Recent Advances of Nanostructured Materials for Photoelectrochemical Bioanalysis

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Abstract: Nowadays, the emerging photoelectrochemical (PEC) bioanalysis has drawn intensive interest due to its numerous merits. As one of its core elements, functional nanostructured materials play a crucial role during the construction of PEC biosensors, which can not only be employed as transducers but also act as signal probes. Although both chemical composition and morphology control of nanostructured materials contribute to the excellent analytical performance of PEC bioassay, surveys addressing nanostructures with different dimensionality have rarely been reported. In this review, according to classification based on dimensionality, zero-dimensional, one-dimensional, two-dimensional, and three-dimensional nanostructures used in PEC bioanalysis are evaluated, with an emphasis on the effect of morphology on the detection performances. Furthermore, using the illustration of recent works, related novel PEC biosensing patterns with promising applications are also discussed. Finally, the current challenges and some future perspectives in this field are addressed based on our opinions.

Keywords: photoelectrochemical; biosensor; nanostructured materials; biosensing patterns; review

1. Introduction

Photoelectrochemical (PEC) phenomena have drawn intensive interest in many fields since being discovered by Becquerel in 1839 [1–3]. The PEC process typically refers to the conversion of light energy to electrical power due to the light absorption-induced excitation and transport of electrons. As an emerging sensing technology, PEC bioanalysis integrates the PEC process and electrochemical biosensing delicately. As shown in Figure 1, a typical PEC analysis system usually includes three parts: a light-source system, detection system, and signal-reading system. As the core part, the detection system is generally composed of three electrodes and an electrolyte. Therefore, photoactive species-modified working electrodes play an important role in the construction of PEC sensors. Taking commonly used semiconductor nanoparticles (NPs), for example, under illumination, photoactive species generate electron–hole (e-h) pairs through the excitation of photoelectrons from the valence band (VB) to the conduction band (CB). With the neutralization of holes by donors (D) or the capture of electrons by acceptors (A) on the interface of working electrodes, constant and stable anodic or cathodic photocurrent signals can be achieved from the transport of electrons. Once the targets trigger specific biorecognition events alter the abovementioned PEC process, the photocurrent signals change. By monitoring the chronoamperometric I-t responses with a signal-reading system that reflects the concentration variation of target analytes, the biochemical analytes of interest can be detected. Compared with traditional electrochemical bioanalysis, PEC bioassay perceives higher sensitivity due to its significantly improved ratio of signal to noise ascribed to the fact that the excitation source and the
higher sensitivity due to its significantly improved ratio of signal to noise ascribed to the different dimensionality used for high-performance PEC bioanalysis are discussed.

2.1. 0D-Nanostructure Materials

Nanostructured materials typically refer to low-dimensional materials with a submicron or nanoscale size of structural units in at least one direction [6,8]. In order to enhance the PEC biosensing performance, many functional nanostructured materials have been developed to serve as transducers, probes, and carriers due to their numerous unique properties. Except for the chemical component, rational morphology regulating of nanostructure surfaces can effectively increase their active sites and improve their target-capture ability. Therefore, extensive efforts have been devoted to the morphology control of functional nanostructures. Classified by dimensionality, nanostructures include zero-dimensional (0D), one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) structures [8] that exhibit a profound impact on PEC biosensing performance. In this section, various functional nanostructured materials with different dimensionality used for high-performance PEC bioanalysis are discussed.

2.1. 0D-Nanostructure Materials

Over the past several decades, 0D-nanostructure materials, with no dimension greater than 100 nm, have been widely used in the construction of sensitive PEC biosensors. As the simplest nanostructure, 0D nanostructures typically include the most commonly used quantum dots (QDs) and nanoparticles (NPs). Herein, the PEC sensing performance using QDs, carbon-based NPs, and metal-based NPs as functional nanostructured materials is discussed.
2.1.1. QDs

QDs, discovered in the 1980s [9,10], have boomed in many biosensing fields such as optics, electronics, catalysis, and so on by coupling with various biomolecules. In the early 2000s, a QD–biomolecule nanohybrid was first applied to PEC analysis [11], which subsequently stimulated substantial interest in experimental and theoretical research in the field of QD-based PEC biosensing. The tailored physicochemical property is one of the most attractive reasons for favoring QDs in PEC biosensing. For example, different from bulk materials, the bandgaps of QDs can be easily regulated by manipulating their size and composition [12–15]. Besides, compared to many other photoactive materials, QDs are easier to synthesize and surface-modified. Their photoelectric conversion activity and chemical stability are fantastic as well. Additionally, with the comparable size to biomolecules and the multifunctional group-modified interface, it is very easy for QDs to construct various kinds of architectures for advanced PEC biosensing platforms.

As photoactive materials, under illumination, semiconductor QDs can generate stable anode or cathode current signals in the presence of suitable electron donors or electron acceptors. Based on this, numerous semiconductor QD-modified electrodes have been developed for the construction of elegant PEC sensing architectures. Compared to “signal on” PEC analysis, “signal off” PEC sensing patterns often have unsatisfied sensitivity due to the limited background signal of photoactive materials. Addressing this challenge, Zhang et al. reported that a PEC assay of CA125 based on the core-shell nanostructure of SiO$_2$@polydopamine (PDA) served as a quencher to CdTe QD-modified ITO. The high photoelectric activity of CdTe QDs coupled with the highly efficient signal quencher contributed to the ultra-low detection limit of 0.3 U L$^{-1}$ [16]. The combination of two or more semiconductor QDs with matching bandgaps is a good way to enhance their ability to absorb visible light and improve the efficiency of charge separation and charge transfer, as well. For example, by using low-bandgap Ag$_2$S QDs as the photosensitizer, the photocurrent intensity of a ZnS@Ag$_2$S QD-modified electrode improved 10 and 1.4 times more than that of ZnS QDs and Ag$_2$S QDs, respectively. From this, based on Hg$^{2+}$ triggered the formation of HgS/ZnS@Ag$_2$S, Wang et al. proposed a “signal on” PEC sensor for the assay of Hg$^{2+}$, which had the detection limit of 1.0 pM [17].

As mentioned above, in PEC assays, photoactive materials are often directly modified on the sensing interface, which could affect the sensitivity and detection limit during the sensing process due to the high initial photocurrent signal and the unneglected background noise. Faced with this issue, Yuan et al. proposed a near-zero background noise PEC biosensor for the detection of microRNA-141. As seen in Figure 2a, the CdTe QDs-methylene blue (MB)-modified DNA tetrahedron (TET) was brought in close proximity to the surface of a cDNA-anchored golden NP (Au NP) immobilized glassy carbon electrode (GCE) by the target DNA (tDNA)-controlled hybridization of cDNA, output DNA, and DNA TET-CdTe QDs-MB complex. Due to the outstanding photovoltaic performance of the TET–QD–MB complex coupled with the enzyme-assisted target-cycling amplification strategy, the proposed PEC biosensor possessed a low detection limit of 17 aM for the detection of miRNR-141 [18].

Despite the superior PEC response, inorganic semiconductor QDs still suffered from several hurdles during the PEC analysis. For example, the widely used inorganic QD Cd-chalcogenide had a potential risk of metal ion leakage, such as Cd and Se, which might influence its application in vivo analysis. In view of the above problem, our group fabricated a tetraphenylporphyrin (TPP)-doped poly[(9,9-dioctylfluorenyl-2,7-diy)-co-(1,4-benzo-[2,1′,3′]-thiadazole)] (PFBT) polymer dot (Pdot)-modified ITO electrode. By choosing L-cysteine as the PEC detection model molecule, we found that this TPP-doped PFBT Pdot shows excellent photostability and biocompatibility. In this work, the PEC biosensing performance of the TPP-doped PFBT Pdot was demonstrated in detail for the first time [19]. After that, our group reported a series of works based on Pdots [20–24]. For instance, using the energy transfer (ET) process between the Pdots and Au NPs, a “signal on” PEC biosensing platform was constructed by us to probe the telomerase activity in cell.
extracts [23]. Besides, we also originally fabricated the heterojunction of semiconducting Cd-chalcogenide QDs and Pdots, and the corresponding PEC assay property for the detection of L-cysteine was evaluated [24].

2.1.2. Carbon-Based NPs

Carbon is one of the most substantial elements in both organic and inorganic materials. In recent years, carbon-based nanostructured materials have attracted increasing research interests in the PEC biosensing field due to their unique physicochemical and electronic characteristics [25–30]. Of the various carbon-based nanostructured materials, such as graphene, fullerene C_{60}, and g-C_{3}N_{4}, only 0D nanostructures will be presented in this part.

Compared to other metallic-based NPs or organic photosensitizers, carbon nanodots (C-dots) are widely used in bioimaging [31–33] and biosensing areas [34–36] because of their low cytotoxicity, resistance to photobleaching, and fascinating biocompatibility [37]. For example, Yu et al. reported on an N-doped C-dot-immobilized TiO_{2}-Pt NP-modified paper-based PEC sensor for the detection of carcinoembryonic antigen (CEA) in human serum. Benefiting from this fabricated hierarchical nanostructure, the significantly enhanced conductivity and the greatly widened visible light absorption range were acquired by the prepared photoelectrode. In addition, though incubation with MCF-7 cells, the cytotoxicity test demonstrated that this C-dot-based nanomaterial had low toxicity to live cells [38]. Using boronic acid-modified C-dots (B-CDs) as the signal probe, Yin et al. proposed that a “signal on” multifunctional PEC biosensor for the assay of 5hmC or β-GT based on the T4-β-glucosyltransferase (β-GT) triggered the glycosylation of 5hmC, which brought a B-CD probe to the surface of a WS_{2} nanosheet-fabricated ITO electrode and led to an increase in photocurrent responses [39].

As a metal-free semiconductor, nitrogen graphene quantum dots (NGQDs) have drawn special attention in various PEC applications due to their size-dependent and edge-sensitive photoluminescence characteristics [40–42]. For instance, Wang et al. found that, loaded with the right amount of NGQDs, the photogenerated charge carriers of semiconductor nanomaterials would get extended lifetimes. In this ground, they fabricated NGOD-modified MoS_{2} nanohybrids used for label-free PEC aptasensing [43]. Instead of being immobilized on the electrode surface, GQDs can also be treated as signal probes by labeling various biomolecules. As shown in Figure 2b, the PEC performance of the ZnO/CdTe QD nanohybrid was improved significantly by the local surface plasmon resonance resulting from the nanogold-assembled mesoporous silica NPs (GMSNs). Subsequently, the N-glycan expression on the cell surface was evaluated by the immobilization of concanavalin A-conjugated GQD (GOD@Con-A) near the as-prepared photoelectrode’s surface based on the Con A-triggered specific recognition with target cells. With horseradish peroxidase (HRP) labeled on multiple branched-arm double-helix DNA (HRP-mdhDNA), a luminol-based chemiluminescent (CL) system was used as the inner light source for the sensitive sensing of N-glycan expression on the target cell surface [44].

Compared with 2D bulk g-C_{3}N_{4}, the g-C_{3}N_{4} QDs with unique quantum size achieve more active sites and enhanced visible light absorption ability [45]. Specially, the hybridization of g-C_{3}N_{4} with other nano semiconductors with suitable bandgaps could further accelerate the electron transfer and inhibit the recombination of charge carriers. Using g-C_{3}N_{4} as the sensitizer, Tan et al. constructed a PEC biosensor for the assay of a CCRF-CEM cell by employing g-C_{3}N_{4}-combined ZnO nanodisks (ZnO NDs@g-C_{3}N_{4}) as the photoactive material. The interfacial built-in fields of the as-prepared heterojunction had consistent direction with the diffusion path of minority charge, which significantly increased the separation of photon-induced electron–hole pairs instead of their recombination, and the detection limit was calculated to be 20 cell/ml [46].

2.1.3. Noble-Metal NPs

Owing to their fantastic optical and electrical properties, noble-metal NPs have attracted great interest in nanostructure-based PEC analysis. Among various noble-metal
NPs, Au NPs and Ag NPs are the most relevant in bioanalysis because they are easy to prepare in different sizes and have excellent stability and biological compatibility.

Addressing to improve the visible light-harvesting and photoelectric conversion ability, noble metal NPs, such as Au NPs, Ag NPs, or a combination of both, are often used for the modification of various semiconductors due to their surface plasmon resonance (SPR) effects. Under illumination, hot electrons would generate from the oscillation of conduction electrons at the surface of plasmonic metal nanomaterials. Due to the higher energy, these hot electrons would easily inject the semiconductors with the matched bandgap. By further transferring to the external circuit, an enhanced photocurrent signal would be obtained. In addition, the density of carriers and the intensity of the photocurrent could also be improved by transferring energy from plasmonic metal NPs to semiconductors [47]. Therefore, based on the SPR effect, numerous PEC bioassay methods have been reported in recent years [48]. For instance, Zhang et al. developed a PEC analysis for M.Sssl methyltransferase (M.Sssl MTase) through target-triggered photocurrent enhancement resulting from the Au NP-mediated SPR effect. The significant increment of the photocurrent was mainly ascribed to the fantastic conductivity and SPR effect of Au NPs coupled with the sensitization of rhodamine (RhB) [49]. Ding et al. studied the PEC property of Au nanoclusters (Au NPs) affected by the Ag NP-based SPR. As displayed in Figure 2c, an Au NC-decorated Ag@SiO₂-modified FTO photoelectrode (Au NC-Ag@SiO₂/FTO) was fabricated. By adjusting the distance between the Au NCs and Ag NPs by changing the thickness of the silica shell, the photocurrent intensity of the fabricated Au NC-Ag@SiO₂/FTO electrode increased to 3.8 times higher than that of the Au NC-modified FTO photoelectrode, which might be attributable to the synergistic effect of hot electron transfer, light-scattering effects, and energy transfer [50]. With regard to the application of SPR in PEC biosensing, our group has a series of reports, as well [51–53]. For example, to improve the visible light-harvesting property of TiO₂ nanorod-modified FTO electrodes, further decoration with Au@Ag NPs was employed. Thanks to the excellent SPR effect of the Au@Ag nanohybrid, threefold enhancement of the photocurrent intensity was acquired [52].

Noble-metal NPs with an SPR effect have also been demonstrated to be powerful nanoprobe of PEC biosensing applications based on the interparticle resonance energy transfer (RET) between energy-donor semiconductors and plasmonic noble-metal NPs. When the SPR absorption spectrum of plasmonic NPs has a large overlap with the emission spectrum of semiconductors, the RET takes place, which accelerates the recombination of photogenerated electrode–hole pairs and quenches the photocurrents. Incidentally, the distance from energy donor to energy acceptor is another critical factor to improve the quenching efficiency of photocurrents [54]. Attracted by the high sensitivity of the RET strategy, many noble-metal NP-based nanoprobe have recently been developed to translate the biomolecular recognition process to the change of photocurrent signals. For example, based on the RET between Ce-TiO₂@MoSe₂ and Au NPs, Liu et al. proposed a “signal on” PEC aptasensor for highly sensitive sensing of AFB1 [55]. Also using Au NPs as the energy acceptor, our group realized the ultrasensitive PEC detection of miRNA-141 by coupling an entropy-driven toehold-mediated DNA strand displacement (ETSD) reaction with an RET signal strategy [21]. Another interesting example is that the RET process of oligonucleotide-encapsulated Ag nanoclusters (Ag NCs) against CdS QDs was estimated, and by detecting the target-induced changes of photocurrent intensity, a DNA PEC sensor with a limit detection of 0.3 pM was developed by us [56]. After that, we synthesized the Ag@Au asymmetric core–satellite nanostructures (Ag@Au ACS), which were used to implement the PEC immunoassay of prostate-specific antigen (PSA) on the basis of the RET interaction between Ag@Au ACS and CdS QDs [57].
of oligonucleotide-encapsulated Ag nanoclusters (Ag NCs) against CdS QDs was estimated, and by detecting the target-induced changes of photocurrent intensity, a DNA PEC sensor with a limit detection of 0.3 pM was developed by us [56]. After that, we synthesized the Ag@Au asymmetric core–satellite nanostructures (Ag@Au ACS), which were used to implement the PEC immunoassay of prostate-specific antigen (PSA) on the basis of the RET interaction between Ag@Au ACS and CdS QDs [57].

Figure 2. (a) Schematic diagrams of the PEC biosensor for miRNA-141 determination. Reprinted with permission from ref. [18]. Copyright 2018, American Chemical Society; (b) schematic representation of the PEC analytical principle for N-glycan expression. Reprinted with permission from ref. [44]. Copyright 2017, American Chemical Society; (c) the energy diagram of the SPR effect-induced photocurrent enhancement system. Reprinted with permission from ref. [50]. Copyright 2020, American Chemical Society.

2.2. 1D-Nanostructure Materials

1D nanostructures are typically defined as high-aspect-ratio nanomaterials with diameters ranging from 1 to 100 nm, such as the morphologies of nanorods, nanowires, nanotubes, nanobelts, nanoneedles, and so on [58]. Compared to bulk semiconductors, the
high surface-to-volume ratio significantly increases the binding sites for various surface reactions and greatly accelerate the transfer speed of photogenerated charge carriers from the interior to the surface of 1D nanomaterials. Besides, the quantum effects can expect to be modulated by adjusting the diameter of the 1D nanostructures [39]. For the past few years, these fascinating characteristics have attracted extensive concerns in PEC analysis applications [60–63]. Here, we will discuss the commonly used 1D nanomaterials in PEC bioanalysis according to the difference in chemical composition.

2.2.1. Metal Oxide-Based Semiconductors

Because of the strong light-absorption property, low toxicity, and high chemical stability and biocompatibility, metal oxide-based 1D nanomaterials such as TiO$_2$, ZnO, CuO, and Fe$_2$O$_3$ have broad application prospects in PEC transducing or signal probing, which are usually immobilized or grown in situ on the surface of substrate materials.

Among various oxide nanostructures, different morphologies of TiO$_2$ nanostructures have attracted much attention [64–67]. Compared to NP-based PEC biosensors, TiO$_2$ nanowire (NW) structures can significantly reduce the transfer time of charge carriers from the interior to the surface due to the tiny radial transmission distance and provide a more favorable transfer route for charge carriers, which are beneficial for the efficient separation of photogenerated charge carriers [68,69]. Feng et al. proposed a triphase bio-photoelectrode (TBP) system for the PEC detection of glucose by immobilizing glucose oxidase on the surface of TiO$_2$ NWs grown in situ on a superhydrophobic carbon textile substrate. In Figure 3a, a solid–liquid–air triphase interface was presented on the surface of this fabricated nanowire array immobilized superhydrophobic substrate, which supplied oxygen for the enzymatic reaction with much higher transport efficiency than a solid–liquid diphase interface. As a result, the biosensing performance was significantly improved. In addition, the photocurrent response properties of TiO$_2$ NWs and TiO$_2$ NPs were evaluated. The results demonstrate that the former electrode had higher sensitivity and a lower detection limit than the latter [70].

In order to improve the visible light absorption performance of metal oxide-based semiconductors without an input offset voltage, coupling them with a narrow bandgap of nanomaterials or sensitizers is the most commonly used and most efficient strategy. For instance, Wei et al. fabricated MoO$_3$-modified TiO$_2$ nanoneedle photoelectrodes with carbon cloth as the substrate. The well-matched alignment of the as-prepared nanocomposites efficiently depressed the recombination of photogenerated electron–hole pairs and a fourfold increase in photocurrent intensity was accomplished [71]. As for QD sensitization, based on the fact that carboxyl- and amino-modified CdTe QD-immobilized ZnO NRs could significantly accelerate the transport of photogenerated electrons, Yu et al. proposed a cascaded photoactive nanostructure that significantly improved the visible light absorption property and amplified photocurrent responses [72]. Zhang et al. developed a highly selective PEC sensor for lysozyme by using Au NP-decorated Fe$_2$O$_3$ NRs as photoactive electrodes. The high aspect ratio of the fabricated nanorod structure was beneficial to shortening the transport distance of the photogenerated electrons. Meanwhile, the Au NPs on the surface of Fe$_2$O$_3$ NRs facilitated the depression of the recombination of charge carriers as well. As a result, the detection limit of lysozyme was shown to be 3 pM [73].

2.2.2. Metal Chalcogenide-Based Semiconductors

As narrow-bandgap semiconductors, CdS nanostructures with various morphologies have been fabricated for PEC biosensing [74,75]. Among them, 1D-structure CdS NRs have shown tremendous potential in PEC applications due to the significantly increased binding sites and the decreased radial transport distance [76]. For example, Lei et al. fabricated beta-cyclodextrin-modified CdS NRs ($\beta$-CD@CdS NRs), which were applied to detect human immunodeficiency virus (HIV) DNA based on a target triggered biocatalytic precipitation (BCP) signal strategy combined with catalytic hairpin assembly (CHA) for signal amplification. Due to the excellent photovoltaic property of CdS NRs and
the reliable biorecognition peculiarity of β-CD, the detection limit of the biosensor was calculated to be 1.16 fM [77]. Illuminated by the outstanding catalytic performance of single-atom metal materials [78,79], using CdS NRs decorated with platinum single atoms (Pt SAs/CdS) as the photosensitive material, Zhu et al. constructed an ultrasensitive PEC immunoassay system for PSA detection based on an ion-exchange reaction (Figure 3b). As shown, although the decoration of both Pt NPs and Pt SAs can significantly increase the photoconversion efficiency, the photocurrent intensity of Pt SA/CdS NR electrodes was almost twofold higher than that of Pt NPs/CdS NRs due to the increase in exited charge-carrying concentration of CdS NRs induced by Pt SAs [80].

![Figure 3. (a) Schematic illustration of the solid–liquid–air triphase bio-photoelectrode (TBP-electrode). Reprinted with permission from ref. [70]. Copyright 2018, John Wiley and Sons; (b) schematic illustration of the mechanism of Pt SA-CdS-based PEC biosensing platform and photocurrent responses for CdS, Pt SAs-CdS, and Pt NP-CdS. Reprinted with permission from ref. [80]. Copyright 2021, American Chemical Society.](image)

Due to the notable near-IR and visible light-harvesting characteristic and the regulable bandgap, bismuth sulfide (Bi$_2$S$_3$) exhibits great potential in PEC bioanalysis applications [81,82]. For instance, Zhang et al. used Bi$_2$S$_3$ NRs as the photosensitive material to develop a “signal on” PEC sensing platform for the detection of polynucleotide kinase (PNK). Because of the high visible light absorption coefficient of Bi$_2$S$_3$ NRs and the dual-signal amplification strategy of steric hindrance coupled with nanozyme catalytic precipitation, the biosensor presented a low detection limit of 1.27 × 10^{-5} U·mL$^{-1}$ [83]. NW structures with very small diameters (typically smaller than Bohr radii) are often called ultrathin semiconductor nanowires (USNWs). Contrasted with regular NWs, USNWs demonstrated outstanding PEC performance due to their higher aspect ratio and quantum size effect. From this, Li et al. synthesized ZnSe USNWs with different diameters, and their size-controlled bandgaps and photoelectrical properties were evaluated [84].

2.2.3. Carbon Nanotube-Based Semiconductors

With the appearance of various allotropes of carbon, carbon nanomaterials have attracted close attention in many biosensing applications due to their special electronic structures and fascinating physicochemical properties. As one kind of promising 1D nanostructure, carbon nanotubes (CNTs) demonstrate excellent photogenerated electrons transfer property, which would significantly enhance the photocurrent signals of photoactive nanomaterials during PEC analysis.

For instance, Yang et al. fabricated a TiO$_2$ NP-coated multiwalled CNT nanohybrid on the substrate of FTO for the biosensing of microcystin-LR (MC-LR). The photocurrent of TiO$_2$@CNT/FTO is about eight times than that of TiO$_2$ (P25)/FTO due to the notable
enhancement in conductivity of the core–shell nanostructure, and the sufficient covalent bonds induced electronic coupling and interaction on the nanohybrid interface [85]. Considering the fact that the heterojunction of an amine-modified MIL-68(In) coupled with CdS NPs could efficiently suppress the recombination of photogenerated electron–hole pairs and the multiwalled CNTs could dramatically enhance the photoelectric conversion properties by regulating the electrons transport paths, Yan et al. constructed a sensitive PEC aptamer biosensor for tetracycline (Tc) sensing by employing MIL-68(In)-NH$_2$/MWCNT/CdS/ITO as the photoactive electrode. Under the optimal condition, the photocurrent intensity of the fabricated MIL-68(In)-NH$_2$/MWCNT/CdS hybrid was improve to 7.8 times and 5.5 times higher than that of ITO/CdS and ITO/MIL-68(In)-NH$_2$/CdS, respectively. The detection limit of the proposed biosensor was calculated to be 0.015 nM [86]. In order to avoid the possible effect of the photoelectric conversion process on biological recognition events, Fan and Shi et al. constructed a double-electrode PEC carcinoembryonic antigen (CEA) bioassay platform with ZnIn$_2$S$_4$-immobilized Fe$^{3+}$-doped TiO$_2$ NTs (ZIS/Fe:TiO$_2$ NTs) as the photoanode and Ag NP-decorated CNTs (Au/CNTs) as the cathode for biological recognition. The result demonstrate that the photogenerated electrons that came from the ZIS/Fe:TiO$_2$ NT photoanode were collected rapidly by the Au/CNT-anchored cathode due to the excellent electrical conductivity of Au NPs and CNTs, which greatly improved the sensitivity and selectivity of the developed PEC biosensor [87].

2.3. 2D-Nanostructure Materials

Since graphene sheets were successfully exfoliated from graphite in 2004 [88], 2D-nanostructure materials have attracted great concern in biosensing applications. 2D materials have many unique physicochemical properties, such as large surface area, abundant active sites, excellent electrical conductivity, tunable bandgap, and outstanding biocompatibility [89]. As a result, these nanostructures have exhibited promising prospects in the PEC bioassay field. Generally, the most commonly used 2D nanostructure for the construction of PEC detection platforms is nanosheets, which are a planar structure with a thickness of less than 100 nm. Except for graphene, other 2D nanosheets, such as graphene-like carbon nitride (g-C$_3$N$_4$), transition metal dichalcogenides, bismuth oxyhalides, 2D organic frameworks, MXenes (e.g., Ti$_3$C$_2$), and Xenes (e.g., black phosphorus), have enriched the family of photoelectrical nanomaterials. Given space limitations, only several typical types of 2D nanostructures will be introduced here.

2.3.1. Carbon-Based Graphene-like 2D Nanostructures

As the typical representative of 2D nanostructures, graphene has excellent electron-transport ability and satisfactory surface functionalization properties because of its outstanding electron conductivity and high specific surface area. Therefore, the sensitivity and selectivity of PEC bioanalysis are expected to be highly improved by the introduction of graphene nanomaterials. Because the bandgap of graphene is zero, this material is usually used to collect and transport electrons. Therefore, numerous graphene-based nanohybrids have been fabricated to improve their photoelectric conversion efficiency. For instance, contrasted with Mo-doped BiVO$_4$ (Mo-BiVO$_4$), the further incorporation of graphene achieved six times more enhancement in photocurrent signals, which demonstrates that graphene efficiently accelerated the transfer of electrons and facilitated the separation of photogenerated electrons and holes. In addition, the π–π stacking interaction of graphene also showed great potential in coupling with targets [90].

In order to improve the photoelectric conversion activity of graphene, heteroatom doping is another widely accepted way, by which the graphene adjusts its bandgap to improve its visible light-harvesting ability. In particular, boron (B) and nitrogen (N) are the most reported dopants because both of them have similar electronegativity to C, which makes graphene exhibit a special electronic structure. From this, Wang fabricated a high-performance photoactive electrode by attaching Bi$_2$MoO$_6$ to the N and B co-doped graphene (BNG). Due to the synergy of oxygen vacancy generated form the reduction of Bi$_2$MoO$_6$
and BNG in contrast with Bi$_2$MoO$_6$, Bi$_2$MoO$_6$/graphene, Bi$_2$MoO$_6$-N-doped graphene and Bi$_2$MoO$_6$/B-doped graphene, the Bi$_2$MoO$_6$/BNG exhibited increases of about elevenfold, sevenfold, threefold, and twofold in photocurrent intensities, respectively [91].

Aside from doping heteroatom-doped graphene, graphene oxide (GO) fabricated from the oxidation of graphite has also received growing interest in PEC analysis due to its abundant active sites, aqueous dispersity, and narrow bandgap [92]. For example, Lin et al. fabricated a photoactive electrode with excellent PEC performance by coupling a porphyrinic metal–organic framework with reduced graphene oxide (PCN-224/rGO), which was used to detect p-arsanilic acid (p-ASA) depending on the strong affinity between the target and the as-prepared transducer. Because the recombination of photogenerated charge carriers was inhibited efficiently, the sensitivity of the biosensor was significantly improved and a detection limit of 5.47 ng L$^{-1}$ was obtained [93]. Apart from being used as transducers, GO can also be treated as a nanoprobe [94]. As shown in Figure 4a, considering the attractive PEC performance of alkylated C$_{60}$ (AC$_{60}$), the excellent electronic conductivity of graphite flakes (Gr), and the abundant carboxyl active sites of GO, Zhang et al. fabricated a photoactive nanohybrid of AC$_{60}$-Gr-GO. Under illumination with visible light, the photocurrent signal of AC$_{60}$-Gr-GO was 35 times higher than that of AC$_{60}$, whereafter, using the as-prepared AC$_{60}$-Gr-GO as the labeled conjugate, a high-performance immunosensor for alpha-fetoprotein (AFP) was developed [95].

As the most stable allotrope of carbon nitride polymer, g-C$_3$N$_4$ constructed from the sp$^2$ hybridization of carbon atoms possesses a similar layer structure as graphene and metal-free covalent semiconductor properties [96]. Due to the comparatively small bandgap of 2.7 eV, easy fabrication, low toxicity, and high photoelectric conversion ability, g-C$_3$N$_4$ has attracted much interest in the construction of PEC biosensors. However, the relatively poor charge conductivity and the higher recombination rate of electrons and holes substantially influence the applications of g-C$_3$N$_4$ in PEC sensing.

Addressing the aforementioned issues, various approaches have been proposed to improve the photoelectric properties of g-C$_3$N$_4$ nanomaterial. For instance, ultrathin 2D nanosheets that were reduced from bulk C$_3$N$_4$ were used for the sensitive PEC detection of small molecular compounds [97]. Considering the fact that the recombination of photogenerated charge carriers generated from graphitic carbon nitride can be efficiently inhibited by the nitrogen vacancies, Li et al. prepared nitrogen-deficient graphitic carbon nitride-modified ITO electrodes, which were used for highly sensitive PEC analysis of ciprofloxacin [98]. Besides, heteroatom doping is another valid strategy to enhance the photoelectric activity [99]. To do so, Wang et al. prepared Cd/N@g-C$_3$N$_4$ photoactive nanomaterial by coupling the N$_2$ plasma treatment with the deposition of a Cd probe. The doping of N significantly enhanced the PEC response of the yielded nanocomposite to H$_2$S, due to the formation of an efficient Z-scheme heterojunction followed by the in-situ generation of CdS. On the basis of the high charge transport property between CdS and g-C$_3$N$_4$ resulting from the doped N, a “signal on” PEC sensing platform for H$_2$S was constructed [100]. Using a similar strategy, and employing a Z-scheme heterojunction of CoO/Au@g-C$_3$N$_4$ as the photoactive nanomaterial, Zeng et al. proposed a PEC aptasensor for the sensing of MC-LR based on the increment of photocurrent responses caused by the target-triggered consumption of photogenerated holes. In this work, the Z-scheme heterojunction was well fabricated because of the perfectly matched bandgaps of CoO and g-C$_3$N$_4$ coupled with the imbedded Au NPs, which not only served as the electron transfer media but also improved the visible light-harvesting ability of the nanohybrid because of the SPR effect [101]. Apart from the abovementioned binary heterostructures, multiple g-C$_3$N$_4$-based heterostructures have also been reported to develop PEC biosensors [102]. For instance, through the in-situ growth approach, Wei et al. fabricated an SnO$_2$- and SnS$_2$-modified mesoporous g-C$_3$N$_4$ (SnO$_2$/SnS$_2$/mpg-C$_3$N$_4$) nanohybrid, which resulted in the significant improvement of PEC responses in contrast with their single materials or binary hybrids due to the well-matched bandgaps. By employing PbS/SiO$_2$-labelled secondary antibody as the quencher, a PEC immunosensor with a sandwich structure was
constructed for the sensing of N-terminal pro-brain natriuretic peptide [103]. Similarly, using TiO$_2$/g-C$_3$N$_4$/CdS composite to serve as a photoactive matrix and CdSe QDs as a sensitizer, Zhu et al. fabricated an ultrasensitive PEC detection platform for the sensing of T4 polynucleotide kinase based on the target-triggered sensitization of CdSe QDs, and the detection limit was shown to be 2.5 pg·mL$^{-1}$ [104]. In addition to coupling with inorganic semiconductors, the photoelectric property of g-C$_3$N$_4$ can also be enhanced by organic modification. For instance, on the basis of π-stacking interactions, Song et al. fabricated a g-C$_3$N$_4$-functionalized two-dimensional (2D) pyrene covalent organic framework (PAF-130/g-C$_3$N$_4$), which exhibited substantially enhanced photoelectric conversion efficiency due to the efficient separation of photogenerated electrons and holes resulting from the well-matched bandgaps between PAF-130 and g-C$_3$N$_4$. This novel nanohybrid demonstrated promising potential in the construction of ultrasensitive PEC bioassay platforms [105].

![Figure 4](image-url)  
**Figure 4.** (a) Scheme of general processes for the assembly of AC$_{60}$-Gr-GO and construction of the PEC AFP immunosensor. Reprinted with permission from ref. [95]. Copyright 2018, American Chemical Society; (b) schematic illustration of the construction of a PEC biosensor based on the MoS$_2$/AAO photoactive nanochannel. Reprinted with permission from ref. [106]. Copyright 2020, John Wiley and Sons.

### 2.3.2. Transition Metal Dichalcogenides (TMDs)

As one of the most important families of 2D nanostructures, TMDs present a laminated structure with a chemical formula of MX$_2$. Here, M represents the transition metal elements, particularly molybdenum (Mo) and tungsten (W), and X refers to chalcogen, such as sulfur (S), selenium (Se), and tellurium (Te). Similar to graphene, TMDs exhibit layered 2D nanostructures, which highly increases their surface area and efficiently closes the transfer distance of photogenerated charge carriers.
Among the various TMD nanomaterials, MoS$_2$ has received the most concerns in the applications of PEC bioassay due to its high optical absorption coefficient and long photoexcited carrier lifetimes. However, limited by the high recombination efficiency of photogenerated electron–hole pairs, hybridizing 2D nanostructures of MoS$_2$ with another compound is a great approach to acquire higher analytical performances. Taking Au NPs, for example, Liu et al. proposed an ultrasensitive PEC sensor for miRNA-155 assay using the photoactive anodic aluminum oxide (AAO) nanochannels functionalized with Au NP-deposited MoS$_2$ flakes. As shown in Figure 4b, MoS$_2$ film was modified on the channel of AAO by atomic layer deposition (ALD). The following decorating of Au NPs not only enhanced the photoelectric conversion efficiency of MoS$_2$ because the Au/MoS$_2$ Schottky junction might have efficiently improved the separation of electron–hole pairs, but also facilitated the immobilization of bio-recognition elements. Employing MoS$_2$/AAO-connected silver mesh as the working electrode, the detection limit of the proposed biosensor was shown to be 3 aM based on the steric effect [106]. Except for the noble-metal NPs sensitization, the constructing heterojunction is another way to improve the performances of MoS$_2$-based PEC biosensing applications. With this consideration, based on the well-matched bandgaps of n-type ZnO nanorods and p-type MoS$_2$ flakes, Niu et al. fabricated a MoS$_2$/ZnO heterostructure that greatly improved its light-harvesting ability and accelerated the separation of photogenerated charge carriers as well. The as-prepared heterostructure-modified ITO photoactive electrodes were successfully used for the highly selective detection of propyl gallate (PG) on account of the specific recognition of Zn (II) and oxygen atoms of PG [107]. As we know, dye sensitization is beneficial for nanomaterials to improve their light-response activities [108]. For example, the bandwidth of MoS$_2$ nanosheets would be obviously narrowed after being decorated with zinc phthalocyanine NPs (ZnPc NPs). Furthermore, in contrast with MoS$_2$, the fabricated ZnPc/MoS$_2$ nanohybrid received a two-fold improvement in carrier lifetime and a 24-fold increment in photocurrent intensity under visible light illumination [109]. In addition, 2D MoS$_2$ can also serve as the nanoprobe for the construction of PEC bioassay architectures [110].

A layered WS$_2$ nanostructure is another kind of TMD of extensive interest that has a narrow bandgap of about 1.3 eV and a large specific area [111]. However, its relatively high recombination efficiency of photogenerated charge carriers is still a tough challenge in PEC biosensing applications. Addressing this issue, combining WS$_2$ with appropriate functional nanomaterials is a widely reported approach to improve the PEC performance [112,113]. For example, considering the well-matched bandgaps of WS$_2$ and g-C$_3$N$_4$, Yin et al. fabricated an Au NP-decorated C$_3$N$_4$-WS$_2$ heterojunction that demonstrated a significantly improved photoelectric conversion property resulting from the notably improved transport rate of electrons and the efficient separation of photogenerated electrons and holes. The as-prepared photoactive nanohybrid was employed to construct a sensitive PEC DNA-sensing platform based on the target DNA, which induced the capture of MnO$_2$ nanoflowers that suppressed the photocurrents by the consumption of the electron donor [114]. For another example, Yin et al. employed the composition of MoS$_2$ nanosheet- and WS$_2$ nanosheet-modified ITO as the photoactive electrodes and the amino-functionalized Fe$_2$O$_3$ and SMCC as the linker. After 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT) was fixed on the photoelectrode, the target 5-formylcytosine (5fC) was captured by AHMT, which further triggered the immobilization of black TiO$_2$ (B-TiO$_2$) on the surface of the WS$_2$/MoS$_2$/ITO electrode. In this way, the target-controlled increase in photocurrent signal was monitored due to the generation of a B-TiO$_2$/WS$_2$/MoS$_2$/ITO ternary heterojunction, which significantly enhanced the transport rate of the photogenerated charge carriers and extended their lifetimes, as well [115].

2.4. 3D-Nanostructure Materials

The 3D-nanostructure materials discussed here typically refer to the single architecture constructed by 0D-, 1D-, and 2D-nanoscale materials [6]. Currently, 3D architectures have attracted growing interest in PEC bioassays [116–118] because their carefully fabricated
hierarchical and biomimetic architectures usually exhibit many attractive advantages, such as excellent target-capture ability, outstanding signal-amplification properties, higher catalytic activity, and notable sensing performances. Structured according to the chemical compositions, different 3D architectures with morphologies like mimicking flowers, coral, and rich wrinkles, which have been widely reported in PEC biosensing, will be discussed in the following part.

Under elaborated preparation, various metal chalcogenides with 3D biological hierarchical architectures have been employed in PEC sensing applications due to their fantastic electrical and optical properties [119,120]. As demonstrated in Figure 5, a peony-like MoS$_2$ nanosheet directly growing on the nanohole-patterned TiO$_2$ was fabricated by Mahshid et al. After the further deposition of Au NPs, the light-absorption region of the hybrid was broadened to 700 nm, resulting from the outstanding electronic conductivity and SPR effect of the Au NPs. Based on the special morphology and the excellent PEC property of the prepared 3D MoS$_2$/Au hybrid, the detection limit for glucose was 1.3 nM [121].

![Figure 5. Nanopattern-assisted direct growth of binary MoS$_2$/gold electrode and the PEC process for glucose detection by peony-like 3D MoS$_2$/Au. Reprinted with permission from ref. [121]. Copyright 2020, American Chemical Society.](image)

Other examples of 3D architectures that have been reported for use in PEC biosensing are ternary compound semiconductors. For example, ZnIn$_2$S$_4$ with 3D architectures have drawn much interest in PEC bioassay applications due to their low cost, high chemical stability, and great visible light-harvesting properties. However, the relatively short lifetime of photogenerated charge carriers is still a great challenge that 3D ZnIn$_2$S$_4$ architectures face. Addressing this issue, Li et al. proposed a lanthanum-doped CdS-immobilized flower-like ZnIn$_2$S$_4$-modified Au@ZnO photoactive electrode (La-CdS/ZnIn$_2$S$_4$/Au@ZnO) for the sensitive detection of aminoterminal pro-brain natriuretic peptides (NT-proBNP) based on the steric effect. The La-CdS with a narrow bandgap significantly improved the visible light-absorption efficiency and efficiently suppressed the recombination of photogenerated electron–hole pairs, which greatly enhanced the PEC biosensing performances of the 3D ZnIn$_2$S$_4$-based photoactive materials [122]. Zhang et al. fabricated a Schottky junction by decorating Bi NPs on the hollow porous ZnSnO$_3$ microspheres (Bi/ZnSnO$_3$), which have much more electron-transport channels and abundant active sites for surface recognition. On account of the SPR effect of metallic Bi NPs, the number of photogenerated charge carriers of the Bi/ZnSnO$_3$ composite was significantly increased, which greatly improved the photocurrent intensity of the as-prepared photoactive material. Furthermore, compared with pure ZnSnO$_3$, the light-absorption edge of Bi/ZnSnO$_3$ was broadened from 397 nm to the near-infrared region, as well. Based on the specific chelating coordination interaction between the target of norepinephrine (NE) and Zn$^{2+}$ coupled with the high photoelectric activity of the Bi/ZnSnO$_3$ composite, an ultrasensitive and highly selective PEC biosensing platform was successfully constructed [123].
Apart from the abovementioned metal-based 3D architectures, various carbon-based 3D architectures also presented promising prospects. Taking 3D nitrogen-doped graphene hydrogel (3DNGH), for example, the analyte-trapping performance was dramatically improved because of its porous hierarchical structure, which offers abundant active sites. Besides, the doping of N is beneficial for enhancing its light-harvesting property and accelerating the separation of photogenerated electrons and holes [124]. Another interesting example is Gong et al., who proposed a coral-like g-C₃N₄ that had been applied to PEC detection of metronidazole (MNZ) in medicine samples. Through the morphology control, the coral-like g-C₃N₄ with an interlaced porous network structure demonstrated particular effects on antibody anchoring, electron transport, and the separation of electron–hole pairs [125]. In another study, flower-like Au-decorated 3D reduced graphene oxide (3D-rGO) was fabricated on the substrate of paper cellulose fiber for the construction of a chemiluminescence-driven PEC lab on a paper platform. In contrast with 2D architecture, the enlarged specific surface area of the 3D-rGO greatly facilitated the immobilization of functional groups. Furthermore, the corrugated 3D architecture was beneficial for efficient mass and electron transfer, which provided a guarantee to construct the ultrasensitive PEC thrombin biosensor [126].

Some examples of 0D-, 1D-, 2D-, and 3D-nanostructure materials for PEC sensing applications are summarized in Table 1 for intuitive comparison.

### Table 1. Summary of representative nanostructure materials for PEC analysis discussed in this review.

<table>
<thead>
<tr>
<th>Nanostructure</th>
<th>Feature</th>
<th>Material</th>
<th>Target</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>QDs</td>
<td>Easily regulated bandgaps, excellent surface modification property and similar size to many biomolecules</td>
<td>CdTe QDs, ZnSe@CuS QDs, DNA-TET-CdTe QDs, TFP doped Pdots</td>
<td>CA125, Hg²⁺, miRNA-141, Telomerase</td>
<td>[16], [17], [35], [23]</td>
</tr>
<tr>
<td>Carbon-based NPs</td>
<td>Low cytotoxicity, resistance to photobleaching, and fascinating biocompatibility</td>
<td>N-doped C-dots, GOD@Mo-Av-A</td>
<td>CEA, N-glycan expression on the surface of MC7 cells, CCRF-CEM cell</td>
<td>[38], [44], [46]</td>
</tr>
<tr>
<td>Noble-metal NPs</td>
<td>Easy to synthesize in different sizes, excellent stability and biocompatibility</td>
<td>Au NCs–Ag@SiO₂, Au@Ag NPs–TiO₂ NRs, Ag@Au asymmetric core–satellite</td>
<td>Alkaline phosphatase activity, DNA, PNA</td>
<td>[50], [52], [57]</td>
</tr>
<tr>
<td>Metal-oxide NWs</td>
<td>Strong light absorption property, low toxicity, and high chemical stability and biocompatibility</td>
<td>TiO₂ NWs, TiO₂ nanowires @MoO₃, ZnO NRs–CdTe QDs, Fe₂O₃ NRs–Au NPs</td>
<td>Glucose, RAW264.7 cells, DNA sequence of HIV-1 lysozyme</td>
<td>[70], [77], [80]</td>
</tr>
<tr>
<td>Metal-chalcogenide NRs</td>
<td>Narrow bandgaps, significantly increased binding sites, and decreased radial transport distance</td>
<td>β-CdTe@CdS NRs, Pt/SnO/Cds NRs, Bi₂S₃ NRs</td>
<td>DNA sequence of HIV, PNA, PNA</td>
<td>[79], [83], [86]</td>
</tr>
<tr>
<td>Carbon NTs</td>
<td>Excellent photogenerated electron-transfer property</td>
<td>TiO₂, NPs@CNTs, MIL-68(In), NH₂–MWNT/CdS NPs, Au NPs@CNPs</td>
<td>MC–LR, Tc, CEA</td>
<td>[85], [87], [97]</td>
</tr>
<tr>
<td>Carbon-based graphene-like nanostructures</td>
<td>Outstanding electron conductivity and high specific surface area</td>
<td>BiMoO₆/BNG, PCN-224/GO, AC₇₁/Cd-GO, CoO/Au NPs–g-C₃N₄, SnS₂/NbS₂–mpg-C₃N₄, TiO₂/g-C₃N₄/CdS, PAF-β-3D-g-C₃N₄</td>
<td>Lincomycin, p–ASA, α-FP, MC-LR, N-terminalpro-brain natriuretic peptide, 14-polymeroduct ketone kinase, α-synuclein</td>
<td>[93], [95], [98], [100], [104], [105]</td>
</tr>
<tr>
<td>TMDs</td>
<td>High specific surface area and short photogenerated charge-carrier transfer distance</td>
<td>AAO-MoS₂ film, MoS₂, ZnO–MoS₂ NPs, Nanosheets, Au NPs–g-C₃N₄, WS₂ NPs, MoS₂ nanosheets</td>
<td>miRNA-155, p–ASA, Edelfosph, SiC, SiC</td>
<td>[100], [107], [109], [114], [115]</td>
</tr>
<tr>
<td>Metal chalcogenides</td>
<td>Mimicking flower-like morphologies and excellent target-capture ability</td>
<td>Peony-like MoS₂ Nanosheet Au NPs</td>
<td>Glucose</td>
<td>[121]</td>
</tr>
<tr>
<td>Ternary compound semiconductors</td>
<td>Low cost, high chemical stability, and great visible light-harvesting property</td>
<td>La₃–CdS–ZnIn₂S₄, Au@ZnO, Bi NPs–ZnO, SiO₂, microspheres</td>
<td>miRNA-141, NE, NT-proBNP</td>
<td>[122], [123], [125]</td>
</tr>
<tr>
<td>Carbon-based architectures</td>
<td>Abundant active sites, high light-harvesting property, and fast charge-carrier transfer speed</td>
<td>Coral-like g-C₃N₄, Flower-like Au-3D-rGO</td>
<td>MNZ, Thrombin</td>
<td>[126]</td>
</tr>
</tbody>
</table>
3. Novel PEC Biosensing Patterns

In the past few decades, PEC biosensing has made great advances due to its distinct advantages of high sensitivity, easy operation, low cost, and increased flexibility. However, considering the increasing demands for portability and the integration of sensing devices, the development of novel PEC biosensing patterns with even greater performance has become more and more urgent. Therefore, a series of relevant reported research works will be discussed in this part.

3.1. Mimic Enzyme Assay

Due to the excellent specificity, natural enzymes have been widely used in PEC bioassays. However, the poor stability, tedious preparation, and relatively high prices are the main challenges to their further application. In recent years, it has been found that some nanomaterials such as metal NPs [127], carbon materials [128,129], and metal oxides [130] can demonstrate enzyme-like characteristics. Because of the excellent stability, high catalytic activity, and low cost, nanomaterial-based mimic enzymes have attracted great research interest in the construction of PEC analytical platforms.

Among various artificial enzymes, horseradish peroxidase (HRP)-like mimic enzymes are the most commonly used catalysts during PEC biosensing applications. For instance, Zhang et al. synthesized manganese-based mimic enzyme (MnME) that could catalyze the oxidation of DAB to produce insulating precipitate due to its excellent peroxidase-like activity. Using Au NP-decorated MnME as the nanoprobe and Au NP-modified Bi$_2$S$_3$ nanorods as the photoactive material, a highly sensitive “signal off” PEC biosensor for polynucleotide kinase activity was proposed (Figure 6a) [83]. Based on the similar mimic enzyme catalytic precipitation strategy, numerous advances in nanozyme research have been reported [112,131–134]. In addition, according to mimic enzyme-induced in situ generation of electron acceptors or donors, Dai et al. proposed a “signal on” PEC biosensor for the detection of H$_2$O$_2$ in vitro by using G-quadruplex/hemin/Pt NP nanocomposite as the catalyst. Specifically, after being anchored on the surface of C$_3$N$_4$ nanosheet/PbS QDs/ITO electrode, the as-prepared mimic enzyme catalyzed the oxidation of H$_2$O$_2$ to generate the electron donor of O$_2$, which accelerated the separation of photogenerated electrons and holes and eventually improved the photocurrent intensities [135].

Recently, except for the abovementioned peroxidase mimic enzymes, RNA-cleaving DNA enzymes (DNAzymes) have demonstrated promising results due to their advantages of great stability and excellent specificity [136]. With the activation of metal ions, DNAzymes catalyzed substrate strands to cleave into two fragments at the position of RNA [136,137]. For instance, on the basis of target Pb$^{2+}$ triggering the cleave of substrate strand DNA1 resulting from the catalysis of CdS QD-modified DNAzyme (DNA2-Cd QD), which significantly suppressed the photocurrent signals due to the disassembly of the TiO$_2$/Au/CdS QD-constructed “Z-scheme”, Chen et al. developed a novel PEC sensor for the detection of Pb$^{2+}$ with the detection limit calculated to be 0.13 pM [138].

3.2. Self-Powered Sensing

With the constantly emerging numerous splendid signal strategies, PEC analysis has attracted great interest in the biosensing field. However, to meet the request of clinical applications, the development of portable and simple instruments is urgently needed. Recently, self-powered sensors, which can work without external power supplies, have aroused increasing interest in biosensing applications. Different from traditional PEC sensing devices, self-powered PEC biosensors usually employ two electrodes that serve as the photoanode and photocathode. Under illumination, the current intensities, which result from the oxidation occurring on the photoanode coupled with the reduction occurring on the photocathode, respond to concentrations of the targets and highly sensitive self-powered assays can be achieved. For example, with the tyrosinase (Tyr)-triggered oxidation of phenol, the generation of catechol serving as the electron donor significantly improved the photocurrent response of the g-C$_3$N$_4$-Bi$_2$S$_3$ photoanode. Based on this, and combined
with the tris (2-carboxyethyl) phosphine (TCEP)-induced chemical redox cycling of catechol, an ultrasensitive and selective self-powered PEC biosensor for Tyr was developed [139]. The traditional PEC assay in particular can be converted into a self-powered PEC assay by the regulation of electron acceptors or donors. For instance, as the working electrode, Au NP @ZnSe nanosheet-modified gold electrodes (Au NPs @ZnSe NSs/GE) only had very low photocurrent signals, as the bias voltage was at 0 V. However, with the electron acceptor of methylene blue (MB) and p-nitrophenol (p-NP) introduced into the electrolyte solution, the photocurrent intensities of the electrodes were greatly enhanced. Based on this, Hun et al. proposed a novel self-powered PEC sensor for sensing KLK2 and PCA3 [140]. Except for the regulation of the electrolyte solution, well-designed photoactive electrodes have been proven to be another efficient approach to constructing self-powered PEC sensors. As exhibited in Figure 6b, Tang et al. proposed a self-powered PEC biosensor for the detection of oxytetracycline (OTC) with a two-electrode system that included the Z-scheme nanostructure photoanode of WO₃/g-C₃N₄/MnO₂ and the aptamer-based photocathode of Au NP-decorated rGO. Owing to the excellent light-harvesting ability and the high separation efficiency of the electrons and holes of the prepared hybrid, the great electrical conductivity of Au NPs, and the abundant active sites of rGO, the detection limit of the as-fabricated self-powered PEC sensor was shown to be 0.1 pM [141].

### 3.3. Dual-Readout Assay

In contrast with single-readout protocol, dual-readout assays that couple PEC analysis with other simple and convenient sensing modes have attracted increasingly intense interest due to their highly improved accuracy resulting from intercalibration and widened linear ranges. Taking photothermal sensing, for example, and considering the target-induced concentration changes of the photothermal materials aroused notable changes in temperature, Dai et al. proposed a simple dual-readout sialic-acid sensor by employing TPP-Pdots as the integrated signal probe of PEC and photothermal sensing. On the basis of sandwich-type immune recognition, the targets were able to trigger an increment in photocurrent intensities and temperature signals [142]. Combining PEC sensing with other electrochemical detection modes is also an efficient approach to constructing dual-readout biosensors. For example, it was found that the catalytic precipitation property of AuPtPd nanozymes could be regulated by urease-induced proton-consuming reactions. Inspired by this, and employing BiVO₄-modified ITO as the photoelectrode and AuPtPd nanospheres as peroxidase-like nanozymes, a photocurrent and electrochemical impedance signal-based dual-readout biosensor for the detection of urease activity was proposed [143].

In regard to coupling PEC sensing with electrochemiluminescence (ECL) strategy, based on the glutathione-triggered Mn²⁺-dependent DNAzyme amplification and DNA walker-controlled immobilization of CdS:Mn-streptavidin probs, Jie et al. fabricated a versatile dual-readout ECL and PEC biosensor for glutathione determination in human serum samples [144]. Visual detections have drawn increasing interest due to the simple devices and convenient operation. Coupling naked-eye readout with PEC sensing not only guarantees the accuracy of results but also improves the detection efficiency. For example, using CuInS₂ microflower-modified ITO as photoactive electrodes and antibody-labelled chlorin e6 (Ce6) as upconversion nanomaterials, which served as both PEC and visible signal probes, a PEC and near-infrared (NIR)-driven fluorescence visualization biosensor for the immunoassay of PSA was constructed by Ouyang et al. based on target-triggered sandwich immunobinding (Figure 6c) [145].
Figure 6. (a) Schematic illustration of a signal-on PEC biosensing for PNK assay based on the strategy of MnME@Au NP-P2 catalytic precipitation. Reprinted with permission from ref. [83]. Copyright 2018, American Chemical Society; (b) PEC mechanism of the self-powered photoanode-supported PEC cathodic sensing. Reprinted with permission from ref. [141]. Copyright 2021, American Chemical Society; (c) schematic illustration of the mechanism for the integrated NIR fluorescence-induced naked-eye readout and photoelectrochemical sensing. Reprinted with permission from ref. [145]. Copyright 2021, American Chemical Society.

4. Conclusions and Perspectives

With the substantial development of material technology and the high customization of biological systems, PEC biosensing has achieved great progress. According to remarkable advances in the last five years, this review summarized the functional nanostructured materials with different dimensionality employed in PEC bioanalysis. The morphology regulation of nanostructures plays a crucial role in the improvement of sensing performance. Compared with 0D, 1D, and 2D nanostructures, 3D-aligned arrays have more favorable electron transfer routes due to the multi-dimensional space and can offer larger surface area as well as uniform pore size for target capture and detection. Several novel PEC biosensing patterns with outstanding advantages in low cost, miniaturization, and high accuracy were also discussed. We sincerely hope this overview can serve as a useful source for interested readers.

As a newly developed sensing technology, PEC bioanalysis still has some challenges to overcome for the practical applications. For example, the fabrication of photoactive electrodes with more stable and higher performance will still be the focus of future works. Easy-operating and highly reproductive sensing patterns remain to be constructed to meet the demand of clinical diagnosis and industrial monitoring. Besides, in a way, the introduction of physical light sources into the PEC analysis system is still a challenge for the commercialization of PEC biosensors. According to the practical application demand and the recent research status, further research in PEC sensing has some trends, as follows. (1) Constructing a photoelectric functional interface by using novel photoactive materials or advanced preparation techniques—for instance, tailoring photoactive nanostructures with specific morphologies to improve the target-capture ability and exploit materials...
with long-wavelength response to minimize the damage to biomolecules during the optical excitation. In addition, using novel fabrication techniques, such as screen printing and microelectromechanical systems for the preparation of electrodes, could efficiently suppress the interelectrode interferences and make it easy to carry out mass production.

(2) Miniaturizing and highly integrating the PEC sensing instruments. In order to meet the requirements of low cost and fast and simple operation, great efforts are needed to streamline PEC sensing devices and further improve their portability. In this regard, many interesting attempts have been made, such as displacing a physical light source with chemiluminescence (CL) for the excitation of photoactive materials [146], employing a self-powered detection model without external power supplies [147], and developing a portable PEC sensing device by integrating a CL-based signal-generating system and a paper-based supercapacitor coupled with a digital multimeter reading system [148]. Even so, most of these studies are still in their preliminary exploratory stage.

(3) Combining a PEC biosensor with multidisciplinary techniques to construct an integrated bioanalysis system. To meet the increasing demand for practical applications, integrating PEC analysis with other electronics or micromachining technologies is an effective approach. For instance, based on the inherent signal-amplification ability of organic electrochemical transistors (OECTs), Zhao et al. integrated one with a PEC biosensor and successfully realized the ultrasensitive detection of C-reactive protein. In contrast with traditional PEC biosensors, targets induced a change in the current signal of the novel OECT-PEC biosensor that was enhanced by nearly two orders of magnitude [149]. Nevertheless, as a developing field, there are many areas that need to be studied and improved, especially the signal mechanism under the irradiation of light.

(4) Developing PEC nanotools for single-cell research. Considering the cell-to-cell differences, single-cell analysis is quite essential to reveal the behaviors and functions of each individual cell. Recently, a nanopipette whose tip was modified with an organic probe/NiO/Ni film was developed for intracellular drug delivery and further PEC assay of oxidative stress [150]. Although materials with high charges might not be loaded into cells by electroosmosis, this work still shows the bright prospects of PEC in single-cell analysis. In all, with the continuous advancement of science and substantial attempts, the present issues PEC assay is facing have the potential to be solved in the coming years.

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