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An Overview of Biosensors Based on Glutathione Transferases and for the Detection of Glutathione

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Abstract: Glutathione transferases are enzymes involved in the detoxification against xenobiotics and noxious compounds. These enzymes catalyse a variety of reactions on many physiological and xenobiotic compounds using glutathione as a co-substrate. Moreover, many compounds are inhibitors of such enzymes. A wide array of biosensors based on glutathione transferases have been developed for analysing a variety of noxious compounds, as well as several biosensors devoted to the detection and quantification of glutathione and of glutathione transferases themselves. Here, we review the state of the art in this active field of research, highlighting the possible applications of such devices.

Keywords: Biosensor · glutathione transferase · glutathione · recalcitrant substances · environmental pollution

1 Introduction

Glutathione transferases (GSTs) are a family of multifunctional enzymes that play a crucial role in the cellular detoxification against exogenous and noxious molecules, including drugs and environmental pollutants [1,2]. GSTs are able to conjugate glutathione (GSH) to a wide range of hydrophobic and electrophilic compounds, making them less toxic and predisposed to further modification for discharge from the cells [2,3]. They are also involved in other catalytic processes and are able to bind numerous molecules non-catalytically [2,4]. GSTs are widely distributed in nature and they are found in both eukaryotic and prokaryotic organisms [1,2,4–9]. GSTs have been grouped into different classes based on their physical, chemical, and structural properties and are considered important biomarkers for several human pathologies, including cancer and neurodegenerative diseases [1]. In the recent years, given their versatility in catalysing different reactions and in binding several substrates and inhibitors, GSTs have been successfully utilized for the development of biosensors with several applications [10].

Biosensors are analytical devices consisting of a biological recognition element coupled to a chemical or physical transducer that produces an electronic signal which is then transmitted to a data acquisition system for signal processing and rendition into a graphical display or a plain numerical value [11,12] (Figure 1). In general, biosensors are categorized on the basis of their biological recognition elements including enzymes, antibodies, nucleic acids, bacteriophages, aptamers, as well as both eukaryotic and prokaryotic whole cells [13–20]. Biosensors are also classified into different groups depending on the method of signal transduction such as electrochemical, optical, calorimetric or piezoelectric transducers [21–23]. These devices have an extensive range of applications including pathogens detection, environmental monitoring, food safety, drug discovery and biomarker detection [24–32]. Recently, new generations of biosensors are constantly being developed that use nanomaterials in view of their excellent multiplicity properties [31–33]. Indeed, nanotechnology plays an increasingly important role in this field due to the chemical and physical characteristics of nanomaterials and the possibility to miniaturize the devices [34,35].

Among biosensors, those based on enzymes are the most popular and have been extensively developed thanks to the easy of construction, the affordability of the instrumentation, the simple operation procedure and the high sensitivity [16,35,36]. The working mechanisms of these biosensors may be based on direct substrate detection or either reversible or irreversible inhibition of enzymes' activities [37,38]. Substrate detection is based on the enzymatic conversion of the analyte into a sensor-detectable product (Figure 2A). Reversible and irreversible inhibitor detection is based on the quantification of

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the inhibitor by measuring the enzymatic activity on a conventional substrate in its absence and presence (Figure 2B). Moreover, it is often possible to improve the sensitivity and specificity of enzymatic biosensors by modifying enzymes' sequences to obtain protein variants with the desired molecular properties [39,40].

All the above-mentioned developments and opportunities have been used in the construction of biosensors based on glutathione transferases for the detection of a large number of compounds, including glutathione. Here we review the state-of-the-art in this active field of research, highlighting the many possible applications of such devices.

2 GST-based Biosensors for the Detection of Pesticides

GST-based biosensors are encouraging tools for the detection of pesticides whose residues in the environment can cause long-term damage to human health [10]. Indeed, up to twenty-seven different pesticides were screened based on their enzymatic inhibition of human GSTs belonging to different classes. All of them were shown to act as inhibitors of one of more GSTs showing the feasibility of this approach [41]. Here the most important examples of GST-based biosensors for the detection of pesticides are summarized.

2.1 Captan

The mammalian GSTP1-1 was used to develop an optical biosensor for the detection of captan in contaminated waters [42]. Captan is a fungicide used to control a broad spectrum of plant pathogenic microorganisms and it is a strong inhibitor of GSTs [43]. The optical biosensor was developed on the basis of the inhibition of GSTP1-1 by captan. GSTs catalyse the conjugation of the substrate 1-chloro-2,4-dinitrobenzene (CDNB, Figure 3) and GSH to form 1-(S-glutathionyl)-2,4-dinitrobenzene (GS-DNB), a yellow product with an absorption peak at 340 nm. The proposed mechanism of inhibition is based on captan-mediated oxidation of up to four GSTP1-1 cysteine residues leading to both intra and intermolecular disulphide bond formation [43]. In the presence of captan, a decrease in product formation is observed due to the inhibition of the GST catalysed reaction by the fungicide (Figure. 4 A). The biosensor could successfully detect captan up to a concentration of 2 ppm with a response time to steady sensor signal of about 15 min [42]. A GST-based electrochemical biosensor to measure captan has also been developed [44]. This new biosensor could detect captan concentrations lower than 1 ppm and also its metabolites. Furthermore, this device exhibited a sensitivity of 4.5 uA/ppm.

2.2 Molinate

An electrochemical biosensor was successfully realized to monitor molinate in environmental samples [45]. Molinate is a thiocarbamate herbicide used to control the germination of weeds in rice fields and other crops [46]. GST from equine liver was immobilized onto a glassy carbon electrode via an aminosilane-glutaraldehyde covalent bond. Electrochemical detection of molinate is based on the inhibition of GST enzymatic activity measured in the

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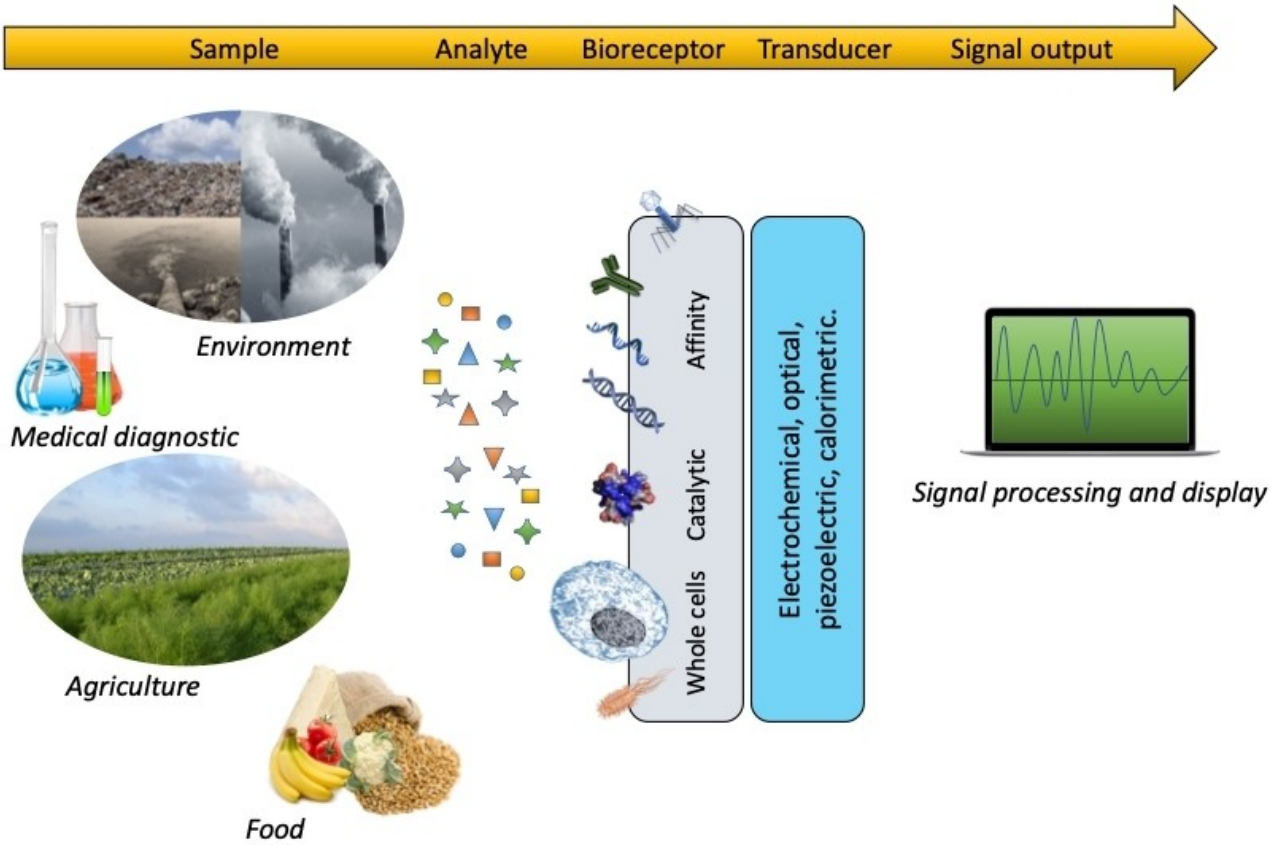

Fig. 1. General structure of a biosensor. It contains a biological recognition element (such as nucleic acids, enzymes, proteins, whole cells and aptamers) coupled to a signal transduction element.

reaction with CDNB and GSH as substrates [47] (Figure 4B). The inhibition of the enzyme leads to a decrease in current peak measurement by differential pulse voltammetry [45]. Compared with other analytical methods for the quantification of molinate, this sensor showed high accuracy, good selectivity and a low detection limit of 64 µg/L [45].

2.3 Captan and Molinate Detection through Vegetative-mycelium-specific Hydrophobin 2 Based Biosensor

Recently, a novel GST-based biosensor – more sensitive than the ones reported above – has been developed and tested in the detection and quantification of both captan and molinate molecules in aqueous environmental samples [48]. This biosensor was developed using the self-assembling characteristic of vegetative-mycelium-specific hydrophobin 2 (Vmh2), purified from the mushroom *Pleurotus ostreatus* [49]. Hydrophobins are small proteins produced by filamentous fungi that can self-assemble into amphiphilic films, like amyloid structures. Hydrophobins are of great interest in the production of new materials, in particular in the nanobiotechnological field. *Schistosoma japonicum* GST (SjGST) was fused to the Vmh2 protein and the fusion product was immobilized via hydrophobin film on the polystyrene surface of multiwell plates [48]. Detection of both pesticides is based on the inhibition of SjGST activity in the presence of GSH and CDNB substrates. The inhibition of the enzymatic activity results in a decrease of the yellow GS-DNB product. The detection limits for molinate and captan were 5 µg/L and 1 µg/L respectively. The main characteristics of this biosensor are the simplicity and speed of preparation, high sensitivity, accuracy and reusability.

2.4 Malathion

Organophosphorus compounds are used in public health, household and agricultural environment to control a wide spectrum of pests. Toxic effects of these molecules and their residues were observed in several organisms, including humans, and the risk of exposure is very high considering the extensive use of organophosphates, in particular in the agricultural field. Furthermore, the excessive use of organophosphates leads to the contamination of the environment and food. Organophosphates irreversibly inhibit acetylcholinesterase that catalyses the breakdown of the neurotransmitter acetylcholine. Previous data demonstrated that GSTs can detoxify organophosphate insecticides by a *O*-dealkylation mechanism

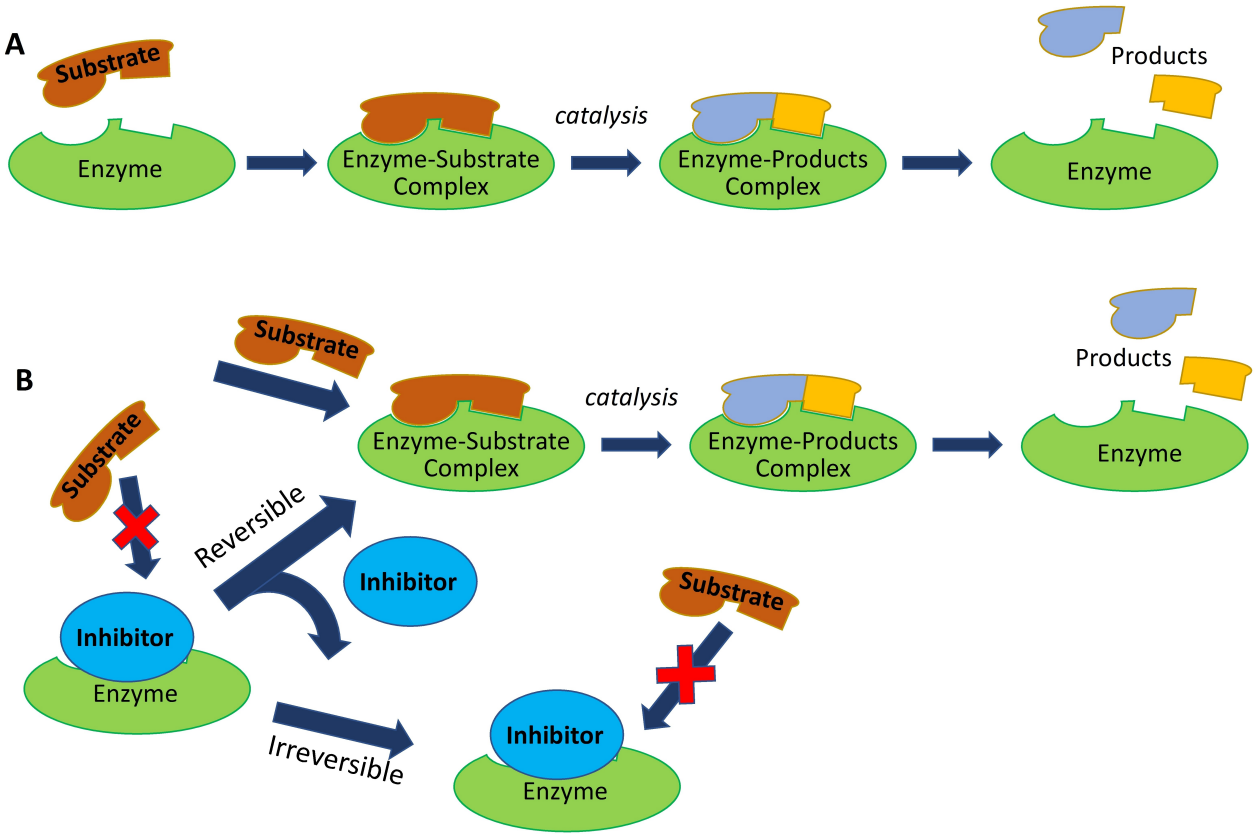

Fig. 2. Main working mechanisms of enzyme-based biosensors. A. Substrate detection. B. Reversible and irreversible inhibitor detection.

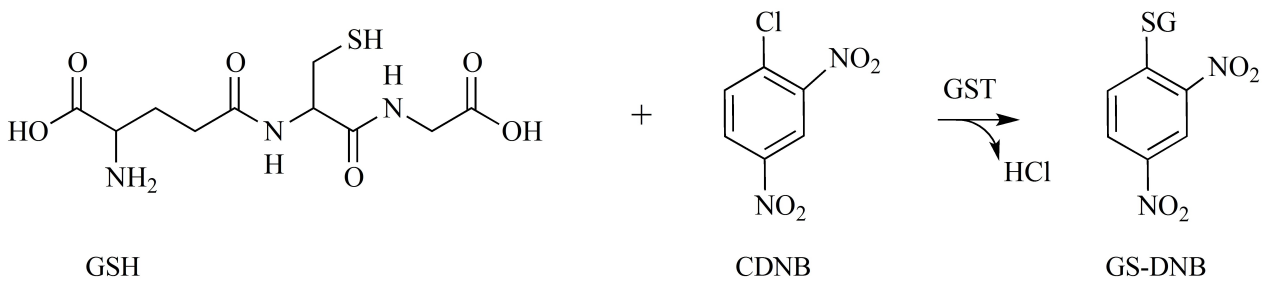

Fig. 3. The canonical enzymatic activity of GSTs consists in the conjugation of GSH to a wide range of hydrophobic and electrophilic compounds. Here the conjugation reaction with 1-chloro-2,4-dinitrobenzene (CDNB) as second substrate to form 1-(S-glutathionyl)-2,4-dinitrobenzene (GS-DNB) is shown.

[50]. Indeed, in insects, epsilon class GSTs play a key role in the resistance to organophosphate insecticides [51].

Malathion, a widely used organophosphorus compound [52], exhibited a reversible competitive inhibition of ZmGSTF1 (an isoenzyme of GST from maize) with respect both CDNB and GSH, indicating that the molecule binds to the substrate binding site of the enzyme [53] (Figure 4C). Furthermore, the ZmGSTF1 mutant Gln53Ala (Gln53 is one of the amino acid residues involved in binding of CDNB and other xenobiotics) was shown to be more sensitive than the wild-type enzyme to malathion, suggesting higher affinity for the molecule. In fact, the mutant enzyme exhibited about 100 % inhibition by malathion. Therefore, immobilized Gln53Ala mutant GST was used to develop a potentiometric biosensor for the quantification of malathion [53]. The biosensor measured the pH change caused by the H^+ ions released during the CDNB/GSH conjugation reaction catalysed by GST.

2.5 Atrazine

Atrazine is one of the most widely used herbicides to control unwanted plant growth. Because of its persistence

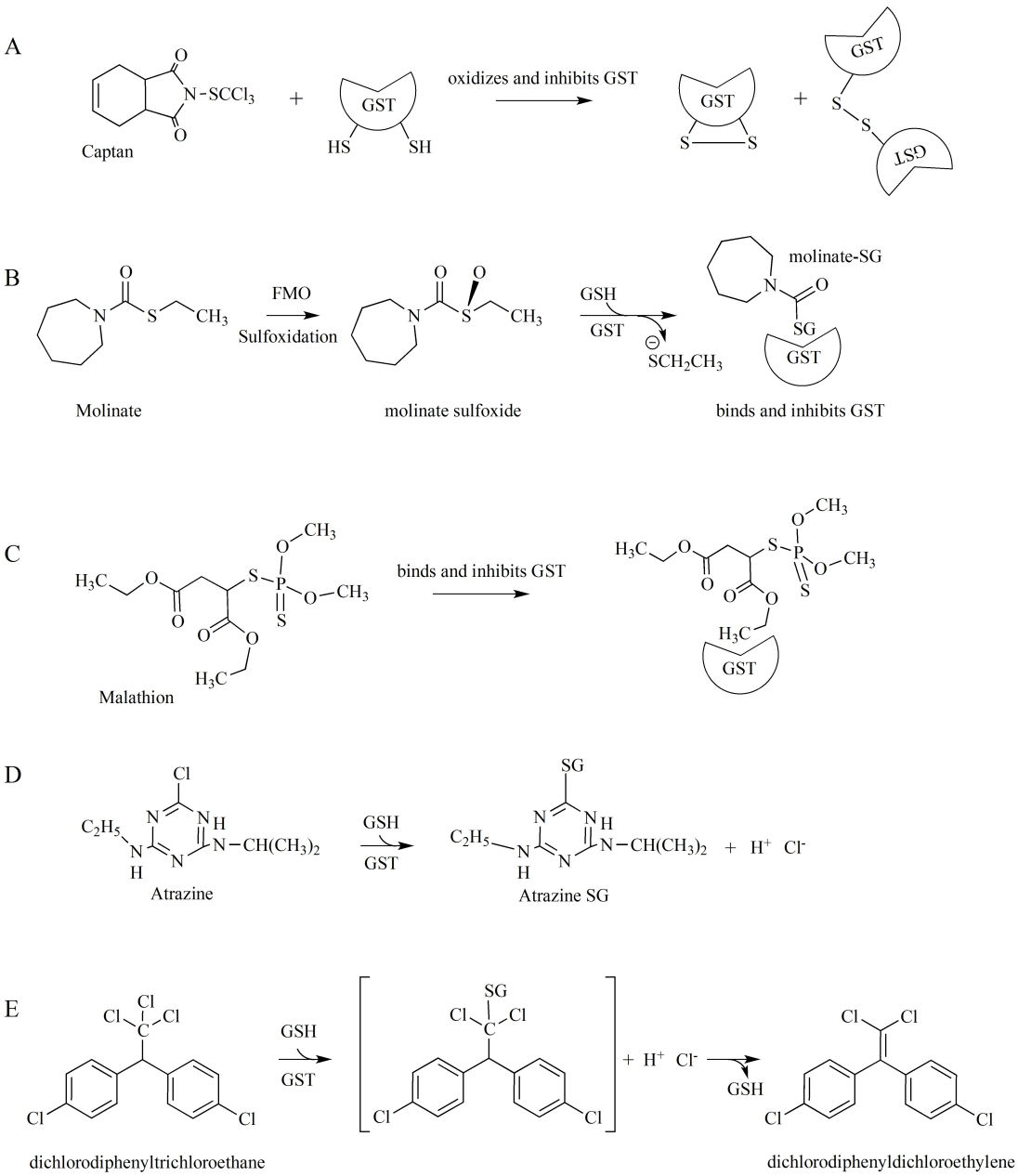

Fig. 4. Chemical structures of pesticides and their interaction with GSTs. A. Captan structure and its inhibitory effect on GSTP1-1 through cysteine oxidation; B. Molinate is sulphoxidized to molinate sulfoxide and forms a conjugate with GSH that exerts inhibition of GST activity; C. Inhibitory binding of malathion to GST; D. Biodegradation of atrazine; E. Dehydrochlorination of DDT.

in soil and water and to several adverse effects in humans, it is a major public health issue in areas of heavy application.

A fiber-optic biosensor for the detection and determination of atrazine was developed based on maize GST–I expressed as recombinant protein in *E. coli*. The enzyme was immobilized on the outer layer of a three-layer minisandwich. GSTs are involved in the first step of atrazine biodegradation causing the removal of the chlorine and a hydrogen ion as produced by the atrazine–GSH conjugation [4,54] (Figure 4D). The release of hydrogen ions results in a decrease of the pH of the bulk electrolyte solution that is transduced to an optically measurable signal. The optical signal is equivalent to the rate of decrease of absorbance, measured at 625 nm [55]. The sensor exhibited satisfactory enzyme stability and reproducibility with a concentration range for atrazine determination of 2.52–125 µM. No interference from other pesticides was observed in the absence of atrazine.

2.6 Dichlorodiphenyltrichloroethane

Commonly known as DDT, it is an organochlorine insecticide primarily used to control malaria, typhus, and

bubonic plague. In addition, DDT was also used on a variety of food crops worldwide. DDT was the weapon used to eradicate malaria in Italy and USA. Although the use of DDT was banned in many countries since 1972 because of dread over carcinogenicity, bioaccumulation, and health effects on wildlife, it is still used in several areas of the world for disease vector control [56]. In particular, DDT is recommended by the World Health Organization for use in indoor residual spraying in stable endemic area. Several studies have highlighted the association between exposure to DDT and the development of tumours in humans [56–58]. Furthermore, DDT and its metabolites are persistent in the environment with a half-life up to 150 years. Thus, a simple assay to monitor insecticide levels for vector control spraying programs could have an important impact on disease control.

An assay to measure DDT levels on sprayed surfaces has been developed using an epsilon class GST, purified from the parasitic vector *Aedes aegypti*, that possesses high DDT dehydrochlorinase activity [59,60] (Figure 4E). Detection was based on the pH change that occurs in a suitable buffer system by the concomitant release of hydrogen ions during the GST-catalysed DDT dehydrochlorination reaction and was measured both potentiometrically and colorimetrically. The range of quantification of the potentiometric assay was of 12 to 250 μg/ml, whereas with the colometric method was narrower: 20 to 100 μg/ml. The biosensor was found to be reproducible and reliable as compared to HPLC methods.

In agreement with previous results, a recent paper described molecular docking and dynamics studies on the interaction of several pesticides with potential biosensing enzymes (including DDT and GST) in order to identify possible enzyme candidates to the effective biosensing of the different pesticides [61]. It was observed that DDT was docked into the active site of the GST with a very high docking score. Furthermore, GST showed the highest values for the calculated inhibition constants, indicating its strong binding efficiency with many pesticides, including DDT [61].

More recently, biosensor probes were constructed through the immobilization of a cytosolic GST from equine liver on a platinum electrode using a graphene oxide-gelatin matrix for the detection and quantification of a wide variety of pesticides [62]. The biosensor was able to detect pesticides from four different classes including benzamidazole, organochlorine, organothiophosphate and polyphenol substrates. Although the biosensor offers slightly higher limits of detection as compared to other techniques, the strong point of this product is its high sensitivity for residues determination of selected pesticides. This method could be applied for checking the maximum residue levels of pesticides tested in several types of food and agricultural commodities present in the EU pesticides database, satisfying the validation criteria required by the European Food Safety Authority [62].

3 Heavy Metal Ions

Environmental contamination by heavy metals is a serious concern to human health because these substances are non-biodegradable. Heavy metals are not metabolized by the body and accumulate in the tissues. Many of them – such as lead, mercury, cadmium and arsenic–are highly toxic even in small concentrations and may enter the human body through food, water and air or absorption by the skin [63]. Bovine liver GST theta 2–2, rich in histidine residues and a *S. japonicum* recombinant GST–C-terminal His(6)-tagged enzyme, that contains three cysteine and three glycine residues on the surface of the protein, were used and compared to develop biosensors for the quantitative determination of heavy metals like Cd^{2+}, Zn^{2+} and Hg^{2+} [64]. These two GSTs were used because previous studies indicated a strong binding capacity of glycine, cysteine or histidine residues for heavy metals. Electrochemical capacitive biosensors were prepared by immobilizing the corresponding proteins on a gold surface. Detection of heavy metals was based on conformational changes in the structure of the GSTs when the metal ion binds the enzyme. Both biosensors were sensitive against all metal ions with detection limits in the range of 1 fM to 1 mM. However, bovine liver GST showed higher selectivity for Zn^{2+} with respect to the recombinant GST. The differences in selectivity of the two biosensors could be explained by their different amino acid composition. Future development will allow to identify different GST isoforms, or site-specific variants of GSTs, to obtain biosensors specific for the detection of a given heavy metal in complex samples. Furthermore, different biosensors could be made considering that several metals at toxic concentrations affect GSTs by direct inhibition of enzymatic activity [65] (see also *GST-SmtA fusion protein).*

4 Titanium Dioxide Nanoparticles

Titanium dioxide nanoparticles (TiO_2 NPs) are among the most widely used nanoparticles because of their high stability and anticorrosive and photocatalytic properties [66]. However, the increase in the production and use of TiO_2 NPs will inevitably cause damage to organisms and to environment. Indeed, the harmful effects of TiO_2 NPs on microorganisms and organisms, including animal cells, have been confirmed in several studies [66]. Their toxicity is probably due to the size-dependent interaction between nanoparticles and intracellular biomolecules adsorbed onto TiO_2 NPs, resulting in the generation of ROS, cell membrane damage and nanoparticles attachment to intracellular organelles. The most active field in the use of TiO_2 NPs photocatalysis, given the photodegradation of a wide range of hazardous compounds, is in the cleaning up of polluted environments [67]. It was observed that some organic compounds are not completely mineralized and maintain residual products that have the potential to produce oxidative injury to the environment. Therefore, it

is important to evaluate the efficacy of the treatment by determining the toxicity during degradation processes. Several approaches for monitoring the toxicity of solutions subjected to TiO_2 NPs photocatalysis were described [68]. A method based on a GST-based biosensor has been developed using rat liver microsomal GST (mGST) a membrane-bound enzyme with GST and glutathione peroxidase activities [9]. Phenol and its photocatalytic products were taken as reference molecules to test the method. mGST was challenged both directly, incubating the microsomes with possible reactive intermediates of phenol, and after decomposition of the molecule by TiO_2 NPs photocatalysis. The results obtained suggest that the mGST assay is sensitive enough to respond to micromolar levels of reactive molecules and it is a promising approach for evaluating the effectiveness of wastewater treatment processes.

5 Anticancer Drugs

The acquisition of resistance to chemotherapics is in part attributed to the detoxification activity played by GSTs through their conjugation with GSH which in turn enables their active extrusion from cancerous cells [1]. Monitoring the concentration of anticancer drugs can optimize therapy and avoid over or under dosages. Moreover, many chemotherapics also bind to GSTs non-catalytically, suggesting the development of inhibition-based biosensors. In particular, the anticancer drug cisplatin was investigated as model molecule. The measurement of cisplatin was based on the competition between CDNB and this anticancer drug for GST using colorimetric or electrochemical methods [69]. Colorimetric detection was obtained by spectrophotometric measurements. Limits were observed against blood serum samples due to interference from light scattering. An electrochemical biosensor was developed by immobilizing equine liver GST on a carbon past electrode [69]. The inhibitory effect following the addition of cisplatin was detected by the decrease of the electric signal due to competition with CDNB. The limit of detection for cisplatin was 8.8 μM. Furthermore, this electrochemical biosensor showed a good sensitivity and acceptable stability against cisplatin and could be also used for other anticancer drugs.

6 Biosensors for the Detection and Quantification of GSTs

GSTs are implicated in a variety of physiological and pathological processes and their detection and quantification in living systems and biological fluids may be important for diagnostic purposes.

6.1 GST Assay with FRET System

A fluorescent turn-on device based on fluorescence resonance energy transfer (FRET) from GSH functionalized Mn-doped ZnS quantum dots (QDs; as energy donor) to graphene oxide (a highly efficient energy transfer acceptor), was developed to detect GSTs in living cells and human urine [70].

In the presence of graphene oxide, the fluorescence of QDs@GSH was efficiently quenched. The addition of the enzyme leads to the fluorescence recovery due to the strong interaction between GST and GSH that causes dissociation of the complex from the graphene oxide surface. This sensor showed high selectivity and sensitivity for GST determination and a low limit of detection of 0.21 nM [70].

6.2 GSTP1-1 Assay in Prostate Cancer

Human Pi-class GST (GSTP1-1) is overexpressed in several types of cancers where it contributes to resistance to apoptosis and metabolism of chemotherapeutic drugs. Therefore, it is considered as a promising marker of malignant and premalignant cells and a target for drug development [71].

Screening tests for prostate cancer at an early stage rely on blood detection of prostate-specific antigen (PSA) and digital rectal exam (DRE). However, these tests often lead to false positive and false negative results due to both low sensitivity and specificity. If these exams are anomalously abnormal, further invasive tests, such as prostate biopsy are often done. Unlike PSA and DRE, hypermethylation of the GSTP1-1 promoter region has been described as a highly specific and sensitive biomarker for prostate cancer [72]. Indeed, under the prostatic carcinoma setting, GSTP1-1 expression is silenced due to aberrant hypermethylation in its gene promoter. Different sensitive biosensors were developed and used for the determination of GSTP1-1 hypermethylation as a prostate cancer biomarker [73–75].

An efficient DNA biosensor was proposed using the DNA hybridization technique and GSTP1-1 promoter hypermethylation was subsequently detected by electrochemical methods [73]. DNA hybridization was monitored by the decrease in magnitude of the guanine oxidation peak as measured by differential pulse voltammetry. An impedimetric system was used for direct electrochemical detection by comparing the resistance before and after hybridization [73]. Another electrochemical approach was described using DNA hybridization technique coupled to multi-walled carbon nanotubes modified screen printed electrodes for detection [74]. This biosensor presented specificity and reproducibility, with low detection limits, as well as being disposable and regenerable. More recently, a label-free voltametric assay was also developed [75]. The biosensor used a 16-channel platinum microelectrode array chip. The electrochemical DNA hybridization sensor was tested with methylated and non-methylated complementary single stranded DNA. The device was appropriate to detect methylation in GSTP1-1 with good sensitivity and high specificity. Furthermore, it is suitable for a *yes* or *no*

response in order to obtain a rapid diagnostic test [75]. A fluorogenic substrate that allows direct and selective detection of GSTP1-1 activity in living cells was also developed [71]. It was observed that fluorescein diacetate (FDA), a cell-staining fluorescent probe, is a good substrate with selectivity for GSTP1-1 among cytosolic GSTs. GSTP1-1 efficiently promoted fluorescence activation catalysing deacetylation of FDA and its derivatives in a GSH-dependent manner. Fluorescein is presumed to be formed by nucleophilic attack of the thiolate anion, formed at the active site of GSTP-1, on the acetyl moieties of FDA [71].

7 Biosensors Based on GST-Fusion Proteins

Biosensors that do not require GST catalytic activity have also been described. Indeed, GST is widely used as an affinity tag to facilitate the purification and detection of the proteins of interest. The DNA sequence of a GST is integrated into expression vectors to produce high levels of recombinant proteins. The result of expression from this vector is a GST-tagged fusion protein in which the functional GST protein is fused to the recombinant protein. Then, the GST-tagged fusion proteins can be purified or detected based on the strong affinity of GST for immobilized GSH. Below are some examples of biosensors developed using GST-tag fusion proteins.

7.1 Detection of Methyl Parathion

To obtain an optical biosensor for the detection of methyl parathion, a recombinant methyl parathion hydrolase (MPH) fused with GST was used. MPH is a metal ion-dependent enzyme capable of hydrolysing a wide range of organophosphorus substrates like methyl parathion, a commonly used pesticide. MPH catalysed the degradation of methyl parathion into dimethylthiophosphoric acid and the yellow-coloured *p*-nitrophenol. To detect methyl parathion, an MPH-GST fusion protein was covalently immobilized onto a chitosan film-coated polystyrene microplate [76]. The MPH-GST biosensor detects *p*-nitrophenol at 410 nm with a low detection limit of 0,1 μM. Furthermore, it proved to be suitable for quick and easy screening, and capable of simultaneous multiple sample detection.

7.2 Detection of Heavy Metal Ions

GST was fused to the synechococcal metallothionein SmtA (GST-SmtA fusion protein) for detecting and quantifying different heavy metal ions [77,78]. Metallothioneins are proteins characterized by a high content of cysteine residues, low molar mass and are able to bind a wide range of metals [78]. The GST-SmtA fusion protein was immobilized on the surface of a modified gold electrode and a capacitive signal transducer was used. The biosensor was based on the direct interaction between SmtA and the target analytes. The detection principle is based on the protein conformational change when a heavy metal ion is bound to SmtA. GST-SmtA fusion protein-based biosensor showed selectivity and sensitivity for zinc, cadmium, copper and mercury ions with detection limits of about 0.1 nM. In particular, higher sensitivity was found for mercury. GST-SmtA electrodes could be regenerated with EDTA and were stable over 16 days [77,78].

7.3 Detection of PI3P and PIP_3 in *Entamoeba histolytica*

The intestinal protozoan parasite *E. histolytica* is the causative agent of amoebic dysentery and liver abscess. Phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), two products of the signalling PI 3-kinase, have been shown to be required for endocytosis, that plays an essential role in the pathogenesis of the parasite. PI3P and PIP_3 modulate phagocytosis by recruiting proteins with specific lipid-binding domains. Proteins with FYVE-finger domains and pleckstrin homology (PH) domains can bind to PI3P and PIP_3 respectively, with high affinity and specificity. The FYVE domains are predominantly found in several proteins involved in various endocytic trafficking pathways, and they are highly specific for PI3P. To better clarify the role of PI3P parasitic cellular function, a biosensor was developed with a GST-tagged fusion protein containing two FYVE-finger domains in tandem (GST-2xFYVE), to localize PI3P inside cells during endocytosis [79]. *E. histolytica* cells were tested (stained) with GST-2xFYVE and GST alone as a control and detection was performed with a polyclonal anti-GST antibody conjugated to green-fluorescent dye. This biosensor was useful for describing the microscopic localization of PI3P on phagosomal compartments of *E. histolytica*, supporting a role for this lipid in phagocytosis [79]. Furthermore, to examine the subcellular localization of PIP_3 in the parasite cells during endocytosis, a GST-tagged biosensor containing a PH domain derived from Bruton's tyrosine kinase (GST-PH^{Btk}) was constructed. The PH domain is a regulatory module that is present in a variety of proteins involved in signal transduction. Similarly to the previously reported procedure, a green-fluorescent dye conjugated anti-GST antibody was used for GST-PH domain immunodetection [80]. The results obtained indicate that PIP_3 is involved in the early stages of phagosome formation in *E. histolytica.*

7.4 White Spot Syndrome Virus

A capacitive biosensor was achieved to detect quantitatively the white spot syndrome virus (WSSV) which is common in most shrimp farming aquaculture facilities of the world, causing large economic losses. A GST fused with the white spot binding protein (WBP) was obtained and was immobilized on a gold electrode through a self-assembled monolayer [81]. The detected capacitance signal was produced by the binding between immobilized GST-WBP and WSSV through protein-protein recogni-

tion. WBP bind specifically to VP26 a of major envelope protein of WSSV. Under optimum conditions, the biosensor detected WSSV over a linear range from 1 to 1×10^5 copies/μl, with a low detection limit of 1 copy/μl. The system was found to be highly sensitive and specific for this virus, with a good reproducibility, and the results were in good agreement with those obtained by real-time PCR.

8 GST and non-GST based Biosensors for the Detection and Quantification of GSH

GSH is the major thiol in most living organisms and is widely distributed from mammals to many prokaryotic species. GSH was found to be involved in numerous cellular processes and to play a key role in defence against oxidative stress, the neutralization of free radicals, and in the detoxification of xenobiotics. Under normal conditions, cellular glutathione is prevalent in the reduced form (GSH) as compared to the oxidized form (GSSG; Figure 5A), with glutathione redox potential (E_{GSH}) more negative than −310 mV. When oxidized, for instance by the activity of selenium-dependent glutathione peroxidase, it is rapidly reduced by glutathione reductase (GR) (Figure 5B). GR is a ubiquitous flavoenzyme that recycles GSSG back to the reduced form and is essential to maintain the redox status of cells during oxidative stress [82]. Anomalous cellular levels of the tripeptide in organisms are related to a variety of human diseases, including neurodegenerative diseases, epilepsy, and hearth illnesses [83]. Also, an increase or decrease in concentration relative to normal GSH values have been observed in numerous types of cancer. Thus, the importance of monitoring GSH concentrations in physiological systems for medical diagnosis is clear. Several different biosensors have been developed for the detection and quantification of GSH including electrochemical, FRET- and photoelectrochemical (PEC)-based biosensors [84–90].

8.1 GST-ZnO Nanocomposite

An electrochemical method for detection of sub-nM concentrations of GSH was developed [85]. The device was achieved using a GST linked to zinc oxide nanoparticles and dropcasted onto an oxide wafer to form a chemiresistive channel. ZnO nanoparticles not only act as a substrate for binding and immobilization of the GST onto the substrate surface, but also as a semiconducting layer that allows the movement of current. GSH measurement was obtained using CDNB as a second substrate in the classic conjugation reaction catalysed by GSTs. Conformational changes due to the enzymatic reaction led to a significant change in the resistance of the GST-ZnO nanocomposite, changing the current flow that could be measured and related to a quantitative measure of the increase or decrease of GSH concentration. This method displayed satisfactory sensitivity and detection limit of 5.68 nA/μM and 41.9 nM respectively. This device could be used in detecting some types of cancer at an early stage.

8.2 GST-MoS_2-based Biosensor

A new electrochemical biosensor based on GST immobilized on multilayer MoS_2 (molybdenum disulphide) nanosheet, based on GSH and CDNB conjugation reaction, was developed [84]. Unlike the previous device, it exhibited satisfactory repeatability and stability that encourages its use for cancer diagnostics. Moreover, the electrochemical device exhibited a sensitivity of 704 pA/

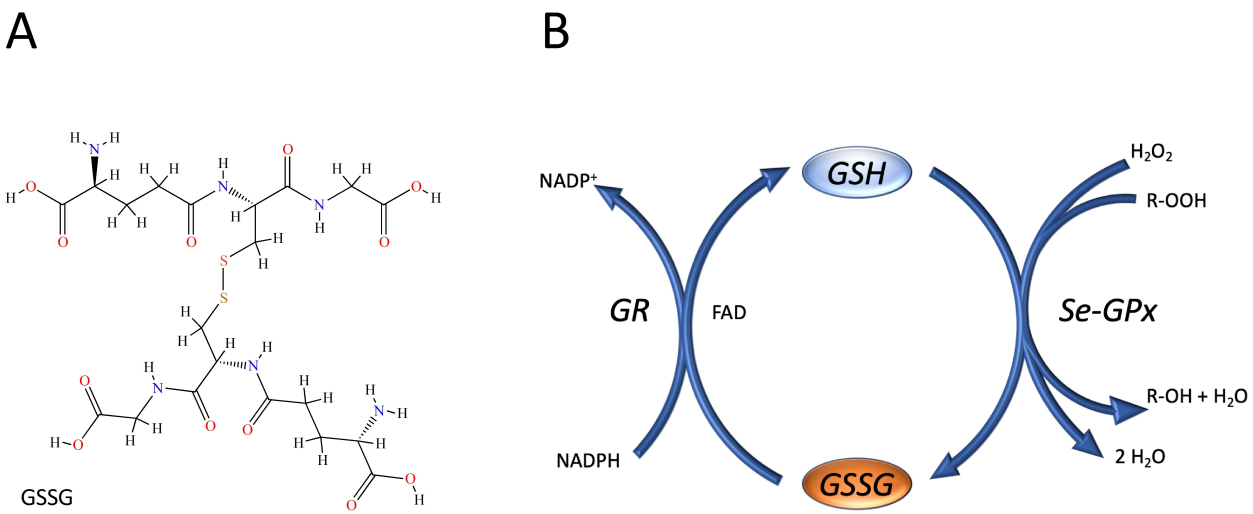

Fig. 5. Selenium-dependent glutathione peroxidase (Se-GPX) and glutathione reductase (GR) enzymatic activities. A) Structure of the oxidized form of GSH (GSSG). B) A glutathione redox cycle is shown where peroxides reduction by Se-GPX is coupled to GSH oxidation to GSSG. The latter is in turn reduced to GSH by GR using NADPH as an electron donor.

Table 1. Overview of the main characteristics of the biosensors described.

	Target analyte	Recognition element	Transducing mechanism	Linear range/ *LOD	Sample	Ref.
GST-based biosensor						
Pesticide	Captan	human placenta GST	Optical	0–2.0 ppm/2 ppm	water	[42]
			Electrochemical	0.25–16 ppm/ < 1ppm	water	[44]
	Molinate	equine liver GST	Electrochemical	0.19-7.9 mg/L / 0.064 mg/L	water	[45]
	Captan Molinate	SjGST	Optical	–/ 1 µg/L –/ 5 µg/L	water	[48]
	Malathion	maize GST	pH electrode	0–20 µM/–	Environmental	[53]
	Atrazine	maize GST	Optical	2.52–125 µM/0.84 µM	–	[55]
	DDT	A. aegypti GST	pH electrode	12 –250 µg/mL/3.8 µg/mL	–	[59]
		equine liver GST	Electrochemical	40 - 150 ppb/40 ppb		[62]
Heavy metal ions	Zn^{2+}, Cd^{2+}, Hg^{2+}	bovine liver GST SjGST $(His)_6$	Electrochemical	1 fM-1 mM**/–	–	[64]
TiO_2 NPs	Phenols	microsomal GST	Colorimetric	–/–	water	[68]
Anticancer drugs	Cisplatin	equine liver GST	Electrochemical Colorimetric	0.05–0.14 mM/8.8 µM		[69]
Biosensors for the detection and quantification of GSTs						
FRET system	GST	***QDs@GSH	Fluorescence	0–100 nM/0.21 nM	Live cells human urine	[70]
Prostate cancer	GSTP1-1	DNA	Electrochemical	–/2,92 pmol	Plasma	[73]
				–/2,7 pmol	–	[74]
Biosensors based on GST fusion proteins						
MPH-GST	methyl parathion	methyl parathion hydrolase	Optical	0.1–10 µM/0,1 µM	–	[76]
GST-SmtA	Heavy metal ions	synechococcal metallothionein	Capacitance electrode	–/0,1 nM	–	[78]
E. histolytica	PI3P PIP_3	2xFYVE domain PH domain	Colorimetric	–/–	E. histolytica cells	[79]
GST-fusion WBP	White spot syndrome virus	white spot binding protein	Capacitance electrode	1–1x10^5 copies/µL/ 1 copy/µL of virus	–	[81]
GST and non-GST based biosensors for the detection and quantification of GSH						
GST-ZnO nanocomposite	GSH	GST	Electrochemical	100 nM–10 mM/41.9 nM	–	[85]
MoS_2-based biosensor	GSH	GST	Electrochemical	10 µM–500 mM/703 nM	–	[84]
MnO_2-based nanosensor	GSH	MnO_2	Fluorescence	0.07–70 µM/48 nM	human serum	[88]
		MnO_2		0.5–300 µM/0.26 µM	HeLa cells	[89]
FRET sensor	GSH	Cu^{2+}	Fluorescence	0–50 µM/1,84 µM 0–50 µM/0.19 µM	human serum water solution	[91]
GPx-based biosensor	GSH	Bovine erythrocytes GPx	Electrochemical	10–250 µM/25 µM	Serum	[95]
Self-powered enzyme-based biosensor	GSH	Bilirubin oxidase	Electrochemical	–/43 µM	Bovine serum	[96]
Photoelectrochemical biosensors	GSH	IrO_2	Photoelectrochemical	1–10 µM/0.80 µM	–	[90]
roGFP2 biosensor	GSH	human GRX1	Fluorescence	-205–−275 mV/–	Plant cytosol	[101]

* LOD, Limit of Detection. **Higher selectively for Zn^{2+}. ***GSH-functionalized Mn-doped ZnS QDs.

μM and a detection limit of 703 nM with a wide linear detection range of 10 μM – 500 mM.

8.3 MnO_2-based Nanosensor

A FRET-based biosensor was developed using graphene quantum dots (GQDs) wrapped MnO_2 nanocomposite with turn-off-on process [88]. GQD is a fluorescent material and MnO_2 has an excellent optical absorption. Through an efficient FRET process between GQDs and MnO_2, the fluorescence of GQDs was quenched. The presence of GSH degraded MnO_2 and caused fluorescence recovery of GQDs. GSH was determined in samples of cancer cells as compared to normal cells. The recognition by fluorescence of cancer cells was achieved because of the higher GSH content in cancer cells than in normal cells. GSH detection was satisfactory in the range of 0.07-70 μM with a limit of about 48 nM [88].

A new FRET-based biosensor was also developed using a near-infrared (NIR) fluorescent semiconducting polymer coated with MnO_2 [89]. NIR fluorescence improves the analytical and imaging performance for GSH sensing. Most used fluorescent probes emit fluorescence in the visible spectrum, in the 400–600 nm range, which may be affected by the relatively weak light penetration into tissues, light scattering in turbid media, and interference of autofluorescence from cells or biological samples in the detection process. In this work, the developed biosensor is able to offer enhanced NIR fluorescence brightness and prohibit the potential leakage of embedded NIR dye, which makes it an improvement of detection reliability and sensitivity. It was successfully applied in monitoring GSH in living cells, revealing a great potential in biomedical applications [89].

8.4 FRET-based GSH Detection in Serum

An interesting FRET sensor was also recently developed using a portable smartphone-sensing platform, a ratiometric fluorescence sensor combined with the 3D-printed smartphone device for rapid, sensitive, quantitative, and on-the-spot determination of GSH [91]. The device was constructed based on the fluorescence "off – on" approach using a ratiometric fluorescence nanoprobe by the combination of blue carbon dots and orange gold nanoclusters with the addition of copper ions. The quenched fluorescence could be rapidly restored upon addition of GSH, showing a distinct colour variation from blue to purple to orange. The portable sensor exhibited a sensitive and selective detection of GSH in the complex human serum system. Thanks to these features, the authors plan to develop a portable home medical equipment to realize convenient and rapid preliminary monitoring of abnormal levels of GSH for disease diagnosis at an early stage [91].

8.5 Glutathione Peroxidases-based Biosensors

The family of glutathione peroxidases (GPxs) includes several enzymes involved in different physiological roles, including the detoxification of hydroperoxides [92]. Moreover, the involvement of GPxs in the occurrence and development of malignant tumours was shown [93]. Selenium-dependent GPxs – with a selenocysteine in the catalytic centre – contribute to the maintenance of redox homeostasis in cells by catalysing the reduction of peroxides with GSH as an oxidizable co-substrate (Figure 5B). Changes in GSH concentration in biological fluids or tissues may be a useful marker in several diseases and in cancers. To date, two biosensors based on GPx to analyse GSH levels were developed. An amperometric biosensor for GSH determination in serum samples was described [94]. Se-GPx immobilized on pyrolytic graphite exhibited high affinity for GSH without loss of enzymatic activity. In the presence of hydroperoxide, GSH was converted into oxidized glutathione which was monitored amperometrically. The biosensor lifetime was approximately two months with continuous use. The second device was developed using platinum nanoparticles electrodeposited on the surface of a glassy carbon paste electrode, followed by the immobilization of Se-GPx enzyme onto the electrode surface [95]. The measurement was based on the electrochemical oxidation of GSH to GSSG in the presence of hydrogen peroxide. The system was tested for GSH detection in synthetically prepared plasma solution. The promising data obtained demonstrate that the developed biosensor can be easily adapted to biological samples.

8.6 Self-powered Enzyme-based Biosensor

A self-powered biosensor utilizing a biofuel cell was developed as a quick and inexpensive alternative to widely used methods [96]. Self-powered sensors are essentially fuel cells where the analyte is detected by either an increase or decrease of current and power output. Enzymatic biofuel cells generate bioelectricity through the oxidation of renewable energy sources such as organic acids and sugars, coupled with the reduction of oxygen to water [97]. The detection of GSH concentration was based on its ability to inhibit bilirubin oxidase, an oxidoreductase that uses oxygen as electron acceptor and reduces it to water. Bilirubin oxidase was incorporated into the cathode. At the anode was incorporated a glucose oxidase which oxidizes glucose (the fuel) by O_2 producing electrical power. The current produced by the biosensor decreases with increasing amounts of GSH due to inhibition of bilirubin oxidase making it possible to determine GSH concentration. Moreover, the sensor showed a sensitivity of $22.1 \pm 0.3\ \mu A\,cm^{-2}\ Mm^{-1}$ and a detection limit of 43 μM [96].

8.7 Photoelectrochemical Biosensors (PEC)

A PEC biosensor to detect GSH was developed [90]. PEC detection is a highly sensitive technique, with response speed and simple instrumentation. In these devices, photocurrent is produced by the physical and chemical interactions between biomolecules; photoactive species are identified as detection signal. TiO_2 nanomaterials have been initially used as photoelectrodes. Then, TiO_2 nanotube arrays, growing on Ti substrates, were modified with Pt and IrO_2 nanoparticles to obtain a better performance and a significantly higher PEC activity. Results obtained showed a linear signal at GSH concentrations in the range of 1–10 μM [90].

8.8 Redox sensitive GFPs Biosensors

Redox probes derived from green fluorescent proteins (GFPs) – by replacing two residues with cysteines – were developed to monitor and to measure dynamic E_{GSH} changes in living cells both in the cytosol and in several other subcellular compartments [98,99]. The cysteine residues form a disulphide bond upon oxidation resulting in conformational changes of the chromophore followed by fluorescence changes that allows for ratiometric measurements. The redox-sensitive GFPs (roGFPs) sensor exhibits an excitation maximum at 475–490 and about 400 nm in the reduced and oxidized form, respectively. Glutaredoxin (GRX) catalyses the reduction of roGFPs in the presence of GSH. The engineering fusion between roGFPs and human GRX1 provided specificity and higher efficiency to a redox sensing process increasing the kinetic response of the biosensors and suppressing the dependency of the device on endogenous GRX [99,100]. For the GRX1-roGFP2 biosensor, the response time is much faster, within seconds, for nanomolar concentrations of GSSG as compared to unfused roGFP2.

This first GRX1-roGFP biosensor–with midpoint potentials between −280 and −290 mV – in the presence of a strongly oxidized environment such as the endoplasmic reticulum or under highly severe stress conditions activated by pathological processes, would still be fully oxidized. Indeed, in these conditions E_{GSH} is outside the measuring range of these probes thus limiting their application. To overcome this limit, it was developed a new GRX1-roGFP2-iL biosensor with a less negative midpoint potential of about −238 mV [101]. In comparison with GRX1-roGFP2, the new biosensor was used to determine E_{GSH} in the cytosol of *Arabidopsis* rml1 seedlings, a mutant with severe depletion of GSH. Although both biosensors measured an E_{GSH} of about −260 mV, the value of GRX1-roGFP2 is at limits of the linear measurement range with an oxidation of 93 %, while the value of GRX1-roGFP2-iL falls in the linear range of −205 – −275 mV with an oxidation of 14 % [101].

A biosensor was also developed to measure mitochondrial redox homeostasis in model nonmammalian organisms. In this case the probe was designed by inverting the components and fusing GRX1 to the C-terminus of roGFP2. The roGFP2-GRX1 biosensor was successfully imported in *Drosophila melanogaster* and *Arabidopsis thaliana* mitochondria without compromising its function [102].

A summary of the main characteristics of all biosensors reported in this work is presented in Table 1.

9 Conclusion

The biosensors field is ever growing and find applications in the many areas of analytical chemistry and clinical biochemistry where good sensitivity and specificity must be coupled with rapid detection, ease of use also for non-expert operators, affordability and portability. Under this light we have seen, for instance, the development of several biosensors based on GST activity, or its reversible and irreversible inhibition, or that use GST as a fusion tag, that may compete and often outperform as compared to traditional analytical methods in the detection and quantification of many different molecules, even in complex matrices. Both optical and electrochemical transducers were successfully used, with the latter being often the preferential choice due to their unique ability of taking advantage of microfabrication electronics. Devices such those cited in this review may find applications in pharmacology, biomarkers and noxious compounds detection in diverse biological fluids, cancer diagnosis and stratification, virus detection not to mention the wide opportunities they offer in the bioremediation field.

10 Future Directions

To make these biosensors effectively progress from the research stage to the routine applications, however, improvements can be made on different aspects of biorecognition element and transducer architecture as well as on the utilized materials. Under this light, the more recent biosensors we cite in this work make ample use of different novel nanomaterials, perfectly fitting within a main trend in the biosensor field. Indeed, nanotechnology offers the possibility to synthesize materials according to the planned needs in terms of chemical-physical properties, size and shape thus greatly increasing the possibility to miniaturize the biosensor in a lab-on-chip fashion. However, efforts have also to be spent on making these materials cost-effective if we aim at using them in the "real world", for instance in routine clinical diagnosis. Finally, before such devices become routinely used more work has to be done on the validation of enzymatic biosensors results on real samples of complex matrices, to effectively assess eventual losses in sensitivity due to interfering compounds. These may be particularly important when using versatile enzymes like GSTs, capable of binding dozens of molecules often with unrelated structures. We anticipate that what has been done so far in the field of GST-based biosensors is only the tip of the iceberg and that many more such biosensors

will be developed and successfully tested in the future for many analytical purposes.

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Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

[1] N. Allocati, M. Masulli, C. Di Ilio, L. Federici, *Oncogenesis* **2018**, *7*, 8.
[2] J. D. Hayes, J. U. Flanagan, I. R. Jowsey, *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 51–88.
[3] P. E. Hanna, M. W. Anders, *Crit. Rev. Toxicol.* **2020**, 1–111.
[4] N. Allocati, L. Federici, M. Masulli, C. Di Ilio, *FEBS J.* **2009**, *276*, 58–75.
[5] A. Di Matteo, L. Federici, M. Masulli, E. Carletti, D. Santorelli, J. Cassidy, F. Paradisi, C. Di Ilio, N. Allocati, *Front. Microbiol.* **2019**, *10*, 9.
[6] N. Allocati, L. Federici, M. Masulli, C. Di Ilio, *Biochimie* **2012**, *94*, 588–596.
[7] N. Allocati, M. Masulli, P. Del Boccio, D. Pieragostino, D. D'Antonio, D. Sheehan, C. Di Ilio, *Folia Microbiol. (Praha)* **2014**, *59*, 107–113.
[8] I. Cummins, D. P. Dixon, S. Freitag-Pohl, M. Skipsey, R. Edwards, *Drug Metab. Rev.* **2011**, *43*, 266–280.
[9] A. Bresell, R. Weinander, G. Lundqvist, H. Raza, M. Shimoji, T. H. Sun, L. Balk, R. Wiklund, J. Eriksson, C. Jansson, B. Persson, P–J. Jakobsson, R. Morgensten, *FEBS J.* **2005**, *272*, 1688–1703.
[10] A. Bocedi, A. Noce, G. Marrone, G. Noce, G. Cattani, G. Gambardella, M. Di Lauro, N. Di Daniele, G. Ricci, *Nutrients* **2019**, *11*, 1741.
[11] M. Farré, L. Kantiani, D. Barceló, In *Chemical Analysis of Food: Techniques and Applications* (Ed.: Y. Picó), Academic Press, Boston, **2012**; pp. 177–217.
[12] N. Bhalla, P. Jolly, N. Formisano, P. Estrela, *Essays Biochem.* **2016**, *60*, 1–8.
[13] Y. Zhang, B. S. Lai, M. Juhas, *Molecules* **2019**, *24*, 941.
[14] U. Farooq, Q. Yang, M. W. Ullah, S. Wang, *Biosens. Bioelectron.* **2018**, *118*, 204–216.
[15] S. Sharma, H. Byrne, R. J. O'Kennedy, *Essays Biochem.* **2016**, *60*, 9–18.
[16] H. N. Nguyen, S. H. Lee, U. J. Lee, C. D. Fermin, M. Kim, *Materials (Basel)* **2019**, *12*, 121.
[17] Y. Du, S. Dong, *Anal. Chem.* **2017**, *89*, 189–215.
[18] Q. Liu, C. Wu, H. Cai, N. Hu, J. Zhou, P. Wang, *Chem. Rev.* **2014**, *114*, 6423–6461.
[19] Q. Gui, T. Lawson, S. Shan, L. Yan, Y. Liu, *Sensors* **2017**, *17*,1623.
[20] J. C. Gutierrez, F. Amaro, A. Martin-Gonzalez, *Front. Microbiol.* **2015**, *6*, 48.
[21] P. Damborsky, J. Svitel, J. Katrlik, *Essays Biochem.* **2016**, *60*, 91–100.
[22] M. Nirschl, F. Reuter, J. Voros, *Biosensors* **2011**, *1*, 70–92.
[23] P. Mehrotra, *J. Oral Biol. Craniofac. Res.* **2016**, *6*, 153–159.
[24] T. Chen, G. Cheng, S. Ahmed, Y. Wang, X. Wang, H. Hao, Z. Yuan, *Talanta* **2017**, *175*, 435–442.
[25] N. Virolainen, M. Karp, *Adv. Biochem. Eng./Biotechnol.* **2014**, *145*, 153–185.
[26] Z. Zhang, J. Zhou, X. Du, *Micromachines* **2019**, *10*, 222.
[27] V. Jayanthi, A. B. Das, U. Saxena, *Biosens. Bioelectron.* **2017**, *91*, 15–23.
[28] A. M. Davis, A. T. Plowright, E. Valeur, *Nat. Rev. Drug Discovery* **2017**, *16*, 681–698.
[29] R. S. Burlage, J. Tillmann, *J. Microbiol. Methods* **2017**, *138*, 2–11.
[30] S. C. Barman, M. F. Hossain, H. Yoon, J. Y. Park, *J. Electrochem. Soc.* **2018**, *165*, B296-B301.
[31] S. C. Barman, M. F. Hossain, H. Yoon, J. Y. Park, *Biosens. Bioelectron.* **2018**, *100*, 16–22.
[32] S. C. Barman, M. A. Zahed, M. Sharifuzzaman, S. G. Ko, H. Yoon, J. S. Nah, X. Xuan, J. Y. Park, *Adv. Funct. Mater.* **2020**, *31*, 1907297.
[33] M. Holzinger, A. Le Goff, S. Cosnier, *Front. Chem.* **2014**, *2*, 63.
[34] A. B. Dahlin, *Sensors* **2012**, *12*, 3018–3036.
[35] S. Kurbanoglu, S. A. Ozkan, A. Merkoci, *Biosens. Bioelectron.* **2017**, *89*, 886–898.
[36] E. A. Songa, J. O. Okonkwo, *Talanta* **2016**, *155*, 289–304.
[37] M. Asal, O. Ozen, M. Sahinler, I. Polatoglu, *Sensors* **2018**, *18*, 1924.
[38] A. Amine, F. Arduini, D. Moscone, G. Palleschi, *Biosens. Bioelectron.* **2016**, *76*, 180–194.
[39] M. Campas, B. Prieto-Simon, J. L. Marty, *Semin. Cell Dev. Biol.* **2009**, *20*, 3–9.
[40] A. Jimenez-Rosales, M. V. Flores-Merino, *Mol. Biotechnol.* **2018**, *60*, 946–974.
[41] E. G. Chronopoulou, A. C. Papageorgiou, A. Markoglou and N. E. Labrou, *J. Mol. Catal. B* **2012**, *81*, 43–51.
[42] J. W. Choi, Y. K. Kim, S. Y. Song, I. H. Lee, W. H. Lee, *Biosens. Bioelectron.* **2003**, *18*, 1461–1466.
[43] C. Di Ilio, P. Sacchetta, S. Angelucci, T. Bucciarelli, A. Pennelli, A. P. Mazzetti, M. Lo Bello, A. Aceto, *Biochem. Pharmacol.* **1996**, *52*, 43–48.
[44] R. P. Singh, Y. J. Kim, B. K. Oh, J. W. Choi, *Electrochem. Commun.* **2009**, *11*, 181–185.
[45] T. I. S. Oliveira, M. Oliveira, S. Viswanathan, M. F. Barroso, L. Barreiros, O. C. Nunes, J. A. Rodrigues, P. de Lima-Neto, S. E. Mazzetto, S. Morais, C. Delerue-Matos, *Talanta* **2013**, *106*, 249–254.
[46] T. E. Stoker, R. J. Kavlock, In *Hayes' Handbook of Pesticide Toxicology.* (3rd Ed.: R. Krieger), Elsevier, **2010**; pp. 551–569.
[47] A. Campbell, D. Holstege, R. Swezey, D. Medina-Cleghorn, *J. Toxicol. Environ. Health Part A* **2008**, *71*, 1338–1347.
[48] A. Piscitelli, A. Pennacchio, S. Longobardi, R. Velotta, P. Giardina, *Biotechnol. Bioeng.* **2017**, *114*, 46–52.
[49] M. M. Penas, B. Rust, L. M. Larraya, L. Ramirez, A. G. Pisabarro, *Appl. Environ. Microbiol.* **2002**, *68*, 3891–3898.
[50] E. L. Abel, T. K. Bammler, D. L. Eaton, *Toxicol. Sci.* **2004**, *79*, 224–232.
[51] X. P. Lu, L. L. Wang, Y. Huang, W. Dou, C. T. Chen, D. Wei, J. J. Wang, *Comp. Biochem. Physiol. Part C* **2016**, *180*, 40–48.
[52] B. Singh, J. Kaur, K. Singh, *Crit. Rev. Microbiol.* **2014**, *40*, 146–154.
[53] P. Kapoli, I. A. Axarli, D. Platis, M. Fragoulaki, M. Paine, J. Hemingway, J. Vontas, N. E. Labrou, *Biosens. Bioelectron.* **2008**, *24*, 498–503.

[54] L. Prade, R. Huber, B. Bieseler, *Structure* **1998**, *6*, 1445–1452.
[55] V. G. Andreou, Y. D. Clonis, *Anal. Chim. Acta* **2002**, *460*, 151–161.
[56] IARC-Working-Group. DDT, Lindane, and 2,4-D. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* 2018, pp 37–266.
[57] K. M. Rodgers, J. O. Udesky, R. A. Rudel, J. G. Brody, *Environ. Res.* **2018**, *160*, 152–182.
[58] T. VoPham, K. A. Bertrand, J. E. Hart, F. Laden, M. M. Brooks, J. M. Yuan, E. O. Talbott, D. Ruddell, C. H. Chang, J. L. Weissfeld, *Cancer Causes Control* **2017**, *28*, 177–190.
[59] E. Morou, H. M. Ismail, A. J. Dowd, J. Hemingway, N. Labrou, M. Paine, J. Vontas, *Anal. Biochem.* **2008**, *378*, 60–64.
[60] F. Matsumura, *Toxicology of insecticides* **1985**, 215–216.
[61] M. Shahbaaz, S. Kanchi, M. Sabela, K. Bisetty, *J. Biomol. Struct. Dyn.* **2018**, *36*, 1402–1416.
[62] H. Borah, S. Gogoi, S. Kalita, P. Puzari, *J. Electroanal. Chem.* **2018**, *828*, 116–123.
[63] V. Masindi, K. L. Muedi, In *Heavy Metals*, IntechOpen: Aglan, France **2018**, 115–133.
[64] E. Saatci, M. Nistor, S. Gaspar, E. Csoregi, M. Iscan, *Int. J. Environ. Anal. Chem.* **2007**, *87*, 745–754.
[65] D. Dobritzsch, K. Grancharov, C. Hermsen, G. J. Krauss, D. Schaumloffel, *J. Trace Elem. Med. Biol.* **2020**, *57*, 48–56.
[66] J. Hou, L. Wang, C. Wang, S. Zhang, H. Liu, S. Li, X. Wang, *J. Environ. Sci.* **2019**, *75*, 40–53.
[67] O. Carp, C. L. Huisman, A. Reller, *Progr. Solid State Chem.* **2004**, *32*, 33–177.
[68] S. Tanvir, F. Merlier, S. Pulvin, *J. Photochem. Photobiol. B* **2012**, *110*, 22–27.
[69] E. M. Materon, P. J. J. Huang, A. Wong, A. A. P. Ferreira, M. D. T. Sotomayor, J. W. Liu, *Biosens. Bioelectron.* **2014**, *58*, 232–236.
[70] L. F. Chang, X. W. He, L. X. Chen, Y. K. Zhang, *Nanoscale* **2017**, *9*, 3881–3888.
[71] Y. Fujikawa, T. Nampo, M. Mori, M. Kikkawa, H. Inoue, *Talanta* **2018**, *179*, 845–852.
[72] I. Meiers, J. H. Shanks, D. G. Bostwick, *Pathology* **2007**, *39*, 299–304.
[73] S. N. Topkaya, D. Ozkan-Ariksoysal, B. Kosova, R. Ozel, M. Ozsoz, *Biosens. Bioelectron.* **2012**, *31*, 516–522.
[74] S. N. Topkaya, D. Ozkan-Ariksoysal, *Electroanalysis* **2016**, *28*, 1077–1084.
[75] K. Meronard, M. Josowicz, A. Saheb, *Anal. Lett.* **2018**, *51*, 2612–2625.
[76] W. Senbua, J. Mearnchu, J. Wichitwechkarn, *Biotechnol. Rep.* **2020**, *27*, e00495.
[77] P. Corbisier, D. Lelie, B. Borremans, A. Provoost, V. Lorenzo, N. L. Brown, J. R. Lloyd, J. L. Hobman, E. Csoregi, G. Johansson, B. Mattiasson, *Anal. Chim. Acta* **1999**, *387*, 235–244.
[78] I. Bontidean, C. Berggren, G. Johansson, E. Csoregi, B. Mattiasson, J. R. Lloyd, K. J. Jakeman, N. L. Brown, *Anal. Chem.* **1998**, *70*, 4162–4169.
[79] R. R. Powell, B. H. Welter, R. Hwu, B. Bowersox, C. Attaway, L. A. Temesvari, *Exp. Parasitol.* **2006**, *112*, 221–231.
[80] Y. A. Byekova, R. R. Powell, B. H. Welter, L. A. Temesvari, *Infect. Immun.* **2010**, *78*, 125–137.
[81] S. Samanman, P. Kanatharana, W. Chotigeat, P. Deachamag, P. Thavarungkul, *J. Virol. Methods* **2011**, *173*, 75–84.
[82] N. Couto, J. Wood, J. Barber, *Free Radical Biol. Med.* **2016**, *95*, 27–42.
[83] J. M. Estrela, A. Ortega, E. Obrador, *Crit. Rev. Clin. Lab. Sci.* **2006**, *43*, 143–181.
[84] B. Rawat, K. K. Mishra, U. Barman, L. Arora, D. Pal, R. P. Paily, *IEEE Sens. J.* **2020**, *20*, 6937–6944.
[85] U. Barman, G. Mukhopadhyay, N. Goswami, S. S. Ghosh, R. P. Paily, *Ieee T. Nanobiosci.* **2017**, *16*, 271–279.
[86] M. Hanko, L. Svorc, A. Plankova, P. Mikus, *Anal. Chim. Acta* **2019**, *1062*, 1–27.
[87] W. J. Dong, R. P. Wa, X. J. Gong, C. Dong, *Anal. Bioanal. Chem.* **2019**, *411*, 6687–6695.
[88] Q. Wang, L. F. Li, X. D. Wang, C. Dong, S. M. Shuang, *Talanta* **2020**, *219*, 121180.
[89] C. Zheng, L. Ding, Y. N. Wu, X. H. Tan, Y. Y. Zeng, X. L. Zhang, X. L. Liu, J. F. Liu, *Anal. Bioanal. Chem.* **2020**, *412*, 8167–8176.
[90] J. Tian, P. Zhao, S. S. Zhang, G. A. Huo, Z. C. Suo, Z. Yue, S. M. Zhang, W. P. Huang, B. L. Zhu, *Nanomaterials* **2020**, *10*, 522.
[91] S. Y. Chu, H. Q. Wang, Y. X. Du, F. Yang, L. Yang, C. L. Jiang, *ACS Sustainable Chem. Eng.* **2020**, *8*, 8175–8183.
[92] R. Brigelius-Flohe, M. Maiorino, *Biochim. Biophys. Acta* **2013**, *1830*, 3289–3303.
[93] M. L. Zhang, H. T. Wu, W. J. Chen, Y. Xu, Q. Q. Ye, J. X. Shen, J. Liu, *J. Transl. Med.* **2020**, *18*.
[94] L. Rover, Jr, L. T. Kubota, N. F. Hoehr, *Clin. Chim. Acta* **2001**, *308*, 55–67.
[95] U. Anik, M. Cubukcu, F. N. Ertas, *Artif. Cells, Nanomed., Biotechnol.* **2016**, *44*, 971–977.
[96] B. G. Roy, J. L. Rutherford, A. E. Weaver, K. Beaver, M. Rasmussen, *Biosensors* **2020**, *10*, 114.
[97] E. Katz, A. F. Buckmann, I. Willner, *J. Am. Chem. Soc.* **2001**, *123*, 10752–10753.
[98] A. J. Meyer, T. P. Dick, *Antioxid. Redox Signal.* **2010**, *13*, 621–650.
[99] M. A. Comini, *Free Radical Res.* **2016**, *50*, 246–271.
[100] M. Gutscher, A. L. Pauleau, L. Marty, T. Brach, G. H. Wabnitz, Y. Samstag, A. J. Meyer, T. P. Dick, *Nat. Methods* **2008**, *5*, 553–559.
[101] I. Aller, N. Rouhier, A. J. Meyer, *Front. Plant Sci.* **2013**, *4*: 506.
[102] S. C. Albrecht, M. C. Sobotta, D. Bausewein, I. Aller, R. Hell, T. P. Dick, A. J. Meyer, *J. Biomol. Screening* **2014**, *19*, 379–386.

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