TÜBİTAK

Turkish Journal of Chemistry

http://journals.tubitak.gov.tr/chem/

Research Article

Turk J Chem
(2018) 42: 346 – 354

doi:10.3906/kim-1708-68

Development of a molecularly imprinted polymer-based electrochemical sensor for tyrosinase

Aysu YARMAN[1,2,*]

[1]Department of Molecular Biotechnology, Faculty of Science, Turkish-German University, İstanbul, Turkey
[2]Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany

Received: 28.08.2017 • **Accepted/Published Online:** 06.11.2017 • **Final Version:** 27.04.2018

Abstract: For the first time a molecularly imprinted polymer (MIP)-based sensor for tyrosinase is described. This sensor is based on the electropolymerization of scopoletin or o-phenylenediamine in the presence of tyrosinase from mushrooms, which has a high homology to the human enzyme. The template was removed either by treatment with proteinase K or by alkaline treatment. The measuring signal was generated either by measuring the formation of a product by the target enzyme or by evaluation of the permeability of the redox marker ferricyanide. The o-phenylenediamine-based MIP sensor has a linear measuring range up to 50 nM of tyrosinase with a limit of detection of 3.97 nM ($R^2 = 0.994$) and shows good discrimination towards other proteins, e.g., bovine serum albumin and cytochrome c.

Key words: Molecularly imprinted polymers, biomimetic sensors, tyrosinase, electropolymerization, scopoletin, o-phenylenediamine

1. Introduction

The high specificity of enzymes and antibodies has been exploited for more than 50 years in clinical analysis, food control, and environmental analysis.[1,2] In order to overcome inherent problems of biomolecules, especially their instability during storage and long-term use, biomimetic binders and catalysts have been generated. Two major concepts have been used: "evolution in the test tube" of nucleotides (aptamers) or total chemical synthesis of (molecularly imprinted) polymers.[3–12]

Molecular imprinting is a methodology used to create recognition sites in synthetic polymers by polymerizing a functional monomer and a cross-linker in the presence of the target analyte, so-called "template". Subsequent removal of the template leads to the formation of binding sites that partially mirror the size, shape, and functionality of the template.[3,10–12] In addition to binding molecularly imprinted polymers (MIPs), also catalytically active MIPs have been developed as mimics of enzymes. In analogy to the generation of catalytically active antibodies a stable analogue of the transition state is used as the template and/or transition state metals or redox active prosthetic groups are integrated into the MIP scaffold as catalytic center.[6,12–16] Whilst classical bulk imprinting techniques have been widely applied for small molecules, it is still a challenge to prepare MIPs for proteins, due to their size and structural complexity.[13–29] Electropolymerization is especially appropriate for the synthesis of protein MIPs because no cross-linker is required, which could react with surface groups of the target protein. Furthermore, the thickness of the MIP layer can be controlled by the charge during the electropolymerization.[3,28,30] In this process the protein should be only partially trapped by

*Correspondence: yarman@tau.edu.tr

the polymer film in order to allow an efficient exchange of the macromolecule with the solution phase during template removal and rebinding. At present almost 1200 papers per year are published on different MIPs, with some 10% covering MIPs for proteins.[3,28] In total, MIPs for almost 15 enzymes have been published. So far no MIP for tyrosinase has been presented in the literature.

Tyrosinase is a copper enzyme that oxidizes both monophenols and diphenolic compounds to the respective quinones.[31,32] Due to the relevance of these substances in environmental analysis, a large spectrum of tyrosinase-based sensors has been described in the literature. Among them, electrochemical sensors clearly dominate. In addition to the classical immobilization methods, entrapment of the enzyme via electropolymerization has been also used. The voltammetric sensors exhibited linear measuring ranges from the lower nM to mM concentrations. Electroenzymatic or bienzyme recycling allowed shifting the measuring range into the subnanomolar region.[33–42]

Tyrosinase is inhibited by several substances with relevance especially in nutrition, e.g., kojic acid or pesticides, and thus sensors for these inhibitors have been developed for food control and environmental analysis.[43–45] Furthermore, it is an established marker for skin cancer. According to the World Health Organization (WHO), 132,000 melanoma skin cancers occur globally each year (www.who.int/uv/faq/skincancer/en/index1.html).[46]

In this paper for the first time a molecularly imprinted polymer-based sensor for tyrosinase is described. It is based on the electropolymerization (EP) of scopoletin or o-phenylenediamine (o-PD) in the presence of tyrosinase from mushroom, which has a high homology to the human enzyme. The template was removed either by treatment with proteinase K or by alkaline treatment. The measuring signal was generated either by measuring the formation of a product by the target enzyme or by evaluation of the permeability of the redox marker ferricyanide. The o-PD based MIP sensor has linear measuring up to 50 nM of tyrosinase and shows good discrimination towards other proteins.

2. Results and discussion

MIP-based sensors were prepared as shown in Figure 1. The functional monomer scopoletin or o-phenylenediamine was electropolymerized in the presence of the target tyrosinase on gold or glassy carbon (GC) electrodes.

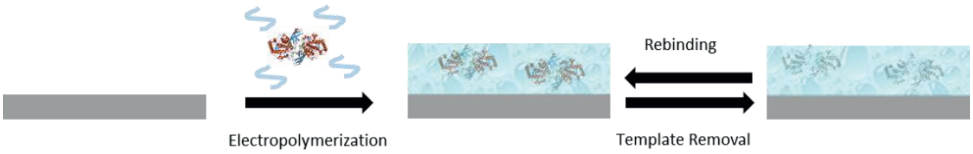

Figure 1. Workflow of the preparation of MIPs for tyrosinase.

2.1. Method I: Scopoletin as functional monomer

At first the thickness of the MIP layer on the gold electrode was controlled by adjusting the number of cycles during electropolymerization. This influences the sensor performance and the stability.[28,30,47] Whilst thicker films provide incorporation of larger amounts of enzyme at the electrode surface, it is more difficult to remove the template molecules from thicker polymer films. Easier removal can be achieved with thinner films. However, the polymer layer was less stable. Therefore, electropolymerization is a crucial step to obtain stable but sufficiently thin layers to remove the template. As a compromise between the lower stability of thin layers and the restriction of target accessibility with increasing film thickness, 30 cycles were chosen for the preparation of the tyrosinase-imprinted electrode (Figure 2).

Ferricyanide was used as a redox probe to characterize the permeability of the layer after each step. After EP the current for ferricyanide was almost completely suppressed for both the MIP (Figure 3) and control NIP (not shown). The MIP-modified electrode gave a markedly increased ferricyanide signal after the removal of the template by the treatment with proteinase K and washing with 1X PBS, indicating the formation of cavities through which ferricyanide can diffuse. This signal was subsequently suppressed after incubation in tyrosinase-containing solutions as expected by the filling of cavities with the target protein.

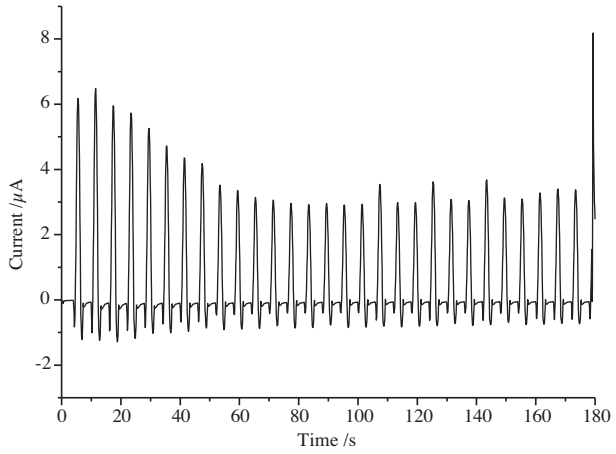

Figure 2. Current-time curves for the preparation of the tyrosinase MIP on the gold electrode in 0.5 mM scopoletin solution containing 10 μM tyrosinase , 0 V = 5 s, 0.9 V = 1 s, 30 cycles.

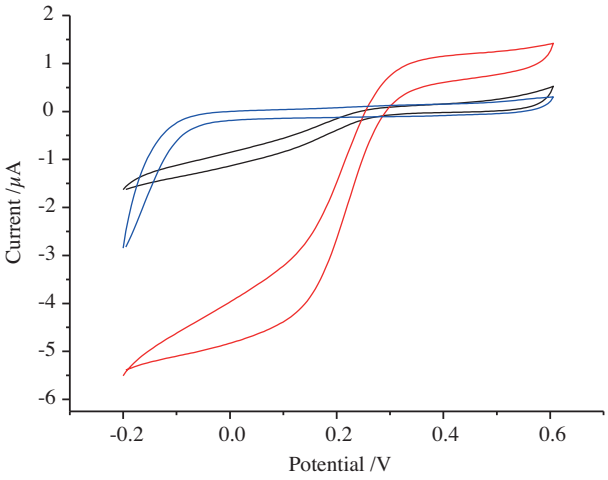

Figure 3. CVs of the different steps of MIP synthesis in 10 mM ferricyanide (100 mM KCl): a) After EP (blue), b) After 30 min incubation in 100 μg/mL proteinase K (in 1X PBS) + 30 min washing in 1X PBS (red), and c) After rebinding of 50 μM tyrosinase (black).

In addition to signal suppression with the redox marker, the detection of the rebinding of the enzyme to the MIP layer was also confirmed by its activity towards its substrate, paracetamol. Figure 4 shows amperometric current-time curves at –100 mV on stepwise addition of paracetamol to a tyrosinase-rebound MIP. Stepwise increase in cathodic current was obtained due to the cathodic reduction of N-acetyl-p-quinoneimine, which was formed in the enzymatic reaction.[48]

2.2. Method II: o-Phenylenediamine as functional monomer

In order to characterize the influence of the functional monomer, a second method was developed for a tyrosinase sensing MIP: o-Phenylenediamine was used as the functional monomer, which has been widely applied for MIP-based sensors not only for low molecular weight targets like drugs and pesticides, but also for high molecular weight targets like proteins.[28,49]

EP was performed in a mixture containing 10 μM tyrosinase and 5 mM o-PD (acetate buffer, pH 5.2) by scanning between 0 V and 0.8 V with a scan rate of 50 mV/s (Figure 5a). As with scopoletin, the thickness of the MIP layer was controlled by adjusting the number of cycles during electropolymerization.

The oxidation currents at around 450 mV decreased in the following sweeps, which is typical of a nonconducting polymer. It can be seen from the CVs of MIP-modified electrodes in ferricyanide/ferrocyanide solution that the increase in the number of scans during EP causes inefficient template removal by NaOH and

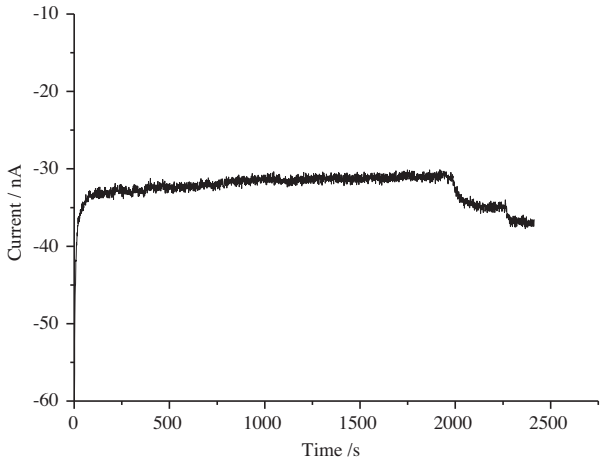

Figure 4. Amperometric responses of the scopoletin-based tyrosinase MIP at –100 mV on stepwise addition of 2 × 100 μM paracetamol into 50 mM phosphate buffer, pH 7, under 300 rpm stirring.

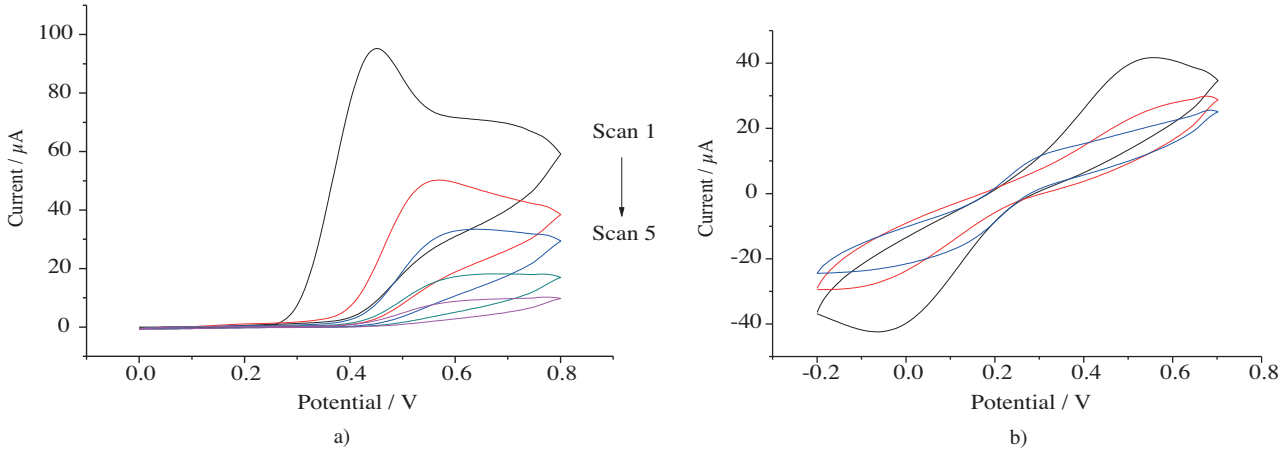

Figure 5. a) CVs showing the MIP preparation on GCE by using 5 scans, cycling between 0 V and 0.8 V with a scan rate of 50 mV/s in 5 mM o-PD solution containing 10 μM tyrosinase (pH 5.2) and b) CVs of the MIP-covered GCEs in 5 mM ferricyanide/ferrocyanide solution (in 100 mM KCl) after incubation in 0.1 M NaOH: black curve: EP by 5 scans and overnight removal of the template, red curve: EP by 10 scans and after 2 days removal of the template and blue curve: EP by 20 scans and after 2 days removal of the template.

requires a longer time (Figure 5b). In order to reduce the time for template removal, 0.1 M sodium dodecyl sulfate solution in 0.1 M NaOH was also applied. However, no success was obtained. Therefore, 5 scans were chosen for the preparation of the tyrosinase-imprinted sensor.

In the next step, the influence of rebinding time was investigated. Two hours were needed for reaching a steady state.

Differential pulse voltammetry (DPV) was used under optimum conditions to prepare the calibration graphics. Although three different electrodes from the same company were used, the current for bare electrodes differed by up to 30%. In order to normalize the differences between the electrodes, the relative current decrease (in %) was evaluated, a procedure that has been frequently applied for sensors that measure the suppression of the diffusional permeability of ferricyanide. The current decreased linearly up to 50 nM tyrosinase with

a limit of detection (LOD) of 3.97 nM (R^2 = 0.994) and reached saturation above 100 nM upon incubation in increasing amounts of tyrosinase (Figure 6). As a control, non-imprinted polymer-modified electrodes were prepared by EP with 20 scans and 2 days removal of the template, but in the absence of tyrosinase. However, when only 5 CV scans were applied for the preparation, it was found that the layer was not tight. On the other hand, at higher numbers of scans (20 scans) tight films were obtained and at saturation of rebinding an imprinting factor of 70 was calculated.

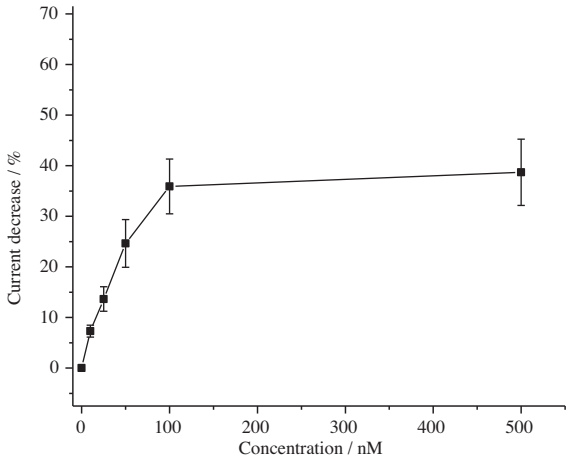

Figure 6. Concentration dependence of rebinding of tyrosinase to the MIP measured by the current decrease in the redox marker by DPV (number of measurements for each concentrations is 3).

Furthermore, the catalytic activity was used as a tool to characterize the tyrosinase rebound to the MIP. Figure 7 shows the amperometric response and calibration graphic of the MIP electrode after rebinding of tyrosinase on stepwise addition of catechol. At –100 mV, o-quinone, which was formed by the enzymatic reaction, was reduced (Figure 7a). The cathodic current increased linearly up to 15 μM (R^2 = 0.982) with a LOD of 58.2 nM (Figure 7b). In spite of the submonolayer enzyme amount in the MIP these values are in the same range as those in the literature.

One of the most important parameters of bio(mimetic)sensors is the specific recognition of the analytes. In order to check the specificity of the tyrosinase MIP, binding of other proteins was studied and compared with the binding of the proteins to a bare GCE. The linear part of the concentration dependence (10 nM) of tyrosinase and the other proteins was compared. Signal suppression of the redox marker is 4-fold higher after incubation with BSA at the bare GCE than for tyrosinase and Cyt c. On the other hand, the tendency is different at the MIP-covered GCE: the signal suppression by tyrosinase (pI 4.7–5) is 3.5-fold and 2.5-fold higher than for BSA (BSA, pI 4.8) and Cyt c (pI 10–10.5), respectively. The proteins used for cross-reactivity studies represent the most critical candidates because both Cyt c and BSA are smaller than the target. Furthermore, the influence of the electrostatic interaction was included because BSA is negatively charged like the target, whilst Cyt c carries a positive overall charge. In addition, it was found that ferritin (MW = 450 kDa), which is bigger than tyrosinase, does not bind to scopoletin-based MIPs. This result is proof of preferential binding of tyrosinase to the MIP and a significant difference to the nonspecific adsorption of proteins to the bare electrode.

Summing up, in this work, molecularly imprinted polymer films for the recognition of tyrosinase were prepared by electropolymerizing either scopoletin or o-PDA. In contrast to our earlier protein MIPs for hexameric tyrosine-coordinated heme protein (HTHP) and acetylcholinesterase (AChE), the MIP was deposited

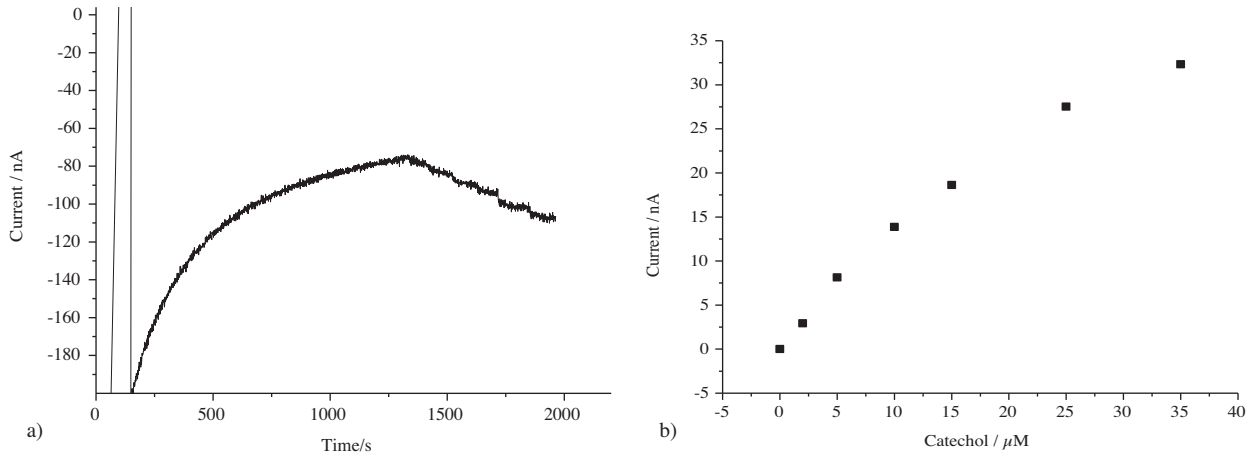

Figure 7. a) Amperometric responses at –100 mV of the MIP-covered GCE after 100 nM tyrosinase rebinding on stepwise addition of catechol (1 × 2 μM Cat, 1 × 3 μM Cat, 2 × 5 μM Cat, and 2 × 10 μM) into 50 mM phosphate buffer, pH 7 and b) Current-concentration dependence.

direct on the electrode surface without an additional self-assembled monolayer (SAM).[50,51] Furthermore, an indirect method, depression of the permeability of the redox marker ferricyanide, was applied for the evaluation of rebinding of the enzyme. This method was very useful to characterize the different steps of MIP preparation. A disadvantage is that at low target concentrations very small decreases in a large current have to be evaluated.

For both MIPs prepared by electropolymerizing scopoletin and o-PDA, concentration dependent rebinding of the target was clearly demonstrated by the indication of substrate conversion by tyrosinase and the suppression of the permeability of the redox marker ferricyanide. However, the stability of the scopoletin-based MIP film was lower. The o-PD-based MIP exhibited good stability and a measuring range for its target in the lower nanomolar concentration range was demonstrated. This range is comparable with that for MIPs described in the literature, e.g., ferritin, HTHP, concanavalin A, and human serum albumin.[47,50,52,53] On the other hand, a few papers describing MIPs for proteins in combination with redox marker-based readout found considerably higher sensitivities and measuring ranges over several orders of magnitudes. The mechanism that leads to the extremely high sensitivity has not yet been elucidated.[54–56]

Interestingly, BSA and Cyt c showed 3.5- and 2.5-fold lower suppression of the ferricyanide permeability as compared with the target. Because both proteins are considerably smaller than tyrosinase, it could be expected that they could simply "fill" the binding pockets and suppress the permeability for the redox marker. Their smaller effect demonstrates the preference of the interactions between the target and the polymer scaffold.

In summary, it can be concluded that the electrosynthesized o-PDA film acts on the GCE as a specific adsorbent that modulates the permeation of the redox probe. This conclusion is in accordance with the enzymatic activity of the rebound protein.

3. Experimental

3.1. Materials

Tyrosinase (EC 1.14.18.1, from mushroom), bovine serum albumin (BSA), cytochrome c (cyt c, from horse heart), proteinase K (EC 3.4.21.64, from *Tritirachium album*), scopoletin, o-phenylenediamine dihydrochloride (o-PD), catechol (Cat), and paracetamol were purchased from Sigma-Aldrich (Steinheim, Germany).

All reagents were of analytical grade and used without further purification.

3.2. Methods

In this work either gold disk electrodes (2 mm in diameter, CHI) or glassy carbon disk electrodes (GCE) (3 mm in diameter, CHI) were used for the MIP-synthesis and for the voltammetric and amperometric measurements.

Prior to electropolymerization, Au electrodes were cleaned with 1.0, 0.3, and 0.05 μm alumina slurry, consecutively, sonicated in Millipore water, and then scanned in 0.5 M H_2SO_4 (–0.35 V–1.7 V, 0.2 V/s) until a typical CV for a clean gold surface was obtained.

The GCEs were cleaned with ethanol and treated with 60% nitric acid for 15 min. After this procedure, mechanical cleaning was performed with 1.0, 0.3, and 0.05 μm alumina slurry, consecutively, and the electrodes were rinsed with Millipore water by sonication.

Two methods were used for the preparation of MIP-covered electrodes:

i) Method I: Electropolymerization on a gold electrode was performed in a mixture of 0.5 mM scopoletin (10 mM stock solution in ethanol), 10 mM NaCl (100 mM stock in water), and 10 μM tyrosinase (100 μM stock solution in 50 mM phosphate buffer, pH 6.5) solutions by using a 2-mL handmade cell. End concentrations in the mixture were obtained by dilution with water. Multistep amperometry was applied, involving a number of cycles (30–100) in which the Au working electrode was polarized at 0 V for 5 s and at 0.9 V for 1 s.

ii) Method II: Electropolymerization was performed in 5 mM o-phenylenediame solution (in 100 mM acetate buffer, pH 5.2) containing 10 μM tyrosinase (the stock solution was prepared as described above) by scanning the potential of the GCE between 0 V and 0.8 V with a scan rate of 50 mV/s. Different numbers of CV scans (5, 10, and 20 scans) were applied. Tyrosinase was removed from the polymer by the incubation of 0.1 M NaOH solution.

Voltammetric and amperometric measurements were performed using a PalmSens potentiostat (Netherlands).

Acknowledgments

This paper is dedicated to the 75th birthday of Prof Dr Frieder W Scheller. Deutsche Forschungsgemeinschaft (DFG) within the framework of the German Excellence Initiative (EXC 314) and Turkish-German University Scientific Research Projects Commission under the grant no. 2016BF0011 are acknowledged for the financial support.

References

1. Scheller, F.; Schubert, F. *Biosensors, Volume 11;* Elsevier Science Publishers: Amsterdam, Netherlands, 1992.
2. Turner, A. P. F. *Chem. Soc. Rev.* **2013**, *42*, 3184-3196.
3. Menger, M.; Yarman, A.; Erdőssy, J.; Yildiz, H. B.; Gyurcsányi, R. E.; Scheller, F. W. *Biosensors* **2016**, *6*, 35.
4. Tuerk, C.; Gold, L. *Science* **1990**, *249*, 505-510.
5. Bunka, D. H.; Stockley, P. G. *Nat. Rev. Microbiol.* **2006**, *4*, 588-596.
6. Wulff, G.; Sarhan, A. *Angew. Chem. Int. Ed. Engl.* **1972**, *11*, 341-344.
7. Shea, K.; Thompson, E. *J. Am. Chem. Soc.* **1980**, *102*, 3148-3156.
8. Arshady, R.; Mosbach, K. *Macromol. Chem. Phys.* **1981**, *182*, 687-692.

9. Hayden, O.; Lieberzeit, P. A.; Blaas, D.; Dickert, F. L. *Adv. Funct. Mater.* **2006**, *16*, 1269-1278.
10. Saylan, Y.; Yilmaz, F.; Özgür, E.; Derazshamshir, A.; Yavuz, H.; Denizli, A. *Sensors* **2017**, *17*, 898.
11. Haupt, K.; Mosbach, K. *Trends Biotechnol.* **1998**, *16*, 468-475.
12. Wulff, G. *Angew. Chem.* **1995**, *107*, 1958-1979.
13. Berti, F.; Todros, S.; Lakshmi, D.; Whitcombe, M. J.; Chianella, I.; Ferroni, M.; Piletsky, S. A.; Turner, A. P. F.; Marrazza, G. *Biosens. Bioelectron.* **2010**, *26*, 497-503.
14. Huang, X.; Yin, Y.; Liu, Y.; Bai, X.; Zhang, Z.; Xu, J.; Shen, J.; Liu, J. *Biosens. Bioelectron.* **2009**, *25*, 657-660.
15. Neto, J. R. M.; Santos, W. J. R.; Lima, P. R.; Tanaka, S. M. C. N.; Tanaka, A. A.; Kubota, L. T. *Sensor. Actuat. B-Chem.* **2011**, *152*, 220-225.
16. Antuña-Jiménez, D.; Blanco-López, M. C.; Miranda-Ordieres, A. J.; Lobo-Castañón, M. J. *Polymer* **2014**, *55*, 1113-1119.
17. Yarman, A.; Scheller, F. W. *Sensors* **2014**, *14*, 7647-7654.
18. Özcan, L.; Şahin, Y. *Sensor. Actuat. B-Chem.* **2007**, *127*, 362-369.
19. Yarman, A.; Scheller, F. W. *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 11521-11525.
20. Yarman, A.; Scheller, F. W. *Electroanalysis* **2016**, *28*, 2222-2227.
21. Altintas, Z.; Chianella, I.; Da Ponte, G.; Paulussen, S.; Gaeta, S.; Tothill, I. E. *Chem. Eng. J.* **2016**, *300*, 358-366.
22. Uygun, Z. O.; Dilgin, Y. *Sensor. Actuat. B-Chem.* **2013**, *188*, 78-84.
23. Lach, P.; Sharma, P. S.; Golebiewska, K.; Cieplak, M.; D'Souza, F.; Kutner, W. *Chem. Eur. J.* **2017**, *23*, 1942-1949.
24. Uludağ, Y.; Piletsky, S. A.; Turner, A. P. F.; Cooper, M. A. *FEBS J.* **2007**, *274*, 5471-5480.
25. Gomez, L. P. C.; Spangenberg, A.; Ton, X. A.; Fuchs, Y.; Bokeloh, F.; Malval, J. P.; Tse Sum Bui, B.; Thuau, D.; Ayela, C.; Haupt, K.; et al. *Adv. Mater.* **2016**, *28*, 5931-5937.
26. Takeuchi, T.; Hishiya, T. *Org. Biomol. Chem.* **2008**, *6*, 2459-2467.
27. Li, S.; Cao, S.; Whitcombe, M. J.; Piletsky, S. A. *Prog. Polym. Sci.* **2014**, *39*, 145-163.
28. Erdőssy, J.; Horváth, V.; Yarman, A.; Scheller, F. W.; Gyurcsányi, R. E. *TrAC Trends Anal. Chem.* **2016**, *79*, 179-190.
29. Yarman, A.; Jetzschmann, K. J.; Neumann, B.; Zhang, X.; Wollenberger, U.; Cordin, A.; Haupt, K.; Scheller, F. W. *Chemosensors* **2017**, *5*, 11.
30. Sharma, P. S.; Pietrzyk-Le, A.; D'Souza, F.; Kutner, W. *Anal. Bioanal. Chem.* **2012**, *402*, 3177-3204.
31. Peter, M. G.; Wollenberger, U. In *Frontiers in Biosensorics I*; Scheller, F. W; Schubert, F.; Fedrowitz, J., Eds. Birkhäuser: Basel, Switzerland, 1997, pp. 63-82.
32. Shleev, S.; Tkac, J.; Christenson, A.; Ruzgas, T.; Yaropolov, A. I.; Whittaker, J. W.; Gorton, L. *Biosens. Bioelectron.* **2005**, *20*, 2517-2554.
33. Yildiz, H. B.; Castillo, J.; Guschin, D. A.; Toppare, L.; Schuhmann, W. *Microchim. Acta* **2007**, *159*, 27-34.
34. Liu, S.; Yu, J.; Ju, H. *J. Electroanal. Chem.* **2003**, *540*, 61-67.
35. Vicentini, F. C.; Garcia, L. L.; Figueiredo-Filho, L. C.; Janegitz, B. C.; Fatibello-Filho, O. *Enzyme Microb. Technol.* **2016**, *84*, 17-23.
36. Pena, N.; Reviejo, A. J.; Pingarron, J. M. *Talanta* **2001**, *55*, 179-187.
37. Vidal, J. C.; Esteban, S.; Gil, J.; Castillo, J. R. *Talanta* **2006**, *68*, 791-799.
38. Biegunski, A. T.; Michota, A.; Bukowska, J.; Jackowska, K. *Bioelectrochemistry* **2006**, *69*, 41-48.
39. Takashima, W.; Kaneto, K. *React. Funct. Polym.* **2004**, *59*, 163-169.

40. Védrine, C.; Fabiano, S.; Tran-Minh, C. *Talanta* **2003**, *59*, 535-544.

41. Guan, Y.; Liu, L.; Chen, C.; Kang, X.; Xie, Q. *Talanta* **2016**, *160*, 125-132.

42. Fritea, L.; Le Goff, A.; Putaux, J. L.; Tertis, M.; Cristea, C.; Săndulescu, R.; Cosnier, S. *Electrochim. Acta* **2015**, *178*, 108-112.

43. Streffer, K.; Kaatz, H.; Bauer, C.G.; Makower, A.; Schulmeister, T.; Scheller, F. W.; Peter, M. G.; Wollenberger, U. *Anal. Chim. Acta* **1998**, *362*, 81-90.

44. Tortolini, C.; Bollella, P.; Antiochia, R.; Favero, G.; Mazzei, F. *Sens. Actuat. B-Chem.* **2016**, *224*, 552-558.

45. Kurbanoglu, S.; Ozkan, S. A.; Merkoçi, A. *Biosens. Bioelectron.* **2017**, *89*, 886-898.
Mossberg, M.; Vernick, S.; Ortenberg, R.; Markel, G.; Diamand, Y. S.; Rishpon, J. *Electroanalysis* **2014**, *26*, 1671-1675.

46. Bosserdt, M.; Erdőssy, J.; Lautner, G.; Witt, J.; Köhler, K.; Gajovic-Eichelmann, N.; Yarman, A.; Wittstock, G.; Scheller, F. W.; Gyurcsányi, R. E. *Biosens. Bioelectron.* **2015**, *73*, 123-129.

47. Valero, E.; Varón, R.; García-Carmona, F. *Biol. Chem.* **2002**, *383*, 1931-1939.

48. Yarman, A.; Turner, A. P. F.; Scheller, F. W. In *Nanosensors for Chemical and Biological Applications*, Honeychurch K. C., Ed. Woodhead Publishing Limited: Amsterdam, Netherlands, 2014, pp. 125-149.

49. Peng, L.; Yarman, A.; Jetzschmann, K. J.; Jeoung, J. H.; Schad, D.; Dobbek, H.; Wollenberger, U.; Scheller, F. W. *Sensors* **2016**, *16*, 272.

50. Jetzschmann, K. J.; Jágerszki, G.; Dechtrirat, D.; Yarman, A.; Gajovic-Eichelmann, N.; Gilsing, H. D.; Schulz, B.; Gyurcsányi, R. E.; Scheller, F. W. *Adv. Funct. Mater.* **2015**, *25*, 5178-5183.

51. Dechtrirat, D.; Gajovic-Eichelmann, N.; Bier, F. F.; Scheller, F. W. *Adv. Funct. Mater.* **2014**, *24*, 2233-2239.

52. Stojanovic, Z.; Erdőssy, J.; Keltai, K.; Scheller, F. W.; Gyurcsányi, R. E. *Anal. Chim. Acta* **2017**, *977*, 1-9.

53. Cai, D.; Ren, L.; Zhao, H.; Xu, C.; Zhang, L.; Yu, Y.; Wang, H.; Lan, Y.; Roberts, MF.; Chuang J. H.; et al. *Nat. Nanotechnol.* **2010** *5*, 597-601.

54. Cieplak, M.; Szwabinska, K.; Sosnowska, M.; Chandra, B. K. C.; Borowicz, P.; Noworyta, K.; D'Souza, F.; Kutner, W. *Biosens. Bioelectron.* **2015** *74*, 960-966.

55. Karimian, N.; Vagin, M.; Zavar, M. H. A.; Chamsaz, M.; Turner, A. P. F.; Tiwari, A. *Biosens. Bioelectron.* **2013** *50*, 492-498.