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Review

Gold Nanozymes: Smart Hybrids with Outstanding Applications

Sandra Jimenez-Falcao [1,2,*], Jose M. Méndez-Arriaga [1], Victoria García-Almodóvar [1], Antonio A. García-Valdivia [1,3] and Santiago Gómez-Ruiz [1,*]

[1] COMET-NANO Group, Departamento de Biología y Geología, Física y Química Inorgánica, Universidad Rey Juan Carlos, Móstoles, 28933 Madrid, Spain
[2] Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain
[3] Departamento de Química Inorgánica, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain
* Correspondence: sandraji@ucm.es (S.J.-F.); santiago.gomez@urjc.es (S.G.-R.)

Abstract: Nanozymes are nanostructured artificial enzymes that have attracted great attention among researchers because of their ability to mimic relevant biological reactions carried out by their natural counterparts, but with the capability to overcome natural enzymes' drawbacks such as low thermostability or narrow substrate scope. The promising enzyme-like properties of these systems make nanozymes excellent candidates for innovative solutions in different scientific fields such as analytical chemistry, catalysis or medicine. Thus, nanozymes with different type of activities are of special interest owing to their versatility since they can reproduce several biological reactions according to the substrates and the environmental conditions. In this context, gold-based nanozymes are a representative example of multifunctional structures that can perform a great number of enzyme-like activities. In addition, the combination of gold-based materials with structures of organic and inorganic chemical nature yields even more powerful hybrid nanozymes, which enhance their activity by providing improved features. This review will carry out a deep insight into gold-based nanozymes, revisiting not only the different type of biological enzymatic reactions that can be achieved with these kinds of systems, but also structural features of some of the most relevant hybrid gold-based nanozymes described in the literature. This literature review will also provide a representative picture of the potential of these structures to solve future technological challenges.

Keywords: nanozyme; gold nanoparticles; hybrid materials; enzyme activity

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1. Introduction

Enzymes are natural biological catalysts that promote biochemical transformations through the formation of a complex between the enzyme and the reactants (known as *substrates*), under normal physiological conditions. According to their performance, enzymes share many features with classic heterogeneous catalysts [1–3]:

- The chemical equilibrium of the reaction is not affected since the catalyst is recovered unaltered after the reaction is finished.
- The role of the catalyst is to lower the activation energy required for the reactants (or substrates) to be converted into products, increasing the reaction rate.
- The reaction takes place near the catalyst's surface.
- Each catalytic reaction presents a specific mechanism.

For these reasons, enzymes have been widely used in many scientific and industrial fields such as food and beverages, agriculture, pharmaceutical, bioenergy or environmental monitoring [4–6]. Nevertheless, because of their bio-organic-based composition (enzymes are mainly composed of amino acids and additional saccharides or metal atoms), they present intrinsic limitations that have resulted in a great effort to find inert alternative materials capable of performing enzyme-mimicking reactions. Early research in this field

resulted in the development of catalytic polymers called *synzymes* [7,8]. Soon after, this concept was expanded to include nanomaterials, which emerged as a great alternative, giving rise to *nanozymes*: entities in the nano scale with catalytical activity that exhibit enzyme-like features [9]. This term was first used in the early 2000s to describe a gold nanoparticle system able to achieve transphosphorylation reactions [10]. In this context, ease of preparation, large scale production, diverse of activities, high stability towards denaturation, recyclability, ability to work in a wider range of temperature and pH, and additional physicochemical features (photodynamic, photothermal or magnetic properties), are some of the benefits of nanozymes in comparison to their natural counterparts [11].

A variety of nanomaterials, such as metal nanoparticles, metallic derivatives (metal oxides, metal sulfides, MnSe), carbon-based systems or polymeric materials, have shown enzyme-like properties [12]. In addition, the potential to design tailor-made synthetic protocols by surface coating, chemical doping or size and morphology tuning, renders as many nanozyme systems as can be imagined, providing new functionalities, or improving their catalytic features. This compositional and morphological versatility make them excellent tools in a variety of scientific fields, such as biomedicine [13], sensing [9,14] or environmental engineering [15].

One of the most outstanding elements for the design of advanced nanozymes is gold. Its physical properties (including surface plasmon resonance), its effectiveness as a catalyst and its capacity of mimicking a great number of enzymatic reactions make this metal an interesting subject of study for the scientific community [16,17].

The aim of this review is to explore the catalytic activity of gold nanozymes, and, in particular, hybrid gold nanozyme systems that combine gold with different nano or biomaterials in order to improve their performance. Despite the great number of advantages that nanozymes present, low specificity and relatively poor activity are undeniable features that arise from the lack of sophisticated active sites in comparison to natural enzymes. These enzymes possess a combination of different amino acid residues that not only bind the substrates, but also catalyze the reaction for a specific molecule, while nanozymes can mimic the reaction of a specific substrate, but also transform other compounds. However, the scientific community is making a great effort to promote nanozyme catalytic reactions or to consider their multienzyme mimetic capacity. A smart strategy to accomplish this goal is the combination of different nanomaterials, resulting in nanohybrids with synergistic effects. Some of the most relevant hybrid gold-based nanozymes synthesized to date are collated in Table 1.

Table 1. Relevant hybrid gold-based nanozymes described in the literature.

Inorganic Biohybrids				
Carbon-Based				
Carbon-Based Material	***Enzymatic Activity***	***Application***		***Ref.***
Carbon dots	Oxidase	Sensing	Biothiols	[18]
		Antitumoral	Liver cancer	[19]
Nanoporous carbon	Oxidase	Sensing	Oxidase	[20]
Carbon nanoshell	HRP [a]	Antitumoral	Gastric cancer	[21]
			-	[22]
Graphite	HRP	Sensing	H_2O_2 and glucose	[23]
Carbon dots	HRP	Sensing	Glucose	[24]
	HRP	Sensing	Tert-butyl hydroquinone and formaldehyde	[25]
	HRP	Catalysis	Oxidation of tert-butyl hydroquinone	

Table 1. *Cont.*

Porous carbon @PDAGCU	HRP	Sensing	PSA [b]	[26]
Carbon nanotubes	HRP	Sensing	H_2O_2	[27]
Graphene oxide@CeO_2	HRP	Sensing	Nitrites	[28]
Yolk shell carbon	Oxidase + HRP	Antitumoral	Colorectal cancer	[29]
MOFs				
MOF	***Enzymatic Activity***	***Application***		***Ref.***
MIL-101	HRP	Sensing	Glucose and lactate	[30]
NH2-MIL-125 (Ti)	HRP	Sensing	Cysteine, H_2O_2 and Hg^{2+}	[31]
Al-MOF-2D	HRP	Antibacterial	*Staphylococcus aureus* and *Escherichia coli*	[32]
Fe-MOF	HRP	Degradation	Methylene blue	[33]
	HRP	Sensing	Hydroxyl radical	
Co-MOF	HRP	Sensing	*Burkholderia pseudomallei*	[34]
Metals				
Metal	***Enzymatic Activity***	***Application***		***Ref.***
Tubular TiO_2	HRP	Sensing	H_2O_2	[35]
Ag alloy	HRP	Antibacterial	*Mycobacterium tuberculosis*	[36]
Pd@Ir core–shell	HRP	Sensing	PSA	[37]
Pt core–shell	HRP	Sensing	Improving ELISA	[38]
Co-Fe core–shell	HRP	Sensing	*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus cereus*	[39]
Yolk shell TiO_2	HRP	Sensing	H_2O_2 and glucose	[40]
Fe_2O_3 nanocubes	HRP	Sensing	Improving ELISA	[41]
	HRP	Sensing	P53 autoantibodies	[42]
Pt core–shell	HRP	Sensing	Glucose	[43]
		Organic Hybrids		
Aminoacids (aa)				
aa	***Enzymatic Activity***	***Application***		***Ref.***
Various	HRP	Sensing	Cu^{2+}, histidine	[44]
Histidine	HRP	Sensing	Nitrite	[45]
Cysteine	HRP	Enantioselectivity	Dopamine	[45]
Peptide	HRP	Optical imaging	Cancer cells (HEL cells)	[30]
Histidine	Oxidase	Sensing	Doxycycline	[46]
	Glucose oxidase	Sensing	Glucose	[47]
Polymers				
Polymer	***Enzymatic Activity***	***Application***		***Ref.***
PEG-SH	HRP	Sensing	H_2O_2	[48,49]

Table 1. *Cont.*

PEG/Carboxylate	HRP	Sensing	Proteins	[50]
PAM-4	HRP	Sensing	Ciprofloxacin	[51]
Heparin	HRP	Microdialysis	Cytokines	[52]
PCL/Gelatin	HRP	Antibacterial + Wound healing	MDR Bacteria	[53]
Hyaluronic acid	HRP	Anticancer	4T1 breast cancer cells	[54]
Biohybrids				
Proteins				
Protein	***Enzymatic Activity***	***Application***		***Ref.***
Ab [c]	HRP	Sensing	*Trichinella spiralis*	[55]
	HRP	Sensing	Influenza A virus	[56]
	HRP	Sensing	Ebola	[57]
	HRP	Sensing	Influenza virus	[58]
Apoferritin	HRP	Sensing	Glucose	[59]
	SOD [d], Catalase, HRP	ROS Scavenger	O_2^-	[60]
BSA [e]	HRP	Sensing	Tea polyphenols	[61]
	HRP	Sensing	Xanthine	[62]
	Glucose oxidase + HRP	Sensing	Glucose	[63]
β-Cas [f]	HRP	Sensing	Protease enzyme	[64]
Nucleic acids				
Nucleic Acid	***Enzymatic Activity***	***Application***		***Ref.***
Apt [g]	HRP	Sensing	C Reactive protein	[65]
	HRP	Sensing	CA125 Ovarian cancer biomarker	[66]
	HRP	Sensing	Ampicillin	[67]
	HRP	Sensing	Norovirus	[68]
	HRP	Sensing	Acetamiprid pesticide	[69]
	HRP	Sensing	Kanamycin	[70]
	HRP	Sensing	Zearalenone	[71]
	HRP	Sensing	Streptomycin	[72]
Polysaccharides				
Polysaccharide	***Enzymatic Activity***	***Application***		***Ref.***
Chitosan	Oxidase + HRP	Antibacterial + Bacterial imaging	*Helicobacter pylori*	[73]
	HRP	Sensing	H_2O_2 and glucose	[74]
	HRP	Sensing	Hg^{2+}	[75]
	HRP	Sensing	Glucose	[76]
	HRP	Sensing	Melamine	[77]

[a] Horseradish peroxidase; [b] Prostate Specific Antigen; [c] Antibody; [d] Superoxide Dismutase; [e] Bovine Serum Albumin; [f] β-Casein; [g] Aptamer.

2. Types of Gold Nanozyme Activity

Since there is a great variety of proteins that perform catalytic tasks, the number of activities that enzymes can carry out is almost unlimited. They can be classified in seven different groups; namely, oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and translocases, which can be subdivided according to the concerned reaction mechanism or the substrate [78]. Nanozymes accomplish a vast number of these typical enzymatic tasks, which is possible because of the presence of high energy atoms on the surface of nanozymes. Moreover, nanomaterials' surface can be grafted with ligands that present functional groups which are typical in enzymes, favoring classic enzymatic catalytic reactions [16].

Nevertheless, compositional, and structural differences between them are responsible for the different catalytic properties exhibited by these kinds of systems. Natural enzymes present different mechanisms to catalyze chemical reactions, employing usually more than one to complete the conversion from substrate to desired products. The mechanism of the enzyme depends on two factors: specificity of the enzyme and the transition state of the reactants or substrates [79]. The main mechanisms are covalent catalysis, acid-base catalysis, electrostatic catalysis and cofactor catalysis.

Covalent catalysis [80] involves the formation of a covalent bond between the active site and at least one of the substrates, while acid-base catalysis [81] involves a proton transfer. In addition, electrostatic catalysis [82] is based on a stabilization of the transition state with electrostatic interactions and, finally, cofactor catalysis [83] relies on the interaction with compounds that are not substrates, but that are necessary for the transformation, desolvation, approximation or strain distortion in specific cases.

Whereas enzymes present a limited number of active sites, nanozymes possess a high number of them. In addition, the presence of multivalent elements or a great number of coordination structures permit the coexistence of different catalytic types of activity in a single nanozyme, which is unusual in natural enzymes [84]. Despite nanozymes presenting lower substrate specificity, this weakness can be overcome by grafting chiral amino acids, providing stereoselectivity [85].

In the case of noble metal nanozymes, catalytic activity relies on the adsorption, activation and electron transfer processes on the catalytic surface, which is possible owing to the variable oxidation states of the metal atoms [15].

Despite the great number of enzyme activity-like reactions, nanozymes are mainly focused on performing the tasks of hydrolases and oxidoreductases, which consist of the hydrolysis of chemical bonds and the accomplishment of redox reactions, based on the transference of electrons and hydrogen or oxygen atoms between molecules, respectively [15] (Figure 1).

Gold-based nanozymes are able to mimic a great variety of enzyme-like reactions. The enzymatic activity of the nanozymes depends on the environmental reaction conditions (availability of substrates, pH), synthetic conditions of the gold nanosystem [86], surface coating [63], or presence of certain substances [87].

2.1. Peroxidase (HRP)

The enzymatic activity that enables the reduction of hydroperoxides (generally hydrogen peroxide) to water is known as peroxidase. This type of enzymatic activity is commonly abbreviated to HRP, since the most used enzyme used for biotechnological purposes is extracted from horseradish peroxidase [88]. Most of the main applications of gold-based nanozymes with HRP activity are in the field of sensing for the determination of H_2O_2 or glucose [89], *Pseudomonas aeruginosa* [90] or CA125 cancer biomarker [66].

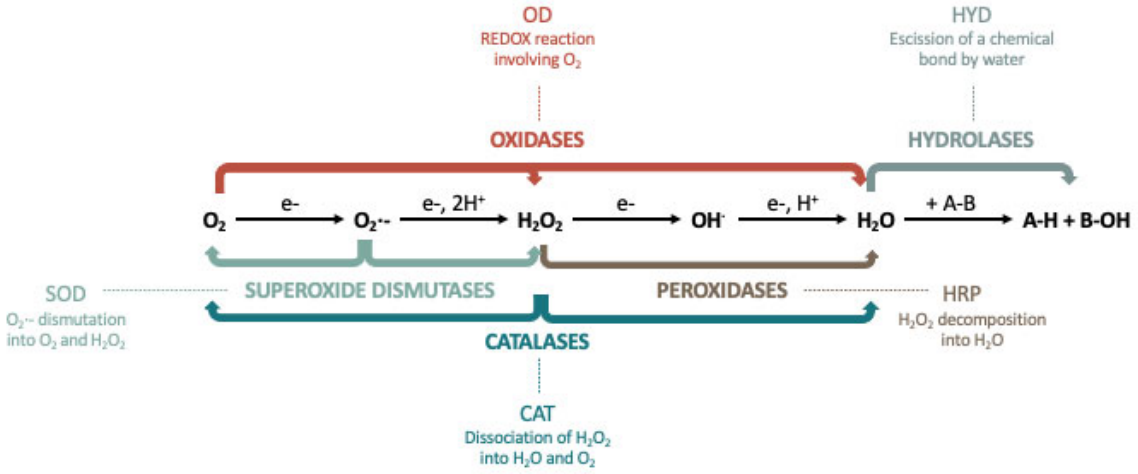

Figure 1. Scheme of the major reactions catalyzed by oxidoreductases (oxidases, superoxide dismutases, HRPs and catalases) and hydrolases. Adapted with permission from [15].

2.2. Superoxide Dismutase (SOD)

Removal of superoxide radicals throughout their transformation to H_2O_2 and O_2 is catalyzed by superoxide dismutase activity. Therefore, this reaction is tightly related to oxidative stress [91]; for example, a gold nanozyme-based system presenting SOD activity [92] is useful in cryopreservation [60].

2.3. Catalase (CAT)

The transformation of H_2O_2 to H_2O and O_2 (a trigger of oxidative stress) is accomplished by catalase activity [93]. Catalase-like gold nanozymes are suggested to be promising tools for the removal of H_2O_2 in industrial applications [94], but also great agents against cancer cell hypoxia [95].

2.4. Glucose Oxidase (GOD)

Glucose oxidase is a specific type of oxidase activity, which promotes the oxidation of glucose to gluconic acid and H_2O_2 [96]. As a consequence of gold-based nanozymes' responsiveness to glucose [97], they are excellent candidates for glucose sensing [98,99]. Nevertheless, they also present additional applications, for example, in tumor ablation therapy [100].

2.5. Esterase

Esterase catalytic reactions describe the hydrolysis of an ester group, which is released as an acid [101]. Although less frequent, some examples can be found in the literature of gold-based nanozymes presenting this kind of special reactivity [102] for gold systems.

2.6. Nuclease

The function of nucleases is to split the phosphodiester bonds of DNA and RNA nucleic acids [103]. The first publication regarding nanozymes reported a gold-based system with phosphate diester-cleavage ability [10]. Interestingly, specific applications of gold-based nuclease nanozymes include DNAse activity to avoid the formation of bacterial biofilms [104] or with the ability to break plasmid DNA [105].

2.7. Combined Activity

It is frequent to find gold-based systems able to perform more than one enzymatic activity. The usual way to control the type of reaction that takes place is by switching the pH; superoxide dismutase and catalase activities perform best at basic to neutral pH, glucose oxidase at neutral to acidic pH [63], and HRP activity at acidic pH [106–108]. Therefore,

by modulating the reaction conditions and environment, gold-based nanozymes can be multifunctional systems with various applications in different biological contexts.

3. Nanozymes Based on Gold Hybrids

3.1. Inorganic Hybrids

In recent years, inorganic hybrid materials have received attention for their use as an enzyme substitute due to their mimetic active centers throughout the structure. Among them, noble metal-based nanozymes exhibit special optical properties, excellent chemical stability, adjustable enzyme-like activity and superior biocompatibility, being considered one of the top research materials in diverse fields such as nanotechnology and medicine [109]. Specifically, Au and Pt nanozymes can show HRP [89], SOD and CAT activities [110]. Au quantum dots also present glucose oxidase (GOD) potential, with an enzymatic activity negatively correlated with particle size (the smaller the particle size, the higher the GOD activity), demonstrating that surface area is a critical parameter in non-enzymatic activity [111]. A classification based on the material supporting Au nanosystems will be presented in this section.

3.1.1. Carbon-Based Supports

Carbon structures have been widely used for different applications, especially energy storage and sensors [112] due to their large pore volume and high specific surface. After modification with gold, one of the more extended applications of carbon/Au-based scaffolds is their use as sensors [18,27], although without nanozyme behavior in most of the cases. Nevertheless, carbon-based gold hybrids (C@Au) can also be applied in cancer treatment by taking advantage of the nanozyme characteristics of the nanomaterial. For example Lei Fan et al. [29] proposed the use of a single gold nanoparticle core with a porous hollow carbon shell nanosphere (HCNs@Au). This hybrid possesses great HRP and oxidase enzymatic activity, leading to reactive oxygen species (ROS) generation under an acidic environment and NIR radiation at 808 nm (Figure 2).

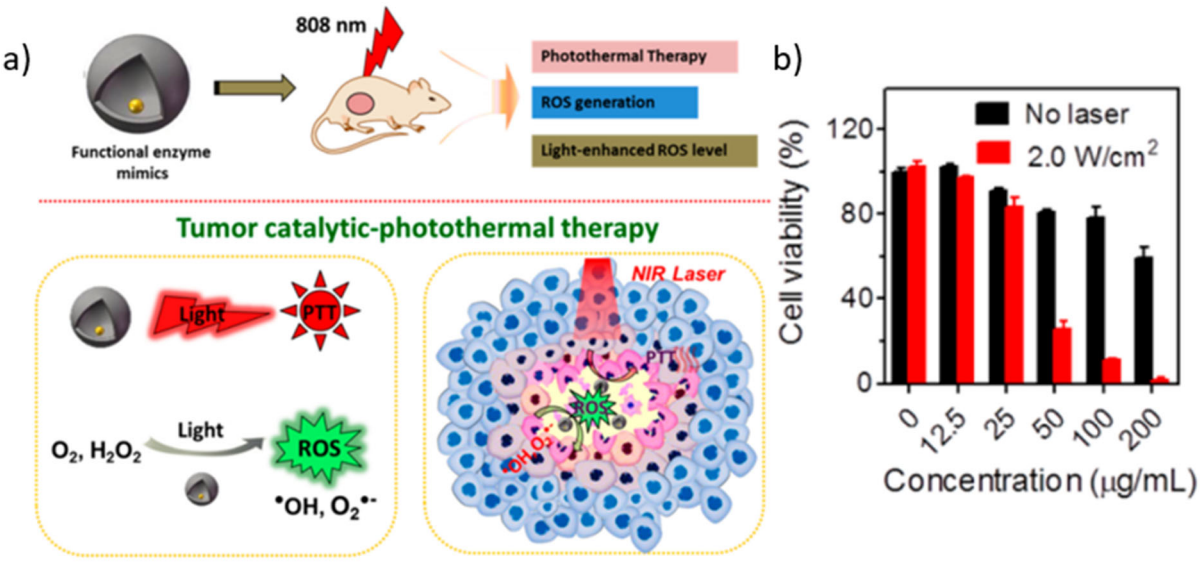

Figure 2. (**a**) Scheme of the action of Au@HCNs under 808 nm radiation. (**b**) Relative viabilities of CT26 cell incubated with Au@HCNs under 808 nm laser irradiation for 10 min. Reprinted with permission from [29]. Copyright © 2018 American Chemical Society.

Novel C@Au nanohybrids have been developed in recent years, performing enzymatic activity in similar conditions to the example cited before [21,22]. Ningqiang Gong et al. [19], for example, synthesized a new carbon dot-supported atomic gold (CAT-g) presenting sensitivity to acid pH inside cancer cells without the presence of any kind of radiation. CAT-

g resulted in being highly toxic to liver cancer cell (HepG-2, BEL-7404 and HCCC-9810), while it was harmless to normal liver cells (L02, QSG-7701) and primary cells.

The use of C@Au hybrid nanozymes have special importance as sensors in the detection of small molecules involved in metabolic processes (like uric acid [20] and mostly glucose) due to their capacity to mimic HRP activity [23,24]. In several recent studies, Qiulan Li et al. and Qing Shang et al. developed C@Au-based systems for the determination of contaminants in food [25] and cancer biomarkers [26], respectively.

Finally, it is mandatory to highlight graphene-based nanozymes, owing to the excellent properties of graphene as supporting systems. Those graphene@Au materials have proven to be great candidates for diverse applications, such as fast and highly selective colorimetric sensors against carcinogenic agents (Figure 3) [28]. In this example, a multimaterial hybrid nanozyme based on chemically modified gold nanoparticle (AuNP)-cerium oxide (CeO_2) NP-anchored graphene oxide (GO) was proposed for the determination of nitrite. Graphene@Au hybrids are also useful as adsorbents for pollutants, or catalysts for the transformation of H_2O_2 to $^{\bullet}OH$ radicals, subsequently exhibiting outstanding removal performance toward different organic dyes [113]. This multivalent behavior of graphene-Au nanozymes makes them one of the most promising alternatives for the next decade.

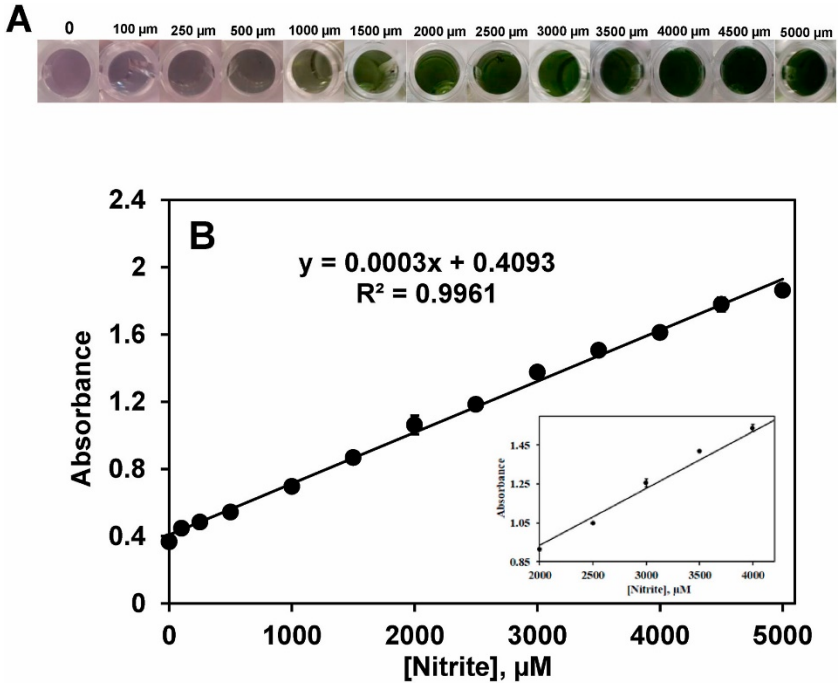

Figure 3. (**A**) Photographic colorimetric response of the AuNP-CeO_2 NP@GO nanozyme to nitrite detection at different incubation times and the corresponding catalytic absorbance signals generated at the different detection times (**B**). Reprinted with permission from [28]. Copyright © 2020 Elsevier.

3.1.2. MOF-Based Supports

Despite the use of metalorganic frameworks (MOFs) being relatively recent in this field, they are one of the most versatile materials to combine with gold in order to obtain nanozymatic hybrids. The presence of a metallic center and bridging linkers favors post-synthetic modifications [114], making these systems excellent candidates to be used as enzyme-substitutes in a wide range of applications such as luminescence, magnetism, catalysis or biomedicine [115–117].

One of the first structures used as a nanozyme was synthesized by Yihui Hu et al. in 2017 [30]. This MOF@Au hybrid, composed by MIL-101 MOF doped with AuNPs, presents HRP enzymatic activity to detect glucose and lactate in living tissues (Figure 4).

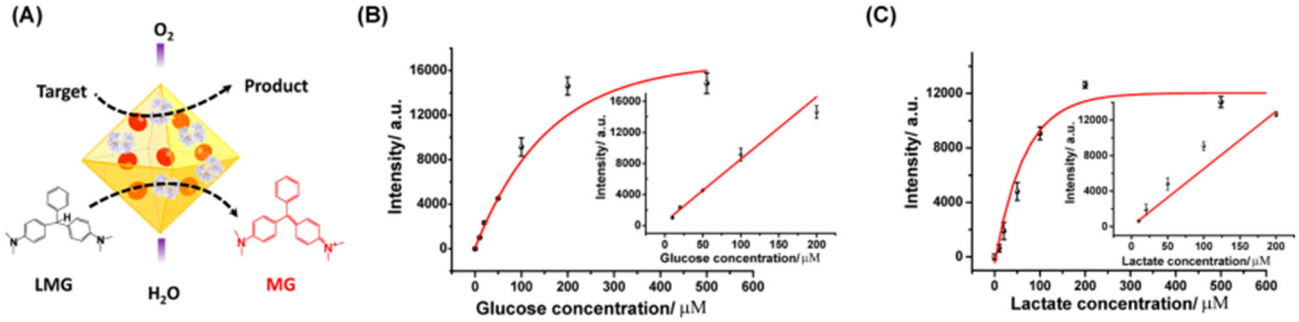

Figure 4. (**A**) Schematic illustration of in vitro detection of glucose (or lactate). (**B**) Plots of Raman intensity of malachite green at 1615 cm^{-1} vs. glucose concentrations. Inset: linear response to glucose concentrations. (**C**) Plots of Raman intensity of malachite green at 1615 cm^{-1} vs. lactate concentrations. Inset: linear response to lactate concentrations. Reprinted with permission from [30]. Copyright © 2017 American Chemical Society.

In the last few years, MOF@Au-based nanozymes have been widely explored for biomedical applications [118]. One of the most interesting approaches was proposed by Yanmei Zhang et al., who developed a MOF-MIL-125(Ti)@Au hybrid able to detect a broad range of biomolecules such as cysteine or H_2O_2 and some metallic cations such as Hg^{2+} [31]. HRP-like behavior permitted the evaluation of the presence of the analytes with this colorimetric tool. In addition, another interesting study was carried out by Wen-Chao Hu et al. who reported a 2D-MOF@Au system useful for antibacterial therapy, using its HRP action to generate OH· radicals to fight *Staphylococcus aureus* [32].

Finally, it is important to note that the very high sensitivity of these materials, specifically those with HRP activity, has permitted its wide usage for the development and improvement of chemical sensors [33,34].

3.1.3. Metal-Based Supports

Metals and metallic derived alloys have shown excellent properties in electrochemistry and photochemistry, making them attractive candidates for catalysis and energy conversion [35,36,39,119]. On the other hand, metallic nanoparticles have been mainly used for biological purposes and sensing, providing high sensitivity for the detection of biomarkers [120]. In this context, Jianbo Liu et al. reported using a AuNP@Pt system with HRP and oxidase-like enzymatic behavior as an electronic biosensor for the simultaneous determination of H_2O_2 and glucose [43]. Following this research direction in the sensing applications, Haihang Ye et al. [38] and Zhuangqiang Gao et al. [37] published gold core—metal shell nanoparticles for the determination of biomarkers by enzymatic colorimetry, improving the classic colorimetric ELISA assay (Figure 5).

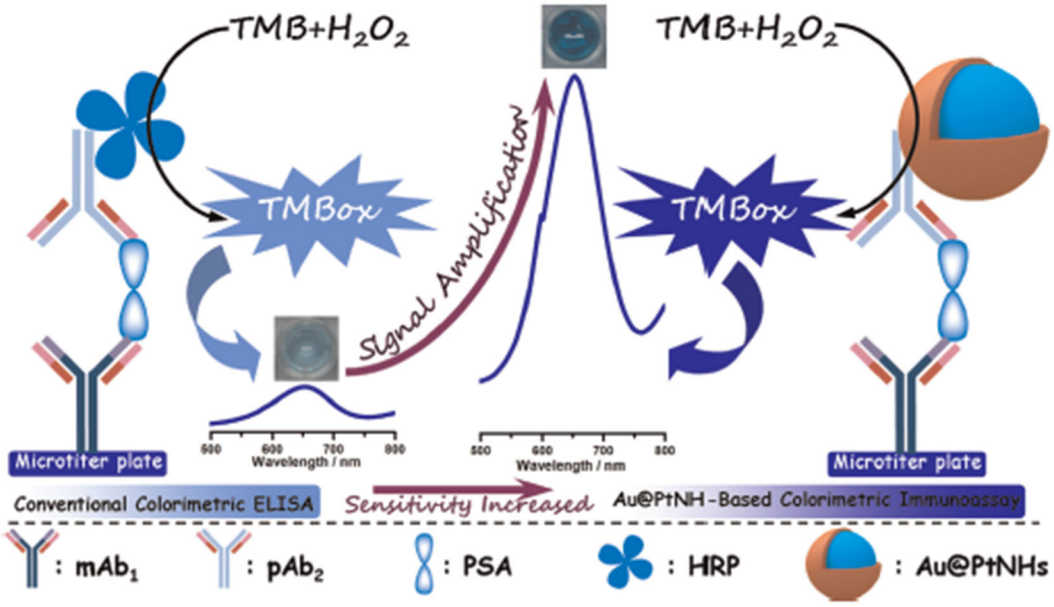

Figure 5. Schematic illustration of conventional colorimetric enzyme-linked immunosorbent assay (left) and Au@Pt nanohybrid-based immunoassay. Reprinted with permission from [37]. Copyright © 2015 Elsevier.

Metal oxide-based nanozymes, in particular, iron and titanium oxides, have shown to be promising materials for sensing applications [40–42,121]. No significant biomedical applications have been reported for gold-core metal hybrid nanozymes to date.

3.2. Organic Hybrids

Organic AuNP hybrids are one of the most employed nanozymatic materials in recent years. The extraordinary variety of organic ligands available makes it necessary to limit the development of this review work to just a few options. Only the versatility of applications of amino acids and the potential to modify the physical properties of hybrid organic polymers will be discussed in this section.

3.2.1. Amino Acids

The use of chemical reducing agents in the presence of surfactants, polymers, or other biomolecules is usually the standard method to obtain metal nanoparticles. However, amino acids (aa) as reducing and functionalizing molecules are becoming an environmentally benign and green alternative for making metal nanoparticles and even functionalize their surface [122–124]. The adsorption of different amino acids on the gold nanoparticle surface depends on the peptide length [125]. Aspartic acid (Asp), lysine (Lys), tryptophan (Trp) or tyrosine (Tyr) present interesting functionals groups along with amine and carboxyl groups, which thus provide an alternative route to synthesize nanoparticles with functionalized surfaces. The reducing ability of histidine from its imidazole group can also lead to gold nanoclusters biocompatible with bio-organisms [126]. Coupled with their photoluminescence properties, this method allows the use of noble metal nanoclusters as biological labels or biosensors. Gold nanoparticle sizes are a critical parameter to determine the affinity of diverse types of amino acids to bind the nanomaterial surface, as supported by recent molecular simulation studies by Qing Shao et al. [127].

For nanozyme-based biosensing applications, current research is mostly oriented towards HRP mimics. One of the main applications of amino acid gold nanozyme materials is focused on ion sensing and detection. The HRP-like catalytic ability of histidine-Au nanoclusters (His@AuNCs) can be inhibited by the addition of Cu^{2+} [44] (Figure 6). In the presence of Cu^{2+}, the enzyme-like activity of His@AuNCs can be efficiently restrained. Upon addition of His, the chelation between Cu^{2+} and the imidazole group of histidine

leads to Cu^{2+} liberation from His@AuNCs, and subsequently results in a dramatic enzyme activity enhancement of His@AuNCs. The ambidentate nature of His triggers the selective recognition of Cu^{2+} to enzyme inhibition of His@AuNCs, being fully reversible by the addition of more His.

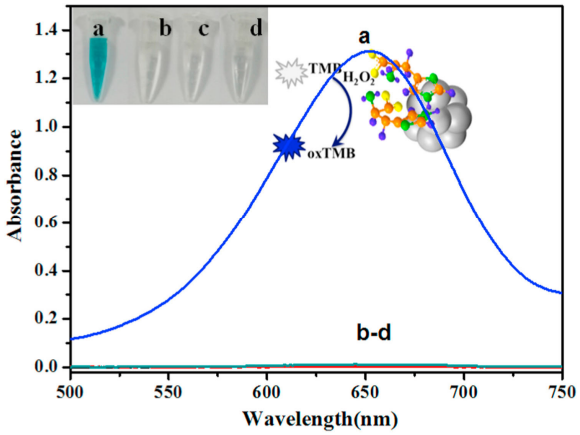

Figure 6. UV–visible absorption spectra of different system: (**a**) TMBHis-AuNC-H_2O_2, (**b**)TMB-H_2O_2, (**c**) TMB-His-AuNC and (**d**) TMB-His-H_2O_2. Reprinted with permission from [44]. Copyright © 2017 Elsevier.

The use of this aa@Au hybrids is not limited to the detection of metal cations. There are also several works where the hybrid nanozyme is sensitive to anions such as nitrites [45]. It is interesting that nitrite inhibits the catalytic and electrocatalytic processes of His@AuNCs/RGO in the oxidation of TMB, and the results shown by the authors indicate that TMB and nitrite may share the same catalytic active sites. The use of His@Au as a sensor has also been extended to the detection of glucose [47] and biological active drugs, such as doxycycline, by colorimetric techniques [46].

In addition to sensing applications, enantioselective nanomaterials can be also produced by the aa@Au combination [128]. In the oxidation of chiral DOPA, the gold nanozyme with D/L-Cysteine (Cys) shows preference over L/D-Dopamine (Dopa) (Figure 7). Molecular simulations showed that the different affinity precipitated by hydrogen bond formation between chiral Cys and Dopa is the origin of the chiral selectivity.

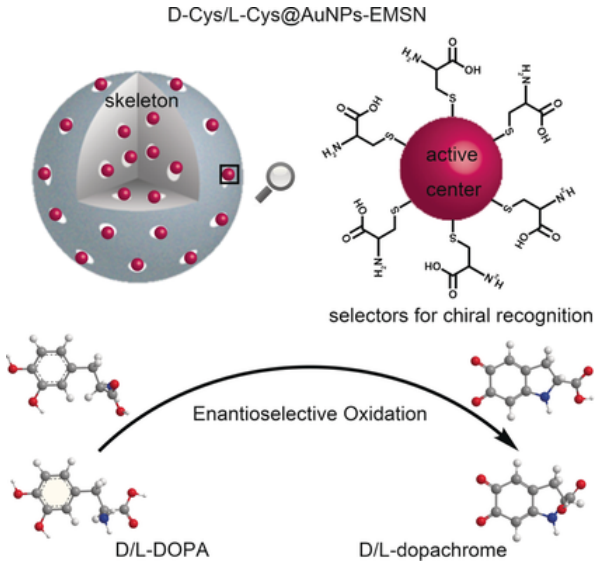

Figure 7. Scheme of the enantioselective D/L-Cys@Au nanozyme. Reprinted with permission from [128]. Copyright © 2020 Wiley.

On the other hand, different uses of amino acid metal nanozymes with biological activities can be found in the literature. For example, a leucine/GO_x/Fe^{2+} material [129] can be internalized by cancer cells and exhibits excellent antibacterial efficiency without additional H_2O_2, which indicates the occurrence of cascade reactions from H_2O_2 generation by glucose oxidation to the production of highly active ·OH via the Fenton reaction.

Focusing on amino acid-gold hybrids, we note that their use in antimicrobial therapy has been spreading in the last decade [130,131]. The combinations between gold nanozymes and peptides have led to biological applications such as the improvement of optical imaging in cancer cells. Accurate cancer cell immunoassays require rational cell-labeling efficiency and HRP-like nanozymes have demonstrated much potential in quantifying tumor cells by aiding its efficient targeting capacity to special antigens or receptors on target cells [132] (Figure 8). With the aid of bioconjugation, peptide gold nanoparticles as nanoprobes can selectively recognize integrin on HEL cell membranes.

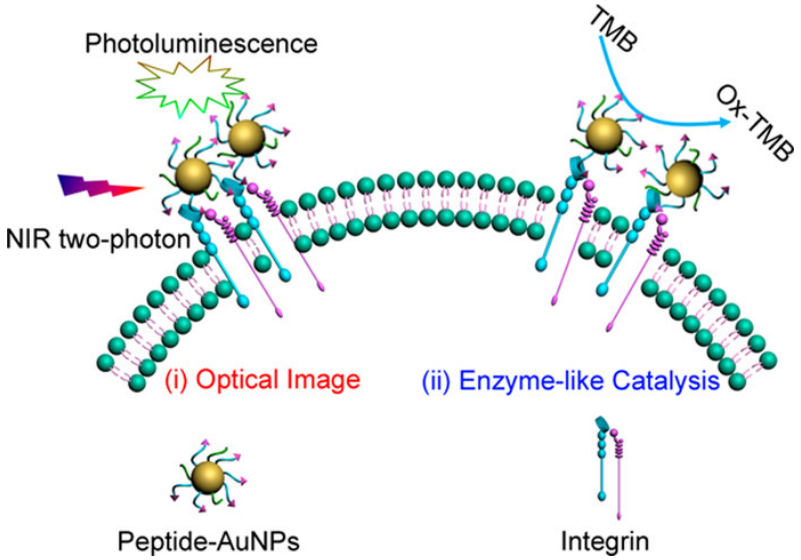

Figure 8. (**i**) Selective recognition-mediated coupling of gold nanoparticles inducing two-photon photoluminescence to display GPIIb/IIIa integrin on the surface of HEL cell. (**ii**) Intrinsic enzyme-like catalysis amplifying signal to sensitively and accurately quantify GPIIb/IIIa expression. Reprinted with permission from [132]. Copyright © 2015 American Chemical Society.

The use of coated gold nanoparticles with peptide chains, mainly with Tyr or Trp residues, as antibiotic molecules has gained interest in the last years, as described by Parvesh Wadhwani et al. [133], who showed that the modification confers stability against trypsin. In addition, Bruno Casciaro et al. [134] proposed the use of an engineered peptide-Au conjugated PEG@Au to enhance the anti-pseudomonal activity of the membrane-active peptide without being toxic to human cells.

3.2.2. Organic Polymers

The use of organic polymers for coating gold nanoclusters and nanoparticles has been very extensive in the last decade. The possibility of modifying the surface of an organic matrix, improving the biocompatibility of the hybrid, and increasing its adherence capability, has led to interesting sensing and biological applications [135].

The synthesis of assembled Au nanorices induced by polyaniline (PANI) led to highly sensitive nanosystems for the detection of H_2O_2 [136]. Owing to their high catalytic activity and unique Surface-enhanced Raman scattering (SERS) properties, the PANI@Au nanorices display promising potential applications in the fields of biocatalysis, disease diagnosis and environmental monitoring. Additional examples can be found with nanorods mimicking

HRP activity as potential tools for H_2O_2 detection [48,49]. Poly(ethylene glycol) and carboxylate coatings on AuNP are also used for the plasmonic detection of proteins [50].

One of the biological applications of polymer gold hybrids which is receiving more attention is their use as nanocarriers for cancer therapy [137]. Nevertheless, the versatility of different biocompatible polymers, such as heparin [138] or hyaluronic acid [54], make these hybrid nanozymatic systems excellent candidates not only as anticancer or sensing agents, but also for many other applications. Moreover, the combination of the above-mentioned polymers to coat/decorate gold nanoparticles has receivedf great attention in the last few years [139]. As an example, a composite integrated by 6-aminopenicillanic acid (APA)-coated-AuNP with fibers of poly(ε-caprolactone) (PCL)/gelatin was developed with good performance against multidrug resistant (MDR) bacteria wound infection, which is a major challenge due to the inability of conventional antibiotics to treat such infections [53] (Figure 9).

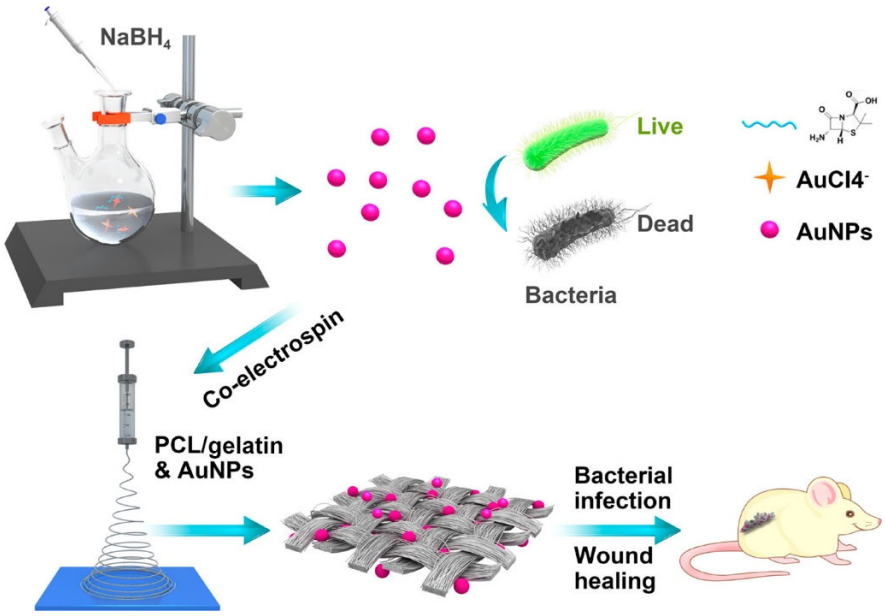

Figure 9. Schematic representation of the synthesis of APA-coated AuNP for wound healing treatment in bacterial infection. Reprinted with permission from [53]. Copyright © 2017 American Chemical Society.

Chang et al. [51] developed recently a highly selective and sensitive colorimetric assay for the monitoring of ciprofloxacin. The resultant AuNPs coated with polyacrylamide (PAM-4) exhibited better HRP-like activity than other PAM ligands with shorter or longer chain in the TMB-H_2O_2 assay.

3.3. Biohybrids

Materials composed of two or more elements, in which at least one is a biomolecule, are known as biohybrids. The study of this type of hybrid compound is currently booming as there are multitude of combinations which can be applied to catalytic, biological or detection processes or even as substitutes for cells in regenerative medicine. The most remarkable feature of protein@Au nanozymes is the utilization of natural entities that ensure biocompatibility of the nanosystems.

3.3.1. Protein

Antibodies

As a consequence of the intrinsic capacity of antibodies to participate in biorecognizing events, most antibody (Ab) Ab@AuNP nanozymes found in the literature are used for sensing purposes.

The use of natural enzymes, such as HRP or alkaline phosphatase, as labelling agents for the preparation of immunoassays is a widespread strategy in the development of sensing platforms. Owing to the progress of nanotechnology and to the intrinsic HRP-like activity of AuNP, several examples can be found in the literature in which gold nanozymes have replaced their natural counterparts in sensing platforms such as enzyme-linked immunosorbent assays (ELISA) [140]. As an example, Goma compared the results using a traditional ELISA and a nano-based ELISA in which Au nano probes substituted the HRP conjugate for the detection of *Trichinella spiralis*; better sensitivity and accuracy were obtained with the nanozyme-based system [55].

Some of the requirements for the design of sensing devices is robustness, speed, simplicity and ease of use. In order to achieve these goals, different strategies can be explored. Modulating the support where capture antibodies are immobilized may facilitate handling, contributing to the obtention of point of care diagnosis tools. Sangjin Oh et al. proposed the use of silica-shelled magnetic nanobeads to develop a nanozyme-linked immunosorbent assay, which provides ultrasensitive detection of *Influenza A* virus [56] (Figure 10). The authors used an immobilized antibody on the positively charged AuNPs via electrostatic attraction, employing also monodispersed Fe_3O_4 nanoclusters (FNCs) capture probes modified with silica shells to prohibit enzyme activity from the surface of iron oxide.

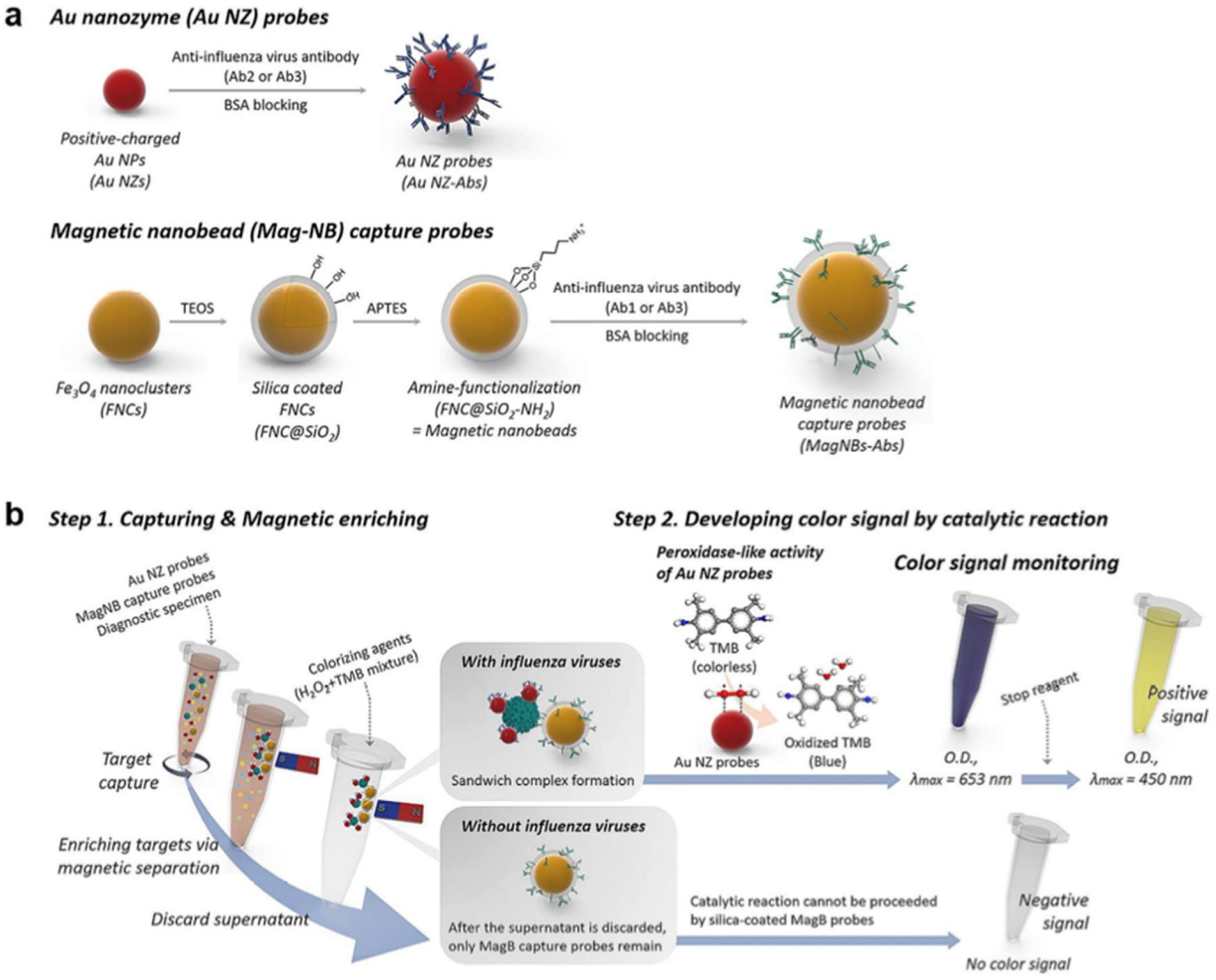

Figure 10. (**a**) Capture probe and Ab-Au nanozyme preparation for a magnetic nanobead-based nanozyme-linked immunosorbent assay (MagLISA). (**b**) Methodology for the determination of *Influenza* virus using the MagLISA strategy. Reprinted with permission from [56]. Copyright © 2018 American Chemical Society.

Cellulose for the development of immunochromatographic strips (ICS) is an interesting support as well. This alternative is simple, cheap, does not either require qualification for the accomplishment of the assay, or a special facility or electricity, and is amenable to mass production. Therefore, some research groups have considered cellulose as support for the capture antibody. Demin Duan et al. proposed a simple nanozyme strip design for the rapid local diagnosis of Ebola [57] (Figure 11).

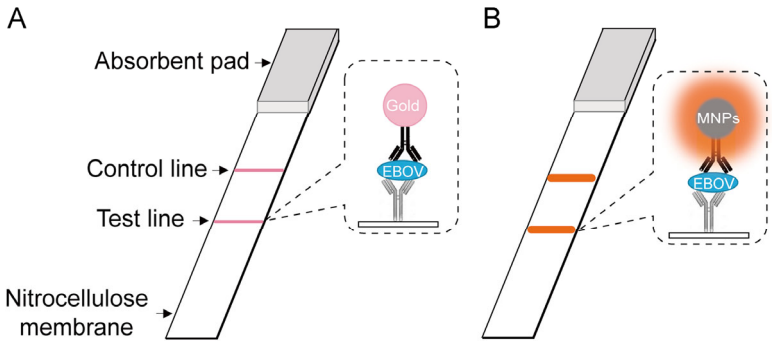

Figure 11. Nanozyme-strip constituents for the rapid diagnosis of Ebola. (**A**) Standard colloidal gold strip. (**B**) Nanozyme gold probe placed in the nanozyme strip. Reprinted with permission from [57]. Copyright © 2015 Elsevier.

The development of naked-eye readout tools also contributes to the massive use of sensors. In this sense, gold-based materials are good candidates since they possess size-dependent optical properties and high extinction coefficients, which permit the detection of bio-recognition events as a change in AuNPs' suspension colour. This alternative to conventional detection techniques such as fluorescence or electrochemical assays is very attractive, since it facilitates detection of biomolecules of interest or pathogens without sophisticated instrumentation. In this context, Qian Zhao et al. developed a sandwich-antigen-antibody structure for an original detection strategy: gold nanoclusters (AuNCs) modifying the outer antigen serve as triggers for the on-site reduction of $HAuCl_4$ into AuNP. This platform was used for the determination of different molecules of biological interest [140] (Figure 12).

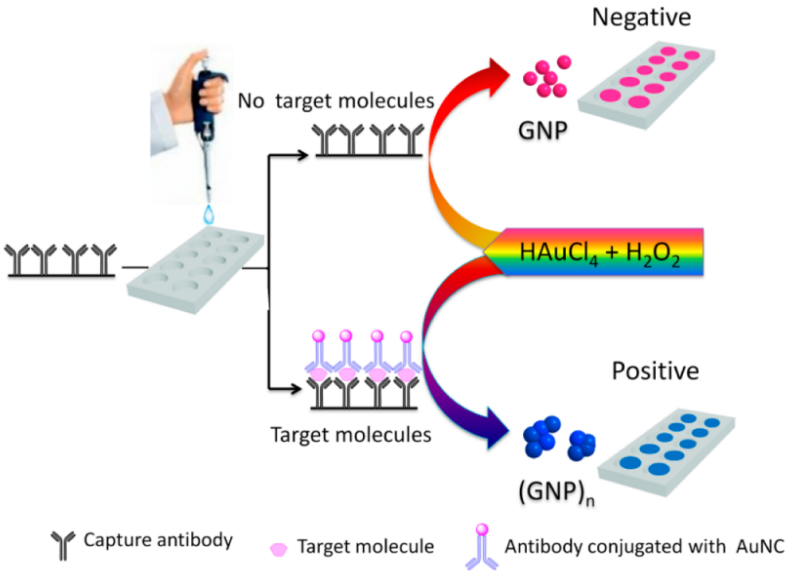

Figure 12. Sandwich-based plasmonic Ab-Au nanozymatic sensor for the determination of different molecules of biological interest. Reprinted with permission from [140]. Copyright © 2016 American Chemical Society.

Ahmed et al. used the same principle for the detection of H5N1 Virus [58] (Figure 13).

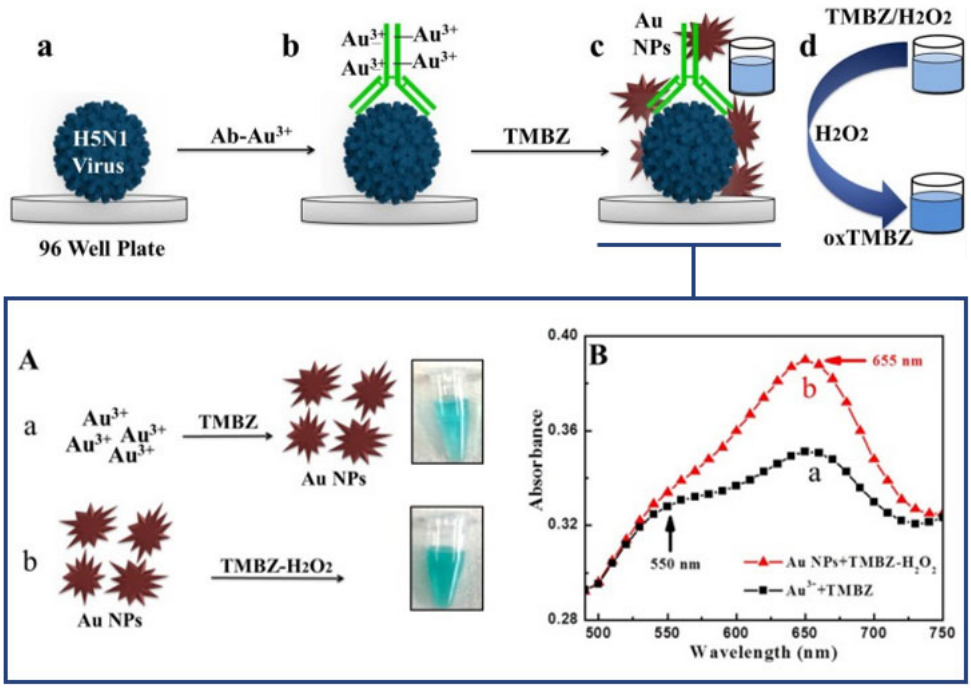

Figure 13. Platform preparation and determination principle for the determination of H5N1. (**a**) Virus deposition; (**b**) Recognition of H5N1 virus by a selective antibody modified with Au ions; (**c**) Au nanostructures formation upon TMBZ addition, resulting in a bluish-green color; (**d**) Color change of the solution as a consequence of TMBZ oxidation upon the presence of H_2O_2. Inset: (**A**) Principle for the appearance of the bluish-green color; and (**B**) resulting UV-Vis spectra. Reprinted with permission from [58]. Copyright © 2017 Nanotheranostics.

Apoferritin

Apoferritin (Ft) is a widely present protein in most organisms, including vertebrates, invertebrates, microorganisms, or plants. It is an essential protein, since one of its main functions is to avoid hazardous accumulation of iron by removing the ion as ferrihydrite phosphate, to be further used as an enzymatic cofactor [141,142]. This spherical protein presents a nanoscale hollow interior which can be used for biotechnological purposes. Many authors have taken advantage of this natural nanocontainer and have used it as a template to synthesize nanomaterials in a limited-growth field, avoiding aggregation and providing homogeneity to the synthetic systems. In particular, the histidine amino acid (one of the six amino acid residues that constitute the ferroxidase centre) permits the strong binding of Au clusters, resulting in an Ft@Au nanozyme which presents HRP activity. In this context, Xin Jiang et al. developed an Ft–Au nanozyme with higher HRP activity than the natural protein which was able to catalyze the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) for the development of a highly sensitive and reproducible glucose sensor [59]. However, Ft@Au nanozymes not only present HRP activity; for example, Fariba Dashtestani, et al. prepared a silver–gold nanohybrid with SOD, catalase and HRP activities, and this hybrid nanozymatic system was used as a ROS scavenger against oxidative damage [59,60] (Figures 14 and 15).

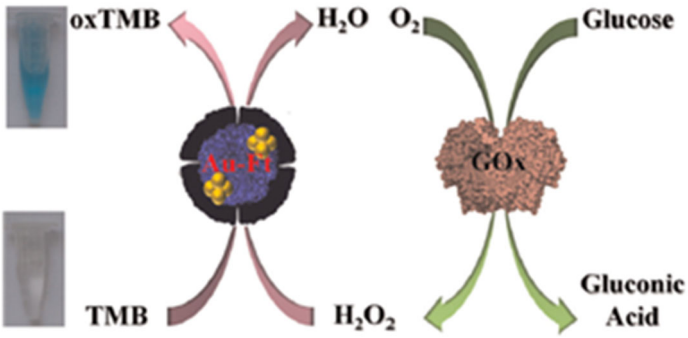

Figure 14. Glucose determination principle using a Ft–Au nanozyme. Reprinted with permission from [59]. Copyright © 2015 Elsevier.

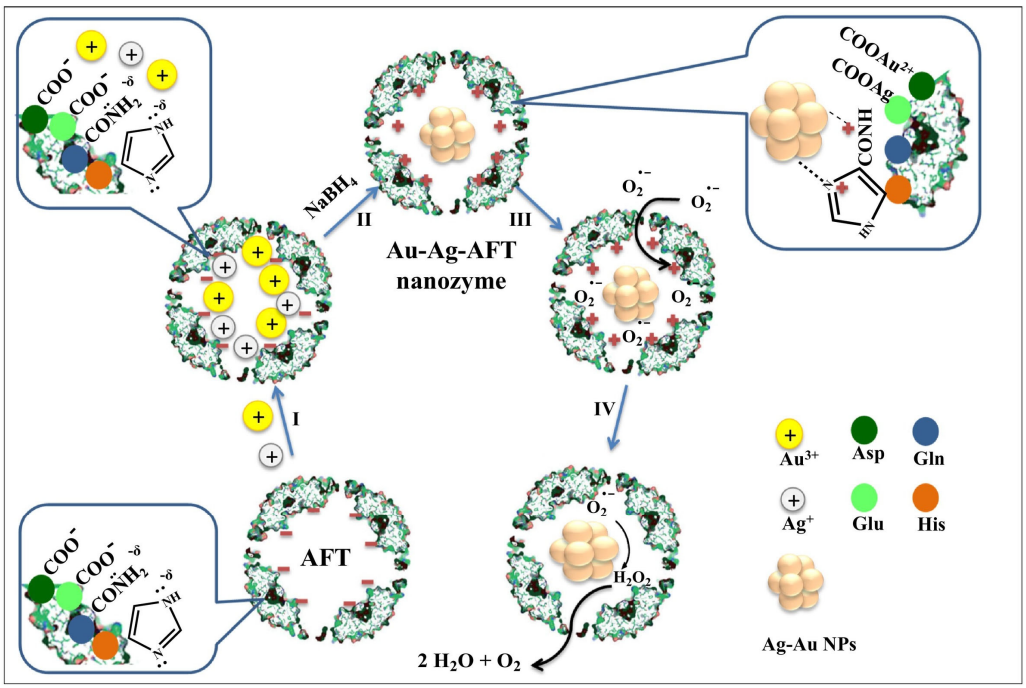

Figure 15. Ft–Au–Ag nanozyme synthesis procedure and $O_2^{\bullet-}$ scavenging and H_2O_2 reduction by the triple enzyme-like activity of the proposed nanozyme. Reprinted with permission from [60]. Copyright © 2019 Elsevier.

Bovine Serum Albumin

Bovine serum albumin (BSA) is one of the most abundant bovine plasma proteins. Owing to its low cost, aqueous solubility, and easy purification [143], it has been widely used for biotechnological purposes, especially for the development of BSA@Au systems, providing biocompatibility, stability and robustness in aqueous environments [115–118].

While some authors suggest that surface modification of AuNP may provoke an inhibition of the nanozymatic activity, Haijiao Zhang et al. proved that Au catalytic activity could be retained in spite of grafting a BSA protein, since dual HRP and GOD-like activity were achieved [63] (Figure 16).

The functionalization of AuNPs capable of mimicking GOD activity with BSA enables an increase of the enzymatic activity, making this biohybrid an interesting option to be used as a sensor for the determination of H_2O_2 and xanthine oxidase (XOD) in urine and human serum samples [62] (Figure 17).

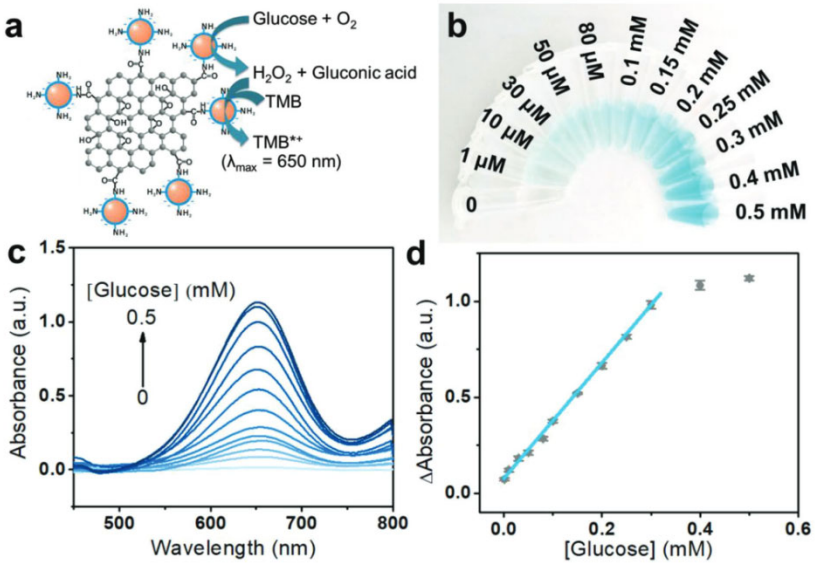

Figure 16. Colorimetric glucose determination by a BSA–Au/graphene oxide composite. (**a**) Working principle of the nanozymatic system that accomplishes TMB oxidation to TMB^{*+}; (**b**) Reaction solutions of different glucose concentration stocks; (**c**) Resulting UV-vis spectra; and (**d**) Linear correlation between absorbance and [Glucose]. Reprinted with permission from [63]. Copyright © 2018 Wiley.

This biohybrid is also useful for the development of a very sensitive method for the determination of tea polyphenols (TP), which has a better performance than the traditional tartaric acid-based determination procedure [61] (Figure 18). The oxidation of TMB by HRP in the presence of H_2O_2 produced by the BSA@Au nanozyme results in a coloured solution, which permits the identification and quantification of relevant components for the food industry such as tyrosol, protocatechuate, chlorogenic acid, theophylline, l-theanine and l-norepinephrine hydrochloride [120].

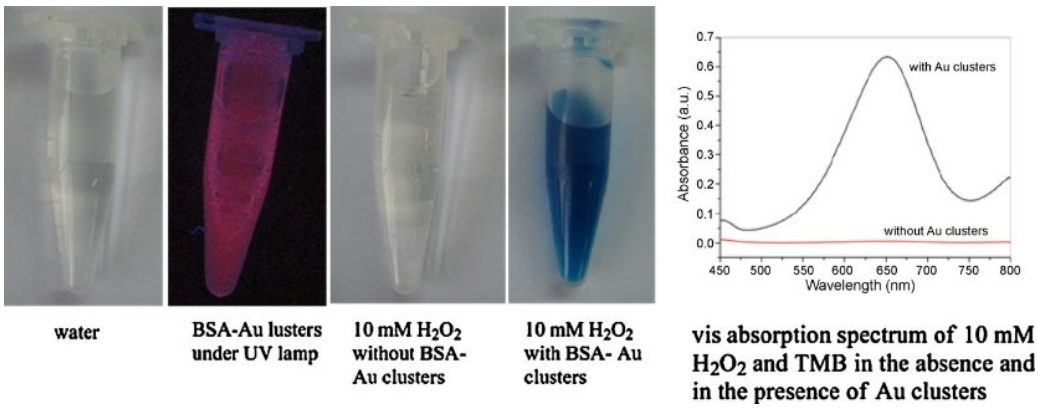

Figure 17. Colorimetric response of BSA–Au clusters under UV radiation and in the presence of H_2O_2. UV–vis spectra of a TMB and H_2O_2 solution in the presence and in the absence of the BSA–Au nanozyme. Reprinted with permission from [62]. Copyright © 2011 Elsevier.

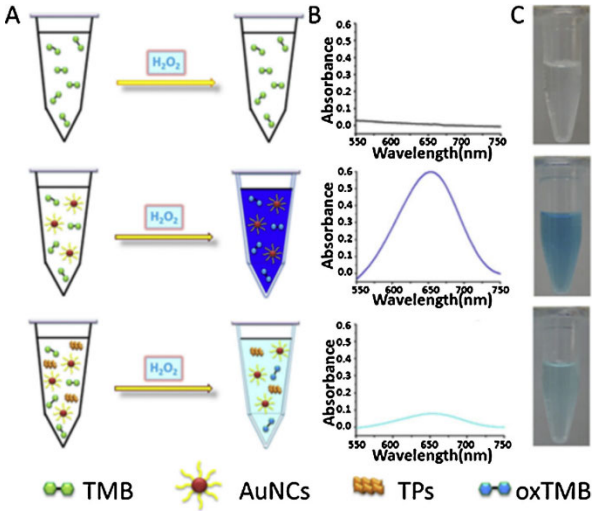

Figure 18. (**A**–**C**) Schematic strategy for the determination of tea polyphenols based on the partial inhibition HRP activity of the BSA–Au nanozymatic system in the presence of tea polyphenols. Reprinted with permission from [61]. Copyright © 2016 Elsevier.

β-Casein

As the most abundant protein in milk, β-Casein (β-Cas) exhibits amphiphilicity in aqueous solution, thus having great capacity for self-assembly into stable micelles. The presence of a high content of acidic amino acid residues, which are negatively charged at neutral pH, causes steric repulsion between polypeptide brushes. These inherent characteristics contribute to the stabilization of the system and improves the affinity of the substrates [144], making this protein a desirable candidate for the preparation of biocompatible β-Cas@Au derivatives. Most β-Cas@Au nanozymes found in the literature are used as biomarker sensors and rely on the same working principle, namely, that β-Cas inhibits intrinsic HRP activity from AuNP and is used as a recognition element for the desired biomarker. As an example, Claire McVey et al. proposed a β-Cas@Au nanozymatic system for the determination of proteolytic biomarkers using TMB as an optical probe [64] (Figure 19).

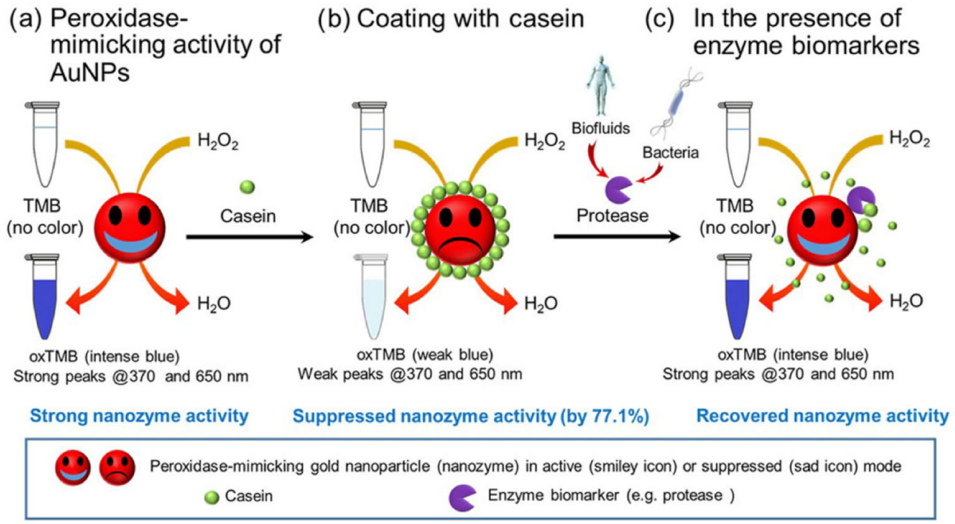

Figure 19. Strategy for the determination of proteolytic biomarkers using the β-Cas@Au nanozyme, based on the partial inhibition of nanozyme activity in the presence of the proteolytic biomarkers. (**a**) Peroxidase-like activity of AuNP; (**b**) Nanozymatic activity inhibition because of casein coating; (**c**) Partial recovery of peroxidase-mimicking activity in the presence of proteolytic biomarkers. Reprinted with permission from [64]. Copyright © 2018 Springer.

3.3.2. Nucleic Acids

Nucleic acids are remarkable biomolecules since they keep and transport genetic information. They are constituted by the polymerization of nucleotides, which are integrated by phosphoric acid, an aldopentose and a nitrogenous base [145]. Although double stranded nucleic acids DNA or RNA are not widely used for the development of Au-based nanozymes, aptamers (which are a special type of nucleic acid) are contributing in a great manner to the development of this technology. Aptamers (Apt) are single-stranded small oligonucleotides (20 to 60 DNA or RNA nucleotides) with high affinity and selectivity for certain molecules to which they can bind.

In this sense, they are like antibodies since they recognize and bind target molecules. Nevertheless, they present some advantages over antibodies, such as lower production cost and time, improved thermal stability due to their chemical synthesis, non-variability between batches, and lower risk of toxicity and immunogenicity since an in vitro screening of the sequence is carried out. For these reasons, they are widely used in biomedicine and sensing [146], and several examples of Apt@Au nanozymes can be found in the literature for the development of sensors that permit the determination of biomolecules or molecules of biological interest. As an example, C-reactive protein, a cardiovascular biomarker associated with the occurrence of cardiovascular events, was determined by Jing Xie et al., who proposed a colorimetric Apt@Au nanozymatic system with HRP activity able to oxidize TMB, replacing the traditional ELISA assay [65].

The oovarian cancer serum biomarker CA125 can also be detected by Apt@Au nanzoymes, as Pranav Tripathi et al. demonstrated with a cost-effective lateral flow assay, with promising applications as a point-of-care device [66] (Figure 20).

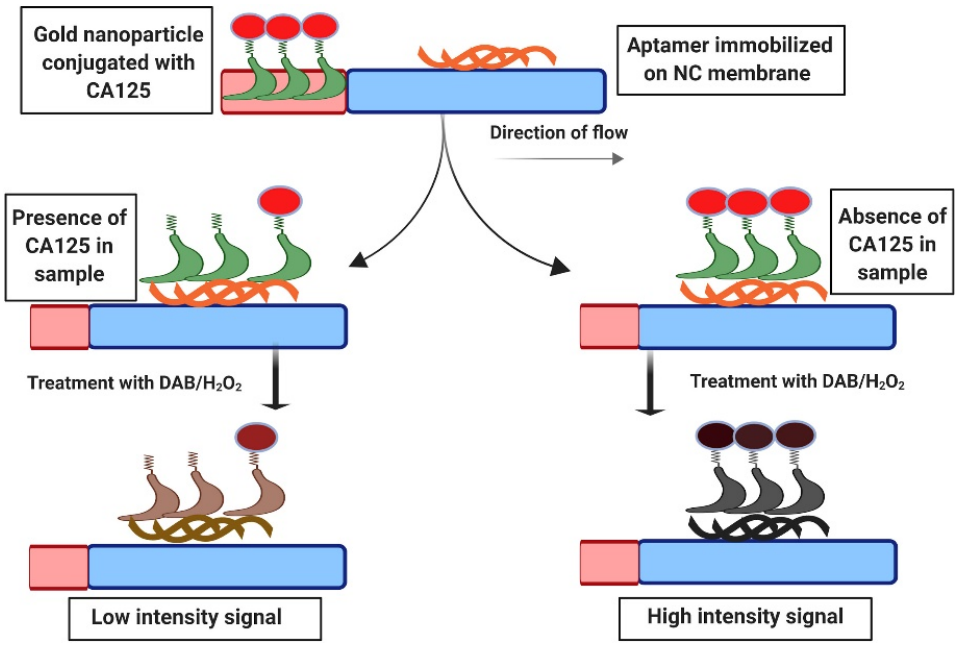

Figure 20. Functioning principle of an Apt–Au nanozyme lateral flow assay for the determination of the CA125 cancer biomarker. Reprinted with permission from [66]. Copyright © 2020 Elsevier.

Besides biomarkers, drugs can also be determined using a nanozymatic strategy based on Apt@Au. Some authors have proposed different strategies for the determination of antibiotics in milk samples; Xuping Zhang et al. developed a sensor using TMB as an optical probe for the determination of ampicillin [67], while Jing Zhao et al. developed a protocol using ABTS as an optical probe for the determination of streptomycin [72]. These examples confirmed that the applicability of these systems is not just limited to biomedical purposes, but they are also useful in other fields like food industry. Hazardous pesticides like acetamiprid, for example, may also be determined using Apt@Au systems. For example,

Pabudi Weerathunge et al. proposed an analogous procedure to the classic enzymatic competitive inhibition process, permitting the rapid determination of this dangerous substance [69].

Not only chemical molecules can be detected with an Apt@Au nanozyme technology; viruses can also be detected. For example, human norovirus (the most frequent cause of viral gastroenteritis) can be tracked, achieving the most sensitive detection of norovirus to date using a biosensing methodology [68] (Figure 21).

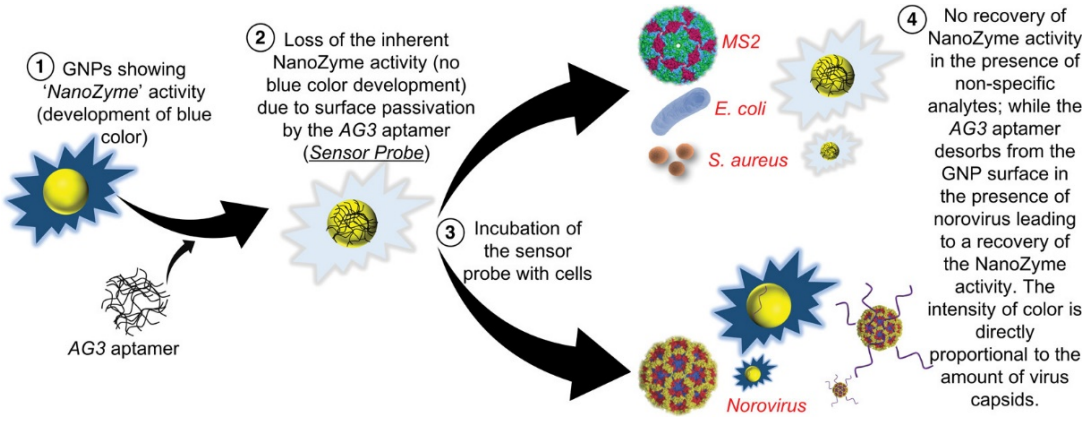

Figure 21. Steps involved in the determination of norovirus using an Apt–Au nanozyme. Reprinted with permission from [68]. Copyright © 2019 American Chemical Society.

3.3.3. Polysaccharides

Despite the enormous variety of polysaccharides that exist in nature, most polysaccharide-based Au nanozymes described in the literature are chitosan-based. Chitosan (Ch) is a positively charged biopolymer obtained by deamination of chitin, which is a fundamental component of crustacean shells' exoskeleton. Its composition (random rearrangement of β-(1–4)-linked D-glucosamine and *N*-acetyl-d-glucosamine) and origin make this material an interesting choice for the development of biotechnological applications, since it is biocompatible, biodegradable, and presents antimicrobial properties [147,148].

Most Ch@Au-based nanozyme systems are used for sensing purposes. Junrong Li et al., who prepared Ch-modified popcorn-like Au–Ag nanoparticles for the detection of melamine in milk powder, used Ch because of its biocompatibility [77] (Figure 22).

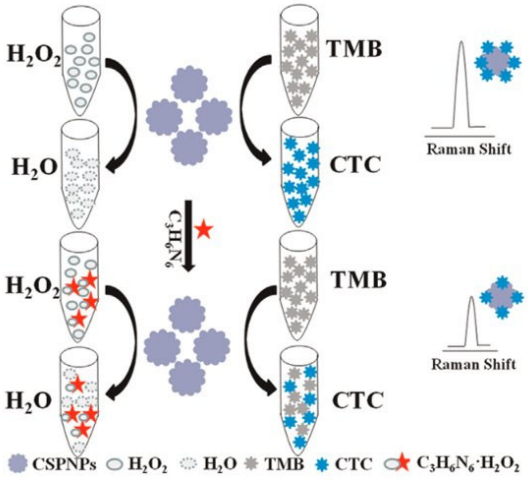

Figure 22. Working mechanism for the determination of melamine in milk powder using a Ch–Au nanozyme with Surface Enhanced Raman Scattering detection (SERS). Reprinted with permission from [77]. Copyright © 2015 Elsevier.

Other authors used chitosan in order to perform a more active role. As an example, Li-Xia Yan et al. prepared mesoporous silica (MS)@AuNP with a chitosan–benzeneboronic acid coating for bacterial targeting, permitting selective imaging and killing of *Helicobacter pylori* [73] (Figure 23).

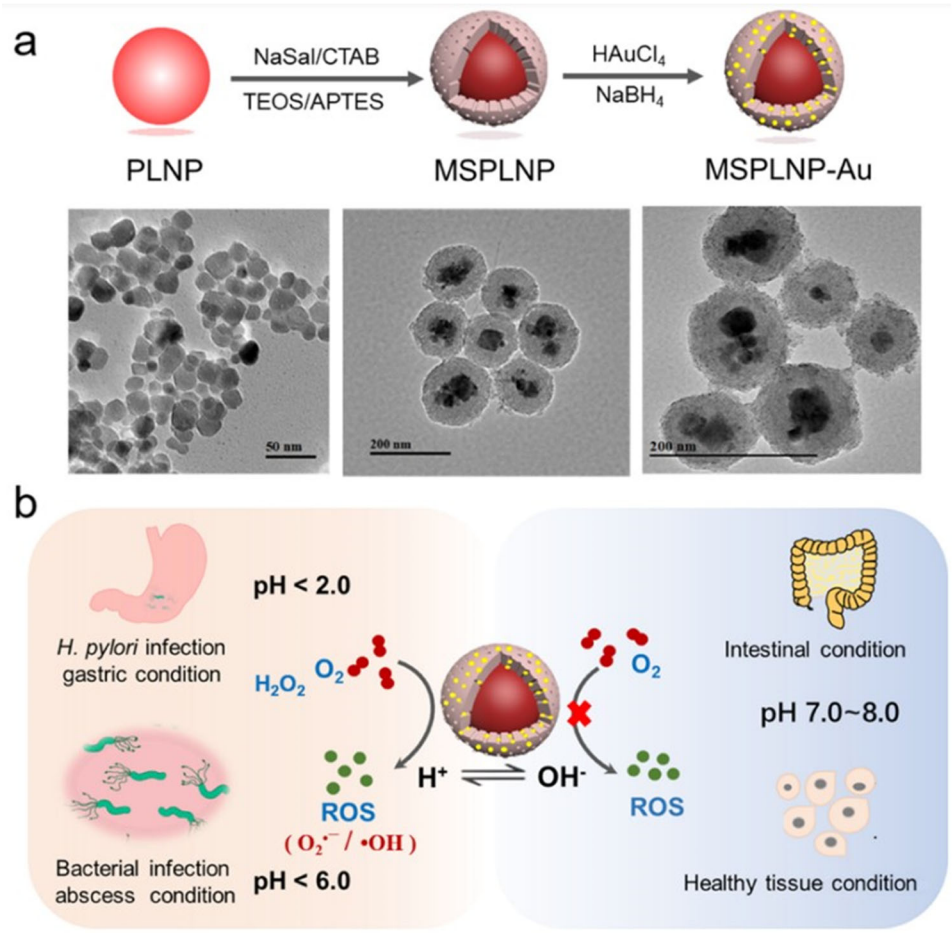

Figure 23. (**a**) Synthesis procedure for the obtention of mesoporous silica (MS) coated with persistent luminescence nanoparticles (MSPLNP) and with Au nanoparticles (AuNP); (**b**) pH responsiveness with OD and HRP-like activity in different biological environments. Reprinted with permission from [73]. Copyright © 2021 American Chemical Society.

Gyubok Lee et al. synthesized a hybrid multimetallic nanozyme, whose HRP activity was improved by the presence of chitosan. The branched morphology of this polysaccharide and its surface charge permitted the uniform distribution of the metal ions [74].

Interestingly, chitosan has also been used in the literature as an Au-reducing agent for the preparation of AuNP. Cuifeng Jiang et al. used this strategy for the synthesis of Ch@AuNP systems, which were applied in the detection of glucose [76]. In a subsequent work, the same group used an identical nanosystem for the determination of Hg^{2+} ions, proving the versatility of Ch@AuNP nanozymes [75].

4. Conclusions

Gold-based nanozymes have proven to be versatile tools for the development of a variety of hybrids with many different applications. From sensing to tumor or infection treatment, catalysis or ROS scavenging are some of the most remarkable fields where gold-based hybrid nanozymes have shown to be valuable instruments. Despite the variety of enzyme-like activities that gold entities can perform, the most remarkable for practical applications are glucose oxidase and mostly HRP-like processes. Tunability of

the enzymatic activity that gold-nanosystems can perform by choosing the appropriate environmental conditions is one of the most interesting features that gold-based nanozymes exhibit in comparison to their natural counterparts. Other remarkable advantages to be highlighted are large-scale production, ease of preparation, recyclability, and high stability. On the contrary, low selectivity towards substrate is one of the most noteworthy limitations of gold-based nanozymes. Nevertheless, selectivity can be ameliorated by gold-surface grafting with adequate molecules.

The number of gold-based nanozymatic hybrids that can be prepared is nearly unlimited, since gold nanoparticles can be combined with almost any nanomaterial. In this context, inorganic@Au hybrids stand out for their excellent chemical stability, while organic@Au hybrids deserve special attention because of the vast number of organic ligands that can be used, widening the number of possibilities imaginable. In addition, the most remarkable feature that biomolecules can provide to Au-biohybrids is the possibility to selectively interact with a specific molecule, and with an excellent biocompatibility. While each family of hybrids can be notable for a certain reason (which is related to the chemical nature of the component of the hybrid), all of them can be applied to similar uses, since the enzyme-like activity originates from the Au constituent.

Nanozymes, therefore, represent an opportunity to face current and future social challenges in fields such as agriculture or energy, that differ from those that have been more extensively explored in recent years (such as sensing, catalysis or biomedicine). Nevertheless, traditional applications may undergo several advances by exploring new technologies too.

Regarding biomedicine, nanozymatic tracking of molecules in the brain or improvement of target therapies by nanozymatic cell vectorization could be future goals capable of being addressed with gold-based nanozymes.

In the case of sensing applications, novel composite hybrids are desirable in order to improve catalytic efficiency, requiring less dosage. Thus, incorporation of biomolecules could also be a solution for future nanozymatic designs that improve signal amplification to ameliorate detection sensitivity. However, the definite step forward would be the incorporation of gold-based nanozymes into micro devices that contributes to advanced lab-on-a-chip technologies and point-of-care testing, democratizing fast, easy and affordable diagnostic tools.

Additionally, there are some examples of multicomponent hybrids that envision a bright future for complex Au-based nanozymes integrated by more than one material. Moreover, the presence of an additional element providing nanozymatic activity would result in advanced systems able to operate in a more autonomous and controlled manner, paving the way for more ambitious applications in several fields of work, not only in catalysis but also in a wide variety of potential therapies.

Author Contributions: S.J.-F. designed the original idea, wrote and reviewed the manuscript. J.M.M.-A. wrote and reviewed the manuscript. V.G.-A. and A.A.G.-V. wrote the manuscript. S.G.-R. designed the original idea and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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