Cell Reports
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Review

Pathogenic Virus Detection by Optical Nanobiosensors

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SUMMARY

The novel coronavirus pandemic is sweeping the world and causing global crises. The lack of effective methods of early diagnosis and accurate detection may result in severe infection as well as mortality. Therefore, it is urgently required that rapid, selective, and accurate techniques for detecting pathogenic viruses are developed. Nanotechnology-based biosensors are finding many applications in biological detection, which may address these issues and realize direct detection of molecular targets in real time. Among various nanoplatforms, optical nanobiosensors have aroused much interest due to their inherent advantages of high sensitivity and direct readout. In this review, a summary of recent progress on the optical biosensors based on nanotechnology for pathogenic virus detection is provided, with focus on quantum dots (QDs), upconversion nanoparticles (UCNPs), noble metal nanoparticles, and organic fluorescent molecules-based nanoprobes and chemiluminescence assays. These representative studies demonstrate appealing performance as biosensors and hold great promise for clinical diagnosis.

INTRODUCTION

Infectious diseases are posing an omnipresent threat to public health, especially the current outbreak of novel coronavirus disease 2019 (COVID-19). It is estimated by World Health Organization (WHO) that there are more than 76,382,044 confirmed COVID-19 cases and more than 1,702,128 deaths in more than 200 countries or territories around the world (as of 23 December 2020).[1,2] One of the underlying reasons for the high prevalence of these diseases is the lack of effective point-of-care detection methods. Therefore, rapid and reliable health technologies for multiple virus diagnosis are key to defend ourselves from grave threats. Detection of viruses or viral biomarkers in human body fluids and lung computed tomography (CT) scanning of patients based on specific imaging features are the two current available methods for viral infection diagnostics. The conventional virus detection methods include viral culture, reverse transcription-polymerase chain reaction (RT-PCR), and enzyme-linked immunosorbent assay (ELISA).[3–7] Nevertheless, most of these diagnostic methods suffer from drawbacks of being time consuming and labor intensive. Table 1 compares the merits and limitations of existing virus detection methods. It is known that virus cultivation is a tedious work, which needs well-trained personnel and specific methodologies. To perform RT-PCR assay, the viral RNA is first extracted and subsequently reverse transcribed into complementary DNA (cDNA). The cDNA is then amplified using polymerase chain reaction (PCR) for the purpose of detection. This RT-PCR technique has high sensitivity but needs expensive specialized equipment, complex sequence alignment primer design, and optimization of assay conditions. Besides, the testing process of RT-PCR technique is time consuming due to repeated cycle heating. ELISA is a rapid detection method that is established by a

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https://doi.org/10.1016/j.xcrp.2020.100288

Table 1. Comparison of Commonly Used Techniques in the Detection of Pathogenic Virus

Technique	Principle	Time	Advantages	Disadvantages	Ref.
PCR	nucleic acid	hours	●well-established ●small number of samples	●ease of contamination ●time consuming	[16,17]
ELISA	viral protein	hours	●highly specific	●low sensitivity ●high-quality sample preparation	[18]
Cell culturing	infectivity assay	days to weeks	●suitable for virus subtyping recovery of novel and divergent strains ●inexpensive	●contamination problems ●time consuming and labor intensive ●unavailable for immediate patient care	[19]
Electron microscopy	viral particles	hours	●rapid method	●well-trained personnel ●low specificity	[20]
Computed tomography (CT)	chest scanning	hours	●good basis for clinical diagnosis and treatment	●technical expertise ●centralized facilities	[21]

solid-phase enzyme immunoassay for detecting the viral antigen, but the relatively low sensitivity and the need for high-quality sample preparation limit its applications for on-site detection.[8] Owing to the lack of kit and false negative rate of RT-PCR at the beginning of the disease outbreak, chest CT scan was temporarily used as the clinical diagnosis of COVID-19. CT scan belongs to a non-invasive tool and involves multiple X-ray scans of the patient's chest at different angles to produce cross-sectional images, which are diverse and depend on the stage of infection after the onset of symptoms. The main disadvantage of CT for COVID-19 detection is its low specificity (25%), because its imaging characteristics overlap with those of other viral pneumonia. Compared with syndromic testing and CT scanning, molecular diagnostic techniques are more suitable for accurate diagnostics because they can directly target and identify specific pathogens.[9]

To overcome the problems associated with conventional diagnostic techniques, alternative robust methods should be developed for virus detection with high sensitivity and accuracy. Benefiting from the facile sensing mechanisms, nanostructure-based biosensors enjoying the superiorities of high specificity and sensitivity, label-free rapid and real-time detection have attracted extensive interest from both nanotechnology and biosensor research communities. Many scientists have been working on presenting new strategies based on advanced nanotechnology for the detection of viruses meticulously.[10] A nanomaterial-based biosensor is an analytical platform usually associated with biological elements, including enzymes, antibodies, nucleic acids, cell receptors, micro-organisms, etc. The device can produce various kinds of signals (electricity, light, pressure, magnetism, or heat) with the binding of detected targets, which can be used to detect low concentration samples with high sensitivity and specificity.[11,12] Among these nanostructured devices, optical nanobiosensor is one of the main techniques in nanobiosensing approaches due to its non-invasive nature, high sensitivity, direct detection, and easy coupling with other technology.[13,14] Numerous studies integrating optical biosensing system with nanotechnology have been reported in recent years. Benefiting from the intrinsic properties, optical nanobiosensors are capable of achieving early diagnosis and enhancing clinical outcomes.[15] The merits of optical nanobiosensors for virtues detection can be summarized as follows: (1) highly sensitive with super low limit of detection (LOD). (2) The naked eye readout assay can be designed, which realizes simple readout and fast diagnosis. (3) Recent development of optical technology lowers down the cost of portable laser and hence the testing instrument is cost

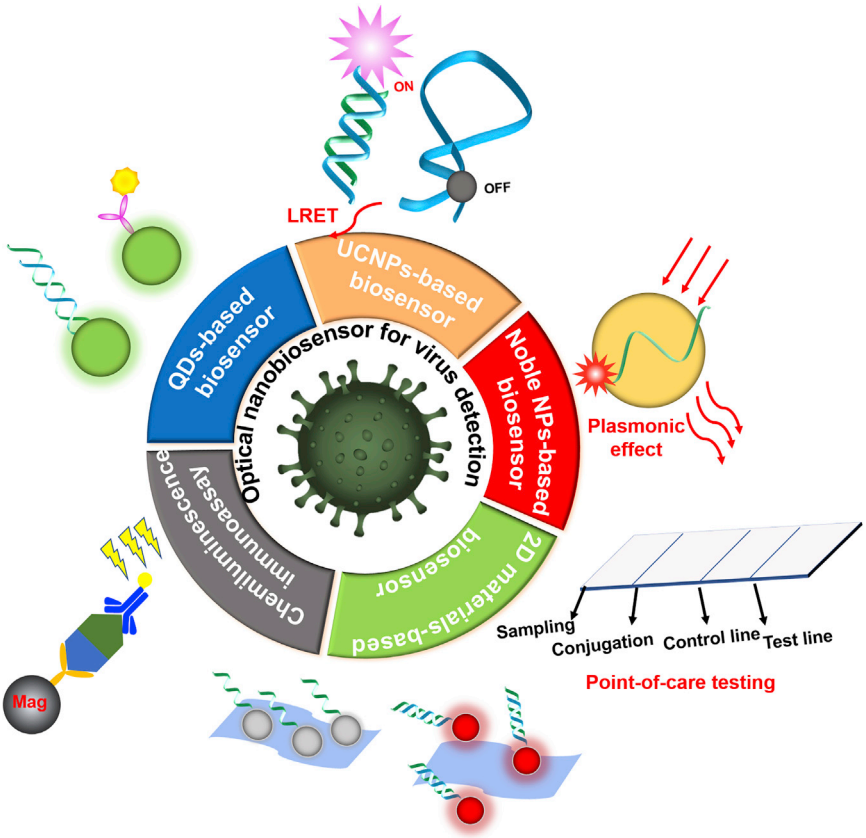


Figure 1. Schematic Diagram of Various Optical Nanobiosensors for Pathogenic Virus Detection
These biosensors are designed based on QDs, UCNPs, noble metal NPs, 2D-fluorescent organic molecules nanoprobes, and chemiluminescence immunoassay.

effective. And thus, in this review, we introduce the recent development of optical nanobiosensors for virus detection, especially those combined with organic fluorescent molecules, quantum dots (QDs), upconversion nanoparticles (UCNPs), gold nanoparticles (AuNPs), and magnetic particles (MPs)-chemiluminescence labels. We also illustrate the mechanism of these optical biosensors and give an overview of the researchers' efforts to improve device performance. Further to the introduction of various optical nanobiosensors, some challenges and outlook of optical nanobiosensors for virus detection are finally provided.

PRINCIPLES AND FIGURES OF MERIT

Fundamental Principles

The design principles for these optical nanobiosensors (Figure 1) primarily rely on the optical and luminescent properties of nanomaterials, luminescence resonance energy transfer (LRET), and surface plasmons (SPs) effects. The nanomaterials, including QDs, UCNPs, AuNPs, and organic dye-based NPs, used in the biosensors have intrinsic luminescence or plasmonic optical absorption characteristics. As for LRET-based biosensors, the fluorescence signal of the acceptor would be quenched or recovered when targeting the analyte. LRET is the process by which energy is transferred from the donor to the acceptor through resonance interaction, resulting in a decreased luminescence intensity of up donors and enhanced that of receptors (Figures 2A and 2B). Because the energy is transferred, the lifetime of the donor is reduced. Manipulating the spectral overlap and the distance

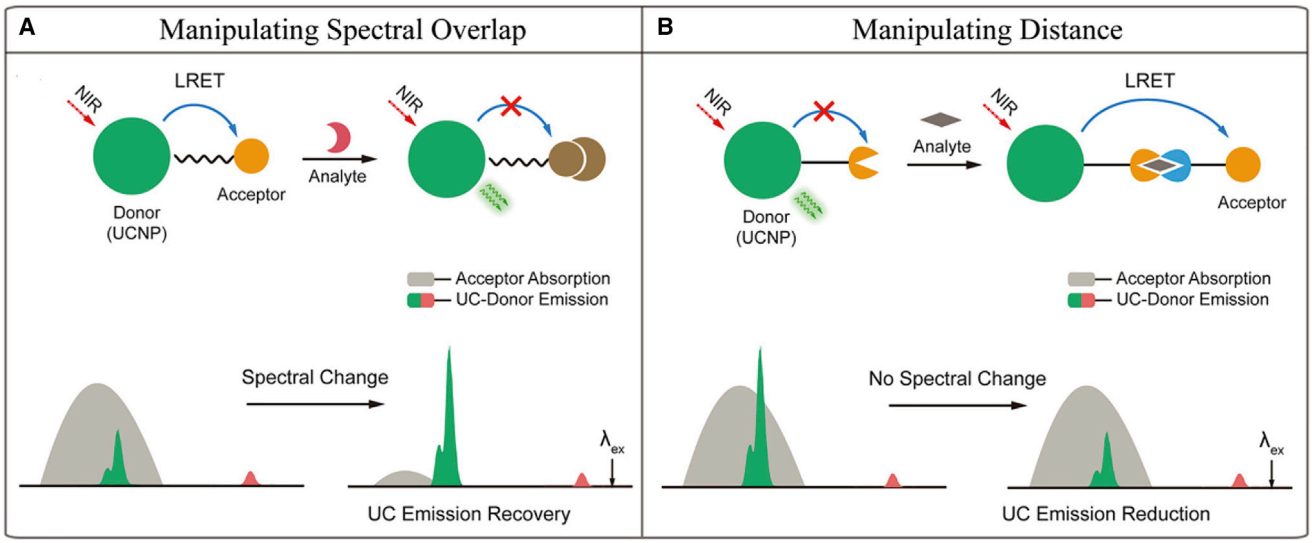


Figure 2. Schematic Diagrams of Main Strategies for the Fabrication of LRET-Based Optical Nanoprobes

(A) The emission is decreased by manipulating the distance between energy donor and acceptor upon addition of the analyte.

(B) The emission is recovered caused by a suppressed LRET process by manipulating the spectral overlap upon addition of the analyte. Reproduced with permission.[32] Copyright 2017, American Chemical Society.

between donor and acceptor are two main approaches for LRET-based biosensor to detect analytes.[22] Recently, surface plasmonic effect has been utilized to enhance the surface sensitivity of several spectroscopic measurements, including fluorescence, Raman scattering, and second-harmonic generation.[23,24] Surface plasmonic biosensors have been widely investigated in the fields of biotechnology, bioimaging, and biochemistry and used as sensing probes for infectious diseases diagnosis, protein-DNA interactions, ion sensing, and biological surface modification.[25–27] The detection mechanism of localized surface plasmon resonance (LSPR) is based on the local refractive index change caused by adsorbed molecules, thereby changing the resonance conditions of surface plasmon waves. Nevertheless, the mechanism of the enhancement effect of Raman scattering is still in debate. There are two primary theories, including electromagnetic theory and chemical theory, to explain the observed phenomena.[28,29] In electromagnetic enhancement, a plasmon is formed when the exciting radiation interacts with the surface electrons. The surface needs to be roughened to give a perpendicular plasmon oscillation and thereby creates plasmon energy, resulting in the Raman process on the analytes. The energy is then transferred back to the plasmon and the scattered radiation shifted in frequency can be detected by a spectrometer. In chemical theory, the molecules are bound to the metal surface to form a charge-transfer complex. By the interaction between the exciting radiation and the metal, an electron-hole pair and energy can be formed and transferred to the analyte. Then, the Raman phenomenon occurs on the analyte molecules and the energy is transferred back into the metal for scattering. The choice of surface metal depends on the plasmon resonance frequency. Chemiluminescence (CL) is the light emission excited by a chemical reaction, and CL immunoassay utilizes the CL principle to label the antibody. According to different physical chemistry mechanism of the light emission, CL immunoassay can be divided into several types based on various labeling mechanisms, including labeling chemical molecules directly involved in the light emission reaction, enzyme-catalyzed light emission reaction, and redox reaction-mediated light emission reaction.[30,31]

Figures of Merit

The assessment of the analytical methods is a crucial step in developing a biosensing system for real applications, and this procedure can be validated by assessing their figures of merit (FOMs). FOMs are constitutive of quantifiable terms that refer to the concepts concerning the methods, including analytes (i.e., sensitivity, selectivity, LOD, and signal-to-noise ratio [SNR]) and final results (i.e., traceability, uncertainty, and representativity).[33] FOMs are often used to characterize the performance of biosensor, and they are required to verify and ensure the quality of results. Specifically, sensitivity and selectivity are mostly used for assessing metrological performance of analytical methods. Besides, reliability and throughput also need to be considered for evaluating the usefulness of a specific biosensing system. The sensitivity of the biosensor is defined as the slope of the calibration curve. If an analytical method is sensitive, it means that a small change in the concentration of the analyte can result in a relatively large change in the response signal. The quality of selectivity is a very important indicator to assess the analysis method. The selectivity of the analytical method is not static and can be improved or enhanced in various ways. LOD of a biosensor is used to assess analytical capacity. LOD is usually the concentration or quantity derived from the minimum signal, which is k times the standard deviation (SD) of the blank. The value of k is a numerical factor selected based on the required confidence. If k is 3, the probability of getting a signal below 3SD from the blank will be higher than 95%. The SNR is one FOM that is the distance from analytical signal of the analyte and the instrumental noise, representing the ability of a biosensor to distinguish the signal from the noise level.[34]

NANOSTRUCTURED OPTICAL BIOSENSORS

Nanotechnology is hardly well defined and usually refers to the study of the properties and applications of materials with structure sizes in the range of 1–100 nm. Owing to the large surface-to-volume ratio of nanoplatforms, it is beneficial for interaction with analytes on the surface.[35] Therefore, it is very intriguing and attractive to detect the analytes with the nanoscale platforms. Nanostructured materials enjoy many attractive features with their quantum confinement effect, multifunctionality, and flexible performance. Nowadays, recent progress on the virus detection based on the optical nanomaterials has been made in the studies aiming at reducing the diagnosis cost and time. Furthermore, the application of nanomaterials in the construction of biosensors enables significant enhancement of the efficiency and sensitivity of these biosensing systems. In this review, we focus on the optical detection of viruses using various types of nanobiosensors, which are listed in Table 2.

QDs-Based Biosensors

QD, known as zero-dimensional (0D) nanomaterials, are semiconductor nanocrystals that have been widely investigated in bioimaging and biosensing.[42,43] QDs have promising characteristics, including (1) intriguing luminescence due to their small size and quantum effect and (2) size-dependent emission due to the quantum confinement effect. Smaller sized QDs exhibit increased confinement of the excitons and thus higher energy bandgap, resulting in the blue-shifted fluorescence. On the contrary, larger sized QDs of the same material demonstrate a smaller energy band gap with red-shifted light emission,[44] (3) superior photostability against blinking and photobleaching in comparison with other organic fluorescence agents, and (4) large surface area suitable for versatile bioconjugation and improvement of their water solubility, sensitivity, and biocompatibility.

Table 2. Comparisons of Optical Nanoprobes Based on Different Nanomaterials in the Detection of Various Viruses

Nanoprobes	Advantages	Disadvantages	Specification	LOD	Time	Ref.
QDs-based biosensors	●large anti-Stokes shifts ●cost effective	●possible breakage and damage of DNA ●highly cytotoxic in the oxidative environment	●rely on hybridization	1–200 nM	minutes to hours	[36,37]
UCNPs-based biosensors	●highly sensitive ●simple readout ●high photostability	●low upconversion luminescence efficiency ●complicated processing technique	●rely on hybridization ●use of spectrometers	60 fM–10 pM	minutes to hours	[38]
Gold immune-chromatographic assays	●safe sampling ●convenient and fast	●not suitable for the early diagnosis ●poor analytical sensitivity	●specific antigen or antibody ●whole blood or serum	500 pM–10 nM	minutes	[39]
Magnetic particle based Chemiluminescence (CL) immunoassays	●high specificity ●relatively fast	●background fluorescence ●tedious incubation and washing steps	●antigen or antibody ●CL labels ●CL substrates ●magnetic beads	50 fM–10 pM	hours	[40]
Organic fluorescent molecules-based assays	●relatively convenient	●high costs ●autofluorescence ●poor photostability	●spectrometers ●rely on efficient nanoquencher	25 pM–1 nM	minutes	[41]

Putting these features together offers an excellent possibility to bind QDs with some biomolecules, such as proteins (peptides, antibodies, or enzymes), oligonucleotides, and polymers.[45] In fact, QDs have been widely applied in biosensing and bioimaging applications, such as cell imaging,[46] tumor diagnosis,[47] genes therapy,[48] and biomarker detection.[49] In terms of virus detection, the luminescent QDs serve as useful platforms to provide rapid and sensitive detection and thus facilitate early diagnostics of pathogenic viral disease. For instance, Chen et al.[36] developed a QDs-based fluoroimmunoassay for rapid and sensitive detection of avian influenza virus (AIV) subtype H5N1 (Figure 3A). Liang and co-workers[50] developed a dual-emission ratiometric fluorescence biosensor for DNA detection based on water-soluble, red-emitting CdTe QDs and blue-emitting carbon dots (CDs) (Figure 3A). Mitoxantrone (MTX) is a synthetic anthraquinone drug that quenches the fluorescence of CdTe QDs by an electron transfer mechanism. However, MTX can be stripped from the surface of CdTe QDs and intercalated into the double helix structure of DNA, resulting in the recovery of QD fluorescence at 599 nm. Meanwhile, blue-emitting CDs serve as a reference for ratiometric detection of double-stranded DNA (dsDNA), which is not sensitive to both MTX and DNA, thereby maintaining constant fluorescence intensity.[50]

A digital single-virus immunoassay for multiplex virus detection was designed by Wu and co-workers,[51] and their virus immunoassay consists of a fluorescent magnetic multifunctional nanosphere, self-assembled by magnetic nanoparticles and QDs with different emission on the surface of copolymer. Due to the strong magnetic response ability of Fe_2O_3 nanospheres and distinguishable fluorescence imaging of QDs, this immunoassay can realize efficient capture and separation of targets without sample pretreatment and single-particle counting for ultrasensitive multiplexed virus detection (Figure 3B).[51] Interestingly, a fluorescent QDs testing strip combined with strand displacement amplification was designed by Deng and co-workers[52] for early diagnostics and management of AIDS. Two hairpin DNA sequences play an important role in their work. Part of the trigger sequence H1 can be complementary to HIV-DNA, and the remaining H1 sequence hybridizes with the QD modified sequence H2. Besides, the biotinylated target DNA and control

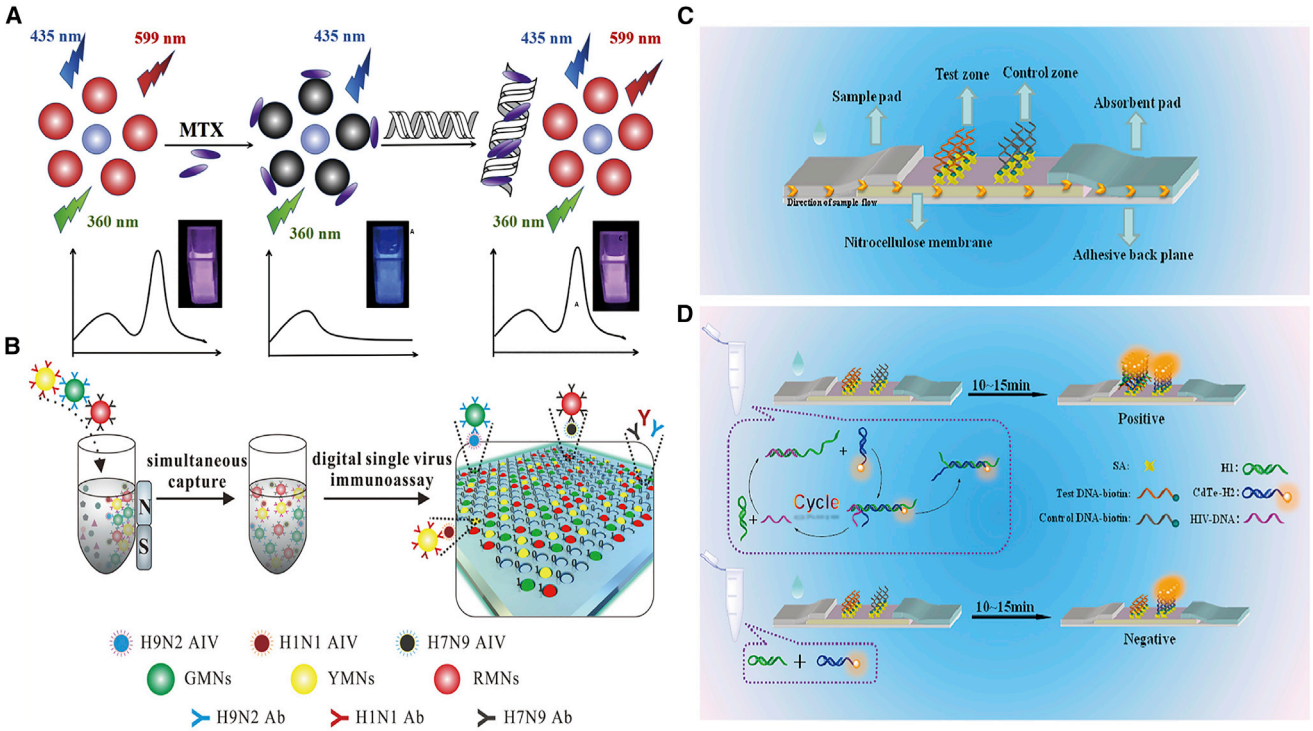


Figure 3. Schematic Diagrams of Different QDs-Based Biosensing Nanoplatforms for Virus Detection

(A) Schematic diagram of the CdTe-CDs-MTX system to detect dsDNA. Reproduced with permission.[50] Copyright 2017, Elsevier.

(B) Schematic diagram of the digital single-virus immunoassay for multiplex avian influenza virus detection. Reproduced with permission.[51] Copyright 2019, American Chemical Society.

(C) The detail of a lateral flow assay strip.

(D) Strand displacement amplification and positive or negative expression on strips.[51] Reproduced with permission.[52] Copyright 2018, Elsevier.

DNA were immobilized on membrane. The dropped sample fluid would get through the whole trip smoothly. When detecting an HIV-DNA target, both test zone and control zone have the signal, which indicates positive result, whereas the fluorescence signal only appearing in the control zone represents a negative result (Figures 3C and 3D). QDs have served as energy donors in virus detection system, and thus the luminous efficiency, photostability, fluorescence background, and energy transfer efficiency should be highly taken into consideration for designing and synthesizing the QDs-based biosensors.

UCNPs-Based Biosensors

In comparison to traditional down-conversion probes (organic dyes, QDs, etc.), the technological superiority of UCNPs-based nanoprobes includes minimal background fluorescence, low photodamage to the virus oligonucleotide probe, high photostability, large anti-Stokes shifts, and low toxicity.[53–57] Furthermore, the availability of relatively cheap and compact near-infrared (NIR) diode lasers as triggering sources further increases the convenience for on-site tests and improves detection limits, owing to the lower levels of autofluorescence and photodamage observed with excitations of short wavelengths.[58–60] Consequently, the UCNPs-based assays feature high sensitivity, facile readout, and rapid detection. In recent years, many researchers have been making their efforts to fabricate UCNPs-based probes for biodetetion.[15,61]

The design principles for upconversion optical sensor usually rely on LRET and the inner filter effect mechanism. The effective LRET generally occurs at approximately

1–10 nm.[62] According to their signal-detection formats, LRET-based upconversion nanobiosensors can be divided into two types, namely homogeneous assay and heterogeneous assay. The homogeneous assay is a liquid-phase assay that primarily relies on the efficiency of the LRET process and can be performed directly in the testing solution through a simple "mix-and-read" step without any tedious washing steps, which is a convenient bioassay method for fast detection in practical applications.[63–65] The heterogeneous assay refers to a solid-phase assay that has the advantages of specific recognition and high binding affinity between target and capture molecules anchored on a solid substrate for detection of trace amounts of targets.[66] To achieve biodetection in luminescent assays, the luminescence energy donor and energy acceptor are usually coated with the complementary oligonucleotide probes to facilitate the hybridization with the viral target oligonucleotide within a few hours. The luminescence intensity at various concentrations of targets manifests the sensing ability of the system. Previously, our team developed a homogeneous biosensor based on LRET from $BaGdF_5$: Yb/Er UCNPs to AuNPs for rapid and sensitive detection of H7 subtypes genes, demonstrating excellent sensitivity with a detection limit of 7 pM (Figure 4A). The quenching of UCNPs' fluorescence is due to the close proximity of the energy donor and acceptor caused by the hybridization process between the complementary strands of the H7 hemagglutinin gene and the nanoprobes.[10] Moreover, a recent study from Liu et al.[67] showed a simple and photostable "head-to-tail" sandwich structure that could efficiently shorten the distance between the donor and acceptor to detect long-chain HIV DNA sequences with high sensitivity and specificity, addressing the challenges of long-chain DNA detection. To improve the detection limit at the femtomolar level, our team developed a heterogeneous assay for Ebola viral gene detection by anchoring the UCNPs and AuNPs on a nanoporous alumina (NAAO) membrane.[38] Specifically, the branched polyetherimide (PEI) as the capping agent was used to modify the $BaGdF_5$:Yb/Er UCNPs to enhance the water dispersity. To engineer a heterogeneous assay, 3-ammopropyltriethoxysilane (APETS)-modified NAAOs were employed for UCNPs conjunction with glutaraldehyde as the linkage molecule. The high surface area to volume ratio of NAAO is beneficial for a large amount of UCNPs to conjugate on the surface of membrane. Under 980-nm-diode laser irradiation, the luminescent intensity of UCNPs at 540 nm was absorbed by AuNPs conjugated with target oligo, which was ascribed to the process of LRET between UCNPs and AuNPs. Interestingly, the detection limit of our developed heterogeneous assay is significantly improved compared to homogeneous one, exhibiting a remarkable value at the femtomolar level. The increased light-matter interaction throughout the nanopore walls of the NAAO membrane is the primary cause for the enhancement of sensitivity (Figure 4B).[38] The specificity test suggests that the optical nanoprobe is specific to Ebola viral gene sequence. Our developed strategy combining UCNPs, AuNPs, and NAAO membrane provides new insights into low-cost, rapid, and ultrasensitive detection of various diseases. Importantly, our team has explored the feasibility of clinical application by using inactivated virus samples. It is noted that the peak absorption of Yb^{3+} at 980 nm may lead to overheating in biological samples and induce DNA damage. Therefore, further work is required to find an effective strategy for avoiding 980-nm laser irradiation during hybridization.

Noble-Nanomaterials-Based Biosensors

Noble metal nanostructures, such as gold nanoparticles, silver nanoparticles, platinum nanoparticles, and palladium nanoparticles, have been extensively used in fabricating the sensing probes, owing to their intrinsic chemical and optical properties and surface plasmon resonance.[68] The noble metal nanostructures possess remarkable optical properties due to their unique interaction with light, which

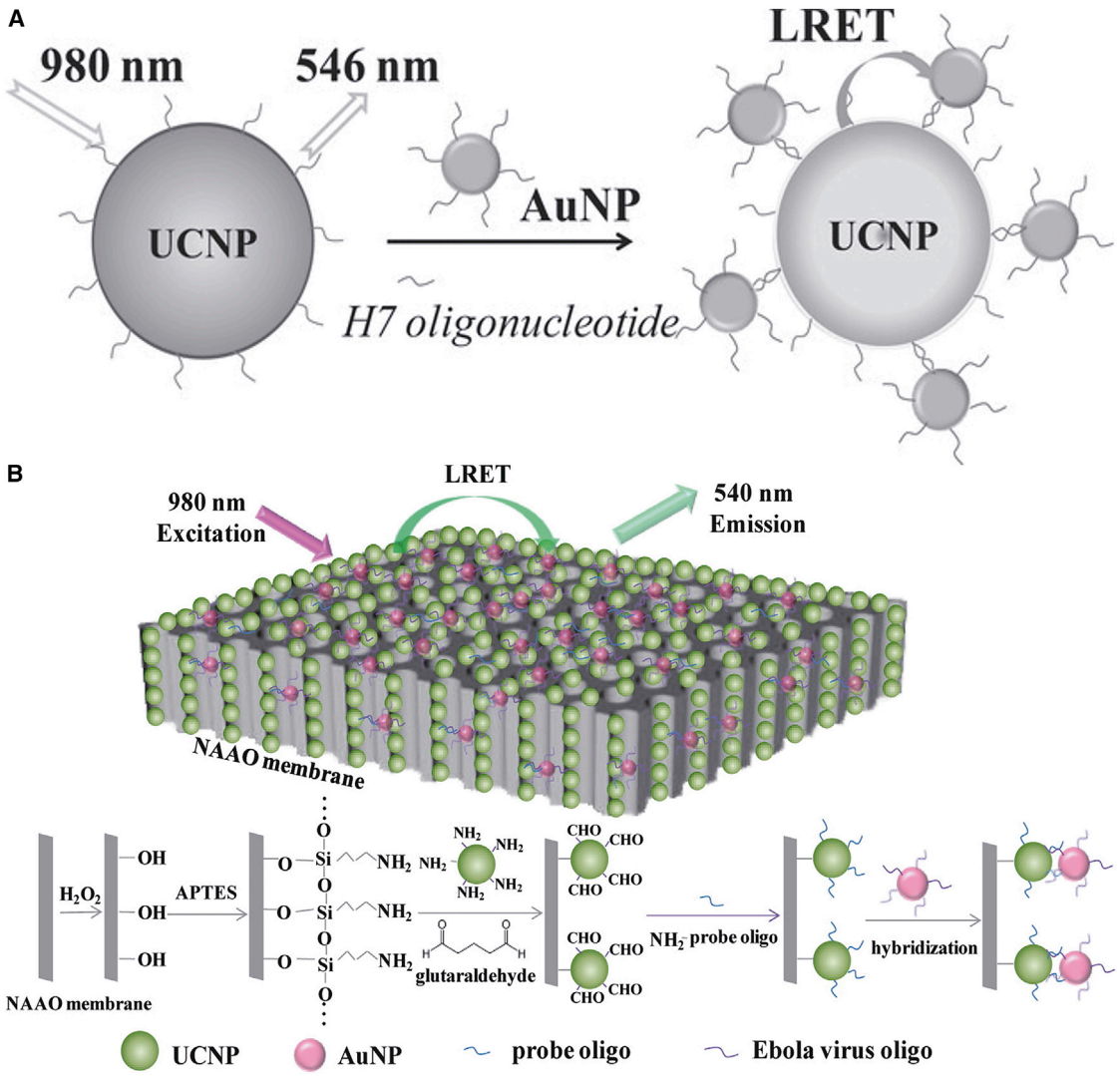


Figure 4. Schematic Diagrams of UCNPs-Based Biosensors Developed by Our Team for Virus Detection

(A) Homogeneous assay of H7 hemagglutinin gene detection by LRET biosensor based on energy transfer from $BaGdF_5$: Yb/Er UCNPs to AuNPs. Reproduced with permission.[10] Copyright 2014, John Wiley and Sons.

(B) Schematic diagram of Ebola target oligo detection based on LRET biosensor with energy transfer from UCNPs to AuNPs on NAAO membrane. Reproduced with permission.[38] Copyright 2016, American Chemical Society.

causes LSPR or the collective coherent oscillation of their free conduction band electrons. It leads to either radiative attenuation with a strong visible scattering of light or nonradiative attenuation that causes the conversion of photon energy into thermal energy. These two attenuation mechanisms have been widely utilized in diagnostics, bioimaging, and therapeutic applications.[69] The excellent surface stability of the above noble metal nanoparticles is one of the most advantageous properties in the biomedical application, which offers facile surface modification with different biological molecules.[70] Surface functionalization of NPs is relatively feasible to be achieved by non-covalent and covalent interactions. This not only replaces the initial stabilizers (such as hexadecyl trimethyl ammonium bromide), which may be potentially toxic, but also allows specific biological targeting modification for biological diagnostics applications.[71] For instance, the latest research has utilized LSPR technique to detect severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2).[72]

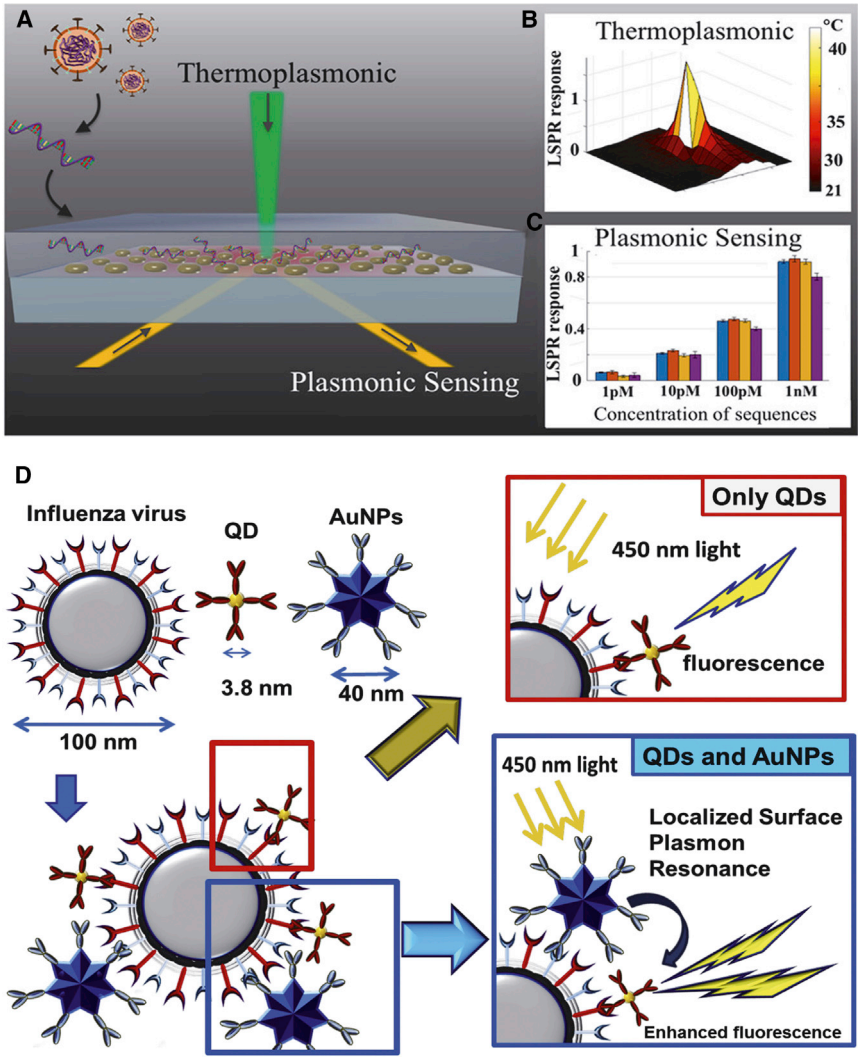


Figure 5. Schematic Diagrams of Surface Plasmonic Nanobiosensors for Virus Detection

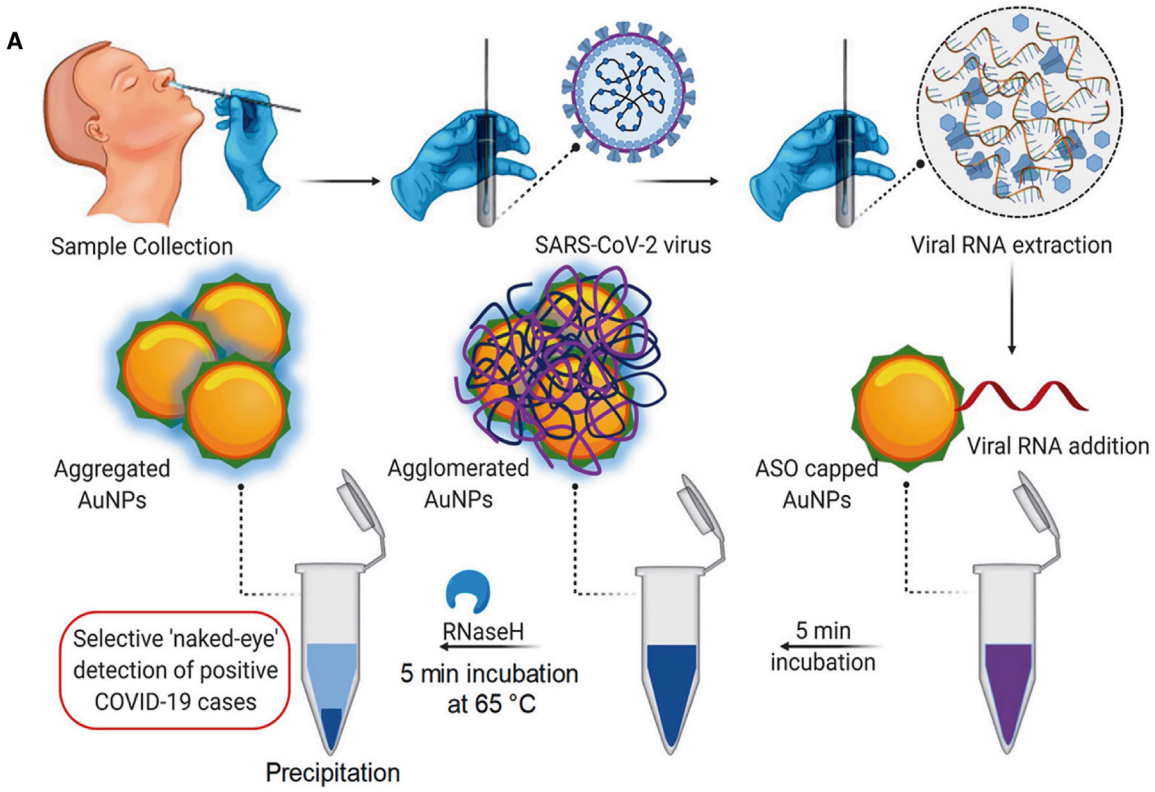
(A) Schematic representation of the dual-functional plasmonic photothermal (PPT) enhanced LSPR biosensing system.

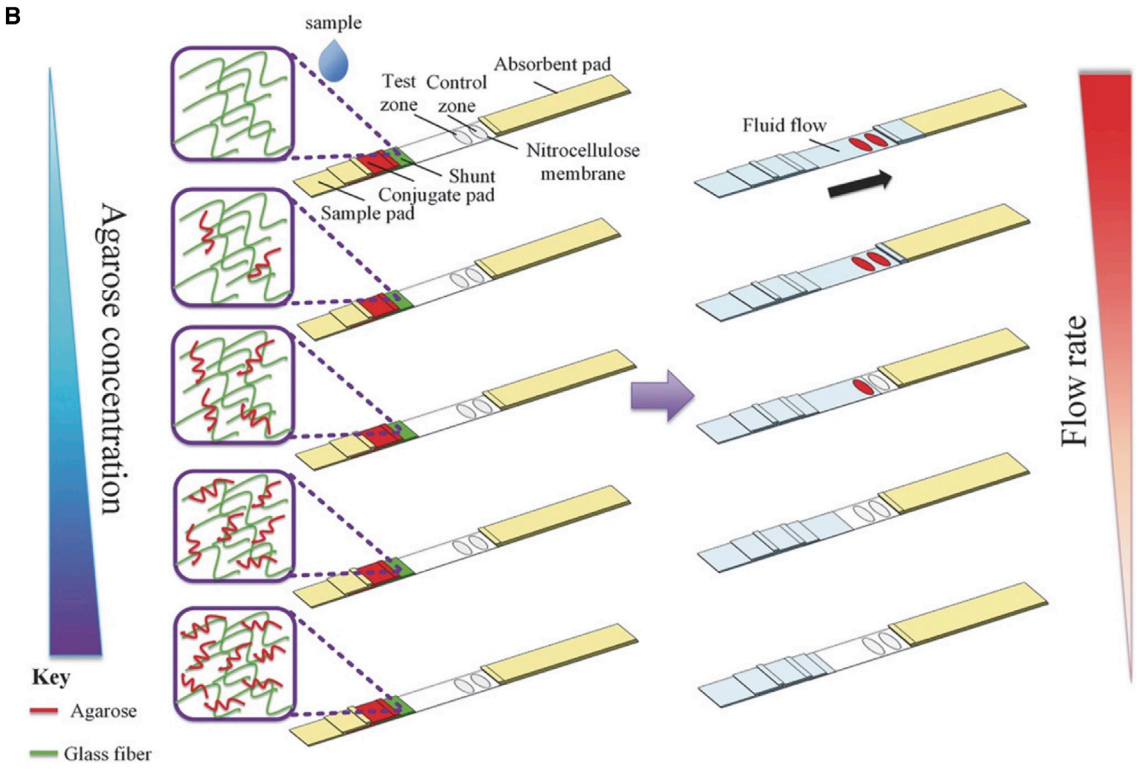
(B) Mapping the temperature distribution around the PPT heat source.

(C) Concentrations of various viral oligos measured using the dual-functional LSPR biosensors. Reproduced with permission.[72] Copyright 2020, American Chemical Society.

(D) Schematic representation of the detection principle for the influenza virus using the LSPR-induced fluorescence nanobiosensor. Reproduced with permission.[73] Copyright 2017, Elsevier.

The gold nanoislands were functionalized with cDNA sequences, realizing sensitive detection of selected sequences from SARS-CoV-2 by nucleic acid hybridization. To achieve better performance, Qiu et al. proposed a promising solution that combines the plasmonic photothermal effect with LSPR for clinical COVID-19 diagnosis. The localized photothermal heat was generated on the gold nanoislands when irradiated at plasmonic resonance frequency, which enhanced the hybridization temperature to facilitate the discrimination of the non-SARS-CoV-2 sequences (Figures 5A–5C). Takemura et al.[73] proposed an ultrasensitive, rapid, and specific LSPR-induced immunofluorescence nanobiosensor for the influenza virus detection based on AuNPs-induced QDs fluorescence signal enhancement. The antigen on the surface of the influenza virus interacts with the anti-neuraminidase antibody-AuNPs and the


A
Sample Collection
SARS-CoV-2 virus
Viral RNA extraction
Aggregated AuNPs
Agglomerated AuNPs
ASO capped AuNPs
Viral RNA addition
Selective 'naked-eye' detection of positive COVID-19 cases
RNaseH
5 min incubation at 65 °C
5 min incubation
Precipitation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Agarose
Glass fiber

anti-hemagglutinin antibody-QDs. The LSPR signal of the adjacent AuNP triggers the fluorescence enhancement of the QDs. The intensity change is proportional to the concentration of the target virus. The LOD of this LSPR-induced immunofluorescence nanobiosensor for influenza virus is 0.03 pg/mL in deionized water and 0.4 pg/mL in human serum (Figure 5D). LSPR is widely used in biosensing systems and applies to a variety of analytes of clinical interest. And surface plasmonic nanobiosensors are promising for detecting viruses in the air, but many future works are needed for further exploration in this research field. For example, it is essential to develop the devices that can inhale air and concentrate aerosols to release RNA from viruses.

The naked-eye readout has great potential for point-of-care diagnostics because of its easy operation for untrained personnel.[74] Many researchers have been dedicated to investigating the naked-eye readout assay for virus detection based on its easy accessibility and simplicity. Recently, DNA hybridization and protein testing are commonly studied in AuNPs-based readout assay. Gold immunochromatographic assay (GICA) is a new type of immunolabeling technology based on the specific antigen-antibody immunoreactions, where colloidal gold is negatively charged in a weak alkaline environment and forms a strong bond with positively charged protein molecules. The diagnosis process can be completed within 30 min. It is worth noting that GICA is not suitable for the early diagnosis of the disease due to the slow pace of the human antibody response to novel coronavirus influenza (about 5–7 days after infection).[75,76] Xia and co-authors[77] developed a smartphone-based point-of-care platform for the detection of avian influenza virus. After the capture of virus by the antibody conjugation, the colorimetric reaction based on Au/Ag NPs can detect the virus by the naked eye with a detection limit of 2.7×10^4 50% embryo infectious dose (EDI_{50})/mL, which is one order of magnitude higher than conventional detection method based on fluorescence ELISA. The smartphone imaging system with data processing capability further improves the detection limit, reaching a limit detection as low as 8×10^3 EID_{50}/mL.[77] In order to realize visual detection of nucleic acid, Moitra et al.[78] reported a colorimetric assay based on antisense oligonucleotides (ASOs)-modified AuNPs for specific detection of N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2 within 10 min from the isolated RNA samples (Figure 6A). In their strategy, four of the ASO sequences were selectively designed according to their binding disruption energies and their binding energies with the target N-gene. ASO1 and ASO3 sequences were functionalized with thiol terminal group at 5′ end, although ASO2 and ASO4 sequences were modified with thiol terminal group at 3′ end. These ASO sequences were utilized for capping AuNPs, resulting in the aggregation in the presence of N-gene sequences of SARS-CoV-2 by their complementary binding. The color change of the solution can be visualized by the naked eye.[78] Choi et al.[39] demonstrated an integrated paper-based biosensor incorporating nucleic acid extraction, amplification, and visual detection of Dengue virus (Figure 6B). In their assay, the biotinylated target DNA would bind to the AuNP-based sensing probes and further interact with streptavidin at the test zone, producing a red signal detectable by the naked eye. They created a hybrid substrate by incorporating agarose into the test strip to achieve flow control for optimal interactions with biomolecules. As compared to the unmodified test strip, the sensitivity of

Figure 6. Schematic Representation of Rapid Point-of-Care Assay for Virus Detection

(A) Schematic diagram for the selective naked-eye detection of SARS-CoV-2 RNA mediated by the suitably designed ASO-capped AuNPs. Reproduced with permission.[78] Copyright 2020, American Chemical Society.

(B) Schematic diagram of lateral flow assay based on paper-hydrogel-AuNPs hybrid material for sensitive point-of-care detection of dengue virus. Reproduced with permission.[39] Copyright 2016, John Wiley and Sons.

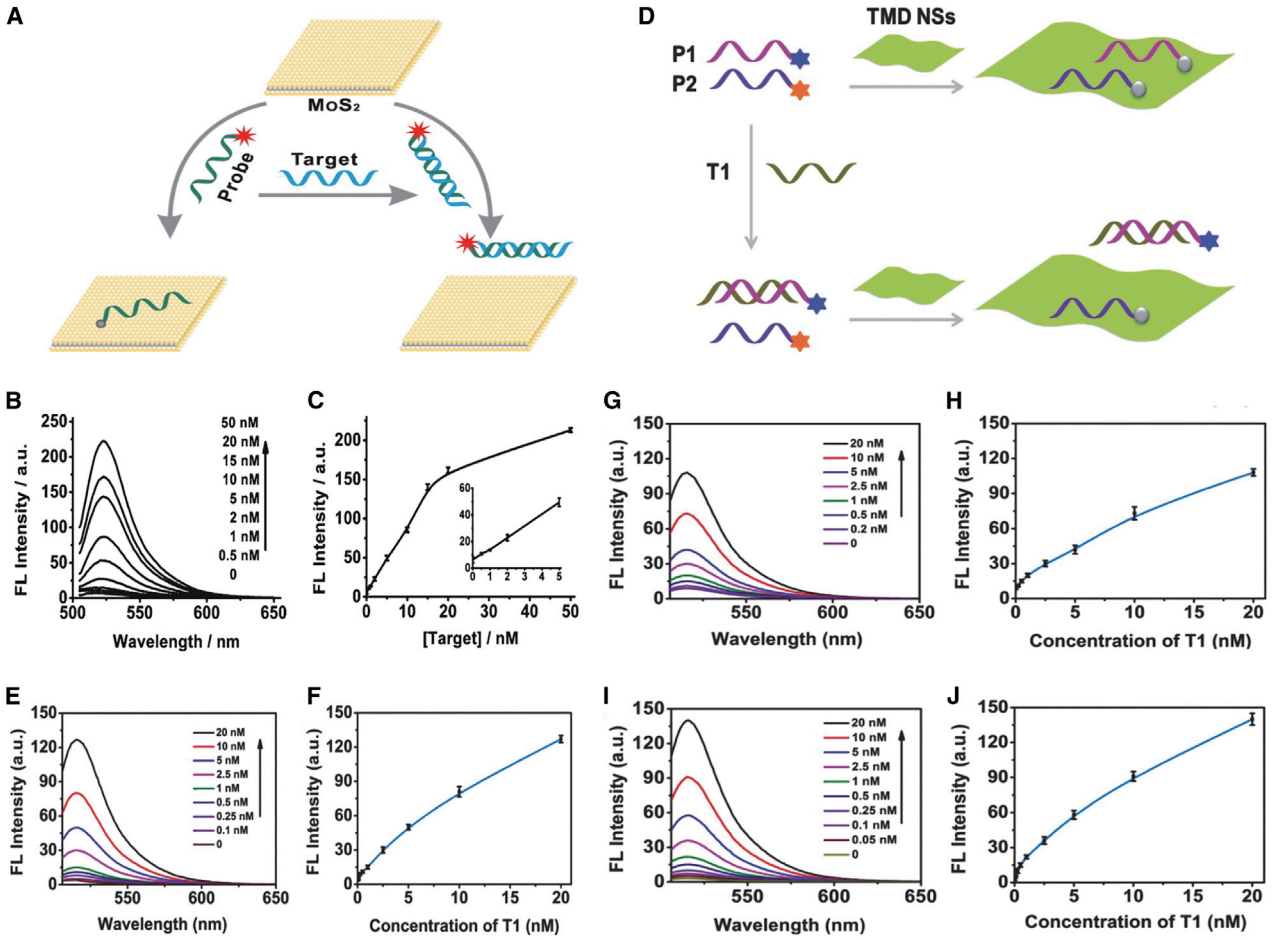

Figure 7. 2D-Materials-Based Fluorescence Nanobiosensor

(A) Schematic illustration of single-layer TMD NS-based fluorometric DNA assay.

(B) Fluorescence spectra of P1 (15 nM) in the presence of different concentrations of the T1.

(C) Calibration curve for DNA detection. Inset: amplification of the low concentration range of the calibration curve is shown. Excitation and emission wavelengths are 494 and 520 nm, respectively. Reproduced with permission.[82] Copyright 2013, American Chemical Society.

(D) Schematic illustration of single-layer TMD NS-based assay for multiplexed fluorescent DNA detection.

(E–J) The sensitivity analysis of three TMD NS-based nanosensors based on the different affinities toward ssDNA and dsDNA of MoS_2 (E and F), TiS_2 (G and H), and TaS_2 (I and J). Reproduced with permission.[41] Copyright 2014, John Wiley and Sons.

target detection was dramatically enhanced.[39] However, the use of the naked readout suffers from some shortcomings, such as low analytical sensitivity and poor quantification capability.

2D-Materials-Based Fluorescence Nanobiosensors

Recently, two-dimensional (2D) materials have received widespread attention due to their advantages of large surface area, good metallic conductivity, excellent biocompatibility, and characteristic absorption peaks, making them desirable for various biological applications.[79–81] On the other hand, dye-labeled single-stranded DNA (ssDNA) probes with high sensitivity and selectivity are usually used for the detection of target DNA or RNA, which mainly rely on the LRET via forming a fluorophore-quencher pair. Based on the excellent fluorescence-quenching ability and different affinity toward ssDNA versus dsDNA, Zhu et al.[82] fabricated the MoS_2 nanosheet (NS) as a sensing platform for the detection of DNA (Figure 7A). MoS_2 could adsorb dye-labeled, ssDNA probe via van der Waals (vdW) force and thus

quench the fluorescence of the dye. Conversely, when a ssDNA probe is hybridized with its complementary target DNA, the dye-labeled probe is detached from the surface of MoS_2 because the nucleobases are buried between the negatively charged helical phosphate backbones, resulting in recovery of the fluorescence signal of the probe. This DNA-sensing nanoplatform demonstrates a linear range from 0 to 15 nM, with a detection limit of 500 pM.[82] Later on, they systematically studied this sensing platform via utilization of single-layer transmembrane domain (TMD) nanosheets (MoS_2, TiS_2, and TaS_2) and dye-labeled ssDNA for the sensitive and selective detection of target DNA. Eventually, they successfully achieved multiplexed fluorescent detection of DNA by using a single-layer TaS_2 NS-based nanoprobe (Figures 7B–7J).[41] However, such optical biosensors based on 2D vdW materials have some drawbacks. For example, the target viral nucleic acid should be pre-labeled with expensive organic dyes, which does not apply to the detection of real virus samples. Besides, the nonuniformity of 2D material is another weakness of these biosensing systems, leading to poor reproducibility. Therefore, the uniform morphology and thickness of material are the priority when constructing 2D vdW materials-based fluorescence nanobiosensors for virus detection.

Chemiluminescence Immunoassays

In comparison to fluorescence, the CL immunoassay has an ultralow background and higher sensitivity. Consequently, CL-based detection is widely used in biosensors due to its high sensitivity, wide calibration range, and simple instrumentation. CL assays for DNA detection are generally performed using microfluidic sensors,[83] capillary electrophoreses,[84] magnetic particles,[85,86] and paper-based nanobiosensors.[87] Here, magnetic particle (MP)-based CL immunoassay biosensor is taken as an example for introducing the detection technique. MP-based CL immunoassay is an emerging analysis method that combines magnetic separation technology, CL technology, and immunoassay technology. Yang and coworkers[88] developed a novel and enhanced detection technique based on CL labels released from long spacer arm-functionalized magnetic particles (LSA-MPs) for the clinical detection of target pathogens. To solve the problem of attenuated CL signals caused by the inner filter-like effect observed with MPs, LSA was introduced to separate targets from MPs for enhanced CL detection. Benefited from its ultrasensitivity and wide detection range, this technique revealed a low detection limit of 50 fM with high selectivity and excellent reproducibility for HBV detection. Moreover, this strategy is also applicable to the detection of other infectious pathogens, such as hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum* (TP) (Figure 8).[88] Nevertheless, this CL-based nanobiosensor has some limitations, mainly related to the possibility that some proposed methods based on this technology could produce false-negative results. Because the human body may not have developed antibodies in the early stages of infection, this method has a limited time window of detection.

CONCLUSIONS AND OUTLOOK

The ongoing COVID-19 crisis has demonstrated the urgency of upgrading the clinical diagnostic technologies for virus detection with high accuracy, reliability, and speed. Optical nanotechnology has great potential in rapid virus detection and identification, genomic analysis, and serological studies. Herein, we outline the recent development of optical nanobiosensors based on QDs, UCNPs, noble metal NPs, 2D-fluorescent organic molecules nanoprobes, and CL immunoassays for the detection of various pathogenic viruses. The detection mechanisms of these nanobiosensors can be categorized according to (1) intrinsic luminescence of NPs or

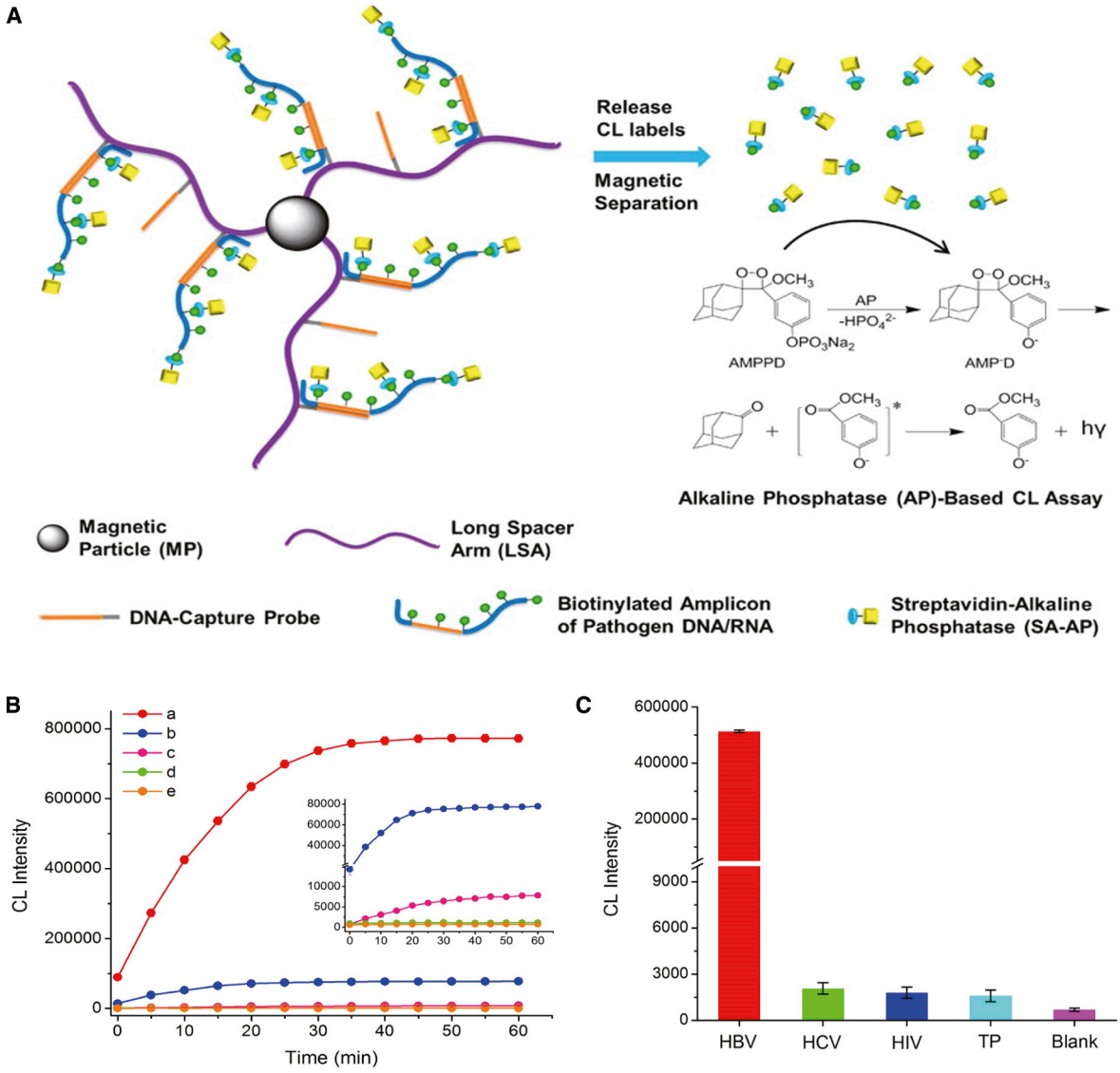

Figure 8. The Chemiluminescence Immunoassay for Virus Detection

(A) Diagram of enhanced chemiluminescent detection of a target pathogen based on released CL labels from long spacer arm-functionalized magnetic particles (LSA-MPs).

(B) CL dynamic curves representing CL detection of HBV amplicons based on LSA-MPs. CL detection of the released CL labels (a), LSA-MPs conjugated with CL labels (b), LSA-MPs remaining after CL labels are released (c), negative-strand HBV DNA (d), and deionized water (e) are shown.

(C) Specificity test of the developed detection technique. HBV, HCV, HIV, and TP were detected. Reproduced with permission.[88] Copyright 2015, American Chemical Society.

molecules, (2) LRET effect, (3) plasmonic effect, (4) colorimetric analysis, and (5) chemiluminescence. All these strategies are capable of enhancing sensitivity for both DNA/RNA and protein detections. Some elaborately designed nanoprobes have presented extremely low detection limits down to femtomolar level, which represents an improvement in sensitivity compared to conventional detection methods. Despite the good performance and successful demonstration for virus detection based on optical nanobiosensors, there are still many challenges that pose significant barriers to implement these diagnosis methods in the clinic applications. For example, the size of NPs should be carefully and precisely manipulated because

the properties of NPs are strongly dependent on the NPs size. Many issues should be taken into consideration for developing optical nanobiosensors, such as minimal background fluorescence, low photodamage to the virus oligonucleotide hybridization with probe, high photostability, and low phototoxicity of probes. Besides these, the issues of the toxicity, reproducibility, and throughput of the biosensors should also be addressed. Optical nanobiosensors have aroused great excitement due to their ability to detect a wide range of analytes at extremely low concentrations. With the development of this field, new types of low-cost, robust, reliable, point-of-care, and ultrasensitive diagnostic techniques are likely to emerge. And thus, a nanomaterials-based optical biosensing system with excellent stability, biocompatibility, reproducibility, and high sensitivity has broad prospects in the future.

ACKNOWLEDGMENTS

This study was supported by National Natural Science Foundation of China (NSFC) (grant no. 31771077), the internal funds of the Hong Kong Polytechnic University (grant no. 1-ZE1E), and the Research Grant Council (RGC) of Hong Kong (RGC GRF no. PolyU 153023/18P).

AUTHOR CONTRIBUTIONS

M.S. wrote the manuscript and prepared the figures. M.Y. and J.H. revised the manuscript and supervised the projects.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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