chemosensors

Supplementary Material

Novel Approach for the Immobilization of Cellobiose Dehydrogenase in PEDOT:PSS Conductive Layer on Planar Gold Electrodes

Esra Cihan [1,2], Eva Melnik [1,*], Steffen Kurzhals [1], Paulina Plata [1,2], Giorgio C. Mutinati [1,*], Rainer Hainberger [1], Alfons K.G. Felice [3], Christopher Schulz [3] and Peter Lieberzeit [2]

[1] Competence Unit Molecular Diagnostics, Center for Health & Bioresources, AIT Austrian Institute of Technology GmbH, 1210 Vienna, Austria
[2] Department of Physical Chemistry, University of Vienna, 1010 Vienna, Austria; peter.lieberzeit@univie.ac.at
[3] DirectSens GmbH, 3400 Klosterneuburg, Austria
* Correspondence: eva.melnik@ait.ac.at (E.M.); giorgio.mutinati@ait.ac.at (G.C.M.)

1. Sensors

In this study, two different sensor designs were used. Photographs of the NaCoS sensor and vapor deposited gold sensor are given in Figure S1. In the NaCoS sensor, the four working electrodes and the counter electrode are made of screen-printed graphite and the reference electrode is made of screen-printed Ag/AgCl. Each of the graphite working electrodes has an area of 0.74 mm^2. In the vapor deposited gold sensor, the working electrode and the counter electrode are made of gold and the reference electrode is made of Ag/AgCl. The electrode dimensions were defined by polyimide acrylic tape as passivating layer with the aim to obtain electrode areas of 1.6 mm^2.

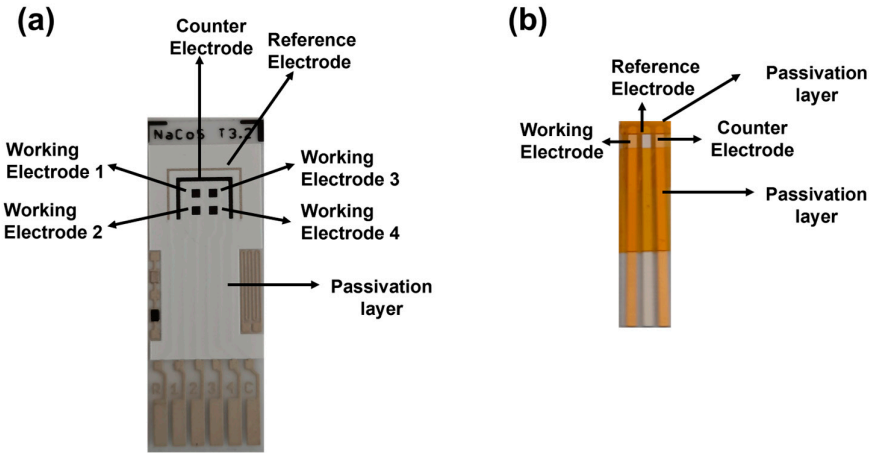

Figure S1. Sensor design of **(a)** NaCoS sensors, **(b)** vapor deposited gold sensor.

2. Measurement Setup

The electrochemical measurements were performed with the Multi EmStat3 potentiostat (Palmsens, Netherlands) using the software Multitrace 3.6.

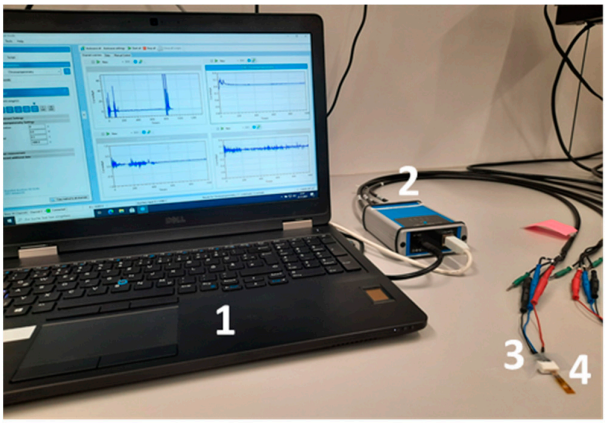

Figure S2. Photograph of the measurement setup: 1) Computer 2) Potentiostat 3) Connector 4) vapor deposited gold sensor.

3. Interference Study

Chronoamperometry measurements were performed using CDH-PEDOT:PSS-PEG-DMA (10kDa) modified NaCoS sensors (see sensor layout in Figure S1) to investigate the interference signals caused by single substances other than glucose (acetaminophen, ascorbic acid, dopamine, maltose and galactose), which can also be present in the body fluid.

The CDH-PEDOT:PSS ink and the PEG-DMA (10 kDa) hydrogel solution were prepared and processed as described in the section "*2.3 Electrode modification*" of the main article. The only difference was that 1.5 µl of CDH-PEDOT:PSS ink and 15 µl of PEG-DMA hydrogel solution were used to modify the NaCoS sensors because the electrode areas of the NaCoS sensors and the physical vapor deposited gold electrodes are different. The solutions with the interference substances were prepared by dissolving the inference compounds in 5 mM glucose solution to keep the glucose concentration constant after the first glucose addition.

Chronoamperometry measurements were performed at 0 V by adding, in 500 s intervals, glucose (5 mM), acetaminophen (20 mg/dL), ascorbic acid (3 mg/dL), dopamine (13 mg/dL), maltose (2650 mg/dL) and galactose (81 mg/dL). The resulting chronoamperometry curves are shown in Figure S3a).

In the section "*3.3 Selectivity test*" of the main article, a mixture of compounds (40 mg/dL acetaminophen, 7.6 mg/dL ascorbic acid, 2 mg/dL dopamine, and 54 mg/dL uric acid) was applied to the vapor deposited gold sensors. In Figure S3b), chronoamperometry measurements performed at 0 V are shown in the case of 5 mM glucose addition and in the case of the interference mixture addition.

Current densities (nA/mm^2) for the glucose response were calculated by dividing the electrical current difference between the average over 100 s of the current 500 s after glucose addition and the average over 100 s of the current 100 s before glucose addition by the respective electrode area.

Current densities observed by the addition of the interference substances were calculated by dividing the current differences between the average over 100 s of the current 500 s after interference solution addition and the average over 100 s of the current 100 s before interference solution addition by the respective electrode area.

The glucose responsive current density for graphite electrodes was calculated to be 84±9 nA/mm^2 (averaging 4 WE) and thus similar to the value observed for gold (97±32 nA/mm^2). The current densities measured for the addition of interference substances were found to be neglectable with respect to the observed glucose detection current density for both NaCoS and physical vapor deposited gold sensors.

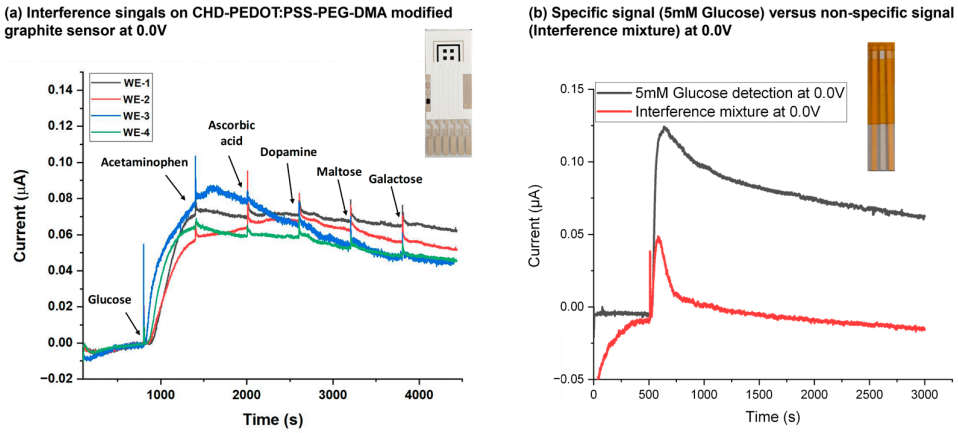

Figure S3. (a) Interference signals on CDH-PEDOT:PSS-PEG-DMA modified carbon sensor at 0.0 V by exchanging liquids containing glucose (5 mM) with either acetaminophen (20 mg/dL), ascorbic acid (3 mg/dL), dopamine (13 mg/dL), maltose (2650 mg/dL) or galactose (81 mg/dL). **(b)** Specific signal (5 mM Glucose) on gold sensors versus non-specific signal (Interference mixture: 40 mg/dL acetaminophen, 7.6 mg/dL ascorbic acid, 2 mg/dL dopamine, and 54 mg/dL uric acid) at 0.0 V chonoamperometry.

4. Adhesion and structural deformation of hydrogel

The adhesion and structural deformation of dried hydrogels having PEG-DMA crosslinkers with different molar masses (1 kDa and 10 kDa) was investigated by drop-casting 50 µl of the PEG-DMA based hydrogel on bare gold surfaces and on 3-mercaptopropionic acid (MPA) modified gold surfaces. The MPA modification was performed to obtain self-assembled monolayers on the gold surface to improve the adhesion. After crosslinking and washing, surfaces were dried at 25 °C for 1 day and the samples were inspected by light microscopy. As can be seen in Figure S4b) and S4d), 10 kDa PEG-DMA based hydrogels were found to be still adhesive to the gold surface after 1 day of drying. In contrast, the 1 kDa PEG-DMA based hydrogels were partially or fully lifted from both the bare and the MPA modified gold surfaces (Figure S4a) and S4c)).

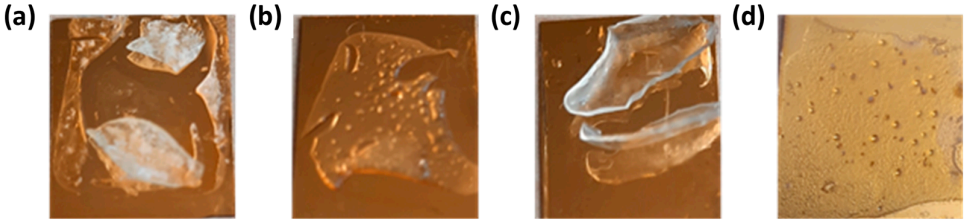

Figure S4. Light microscopic images of drop-casted hydrogels having PEG-DMA with different molar masses after drying at 25 °C for 1 day: **(a)** hydrogel with 1 kDa PEG-DMA on bare gold surface, **(b)** hydrogel with 10 kDa PEG-DMA on bare gold surface, and **(c)** hydrogel with 1 kDa PEG-DMA on MPA-modified gold surfaces, **(d)** hydrogel with 10 kDa PEG-DMA on MPA-modified gold surfaces.

5. Measurement Setup for Long Time Measurement

To investigate the operational stability of the vapor deposited gold sensors modified using CDH-PEDOT:PSS-PEG-DMA with different molar masses (1 and 10 kDa), long term (10h) chronoamperometry measurements were performed (see section "*3.6 Glucose detection in the presence of PEG-DMA crosslinkers with different molar masses*" of the main article). Due to the long measurement times and to avoid evaporation of the measurement solution, the sensors were placed in buffer-filled beakers, which were temperature controlled by a Lauda thermostat. The measurement set-up is shown in Figure S5. The measurement solution was placed in the beakers (3) and the sensors connected to connectors (2) were placed in the measurement solution. To prevent sensor moving

during the measurement, sensors are kept stable with the stand clamps (1). The setup also includes a temperature controller (4) keeping the buffer solutions at 25 °C.

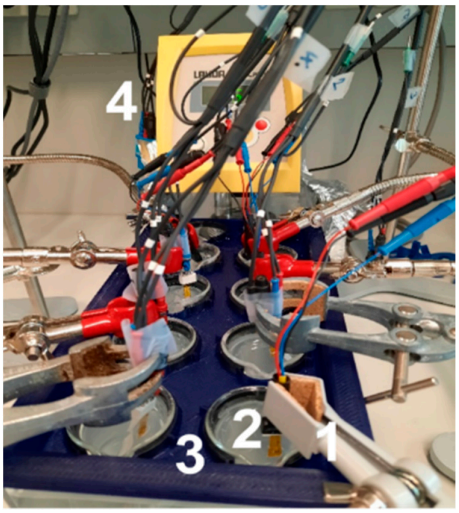

Figure S5. Image of the high volume set up used for the investigation of operational stability of the CDH-PEDOT:PSS-PEG-DMA modified (1 and 10 kDa) sensors.

6. Literature Comparison

Table S1 compares the results of our gold sensor as reported in the main article with the reported results found in the literature. The reference numbers are the same as in the reference list of the main article.

Table S1. Comparison of DET enzymes-based sensors for glucose detection.

Electrode	Modification	Enzyme	Buffer condition	Potential vs. Ag/AgCl	Glucose measurement range in mM	Glucose detection limit [mM]	Linear range (mM)	Ref (as in main article)
C	MWCNT + chitosan +CDH	FAD-GDH	PBS	-412 mV	0-5.5 mM	0.015	0-1	[12]
C	EGDGE/CDH	CDH	PBS	100 mV	0-100 mM	NA	0-5	[18]
Glassy C	AuNP/CDH	CDH	TRIS, 50mM	250 mV	0-200 mM	0.006	0.02-30	[36]
C	MWCNT/ Pyrene-NHS/GDH	FADGDH	Potassium phosphate, 100mM	400 mV	0-50 mM	NA	0.1-5	[37]
C	MWCNT/ Pyrene-NHS/GDH	Glucose dehydrogenase Pcyb-AfGDH	Potassium phosphate, 100mM	100 mV and 400 mV	0-50 mM	NA	0.1-5	[38]

Au	CDH-PEDOT:PSS-PEG-DMA(10kDa)	CDH	physiol. PBS	0 mV	0-80 mM	0.1	0.1-20	This work

References

12. Jeon, W.-Y.; Kim, H.-S.; Jang, H.-W.; Lee, Y.-S.; Shin, U.S.; Kim, H.-H.; Choi, Y.-B. A stable glucose sensor with direct electron transfer, based on glucose dehydrogenase and chitosan hydro bonded multi-walled carbon nanotubes. *Biochem. Eng. J.* **2022**, 187, 108595, doi:10.1016/j.bej.2022.108589.
18. Jayakumar, K.; Reichhart, T.M.B.; Schulz, C.; Ludwig, R.; Felice, A.K.G.; Leech, D. An Oxygen Insensitive Amperometric Glucose Biosensor Based on An Engineered Cellobiose Dehydrogenase: Direct versus Mediated Electron Transfer Responses. *ChemElectroChem* **2022**, 9, doi:10.1002/celc.202200418.
36. Bollella, P.; Gorton, L.; Ludwig, R.; Antiochia, R. A Third Generation Glucose Biosensor Based on Cellobiose Dehydrogenase Immobilized on a Glassy Carbon Electrode Decorated with Electrodeposited Gold Nanoparticles: Characterization and Application in Human Saliva. *Sensors* **2017**, *17(8)*, 1912–1926, doi:10.3390/s17081912.
37. Ito, K.; Okuda-Shimazaki, J.; Mori, K.; Kojima, K.; Tsugawa, W.; Ikebukuro, K.; Lin, C.-E.; La Belle, J.; Yoshida, H.; Sode, K. Designer fungus FAD glucose dehydrogenase capable of direct electron transfer. *Biosens. Bioelectron.* **2019**, *123*, 114–123, doi:10.1016/j.bios.2018.07.027.
38. Ito, K.; Okuda-Shimazaki, J.; Kojima, K.; Mori, K.; Tsugawa, W.; Asano, R.; Ikebukuro, K.; Sode, K. Strategic design and improvement of the internal electron transfer of heme b domain-fused glucose dehydrogenase for use in direct electron transfer-type glucose sensors. *Biosens. Bioelectron.* **2021**, *176*, 112911-112917, doi:10.1016/j.bios.2020.112911.