How Do Enzymes "Meet" Nanoparticles and Nanomaterials?

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Abstract

Enzymes are fundamental biological catalysts responsible for biological regulation and metabolism. The opportunity for enzymes to "meet" nanoparticles and nanomaterials is rapidly increasing due to growing demands for applications in nanomaterial design, environmental monitoring, biochemical engineering, and biomedicine. Therefore, understanding the nature of nanomaterial-enzyme interactions is becoming important. Since 2014, enzymes have been used to modify, degrade or make nanoparticles/nanomaterials; while numerous nanoparticles/nanomaterials have been used as materials for enzymatic immobilization and biosensors and as enzyme mimicry. Among the various nanoparticles and nanomaterials, metal nanoparticles and carbon nanomaterials have received extensive attention due to their fascinating properties. This review provides an overview about how enzymes "meet" nanoparticles and nanomaterials.

Focusing on the Interaction of Enzymes with Nanoparticles and Nanomaterials

The rapid development of nanotechnology has significantly increased the opportunity for enzymes to interact with nanoparticles and nanomaterials. Demands to bring nanoparticles, nanomaterials and enzymes together for applications in biomedicine, biochemical engineering, environmental monitoring, nanoparticle design, and biosensor continue to increase. There has been considerable progress in the field of interactions between enzymes and nanoparticles/nanomaterials since 2014, across a wide variety of interactions such as enzymatic modification or degradation of nanoparticles/nanomaterials, and enzymatic immobilization and biosensors with nanoparticles/nanomaterials. During this period, nanoparticles and nanomaterials have been applied to adjust enzyme activity and to affect enzymatic structures and functions. Simultaneously, reports emerged of enzymes that are capable of modifying nanoparticle/nanomaterial properties to develop their assemblies or conjugates. Enzymatic immobilization on nanoparticles or nanomaterials based on rational design and optimization can significantly enhance enzymatic catalytic performance. Incorporation of nanoparticles or nanomaterials generally increases the stability and sensitivity of enzyme-based biosensors, because the nanoparticles or nanomaterials impart their unique intrinsic properties to the biosensors. In addition to these applications of enzyme/nanomaterial interfaces, the potential environmental risks of nanoparticles or nanomaterials have elicited research interest in the enzymatic degradation of nanoparticles and nanomaterials [1, 2]. Natural enzymes present

inherent drawbacks such as easy inactivation and time-consuming extraction [3], so enzyme nanoparticles and enzyme mimics of nanoparticles have been fabricated to overcome these drawbacks. While a number of previous reviews have focused on the protein-nanoparticle interface and interactions, they did not emphasize enzymes and instead treated them as common proteins [4-7]. However, enzymes are different from common proteins, because they are catalysts in living organisms that play very important roles in biological regulation and metabolism. Thus, in this Review we provide a greater insight into the interactions between enzymes and nanoparticles/nanomaterials and associated variations in enzymes, including which enzymes "meet" nanoparticles and nanomaterials; which nanoparticles and nanomaterials "meet" enzymes; and the main pathways through which enzymes "meet" nanoparticles and nanomaterials.

This review discusses important issues related to the interactions of nanoparticles and nanomaterials with enzymes, with special emphasis on papers published in the past three years. Over more than 3 years, more than 25 nanoparticles/nanomaterials have "met" more than 40 enzymes. Information on each of these enzymes was adapted from the well-known BRENDA enzyme database [8] (http://www.brenda-enzymes.org/), and corresponding links are provided in Table 1. Some of these enzymes overlap according to enzymatic definition in Table 1, such as peroxidase, myeloperoxidase and eosinophil peroxidase, where myeloperoxidase and eosinophil peroxidase belong to peroxidase. However, in this Review, they are

considered different to give a faithful account of what enzymes are in contact with nanoparticles or nanomaterials. For organisms that contain these enzymes, only 1-2 examples are provided for each enzyme.

Table 1. Information on the enzymes interacting with nanoparticles or nanomaterials.

Enzyme	Description	Organisms
Lysozyme[1]	A model protein to investigate the interactions between protein and nanoparticles	*Brassica oleracea, Rattus norvegicus*
Laccase[2]	One of ligninolytic enzymes; can oxide lignin, and various organic pollutants such as polycyclic aromatic hydrocarbons (PAHs).	*Trametes versicolor, Rhizoctonia solani*
Haloalkane dehalogenase[3]	Can degrade β-hexachlorocyclohexane and other compounds.	*Aeromicrobium marinum, Mycobacterium smegmatis*
Lignin peroxidase[4]	One of ligninolytic enzymes.	*Phanerochaete chrysosporium, Trametopsis cervina*
Naphthalene 1,2-dioxygenase[5]	Participating in the degrading pathways of some PAHs such as anthracene and naphthalene.	*Escherichia coli, Pseudomonas putida*
Manganese peroxidase[6]	Catalyzing the reaction "2 Mn(II) + 2 H^+ + H_2O_2 = 2 Mn(III) + 2 H_2O".	*Phanerochaete chrysosporium, Trametes hirsuta*
α-chymotrypsin[7]	Carrying out the hydrolysis of protein and polypeptides.	*Homo sapiens, Spodoptera frugiperda*
Protein tyrosine phosphatase[8]	Catalyzing the reaction "[a protein]-tyrosine phosphate + H_2O = [a protein]-tyrosine + phosphate".	*Drosophila melanogaster, Gallus gallus*
Feruloyl esterase[9]	Catalyzing the hydrolysis of ester bonds in plant cell walls into ferulic acid, and has been applied in medicine, paper-pulp and food industries.	*Anaeromyces mucronatus, Aspergillus niger*
Aminoacylase[10]	Also known as N-acyl-L-amino-acid aminohydrolase, catalyzing several reaction types, such as the hydrolysis of amide bond and N-acetylated amino acids.	*Alcaligenes faecalis, Bos taurus,*
Bovine serum amine oxidase	A copper-containing enzyme composed of a homodimer, being involved in the oxidative deamination of primary amines.	*Bos taurus*
Plant esterase	Can be found in numerous plants and is highly sensitive to organophosphates (EC 3.1.1.X).	*Oryza sativa, Triticum aestivum*
β-Galactosidase[11]	Can convert lactose into glucose and galactose.	*Aspergillus aculeatus,*

		Bacillus circulans
Acetylxylan esterase[12]	Catalyzing the deacetylation of xylans and xylo-oligosaccharides.	*Aspergillus ficuum*, *Bacillus pumilus*,
Glucose oxidase[13]	Catalyzing the reaction "β-D-glucose + O_2 = D-glucono-1,5-lactone + H_2O_2".	*Aspergillus* sp., *Penicillium canescens*
Cytochrome *bd* oxidase[14]	Also known as menaquinol oxidase (H+-transporting); Catalyzing the reaction "2 menaquinol + O_2 = 2 menaquinone + 2 H_2O".	*Bacillus subtilis*, *Escherichia coli*
Sulfite oxidase[15]	Catalyzing the reaction "sulfite + O_2 + H_2O = sulfate + H_2O_2".	Homo sapiens, *Bos taurus*
Peroxidase[16]	Catalyzing the reaction "2 phenolic donor + H_2O_2 = 2 phenoxyl radical of the donor + 2 H_2O".	*Allium sativum*, *Homo sapiens*
Lactoperoxidase	A heme-containing peroxidase.	*Homo sapiens*, *Mus musculus*
Myeloperoxidase[17]	Catalyzing the reaction "Cl^- + H_2O_2 + H^+ = HClO + H_2O".	*Bos taurus*, *Homo sapiens*
Eosinophil peroxidase	A heme peroxidase; catalyzing the oxidation of halides.	*Homo sapiens*, *Mus musculus*
Glucose dehydrogenase	Found in *Ewingella americana*; composed of α, β, γ subunits	*Ewingella americana*
Microbial esterase	Presented in various microbes.	*Enterococcus faecalis* V583, *Sinorhizobium meliloti*
Nitrate reductase[18]	Catalyzing the conversion of nitrite to nitrate.	*Rhodotorula glutinis*, *Candida nitratophila*
DNA methyltransferase[19]	An enzyme family that is involved in DNA methylation.	*Homo sapiens*, *Spiroplasma monobiae*
Protein disulfide-isomerase[20]	Responsible for the rearrangement of -S-S- bonds in proteins.	*Homo sapiens*, *Oryctolagus cuniculus*
DNA ligase	Four DNA ligases are presented in BRENDA: DNA ligase (ATP) (EC 6.5.1.1), DNA ligase (NAD+) (EC 6.5.1.2), DNA ligase (ATP or NAD+) (EC 6.5.1.6) and DNA ligase (ATP, ADP or GTP) (EC 6.5.1.7).	*Homo sapiens*, *E. coli*
Lipase	Can hydrolyze fats.	*Candida rugose*
Glycerol-3-phosphate oxidase[21]	Catalyzing the reaction "sn-glycerol 3-phosphate + O_2 = glycerone phosphate + H_2O_2".	*Mycoplasma pneumoniae*, *Pediococcus* sp.

Glycerol kinase[22]	Catalyzing the reaction “ATP + glycerol = ADP + sn-glycerol 3-phosphate”.	*Candida mycoderma, Elizabethkingia meningoseptica*
Cholesterol oxidase[23]	Catalyzing the reaction “cholesterol + O_2 = cholest-5-en-3-one + H_2O_2”.	*Nocardia erythropolis, Schizophyllum commune*
Cholesterol esterase[24]	Also called sterol esterase; catalyzing the reaction “a steryl ester + H_2O = a sterol + a fatty acid”.	*Candida rugosa, Rattus sp.*
Uricase[25]	Also known as urate oxidase or factor-independent urate hydroxylase; catalyzing the reaction “urate + O_2 + H_2O = 5-hydroxyisourate + H_2O_2”.	*Arthrobacter pascens, Bos taurus*
Superoxide dismutase[26]	Catalyzing the reaction “2 superoxide + 2 H^+ = O^2 + H_2O_2”.	*Allium sativum, Bacillus sp.*
Pullulanase[27]	Catalyzing the hydrolysis of O-glycosyl bond.	*Enterobacter aerogenes, Fervidobacterium pennivorans,*
Inulinase[28]	Catalyzing the endohydrolysis of (2->1)-beta-D-fructosidic linkages in inulin.	*Aspergillus fumigatus, Bacillus safensis*
α-amylase[29]	Catalyzing the hydrolysis of alpha bonds from polysaccharides, such as starch.	*Apis mellifera, Bacillus coagulans*
Cellulase[30]	Catalyzing cellulolysis.	*Acetivibrio cellulolyticus, Bacillus mycoides*
α-galactosidase[31]	Catalyzing the decomposition of glycolipids and glycoproteins whose terminal alpha-galactosyl moieties are hydrolyzed.	*Homo sapiens, Mus musculus*
Diamine oxidase[32]	Catalyzing the reaction “histamine + H_2O + O_2 = (imidazol-4-yl)acetaldehyde + NH_3 + H_2O_2”.	*Bos Taurus, Euphorbia characias*
Monoamine oxidase[33]	Catalyzing the reaction “RCH_2NHR' + H_2O + O_2 = RCHO + $R'NH_2$ + H_2O_2”.	*Avena sativa, Macaca fuscata*

Interactions of Enzymes with Nanoparticles or Nanomaterials and Associated Property Changes

Nanoparticles or nanomaterials have been increasingly used for a variety of applications [9-13]. Many of these applications require the participation of enzymes, which results in favorable or unfavorable changes in physicochemical properties of enzymes or nanoparticles/nanomaterials. For example, the presence of enzymes may impair the intrinsic physical properties of single-walled CNTs (SWCNTs) by nonspecific adsorption onto SWCNTs [14]. Proteins or enzymes can bind to the surface of nanoparticles to form the nanoparticle-protein corona which provides a biological identity to the nanoparticles [7, 15-17]. It is critical to find out how structural and functional changes in enzymes are caused by nanoparticles and nanomaterials. In general, enzymatic adsorption onto nanoparticles and nanomaterials will cause conformational transitions, accompanied by the inhibition or enhancement of enzyme activity. A range of nanoparticles and nanomaterials including carbon nanotubes (CNTs), graphene, fullerene derivatives (Table 2) and metal nanoparticles (Table 3), have been demonstrated to have different effects on enzymatic structures or activities. The differing effects depend on the types and orientations of enzymes, physical properties of nanomaterials (e.g., shape and size), chemical groups attached to nanomaterials and environmental conditions [18-21]. Different enzymes have inconsistent amino acid composition and 3D structures, which will lead to different interactions with nanoparticles and nanomaterials. Enzyme orientations towards

nanoparticles and nanomaterials are critical, because improper orientations could make the enzymatic active sites blocked or hindered by nanoparticles/nanomaterials or other adjacent molecules [19]. The physicochemical properties of nanoparticles and nanomaterials, together with the surrounding environmental conditions (e.g., pH and temperature), will affect their binding orientations and interactions with enzymes, stability of enzymes, or substrate accessibility.

The effects of nanoparticles and nanomaterials on the structures and activity of several enzymes have been investigated, including lysozyme, α-chymotrypsin, protein tyrosine phosphatase 1B, laccase, haloalkane dehalogenase, lignin peroxidase, naphthalene 1,2-dioxygenase, manganese peroxidase, horseradish peroxidase, NADPH oxidase and β-galactosidase. The first enzyme we review here is lysozyme, which has been extensively applied as a model enzyme to explore the interactions between nanoparticles/nanomaterials and enzymes. It was reported that lysozymes' adsorption onto SWCNTs was inhibited by arginine, because arginine inhibited the interactions of SWCNTs with its amino acid residues [14]. Du et al. [22] investigated the adsorption of lysozyme onto three functionalized multi-walled CNTs (MWCNTs) that were graphitized, carboxylated or hydroxylated. Hydroxylated MWCNTs had the maximum adsorption capacity, followed by carboxylated and graphitized MWCNTs. Pan et al. [23] studied the structural basis and adsorption of T4 lysozyme onto silica nanoparticles, and significant activity loss was found. α-chymotrypsin is another enzyme that is often used to investigate the effect of nanomaterials on enzymatic

activity. α-chymotrypsin activity can be regulated by carboxylated SWCNTs. Zhao and Zhou [24] found that the adsorption of α-chymotrypsin onto CNTs would slightly lead to changes in the secondary structure of α-chymotrypsin. The authors further pointed out that the binding of α-chymotrypsin to carboxylated CNTs can inhibit enzymatic activity through a competitive-like mode, while the interaction between α-chymotrypsin and pristine CNTs is non-competitive.

In addition to lysozyme and α-chymotrypsin, other enzymes have also contacted nanoparticles or nanomaterials. One study reported that protein tyrosine phosphatase 1B could be inhibited by fullerene derivatives [25]. Chen et al. found that SWCNT led to significant conformational changes in C-terminuses of several microbial enzymes including laccase, haloalkane dehalogenase, lignin peroxidase, naphthalene 1,2-dioxygenase and manganese peroxidase during their applications for oxidation of organic pollutants or lignin model compounds, and some N-terminuses of the enzymes experienced significant conformational dynamics [26]. Interestingly, different graphene-based nanomaterials (graphene, graphene oxide (GO) and reduced graphene oxide (RGO)) exhibited different effects on the activity or stability of horseradish peroxidase (HRP) [27]. Graphene and GO decreased the enzymatic stability, while RGO enhanced the enzymatic stability. All of these graphene-based nanomaterials induced change in secondary structure of HRP.

The effect of TiO_2 on NADPH oxidase has been investigated as well. TiO_2 cannot activate this enzyme without the classical activator, arachidonic acid. However, in the

presence of arachidonic acid, TiO_2 nanoparticles increased NADPH oxidase production of superoxide anion by 140% [28]. The interaction between copper oxide nanoparticles and β-galactosidase was investigated, showing that the conformation and activity of β-galactosidase was disrupted by copper oxide nanoparticles [29].

In short, the interaction patterns of enzymes with nanoparticles and nanomaterials and associated property change have been investigated. The properties (e.g., shape, size and surface chemistry) of nanoparticles and nanomaterials can improve enzymatic performance if rational design is performed, which have attracted researchers' interest in the utilization of nanoparticles and nanomaterials as supports for enzymatic immobilization.

Table 2. Interactions of enzymes with carbon nanomaterials.

Nanomaterial	Enzyme	Reason for their interactions	Reference
SWCNT	Lysozyme	Investigating lysozymes' adsorption onto SWCNTs	[14]
	Laccase	Exploring the impacts of SWCNT on enzyme-catalyzed oxidation processes	[26]
	Haloalkane dehalogenase	Exploring the impacts of SWCNT on enzyme-catalyzed oxidation processes	[26]
	Lignin peroxidase	Exploring the impacts of SWCNT on enzyme-catalyzed oxidation processes	[26]
	Naphthalene 1,2-dioxygenase	Exploring the impacts of SWCNT on enzyme-catalyzed oxidation processes	[26]
	Manganese peroxidase	Exploring the impacts of SWCNT on enzyme-catalyzed oxidation processes	[26]
	α-chymotrypsin	Investigating α-chymotrypsin inhibition by SWCNTs	[24]
	Horseradish peroxidase	Biodegradation of SWCNTs	[30]
	Lactoperoxidase	Biodegradation of SWCNTs	[31]
	Myeloperoxidase	Biodegradation of SWCNTs	[32]
	Eosinophil peroxidase	Biodegradation of SWCNTs	[33]

MWCNT	Lysozyme	Investigating lysozymes' adsorption onto MWCNTs	[22]
	Human monoamine oxidase B	Establishing a monoamine-sensitive electrochemical biosensor	[34]
	Horseradish peroxidase	Biodegradation of MWCNT	[30]
	Laccase	Enzymatic immobilization	[35]
	Cellulase	Enzymatic immobilization	[36]
Fullerene	Protein tyrosine phosphatase 1B	Analyzing the inhibitory mechanism of protein tyrosine phosphatase 1B towards fullerene derivatives.	[25]
Graphene	Diamine oxidase	A histamine biosensor	[37]
	Plant esterase	Biosensor for detection of methyl parathion and malathion	[38]
	Horseradish peroxidase	Investigating the impacts of graphene-based nanomaterials on the activity or stability of horseradish peroxidase	[27]
	α-galactosidase	Enzymatic immobilization	[39]
Graphene oxide	Horseradish peroxidase	Investigating the impacts of graphene-based nanomaterials on the activity or stability of horseradish peroxidase	[27]
	Lipase	Enzymatic immobilization	[40]
Reduced graphene oxide	Glucose oxidase	Enzymatic immobilization and biosensing of glucose	[41]
	Horseradish peroxidase	Investigating the impacts of graphene-based nanomaterials on the	[27]

Enzymatic Immobilization on Nanoparticles or Nanomaterials to Improve Enzymatic Performance

Enzyme-mediated reactions often require suitable environmental conditions. Free enzymes are easily inactivated in practical applications [42]. In addition, reusing them is very difficult [43]. Enzymatic immobilization on nanoparticles or nanomaterials was expected to solve these problems. However, simple immobilization of enzymes may not achieve the desired enhancement of enzymatic activity, as it may lead to deformation of enzymatic structures and activity loss. Thus, the optimal immobilization conditions need to be explored.

Over the past few years, there has been significant interest in magnetic nanoparticles as supports for enzymatic immobilization because they allow the enzymes to be easily collected for recycling by a magnet device. Magnetic Fe_3O_4 nanoparticles have been adopted as support for aminoacylase. Then, the optimum immobilization conditions for aminoacylase on Fe_3O_4 nanoparticles with 3-(aminopropyl)triethoxysilane were demonstrated [44]. A study from Yang et al. [43] presented a nanohybrid composed of glucose oxidase (GOX) on Fe_3O_4@C-silica. The enzyme-material nanohybrid exhibited a good thermal and operational stability, and GOX immobilized on the nanohybrid can increase thermal range of stable enzyme activity and retention of the activity after re-use. He et al. [42] used magnetic Fe_3O_4 nanoparticles as

immobilization support for feruloyl esterases to improve its thermal and operational stability for the application in production of ferulic acid. The immobilized recombinant acetyl xylan esterase (rAXE) on Fe_3O_4 magnetic nanoparticles coated with chitosan showed better performance than free rAXE; specifically retained stability over broader pH and thermal ranges [45].

Gold nanoparticles, another group of extensively used metal nanoparticles, have attracted intense interest for enzyme immobilization due to their unique physicochemical properties (Table 3). Venditti et al. [46] described the development of a bioconjugation composed of gold nanoparticles, poly(3-dimethylammonium-1-propyne hydrochloride) and bovine serum amine oxidase. It was shown that the enzymatic activity was up to 40% onto this bioconjugation relative to the native enzyme activity. Fournier et al. [47] established a setup for the exploration of catalytic activity and inhibition of *E. coli* cytochromes *bd* oxidase by immobilization on the electrodes modified with gold nanoparticles.

Recent research was also focused on enzymatic immobilization onto nano-silica, nano-ZnO, cobalt oxide nanoparticles and CdS nanoparticles. A novel pullulanase was first purified from *Fontibacillus* sp. Strain DSHK 107, and then was immobilized on nano-silica using a glutaraldehyde spacer arm [48]. Immobilized pullulanase showed better thermal stabilization than the free enzyme (15.6- and 16-folds at 35 ºC and 50 ºC, respectively). This immobilized pullulanase also exhibited a shift in the maximum working pH with a decrease from 6.0 to 5.0. Electroactive

nanobiomolecular multilayered architectures have been constructed by the combination of laccase, cytochrome *c* and silica nanoparticles, where they were used as biocatalyst, electron-shuttle and artificial matrix, respectively [49]. Compared to free enzyme, octadecyl substituted nanoporous silica also enhanced the thermostability and reusability of inulinase from *Aspergillus niger* [50]. Similarly, immobilized diastase α-amylase on nano-ZnO had better thermal stability than the free enzyme [51]. *Bacillus subtilis*-templated cobalt oxide nanoparticles was used to immobilize the microbial esterases. After 15 reuses, the enzyme-based nanostructure retained about 85% of the initial activity [52]. Human sulfite oxidase was assembled on indium tix oxide (ITO) electrode modified with polyethylenimine-entrapped CdS nanoparticles, which facilitates the binding of human sulfite oxidase to the electrode and electron transfer [53].

MWCNTs act as supports for the immobilization of laccase and cellulase (Table 2). The efficiency of immobilized laccase [35] and cellulase [36] reaches an optimal level by reasonably setting the enzyme concentrations, pH and temperature, and contact time. Lignin peroxidase activity of enzymatic extracts from *Ganoderma lucidum* and *Pleurotus ostreatus* increased 27- and 18-fold upon immobilization on CNTs, respectively, as compared to the free enzyme [54]. Besides CNTs, functionalized graphene and GO were also used as supports for the immobilization of cicer α-galactosidase [39] and lipase from *Rhizopus oryzae* [40], respectively. Both graphene and GO increased the thermal stability of enzymes.

Enzymes immobilized on a number of nanoparticles and nanomaterials have shown significant enhancement in enzymatic performance, which strongly depends on enzyme types, support materials, and immobilized conditions. The optimal immobilized conditions for enzymes can be found by repeatedly testing under numerous conditions with different values of pH, temperature, enzymes concentrations, immobilized time, and nanoparticle concentrations. Optimized immobilized enzymes on nanoparticles/nanomaterials often showed four advantages than the native enzymes: (1) wider temperature range; (2) broader working pH; (3) greater thermostability; (4) increased reusability. The high surface-to-volume ratio, and chemical, electrical and optical properties of nanoparticles or nanomaterials can increase enzyme loading, and affect the diffusion of immobilized enzymes and catalytic activity [55]. In addition to the utilization for enzymatic immobilization, nanoparticle and nanomaterials are also assembled into biosensors for the detection of various molecules.

Table 3. Enzymes "meet" inorganic nanoparticles.

Nanoparticle/nanomaterial	Enzyme	Reason for their interaction	Reference
Fe_3O_4	Aminoacylase	Enzymatic immobilization	[44]
	Glucose oxidase	Enzymatic immobilization	[43]
	Feruloyl esterases	Enzymatic immobilization	[42]
	Acetyl xylan esterase	Enzymatic immobilization	[45]
	DNA ligase	Biosensor for detection of genomic DNA	[56]
	Peroxidase	Enzyme mimic	[57, 58]
Au	Bovine serum amine oxidase	Development of a bioconjugation	[46]
	Cytochrome bd oxidase	Detecting the inhibitors of cytochrome bd oxidases	[47]
	Glucose oxidase	Glucose biosensor	[59-61]
	Glucose dehydrogenase	Developing a high current and mediatorless bioanode with low redox potential	[62]
	Glutathione-S transferase	Developing glutathione-S transferase immunosensor	[63]
	Horseradish peroxidase	Developing glutathione-S transferase immunosensor	[63]
	DNA methyltransferase	Electrochemiluminescence biosensor for detection of	[64]

		DNA methyltransferase activity	
	Protein disulfide isomerase	Examination of the hydrodynamic dimension of protein disulfide isomerase	[65]
	DNA ligase	Biosensor for detection of genomic DNA	[56]
	Plant esterase	Biosensor for detection of methyl parathion and malathion	[38]
	DNA methyltransferase	Detection of DNA methyltransferase	[66]
	Peroxidase	Detection of influenza virus	[67]
	Horseradish peroxidase	Biosensors for cyanide measurements	[68]
Ag	Glucose oxidase	Enzymatic immobilization and biosensing of glucose	[41]
	Nitrate reductase	Determination of soil nitrates	[69]
AgX (X is Cl, I or Br)	Peroxidase	Enzyme mimic	[70]
SiO_2	Laccase	Constructing electroactive nanobiomolecular multilayered architectures	[49]
	Lysozyme	Investigating lysozymes' adsorption onto silica nanoparticles	[23]
	Pullulanase	Enzymatic immobilization	[48]
	Inulinase	Enzymatic immobilization	[50]

CdS	Sulfite oxidase	Assembling human sulfite oxidase ITO electrode modified with polyethylenimine-entrapped CdS nanoparticles	[53]
	DNA methyltransferase	Electrochemiluminescence biosensor for detection of DNA methyltransferase activity	[64]
Pt	Peroxidase	Detection of nucleic acids	[71]
	Diamine oxidase	Biosensors for histamine detection	[37]
Cobalt oxide	Microbial esterases	Enzymatic immobilization	[52]
Porous nanorods of ceria	Peroxidase	Enzyme mimic	[72]
CuS	DNA methyltransferase	Detection of DNA methyltransferase	[66]
Cu^{2+}–g-C_3N_4	Horseradish peroxidase	Enzyme mimic	[73]
Gly-$Cu(OH)_2$	Superoxide dismutase	Enzyme mimic	[74]
TiO_2	NADPH oxidase	Studying the impacts of tio_2 nanoparticles on NADPH oxidase	[28]
ZnO	α-amylase	Enzymatic immobilization	[51]

Enzyme-based Biosensors with Nanoparticles or Nanomaterials

Electrochemical biosensors based on enzymes are a class of analytical tools to detect a variety of molecules of interest. Enzyme-based biosensors show new properties such as enhanced selectivity, analytical signal and sensitivity after they are modified by

nanoparticles or nanomaterials, due to the intrinsic properties of nanoparticles or nanomaterials (e.g., large surface area, good electrical conductivity, unique optical properties, and nanoscale structures) [75, 76]. Enzyme-based biosensors with nanoparticles or nanomaterials allow rapid, sensitive, in-time and specific detection of compounds, while traditional detection methods such as chromatography are often very difficult for field operations [77] and their testing speed is relatively slow. Several types of nanoparticles/nanomaterials have been selected for the development of biosensors for the detection of target molecules, including gold nanoparticles, silver nanoparticles, carbon nanomaterials, Fe_3O_4 magnetic nanoparticles, platinum particles, and ceria nanospheres. Meanwhile, there have been various enzymes participated in the construction of the biosensors, including GOX, HRP, DNA methyltransferase, protein disulfide isomerase, DNA ligase, organophorous hydrolases, diamine oxidase, human monoamine oxidase B, and nitrate reductase. The reported target molecules for biosensor detection include glucose, cyanide, histamine, pesticides, and enzymes.

There have been several publications focusing on the use of gold nanoparticles for glucose biosensors, which have been applied in the food industry and in bio-detection and biomedicine, and have represented a reliable method for glucose detection. Gold nanoparticles could improve the performance of glucose biosensor comprising GOX and zinc oxide nanoarrays [59]. The surface conditions of gold nanoparticles are critical to conformation and bioactivity of immobilized GOX on gold nanoparticle

surfaces [60]. The direct electrochemistry of GOX was analyzed after it was immobilized on RGO/silver nanocomposite modified electrode, showing good electrocatalytic activity and sensitivity towards glucose [41]. Ratautas et al. [62] explored catalytic oxidation of glucose by glucose dehydrogenase from *Ewingella americana* onto the functionalized gold nanoparticles using 4-mercaptobenzoic acid and 4-aminothiophenol. To obtain a high-performance 3D bio-platform for glucose detection, GOX was attached to gold nanoparticles assembled polyaniline nanowires. The formed entity has good stability and selectivity with a low detection limit of 0.05 μM [61]. Thus, the incorporation of gold nanoparticles can significantly improve the performance of glucose biosensors by surface functionalization, and cooperation with other materials.

In addition to glucose, gold nanoparticles have received considerable attention for the development of biosensors to detect cyanide, enzymes and genomic DNA (Table 3). A biosensor using HRP and a gold sononanoparticle has been developed for cyanide measurements [68]. The preparation of a GST immunosensor using double-layer gold nanoparticles, GST antibody, and HRP was described by Lu et al. [63], showing a minimum detect limit of 0.03 pg/mL. Zhou et al. [64] established an electrochemiluminescence biosensor to detect DNA methyltransferase activity based on CdS quantum dots and gold nanoparticles. Gold nanoparticles could efficiently enhance the electrochemiluminescence of CdS quantum dots. Another group also achieved the convenient detection of DNA methyltransferase by the cation-exchange

reaction of CuS nanoparticles together with the click chemistry of functionalized gold nanoparticles without the need of sophisticated instruments [66]. Zheng et al. [65] combined citrate-capped gold nanoparticles probes and dynamic light scattering to develop a method for the examination of the hydrodynamic dimension of protein disulfide isomerase. The hydrodynamic size of the enzyme was deduced based on the change in the average diameter of gold nanoparticles before and after protein corona formation. An electrochemical biosensor that integrated gold nanoparticles, Fe_3O_4 magnetic nanoparticles, DNA ligase and a modified gold electrode was found able to efficiently detect genomic DNA [56].

A number of organophorous pesticides (e.g., paraoxon, coumaphos and parathion) can be decomposed by organophorous hydrolases. Large-scale use of organophosphate pesticides has led to a considerable concentration of organophosphates in water and soil. Thus, tools to determine organophosphate levels in the environment are needed urgently. Biosensors based on enzymes and nanoparticles are a good method to monitor them, and have been well practiced. Khaksarinejad et al. [78] reported a paraoxon biosensor consisting of organophosphorus hydrolase and silica-coated magnetic nanoparticles with a detection limit of 5×10^{-6} μM. Via the combination of plant esterase, gold nanoparticles, chitosan and graphene nanosheets, a biosensor that can detect methyl parathion and malathion was created, where the nanocomposite comprising chitosan, gold nanoparticles and graphene nanosheets are helpful in the improvement of electron transfer and electric conductivity [38].

The use of nanoparticles and nanomaterials in biosensors has now made it possible to efficiently detect histamine (2-(4-imidazolyl)ethylamine), total monoamines, putrescine (1,4-diaminobutane) and soil nitrate (NO_3^-). Histamine and putrescine are two amines that are the indicators of food quality. Monoamines such as serotonin and dopamine take part in many physiological functions in the human body and are linked to various diseases. As for nitrate, it is a common pollutant in the environment. Detection of these molecules is of great importance, because their determination can help assess food quality, contribute to disease diagnosis, or achieve environmental monitoring. To detect histamine, a histamine biosensor was constructed based on the modified carbon screen-printed electrode by diamine oxidase, graphene, platinum particles and chitosan, which shows low detection limit and good sensitivity [37]. Aigner et al. [34] established a monoamine-sensitive electrochemical biosensor with MWCNTs and human monoamine oxidase B. Diamine oxidase was immobilized on ceria nanospheres to measure the putrescine content in tiger prawn [79]. Nitrate reductase was immobilized on epoxy glued silver nanoparticles, leading to the formation of epoxy/AgNPs/NR conjugates for the quantities of soil nitrate [69]. Although nanoparticles and nanomaterials provide many benefits to biosensor performance for measurement of molecules of interest, their environmental risks should be concerned.

Enzymatic Degradation of Nanoparticles or Nanomaterials

The potential adverse impacts of nanoparticles and nanomaterials on the ecosystem require their removal from the environment when their working life is ended. Enzyme degradation is an environmentally friendly technique to degrade nanoparticles or nanomaterials, and has been an area of interest since numerous studies reported the potential toxicity of nanoparticles and nanomaterials to animals, plants and microbes [1, 21, 80]. Specifically, the enzymatic degradation of CNTs, graphene and their derivatives have been reviewed recently [1]. HRP [30], lactoperoxidase [31], myeloperoxidase [32], eosinophil peroxidase [33], lignin peroxidase [81] and manganese peroxidase [82] can degrade CNTs and their derivatives, while the main enzymes known for the decomposition of graphene and their derivatives are HRP [83], myeloperoxidase [84] and lignin peroxidase [85] (Table 2). Recently, Chen et al. identified the molecular basis of functionalized-triggered SWCNT degradation by HRP and lactoperoxidase [2]. Carboxylation of the substrate resulted in that enzymes bind to substrates more stably, as showed by the enzyme-substrate interaction energies. Different carboxylated SWCNTs caused significantly different variations in cavity volume of SWCNT-degrading enzymes.

Enzyme Nanoparticles

Interestingly, enzymes can appear in the form of nanoparticles where enzyme molecules aggregate at the nanoscale. In this regard, the interactions between nanoparticles and enzymes are internal; that is, the interactions occur between

themselves (Figure 1). To construct a triglyceride bionanosensor, nanoparticles of lipase, glycerol-3-phosphate oxidase and glycerol kinase were prepared separately, and then were immobilized on an Au electrode [86]. Transmission electron microscopy analyses showed that the average size of lipase, glycerol-3-phosphate oxidase and glycerol kinase nanoparticle aggregates was 134 nm, 45 nm and 221 nm, respectively. In another study, the same group also carried out the immobilization of these three types of enzyme nanoparticles (average size: 20 nm) onto a pencil graphite electrode [87]. In addition to the above enzymes, other enzyme nanoparticles were also reported, including cholesterol oxidase, cholesterol esterase [88], and uricase [89].

Noteworthy, available enzyme nanoparticles are often prepared for the construction of biosensors for the determination of molecules of interest, such as triglyceride and uric acid. They have been immobilized onto a variety of electrodes for the improvement of biosensor performance (sensitivity, stability and activity retainment).

Enzyme Mimics of Nanoparticles or Nanomaterials

Biological enzymes have been extensively used for industrial activities and environmental remediation. They are efficient in practical applications, but present inherent defects, such as easy inactivation and denaturation [57]. These defects encouraged researchers to create enzyme mimics or nanozymes that have enzyme-like activity and simultaneously overcome the limitations of biological enzymes. Nanoparticles and nanomaterials have received considerable interest in the

development of "artificial enzymes" with improved properties as compared to biological enzymes (e.g., higher resistance to extreme environmental conditions, good stability, low cost, and easy storage) [90]. In this regard, enzymes "meet" nanoparticles/nanomaterials through the ways that nanoparticles/nanomaterials mimic enzymes and possess catalytic activities. The activity of nanozymes is determined by their own properties, including size, shape, composition, and functionalized molecules [58]. Thus, their activities can be controlled and regulated by designing or changing the properties of nanoparticles or nanomaterials.

The most widely reported nanozymes are peroxidase mimics (Table 3). Polypyrrole nanoparticles present peroxidase-like activity so they can be adopted for the detection of H_2O_2 [3]. Fe_3O_4 nanoparticles were also reported to exhibit peroxidase-like activity through the interconversion of Fe^{2+} and Fe^{3+}. However, the peroxidase-like activity of Fe_3O_4 nanoparticles is limited by multiple factors. Thus, several studies have tried to improve their performance. For example, cubic Fe_3O_4 nanoparticles loaded on supports comprising CNTs and GO nanosheets were found to have improved peroxidase-like catalysis activity and electrocatalytic activities compared to those on CNT supports and free Fe_3O_4 nanoparticles, where GO nanosheets enhanced the dispersion of CNTs and facilitated the loading of cubic Fe_3O_4 nanocatalysts [57]. Attachment of ATP to Fe_3O_4 nanoparticles improved peroxidase-like activity over a wide range of pH values, allowing it to function even in acid pH [58]. Chitosan modified AgX (X is Cl, I or Br) nanoparticles possess peroxidase-like activity in the

presence of H_2O_2 or in the case of photoactivation [70]. Stable peroxidase-like activity of porous nanorods of ceria (PN-Ceria) has been reported [72]. PN-Ceria could retain stable peroxidase activity in a wide range of temperatures and pHs, and has been used as a new diagnostic tool for breast cancer detection. Moreover, many nanoparticles, including platinum nanoparticles [71], gold nanoparticles [67] and carbon nitride nanoparticles modified by Cu^{2+} (Cu^{2+}–g-C_3N_4) [73], also exhibited peroxidase-like activity.

In addition to peroxidase mimics, nanozymes also exhibit other enzyme-like activity. For example, superoxide dismutase (SOD) activity of Gly-$Cu(OH)_2$ was reported [74]. Interestingly, some nanoparticles are able to simultaneously show multi-enzyme activities. Co_3O_4 nanoparticles showed three enzymes-like activities, including peroxidase, catalase and SOD [91]. These three enzymes-like activities were also observed for Prussian blue nanoparticles [92]. Cu^{2+}-functionalized GO nanoparticles exhibited two enzymes' functions, that is, NADH peroxidase and HRP [93].

Concluding Remarks

Here, we reviewed the main pathways by which enzymes "meet" nanoparticles and nanomaterials, including the enzymatic modification of nanoparticles/nanomaterials, enzymatic immobilization and biosensors with nanoparticles or nanomaterials, enzymatic degradation of nanoparticles or nanomaterials, enzyme nanoparticles and enzyme mimicry by nanoparticles or nanomaterials (Figure 2). The physicochemical properties of enzymes (e.g., structure) and nanoparticles/nanomaterials (e.g., shape,

size, chemistry) determine the interactional patterns between them. It should be noted, however, new pathways may appear through the development of bionanotechnology.

The main consequence of interactions between enzymes and nanoparticles/nanomaterials is a structural change in the enzymes, accompanied by a decrease or enhancement of enzymatic activity and stability. Enzymatic immobilization on nanoparticles/nanomaterials can enhance their catalytic performance by rational design and optimization. Their catalytic performance is determined by the properties of both enzymes and nanoparticles/nanomaterials, together with their attached "decoration". Immobilization methods include physical adsorption, covalent modification, and others. Generally, the immobilized enzymes have better thermal stability and reusability, making them able to retain enzymatic activity in a wide range of temperatures and pHs than native enzymes. It is important to remember that, among various nanoparticles/nanomaterials, Fe_3O_4 nanoparticles and gold nanoparticles are two extensively used support materials for enzymatic immobilization. Enzyme-nanoparticle/nanomaterial-based biosensors are another important application of enzyme-nanoparticle/nanomaterial interactions for the detection of numerous molecules. The cooperation between enzymes and nanoparticles/nanomaterials make biosensors endowed with new properties, and thus significantly enhanced the performance of biosensors for the detection of numerous molecules. The negative impacts of nanoparticles and nanomaterials on the environment can be eliminated by enzymatic degradation. Thus, their "meeting" is

"hostile", and is to "kill" one of them. Up to now, there have been limited enzymes available for the degradation of nanoparticles and nanomaterials. The drawbacks of native enzymes have stimulated the emergence of two inventions, enzyme nanoparticles and nanozymes.

A detailed illustration of interactions between enzymes and nanoparticles/nanomaterials will undoubtedly be helpful in understanding the interaction processes, identifying the environmental fate and consequence of nanoparticles and nanomaterials, and better applications of enzyme-nanoparticles for biosensors, other devices or nanocomposites and biocompatible nanoparticle design. In the future, several key issues need to be overcome (see Outstanding Questions):

1. Understanding the impacts of nanoparticle surface properties on the interactions of biological enzymes with nanoparticles or nanomaterials and associated underlying mechanism. Studies are needed to investigated the change of biochemical and biophysical properties in enzymes before and after they are in contact with nanoparticles. As demonstrated above, some studies have applied several enzymes to decorate nanoparticles or nanomaterials. However, the information about the interactions between nanoparticles and enzymes is still very limited. Still, there are lots of problems to solve.

2. Currently, the variety of molecules that can be detected by enzyme-based biosensors with nanoparticles is very limited. Thus, the range of target molecules for biosensor detection needs to be expanded. Moreover, the adopted

nanoparticle/nanomaterials and enzymes for biosensors are also limited, given that the number and types of nanoparticles/nanomaterials and enzymes are very large.

3. In particular, our knowledge on the correlation between nanomaterial/enzyme interactions and health risks is limited. Determining the link between the toxicity of nanoparticles to biology or other effects and interactions between enzymes and nanoparticles/nanomaterials is urgent.

4. Identifying general rules that govern the biocompatible and safe nanoparticles or nanomaterials by enzymes.

5. Constructing algorithms or models for the prediction of behavior and fate of nanoparticles and nanomaterials, by taking into account the available knowledge of interactions of nanoparticle and nanomaterials with enzymes.

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Resources

BRENDA entries for discussed enzymes

[1]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.17

[2]http://www.brenda-enzymes.org/enzyme.php?ecno=1.10.3.2

[3]http://www.brenda-enzymes.org/enzyme.php?ecno=3.8.1.5

[4]http://www.brenda-enzymes.org/enzyme.php?ecno=1.11.1.14

[5]http://www.brenda-enzymes.org/enzyme.php?ecno=1.14.12.12

[6]http://www.brenda-enzymes.org/enzyme.php?ecno=1.11.1.13

[7]http://www.brenda-enzymes.org/enzyme.php?ecno=3.4.21.1

[8]http://www.brenda-enzymes.org/enzyme.php?ecno=3.1.3.48

[9]http://www.brenda-enzymes.org/enzyme.php?ecno=3.1.1.73

[10]http://www.brenda-enzymes.org/enzyme.php?ecno=3.5.1.14

[11]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.23

[12]http://www.brenda-enzymes.org/enzyme.php?ecno=3.1.1.72

[13]http://www.brenda-enzymes.org/enzyme.php?ecno=1.1.3.4

[14]http://www.brenda-enzymes.org/enzyme.php?ecno=1.10.3.12

[15]http://www.brenda-enzymes.org/enzyme.php?ecno=1.8.3.1

[16]http://www.brenda-enzymes.org/enzyme.php?ecno=1.11.1.7

[17]http://www.brenda-enzymes.org/enzyme.php?ecno=1.11.2.2

[18]http://www.brenda-enzymes.org/enzyme.php?ecno=1.7.1.2

[19]http://www.brenda-enzymes.org/enzyme.php?ecno=2.1.1.37

[20]http://www.brenda-enzymes.org/enzyme.php?ecno=5.3.4.1

[21]http://www.brenda-enzymes.org/enzyme.php?ecno=1.1.3.21

[22]http://www.brenda-enzymes.org/enzyme.php?ecno=2.7.1.30

[23]http://www.brenda-enzymes.org/enzyme.php?ecno=1.1.3.6

[24]http://www.brenda-enzymes.org/enzyme.php?ecno=3.1.1.13

[25]http://www.brenda-enzymes.org/enzyme.php?ecno=1.7.3.3

[26]http://www.brenda-enzymes.org/enzyme.php?ecno=1.15.1.1

[27]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.41

[28]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.7

[29]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.1

[30]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.4

[31]http://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.22

[32]http://www.brenda-enzymes.org/enzyme.php?ecno=1.4.3.22

[33]http://www.brenda-enzymes.org/enzyme.php?ecno=1.4.3.4

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Figure legends

Figure 1. Enzyme nanoparticles for lipase, glycerol-3-phosphate oxidase, glycerol kinase, cholesterol oxidase, cholesterol esterase, and uricase.

Figure 2. Main pathways by which enzymes "meet" nanoparticles and nanomaterials. NP, nanoparticle; NM, nanomaterials.