Recent advances of noble metal aerogels in biosensing

Wei Gao | Dan Wen

Abstract
Noble metal aerogels were first reported in the late 2000s and developed rapidly over the past 10 years. With the structure advantages of ultralow density, high porosity, large specific surface area and hierarchical structure like traditional aerogels, noble metal aerogels with good conductivity and specific chemical properties have attracted great research interests and application potentials in various fields, including biological applications. Here, in this perspective, recent advances about the preparation and application of noble metal aerogels in biosensing are summarized and highlighted. As mimics of bio-active “nanozymes” and as supports of natural enzymes, noble metal aerogel-based biological sensors exhibited much better activity and sensitivity towards the detection of various substrates compared to their counterparts of nanostructured materials. Moreover, the main challenges and perspectives of noble metal aerogels about their biocompatibility, types, surface properties, selectivity, and potential in wearable applications for biosensing are also emphasized and addressed. Overall, as a novel branch of aerogels, noble metal aerogels hold great promises for biological sensing applications and still have many problems to overcome for better utilizations.

KEYWORDS
biosensing, enzyme supports, nanozymes, noble metals aerogels

The term aerogel comes from the combination of aero- and gel, where the gel is defined to be “Non-fluid colloidal network or polymer network that is expanded throughout its whole volume by a fluid,” according to IUPAC, while aero- refers to things connected with air. Thus, aerogel is described as “Gel comprised of a microporous solid in which the dispersed phase is a gas”. From this explanation, aerogel is not a specific material, but a large family of porous hierarchically networked materials with gas as the dispersed fluid. Since the first report of aerogel exhibited by Kistler in the early 1930s to prepare silica and other inorganic and organic aerogels, rising attention is focused on the syntheses, structure investigations, and applications of different aerogels, including inorganic aerogels (such as metal oxides, carbides, metal alloys, and metal chalcogenides), inorganic-organic hybrid aerogels (like polymer- and cellulose-crosslinked metal oxides), organic aerogels (typically resorcinol-formaldehyde and melamine-formaldehyde), and carbon aerogels (including graphene and inorganic/organic doped carbon).
Originating from the hierarchical structure with a large surface-to-volume ratio, aerogels are well-known to have extremely large specific areas, high continuous porosity, and ultra-low mass density. Because of the unique physiochemical and mechanical properties of aerogels with controllable compositions, their applications have been broadened to various fields, like heterogeneous catalysis for the chemical industry, super thermal insulators for buildings and aerospace, chemical sensors for hazardous emission detection, piezoelectricity for energy conversion, permeability for nuclear waste containment, biocompatibility for pharmaceutical and biomedical diagnose and clinical treatments, etc.

Noble metals are acquainted with their excellent chemical properties and low abundances on the earth and drew great research and application interests. Thus, improving the atomic utilization of noble metals is of particular importance for their practical applications. Among various techniques, designing three-dimensional porous materials, typically aerogel structures, is an efficient pathway to make the most of their surface atoms accessible for various applications, while simultaneously decreasing the quantity of noble metals to lower the costs. For noble metal aerogels, the pioneering research of monometallic (Pt, Ag, and Au) and bimetallic (Pt/Ag and Au/Ag) aerogels is exhibited by Eychmüller et al in 2009 for the first time, from the assembly of the corresponding noble metal nanoparticles. As a result, the densities (typically 0.016 g/cm³ for Au/Ag aerogel) of the above noble metal aerogels are about 3 orders of magnitude lower than their corresponding bulk characteristics. The great prosperity and booming of noble metal aerogels during the next 10 years after 2009 witnessed their fast development in their design and growth mechanisms, as well as their potential applications. Especially, the intrinsic physical and chemical properties allow noble metal aerogels to display better properties than their corresponding bulk materials and nanoparticles in the circumstances of heterogeneous catalysis (including photocatalysis, electrocatalysis, and bioelectrocatalysis), sensors, energy harvesting and conversion, optoelectronics, adsorbents, and filters which have been comprehensively reviewed and summarized by others and will not be discussed in detail herein.

Biosensing protocols and biosensors are able to detect a wide range of targets with high sensitivity and selectivity in the applications of bio-diagnosis, security, and environmental safety, where biological recognition elements are of great importance and necessity. Enzymes can efficiently and selectively react with targets and exhibit special signals, therefore are widely employed in biological sensing. When applied in biological devices, enzymes require suitable and stable supports to disperse them with higher utilization and anchor them from exfoliation. Porous materials with three-dimensional structure and higher surface area relative to planar supports would be an attractive choice. They can increase the enzyme loading and reactive surface area and, thus, the sensing signal generated on one hand, and on the other hand, the pores being large enough to allow for easy permeation of the electrolyte and targets, and support their transport to the catalyst reaction sites. Additionally, artificially-synthetic “nanozymes” which are composed of inorganic and inorganic/organic hybrid nanomaterials that mimic enzymatic catalytic properties to various targets.

Besides nonprecious metals and their compounds, noble metals including Au, Ag, Ru, Ir, Pd, and Pt with excellent chemical and catalytic properties have also been explored lately as nanozymes to perform properties similar to natural enzymes of oxidases, peroxidases, hydrolases, and others, in the applications of detection, immunoassays, antibacterial, and antioxidation.

During the past decades, aerogels consisting of inorganics, organics, and their hybrid composites have been studied as functionalized biomaterials for both in vivo and in vitro biological and clinical applications because of their hierarchical porous structures and good biocompatibility and biodegradability. Noble metals with their superb chemical activity hold great potential for biological sensing, while their practical applications are limited by the unsatisfied utilization and high costs. As described above, noble metal aerogels are of high atomic utilization, and have the significant advantages of ultralow mass density, hierarchical porous structure, good conductivity, excellent chemical properties to interact with biomolecules, as well as work as the support for natural enzymes. These structural and chemical features of noble metal aerogels guarantee their potential in biosensing and outperform other organic and inorganic aerogels. Thus, it is not surprising, but expected, that noble metal aerogels can function as nanozymes and/or enzyme supports for in vitro sensing, because very small amounts of noble metal aerogels would be required to perform considerable activity. Five years after the first report of noble metal aerogels, the Eychmüller group furthermore broadened the applications of noble metal aerogels into the electrochemical detection of biomolecules. From then on, series of researches explored and implemented noble metal aerogels and their derived composites as efficient sensors for detecting. Noble metal aerogels are then proposed and employed as not only the support of natural enzymes but also the nanozymes for biological sensors. Especially, the good mimic properties of noble metals allow their high performances for target detection. Here, to better understand the development of noble metal aerogels in biosensing, and comprehend the effects of various factors on the properties of noble metal aerogels, several representative research works
about Pd, Au, and Ag aerogels on the detection of glucose, H$_2$O$_2$, and dopamine are introduced concretely below (Scheme 1).

In 2014, Wen et al demonstrated the first case of incorporating Pd aerogels with natural enzyme for bioelectrocatalytic detection of glucose and further applications in biofuel cells.\textsuperscript{30,31} By Ca$^{2+}$-induced assembly of citrate-coated Pd nanoparticles and followed by supercritical CO$_2$ drying, Pd aerogels with hierarchical porous structure were obtained (Figure 1A) and worked as support electrode of natural enzymes for glucose detection.\textsuperscript{30} Typical scanning electron microscope (SEM) images demonstrated the high porosity and open hierarchical structure of Pd aerogel (Figure 1B). With the decoration of the glucose oxidase (GOD) enzyme in Pd aerogels and ferrocenecarboxylic acid (Fc) as electron mediator to bioelectrochemically oxidize glucose in 0.1 M phosphate buffer solution (PBS, pH 7.4), Pd aerogel-GOD-Nafion/GC (GC = glassy carbon) exhibited much higher bioelectrocatalytic activity compared to Pd nanoparticles-GOD-Nafion/GC and
GOD-nafion/GC (Figure 1C). Also, the Pd aerogel-GOD-nafion/GC electrode also displayed a higher response to different concentrations of glucose (Figure 1C). In this work, the support of Pd aerogel contributed to a better dispersion and adhesion for GOD enzyme, high porosity for substrate and mediator transport, together with good conductivity for electron transfer, and the result of better response to the substrate also confirmed their potential for glucose detection. A further investigation illustrated the membraneless glucose/oxygen biofuel cell built up with noble metal aerogels as enzyme supports. GOD in Pd aerogel as bioanode and bilirubin oxidase (BOD) on Pd-Pt aerogel as biocathode realized the electrocatalytic glucose oxidation and oxygen reduction reactions, respectively. Working in the solution of 0.1 M PBS (pH 7.0) containing 50 mM glucose, this biofuel cell exhibited an open-circuit potential of 0.40 V and a maximum power density of 20 μW cm$^{-2}$, making it promising as an integrated fuel cell in biological applications, especially for self-powered biological sensing devices.

Besides functioning as supports for enzymes due to the high porosity, noble metal aerogels with good mimic activity also allow their biosensing application as nanozymes. Typically, the good biocompatibility of Au promotes its wide applications in biological fields. However, the construction of Au aerogels is difficult from the traditional method because of the tendency to sinter to larger-size aggregates for Au nanoparticles. Recently, Au aerogels composed of three-dimensional nanowire-like networks by a dopamine-induced assembly of Au nanoparticles were developed by Wen et al. in 2016. Employing dopamine as the destabilizing agent, various ligand-capped Au nanoparticles (including β-CD, and citrate as both stabilizers and nonstabilizer) were assembled into aerogels quickly with different functional groups (Figure 2A-D). The effects of various ligands on the interaction with dopamine for Au nanoparticles are explored and explained to clarify the formation of the aerogels. Besides the interaction between dopamine and the Au core, the host–guest interaction between β-CD and dopamine functioned as the main driving force to induce the assembly of Au nanoparticles into fused nanowires with the partial loss of β-CD. Au nanoparticles with rather weakly bound ligands (like citrate) are easily attacked by dopamine for surface ligand exchange and subsequent host–guest interaction, resulting in branched nanowire-like aerogels. Strong ligands on Au nanoparticles cannot be replaced easily, hindering the further formation of aerogels. When employed as sensors without decorating natural enzymes, Au aerogels with various ligands showed different response in alkaline solution without and with glucose (Figure 2E,F). Importantly, the Au$_{β-CD}$ aerogel, among all aerogels, displayed the largest electrochemical active surface area (ECSA) of 18.2 m² g$^{-1}$ and the highest sensitivity of 332.9 ± 7.6 μA mM$^{-1}$ cm$^{-2}$ ($n = 7$) in the range of 0–20 mM glucose (Figure 2G,H). Thus, natural enzyme-free sensors of Au aerogels were successfully prepared by the assembly of Au nanoparticles. Simultaneously, the formation mechanism of Au aerogels and the effect of different ligands are also uncovered. This research work facilitated

**FIGURE 2**  (A) SEM of the Au$_{β-CD}$, TEM of the (B) Au$_{β-CD}$, (C) Au$_{NS}$, and (D) Au$_{Cit}$. CVs of the Au$_{β-CD}$ (black line), Au$_{Cit}$ (red line), and Au$_{NS}$ (blue line) modified electrodes in the (E) absence and (F) presence of 4 mM glucose in 0.1 M NaOH at a scan rate of 50 mV/s. (G) Current densities for 4 mM glucose oxidation on three Au aerogel modified GC electrodes. (H) Plots of the electrocatalytic current of glucose vs concentration (2–30 mM) of the modified electrodes. Reproduced with permission. Copyright 2016, The American Chemical Society.
For noble metal nanoparticles synthesized in organic solvents, the absorbed solvents are not favored for further assembling of nanoparticles into aerogels, while the removing of them should be done carefully to avoid the aggregation of nanoparticles. Lately, Fan et al. proposed a two-phase ligand exchange method to replace the long-chain ligand oleylamine on Au nanoparticles by short sulfide ions and subsequent self-assembling into aerogels (Figure 3a).\textsuperscript{33} Au aerogels with small ligament sizes (ca. 3–4 nm) and tunable surface valence states showed good catalytic activity as peroxidase mimics (Figure 3b). Moreover, the catalytic properties of aerogels with different Au surface states (Au(0) and Au(I)) were explored. By correlating the surface valence of the Au species to apparent steady-state kinetic parameters, it is proven that Au(I) acted as both the catalytically active site for H$_2$O$_2$ reduction and the substrate-binding sites for 3,3′,5,5′-tetramethylbenzidine (TMB), which provided a new understanding of the catalytic mechanism of the mimic enzymatic activity of Au-based catalysts (Figure 3c).

The surface structure of aerogels is another crucial property to regulate their mimic enzymatic activity and further sensing performance. By surface reconstruction, the facet ratios in Au aerogels with different sizes were regulated and their effect on the activity was illustrated, as demonstrated by Wang et al in 2019.\textsuperscript{34} Using two different types of Au nanoparticles at different particle ratios, series of Au$^{m-n}$ aerogels ($m$ and $n$ are the size of nanoparticles) were synthesized, where Au$^{6-50}$ aerogels had the highest ratios of [110] facets. The surface reconstruction between the two differently-sized Au nanoparticles during the Ostwald-ripening process occurred with a size ratio larger than 5 and promoted the formation of exposed [110]-rich facets on the surfaces of the resulting Au$^{m-n}$ aerogels. Further SEM and TEM characterizations confirmed the hierarchical porous structure of the aerogels with rich [110] facets on the surface. The Au$^{6-50}$ aerogels with 35.5% of [110] facets were investigated as a high-performance enzyme-free glucose sensor, which exhibited a good selectivity toward glucose detection. With the successive addition of different glucose concentrations, the response currents can reach the steady state within 2 s, showing a successively steep increase. On the basis of the corresponding calibration curve, the response current of glucose on Au$^{6-50}$ aerogels exhibited a good linearity in the concentration range from 1 μM to 1.59 mM, with a sensitivity of 2044.71 μA cm$^{-2}$ mM$^{-1}$ and a limit of detection of 0.58 μM, which were better than most of the other Au-based materials. Thus, the realization of Au aerogels with controlled facets was shown, and its effect on further regulating the electrochemical response to substrates with better sensitivity and lower limit of detection is also emphasized in this work.

As the cheapest noble metal, Ag is also of excellent electrical conductivity and high mimic peroxide activity,
while only rare references unveiled Ag-based aerogels and their biosensing applications. Very recently, monolithic Ag aerogel assembled from Ag wires are reported by Yang et al., where interconnected, continuous Ag wires allowed the rapid electron transfer from Ag wires to the substrate, to enhance the electrochemical response to H$_2$O$_2$ (Figure 4A-C). By constructing a microfluidic chip H$_2$O$_2$ sensor with Ag aerogel as the working electrode, the concentration of H$_2$O$_2$ solutions flowing through the sensor can be facilely detected with various cathodic current increments (Figure 4D). This Ag aerogel sensor exhibited quick response to H$_2$O$_2$ concentration changes from 0 to 1.0 mM and a stable catalytic current at each fixed H$_2$O$_2$ concentration (Figure 4E). Additionally, the relationship between the cathodic current and the H$_2$O$_2$ concentrations (Figure 4F) showed an excellent linearity of 0.997 and a remarkable sensitivity of 4.178 $\mu$A mM$^{-1}$ mm$^{-2}$ in the range of 0-0.8 mM H$_2$O$_2$. The limit of detection of the sensor was 2.1 $\mu$M based on a signal-to-noise ratio of 3. When applied to the practical case of detecting a diluted dish cleaner solution, the sensor showed a fast and reliable response to confirm its concentration (Figure 4G). Thus, this study realized an efficient integrated setup of a microfluidic chip with Ag aerogel, but also highlighted an excellent performance and the further potential of Ag aerogel for H$_2$O$_2$ sensing.

Considering that the typical environment for biomolecule detection is liquid, noble metal aerogels would be filled by water, electrolytes, and other solutions during biological sensing. From this aspect, porous materials with the dispersion of water are also feasible for biological detection. Hydrogels also have the three-dimensional cross-linked porous structure like aerogels, while water is the dispersion medium. Noble metal hydrogels with similar structural features as aerogels are also very promising as biological enzyme supports and sensing materials, and their typical biosensing applications are also described here. Lately, Zhu et al. presented noble hydrogels of Au and AuPt as nanozyme and enzyme immobilization matrix for the detection of glucose and
organophosphorus pesticide, respectively.\textsuperscript{36,37} Typically, the polydopamine-capped bimetallic AuPt hydrogels with good biocompatibility, porosity, and high surface area served as stable platforms to immobilize acetylcholinesterase for the biosensor fabrication (Figure 5A,B), where this sensor exhibited a wide linearity range from 0.5 to 1000 ng/L with a low detection limit of 0.185 ng/L for organophosphorus pesticide (Figure 5C,D).\textsuperscript{36} Similarly, when working as nanozymes, the hierarchically porous Au hydrogel from the one-step dopamine-induced self-assembly of Au nanoparticles also showed enhanced GOD-like and peroxidase-like activities compared to other nanostructured Au materials (Figure 5E-5H).\textsuperscript{37} Besides the structural advantages, the high conductivity and excellent mass transfer in the noble metal hydrogels also contributed to the improved biological activity to outperform other materials.

With the features of ultralow density, high porosity and high specific surface area, good intrinsic activity, and sensitive response to substrates, noble metal aerogels are rising quickly in the past years to extend their biosensing applications. On the one hand, when acting as supports to incorporate natural enzymes, the large porosity of noble metal aerogels provides rich pores for the loading of natural enzymes with excellent dispersion and adhesion. Meanwhile, the good conductivity of aerogels allows faster charge transfer, and the porous structure promotes the mass transport during the reaction, thus contributing to the enhanced selective sensing performance to specific targets. On the other hand, the structure features and the catalytic activity enable the noble metal aerogels to function beneficially as efficient nanozymes to show promising catalytic activity and response as well. Therefore, noble metal aerogels are not only structure-stable supports for the immobilization of natural enzymes, but also excellent nanozymes to react with different targets like glucose, H$_2$O$_2$, etc., for fast \textit{in vitro} biological detection and sensing. However, limited research on the biological fields for noble metal aerogels have been published over the past years, and the number of studies in this field are still very low. Though the advantages of aerogels and their incorporation with enzymes are very attractive and fascinating, several challenges and perspectives are still inevitable for the further development of noble metal aerogels in biosensing applications, and these points are emphasized and described below.

1. Biocompatibility of aerogels. The biocompatibility of nanomaterials is of great concern for their biological
effects, especially for in vivo and clinical applications. Noble metals and their alloys are known to be generally nontoxic for cells and microorganisms, but their ions are usually toxic in most cases. Considering the complexity of biochemical environments in cells, microorganisms, and living bodies, the chemical/biochemical/electrochemical corrosion of noble metal aerogels may occur in specific conditions to release the corresponding ions. From this aspect, their potential toxicity can greatly impede noble metal aerogels from in vivo diagnosis. As a result, it is not surprising that no work has been reported or demonstrated the in vivo biological application of noble metal aerogels so far. Therefore, to have more options for in vivo biological diagnosis, the modification of noble metal aerogels to improve the biocompatibility is necessary. Many successful examples of organic aerogels and inorganic oxide aerogels (like silica) in clinical applications may guide the design and modulation of noble metal aerogels to have better stability and compatibility in living bodies and promote their in vivo sensing applications.\textsuperscript{24,25}

2. Category of noble metal aerogels. For different noble metals, their unique physiochemical properties suggest their special potentials in biological applications. However, mostly only Au, Ag, Pt, and Pd are studied as single noble metal aerogels for biological sensing applications up to now, as demonstrated above. For other noble metal elements like Ru, and Ir, not only their syntheses, but also the applications in sensing are rarely exhibited or reported. To enrich the diversity and category of aerogels, noble metal aerogels (besides the above mentioned) and the hybrid aerogels (as well as hydrogels) of noble metals with other elements may be developed in the future. Several advantages could be the result of preparing nonprecious and precious hybrid aerogels. First, both the electronic and crystalline structures of aerogels would be significantly regulated in a broad range, which may contribute to unexpected chemical properties and biological responses. Then, more biological substrates can be detected and sensed with the larger diversity of hybrid metal aerogels to satisfy various biochemical circumstances and requirements like vitamins and hormones. Last, noble metals can be utilized more efficiently with the incorporation of nonprecious elements, which furthermore would decrease the cost of aerogels as biosensors.

3. Effects of surface properties on biological properties. Over the past decades, the surface properties like facets, absorbed ligands, hydrophilicity, surface chemical states, etc. have been widely concerned and explored for nanosized materials to unveil their effects on the physical and chemical properties. In the above research results, the effect of ligands, facets, and surface chemical states of noble metal aerogels on their biological performances are discussed, suggesting their importance in biosensing. However, a deep and comprehensive understanding of the surface properties of noble metal aerogels is still lacking. Especially, the underlying correlations among various parameters impede to uncover and clarify their functions. To develop robust and highly active noble metal aerogels as mimic enzymes or supports for various enzymes, the challenges in investigating and regulating the surface properties should be overcome for both scientific and practical views.

4. Selectivity to different substrates. Unlike natural enzymes with high selectivity and sensitivity to specific substrates and detection conditions, nanozymes including noble metal aerogels can efficiently respond to various substrates under different biochemical conditions. The wide applicability on the one hand makes nanozymes robust and widely usable in practice, while on the other hand, it leads to difficulties in recognizing of the specific substrates (like homologues). At present, the selectivity is not listed and considered first as the key points for noble metal aerogels as nanozymes. Generally, experiments are carried out in vitro with known and given substrates and solutions, and therefore leading to a “look-good” selectivity during tests. However, the complex circumstances of in vivo applications with various inorganic salts, saccharides, amino acids, organelles, pH values, and so on may cause false responses and even failures of aerogel based biosensors. Therefore, the selectivity of noble metal aerogels, which can usually react with many different chemicals, should be seriously considered and designed to avoid false signals. Moreover, so far only a few substrates like glucose and H\textsubscript{2}O\textsubscript{2} have been successfully detected as targets for noble metal aerogels, while the selectivity and responses to other substrates are still unknown. From the view of practical applications, the selectivity of noble metal aerogels in biosensing requires further exhaustive exploration.

5. Wearable sensing application. The development of high-performance wearable sensors is of great importance for practical applications in biosensing, which would easily realize a real-time and non-invasive health monitoring out of the clinic. Thus, the flexible sensors working in bent and stretched states are of significance because human skin is highly elastic. Besides the flexible substrates, flexible sensing electrodes play a key role for the wearable sensor design.\textsuperscript{38} The noble metal aerogels composed of three-dimensional, interconnected networks of nanowires show excellent structure stability upon bending, probably promising.
a constant sensing performance during wearable monitoring when the noble metal aerogels are employed as either enzyme supports or nanzymes. To explore the wearable sensing application of such highly active and flexible noble metal aerogels would open up yet another attractive possibility for bio-diagnosis. More, besides the biocompatibility of aerogels to human skins in wearable devices, the compatibility between aerogels and substrates should also be concerned. The good connection and adhesion of aerogels on substrates could guarantee the well transportation of electrochemical signals from aerogels to devices, as well as the reliable mechanical strength to prevent aerogels from shedding during mechanical behaviors like bending, twisting, and rubbing.

In summary, the interconnected, hierarchical, porous structure features of noble metal aerogels provide good electron conductivity, a rich variety of pores for mass transfer, and improve the utilization of noble metals with large surface areas. These advantages further contribute to the fast-growing field of noble metal aerogels in biosensing applications as nanzymes and as supports for natural enzymes via optical, chemical, and electrochemical methods for the sensing of targets. However, the development and applications of noble metal aerogels in biosensing are still in an infant stage such that so far only a limited number of noble metal aerogels are proposed for several substrates detection. Challenges in aerogel types and composites, compatibility for in vitro and in vivo biological sensing applications, effects of surface properties, selective recognition of substrates, and wearable sensing applications should be well concerned and investigated in the future for a better understanding of noble metal aerogels and enhancing their activity. In short, as a large family of porous materials, noble metal aerogels hold attractive promises and potentials for the fabrication of biosensors, and their further study of faster response, lower cost, and more reliable applications are eagerly expected in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORCID

Wei Gao https://orcid.org/0000-0001-7606-7402
Dan Wen https://orcid.org/0000-0001-6879-7982

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**AUTHOR BIOGRAPHIES**

**Wei Gao** received his PhD degree in materials science and engineering from Xi’an Jiaotong University in 2018, and the joint PhD degree from City University of Hong Kong in 2018. He is working in Northwestern Polytechnical University as an associate professor. Now he is focusing on the development, modification, and applications of transition metal-based electrode materials for various electrocatalytic processes like water electrolysis and fuel cells.

**Dan Wen** received her PhD degree in analytical chemistry from Changchun Institute of Applied Chemistry, the Chinese Academy of Sciences in 2012. From 2012 to 2016, she worked at TU Dresden, Germany as a Humboldt Postdoctoral Fellow and a research associate. She is now working in Northwestern Polytechnical University as a full professor. Her current research interests include the synthesis, assembly, and bio-/electrocatalytic and sensing applications of nanostructured materials.

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