Short Communication
doi.org/10.1002/elsa.202100131

Chemistry Europe
European Chemical Societies Publishing

Received: 29 June 2021
Accepted: 15 July 2021

Photoelectrochemical enzymatic penicillin biosensor: A proof-of-concept experiment

Rene Welden[1,2] | **Cindy A. Nagamine Komesu**[1] | **Patrick H. Wagner**[2] | **Michael J. Schöning**[1,3] | **Torsten Wagner**[1,3]

[1] Institute of Nano- and Biotechnologies, Aachen University of Applied Sciences, Jülich, Germany

[2] Laboratory for Soft Matter and Biophysics, Katholieke Universiteit Leuven, Leuven, Belgium

[3] Institute of Biological Information Processing (IBI-3), Forschungszentrum Jülich, Jülich, Germany

Correspondence
Michael J. Schöning, Institute of Nano- and Biotechnologies, Aachen University of Applied Sciences, Heinrich-Mußmann-Str. 1, Jülich, 52428, Germany.
Email: schoening@fh-aachen.de

Funding information
German Federal Ministry of Education and Research, Grant/Award Number: 13N12585; Bundesministerium für Bildung und Forschung, Grant/Award Number: 13N12585

Abstract

Photoelectrochemical (PEC) biosensors are a rather novel type of biosensors that utilize light to provide information about the composition of an analyte, enabling light-controlled multi-analyte measurements. For enzymatic PEC biosensors, amperometric detection principles are already known in the literature. In contrast, there is only a little information on H^+-ion sensitive PEC biosensors. In this work, we demonstrate the detection of H^+ ions emerged by H^+-generating enzymes, exemplarily demonstrated with penicillinase as a model enzyme on a titanium dioxide photoanode. First, we describe the pH sensitivity of the sensor and study possible photoelectrocatalytic reactions with penicillin. Second, we show the enzymatic PEC detection of penicillin.

KEYWORDS
enzymatic biosensor, penicillin, penicillinase, photoelectrochemistry, titanium dioxide photoanode

1 | INTRODUCTION

Light enhances the performance of many materials, as the absorbed energy in form of photons can change fundamental material properties, especially, semiconductors can be applied for solar cells, water splitting, or photocatalysis.[1–3] In photoelectrochemical (PEC) cells, photon absorption leads to the generation of electron-hole pairs inside the semiconductor. When the semiconductor is in direct contact with an analyte, the subsequent photo-induced current can trigger photoelectrocatalytic reactions at the semiconductor-analyte interface.

The same principle holds for the functioning of PEC biosensors, where the sensor operation functionality is only present under illumination. The literature describes a wide range of PEC biosensors, including examples of DNA detection, autosensing, and the detection of metal ions.[4–6] PEC enzymatic biosensors are mostly based on the amperometric principle, leading to reduction/oxidation reactions or direct electron transfer at the working electrode.[7] This principle is used, for example, for the detection of glucose and lactate.[8,9] On the other hand, if ions (protons or hydroxides) serve as a product of the enzymatic catalysis, potentiometric detection principles such as

Short Communication
doi.org/10.1002/elsa.202100131
Chemistry Europe
European Chemical Societies Publishing

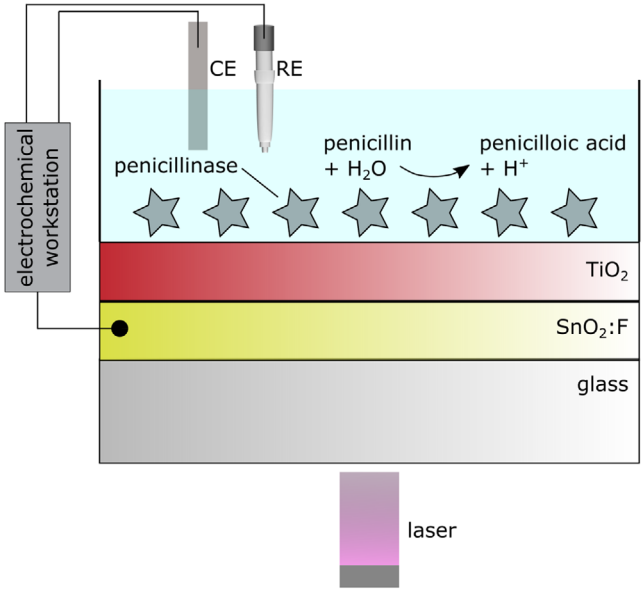

FIGURE 1 Schematic representation of the glass/SnO_2:F/TiO_2 heterostructure connected to an electrochemical workstation utilizing a three-electrode setup. Penicillinase immobilized on the photoelectrochemical (PEC) surface catalyzes the conversion of dissolved penicillin to penicilloic acid and H^+ ions

electrolyte-insulator-semiconductor sensors can be applied.[10,11] Here, in contrast to amperometry, a pH-sensitive transducer layer can detect surface-charge changes as well as variations in the H^+-ion concentration.

Some of the recently discussed materials used in PEC cells are also sensitive to pH changes and influence the resulting photocurrent.[12,13] At the same time, enzymatic PEC biosensors did not utilize this functionality to detect pH changes so far. The extension to this class of H^+-ion generating enzymes would enable the detection of a variety of other analytes that become hydrolyzed by these enzymes. Moreover, the possibility to address different regions of such PEC-sensor surface with defined light spots offers multi-analyte detection with various enzyme classes on a single chip.[9]

To prove this feasibility for detection of enzymatically produced H^+ ions with an amperometric detection principle, penicillinase as a model enzyme was immobilized on the PEC biosensor surface, as it converts penicillin to penicilloic acid, whereby H^+ ions are generated (Figure 1). Although penicillin only acts as a model enzyme in this experiment, the on-field detection of penicillin becomes more and more important, as multidrug-resistant organisms emerge from high antibiotic usage in food and feed productions, especially for livestock treatment.[14]

We used a glass/SnO_2:F/TiO_2 heterostructure (photoanode) with SnO_2:F as a transparent-conductive layer and TiO_2 as the photoconductive material. This configuration allows rear-side illumination, avoiding light-induced influences on the enzyme and the analyte. The sensor (working electrode, WE) is operated in a three-electrode arrangement with the counter electrode (CE) and reference electrode (RE).

2 | MATERIALS AND METHODS

A thin TiO_2 layer (~150–200 nm) was deposited on the glass/SnO_2:F substrate using the pulsed laser deposition technique. Details of fabrication parameters are described in the study by Welden et al.[15]

The enzyme penicillinase from *Bacillus cereus* (Sigma-Aldrich) was immobilized by crosslinking with glutaraldehyde. For this, we have mixed 15 units of penicillinase with glutaraldehyde and bovine serum albumin (2 vol%/2 vol%), drop-coated on the TiO_2 surface, and dried for several hours at room temperature.

For transient photocurrent measurements, the electrode was connected to an electrochemical workstation (ZENNIUM pro; Zahner-Elektrik GmbH & Co. KG, Kronach, Germany) in a three-electrode setup with an Ag/AgCl reference electrode (3 M KCl; Metrohm GmbH & Co. KG, Filderstadt, Germany) and a Pt-counter electrode. The rear side was illuminated with a 405 nm laser diode (CP1TM9/M; Thorlabs, Germany) with a controlled laser power of 2.0 mW. The laser diode had a beam diameter of 200 μm ($1/e^2$).

At the beginning of the transient photocurrent measurements, the dark current was equilibrated for 2 min without illumination. After that, the laser diode was switched on and off for 60 s, respectively. The switching was repeated three times. The net photocurrent (I_{photo}) was calculated by subtracting the dark current from the current with illumination (Eq. (1))

$$I_{photo} = I_{measured} - I_{dark} \quad (1)$$

The stated I_{photo} values were calculated from the last 10 s of each respective illumination phase.

3 | RESULTS AND DISCUSSION

In order to determine changes in the H^+-ion concentration resulting from the enzymatic conversion of penicillin, in the first experiment, the pH sensitivity of the glass/SnO_2:F/TiO_2 transducer structure was studied. Several reports for photoanodes discuss a pH dependence of the photocurrent, which decreases when lowering the pH.[16,17] To assess whether this holds also for the utilized SnO_2:F/TiO_2 heterostructure, we evaluated the transient photocurrent response for 0.33 mM phosphate buffered

Short Communication
doi.org/10.1002/elsa.202100131
Chemistry Europe
European Chemical Societies Publishing

saline (PBS) solution with the pH adjusted in the range from pH 5.0 to 8.0 (titration NaOH, HCl).

Exemplarily, Figure 2a depicts the pH-dependent photocurrent between the photoanode and the counter electrode for an applied bias potential of –100 mV with respect to the reference electrode. After the illumination starts, the photocurrent equilibrated at 441 ± 46 nA for pH 8.0 and decreased to 51 ± 6 nA for pH 5.0. In Figure 2c, the applied potential was set to 300 mV, to evaluate the effect of the bias potential towards the pH sensitivity. Here, during illumination, the photocurrent increased to 1780 ± 146 nA at pH 8.0 and 1550 ± 196 nA at pH 5.0. The general photocurrent increase with higher applied potentials can be explained by an increased band bending inside the semiconductor when applying more positive potentials. Nevertheless, comparison between both applied potentials reveals an average pH sensitivity ($n = 3$ sensors) of 133 ± 12 nA/pH for –100 mV (Figure 2b) and 79 ± 29 nA/pH for 300 mV (Figure 2d) in the range from pH 8.0 to 5.0. Only very slight hysteresis effects occur when measuring upwards from pH 5.0 to 8.0.

Here, the pH sensitivity only slightly changed to 132 ± 5 and 82 ± 39 nA/pH for –100 and 300 mV, respectively, which demonstrates the functionality of this sensor type.

In contrast, for applied potentials lower than –100 mV, due to the proximity to the flat-band potential (~–400 mV), the resulting photocurrent was not stable enough to perform a pH-dependent calibration curve.

Interestingly, for higher applied potentials (300 to 800 mV), the total photocurrent amplitude further increased, however, a difference for varying pH of the solutions was not visible (data not shown). The exact reason for this behavior is currently being studied. A possible explanation for the potential-dependent pH sensitivity can be as follows: for potentials close to the flat-band potential, the energy bands are almost flat. An additional potential, e.g., due to surface protonation/deprotonation, has a greater effect on the band bending than at higher applied potentials, where a larger band bending occurs.

For the detection of penicillin, using the PEC biosensor with penicillinase, it is important to understand the role of photocatalytic effects between the molecules under test and the electrode. When using metal-oxide semiconductors, it is possible to directly photoelectrocatalytically oxidize or reduce the target substrate. Exemplarily, the photocatalytic oxidation of ß-lactam antibiotics, to which penicillin belongs, can be found in the literature.[18,19] To exclude undesired photocurrent changes due to penicillin oxidation without immobilized penicillinase on the transducer structure, transient photocurrent response curves were performed for 0, 0.5, and 1 mM penicillin G in solution as a reference experiment. Figure 3 shows the recorded photocurrents for both applied potentials (–100 and 300 mV). The pH for each penicillin concentration was set to match

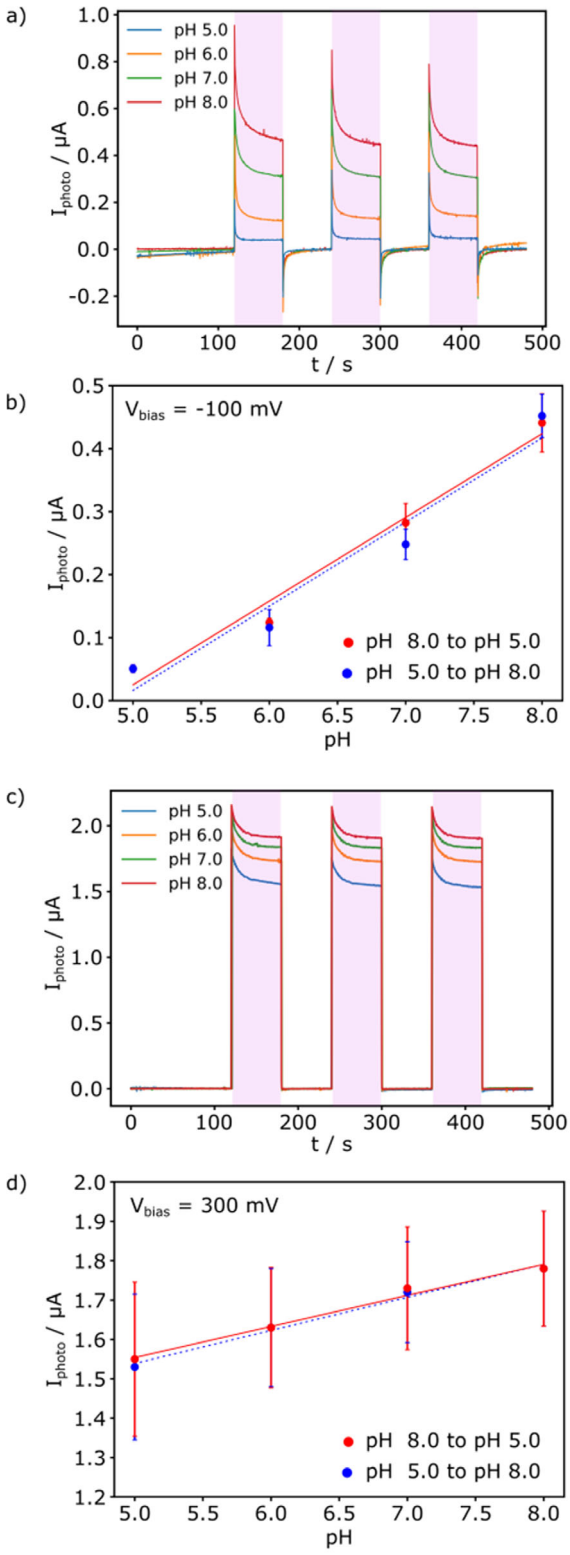

FIGURE 2 Transient photocurrent curves in 0.33 mM PBS buffer, pH 5.0–8.0, for an applied bias potential of (a) –100 mV and (c) 300 mV. Photocurrent-pH calibration curve for applied bias potentials of (b) 100 mV and (d) 300 mV, respectively. The blue curves (dotted) show the pH-dependent photocurrent from pH 5.0–8.0 and the red (solid) curves correspond to the pH-dependent photocurrent for pH 8.0–5.0 ($n = 3$ sensors)

Short Communication
doi.org/10.1002/elsa.202100131

Chemistry Europe
European Chemical Societies Publishing

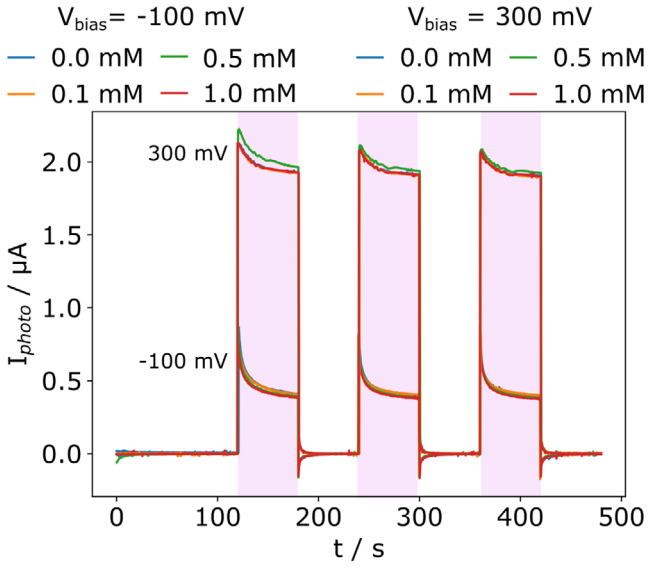

FIGURE 3 Combined transient photocurrent curves for 0.1–1.0 mM penicillin in 0.33 mM PBS, pH 7.3, without immobilized penicillinase for applied bias potentials of –100 and 300 mV

the buffer solution without penicillin (pH 7.3). Compared to the measured photocurrent without penicillin, no significant photocurrent changes for increasing penicillin concentrations were detected for both applied potentials.

As a final experiment, penicillinase was immobilized on the TiO_2 via crosslinking with bovine serum albumin (BSA) and glutaraldehyde for enhanced stability. Penicillin concentrations from 50 μM to 10.0 mM in 0.33 mM PBS buffer were again adjusted to pH 7.3. The measurements were started after 10 min of incubation with the respective penicillin solution. A potential of –100 mV was applied as the experiments described above show that this bias potential results in a higher pH sensitivity than 300 mV (Figure 2).

Figure 4a renders the transient photocurrent curve for various penicillin concentrations having the typical "S-shaped" biosensor characteristic. With increasing penicillin concentration, the photocurrent decreases systematically because the surplus generation of H^+ ions (due to the enzymatic reaction) leads to a decrease in the pH value. The resulting surface protonation can be assigned to the decreasing photocurrent.[20]

With respect to PBS buffer without penicillin, for 0.05 mM penicillin solution, the photocurrent (ΔI_{photo}) decreased by 3 nA. For 0.1 mM, a photocurrent change of 17 nA was measured. For increasing concentrations, up to 5.0 mM, the photocurrent decreased further by 271 nA. For an even higher concentration of 10.0 mM, only a small additional change of 11 nA (282 nA) was detected (Figure 4b). The mean penicillin sensitivity for three experiments was 161 ± 34 nA/dec in the range 0.1–5.0 mM penicillin G (Figure 4b, inset). These results indicate the feasibility of an enzymatic PEC detection of penicillin utilizing a photoanode with an amperometric detection principle.

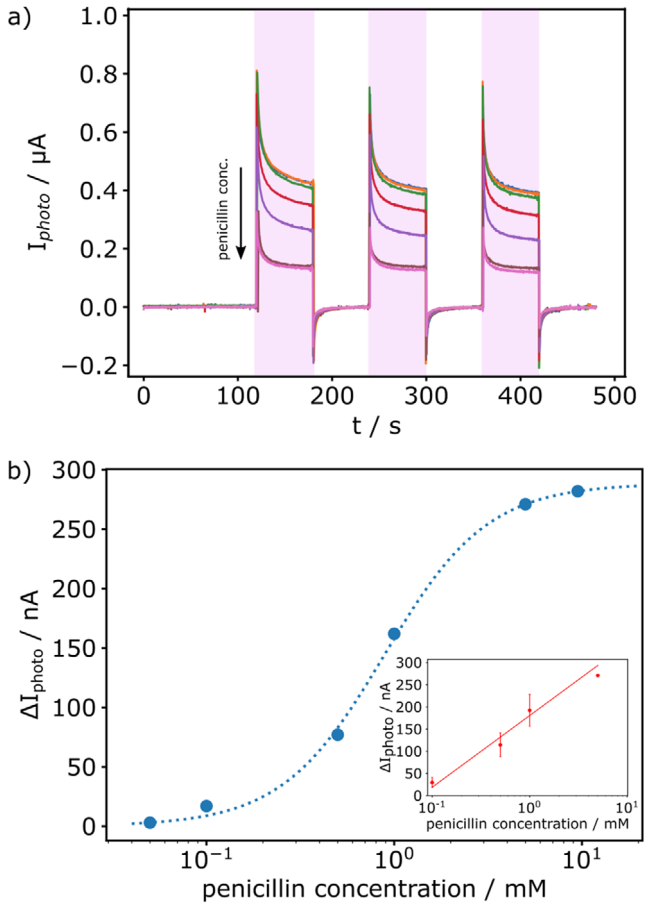

FIGURE 4 (a) Transient photocurrent curves for increasing penicillin concentrations from 0.05 to 10.0 mM penicillin in 0.33 mM PBS, pH 7.3, at an applied potential of –100 mV with immobilized penicillinase. (b) Change in photocurrent for varying penicillin concentrations with respect to 0.33 mM PBS buffer solution without penicillin. The inset shows the ΔI_{photo}-penicillin calibration for 0.1–5.0 mM penicillin ($n = 3$)

4 | CONCLUSION

In this work, we have shown in a proof-of-concept experiment the PEC detection of H^+-ion generation stimulated by an enzymatic reaction. In a first step, the potential-dependent pH sensitivity of TiO_2 was studied with a sensitivity of 133 nA/pH for an applied potential of –100 mV. Without the immobilized enzymes, no significant photocurrent change could be attributed to the photoelectrocatalytic oxidation of penicillin.

For penicillin detection, cross-linked penicillinase was immobilized on the TiO_2 surface. Photocurrent changes were measured between 0.05 and 10.0 mM. Exemplarily, a

penicillin sensitivity of 161 nA/dec was achieved between 0.1 and 5.0 mM. The results underline the feasibility of this novel detection principle. For comparison, potentiometric field-effect sensors show a widely linear penicillin detection range between 0.05 and 20.0 mM.[21,22] On the other hand, the PEC penicillin detection has not yet been optimized with regard to experimental conditions such as enzyme immobilization strategy, temperature, and pH optimum.

The main advantage of the new PEC detection with a light-addressable PEC biosensor is given by its light addressability. Immobilizing a variety of enzymes such as urease or glucose oxidase, in addition to penicillinase, on different positions of the same chip would enable multi-analyte detection, without increasing the complexity of the sensor. Changing the illuminating spot and tuning the bias potential, would be enough to have three sensors in one and further upgrades are always possible.

ACKNOWLEDGMENTS

The authors would like to thank Melanie Jablonski for her valuable discussions. Rene Welden would like to thank the Aachen University of Applied Sciences for financial support.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Michael J. Schöning https://orcid.org/0000-0003-4347-6685

REFERENCES

1. V. Sugathan, E. John, K. Sudhakar, *Renew. Sustain. Energy Rev.* **2015**, *52*, 54.
2. C. Jiang, S. J. A. Moniz, A. Wang, T. Zhang, J. Tang, *Chem. Soc. Rev.* **2017**, *46*, 4645.
3. K. Wenderich, G. Mul, *Chem. Rev.* **2016**, *116*, 14587.
4. W.-W. Zhao, J.-J. A. Xu, H.-Y. Chen, *Chem. Soc. Rev.* **2017**, *44*, 729.
5. W.-W. Zhao, J.-J. Xu, H.-Y. Chen, *Trends Anal. Chem.* **2016**, *82*, 307.
6. W.-W. Zhao, J.-J. Xu, H.-Y. Chen, *Analyst* **2016**, *141*, 4262.
7. W.-W. Zhao, J.-J. Xu, H.-Y. Chen, *Biosens. Bioelectron.* **2017**, *92*, 294.
8. J. Tang, Y. Wang, J. Li, P. Da, J. Geng, G. Zheng, *J. Mater. Chem. A* **2014**, *2*, 6153.
9. M. Riedel, A. Ruff, W. Schuhmann, F. Lisdat, F. Conzuelo, *Chem. Commun.* **2020**, *56*, 5147.
10. A. Poghossian, M. Thust, P. Schroth, A. Steffen, H. Lütz, M. J. Schöning, *Sens. Mar.* **2001**, *3*, 207.
11. A. Poghossian, M. Jablonski, C. Koch, T. S. Bronder, D. Rolka, C. Wege, M. J. Schöning, *Biosens. Bioelectron.* **2018**, *110*, 168.
12. Y. Nakabayashi, Y. Nosaka, *Phys. Chem. Chem. Phys.* **2015**, *17*, 30570.
13. F. Wu, B. Zhou, J. Wang, M. Zhong, A. Das, M. Watkinson, K. Hing, D.-W. Zhang, S. Krause, *Anal. Chem.* **2019**, *91*, 5896.
14. K. L. Tang, N. P. Caffrey, D. B. Nóbrega, S. C. Cork, P. E. Ronksley, H. W. Barkema, A. J. Polachek, H. Ganshorn, N. Sharma, J. D. Kellner, W. A. Ghali, *Lancet Planet. Health* **2017**, *1*, e316.
15. R. Welden, M. Jablonski, C. Wege, M. Keusgen, P. H. Wagner, T. Wagner, M. J. Schöning, *Biosensors* **2021**, *11*, 171.
16. M. Riedel, S. Hölzel, P. Hille, J. Schörmann, M. Eickhoff, F. Lisdat, *Biosens. Bioelectron.* **2017**, *94*, 298.
17. Y. Tu, N. Ahmad, J. Briscoe, D.-W. Zhang, S. Krause, *Anal. Chem.* **2019**, *90*, 8708.
18. D. Klauson, J. Babkina, K. Stepanova, M. Krichevskaya, S. Preis, *Catal. Today* **2010**, *151*, 39.
19. S. Wu, Y. H. Hu, *Chem. Eng. J.* **2021**, *409*, 127739.
20. A. Imanishi, T. Okamura, N. Ohashi, R. Nakamura, Y. Nakato, *J. Am. Chem. Soc.* **2007**, *129*, 11569.
21. J. R. Siqueira Jr., M. H. Abouzar, A. Poghossian, V. Zucolotto, O. N. Oliveira Jr., M. J. Schöning, *Biosens. Bioelectron.* **2009**, *25*, 497.
22. D. Molinnus, S. Beging, C. Lowis, M. J. Schöning, *Sensors* **2020**, *20*, 4924.

How to cite this article: R. Welden, C. A. N. Komesu, P. H. Wagner, M. J. Schöning, T. Wagner, *Electrochem Sci Adv* **2022**, *2,* e2100131. https://doi.org/10.1002/elsa.202100131