Hybrid Nanobioengineered Nanomaterial-Based Electrochemical Biosensors

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Nanobioengineered-based hybrid electrochemical biosensors exploit the synergistic properties of hybrid systems that connect biomolecules with nanomaterials to engineer highly sensitive biosensing platforms for the specific electrochemical detection of different target analytes.

Keywords: hybrid ; nanobiomaterial ; bioreceptor ; bioaffinity ; biocatalytic ; biosensor ; cytosensor ; genosensor ; immunosensor ; electrochemical

1. Introduction

Nanobioengineered-based hybrid electrochemical biosensors exploit the synergistic properties of hybrid systems that connect biomolecules with nanomaterials to engineer highly sensitive biosensing platforms for the specific electrochemical detection of different target analytes. Nanobioengineered platform-based electrochemical biosensors have been implemented in biomedicine, environmental, food, and security industries, demonstrating their versatility and great potential. Notably, in the biomedical field, modern nanobioengineered biosensing devices are escalating their horizon to face multitudinous medical complications, i.e., providing early, accurate, and specific diagnoses of diseases [1][2][3].

Electrochemical nanobiosensors are devices designed as alternatives to conventional laboratory-based detection techniques for disease diagnosis due to their robustness, small size, user-friendly operation, amenability for miniaturization, and potential for personalized diagnosis [4][5][6]. Electrochemical nanobiosensors comprise electroactive transducer platforms that anchor specific and selective bioreceptors, generating a nanobioconjugate. The nanobioconjugate uses different interactions between enzymes, antibodies, DNA(RNA) strands, cell-organelles, proteins, peptides, glycans, etc., with target analytes to report superficial electrochemical changes [7]. The sensing mechanism involves the target analyte–bioreceptor interaction, generating a stimulus transduced (transformed) into a decipherable signal that correlates to the target analyte's concentration in a particular sample [8].

Despite the tremendous promise of electrochemical nanobiosensors in biomolecular analysis, they sometimes suffer from poor sensitivity and short shelf life. The limit of detection (LOD) and narrow dynamic range often limit their practical application, hindering their path toward the market [9]. In this context, current investigations in the biosensing field aim to engineer nanomaterials that, coupled with biomolecules and transducer platforms, can give rise to specific and versatile nanobiohybrid-based biosensors to address the commented limitations, paving the way toward real solutions.

In recent years, researchers have dedicated efforts to harnessing the unique atomic and molecular properties of nanobioengineered nanomaterials, including carbon nanomaterials, semiconductor/conductor polymers, metallic nanoparticles, and their nanoconjugates [10][11][12]. Such nanobiostructures may improve the interaction with the bioreceptors, thus dramatically amplifying the resultant signal, lowering the LOD, extending the linear detection range, shortening the testing time, and increasing the long-term stability of the detection systems [13][14][15].

2. Nanohybrids and Nanocomposites

Nanostructured nanomaterials, both nanohybrids and nanocomposites, have been increasingly exploited in developing electrochemical biosensors [16] and functional interfaces [17][18][19][20][21] with enhanced properties in terms of sensitivity, selectivity, robustness, and simplicity [22].

Nanocomposite materials are prepared by combining two or more different materials with different physicochemical properties, where one of the constituents has dimensions at the nanoscale or, instead, the nanocomposite structure exhibits a nanometric phase separation of the individual components. In preparing nanocomposites, one of the constituent

materials acts as a support matrix in which other materials called reinforcement agents are incorporated [23][24]. Nanocomposites present mixed properties based on the original properties of each constituent nanomaterial, not modified during the preparation process [25].

Similarly, a hybrid nanomaterial combines organic and inorganic building blocks [4], which present a continuous interface between the structural components [25], and new, improved physicochemical properties emerge that are distinct from the specific properties of the components alone [26]. Hybrid nanomaterials can function as novel electrode materials, signal amplifiers, and catalysts of the electrochemical reaction of the product generated in situ during the biorecognition event. To date, the most common hybrid nanomaterials applicable to electrochemical biosensing include metallic nanostructures [27][28], silicon nanomaterials [29][30][31][32], carbon nanostructures [17][33][34], and semiconductor polymers [35][36][37][38][39], with great potential for the development of electrochemical nanobiosensors with enhanced performance [40][41][42], as commented. This section will comment on the main examples of the last ten years (**Table 1**), focused on nanostructured nanomaterials employed in developing nanohybrids for their implementation in electrochemical biosensing.

Table 1. Comparative analytical characteristics of nanomaterial-based electrochemical biosensors focused on the last ten years.

Nanomaterial	Hybrid [a]	Target [b]	Analytical Characteristics		Comments	References
			Linear Range	LOD		
Metallic nanostructures	3D hybrid graphene–GNR.	H_2O_2	0 to 50 mM	2.9 µM	Metallic nanostructures have high catalytic activity, easy preparation, and relatively low cost. However, this kind of nanomaterial can change its oxidation state due to variations in conditions of the medium, such as pH, ionic strength, and temperature upon time.	[43]
	TiO_2 nanoparticles encapsulated ZIF-8	Glucose	2 to 10 mM	80 nM		[44]
	Nanohybrid of VS_2/AuNP and $CoFe_2O_4$ nanozyme	Kana	1 pM to 1 µM	0.5 pM		[45]
	Ag and hybrid Ag–Fe_3O_4 metallic nanoparticles.	AA	0.2–60 µM	74 nM		[46]
Silicon nanomaterials	$mSiO_2$@MWCNT.	Thrombin	0.0001 nM and 80 nM	50 fM	These nanomaterials have high mechanical resistance, thermal stability, long functional life, and versatility; nonetheless, they require long synthetic processes, and their application is limited to certain analytes.	[31]
	MSF/APTES/AgNP	STR	1 to 6.2 ng/mL	0.33 fg/mL		[47]
	Ap–GA–NH_2MCM-41–GCE	hemin and Hb	1.0×10^{-19} to 1.0×10^{-6} M	7.5×10^{-20} M and 6.5×10^{-20} M		[48]
	AuNPs loaded in functionalized MSNPs	CEA	1.0×10^{-3} to 100 ng/mL	9.8×10^{-4} ng/mL		[49]

Nanomaterial	Hybrid [a]	Target [b]	Analytical Characteristics		Comments	References
			Linear Range	LOD		
Carbon nanostructures	MWCNTs and GQDs.	IL-13Rα2	2.7 to 100 ng/mL	0.8 ng/mL	These nanomaterials enjoy thermal stability, large surface area, and a wide range of nanostructures and functional groups. They are the main nanomaterials used in the preparation of electrochemical biosensors.	[50]
	GQDs/AuNPs.	P53	0.000592–1.296 pM	0.065 fM		[51]
	CQDs/AuNps	Glucose	0.05 mM to 2.85 mM	17 µM		[52]
	CoCu-ZIF@CDs	B16-F10 cells	1×10^2 to 1×10^5 cells/mL	33 cells/mL		[53]
Polymers	(Chi-Py) mixture, AuNPs, and MWCNT	Escherichia coli	3×10^1 to 3×10^7 cfu/mL	~30 CFU/mL	These have high biocompatibility, high affinity, strong adsorption ability, low molecular permeability, physical rigidity, and chemical inertness in biological processes. However, functionalizing their surface is necessary for the anchorage of bioreceptors, and some polymers oxidize due to changes in medium conditions.	[54]
	PANI/active carbon and n-TiO_2	Glucose	0.02 mM to 6.0 mM	18 µM		[55]
	PEG/AuNPs/PANI	alpha-fetoprotein	10^{-14} to 10^{-6} mg/mL	0.007 pg/mL		[56]
Other nanostructured nanomaterials	WSe_2 and AuNPs	Thrombin	0–1 ng/mL	190 fg/mL	Other hybrid nanostructures have a large specific surface area, excellent electrical conductivity, and electrocatalytic properties.	[57]
	MoS_2/Ti_3C_2 nanohybrids	miRNA	1 fM to 0.1 nM	0.43 fM		[58]
	AuNPs/Ti_3C_2 MXene 3D	miRNA155	1.0 fM to 10 nM	0.35 fM		[59]

[a] GNR, graphene–gold nanorod; AuNPs, gold nanoparticles; Ap, aptamer; GA, glutaraldehyde; GCE, glassy carbon electrode; MSNPs, mesoporous silica nanoparticles; MWCNTs, multiwalled carbon nanotube; MSF, mesoporous silica thin film; APTES, (3-aminopropyl) triethoxysilane; AgNP, silver nanoparticles; CDs, carbon-dots; Chi-Py, pyrrole branched chitosan; PEG, polyethylene glycols; PANI, polyaniline. [b] AA, ascorbic acid; STR, streptomycin; miRNA; micro-RNA.

3. Conjugation of Nanohybrid Materials with Biomolecules

Biosensors can be label-free and label-based. Briefly, in a label-free mode, the detected signal is generated directly by the interaction of the analyzed (bio)material with the transducer. In contrast, label-based sensing involves chemical or biological compounds that act as labels, generating a detectable signal by analytical techniques such as colorimetry, fluorescence, and electrochemistry [60].

3.1. Bioreceptors

Biological receptors are biomolecules that bind to a specific ligand with a defined structure, commonly through bioaffinity interactions. These biological components are used when assembling nanobiosensors due to their high specificity, differentiating the target molecule from analogous counterparts and even isomers of the same molecule (**Figure 1**). Different bioreceptors can be anchored at electrochemical transducers to confer specificity to nanobioengineered devices. They can generally be classified into five major categories, i.e., enzymes, antibody/antigens, nucleic acids, cellular structures/cells, and biomimetic entities, as depicted in **Figure 1**.

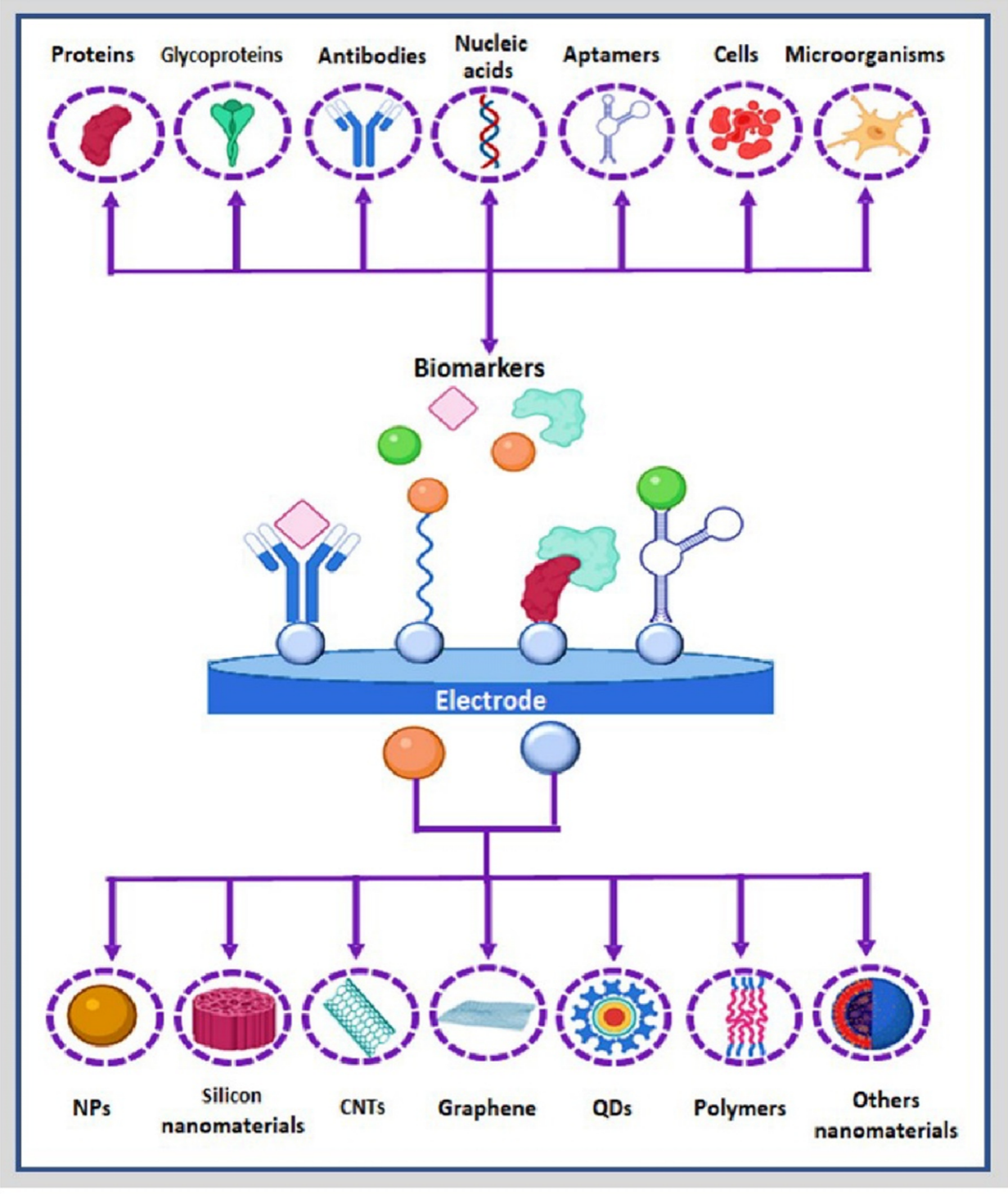

Figure 1. Scheme of electrochemical biosensors based on proteins, glycoproteins, antibodies, nucleic acids, aptamers, cells, or microorganisms at an electrode surface decorated with NPs, silicon nanomaterials, CNTs, graphene, QDs,

polymers, or other nanomaterials.

4. Functional Groups and Conjugation Chemistry

Assembling nano(bio)sensors involves binding the bioreceptor's specific and oriented form to the transducer surface [61] by physical or chemical methods. The physical methods include the following: (i) physical adsorption of the bioreceptor on a matrix based on hydrophobic, electrostatic, and van der Waals attractive forces; (ii) enzyme entrapment in a sol–gel, hydrogel, or paste, confined by semipermeable membranes; (iii) encapsulation—confinement of the biomolecule within a solid matrix. The chemical immobilization methods include (i) covalent binding of the bioreceptor to a solid matrix or directly to the surface of the transducer; (ii) crosslinking employing multifunctional, low-molecular-weight reagents based on the formation of strong covalent bonding between the transducer and the biological material using a bifunctional agent; (iii) affinity binding, exploiting specificity of a bioreceptor to its support under different physiological conditions (**Figure 2**). Conjugation is achieved either by coupling the bioreceptor to the matrix based on affinity interactions or conjugating the bioreceptor to an entity that develops affinity toward the matrix [62].

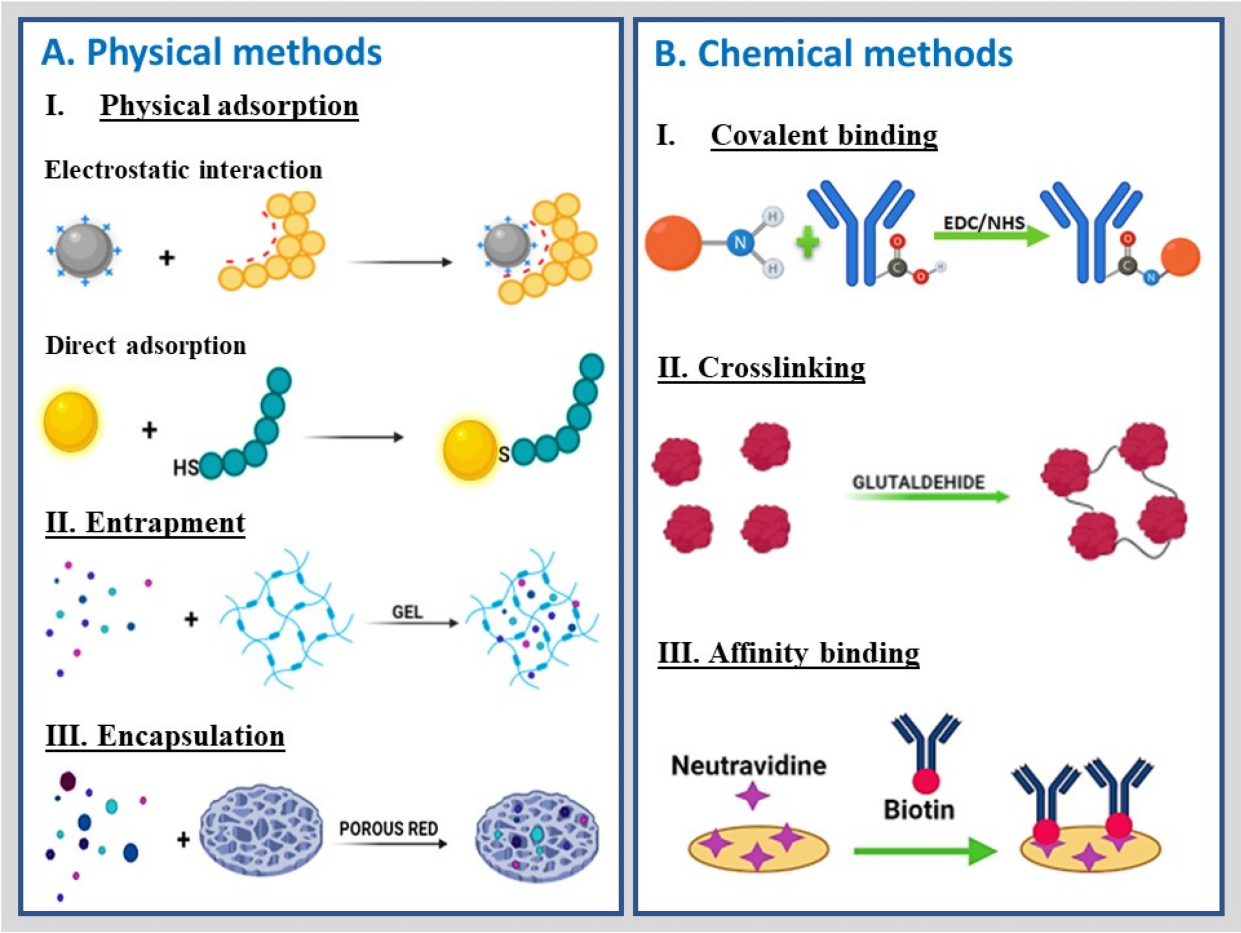

Figure 2. Conjugation of nano(bio)sensors involves binding the bioreceptor's specific and oriented form to the transducer surface by physical (**A**) or chemical methods (**B**). (**A**) The physical methods include (**I**) physical adsorption; (**II**) enzyme entrapment in a sol–gel, hydrogel, or paste, confined by semipermeable membranes; and (**III**) encapsulation. (**B**) The chemical methods include (**I**) covalent binding, (**II**) crosslinking, and (**III**) affinity binding.

The physical methods used in developing nano(bio)sensors are based on weak interactions and, therefore, the most straightforward and affordable. However, they are affected by environmental conditions such as pH, temperature, and ionic strength, generating biomolecule leaching processes during biodetection and storage. For this reason, covalent binding is commonly used as an immobilization alternative. However, this methodology requires modifying the surface of the electrode with a specific functional group that includes carboxylic acid (-COOH), aldehyde (-CHO), amine ($-NH_2$), sulfhydryl (-SH), and azide ($-N_3$) for the anchorage of bioreceptors. Therefore, this methodology requires precise knowledge of the surface chemistry of the electrode surface and the bioreceptor to favor the specific and oriented anchoring of the biomolecules [42].

Modification of the surface of the transducer can be achieved by bifunctional agents such as 4-aminobenzylamine (ABA) [63], 1-pyrenebutanoic acid, acid/basic treatment, or by modification of the electrode surface with affinity agents such as cysteamine [64], cyclodextrin [65], and biotin-avidin [38]. Finally, the modulation of bioreceptor–platform interactions [25]

through specific groups on the nanostructured surface and the bioreceptors determines the methodology of immobilization, promotes the bioreceptor orientation, and increases its compatibility with the platform interface, thus influencing the selectivity, specificity, and stability of the resultant nanobioengineered platforms [28][61][65][66]. Therefore, changes in the surface chemistry of the platforms influence the physicochemical and electrochemical properties of the resultant (bio)sensing devices [67][68].

Bioreceptors can be reversibly adsorbed or trapped and retained or embedded on the surface of electrochemical platforms through ionic, electrostatic, hydrogen bonding, hydrophobic, or van der Waals interactions or irreversibly through covalent bonds. The interactions depend not only on the morphology and reactive functional groups on the electrochemical platform but also on the chemical nature, affinity, isoelectric point, and polarity of the solvent as well as the medium conditions for bioreceptor anchoring. As an illustration, the physical adsorption of bioreceptors can show low reproducibility due to the leaching effect during the analysis and little stability in different medium conditions. In contrast, the binding of biomolecules by covalent bonds through activated functional groups, often including carboxylic acid, amino, thiols, and esters, offers high stability despite possible aggregation, polymerization, and random biomolecule orientation [69]. Conjugation of nano(bio)sensors involves binding the bioreceptor's specific and oriented form to the transducer surface by physical or chemical methods, as depicted in **Figure 2**.

5. Characterization of Nanobioengineered Platforms

Some of the main techniques to characterize nanoengineered platforms include electrochemical and physiochemical techniques such as CV, EIS, and DPV to characterize electrochemical behavior and electron transfer; Fourier transform infrared spectroscopy (FTIR) to characterize the composition and surface chemistry; scanning electron microscopy (SEM) to characterize morphology and composition; and dynamic light scattering (DLS) to determine the surface properties, summarized in **Table 2** [70].

Table 2. Characterization techniques of hybrid nanomaterials, nanobioconjugates and electrochemical biosensors.

Techniques	Physicochemical Characteristics Analyzed
Fourier transform infrared spectroscopy (FTIR).	This technique characterizes the functional groups, surface properties, structure, and conformation of hybrid nanomaterials and nanobioconjugates.
Thermogravimetric analysis (TGA).	Thermogravimetric analysis of nanohybrids determines their thermal stability by estimating organic and inorganic material extent.
Ultraviolet spectroscopy (UV-Vis).	This technique can be used to estimate variables such as K_m and V_{max} in enzyme nanobioconjugates.
Dynamic light scattering (DLS).	This technique can estimate the hydrodynamic size distribution of nanostructures.
Electrophoretic light scattering.	The stability of nanomaterials is highly dependent on the surface charge, among other factors.
X-ray diffraction (XRD).	These techniques characterize hybrid nanomaterials' size, shape, and crystalline structure.
X-ray photoelectron spectroscopy (XPS).	

Techniques	Physicochemical Characteristics Analyzed
Transmission electron microscopy (TEM). Scanning electron microscopy (SEM).	Imaging techniques study size, size distribution, aggregation, dispersion, heterogeneity, morphological characteristics, and compositional analysis of the hybrid nanomaterials and nanobioconjugates.
Electrochemical techniques.	Electrochemical techniques such as CV and EIS are used to evaluate electron transfer before, during, and after the bioreceptors attach to the surface of hybrid nanomaterials. They are also used to characterize the analytical properties of the resultant biosensors.

6. Examples of Nanobioengineered Platforms for Electrochemical Biosensing in the Last Five Years

The significant advance in developing nanobioengineered platforms for electrochemical biosensing has been remarkable in the last five years. However, new 2D and 3D nanomaterials emerge year by year with various improved properties ranging from quantum tunneling, excellent stability, and high conductivity and versatility, which provide new opportunities to develop electrochemical biosensors with high selectivity and extremely low LODs. Furthermore, the appearance of these novel nanostructured materials has led to the implementation of advanced and ultrasensitive biodetection tools (**Table 3**).

Table 3. Examples of nanobioengineered biosensors, indicating the nanobiohybrid (nanomaterial and biomolecules) and analytic characteristics.

Biosensor	Application [a]	Nanobiohybrid: Nanomaterial and Biomolecules [b]	Characterization [c]	Analytical Performance (Linear Range and LOD) [d]	Reference

Immunosensor	PSA	Antibody/HP5@AuNPs@g-C_3N_4 bioconjugated with PSA-Ab2	CV, EIS, and DPV	0.0005 to 0.00 ng/mL with LOD of 0.12 pg/mL	[71]
	HER2	Ab/g-C_3N_4/AuNPs/Cu-MOF	CV and EIS	1.00 to 100.00 ng/mL with LOD of 3.00 fg/mL	[72]
	AXL	Ab/fGQDs	XRD, FTIR, UV-Vis, TEM, EIS, DPV	1.7 to 1000 pg/mL with LOD of 0.5 pg/mL	[73]
	CEA	CdSe-QD-melamine and Ab1-TiO_2-AuNP-ITO	DPV	0.005 - 1000 ng/mL with a LOD of 5 pg/mL	[74]
	CA19-9	CeO_2/FeOx@mC	XPS, TEM, EIS, CV	0.1 mU/mL to 10 U/mL with a LOD of 10 µU/mL	[75]
	NMP-22	Co-MOFs/CuAu NWs/Ab	SEM, XPS, CV, and chronoamperometry	0.1 pg/mL to 1 ng/mL with a LOD of 33 fg/mL	[76]

Genosensor	Zika	Anti-Dig-HRP	Chronoamperometry, CV, EIS	5 to 300 pmol/L with LOD of 0.7 pM	[77]
	Zika genes	AuNPs/ssDNA	SEM, CV, DPV, and chronoamperometry	10 to 600 fM with LOD of 0.2 fM	[78]
	CaMV35S gen	Fe_3O_4-Au@Ag-sDNA on MWCNT/AuNPs/SH-sDNA	TEM, XRD, UV-Vis, CV, and DPV	1×10^{-16} M to 1×10^{-10} M with LOD of 1.26×10^{-17} M	[79]
	mi-R21	3-(trimethoxysilyl)propyl methacrylate/ITO/PET/Fc-hybrid DNA hydrogel	DPV	10 nM to 50 µM with a LOD of 5 nM	[80]
	miRNA-122	rGO/Au/DNA	XRD, TEM, Raman, XPS, CV, and DPV	10 µM to 10 pM with a LOD of 1.73 pM	[81]
	OVA	SiO_2@Au/dsDNA/CeO_2	DPV	1 pg/mL to 1000 ng/mL with a LOD of 0.87 pg/mL	[82]
Enzymatic	Glucose	GOx-Ti_3C_2-PANI	CV and chronoamperometry	0.02 to 6.0 mM with LOD of 18 µM	[83]
	Glucose	Cu-nanoflowers-Gox-HRP/AuNPs-GO-PVA-nanofibers	UV-Vis, SEM, TEM, XDR, CV, and chronoamperometry	0.001 to 0.1 mM with a LOD of 0.019 µM	[84]
	Organophosphate pesticides	acetylcholinesterase/chitosan-transition metals/graphene/GCE	SEM, TEM, XPS, XRD, CV, DPV and EIS	11.31 µM to 22.6 nM with LOD of 14.45 nM	[85]
	β-hydroxybutyric acid	Ti_3C_2Tx nanosheets conjugated with β-hydroxybutyrate dehydrogenase	SEM, CV, and chronoamperometry	0.36 to 17.9 mM with a LOD of 45 µM	[86]

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Based on peptides	Norovirus	Cys/peptide/gold layer	CV and EIS	The LOD was 99.8 nM and 7.8 copies/mL for rP2 and human norovirus, respectively	[87]
	PSA	MXene-Au-MB nanohybrid/peptide	DPV	5 pg/mL to 10 ng/mL with a LOD of 0.83 pg/mL	[88]
	PKA and CK2	Peptide/MSF/ITO	Chronoamperometry	The LODs were 0.083 and 0.095 U/mL, for PKA and CK2, respectively	[89]
	NHE	Cys -PEG-QRRMIEEPA-MB	DPV and SWV	10 and 150 nM with a LOD of 250 pM	[90]
Based on glycoproteins	*Toxoplasma gondii*	Ab/ glycosylphosphatidylinositol/SPAuE	CV, EIS	1.0 to 10.0 IU/mL with a LOD of 0.31 IU/mL	[91]
	MIPs/glycoproteins	Fc/MPBA/AuNPs-SiO_2 nanobioconjugate	FTIR, CV, EIS, DPV, and chronoamperometry	1 pg/mL to 100 ng/mL and reached a LOD of 0.57 pg/mL	[92]

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tumor exosomes extracted from lymph node carcinoma of a prostate cells line	MNPs/aptamer-DNA/double stranded DNA/GCE	DPV	The LOD was 70 particles/μL	[93]
miRNA	DSN/AuNPS/HRP	CV, EIS and chronoamperometry	The LOD was 43.3 aM	[94]
CA125 and living MCF-7 cells	Tb-MOF-on-Fe-MOF	SEM, TEM, XPS, CV and EIS	100 μU/mL to 200 U/mL with a LOD of 58 μU/mL towards CA125. Moreover, biosensor detecting MCF-7 cells with a LOD of 19 cells/mL	[95]
Based on aptamers	Paper-electrode functionalized with amino-modified graphene-Thi-AuNPs and PB-PEDOT	DPV	0.01 to 900 ng/mL for CEA and 0.05 - 500 ng/mL for NSE with a LOD of 2 pg/mL for CEA and 10 pg/mL for NSE, respectively	[96]
CEA and NSE				

Impedimetric biosensor/*Escherichia coli* B	CNT/PEI-T2 virus/GCE	EIS	10^{3} to 10^{10} CFU/mL with LOD of 1.5 × 10^{3} CFU/mL	[97]
Nonenzymatic biosensor/glucose	GS/GNR/Ni	Chronoamperometry	5 nM to 5 mM with a LOD of 2.5 nM	[98]
Mimicking biosensor/H_2O_2 released from H9C2 cardiac cells	AuNFs/Fe_3O_4@ZIF-8-MoS_2	SEM, fluorescence, CV, EIS, and chronoamperometry	5 nM to 120 μM and a LOD of 0.9 μM	[99]
Electrochemical/glucose	CuOx@Co_3O_4 core-shell nanowires/ZIF-67	SEM, TEM, XRD, XPS, CV, and chronoamperometry	0.1 to 3000 μM with a LOD of 36 nM	[100]
Mimicking cytostatic	rGO-Co_3O_4 hybrids	TEM, XPS, XRD, AFM, EIS, CV, and DPV	1.00×10^{-6} to 1.50×10^{-4} mol/L with a LOD of 1.40×10^{-7} mol/L	[101]
Biomimetic biosensor/glucose	Fe_3O_4@PNE-GOx	Chronoamperometry	0.24 to 24 mM with a LOD of 6.1 μM	[102]
PAD/creatinine	CuO/IL/ERGO/SPCE	Chronoamperometry	0.01 to 2.0 mM and a LOD of 0.22 μM	[103]
3D paper-based microfluidic electrochemical biosensor/glucose	rGO-TEPA/PB	SEM, Raman, CV, and chronoamperometry	0.1 mM–25 mM with a LOD of 25 μM	[104]

[a] PSA, prostate-specific antigen; HER2, human epidermal growth factor receptor 2; AXL, tyrosine kinase; CaMV35S, cauliflower mosaic virus 35S; SAMs, self-assembled monolayers; MIPs, molecular imprinted polymers; miRNA, microribonucleic acid; CA19-9, carbohydrate antigen 19-9; CA125, carbohydrate antigen 125; NMP-22, nuclear matrix protein-22; PKA, protein kinase A; CK2, casein kinase II; PAD, paper-based analytical devices; OVA, ovalbumin; CEA, carcinoembryonic antigen; NHE, human neutrophil elastase; NSE, neuron-specific enolase.

[b] HP5, hydroxylpillar[5]arene; AuNPs, gold nanoparticles; Ab, antibody; Gox, glucose oxidase; PANI, polyaniline; Cys, cysteine; MOFs, metal-organic framework; fGQDs, functionalized graphene quantum dots; anti-Dig-HRP, antibody-digoxigenin-horseradish peroxidase; ssDNA, single-strand DNA; MWCNT, multiwalled carbon nanotube; CNT, carbon nanotube; PEI, polyethylenimine; GCE, glassy carbon electrode; SPAuE, screen-printed gold electrode; MNPs, metallic nanoparticles; Fc, ferrocene; MPBA, 4-mercaptophenylboronic acid; QD, quantum dots; ITO, indium tin oxide; DSN, duplex-specific nuclease; PET, polyethylene terephthalate; GO, graphene oxide; PVA, poly(vinyl alcohol); GS, graphene sheet; GNR, graphene-gold nanorod; rGO, reduced graphene oxide; UT, ultrathin; PEG, polyethylene glycol; MB,

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methylene blue; TH, electron-mediating thionin; PB, Prussian blue; PEDOT, poly(3,4-ethylenedioxythiophene); SPCE, screen-printed carbon electrode; MSN, mesoporous silica thin film; PNE, polynorepinephrine; IL, ionic liquid; ERGO, electrochemically reduced graphene.

[c]CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; DPV, differential pulse voltammetry; XRD, X-ray diffraction; XPS, X-ray photoelectron spectroscopy; FTIR, infrared spectroscopy; UV-Vis, ultraviolet visible spectroscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy; SWV, square wave voltammetry.

[d]LOD, limit of detection.

7. Limitations, Opportunities, and Concluding Remarks

Biosensor technology based on nanobiohybrid materials represents a vast field that significantly impacts healthcare, the environment, and food quality control. These functional platforms promote target molecule detection with high specificity and sensitivity, particularly in the biomedical field [105][106][107][108][71][109]. Furthermore, the rational design of the nanobiohybrids has been demonstrated to enhance the response and long-term stability of the resultant devices due to the incorporation of nanomaterials with improved properties that promote a favorable nanoenvironment for bioreceptors anchoring. Besides, the versatility of nanomaterials facilitates the conjugation with molecules by multiple conjugation chemistry, opening options to detect numerous target molecules.

Electrochemical-based nanohybrid biosensors have the potential to solve most of the limitations and concerns of bioanalysis and diagnostic tests while maintaining the required sensitivity, selectivity, and LOD to face real needs. Besides, integrating sample preparation into the device allows the possibility of direct analysis within a sample matrix and offers opportunities for new strategies of long-term analysis in vivo, among many other exciting applications. Electrochemical nanohybrid biosensors are particularly suitable for miniaturization and integration in microfluidic devices, thus reducing the consumption of reagents and samples {Formatting Citation}. Applications include detecting whole cells, cell components, proteins, and small molecules to address diagnostics and food and environmental control tasks online and in real-time, but still require more sophisticated platforms with additional elements, such as sample preparation. Although nanobioengineered biosensors are an affordable analytical strategy relative to gold standard detection methods, the development of large-scale electrochemical nanobiosensors is still challenging because they require state-of-the-art technologies for their production in a reproducible and stable manner, directly influencing the cost of the sensing device [109][110]. This apparent drawback could be overcome by scaling, automation, and mass manufacturing to lower costs through advanced methods in elaborating cost-affordable and disposable electrochemical nanobiosensors based on additive manufacturing, including screen inkjet 3D printing or microfabrication technologies [111][110][112][113].

Overall, this research exemplified nanobiosensors mainly based on screen-printed electrodes modified with nanohybrids conjugated with highly specific bioreceptors for enhanced biosensing. Yet, the richness in the art of biosensors deserves deeper exploration and support of exciting new ideas. Overall, nanobiohybrids are paving the way in the pioneering development of highly sensitive and selective electrochemical nanobiosensors and represent remarkable research advances that are a step forward in increasing the impact of this exciting, cutting-edge technology in the field of biomarker detection of clinical interest [110].

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