Highly Sensitive Amperometric Biosensors Based on Oxidases and CuCe Nanoparticles Coupled with Porous Gold†

Nataliya Stasyuk 1,2, Olha Demkiv 1,3, Galina Gayda 1,*, Roman Serkiz 1, Andriy Zakalskiy 1, Oksana Zakalska 1, Halyna Klepach 2, Galeb Al-Maali 4, Nina Bisko 4 and Mykhailo Gonchar 1,2

Abstract: Metallic nanoparticles are usually applied in biosensors as catalysts and/or mediators of electron transfer. We describe the development of amperometric biosensors (ABSs) based on oxidases and nanoparticles of CuCe (nCuCe). nCuCe, being an electro-active mediator and active peroxidase (PO) mimetic, was used as an H2O2-sensing platform in oxidase-based ABSs. ABSs for glucose, primary alcohols, methyl amine, catechol, and L-arginine, which are based on corresponding oxidases and nCuCe, were developed. These ABSs exhibited improved analytical characteristics in comparison with the appropriate bi-enzyme ABSs containing natural PO. Including electrode-deposited porous gold in the chemo-sensing layer was shown to increase significantly the sensitivities of all constructed ABSs.

Keywords: electroactive nanoparticles; peroxidase-like nanozyme; oxidases; porous gold; amperometric biosensors

1. Introduction

Metallic nanoparticles have wide potential practical applications in various fields of science and industry. In biosensorics, they usually act as mediators in electron transfer and/or catalysts (nanozymes, NZ) [1–5].

NZs are the newest class of functional nanomaterials [3–7] that have enzyme-like activities with different reaction specificities. NZs possess increased stability and greater availability due to their simpler preparation technologies. Most reported NZs are mainly mimetics of oxidoreductases, including peroxidase (PO) [7–9].

PO catalyzes the oxidation of diverse organic compounds, using H2O2 as the electron acceptor [8]. Many natural enzymes (oxidases) produce H2O2 as a byproduct of their enzymatic reactions, so the detection of a target substrate can be performed by measuring H2O2 generation. Over the last few years, a number of reports have described the application of various PO-like NZs for H2O2 detection using different sensors [10–14]. The main peculiarities of PO-like NZs as catalysts are that they have high stability, sensitivity, and selectivity to H2O2 in extra-wide linear ranges. PO-like NZs coupled with natural oxidases are widely used in amperometric oxidase-based biosensors (ABSs) [1–3,7–10].

In our earlier works, different types of chemically and “green” synthesized NZs were described [9,10]. Nanoparticles of CuCe (nCuCe) were chosen as effective...
electroactive PO-mimetics and were characterized using scanning electron microscopy (SEM) coupled with X-ray microanalysis (SEM-XRM) [9,15]. Our results demonstrated that the synthesized nCuCe, having an excellent sensitivity and a wide linear range for H2O2 detection, may be promising artificial POs for the development of oxidase-based ABSs. nCuCe were successfully used for the construction of Arg-sensitive ArgO-based ABSs [15].

A number of approaches have been proposed for improving the analytical characteristics of ABSs. One of them is increasing the effective working surface of the electrode in order to obtain maximal electroactive sites for the immobilization of biocatalysts, including enzymes and NZs [7,8].

Micro/nanoporous gold (npAu), because of its high surface area-to-volume ratio, excellent conductivity, chemical inertness, physical and chemical stability, biocompatibility, high area, electrochemical activity, easily tunable pores, and plasmonic properties, may be promising in medicine for diagnostics and drug delivery, in energy storage, and in sensing and biosensing. A lot of synthetic methods for obtaining npAu have been reported, including deallyling, templating, sputtering, self-assembling, and electrodeposition [16,17]. The last method is the most popular.

The aim of the current study was to fabricate and characterize highly sensitive ABSs using various oxidases as biorecognition elements with nCuCe as an electroactive mimetic of PO and electrodeposited npAu as an effective carrier of enzymes/NZs with a highly advanced surface area [16,17].

2. Materials and Methods

2.1. Reagents

Cerium(IV) bicarbonate(Ce(HCO3)4), copper(II) sulfate (CuSO4), L-arginine (Arg), methylamine (MA), ethanol, methanol, o-dianisidine, hydrogen peroxide (H2O2, 30%), hydrogen tetrachloroaurate(III) H[AuCl4], D-glucose, sodium sulfide (Na2S), ammonia chloride (NH4Cl), Nafion (5% solution in 90% low-chain aliphatic alcohols), Horse radish peroxidase (PO, EC 1.11.1.7) from Armoracia rusticana (500 U·g−1), glucose oxidase (GO, EC 1.1.3.4) from Aspergillus niger (168 U·mg−1), and all other reagents and solvents used in this work were purchased from Sigma-Aldrich (Steinheim, Germany).

All reagents were of analytical grade and were used without further purification. All solutions were prepared using ultra-pure water obtained with the Milli-Q® IQ 7000 Water Purification system (Merck KGaA, Darmstadt, Germany).

2.2. Enzymes Isolation and Purification

Electrophoretically homogeneous yeast enzymes—alcohol oxidase (AO, EC1.1.3.13), L-arginine oxidase (ArgO, EC 1.4.3.25), methylamine oxidase (AMO, EC 1.4.3.21), and laccase (EC 1.10.3.2) were used for amperometric biosensors fabrication.

Yeast AO was isolated from cell-free extract of the selected over-producing strain Ogataea polymorpha C-105 (gcr1 catX) using a two-step ammonium sulfate fractionation (at 30 and 70% saturation), followed by ion exchange chromatography on DEAE-Toyopearl 650 M [18]. Purified AO with specific activity ~20 U·mg−1 of protein was kept as a suspension in 70% sulfate ammonium and 50 mM phosphate buffer (PB) at pH 7.5 at 4°C. Fungal ArgO was isolated from an extract of the fruiting body of the wild forest mushroom Amanita phalloides and partially purified up to ~7.9 U·g−1 of protein using a two-step ammonium sulfate fractionation (at double 70% of saturation), followed by ion exchange chromatography on Toyopearl DEAE-650M resin [15]. Activities of AO, ArgO, or GO were determined by the rate of hydrogen peroxide formation in reaction with substrate (methanol, Arg, or glucose) as monitored by the peroxidative oxidation of o-dianisidine in the presence of PO and correspondent substrates methanol [18], Arg [15], or glucose [10].

Yeast AMO was isolated from the recombinant yeast strain Saccharomyces cerevisiae C13ABYS86 [19]. The (His)6-tagged AMO was purified from the cell-free extract by metal-affinity chromatography on Ni-NTA-agarose. Activity of AMO was determined by the rate of
hydrogen peroxide formation in reaction with MA as monitored by the peroxidative oxidation of 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) in the presence of PO.

Fungal laccase was isolated from a cultural liquid of the fungus *Trametes zonatus* by a two-step ammonium sulfate fractionation (up to 70% of saturation), followed by ion exchange chromatography on Toyopearl DEAE-650M [20]. Fractions with the laccase activity were pooled, concentrated by Millipore filter (10 kDa) up to specific activity of enzyme ≥ 10 U·mg⁻¹ followed by precipitation with 80% sulfate ammonium.

The activity of laccase was determined by the rate of the increase in absorbance monitored spectrophotometrically at 420 nm (Shimadzu, Kyoto, Japan). As a substrate, 0.5 mM ABTS in 50 mM sodium acetate (NaOAc) buffer solution, pH 4.5 was used. One unit of laccase activity was defined as the amount of the enzyme required to oxidize 1 µmole of a substrate (ε₄₂₀ = 36 mM⁻¹·cm⁻¹) per minute at 24 °C.

2.3. Synthesis of Nanoparticles and Estimation of Their Pseudo-Peroxidase Activity

Nanoparticles of CuCe (nCuCe) were synthesized, as described previously [9]. The synthesized nCuCe were collected by centrifugation. The precipitates were rinsed twice with water and were stored as a water suspension at +4 °C until use.

Pseudo-peroxidase (PO-like) activity of the nCuCe was measured using the colorimetric method, with o-dianisidine as a chromogenic substrate in the presence of H₂O₂ [9]. One unit (U) of PO-like activity was defined as the amount of nCuCe releasing 1 µmol H₂O₂ per 1 min at 30 °C under standard assay conditions.

2.4. Apparatus

A piece of Pt wire and an Ag/AgCl/3M KCl electrode were used as the counter and reference electrodes. 3.05 mm graphite rods (type RW001, Ringsdorff Werke, Bonn, Germany) were used as working electrodes. They were sealed in glass tubes with epoxy forming disk electrodes. Before sensor preparation, the graphite electrode (GE) was polished with emery paper. Amperometric measurements were carried out with a potentiostat CHI 1200A (IJ Cambria Scientific, Burry Port, UK) in batch mode under continuous stirring in a standard 40 mL cell at a room temperature.

A SEM microanalyser REMMA-102-02 (Lviv, Ukraine) was used for morphological analyses of the synthesized nAu-film (nAu).

2.5. Electrodeposition of Nanoporous Gold onto Graphite Electrode

A micro/nanoporous gold (npAu) was synthesized on the surface of GE in two stages. In the first stage, npAu was electrodeposited from a solution containing 10 mM of HAuCl₄ in 2.5 M ammonia chloride, using cyclic voltammetry in the range of 0 to +800 mV with a scan rate of 50 mV·min⁻¹ for 25 cycles. In the second stage, the obtained modified electrode (npAu/GE) was re-immersed in a solution of 10 mM of HAuCl₄ in 2.5 M of ammonia chloride using the potentiostatic mode at –1000 mV for 120 s. The obtained npAu/GE was rinsed with water and equilibrated before usage in the appropriate buffer.

2.6. Immobilization of Natural and Artificial Peroxidases onto Electrode

Natural PO and the synthesized nCuCe as an artificial PO were immobilized on the surfaces of GE or npAu/GE using the physical adsorption method. For the development of the nCuCe/npAu/GE, an aliquot of nCuCe solution (5–10 µL) with a PO-like activity of 1 U/mL was dropped onto the surface of npAu/GE. For development of the PO/GE, an aliquot of PO solution (5–10 µL) with an activity of 1 U/mL was dropped onto the surface of GE. After drying the sensing film for 10 min at room temperature, the modified GE was covered with 10 µL of 1% Nafion solution in 50 mM PB, pH 7.5. The modified electrodes were rinsed with 50 mM PB, pH 7.5, and kept in this buffer with 0.1 mM EDTA at 4 °C until used.
2.7. Immobilization of Oxidases onto the Modified Electrodes

To fabricate the oxidase-based amperometric biosensors (ABS), GO, AMO, AO, ArgO, or laccase were immobilized onto the modified GE.

A total of 5–10 µL of enzyme solution was dropped onto the dried surfaces of the PO/GE, nCuCe/GE, or nCuCe/npAu/GE. To develop the ABSs on the base of ArgO, GO, MAO or laccase, the dried composites were covered with a Nafion membrane, as described in Section 2.6. To prepare 1% Nafion solution, the stock 5% solution was diluted with the appropriate buffer: 50 mM NaOAc, pH 4.5 for construction of laccase-based ABS and with 50 mM PB, pH 7.5 in other cases.

It is worth mentioning that, in the AO-based ABS, the biosensing film on the electrode was fixed, not with Nafion, but with a dialysis membrane.

The coated bioelectrodes were rinsed with water and stored in the corresponding buffers until use.

2.8. Measurements and Calculations

Amperometric measurements were carried out using a CHI 1200A potentiostat (IJ Cambria Scientific, Burry Port, UK) connected to a personal computer, used in a batch mode under continuous stirring in an electrochemical cell with a 20 mL volume at 25 °C.

All experiments were carried out in triplicate trials. Analytical characteristics of the proposed electrodes were statistically processed using OriginPro 8.5 software. Error bars represent the standard error derived from three independent measurements. Calculation of the apparent Michaelis–Menten constants (K_{Mapp}) was performed automatically by this program, according to the Lineweaver–Burk equation.

3. Results and Discussion

3.1. Development of Oxidase-Based Biosensors Using nCuCe and Porous Gold

We describe here the development of amperometric biosensors (ABSs) based on oxidases and nCuCe. nCuCe, being an active PO mimetic, was used as a hydrogen peroxide-sensing platform for oxidase-based ABSs.

To improve analytical characteristics of ABSs, namely, sensitivity, we modified the surface of a graphite electrode (GE) with micro/nanoporous gold (npAu).

npAu has a high surface area-to-volume ratio, excellent conductivity, chemical stability, high area, electrochemical activity, easily tunable pores, and plasmonic properties, thus, it may be promising for use in sensing and biosensing.

npAu has the unique properties of chemical stability, a high area, and electrochemical activity, thus it may be promising in biosensing. The principal scheme of bioelectrode construction is presented in Figure 1.
Figure 2 demonstrates the results of morphological characterization of npAu using the SEM technique, which provides information on the size, distribution, and shape of the tested npAu. The XRM images showed the characteristic peaks for the gold.

![SEM images of npAu](image1.png)

(a) (b)

![X-ray spectral microanalysis](image2.png)

(c)

**Figure 2.** Characteristics of the npAu SEM images (a,b); X-ray spectral microanalysis (c).

### 3.2. Analytical Characteristics of the Constructed Biosensors

Using GO, AO, AMO, or ArgO as biorecognition elements, nCuCe as a PO-like NZ or as an electro-active mediator, and npAu as a carrier of enzymes/NZs, the ABSs for glucose, primary alcohols, methyl amine, L-arginine, and catechol, respectively, were constructed and characterized.

Figure 3 demonstrates the amperometric characteristics of the developed GO-based ABS for glucose determination. Using the chronoamperograms at optimal working potentials for the modified and control electrodes, calibration curves were plotted for analyte determination using the developed ABSs. The same experiments were carried out with other oxidase-based ABSs (data not shown). It is worth mentioning that the npAu/GE as a control electrode was also tested, and no amperometric signals were detected with any analyte addition under the chosen conditions (data not shown).

Table 1 summarizes the main bioanalytical characteristics for the developed ABSs, which were based on the usage of various oxidases and nanomaterials. It is worth mentioning that nCuCe plays a dual role in the developed ABSs: for laccase it is a mediator of electron transfer, and for other oxidases it is an artificial PO.

As can be seen in Table 1, nCuCe had a significant positive effect on sensor sensitivity in comparison to electrodes that were not modified with nanomaterials. For example, for AMO/nCuCe/GE and ArgO/nCuCe/GE, the sensitivities were 5-fold higher, than for the corresponding GEs without nCuCe.

The presence of npAu was shown to provide additional contributions to improving the analytical parameters of the ABS, especially in terms of their sensitivities. For example, the sensitivity of the GO/nCuCe/npAu/GE is 9.1-fold higher than that of the GO/PO/GE and 5.5-fold higher in comparison to the GO/nCuCe/GE. The same tendency, but at various levels, was demonstrated for all investigated enzymes. This fact has simple explanation: a highly advanced surface of the npAu, having hierarchical pores of nano- and micro-sizes with different diameters, has an enhanced working 3D surface area of electrode. The increased surface of the modified GE leads to the enhanced adsorption of...
nanomaterials/enzymes and, thus, to improved efficiency of electron transfer in ABS in comparison with unmodified GEs.

Figure 3. Amperometric characteristics of the GO/PO/GE (a,b), GO/nCuCe/GE (c,d) and GO/nCuCe/npAu/GE (e,f): (a,c,e) chronoamperograms (inserted) and dependences of amperometric signal on concentration of glucose; (b,d,f) calibration graphs for glucose determination. Conditions: working potential –50 mV vs. Ag/AgCl/3 M KCl in 50 mM PB, pH 6.0. The sensing layers contain 0.01 U of PO/PO-like activity and 0.01 U of GO.
Table 1. Analytical characteristics of the constructed bioelectrodes based on different oxidases, natural or artificial peroxidases, and npAu.

| Bioelectrode                  | Potential, mV | Sensitivity, A·M⁻¹·m⁻² | Linear Range, µM | LOD, µM |
|-------------------------------|---------------|--------------------------|------------------|--------|
| GO/PO/GE                      | -50           | 44                       | 50–5000          | 150    |
| GO/nCuCe/GE                   | -50           | 73                       | 500–7300         | 150    |
| GO/nCuCe/npAu/GE              | -50           | 400                      | 25–2000          | 75.7   |
| AMO/PO/GE                     | -250          | 7                        | 200–1700         | 130    |
| AMO/nCuCe/GE                  | -250          | 35                       | 60–1700          | 18     |
| AMO/nCuCe/npAu/GE             | -250          | 125                      | 60–500           | 18     |
| AO/PO/GE                      | -50           | 22                       | 130–900          | 39     |
| AO/nCuCe/GE                   | -50           | 32                       | 50–2100          | 15     |
| AO/nCuCe/npAu/GE              | -50           | 102                      | 33–500           | 10     |
| ArgO/PO/GE                    | -150          | 24                       | 75–1150          | 35     |
| ArgO/nCuCe/GE                 | -150          | 113                      | 50–2250          | 15     |
| ArgO/nCeCu/npAu/GE            | -150          | 200                      | 100–500          | 33     |
| Laccase/GE                    | +200          | 2300                     | 8–160            | 2      |
| Laccase/nCuCe/GE              | +200          | 5055                     | 3–40             | 1.5    |
| Laccase/nCuCe/npAu/GE         | +200          | 9280                     | 2–40             | 1      |

4. Conclusions

In the present work, the development of ABSs based on different oxidases and nCuCe was described. nCuCe has a dual role being an active mimetic of PO and a mediator of electron transfer. It was used as an electro-active mediator for laccase-based ABS and as a PO-like NZ in ABSs, based on other oxidases, namely, GO, AO, AMO and ArgO. The ABSs for catechol, glucose, primary alcohols, methyl amine, and L-arginine were constructed and characterized. The developed mono-enzyme ABSs exhibited improved analytical characteristics in comparison with the correspondent bi-enzyme ABSs, which contained natural PO. It was demonstrated, that including electrodeposited nanoporous gold in chemosensing layer on graphite electrode allows a significant additional increase in ABSs sensitivity. This fact may be explained by the highly advanced surface area of npAu due to pores of nano- and micro-sizes. Such a hierarchical porous 3D surface leads to the enhanced adsorption of nanomaterials/enzymes and, thus, to improved efficiency of electron transfer in ABS, in comparison with unmodified GEs.

Author Contributions: Conceptualization, N.S. and G.G.; methodology, N.S., O.D. and G.A.-M.; software, R.S.; validation, O.D. and H.K.; formal analysis, N.B.; investigation, O.D., O.Z., R.S.; resources, O.Z.; data curation, A.Z. and N.B.; writing—original draft preparation, M.G.; writing—review and editing, G.G.; visualization, A.Z.; supervision, M.G.; project administration, M.G.; funding acquisition, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially founded by the National Research Foundation of Ukraine (projects No 2020.02/0100 and No 2021.01/0010), by the National Academy of Sciences of Ukraine (The program “Smart sensor devices of a new generation based on modern materials and technologies”), and Ministry of Education and Science of Ukraine (projects Nos. 0120U103398, 0121U109539 and 0121U109543).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within this article.

Acknowledgments: The authors thank M. Horecha (Institute of Cell Biology NAS of Ukraine, Lviv, Ukraine) for her technical assistance in experiments.

Conflicts of Interest: The authors declare no conflict of interest.
References

1. Naresh, V.; Lee, N. A review on biosensors and recent development of nanostructured materials-enabled biosensors. Sensors 2021, 21, 1109. [CrossRef] [PubMed]

2. Nayl, A.A.; Abd-Elhamid, A.I.; El-Moghazy, A.Y.; Hussin, M.; Abu-Saied, M.A.; El-Shanshory, A.A.; Solman, H.M.A. The nanomaterials and recent progress in biosensing systems: A review. Trends Environ. Anal. Chem. 2020, 26, e00887. [CrossRef]

3. Presutti, D.; Agarwal, T.; Zarepour, A.; Celikkin, N.; Hooshmand, S.; Nayak, C.; Ghomi, M.; Zarrabi, A.; Costantini, M.; Behera, B.; et al. Transition Metal Dichalcogenides (TMDC)-Based Nanozymes for Biosensing and Therapeutic Applications. Materials 2022, 15, 337. [CrossRef] [PubMed]

4. Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes (II). Chem. Soc. Rev. 2019, 48, 1004–1076. [CrossRef] [PubMed]

5. Huang, Y.; Ren, J.; Qu, X. Nanozymes: Classification, Catalytic Mechanisms, Activity Regulation, and Applications. Chem. Rev. 2019, 119, 4357–4412. [CrossRef] [PubMed]

6. Liu, Q.; Zhang, A.; Wang, R.; Zhang, Q.; Cui, D. A Review on Metal- and Metal Oxide-Based Nanozymes: Properties, Mechanisms, and Applications. Nano Micro Lett. 2021, 13, 154. [CrossRef] [PubMed]

7. Stasyuk, N.; Smutok, O.; Demkiv, O.; Prokopiv, T.; Gayda, G.; Nisnevitch, M.; Gonchar, M. Synthesis, Catalytic Properties and Application in Biosensors of Nanozymes and Electronanocatalysts: A Review. Sensors 2020, 20, 4509. [CrossRef] [PubMed]

8. Neumann, B.; Wollenberger, U. Electrochemical Biosensors Employing Natural and Artificial Heme Peroxidases on Semiconductors. Sensors 2020, 20, 3692. [CrossRef] [PubMed]

9. Demkiv, O.; Stasyuk, N.; Serkiz, R.; Gayda, G.; Nisnevitch, M.; Gonchar, M. Peroxidase-Like Metal-Based Nanozymes: Synthesis, Catalytic Properties, and Analytical Application. Appl. Sci. 2021, 11, 777. [CrossRef]

10. Gayda, G.Z.; Demkiv, O.M.; Gurianov, Y.; Serkiz, R.Y.; Klepach, H.M.; Gonchar, M.V.; Nisnevitch, M. “Green” Prussian Blue Analogues as Peroxidase Mimetics for Amperometric Sensing and Biosensing. Biosensors 2021, 11, 193. [CrossRef] [PubMed]

11. Komkova, M.A.; Pasquarelli, A.; Andreev, E.A.; Galushin, A.A.; Karyakin, A.A. Prussian Blue modified boron-doped diamond interfaces for advanced H2O2 electrochemical sensors. Electrochim. Acta 2020, 339, 135924. [CrossRef]

12. Keihan, A.H.; Karimi, R.R.; Sajad, S. Wide dynamic range and ultrasensitive detection of hydrogen peroxide based on beneficial role of gold nanoparticles on the electrochemical properties of Prussian blue. J. Electroanal. Chem. 2020, 862, 114001. [CrossRef]

13. Tripathi, A.; Harris, K.D.; Elias, A.L. High surface area nitrogen-functionalized Ni nanozymes for efficient peroxidase-like catalytic activity. PLoS ONE 2021, 16, e0257777. [CrossRef] [PubMed]

14. Fu, Z.; Zeng, W.; Cai, S.; Li, H.; Ding, J.; Wang, C.; Chen, Y.; Han, N.; Yang, R. Porous Au@Pt nanoparticles with superior peroxidase-like activity for colorimetric detection of spike protein of SARS-CoV-2. J. Colloid Interface Sci. 2021, 604, 113–121. [CrossRef]

15. Stasyuk, N.; Gayda, G.; Demkiv, O.; Darmohray, L.; Gonchar, M.; Nisnevitch, M. Amperometric biosensors for L-arginine determination based on L-arginine oxidase and peroxidase-like nanozymes. Appl. Sci. 2021, 11, 7024. [CrossRef]

16. Stine, K.J.; Jefferson, K.; Shulga, O.V. Nanoporous gold for enzyme immobilization. Methods Mol. Biol. 2017, 1504, 37–60. [PubMed]

17. Bhattachar, J.K.; Neupane, D.; Nepal, B.; Mikhaylov, V.; Demchenko, A.V.; Stine, K.J. Preparation, Modification, Characterization, and Biosensing Application of Nanoporous Gold Using Electrochemical Techniques. Nanomaterials 2018, 8, 171. [CrossRef] [PubMed]

18. Klepach, H.M.; Zakalskiy, A.E.; Zakalska, O.M.; Gayda, G.Z.; Smutok, O.V.; Gonchar, M.V. Alcohol Oxidase from the Methylotrophic Yeast Ogataea polymorpha: Isolation, Purification, and Bioanalytical Application. Flavins Flavoproteins 2021, 2280, 231–248.

19. Stasyuk, N.E.; Smutok, O.V.; Zakalskiy, A.E.; Zakalska, O.M.; Gonchar, M.V. Methylamine-Sensitive Amperometric Biosensor Based on (His)6-Tagged Hansenula polymorpha Methylamine Oxidase Immobilized on the Gold Nanoparticles. Biomed. Res. Int. 2014, 2014, 480494. [CrossRef]

20. Demkiv, O.; Stasyuk, N.; Gayda, G.; Gonchar, M. Highly sensitive amperometric sensor based on laccase-mimicking metal-based hybrid nanozymes for adrenaline analysis in pharmaceuticals. Catalysts 2021, 11, 1510. [CrossRef]