Chapter 5

Supramolecular Materials for Optical and Electrochemical Biosensors

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Abstract

It is incontestable that the interactions and bonds that keep molecules united to generate unique supramolecular compounds, with individual properties, morphologies and behaviour, are of special dynamics and singular forces. Therefore, it is necessary to discuss and consider the types of interactions that may occur in a determined system, their dynamics and number, which directly act on the energetic balance that strengthen the union between participants and give rise to a supramolecule.

In this chapter, a number of such supramolecular systems that find application as any component of a biosensor are presented and discussed, considering intermolecular interaction forces that confer them shape, function and unique properties. To better understand their structural dynamics and the mechanisms through which they can be used in biosensing, a brief explanation on the interaction thermodynamics, types of intermolecular interactions that compete against each other and the energetic equilibrium that originate and stabilize supramolecular systems is given. To explain how this balance of forces can be extensively exploited to develop methods to produce supramolecular compounds, an overview on supramolecular strategies is presented and their contribution is explored in each example presented in this text, to evidence the importance of planning and developing methodologies of preparation, based on

accurate information on structural characteristics and properties of the initial compounds. Thus, an approach to different procedures of preparation or adaptation methods applied to supramolecular compounds to fit a determined application, as well as newly developed materials, is the focus of the following discussions. For instance, supramolecules presenting interesting and singular characteristics that can be advantageous in biosensors, such as high quantum yield luminescence, high efficiency of energy conversion, which enable charge transfer and migration processes, adaptive and mimetic properties, among others, are discussed on preparation and function basis. A variety of supramolecules and supramolecule-based materials produced via distinct supramolecular strategies are discussed and examples of their applications are presented. Specifically, new and extraordinary supramolecular materials that present properties and characteristics that could not occur in ordinary organic/inorganic compounds, but notoriously enhance optical and electrochemical biosensor performance, will be presented and their properties, detailed. Also, the mechanisms of action of supramolecule-based optical and electrochemical biosensors and their dependence on singular properties of each type of supramolecular system are discussed to provide an understanding of the most prominent aspects that govern a compound behaviour in a device and guide the scientist through the choice of the appropriate material for biosensing applications in the wide field of available supramolecular compounds. In an attempt to provide accurate information to those who desire to understand the importance of supramolecular materials in biosensor applications, an overview on the recent progress on materials for optical, electrochemical and photoelectrochemical biosensors are presented, in order to inspire research on new and even more delicate and effective supramolecular materials for biosensing applications. Moreover, we discuss some of our recent results, which are provided as examples, that highlight the advantages of producing supramolecules based on peptides for applications in optical biosensors and introduce some interesting methods to anchor enzymes in electrodes to enhance device performances, as well as provide new technologies for distinct device production.

Keywords: Self-assembled materials, optical biosensors, electrochemical biosensors, fluorescence, cyclic voltammetry, electronic energy transfer, peptides

1. Introduction

1.1. Recognition mechanism — The fundamental concept of supramolecular chemistry

The property of molecular recognition is of great importance to describe most biological processes, such as DNA replication, enzyme activity, among several others, and consequently, it has played a significant role in supramolecular chemistry development. Among them, the implementation of molecular recognition as a method to control the production of supramolecular species, since it is based on the combination of small components, was a breakthrough.

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Supramolecular control of synthesis of compounds is achieved by the development of methods that combine the design of the spontaneously generated complex molecular structures, based on self-assembly of chosen components, with pre-determined experimental conditions. As these self-assembled structures are obtained with impressive success, self-assembly property has been exploited in a distinguished number of organic and inorganic systems, which gave rise to the known *bottom-up* and *top-down* supramolecular built-up strategies.

The first step on producing a supramolecular compound is to have in mind the properties expected for this compound to present. It will both, to justify its applications and to enable the selection of the adequate experimental steps to produce it with high quality, yield and purity. Bottom-up strategies are preferred when a property found in a single molecule is desired in the supramolecular complex, since they tend to preserve such molecular characteristics. On the other hand, top-down strategies are preferred when the objective is to prepare representative small structures of major ones, presenting the desired structure and properties, being important on miniaturization processes.

1.2. Supramolecular strategies — Towards excellent structures for biosensors

1.2.1. Bottom-up strategies

In comparison to top-down strategies, bottom-up ones enable better chemical control of the produced supramolecular surface, better size control and higher degree of purity. They correspond to synthetic processes that involve the supramolecular growth from atoms, achieving molecules and further to aggregate with the desired dimensions and presenting the desired properties. Most of the bottom-up strategies occur in vapour and liquid phases. Among them are the following:

- Condensation methods, usually occurring in sealed chambers in which the precursor compound is vaporized and condensed in the substrate. The sublimation of the compound can be processed either by laser ablation or by thermal heating.
- Vapour phase nucleation methods, in which structure growth occurs usually in a vapour phase reactor. These methods present advantages, such as the use of precursors in solid, liquid and gas phase, mild conditions of preparation and homogeneous multi-component supramolecular structures production.
- Liquid phase nucleation methods, in which structures are prepared in a wide variety of conditions, depending on the desired supramolecular structure.
- Layer-by-layer methods of film preparation, which enable film production from large compounds such as polymers, proteins, even DNA or from small molecules and atoms. Figure 1 illustrates some bottom-up strategies to obtain supramolecules.

1.2.2. Top-down strategies

To particulate materials is the fundamental conception of any top-down preparation method that can be conceived. Especially, physical methods of fragmentation or disaggregation, in

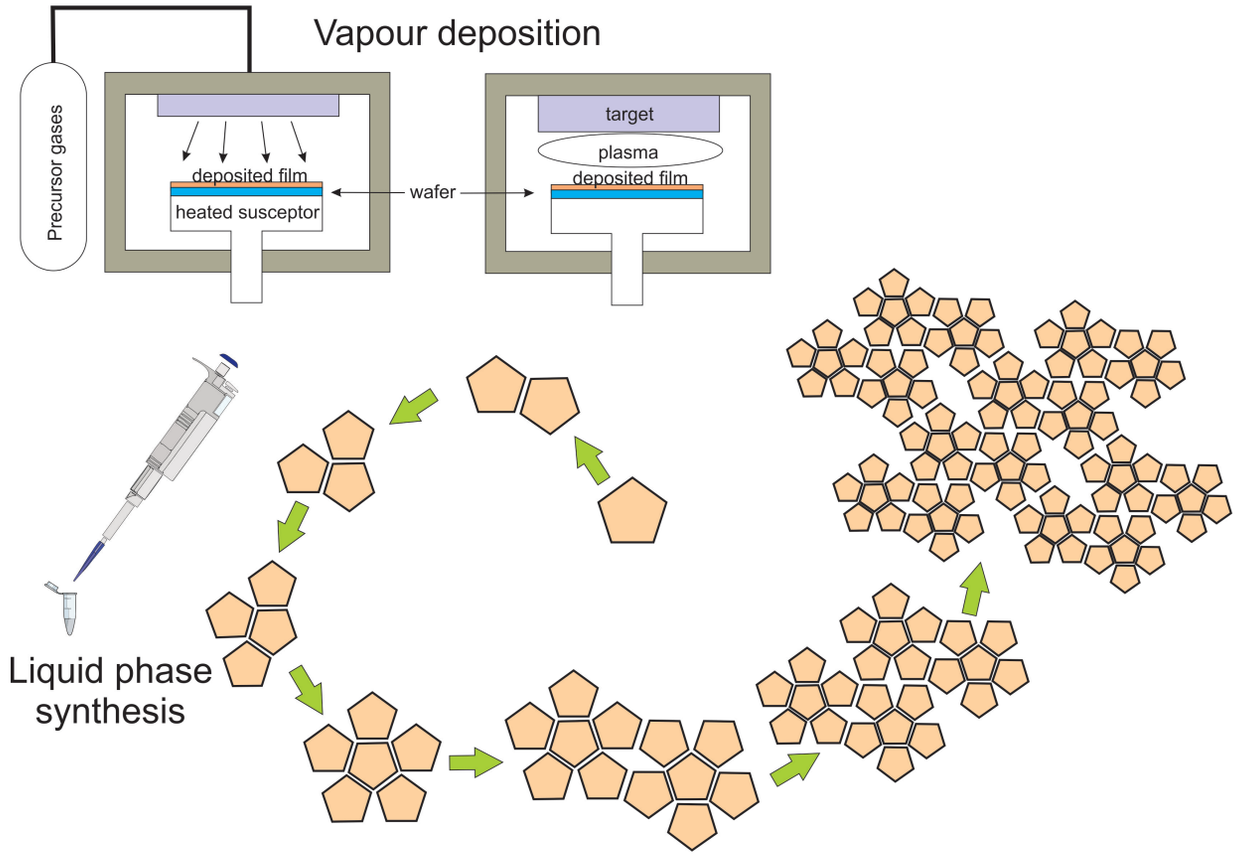

Figure 1. Schematic illustration of distinct bottom-up strategies to produce supramolecular compounds.

which the major structure is broken, nevertheless conserving its organization and physical-chemical properties, are based on the basic principle of top-down methods. Among the most employed top-down methods are the following:

- Laser ablation, in which supramolecular compounds of distinct nature are obtained by a surface irradiation process that promotes particle removal.
- Ball milling, in which highly energetic collisions between small balls in the milling chamber and the material repeatedly occur, resulting in a fine and uniform material dispersion and conserving all physicochemical characteristics of the initial material.
- Atomic force microscopy (AFM), which has been recently proved to be adequate to manipulate particles of a surface [1], due to an attraction/repulsion force balance between the surface and the cantilever of the microscope.
- Thermal treatment of a compound to weaken interaction forces that keep the major structure together and sonication of the material solution in an appropriate solvent are simple methods of physical separation that are employed to separate the aggregation units and generate the desired supramolecules. Figure 2 illustrates some top-down supramolecular strategies.

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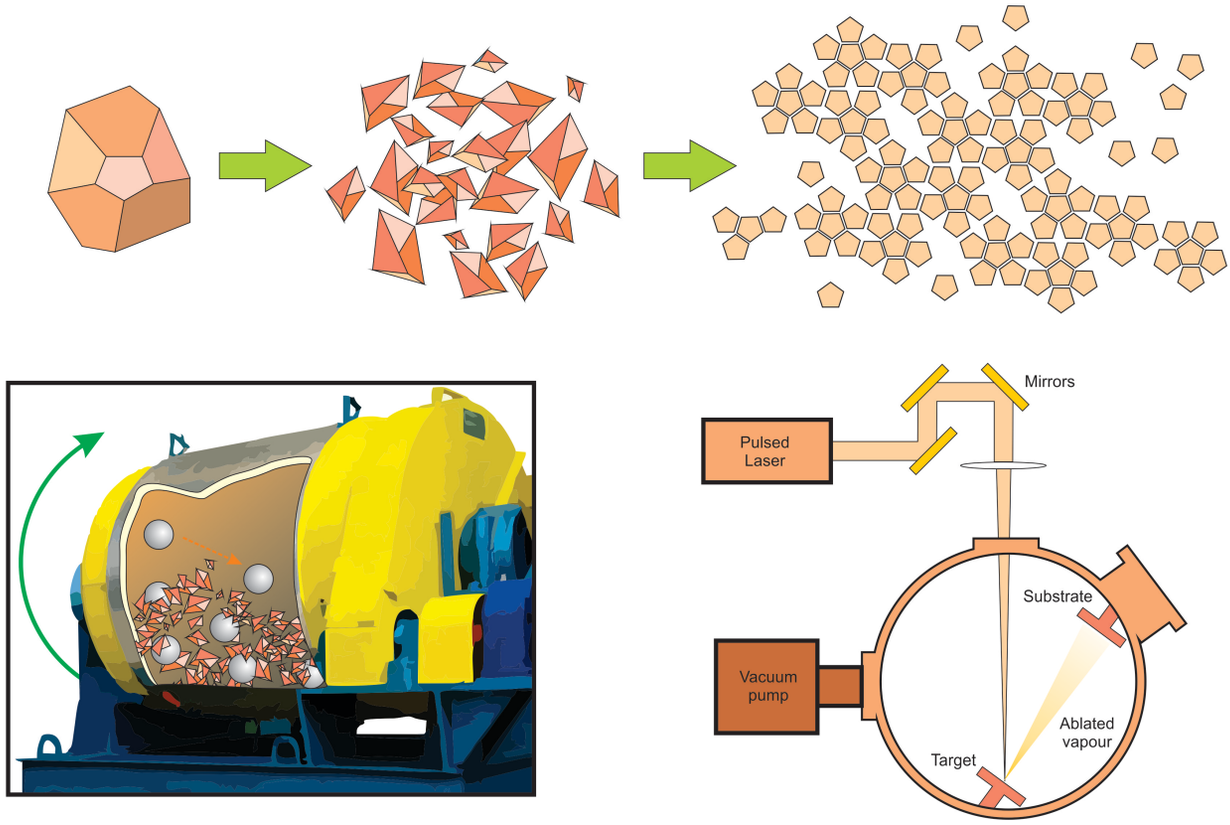

Figure 2. Scheme of supramolecular top-down strategies based on ball mills and ablation techniques.

2. Supramolecular interactions

Whatever strategy is considered adequate to build a super compound, the nature of interactions that govern the self-organization of primary compounds into the complex one is usually of the weak kind. This means that the units responsible for super compounds or supramolecules are kept together by non-covalent interactions, rather than covalent bonds.

It is clear that covalent bonds are, and always will be, the fundamental occurrence between atomic orbitals that originate molecules, structures, tissues and, therefore, materials and life. Nevertheless, the participation of weak forces on conferring identity and possibilities of change to every molecule formed cannot be neglected. In supramolecular chemistry, the modelling characteristics of compounds are derived from weak interaction forces that are exploited to confer them the abilities of formation and change, by accumulation of interaction forces. In fact, to generate a supramolecular compound, weak interaction forces work synergistically to create alternative forms of bonds that give rise to functional structures, in dynamic environments and mild conditions. As predicted by Steed and Atwood [2], very stable complexes can be formed based on non-covalent interactions that present, each one, a small contribution to the supramolecular compound stabilization, which, when allied to others, become important and overwhelms any environmental disturb that could lead to the compound dissociation.

Indeed, to have an idea of the binding forces and which supramolecular structures are energetically favourable, it is important to correctly identify the non-covalent bonds that dominate the formation processes and their comparative forces. In an attempt to better identify the most prominent processes and the equilibrium that is formed between components and the environment, the most important non-covalent interactions that are present in such complexes are as follows:

- **Charge interactions**

Due to their characteristic of making use of the strong effect that charged group may exert upon uncharged molecules, these interactions are favourable, since they promote a temporary completion of orbital charges. They involve positively or negatively charged entities affecting uncharged molecules or groups that present surface charge. Among them are the following:

- *Ion-ion interaction:* in compounds structured upon these interactions, there is a strong electrostatic interaction of an attractive character that enables the strong interaction that holds the isolated participants together, although at a distance from each other, which preserves their individual identities. These interactions present strength similar to those of covalent bonds with energetic values around 100-350 kJ mol^{-1}.

- *Ion–Dipole Interactions:* these interactions occur between an ion and a polar molecule, also susceptible to electrostatic effects. Since one of the components is uncharged, these interactions are weaker than the former, presenting binding energies of 50 – 200 kJ mol^{-1}.

- *Cation/Anion–π,π Interactions:* these are examples of electrostatic interactions that occur between charged moieties, mostly aromatic rings, although they are of the same nature of those observed between positively charged elements and carbon-carbon double bonds (C=C). They are of moderate intensity, presenting binding energies of 30-80 kJ mol^{-1} [3].

- *Hydrogen Bonding:* Linus Pauling described it [4] as a versatile interaction, presenting energy of moderate intensity (4 – 100 kJ mol^{-1}) and occurring between a compound that possesses a hydrogen atom adjacent to an electronegative or electron withdrawing group and the dipole portion of a neighbouring molecule.

- **Induced charge interactions**

These are interactions that occur whenever neutral groups or molecules approximate to each other, being perceived due to their distinct electron densities. They occur with small energetic changes and are more important when numerous in a given structure. They are modulated by the identity of participants and, therefore, by the singular characteristics of each molecular combination. They may present a wide range of lengths, angles and can be classified depending on the main characteristics of their participants:

- *π–π Interactions:* they involve π-orbitals of any compound and are common in aromatic rings, being very important in self-assembling processes. Presenting energies of 1-10 kJ mol^{-1}, their occurrence is justified by an electron density divergence between the participants.

- *Dipole–Dipole Interactions:* When a dipole is nearby another, they tend to align, due to their perception of each other, by attractive and/or repulsive interaction forces. They are of moderate intensity, presenting energies of 5-50 kJ mol^{-1}.
- *Van der Waals Interactions:* they are weak interactions of approximately 1-5 kJ mol^{-1} that arise from the perception of polarizable molecules on charged or possessing charge character compounds.

All these interactions occur in any supramolecular system, and their balance of forces and the role that each interaction plays in the configuration and stability of each system depends exclusively upon the preparation methods, the individual characteristics of the participants and the proposed molecular designs. Some of the above-mentioned interactions are illustrated in figure 3, in which water interacts with poly[5-methoxy-2-(3-sulfopropoxy)-1,4-phenylene-vinylene] (MPS-PPV) moieties through hydrogen bonds and the polymer moieties interact with other molecules through π- π interactions.

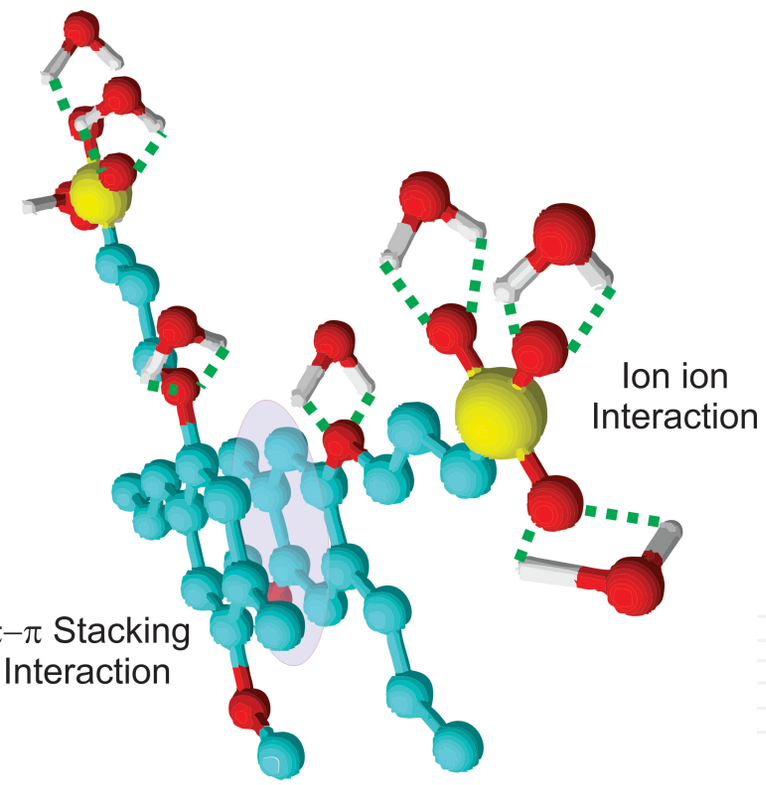

Figure 3. Examples of ion-ion and π- π interactions as they occur in an aqueous solution of MPS-PPV.

It is important to consider that all these interactions work synergistically in order to form a compound with some ideal characteristics that enhance the effects of the environment on their ability to act as host/guest system, such as the structural characteristics that are assumed when solvation is possible, participation in lattices, component changes, etc. Regarding these abilities, supramolecular chemistry has played a fundamental role in the biosensors development.

3. Optical and electrochemical biosensors

Since biosensors are devices that can perform a rigorous exploration of a system, under conditions that require the minimum possible human intervention, they can be constructed upon a variety of compounds, depending on their application and environmental conditions they will be subject to. The selection of the appropriate recognition element considers not only what the information needed is, but also the ease of construction of the devices employing such elements and their durability.

Once the recognition component is selected and, thus, the mechanism in which it interacts with the analyte is selected, it is important to find an appropriate mechanism to transform the resulting perturbation, or signal, into comprehensible information. The selection of the appropriate transducer determines the type of the biosensor to be constructed. Its selection enables a fast and precise data interpretation, which is an important requirement. Examples of biosensor components are displayed in figure 4.

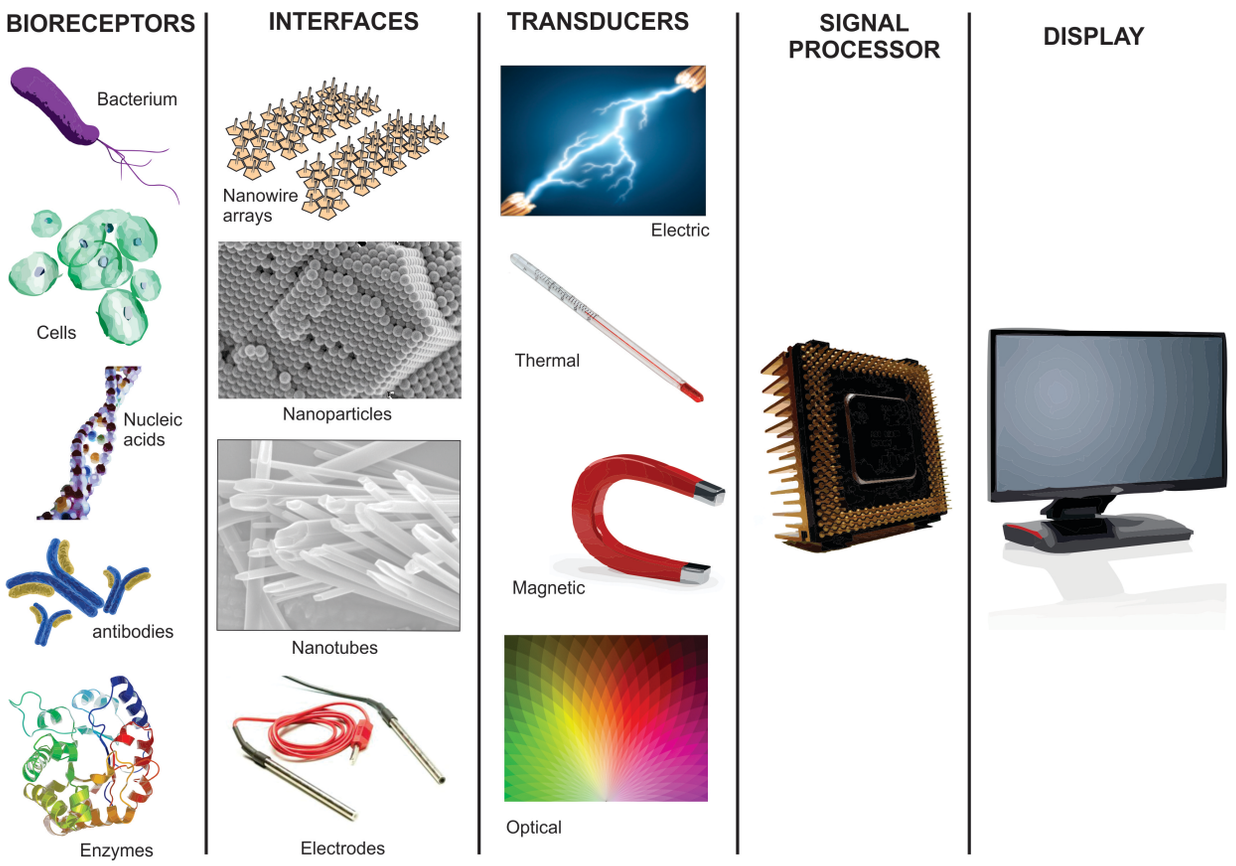

Figure 4. Illustration of materials commonly used in biosensor component proposals.

Transducers are available based on a variety of sensing techniques. In optical biosensors they include Fluorescence Spectroscopy, intensity/lifetime measurements of luminescence, light absorption or light reflectance in the ultraviolet (UV), visible, or near-infrared (NIR), spectral

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regions and Surface Plasmon Resonance (SPR). Also included are techniques based on microscopy, such as Atomic Force Microscopy (AFM), Fluorescence Confocal Microscopy, Raman Confocal Microscopy or Surface Enhanced Raman Spectroscopy (SERS). Also based on the transducer choice, electrochemical biosensors are classified into amperometric, conductometric, potentiometric and voltammetric categories. The variety of electrochemical and optical techniques that are exploited as transducers confer to the devices improvements on selectivity, sensibility, configuration versatility, ease of construction, possibility of miniaturization. The combination of optical to electrochemical transducers give rise to optical-electrochemical biosensors, which deliver either electrical or optical signals that are interpreted together or separately.

Independently of the chosen components, an efficient biosensor must effectively recognize the analyte and selectively interact with it, to deliver comprehensible data in short time, to avoid loss of information, mainly by analyte´s degradation. This means that the guest-host ability of the biosensor is the key factor for a device to show high selectivity and sensibility and, thus, this ability may be modulated by the recognition of material choice. As depicted by Lehn, molecular recognition is related to the binding energy and the interaction ability between host and guest [5]. When it involves compounds with unique structures, it can occur in a specific way, and implies in the union of the spatial and functional characteristics of the molecular groups, that interact to each other and shapes the selectivity and sensibility characteristics of a compound. As predicted by Araki [6], selectivity and specificity are measures of the interaction of free energy between the binding moieties and, thus, the role of surface and electronic aspects is fundamental to modulate the free energy. In fact, shape, size, conformation changes of binding sites in host-guest systems, polarity, polarizability, charge density of groups and terminations are the most important features to be determined and considered when evaluating the selectivity of a host. Those are also the most important features when designing an efficient recognition element for a biosensor.

Indeed, with the rise of a variety of supramolecular structures and the number of studies that aim to describe their singular properties and performance in several applications, the diversity of compounds that showed perspective of use in biosensors arose significantly, and the resulting devices have enhanced its selectivity, sensibility and usage spectra.

4. Supramolecular compounds for optical biosensors

In optical biosensors, due to the wide range of compounds that can be employed in their construction, to result in selective recognition systems that converts the optical signal by several techniques, significant development have been achieved in the past twenty years. In particular, biosensors based on fluorescent responses greatly benefit from supramolecular compounds used as recognition systems and transducers, since they are able to significantly enhance charge transfer processes and this property can be exploited to label the analyte and, hence, enable detection via fluorescence imaging or enabling FRET, which can be efficiently detected using special biosensors [7-9].

A wide range of luminescent supramolecular compounds are available for biosensor construction, and the reason for this diversity is that supramolecular compounds can self-assemble. Self-assembling is the characteristic that distinguishes large molecules and supramolecules. Due to it, the alternatives for the design of a supramolecule are almost unlimited and production of supramolecules is becoming cheaper as they become comprehensible to the scientists [10]. Based on the variety of interactions that is possible in self-assembled structures and due to their lability, supramolecular materials can be designed based on organic and inorganic polymers, hybrid materials, charged molecules and macromolecules, crystals, liquid crystals, gels, metallic nanoparticles, some alternatives containing rare-earths [11], giving rise to new materials and nanomaterials presenting so many distinguished properties that confer them new applications and classifications, such as adaptive and self-healing materials.

In the past five years, much has been produced in the supramolecular and new materials research themes. New materials are often proposed to improve devices and technologies, but sometimes they carry so many innovations that new applications must be proposed. Due to that, the sensing development has achieved this great status and is still on its way up!

Earlier, the secondary interactions that predominate in supramolecular chemistry and their associated energies were discussed; nevertheless, it is important to reinforce that supramolecular compounds are formed by a balance of interaction forces that form a dynamic system, formed by distinct structural parts that struggle to remain in their possible less energetic conformation. In self-assembling processes, the major influence of one or a couple interaction types is often recognizable. When electrostatic interactions dominate, ionic self-assembling (ISA) is expected to occur. This type of self-assembling process gives rise to an enormous variety of supramolecules, each presenting its own characteristics [10, 12]. An example is the result of the combination of two dyes synthesized by Bohm et al. [13], 1,4-dihydroxyanthraquinone (AQ-OH) and 1,4-di- N –adamantyl-amino-anthraquinone (AQ-H), with the hyperbranched polyethylenimine attached to β –cyclodextrin (β -CD-PEI), which is a spontaneously formed host–guest complex with high hydrodynamic diameter. In their work, to demonstrate enlarged networks produced on supramolecular basis, due to host–guest interactions, they had measured the initial and final hydrodynamic diameters by dynamic light scattering, and they found that, after the assembling process, the hydrodynamic diameter presented a 40-fold increase.

Supramolecular compounds formed upon electrostatic interactions find many applications, including in optical and electrochemical biosensors. In their work, Wang et al. [14] produced a H_2O_2 non-enzymatic biosensor based on a self-assembled peptide nanofiber that was, then, metalized to give silver nanowires. This material was supported in graphene nanosheets to build the device, which presented high sensitivity, high selectivity and low detection limit. The use of this interesting designed peptide presented two important breakthroughs: it enabled the production of 1D peptide nanofibers by self-assembly and it rendered metal nanowires by metallization. The authors claim that this system may present potential applications in nanodevices, especially other biosensors, as well as in biomedicine and in Raman analysis.

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Also, the self-assembling properties presented by polyelectrolytes have been widely explored to develop a series of multifunctional compounds. In their work, Habibi et al. [15] presented a self-assembled system based on anionic poly(styrene sulfonate) (PSS) and cationic poly(allylamine hydrochloride) (PAH) modified with S-protein to promote the loading and release of macromolecules from the interior of the obtained capsule. The permeability of this system was pH tuned and the encapsulation and release of macromolecules were promoted by controlled pH change. The functionality is based on the fact that S- proteins self-assemble into monomolecular arrays at different interfaces, which provide a regular arrangement of functional groups in the final structure, following a bottom-up self-assembly strategy to generate functional supramolecular structures and devices. As they showed that this system is biocompatible [16, 17], they suggest that its application in biosensing, in drug delivery and as micro-reactors, among several others, is highly probable.

Nanostructured materials have been studied aiming the biosensing application due to their specific physical-chemical properties and to their quantum-size effects when compared to bulk [18]. Specifically, highly luminescent compounds and charge transporter materials have also been widely employed to generate supramolecular structures for biosensing applications. This class of materials includes quantum dots, metallic or magnetic nanoparticles, nanostructured materials into one-dimensional or bi-dimensional structures such as nanowires, nanoribbons, nanospheres or nanosheets and may improve device sensitivities due to their large surface-to-volume ratios. Also, due to their small sizes, they can be delivered into living cells, enabling *in vivo* sensing applications. Among the devices that can be thought for *in vivo* applications, fluorescence-based biosensors have been developed, possessing several configurations, sizes and, thus, functions. Tunceroglu et al. [19] described a time-dependent fluorescence-based biosensor with the ability to evaluate drug resistance, by monitoring Bcr-Abl activity in patients diagnosed with chronic myelogenous leukemia (CML), and Gulyani et al. [20] developed a device that analyses the fluorescence enhancement when it binds to the target kinase with the determined conformation, becoming a Src merobody biosensor.

Rare-earth-based materials and quantum dots (QD) are of great interest due to their high quantum yield of luminescence at UV/Vis solar spectrum region [21, 22]. They also present narrow and tunable emission spectra and usually present good photostability, which substantially increase their hall of applications. Nguyen et al. [23] described a CdTe quantum dot–based biosensor, developed specifically to detect flu virus H5N1. They reported that the biosensors consisted of CdTe/CdS quantum dots of high quantum yield, chromatophores extracted from a bacteria and of β-subunit antibody, all connected to a protein, which was, then, connected to the H5N1 antibody. They recorded the changes in the QD photoluminescence spectra and collected images of the QD-labelled chromatophores in an optical microscope to evaluate the specificity of the biosensor [23].

Hybrid materials are often thought as interesting materials for biosensing, since nanoparticles, which present high surface-to-volume ratio, can be attached to biomolecules, resulting in a biologically active compound with improved characteristics. Semiconductor QDs are frequently a good alternative for this purpose. Boeneman et al. [24] developed a hybrid material based on CdSe/ZnS core/shell quantum dot conjugated to fluorescent proteins and to a light-

harvesting compound. The activity of the resulting hybrid is based on the QDs electron-donor characteristic for Förster resonance energy transfer (FRET) and on the fact that the fluorescent proteins are FRET acceptors. Their results showed that QDs and fluorescent proteins can be conjugated in cellular environment, inclusively, which confer them the potential for live-cell imaging and biosensing applications. Brunetti et al. [25] also successfully produced a Cadmium-free QD, with *in vivo* and *in vitro* toxicity testing done.

Since Cadmium-based quantum dots may present a risk for living organisms, their substitution for more biocompatible elements has leveraged quantum dots research. Recently, biocompatible quantum dots have been developed, and for biosensors application and *in vivo* imaging, silicon QDs (Si QDs), which are expected to be less toxic than the common group II–VI based QDs, are good alternatives. [26]. Notwithstanding, an interesting cadmium-free quantum dots class based on carbon was rapidly suggested as biocompatible and environmentally friendly, with its multi-coloured luminescence, as presented by Sun et al. [27], being its most attractive characteristic. It is prepared by the supramolecular top-down strategy of laser ablation of a carbon target in water vapour, employing Argon as the carrier gas. The surface passivation is necessary and it is held by attaching organic biocompatible polymers such as $PEG_{1500}N$, as they purposed, since this polymer is hydrophilic and can be readily conjugated to proteins, antibodies and several others bioactive molecules.

Although proteins are very important in selective QD-based biosensor design, they are often too large, which causes sterical problems that restrict the biosensor activity. As an alternative, peptides have been thought of as substitutes, since they also can provide biological activity to the device with the advantage of size accommodation. They are also of facile synthesis, biocompatible, commercially available and, as a chemical advantage, they present a well-known structure. Also, their ability to self-assemble enables their use in strategically designed biosensors. In their work, Nagy et al. [28] presented biosensors based on peptide-functionalized quantum dots, exploring the Forster Resonance Energy Transfer that occurs between the peptide and the Quantum Dot. Choulier et al. [29] compared the sensibility of protein-based and peptide-based fluorescent biosensors and they found that peptides perform as good as protein receptors; nevertheless, the ability of forming complexes with the target provided a significant difference due to size interference. They employed a 3-hydroxychromone (3HC) derivative as dye in this system, since it presents a two-band fluorescence emission, whose ratio is susceptible to environmental changes. They observed that a quantitative target determination is more efficient in the peptide–target interface than in the protein–target interface, due to its smaller size and high flexibility [29]. Another important advantage of using peptides in biosensors, with no doubt, lies on their ability to self-assemble. Kim et al. [30] produced a hydrogel based on diphenylalanine dipeptide that encapsulates enzymatic bioreceptors along with fluorescent probes based on Cadmium quantum dots. They argued that enzymes and quantum dots (QDs) physically immobilized in a self-assembled hydrogel peptide result in efficient biosensors due to the three-dimension network formed of 70–90 nm diameter nanofibers. This system was tested for detection of glucose and phenols by means of photoluminescence quenching of the hybridized QDs.

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Recently, our research group showed that fluorescent environmental biosensors can be produced with self-assembled peptides and they can be equally active in solution or solid phase, with high sensibility, reproducibility, durability and low costs of fabrication. Souza [31] registered a fluorescent system consisting of diphenylalanine self-assembled nanotubes doped with a coumarin dye derivative and supported in glass substrate. It was tested regarding efficiency, reproducibility and durability, with good results. The sensor was tested for water dissolved Oxygen (O_2) after respiration processes of living bodies in the environment. Coumarin fluorescence is modulated by O_2 presence in any environment, since its populated electronic excited state is resonant with O_2 triplet electronic state. An energy transfer process occurs to convert O_2 triplet into O_2 singlet, resulting in the coumarin fluorescence intensity decreases. Very low limits were detected, which suggest that this system can be used to explore cellular environments. Since its function is based on singlet O_2 generation and since it is constructed with biocompatible components, this system may also be used in photodynamic strategies for cancer treatment.

Other self-assembled structures find application in biosensors, such as those produced with carbon. An example is the work by Robinson et al. [32], which applied single-walled carbon nanotubes (SWNTs) for *in vivo* imaging of tumour cells in mice. Their system took advantage of the unique optical properties of SWNTs to perform 3D reconstructions of *in vivo* fluorescent imaging. They functionalized it with an amphiphilic surfactant to turn the SWNTs biocompatible and performed video imaging of tumours. The tumour was pointed within ~20 s after injection!

In fact, the use of carbon nanotubes in biosensors presents several advantages, mainly due to their photophysical properties that enable to explore phenomena at the NIR emission range, which is coincident to the tissue transparency window and guarantee *in vivo* security of exploration. They do not suffer bleaching or blinking and present a large Stokes Shift, turning them ideal materials for long-term sensing applications. Nevertheless, they cannot be easily regenerated due to their facile functionalization [33]. Some other carbon structures are being currently tested in biosensors construction. One of the most interesting is graphene oxide, due to its ability of charge transfer quenching. In their work, Shi et al. [34] reported a biosensor consisting of a peptide supported in 2D graphene oxide film to monitor, via fluorescence resonance energy transfer (FRET), the BoNT serotype-A protease activity. The system was able to selectively detect it. Tao et al. [35] produced biosensors based on self-assembled graphene modified with DNA, whose sensitivity is such that they claim it could be a universal biosensor. It consists of a colorimetric label-free sensor able to detect distinct compounds, such as metal ions, DNA and small molecules as well. Several other optical and electrochemical biosensors using graphene were already reported [36, 37].

Strategies with good results are often based on combining materials. Metallic nanoparticles are interesting due to the possibility of modulating their properties depending on size. Combining it with carbon nanostructures have been widely explored for biosensing applications. Among the nanoparticles with application in sensing, those of gold are highlighted due to their high surface-volume ratio, stability, biocompatibility and their unique optical properties, such as their intense colour changes upon aggregation, enabling their use in optical

biosensors. An example is the work by Kim et al. [38], in which these nanoparticles were assembled to distinct cathepsins to show selectivity to the B variety, which is related to distinct types of cancer, such as brain, breast, gastric, thyroid cancers, among others.

Metallic nanoparticles are also interesting in biosensing and therapies due to their property of magnetism. Since their magnetic properties can be modulated by controlling nanoparticle size and by coating them to avoid irreversible aggregation, biosensors based on distinct transduction methods such as electrochemical, optical, piezoelectric or exploiting magnetic fields, can be built. In a recent work, Wang et al. [39] presented a biosensor constructed upon magnetic nanoparticle composites, while another researcher Wang produced biosensors for rabbit IgG detection employing magnetic nanoparticle supported on SiO_2 surfaces [40].

In our research activities, Dias prepared a fluorescent self-assembled peptide nanotube combined to iron nanoparticles and to a highly fluorescent cadmium-based quantum dot via liquid synthesis. The resulting system presented high fluorescence quantum efficiency dependent of the dominant interactions between the system components, being tunable and occurring in a wide spectral range, from near UV to visible red-region, including the therapeutic window (621 nm), which indicated that this system is adequate for biosensing and therapeutic applications [41].

Polymers are also widely used in several types of devices, including biosensors, in which they are used to immobilize proteins, enzymes, DNA [42], as coatings and traps [43, 44] and as electron donor/acceptor in transducers [45]. They are also combined to metallic nanoparticles [46] and carbon nanotubes [47] to enhance the biosensor sensitivity and to diversify the analytical routine that can be conducted.

New supramolecular compounds have been developed in order to facilitate devices fabrication and to turn sampling, sensing and fabrication procedures as much sustainable as possible. In this path, non-covalent interactions are being stimulated in order to create new conceptions of compounds. It had its start on the behaviour characterization of nanoscaled materials and, now, it has been applied to achieve complex and ordered non-covalent systems. Due to that, some interesting characteristics have been achieved such as multi-functions, self-healing, adaptativity, recycling ability, self-assembling, characteristics that enable a variety of biomedical, technological and scientific applications. Adaptive materials are able to respond to externally imposed situations by implementing changes in structure or function. In supramolecular compounds, this is possible due to the lability of non-covalent interactions, which can quickly change upon environmental changes. In biosensor development, this characteristic can be of great importance [48]. Recently, Zhang et al. [49] reported a biosensor based on an adaptive material composed by an electro-active supramolecular ionic material of imidazolium di-cation and di-anionic dyes. These compounds self-assemble, resulting in a structure with unique optical and electrochemical properties and adaptive encapsulation properties in the presence of cationic dyes. It was tested to sense NADH at a low potential of -0.07 V.

Self-healing materials are, in a simple description, stretchable materials in which non-covalent interactions enable them to recover their form, even after severe stress. This characteristic turns them into convenient materials for almost any application one can imagine. When this physical

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property is associated to electrochemical sensibility or energy transfer, the material can be explored in sensing, biomedical, biophysical and photovoltaic applications. Kim et al. [50] reported a biosensor designed upon self-healing materials that are able to conveniently mould themselves into tissues of complex geometry. They were applied in sensors for cardiac muscles, while Ramuz et al. [51] described their optical and pressure biosensor as having activity similar to electronic skin, since it is suitable for covering large areas. Figure 5 illustrates the self-healing behaviour presented by some supramolecular compounds.

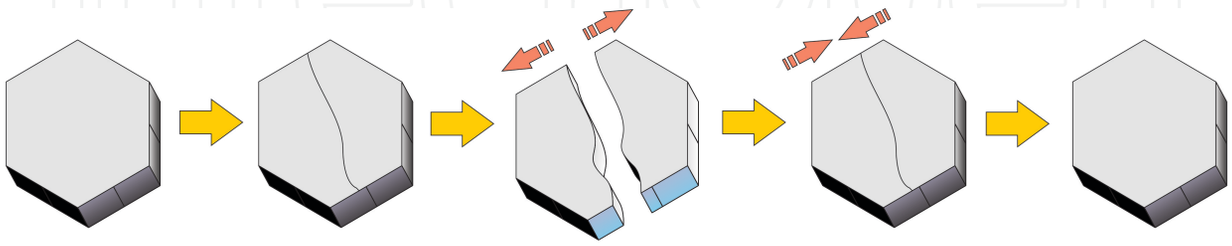

Figure 5. Schematic concept of self-healing activity of some supramolecular materials.

5. Supramolecular compounds for electrochemical biosensors

An electrochemical sensor is a device that provides an electrochemical response in the presence of an analyte such as amperometric, potentiometric, conductometric or impedimetric responses, which can be, thus, detected by electrochemical techniques [52]. Electrochemical biosensors consist of electrochemical sensors that use a biomolecule in its conception, either as recognition element or as transducer. For biosensing applications, it is common to employ enzymes as recognition element, adding simplicity to analysis, reproducibility, selectivity, low-cost, high sensitivity and short response time to the device. Durability is often observed in enzyme-based electrochemical biosensors. In such biosensors, the enzyme catalyses a chemical reaction, generating an electron flow that is identified by the device as dependent on analyte concentration. Therefore, the enzyme must be in contact with a transducer to conveniently enable the electron transfer to an electrode and generate a comprehensible signal.

The enzymes catalyse electrochemical reactions in biosensors by two main mechanisms as described below and illustrated in figure 6:

- Through substrate modification, producing an intermediate that can be reduced or oxidized at lower potentials, compared with those of the original substrate. In this system, the electron transfer is promoted by an electron carrier, which is immobilized in the electrode surface and in contact with the activated substrate, modified by an enzymatic reaction. This is a common process, exploited for instance, on the ethanol oxidation process, enabled by the enzymatic NADH/NAD+ reduction couple. In their work, Neto et al. [53] developed a supramolecular complex based on gold nanoparticle coupled to carbon nanotubes, whose function is to enhance NADH/NAD+ conversion rates, leading to lower potentials.

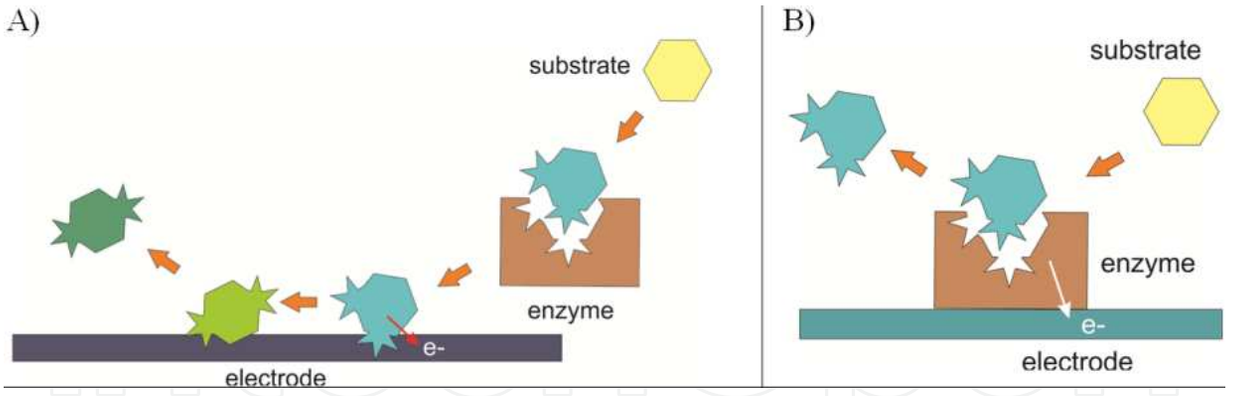

Figure 6. Scheme of the catalytic activity of an enzyme A) through electrochemical process of involving substrate modification and B) through enzyme-electrode interactions.

- Through enzyme immobilization in the electrode surface, enabling direct electron transfer to the electrode and eliminating the carrier.

Although many forms of attaching an enzyme to the electrode have been developed in the last years, it still remains the limitation of the bioelectrochemical devices. The most common method is the physical adsorption of an enzyme onto an electrode surface, nevertheless it usually results in enzymatic activity loss and, since the enzyme- electrode interaction is weak, the enzyme can be dragged by the electrolyte, which leads to a low efficient electron transfer [54] and may also result in biomolecule inactivation [55]. Supramolecular strategies of immobilization are at the forefront of the enzyme-based biosensors [56-60] in an attempt to repair the hazardous effects of binding-immobilization, such as reduction of enzymatic activity or inactivation and loss of electrode due to irreversible modifications on its surface. The common strategy of permanent modification is cross-linking of enzymes, which results in immobilization with no significant activity loss and stability, therefore, there are several strategies available for the fabrication and characterization of cross-linked enzymes [61]. Glutaraldehyde, is the most popular cross-linking agent for enzymes, however cross-linking conditions must be optimized and the best results are often obtained at glutaraldehyde concentrations of 0.1 to 5%. Even in supramolecular methods, this is popular, as in Alves et al. [62] work, which prepared a glucose biosensor by immobilizing glucose oxidase onto platinum electrode, executing the enzyme immobilization via cross-linking process, using a 2.5 % glutaraldehyde solution.

Arya et al. [63] proposed a biosensor for human influenza virus, which employed a self-assembled peptide interacting with the virus hemagglutinin antibody, in a microelectrode design that is claimed to be more sensitive than common macro-electrodes, due to its radial diffusion profile, as opposed to planar diffusion in macroelectrodes. Electrochemical impedance and cyclic voltammetry techniques were used to characterize the electrode and to estimate human Influenza virus hemagglutinin antibody concentration.

Among the enzymes that can be immobilized in biosensor electrodes, peroxidases are by far the most popular to detect distinct organic compounds, such as phenol, peroxides, poisons, hormones, gases, etc. [64]. Due to its powerful catalytic activity, through electron transfer mechanisms, peroxidase has found application in a variety of biosensors, mainly electrochem-

ical ones, in which distinct types of responses can be recorded and treated to give accurate information on a specific analyte. Attar et al. [65] constructed a biosensor based on horseradish peroxidase to detect cyanides, which consist of extremely poisonous substances and is present in surface and ground-waters. In their proposal, peroxidase enzymatic activity is inhibited, and the decrease is inversely proportional to cyanide concentration increase. This process is followed by current density measurements, in an amperometric biosensor. Oliveira et al. [66] prepared a bi-enzymatic biosensor based on laccase and tyrosinase to detect carbamates, which are dangerous pesticides largely used to increment crop yields. The biosensor consisted of a hybrid film self-assembled onto a grapheme-doped carbon paste electrode, which presented good sensitivity and fast response.

Another strategy for biosensors development is to consider the electrode modification. Krzyczmonik et al. [67] produced a glucose oxidase biosensor in which the electrode was modified with poly(3,4-ethylenedioxythiophene) and polyacrylic acid doped with poly(4-lithium styrene-sulfonic acid), conferring good sensitivity and durability to the electrode.

Pure enzymes are highly selective, but they are costly and present low stability. As alternatives, crude enzyme preparations have been commercially available and scientifically exploited for devices application [61]. In our recent work [68], crude Brazilian zucchini (*Cucurbirta pepo*) extract was used as peroxidase source. The procedure to extract the enzyme was adapted from Fatibello and Vieira [69] and consisted of the adequate preparation of *C. pepo* aqueous suspension that is added to active carbon. To carry out the electrochemical experiments, carbon paste was prepared by with crude enzyme extract, carbon Vulcan XC 72R and mineral oil ca. Nujol®. The resulting emulsion was used to coat a carbon electrode. The bioelectrocatalytic property was characterized by cyclic voltammetry in the presence and absence of hydrogen peroxide. Figure 7A shows cyclic voltammetry data registered in this condition.

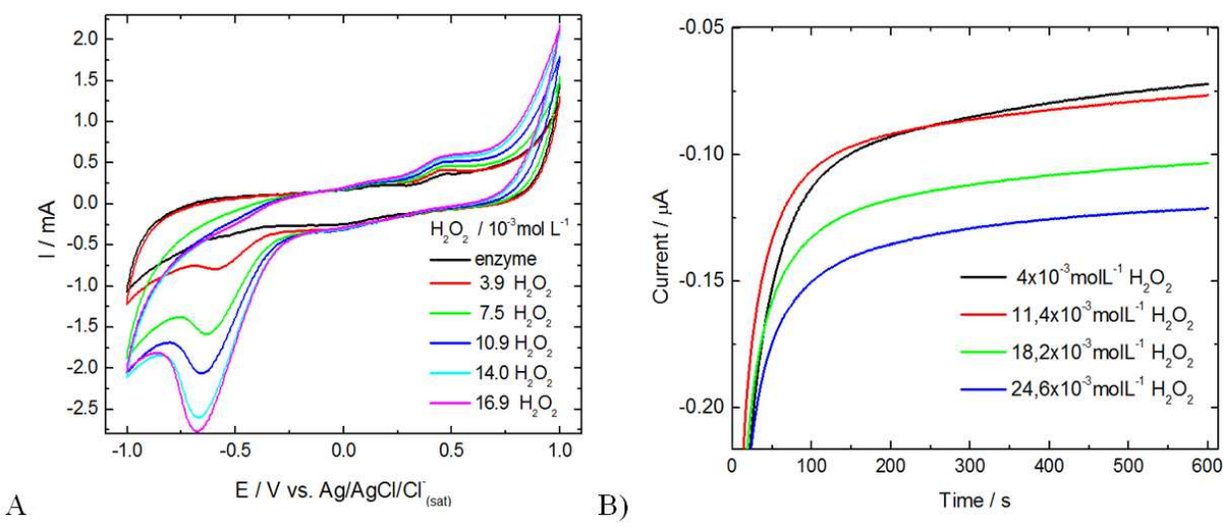

Figure 7. A) Cyclic Voltammetry of crude extract source peroxidase enzyme in buffer phosphate (pH = 6.5) in absence and in presence of H_2O_2 at distinct concentrations, v = 100m V s^{-1}, T = 25°C; B) Cronoamperometry of crude extract source of peroxidase enzyme catalysing the peroxide reduction reaction in buffer phosfate (pH = 6.5) with H_2O_2 at distinct concentrations. Electrode polarized at -0.5V vs. Ag/AgCl

In figure 7A, data shows no Faraday current in the positive scan until 0.45 V vs. Ag/AgCl/Cl-(sat). At 0.5 V, a small peak related to oxidation of some electroactive species is observed, above this potential the current increases due to enzyme oxidation process. Negative scan shows a current peak related to hydrogen peroxide reduction. The onset potential is -0.2 V and the peak current is 0.65 V. Moreover, with increase of H_2O_2 concentration in the electrolyte, the current peak increases due to the catalytically activated peroxide reduction, enabling electron transfer from the electrode to the electrochemical active species formed (reduced), which is H_2O. Since the electrode contains the crude enzymatic extract, cofactor and coenzymes are present and they enable the electron transfer. Regarding stability, cronoamperometry experiments were carried out in crude extract polarized at 0.5 V (half peak) during 10 minutes and the current decrease in different hydrogen peroxide concentrations was recorded. No current changes were observed during the experiment, as observed in Figure 7B. The crude peroxidase extract-based biosensor is easily prepared, presents low cost and is ideal to determine peroxide. Yet, in this device, reproducibility is a challenge.

Lima et al. [70] developed a biosensor based on the enzymatic extract of the Brazilian fruit pequi (Caryocar brasiliense), suitable for thiodicarb determination. In this case, the crude enzymatic extract is the source of polyphenol oxidase.

The development of an enzymatic biosensor for 3,4-dihydroxyphenyl ethylamine (dopamine) determination, based on functionalized multi-walled carbon nanotubes and horseradish peroxidase obtained from the crude extract of zucchini (Cucurbita pepo L.) as supramolecular active interface, was proposed by Ribeiro et al. [71]. In the procedure, C. pepo L. was homogenized in phosphate buffer (pH 6.5) and Polyclar SB-100 to produce the enzymatic source. In a similar procedure, Moccelini et al. [72] constructed a biosensor based on alfalfa sprout (*Medicago sativa*) as peroxidase source, also for thiodicarb determination. This enzyme was immobilized by self-assembled monolayers of l-cysteine on gold electrode.

Depending on the biosensor finality, the immobilization technique may vary, making use of a wide range of material classes, from organic polymers to metallic nanoparticles, biological materials, self-assembled materials, among others. This variety is stimulated by supramolecular approaches. Besides the above-mentioned surfaces with enzymes immobilized, carbon structures are widespread [52]. In their work, Puri et al. [47] proposed a biosensor for myoglobin detection, presenting high sensibility of 118% per decade, with high specificity. Their device consisted of a single-walled carbon nanotube (SWNT)-based label-free biosensor, containing poly(pyrrole-co-pyrrolepropylic acid) with pendant carboxyl groups electrochemically deposited on carbon structures to act as a conducting linker for immobilization, specifically of cardiac myoglobin antibodies. They performed the device characterization by source-drain current-voltage (I-V) and charge transfer studies. It presented a linear change in conductance in SWNT channel towards a wide range of myoglobin concentrations.

Lu et al. [58] proposed a graphene-functionalized self-assembled cyclodextrin for horseradish peroxidase immobilization by host-guest supramolecular strategy, to construct a biosensor for H_2O_2 determination. It was evaluated by means of electrochemical impedance spectroscopy and cyclic voltammetry, while Díez et al. [59] employed cyclodextrin combined with (3-aminopropyl)triethoxysilane-coated superparamagnetic Fe_3O_4 nanoparticles to construct

amperometric biosensors towards catechol and xanthine, since the capped- magnetic nanoparticles are able to support the host–guest supramolecular immobilization of two different enzymes, tyrosinase and xanthine oxidase. Also, Ozturk et al. [73] developed a supramolecular version of an amperometric biosensor to determine xanthine in urine samples, consisting of xanthine oxidase immobilized into Fe_3O_4 nanoparticles-modified carbon paste. Its activity is based on the electron transfer properties between the electrode components and it was characterized by cyclic voltammetry and electrochemical impedance spectroscopy. Its activity was amplified due to an increase of the electroactive surface area of the electrode with the addition of Fe_3O_4 nanoparticles and to the resulting efficient electron transfer that occurs at the solution/electrode interface. A composition of these self-assembled materials with distinct characteristics can be also explored to result in better properties and innovative biosensor applications [56, 60].

6. Photoelectrochemical biosensors

The photon-to-electricity conversion process consists of a versatile phenomenon presented by many supramolecular structures and, nowadays, have been extremely advantageous to several technological and scientific areas of application. It is based on the charge separation and subsequent charge transfer that occurs in a photoactive material after its interaction with photons of light presenting the proper energy. The electron–hole pairs that rise in the material interface are responsible for several photo-physical-chemical processes that go into the materials towards its ground and stable electronic state. Due to the increasing demand for faster and reliable bioanalysis, supramolecular compounds have been thought of as facilitators in the process to integrate new and even more sensitive and analysis and imaging techniques, in such a way that gave rise to a new sensing area, which integrates the photoelectrochemistry to bioanalysis, the photoelectrochemical (PEC) bioanalysis [74, 75].

Research in this area is recent and in the past 5 years, a variety of contributions were registered. Freeman et al. [76] produced a hybrid system based on quantum dots as doping agents of nucleic acids for photoelectrochemical biosensor application. In their approach, the recognition and catalytic properties of nucleic acids were added to the photophysical properties of a quantum dot (QD), which enabled different photophysical mechanisms, such as fluorescence emission, electron transfer quenching, as well as fluorescence resonance energy transfer (FRET) and chemiluminescence resonance energy transfer (CRET), which were exploited in a multi-parameter biosensor construction. These processes are schematically represented in figure 8.

Moreover, Zhang et al. [77] constructed a bi-enzyme-based biosensor electrostatically interacting with multi-walled carbon nanotubes (MWCNT), along with a set of bilayers consisting of MWCNT-polyethyleneimine and MWCNT-DNA supported on glassy carbon electrode for selectively detect pesticides. Using apple samples, they reported remarkable detection results, with highly discriminative signals obtained for organophosphorus pesticides towards non-organophosphorus pesticides. They employed cyclic voltammetry and UV-Vis absorption as detection methods.

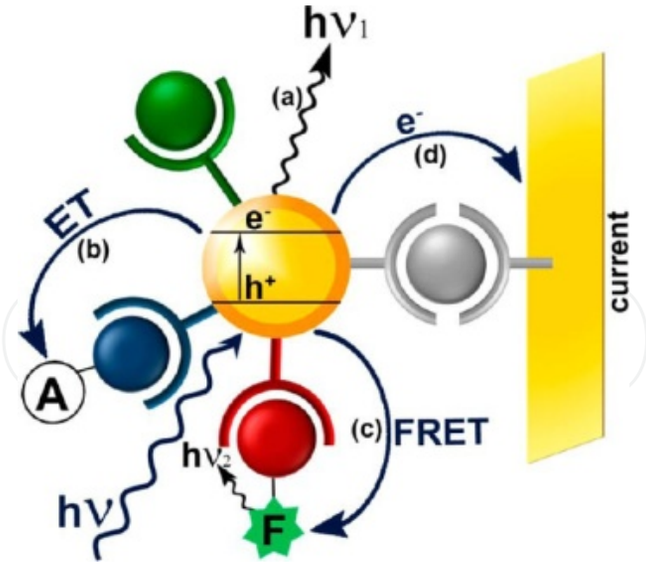

Figure 8. Scheme of QD-based sensing processes suitable for detection in Freeman´s biosensor: (a) luminescence, (b) electron transfer, (c) FRET, (d) photocurrent generation. Reprinted with permission from ref [76] *Freeman R, Girsh J, Willner I. Nucleic Acid/Quantum Dots (Qds) Hybrid Systems for Optical and Photoelectrochemical Sensing. ACS Applied Material and Interfaces. 2013, 5 (8) 2815-2849*. Copyright 2015 American Chemical Society.

These multiple detection method devices are the most reliable perspectives for biosensing application. It opens several opportunities for device designs, conserving the integrity of organisms and the individuality of each assessed environment. The detection principle is that the change of photocurrent into photopotential could be driven by biological interactions between recognition elements of distinct classes and chemical constitutions and any analyte.

In a crude way, photoelectrochemical detection is the reverse of the electrochemiluminescence process, which is applied to photovoltaics, photocatalysis, and photosynthesis, since light excites a compound and the generated photocurrent results in the detected signal. Therefore, as the same principles are applied to these distinct applications, the developments achieved in photovoltaic device fabrication could be brought about to photoelectrochemical biosensor fabrication, in an attempt to achieve low cost fabrication, enabling their commercial production and practical use [78].

Due to the versatility of excitation sources and the variety of detection techniques that can be exploited in such biosensors, they are advantageous and attractive alternatives for bioanalysis. They are becoming more popular than conventional electrochemical methods, as they present higher sensitivity and selectivity and are more simple and cheaper than optical techniques, which usually need complicated and expensive equipment.

7. Conclusion: What about the future?

Supramolecular materials-based biosensors provide a new and powerful enhancement of biosensor functionalities and construction, which encompasses distinct applications in

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diagnosis and therapeutics, as well as contribute to biological and biochemical research areas. Several electrochemical, photophysical and photochemical biosensing strategies are enabled by the unique physical-chemical properties such supramolecular compounds present, which is the reason for the rapid development of this research area.

From the achievements commented in this chapter, the share of supramolecular compounds in biosensing development is incontestable. Because of them, optical and electrochemical biosensors constitute potent and sensitive devices for *in vitro*, cellular environment and *in vivo* sensing, making use of innumerous processes that enable monitoring dynamic molecular events, living processes, degradation processes and, also, enable accurate imaging of organisms and biological processes with high precision and sensibility. The possibilities incremented by, for instance, the fluorescence spectroscopic development, giving rise to a variety of techniques that explore specific aspects of ground and excited, electronic and vibrational energy levels, open the perspective for many other materials for biosensor construction and to several other applications. Also, electrochemical sensors constructed on the supramolecular basis have enhanced the current molecular abilities of diagnosis, by enabling rapid and highly accurate responses delivery and it is thought, in the near future, to contribute to the integration of diagnostic and therapeutic techniques, enhancing medical care procedures and reliability. The possibility of combining detection techniques provide the proposal of versatile devices and enable a variety of designs and materials for biosensors, fitting clinical needs. With the analytical techniques development and the simplicity of the synthetic routines enabled by supramolecular chemistry in order to obtain pure and diverse compounds, improvements on key issues, such as sensitivity and signal-noise ratio have been fixed and the biosensors confidence have been exponentially enhanced. The perspective for the near future of biosensors application senses no limits and comprehends distinct areas such as drug delivery, therapeutics, biophysics, artificial tissue engineering, imaging, diagnosis, cellular and molecular levels exploration, as well as complete organisms, environmental studies, recycling processes development, energy harvesting and production and its limits depend almost exclusively on scientists' imagination.

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References

[1] Darwich S., Mougin K., Rao A., Gnecco E., Jayaraman S., Haidara H., Manipulation of Gold Nanoparticles with Atomic Force Microscopy in Dynamic Mode: Influence of Particle-Substrate Chemistry and Morphology, and of Operation Conditions. Nanotechnology. 2011; 2: 85–98.

[2] Steed J. W., Atwood J. L. editors. Supramolecular Chemistry. 2nd ed. Chichester: John Wiley & Sons; 2009. 970 p.

[3] Ma J. C., Dougherty D. The Cation-π Interaction. Chemical Reviews. 1997; 97: 1303–1324.

[4] Pauling L. The Nature of Chemical Bond and the Structure of Molecules and Crystals: An Introduction to Modern Structural Chemistry. Ithaca: Cornell University Press; 1939. 664 p.

[5] Lehn J. M. Cryptates: Inclusion Complexes of Macropolycyclic Receptor Molecules. Pure and Applied Chemistry. 1978; 50: 871–892.

[6] Araki K., Toma H. E. Química de Sistemas Supramoleculares Constituídos por Porfirinas e Complexos Metálicos. Química Nova. 2002; 25(6): 962–975.

[7] Dang Y. Q., Li H. W., Wu. Y. Construction of a Supramolecular Föster Resonance Energy Transfer System and Its Application Based on the Interaction Between Cy3-Labeled Melittin and Phosphocholine Encapsulated Quantum Dots. ACS Applied Materials and Interfaces. 2012; 4: 1267–1272.

[8] Ghadiali J. E., Lowe S. B., Stevens M. M. Quantum-Dot-Based FRET Detection of Histone Acetyltransferase Activity. Angewandte Chemie – International Edition. 2011; 50: 3417−3420.

[9] Biswas P., Cella L. N., Kang S. H., Mulchandani A., Yates M. V., Chen W. A Quantum-Dot Based Protein Module for *in Vivo* Monitoring of Protease Activity Through Fluorescence Resonance Energy Transfer. Chemical Communications Cambridge. 2011; 47: 5259−5261.

[10] Faul C. F. J., Antonietti M. Ionic Self-Assembling: Facile Synthesis of Supramolecular Compounds. Advanced Materials. 2003; 15(9): 673–683.

[11] Wang S., Wang L. Lanthanide-Doped Nanomaterials for Luminescence Detection and Imaging. Trends in Analytical Chemistry. 2014; 62: 123–134.

[12] Guan Y., Yu S. H., Antonietti M., Bottcher C., Faul C. F. J. Synthesis of Supramolecular Polymers by Ionic Self-Assembly of Oppositely Charged Dyes. Chemistry: An European Journal. 2005; 11: 1305–1311.

[13] Böhm I., Kreth S. K., Ritter H., Branscheid R., Kolb U. Switchable Supramolecular Crosslinking of Cyclodextrin-Modified Hyperbranched Polyethylenimine Via Anthraquinone Dyes. Macromolecular Chemistry and Physics. 2012; 213: 243–248.

[14] Wang J., Zhao X., Li J., Kuang X., Fan Y., Wei G., Su Z. Electrostatic Assembly of Peptide Nanofiber–Biomimetic Silver Nanowires onto Graphene for Electrochemical Sensors. ACS Macroletters. 2014; 3: 529–533.

[15] Habibi N., Pastorino L., Sandoval O. H., Ruggiero C. Polyelectrolyte Based Molecular Carriers: The Role of Self-Assembled Proteins in Permeability Properties. Journal of Biomaterials Applications. 2012; 28(2): 262–269.

[16] Pastorino L., Erokhina S., Soumetz F. C., Bianchini P., Konovalov O., Diaspro A., Ruggiero C., Erokhin V. Collagen Containing Microcapsules: Smart Containers for Disease Controlled Therapy. Journal Colloid Interface Science. 2011; 4: 56–62.

[17] Habibi N., Pastorino L., Soumetz F. C., Sbrana F., Raiteri R., Ruggiero C. Nanoengineered Polymeric S-layers Based Capsules with Targeting Activity. Colloids and Surfaces B: Interfaces. 2011; 88(1): 366–372.

[18] Sagadevan S., Periasamy M. Recent Trends in Nanobiosensors and Their Applications – A Review. Reviews on Advanced Materials Science. 2014; 36: 62–69.

[19] Tunceroglu A., Matsuda M., Birge R. B. Real-Time Fluorescent Resonance Energy Transfer Analysis to Monitor Drug Resistance in Chronic Myelogenous Leukemia. Molecular Cancer Therapeutics. 2010; 9: 3065–3073.

[20] Gulyani A., Vitriol E., Allen R., Wu J., Gremyachinskiy D., Lewis S., Dewar B., Graves L. M., Kay B. K., Kuhlman B., Elston T., Hahn K. M. A Biosensor Generated Via High-Throughput Screening Quantifies Cell Edge Src Dynamics. Nature Chemical Biology. 2011; 7: 437–444.

[21] Holzinger M., Le Goffand A., Cosnier S. Nanomaterials for Biosensing Applications: A Review. Frontiers in Chemistry. 2012; 2(article 63): 1–10.

[22] Liab J., Zhu J. J. Quantum Dots for Fluorescent Biosensing and Bio-imaging Applications. Analyst. 2013; 138: 2506–2515.

[23] Nguyen T. H., Ung T. D. T., Vu T. H., Tran T. K. C., Dong V. Q., Dinh D. K., Nguyen K. L. Fluorescence Biosensor Based on CdTe Quantum Dots for Specific Detection of H5N1 Avian Influenza Virus. Advances in Natural Sciences: Nanoscience and Nanotechnology. 2012; 3: 035014.

[24] Boeneman K., Delehanty J. B., Susumu K., Stewart M. H., Deschamps J. R., Medintz I. L. Quantum Dots and Fluorescent Protein FRET-Based Biosensors. Advances on Experimental Medicine and Biology. 2012; 733: 63–74.

[25] Brunetti V., Chibli H., Fiammengo R., Galeone A., Malvindi M. A., Vecchio G., Cingolani R., Nadeau J. L., Pompa P. P. InP/ZnS as a Safer Alternative to CdSe/Zns Core/

Shell Quantum Dots: *In Vitro* and *In Vivo* Toxicity Assessment. Nanoscale. 2012; 5: 307–317.

[26] Zhu J. J. Quantum Dots for Fluorescent Biosensing and Bio-imaging Applications. Analyst. 2013;138: 2506–2515.

[27] Sun Y. P., Zhou B., Lin Y., Wang W., Fernando K. A. S., Pathak P., Meziani M. J., Harruff B. A., Wang X., Wang H. F., Luo P. G., Yang H., Kose M. E., Chen B., Veca L. M., Xie S. Y. Quantum-Sized Carbon Dots for Bright and Colorful Photoluminescence. Journal of the American Chemical Society. 2006; 128: 7756–7757.

[28] Nagy A., Gemmill K. B., Delehanty J. B., Medintz I. L., Sapsford K. E. Peptide-Functionalized Quantum Dot Biosensors. IEEE Journal of Selected Topics in Quantum Electronics. 2014; 20(3): article 6900512, 1–12.

[29] Choulier L., Shvadchak V. V., Naidoo A., Klymchenko A. S., Mély Y., Altschuh D. A Peptide-Based Fluorescent Ratiometric Sensor for Quantitative Detection of Proteins. Analytical Biochemistry. 2010; 401: 188–195.

[30] Kim J. H., Lim S. Y., Nam D. Y., Ryu J., Ku S. H., Park C. B. Self-Assembled, Photoluminescent Peptide Hydrogel as a Versatile Platform for Enzyme-Based Optical Biosensors. Biosensors and Bioelectronics. 2011; 26: 1860–1865.

[31] Souza G. A. Caracterização Fotofísica e Morfológica de Estruturas Peptídicas Contendo Composto Fluorescente para Aplicação Ambiental. [dissertation]. Goiânia, Brazil. Federal University of Goiás; 2014. 110 p.

[32] Robinson J. T., Hong G., Liang Y., Zhang B., Yaghi O. K., Dai H. *In Vivo* Fluorescence Imaging in the Second Near-Infrared Window with Long Circulating Carbon Nanotubes Capable of Ultrahigh Tumor Uptake. Journal of American Chemical Society. 2012; 134: 10664–10669.

[33] Kruss S., Hilmer A. J., Zhang J., Reuel N. F., Mu B., Strano M. S. Carbon Nanotubes as Optical Biomedical Sensors. Advanced Drug Delivery Reviews. 2013; 65: 1933–1950.

[34] Shi J., Guo J., Bai G., Chan C., Liu X., Ye W., Hao J., Chen S., Yang M. A Grapheme Oxide Based Fluorescence Resonance Energy Transfer (FRET) Biosensor for Ultra Sensitive Detection of Botulinum Neurotoxin A (Bont/A) Enzymatic Activity. Biosensors and Bioelectronics. 2015; 65: 238–244.

[35] Tao Y., Lin Y., Re J., Qu X. Self-Assembled, Functionalized Graphene and DNA as a Universal Platform for Colorimetric Assays. Biomaterials. 2013; 34: 4810–4817.

[36] Ma H., Wu D., Cui Z., Li Y., Zhang Y., Du B., Wei Q. Graphene-Based Optical and Electrochemical Biosensors: A Review. Analytical Letters. 2012; 46(1): 1–17.

[37] Shao Y., Wang J., Wu H., Liu J., Aksay I. A., Lina Y. Graphene Based Electrochemical Sensors and Biosensors: A Review. Electroanalysis. 2010; 22(10): 1027–1036.

[38] Kim C. J., Lee D. I., Kim C., Lee K., Lee C. H., Ahn I. S. Gold Nanoparticles-Based Colorimetric Assay for Cathepsin B Activity and the Efficiency of Its Inhibitors. Analytical Chemistry. 2014; 86: 3825−3833.

[39] Wang J., Song D., Zhang H., Zhang J., Jin Y., Zhang H., Zhou H., Sun Y. Studies of Fe_3O_4/Ag/Au Composites for Immunoassay Based on Surface Plasmon Resonance Biosensor. Colloids and Surfaces B: Biointerfaces. 2013; 102: 165–170.

[40] Wang L., Sun Y., Wang J., Wang J., Yu A., Zhang H., Song D. Preparation of Surface Plasmon Resonance Biosensor Based on Magnetic Core/Shell Fe_3O_4/SiO_2 and Fe_3O_4/Ag/SiO_2 Nanoparticles. Colloids and Surfaces B: Biointerfaces. 2011; 84: 484–490.

[41] Dias D. L. Espectroscopia de Fluorescência Aplicada ao Estudo das Interações de Formação de Nanoestruturas de Peptídeos Contendo Maguemita. [dissertation]. Goiânia, Brazil. Federal University of Goiás; 2014. 92 p.

[42] Wang C., Tang Y., Liu Y., Guo Y. Water-Soluble Conjugated Polymer as a Platform for Adenosine Deaminase Sensing Based on Fluorescence Resonance Energy Transfer Technique. Analytical Chemistry. 2014; 86(13): 6433–6438.

[43] Wong L. S., Wong C. S. A New Method for Heavy Metals and Aluminum Detection Using Biopolymer-Based Optical Biosensor. IEEE Sensors Journal. 2015; 15(1): 471–475.

[44] Hosseini S., Ibrahim F., Djordjevic I., Rothan H. A., Yusof R., Van der Marel C., Benzinae A., Koole L. H. Synthesis and Characterization of Methacrylic Microspheres for Biomolecular Recognition: Ultrasensitive Biosensor for Dengue Virus Detection. European Polymer Journal. 2014; 60: 14–21.

[45] Wang C., Tang Y., Guo Y. Adenosine Deaminase Biosensor Combining Cationic Conjugated Polymer-Based FRET with Deoxyguanosine-Based Photoinduced Electron Transfer. ACS Applied Materials & Interfaces. 2014; 6(23): 21686–21691.

[46] Chen B., Liu C., Hayashi K. Selective Terpene Vapor Detection Using Molecularly Imprinted Polymer Coated Au Nanoparticle LSPR Sensor. IEEE Sensors Journal. 2014; 14(10): 3458–3464.

[47] Puri N., Niazi A., Biradar A. M., Mulchandani A., Rajesh. Conducting Polymer Functionalized Single-Walled Carbon Nanotube Based Chemiresistive Biosensor for the Detection of Human Cardiac Myoglobin. Applied Physics Letters. 2014; 105(15): 153701–153705.

[48] Rybtchinski B. Adaptive Supramolecular Nanomaterials Based on Strong Noncovalent Interactions. ACS Nano. 2011; 5 (9): 6791–6818.

[49] Zhang L., Qi H., Hao J., Yang L., Yu P., Mao L. Water-Stable, Adaptive, and Electroactive Supramolecular Ionic Material and Its Application in Biosensing. ACS Applied Materials and Interfaces. 2014; 6: 5988−5995.

[50] Kim D. H., Ghaffari R., Lu N., Wang S., Lee S. P., Keum H., D'Angelo R., Klinker L., Su Y., Lu C., Kim Y. S., Ameen A., Li Y., Zhang Y., Graff B., Hsu Y. Y., Liu Z., Ruskin J., Xu L., Lu C., Omenetto F. G., Huang Y., Mansour M., Slepian M. J., Rogers J. A. Electronic Sensor and Actuator Webs for Large-Area Complex Geometry Cardiac Mapping and Therapy. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109: 19910–19915.

[51] Ramuz M., Tee B. C., Tok J. B., Bao Z. Transparent, Optical, Pressure-Sensitive Artificial Skin for Large-Area Stretchable Electronics. Advanced Materials. 2012; 24: 3223–3230.

[52] Bahadir E. B., Szgintürk M. K. Electrochemical Biosensors for Hormone Analyses. Biosensors and Bioelectronics. 2015; 68: 62–71.

[53] Neto S. A., Almeida T. S., Belnap D. M., Minteer S. D., Andrade A. R. Enhanced Reduced Nicotinamde Adenine Dinucleotide Eletrocatalysis onto Multi-Wall Carbon Nanotubes-Decorated Gold Nanoparticles and Their Use in Hybrid Biofuel Cell. Journal of Power Sources. 2015; 273: 1065–1072.

[54] Albareda-Sirvent M., Merkoçi A., Alegret S. Configurations Used in the Design of Screen-Printed Enzymatic Biosensors. A Review. Sensors and Actuators B. 2000; 69: 153–163.

[55] [55]Mendes R. K., Carvalhal R. F., Kubota L. T. Effects of Different Self-Assembled Monolayer on Enzyme Immobilization Procedures in Peroxidase-Based Biosensor Development. Journal of Electroanalytical Chemistry. 2008; 612(2): 164–172.

[56] Diez P., Piuleac C. G., Martinez-Ruiz P., Romano S., Gamella M., Villalonga R., Pingarrón J. M. Supramolecular Immobilization of Glucose Oxidase on Gold Coated with Cyclodextrin-Modified Cysteamine Core PAMAM G-4 Dendron/Pt Nanoparticles for Mediatorless Biosensor Design. Analytical and Bioanalytical Chemistry. 2013; 405(11): 3773–3781.

[57] Sioniewska A., Palys B. Supramolecular Polyaniline Hydrogel as a Support for Urease. Electrochimica Acta. 2014; 126 (Special Issue-SI): 90–97.

[58] Lu L. M., Qiu X. I., Zhang X. B., Shen G. L., Tan W., Yu R. Q. Supramolecular Assembly of Enzyme on Functionalized Graphene for Electrochemical Biosensing. Biosensors and Bioelectronics. 2013; 45: 102–107.

[59] Díez P., Villalonga R., Villalonga M. L., Pingarrón J. M. Supramolecular Immobilization of Redox Enzymes on Cyclodextrin-Coated Magnetic Nanoparticles for Biosensing Applications. Journal of Colloid and Interface Science. 2012; 386: 181–188.

[60] Villalonga R., Diez P., Eguilaz M., Martínez P., Pingarrón J. M. Supramolecular Immobilization of Xanthine Oxidase on Electropolymerized Matrix of Functionalized Hybrid Gold Nanoparticles/Single-Walled Carbon Nanotubes for the Preparation of

Electrochemical Biosensors. ACS Applied Materials & Interfaces. 2012; 4(8): 4312–4319.

[61] Roy J. J., Abraham T. E. Strategies in Making Cross-Linked Enzime Crystals. Chemical Reviews. 2004; 104(9): 3705–3727.

[62] Alves W. A., Fiorito P. A., Torresi S. I. C., Torresi R. M. Design of Molecular Wires Based on Supramolecular Structures for Application in Glucose Bionsensors. Biosensors and Bioelectronics. 2006; 22: 298–305.

[63] Arya S. K., Kongsuphol P., Wong C. C., Polla L. J., Park M. K. Label Free Biosensor for Sensitive Human Influenza Virus Hemagglutinin Specific Antibody Detection Using Coiled-Coil Peptide Modified Microelectrode Array Based Platform. Sensor and Actuators B. 2014; 194: 127–133.

[64] Barton S. C., Gallaway J., Atanassov P. Enzimatic Biofuel Cells for Implantable and Microscale Devices. Chemical Reviews. 2004; 104(9): 4867–4886.

[65] Attar A., Cubillana-Aguilera L., Naranjo-Rodríguez I., de Cisceros J. L., Palacios-Santander J. M., Amine A. Amperometric Inhibition Biosensors Based on Horseradish Perxidase and Gold Sononanoparticles Immobilized onto Different Electrodes for Cyanide Measurements. Bioelectrochemistry. 2015; 101: 84–91.

[66] Oliveira T. M. B. F., Barroso M. F., Morais S., Araújo M., Freire C., Lima-Neto P., Correia A., Oliveira M. P. P. P., Delerue-Matos C. Sensitive Bi-enzymatic Biosensor Base on Polyphenoloxidases-Gold Nanopartilcles-Chitonan Hybrid Film-Graphene Doped Carbon Paste Electrode for Carbamates Detection. Bioelectrochemistry. 2015; 98: 20–29.

[67] Krzyczmonik P., Socha E., Skrzyped S. Immobilization of Glucose Oxidase on Modified Electrode with Composite Layers Based on Poly(3, 4-Ethylenedioxythiophene). Bioelectrochemisty. 2015; 101: 8–13.

[68] YI L. H., Colmati F. Estudo da Bioeletroredução de Peróxido de Hidrogênio Catalisada por Extrato Bruto Fonte de Enzima Peroxidase. In: I Seminário de Iniciação em Desenvolvimento Tecnológico e Inovação/VIII Congresso de Pesquisa, Ensino e Extensão da UFG/63a Reunião Anual da SBPC, 10–15 July 2011. Goiânia, Brazil. editor. VIII Congresso de Pesquisa, Ensino e Extensão da UFG/63a Reunião Anual da SBPC; 2011.

[69] Fatibello-Filho O., Vieira I. C. Uso Analítico de Tecidos e de Extratos Brutos Vegetais como Fonte Enzimática. Química Nova. 2002; 25(3): 455–464.

[70] Lima F., Lucca B. G., Barbosa A. M. J., Ferreira V. S., Moccelini S. K., Franzoi A., Vieira I. Biosensor Based on Pequi Polyphenol Oxidase Immobilized on Chitosan Crosslinked with Cyanuric Chloride for Thiodicarb Determination. Enzyme and Microbial Technology. 2010; 47: 153–158.

[71] Ribeiro F. A. S., Tarley C. R. T., Borges K. B., Pereira A. C. Development of a Square Wave Voltammetric Method for Dopamine Determination Using a Biosensor Based

on Multiwall Carbon Nanotubes Paste and Crude Extract of Cucurbita Pepo L. Sensors and Actuators B. 2013; 185: 743–754.

[72] Moccelini S. K., Vieira I. C., Lima F., Lucca B. G., Barbosa A. M. J., Ferreira V. S. Determination of Thiodicarb Using a Biosensor Based on Alfalfa Sprout Peroxidase Immobilized in Self-Assembled Monolayers. Talanta. 2010; 82: 164–170.

[73] Öztürk F. O., Erden P. E., Kaçar C., Kiliç E. Amperometric Biosensor for Xanthine Determination Based on Fe_3O_4 Nanoparticles. Acta Chimica Slovenica. 2014, 61, 19–26.

[74] Zhao W. W., Xu J. J. Chen H. Y. Photoelectrochemical DNA Biosensors. Chemical Reviews. 2014; 114(15): 7421−7441.

[75] Zhao W. W., Xiong M., Li X. R., Xu J. J., Chen H. Y. Photoelectrochemical Bioanalysis: A Mini Review. Electrochemistry Communications. 2014; 38: 40–43.

[76] Freeman R., Girsh J., Willner I. Nucleic Acid/Quantum Dots (QDs) Hybrid Systems for Optical and Photoelectrochemical Sensing. ACS Applied Material and Interfaces. 2013; 5(8): 2815–2849.

[77] Zhang Y., Arugula M. A., Wales M., Wild J., Simonian A. L. A Novel Layer-by-Layer Assembled Multi-enzyme/CNT Biosensorfor Discriminative Detection Between Organophosphorus and Non-organophosphrus Pesticides. Biosensors and Bioelectronics. 2014; S0956-5663(14): 00624–00625.

[78] Yue Z., Lisdat F., Parak W. J., Hickey S. G., Tu L., Sabir N., Dorfs D., Bigall N. C. Quantum-Dot-Based Photoelectrochemical Sensors for Chemical and Biological Detection. ACS Applied Materials and Interfaces. 2013; 5: 2800−2814.