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ADVANCES IN NANOZYMES AS A PARADIGM FOR VIRAL DIAGNOSTICS AND THERAPY

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Running Title Page

Nanozymes for viral diagnosis and therapy

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ABBREVIATIONS :

AgNPs, Silver nanoparticles; AuNP, Gold nanoparticle; AuNP-LF, gold nanoparticle-based lateral-flow; AuNRs, Gold nanorods; AuNZs, Gold nanozymes; BSA, bovine serum albumin; CS, chitosan; CuNFs, copper nanoflowers; CuO, copper oxide; CeO_2, cerium oxide; EVD, Ebola virus disease; Fe_3O_4, iron oxide; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; HA, hemagglutinin; HRP, horseradish peroxidase; HCV, Hepatitis C virus; HEV, Hepatitis E virus; HIV, acquired immunodeficiency syndrome; IgM, immunoglobulin M; IgG, immunoglobulin G; IIF, indirect immunofluorescence; IAVs, influenza A viruses; *IL-6, interleukin-6;* LOD, limit of detection; mAbs, monoclonal antibodies; Mn_3O_4, Trimanganese tetraoxide; MnO_2, manganese dioxide; MNP, magnetic nanoparticle; MREs, molecular recognition elements; MagLISA, magnetic nanozyme-linked immunosorbent assay; 5′ NTR, 5′ nontranslated region; O_2, oxygen; O^{2-}, superoxide; PtNPs, Platinum nanoparticles; PDT, photodynamic therapy; POCT , point-of-care testing; Pd-Ir, Palladium-Iridium; PLGA, Poly(lactic-co-glycolic acid); ROS : reactive oxygen species; RT-qPCR, real-time quantitative polymerase chain reaction; RT-LAMP, RT-loop-mediated amplification; SARS-CoV-2 NP, SARS-CoV-2 nucleoprotein; SERS, surface-enhanced Raman scattering; SANs, single-atom Nanozymes; SPR, surface plasmonic resonance; SOD, superoxide dismutase; TMB, 3,3′,5,5′-tetramethylbenzidine; TNF-α , tumor necrosis factor-α; TiO_2, titanium oxide; V_2O_5 , vanadium pentaoxide; WIV, whole inactivated virus; ZIKV, Zika virus

Abstract

Over the last few decades, humankind has constantly encountered new viral species that create havoc in the socio-economic balance worldwide. Among the method to combat these novel viral infections, fast and Point-of-care diagnosis is of prime importance to contain the spreading of viral infections. However, most sensitive diagnostic systems for viral infections are time-consuming and require well-trained professionals, making it difficult for the patients. In recent years nanozymes emerged as promising therapeutic and fast diagnostic tools due to their multienzyme-like catalytic performance. Nanozymes can be designed using inorganic or organic components with tailorable physicochemical surface properties, enabling the attachment of various molecules and species on the surface of the nanozyme for specific recognition. In addition to the composition, the multienzyme-like catalytic performance can be modulated by the shape and size of the nanoparticles. Due to their multicatalytic abilities, nanozymes can be used for fast diagnosis and therapy for viral infections. Here we attempt to focus on the insights and recent explorations on the advances in designing various types of nanozymes as a theranostic tool for viral infections. Thus, this review intends to generate interest in the clinical translation of nanozymes as a theranostic tool for viral infections by providing knowledge about the multidisciplinary potential of nanozyme.

Significance Statement

The multienzyme-like properties of nanozymes suggest their role in diagnosing and treating various diseases. Although the potential roles of nanozymes for various viral infections have been studied in the last few decades, no review provides recent explorations on designing various types of nanozymes for the detection and treatment of viral infections. This review will provide insights into designing nanozymes to diagnose and treat viral infections, assisting future researchers in developing clinically translatable nanozymes to combat novel viral infections.

1. Introduction

Nanozymes are a class of synthetic nanoparticulate enzymes that possess the unique properties of nanomaterials and conventional enzymes due to their surface properties. Since it was discovered that the magnetic nanoparticles possess horseradish peroxidase (HRP) activity (Gao *et al.*, 2007), nanozymes have been widely explored for their various types of enzyme-mimicking activities, such as oxidase, catalase, and superoxide dismutase (SOD). It is reported that nanozymes are more efficient and catalytic than natural enzymes. In addition, the special characteristics exhibited by the nanozymes are extremely potent and functional in various fields (Zhang *et al.*, 2022). Thus, nanozymes are explored and used for their industrial applications.

The manufacture of natural enzymes has always been tedious owing to their fast denaturation in harsh chemical environments. Besides, manufacturing natural enzymes requires huge capital (Fedeli *et al.*, 2021). Nanozymes have successfully overcome the hindrances faced during the manufacture of natural enzymes. They maintain their catalytic activity even in a stressful environment. They are even cheaper than natural enzymes (Wu *et al.*, 2019; Ren *et al.*, 2022). They also show a very high yield and, unlike natural enzymes, do not get denatured easily. They also offer a greater surface area that increases their functionalities (Wei and Wang, 2013; P Wang *et al.*, 2020; Ma *et al.*, 2021). Thus, nanozymes are becoming more prevalent than natural biocatalysts (Niu *et al.*, 2020). Furthermore, the use of nanocomposites in these nanozymes helps to make these enzymes extraordinarily versatile. Thus, nanozymes are not only overcoming the limitations of natural enzymes but also giving the insight to possess and make the nanomaterials useful in a broader aspect in therapeutics and other domains of biomedical sciences (Jin *et al.*, 2021).

The nanozymes are structurally very flexible, and the major constituents of most of the nanozymes are carbon-based, metal-based, and metal oxides-based. The composition of nanozymes can be varied according to the needs by introducing several nanomaterials to modulate the catalytic activities of the nanozymes. In addition, environment-responsive nanozymes can also be designed by modulating their composition (Zhang *et al.*, 2021). Although nanozymes show various intrinsic enzyme-mimicking properties, the specificity and sensitivity of the nanozymes can be further altered by various additional modifications. For instance, nanozymes can be particularly targeted to act on specific disease sites, rendering greater specificity and reduced side effects (Y Jiang *et al.*, 2016; Y Huang *et al.*, 2019).

Viruses are the causative agents of various life-threatening diseases (Tobin *et al.*, 2011; Doss *et al.*, 2017; Bhattacharya *et al.*, 2020; Chakraborty, 2021; Chakraborty *et al.*, 2021). Almost 200 viruses have been known to date that are responsible for human diseases (Woolhouse *et al.*, 2012). In addition to treatment, containment of viral infection is a promising strategy to counter viral infections. A fast, sensitive, and specific diagnosis is the best possible route to contain the spreading virus. The viruses are composed of nucleic acids (DNA or RNA) enveloped with capsid proteins or lipid-protein complex membranes specific to the target virus (Wigginton and Kohn, 2012). Thus, the first approach to identifying viruses is based on their envelope proteins. The second approach to identifying viral infection is based on the specific immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies produced by viruses in the human body. For diagnostic purposes, nanozymes can be conjugated to the antibodies or with antigens binding to specific capsid proteins or IgM and IgG antibodies, respectively. Later, the enzyme-mimicking activities of the nanozymes can perform a colorimetric immunoassay to detect the viral load in the samples. This makes nanozymes suitable for the therapy and diagnosis of viral infections.

This review mainly aims to discuss the potent applications of nanozymes as an efficient therapeutic and diagnostic agents for various viral infections. It also informs the readers about the various classifications of nanozymes and their superiority and advantages compared to natural enzymes. Due to certain features of nanozymes, they can act as a therapeutic agent in treating a broad range of diseases other than viral infections. The broader aspect of enzyme mimicking and the colorimetric detection of pathogens have made them suitable for treating several viral infections.

II. Nanozymes based on composition

A. Metal nanoparticle-based nanozymes

As the name suggests, these nanozymes are composed of metal nanoparticles like gold (Au), platinum (Pt), silver (Ag), zirconium (Zr), and palladium (Pd). The metals mimic the role of cofactors of natural enzymes and provide the nanostructural base of the nanozymes (R Zhang *et al.*, 2020; Y Liu, Chen, *et al.*, 2021). We observed that similar to natural enzymes, metal nanozymes helped to neutralize environmental pollution (Alle *et al.*, 2021; Dadigala *et al.*, 2022) and speed up biochemical reactions to a greater extent for immunoassays (Alle *et al.*, 2022). In addition, metal-derived nanozymes can also be employed as an efficient anti-inflammatory agent and perform tumor catalytic therapy (Xu *et al.*, 2022).

Moreover, metal nanozymes are easy to synthesize and can be altered without much hindrance. However, metal nanozymes have some limitations because they are toxic and tend to aggregate quickly (Pattadar *et al.*, 2021; Wu *et al.*, 2021). To avoid the aggregation and toxicity of metal nanoparticles, they are grown on biocompatible supports or encapsulated by biomolecules (Alle *et al.*, 2022). Nevertheless, these nanozymes can still perform enzymatic activities to their full potential. Due to these properties, metals coupled with several organic ligands also show great

superiority because these metals are present in the catalytic cores of the nanozymes and play an important role in transferring electrons (L Wang *et al.*, 2020), enabling versatile enzymatic properties to metal-based nanozymes. Some of the metal-based nanozymes are discussed below.

1. Gold nanoparticle-based nanozymes. Among metal-based nanozymes, Au nanozymes are the most extensively explored due to their ability to generate ROS. The catalytic property of gold nanoparticles (AuNPs) was first reported in the 1970s by Parravano's group (Cha and Parravano, 1970). Since then, the AuNPs have been explored as nanozymes for various catalytic activities (Lin *et al.*, 2014; Lou-Franco *et al.*, 2020), such as peroxidase (Jv *et al.*, 2010), oxidase (Tao *et al.*, 2015), glucose oxidase (Comotti *et al.*, 2004; Luo *et al.*, 2010), SOD (He *et al.*, 2013), catalase (Tseng *et al.*, 2012), and reductase (Pradhan *et al.*, 2001). The exceptional property of the Au nanozymes to mimic the properties of a wide range of enzymes makes them new candidates to be employed in several fields (Saha *et al.*, 2012; Lou-Franco *et al.*, 2020; Sharifi *et al.*, 2020).

2. Silver nanoparticle-based nanozymes. Besides Au, Ag-based nanozymes have also been reported to possess an intrinsic enzymatic property. For example, it has been shown that Ag-based nanozymes mimic the peroxidase activity and thus can be used in ELISA assay (Sloan-Dennison *et al.*, 2017). Besides, the catalytic mechanism of these AuNPs also helps in colorimetric assays. Furthermore, the Ag nanozymes also possess a unique light scattering phenomenon known as Rayleigh resonance scattering, which also helps in sensing ions for detecting biological molecules (G-L Wang *et al.*, 2012).

3. Platinum nanoparticle-based nanozymes. It has been found that Pt nanoparticles (PtNPs) also possess various types of enzyme-like activities, such as SOD-like, catalase-like, oxidase-like, and peroxidase-like (Ma *et al.*, 2011), which are affected by the size and shape of the nanoparticles (Narayanan and El-Sayed, 2004). The Pt-based nanozymes show unique scavenging

mechanisms of cellular ROS (Moglianetti *et al.*, 2016). The hydroxyl radical is a strong oxidant among ROS, which is also scavenged by the Pt nanozymes. Thus, protecting the cells from the oxidative stress generated due to SOD and catalase-mimicking activity (Gunes *et al.*, 2021). Thus, owing to the specialized mechanism of Pt nanozymes to enhance the decomposition of H_2O_2 and ROS, these nanozymes are employed as antioxidants (Gunes *et al.*, 2021). However, despite many advantages, PtNPs are less used due to their unstable nature, causing agglomeration. To avoid the aggregation of PtNPs, the surface of PtNPs can be fabricated with Au clusters, which also increases the oxidation potential of Pt nanoparticles (Zhang *et al.*, 2007).

4. Bimetallic nanoparticle-based nanozymes. The bimetallic nanoparticles have unique biological and physicochemical properties. It has been suggested that the catalytic activities of metallic Nanozymes are enhanced by fabricating bimetallic or multimetallic Nanozymes due to the synergistic or additive effects (Toshima and Yonezawa, 1998). Further studies showed that the bimetallic core-shell (Gao *et al.*, 2015; T Jiang *et al.*, 2016) and alloy (Wang *et al.*, 2016; He *et al.*, 2017) nanostructures could enhance the enzymatic activity of the metallic nanozyme, which depends on their composition, size, and morphology.

They can be synthesized through various methods, including thermal decomposition (Toshima and Yonezawa, 1998), microwave (Toshima and Yonezawa, 1998), seeded growth (Chen *et al.*, 2013), co-reduction (Toshima and Yonezawa, 1998), and galvanic displacement (Bansal *et al.*, 2008; Xia *et al.*, 2013; Anderson *et al.*, 2019; Bhanushali *et al.*, 2020), which can affect the catalytic activity of the nanocomposite. For example, Liu et al. demonstrated that the introduction of copper (Cu) in the Pt bimetallic system, i.e., PVP–Pt–Cu nanoparticle clusters, enhances the catalytic activity of the Pt nanozymes to scavenge H_2O_2, $^{\cdot}OH$, and superoxide anions due to the synergistic effect of Cu and Pt (Y Liu, Qing, *et al.*, 2021). In another study, Cu-Pt

nanozyme was fabricated for colorimetric glucose detection in urine using the galvanic displacement method, where Cu was used as a sacrificial template to enhance the ambient stability of the nanozyme (Naveen Prasad *et al.*, 2022).

Covering the surface of Au with Pt to form Au-Pt core-shell nanoparticles is also considered a well-known bio-materials for many applications (Wu *et al.*, 2018). For example, Pt nanodots coated on Au nanorods (AuNRs) (Au@Pt nanostructures) exhibited intrinsic enzyme-like (peroxidase-, oxidase-, ferroxidase-, and catalase-like) activities (He *et al.*, 2011; Liu *et al.*, 2011, 2012). Thus, it can be suggested that bimetallic nanozymes are among the best-suitable enzyme-mimic candidates in immunoassays and therapeutics due to their better stability, easy preparation, low cost, and tunable catalytic activities. However, there is still a scope to understand the more comprehensive mechanism to fully exploit the biocatalytic capacities and fulfill the growing demands in clinical practices.

B. Metal oxide nanoparticles-based nanozymes.

The metal oxide-based nanozymes can also perform oxidases, catalases, or peroxidase activities. Compared to meta-based nanozymes, metal oxides possess additional functional groups that could increase their catalytic efficiency. These functional groups are mostly situated at the surface of the nanozymes, making it easier to amend according to the needs. Besides, as they are oxides, many additional functional groups can be added to increase the catalytic efficiency of the nanozymes. Furthermore, metal oxide nanoparticles have various advantages, such as the properties like solubility, biodegradable nature, and several other mechanical characteristics of metal oxide nanoparticles can be altered, making them suitable as an efficient therapeutic and diagnostic agent (Q Liu, Zhang, *et al.*, 2021). Some of the examples of this subclass include

manganese dioxide (MnO_2) (Zhao *et al.*, 2022), copper oxide (CuO) (Chen *et al.*, 2022), iron oxide (Fe_3O_4) (Guo *et al.*, 2022), vanadium pentaoxide (V_2O_5) (Li et al., 2022), and nanoceria (Singh *et al.*, 2017).

C. Carbon nanostructures-based nanozymes.

The nanoparticles derived from carbon, namely graphene, carbon nanodots, carbon nanotubes, carbon nanospheres, and fullerene, are among the most efficient class of enzyme-mimicking nanozymes. Carbon-based nanozymes can show peroxidase-like and SOD-like activities (Song *et al.*, 2010; Wei and Wang, 2013). The presence of carbon in these nanozymes allows for many modifications, making them suitable for environmental biotechnology. Furthermore, carbon-based nanomaterials have high catalytic and extraordinary physicochemical properties due to their high mechanical strength, biocompatibility, unique structure, large surface area, and chemical stability. As a result, carbon-based nanomaterials are extremely versatile in nature and promising materials for several advanced biomedical applications.

The several modifications in graphene and carbon nanospheres with the heme groups or the introduction of nitrogen atoms make these nanozymes functional in determining the presence of glucose. The introduction of the heme groups also increases the loading volume of the nanozymes (Xue *et al.*, 2012). One of the leading examples of nanozyme made from carbon is the carbon dots. The carbon dots loaded with the nanoparticles make it easier for surface manipulation and doping (Bandi *et al.*, 2022). Due to these extraordinary properties and stable structural conformation, carbon-based nanozymes are widely used in biomedicine (C Yang *et al.*, 2020; Jin *et al.*, 2021).

III. Nanozymes based on enzymatic activities

Based on the types of activities, nanozymes can again be broadly segregated into five major subclasses: peroxidase, catalase, oxidase, SOD, and cleavage. Nanozymes with peroxidase and oxidase activities generate ROS, while nanozymes with SOD and catalase activities scavenge ROS. Below is a brief account of each type of enzymatic activity and its therapeutic applications.

A. Peroxidase and oxidase.

Peroxidase activity of nanozymes is the most widely studied activity, followed by oxidase activity. The first insight into the nanozymes showing peroxidase activity was reported in 2007 by Gao et al. (Gao *et al.*, 2007). They reported that the magnetite nanoparticles have an intrinsic enzyme mimetic activity similar to the natural peroxidases. Thus, the magnetic nanoparticles can catalyze the oxidation of organic substrate to produce colour change which can be used as a detection tool. Furthermore, several nanomaterials made up of transition elements like vanadium, Au, Ag, Pt, Cu, and molybdenum also showed peroxidase-like activities, enhancing the catalytic efficiencies of the nanozymes. Besides peroxidase, oxidases also hold promising prospects as facile and biocompatible components in biosensor design since they can catalyze the oxidation of substrates by employing ambient oxygen as an electron acceptor.

Further, it was suggested that the peroxidase-mimicking Nanozymes (i.e., metal oxides, carbon materials, and noble metal nanoparticles) could overcome the drawbacks of protein-based enzymes, such as HRP. The HRP is a well-known protein-based enzyme that can convert 3,3′,5,5′-tetramethylbenzidine (TMB) into a blue-colored product. Although HRP, in conjugation with antibodies, is a widely used protein enzyme in immunoassays, it has inherent drawbacks, such as

the complexity of purification and preparation, susceptibility to protease digestion, denaturation, and loss of catalytic activity upon storage (Li and Li, 2021).

It has been shown that the peroxidase-like activity of silver nanoparticles (AgNPs), which form the core of Ag nanozymes, oxidize TMB and hydrogen peroxide (H_2O_2) by a two-step oxidation process involving two electrons (Josephy *et al.*, 1982; Sloan-Dennison *et al.*, 2017). This mechanism of peroxidase mimicking nanozymes is paving the way to replace conjugated antibodies employed in conventional ELISA. Wang *et al.* found that bovine serum albumin (BSA)-protected Ag nanoclusters are stimulated by Hg^{2+} to mimic oxidase-like activity by generating superoxide anions for TMB oxidation (G-L Wang *et al.*, 2015). Thus, the peroxidase-like and oxidase-like activity of the Ag nanozymes can be utilized for specific colorimetric assays and thus can be used as a diagnostic agent for detecting several disease biomarkers (Fan *et al.*, 2012).

AuNPs also possess intrinsic peroxidase-like activity that oxidizes TMB in the presence of H_2O_2. Moreover, due to the high affinity of AuNPs towards the TMB substrate, Au nanozymes displayed higher catalytic activity than HRP (Das et al., 2022). Similarly, AuNPs also possess oxidase-like activity that can generate singlet oxygen ($^1O_2$), hydroxyl radicals (·OH), and superoxide (O_2^-) (Lou-Franco *et al.*, 2020). Au nanozymes can exploit their oxidase-mimicking activity to oxidize glucose, similar to glucose oxidase enzymes (Chen *et al.*, 2021). The reaction of hydrated glucose molecules with the surface of gold atoms results in the formation of an electron-rich gold atom which undergoes a nucleophilic reaction and activates molecular oxygen. Thus, a dioxogold intermediate is formed, which acts as a bridge between electron transfer from glucose to dioxygen (Comotti *et al.*, 2006). These studies suggest that Au nanozymes could be highly sensitive and specific cost-effective peroxidase- and oxidase-mimicking colorimetric biosensing platforms to detect analytes with the naked eye.

The nanoceria or Ce-based nanozyme derived from metal oxide also showed to mimic different enzymatic activities, such as peroxidase and oxidase. It facilitates the oxidation of several moieties under an acidic environment (Asati *et al.*, 2009). However, the enzymatic activities of nanoceria are dependent on their size and morphology. Besides nanoceria, Fe_3O_4-based and V_2O_5-based nanoparticles mimic peroxidase-like and GPX-like activity, respectively (Vernekar *et al.*, 2014; Gao *et al.*, 2016). Another nanozyme derived from molybdenum, i.e., Molybdenum trioxide nanoparticles (MoO_3 nanoparticles), shows oxidase-like activities. These nanozymes were efficient in treating diseases caused due to the lack of sulfite oxidase, which catalyzes sulfite oxidation to sulfate in the amino acid and lipid metabolism (Ragg *et al.*, 2014).

It has been evidenced that carbon-based nanozymes also possess peroxidase activities. Quantum dots, composed of graphenes, are among the most commonly used carbon-based nanozymes that have shown considerable peroxidase activities, which can be illustrated in detecting diseases (Song *et al.*, 2010; Garg and Bisht, 2016; Sun *et al.*, 2018). In addition to metal-based and carbon-based nanomaterials, the oxides of graphene are also capable of imitating the peroxidase property of natural enzymes (C Liu, Zhao, *et al.*, 2021). It is reported that peroxidase-mimicking nanozymes are more stable, can enhance the peroxidase activity, and can easily be used as biosensors by conjugating with selective aptamers or antibodies (Tao *et al.*, 2020).

B. Catalase and Superoxide dismutase.

The enzyme catalase naturally occurs in aerobic organisms that convert H_2O_2 into O_2 (Guan and Scandalios, 1995). Therefore, catalase is used as a biomarker to determine the status of various pathological conditions and toxicities (Fransen *et al.*, 2012). In addition to naturally occurring catalase, nanozymes with catalase-like activities have gained interest in therapies and diagnostics

due to their easy synthesis procedure, low cost, and high stability (Jiang *et al.*, 2019; Wu *et al.*, 2019). Owing to these advantages, nanozymes have been studied against various oxidative-related diseases (Y Zhang *et al.*, 2020). For example, the Pt nanozymes have successfully mimicked the catalase enzymes by reacting with H_2O_2, producing oxygen which can serve as an effective therapeutic agent against tumor cells (Y Li *et al.*, 2019). A closer look at the mechanism of Pt nanozymes revealed its capability of mimicking catalase and SOD, which alters the activities of several cytokines, including the excessive production of tumor necrosis factor-α (TNF-*α*), interleukin-6 (IL-6), etc. (Zhu *et al.*, 2019).

Nanozymes with catalase activities can be designed using organic and inorganic nanomaterials. These nanozymes play a therapeutic role and can be used for cellular protection via ROS scavenging (Q Wang *et al.*, 2021). Recently, metal-organic frameworks were designed as catalase-mimicking nanozymes to alleviate tumor hypoxia by generating O_2, increasing the nutrient consumption in the tumor, and resulting in an increased apoptotic rate in tumor cells (Zhang *et al.*, 2013; Y Zhang *et al.*, 2018). In addition, various inorganic nanoparticles, such as MnO_2 (Zhu *et al.*, 2016), Pt (Y Zhang *et al.*, 2018), and Au (Liu *et al.*, 2017) nanoparticles-based nanozymes have been explored for anticancer therapy *via* producing O_2 to enhance tumor-specific photodynamic therapy (PDT). Furthermore, besides cancer, the catalase-like activity of Mn_3O_4-nanoflower (Yao *et al.*, 2018) and cerium oxide (CeO_2) (Pirmohamed *et al.*, 2010; Kwon *et al.*, 2018) based nanozyme can remove the intracellular ROS to alleviate neurodegenerative disorders, such as Parkinson's disease.

In addition to therapy, the nanozyme imitating the catalase activity has also illustrated a typical role in biomedical image analysis. For example, dual enzyme species superparamagnetic Fe_3O_4 particles with catalase and SOD activities are used for dual-mode tumor imaging as an

efficient and sensitive diagnostic nanozyme (X Wang *et al.*, 2015). Furthermore, another nanozyme coated with iridium oxide has an immense role in the photoacoustic imaging of tumor cells and protects normal cells against H_2O_2-induced reactive oxygen pressure and inflammation (Zhen *et al.*, 2018). Thus catalase-like nanozymes can function as a theranostic agent for the therapy and diagnosis of various pathological conditions.

The SOD is a major class of nanozyme that is functional against the prevailing oxidative stress in the human body. The SOD catalyzes the dismutation of superoxide (O_2^-) into O_2 and H_2O_2. The H_2O_2 is further eliminated by catalase. Thus, SOD and catalase enzymes work in combination to scavenge superoxide. Generally, SOD enzymes are metalloproteins and can be subdivided according to various cofactors present and the types of folding of those metalloproteins. The three categories of nanozymes that exhibit superoxide radical scavenging activity contain zinc and copper, manganese and iron, and nickel derivatives (Mu *et al.*, 2016; Zhang *et al.*, 2016). As discussed earlier, the nanoceria is also potent in showing SOD-like and catalase-like activities that protect animal cells from ROS. Sometimes depending on the enzyme kinetics and physiological conditions like pH, some of the nanozymes also have the property of switching from one class to another. For instance, in addition to peroxidase activity, nanoceria also possesses SOD-, and catalase-like enzymatic activities (Singh *et al.*, 2017; P Wang *et al.*, 2020)

Similarly, AuNPs exhibit SOD-like and catalase-like activities determined by the environment's pH values (He *et al.*, 2013). He *et al*. reported that AuNPs show SOD-like activity at physiological pH, while AuNPs show catalase-like activity at alkaline pH. In contrast, the SOD-like and Catalase-like activities of AuNPs are significantly diminished in acidic pH. Moreover, in acidic conditions and in the presence of H_2O_2, AuNPs induce apoptosis by generating hydroxyl radicals that facilitate the oxidation of cellular components (He *et al.*, 2013). Besides pH, the

catalytic activities of AuNPs also depend on the size and surface properties of AuNPs, and the electron transfer and adsorption of superoxide with AuNPs (Lin *et al.*, 2014). However, there is a need to explore more the SOD-mimicking mechanism of AuNPs involving the adsorption of O_2^{2-} by the gold nanostructures resulting in the transfer of electrons for evaluating risks associated with AuNPs-based nanozymes for their functional applications.

IV. Role of nanozymes as a diagnostic and therapeutic agent against viral infections

Owing to the intrinsic and tunable physicochemical properties of nanozymes, they have been very successful in treating and detecting several viral infections. Besides being a therapeutic agent, nanozymes have been a potent agent implying they are to be used for detecting the antigens of this virus. Moreover, nanozymes can also enhance the sensitivity of biosensing and biomedical devices against viral infections. Nowadays, most kits used for this viral detection purpose employ nanozymes (Chakravarty and Vora, 2021). **Table 1** summarizes the diagnostic and therapeutic applications of various nanozymes reported for viral infections.

A. SARS-CoV-2 and other respiratory viruses

One of the recent outbreaks that have disrupted the World in many ways is the pandemic situation caused by the virus SARS-CoV-2. To combat the pandemic, a fast and accurate diagnosis is required. Currently, two methods are employed for detecting SARS-CoV-2, i.e., serological virus-induced antibody tests and real-time quantitative polymerase chain reaction (RT-qPCR). However, these methods have some disadvantages. For example, RT-qPCR is time-consuming and needs certified laboratories (Fleige and Pfaffl, 2006). Furthermore, although the serological detection of antibodies is cheaper and faster, the antibodies need weeks to develop in the serum

leading to false negative results due to a lack of sufficient antibody load in the serum (Mavrikou *et al.*, 2020). Therefore, the detection of spike glycoproteins is suggested as an alternative to antibodies as a low-cost, high-accuracy, and fast method because of the high specificity of spike protein compared to other proteins (Lu *et al.*, 2004).

AuNPs have been widely explored for detecting viral infections due to their biocompatibility and unique optical properties, which make them a suitable candidate for colorimetric assays. A colloidal solution of AuNPs functionalized with antibodies against SARS-CoV-2 surface proteins (spike, envelope, and membrane) was developed by Ventura *et al*. as reliable, cheap, and fast nanozymes. This nanozyme acts as a colorimetric biosensing tool to detect SARS-CoV-2 from nasal and throat swabs within 3 minutes **(Fig. 1)** (Ventura *et al.*, 2020). This method is sensitive to the viral particles rather than the viral RNA, which could measure viral particle concentration by a visibly recognizable color change from red to purple. The detection limit of SARS-CoV-2 using the AuNPs-based colorimetric method was quite similar to the RT-qPCR method, indicating the great potential of nanozymes as biosensors. In addition, Lew *et al*. developed a colorimetric serological assay in which AuNPs were conjugated to short antigenic epitopes on spike and nucleocapsid proteins of SARS-CoV-2 **(Fig. 1)** (Lew *et al.*, 2021)**.** These epitope-conjugated AuNPs bivalently bind to IgG in the plasma of COVID patients, triggering aggregation of AuNps that leads to the optical transition in the plasmon of AuNPs within 30 min. The specificity and sensitivity of this method were 100% and 83%, respectively.

Another study reported a colloidal AuNPs-based lateral-flow (AuNP-LF) assay in which an analytical membrane was coated with SARS-CoV-2 nucleoprotein (SARS-CoV-2 NP) to catch and detect IgM antibody within 15 min against the SARS-CoV-2 virus in the serum samples of COVID-19 patients **(Fig. 1)** (Huang *et al*., 2020). During the acute infection phase, the amount of

IgM antibodies are increased in the blood of the patients (Boyle *et al.*, 2019). Since it is possible to detect IgM antibodies against the SARS-CoV-2 virus in the blood samples even after several days of infection, developing fast and accurate technology to detect IgM could be of clinical advantage. In addition, to date, AuNP-LF technology has been widely used to detect various drugs, viruses, pathogens, and proteins (Liu *et al.*, 2018; L Huang *et al.*, 2019; F Yang *et al.*, 2020; Ren *et al.*, 2020). Thus, AuNP-LF assay-based point-of-care testing (POCT) strip could be a convenient, economic, and consumer-friendly SARS-CoV-2 detection system that can provide positive or negative results on the spot. Compared to RT-qPCR, the sensitivity and specificity of the AuNP-LF assay were determined to be 100 and 93.3%, respectively.

In another modified- AuNP-LF immunoassay, AuNPs were immobilized with antibodies and 4-mercaptobenzoic acid to quantitatively detect surface-enhanced Raman scattering (SERS) signal and increase the sensitivity of common AuNP-LF immunoassays. The detection limit and time of this method were 0.1 ng/mL and 20 min, respectively, compared to 1 ng/mL and 15 min, respectively, for qualitative LF immunoassay and 0.4 ng/mL and 3.5 h, respectively, for ELISA (Serebrennikova *et al.*, 2021). Bradbury *et al*. recently designed a user-friendly all-in-one 3D printable casing device to detect N-protein of SARS-CoV-2 in the serum at a 0.1 ng/mL concentration within 40 min. This LF immunoassay test kit consists of a liquid enhancement buffer stored in a chamber and dehydrated signal enhancement reagents. In this device, a single button push is sufficient to enhance the signal after the LF immunoassay detection step. This device could detect SARS-CoV-2 infection at the early stages of infection (Bradbury *et al.*, 2021).

SARS-CoV-2 can also be detected using Co–Fe@hemin-peroxidase nanozyme core-based chemiluminescence paper assay within 16 min, which is less than methods generally required for nucleic acid tests. The nanozyme catalyzed chemiluminescence and amplified immune reaction

signals similar to HRP. The sensitivity of this approach was comparable with the ELISA method (i.e., 360 TCID50/mL), and the limit of detection (LOD) for recombinant spike antigen of SARS-CoV-2 was 0.1 ng/mL. In addition, the smartphone can also be used to record signal detection. Thus, it could be a feasible, fast, and reliable approach for high-sensitive POCT detection for SARS-CoV-2 antigen (D Liu *et al.*, 2020).

Fu *et al*. developed spike (S1) protein detection polyclonal antibodies conjugated Au@Pt core-shell NPs that possess peroxidase-like properties to calorimetrically detect the S1 protein of SARS-CoV-2 (Fu *et al.*, 2021). This technique is a simple, selective, and specific tool where the fast electron transfer in the electron-rich porous Pt shells enhances the catalytic activity having LOD as low as 11 ng/mL, which is lower than the HRP-conjugated bi-specific monoclonal antibody (F157) (i.e., 19 ng/mL) **(Fig. 2)** (Sunwoo *et al.*, 2013).

In addition to the S1 protein, nucleocapsid protein is also suggested as a potential candidate for antigen-based immunoassays. In a study, the monoclonal antibodies (mAbs)-labeled Pt@AuNPs shell-core were used as a nanozyme to detect the nucleocapsid phosphoprotein of SARS-CoV-2 in 37 serum samples from 20 COVID-19 patients using a smartphone. The LOD of this system was as low as 10 pg/mL compared to 100 pg/mL of conventional ELISA **(Fig. 2)** (B Liu *et al.*, 2021). This device could be an effective POCT cost-effective system with added advantages such as being easy to operate at home, lacking the need for trained technicians, and wifi-mediated data transfer.

Another noble metals-based peroxide-mimicking nanozyme reported for detecting nucleocapsid protein from SARS-CoV-2 is composed of ultrafine-tuned Palladium-Iridium (Pd-Ir) nanocubes whose catalytic constant is much higher than other Nanozymes that are composed of metal oxides nanoparticles, other noble metals-based nanomaterials, and carbon nanomaterials. It

was found that the Pd-Ir nanocubes-based Nanozymes display almost two times higher detection limits on immunoassays. Since both Pd and Ir are inert noble metals, they are stable under acidic and alkaline conditions. The advantage of the Pd-Ir nanocubes-based nanozyme is that their catalytic property can be modulated based on the thickness of the Ir deposition on the Pd core (Li and Li, 2021).

Self-assembled copper nanoflowers (CuNFs) were also reported to act as peroxidase-mimicking Nanozymes to detect respiratory viruses (Khoris *et al.*, 2021). This study showed that the captured virus could be recognized by the bound TMB-NP-encapsulated Poly(lactic-co-glycolic acid (PLGA) nanovesicles and release encapsulated TMB-NPs, which self-assembled CuNFs can oxidize to produce amplified colorimetric signal in the presence of H_2O_2. Furthermore, the CuNFs Nanozymes could detect the clinically isolated IV/A/H3N2 and spike protein of SARS-CoV-2 with high efficiency at the femtogram level (Khoris *et al.*, 2021). These results show that Nanozymes could effectively detect SARS-CoV-2 from the serum and throat or nasal samples at a detection limit lesser than the conventional ELISA method and thus could be used in clinical samples.

Wang *et al*. showed that Ag atoms that are atomically dispersed on titanium oxide (TiO_2) supported single-atom Nanozymes (SANs) strongly bind to the surface of spike 1 RBD via amino acids (i.e., asparagine and cysteine). Thus, Ag-TiO_2 SAN can adsorb SARS-CoV-2 to make a complex phagocytosed by macrophages and reaches lysosomes where the acidic environment assists in the O_2 reduction reaction through the intrinsic peroxidase-like activity of Ag-TiO_2 SAN. This results in the elimination of the virus. Thus, the SANs with peroxidase-like activities can serve as an efficient anti-viral composite **(Fig. 3)** (D Wang *et al.*, 2021)**.**

Thus, Nanozymes have been studied as a successful therapeutic/diagnostic agent for treating this global pandemic in many ways (Ali *et al.*, 2021). Furthermore, since nanomaterials composed of ZnO, AgNPs, Ag nanowires, and glutathione-coated AgS_2 nanoclusters have shown anti-viral effects after coronaviruses, the use of these nanomaterials as Nanozymes could also be explored for inhibiting the viral entry inside the host, disrupting its attachment with the spike protein, and blocking the SARS-CoV-2 transcription once it is inside the host cell. Thus, in these ways, Nanozymes can also stimulate the defensive mechanism of the human body to combat SARS-CoV-2 infection and can be employed in providing vaccination. However, more studies are required for the clinical translation of Nanozymes to combat SARS-CoV-2.

B. Influenza virus

The development of an effective therapeutic agent for the influenza virus has always been challenging for scientists for several reasons, mainly the prevalence of a higher mutation frequency and the variety of the influenza virus antigenic subtypes. Nanozymes could be a possible tool to overcome these challenges. Nanozymes have shown diagnostic potential for the influenza virus using colorimetric substrates. Ahmed *et al*. developed a highly sensitive, simple, low-cost, and specific enhanced colorimetric immunoassay that can detect commercially available avian influenza A (H5N1) virus hemagglutinin (HA) protein with the detection limit of 1.11 pg/mL, compared to 909 pg/mL while using conventional ELISA method (Ahmed, Corredor, *et al.*, 2017). This method utilized a conjugate of Au ion and anti-HA H5N1 antibodies (Ab 135382). The H5N1 virus binds to the specific antibodies present in this bioconjugate solution. The AuNPs are *in situ* synthesized from Au ions present in this bioconjugate solution after adding TMBZ, resulting in bluish green color. Furthermore, the *in situ* synthesized AuNPs intensifies bluish green color by

oxidizing TMBZ due to peroxidase-like activity after the addition of H_2O_2/TMBZ solution **(Fig. 4)**. This approach proved to be superior to other available diagnostic methods in terms of sensitivity by having detection limits of 0.0269 HAU and 0.0331 HAU for avian influenza A (H4N6) and A (H9N2) virus, respectively, in the blood samples, proposing its applicability in the clinical field.

The enzymatic activities of Au nanozymes (AuNZs) can also be applied to develop a magnetic nanozyme-linked immunosorbent assay (MagLISA) by combining them with silica-shelled magnetic nanobeads (MagNBs). The MagNBs recognize and capture the target virus for separation by applying an external magnetic field. The virus particles captured by MagNBs also interact with the antibodies conjugated with the AuNZs (AuNZ-Abs) to form a sandwich-like structure that can be magnetically collected, generating colorimetric signals by AuNZs-mediated oxidation reaction of TMB, facilitating specificity and reducing the assay time. The lowest recorded detection limit for influenza virus A (New Caledonia/20/1999) by MagLISA was 44.2×10^{-15} g/mL by a microplate reader and 5.0×10^{-12} g/mL only by human eyes. Moreover, even human serum samples also showed a detection limit of 2.6 PFU/mL, indicating a sensitive POCT virus detection platform **(Fig. 4)** (Oh *et al.*, 2018).

In addition to diagnosis, IONzymes are also used for therapy by targeting the envelope protein of the influenza virus, which is rich in lipids. It was observed that IONzymes could catalyze lipid peroxidation in influenza A viruses (IAVs) envelope, resulting in the inactivation of the virus and altering its infectivity by destroying the integrity of proteins such as neuraminidase, HA, and matrix protein 1. Furthermore, when tested against 12 subtypes of IAVs (H1~H12), IONzymes showed anti-viral activity against the three most threatening viral subtypes, i.e., H1N1, H5N1, and H7N9 **(Fig. 5)** (Qin *et al.*, 2019).

Another study reported the use of chitosan (CS) functionalized IONzyme as a catalytic mucosal adjuvant tool for the whole inactivated virus (WIV) influenza nasal vaccine **(Fig. 5)** (Qin *et al.*, 2020)**.** This CS-IONzyme-based WIV vaccine proved to be a successful strategy by increasing the adhesion of HIN1 WIV antigen to the mucosal membrane of nasal passage by 30-fold compared to HIN1 WIV alone. In addition, the CS-IONzyme-based WIV vaccine showed enhanced peroxidase-like activity for ROS-dependent maturation and migration of submucosal H1N1 WIV-loaded dendritic cells into the draining lymph nodes, resulting in the initiation of the antigen-specific immune response. Thus, these studies signify the importance of Nanozymes in diagnostics, therapy, and vaccination against the influenza virus. Based on these observations, it is possible to develop Nanozymes-based anti-influenza strategies. Furthermore, since IONzymes show anti-viral activity by performing lipoxidase-like activity, IONzymes can also be used against other life-threatening viral infections, such as influenza, HIV, Ebola virus, Zika virus, etc.

C. Mumps virus

One of the most common diseases in children is mumps which is caused by the mumps virus. The overall effects in the case of mumps can be minimal, but in some cases, the effect is detrimental. It leads to severe conditions of health leading to prolonged disabilities (Galazka *et al.*, 1999). In general, laboratories use the PCR method to detect the nucleic acid of the mumps virus from human samples. In addition, serological markers can also be used for the diagnosis of mumps using specific immunoglobulin M (IgM) class antibodies. However, the possibility of false negative results in the samples is high due to the low or even undetectable IgM levels during the early stages of infection. Thus, a sensitive and simple diagnosis method is needed to detect mumps infection at its early stages. Due to the plethora of applications, nanozymes are also capable of

detecting the presence of the mumps antigen in a sample and have been a reliable method of detection.

The Au@Pt core/shell nanostructures, where AuNRs provide attachment sites for Pt nanodots, are highly desirable for catalysis as they have been known to exhibit peroxidase-like activity (Liu *et al.*, 2012). A nanostructure designed by modifying Au@Pt NRs with mesoporous SiO_2 shells (APMSNs) worked as a nanoprobe for the serodiagnosis of the mumps virus. The use of SiO_2 in the nanozyme has successfully retained the catalytic property of the nanozyme, which may get inhibited due to the certain interaction with the antigen. This is possible because the assembly of nanozyme in the silica core creates an obstacle, rendering a good catalytic property. On the other hand, these nanozymes have retained their functionality even in extreme conditions like high pH or temperature. These nanoprobes could detect IgM antibodies specific to mumps at as low as 10 ng/mL level in the samples, confirming that APMSNs could be used as an efficient immunological probe for detecting viral load in clinical samples (**Fig. 6**) (Long *et al.*, 2020).

D. Measles virus

Measles is an acute viral respiratory illness caused by the measles virus. Although vaccines can prevent measles virus infection, accurate diagnosis is essential to eliminate measles. In clinics, ELISA and indirect immunofluorescence (IIF) methods to detect IgG and IgM specific to measles (de Ory *et al.*, 2015). However, it has been seen that replacing HRP with nanozymes antigen conjugate composed of Au@Pt NRs can improve the conventional ELISA method for measles virus serodiagnosis (**Fig. 6**) (Long *et al.*, 2018). As discussed earlier, the Au@Pt NRs (AuNR core/Pt shell) possess an intrinsic peroxidase-like activity. Therefore, in this case, AuNRs act as a support system to prevent the aggregation of PtNPs owing to the simple synthesis method of

AuNPs with tailorable and well-controlled surface plasmonic resonance (SPR) features. Furthermore, the catalytic activity can also be enhanced by using an additional ligand effect. Long *et al*. showed that when Au@Pt NRs conjugate with measles antigen, they can be used as a nanozyme probe to detect the IgM isotype of captured measles virus-specific antibody *via* its interaction with measles antigen (Long *et al.*, 2018). This nanozyme-antigen conjugate could be a robust and sensitive diagnostic tool for diagnosing measles infection.

E. Zika Virus

Zika virus (ZIKV), a flavivirus, is challenging to diagnose due to symptoms similar to chikungunya, dengue, and yellow fever. Currently, highly sensitive and specific real-time RT-PCR is used to detect ZIKV. However, the real-time RT-PCR technique is expensive, complicated, and requires highly specialized professionals and laboratories. Further, an RT- loop-mediated amplification (RT-LAMP) was developed to reduce the instrumentation requirement (Song *et al.*, 2016). Still, using a microfluidic cassette and specific primers in RT-LAMP limits its use as a POC ZIKV detection kit. Therefore, in search of a rapid, inexpensive, simple, and instrument-free detection method, a strip-based LF immuno-chromatographic assay was developed to detect ZIKV (Hristov *et al.*, 2019; Rong *et al.*, 2019). Since the use of smartphones is increasing worldwide, these detection systems were made smartphone-friendly. Although these detection systems proved to be user-friendly POC approaches, they may lead to false results as they were less specific and less sensitive due to the cross-reactivity of NS1 antibodies with another flavivirus (e.g., dengue virus).

To overcome these issues, Hsu *et al*. developed a smartphone-friendly nanozyme, composed of antibody-modified Pt@AuNPs, based POC immunosensor system for specific and sensitive detection of ZIKV in whole blood. This system avoids the cross-reactivity of antibodies

with other flaviviruses (Hsu *et al.*, 2020). In this method, a few drops of blood are directly added into an antibody-modified Pt@AuNPs immunosensor vial, and the result is analyzed using a smartphone. This is a convenient approach at airports and other travel platforms to detect the presence of ZIKV in passengers traveling from endemic geographical locations.

F. Hepatitis C virus (HCV)

Hepatitis C virus (HCV), a positive-strand RNA virus, causes chronic liver diseases, such as cirrhosis, hepatitis, and cancer. Presently, the therapy for HCV is based on interferon that is non-specific to HCV and accounts for only viral clearance in 50% of patients with significant side effects (Lanford *et al.*, 2010). The initiation of HCV RNA translation is regulated by a highly conserved 5′ nontranslated region (5′ NTR) that provides the ribosome entry site. It has been reported that the replication of HCV can be inhibited by siRNA 331, which targets the 5′ NTR region (Yokota *et al.*, 2003), suggesting that enzymatic cleavage of the 5′ NTR region could potentially inhibit the replication of HCV. Following this concept, Wang *et al*. developed a nanozyme that consists of AuNPs as the backbone of the nanozyme because of their alkylthiol functionalization property. The AuNPs backbone binds to catalytically active non-specific robust endoribonucleases (RNase A) through noncovalent adsorption. Close to RNase A, AuNPs hold a single-stranded DNA oligonucleotide complementary to the target RNA. In this nanozyme, DNA oligonucleotide and RNase A act in synergy and could effectively cleave the 5′ NTR region in HCV in a sequence-specific manner (**Fig. 7**) (Z Wang *et al.*, 2012). It was found that this nanozyme displayed an anti-viral effect in cultured HCV cells and mouse models.

Thus, nanozyme-based cleavage at the 5′ NTR region to prevent RNA translation could become a potential tool to regulate gene expression of viral machinery. Furthermore, the functionalities of such nanozymes can be further increased by adding functional moieties to the nanozymes for organ-specific and subcellular organelle targeting. Although nanozymes have been frequently explored for detecting various viruses, studies related to nanozymes-based rapid detection of HCV are not available. This suggests a high scope in studies on developing nanozymes-based HCV detection technologies.

G. Hepatitis E virus (HEV)

Similar to HCV, the Hepatitis E virus (HEV) is also associated with acute liver diseases (Navaneethan *et al.*, 2008). The most common HEV detection methods are by detecting IgG, IgM, and IgA antibodies post-infection and RNA-targeting RT-PCR (Melgaço *et al.*, 2018). However, it is well-discussed that natural enzyme-based colorimetric ELISA detection methods are fast and simple but less sensitive and less reliable than RT-PCR in detecting the presence of HEV in samples at very low concentrations (Chen *et al.*, 2018). Thus, Khoris *et al*. developed an upgraded and sensitive nanozyme-based ELISA biosensor to detect HEV in the fecal samples from HEV-infected monkeys (Khoris *et al.*, 2020). They used Ag deposition on Au nanozymes (AuNPs@Ag) as a signal amplification strategy for colorimetric analysis by enhancing the catalytic activity towards TMB compared to bare Au nanozyme. In this technique, AuNPs@Ag nanozyme-based immunoassay, the Au nanozyme was conjugated with IgG antibody against HEV that could enhance the virus detection sensitivity in the samples. Later, the deposition of Ag ions in Au nanozyme significantly amplifies the colorimetric analysis compared to bare Au nanozyme. The

efficiency and reliability of this technique were equivalent to RT-PCR, and thus, it can be a promising method for rapid and easy detection of HEV in clinical samples.

H. Rubella virus

Rubella virus causes German measles or Rubella disease. As discussed in previous sections, Au@Pt NRs have various intrinsic enzymatic abilities. The peroxidase-like activity of rubella antigen-conjugated Au@Pt NRs-based nanozymes has also been studied to detect rubella-specific IgM or IgG in rubella virus-positive human serum samples. It was found that rubella antigen-conjugated Au@Pt NRs-based Nanozymes could detect rubella-specific IgM antibodies at as low as 10 ng/mL concentration, which is 1000 times more sensitive than the commercially available ELISA method. This might be because of the homogenous distribution of Pt nanodots AuNRs surface, resulting in a large surface area of AuNRs covered with fine Pt nanodots. Moreover, rubella antigen-conjugated Au@Pt NRs-based Nanozymes showed high specificity for rubella-specific IgM antibodies by not detecting other infectious viruses in the serum (T Zhang *et al.*, 2018).

However, in Au@Pt NRs-based Nanozymes, the functional activity of AuNRs core is shielded by the functionalizing chemicals, reducing its catalytic activity. Thus, coating the Au@Pt NRs-based Nanozymes with chemically inert porous shells can provide channels through which chemical species can reach the functionally active nanoparticle core. Based on this concept, mesoporous silica encapsulated Au@Pt NRs-based Nanozymes (Au@Pt@SiO_2) were developed for immunoassays for ultrasensitive colorimetric detection of rubella IgM antibodies **(Fig. 8)** (A Li *et al.*, 2019). Although the sensitivity of Au@Pt@SiO_2 against rubella IgM antibodies was similar to Au@Pt NRs (i.e., 10 ng/mL), Au@Pt@SiO_2 are supposed to be more robust against the

harsh chemical environment and high peroxidase-like activity. Thus, Au@Pt@SiO_2 could be a highly sensitive immunoassay ELISA method to detect viral infections in future clinical applications under robust and harsh conditions.

I. Ebola

Ebola virus disease (EVD) was first identified in 1976 and caused a major outbreak in West Africa in 2014. Because no efficient treatment for EVD is available to date, rapid diagnosis and quarantine of the infected is the best approach to controlling Ebola spread. Although various diagnostic methods for ebola are available in the market, such as RT-PCR and colloidal gold immunochromatographic strip, there is an urgent need to extend the POC diagnostic methods to detect Ebola infection soon after the onset of EVD symptoms. This could be achieved by enhancing the sensitivity of the ELISA method using nanozymes compared to the conventional natural enzyme-based ELISA method. It was found that a nanozyme-strip made up of Fe_3O_4 magnetic nanoparticle (MNP) as a nanozyme probe could detect the presence of Ebola glycoprotein at the minimum limit of 1 ng/mL through the naked eye. This MNP-based nanozyme probe was conjugated with anti-Ebola antibodies and performed recognition, separation, and colorimetric visualization on a strip through intrinsic peroxidase-like activity.

Although the sensitivity of MNP-based nanozyme was almost comparable to clinically available colloidal gold strip ELISA methods, MNP-based nanozyme was easy to use and was much faster (within 30 min) than any other method (Duan *et al.*, 2015). Thus, it has a vast potential in the diagnostic field as a POC system in the rural area of West Africa to detect the presence of viral infections.

J. Norovirus

Norovirus, a highly contagious virus, is a member of the Caliciviridae family and causes acute viral gastroenteritis. The norovirus spreads through contaminated food, water, personal contact, or surfaces. Due to the contagious nature of norovirus, it is important to develop a rapid and accurate detection method for norovirus. Because graphene-based and Au-based materials have emerged as interesting biomaterials with peroxidase-mimicking ability, Ahmed *et al*. designed a hybrid nanoprobe composed of antibodies-conjugated graphene-AuNPs to detect norovirus-like particles in the human serum using colorimetric immunoassays. In this design, AuNPs were reduced and stabilized on the graphene surface to achieve the combinational peroxidase-like activity of both graphene and AuNPs. The detection limit of antibodies-conjugated graphene-AuNPs nanoprobe was 92.7 pg/mL for norovirus-like particles, which is 112 times lower than the conventional ELISA method. In contrast, the sensitivity of the antibodies-conjugated graphene-AuNPs nanoprobe was 41 times greater than the commercially available diagnostic kit (Ahmed, Takemeura, *et al.*, 2017).

Furthermore, the addition of target-specific molecular recognition elements (MREs), such as MNV AG3 aptamer, to the Nanozymes can enhance the sensitivity and allow specific detection of the MNV virion **(Fig. 9)** (Weerathunge *et al.*, 2019). This suggests that nanozyme has great potential for future real-life diagnostic applications, which can be further enhanced by adding a target-specific aptamer to the nanozyme.

K. Human Immunodeficiency virus

HIV, a causative agent of acquired immunodeficiency syndrome (AIDS), has no effective cure, but it can be controlled with proper medical care by extending its latency period. The intact viral genome of HIV resides in the infected cells for a latent period in which it is transcriptionally

silent. However, upon oxidative stress, viral long terminal repeat (LTR) in the HIV genome becomes transcriptionally active via NF-κB-mediated signaling pathways in the latent reservoirs (Pyo *et al.*, 2008). In contrast, cellular antioxidant responses prolong the latency period (Shytaj *et al.*, 2015). Bhaskar *et al*. suggested that alteration in the GPx enzyme family is associated with the reactivation and replication of HIV (Bhaskar *et al.*, 2015). Singh *et al*. synthesized three types of V_2O_5 nanomaterials, i.e., nanowires, nanosheets, and ultrathin nanosheets, and showed that the GPx –like activity of V_2O_5 nanomaterials was dependent on their morphologies (Singh *et al.*, 2021). The V_2O_5 nanosheets mimic GPx-like activity to block HIV reactivation by catalytically neutralizing ROS in HIV-infected cells. Furthermore, they showed that the V_2O_5 nanosheets were very selective toward H_2O_2 and non-toxic to monocytic cell line (U1) latently infected with HIV-1, compared to the other two types of V_2O_5 nanowires and nanosheets. They further showed that a combination of V_2O_5 nanosheets and BAY11-7082 (a pharmacological inhibitor of NF-κB) suppressed the virus reactivation (Singh *et al.*, 2021). Thus, nanozymes could be a possible therapeutic platform against infectious diseases. However, more underlying mechanistic details need to be explored.

V. Limitations of using nanozymes as a therapeutic agent

The use of nanozymes in the medical field is a new realm of exploration. Due to some of their excellent therapeutic properties, nanozymes have been a potential therapeutic agent. However, despite the superiority, this domain also has certain limitations. The most important one is the catalytic ability of the nanozymes in comparison to the natural enzymes. Therefore, studies have implied the idea of creating a cascade of these nanozymes so that the catalytic efficiency can be retained to a greater extent (Y Liu *et al.*, 2020; Ren *et al.*, 2022).

Moreover, the specificity of the nanozymes is also a big issue. Implementing single atoms might be a solution in this case (Jiang *et al.*, 2019; Ren *et al.*, 2022). The mimicking ability of the nanozymes is highly praiseworthy in some cases. Yet, some challenges are affecting its efficiency in some cases. The nanozymes, due to this property, can be accessible to several substrates, raising the question of their specificity. Therefore, a specific computational analysis should be performed before preparing such nanozymes (Jiang *et al.*, 2019). The cytotoxicity of the nanozymes can be ignored in all cases. Sometimes it is also a responsible factor for the generation of ROS, which deteriorates the efficiency of the nanozymes (Yang *et al.*, 2019). After using nanozymes for treating diseases, there are several issues regarding their accumulation. First, the metabolic activities of the nanoparticles are blocked by some means, which can be problematic for the human body. Second, the accumulation of these nanoparticles is very harmful, leading to hepatotoxicity (Alexis *et al.*, 2008; Jun *et al.*, 2008). Notably, nanozymes are mostly found to mimic oxidoreductase or hydrolase activities. However, the evidence for lyase or transferase remains unexplored. Besides, the catalytic behaviors of the nanozymes should be studied well to get a clear idea about the mechanism of action because several ancillary factors govern the working of the nanozymes (Ma *et al.*, 2021). However, knowing all these factors and their catalytic role is of utmost importance.

Vl. Conclusion

The application of nanotechnology in the field of medicine is highly commendable. The various extraordinary features of nanoparticles have led to their use in manufacturing nanozymes. The several advantages of using nanozymes is a paradigm shift from the primitive treatment methods. Besides, they are suitable for the early detection of several disease biomarkers and for

imaging several parts of the human anatomy. These applications are very suitable for clinicians to identify the biomarkers of a particular disease and initiate the treatment at an early stage, which can save many lives. However, there is still scope for the modification of the inherent properties of the nanozymes to enhance their ability as therapeutic agents. Although nanozymes present a modified landscape in terms of functionality and biocompatibility compared to natural enzymes, it has some limitations in use. Enhancing some of the features of nanozymes will surely be a boon for the biomedical field. Specific issues that could hinder treating a particular disease using the commonly known therapeutics are being well handled by administering a particular dose of nanozyme. An accelerated move is currently required to eliminate certain constraints and make nanozymes one of the best therapeutic agents.

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Conflict-of-interests

No author has an actual or perceived conflict of interest with the contents of this article

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Figures legends

Fig. 1 Schematic presentation of functionalized AuNPs-mediated detection of SARS-CoV-2. (A) Using antibodies-functionalized AuNPs against spike, envelope, and membrane surface proteins of SARS-CoV-2. Reproduced with permission from Ventura et al. (2020). (B) Using epitope-functionalized AuNPs for detecting SARS-CoV-2 IgG antibody. Reproduced with permission from Lew et al. (2021) (Copyright © 2021, American Chemical Society). (C) Lateral flow assay for colloidal AuNPs-based detection of SARS-CoV-2 IgM antibodies. Adapted with permission from Huang et al. (2020).

Fig. 2 Schematic presentation of functionalized Au@Pt core-shell NPs-mediated detection of SARS-CoV-2. (A) Detection of S1 protein *via* polyclonal antibody (Ab)-coated wells. (B) Detection of nucleocapsid phosphoprotein antigen (Ag) from the serum of patient *via* monoclonal Ab(mAb)-magnetic beads (MBs)-coated wells using smartphone. Created with BioRender.com.

Fig. 3 Schematic presentation of SARS-CoV-2 elimination using nanozymes composed of TiO_2 supported single Ag atoms. Reproduced with permission from Wang et al. (2021) (Creative Commons Attribution License (CC BY)).

Fig. 4 Schematic presentation of functionalized AuNPs-mediated detection of Influenza virus. (A) Dual enhanced colorimetric detection *via* a conjugate of Au ion and anti- hemagglutinin (HA) H5N1 antibodies (Ab). Reproduced with permission from Ahmed et al. (2017) (https://creativecommons.org/licenses/by-nc/4.0/) (B) Magnetic Nanobead-based Nano(e)zyme-Linked Immunosorbent Assay (MagLISA) to detect influenza virus. Adapted with permission from Oh et al. (2018) (Copyright © 2018, American Chemical Society).

Fig. 5 (A) Schematic presentation of IONzymes-mediated viral lipid peroxidation for influenza virus inactivation that produces free radicals to destroy the neighboring proteins. Reproduced with permission from Qin et al. (2019) (https://creativecommons.org/licenses/by/4.0/). (B) Schematic presentation of CS-IONzyme-based influenza vaccine by enhancing antigen-specific immune response. Reproduced with permission from Qin et al. (2020) (https://creativecommons.org/licenses/by/4.0/)

Fig. 6 (A) Schematic presentation of the immunoassay of antigens-conjugated Au@Pt@mesoporous SiO_2 nanozyme based ELISA system for detecting Mumps-specific IgM antibody. Adapted from Long et al., (2020) (Creative Commons Attribution License (CC BY). (B) Schematic presentation of immunoassay of Poly-(sodium 4-styrenesulfonate)(PSS)-coated Au@Pt NR-antigen conjugates based ELISA system for detecting Measles virus (MV)--specific IgM antibody. Reproduced with permission from Long et al. (2018) (http://creativecommons.org/licenses/by/4.0/)

Fig. 7 Schematic representation describing the design and function of a nanozyme based on artificial RNA silencing machinery for antiviral therapy against Hepatitis C virus. Adapted with permission from Wang et al. (2012) (Copyright © 2012 Proceedings of the National Academy of Sciences, U.S.A.)

Fig. 8 Schematic presentation of the immunoassay of antigen-labeled Au@Pt@SiO_2 nanozyme based ELISA system to detect Rubella IgM antibody. Adapted with permission from Li et al. (2019) (http://creativecommons.org/licenses/by/4.0/)

Fig. 9 Schematic presentation of the NanoZyme Aptasensor-based Ultrasensitive Colorimetric Detection of Murine Norovirus. Reproduced with permission from Weerathunge et al. (2019) (Copyright © 2019, American Chemical Society).

Table 1 Nanozymes for diagnosis and therapy for viral infections

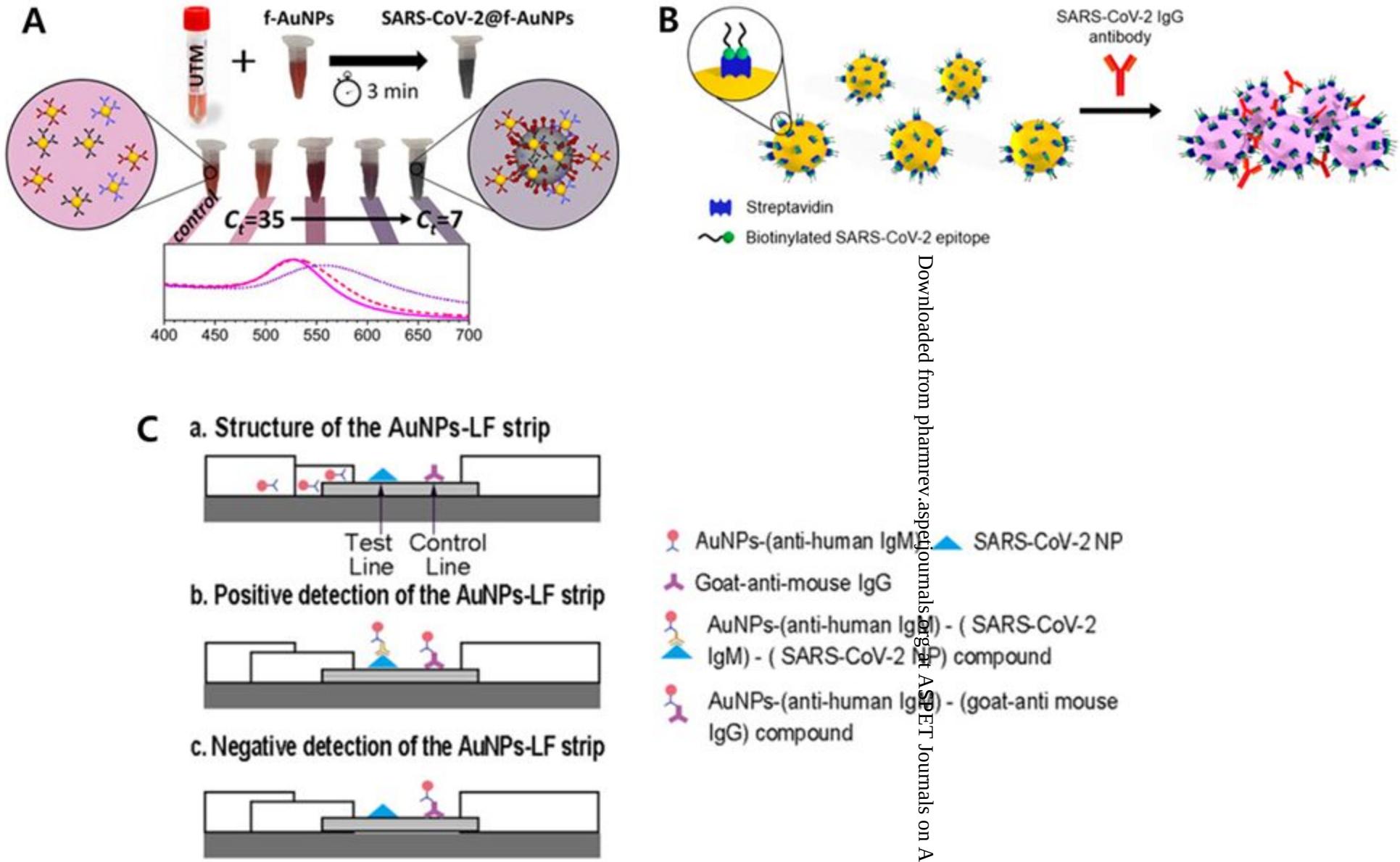
S. No.	Viral Disease	Composition /system of Nanozyme	Analyte	Detection limit	Sensitivity	Purpose of use (Diagnostic/ Therapeutic)	Details	Reference
1.	SARS-CoV-2	AuNP/ colloidal gold nanoparticle-based lateral-flow assay	Nucleoprotein	1 ng/mL	100%	Diagnostic	It catches and detects IgM antibody within 15 min in the serum samples of COVID-19 patients	Huang *et al*., 2020, Serebrennikova *et al*., 2021
		Co–Fe@hemin-peroxidase/ chemiluminescence paper assay	Spike protein	0.1 ng/mL	360 TCID50/mL (similar to ELISA)	Diagnostic	It catalyzes chemiluminescence and amplifies immune reaction by detecting the spike protein with 16 minutes	Liu *et al*., 2020
		Au@Pt core-shell NP/ monoclonal/polyclonal antibodies conjugated system	S1 subunit of Spike protein and Nucleoprotein	11 ng/mL (for S1 protein) and 10 pg/mL (for nucleoprotein)	-	Diagnostic	It possess peroxidase-like properties to calorimetrically detect S1 protein of SARS-CoV-2. Besides, it also	Fu *et al*., 2021, Sunwoo *et al*., 2013, Liu *et al*., 2021

							detects the nucleocapsid phosphoprotein of SARS-CoV-2 using smartphones.	
		Pd-Ir/ ultrafine tuned nanocubes	Nucleocapsid protein	Extremely high limit of detection in comparison to the other immunoassays	-	Diagnostic	It detects the nucleocapsid protein of SARS-CoV-2 virus	Li and Li, 2021
		CuNF/ Self-assembled copper nanoflower system	Spike protein	Detection limit is lesser than the conventional ELISA	-	Diagnostic	It detects the clinically isolated IV/A/H3N2 and spike protein of SARS-CoV-2 with high efficiency.	Khoris *et al*., 2021
		Ag-TiO_2 SAN dispersed on titanium oxide (TiO2)	Spike RBD	-	-	Therapeutic	It eliminates the virus by utilizing its peroxidase activity by enhancing the process of phagocytosis.	Wang *et al*., 2021
2.	Influenza virus	AuNP/ colorimetric immunoassay	Hemagglutinin protein	1.11 pg/mL normally; 0.0269	-	Diagnostic	It detects commercially available avian	Ahmed, Corredor,

				HAU and 0.0331 HAU for avian influenza A (H4N6) and A (H9N2) virus, respectively upon the addition of TMBZ			influenza A (H5N1) virus hemagglutinin protein	*et al*., 2017
		CS-IONzyme / Chitosan functionalized IONzyme	Whole inactivated influenza virus	-	-	Therapeutic	It is used as a catalytic mucosal adjuvant tool in WIV vaccine.	Qin *et al*., 2020
3.	Mumps virus	Au@Pt core-shell NP introduced in mesoporous SiO_2 shell	IgM antibodies specific to mumps	10 ng/mL	-	Diagnostic	The Au@Pt NRs with mesoporous SiO_2 shell can be used as a nanoprobe for the serodiagnosis of mumps virus	Long *et al*., 2020
4.	Measels virus	Au@Pt NP/ antigen conjugated system	Measels antigen	-	-	Diagnostic	It acts as a nanoprobe to detect the IgM isotype of captured measles	Long *et al*., 2020

							virus-specific antibody through specific antibody interactions	
5.	Zika virus	Pt@AuNP/ POC immunosensor system	Antigenic proteins of the zika virus	-	-	Diagnostic	It detects the presence of Zika virus using a smartphone.	Hsu *et al*., 2020
6.	Hepatitis C virus	AuNP introduced as a backbone for the alkylthiol functionalization property	RNase A and DNA oligonucleotide	-	-	Therapeutic	It cleaves the 5′ NTR region of HCV in a sequence-specific manner, possessing an anti-viral activity.	Wang *et al*., 2012
7.	Hepatitis E virus	AuNPs@Ag nanozyme-based ELISA biosensor	Hepatitis E virus	-	-	Diagnostic	It helps in the rapid detection of HEV in fecal samples	Khoris *et al*., 2020
8.	Rubella virus	Au@Pt NP/ rubella antigen-conjugated system	Rubella antigen	10 ng/mL	1000 folds more sensitive than ELISA	Diagnostic	It detects rubella-specific IgM or IgG in rubella virus-positive human serum samples	T Zhang *et al*., 2018

9.	Ebola virus	Fe_3O_4 magnetic nanoparticle (MNP) strips conjugated with anti-Ebola antibodies	Ebola glycoprotein	1 ng/mL through naked eyes	Similar to colloidal gold strip ELISA method	Diagnostic	It detects the presence of Ebola virus within 30 minutes	Duan *et al*., 2015
10.	Norovirus	AuNPs/ antibodies-conjugated graphene system	Norovirus-like particles	92.7 pg/Ml (112 times lower than conventional ELISA methods)	41 folds more sensitive than the existing diagnostic kit	Diagnostic	It detects the presence of Norovirus by utilizing the peroxidase-mimicking activity	Ahmed, Takemeura, *et al*., 2017
11.	Human Immunodeficiency Virus	V_2O_5 nanosheets	HIV-infected cells	-	-	Therapeutic	It hinders HIV reactivation by imitating the activities of GPx enzyme	Singh *et al*., 2021



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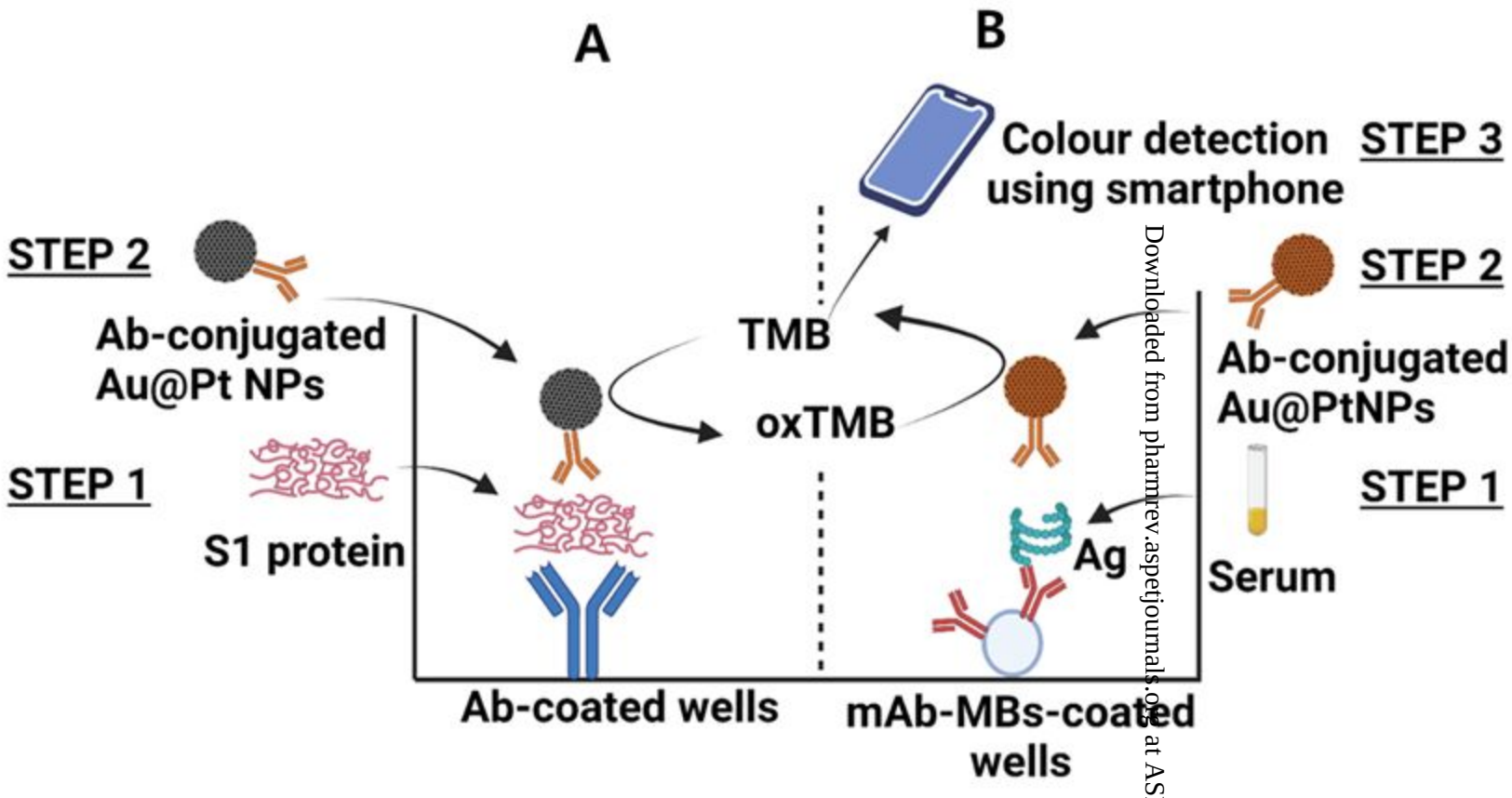
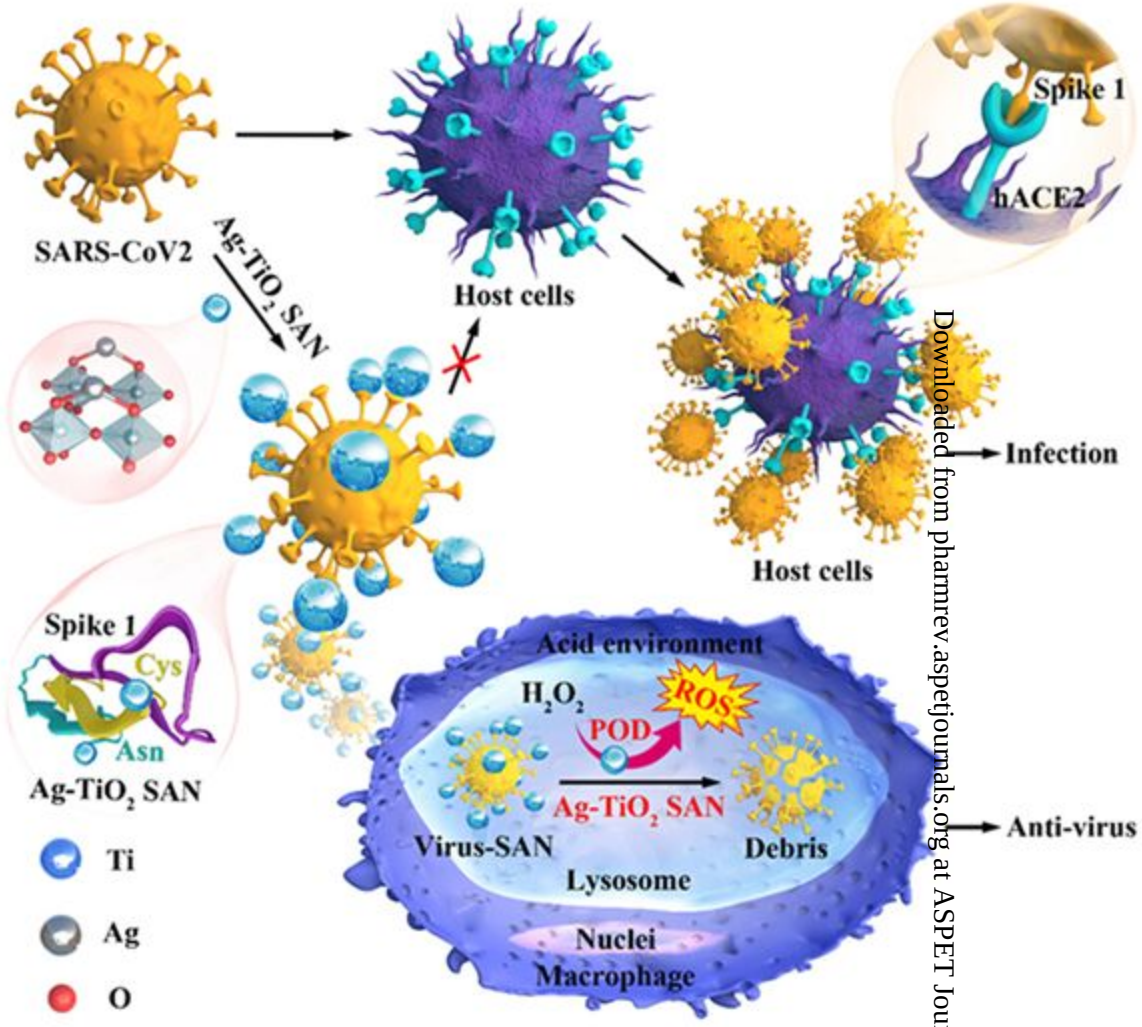


Fig. 2



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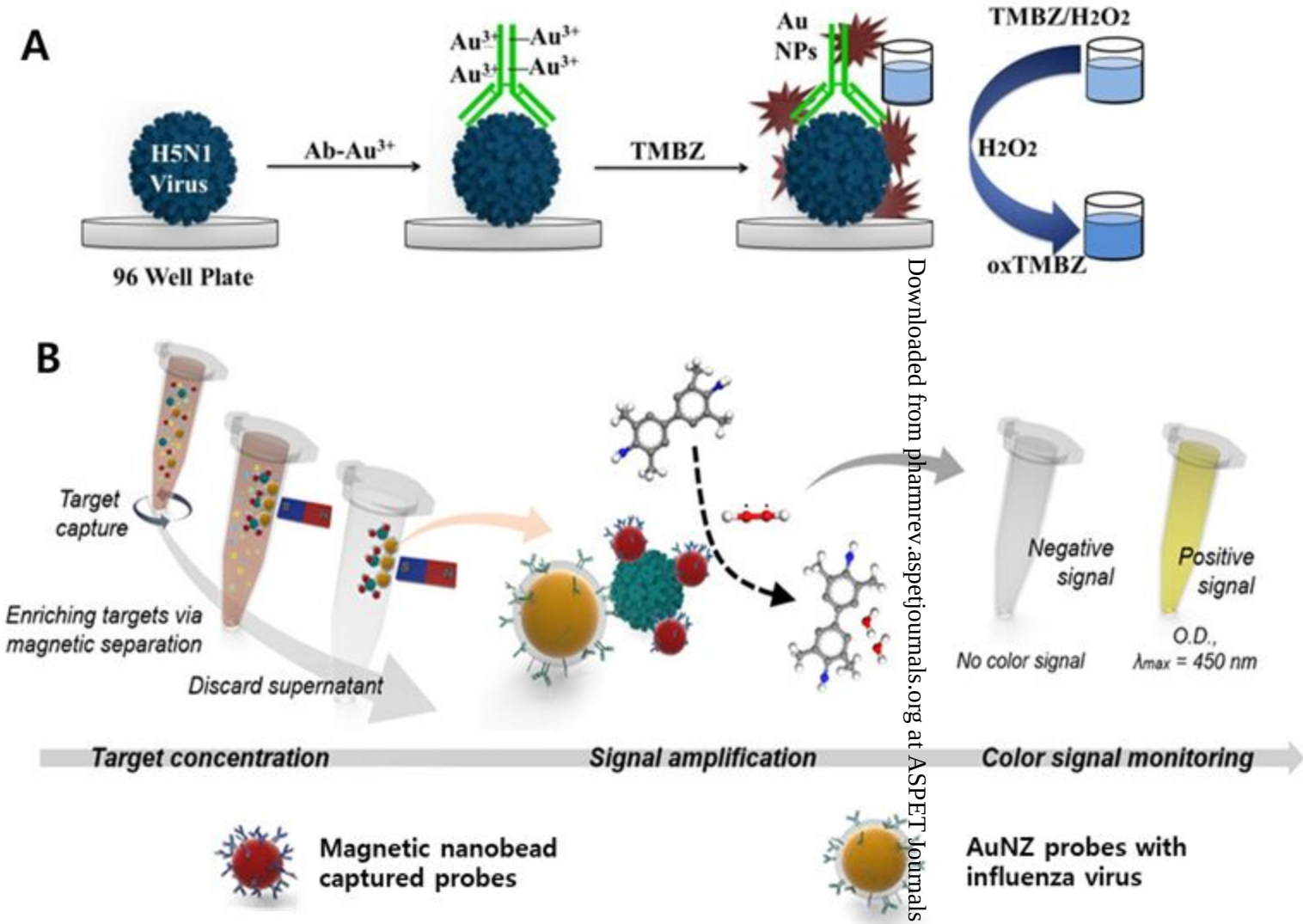

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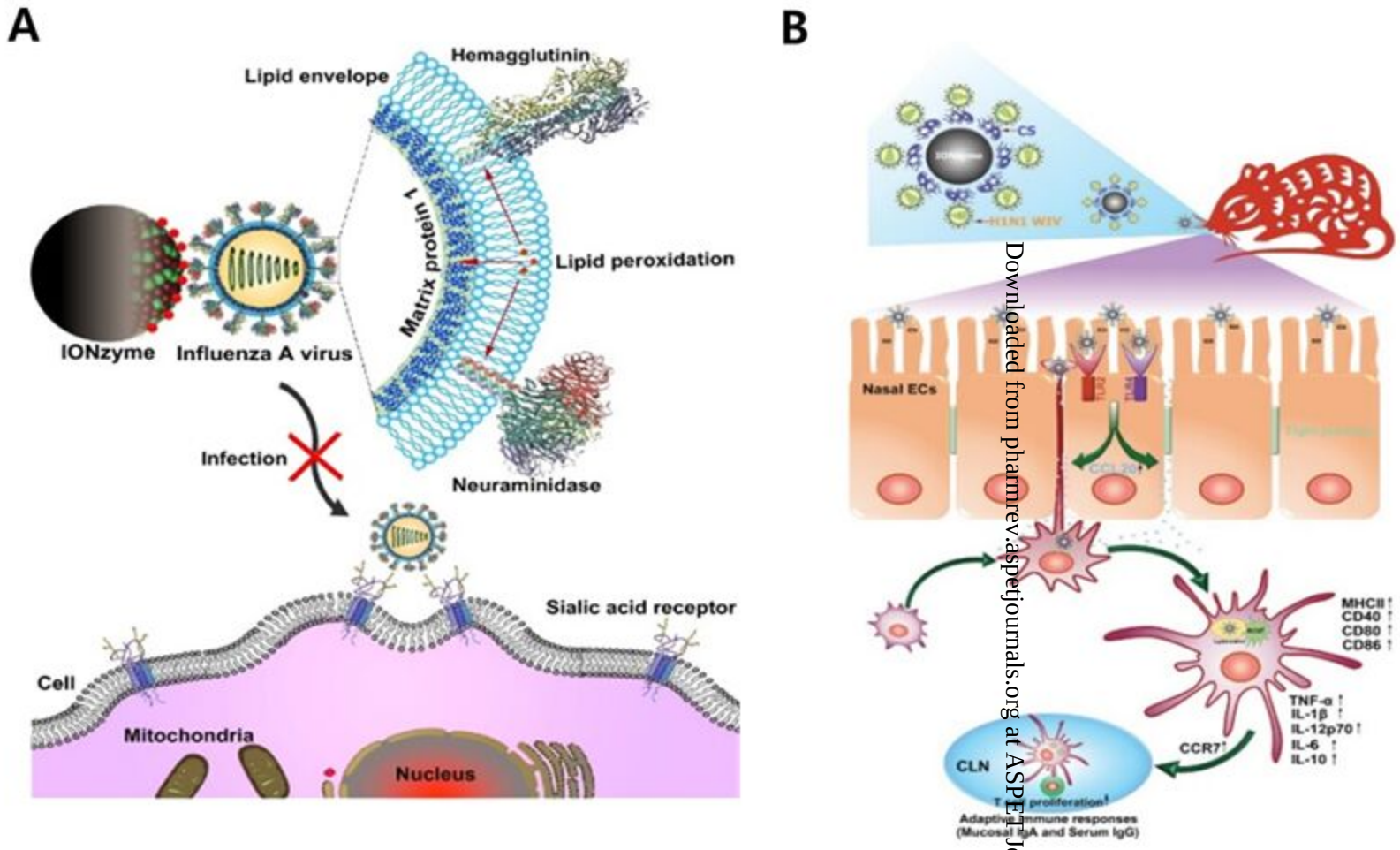

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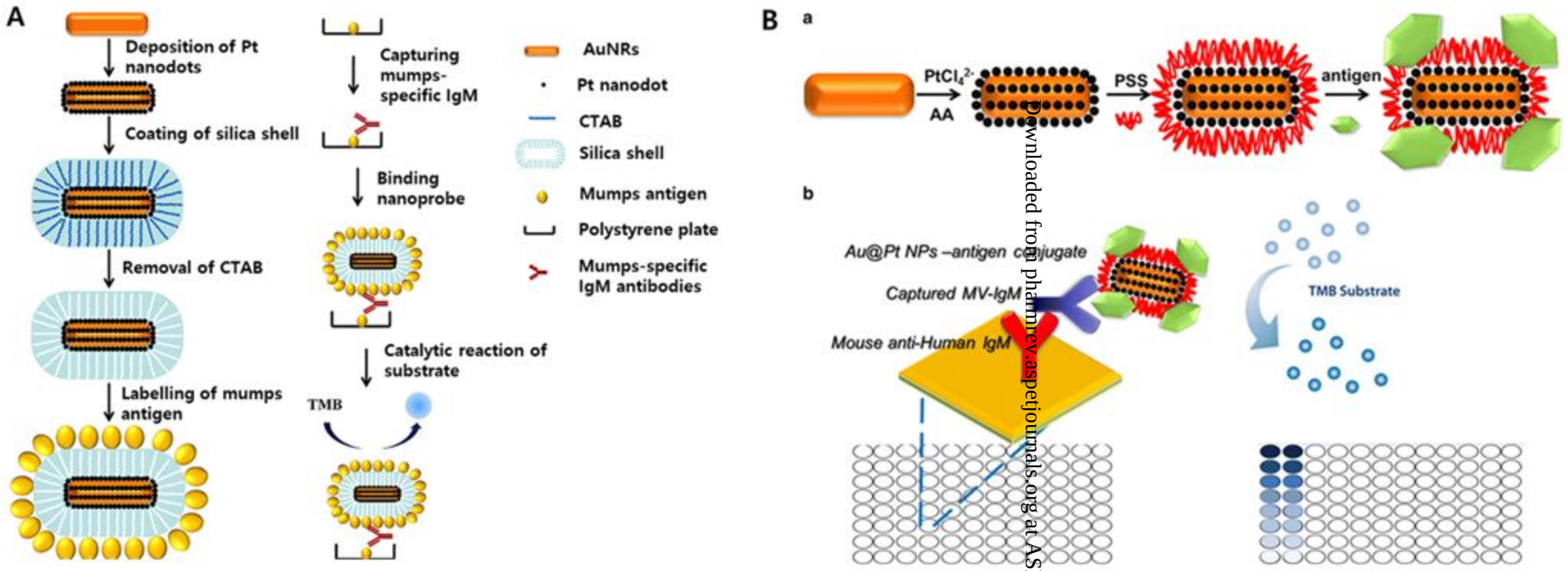

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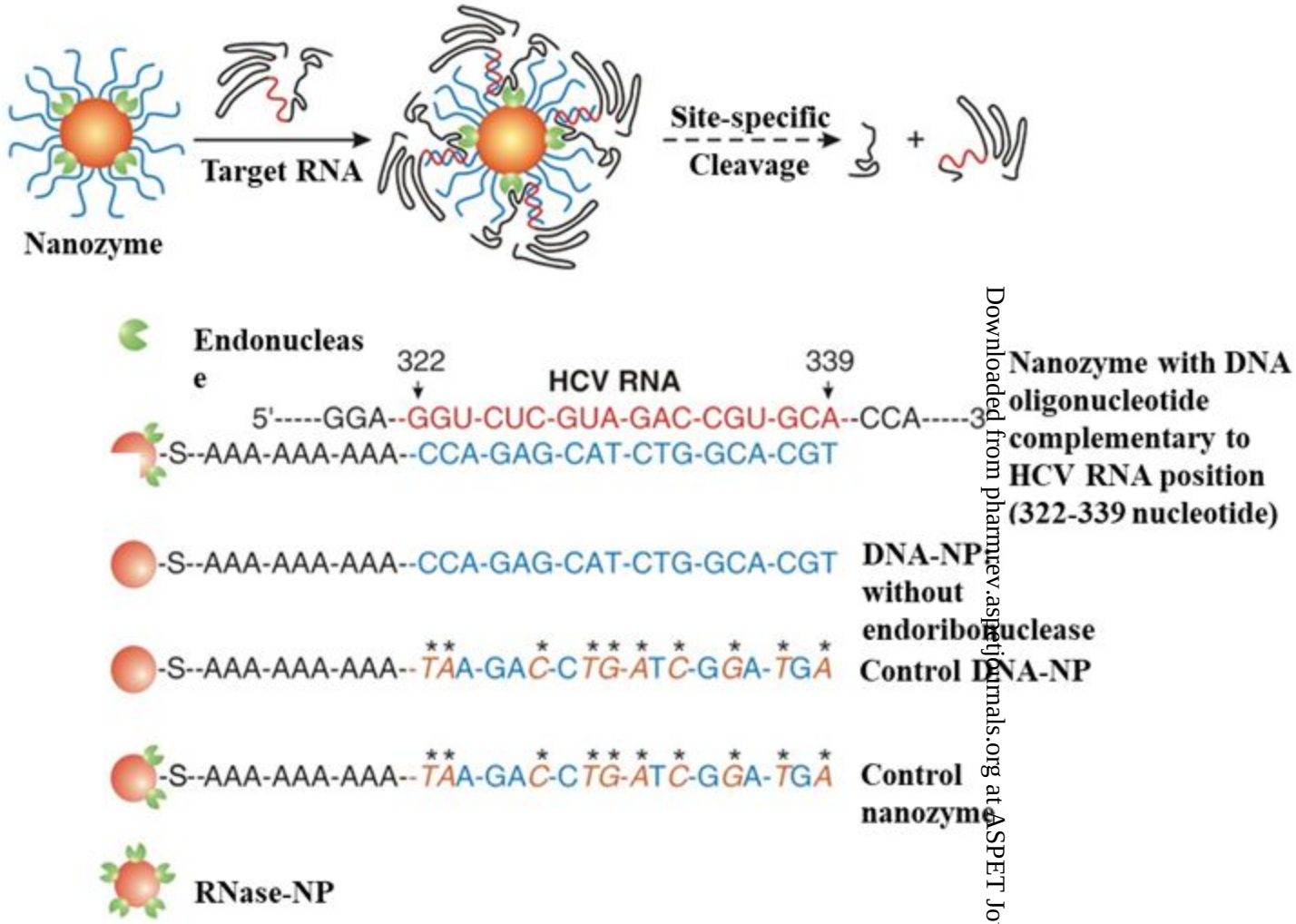

Fig. 7

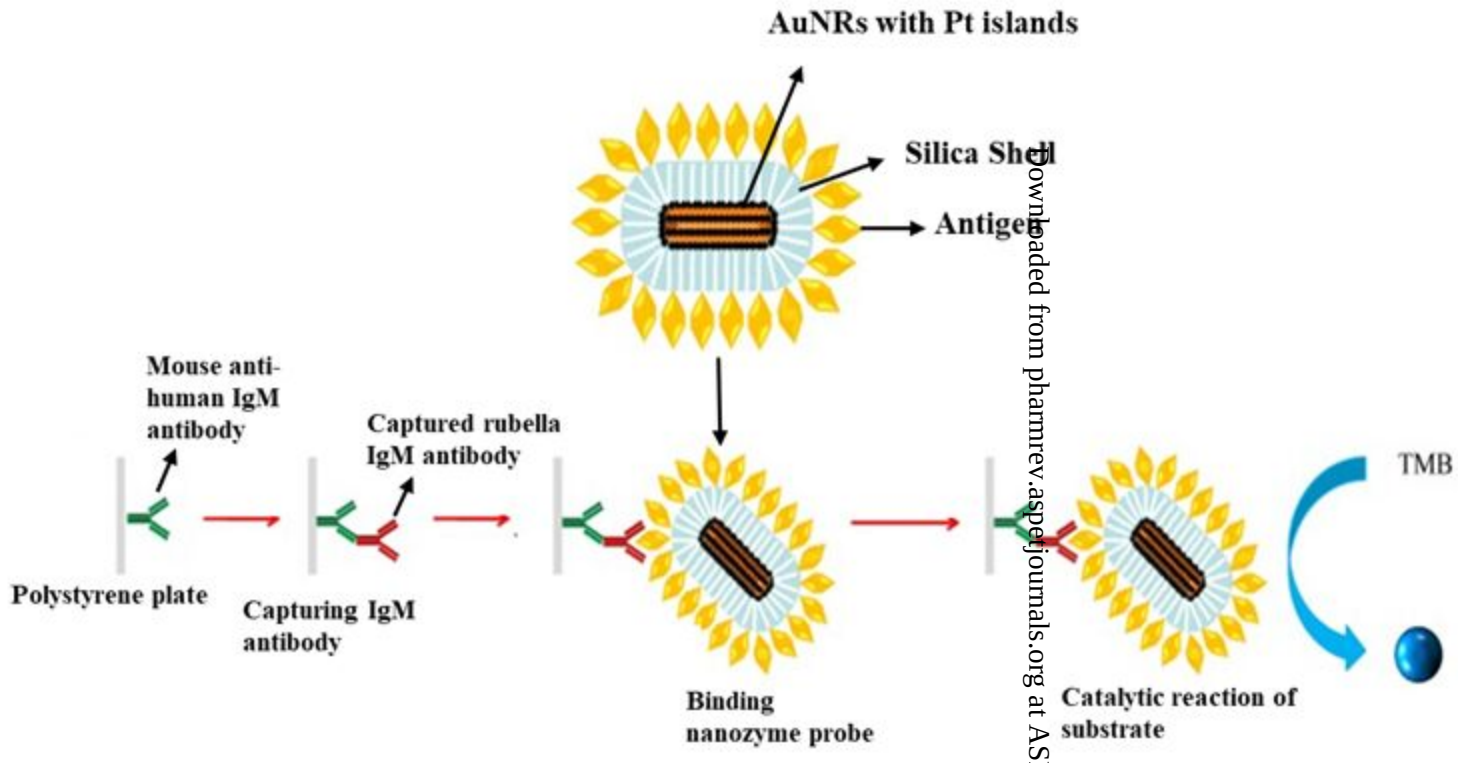

Fig. 8

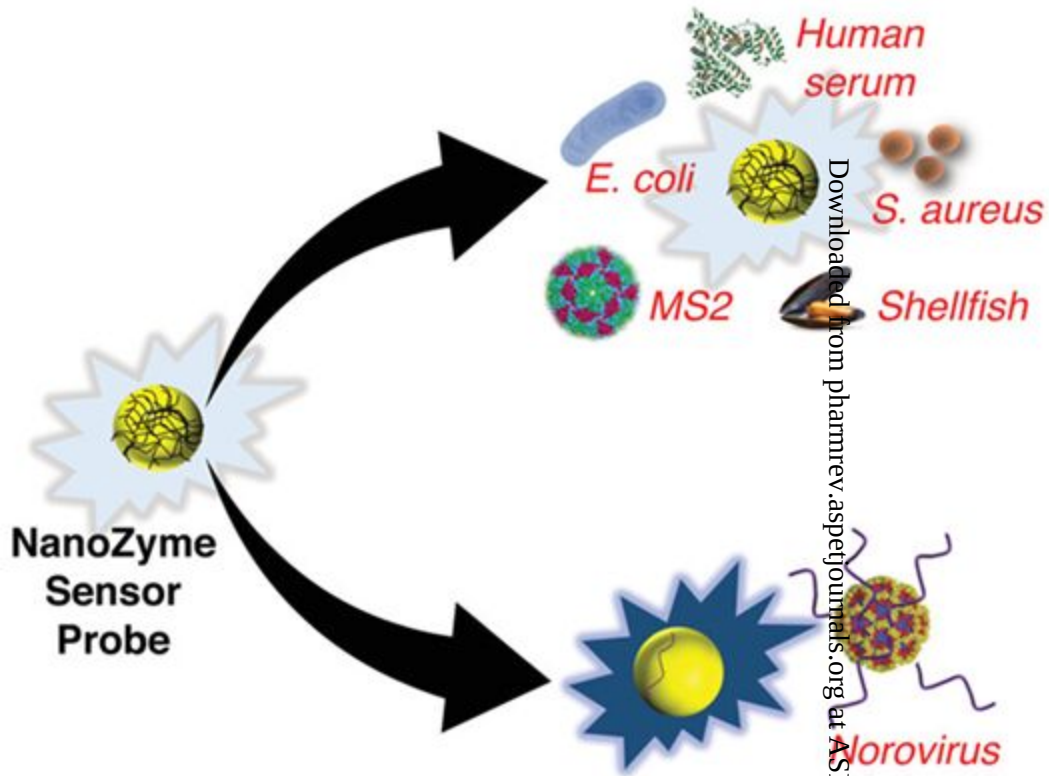

Fig. 9