MINI-REVIEW

Advances in nanoparticle-based lateral flow immunoassay for point-of-care testing

Doudou Lou | Lin Fan | Tao Jiang | Yu Zhang

1 Jiangsu Institute for Food and Drug Control, 17 Kangwen Road, Nanjing, P. R. China
2 School of Geographic and Biologic Information, Nanjing University of Posts and Telecommunications, Nanjing, P. R. China
3 Army of Reserve Infantry Division in Heilongjiang Province, Harbin, Heilongjiang Province, P. R. China
4 State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering & Collaborative Innovation Center of Suzhou Nano Science and Technology, Southeast University, Nanjing, P. R. China

Correspondence
Yu Zhang, State Key Laboratory of Bioelectronics, Jiangsu Key Laboratory for Biomaterials and Devices, School of Biological Science and Medical Engineering & Collaborative Innovation Center of Suzhou Nano Science and Technology, Southeast University, Nanjing, P. R. China. Email: zhangyu@seu.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 61821002, 82072067; Science and Technology Support Project of Jiangsu Province, Grant/Award Number: BE2017763; Medical Research Project of Jiangsu Province Health Committee, Grant/Award Number: K2019020; Transformation of Scientific and Technological Achievements of Jiangsu Province, Grant/Award Number: BA2020016; the Central Universities and Wenzhou Basic Research Projects, Grant/Award Numbers: Y2020916, Y20201013; NUPTSF, Grant/Award Number: NY220167; Jiangsu Provincial Double-Innovation Doctor Program, Grant/Award Number: JSSCBS20210506

Abstract
As a representative technology for point-of-care testing (POCT), lateral flow immunoassay (LFIA) has been broadly used to detect analytes in many fields. However, its clinical application is severely limited by the unsatisfactory sensitivity, which makes it difficult to obtain accurate results when detecting biomarkers of trace levels, especially in complex matrices. Nanoparticles have been introduced into LFIA for years and become an indispensable part, acting not only as carriers that load and enrich biomolecules, such as antibodies and dyes, but also a miniature platform applied for creative design and construction of nanoprobes. Due to the unique properties at the nanoscale, including the mimetic enzyme activity, the characteristic plasma resonance spectrum and so on, nanomaterials exhibit great potential in the development of novel LFIA and high-sensitivity detection.

KEYWORDS
antibody orientation, high-sensitivity detection, lateral flow immunoassay, nanoparticle, point-of-care testing

1 | INTRODUCTION

Lateral flow immunoassay (LFIA) is the most representative point-of-care testing (POCT) technology that has been widely used in various fields such as rapid in vitro diagnosis (IVD), environmental monitoring, and food safety detection.\[1,2\]

Besides proteins, the most common targets, LFIA is applicable for numerous different analytes such as small molecules,\[3\] nucleic acids,\[4\] etc. As an important...
component of the clinical laboratory diagnosis, it has obvious advantages and irreplaceability such as higher population coverage, lower costs, easier transportation, and shorter detection time. LFIA is suitable for the early screening, exclusion and triage of many diseases, thus, greatly sharing the sample testing pressure of large instruments in clinical laboratories. In recent years, LFIA has become more and more important due to its expanded application for the early diagnosis of major diseases (such as acute myocardial infarction and cancer) and infectious diseases (such as hepatitis B and coronavirus disease 2019 [COVID-19]). In the prevention and control of COVID-19, the LFIA-based IgG/IgM detection has been proven to be effective for the rapid detection and screening of large-scale population, with very few restrictions on the operators and the sites. Besides, the LFIA platform for the quantitative detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA has also been developed. The intelligent development of LFIA also makes it play a greater value such as the combination of smart phones and network platform.

Nanoparticles have been recruited in LFIA for years. In fact, nanoparticles are contained in almost all commercial LFIA kits. In most cases, nanoparticles serve as the carrier of antibodies and signal tags or exist as the signal donor themselves. The conjugation and enrichment of bioactive proteins and dyes as well as the unique physicochemical properties of nanoparticles result in the increase of the detection sensitivity. Besides, the diversity of signal types also greatly expands the applicability of LFIA, which mainly includes color, luminescent, and magnetic signals. Therefore, nanoparticles have the potential to be exploited for the development of high-sensitivity LFIA.

However, lack of surface design, random conjugation of biomolecules, and underutilization of nanoparticles’ properties make the integration of nanoparticles and LFIA remain rough and primary, thus, limiting its clinical potential, especially for the quantitative and sensitive detection of trace level biomarkers in complex matrices. Novel nanoparticle-based signal enhancement strategies for highly sensitive LFIA have been studied to cope with the challenge, which has attracted a lot of attention.

Here, we will mainly focus on the development of signal enhancement strategies based on nanoparticles and their application in LFIA, and the representative ideas and progresses have been summarized in Table 1. The representative progresses in the aspects of the selection of nanoparticles, the surface functional design, and the antibody immobilization of nanoprobes, the combination of multiple nanomaterials and signals, as well as the secondary signal amplification are discussed in this review (Figure 1).
| Nanoparticle     | Sample                        | Analytes                  | Signal types      | LOD                  | Assay time | Unique strategies                  | Detection steps | Refs. |
|------------------|-------------------------------|---------------------------|-------------------|----------------------|------------|------------------------------------|-----------------|-------|
| GNPs             | Serum                         | Troponin                  | Color             | 0.001-0.01 pg/mL     | 10 min     | Dual GNPs                          | 1               | [51,52]|
| GNPs             | PBS                           | Bisphenol A              | Color             | 0.5 ng/mL            | 10 min     | Dual GNPs                          | 1               | [34]  |
| GNPs             | Serum, whole blood            | PCT                       | Color, chemi-luminescence | 0.01 pg/mL          | 30 min     | HRP                               | 3               | [57]  |
| GNPs             | Serum                         | CEA                       | Color             | 2.9 fg/mL            | 15 min     | Dual gold, HRP                     | 3               | [58]  |
| GNPs             | Fetal bovine serum            | HCG                       | Color             | 0.3 mIU/mL           | ~40 min    | Gold deposition                    | 3               | [6]   |
| GNPs             | Standard solution             | PSA                       | Color             | 0.1 ng/mL            | 20 min     | Dual gold/silver deposition, “self contained” strategy | 2               | [66]  |
| Gold nanostar    | Whole blood                   | CEA                       | SERS              | 1 ng/mL              | >20 min    | Spiked morphology                  | 2               | [13]  |
| Polystyrene      | Serum                         | Troponin                  | Fluorescence      | 0.049 ng/mL          | 15 min     | SA-biotin                          | 1               | [3]   |
| Polystyrene      | Serum                         | Troponin                  | Fluorescence      | 0.032 ng/mL          | 15 min     | Protein G-mediated antibody orientation | 1               | [8]   |
| Polystyrene      | Serum                         | Troponin                  | Fluorescence      | 0.097 ng/mL          | 15 min     | pH-mediated antibody orientation   | 1               | [42]  |
| QDs              | Serum                         | HBsAg                     | Fluorescence      | 0.05 ng/mL           | 15 min     | Hydrazide-mediated antibody orientation | 1               | [42]  |
| Magnetic beads   | Plasma                        | Troponin                  | Color             | 1-10 ng/mL           | 15-25 min | External magnetic field             | 3-5             | [3]   |
| MNPs             | Serum                         | Ebola virus               | Color             | 1 ng/mL              | <30 min    | Mimetic enzyme                     | 3               | [60]  |
| Fe3O4@GNPs       | *Lupinus albus*               | β-conglutinin             | Color             | 8 fM                 | <20 min    | External magnetic field, HRP       | 2               | [34]  |
| GNPs and polymer dots | Swine urine and muscle tissue | Ractopamine               | Fluorescence      | 0.16 ng/mL           | 20 min     | Fluorescent quenching              | 1               | [1]   |
| Goldmag nanoparticles | Whole blood                | Alleles                   | Color             | 5 ng DNA template    | 75 min     | Gold-coated MNPs                   | 3               | [56]  |
| GNPs, QDs, mesoporous silicon particles | Serum          | Cystatin C                | Color, fluorescence | 0.61 and 0.24 ng/mL | 8 min     | Dual-mode detection                | 1               | [20]  |
| Pt-Pd nanoparticles | Milk, ice cream             | Salmonella Enteritidis and E. coli O157:H7 | Color | ~20 and ~34 CFU/ml | 11 min     | Mimetic enzyme                     | 2               | [59]  |
| GNPs, MNPs       | Plasma                        | Troponin                  | Color             | 0.1 ng/ mL           | 20 min     | GNPs-coated MNPs, magnetic separation, gold deposition | 3               | [61]  |

*Abbreviations:* CEA, carcinoembryonic antigen; SERS, surface enhanced Raman scattering; GNPs, gold nanoparticles; HRP, horse radish peroxidase; SA, streptavidin; QDs, quantum dots; HBsAg, hepatitis B surface antigen; MNPs, magnetic nanoparticles; PCT, procalcitonin; HCG, human chorionic gonadotropin; PSA, prostate specific antigen.
1.1 | Nanoparticles in LFIA

Nanoparticles are an indispensable component in nearly all the LFIA platforms. The selection of nanoparticles primarily depends on the signal type that the researcher choose according to the analyte and detection performance requirements. A variety of signal labels, such as color, luminescence, surface enhanced Raman scattering (SERS), magnetic and electrochemical signals, have been recruited for the development of LFIA. Here, we mainly discuss the relationship between the employed nanoparticles and the signal types based on recent works.

As well known, gold nanoparticles (GNPs) are the most used nanomaterial for color-based LFIA. GNPs have characteristic plasmonic absorption peaks in the visible region that shows slightly red shift with the increase of the size. Generally, GNPs with sizes between 20 and 40 nm are preferred since they easily generate clear rose red color after accumulating on the test zone, which can be observed with naked eyes or recorded by image readers for qualitative and quantitative detection, respectively. In addition to GNPs, gold nanomaterials with anisotropic or spiked morphologies, such as nanostars, nanoflowers and nanoplates, have also been synthesized and many of them have been reported to be used in LFIA. Compared to GNPs, these special morphologies often coexist with red-shifted and enhanced absorption peaks, thus, can be used for optical sensitivity enhancement. Gold nanomaterials are also widely used in SERS since the nanoscale rough surfaces lead to the strong optical enhancement caused by local surface plasma resonance. Similarly, the tips of spiked materials greatly enhance the intensity of the surrounding electromagnetic field, thus, leading to amplified SERS signal and higher detection sensitivity. Gao et al. employed gold nanostars to develop SERS probes and detect carcinoembryonic antigen (CEA) from 30 μL of whole blood using a portable Raman reader, achieving a limit of detection of 1.0 ng/mL.

Organic fluorescent dyes are capable of emitting fluorescence when stimulated by exciting light. In addition to monomer form, dyes are often doped in polymer nanocarriers or immobilized on the surface of carriers before combining with antibodies to obtain higher fluorescence yield. Bamrungsap et al. doped dye Cy5 into silica nanoparticles for the detection of an influenza antigen, proving an eightfold better sensitivity than the commercial color-based LFIA kit. Quantum dots (QDs) make up for some inherent defects of organic fluorescent dyes due to their sharp emission peaks, strong signal intensity, low background, and photostability. It is reported that single QDs have longer fluorescence lifetime and 10–20 times higher signal strength than organic fluorescent dyes. Besides, multiple separated emission peaks can be obtained under the same excitation wavelength of different QDs, which is particularly suitable for simultaneous detection of multiple biomarkers. QDs can also be doped/encapsulated into or coated on the surface of nanocarriers to further improve the chemical and photo stability, signal intensity, hydrophilicity, and dispersion, bioconjugation capability, and reduce the environmental toxicity. Semicontacting polymer dots have the advantages of biocompatibility, luminescent property, and surface activity, thus, developing rapidly in recent years.

Magnetic nanoparticles (MNs) have been widely used in the purification, enrichment, and detection of biomolecules. Both color and magnetic signals can be generated by MNPs due to their dark appearance (brown or black) and superparamagnetic property. Magnetic detection has to be operated with the assistance of a magnetic sensor, such as the giant magneto-resistive sensor or the resonant coil magnetometer, which makes it possible to achieve higher detection sensitivity by measuring the magnetic signals both at the surface and deep within the membrane. Cui’s group utilized tunneling magnetoresistance sensors for quantitation of MNPs in lateral flow strips, achieving high sensitivity and strong anti-interference ability. In addition to generating detection signals, the magnetism of particles has been used for other purposes. In combination with magnetic separation, the target molecules can be separated, enriched, and captured on the nanoparticles before chromatography, which is of great value for the analysis of biomarkers in complicated matrices (e.g., plasma, saliva). External magnet placed in certain sites help to promote the flow and immunoreaction time and enhance the signal intensity. Ren et al. reported a “magnetic focus” LFIA by putting an external magnet below the strip near the test zone, significantly improving the visual detection sensitivity of pathogens.

In addition to the well-known gold, fluorescent and MNPs, some other materials have also been recruited for LFIA. Upconverting nanoparticles (UCNPs) have also been used in LFIA recently, having a typical near-infrared excitation wavelength and a shorter emission wavelength, which greatly reduce the interference of the background noise of biological samples. Ji et al. employed near-infrared to near-infrared UCNPs with an excitation peak at 980 nm and an emission peak at 800 nm for background-free LFIA. Stable and sensitive results could be obtained even in hyperbilirubinemia, hyperlipidemia, and hemolytic plasma samples, free from the interference of autofluorescence and photon scattering. Multiwalled carbon nanotubes could be conjugated with antibodies after solubility improvement and applied in LFIA for the semiquantitative and quantitative assays of methamphetamine, with satisfactory sensitivity and wide linear range. Li et al. developed a “lateral flow assay ruler” and
employed platinum nanoprobes as mimic enzyme to generate oxygen and push ink advancement, successfully measuring the concentration of prostate-specific antigen.\[29\]
In chemiluminescent LFIA, substrate and H$_2$O$_2$ are added after chromatography to generate signals under the catalysis of horse radish peroxidase (HRP), where nanoparticles are not necessary but still used as carriers of HRP and antibodies in some researches.\[30\]
Electrochemical signals are also considered in LFIA, where the electrode is usually indispensable.\[31\] Limited to the technology transfer, cost, stability, professional operation, and other inherent defects, the application of these strategies in LFIA is still rare.

In addition to choosing the appropriate nanoparticles, the functionalization of nanoparticles and construction of nanoprobes are also important for the detection efficacy, which will be described in detail in the following sections.

1.2  | Surface functionalization and antibody orientation regulation of nanoparticles

1.2.1  | Surface functionalization of nanoparticles

In addition to serving as signal tags themselves, nanoparticles are also carriers that load and enrich antibodies and signal molecules on the surface, therefore, scientific and reasonable surface functionalization is worth studying to give full play to the role of the immobilized molecules. Basically, nanoparticles provide a large amount of surface area and are open to surface modification with functional groups (such as carboxy and amino groups), to introduce active sites for subsequent coupling, improve hydrophilicity, and increase stability. Further functionalization methods are more varied and complex. For instance, the signal intensity of the fluorescent dyes on the surface of gold nanorods depends on the distance between the dye and the substance. When the longitudinal localized surface plasmon resonance overlaps with the excitation or emission peak, the fluorescence will be enhanced within a certain range of distance. Singamaneni’s group adsorbed 800CW fluorophore labeled albumin onto gold nanorods and each 800CW was enhanced by nearly 30-fold.\[32\] Nanorods were previously coated with 2.3 nm of siloxane copolymer layer, which serves as a spacer layer between the metal surface and the fluorophore to prevent fluorescence quenching. Conversely, in the absence of spacer, the fluorescence resonance energy transfer between the polymer dots and the surface of GNPs leads to fluorescence quenching, which has also been utilized in LFIA for the fast detection of ractopamine.\[33\]

Biotin-streptavidin (SA) system is another classical nanoparticle surface functionalization example. The application of biotin and SA makes the conjugation easier and firmer, increases the surface area and the number of binding sites since one SA molecule can bind up to four biotins, and further reduces the steric hindrance by modifying biotin with spacer arm.\[33,34\]

1.2.2  | Antibody orientation

Antibodies are necessary for the construction of nanoprobes in LFIA. The immune activity of the nanoprobes is determined by the quantity, orientation, steric hindrance, and bonding strength of immobilized antibodies together. Traditional physical adsorption and chemical coupling lead to the random immobilization of antibodies, as a result, antigen binding sites are very likely to be occupied by chemical bonds or become inaccessible due to the steric hindrance. To solve this problem, it’s essential to bind the antibodies onto the surface in a specific direction to fully expose the antigen binding fragments (Fab) and retain its biological activity to the maximum. The structure of antibodies is highly conserved and similar, known to be Y-shaped consisting of two heavy chains and two light chains. The upper and lower halves of the “Y” are respectively two Fab regions and a conservative crystallizable fragment (Fc). Except the special structure, antibodies share many unique sites and properties that can be used to develop antibody orientation schemes which have been used or have the potential to be used in LFIA.

The end of the Fab region is highly variable, while the structure of the Fc region is extremely stable. Protein A from Staphylococcus aureus and protein G from Streptococcus are found to specifically target the Fc region.\[36\] Natural or recombinant protein A or G has been shown to significantly improve the performance of biosensors.\[37\] In our previous work, protein G-mediated antibody orientation on the fluorescent nanoprobes greatly enhance the detection sensitivity of cardiac troponin I in serum.\[38\]

Disulfide bonds connect the heavy chains and light chains together, which breaks into sulfhydryl groups after degradation. By selectively reducing the disulfide bonds in the hinge region, a whole antibody turns into two identical halves, each consists of a light chain and a heavy chain and provides available sulfhydryl groups in the original hinge region, which have been used for the orientation of antibodies on the sulfhydryl specific surfaces. Baniukevic’ et al. adsorbed the half-antibody onto the gold coated surface in a site directive way based on the strong affinity of sulfur element and gold atoms, which significantly improved the detection sensitivity of bovine leukemia virus.\[39\] As one of the strongest noncovalent interactions,
SA-biotin is commonly used for protein labeling. The maleimide-modified biotin targets the hinge region of the antibody fragments, so that the fragments bind to SA-modified surface in a certain orientation.\[140\] The CH2 domain of the Fc region carries oligosaccharide chains. Adak et al. immobilized the Fc end of the antibody on the meta-aminophenylboronic acid (mAPBA)-modified surface and left the Fab region fully exposed by taking advantage of the mAPBA’s ability to recognize ortho hydroxyl groups of sugar chains.\[41\] Further, they introduced photo-coupling technology to strengthen the reversible binding. There are also studies that modified hydrazide groups onto the surface of nanoparticles that specifically bind aldehyde groups of antibodies’ sugar chains, and form strong chemical bonds.\[42\] The nucleotide binding site is a conserved region located at the Fab region of all antibodies that has a strong affinity with nucleotides and some aromatic amino acids. Alves et al. modified this site with indole-3-butryic acid (IBA) labeled biotin, having the aromatic rings of IBA recognized by the nucleotide binding site and the biotin captured by the SA-modified substrate.\[43\] The binding would not weaken the antigen binding activity since there is enough space between the nucleotide binding site and the antigen binding site.

By the use of protein engineering, specific positions of antibodies can be modified with histidine tags (His-tags).\[44,45\] The tag site preferentially binds to surface modified by various metal ions represented by nickel ions. Some studies have also fused His-tags to the N-terminus or C-terminus of protein A or G, so that protein A or G bind to the substrate in a preset direction to realize the dual orientation of both protein A/G and antibodies.\[46\]

The anisotropic distribution of charge and hydrophobic sites of antibodies have also been utilized to adjust their orientation by simply adjust the reaction environment such as the pH and ion concentration of the buffer and the net charge and the hydrophobicity of the nanocarrier,\[47\] as well as the application of external electric field.\[48\] In our previous work, the coupling orientation of antibodies on the carboxyl surface of nanocarriers were significantly improved by lowering the pH of the buffer and adjusting the surface density of antibodies.\[47\] Johnson et al. found that the orientation of protein G on the substrate was also affected by pH, thus, further improving the antibody binding efficiency.\[49\]

Antibodies often need to be reduced or oxidized for the specific binding of disulfide bonds or aldehyde groups, which is prone to cause over-reduction or oxidation to denature and inactivate the antigen binding sites. The Fc binding strength and capacity of protein A and G are limited by the source and subtype of antibodies. The binding between protein A or G and antibodies is reversible, which is broadly applied for antibody purification. However, firmer conjugation between protein A or G and antibodies is needed to improve the stability and bioactivity of nanoprobes, since nanoprobes have to be sprayed onto the conjugation pad, dried, and often stored for a long time before use. Antibody orientation strategies based on its own physicochemical properties are free of introducing new reagents or pretreating the antibody, but the accuracy of the regulation is compromised more or less. Researchers need to comprehensively consider the detection requirements, production technology, and other necessary factors before determining the antibody orientation schemes.

### 1.3 | Combination and collocation of nanoparticles and signals

Because of the need of higher sensitivity, wider linear range, broader application scenarios, or detecting multiple biomarkers simultaneously, researchers have made great efforts to integrate two kinds of nanomaterials or signals into a single test strip for the development of new LFIA methods.

#### 1.3.1 | Simultaneous detection of multiple biomarkers

For the detection of multiple biomarkers with a single test strip at the same time, several detection lines are usually set parallelly, each for the detection of one biomarker.\[50\] QDs have unique advantages due to their size-dependent, well-separated emission peaks that can be excited by light source of the same wavelength.\[16\] As a result, more than one biomarkers can be recognized on the same test line by measuring the intensity of the corresponding peaks. The above methods are developed to decrease sample consumption, simplify operation steps, shorten test duration, and reduce production and use costs.

#### 1.3.2 | Multimode detection of one single biomarker

For multimode detection of one biomarker, two different kinds of signal types are detected from the same test line. Generally, one is equipment free and qualitative, while the other is quantitative and more sensitive. The former is usually color signal and the latter can be stronger color, fluorescence, SERS, magnetic signal, and so on. Dual GNP-based visible optical signal amplification has been utilized by several studies. Two GNP s with different sizes are loaded with different functional molecules and sprayed
onto two adjacent conjugation pads. The large-sized particles target the small ones before or after the formation of antigen-antibody sandwich structure, significantly enhance the color of the test line.\cite{51–53} Mei et al. constructed the anti-bovine serum albumin (BSA)-coated second GNPs to target the BSA blocking first GNPs, improving the limit of detection of bisphenol A from 0.5 to 0.076 ng/mL.\cite{54} The color of MNPs can also be notably darkened by the immobilization of smaller metal particles on the surface. Gold surrounded magnetic particles have been shown to have higher extinction coefficient, no matter via reduction of gold ions or adsorption of GNPs.\cite{55,56} GNPs can also provide SERS signals, so it is possible to detect both color and SERS signals by simply employing GNPs, or anisotropic gold nanomaterials for higher SERS intensity. Similarly, MNPs generate color and magnetic signal at the same time. Huang et al. integrated both GNPs and QDs into the mesoporous silicon particles to prepare “all-in-one” labels, generating color, and fluorescent signals, respectively, in LFIA without interfering with each other.\cite{20}

Secondary signal amplification is usually conducted after the first-stage lateral chromatography, which aims to enhance the existing color signals or produces sensitive chemiluminescent signals. The HRP enzyme loaded on the surface of nanoparticles catalyze the substrate to deposit deepened color or generate chemiluminescent signals on the test line.\cite{24,57} In another study, researchers set up a dual-GNP-based LFIA platform to detect CEA. One of the GNPs is coated with HRP to further induce enzyme-catalyzed reactions for stronger color signals.\cite{58} Noble metal and alloy nanoparticles of small diameter have the mimetic enzyme properties themselves, thus, can be used instead of natural HRP.\cite{59} MNPs have also been found to show the catalytic activity. Yan’s group developed nanozyme-strip for rapid local diagnosis of Ebola by taking advantage of the mimetic enzyme property of Fe$_3$O$_4$ MNPs, 100 times more sensitive detection than the colloidal gold strip.\cite{60} Metal ions (such as gold, silver, and platinum ions) form deposits onto the surface of GNPs under the action of additional reducing reagents after the chromatography process, which also darkens the color of the test zone.\cite{61,62} Sharma et al. improved the visual detection limit of the plasma troponin to 0.1 ng/mL within 20 min by combining more than one of the above-mentioned means.\cite{63} Briefly, they conjugated 10 nm GNPs on the surface of MNPs, preincubated nanoprobe with the analyte and removed unbound impurities with magnetic separation before sample loading. After the chromatography process, chloroauric acid was reduced by hydroxylamine hydrochloride to form gold clusters onto the surface of GNPs for stronger color signals. Despite the enhancement of the detection sensitivity, secondary amplification methods need additional operation after regular steps. To solve this problem, some researchers have proposed the concept of “self-contained.” All or part of the additional reagents are fixed and dried on the test strip together with the conventional components before detection to integrate the immunoreaction and signal amplification modules.\cite{64,65}

In addition to improve the sensitivity and expanding the linear range, dual-mode detection is also good for the two signals to verify each other to eliminate systematic errors and improve accuracy. It is noteworthy that gold nanomaterials are not only mature in color-based LFIA platform, but also have promising prospects in new devices. Gold nanomaterials can generate visible, SERS, and even photothermal signals. Their mimetic enzyme property can be used to catalyze substrate to generate enhanced color or luminescent signals. The fluorophores near the surface of gold nanomaterials can either be enhanced or quenched easily. In addition, it is easy to obtain gold nanomaterials with a variety of morphologies. The distinction of material morphology contributes to the difference of physicochemical features, having the potential to meet various needs.

## 2 SUMMARY AND OUTLOOK

In summary, the selection and collocation of nanoparticles, the functionalization and modification of their surfaces, as well as the proper integration with other signal amplification methods greatly promote the detection performance of LFIA, which expands its application boundary. A complete LFIA test strip is basically composed of cassette, back pad, sample pad, conjugation pad, nitrocellulose membrane according to certain rules, with nanoprobe and antibodies loaded on specific sites. Each part undertakes the important function that cannot be replaced. Meanwhile, there exist close connections and mutual influences between different parts. As a result, researchers should take full account of the compatibility of all the parts before conducting innovative improvement of nanoparticles. It is worth noting that some studies focus on other engineering techniques such as the modification of membrane and the design of cassette structure. The influence of these modifications on nanoparticles should also be considered. Besides, researchers need to consider that how to take full advantage of the selected materials’s properties before developing signal enhancement strategies. As a platform with strong compatibility, LFIA exhibits superior combination potential with new technologies and materials. However, the practicability of new developed LFIA, including the dependence on equipment, the time consumption, the professional requirements for operators, as well as the cost and stability of manufacturing are all issues to be considered for future.

---

[307] LOU ET AL. VIEW
advances and a lot of work still needs to be done. For example, chemiluminescence-based LFIA has the potential to achieve higher detection sensitivity, but requires more operations, such as the addition of H$_2$O$_2$ and substrate, and longer time is required for antigen–antibody recognition and signal generation. In conclusion, it is necessary to evaluate the comprehensive performance of a new approach for better adaption to clinical analysis.

ACKNOWLEDGMENTS
The authors would greatly acknowledge the financial support by National Natural Science Foundation of China [Nos. 61821002, 82072067], Science and Technology Support Project of Jiangsu Province [No. BE2017763], Medical Research Project of Jiangsu Province Health Committee [No. K2019020], Special Fund for Transformation of Scientific and Technological Achievements of Jiangsu Province [No. BA2020016], Fundamental Research Funds for the Central Universities and Wenzhou Basic Research Projects (Nos. Y2020916, Y20201013), NUPTSF (No. NY220167); Jiangsu Provincial Double-Innovation Doctor Program (No. JSSCBS20210506).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ORCID
Yu Zhang @ https://orcid.org/0000-0002-0228-7979

REFERENCES
1. J. H. Soh, H. M. Chan, J. Y. Ying, Nano Today 2020, 30, 100831.
2. T. Mahmoudi, M. de la Guardia, B. Shirdel, A. Mokhtarzadeh, B. Baradaran, TrAC Trends Anal. Chem. 2019, 116, 13.
3. C. Y. Shi, N. Deng, J. J. Liang, K. N. Zhou, Q. Q. Fu, Y. Tang, Anal. Chim. Acta 2015, 854, 202.
4. L. Zhuang, Y. Ji, P. Tian, K. Wang, C. Kou, N. Gu, Y. Zhang, BMC Veterinary Research 2019, 15, 30.
5. K. Koczuła, A. Gallotta, Essays Biochem. 2016, 60, 111.
6. J. L. Wu, W. P. Tseng, C. H. Lin, T. F. Lee, M. Y. Chung, C. H. Huang, S. Y. Chen, P. R. Hsu, S. C. Chen, J. Infect. 2020, 81, 435.
7. A. Parihar, P. Ranjan, S. K. Sanghi, A. K. Srivastava, R. Khan, ACS Applied Bio Materials 2020, 3, 7326.
8. D. Wang, S. He, X. Wang, Y. Yan, J. Liu, S. Wu, S. Liu, Y. Le, M. Chen, L. Li, J. Zhang, L. Zhang, X. Hu, X. Zheng, J. Bai, Y. Zhang, Y. Zhang, M. Song, Y. Tang, Nature Biomedical Engineering 2020, 4, 1150.
9. G.-R. Han, H. J. Koo, H. Ki, M.-G. Kim, ACS Appl. Mater. Interfaces 2020, 12, 34564.
10. R. Banerjee, A. Jaiswal, Analyst 2018, 143, 1970.
11. S. H. Lee, J. Hwang, K. Kim, J. Jeon, S. Lee, J. Ko, J. Lee, M. Kang, D. R. Chung, J. Choo, Anal. Chem. 2019, 91, 12275.
12. X.-T. Kong, Z. Wang, A. Govorov, Adv. Opt. Mater. 2016, 5, 15.
13. X. Gao, J. Boryczka, S. Kasani, N. Wu, Anal. Chem. 2021, 93, 1326.
14. S. Bamrungsap, C. Apiwat, W. Chantima, T. Dharakul, N. Wiriyachaiporn, Microchim. Acta. 2014, 181, 223.
15. L. D. Chen, Y. Li, H. Y. Yuan, D. W. Pang, Ai Zheng 2006, 25, 651.
16. N. A. Taranova, A. N. Berlina, A. V. Zherdev, B. B. Dzantiev, Biosens. Bioelectron. 2015, 63, 255.
17. S. T. Selvan, P. K. Patra, C. Y. Ang, J. Y. Ying, Angew. Chem. Int. Ed. 2007, 46, 2448.
18. L. Ma, C. Tu, P. Le, S. Chitoor, S. J. Lim, M. U. Zahid, K. W. Teng, P. Ge, P. R. Selvin, A. M. Smith, Journal of American Chemistry Society 2016, 138, 3382.
19. Y. Ma, Y. Li, S. Ma, X. Zhong, Journal of Materials Chemistry B 2014, 2, 5043.
20. L. Huang, J. Jin, L. Ao, C. Jiang, Y. Zhang, H. M. Wen, J. Wang, H. Wang, J. Hu, ACS Appl. Mater. Interfaces 2020, 12, 58149.
21. Y. Yuan, W. Hou, W. Qin, C. Wu, Biomaterials Science 2021, 9, 328.
22. H. Lei, K. Wang, X. Ji, D. Cui, Sensors 2016, 16, 2130.
23. A. Sharma, A. I. Y. Tok, C. Lee, R. Ganapathy, P. Alagappan, B. Liedberg, Sens. Actuators B 2019, 285, 431.
24. Z. Wu, D. He, E. Xu, A. Jiao, M. F. J. Chughtai, Z. Jin, Food Chem. 2018, 269, 375.
25. W. Ren, I.-H. Cho, Z. Zhou, J. Irudayaraj, Chem. Commun. 2016, 52, 4930.
26. F. Mehdi, D. Rohila, Anal. Chem. 2020, 92, 15766.
27. T. Ji, X. Xu, X. Wang, N. Cao, X. Han, M. Wang, B. Chen, Z. Lin, H. Jia, M. Deng, Y. Xia, X. Guo, M. Lei, Z. Liu, Q. Zhou, G. Chen, ACS Nano 2020, 14, 16864.
28. W. Sun, X. Hu, J. Liu, Y. Zhang, J. Lu, L. Zeng, Biosci. Biotechnol. Biochem. 2017, 81, 1874.
29. Z. Li, H. Chen, P. Wang, Analyst 2019, 144, 3314.
30. H. Jung, S. H. Park, J. Lee, B. Lee, J. Park, Y. Seok, J.-H. Choi, M.-G. Kim, C.-S. Song, J. Lee, Anal. Chem. 2021, 93, 792.
31. P. D. Sinawang, Y. Rai, R. E. Ionescu, R. S. Marks, Biosens. Bioelectron. 2016, 77, 400.
32. J. Luan, A. Seth, R. Gupta, Z. Wang, P. Rathi, S. Cao, H. Gholami Derami, R. Tang, B. Xu, S. Achilefu, J. J. Morrissey, S. Singamaneni, Nature Biomedical Engineering 2020, 4, 518.
33. D. Lou, L. Fan, Y. Ji, N. Gu, Y. Zhang, Anal. Methods 2019, 11, 3906.
34. L. Zhuang, J. Gong, Y. Ji, P. Tian, F. Kong, H. Bai, N. Gu, Y. Zhang, Analyst 2020, 145, 2367.
35. A. K. Trilling, J. Beekwilder, H. Zuilhof, Analyst 2013, 138, 1619.
36. K. Treerattrakoon, W. Chanthima, C. Apiwat, T. Dharakul, S. Bamrungsap, Microchim. Acta 2017, 184, 1941.
37. M. Iijima, S. I. Kuroda, Biosens. Bioelectron. 2017, 89, 810.
38. D. Lou, L. Fan, Y. Cui, Y. Zhu, N. Gu, Y. Zhang, Anal. Chem. 2018, 90, 6502.
39. J. Baniukevic, J. Kirlyte, A. Ramanavicius, A. Ramanaviciene, Sens. Actuators B 2013, 189, 217.
40. J.-W. Park, I.-H. Cho, D. W. Moon, S.-H. Paek, T. G. Lee, Surf. Interface Anal. 2011, 43, 285.
41. A. K. Adak, B. Y. Li, L. D. Huang, T. W. Lin, T. C. Chang, K. C. Hwang, C. C. Lin, ACS Appl. Mater. Interfaces 2014, 6, 10452.
42. J. Hu, S. Zhou, L. Zeng, Q. Chen, H. Duan, X. Chen, X. Li, Y. Xiong, Talanta 2021, 223, 121723.
43. N. J. Alves, N. Mustafaoglu, B. Bilgicer, Biosens. Bioelectron. 2013, 49, 387.
44. H. Chen, Q. Mei, Y. Hou, K. Koh, J. Lee, B. Chen, L. Fang, X. Zhao, *Sens. Actuators B* 2013, 181, 38.
45. W. Zhang, J. Dong, Y. Wu, P. Cao, L. Song, M. Ma, N. Gu, Y. Zhang, *Colloids Surf. B* 2017, 154, 55.
46. G. Bodelón, S. Mourdikoudis, L. Yate, I. Pastoriza-Santos, J. Pérez-Juste, L. M. Liz-Marzán, *ACS Nano* 2014, 8, 6221.
47. D. Lou, L. Ji, L. Fan, Y. Ji, N. Gu, Y. Zhang, *Langmuir* 2019, 35, 4860.
48. S. Emaminejad, M. Javanmard, C. Gupta, S. Chang, R. W. Davis, R. T. Howe, *Proc. Natl. Acad. Sci.* 2015, 112, 9295.
49. B. N. Johnson, R. Mutharasan, *Langmuir* 2012, 28, 6928.
50. O. A. Goryacheva, C. Guhrrenz, K. Schneider, N. V. Beloglazova, I. Y. Goryacheva, S. De Saeger, N. Gaponik, *ACS Appl. Mater. Interfaces* 2020, 12, 24575.
51. J. Zhu, N. Zou, D. Zhu, J. Wang, Q. Jin, J. Zhao, H. Mao, *Clin Chem* 2011, 57, 1732.
52. D. H. Choi, S. K. Lee, Y. K. Oh, B. W. Bae, S. D. Lee, S. Kim, Y. B. Shin, M. G. Kim, *Biosens. Bioelectron.* 2010, 25, 1999.
53. N. Wiriyachaiporn, W. Maneeprakorn, C. Apiwat, T. Dhakaruk, *Microchim. Acta* 2015, 182, 85.
54. Z. Mei, W. Qu, Y. Deng, H. Chu, J. Cao, F. Xue, L. Zheng, H. S. El-Nezamic, Y. Wu, W. Chen, *Biosens. Bioelectron.* 2013, 49, 457.
55. X. Liu, C. Zhang, K. Liu, H. Wang, C. Lu, H. Li, K. Hua, J. Zhu, W. Hui, Y. Cui, X. Zhang, *Anal. Chem.* 2018, 90, 3430.
56. S. Zhang, Y. Cai, J. Zhang, X. Liu, L. He, L. Cheng, K. Hua, W. Hui, J. Zhu, Y. Wan, Y. Cui, *Nanoscale* 2020, 12, 10098.
57. Y. Chen, J. Sun, Y. Xianyu, B. Yin, Y. Niu, S. Wang, F. Cao, X. Zhang, Y. Wang, X. Jiang, *Nanoscale* 2016, 8, 15205.
58. C. Qin, W. Wen, X. Zhang, H. Gu, S. Wang, *Chem. Commun.* 2015, 51, 8273.
59. N. Cheng, Y. Song, M. M. A. Zeinhom, Y. C. Chang, L. Sheng, H. Li, D. Du, L. Li, M.-J. Zhu, Y. Luo, W. Xu, Y. Lin, *ACS Appl. Mater. Interfaces* 2017, 9, 40671.
60. D. Duan, K. Fan, D. Zhang, S. Tan, M. Liang, Y. Liu, J. Zhang, P. Zhang, W. Liu, X. Qiu, G. P. Kobinger, G. Fu Gao, X. Yan, *Biosens. Bioelectron.* 2015, 74, 134.
61. E. Fu, T. Liang, J. Houghtaling, S. Ramachandran, S. A. Ramsey, B. Lutz, P. Yager, *Anal. Chem.* 2011, 83, 7941.
62. Z. Ma, S.-F. Sui, *Angew. Chem. Int. Ed.* 2002, 41, 2176.
63. A. Sharma, A. I. Y. Tok, P. Alagappan, B. Liedberg, *Sens. Actuators B* 2020, 312, 127959.
64. M. O. Rodríguez, L. B. Covian, A. C. Garcia, M. C. Blanco-López, *Talanta* 2016, 148, 272.
65. J. Deng, M. Yang, J. Wu, W. Zhang, X. Jiang, *Anal. Chem.* 2018, 90, 9132.

**Author Biographies**

**Doudou Lou** received her PhD degree at Southeast University. She is now a young researcher at Wenzhou Medical University. Her research is focused on the design and development of LFIA devices for POCT application.

**Yu Zhang** received his PhD degree and is now a full professor at Southeast University. His current research interests include the design and transformation of LFIA devices for POCT application.

**How to cite this article:** D. Lou, L. Fan, T. Jiang, Y. Zhang, *VIEW* 2022, 3, 20200125.

https://doi.org/10.1002/VIW.20200125