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Detection Mechanism and Principles of the Multinanozyme Systems as the New Generation of Nanozyme-Mediated Sensing Assays: A Critical Review

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Abstract

Nanozymes are defined as nanoscale materials with enzyme-like properties. Due to their higher stability and lower cost than native enzymes, nanozymatic systems have been utilized for several practical applications, especially for sensing and detection. Most of the 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 multinanozyme systems have been developed and utilized for highly sensitive and selective sensing aims. The main advantages of multinanozyme 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. 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 multinanozyme detection system was also described and reviewed.

Keywords: Nanozymes, Multinanozyme System, Nanozyme-Based Sensing and Detection.

Introduction

An interesting research field is the synthesis of novel nanoparticles with high enzyme-like activity formally called nanozymes [1, 2]. 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) for overcoming and resolving the above-mentioned drawbacks of native enzymes, the development of the enzyme immobilization processes is attracted good attention [3-7]. The recent progress in nanochemistry and material science opens a new door for developing high-performance materials such as MOFs, catalytic materials nanoparticles with unique optical properties and nanoparticles with enzyme-like activity [08-20]. For the first time, Gao and his coworkers reported the enzyme-like activity of nanoparticles in 2007 [21]. They investigated the peroxidase mimicking characteristics of the iron oxide nanoparticles as the peroxidase mimics 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 are formally known as "nanozymes". The majority of the enzyme-like nanoscale materials reveal the peroxidase-like activity. It means 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 the design and development of innovative catalyst-based analytical sensors which are currently known as nanozyme-based sensors [22,23]. Regarding the design and explore of the nanozymatic sensors, up to date, different types of nanozyme-based sensors have been designed and constructed for the chemi-quantification and bio-quantification of a variety of compounds such as glutathione (GSH) folic acid, xanthine, metal cations, glucose, H2O2 and explosives as well as cysteine 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 colorimetric nanozyme-based sensors by employing the n-electron irreversible oxidation reaction of the high stable 3,3′-diaminobenzidine (DAB) to its corresponding

stable brown-colored indamine polymer and used this resulted in indamine polymer as the analytical probe instead of the common cation radicals resulting from TMB and OPD [24-34]. Besides the sensing applications, recently nanozymes have also been used in the biocatalysis of reactions instead of natural enzymes, water treatment, and dye degradation [35-37]. Moreover, since the first report of COVID-19 in 2019 nanozyme-based sensors have been employed for the diagnosis of COVID-19 [38- 41]. Regarding the nanozymes application in sensing and detection, the most 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 multinanozyme systems have been developed and utilized for highly sensitive and selective sensing aims. The main advantages of multinanozyme systems compared to common single-nanozymatic sensors are their impact on simultaneously enhancing the selectivity and sensitivity of detection systems along with improving the kinetics performances of the 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 are not well-described in the literature, the aim of this article is a fast review of the principles of design of this new generation of nanozyme-based sensing and detection. Besides, the mechanism of the multinanozyme 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 significant visible region absorbances, they have been used for 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 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, hydrogen peroxide detection is demanded, the native absorbance of the colored product of the 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 nanozymatic sensors [29,42]. In these cases, the analytical response can be $\Delta A=A0-A$, the relative ratio of $(A0 - A)/A0$ (i.e., relative absorbance) or the ratio of A0/A which A0 is the absorbance of blank (i.e., Abs. in the absence of analyte) and A is the absorbance of the sample (i.e., Abs. in the presence of analyte) [47-50].

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 as represented in Figure 1. Besides, enzyme-mimic peroxidase hybrids are divided into 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 as an index for indirect detection of the analyte. In the later subclass, the variation pH of reaction media and consequently its effect on the oxidation process of enzyme substrates (e.g., TMB) is considered the analytical index for indirect detection of some analytes, for instance, the released NH3 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 NH3 [51]. As can be seen in Figure 1, the HP-based sensors are divided into oxidase-mimic peroxidase systems, acidic cleavage-mimic peroxidase systems, and UV irradiation-mimic peroxidase systems 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 systems/sensors [52,53].

Besides the common classes of nanozyme-based sensors, in 2020 a novel generation of nanozyme-based sensors called multinanozyme sensor was explored by Hormozi Jangi et al. toward enhancing both sensitivity and selectivity of the nanozymatic sensor via the synergetic effect of multinanozyme system on the catalytic activity and specificity. Since the principles of design and detection mechanism of this new generation are not well-described in the literature, the aim of this article is a fast review of the principles of design of this new generation of nanozyme-based sensing and detection [42]. Besides, the mechanism of the multinanozyme detection system was also described and reviewed.

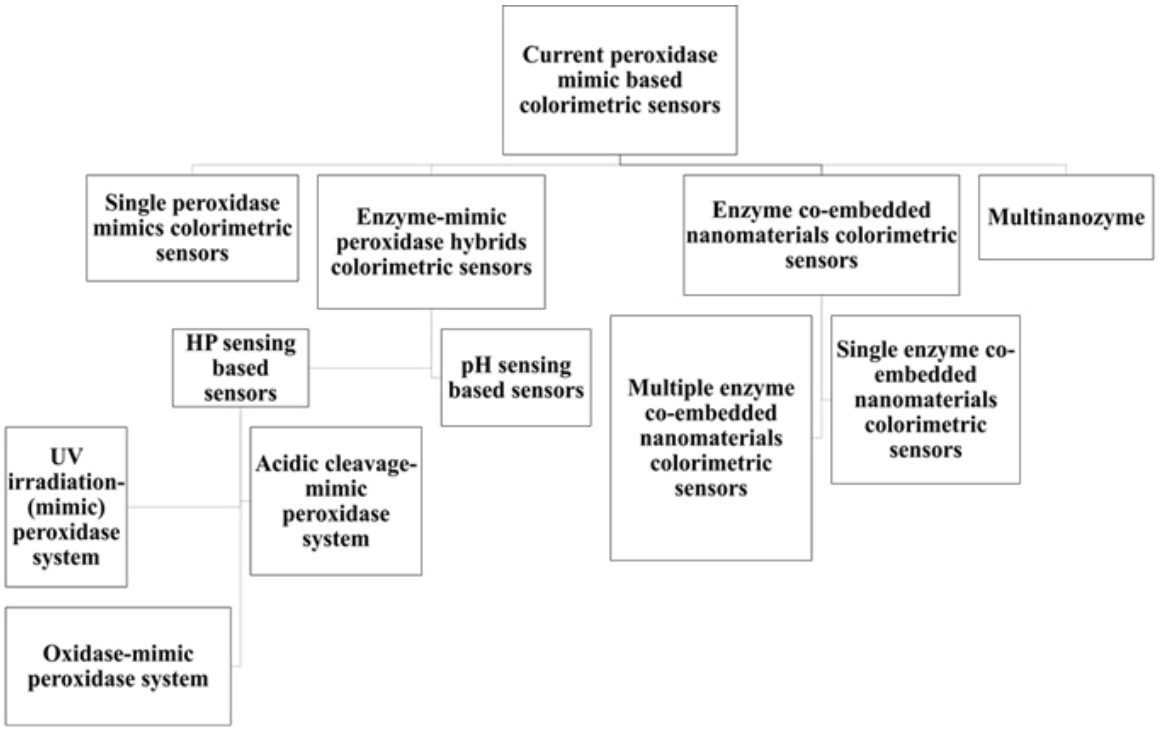

Figure 1: Current classes of nanozymatic sensors based on sensor design.

4. Multinanozyme Systems

Generally, multinazymes systems are defined as nanozymatic systems developed by the simultaneous use of two identical pseudo-enzymes (e.g., both mimic-peroxidase) in a nanozymatic system [42,54,55]. 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. They developed a novel multinanozyme colorimetric method for glutathione (GSH) detection. They pointed out that MnO2-nanozymes can catalyze the oxidation reaction of 3, 3′-diaminobenzidine (DAB) and produce a brown indamine polymer. In the presence of GSH, this reaction slowly proceeds. When Au-nanozymes were used as peroxidase mimics along with MnO2-nanozymes, the analytical signal and selectivity (particularly, over Cys and AA) were significantly improved for GSH detection (Figure 2). In fact in this system, as shown in Figure 2A, in the blank, Au-nanozymes catalyzed the oxidation of DAB by HP to a brown-colored indamine polymer along with Au-nanozymes, MnO2-nanozymes also directly interacted with residual DAB to producing additional indamine polymer via the future oxidative cycles. A different scenario is underway in the sample solution. First, the pre-incubated Au-nanozymes interacted with GSH to produce a GSGS-Au hybrid 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 with the DAB oxidation by HP resulting in a considerable decrease in the indamine polymer (probe) formation. It should be mentioned that the single Au-nanozymes are poor in sensitivity (Figure 2B) 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 (Figure 2C).

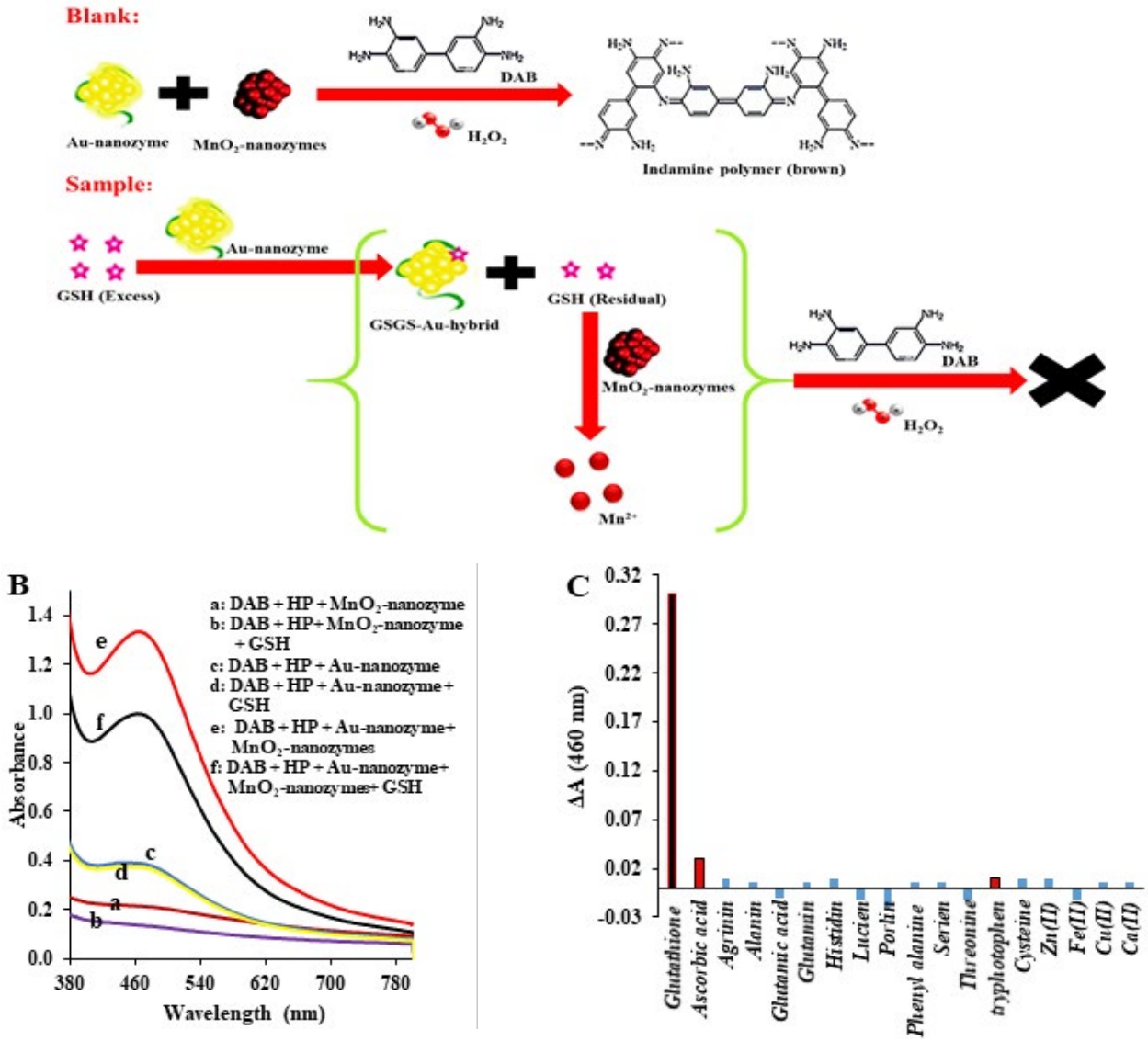

Figure 2: (A) Possible mechanism of Au/MnO_2 multinanozyme system for GSH detection (adapted from Hormozi Jangi et al. (2020) [42]), (B) sensitivity of multinanozyme system compared of single nanozymatic systems for GSH detection, and (C) selectivity of the Au/MnO_2 multinanozyme system for GSH detection, inset: poor selectivity of single MnO_2 nanozymatic system for GSH detection.

After the first report, another multinanozyme system was also developed by Hormozi Jangi et al. [54] utilizing SiO_2@Fe_3O_4 and MnO_2 nanozymes which were simultaneously used for DAB oxidation. They utilized the developed system for hydrogen peroxide detection in milk and organic dye degradation in water media (Figure 3A). They also checked the significance of the multinanozyme system for hydrogen peroxide detection compared to the single nanozyme system (Figure 3B), the results showed that when SiO_2@Fe_3O_4 and MnO_2 nanozymes were simultaneously used for DAB oxidation, the analytical signal was significantly higher than the single nanozyme system (only SiO_2@Fe_3O_4 or MnO_2 nanozymes). Hence, the multinanozyme system can be used for the enhancement of the sensitivity of the detection system compared to the single nanozyme systems [54].

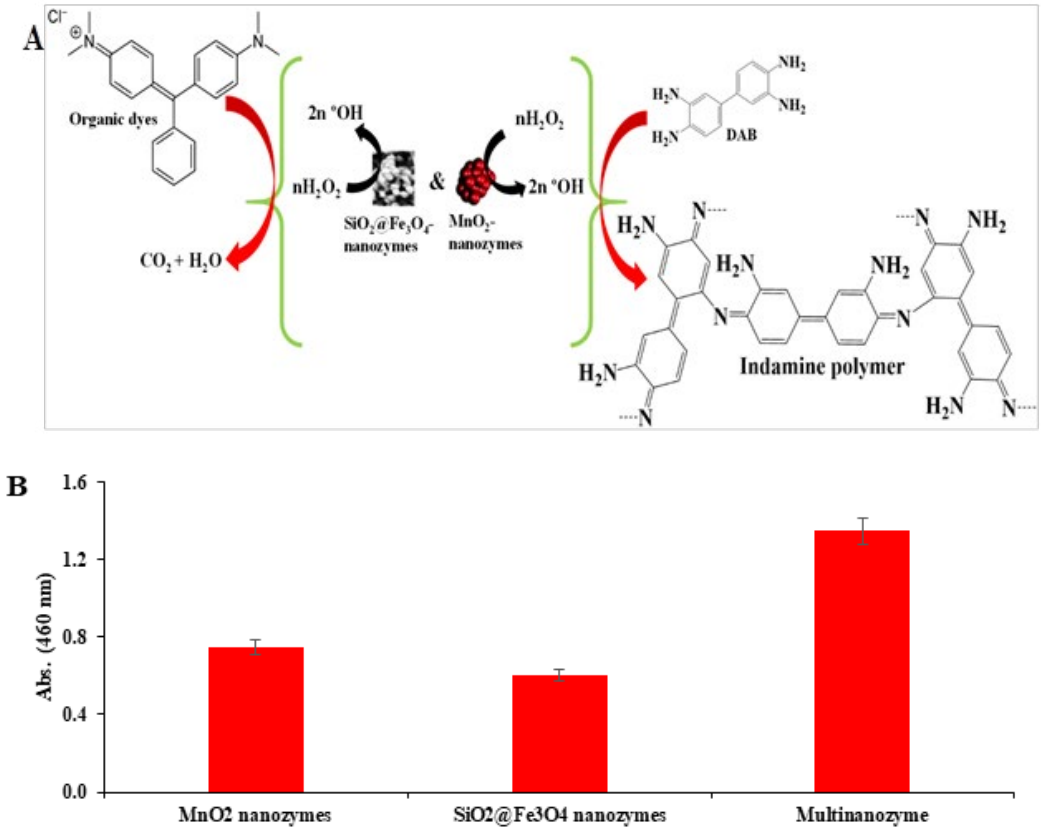

Figure 3: (A) Possible mechanism of SiO_2@Fe_3O_4/MnO_2 multinanozyme system toward hydrogen peroxide detection and organic dye biodegradation and (B) significance of multinanozyme system compared of its corresponding single nanozymatic systems (adopted from Hormozi Jangi et al. (2020) [54]).

The last report on multinanozyme systems was published by Tang et al. (2022) [55]. They developed a novel Au NPs/GeO_2 multinanozyme system with the promising prospect for the detection of choline iodide (ChI). In fact, they discovered that both Au NPs and GeO_2 nanozymes have peroxidase-like activity, catalyzing the oxidation of TMB. Interestingly, compared with single Au NPs or GeO_2 nanozymes, the Au NPs/GeO2 multinanozyme system shows stronger peroxidase-like activity (Figure 4).

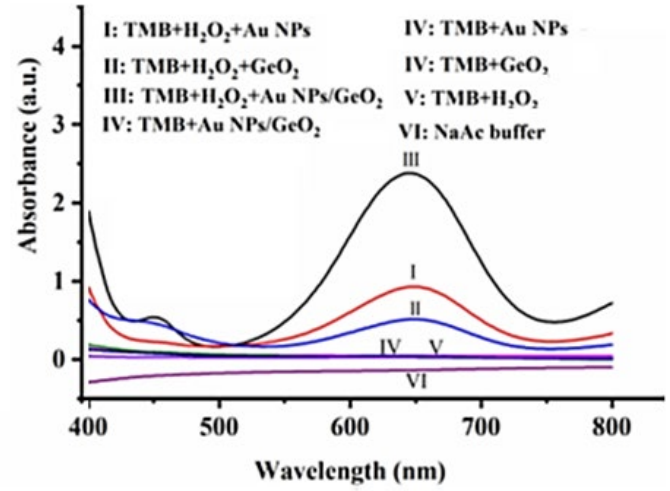

Figure 4. Nanozymatic performances of Au NPs/GeO_2 multinanozyme system compared to its corresponding single nanozyme systems (adopted from Tang et al. (2022) [55]).

The mentioned two enhancements are ascribed to a positive synergistic function of Au NPs/GeO_2 nanozymes. Surprisingly, choline iodide (ChI) can inhibit the positive synergy in Au NPs/GeO_2 nanozymes, and slow down the reaction of TMB-H_2O_2-Au NPs/GeO_2 system [55]. On this foundation, a new Au NPs/GeO_2 SERS technique with high sensitivity, a label-free detection method of choline iodide (ChI) was established (Figure 5). As can be seen from Figure 12, 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 peroxidase-like activity, hence, by introducing TMB and hydrogen peroxide into this solution, the oxidation process of TMB cannot be successfully catalyzed and consequently, the color intensity was inhibited [53].

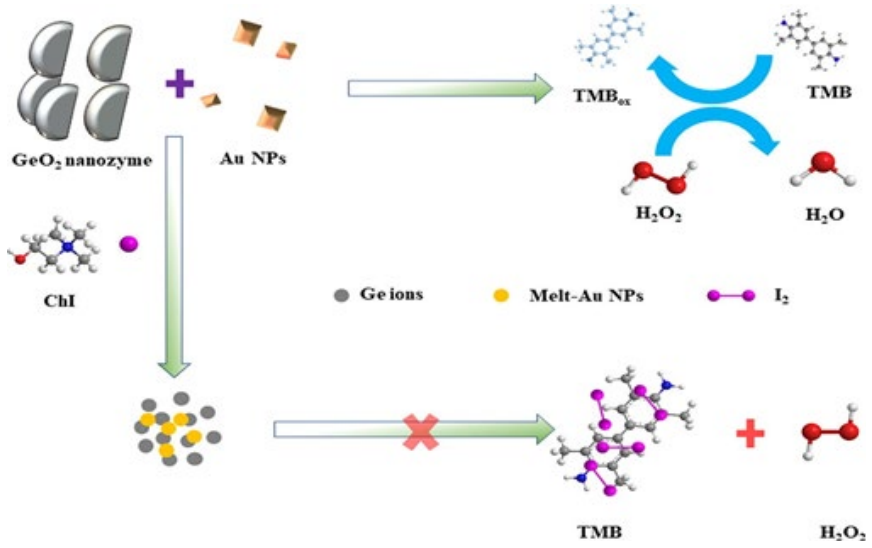

Figure 5. A novel Au NPs/GeO_2 multinanozyme system with the promising prospect for detection of ChI (adopted from Tang et al. (2022) [55]).

4. Multinanozyme System vs Single Nanozymatic Systems

The multinanozyme systems reveal several advantages compared to common nanozyme-based sensors: (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 [42,54,55]. 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

Nanozymes are defined as nanoscale materials with enzyme-like properties. Due to their higher stability and lower cost than native enzymes, nanozymatic systems have been utilized for several practical applications, especially for sensing and detection. Most of the 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 multinanozyme systems have been developed and utilized for highly sensitive and selective sensing aims. The main advantages of multinanozyme systems compared to common single-nanozymatic sensors are their impact on simultaneously enhancing the selectivity and sensitivity of detection systems along with improving the kinetics performances of the system via applying two nanozymes with identical enzyme mimic activity (e.g., two peroxidase mimics) in a well-designed

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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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