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Review—Recent Advances in the Development of Nanoporous Au for Sensing Applications

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In the fields of medicine, environmental protection, and food safety, sensors are imperative for the detection of biomarkers, contaminants, and preservatives. The use of nanoporous gold (NPG) as a sensing platform may greatly enhance performance due to its stability, high surface area, and catalytic abilities. There are many methods reported in the literature for fabricating NPG, including chemical strategies and various electrochemical techniques. The primarily use of NPG in sensing applications may be classified into three categories: electrochemical, bioelectrochemical, and optical. Although both electrochemical and bioelectrochemical sensors are based on the electrical signal produced by a specific analyte, a biological recognition element is involved in the bioelectrochemical sensing process. On the other hand, optical sensors exploit NPG through unique surface plasmon resonance properties that can be monitored by UV-Vis, Raman, or fluorescence spectroscopy. For this review, the primary strategies for fabricating NPG, including dealloying, electrochemical, and dynamic hydrogen bubble template (DHBT), are discussed. In addition, advances made over the last decade towards the detection of biomarkers, pollutants, contaminants, and food additives are highlighted. The future development of NPG based sensors for medical, environmental, and food safety applications is discussed. © 2020 The Author(s). Published on behalf of The Electrochemical Society by IOP Publishing Limited. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, http://creativecommons.org/licenses/ by/4.0/), which permits unrestricted reuse of the work in any medium, provided the original work is properly cited. [DOI: 10.1149/ 1945-7111/ab64c0] $(\mathbf{\hat{P}})$

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The ability to rapidly detect target molecules with high sensitivity and selectivity has significant and far-reaching impact in the fields of medicine, environmental monitoring, and food safety. Contingent on their composition and structure, sensors have the capacity to detect various analytes, such as glucose, metal ions in water, and preservatives in food.^{1–5} The significant diversity in sensor applications has led to a substantial level of research over the last decade. A good quality sensor must be highly stable, sensitive, selective, and economically viable.

A wide variety of materials have been explored for sensor applications, with much interest being directed to gold, as it exhibits biocompatibility, high stability, and conductivity.^{6–8} However, its high cost makes bulk gold economically prohibitive for some applications.^{9–11} Thus, the implementation of nanostructured Au is attractive as it has an exponentially increased surface area to volume ratio and sensitivity at a reduced cost due to less material needed for the same surface area. The fabrication of Au nanoparticles (NP) has been widely employed as a facile technique to achieve the desired surface area optimization; however, utilizing NPs requires their immobilization on an electrode surface, which may lead to issues insofar as their delamination and surface loss.

Another option is the nanostructuring of Au metal surfaces to create freestanding porous frameworks. This enables nanoscale surface area optimization without the recovery issues. A commonly utilized morphology is nanoporous gold (NPG). The increased catalytic activity of NPG over bulk gold has been attributed to the unique highly curved morphology that permeate the material, exhibiting a high density of steps, holes, and kinks.¹² These areas are host to low coordination atoms, in contrast to the normal high coordination associated with a close-packed surface. These low coordination sites interact more strongly with target molecules due to their local upshift and narrowed d-band, which are generally involved in Au bonding.^{12,13}

With a myriad of studies published on sensors due to their wide applications, each with a number of variations that can be optimized under specific conditions, it would be a gargantuan task to complete an exhaustive list of the published research. Here we have endeavored to select a number of recent works that best represent the most common approaches of NPG for sensing, including the prevalent fabrication methods, for the detection of target analytes in electrochemical, bioelectrochemical, and optical sensing.

Electrochemical sensors operate by the target material coming into contact with the electrode surface and undergoing a redox reaction, which produces an electrical signal that can then be attributed to the specific analyte material. Bioelectrochemical sensors utilize biomolecular receptors to specifically bind to the target analyte. Generally, the fabrication of bioelectrochemical sensors is more complex as they require the immobilization of enzymes or aptamers; however, this may make them more selective than their electrochemical counterparts.

The unique optical properties of NPG may be utilized in the form of optical sensors. When the dimensions of metals such as Au are reduced to the scale of the mean free path of electrons within the material, the oscillations of electrons generate localized surface plasmon resonance (LSPR).¹⁴ These electron waves may interact with their surroundings, which can be used to enhance spectroscopic characterization techniques, such as surface enhanced Raman spectroscopy (SERS).¹⁵

With the resonant frequency dictated by the size of the materials, UV-vis absorption can be employed to determine both the resonant wavelengths and dimensions. In the case of NPs, there is generally a single peak observed in the UV-vis spectrum, which is related to the nanoparticle size.¹⁶ In the case of NPG, multiple absorption peaks may be observed due to the different ligament aspect ratios. Stronger enhancements are observed as the dimensions of the nanopores of the NPG structures are reduced.¹⁷

The use of NPG for electrochemical catalysis, sensors, capacitance, and medical applications has been widely reported in literature. Its high surface area interfaced with a conductive substrate lends itself very well toward its implementation as an electrode for electrochemical studies. These, coupled with a variety of simple fabrication methods, are some of the reasons behind its widespread interest. In this review, we will focus on some common methods for the fabrication of NPG, as well as recent advances for its use in electrochemical, bioelectrochemical, and optical sensing applications that have been made over the last decade.

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Synthesis of NPG

There are various strategies available for the creation of NPG for sensing applications. Three primary techniques currently employed for the synthesis of NPG, including dealloying, electrochemical, and dynamic hydrogen bubble templating, are presented in this section.

Dealloying.—The process of dealloying is widely utilized in the formation of NPG due to its facile process and the availability of commercial high purity gold alloys.^{18,19} By varying leaching conditions such as time and temperature, the size, shape, and density of nanopores can be tuned, which are critical to the performance of sensors.^{20,21} The starting Au–M alloys can either be cast or cosputtered onto a substrate for dealloying via an electrochemical method, or chemical corrosion under acidic conditions.²² The selective dissolution of less noble metals (e.g., Zn, Cu, Ag) in the alloy has been extensively studied, both experimentally and theoretically.^{2,23,24}

A general schematic of the corrosion method is illustrated in Fig. 1, where Au-Cu thin films with different Au-Cu ratios were cosputtered on glass and silicon. The formed Au-Cu alloys were then dealloyed by the selective dissolution of Cu in nitric acid, forming a NPG thin film.²⁵ This study revealed that an Au-Cu thin film with a high percentage of Au exhibited slow kinetics in NPG formation, and that longer dealloying times increased porosity.²⁶

The propagation of subsurface cracks throughout the Au–M alloy is quintessential to the mechanism of pore formation, and is contingent on the relative composition of the alloy, which is imperative to facilitate the dealloying process.²⁶ There must be sufficient quantities of the less noble metals available to achieve the partitioning limit of the alloy. The partitioning limit was found to be 60% in the case of Au–Ag alloys, and 40% for Au–Cu alloys.²⁵ While the relative compositions of alloys impact NPG formation, the formed crystal phase and defects sites also depend on the free corrosion mechanism.^{27,28} This leads to variations between the NPG prepared from homogeneous or non-homogeneous alloys. While there are a significant number of variables that introduce complexity, which needs to be controlled to fabricate reproducible NPG surfaces, the manipulation of these variables can be utilized to tune the film thickness, pore size, Au aspect ratio, and pore density.

Electrochemical.—Electrochemical techniques are commonly employed to create highly porous structures. A variety of approaches can fall under this category, such as electrochemical alloying/ dealloying and anodization.^{29–32} The inclusion of a potentiostat to control and monitor the conditions leads to a higher level of control of the NPG pore dimensions and uniformity over the free corrosion process. Electrochemical approaches can effectively eliminate the requirement for the preformation of Au-M alloys via an in situ electrochemical alloying/dealloying process.

The electrochemical alloying/dealloying technique is a one-step process to produce NPG, which is widely utilized in the fabrication of gold wire microelectrodes.³³ An Au metal electrode is placed in a three-electrode cell that contains an electrolyte, which alloying metal ions, such as Zn, Al, Ag, etc.³⁴ Cyclic voltammetry (CV) is performed, typically with a sweep rate between 5 and 50 mV s⁻¹ over a potential window tailored to enable the electrochemical alloying/dealloying process. The cathodic sweep reduces metal ions in solution at the Au working electrode, forming an Au-M alloy. The anodic sweep then oxidizes the alloyed metal and removes it from the Au electrode, which leaves a pitted surface behind. By continuing this alloying/dealloying process, the pitting deepens and widens, thus creating a nanoporous structure.

An example of this process is shown in Fig. 2a, where an Au-Zn alloying/dealloying process was employed to fabricate a NPG microelectrode in a 1.5 M ZnCl₂ solution at a potential range between -0.80 and +1.80 V (vs Zn) at a scan rate of 10 mV s⁻¹ at 110 °C.³⁵ Energy dispersive X-ray spectroscopy (EDX) and X-ray diffraction (XRD) analysis (Figs. 2b and 2c) revealed that some Zn was present following the alloying/dealloying process. A treatment in a H₂SO₄ solution could be used to remove the residual Zn.

Another electrochemical process that is commonly performed to fabricate NPG is the direct oxidation/reduction of the Au electrode without the requirement of a metal ion electrolyte to facilitate alloying/dealloying. This was shown in work of Sukeri et al., where an Au electrode was held at a potential ~ 2.0 V vs a Ag/AgCl reference electrode in a 0.5 M H₂SO₄ solution.³⁶ This anodization generated an oxide film on the gold surface.³⁷ The reduction of this electrode led to the pitting of the oxide layer, which resulted in a NPG surface. This anodization process is an excellent example of a green chemistry initiative, where fabrication/synthesis processes are optimized to reduce the quantity of reagents consumed.

Dynamic hydrogen bubble template.—Most of the processes discussed up to this point have been well documented and utilized for the fabrication of NPG. One newer approach of producing NPG is known as the dynamic hydrogen bubble template (DHBT) method. This strategy was initially utilized for metals that are more active according to the volcano curve, in terms of activity toward the cathodic portion of the water splitting reaction.^{38–40} Metals such as Zn, Cu, Sn, and Ag have been extensively employed to produce multimodal nanoporous foams via the DHBT method. More recently, this process has been expanded to include metals such as gold.^{41–43}

For this process, metals may be classified as one of three distinct groups, referred to as normal metals (Cd, Zn, Sn, Ag), which have a



Figure 1. General process of free corrosion chemical dealloying. Co-deposited Au-Cu alloy immersed in concentrated nitric acid. Reprinted with permission from Ref. 25 Copyright 2015 American Chemical Society.



Figure 2. Process of electrochemical alloying/dealloying. Polycrystalline Au (a) undergoes cycling in ZnCl electrolyte, resulting in a nanoporous morphology. Further acid treatment removes excess Zn. XRD diffractograms (b) and EDX (c) of the substrate at various stages of the process confirm the total removal of Zn from the sample. Reprinted with permission from Ref. 35 Copyright, 2018 Elsevier.

high exchange current density and a low melting point; intermediate metals (Au, Cu, Ag), with an intermediate exchange current density and melting point; and inert metals (Fe, Ni, Co, Pt, Cr, Mn), which exhibit a low exchange current density and a relatively high melting point. It has been shown that for metals with low and intermediate exchange currents, dendrite formation may occur if the overpotential (η) for deposition is higher than the minimum initiation overpotential (η_i) denoted by the equation:

$$\eta > \eta_i = \frac{RT}{nF} \frac{j_L}{j_i} \tag{1}$$

where j_L and j_i are the limiting and exchange current densities for the reduction of the metal ion, and *R*, *T*, *n*, and *F* are the temperature, moles, and Faraday constant, respectively. Chung et al. postulated that only metals categorized as normal or intermediate classes, as defined above, can be utilized in the electrodeposition of multimodal nanoporous foams.³⁸

The fundamental mechanism behind this process is as follows: a low concentration of chloroauric acid (~0.1 M) with a quantity of ammonium, composes the electrolyte in a three-electrode cell, with a Pt or Ti working electrode typically against a Pt counter electrode. A potential within the H₂ evolution regime, normally between -4 to -8 V, is then applied to the working electrode. The evolution of H₂ and the reduction of Au ions occur simultaneously at the electrode surface. Continuous gas nucleation, coalescence, and desorption at the electrode surface results in a self-supporting Au thin film composed of micrometer pores with nanoporous features.

In addition, Wei et al. modified this process by repeating gold oxidation and reduction/ H_2 evolution at an Au disk working electrode, using square wave potential pulse (SWPP) voltammetry,

with the potential being switched between +0.8 and -5 V at 50 Hz.⁴⁴ When the applied pulse potential was held at 0.8 V, the gold was oxidized, accompanied by a release of oxygen gas. When the potential was switched to -5 V, the formed oxide layer was reduced to gold metal, which self-assembled under the action of intense hydrogen bubbles. As the cycle was repeated, 2D and 3D porous structures were developed, as shown in Figs. 3a-3c.⁴⁴

Overall, the common fabrication techniques described in literature trend toward favoring simplicity, as well as control over pore size, pore density, and film thickness. Of the three aforementioned common processes, dealloying is the most passive approach. The free corrosion process and initial conditions dictate the NPG formation without monitoring further manipulation. This method is often employed for the facile production of NPG from prefabricated Au-M alloy leaves, which are widely available for purchase. Synthetic methods are imperative to the reproducibility of samples; however, reproducibility of methods used is rarely reported in literature, making it difficult to compare the optimal approach. Additional control and the specificity of NPG morphologies can be dictated by more active electrochemical fabrication methods, such as alloying/dealloying and DHBT methods. Progress in these processes has trended towards green chemistry, with the reduction and elimination of consumable reagents wherever possible.

NPG Based Electrochemical Sensor

The high specific surface area, stability, facile preparation and modification, and high electrochemical response make NPG an ideal candidate as an electrochemical sensor. Due to these unique properties, NPGs have been extensively used for the direct electrochemical sensing of various molecules and ions for medical,



Figure 3. Schematic of SWPP approach to the dynamic hydrogen bubble template synthetic route to NPG formation. Repeated oxidation and reduction/ hydrogen gas formation leads to the accretion of Au metal around the H_2 gas bubbles. This results in a multimodal nanoporous structure (a)–(c) along the substrate. Reprinted with permission from Ref. 44 Copyright 2009 American Chemical Society.

environmental, and food safety applications. The performance of the sensors being reviewed is compared in Table I.

Electrochemical sensing of biomarkers.—The monitoring of biomarkers such as glucose (Glu), dopamine (DA), ascorbic acid (AA), and nitric oxide (NO) is essential in medicine. These can be detected by monitoring for their direct oxidation at the electrode surface with the electrochemical technique being implemented.⁶⁷ Among these, significant efforts have been invested in the none-nzymatic detection of glucose concentrations in blood due to its importance in clinical diagnoses, and the monitoring of glucose levels for those afflicted with diabetes. Recently, NPG and modified NPG, fabricated by various means, were used as promising platforms for the electrochemical sensing of glucose. Li et al. developed a simple and quick electrochemical step (Fig. 4A) in the initial passivation region of pure gold in a HCl or KCl solution.^{68,69}

The preparation process, as probed by in situ Raman spectroelectrochemistry, involved anodic dissolution, Au(I) disproportionation, and Au deposition.⁴⁵ At the preparation potential, reduced gold atoms from Au(I) disproportionation may be easily passivated, aggregating onto a surface to form a nanoporous gold film. A nonenzymatic glucose sensor based on the formed NPG exhibited a high sensitivity of 232 μ A mM⁻¹ cm⁻² and a wide linear range, of from 1 mM to 14 mM, with a limit of detection of 53.2 μ M (*S IN* = 3) in the absence of Cl⁻. Further, a sensitivity of 66 μ A mM⁻¹ cm⁻² and a linear range from 10 μ M to 10 mM with a limit of detection of 8.7 μ M (*S*/*N* = 3) in the presence of 0.1 M NaCl (Figs. 4A–4b) were observed.

More recently, in combination with a dealloying process, Xia et al. fabricated a NPG film modified with $Au_{80}Sn_{20}$ (wt.%) in a HCl medium by applying an anodic potential step for 80 s at its initial passivation region (Fig. 4B).⁴⁶ The prepared NPG/AuSn

demonstrated enhanced performance for the oxidation of glucose in an alkaline electrolyte (Figs. 4B–4b and 4c) with rapid response (4 s), high sensitivity (4374.6 μ A mM⁻¹ cm⁻²), a wide linear detection range (2 μ M to 8.11 mM), a low detection limit (0.36 μ M, *S* /*N* = 3), and good selectivity. The incorporation of additional components into/on NPG films may enhance the electrochemical detection of glucose.⁴²

As depicted in Fig. 4C, the incorporation of Ni(OH)₂ on the NPG film formed two pairs of efficient electron mediators, Ni(II)/Ni(III) and Au/Au(I). Their coupling (Ni(III) + Au = Ni(II) + Au(I)) resulted in high electrocatalytic activity toward the oxidation of glucose, and excellent performance for glucose sensing.⁴² Lang et al. prepared a three-dimensional (3D) bicontinuous NPG layer directly onto gold microwires, initially though an electrochemical alloying/dealloying approach, and then decorated the surface with cobalt oxide using a hydrothermal method.³⁴ The formed NPG/Co₃O₄ microelectrode exhibited synergistic electrocatalytic activity for glucose sensing with high sensitivity (up to 12.5 mA mM⁻¹ cm⁻²), rapid response (less than 1 s), and a low detection limit (5 nM).

The electrochemical detection of NO, DA, and AA has also received much attention using NPG as a sensor platform due to their biological significance. Recently, Liu et al. fabricated a hierarchical 3D NPG through the electrochemical alloying/dealloying of an Au microwire (Figs. 5A-5a).⁵² The NPG demonstrated enhanced electrocatalytic activity for the oxidation of NO, 6.23 times higher than the bare gold microwire electrode, (Figs. 5A-5b) and exhibited excellent performance for the detection of NO by differential pulse voltammetry (DPV) (Figs. 5A-5c) and amperometry (Figs. 5A-5d). The developed NPG was further tested for the in situ monitoring of NO release from different cells.⁵²

DA plays a critical role in facilitating the operation of the mammalian central nervous system. It is essential to accurately

Sensor type	Electrode	Analyte	Technique	Linear range	LOD	References
Electrochemical	NPG	glucose	CA	1–14 mM	53.2 µM	45
	NPG	C	CA	2 μM-8.11 mM	0.36 µM	46
	Ni(OH) ₂ / NPG		CA	$\frac{1}{2}$ μ M–7 mM	0.73 μM	42
	AuNP/SWCNT Hybrid gold film	dopamine	CV	50 nM-1 mM	10 nM	47
	, e	serotonin		250 nM-1 mM	50 nM	
	NPG	dopamine	SWV	$0.1 - 10 \ \mu M$	0.030 µM	48
	NPG	ascorbic acid	CA	0.1 μM–13 mM	0.06 μM	49
	NPG/SWCNT	BPA	DNPV	, <u> </u>	$0.10 \ \mu M$	50
	SH- β -CD/ NPGL		SW	0.3–100 µM	0.06 μM	51
	NPG	NO	DPV	0.360–18.0 μM	$0.018 \ \mu M$	52
	NPG	N_2H_4 SO2 ²⁻	DPV	5.0–4000 µM	0.911 μM 0.377 μM	5
		NO_2^-		0.0.110.1.14	$1.44 \ \mu M$	53
	NPG@N1	N_2H_4 H_2O_2	CA	0.2–110.1 μM 20–9740 μM	0.033 μM 10.0 μM	
	Pt NPs/NPG	H_2O_2	CA		0.3 nM	54
	NPG	O_2	CA	0.054–0.177v/v %	0.0075%	55
	FeOOH/ NPG	Hg^{2+}	SWV	0.02–2.2 μM	$0.0078 \ \mu M$	4
	NPG	Hg^{2+}	CV	0.1-100 mM	5 nM	56
		N_2H_4		0.01–2 mM	0.62 μM	
		nitrobenzene		0.01–5 mM	0.585 μM	
Bioelectrochemical	GOx/NPG	glucose	LSV	50 µM-10 mM	$1.02 \ \mu M$	57
	GOx/NPG	glucose	CA	$50 \ \mu M$ – $10 \ mM$	25 µM	58
	GOx/Thiol/NPG	glucose	CA	1 mM-19 mM	$10 \ \mu M$	59
	GOx/PEDOT/GOx	glucose	CA	0.1mM-15 mM		60
	ChOx-HRP-ChE/NPG	cholesterol	CV	0.97 mM-7.8 mM	0.64 mM	61
	NPG & PANI-Au AMNPs	CA72-4	CA	$2-200 \text{ U mL}^{-1}$	0.1 U mL^{-1}	62
	GR-5DNAzyme/NPG	Pb^{2+}	DPV	1.0 nM-120 nM	20 pM	63
	S1-S2-S3/NPG	Ag^+	SWV	$0.1 \text{ nM} - 1 \mu \text{M}$	48 nM	64
	Thymine-Hg ²⁺ -thymine/NPG	Hg^{2+}	DPV	0.01 nM-5000 nM	3.6 pM	65
Optical	Cy5/NPG	Hg^{2+}	Raman	N/A	1.0 pM	17
	R6G/NPG	Hg^{2+}	Fluorescence	1 nM–1 pM	<1.0 pM	66

CA-chronoamperometry, CV-cyclic voltammetry, DPV-differential pulse voltammetry, SWV-square wave voltammetry, SW-AdSV-square wave anodic stripping voltammetry, DNPV-differential normal pulse voltammetry, SWASV-square wave anodic stripping voltammetry, BPA-bisphenol A, μ M-micromolar, mM-millimolar, nM-nanomolar, HNPG-highly surface-roughened nanoporous gold film, S/NPG/Co₃O₄-seamless solid/nanoporous Au/Co₃O₄, MIP/NPAMR-molecularly imprinted polymer/nanoporous AuAg alloy microrod, SH- β -CD/ NPGL-thiolated beta-cyclodextrin (SH- β -CD) self-assembled NPG leaf, NPG@Ni-three-dimensional nanoporous gold supported on three dimensional Ni foam.

detect DA for the diagnosis of cognitive diseases. Recently, Xiao et al. fabricated a highly conductive, paper-based NPG films composed of gold NPs layered atop a matrix of metallic single walled carbon nanotubes (SWCNTs) on mixed cellulose ester filter paper (Fig. 5B).⁴⁷ The as-prepared hybrid gold film was applied to detect DA and serotonin (5-HT) by CV, exhibiting a linear range from 50 nM to 1 mM with a detection limit of 10 nM for DA (Figs. 5B-5b) and from 250 nM to 1 mM with a detection limit of 50 nM for 5-HT (Figs. 5B-5c). More recently, Espinoza-Montero et al. developed an electrochemical DA sensor based on a NPG microelectrode, where the NPG was prepared via the electrochemical anodization-reduction of a gold microelectrode in a H2SO4 solution.⁴⁸ Square wave voltammetry (SWV) revealed a linear range of from 0.1–10 μ M, detection limit of 30 nM, and sensitivity of 1.18 mA μ M⁻¹ cm⁻². In addition, a proof-of-concept for the sensor application in the detection of released DA using scanning electrochemical microscopy (SECM) was demonstrated.

An important role in the body is also played by AA, as it is an essential nutrient for animals and humans, which is involved in metabolism. The determination of AA is beneficial for medical diagnostics and food safety applications. Huang et al. reported on the synthesis of small Au NPs ($2 \sim 5$ nm) by anodically dispersing a gold wire in an acidic solution containing Cl⁻ and SnCl₂ (Figs. 5C–5 a).⁴⁹ The modification of the Au NPs onto a glassy carbon electrode (AuNPs/GCE) resulted in a hierarchical NPG structure (Figs. 5C–5b) due to a protective residual Sn oxide layer. The formed NPG

sensor exhibited high electrocatalytic activity for AA oxidation, and excellent performance for the amperometric detection of AA, with a wide linear range of from 0.1 μ M to 13 mM, high sensitivity of 1033 μ A mM⁻¹ cm⁻², and a low detection limit of 0.06 μ M (Figs. 5C–5c and 5d).

Electrochemical sensors for food safety.—Food safety has received considerable attention due to the potential existence of variety of chemical additives and contaminants. Electrochemistry provides facile and effective analytical methods to assess food safety and quality, where the electrode material plays a key role in the design of sensors. NPG has been explored as an electrochemical sensing platform for the detection of a variety of food additives and contaminants, such as bisphenol A (BPA), hydrazine (N₂H₄), sulfite (SO₃^{2–}), and nitrite (NO₂[–]). Fang et al. fabricated NPG (Fig. 6A) through the anodic etching of gold in a mixed solution of hydrofluoric acid (HF) and dimethylformamide (DMF).⁵⁰ Following modification with SWCNTs, the prepared BPA sensor showed a low detection limit of 100 nM (*S/N* = 3).⁵⁰

Recently, Li et al. developed a dual-signal BPA sensor by selfassembling of thiolated beta-cyclodextrin (SH- β -CD) on NPG leaf.⁵¹ The fabricated sensor revealed a wide linear range of from 0.3–100 μ M and a low detection limit of 60 nM. It was tested for the detection of BPA in milk and tap water with high accuracy.⁵¹ Manikandan et al. employed an electrochemical alloying/dealloying method to create a NPG microelectrode (Figs. 6B–6a), and



Figure 4. (A) (a) LSV (solid line) at 5 mV s⁻¹ and current–time transient (dashed line) at 1.29 V (indicated with vertical dotted line, vs SCE) for a polished gold electrode ($\phi = 2 \text{ mm}$) in 1 M KCl, and (b) Amperometric responses of the nanoporous gold film (NPGF) electrode at 0.2 V to the successive addition of glucose in a continuously stirred solution of 0.1 M PBS (pH 7.4) + 0.1 M NaCl. Reprinted with permission from Ref. 69 Copyright 2011 Elsevier (B) (a) A schematic diagram regarding the formation of HNPG/AuSn and NPG/Au during potentiostatic anodization vs SCE), (b) Effect of the detection potentials on the amperometric responses of HNPG/AuSn for 1 mM glucose, 0.1 mM AA and 0.02 mM UA in 0.1 M NaOH and (c) Amperometric responses to the successive addition of different concentrations of glucose in 0.1M NaOH under continuous stirring at 0.1 V. Reprinted with permission from Ref. 46 Copyright 2018 Elsevier (C) (a) SEM and TEM (the inset) of the Ni(OH)₂-coated NPGF electrode and (b) Calibration curve corresponding to the amperometric responses of the Ni(OH)₂/NPGF electrode to the successive addition of glucose in a continuously stirred solution of 0.1 M NaOH at 0.1 V (vs Hg/HgSO₄). Reprinted with permission from Ref. 42 Copyright 2014 Elsevier.

employed it for the simultaneous detection of N₂H₄, SO₃²⁻ and NO₂⁻ by DPV (Figs. 6B–6b).⁵ The NPG sensor exhibited a wide linear range of from 5.0 to 4000 μ M and low detection limits of 0.911, 0.337, and 1.44 μ M for the detection of N₂H₄, SO₃²⁻, and NO₂⁻, respectively. The sensor was further tested with real samples of water, wine, apple cider beer, and beef with a high level of accuracy.

Electrochemical sensing for environmental applications.—The presence of toxic heavy-metal ions (Hg(II), As(III), etc) in the environment has led to increasingly serious global threats to human and animal health, as well as natural ecosystems. It is thus urgent that simple and reliable analytical methods for the sensitive detection of these toxic ions be developed. Electrochemical determination is a promising approach between various analytical methods. Recently, Liu et al. developed a Hg(II) sensor based on a FeOOH/NPG microelectrode (Fig. 7A).⁴ The NPG microelectrode with a hierarchical porous structure (Figs. 7A–7b) was prepared by electrochemical alloying/dealloying and then decorated with FeOOH nanoflakes (Figs. 7A–7c) by electrodeposition.⁴

The developed FeOOH/NPG microelectrode possessed a 3D network and a high specific surface area, which facilitated the transport and accumulation of analyte Hg(II) ions. It exhibited excellent detection performance (Figs. 7A–7d) with a sensitivity of 123.5 μ A μ M⁻¹ cm⁻², a linear range of 0.02–2.2 μ M, a detection limit of 7.81 nM, and good anti-interference from a number of common ions. Fang et al. prepared NPGs by alloying and dealloying Au–Zn alloy in ZnCl₂–urea deep eutectic solvent (Figs. 7B–7a). The NPs showed an excellent performances for the detection of Hg²⁺ (Figs. 7B–7b) and nitrobenzene (Figs. 7B–7c) by CV.⁵⁶

Other electrochemical sensing applications.—Thin films comprised of NPG might serve as sensor platforms for the detection of other important species. Cui et al. prepared a hybrid NPG@Ni foam electrode by dealloying an electrodeposited AuSn film on a Ni foam.⁵³ The NPG@Ni foam was applied for the detection of H_2O_2 and N_2H_4 with a high sensitivity of 2.88 and 10.687 mA mM⁻¹ cm⁻², and detection limits of from 10 μ M and 33 nM, respectively.

Huang et al. fabricated a self-supported NPG microelectrode by electrochemical alloying/dealloying method and then decorated with tiny Pt NPs on it.⁵⁴ The sensor based on the Pt NPs/NPG has high sensitivity and selectivity towards the reduction of hydrogen peroxide with a low detection limit of 0.3 nM. Hu et al. developed a facile approach to create NPG electrode arrays for the electrochemical sensing of O₂ in an ionic liquid (Fig. 8).⁵⁵ The O₂ sensor possessed high sensitivity, a linear range of from 0.054–0.177 v/v %, a low detection limit (0.0075%), and a quick response time (< 10 s).

Electrochemical sensors based on NPG are promising toward the detection of biomarkers, contaminants, and additives in food, environmental pollutants, and more, due to their extensive active surface areas and high stability. In instances where there are competing molecules along with the target analyte, there are cases where the target will be screened by other species. This may result in false positives or non-detection of the intended analyte. The incorporation of biomolecules such as enzymes and DNA may improve the selectivity of NPG.

NPG Based Electrochemical Biosensor

NPG has been widely used as a platform for the immobilization of enzymes and other biomolecules in the development of electrochemical biosensors.^{2,7,31,57–59,62–65,70–74} In this section, the NPG based electrochemical biosensors for the detection of metal ions, glucose, and other biological molecules are discussed.

Bioelectrochemical sensing of biomarkers.—Glucose biosensors are one of the most widely investigated and commercially applied sensor devices worldwide. Most of glucose biosensors are based on the GOx enzymatic reaction, shown in the following reaction:



Figure 5. (A) (a) SEM images of hierarchical nanoporous gold (HNG) microwire treated by electrochemical alloying/dealloying in a mixed electrolyte of benzyl alcohol (BA) and ZnCl₂, (b) CV response to 5.0 μ M NO recorded on a gold microwire electrode and the HNG microelectrode in 0.1 M PBS (pH 7.2) at a scan rate of 20 mV s⁻¹, (c) DPV responses and (d) Amperometric responses at 0.8 V of the HNG microelectrode for the analysis of different concentrations of NO in 0.1 M of PBS (pH 7.2). The potentials in (b)–(d) refer to the Ag/AgCl (1 M KCl). Reprinted with permission from Ref. 52 Copyright 2017 American Chemical Society. (B) (a) Schematic representation of surface morphology and photograph of AuNP/SWCNT hybrid gold film and its application in the electrochemical discrimination between dopamine (DA) and serotonin (5-HT). (b)–(c) Calibration curve for CV current of various concentrations of (b) DA at 0.19 V and (c) 5-HT a 0.39 V in 0.1 M PBS (pH 7.4). The potentials refer to Ag/AgCl. Reprinted with permission from Ref. 47 Copyright 2015 American Chemical Society. (C) (a) Dispersion mechanism of an Au wire by anodic potential step in an acidic solution, containing Cl⁻ and SnCl₂, (b) SEM and TEM images for the nanoporous of AuNPs/GCE surface, and (c) Amperometric curve for the AuNPs/GCE at 0.05 V and (d) Corresponding calibration curve for the different concentrations of AA in 0.2 M PBS (pH 7.4). Potentials refer to Hg/HgSO₄ (sat. K₂SO₄). Reprinted with permission from Ref. 49

$Glucose + O_2 + H_2O \rightarrow Gluconic \ acid + H_2O_2$

GOx can selectively interact with glucose and catalyze the oxidation of glucose to gluconic acid and H_2O_2 . Therefore, the signal from the electrochemical measurement of the H_2O_2 oxidation is directly correlated to the concentration of glucose present in the electrolyte. NPG with continuous 3D nanostructures with immense surface areas provides an ideal environment for the immobilization of biological molecules and exhibits good performance for glucose sensing.

Ahmadalinezhad et al. developed a biosensor of immobilization of glucose oxidase on a Prussian blue modified nanoporous gold surface.⁷⁵ The electrochemical behavior and the response of the glucose biosensing are shown in Fig. 9. This biosensor with nanoporous gold as the signal amplifier exhibited a very low detection limit of 2.5 μ M glucose and high sensitivity of 177 μ A mM-1. Chen et al. investigated the effects of a thiol-linker and the NPG nanopore size on the sensitivity of a glucose biosensor.⁵⁹ The thiol-linker enhanced biosensor demonstrated a significantly higher sensitivity due to an enhanced GOx distribution density and non-destructive enzyme bioactivity. When comparing sensitivity in relation to the NPG pore size (from 18 nm to 50 nm), the highest sensitivity of 8.6 μ A cm⁻² mM⁻¹ with a detection limit of 10 μ M was achieved for the NPG, with a pore size of 30 nm, which may have been related to the feasibility of the macromolecule enzyme immobilization and free access of the small molecules.

Xiao et al. integrated poly(3.4-ethylenedioxythiophene) (PEDOT) as an electrochemically stable conducting medium with

the NPG/GOx biosensor for the improved detection of glucose.⁶⁰ The optimized NPG/PEDOT/GOx biosensor mediated by p-benzoquinone (BQ) revealed a sensitivity of 7.3 μ A cm⁻² mM⁻¹ and a linear range from 0.1 to 15 mM, which demonstrated improved performance over an Au/PEDOT/GOx biosensor with a smooth gold surface. In another of Xiao's studies, two redox mediators (BQ and ferrocenecarboxylic acid (FCA)) were explored for shuttling electrons between the enzyme redox centre within the GOx and the NPG electrode.⁷³ Stronger electronic interactions with the electrode surface was achieved for the electrochemical electron transfer (ET) of BQ compared with FCA, which resulted in both higher interfacial electrochemical ET rates and more efficient functionality toward mediated GOx electrocatalysis.

Further, NPG has been frequently employed for the detection of other biological molecules such as cholesterol, adenosine triphosphate (ATP) and carbohydrate antigen 72-4 (CA72-4).^{31,71,62} Ahmadalinezhad et al. fabricated a biosensor using three enzymes, including cholesterol oxidase (ChOx), cholesterol esterase (ChE), and horseradish peroxidase (HRP), which were immobilized on NPG for the detection of cholesterol, where ChE catalyzed the hydrolysis of esterase-esterified cholesterol, and ChOx catalyzed the oxidation of cholesterol to form H_2O_2 .

The HRP catalyzed H_2O_2 reduction reaction may be electrochemically monitored by CV, which is indirectly dependant on the quantities of cholesterol and esterase-esterified cholesterol (Fig. 10).⁶¹ The designed biosensor exhibited a high sensitivity of 29.33 μ A mM⁻¹ cm⁻² and a wide linear detection range of up to 300 mg dL⁻¹. Kashifi-Kheyrabadi et al. also selected NPG as a platform for the development of an electrochemical ATP



Figure 6. (A) (a) Nanoporous gold (NpAu) film fabricated by electrochemical etching of gold in a solution of hydrofluoric acid and dimethylformamide, (b) DNPV curves obtained on the NpAu-SWCNT electrode in the presence of different concentrations of BPA (including a DNPV curve obtained on the NpAu electrode (10 mM BPA)) and corresponding calibration curve. Reprinted with permission from Ref. 50 Copyright Royal Society of Chemistry. (B) (a) SEM image of gold microelectrode following the alloying/dealloying process, and (b) DPV responses of the nanoporous gold microelectrode in 0.1 M PBS (pH 6.5) without (dashed line) and with (solid line) N_2H_4 , SO_3^{2-} , and NO_2^{-} (500 μ M each). Reprinted with permission from Ref. 5 Copyright 2018 Elsevier.

aptasensor and signal amplifier.³¹ The first fragment of the ATP binding aptamer was immobilized on NPG via a self-assembled alkanethiol monolayer. The second fragment of the aptamer was bound to 3,4-diaminobenzoic acid (DABA).

The two aptamer fragments interacted with each other only in the presence of ATP, which resulted in increments of the amounts of DABA on the electrode surface, which was monitored using DPV. The fabricated aptasensor exhibited detection limits at the submicromolar level, along with good stability. Fan et al. designed a sensitive electrochemical immunosensor for CA72-4 detection by employing a NPG film as the substrate, to immobilize a large population of antibodies and polyaniline-Au asymmetric multicomponent nanoparticles, with anti-CA72-4 adsorbed (PANi-Au AMNPs-Ab2) as labels.⁶² This immunosensor exhibited a detection limit of 0.10 U/ml CA72-4 and a linear range of from 2 to 200 U ml⁻¹, which were attributed to synergetic effects between the NPG film and polyaniline-Au nanoparticles for signal enhancement.

The use of NPG based bioelectrochemical sensors for the detection of biomarkers, environmental pollutants, and other targets showed excellent selectivity and sensitivity. However, the addition of biomolecules to the electrode surface for increased selectivity towards the desired analyte may have had a trade-off with stability. These molecules are susceptible to extreme pH values, as well as the possibility of denaturing at high temperatures.

Bioelectrochemical sensors for environmental applications.— Due to increased industrial activities, metal ions (e.g., Pb^{2+} , Ag^+ , and Hg^{2+}) have reached threshold levels in the environment that pose serious risks to plants, animals, and human health, both directly and by accumulation through the food chain. The accurate detection of metal ions is a prerequisite to the treatment and removal of contaminants from the environment. A label-free electrochemical biosensor was designed by Zhou et al. for the detection of Hg^{2+} , which employed NPG as the signal amplifier, and an immobilization platform.⁶³ A thiolated DNA hybridized GR-5DNAzyme was employed as the probe, and disodium-anthraquinone-2,6-disulponate (AQDS) as the DNA intercalator. When the prepared biosensor was immersed into an AQDS solution, the AQDS intercalated into the double stranded DNA probe and generated a reversable redox reaction in DPV measurements.

When Pb^{2+} was introduced into the solution, the trans-acting catalytic strand cleaved the phosphodiester into two fragments, resulting in a decrement of the redox reaction of AQDS in the DPV measurement. The changes in the DPV signal due to the interactions between DNA and Pb^{2+} depended on the concentration of Pb^{2+} that was present in the solution, which allowed for the monitoring of Pb^{2+} . A linear regression of the current vs the Pb^{2+} concentration, with a correlation coefficient of $R^2 = 0.9906$ was obtained. This biosensor demonstrated a wide dynamic working range of from 1 to 120 nM and a detection limit of 0.02 nM.



Figure 7. (A) (a) Schematic of the effective electrochemical detection of Hg(II) using the FeOOH/NPG microelectrode and (b) Typical SWV responses of Hg (III) on the FeOOH/NPG microelectrode over a concentration range of from 0.02 to 2.2 μ M in a PBS solution (pH 5.0). Reprinted with permission from Ref. 4 Copyright 2019 Elsevier. (B) Schematic illustration showing the anodization process of Au electrode in the oxalic acid solution and its detection of *p*-NP with electrochemical control. Reprinted with permission from Ref. 56 Copyright 2018 Royal Society of Chemistry.

Figure 11 illustrates the NPG based electrochemical biosensor for the detection of $Ag^{+,64}$ where the NPG was deposited on a GCE as the transducer. Three DNA probes, labelled: S1 (5'-HS-(CH₂)₆-SS-(CH₂)₆-TCA-GAC-TAGC-CCC-CCC-CCC-CCC-CGG-ACG-3'), S2 (5'-CC-TGC-TTT-CGT-CC-3') and S3(3'-AGT-CTG-ATCG-CCC-CCC-CCC-CCC-GG-ACG-5'), were self-assembled on the NPG to form a hairpin structure as the probe. Due to its good electrochemical performance, AQDS was utilized as the electroactive signal indicator. Square wave voltammograms were recorded as a function of the Ag^+ concentrations.

recorded as a function of the Ag^+ concentrations. The logarithm of the Ag^+ concentration vs the obtained oxidation current signal exhibited a linear relationship in the range of from 10^{-6} M to 10^{-10} M with a detection limit of 4.8×10^{-11} M. Furthermore, a reusable electrochemical aptasensor was constructed with NPG as the signal amplifier and thymine- Hg^{2+} -thymine as the probe, for the sensitive and selective detection of mercury ions.⁶⁵ Due to the significant amplification effect of the NPG used in the designed aptasensor, a wide detection range of from 0.01 to 5000 nM, with a detection limit as low as 0.0036 nM was achieved for the detection of Hg^{2+} . The NPG played a substantial role in the fabrication of the probes and for signal amplification.

NPG Based Optical Sensors

Taking advantage of the unique optical properties that arise when metals are reduced to the nanoscale, NPG may be employed as an optical sensor when combined with fluorescence, UV-Vis, or Raman spectroscopy.



Figure 8. Inkjet printing of nanoporous gold electrode arrays on cellulose membranes for amperometric oxygen sensors with high sensitivity using 1.0 μ l of BMIMPF₆ electrolyte. Reprinted with permission from Ref. 55 Copyright 2012 American Chemical Society.



Figure 9. (A) Cyclic voltammograms of the Ti/Au PB electrode (Plot a), the Ti/NP Au/PB electrode (Plot b) and the Ti/NP Au/PB/GOx electrode (Plot c) recorded in a 0.1 M PBS (pH = 7.0) at a scan rate of 10 mV s⁻¹; (B) the Ti/NPAu/PB/GOx electrode in the absence (dashed line) and in the presence of 1mM glucose (solid line); (C) Amperometric response to successive additions of 0.12 mM glucose into a 0.1 M pH 7.0 PBS under the applied electrode potential -0.1 V; (D) the corresponding calibration curve for the response of: (i) the Ti/Au/PB/GOx and (ii) Ti/NPAu/PB/GOx electrode. Reprinted with permission from Ref. 75 Copyright 2009 Elsevier.

Optical properties.—Interestingly, Au exhibits intrinsic optical properties when fabricated with dimensions at the nanoscale.²¹ When photonics are coupled with electronic and catalytic properties, the surface plasmons of NPG can be utilized as optical sensors for the detection of a variety of analytes.^{14,36,76–78} The interconnected framework of Au ligaments and pores provides suitable channels for the transport of solvents, which results in an extensive surface area for metal-dielectric interactions. As the dimensions of the metallic ligaments in the NPG network are at the same length scale as the

mean free path of electrons within the metal, localized surface plasmon resonance (LSPR) can be activated by visible light.⁷⁹ While this phenomenon is well documented for a variety of metallic nanomaterials, unique bimodal enhancement has been observed in porous structures such as NPG, which can be utilized by UV-Vis absorption, Raman, or fluorescence spectroscopy.⁷⁹

The wavelength absorbed by LSPR is contingent on both the size and shape of nanostructures coupled with the dielectric environment. By monitoring the peak shift in SPR, NPG may be employed as a



Figure 10. (A) (a) SEM image of NPG grown directly on a titanium substrate, (b) Cyclic voltammograms and (c) Calibration curve of the biosensor upon addition of different concentrations of cholesterol in a phosphate buffer (pH 7.4). Potential scan rate: 50 mV s⁻¹. Reprinted with permission from Ref. 61 Copyright 2011 Elsevier.



Figure 11. (A) Scheme of the NPG-DNA sensor, (B) SWV curves following the addition of various concentrations of Ag^+ , and (C) Linear relationship between the peak current and common logarithm of target concentration. Reprinted with permission from Ref. 64 Copyright 2015 Royal Society of Chemistry.

plasmonic sensor.⁸⁰ Extensive investigations have been conducted to elucidate the dependence of SPR on the surface morphology and nanopore dimensions of these complex 3D structures.^{21,80-82} It was revealed in UV-Vis absorption/transmission experiments (Fig. 12A) that the aspect ratio of the Au ligaments in the porous framework gave rise to a bimodal absorption spectrum in the visible range. Due to the fabrication and nanopore size distribution, the equatorial widths of the ligaments were consistent throughout the sample, leading to a specific SPR peak observed, which was related to this width (490 nm).

The second SPR peak, related to the longitudinal dimension of the Au ligaments, appeared at a longer wavelength than its sister peak. This peak showed a consistent shift related to aspect ratio, as its length was variable throughout the sample. Individually, these features exhibited two distinct peaks related to these dimensions; however, as a continuous network making up the surface, the longer wavelength peak demonstrated a broadening, which corresponded to varying ligaments throughout the porous surface. The dependence of the optical properties of NPG on its nanopore dimensions was further investigated (Fig. 12B), which revealed that as the nanopore size of the NPG was increased, the second SPR peak, λ_2 , associated with the Au ligament length, was shifted to a longer wavelength, while the axial peak, λ_1 , remained constant.

LSPR effects are known to offer a significant augmentation for Raman spectroscopy, in a technique known as surface enhanced Raman spectroscopy.⁸³ SERS effects induced by NPG have also been investigated (Fig. 12C) by varying nanopore sizes, revealing that the intensity of the Raman signal was increased as the nanopore sizes of the NPG were reduced. Understanding these fundamental optical properties of NPG allows for the fine-tuning of this versatile material for optical sensing applications.

Optical detection by Raman and visible light.—As described previously, NPG was shown to be an excellent plasmonic substrate due to its capacity for generating LSPR effects from its gold

framework and nanopores, which can be used to amplify the Raman scattering of molecules. Utilizing SERS on NPG allows for highly reproducible enhancement from any position on the film due to its continuous nanoporous framework. The use of SERS for the detection of heavy metal ions has been performed by Zheng et al. with the sub ppt detection of Hg^{2+} (Fig. 13A).¹⁷ By anchoring highly selective sulphur terminated Cy5-labeled aptamers on the surface of NPG, a 1 pM (0.2 ppt) Hg^{2+} sensitivity was achieved by Raman detection, which was a four order of magnitude lower Hg detection than the maximum level permitted by the EPA in water.

More recently, Wang et al. utilized NPG for the optical detection of Hg employing fluorescing molecules of rhodamine 6G (R6G) and 3-mercaptopropionic acid (MPA) anchored on the NPG surface.⁶⁶ These highly fluorescent molecule ligands could then be monitored by fluorimetry measurements, with their intensity being further enhanced by the plasmonic interactions from the NPG substrate. The interactions between Hg^{2+} and Au were stronger than that of R6G and Au, which resulted in the desorption of R6G in the presence of mercury ions. This, in turn, lowered the fluorescence of material with increasing mercury ion concentrations (Fig. 13B), which could be correlated to the concentration of mercury in solution.

This process was found to be effective for the detection of both organic and non-organic mercury ions found in the environment. This sensor was then tested using solutions with mixtures of ions and real-world samples. The detection of mercury was consistent regardless of potential screening ions, which indicated the high selectivity of this sensor for mercury ions. Detection was achieved down to 1 pM of mercury ions, which was consistent with the Raman method discussed previously. These processes can be tuned and employed to develop simple tools for the detection of metal ion pollutants for environmental and health monitoring applications. Fabrication of electrochemical sensors that take advantage of optical properties has been explored recently in literature, known as spectroelectrochemical sensors.^{84,85}



Figure 12. (A) Simultaneous shifts of two plasmonic peaks in opposite directions. (a) Short-wavelength peak shifts slightly to the blue (up to 3 nm), while the long-wavelength peak shifts to the red (up to 32 nm). The AR is defined as the ratio of the average ligament length to the average ligament diameter. (b) Illustration of how ligament length and diameter are measured. Reprinted with permission from Ref. 83 Copyright AIP Publishing. (B) SEM images of NPG (a, b) UV-Vis extinction spectra (c) Resonant peak position (d) $\lambda 1$ and $\lambda 2$ of NPG with the pore sizes of 10–50 nm in water. For comparison, the dashed line represents the size dependence resonant band of gold nanoparticles. Reprinted with permission from Ref. 78 Copyright AIP Publishing. (C) SERS spectra of nanoporous gold with different pore sizes for (a) 10^{-7} mol 1^{-1} R6G aqueous solution and (b) 10-5 mol 1^{-1} CV methanol solution. (c) Nanopore size dependence of the integrated intensities of 1650 cm⁻¹ Raman band at concentrations of 1×10^{-7} mol 1^{-1} R6G and 632.8 nm for CV. Reprinted with permission from Ref. 79 Copyright 2007 AIP Publishing.

well suited for NPG, which possesses both of these properties, making it a strong candidate for this technique going forward which may lead to improved selectivity and sensitivity.

Summary and Outlook

NPG has been used extensively for the development of electrochemical, bioelectrochemical, and optical sensors. The fabrication of NPG can be achieved by several different methods, and three widely used techniques that follow green chemistry strategies were discussed. The most common process (due to simplicity) is dealloying via a free corrosion pathway. Electrochemical fabrication processes such as alloying/dealloying, SWPP, and DHBT have seen increased use as they have the capacity to eliminate reagents via one-pot synthesis, while maintaining a higher level of control of the formed NPG. The optimization of NPG morphologies generally demands the highest surface to volume ratio possible. This favours small pores with high density in the material, which reduces the quantity of expensive gold required, while maximizing the surface area. DHBT is particularly suited in this regard, as it results in unique bimodal nanoporous structuring.

With a significant level of variation in the fabrication and modification of NPG between lab groups and applications, it is prudent to initially categorize these by the analysis technique that they are intended for. In this case, we have selected to explore electrochemical, bioelectrochemical, and optical sensors. Within these categories, further classification can be easily segregated by the type of analyte that the sensor is designed to detect, such as biomarkers for medical applications, heavy metal ions for environmental remediation, or preservatives in food safety, as summarised in Table I. Generally, bioelectrochemical sensors are more complex to fabricate as they require the immobilization of enzymes or aptamers that may selectively bind to targets. This makes them more sensitive than their electrochemical counterparts; however, it



Figure 13. (A) Characterization of NPG/aptamer hybrid SERRS sensor. Schematic (a) NPG used by SERRS sensing of Hg²⁺ based on aptamer modified NPG. (b) Quantitative detection of Hg^{2+} by measuring SERRS signal drop of Cy5 tags, using Apt15@NPG sensor. Inset shows the experimental setup for the Hg^{2-} detection. Reprinted with permission from Ref. 17 Copyright 2013 American Chemical Society (B) Photoemission spectra (a) of R6G/MPA-NPG in the presence of Hg^{2+} with different concentrations. (b) Normalized fluorescence intensity variation (I/I₀) of R6G/MPA-NPG as a function of Hg^{2+} concentration in ultrapure water, Yangtse river and tributary of the Huangpu river water. I₀ is the fluorescence intensity of R6G from the sensor in water only. Excitation wavelength is 532 nm. (c) Selectivity test of the R6G/MPA-NPG sensor. Normalized fluorescence change of R6G with individual metal ions at various concentrations. (d) Normalized fluorescence intensities with various mixed instant different concentrations. Rhomb dots indicate that the solution contains Hg^{2+} , Ag^+ , Mn^{2+} , Mg^{2+} , Ca^{2+} , K^+ , and Ba^{2+} at the same concentrations, and square dots (super-mixed) indicate that the solution contains Hg^{2+} , Ag^+ , Mn^{2+} , Mg^{2+} , Ca^{2+} , K^+ , an^+ , Co^{2+} , Zn^{2+} , and Pb^{2+} at the same concentration. Excitation wavelength is 532 nm. Reprinted with permission from Ref. 66

leaves them prone to degradation and fouling. It is generally considered that simpler, non-enzymatic approaches are more favourable if they can selectively identify target analytes. Nevertheless, this is sometimes not possible due to the obstruction of the target by interferent molecules. In these cases, bioelectrochemical sensors with improved selectivity are more aptly suited. As an optical sensor, NPG is unique from other techniques, as it utilizes the intrinsic optical enhancement of LSPR found in nanostructured gold. This process can be exploited by Raman, UV-Vis and fluorescence spectroscopy techniques. This LSPR enhancement allows for the pM detection of Hg²⁺ ions, which is several orders of magnitude more sensitive than the maximum limit set out by the EPA. This is a testament to how useful these powerful techniques might be in environmental remediation applications.

Overall, the extensive amount of literature that articulates the use of NPG in sensors confirms that its morphological and chemical features are well suited to sensing applications. It is anticipated that research in this field will continue and expand, with a focus on optimizing fabrication to further enhance the targeting of molecules of interest. Since there remains a significant disconnect between these studies and their implementation in industry, more effort should be made toward the development and implementation of NPG based devices for practical sensing applications in environmental monitoring, biomarker detection and food safety & quality control.

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