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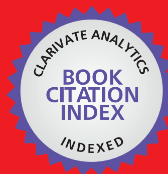

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Chapter 1

Recent Progress in Optical Biosensors for Environmental Applications

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Additional information is available at the end of the chapter

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1. Introduction

The rapid screening and sensitive monitoring of environmental pollutants, such as pesticides, persistent organic pollutants (POPs), endocrine disrupting chemicals (EDCs), explosives, and toxins, is indeed essential to ensure environmental quality, and therefore, human health. Until recently, the quantification of most contaminants has been limited to the traditional chromatographic and spectroscopic technologies. These methods, although accurate with low detection limits, are labor-intensive and require expensive and sophisticated instrumentation, as well as complicated and multistep sample preparation, which prohibits frequent and real-time on-site monitoring of contaminants in environment.[1] Considerable research interests, therefore, have risen for detecting low levels of environmental pollutants in biosensor development because of their simplicity, robustness, sensitivity, specificity and cost-effectiveness. [2]

A biosensor is an analytical device combined a biological sensing element with a physical transducer, in which the binding or reaction between the target and the recognition element is translated into a measurable electrical signal. [3] Among them, optical biosensors are powerful alternative to conventional analytical techniques due to their cost-effective, fast and portable detection, which makes on-site and real-time monitoring possible without extensive sample preparation. Optical biosensors have vast potential applications in environmental monitoring, food safety, drug development, and medical diagnosis.[4] Although the use of optical biosensors in water quality early-warning and pollution control is still in its infancy, research on this topic is an active area and the remarkable technological progress has been made.

The present article gives an overview of the recent advances in optical biosensors and their applications in the environmental field. Functional biorecognition materials (e.g. enzyme,

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antibody, aptamer, and DNAzyme), a key component of biosensor and specifically binding a broad range of analytes including inorganic, organic, and biomolecules, will be first reviewed. Then, nanomaterials such as quantum dots, graphene, nanogold particles, carbon nanotubes, and magnetic nanoparticles will be introduced, which have been successfully incorporated into optical biosensors to improve the sensibility, sensitivity, and selectivity due to their unique physicochemical properties. In addition, the recent significant improvements in instrumentation will also be discussed, which have allowed a wider variety of pollutants to be analysed in details, and led to the increasing application of optical biosensor technology throughout the environmental detection field. Finally, recent developments of optical biosensors for pollution control and early-warning will be highlighted.

2. Functional biorecognition materials

Functional biorecognition materials are key components of biosensors, and generally have high affinity (low detection limit), high specificity (low interference), wide dynamic range, fast response time, and long shelf life. The antibodies are most frequently used biorecognition molecules in the optical biosensor community. However, enzymes were the first recognition elements used in biosensors. Another frequently used recognition elements are nucleic acids such as aptamer and DNAzyme for the monitoring of environmental pollutants.

2.1. Antibody

Immunosensors, based on specific antigen-antibody interactions, have become the gold-standard technique in clinical diagnostics and environmental monitoring.[4-9] Antibody is a large Y-shaped protein used by the immune system to identify and neutralize a unique part of foreign target, called an antigen, and is produced by white blood cell (a plasma cell). Antibodies are typically made of basic structural units: each with two larger heavy chains and two shorter light chains. The IgG molecule (see Figure 1) is the most used antibody type and is about 150-kDa protein composed of four polypeptide chains.[8] Antibodies are produced as monoclonal and polyclonal varieties, with monoclonal antibodies binding to a single epitope and polyclonal antibodies being capable of binding to multiple epitopes.[9,10] In immunoassay, two antigen binding sites of antibody have a highly specific interaction for one particular target, and this immunochemical reaction can be detected by the transducer (e.g. optical, electronical). [5-9] Therefore, the immunosensor assay provides a highly repeatable and highly specific reaction format, and the capacity for specific recognition of environmental contaminants.

Due to most of the environmental pollutants have the low molecular weight (<1 kDa) and are called haptens which are non-immunogenic, it has to be conjugated to carrier proteins to make them immunogenic.[11] Preparation of antibodies against haptens, such as pesticides, persistent organic pollutants (POPs), and endocrine disrupting chemicals (EDCs), is based on covalent binding of the hapten to a carrier protein and immunisation of animals by the synthesised immunogens. The specificity of antibody is important for immunoassay, while the specificity and quality of antibody is mostly determined by the manner of chemical

binding of the hapten to the carrier protein, called complete antigen. In our group, the complete antigen of microcystin-LR (MC-LR-BSA), the most frequent and most toxic hepatotoxin, was synthesized by introducing a primary amino group in the seventh N-methyldehydroalanine residue, and then the product aminoethyl-MC-LR was coupled to bovine serum albumin (BSA) by glutaraldehyde.[11] The residue is located most distantly from both of the variable amino acid residues and Adda, promising active and possibly more specific immunoreactivity. Polyclonal antibodies and a monoclonal antibody (Clone MC8C10) against MC-LR were generated by immunization with MC-LR-BSA, respectively. An indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) with MC8C10 was established to detect the MCs in waters, which showed highly specificity with MC-LR and have a detection limit for MC-LR 0.1 μg L^{-1}.[12]

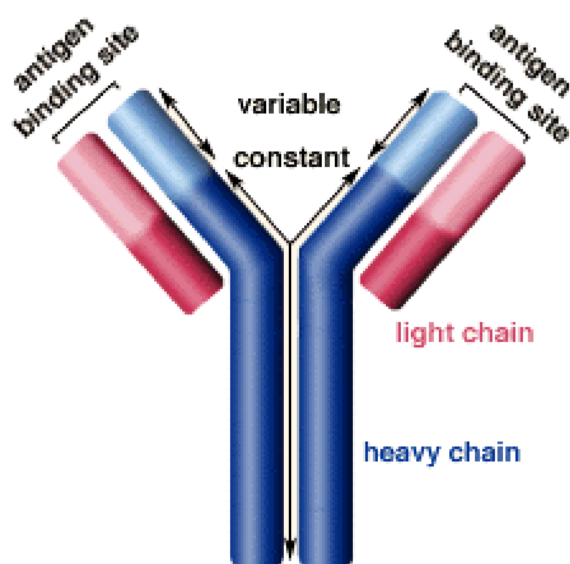

Figure 1. The structure of antibody

A compact, portable, multichannel fiber-optic instrument was reported to detect four targets simultaneously using antibody immobilized fiber-optic probes. [13] This biosensor was simultaneously able to determine 10^5 cfu/mL of *Bacillus globigii*, 10^7 cfu/mL of *Erwinia herbicola*, and 10^9 pfu/mL of MS2 coli phages. A biosensor platform (Analyte 2000) developed by the Naval Research Laboratory (USA) was used to simultaneous determine both the explosives 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). [14] The limit of detection of TNT and RDX was 5 μg/L and 2.5 μg /L, respectively.

The main advantage of immunosensors over other immnunological methods (e.g., ELISA formats) is the better regeneration and binding properties of the sensing surface, which is critical for the successful reuse of the same sensor surface and the accuracy of detection results.[15] In environmental analysis, targets interest are usually small molecule substances (molecular weight <1kDa), which are greatly difficult to be directly immobilized onto the biorecognition sensing surface. Therefore, antibody immobilisation is always utilized in preparing sensor surface of immunosensors.[5-8,16] However, the control over the number of

antibodies and their orientation and position relative to the sensor surface is very difficult. Because of the possibility of inadvertently disrupting the binding site when conjugating antibody with active surface of sensor, the activity loss of antibody is inevitable.[17,18] Most important, due to strong acid being usually used in regeneration process, the recognition ability of antibodies immobilized may be lower after senor surface reuse, which will affect the stability and reliability of the immunosensor. The cycles of regeneration are usually no more than fifteen times and in each cycle, antibody activity decreased, which leads to inaccurate detection results. Therefore, hapten-carrier-protein conjugates as bio-recognition molecules were immobilized onto the surface of immunosensor for obtaining the stable reusable sensor. For example, a reusable immunosurface is provided via the covalent attachment of the 2,4-D-BSA and MC-LR-OVA to a self-assembled monolayer formed onto the fiber optic sensor.[19] The regeneration of the sensor surface allows the performance of more than 100 assay cycles.

2.2. Enzyme

Enzymes are biological molecules that catalyze (i.e., increase the rates of) chemical reactions, and are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Enzymes are historically the first molecular recognition elements included in biosensors and continue to be the basis for a significant number of publications reported for biosensors in general as well as for environmental applications.[4] Enzyme biosensors have several advantages, such as a stable source of material, the possibility of modifying the catalytic properties or substrate specificity by means of genetic engineering, and catalytic amplification of the biosensor response by modulation of the enzyme activity with respect to the target analyte. [20]

Most of enzyme biosensors normally use enzymes as the bioreceptors and achieved pollutants detection based on the enzyme inhibition mechanism.[4][20] Due to ChE enzymes can be inhibited by several toxic chemicals such as organophosphate and pesticides, heavy metals, and toxins, the ChEs biosensors is of particular interest in the area of global toxicity monitoring.[4,21,22]

Due to various pollutants that inhibit the activity of enzymes in a different ways, multi-analytes detection can be achieved by enzyme sensors. For example, simultaneous detection both pesticides and heavy metal ions in a sample solution is possible due to selective inhibition of butyrylcholine esterase by pesticides and urease by heavy metals ions.[4-6,23] Comparing with the inhibition level of urease or butyrylcholine esterase, respectively, the pesticides or heavy metal ions can be determined. The enzyme-inhibition based biosensors array could achieve the simultaneous determination of various pollutants in water samples.

Enzyme biosensors have some limitations for the detection of environmental pollutants, which include the limited number of substrates for which enzymes have been evolved, the limited interaction between environmental pollutants and specific enzymes, and the lack of specificity in differentiating among compounds of similar classes.[6,23] However, artificial or synthetic enzymes could be a useful alternative to natural enzymes for the development

of new biosensors, which are more robust, available, chemically malleable and cheap, in comparison with their natural analogues.

No	Target	Aptamer type	Sequence	Reference
1	2,3',5,5'-Tetrachlorobiphenyl; 2,3,3',4,5-Pentachlorobiphenyl	DNA	9.1:CGCTACACCT,CGCCAGCAAA,TTGCCGCCCG,CAGCCCTCTA 9.2:GGGACTCGAG,ACCCGTTCCG,TTCTCCGCTT,GCCCCACAAT	[26]
2	4,4'-methylenedianiline(MDA)	RNA	M1:CUGCGAUCA,GGGGUAAAUU,UCCGCGCAGG,CUCCACGCCG,C M2:CUCGA,GUCCUCUUGA,GCGGUUCCUA,CUUCCCUCUG,CUGUG	[27]
3	Organophosphorus Pesticides:phorate,profenofos, isocarbophos, omethoateas	DNA	1: AAGCTTGCTTTATAGCCTGCAGCGAT TCTTGATCGGAAAAGG CTGAGAGCTACGC 2:AAGCTTTTTTGACTGACTGCAGCGATTCTTGATCGCCACGGTCTGGAAAA AGAG	[28]
4	bisphenol A	DNA	CCGGTGGGTGGTCAGGTGGGATAGCGTTCCGCGTATGGCCCAGCGCATC ACGGGTTCGCACCA	[29]
5	17β-estradiol	DNA	GCTTCCAGCTTATTGAATTACACGCAGAGGGTAGCGGCTCTGCGCATTCA ATTGCTGCGCGCTGAAGCGCGGAAGC	[30]
6	Chloramphenicol	DNA	1:ACTTCAGTGA,GTTGTCCCAC,GGTCGGCGAG,TCGGTGGTAG 2:CACCAAGCGC,AGGGAATTAC,ATTGAAGTGT,GGGATTGGCT	[31]
7	Oxytetracycline	DNA	1:CGACCGCAGGTGCACTGGGCGACGTCTCTGGTGTGGTGT 2:CGACGCGCGTTGGTGGTGGATGGTGTGTTACACGTGTTGT	[32]
8	Tetracycline	DNA	T7:GGGCAGCGGTGGTGTGGCGGGATCTGGGGT,TGTGCGGTGT T15:GGAGGAACGGGTTCCAGTGTGGGGTCTATC,GGGGCGTGCG	[33]
9	Kanamycin	DNA	TGGGGGTTGAGGCTAAGCCGA	[34]
10	ricin B chain	DNA	ACACCCACCGCAGGCAGACGCAACGCCTCGGAGACTAGCC	[35]
11	Ochratoxin A	DNA	GATCGGGTGTGGGTGGCGTAAAGGGAGCATCGGACA	[36]
12	E. coli	DNA	ATCCGTCACACCTGCTCTACGGCGCTCCCAACAGGCCTCTCCTTACGGCAT ATTA TGGTGTTGGCTCCCGTAT	[37]
13	Staphylococcus aureus Enterotoxin B	DNA	GGTATTGAGGGTCGCATCCACTGGTCGTTG TTGTCTGTTGTCTGTTATGTTGTTTCGTGATGG CTCTAACTCTCCTCT	[38]
14	Salmonella entericaserovars	DNA	23:CCGCCTTTACTAAATTGACGAACATAGGAATCAATGAAGC 24:GGGAGTCAGAACGCCTGGGCAAGCATAGTACTCGCCGGAA	[39]
17	Ibuprofen	DNA	IBA2:ACAGTAGTGAGGGGTCCGTCGTGGGGTAGTTGGGTCGTGG IBA8:GCGAACGACTTCATAAAATGCTATAAGGTTGCCCTCTGTC	[40]
18	Arsenic	DNA	TTACAGAACAACCAACGTCGCTCCGGGTACTTCTTCATCG	[41]

Table 1. DNA/RNA aptamers

2.3. Aptamer

Aptamer is a single-stranded oligonucleotide that folds into complex three-dimensional structure and bind strongly and selectively to one certain kind of target or one class of targets. [4-6] The aptamer is selected using an *in vitro* process called Systematic Evolution of Ligands by EXponential enrichment (SELEX), which was first put forward by Ellington et al. and Tuerk et al. in 1990.[24,25] For the selection of DNA aptamer, the SELEX starts from the construction of a random pool of DNA sequences (~10^{15}), and then the selection procedure could take place, including: (a) binding between the library and the target; (b) separation of the unbound ssDNA and the ssDNA-target complex; (c) elution the bound ssDNA from the ssDNA-target complex; (d) amplification of the bound ssDNA, usually using the PCR method; (e) generation of single strand DNA from the double strand PCR products. Generally, a traditional aptemer selection process need 10-12 cycles. The ssDNA pool from the first selection cycle is used as the starting library for the second selection cycle, then repeating procedure (a), (b),(c),(d) and (e). After the last selection cycle, molecular cloning and DNA sequencing was applied, and the aptamer with high affinity and specifity is obtained.

Aptamers offer a useful alternative to antibodies as sensing molecules and have opened a new era in development of affinity biosensing due to their unique characterizations. In vitro selected aptamers could be produced for any targets such as proteins, peptides, amino acids, nucleotides, drugs, heavy metal ions, and other small organic and inorganic compounds. [26-41] Aptamers could be chemically synthesized without the complicated and expensive purification steps by eliminating the batch-to-batch variation found when using antibodies. Furthermore, modifications in the aptamer through chemical synthesis can be introduced enhancing the stability, affinity and specificity of the molecules. In addition, aptamers are more stable than antibodies and thus are more resistant to denaturation and degradation. Often the affinity parameters of aptamer-target complex can be changed for higher affinity or specificity. In addition, aptamers have the higher temperature stability and can recover their native active conformation after denaturation, whereas antibodies are large, temperature-sensitive proteins that can undergo irreversible denaturation. Recently, several DNA/RNA aptamers, selected for POPs, EDCs, organophosphorus pesticides, antibiotics, biotoxins, and pathogenic microorganisms, are listed in Table 1.

Aptamers have become increasingly important molecular tools for environmental bioassay. EDCs are contaminants of emerging concern and required routine monitoring in water samples, as posed by EPA Unregulated Contaminant Regulation (UCMR3). Gu et al. [42] reported a reusable evanescent wave aptamer-based biosensor for rapid, sensitive and highly selective detection of 17β-estradiol, frequently detected in environmental water samples. In this system, the capture molecular, β-estradiol 6-(O-carboxy-methyl) oxime-BSA, was covalently immobilized onto the optical fiber sensor surface. With an indirect competitive detection mode, the limit of detection of 17β-estradiol was determined as 2.1 nM. Kim et al. [41] developed a high affinity DNA aptamer for arsenic that can bind to arsenate [(As(V)] and arsenite [As(III)] with a dissociation constant of 5 and 7 nM, respectively. Through the “signal-on” mode or the “signal-off” mode, reflecting the extent of the binding process thereby allowing for quantitative measurement of target concentration, several DNA aptamer fluo-

rescence-based sensors have been developed for the detection of Hg^{2+}, Pb^{2+} and other trace pollutants.[43] Although a variety of aptamer has been successfully selected for environmental contaminants, the detection of the real water samples using the right aptamer is still in the cradle.

2.4. DNAzyme

DNAzymes are small single-stranded nucleic acids that fold into a well-defined three-dimensional structure with high specificity to various ligands, such as low-molecular-weight organic or inorganic substrates or macromolecules or metal ions.[43] DNAzymes have a promising capacity to selectively identify charged organic and inorganic compounds at ultratrace levels in environmental samples or biological systems. Furthermore, DNAzymes can perform chemical modifications on nucleic acids, while aptamers can bind a broad range of molecules. A combination of the two has generated a new class of functional nucleic acids known as allosteric DNAzymes or aptazymes. Combining the specificity of nano-biological recognition probes and the sensitivity of laser-based optical detection, DNAzymes are capable of provide unambiguous identification and accurate quantification of environmental pollutants, ranging from small ions to large molecules. RNA-cleaving DNAzymes are the most widely used due to their simple reaction conditions, fast turnover rates and significant modifications of their substrate lengths.

Using the in vitro selection of specifical DNAzymes, several fluorescence biosensors have extensively been developed for the detection of various heavy metal ions, such as Pb^{2+}, Cu^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, UO_2^{2+}, Hg^{2+} and Ag^{+}, etc.[43,44] Moreover, DNAzymes and aptazymes have already found many applications in almost every aspect of DNA nanotechnology, which result to new materials and devices that may penetrate into many other fields for practical applications, including environmental monitoring.

3. Nanomaterials in optical biosensors

Nanomaterials exhibit unique size-tunable and shape-dependent physicochemical properties that are different from those of bulk materials.[45-50] Specially, the interaction of nanomaterials and functional biomaterials opens a new door to develop various novel optical biosensors. NPs such as gold NPs (AuNPs), quantum dots (QDs), magnetic NPs (MNPs), graphene and carbon nanotubes have specific optical, fluorescence and magnetic properties, and interactions between these properties give NPs great potential for environmental screening.[45-51] The extremely high surface-to-volume ratios and exceptional nanoscale properties make NPs useful for next-generation environmental detection.

3.1. Quantum dots

Semiconductor quantum dots (QDs), nanocrystals of inorganic semiconductors, have emerged as promising alternative bioanalytical tools because of their unique optical properties including high quantum yield, photostability, narrow emission spectrum, and broad ab-

sorption.[52,53] QDs' band gap depends on the size of the nanocrystal. That is to say, the smaller the nanocrystal, the larger the difference between the energy levels and, therefore, the wider the energy gap and the shorter thewavelength of the fluorescence. [52,53]

The main application of QDs as sensors exploits the Forster resonance energy transfer effect (FRET) due to their narrow, size-tuned, and symmetric emission spectra, which has made them excellent donors for fluorescence resonance energy transfer (FRET) sensors, and greatly reduces the overlap between the emission spectra of donor and acceptor and circumvents the cross-talk in such FRET pairs.[52,53] Meanwhile, QDs have broad excitation spectra as donor, and allow excitation at a single wavelength far removed (>100nm) from their respective emissions, which enables QDs to be used in multiplex assays without the need for multiple excitation sources. In addition, the high photobleaching threshold and good chemical stability of QDs greatly improve the detection sensitivities and detection limits. Therefore, QD-based FRET biosensors have been widely used in environmental monitoring, medical imaging, clinical/diagnostic assays, and biomolecular binding assay. Among available QDs, CdSe/ZnS core-shell quantum dots are most commonly used for biosensing applications. Antibody (or aptamer) bioconjugates of QDs, prepared using covalent or non-covalent linking approaches, are the most developed and widespread detection bioprobes to integrating QDs into bioanalyses. The fluorescent detection of pathogens such as respiratory syncytial virus,[54] E. coli O157:H7,[55] and Bacillus thurinigensis,[56] has been performed based on QDs-FRET.

However, the control over the number of antibodies (or aptamers) per QD and their orientation and position relative to the QD is very difficult. Due to the possibility of inadvertently disrupting the binding site when conjugating QD with antibody, the activity loss of antibody is inevitable.[52,57] Additionally, antibodies usually need to be cryopreserved but QDs cannot be freezed, which makes the storage of QD-antibody a major obstacle for its practical applications. To effectively address these challenges, we have developed carrier-protein-haptens-coupled quantum-dot nanobioprobe protocols to perform rapid and sensitive detection of small targets in real water samples.[58] 2,4-Dicholrophenoxyacetic acid (2,4-D), one of the most widely used pesticides worldwide, was selected as a model target. QD nano-immunoprobe were prepared through conjugating carboxyl quantum dots with 2,4-D-BSA conjugate, which regarded as the immunological recognition of anti-2,4-D antibody as well as for optical transducer. With a competitive detection mode, samples containing different concentrations of 2,4-D were incubated with a given concentration of QD immunoprobe and fluorescence-labeled antibody, and then detected by an all-fiber microfluidic biosensing platform developed by our group. A higher concentration of 2,4-D led to less fluorescence-labeled anti-2,4-D antibody bound to the QD immunoprobe surface, and thus to lower fluorescence signal. The quantification of 2,4-D over concentration ranges from 0.5 nM to 3 μM with a detection limit determined as 0.5 nM. The structure of multiplex-haptens/BSA conjugate coupling to QD greatly improves the FRET efficiency and nanosensor's sensitivity. With the use of different QD immunoprobes modified by the conjugates of other haptens/ carried protein, the methodology presented here has the potential to extend to toward the

on-site monitoring other small analytes in a variety of application fields ranged from environmental to biomedical areas.

3.2. Nanogold particles

Gold nanoparticles (AuNPs) typically have dimensions ranging from 1-100 nm and display many interesting electrical and optical properties. Nanogold particles based optical biosensors commonly take use of the fluorescence quenching through fluorescence resonance energy transfer (FRET) or a visible color change due to the aggregation of AuNPs of appropriate sizes.[59] Over the past decades, AuNPs based optical sensors have an important role in the detection of environmental pollutants such as toxins, heavy metals and other pollutants due to their typically high signal-to-noise ratios.[51]

Uzawa et al.[60] developed sugar-coated GNPs for the detection of ricin with visual readout using the naturally occurring infection mechanism and the strong affinity of the toxin ricin to sugar. Many kinds of immunoassays using GNP-antibody conjugates have been developed for detection of ochratoxin A (OTA), zearalenone (ZEA), and aflatoxin B1 (AFB1).[51,61] AuNP-based biosensors have also been used to highly competitive assay technologies for the detection of oligonucleotide targets.[51]

Heavy metal contamination is an ongoing concern worldwide, and it is vital for rapid and simple monitoring technologies of heavy metal ions in environment. Darbha et al.[62] developed a GNP-based sensor for rapid, easy and reliable detection of Hg^{2+} ions in aqueous solutions, which had a detection limit of 5 ng/ml (ppb) through non-linear optical properties. A visual detection methode of Cu^{2+} was reported by Lcysteine-functionalized GNPs in aqueous solution.[63] This colorimetric nanosensor allows rapid, quantitative detection of Cu^{2+} with a sensitivity of 10^{-5} M. Similarly, Xue et al.[64] developed a novel and practical system for room temperature colorimetric detection of mercury based on T-Hg^{2+}-T structure with a sensitivity of 3.0 ppb. Several AuNPs-based optical sensors have been developed on the basis of the principle of FRET. Based on modulating photoluminescent-quenching efficiency between a perylene bisimide chromophore and GNPs in the presence of Cu^{2+}, a homogeneous assay to detect Cu^{2+} was reported.[65] Chen et al.[66] developed a GNP-rhodamine 6G-based fluorescent sensor for detecting Hg^{2+} in aqueous solution with a detection limit of 0.012 ppb. Li et al.[67] used a T-Hg^{2+}-T structure to develop a detection method of aqueous Hg^{2+} with the limit of detection of 50 nM. Freeman et al.[68] showed a multiplex assay for detecting Hg^{2+} and Ag^{+} using FRET.

Small molecules, such as hydrogen, carbon dioxide, TNT, and ammonium ions, can also be detected by AuNPs. Dasary et al.[69] developed a cysteine-modified GNP-based label-free surface enhanced Raman spectroscopy probe based on the reaction between TNT and cysteine on the GNP surface. An AuNPs color change is induced in the presence of TNT with a detection limit of 2 pM in water samples.

3.3. Graphene

Graphene, a true two-dimensional material, has received increasing interest due to its unique physicochemical properties such as high surface area, fast electron transportation, high thermal conductivity, high mechanical strength, and excellent biocompatibility.[70] These properties of graphene give it potential applicability in biosensors, especially for electrochemical biosensors. However, the optical properties of graphene have received considerable attention. Wen et al.[71] reported a fluorescence sensor for Ag(I) ions based on the target-induced conformational change of a silver-specific cytosine-rich oligonucleotide (SSO) and the interactions between the fluorogenic SSO probe and graphene oxide. He et al.[72] developed a SERS-based biosensor for DNA detection. The Raman signals of dye were dramatically enhanced by the substrate based on gold nanoparticles-decorated graphene. This platform showed extraordinarily high sensitivity and excellent specificity for DNA detection with a detection limit as low as 10 pM.

Lee et al.[73] reported on a platform based on chemiluminescence resonance energy transfer (CRET) between graphene nanosheets and chemiluminescent donors. In contrast to FRET, CRET occurs via nonradiative dipole-dipole transfer of energy from a chemiluminescent donor to a suitable acceptor molecule without an external excitation source. This graphene-based CRET platform was used for immunoassay of C-reactive protein (CRP) using a luminol/hydrogen peroxide chemiluminescence (CL) reaction catalyzed by horseradish peroxidase with a LOD of 1.6 ng mL^{-1}.

Graphene oxide (GO), a promising precursor for graphene, has great potential for use in biosensors due to its unique characteristics such as facile surface modification, high mechanical strength, good water dispersibility, and photoluminescence.[74,75] The GO has negatively charged functional groups such as carboxylic acids, hydroxy groups, and epoxides, which benefit to the biomolecules bound to the GO sheets. A GO-based immuno-biosensor system has been developed for the detection of rotavirus.[76] The anti-rotavirus antibodies are immobilized on the GO array, and captured the rotavirus cell by specific antigen-antibody interaction. The capture of a target cell was verified by observing the fluorescence quenching of GO by FRET between the GO and AuNPs. AuNP-linked antibodies were bridged with 100-mer single stranded DNA molecules, which provide facile control of distance between Ab and AuNPs. When the Ab-DNA-AuNP complexes were selectively bound to the target cells that were attached to the GO arrays, the fluorescence emission of GO decreased by AuNP quenching, which enabled the identification of pathogenic target cells.

3.4. Carbon nanotubes

Carbon nanotubes (CNTs) are molecular-scale tubes of graphitic carbon with outstanding properties such as high aspect ratios, high mechanical strength, high surface areas, excellent chemical and thermal stability, and rich electronic and optical properties.[77] Carbon nanotubes (CNTs) have been explored for highly sensitive biosensing assay of various types of targets such as cells, proteins, DNA, heavy metal ions, small molecules, and so on.

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Compared with biosensors using CNTs' electrochemical or electrical properties, the number of the CNTs' biosensors that exploit the optical properties of CNTs is small. However, several CNTs-based optical biosensing platforms have been developed by the use of the ability of CNTs to quench fluorescence or the near-infrared (NIR) photoluminescence exhibited by semiconducting nanotubes. Due to NIR radiation is not absorbed by biological materials, luminescence of SWNTs is particularly interesting for biosensing, especially within biological samples or organisms. With the ability of CNTs to quench fluorescence, Yang et al.[78] demonstrated a DNA detection system using the preference for single stranded oligonucleotides to wrap around SWNTs compared with the related duplexes. Without the complementary DNA (cDNA), the oligonucleotides labeled with the fluorophore 6-carboxyfluorescein wrap around the SWNTs and the fluorescence will be quenched. With the present of complementary DNA, the fluorescence labeled DNA probes has hybridization with cDNA and forms a rigid duplex, which does not wrap around the nanotubes and hence a fluorescence signal will be observed. The similar methods have also been used to detect the heavy metal ions.[79]

3.5. Magnetic nanoparticles

Magnetic nanoparticles (MNPs), consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds, are a class of nanoparticle which can be manipulated using magnetic field.[80] MNPs are usually prepared in the form of superparamagnetic magnetite (Fe_3O_4), greigite (Fe_3S_4), and various types of ferrites (MeO Fe_2O_3). MNPs provide attractive possibilities in environmental monitoring. On the one hand, MNPs bound to biorecognitive molecules (e.g. DNA or antibody) can be used to enrich the analyte to substantially improve the sensitivity of the biosensors. On the other hand, many of the MNPs are superparamagnetic, which can immediately be magnetised with an external magnetic field and resuspended immediately once the magnet is removed. It is greatly useful for the separation of targets from the complex matrix of environmental samples when developing sensitive and selectively biosensors.

Chemla et al.[81] used MNPs labelled antibodies for detecting biological targets, in which the sensitive superconducting quantum interference device was used to only detect the antigen-antibody magnetic NPs. A good relationship between the luminescence and the mouse IgG concentration was obtained in the 1-10^5 fg/cm^3 range. Moreover, using magnetic NPs substantially shortened the assay time. Tudorache et al.[82] reported a magnetic-beads-based immunoassay strategy for sensitive atrazine with a limit of detection of 3pg/L.

4. Optical biosensing platform for environmental applications

4.1. Optical waveguide based biosensors

Optical waveguide (e.g. fiber optic and planar waveguide) transmit light on the basis of the principle of total internal reflect (TIR). When the incident light is totally reflected, the evanescent wave that penetrates essentially into the surrounding cladding of lower refractive index, decays exponentially with distance[83] (Figure 3):

$$E(z) = E_0 \exp(- d / d_p) \tag{1}$$

Where $\delta$is the distance from the interface. For multimode waveguides, the penetration depth d_p, is a function of the two refractive indices, the angle of incidence of the light, and the wavelength, is given by:

$$d_p = \frac{\lambda_{ex}}{2\pi}\left[(n_2)\ \sin^2\alpha - (n_1)\right]^{2-1/2} \tag{2}$$

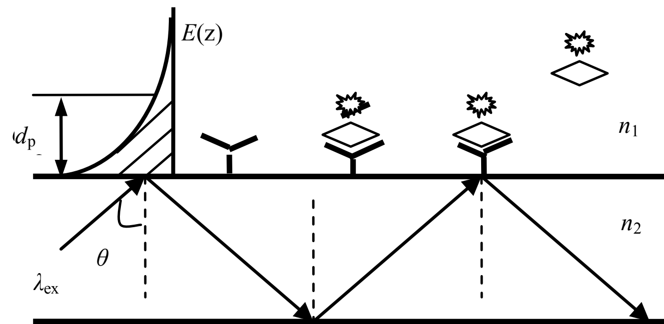

Figure 2. The principle of evanescent wave fluorescence biosensor

In evanescent wave fluorescence biosensor, the evanescent wave can excite fluorescence primarily from the fluorescently labelled analyte complexes that have been bound to the surface through affinity recognition interactions. Not only does this decreases the need for washing or separation procedures to avoid optical interference or contribution from free components, but the signal obtained is directly related to the binding kinetics of the detection interaction.[84]

4.1.1. Evanescent wave fiber-optic biosensor

Evanescent wave fiber optic biosensors, one of the most promising detection technologies to achieve the rapid, specific, sensitive, cost-effective, and real-time on-site detection of the environmental pollutants. They have been applied to detect a wide variety of analytes such as TNT, 2,4-D, atrazine, Escherichia coli O157:H7, and Staphylococcal enterotoxin B, etc.[19] Despite the technological leaps made in the past decades, Evanescent wave fiber optic immunosensor, based on the selective interaction between antigen and antibody, has few actual applications to routine analysis. The following problems should be responsible for this situation. The conventional evanescent wave fiber optic biosensor always have the large size, number of optic components, such as chopper, off-axis parabolic reflector, biconvex silica lens and so on. Such a conventional bulk optics arrangement is costly and requires crucial optical alignment. Once the direction of any element appears inaccurate, the whole system will be destroyed and be difficult to reconvert.

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Recently, we reported a portable evanescent wave all-fiber biosensor (EWAB) (Figure 3),[85] whose configuration is simple, compact and portable, has been developed.With a single–multi-fiber optic coupler, both the transmission of the excitation light and the collection and transmission of the fluorescence are achieved by fiber optic in this system, which reduces optical components required and does rarely need optical alignment. Meanwhile, the efficiency of light transmission is higher, light loss lower, and the S/N ratio improved.

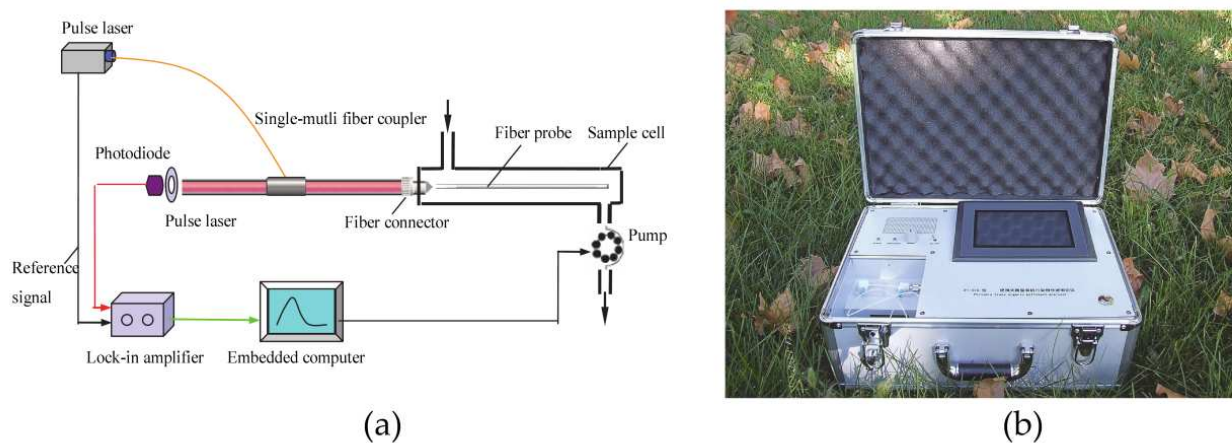

Figure 3. (a) Schematic of EWAB; (b) Photograph of EWAB.

Fast and sensitive detection of microcystin-LR (MC-LR) was conducted with this portable evanescent wave biosensor based on the principle of immunoassay and total internal reflection fluorescence. The reusable biosensing surface was produced by covalently immobilizing a MC-LR-ovalbumin (MC-LR-OVA) conjugate onto a self-assembled thiol-silane monolayer of fiber optic sensor through a heterobifunctional reagent. The MC-LR-OVA immobilized fiber optic probe is highly resistive to non-specific binding of proteins, and can be reused more than 150 times. The limit of detection (LOD) of MC-LR is 0.03 μg/L. The developed immunosensor method was applied to the monitoring of MC-LR in various types of water with the recovery ratio ranged from 80 to 110%. The sensitive and rapid detection of the herbicide 2,4-D has also been achieved with the EWAB. Under optimum conditions, calibration curve obtained for 2,4-D had detection limits of 0.07μg/L. The portable biosensor is commercially obtained from the company JQ-environ Co. Ltd. (China).

Ultrasensitive DNA detection was achieved by the EWAB based on quantum dots (QDs) and total internal reflection fluorescence (TIRF), which featured an exceptional detection limit of 3.2 amol of bound target DNA.[86] The ssDNA coated fiber probe was evaluated as a nucleic acid biosensor through a DNA-DNA hybridization assay for a 30-mer ssDNA, the segments of the *uidA* gene of *Escherichia coli*, labeled by QDs using avidin/biotin interaction. Based on our proposed theory, the quantitative measurement of binding kinetics can be achieved with high accuracy, indicating 1.38×10^{6} $M^{-1}s^{-1}$ for association rate and 4.67×10^{-3} s^{-1} for dissociation rate.

Moreover, based on a direct structure-competitive detection mode, we report a rapid and highly sensitive Hg^{2+} detection method using the EWAB.[87] In this system, a DNA probe covalently immobilized onto a fiber optic sensor contains a short common oligonucleotide sequences that can hybidize with a fluorescently labeled complementary DNA. The DNA probe also comprises a sequence of T-T mismatch pairs that binds with Hg^{2+} to form a T-Hg^{2+}-T complex by folding of the DNA segments into a hairpin structure. With a structure-competitive mode, higher concentration of Hg^{2+} lead to less fluorescence-labelled cDNA bound to the sensor surface and thus in lower fluorescence signal. The total analysis time for a single sample, including the measurement and surface regeneration, was <6 min with a detection limit of 2.1 nM.

4.1.2. Surface Plasmon Resonance (SPR) biosensors

SPR is a surface sensitive optical technique for monitoring biomolecular interactions exploiting special electromagnetic waves due to fluctuations in the electron density at the boundary of two materials.[88] SPR has given it a great potential for the real-time and label-free study of the binding interactions between a biorecognition molecules immobilized on sensor surface with its special receptors (analyte). The SPR biosensors have been used to investigate protein binding, association/ dissociation kinetics, and affinity constants, and have wide applications such as clinical diagnosis, drug discovery, food analysis, environmental monitoring. [89]

The principle of SPR biosensor was shown in Figure 4. Using a Kretschmann configuration, SPR detects a small refractive index change at the metal/analyte interface, and the information of the molecular interactions can be obtained by measuring the optical intensity (or phase/polarization) of light reflected from the optical instrument.[88,89] In SPR sensors, changes in the plasmonic resonance signals at a thin metal film are strongly dependent on the refractive index (RI) of the medium. SPR biosensors containing a biorecognition molecule layer can detect minute changes in RI on binding of the special receptors. The sensitivity of the SPR biosensor is limited by the magnitude of the refractive index change at the metal surface, and the minimum SPR shift is detectable by the instrument as a result of recognition events occurring between a surface-bound receptor and analyte of interest.

Recently, there is a growing interest to use indirect competitive SPR immunoassays for detection of environmental contaminants including atrazine, dichloro diphenyl trichloroethane (DDT), 2,4-D, Benzo(a)pyrene (BaP), biphenyl derivatives, carbaryl, 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD), TNT, and so on.[89] BaP, a potential marker of environmental pollution, is a carcinogenic endocrine disrupting chemical and its content well correlates with the total amount of polycyclic aromatic hydrocarbons (PAHs) in the environment. An SPR immunosensor for BaP was reported using the indirect competitive immunoreaction principle with a detection limit of 10 ppt.[90] Svitel et al.[91] showed the sensitive detection of 2,4-D by exploring the binding interaction of dextran matrix with D-glucose and concanavalin A. Shimomura et al.[92] developed an immunosensor for the detection of TCDD, polychlorobiphenyl (PCB) and atrazine, and found a higher sensitivity with the indirect competitive assay than the direct assay. A possibility of ultra-highly sensitive detection of

TNT has been shown by an indirect competitive SPR immunoassay using commercial and home-made antibodies. Mauriz et al.[93] showed the detection of carbaryl, DDT and chlorpyrifos using a portable SPR instrument, where the immunosensor fabricated by a self-assembly method is highly stable and regenerable for more than 250 cycles. Despite the progress has been made, the complex matrix of environmental water samples will still be a great challenge for the practical applications of SPR biosensor.

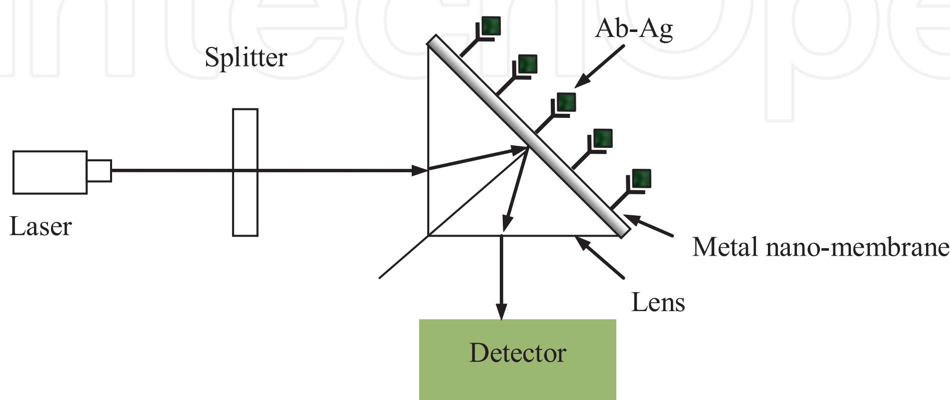

Figure 4. The schematic of SPR biosensor

4.2. Optical biosensor arrays

Analytical microarrays have emerged as powerful tools for high-throughput and rapid analysis of multiple analytes.[94] Antibody and hapten arrays are specific quantitative analytical techniques using antibodies/antigens as highly specific biological recognition elements. They possess the capability to simultaneously detect numerous analytes in low sample volumes. Because antibodies have been generated which specifically bind to individual compounds or groups of structurally related compounds with a wide range of affinities, immunosensors are inherently more versatile than enzyme-based biosensors. Recent advances reported for immunosensor arrays for environmental applications have primarily been focused on using analyte derivatives as immobilized recognition molecules. For example, Jin et al.[95] have developed a fluorescent immunosensor system for the detection of bioterrorism agents with high sensitivity, specificity, and reproducibility.

We developed a proof-of-concept development of a novel optic fiber-based immunoarray biosensor for the detection of multiple small analytes.[96] This was developed through immobilization of two kinds of hapten conjugates, MC-LR-OVA and NB-OVA, onto the same fiber optic probe. The technique is significantly different from conventional immunoarray sensors. Microcystin-LR and trinitrotoluene (TNT) could be detected simultaneously and specifically within an analysis time of about 10 min for each assay cycle. The limits of detection for MC-LR and TNT were 0.04 μg/L and 0.09mg/L, respectively. Good regeneration performance, binding properties, and robustness of the sensor surface of the proposed immunoarray biosensor ensure the cost-effective and accurate measurement of small analy-

tes. This compact and portable quantitative immunoarray provides an excellent multiple-assay platform for clinical and environmental samples.

There are, however, several limitations in the use of immunosensors for environmental monitoring applications. For example, the complexity of assay formats; and the number of specialized reagents (e.g., antibodies, antigens, tracers, etc.) that must be developed and characterized for each compound; and the limited number of compounds typically determined in an individual assay as compared to the multiple compounds that contaminate environmental samples.[4-8]

4.3. Emerging optical biosensors

Label-free optical biosensing is a rapidly emerging research area with potential applications ranging from medical and clinical diagnostics to food safety and environmental detection, especially for portable, easy-to-use devices.[88,89] Without the use of radioactive or fluorescent labels, the complexity in the detection and screening process significantly reduces and the intrinsic properties of the target molecules have been few influenced.[88,89] To dated, the most well established technique for label-free optical biosensing is surface plasmon resonance (SPR) based biosensor. Recently, several novel optical biosenors such as optical ring resonator based biosensor, photonic crystal biosensors, and optical nano-biosensors, have been developed in very small dimensions and allowed to fabricate with standard CMOS techniques. Although they have little applications in the monitoring of environmental pollutants, these biosensors have great potential posibilities for on-site real-time detection of micro-environment.

4.3.1. Optical ring resonator based biosensors

Optical ring resonator is an emerging sensing technology, in which at least one is a closed loop coupled some sort of light input and output (see Figure 5).[97] In a ring resonator, the light propagates in the form of whispering gallery modes (WGMs) or circulating waveguide modes. When light of the resonant wavelength transports through the loop from input waveguide, it builds up in intensity over multiple round-trips due to constructive interference and is output to the output bus waveguide which serves as a detector waveguide. [97]

The WGM spectral position is related to the refractive index (RI) through the resonant condition:$\theta = 2\pi r n_{eff} / m$, where r is the ring outer radius, n_{eff} the effective RI experienced by the WGM, and m is an integer. n_{eff} changes when the RI near the ring resonator surface is modified due to the capture of target molecules on the surface, which in turn leads to a shift in the WGM spectral position.[98] Thus, by directly or indirectly detecting the WGM spectral shift, the quantitative detection of targets will be achieved.

4.3.2. Photonic crystal biosensors

Photonic crystal fibres have wavelength-scale morphological microstructures that run along the entire fiber length by corralling it within a periodic array of microscopic air holes.[99] Overcoming the limitations of conventional fiber optics, photonic crystal fibers are proving

to have a multitude of important technological and scientific applications including biosensors. Due to their well-defined physical properties such as reflectance/ transmittance, photonic crystal biosensors are enabled superior levels of sensitivity resulting in precise detection limits. Photonic crystal biosensors are very small and are possible through coupling the incident and reflected/transmitted light to optical fibers and analyzing them in remote locations.

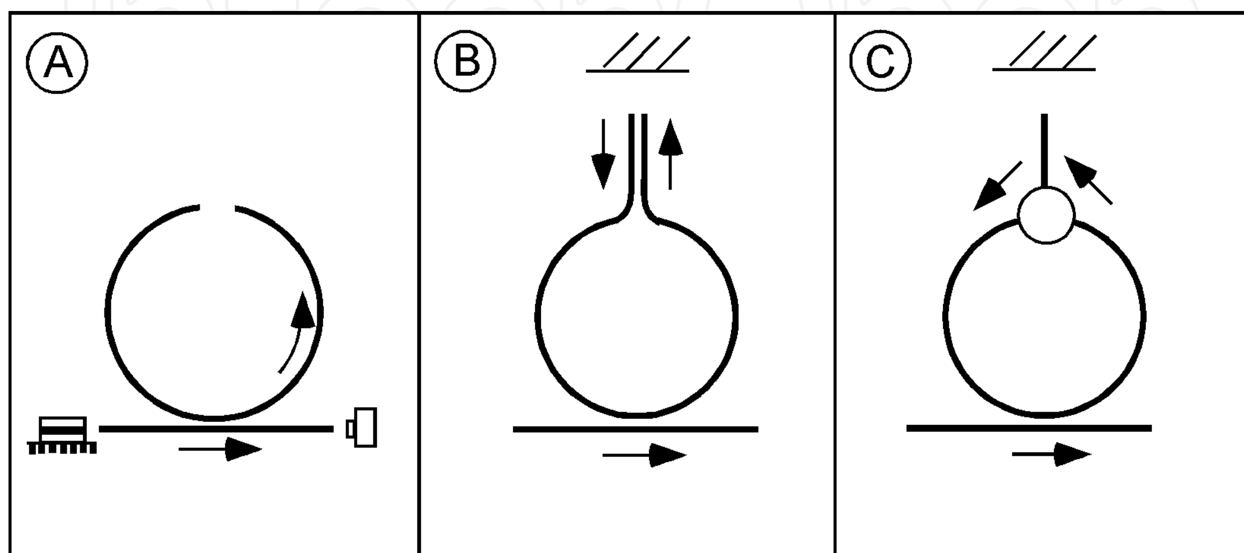

Figure 5. Various ring resonator biosensors

To design a photonic crystal biosensor, some portion of the resonant electric field must be in contact with liquid media that contains the analyte, providing a surface on which biorecognition molecules may be adsorbed. Label-free photonic crystal biosensors generally detect shifts in resonant wavelength or coupling angle caused by the interaction between the target molecule and the evanescent wave.[99] The narrow spectral linewidth achieved by using high Q factor passive optical resonators enables sensor systems to resolve smaller wavelength shifts associated with the detection of analytes at low concentration, such as environmental pollutants. Photonic crystals biosensors have been applied for sensing the pH and ionic strength of solutions, metal ions and trace orgnic pollutants. [100]

4.3.3. Optical nano-biosensors

Recent developments have greatly improved the sensitivity of optical sensors based on nano-structures and nanoparticles.[101] Optical biosensors have been used to provide a reliable method of monitoring various chemicals in microscopic environments and to detect different entities within single cells.

Vo-Dinh et al.[102] have designed fiber-optic nanosensors for environmental and biochemical monitoring. The nanosensors were fabricated with tapered optical fibers with distal ends with a 20~500m diameter. Biorecognition molecules, such as antibody, peptides, and nucleic acids, are immobilized on the fiber tips and designed to be selective to bind target molecules

(analyte) of interest. This fiber-optic nanosensor has become a powerful tool for measurements in submicron environments and for probing individual chemical species in specific locations throughout a living cell due to their small nanoscale sizes. In their previous work,[103] the nanosensors have been developed for in situ measurements of the carcinogen BaP inside single cells using the antibody probe. In another study,[102] nanosensors have been used for the measurement of intracellular concentrations of benzopyrene tetrol (BPT) in the cytoplasm of human mammary carcinoma cells and rat liver epithelial cells. They performed calibration measurements of solutions containing different BPT concentrations ranging from 1.56×10^{-10} to 1.56×10^{-8} M. Fiber-optic nanosensors for monitoring single cells have opened up new applications due to their small sizes, which provide important tools for minimal invasive analysis at single cellular or subcellular level.

5. Optical biosensors for pollution control and early-warning

The new technologies for environment pollutants, which are rapid, specific, sensitive, cost-effective, and suitable for real-time on-site detection, have a strong demand due to a large number of pollutants and their derivates present in surface and ground waters and stricter regulations for the detection of these pollutants set out by the legislative bodies.[104] Existing analysis methods, such as HPLC or GC/MS, are very sensitive at detecting these toxic targets, however, the analytical procedure are rather complicated and therefore labour-intensive and time-consuming. Moreover, contaminant concentrations in water courses are dynamic, changing both as a result of inputs and changes in water flow. With monthly sampling and analysis, it is extremely unlikely that the maximum concentration for a period of time can be detected. The need for cheap and general network system (multiple autonomous analytical stations that extensively control the sites of interest in rivers and lakes) for pollution control and early warning has generated great interest.

The EWS is an integrated system for monitoring, analyzing, interpreting, and communicating monitoring data, which identify low probability/high-impact contamination events in sufficient time to be able to safeguard the public health. The ideal integrated EWS should demonstrate a number of characteristics as following: [104]

- provide a rapid response and warning in sufficient time for action
- covers all potential threats
- exhibit a significant degree of automation, including automatic sampling
- allows acquisition, maintenance, and upgrades at an affordable cost
- require low skill and training
- identify the source of the contaminant
- demonstrate sufficient sensitivity
- give minimal false-positives/false-negatives

- exhibit robustness and ruggedness in long-term monitoring
- reproducible and verifiable
- allow remote operation

Single device alone may not satisfy all of these requirements. Therefore, an early warning system network (EWSN) including various detection technologies will be useful for homogeneous environments such as for rivers and coastal areas. A new generation of monitoring tools based on sensor technology has emerged in the last decades. Optical biosensors have proven advantages over other types of sensors for multitarget sensing and continuous real-time on-site monitoring. Optical biosensors have been integrated into many early warning systems (EWS) that can provide easy, rapid and on-site measurements. These EWS are useful for mapping of contamination such as after accidental spills or pollution events.

A number of early warning systems have been developed. For example, J-Mar Biosentry™ can perform low density microbial suspension detection in drinking water using eight on-line sensors and instruments.[105] This system was able to indicate significant visual responses to the introduction of *E. coli* and *B. globigii* down to concentrations of 600 cfu/mL. The YSI Sonde™ system can simultaneously achieve measurement of conductivity, salinity, temp, depth, pH, dissolved oxygen, turbidity, chlorophyll and blue-green algae, which is ideal as early warning of algae blooms with good sensitivity at natural levels. [106]

TOXcontrol™ uses freshly cultivated light emitting bacteria (*Vibrio fischeri*) as a biological sensor,[107] which combines the advantages of whole organism toxicity testing and instrumental precision. The luminescence is measured before and after exposition to calculate the inhibition in percent. The more toxic the sample, the greater the percent light loss from the test suspension of luminescent bacteria.

The DaphTox II[108] is a new sensitive system to detect hazardous compounds in water from rivers (source-water protection) based on the Extended Dynamic Daphnia Test. Sample water (0.5-2 L/h) continuously runs through the measuring chamber containing the daphnia. The live images obtained using a CCD-camera are evaluated online with an integrated PC to analyse changes in the behaviour of the daphnia. If the change is statistically significant, an alarm is triggered. The method of image analysis enables a series of measurement methods and plausibility tests to assess the daphnia's behaviour using different criteria.

Supported by the Water Framework Directive (WFD), the automated water analyser computer-supported system (AWACSS)[109], based on an optical immunosensor, was the establishment of an early-warning system by means of a network of measurement and control stations. The AWACSS system included four major components: the AWACSS instrument with fluidics control and optical transducer chip, the HTC PAL autosampler for sample preparation, the personal computer at the sampling site and the server with database and web site. The sampling site software allows for bi-directional autosampler control. Using fluorescence-based immunoassay technology, this system can measure several organic pollutants at low ng/L in a single few-minutes analysis without any prior sample

neither pre-concentration nor pre-treatment steps. A web-based AWACSS system allows for the internet-based networking between the measurement and control stations, global management, trend analysis, and early-warning applications.

6. Key trends and perspectives

There is no doubt that the progress of biosensor technology in recent years makes an important contribution to protect human health and local ecosystems.[2-5] However, biosensors are not as successful as was expected initially, and there is a challenge to creating improved, cost-effective, and more reliable instruments. There are many reasons for this, and a few of them are mentioned here. First, most of the biosensor systems commercially available today are either prohibitively costly or highly inflexible. Second, the content of the environmental samples is complex and vary, which is not like that of clinical samples. It is essential to reduce the effect of matrix of environmental samples on the bioassay. Third, the storage of bio-reagents is one of the key issues to be resolved in long-term monitoring. Finally, the stability and reliability of biosensors should be improved to satisfy the practical applications.

Optical biosensors have proven advantages over other types of sensors for multi-target sensing and continuous monitoring.[4,6-8] Development of new functional materials allows the optical biosensor to have more practical applications. The unique properties of nano-materials offer excellent prospects for interfacing biological recognition events with optical transductor and for designing next-generation of biosensors exhibiting novel functions. Recent technological developments in miniaturizing optical biorecognition elements and wireless-communication technology have led to the emergence of environmental sensor networks, which will greatly enhance on-site long-term monitoring ability of the natural environment and provide more effective way to deal with the pollution incidents with less effort and cost. The trend toward multianalyte sensing and toward biosensor arrays allow optical biosensors become more compact, robust, smaller and adaptable for rapid toxicity screening, multianalyte testing, and continuous on-site monitoring of environmental pollutants. Moreover, biosensor will offer strong potential for researchers to more effectively investigate and understand diverse environmental phenomena, including the fate and transport of contaminants, which provide novel insights into the mechanisms of remediation. The number of opportunities to incorporate new science and technology into optical biosensor systems is almost overwhelming. In the near future, we believe that optical biosensor will provide the most productive paths to solve real problems in everyday life.

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