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In situ electrochemical synthesis of a conducting polymer composite for multi-metabolite sensing

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Abstract:

Electrochemical polymerization is a versatile method for rapid deposition of conducting polymer (CP) films. In addition to the fine control over the geometry of coatings, the technique enables to incorporate various biomolecules and additives in the CP matrix. The target substrates have, however, been limited to planar, metallic surfaces, hence, the devices that integrate electropolymerized CP films have predominantly been passive electrodes. In this work, we show that electrochemical polymerization has a high degree of freedom, allowing to grow biofunctionalized CP films in microscale transistor channels. We electrochemically deposit CP films from two monomers, namely, 3,4-ethylenedioxythiophene (EDOT) and hydroxymethyl EDOT (EDOTOH), inside the channel of an organic electrochemical transistor (OECT). When operated in aqueous electrolytes, the copolymer p(EDOT-*ran*-EDOTOH) shows charging capability and OECT performance superior to the homopolymer pEDOT that is electropolymerized under the same conditions. Moreover, the presence of hydroxyl groups facilitates stable incorporation of catalytic enzymes in the copolymer matrix during electropolymerization, rendering OECT channels biologically-functionalized, as confirmed by X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR). The OECT channels which are made of CP films with the entrapped enzyme glucose oxidase show output characteristics that change with respect to a broad range of glucose concentrations. Designed as a single, miniaturized chip, the multi-transistor platform comprising p(EDOT-*ran*-EDOTOH) derivatives that incorporate two other catalytic enzymes simultaneously measures glucose, cholesterol and lactate concentrations of a given fluid. With the ability to grow and pattern CPs functionalized with biorecognition units on miniaturized areas, this technique promises for the development of multiplexed platforms for electronic sensing of analytes.

Main Text:

The last decade has seen a tremendous growth in the development of conducting polymers (CPs) applied at the interface with biological milieu. These materials and devices made thereof have received particular interest for sensing weak biological signals and detecting small concentrations of biologically relevant molecules.^[1-2] Amongst all, poly(3,4-ethylenedioxythiophene) (PEDOT) is the most popular conjugated polymer due to its high electrical conductivity, optical transparency, soft mechanical properties, electrochemical and thermal stability, and biocompatibility.^[3-5] The most common form of PEDOT is the aqueous dispersion of the polymer that is doped and stabilized with polystyrenesulfonate (PSS), i.e., PEDOT:PSS. The commercial availability of PEDOT:PSS has further contributed to the success of this family, rendering the material the gold standard for bioelectronics as well as industrial applications. Despite its favorable characteristics, PEDOT:PSS has one major drawback, that is, the difficulty to tailor the polymer and add a functionality to meet the needs of a particular application.^[6-7] Synthetic efforts have thus focused on routes to develop EDOT derivatives that carry the functionality required for the intended application such as biochemical sensing,^[8-9] interfacing with living systems,^[10-11] electrochromism,^[12-13] and energy storage.^[14] Chemical oxidative polymerization enabled bulk production of PEDOT based CPs furnished with various functionalities coexisting with conductivity, however, the chemistry behind is not easily adaptable, limiting their wide spread use. A straightforward method omitting multiple and long steps of synthesis as well as hazardous chemicals and solvents used throughout the process is highly desirable to tailor the properties of PEDOT to suit numerous applications.

Electrochemical polymerization (or electropolymerization) is a strong candidate along this direction.^[15-16] Electropolymerization of CPs involves the electrochemical oxidation of the p-type monomer in the presence of its counterion in the reaction solution.^[17] The polymerization requires only small amounts of the monomer and the deposition is fast and localized on the

selected area of a conducting substrate. In addition to the nature of the counterion, the magnitude and type of the oxidation potential as well as the solvent used for the reaction have a strong effect on the characteristics of the resulting film and as such they stand as specific process parameters that can be employed to modify the resulting film properties.^[18] When biologically relevant molecules (such as proteins) are added into the reaction mixture, they typically end up incorporated into the CP network without chemical modifying the conjugated backbone. Despite being a small area coating technique, electropolymerization has thus rendered itself valuable for niche applications in bioelectronics as it allows for easy biofunctionalization of CPs. Biological molecules were either physically entrapped inside the polymeric matrix or those that carry negative charges were incorporated acting as the polyanion dopant.^[19-21] When electropolymerized in the presence of catalytic enzymes reacting with specific metabolites, CP films showed an electrical output proportional to the metabolite concentration.^[19-22]

Electropolymerization has almost exclusively been used to deposit CP films on electrode surfaces, which has led to the perception that the deposition is limited to electrode configuration. The first organic electrochemical transistor (OECT), the state-of-the-art device of organic bioelectronics, is, however, made of a CP film electropolymerized onto and within the gaps of interdigitated gold contacts.^[23] The OECT is an electrolyte gated transistor consisting of a (semi)conducting polymer film deposited between two metal electrodes (the source and the drain), and a gate electrode that is physically separated from the channel via an electrolyte.^[24] Because of the high gain of these devices resulting from the volumetric charging of the CP channel upon application of a gate voltage, the OECT configuration has been explored to sense a variety of biologically relevant species such as metabolites,^[25-26] ions,^[27-28] neurotransmitters^[29] and proteins such as Immunoglobulin G^[30] and amyloid beta.^[31] Ever since this first report of the electrodeposited OECT channel, only a recent work has demonstrated

that a CP can be electropolymerized within the source and drain electrodes separated by a few tens of micrometers.^[32] The authors showed that the channel was formed within minutes of application of an oxidizing source-drain voltage, generating a device with short- term and long- term memory functionalities.

Scheme 1. The conducting polymer composite grown in the organic electrochemical transistor (OECT) channel. The enzyme is physically entrapped within the conducting polymer network. The electropolymerization continues until the source and drain contacts are both covered with the polymer.

In aspiration of combining the features of electropolymerization with the performance of OECTs in biochemical sensing, in this work, we electropolymerize enzyme-functionalized PEDOT derivatives in microscale OECT channels. We grow conducting polymers by electrochemically addressing the drain contact of the channel and continue the polymerization until the polymer makes a physical contact with the source contact (Scheme 1). Adding catalytic enzymes such as glucose oxidase into the reaction mixture generates conducting polymers with

enzymes entrapped therein. We design a platform where each microscale channel is functionalized with a different enzyme, assembling altogether a multi-metabolite sensor. With the ability to form OECT channels which show a current response scaling with the concentration of the target metabolite, we explore the performance of this platform for sensing three metabolites at once from the mixture sample.

Firstly, we examine the channel forming ability of the monomers that we chose. We use hydroxymethyl EDOT (EDOTOH) alongside EDOT as the building blocks of the CP network, while ClO_4^- and water are used as the dopant and the medium, respectively (Figure 1a). Our polymers contain EDOTOH because the monomer introduces hydroxyl (-OH) groups to the polymer network which are envisaged to interact with target enzymes but also enable chemical coupling reactions for further modification.^[33-34] We chose ClO_4^- as the dopant instead of the more commonly used PSS^- because of the hygroscopicity and acidic nature of the latter leading to CPs with aqueous instability and sensitivity to humidity.^[35] Moreover, ClO_4 is known to lead to CP films with high porosity.^[36] Such a loose architecture should provide more space to the enzyme, increasing the amount of the protein accommodated therein. We electropolymerized three polymers under identical conditions: homopolymer of EDOT ($\text{pEDOT}:\text{ClO}_4$), homopolymer of EDOTOH ($\text{pEDOTOH}:\text{ClO}_4$) and the random copolymer made from these two monomers, $\text{p(EDOT-ran-EDOTOH)}:\text{ClO}_4$ (Figure 1a). The copolymer was prepared from a co-monomer feed ratio of 1:1. The channel was patterned to have a width of 100 μm and a length of 10 μm , and we used a 1.5 μm thick Parylene film to insulate the gold contacts/interconnects (see Experimental section for details on device fabrication). To facilitate electropolymerization, we designed the channel with a large overlap on gold contacts: the uninsulated area on top of each contact is about 1000 μm^2 (Figure 1b). The channel was formed within 120 seconds of a continuous potential at 1 V applied at the drain contact. We monitored the formation of the polymer in the channel using a microscope (Movie S1) and show selected

snapshots in Figure 1b taken before the polymerization starts, at the 1st minute of the polymerization and once the bias was stopped. Since the polymerization starts on one contact and proceeds towards the second one, the film thickness throughout the channel is not homogenous (Figure S1). The typical photolithographic fabrication of OECTs requires a second, sacrificial layer of Parylene coating and its removal (known as peel-off) to pattern the spin casted, drop casted or vapor deposited PEDOT films in the channel.^[37] By electropolymerizing the CP film in the channel, we omit this step as well as the antiadhesive coating before that. The thickness of the polymer grown in the channel can be up to 7 μm as revealed by cross-sectional FIB-SEM studies (Figure 1c). Moreover, the technique is compatible with smaller or larger channel dimensions (*e.g.* 10 μm x 10 μm and 50 μm x 50 μm for width x length) as long as the polymerization time is regulated.

Figure 1. **a)** Chemical structure of the polymers. **b)** Microscope images showing the growth of p(EDOT-ran-EDOTOH):ClO₄ in the OEET channel. Electropolymerization lasts for 2 mins and the images were taken at t=0 min (before the bias), at t=1 min (half time of polymerization) and t=2 min (from top to bottom). The polymerization starts at one gold contact by applying a constant potential at 1 V and continues until the two contacts are bridged by the polymer. The channel width and length are 100 μm and 10 μm, respectively, and the uninsulated area on each contact is 1010 μm². The scale bar is 50 μm. **c)** FIB-SEM image of p(EDOT-ran-EDOTOH):ClO₄ polymerized in situ. The Pt (1) and Carbon (2) layers were used as protective coatings, while (3) is the polymer, (4) is the gold and (5) is the glass substrate. The scale bar is 5 μm. **d)** The transfer characteristics of OEETs with electropolymerized channels recorded

using an Ag/AgCl electrode immersed in PBS (10 mM). The dashed lines represent the transconductance (g_m) as a function of V_G , measured at $V_D = -0.6$ V.

All the three polymers electropolymerized in situ exhibit clear transistor characteristics with well-defined linear and saturation regimes (Figure S2). Since the polymers are intrinsically doped, the transistors operate in depletion mode. The electropolymerized films in the channel are electrochemically de-doped upon application of a positive voltage at the gate electrode (V_G) and the drain current (I_D) decreases further with an increase in V_G (Figure 1d). Among the three polymers electropolymerized under the same conditions, pEDOTOH:ClO₄ exhibits the highest current at $V_G = 0$ V and the largest modulation in the I_D : the maximum transconductance (g_m , measured at $V_D = -0.6$ V) is 2.36 mS at $V_G = 0$ V (Figure 1e). The copolymer based OEETs, on the other hand, outperform those comprising the pEDOT homopolymer.

Figure 2. **a)** Cyclic voltammetry (CV) curves and **b)** electrochemical impedance spectra (Bode plot) of the polymers deposited on Au electrodes (500 μm x 500 μm). CV measurements were performed at a scan rate of 0.1 mV s⁻¹ over a potential window of -0.6 to 0.6 V vs. Ag/AgCl,

while the impedance spectra were acquired at 0 V vs the open-circuit potential (V_{oc}). The electrolyte is 10 mM PBS. Scanning electron microscopy (SEM) images of these films: **c**) pEDOT:ClO₄, **d**) pEDOTOH:ClO₄, and **e**) p(EDOT-*ran*-EDOTOH):ClO₄. The scale bar is 1 μ m. pEDOT:ClO₄, pEDOTOH:ClO₄ and p(EDOT-*ran*-EDOTOH):ClO₄ films have a thickness of 2.7 μ m, 3.9 μ m and 3.2 μ m, respectively, deposited on Au electrode.

To understand the differences in the OECT performance, we investigated the electrochemical properties of the films. Cyclic voltammetry curves of the films recorded in PBS show the typical capacitive behavior of PEDOT based CPs,^[38] with pEDOTOH exhibiting the highest current density values (Figure 2a). The electrochemical impedance spectra show that pEDOTOH film has the lowest impedance magnitude at all the frequencies scanned (Figure 2b). As we cannot determine the molecular weight and the number of repeat units of the polymers, we report the volume normalized capacitance of the films. By using the complex impedance formula, we construct capacitance-frequency plots (Figure S3) and extract the volumetric capacitance by dividing the capacitance at 0.1 Hz with the volume of the corresponding film. We calculate the volumetric capacitance of pEDOT, pEDOTOH, and p(EDOT-*ran*-EDOTOH) to be 102 Fcm⁻³, 227 Fcm⁻³, and 153 Fcm⁻³, respectively. The films that contain EDOTOH exhibit a higher electrochemical capacitance than the pEDOT, in agreement with previous work reporting that hydroxymethyl groups increase the electropolymerization efficiency resulting in polymers with low electrochemical impedance.^[39-44] The capacitance of a polymer film is governed by the charge carrier density (depending on the length of conjugated backbone and the delocalization of the polaron along the chain) and the ability of ions to penetrate the film. A dense network can impose a mechanical barrier for ion penetration whereas a porous film will typically allow for facile penetration as well as enhanced water uptake which will improve ion transport in water swollen channels.^[45] The PEDOT film exhibits a relatively smooth surface, while EDOTOH based film surfaces are rougher with a larger area, which is expected to contribute to

the high capacitance (compare Figure 2c with d and e). The dense population of OH groups in the homopolymer pEDOTOH, however, may lead to steric hindrance during bioconjugation, providing a low degree of conformational freedom for biorecognition units.^[9,28,33,46] Secondly, EDOTOH monomer has low solubility in water with aggregates visible to bare eye in the reaction mixture. Despite the superior electrochemical performance of pEDOTOH, we thus chose to further work with the copolymer p(EDOT-*ran*-EDOTOH). While exhibiting high transconductance, p(EDOT-*ran*-EDOTOH) also benefits from the presence of OH groups. For instance, while a fluorescent dye labeled insulin could readily adhere on the p(EDOT-*ran*-EDOTOH) film surface, it does not show an affinity to pEDOT film (Figure S4). Hydroxyl-rich network of the copolymer enables hydrogen bonding and electrostatic interactions with biomolecules.^[11, 41, 46-48] Taken altogether, we conclude that p(EDOT-*ran*-EDOTOH) is the ideal polymer to incorporate enzymes via electropolymerization and for use in an OECT channel.

Figure 3. Compositional characterization of the p(EDOT-*ran*-EDOTOH) films prepared in the presence (red) and absence (green) of the enzyme (glucose oxidase, GO_x) in the reaction mixture. **a)** High-resolution of XPS C 1s spectra of the polymer films. The peak at 287.6 eV is attributed to the characteristic bond of C=O originating from the amino acids. **b)** High-resolution of N 1s XPS spectra of the polymer films. The spectrum of the enzyme entrapped

polymer film demonstrates a characteristic peak for NH_2 groups. **c)** FTIR spectra of the polymer films.

We next electropolymerized p(EDOT-*ran*-EDOTOH) films in the presence of the catalytic enzyme, glucose oxidase (GO_x), in the reaction mixture. To investigate the incorporation of the enzyme in the CP matrix, we used X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FTIR). The high-resolution XPS C 1s spectrum of the enzyme-functionalized copolymer shows a peak at 287.6 eV corresponding to C=O bond (Figure 3a).^[49] The N 1s spectrum of this polymer film displays a distinct peak at 399.6 eV attributed to NH_2 groups (Figure 3b). Neither of these peaks are present in the spectra of the pristine copolymer and both C=O and $-\text{NH}_2$ groups originate from the enzyme. FTIR spectra support these findings: we detect C=O bonds only in the spectrum of the copolymer polymerized in the presence of the enzyme (Figure 3c). These results suggest the GO_x molecules are incorporated into the polymer network, and not only bound on the surface of the film but also in its bulk.

Figure 4. Sensing performance of the OECT with GO_x -entrapped p(EDOT-*ran*-EDOTOH) channel. **a)** Real-time response of the channel to successive additions of glucose from 10 μM to 40 mM. Inset shows the changes in the I_D of a pristine (GO_x -free) p(EDOT-*ran*-EDOTOH) channel in response to glucose. **b)** The change in channel current (ΔI_D) as a function of glucose concentrations. Inset shows the same plot in logarithmic scale. **c)** Real-time response of the sensor to the additions of uric acid (UA), ascorbic acid (AA), dopamine (Dop) and glucose

(Glu) into the measurement solution and to subsequent rinsing with PBS. The error bars were derived from measurements with 3 devices. The gate electrode is Ag/AgCl and the electrolyte is PBS (10 mM), $V_D = -0.2$ V and $V_G = 0$ V.

As we verified that polymer channel is successfully functionalized with GO_x , we next investigated whether the enzyme incorporated is catalytically active and the performance of the OECTs in detecting glucose. An active enzyme will electronically communicate with the polymer, transferring electronic charges and byproducts generated upon its reaction with the metabolite to the conducting network. We first recorded the transfer curves of an enzyme-free and an enzyme-entrapped channel in PBS containing a variety of glucose concentrations (Figure S5). While the biofunctionalized device showed an increase in its current scaling with glucose content of the solution at all gate voltages applied, the pristine (enzyme-free) device had no response to glucose. The changes in the drain current in response to glucose are maximized at $V_G = 0$ V, which is also the gate voltage that leads to the highest transconductance (Figure 1e). We, therefore, monitored the current of the channel when it is gated continuously with V_G at 0 V while changing the glucose concentration in the electrolyte. Figure 4a displays the real time changes in channel current in response to different glucose concentrations. The current increases with the glucose content of the electrolyte from 10 μ M to 40 mM. In contrast, enzyme-free channel is insensitive to glucose (inset to Figure 4a), suggesting that the entrapped enzyme is responsible from the current response. Note that during these measurements the gate current is in the range of few nA and seems to be independent of the glucose content of the electrolyte (Figure S6a), indicating that glucose detection happens in the channel. In the conventional three-electrode electrochemical configuration, the current measured from this film in the microscale channel is much lower and the change that the enzymatic reaction causes is very small when monitored in chemiresistor configuration (Figure S6b-c). Despite the inherently low current of such a microscale pattern, we are able to detect the glucose dependent changes

therein thanks to the OECT configuration. OECT provides a high channel current right at the point of detection, making the measurement much less sensitive to noise associated with external amplifiers.^[50] Note that the gate voltage applied is zero, meaning that the system is practically biased with just one power supply. These results justify the use of OECT configuration which allows to record signals from microfabricated glucose-responsive CPs without the need of external amplifiers.

We attribute the increase in I_D upon enzymatic reaction to the oxidation of the CP film with hydrogen peroxide (H_2O_2), a by-product of the enzymatic reaction. To verify this hypothesis, we recorded the impedance spectrum, CV curve and drain current of the GO_x -entrapped channel as H_2O_2 is added into the measurement solution (Figure S6d-f). With H_2O_2 present in the solution, we observe a lower impedance at the 1-100 Hz regime accompanied with an increase in the anodic current. The current flowing in the channel increases with H_2O_2 , particularly at high concentrations (10 mM). Recent work reported PEDOT:PSS to be an effective catalyst for H_2O_2 , i.e., neutral PEDOT is oxidized by H_2O_2 to its charged bipolaronic form in acidic conditions.^[51] We thus hypothesize that the detection mechanism of these OECT sensors is based on H_2O_2 catalysis at the EDOT comprising channel. Note that the increase in the channel current caused by H_2O_2 is not as significant as that triggered by glucose. We attribute this to the limited interactions between H_2O_2 and EDOT when H_2O_2 is introduced externally to the solution. During the enzymatic reaction, H_2O_2 is released locally at the reaction site in the channel, and as such it effectively reacts with pEDOT chains. On the other hand, when H_2O_2 is introduced into the solution by pipetting, its interactions with the polymer are restricted mostly onto the surface of the film and limited by diffusion.

Figure 4b shows that the change in the channel current is proportional to the concentration of glucose, allowing for its quantification. The sensitivity of the device is $4.04 \mu A m M^{-1}$ and 0.35

$\mu\text{A mM}^{-1}$ in the range of 0.1 mM to 1 mM and 1 mM to 10 mM, respectively. Moreover, the device shows negligible response to endogenous electroactive species commonly found in biological media such as uric acid, dopamine and ascorbic acid (Figure 4c). Besides its compatibility with biorecognition elements that can be sensitive to high bias, the low gate bias that we use ($V_G = 0$ V) minimizes signals from these species which can interfere with those arising from the oxidation of glucose by the enzyme. Once rinsed with PBS, the current reverts to its initial value and the device can be used again for glucose detection. The OECT can indeed be continuously operated with square shaped pulses at the gate electrode for more than 6 hours with negligible decay of performance (Figure S7). We evaluated the stability of the device response for a period of two months by recording the current output in response to 100 μM of glucose. The devices used for this test have been stored in sealed vacuum bags at 4 °C after each measurement. After two months, 65 ± 4 % of the current response is maintained (Figure S8) however the sensors need to be re-calibrated before use.

Figure 5. Multi-metabolite sensing using enzyme-entrapped OECT sensors. **a)** Photograph of a 4-channel OECT array. Each channel has width of 100 μm and length of 10 μm . The scale bar is 200 μm . **b)** Calibration curves of the sensors to different concentrations of cholesterol and lactate. **c)** The response of the 4-channel OECT array to 100 μM of glucose, 100 μM of lactate and 100 μM of cholesterol as well as a mixture containing all these metabolites. $V_D = -0.2$ V and $V_G = 0$. Each color represents the response of a channel functionalized with one enzyme alongside the response of bare p(EDOT-*ran*-EDOTOH) channel (green).

Glucose oxidase is not the only enzyme which can be incorporated into p(EDOT-*ran*-EDOTOH) based OECT channels. To demonstrate the applicability of the approach, we entrapped two other catalytic enzymes, cholesterol oxidase (ChO_x) and lactate oxidase (LO_x) in two other channels of one OECT array platform, for cholesterol and lactate detection, respectively (Figure 5a). We fabricated 4 OECT channels in a 1x1 mm area, where channel 1, 2 and 3 are assigned for glucose, cholesterol and lactate sensing, respectively, and they contain

the respective enzyme. Channel 4 is set as the reference sensor to monitor any change in current that is triggered by interferences, for instance, H_2O_2 generated during the enzymatic reactions happening in the neighbor channels as it diffuses out to the solution. The enzyme-entrapped OECTs have a current response linearly scaling with the respective analyte and the channels are not responsive to non-target metabolites. The sensitivity to cholesterol and lactate is $1.94 \mu\text{A mM}^{-1}$ and $2.31 \mu\text{A mM}^{-1}$ in the range of 0.1 to 1 mM, respectively (Figure 5b). The superior performance of glucose sensing is a result of the more robust nature of the enzyme glucose oxidase compared to others.^[52] Although electropolymerization conditions are set to induce minimal interference on enzyme structure (the aqueous reaction medium, the low magnitude of the voltage applied and its duration), denaturation is unavoidable as multiple interactions can take place between p(EDOT-*ran*-EDOTOH) and the protein. Note that while these interactions may reduce the activity of the enzyme, they are also required to prevent its leakage.^[53] Figure 5c displays the current response of these four channels to different solutions. Each channel that is functionalized with a particular enzyme exhibits the highest current response to the corresponding, target metabolite. For example, GO_x -functionalized channel is most sensitive to glucose in the solution, similar to LO_x - and ChO_x -functionalized channels exhibiting highest current changes in response to lactate and cholesterol, respectively. The functionalized channels show a current response to non-target metabolites; however, the values are much lower than the signals generated by target metabolites. Such interference can be accounted for by recording the response of the reference channel. Future work will focus on improving the readout accuracy of this platform by involving microfluidics which will avoid crosstalk between the channels.

In this work, we presented an easy approach to electrochemically grow conducting polymers within the microscale channel of an organic electrochemical transistor (OECT). The hydroxyl containing copolymer p(EDOT-*ran*-EDOTOH) showed remarkable OECT performance and

strong affinity to biomolecules. We entrapped catalytic enzymes inside the matrix of this copolymer during electropolymerization and used the bio-functionalized channels to detect metabolites. The glucose oxidase-functionalized channel showed a current response scaling with the glucose content of solution, within the range of 10 μM to 10 mM, and with stability up to 2 months. We designed a multi-channel platform where each microscale channel is functionalized with a different enzyme, assembling altogether a multi-metabolite sensor. With the ability to form OECT channels which show a current response specific to the concentration of the target metabolite, we explored the performance of this platform for sensing three metabolites at once from complex media. Conducting polymers developed in this work can be integrated into different bioelectronic device configurations, while the electropolymerization method can easily be adapted to functionalize polymers with various biomolecules via molecular entrapment or chemical bonding through reactive groups such as hydroxyls.

Experimental Section

Materials

We purchased 3,4-ethylenedioxythiophene (EDOT), hydroxymethyl EDOT (EDOTOH), lithium perchlorate (LiClO_4), glucose oxidase (GO_x), lactate oxidase (LO_x), cholesterol oxidase (ChO_x), fluorescein isothiocyanate (FITC) labeled insulin, human serum from human male AB plasma, and phosphate buffer solution (PBS) from Sigma-Aldrich, and used all as received. We purchased S1813 and AZ9260 photoresists as well as MF-319 and AZ developers from Microchemicals GmbH. The aqueous solutions were prepared using ultrapure water (Millipore Milli-Q).

Fabrication of organic electrochemical transistor (OECT)

We fabricated the OECTs on glass wafers (D 263® T eco, Schott) using photolithography. We started the device fabrication by patterning of Au contacts and interconnects. We deposited the photoresist S1813 on cleaned substrates, followed by the exposure to UV light using contact

aligner and development with MF-319 developer. We next sputtered 10 nm of Cr and 100 nm of Au on the substrates and patterned the metal through a standard lift-off process. The second layer of photoresist AZ9260 was then spin-coated on the substrate and developed using AZ developer to define the channel areas. A Parylene-C layer (ca. 1.5 μm) was evaporated to insulate the gold interconnects. The channel patterns and the contact pads were opened via reactive ion etching. The polymer was electropolymerized in the channel as described above.

Electropolymerization of the conducting polymer and enzyme entrapment

We prepared an aqueous dispersion of EDOTOH (20 mM) and of EDOT (20 mM) for the polymerization of the homopolymers, and an aqueous mixture of EDOTOH (10 mM) and EDOT (10 mM), i.e. the feed ratio of 1:1, for the copolymer. We added 100 mM of LiClO_4 as the counter ion and sonicated the dispersion for 30 min at room temperature. This dispersion was then used as the reaction solution placed on top of the transistor channel. An Ag/AgCl and a platinum wire were immersed into the solution and used as the reference and counter electrode, respectively. Potentiostatic electropolymerization mode at 1 V was performed for 120 s until the polymer grew and covered the uninsulated area of both source and drain contacts of the channel that were in contact with the solution. We then washed the electropolymerized film with deionized water and dried the samples with N_2 spray to remove any material that has weakly bound to the surface as well as unreacted monomers. For the functionalization of the film with the enzyme, the respective enzyme was added into the monomer dispersion (5 mg/mL) and a portion of it got entrapped into the polymer network during the electro-synthesis.

Physicochemical characterization

We examined the surface morphology of the polymer films using FEI Nova nano scanning electron microscope (SEM) with an accelerating voltage of 3 kV and a working distance of 5 mm. The films were mounted onto aluminum stubs and attached with conductive double-sided tape. Cross-sectional images were taken with a focused ion beam (FIB)/SEM (FEI Helios NanoLab 400S). FIB/SEM dual-beam system was equipped with a Ga^+ ion source. Carbon and

platinum layers were deposited on the surface region of interest by Electron & Ion beam for sample protection. To characterize the elemental composition of the films, we performed X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared (FTIR) studies. XPS was carried out using a KRATOS Analytical AMICUS instrument equipped with an achromatic Al K α X-ray source (1468.6 eV). The source was operated at a voltage of 10 kV and a current of 10 mA generating a power of 100 Watts. The high-resolution XPS spectra were acquired with a step of 0.1 eV. The pressure in the analysis chamber was in the range of $\times 10^{-7}$ Pa during the course of the measurements. We recorded the FTIR spectra in the range 550-4000 cm $^{-1}$ at room temperature using Thermo Scientific Nicolet iS10. We used attenuated total reflection (ATR) FTIR mode to obtain the reflectance infrared spectra of the polymer film. We signal-averaged 64 scans to form a single spectrum which was then displayed in terms of transmittance. The baseline was corrected using OMNIC FTIR software.

Electrochemical characterization

We evaluated the electrochemical performance of each polymer film using a potentiostat (Autolab PGstat128N, MetroOhm) and a three-electrode configuration. The polymer films were grown on top of Au coated electrode (500 μ m x 500 μ m) as described above. A three-electrode electrochemical cell was used with a 10 mM PBS solution (pH = 7.4) used as the electrolyte and a platinum wire as the counter electrode and Ag/AgCl electrode (in 3 M KCl) as the reference electrode. For the CV measurements, the potential was swept from -0.6 to +0.6 V at a scan rate of 10 mV/s. The impedance measurements were performed by applying 10 mV sinusoidal perturbations onto a DC potential fixed at 0 V, -0.2 V and 0.2 V vs. Ag/AgCl (or vs. open circuit potential (V_{OC})) in the range of 0.1 to 10000 Hz. All measurements were performed at room temperature inside a grounded Faraday cage and in air unless stated otherwise. The capacitance-frequency plots were constructed by using the complex impedance formula as described in equation 1.

$$Z = \sqrt{R^2 + \frac{1}{\omega^2 C^2}} \quad (1)$$

OECT characterization and evaluation of sensor performance

We characterized OECTs using a semiconductor analyzer (Keithley 2612 A) controlled with customized LabVIEW software. The output and transfer characteristics were recorded by sweeping the drain voltage (V_D) from 0 to -0.6 V and gate voltage (V_G) from 0 to +0.6 V. For the pulsed V_G measurements ($V_G = 0$ V applied for 5 seconds), a constant V_D of -0.2 V was used. The gate electrode was Ag/AgCl, the electrolyte was 10 mM PBS and we used a poly(dimethylsiloxane) (PDMS) well to confine the electrolyte on top of the transistor. For the sensing measurements, after stabilization of the background current, aliquots of metabolite solution at different concentrations were introduced to the solution while maintaining a constant electrolyte volume (30 μ L) and the current changes were recorded in real-time. The response of the OECT to the corresponding metabolite (ΔI_D) was calculated by the following equation:

$$\Delta I_D = I - I_0 \quad (2)$$

where I and I_0 are the current outputs of the OECT in the presence and absence of the metabolite, respectively.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Supplementary Information

for

In situ electrochemical synthesis of a conducting
polymer composite for multi-metabolite sensing

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Figure S1. SEM image of the copolymer p(EDOT-*ran*-EDOTOH) electropolymerized in the OECT channel. The scale bar is 5 μm .

Figure S2. Output characteristics of **a)** pEDOT, **b)** pEDOTOH, and **c)** p(EDOT-*ran*-EDOTOH) OECTs gated with an Ag/AgCl pellet immersed in PBS (10 mM).

Figure S3. The capacitance versus frequency plots of the polymer films deposited on Au electrodes ($500\ \mu\text{m} \times 500\ \mu\text{m}$). The electrochemical impedance spectra were recorded at $0\ \text{V}$ vs V_{oc} in PBS.

Figure S4. Fluorescence microscopy images of pEDOT (a,c) and p(EDOT-ran-EDOTOH) (b,d) deposited into the OECT channel ($100\ \mu\text{m} \times 10\ \mu\text{m}$, width x length, top) and on the Au patterns ($500\ \mu\text{m} \times 500\ \mu\text{m}$, bottom). The fluorescein isothiocyanate (FITC) labelled insulin molecules (green) were entrapped within the polymer matrix during electropolymerization.

Figure S5. Transfer characteristics of p(EDOT-ran-EDOTOH) OECTs in the presence of glucose in PBS solution. a) The channel comprises GO_x and b) the channel is GO_x -free

Figure S6. **a)** Gate current (I_G) of GO_x-entrapped p(EDOT-ran-EDOTOH) OEECT during chronoamperometric sensing measurements. The spikes indicate the time points where we introduce a new concentration of glucose into the solution. **b)** CV curve and **c)** the amperometric response of GO_x-entrapped p(EDOT-ran-EDOTOH) film in the channel in the presence of glucose in PBS. **d)** Electrochemical impedance spectra and **e)** CV curve of the GO_x-entrapped p(EDOT-ran-EDOTOH) film recorded in the presence of different concentrations of H₂O₂. **f)** Drain current of GO_x-entrapped p(EDOT-ran-EDOTOH) OEECT in response to H₂O₂ addition and subsequent rinsing with PBS.

Figure S7. The operational stability of a p(EDOT-*ran*-EDTOH) OEET upon square-shaped pulses continuously applied at $V_G = 0.1$ V for about 6 hours. $V_D = -0.2$ V.

Figure S8. The stability of the OEET sensors that have been stored for up to two months. The current response to 100 μM of glucose was tested multiple times at each day.