# Measurement of Barrier Tissue Integrity with an Organic Electrochemical Transistor

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The ability of organic electronic materials to interface with biological systems has been much vaunted of late, with examples showing the elegant use of these materials to deliver neurotransmitters in vivo,[1] control cell adhesion[2,3] and migration[4] and measure neuronal activity in vivo.[5,6] With the unique ability of organic electronic materials to conduct both electronic and ionic carriers, they act as an ideal platform for the integration of electronic and biological systems. The organic electrochemical transistor (OECT) has been recently employed as a sensor for DNA,[7] enzymes[8] and cell attachment.[9] Here, we show for the first time the integration of OECTs with human cells for assessing barrier tissue layer integrity. We are able to measure minute variations in paracellular ionic flux induced by toxic compounds in real time, with unprecedented temporal resolution and extreme sensitivity. The ability to measure paracellular transport is important as it provides a wealth of information about barrier tissue function, and disruption or malfunction of the structures involved in transport through barrier tissue is often indicative of toxicity or disease. Development of this technology will have implications for toxicology, drug development, infectious disease diagnostics and basic research on the molecular biology and electrophysiology of barrier function.

Barrier tissues in multicellular organisms serve as essential functional interfaces, maintaining highly regulated fluid compartments by limiting the diffusion of ions, macromolecules and other cells, while selectively absorbing nutrients, electrolytes and water that sustain the host.[10] Examples of barrier tissue in the body include the intestinal epithelium, kidney epithelium and the blood brain barrier endothelium. Epithelial and endothelial cells generally exist in single layers and are joined to each other by specialized complexes that include the adherens junction and the tight junction (TJ).[11] TJs restrict paracellular diffusion[12,13] by regulating the passage of molecules across the barrier as they selectively open and close in response to signals from inside and outside the cells. The degree of barrier tissue integrity serves as an indicator for disease state[14,15] and also of the appropriateness of a particular in vitro model for use in toxicology and drug screening. Accurate in vitro cell models are urgently required, as current in vivo methods for evaluating toxic compounds or drugs are expensive and time consuming. Efficient methods of characterization will help to validate and develop models as replacements for animal testing, an essential component of the European REACH regulation to handle toxicological profiling of more than 30 000 chemical substances imported into Europe.

Currently, two main parameters are used for assessing the integrity of barrier tissue: permeability and trans-epithelial/endothelial electric resistance (TER or TEER). In general, a correlation between permeability of a cell layer and the TER exists, with tight cell layers exhibiting high TER and low permeability.[10,13] Permeability is typically measured by monitoring the flux of radiolabeled/fluorescent compounds across cells cultured on suspended porous filters (Transwell). TER can be measured with a handheld epithelial volt-ohm meter. Unfortunately, data collection is associated with slow temporal resolution, poor measurement reproducibility and incompatibility with high throughput methods. Impedance spectroscopy can be employed to measure the capacitance and resistance of the cell layer in a more automated and reproducible fashion.[16,17] This technology has provided significant contributions to the field, but requires expensive equipment and still presents limitations in temporal resolution and sensitivity. Here, we present a method for monitoring in situ barrier tissue integrity using an organic electrochemical transistor (OECT). With the proposed device, we introduce a novel mechanism for sensing barrier tissue integrity achieved with a simple device format, compatible with low cost solution-based fabrication.[18] The OECT presents an exciting new avenue for assessing barrier tissue cell layers, with their exquisitely tuned ionic transport systems.[10]

The integration of barrier tissue with an OECT is illustrated in **Figure 1**a. PEDOT:PSS, a conducting polymer (CP) with demonstrated biocompatibility and stability,[8,9] is used as the active material in the transistor channel. The cell layer is grown on a Transwell membrane and incorporated into the device prior to measurement. In order to understand the sensing mechanism of the device, it is necessary to briefly review the operating mechanism of an OECT. The OECT comprises a degenerately doped CP channel in contact with an electrolyte.[6,19] A gate electrode is immersed in the electrolyte, and source and drain contacts at either side of the transistor channel measure the drain current ($I_D$). On application of a positive gate voltage ($V_G$), cations from the electrolyte enter the channel, de-doping the CP and decreasing the drain current ($I_D$). The steady state

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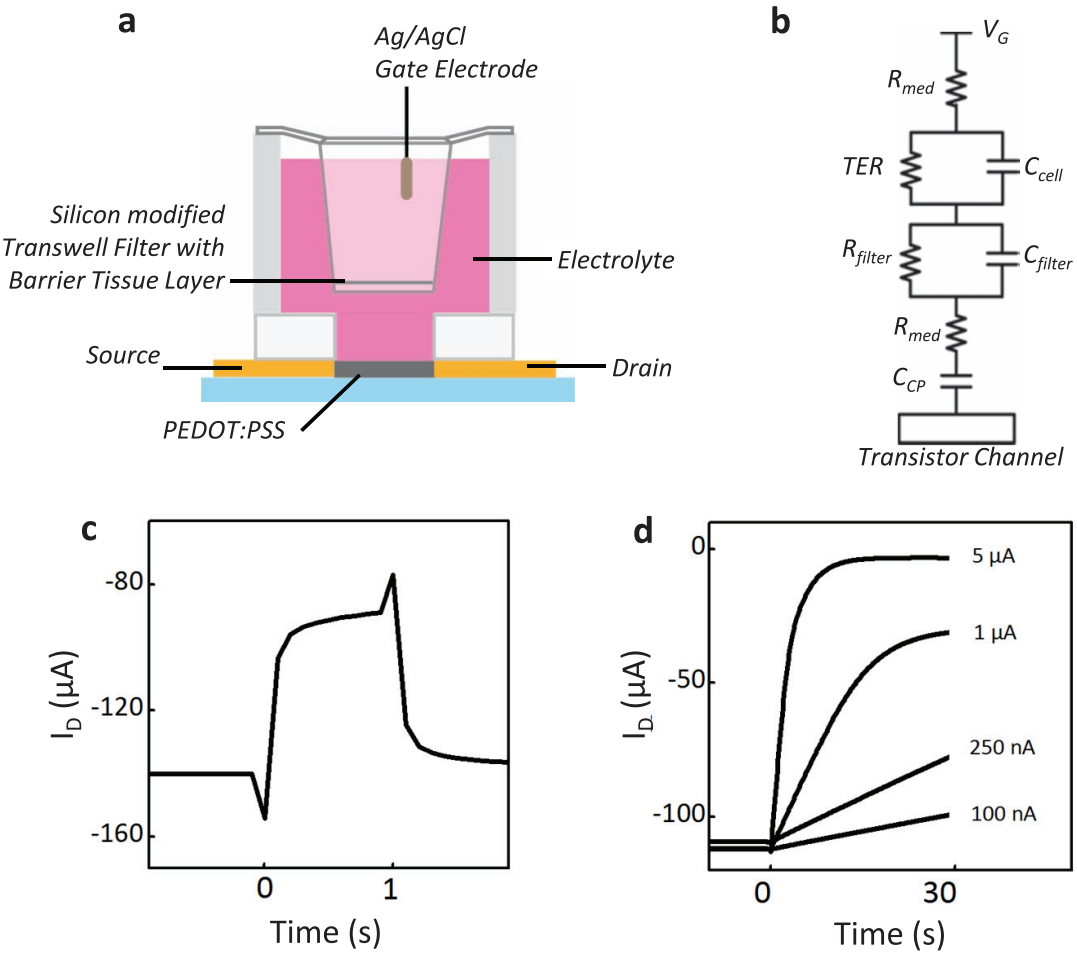


**Figure 1.** The integration of barrier tissue with an OECT. a) Device architecture. b) Equivalent circuit describing ionic transport between gate electrode and transistor channel. TER refers to the transepithelial resistance of the cell layer, $C_{cell}$ refers to the capacitance of the cell layer, $R_{filter}$ and $C_{filter}$ refer to the resistance and capacitance of the porous filter, respectively, $R_{med}$ refers to the resistance of the media, and $C_{CP}$ refers to the capacitance at the CP and electrolyte layer. c) OECT $I_D$ transient response to a $V_G$ pulse. d) OECT transient $I_D$ response to a 30 s pulse of constant $I_G$ of varying magnitudes, as labeled. As with other experiments, $V_D = -0.1$ V.

$I_D$ is linearly proportional to the total number of ions that have entered the polymer film, and the ionic flux determines the speed at which the transistor reaches steady state.[20] Thus, the OECT acts as a transducer of ionic signal to electronic current. A key difference in the use of a transistor as the sensing element, opposed to a conventional electrode,[16,17,21] is the inherent amplification associated with the former, where small changes in the ionic flux are integrated and amplified in the drain current. It has been shown that the ionic current in an OECT can be modeled using a combination of linear circuit elements.[20] The modification of the OECT ionic circuit on the addition of barrier tissue is shown schematically in Figure 1b, with the cell layer represented as a resistor and capacitor in parallel.[16] The resistance of the cell layer (the TER) influences the ionic flux into the channel: a higher TER will result in a lower ionic flux, and vice versa. Figure 1c shows the OECT $I_D$ response to a square voltage pulse in the absence of a cell layer. The hypothesized effect of TER is illustrated in Figure 1d by subjecting the device to pulses of constant gate current ($I_G$): as $I_G$ is decreased, the transient $I_D$ response is slower. The influence of the cell layer on the ionic flux is thus reflected in $I_D$. The sensing mechanism presented here is in contrast to a previous application of an OECT as a whole cell sensor for measurement of cell adhesion and detachment.[9] With cells grown directly on the CP channel, authors demonstrated a change in steady state transistor performance as a function of cell coverage, attributed to electrostatic modification of the cell/CP interface. While this device was able to detect gross cell detachment, our device is designed to dynamically address the health of a tissue layer that may be reversibly or temporarily damaged. Furthermore, the use of Transwell filters as the support for the cell layer renders our device completely compatible with existing barrier tissue characterization and toxicology techniques.

To demonstrate the application of an OECT as a sensor for barrier tissue integrity, we chose to use a well-known model for the gastrointestinal tract: the Caco-2 cell line.[22] **Figure 2**a shows a cartoon monolayer of polarized Caco-2 cells with tight junction protein complexes (left) and without (right). In Figure 2b (left), we demonstrate that in the presence of cells with TJs, the barrier properties slow the OECT $I_D$ response time, compared

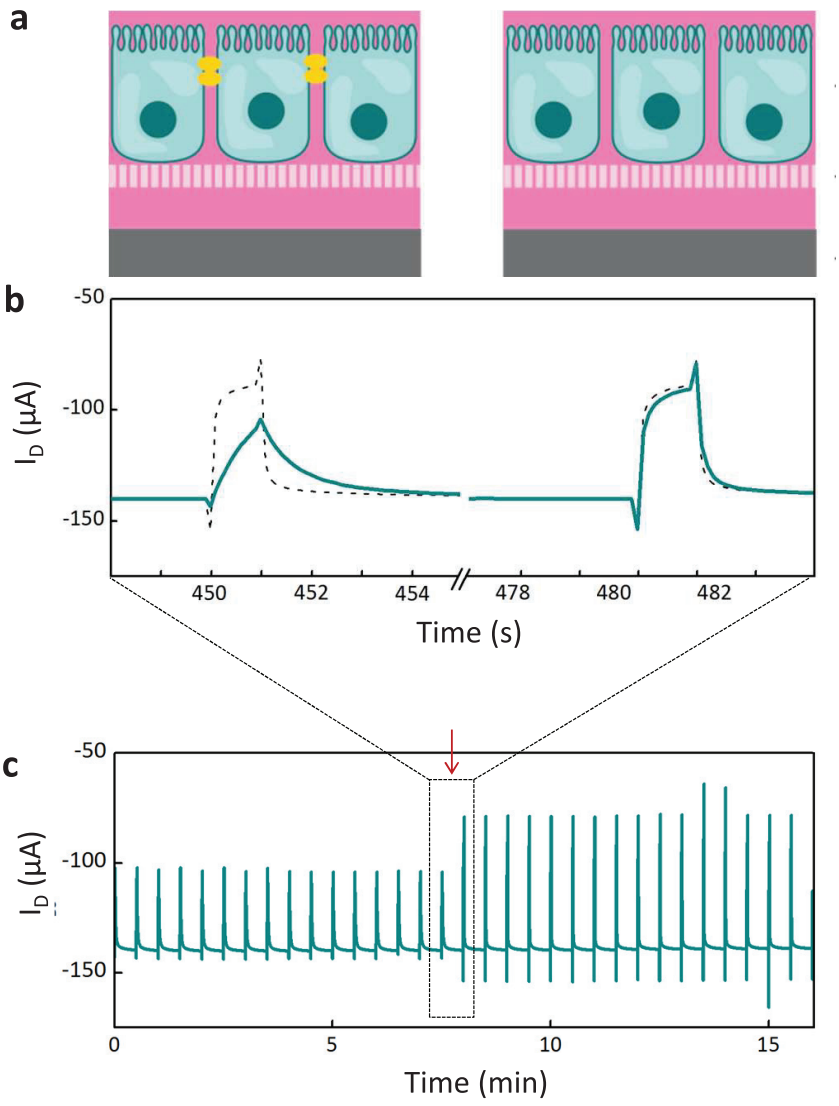


**Figure 2.** Monitoring barrier tissue integrity with an OECT. a) Cartoon showing polarized Caco-2 cells with tight junctions (left) and without (right), sitting on a porous cell culture membrane, above a PEDOT:PSS transistor channel. Tight junctions are shown in yellow. b) OECT $I_D$ transient response with cells before (left) and after (right) the addition of 100 mM $H_2O_2$, (solid lines). OECT $I_D$ response in the absence of cells is overlaid (dashed lines). c) In situ OECT response to periodic square $V_{GS}$ pulses. As indicated, data here correspond to the same measurement as shown in (b), but for an expanded time scale. The red arrow indicates the point of $H_2O_2$ introduction. The immediate change in OECT response to the addition of $H_2O_2$ is evident by the drastic change in $\Delta I_D$.

to the $I_D$ response when no cells are present. To induce a change in tissue layer integrity, hydrogen peroxide ($H_2O_2$), a reactive oxygen species known to disrupt TJs,[23] was introduced. The disruption of barrier tissue integrity results in an increase in ionic transport across the cell layer and therefore a faster $I_D$ response (Figure 2b, right). Control experiments were carried out to confirm that what we observe is not a parasitic effect of $H_2O_2$ reacting with the gate electrode or the polymer channel (Supporting Information). A key advantage of our sensor compared to common characterization methods is the ability to measure the response of the cell layer to a destructive species with such high temporal resolution. Figure 2c shows the in situ OECT response on addition of $H_2O_2$. In previous reports, effects of $H_2O_2$ on Caco-2 barrier integrity were observed after 30 to 60 min,[23] whereas we show an effect within 30 s. Detection of such an immediate response has enormous potential for toxicology purposes. In addition to high temporal resolution, we also observe high sensitivity: **Figure 3**a shows a normalized response (NR) of OECT detection for a range of $H_2O_2$ concentrations. From 100 mM to 5 mM $H_2O_2$, an extremely rapid response is detected, with complete barrier disruption detected within 1 min. On exposure to 1 mM $H_2O_2$, barrier tissue degradation proceeds for approximately 10 min and plateaus before complete barrier disruption. Figure 3b shows control measurements of the OECT response +/− cells for easy comparison of NR.

We also monitor barrier tissue disruption in the presence of ethanol using the OECT sensor (Figure 3c). Ethanol is known to cause functional damage to barrier tissue by producing progressive disruption of TJ proteins. High doses of ethanol (40%) are known to be directly cytotoxic, while lower doses have been reported to disrupt TJs in a reversible way.[24] The normalized responses of the OECT in the presence of ethanol are strikingly different compared to that of $H_2O_2$. While 30% ethanol disrupts barrier function within 45 min, the disruption occurs in a very gradual manner. Incubation with 20% ethanol disrupts the barrier function over the course of 1 h, while 10% EtOH results in no measurable disruption.

When comparing results from the literature, it is important to recognize differences in experimental conditions, notably the method used to introduce the destructive species, as this dramatically affects sensitivity. Experiments discussed here were performed using complete cell culture media as the delivery vehicle. The fact that the OECT operates so well in such a complex environment is an advantage, and we believe that we are sensing a more subtle disruption of barrier tissue than previously demonstrated.[25] Figure 3d shows apparent permeability data of Caco-2 layers exposed to $H_2O_2$ and ethanol, in complete cell culture media. While permeability assays after exposure to $H_2O_2$ show no effect up to 50 mM, the OECT is sensitive at 1 mM. It should be noted that we observe a continuous cell monolayer after $H_2O_2$ exposure, up to concentrations of 50 mM (immunofluorescence staining, data not shown). This is in agreement with reports in the literature that observe TER disruption on addition of modest amounts of $H_2O_2$ (10–40 mM), without gross disruption of monolayer integrity.[25,26] Permeability assays after exposure to ethanol

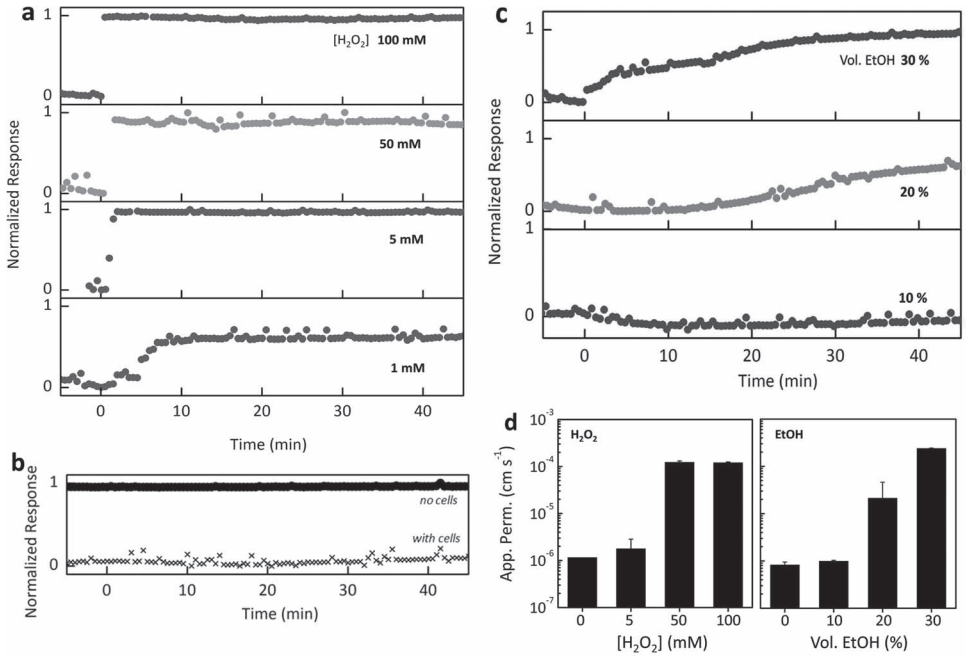


**Figure 3.** In situ OECT response and sensitivity. a) In situ normalized response of the OECT with barrier tissue on introduction of 100, 50, 5, and 1 mM $H_2O_2$, as labeled. $H_2O_2$ was introduced at $t = 0$. Here, NR = 0 corresponds to full barrier properties of a confluent monolayer and a NR = 1 corresponds to a cell layer with no barrier properties. b) NR controls corresponding to the OECT alone, with no cells (•), and a control corresponding to OECT with cells, but no added destructive species (×). c) NR of OECT with barrier tissue on introduction of ethanol (EtOH) at volume concentrations of 30, 20, and 10%, as labeled. Ethanol was introduced at $t = 0$. d) Apparent permeability of Lucifer Yellow across Caco-2 cells after a 24 h incubation with either $H_2O_2$ (left) or ethanol (right).

indicate that barrier properties are disrupted for concentrations over 20%. We demonstrate that the OECT is equally as sensitive in the case of ethanol and more sensitive with respect to $H_2O_2$ detection, but considerably faster than this standard assay used for toxicology. Moreover, OECT yields dynamic information: an important consideration in light of the emerging idea of tight junctions as dynamic structures, characterized by continuous engagement and release[10] and subject to rapid regulation in response to a variety of stimuli (pharmacological, physiological). Even impedance scans, the state-of-the-art for dynamic TER measurements, require 1 to 10 min for data collection. The ability of the OECT to measure variation in tissue dynamics in such a sensitive manner suggests that it may be possible to associate a "temporal signature" with toxic compounds that act on tissue layers using different molecular mechanisms. Identification of attacking species based on dynamic barrier properties alone could provide a means to achieve label-free detection of toxins and pathogens.

The use of barrier tissue models in combination with different measurement technologies has been hampered by invasive, expensive, slow to respond and difficult to implement methods. The integration of the OECT with human cells demonstrates a sensing device in a format that is compatible with low cost manufacturing techniques, of great importance for high throughput screening. While there have been other reports of the use of organic electronic devices integrated with cells to assess cytotoxicity, none have achieved the depth of information shown here. We believe this work represents a breakthrough in the field of organic bioelectronics as it marks the first time that an organic electronic device has been used to detect minute disruptions in barrier function. Pushing the device towards greater sensitivity levels will have implications for toxicology purposes and fundamental research, as the OECT provides a tool to assess minute, transient effects on tight junctions during both normal physiological function, and during disease.

## Experimental Section

*Cell Culture*: Caco-2 cells were seeded at $5\times10^4$ cells/insert (1.1 $cm^2$). Cells were routinely maintained at 37 °C in a humidified atmosphere of 5% $CO_2$, in DMEM (Advanced Dulbecco's Modified Eagle Medium, Reduced Serum 1X, Invitrogen) with 2 mM Glutamine (Glutamax-1, Invitrogen), 10% FBS (fetal bovine serum, Invitrogen) and Pen-strep (5000 [U/mL] penicillin–5000 [μg/mL] streptomycin, Invitrogen). For all OECT and permeability experiments, Caco-2 cell layers were used at 21 days in culture, corresponding to a TER of 400–500 $\Omega\cdot cm^2$ and an apparent permeability of $1 \times 10^{-6}$ $cm\cdot s^{-1}$, consistent with literature reports.[27] Cells were cultured on Transwell filters with a 0.4 $\mu m^2$ pore size and area of 1.1 $cm^2$. For OECT measurements only, cell layer area was reduced by a factor of ten using silicone on the back side of the filter, for purposes of increasing effective cell layer resistance.

*Permeability Assays*: Prior to permeability measurements, cells were exposed to various concentrations of ethanol and $H_2O_2$ in complete DMEM for 24 h. The value of the apparent permeability ($P_{app}$) was calculated according to the following relationship: $P_{app} = ((Flux \times V_{bas})/t)(1/C_oA)$ and $Flux = 100(LY_{bas}V_{bas})/(LY_{api}V_{api})$, *where* $LY_{bas}$ and $LY_{api}$ are the concentration of Lucifer yellow in the basal and apical sides of the hanging porous filter, respectively, $V_{bas}$ and $V_{api}$ are the volume in the basal and apical sides, respectively, $t$ is the time of incubation,

$C_0$ is the initial concentration of Lucifer yellow on the apical side and $A$ is the area of the filter. At least two samples were measured for each condition.

*Device Fabrication*: PEDOT:PSS (Heraeus, Clevios PH 1000) was used as the conducting polymer active layer. Ethylene glycol (Sigma Aldrich) was added in a volume ratio of 1:4 (ethelyne glycol to PEDOT:PSS) to increase conductivity. Dodecylbenzenesulfonic acid (DBSA) (0.5 µL/mL) was added as a surfactant to improve film formation, and 3-glycidoxypropyltrimethoxysilane (GOPS) (10 mg/mL) was added as a cross-linker to improve film stability. Thermally evaporated gold source and drain contacts were defined via lift-off lithography. Channel dimensions were patterned using a parylene peel-off technique described previously,[6,28] resulting in a PEDOT:PSS channel width of 1 mm. Following PEDOT deposition, devices were baked for 1 h at 140 °C at atmospheric conditions. A PDMS well defined active area, resulting in a channel area of approximately 8 $mm^2$.

*Device Measurements*: For OECT measurements, Ag/AgCl was used as the gate electrode and (DMEM) cell culture media was used as the electrolyte All measurements were made with Keithley 2612 SourceMeter and customized Labview software. In all cases, cell media (as described above) was used as the electrolyte. Measurements were performed at ambient temperature, but controls were conducted to ensure that temperature effects do not dominate changes in OECT response within the time required for measurements. $H_2O_2$ was added to the apical side of the cell filter, without changing media. Because larger amounts of EtOH were needed to achieve compositions of 10, 20, and 30%, half of the apical media was removed before introducing EtOH. In both cases, we confirmed that the act alone of addition/removal of liquid did not disrupt the barrier tissue layer. Throughout these experiments, measurement parameters were chosen to avoid exposing the cell layers to a voltage drop above 0.4 V, as high voltages have been shown to damage bilayer membranes.[28] Unless otherwise stated, OECT data was collected using the following parameters: $V_{GS}$ = 0.3 V, $V_{DS}$ = –0.1 V, $V_{GS}$ on time = 1 s, off time = 29 s, duty cycle = 3.33%. In some cases, OECT data is shown in the form of a normalized response (NR). NR is obtained by calculating $\Delta I_D/I_o$ (where $\Delta I_D$ refers to drain current modulation in response to the application of the gate voltage, and $I_o$ refers to the drain current when $V_G$ is off) and subsequently normalizing the dataset to [0,1].

## Supporting Information

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

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