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Review

Biomimetic two-dimensional nanozymes: Synthesis, hybridization, functional tailoring, and biosensor applications

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Biological enzymes play important roles in mediating the biological reactions *in vitro* and *in vivo* due to their high catalytical activity, strong bioactivity, and high specificity, however, they have also some disadvantages such as high cost, low environmental stability, weak reusability, and hard production. To overcome these shortcomings, functional nanomaterials including metallic nanoparticles, single atoms, metal oxide, alloys, and others have been utilized as nanozymes to mimic the properties and functions of natural enzymes. Thanks to the development of the synthesis and applications of two-dimensional (2D) materials, 2D nanomaterials have shown high potential for using as novel nanozymes in biosensing, bioimaging, therapy, logic gates, and environmental remediation due to their unique physical, chemical, biological, and electronic properties. In this work, we summarize recent advance in the preparation, functionalization, as well as biosensor and immunoassay applications of various 2D material-based nanozymes. To achieve this aim, first we demonstrate the preparation strategies of 2D nanozymes such as the chemical reduction, templated synthesis, chemical exfoliation, calcination, electrochemical deposition, hydrothermal synthesis, and many others. Meanwhile, the structure and properties of the created 2D nanozymes by conjugating 2D materials with nanoparticles, metal oxide, biomolecules, polymers, ions, and 2D heteromaterials are introduced and discussed in detail. Then, the applications of the prepared 2D nanozymes for colorimetric, electrochemical, fluorescent, and electrochemiluminescent sensors are demonstrated.

1. Introduction

Natural enzymes play important roles in converting various substrates into products in biochemical reactions due to their high catalytic activity, strong biological activity, and high substrate specificity, making them versatile biomaterials in the applications of biomedicine, tissue engineering, biosensing, energy science, catalysis, and others.[1] Excepting these advantages and wide applications, natural enzymes have suffered from several shortcomings including hard extraction and purification, high cost, low environmental stability, and weak reusability, which have limited their applications in many fields.[2, 3]

With the development of biotechnology and nanotechnology, it has been found that some nanomaterials revealed enzyme-similar properties to that of peroxidase, oxidase, catalase, superoxide dismutase, and many others.[4, 5] Due to the combined properties of both nanomaterials and natural enzymes, those functional nanomaterials with biomimetic enzymatic properties have been called as "Nanozymes" by Wei and Wang firstly.[6, 7] A lot of nanozymes such as metal nanoparticles (NPs),[8] single atoms,[9] metal oxide,[10] alloys,[11] carbon materials,[12] and nanoscale hybrids[13] have been synthesized and served as nanozymes, which showed the advantages of similar catalytic activity, lower cost, higher stability, longer durability, and easier production compared to natural enzymes,[14] and therefore exhibited high potential in the applications of sensing, imaging, therapy, logic gates, environmental remediation, and others.[15, 16]

Two-dimensional (2D) nanomaterials such as graphene, metal nanosheets, transition metal oxide/dichalcogenide (TMO/TMD) nanosheets, MXene, and metal-organic frameworks (MOFs) have attracted remarkable attentions in recent years due to their unique physical, chemical, biological, and electronic properties.[17-19] 2D nanomaterials possess high specific surface area, numerous active sites, and good electronic conductivity, revealing great advantages as nanoscale supporting components for the fabrication of various 2D material-based functional nanozymes.[20-25] For instance, Cai *et al.* reported the synthesis of 2D Pd nanosheet-supported gold nanoparticles (AuNPs) with enhanced nanozyme catalysis,[20] Huang *et al.* demonstrated the peroxidase-like nanozymatic activity of layered VS_2 nanosheets,[21] Qin *et al.* presented the preparation of 2D MOFs (M-TCPP(Fe)) for the fabrication of nanozyme sensors,[22] and Kim *et al.* explored the fabrication of F-N single site-embedded graphene nanozymes with improved catalytic activity and sensitivity.[25] All these 2D material-based nanozymes have shown promising applications in highly sensitive and selective sensing towards various analytes.

Compared to natural enzymes, 2D nanozymes are easier to synthesise and have lower cost. In addition, 2D nanozymes can be used in some other non-biological systems, such as in the

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environments of high temperature, high pressure, low/high pH, and organic solvents. Meanwhile, 2D nanozymes reveal a few advantages than other nanomaterial-based nanozymes. For instance, 2D nanozymes have greater compelling electronic properties, higher specific surface area, more exposed active sites for catalytic reactions, and improved catalytic actives and functions due to the synergistic effects of 2D nanomaterials and other conjugated nanoscale building blocks.

As a lot of studies on the synthesis of 2D material-based nanozymes for sensors and biosensors have been reported, it is realized that a summary on recent advance in the synthesis and biosensor applications of 2D nanozymes is necessary to fill in the space in this important research field, however, to the best of our knowledge until now corresponding efforts have not been performed. Therefore, in this work we present recent progress of the synthesis of functional nanozymes based on various 2D materials, such as graphene, TMD, TMO, graphic carbon nitride, MOFs, and others by using their biomimetic enzymatic activities, as indicated in **Fig. 1**. We hope that the introduction and discussions on the advanced structures, functions, properties, and biosensor applications of 2D nanozymes will be helpful for readers to understand the main innovations and advantages of 2D materials for expanding and inspiring the applications of nanozymes in various research fields.

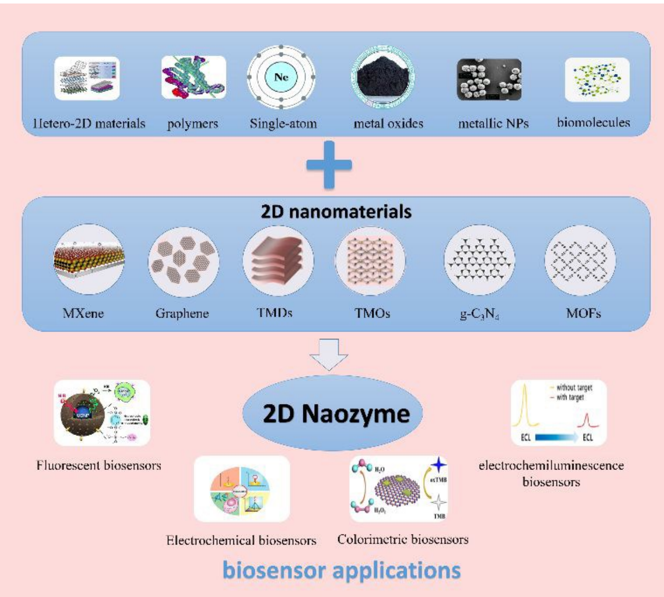

Fig. 1 Schematic presentation of 2D material-based nanozymes and complex nanozymes with biomimetic enzymatic activities.

2. How to prepare biomimetic 2D nanozymes?

Many studies have confirmed 2D nanozymes had unique enzyme-like properties for biological applications. Meanwhile, 2D nanomaterials are also excellent carriers for incorporating with metals, metal oxides, biomolecules, polymers, ions, and other 2D materials to improve their performance in catalysis. In this part, we mainly introduce the preparation strategies and properties of 2D nanozymes and complex 2D nanozymes formed by the combination between 2D nanozymes and other functional nanoscale building blocks.

2.1 2D materials as nanozymes

In this section, potential methods, such as exfoliation, hydrothermal/solvothermal reaction, surfactant-assisted synthesis, and wet chemical synthesis, for the preparation of pure 2D materials as nanozymes are presented.

2.1.1. Exfoliation

Exfoliation is a common approach for the preparation of 2D nanomaterials as nanozymes. According to different peeling mechanisms, it can be divided into mechanical exfoliation, liquid exfoliation, intercalation, and chemical exfoliation. In this section, the specific principles of representative exfoliation methods are described. Then, in the process of preparing various 2D nanomaterials as nanozymes, the employed exfoliation methods are summarized briefly.

Mechanical exfoliation refers to a technique that placing the 2D material with layered sheets to be prepared on the transparent adhesive tape and peeling off the bulk material by repeated adhesion, thereby preparing required 2D materials. Geim and co-workers are the first to separate a single graphite atomic layer, i.e., graphene, from block graphite through mechanical exfoliation successfully.[26] The 2D material prepared by mechanical exfoliation has the advantages of flat surface and little defects, whereas it has low production efficiency and cannot achieve in large-scale production. Generally, the production of graphene by stripping graphite can be divided into two mechanical pathways, namely the normal force and the lateral force,[27] as shown in **Fig. 2a**. High-quality graphene can be produced by controlling the process route and the fragmentation effect during the peeling step (**Fig. 2 b and c**). In addition to graphene, a class of 2D materials with layered structure, such as graphite nitride, TMD, TMO, and metallic organic skeleton have been produced based on this approach.[28]

Liquid exfoliation is a method to produce graphene that was explored primarily by Coleman.[29] Through ultrasonic treatment of graphite in certain liquids, the effect of ultrasonic wave gives rise to peeling off from the graphite crystallite, thereby obtaining graphene nanosheets. To stabilize these nanosheets, solvent interactions and adsorbed surfactants or polymers are often used. This approach provides new ideas for the synthesis of other 2D materials. For example, Zhang *et al.* provided a synthetic pathway of ultra-thin graphite phase C_3N_4 (g-C_3N_4) nanocrystals,[30] which is a green liquid exfoliation method of ultra-thin g-C_3N_4 nanocrystals that prepared from large chunks of g-C_3N_4 in water, as shown in **Fig. 2d**. The transmission electron microscope (TEM) image of the exfoliated individual nanoflakes with a diameter of ~120 nm is

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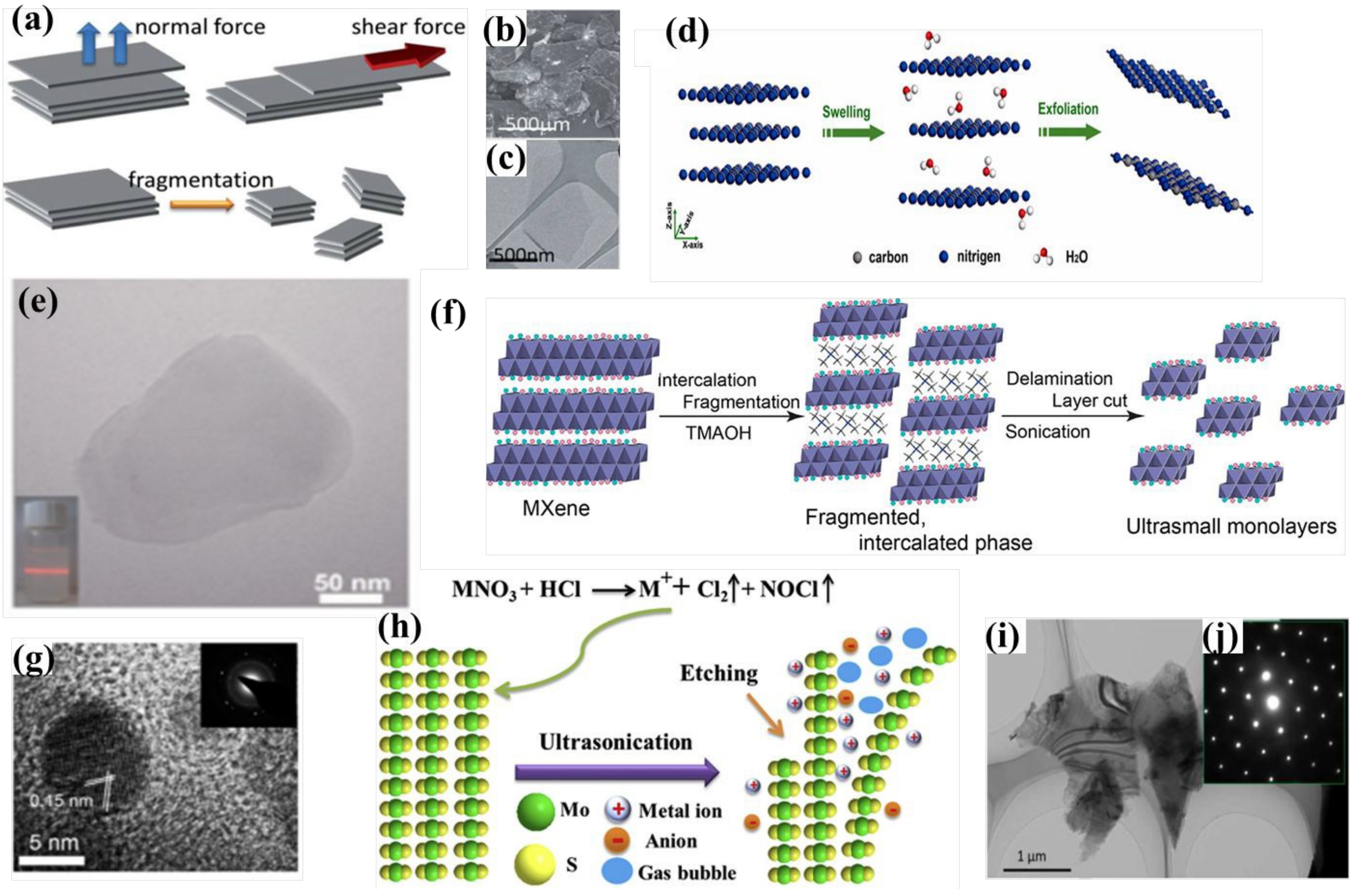

Fig. 2 Synthesized 2D materials as nanozymes by exfoliation. (a-c) Graphene nanosheets prepared by normal and auxiliary exfoliation of flakes: (a) preparation process, (b) SEM image of graphene flakes, and (c) TEM image of exfoliated graphene. Reprinted images with permission from Ref. 27. Copyright 2015, Royal Society of Chemistry. (d,e) g-C_3N_4 nanosheets: (d) liquid exfoliation for synthesizing g-C_3N_4, and (e) TEM image of ultrathin g-C_3N_4. Reprinted images with permission from Ref. 30. Copyright 2013, American Chemical Society. (f,g) Ultrasmall TiC nanosheets synthesized by intercalation stripping: (f) Synthesis process and (g) high-resolution TEM image of TiC nanosheets. Reprinted images with permission from Ref. 37. Copyright 2017, American Chemical Society. (h-j) MoS_2 and WS_2 nanosheets synthesized by chemical exfoliation: (h) Chemical exfoliation process, (i) TEM image of WS_2 nanosheets and (j) corresponding SAED pattern. Reprinted images with permission from Ref. 39. Copyright 2017, Elsevier BV.

shown in **Fig. 2e**. In another case, Tian *et al.* synthesized ultra-thin g-C_3N_4 nanosheets through liquid exfoliation, and further confirmed that the as-prepared ultra-thin g-C_3N_4 nanosheets could catalyze the peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB) in the presence of the hydrogen peroxide (H_2O_2).[31]

Besides, 2D MOF nanosheets can also be prepared through liquid exfoliation in a variety of solvents,[32-34] but the method was limited by low yield of nanosheets. Recently, Coleman *et al.* reported a surfactant-free liquid exfoliation method for the production of inorganic graphene analogs with several layers of nanosheets that dispersed in various organic solvents.[35] Also, this reported new approach was found to be able to produce TMD materials, including MoS_2 and WS_2. Zhou *et al.* demonstrated a versatile and scalable hybrid solvent strategy for liquid exfoliation of inorganic graphene analogs in volatile solvents, including WS_2, MoS_2, and BN.[36] By selecting the appropriate solvent composition, a highly stable suspension of inorganic graphene analogues can be obtained in a solvent mixture with low boiling points.

The intercalation stripping is a method that has been developed for the synthesis of 2D MXene nanosheets.[37] In this approach, multilayer MXene, i.e., the phase of Al between high concentration HF extraction layers, was used as the starting crystal. Tetramethylammonium (TMA) ions were inserted in the corridor space, and the host layer was cut into smaller fragments. After ultrasonic processing, these fragments were

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broken in the plane and split vertically, resulting in targeted ultra-thin slices. This mild and efficient synthesis method maintains the chemical structure of the parent phase in the ultrafine sheet. For example, a most studied nanosheet in MXene family, i.e., Ti_3C_2 nanosheet, has been synthesized through the intercalation stripping with a lateral size of 4 nm and an average thickness of 1 nm.[37] The as-prepared ultrafine Ti_3C_2 nanosheets exhibited strong light absorption and excitation emission characteristics. As shown in **Fig. 2f**, the preparation process of the ultra-small Ti_3C_2 monomer layer involves a simple and gentle reaction in aqueous TMAOH, followed by ultrasonic treatment. Meanwhile, this method can also be extended to the synthesis of ultra-small flakes of other members of the family. In another work, Desai and co-workers prepared the subminiature 2D Ti_3C_2 nanosheets by the intercalation stripping method.[38] The high-resolution TEM (HRTEM) image showed clear striations in each nanocrystal, indicating that the resulting ultrafine flakes had a single-crystal characteristic (**Fig. 2g**). The synthesized subminiature Ti_3C_2 nanosheets exhibited good stability, bright blue fluorescence, high quantum yield, satisfied dispersity, and excitation dependent emission spectrum, and therefore have been further applied for the detection of Ag^+ and Mn^{2+}. Here it should be noted that 2D MXene materials such as Ti_3C_2Tx cannot be used directly as nanozymes because of their low enzyme-catalytic activity, however they can serve as good supports to bind with other nanozymes for the formation of hybrid 2D nanozymes with improved enzyme-like performance.

Chemical exfoliation is known as a rapid and efficient method for the preparation of 2D materials, owing to the synergistic effect of surface etching, ion insertion, and bubble eruption. For example, Lin *et al.* prepared high-yield MoS_2 and WS_2 nanocrystals through chemical exfoliation.[39] It has been found that the reaction of HCl and NO_3^- ions in aqueous solution resulted the formation of NOCl and Cl_2, which could be utilized for controlling the formation of high-quality MoS_2 and WS_2 nanosheets through adjusting their concentrations simply. The mechanism of chemical exfoliation is illustrated in **Fig. 2h**. The TEM image of WS_2 nanocrystals is presented in **Fig. 2i**, and the characteristic honeycomb lattice indicated good crystallization of the exfoliated WS_2 nanocrystals (**Fig. 2j**). Moreover, the as-prepared MoS_2 nanocrystals exhibited significant oxygen reduction reaction (ORR) activity and corrosion resistance.

Du and co-workers reported a low-cost and facile chemical exfoliation method for the synthesis of large-size BN nanoparticles, which could be further used for the preparation of BN nanosheets.[40] The reported approach does not require high temperature or vacuum, only wet chemical treatment is needed, therefore the operation is simple and the cost is low. One recent study has verified the production of the g-C_3N_4 nanosheets via the chemical exfoliation method. For instance, Xu *et al.* successfully obtained single-atomic-layer g-C_3N_4 nanosheets simply by chemical exfoliation of bulk g-C_3N_4.[41] Although there were still 40% multilayer nanocrystals in the final product, this was the first attempt to produce single-layer g-C_3N_4 nanocrystals. Compared with block g-C_3N_4, single-layer g-C_3N_4 nanocrystals possess larger specific surface area and greater photoelectric carrier transport advantages. Moreover, the photocatalytic activity and photocurrent of single-layer g-C_3N_4 nanocrystals were significantly improved.

2.1.2. One-step solvothermal/hydrothermal reaction

Hydrothermal synthesis refers to the preparation of a material by employing a tailor-made closed reactor (such as autoclave) with aqueous solutions as the reaction system. By heating and pressurizing the reaction system, object materials can be produced under relatively high temperature and pressure. The principle of solvothermal method is similar to that of hydrothermal method, except that the aqueous solution in hydrothermal method is replaced with organic solvent or non-aqueous solvent. In recent years, the hydro/solvothermal techniques have been widely used for the synthesis of various nanomaterials with high purity and well-developed grain.

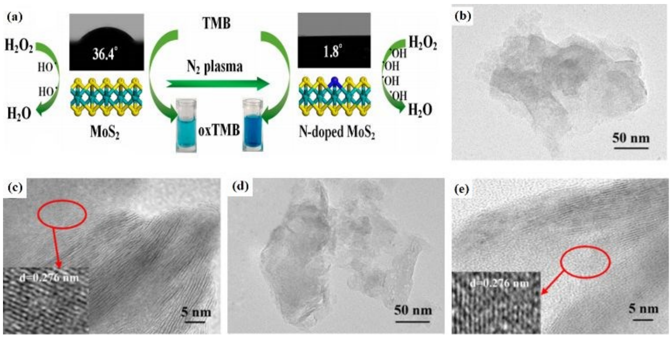

Fig. 3 Hydrothermal synthesis of 2D N-doped MoS_2 nanozymes: (a) Synthesis mechanism and peroxidase-like activity of both MoS_2 and N-doped MoS_2. (b) TEM image of MoS_2, (c) High-resolution TEM image of MoS_2, (d) TEM image of N-doped MoS_2, and (e) High-resolution TEM image of N-doped MoS_2. Reprinted images with permission from Ref. 42. Copyright 2020, American Chemical Society.

Huang *et al.* reported the preparation of layered VS_2 nanosheets, a common TMD material, by using the hydrothermal synthesis method.[21] The VS_2 nanosheets showed intrinsic peroxidase-like activity, which could catalyze the oxidation of substrates in the presence of H_2O_2, yielding colored reaction products. In addition, as a biocatalyst of peroxidase enzyme, VS_2 nanocrystals possess good catalytic performance, stability and reusability, which are comparable to many other peroxidase mimics and natural horseradish peroxidase (HRP).

In addition to traditional hydrothermal synthesis, some other techniques can be further used to enhance the catalytic activity of 2D nanozymes. For instance, Feng and co-workers utilized N_2 plasma to conduct room temperature N doping on MoS_2 to form a novel 2D nanozyme through hydrothermal synthesis.[42] The main synthesis process and principle are shown in **Fig. 3a**. With the prolongation of the treatment period of plasma, the catalytic activity of nanozymes was increased significantly. It was found that by microscopy analysis the N-doped MoS_2 and pristine MoS_2 have similar morphologies (**Fig. 3 b and d**), indicating that the plasma-assisted N-doping was effective without affect the 2D structure of MoS2 nanosheets. In addition, the obtained MoS_2 material showed almost transparent characteristics. The HRTEM images of the detailed structure of the nanosheets are shown in **Fig. 3e** and **e**. Moreover, the XPS analysis indicated that plasma treatment promotes the covalent doping of N element into MoS_2 through the formation of Mo-N bond, thereby N-doped MoS_2 presented higher catalytic stability in water. Here, we suggest that the plasma treatment can greatly improve the surface wettability and substrate affinity of 2D nanozymes, making electrons and reaction substrates readily for acquisition.

2.1.3. Wet chemical synthesis and surface modification

For the preparation of 2D nanomaterials as nanozymes, there are many other methods aside from the afore-mentioned methods. However, these methods are not universal or performs better for the preparation of some specific 2D materials. Here, a brief summary of these methods is given.

Recently, Zhao *et al.* developed a new strategy for preparing 2D MOF (M-TCPP, M=Zn, Cu, Cd, or Co) nanosheets through a surfactant-assisted synthesis, which was effective to achieve mass production of 2D MOF nanosheets with a thickness of around 10 nm.[43] For the first time, they reported surfactant-assisted method by using tetrakis(4-carboxyphenyl) porphyrin (TCPP) to synthesize ultrathin 2D bimetallic MOF nanosheets with a thickness of less than 10 nm. A series of 2D M-TCPP(Fe) nanosheets (M=Co, Cu and Zn) were produced by surfactant-assisted synthesis with three types of metal nodes, such as Co, Cu, and Zn. This novel approach provided alternative way to synthesize ultrathin bimetallic MOF nanosheets with high yield.

In another work, Tang *et al.* proposed a novel one-step wet chemistry method for directed and controlled synthesis of 2D MnO_2 nanosheets.[44] By changing the ratio of bovine serum albumin (BSA) to Mn^{2+}, the size and thickness of the created nanosheets could be adjusted. In addition, 2-S-(4- benzoyl isothiocyanate)-1,4,7-triazacyclic nonane-1,4,7-triacetic acid-modified BSA(BSA-NOTA) was used to modify the surface of the as-prepared MnO_2 nanosheets, allowing the obtained 2D nanosheet materials with high stability and dispersion ability. Through a unique wet chemistry method, 2D ultrathin MnO_2 nanosheets with glucose oxidase (GOx) analogues activity have been synthesized, which were capable of converting glucose and oxygen into gluconic acid and H_2O_2. The designed novel 2D nanozymes could be potentially utilized for the cancer therapy through inducing the starvation of cancer cells by decreasing the glucose concentration level.

2.1.4. Enzyme-like properties of 2D nanozymes

As introduced above, a lot of 2D nanozymes have been synthesized with the mentioned methods, which revealed enzyme-like properties based on the type of applied nanomaterials. According to the catalytic mechanisms and kinetics, nanozymes could be divided to the families of oxidase, peroxidase, catalase, superoxide dismutase, and others.[4, 7] For instance, FeP@C nanosheets have revealed oxidase-like activity,[45] and other 2D nanomaterials such as Co_3O_4 nanoplates and MnFe-layered double hydroxide (LDH) have exhibited catalase-like activity.[46, 47] However, it was found that most of the studies on 2D nanozymes currently have been focused on the utilization of enzyme-like peroxidase and oxidase activities for various applications.

2.2. 2D materials decorated with metallic NPs as nanozymes

2D hybrid nanomaterials as nanozymes have attracted much attention due to their composite properties and enhanced catalytic ability.[48, 49] Because of the unique electronic, optical, and catalytic properties of metal NPs, the hybridization of 2D nanomaterials and metal NPs can improve their properties greatly.[50, 51] In this section, the in-corporation of 2D materials with monometallic and bimetal NPs as nanozymes are introduced.

2.2.1 Hybridization of 2D nanomaterials with monometallic NPs

Metalloporphyrin-based MOF materials can be used as the biomimetic enzymes for peroxidase, but usually only one kind of enzyme can be simulated. How to use MOF hybrid nanomaterials to simulate the cascade reaction of enzymes has not been reported.

Recent studies have confirmed that the combination of 2D MOF nano-flakes with other functional materials can be an effective method for the synthesis of 2D MOF hybrid nanomaterials.[52] Huang *et al.* reported the synthesis of 2D AuNPs/Cu-TCPP(M) hybrid nanosheet by using the 2D copper TCPP(M) (TCPP=tetra(4-carboxyphenyl) porphyrin, M=Fe, Co) nanosheet as the template for the growth of AuNPs,[53] as shown in **Fig. 4a**. 2D water-soluble metal porphyrins were prepared using TCPP(M) as ligand and $Cu_2(COO)_4$ paddle wheel cluster as metal nodes. MOF nanoplates of Cu-TCPP(M) were prepared by growing AuNPs on 2D Cu-TCPP(M) nanoplates. The as-synthesized hybrid nanosheets could be used to detect glucose and other biomolecules, providing a novel strategy for monitoring the cascade reactions by using nanozymes.

2D boron nitride (hBN), with the characteristics of thermal conductivity, high-temperature stability, mechanical resistance, as well as acid and alkali oxidant resistance, could be used as a support for various NPs.[54-57] However, hBN holds an inert surface and lacks interaction with metal precursors, which made it hard to conjugate with metal NPs. A recent study indicated that the liquid-phase stripping can be performed on hBN by ultrasound to produce thin hBN nanocrystalline (hBNNSs) colloidal dispersion, and the produced hBNNSs were adopted to further deposit metals. By this method, hBN nanosheets doped with metal NPs could be obtained. For instance, Ivanova *et al.* synthesized Pt/hBNNS nanocomposites with chemically exfoliated hBN nanosheets modified by platinum (Pt) NPs successfully.[24] Meanwhile, hydrophilic functional groups (mainly -OH) of H_2O_2 were introduced onto the hBN surface, thus promoting hBNNSs hydrocolloids to be spalled into chemically modified hBNNSs. These water-soluble Pt/hBNNSs nanocomposites exhibited good peroxidase-like catalytic activity during the oxidation of TMB (**Fig. 4b**).

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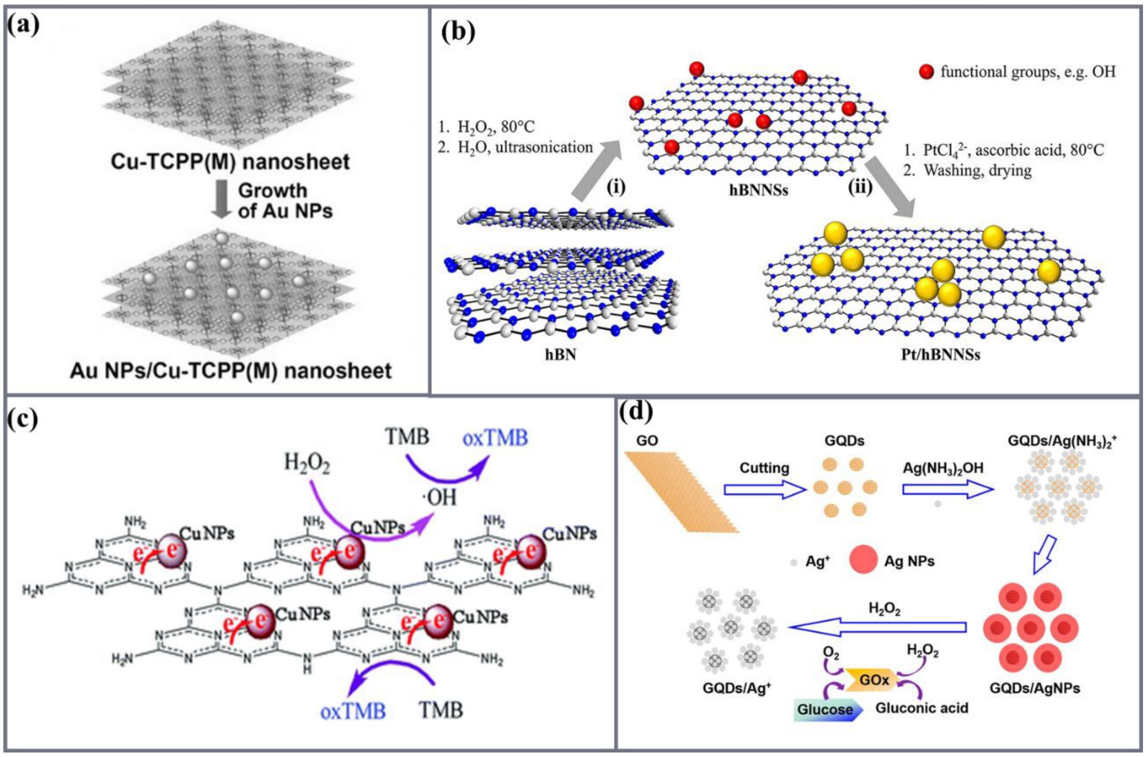

Fig. 4 Synthesis strategies of 2D materials decorated with single metal NPs as nanozymes: (a) MOF/AuNPs. Reprinted image with permission from Ref. 53. Copyright 2017, Wiley. (b) hBN/PtNPs. Reprinted image with permission from Ref. 24. Copyright 2019, American Chemical Society. (c) CuNPs/g-C_3N_4. Reprinted image with permission from Ref. 59. Copyright 2015, Royal Society of Chemistry. (d) GQDs/AgNPs. Reprinted image with permission from Ref. 61. Copyright 2014, American Chemical Society.

2D g-C_3N_4 nanosheets and their nanocomposites holding peroxidase- or oxidase-like activity offer great advantages as compared to other nanomaterials like composite metal NPs. Recently, Wang *et al.* demonstrated the synthesis of g-C_3N_4/PtNPs composites through employing ultrasonic-assisted chemical reduction and using g-C_3N_4 as the stabilizer.[58] Pt ions were chemically reduced to PtNPs, which were deposited on the surface of g-C_3N_4 uniformly, thereby improving the catalytic activity of the formed 2D nanozymes. The prepare g-C_3N_4/PtNPs composites could be further utilized to fabricate colorimetric sensors for Hg^{2+} detection. In another work, Wang and co-workers reported the formation of CuNPs-modified g-C_3N_4 nanosheets by calcining dicyandiamide-Cu^{2+} complexes under humic acid reduction conditions.[59] The as-prepared CuNPs/g-C_3N_4 nanosheets possessed inherent enhanced peroxidase-like activity, which was significantly better than that of pristine CuNPs and g-C_3N_4 for promoting the reaction between H_2O_2 and TMB (**Fig. 4c**). Moreover, due to the synergistic effects of CuNPs and g-C_3N_4, the prepared CuNPs/g-C_3N_4 nanozymes exhibited high sensitivity to H_2O_2 and glucose, revealing potential prospects in biocatalysis and clinical diagnosis.

Silver nanoparticles (AgNPs) have excellent antibacterial properties, while they suffer from easy oxidization and agglomeration. Graphene is known as an excellent carrier, and recent work have demonstrated that the incorporation of graphene with AgNPs improved the stability of AgNPs.[60] Chen *et al.* proposed a facile process for the synthesis of GQDs/AgNPs complex by in-situ growth of AgNPs onto the surface of GQDs (**Fig. 4d**).[61] In this process, GQDs served as an excellent support for allowing the stabilization of GQDs/AgNPs mixture for at least 7 days. Using $Ag(NH_3)_2OH$ as the precursor and GQDs as the substrate, the in-situ growth of AgNPs on the surface of GQDs resulted in the formation of well-dispersed AgNPs with highly improved stability in aqueous media. Consequently, the prepared GQDs/AgNPs nanohybrids exhibited excellent peroxidase-like catalytic activity.

2.2.2 Hybridization of 2D nanomaterials with bimetallic NPs

In previous section, we introduced various examples of 2D nanomaterials that decorated with single metal NPs as functional nanozymes. It has been reported that noble metals, bimetals, and polymetallic nano-alloys exhibited better catalytic performance than single metal due to the synergistic and electronic effects.[62, 63] Therefore, the catalytic activity can be further regulated by adjusting the combination of ingredients. Although the combination of different noble metal NPs contributed to the improvement of the catalytic performance,

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it also induced high cost. Therefore, two strategies can be utilized for reducing the cost and improving the catalytic activity of 2D material/single metal nanozymes. Here we would like to introduce several examples based on the combination of bimetals and 2D nanomaterials, and further discuss the synthetic methods used between them.

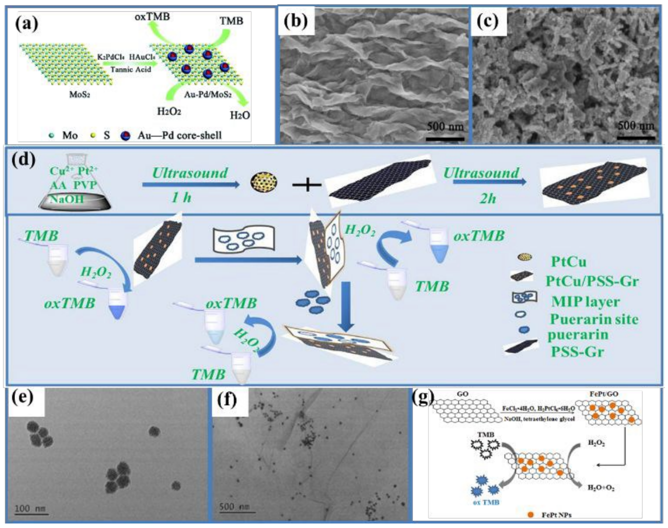

Fig. 5 Synthesis of 2D materials decorated with bimetallic NPs as nanozymes. (a-c) AuPd/MoS_2 with peroxidase-like activity: (a) synthesis process and biomimetic enzymatic activity, (b) SEM of MoS_2, and (c) SEM of AuPd/MoS_2. Reprinted images with permission from Ref. 65. Copyright 2015, Royal Society of Chemistry. (d-f) Synthesis of PtCu/PSS-Gr 2D nanozyme: (d) Synthesis process and enzymatic catalysis mechanism, (e) TEM image of PtCu bimetallic NPs, and (f) TEM image of PtCu/PSS-Gr. Reprinted images with permission from Ref. 66. Copyright 2020, Elsevier BV. (g) Synthesis and biomimetic enzymatic activity of FePt/GO nanocomposites. Reprinted image with permission from Ref. 68. Copyright 2018, Elsevier BV.

One effective strategy to enhance the catalytic activity of 2D nanozymes has been raised by combining 2D materials with non-noble bimetallic NPs metals (such as Cu, Fe, and Ni), thereby reducing cost of the catalysts and making full use of non-noble metals while maintaining their excellent catalytic activity. The introduction of single metal to 2D nanomaterials were found to improve the catalytic activity of corresponding nanozymes. The addition of a second metal to the nanozyme systems can change the electronic and geometric properties of bimetallic NPs, which may further improve the catalytic activity and stability of nanozymes. Previously, Zhong and co-workers reported the synthesis of NiFe alloy-decorated MoS_2 nanosheets with improved oxidation activity to hydrazine through the electrodeposition.[64] However, how to use a simpler method to disperse bimetallic NPs with different heterostructures on MoS_2 has been an open question and challenge. To solve this problem, Sun *et al.* reported a facile co-reduction method for the synthesis of MoS_2-supported AuPd NPs with core-shell structure (AuPd/MoS_2), which could be applied for the oxidation of TMB by adding H_2O_2 (**Fig. 5a**).[65] By using tannic acid to reduce $HAuCl_4$ and K_2PdCl_4 mixture simultaneously, AuPd alloy NPs were successfully formed and dispersed on MoS_2 nanosheets at room temperature. The morphology of MoS_2 nanosheets clearly illustrated that the re-stacked layer of MoS_2 exfoliated with wrinkle characteristics (**Fig. 5b**). The layered MoS_2 served as a support to decorate AuPd NPs, as shown in **Fig. 5c**. The as-prepared AuPd/MoS_2 hybrids exhibited excellent peroxidase-like performance. Besides, the synergistic effect of the added bimetallic AuPd NPs and the MoS_2 support further improved the catalytic activity of the formed AuPd/MoS_2 nanozyme.

The other strategy to improve the catalytic enzymatic activity of 2D nanozymes is to combine noble and non-noble metal NPs together, which can not only make full use of noble metals and exert their catalytic properties, but also further reduce the cost. For example, Guo *et al.* demonstrated the deposition of PtCu bimetallic NPs on poly(styrene sulfonate) (PSS)-modified graphene (PSS-Gr) to form a novel 2D nanozyme with peroxidase-like activity, as shown in **Fig. 5d**.[66] PtCu bimetallic NPs were deposited on the surface of PSS-Gr by a simple deposition method, and the resulting PtCu/PSS-Gr nanocomposite was wrapped on the molecularly imprinted polymer. In addition, a selective analysis was established by means of the high peroxidase-like activity of PtCu/PSS-Gr nanocomposites for the fabrication of highly sensitive sensor of puerarin. The morphology of the PtCu bimetallic NPs was confirmed by TEM image with an average diameter of about 25 nm, as shown in **Fig. 5e**. After being loaded onto the PSS-Gr nanosheet, the PtCu bimetallic NPs were uniformly distributed on the transparent PSS-Gr nanosheets (**Fig. 5f**).

CuPd bimetallic NPs have endogenous peroxidase-like activity and can catalyze the oxidation of TMB and o-phenylenediamine (OPD) in the presence of H_2O_2. Therefore, the deposition of CuPd bimetallic NPs on functionalized graphene could greatly enhance their catalytic activity and promote the applications of 2D nanozymes. Darabdhara *et al.* synthesized CuPd bimetallic NPs on a few 2D nanomaterials, including reduced graphene oxide (rGO), g-C_3N_4, and MoS_2, all of which exhibited peroxidase- and oxidase-like enzymatic behavior.[67]

Unlike the above-mentioned method for synthesizing bimetallic NPs on 2D graphene, Chen and co-workers presented the decoration of GO nanosheets with well-dispersed FePt NPs through s simple polyol method.[68] As shown in **Fig. 5g**, the designed FePt/GO nanocomposites exhibited good affinity and excellent catalytic activity to H_2O_2. Due to the synergistic effect of both FePt NPs and GO nanosheets, the formed 2D hybrid nanozymes have been applied for the fabrication of an ultrasensitive colorimetric sensor of H_2O_2 with a detection limit of 22 μM.

2.3. 2D materials decorated with metal oxides as nanozymes

Besides metallic NPs, metal oxides can be used for the decoration of 2D materials for functional nanozymes with improved performance. Murugan *et al.* for the first time reported the decoration of flower-like MoS_2 nanoflakes with nanoscale CeO_2 ($NCeO_2$) for selective killing cancer cells.[69] The flower-like MoS_2 nanosheets were synthesized by hydrothermal reaction and covalent modification to form S-Mo-S three-layer structure. As indicated in **Fig. 6a**, MoS_2 nanoflake was functionalized with cysteine (Cys) to form MoS_2-Cys, which was then activated with (N-ethyl-N'-(3-(dimethylamino) propyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) to covalently link polyethylenimine (PEI) to form MoS_2-PEI nanohybrids. Finally, $NCeO_2$-PEI-MoS_2 nanoflakes were synthesized through the electrostatic interactions between negative-charged $NCeO_2$ and positive-charged MoS_2-PEI nanohybrids. The introduction of $NCeO_2$ to 2D materials improved the pharmacological properties on the one hand, and on the other hand changed the oxidation state between Ce^{4+} and Ce^{3+} to mimic the properties of biological enzymes. This novel 2D nanozymes could be used for the photothermal therapy of cancers due to their high photostability and biocompatibility.

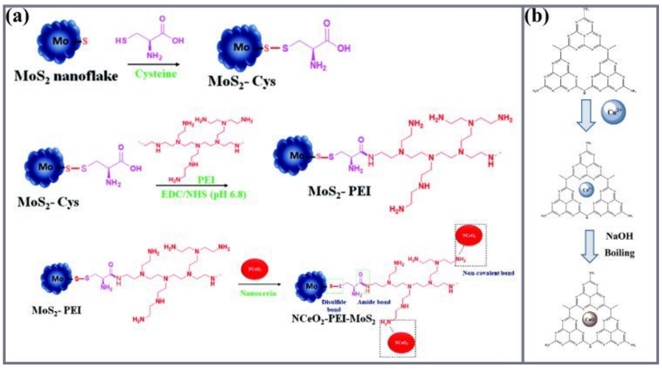

Fig. 6 2D material-metal oxide hybrids as nanozymes: (a) Synthesis process of MoS_2-CeO_2 nanozyme. Reprinted image with permission from Ref. 69. Copyright 2019, Royal Society of Chemistry. (b) Synthesis process of CuO-g-C_3N_4 nanozyme. Reprinted image with permission from Ref. 70. Copyright 2018, Elsevier BV.

The g-C_3N_4 is an excellent support for conjugating metal oxides for forming 2D nanozymes. For example, Zhu *et al.* demonstrated the in-situ synthesis of CuO NPs embedded in g-C_3N_4 for the formation of CuO-g-C_3N_4 nanozymes with HRP-like activity.[70] In the synthesis process, the as-prepared Cu^{2+} and g-C_3N_4 were used as the precursors for the preparation of CuO-g-C_3N_4 nanocomposites, as shown in **Fig. 6b**. To promote their binding, g-C_3N_4 and Cu^{2+} are first mixed and ultrasonically treated in advance. Therefore, through the coordination, Cu^{2+} can be closely embedded onto the surface of g-C_3N_4. As the g-C_3N_4 surface has a variety of chemical groups that can be used to modify additional chemical units, CuO NPs can be synthesized uniformly on g-C_3N_4. The fabricated 2D nanozymes exhibited improved catalytic activity and typical HRP-like activity, and could be used to construct non-enzymatic H_2O_2 electrochemical and colorimetric sensors.

2.4. 2D materials modified with biomolecules as nanozymes

The decoration of 2D materials with biomolecules as nanozymes can enhance the bioactivity and biocompatibility of materials as well as the affinity/specificity to analytes, revealing increasing interest in biomedical applications.

MOF is a porous and crystalline material formed by metal nodes and multidentate ligands, which has been a good nanoscale support for the fabrication of 2D nanozymes.[71, 72] In order to further improve the catalytic performance of MOF nanozymes and expand their bioapplications, MOF materials were further transformed into ultrathin nanosheets to bind biomolecules. Cheng *et al*. tailored the peroxidase-like activity of MOF (TCPP(Fe)) nanosheets by adsorbing Hep-specific AG73 peptides.[73] A series of 2D MOFs that mimic the peroxidase activity have been synthesized based on the TCPP ligand. As shown in **Fig. 7a**, the Hep-specific AG73 peptide, which can recognize the motif, is bound to the MOF nanosheet by physical adsorption, which realizes the specific binding of biomolecules to the 2D MOF nanomaterials. In addition, this binding blocked the non-specific substrate-catalyst interactions on the active sites. Therefore, the designed 2D nanozymes in this way provided a universal *in vivo* diagnostic platform, which could potentially be used for direct detection of many other biological targets besides Hep.

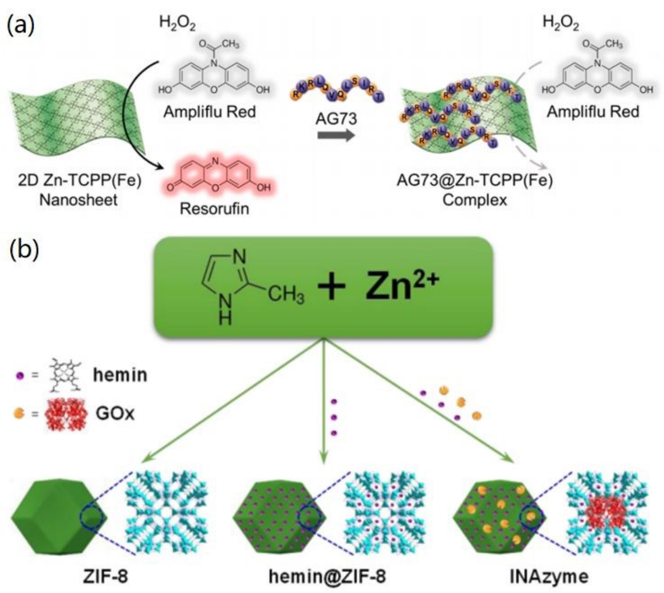

Fig. 7 MOF functionalized with biomolecules as 2D nanozymes: (a) MOF-peptide nanohybrids as nanozyme. Reprinted image with permission from Ref. 73. Copyright 2017, American Chemical Society. (b) MOF-hemin-GOx as nanozyme. Reprinted image with permission from Ref. 75. Copyright 2016, American Chemical Society.

Nanozymes, using nanomaterials to mimic natural enzymes, have better stability under harsh conditions. However, natural enzymes, as a biological molecule in the human body, certainly have great advantages compared to nanozymes. Recently, the design of a high-performance hybrid nanozyme system that combines nanozymes and natural enzymes for cancer diagnosis and treatment has attracted more and more attention.[74] How to control natural enzymes in the body and how to synergistically catalyse nanozymes and natural enzymes have become the focus of this field.

Previously, Cheng and co-workers reported the design of integrated nanozymes (INAzyme) by introducing two cascade catalysts (hemin and GOx) into the MOF (ZIF-8), as indicated in **Fig. 7b**.[75] In the synthesized MOF hybrids, hemin and GOx served as molecular catalyst and a natural enzymatic catalyst, respectively, and therefore the formed 2D nanozymes exhibited great advantages with improved catalytic efficiency through subsequent enzymatic catalysis, which overcame the diffusion-affected kinetic and low stability of products. In another study, Yang *et al.* constructed an efficient biomimetic hybrid nanozyme by the combination of natural enzyme GOx and MnO_2 nanozyme together.[76] It was found that the synthesized GOx-MnO_2 nanozymes could self-supply H^+ and accelerate the production of O_2, thereby alleviating the tumor hypoxia and improving the cancer cell-killing efficiency by the starvation therapy. The uniqueness of this biomimetic hybrid nanozyme lies in the design of a nanosystem that promotes each other in the complex tumor microenvironment and maximizes the enzymatic activity of nanozymes, MnO_2, and GOx.

2.5. 2D materials modified with polymers as nanozymes

Polymer nanozymes have been developed for biosensor and biomedical applications due to their good aqueous dispersion, high stability, and enzyme-mimic activity.[77] Therefore, it is possible to conjugate 2D materials with some polymers together to achieve in enhanced enzymatic catalysis performance.

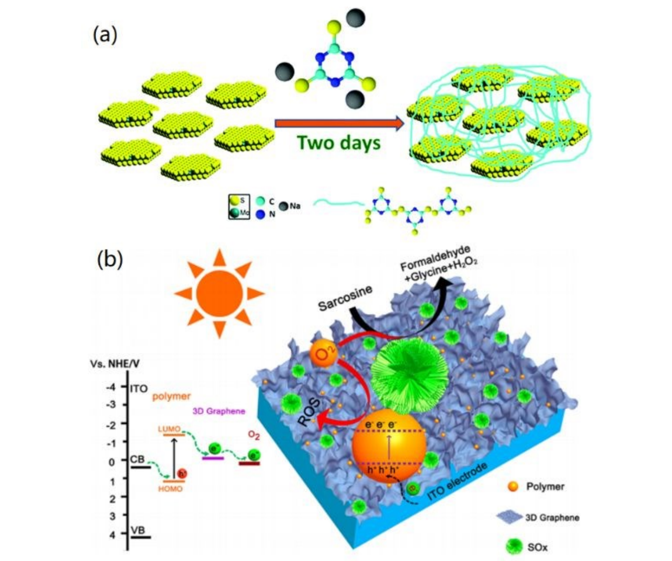

Fig. 8 2D material-polymer hybrids as nanozymes: (a) PTCA-MoS_2 hybrid nanosheets as nanozyme, Reprinted image with permission from Ref. 78. Copyright 2020, Royal Society of Chemistry. (b) 3D structure of polymer/graphene nanozyme. Reprinted image with permission from Ref. 79. Copyright 2018, American Chemical Society.

For instance, Sreeramareddygari and co-workers reported the in-situ polymerization of trithiocyanuric acid (TCA) on MoS_2 nanosheets for the formation of a novel nanozyme with enhanced peroxidase activity.[78] As shown in **Fig. 8a**, the binding of TCA onto the edges of MoS_2 and subsequent polymerization resulted in the formation of PTCA-MoS_2 composites with continuous, porous, polymeric network, which exhibited enhanced peroxidase-like activity compared to native MoS_2 nanosheets. Therefore, the synthesized PTCA-MoS_2 nanozyme could be used for the fabrication of a colorimetric sensor for direct detection of H_2O_2 and indirect detection of glucose, ascribing to their high catalytic activity.

In another study, Shi *et al.* demonstrated the synthesis of 3D networks of semiconducting polymer (SP) and graphene through the hydrothermal and freeze-drying preparation of porous graphene and subsequent binding with SP.[79] The as-prepared SP/graphene hybrids were immobilized onto the indium tin oxide (ITO) substrate for the fabrication of a photocathodic device for sensitive and selective determination of sarcosine after introducing sarcosine oxidase into the nanosystem (**Fig. 8b**). This study proved the potential of SP/graphene hybrid for photocathodic enzymatic bioanalysis in one way, and guided a new horizon for the design of functional platforms for photoelectrochemical applications.

2.6. Hetero-2D materials as nanozymes

2D materials, such as g-C_3N_4, graphene (GO and rGO), and MoS_2 nanosheets, are considered as various nanozymes with high enzymatic activity due to large specific surface area and numerous catalytic active sites.[80, 81] It is also a potential way to form hetero-2D materials as functional nanozymes for further enhancing their functions and expanding their bioapplications.

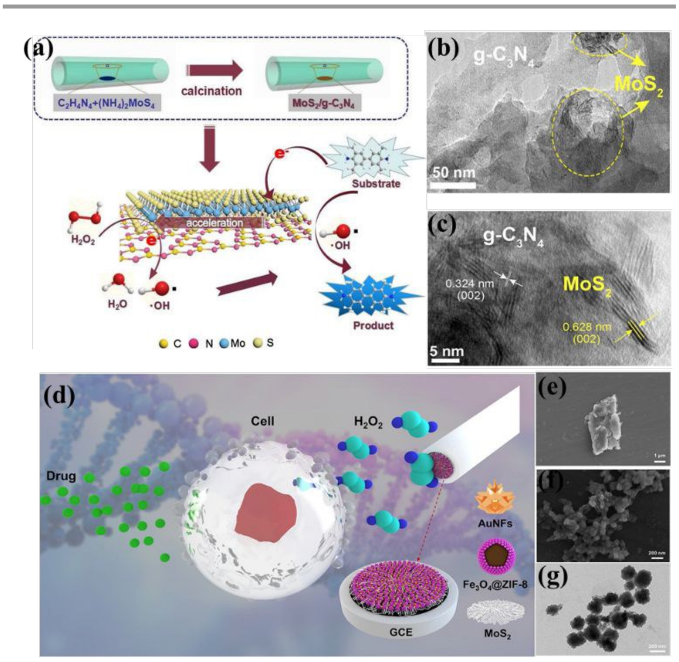

Fig. 9 Hetero-2D nanozymes. (a-c) 2D MoS_2/g-C_3N_4 heterojunction: (a) synthesis scheme, (b) TEM and (c) HRTEM image. Reproduced images with permission from Ref. 82. Copyright 2020, Elsevier BV. (d-g) Sandwich-like AuNFs/Fe_3O_4@ZIF-8/MoS_2: (d) Structure and sensing mechanism of H_2O_2, (e) SEM image of MoS_2 nanosheet, (f) SEM and (g) TEM of Fe_3O_4@ZIF-8. Reproduced images with permission from Ref. 83. Copyright 2020, Elsevier BV.

Recently, a hetero-2D material complex with heterojunction sites has been developed as an artificial nanozyme to improve the peroxidase-like activity of 2D nanozymes. For instance, Liu *et al.* constructed 2D MoS_2/g-C_3N_4 heterojunction with the combination of MoS_2 and g-C_3N_4 nanosheets by one-pot bottom-up calcination.[82] The formed hetero-2D nanozymes exhibited enhanced peroxidase-like activity due to the synergistic effects and promoted the electron transport of the formed heterojunction structure (**Fig. 9a**). The TEM characterization of the as-prepared 2D MoS_2/g-C_3N_4 heterojunction (**Fig. 9b**) indicated that the MoS_2 nanosheets were horizontally anchored and evenly dispersed on the g-C_3N_4

nanosheets and there was close interface contact between the g-C_3N_4 nanosheets and the MoS_2 nanosheets. The HRTEM image (**Fig. 9c**) demonstrates that the lattice spacing of two types was 0.324 and 0.628 nm, respectively, which is consistent with the structure of g-C_3N_4 and MoS_2. The hetero-2D MoS_2/g-C_3N_4 nanosheets prepared this strategy could accelerate the electron transfer during the catalytic reaction process. Meanwhile, the synergistic effect between MoS_2 and g-C_3N_4 resulted in high affinity for the substrate and excellent peroxidase activity.

Beside the calcination process, the electrodeposition is another potential way for the fabrication of hetero-2D nanozymes. Through the combination of MOFs (ZIF-8) and magnetic Fe_3O_4 NPs, novel magnetic MOF (MMOF, Fe_3O_4@ZIF-8) nanocomposites could serve as highly active nanozymes, in which MOFs were modified with Fe_3O_4 NPs and MOFs prevented the aggregation of NPs and maintained their stability. However, one clear problem of MMOFs was the poor contact between the electrode surface and the micro-MMOFs. Therefore, to obtain stable interface nanostructure on the electrode surface, MoS_2 nanosheets were added as supports. On the other hand, the synthesized MMOFs were used as a signal enhancer and inserted between the MoS_2 nanosheets and a thin layer of electrodeposited gold nanoflowers (AuNFs).[83] With the help of MoS_2 nanosheets and electrodeposited AuNF thin layer, the formed MMOF was inserted to form a sandwich-like structure (AuNFs/MMOF/MoS_2), which kept the electrocatalytic activity and stabilized the surface states of MMOFs. Hence, the formed AuNFs/MMOF/MoS_2 nanozyme showed high selectivity and good sensitivity when using as electrochemical biosensors of H_2O_2 (**Fig. 9d**). The fabricated sensors have been successfully used to monitor extracellular releasing of H_2O_2 that assisted by H9C2 cardiomyocytes for drug evaluation. The microscopy characterization proved that the used MoS_2 has a typical lamellar structure and the Fe_3O_4@ZIF-8 endows a Fe_3O_4 core and MOF shell (**Fig. 9e-g**).

2.7. Single-atom-modified 2D materials as nanozymes

Besides the above-mentioned components for the modification of 2D materials as functional nanozymes, single atom could be applied for the modification of 2D nanomaterials as a new type of nanozymes. Previous studies have indicated that single-atom nanozymes were a rising star recently, which have atomically dispersed enzyme-like active sites with enhanced catalytic performance.[9, 84, 85] Therefore, improved enzyme-like properties can be easily achieved by conjugating single atom with 2D nanomaterials like graphene, g-C_3N_4, MoS_2, and MOF.[86]

For instance, Kim *et al.* reported the synthesis of graphene embedded with Fe-N single site, which could resemble the heme cofactor of the natural HRP and therefore the formed nanozyme revealed enhanced peroxidase-like catalytic activity.[25] Zhu *et al.* demonstrated the modification of graphene with heteroatom for the fabrication of the nanozyme sensor arrays.[87] It was found that the created nanozyme sensor exhibited high potential for the detection of aromatic pesticides. Wang and co-workers presented the synthesis of heterogeneous single-atom Co-MoS_2 nanozyme, which exhibited peroxidase-like activity similar to the natural enzymes.[88] In a very recent study, Ma *et al.* reported the synthesis and microwave enhancing dynamic therapy application of Fe-based MOF nanozyme with biodegradable function.[89] These interesting studies proved the possibility for using MOF materials and single-atom to form functional hybrid nanozymes for advanced applications, however the corresponding studies are just in a very preliminary stage and more deeper investigations should be done in the future.

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2.8. Summary on the synthesis of functional 2D nanozymes

In the above introduction and discussions, we presented the preparation of various pure and hybrid 2D nanozymes. 2D pure nanozymes can be synthesized through mechanical, chemical, and intercalation exfoliation, as well as wet/sono-chemical synthesis. In addition, the hybrid 2D nanozymes can be fabricated by decorating one 2D material with various materials such as metallic NPs, metal oxides, biomolecules, polymers, and another 2D material. The formed 2D nanozymes exhibited multi-enzymatic activity. Here it should be noted that sometimes 2D material-based nanozymes exhibited dual enzymatic activities. For instance, protein (BSA)-directed MnO_2 nanoflakes showed dual GOx-like and peroxidase-like activities,[90] and a metal-free nanozyme based on modified g-C_3N_4 showed bifunctional enzyme-mimicking behaviours of GOx and peroxidase.[91]

Previously, the enzymatic activity of peroxidase and oxidase have been utilized widely in the biosensor and immunoassay fields. Therefore, here more attention is focused on the design, synthesis, and applications of 2D nanozymes with enhanced peroxidase and oxidase activities. To help the readers to well understand these synthesis methods, we summarize the types, synthesis process, enzymatic activity and potential applications of 2D nanozymes.

Table 1. Summary of the types, synthesis methods, mimetic enzymatic activity, and potential applications of 2D nanozymes.

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Strategy	Synthesis method	2D nanozyme	Mimetic activity	Application	Ref.
Exfoliation	Mechanical exfoliation/ CVD/reduction of GO	Graphene	Peroxidase	Tissue engineering, drug delivery, and biosensors	26
	Liquid exfoliation	g-C_3N_4	Peroxidase	Bioimaging	30
		g-C_3N_4	Peroxidase	H_2O_2 and glucose biosensors	31
	Intercalation exfoliation	MXene nanosheets	-	Ag^+/Mn^{2+} sensors	38
	Chemical exfoliation	HBN/PtNPs	Peroxidase	PDA biosensor	24
One-step solvothermal/hydrothermal reaction	hydrothermal reaction	VS_2 Nanosheets	Peroxidase	Biocatalyst of peroxidase enzyme	21
	hydrothermal synthesis	MoS_2	Peroxidase	-	42
	Hydrothermal synthesis and covalent linking	MoS_2/CeO_2	Bioenzyme	PTT	69
	hydrothermal and freeze-drying	GO/SP	-	Determination of sarcosine	79
Synthesis and surface modification	Surfactant-assisted synthesis	MOFs	Peroxidase	Fluorescent DNA biosensors	43
	Wet/Sono-Chemical synthesis	MnO_2 Nanosheets	Oxidase	Catalysis-Enhanced Phototheranostics	44
	Templated synthesis	MOF/Metalloporphyrinic/ AuNPs	Oxidase and peroxidase	Glucose biosensor	50
	In-situ synthesis	GQDs/AgNPs	Peroxidase	H_2O_2 and glucose biosensors	58
Reduction	Microwave-mediated chemical reduction	g-C_3N_4/PtNPs	Oxidase	Colorimetric Hg^{2+} sensor	58
	CO reduction	MoS_2/Au-Pd	Peroxidase	catalysis	66
Others	Calcination	FeP/ C nanosheets	oxidase	cysteine and Cu^{2+} biosensors	45
		g-C_3N_4/CuNPs	Peroxidase	H_2O_2 and glucose biosensors	59
		MoS_2/g-C_3N_4	Peroxidase	Sulfide ion sensor	82
	Soft template method	Co_3O_4	Catalase and peroxidase	Glucose biosensor	46
	Co-precipitation method	Mn–Fe	Catalase	photodynamic cancer therapy.	47
	Deposition	RGO/Pd-Cu	Peroxidase	Glucose biosensor	67
	Polyol protocol	GO/Fe-Pt	Peroxidase	H_2O_2 biosensor	68
	In-situ growth	g-C_3N_4/CuO	Peroxidase	H_2O_2 biosensor	70
	Physical binding	MOF/AG73	Peroxidase	Hep detection	73
	-	MnO_2/GOx	Oxidase	Catalysis and PTT	76
	In-situ polymerization	MoS_2/TCA	Peroxidase	H_2O_2 and Glucose biosensor	78
	Electrochemical deposition	MOF/MMOF/MoS_2	Peroxidase	Electrochemical biosensors	83
	-	Fe-N single site/GO	Peroxidase	Pesticides sensor	87
	-	Co-MoS_2	Peroxidase		88

3. Biosensor applications

Due to the unique 2D structure and improved catalytical activity, 2D nanozymes have been widely applied for the biosensor applications. Hence, in this part, we demonstrate the biosensor applications of 2D nanozyme-based colorimetric, electrochemical, fluorescent, and electrochemiluminescent (ECL) sensors.

3.1. Colorimetric biosensors

One of the unique functions of nanozymes is the fabrication of colorimetric biosensors with high sensitivity and selectivity. 2D nanozyme-based colorimetric biosensors have been widely

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used for the determination of heavy metal ions, small molecules, and biomolecules.

Environmental pollution caused by heavy metal ions has become a serious problem to affect the human living. Because of their accumulation and high toxicity, they can cause health

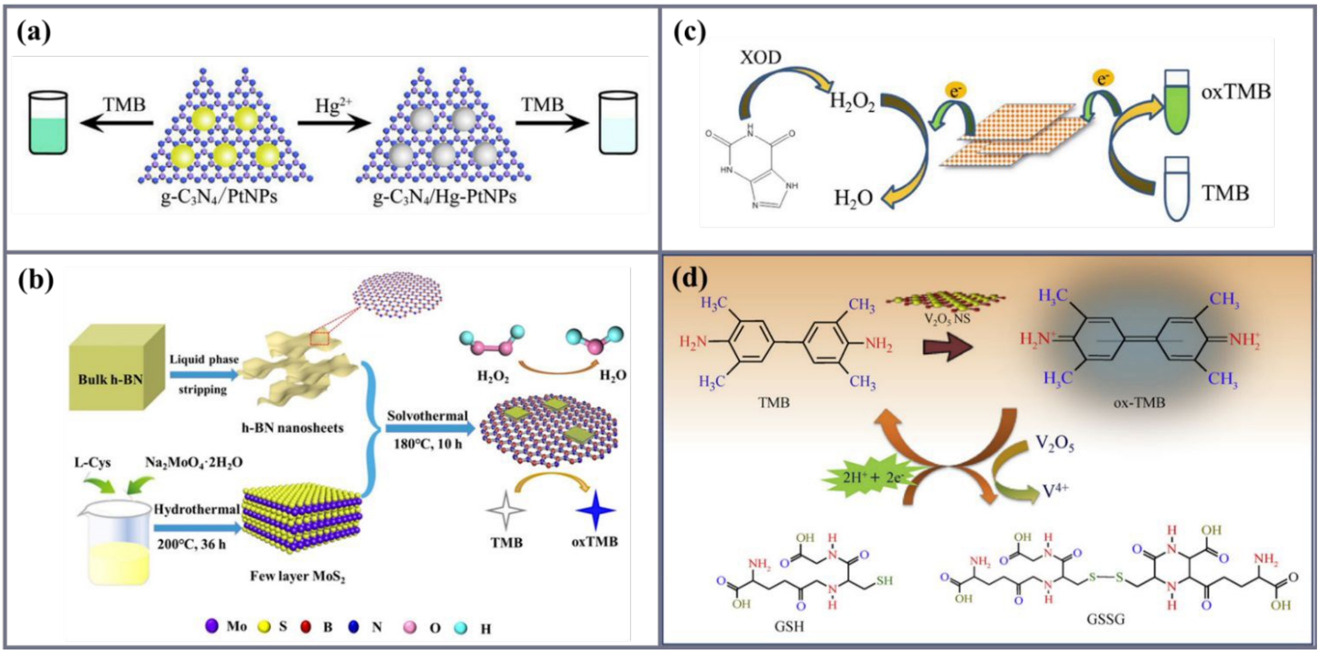

Fig. 10 2D nanozyme-based colorimetric biosensors: (a) $g\text{-}C_3N_4$/PtNPs-based Hg^{2+} biosensor. Reprinted image with permission from Ref. 58. Copyright 2017, Elsevier BV. (b) BN/N-doped MoS_2 heterostructured nanozyme for H_2O_2 sensor. Reprinted image with permission from Ref. 93. Copyright 2020, Wiley BV. (c) 2D WO_3 nanosheets for xanthine sensor. Reprinted image with permission from Ref. 95. Copyright 2019, Elsevier BV. (d) Layered V_2O_5 for glutathione sensor. Reprinted image with permission from Ref. 97. Copyright 2018, Elsevier BV.

hazards to the human body even at low concentrations. Therefore, it is of great significance to establish a sensitive and rapid method for the determination of trace heavy metal ions in environmental samples. For this aim, Wang *et al.* used a one-step method to prepare a new type of $g\text{-}C_3N_4$/PtNPs 2D nanozyme material,[58] which exhibited the advantages of high stability, good dispersibility, and enhanced oxidase-like activity. Unlike the most reported peroxidase biomimetic nanozymes that require the addition of an oxidant for assisting the catalytic reaction, the designed $g\text{-}C_3N_4$/PtNPs could effectively catalyse the oxidation of TMB without adding H_2O_2. Interestingly, it was found that Hg^{2+} could significantly inhibit the catalytic activity of $g\text{-}C_3N_4$/PtNPs, and therefore could be used for rapid and selective detection of trace Hg^{2+} in aqueous solutions (**Fig. 10a**). It should be noted that the rigid 2D structure of $g\text{-}C_3N_4$ made most of the attached PtNPs to the detection environment, which was beneficial to improve the sensitivity of the fabricated colorimetric biosensors. In another study, Ni and co-workers synthesized MoS_2 nanofilms by using thiourea and sodium molybdate as precursors under a hydrothermal reaction.[92] Their results showed that the luminescent MoS_2 nanosheets have catalytic activity similar to peroxidase, and can effectively catalyse the colorimetric reaction of H_2O_2 to oxidize the colorless substrate, o-phenylenediamine (OPD). After mixing with Fe^{2+}, MoS_2 nanosheets revealed enhanced peroxidase-like catalytic activity compared to that without Fe^{2+}. Therefore, the synergistically enhanced catalytic performance was used to construct a label-free colorimetric sensor for detecting Fe^{2+}.

2D nanozymes have high potential for the fabrication of small molecule colorimetric biosensors. H_2O_2 is an important intermediate in many biological processes and there is an urgent need to develop fast and sensitive H_2O_2 detection methods in various fields such as biological analysis, medicine, environmental protection, and food safety. Zhang *et al.* synthesized a 2D h-BN/N-doped MoS_2 heterostructured nanozyme catalyst for fabricating H_2O_2 biosensors.[93] Compared with pure h-BN and MoS_2, the prepared hybrid catalyst showed larger specific surface area and layer spacing, which greatly improved the catalytic performance towards H_2O_2. The synthesis of BN/N-doped MoS_2 heterostructured nanozyme and its sensing mechanism of H_2O_2 that shown **in Fig. 10b** indicated that the designed nanozymes had high catalytic activity toward the TMB-H_2O_2 colorimetric systems. In this case, the nanozyme has been used to accurately determine of H_2O_2 due to main advantages. First, h-BN and MoS_2 formed a heterogeneous doped structure, which enlarged the layer spacing and specific surface area of MoS_2, which is beneficial to the exposure of its active center. Second, h-BN and MoS_2 enhanced the catalytic ability of H_2O_2 due to their synergistic effect. This simple, direct colorimetric sensor system based on BN/N-doped MoS_2 nanozyme exhibited wider linear range (1-1000 μM) and lower detection limit (0.4 μM) than most Mo-based nanozymes, which was expected to broaden the applications fields of Mo-based nanozymes in highly sensitive detection of small molecules.

In addition, 2D nanozymes showed great advantages for the colorimetric sensing of biomolecules. Xanthine plays an important role in animal blood-brain barrier and is particularly important for the determination of its concentration in humans. Xanthine oxidase (XOD) can selectively catalyse the oxidation of xanthine to generate H_2O_2. Based on this principle, many peroxidase-mimetic nanozymatic colorimetric sensors have been developed for the detection of xanthine. For instance, Yang *et al.* used $MoSe_2$ nanosheets as highly effective nanozymes to colorimetrically determine xanthine in human serum samples.[94] In another study, Li and co-workers developed a simple and environmentally friendly ultrasonic peeling method to prepare 2D WO_3 nanosheets with intrinsic peroxidase-like activity.[95] The prepared 2D nanozyme exhibited highly catalytic activity for the oxidation of TMB (**Fig. 10c**), and a simple, selective, and sensitive colorimetric xanthine sensor was fabricated by the incorporation with XOD, which revealed a detection limit of 1.24 μM and a linear detection range of 25-200 μM.

Acid phosphatase (ACP) is a phosphatase that is widely present in mammalian tissues and body fluids. Its role in humans is to catalyse the dephosphorization of orthophosphate monoesters under acidic conditions. Abnormally elevated serum ACP levels may indicate various diseases such as prostate disease, kidney disease, hyperparathyroidism, and multiple myeloma. For the determination of ACP activity, one usual way is to use the Pd-based enzyme catalysts, but only limited studies have been performed for the detection of ACP with 2D nanozymes. Chen *et al.* found that the 2D Pd nanoplates on rGO (PdSP@rGO) exhibited a facet-dependent oxidase-like activity.[96] Compared with pure Pd nanocubes, 2D-PdSP@rGO showed stronger activity due to the effects of the (111)-facets of hybrid 2D nanozymes and the catalysis of Pd nanocubes to TMB. By using ACP as the substrate and TMB as the signal amplifier, the fabricated colorimetric ACP sensor showed a detection limit of 8.3 μU/mL with a good linear detection range from 0.01 to 6.0 mU/mL.

Glutathione is one of the most ubiquitous non-protein biological thiols in cells and it plays an important role in maintaining intracellular redox, signal transduction, exogenous metabolism, and gene regulation. In a recent study, Ganganboina *et al.* proved that layered V_2O_5 as nanozyme had the properties of oxidase for the detection of glutathione in the presence of TMB.[97] **Fig. 10d** shows the sensing mechanism of the layered V_2O_5 nanozyme for glutathione by using TMB as the indicators of nanozyme. The sensing principle of this system is based on the oxidation of TMB to ox-TMB in the presence of V_2O_5, inducing the color change of TMB from original light yellow to blue (ox-TMB). After adding glutathione, the reducing ability of biothiols caused the color of the solution to change from blue to light yellow again. In this sensor system, V_2O_5, as a biomimetic 2D nanozyme, do not require any functional modification, making this type of nanozyme well suitable for the highly sensitive and selective determination of glutathione in biological samples. However, due to the strong affinity of sulfhydryl groups with metals/metal oxides, when the solution contained other biothiols, the selectivity of the colorimetric sensors based on TMB oxidation was not high.[98]

Dopamine (DA) plays an indispensable role in hormones, kidneys, and central nervous system as a neurotransmitter. Changes in the concentration of DA can cause neurological syndromes, therefore how to develop methods to detect the concentration of DA in the human body quickly has high importance. Ivanova *et al.* reported the synthesis of Pt-decorated NB nanosheets as 2D nanozymes for the colorimetric detection of DA with a detect limit of 0.76 μM.[24] Due to the influence of DA during this oxidation process, this study could be used to detect the concentration of DA in the human body.

Enzyme-linked immunosorbent assay (ELISA) is a commonly used and effective analysis method. Because of its unique advantages such as easy operation, high sensitivity, and strong specificity, it has been widely used in disease diagnosis, environmental analysis, food safety, and other bio-related applications. In previous studies, natural enzymes were used for ELISA usually, but the inherent limitations of natural enzymes make the detection efficiency very low. 2D MOF nanozyme materials have higher encapsulation efficiency and can provide better shielding to the loaded enzymes to resist harsh environments.[99] Wang *et al.* proposed a new strategy for preparing enzyme-antibody conjugates by binding antibody onto Cu-MOF to form bifunctional complex nanozymes.[100] In order to prove its feasibility, rabbit anti-mouse immunoglobulin G antibody (RIgG) and Cu-MOF with peroxidase-like activity as a model, were used to prepare the RIgG@Cu-MOF composite, in which Cu-MOF served as a host to protect RIgG from the effects of long-term storage, high temperature, and biodegradation without affecting their specific binding with antigen (mIgG). More importantly, 2D nanozyme (Cu-MOF) could be used as a signal amplification unit for this colorimetric immunosensor of mIgG after the formation of sandwich-like RIgG@Cu-MOF-mIgG-antibody structure. The fabricated immunosensor exhibited a detection limit of 0.34 ng/mL towards mIgG, which was 3-fold lower than the methods based on the HRP-labelled RIgG. In other cases, 2D MOF materials have been functionalized with Ni,[23] Ce,[101] Zn,[102] Co/2Fe,[103] and GOx[104] to form functional nanozymes for colorimetric sensing of H_2O_2, ascorbic acid, alkaline phosphatase, glucose, and other biomolecules.

HCG is not only an important marker for the diagnosis of pregnancy, but also a tumor marker for trophoblastic carcinoma, testicular germ cell tumors, and prostate cancer. Therefore, the sensitive detection of HCG plays important role in biomedical fields. Previously, MnO_2 has been proved to endow high peroxidase-like activity,[105] and MnO_2-based nanozymes have been utilized as tags for the fabrication of sandwich-like immunosensors.[106-108] For instance, Zhang and co-workers designed a HCG colorimetric detection sensor platform based on 2D MnO_2 nanorods (NRs).[109] In their study, nine 6-well plastic microplates were selected as flat carriers, and bovine serum albumin (BSA) was coated onto the inner surface of the microwells to generate reactive functional groups. Chitosan (CS) was used to disperse MnO_2 NRs. The

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amino groups in CS were covalently bound to the functional groups in the wells of the microplate. Then streptavidin was immobilized in the micropores and selectively bound to the biotinylated β-HCG

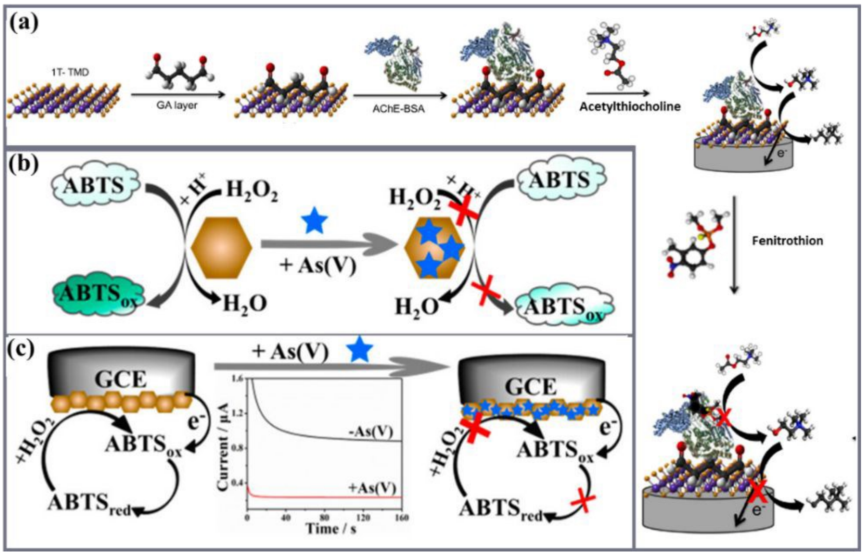

Fig. 11 2D nanozyme-based electrochemical biosensors: (a) 2D 1T-phase TMDs for fenitrothion detection. Reproduced image with permission from Ref. 112. Copyright 2017, American Chemical Society. (b,c) 2D CoOOH nanosheets for Arsenate detection. Reprinted images with permission from Ref. 113. Copyright 2019, American Chemical Society.

antibody. In this scheme, after the antigen-antibody immune complex was incubated with the antigen sample in the microwell, the antigen-antibody immune complex formed on the sensing platform inhibited the reaction of MnO_2 NRs with the colorimetric substrate due to the steric hindrance. Therefore, the measured absorbance signal decreased with the increase of the antigen concentration, thus constructing an immunoassay system without any target labelling, providing a new way for detecting the pregnancy biomarker of HCG with a detection limit of 0.36 mIU/mL.

3.2. Electrochemical biosensors

2D nanozymes can be utilized as very good materials for the modification of electrodes to fabricate electrochemical biosensors. Electrochemical biosensors have the advantages of on-site detection, fast response, high selectivity, and high sensitivity compared to the colorimetric biosensors.

Due to their excellent electrocatalytic properties, MoS_2 nanosheets have been designed as nanozymes for the modification of the electrode surface to fabricate H_2O_2 sensors. The nanosheets can be reasonably used to promote the interface electron transfer and improve the stability of electrochemical biosensors.[110] However, the sensitivity of sensors by just using MoS_2 nanosheets as nanozymes is not high.

To improve the sensitivity of sensor, Lu *et al.* reported the direct electrodeposition of AuNFs on Fe_3O_4@ZIF-8-MoS_2 nanocomposites to fabricate a highly sensitive electrochemical H_2O_2 biosensor.[83] This electrochemical biosensor had high selectivity and sensitivity in neutral solutions, and exhibited a fast electrochemical response to the detection of H_2O_2 with ultra-wide linear detection range of 5 μM to 120 mM and low detection limit of 0.9 μM. This kind of 2D nanozyme-modified electrode can be used as a universal and powerful platform for the detection of other disease biomarkers for drug evaluation.

Currently electrochemical biosensors are mainly focused on graphene and other carbon-based materials to modify electrodes to improve the sensitivity and amplification effects. Some other layered graphene-like 2D materials, such as TMDs, have not been widely used for enzymatic biosensors. Compared with graphene, TMDs have higher cell tunability and lower cytotoxicity, and the metal abundance of stripped TMDs is expected to improve the response signal of the fabricated biosensor system.[111] Nasir *et al.* demonstrated the fabrication of a electrochemical pesticide sensor by using 2D 1T-phase TMDs as nanosubstrates to enhance and stabilize the enzymatic activity.[112] As shown in **Fig. 11a**, 1T-TMD was first modified with glutaraldehyde (GA) layer for binding acetylcholinesterase-bovine saline albumin (AChE-BSA), and then enzymatic hydrolysis of AChE through electrochemical oxidation to form thiocholine. However, the addition of fenitrothion could inhibit the oxidation of AChE. Therefore, the current revealed obvious decrease after the addition of fenitrothion into the electrochemical system.

2D CoOOH nanosheets have good electrical conductivity and catalytic properties, and are a promising nanozymes for the fabrication of electrochemical sensors. Based on the simple preparation process, excellent oxidation and chemical properties of 2D CoOOH, it is possible to fabricate high-performance sensors by using CoOOH as a good component. For instance, Wen *et al.* reported the dual-mode analysis of arsenate by using CoOOH nanosheets with excellent peroxidase activity.[113] In the presence of H_2O_2, this 2D nanozyme can convert the chromogenic substrate 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) into an green oxidation product (ABTSox), as indicated in **Fig. 11b**. The presence of As(V) can weaken the peroxidase activity of CoOOH nanozymes, resulting in the decreasing of catalytic efficiency and green oxidation products. Based on this mechanism, CoOOH nanosheets were designed as colorimetric probes for the on-site determination of As(V). In addition, by using the redox reaction between ABTS and ABTSox on the surface of CoOOH-modified electrode, an electrochemical method was further developed to detect the trace As(V) (**Fig. 11c**). Through the analysis of real samples, the practicability of the dual-modal method of 2D CoOOH nanozyme has been verified successfully.

Feng *et al.* designed a new type metal ion-functionalized titanium phosphate nanospheres (TiP-metal-ion) as a tracer to label the antibody (Ab2),[114] which was then fixed onto the graphene oxide nanoribbons (GONRs) that captured antibody (Ab1). The designed hybrid nanostructure could be utilized for the construction of an ultrasensitive immunoassay platform for detecting HIgG. Due to the excellent metal ion exchange performance, a large amount of metal ions such as Cd^{2+} and Zn^{2+} are introduced into the as-prepared TiP nanospheres to form TiP-metal ion hybrids, by which the anti-cTnI and anti-fatty-acid-binding protein (anti-FABP) were bound onto TiP-Cd^{2+} and TiP-Zn^{2+} to form anti-cTnI-TiP-Cd^{2+} and anti-FABP-TiP-Zn^{2+} bioconjugates. By using these bioconjugates as probes, a new method for simultaneous detection of AMI biomarkers has been developed. After replacing FABP with HIgG, it was possible to fabricate a multiplex immunoassay method with 2D nanozyme materials for the detection of other multiple proteins.

3.3. Fluorescent biosensors

2D material-based nanozymes can be utilized for the fabrication of fluorescent biosensors by introducing fluorescent signals into the detection systems. Fluorescence biosensors have the advantages of simple preparation, fast response, and high sensitivity.

2D g-C_3N_4 has peroxidase-like catalytic activity to promote the oxidization of TMB into colored products. Heo *et al.* prepared a supramolecular g-C_3N_4 assembly through a simple reaction of melamine and cyanuric acid.[115] The formed rosette-shaped g-C_3N_4 revealed large surface area and high porosity. Importantly, it exhibited high peroxidase-like activity and could produce intense fluorescence from the oxidation of Amplex UltraRed (AUR) in the presence of H_2O_2 (**Fig. 12a**). The Rosette-shaped g-C_3N_4 also showed higher activity in a wide pH range, including physiological pH conditions. Therefore, it can be used for one-step fluorescence detection of glucose with a detection limit of 1.2 μM.

Based on traditional fluorescence method, Guo *et al.* developed a simple, green, and label-free fluorescence strategy based on dual-functional MOF (MIL-101 (Fe)), as a nanozyme for sensitive and selective detection of choline and acetylcholine.[116] It was found that the synthesized MIL-101 (Fe) has excellent catalase activity, which was generated effectively by H_2O_2 under the catalysis of the MIL-101 (Fe) nanozyme. Therefore, in the presence of H_2O_2 the used 2D MOF nanozyme could be used as a bifunctional material to catalyse the oxidation of its non-fluorescent bridging ligand terephthalic acid (TA) to produce highly fluorescent 2-hydroxy terephthalic acid (HTA). It is known that AChE can catalyse the decomposition of acetylcholinesterase (ACh) into choline and acetic acid, and the produced choline can be further converted into H_2O_2 and betaine under the catalysis of choline oxidase (ChOx), as shown in **Fig. 12b**. Since the concentration of H_2O_2 was proportional to the concentration of choline and acetylcholine, the choline and acetylcholine biosensors have been constructed based on the detection of H_2O_2. This fluorescent sensing strategy does not require the addition of fluorescent substrates, and can provide a green label-free detection method for H_2O_2, choline, and AChE. In addition, this strategy has been successful in the detection of choline in milk and AChE in human plasma, and therefore was highly promising for early disease diagnostics.

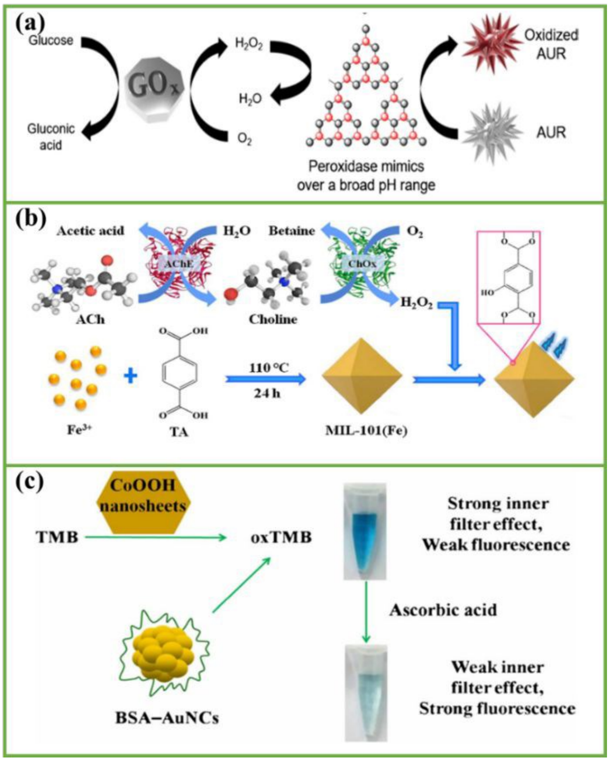

Fig. 12 2D nanozymes for fluorescent biosensors: (a) 2D Rosette shaped g-C3N4 for glucose biosensor. Reprinted image with permission from Ref. 115. Copyright 2020, Springer. (b) MIL-101 (Fe) for ACh, acetic acid, and H_2O_2 biosensors. Reprinted images with permission from Ref. 116. Copyright 2020, Elsevier BV. (c) CoOOH nanosheets and BSA-AuNCs for ascorbic acid biosensor. Reprinted images with permission from Ref. 117. Copyright 2014, American Chemical Society.

Based on blue fluorescence as an energy donor, CoOOH nanosheets have been used as a quencher for fluorescent sensing systems such as ascorbic acid.[117] Cui and co-workers reported that CoOOH nanosheets can catalyse the oxidation of TMB under weakly acidic conditions to produce blue solution without adding H_2O_2.[118] Meanwhile, CoOOH nanosheets have typical oxidase-like activity and can undergo specific redox reactions with ascorbic acid. Based on these features, a fluorescent assay for the determination of ascorbic acid has been developed.[117] As shown in **Fig. 12c**, the blue oxidation

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product of TMB (oxTMB) acts as a quencher of BSA-stabilized gold nanoclusters (BSA-AuNCs). Because the absorption band of oxTMB is similar to that of BSA, their

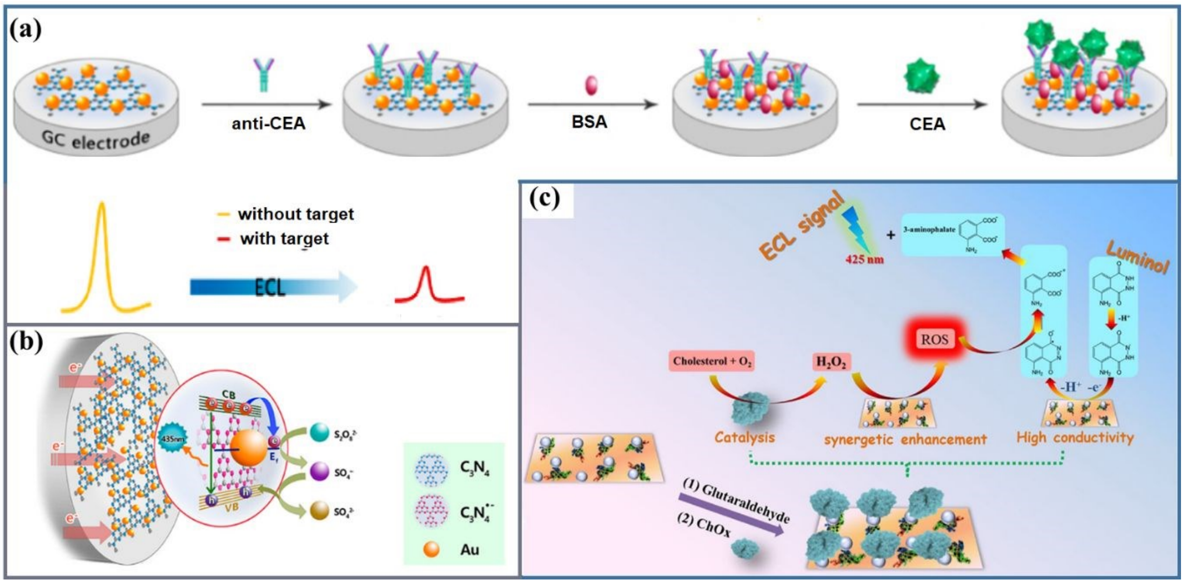

Fig. 13 2D nanozymes for ECL biosensor applications: (a,b) Au-g-C_3N_4NHs for ECL CEA. (a) Fabrication of ECL sensor platform. (b) ECL amplify mechanism. Reprinted images with permission from Ref. 124. Copyright 2014, American Chemical Society. (c) 2D hybrid Ag-BSA-MnO_2 hybrid system for ECL sensing cholesterol. Reprinted image with permission from Ref. 126. Copyright 2018, Elsevier BV.

emission peaks overlap with that of BSA-AuNCs. Due to the internal filter effect, the oxTMB absorbed the emission of BSA-AuNCs and reduced their fluorescence. However, when ascorbic acid was present, it destroyed the CoOOH nanosheets, thereby delaying the oxidation process and reducing the fluorescence. The fabricated fluorescent biosensor exhibited a detection limit of 0.2 μM for the sensing of ascorbic acid.

2D nanomaterials have a wide range of applications in fluorescence detection ascribed to their more active sites and fewer diffusion barriers. MOFs have been utilized to mimic natural enzymes for fluorescence detection. For example, PCN-600(Fe) has used as a nanozyme to catalyse the oxidation reaction,[119] MIL-100(Fe) and MIL-68(Fe) have been used as highly efficient peroxidase-like enzymes to catalyse the oxidation of different peroxidase substrates through H_2O_2,[120] and HKUST-1 has been use to catalyse the oxidation of thiamine by H_2O_2.[121] Based on these studies, Hu *et al.* developed a new peroxidase mimetic Cu(II)-based 2D MOF with nanosheet structure (CuBDC NS) for the fabrication of a fluorescent biosensor of inorganic pyrophosphatase.[122] In the presence of sodium pyrophosphate, the coordination of Cu^{2+} in CuBDC NS affects its peroxidase (POx) activity and structure. In the presence of pyrophosphatase, sodium pyrophosphate was hydrolyzed into phosphate that is inconsistent with Cu^{2+}, while the structure of CuBDC NS is maintained well. CuBDC NS acted as a POx mimic enzyme to catalyse the production of hydroxyl radicals (•OH) from H_2O_2. Since the oxidation ability of •OH is much stronger than that of H_2O_2, it promotes the oxidation of terephthalic acid (H2BDC) to oxidized BDC, which leads to the increase of fluorescence intensity.

3.4. Electrochemiluminescence biosensors

ECL is a process in which species are generated on the electrode surface to form a luminescent emission state through the electron transfer reaction.[123] ECL has the advantages of lower background emission, higher sensitivity, and easier operation compared with photoluminescence (PL) technique. In this section, the design of 2D nanozymes for ECL sensor applications is presented.

Chen *et al.* prepared AuNP-modified g-C_3N_4 nanosheets nanohybrid (Au-g-C_3N_4NHs) as a ECL sensing material.[124] The synthesized Au-g-C_3N_4NHs has strong and stable cathodic ECL activity. Based on the designed material, a sensitive, selective, and label-free ECL sensor has been developed to detect carcinoembryonic antigen (CEA) (**Fig. 13a**). Since g-C_3N_4 nanosheets have excellent film-forming ability and are easy to combine with AuNPs to form Au-g-C_3N_4NHs, the hybridization of g-C_3N_4 nanosheets with AuNPs may prevent g-C_3N_4 nanosheets by transferring some electrons to AuNPs. Meanwhile, Au-g-C_3N_4NHs could catalyse the reduction of persulfate ($S_2O_8^{2-}$) to hole donor (SO_4^-), thereby mediating the excessive injection of conductive electrons into the conduction band (CB) of g-C_3N_4 nanosheets. Therefore, the ECL emission of nanomaterial was enhanced, as shown in **Fig. 13b**. The fabrication of this ECL biosensor was simple and did not need any complex chemical modification of 2D nanozymes for the binding of ligands.

In Part 3.1, a colorimetric assay using V_2O_5 to detect glutathione was introduced. Here, an ECL method for glutathione detection is demonstrated. Gao *et al.* developed the cathode ECL of lucigenin on a glassy carbon electrode to detect glutathione in the presence of MnO_2 nanosheets.[125] Lucigenin (N,N'-dimethylbicyclopyridine dinitrate) is a chemiluminescent material that can generate ECL signals without the need for other cofactors, revealing great advantage in sensitive bioanalysis. At low concentrations, MnO_2

nanosheets have little effect on the ECL. However, glutathione can reduce MnO_2 nanosheets to Mn^{2+}, significantly inhibiting the ECL of lucigenin, which makes the ultra-sensitive detection of glutathione possible. This detection method did not require labelling and electrode modification, and could be used for the sensitive ECL detection in biological systems.

Cholesterol, as a part of fat intake in a healthy diet, is an important biomarker for several diseases. Abnormal levels of cholesterol may cause diseases such as heart disease, so sensitive and accurate detection of cholesterol is particularly important. ECL-based sensors have been alternative platforms for determining cholesterol. Yang *et al.* reported the design and synthesis of a novel 2D hybrid Ag-BSA-MnO_2 hybrid system for ECL sensing cholesterol.[126] In this hybrid system, the introduction of BSA significantly improved the biocompatibility of MnO_2 nanosheets by providing different functional groups. Importantly, the synergistic effect of AgNPs and MnO_2 nanosheets provided high conductivity for the electron transfer and good catalytic ability for the generation of reactive oxygen species (ROS), as shown in **Fig. 13c**. Hence, an ultra-sensitive cholesterol ECL sensor based on Ag-BSA-MnO_2 nanosheets was developed, which exhibited a detect limit of 0.07 µM and a linear detection range from 0.21 to 1667 µM.

To make it more clear for the above introduction, here we provide a table (**Table 2**) to summarize the sensor type, 2D nanozyme, and corresponding sensing performances of the demonstrated biosensors.

Table 2. Summary of the sensor type, used 2D nanozyme, analyte, detection limit, and detection range of the fabricated 2D nanozyme-based biosensors.

Type	Nanozyme	Analyte	Detection limit	Detection range	Ref.
Colorimetric biosensors	g-C_3N_4 / PtNPs	Hg^{2+}	1.23 nM	5-500 nM	58
	MoS_2 nanofilms	Fe^{2+}	7 nM	0.01-0.8 µM	92
	h-BN/N-doped MoS_2	H_2O_2	0.4 µM	1-1000 µM	93
	$MoSe_2$	xanthine	1.964 µM	0.01 mM-0.32 mM	94
	$MoSe_2$	H_2O_2	0.408 µM	0.01 mM-0.16 mM	94
	WO_3 nanosheets	xanthine	1.24 µM	25-200 µM	95
	RGO/Pd nanoplates	acid phosphatase	8.3 µU/mL	0.01-6.0 mU/mL	96
	V_2O_5	glutathione	2.4 nM	10-500 nM	97
	NB nanosheets	dopamine	0.76 µM	2-50 µM	24
	RIgG/Cu-MOF	antigen	0.34 ng/mL	1-100 ng/mL	100
	Ni-MOF	H_2O_2	0.008 µM	0.04–160 µM	23
	Ce-MOF	ascorbic acid	0.28 µM	1-20 µM。	101
	MOF	alkaline phosphatase	2.5 U L^{-1}	2.5–200 U L^{-1}	102
	MOF/(Co/2Fe)	H_2O_2	5 µM	10-100 µM	103
	MOF/Fe(III)-BTC	glucose	2.4 µM	5.0–100 µM	104
	MnO_2	HCG	0.36 mIU/mL	0.5-400 mIU/mL	109
Electrochemical biosensors	AuNFs/ Fe_3O_4@ZIF-8-MoS_2	H_2O_2	0.9 µM	5 µM-120 mM	83
	1T-TMD	pesticide	2.86 nM	1-1000 nM	112
	CoOOH nanosheets	arsenate	3.72 ppb	0.1-200 ppb	113
	TiP nanospheres	cardiac troponin I	1 fg/mL	0.05 pg/mL-50 ng/mL	114
Fluorescent biosensors	g-C_3N_4	glucose	1.2 µM	5.0-275.0 µM	115
	MOF	choline	20 nM	0.1-10 µM	116
	MOF	acetylcholine	8.9 nM	0.01-100 µM	116
	CoOOH nanosheets	ascorbic acid	0.2 µM	-	118
	Cu(II)-based 2D MOF	inorganic pyrophosphatase	0.6 mU/mL	1-50 mU/mL	122
ECL biosensors	AuNP/g-C_3N_4	carcinoembryonic antigen	6.8 pg/mL	0.02-80 ng/mL	124
	MnO_2 nanosheets	glutathione	3.7 nM	10-2000 nM	125

$Ag/BSA/MnO_2$	cholesterol	0.07 μM	0.21-1667 μM	126

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4. Conclusions and perspectives

Overall, we summarized recent advance in the design, synthesis, and biosensor applications of 2D material-based nanozymes. We first introduced various methods for the synthesis of 2D material-based nanozymes, including physical, chemical, and intercalation exfoliation, as well as other hydrothermal, and wet/sono-chemical synthesis. Due to their high specific surface area and numerous active sites, 2D materials self and their composites with other nanoscale building blocks such as metal NPs, metal oxides, biomolecules, polymers, and others exhibited enhanced catalytic activity, which is beneficial for the fabrication of biosensor platforms. To present the advantages of 2D nanozymes in the design and fabrication of colorimetric, electrochemical, fluorescent, ECL, and immunoassay sensors, the synthesis of 2D nanozymes with specific biomimetic enzymatic activities, the design of functional sensor systems, the sensing performance, and the potential sensing mechanisms were analyzed and discussed in detail. It is expected this work will inspire the design and synthesis of other novel 2D nanozymes for expanding applications.

Still, 2D nanomaterial-based nanozymes have a few limitations in the aspects of synthesis, function, and applications. For example, 2D nanozymes have no similar high activity to the natural enzymes. 2D nanozymes show multiple enzymatic functions, which are different from the specific catalytic activity of the natural enzymes. Therefore, it is difficult to apply 2D nanozymes for some biological enzymatic catalysis that induced by natural enzymes easily. In addition, the stabilization and dispersity of 2D nanozymes in biological buffer systems should be improved to meet the requirements of nanozymes for in vivo biosensor applications as in some cases the dissolution and modification of 2D nanomaterials in aqueous solutions are not easy.

Here we would like to point out several aspects that could be paid more focus in the near future. First, more theoretical analysis should be done to understand the catalytic dynamics and detailed enhancement mechanisms of 2D hybrid nanozymes. Previously, a lot of studies have been carried out to combine 2D with various nanomaterials to improve the nanozymatic catalysis activity, however limited theoretical studies on the 2D nanozymes have been done. Second, most of the synthesized 2D nanozymes (including other nanozymes) showed weak substrate specificity, which is very far from that of the natural enzymes. Some other biomolecules with high bioactivity and bioaffinity, such as aptamer and peptides could be a potential way to improve the substrate specificity of nanozymes. Third, recently more focus has been attracted onto the single-atom catalysis,[9, 85] The synthesis of 2D nanozymes with single-atom decoration will attract great interest ascribed to the unique properties and functions of single atoms. Fourth, other kinds of 2D materials, such as black phosphorus and layered double hydroxide (LDH), could be utilized for the combination with other nanomaterials to form novel 2D nanozymes. Finally, we suggest that 2D nanozymes can also be used for other kind of applications, such as the photothermal therapy, drug delivery, environmental remediation, and others.

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Notes

The authors declare no interest of conflict.

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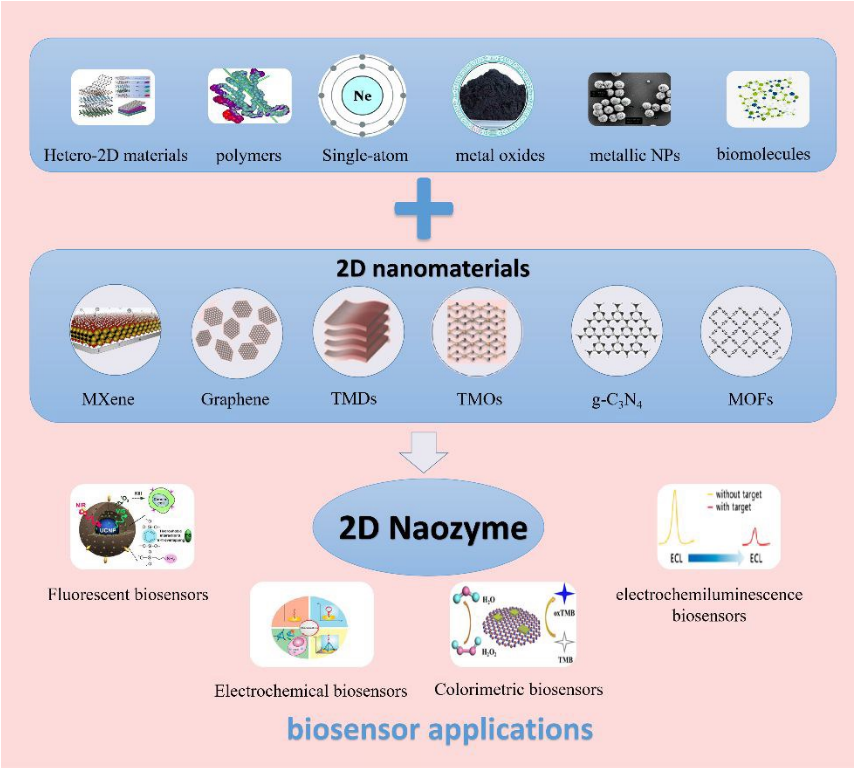
Graphical Abstract



2D material-based nanozymes exhibited high potential for the applications in biosensors and immunoassays.