Supporting Information for
Tyrosine-specific Bioconjugation Allowing Hole Hopping along Aromatic Chains of Glucose Oxidase

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Details of the chemical and electrochemical experiments

Materials

Lyophilized glucose oxidase (GOx) type VII from *Aspergillus niger* and horseradish peroxidase (HRP) type I were purchased from Sigma Aldrich. Organic solvents acetonitrile (ACN), chloroform (CHF) and tetrahydrofuran (THF) were purchased from ChemPur. For MS analysis, trifluoroacetic acid (TFA) and acetonitrile (ACN) were from Merck (Germany), formic acid (FA) was from Riedel de Haën (Germany). Ammonium bicarbonate (AB), polyethylene glycol 20,000 (PEG), and SOLu-Trypsin dimethylated were obtained from Sigma-Aldrich (Germany). Buffer reagents dipotassium phosphate, monopotassium phosphate, and potassium chloride were purchased from Carl Roth GmbH+ Co, KG, 50 mM pH 7.0 potassium phosphate buffer (PPB) with 100 mM KCl was used in the electrochemical experiments. All the manipulations with the enzymes and their bioconjugates were carried out in the PPB without KCl. The reagents used for the enzyme modification phenothiazine (PTZ) and phenoxazine (PXZ) were purchased from Alfa Aesar. 4-Mercaptobenzoic acid (MBA), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) from Sigma Aldrich were used for immobilization on the gold electrode surface. *o*-Dianizidine from SERVA FEINBIOCHEMICA GmbH & Co. was used during enzyme activity tests. *D*-Glucose (GLU), *D*-galactose, *D*-mannose, *D*-fructose, *D*-xylose were supplied by Carl Roth GmbH + Co, D-ribose was purchased from Acros Organics, and lactate, urea, uric acid, and ascorbic acid were purchased from Sigma Aldrich. Argon gas was purchased from ELME MESSER GAAS. All the experiments were conducted using type II water (R > 18 MΩ) purified in a Milli-Q system. Gold nanoparticles (AuNP) of a diameter of around 10 nm were prepared using the Turkevich's method.[1]

Instrumentations

All the voltammetry (CV) and chronoamperometry (CA) measurements were performed using PalmSense4 potentiostat and a conventional three-electrode system in a 10 mL phosphate buffer solution. Electrochemical data were analyzed with the PSTrace program (version 5.9). Gold (Au) working electrodes (0.031 cm^2), Ag/AgCl as the reference electrode and titanium (~0.5 cm^2) as the counter electrode were used for the electrochemical measurements.

The Evolution 300 Security UV-Vis spectrophotometer (Thermo Fisher Scientific) was used to record UV-Vis spectra and determine the GOx kinetic constants. The J-815 Circular Dichroism spectrometer (JASCO) was used to record circular dichroism (CD) spectra, hydrodynamic particle sizes were determined using a Zetasizer μV (Malvern) device. Emission spectra of the samples were measured using an FS5 fluorescence spectrometer (Edinburgh Instruments), Professional Grade Ultrasonic Cleaner P4820-WPT ultrasonic bath (iSonic), Centrifuge 5418 centrifuge (Eppendorf) and IKA RV 10 rotary evaporator (IKA) were used for sample preparation.

Electrochemical synthesis of the GOx-PTZ and GOx-PXZ bioconjugates

For the modification using PTZ and PXZ, the mixture of 5.0 mg GOx and 6.2 mg PTZ or 5.7 mg PXZ were prepared at a molar ratio of 1:1000, with an initial enzyme concentration of 1 mg mL^{-1}. The mixtures were dissolved in a mixture of 5 mL of ACN and pH 7.0 PPB in a volume ratio of 3:2. To make the mixtures more homogeneous, the solutions were kept in an ultrasonic bath for several minutes. For the electrochemical modification of the samples, a cell with a working graphite electrode (diameter 4.57 mm), a reference Ag/AgCl electrode, and an auxiliary 0.5 cm^2 Ti plate electrode were used. Chronopotentiometry at a current 10 mA for 20 min was employed to perform the reaction. During the modification, argon gas was allowed into the electrochemical cell to remove oxygen. After the reaction, the samples were evaporated using a rotary evaporator with the bath temperature maintained at 40 °C until the ACN was evaporated from the solutions. The remaining solution was diluted to 5.0 mL with the PPB buffer and filtered through a membrane syringe (CA 0.45 μm) to remove excess water-insoluble organic compounds. ROTI®Spin, MINI-30 centrifuge tubes with a molecular weight cut-off of 30 kDa were used to collect (dialyzed) the enzymatic fraction on the filter of the tube. Samples of 0.5 mL were added to the test tubes and centrifuged for 7 min at 8000 rpm. The process was repeated until the entire volume of the samples (5 mL) was filtered. For data comparison, the solutions of pure substances (PTZ and PXZ) in ACN/PPB mixture (3:2) were also prepared at a concentration equal to 1 mg mL^{-1}.

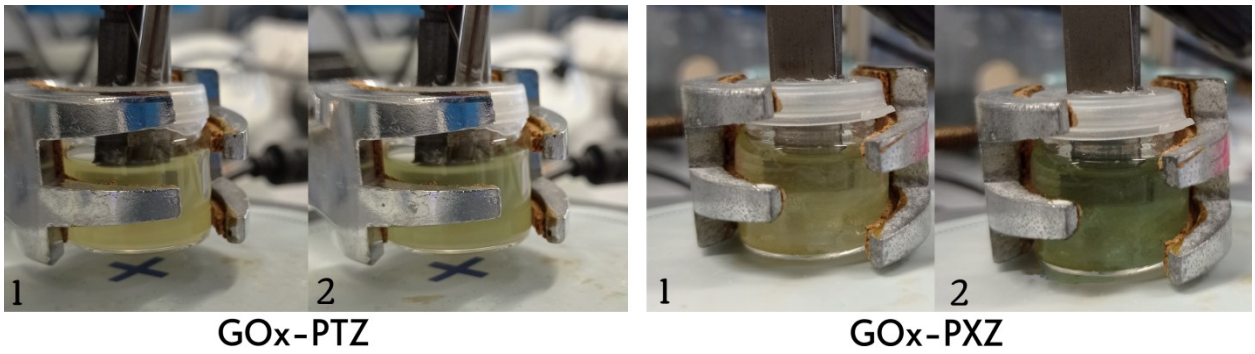


Figure S1. Illustrations of the electrochemical reaction mixtures with the electrodes before reaction (1) and after reaction (2) for the synthesis of GOx-PTZ(SO) and GOx-PXZ, respectively

Biocatalytic properties of the GOx-PTZ and GOx-PXZ bioconjugates

The biocatalytic measurements were performed based on the literature.[2] The hydrogen peroxide formed in the GOx-catalyzed reaction is detected using HRP and *o*-dianisidine by recording the increase in absorption of the oxidation product at a wavelength of 460 nm. The temperature in the measuring cell of the spectrophotometer was maintained at 25 °C. The activity measurement mixture in a quartz cuvette consisted of 10 µL of a solution 20 mM *o*-dianizidine, 50 µL of 5 mg mL^{-1} HRP, and the volume of 10 µL of a glucose (GLU) solution (1 M), and PPB (50 mM, pH 7.0) added to 2 mL total volume. The background level (baseline) is set in the program, and then 10 µL of a GOx solution (1 mg mL^{-1}) was added to the cuvette, the mixture was stirred manually within several seconds, and the program was started. The increase in absorbance was recorded for 200 seconds. A linear part of the slope was used to determine the enzymatic activity in enzyme units (U = 1 µmol min^{-1}) for 1 mg of enzyme:

$$U\ \mathrm{mg}^{-1} = \frac{dA}{dt}\frac{60}{\varepsilon\, l\, C(GOx)\, V} \qquad \text{(S1)}$$

where $\frac{dA}{dt}$ is a mathematical differential of UV-Vis absorption A versus time or the slope, where both points align on the same trend line, C(GOx) are the concentrations of enzyme (mol mL^{-1}) and the enzyme in the sample. ε is the molar extinction coefficient of oxidized o-dianisidine at 460 nm (11300 L mol^{-1} cm^{-1}); l equals the path length (1.0 cm), V is volume of the cell. These biocatalytic activities for the products were calculated by employing the OriginPro 2015 software.

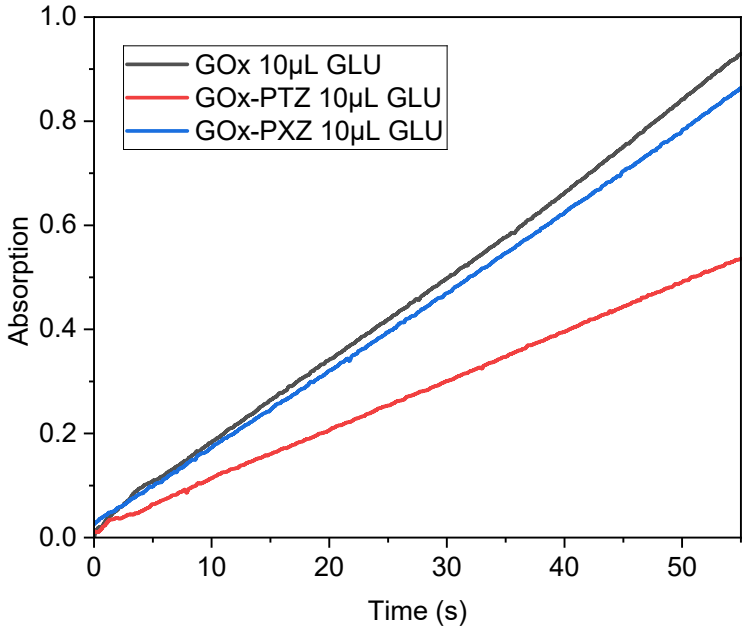
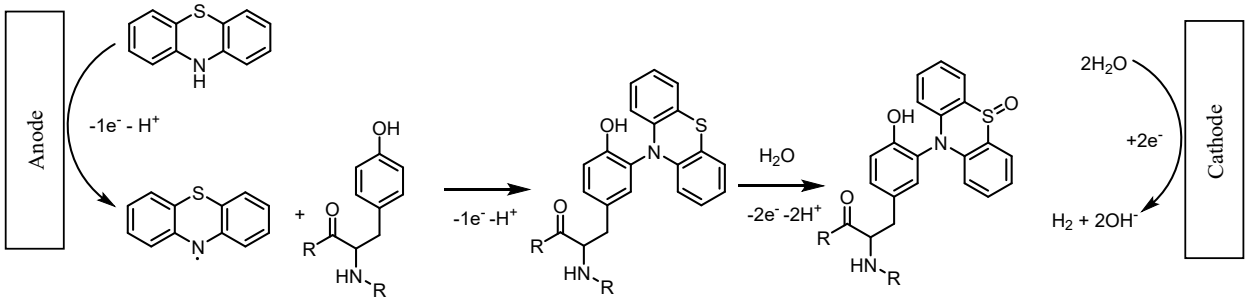


Figure S2. Biocatalytic activity curves of 10 µL of the native GOx, and the GOx-PTZ, GOx-PXZ bioconjugates (1.0 mg mL^{-1}) in a cuvette consisted of 10 µL of a solution 20 mM *o*-dianizidine, 50 µL of 5 mg mL^{-1} HRP, 10 µL of GLU solution (1.0 M), and PPB (50 mM, pH 7.0) added to 2 mL total volume

Measurement of the enzymatic activity during the GOx-PTZ synthesis

A 10 mL reaction mixture was prepared consisting of 10 mg GOx, 12.4 mg PTZ and ACN:PPB solutions in a ratio of 6:4. A first sample of 100 µL corresponding to 0 min GOx-PTZ was taken before the start of the electrochemical reaction. Chronopotentiometry was performed at 10 mA current for 3600 s (1 hour). Samples were taken at 5 min, 10 min, 20 min, 30 min, 40 min and 1 h and evaporated using a rotary evaporator with the bath temperature maintained at 40 °C until the solvents had evaporated from the solutions. The remaining solution was diluted to 200 µL with PPB buffer and centrifuged at 16,000 rcf for 10 min. The supernatant was separated and used for spectroscopic measurements of the enzyme activity. The illustrations of the electrochemical reaction mechanism and the reaction mixtures are presented in Scheme S1 and Figure S3, respectively.



Scheme S1. Mechanism of the electrochemical reaction of the GOx-PTZ(SO) bioconjugate on anode and cathode

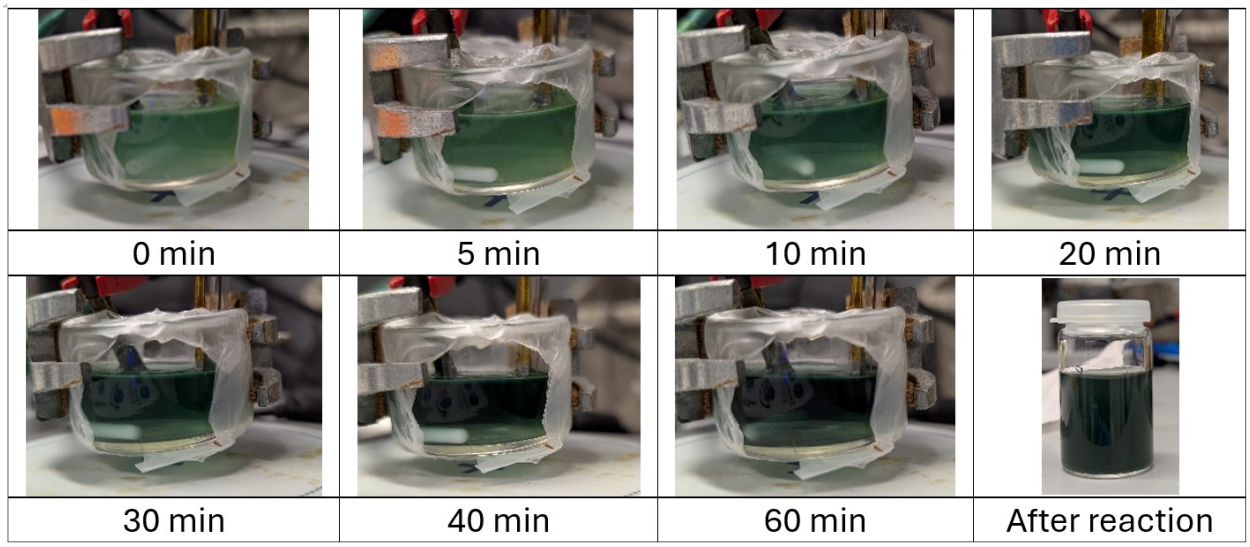


Figure S3. The illustrations of the mixtures of the electrochemical synthesis

The mixture for measuring the activity of GOx-PTZ by time (before reaction, after 5 min, 10 min, 20 min, 30 min, 40 min and after one hour) in a quartz cuvette consisted of 10 µL of a solution of 20 mM *o*-dianizidine, 50 µL of 5 mg/mL HRP and 10 µL of a GLU solution (1 M) and PPB (50 mM, pH 7.0) added to a total volume of 2 mL. Spectroscopy was performed at a wavelength of 460 nm for approximately 45 s,, and the bioconjugate (enzyme) was added after the first 5 seconds. Each sample was measured and the activity (U/mg) calculated from the slope of the kinetic curve multiplied by the number of dilutions. Figures S4 and S5 show the results of the activities during the electrochemical reaction.

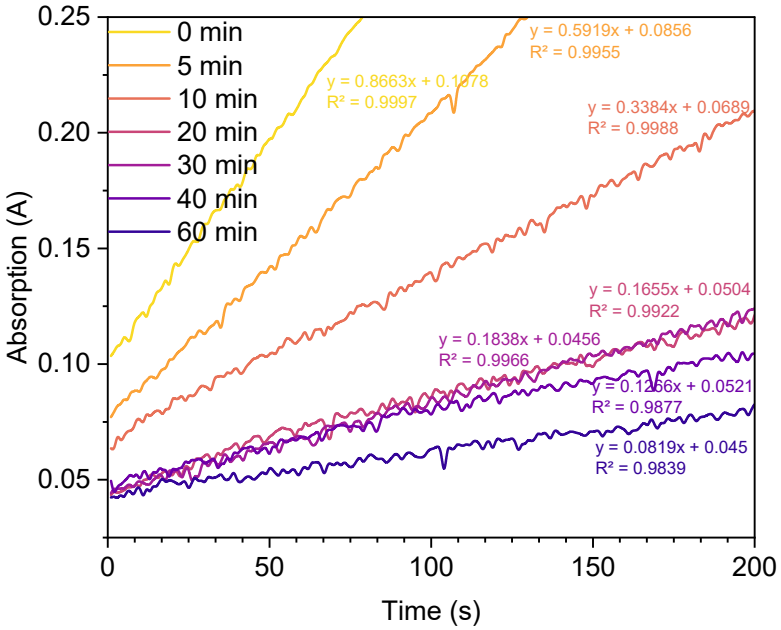


Figure S4. Curves of biocatalytic activity of 10 µL of the GOx-PTZ samples taken from the reaction mixture

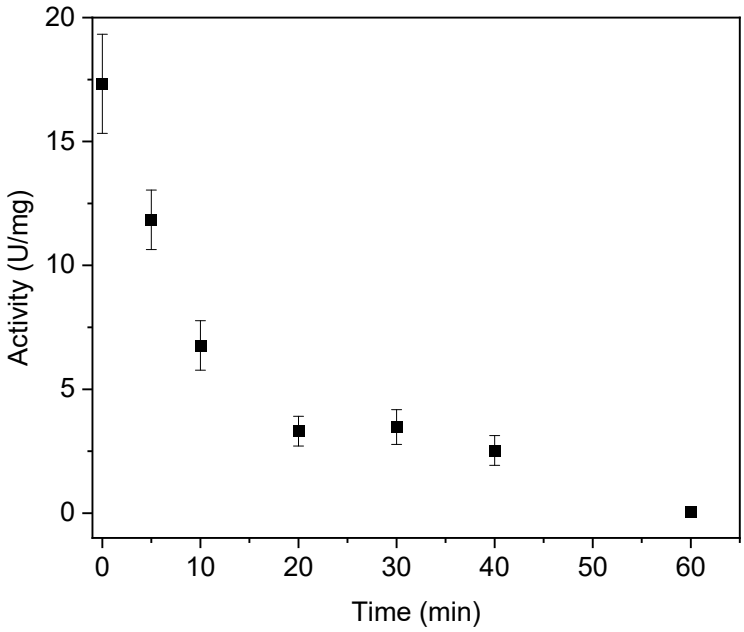
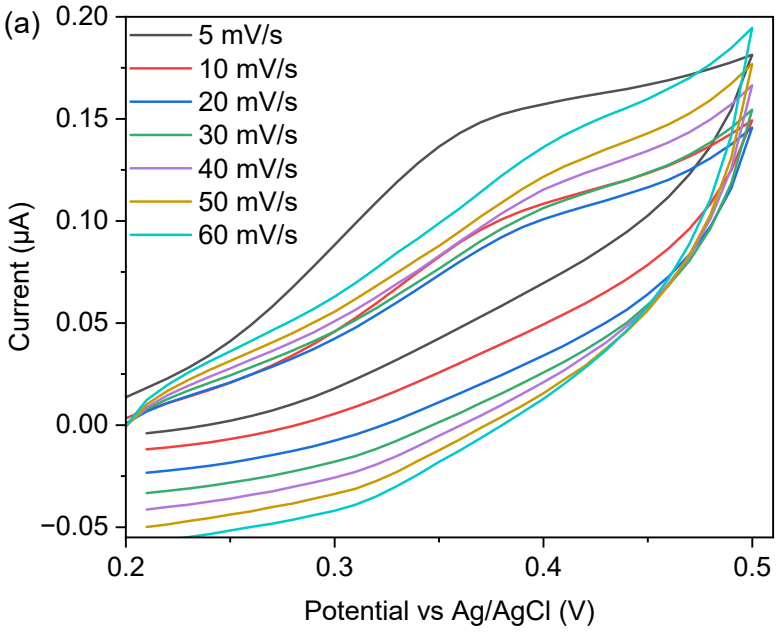


Figure S5. The calculated enzymatic activity during the electrochemical synthesis of the GOx-PTZ bioconjugate

Electrochemical measurements and immobilization of the bioconjugates on the working electrodes

Initially, bioelectrochemical cyclic voltammetry (CV) tests of the samples of the bioconjugates and starting enzyme were performed in the PPB (50 mM) with 100 mM KCl solution using an electrochemical cell with a glass graphite working electrode (diameter of 2 mm), Ag/AgCl reference and the Ti plate (~0.5 cm^2) auxiliary electrodes enzyme and without and with 100 mM GLU. The bioconjugates and starting GOx enzyme were dropped on the glassy carbon electrode, dried and covered with membrane. The CV scan interval was carried out from 0 to 500 mV vs Ag/AgCl, the scan rate was 10 mV s^{-1} in the PPB buffer with 100 mM KCl without and with 100 mM GLU, respectively. Two scans were performed and the second scan was used for the visualization. In this experiment, samples were selected in which changes in current strength were observed, and then they were immobilized on the Au electrodes. The relationship between the current and the scan rate of the potential was measured using the scan rates from 5 to 60 mV s^{-1} (Figure 6a and b).



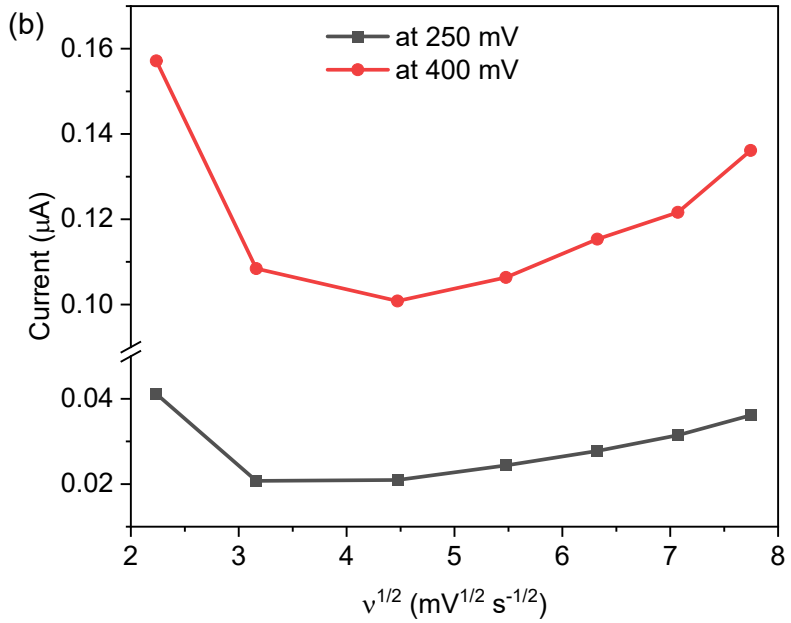


Figure S6. The relationship between the current and the scan rate of the potential: (a) CV scan-rate dependence measurements conducted using the GOx-PTZSO modified electrode in a 100 mM GLU and PPB solution; (b) the relationships between the square root of the scan rate ($v^{0.5}$) and the currents observed at 250 mV and 400 mV versus Ag/AgCl

For the immobilization of the bioconjugates, the gold (diameter of 2 mm) electrodes were used. Initially, the Au electrodes were kept for several minutes in piranha (a mixture of conc. H_2SO_4 and 30% H_2O_2 in the ratio 3:1), and washed with distilled water. Then, the electrodes were cleaned with a MicroPolish alumina 0.3 μm gel, immersed in distilled water and kept in an ultrasonic bath for 5 min. Each electrode was cleaned electrochemically using the 50 mM KOH (40 cycles from 0 to –2.6 V vs. Ag/AgCl reference electrode at scan rate 0.3 V s^{-1}) and 500 mM H_2SO_4 (40 cycles from –0.2 to 1.75 V vs. Ag/AgCl at scan rate 0.3 V s^{-1}) solutions using CV. The cleanliness of the electrode is judged from the cyclic voltammogram during acid cleaning, three oxidation signals and one reduction peak should be visible in the range of 1.15 – 1.45 V vs Ag/AgCl.

The clean Au electrodes were washed with distilled water and covered with 2 μL of a solution of 20-25 nm gold nanoparticles (AuNP). The Au/AuNP electrodes were cleaned electrochemically using 500 mM H_2SO_4 (20 cycles from –0.2 to 1.75 V vs. Ag/AgCl at scan rate 0.3 V s^{-1})

solutions using CV. After this layer dried, the electrodes were soaked in 5 mM 4-mercaptobenzoic acid (MBA) in methanol. The electrodes were stored at +4 °C for 12 h. After 12 hours, the unreacted MBA part was washed with water. Then, 3 µL of the mixture of EDC and NHS (2 mM and 5 mM, respectively) was dropped on the Au/AuNP/MBA electrodes. After 30 min, 3 µL of the bioconjugates or starting GOx was dropped onto the electrodes. Before the electrochemical experiments, the Au/AuNP/MBA/enzyme electrodes were washed with ultra-high-quality water. The electrolyte of the electrochemical cell was continuously stirred with a magnetic stirrer during the experiments. The prepared electrodes were stored in sealed containers with the buffer at +4 ◦C between experiments, and their surface was rinsed with ultra-high-quality water before each measurement.

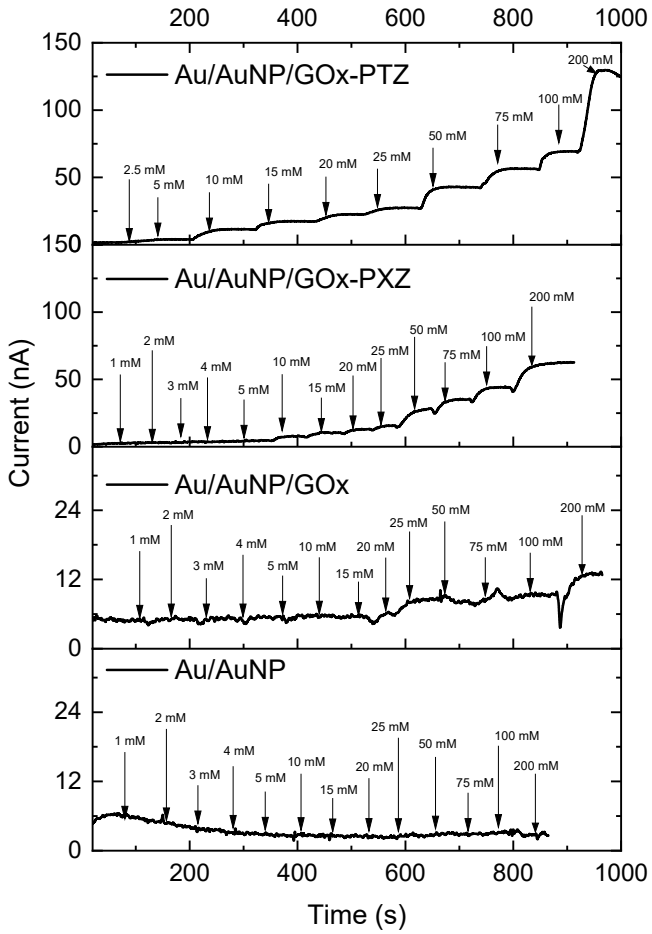


Figure S7. The titration of the Au/AuNP/enzyme and control Au/AuNP electrodes

Prior to the chronoamperometry experiments, the Au/AuNP/GOx-PTZ electrodes in 10 mL of the 50 mM PBB were polarized for 10 s at 900 mV, then at 250 mV for 5 s, and further titrations were carried out at 250 mV vs Ag/AgCl. To determine the sensitivity of the electrode to GLU, the measurements were performed with increasing concentration (0.1 mM – 200 mM GLU) at the potential of 250 mV vs Ag/AgCl. Analogous measurements were also performed in an oxygen-free environment. i.e., argon gas was passed through the solution for 5 min. Figure S7 shows the titration of the electrodes with monolayers of the bioconjugates, native GOx, Au/AuNP as a control.

For characterization of the interferences, the response of other samples was studied chronoamperometrically. Other analytes such as D-galactose, D-mannose, D-ribose, D-fructose, D-xylose, L-lactic acid, urea, uric acid at a concentration of 5 mM and L-ascorbic acid at 1 mM were added in the cell at same condition with 5 mM GLU to check the selectivity of the developed biosensor. The limit of detection (LoD) for the corresponding electrode was calculated using the Equation (S2):

$$LoD = \frac{3.0\,\sigma}{a} \qquad \text{(S2)}$$

where a is a slope and σ is a standard deviation of the linear curve and a signal to a noise (S/N) ratio of 3.0 was used. These parameters were calculated using a Data analysis tool from MS 2007 Excel.

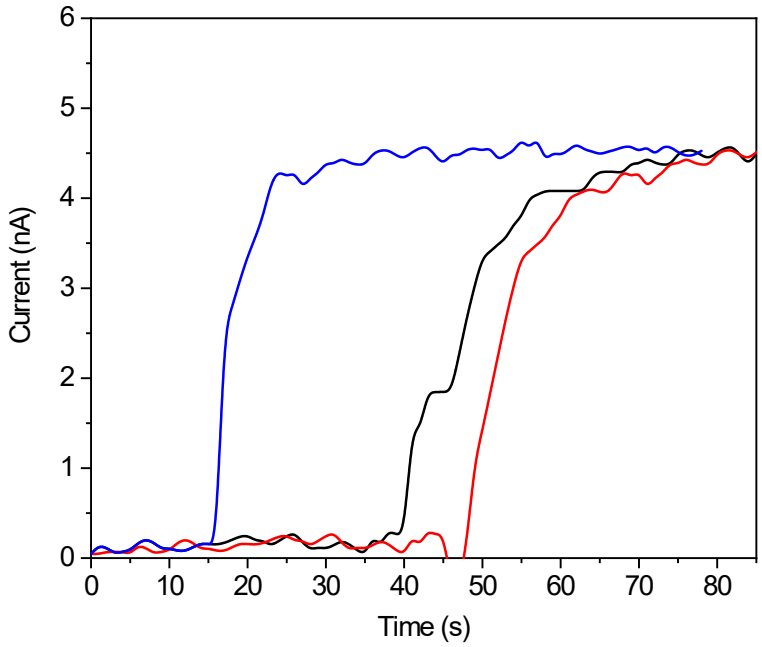


Figure S8. Reproducibility of the Au/AuNP/GOx-PTZ bioelectrode response after addition of 5 mM GLU at 250 mV versus Ag/AgCl.

Mass spectrometry measurements

Sample preparation for LC-MS/MS

Samples in a volume of 1 µL were subjected to 2 h proteolysis at 40 °C in 20 µL of 50 mM AB by 50 ng of trypsin. The digests were vacuum-dried and re-constituted in 1% FA and 0.001% PEG.

LC MS/MS setup

The LC-MS/MS analyses of the digests were done using the UltiMate 3000 RSLCnano system (Thermo Fisher Scientific) connected to the timsTOF Pro mass spectrometer (Bruker). Prior to LC separation, tryptic digests were online concentrated and desalted using a trapping column (Acclaim™ PepMap™ 100 C18, dimensions 300 µm ID, 5 mm long, 5 µm particles, Thermo Fisher Scientific). After washing the trapping column with 0.1% TFA, the peptides were eluted (flow rate – 150 nL min^{-1}) from the trapping column onto an analytical column (Aurora C18, 75

μm ID, 250 mm long, 1.7 μm particles, heated to 50 °C, PN AUR3-25075C18-CSI, Ion Opticks) by 30 min linear gradient program (4-42% of mobile phase B; mobile phase A: 0.1% FA in water; mobile phase B: 0.1% FA in 80% ACN). Equilibration of the trapping column and the analytical column was done prior to sample injection into the sample loop. The analytical column was placed inside Column Toaster heater (Bruker) and its emitter side was installed inside the CaptiveSpray ion source (Bruker) according to the manufacturer's instructions with the column temperature set to 50 ºC. Spray voltage of 1.5 kV was used. MS^n data were acquired in *m/z* range of 100-1700 and $1/k_0$ range of 0.6-1.4 V s cm^{-2} using the data-dependent acquisition parallel accumulation serial fragmentation (DDA-PASEF) method acquiring 10 PASEF scans with scheduled target intensity of 20,000 and intensity threshold of 2,500. Active exclusion was set for 0.4 min with precursor reconsideration for 4-fold more intense precursors.

LC-MS/MS data processing

Mascot MS/MS ion searches (Matrixscience, London, UK; version 2.5.1) were done against in-house database containing expected protein sequence of GOx and cRAP contaminant database (downloaded from http://www.thegpm.org/crap/) to exclude contaminant spectra. Mass tolerance for peptides and MS/MS fragments was 10 ppm and 0.1 Da, respectively, with the option of one ^{13}C atom to be present in the parent ion. To account for anticipated products and common artefacts arising from sample processing, the following modifications were specified during database searching: tyrosine modification by PXZ and oxidized forms of PTZ(SO), methionine oxidation, and the deamidation of asparagine and glutamine residues. All searches employed semi-tryptic specificity, allowing for up to one missed cleavage. Mass spectra corresponding to conjugated enzymes underwent manual validation. A tryptic digest of unmodified GOx served as a negative control.

Details of the theoretical simulation

Details of DFT computations

The X-ray structure analysis of GOx from *Aspergillus niger* (a code in Protein Data Bank is 3qvp) was used. The Trp residues with the shortest distances from the FAD cofactor and the bioconjugated Tyr residues were selected, and the structure with only these residues was

generated, and hydrogen atoms were added using UCSF Chimera (version 1.16). FAD was replaced with $FADH_2$ as the reduced forms of the cofactor. In the generated structure, phenothiazine (PTZ), and its PTZSO, PTZSO2 oxidized forms, and phenooxazine (PXZ) groups were connected to the Tyr residue. These functionalized residues are visualized in Figure S9. For the simulations, aliphatic fragments of the amino acid residues were replaced with methyl groups. These structures were optimized using the B3LYP functional and the 6-31G(d, p) basis set, and the conductor-like polarisable continuum model (C-PCM) of the apolar solvent (ε_{st} = 3.2) media, which describes the dielectric continuum of the enzyme. At these structures, the dispersion corrected hybrid density B3LYP-D3 functional and the 6-31G(d,p) basis set were used to compute the site energies. The density function theory (DFT) computation was carried out employing the Sapratn'24 software (Wavefunction, Inc., Irvine, USA).

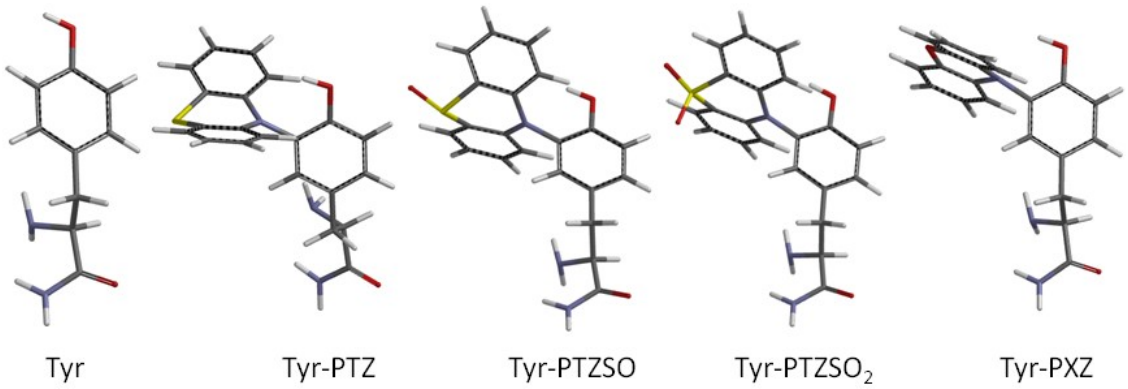


Figure S9. Optimized structures of the functionalized Tyr with the PTZ, PTZSO, PTZSO2, and PXZ groups.

Details of calculations of the transfer constants

To calculate charge carrier (hole) transfer constants between the immobilized GOx-PTZ, GOx-PTZSO, and GOx-PXZ bioconjugates and the gold surface, our modified Marcus–Bagdziunas eqn (S3) was used:

$$k_i = \frac{2\pi}{\hbar}|H_i|^2 \frac{1}{\sqrt{4\pi\lambda_{tot}k_bT}} \exp\left(-\frac{(\lambda_{tot}+\Delta G^0)^2}{4\lambda_{tot}\,k_bT} - \frac{U}{\varepsilon_{st}}\right) \quad \text{(S3)}$$

where k_i is charge transfer rate constant for pathway i, $|H_i|$ is a module of electronic coupling between the initial and final states, λ_{tot} corresponds the total reorganization energy, k_b is the Boltzmann's constant and T is a absolute temperature (298 K), e is the elementary charge (1.60×10^{-19} C). The calculations using this Eqn (S3) can give more accurate results at the electrical field. Therefore, U is an applied potential versus the standard hydrogen reference electrode, which is a measure of the applied electric field (for these calculations, values of U were re-calculated to the Ag/AgCl electrode as $U = U_{Ag/AgCl} + 0.20$ V), ε_{st} is a static dielectric constant (permittivity) of the peptide environment and d_i is the distance for corresponding pathway i between the neighbouring aromatic amino acid fragments (i.e., centre of their frontiers orbitals) in the dimers or the gold surface, respectively. The ε_{st} value of the peptide environment was set to 3.2 for the calculations based on the literature.[3]

The standard free-energy change ($\Delta G°$) of the initial and final states for the hole transfer reaction was calculated. In this case, this energy is equal to the difference of the vertical ionization potentials of the hole acceptor and donor (i.e., $IP_A - IP_D$) and the effect of applied potential, U:

$$\Delta G_i^0 = (IP_A - IP_D) - \frac{U}{\varepsilon_{st}} \qquad \text{(S4)}$$

The values of the internal reorganization energies of these residues were calculated using a model of four points from adiabatic potential energy surface with the B3LYP/6-31G(d,p) level of theory and using C-PCM of the apolar solvent ($\varepsilon_{st} = 3.2$)[3] media using Eqn S5:[4]

$$\lambda_{int} = [E^+(g^0) - E^+(g^+)] + [E^0(g^+) - E^0(g^0)] \qquad \text{(S5)}$$

where E corresponds to the energy of neutral residue (g^o) in the geometry of cation radical (g^+) and vice versa. The external reorganization energies, λ_{out}, for all the pathways of carrier transfer in the enzymatic media were estimated as a charge carrier transfer work between the neighbouring residues:[4]

$$\lambda_{out} = \frac{\Delta z\,(1 - \Delta z)\,e^2}{4\pi\varepsilon_{st}\varepsilon_0 d_i} \qquad \text{(S6)}$$

where Δz is a part of transferred charge, ε_0 is the electric permittivity of vacuum and the distance d_i for pathway i, respectively. In this work, value of Δz was estimated to be 0.5 from the differences of natural charge of the charged dimers of the residues. Due to the low electric field, the half of the charge carriers is transferred. The λ-values for a cross-reaction between the

different fragments and additional external λ_{out} of corresponding pathway i can be estimated according to the self-exchange reorganization energies for each m and n fragment:[4]

$$\lambda_{tot} = \frac{1}{2}(\lambda_{mm} + \lambda_{nn}) + \lambda_{out} \quad \text{(S7)}$$

To determine the electronic coupling ($|H_i|$), we used a simpler model for the estimation of $|H_i|$ based on the computation of frontier molecular orbitals of all the residues in one input using DFT and the charge carrier tunnelling theory because the coupling integrals at large distance between the sites are often overestimated using DFT.[5] Therefore, the values of $|H_i|$ integrals with the tunnelling effects for the pathways between the fragments m and n were approximated using the fragment (residue) charge difference scheme (Eqn. S8):[6]

$$H_i = \frac{(E_m - E_n)|\Delta z_{mn}|}{\sqrt{(\Delta z_m - \Delta z_m)^2 + 4\Delta z_{mn}{}^2}} \exp\left(-\frac{1}{2}\beta d_i\right) \quad \text{(S8)}$$

where E_m and E_n are the energies of corresponding frontier orbitals (i.e., HOMO and/or HOMO-1) of m and n residues, which can be derived from a one-electron theory. All these values were computed by using the B3LYP-D3 functional and the 6-31G(d,p) basis set and C-PCM of the apolar solvent (ε_{st} = 3.2) media. β is a distance-decay constant. The values of β were used to be 1.1 $Å^{-1}$ and 1.6 $Å^{-1}$ for the tunnelling through peptide matrix and though water media (i.e., though water hydration shell of the enzyme), respectively.[7] Moreover, the site energy of the gold nanoparticles and the energy of reorganization were used to be 5.30 eV versus vacuum and ~0 eV, respectively.[8] Table S1 summarises all the calculated rate constants.

Table S1. Estimated hole, h^+, transfer lengths, coupling integrals, total reorganization energies calculated for various pathways of hole injection and hopping from the gold surface and residues of the bioconjugated GOx.

Entry	Pathway (dimer)	d_i (Å)	ΔG^0 (eV)	$\lvert H_i \rvert$ for h^+ (eV)	λ_{tot} for h^+ (eV)	k_i (s^{-1})
1	Tyr182PTZSO → $FADH_2$	21.3	–1.39	4.0×10^{-6}	0.51	0.12
2	Tyr54PTZSO → $FADH_2$	21.4	–1.39	3.8×10^{-6}	0.51	0.11
3	Tyr237PTZSO → FADH2	29.0	–1.39	5.8×10^{-8}	0.49	1.1×10^{-5}
4	Tyr139TZSO → $FADH_2$	22.4	–1.39	2.2×10^{-6}	0.51	3.1×10^{-2}
5	Tyr539TZSO → $FADH_2$	27.8	-1.39	1.1×10^{-7}	0.50	4.4×10^{-4}

6	Tyr435TZSO → $FADH_2$	23.9	-1.39	9.6×10^{-7}	0.50	4.9×10^{-3}
7	Tyr300TZSO → $FADH_2$	26.0	-1.39	3.0×10^{-7}	0.50	3.8×10^{-4}
8	Tyr280TZSO → $FADH_2$	33.5	-1.39	4.9×10^{-9}	0.49	5.2×10^{-8}
9	Tyr22TZSO → $FADH_2$	26.6	-1.39	2.2×10^{-7}	0.50	1.9×10^{-4}
10	Tyr579TZSO → $FADH_2$	33.3	-1.39	5.4×10^{-9}	0.49	6.6×10^{-8}
11	Trip426 → $FADH_2$	10.3	-1.12	1.4×10^{-3}	0.66	1.7×10^{9}
12	Trip111 → $FADH_2$	12.1	-1.12	5.2×10^{-4}	0.64	1.8×10^{8}
13	Trip232 → $FADH_2$	15.3	-1.12	8.9×10^{-5}	0.62	3.5×10^{6}
14	Trip111 → Trip133	11.0	-1.41	4.7×10^{-6}	0.59	1.7×10^{4}
15	Trip122 → Trip131	7.1	-1.41	4.0×10^{-5}	0.65	7.1×10^{5}
16	Trip232 → Trip111	10.4	-1.41	1.0×10^{-9}	0.60	3.1×10^{4}
17	Tyr539PTZSO → Trip131	7.8	-0.41	1.2×10^{-3}	0.54	2.5×10^{10}
18	Tyr539PTZ → Trip131	7.8	-0.011	1.9×10^{-4}	0.68	6.1×10^{6}
19	Tyr539PXZ → Trip131	7.8	0.16	1.8×10^{-4}	0.60	$4{,}6\times10^{8}$
20	Tyr539PTZSO2 → Trip131	7.8	0.13	3.3×10^{-3}	0.49	1.3×10^{8}
21	Tyr539 → Trip131	8.5	0.46	2.4×10^{-3}	0.64	1.3×10^{3}
22	Au → Tyr139PTZSO	16	0.12	2.9×10^{-7}	0.16	30
23	Au → Tyr139PTZ	16	-0.28	8.3×10^{-9}	0.31	2.0
24	Au → Tyr139PXZ	16	-0.45	9.7×10^{-9}	0.25	0.37
25	Au → Tyr139PTZSO2	16	-0.40	7.2×10^{-7}	0.12	42
26	Au → Tyr	17	0.45	3.4×10^{-7}	0.27	3.2×10^{-5}
27	Au → Trp	19	-0.15	4.1×10^{-9}	0.26	0.37
28	Au → $FADH_2$	37	1.13	5.4×10^{-14}	0.31	6.8×10^{-20}

Based on the Faraday's laws of electrolysis, the experimental first order rate constant of the hole transfer, k_{ht}, was calculated using Eq. (S9):

$$k_{ht} = \frac{I}{nF\Gamma A} \qquad \text{(S9)}$$

where I is bioelectrochemical current of the electrode, A is an area of the electrode, n is the number of holes (electrons) involved in the bioelectrocatalytic process (in our case n = 1, we consider that the hole (electron) was transported through the enzyme one after the other, not two

electrons synchronously), F is the Faraday constant, and Γ is the effective surface coverage on the electrode surface (~10^{-11} mol cm^{-2})[9].

To estimate charge carrier (hole) mobility, the equation (S10) combining the Pauli master and Einstein equations was used:[10]

$$\mu = \frac{d^2 k_i e}{2N\, k_b T} \qquad \text{(S10)}$$

where N is the dimensionality (N = 1, because the charge carriers migrate in one direction of the electric field) and d_i is the distance between the neighboring moieties of rate limiting step in dimer i, e is an electron charge.

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