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Enzyme assays using sensor arrays based on ion-selective carbon nanotube field-effect transistors

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ABSTRACT

In the fields of clinical diagnostics and point-of-care diagnosis as well as food and environmental monitoring there is a high demand for reliable high-throughput, rapid and highly sensitive assays for a simultaneous detection of several analytes in complex and low-volume samples. Sensor platforms based on solution-processable electrolyte-gated carbon nanotube field-effect transistors (CNT-FETs) are a simple and cost-effective alternative for conventional assays. In this work we demonstrate a selective as well as direct detection of the products of an enzyme-substrate interaction, here the for metabolic processes important urea-urease system, with sensors based on spray-coated CNT-FETs. The selective and direct detection is achieved by immobilizing the enzyme urease via certain surface functionalization techniques on the sensor surface and further modifying the active interfaces with polymeric ion-selective membranes as well as pH-sensitive layers. Thereby, we can avoid the generally applied approach for a field-effect based detection of enzyme reactions via detecting changes in the pH value due to an on-going enzymatic reaction and directly detect selectively the products of the enzymatic conversion. Thus, we can realize a buffering-capacity independent monitoring of changes in the substrate concentration.

1. Introduction

In the fields of clinical diagnostics, point of care diagnosis systems, food quality management and water analysis there is a high demand for reliable high-throughput, rapid and highly sensitive assays for a simultaneous detection of multiple chemical as well as biological species in complex samples (Turner, 2013). Current detection methods for biological and chemical species are mostly based on spectroscopic, voltammetric or chromatographic assays, which are either limited in sensitivity or require expensive equipment with complicated data acquisition systems. Most applications additionally require a simultaneous and fast detection and analysis of multiple species in a small test volume. The availability of a sensor array with different functionalities could prevent a loss of information about time varying concentrations of different species in the test sample. The parallel read-out of all sensors can further reduce the assay time drastically. Additionally using an array configuration of several sensors with the same functionality could be an efficient solution for a simultaneous spatiotemporal recording of concentration gradients.

Sensors based on field-effect transistors (FETs) provide a simple and cost-effective approach for the recognition of very small concentrations of biological as well as chemical species (Goda and Miyahara, 2013; Hideshima et al., 2013; Huang et al., 2013; Jung et al., 2014; Li et al., 2014; Mu et al., 2014; Nguyen et al., 2014). By using highly sensitive and solution-processable nanomaterials like carbon nanotubes as active semiconducting material, the fabrication process of such FET-based sensors can be designed to be easy and cost-effective by using highly-reproducible scalable deposition techniques like spray-coating (Abdellah et al., 2011, 2013). FET-based sensors with carbon nanotubes as semiconducting layer, which are capable of identifying chemical and biological species, have been demonstrated previously (Choi et al., 2012, 2013; Kulkarni and Zhong, 2012; Lerner et al., 2013; Münzer et al., 2013a; Münzer et al., 2015), but are still limited to the detection of one certain species at a time.

By using multiple of these sensors in an array configuration a simultaneous identification of different chemical as well as biological species can be realized. The use of scalable deposition processes allows the fabrication of large sensor arrays as well as sensor arrays with a small sensor feature size, which can further increase the number of assay types performed at the same time or makes the recording of spatial concentration gradients possible. Due to the solution-processability of carbon nanotubes these

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arrays can be fabricated on rigid as well as flexible substrates (Abdellah et al., 2011; Haeberle et al., 2012; Melzer et al., 2015), which further opens the possibility to integrate these sensor arrays directly in microfluidic channels or lab-on-a-chip systems and allows a fabrication of cheap and disposable sensor systems.

In this work we demonstrate the possibility of the direct and simultaneous detection of multiple products of an enzyme-substrate interaction with sensor arrays based on carbon nanotube field-effect transistors modified with polymeric ion-selective membranes used in a planar electrolyte-gated configuration. As a model enzyme-substrate system we use the urea-urease couple since urea is one of the most important compounds generated in metabolic processes and therefore enzyme assays for a direct and fast detection changes in the urea concentration in physiological solutions like blood or urine are an extremely important tool for the diagnosis of several diseases (Koncki, 2007).

2. Material and methods

2.1. Fabrication of CNT-FET

For the water-based carbon nanotube ink 0.3 mg/ml of single-walled carbon nanotubes (SouthWest NanoTechnologies SweNT, SG65) and 10 mg/ml of sodium dodecyl sulfate (SDS, Sigma-Aldrich) were dispersed in deionized water (DI-H_2O, Millipore, resistivity of 18.2 MΩ cm). On a silicon wafer with a 200 nm thick silicon dioxide (SiO_2) layer on top (SiMat, $d_{ox}(SiO_2)=200$ nm) interdigitated gold (5 nm Cr, 40 nm Au) source and drain electrodes with a channel width (W) of 45 mm and length (L) of 50 μm (W/L=900) and the platinum (10 nm Ti, 40 nm Pt) gate electrode were patterned via photolithography, physical vapor deposition, and a subsequent lift-off process. Spray-coating of the water-based carbon nanotube ink through a shadow mask on the substrate with the pre-patterned electrodes forms a highly uniform carbon nanotube network in the channel region. A more detailed explanation of the spray-coating process can be found in references (Abdellah et al., 2011, 2013). Afterwards the samples are placed in DI-H_2O for 20 min at room temperature to remove the remaining surfactant and subsequently the devices are dried with nitrogen.

To cover the gate electrode with MW-CNTs a 0.16 mg/ml solution of multi-walled carbon nanotubes (Hanos CM 250) in isopropanol was prepared and 15 μl of the CNT solution were drop-casted on the planar gate electrode.

2.2. Functionalization of the CNT-FETs with urease

2.2.1. Modification of CNTs with urease via covalent sidewall functionalization

22.1 mg of MWCNTs-COOH were added to 4 ml of acetate buffer (0.1 M, pH =4.54). Dispersion of carbon nanotubes was prepared using a tip sonicator. After obtaining suspension, the MWCNTs-COOH were left on the magnetic stirrer (600 rpm), and then 20.1 mg of urease and 20 μl of EDC (1-ethyl-3–3-dimethylaminopropyl carbodiimide) were added. Suspension was left on the magnetic stirrer for 2.5 h. Suspension of MWCNTs-COOH with covalently bound urease was centrifuged (18,000 rpm) to separate modified CNTs from unbound urease. The MWCNTs-COOH modified by urease were dispersed in deionized water and again centrifuged.

2.2.2. Modification of CNTs with urease via π–π stacking

To attach the linking pyrene molecule on the carbon nanotubes the devices were put in a 2.3 mg/ml 1-pyrenebutanoic acid succinimidyl ester (Molecular Probes, Inc., USA) solution in dimethylsulfoxide (DMSO) for 6 h. Afterwards they were washed with clean DMSO and rinsed with DI-H_2O. To covalently bind the enzyme through an amide bond on the linking pyrene molecule the samples were immersed in DI-H_2O containing 10 mg/ml urease (Sigma-Aldrich) for 16 h, and afterwards washed thoroughly with DI-H_2O.

To guarantee that just either the MWCNTs on the gate electrode or the semiconducting CNTs in the channel area are functionalized with the enzyme, both functionalization solutions were just in contact with the respective area.

2.3. Electrical characterization

All electrical measurements were performed under ambient conditions at room temperature. For all electrical measurements a PDMS chamber was mounted around the active area of the sensor array to serve as a compartment for 100 μl of the sample solution, which was exchanged manually using a pipette (Gilson, Pipetman NEO P-1000N). Transfer curves (I_{SD} vs. V_{EG}, V_D constant) and output curves (I_D vs. V_{DS}, V_{EG} constant) were recorded using a Keithley Sourcemeter (2636 series) together with a customized automated data acquisition system. The gate voltage for the online measurements was chosen to be in the linear operation range of the transistor (I_{DS} vs. V_{EG}) to have the biggest modulation in the drain current due to changes in the analyte concentration. The drain voltage was chosen at $V_D=-0.4$ V to achieve a higher drain current recorded during the online measurements and therefore have a better signal-to-noise ratio.

All data points shown for the online measurements (I_D vs. time, V_{DS} and V_{EG} constant) correspond to the mean value and the standard deviation of 250 data points recorded under the respective conditions (changing pH value, changing ion concentration or changing urea concentration).

To read-out the two individual sensors of the array in parallel, two Keithley Sourcemeters (2636 series) were used. The common gate electrode was controlled with one channel and the two drain voltages were applied with two other channels, while reading out drain and gate currents. For both individual CNT-FETs the same source contact was used and grounded.

2.4. Sample preparation and handling

For details on the preparation of the Britton-Robinson buffer solutions and the buffer solutions used for the characterization of the H^+-selective CNT-FET please check Section 7 in the Supporting Information. The pH value and the conductivity of the samples were controlled with a conventional pH-meter (pHenomenal, VWR). Urea (ACS reagent, Sigma Aldrich) solutions with different concentrations ranging from 0.01 mM to 100 mM were prepared in a PBS buffer (pH 7.4, BioReagent, Sigma Aldrich). The concentration of the PBS buffer was changed by diluting the stock buffer solution (0.02 M phosphate buffered saline, 0.276 M NaCl, 0.0054 M KCl, pH 7.4 at 25° C) with DI-H_2O.

3. Results and discussion

Since many of the known enzyme-substrate reactions are based on an enzymatic catalysis of the substrate into certain products with the contribution of acidic or basic molecules, this can change the pH value of the surrounding electrolyte solution (Huang et al., 2015; Mu et al., 2014). Therefore electrolyte-gated carbon nanotube field-effect transistors (CNT-FETs), which are well-known to be in their non-functionalized state pH- as well as ion-sensitive (Haeberle et al., 2012; Heller et al., 2010; Münzer et al., 2013b; Münzer et al., 2013a) are an optimal sensor platform to detect different concentrations of the substrate. Moreover, to allow a