Clinical Images and Case Reports

Received 19 Jun 2024 | Revised 22 Jun 2024 | Accepted 05 Jul 2024 | Published Online 24 Jul 2024

Published By: Vision Publisher

CICR 02 (7), 67-79

ISSN 2942-0776

Biosensors: Carbon Nitride–Based Biosensors, Opportunities for Biosensors by Using of Carbon Nitride, Engineering of Micro/Nanostructures (Nanosheets) and Electro-Chemiluminescent Bio-Sensing

KADHIM JAWAD KADHIM[1], GHASSAQ MAHDI RATEB[2], YOUSIF THAMER ABED OUN[3]

[1,2,3]Hussain University College, Medical Device Engineering, Iraq

Abstract: Outstanding photovoltaic effects were demonstrated by polymeric carbon nitride (CN), a type of potential two-dimensional conjugated matter. The attractive properties of CN, such as its photoelectrochemical features, abundance of surface functional groups, remarkable thermal and physicochemical stability, responsiveness to visible light, and facile synthesis from inexpensive raw materials with modulable electronic structures, have recently attracted increasing attention in the construction of biosensors. In addition to outlining the benefits of CN for biosensing applications, this chapter provides an introduction to the synthesis and structural engineering of CN materials. A lot of work has gone into improving the biosensing properties of CN materials, including as their solubility, signal conversion efficiency, and active surfaces/sites that interact with proteins. Numerous signal output modes and biosensor designs benefit from CN's exceptional optical properties, including photoluminescence, chemiluminescence, electrochemiluminescence, and photoelectrochemistry, all of which are the result of its narrow band gap and status as a metal-free semiconductor. Interestingly, in the last chapter, we present a few recent instances that utilise CN's unique features. These examples go beyond traditional configurations and sensing processes, and they further showcase the promising future of CN materials in biosensing.

Keywords: Biosensors, Carbon Nitride–Based Biosensors, Carbon Nitride, Nanostructures, Nanosheets, Electro-Chemiluminescent, Bio-Sensing

Corresponding Author: KADHIM JAWAD KADHIM†, Hussain University College, Medical Device Engineering, Iraq

Introduction

The compound carbon nitride (CN) is one of the initial polymer synthesis acknowledged by chemists; Liebig later renamed this compound ‘melon’ after their discovery by Berzelius in the year 1834. It was anticipated that CN may be separated into five distinct phases as research progressed: It belongs to five polymorphs namely; α-C_3N_4, β-C_3N_4, cubic -C_3N_4, pseudocubic - C_3N_4 and graphitic C_3N_4 (g-C_3N_4). g-C3 N4 is the most stable allotrope under the ambient conditions due to the Layered structure that is similar to graphite and hence it has attracted a lot of attention among the other phases that have been discussed. The aromatic planes formed by triazine or tri-s-triazine elements are found out to be arranged by very weak van der Waals’ forces [1, 2]. Actually, it has been pointed out that because of the de-amination or polymerisation of the nitrogen containing precursors not yet performed to completion, the most genuine g-C_3N_4 is in fact a defect rich N-bridged “poly(tri-s-triazine)/poly(triazine)” not corresponding to ideal C3N4 composition. Phenomena, biosensing, advancement in solar cell, nano architecture, NV memory and so on have experienced a marked increased interest towards CN in the recent few years because of its inherent properties and structural tunability.

Carbon Nitride's Alluring Qualities

It has already been elaborated that various exceptional qualities are revealed by CN materials. The basics of CN are carbon and nitrogen and both are naturally available on the Earth. As a consequence, CN does not require expensive or hard to find raw materials to be produced. Also, CN does not contain heavy metal ions and hence is considered easy to handle and manage when it comes to disposal [3-5]. Also, g-C3N4 and other CN materials possess excellent thermal and physicochemical stability and therefore their practical applications are seemingly unlimited. Specifically, thermal gravimetric analysis (T) proved that g-C_3N_4 was not decomposed and did not volatilise at up to 600°C in indoors. It oxidized and then disintegrated and the hardness of it was 700°C. G-C3N4 was also found to be resistant to extreme chemical environment such as acid, base, and organic solvents and thermal stability. Compared with other types of semiconductors, g-C3N4 possesses a moderate bandgap, which is regarded as the medium-bandgap semiconductor, and the conjugated polymeric network is also for its electrical benefits. 16 Due to this quality, it was widely used in photocatalysis, photoelectric transformation, and other vital catalytic processes, quite a number of which are linked with renewable power sources [6-8]. Subsequently, CN began to employ these semiconductor-driven properties—photoluminescence (PL), chemiluminescence (CL), electrochemiluminescence (ECL), and photoelectrochemistry (PEC)—in the development of novel sensors. The exceptional ability to convert light into electricity is one of the latter two processes that significantly raises the sensitivity of sensors based on g-C_3N_4. At the same time, g-C3N4's distinctive catalytic characteristics have allowed for the successful construction of a number of colorimetric sensors. In addition to the aforementioned intrinsic features, CN's aromatic, p-conjugated framework, intra-layer hydrogen bonds, and mild interface reactivity make it an easy molecular target for a wide range of optoelectronic property modulation [9, 10]. A realistic technique for constructing molecular structures allows for the incorporation of any desired molecules, components, and functional groups into the final CN structures. This improves the fundamental characteristics of the materials and makes them more suitable for task-specific applications. There has been a lot of work to fully utilise CN in several industries because of all these enticing features.

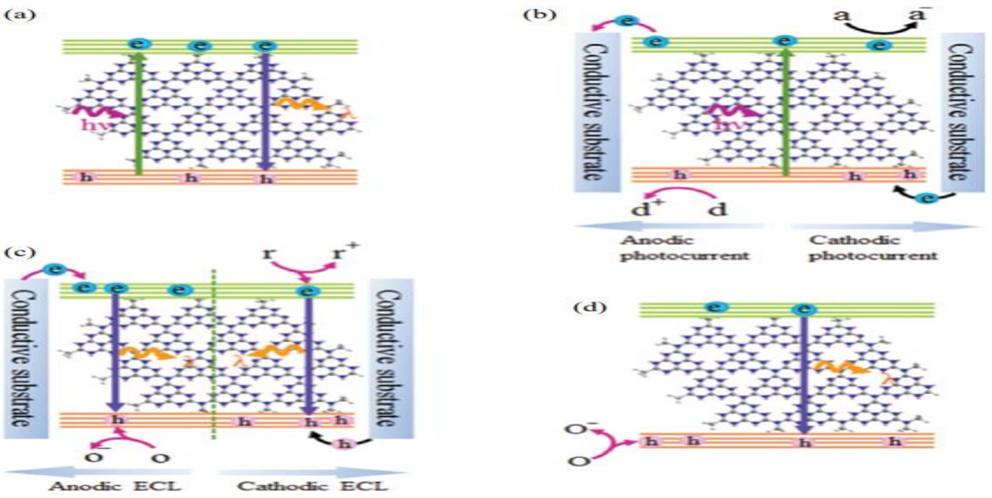

Figure 1. Sensing mechanisms employing CN. (a) Rapid recombination of photoinduced electrons and holes produces photoluminescent emission. (b) Light illumination in the presence of electron donors or acceptors that have been solubilized produces an anodic or cathodic photocurrent, respectively. (c) Electrochemical injection of electrons or holes in the presence of oxidative or reductive coreactants, respectively, generates cathodic or anodic electrochemiluminescence. (d) The emission of light by chemical entities that have had electrons or holes injected into them.

Opportunities of Carbon Nitride Biosensors

The properties of carbon nitride have placed it recently in gradual revelation of enormous potential in biosensing. First, it needs to be pointed out that the detecting processes can occur in a complex environment. Therefore, the stability could be undoubtedly regarded as an important factor to realise a superior biosensor. Non-toxic, flavourless, chemically and thermally stable, CN is also an ideal material. Based on this account, g-C_3N_4, recognized as the most thermodynamically stable form of the whole CN family, does not degrade at temperature as high as 600°C in air and under certain specific chemical conditions, therefore, the fabrication of fairly stable biosensors can be applied even if the devices are exposed to rather complex and realistic matrices [11-13]. This is because CN is mostly constituted with elements that are constituent on the earth in great abundance and cheap in terms of cost. Also, photoluminescence, chemiluminescence, electrochemiluminescence and photo electrochemical properties and the feature of CN as metal-free semiconductor with a small band-gap pave very wide applications in the fields of biosensors designing. While ECL and PEC differ from other forms of photoluminescence, the two can use the energy conversion process to isolate themselves from the interference of the input signal. This way, the versatility of the biosensors in terms of the input and output signals which can be processed has increased greatly [14, 15]. Furthermore, it is a favourable quality that CN's growth is organic, allowing for convenient tweaking of molecule structures as well as the optoelectronic properties of the product. Therefore, CN molecular structure engineering also enhanced the versatility of CN-based biosensors and the optimization of CN's favourable characteristics based on requirement.

Carbon Synthesis Molecular Structure Engineering using Nitride

Chemical Intake

Doping is the process of deliberately introducing impurities in semiconductors in order to control the electrical structures and hence change the conductivities, optical, luminescent, magnetic and the other physical features. The characteristics of CN can be improved by the chemical doping that is defined as the process of incorporating a dopant into a material matrix. Heteroatoms have the attribute of modifying the nature of electrical structure of CN. P is one of such atoms that have been demonstrated to enhance and control the CN performance in photoelectrochemical and photocatalytic applications, bioimaging and other areas. When our group first stated that phosphorus atoms created P-N bonds in the doped CN [16, 17], it probably substituted the structure's corner/bay carbon atom. By doping the heteroatoms P, CN electronic structure was apparently tuned, which made the light absorption range rather wide compared to other materials, almost encircling the whole range. As a result, P-doped CN processed a 5 times improvement in photocurrent generation besides exhibiting four orders of improvement in electrical conductivity. Doping developed all these great characteristics which enabled CN to construct biosensors with higher sensitivity and with strong signal feedback. The same way that S another typical doping heteroatom can also impart a noteworthy impact on the CN's characteristics. For instance, a study has established that the incorporation of S atom increases the ability to control the optical properties of CNNS. To fabricate S-CNNS with a red-shift PL and an ECL emission peak at 531 nm, CN was copolymerized with the bulk CN dopant of 2-aminothiophene-3-carbonitrile. As for the sensing properties of ECL biosensors, we were also constructing a wavelength resolved ECL biosensor for the parallel detection of multiple biomarkers at one electrode substrate in the lab since the emission wavelength of S-doped CN can be tuned. By trying to modulate the doping in the CNNS a possibility to use CNNS as metal-free monochromatic luminophores with different emission wavelength but the same coregent and excitation potential was examined [18-21]. This could lead to an improvement of the types of biocompatible monochromatic ECL luminophores for the detection of multiple biomarker. Other than the changed wavelength, Ma et al have as well demonstrated that S doping can alter the CNQD surface states and generate new element vacancies. This in turn enhances the sensitivity of the gene detected and enhances the ECL by a tune of 2. 5 times. 51 Besides the above mentioned heteroatom doping, Zhang and colleagues also found that in APG, carbon influenced the self-doping replacing the bridging N atoms. The former could result in the formation of delocalized p bonds in between the doped C atoms and (other) aromatic heterocycles; this would enhance the transmission of electrons, thus decreasing the bandgap. The method of doping for CN materials involves the alteration of their characteristics for wider applications in the field of bio-sensing.

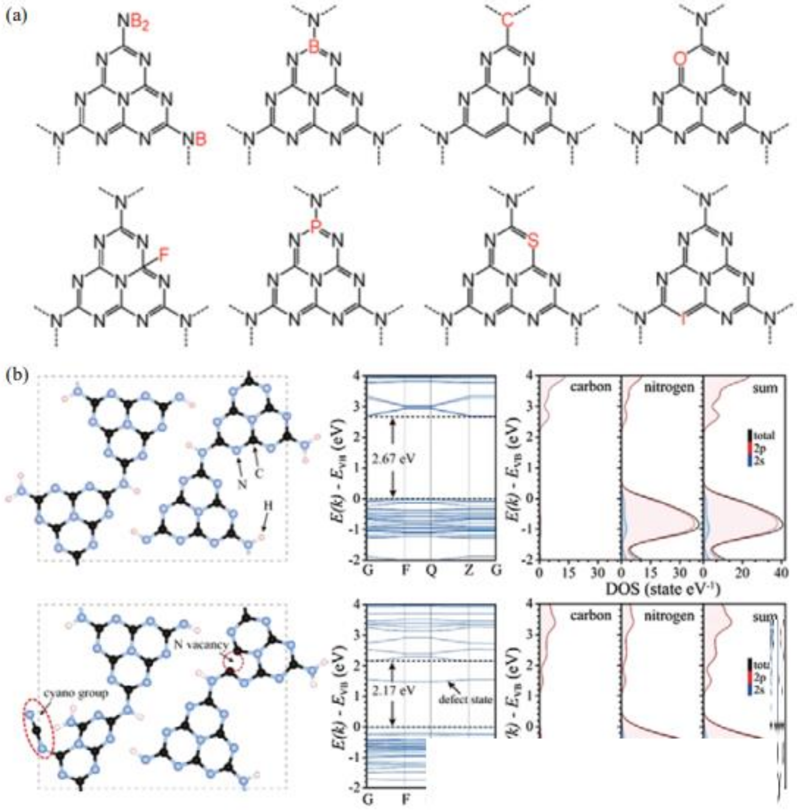

Figure 2. Engineering of CN molecule structures via doping and flaws. (a) Molecular motif of doped CN that is representative.32 (b) Components of the CN and CN with N defects structures, together with the similar band structures and partial density of states computed for them.

The modulation of defects generally greatly influences the characteristics of semiconductor electronic structures. Nitrogen faults appear to have big impact on the band gap structure and optoelectronic properties of CN. A recent area in consideration for CN materials is nitrogen defect engineering since it can be utilized to improve the desirable characteristics of the material for various applications. Conclusively, the impact that arises from the introduction of N defects into CN networks was established, with regard to the local coordination environment, electron sharing locally, and p-electron sharing [22-25]. Hence it can be deduced that just as adding N flaws improved the architecture of CN, it also had a positive impact on CN's performance. Out of the nitrogen defects, cyano groups in the nitrogen vacancies in the bulk and surface nitrogen vacancies are the two varieties that were selectively introduced. Nitrogen defects shifted CN absorption edge to the longer wavelengths and better separated the photoexcited charge carriers as per the reports from the theory and experiments. It was thus possible to observe that the photoelectrochemical and photocatalytic hydrogen evolution performance was better in the as-prepared CN. Hence, it cannot be doubted that the inclusion of flaws into CN biosensors enhances only their sensitivity and their bio-sensing potential as a whole.

An overview about Abstract Concept of Topological Structure

Apart from the structural engineering through doping or defect introduce, the physical and chemical properties of CN can also be tuned through the build up architectures with specific topological structures, which have strong correlation with the electronic band structures and molecular orbitality. In accordance with the above mentioned C/N ratios in the CN framework, new research work for various diverse applications such as sustainable photocatalytic reactions and highly sensitive optoelectronic biosensing has been started. As a result, various new CxNy materials have been predicted and/or synthesized with different prosperous topologies and stoichiometries. As a result, there has arisen a lot of concern on the molecular structural change of the majority of C_3N_4 reports. For instance, earlier in the thermal deammoniation process, Kumar and coworkers [26, 27] have synthesized a novel carbon nitride architecture C_3N_5. Melem hydrazine can be synthesized by the replacement chemical reaction or pre-treatment of the precursor melem with hydrazine; this results in the formation of a new bridging azo (-C=C-) bond. From this it was established that the bandgap was lowered to 1. A new series of compounds was synthesized from tri-s-triazine heptazine core and -C=C- linkage… These extension led to the increase in eV and the electron density of the material due to the extended conjugation. As in the case of the previous examples, to change CN in another simple way, one can change the repeating units. Vinu and collegues have synthesized a new topological C3N5 with a repeating unit of two six-membered ring triazines and one five-membered ring triazole. This compound can be synthesised easily by the self-assembly of the precursor, 5-amino - 1H-tetrazole (5-ATTZ). The redox reaction activity and diffusion current density properties of the as-prepared C_3N_5 were higher or were compared with those of bulk g-C_3N_4. Moreover, Zhou and colleagues also prepared C4N3, in which the bridging groups are replaced with carbon atoms. Its optical absorbance is much better; it located at more dangling bonds – it may originated from free electrons on the C atoms that bridging the two C atoms. It determines that the high relative energy level of the lone pair electrons on N atoms is due to the effective n → p * transition in most of the CxNy materials. Therefore, they are able to capture visible light to aid in their sensitive in vivo biosensing. Similar to one another, both CxNy's are capable of promoting a numerous amount of prospective applications that require geometric, electrical, and interfacing properties because of the recurring units, bridging groups, and defect sites. Besides the six-membered rings, our lab has employed the that we produced a C3N2 material based on a five-membered aromatic ring through the synthesis of the zeolite imidazole framework [28-31]. The rich feature of dangling bonds and the asymmetry of the five-membered rings in the condensed framework reduce the initial excitation energy to a low value of 0. 8 eV, making possible PEC biosensing of the turbid biological materials using near IR light for the first time..

Micro/Nanostructure Engineering: Nanosheets

As it can be seen from the polled data, large quantities of cheap CN can already be produced. The methods that have been used in syntheses of CN include solvent synthesis, solid-state process, electrodeposition, and thermal decomposition. Among these, thermal decomposition is widely used to prepare bulk CN from various precursors ophysical and chemical properties of CN. The imperfect CN which prepared by high-temperature solid–state synthesis is a big material with low solubility in most solvents and with large surface area. However, sensitive biosensors that can effectively respond promptly, often look for surfaces with many available sites of adsorption, many dispersion of water, and a large surface area. Sadly, this implies that despite being a completely native language, pure CN is not a perfect choice for the biosensing application. Different strategies have emerged to transform the original bulk CN into micro/nanostructures with improved biosensing properties as well as larger surface area, better water-solubility, etc. – all of them nullifying these practical application issues. Closely related to graphite in structure, graphitic C3N4 was named after graphite. Some scientific favorable assumption rationalizes that using the bulk g-C3N4 as the starting material and following the same strategy in the preparation of graphene, the CNSS could be prepared as CN nanosheets. Abundant pioneering attempts have been described to achieve this in different manners including heat oxidation and liquid exfoliation. The disadvantage of low carrier mobility and poor stability of the bulk CN can be easily compensated by the unique optoelectronic properties, enhanced water solubility, and larger surface area of the ultrathin two-dimensional CNNSs, while the biocompatibility of the bulk CN remains unbeaten. This approach has received a lot of attention from biosensing because it achieves the integration of a close contact between the guest molecules and CN materials. Thermal oxidation, which aimed at using heat to break the hydrogen bonds between layers and then oxidise with air, also discovered to be rather inefficient, however. This problem was solved by Qiao and colleagues through developing of CNNS using the method of sonication-exfoliation of bulk g-C3 N4 in an acidic solution. This process reduced the time of exfoliation to 2hrs and made the g-C3 N4 to have a positive charge by using HCl to protonate the surface. 64 First, CN has modest efficiency second, and the exercise is challenging due to CN's poor solubility in most solvents. It is also rather difficult to achieve the formation of a chemical bond between guest molecules and chemically inactive, defect-free CNNS. This is why, as shown in Figure 3(b), our group has devised a simple method for mechanically grinding aromatic molecules into bulk CN, which allows for simultaneous modification and exfoliation [32, 33]. In addition to preserving the perfect optoelectronic characteristics of bulk CN, the produced CNNSs enhanced a biomolecule-friendly interface, making them ideal for future coupling experiments.65 The emergence of CNNS not only increases the bio-sensing potential of CN materials by inheriting their distinctive characteristics, but it also mitigates some of the drawbacks of bulk CN.

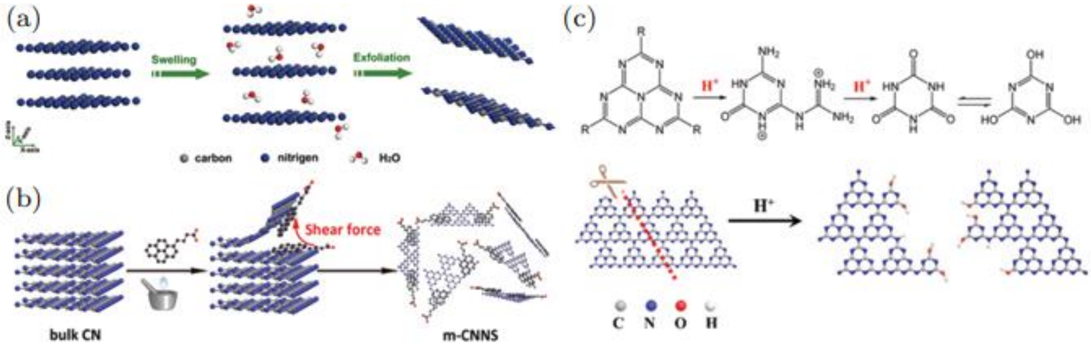

Figure 3. Nanostructure engineering with CN. (a) Converting bulk CN into ultrathin nanosheets by a liquid-exfoliation technique. (b) The process of mechanically grinding bulk CN into nanosheets while simultaneously exfoliating and modifying it.65 (c) Creating CN quantum dots through chemical modification in an acidic water solution.

Dots

Fluorescent semiconductor quantum dots (QD) are used across several industries because of the outstanding stability and customizable features of their optical and electrical properties in addition to big impacts on biomedicine, photovoltaics, optical equipment and much more. Other kinds of semiconductor QDs with differing photoluminescence properties have also been developed; these are; CdS/Se, PbS/Se, ZnS/Se, AgS/Se and etc. These metal based QDs are indeed superior to the organic dyes in terms of photobleaching broad emission, and poor photo stability but they are not yet suitable for biosensing because of their issues like high toxicity, poor water solubility and complex synthesis process. For these reasons, carbon based QDs, belonging to metal free quantum dots, have been gaining quite a lot of interest recently and are believed that they may be prospective biosensing materials. However, for all the quantum dots made from metals, carbon quantum dots are displayed to possess better optical properties and they are easy to synthesize, non-toxic, and biocompatible. In the case of biosensing, previous work proves that materials with CN have immense potential in this area as demonstrated above. Nevertheless, due to the wide size dispersion and low dispersibility, CN could not be used practically. That is why much effort has been directed to the preparation of homogenous CNQDs since they are more suitable for biosensing application. This has been a regular practice for some time now and therefore, defining the synthesis methods of CNQD as either top-down or bottom-up. The top-down method concerns preliminary modification of CN bulk with different processing that include chemical oxidation, ultrasonication, chemical functionalization hydrothermal treatment, electrochemical oxidation and hot-tailoring before slicing into smaller Nam CNQDs. In contrast to the multi-step top-down approach, the bottom-up synthesis just requires a single step to produce CNQDs: the sol–gel process and the polymerisation of small organic molecules containing a lot of nitrogen. Some of the well-known bottom-up approaches are hydrothermal, solvothermal, and the solid-phase processes. Compared with the bulk CN, CNQDs display better biosensing performance due to their fairly narrow size distribution, good solubility in water, non-toxicity and high biocompatibility according to the synthesis process used. Thus, due to the tri-s-triazine structural unit, CNQDs demonstrate even further stability under various physical and chemical impacts including [33, 34] heightened physical conditions. Additionally, CNQDs outperform carbon-based QDs in terms of their broad emission spectra, high quantum yield, and clear mechanism.66 The aforementioned benefits of CNQDs have led to their potential use as optical biosensing probes.

The Carbon Nitride Photoluminescence Signal Conversion Process

The CN material is free of metal and has the ability to apply for biosensing, where different signals will be generated based on the variation of the fluxion and recombination rate of electron hole pairs in relation to the CB and VB as a result from external stimuli. As the majority of ordinary semiconductors do, bulk CN materials initially exhibited first Order photoluminescence properties. Most of the times, when high energy photons affects CN, the electrons in the VB get transferred to the CB creating an equivalent number of holes. Such electron-hole pairs can emit luminescence at the desired wavelength after the fast recombination of these electron-hole pairs. Thus, the regulation of the PL properties of CN is easier due to the flexibility of its molecular and nanostructures of CN materials in terms of the degree of polymerisation, morphological modification or the formation of nanocomposites. According to the findings made, it emerges that emission wavelengths depend with the extent to which polymerisation occurs during the process of precursor condensation. Notably, for pure melem, a PL peak of approximately 366 nm is created. When the synthesized CN materials have been heated to 450°C for an hour to remove the surface doping carbon and then cooled to the bulk CN, the PL peak started to red shift and approaches 435 nm. Depending on the condensation temperature and time the PL peak shifts to 480 nm as a result of transition from lone pair of nitrogen atoms to p* conduction band at relatively higher condensation temperature 550 C for 4 hrs-degrees of condensation. In contrast, by heating the precursors to 600°C the value of the PL peak shifted to a higher energy [34, 35] value, which means that heating affected the electrical contact between the layers. Since the water solubility and surface area and other properties are enhanced when transformed into nanoscale forms in practical biosensing applications, the CN materials are often altered to nanoscale in the second section. This work explains the blue-shifted PL of CNNS with the larger poor gap due to the quantum size effect of the material. CN has defect and termination sites which reduces the increase in the PL intensity of CNNS. It results in movement of electrons and participates in the process of photogenerated electron hole pair recombination. The anantomy of CNNS is demonstrated as follows: Under the illumination of near-infrared excitation light (λex = 760 nm), CNNS exhibited excellent two-photon PL intensity at 438 nm, and relatively large two-photon absorption cross-section, and nearly negligible photobleaching. By absorbing two near-infrared photons at the same time, it was confirmed that CNQDs could also release a strong green PL emission.69 Thanks to its high biocompatibility and exceptional near-infrared excitation permeability, nanosized CN structures can be utilised for in vivo biosensing and in-depth imaging by utilising two-photon PL emission. Another important CN nanostructure, CN quantum dots (CNQDs), have shown great promise as fluorescent tags because of their distinctive optical properties, including a high PL quantum yield and an unmistakable PL emission mechanism. The quantum size effect caused CNQDs to emit PLs that were blue-shifted, just like CNNSs. Nevertheless, using a simple hot-tailoring top-down production process, our group reported a type of CNQDs with an anomalous redshifted PL emission. Possible causes for this effect include functionalized oxygen-containing groups and an enhanced C/N ratio during the liquid-collection step. The biosensing applications of CNQDs are likely to be even more expansive due to the tuneable PL emission.

Advanced photovoltaics

Biosensing has benefited from PEC because, being a hybrid of optical and electrochemical methods, it uses new semiconductor-based photoactive materials. PEC biosensing is highly sensitive because of the signal to electricity conversion and has low background noise. As a typical metal-free semi-conductor which CN is categorized due to the differentiate of the highly delocalized p-conjugated structure, it has recently emerged as a novel and potentially promising transducer for PEC biosensing. Light stimulates CN to separate and transfer charges, which results in the generation of an electrical signal. Namely, anodic photocurrent is obtained when electron donors dissolved in the electrolyte consumes holes on the VB, subsequently, electrons move from the CB of the CN to the electrode. For cathodic photocurrents where electrons generated from the light on the conduction band of CN are injected to the electron acceptors in the electrolyte the reverse is the case. The movement of photo-induced electrons and holes throughout the entire electro-circuit can be regulated by the redox effect to various targets of the electrolyte for sensing uses. However, pure CN material has a short photo-induced electron and hole pair recombination time this decreases the photocurrent. Besides, pure CN only interacts with ultraviolet light and a part of visible light with the wavelength shorter than 460 nm. Short-wave light cannot [36, 37] penetrate tissues for depths biosensing; the light induces production of ROS damaging DNA, proteins, cells thus limiting the use of CN in biosensing. As a result of the requirement for higher efficiency of CN-based PEC biosensing, numerous strategies have been employed to enhance carrier separation and migration as well as charge rectification along with CN's optoelectronic characteristics. The findings revealed that composites created from CN and some of the other interacting compounds might lead to a marked enhancement of the photocurrent. While enhancing the interfacial electron transport and avoiding the electrons from the CN conduction band, highly conductive materials including conducting polymers as well as noble metal nanoparticles can increase the photocurrent. The charge separation and PEC signals can be strengthened by coating semiconductors with high band gap on the CN nanostructures, in this way CN only need act as the electron supplier and transporter of the photoinduced electrons. On similar lines, CN can increase the photocurrent by trapping photo generated electrons from the conduction band of narrow band gap semiconductors. In addition, by transfer of appropriate dyes on CN, it is possible to obtain photocurrent that is increased to the conventional semiconductors level, or change the wavelength of the responsive light by means of the D-A connection. The other factor that will influence the photocurrent relates to the contact state between the CN material and the conductive substrate besides formation of the heterojunction. Modifying CN with P doping to increase visible light irradiation, coupling a hole/electron-transfer layer and redox shutter species to increase the charge separation efficiency, controlling the morphology to enlarge the surface area, and improving crystallinity to suppress unwanted charge recombination are some of the effective methods that our group recently developed to enhance the photocurrent signal. In addition, our team put forward a flexible microwave-assisted technique for producing a top-notch CN photoelectrode. This process produced an electrode with a highly adhesive CN layer, a carbon-rich texture gradient, reduced grain boundaries, and exceptional electron mobility, all of which contributed to the enhanced PEC response. Because of its exceptional photon-harvesting capabilities and distinctive optoelectronic characteristics, CN, a polymetric semiconductor, shows tremendous promise in PEC biosensing.

Using an electric current to produce light

PEC's inverse process, known as electrochemiluminescence (ECL), enjoys the same level of recognition as another remarkable method for biosensing because of its such characteristics as low background emission, low cost, higher control, and sensitivity. It also mentioned that another method is electrochemical injection in order to produce electron-hole pairs in CN apart from illumination. Mainly, there are two broad categories of procedures that are mostly applied in the production of ECL signals. For instance in the annihilation ECL, one electrode is employed in generating the oxidation and reducing states of the ECL-reagent which in turn are mutually annihilated to create the emitted excited state and the ECL signal. What is more, while injection of holes produces oxidised CN•+ and injection of electrons creates reduced CN• −, the ECL of pure CN is not observed, which means that CN•+ is not very stable in solutions. Instead of that, there is coreactant ECL where a coreactant supports redox process by which the ECL-reagent attains the exited state. CN materials have shown fairly inspiring coreactant ECL properties and the examples of their usage show it. Upon electrochemical injection of electrons or holes to the CN materials either linked to the working electrode, luminescence is generated by means of electron transfer between g-CN and redox-active coreactants and visible light.

Another significant parameter that at the same time has been vastly studied to enhance the biosensor's sensitivity is the ECL efficiency of CN materials. At first, change on ECL efficiency may be achieved by alteration of CN itself and by adding, for instance, the vacancy to CN. One such study is the one done by Wang and his team, where successfully, they inserted a vacancy into the structure of CN with added Eu. Since O vacancy was present in the case of the catalyst used, energy transfer was quicker from the catalyst to the ECL reagents. The mentioned use of CN combined with other conductive materials is also regarded as another effective method of increasing ECL efficiency of CN. The one beneficial impact helpful is that it can improve the electron transport between the electrodes and diminish the CN passivation resulting from high-energy electrons. However, a better ECL signal can be attained either by fixating CN onto other areas of large surface, in which CN can be loaded in larger quantities. When the property of catalytic activity is incorporated in the substrates and composites, there are possibilities of improving ECL efficiency in several ways which include but are not limited to the acceleration of the over all ECL reaction88 or providing more of the coreactants apart from modifying the ECL reagents. The substrates may also influence the efficiency of ECL which may result in alterations in electro-conductivity of the substrates, formation of the coreactant, or load of the ECL reagent. Strikingly, it is revealed that the quality of the CN materials formed on the electrodes also greatly influences the ECL efficiency, apart from the heterogeneous composites. For instance, the cathodic efficiency of ECL recorded at our group was at a record level with the help of a high quality CN photoelectrode prepared in using the microwave assisted technique.

To improve interfaces, the concept of biomolecules is employed

CN materials' recognition components or analytes conjugation is the primary strategy for the diversified design of other signal types and sensing systems in an integrated biosensor. The advantages of biosensor stability, selectivity, and sensitivity are thus evident through the interaction between biomolecules and CN materials that are easy and do not distort the basic biomolecules' properties. It has been mentioned before, the interaction between CN and numerous biomolecules and analytes has been a matter of substrate engineering. As a result, CN materials can directly place themselves as recognition units due to the presence of intrinsic ligands such as amino groups, imino groups and nitrogen atoms. Thus, chelating with lots of different function groups on the surface of CN, Ag+, Cu2+ and Fe3+ions could be detected with CN-based sensors, which enhanced the selectivity and stability significantly. Said previously, CN can be rolled down to the various nanostructures to make a large surface area that will increase the number of recognition sites. As for the exchanging ions with cavity sites in CNNS, for example, Chen and colleagues have described that Ag+ can coordinate with the four-edge nitride atoms so that PL of Ag+ can be detected. Apart from that, in the experiments conducted in our lab, it was discovered that CNQDs are able to coordinate with Fe3+ through hydroxyl and nitrogen atoms. Another useful use of EDC/NHS activation beyond metal ion chelation is the easy conjugation of amino-terminated biomolecules to carboxylated CN nanostructures. As an example, Wei and colleagues have detailed a method for covalently modifying antibodies on CNNS with carboxyl groups. This allowed for the realisation of the usual sandwich-type biosensing for carcinoembryonic antigen, which is achieved by the specific binding of the antigen and antibody. Nanocomposites give CN more opportunities to bond with biomolecules, in addition to the functional groups that are intrinsic to CN. The most typical method for depositing gold nanoparticles (AuNPs) onto CN is to take use of the numerous nitrogen atoms present in the CN framework to anchor the AuNPs. Interestingly, AuNPs may boost the bioconjugation reliability of several biomolecules, including single-strand DNA, DNA hairpin probes, DNA tetrahedron nanostructures, antibodies, peptides102, and more. They can also improve the conductivity of CN in PEC and ECL sensing. As an additional noble metal, platinum (Pt) can facilitate the Pt-N link, which allows CN materials to covalently conjugate with biomolecules that have amino groups attached to them. It is possible to immobilise biomolecules using semiconductors as well as metal nanoparticles. In a TiO2 and CN material complex, for instance, it was shown that TiO2 could conjugate the phosphate groups of peptide 103 and the carboxyl groups of DNA. To make the binding with DNA probes a reality, CNNS was additionally modified using CdS quantum dots. In order to achieve a more intense and targeted interaction with biomolecules, CN materials have been functionalized using compounds like β-cyclodextrin and polyethylenimine108, which contain several recognition sites. The electrostatic interaction, hydrophobic interaction, hydrogen bonding contact, p-p stacking, and covalent combinations are not the only methods for non-covalent conjugation of CN compounds. For example, using the affinity between single-stranded DNA and CNNSs, Ju and colleagues have built a flexible PL sensing platform.

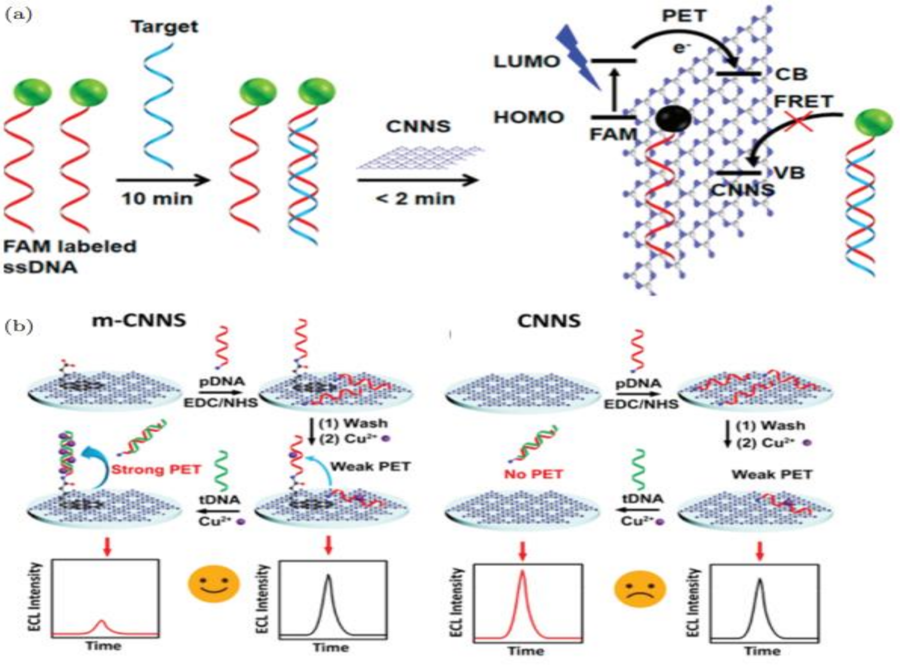

Figure 4. Conversational neural networks and their interactions with biological materials. (a) The p-p stacking interaction between DNA and CNNS. (a) DNA-CNNS covalent binding with aromatic molecule-modified CNNS.

Carbon Nitride Biosensors: Common Examples

Biosensing using Photoluminescence

Numerous studies have effectively utilised CN in biosensing to create novel and sensitive sensors, because to its exceptional photoluminescent characteristics. The sensing mechanisms often comprise ratiometric schemes, "on-off-on" and "on-off-on-on" patterns. When it comes to the "on-off" sensing process, CN can be used as a luminophore because of its unique optical properties and great biocompatibility. It interacts closely with targets, causing their photoluminescence to be quenched through numerous methods. To begin, one way to efficiently quench CN materials is through photoinduced electron transfer (PET) between targets in corporate nanocomposites and nanosized CN. The PL signal of nanosized CN can decline significantly if the linking targets' redox potential is precisely between the VB and CB of CN. This is because electrons can transfer from the CB of CN to the neighbouring targets. The levels of the relevant targets are reflected in the decrease of PL of CN under specific situations because of the strong relationship between the two. In a groundbreaking development, Lee and colleagues have created a Cu2+ "onoff" PL sensor using cubic mesoporous graphic (mpg)-CN. This novel material has a large surface area, which allows it to bind to metal ions efficiently. What's more, it shows a highly selective and sensitive PL response to Cu2+ alone, without any interference from other metal ions. It was thought that this happened because Cu2+ was coordinated to the intrinsic ligands during the PET process from cubic (mpg)-CN to Cu2+. In a similar vein, the same PET process has been used to sensitively detect a range of metal ions based on CN in various nanostructures. When it comes to detecting Ag+, one example is the work of Chen and colleagues. They used acetaldehyde-modified CNNS as the luminophore, and they were able to selectively and effectively quench the photoluminescent signal. In addition, Chen and colleagues have developed a CNNS nanofilm-based sensor that can detect Ag+ and Cu^{2+} ions as well as differentiate between these two types of metal ions in conjunction with glutathione. The great adsorption ability to metal ions, particularly Ag+ and Cu^{2+}, was shown by the numerous functional groups on the surface of CN. Even more crucially, these two metal ions' redox potentials fall squarely inside the CNNS VB–CB range. The PET method could come to fruition because of these two benefits. For PET sensing, not only were CNNS used, but also CNQDs with a high PL quantum yield. Our team found that chemically cleaved CNQDs have remarkable optical characteristics. The PET technique caused a clear decrease in the PL of CNQDs when Fe3+ was present, allowing for the selective detection of Fe3+ with a lower detection limit of 1μM compared to C-dots. In addition to detecting metal ions that coordinate with CN, PET sensing can also involve a variety of analytes that are easily bound to the surface of CN. It is worth noting that a highly selective tetracycline sensor has been developed by Li and colleagues using CN nanoseaweeds. The surface-interaction of tetracycline might trap the photoinduced electron of CN nanoseaweeds. Both the redox potentials that are appropriated and the degree to which targets and nanosized CN materials come into close proximity are crucial for PET sensing of CN. Applications based on CN have also made use of the inner filter effect (IFE), another crucial method for "turn-off" sensors. As an example, our team suggested a solid-state PAH sensor that uses polymethyl acrylic acid as a platform for uniformly dispersed CNNSs. The IFE procedure allowed PAHs that the b-cyclodextrin was designed to target to dampen the powerful PL signal of CNNSs. Additionally, it has been shown that 2,4,6-trinitrophenol (TNP) can be recognised when coupled to convolutional neural networks (CNNs) using the IFE process in a standard "on-off" paradigm. The sensitivity of this sensor was increased due to the improved IFE caused by the intensive interaction between the TNP and CNNSs, which included electrostatic, p-p, and hydrogen bonding interactions. Additionally, CN materials can be used in the PET process for purposes other than electron donation. In addition to being PL quenchers, they can act as electron acceptors for other fluorophores. From excited fluorophores to the conductive band of CNNSs, Ju and colleagues used the PET technique to build a DNA and Hg+ sensing platform. The aggregation of CN nanostructures can trigger the PL quenching, just like PET and IFE. In addition to altering the electrical distribution in the CNNSs planar framework, heparin binding to CNNSs caused the CNNSs to aggregate, which in turn caused self-quenching and collisional quenching. A "on-off-on" technique has evolved to prevent false signals caused by interference in the sensing environment, which further enhances the sensitivity of CN-based sensors. To alter the "always-on" state of CN nanostructures, it is common practice to first quench the PL of CN using a variety of chemicals. After that, target identification is achieved since the PL signal simply tends to recover when targets are present. One example is the work of Lv and colleagues, who found that persistent luminescent CNNSs may be used to build a sensitive sensing approach for biothiol detection with Ag+ as the quencher. First, Ag+ was used to quench the PL of CNNSs through the PET. Then, biothiols were introduced to disrupt the PL, which increased sensitivity because they evidently amplified the impacts of contaminants. Using a PET-based "on-off-on" method using metal ions and CN nanostructures, the detection of numerous compounds, including ascorbic acid, hyaluronidase, alkaline phosphatase, and cyanide116, has been achieved. Yang and colleagues found that, in addition to metal ions, MnO2 can reduce the PL of CNNSs; this effect is reversed when glutathione is present, as MnO2 is reduced to Mn2+; consequently, glutathione concentration is monitored. The ratiometric PL method with dual-output signals was introduced to further improve the sensitivity of CN-based sensors by normalising the fluctuation caused by environmental changes and increasing the sensitivity of biosensors. To detect and collect circulating tumour cells (CTCs), for instance, Yin and colleagues have built a ratiometric PL probe out of CNQDs and gold nanoclusters (AuNCs). Two fluorophores were CNQDs and AuNCs, respectively. In the absence of CTCs, the former remained unaltered, but the latter showed improvement as a result of reduced CNQD aggregation.

Biosensing using Electrochemiluminescence

Due to its appealing qualities, such as its ability to avoid photoexcitation background and its primarily high signal-to-noise ratio, electrochemical generation of excited states (ECL), the inverse process of photoexcitation coupling (PEC), has attracted increasing interest for use in biosensors. A wide variety of ECL sensing techniques are possible, depending on the many stages of the ECL process that convert electrons to photons. The ability of certain analytes to quench the ECL signal by accepting excited-state electrons from the CB of CN materials has led to their widespread application in the construction of dependable sensors, particularly for metal ions. In order to detect copper ions, Choi and colleagues built the first "turn-off" ECL sensor. This sensor allowed them to see how electron transfer from CN, in conjunction with copper ions coordinated with the intrinsic ligands of CN, may quench the ECL. Our group successfully detected Cu^{2+} using CNNS that were made by liquid-exfoliating bulk g-CN with varying degrees of polymerisation. The redox potential of Cu^{2+}/Cu, which lies between the CB and VB of CN materials, is responsible for copper's selectivity. Our lab has achieved a breakthrough in DNA detection. Instead of just sensing metal ions, we are able to detect the target DNA by binding Cu2+ to the N7 position of DNA bases and quenching the ECL of CNNS. The ECL resonance energy transfer (ECL-RET) is a potential alternate method for reducing the CN ECL signal; this method involves efficiently transferring the energy of excited CN to nearby substances in order to achieve "turn-off" biosensing. As an example, Xiao and colleagues have introduced a rutin ECL sensor, which takes advantage of the fact that the oxidation product of rutin can efficiently reduce the anodic ECL of CN by energy transfer. A number of ratiometric assays built on the ECL-RET technique have been developed to further improve sensitivity; these assays can successfully normalise environmental variations by comparing the ratio of two signals. Also, CN nanostructures can be used as ECL tags in biosensing to detect biomolecules upon arrival at electrode surfaces, allowing for "turn-on" detection. As an example, Yuan and colleagues published a cardiac troponin I ECL sensor that increased the ECL signals of CNNSs via the antigen-antibody interaction (MIL(Fe)). In order to detect DNA methyltransferase, our group created a sandwich-assembled ECL biosensor that used the affinity of single-stranded DNA and CNNSs. To improve the biosensor's dependability, multifunctional CNNSs were used as both the analyte reporter and the self-test reporter. Since coreactants are essential to CN-based ECL biosensing, variations in the concentration of coreactants surrounding CN materials caused by different target recognition contacts might indirectly disrupt ECL signals. One example is the DNA ECL biosensor that Ju and colleagues have developed. This biosensor uses hemin-labeled DNA strands, which may absorb dissolved oxygen and prevent the production of H_2O_2, an endogenous coreactant for CNNSs. Aside from chemical interactions, another effective method for controlling the diffusion of coreactants or electrons to the surface of CN materials is the steric barrier of biomolecules with large molecular weights .

References

1. Liebig, J. Uber einige Stickstoff — Verbindungen. Ann. Pharm. 1834, 10, 1–47.

2. Thomas, A.; Fischer, A.; Goettmann, F.; Antonietti, M. Graphitic Carbon Nitride Materials: Variation of Structure and Morphology and their Use as Metal-free Catalysts. J. Mater. Chem. 2008, 18, 4893.
3. Wang, X.; Maeda, K.; Thomas, A.; Takanabe, K.; Xin, G.; Carlsson, J. M.; Domen, K.; Antonietti, M. A Metal-free Polymeric Photocatalyst for Hydrogen Production from Water Under Visible Light. Nat. Mater. 2008, 8, 76–80.
4. Cohen, A. Y. L. a. M. L. Prediction of Low Compressibility Solids. Science 1989, 245, 841–842.
5. Teter, D. M.; Hemley, R. J. Low-Compressibility Carbon Nitrides. Science 1996, 271, 53–55
6. Liu, J.; Liu, Y.; Liu, N.; Han, Y.; Zhang, X.; Huang, H.; Lifshitz, Y.; Lee, S.-T.; Zhong, J.; Kang, Z. ChemInform Abstract: Metal-Free Efficient Photocatalyst for Stable Visible Water Splitting via a TwoElectron Pathway. ChemInform 2015, 347, 970–974.
7. Zhou, Z.; Wang, J.; Yu, J.; Shen, Y.; Li, Y.; Liu, A.; Liu, S.; Zhang, Y. Dissolution and Liquid Crystals Phase of 2D Polymeric Carbon Nitride. J. Am. Chem. Soc. 2015, 137, 2179–2182.
8. Cui, Q.; Xu, J.; Wang, X.; Li, L.; Antonietti, M.; Shalom, M. PhenylModified Carbon Nitride Quantum Dots with Distinct Photo luminescence Behavior. Angew. Chem. Int. Ed. 2016, 55, 3672–3676.
9. Zhang, J.; Zhang, M.; Yang, C.; Wang, X. Nanospherical Carbon Nitride Frameworks with Sharp Edges Accelerating Charge Collection and Separation at a Soft Photocatalytic Interface. Adv. Mater. 2014, 26, 4121–4126.
10. Zhou, Z.; Shen, Y.; Li, Y.; Liu, A.; Liu, S.; Zhang, Y. Chemical Cleavage of Layered Carbon Nitride with Enhanced Photoluminescent Performances and Photoconduction. ACS Nano 2015, 9, 12480–12487.
11. Zhou, Z.; Shang, Q.; Shen, Y.; Zhang, L.; Zhang, Y.; Lv, Y.; Li, Y.; Liu, S.; Zhang, Y. Chemically Modulated Carbon Nitride Nanosheets for Highly Selective Electrochemiluminescent Detection of Multiple Metal-ions. Anal. Chem. 2016, 88, 6004–6010.
12. Zhao, F.; Cheng, H.; Hu, Y.; Song, L.; Zhang, Z.; Jiang, L.; Qu, L. Functionalized Graphitic Carbon Nitride for Metal-free, Flexible and Rewritable Nonvolatile Memory Device via Direct Laser-Writing. Sci. Rep. 2014, 4, 5882.
13. Kessler, F. K.; Zheng, Y.; Schwarz, D.; Merschjann, C.; Schnick, W.; Wang, X.; Bojdys, M. J. Functional Carbon Nitride Materials — Design Strategies for Electrochemical Devices. Nat. Rev. Mater. 2017, 2, 17030.
14. Gillan, E. G. Synthesis of Nitrogen-Rich Carbon Nitride Networks from an Energetic Molecular Azide Precursor. Chem. Mater. 2000, 12, 3906–3912.
15. Liu, J.; Wang, H.; Antonietti, M. Graphitic Carbon Nitride “Reloaded”: Emerging Applications Beyond (Photo)Catalysis. Chem. Soc. Rev. 2016, 45, 2308–2326.
16. Xu, J.; Antonietti, M.; Shalom, M. Moving Graphitic Carbon Nitride from Electrocatalysis and Photocatalysis to a Potential Electrode Material for Photoelectric Devices. Chem. Asian J. 2016, 11, 2499–2512.
17. Zhang, J.; Zhang, M.; Sun, R.-Q.; Wang, X. A Facile Band Alignment of Polymeric Carbon Nitride Semiconductors to Construct Isotype Heterojunctions. Angew. Chem. Int. Ed. 2012, 51, 10145–10149.
18. Wang, X.; Blechert, S.; Antonietti, M. Polymeric Graphitic Carbon Nitride for Heterogeneous Photocatalysis. ACS Catal. 2012, 2, 1596–1606.
19. Lee, E. Cubic Mesoporous Graphitic Carbon(IV) Nitride: An All-in-One Chemosensor for Selective Optical Sensing of Metal Ions. Angew. Chem. Int. Ed. 2010, 49, 9706–9710.
20. Tang, Y.; Su, Y.; Yang, N. Carbon Nitride Quantum Dots: A Novel Chemiluminescence System for Selective Detection of Free Chlorine in Water. Anal. Chem. 2014, 86, 4528–4535.

21. Lv, Y.; Chen, S.; Shen, Y.; Ji, J.; Zhou, Q.; Liu, S.; Zhang, Y. Competitive Multiple-Mechanism-Driven Electrochemiluminescent Detection of 8-Hydroxy-2′-deoxyguanosine. J. Am. Chem. Soc. 2018, 140, 2801–2804.
22. Zhang, Y.; Wang, F.; Zhang, H.; Wang, H.; Liu, Y. Multivalency Interface and g-C3N4 Coated Liquid Metal Nanoprobe Signal Amplification for Sensitive Electrogenerated Chemiluminescence Detection of Exosomes and Their Surface Proteins. Anal. Chem. 2019, 91, 12100–12107.
23. Wang, Y.; Xia, L.; Wei, C.; Wang, H.; Wang, H.; Yuan, R.; Wei, S. Ultrasensitive Photoelectrochemical MicroRNA Biosensor Based on Doxorubicin Sensitized Graphitic Carbon Nitride Assisted by a Targetactivated Enzyme-free DNA Walker. Chem. Commun. 2019, 55, 13082–13084.
24. Sengupta, P.; Pramanik, K.; Datta, P.; Sarkar, P. Chemically Modified Carbon Nitride-chitin-acetic Acid Hybrid as a Metal-free Bifunctional Nanozyme Cascade of Glucose Oxidase-peroxidase for “Click Off” Colorimetric Detection of Peroxide and Glucose. Biosens. Bioelectron. 2020, 154, 112072.
25. Qiu, H.; Pu, F.; Ran, X.; Liu, C.; Ren, J.; Qu, X. Nanozyme as Artificial Receptor with Multiple Readouts for Pattern Recognition. Anal. Chem. 2018, 90, 11775–11779.
26. Wang, Y.-M.; Liu, J.-W.; Adkins, G. B.; Shen, W.; Trinh, M. P.; Duan, L.-Y.; Jiang, J.-H.; Zhong, W. Enhancement of the Intrinsic Peroxidase-Like Activity of Graphitic Carbon Nitride Nanosheets by ssDNAs and Its Application for Detection of Exosomes. Anal. Chem. 2017, 89, 12327–12333.
27. Lu, Q.; Wang, H.; Liu, Y.; Hou, Y.; Li, H.; Zhang, Y. Graphitic Carbon Nitride Nanodots: As Reductant for the Synthesis of Silver Nanoparticles and its Biothiols Biosensing Application. Biosens. Bioelectron. 2017, 89, 411–416.
28. Zhou, Z.; Zhang, Y.; Shen, Y.; Liu, S.; Zhang, Y. Molecular Engineering of Polymeric Carbon Nitride: Advancing Applications from Photocatalysis to Biosensing and More. Chem. Soc. Rev. 2018, 47, 2298–2321.
29. Chen, L.; Song, J. Tailored Graphitic Carbon Nitride Nanostructures: Synthesis, Modification, and Sensing Applications. Adv. Funct. Mater. 2017, 27, 1702695.
30. Lin, Z.; Wang, X. Nanostructure Engineering and Doping of Conjugated Carbon Nitride Semiconductors for Hydrogen Photosynthesis. Angew. Chem. Int. Ed. 2013, 52, 1735–1738.
31. Wang, Y.; Zhang, J.; Wang, X.; Antonietti, M.; Li, H. Boron- and Fluorine-Containing Mesoporous Carbon Nitride Polymers: MetalFree Catalysts for Cyclohexane Oxidation. Angew. Chem. Int. Ed. 2010, 49, 3356–3359.
32. Dong, G.; Zhao, K.; Zhang, L. Carbon Self-doping Induced High Electronic Conductivity and Photoreactivity of g-C3N4. Chem. Commun. 2012, 48, 6178.
33. She, X.; Wu, J.; Zhong, J.; Xu, H.; Yang, Y.; Vajtai, R.; Lou, J.; Liu, Y.; Du, D.; Li, H.; Ajayan, P. M. Oxygenated Monolayer Carbon Nitride for Excellent Photocatalytic Hydrogen Evolution and External Quantum Efficiency. Nano Energy 2016, 27, 138–146.
34. Liu, J.; Li, W.; Duan, L.; Li, X.; Ji, L. A Graphenelike Oxygenated Carbon Nitride Material for Improved Cycle-Life Lithium/Sulfur Batteries. Nano Lett. 2015, 15, 5137–5142.
35. Wang, Y.; Di, Y.; Antonietti, M.; Li, H.; Chen, X.; Wang, X. Excellent Visible-Light Photocatalysis of Fluorinated Polymeric Carbon Nitride Solids. Chemistry of Materials 2010, 22, 5119–5121.
36. Ma, T. Y.; Ran, J.; Dai, S.; Jaroniec, M.; Qiao, S. Z. PhosphorusDoped Graphitic Carbon Nitrides Grown In Situ on Carbon-Fiber Paper: Flexible and Reversible Oxygen Electrodes. Angew. Chem. Int. Ed. 2015, 54, 4646–4650.
37. Guo, S.; Deng, Z.; Li, M.; Jiang, B.; Tian, C.; Pan, Q.; Fu, H. Phosphorus-Doped Carbon Nitride Tubes with a Layered Micronanostructure for Enhanced Visible-Light Photocatalytic Hydrogen Evolution. Angew. Chem. Int. Ed. 2016, 55, 1830–1834.