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# Dynamic Luminescent Biosensors Based on Peptides for Oxygen Determination

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and Flavio Colmati*

## Abstract

This chapter summarizes the fundamentals of biosensing techniques based on fluorescence spectroscopy and the protagonism of state-of-the-art luminescent biosensors in a wide range of scientific areas, from environmental monitoring to diagnostics and disease treatment, focusing on the paramount contribution of biosensors based on the Förster Resonance Energy Transfer (FRET) transducing mechanism. State-of-the-art FRET biosensors are specially characterized by outstanding sensitivity toward a number of environmental pollutants and dissolved oxygen in aquatic ecosystems, capable of detecting concentrations in the nano and picomolar scales. These biosensors have also been showing impressive performance over other methods in the study of real-time biological processes *in vivo* relevant to help understanding disease progression like cancer.

**Keywords:** fluorescent biosensors, ROS detection, sensitivity, FRET biosensors, new materials

## 1. Introduction

Fluorescence spectroscopy has been revolutionizing the field of life sciences and clinical routines such as diagnostics and biosensing, due to its impressive sensitivity and the biocompatibility of many fluorescent organic compounds, which allows one to probe biological processes *in vivo* in noninvasive bioimaging procedures. The improvement of instrumentation has granted optical-based sensing routines a new level of sensitivity, accuracy, and reliability. Very subtle changes in fluorescence intensity, or even extremely low levels of light that might result from an interaction between the fluorescent probe or sensor with the environment under study, are easily detectable nowadays thanks to modern instrumentation such as photomultiplier tubes, in which electronic impulses are created by just a single photon. In the time-resolved fluorescence approach, in which the fluorescence lifetime of the fluorophore is monitored, with current femtosecond pulsed lasers, even more sensitive and reliable measurements are possible, since the intensity decay is not affected by a number of possible undesired factors that interferes in the steady-state intensity. These advantages make the fluorescence spectroscopy a powerful technique, paving the way to the most wished rapid and low-cost sensing in a wide range of biological and environmental applications and point-of-care diagnostics for real-time

monitoring of physiological conditions. Sensing methods of remarkable sensitivity, reliability, and selectivity based on fluorescence spectroscopy dominates the field of sensing and biosensing. DNA sequencing and fragment analysis, fluorescence staining for bioimaging and fluorescence immunoassays are all based on fluorescence techniques.

The countless possibilities of combinations of biorecognition element, support matrix, and the transducing method in biosensors constituted of nanomaterials make it possible to design versatile and selective biosensors. In this review, particular attention is centered on luminescent biosensors based on the Förster resonance energy transfer, or FRET, biosensing transducing method, which encompasses a huge variety of biosensors, due to their unique sensitivity, selectivity, and fast response. For this reason, FRET-based sensors have enabled, for example, intracellular monitoring of ROS kinetics and oxygen sensing, which is vital for elucidating how tumor cells respond to treatment, in order to develop better therapeutic strategies. Before FRET sensors were introduced, these practices were hampered by difficulties and unreliability in real-time monitoring intracellular ROS. In fluorescence bioimaging, thanks to its high spatial and temporal resolution, it is becoming possible to probe in real time biological elements and processes, such as enzymatic reactions, protein/protein, protein/nucleic acid, protein/substrate, and biomembrane interactions.

In the context of environmental monitoring, with the possibility of miniaturization of biosensors based on nanomaterials, it is becoming possible to perform fast and accurate field analysis and real-time surveillance of analytes relevant in the assessment of water quality. A variety of FRET-based biosensors for water pollutants, such as heavy metal ions, pesticides, antibiotics, and halogenated compounds, is reported, some of them capable of detecting concentrations in the pico and nanomolar scales. Such sensitivity levels are far from reach with conventional analytical methods.

## **2. Principles and evolution of biosensing techniques**

Biosensors are devices that can perform the measurement of a physiological activity in living organisms or that are constructed upon biological components. They determine chemical or biological analytes in systems where the minimum human intervention is present. They generate optical and/or electrochemical signals that are transduced by a variety of transducers, and depending on their operation, biosensors are classified.

To fulfill an application, biosensors can be constructed by using a wide variety of bioreceptors, that can deliver distinct types of signals and the choice of transducers and interfaces will respond for their selectivity and sensibility, as well as their configuration versatility and possibility of miniaturization. Techniques such as voltammetry, amperometry, potentiometry, among others, are exploited to transduce electrochemical signals, whereas fluorescence, light absorption or light reflectance in the ultraviolet (UV), visible, or near-infrared (NIR) spectral regions can have their intensity or lifetime changes determined to efficiently detect optical responses in optical biosensors. Also, there is a variety of biosensors that can explore dual or multiple transducers to deliver electrical and luminous signals that can be interpreted together or separately, giving rise for more versatile and usable biosensors.

Independent of their usage and characteristics, the basic configuration of the biosensors is similar. They must be composed of a sample holder, which is adapted to the sample physical characteristics; a biological recognition element, which must

be highly selective; and a physical transducer to generate a measurable signal, a signal processor, and an interface that is able to communicate the data to the operator. The types of biosensor that can be constructed are based on the recognition elements employed, which can be any biological system, from antibodies to microbes and cells. It is selected considering the information to be obtained and the physical characteristics of the biosensor, which in its turn, determines the durability and the more applicable processes to construct the biosensor.

The specificity and the sensitivity of biosensors have been the concern of several scientists due to the variety of biochemical processes that need to be evaluated and followed, the increase of accuracy and reproducibility of measurements that is fundamental for the spread of usage of such devices, and due to the need for miniaturizing and automatizing devices, in order to turn them more applicable to distinct regions of a living organism.

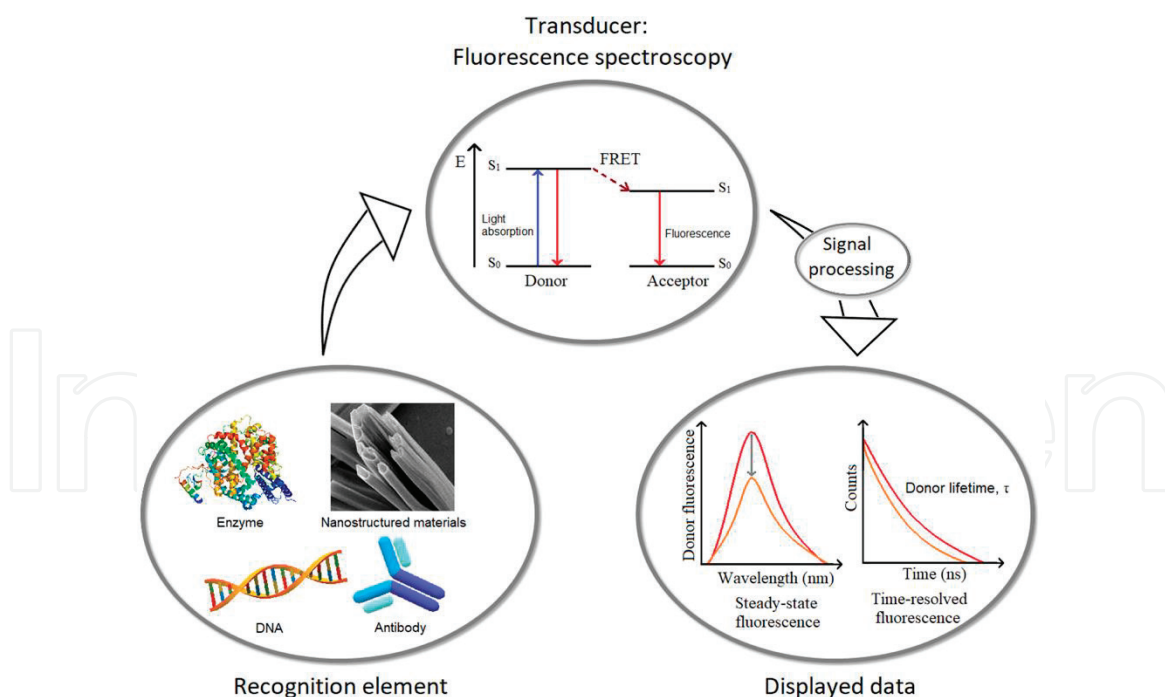
In special, optical biosensors can be widely applied if they accompanied the development of the spectroscopic and microscopic technique development to improve their transducing methods, signal processing, and interface. If these components are well developed, optical biosensors and, in special, fluorescent biosensors can present high sensitivity, perform real-time measurements with high frequency of detection, which enable them to find application in diagnostics and therapeutics, with the right transducers, it is possible to image disease progression and to monitor the organism response to therapeutics, and also, they can be thought for drug discovery programs development, as well as for clinical evaluation of new drugs [1]. In recent years, recognition elements based on graphene had been widely used due to their excellent electrical and optical properties. In biosensing, materials based on graphene had promoted efficient detection of biomarkers and have proportioned an important technological advance, due to the perspective of developing new and interesting materials, such as graphene-like 2D materials and the impressive single-atom-thick layers of van der Waals materials [2].

Most of the fluorescent biosensors are small molecules that are arranged on a receptor that identifies a specific target, and its fluorescent response is readily transduced. In this case, the signal recognition is based on the distinct fluorescence emitted by the biosensor in the presence of the analyte, which can be metabolites, proteins, ions, or antibodies. These biosensors are based on the steady-state fluorescence that the device can produce. They find effective application for early biomarker detection, for instance, in clinical diagnostics and ordinary biochemical processes, as well as for tissue imaging and, as an extrapolation, in image guided surgery.

Biosensors based on time-resolved fluorescence are promising because they are able to promote improvements on selectivity, specificity, and sensibility, becoming ultrasensitives, capable of determining minimal local variations of the analyte concentration, and can be combined to several other analytical techniques. In this context, there are the nanopores, which are highly sensitive biosensors, able to detect the analyte at the range of nanomolar of concentration, due to their characteristic structure of a nanometer scale pore that is similar to the size of the target molecule, enabling a single-molecule analysis [3].

Any of the fluorescence properties of the recognition element, namely, the intensity, wavelength, anisotropy or lifetime, can be exploited in optical sensing. One straightforward mechanism to consider is collisional quenching, in which a fluorophore has its fluorescence quenched upon collision with the analyte molecule. Nevertheless, the most encountered and relevant mechanism in sensing is the Förster Resonance Energy Transfer (FRET), which occurs via long range dipole-dipole interaction when an energy donor, in an excited state, and an acceptor are brought into close proximity, but not necessarily into contact. In FRET, the



**Figure 1.**

*Illustration of the components and function of biosensors based on fluorescence spectroscopy transducing.*

fluorescence intensity and decay time of the donor are decreased, as depicted in the Jablonski diagram and spectrum models in **Figure 1**. The energy transfer efficiency in FRET depends on the distance between donor and acceptor: when the donor-acceptor pair is in between 20 and 60 angstroms apart, which is called the Förster radius, the efficiency of energy transfer is around 50%. The efficiency also depends on the spectral overlap between the absorption spectrum of the acceptor and the emission spectrum of the donor: the greater the overlap, the more efficient the process.

Since FRET does not require contact between the electronic clouds of donor and acceptor, it can occur over macromolecular distances. This is one of the reasons, along with energy transfer efficiency, responsible for the high sensitivity of FRET-based sensors. Low concentrations of analyte that would result in great distances from the donor-acceptor pair would most likely involve FRET rather than collisional quenching, which requires physical contact, in order to bring about a change in the FRET process necessary for sensing. Besides, since the donor and acceptor in a FRET sensor do not need to be bound molecules, it simplifies the design of the fluorophore because the donor is not required to be intrinsically sensitive to the analyte and can be chosen according to the desired light source [4].

A great variety of FRET sensors and biosensors can be found in the literature. The major advantages that make them special are high sensitivity and fast response. Besides, due to the biocompatibility of FRET-based biosensors, allied to the inherent sensitivity of optical sensing techniques, they are becoming ubiquitous in clinical applications and in the field of biomedical research. A recent example is a fluorescent peptide/GO sensor containing a fluorophore-labeled peptide sequence that proved versatile for measuring the activity of different protein kinases. Kinases are group of proteins that regulates intracellular phosphorylation pathways. Several diseases such as cancer, diabetes, Alzheimer's, etc. are related to anomalous activity of kinase proteins. In their sensor, the fluorophore-labeled peptide sequence is cleaved by the carboxypeptidase, in the absence of phosphorylation, and is separated from the GO, resulting in recovery of fluorescence [5].

Quantum dots (QDs) comprise another class of intensively researched materials for biosensing application due to their advantages such as high photostability and large extinction coefficient. Additionally, their broad absorption spectra and the possibility of tuning emission wavelength make them suitable as sensors based on FRET energy transfer, in which the spectral overlap is important for efficiency of the process, and, ultimately, the sensitivity of the biosensor. Their broad absorption spectrum also enables selective excitation of QD donors without exciting the acceptors and allows excitation of different donors at once, making them useful for multiplexing applications. Their high luminescence and nanoscale dimensions also make them ideal for bioimaging applications as fluorescent probes [6].

Despite their versatility and large scope of possibilities for biosensing, one major disadvantage of QDs fabricated with inorganic materials is their toxicity, which limits their clinical applications. Graphene and other carbon-based QDs, unlike the conventional heavy-metals DQs, are biocompatible, environment friendly, and easier to prepare [7, 8], and for that reason, they have gained considerable attention. Among the most recent examples of CQD-based is a fluorescent CQD biosensor for hydrogen peroxide detection and simultaneous monitoring of the acetylcholinesterase reaction system [7]. A great number of QD-based biosensors for environmental monitoring are found in the literature. A few recent contributions are described in Section 4.

Similarly to graphene QDs, the high efficiency of energy transfer from dyes to graphene oxide, GO, along with GO's intrinsic properties, opened up a new avenue for designing a lot of FRET-based biosensors. Thanks to pi stacking and hydrogen bonding interactions, GO is capable of strong binding with biomolecules, such as fluorescent dyes, which are quenched by GO via the FRET process. In the past few years, a number of GO-based biosensors using DNA as a probe are reported [9, 10].

As a special case of FRET-based biosensors, there is the bioluminescence resonance energy transfer (BRET) principle, which has been used to produce new and ultrasensitive biosensors. In these biosensors, a bioluminescent enzyme is the energy donor and a specific fluorescent molecule, chosen by spectral overlapping, acts as an acceptor. This process is extensively used to monitor and study molecular interactions between proteins and other metabolites, in vitro and in living cells [11].

### **3. ROS and oxygen sensing**

One very important class of compounds that play a major role in regulating biological processes, which also have a close relationship with the differentiated metabolism profile of tumor cells, is the reactive oxygen species, ROS, and for that reason, they receive significant attention in sensing/biosensing research. ROS are very reactive free radicals that act as electron acceptors, thus being strong oxidizing agents, which react with any neighboring molecule in order to attain a stable configuration. Hydrogen peroxide, the superoxide anion, and the singlet excited states of oxygen are examples of ROS. These molecules are produced physiologically mainly as a by-product of oxygen metabolism during electron transfer events in respiratory chain processes. Since they are highly reactive, ROS are harmful for the cells, and antioxidant enzymes located in the cytosol and mitochondria are responsible for a delicate regulation process that control the oxidative stress generated by ROS. Despite their toxic effects, moderate levels of ROS play a role in vital biological processes, such as biological signaling, chemical defense, biosynthetic reactions, etc. [12]. For instance, in biological signaling, the ROS act as secondary messengers in cellular adhesion, spreading, and migration, thus governing the proliferation and, ultimately, the survival of cells [13].

Multicellular organisms also maintain a homeostasis of  $O_2$  levels by regulating the distribution of  $O_2$  according to the demands of each organ. In this context, the cells also respond to hypoxia condition, a state of insufficient levels of  $O_2$  necessary for maintaining nominal cellular function, in an effort to adapt to such condition and ensure its survival. This condition is always present in tumor cells, and understanding the cell mechanisms of coping with hypoxia is crucial for understanding tumor growth and survival. For that to work, the cells have a sensing mechanism for  $O_2$ . The hypoxia-inducible factors HIF-1 and HIF-2 are oxygen sensitive transcriptional complexes that mediate the response of cells to  $O_2$  levels [14].

It has been reported that the level of ROS in the cells is related to the activity of HIF- $\alpha$  factor. However, elucidation of cell metabolism related to or governed by ROS, as well as the cellular oxygen sensing mechanisms, has been hampered by the difficulties in tracking intracellular ROS kinetics with reliability using fluorescent dyes. FRET-based sensors have demonstrated to overcome these obstacles [14–16]. Guzy et al. suggested that mitochondria functions as an  $O_2$  sensor and signal hypoxia-induced HIF stabilization by releasing ROS to the cytosol. To confirm this debated hypothesis, they fabricated a reliable and accurate redox-sensitive FRET protein sensor for intracellular ROS determination that consists of a cyan and yellow fluorescent protein as the donor-acceptor pair linked by a redox-regulated HPS33 protein domain. Oxidation of the protein domain by ROS causes a conformational change, which alters the donor-acceptor distance required for FRET efficiency, thus leading to fluorescence recovery [14]. More recently, Bernardini et al. also used this same sensor configuration for monitoring the kinetics of ROS in cultured cells of mouse carotid body, seeking to elucidate the role of a NADPH oxidase subunit on cell response to  $O_2$  levels [15].

Overall, cyan and yellow fluorescent protein FRET sensors containing an internal metabolite-binding protein are successfully used for monitoring biomolecules in real time, metabolite dynamics, protein interaction, and signal transduction, due to the fast response ability of these sensors. Just like in aptamer sensors, the choice of the right metabolite-binding protein is the key for obtaining a highly selective FRET sensor for the desired target [16].

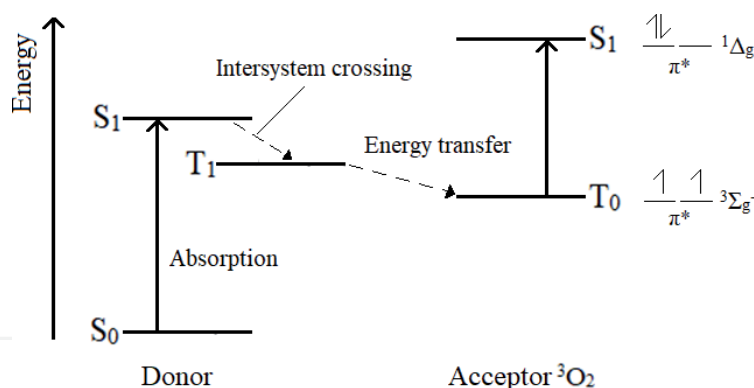
Apart from reactive oxygen species, the  $O_2$  itself is also an important indicator of biological processes, including the monitoring of disease progression. A variety of optical biosensors for oxygen determination are found in the literature, given the importance of this molecule in the study of biological systems.

Recently, fluorescence biosensors based on QDs are proving their potential for monitoring tumor activity, thanks to advantages such as the ability to penetrate solid tumor cells, high photoluminescence quantum yields, photostability, and the other photophysical properties already mentioned. Oxygen-sensitive phosphorescent molecules are particularly interesting for detecting oxygen in biological systems, because it is noninvasive and has high spatiotemporal resolution [17].

The underlying energy transfer mechanism involved in oxygen sensing is mostly a triplet-triplet annihilation process. After absorption of light, the sensing molecule is excited to a singlet excited state,  $S_1$ . Subsequently, it can return to the ground state,  $S_0$ , through fluorescence or undergo intersystem crossing to a triplet excited state,  $T_1$ , and then return to the  $S_0$  state by phosphorescence. Alternatively, in a competing process, the molecule in the  $T_1$  state can interact with molecular oxygen in the ground triplet state via collisional quenching. When this happens, a triplet-triplet annihilation process occurs, which is characterized by generation of excited-state singlet oxygen, as illustrated in the Jablonsky diagram in **Figure 2**. This process involving the  $O_2$  is also called a photosensitization effect.

Consequently, a quenching of fluorescence or phosphorescence (depending on what radiative process is being monitored) is observed. The lifetime is





**Figure 2.** Jablonsky energy diagram illustrating a T-T annihilation process involving a photosensitizer molecule and molecular oxygen.

also decreased. The oxygen concentration can be determined in a steady-state or time-resolved manner, in which the intensity or lifetime decrease of the quenched molecule is related to the concentration of oxygen by the Stern-Volmer kinetics of collisional quenching, as shown below.

$$\frac{I_0}{I} \text{ or } \frac{\tau_0}{\tau} = 1 + kq \tau_0 [Q] \quad (1)$$

In this expression,  $I_0$  and  $I$  are the fluorescence intensities in the absence and presence of  $O_2$ , respectively,  $\tau_0$  is the natural fluorescence lifetime of the molecule in the absence of  $O_2$ ,  $\tau$  is the lifetime at a given oxygen concentration,  $[Q]$ , and  $kq$  is the bimolecular quenching rate constant [4].

However, intersystem crossing to  $T_1$  state is generally a slow process, and in order to achieve an oxygen sensor of high sensitivity, the population of the triplet state must be maximized. The addition of heavy metal atoms can circumvent this limitation by increasing spin-orbit coupling, which favors the forbidden transition between electronic states of different spin multiplicities. Still, once in the  $T_1$  state, the phosphorescence competes with energy transfer to  $O_2$ , which further limits the sensitivity of the sensor. The coupling of an oxygen-responsive phosphorescent molecule with a QD results in a sensor with enhanced sensitivity toward oxygen. FRET readily occurs between the molecule and QD, which form the donor-acceptor pair, and emission becomes oxygen-dependent due to the analyte interference in the FRET process.

In addition to FRET, QD sensors can also function through Dexter exchange interactions, another mechanism of nonradiative energy transfer between an acceptor-donor pair, which involves the donation of an electron from the LUMO orbital of the donor followed by the transfer of an electron from the acceptor HOMO orbital to the HOMO of the donor. Unlike the FRET, Dexter interactions require physical contact between donor and acceptor. Both mechanisms can compete depending on the degree of spectral overlap. It has been found that the size of QD determines the predominant energy transfer mechanism in QD-based sensors: smaller QD, which exhibit low spectral overlap and high orbital overlap with the donor, favors Dexter energy transfer, whereas in larger QDs, FRET dominates [17].

Belonging to the realm of QD-based sensors for oxygen, self-assembled sensors have interesting advantages including ease of preparation and allow easy fine-tuning of donor-acceptor ratio. In general, these sensors are comprised of a metal ion bound to functional groups such as imidazole, amines, etc. and an acceptor molecule attached to the functional group. For instance, Lemon et al. managed to red shift the emission of a CdSe QD sensor reported previously in order to increase



its sensitivity at physiological  $O_2$  pressure by pairing Pd(II) porphyrins, which emits at 690 nm, with the CdSe core-shell QDs that emit at 519 nm. The QD was chosen to maximize spectral overlap with Pd(II) porphyrin absorption, thereby increasing FRET efficiency to 94%, greatly improving sensitivity [18]. Later on, other authors have used Au(III) corroles to shift the emission even further to the NIR [19]. Red emitting sensors are interesting for biomedical applications due to the greater penetration of red light into organic tissues and less scattering. These contributions reveal the versatility of QD-based sensors, which can be easily designed and adjusted to fulfill the desired purpose.

Fluorescence microscopy is another common technique in biological and clinical fields for visualizing intracellular structures, both *in vitro* and *in vivo*, which is based on the staining of a cell with a fluorescent probe. It is also possible to determine intracellular concentration of analytes of interest and monitor reactions. However, one major problem of microscopy based on steady-state intensity is the intensity dependence on the probe concentration. The difficulty in knowing the probe concentration within the different regions of the cell impedes quantitative measurements with reliability. Fluorescence lifetime imaging, on the other hand, circumvents this problem because the lifetime of the fluorophore probe is independent on its concentration. Therefore, variations in lifetime due to interactions of the probe with biomolecules can be correlated to analyte concentration regardless of the probe concentration. For this reason, high fidelity images with improved contrasts can be achieved. Lifetime imaging is employed, for example, in intracellular oxygen sensing, which is not possible via any microscopic method based on intensity measurements [4].

#### **4. FRET applied to environmental biosensing**

Due to the outstanding selectivity and sensitivity of optical biosensors, especially those based on FRET transducing, they meet the requirement of trace and even ultratrace detection of a great variety of environment pollutants, such as pesticides, antibiotics, halogenated contaminants [20], etc., which represent a major concern of the modern era due to the threat they pose to ecosystems and human health. In addition to this, the portability offered by the possibility of miniaturization of biosensor platforms enables the fast and low-cost field analysis, which is not possible through expensive conventional analytical methods like chromatography, mass spectrometry, and others.

Aptamer FRET sensors, or aptasensors, for instance, comprise a class of versatile and very sensitive biosensors, capable of detecting concentrations in nano and picoscales. Ultrasensitive FRET aptasensors for trace detection of metal ions [21, 22] and antibiotics [23] are reported. In a multiplexed detection system for Pb(II), Hg(2), and Ag(II) ions, the binding of an ion or multiple ions to DNA sequences triggers the DNA self-assembly. Subsequently, a cascade FRET event results in a fluorescent spectrum that can be interpreted as a fingerprint of the presence of a single or multiple metal ions [21]. In the sensor for the kanamycin antibiotic, an aptamer sensor, upconversion nanoparticles bound to an aptamer for kanamycin act as energy donor and the graphene as acceptor, in which the FRET is blocked in the presence of kanamycin, resulting in fluorescence. An impressive lower detection limit of 9.0 picomolar concentration is reported in aqueous buffer solution [23]. Indeed, by designing the suitable aptamer, versatile and selective FRET sensors for countless targets can be constructed.

Quantum dot and nanoparticle biosensors of equally impressive sensitivity for molecules of a wide range of sizes, from ions to large proteins, temperature, pH,

and oxygen, also have potential application in environmental monitoring [24–26]. Most recent examples include a sensor for edifenphos fungicide, comprised of a ZnS QD conjugated to a single-stranded DNA aptamer immobilized on a graphene oxide sheet. In this sensor, FRET occurs between the QD and graphene sheet, and the fluorescence quenching is proportional to analyte concentration. Interestingly, their sensor showed remarkable selectivity, even when comparing to other pesticides of similar molecular structure [27]. In a chlorophyll-containing carbon QD of tunable fluorescence for organophosphate pesticide determination, the fluorescence of the QD is quenched via a FRET process by gold nanoparticles when the analyte is not present [28]. In another recent contribution, Luo et al. constructed a highly sensitive fluorescent sensor of Au/Ag nanoparticles containing rhodamine B for the detection of organophosphorus pesticides, which showed a detection limit of  $1.8\text{-pg ml}^{-1}$  in fruit and water samples [25]. A similar biosensor of Au/Ag core-shell nanoparticles for the detection of arsenic has a lower detection limit of 0.1 ppb (parts per billion) [29]. Another FRET sensor constituted of Au upconversion nanoparticles for fluoroquinolones detection showed a sensitivity of  $0.19\text{--}0.32\text{ ng ml}^{-1}$ . Fluoroquinolones are a class of antibiotics that have become serious water contaminants [30].

Other prominent biosensors for environmental surveillance are the nano-photonic biosensors, which are devices constituted of biological receptor layers immobilized onto the core surface of a waveguide, to detect evanescent waves [31]. Their functioning mechanism is based on the exposure of the waveguide surface to the analyte, resulting in a biochemical interaction that promotes a local change in the optical properties of the waveguide transducer, which can be detected, and its amplitude is modulated by the concentration of the analyte. An advantage of photonic biosensors is that they can be integrated to lab-on-a-chip platforms, enhancing their application possibilities.

Oxygen sensing is not limited to the biomedical field. It is also a valuable analyte in environmental monitoring. Determination of  $\text{O}_2$  levels in aqueous ecosystems, such as rivers and lakes, is a common routine for evaluating the habitability conditions of these waters. Biochemical oxygen demand (BOD) and chemical oxygen demand (COD) analyses are the standard quantitative analytical methods applied for that purpose. BOD is the amount of dissolved oxygen demanded by aerobic microorganisms to decompose organic matter in a given water sample during an incubation time, usually 5 days. BOD expresses the concentration of consumed oxygen during this time period. In rivers polluted with high levels of organic waste, aerobic bacteria consume the dissolved  $\text{O}_2$  during decomposition of organic matter, which results in a drastic reduction of available  $\text{O}_2$  that aquatic animals need to survive. Analogous to BOD, the COD is a more general method, which gives the amount of oxygen needed for oxidation of any chemically oxidizable material, apart from organic matter [32].

Biosensors for oxygen and organic pollutants sensing for environmental surveillance are evolving rapidly due to many advantages they offer over the traditional methods of BOD and COD analyses, such as faster and more accurate results and the possibility of online and real-time monitoring of water quality [32–34]. One recent contribution in this field includes a microbial fuel cell biosensor for real-time BOD analysis that was tested for urine sensing. The device emits a sound alarm whenever the concentration of the analyte exceeds a given concentration threshold and is self-powered by the electroactive microorganisms of the microbial fuel cell [34].

Regarding oxygen sensing, both electrochemical and optical biosensors of noticeable sensitivity are found in the literature. In a recent electrochemical biosensor, peptide micro/nanostructures are self-assembled with a complex of copper

that acts as the oxygen reduction catalyst, immobilized onto a glassy carbon. This biosensor showed a lower detection limit of  $0.1 \text{ mg l}^{-1}$  [35]. Most recent optical biosensors based on FRET transducing include a BOD biosensor chip and a ratio-metric FRET sensor. In the biosensor chip for BOD analysis, an oxygen sensitive ruthenium complex coated with a polyethylene-polypropylene film permeable only by oxygen avoids the interference of pollutants from the sample. In this biosensor, the fluorescence intensity is correlated with oxygen concentration [36]. Another ratiometric FRET oxygen sensor consists of a Pt(II)-5,10,15,20-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin oxygen probe entrapped in a copolymer matrix that is capable of real-time monitoring of extra-cellular  $\text{O}_2$  consumption by *E. coli* bacteria and Hela cells. This biosensor showed a sensitivity of  $0.08 \text{ mg l}^{-1}$  [37]. The coating of the sensing unit or its immobilization in a matrix selective to oxygen permeability is a commonly adopted strategy in the design of optical sensors for oxygen in order to ensure its selectivity. Additionally, transition metal complexes, especially those of ruthenium and platinum, have a long phosphorescence lifetime, a requirement for efficient energy transfer from the sensing unit to molecular oxygen through collisional quenching, as described in Section 3, necessary for achieving high levels of sensitivity [4, 38, 39].

Our group has also developed a colorimetric sensor for dissolved  $\text{O}_2$ . Our sensor, comprised of a self-assembled peptide containing a fluorescent dye, is based on a FRET energy transfer between the constituents of the system that arises from the formation of a charge transfer complex. It showed remarkable sensitivity and selectivity toward dissolved  $\text{O}_2$ , both in steady-state and time-resolved fluorescence measurements. This self-assembled sensing platform, which was tested in fish breeding environment and showed good reproducibility, might be useful in analytical methods for determination of  $\text{O}_2$  levels in polluted water samples.

Additionally, our material, when allied with an antioxidant drug used in cancer treatment, showed antioxidant activity by sensing singlet oxygen, as well as pro-oxidant behavior by generating that same reactive oxygen species when irradiated with light, which makes it promising for photodynamic therapy as well [40]. The singlet excited state of  $\text{O}_2$  is perhaps the most important of the ROS molecules. Due to its considerable lethal effect for cells, it is exploited in photodynamic therapy, an alternative approach for a number of cancers that has proven to be efficient and far less invasive and harmful than the side effects of conventional treatment protocols. It is based on the same photosensitization process as described earlier.

## 5. Conclusions

The state-of-the-art contributions summarized in this chapter reveal the undoubted vanguard of luminescent biosensors in the race toward new low-cost, biocompatible, and smart materials to help solving some of the most relevant problems the modern civilizations face, such as environmental pollution and diseases like cancer.

Biosensors based on optical techniques, allied with biological molecules and nanomaterials, such as quantum dots, are constantly bringing a new family of versatile sensors and biosensors that are providing unprecedented levels of accuracy, sensitivity, and control in the study of biological processes relevant in disease treatments and point-of-care devices for environmental monitoring. Regarding versatility, the design of aptamer sensors and quantum dot conjugate systems, for instance, allows countless modifications and combinations that can be easily carried out in order to fulfill the specificity of the desired purpose. The current pace in the development of this new generation of versatile, adaptive biosensors based

on nanoscale and biological materials certainly promises to solve the main issues in biosensing development, which is the specificity, sensitivity, and portability requirements for the spread use of biosensing devices.

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## Conflict of interest

The authors acknowledge that there is no conflict of interests in this work.

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