Recent Progress on the Applications of Nanozyme in Surface-Enhanced Raman Scattering

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Abstract: Nanozymes are nanomaterial with natural enzyme-like activity and can catalyze specific reactions for analyte identification and detection. Compared to natural enzymes, they have several benefits, including being steady, low-cost, easy to prepare and store. Based on the promising development of nanozymes in surface-enhanced Raman scattering (SERS), this paper reviews the classification of different types of nanozymes in SERS, including metal-based nanozyme, carbon-based nanozyme, metal-organic framework (MOF)/covalent organic framework (COF)-based nanozyme, and semiconductor-based nanozyme, followed by a detailed overview of their SERS applications in disease diagnosis, food safety, and environmental safety. Finally, this paper discusses the practical shortcomings of nanozymes in SERS applications and makes some suggestions for further research.

Keywords: nanozyme; surface-enhanced Raman scattering (SERS); substrate; application

1. Introduction

Natural enzymes, being macromolecules with efficient selective catalytic activity, play a vital role in many biological processes [1,2]. However, their widespread application is severely constrained by low stability, harsh preparation, and high costs [3–5]. With the rapid progress and development of nanotechnology, researchers have become interested in a nanomaterial known as nanozyme, which possesses catalytic activity comparable to that of the natural enzyme. Since the discovery of Fe₃O₄ nanoparticles with peroxidase-like activity in 2007 [6], an increasing number of nanomaterials have been demonstrated to exhibit nanozyme activity, including metal oxide nanoparticles [7], carbon nanomaterials [8], and noble metal nanoparticles [9]. Different from natural enzymes, nanozymes offer outstanding benefits including being low cost, and having high stability and ease of storage [10,11], and have promising application potential [12–15].

Surface-enhanced Raman scattering (SERS) is a powerful molecular spectroscopy technique [16,17], which can be utilized for structural characterization and monitoring of analytes with the benefits of high selectivity, high sensitivity, quick response, and nondestructive detection [18]. It is currently extensively employed in various domains, including food safety [19], biomedicine [20], and environmental safety [21], which is regarded as one of the most promising analytical techniques. Given its sensitivity down to the single-molecule level, it is an ideal technique for researching the catalytic behavior of nanozymes. On the other hand, since nanozymes generally lack selectivity, the unique fingerprint peaks of SERS can also provide more information about the analyte. Different from the current conventional colorimetric methods used to study the catalytic performance of nanozymes, SERS can precisely monitor the changes in adsorbed molecules on the catalyst surface as well as the catalytic process [22], while the colorimetric method can only reflect the reaction process in solution [23], not at the catalyst interface, and its sensitivity is also rather limited. SERS provides a novel and powerful method for studying the reaction kinetics of nanozymes [24]. It is obvious that developing nanozymes compatible with SERS is of utmost significance to utilize SERS in the study of catalytic kinetics at catalyst surfaces.
The distinct physicochemical characteristics of nanomaterials endow nanozymes with several possibilities for modification and functionalization [25,26], and SERS substrates are also nanomaterials. Therefore, nanozymes also have the potential to be designed as nanozyme SERS substrates to possess the dual properties of nanozymes and SERS substrates [27]. In short, nanozymes can be synthesized via top-down or bottom-up strategies. They can also be combined with SERS active ingredients like gold and silver to generate nanozyme SERS substrates (Figure 1). Therefore, the strategy of nanozyme applications for SERS can be divided into two categories: since nanozyme SERS substrates are typically constructed by combining SERS active materials with nanomaterials that have nanozyme activity [28,29], they do not need to add additional SERS substrates to provide SERS signal when performing SERS application. On the other hand, the majority of nanozyme just possess catalytic activity. Thus, the addition of SERS substrates after the enzyme-catalyzed reaction has taken place is also a form of nanozyme SERS application [30]. In recent years, research in the field of nanozymes in SERS has made significant progress, and its related research is also increasing year by year (Figure 2). In this study, we integrate recent research in the past 10 years to detail the classification of nanozymes in SERS and their latest application advances in disease diagnostics, food safety, and environmental safety. Finally, the outlook and future trends for the development of nanozymes in SERS applications were prospected.

Figure 1. (a) Top-down synthesis and (b) bottom-up synthesis of nanozymes in SERS.

2. Classification of Nanozymes in SERS

Nanozymes have undergone rapid development since their discovery. Currently, nanozymes for SERS applications are roughly divided into the following four categories: metal-based nanozymes, carbon-based nanozymes, MOF/COF-based nanozymes, and semiconductor-based nanozymes. Some of them can also be mixed with SERS-active substances to create nanozyme SERS substrates, which have the characteristics of nanozymes as well as being SERS substrates.
2.1. Metal-Based Nanozymes

Metal nanoparticles have been a hot topic in SERS due to their remarkable electronic characteristics, and there have been reports of enzyme-like catalytic properties in metal nanomaterials such as Au, Ag, Pt, and Cu, as well as their polymeric nanoparticles and nanostructures [31,32]. However, researchers did not initially find them to have SERS activity and only used them as catalysts in SERS applications. For example, Liang and co-workers [33] used NaBH₄ and sodium citrate as reducing agents to prepare enzymatically active gold nanoparticles (AuNPs) at room temperature, and it was found that AuNPs had a robust catalytic activity on the \( \text{H}_2\text{O}_2\)-\( \text{HAuCl}_4 \) reaction in acidic conditions. According to research on its catalytic mechanism, it was found that the surface of positively charged AuNPs will first adsorb a substantial amount of \( \text{H}_2\text{O}_2 \) and \( \text{HAuCl}_4 \) as the reaction progresses. On the other hand, the abundance of free electrons on the surface of AuNPs will promote the electron transfer between \( \text{H}_2\text{O}_2 \) and \( \text{HAuCl}_4 \), therefore increasing the reduction of \( \text{Au}^{3+} \).

Silver nanoparticles (AgNPs) have an enzymatic activity that is comparable to those of AuNPs. It has been demonstrated that, when \( \text{H}_2\text{O}_2 \) is adsorbed on the surface of AgNPs, \( \text{Ag}^0 \) will be oxidized to \( \text{Ag}^+ \) while \( \text{H}_2\text{O}_2 \) will decompose to form \( \text{OH}^- \), thus, the catalytic activity of AgNPs is strongly reliant on the initial adsorption of \( \text{H}_2\text{O}_2 \) [34]. Considering that the morphology of silver nanoparticles might impact their enzymatic activity, Jiang et al. [35] studied the enzymatic activity of silver nanoparticles with different morphologies, such as silver nanorods (AgNRs), spherical silver nanoparticles (AgNPs), and triangular silver nanoparticles (AgNT). The results indicated that AgNRs > AgNPs > AgNT, and the AgNRs exhibited the strongest enzymatic activity. Other metal nanozymes such as Ag-Au alloy nanoparticles [36] and Pd-Cu bimetallic nanomaterials [37] also have similar properties. The catalytic mechanism of metal-based nanozymes has been demonstrated to be attributed to the adsorption, activation, and electron transfer of the substrate on the metal surface, which is generated by the change of the metal valence state of the nanomaterials [38].

In recent years, noble metals such as Au and Ag have been extensively studied due to their distinctive plasmonic properties at the nanoscale, and their potential as SERS substrates has also been fully exploited. Using a hybridized double-strand poly adenine DNA-directed self-assembly approach, Li et al. [39] employed ascorbic acid as the reducing agent and two different sizes of AuNPs as the core to construct a sea urchin-shaped gold nanocrystals (AuNCs) and gold nanospheres (AuNSs), it is clearly that sea urchin-shaped AuNCs (line a in SERS spectra) demonstrated much higher SERS sensitivity than AuNSs (line b in SERS spectra) (Figure 3). The synthesized AuNCs had a homogeneous nanoprotuberance structure with relatively large surface areas, which could be used as SERS substrates and a catalytic for SERS analysis of food colorants and catalytic degradation of potential pollutants. Hu et al. [40] designed chitosan-modified Au-Ag nanoparticles (CSPNPs) by reducing \( \text{HAuCl}_4 \) with ascorbic acid in the presence of chitosan (CS) and \( \text{AgNO}_3 \). The prepared CSPNPs are ecologically friendly and biocompatible since CS is a natural nontoxic polysaccharide. Furthermore, CSPNPs could also catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by \( \text{H}_2\text{O}_2 \). It was found that the high surface area of CSPNPs and the presence of Au in CSPNPs greatly promoted the catalytic reaction; meanwhile, the positive charge of CS will affect the adsorption of \( \text{H}_2\text{O}_2 \) by CSPNPs. By affecting the electron transfer process mediated by CSPNPs, their catalytic ability can be enhanced. CSPNPs can play the dual role of SERS substrate and nanozyme. The application of Pt-based noble metal nanozymes has also been reported; however, the high cost and scarcity of Pt limit their widespread application [41]. As a result, scientists begin to concentrate on reducing the Pt content of Pt-based nanozymes. An efficient and less expensive option is to substitute Pt in the manufacture of nanozymes with Au or Ag, which not only retains the material’s enzymatic activity but also shows unique optical, electrical, and thermal characteristics. Using multi-branched AuNPs as seeds, Wei et al. [42] synthesized Au@Pt nanoparticles and discovered that varied Pt concentrations might result in Au@Pt nanoparticles with various shapes and surface plasmon resonance properties. It was possible to
obtain both SERS activity and enzyme-mimicking activity by adjusting the Pt concentration. Hu et al. [43] successfully fabricated well-dispersed Au@Ag@Pt nanoparticles (FBNPs) by mediating the multi-branch epitaxial growth of Pt on gold-core-silver-shell nanoparticles using CS. The oxygen-oxygen bond of H$_2$O$_2$ will be rapidly broken by the enzymatic activity of the Pt shell, resulting in the formation of OH$^-$. The produced OH$^-$ was stabilized on the surface of the Pt shell, allowing the conversion of TMB into TMB$_{OX}$. Among these, the Au core improves the chemical stability and durability of the Au@Ag@Pt, while CS will generate a positive charge in acidic circumstances due to the presence of the -NH$_3^+$ group, giving the FBNPs extremely strong peroxidase-like activity. Finally, it was employed to detect glucose.

**Figure 3.** Schematic of the synthesis of sea urchin-shaped AuNCs for SERS analysis and catalytic degradation. Reprinted with permission from [39]. Copyright 2021 IOP Publishing Ltd. Printed in the UK.

### 2.2. Carbon-Based Nanozymes

For carbon nanomaterials, it is difficult to engage in catalytic reactions because carbon nanomaterials do not have unoccupied orbitals. However, by modifying the surface of carbon materials, it may also display catalytic activity [44]. There are several carbon-based nanomaterials that are employed in catalysis, including graphene oxide [45], fullerene [46], and carbon dots [29], which have received a lot of interest due to their low cost, high stability, and simple synthesis procedure [47,48]. In the first few years, carbon-based nanozymes were also used only as catalysts in SERS.

Graphene oxide (GO) was a novel species of carbon material [49]. Jiang and co-workers [50] examined the catalytic activity of GO and discovered that its surface is rich in π-electrons, which may catalyze the reduction of HAuCl$_4$ by H$_2$O$_2$. Fullerenes is also a crucial carbon nanomaterial, although its use is constrained by its poor solubility. By altering the surface of the fullerene, Jiang et al. [51] increased its water solubility. It was then surprisingly discovered that fullerenes had a potent enzymatic impact on the reaction between HAuCl$_4$ and trisodium citrate, which can lead to the formation of AuNPs with SERS activity. They found that fullerenes were effective electron acceptors, that is, AuCl$_4^-$ ions and citrate will adsorb on its surface, facilitating electron transfer from citrate to AuCl$_4^-$ ions and the formation of AuNPs. Carbon dots (CDs), after graphene oxide and fullerenes, have become the most popular carbon nanomaterials due to their outstanding optical characteristics, chemical stability, and simplicity of modification. Ouyang et al. [52] synthesized CDs by hydrothermal treatment utilizing various carbon sources such as fructose, sucrose, and citric acid, and the resulting CDs could catalyze the AgNO$_3$-trisodium citrate reaction to form AgNPs with SERS activity. It has been demonstrated that CDs contain electron-rich surfaces that can speed up the flow of electrons between oxidant and reductant, making redox reactions simple to carry out, among which fructose-based CDs have the highest catalytic activity. The catalytic activity of carbon nanomaterials may be
significantly increased by doping with non-metallic atoms. It should be emphasized that dopant type, content, and modification site all have a significant impact on nanozyme activity. For example, nitrogen-doped carbon dots (CD$_N$) with high catalytic activity were prepared using a hydrothermal approach [53]. The prepared CD$_N$ has a strong catalytic impact on the interaction of HAuCl$_4$ with H$_2$O$_2$. It has also been reported that the catalytic activity of nanozymes is also affected by metal atom doping, such as Zn-doped carbon dots [54]. Recently, different doping elements Ca, Au, and Ag have been compared in some of this research for their impact on CD nanozyme activity [55]. The results demonstrated that Ag-doped CDs (CD$_{Ag}$) had the highest level of nanozyme activity. Additionally, there are nanozymes that have co-doped with non-metal and metal atoms, such as nitrogen-silver co-doping [56] and nitrogen-iron co-doping [57], among others.

Carbon-based nanozymes can also be used to prepare nanozyme SERS substrates by combining the SERS activity of noble metals [58]. Reduced graphene oxide (rGO), a two-dimensional (2D) carbon material, is frequently utilized as a carrier for functional nanomaterials. He and co-workers [59] designed an Ag-Cu$_2$O/rGO nanozyme by employing a simple two-step in situ reduction method. Ag-Cu$_2$O/rGO nanocomposites have inherent peroxidase-like activity and SERS activity, which could quickly oxidize the peroxidase substrate TMB when H$_2$O$_2$ is present. Song and colleagues [60] use a one-pot solvothermal procedure to fabricate an Au-NiFe layered double hydroxide (LDH)/rGO nanocomposite (Au-NiFe (LDH)/rGO). The solvothermal treatment concurrently produces the crystallization of NiFe LDH, the reduction of GO, and the creation of Au nanoparticles from the reduction of HAuCl$_4$. Following that, mono-dispersed Au nanoparticles and NiFe LDH crystals are deposited on the surface of rGO nanosheets (Figure 4). In addition to being an effective nanozyme with oxidase-like activity, the resulting Au-NiFe (LDH)/rGO is also an effective SERS substrate for determining organic mercury. CDs are also excellent carriers, Zhao et al. [61] prepared Ag@CDs NPs with core–shell nanostructures through direct reduction of AgNO$_3$ with CDs acting as a reducing agent and stabilizer. The Ag@CDs NPs had an ultrathin continuous carbon shell with a thickness of approximately 2 nm and an AgNPs core with an average diameter of around 40 nm. Since CDs possess plentiful -OH and -NH$_2$ groups, this approach may directly reduce Ag$^+$ to AgNPs utilizing CDs without the use of an external light irradiation procedure or any reducing agent. Surprisingly, the produced Ag@CDs NPs can efficiently quench the fluorescence of CDs, which is useful for studying their SERS performance. Wang et al. [62] prepared chain-like Au/carbon dot nanocomposites (Au/CD) by a self-assembly method. Briefly, negatively charged Au nanoparticles and positively charged CDs nanoparticles were first linked together by electrostatic interactions, and under the catalytic effect of Au/CD, the amino and carboxyl groups on CDs cross-linked to form one-dimensional chain nanocomposites. It has been demonstrated that the significant catalytic action of Au/CD nanocomposites is caused by the electron transport between Au and CDs. Meanwhile, the aggregation of AuNPs enhances the electromagnetic field of Au/CD, which enhances the SERS signal. Recently, a homogeneous core–shell Ag/oxidized graphene quantum dots (Ag/o-GQDs) nanozyme was reported [63]. The Ag/o-GQDs inherit distinctive properties of o-GQDs, possessing the growing and widening plasmon adsorption. Additionally, the Ag/o-GQDs exhibit outstanding biocompatibility and effective enzymatic activity, which may be used to catalyze peroxidase-like reactions for precise sensing of the intracellular H$_2$O$_2$ at the subcellular level.
2.3. COF/MOF-Based Nanozymes

Covalent organic frameworks (COFs) are a new type of porous organic material linked by strong covalent bonds [64]. It has the characteristics of a large specific surface area, orderly channels, and ease of functionalization, which can accurately regulate the structure and physicochemical characteristics of COFs by chemical modification or surface functionalization [65], and it has also been recently found to have enzymatic activity. Liang et al. [66] used melamine (Ma) and p-benzaldehyde (Bd) as raw materials to synthesize a COF-based nanozyme MaBd by polycondensation, and the resulting MaBd demonstrated effective catalysis for the ethylene glycol-HAuCl₄ reaction. Then, by growing AuNPs in situ on COFs, Wu and co-workers [67] effectively constructed AuNPs-doped COF (COFs@AuNPs), and the COFs@AuNPs demonstrated strong simulated nitroreductase activity. In the presence of COFs@AuNPs, 4-nitrothiophene (4-NTP) may be transformed into 4-aminothiophene (4-ATP) with SERS signal, which can activate the Raman “hot spot” with the assistance of nanozyme-mediated signal amplification and nanozyme products. Palladium nanoclusters (PdNCs) also possess remarkable catalytic performance; however, PdNCs tend to agglomerate and lose their catalytic activity. Jiang et al. [68] loaded PdNCs onto the surface of COF while employing COF as a catalytic carrier, thus successfully preparing a stable COF-based nanozyme named PdTpPa to detect oxytetracycline (Figure 5). When compared to PdNCs with no carrier, PdNCs in PdTpPa had smaller and more evenly dispersed particles, and their catalytic activity had increased by about 300%. The addition of COF increased the stability of the nanozyme as well as its catalytic activity. Similarly, metal-organic frameworks (MOFs) also have catalysis activity [69]. By using 1H-pyrazole-3,5-dicarboxylic acid (H₃pdc) and 2-pyrazincarboxylic acid as ligands, Jiang et al. [70] first synthesized a neodymium MOF (MOFNd). Following that, a new MOF-based nanozyme Au@MOFNd with good stability and high catalytic activity was made by employing NaBH₄ to reduce HAuCl₄, and the introduction of MOF increased the stability and enzymatic activity of the nanozyme. Jiang et al. [71] then used trimesic acid, cerium nitrate, silver nitrate, and NaBH₄ as raw materials to prepare the silver nanocluster-loaded cerium MOF (MOFCeAgNC) via a simple stirring process. In the HAuCl₄-sodium lactate catalytic reaction, the MOFCeAgNC showed significant catalytic activity. Additionally, the enzymatic activity of cerium MOF (MOFCe), silver nanoclusters (AgNC), and MOFCeAgNC were evaluated. The catalytic impact of MOFCeAgNC was shown to be much greater than that of MOFCe and AgNC. The doping of AgNC promotes the aggregation of MOFCeAgNC, resulting in an increase in the particle size of the MOFCeAgNC, and the particles in the solution tend to be stabilized. In other words, doping with AgNCs dramatically increased the stability of MOFCeAgNC.
MOFs are not only excellent adsorbents but also can be used as carriers for catalysts [72,73]. The combination of metal nanoparticles with MOFs as a nanozyme SERS substrate has a wide range of application prospects and has attracted the interest of many researchers. Wei et al. [74] embedded AuNPs into the MOF called MIL-101 by an in situ reduction strategy, where MIL-101 has high porosity and thermal stability to prevent AuNPs aggregation. The obtained AuNPs@MIL-101 nanozyme can act not only as a peroxidase-like enzyme to catalyze the conversion of inactive Raman reporter molecules into active Raman reporter molecules, but also as a SERS substrate to enhance the Raman signal of the reporter molecules. Notably, AuNPs@MIL-101 can be further modified by oxidase to form AuNPs@MIL-101@oxidase-integrated nanozyme, which enables an efficient enzyme cascade reaction. Yang et al. [75] prepared inositol hexakisphosphate (IP$_6$)-modified MIL-101 (IP$_6$-MIL-101) by a solvothermal method, and successfully prepared Ag-Au-IP$_6$-MIL-101 by in situ synthesis of Ag and Au on the surface of MIL-101 with the assistance of IP$_6$. Among them, IP$_6$ are stabilizers and bridging agents between metal nanoparticles and MIL-101. Meanwhile, gold and silver can be grown in situ on the MIL-101 surface by the action of IP$_6$, thus preserving the MIL-101 structure and finally improving the stability of the product. Song et al. [76] constructed a multifunctional magnetic MOF-based composite Fe$_3$O$_4$@Au@MIL-100(Fe). Due to its unique MOFs shell layer structure, cationic dyes can be selectively adsorbed and enriched inside the composite through the pore channels of the MOF shell layer. The internal gold nanoparticles can enhance the Raman signal of these cationic dye molecules and realize the ultra-sensitive SERS detection of cationic dyes. In addition, the composite can be used as a SERS substrate to detect and differentiate mixtures of cationic dyes with no additional processing. The cationic dyes adsorbed to the interior of the composites can be catalytically degraded with the assistance of H$_2$O$_2$, and the degradation mechanism was confirmed to be an enzyme-catalyzed oxidation process.

2.4. Semiconductor-Based Nanozymes

Semiconductor materials have the advantages of high chemical stability and biocompatibility, and their enzymatic activities have been reported recently [77]. In SERS applications, semiconductor materials usually appear as carriers, and by combining with gold, silver, and other noble metal nanoparticles, new nanozyme composites with high SERS activity can be formed.
Song et al. [78] prepared an excellent semiconductor SERS substrate, reduced MnCo$_2$O$_4$ nanotubes (R-MnCo$_2$O$_4$), by electrostatic spinning, calcination, and NaBH$_4$ in situ reduction processes. R-MnCo$_2$O$_4$ nanotubes exhibited good oxidase-like activity assisted by molecular oxygen. It was shown that the good SERS sensitivity of R-MnCo$_2$O$_4$ was mainly attributed to the introduction of oxygen vacancies, which promoted the charge transfer between R-MnCo$_2$O$_4$ and TMB, resulting in defective state energy levels. In addition, the surface of R-MnCo$_2$O$_4$ is rich in Mn$^{2+}$/Mn$^{3+}$ and Co$^{2+}$/Co$^{3+}$ oxidation–reduction states, which also makes it an excellent electron transfer medium and helps to promote the chemical enhancement of SERS. However, the enhancement factors of semiconductor substrates are still not as high as those of noble metal substrates. Therefore, more and more semiconductor-noble metal hybrid nanostructures are being synthesized and show strong SERS performance. Zhao et al. [79] synthesized R-MnCo$_2$O$_4$ nanotubes with abundant oxygen vacancies by NaBH$_4$ reduction reaction using MnCo$_2$O$_4$ nanotubes as raw material. On the surface of R-MnCo$_2$O$_4$ nanotubes, defective state carriers induced by oxygen vacancies can reduce AuCl$_4^-$ to Au$^0$, thus successfully preparing an excellent composite nanozyme R-MnCo$_2$O$_4$/Au NTs. In R-MnCo$_2$O$_4$/Au NTs, R-MnCo$_2$O$_4$ provides peroxidase-like activity; on the other hand, Au NPs were introduced to further enhance the SERS activity of R-MnCo$_2$O$_4$/Au NTs.

In recent years, transition metal-sulfide semiconductors have attracted attention for their excellent optical, catalytic, and electrochemical properties. A series of sheet FeS and CuS with hierarchical structures have been found to have peroxidase activity similar to that of Fe$_3$O$_4$ nanoparticles. Shao et al. [80] prepared Au/FeS bifunctional composites using in situ reduction of Au nanoparticles onto the FeS surface. Among them, FeS has a layered structure with Au nanoparticles of an average size of 47.5 nm wrapped around its surface. The prepared Au/FeS composites exhibited excellent SERS properties and were able to enhance the Raman signals of R6G molecules. In addition, the Au/FeS composites were also shown peroxidase-like activity, which can decompose H$_2$O$_2$ into OH$^-$ and then degrade organic pollutants into small molecules. Li et al. [81] prepared Au/CuS composites using a solvothermal and in situ reduction method. It was found that the gold nanoparticles are a common SERS substrate, which can significantly enhance the signal of Raman reporter molecules adsorbed on the surface of the substrate. The simulation results show that the “hot spot” is not only confined to the interparticle of Au nanoparticles, but also exists in the gap between Au nanoparticles and CuS nanoplates. That is, not only the large electromagnetic field coupling between Au nanoparticles, but also the electromagnetic field coupling between Au nanoparticles and CuS nanoplates leads to high SERS activity. The results also show that the Au/CuS composites have good peroxidase-like activity, and their enzyme-like activity may come from the Cu$^{2+}$ on the surface of the nanoparticle. Au/CuS is not only an excellent peroxidase-like enzyme but also has good SERS performance.

3. Nanozyme SERS Application

Various SERS applications based on nanozymes have been widely explored, including disease diagnosis and treatment, food safety, environmental safety, and kinetics research. Below, we briefly introduce the progress of nanozymes in these fields.

3.1. Disease Diagnosis

Nanozymes overcome the disadvantages of high-cost, poor stability, and difficult storage of natural enzymes and turn out to be promising tools for disease diagnosis. As we know, the levels of molecular components in the blood are directly and intimately tied to the physiological condition of the organism [82–84]. The detection of compounds such as glutathione (GSH) and cholesterol in serum can be employed to prevent, diagnose, and treat a wide range of disorders [85]. By modifying magnetic Fe$_3$O$_4$ with gold nanoparticles, Qu et al. [86] developed a new peroxidase-like enzyme R-Fe$_3$O$_4$/Au to detect GSH and cholesterol in human blood. It was shown that R-Fe$_3$O$_4$/Au could catalyze the oxidation of TMB by H$_2$O$_2$ and also function as a SERS substrate to detect the Raman signal of
the oxidation product of TMB (TMB$_{\text{OX}}$), giving a valuable approach to examining the reaction kinetics of the nanozyme. In addition, without complex sample preparation, the nanozyme can quickly detect glutathione and cholesterol in serum. Even after five cycles, the R-Fe$_3$O$_4$/Au still demonstrated strong peroxidase activity. With its high accuracy and repeatability, the suggested colorimetric-SERS dual-mode sensor provides an innovative method for fabricating multifunctional sensors with exciting potential for use in biosensing. Important neurochemicals like glucose and lactate are also involved in a variety of physiological and pathological brain processes including ischemia, learning, and memory. By growing AuNPs in situ on MIL-101, Wei et al. [74] produced the nanozyme AuNPs@MIL-101 that resembles peroxidase. Then, they modified glucose oxidase (GOx) and lactate oxidase (LOx) onto AuNPs@MIL-101 to create AuNPs@MIL-101@GOx and AuNPs@MIL-101@LOx integrated nanozymes for in vitro SERS detection of glucose and lactate. Additionally, they can determine whether an ischemic stroke is present in a living organism by observing changes in glucose and lactic acid levels and then assess the therapeutic impact of astaxanthin on preventing ischemic stroke (Figure 6). This work offers a fascinating method for creating nanozymes for biomedical and catalytic applications. Tumor-associated biomarkers, such as proteins and metabolites, are linked to genetic mutations or epigenetic alterations and are crucial in the development, progression, and metastasis of cancer. For early cancer diagnosis, prognosis, and epidemiology, examination of these circulating biomarkers may be beneficial. A hollow Janus hybrid nanozyme carrier (h-JHNzyme) based on Ag-Au nanozyme was presented by Zhu et al. [87], and the targeted h-JHNzyme was employed as a signal amplification tag for quantitative SERS liquid biopsy of multiple tumor-associated biomarkers such as CEM cells and microRNA-21 (miR-21). It is an important development in the direction of a trustworthy quantitative SERS platform for clinical diagnosis. Other compounds, such as glucose [88], caffeine [89], H$_2$O$_2$ [90], etc., are also strongly connected to the diagnosis of disease.

![Figure 6](http://example.com/figure6.png)

**Figure 6.** Schematic illustration of AuNPs@MIL-101@oxidases for efficient enzymatic cascade reactions. Reprinted with permission from [74]. Copyright 2017 American Chemical Society.

### 3.2. Food Safety

In addition to disease diagnosis, SERS applications of nanozymes in food safety have also gained increasing attention, and have shown great potential in the detection of foodborne pathogens, toxins, and small molecules in food recently [39,91]. Campylobacter jejuni (C. jejuni), a Gram-negative microbe, is widely regarded as the leading cause of severe bacterial gastroenteritis in humans. Jin et al. [92] created a colorimetric/SERS dual-mode lateral flow detection approach based on artificial Pt-coated gold nanorods (AuNR@Pt) with peroxidase-like activity and SERS activity for the sensitive, quick, and specific detection of C. jejuni. The technique was used to identify C. jejuni in milk samples with recoveries ranging from 89.33% to 107.62%. Staphylococcus aureus (S. aureus) is also a common foodborne pathogen that can survive in a variety of dry environments and is easily airborne. Additionally, dermatitis, gastrointestinal infections, bacteremia, and infective...
endocarditis have all been linked to it. A signal amplification system for the specific detection of *S. aureus* was built by Chen et al. [93], and *S. aureus* was first magnetically isolated from the spiked solution after being enriched by a capture probe. Then, to enrich for *S. aureus*, a quantity of *S. aureus* aptamer was added. After being separated magnetically, the supernatant containing the *S. aureus* aptamer residue was added to the nanozyme Fe-MIL-88 to alter its catalytic activity. The more *S. aureus* was enriched, the less *S. aureus* aptamer residue was present in the supernatant, which led to a greater catalytic activity of Fe-MIL-88 following incubation. As a result, following the addition of the reaction’s catalytic substrate, the SERS and UV-Vis signals of the catalytic product were proportional to the amount of *S. aureus*. Finally, the developed technique was employed to identify *S. aureus* in milk and chicken.

Natural toxins, commonly referred to as biotoxins, are toxic elements that are pervasive in the natural environment and are lethal to both humans and other animals. Ochratoxin (OTA) is a naturally occurring toxin that is present in food and is nephrotoxic, mutagenic, and possibly carcinogenic. Yang et al. [94] employed Pd-Pt NRs linked with ochratoxin aptamer (Apt) as the recognition probe and magnetic nanoparticles (MNPs) coupled with capture aptamer (cApts) as the capture probe. The binding of the recognition probe and the capture probe was prevented in the presence of OTA due to the specific recognition between OTA and Apt. On the contrary, because of the interaction between Apt and cApt, the recognition probe will bind to the capture probe and have a significant catalytic impact on TMB. The produced TMB$_{OX}$ had a stronger Raman signal when AuNPs were added as the SERS substrate. The technique was used to identify OTA in wine and grapes, and the detection findings agreed well with those obtained by high-performance liquid chromatography-tandem mass spectrometry, which offered a new technique for the quick and accurate detection of OTA.

As a typical oxidant in food, H$_2$O$_2$ is also frequently utilized in biomedical, pharmacological, industrial, environmental, and enzymatic operations. In fact, intracellular H$_2$O$_2$ concentrations over 700 nmol/L or large levels of ingested H$_2$O$_2$ can be harmful to human health. A unique H$_2$O$_2$ dual-mode sensor was created by Zhong et al. [95] using colorimetry and SERS. The Fe$_3$O$_4$@AuNPs nanozyme employed in this study maintains the amazing catalytic characteristics of Fe$_3$O$_4$ while also having the ability to tune the SERS signal by regulating the growth of AuNPs on the surface of Fe$_3$O$_4$. Fe$_3$O$_4$@AuNPs may accelerate the oxidation of TMB to TMB$_{OX}$ in the presence of H$_2$O$_2$. Finally, the developed colorimetric/SERS dual-mode sensor was successfully used to detect H$_2$O$_2$ in milk and plasma, exhibiting good sensitivity and selectivity (Figure 7).

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Schematic illustration for the synthesis of Fe$_3$O$_4$@AuNPs and principle of colorimetric/SERS dual-sensor of H$_2$O$_2$ constructed via TMB-Fe$_3$O$_4$@AuNPs. Reprinted with permission from [95]. Copyright 2021 Elsevier B.V.
3.3. Environmental Safety

Environmental pollutants, including heavy metal ions and herbicides, may have harmful effects on human health and the environment. Nanozymes, as efficient catalysts, have been widely used in the detection and degradation of pollutants [96]. For example, Hg$^{2+}$ are hazardous metal ions that once build in the body and are difficult to eliminate through metabolism, which will cause heart, liver, and thyroid illnesses [97]. Therefore, the quick and accurate detection of Hg$^{2+}$ is crucial. By coating hexahedral gold nanoparticles with platinum, Wang et al. [98] synthesized nanozyme Au@AgPt NPs, which combined the greater SERS activity of Au with the stronger enzymatic activity of Pt. Au@AgPt NPs were then surface-modified with mercaptohexanol to construct the colorimetric/SERS dual-mode probe Au@AgPt@MCH. Hg$^{2+}$ will be captured specifically by Au@AgPt@MCH, where it combines with Pt atoms to produce Pt$^{2+}$ and Hg atoms. The catalytic activity of Au@AgPt@MCH is decreased due to the depletion of Pt atoms on nanoparticles, which effectively limits the oxidation of TMB and affects the yield of TMB$_{OX}$. As a result, the selective detection of Hg$^{2+}$ is successfully accomplished. Glyphosate (Gly) is a non-selective, highly effective, and low toxicity herbicide frequently used to control annual or perennial weeds. Recent findings suggest that the accumulation of Gly in the environment may pose a carcinogenic risk to humans. Monitoring the amount of Gly in environmental samples is therefore crucial and necessary. With the use of chain-like Au/Ag nanoparticles as SERS substrates and Au-Pt nanoparticles as nanozymes, Zheng et al. [99] created a SERS approach for the detection of Gly in tap water. The specific principle is as follows: the addition of L-cysteine (L-Cys) limits the activity of Au-Pt nanoparticles, preventing TMB from being oxidized to TMB$_{OX}$. When Cu$^{2+}$ is present, Gly binds to L-Cys to lessen its inhibitory effect on Au-Pt nanoparticles. Using the SERS signal produced by the conversion of TMB to TMB$_{OX}$ catalyzed by Au-Pt nanozyme, the indirect detection of glyphosate content was finally accomplished.

Aside from detecting harmful pollutants like Hg$^{2+}$ and insecticides, nanozymes have a wide range of uses. It has been demonstrated that nanozymes may eliminate pollutants from water and the environment through Fenton-like reactions. Ni@MIL-100(Fe)@Ag, an MOF-based SERS probe created by Cui et al. [100], has high sensitivity and good recyclability for detecting a range of cationic dyes. The authors initially used a magnetic-assisted chemical reduction technique to create Ni nanowires with a spiky form. On top of the Ni nanowires, layers of MIL-100(Fe) with peroxidase activity were then produced. Finally, the $[\text{Ag(NH}_3\text{)}_2]^{+}$ ions that permeated MIL-100(Fe) were converted by glucose in situ to AgNPs. MOF may be employed not only as a carrier and adsorbent to boost SERS activity but also as a peroxidase-like enzyme for self-cleaning. That is, the OH· produced by the interaction of H$_2$O$_2$ with MIL-100 can quickly degrade organic pollutants on Ni@MIL-100(Fe)@Ag. This reusable SERS probe has considerable promise for use in aquaculture, biomedicine, and chemical analysis. Nitrophenol ramifications are very dangerous and a poisonous organic pollutant, which poses a hazard to both the environment and people. Li et al. [69] created Au/MOF-74 composite nanoparticles with SERS activity and catalytic capabilities, which were effectively applied for sensitive SERS detection and nitrophenol compound degradation. They discovered that the open metal sites in the MOF-74 shell might speed up the electron transfer process. As a result, the Au/MOF-74 can speed up the 4-NTP degradation reaction with a high kinetic reaction rate (Figure 8).
3.4. Others

Traditional liquid-phase mass spectrometry and infrared spectroscopy have been developed for kinetics research. These methods, however, cannot be used to analyze and measure complex multiphase reaction systems in real time. In recent years, a growing variety of nanozyme SERS substrates have been utilized for kinetics research in situ and in real time, bringing new insights for understanding the kinetics and mechanisms of the reactions [101]. Zhao et al. [61] created core–shell Ag@CDs SERS substrates by directly reducing Ag\(^{+}\) to Ag NPs using CDs. It was discovered that Ag@CDs NPs also displayed strong peroxidase-like activity, which may be exploited for in situ, real-time SERS monitoring of the TMB oxidation process. Furthermore, the reduction of p-nitrothiophenol (PNTP) to p-aminothiophenol (PATP) in the presence of NaBH\(_4\) could also be studied by Ag@CDs NPs in real time (Figure 9). This research was effective in learning about the molecular changes that occur during catalysis and expanded the use of CDs for in situ real-time monitoring of multiphase catalytic processes in SERS. The material preparation method was then improved by Song and her co-workers [102]. In their research, AgNPs/CDs nanozyme was formed by chemically combining CDs and AgNPs. During the synthesis procedure, carboxyl groups on the surface of Ag NPs reacted with amino groups of CDs. One benefit of this method is that it produces uniformly dispersed, individual CDs on the surface of Ag NPs. The produced Ag@CDs NPs could be utilized as SERS substrates for in situ study of the kinetic process of catalytic reactions. Lu et al. [103] created polyaniline (PANI)/Au nanoparticles by self-assembling HAuCl\(_4\) and aniline in the presence of 3-(aminopropyl)triethoxysilane. PANI/Au showed stronger peroxidase-like activity than Au nanoparticles and PANI alone because of the synergistic interaction between Au nanoparticles and PANI. The produced PANI/Au turned out to be fantastic SERS substrates for in situ monitoring of the TMB oxidation process. By using a three-step process of solvothermal reaction, Au seed-induced growth, and low-temperature cyclic self-assembly, Song et al. [73] synthesized the magnetic MOF-based nanozyme Fe\(_3\)O\(_4\)/Au@MIL100(Fe). The as-prepared Fe\(_3\)O\(_4\)/Au@MIL100(Fe) exhibits peroxidase-like activity, and the system may be utilized to continuously measure the concentration of H\(_2\)O\(_2\) while also in situ studying the kinetic process of peroxidase-like processes. This study proposes a novel method for increasing the catalytic activity of iron-based MOFs and applies SERS technology to in situ studying the peroxidase-like catalyzed reactions.
Figure 9. Schematic illustration of the Ag@CDs NPs catalyzed reduction of PNTP to PATP. Reprinted with permission from [61]. Copyright 2016 American Chemical Society.

4. Conclusions and Prospects

In the last ten years, researchers have been increasingly interested in the application of nanozymes in SERS. In this paper, the classification of nanozymes utilized in SERS research is reviewed, including metal-based nanozymes, carbon-based nanozymes, MOF/COF-based nanozymes, and semiconductor-based nanozymes; it also introduces the SERS applications of nanozymes in food safety, disease diagnosis, environmental safety, and others. Even though the SERS research on nanozymes has advanced significantly, some important problems still need to be resolved:

(1) Boost the catalytic performance of nanozymes in SERS. The majority of nanozymes in SERS offer both SERS properties as well as enzyme-like catalytic properties. Among them, the introduction of SERS active materials may obstruct the catalytic active sites on the surface of nanozymes, decreasing the catalytic effectiveness; on the other hand, the introduction of nanozyme components and even the occurrence of enzymatic processes may also alter the SERS signal. Even though numerous methodologies for the rational design of nanozymes in SERS have been devised, the catalytic efficiency of existing nanozymes in SERS is still typically lower than that of natural enzymes. Future research may focus on merging machine learning with high-throughput trials to hasten the discovery of highly catalytically active nanozymes in SERS.

(2) Although nanozyme-based SERS analysis technology has been developed in a variety of analysis areas, the reported research mostly focuses on the detection of simple environmental and biological samples, with the detection of complex system samples being rare. For this reason, expanding the use of nanozymes in SERS requires significant improvements in the pretreatment of analytes during sample preparation. The application of nanozymes in SERS for complex systems can be promoted by designing devices with integrated sample pretreatment functions or by designing “all in one” nanozymes that integrate multiple functions such as separation and enrichment capabilities. In addition, realizing the detection of multiple targets in complicated systems is likewise a foreseeable tendency.

(3) Expand the variety of nanozymes in SERS. Currently, the published nanozymes in SERS are dominated by peroxidase-like enzymes, which are inadequate to cover all major enzymatic reactions. Therefore, it is essential for researchers to widen the
kinds of nanozymes in SERS as well as further clarify the definition and properties of related nanozymes.

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