ISSN 2993-5407

Review Article

Translational Medicine : Open Access

A Brief Overview of Nanozyme-Based Colorimetric and Fluorometric Sensors for Early Diagnosis of COVID-19

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Submitted: 2023, Aug 28; **Accepted:** 2023, Sep 26; **Published:** 2023, Oct 30

Citation: Hormozi Jangi, S. R. (2023). A Brief Overview of Nanozyme-Based Colorimetric and Fluorometric Sensors for Early Diagnosis of COVID-19. *Trans Med OA, 1*(2), 76-84.

Abstract

Currently, there are several methods for diagnosis of COVID-19 such as real-time reverse transcription-polymerase chain reaction, hematology examination, polymerase chain reaction, diagnostic guidelines based on clinical features, Chest CT scans, etc. However, yet actual testing methods to identify SARS-CoV-2 are limited. Besides, diagnosis of this new pandemic over SARS, MERS, and H1N1 is one of the most challenges of this field due to their very similar clinical characteristics. To overcome these difficulties, recently, nanozmyes-based systems have been applied for fast, accurate, reliable, and cost-effective early diagnosis of COVID-19. The aim of this review is quick overview of the nanozyme-based sensing and detection colorimetric and fluorometric methods toward early diagnosis of COVID-19. In this regard, the historical background of COVID-19 and its current diagnostic methods were reviewed. Afterward, the nanozymes were introduced and their biomedical applications were discussed. Finally, the recent progress of early diagnosis of COVID-19 based on nanozymatic systems was reviewed.

Keywords: COVID-19, Nanzoyems; Early Diagnosis of COVID-19, Nanozyme-Based Sensing and Detection, Infectious Disease, Colorimetric, Fluorometric Methods

1. Introduction

On December 31, 2019, the first case of a novel infectious disease with unknown origin (causative agent), features, duration of human transmission, and epidemiological parameters was confirmed in a designated hospital in Wuhan, a major city in China [1,2]. The studies on this new infectious disease revealed that a new generation of coronavirus, SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), is its causative agent [3-5]. Coronaviruses are a group of Coronaviridae families with a broad distribution in mammals which are known as the non-segmented positive-sense RNA viruses [6]. This novel disease caused by SARS-CoV-2 was called Coronavirus disease 2019 and termed COVID-19 by WHO on 11 Fed 2020 [7]. Although the human infections resulting from coronavirus are mild in most cases, shortly after the first report of COVID-19, the novel COVID-19 exhibited a high potential for outbreaks and becoming an epidemic disease and even a pandemic, as now we see in the world [6,8-10]. Currently, there are several methods for diagnosis of COVID-19 including real-time reverse transcription-polymerase chain reaction (rRT-PCR), hematology examination, polymerase chain reaction (PCR), diagnostic guidelines based on clinical features, Chest CT scans [1]. However, early diagnosis of any disease is crucial for an appropriate treatment, yet actual testing methods to identify SARS-CoV-2 are limited. Here, it is challenging to develop effective diagnostics and therapeutics against SARS-CoV-2 [11]. Besides The diagnosis of this new pandemic over SARS, MERS, and H1N1 is one of the most challenges due to their very similar clinical characteristics. To overcome these difficulties, nanozmyes have been applied for fast, accurate, reliable, and cost-effective early diagnosis of COVID-19 [1]. Hence, the aim of this review is a quick overview of the nanozyme-based sensing and detection colorimetric and fluorometric methods for early diagnosis of COVID-19.

2. Nanozymes: Nanomaterials with Enzyme-Like Activity

The fast development of nanoscience and material chemistry has increased interest in researching new and innovative synthesis methods to produce new nanomaterials with unique catalytic activity, unique optical properties, high active area, antibacterial properties, and high biocompatibility [12-19]. The new field of nanozyme-based catalysis, which has been introduced as an alternative to enzyme-based catalysis, is called nanozyme chemistry. On the other hand, nanozymes are known as nanomaterials with high enzyme-like activity and can be used to simulate enzymatic reactions in harsh environmental conditions (for example, higher temperature or wider pH range) [20-27]. As previously reported in the literature, native enzymes, for instance, native peroxidases or ureases suffer from several disadvantages and drawbacks such as low pH stability, low thermal stability,

low recoverability, and no reusability. Commonly, to solve these difficulties and drawbacks of native enzymes, the development of enzyme immobilization protocols has been widely considered in the literature [27-30]. Although enzyme immobilization can enhance enzyme stability, however, the immobilized enzymes reveal very lower activity than the native enzymes due to the enzyme inactivation during the immobilization process [27]. Hence to solve these difficulties, the design and development of low-cost nanozymes with higher stability than the native enzymes along with high enzyme-like/mimic activity were considered as an interesting way for performing enzyme-catalyzed reactions in harsh conditions [21,31].

3. Biomedical Application of Nanozymes

Nanozymes are intensively studied for biomedical applications thanks to their superior intrinsic and tunable enzyme-like activities, for instance, nanozymes with peroxidase, oxidase, and catalase-like activity have been used for several applications in catalysis, biomedical imaging, tumor therapy, and sensing and detection [32-38]. Regarding biosensing applications of nanozymes, up to date, different types of nanozyme-based sensors such as single nanozymatic sensors, enzyme-nanozyme hybrid sensors, etc. have been developed [39]. The majority of nanozyme-based sensors are single-nanozyme-based systems, however, recently a new generation of nanozyme-based systems called "multinanozyme system' was introduced by Hormozi Jangi et al. (2020) [40,41]. During the last years, a wide variety of nanozyme-based colorimetric sensors have been developed for the detection and quantification of a variety of analytes for instance, tryptophan, glutathione (GSH), dopamine, tetracycline, metal cations, glucose, H_2O_2, explosives, and cysteine through the nanozyme-catalyzed oxidation of chromogenic peroxidase substrates including 3, 3′, 5, 5′-tetramethylbenzidine (TMB), 3,3′-diaminobenzidine (DAB), and o-phenylenediamine (OPD) as system-substrate and their colored oxidation products as analytical probes [39,42-50]. Besides, some of the dual-mode nanozyme-based sensors with fluorescence-based response had been developed and utilized for detecting several analytes [51,52].

4. Early Diagnosis of COVID-19 Using Nanozymes

To determine the occurrence and development of COVID-19, in vitro diagnostics based on nanozymatic systems via colorimetric detection were introduced after the first report of COVID-19 in 2019. In this regard, nanozymes with peroxidase-like activity, especially nanozymes with various chemical designs, are inspired to catalyze a colored reaction for fast, accurate, reliable, and cost-effective early diagnosis of COVID-19. For example, Liang et al. (2021) developed a nanozyme-linked nanosensor for the rapid and quantitative diagnosis of COVID-19 by detecting the SARS-CoV-2 nucleocapsid protein in human blood [53]. In this system, immunoreaction and enzyme-catalyzed substrate color reaction were carried out on the chromatographic strip in a device, of which the light signal was read by a photometer through a biosensor channel, and the data was synchronously transmitted via the Bluetooth to the app in-stored smartphone for reporting the result (Figure 1).

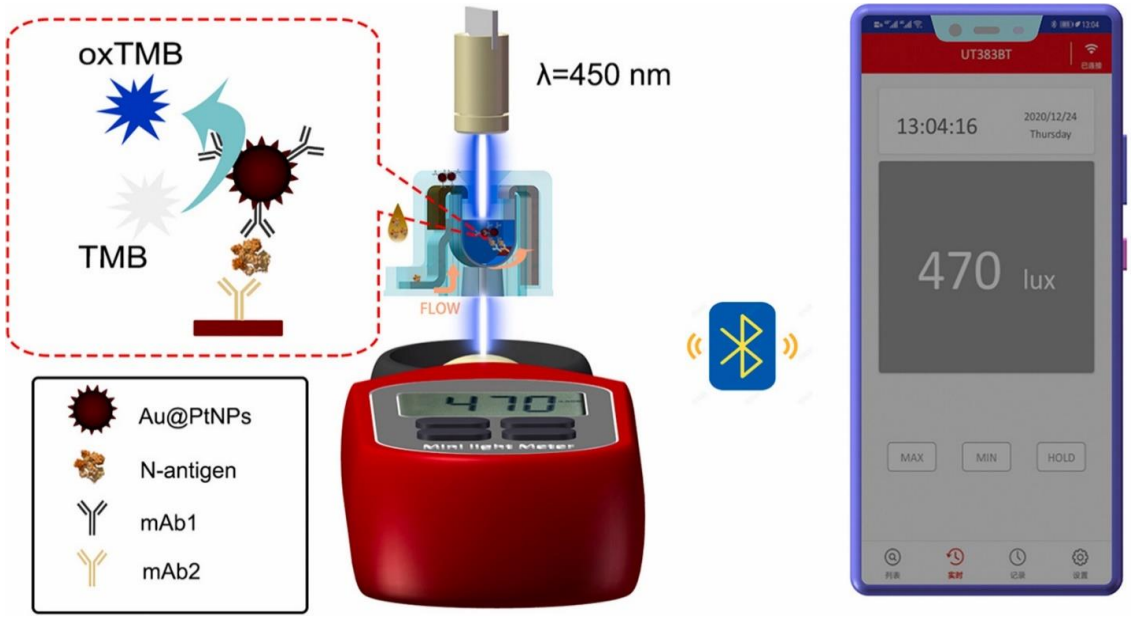

Figure 1; A Strip Nanozyme-Linked Nanosensor for the Rapid and Quantitative Diagnosis of COVID-19 (Adopted from Liang et al. (2021) [53]).

Besides, Fu et al. (2021) used porous metallic gold@platinum nanozymes for the diagnosis of COVID-19 via colorimetric detection of spike (S1) protein of SARS-CoV-2, obtaining a wide linear working range over 10–100 ng mL^{-1} along with a low limit of detection (LOD) of 11 ng mL−1 [54]. The schematic representation of this nanosensor is shown in Figure 2. As can be seen from this figure, in this system, the bimetallic nanozymes were synthesized by successive reduction of Au(III) and Pt(IV) ions, followed by conjugation of antibody of COVID-19 on the surface of the as-prepared nanozymes. Thereafter, the nanozymes

were used for the oxidation of TMB to produce a blue-colored product. In the presence of spike (S1) protein of SARS-CoV-2, the color intensity was inhibited which was used as a basis for colorimetric diagnosis of COVID-19 [54].

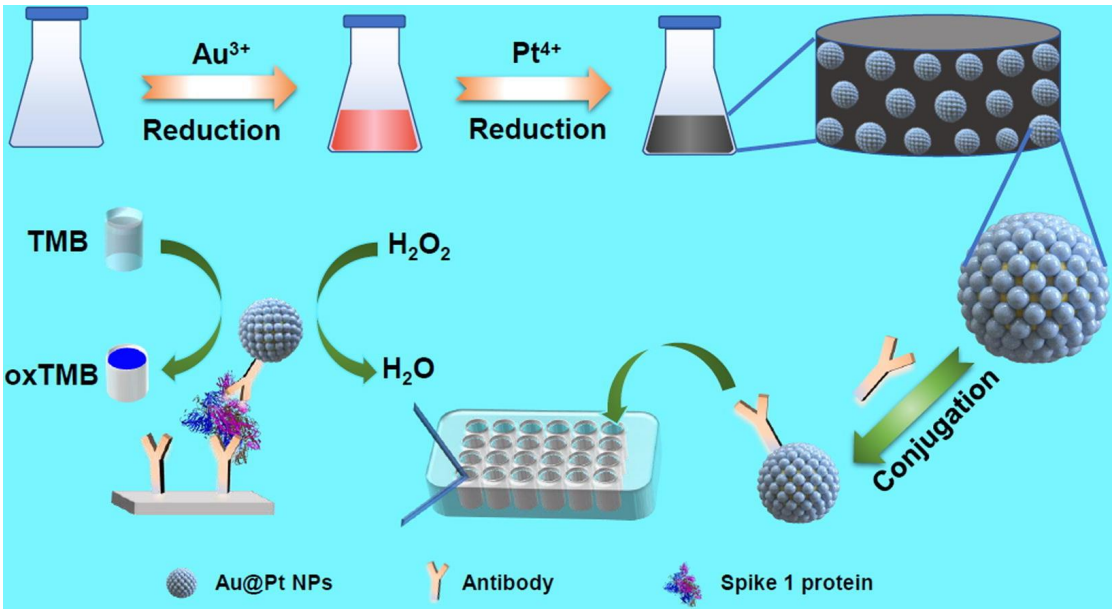

Figure 2: A Novel Strategy for Diagnosis of COVID-19 Based on Metallic Nanozyme-Catalysis (adopted from Fu et al. (2021) [54]).

In 2021, Liu et al. developed a paper-based chemiluminescence nanozymatic strip test for sensitive detection of SARS-CoV-2 antigen which the core of their paper test was a Co–Fe@hemin-peroxidase nanozyme that can catalyze chemiluminescence and amplify immune reaction signal (Figure 3), providing a very low detection limit of 0.1 ng/mL of antigen of COVID-19 and a wide linear range of 0.2-100 ng/mL with a short test time as low as 16.0 min [55].

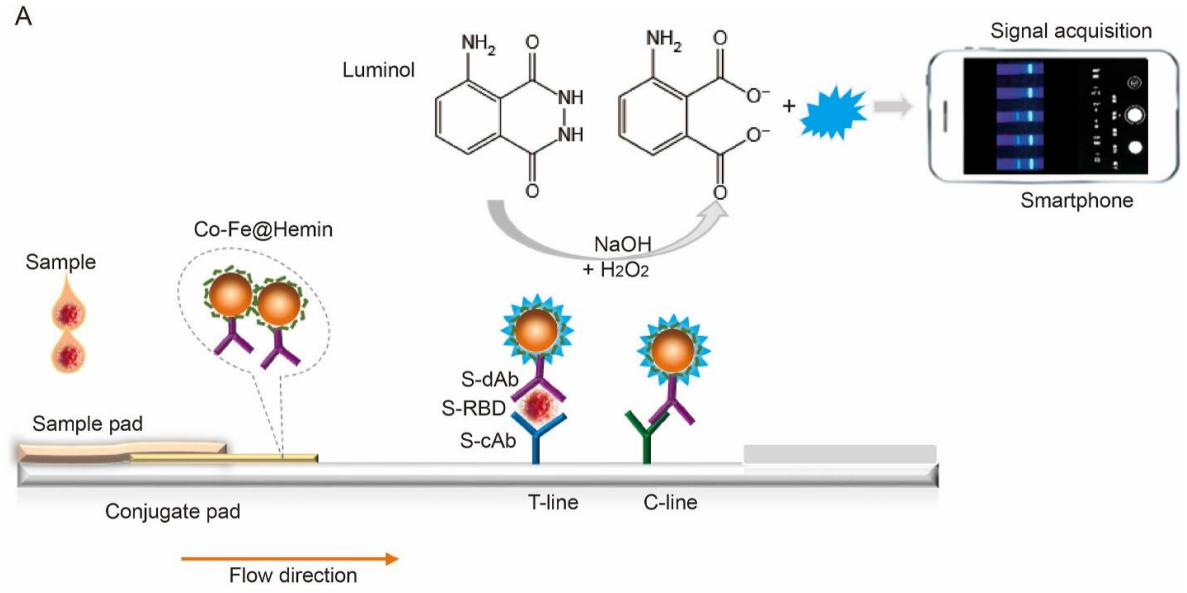

Figure 3: A Paper-Based Chemiluminescence Nanozymatic Strip Test for Sensitive Detection of SARS-CoV-2 Antigen (adapted from Liu et al. (2021) [55]).

Liu et al. (2021) introduced a smartphone-based nanozyme-linked immunosorbent assay for quantitative sensing of SARS-CoV-2 nucleocapsid phosphoprotein in 37 serum samples from 20 patients infected with COVID-19 [56]. The system was based on probing the light intensity of the colored product of oxidation of TMB using a Lux meter or an android-based Lux meter application. The schematic representation of this smartphone-based nanozyme-linked immunosorbent assay is shown in Figure 4. As can be seen from this figure, in the first step, the SARS-CoV-2 nucleocapsid phosphoprotein was separated from the blood via its interaction with magnetic beads modified with antibody#1 and applying a magnetic field. The magnetic beads were then introduced into a nanozymatic system. The SARS-CoV-2 nucleocapsid phosphoprotein presented on the surface

of the magnetic beads interacted with antibody#1 presented on the nanozymes surface. Afterward, the linked nanozymes were applied to catalyze the oxidation of TMB by hydrogen peroxide. Thereafter, the Lux meter was utilized for detecting the variation of light intensity as an index for detecting the SARS-CoV-2 NP antigen [56].

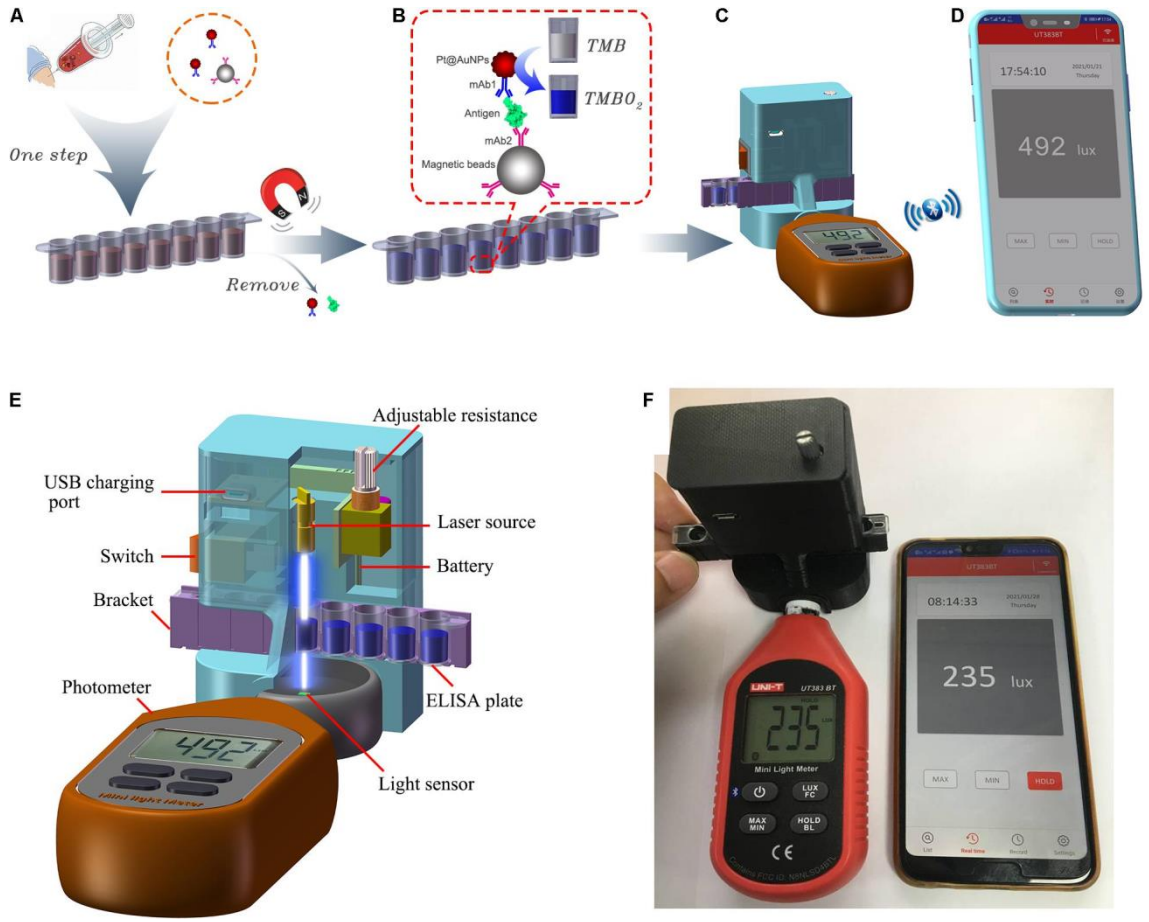

Figure 4: A Smartphone-Based Nanozyme-Linked Immunosorbent Assay for Quantitative Sensing of SARS-CoV-2 Nucleocapsid Phosphoprotein (Adapted from Liu et al. (2021) [56]).

In 2022, Ali and Omer introduced an ultrasensitive aptamer-functionalized Cu-MOF fluorescent nanozyme and utilized it for optical detection of C-reactive protein toward the diagnosis of COVID-19 via colorimetric and fluorometric dual mode responses of a nanozymatic process based on TMB oxidation for colorimetric and variation of fluorescence intensity of Cu-MOF for fluorometric mode detection of COVID-19 [57]. It is notable that the immobilized RNA on Cu-MOFs can block the peroxidase activity and fluorescence of the signal traducer probe while upon addition of C-reactive protein, the RNA will release from the surface of nanozyme and consequently both the fluorescence and peroxidase activity of Cu-MOFs will recover which was used as a basis for diagnosis of COVID-19 (Figure 5).

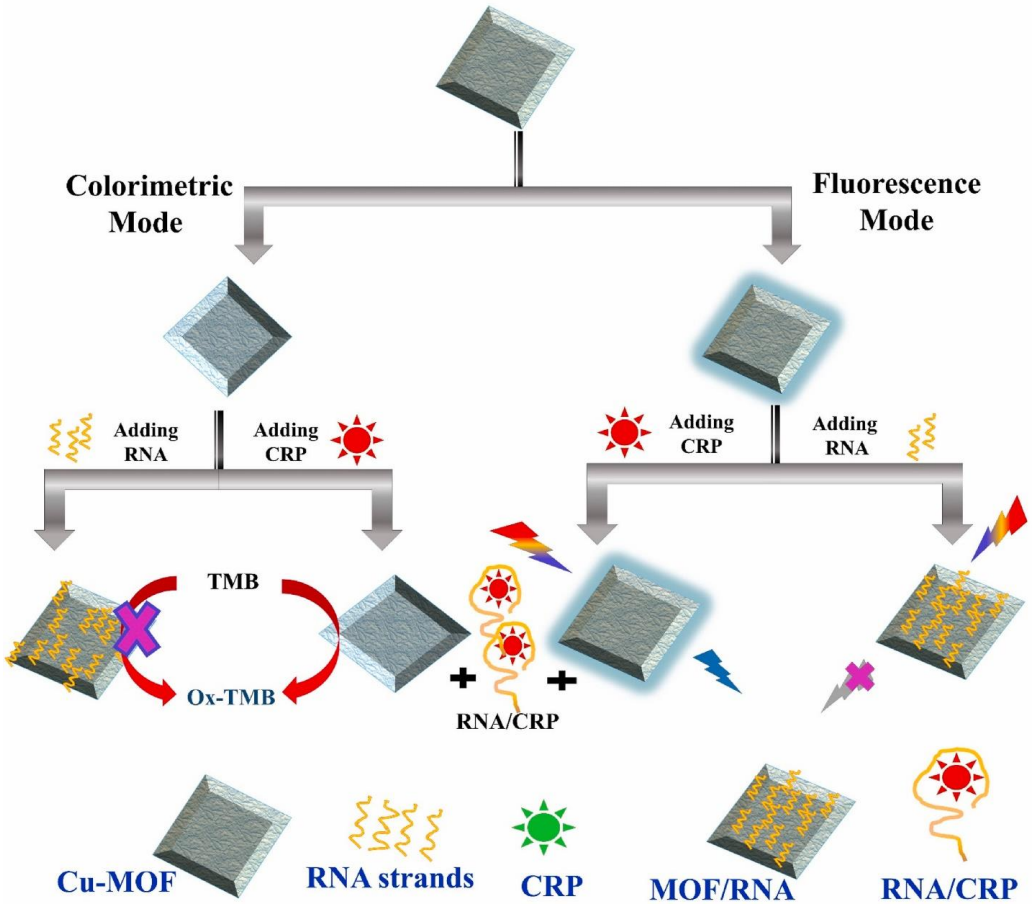

Figure 5: An Ultrasensitive Aptamer-Functionalized Cu-MOF Fluorescent Nanozyme for Optical Dual Mode Diagnosis of COVID-19 (Adopted from Ali and Omer (2022) [57]).

Zhao et al. (2022) employed MIL-101(CuFe) nanozymes for accurate visual naked-eye diagnosis of COVID-19 via detecting the universal receptor of CD147, providing a very low detection limit of 3 PFU/mL and a detection time as short as 30 min [58]. The sensor was based on the inhibition of the peroxidase-like activity of as-prepared nanozymes in the presence of the universal receptor of CD147. This inhibition was probed by detecting the color intensity of the oxidation product of TMB in the presence and the absence of the universal receptor of CD147. The principles of this nanobiosensing method are shown in Figure 6.

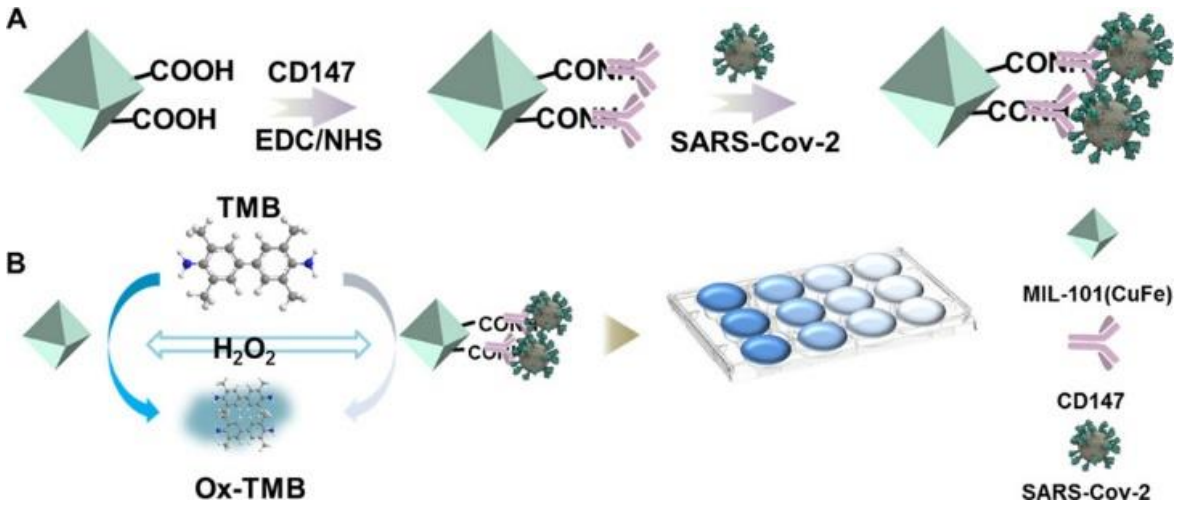

Figure 6: MIL-101(CuFe) Nanozymes for Accurate Visual Naked-Eye Diagnosis of COVID-19 via Detecting the Universal Receptor of CD147 (Adopted from Zhao et al. (2022) [58]).

Besides, Wu et al. (2022) developed a MnO2 nanozyme-mediated CRISPR-Cas12a system for naked-eye diagnosis of COVID-19 [59]. In this system, the MnO2 nanorods were initially linked to magnetic beads using a single-stranded DNA (ssDNA). The as-prepared nanozymes show high oxidase-like activity and can catalyze the oxidation of TMB to a blue-colored product. However, the detection color will change by activation of Cas12a by SARS-CoV-2 and cleaving the ssDNA which was used as a basis for the detection of SARS-3CoV-2 (Figure 7).

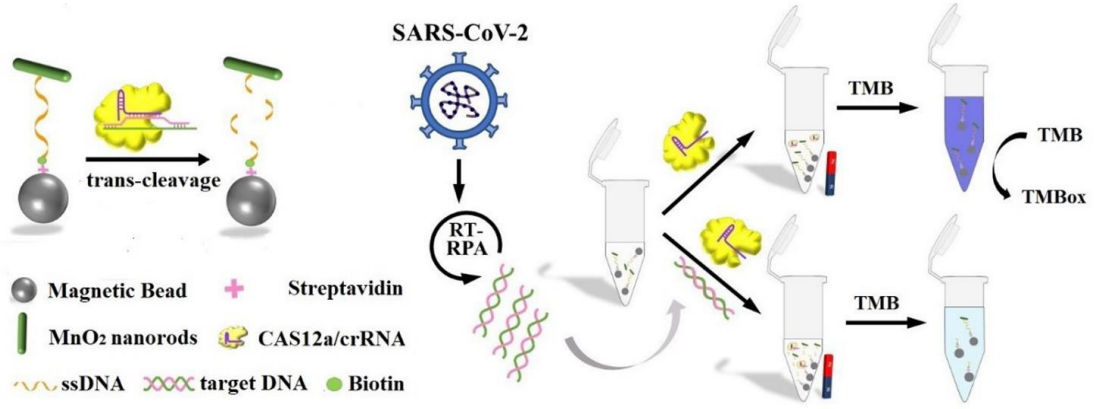

Figure 7: A MnO_2 Nanozyme-Mediated CRISPR-Cas12a System for Naked-Eye Diagnosis of COVID-19 (Adopted from (Wu et al. 2022) [59]).

In 2023, He et al. performed a nanozyme-based colorimetric method for naked-eye diagnosis of COVID-19 by iron manganese silicate nanozymes as peroxidase-like nanozymes [60]. The nanozymes activity can be inhibited by introducing the pyrophosphate ions which are generated by amplification processes and can be used for optical diagnosis of COVID-19. Besides, Chu et al. developed a robust colorimetric immunosensing method using liposome-encapsulated MnO2 nanozymes for diagnosis of COVID-19 via detection of SARS-CoV-2 antigen using TMB as the chromogenic substrate [61]. Moreover, Vafabakhsh et al. reported a paper-based colorimetric nanozyme-based sensor for diagnosis of COVID-19 using aptamer-modified ChF/ZnO/CNT nanohybrids as peroxidase mimics and TMB as the chromogenic substrate [62]. The main nanocomposite platform was constructed and functionalized with a specific COVID-19 aptamer, providing a linear range of 1–500 pg/mL and a detection limit of 0.05 pg/mL. Also, Sun et al. used Fe(II)-doped ZIF-67 derivatives-based composites as nanozymes for eye diagnosis of COVID-19 via dual-mode colorimetric and fluorescent detection of SARS-CoV-2 nucleocapsid protein, providing a limit of detection of 0.022 ng/mL and 0.018 ng/mL for colorimetric and fluorescent nanozymatic sensors, respectively [63]. The schematic representation of the introduced sensor is shown in Figure 8. As can be seen in this scheme, TMB was used as a chromogenic substrate and CDs were utilized as fluorescent nanoprobes for the detection of SARS-CoV-2 nucleocapsid protein.

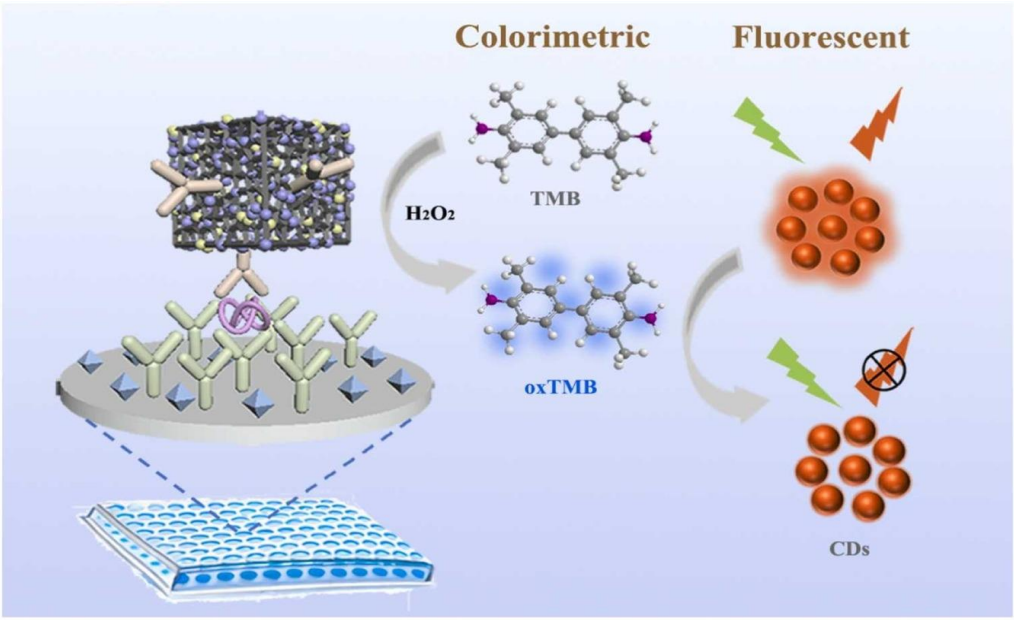

Figure 8: Fe(II)-doped ZIF-67 Derivatives-Based Composites as Nanozymes for Eye Diagnosis of COVID-19 Via Dual-Mode Colorimetric and Fluorescent Detection of SARS-CoV-2 Nucleocapsid Protein (Adopted from Sun et al. (2023) [63]).

5. Conclusions

Currently, there are several methods for diagnosis of COVID-19 such as real-time reverse transcription-polymerase chain reaction, hematology examination, polymerase chain reaction, diagnostic guidelines based on clinical features, Chest CT scans, etc. However, yet actual testing methods to identify SARS-CoV-2 are limited. Besides, diagnosis of this new pandemic over SARS, MERS, and H1N1 is one of the most challenges of this field due to their very similar clinical characteristics. To overcome these difficulties, recently, nanozmyes-based systems have been applied for fast, accurate, reliable, and cost-effective early diagnosis of COVID-19. The aim of this review is a quick overview of the nanozyme-based sensing and detection colorimetric and fluorometric methods toward early diagnosis of COVID-19. In this regard, the historical background of COVID-19 and its current diagnostic methods were reviewed. Afterward, the nanozymes were introduced and their biomedical application was discussed. Finally, the recent progress of early diagnosis of COVID-19 based on nanozymatic systems was reviewed.

Acknowledgments

The authors gratefully thank the Hormozi Laboratory of Chemistry and Biochemistry (Zabol, Iran) for the support of this work.

Conflict of Interest

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

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