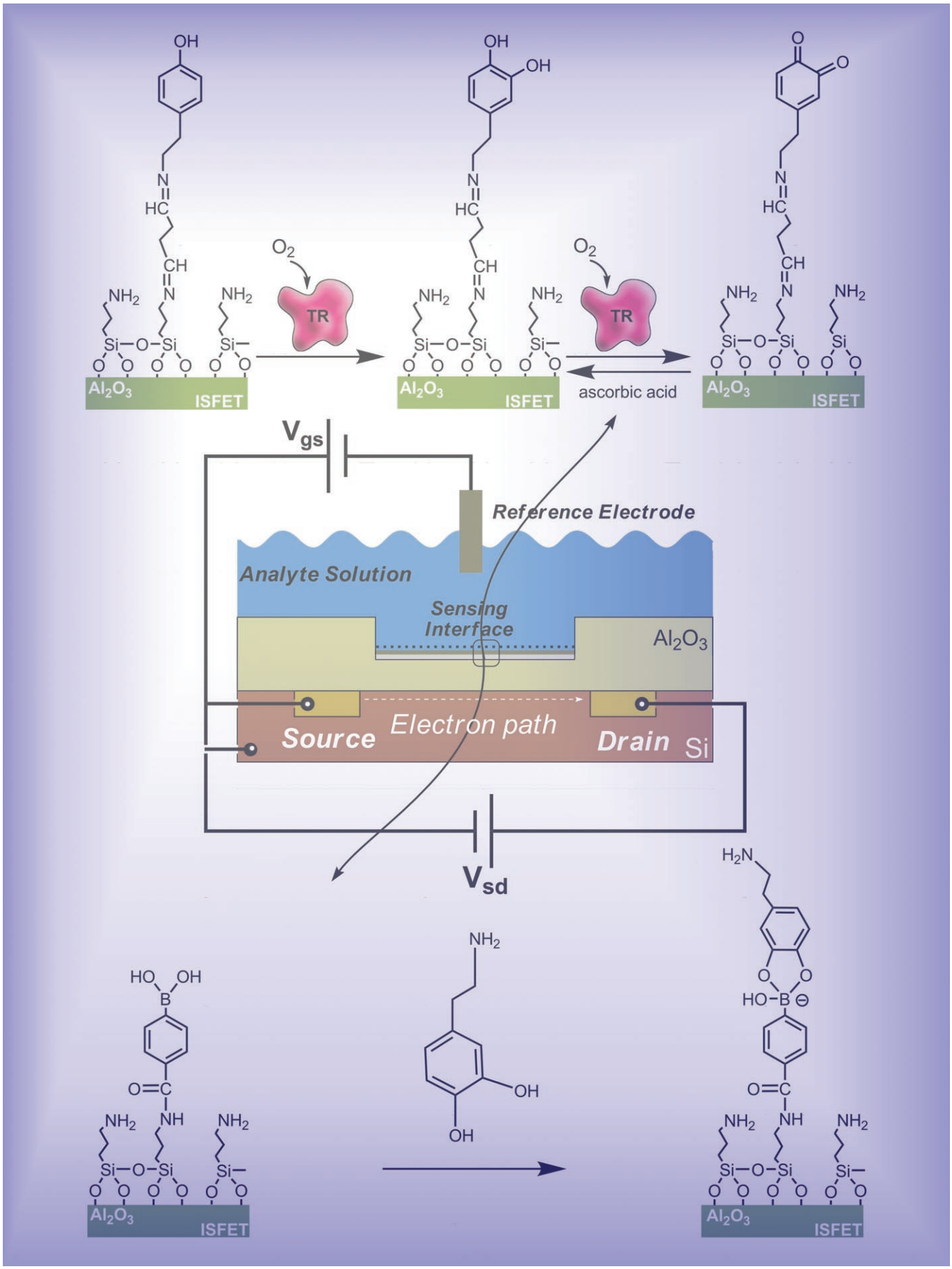
DOI: 10.1002/chem.200700734



Analysis of Dopamine and Tyrosinase Activity on Ion-Sensitive Field-Effect Transistor (ISFET) Devices

Ronit Freeman, Johann Elbaz, Ron Gill, Maya Zayats, and Itamar Willner*[a]

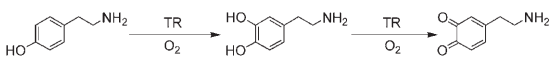
Abstract: Dopamine (**1**) and tyrosinase (TR) activities were analyzed by using chemically modified ion-sensitive field-effect transistor (ISFET) devices. In one configuration, a phenylboronic acid functionalized ISFET was used to analyze **1** or TR. The formation of the boronate–**1** complex on the surface of the gate altered the electrical potential associated with the gate, and thus enabled **1** to be analyzed with a detection limit of 7×10^{-5} M. Similarly, the TR-induced formation of **1**, and its association with the boronic acid ligand allowed a quantitative assay of TR to be performed. In another configuration, the surface of the ISFET gate was modified with tyramine or **1** to form functional surfaces for analyzing TR activities. The TR-induced oxidation of the tyramine- or **1**-functionalized ISFETs resulted in the formation of the redox-active dopaquinone units. The control of the gate potential by the redox-active dopaquinone units allowed a quantitative assay of TR to be performed. The dopaquinone-functionalized ISFETs could be regenerated to give the **1**-modified sensing devices by treatment with ascorbic acid.

Keywords: biosensors · enzymes · ion-sensitive field-effect transistors · monolayers · tyrosinase

Introduction

There has been growing interest in ion-sensitive field-effect transistors (ISFETs) as label-free transducers for biorecognition events.[1] Different ISFET-based biosensors have been developed, which include devices that monitor biocatalytic transformations,[2] that probe antigen–antibody binding processes[3] and DNA hybridization,[4] and very recently, that follow the formation of complexes between aptamers and low molecular weight substrates.[5] In all of these biosensor systems the biocatalytic processes or the biorecognition events alter the gate potential of the ISFET devices, thus allowing the electronic transduction of the sensing processes.

In the present study we demonstrate the detection of dopamine (**1**) by a functionalized ISFET device, and also demonstrate the use of three different ISFET systems to follow the activity of tyrosinase (TR). TR oxidizes phenol derivatives, such as tyrosine or tyramine (**3**), in the presence of O_2 to form their respective catechol derivatives, for example, L-3-(3,4-dihydroxyphenyl)alanine (L-dopa) or **1**, which are further oxidized by the enzyme to form their respective quinone products (Scheme 1).



Scheme 1. Oxidation of **3** to **1**, and subsequently to dopaquinone by the enzyme tyrosinase.

Elevated amounts of TR were detected in melanoma cancer cells, and the enzyme is considered to be a marker for this type of malignant cells.[6] It was also reported that the loss of **1** in neurons might cause diseases such as Parkinson's disease.[7] Compound **1** is a central neurotransmitter, and its sensitive detection, particularly by using integrated miniaturized devices, could be valuable for the invasive monitoring of neural responses. Different methods to analyze **1** were reported, which included electrochemical[8] means or optical methods that involved the **1**-induced formation of Au nanoparticles.[9] TR was probed electrochemically by the enzyme-stimulated formation of **1** on electrodes and the voltammetric detection of the product by labeling it with the redox-active ferrocene–boronic acid.[10] Similarly, TR activity was monitored through the biocatalyzed oxidation of tyrosine to form L-dopa and the product-mediated formation of Au nanoparticles, which were optically detect-

[a] R. Freeman, J. Elbaz, R. Gill, Dr. M. Zayats, Prof. I. Willner
Institute of Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904 (Israel)
Fax: (+972) 2-652-7715
E-mail: willnea@vms.huji.ac.il

ed.[9] Alternatively, CdSe quantum dots (QDs) were functionalized with methyl ester of tyrosine, and the enzyme-catalyzed oxidation of the substrate to dopaquinone resulted in the fluorescence quenching of the QDs, thus enabling the optical biosensing of TR activity.[11]

Results and Discussion

Scheme 2 shows one configuration for detecting **1** and analyzing TR activity. The Al_2O_3 gate of the ISFET device was functionalized with an aminopropylsiloxane film that was further treated with 4-carboxyphenyl boronic acid (**2**). The resulting **2**-functionalized gate was then employed as the active surface for the association of **1** (Scheme 2, route a). The formation of the charged boronate complex on the surface of the gate alters the gate potential, thus enabling the quantitative analysis of **1** by the ISFET device. Figure 1A shows the gate-to-source potential changes of the **2**-functionalized device after interaction with different concentrations of **1** for 20 min. The detection limit for analyzing **1** is 7×10^{-5} M, which is very similar to the detection limits for electrochemical[8] or optical[9] methods previously reported.

The **2**-functionalized ISFET device was also employed to analyze TR activity. To achieve this aim, **3** was treated with different concentrations of TR for a fixed time interval of 20 min before the enzyme was thermally deactivated. The resulting mixture that consisted of **1** and dopaquinone was then reduced with citric acid to give **1** as the sole product, and the resulting solution was analyzed by using the ISFET device (Scheme 2, route b). The association of **1** with the boronic acid ligand resulted in the changes in potential on the surface of the gate (Figure 1B). As the concentration of TR increased, the concentration of **1** generated also increased and the potential changes were enhanced. The gate potential leveled off at a concentration of around 1.2 U of TR, and the detection limit for the analysis of TR was around 0.25 U. It should be noted that treatment of the **2**-functionalized gate with thermally deactivated TR within the same concentration range resulted in minute potential changes ($\Delta V = 2$ mV), which implies that nonspecific adsorption of TR to the gate is not occurring, and thus the potential changes are as a result of the formation of the **1**–boronate complex.

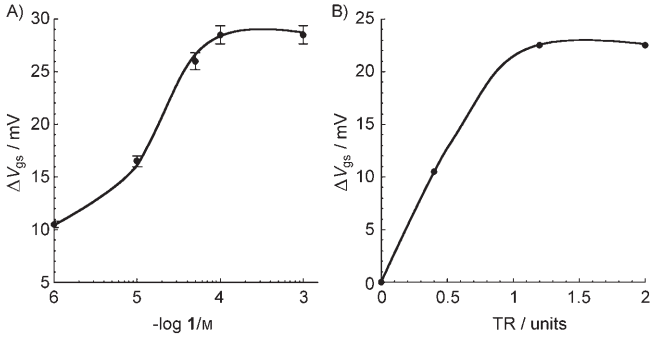
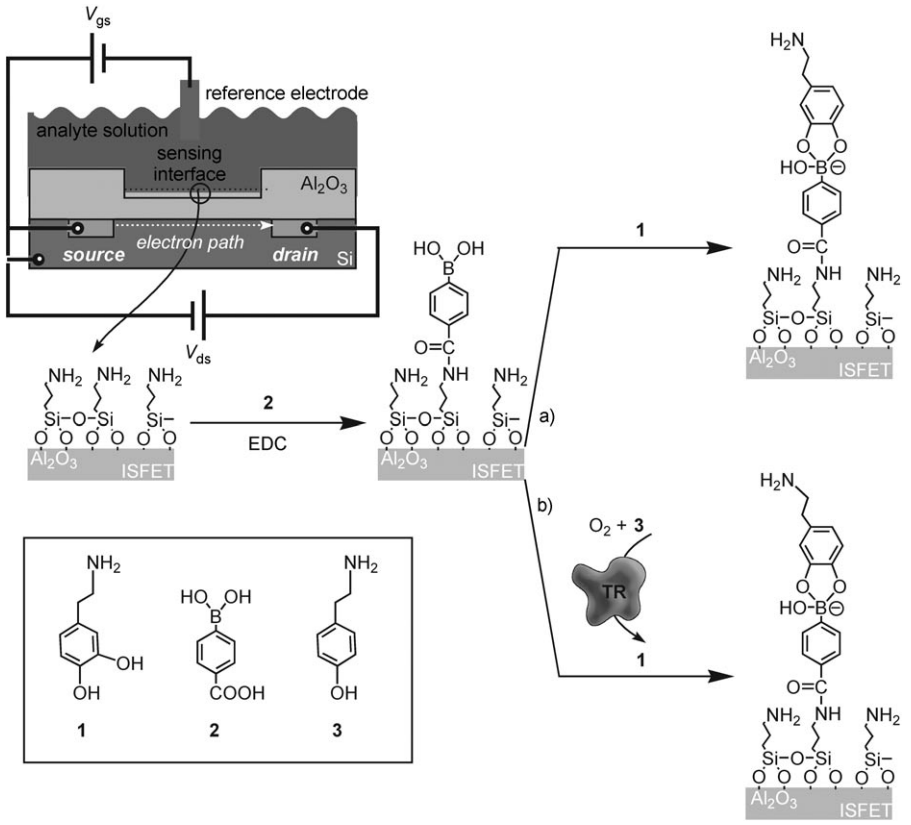
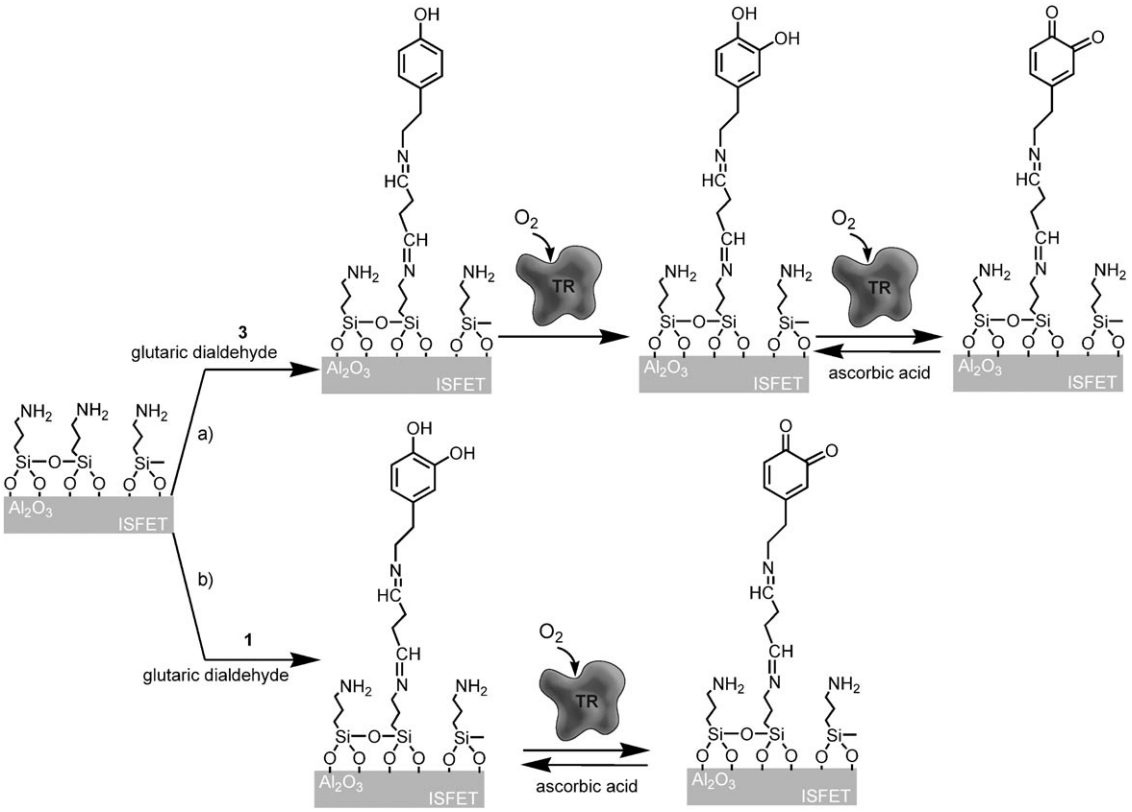


Figure 1. A) Calibration curve that corresponds to the analysis of **1** by the **2**-functionalized ISFET device. B) Calibration curve that corresponds to the analysis of different concentrations of TR.

Although the analysis of TR by the **2**-functionalized ISFET device is feasible, the activation of the biocatalytic oxidation of **3** in solution, the need to thermally deactivate the enzyme, and the subsequent association of the reaction product onto the gate surface, turn the analytical protocol into a complex procedure. To facilitate the analysis of TR, we looked to design a surface-confined process that would enable the separation of the reaction product from the enzyme, and would eliminate the time interval required to bind the reaction product to the functionalized



Scheme 2. Schematic representation of the detection of **1** (route a) and the analysis of TR activity (route b) by using a **2**-functionalized ISFET device.



Scheme 3. Analysis of TR activity by using a **3**-functionalized ISFET device (route a) and a **1**-functionalized ISFET device (route b).

gate. Scheme 3 shows the configuration of the ISFET device for the analysis of TR on the surface of the gate. The aminopropylsiloxane-functionalized gate was treated with glutaric dialdehyde, and subsequently with **3** (Scheme 3, route a). The modified surface was then treated with TR (under air) to yield the **1**–dopaquinone mixed film. The subsequent reduction of the dopaquinone units with ascorbic acid yielded the **1**-modified gate, in which the gate potential was controlled by the quantity of redox-active **1** present. Note that the biocatalytic transformation on the surface of the gate, and the subsequent reduction of the surface-confined reaction product by ascorbic acid allowed the stepwise separation of the **1**-modified gate from the enzyme and the reducing agent, and the rapid generation of clean **1**-modified ISFET devices.

Figure 2 shows the changes in the gate potentials upon treating the **3**-modified ISFET with TR (2 U) for different time intervals, according to route (a) in Scheme 3. As the reaction time increased, the changes in the gate potential were enhanced, and they leveled off to a saturation value of $\Delta V_{gs} = 70$ mV after about 15 min. These results are consistent with the increase in the concentration of **1** on the surface of the gate as the reaction with TR is prolonged. When **3** is fully oxidized, the gate potential reaches its saturation value. To prove that the TR-induced oxidation of **3** leads to the formation of dopaquinone and that the reduction of the latter by ascorbate is essential to yield the **1**-functionalized gate, the gate of an ISFET device was directly modified

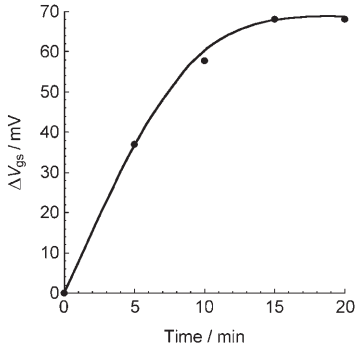


Figure 2. Time-dependent gate-to-source potential changes (ΔV_{gs}) upon analyzing the activity of TR (2 U). Measurements were performed in phosphate buffer (0.01 M, pH 6.3).

with **1** (Scheme 3, route b). This device was fabricated by the covalent attachment of **1** to the glutaric dialdehyde modified gate (instead of **3**).

Curve a in Figure 3A shows the changes in the potentials of the gate modified with **1** upon treatment with different concentrations of TR for a fixed time interval of 20 min. The changes in the gate potentials increase as the concentration of TR increases, and they level off at a TR concentration of 1.6 U. Each of the **1**-modified gates treated with TR (results shown in Figure 3A, curve a) was subsequently treated with ascorbate. The base potential of the **1**-modified gates was observed for all of the devices (Figure 3A, curve

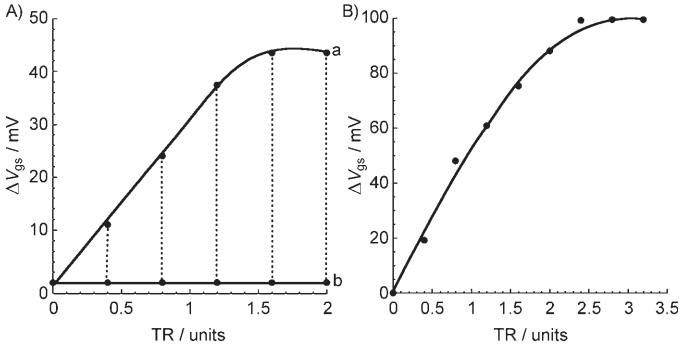


Figure 3. A) Gate-to-source potential changes upon analyzing different concentrations of TR by using the same ISFET device functionalized with **1**. Curve a) shows the potential changes as a result of oxidation of **1** by different concentrations of TR that yield the dopaquinone. Curve b) shows the changes in the gate-to-source potential after treatment of the dopaquinone-modified ISFET with ascorbic acid (1 mM) in phosphate buffer (0.01 M, pH 6.3). The results indicate the regeneration of the base potential of the ISFET device prior to analysis of any of the TR concentrations. B) Gate-to-source potential changes upon analyzing different concentrations of TR by using the **3**-modified ISFET. Measurements were performed in phosphate buffer (0.01 M, pH 6.3).

b), which implies that all of the enzyme-generated dopaquinone was reduced to form the **1**-functionalized surfaces. In fact, the TR-stimulated oxidation of **3** to dopaquinone and the back reduction of dopaquinone to **1** are fully reversible (Figure 3A). These results confirm that the oxidation of **3** on the surface lead to the formation of dopaquinone, which is reduced by ascorbate to form the **1**-functionalized surfaces. Furthermore, the results imply that the **1**-functionalized ISFET devices may be used as biosensors to follow TR activity, and that the reduction of the dopaquinone product may be used as a means of regenerating the interface.

The development of the **3**-modified ISFET device as a bioelectronic device that transduces the biocatalyzed formation of **1** on the surface of the gate enabled us to construct a new biosensor configuration for analyzing TR activity. Figure 3B shows the changes in the potential of the gate modified with **3** upon the analysis of different concentrations of TR. In these experiments, the **3**-modified ISFETs were treated with different concentrations of TR (for a fixed time interval of 20 min), and the resulting dopaquinone-functionalized gates were reduced with ascorbate (Scheme 3, route a). As the concentration of TR was increased, the change in the gate-to-source potential (ΔV_{gs}) was enhanced, which is consistent with the fact that the coverage of **1** on the surface of the gate was increased.

Conclusion

In conclusion, the present study has demonstrated the novel use of ISFET devices for the analysis of **1** or for the assay of TR. The **2**-functionalized ISFET could provide a useful miniaturized implantable electronic system for monitoring the neurotransmitter **1**. Similarly, **3**- or **1**-functionalized ISFETs could be useful sensing devices to monitor TR activity for the rapid detection of melanoma cancer cells. In the present studies we used two different principles to control the gate potential. The first approach involved the alteration of the change associated with the gate by linking the product of the biocatalytic transformation with the boronic acid ligand that was attached to the ISFET device. The second method to control the gate potential included the generation of a redox-active product (dopaquinone) that gave an electrochemical potential on the surface of the gate. All previous methods for the analysis of TR activity involved metallic nanoparticles,[9] semiconductor QDs,[11] or functionalized redox molecules,[10] as labels for the optical or electrochemical detection of TR activity. The present study demonstrates a label-free procedure for analyzing TR activities with comparable sensitivities to previous methods.

Experimental Section

Materials: Compounds **1**, **2**, **3**, TR, 3-aminopropyltriethoxysilane, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), ascorbic acid, citric acid, and glutaric dialdehyde were purchased from Sigma or Aldrich and used as supplied. Ultrapure water obtained from Barnstead NANOpure DIamond was used in all experiments.

Preparation of the modified ISFET devices: The primary modification of the Al_2O_3 gate of an ISFET device was achieved by treating the ISFET with a solution of 3-aminopropyltriethoxysilane (0.2 μL, 10% (v/v) in toluene) at room temperature for 12 h.

*Preparation of the **2**-functionalized ISFET*: The silylated chips were thoroughly rinsed with toluene followed by a HEPES buffer solution (10 mM, pH 6.3), and were subsequently dried in air at room temperature for 30 min. Compound **2** was then covalently linked to the aminosiloxane-functionalized gate interface by treating the gate with HEPES buffer (0.2 μL) solution that contained **2** (1 mM) and EDC (10 mM) for 2 h. The resulting modified ISFET devices were thoroughly rinsed with the HEPES buffer solution and dried in air for 30 min.

*Preparation of the **1**- or **3**-functionalized ISFETs:* Compound **1** was covalently linked to the aminosiloxane-functionalized gate interface by treating of the gate with glutaric dialdehyde (0.2 μL, 10% (*v*/*v*) solution in water) at room temperature for 20 min. The chips were rinsed with water and then with a HEPES buffer solution (10 mM, pH 6.3) and then treated with **1** (0.2 μL, 1 mM in HEPES buffer solution) for 20 min. The **3**-modified gates were prepared by the same procedure as described for **1**.

Potentiometric ISFET measurements: ISFET devices (IMT, Neuchâtel, Switzerland) with Al_2O_3 gates (20 μm × 700 μm) were used in all experiments. A conventional Ag/AgCl electrode was used as a reference electrode. The **2**-functionalized gate was immersed in a working cell composed of phosphate buffer solution (0.8 mL, 0.01 M, pH 8.8), which contained **1** (1 mM). To follow the time-dependent potential changes on the surface of the gate, the measurement of the gate-to-source potentials were monitored for a time interval of 30 min. To extract the calibration curve for **1**, the **2**-functionalized ISFETs were exposed to various concentrations of **1** for 20 min, and the resulting devices were immersed in a working cell composed of phosphate buffer solution (0.8 mL, 0.01 M, pH 8.8).

To follow the activity of TR, a solution of **3** (1 mM) was treated with variable concentrations of TR in phosphate buffer (200 μL, 10 mM, pH 6.3) for a fixed time interval of 20 min. Subsequently, the enzyme was thermally deactivated by heating the sample at 80° for 2 min. The resulting mixture consisted of **1** and dopaquinone. The mixture was treated with citric acid (1 mM) for 5 min to reduce to **1** any dopaquinone that had been generated. The **2**-functionalized ISFET devices were then treated with the mixtures (0.2 μL) for a time interval of 20 min. The resulting de-

vices were immersed in a working cell composed of phosphate buffer (0.8 mL, 10 mM, pH 6.3).

To follow the TR activity by using the **3**-functionalized ISFET, the modified device was immersed in a working cell composed of phosphate buffer (0.8 mL, 10 mM, pH 6.3) that contained TR (2 U), and the response of the device was monitored for a time interval of 20 min. The calibration curve for analyzing TR activity by using the **3**-modified ISFET devices was extracted by interacting the modified sensing devices with different concentrations of TR in phosphate buffer (0.8 mL, 10 mM, pH 6.3) for 20 min. The resulting devices were then treated with ascorbic acid (1 mM) for 5 min to transform all of the dopaquinone that was generated to **1**. The resulting gate was then immersed in a working cell composed of phosphate buffer (0.8 mL, 10 mM, pH 6.3), and the potentiometric responses of the devices were monitored.

The analysis of TR activity by the **1**-functionalized ISFET was achieved by exposing the modified device to samples that contained different concentrations of TR outside of the working cell for 20 min. The resulting device was immersed in a working cell composed of phosphate buffer (0.8 mL, 10 mM, pH 6.3) and its potentiometric response was monitored. To regenerate the **1**-functionalized sensing ISFET device, the TR-treated ISFET was then treated with ascorbic acid (1 mM) for 5 min. In all of the ISFET devices, the potentiometric signal between the source of the ISFET and the reference electrode was recorded by using a semiconductor parameter analyzer (HP 4155B). The parameter that was monitored was the change in the gate-to-source voltage (V_{gs}), whereas the drain current (I_d) or the drain-to-source voltage (V_{ds}) remained at a constant value ($I_d = 100$ μA or $V_{ds} = 1.5$ V). All of the measurements were carried out for 5 min at ambient temperatures without stirring to simulate the conditions for possible future in vivo applications. Reproducibility of the measurements was ±2 mV in a number of experiments ($n = 3$). The detection limits were estimated according to IUPAC recommendations[12] as the intercept of the two linear segments in the calibration curves.

Acknowledgements

This study is supported by the Israel Ministry of Science as an Infrastructure Project and by the Intel Cooperation.

[1] a) P. Bergveld, *Sens. Actuators B* **2003**, *88*, 1–20; b) S. V. Dzyadevych, A. P. Soldtakin, A. V. El'skaya, C. Martelet, N. Jaffrezic-Renault, *Anal. Chim. Acta* **2006**, *568*, 248–258; c) M. J. Schçning, A. Poghossian, *Electroanalysis* **2006**, *18*, 1893–1900.

[2] a) S. V. Pogorelova, M. Zayats, A. B. Kharitonov, E. Katz, I. Willner, *Sens. Actuators B* **2003**, *89*, 40–47; b) J. J. Xu, W. Zhao, X. L. Luo, H. Y. Chen, *Chem. Commun.* **2005**, 792–794; c) O. A. Biloivan, S. V Dzyadevych, O. A. Boubriak, A. P. Soldtakin, A. V. El'skaya, *Electroanalysis* **2004**, *16*, 1883–1889.

[3] a) M. Zayats, O. A. Raitman, V. I. Chegel, A. B. Kharitonov, I. Willner, *Anal. Chem.* **2002**, *74*, 4763–4773; b) G. A. J. Besselink, R. B. M. Schasfoort, P. Bergveld, *Biosens. Bioelectron.* **2003**, *18*, 1109–1114.

[4] a) J.-K. Shin, D.-S. Kim, H.-J. Park, G. Lim, *Electroanalysis* **2004**, *16*, 1912–1918; b) F. Uslu, S. Ingebrandt, D. Mayer, S. Böcker-Meffert, M. Odenthal, A. Offenhäusser, *Biosens. Bioelectron.* **2004**, *19*, 1723–1731; c) J. Fritz, E. B. Cooper, S. Gaudet, P. K. Sorger, S. R. Manalis, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 14142–14146.

[5] M. Zayats, Y. Huang, R. Gill, C. A. Ma, I. Willner, *J. Am. Chem. Soc.* **2006**, *128*, 13666.

[6] C. Angeletti, V. Khomitch, R. Halaban, D. L. Rimm, *Diagnostic Cytopathology* **2004**, *31*, 33–37.

[7] C. W. Olanow, *Neurology* **1990**, *40*, 32–37.

[8] a) M. Lahav, A. N. Shipway, I. Willner *J. Chem. Soc. Perkin Trans. 2* **1999**, *9*, 1925–1931; b) S. M. Strawbridge, S. J. Green, J. H. R. Tucker, *Chem. Commun.* **2000**, 2393–2394; c) S. Tembe, M. Karve, S. Inamdar, S. Haram, J. Melo, S. F. D'Souza, *Anal. Biochem.* **2006**, *349*, 72–77.

[9] a) R. Baron, M. Zayats, I. Willner, *Anal. Chem.* **2005**, *77*, 1566–1571; b) X. M. Feng, C. J. Mao, G. Yang, W. H. Hou, J. J. Zhu, *Langmuir* **2006**, *22*, 4384–4389.

[10] D. Li, R. Gill, R. Freeman, I. Willner, *Chem. Commun.* **2006**, 5027–5029.

[11] R. Gill, R. Freeman, J. P. Xu, I. Willner, S. Winograd, I. Shweky, U. Banin, *J. Am. Chem. Soc.* **2006**, *128*, 15376–15377.

[12] R. P. Buck, E. Lindner, *Pure Appl. Chem.* **1994**, *66*, 2527–2536.

Received: May 15, 2007
Published online: August 8, 2007

