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Review

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Electrochemical Biosensing of Algal Toxins in Water: The Current State-of-the-Art

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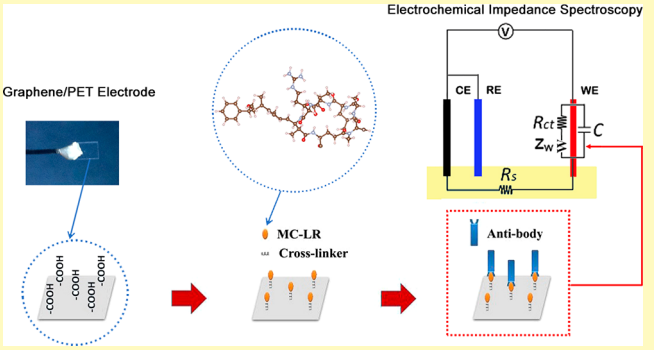
ABSTRACT: Due to increasing stringency of water legislation and extreme consequences that failure to detect some contaminants in water can involve, there has been a strong interest in developing electrochemical biosensors for algal toxin detection during the past decade, evidenced by literature increasing from 2 journal papers pre-2009 to 24 between 2009 and 2018. In this context, this review has summarized recent progress of successful algal toxin detection in water using electrochemical biosensing techniques. Satisfactory detection recoveries using real environmental water samples and good sensor repeatability and reproducibility have been achieved, along with some excellent limit-of-detection (LOD) reported. Recent electrochemical biosensor literature in algal toxin detection is compared and discussed to cover three major design components: (1) biorecognition elements, (2) electrochemical read-out techniques, and (3) sensor electrodes and signal amplification strategy. The recent development of electrochemical biosensors has provided one more step further toward quick in situ detection of algal toxins in the contamination point of the water source. In the end, we have also critically reviewed the current challenges and research opportunities regarding electrochemical biosensors for algal toxin detection that need to be addressed before they attain commercial viability.



KEYWORDS: *electrochemical, biosensor, algal toxin, enzymes, nanomaterials*

Drinking water security and sufficient treatment are of paramount importance to the health of a community and the quality of life in any country.[1,2] Episodes of harmful algal blooms (HAB), usually involving cyanobacteria or dinoflagellates, occur frequently in fresh or marine water bodies worldwide as a consequence of eutrophication resulting from anthropogenic activities, such as agricultural runoff, urban waste, detergent manufacture, and global warming.[3,4] HAB often produce undesirable color, odor, and taste, but most importantly can produce harmful algal toxins, such as hepatotoxins (i.e., microcystins, cylindrospermopsin, and nodularin), neurotoxins (i.e., anatoxins, brevetonxins and saxitoxins), and dermatoxins (i.e., lyngbyatoxins and lipopolysaccharides). It has been estimated that 50−70% of metabolites produced by HAB are harmful toxins (e.g., cyanotoxins),[5] which is a significant hazard for human health and the ecosystem in drinking water, recreational water, and aquaculture. Exposure to algal toxins occurs through drinking water, recreational activities, or consuming foods in which algal toxins have accumulated. The concentrations observed in various freshwater and marine water bodies range from undetectable at the ng/L to lower ng/L and can reach up to several hundreds of μg/L. For example, residents in the city of Toledo, OH, USA could not use or drink tap water in the summers of 2013 and 2014 due to the presence of cyanotoxins detected in their drinking water supplies.[6] Following a significant HAB event, there is an urgent need to develop appropriate early alarming systems and establish when a water source is safe to use or to evaluate the level of treatment required to make a source safe.

CYANOBACTERIA TOXINS IN WATER

Microcystins belong to a group of cyclic heptapeptides, which are potent and specific in inhibiting protein phosphatases 1 and 2A (PPl, PP2A).[7] Acute or prolonged exposure to microcystins will cause liver damage, followed by a massive intrahepatic hemorrhage probably leading to death. Microcystins are some of the most frequently detected cyanotoxins in freshwater throughout the world. To date, 80 variants of microcystins have been isolated depending on the amino acids and identified from freshwater cyanobacteria genera, among

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which microcystin-LR (MC-LR, MW 995.19) is the most toxic variant,[8] with an LD_{50} value of 43 μg/kg for mouse bioassay.[9] MC-LR is mainly produced by *Microcystis*, but may also be produced by other genera, such as *Anabaena*, *Nostoc*, *Phormidium*, and *Planktothrix*. It has been confirmed that microcystins were responsible for some poisoning of animals and humans where water sources contained toxic cyanobacteria blooms.[10]

Nodularins (NOD, MW 824.98) are cyclic pentapetides of a structure similar to MC-LR with strong hepatotoxic activity; however, they are only produced by *Nodularia spumigena* in brackish water.[11] Although MC-LR and NOD are chemically and toxicologically very similar, NOD does not bind covalently to PP1, as in the case of MC-LR.[12] Only six variants of nodularins have been identified. Beyond protein inhibition, other adverse toxicological effects have been reported concerning MC-LR and NOD exposure, such as intracellular glutathione alteration, reactive oxygen species production, and lipid peroxidation.[13,14] The mouse bioassay study shows slightly less toxicity when compared with MC-LR with a LD_{50} value of 30 μg/kg.

Another major group of cyanotoxins are alkaloids, including cylindrospermopsins and anatoxins. Cylindrospermopsin (CYN, MW 415.43) is readily emerging as a globally important cyanobacterial freshwater toxin, since the human CYN poisoning accident in 1979 (Queensland, Australia).[15] CYN is water-soluble and heat-stable, produced by a variety of cyanobacteria species such as *Cylindrospermopsis raciborskii*[16] and *Anabaena bergii*.[17] CYN has two natural variants, deoxycylindrospermopsin (deoxy-CYN) and 7-epicylindrospermopsin (7-epi-CYN),[18] which have very similar structures (the position of the hydroxyl group being the only difference). A guideline value for safe water supply of 1 μg/L of CYN has been recommended based on toxicity studies.[19] While hepatotoxicity is the main effect of CYN, it also affects lungs, kidneys, intestinal tract, stomach, and vascular and lymphatic systems.[20] Moreover, CYN is proved to be cytotoxic and genotoxic.[21] Unlike microcystins, CYN is typically extracellular in water; thus, the removal of the bacterial filaments during water treatment does not remove the toxin.[22]

Anatoxin-a (ATX) is the smallest potent neurotoxin (MW 165.23 Da) with an LD_{50} value of 200 μg/kg in the mouse bioassay study, produced by a variety of cyanobacteria of *Anabena flos-aquae*, *Aphanizomenon flos-aquae*, and *Oscillatoria*.[23] Anatoxin-a(s) (MW 252.21), a phosphate ester of *N*-hydroxyguanidine, is another *Anabena flos-aquae* produced cyanotoxins, of which the toxicity is much higher, with an LD_{50} value of 50 μg/kg in the mouse bioassay study.[24] Although they are structurally quite different, they are both potent quasi-irreversible inhibiters of acteylcholinesterase activity in the neuromuscular junctions, thus resulting in serious nervous dysfunction.[25] The exposure to anatoxin-a and anatoxin-a (s) commonly occurs through swallowing or drinking contaminated water and causes the symptoms of cardiac arrhythmia and respiratory paralysis. A mouse toxicity study has proposed a guideline value of 1 μg/L for anatoxin-a in drinking water.[26]

DINOFLAGELLATE TOXINS IN WATER

HABs caused by dinoflagellates, often referred to as red tides, can also produce a range of neurotoxins. Among these, saxitoxins (STX, MW 299.29) are one of the most toxic nonprotein substances with an LD_{50} value of 10 μg/kg in mouse bioassay study produced by dinoflagellates species of *Alexandrium* spp. and *Gymnodinium* spp. They consist of a family of carbamate alkaloid neurotoxins that are nonsulfated, singly sulfated, or doubly sulfated. Saxitoxins selectively block the sodium channels on excitable cells in a high affinity specific receptor binding process and reduce the number of conducting Na^+ channels.[27] They are rapidly absorbed through the gastrointestinal tract and can diffuse through the blood–brain barrier,[28] resulting in a variety of neurological symptoms culminating in respiratory arrest and cardiovascular shock.

Brevetoxins (PbTx) are a group of potent lipid soluble cyclic polyether neurotoxins naturally produced by the dinoflagellate *Karenia brevis*. BTXs are tasteless, odorless acids and are heat stable (up to 300 °C). BTX-2 (MW 895.08) and −3 (MW 897.2) are the most predominant forms among ten BTXs that have been isolated and characterized from aerosols, field blooms, and *Kareniabrevis* cultures.[29] BTXs as depolarizing substances can open the voltage-gated sodium channels in nerve cell walls, leading to uncontrolled Na^+ influx into the cell and subsequent neurologic poisoning.[30]

The US EPA currently suggests three cyanotoxins on contaminant candidate list 3: namely, anatoxin-a, microcystin-LR, and cylindrospermopsin.[31] After its first fatal incident in Brazil,[10] MC-LR was the first algal toxin assigned a provisional drinking water guideline value (i.e.,1 μg/L) by the World Health Organization (WHO) in 1998.[32] The WHO drinking water concentration limit for nodularins is 1.5 μg/L, which is extended from microcystins-LR. In spite of serious harmful effects, monitoring for any other cyanotoxins is not routinely performed in fresh water and WHO has not declared their drinking water guideline values due to inadequate toxicological data. However, few countries were expanding their monitoring programs and leading the way to developing provisional guidelines for other algal toxins. For example, Australia has suggested provisional drinking water guidelines of 3 μg/L, 3 μg/L, and 1−13 μg/L for anatoxin-a, saxitoxin, and cylindrospermopsin, respectively.[33]

CURRENT STATUS OF DEVELOPING HAB TOXIN MONITORING TECHNIQUE IN WATER

The development of reliable methods for monitoring HAB toxins in water resources is of great interest to determine the occurrence and to prevent human exposure. High-performance liquid chromatography/mass spectrometry (HPLC-MS) is a U.S. EPA approved analytical method for detection of a wide range of algal toxins, including microcystins,[34] nodularins,[35] and cylindrospermopsin,[36] which are in common use with a low detection limit of 0.1−1 μg/L. In contrast, the analysis of saxitoxins,[37] anatoxin-a, and anatoxin-a(s)[38,39] often require nonstandard analytical techniques, such as capillary electrophoresis or chemical derivatization in combination with chromatographic techniques. Overall, well-established laboratory techniques, while being competent, often require complex instrumentation and procedures, highly trained technicians, long turn-around time, high processing cost, and sample pretreatment, and are only practical in the laboratory, not in situ. Currently, various commercial enzyme-linked immunosorbent assay (ELISA) kits are also available to monitor and quantify HAB toxins in water samples;[40] however, concentrations determined by ELISA were systematically higher than concentrations determined by LC-MS, which was attributed to matrix effects and cross-reactivity with other unidentified derivatives.

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Electrochemical sensors have become a mature discipline with some outstanding commercial success, since they are suitable devices for in situ monitoring, due to their possible miniaturization toward the fabrication of an implantable biosensor, portability (e.g., hand-held), and automation (e.g., continuous monitoring).[41] Other advantages of electrochemical sensors include high specificity, low detection limits, relative freedom from matrix interference, and low cost over other types of sensors.[42] A recent analysis indicated that electrochemical biosensors dominate the biosensor industry contributing to 71% of market share (USD 15.96 Billion) in 2016 and anticipated to exceed USD 21 billion by 2024 (*Biosensors Market Forecast - Industry Size, Share Report 2018-2024* from https://www.gminsights.com/industry-analysis/biosensors-market). Based on their application, the environmental biosensor segment is small but is growing fast (>14% p.a.), partly spurred by increased stringency of water legislation, due to the extreme consequences that failure to detect some contaminants in water can involve. In this context, there has been significant progress over the past decade in developing highly sensitive and specific electrochemical biosensors/immunosensors for environmental monitoring, especially for algal toxins.[43] As the only type of algal toxin currently governed by WHO drinking water guidelines, electrochemical sensor detection of MC-LR in water has received by far the most attention in the literature (i.e., 18 out of 26 total studies surveyed in this review), which are summarized in Table 1.

ELECTROCHEMICAL BIOSENSING OF ALGAL TOXINS: PRINCIPLE AND APPLICATIONS

Electrochemical sensing has become one of the most promising analytical techniques because of the high sensitivity, ease of miniaturization, low cost, and relative simplicity.[70] Electrochemical biosensors developed for algal toxin detection usually consist of three major components: biorecognition elements, signal transduction (i.e., electrochemical read-out) mechanism, and sensor electrodes.

Biorecognition Elements. To offer detection selectivity and specificity, biorecognition receptors are essential for electrochemical biosensors, including antibodies (Ab), DNA/aptamers, carbohydrates, and antimicrobial peptides.[71] So far, antibodies are the most reported receptors in electrochemical biosensing of algal toxins, because they are readily available and provide highly specific molecular recognition without any preconcentration or pretreatment. Indeed, due to their large size (MW ∼ 150k Da), the electrochemical sensing depending on conformation changes of antigen/antibody conjugation is more suitable for the detection of large analytes. In the case of algal toxins (<1k Da), many studies adopted a competitive or displacement immunoassay design, in which sensor electrode surfaces were bound with algal toxins first and then incubated in solutions of a fixed amount of antibodies and algal toxins of various concentrations.[50,51,55,56,58−60] As a result, algal toxins in sample solution would compete with algal toxins immobilized on the electrode to interact with antibodies. Electrochemical responses (i.e., redox peak currents or electron transfer resistance) upon this biorecognition event are much higher than a direct immunoassay procedure (i.e., antibodies onto the electrode surfaces first and then algal toxins binding to them), leading to better biosensing sensitivity (i.e., response per analyte concentration change).

Aptamers belong to a group of synthetic small single-stranded (ss) DNA/RNA molecules that recognize various target molecules, including cells, proteins, peptides, and amino acids.[72] Compared with antibodies, aptamers are of much smaller size, and higher affinity, specificity, and stability with target molecules under a variety of conditions. Aptamers are normally prepared with terminal moieties such as thiol,[48,54] amino,[66] ferrocene,[57] and disulfide groups,[53,62] which can readily bind to various electrode surfaces. In the case of gold surface binding, 6-mercapto-1-hexanol (MCH) can be used to remove weakly adsorbed aptamer nucleotides from gold electrode surfaces in order to free up their secondary structure (e.g., stem and loop segments) for greater access to target detection (i.e., algal toxins).[73] The amount of aptamer loaded onto the sensor electrode surface during or before a biorecognition event should be controlled or optimized, since cross-hybridization may occur between neighboring aptamers resulting in unnecessary interfering read-out signals. A few studies showed that increasing Mg^{2+} concentration enhanced the binding affinity of three algal toxins (i.e., MC-LR, CYN, and PbTx-2) to their aptamers until it plateaued, while Na^{+} had insignificant effects.[57,62,69] It is speculated that aptamer conformation can be controlled through the interaction with Mg^{2+}, which in turn stabilizes their secondary structure for easy recognition of its target.[74] Low pH (<3.5) was also found to affect the conformation of DNA and thus reduce their binding affinity to aptamer.[57,69] In light of these findings, pH and Mg^{2+} concentration of water samples should be preconditioned to avoid any possible interference with algal toxin/aptamer binding. It is noteworthy that aptamers as biorecognition materials for algal toxin electrochemical detection currently offer the best promise for commercialization in terms of the storage stability, durability, and quick response time of the sensor.

There are also some less conventional biorecognition receptors reported for toxin detection in the literature, such as peptides[75,76] and cells.[77] In the case of algal toxin, cultured frontal cortex neuronal networks—a cell recognition type receptor—were grown over microelectrode arrays for detection of STX and PdTx-3 with action potentials or spikes as electrochemical response signal. LOD of 0.031 and 0.33 μg/L were achieved for STX and PdTx-3 in buffer solutions, while in 25-fold-diluted seawater, they increased to 0.076 and 0.48 μg/L, respectively.[78]

To facilitate the biomolecules (i.e., algal toxins, antibodies, or aptamers) binding to the electrode surface, various types of cross-linkers are frequently employed. Cysteamine with one amine and thiol group is useful for binding a gold or silver electrode surface with a carboxyl group, another amine group (e.g., MC-LR) when coupled with glutaraldehyde,[50] hydroxyl group (e.g., PbTx-2) when coupled with 1,4-phenylene diisocyanate (PDIC),[69] and carbonyl group (e.g., ATX) when coupled with sodium borohydride to form a Schiff-base bond.[65] Glutaraldehyde with two carbonyl groups is used for binding an amine group (e.g., MC-LR), and with assistance of (3-aminopropyl) triethoxysilane (APTES), it was used to conjugate MC-LR antibody with a hydroxyl group containing CNT electrode.[56] 1-Pyrenebutanoic acid succinimidyl ester (PBASE) can facilitate conjugation with graphene and CNT electrodes through $\pi-\pi$ stacking, and its succinimidyl ester group can covalently bind with an amine group (e.g., MC-LR).[55] Polydopamine (PDA) is a natural pigment and major component of animal melanin, which would undergo

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Table 1. Reported Studies of Algal Toxins Detection in Water Using Various Electrochemical Biosensors

toxins	electrodes	signal labels	blocking agent	cross-linkers	detection range (LOD)	recovery (%)	detection techniques	electroactive probes	refs
MC-LR	CNT	antibody	-	-	10 to 40 ng/L (0.6 ng/L)	94.05−101.32	Amperometry	-	44
MC-LR	GCE/GS	Ab_1 and PtRu-Ab_2	-	EDC-NHS	0.01 to 28 μg/L (9.63 ng/L)	99.5−102	Chronoamperometry	H_2O_2	45
MC-LR	CNx-MWNTs/Au/GCE	Antibody	-	-	-	95.1−96.2	DPV	*o*-PD and H_2O_2	46
MC-LR	Gold-GNPs	Monoclonal antibody	BSA	L-cysteine	0.05 to 15 μg/L (20 ng/L)	95.6−105	DPV	hydroquinone	47
MC-LR	Gold	5′-thiolated aptamer	-	-	0.05 to 100 μg/L (18 ng/L)	91.2−113.7	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	48
MC-LR Nodularin	Carbon/UltraBind membrane	IgG	Gelatin	Poly(ether sulfone)	0 to 10 μg/L (0.5 μg/L)	-	Chronoamperometry	HRP and H_2O_2	49
MC-LR	AuNPs/GCE	HRP-mAb	4-chloro-1-naphthol with the aid of H_2O_2	Cysteamine/glutaraldehyde	0.01 to 100 μg/L (4 ng/L)	94.1−98.1	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	50
MC-LR	CNFs/PEG/GCE	Ab_1 and Au-Ab_2	BSA	-	0.0025 to 5 μg/L (1.68 ng/L)	98−99.2	DPV	AuNPs in HCl	51
MC-LR	AuNp-polyDPB-G-AuNP/GCE	Polyclonal Ab	BSA	EDC-NHS	0.1 pg/L to 8 pg/L (0.037 pg/L)	96.3−105.8	DPV	-	52
MC-LR	Gold	5′-disulfide terminated Aptamers	-	Aminoethanethiol/NHS	0.01 to 10 μg/L (7.5−11.8 ng/L)	-	SWV	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	53
MC-LR Nodularin-R	GCE/SDD-Co(II)/AgNPs	5′-thiolated aptamer	6-mercapto-1-hexanol (MCH)	-	0.1 to 1.1 μg/L (0.04 μg/L)	94.3−115	CV	-	54
MC-LR	SWCNT/SiO_2/Gold	Monoclonal Ab	TW20	APTES-PBASE	1 to 1000 ng/L (0.6 ng/L)	84.7−124.2	Electrical resistance	-	55
MC-LR	CNT@Co silicate	Multi-HRP-(Fe_3O_4@PDA-Au)-Ab	Skim milk	PDA, APTES-glutaraldehyde	0.005 to 50 μg/L (4 ng/L)	91.6−110.7	CV	HRP and H_2O_2	56
MC-LR	GSPE	Ferrocene and disulfide labeled aptamer	-	Physical adsorption	0.1 to 1000 ng/L (1.9 ng/L)	91.7	SWV	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	57
MC-LR	GSs-CS/CGCE	HRP-CNS-Ab	-	EDC-NHS	0.05 to 15 μg/L (16 ng/L)	88−107.8	DPV	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	58
MC-LR	CNT	Monoclonal antibody	-	EDC-NHS	0.05 to 20 μg/L	-	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	59
MC-LR	GF	Monoclonal antibody	-	EDC-NHS	0.05 to 100 μg/L	92.7	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	60
MC-LR	GFC	Monoclonal antibody	-	EDC-NHS	0.005 to 10 μg/L	93.5−98.2	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	61
Cylindrospermopsin	Gold	Disulfide-modified aptamer	6-mercapto-1-hexanol (MCH)	-	0.1 to 80 μg/L (0.1 μg/L)	-	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	62
Cylindrospermopsin	Thionine-graphene	Aptamer	-	glutaraldehyde	0.39 to 78 μg/L (0.117 μg/L)	-	EIS	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	63
Anatoxin-a(s)	7,7,8,8-tetracyanoquinodimethane (TCNQ)-graphite	Acetylcholinesterase (AChE)	-	-	1 to 10 μg/L (1 μg/L)	95.8−103.2	Amperometry	Actylthiocholine chloride	64
Anatoxin-a	Gold	Aptamer	6-mercapto-1-hexanol (MCH)	Cysteamine/Sodium borohydride	1 to 100 μg/L (0.5 μg/L)	94.8−108.6	EIS in	$[Fe(CN)_6]^{4-}$/$[Fe(CN)_6]^{3-}$	65
Saxitoxin	MWCNT/Au	3′-amino-modified aptamer	ODT	EDC-NHS	0.9 to 30 μg/L (0.38 μg/L)	63−20.5	DPV	MB	66
Saxitoxin	Cu/GNS/lipid layer	Antibody	-	-	1 to 1000 μg/L (1 μg/L)	92−103	Voltage	-	67

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Table 1. continued

toxins	electrodes	signal labels	blocking agent	cross-linkers	detection range (LOD)	recovery (%)	detection techniques	electroactive probes	refs
Saxitoxin and bevetoxin-3	Hydrogen peroxide/Immobilon AV membrane	Glucose oxidase-labeled antibody	BSA	Succinic anhydride/EDC and glutaraldehyde	(15 μg/L)	-	Amperometry	Glucose	68
Bevetoxin-2	Gold	Aptamer	ethanolamine	Cysteamine/1,4-phenylene diisocyanate (PDIC)	0.01 to 2000 μg/L (106 ng/L)	-	EIS	$[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$	69

spontaneous self-polymerization of dopamine in the presence of oxygen under alkaline conditions, providing excellent adhesion to most electrode surfaces. Upon adhesion, its catechol group can then covalently bind with amino groups or thiol groups of any biomolecules via Schiff base reaction or Michael addition,[79] while the noncovalent immobilization was also found through hydrogen bonding,[80] metal coordination or chelating and $\pi-\pi$ interaction.[81] EDC (1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride) and NHS (*N*-hydroxysuccinimide) are frequently used to assist the covalent conjugation between the thiol-group or amine-group of biomolecules and carboxyl group containing electrodes.[82] NHS or its water-soluble sulfo-NHS was added to increase the stability of the formed amine-reactive intermediate by converting it to an amine-reactive sulfo-NHS ester and thus prevent its rapid hydrolysis in aqueous solutions. They are particularly useful for the electrochemical detection process involving interfacial electron-charge transfer, since they introduce no spacer molecules (hence the name "zero-length cross-linker") that are nonconductive.[45,52,58−60,66] Nevertheless, it is noteworthy that cross-linkers induced by coupling could potentially be hydrolyzed during storage or reaction, which may lead to a low efficiency of biocompatibility.[83]

Some studies simply resorted to physical adsorption for immobilizing algal toxins or their antibodies to the electrodes,[57] although the stability of such binding (e.g., long-term storage) should be subject to further scrutiny. In addition, commercially available affinity membrane (UltraBind US-800) (preactivated by poly(ether sulfone) to generate aldehyde group) was also employed for binding biomolecules (e.g., MC-LR antibody) on a screen-printed carbon electrode.[49] Bratakou et al. reported a lipid membrane self-polymerized on a glass fiber filter for immobilization of STX antibody upon physical absorption.[67]

Prior to a biorecognition event, applying blocking agents is also essential to obtain higher sensitivity by minimalizing nonspecific binding of analytes to sensor electrodes. BSA[47,51,52,68] and ethanolamine[69] were used to block the unbound carboxyl sites, while 6-mercapto-1-hexanol (MCH),[62,65] L-cysteine,[47] and octadecanethiol (ODT)[62] formed self-assembled monolayers (SAM) on a gold surface and restricted its access to further binding. However, the coverage of blocking agents or SAM layers on the sensor electrode can be difficult to characterize or quantify. At the moment, some authors suggest that the amount of SAM layers can be estimated by measuring their one-electron reductive desorption from sensor electrodes in CV analysis (i.e., integration of first reduction peak).[84]

The biorecognition process can also be improved by a sandwich type design (see Figure 1), where an enzyme-antibody or double antibodies Ab_1-Ab_2 can be configured on a carrier agent. Nanomaterials such as carbon nanospheres (CNS) with larger surface area and good biocompatibility were demonstrated as good carrier agents to increase the loading of biomolecules (i.e., enzymes and MC-LR antibody).[58] Wei et al. provided a double antibody-type sandwich electrochemical biosensor using nanometal alloys PtRu as the carrier agent to detect MC-LR.[45] Fe_3O_4 nanoclusters are another promising candidate as carrier agents for this type of biosensor due to easy magnetic collection without any impurities, which has been demonstrated in electrochemical detection of MC-LR.[56]

Electrochemical Read-Out Techniques. Electrochemical read-out techniques typically involve sensor electrode surface-

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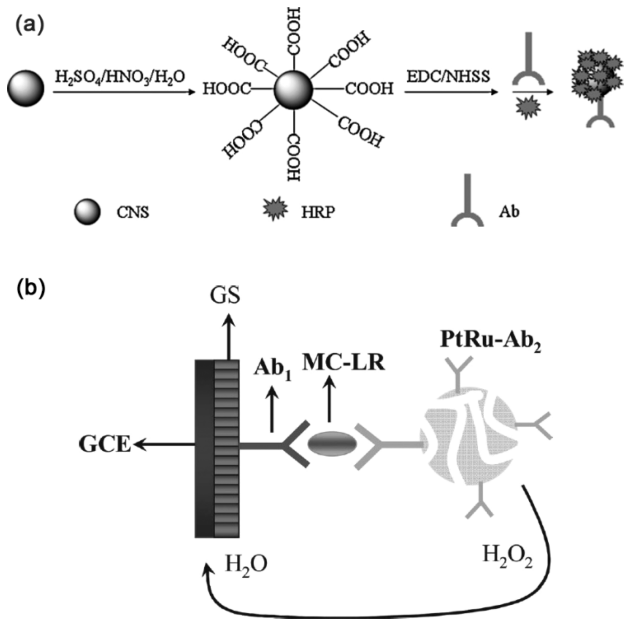

Figure 1. Preparation schematics of carbon nanosphere (CNS) (a) (Reproduced with permission from ref 58, Copyright 2013 Elsevier) and PtRu (b) as carrier agents in sandwich type electrochemical biosensors (Reproduced with permission from ref 45, Copyright 2011 Wiley).

bound receptors that can change its responses to electroactive probe species in the solution, leading to measurable currents (i.e., voltammetry and amperometry), potential changes (i.e., potentiometry), or charge accumulation resulting in changes in capacitance or impedance measured by impedance spectroscopy or electric-field effects upon a specific biorecognition event. For easy interpretation and comparison, most of the electrochemical techniques used for algal toxin detection reported in the literature can be broadly classified into two categories: (1) voltammetry or amperometry, and (2) electrochemical impedance spectroscopy, of which typical response plots are shown in Figure 2, respectively.

Voltammetry or amperometry is a major electrochemical technique used for algal toxin detection in water. Based on the waveform in which the potential is varied as a function of time, it can be further divided into cyclic voltammetry (CV),[54,56] square wave voltammetry (SWV),[53,57] and differential pulse voltammetry (DPV).[46,47,51,52,58,66] The voltammetry method concept generally replies on electroactive redox probe compounds present in solution that are denied access to the electrode surface upon binding of the target analyte (e.g., algal toxins) to a surface-confined receptor (e.g., antibodies). This denial of access could be the result of steric hindrance due to antigen−antibody complex formation resulting in increased mass transfer resistance of the redox probes. This coupled with the insulating nature of the formed biocomplex layer on the electrode surface generally causes a decrease in the corresponding redox currents that can be measured as a function of target analyte concentration. A range of aqueous electroactive redox probes have been applied to facilitate voltammetry detection of algal toxins, including methylene blue,[66] hydroquinone,[47] $Au/AuCl_4^-$,[51] $[Ru(NH_3)_6]^{3+}$,[53] and $[Fe(CN)_6]^{3-/4-}$.[52,57] Without using any electroactive probes/

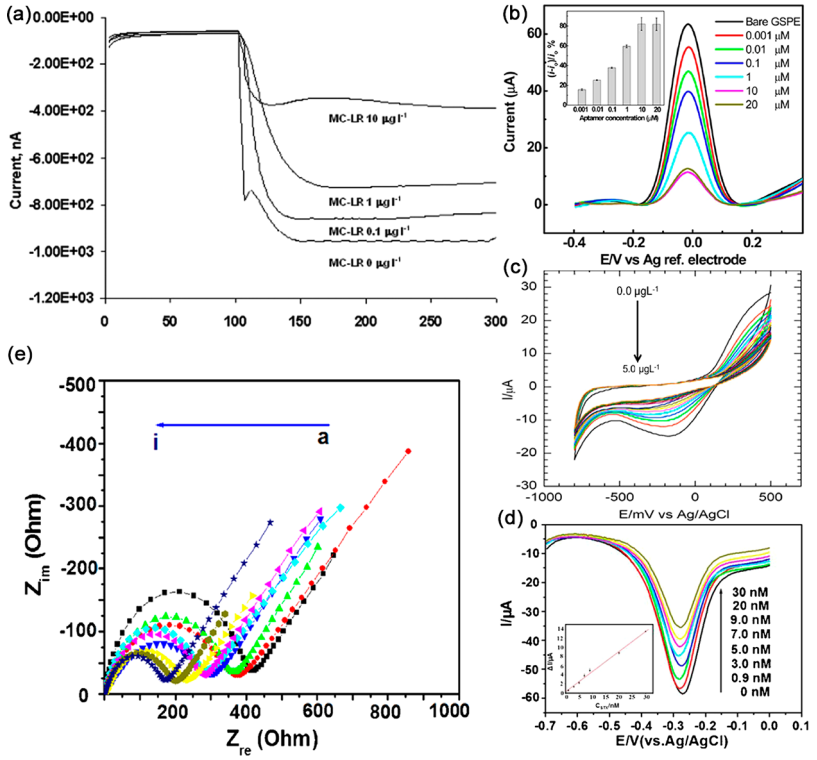

Figure 2. Typical biosensor responses to algal toxin in different concentrations using various electrochemical detection techniques: (a) Amperometry (Reproduced with permission from ref 49, Copyright 2012 American Chemical Society), (b) SWV (Reproduced with permission from ref 57, Copyright 2014 American Chemical Society), (c) CV (Reproduced with permission from ref 54, Copyright 2016 MDPI), (d) DPV (Reproduced with permission from ref 66, Copyright 2016 Springer), and (e) EIS, where a−i represent 0.01, 0.05, 0.1, 0.25, 0.75, 1, 10, 50, and 100 μg/L MC-LR (Reproduced with permission from ref 50, Copyright 2016 Elsevier).

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mediators in the solution, one study demonstrated that the linear range of MC-LR concentration to CV current response was very limited (i.e., 0.1 to 1.1 μg/L) before it began to plateau.[54] It should also be noted that in the case of voltammetry sensor using negatively charged aptamers, their electrostatic interaction with electroactive probes chosen (i.e., repulsion or attraction) could play a more important role than steric/conformational changes into locked structure upon binding with algal toxins. For example, when using negatively charged $[Fe(CN)_6]^{3-/4-}$ as electroactive probes, it was observed that redox current increased with the amount of MC-LR binding to aptamers.[57] On the other hand, one study reported the opposite for STX when positively charged MB was used as probe.[66] In some cases, the voltammetry detection process can be further assisted by coupling redox probes with different enzymes as mediators, e.g., H_2O_2 with horseradish peroxidase (HRP) and *o*-phenylenediamine (*o*-PD),[46] H_2O_2 with HRP,[56,58] actylthiocholine chloride with acetylcholinesterase (AChE),[60] and glucose with glucose oxidase.[68]

Chronoamperometry is another subclass of amperometry techniques, which has also been employed in a couple of studies on electrochemical detection of algal toxins using electro-active probes such as H_2O_2,[42] ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), and H_2O_2 with HRP/hydroquinone as mediators.[46] Since the current is measured as a function of time at a fixed voltage, the key to sensitive amperometry detection of target biomolecule markers (i.e., algal toxins) in the solution is to optimize the concentration and redox potential of redox-active probe compounds for a maximum target analyte signal and minimal background response.

Electrochemical impedance spectroscopy (EIS) is another frequently used method in electrochemical sensing for monitoring the interaction between antibodies and biomolecules at an electrode surface, which impart excellent advantages such as high sensitivity, ease of performance, and use of simple equipment.[85] Consequently, EIS has attracted a lot of attention to establish bioelectronic devices for detection and quantification of algal toxins.[48,50,59,60,62,63,65,69] These sensors measure changes in electrode impedance in a reaction solution when target biochemical molecules (i.e., algal toxins) are captured by probes (e.g., antibodies) attached on the electrode surface. Changes in electrochemical impedance are due to changes in capacitance or charge-transfer resistance (or combination of both) on the interface between electrode surface and bulk. Electrode impedance variations are highly dependent on the surface coverage of the electrodes by target biomolecules. The formation of such bioaffinity complexes commonly leads to an insulating layer that retards the interfacial electron transfer kinetics between the redox probe and the electrode and increases the electron-transfer resistance. A small alternating current (AC) voltage (i.e., in the order of millivolts) is essential in EIS setup to avoid denaturing of large biomolecules. The ferric/ferrous cyanide ion pair $[Fe(CN)_6]^{3-/4-}$ is the most applied electro-active redox probe for EIS measurement of algal toxins in water so far. Typical EIS spectra consist of a semicircle portion at higher frequencies corresponding to the electron-transfer-limited process, of which the diameter equals the electron-transfer resistance, and a linear portion at lower frequencies represents the diffusion-limited process (see Figure 1e). Sensitivity of EIS techniques is higher compared to other voltammetry-based techniques (e.g., CV and DPV) and amperometry, since EIS can effectively measure small changes upon biorecognition or binding events on the surface of the electrodes[86,87] and the response time is significantly faster.[88,89] Enzyme assisted biocatalytic precipitation (BCP) can also be used to further enhance the EIS detection sensitivity.[90,91] For example, the 4-chloro-1-naphthol (4-CN) with HRP labeled antibody and H_2O_2 was used to generate insulating precipitates (i.e., benzo-4-chlorohexadienone) on biosensor electrode surface and thus restrict the access of an electroactive redox probe (i.e., $[Fe(CN)_6]^{3-/4-}$).[50] Using fixed frequency (usually less than 1 Hz) to measure impedance can lead to even faster analysis, and possible real-time monitoring tools. Contrary to EIS-based biosensors using antibodies with negatively charged $[Fe(CN)_6]^{3-/4-}$ as redox probe, many aptamer sensor studies observed that the aptamer−algal toxin interaction actually lead to a decrease (rather than the common increase) in the electron transfer resistance,[48,62,65] of which the mechanism is illustrated in Figure 3.

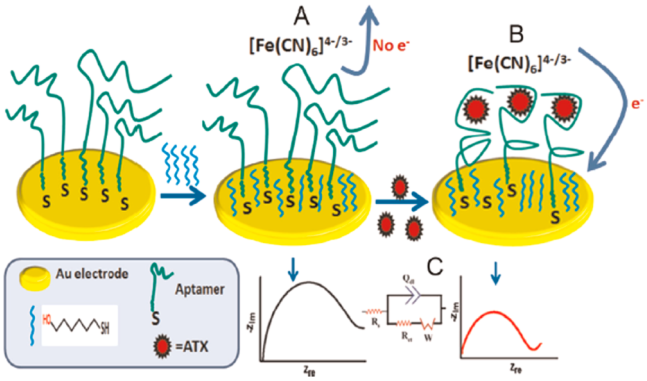

Figure 3. Fabrication of the label-free impedimetric aptasensor of anatoxin-a. Without toxin, the negatively charged $[Fe(CN)_6]^{4-/3-}$ redox probe is repelled from the surface and its redox reaction is hindered (A). Upon toxin recognition to the aptasensor, the aptamer induced switching results in compact structure, allowing accessibility of the $[Fe(CN)_6]^{4-/3-}$ marker to the surface and the resistance to electron transfer is decreased (B). Equivalent circuit R_s ($Q_{dl}[R_{et}W]$) is used to fit the frequency scans along with an impedance spectra (C). Figure reproduced with permission from ref 65, Copyright 2015 Elsevier.

There are also a couple of studies reported on less used electrochemical methods for algal toxin detection. Direct measurement of field-effect transistors (FET) electrical resistance variations upon MC-LR and antibody binding to its surface was carried out using the slopes of $I-V$ curves between +0.1 V and −0.1 V.[55] In another study, the potential difference between working and reference electrodes was directly measured as a response to STX concentration changes.[67] This potential change was brought about by induced lipid crystallinity when STX binds to its antibody precoated on a lipid film layer (see Figure 4). This crystal phase alteration can lead to electrostatic interactions,[92] dynamic conformational and fluidity changes of the lipid film.[93]

Sensor Electrodes and Signal Amplification. Conventional sensor electrodes reported for electrochemical detection of algal toxins include glass carbon electrodes (GCE)[45,46,50−52,54,58] and gold[48,53,55,57,62,65,69] prepared using screen printing techniques. More recently, signal amplification by utilizing various nanomaterials is regarded as an ideal strategy to produce highly sensitive sensor electrodes during

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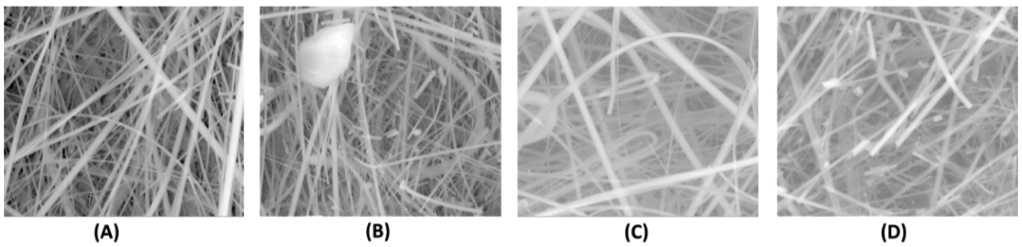

Figure 4. SEM images of (A) polymer without the lipid, (B) polymer with the lipid, (C) polymer with the lipid after the incorporation of Anti-STX, and (D) same as (C) but with a drop of toxin placed on the filter. Magnification is 2000 (nm). Figure reproduced with permission from ref 67, Copyright 2017 Wiley.

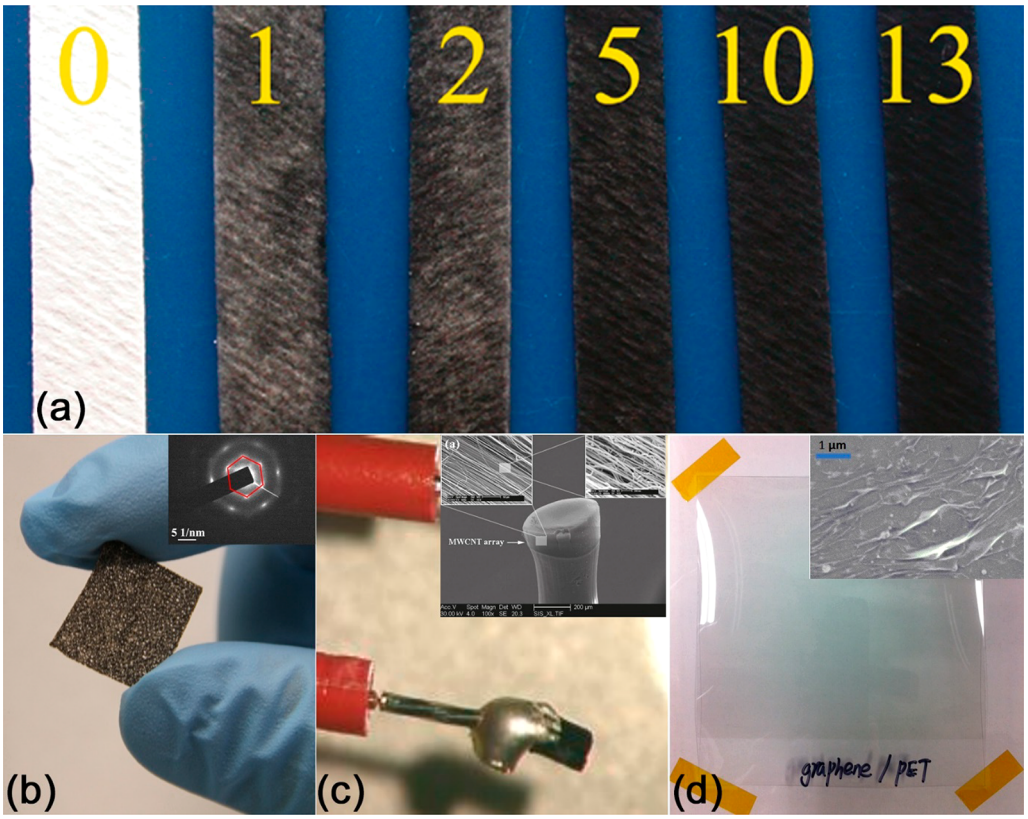

Figure 5. Photographs of various nanomaterials electrodes for electrochemical biosensing of algal toxins in water: (a) SWNT on filtration paper, where the numbers represent SWNT deposition cycles (Reproduced with permission from ref 44, Copyright 2009 American Chemical Society), (b) Graphene foam (GF) (Reproduced with permission from ref 60, Copyright 2017 Elsevier), (c) MWNT (Reproduced with permission from ref 59, Copyright 2013 Wiley), and (d) Graphene film/PET composite (GFC) (Reproduced with permission from ref 61, Copyright 2018 Nature).

the electrochemical sensing process resulting from their unique structural and electronic features. Facile surface modification can be achieved, giving many opportunities for noncovalent and covalent immobilization of small or large biomolecules, which has been widely employed in clinical diagnostics, environmental control, and other fields.[94]

To facilitate efficient signal amplification, there are several studies using carbon nanotubes (CNT) to fabricate the sensor electrodes. As one-dimensional (1D) materials, CNTs offer better sensitivity and faster response speed toward the change in the surface microenvironment of the transfer channels derived from the adsorption/modification of extraneous molecules. The earliest example of using CNTs as electrodes for MC-LR electrochemical detection was demonstrated by Wang et al. in 2009, in which simple disposable sensor strips were fabricated by filtering single-walled carbon nanotube (SWNT) out on normal filtration paper (see Figure 5a); amperometry measurement by directly oxidizing target toxins (i.e., MC-LR) without using any receptors such as antibodies was carried out.[44] Although decent LOD was obtained, one would expect that it would give rise to serious cross-reactivity issues with other algal toxins present in water. Since then, Han et al. has demonstrated a multiwalled carbon nanotubes (MWNT)-based sensor electrode for MC-LR detection using EIS technique (see Figure 5c).[59] Tan et al. demonstrated SWNT enabled electrochemical detection of MC-LR by measuring electrical resistance variations.[55] Zhang et al. reported a nitrogen-doped multiwalled carbon nanotube (CNx-MWNT) based electrode, of which the doping process was claimed to provide more surface-active sites and better biocompatibility.[46] Gan et al. provided a study on CNT electrode modified with core−shell hierarchical three-dimensional villiform-like nanostructures (CNT@Co silicate).[56] Hou et al. constructed a MWCNT modified gold sensor electrode

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for electrochemical detection of saxitoxin.[66] In addition to CNT, Zhang et al. showcased an electrochemical sensor based on carbon nanofiber (CNF)[51] for MC-LR detection in water. Compared with CNTs, CNFs possess much larger functionalized surface area and more edge sites on the outer wall,[95] and thus they are more suitable for immobilization and stability of biomolecules.[96]

The unique physical and electrochemical properties (e.g., high electrical conductivity, ease of functionalization, high electrochemically active surface area, and broad range of working potentials in aqueous solutions) of graphene would make it a candidate material for developing novel and fit-for-purpose electrochemical biosensors/immunosensors as alternatives to the time-consuming, expensive, nonportable, and often skills-demanding conventional methods of analysis involved in water quality assessment.[97,98] Many preparation methods have been reported to produce high-quality graphene nanoplatelets, nanoflakes, or even single graphene sheets, e.g., mechanical exfoliation of graphite and chemical reduction of graphene oxide. So far, there are a few studies reporting on graphene enabled electrochemical chemical sensors for algal toxin detection, including commercially available graphene-modified screen-printed carbon electrodes (GSPE) (Dropsens, Inc. 110GPH),[57] in situ electrodeposition and reduction of graphene oxides[52,58] and drop-casting of graphene dispersion on electrode surfaces.[45,67] Zhao et al. even conducted a direct comparison study between chitosan-coupled graphene and CNT biosensor electrodes in a controlled experiment of MC-LR electrochemical detection, where the former achieved 2.3 times higher DPV signal than the latter.[58] However, these graphene preparation methods are usually a labor-intensive, low-yield, nonscalable process and produce very dispersive forms of graphene. Therefore, special emphasis has been placed to find economic and scalable routes of large-sized monolithic graphene material fabrication, which are more suitable for commercially viable devices in this direction. Recently, a method using modified chemical vapor deposition (CVD) to grow large-sized monolithic graphene has been developed, such as macroporous foam-like 3D graphene network[99] and graphene/polymer sheets featuring multilayer graphene deposition (i.e., the roll-to-troll method).[100] In this context, Zhang et al. have been among the first to demonstrate MC-LR electrochemical sensors using these two types of graphene-enabled electrodes (see Figure 5b and d).[60,61] Coupled with EIS, broad linear detection range of MC-LR was achieved between 100 and 0.005 μg/L. In addition, the scalability and the processability of CVD provides a step closer to developing practical graphene-based biosensors on the large scale as the resulting graphene material is free-standing, scalable, and macroscopic with easily tunable size rather than previously reported two-dimensional nanoflakes or their agglomerates often in fine powder form.

Usage of nanosized noble metals to modify sensor electrode for electrochemical algal toxin detection have also been attempted to facilitate surface electron transfer due to their high conductivity and large specific surface area, such as gold nanoparticles (AuNP).[47,50] When immobilized on the sensor electrodes, AuNPs can firmly bind antigens/antibodies through hydrophobic, electrostatic interactions, or covalent bond formation with the help of cross-linkers.[101,102] Bilibana et al. investigated a nanocomposite sensor fabricated by electrosynthesis of silver nanoparticles (AgNP) on the surface of cobalt(II) salicylaldimine metallodendrimer (SDD-Co(II)) dendrimer for electrochemical detection of MC-LR.[51] Dendrimers belong to a group of well-ordered and symmetrical polymers with a core, self-replicating branching units and peripheral surface group at different positions. Using them as electrode substrates was shown to provide additional functional groups and internal cavities leading to much improved sensitivity.[103] Furthermore, potential synergistic effects were also demonstrated when combined with other nanomaterials, such as graphene[52] or CNT.[43] For example, Li et al. electrodeposited graphene alternately with AuNPs and 2,5-di(2-hienyl)-1-pyrrole-1-(*p*-benzoic acid) (DPB) for multiple cycles (i.e., 20 times) and obtained the lowest LOD (i.e., 0.037 pg/L) for MC-LR among all the studies.[52]

CURRENT CHALLENGES AND FUTURE OPPORTUNITIES

A sensitive, specific, simple, and rapid method for monitoring algal toxins in water could help to prevent human exposure especially in a contamination event. Over the past decade, significant progress has been made toward developing commercial electrochemical biosensor for algal toxins detection, of which great sensitivity and fast response were achieved by the nanomaterials related signal transduction upon a biorecognition event. According to the 2005 ICH guidelines for analytical validation Q2 (R1), apparent recoveries of 80−115% are acceptable. In this context, most of electrochemical biosensing studies for algal toxins was reported to demonstrate satisfactory recoveries using real environmental water samples, as well as good repeatability and reproducibility. Coupled with some excellent LODs reported, developed electrochemical biosensor in these works has provided one more step toward quick in situ detection of algal toxins in the contamination point of the water source. Current challenges and opportunities regarding the electrochemical biosensor for algal toxins that needed to be addressed before they attain the commercial viability include the following:

1. There are no reported studies on developing electrochemical biosensor for algal demertoxins detection, including lyngbyatoxins, aplysiatoxins, and lipopolysaccharides. Some pioneering work in this particular area is much needed.

2. Using of conductive polymer as binder or underlay (e.g., polythiophene, polyaniline, and polypyrrole) could potentially enhance the signal and sensitivity. Only study carried out was using 2,5-di(2-hienyl)-1-pyrrole-1-(*p*-benzoicacid) (DPB) and reported with a much improved LOD for MC-LR.[52] However, they may suffer from possible conductivity loss issue (or aging effect) over long time storage.[104] With fast progress made in this field every year, opportunities may rise to overcome this issue in the near future.

3. Most of work reported in electrochemical biosensing of algal toxins are disposable biosensor. On the other hand, regeneration of used sensor electrode can be carried out by using different chaotropic agents,[105] which have been demonstrated in some of the reported studies, including urea,[47] and glycine-HCl[51,52,68] solutions. However, their effectiveness and potential side effects on biomolecule immobilization have not been assessed across the studies systematically.

4. Response times of electrochemical biosensors for algal toxin detection were not widely reported and discussed in most of the studies. In one immunosensor study, an optimal 60 min response time was recommended using HRP enzyme assisted CV detection of MC-LR.[56] In another immunosensors design,

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a much-reduced response time of 5−20 min was reported using voltage changes as electrochemical signal for saxitoxin detection.[67] This necessitates more feasibility and comparison studies across the different biosensor designs systematically, since <20 min of response time is the rule-of-thumb requirement for any realistic in situ monitoring or being commercially meaningful.

5. Recently, the use of microfluidics chips (MFCs) have gained in popularity for pathogen or toxin detection. Since MFC-enabled reaction chambers are usually on the micro- or nanoscale, both the volume and the distance of diffusion inside the microchannels are significantly reduced, and so are analysis time and reagent consumption. This has proven to be cost-effective due to the ease of automation, integration (i.e., sample preparation, amplification, and signal detection), miniaturization, and multiplexing. There are few studies reported on using MFCs to facilitate algal toxin sensing, such as crystal microbalance (QCM) sensor[106] and immunosensor.[107] One can expect that integration of MFCs into electrochemical biosensing protocols of algal toxins would certainly be advantageous based on the same principle.

6. Until now, most electrochemical sensors reported in the literature utilized Faradaic process, that is, after incubation in analyte solutions for biorecognition (i.e., antigen/antibody binding), biosensors are then transferred to a secondary solution containing electroactive species (e.g., $Fe(CN)_6]^{3-/4-}$) for electrochemical signal measurement. This changing of solutions makes the Faradaic process based electrochemical detection impractical for continuous monitoring in situ. On the other hand, a non-Faradaic process could potentially allow continuous in situ monitoring since no electroactive redox species are required in the analyte solution. In a typical non-Faradaic EIS measurement, phase offset or shift (degree) between the input voltage and output current is commonly used as electrochemical sensing signal. This is an electrode surface area independent signal that directly reflects a change in interfacial capacitance of sensor electrode. In other words, any physical change (i.e., a biorecognition event) occurring on the electrode interface will likewise generate a detectable change in phase shift over a range of frequencies.[108,109] So far, nonfaradaic EIS technique has been reported to detect different diseases with satisfactory performance, such as diabetes, cancer, and cardiovascular diseases.[110−113] It could be a valuable tool in developing EIS-based algal toxin biosensor as well. For this type of EIS biosensors, a full coverage of insulating self-assembly monolayer (SAM) on the surface of sensor electrodes is essential and needs to be meticulously carried out.

7. In the past decade, synthetic receptors produced by various surface-imprinting technologies, such as self-assembly, molding, and stamping methods, have become a promising area of research in specific biomolecule or bioparticle detection.[114] A typical surface-imprinting process involves two essential steps: first, target molecules are imbedded as template into formed SAM substrate, and second, these molecule templates are extracted from SAM surface to create microcavities, which can offer highly specific target molecule rebinding through intermolecular interactions like hydrogen bonds and dipole−dipole and ionic interactions between the template molecule and functional groups present in SAM. As alternatives to natural receptors, such as antibodies or aptamers, synthetic receptors made from polymers are more chemically and thermally robust, and thus can offer multiple advantages, such as online continuous monitoring, easy electrode regeneration, and unlimited shelf life at room temperature without concerns of receptor deterioration. Coupled with synthetic receptors, amperometry, potentiometry, and EIS methods have been shown to detect biomolecules with decent LODs down to the ng/mL level, including protein glycoprotein (gp51),[115] carcinoembryonic antigen (CEA),[116] human ferritin, and human papillomavirus derived E7 protein.[117] At the moment, the main challenge of applying synthetic receptors in electrochemical detection of algal toxins could be low sensitivity of electrochemical response and increased difficulty of imprinting template molecule extraction, considering the exceptionally small size of algal toxin molecules (less than 1k Da) and thickness of imprinted SAM layers. However, obvious advantages of synthetic receptors cannot afford to be overlooked, and it is certainly worthy of more concerted efforts in this direction.

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Notes

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VOCABULARY

Harmful algal blooms (HAB), colonies of algae grow out of control due to an overabundance of nutrients (i.e., nutrient and phosphorus) present in the water. HABs can severely lower oxygen levels, killing marine life, and release algal toxins in natural waters; Electrochemical impedance spectroscopy (EIS), a frequently used method for monitoring change of electrochemical response upon a biorecognition event at an electrode surface with high sensitivity; Aptamers, a group of biorecognition materials for cells, proteins, peptides, and amino acids, which is synthesized from small single-stranded (ss) DNA/RNA molecules. Aptamer-based biosensors offer the best promise for commercialization in terms of the storage stability, durability, and quick response time; Self-assembled monolayers (SAM), formed by the chemisorption of organic molecules onto a substrate from either the vapor or liquid phase followed by a slow and orderly organization into a thin monolayer deposit. SAMs have wide applications in electrochemistry and electronics as effective insulators or blockers; Microfluidics chips (MFCs), feature a set of microchannels etched or molded into a substrate (glass, silicon or polymer). These features enable MFCs to create biosensors that are quick, efficient, and easy to manipulate as well as portable.

REFERENCES

(1) Zhang, W.; Jia, B.; Wang, Q.; Dionysiou, D. Visible-light sensitive TiO_2 synthesis via wet chemical N-doping for the

DOI: 10.1021/acssensors.8b00359
ACS Sens. XXXX, XXX, XXX−XXX

degradation of dissolved organic compounds in wastewater treatment: A review. *J. Nanopart. Res.* **2015**, *17*, 221.
(2) Zhang, W.; Zou, L.; Wang, L. Visible-light assisted methylene blue (MB) removal by novel TiO_2/adsorbent nanocomposites. *Water Sci. Technol.* **2010**, *61*, 2863−2871.
(3) Anderson, D. M.; Boerlage, S.; Dixon, M. *Harmful algal blooms (HABs) and desalination: A guide to impacts, monitoring and management*; UNESCO, 2017.
(4) Zhang, W.; Zhang, Y.; Fan, R.; Lewis, R. A facile TiO_2/PVDF composite membrane synthesis and their application in water purification. *J. Nanopart. Res.* **2016**, *18*, 31.
(5) Carmichael, W. W. Cyanobacteria secondary metabolites-the cyanotoxins. *J. Appl. Bacteriol.* **1992**, *72*, 445−459.
(6) http://www.ecowatch.com/toxic-algae-bloom-leaves-500-000-without-drinking-water-in-ohio-1881940537.html.
(7) Yoshizawa, S.; Matsushima, R.; Watanabe, M. F.; Harada, K.; Ichihara, A.; Carmichael, W. W.; Fujiki, H. Inhibition of protein phosphatases by microcystis and nodularin associated with hepatotoxicity. *J. Cancer Res. Clin. Oncol.* **1990**, *116*, 609−614.
(8) Lawton, L. A.; Chambers, H.; Edwards, C.; Nwaopara, A. A.; Healy, M. Rapid detection of microcystins in cells and water. *Toxicon* **2010**, *55*, 973−978.
(9) Gupta, N.; Pant, S.; Vijayaraghavan, R.; Rao, P. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. *Toxicology* **2003**, *188*, 285−296.
(10) Azevedo, S. M.; Carmichael, W. W.; Jochimsen, E. M.; Rinehart, K. L.; Lau, S.; Shaw, G. R.; Eaglesham, G. K. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology* **2002**, *181−182*, 441−446.
(11) Pearson, L.; Mihali, T.; Moffitt, M.; Kellmann, R.; Neilan, B. On the Chemistry, Toxicology and Genetics of the Cyanobacterial Toxins, Microcystin, Nodularin, Saxitoxin and Cylindrospermopsin. Mar. *Mar. Drugs* **2010**, *8*, 1650−1680.
(12) Annila, A.; Lehtimaki, J.; Mattila, K.; Eriksson, J. E.; Sivonen, K.; Rantala, T. T.; Drakenberg, T. Solution structure of nodularin. An inhibitor of serine/threonine-specific protein phosphatases. *J. Biol. Chem.* **1996**, *271*, 16695−16702.
(13) Bouaicha, N.; Maatouk, I. Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes. *Toxicol. Lett.* **2004**, *148*, 53−63.
(14) Jayaraj, R.; Anand, T.; Rao, P. V. L. Activity and gene expression profile of certain antioxidant enzymes to microcystin-LR induced oxidative stress in mice. *Toxicology* **2006**, *220*, 136−146.
(15) Bourke, A. T. C.; Hawes, R. B.; Neilson, A.; Stallman, N. D. An outbreak of hepato-enteritis (the Palm Island mystery disease) possibly caused by algal intoxication. *Toxicon* **1983**, *21*, 45−48.
(16) Falconer, I. R.; Humpage, A. R. Cyanobacterial (blue-green algal) toxins in water supplies. *Environ. Toxicol.* **2006**, *21*, 299−304.
(17) Schembri, M. A.; Neilan, B. A.; Saint, C. P. Identification of genes implicated in toxin production in the cyanobacterium Cylindrospermopsis raciborskii. *Environ. Toxicol.* **2001**, *16*, 413−421.
(18) Norris, R. L.; Eaglesham, G. K.; Pierens, G.; Shaw, G. R.; Smith, M. J.; Chiswell, R. K.; Seawright, A. A.; Moore, M. R. Blooms of the cylindrospermopsin containing cyanobacterium, Aphanizomenon ovalisporum (Forti), in newly constructed lakes, Queensland, Australia. *Environ. Toxicol.* **1999**, *14*, 163−165.
(19) Humpage, A. R.; Falconer, I. R.; Humpage, A. R. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.* **2003**, *18*, 94−103.
(20) Hawkins, P. R.; Runnegar, M. T.; Jackson, A. R.; Falconer, I. R. Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) Cylindrospermopsis raciborskii (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Appl. Environ. Microbiol.* **1985**, *50*, 1292−1295.
(21) Bazin, E.; Huet, S.; Jarry, G.; Hegarat, L. L.; Munday, J. S.; Humpage, A. R.; Fessard, V. Cytotoxic and genotoxic effects of cylindrospermopsin in mice treated by gavage or intraperitoneal injection. *Environ. Toxicol.* **2012**, *27*, 277−284.
(22) Svrcek, C.; Smith, D. W. Cyanobacteria toxins and the current state of knowledge on water treatment options: a review. *J. Environ. Eng. Sci.* **2004**, *3*, 155−185.
(23) Bogialli, S.; Bruno, M.; Curini, R.; Di Corcia, A.; Lagana, A. Simple and rapid determination of anatoxin-a in lake water and fish muscle tissue by liquid-chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2006**, *1122*, 180−185.
(24) Mahmood, N. A.; Carmichael, W. W. The pharmacology of anatoxin-a(s), a neurotoxin produced by the freshwater cyanobacterium Anabaena flos-aquae NRC 525−17. *Toxicon* **1986**, *24*, 425−434.
(25) Carmichael, W. W. Toxins of cyanobacteria. *Sci. Am.* **1994**, *270*, 78−86.
(26) Fawell, J. K.; Mitchell, R. E.; Everett, D. J. The toxicity of cyanobacterial toxins in the mouse: I microcystin-LR. *Hum. Exp. Toxicol.* **1999**, *18*, 168−173.
(27) Su, Z.; Sheets, M.; Ishida, H.; Li, F.; Barry, W. H. Saxitoxin blocks LType/Ca. *J. Pharmacol. Exp. Ther.* **2003**, *308*, 324−329.
(28) Llewellyn, L. E.; Bell, P. M.; Moczydlowski, E. G. Phylogenetic survey of soluble saxitoxin-binding activity in pursuit of the function and molecular evolution of saxiphilin, a relative of transferrin. *Proc. R. Soc. London, Ser. B* **1997**, *264*, 891−902.
(29) Landsberg, J. H. The Effects of Harmful Algal Blooms on Aquatic Organisms. *Rev. Fish. Sci.* **2002**, *10*, 113−390.
(30) Baden, D. G.; Bourdelais, A. J.; Jacocks, H.; Michelliza, S.; Naar, J. Natural and derivative brevetoxins: historical background, multiplicity, and effects. *J. Environ. Health Perspect.* **2005**, *113*, 621−625.
(31) Creating a cyanotoxin target list for the unregulated contaminant monitoring rule; USEPA, 2001.
(32) *Guidelines for Drinking-Water Quality*, 2nd ed., Addendum to Vol. *1*. Recommendations; World Health Organization: Geneva, 1998; p 13.
(33) Chorus, I.; Bartram, J., Eds. *Toxic cyanobacteria in water*; World Health Organization: London, 1999.
(34) U.S. EPA Method 521; https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=103912.
(35) Trifirò, G.; Barbaro, E.; Gambaro, A.; Vita, V.; Clausi, M. T.; Franchino, C.; Palumbo, M. P.; Floridi, F.; De Pace, R. Quantitative determination by screening ELISA and HPLC-MS/MS of microcystins LR, LY, LA, YR, RR, LF, LW, and nodularin in the water of Occhito lake and crops. *Anal. Bioanal. Chem.* **2016**, *408*, 7699−7708.
(36) Guzman-Guillen, R.; Prieto, A. I.; Gonzalez, A. G.; Soria-Diaz, M. E.; Camean, A. M. Cylindrospermopsin determination in water by LC-MS/MS: Optimization and validation of the method and application to real samples. *Environ. Toxicol. Chem.* **2012**, *31*, 2233−2238.
(37) Papageorgiou, J.; Nicholson, B. C.; Linke, T. A.; Kapralos, C. Analysis of cyanobacterial-derived saxitoxins using high-performance ion exchange chromatography with chemical oxidation/ fluorescence detection. *Environ. Toxicol.* **2005**, *20*, 549−559.
(38) Vasas, G.; Gaspar, A.; Pager, C.; Suranyi, G.; Mathe, C.; Hamvas, M. M.; Borbely, G. Analysis of cyanobacterial toxins (anatoxin-a, cylindrospermopsin, microcystin-LR) by capillary electrophoresis. *Electrophoresis* **2004**, *25*, 108−115.
(39) James, K. J.; Sherlock, I. R. Determination of the cyanobacterial neurotoxin, anatoxin-a, by derivatisation using 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F) and HPLC analysis with fluorimetric detection. *Biomed. Chromatogr.* **1996**, *10*, 46−47.
(40) Zhang, C.; Zhang, J. Current Techniques for Detecting and Monitoring Algal Toxins and Causative Harmful Algal Blooms. *J. Environ. Anal. Chem.* **2015**, *2*, 123.
(41) Ronkainen, N. J.; Halsall, H. B.; Heineman, W. R. Electrochemical biosensors. *Chem. Soc. Rev.* **2010**, *39*, 1747−1763.
(42) Zhu, C.; Yang, G.; Li, H.; Du, D.; Lin, Y. Electrochemical Sensors and Biosensors Based on Nanomaterials and Nanostructures. *Anal. Chem.* **2015**, *87*, 230−249.

DOI: 10.1021/acssensors.8b00359
ACS Sens. XXXX, XXX, XXX−XXX

(43) Weller, M. G. Immunoassays and biosensors for the detection of cyanobacterial toxins in water. *Sensors* **2013**, *13*, 15085−15112.
(44) Wang, L.; Chen, W.; Xu, D.; Shim, B. S.; Zhu, Y.; Sun, F.; Liu, L.; Jin, Z.; Xu, C.; Kotov, N. A. Simple, rapid, sensitive, and versatile SWNT-paper sensor for environmental toxin detection competitive with ELISA. *Nano Lett.* **2009**, *9*, 4147−4152.
(45) Wei, Q.; Zhao, Y.; Du, B.; Wu, D.; Cai, Y.; Mao, K.; Li, H.; Xu, C. Nanoporous PtRu alloy enhancer nonenzymatic immunosensor for ultrasensitive detection of microcystin-LR. *Adv. Funct. Mater.* **2011**, *21*, 4193−4198.
(46) Zhang, J.; Lei, J.; Pan, R.; Leng, C.; Hu, Z.; Ju, H. In situ assembly of gold nanoparticles on nitrogen-doped carbon nanotubes for sensitive immunosensing of microcystin-LR. *Chem. Commun.* **2011**, *47*, 668−670.
(47) Tong, P.; Tang, S. R.; He, Y.; Shao, Y. H.; Zhang, L.; Chen, G. N. Label-free immunosensing of microcystin-LR using a gold electrode modified with gold nanoparticles. *Microchim. Acta* **2011**, *173*, 299−305.
(48) Lin, Z.; Huang, H.; Xu, Y.; Gao, X.; Qiu, B.; Chen, X.; Chen, G. Determination of microcystin-LR in water by a label-free aptamer based electrochemical impedance biosensor. *Talanta* **2013**, *103*, 371−374.
(49) Lotierzo, M.; Abuknesha, R.; Davis, F.; Tothill, I. E. A membrane-based ELISA assay and electrochemical immunosensor for microcystin-LR in water samples. *Environ. Sci. Technol.* **2012**, *46*, 5504−5510.
(50) Hou, L.; Ding, Y.; Zhang, L.; Guo, Y.; Li, M.; Chen, Z.; Wu, X. An ultrasensitive competitive immunosensor for impedimetric detection of microcystin-LR via antibody-conjugated enzymatic biocatalytic precipitation. *Sens. Actuators, B* **2016**, *233*, 63−70.
(51) Zhang, J.; Sun, Y.; Dong, H.; Zhang, X.; Wang, W.; Chen, Z. An electrochemical non-enzymatic immunosensor for ultrasensitive detection of microcystin-LR using carbon nanofibers as the matrix. *Sens. Actuators, B* **2016**, *233*, 624−632.
(52) Li, R.; Xia, Q.; Li, Z.; Sun, X.; Liu, J. Electrochemical immunosensor for ultrasensitive detection of microcystin-LR based on graphene-gold nanocomposite/functional conducting polymer/gold nanoparticle/ionic liquid composite film with electrodeposition. *Biosens. Bioelectron.* **2013**, *44*, 235−240.
(53) Ng, A.; Chinnappan, R.; Eissa, S.; Liu, H.; Tlili, C.; Zourob, M. Selection, charaterization, and biosensing application of high affinity congener-specific microcystin-targeting aptamers. *Environ. Sci. Technol.* **2012**, *46*, 10697−10703.
(54) Bilibana, M. P.; Williams, A.; Rassie, C.; Sunday, C. E.; Makelane, H.; Wilson, L.; Ntshongontshi, N.; Jijana, A. N.; Masikini, M.; Baker, P. G. L.; Iwuoha, E. I. Electrochemical aptatoxisensor responses on nanocomposites containing electro-deposited silver nanoparticles on poly(propyleneimine) dendrimer for the detection of microcystin-LR in freshwater. *Sensors* **2016**, *16*, 1901.
(55) Tan, F.; Saucedo, N. M.; Ramnani, P.; Mulchandani, A. Label-free electrical immunosensor for highly sensitive and specific detection of microcystin-LR in water samples. *Environ. Sci. Technol.* **2015**, *49*, 9256−9263.
(56) Gan, C.; Ling, L.; He, Z.; Lei, H.; Liu, Y. In-situ assembly of biocompatible core-shell hierarchical nanostructures sensitized immunosensor for microcystin-LR detection. *Biosens. Bioelectron.* **2016**, *78*, 381−389.
(57) Eissa, S.; Ng, A.; Siaj, M.; Zourob, M. Label-free voltammetric aptasensor for the sensitive detection of microcystin-LR using graphene-modified electrodes. *Anal. Chem.* **2014**, *86*, 7551−7557.
(58) Zhao, H.; Tian, J.; Quan, X. A graphene and multienzyme functionalized carbon nanosphere-based electrochemical immunosensor for microcystin-LR detection. *Colloids Surf., B* **2013**, *103*, 38−44.
(59) Han, C.; Doepke, A.; Cho, W.; Likodimos, V.; de la Cruz, A. A.; Back, T.; Heineman, W. R.; Halsall, H. B.; Shanov, V. N.; Schulz, M. J.; Falaras, P.; Dionysiou, D. A multiwalled-carbon-nanotube-based biosensor for monitoring microcystin-LR in sources of drinking water supplies. *Adv. Funct. Mater.* **2013**, *23*, 1807−1816.
(60) Zhang, W.; Han, C.; Jia, B.; Saint, C.; Nadagouda, M.; Falaras, P.; Sygellou, L.; Vogiazi, V.; Dionysiou, D. A 3D graphene-based biosensor as an early microcystin-LR screening tool in sources of drinking water supply. *Electrochim. Acta* **2017**, *236*, 319−327.
(61) Zhang, W.; Jia, B.; Furumai, H. Fabrication of graphene film composite electrochemical biosensor as a pre-screening algal toxin detection tool in the event of water contamination. *Sci. Rep.* **2018**, *8* (10), 10686.
(62) Elshafey, R.; Siaj, M.; Zourob, M. *In Vitro* selection, characterization, and biosensing application of high-affinity cylindrospermopsin-targeting aptamers. *Anal. Chem.* **2014**, *86*, 9196−9203.
(63) Zhao, Z.; Chen, H.; Ma, L.; Liu, D.; Wang, Z. A label-free electrochemical impedance aptasensor for cylindrospermopsin detection based on thionine−graphene nanocomposites. *Analyst* **2015**, *140*, 5570−5577.
(64) Villatte, F.; Schulze, H.; Schmid, R. D.; Bachmann, T. T. A disposable acetylcholinesterase-based electrode biosensor to detect anatoxin-a(s) in water. *Anal. Bioanal. Chem.* **2002**, *372*, 322−326.
(65) Elshafey, R.; Siaj, M.; Zourob, M. DNA aptamers selection and characterization for development of label-free impedimetric aptasensor for neurotoxin anatoxin-a. *Biosens. Bioelectron.* **2015**, *68*, 295−302.
(66) Hou, L.; Jiang, L.; Song, Y.; Ding, Y.; Zhang, J.; Wu, X.; Tang, D. Amperometric aptasensor for saxitoxin using a gold electrode modified with carbon nanotubes on a self-assembled monolayer and methylene blue as an electrochemical indicator probe. *Microchim. Acta* **2016**, *183*, 1971−1980.
(67) Bratakou, S.; Nikoleli, G. P.; Siontorou, C. G.; Nikolelis, D. P.; Karapetis, S.; Tzamtzis, N. Development of an electrochemcial biosensor for the rapid detection of saxitoxin based on air stable lipid films with incorporated anti-STX using graphene electrodes. *Electroanalysis* **2017**, *29*, 990−997.
(68) Carter, R. M.; Poli, M. A.; Pesavento, M.; Sibley, D. E. T.; Lubrano, G. J.; Guilbault, G. G. Immunoelectrochemical biosensors for detection of saxitoxin and brevetoxin. *ImmunoMethods* **1993**, *3*, 128−133.
(69) Eissa, S.; Siaj, M.; Zourob, M. Aptamer-based competitive electrochemical biosensor for brevetoxin-2. *Biosens. Bioelectron.* **2015**, *69*, 148−154.
(70) Bakker, E.; Qin, Y. Electrochemical sensors. *Anal. Chem.* **2006**, *78*, 3965−3983.
(71) Vikesland, P. J.; Wigginton, K. R. Nanomaterial enabled biosensors for pathogen monitoring-a review. *Environ. Sci. Technol.* **2010**, *44*, 3656−3669.
(72) Clark, S. L.; Remcho, V. T. Aptamers as analytical reagents. *Electrophoresis* **2002**, *23*, 1335−1340.
(73) Liu, Y.; Zhou, Q.; Revzin, A. An aptasensor for electrochemical detection of tumor necrosis factor in human blood. *Analyst* **2013**, *138*, 4321−4326.
(74) Deng, Q.; Watson, C. J.; Kennedy, R. T. Aptamer affinity chromatography for rapid assay of adenosine in microdialysis samples collected in vivo. *J. Chromatogr. A* **2003**, *1005*, 123−130.
(75) Liu, Q.; Wang, J.; Boyd, B. J. Peptide-based biosensors. *Talanta* **2015**, *136*, 114−127.
(76) Xiao, X.; Kuang, Z.; Slocik, J. M.; Tadepalli, S.; Brothers, M.; Kim, S.; Mirau, P. A.; Butkus, C.; Farmer, B. L.; Singamaneni, S.; Hall, C. K.; Naik, R. R. Advancing Peptide-Based Biorecognition Elements for Biosensors Using in-Silico Evolution. *ACS Sensors* **2018**, *3*, 1024−1031.
(77) Velusamy, V.; Arshak, K.; Korostynska, O.; Oliwa, K.; Adley, C. An overview of foodborne pathogen detection: In the perspective of biosensors. *Biotechnol. Adv.* **2010**, *28*, 232−254.
(78) Kulagina, N. V.; Mikulski, C. M.; Gray, S.; Ma, W.; Doucette, G. J.; Ramsdell, J. S.; Pancrazio, J. J. Detection of marine toxins, bevetoxin-3 and saxitoxin, in seawater using neuronal networks. *Environ. Sci. Technol.* **2006**, *40*, 578−583.
(79) Lee, B. P.; Chao, C.; Nunalee, F. N.; Motan, E.; Shull, K. R.; Messersmith, P. B. Rapid gel formation and adhesion in photocurable

DOI: 10.1021/acssensors.8b00359
ACS Sens. XXXX, XXX, XXX−XXX

and biodegradable block copolymers with high DOPA content. *Macromolecules* **2006**, *39*, 1740−1748.
(80) Li, S. C.; Chu, L. N.; Gong, X. Q.; Diebold, U. Hydrogen bonding controls the dynamics of catechol adsorbed on a TiO2(110) surface. *Science* **2010**, *328*, 882−884.
(81) Liu, Y.; Ai, K.; Lu, L. Polydopamine and its derivative materials: synthesis and promising applications in energy, environmental, and biomedical fields. *Chem. Rev.* **2014**, *114*, 5057−5115.
(82) Grabarek, Z.; Gergely, J. Zero-length crosslinking procedure with the use of active esters. *Anal. Biochem.* **1990**, *185*, 131−135.
(83) Gong, P.; Grainger, D. W. Comparison of DNA immobilization efficiency on new and regenerated commercial amine-reactive polymer microarray surfaces. *Surf. Sci.* **2004**, *570*, 67−77.
(84) Yang, W. R.; Gooding, J. J.; Hibbert, D. B. Characterisation of gold electrodes modified with self-assembled monolayers of L−cysteine for the adsorptive stripping analysis of copper. *J. Electroanal. Chem.* **2001**, *516*, 10−16.
(85) Ramanavicius, A.; Finkelsteinas, A.; Cesiulis, H.; Ramanaviciene, A. Electrochemical impedance spectroscopy of polypyrrole based electrochemical immunosensor. *Bioelectrochemistry* **2010**, *79*, 11−16.
(86) Park, J. − Y.; Lee, Y. − S.; Chang, B. − Y.; Karthikeyan, S.; Kim, K. S.; Kim, B. H.; Park, S. − M. (R)-lipo-diaza-18-crown-6 self-assembled monolayer as a selective serotonin receptor. *Anal. Chem.* **2009**, *81*, 3843−3850.
(87) Daniels, J. S.; Pourmand, N. Label-free impedance biosensors: Opportunities and challenges. *Electroanalysis* **2007**, *19*, 1239−1257.
(88) Yang, L.; Bashir, R. Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. *Biotechnol. Adv.* **2008**, *26*, 135−150.
(89) Ruan, C.; Yang, L.; Li, Y. Immunobiosensor chips for detection of Escherichia coli O157:H7 using electrochemical impedance spectroscopy. *Anal. Chem.* **2002**, *74*, 4814−4820.
(90) Patolsky, F.; Zayats, M.; Katz, E.; Willner, I. Precipitation of an insoluble product on enzyme monolayer electrodes for biosensor applications: characterization by faradaic impedance spectroscopy, Cyclic Voltammetry, and Microgravimetric Quartz Crystal Microbalance Analyses. *Anal. Chem.* **1999**, *71*, 3171−3180.
(91) Yang, F.; Han, J.; Zhuo, Y.; Yang, Z.; Chai, Y.; Yuan, R. Highly sensitive impedimetric immunosensor based on single-walled carbon nanohorns as labels and bienzyme biocatalyzed precipitation as enhancer for cancer biomarker detection. *Biosens. Bioelectron.* **2014**, *55*, 360−365.
(92) Durán-Riveroll, L. M.; Cembella, A. D.; Band-Schmidt, C. J.; Bustillos-Guzmán, J. J.; Correa-Basurto, J. Docking simulation of the binding interactions of saxitoxin analogs produced by the marine dinoflagellate gymnodinium catenatum to the voltage-gated sodium channel Nav1.4. *Toxins* **2016**, *8*, 129.
(93) Tobi, D.; Bahar, I. Structural changes involved in protein binding correlate with intrinsic motions of proteins in the unbound state. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 18908−18913.
(94) Campàs, M.; Garibo, D.; Prieto-Simón, B. Novel nano-biotechnological concepts in electrochemical biosensors for the analysis of toxins. *Analyst* **2012**, *137*, 1055−1067.
(95) Kim, S. U.; Lee, K. H. Carbon nanofiber composites for the electrodes ofelectrochemical capacitors. *Chem. Phys. Lett.* **2004**, *400*, 253−257.
(96) Wu, L. N.; Zhang, X. J.; Ju, H. X. Detection of NADH and ethanol based oncatalyticactivity of soluble carbon nanofiber with low overpotential. *Anal. Chem.* **2007**, *79*, 453−458.
(97) Novoselov, K. S.; Geim, A. K.; Morozov, S. V.; Jiang, D.; Zhang, Y.; Dubonos, S. V.; Grigorieva, I. V.; Firsov, A. A. Electric field effect in atomically thin carbon films. *Science* **2004**, *306*, 666−669.
(98) Pumera, M. Graphene in biosensing. *Mater. Today* **2011**, *14*, 308−315.
(99) Chen, Z.; Ren, W.; Gao, L.; Liu, B.; Pei, S.; Cheng, H. Three-dimensional flexible and conductive interconnected graphene networks grown by chemical vapour deposition. *Nat. Mater.* **2011**, *10*, 424−428.
(100) Bae, S.; Kim, H.; Lee, Y.; Xu, X.; Park, J.; Zheng, Y.; Balakrishnan, J.; Lei, T.; Kim, H.; Song, Y.; Kim, Y.; Kim, K.; Özyilmaz, B.; Ahn, J.; Hong, B.; Iijima, S. Roll-to-roll production of 30-in. graphene films for transparent electrodes. *Nat. Nanotechnol.* **2010**, *5*, 574−578.
(101) Cui, R. J.; Pan, H. C.; Zhu, J. J.; Chen, H. Y. Versatile immunosensor using CdTe quantum dots as electrochemical and fluorescent labels. *Anal. Chem.* **2007**, *79*, 8494−8501.
(102) Zhuo, Y.; Yuan, P. X.; Yuan, R.; Chai, Y. Q.; Hong, C. L. Bienzyme functionalized three-layer composite magnetic nanoparticles for electrochemical immunosensors. *Biomaterials* **2009**, *30*, 2284−2290.
(103) Hirao, A.; Yoo, H. S. Dendrimer-like-star-branched polymers: Novel structurally well-defined hyperbranched polymers. *Polym. J.* **2011**, *43*, 2−17.
(104) Dhand, C.; Das, M.; Datta, M.; Malhotra, B. D. Recent advances in polyaniline based biosensors. *Biosens. Bioelectron.* **2011**, *26*, 2811−2821.
(105) Locascio-Brown, L.; Plant, A. L.; Horvath, V.; Durst, R. A. Liposome flow injection immunoassay: implications for sensitivity, dynamic range, and antibody regeneration. *Anal. Chem.* **1990**, *62*, 2587−2593.
(106) Han, J.; Zhang, J.; Xia, Y.; Li, S.; Jiang, L. An immunoassay in which magnetic beads act both as collectors and sensitive amplifiers for detecting antigens in a microfluidic chip (MFC)-quartz crystal microbalance (QCM) system. *Colloids Surf., A* **2011**, *379*, 2−9.
(107) Zhang, J.; Liu, S.; Yang, P.; Sui, G. Rapid detection of algal toxins by microfluidic immunoassay. *Lab Chip* **2011**, *11*, 3516−3522.
(108) Cai, X.; Baldelli, S. Surface barrier properties of self-assembly monolayers as deduced by sum frequency generation spectroscopy and electrochemistry. *J. Phys. Chem. C* **2011**, *115*, 19178−19189.
(109) Boubour, E.; Lennox, R. B. Insulating properties of self-assembled monolayers monitored by impedance spectroscopy. *Langmuir* **2000**, *16*, 4222−4228.
(110) Luo, X. L.; Xu, M. Y.; Freeman, C.; James, T.; Davis, J. J. Ultrasensitive Label Free Electrical Detection of Insulin in Neat Blood Serum. *Anal. Chem.* **2013**, *85* (8), 4129−4134.
(111) Jacobs, M.; Selvam, A. P.; Craven, J. E.; Prasad, S. Antibody-conjugated gold nanoparticle based immunosensor for ultra-sensitive detection of troponin-T. *J. Lab. Autom* **2014**, *19* (6), 546−554.
(112) Jacobs, M.; Muthukumar, S.; Selvam, A. P.; Craven, J. E.; Prasad, S. Ultra-sensitive electrical immunoassay biosensors using nanotextured zinc oxide thin films on printed circuit board platforms. *Biosens. Bioelectron.* **2014**, *55*, 7−13.
(113) Assaifan, A. K.; Lloyd, J. S.; Samavat, S.; Deganello, D.; Stanton, R. J.; Teng, K. S. Nanotextured surface on flexographic printed ZnO thin films for low-cost noon-faradaic biosensors. *ACS Appl. Mater. Interfaces* **2016**, *8*, 33802−33810.
(114) Eersels, K.; Lieberzeit, P.; Wagner, P. A review on synthetic receptors for bioparticle detection created by surface-imprinting techniques-from principles to applications. *ACS Sens.* **2016**, *1*, 1171−1187.
(115) Ramanaviciene, A.; Ramanavicius, A. Molecularly imprinted polypyrrole-based synthetic receptor for direct detection of bovine leukemia virus glycoproteins. *Biosens. Bioelectron.* **2004**, *20* (6), 1076−1082.
(116) Wang, Y.; Zhang, Z.; Jain, J.; Yi, J.; Mueller, S.; Sokolov, J.; Liu, Z.; Levon, K.; Rigas, B.; Rafailovich, M. H. Potentiometric sensors based on surface molecular imprinting: detection of cancer biomarkers and viruses. *Sens. Actuators, B* **2010**, *146* (1), 381−387.
(117) Cai, D.; Ren, L.; Zhao, H.; Xu, C.; Zhang, L.; Yu, Y.; Wang, H.; Lan, Y.; Roberts, M. F.; Chuang, J. H.; Naughton, M. J.; Ren, Z.; Chiles, T. C. A molecular-imprint nanosensor for ultrasensitive detection of proteins. *Nat. Nanotechnol.* **2010**, *5*, 597−601.

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ACS Sens. XXXX, XXX, XXX−XXX