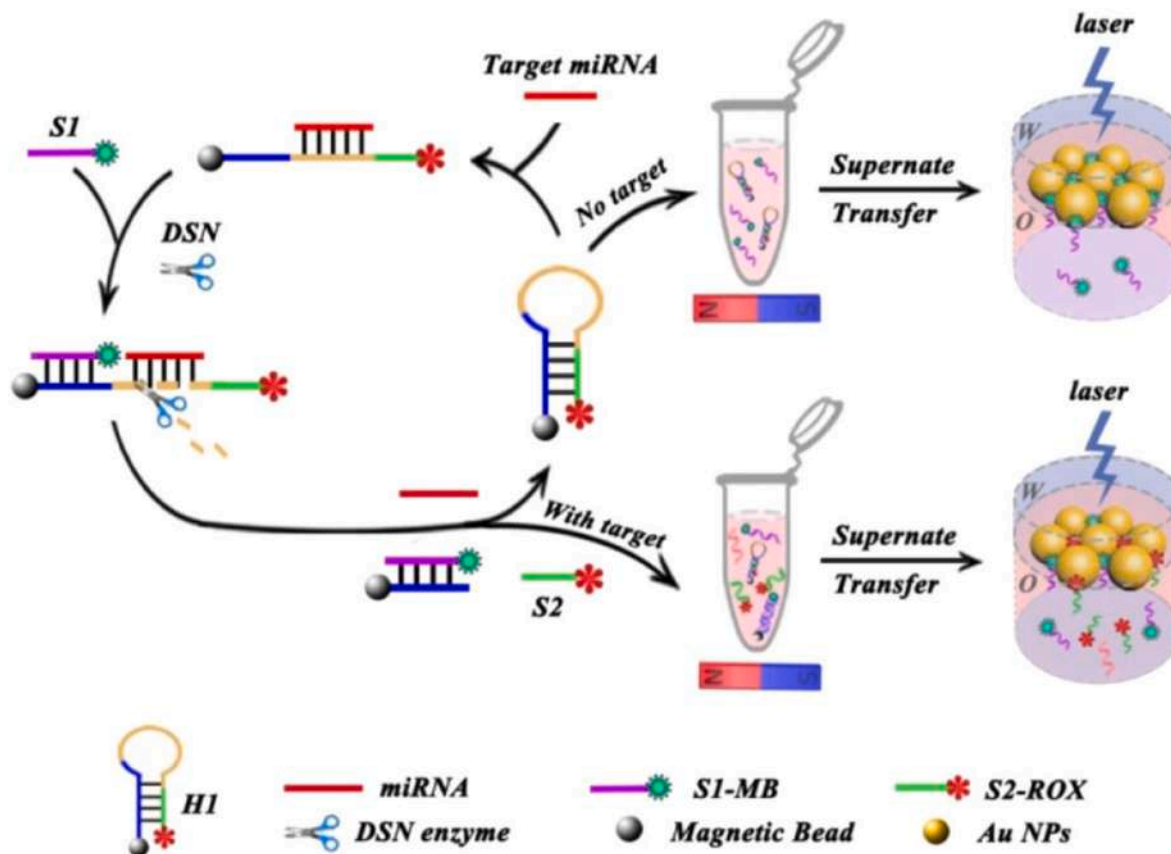


Fig. 8. Magnetic separation for the detection of miRNAs. Reprinted with permission from Luo et al. [165]. Copyright 2020 American Chemical Society.

substrates, which resulted in a LOD of 10^{-17} M. The protocol has not been tested directly in body fluids but has been applied for the measurement of miRNA-10a and miRNA-21 extracted from urinal exosomes of prostate cancer patients.

Another paper that applies the sandwich-based protocol for the exosomal miRNAs detection is presented by Jiang et al. [131]. In this paper, the miRNA expression is measured inside the exosome directly in serum, thus showing more advanced applicability. The AuNPs, labeled and modified with the capture DNAs, are able to penetrate into the exosome, where they hybridize with the opposite ends of the target miRNA, forming a Y-shaped structure, as shown in Fig. 7. The reporter in the hot spot between two conjunct NPs produces the SERS response signaling the presence of the target. The magnetic separation can be used to improve the signal by modifying the exosomes with the magnetic NPs in order to concentrate all exosomes in the measurement spot. The protocol has been implemented for the comparison of the concentration of miRNA-10b in the serum of pancreatic cancer patients and healthy people and reached a LOD of 2.1×10^{-16} M.

Luo et al. have used the enzyme-assisted protocol with the hairpin DNAs for dual signal detection [165]. Fig. 8 illustrates the hairpin DNAs initially labeled by the 6-Carboxyl-X-Rhodamine (ROX) reporter and attached to the magnetic bead. In the absence of the targets, the hairpins stay closed and no further reactions are activated. Addition of the target miRNAs results in their hybridization with the hairpins that further hybridize with other DNAs labeled by the methylene blue (MB) reporter. The miRNA-DNA parts activate the cleavage of the sequences by the DSN so that the miRNAs are released for a new cycle and the strands with ROX are detached from the magnetic beads. When the magnet is applied to the solution, the magnetic beads with their attachments are left in the original tube, while the supernatant is mixed with NPs for further SERS measurement. In the absence of target miRNAs, the ROX reporters remain attached to the beads, but free MB molecules are transferred into

the analyzed sample. On the contrary, in the presence of the targets, most MBs are left in the original solution while the released ROX molecules can enter the analyzed sample. As a result, the ROX's signal signifies the presence of the target miRNAs, while MB's signal is used to quantify the amount of hairpins not activated by the targets. This protocol resulted in a good LOD of 1.1×10^{-18} M for the detection of miRNA-155 artificially diluted in human serum to simulate liquid biopsy application, which is called spiked serum.

Fig. 9 shows the specialized protocol that involves the formation of the closed capsule [177]. As can be seen, there is an enzyme-assisted pre-amplification step that from a single target miRNA produces multiple ssDNAs used later as triggers for further reaction in the protocol. As a substrate, the authors used a leaf with natural pores covered with AgNPs. The capsule is created by attaching two types of small DNA strands to the acrylamide, then adding a toluidine blue (TB) reporter, and then closing the capsule with the capture DNAs from two sides. The closed capsule is placed on the substrate but no signal is detected initially because the reporters are trapped inside the capsule. Then, the trigger DNAs from the pre-amplification step hybridize with the capture DNAs, which causes the opening of the capsule, the release of the reporters on the NPs, and the SERS response. The authors have tested this protocol for the detection of miRNA-155 in spiked serum and achieved a LOD of 0.83×10^{-16} M.

4.2. Detection of DNA point mutation

Fig. 10 presents an illustration from Zhang et al. who used a pre-amplification by the duplex-specific enzyme before the frequency shift-based detection [75]. The hairpin DNA, initially attached to the DSNB reporter on the substrate, hybridizes with the target DNA. The formed dsDNA activates the enzyme which cleaves the hairpin, releases the target for a new cycle, and leaves a small strand on the substrate.

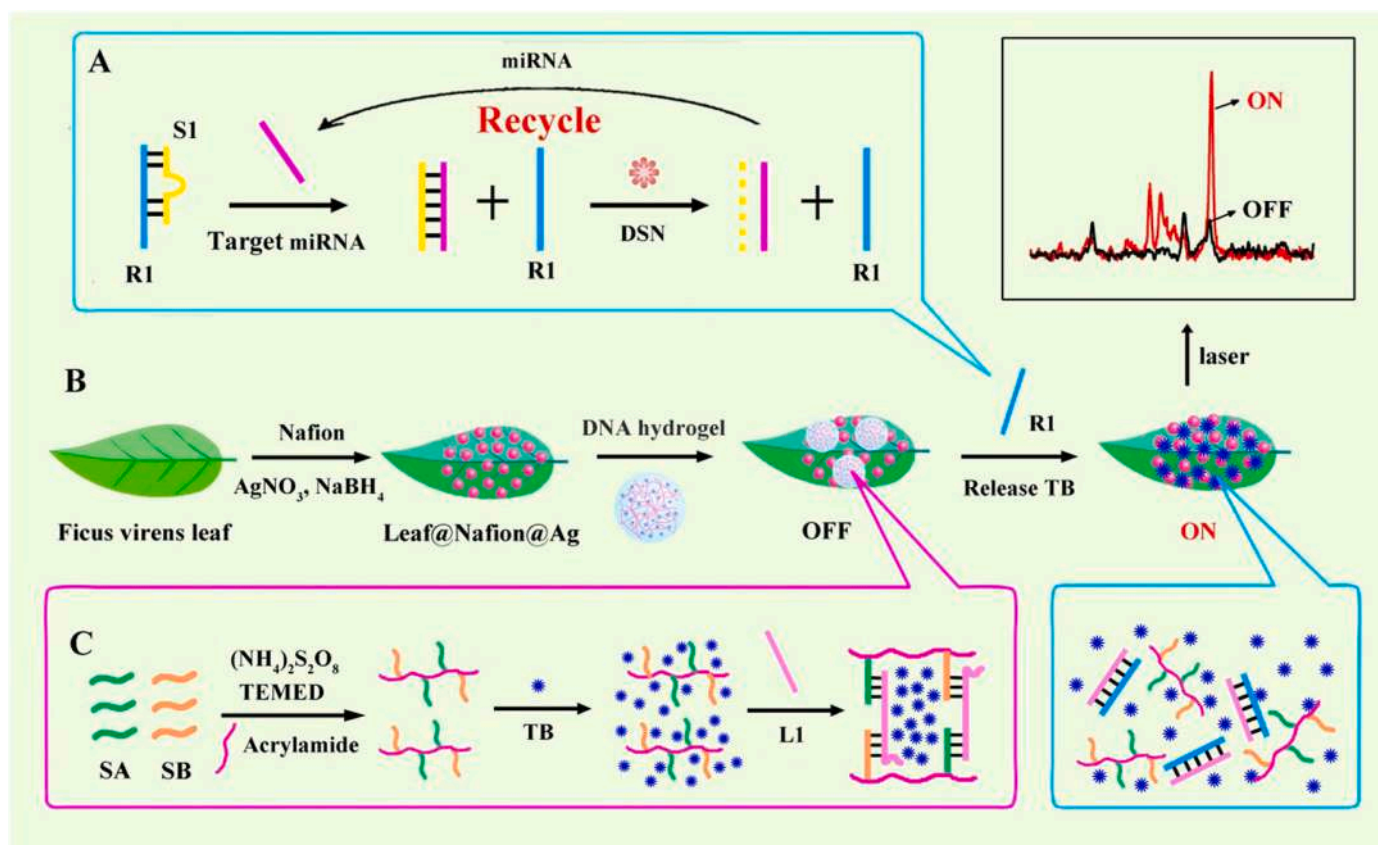


Fig. 9. Sandwich-based protocol for the detection of miRNAs. Reprinted with permission from He et al. [177]. Copyright 2017 American Chemical Society.

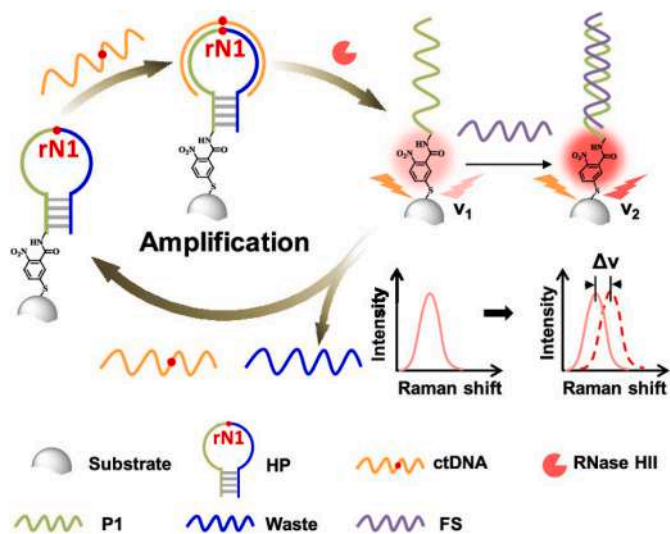


Fig. 10. Frequency shift-based protocol for the detection of DNA point mutation. Reprinted with permission from Zhang et al. [75]. Copyright 2019 American Chemical Society.

Special probe DNAs complementary to the left strand are captured by it, reproducing the frequency shift-based protocol. The spectrum shifted by $0.19\text{--}0.37\text{ cm}^{-1}$ in response to about $10^{-14}\text{--}10^{-12}\text{ M}$ of the target DNA. In the case of no target, the enzyme is not activated, so the hairpin stays inactive, and no change in SERS signal is observed. The protocol resulted in the LOD of $1.2 \times 10^{-16}\text{ M}$ and has been used for the detection of 17 nt KRAS G12D mutation in the serum and plasma of lung cancer patients.

Fig. 11 presents the enzyme-assisted protocol developed by Miao

et al. [171]. The method involves hairpin DNAs labeled by a Cy5 reporter, which hybridize with the target DNAs to activate the Exo III enzyme. After the cleavage, the targets participate in new cycles, while the free labeled strands from the hairpins are caught further by the capture DNAs. Thus, the reporter is brought close to the AuNPs-covered substrate, producing a SERS signal. In the case of no target, the initial hybridization with the hairpin does not occur, so the labeled strand is not attached to the substrate. This protocol reached a LOD of $0.91 \times 10^{-14}\text{ M}$ for the sensing of the single-base-mutated 22 nt long DNA of diffuse intrinsic pontine gliomas in spiked blood.

Huang et al. presented an application of ligation, polymerase, and nicking enzyme for the detection of a DNA point mutation [187]. According to Fig. 12, the protocol originally involves two capture DNAs, one hairpin and one single-stranded. The target and single-mismatched DNAs hybridize with both capture DNAs in such a way that the potential point of mutation is in between the two captures. In the case of a match, ligation occurs and the polymerase can prolong the upper DNA strand. The duplex-specific enzyme is activated to cleave the end of the dsDNA, creating trigger DNAs. These triggers are used in order to capture the labeled DNA probes and attach them to the AuNPs to produce the SERS signal. In the case of mismatched DNA, the point of mutation does not allow the ligation and further creation of the dsDNAs, so the trigger DNAs are not obtained, the labeled DNAs are not captured by the NPs, and no signal is observed. The achieved LOD is $1.4 \times 10^{-12}\text{ M}$ for the detection of the 44 bp long KRAS gene artificially added into the cell lysate.

4.3. Detection of DNA methylation

Fig. 13 illustrates the capture-based method presented by Hu et al. [72]. The methylated and unmethylated target DNAs are exposed to bisulfite pre-treatment to convert unmethylated cytosine to uracil. The

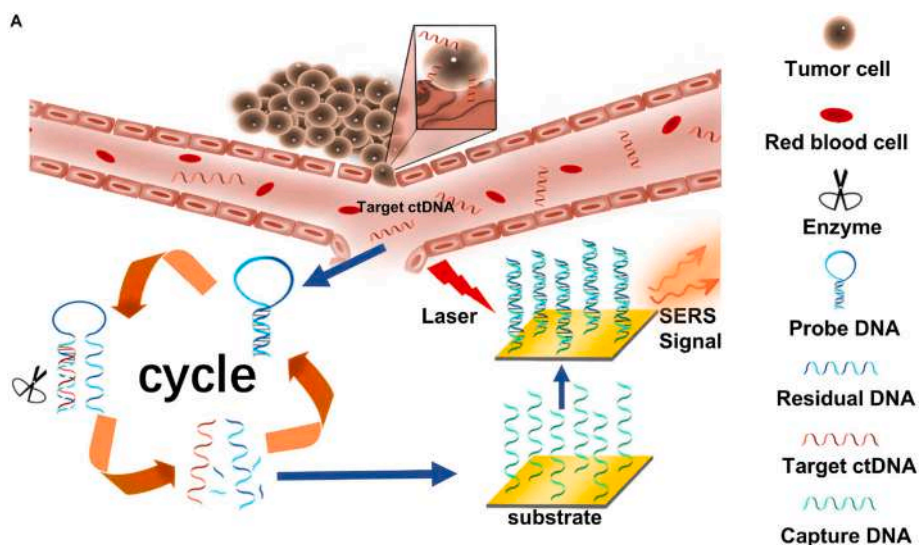


Fig. 11. Enzyme-assisted protocol for the detection of DNA point mutation. Reprinted with permission from Miao et al. [171]. Copyright 2021 Frontiers in Molecular Biosciences.

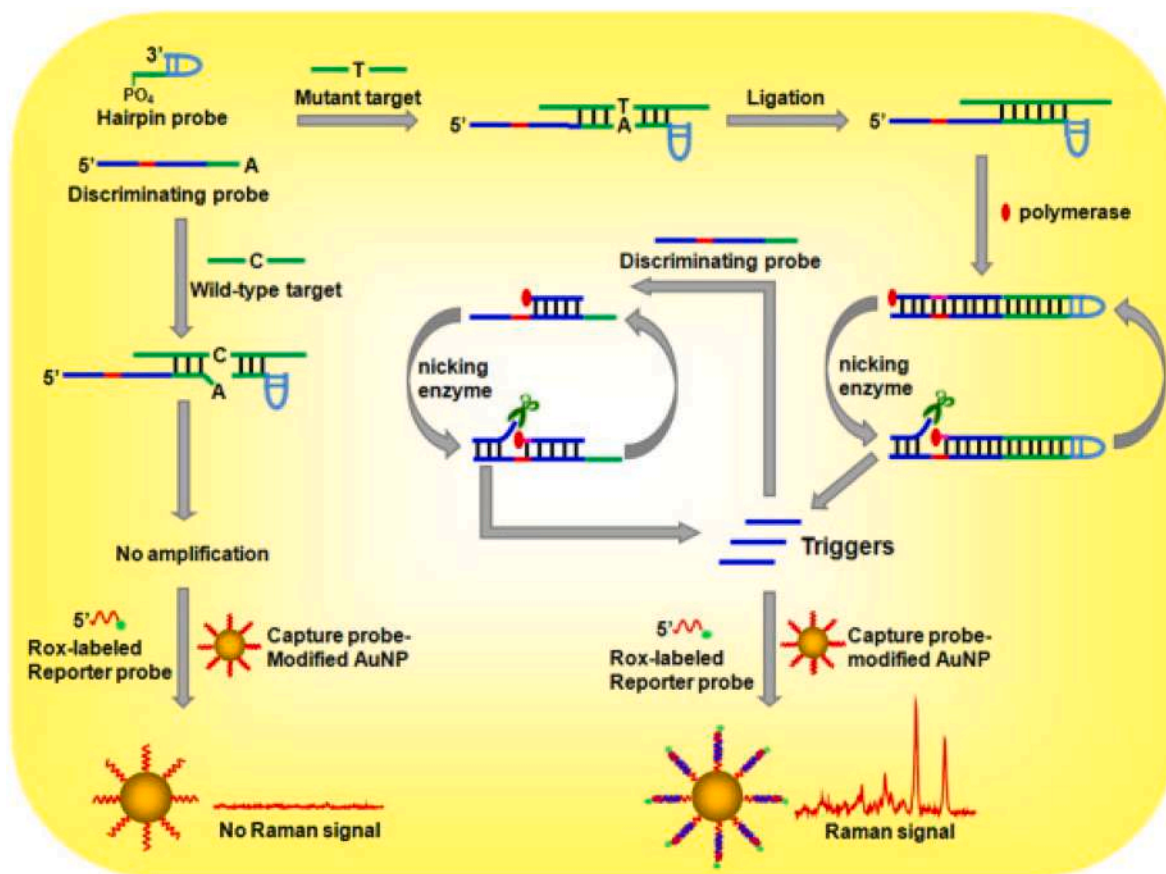


Fig. 12. Polymerase-assisted protocol for the detection of DNA point mutation. Reprinted with permission from Huang et al. [187]. Copyright 2014 Elsevier.

AuNPs are modified with capture DNAs, which can hybridize with both target DNAs. Since the methylated cytosines have not been converted to uracil, they can capture free labeled guanines, producing a SERS signal. The unmethylated DNA is left without the cytosines after the bisulfite treatment, so it can not capture labeled guanines. The protocol showed a LOD of $3 \times 10^{-12}M$ and can distinguish a 1% change in methylation level. The protocol has been tested for DNAs diluted in water.

Fig. 14 illustrates the protocol, which does not require bisulfite treatment because it applies methylation-specific antibodies [188]. AuNPs are modified with antibodies that can capture the target DNAs through their methyl groups. The targets are labeled by the reporters and placed on the AgNPs-based SERS substrate for the SERS measurement. Unmethylated DNAs are not captured and thus detected. The protocol has been tested for the measurement of the methylation level of

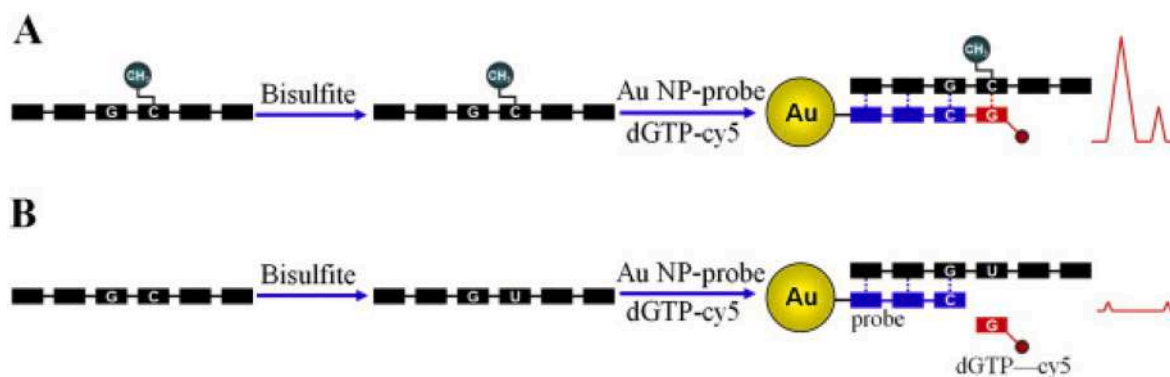


Fig. 13. Capture-based protocol for the detection of DNA point mutation. Reprinted with permission from Hu et al. [72]. Copyright 2011 Elsevier.

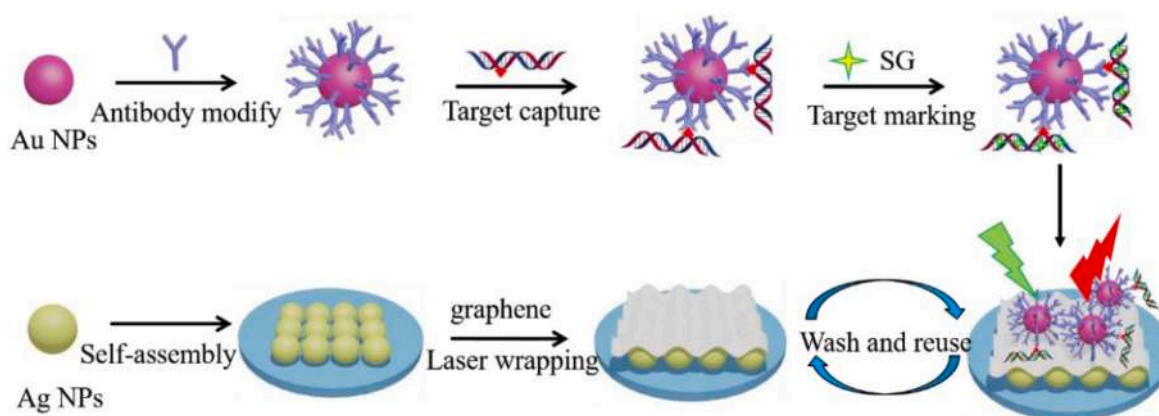


Fig. 14. The specialized protocol for the detection of DNA point mutation. Reprinted with permission from Ouyang et al. [188]. Copyright 2017 Elsevier.

DNAs extracted from cells. It reached a LOD of $1.8 \times 10^{-12}M$ and can distinguish a change in methylation as low as 0.1%.

It can be noticed that highly sensitive miRNA detection can be achieved with some relatively simple assays. For example, high LODs are achieved with one-to-two-step detection with the frequency-shift capture protocol of Cheng et al. [74], the displacement-based method of Kim et al. [97], and the label-free sandwich of Kim et al. [118]. For comparison, similar protocols for the DNA point mutation require either a pre-amplification step (the frequency-shift capture protocol of Zhang et al. [75]) or post-dehybridization by electric potential or wash. Moreover, the highly sensitive detection of miRNA can be reached even with label-free methods if the sensitive and reproducible SERS substrates are used [118]. The protocols developed for miRNA detection have been integrated with the LFA devices [89] and are more often used for the multiplex sensing of several target sequences [89,97] and even of different types of biomarkers [74].

5. Discussion

The review has shown that there is significant research attention being paid to SERS-based methods for the detection of genomic biomarkers, especially for changes in miRNA expression levels. However, to reach a clinical level, the presented protocols should show promising detection ability and applicability in body fluids. Thus, a LOD should be sufficient to detect the physiological concentration of miRNAs (about $10^{-14}M$) and DNAs (about $10^{-10}M$). Moreover, the biological protocols should be successfully tested in the environment as close as possible to real body fluids. Some papers have presented the detection of targets in complex media, such as the urine, serum, or plasma of cancer patients. However, others have tested the protocols in water/buffer solutions, spiked serum, or total RNAs/DNAs extracted from cancer cells. Table 1

summarizes the protocols for detecting all three types of genomic biomarkers and provides the best-performed example for each protocol. The examples are selected according to the two parameters, a LOD and the complexity of the tested matrix. Firstly, works that reached LODs sufficient for the detection of the required target have been filtered, and then among them, the paper that tested the detection in the most complex matrix has been selected.

According to Table 1, the best-performed examples for the majority of the protocols are labeled techniques. A variety of reporters are implemented in the protocols, including the already mentioned MBA, DSNB, MB, ROX, and TB, as well as others: 4-aminobenzenethiol (ABT), 6-Carboxyfluorescein (FAM), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), 3-mercaptophenylboronic acid (MPBA), Cyanine-5 (Cy5), Cyanine-3 (Cy3), 1-tetradecanol (TD), crystal violet (CV), 5-Carboxy-tetramethylrhodamine (TAMRA), texas red (TR), 1,3,3,1',3',3'-hexamethyl-2,2'-indotricarbocyanine iodide (HITC), and 4-mercapto-3-nitro benzoic acid (MNBA). Most labeled methods have achieved sufficient LODs, except the hairpin-assisted displacement method for the detection of miRNAs ($4.6 \times 10^{-12}M$ [108]) and the affinity difference-based capture protocol applied to the DNA point mutation recognition ($0.5 \times 10^{-8}M$ [79]). In terms of applicability, the detection of miRNAs has shown promising potential: all labeled capture and sandwich methods have been applied in real serum, the displacement and cleavage-based protocols have been tested in total RNAs, and only the specialized protocol has been checked in spiked serum. The applicability of DNA point mutation is at a lower level: only three protocols, such as the simple and frequency shift-based capture and displacement, have been tested in body fluids (plasma, serum, and blood), and only the hairpin-assisted capture method has been checked in total RNAs. Labeled protocols for DNA methylation measurement have been only tested in total RNA (sandwich), spiked serum (specialized), and buffer (hairpin-assisted

Table 1

Performance of the SERS-based methods for the detection of miRNAs, DNA point mutation, and DNA methylation level. (LOD - limit of detection).

Protocol	Scheme	Label	Matrix	LOD (M)	Ref.
miRNAs					
Capture	Fig. 2 (a1)	label-free	buffer	4.85×10^{-10}	[61]
	Fig. 2 (a2)	MBA	serum	10^{-17}	[74]
	Fig. 2 (a3)	label-free	total RNA	5×10^{-12}	[78]
	Fig. 2 (a4)	ROX,Cy5, FAM	serum	10^{-18}	[85]
Displacement	Fig. 2 (b1)	Cy3	total RNA	5×10^{-17}	[96]
	Fig. 2 (b2)	Cy5	total RNA	4.6×10^{-12}	[108]
Sandwich	Fig. 2 (c1)	Cy5	serum	2.1×10^{-16}	[131]
	Fig. 2 (c2)	R6G,CV,4-ATP	serum	3×10^{-16}	[140]
	Fig. 2 (c3)	R6G	serum	3×10^{-17}	[158]
Cleavage	Fig. 2 (d1)	MBA	total RNA	3.4×10^{-16}	[167]
	Fig. 2 (d2)	DTNB	total RNA	0.84×10^{-16}	[175]
Specialized	Fig. 2 (e1)	MPBA	spiked serum	0.775×10^{-17}	[178]
DNA point mutation					
Capture	Fig. 2 (a1)	Cy3,TAMRA	plasma	5.15×10^{-11}	[68]
	Fig. 2 (a2)	DSNB	serum	1.2×10^{-16}	[75]
	Fig. 2 (a3)	TR	buffer	0.5×10^{-8}	[79]
	Fig. 2 (a4)	FAM	total DNA	0.5×10^{-13}	[92]
Displacement	Fig. 2 (b1)	Cy5	blood	4×10^{-13}	[100]
Sandwich	Fig. 2 (c1)	label-free	buffer	10^{-6}	[122]
	Fig. 2 (c2)	HITC,TD	buffer	10^{-16}	[142]
Cleavage	Fig. 2 (d1)	DTNB	spiked serum	3×10^{-16}	[172]
Specialized	Fig. 2 (e2)	ABT	buffer	3×10^{-14}	[184]
DNA methylation					
Capture	Fig. 2 (a1)	label-free	water	10^{-10}	[71]
	Fig. 2 (a4)	Cy5	buffer	3×10^{-12}	[72]
Sandwich	Fig. 2 (c1)	MNBA, MBA	total RNA	0.5×10^{-12}	[124]
Specialized	Fig. 2 (e3)	R6G	spiked serum	1.8×10^{-12}	[189]

capture). The applicability of these protocols to DNA detection is further complicated by the length of DNAs. Most of the studied papers tested their protocols for the detection of 22–23 nt long miRNAs and 17–53 nt long DNAs. The length of miRNAs in body fluids is usually within the studied ranges, but circulating DNAs are longer. DNAs with a sufficient length of more than 150 nt have been only tested by the hairpin-assisted capture and displacement protocols [85,97].

Some protocols shown in Table 1, have label-free methods presented as the best-performing examples. It can be seen that LODs of these protocols are not sufficient or are at a bare minimum for the miRNAs/DNAs detection. Thus, the simple and affinity difference-based capture protocols for miRNA detection have LODs of $4.85 \times 10^{-10}M$ [61] and $5 \times 10^{-12}M$ [78], respectively, the sandwich-based assay for the DNA point mutation recognition has a LOD of $10^{-6}M$ [122], and the LOD of the simple capture protocol for DNA methylation measurement is $10^{-10}M$ [71]. Three out of four protocols have only been tested in

buffer/water, and only the affinity difference-based method for the miRNAs detection has been applied in total RNAs. There is also the label-free sandwich protocol for miRNAs detection developed by Kim et al. and mentioned in the previous section [118]. It can be the best-performed example and is listed in Table 1 because of a very good LOD ($10^{-17}M$), but it has been tested only in total RNAs.

Thus, there are some promising label-free protocols, but more work should be conducted to consider them for liquid biopsy application. Currently, the labeled techniques have a better potential for liquid biopsy detection.

6. Conclusion

In conclusion, the literature review has shown that the SERS-based detection of genomic biomarkers has achieved a high level of research attention during the last decade. Currently, labeled protocols mostly outperform label-free methods. Thus, sufficient LODs and applicability in body fluids have been achieved by the labeled capture and sandwich protocols for the detection of miRNAs and labeled capture and displacement protocols for DNA point mutation recognition. However, some papers have presented label-free protocols that achieved LODs sufficient or nearly sufficient for the detection of genomic biomarkers. More work should be performed on further improving LODs and on detection in body fluids.

Among all three genomic biomarkers, SERS-based methods have been found to be particularly compatible with miRNAs due to their shorter length and easier manipulations. The protocols are used more often for the multiplex detection of miRNAs and even simultaneous measurement of miRNA and another type of biomarkers (such as proteins). Moreover, the LOD of the miRNA detection has been improved due to the application of more advanced SERS substrates and LFA devices. In addition, SERS-based methods can be used not only for the detection of free miRNAs in body fluids but also for miRNAs in exosomes, which expands the area of application. In comparison, the detection of DNA point mutation with good LOD and applicability has been achieved with frequency-shift capture- and displacement-based protocols, and only the latter has also been applied to the DNA with a realistic length. Moreover, the most successful protocols for DNA point mutation measurement usually apply pre-amplification or post-washing, which again makes the assay more complex. The SERS-based detection of DNA methylation is the least developed among the three: it is presented in the smallest number of papers, and no protocol has been tested in body fluids yet (although an achieved LOD is promising). Their SERS-based potential is also lower because of the bisulfite pre-treatment, which again makes the protocols more complicated.

Although the attractive direct detection without any biological pre-treatment was found to be not suitable for liquid biopsy application due to its inability to selectively capture the target, SERS-based methods still have some potential advantages over currently applied fluorescence-based detection. First, there is a possibility to increase the sensitivity of the label-free techniques by designing appropriate reproducible and sensitive SERS sensors, especially for miRNA detection. Moreover, even some labeled protocols are based on one-to-two-step detection (capture and displacement) and do not require amplification for miRNA detection, which simplifies the complexity of the assay. In addition to potentially more straightforward assays, the labeled SERS techniques can provide good multiplexing abilities, so the diagnostic accuracy can be improved by relying on multiple biomarkers. Finally, the labeled SERS can be combined with fluorescence-based methods to overcome the limitations of both techniques and reach more accurate detection.

This paper has shown that the potential of SERS for liquid biopsy application is very promising, especially for the detection of miRNA-based biomarkers. Some protocols already achieved sufficient LODs and applicability to be implemented in practice. Further research work in this direction should be conducted toward the development of more

reproducible, sensitive, and applicable SERS sensors and the application of simple protocols for the target capturing. This work can hopefully contribute to attracting the interest of many researchers with different educational backgrounds on this topic and stimulate work in this direction.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgements

The project is a part of the PHAST-ETN project that received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant.

References

- [1] H. Sung, J. Ferlay, R. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *Cancer J. Clin.* 71 (2021) 209–249.
- [2] J. Marrugo-Ramirez, M. Mir, J. Samitier, Blood-based cancer biomarkers in liquid biopsy: a promising non-invasive alternative to tissue biopsy, *Int. J. Mol. Sci.* 19 (2018) 1–21.
- [3] V. Levenson, Biomarkers for early detection of breast cancer: what, when, and where? *Biochim. Biophys. Acta* 1770 (2007) 847–856.
- [4] I. Avital, R. Langan, S.S.T.A. Summers, S. Waldman, V. Backman, J. Yee, A. Nissan, P. Young, C. Womeldorph, P. Mancusco, R. Mueller, K. Noto, W. Grundfest, A.J. Bilchik, M. Protic, M. Daumer, J. Eberhardt, Y. Man, B. Brücher, A. Stojadinovic, Evidence-based guidelines for precision risk stratification-based screening (prsbs) for colorectal cancer: lessons learned from the us armed forces: consensus and future directions, *J. Cancer* 4 (2013) 172–192.
- [5] S. Abalde-Cela, L. Wu, A. Teixeira, K. Oliveira, L. Diéguez, Multiplexing liquid biopsy with surface-enhanced Raman scattering spectroscopy, *Adv. Opt. Mater.* 9 (2021) 1–21.
- [6] B. Sadikovic, K. Al-Romaih, J. Squire, M. Zielenska, Cause and consequences of genetic and epigenetic alterations in human cancer, *Curr. Genom.* 9 (2008) 394–408.
- [7] G. Novelli, C. Ciccacci, P. Borgiani, M. Amati, E. Abadie, Genetic tests and genomic biomarkers: regulation, qualification and validation, *Clinical Cases Miner. Bone Metabol.* 5 (2008) 149–154.
- [8] X. Zhang, S. Shi, B. Zhang, Q. Ni, X. Yu, J. Xu, Circulating biomarkers for early diagnosis of pancreatic cancer: facts and hopes, *Am. J. Cancer Res.* 8 (2018) 332–353.
- [9] Y. Chu, Y. Gao, W. Tang, L. Qiang, Y. Han, J. Gao, Y. Zhang, H. Liu, L. Han, Attomolar-level ultrasensitive and multiplexed microRNA detection enabled by a nanomaterial locally assembled microfluidic biochip for cancer diagnosis, *Anal. Chem.* 93 (2021) 5129–5136.
- [10] Y. Wang, S. Kong, X. Su, A centrifugation-assisted visual detection of snp in circulating tumor dna using gold nanoparticles coupled with isothermal amplification, *RSC Adv.* 10 (2020) 1476–1483.
- [11] J.C.S.S. Kim, J.W. Eun, H. Woo, H. Cho, H. Ahn, C. Suh, G. Baek, S. Cho, J. Cheong, Mlh1 single-nucleotide variant in circulating tumor dna predicts overall survival of patients with hepatocellular carcinoma, *Sci. Rep.* 10 (2020) 17862–17870.
- [12] A. Hussein, T. Forouzanfar, E. Bloemena, J. Visscher, R. Brakenhoff, C. Leemans, M. Helder, A review of the most promising biomarkers for early diagnosis and prognosis prediction of tongue squamous cell carcinoma, *Br. J. Cancer* 119 (2018) 724–736.
- [13] A. Alzahrani, A. Murugan, E. Qasem, M. Alswailem, H. Al-Hindi, Y. Shi, Single point mutations in pediatric differentiated thyroid cancer, *Thyroid* 27 (2017) 189–198.
- [14] R. Daum, E. Brauchle, D. Berrio, T. Jurkowski, K. Schenke-Layland, Non-invasive detection of dna methylation states in carcinoma and pluripotent stem cells using Raman microspectroscopy and imaging, *Sci. Rep.* 9 (2019) 1–13.
- [15] W. Locke, D. Guanzone, C. Ma, Y. Liew, K. Duesing, K. Fung, J. Ross, Dna methylation cancer biomarkers: translation to the clinic, *Front. Genet.* 10 (2019) 1–22.
- [16] T. Mikeska, J. Craig, Dna methylation biomarkers: cancer and beyond, *Genes* 5 (2014) 821–864.
- [17] J. Ye, M. Xu, X. Tian, S. Cai, S. Zeng, Research advances in the detection of mirna, *J. Pharm. Anal.* 9 (2019) 217–226.
- [18] C. Delaney, S. Garg, R. Yung, Analysis of dna methylation by pyrosequencing, *Methods Mol. Biol.* 1343 (2015) 249–264.
- [19] J. Plou, P. Valera, I. Garcia, C. de Albuquerque, A. Carracedo, L. Liz-Marzan, Prospects of surface-enhanced Raman spectroscopy for biomarker monitoring toward precision medicine, *ACS Photonics* 9 (2022) 333–350.
- [20] J. Langer, D. de Aberasturi, J. Aizpurua, R. Alvarez-Puebla, B. Auguie, J. Baumberg, G. Bazan, S. Bell, A. Boisen, A. Brolo, J. Choo, D. Cialla-May, V. Deckert, L. Fabris, K. Faulds, F. de Abajo, R. Goodacre, D. Graham, A. Haes, C. Haynes, C. Huck, T. Iton, M. Kall, J. Kneipp, N. Kotov, H. Kuang, E.L. Ru, H. Lee, J. Li, X. Ling, S. Maier, T. Mayerhofer, M. Moskovits, K. Murakoshi, J. Nam, S. Nie, Y. Ozaki, I. Pastoriza-Santos, J. Perez-Juste, J. Popp, A. Pucci, S. Reich, B. Ren, G. Schatz, T. Shegai, S. Schlucker, L. Tay, K. Thomas, Z. Tian, R. V. Duyn, T. Vo-Dinh, Y. Wang, K. Willets, C. Xu, H. Xu, Y. Xu, Y. Yamamoto, B. Zhao, L. Liz-Marzan, Present and future of surface-enhanced Raman scattering, *ACS Nano* 14 (2020) 28–117.
- [21] M. Gaidi, K. Daoudi, A. Tlili, S. Columbus, J. Leblanc-Lavoie, K. Ramachandran, B. Suleiman, A. Alhazaa, M.E. Khakani, Fast, highly sensitive and label free detection of small genetic sequence difference of dna using novel surface-enhanced Raman spectroscopy nanostructured sensor, *Sensing and Bio-Sensing Res.* 32 (2021) 1–10.
- [22] J. Wang, K. Koo, Y. Wang, M. Trau, Engineering state-of-the-art plasmonic nanomaterials for sers-based clinical liquid biopsy applications, *Adv. Sci.* 6 (2019) 1–35.
- [23] A. Perez-Jimenez, D. Lyu, Z. Lu, G. Liu, B. Ren, Surface-enhanced Raman spectroscopy: benefits, trade-offs and future developments, *Chem. Sci.* 11 (2020) 4563–4577.
- [24] G. Auner, S. Koya, C. Huang, B. Broadbent, M. Trexler, Z. Auner, A. Elias, K. Mehne, M. Brusatori, Applications of Raman spectroscopy in cancer diagnosis, *Cancer Metastasis Rev.* 37 (2018) 691–717.
- [25] C. Haynes, A. McFarland, R.V. Duyn, New substrates and single-molecule detection are just two of the advances that are fueling interest in sers, *Anal. Chem.* (2005) 338–346.
- [26] M. Jahn, S. Patze, I. Hidi, R. Knipper, A. Radu, A. Muhligh, S. Yuksel, V. Peksa, K. Weber, T. Mayerhofer, D. Cialla-May, J. Popp, Plasmonic nanostructures for surface enhanced spectroscopic methods, *Analyst* 141 (2012) 756–793.
- [27] E. Ru, P. Etchegoin, Principles of Surface-Enhanced Raman Spectroscopy: and Related Plasmonic Effects, Elsevier, 2008.
- [28] C. Yuan, J. Fang, M. Chapelle, Y. Zhang, X. Zeng, G. Huang, X. Yang, W. Fu, Surface-enhanced Raman scattering inspired by programmable nucleic acid isothermal amplification technology, *Trends Anal. Chem.* 143 (2021) 1–15.
- [29] P. Mosier-Boss, Review of sers substrates for chemical sensing, *Nanomaterials* 7 (2017) 1–30.
- [30] T. Vo-Dinh, K. Houck, D. Stokes, Surface-enhanced Raman gene probes, *Anal. Chem.* 66 (1994) 3379–3383.
- [31] D. Cialla, S. Pollock, C. Steinbrücker, K. Weber, J. Popp, Sers-based detection of biomolecules, *Nanophotonics* 3 (2014) 383–411.
- [32] S. Bell, N. Sirimuthu, Surface-enhanced Raman spectroscopy (sers) for sub-micromolar detection of dna/rna mononucleotides, *J. Am. Chem. Soc.* 128 (2006) 15580–15581.
- [33] K. Kneipp, H. Kneipp, V. Kartha, G.D.R. Manoharan, I. Itzkan, R. Dasari, M. Feld, Detection and identification of a single dna base molecule using surface-enhanced Raman scattering (sers), *Phys. Rev.* 57 (1998) 6281–6284.
- [34] E. Prado, N. Daugey, S. Plumet, L. Servant, S. Lecomte, Quantitative label-free rna detection using surface-enhanced Raman spectroscopy, *Chem. Commun.* 47 (2011) 7426–7427.
- [35] M. Muniz-Miranda, C. Gellini, M. Pagliai, M. Innocenti, P. Salvi, V. Schettino, Sers and computational studies on microrna chains adsorbed on silver surfaces, *J. Phys. Chem. C* 114 (2010) 13730–13735.
- [36] D. Li, L. Xia, Q. Zhou, L. Wang, D. Chen, X. Gao, Y. Li, Label-free detection of mirna using surface-enhanced Raman spectroscopy, *Anal. Chem.* 92 (2020) 12769–12773.
- [37] J. Driskell, A. Seto, L. Jones, S. Jokela, R. Dluhy, Y. Zhao, R. Tripp, Rapid microRNA (mirna) detection and classification via surface-enhanced Raman spectroscopy (sers), *Biosens. Bioelectron.* 24 (2008) 917–922.
- [38] X. Cao, M. Bao, Y. Shan, W. Li, H. Shi, Rapid detection and identification of mirnas by surface-enhanced Raman spectroscopy using hollow au nanoflowers substrates, *J. Nanomater.* (2017) 1–12, 2017.
- [39] J. Cui, G. Guan, H. Lv, K. Fu, R. Fu, Z. Feng, F. Chen, G. Zhang, Three-dimensional nanorod array for label-free surface-enhanced Raman spectroscopy analysis of microRNA pneumoconiosis biomarkers, *Spectrochim. Acta Mol. Biomol. Spectrosc.* 261 (2021) 1–7.
- [40] J. Morla-Folch, H. Xie, R. Alvarez-Puebla, L. Guerrini, Fast optical chemical and structural classification of rna, *ACS Nano* 10 (2016) 2834–2842.
- [41] E. Papadopoulos, S. Bell, Label-free detection of single-base mismatches in dna by surface-enhanced Raman spectroscopy, *Angew. Chem. Int. Ed.* 50 (2011) 9058–9061.
- [42] L. Xu, Z. Lei, J. Li, C. Zong, C.J. Yang, B. Ren, Label-free surface-enhanced Raman spectroscopy detection of dna with single-base sensitivity, *J. Am. Chem. Soc.* 137 (2015) 5149–5154.
- [43] Y. Li, T. Gao, G. Xu, X. Xiang, B. Zhao, X. Han, X. Guo, Direct approach toward label-free dna detection by surface-enhanced Raman spectroscopy: discrimination of a single-base mutation in 50 base-paired double helices, *Anal. Chem.* 91 (2019) 7980–7984.
- [44] J. Zeng, M. Dong, B. Zhu, D. Chen, Y. Li, A new method towards the detection of dna mutation by surface-enhanced Raman spectroscopy, *Talanta* 223 (2021) 121746–121753.

- [45] J. Zeng, M. Dong, B. Zhu, X. Gao, D. Chen, Y. Li, Label-free detection of ct mutations by surface-enhanced Raman spectroscopy using thiosulfate-modified nanoparticles, *Anal. Chem.* 93 (2021) 1951–1956.
- [46] J. Morla-Folch, P. Gisbert-Quilis, M. Masetti, E. Garcia-Rico, R. Alvarez-Puebla, L. Guerrini, Conformational sers classification of k-ras point mutations for cancer diagnostics, *Angew. Chem. Int. Ed.* 129 (2017) 2421–2425.
- [47] D. Lin, Q. Wu, S. Qiu, G. Chen, S. Feng, R. Chen, H. Zeng, Label-free liquid biopsy based on blood circulating dna detection using sers-based nanotechnology for nasopharyngeal cancer screening, *Nanomed. Nanotechnol. Biol. Med.* 22 (2019) 102100–102109.
- [48] J. Morla-Folch, R. Alvarez-Puebla, L. Guerrini, Direct quantification of dna base composition by surface enhanced Raman scattering spectroscopy, *J. Phys. Chem. Lett.* 7 (2016) 3037–3041.
- [49] A. Hobro, S. Abdali, E. Blanch, Sers study of methylated and nonmethylated ribonucleosides and the effect of aggregating agents, *J. Raman Spectrosc.* 43 (2012) 187–195.
- [50] A. Barhoumi, N. Halas, Detecting chemically modified dna bases using surface-enhanced Raman spectroscopy, *J. Phys. Chem. Lett.* 2 (2011) 3118–3123.
- [51] L. Guerrini, Z.K.D. Lierop, R. Alvarez-Puebla, D. Graham, Direct surface-enhanced Raman scattering analysis of dna duplexes, *Angew. Chem. Int. Ed.* 53 (2014) 1–6.
- [52] S. Ganesh, K.V. annd, B. Tan, Quantum scale organic semiconductors for sers detection of dna methylation and gene expression, *Nat. Commun.* 11 (2020) 1135–1150.
- [53] S. Hasan, Y. He, T. Chang, J. Wang, M. Gartia, Detecting dna methylation using surface-enhanced Raman spectroscopy, *J. Phys. Chem.* 123 (2019) 698–709.
- [54] X. Luo, L. Jiang, T. Kang, Y. Xing, E. Zheng, P. Wu, C. Cai, Q. Yu, Label-free Raman observation of tet1 protein-mediated epigenetic alterations in dna, *Anal. Chem.* 91 (2019) 7304–7312.
- [55] X. Luo, Y. Xing, D. Galvan, E. Zheng, P. Wu, C. Cai, Q. Yu, Plasmonic gold nanohole array for surface-enhanced Raman scattering detection of dna methylation, *ACS Sens.* 4 (2019) 1534–1542.
- [56] V. Moisoiu, V. Sas, A. Stefanu, S. Iancu, A. Jurj, S. Pasca, S. Iluta, A. Zimta, A. Tigu, P. Teodorescu, C. Turcas, C. Blag, D. Dima, G. Popa, S. Arghirescu, S. Man, A. Colita, N. Leopold, C. Tomuleasa, Sers-based evaluation of the dna methylation pattern associated with progression in clonal leukemogenesis of down syndrome, *Front. Bioeng. Biotechnol.* 9 (2021) 703268–703275.
- [57] J. Kelly, G. Najand, F. Martin, Characterisation of dna methylation status using spectroscopy (mid-ir versus Raman) with multivariate analysis, *J. Biophot.* 4 (2011) 345–354.
- [58] V. Moisoiu, A. Stefanu, S. Iancu, T. Moisoiu, L. Loga, L. Dican, C. Alecsa, I. Boros, A. Jurj, D. Dima, C. Bagacean, R. Tetea, E. Burzo, C. Tomuleasa, F. Elec, N. Leopold, Sers assessment of the cancer-specific methylation pattern of genomic dna: towards the detection of acute myeloid leukemia in patients undergoing hematopoietic stem cell transplantation, *Anal. Bioanal. Chem.* 411 (2019) 7907–7913.
- [59] A. Stefanu, V. Moisoiu, M. Desmirean, S. Iancu, A. Tigu, B. Petrushev, A. Jurj, R. Cozan, L. Budisan, B. Fetica, A. Roman, G. Dobie, C. Turcas, M. Zdrenghea, P. Teodorescu, S. Pasca, D. Picio, D. Dima, Z. Balint, N. Leopold, C. Tomuleasa, Sers-based dna methylation profiling allows the differential diagnosis of malignant lymphadenopathy, *Spectrochim. Acta Mol. Biomol. Spectrosc.* 264 (2022) 120216–120223.
- [60] X. Li, T. Yang, C. Li, Y. Song, D. Wang, L. Jin, H. Lou, W. Li, Polymerase chain reaction – surface-enhanced Raman spectroscopy (pcr-sers) method for gene methylation level detection in plasma, *Theranostics* 10 (2020) 898–909.
- [61] A. Chiado, C. Novara, A. Lamberti, F. Geobaldo, F. Giorgis, P. Rivolo, Immobilization of oligonucleotides on metal-dielectric nanostructures for mirna detection, *Anal. Chem.* 88 (2016) 9554–9563.
- [62] J. Abell, J. Garren, J. Driskell, R. Tripp, Y. Zhao, Label-free detection of micro-rna hybridization using surface enhanced Raman spectroscopy and least-squares analysis, *J. Am. Chem. Soc.* 134 (2012) 12889–12892.
- [63] K. Koo, J. Wang, R. Richards, A. Farrell, J. Yaxley, H. Samarantunga, P. Teloken, M. Roberts, G. Coughlin, M. Lavin, P. Mainwaring, Y. Wang, R. Gardiner, M. Trau, Design and clinical verification of surface-enhanced Raman spectroscopy diagnostic technology for individual cancer risk prediction, *ACS Nano* 12 (2018) 8362–8371.
- [64] J. Wang, K. Koo, E. Wee, Y. Wang, M. Trau, A nanoplasmonic label-free surface-enhanced Raman scattering strategy for non-invasive cancer genetic subtyping in patient samples, *Nanoscale* 9 (2017) 3496–3503.
- [65] A. Kamińska, A. Sivanesan, E. Witkowska, J. Golab, M. Winiarska, D. Nowis, I.D. J. Weyher, J. Waluk, Detection of dna mutations using novel sers (surface-enhanced Raman spectroscopy) diagnostic platform, *J. Chem. Chem. Eng.* 7 (2013) 972–978.
- [66] T. Abdallah, H. Awad, H. Talaat, Sers detection of dna cancer with single-base mutation for kras-gene, *Egypt. J. Solid.* 43 (2021) 144–157.
- [67] Y. Kayran, N. Cinar, D. Jambrec, W. Schuhmann, Monitoring potential-induced dna dehybridization kinetics for single nucleotide polymorphism detection by using in situ surface enhanced Raman scattering, *Chemelectrochem* 5 (2018) 756–760.
- [68] X. Li, T. Yang, C. Li, Y. Song, H. Lou, D. Guan, L. Jin, Surface enhanced Raman spectroscopy (sers) for the multiplex detection of braf, kras, and pik3ca mutations in plasma of colorectal cancer patients, *Theranostics* 8 (2018) 1678–1689.
- [69] S. Mahajan, J. Richardson, T. Brown, P. Bartlett, Sers-melting: a new method for discriminating mutations in dna sequences, *J. Am. Chem. Soc.* 130 (2008) 15589–15601.
- [70] S. Mahajan, J. Richardson, N. Gaied, Z. Zhao, T. Brown, P. Bartlett, The use of an electroactive marker as a sers label in an e-melting mutation discrimination assay, *Electroanalysis* 21 (2009) 2190–2197.
- [71] J. Kim, H. Park, J. Kim, B. Chang, H. Park, Label-free detection for a dna methylation assay using Raman spectroscopy, *Chinese Med J* 130 (2017) 1961–1967.
- [72] J. Hu, C. Zhang, Single base extension reaction-based surface enhanced Raman spectroscopy for dna methylation assay, *Biosens. Bioelectron.* 31 (2012) 451–457.
- [73] W. Zhu, L. Cheng, M. Li, D. Zuo, N. Zhang, H. Zhuang, D. Xie, Q. Zeng, J. Hutchison, Y. Zhao, Frequency shift Raman-based sensing of serum micrnas for early diagnosis and discrimination of primary liver cancers, *Anal. Chem.* 90 (2018) 10144–10151.
- [74] L. Cheng, Z. Zhang, D. Zuo, W. Zhu, J. Zhang, Q. Zeng, D. Yang, M. Li, Y. Zhao, Ultrasensitive detection of serum microrna using branched dna-based sers platform combining simultaneous detection of alpha-fetoprotein for early diagnosis of liver cancer, *ACS Appl. Mater. Interfaces* 10 (2018) 34869–34877.
- [75] J. Zhang, Y. Dong, W. Zhu, D. Xie, Y. Zhao, D. Yang, M. Li, Ultrasensitive detection of circulating tumor dna of lung cancer via an enzymatically amplified sers-based frequency shift assay, *ACS Appl. Mater. Interfaces* 11 (2019) 18145–18152.
- [76] J. Driskell, R. Tripp, Label-free sers detection of microrna based on affinity for an unmodified silver nanorod array substrate, *Chem. Commun. J.* 46 (2010) 3298–3300.
- [77] Y. Song, T. Xu, L. Xu, X. Zhang, Nanodendritic gold/graphene-based biosensor for tri-mode mirna sensing, *Chem. Commun. J.* 55 (2019) 1742–1745.
- [78] J. Zheng, J. Bai, Q. Zhou, J. Li, Y. Li, J. Yang, R. Yang, Dna-templated in situ growth of agnps on swnts: a new approach for highly sensitive sers assay of microrna, *Chem. Commun. J.* 51 (2015) 6552–6555.
- [79] E. Ganbold, T. Kang, K. Lee, S. Lee, S. Joo, Aggregation effects of gold nanoparticles for single-base mismatch detection in influenza a (h1n1) dna sequences using fluorescence and Raman measurements, *Colloids Surf. B Biointerfaces* 93 (2012) 148–153.
- [80] J. Xia, Y. Liu, M. Ran, D. Lu, X. Cao, Y. Wang, Sers platform based on bimetallic au-ag nanowires-decorated filter paper for rapid detection of mir-196a in lung cancer patients serum, *J. Chem.* 2020 (2020) 1–14.
- [81] C. Song, Y. Yang, B. Yang, Y. Sun, Y. Zhao, L. Wang, Ultrasensitive sers sensor for simultaneous detection of multiple cancer-related mirnas, *Nanoscale* (2016) 1–9, 2016.
- [82] Z. Wang, S. Zong, Z. Wang, L. Wu, P. Chen, B. Yun, Y. Cui, Microfluidic chip based mirna detection through the combination of fluorescence and surface enhanced Raman scattering techniques, *Nanotechnology* 28 (2017) 1–8.
- [83] T. Kang, J. Zhu, X. Luo, W. Jia, P. Wu, C. Cai, Controlled self-assembly of a close-packed gold octahedra array for sers sensing exosomal micrnas, *Anal. Chem.* 93 (2021) 2519–2526.
- [84] L. Liu, C. Shangguan, J. Guo, K. Ma, S. Jiao, Y. Yao, J. Wang, Ultrasensitive sers detection of cancer-related mirna-182 by mxene/mos2@aunps with controllable morphology and optimized self-internal standards, *Adv. Opt. Mater.* 8 (2020) 2001214–2001224.
- [85] S. Meng, R. Chen, J. Xie, J. Li, J. Cheng, Y. Xu, H. Cao, X. Wu, Q. Zhang, H. Wang, Surface-enhanced Raman scattering holography chip for rapid, sensitive and multiplexed detection of human breast cancer-associated micrnas in clinical samples, *Biosens. Bioelectron.* 190 (2021) 113470–113480.
- [86] J. Chen, Y. Wu, C. Fu, H. Cao, X. Tan, W. Shi, Z. Wu, Ratiometric sers biosensor for sensitive and reproducible detection of microrna based on mismatched catalytic hairpin assembly, *Biosens. Bioelectron.* 144 (2019) 11619–11627.
- [87] J. Wi, C. Chung, H. Na, T. Oh, Easy-to-make-and-use gold nanotrench arrays for surface-enhanced Raman scattering, *Opt. Mater. Express* 11 (2021) 3363–3369.
- [88] X. Cao, Y. Sun, Y. Mao, M. Ran, Y. Liu, D. Lu, C. Bi, Rapid and sensitive detection of dual lung cancer-associated mirna biomarkers by a novel sers-lfa strip coupling with catalytic hairpin assembly signal amplification, *J. Mater. Chem. C* 9 (2021) 3661–3671.
- [89] Y. Mao, Y. Sun, J. Xue, W. Lu, X. Cao, Ultra-sensitive and high efficiency detection of multiple non-small cell lung cancer-related mirnas on a single test line in catalytic hairpin assembly-based sers-lfa strip, *Anal. Chim. Acta* 1178 (2021) 338800–338814.
- [90] H. Zhang, C. Fu, S. Wu, Y. Shen, C. Zhou, J. Neng, Y. Yi, Y. Jin, Y. Zhu, Magnetic-capture-based sers detection of multiple serum microrna biomarkers for cancer diagnosis, *Anal. Methods* 11 (2019) 783–793.
- [91] M. Wabuyele, F. Yan, T. Vo-Dinh, Plasmonics nanoprobes: detection of single-nucleotide polymorphisms in the breast cancer brca1 gene, *Anal. Bioanal. Chem.* 398 (2010) 729–736.
- [92] L. Wu, A. Garrido-Maestu, J. Guerreiro, S. Carvalho, S. Abalde-Cela, M. Prado, L. Diéguez, Amplification-free sers analysis of dna mutation in cancer cells with single-base sensitivity, *Nanoscale* 11 (2019) 7781–7789.
- [93] S. Restaino, I. White, Inkjet-printed paper surface enhanced Raman spectroscopy (sers) sensors, in: *IEEE Proceeding*, 2016, pp. 1–3.
- [94] S. Restaino, I. White, Surface enhanced Raman spectroscopy (sers) methods for endpoint and real-time quantification of mirna assays, *Optics and Biophotonics in Low-Resource Settings III* 10055 (2017) 1005502–1005511.
- [95] T. Lee, S. Kwon, H. Choi, H. Lim, J. Lee, Highly sensitive and reliable microrna detection with a recyclable microfluidic device and an easily assembled sers substrate, *ACS Omega* 6 (2021) 19656–19664.
- [96] T. Lee, J. Wi, A. Oh, H. Na, J. Lee, K. Lee, T. Lee, S. Haam, Highly robust, uniform and ultra-sensitive surface-enhanced Raman scattering substrates for microrna detection fabricated by using silver nanostructures grown in gold nanobowls, *Nanoscale* (2013) 1–9, 2017.

- [97] J. Kim, J. Park, J. Ki, H. Rho, Y. Huh, E. Kim, H. Son, S. Haam, Simultaneous dual-targeted monitoring of breast cancer circulating miRNA via surface-enhanced Raman spectroscopy, *Biosens. Bioelectron.* 207 (2022) 114143–114152.
- [98] H. Zhou, J. Zhang, B. Li, J. Liu, J. Xu, H. Chen, Dual-mode SERS and electrochemical detection of miRNA based on popcorn-like gold nanofilms and toehold-mediated strand displacement amplification reaction, *Anal. Chem.* 93 (2021) 6120–6127.
- [99] T. Lee, M. Mohammadniaei, H. Zhang, J. Yoon, H. Choi, S. Guo, P. Guo, J. Choi, Single functionalized prna/gold nanoparticle for ultrasensitive miRNA detection using electrochemical surface-enhanced Raman spectroscopy, *Adv. Sci.* 7 (2020) 1902477–1902488.
- [100] S. Kim, S. Jeong, S. Bae, H. Chung, S. Yoo, Sensitive surface enhanced Raman scattering-based detection of a high3 point mutation associated with avellino corneal dystrophy, *Anal. Chem.* 88 (2016) 11288–11292.
- [101] T. Kang, H. Kim, J. Lee, H. Lee, Y. Choi, G. Kang, M. Seo, B. Chung, Y. Jung, B. Kim, Ultra-specific zzeptomole miRNA detection by plasmonic nanowire interstice sensor with bi-temperature hybridization, *Small Biomarkers* 10 (2014) 4200–4206.
- [102] C. Novara, A. Chiado, N. Paccotti, S. Catuogno, C. Esposito, G. Condorelli, V. Franciscis, F. Geobaldo, P. Rivolo, F. Giorgis, SERS-active metal-dielectric nanostructures integrated in microfluidic devices for label-free quantitative detection of miRNA, *The Royal Soc. Chem.* 205 (2017) 271–289.
- [103] Y. Han, L. Qiang, Y. Gao, J. Gao, Q. He, H. Liu, L. Han, Y. Zhang, Large-area surface-enhanced Raman spectroscopy substrate by hybrid porous gan with au/ag for breast cancer miRNA detection, *Appl. Surf. Sci.* 541 (2021) 148456–148465.
- [104] S. Mabbott, S. Fernandes, M. Schechinger, G. Cote, K. Faulds, C. Mace, D. Graham, Detection of cardiovascular disease associated mir-29a using paper-based microfluidics and surface enhanced Raman scattering, *Analyst* 145 (2020) 983–991.
- [105] L. Ye, J. Hu, L. Liang, C. Zhang, Surface-enhanced Raman spectroscopy for simultaneous sensitive detection of multiple miRNAs in lung cancer cells, *Chem. Commun. J.* 50 (2014) 11883–11886.
- [106] Y. Song, T. Xu, L. Xu, X. Zhang, Superwetttable nanodendritic gold substrates for direct miRNA SERS detection, *Nanoscale* 10 (2018) 20990–20994.
- [107] B. Crawford, H. Wang, A. Fales, M. Bowie, V. Seewaldt, T. Vo-Dinh, SERS-based inverse molecular sentinel (ims) nanopores for multiplexed detection of miRNA cancer biomarkers in biological samples, *Plasmonics Biol. Med.* XIV 10080 (2018) 1008008–1008020.
- [108] B. Crawford, H. Wang, C. Stolarchuk, R. Furstenberg, P. Strobbia, D. Zhang, X. Qin, K. Owzar, K. Garman, T. Vo-Dinh, Plasmonic nanobiosensors for detection of miRNA cancer biomarkers in clinical samples, *Analyst* 145 (2020) 4587–4594.
- [109] H. Wang, B. Crawford, A. Fales, M. Bowie, V. Seewaldt, T. Vo-Dinh, Multiplexed detection of miRNA biomarkers using SERS-based inverse molecular sentinel (ims) nanopores, *J. Phys. Chem.* 120 (2016) 21047–21055.
- [110] Y. He, X. Yang, R. Yuan, Y. Chai, off to “on” surface-enhanced Raman spectroscopy platform with padlock probe-based exponential rolling circle amplification for ultrasensitive detection of miRNA 155, *Anal. Chem.* 89 (2017) 2866–2872.
- [111] Y. Wu, Y. He, X. Yang, R. Yuan, Y. Chai, A novel recyclable surface-enhanced Raman spectroscopy platform with duplex-specific nuclease signal amplification for ultrasensitive analysis of miRNA 155, *Sensor. Actuator. B Chem.* 275 (2018) 260–266.
- [112] B. Guven, F. Dudak, I. Boyaci, U. Tamerc, M. Ozsoz, SERS-based direct and sandwich assay methods for mir-21 detection, *Analyst* 139 (2014) 1141–1147.
- [113] J. Su, D. Wang, L. Norbel, J. Shen, Z. Zhao, Y. Dou, T. Peng, J. Shi, S. Mathur, C. Fan, S. Song, Multicolor gold-silver nano-mushrooms as ready-to-use SERS probes for ultrasensitive and multiplex DNA/miRNA detection, *Anal. Chem.* 89 (2017) 2531–2538.
- [114] L. Peng, J. Zhou, Z. Liang, Y. Zhang, L. Petti, T. Jiang, C. Gu, D. Yang, P. Mormile, SERS-based sandwich bioassay protocol of miRNA-21 using Au@Ag core-shell nanoparticles and a Ag/tio2 nanowires substrate, *Anal. Methods* 11 (2019) 2960–2968.
- [115] L. Qi, M. Xiao, X. Wang, C. Wang, L. Wang, S. Song, X. Qu, L. Li, J. Shi, H. Pei, Dna-encoded Raman-active anisotropic nanoparticles for miRNA detection, *Anal. Chem.* 89 (2017) 9850–9856.
- [116] W. Zhou, Y. Tian, B. Yin, B. Ye, Simultaneous surface-enhanced Raman spectroscopy detection of multiplexed miRNA biomarkers, *Anal. Chem.* 89 (2017) 6120–6128.
- [117] W. Kim, J.U. Lee, S. Song, S. Kim, Y. Choi, S. Sim, A label-free, ultra-highly sensitive and multiplexed SERS nanoplasmonic biosensor for miRNA detection using a head-flocked gold nanopillar, *Analyst* 144 (2019) 1768–1776.
- [118] W. Kim, J. Lee, M. Jeon, K. Park, S. Sim, Three-dimensional hierarchical plasmonic nano-architecture based label-free surface-enhanced Raman spectroscopy detection of urinary exosomal miRNA for clinical diagnosis of prostate cancer, *Biosens. Bioelectron.* 205 (2022) 114116–114125.
- [119] Z. Liang, J. Zhou, L. Petti, L. Shao, T. Jiang, Y. Qing, S. Xie, G. Wua, P. Mormile, SERS-based cascade amplification bioassay protocol of miRNA-21 by using sandwich structure with biotin-streptavidin system, *Analyst* 144 (2019) 1741–1750.
- [120] J. Lee, W. Kim, H. Lee, K. Park, S. Sim, Quantitative and specific detection of exosomal miRNAs for accurate diagnosis of breast cancer using a surface-enhanced Raman scattering sensor based on plasmonic head-flocked gold nanopillars, *Small* 15 (2019) 1804968–1804978.
- [121] Y. Zhao, X. Fang, M. Bai, J. Zhang, H. Yu, F. Chen, Y. Zhao, A microfluidic surface-enhanced Raman scattering (SERS) sensor for miRNA in extracellular vesicles with nucleic acid-tyramine cascade amplification, *Chin. Chem. Lett.* 33 (2022) 2101–2104.
- [122] B. Moody, G. McCarty, Statistically significant Raman detection of midsequence single nucleotide polymorphisms, *Anal. Chem.* 81 (2009) 2013–2016.
- [123] B. Moody, J. Leotaud, G. McCarty, Using surface-enhanced Raman spectroscopy to probe for genetic markers on single-stranded DNA, *J. Biomed. Opt.* 15 (2010), 027014–1–027014–6.
- [124] Y. Wang, E. Wee, M. Trau, Highly sensitive DNA methylation analysis at CpG resolution by surface-enhanced Raman scattering via ligase chain reaction, *Chem. Commun.* 51 (2015) 10953–10956.
- [125] L. Xu, Y. Gao, H. Kuang, L. Liz-Marzan, C. Xu, MiRNA-directed intracellular self-assembly of chiral nanorod dimers, *Angew. Chem. Int. Ed.* 57 (2018) 10544–10548.
- [126] M. Schechinger, H. Marks, A. Locke, M. Choudhury, G. Cote, Development of a miRNA surface-enhanced Raman scattering assay using benchtop and handheld Raman systems, *J. Biomed. Opt.* 23 (2018), 017002–017013.
- [127] M. Schechinger, H. Marks, S. Mabbott, M. Choudhury, G. Cote, A SERS approach for rapid detection of miRNA-17 in the picomolar range, *Analyst* 144 (2019) 4033–4044.
- [128] W. Lee, K. Kim, M. Hossain, H. Cho, J. Choi, DNA-gold nanoparticle conjugates for intracellular miRNA detection using surface-enhanced Raman spectroscopy, *BiChip Journal* 16 (2022) 33–40.
- [129] K. Shin, J. Choi, Y. Kim, Y. Lee, J. Kim, S. Lee, H. Chung, Feasibility study for combination of field-flow fractionation (FFF)-based separation of size-coded particle probes with amplified surface enhanced Raman scattering (SERS) tagging for simultaneous detection of multiple miRNAs, *J. Chromatogr. A* 1556 (2018) 97–102.
- [130] M. He, S. Chen, K. Yao, K. Wang, Y. Yu, J. Wang, Oriented assembly of gold nanoparticles with freezing-driven surface DNA manipulation and its application in SERS-based miRNA assay, *Small Methods* 3 (2019) 1900017–1900024.
- [131] S. Jiang, Q. Li, C. Wang, Y. Pang, Z. Sun, R. Xiao, In situ exosomal miRNA determination by target-triggered SERS and Fe3O4@TiO2-based exosome accumulation, *ACS Sens.* 6 (2021) 852–862.
- [132] H. Wang, T. Vo-Dinh, Plasmonic coupling interference (PCI) nanopores for nucleic acid detection, *Small Biosensors* 7 (2011) 3067–3074.
- [133] H. Wang, T. Vo-Dinh, Plasmonic coupling interference: a new approach for cancer diagnostics using SERS detection, *Adv. Biomed. Clin. Diagnostic Syst. X* 8214 (2012) 821405–821413.
- [134] H. Wang, B. Crawford, S. Norton, T. Vo-Dinh, Direct and label-free detection of miRNA cancer biomarkers using SERS-based plasmonic coupling interference (PCI) nanopores, *J. Phys. Chem.* 123 (2019) 10245–10251.
- [135] M. Hou, D. He, H. Wang, J. Huang, H. Cheng, K. Wan, H. Li, Z. Tang, X. He, K. Wang, Simultaneous and multiplex detection of exosomal miRNAs based on the asymmetric Au@Au@Ag probes with enhanced Raman signal, *Chin. Chem. Lett.* 51 (2021), 01046–01055.
- [136] M. Muhammad, C. Shao, C. Liu, Q. Huang, Highly sensitive detection of elevated exosomal miR-122 levels in radiation injury and hepatic inflammation using an aptamer-functionalized SERS-sandwich assay, *ACS Appl. Bio Mater.* 4 (2021) 8386–8395.
- [137] Y. Yang, X. Jiang, J. Chao, C. Song, B. Liu, D. Zhu, Y. Sun, B. Yang, Q. Zhang, Y. Chen, L. Wang, Synthesis of magnetic core-branched Au shell nanostructures and their application in cancer-related miRNA detection via SERS, *Sci. China Mater.* 60 (2017) 1129–1144.
- [138] H. Zhang, Y. Yi, C. Zhou, G. Ying, X. Zhou, C. Fu, Y. Zhu, Y. Shen, SERS detection of miRNA biomarkers for cancer diagnosis using gold-coated paramagnetic nanoparticles to capture SERS-active gold nanoparticles, *RCS Advances* 7 (2017) 52782–52793.
- [139] H. Zhang, C. Fu, Y. Yi, C. Zhou, G. Ying, Y. Shen, Y. Zhu, Magnetic-based SERS approach for highly sensitive and reproducible detection of cancer-related serum miRNAs, *Anal. Methods* 10 (2018) 624–633.
- [140] J. Wu, X. Zhou, P. Li, X. Lin, J. Wang, Z. Hu, P. Zhang, D. Chen, H. Cai, R. Niessner, C. Haisch, P. Sun, Y. Zheng, Z. Jiang, H. Zhou, Ultrasensitive and simultaneous SERS detection of multiplex miRNA using fractal gold nanotags for early diagnosis and prognosis of hepatocellular carcinoma, *Anal. Chem.* 93 (2021) 8799–8809.
- [141] H. Ngo, N. Gandra, A. Fales, S. Taylor, T. Vo-Dinh, Sensitive DNA detection and SNP discrimination using ultrabright SERS nanorattles and magnetic beads for malaria diagnostics, *Biosens. Bioelectron.* 81 (2016) 8–14.
- [142] H. Ngo, N. Gandra, A. Fales, S. Taylor, T. Vo-Dinh, DNA detection and single nucleotide mutation identification using SERS for molecular diagnostics and global health, *Proc. SPIE* 10054 (2017), 100540C–1–100540C–9.
- [143] N. Lyu, V. Rajendran, J. Li, A. Engel, M. Molloy, Y. Wang, Highly specific detection of KRAS single nucleotide polymorphism by asymmetric PCR/SERS assay, *Analyst* 146 (2021) 5714–5721.
- [144] M. Zhu, Z. Sun, Z. Zhang, S. Zhang, Ultrasensitive SERS detection of highly homologous miRNAs by generating 3D organic-nanoclusters and a functionalized chip with locked nucleic acid probes, *Chem. Commun. J.* 54 (2018) 13431–13434.
- [145] Y. Wu, Y. Li, H. Han, C. Zhao, X. Zhang, Dual cycle amplification and dual signal enhancement assisted sensitive SERS assay of miRNA, *Anal. Biochem.* 564 (2019) 16–20.
- [146] S. Ye, M. Wang, Z. Wang, N. Zhang, X. Luo, A DNA-linker-DNA bifunctional probe for simultaneous SERS detection of miRNAs via symmetric signal amplification, *Chem. Commun. J.* 54 (2018) 7786–7789.

- [147] X. Du, S. Wu, X. Huang, J. Sun, Ag nanocubes coupled with heating-enhanced dsb-assisted cycling amplification for surface-enhanced Raman spectroscopy detection of microRNA-21, *ACS Appl. Nano Mater.* 4 (2021) 2565–2574.
- [148] H. Zhang, Y. Liu, J. Gao, J. Zhen, Sensitive sers detection of microRNA using a label-free multifunctional probe, *Chem. Commun. J.* 51 (2015) 16836–16839.
- [149] S. Ye, X. Li, M. Wang, B. Tang, Fluorescence and sers imaging for the simultaneous absolute quantification of multiple miRNAs in living cells, *Anal. Chem.* 89 (2017) 5124–5130.
- [150] Y. Sun, T. Li, Composition-tunable hollow Au/Ag sers nanoparticles coupled with target-catalyzed hairpin assembly for triple-amplification detection of miRNA, *Anal. Chem.* 90 (2018) 11614–11621.
- [151] C. Liu, C. Chen, S. Li, H. Dong, W. Dai, T. Xu, Y. Liu, F. Yang, X. Zhang, Target-triggered catalytic hairpin assembly-induced core-satellite nanostructures for high-sensitive “off-to-on” sers detection of intracellular microRNA, *Anal. Chem.* 90 (2018) 10591–10599.
- [152] Y. Si, L. Xu, T. Deng, J. Zheng, J. Li, Catalytic hairpin self-assembly-based sers sensor array for the simultaneous measurement of multiple cancer-associated miRNAs, *ACS Sens.* 5 (2020) 4009–4016.
- [153] Z. Wang, S. Ye, N. Zhang, X. Liu, M. Wang, Triggerable mutually amplified signal probe based sers-microfluidics platform for the efficient enrichment and quantitative detection of miRNA, *Anal. Chem.* 91 (2019) 5043–5050.
- [154] J. Liu, T. Zheng, Y. Tian, Functionalized h-bn nanosheets as a theranostic platform for sers real-time monitoring of microRNA and photodynamic therapy, *Angew. Chem. Int. Ed.* 58 (2019) 7757–7761.
- [155] Z. Wang, J. Xue, C. Bi, H. Xin, Y. Wang, X. Cao, Quantitative and specific detection of cancer-related miRNAs in living cells using surface-enhanced Raman scattering imaging based on hairpin dna-functionalized gold nanocages, *Analyst* 144 (2019) 7250–7262.
- [156] J. Zhang, H. Zhang, S. Ye, X. Wang, L. Ma, Fluorescent-Raman binary star ratio probe for microRNA detection and imaging in living cells, *Anal. Chem.* 93 (2021) 1466–1471.
- [157] X. Cao, Z. Wang, L. Bi, C. Bi, Q. Du, Gold nanocage-based surface-enhanced Raman scattering probes for long-term monitoring of intracellular microRNA during bone marrow stem cell differentiation, *Nanoscale* 12 (2020) 1513–1527.
- [158] H. Shao, H. Lin, Z. Guo, J. Lu, Y. Jia, M. Ye, F. Sud, L. Niu, W. Kang, S. Wang, Y. Hu, Y. Huang, A multiple signal amplification sandwich-type sers biosensor for femtomolar detection of miRNA, *Biosens. Bioelectron.* 143 (2019) 111616–111623.
- [159] J. Wang, C. Zhang, Z. Liu, S. Li, P. Ma, F. Gao, Target-triggered nanomaterial self-assembly induced electromagnetic hot-spot generation for sers-fluorescence dual-mode in situ monitoring miRNA-guided phototherapy, *Anal. Chem.* 93 (2021) 13755–13764.
- [160] S. Ye, Y. Wu, X. Zhai, B. Tang, Asymmetric signal amplification for simultaneous sers detection of multiple cancer markers with significantly different levels, *Anal. Chem.* 87 (2015) 8242–8249.
- [161] X. Li, S. Ye, X. Luo, Sensitive sers detection of miRNA via enzyme-free dna machine signal amplification, *Chem. Commun. J.* 52 (2016) 10269–10272.
- [162] J. Zheng, D. Ma, M. Shi, J. Bai, Y. Li, J. Yang, R. Yang, A new enzyme-free quadratic sers signal amplification approach for circulating microRNA detection in human serum, *Chem. Commun. J.* 51 (2015) 16271–16274.
- [163] X. Luo, J. Zhu, W. Jia, N. Fang, P. Wu, C. Cai, J. Zhu, Boosting long-range surface-enhanced Raman scattering on plasmonic nanohole arrays for ultrasensitive detection of miRNA, *ACS Appl. Mater. Interfaces* 13 (2021) 18301–18313.
- [164] L. Ma, S. Ye, X. Wang, J. Zhang, Sers-microfluidic approach for the quantitative detection of miRNA using dnzyme-mediated reciprocal signal amplification, *ACS Sens.* 6 (2021) 1392–1399.
- [165] W. Luo, C. Wu, S. Huang, X. Luo, R. Yuan, X. Yang, Liquid phase interfacial surface-enhanced Raman scattering platform for ratiometric detection of microRNA 155, *Anal. Chem.* 92 (2020) 15573–15578.
- [166] Y. Pang, C. Wang, J. Wang, Z. Sun, R. Xiao, S. Wang, Fe₃O₄@Ag magnetic nanoparticles for microRNA capture and duplex-specific nuclease signal amplification based sers detection in cancer cells, *Biosens. Bioelectron.* 79 (2016) 574–580.
- [167] S. Wen, Y. Su, C. Dai, J. Jia, G. Fan, L. Jiang, R. Song, J. Zhu, Plasmon coupling-enhanced Raman sensing platform integrated with exonuclease-assisted target recycling amplification for ultrasensitive and selective detection of microRNA-21, *Anal. Chem.* 91 (2019) 12298–12306.
- [168] Y. Hu, C. Wu, S. Huang, X. Luo, R. Yuan, X. Yang, A novel sers substrate with high reusability for sensitive detection of miRNA 21, *Talanta* 228 (2021) 122240–122247.
- [169] Y. Cui, J. Zheng, W. Zhuang, H. Wang, A target-activated plasmon coupling surface-enhanced Raman scattering platform for the highly sensitive and reproducible detection of miRNA-21, *New J. Chem.* 45 (2021) 10907–10913.
- [170] S. Yoo, T. Kang, B. Kim, S. Lee, Detection of single nucleotide polymorphisms by a gold nanowire-on-film sers sensor coupled with s1 nuclease treatment, *Eur. J. Med. Chem.* 17 (2011) 8657–8662.
- [171] X. Miao, Q. Fang, X. Xiao, S. Liu, R. Wu, J. Yan, B. Nie, J. Liu, Integrating cycled enzymatic dna amplification and surface-enhanced Raman scattering for sensitive detection of circulating tumor dna, *Front. Mol. Biosci.* 8 (2021) 676065–676074.
- [172] Y. Pang, Q. Li, C. Wang, S. Zhen, Z. Sun, R. Xiao, Crispr-cas12a mediated sers lateral flow assay for amplification-free detection of double-stranded dna and single-base mutation, *Chem. Eng. J.* 429 (2022) 132109–132118.
- [173] Y. Yao, H. Zhang, T. Tian, Y. Liu, R. Zhu, J. Ji, B. Liu, Iodide-modified Ag nanoparticles coupled with dsb-assisted cycling amplification for label-free and ultrasensitive sers detection of microRNA-21, *Talanta* 235 (2021) 122728–122735.
- [174] M. Li, J. Li, X. Zhang, M. Yao, P. Li, W. Xu, Simultaneous detection of tumor-related miRNA and miRNA in cancer cells with magnetic sers nanotags, *Talanta* 232 (2021) 122432–122441.
- [175] W. Xu, A. Zhao, F. Zuo, R. Khan, H. Hussain, J. Chang, Au@Ag core-shell nanoparticles for microRNA-21 determination based on duplex-specific nuclease signal amplification and surface-enhanced Raman scattering, *Microchim. Acta* 187 (2020) 384–393.
- [176] D. Ma, C. Huang, J. Zheng, J. Tang, J. Li, J. Yang, R. Yang, Quantitative detection of exosomal microRNA extracted from human blood based on surface-enhanced Raman scattering, *Biosens. Bioelectron.* 101 (2018) 167–173.
- [177] Y. He, X. Yang, R. Yuan, Y. Chai, Switchable target-responsive 3d dna hydrogels as a signal amplification strategy combining with sers technique for ultrasensitive detection of miRNA 155, *Anal. Chem.* 89 (2017) 8538–8544.
- [178] Y. He, X. Yang, R. Yuan, Y. Chai, A novel ratiometric sers biosensor with one Raman probe for ultrasensitive microRNA detection based on dna hydrogel amplification, *J. Mater. Chem. B* 7 (2019) 2643–2647.
- [179] Y. Si, L. Xu, N. Wang, J. Zheng, R. Yang, J. Li, Target microRNA-responsive dna hydrogel-based surface-enhanced Raman scattering sensor arrays for microRNA-marked cancer screening, *Anal. Chem.* 92 (2020) 2649–2655.
- [180] X. Yang, S. Wang, Y. Wang, Y. He, Y. Chai, R. Yuan, Stimuli-responsive dna microcapsules for sers sensing of trace microRNA, *ACS Appl. Mater. Interfaces* 10 (2018) 12491–12496.
- [181] Y. Huh, A. Lowe, A. Chung, B. Cordovez, A. Strickland, C. Batt, D. Erickson, Optofluidic surface enhanced Raman scattering based detection of single nucleotide polymorphism, in: *Conference on Miniaturized Systems for Chemistry and Life Sciences*, 13, 2009, pp. 281–283.
- [182] Y. Huh, A. Lowe, A. Strickland, C. Batt, D. Erickson, Surface-enhanced Raman scattering based ligase detection reaction, *J. Am. Chem. Soc.* 131 (2009) 2208–2213.
- [183] A. Lowe, Y. Huh, A. Strickland, D. Erickson, C. Batt, Multiplex single nucleotide polymorphism genotyping utilizing ligase detection reaction coupled surface enhanced Raman spectroscopy, *Anal. Chem.* 82 (2010) 5810–5814.
- [184] M. Shi, J. Zheng, Y. Tan, G. Tan, J. Li, Y. Li, X. Li, Z. Zhou, R. Yang, Ultrasensitive detection of single nucleotide polymorphism in human mitochondrial dna utilizing ion-mediated cascade surface-enhanced Raman spectroscopy amplification, *Anal. Chem.* 87 (2015) 2734–2740.
- [185] J. Barea, D. Kang, Integration of surface-enhanced Raman spectroscopy with pcr for monitoring single copy of kras g12d mutation, *Bull. Kor. Chem. Soc.* 42 (2021) 945–951.
- [186] N. Lyu, V. Rajendran, R. Diefenbach, K. Charles, S. Clarke, A. Engel, S.C.C. S. Investigators, H. Rizos, M. Molloy, Y. Wang, Multiplex detection of ctDNA mutations in plasma of colorectal cancer patients by pcr/sers assay, *Nanotheranostics* 4 (2020) 224–232.
- [187] S. Huang, J. Hu, G. Zhu, C. Zhang, Sensitive detection of point mutation using exponential strand displacement amplification-based surface enhanced Raman spectroscopy, *Biosens. Bioelectron.* 65 (2015) 191–197.
- [188] L. Ouyang, Y. Hu, L. Zhu, G. Cheng, J. Irudayaraj, A reusable laser wrapped graphene-Ag array based sers sensor for trace detection of genomic dna methylation, *Biosens. Bioelectron.* 92 (2017) 755–762.
- [189] A. Nguyen, J. Lee, S. Sim, Plasmonic coupling-dependent sers of gold nanoparticles anchored on methylated dna and detection of global dna methylation in sers-based platforms, *J. Opt.* 17 (2015) 114022–114034.
- [190] Y. Wang, E. Wee, M. Trau, Accurate and sensitive total genomic dna methylation analysis from sub-nanogram input with embedded sers nanotags, *Chem. Commun.* 52 (2016) 3560–3563.
- [191] Z. Li, H. Chen, P. Wang, Lateral flow assay ruler for quantitative and rapid point-of-care testing, *Analyst* 144 (2019) 3314–3322.