Conjugated and nonconjugated redox polymers for immobilization and charge transfer in oxidoreductase-based electrochemical enzymatic biosensors

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7.1 Introduction

Biosensors have a crucial role in modern life in detecting chemical compounds, biomolecules, or cells in complex media [1,2]. Traditional analytical methods have limitations, such as the need for qualified personnel, expensive equipment, and large sample volumes [1]. Biosensors have emerged as a solution to these challenges, enabling complex analytical measurements to be carried out in simple and easy-to-use formats. The presence of a bioreceptor allows for high selectivity and specificity whereas the transducer converts the recognition event into a measurable signal and determines the sensitivity of the device (Fig. 7.1a–c) [3–5]. There are various transduction mechanisms for biosensors, based on optical, thermal, mechanical, magnetic, and electrochemical signals, among others [6–8]. Electrochemical biosensors have become popular owing to their advantages, such as robustness, stability, reproducibility, low detection limits, a fast response, a low sample volume, easy miniaturization, and the ability to be used in complex media (Fig. 7.1d and e) [6,9–11]. Electrochemical biosensors can detect electrical signals generated by interactions between biomolecules and target analytes in solution. Amperometry and potentiometry are the most commonly used transduction methods, followed by conductometric and impedance spectroscopy [7,11,12]. These electrochemical devices have emerged as a promising technology for various applications, including food safety, clinical diagnosis, and pathogen and toxin detection, and can be integrated into wearable, portable,

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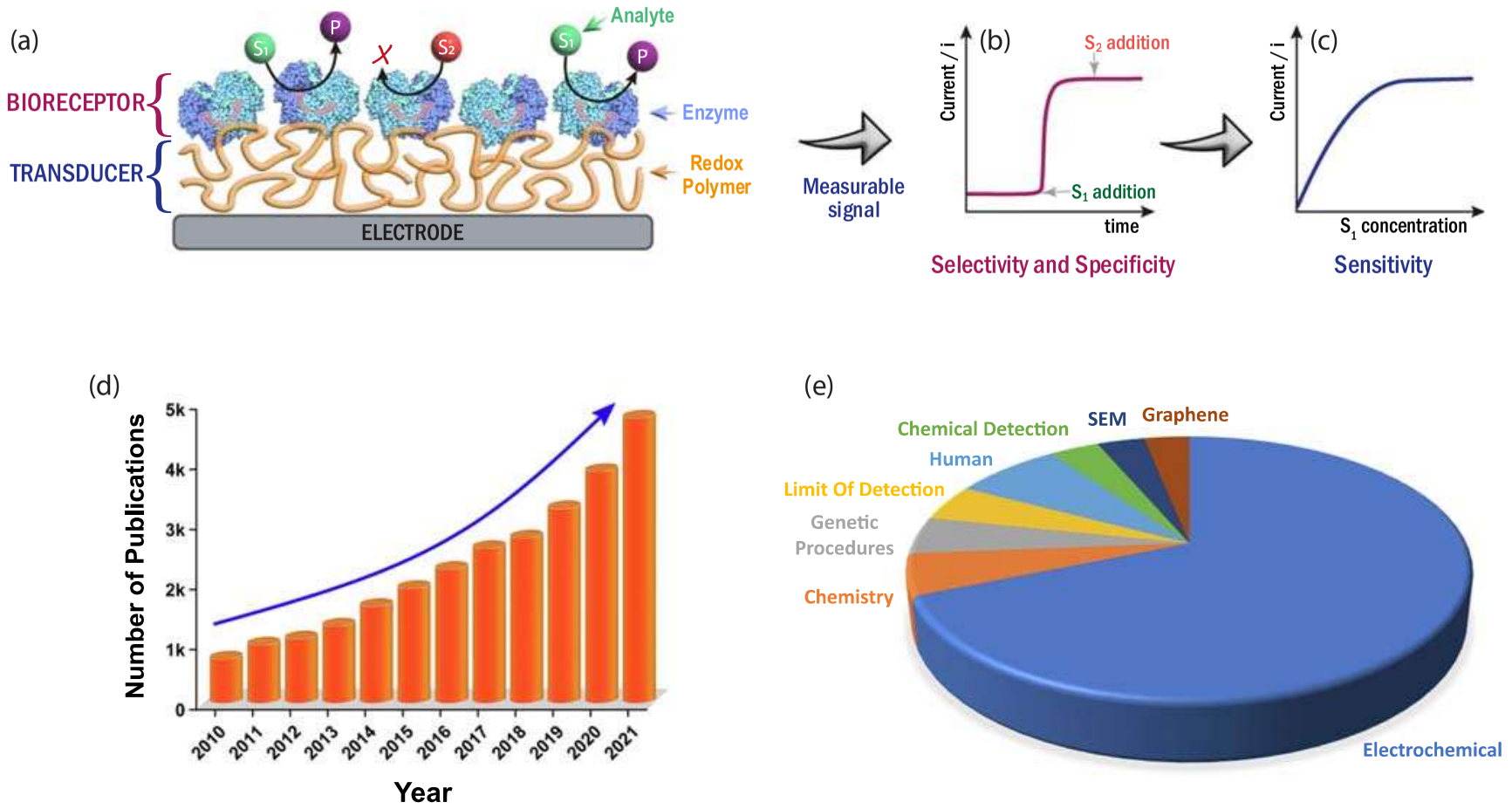

Figure 7.1 (a) Components of an amperometric biosensor based on enzymes and redox polymers. (b) The enzyme catalyzes the reaction of S_1 to P, yielding a current response, whereas no response is observed upon the addition of S_2. (c) The variation in current with the concentration typically yields curves that reflect Michaelis–Menten kinetics. (d) Number of publications in electrochemical biosensors since 2010. (e) Pie chart of main keywords in biosensor publications. *SEM,* scanning electron microscopy.
Data were obtained from the Scopus database since 2010 with "electrochemical biosensor" and "biosensor" queries.

and reusable devices. However, a significant challenge facing electrochemical biosensors is their ability to function accurately in real-world samples while maintaining all of their attributes and capabilities.

Enzymes are essential biological molecules that catalyze specific biochemical reactions. Their unique properties make them ideal bioreceptors in biosensors. Among the various classes of enzymes, oxidoreductases are particularly useful for electrochemical biosensors because they facilitate reactions involving electron transfer [13]. The first commercial glucose amperometric biosensor, which revolutionized the health care industry, used glucose oxidase (GOx) as the bioreceptor [14]. Likewise, electrode materials have undergone significant developments over the years. Precious metals such as gold and platinum were initially used, followed by conductive doped ceramics such as indium tin oxide (ITO). The advent of screen-printed electrodes allowed for easily mass-produced carbon materials to be employed [15]. However, enzymes have a limited life span and are susceptible to changes in temperature, pH, and ionic strength, which makes electrode modification a crucial process in electrochemical biosensors [16]. This is why the combination of enzymes and polymeric matrices is greatly relevant. To date, polymer-based electrodes with different biorecognition elements and immobilization techniques have been extensively studied, in which polymers containing redox molecules are the most relevant. Redox polymers, with their ability to facilitate charge transport, are an attractive alternative to traditional transducer materials in electrochemical devices. These polymers are classified by the International Union of Pure and Applied Chemistry (IUPAC) into two categories based on their chemical structure and electron transport mechanisms: polymers with a conjugated backbone (also called conducting or semiconducting polymers) and polymers with redox-active pendant groups (also known as plain redox polymers) [17–19]. For clarity, this chapter will use the terms conjugated redox polymer (CRP) and nonconjugated redox polymer (NCRP) to refer to these respective categories. This chapter compares the two categories of redox polymers when used with oxidoreductases in biosensing, beginning with their respective charge transport mechanisms and immobilization on electrode surfaces with enzymes. The chapter then explores interactions between the enzymes and redox polymers, specifically in their contribution to different sensing mechanisms. Finally, we present several examples of bioanalytical systems that use different types of enzymes in conjunction with redox polymers for biosensing.

7.2 Charge transport mechanisms

7.2.1 Charge transport in conjugated redox polymers

In polymers with conjugated backbones, electrons can delocalize, offering the possibility of charge mobility [20]. In the ground state, however, most of these polymers are not intrinsically conductive, but rather semiconductors with bandgaps ranging from $\sim$1.5 eV for polyacetylene to $\sim$3.2 eV for polyaniline (PANI), for example [21]. This bandgap arises from the Peierls distortion, which establishes that, despite having an unpaired electron per atom, the most stable configuration for these

unidimensional systems is an alternate sequence of single and double bonds, as opposed to all bonds being of equal energy (and length) [22]. If switching which bonds are single and which are double does not affect the ground state energy of the polymer, it is said to be degenerate (like polyacetylene). If, on the other hand, the chemical structure of the polymer causes differences in energy when switching the location of the double and single bonds, the polymer is said to be nondegenerate (e.g., polythiophene, polypyrrole [PPy], and PANI) [23].

Upon oxidation or reduction, charges can be generated in the conjugated chain. For degenerate conjugated polymers, anions (negative solitons) or cations (positive solitons) are formed with states in the center of the energy bandgap [23]. In nondegenerate polymers, redox processes produce radical cations (positive polarons), or radical anions (negative polarons) [20]. Further oxidation or reduction can create dications (positive bipolarons) or dianions (negative bipolarons). In these cases, two bands are created in the space between the conduction and valence bands of the ground state. All of these entities (classified by physics as quasiparticles), can serve as charge carriers for band conduction in a CRP. Therefore, oxidized or reduced conjugated polymers have electrical conductivities orders of magnitude higher than their neutral counterparts and are said to be doped. Doping can take place chemically or electrochemically. Either way, the charge acquired by the polymer must be balanced by a counterion that forms part of the doping process.

The effect of electrochemical doping on the conductivity is usually characterized by depositing a film of the conjugated polymer over two (commonly interdigitated) electrodes. A small potential difference is applied between them to measure the film conductivity. Simultaneously, the potential of both electrodes is changed with respect to a reference electrode to oxidize or reduce the polymer, measuring the faradaic current using a separate counter-electrode [24,25]. Usually, the potential is swept in the positive direction, causing an increase in conductivity concomitant with the oxidation current (Fig. 7.2a). If excessive oxidation is carried out, the polymer becomes nonconductive. This decrease in conductivity can be reversibly caused by electrostatic interactions associated with highly packed charge carriers. At extremely high potentials, irreversible degradation of the polymers causes a permanent loss of conductivity.

Owing to their conductivity, these materials have been referred to as semiconducting, conducting, and even metallic polymers. An exploration of the temperature dependence of their electrical conductivity helps in the discussion of the most appropriate designation. Most conjugated polymers show an increase in conductivity when heated, which is expected for semiconductor materials [26]. Only a few reports have shown a decrease in resistivity when lowering the temperature, as expected for true metallic behavior [27,28]. This can be rationalized in terms of the degree of order in the conjugated polymers. Compared with metals, which are mostly crystalline, conjugated polymers have significantly more disorder and defects. These, together with the finite length of the polymer chains, create the localization of some of the states. Electron movement between these localized states does not take place through band conduction, but rather through hopping [29]. This is a slower process and ultimately limits conductivity in conjugated polymers. Besides in-chain defects, spatial order between chains affects conductivity, in which more crystalline polymers are more conductive than

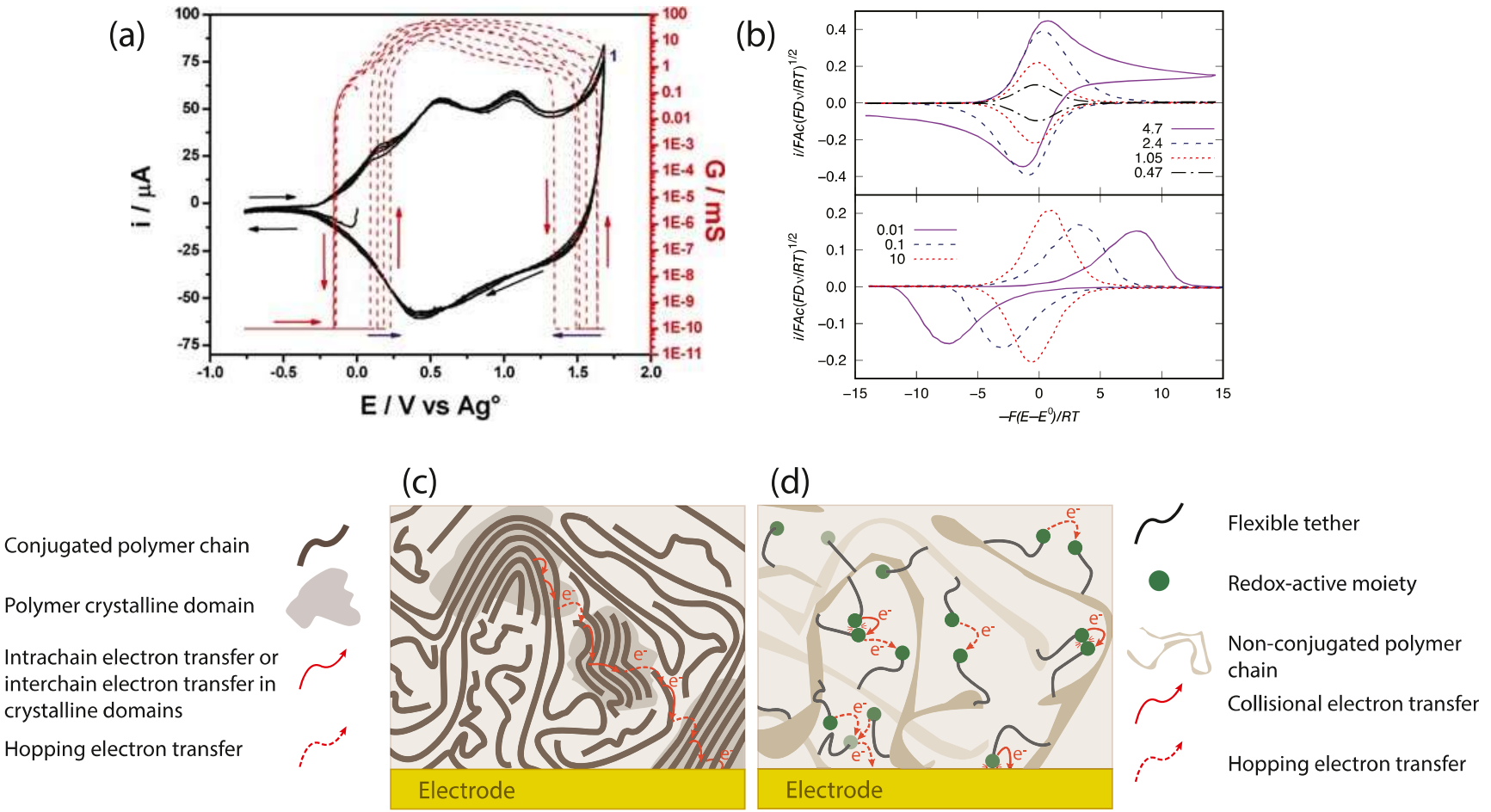

Figure 7.2 (a) Experimental cyclic voltammograms and conductance measurements of a poly-3,4-*ortho*-xylendioxythiophene layer deposited on a Pt interdigitated electrode microarray. (b) Calculated cyclic voltammograms of a nonconjugated redox polymer layer expressed in terms of dimensionless currents and potentials, showing the transition between thin-layer and diffusion-limited regimes. The top panel shows the reversible case for different values of the parameter $\delta(Fv/RTD_e)^{1/2}$. The bottom panel shows the quasireversible case for different values of the parameter $k_s(RT/D_e v)^{1/2}$. k_s is the standard rate constant of electron transfer at the electrode surface. (c and d) Schematics of electron transport across conjugated (c) and nonconjugated (d) redox polymers deposited on an electrode.
(a) Reprinted from G. Salinas, J.-A. Del-Oso, P.-J. Espinoza-Montero, J. Heinze, B.A. Frontana-Uribe, Electrochemical polymerization, characterization and in-situ conductivity studies of poly-3,4-ortho-xylendioxythiophene (PXDOT), *Synth. Met.* 245 (2018) 135−143, Copyright (2018), with permission from Elsevier. (c) Adapted from C.P. Andrieux, J.M. Savéant, Electron transfer through redox polymer films, *J. Electroanal. Chem.* 111 (1980) 377−381, Copyright (1980), with permission from Elsevier.

amorphous ones [30]. In fact, the structure of conjugated polymers has been described as metallic (ordered) islands in a semiconducting (disordered) matrix [24]. Electron transfer in a delocalized polymer chain and between chains in a π-stacked ordered region is faster than between ordered regions or between an ordered region and a chain in a disordered region (Fig. 7.2c) [31]. Further support for this view comes from the observation that adding solvents to the polymers (solvent annealing) can increase the conductivity by several orders of magnitude. This procedure has also been shown to shift the transition between an insulating/conductive character to more negative potentials, increasing the useful potential window [32].

7.2.2 Charge transport in nonconjugated redox polymers

NCRPs include a polymeric backbone with redox moieties as pendant groups. Vinyl polymers, poly(ethylenimine) (PEI), poly(allylamine), and even polysiloxanes [33] and polymeric ionic liquids [19] have been reported. In NCRPs, spatially localized redox sites are separated by a given distance (d). These redox sites are not electronically connected by the conjugation of bonds described in the previous section. Therefore, band conduction is not possible. Rather, self-exchange reactions between similar redox sites (except for their oxidation state) are responsible for electron transport. Dahms and Ruff laid the foundations of this theory by describing electron transfer reactions between redox sites of molecules dissolved in solution [34–37]. They observed that the self-exchange resembled a diffusive process that, added to the actual physical movement of these redox molecules in the solution, contributed to their apparent diffusion. Because of the high mobility of the molecules in solution, electron exchange was explained in terms of collision theory. Subsequently, others applied this theory to experiments on polymer layers that included electrostatically trapped coordination ions. Considering that these redox centers could be fixed or have a limited movement compared with ions in solution, the contribution to the self-exchange reaction was dominant over actual molecular diffusion [38–41]. Moreover, because of the reduced mobility, collisions between electroactive molecules were less likely than in solution, and the researchers hypothesized that electron hopping between neighboring redox sites could be an important mechanism contributing to charge transfer in these films [42,43]. Nowadays, both collisions and electron hopping are regarded as contributing to the charge transport mechanism in polymers containing electroactive moieties as pendant groups (Fig. 7.2d). The contribution of each mechanism depends on factors such as the mobility of the redox moieties (via the flexible tether attaching it to the polymer backbone) and the separation between them [18].

Self-exchange charge transport is an outer sphere phenomenon that is feasible because the products and reactants are the same molecule, except for the different oxidation states [44]. Self-exchange electron transfer reactions occur in random directions throughout the polymeric network as a response to concentration gradients or, more appropriately, to electrochemical potential gradients generated by the potential imposed on the electrode, phenomenologically obeying Fick's laws derived for freely diffusing molecules in solution [43]. The rate at which this process occurs is defined by

the apparent electron diffusion coefficient (D_e) and is limited by the separation between redox sites. Despite numerous reports in the literature and the use of simulation models [45–47], this charge transport phenomenon is not fully understood. It is known that some of the rate constants of the redox sites in these polymeric matrices can decrease up to 10^4 times compared with their rates in solution, regardless of the type of redox center used [46]. In addition, when redox polymers are incorporated into more structured systems (e.g., hydrogels), the electron transfer mechanism can become more complex. Because these hydrogels are three-dimensional (3D) matrices that swell and shrink depending on their water content, inertial and surface forces have an important role in the dynamics of the polymer chains, generating cooperative diffusion [46,48].

As in electroactive molecules diffusing in solution, charge transfer in electrochemical experiments using electrodes modified with NCRPs can be limited by the depletion of the reactant (so-called thin-layer regime [49]) or diffusion. The simplest and most commonly used method to determine the process that limits the system is to perform cyclic voltammetry measurements at different scan rates. If there is a linear dependence of the peak current (i_p) with the scan rate (v), the system is bounded by a thin-layer process, following the equation [50]:

$$i_p = \frac{n^2F^2}{4RT} vVc \tag{7.1}$$

where n is the number of electrons, F is the Faraday constant, R is the gas constant, T is the temperature, V is the volume of the thin (polymer) layer, and c is the concentration of electroactive pendant groups. This equation is similar to the one employed for the case of reactants immobilized (typically adsorbed) on the electrode surface [51]. The only difference is the replacement of the product Vc for $A\Gamma$, where A is the electrode area and Γ is the surface concentration of redox moieties. Obviously, both quantities are equal to the total number of redox sites in the film. Another characteristic of this regime is that the peak potential difference (ΔE_p) tends toward zero (Fig. 7.2b).

Conversely, if the dependence of i_p is linear with respect to the square root of the scan rate, the process is diffusion-limited and follows the Randles-Sevcik equation:

$$i_p = 2.69x10^5 n^{3/2} F^{3/2} AcD_e{}^{1/2} v^{1/2} \tag{7.2}$$

This diffusive-like charge transport is more commonly observed because the thickness of polymeric films (δ) tends to be larger than the thickness of the diffusion layer in most practical cases. Because of the difficulty of quantifying the concentration of redox sites in the polymer layer, the product $c(D_e)^{1/2}$ is commonly used. There are several ways to calculate this product. Among the most common, we can find (a) cyclic voltammetry, plotting the dependence of the peak current (i_p) versus the scan rate, using Eq. (7.2); (b) electrochemical impedance spectroscopy, analyzing the behavior of the Warburg element; and (c) chronoamperometry using the Cotrell

equation. A given system can switch between thin layer and diffusion-limited regimes, depending on parameters such as the layer thickness, the apparent electron diffusion coefficient, and the time scale of the experiment.

7.2.3 Immobilization and deposition of enzymes and redox polymers on electrode surfaces

In biosensor design and fabrication, immobilization can be interpreted as the confinement of molecules in a defined space, preserving their functionality. Electrode modification with biological receptors is an important process to improve sensitivity and selectivity in the construction of biosensors. The method used for immobilization depends on the type of receptor [52,53]. In the case of enzymes, maintaining catalytic activity for repeated and/or continuous use is highly desirable. Compared with redox polymers, the higher complexity of the enzymes makes them more vulnerable to the impacts of the immobilization process. Some adverse effects are changes in their 3D conformation or modification of their reactivity, all of which can result in a decrease in their catalytic activity. On the other hand, benefits include enzyme reuse, increased stability or resistance to factors such as temperature, pH, and solvents, higher activity per unit volume, and, in some cases, improved selectivity.

It is possible to immobilize the enzymes and redox polymers on the electrode surface through a variety of physical or chemical interactions. These include van der Waals and hydrophobic forces, electrostatic attraction, and covalent bonds. In general, physical methods are considered to be the least aggressive option toward the molecular structure. Their most representative examples in enzyme immobilization include adsorption, encapsulation, and entrapment in matrices of different natures (often polymeric). Using these methodologies, the enzyme is as close as possible to its native state. However, interactions between the enzyme and the surrounding matrix are not strong. These systems are prone to enzyme leakage when the device is in contact with aqueous media during use. Compared with physical methods, chemical methods bind molecules to the electrode surface by employing stronger (covalent) interactions. This can lead to structural alterations and, in the case of enzymes, a loss of catalytic activity if necessary care is not taken. Direct covalent bonding, cross-linking, and affinity ligand conjugation are some examples of this group of immobilization strategies.

7.2.4 Covalent bonding

Covalent and coordination bonds are strong interactions between atoms that share electrons in their valence orbitals. In polymer and enzyme immobilization, covalent bonding is used in two different strategies: covalent cross-linking and direct covalent bonding.

Covalent cross-linking is one of the most widely used methods for enzyme immobilization on electrodes. In general, this method is based on the union of two or more molecules through covalent bonds, forming hierarchical structures at the nanoscale and microscale. 3D structures can provide many benefits to the immobilized enzymes, but

they can also be detrimental if the proper conditions are not used [54,55]. The reagents used for this method are cross-linking molecules that contain two or more terminal groups that are highly reactive toward specific functional groups (e.g., primary amines, hydroxyls, sulfhydryls, carboxylic acids) found in proteins or polymeric structures [56,57]. Cross-linkers can generate intramolecular and intermolecular (enzyme−enzyme, enzyme−polymer, or polymer−polymer) bonds. The selection of cross-linkers will depend on the characteristics of the system and its own properties, such as chemical specificity and spontaneity, chain length, and water solubility [58].

Normally, the combination of cross-linkers in conjunction with nonconjugated polymers is useful for incorporating enzymes into the matrices formed to provide greater protection and favorable microenvironments, although conjugated polymers are also used to a lesser extent. Upon cross-linking at the electrodes, the matrix formed becomes insoluble, and a variety of structures can be obtained, ranging from soft jelly-like structures (hydrogels) to more rigid membrane-like structures, depending on the degree of cross-linking [59]. Regardless of the rigidity, these matrices can hydrate and swell to some degree in water, improving their electron transport compared with their dry state.

A large variety of cross-linking strategies have been developed in polymer science [60]. In electrodes modified with enzymes and redox polymers, the focus has been on the reactivity of amine and carboxyl groups present in these molecules [58,61]. Dialdehydes, such as terephthalaldehyde and glutaraldehyde (GA), are frequently used cross-linkers for these groups, and GA is the most popular [62]. GA reacts aggressively mainly with primary amines, forming Schiff bases and/or secondary amines from addition reactions with GA oligomers [63−65]. These characteristics make GA one of the best options for creating stable polymeric matrices. However, its high toxicity has prompted the search for alternatives when working with biological or cellular systems. Therefore, diepoxides such as ethylene glycol diglycidyl ether and poly(ethylene glycol) diglycidyl ether (PEGDGE), have become the reference standard as cross-linking agents. Although their epoxy end groups are less reactive with primary amines than GA, they have the advantage of being biocompatible. A less popular strategy, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in combination with *N*-hydroxysuccinimide (NHS), generates the activation of carboxylated molecules to conjugate them with primary amines [66]. Because the EDC and NHS moieties are not retained in the final product, this strategy generates a smaller separation between molecules compared with dialdehydes and diepoxides [67].

Many combinations using different NCRPs and CRPs have been evaluated with these cross-linkers. Some representative examples of nonconjugated polymers are poly(allylamine) (PAH) [68,69], PEI in its linear form [70], or branched form [71,72], poly(vinyl pyridine) [73], poly(vinyl alcohol) [74], and chitosan [75,76], modified with osmium complexes [77] or ferrocene redox centers. Most of these polymers contain amino groups that facilitate cross-linking at room temperature. Also, the amino groups in the enzyme can yield cross-linking of both molecules in a single step (Fig. 7.3a). In addition, incorporating nanomaterials such as nanoparticles of different natures, and also carbon nanotubes (CNTs) can generate synergies with these

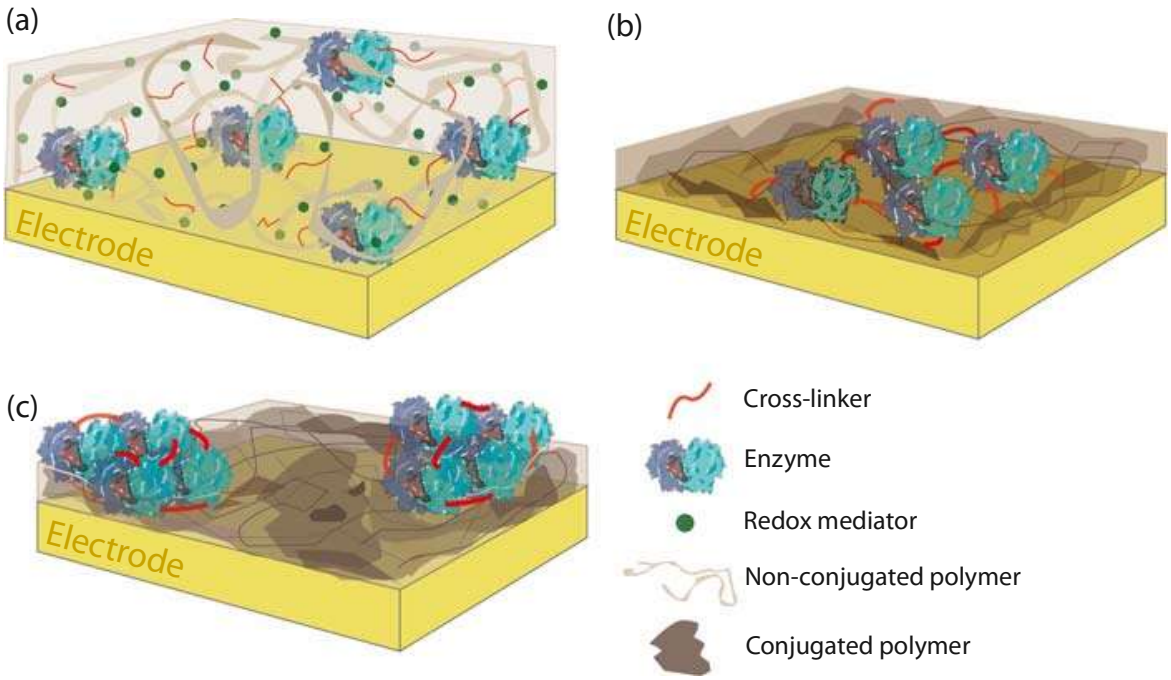

Figure 7.3 Cross-linking in enzymes and redox polymers. (a) Hydrogel composed of a nonconjugated redox polymer, an enzyme, and a cross-linker. (b) Conjugated redox polymer cross-linked to enzymes via a covalent bond. (c) Cross-linked enzymes interacting with a conjugated polymer that does not have functional groups reactive to the cross-linkers.

polymeric matrices, improving their mechanical properties, stability, and response to stimuli, among other aspects [78,79].

In contrast with NCRPs, with a few exceptions [80], the literature for cross-linking conjugated polymers with enzymes describes only the use of GA. These reports usually do not specify which groups in the polymer react with the cross-linker. PPy and PANI have secondary amines that are suitable for reaction with GA (Fig. 7.3b) [81–83]. Nevertheless, several publications state that GA is employed to cross-link the enzymes to other conjugated polymers that do not have primary or secondary amines, or any other group that will react readily with GA [84,85]. If any, some of them contain tertiary amines that are not reactive with this cross-linker [65]. The improvements upon addition of GA may be caused by intramolecular bonds in an enzyme and cross-linking between different enzymes (Fig. 7.3c) [86]. Alternatively, composites have been deposited, mixing the conjugated polymer with another one that has reactive groups suitable for cross-linking [87].

A different approach, direct covalent bonding, consists of the immobilization of a molecule to the support (an electrode in this case) via covalent or coordination bonds. Some polymers can generate bonds with the support by themselves. PEIs, for example, naturally have amino functional groups that can adsorb on metal surfaces via the coordination of their lone electron pair [88,89]. This takes place preferentially in branched PEIs because of their higher content in primary amines and is analogous to the chelating properties displayed with metal ions [90,91]. Alternatively, the electrode surface can be premodified with functionalized nanomaterials or by other methodologies that generate specific functional groups to bind the enzymes or polymers

covalently. Self-assembled monolayers (SAMs) have become a popular choice for modifying electrode surfaces because there is a wide variety of terminal functional groups. Carboxylic acid terminated SAMs, for example, are usually covalently bonded to amino groups using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry [92]. Alternatively, the cross-linkers described earlier can be used between amino groups generated on the surface and those in the polymer or enzyme. Enzyme immobilization via direct covalent bonding in the absence of a polymer matrix can increase the risk of denaturation [93] owing to the interaction between the enzyme and the support surface. Therefore, it is comparatively less popular than cross-linking. Finally, the enzyme can be covalently bonded to the monomer [94] or the dopant [95] before electrodeposition of the conjugated polymer is performed.

7.2.5 Noncovalent interactions

Although weaker than covalent bonds, noncovalent interactions can be used to immobilize molecules on an electrode surface. Among them, electrostatic attraction and repulsion have been the most exploited to immobilize enzymes and polymers, which can have ionizable groups bearing different charges, depending on the pH of the medium. This character is described by their isoelectric point, defined as the pH at which the total charge on the molecule is zero. At higher pH values, the molecule bears a negative charge, whereas positive charges are observed at more acidic values. When enzyme and polymer charges are opposite, these molecules can attract each other, forming adducts. In conjugated polymers, the charge of the doping agents can affect the effective potential with which the protein interacts [96]. Besides immobilizing the enzymes to the polymer, it has been shown that these interactions can increase the charge transfer between oxidoreductases and pendant electroactive groups in NCRPs [97].

A common strategy for exploiting the power of electrostatic interactions is the self-assembly technique known as layer-by-layer deposition (LbL) [98,99]. The general process is based on the sequential deposition of macromolecules from their diluted solutions on a solid substrate, forming a multilayer thin film. An LbL system can be stabilized by noncovalent interactions (either electrostatic or nonelectrostatic) between molecular building blocks. Tuning these interactions modifies the position and defines the orientation of molecular components in a supramolecular architecture [100,101]. LbL has emerged as an attractive technique to modify the surface of substrates, producing a highly ordered nanostructured multilayer system. This technique offers great versatility because it provides control over the construction, applies to any substrate shape, and can be adapted for automatization. LbL assembly is an efficient, robust, flexible, simple, inexpensive, and fast tool to fabricate modified surfaces that can be applied in optics, energy, catalysis, separation, biomedicine, and sensor technologies [100−105]. With this method, it is possible to build nanostructured materials with fine control over film thickness, stiffness, roughness, morphology, film composition, and mechanical, electrical, and chemical properties [99−101,103−108]. Several deposition methods for LbL assemblies (Fig. 7.4) can be used, such as spin-coating,

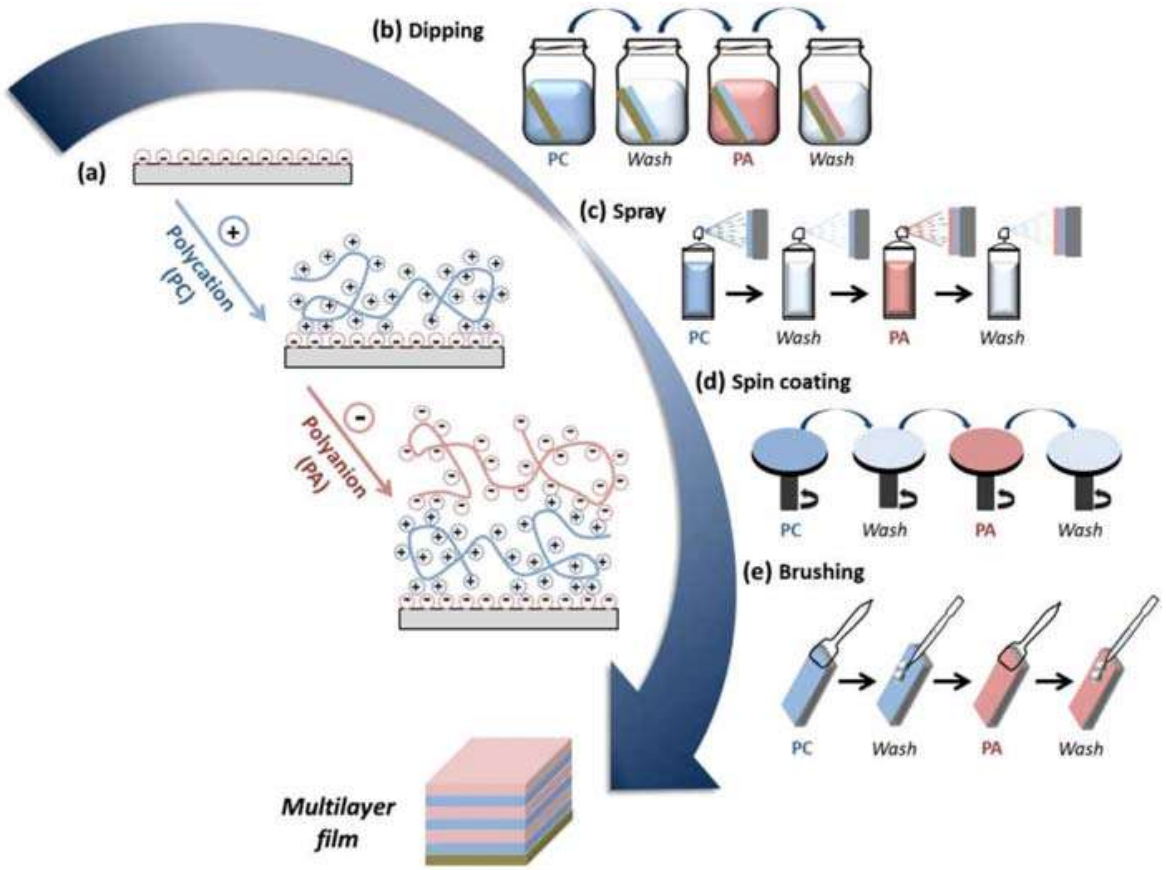

Figure 7.4 (a) Principle of layer-by-layer self-assembly. (b–e) Different methods for layer-by-layer deposition.
Reprint from M. Criado-Gonzalez, C. Mijangos, R. Hernández, Polyelectrolyte multilayer films based on natural polymers: from fundamentals to bio-applications, *Polymers* 13 (2021) 2254, which is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

spraying, perfusion, and dip-coating, which is the most popular. However, spin-coating is an alternative because it can be used with supports that present more complex geometries. Hydrodynamic dip-coating, high-gravity field, and inkjet printing are being explored as deposition techniques [100]. To avoid the cross-contamination of deposition solutions, a rinsing step with the solvent is necessary. Also, these steps between each macromolecule deposition remove weakly adsorbed or excess macromolecules on the surface [99–101,104,106].

Because of the predictable attraction between molecules and/or surfaces of opposite charge densities, the most well-studied systems in LbL assemblies are based on electrostatic interactions [100]. Besides the pH effects explained earlier, ionic strength has an important effect by screening the charge density, decreasing the intensity of electrostatic interactions. Other solution properties such as the temperature, concentration, or polarity, also modify this type of interaction. Also, the molecular weight and structure of the polymer influence the observed behavior. The intensity of polyelectrolyte–surface and polyelectrolyte–polyelectrolyte interactions in LbL assemblies influences the growth and thickness of the multilayer film [101,106]. In addition, the use of redox

polyelectrolytes in electrodes modified by LbL assemblies allows for the fabrication of an electroactive platform with powerful applications in sensors, biosensors with redox enzymes, and electrochromic devices [102,105,108]. The properties of the medium surrounding the adsorbed layers can be significant for their stability and even induce their desorption. Therefore, it is important to control variables such as the electrical field, light, temperature, humidity, and contaminants [100,102].

Derivative polymers with osmium, ferrocene, or ruthenium redox centers are the most well-studied nonconjugated redox polyelectrolytes used in LbL. Electrostatic interactions between these molecules and polyelectrolytes [102,105,109], CRPs [110], surfactants [111−113], or redox enzymes [114−118] have been used in bioelectrodes for sensing or energy applications (Table 7.1).

CRPs can also be used as the molecular building block of LbL assemblies. The incorporation of this type of polymers depends on their electrical conductivity and the charge of the oxidized/reduced states. CRPs can be assembled with another CRP, nanoparticles, or polyelectrolytes. The spontaneous process of LbL assembly could be stimulated using the substrate as electrodes in an electrodeposition process. This method provides an increase in the density and thickness of polymer deposition. Table 7.1 shows examples of LbL systems containing conjugated redox polyelectrolytes and their main applications.

Table 7.1 Examples of layer-by-layer deposition systems containing redox polyelectrolytes and their main applications.

Polymer type	Polymer	Application	Reference
Nonconjugated redox polymer	Branched poly(ethylenimine) with ferrocene/poly(3,4-ethylenedioxythiophene)	Biosensor	[110]
	Polyallylamine with osmium	Molecular and stimulus-responsive devices	[111]
		Biosensor	[114]
		Bienzymatic sensor	[115]
		Biocathode	[118]
	Linear poly(ethylenimine) with ferrocene	Biobattery and supercapacitor	[116]
Conjugated redox polymer	Poly(p-phenylene vinylene)	Nanoparticle preservation	[107]
	Poly(3,4-ethylenedioxythiophene) and poly(N-methylpyrrole)	Supercapacitor	[119]
	Polyaniline nanofibers	Energy storage device	[120]
	Polyaniline	Electrochemical capacitor	[121]
	Poly(3-aminobenzylamine)	Sensor	[122]

Vyas et al. [107] performed a CRP LbL assembly with alternating dip-coating of a modified gold substrate in poly(p-phenylene vinylene) and poly(sodium 4-styrenesulfonate) solutions. A similar process with poly(3,4-ethylenedioxythiophene) and poly(N-methylpyrrole) was used by Aradilla et al. to fabricate a supercapacitor [119]. Yun et al. [120] described the deposition of positively charged PANI nanofibers and negatively charged 2D $Ti_3C_2T_x$ MXene nanosheet multilayer films as an alternative material for energy storage devices. Hyder et al. [121] used functionalized multiwalled CNTs and doped PANI LbL assemblies for electrochemical capacitors. Furthermore, Marmisollé et al. integrated poly(3-aminobenzylamine) with polyanions through electrostatic interactions in modified electrodes that showed a catalytic response for ascorbic acid [122].

Enzymes and proteins are polyampholytes with a heterogeneous spatial charge distribution, which allows them to form electrostatic interactions with other compounds [123,124]. Owing to its characteristics, an LbL assembly generates a supramolecular structure that prevents enzyme release and inactivation by degradation or denaturation [53,124]. Heller's group [125] pioneered the electrostatic LbL assembly of an NCRP and GOx, whereas Onda [126] studied enzymes and polymers in multilayer systems, and Hodak [127] described effective electrical wiring between NCRP and GOx [114,115,128]. The formed structure has a defined distribution of the components, improving mass transport and allowing molecular recognition and signal generation by redox mediation. With this methodology, both the enzyme and polymer could be quantified by spectroscopic and/or electrochemical techniques [115].

Because of the pH dependence of the enzyme and polymer charge, there is a (sometimes narrow) pH range in which the desired enzyme and polymer can form electrostatic LbL assemblies. Furthermore, the pH value changes not only the charge sign but also the charge density of both components, which can modify the intensity of enzyme−polymer interactions [52,106,123,129]. In addition, the bioactivity of the immobilized enzyme and the polymer conformation can be pH-dependent, which may result in less effective electron transfer between redox centers [106,123]. The formation of enzyme−polymer complexes (EPCs) has been studied as an alternative to avoid electrostatic repulsion between enzymes and polymers with the same charge sign. EPCs are formed by electrostatic interactions using polymer A, which has an electrical charge opposite that of the enzyme at a certain pH. This polymer surrounds the enzyme forming an EPC, which then forms an electrostatic LbL with polymer B of a charge opposite that of A (Fig. 7.5a). This strategy has been evaluated for different enzymes and proteins such as lysozyme, insulin, and GOx, with several polyanions and polycations [123,124]. LbL assemblies are not limited to NCRPs. Some CRPs, such as PPy and PANI, have high electron transfer capacity between enzymes and conducting surfaces [130]. Xu et al. [131] adsorbed GOx over PPy by electrostatic interactions between amine and carboxylic groups, and Xue et al. [132] immobilized GOx on positively charged PANI-polyisoprene for the determination of glucose.

Not only ion concentration but also its nature is important in the construction of LbL self-assemblies. Dressick et al. studied multilayer systems in the presence of phosphate, sulfate, acetate, chloride, and fluoride, among other ions [133]. They found that the properties of the LbL system, such as absorbance or thickness, changed according to the position of the anion in the Hofmeister series, which considers the capacity of the ion to

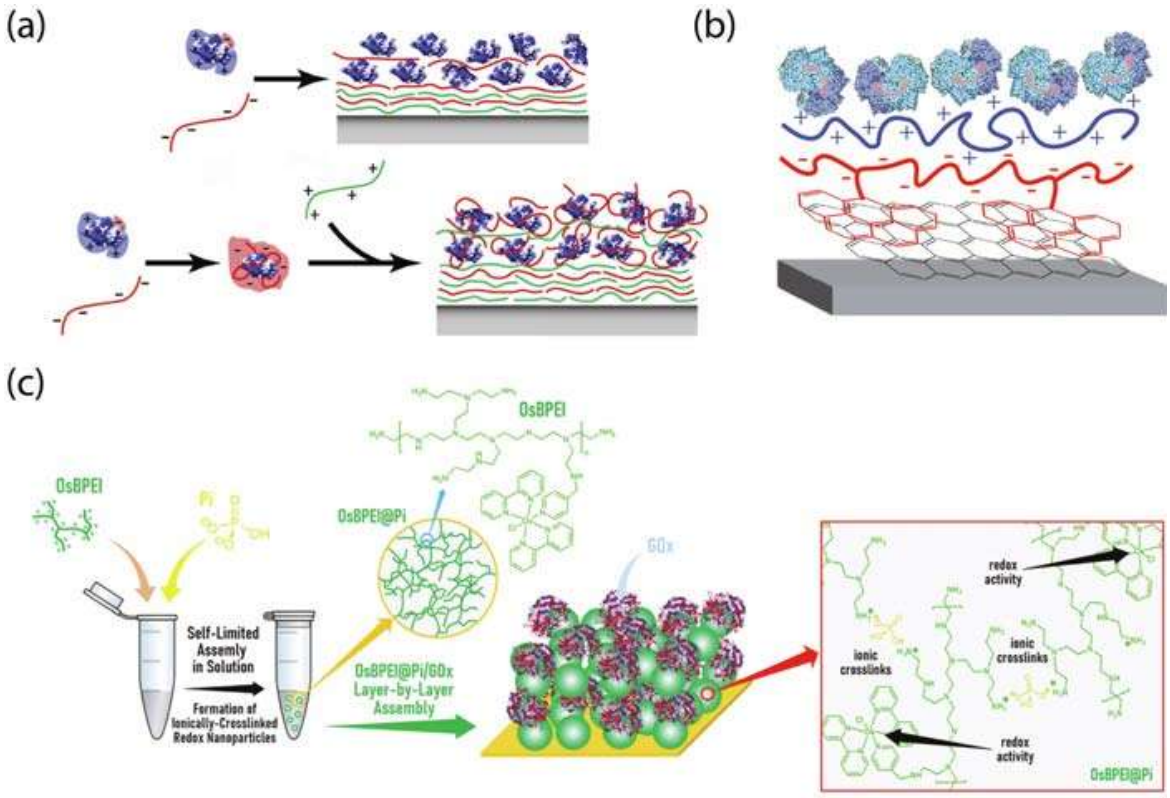

Figure 7.5 Different methods for enzyme immobilization by nonelectrostatic interactions. (a) Immobilization of a positive enzyme by itself and forming enzyme−polymer complexes with a polyanion. (b) Enzyme immobilized using layer-by-layer deposition polymer by π−π stacking interactions. (c) Enzyme ionic cross-linking by phosphate ions. *BPEI*, branched poly(ethylenimine); *GOx*, glucose oxidase.
(a) Adapted with permission from C. Vranckx, L. Lambricht, V. Préat, O. Cornu, C. Dupont-Gillain, A. Vander Straeten, Layer-by-layer nanoarchitectonics using protein−polyelectrolyte complexes toward a generalizable tool for protein surface immobilization, *Langmuir* 38 (2022) 5579−5589, Copyright 2022, American Chemical Society. (c) Reprinted with permission from L.L. Coria-Oriundo, M.L. Cortez, O. Azzaroni, F. Battaglini, Enzymes hosted in redox-active ionically cross-linked polyelectrolyte networks enable more efficient biofuel cells, *Soft Matter*. 17 (2021) 5240−5247.

organize or disorganize the structure of water [133,134]. For polyamines, this effect is even more complex because amines can form hydrogen bonds with sulfate or phosphate ions, which act as ionic cross-linkers [133,135]. Hydrogen phosphate, for example, has been reported to form colloidal structures with polyelectrolytes and redox polyelectrolytes in LbL assemblies [108,136−138]. The degree of polyelectrolyte protonation and the phosphate ion concentration determined the degree of phosphate-amine cross-linking. Also, high ionic strength produces a charge screening effect, intensifying hydrogen bond interactions [133,136,138]. This principle was applied to construct biosensors and biofuel cells based on enzyme-redox polymer self-assemblies using phosphate at high ionic strength, showing the formation of colloidal particles that can be adsorbed on an electrode surface (Fig. 7.5c) [136,139]. The phosphate ions can also interact with the enzyme's basic residues such as lysine or arginine [140−142]. Furthermore, the amount of adsorbed enzyme increased in these assemblies, yielding a higher

catalytic current owing to a gel-like structure in which the redox centers move freely, generating more effective electronic transfer [136,139].

This discussion clearly shows that the stability of these LbL structures also results from entropic factors and nonelectrostatic interactions. Thus, researchers began to explore interactions such as hydrogen bonding, hydrophobic bonding, π−π stacking, and host−guest interactions present in LbL assemblies that do not depend on the charge of the molecular blocks [106,128,143,144]. Stockton and Rubner formed an LbL assembly by H-bonding interactions using poly(vinylpyrrolidone), poly(vinylalcohol), poly(acrylamide), and poly(ethylene oxide) with good reversibility and resistance to high ionic strength [145]. On the other hand, Zeng et al. [144] modified reduced graphene oxide with pyrene-grafted poly(acrylic acid) (PAA) by π−π stacking interactions (Fig. 7.5b). The PAA film can be used to deposit a polycation or a positively charged enzyme. Hydrophobic interactions have also been studied in the formation of LbL assemblies. Cortez et al. [146] explored introducing an anionic surfactant in a polyelectrolyte-enzyme assembly. The surfactant and the polyelectrolyte interact electrostatically, whereas hydrophobic interactions with the enzyme are enhanced. In another work, Cortez et al. [147] reported the structure of a supramolecular system formed by a redox polyelectrolyte with lectin units assembled with surfactant and concanavalin A, on which horseradish peroxidase (HRP) is immobilized by carbohydrate−lectin interactions.

7.2.6 Mechanical immobilization

Some techniques to immobilize molecules on electrodes are based on either the size or shape of the material with respect to its surrounding environment. The most common example of these methods is enzyme entrapment. The enzyme is physically trapped in a matrix that has pores smaller than the enzyme but larger than its substrate. This matrix can be prepared in a variety of ways, including sol-gel, cross-linking and polymerization methods [148]. Alternatively, the enzyme can be trapped in structured voids covered by semipermeable films [149]. Covalent cross-linking of enzymes to the polymer structure is referred to as entrapment in some publications [150]. However, we classify those systems as covalent cross-linking based on the main immobilization principle at play.

CRPs have been used to entrap enzymes because they can be incorporated in the same step as polymerization. This process is commonly carried out by electrodeposition using either potentiostatic (controlled potential) or galvanostatic (controlled current) techniques. In both cases, electrodeposition can take place continuously or at short intervals (pulses). Electrodeposition is advantageous because polymer synthesis and deposition are simultaneous. Besides, accurate control or measurement of the current is simple and easily related to the amount of deposited polymer. Integrating the current, one can find the electric charge employed in the deposition (Q) and relate it to the film's thickness (d) according to:

$$d = \alpha \frac{Q}{A} \tag{7.3}$$

where A is the electrode area and α a constant that is determined for each polymer [84]. When using these calculations, however, one must keep in mind that electrodeposition does not always form smooth layers. Under some circumstances, polymer particles can be formed, resulting in a rough surface (Fig. 7.6a–c). An advantage of electrodeposition is that the polymers will deposit on the electrode surfaces only where oxidation takes place. Other methods, such as drop casting, will deposit the polymer on all the surfaces in contact with the deposition solution. Furthermore, chemical and even biochemical synthesis and deposition of CRPs have been reported [15,151].

In general, the monomers required to prepare conjugated polymers are poorly soluble in water. Therefore, polymerization is preferably carried out in nonaqueous solvents such as propylene carbonate, acetonitrile, and dichloromethane [84]. However, these solvents might damage the enzymes. Thus, when enzyme entrapment is carried out during polymerization, aqueous solutions of moderate pH are required. Pyrrole, N-methylpyrrole, ortho-phenylenediamine, and 3,4-ethylenedioxythiophene have comparatively higher solubility in water and therefore have been used to entrap enzymes from aqueous buffer solutions [152–155]. When the enzymes are trapped in the electropolymerized matrix, they are not covalently bonded to the polymer and are more likely to retain their structure and therefore their activity [156,157]. Although they are not covalently linked, some interactions can take place between the enzymes and polymer. However, these are, not fully understood and remain an active field of study [158]. Among these forces, some ionic attraction can be present because the oxidized polymers are usually cationic. Enzymes with isoelectric point lower than the solution pH will therefore be negatively charged and favorably attracted to the polymer. Evidence for these interactions is that it is more difficult to trap cationic enzymes in conjugated polymers [152]. Because of the absence of covalent links, trapped enzymes are susceptible to leaching. This might be the reason behind the enhanced stability of poly(ethylene glycol)-conjugated enzymes trapped in a CRP matrix, compared with the unconjugated enzyme [155].

Some electrodeposited CRP films are robust enough that they can be peeled from the electrode on which they were deposited. Thus, they can be used as standalone electrodes. Furthermore, some special formulations have been created incorporating polyelectrolyte counterions during electrodeposition to obtain thermoplastic conjugated polymers [159]. These can be shaped arbitrarily by melting them into molds [160]. These standalone electrodes can be used by themselves, or they may have another material deposited on their surface. Khan and Wernet, for example, deposited platinum black on a PPy film doped with polyanions and used it for single and multiple enzyme immobilization [160,161].

NCRPs are not generally suitable for enzyme entrapment because when they are cross-linked, they form networks with larger pores and severe enzyme leaching. Instead, these polymers have been entrapped in other polymers. Schuhmann's group, for example, trapped Os-modified poly(vinyl-imidazole) and alcohol dehydrogenase (ADH) inside a Resydrol matrix [162]. This strategy allowed them to use the electrochemical wiring of the Os complexes without cross-linking the enzyme to the polymer. Resydrol deposition was triggered by electrochemical acidification near the electrode,

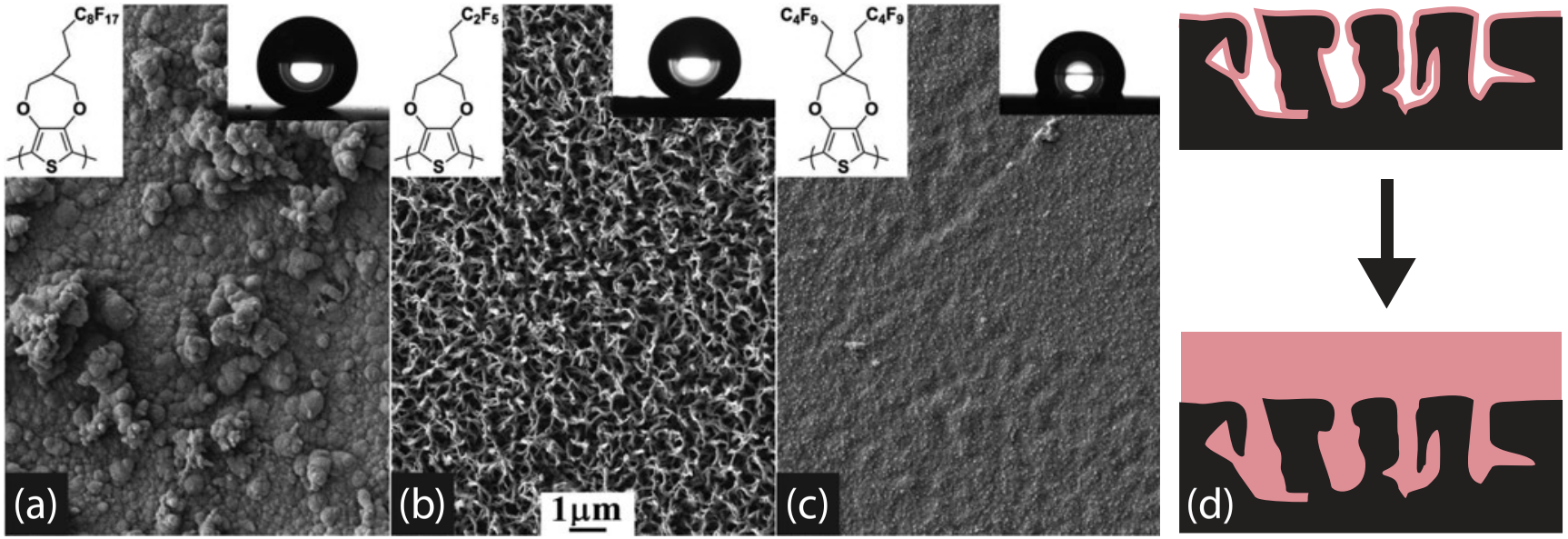

Figure 7.6 (a–c) Scanning electrochemical microscopy images of fluorinated derivatives of 4-propylenedioxythiophene electrodeposited on a gold electrode. (d) Schematic of mechanical interlocking of an electrodeposited polymer layer on a porous electrode.
(a–c) Images were used with permission of the Royal Society of Chemistry, from J. El-Maiss, T. Darmanin, F. Guittard, Controlling electrodeposited conducting polymer nanostructures with the number and the length of fluorinated chains for adjusting superhydrophobic properties and adhesion, *RSC Adv.* 5 (2015) 37196–37205; permission conveyed through Copyright Clearance Center, Inc.

allowing significantly more spatial control compared with a drop-cast gel using the same NCRP.

Finally, polymer layers can mechanically interact with the supports on which they are deposited. Polymer chains can be entrapped in porous media [163]. Furthermore, when polymers are prepared while in contact with a surface, they can acquire their complementary shape. If the surface has voids or pores, the polymer can fill them creating interlocking structures (Fig. 7.6d). This mechanical interlocking is a known adhesion mechanism [164] and can increase the stability of the deposit compared with smooth surfaces. Depending on the nature of the electrode material, these interactions can have a larger role. Highly porous supports made of carbonaceous materials [165] and metals [166] are particularly suitable for exploiting this potential and reducing the risk of polymer detachment with use.

7.3 Sensing mechanisms

First-generation biosensing involves a faradaic reaction of one of the reagents or products of the enzymatic reaction (Fig. 7.7a). This strategy has been widely applied in oxidases, usually after H_2O_2 production [153], although O_2 consumption can also be employed. In electrodes modified with conjugated polymers, there has been discussion regarding whether the electrochemical reaction takes place at the polymer surface or the underlying electrode support [167]. In some cases, metal nanoparticles are deliberately added to the polymer to improve the electrochemical reaction rate [168]. The mechanism is usually demonstrated by obtaining the same response without an enzyme substrate but with H_2O_2 additions.

Another mechanism is based on the catalytic regeneration of a redox-active mediator at the electrode surface (Fig. 7.7b). This is the most common mechanism in NCRPs and is referred to as second-generation amperometric biosensing. We will use an oxidase-based anode as an example. In an enzymatic cathode, the direction of the processes (i.e., oxidation vs. reduction) would simply be inverted. In an anode, the surface electrode reaction is the oxidation of the electroactive mediator. This oxidized mediator can replace the enzyme's natural electron acceptor (O_2 for oxidases) and react with the reduced enzyme, producing an oxidized enzyme and a reduced mediator. This reduced mediator can then be oxidized again at the electrode. This cycle continues as long as the substrate, enzyme, and mediator are present. This system is similar to a homogeneous catalyst that regenerates the reactant of the electrode reaction. The characteristic response is an increase in the current of the forward electrochemical reaction (oxidation of the mediator) with respect to its value in the absence of the substrate. On the other hand, the reverse electrochemical reaction (reduction of the mediator) has a decrease in its peak current until it disappears [169]. In the completely catalytic system, a sigmoidal voltammogram is therefore obtained. This response is favored at low potential scan rates and/or high catalytic rates. In the case of oxidoreductases immobilized along with NCRPs, this catalytic rate includes the enzyme kinetics as well as the rates of both the enzyme−mediator and

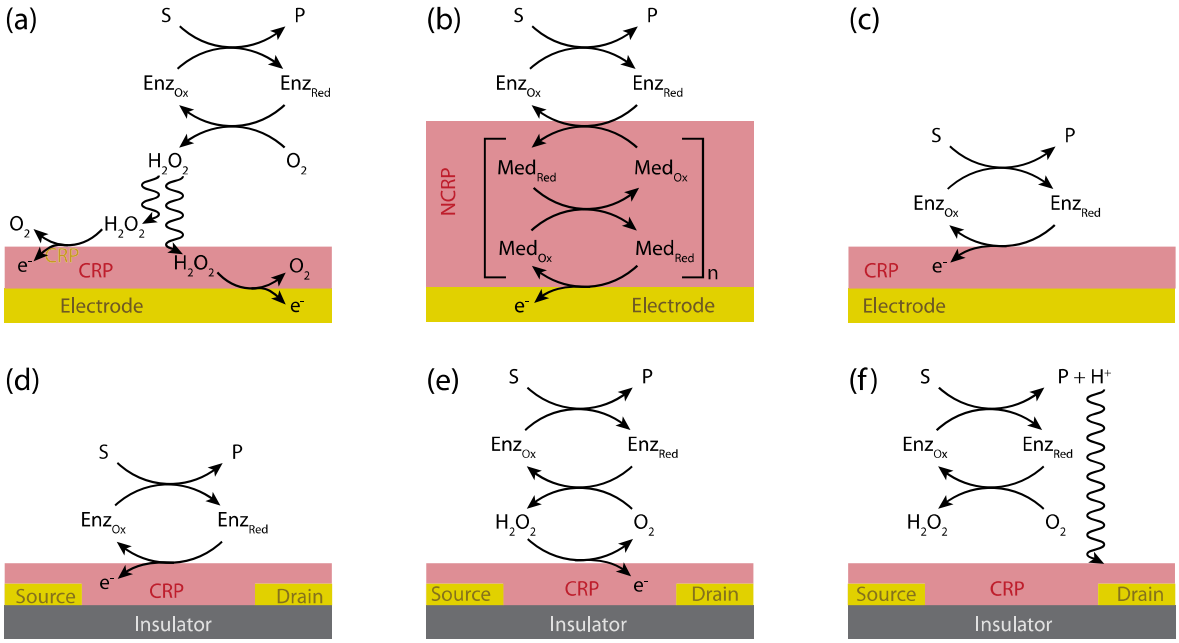

Figure 7.7 Different amperometric (a–c) and conductimetric (d–f) sensing strategies using oxidoreductases and redox polymers. (a) Faradaic reaction of enzymatic product on the conjugated redox polymer (CRP) or the underlying electrode (first generation). (b) Mediated electron transfer through a nonconjugated redox polymer (NCRP) (second generation). (c) Direct electron transfer from the enzyme to the CRP (third generation). (d) CRP conductivity change by enzymatic oxidation. (e) CRP conductivity change by oxidation with an enzymatic product. (f) CRP conductivity change by environmental pH change.

mediator–mediator electron transfer until the electrons reach the electrode surface. Although cyclic voltammetry is useful for demonstrating the principle of this mechanism, chronoamperometry is usually employed for sensing, following the forward reaction at values slightly higher (lower for the cathode) than the peak found in the cyclic voltammogram.

Direct electron transfer (DET) between oxidoreductases and the electrode has been employed as a sensing strategy termed third-generation faradaic enzymatic biosensing (Fig. 7.7c) [170–172]. In this case, the enzyme cofactor must be close enough to the electrode surface for the process to be feasible with appreciable kinetics. The addition of a CRP increases the electrically conductive area and therefore the amount of enzyme that can be immobilized nearby [173]. The sensing mechanism relies on the electrochemical regeneration of the enzyme cofactor to its active state (e.g., oxidized for an anode). Evidence for this mechanism includes cyclic voltammograms showing peaks attributed to the cofactor of immobilized enzymes. However, it has been suggested that complementary evidence such as product analysis, inhibition studies, and even mutations are required to prove DET unequivocally [174].

Another sensing mechanism that is popular for CRPs varies the conductivity of a thin layer of polymer placed between two electrodes, termed source and drain, similar to that for transistors. These electrodes can be polarized with respect to a reference and counter-electrodes (collectively termed gate) that are immersed in the same electrolyte solution. This setup is sometimes referred to as a class of field effect transistors (FETs).

However, it has substantial differences from real FETs and bio-FETs. First, the gate is not employed to apply an electric field directly, but rather to control the redox state (doping) of the conjugated polymer before or during the experiment. Second, changes in the conductivity of the polymer layer (the channel) are not the direct result of a change in the surrounding electric field. Instead, they are brought about by chemical changes in the conjugated polymer.

A few different strategies have been proposed to create a dependence of the conductivity on the analyte concentration (enzyme substrate). Bartlett and Birkin devised a mediated system in which the polymer served as the final electron acceptor of GOx (Fig. 7.7d). Therefore, the conjugated polymer became reduced as the enzyme reaction took place, changing its conductivity [175]. Other approaches have relied on the effects of the enzymatic reaction by-products. Oxidants (such as H_2O_2) have been suggested to oxidize the conjugated polymer, increasing its doping (Fig. 7.7e) [176]. However, care must be taken because the conductivity or PPy can be irreversibly lost owing to excessive oxidation by H_2O_2 [167]. Another strategy exploits the dependence of polymer conductivity on the pH owing to the accumulation of a reaction product with acid/base properties near the electrode (Fig. 7.7f) [177].

7.4 Practical examples in biosensing

7.4.1 Oxidases

Oxidases are the most widely studied group of oxidoreductases for the development of electrochemical biosensors. These enzymes catalyze oxidation/reduction reactions using molecular oxygen as the final electron acceptor, resulting in the production of water and hydrogen peroxide [178]. Combining oxidases with CRPs and NCRPs has greatly improved the efficiency of enzyme immobilization, electronic transfer, sensitivity, and other aspects of the developed devices. GOx is the most reported oxidase in the literature, followed by laccases.

GOx is the reference model for the research and development of oxidase-based biosensors. Most GOx-based devices for glucose measurement are amperometric. Singh [179], Zhao [180], and Lai [181] reviewed the integration of GOx with popular CRPs. Conjugated polymers have been used by themselves as enzyme immobilization matrices on first-generation electrodes [81,82,167] or mixed with nanoparticles, CNTs, and other nanostructures to increase the surface area, allowing for higher GOx loadings and better electron transfer [182]. Incorporating Pt nanoparticles in PANI [168] or together with CNTs (Fig. 7.8) [183] has been shown to improve the sensitivity, stability, and response time of first-generation systems by catalyzing H_2O_2 oxidation.

In other cases, GOx has been employed in second-generation biosensors using artificial electron mediators such as Os complexes, ferrocene derivatives, and methylene blue. To work with conjugated polymers, these mediators have been entrapped in the polymer [83,184], deposited as a separate layer [80], or simply absorbed in the porous structure [185]. Similarly, nonconjugated polymers that have these mediators in their

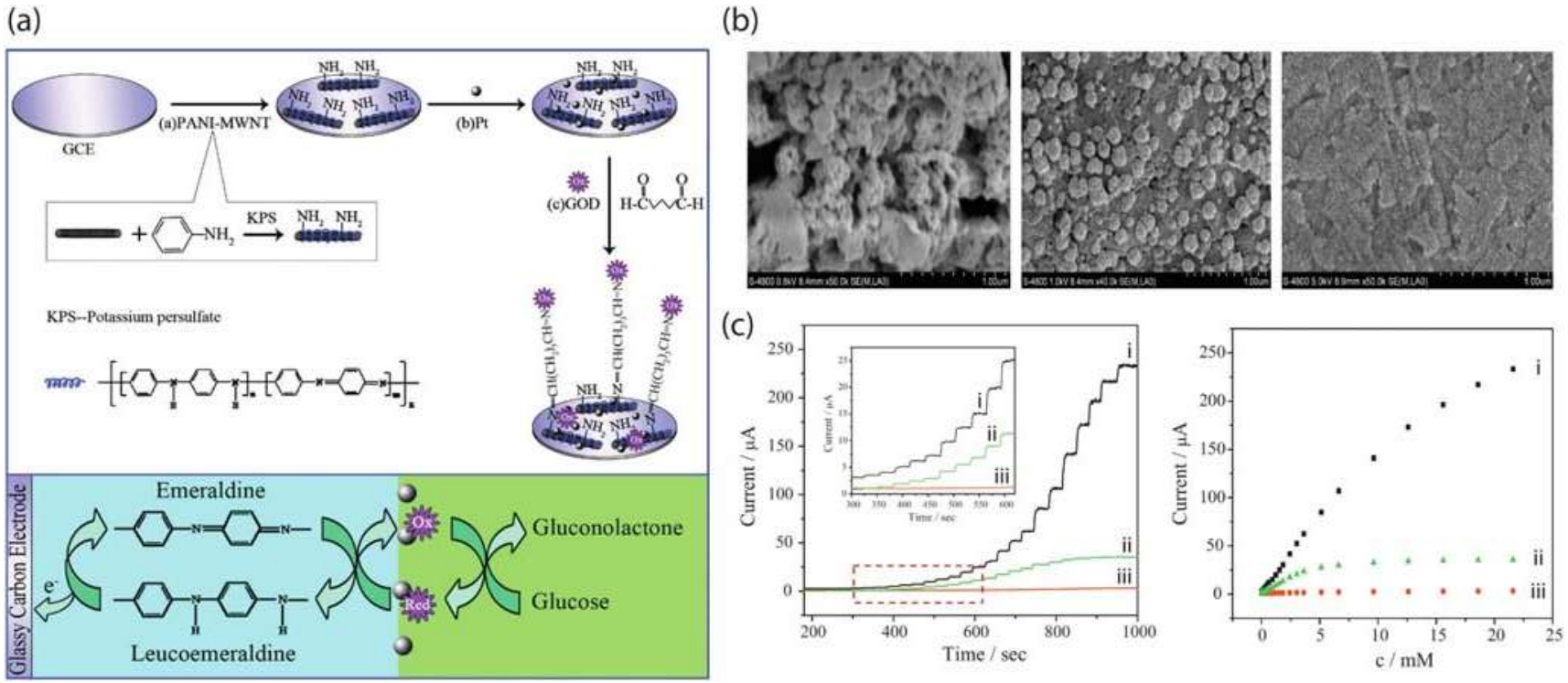

Figure 7.8 (a) Preparation process and detection mechanism of glucose amperometric biosensor based on multiwall carbon nanotube (MWNT)−polyaniline (PANI) nanocomposites, platinum nanoparticles, and glucose oxidase (GOD) proposed by Zhong et al. (b) Scanning electron microscopy images of different glassy carbon electrode (GCE) modification steps: MWNT-PANI (*left*), Pt/MWNT-PANI (*center*), and GOD/Pt/MWNT-PANI (*right*). (c) Amperometric response and calibration curve of glucose of GOD/Pt/MWNT-PANI/GCE (i), GOD/Pt/GCE (ii), and GOD/MWNT-PANI/GCE (iii).

Reprinted from H. Zhong, R. Yuan, Y. Chai, W. Li, X. Zhong, Y. Zhang, In situ chemo-synthesized multi-wall carbon nanotube-conductive polyaniline nanocomposites: characterization and application for a glucose amperometric biosensor, *Talanta*. 85 (2011) 104−111. Copyright (2011), with permission from Elsevier.

structure have been employed [186−191]. Incorporating artificial mediators into the system enables the reoxidation of reduced GOx, generating a reuse cycle and improving charge transport, which translates into better sensitivity.

Efforts have been made to reduce manufacturing costs and system complexity by eliminating the use of redox mediators [155]. However, the active site of GOx is inadequate for DET because it is deeply embedded within the enzyme and protected by a glycoprotein shell. Nevertheless, several groups reported DET between the flavin adenine dinucleotide (FAD) cofactor in GOx and the surface of electrodes containing CRPs [192−196]. These claims have been based, among other evidence, on cyclic voltammograms showing a pair of redox peaks assigned to the FAD in the active enzyme. However, the significance of the experimental results has been questioned [174,197−199]. Critics argue that the observed response may be explained by the release of cofactors from inactivated enzymes or impurities, rather than by DET. This issue is not limited to conjugated polymer electrodes; it is a general problem with DET claims in GOx. Therefore, readers should exercise caution when interpreting the presented evidence.

GOx has also been employed in nonfaradaic sensors. Potentiometric sensors, for instance, use the glucose-dependent potential of PPy/GOx films as a sensing mechanism, which can be explained by pH changes caused by gluconic acid produced by the enzymatic reaction or by the effect of the produced H_2O_2 [200]. GOx has also been incorporated into thin film transistors made of CRPs, in which the production of H_2O_2 increases the oxidation state of the polymer, resulting in higher currents between the source and drain electrodes [176,201]. Hoa et al. immobilized GOx in a PANI electrode and attributed the change in resistance to the pH shift owing to the accumulation of gluconic acid near the polymer [177].

Laccases belong to the multicopper oxidase family [202−204], which contains four copper atoms in the active site. A mononuclear center contains one type I Cu atom and a trinuclear cluster is formed by one type II Cu atom and two type III Cu atoms. Whereas substrate oxidation occurs at the mononuclear center, the trinuclear cluster is responsible for the four-electron reduction of oxygen to water [205−207]. Laccases can oxidize a wide range of phenolic and nonphenolic compounds, with and without redox mediators, which makes them attractive for various applications, including biosensors [202,207−210]. The redox potential of laccases varies according to their origin, which is mainly associated with the ligand of the type I Cu atom. High potential laccases are found only in Basidiomycete fungi, medium-potential laccases in fungi and bacteria, and low-potential laccases in plants and bacteria [131,203,211].

Chawla et al. cross-linked *Ganoderma* sp. laccase with GA on a modified electrode with CNTs, PANI, Cu nanoparticles, and chitosan for polyphenol biosensing [210]. Another study reported the covalent immobilization of laccase on an electrode with PANI bonded to CNTs and Ni nanoparticles [212]. Kushwah et al. electrostatically immobilized *Pleurotus ostreatus* laccase to PANI films over ITO for use as phenol amperometric biosensors in industrial effluents [213]. Soylemez et al. prepared an amperometric biosensor for catechol determination in environmental samples [214]. They modified the electrode with the conjugate polymer thienothiophene-bezoxadiazole-alt-benzodithiophene, to which *Trametes versicolor* laccase was

cross-linked. Cevher et al. proposed an indenoquinoxalinone-based CRP as a matrix to immobilize *T. versicolor* laccase to obtain a catechol amperometric biosensor for tap water [215]. Ferry and Leech reported the performance of a catecholamine amperometric biosensor using an Os complex bonded to poly-(N-vinylimidazole) and *T. versicolor* laccase cross-linked with PEGDGE [216].

Other oxidases have been reported in combination with different conjugated and nonconjugated polymers for use in electrochemical biosensors. Examples include the use of chitosan-PPy-Au nanoparticles and xanthine oxidase for the determination of xanthine in fish, chicken, and beef [217]. Also, cholesterol detection has been carried out using cholesterol oxidase and polymers of thiophene [218,219], aniline [219], and pyrrole [220] derivatives. Catechol has been quantified using polyphenol oxidase immobilized into PANI [221]. Lactate oxidase has been extensively studied in combination with different redox polymers [222]. Pyranose oxidase, which can recognize both α- and β-anomers of glucose, has been used in combination with poly(1-vinylimidazole)- and poly(vinylpyridine)-based polymers modified with osmium complexes [223]. Finally, urate oxidase incorporated in poly(*N*-methylpyrrole) has been employed to detect uric acid in a bipotentiostatic arrangement to record background currents simultaneously [153].

7.4.2 Dehydrogenases

The presence of oxygen can affect the response of bioelectrodes modified with oxidases that use oxygen as a natural electron acceptor. In these systems, O_2 competes with the redox mediator as an electron acceptor, affecting the current density output and their performance [224,225]. Therefore, glucose dehydrogenase (GDH), pyranose dehydrogenase, ADH, lactate dehydrogenase (LDH), and cellobiose dehydrogenase have been used instead of their corresponding oxidases [225−229].

The group of GDHs includes enzymes that employ pyrroloquinoline (PQQ), β-nicotinamide adenine dinucleotide (NAD), and FAD as cofactors. These dehydrogenases have different properties such as the redox potential or substrate selectivity [230,231]. Unlike the FAD cofactor in GOx, cofactors in GDHs do not become oxidized in the presence of oxygen. Antiochia and Gorton immobilized NAD-GDH with an Os-based redox polymer over CNT paste, using PEGDGE as a cross-linker to obtain a glucose biosensor [232]. Du et al. employed NAD-GDH immobilized on CNTs functionalized with a CRP (poly[nile blue A]) as a dehydrogenase-based glucose amperometric biosensor [233]. Flexer and Mano modified electrodes immobilizing PQQ-GDH and osmium-poly(4-vinylpyridine) as a redox polymer by cross-linking with PEGDGE [234]. The electrode was used as a glucose biosensor and produced a higher response than a similar system with GOx. Habermuller et al. described using an osmium complex-pyrrole derivative/pyrrole conducting copolymer with PQQ-GDH as a glucose biosensor [235]. Gladisch et al. fabricated a bioelectrode for glucose concentration control using sulfonated PANI-conducting fibers covalently bonded to PQQ-GDH [236]. Calvo et al. built a bienzymatic electrode with GOx, NAD-GDH, and an NCRP (Os complex bonded to polyallylamine) through LbL assembly [115]. The authors found that NAD-GDH catalyzed the conversion of gluconic

acid back to glucose, generating signal amplification through continuous substrate renovation. Pyranose dehydrogenase has been used with an Os-based polymer to oxidize aldopyranoses, including glucose, cellobiose, maltotriose, trehalose, and xylose without anomer selectivity [229].

On the other hand, an enzyme commonly used for ethanol oxidation is ADH, which uses NAD or PQQ as a cofactor [237,238]. Bilgi et al. modified screen-printed electrodes with CNTs, gold nanoparticles, CRP polyneutral red, and NAD-ADH cross-linked with GA, for use as an ethanol biosensor [237]. Niculescu et al. developed an ethanol biosensor using a hydrogel formed with an Os complex bonded to (1-vynilimidazole) and PQQ-ADH cross-linked with PEGDGE [239]. The authors used this biosensor to analyze wine and integrated it into an automatic analyzer, improving its performance for industrial conditions. Ramanavicius et al. reported DET between PPy and an ADH that used PQQ and heme groups as cofactors [173].

The determination of lactate in plasma is important to diagnose different diseases [240–242]. Malhotra's group studied the immobilization of LDH on PPy-polyvinylsulfonate composites and PANI films, by cross-linking with GA and by electrostatic interactions, respectively, for lactate biosensing [240,241]. Rahman et al. covalently immobilized NAD and LDH on CRP poly-5,2′-5′,2″-terthiophene-3′carboxylic acid [242]. The bioelectrode was used to determine lactate concentrations in commercial milk and human serum samples by an amperometric technique. Hussain et al. (Fig. 7.9) fabricated a lactate amperometric nano-biosensor immobilizing NAD^+ and LDH on a bifunctionalized conjugated polymer (poly 3-[(2,2′:5′,2″-terthiophen)-3′-yl]-5-aminobenzoic acid) [243].

7.4.3 Peroxidases

Peroxidases are enzymes that catalyze the reduction of peroxides. Among them, the most employed is HRP. Unsurprisingly, many H_2O_2 sensors have been reported using HRP and redox polymers. For example, second-generation sensors have been designed using poly(4-vinylpyridine) derivatives with $Os(bipy)_2^{2+}$ pendant groups. Different tethers have been tested, with the result that longer ones yielded higher currents because of their higher flexibility, reflected in better self-exchange reactions [244]. Owing to an exposed active center, this enzyme has been reported to undergo DET with the electrode. The addition of a conjugated polymer 5,2′:5′,2″-terthiophene-3′-carboxylic acid polymer layer on a carbon electrode improved the electron transfer rate constant [245]. Peroxide reduction by HRP has also been exploited to design multienzyme assays in which HRP is used to reduce the H_2O_2 produced by oxidases. This is particularly advantageous for overcoming the problem of coupling oxidases that are highly selective to O_2 as an electron acceptor to a redox mediator in a polymer. This is the case, for example, of choline oxidase [246].

However, the more interesting uses of this enzyme come from the concomitant oxidation of organic compounds that takes place during peroxide reduction. The exposed active center makes HRP able to oxidize a large variety of organic compounds. This poor selectivity is useful in competition assays in which the presence

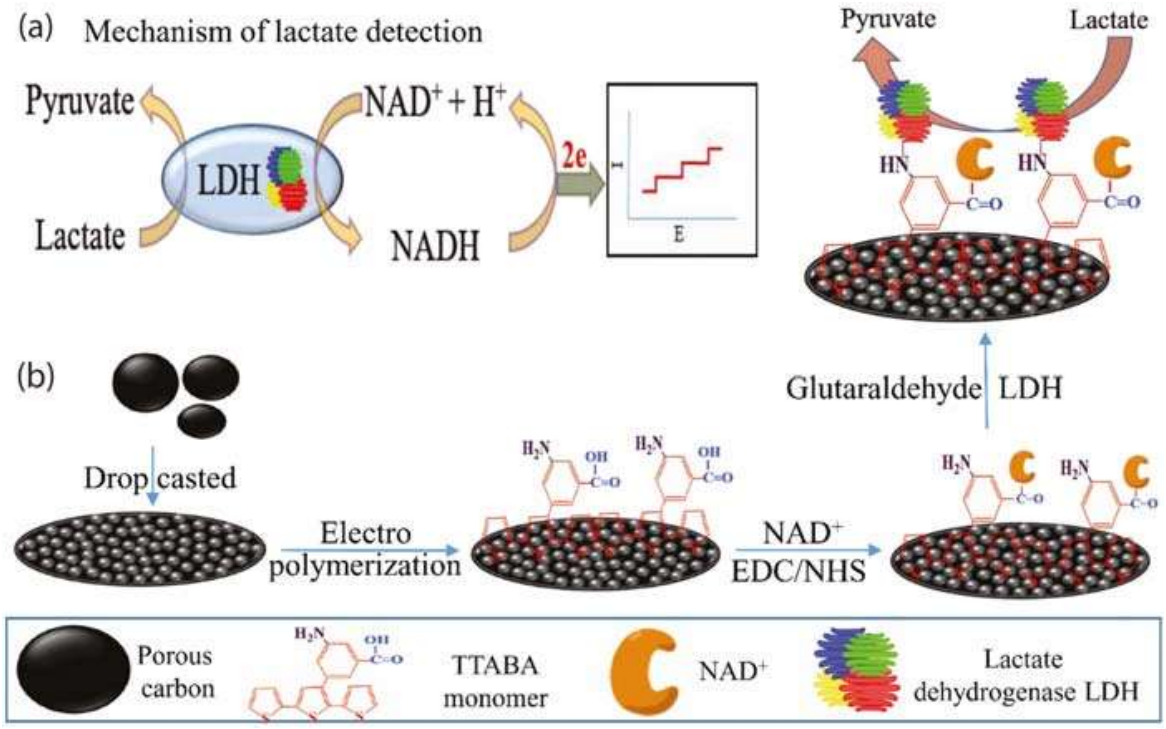

Figure 7.9 Detection mechanism (a) and preparation (b) of a lactate amperometric nanobiosensor in which lactate dehydrogenase (LDH) and β-nicotinamide adenine dinucleotide (NAD^+) are immobilized in the amine and carboxylic groups of a conjugated redox polymer, respectively. *EDC*, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. *NHS*, N-hydroxysuccinimide; *TTABA*, poly 3-((2,2′:5′,2″-terthiophen)-3′-yl)-5-aminobenzoic acid. Reprinted from K.K. Hussain, N.G. Gurudatt, M.H. Akhtar, K.D. Seo, D.S. Park, Y.B. Shim, Nano-biosensor for the in vitro lactate detection using bi-functionalized conducting polymer/N, S-doped carbon; the effect of αCHC inhibitor on lactate level in cancer cell lines, *Biosens. Bioelectron*. 155 (2022) 112094; Copyright (2022), with permission from Elsevier.

of one of the substrates modifies the rate at which the other one is oxidized. For example, Spychalska [84] adapted a sensor developed by Monerris [247], in which 17β-estradiol (17β-E) and pyrocatechol (H_2Q) are oxidized by HRP. The product of the H_2Q oxidation is benzoquinone, which can be reduced back to H_2Q on the electrode surface. Therefore, this reduction current is inversely proportional to the 17β-E concentration in the original sample. In Spychalska's approach, enzyme immobilization on a conjugated polymer is simpler and less expensive than the antibody/nanoparticle/SAM structure in Monerris' work.

7.5 Conclusion and perspectives

Throughout this text, we have explored approaches in which two kinds of redox polymers have been used for oxidoreductase-based biosensing. NCRPs excel in their application as mediators in second-generation faradaic biosensors. On the other hand, CRPs are more versatile. They are used as supports in first- or third-generation faradaic

biosensors, or in conductimetric transistor-like biosensors. Future developments may combine both charge transport principles by having redox-active entities in conjunction with conjugated backbones. This can be achieved by mixing conjugated polymers with free mediators or with nonconjugated polymers containing redox pendant groups. Furthermore, the inclusion of redox pendant groups in conjugated polymers has been reported [248−252]. Although most studies focus on energy applications, they can be applied in biosensors. It is expected that the number of publications in this field will increase. The ability to deposit conjugated polymers by electropolymerization is also expected to have an important role in miniaturization. In particular, incorporating electrochemical biosensors in microfluidic and lab-on-a-chip systems demands the continuous miniaturization of sensing electrodes. Compared with photolithography, electropolymerization can be a simpler strategy to achieve reproducible electrode modification at the required scale.

References

[1] B. Pérez-Fernández, A. de la Escosura-Muñiz, Electrochemical biosensors based on nanomaterials for aflatoxins detection: a review (2015−2021), Anal. Chim. Acta 1212 (2022) 339658, https://doi.org/10.1016/j.aca.2022.339658.

[2] A. Villalonga, A. Sánchez, B. Mayol, J. Reviejo, R. Villalonga, Electrochemical biosensors for food bioprocess monitoring, Curr. Opin. Food Sci. 43 (2022) 18−26, https://doi.org/10.1016/j.cofs.2021.09.006.

[3] A.P.F. Turner, Biosensors: sense and sensibility, Chem. Soc. Rev. 42 (2013) 3184−3196, https://doi.org/10.1039/c3cs35528d.

[4] L.M. Schmidt-Speicher, K. Länge, Microfluidic integration for electrochemical biosensor applications, Curr. Opin. Electrochem. 29 (2021) 100755, https://doi.org/10.1016/j.coelec.2021.100755.

[5] Y. Toum Terrones, V.M. Cayón, G. Laucirica, M.L. Cortez, M.E. Toimil-Molares, C. Trautmann, W.A. Marmisollé, O. Azzaroni, Ion track-based nanofluidic biosensors, in: Miniaturized Biosensing Devices, Springer Nature Singapore, Singapore, 2022, pp. 57−81, https://doi.org/10.1007/978-981-16-9897-2_3.

[6] M. Elbadawi, J.J. Ong, T.D. Pollard, S. Gaisford, A.W. Basit, Additive manufacturable materials for electrochemical biosensor electrodes, Adv. Funct. Mater. 31 (2021) 2006407, https://doi.org/10.1002/adfm.202006407.

[7] P. Kassal, M.D. Steinberg, I.M. Steinberg, Wireless chemical sensors and biosensors: a review, Sens. Actuators B Chem. 266 (2018) 228−245, https://doi.org/10.1016/j.snb.2018.03.074.

[8] M.Z.H. Khan, M.R. Hasan, S.I. Hossain, M.S. Ahommed, M. Daizy, Ultrasensitive detection of pathogenic viruses with electrochemical biosensor: state of the art, Biosens. Bioelectron. 166 (2020) 112431, https://doi.org/10.1016/j.bios.2020.112431.

[9] Z. Wang, Z. Dai, Carbon nanomaterial-based electrochemical biosensors: an overview, Nanoscale 7 (2015) 6420−6431, https://doi.org/10.1039/c5nr00585j.

[10] E.B. Bahadir, M.K. Sezgintürk, Electrochemical biosensors for hormone analyses, Biosens. Bioelectron. 68 (2015) 62−71, https://doi.org/10.1016/j.bios.2014.12.054.

[11] J.L. Hammond, N. Formisano, P. Estrela, S. Carrara, J. Tkac, Electrochemical biosensors and nanobiosensors, Essays Biochem. 60 (2016) 69−80, https://doi.org/10.1042/EBC20150008.
[12] A.A. Ansari, B.D. Malhotra, Current progress in organic−inorganic hetero-nano-interfaces based electrochemical biosensors for healthcare monitoring, Coord. Chem. Rev. 452 (2022) 214282, https://doi.org/10.1016/j.ccr.2021.214282.
[13] Y.-F. Fan, Z.-B. Guo, G.-B. Ge, Enzyme-based biosensors and their applications, Biosensors 13 (2023) 476, https://doi.org/10.3390/bios13040476.
[14] M. Pohanka, Glucose electrochemical biosensors: the past and current trends, Int. J. Electrochem. Sci. 16 (2021) 1−12, https://doi.org/10.20964/2021.07.52.
[15] N. Fourati, N. Blel, Y. Lattach, N. Ktari, C. Zerrouki, Chemical and biological sensors from conducting and semiconducting polymers, in: Reference Module in Materials Science and Materials Engineering, Elsevier, Amsterdam, 2016, pp. 1−35.
[16] G. Rocchitta, A. Spanu, S. Babudieri, G. Latte, G. Madeddu, G. Galleri, S. Nuvoli, P. Bagella, M. Demartis, V. Fiore, R. Manetti, P. Serra, Enzyme biosensors for biomedical applications: strategies for safeguarding analytical performances in biological fluids, Sensors 16 (2016) 780, https://doi.org/10.3390/s16060780.
[17] K. Horie, M. Barón, R.B. Fox, J. He, M. Hess, J. Kahovec, T. Kitayama, P. Kubisa, E. Maréchal, W. Mormann, R.F.T. Stepto, D. Tabak, J. Vohlídal, E.S. Wilks, W.J. Work, Definitions of terms relating to reactions of polymers and to functional polymeric materials: (IUPAC Recommendations 2003), Pure Appl. Chem. 76 (2004) 889−906, https://doi.org/10.1351/pac200476040889.
[18] F. Mao, N. Mano, A. Heller, Long tethers binding redox centers to polymer backbones enhance electron transport in enzyme "wiring" hydrogels, J. Am. Chem. Soc. 125 (2003) 4951−4957, https://doi.org/10.1021/ja029510e.
[19] V. Skrypnik, D. Rochefort, Electron transfer properties of a redox polyelectrolyte based on ferrocenated imidazolium, Electrochim. Acta. 305 (2019) 155−163, https://doi.org/10.1016/j.electacta.2019.02.119.
[20] P. Misra, Physics of Condensed Matter, Academic Press, Burlington, 2011.
[21] H. Kumar, A. Boora, A. Yadav, R. Rajni, Polyaniline-metal oxide-nano-composite as a nano-electronics, opto-electronics, heat resistance and anticorrosive material, Results Chem. 2 (2020) 100046, https://doi.org/10.1016/j.rechem.2020.100046.
[22] A.M. Bryan, L.M. Santino, Y. Lu, S. Acharya, J.M. D'Arcy, Conducting polymers for pseudocapacitive energy storage, Chem. Mater. 28 (2016) 5989−5998, https://doi.org/10.1021/acs.chemmater.6b01762.
[23] M. Wan, Conducting Polymers with Micro or Nanometer Structure, Springer Science & Business Media, Beijing, 2009.
[24] T.M. Swager, , 50th anniversary perspective: conducting/semiconducting conjugated polymers. A personal perspective on the past and the future, Macromolecules 50 (2017) 4867−4886, https://doi.org/10.1021/acs.macromol.7b00582.
[25] G. Salinas, J.-A. Del-Oso, P.-J. Espinoza-Montero, J. Heinze, B.A. Frontana-Uribe, Electrochemical polymerization, characterization and in-situ conductivity studies of poly-3,4-ortho-xylendioxythiophene (PXDOT), Synth. Met. 245 (2018) 135−143, https://doi.org/10.1016/j.synthmet.2018.08.020.
[26] V. Shaktawat, N. Jain, R. Saxena, N.S. Saxena, K. Sharma, T.P. Sharma, Temperature dependence of electrical conduction in pure and doped polypyrrole, Polym. Bull. 57 (2006) 535−543, https://doi.org/10.1007/s00289-006-0580-9.
[27] K. Lee, S. Cho, S. Heum Park, A.J. Heeger, C.-W. Lee, S.-H. Lee, Metallic transport in polyaniline, Nature 441 (2006) 65−68, https://doi.org/10.1038/nature04705.

[28] N. Massonnet, A. Carella, A. de Geyer, J. Faure-Vincent, J.-P. Simonato, Metallic behaviour of acid doped highly conductive polymers, Chem. Sci. 6 (2015) 412–417, https://doi.org/10.1039/C4SC02463J.
[29] S. Roth, Hopping conduction in electrically conducting polymers, Mod. Probl. Condens. Matter Sci. 28 (1991) 377–395, https://doi.org/10.1016/B978-0-444-88037-6.50017-8.
[30] K.P. Ramesh, NMR studies of disorder in condensed matter systems, Annu. Rep. NMR Spectrosc. 71 (2010) 139–175, https://doi.org/10.1016/B978-0-08-089054-8.00005-8.
[31] M.N. Gueye, A. Carella, J. Faure-Vincent, R. Demadrille, J.-P. Simonato, Progress in understanding structure and transport properties of PEDOT-based materials: a critical review, Prog. Mater. Sci. 108 (2020) 100616, https://doi.org/10.1016/j.pmatsci.2019.100616.
[32] H.-S. Park, S.-J. Ko, J.-S. Park, J.Y. Kim, H.-K. Song, Redox-active charge carriers of conducting polymers as a tuner of conductivity and its potential window, Sci. Rep. 3 (2013) 2454, https://doi.org/10.1038/srep02454.
[33] L. Boguslavsky, P.D. Hale, L. Geng, T.A. Skotheim, H.-S. Lee, Applications of redox polymers in biosensors, Solid State Ion. 60 (1993) 189–197, https://doi.org/10.1016/0167-2738(93)90294-D.
[34] H. Dahms, Electronic conduction in aqueous solution, J. Phys. Chem. 72 (1968) 362–364, https://doi.org/10.1021/j100847a073.
[35] I. Ruff, I. Korosi-Odor, Application of diffusion constant measurement to the determination of the rate constant of electron-exchange reactions, Inorg. Chem. 9 (1970) 186–188, https://doi.org/10.1021/ic50083a037.
[36] I. Ruff, V.J. Friedrich, Transfer diffusion. I. Theoretical, J. Phys. Chem. 75 (1971) 3297–3302, https://doi.org/10.1021/j100690a016.
[37] I. Ruff, V.J. Friedrich, K. Demeter, K. Csillag, Transfer diffusion. II. Kinetics of electron exchange reaction between ferrocene and ferricinium ion in alcohols, J. Phys. Chem. 75 (1971) 3303–3309, https://doi.org/10.1021/j100690a017.
[38] C.P. Andrieux, J.M. Savéant, Electron transfer through redox polymer films, J. Electroanal. Chem. Interfacial Electrochem. 111 (1980) 377–381, https://doi.org/10.1016/S0022-0728(80)80058-1.
[39] E. Laviron, A multilayer model for the study of space distributed redox modified electrodes: Part I. Description and discussion of the model, J. Electroanal. Chem. Interfacial Electrochem. 112 (1980) 1–9, https://doi.org/10.1016/S0022-0728(80)80002-7.
[40] D.A. Buttry, F.C. Anson, Electron hopping vs. molecular diffusion as charge transfer mechanisms in redox polymer films, J. Electroanal. Chem. Interfacial Electrochem. 130 (1981) 333–338, https://doi.org/10.1016/S0022-0728(81)80402-0.
[41] H.S. White, J. Leddy, A.J. Bard, Polymer films on electrodes. 8. Investigation of charge-transport mechanisms in Nafion polymer modified electrodes, J. Am. Chem. Soc. 104 (1982) 4811–4817, https://doi.org/10.1021/ja00382a013.
[42] N.A. Surridge, J.C. Jernigan, E.F. Dalton, R.P. Buck, M. Watanabe, H. Zhang, M. Pinkerton, T.T. Wooster, M.L. Longmire, J.S. Facci, R.W. Murray, The Electrochemistry Group Medal Lecture. Electron self-exchange dynamics between redox sites in polymers, Faraday Discuss. Chem. Soc. 88 (1989) 1, https://doi.org/10.1039/dc9898800001.
[43] E.F. Dalton, N.A. Surridge, J.C. Jernigan, K.O. Wilbourn, J.S. Facci, R.W. Murray, Charge transport in electroactive polymers consisting of fixed molecular redox sites, Chem. Phys. 141 (1990) 143–157, https://doi.org/10.1016/0301-0104(90)80026-T.

[44] R.A. Marcus, Electron transfer reactions in chemistry: theory and experiment (Nobel lecture), Angew. Chem. Int. Ed. Engl. 32 (1993) 1111−1121, https://doi.org/10.1002/anie.199311113.
[45] D.A. Buttry, F.C. Anson, Effects of electron exchange and single-file diffusion on charge propagation in Nafion films containing redox couples, J. Am. Chem. Soc. 105 (1983) 685−689, https://doi.org/10.1021/ja00342a001.
[46] K. Sato, R. Ichinoi, R. Mizukami, T. Serikawa, Y. Sasaki, J. Lutkenhaus, H. Nishide, K. Oyaizu, Diffusion-cooperative model for charge transport by redox-active nonconjugated polymers, J. Am. Chem. Soc. 140 (2018) 1049−1056, https://doi.org/10.1021/jacs.7b11272.
[47] K. Zojer, Simulation of charge carriers in organic electronic devices: methods with their fundamentals and applications, Adv. Opt. Mater. 9 (2021), https://doi.org/10.1002/adom.202100219.
[48] T. Fujiyabu, F. Toni, X. Li, U.I. Chung, T. Sakai, Three cooperative diffusion coefficients describing dynamics of polymer gels, Chem. Commun. 54 (2018) 6784−6787, https://doi.org/10.1039/c8cc01357h.
[49] A.T. Hubbard, D.G. Peters, Electrochemistry in thin layers of solution, CRC Crit. Rev. Anal. Chem. 3 (1973) 201−242, https://doi.org/10.1080/10408347308542661.
[50] A.T. Hubbard, Study of the kinetics of electrochemical reactions by thin-layer voltammetry: I. theory, J. Electroanal. Chem. Interfacial Electrochem. 22 (1969) 165−174, https://doi.org/10.1016/S0022-0728(69)80247-0.
[51] N. Elgrishi, K.J. Rountree, B.D. McCarthy, E.S. Rountree, T.T. Eisenhart, J.L. Dempsey, A practical beginners guide to cyclic voltammetry, J. Chem. Educ. 95 (2018) 197−206, https://doi.org/10.1021/acs.jchemed.7b00361.
[52] A. John, L. Benny, A.R. Cherian, S.Y. Narahari, A. Varghese, G. Hegde, Electrochemical sensors using conducting polymer/noble metal nanoparticle nanocomposites for the detection of various analytes: a review, J. Nanostruct. Chem. 11 (2021) 1−31, https://doi.org/10.1007/s40097-020-00372-8.
[53] S. Wu, J. Hao, S. Yang, Y. Sun, Y. Wang, W. Zhang, H. Mao, X.-M. Song, Layer-by-layer self-assembly film of PEI-reduced graphene oxide composites and cholesterol oxidase for ultrasensitive cholesterol biosensing, Sens. Actuators B Chem. 298 (2019) 126856, https://doi.org/10.1016/j.snb.2019.126856.
[54] H. Yamaguchi, Y. Kiyota, M. Miyazaki, Techniques for preparation of cross-linked enzyme aggregates and their applications in bioconversions, Catalysts 8 (2018) 174, https://doi.org/10.3390/catal8050174.
[55] E.M. Papamichael, P.-Y. Stergiou, Enzyme immobilization strategies and bioprocessing applications, in: Biomass, Biofuels, Biochemicals, Elsevier, Netherlands, 2020, pp. 217−241, https://doi.org/10.1016/B978-0-12-819820-9.00012-0.
[56] S. Kasapis, I.T. Norton, J.B. Ubbink, Modern Biopolymer Science: Bridging the Divide between Fundamental Treatise and Industrial Application, Elsevier/Academic Press, Amsterdam Boston, 2009.
[57] A. Sinz, Chemical cross-linking and mass spectrometry for mapping three-dimensional structures of proteins and protein complexes, J. Mass Spectrom. 38 (2003) 1225−1237, https://doi.org/10.1002/jms.559.
[58] M. Nair, S.M. Best, R.E. Cameron, Crosslinking collagen constructs: achieving cellular selectivity through modifications of physical and chemical properties, Appl. Sci. 10 (2020) 6911, https://doi.org/10.3390/app10196911.
[59] J. Maitra, V.K. Shukla, Cross-linking in hydrogels − a review, Am. J. Polym. Sci. 4 (2014) 7, https://doi.org/10.5923/j.ajps.20140402.01.

[60] G. Tillet, B. Boutevin, B. Ameduri, Chemical reactions of polymer crosslinking and post-crosslinking at room and medium temperature, Prog. Polym. Sci. 36 (2011) 191–217, https://doi.org/10.1016/j.progpolymsci.2010.08.003.
[61] C.-M. Jannu, A. Noé, C. Bibiana, Bioelectrocatalysis for biofuel cells, in: Biofuel Cells, John Wiley & Sons, Ltd, Beverly, 2021, pp. 1–52.
[62] Y. Chung, M. Christwardana, D.C. Tannia, K.J. Kim, Y. Kwon, Biocatalyst including porous enzyme cluster composite immobilized by two-step crosslinking and its utilization as enzymatic biofuel cell, J. Power Sources 360 (2017) 172–179, https://doi.org/10.1016/j.jpowsour.2017.06.012.
[63] A.R. Pereira, G.C. Sedenho, J.C.P.D. Souza, F.N. Crespilho, Advances in enzyme bioelectrochemistry, An. Acad. Bras. Ciênc. 90 (2018) 825–857, https://doi.org/10.1590/0001-3765201820170514.
[64] I. Delrue, D. Verzele, A. Madder, H.J. Nauwynck, Inactivated virus vaccines from chemistry to prophylaxis: merits, risks and challenges, Expert Rev. Vaccines 11 (2012) 695–719, https://doi.org/10.1586/erv.12.38.
[65] T.J.A. Johnson, Glutaraldehyde cross-linking, in: Biocatalysy Design for Stability and Specificity, American Chemical Society, Washington, 1993, pp. 283–295, https://doi.org/10.1021/bk-1993-0516.ch023.
[66] C. Wang, Q. Yan, H.-B. Liu, X.-H. Zhou, S.-J. Xiao, Different EDC/NHS activation mechanisms between PAA and PMAA brushes and the following amidation reactions, Langmuir 27 (2011) 12058–12068, https://doi.org/10.1021/la202267p.
[67] W.E. Hennink, C.F. van Nostrum, Novel crosslinking methods to design hydrogels, Adv. Drug Deliv. Rev. 64 (2012) 223–236, https://doi.org/10.1016/j.addr.2012.09.009.
[68] P. Ó Conghaile, S. Kamireddy, D. MacAodha, P. Kavanagh, D. Leech, Mediated glucose enzyme electrodes by cross-linking films of osmium redox complexes and glucose oxidase on electrodes, Anal. Bioanal. Chem. 405 (2013) 3807–3812, https://doi.org/10.1007/s00216-012-6628-9.
[69] N. Wathoni, L. Fauziah, D. Gozali, A. Hasanah, Y. Wahyuni, Determination of uric acid level by polyaniline and poly (allylamine): based biosensor, J. Adv. Pharm. Technol. Res. 5 (2014) 13, https://doi.org/10.4103/2231-4040.126981.
[70] J.M. Díaz-González, Effects of the cross-linker on the performance and stability of enzymatic electrocatalytic films of glucose oxidase and dimethylferrocene-modified linear poly(ethyleneimine), Electrochim. Acta (2020) 11.
[71] V.M. Pyeshkova, O.Y. Dudchenko, O.O. Soldatkin, B.O. Kasap, F. Lagarde, B.A. Kurç, S.V. Dzyadevych, Application of silicalite-modified electrode for the development of sucrose biosensor with improved characteristics, Nanoscale Res. Lett. 10 (2015) 149, https://doi.org/10.1186/s11671-015-0853-z.
[72] K. Reybier, The use of polyethyleneimine for fabrication of potentiometric cholinesterase biosensors, Talanta 56 (2002) 1015–1020, https://doi.org/10.1016/S0039-9140(01)00588-4.
[73] P.P. Joshi, S.A. Merchant, Y. Wang, D.W. Schmidtke, Amperometric biosensors based on redox polymer–carbon nanotube–enzyme composites, Anal. Chem. 77 (2005) 3183–3188, https://doi.org/10.1021/ac0484169.
[74] F.-L. Wong, A. Abdul-Aziz, Comparative study of poly(vinyl alcohol)-based support materials for the immobilization of glucose oxidase, J. Chem. Technol. Biotechnol. 83 (2008) 41–46, https://doi.org/10.1002/jctb.1774.
[75] Y. Zhang, C. Ji, Electro-Induced covalent cross-linking of chitosan and formation of chitosan hydrogel films: its application as an enzyme immobilization matrix for use in a phenol sensor, Anal. Chem. 82 (2010) 5275–5281, https://doi.org/10.1021/ac100714s.

[76] W. Suginta, P. Khunkaewla, A. Schulte, Electrochemical biosensor applications of polysaccharides chitin and chitosan, Chem. Rev. 113 (2013) 5458−5479, https://doi.org/10.1021/cr300325r.
[77] G.R. VandeZande, J.M. Olvany, J.L. Rutherford, M. Rasmussen, Enzyme immobilization and mediation with osmium redox polymers, in: S.D. Minteer (Ed.), Enzyme Stabilization and Immobilization: Methods and Protocols, Springer, New York, NY, 2017, pp. 165−179, https://doi.org/10.1007/978-1-4939-6499-4_13.
[78] T. Chen, K. Hou, Q. Ren, G. Chen, P. Wei, M. Zhu, Nanoparticle-polymer synergies in nanocomposite hydrogels: from design to application, Macromol. Rapid Commun. 39 (2018) 1800337, https://doi.org/10.1002/marc.201800337.
[79] V.A. Arlyapov, A.S. Kharkova, S.K. Kurbanaliyeva, L.S. Kuznetsova, A.V. Machulin, S.E. Tarasov, P.V. Melnikov, O.N. Ponamoreva, V.A. Alferov, A.N. Reshetilov, Use of biocompatible redox-active polymers based on carbon nanotubes and modified organic matrices for development of a highly sensitive BOD biosensor, Enzyme Microb. Technol. 143 (2021) 109706, https://doi.org/10.1016/j.enzmictec.2020.109706.
[80] S. Kakhki, M.M. Barsan, E. Shams, C.M.A. Brett, New robust redox and conducting polymer modified electrodes for ascorbate sensing and glucose biosensing, Electroanalysis 25 (2013) 77−84, https://doi.org/10.1002/elan.201200402.
[81] B. Jugović, B. Grgur, M. Antov, Z. Knežević-Jugović, J. Stevanović, M. Gvozdenović, Polypyrrole-based enzyme electrode with immobilized glucose oxidase for electrochemical determination of glucose, Int. J. Electrochem. Sci. 11 (2016) 10.
[82] M.M. Gvozdenović, B.Z. Jugović, D.I. Bezbradica, M.G. Antov, Z.D. Knežević-Jugović, B.N. Grgur, Electrochemical determination of glucose using polyaniline electrode modified by glucose oxidase, Food Chem. 124 (2011) 396−400, https://doi.org/10.1016/j.foodchem.2010.06.046.
[83] R. Antiochia, F. Mazzei, L. Gorton, D. Leech, G. Favero, Composite material based on macroporous polyaniline and osmium redox complex for biosensor development, Electroanalysis 26 (2014) 1623−1630, https://doi.org/10.1002/elan.201400021.
[84] K. Spychalska, D. Zając, J. Cabaj, Electrochemical biosensor for detection of 17β-estradiol using semi-conducting polymer and horseradish peroxidase, RSC Adv. 10 (2020) 9079−9087, https://doi.org/10.1039/c9ra09902f.
[85] S. Söylemez, Investigation the effect of dioctylfluorenyl-co- benzothiadiazole bearing polymer and MWCNTS on biosensor performance, Mugla J. Sci. Technol. 5 (2019) 17−23, https://doi.org/10.22531/muglajsci.495209.
[86] E.M.I.M. Ekanayake, D.M.G. Preethichandra, K. Kaneto, An amperometric glucose biosensor with enhanced measurement stability and sensitivity using an artificially porous conducting polymer, IEEE Trans. Instrum. Meas. 57 (2008) 1621−1626, https://doi.org/10.1109/TIM.2008.922068.
[87] P. Krzyczmonik, E. Socha, S. Skrzypek, Immobilization of glucose oxidase on modified electrodes with composite layers based on poly(3,4-ethylenedioxythiophene), Bioelectrochemistry 101 (2015) 8−13, https://doi.org/10.1016/j.bioelechem.2014.06.009.
[88] R. Gorejová, N. Podrojková, K. Sisáková, J. Shepa, I. Shepa, A. Kovalčíková, I. Šišoláková, F. Kaľavský, R. Oriňaková, Interaction of thin polyethyleneimine layer with the iron surface and its effect on the electrochemical behavior, Sci. Rep. 12 (2022) 3460, https://doi.org/10.1038/s41598-022-07474-z.
[89] K. Bali, M. Bak, K. Szarka, G. Juhász, G. Sáfrán, B. Pécz, J. Mihály, R. Mészáros, Controlling the morphology of poly(ethyleneimine)/gold nanoassemblies through the variation of pH and electrolyte additives, J. Mol. Liq. 322 (2021) 114559, https://doi.org/10.1016/j.molliq.2020.114559.

[90] S. Kobayashi, T. Saegusai, Chelating properties of linear and branched Poly(-ethylenimines), Macromolecules 20 (1987) 5.
[91] Y.R. Dangi, J.K. Bediako, X. Lin, J.-W. Choi, C.-R. Lim, M.-H. Song, M. Han, Y.-S. Yun, Polyethyleneimine impregnated alginate capsule as a high capacity sorbent for the recovery of monovalent and trivalent gold, Sci. Rep. 11 (2021) 17836, https://doi.org/10.1038/s41598-021-97228-0.
[92] S. Suriyanarayanan, H.-H. Lee, B. Liedberg, T. Aastrup, I.A. Nicholls, Protein-resistant hyperbranched polyethyleneimine brush surfaces, J. Colloid Interface Sci. 396 (2013) 307−315, https://doi.org/10.1016/j.jcis.2012.12.076.
[93] N. An, C.H. Zhou, X.Y. Zhuang, D.S. Tong, W.H. Yu, Immobilization of enzymes on clay minerals for biocatalysts and biosensors, Appl. Clay Sci. 114 (2015) 283−296, https://doi.org/10.1016/j.clay.2015.05.029.
[94] B.F.Y. Yon-Hin, M. Smolander, T. Crompton, C.R. Lowe, Covalent electro-polymerization of glucose oxidase in polypyrrole. Evaluation of methods of pyrrole attachment to glucose oxidase on the performance of electropolymerized glucose sensors, Anal. Chem 65 (1993) 2067−2071, https://doi.org/10.1021/ac00063a022.
[95] W.J. Sung, Y.H. Bae, A glucose oxidase electrode based on electropolymerized conducting polymer with polyanion−enzyme conjugated dopant, Anal. Chem. 72 (2000) 2177−2181, https://doi.org/10.1021/ac9908041.
[96] M.J. Higgins, G.G. Wallace, Surface and biomolecular forces of conducting polymers, Polym. Rev. 53 (2013) 506−526, https://doi.org/10.1080/15583724.2013.813856.
[97] I. Katakis, L. Ye, A. Heller, Electrostatic control of the electron-transfer enabling binding of recombinant glucose oxidase and redox polyelectrolytes, Anal. Chem. 116 (1994) 3617−3618, https://doi.org/10.1021/ja00087a065.
[98] M. Aliofkhazraei, Anti-Abrasive Nanocoatings, Elsevier, Cambridge, 2015, https://doi.org/10.1016/C2013-0-16186-7.
[99] S. Kulandaivalu, Y. Sulaiman, Recent advances in layer-by-layer assembled conducting polymer based composites for supercapacitors, Energies 12 (2019) 2107, https://doi.org/10.3390/en12112107.
[100] J. Borges, J.F. Mano, Molecular interactions driving the layer-by-layer assembly of multilayers, Chem. Rev. 114 (2014) 8883−8942, https://doi.org/10.1021/cr400531v.
[101] G. Decher, J.B. Schlenoff (Eds.), Multilayer Thin Films: Sequential Assembly of Nanocomposite Materials, 1st ed., Wiley, Weinheim, 2012 https://doi.org/10.1002/9783527646746.
[102] M. Tagliazucchi, D. Grumelli, E.J. Calvo, Nanostructured modified electrodes: role of ions and solvent flux in redox active polyelectrolyte multilayer films, Phys. Chem. Chem. Phys. 8 (2006) 5086, https://doi.org/10.1039/b609341h.
[103] X. Zhu, D. Jańczewski, S.S.C. Lee, S.L.-M. Teo, G.J. Vancso, Cross-linked polyelectrolyte multilayers for marine antifouling applications, ACS Appl. Mater. Interfaces 5 (2013) 5961−5968, https://doi.org/10.1021/am4015549.
[104] J.J. Richardson, M. Björnmalm, F. Caruso, Technology-driven layer-by-layer assembly of nanofilms, Science 348 (2015) aaa2491, https://doi.org/10.1126/science.aaa2491.
[105] M. Tagliazucchi, E.J. Calvo, Charge transport in redox polyelectrolyte multilayer films: the dramatic effects of outmost layer and solution ionic strength, ChemPhysChem 11 (2010) 2957−2968, https://doi.org/10.1002/cphc.201000172.
[106] E. Guzmán, F. Ortega, R.G. Rubio, Layer-by-layer materials for the fabrication of devices with electrochemical applications, Energies 15 (2022) 3399, https://doi.org/10.3390/en15093399.

[107] R.N. Vyas, B. Wang, Electrochemical analysis of conducting polymer thin films, Int. J. Mol. Sci. 11 (2010) 1956−1972, https://doi.org/10.3390/ijms11041956.
[108] S.E. Herrera, M.L. Agazzi, M.L. Cortez, W.A. Marmisollé, C. Bilderling, O. Azzaroni, Layer-by-layer formation of polyamine-salt aggregate/polyelectrolyte multilayers. Loading and controlled release of probe molecules from self-assembled supramolecular networks, Macromol. Chem. Phys. 220 (2019) 1900094, https://doi.org/10.1002/macp.201900094.
[109] E.J. Calvo, A. Wolosiuk, Donnan permselectivity in layer-by-layer self-assembled redox Polyelectrolye thin films, J. Am. Chem. Soc. 124 (2002) 8490−8497, https://doi.org/10.1021/ja020107h.
[110] J.-Y. Wang, L.-C. Chen, K.-C. Ho, Synthesis of redox polymer nanobeads and nanocomposites for glucose biosensors, ACS Appl. Mater. Interfaces 5 (2013) 7852−7861, https://doi.org/10.1021/am4018219.
[111] M.L. Cortez, A. Lorenzo, W.A. Marmisollé, C. von Bilderling, E. Maza, L. Pietrasanta, F. Battaglini, M. Ceolín, O. Azzaroni, Highly-organized stacked multilayers via layer-by-layer assembly of lipid-like surfactants and polyelectrolytes. Stratified supramolecular structures for (bio)electrochemical nanoarchitectonics, Soft Matter 14 (2018) 1939−1952, https://doi.org/10.1039/C8SM00052B.
[112] M.L. Cortez, A.L. Cukierman, F. Battaglini, Surfactant presence in a multilayer polyelectrolyte−enzyme system improves its catalytic response, Electrochem. Commun. 11 (2009) 990−993, https://doi.org/10.1016/j.elecom.2009.02.041.
[113] M.L. Cortez, M. Ceolín, O. Azzaroni, F. Battaglini, Formation of redox-active self-assembled polyelectrolyte−surfactant complexes integrating glucose oxidase on electrodes: influence of the self-assembly solvent on the signal generation, Bioelectrochemistry 105 (2015) 117−122, https://doi.org/10.1016/j.bioelechem.2015.06.001.
[114] V. Flexer, E.J. Calvo, P.N. Bartlett, The application of the relaxation and simplex method to the analysis of data for glucose electrodes based on glucose oxidase immobilised in an osmium redox polymer, J. Electroanal. Chem. 646 (2010) 24−32, https://doi.org/10.1016/j.jelechem.2009.11.017.
[115] E.J. Calvo, F. Battaglini, C. Danilowicz, A. Wolosiuk, M. Otero, Layer-by-layer electrostatic deposition of biomolecules on surfaces for molecular recognition, redox mediation and signal generation, Faraday Discuss 116 (2000) 47−65, https://doi.org/10.1039/b001665i.
[116] K.L. Knoche, D.P. Hickey, R.D. Milton, C.L. Curchoe, S.D. Minteer, Hybrid glucose/O_2 biobattery and supercapacitor utilizing a pseudocapacitive dimethylferrocene redox polymer at the bioanode, ACS Energy Lett. 1 (2016) 380−385, https://doi.org/10.1021/acsenergylett.6b00225.
[117] R. Szamocki, V. Flexer, L. Levin, F. Forchiasin, E.J. Calvo, Oxygen cathode based on a layer-by-layer self-assembled laccase and osmium redox mediator, Electrochim. Acta 54 (2009) 1970−1977, https://doi.org/10.1016/j.electacta.2008.09.002.
[118] P. Scodeller, R. Carballo, R. Szamocki, L. Levin, F. Forchiassin, E.J. Calvo, Layer-by-Layer self-assembled osmium polymer-mediated laccase oxygen cathodes for biofuel cells: the role of hydrogen peroxide, J. Am. Chem. Soc. 132 (2010) 11132−11140, https://doi.org/10.1021/ja1020487.
[119] D. Aradilla, F. Estrany, C. Alemán, Symmetric supercapacitors based on multilayers of conducting polymers, J. Phys. Chem. C 115 (2011) 8430−8438, https://doi.org/10.1021/jp201108c.

[120] J. Yun, I. Echols, P. Flouda, S. Wang, A. Easley, X. Zhao, Z. Tan, E. Prehn, G. Zi, M. Radovic, M.J. Green, J.L. Lutkenhaus, Layer-by-Layer assembly of polyaniline nanofibers and MXene thin-film electrodes for electrochemical energy storage, ACS Appl. Mater. Interfaces 11 (2019) 47929−47938, https://doi.org/10.1021/acsami.9b16692.
[121] M.N. Hyder, S.W. Lee, F.Ç. Cebeci, D.J. Schmidt, Y. Shao-Horn, P.T. Hammond, Layer-by-layer assembled polyaniline nanofiber/multiwall carbon nanotube thin film electrodes for high-power and high-energy storage applications, ACS Nano 5 (2011) 8552−8561, https://doi.org/10.1021/nn2029617.
[122] W.A. Marmisollé, E. Maza, S. Moya, O. Azzaroni, Amine-appended polyaniline as a water dispersible electroactive polyelectrolyte and its integration into functional self-assembled multilayers, Electrochim. Acta 210 (2016) 435−444, https://doi.org/10.1016/j.electacta.2016.05.182.
[123] A. vander Straeten, A. Bratek-Skicki, A.M. Jonas, C.-A. Fustin, C. Dupont-Gillain, Integrating proteins in layer-by-layer assemblies independently of their electrical charge, ACS Nano 12 (2018) 8372−8381, https://doi.org/10.1021/acsnano.8b03710.
[124] C. Vranckx, L. Lambricht, V. Préat, O. Cornu, C. Dupont-Gillain, A. vander Straeten, Layer-by-layer nanoarchitectonics using protein−polyelectrolyte complexes toward a generalizable tool for protein surface immobilization, Langmuir 38 (2022) 5579−5589, https://doi.org/10.1021/acs.langmuir.2c00191.
[125] Y. Degani, A. Heller, Electrical communication between redox centers of glucose oxidase and electrodes via electrostatically and covalently bound redox polymers, J. Am. Chem. Soc. 111 (1989) 2357−2358, https://doi.org/10.1021/ja00188a091.
[126] M. Onda, Y. Lvov, K. Ariga, T. Kunitake, Sequential actions of glucose oxidase and peroxidase in molecular films assembled by layer-by-layer alternate adsorption, Biotechnol. Bioeng. 51 (1996) 163−167, https://doi.org/10.1002/(SICI)1097-0290(19960720)51:2<163::AID-BIT5>3.0.CO;2-H.
[127] J. Hodak, R. Etchenique, E.J. Calvo, K. Singhal, P.N. Bartlett, Layer-by-layer self-assembly of glucose oxidase with a poly(allylamine)ferrocene redox mediator, Langmuir 13 (1997) 2708−2716, https://doi.org/10.1021/la962014h.
[128] J. Zhang, X. Huang, L. Zhang, Y. Si, S. Guo, H. Su, J. Liu, Layer-by-layer assembly for immobilizing enzymes in enzymatic biofuel cells, Sustain. Energy Fuels 4 (2020) 68−79, https://doi.org/10.1039/C9SE00643E.
[129] N.P. Godman, J.L. DeLuca, S.R. McCollum, D.W. Schmidtke, D.T. Glatzhofer, Electrochemical characterization of layer-by-layer assembled ferrocene-modified linear poly(ethylenimine)/enzyme bioanodes for glucose sensor and biofuel cell applications, Langmuir 32 (2016) 3541−3551, https://doi.org/10.1021/acs.langmuir.5b04753.
[130] S. Ramanavicius, A. Ramanavicius, Conducting polymers in the design of biosensors and biofuel cells, Polymers 13 (2020) 49, https://doi.org/10.3390/polym13010049.
[131] G. Xu, S.B. Adeloju, Y. Wu, X. Zhang, Modification of polypyrrole nanowires array with platinum nanoparticles and glucose oxidase for fabrication of a novel glucose biosensor, Anal. Chim. Acta 755 (2012) 100−107, https://doi.org/10.1016/j.aca.2012.09.037.
[132] H. Xue, Z. Shen, Y. Li, Polyaniline−polyisoprene composite film based glucose biosensor with high permselectivity, Synth. Met. 124 (2001) 345−349, https://doi.org/10.1016/S0379-6779(01)00381-2.
[133] W.J. Dressick, K.J. Wahl, N.D. Bassim, R.M. Stroud, D.Y. Petrovykh, Divalent−anion salt effects in polyelectrolyte multilayer depositions, Langmuir 28 (2012) 15831−15843, https://doi.org/10.1021/la3033176.

[134] M. Salomäki, P. Tervasmäki, S. Areva, J. Kankare, The Hofmeister anion effect and the growth of polyelectrolyte multilayers, Langmuir 20 (2004) 3679−3683, https://doi.org/10.1021/la036328y.
[135] Y. Lapitsky, Ionically crosslinked polyelectrolyte nanocarriers: recent advances and open problems, Curr. Opin. Colloid Interface Sci. 19 (2014) 122−130, https://doi.org/10.1016/j.cocis.2014.03.014.
[136] D. Zappi, L.L. Coria-Oriundo, E. Piccinini, M. Gramajo, C. von Bilderling, L.I. Pietrasanta, O. Azzaroni, F. Battaglini, The effect of ionic strength and phosphate ions on the construction of redox polyelectrolyte−enzyme self-assemblies, Phys. Chem. Chem. Phys. 21 (2019) 22947−22954, https://doi.org/10.1039/C9CP04037D.
[137] S.E. Herrera, M.L. Agazzi, M.L. Cortez, W.A. Marmisollé, M. Tagliazucchi, O. Azzaroni, Polyamine colloids cross-linked with phosphate ions: towards understanding the solution phase behavior, ChemPhysChem 20 (2019) 1044−1053, https://doi.org/10.1002/cphc.201900046.
[138] G. Laucirica, W.A. Marmisollé, O. Azzaroni, Dangerous liaisons: anion-induced protonation in phosphate−polyamine interactions and their implications for the charge states of biologically relevant surfaces, Phys. Chem. Chem. Phys. 19 (2017) 8612−8620, https://doi.org/10.1039/C6CP08793K.
[139] L.L. Coria-Oriundo, M.L. Cortez, O. Azzaroni, F. Battaglini, Enzymes hosted in redox-active ionically cross-linked polyelectrolyte networks enable more efficient biofuel cells, Soft Matter 17 (2021) 5240−5247, https://doi.org/10.1039/D1SM00221J.
[140] W.A. Marmisollé, J. Irigoyen, D. Gregurec, S. Moya, O. Azzaroni, Supramolecular surface chemistry: substrate-independent, phosphate-driven growth of polyamine-based multifunctional thin films, Adv. Funct. Mater. 25 (2015) 4144−4152, https://doi.org/10.1002/adfm.201501140.
[141] C. Peng, J. Liu, Y. Xie, J. Zhou, Molecular simulations of cytochrome c adsorption on positively charged surfaces: the influence of anion type and concentration, Phys. Chem. Chem. Phys. 18 (2016) 9979−9989, https://doi.org/10.1039/C6CP00170J.
[142] D.A. Capdevila, W.A. Marmisollé, F.J. Williams, D.H. Murgida, Phosphate mediated adsorption and electron transfer of cytochrome c. A time-resolved SERR spectroelectrochemical study, Phys. Chem. Chem. Phys. 15 (2013) 5386−5394, https://doi.org/10.1039/C2CP42044A.
[143] A. Rodriguez-Abetxuko, D. Sánchez-deAlcázar, A.L. Cortajarena, A. Beloqui, A versatile approach for the assembly of highly tunable biocatalytic thin films, Adv. Mater. Interfaces. 6 (2019) 1900598, https://doi.org/10.1002/admi.201900598.
[144] G. Zeng, Y. Xing, J. Gao, Z. Wang, X. Zhang, Unconventional layer-by-layer assembly of graphene multilayer films for enzyme-based glucose and maltose biosensing, Langmuir 26 (2010) 15022−15026, https://doi.org/10.1021/la102806v.
[145] W.B. Stockton, M.F. Rubner, Molecular-level processing of conjugated polymers. 4. Layer-by-layer manipulation of polyaniline via hydrogen-bonding interactions, Macromolecules 30 (1997) 2717−2725, https://doi.org/10.1021/ma9700486.
[146] M.L. Cortez, N. De Matteis, M. Ceolín, W. Knoll, F. Battaglini, O. Azzaroni, Hydrophobic interactions leading to a complex interplay between bioelectrocatalytic properties and multilayer meso-organization in layer-by-layer assemblies, Phys. Chem. Chem. Phys. 16 (2014) 20844−20855, https://doi.org/10.1039/C4CP02334J.
[147] M.L. Cortez, D. Pallarola, M. Ceolín, O. Azzaroni, F. Battaglini, Ionic self-assembly of electroactive biorecognizable units: electrical contacting of redox glycoenzymes made easy, Chem. Commun. 48 (2012) 10868, https://doi.org/10.1039/c2cc35949a.

[148] H.T. Imam, P.C. Marr, A.C. Marr, Enzyme entrapment, biocatalyst immobilization without covalent attachment, Green Chem. 23 (2021) 4980–5005, https://doi.org/10.1039/D1GC01852C.
[149] S.-H. Jung, Y.-K. Lee, Y.-K. Son, Improved sensitivity of a glucose sensor by encapsulation of free GOx in conducting polymer micropillar structure, J. Electrochem. Sci. Technol. 2 (2011) 124–129.
[150] J.J. Mitala, A.C. Michael, Improving the performance of electrochemical microsensors based on enzymes entrapped in a redox hydrogel, Anal. Chim. Acta 556 (2006) 326–332, https://doi.org/10.1016/j.aca.2005.09.053.
[151] S. Ramanavicius, A. Ramanavicius, Charge transfer and biocompatibility aspects in conducting polymer-based enzymatic biosensors and biofuel cells, Nanomaterials 11 (2021) 1–22, https://doi.org/10.3390/nano11020371.
[152] P.N. Barlett, J.M. Cooper, A review of the immobilization of enzymes in electropolymerized films, J. Electroanal. Chem. 362 (1993) 1–12, https://doi.org/10.1016/0022-0728(93)80001-X.
[153] R. Dobay, G. Harsányi, C. Visy, Detection of uric acid with a new type of conducting polymer-based enzymatic sensor by bipotentiostatic technique, Anal. Chim. Acta 385 (1999) 187–194, https://doi.org/10.1016/S0003-2670(98)00662-X.
[154] S.A. Rothwell, S.J. Killoran, R.D. O'Neill, Enzyme immobilization strategies and electropolymerization conditions to control sensitivity and selectivity parameters of a polymer-enzyme composite glucose biosensor, Sensors 10 (2010) 6439–6462, https://doi.org/10.3390/s100706439.
[155] B. Piro, L.A. Dang, M.C. Pham, S. Fabiano, C. Tran-Minh, A glucose biosensor based on modified-enzyme incorporated within electropolymerised poly(3,4-ethylenedioxythiophene) (PEDT) films, J. Electroanal. Chem. 512 (2001) 101–109, https://doi.org/10.1016/S0022-0728(01)00595-2.
[156] H.H. Nguyen, M. Kim, An overview of techniques in enzyme immobilization, Appl. Sci. Converg. Technol. 26 (2017) 157–163, https://doi.org/10.5757/asct.2017.26.6.157.
[157] S. Cosnier, M. Holzinger, Electrosynthesized polymers for biosensing, Chem. Soc. Rev. 40 (2011) 2146–2156, https://doi.org/10.1039/C0CS00090F.
[158] F.R.R. Teles, L.P. Fonseca, Applications of polymers for biomolecule immobilization in electrochemical biosensors, Mater. Sci. Eng. C. 28 (2008) 1530–1543, https://doi.org/10.1016/j.msec.2008.04.010.
[159] W. Wernet, Thermoplastic and elastic conducting polypyrrole films, Synth. Met. 41 (1991) 843–848, https://doi.org/10.1016/0379-6779(91)91508-8.
[160] G.F. Khan, W. Wernet, Platinization of shapable electroconductive polymer film for an improved glucose sensor, J. Electrochem. Soc. 143 (1996) 3336–3342, https://doi.org/10.1149/1.1837208.
[161] G.F. Khan, W. Wernet, A highly sensitive amperometric creatinine sensor, Anal. Chim. Acta 351 (1997) 151–158, https://doi.org/10.1016/S0003-2670(97)00362-0.
[162] A. Vilkanauskyte, T. Erichsen, L. Marcinkeviciene, V. Laurinavicius, W. Schuhmann, Reagentless biosensors based on co-entrapment of a soluble redox polymer and an enzyme within an electrochemically deposited polymer film, Biosens. Bioelectron. 17 (2002) 1025–1031, https://doi.org/10.1016/S0956-5663(02)00095-7.
[163] G.P. Willhite, J.G. Dominguez, Mechanisms of polymer retention in porous media, in: D.O. Shah, R.S. Schechter (Eds.), Improved Oil Recovery by Surfactant and Polymer Flooding, Academic Press, New York, 1977, pp. 511–554.

[164] H. Wei, J. Xia, W. Zhou, L. Zhou, G. Hussain, Q. Li, K. Ostrikov, Adhesion and cohesion of epoxy-based industrial composite coatings, Compos. B Eng. 193 (2020) 108035, https://doi.org/10.1016/j.compositesb.2020.108035.
[165] A. Casanova, J. Iniesta, A. Gomis-Berenguer, Recent progress in the development of porous carbon-based electrodes for sensing applications, Analyst 147 (2022) 767−783, https://doi.org/10.1039/D1AN01978C.
[166] S. Xiao, S. Wang, X. Wang, P. Xu, Nanoporous gold: a review and potentials in biotechnological and biomedical applications, Nano Sel. 2 (2021) 1437−1458, https://doi.org/10.1002/nano.202000291.
[167] D. Bélanger, J. Nadreau, G. Fortier, Electrochemistry of the polypyrrole glucose oxidase electrode, J. Electroanal. Chem. Interfacial Electrochem. 274 (1989) 143−155, https://doi.org/10.1016/0022-0728(89)87036-6.
[168] D. Zhai, B. Liu, Y. Shi, L. Pan, Y. Wang, W. Li, R. Zhang, G. Yu, Highly sensitive glucose sensor based on Pt nanoparticle/polyaniline hydrogel heterostructures, ACS Nano 7 (2013) 3540−3546, https://doi.org/10.1021/nn400482d.
[169] A. Molina, J. González, E. Laborda, Y. Wang, R.G. Compton, Catalytic mechanism in cyclic voltammetry at disc electrodes: an analytical solution, Phys. Chem. Chem. Phys. 13 (2011) 14694−14704, https://doi.org/10.1039/C1CP21181A.
[170] W. Zhang, G. Li, Third-generation biosensors based on the direct electron transfer of proteins, Anal. Sci. 20 (2004) 603−609, https://doi.org/10.2116/analsci.20.603.
[171] P. Das, M. Das, S.R. Chinnadayyala, I.M. Singha, P. Goswami, Recent advances on developing 3rd generation enzyme electrode for biosensor applications, Biosens. Bioelectron 79 (2016) 386−397, https://doi.org/10.1016/j.bios.2015.12.055.
[172] R.S. Freire, C.A. Pessoa, L.D. Mello, L.T. Kubota, Direct electron transfer: an approach for electrochemical biosensors with higher selectivity and sensitivity, J. Braz. Chem. Soc. 14 (2003) 230−243, https://doi.org/10.1590/S0103-50532003000200008.
[173] A. Ramanavicius, K. Habermüller, E. Csöregi, V. Laurinavicius, W. Schuhmann, Polypyrrole-entrapped quinohemoprotein alcohol dehydrogenase. Evidence for direct electron transfer via conducting-polymer chains, Anal. Chem. 71 (1999) 3581−3586, https://doi.org/10.1021/ac981201c.
[174] R.D. Milton, S.D. Minteer, Direct enzymatic bioelectrocatalysis: differentiating between myth and reality, J. R. Soc. Interface. 14 (2017) 20170253, https://doi.org/10.1098/rsif.2017.0253.
[175] P.N. Bartlett, P.R. Birkin, The application of conducting polymers in biosensors, Synth. Met. 61 (1993) 15−21, https://doi.org/10.1016/0379-6779(93)91194-7.
[176] H. Yoon, S. Ko, J. Jang, Field-effect-transistor sensor based on enzyme-functionalized polypyrrole nanotubes for glucose detection, J. Phys. Chem. B 112 (2008) 9992−9997, https://doi.org/10.1021/jp800567h.
[177] D.T. Hoa, T.N.S. Kumar, N.S. Punekar, R.S. Srinivasa, R. Lal, A.Q. Contractor, A biosensor based on conducting polymers, Anal. Chem. 64 (1992) 2645−2646, https://doi.org/10.1021/ac00045a031.
[178] P.S. Phale, A. Sharma, K. Gautam, Microbial degradation of xenobiotics like aromatic pollutants from the terrestrial environments, in: Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology, Elsevier, Oxford, 2019, pp. 259−278.
[179] M. Singh, P.K. Kathuroju, N. Jampana, Polypyrrole based amperometric glucose biosensors, Sens. Actuators B Chem. 143 (2009) 430−443, https://doi.org/10.1016/j.snb.2009.09.005.

[180] Y. Zhao, L. Cao, L. Li, W. Cheng, L. Xu, X. Ping, L. Pan, Y. Shi, Conducting polymers and their applications in diabetes management, Sensors 16 (2016) 1787, https://doi.org/10.3390/s16111787.
[181] J. Lai, Y. Yi, P. Zhu, J. Shen, K. Wu, L. Zhang, J. Liu, Polyaniline-based glucose biosensor: a review, J. Electroanal. Chem. 782 (2016) 138−153, https://doi.org/10.1016/j.jelechem.2016.10.033.
[182] N. German, A. Ramanaviciene, A. Ramanavicius, Dispersed conducting polymer nanocomposites with glucose oxidase and gold nanoparticles for the design of enzymatic glucose biosensors, Polymers 13 (2021) 2173, https://doi.org/10.3390/polym13132173.
[183] H. Zhong, R. Yuan, Y. Chai, W. Li, X. Zhong, Y. Zhang, In situ chemo-synthesized multi-wall carbon nanotube-conductive polyaniline nanocomposites: characterization and application for a glucose amperometric biosensor, Talanta 85 (2011) 104−111, https://doi.org/10.1016/j.talanta.2011.03.040.
[184] P.A. Fiorito, S.I.C. de Torresi, Glucose amperometric biosensor based on the Co-immobilization of glucose oxidase (GOx) and ferrocene in poly(pyrrole) generated from ethanol/water mixtures, J. Braz. Chem. Soc. 12 (2001), https://doi.org/10.1590/S0103-50532001000600007.
[185] L.H. Nguyen, H.B. Nguyen, N.T. Nguyen, T.D. Nguyen, D.L. Tran, Portable cholesterol detection with polyaniline-carbon nanotube film based interdigitated electrodes, Adv. Nat. Sci. Nanosci. Nanotechnol. 3 (2012) 015004, https://doi.org/10.1088/2043-6262/3/1/015004.
[186] J. Fei, Y. Wu, X. Ji, J. Wang, S. Hu, Z. Gao, An amperometric biosensor for glucose based on electrodeposited redox polymer/glucose oxidase film on a gold electrode, Anal. Sci. 19 (2003) 1259−1263, https://doi.org/10.2116/analsci.19.1259.
[187] S.A. Merchant, T.O. Tran, M.T. Meredith, T.C. Cline, D.T. Glatzhofer, D.W. Schmidtke, High-sensitivity amperometric biosensors based on ferrocene-modified linear poly(-ethylenimine), Langmuir 25 (2009) 7736−7742, https://doi.org/10.1021/la9004938.
[188] J. Liu, S. Sun, C. Liu, S. Wei, An amperometric glucose biosensor based on a screen-printed electrode and Os-complex mediator for flow injection analysis, Measurement 44 (2011) 1878−1883, https://doi.org/10.1016/j.measurement.2011.09.001.
[189] A. Mugweru, R. Mahmud, K. Ghosh, A. Wanekaya, Carbon nanofiber modified with osmium based redox polymer for glucose sensing, J. Electrochem. Sci. Eng. 7 (2017) 181−191, https://doi.org/10.5599/jese.422.
[190] Z. Jiang, Y. Shangguan, Q. Zheng, Ferrocene-modified polyelectrolyte film-coated electrode and its application in glucose detection, Polymers 11 (2019) 551, https://doi.org/10.3390/polym11030551.
[191] K. Jayakumar, R. Bennett, D. Leech, Electrochemical glucose biosensor based on an osmium redox polymer and glucose oxidase grafted to carbon nanotubes: a design-of-experiments optimisation of current density and stability, Electrochim. Acta 371 (2021) 137845, https://doi.org/10.1016/j.electacta.2021.137845.
[192] Y. Oztekin, A. Ramanaviciene, Z. Yazicigil, A.O. Solak, A. Ramanavicius, Direct electron transfer from glucose oxidase immobilized on polyphenanthroline-modified glassy carbon electrode, Biosens. Bioelectron. 26 (2011) 2541−2546, https://doi.org/10.1016/j.bios.2010.11.001.
[193] C.X. Guo, C.M. Li, Direct electron transfer of glucose oxidase and biosensing of glucose on hollow sphere-nanostructured conducting polymer/metal oxide composite, Phys. Chem. Chem. Phys. 12 (2010) 12153−12159, https://doi.org/10.1039/C0CP00378F.

[194] M. Zhao, X. Wu, C. Cai, Polyaniline nanofibers: synthesis, characterization, and application to direct electron transfer of glucose oxidase, J. Phys. Chem. C 113 (2009) 4987−4996, https://doi.org/10.1021/jp807621y.
[195] B.C. Thompson, O. Winther-Jensen, J. Vongsvivut, B. Winther-Jensen, D.R. MacFarlane, Conducting polymer enzyme alloys: electromaterials exhibiting direct electron transfer, Macromol. Rapid Commun. 31 (2010) 1293−1297, https://doi.org/10.1002/marc.201000064.
[196] Z. Wang, S. Liu, P. Wu, C. Cai, Detection of glucose based on direct electron transfer reaction of glucose oxidase immobilized on highly ordered polyaniline nanotubes, Anal. Chem. 81 (2009) 1638−1645, https://doi.org/10.1021/ac802421h.
[197] P.N. Bartlett, F.A. Al-Lolage, There is no evidence to support literature claims of direct electron transfer (DET) for native glucose oxidase (GOx) at carbon nanotubes or graphene, J. Electroanal. Chem. 819 (2018) 26−37, https://doi.org/10.1016/j.jelechem.2017.06.021.
[198] P. Bollella, E. Katz, Enzyme-based biosensors: tackling electron transfer issues, Sensors 20 (2020) 3517, https://doi.org/10.3390/s20123517.
[199] G.S. Wilson, Native glucose oxidase does not undergo direct electron transfer, Biosens. Bioelectron. 82 (2016) vii−viii, https://doi.org/10.1016/j.bios.2016.04.083.
[200] S.B. Adeloju, A.N. Moline, Fabrication of ultra-thin polypyrrole−glucose oxidase film from supporting electrolyte-free monomer solution for potentiometric biosensing of glucose, Biosens. Bioelectron. 16 (2001) 133−139, https://doi.org/10.1016/S0956-5663(00)00117-2.
[201] J. Liu, M. Agarwal, K. Varahramyan, Glucose sensor based on organic thin film transistor using glucose oxidase and conducting polymer, Sens. Actuators B Chem. 135 (2008) 195−199, https://doi.org/10.1016/j.snb.2008.08.009.
[202] M.M. Rodríguez-Delgado, G.S. Alemán-Nava, J.M. Rodríguez-Delgado, G. Dieck-Assad, S.O. Martínez-Chapa, D. Barceló, R. Parra, Laccase-based biosensors for detection of phenolic compounds, TrAC Trends Anal. Chem. 74 (2015) 21−45, https://doi.org/10.1016/j.trac.2015.05.008.
[203] D.M. Mate, M. Alcalde, Laccase engineering: from rational design to directed evolution, Biotechnol. Adv. 33 (2015) 25−40, https://doi.org/10.1016/j.biotechadv.2014.12.007.
[204] D.M. Mate, M. Alcalde, Laccase: a multi-purpose biocatalyst at the forefront of biotechnology, Microb. Biotechnol. 10 (2017) 1457−1467, https://doi.org/10.1111/1751-7915.12422.
[205] U.N. Dwivedi, P. Singh, V.P. Pandey, A. Kumar, Structure−function relationship among bacterial, fungal and plant laccases, J. Mol. Catal. B Enzym. 68 (2011) 117−128, https://doi.org/10.1016/j.molcatb.2010.11.002.
[206] C.M. Rivera-Hoyos, E.D. Morales-Álvarez, R.A. Poutou-Piñales, A.M. Pedroza-Rodríguez, R. RodrÍguez-Vázquez, J.M. Delgado-Boada, Fungal laccases, Fungal Biol. Rev. 27 (2013) 67−82, https://doi.org/10.1016/j.fbr.2013.07.001.
[207] P. Giardina, V. Faraco, C. Pezzella, A. Piscitelli, S. Vanhulle, G. Sannia, Laccases: a never-ending story, Cell. Mol. Life Sci. 67 (2010) 369−385, https://doi.org/10.1007/s00018-009-0169-1.
[208] M.T. Meredith, S.D. Minteer, Biofuel cells: enhanced enzymatic bioelectrocatalysis, Annu. Rev. Anal. Chem. 5 (2012) 157−179, https://doi.org/10.1146/annurev-anchem-062011-143049.
[209] M. Di Fusco, C. Tortolini, D. Deriu, F. Mazzei, Laccase-based biosensor for the determination of polyphenol index in wine, Talanta 81 (2010) 235−240, https://doi.org/10.1016/j.talanta.2009.11.063.

[210] S. Chawla, R. Rawal, C.S. Pundir, Fabrication of polyphenol biosensor based on laccase immobilized on copper nanoparticles/chitosan/multiwalled carbon nanotubes/polyaniline-modified gold electrode, J. Biotechnol. 156 (2011) 39−45, https://doi.org/10.1016/j.jbiotec.2011.08.008.

[211] A.M. Garzillo, M.C. Colao, V. Buonocore, R. Oliva, L. Falcigno, M. Saviano, A.M. Santoro, R. Zappala, R.P. Bonomo, C. Bianco, P. Giardina, G. Palmieri, G. Sannia, Structural and kinetic characterization of native laccases from *Pleurotus ostreatus*, *Rigidoporus lignosus*, and *Trametes trogii*, J. Protein Chem. 20 (2001) 191−201, https://doi.org/10.1023/A:1010954812955.

[212] S. Chawla, R. Rawal, S. Sharma, C.S. Pundir, An amperometric biosensor based on laccase immobilized onto nickel nanoparticles/carboxylated multiwalled carbon nanotubes/polyaniline modified gold electrode for determination of phenolic content in fruit juices, Biochem. Eng. J. 68 (2012) 76−84, https://doi.org/10.1016/j.bej.2012.07.008.

[213] B.S. Kushwah, S.C. Upadhyaya, S. Shukla, A. Singh Sikarwar, R.M.S. Sengar, S. Bhadauria, Performance of nanopolyaniline-fungal enzyme based biosensor for water pollution, Adv. Mater. Lett. 2 (2012) 43−51, https://doi.org/10.5185/amlett.2010.8149.

[214] S. Soylemez, S.A. Bekmezci, S. Goker, L. Toppare, Fabrication of a novel polymeric scaffold for amperometric laccase biosensor, J. Polym. Sci. Polym. Chem. 57 (2019) 2333−2339, https://doi.org/10.1002/pola.29537.

[215] Ş.C. Cevher, S.A. Bekmezci, S. Soylemez, Y.A. Udum, L. Toppare, A. Cirpan, Indenoquinoxalinone based conjugated polymer substrate for laccase biosensor, Mater. Chem. Phys. 257 (2021) 123788, https://doi.org/10.1016/j.matchemphys.2020.123788.

[216] Y. Ferry, D. Leech, Amperometric detection of catecholamine neurotransmitters using electrocatalytic substrate recycling at a laccase electrode, Electroanalysis 17 (2005) 113−119, https://doi.org/10.1002/elan.200403069.

[217] M. Dervisevic, E. Dervisevic, E. Çevik, M. Şenel, Novel electrochemical xanthine biosensor based on chitosan−polypyrrole−gold nanoparticles hybrid bio-nanocomposite platform, J. Food Drug Anal. 25 (2017) 510−519, https://doi.org/10.1016/j.jfda.2016.12.005.

[218] E. Cevik, A. Cerit, N. Gazel, H.B. Yildiz, Construction of an amperometric cholesterol biosensor based on DTP(aryl)aniline conducting polymer bound cholesterol oxidase, Electroanalysis 30 (2018) 2445−2453, https://doi.org/10.1002/elan.201800248.

[219] M. Dervisevic, E. Çevik, M. Şenel, C. Nergiz, M.F. Abasiyanik, Amperometric cholesterol biosensor based on reconstituted cholesterol oxidase on boronic acid functional conducting polymers, J. Electroanal. Chem. 776 (2016) 18−24, https://doi.org/10.1016/j.jelechem.2016.06.033.

[220] B.O. Özer, S. Çete, Development of a novel biosensor based on a polypyrrole−dodecylbenzene sulphonate (PPy−DBS) film for the determination of amperometric cholesterol, Artif. Cells Nanomed. Biotechnol. 45 (2017) 824−832, https://doi.org/10.1080/21691401.2016.1178133.

[221] Y. Tan, X. Guo, J. Zhang, J. Kan, Amperometric catechol biosensor based on polyaniline−polyphenol oxidase, Biosens. Bioelectron. 25 (2010) 1681−1687, https://doi.org/10.1016/j.bios.2009.12.007.

[222] K. Rathee, V. Dhull, R. Dhull, S. Singh, Biosensors based on electrochemical lactate detection: a comprehensive review, Biochem. Biophys. Rep. 5 (2016) 35−54, https://doi.org/10.1016/j.bbrep.2015.11.010.

[223] S. Timur, Y. Yigzaw, L. Gorton, Electrical wiring of pyranose oxidase with osmium redox polymers, Sens. Actuators B Chem. 113 (2006) 684−691, https://doi.org/10.1016/j.snb.2005.07.017.

[224] R. Cohen, R.E. Bitton, N.S. Herzallh, Y. Cohen, O. Yehezkeli, Utilization of FAD-glucose dehydrogenase from *T. emersonii* for amperometric biosensing and biofuel cell devices, Anal. Chem. 93 (2021) 11585−11591, https://doi.org/10.1021/acs.analchem.1c02157.

[225] M.M. Barsan, M.E. Ghica, C.M.A. Brett, Electrochemical sensors and biosensors based on redox polymer/carbon nanotube modified electrodes: a review, Anal. Chim. Acta 881 (2015) 1−23, https://doi.org/10.1016/j.aca.2015.02.059.

[226] D. MacAodha, P.O. Conghaile, B. Egan, P. Kavanagh, D. Leech, Membraneless glucose/oxygen enzymatic fuel cells using redox hydrogel films containing carbon nanotubes, ChemPhysChem 14 (2013) 2302−2307, https://doi.org/10.1002/cphc.201300239.

[227] N.B. Duong, V.M. Truong, Y.-S. Li, C.-L. Wang, H. Yang, Improving the immobilization of glucose oxidase on carbon cloth via a hybrid approach of cross-linked chitosan/TPP matrices with Na polymers for high-performance self-pumping enzyme-based biofuel cells, Energy Fuels 34 (2020) 10050−10058, https://doi.org/10.1021/acs.energyfuels.0c01033.

[228] N. Mano, A. de Poulpiquet, O_2 reduction in enzymatic biofuel cells, Chem. Rev. 118 (2018) 2392−2468, https://doi.org/10.1021/acs.chemrev.7b00220.

[229] F. Tasca, S. Timur, R. Ludwig, D. Haltrich, J. Volc, R. Antiochia, L. Gorton, Amperometric biosensors for detection of sugars based on the electrical wiring of different pyranose oxidases and pyranose dehydrogenases with osmium redox polymer on graphite electrodes, Electroanalysis 19 (2007) 294−302, https://doi.org/10.1002/elan.200603740.

[230] E.-H. Yoo, S.-Y. Lee, Glucose biosensors: an overview of use in clinical practice, Sensors 10 (2010) 4558−4576, https://doi.org/10.3390/s100504558.

[231] S. Ferri, K. Kojima, K. Sode, Review of glucose oxidases and glucose dehydrogenases: a birds eye view of glucose sensing enzymes, J. Diabetes Sci. Technol. 5 (2011) 1068−1076, https://doi.org/10.1177/193229681100500507.

[232] R. Antiochia, L. Gorton, Development of a carbon nanotube paste electrode osmium polymer-mediated biosensor for determination of glucose in alcoholic beverages, Biosens. Bioelectron. 22 (2007) 2611−2617, https://doi.org/10.1016/j.bios.2006.10.023.

[233] P. Du, P. Wu, C. Cai, A glucose biosensor based on electrocatalytic oxidation of NADPH at single-walled carbon nanotubes functionalized with poly(nile blue A), J. Electroanal. Chem. 624 (2008) 21−26, https://doi.org/10.1016/j.jelechem.2008.07.031.

[234] V. Flexer, N. Mano, Wired pyrroloquinoline quinone soluble glucose dehydrogenase enzyme electrodes operating at unprecedented low redox potential, Anal. Chem. 86 (2014) 2465−2473, https://doi.org/10.1021/ac403334w.

[235] K. Habermüller, A. Ramanavicius, V. Laurinavicius, W. Schuhmann, An oxygen-insensitive reagentless glucose biosensor based on osmium-complex modified polypyrrole, Electroanalysis 12 (2000) 1383−1389, https://doi.org/10.1002/1521-4109(200011)12:17<1383::AID-ELAN1383>3.0.CO;2-0.

[236] J. Gladisch, D. Sarauli, D. Schäfer, B. Dietzel, B. Schulz, F. Lisdat, Towards a novel bioelectrocatalytic platform based on "wiring" of pyrroloquinoline quinone-dependent glucose dehydrogenase with an electrospun conductive polymeric fiber architecture, Sci. Rep. 6 (2016) 19858, https://doi.org/10.1038/srep19858.

[237] M. Bilgi, E. Ayranci, Biosensor application of screen-printed carbon electrodes modified with nanomaterials and a conducting polymer: ethanol biosensors based on alcohol dehydrogenase, Sens. Actuators B Chem. 237 (2016) 849−855, https://doi.org/10.1016/j.snb.2016.06.164.

[238] M. Bilgi, E. Ayranci, Development of amperometric biosensors using screen-printed carbon electrodes modified with conducting polymer and nanomaterials for the analysis of ethanol, methanol and their mixtures, J. Electroanal. Chem. 823 (2018) 588−592, https://doi.org/10.1016/j.jelechem.2018.07.009.
[239] M. Niculescu, T. Erichsen, V. Sukharev, Z. Kerenyi, E. Csöregi, W. Schuhmann, Quinohemoprotein alcohol dehydrogenase-based reagentless amperometric biosensor for ethanol monitoring during wine fermentation, Anal. Chim. Acta 463 (2002) 39−51, https://doi.org/10.1016/S0003-2670(02)00344-6.
[240] A. Chaubey, K.K. Pande, V.S. Singh, B.D. Malhotra, Co-immobilization of lactate oxidase and lactate dehydrogenase on conducting polyaniline films, Anal. Chim. Acta 407 (2000) 97−103, https://doi.org/10.1016/S0003-2670(99)00797-7.
[241] A. Chaubey, M. Gerard, R. Singhal, V.S. Singh, B.D. Malhotra, Immobilization of lactate dehydrogenase on electrochemically prepared polypyrrole−polyvinylsulphonate composite films for application to lactate biosensors, Electrochim. Acta 46 (2001) 723−729, https://doi.org/10.1016/S0013-4686(00)00658-7.
[242] M.M. Rahman, M.J.A. Shiddiky, MdA. Rahman, Y.-B. Shim, A lactate biosensor based on lactate dehydrogenase/nictotinamide adenine dinucleotide (oxidized form) immobilized on a conducting polymer/multiwall carbon nanotube composite film, Anal. Biochem. 384 (2009) 159−165, https://doi.org/10.1016/j.ab.2008.09.030.
[243] K.K. Hussain, N.G. Gurudatt, M.H. Akhtar, K.-D. Seo, D.-S. Park, Y.-B. Shim, Nanobiosensor for the in vitro lactate detection using bi-functionalized conducting polymer/N, S-doped carbon; the effect of αCHC inhibitor on lactate level in cancer cell lines, Biosens. Bioelectron. 155 (2020) 112094, https://doi.org/10.1016/j.bios.2020.112094.
[244] D.A. Guschin, Y.M. Sultanov, N.F. Sharif-Zade, E.H. Aliyev, A.A. Efendiev, W. Schuhmann, Redox polymer-based reagentless horseradish peroxidase biosensors: influence of the molecular structure of the polymer, Electrochim. Acta 51 (2006) 5137−5142, https://doi.org/10.1016/j.electacta.2006.03.056.
[245] Y.-T. Kong, M. Boopathi, Y.-B. Shim, Direct electrochemistry of horseradish peroxidase bonded on a conducting polymer modified glassy carbon electrode, Biosens. Bioelectron. 19 (2003) 227−232, https://doi.org/10.1016/S0956-5663(03)00216-1.
[246] M.G. Garguilo, N. Huynh, A. Proctor, A.C. Michael, Amperometric sensors for peroxide, choline, and acetylcholine based on electron transfer between horseradish peroxidase and a redox polymer, Anal. Chem. 65 (1993) 523−528, https://doi.org/10.1021/ac00053a007.
[247] M.J. Monerris, F.J. Arévalo, H. Fernández, M.A. Zon, P.G. Molina, Development of a very sensitive electrochemical immunosensor for the determination of 17β-estradiol in bovine serum samples, Sens. Actuators B Chem. 208 (2015) 525−531, https://doi.org/10.1016/j.snb.2014.11.048.
[248] H. Wang, R. Emanuelsson, H. Liu, K. Edström, F. Mamedov, M. Strømme, M. Sjödin, Redox-state-dependent interplay between pendant group and conducting polymer backbone in quinone-based conducting redox polymers for Lithium ion batteries, ACS Appl. Energy Mater. 2 (2019) 7162−7170, https://doi.org/10.1021/acsaem.9b01130.
[249] K. Oka, C. Strietzel, R. Emanuelsson, H. Nishide, K. Oyaizu, M. Strømme, M. Sjödin, Conducting redox polymer as a robust organic electrode-active material in acidic aqueous electrolyte towards polymer−air secondary batteries, ChemSusChem 13 (2020) 2280−2285, https://doi.org/10.1002/cssc.202000627.
[250] R. Emanuelsson, C. Karlsson, H. Huang, C. Kosgei, M. Strømme, M. Sjödin, Quinone based conducting redox polymers for electrical energy storage, Russ. J. Electrochem. 53 (2017) 8−15, https://doi.org/10.1134/S1023193517010050.

[251] K. Oka, R. Löfgren, R. Emanuelsson, H. Nishide, K. Oyaizu, M. Strømme, M. Sjödin, Conducting redox polymer as organic anode material for polymer-manganese secondary batteries, Chemelectrochem 7 (2020) 3336−3340, https://doi.org/10.1002/celc.202000711.

[252] L. Yang, X. Huang, A. Gogoll, M. Strømme, M. Sjödin, Conducting redox polymer based anode materials for high power electrical energy storage, Electrochim. Acta 204 (2016) 270−275, https://doi.org/10.1016/j.electacta.2016.03.163.