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Posted Date: 1 September 2023

doi: 10.20944/preprints202309.0022.v1

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Review

Multinanozyme Systems: The New Generation of Nanozymatic Systems

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Abstract: Considering higher stability and lower cost of nanozymes than native enzymes, the nanozymatic systems had been utilized for several practical application, especially for sensing and detection. Most of common nanozymatic 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). Since the first report of multinanozyme systems, several multinaozyme systems have been developed and utilized for highly sensitive and selective sensing aims. The main advantages of multinaozyme systems compared of common nanozymatic sensors are their impact on simultaneous enhancing selectivity and sensitivity of sensor in a well-designed detection process. Since, the principles of design and detection mechanism of this new generation is not well-described in the literature, the aim of this article is the fast review of the principles of design of this new generation of nanozyme-based sensing and detection.

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1. Introduction

An interesting research field is the synthesis of novel nanoparticles with high enzyme-like activity formally called nanozymes [1,2]. The natural (native) enzymes suffer from several drawbacks and disadvantages for example, low stability (i.e., a narrow thermal range or a narrow working pH range) [3,4], for overcoming and resolving the above-mentioned drawbacks of native enzymes, the development of the enzyme immobilization processes is attracted good attention [5–7]. The recent progress in nanochemistry and material science opens a new door for developing high-performance materials such as MOFs [8], catalytic materials [9–11], nanoparticles with unique optical properties [12–16], and nanoparticles with enzyme-like activity [17–20]. For the first time, Gao and his coworkers reports the enzyme-like activity of nanoparticles in 2007 [21]. They investigated the peroxidase mimicking characteristics of the iron oxide nanoparticles as the peroxidase mimic nanoscale materials. After this first report by Gao (i.e., pioneer of the nanozyme field), different types of nanoscale materials (nanoparticles) for instance, metal oxides nanoparticles, noble metal-based nanoparticles, and carbon-based nanomaterials were designed and introduced as enzyme mimetics which formally known as "nanozymes". The majority of the enzyme-like nanoscale materials reveal the peroxidase-like activity. It is mean that most of the introduced nanozymes are peroxidase mimetic materials with higher stability than the native peroxidase. Thanks to the significant and characteristic peroxidase-like activity of these nanozymes, the nanoscale peroxidase mimic materials can be utilized for design and development of the innovative catalyst-based analytical sensors which currently known as nanozyme-based sensors [22,23]. Regarding the design and explore of the nanozymatic sensors, up to date, different types of the nanozyme-based sensors have been designed and constructed for the chemi-quantification and bio-quantification of a variety of compounds such as glutathione (GSH) [24–26], folic acid [27], xanthine [28], metal cations [29], glucose [30], H_2O_2 [31,32], and explosives [33], as well as cysteine [34] using the nanozyme-catalyzed/mediated oxidation of the common chromogenic substrates of peroxides enzyme such as 3, 3′, 5, 5′-tetramethylbenzidine (TMB) and o-phenylenediamine (OPD) to their colored cation radicals [22]. Besides the OPD and TMB-based sensing methods, in 2020, Hormozi Jangi et al. explored a new type of the colorimetric nanozyme-based sensors by employing the n-electron irreversible oxidation reaction of the high

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stable 3,3′-diaminobenzidine (DAB) to it's corresponding stable brown-colored indamine polymer and used this resulted indamine polymer as the analytical probe instead of the common cation radicals resulting from TMB and OPD [29]. Besides the sensing applications, recently nanozymes had also been used biocatalysis of reactions instead of natural enzymes, water treatment, and dye degradation [35–37]. Moreover, since the first report of COVID-19 on 2019 [38,39], the nanozyme-based sensors have been employed for diagnosis of COVID-19 [40,41]. Regarding the nanozymes application in sensing and detection, most of common nanozymatic 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) [42]. Since the first report of multinanozyme systems, several multinaozyme systems have been developed and utilized for highly sensitive and selective sensing aims. The main advantages of multinaozyme systems compared of common single-nanozymatic sensors are their impact on simultaneous enhancing selectivity and sensitivity of detection systems along with improving the kinetics performances of system via applying two nanozymes with identical enzyme mimic activity (e.g., two peroxidase mimics) in a well-designed detection process [42]. Since, the principles of design and detection mechanism of this new generation is not well-described in the literature, the main aim of this review article is the fast and quick review of the principles of design of this new generation of nanozyme-based sensing and detection. Besides, the mechanism of the multinaozyme detection system was also described and reviewed.

2. Analytical Probe and Analytical Response of Nanozyme-Based Sensors

Since nanoscale enzyme-like materials or formally, nanozymes can catalyze the oxidation reaction of the common peroxidase substrates to form some colored products with the significant visible region absorbances, they have been used for the analytical purposes [43–45]. Usually the common enzyme substrates including 3,3',5,5'-tetramethylbenzidine (TMB), 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), o-phenylenediamine (OPD), and 3,3'-diaminobenzidine (DAB) are employed as the analytical system substrates, and their corresponding colored oxidation products were utilized as the analytical probes for sensing aims [22]. The sensing is based on probing the absorbance of colored analytical probes as a function of analyte concentration for the hydrogen peroxide (HP) detection and for the HP-based nanozymatic sensors. In fact, the HP-based nanozymatic sensors are a class of nanozyme-based sensors for indirect detection of some analytes (e.g., triacetone-triperoxide and glucose) via their conversion to hydrogen peroxide or via monitoring the hydrogen peroxide released from the reaction of the analyte with a certain reactant, as reported [32,33,46]. Hence, when, the hydrogen peroxide detection is demanded, the native absorbance of the colored product of oxidation process is used as the analytical response for direct detection of HP or indirect detection of some analytes via detecting HP resulting from a certain reaction (e.g., analyte decomposing by UV light, hydrolysis of analyte by acids, etc.) [22].

In addition, the variation of the absorbance in the presence and the absence of a certain analyte can be used as an analytical index for the quantification of different analytes using the nanozymatic sensors [29,42]. In these cases, the analytical response can be $\Delta A=A_0-A$ [47,48], the relative ratio of $(\mathbf{A_0}-\mathbf{A})/A_0$ (i.e., relative absorbance) [49] or the ratio of A_0/A [50] which A_0 is the absorbance of blank (i.e., Abs. in the absence of analyte) and A is the absorbance of sample (i.e., Abs. in the presence of analyte).

3. Current Classes of Nanozymatic Sensors Based on Sensor Design

Based on the sensor design and detection mechanism, the colorimetric nanozyme-based sensors are classified into three common subclasses including single peroxidase mimic, enzyme-mimic peroxidase hybrids, and enzyme co-embedded nanomaterials [22] as represented in Figure 1. Besides, enzyme-mimic peroxidase hybrids are divided to two subclasses including HP-based sensors and pH-based sensors. In the first subclass, the released hydrogen peroxide from an enzymatic reaction of analyte (glucose, Xanthine) is used an index for indirect detection of analyte. While in the later subclass, the variation pH of reaction media and consequently its effect on the

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oxidation process of enzyme substrates (e.g., TMB) is considered the analytical index for indirect detection of some analytes, for instance, the released NH_3 from urease-mediated hydrolysis of urea can affect the color intensity of the oxidation product of TMB which can be used as an index for indirect determination of urea via mentoring the released NH_3 [51]. As can be seen in Figure 3, the HP-based sensors are divided into oxidase-mimic peroxidase systems, acidic cleavage-mimic peroxidase systems, and UV irradiation-mimic peroxidase system which the first part of the names of these systems (i.e., oxidase, acidic cleavage, and UV irradiation) is pointed to the reaction that leads to releasing hydrogen peroxide in the sensing media via acting a reactant (e.g., enzyme, acid, or light) on analyte [22]. Notably, in all of these types of sensors, one nanozyme is involved and basically can be considered as single nanozyme-based sensors.

Besides the common classes of nanozyme-based sensors, in 2020, by Hormozi Jangi et al. a novel generation of the nanozyme-based colorimetric sensors was explored and developed which currently called multinanozyme sensors [42]. The main aim of this design was enhancement of both sensitivity and selectivity of the nanozymatic sensor via the utilizing synergetic effect of multinanozyme system on the catalytic activity and specificity for the biosensing of some analytes. Since, the principles of design and detection mechanism of this new generation is not well-described in the literature, the aim of this article is the fast review of the principles of design of this new generation of nanozyme-based sensing and detection. Besides, the mechanism of the multinaozyme detection system was also described and reviewed.

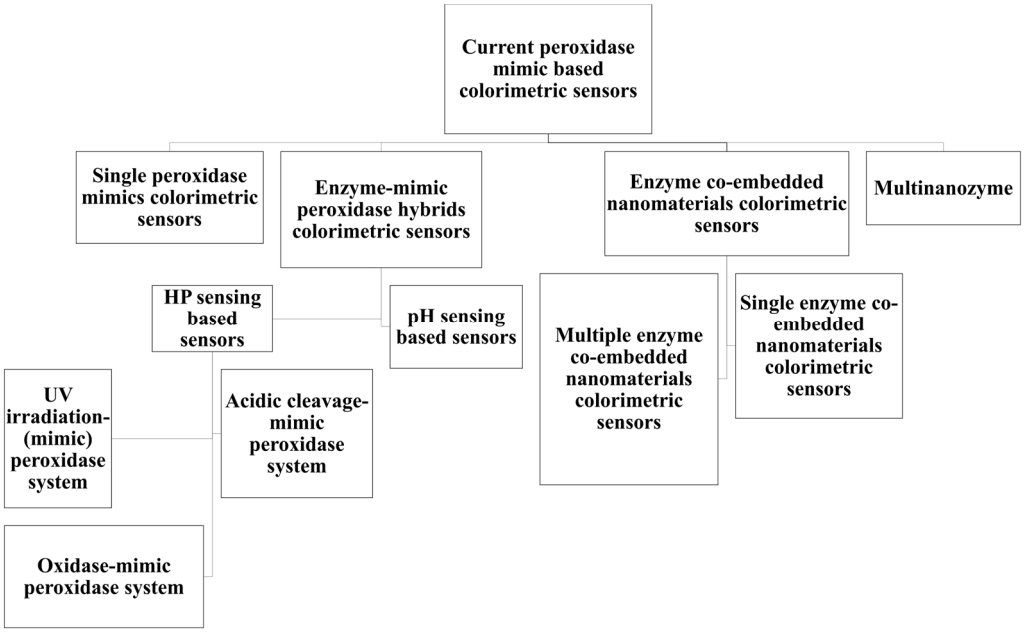

Figure 1. Current classes of nanozymatic sensors based on sensor design.

4. Multinanozyme Systems

Generally, multinazymes systems are defined as nanozymatic systems which developed by simultaneous use of two identical pseudo-enzymes (e.g., both mimic-peroxidase) in a nanozymatic system [42,52,53]. There are few reports about the positive synergistic function of dissociative multinanozyme systems. The multinanozyme sensors were introduced by Hormozi Jangi et al. in 2020 [42], for the first time as a new generation of nanozyme-based sensors which had been utilized for the nanozyme-medaited colorimetric detection for glutathione in real blood media. The mechanism of this novel innovative system was as follows; initially, MnO_2-nanozymes catalyze the irreversible oxidation DAB for formation of the corresponding brown-colored indamine polymer of DAB which this process can be inhibited by introducing GSH. However, by employing gold-nanozymes along with the MnO_2-nanozymes for catalyzing the oxidation process of DAB, the analytical signal was enhanced due to higher catalytic efficient of the multinanozyme system tan the

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single-nanozyme system. Besides, the selectivity of the method was improved toward GSH, particularly, over Cys and AA. In fact on this system, in the blank, Au-nanozymes catalyzed the oxidation of DAB by HP to a brown-colored indamine polymer along with Au-nanozymes, MnO_2-nanozymes also directly interacted with residual DAB to producing additional indamine polymer via the future oxidative cycles. While a different scenario is underway in the sample solution. First, the pre-incubated Au-nanozymes were interacted with GSH to producing GSGS-Au hybrid [42], then, MnO_2-nanozymes was introduced to the mixture and reduced to Mn^{2+} by the excess GSH [42]. These inhibited Au-nanozymes and oxidized MnO_2-nanozymes cannot proceed the DAB oxidation by HP resulted in a considerable decrease in the indamine polymer (probe) formation. It should be mentioned that the single Au-nanozymes are poor in sensitivity while the simultaneous use of two peroxidase-mimics in the developed multinanozymes system causes a considerable improvement in the sensitivity of GSH detection compared of the corresponding single nanozyme sensor. Besides, the single MnO_2-nanozymes system is poor in selectivity while the multinanozyme system with both MnO_2- and Au-nanozymes revealed an excellent selectivity for GSH detection.

After the first report, another multinaozyme system was also designed and developed by research group of Hormozi Jangi [52] by utilizing $SiO_2@Fe_3O_4$ and MnO_2 nanozymes which were simultaneously applied for the irreversible DAB oxidation toward formation of the colored indamine polymer which then utilized as the visible-probable analytical probe system. They utilized the developed system for the detection and quantification of hydrogen peroxide content of the food samples (milk) as well as for the organic dye degradation in water media. The experimental results of their work revealed that the sensitivity of the developed $SiO_2@Fe_3O_4/MnO_2$ multinanozyme system was higher than those of the corresponding single nanozyme systems of both $SiO_2@Fe_3O_4$ and MnO_2 nanoparticles. Besides, the yield of the multinanozyme system was found to be higher than those of the corresponding single nanozymatic systems. Hence, the based on the results of their work, it can be deduced that multinanozyme systems can be applied for the enhancement of the analytical sensitivity of the detection system as well as for improving the yield of a catalytic reaction (e.g., dye degradation) [52].

The last report on multinaozyme systems was published by Tang et al. (2022) [53]. They developed a novel Au NPs/GeO_2 multinanozyme system for the detection and bioquantification of the choline iodide as a model analyte (Figure 2). In fact, although both gold nanoparticles and GeO_2 nanozymes exhibited unique peroxidase-like activity for proceeding the TMB oxidation, however, the Au NPs/GeO_2 multinanozyme system shows stronger peroxidase-like activity and consequently higher catalytic efficiency that the corresponding single gold nanoparticles and GeO_2 nanozymes. The peroxidase-like activity of the Au NPs/GeO_2 multinanozyme system was inhibited by introducing choline in the reaction media which can be used for the detection of choline in real sample. In the presence of hydrogen peroxide, the designed multinanozyme system can significantly catalyze the oxidation of TMB to its corresponding blue-colored oxidation product (i.e., TMB-ox). However, by introducing the ChI into the multinanozyme mixture, the GeO_2 was converted to Ge ions by ChI and the Au NPs were converted to melt-Au NPs. The products cannot show the peroxidase-like activity, hence, by introducing TMB and hydrogen peroxide into this solution, the oxidation process of TMB cannot successfully catalyzed and consequently the color intensity was inhibited [53].

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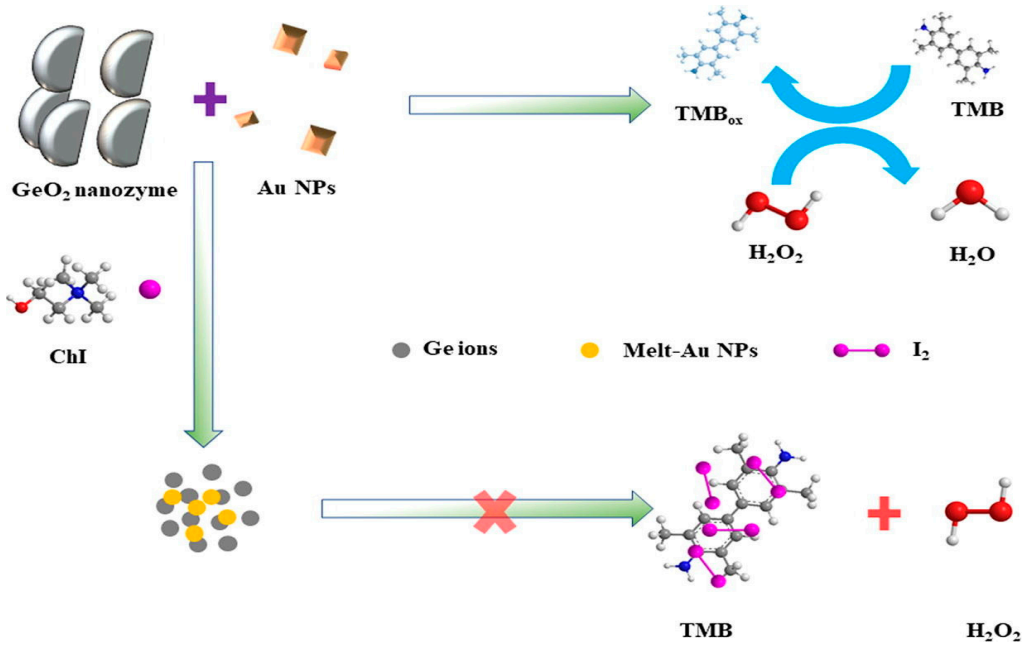

Figure 2. A novel Au NPs/GeO_2 multinanozyme system with the promising prospect for detection of ChI (adopted from Tang et al. (2022) [53]).

4. Multinanozyme System *vs.* Single Nanozymatic Systems

The multinanozyme systems reveal several advantages toward compared of common nanozyme-based sensors [42,52,53]: (i) The multinanozyme systems reveal more selective responses than the corresponding single-nanozymatic systems, (II) The comparative data exhibited that the K_m value (substrate affinity constant) of the multinanozyme systems is extremely lower than those of the native enzymes and the single-nanozymatic sensors, as reported. Hence, by applying multinanozyme systems the substrate affinity for binding to the active nodes (sites) of nanozymes will increased compared of the common single-nanozymatic sensors, (iii) The multinanozymes systems exhibited higher sensitivity than the corresponding single nanozymatic-based sensors, (iv) The positive synergetic effect of multinanozymes systems on the overall catalytic efficiency (V_{max}) of nanozymatic process was also proved may be due to more effective capture of substrate active agents on the surface of the nanozymes compared of single nanozymatic systems, (v) The reaction time of the multinanozyme systems is significantly shorter than the time of corresponding single nanozymatic systems, (vi) Using the advantages of two nanozymes and reducing the drawbacks of these nanozymes via combination of two nanozymes with different catalytic efficiency in a one simple multinanozyme system, (vii) providing more active surface area and consequently more available active nodes/ sites for the enzymatic reaction by developing the efficient multinanozyme systems comapared their corresponding single nanozyme systems, and (viii) improving the adsorption capacity of the system toward substrate adsorption on the active surface of the nanozymatic system and consequently producing more products by developing the multinanozyme systems.

5. Conclusions

Considering higher stability and lower cost of nanozymes than native enzymes, the nanozymatic systems had been utilized for several practical application, especially for sensing and detection. Most of common nanozymatic 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). Since the first report of multinanozyme systems, several multinaozyme systems have been developed and utilized for highly sensitive and selective sensing aims. The main advantages of multinaozyme systems compared of common nanozymatic sensors are their impact on simultaneous enhancing selectivity and sensitivity of sensor in a well-designed detection process.

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Acknowledgments: The authors gratefully thank the Hormozi Laboratory of Chemistry and Biochemistry for the support of this work.

Conflict of Interest: None.

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