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A Mini-Review on Nanozyme Chemistry with Focus on Analytical and Bioanalytical Sensing Applications

Saeed Reza Hormozi Jangi

Hormozi Laboratory of Chemistry and Biochemistry, Zabol 9861334367, Iran

***Corresponding author**

Saeed Reza Hormozi Jangi,
Hormozi Laboratory of Chemistry and Biochemistry,
Zabol 9861334367,
Iran
ORCID : https://orcid.org/0000-0003-0206-4094

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Abstract

The fast development of nanoscience and material chemistry has increased interest in researching new and innovative synthesis methods to produce new nanomaterials. Among different nanomaterials, a wide variety of these materials reveal high intrinsic enzyme-like activity. Due to their high catalytic efficiency and stability, 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. This article aimed to present a brief introduction on the nanozyme-based chemistry with emphasizing on the historical overview of recent nanozymatic sensors.

Keywords: Nanozymes; Nanozyme-based chemistry; Nanozymes applications; nanozymatic sensors.

Nanozyme Chemistry and Nanozymes Application

Most of identified enzymes are proteins which are commonly introduced as catalysts of chemical reactions in biological environments (i.e., bioctatalysts). The key feature of these biocatalysts is their high catalytic efficiency and substrate specificity which make them suitable for playing a specific role in biochemistry. Among different types of enzymes, peroxidase enzymes, especially horseradish peroxidase (HRP), are attractive enzymes from both industrial and clinical points of view. In real world, practical application of peroxidase enzyme in industrial reactions as the biocatalyst is an interesting filed. Up to now, several researches on these enzymes are carried out to provide useful information about the enzyme structure, and its functional groups, reaction pathway, and active sites (Huang et al., 2019; Wu et al., 2019; Liang & Yan, 2019; Wei & Wang, 2013; Wang et al., 2020; Zhang et al., 2021; Wang et al., 2016; Jiao et al., 2020; Liu & Liu, 2017; Wang et al., 2020; Dong et al., 2020; Hormozi Jangi, 2023; HORMOZI JANGI & Akhond, 2020; Dehghani et al., 2024; Hormozi Jangi & Gholamhosseinzadeh, 2023; Jangi & Akhond, 2021; Thakkar et al., 2010; Hajipour et al., 2012; Hormozi Jangi, 2023; Hormozi Jangi, 2023). Regarding the peroxidase enzymes, the enzyme specific substrate is hydrogen peroxide (HP) while their function is catalyzing the oxidation of a hydrogen-donating substrate (for example, benzidine). More precisely, hydrogen peroxide is the initiator of the peroxidase-mediated reactions. In fact, oxidation of a wide range of organic compounds (substrates) including aromatic amines, phenols, and their mixtures can be initiated in the presence of hydrogen peroxide or other hydroperoxides and HRP as enzymes. Many chromogenic substrates have been defined as secondary substrates of horseradish peroxidase due to its low selectivity to electron-donating compounds. These chromogenic substrates are called chromogenic electron donors because these compounds show a distinct color change when oxidized by hydrogen peroxide in the presence of the peroxidase enzyme. It is noteworthy that peroxidase and other natural enzymes, show some of the following serious disadvantages including:

1. They are sensitive to environmental changes such as pH and temperature changes and are easily denatured.
2. They are digested by protease enzyme.
3. Their preparation and purification are complicated and expensive.

Fixing these disadvantages is possible through the development of some stable artificial enzymes with high catalytic ability. In this regard, nanotechnology has opened the doors for the development of new enzyme mimetic materials. In 2007, it was explored that Fe_3O_4 magnetic nanoparticles (NPs) exhibited significant peroxidase-like activity. This research opened the door for a new branch of nanochemistry called "nanozyme chemistry" (Huang et al., 2019; Wu et al., 2019; Liang & Yan, 2019; Wei & Wang, 2013; Wang et al., 2020; Zhang et al., 2021; Wang et al., 2016; Jiao et al., 2020; Liu & Liu, 2017; Wang et al., 2020; Dong et al., 2020; Hormozi Jangi, 2023; HORMOZI JANGI & Akhond, 2020; Dehghani et al., 2024; Hormozi Jangi & Gholamhosseinzadeh, 2023; Jangi & Akhond, 2021; Thakkar et al., 2010; Hajipour et al., 2012; Hormozi Jangi, 2023; Hormozi Jangi, 2023; Hormozi Jangi, 2023; Jangi, 2023; Hormozi Jangi & Dehghani, 2023; Jangi, 2023; Hormozi Jangi, 2023; Ahmadi-Leilakouhi et al., 2023; Hormozi Jangi, 2023; Jangi & Akhond, 2021; Jangi & Akhond, 2022; Jangi et al., 2020; Wang et al., 2018; Lu et al., 2022; Ren et al., 2022; Tang et al., 2021; Li et al., 2020; Yu et al., 2021; Chang et al., 2020; Arshad et al., 2022; Jangi et al., 2020). Nanozyme chemistry is -consisted of design, synthesis, modification, biochemical characterization, structural characterization, and application of nanoscale artificial enzymes as well as evaluation of mechanism of nanozyme-based systems (Figure 1) (Huang et al., 2019; Wu et al., 2019; Liang & Yan, 2019; Wei & Wang, 2013; Wang et al., 2020; Zhang et al., 2021; Wang et al., 2016; Jiao et al., 2020; Liu & Liu, 2017; Wang et al., 2020; Dong et al., 2020). In fact, 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 (Hormozi Jangi, 2023; HORMOZI JANGI & Akhond, 2020), unique optical properties (Dehghani et al., 2024; Jangi & Akhond, 2021), high active area (Thakkar et al., 2010), antibacterial properties (Hajipour et al., 2012), and high biocompatibility (Hormozi Jangi, 2023). 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) (Hormozi Jangi, 2023; Hormozi Jangi, 2023; Jangi, 2023; Hormozi Jangi & Dehghani, 2023; Jangi, 2023; Hormozi Jangi, 2023; Ahmadi-Leilakouhi et al., 2023; Hormozi Jangi, 2023). As previously reported in the literature (Hormozi Jangi, 2023), 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 (Hormozi Jangi, 2023; Jangi & Akhond, 2021; Jangi & Akhond, 2022; Jangi et al., 2020). Hence to solve these difficulties, the design and development of low-cost nanozymes were considered as an interesting way for performing enzyme-catalyzed reactions in harsh conditions (Hormozi Jangi, 2023; Wang et al., 2018). Nanozymes have been used for several applications in catalysis (Lu et al., 2022), biomedical imaging (Ren et al., 2022), diagnosis of infection diseases (e.g., COVID-19), treatment of diseases, tumor therapy (Tang et al., 2021; Li et al., 2020), and sensing and detection (Yu et al., 2021; Chang et al., 2020; Arshad et al., 2022) (Figure 2).

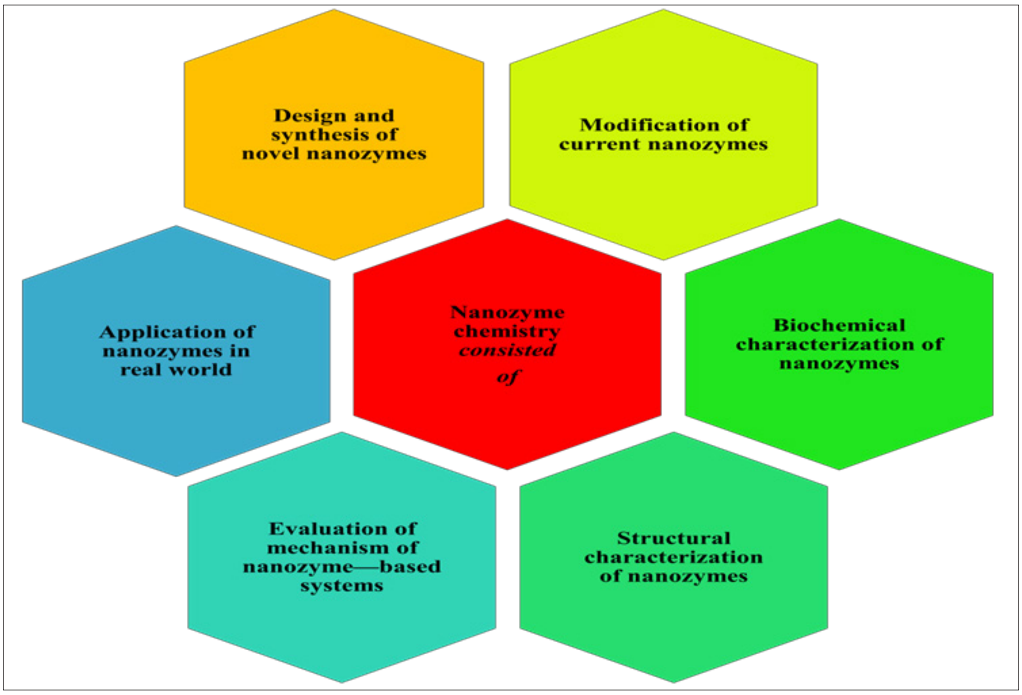

Figure 1: Nanozyme chemistry consisted of several parts.

For instance, up to date, different types of nanozyme-based sensors such as single nanozymatic sensors, enzyme-nanozyme hybrid sensors, etc. have been developed [39]. Recently a new generation of nanozyme-based systems called "multinanozyme system' was introduced by (Hormozi Jangi et al., 2020) (Jangi et al., 2020; Jangi et al., 2020). 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 (Xu et al., 2023), glutathione (GSH) (Jangi & Akhond, 2020), dopamine (Ray et al., 2020), tetracycline

(Shen et al., 2022), metal cations (Akhond et al., 2020), glucose (Chen et al., 2019), H_2O_2 (Hormozi Jangi & Dehghani, 2023), explosives (Hormozi Jangi et al., 2020), and cysteine (Singh et al., 2017). Besides, some of the nanozyme-based sensors with fluorescence-based response had been developed and utilized for detecting several analytes (Wang et al., 2022; Heo et al., 2020). It is notable that after the first report of COVID-19 on 2019 (Hormozi Jangi, 2023; Jangi, 2023), the nanozymes had been utilized for detection of SARS-CoV-2 (Liang et al., 2021).

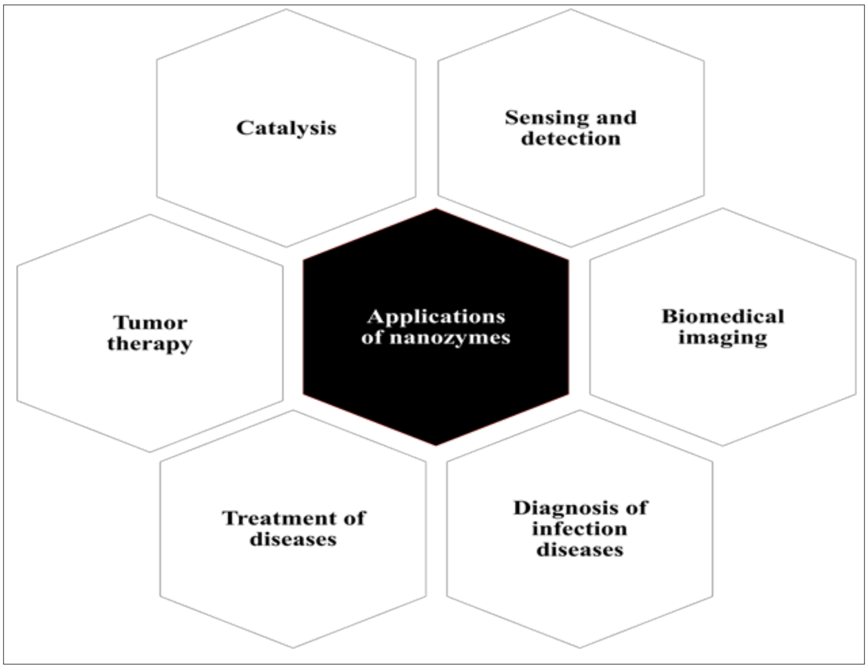

Figure 2: Different applications of nanozymes in real world.

Historical overview of nanozymatic sensors

Up to now, several reports on nanozymes application in the field of sensing and detection were published as we mentioned above. In this section, the recent published works were reviewed. In 2016, (Lu et al., 2016) fabricated a new nanozyme based sensor for colorimetric quantification of hydrogen peroxide utilizing a new 3D porous dendrites of PtCu three-dimensional (3D) hierarchical porous. The peroxidase mimic properties of the dendrites were checked by the standard method of 3,3′,5,5′-tetramethyl-benzidine oxidation by hydrogen peroxide. The sensor provided a linear range of over 0.3–325 µM along with a LOD of 0.1 µM for hydrogen peroxide determination which is lower than the accepted level of hydrogen peroxide reported by US FDA (i.e., 15 µM). The method showed good selectivity and was applied for the determination of the hydrogen peroxide content of milk samples (Figure 3).

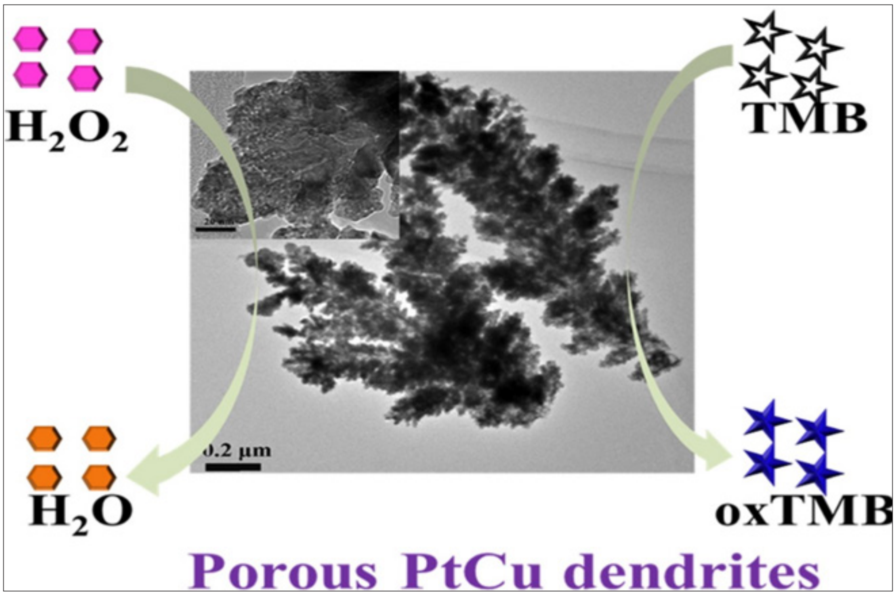

Figure 3: A nanozyme based sensor for colorimetric quantification of hydrogen peroxide utilizing a new 3D porous dendrites of PtCu three-dimensional (3D) hierarchical porous (adapted from (Lu et al., 2016).

In 2017, (Singh et al., 2017) reported a selective nanozyme-based method for the quantification of malathion utilizing the peroxidase-mimicking properties of palladium-gold nanorods. The O- phenylenediamine was used as the chromogenic compound for the detection purpose upon its oxidation by hydrogen peroxide catalyzed by palladium-gold nanozyme. A LOD as low as 60 ng mL^{-1} was obtained for the detection of malathion along with a recovery percentage of 80–106%. The reproducibility of the sensor was found to be 2.7-6.1% and 3.2-5.9% intra and inter-assay, in turn. In 2017, (Zhang et al., 2018) immobilized modified the Cu(II)-based MOF-74 and employed them for the sensitive electrochemical detection of 2,4,6-trichlorophenol. XRD, FT-IR, SEM, UV-Vis., and CV measurements were performed for investigation of composite properties. A wide linear range over 0.01-9 μM and LOD of about 0.005 μM was achieved for the determination of 2, 4, 6-trichlorophenol. The repeatability studies showed an RSD% of 4.6% for determination of 0.5 5 μM 2, 4, 6-trichlorophenol. In 2018, (Chen et al., 2018) synthesized honeycomb-like zinc-doped Ni(II)-based MOF with spherical particles using HCl as the modulator upon a microwave-assisted method. The resulting MOFs were used as electrode materials, showed a specific capacity of 237.4 mA h g^{-1} for 1 A g^{-1} which can be used as supercapacitor material. In 2018, (Yu et al., 2018) designed a new sensor for the Pb(II) detection using a Fe(II)-MOFs/Pd-Pt alloys composites via a target-triggered nuclear acid cleavage of Pb^{2+}-specific DNAzyme. Moreover, the DNAzyme was immobilized on streptavidin-modified reduced graphene oxide-tetraethylene pentamine-gold nanoparticles for use as the sensor platform. By introducing the Pb(II), the DNA was cleaved by the DNAzyme and a new single strand of DNA was produced. In the presence of Pb^{2+}, the substrate DNA strand can be specifically cleaved at the ribonucleotide site by DNAzyme to produce a new single-DNA on the interface. Then, the single-strand DNA was used for modification of Fe-MOFs/PdPt NPs for signal amplification. The sensor showed a linear range over 0.005-1000 nM and a LOD of 2 pM (S/N =3) for Pb(II) determination in drinking water. In 2018, (Li et al., 2018) synthesized and characterized a new iron-base MOF@palladium nanoparticles composite via assembly palladium nanoparticles on the surface of NH_2-Fe-MIL-88. The composite was used for the determination of microRNA-122 by the electrocatalytic oxidation of 3,3′,5,5′-tetramethylbenzidine in the presence of H_2O_2 catalyzed by the developed nanocomposite with intrinsic peroxidase-like activity. A working range over 0.01 fM-10 pM along with a LOD of 0.003 fM (S/N =3) was obtained. In 2018, (Lopa et al., 2018) used a microwave-assisted solvothermal route for the synthesis of a novel base-stable Cr(III)-MOF and utilized it for the non-enzymatic quantification of hydrogen peroxide via its electro-reduction in 0.1 M NaOH through the redox process of Cr(III)/Cr(II) in the Cr (III)-MOF. A working range of 25-500 mM and a LOD of 3.52 mM was provided for hydrogen peroxide determination. In 2018, (Wang et al., 2018) used the Ru, Ir, and Pt-based nanozymes for developing a nanozyme sensor array toward biothiols and proteins determination as well as cancer cells discrimination. The sensor array can accurately identify 42 of 45 proteins and 28 of 30 biothiols which makes it applicable for biothiols detection in blood and protein discrimination in urine samples. In 2019, (Xue et al., 2019) reported a new nanocomposite of silver nanoparticles with amino-functionalized multi-walled carbon nanotubes with high water-processibility, environmental stability, and electrocatalytic capacity via the ultrasonic-assisted liquid-phase exfoliation method. The nanocomposite was then dispersed in carboxymethyl cellulose sodium and applied for the development of electrochemical sensors for single/simultaneous determination of xanthine, uric acid, and hypoxanthine, showing a working range of 0.5–680 μM (LOD=0.021 μM), 0.1–800 μM (LOD= 0.052 μM), and 0.7–320 μM (LOD=0.025 μM), in order. In 2020, (Zhu et al., 2020) fabricated a nanozymatic sensor array for the detection of aromatic pesticides using heteroatom-doped graphene. The enzyme-like activity of nanozyme was inhibited in the presence of the different pesticides with a characteristic distinguish between them. This sensor array can determine the lactofen, bensulfuron-methyl, fluoroxypyr-meptyl, diafenthiuron, and fomesafen over 5-500 μM. The array was practically applied for the analysis of soil samples. In 2020, (Hormozi Jangi et al., 2020) developed a novel naked-eye method for field detection of notorious explosive triacetone triperoxide via the oxidation of 3, 3′-diaminobenzidine in the presence of hydrogen peroxide produced from the acidic decomposition of triacetone triperoxide catalyzed by MnO_2 nanozymes. A linear range of 1.57-10.50 mg L−1, a LOD of 0.34 mg L−1, and a fast spot test analyzing time of 5 s were provided. Since the DAB oxidation was selectively proceeded by hydrogen peroxide not, by molecular oxygen, hence, this method can be eliminated the common false-positive results from laundry detergents (Figure 4).

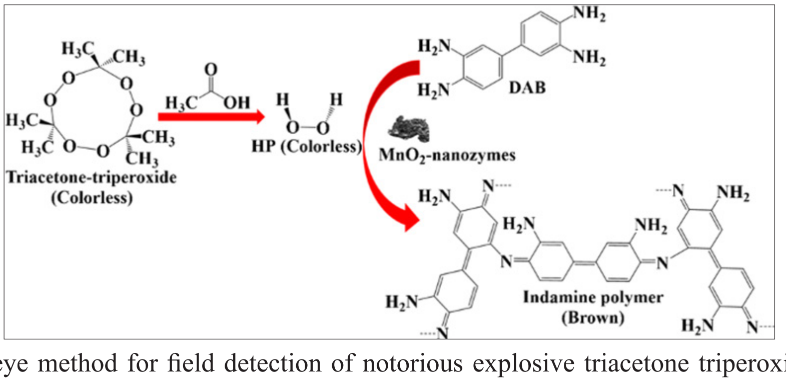

Figure 4: A novel naked-eye method for field detection of notorious explosive triacetone triperoxide via the oxidation of 3, 3′-diaminobenzidine in the presence of hydrogen peroxide produced from the acidic decomposition of triacetone triperoxide catalyzed by MnO_2 nanozymes (adapted from Hormozi Jangi et al., 2020).

In 2020, (Lin et al., 2020) synthesized the gold alloy-based nanozymes which showed better catalytic performances than the common gold nanoparticles. The developed nanozymes were used for the discrimination of cysteine, glutathione, mercaptoacetic acid, dithiothreitol, mercaptosuccinic acid, and mercaptoethanol in the human serum samples. In 2021, (Soltani et al., 2021) synthesized and characterized a carboxylic acid-functionalized layered double hydroxide/MOF nanocomposite via growing the UiO-66-(Zr)-$(COOH)_2$ MOF on the surface of COOH-functionalized $Ni_{50}Co_{50}$-layered double hydroxide nanosheets at 100°C. The product was used for Cd(II) and Pb(II) removal from the water via surface adsorption mechanism, showing an adsorption capacity of 415.3 and 301.4 mg g^{-1} for Cd(II) and Pb(II), in turn. The method showed a Langmuir adsorption isotherm and a pseudo-first-order kinetic model. In 2021, (Butova et al., 2021) reported a scalable route for the synthesis of MOF-801. They evaluated the effect of the concentration of mono-carboxylic acids on the water and hydrogen uptake, porosity, crystallinity, size, and shape of particles. They revealed that heating and small grains in powders are suitable for the fast release of water. The properties of the MOF-801 can enhance by both formic and acetic acid. The resulted MOF-801 showed 1.1 wt% hydrogen uptake at 750 mmHg and 20% water uptake at ambient temperature. In 2021, (He et al., 2021) prepared stable MOF based on porphyrinic for the encapsulation of metal nanoparticles via stirring at ambient temperature. In this regard, Pt NPs encapsulated into the MOF can be produced by stirring the Pt NPs solution in the presence of the MOFs. In 2021, (Kang et al., 2021) reported a nanozyme based sensor for the determination of dopamine using hemin-doped-HKUST-1. The hemin-doped-HKUST-1 was prepared using a one-pot hydrothermal method and combined with reduced graphene oxide modified on a glassy carbon electrode. The composite exhibited high electrocatalytic activity toward electro-oxidation of dopamine. Using this sensor, a linear range of 0.03–10 μM and a LOD of 3.27×10^{-8} M (S/N = 3) was obtained for the quantification of dopamine. In 2021, (Hermosilla et al., 2021) proposed a new colorimetric method for assaying the oxidase-mimicking properties of $MnFe_2O_4$ NPs (size, 3.19 nm). The protocol was based on the oxidation of 3-methyl-2-benzothiazolinone-hydrazone to 3-(dimethylamino) benzoic acid. The pH and temperature effect on the assay response was evaluated, revealing an optimum pH of 3.9 at 30 °C. The Michaelis Menten model supported the kinetic behavior of the nanozyme catalyzed reaction, obtaining a Km of 13.59 μM and a k_{cat} of 5.25×10^7 s^{-1} along with a k_{cat}/Km ratio of 3.86×10^{12} M^{-1} s^{-1}. In 2022, (Wu et al., 2022) developed a MnO_2 nanozyme-mediated CRISPR-Cas12a system for naked-eye diagnosis of COVID-19. In this system, the MnO_2 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 5).

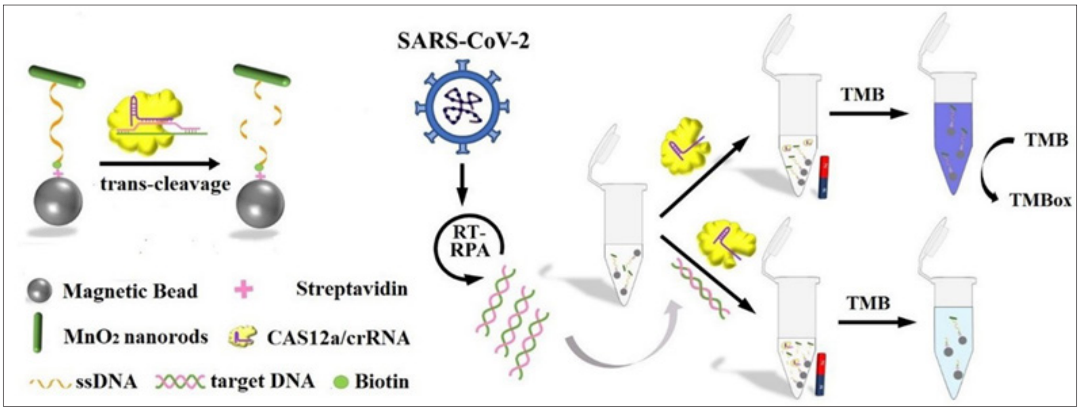

Figure 5: A MnO_2 nanozyme-mediated CRISPR-Cas12a system for naked-eye diagnosis of COVID-19 (adopted from (Wu et al., 2022).

In 2023, (He et al., 2023) performed a nanozyme-based colorimetric method for naked-eye diagnosis of COVID-19 by iron manganese silicate nanozymes as peroxidase-like nanozymes. 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., 2023) developed a robust colorimetric immunosensing method using liposome-encapsulated MnO_2 nanozymes for diagnosis of COVID-19 via detection of SARS-CoV-2 antigen using TMB as the chromogenic substrate. Moreover, (Vafabakhsh et al., 2023) 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.

Perspectives

The nanozyme chemistry is new filed and on its initial steps (Hormozi Jangi, 2023; Hormozi Jangi, 2023; Jangi, 2023; Hormozi Jangi et al., 2023; Hormozi Jangi et al., 2023; Hormozi Jangi et al., 2023). As a perspective to future of this filed, it can be pointed to the following items;

- Developing nanozymes with higher catalytic efficiency and higher substrate affinity in their native form.
- Developing nanozymes with intrinsic activity of commercial enzymes such as lipase and urease for application in industrial process in real world.
- Extending the multinanozyme systems for improving sensitivity and selectivity of nanozymatic sensors
- Design of biocompatible nanozymes with drug-like properties for treatment of diseases with minimal side effects.
- Developing simple surface modification of nanozymes for enhancing their specificity.
- Evaluating biochemical behavior of nanozymes for better understanding their best performances
- Developing reusable nanozymes with high cycling stability and simple recovery suitable for real practical applications
- Design of specific nanozyme-based sensors compared of current selective sensors
- etc.

Conclusions

The fast development of nanoscience and material chemistry has increased interest in researching new and innovative synthesis methods to produce new nanomaterials. Among different nanomaterials, a wide variety of these materials reveal high intrinsic enzyme-like activity. Due to their high catalytic efficiency and stability, 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. This article aimed to present a brief introduction on the nanozyme-based chemistry with emphasizing on the historical overview of recent nanozymatic sensors.

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Conflict of Interest

None.

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