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Peer Review File

Reconfiguration of organic electrochemical transistors for high-accuracy potentiometric sensing

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In this manuscript, the authors introduced a reference electrode into traditional OECT to fabricate a potentiometric-OECT (pOECT). The pOECT can get larger channel current modulation than that of traditional OECT when using polarized electrodes (Pt or Au) as gate electrodes. This result is obvious. If we want to get larger channel current modulation, we can still use traditional OECT with Ag/AgCl gate electrode. Although the channel current modulation is not high in traditional OECT with Pt or Au gate electrodes, the surface potential change on the gate electrode can still be detected and can be applied for many types of sensing applications. So what are the distinguished advantages of pOECT for sensing applications? Can its application areas be limited? Furthermore, using Ag/AgCl as the reference electrode still limits the miniaturization of the device, although the size of Gs is not very important.

Reviewer #2 (Remarks to the Author):

In this manuscript, the authors present a novel OECT configuration, termed pOECT, that is suitable for potential measurement. They assert that the sensing performance has been significantly improved by incorporating an additional reference gate electrode into the conventional OECT. The data and analysis provided in the manuscript appear valid, and I can support the concept of enhancing OECT's sensing performance by introducing a reference gate electrode. However, as the authors acknowledge, miniaturizing OECT is a critical goal, yet adding another gate electrode could increase the device's dimensions. Moreover, despite the claim that OECT is a robust sensing platform capable of attomole-femtomole scale detection, the presented data only pertains to the micromole scale. Consequently, I believe that the paper's concept and ideas may not align with the high standards of Nature Communications.

1. I agree with the authors idea about miniaturizing OECT dimensions. Thus, I could not agree with the idea of introducing another gate electrode for reference electrode. Additional gate electrode would inevitably increase the dimension of the devices. To address this issue, the authors need to demonstrate that the area occupied by the proposed gate electrode configuration is smaller than that of a conventional single gate electrode configuration.
2. I acknowledge that the authors' new gate electrode configuration improves the sensing performance. However, demonstrating sensing performance at the micromole scale alone is insufficient. I recommend that the authors present data on the detection of lower concentrations by the sensors to substantiate the superiority of the new methods.
3. The full name of the NR is missing in the main text and GR in Figure S2 seems to be mislabeled.
4. At right panel of Figure S13, leakless RE shows large fluctuations during the transfer curve while transfer curve of left panel seems stable. Please give additional information about this phenomenon. Also, I suggest authors to set x axis of right panel to Vgs.
5. In Supplementary Discussion 9, the authors mention that all electrodes will vary their potential over time, yet no evidence is provided to support this claim. Additionally, there is a lack of information explaining why the pOECT exhibits negligible change when exposed to both fresh and old media. It is unclear if measurements were taken following a 3-day incubation in cell media.
6. The authors assert that the pOECT has size efficiency advantages over conventional OECT due to the lack of need for size control of the gate. However, I disagree with this perspective because the size of the Ag/AgCl pellet is not necessarily smaller compared to an Au electrode. Additionally, the size of the electrode could be minimized by selecting alternative gate materials.

Peer review file

Manuscript: Reconfiguration of organic electrochemical transistors for high-performance potentiometric sensing, NCOMMS-24-09539-T

We thank the reviewers for their valuable comments and for giving us the opportunity to improve this work and communicate our results more effectively. We are excited to address their concerns and revise our manuscript based on the questions. We have addressed each reviewer's comments (in bold) in a point-by-point response, as detailed below. When we referred to an existing figure, we underlined it. All modifications to the text and figures are highlighted in yellow here and in our manuscript.

On behalf of all authors,

Sahika Inal

Reviewer 1:

- In this manuscript, the authors introduced a reference electrode into traditional OECT to fabricate a potentiometric-OECT (pOECT).

We thank the reviewer for this comment. However, we did not introduce a reference electrode into the traditional OECT. The extra element introduced in the pOECT is a simple electrode (gating gate, G_G). The Ag/AgCl pellet depicted in Figure 1 is a regular type of electrode used to gate the OECTs (it could as well be a polarizable electrode). It was shown to be connected to the reference electrode cable of the potentiostat. We realize that this schematic might have misled the reviewer to think that we introduced an Ag/AgCl reference electrode (or pellet) to the pOECT. We understand this confusion and have, therefore, modified the figure.

The new Figure 1 now shows an arbitrary functionalized electrode as the sensing electrode introduced to the electrolyte from the top. As for the newly added element, the gating gate, we show a Pt coil, which is a very common type of counter electrode, to facilitate the understanding of the role of this electrode. One can also use a different electrode material instead of Pt coil, as was shown in the experiments depicted in Figure 2c or in the new Figure 1c-bottom and new Figure S7. From the 3D illustration of the modified Figure 1, it is hopefully now clear that the only physical difference between the OECT and the pOECT is the gating gate (G_G).

To avoid any misunderstanding, we now separate the original Figure 1 into two parts. We first introduce the schematics and wiring (Figure 1) and move on to the results from Figure 2 onwards. The new Figure 1 with modified gate electrode is as follows:

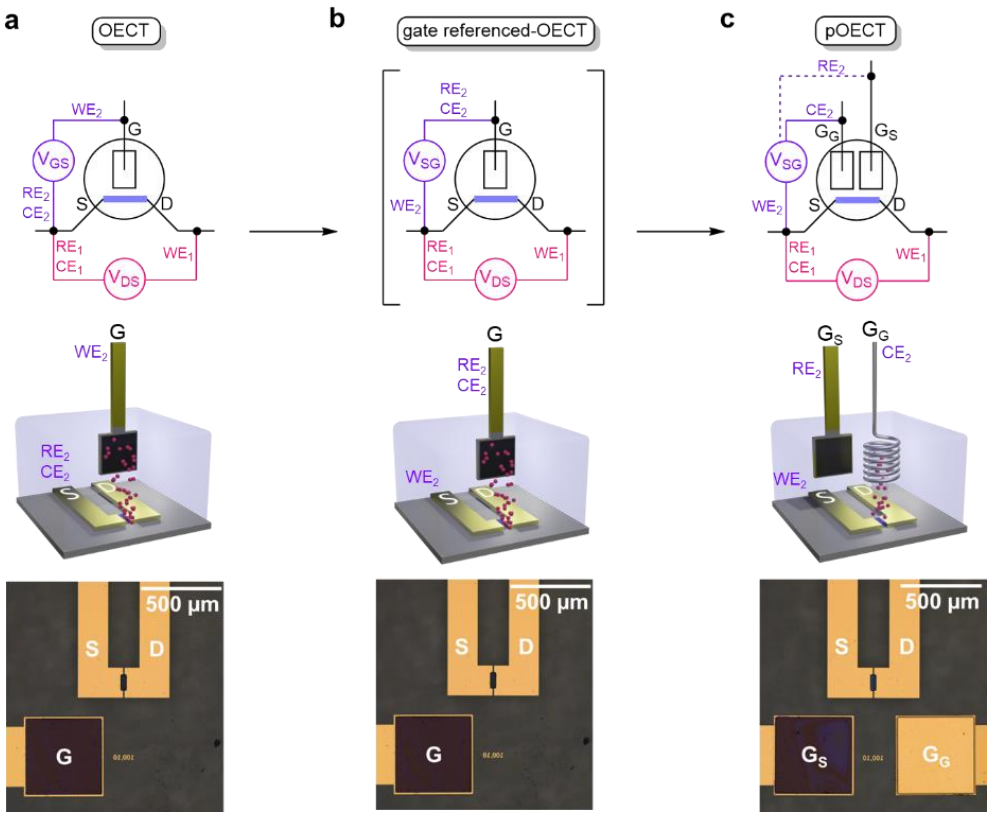


Figure 1. The p-OECT configuration. Comparison of the **a)** OECT, **b)** gate referenced-OECT and **c)** pOECT configurations. In the top schematic, the black circle represents the electrolyte, and the dashed line represents the high-impedance connection of the potentiostat. The pink legend represents the cable labeling for the V_{DS} application, and the purple legend represents the cable labeling for the V_{GS}/V_{SG} application. The 3D illustrations of the setups are in the middle panel, with G, G_G, and G_S placed vertically with respect to the channel. The red spheres represent the ionic flux of the leakage current….

To clarify that the new element in the pOECT is a simple counter electrode, and the sensing electrode connection is changed from WE_2 to RE_2 when we switch from OECT to pOECT, we modified the text in *Result and Discussion – the pOECT configuration* section. Some changes we made are:

on page 6:

"The G, which is now a combined RE_2/CE_2, can be decomposed into two independent electrodes, i.e., RE_2 and CE_2 (**Fig.1c**). The sensing electrode remains connected to RE_2. A new electrode is introduced, which can be any type of counter electrode, such as a Pt coil (**Fig.1c-middle**) or an Au electrode (**Fig.1c-bottom**), and connected to CE_2. Together, RE_2 and CE_2 represent the new "gating system". This configuration is referred to as the "*potentiometric-OECT*" (pOECT). The pOECT does not require any additional connections. While the conventional OECT operation involves shorting the RE_2 and CE_2 cables and connecting them to the same electrode (S), in the pOECT, these cables are separated and linked to electrodes with distinct functions."

"Both RE_2 and CE_2 gate the channel but serve different roles. RE_2 (sensing electrode) serves as the reference point for the electrochemical potential of the S. CE_2, on the other hand, actively

provides the voltage and current to bring the S to the required potential difference with respect to RE_2, thereby doping/dedoping the channel."

It is important to understand the difference between the reference (RE), counter (CE), and working (WE) labels of the potentiostat cable connections (for the application of the operating voltages V_{DS} and V_{GS}) and the actual electrodes fabricated for use as a reference electrode, counter electrode or working electrode. For example, while a Pt coil is manufactured and sold for use as a counter electrode in an electrochemical cell, we have the freedom to connect this element to any potentiostat cable (RE, WE, CE) that we consider appropriate for our experiment. Similarly, a sensing electrode, such as an ISE, normally commercialized for use as a working electrode in an electrochemical cell, is connected to the WE cable of the potentiostat. However, in a pOECT, this same electrode is connected to the RE cable of the potentiostat channel to apply the gating voltage.

We modified the caption of Figure 1 (pls see above) to clearly designate the potentiostat connections of each OECT component. To prevent any confusion between the cable labeling and the purpose of the employed electrodes, we also modified the text as the following on page 6:

"The top panel in **Fig.1a** shows the electrical connections of a bipotentiostat operating a conventional OECT. To apply V_{DS}, the D is connected to the WE_1, while the S is connected to a combined RE_1/CE_1. The electrochemical potential of the D is defined with reference to S (due to the RE_1 connection), and the current flowing between WE_1 and CE_1 is the I_{DS}. Simultaneously, V_{GS} is applied between the G (used as the sensing electrode in OECTs), which is connected to the WE_2, and the S, connected to the RE_2/CE_2. The current between G and S is I_{GS}, also called *leakage* current."

- The pOECT can get larger channel current modulation than that of traditional OECT when using polarized electrodes (Pt or Au) as gate electrodes. This result is obvious. If we want to get larger channel current modulation, we can still use traditional OECT with Ag/AgCl gate electrode.

The reviewer is correct in observing that the pOECT can generate larger channel current modulation when using polarizable gate electrodes. However, we are unsure how this result was obvious to them as the pOECT configuration is for the first time presented in our work. Nevertheless, the purpose of the pOECT is not to get larger current modulation but to stop the polarization of the sensor surface and eliminate leakage currents on the sensing electrode. The larger current generation is a secondary positive outcome of this configuration.

Direct biasing the sensing electrode in the OECT polarizes it, causing artifacts as well as possible damage to the fragile biorecognition units, especially when the device is pushed for full modulation. As the reviewer states, traditional OECT with an Ag/AgCl gate will generate larger currents in enhancement mode devices than a polarizable gate such as gold. However, this statement is irrelevant for potentiometric biosensors as with a traditional Ag/AgCl gate electrode; it is not possible to perform potentiometric biosensing through the gate, which must be a material functionalize with the recognition unit. Moreover, Ag/AgCl is a non-polarizable electrode with a constant surface potential hence, even if a functionalization were possible, potentiometric sensing would not be possible since the surface potential of Ag/AgCl cannot change.

We edited the text to describe why we show the transfer curve comparisons of pOECT and OECT.

On page 9:

"Despite the use of a polarizable electrode as the G_S, the pOECT behaves as if the channel is gated with a non-polarizable electrode. When using a polarizable gate electrode, it is not possible to obtain the same I_{DS} modulation with the OECT in an analogous biasing range, requiring to apply a much higher V_{GS} (x2.5) up to 1.26 V, as detailed in **Supplementary Discussion 2**. This marks the first advantage of the pOECT: it enables the safe use of polarizable gate electrodes to attain stable and full modulation of the channel. Secondly, in the pOECT, negligible current (pA or less) flows through the G_S due to the high impedance of the RE_2 connection (100 TΩ). We thus secure a nondestructive biasing method for the sensing interface, which often contains the biorecognition units that may be damaged due to the leakage current (I_{GS}). Moreover, high voltages applied for better modulation in the OECTs push the sensing electrode to electrochemical potentials, which can be detrimental to the biofunctionalized gate electrode (0.9 V vs. Ag/AgCl, **Fig.S3**)."

- Although the channel current modulation is not high in traditional OECT with Pt or Au gate electrodes, the surface potential change on the gate electrode can still be detected and can be applied for many types of sensing applications.

This is a correct statement. A full channel current modulation may, for some cases, not be required as devices can be used in the subthreshold region for potentiometric detection, as we also observed from some of our own biosensing work. However, as described above, the purpose of the pOECT is not to increase channel modulation but to avoid gate polarization, which otherwise leads to inaccurate results. The increased modulation is a natural outcome of the configuration, which is found to be highly favorable (see the next paragraph). We highlight the purpose of the configuration in several different sections of the manuscript. We also hope that the edits we made in the text above have clarified the point of showing a comparison of OECT vs. pOECT transfer curves.

Moreover, in Figure 4b, we have already demonstrated that the OECT with a functionalized Au gate is unable to detect a change in surface potential of 59 mV, while the same sensing interface can easily do this in a pOECT configuration. 59 mV is a significantly high value for pH sensing since it corresponds to 1 pH unit. This value is also close to the potentiometric change at saturation target concentrations if we consider the EGOFET publications involving protein sensing. However, the OECT in this geometry shows no modulation even when the gate undergoes a 59 mV change in its potential. The same channel, on the other hand, becomes an excellent sensor in the pOECT configuration. Here, one can clearly see how the "higher current modulation" of the pOECT allows the channel to operate effectively in a biosensor, without redesigning the gate electrode or doing geometry optimizations, etc.

Furthermore, the negligible OECT current modulation in Figure 4b cannot be overcome by simply increasing the gating voltage. In fact, a gating voltage higher than -0.7 V could not be applied to the OECT to reach modulation similar to the pOECT. As we have already demonstrated in Figure S3, such biasing would polarize the gate to overoxidizing potentials that damage the electrode.

The I_{DS} modulation is naturally not always low for OECTs, as in Figure 4b. However, in cases in which the OECT can provide a good modulation, as in Fig.S7 (see Reviewer 2, comment 2 for an explanation of this figure), the G is still significantly polarized, erasing the potentiometric analytical input signal, as explained in detail in *Results and Discussion - Thermodynamic stability of the pOECT - comparison with the OECT* section. Please see Reviewer 2, comment 2 for the demonstration that even a large area gate cannot compensate for the gate polarization when OMIECs are used in the transistor channel.

- So what are the distinguished advantages of pOECT for sensing applications?

We presented the advantages of the pOECT for sensing applications evidenced by various experimental results throughout the manuscript, discussed them after each section, and summarized them in detail in the Conclusions section. To summarize, the main advantage of the pOECT is the removal of the gate current/leakage current from the sensing electrode (gate). This is very important because the gate current has two main negative effects: (i) it can damage the functionalization layer, causing parasitic faradaic reactions or protein unfolding. This is a well-known effect and is mentioned in several publications, especially in the methods section when the applied operating voltages are justified; (ii) the gate polarization disturbs the thermodynamic equilibrium. The equilibrium is perturbed by the currents and is required for a reliable correlation between the electrode surface potential and the target concentration. By mitigating these two negative effects of the gate current, the pOECT leads to better stability of the sensor surface (see (i)) and to higher accuracy of the device response (see (ii)) compared to the OECT.

To reflect better the main compelling advantage of the work, we now edited the manuscript title as "**Reconfiguration of organic electrochemical transistors for high-accuracy potentiometric sensing".** We previously used the word "performance".

These are the two main reasons that led us to develop this configuration. However, the removal of the gate current from the sensing interface proved to be useful for other cases, as we explained in the manuscript. One that we would like to highlight to the reviewer is the possibility to use high impedance electrodes as sensing gate, which is not possible with OECTs. This is extremely interesting as it allows us to use different sensing materials that are not necessarily metals/metal oxides. We modified the *Results and Discussion - The operation of the pOECT as a sensor* section to better highlight this feature:

On page 12:

"In addition to this high response, the pOECT demonstrates exceptional modularity, enabling the integration of virtually any type of potentiometric sensing interface as Gs, even materials with extremely high impedance. This capability surpasses that of traditional OECTs, which cannot accommodate high-impedance materials as the G."

To clarify better the many advantages of the system over the state of the art, we edited the manuscript in various sections. We would like to refer the reviewer to these highlighted sections. Some examples are:

On page 9:

"In the pOECT, we bypass any possible polarization of the Gs by redirecting the polarizing current to the GG. Importantly, the choice of the GG in the pOECT design does not affect device characteristics."

On page 22:

"The number of transistor channels is virtually unlimited, as long as the GG is capacitive enough not to be polarized at too high or low potentials (due to the I_{GS}). Considering the usually small size of the pOECT channels, a regular CE, for example at Pt wire, easily serves the purpose. On the other hand, with a conventional OECT, increasing the number of devices progressively increases the IGS, leading to G polarization and lower reproducibility of the biasing conditions."

We included more advantages of the pOECT over the state-of-the-art OECTs used in potentiometric sensing in a Supplementary Discussion 6:

"Supplementary Discussion 6: Details on the thermodynamic advantages of pOECT over OECT

To the best of our knowledge, the first example of OECT sensor designed to respond to variations of the electrochemical potential of the gate in a Nernstian manner (hence thermodynamic response) is reported by Gualandi et al. (Nanoparticle gated semiconducting polymer for a new generation of electrochemical sensors. Sens. Actuators B Chem. 273, 834–841 (2018)). Their OECTs represent the closest analogous to classical potentiometric sensing in a 2-electrode setup, and for this reason, this work will be taken as a reference point. The authors showed how an Ag/AgCl electrode can modulate the I_{DS} of an OECT depending on the concentration of Cl^- in the electrolyte in contact with the AgCl wire, following the Nernst equation. The same group developed other OECT-based potentiometric sensors, for the detection of other ions (Gualandi, I. et al. Organic Electrochemical Transistors as Versatile Analytical Potentiometric Sensors. Front. Bioeng. Biotechnol. 7, 354 (2019)) and pH (Mariani, F., Gualandi, I., Tessarolo, M., Fraboni, B. & Scavetta, E. PEDOT: Dye-Based, Flexible Organic Electrochemical Transistor for Highly Sensitive pH Monitoring. ACS Appl. Mater. Interfaces 10, 22474–22484 (2018), Mariani, F. et al. Design of an electrochemically gated organic semiconductor for pH sensing. Electrochem. Commun. 116, 106763 (2020), Mariani, F. et al. Advanced Wound Dressing for Real-Time pH Monitoring. ACS Sens. 6, 2366–2377 (2021)) with different OECT architectures, such as dual terminal and Wrighton-configuration. In these works, the authors list or indirectly show a series of limitations that our pOECT can overcome. For example, the use of redox active species as a sensing element narrows down the range of possible voltages that can be applied to the OECT because of the faradaic reactions from the sensing material, leading to its oxidation or reduction. This problem is avoided in the pOECT because the sensing interface is maintained in an OCP configuration; hence, no voltage is applied to it.

The authors also introduced in the OECT field the concept of electrochemical gating (Ng, A. L. et al. Chemical Gating of a Synthetic Tube-in-a-Tube Semiconductor. J. Am. Chem. Soc. 139, 3045–3051 (2017)), where the conductivity of a channel, electrically shorted with the gate, or inside which the gating material is dispersed, can be modulated simply through spontaneous electrochemical reactions without a gate voltage bias. This configuration simplifies the OECT design because it removes the application of the gate voltage or completely removes the gate electrode[1] but, at the same time, is equivalent to operating the OECT at $V_{GS} = 0$ V. This operating condition, however, often does not represent the voltage at which the OECT guarantees the highest sensor response, as shown for example in Fig.3f. Moreover, while the sensitivity of an ISE depends only on the charge of the detected ion (according to the Nernst equation), in an electrochemically gated OECT, the behavior is different. Since the electrochemical potential of the S is equal to the electrochemical potential of the gate ($E_S = E_G$) at any time, the channel is biased around different electrochemical potentials (doping states), depending on the k of the Nernst equation (equation 1) that describes the specific potentiometric interface, which then corresponds to different sensitivity regimes for the OECT. This leads to anomalous behavior if compared to the Nernstian one, where despite the same charge of the ion, different ionic species (such as Cl^-,Br^-, I^-) show different sensitivity due to different k values. With the pOECT configuration instead, it is still possible to apply any desired V_{GS}, not only 0 V, because this voltage is provided by an independent CE (G_G), and bias the channel always at the voltages where the pOECT shows the highest response, regardless the k of the Nernst equation that describes the thermodynamic equilibrium of the G_S.

This allows to operate the device at an electrochemical potential close to the onset potential of the OMIEC, maximizing the transconductance efficiency (Tang, W. et al. Solution processed low power organic field-effect transistor bio-chemical sensor of high transconductance efficiency. Npj Flex. Electron. 6, 1–8 (2022)). The authors also point out that the electrochemical gating can work only if the gate or the gating material has a much higher capacitance than the channel, in order to bring the Fermi level of the channel to the same level as the G, without affecting the Fermi level of the G itself. This requirement can be reached only with a large size or a non-polarizable G. Also, in this case, with the pOECT, the modulation of the Fermi level of the channel is provided by an independent electrode (G_G), whose characteristics do not affect the pOECT performance, removing the requirement of non-polarizability of the G. They also show that a voltage applied to the potentiometric electrode hampers the spontaneous redox processes required to reach the thermodynamic equilibrium and stable potential."

- Can its application areas be limited?

The main limitation of this setup was explained in Supplementary Discussion 3. We will summarize it here for the reviewer. Despite the fewer limitations on the sensing gate electrode size compared to the standard OECT, which allows for the use of a sensing electrode of a smaller area (important for miniaturization), the sensing gate cannot be infinitesimally small. In fact, a minimal leakage current in the order of a few units of pA still flows through the sensing gate (Figure S5), the same as it flows through the reference electrode in a normal cyclic voltammetry experiment (Figure S4). This leakage current can polarize the sensing gate if its impedance is high (due to either an extremely small size (µm range or lower) or low conductivity). However, we showed how even with a gate electrode of high impedance, such as 500 MΩ, the polarization obtained with the pOECT (±1mV) does not significantly affect the thermodynamic stability of the sensing gate (Figure S6 and Figure 4a). Therefore, this is not a real limitation if compared to a standard OECT –it is a limitation in absolute terms but an advantage compared to the OECT.

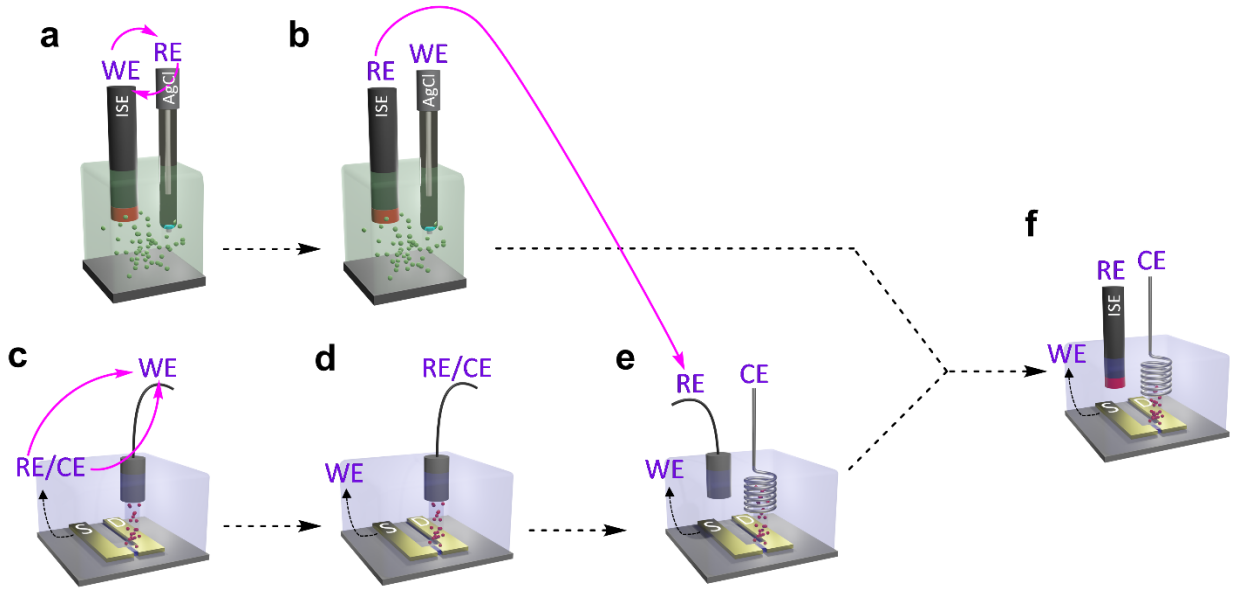
We noticed that we did not emphasize enough another limitation of the pOECT, which was mentioned in the Results and Discussion - Multichannel pOECT for cell monitoring at constant OMIEC thermodynamic conditions) section. Since with the pOECT, the sensing electrode is in an open circuit potential (OCP) condition, it is not possible to perform faradaic or impedimetric sensing, due to the complete absence of current passing through it. However, this is not really a limitation for our application since we developed this configuration for performing potentiometric sensing. In any case, we show how impedimetric or faradaic sensing is still possible with the pOECT if the detection is performed in the channel instead of the gate (Figure 7). Here, the only design limitation may be the necessity to use a leakless Ag/AgCl electrode, but this is, anyhow, the most optimal condition for the particular sensing experiment reported in the manuscript.

In order to detail this point, we added the following on page 21:

"The devices demonstrated above used the pOECT configuration to amplify the electrochemical potential changes that an external electrode (G_S) undergoes during sensing. While the pOECT is not designed for faradaic or impedimetric sensing on the gate due to the constant OCP condition of this electrode, it is still possible to use the configuration to perform such sensing directly on the channel. Here, we report a peculiar example of OECT-based impedimetric sensing, where the pOECT provides a fundamental advantage over the traditional OECT."

-Furthermore, using Ag/AgCl as the reference electrode still limits the miniaturization of the device, although the size of Gs is not very important.

We thank the reviewer for this comment, as it gave us the opportunity to highlight the features of this platform better. We do not use an Ag/AgCl as the reference electrode when we perform potentiometric sensing with the pOECT. In fact, in the 3D illustrations that we already reported, the Ag/AgCl as the reference electrode does not appear in the pOECT setup (see Figure 3a, Figure 3b and ex-Figure S7f). We use one of our previous figures as an example:



Previous Figure S7. Schematics of a) the conventional 2-electrode setup for open circuit potential (OCP) measurement and b) reversed potentiometric setup. Schematic of OECT/pOECT configurations with only the electrodes involved in the application of the gate voltage labeled: c) Classical OECT configuration with an Ag/AgCl pellet as G, d) Referenced gate OECT configuration, e) pOECT configuration with an Ag/AgCl pellet as GS and a Pt spiral as GG, f) pOECT with an ISE as GS and a Pt spiral as GG, i.e., IS-pOECT.

Figure 6a must have mistaken the Reviewer, which shows the use of Ag/AgCl. However, here, we use Ag/AgCl as floating gates, not as reference electrodes. The choice of Ag/AgCl and the configuration was solely to show the versatility of the pOECT setup, which can be operated in a single chamber, a dual chamber with a microfluidic connection, or a dual chamber with floating gates. The floating Ag/AgCl reference electrode is not essential; the floating gate connection can be achieved not necessarily with these electrodes but with simple Au microfabricated electrodes as has been shown in previous work (for example, S. P. White, et al., Label-Free DNA Sensing Platform with Low-Voltage Electrolyte-Gated Transistors, Anal. Chem. 87, 1861–1866 (2015)).

To clarify this point better, we modified the text on page 19:

"In **Fig.6a**, we depict the complementary pOECT with a pH-sensitive PANI electrode as the Gs. We used Ag/AgCl electrodes as floating gates that establish communication between the sensing chamber and the channels' chamber. The two Ag/AgCl electrodes play the role of a floating gate and not of conventional reference electrodes. The integration of the floating gates here is equivalent to that of a salt bridge or a microfluidic channel. The same floating gate configuration

can also be achieved by using two microfabricated planar Au electrodes instead of Ag/AgCl (Anal. Chem., 2015, 87, 1861–1866)."

The only pOECT setup that used an Ag/AgCl as a reference electrode is the tissue permeability sensing reported in Figure 7. However, this is not potentiometric sensing; it is an impedimetric sensing measurement. The purpose of this experiment is to show that the pOECT is able to provide advantages over the OECT also for categories of sensing for which the pOECT was not designed. As explained in this section, the use of a reference electrode, leakless in particular, is essential for this type of measurement, and this kind of electrode can be used exclusively with the pOECT. Please see Reviewer 1, comment 5 for further details regarding this experiment.

In a further effort to avoid any misunderstanding, we removed the Ag/AgCl pellet from the figures, (and deleted the ex-Supplementary Discussion 4 since it became redundant). The new Figure 3a is as follows:

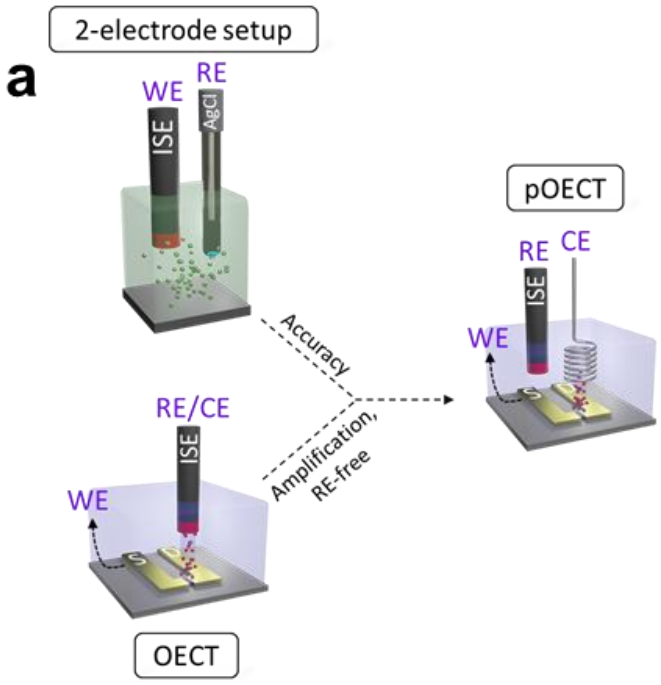


Figure 3. The pOECT as a potentiometric sensor a) Combination of a 2-electrode potentiometric setup with the OECT to obtain a reference electrode-free IS-pOECT.

The description of Figure 3a has been modified as follows at page 11:

"In Fig.3a, we schematically represent how the 2-electrode setup and the OECT are merged into a single configuration in the pOECT. By using a Na^+ ISE as the G_S, we obtain a Na^+ Ion Selective pOECT (Na^+-IS-pOECT)."

Moreover, to justify the use of Ag/AgCl in one single electrical characterization (no sensing) of this work, we modified the Results and Discussion - The pOECT configuration section:

On page 8:

"**Fig.2a-left** shows the transfer curves of an n-type enhancement-mode (see Scheme S1 for the polymer chemical structure) device wired in the conventional way (dotted lines, "OECT"), and reconfigured as described in **Fig.1c** (solid lines, "pOECT"). First, to characterize the behavior of the pOECT setup as a transistor, we used non-polarizable Ag/AgCl electrodes as G and Gs, and a Pt coil as the G_G."

For the concerns regarding device miniaturization, please see the new Figure 1, which shows a completely miniatured pOECT with all components integrated into one substrate. The experiments done with this device are in Figure S7, providing evidence that the pOECT can be miniaturized as much as an OECT. Please also see Reviewer 2, comment 1, for more details on this topic, where we also demonstrate that pOECT can be even smaller than a conventional OECT.

Reviewer 2:

In this manuscript, the authors present a novel OECT configuration, termed pOECT, that is suitable for potential measurement. They assert that the sensing performance has been significantly improved by incorporating an additional reference gate electrode into the conventional OECT. The data and analysis provided in the manuscript appear valid, and I can support the concept of enhancing OECT's sensing performance by introducing a reference gate electrode. However, as the authors acknowledge, miniaturizing OECT is a critical goal, yet adding another gate electrode could increase the device's dimensions.

We thank the Reviewer for their feedback and the valuable time taken to review our study. First, we would like to clarify that the design does not include a reference gate electrode. What we added to the OECT is an electrode of any sort that is connected to the CE cable of a regular potentiostat to ensure that no current passes through the sensing electrode (which is normally used as the OECT gate electrode).

Please see our response to Reviewer 1, the first and final comments for a detailed explanation of the device configuration and, importantly, the changes we made to avoid any misunderstandings. We feel that the first figure of the manuscript might have misled the reviewer. We have now made the modifications in the figures and in the text to better describe the configuration, components, and geometry of the pOECT and how it compares to the OECT.

Knowing that we don't introduce an Ag/AgCl reference element to the conventional OECT will hopefully further increase the value and the novelty of this work for the reviewer The modifications made will also respond to their major concern, which is about miniaturization, as we detail below.

1. I agree with the authors idea about miniaturizing OECT dimensions. Thus, I could not agree with the idea of introducing another gate electrode for reference electrode. Additional gate electrode would inevitably increase the dimension of the devices. To address this issue, the authors need to demonstrate that the area occupied by the proposed gate electrode configuration is smaller than that of a conventional single gate electrode configuration.

We thank the reviewer for this comment, which allowed us to show how this design can actually be easily miniaturized. Firstly, since we do not rely on an Ag/AgCl, miniaturization is not a concern. The gating gate we introduced can be of any size and introduced on the same substrate lateral to the channel (see the new Figure 1) or to the same substrate with the sensing gate. The G_G size does not matter (as we verified with the experiments in our work). Our main point here is that pOECT removes polarization on the sensing electrode and improves the device's overall performance, which is the most crucial aspect of a biosensor. For example, as we show in Figure 4b and Figure 4c, the OECT can not accurately sense the target (or not all due to unoptimized geometry) because of gate polarization. The pOECT, in the same dimensions, just by the addition of the gating gate (which does not take up extra physical space from the substrate), generates accurate transfer curves from a fully modulated channel. We anyhow feel that it is not fair to consider geometry as the primary specification when accuracy is at stake. A fair comparison has to be made between a pOECT and an OECT of equivalent performance (meaning that there is no polarization in the OECT case, which can be achieved with a very large gate electrode at the expense of miniaturization). We will come back to this point below.

We now show one example of how to miniaturize the pOECT, where we integrated a lateral G_G in micron-scale dimensions. In fact, the pOECT can be easily miniaturized using lateral electrode configuration, just as we do for the OECT. To highlight the miniaturization capabilities of the pOECT setup, we modified Figure 1 by adding below the 3D illustrations of the setups with external vertical electrodes, also the pictures of the microfabricated versions with planar gates:

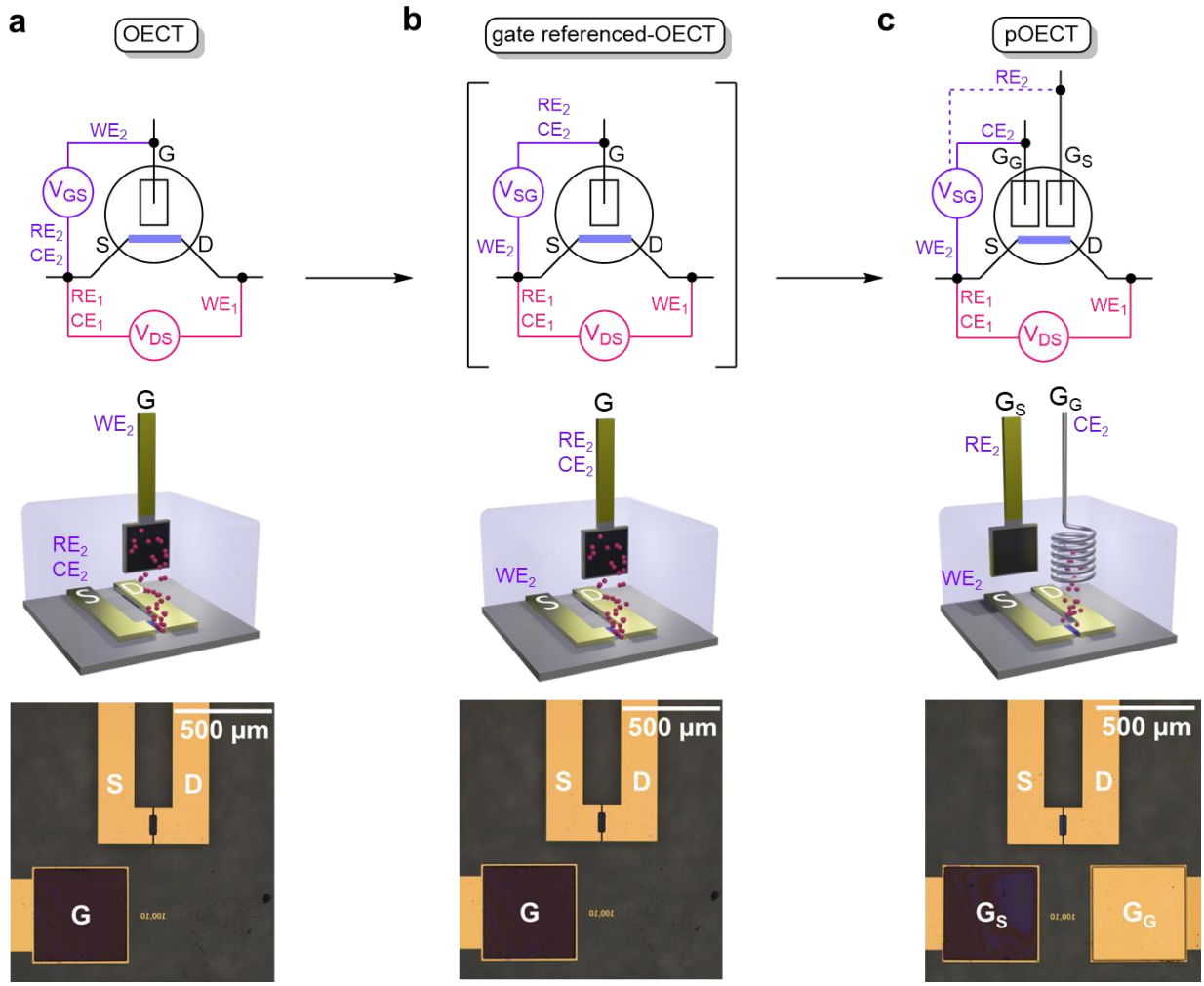


Figure 1. The p-OECT configuration. Comparison of the **a)** OECT, **b)** gate referenced-OECT and **c)** pOECT configurations. In the top schematic, the black circle represents the electrolyte, and the dashed line represents the high-impedance connection of the potentiostat. The pink legend represents the cable labeling for the VDS application, and the purple legend represents the cable labeling for the VGS/VSG application. The 3D illustrations of the setups are in the middle panel, with G, GG, and GS placed vertically with respect to the channel. The red spheres represent the ionic flux of the leakage current. Microscopy images of the microfabricated devices at the bottom panel depict the planar G, GG, and GS. The channel size 100x10 µm, and G, GG, and GS size is 500x500 µm. G and GS represent any type of sensing interface, while GG represents any conducting material that can act as a counter electrode.

The electrical characterization results of pOECT and OECT are now presented in an independent figure (Figure 2).

The following text is added to highlight the miniaturized device dimensions and the flexibility of the device design in terms of materials used but also the geometry:

On page 8:

"In **Fig.1** (middle and bottom), we present schematics and microscope images of the same channel gated in these three configurations. Like in the OECT, the new gating system (G_S and G_G) can be either vertical (**Fig.1-middle**), or the pOECT can be miniaturized, consolidating all the device components into a single substrate by fabricating planar G_G and G_S, thereby maintaining the overall device size in the micrometer range (**Fig.1-bottom**). The additional electrode introduced has, therefore, no impact on device geometry but offers significant advantages in device performance and sensor accuracy, as described below."

On page 9:

"This result underscores the versatility of the pOECT, as any material can be employed as the G_G without imposing limitations based on its polarizability/size or specific electrochemical potential. The absence of specific requirements for the newly introduced electrode in our configuration streamlines device fabrication and design. The results depicted in **Fig. 2** are derived from devices equipped with vertical gating electrodes. A similar enhancement in device performance can also be achieved with fully microfabricated devices, as demonstrated in **Fig.1-bottom** and **Fig.S7**."

We also added the electrical characterization of the miniaturized pOECT in Figure S7 with the text describing the findings:

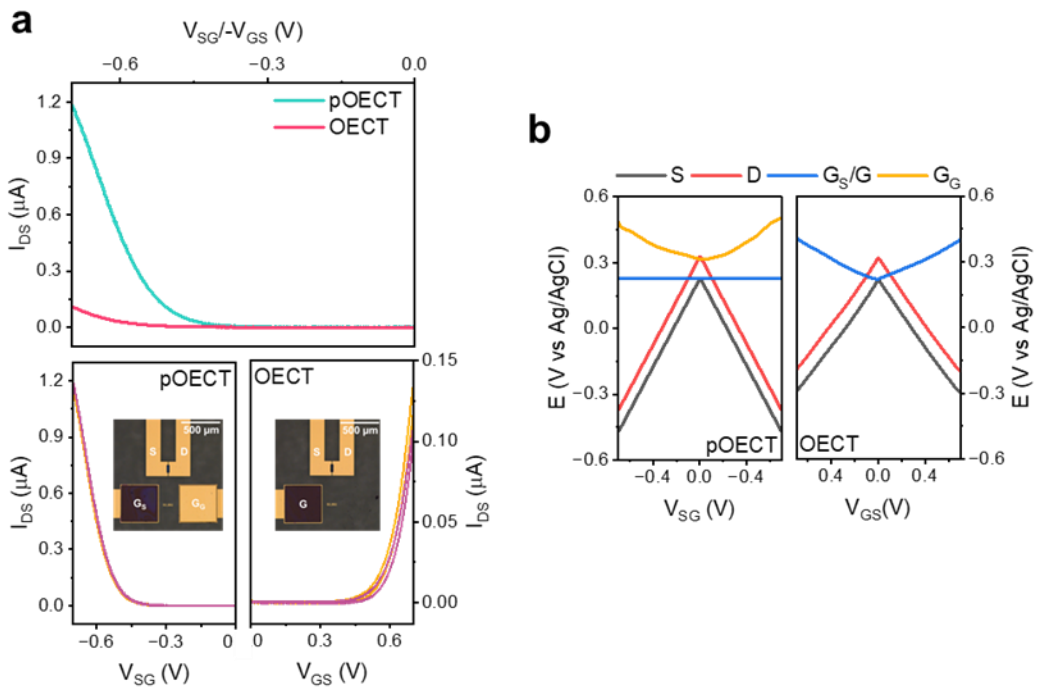


Figure S7. a) Transfer curves of microfabricated pOECT and OECT with a 100x10 µm channel and a 500x500 µm G, G_S and G_G. Top: the plot shows the different I_{DS} modulation capabilities of the two configurations. Bottom: the plots report consecutive transfer curves obtained with the same

device, in particular the 1st and 5th of the sequence. **b)** Electrochemical potentials of the microfabricated pOECT and OECT components during the transfer curve.

"In Fig. S7, we compare the electrochemical performance of microfabricated OECT and pOECT of similar size. The devices have identical channel (100x10 µm) and sensing interface (500x500 µm, G and G_S) dimensions. The only difference for the pOECT is the additional electrode (G_G) of the same size as the sensing interface. The overall substrate size and active area are exactly the same in both devices. The pOECT allows us to reach not only a much larger I_{DS} modulation (Fig.S7a-top) but also a significantly more stable response, as demonstrated by the consecutive repetition of the transfer curves (Fig.S7a-bottom). In the OECT, the G polarizes during the V_{GS} application, even if the electrode has a surface area 250 times higher than the channel (Fig.S7b-right). This polarization is instead efficiently prevented by the pOECT (Fig.S7b-left), where G_S remains unperturbed at its stable equilibrium electrochemical potential, and the auxiliary electrode (G_G) plays the role of doping the OMIEC, being consequently polarized. In addition to its stable and high performance, the pOECT is as miniaturized as the conventional OECT, as exemplified here."

Moreover, we added Supplementary Discussion 4, demonstrating that the overall size of a pOECT potentiometric sensor can be even smaller than that of an OECT potentiometric sensor when we design the sensors such that their performance is comparable.

"Supplementary Discussion 4: pOECT miniaturization capabilities and comparison with OECT dimensions

As demonstrated in Fig.S7 and Fig.4b-c, an OECT with a comparable electrode size as a pOECT (OMIEC and sensing interface area) has an output signal with severe interference from gate polarization. The polarization renders the OECT in this geometry either a poor sensor (due to low modulation) or a sensor with inaccurate output (as the polarization affects the channel current). To achieve the same "accuracy" as that of the pOECT, we need to operate the OECT with a very large G. However, the necessary size to make this electrode non-polarizable exceeds the acceptable dimensions for a portable device. A partial demonstration of this is reported in Fig.2c-right and Fig.S2. Despite the large capacitance of the G_G constituted by a 23 cm long Pt wire, the electrode is still polarized (+65 mV) by the leakage current necessary to dope the OMIEC. Hence, if this electrode is used as G in an OECT, it will still be polarized.

With organic semiconductors employed for EGOFETs, a millimeter gate size is sufficient to prevent polarization and provide reliable performance, as demonstrated in previous work (Nat. Comm. 2018, 9, 3223, Sci. Adv. 2022, 8, 27, Adv. Mater. 2023, 35, 42, 2304102). With OMIECs, the doping mechanism involves the entire bulk of the transistor channel, requiring a larger number of ionic charges and, in turn, a larger I_{GS}. The result is that for OMIEC-based transistors, even the so-called "large area interfaces" (Chem. Rev. 2022, 122, 4, 4636–4699) of EGOFET biosensors are not large enough to prevent the polarization of the G. To achieve non-polarizability, the electrode should be way larger than the ones employed for EGOFETs, and for this reason, a sacrificial electrode such as the G_G of the pOECT is a much more practical solution. For the same reason, we cannot report any OECT measurement with a non-polarizable sensing electrode such as G due to the impractical size of this electrode. However, a size comparison between a pOECT and OECT of equivalent performance can be made if Ag/AgCl reference electrodes are used as G and G_S.

An Ag/AgCl reference electrode is considered a non-polarizable electrode, and commonly used as G for OECTs to either investigate the properties of the OMIECs or perform sensing on the transistor channel. However, the non-polarizability concept is only ideal, and as already represented in Fig.S4a, even an electrode designed to be non-polarizable can deviate from its ideal behavior if the current passing through it (I_{GS} in our case) exceeds a critical value. If we use an Ag/AgCl reference electrode as G in the OECT setup, we obtain a transfer curve, as reported in Fig.S8a-left. However, if we operate the same transistor channel as a pOECT and employ the same Ag/AgCl electrode as G_S, we obtain the transfer curve of Fig.S8a-right. The OMIEC response in the two cases is clearly different, with the OECT providing a smaller current modulation and a significantly larger hysteresis. Note that, as demonstrated in Fig.2c, the nature of the G_G does not affect the pOECT response. Hence, the difference between the OECT and pOECT transfer curves is solely related to the behavior of the Ag/AgCl electrode when employed either as G or G_S.

The reason for this difference in the transfer curves is reported in Fig.S8b, where we show the electrochemical potentials of the OECT components during the transfer curve. From this measurement, it is clear how, with the OECT, the Ag/AgCl gate, even though considered a non-polarizable electrode, deviates from the ideal behavior and becomes polarized at $V_{GS} > +0.2$ V, where the OMIEC significantly increases its doping state. This G polarization prevents the OMIEC from reaching higher doping as with the pOECT (up to 4.5 mA) and leads to hysteresis. The measurement demonstrates that even Ag/AgCl can still be significantly polarized, leading the OECT to reach a lower performance than the pOECT. If we want to reach identical performance as the pOECT (identical current modulation and hysteresis), we have to use six Ag/AgCl reference electrodes connected in parallel, as shown in Fig.S8c. Only in this extreme and unpractical condition does the I_{DS} profile of the OECT match with the one of the pOECT. Note that the OECT measurement of Fig2a was obtained exactly in this condition.

With the pOECT, we have already demonstrated that the non-polarizability of the sensing interface is guaranteed even with a small G_S (5 mm diameter, Fig.2b) and is also possible to use a small G_G (1.6 mm diameter, Fig.2c). On the other hand, Fig.S8 demonstrates that with the OECT the G should have an electroactive area equivalent to the capacitance of 6 Ag/AgCl reference electrodes not to be polarized, requiring to use a G of unpractical size. We can conclude that an OECT with comparable performance as a pOECT requires a gate electrode of unreasonable size, which would definitely exceed the cm range. Hence, the pOECT allows to obtain a device that satisfies the requirements of a potentiometric measurement by using a much smaller size than what would be required with an OECT.

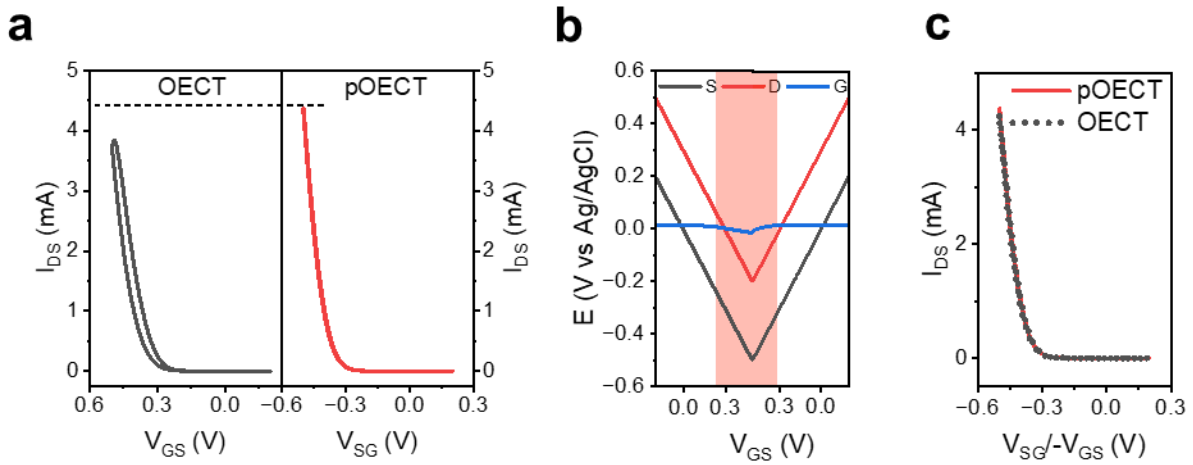


Figure S8. a) Transfer curves of an n-type OECT (left) and pOECT (right) when an Ag/AgCl reference electrode is used as G and G_S, respectively. **b)** Electrochemical potentials of the OECT components during the transfer curve. The red band shows the V_{GS} values at which the Ag/AgCl G is polarized. **c)** Transfer curves of an n-type pOECT and OECT when one Ag/AgCl is used as G_S and six Ag/AgCl in parallel are used as G. The Ag/AgCl electrodes used are commercially available reference electrodes where the Ag/AgCl wire is immersed in a solution of high concentration of Cl^- (3 M) to allow the redox reaction $Ag_{(s)} + Cl^-_{(aq)} \rightleftharpoons AgCl_{(s)}$ to keep the electrode at a constant potential.

This Supplementary Discussion 4 is introduced in the main text (Results and Discussion - Thermodynamic stability of the pOECT - comparison with the OECT) as follows:

"Note that, to construct an OECT with comparable accuracy as the pOECT, the G area could be increased. However, the geometry required to attain the same pOECT performance exceeds any reasonable size for a small and portable device, as discussed in detail in **Supplementary Discussion 4**. The pOECT allows for the use of smaller sensing interfaces, a significant advantage over the OECT in terms of device miniaturization, while increasing device performance and ensuring accuracy."

3. I acknowledge that the authors' new gate electrode configuration improves the sensing performance. However, demonstrating sensing performance at the micromole scale alone is insufficient. I recommend that the authors present data on the detection of lower concentrations by the sensors to substantiate the superiority of the new methods.

A low limit of detection (LoD) is an important parameter for some sensors. For others, a broad dynamic range or a fast speed can be the figure of merit. For all sensors, however, accuracy is the key. Without accuracy, any reported LoD, speed, or dynamic range is prone to artifacts. In particular, potentiometric OECT sensors suffer from issues related to accuracy and sensing interface stability but not from low detection limits. For this reason, the main goal of this work and of the pOECT setup is to improve the accuracy of the measurement and prevent any damage to the sensing interfaces. We did this by eliminating the polarization on the sensing electrode. We discovered that by the new configuration, we not only improved the accuracy by maintaining the

Nernstian behavior but also generated many other compelling advantages, as discussed in different sections in the manuscript. To make our purpose clearer, we modified the title of the manuscript as follows:

"Reconfiguration of organic electrochemical transistors for high-accuracy potentiometric sensing"

Second, focusing on detecting ions such as Na^+, H^+ and Cl^- with the commercially available ion selective membranes at concentrations lower than the μM range is physically impossible but also not of biological interest. As declared by the manufacturer, and demonstrated in Fig.3d, these ISEs are not able to detect concentrations < 100 μM. This is a known issue, and the physical reason is due to the mixed potentials that become the predominant contributors to the faradaic current that establishes the electrode surface potential, when the concentration of the chemical species that define the Nernst equation of interest is too low (see S. J. Percival, et al., Ultra-Sensitive Potentiometric Measurements of Dilute Redox Molecule Solutions and Determination of Sensitivity Factors at Platinum Ultramicroelectrodes, Anal. Chem. 2017, 89, 18, 9843–9849, for more details). It is important to understand that the pOECT can only improve an input signal that already exists (surface potential change of the G_S), but it cannot (and should not) create a new one. If the ISE surface potential does not change for lower ion concentrations, as demonstrated in Fig.3d by using a sophisticated potentiostat with a resolution of 1 μV, this means that there is no variation of input gate voltage for the pOECT hence, no I_{DS} variation should be detected. In fact, other highly cited Nature communication publications on OECT based ion sensors report a similar range of concentrations as our work, and focus on the sensitivity improvement of the sensor rather than its limit of detection, as we did (Romele, P., et al. Multiscale real time and high sensitivity ion detection with complementary organic electrochemical transistors amplifier. Nat Commun 11, 3743 (2020), Ghittorelli, M., et al. High-sensitivity ion detection at low voltages with current-driven organic electrochemical transistors. Nat Commun 9, 1441 (2018)). Moreover, these ions are always present in biological media with a background concentration at least in the μM range hence, their detection at lower concentrations has no significant interest for biosensing.

Third, the few potentiometric OECT biosensors which reported attomole-femtomole LODs used sensing electrodes functionalized with antibodies or complementary strands for the detection of detection protein or DNA. Asking to perform biosensing to demonstrate the possibility of lower LoDs would mean completely changing the type of sensing electrodes used in this study, but without changing any of our conclusions. In fact, as reported in several OECT protein or DNA sensing publications (for example Y. Liang, et al., Amplification of aptamer sensor signals by four orders of magnitude via interdigitated organic electrochemical transistors, Biosens. and Bioelec., 144, 111668, (2019), P. Lin, et al., Organic Electrochemical Transistors Integrated in Flexible Microfluidic Systems and Used for Label-Free DNA Sensing, Adv. Mat., 23, 35, 4035-4040 (2011), H. Liu, et al., Ultrafast, sensitive, and portable detection of COVID-19 IgG using flexible organic electrochemical transistors, Sci. Adv., 7, 38 (2021), W. Tao, et al., A sensitive DNA sensor based on an organic electrochemical transistor using a peptide nucleic acid-modified nanoporous gold gate electrode, RSC Adv., 7, 52118 (2017)), the surface potential change of the sensing electrode is about 10 mV for the detection of atto or femtomolar concentrations. The maximum changes are reported to be ca. 150 mV for the concentrations at sensor saturation. These surface potential changes are analogous to what the ISEs show. Despite the different origins of the surface potential change (ion vs. DNA/protein binding), the response of the pOECT would, therefore, be identical independent of what the target is. The pOECT simply transduces the input voltage.

Fourth, we did not prefer to use such biofunctionalized electrodes to benchmark this system because our configuration is new and should be tested with well established electrodes. With ISEs, we can use the Nernst equation as reference point and compare the OECT and pOECT performance with the expected theoretical behavior and demonstrate the accuracy improvement of the pOECT. With protein or DNA sensing, there is currently no universally accepted and unified thermodynamic equation that describes the sensing mechanism. For example, while the electrochemical potential of an electrode ruled by the Nernst equation does not depend on the electrode size (Anal. Chem., 2013, 85, 964–970), numerous experiments suggest that electrodes functionalized with antibodies for protein detection undergo a surface potential change that depends on the electrode size, and increasing its value can be used to improve the sensitivity and limit of detection (LoD) of the sensor (Chem. Rev. 2022, 122, 4, 4636–4699). Using such systems for the sake of showing low LoDs will not be beneficial, considering the scope of this work.

We highlighted these points in the main text:

On page 10:

"We chose ions as the target instead of other biomarkers, such as proteins or nucleic acids, because ion sensing can be easily described by the thermodynamic Nernst equation. This allows us to compare the pOECT and OECT experimental behavior with the theoretically expected one (Nernstian behavior). Secondly, the potentiometric detection mechanism of OECTs for such biomarkers, despite their much lower limit of detection (LoD) compared to that of ion sensors, is still under debate and not uniquely defined by a thermodynamic equation (Anal. Chem., 2013, 85, 964–970 and Chem. Rev. 2022, 122, 4, 4636–4699). Therefore, to analyze the performance of this new configuration, we opted to work with a well-established sensor surface. Nevertheless, surface potential changes resulting from protein or DNA binding range from 10 to 150 mV for LoD and saturation concentrations, respectively (Y. Liang, et al., Amplification of aptamer sensor signals by four orders of magnitude via interdigitated organic electrochemical transistors, Biosens. and Bioelec., 144, 111668, (2019), P. Lin, et al., Organic Electrochemical Transistors Integrated in Flexible Microfluidic Systems and Used for Label-Free DNA Sensing, Adv. Mat., 23, 35, 4035-4040 (2011), H. Liu, et al., Ultrafast, sensitive, and portable detection of COVID-19 IgG using flexible organic electrochemical transistors, Sci. Adv., 7, 38 (2021), W. Tao, et al., A sensitive DNA sensor based on an organic electrochemical transistor using a peptide nucleic acid-modified nanoporous gold gate electrode, RSC Adv., 7, 52118 (2017)). These values are in the same range as those reported here for the ISEs. Despite the different origins of the surface potential change (ion capture or DNA/protein binding), the response of the pOECT remains the same since the device detects these changes as a simple equivalent gating voltage variation. Therefore, the conclusions drawn regarding the pOECT's operation in ion sensing and its advantages extend to the detection of any other target through a potentiometric mechanism."

On page 15:

"These ion and pH sensing experiments prove that the pOECT combines the accuracy of the 2-electrode setup with the amplification capability of the OECT, all without a conventional RE. Accuracy is the most important trait of all sensors, which is guaranteed for potentiometric OECT sensors through this configuration. We note that the lowest electrochemical potential change upon ion binding to an ISE is about ±10 mV. This is also the lowest value that has been recorded upon protein or nucleic acid binding to the gate of an OECT (Y. Liang, et al., Amplification of aptamer sensor signals by four orders of magnitude via interdigitated organic electrochemical transistors,

Biosens. and Bioelec., 144, 111668, (2019), P. Lin, et al., Organic Electrochemical Transistors Integrated in Flexible Microfluidic Systems and Used for Label-Free DNA Sensing, Adv. Mat., 23, 35, 4035-4040 (2011), H. Liu, et al., Ultrafast, sensitive, and portable detection of COVID-19 IgG using flexible organic electrochemical transistors, Sci. Adv., 7, 38 (2021), W. Tao, et al., A sensitive DNA sensor based on an organic electrochemical transistor using a peptide nucleic acid-modified nanoporous gold gate electrode, RSC Adv., 7, 52118 (2017)). This value translates into an LoD in the micromolar range for ion sensors, and the physical reason that limits the LoD of ISEs has been discussed by A. J. Bard et al (S. J. Percival, et al., Ultra-Sensitive Potentiometric Measurements of Dilute Redox Molecule Solutions and Determination of Sensitivity Factors at Platinum Ultramicroelectrodes, Anal. Chem. 2017, 89, 18, 9843–9849), and an LoD in the attomolar range for protein sensors. An effective strategy to further improve the LoD for OECT-based potentiometric sensors is to optimize the chemistry of the sensing interface for amplifying the input signal, i.e., surface potential change (H. Liu, et al., Ultrafast, sensitive, and portable detection of COVID-19 IgG using flexible organic electrochemical transistors, Sci. Adv., 7, 38 (2021)."

Nevertheless, we are currently working on the use of the pOECT setup for protein sensing based on antibodies and nanobodies, which we aim to focus in a separate study with clinical samples. We are investigating how the pOECT can be used to solve the Debye length issue, while at the same time keeping the OMIEC at the highest performing conditions. We are studying how the leakage current of the OECT can damage the protein functionalization layer by either irreversible chemical reactions or proteins unfolding and at which magnitude the pOECT is able to prevent it. We are also investigating how the additional electrode introduced in the pOECT (G_G) can also be used to (i) improve the functionalization stages, for example, by applying voltage pulses to promote optimal proteins/SAM conformations in a significantly shorter time and (ii) improve the sensitivity and LoD by leveraging electrothermal flow phenomena. As the reviewer can appreciate, the focus of such a study, which shows lower LoD (not because of the pOECT but because of the sensing electrode chosen), is completely different from this one, which is very novel and dense.

4. The full name of the NR is missing in the main text and GR in Figure S2 seems to be mislabeled.

We thank the reviewer for noticing these mistakes. The main text has been modified as follows on page 12:

"In **Fig.3f-left**, we report the normalized response (NR) of the Na^+-IS-pOECT calculated as the change in I_{DS} at different V_{SG}…"

Details on how to calculate the NR are reported in the supplementary material, Experimental Section.

Figure S2 has been modified as follows:

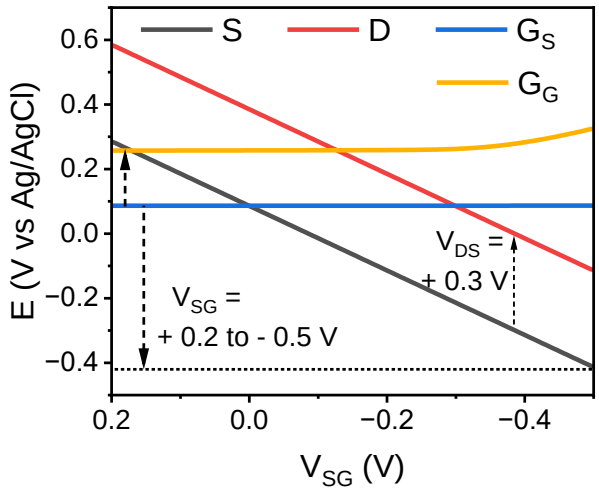


Figure S2. Electrochemical potentials of the pOECT components during the application of the voltages of a transfer curve (V_{DS} = +0.3 V and V_{SG} from +0.2 to -0.5 V).

5. At right panel of Figure S13, leakless RE shows large fluctuations during the transfer curve while transfer curve of left panel seems stable. Please give additional information about this phenomenon. Also, I suggest authors to set x axis of right panel to Vgs.

We thank the reviewer for this comment. The fluctuations of the leakless RE potential are due to the voltage steps of the V_{GS} application. A theoretical linear sweep of the V_{GS} is in reality performed by the instrument as a series of small stair steps, whose step size is defined by the 16-bit resolution Analog-to-Digital converter (ADC) on the circuit board, as represented in Figure R1a. Every time the instrument increases the V_{GS} of a step unit, a voltage spike appears due to the gate electrode polarization and the capacitive current. The voltage step duration of the experiment reported in Figure S13 is 200 ms, while the response time of the OMIEC is about 5 ms, as shown in Figure R1b. Therefore, at the end of the voltage step when the I_{DS} is sampled by the instrument, the current has already reached the equilibrium value for that polarization condition, leading to a transfer curve not as scattered as the electrochemical potential measurement (but with a lower modulation).

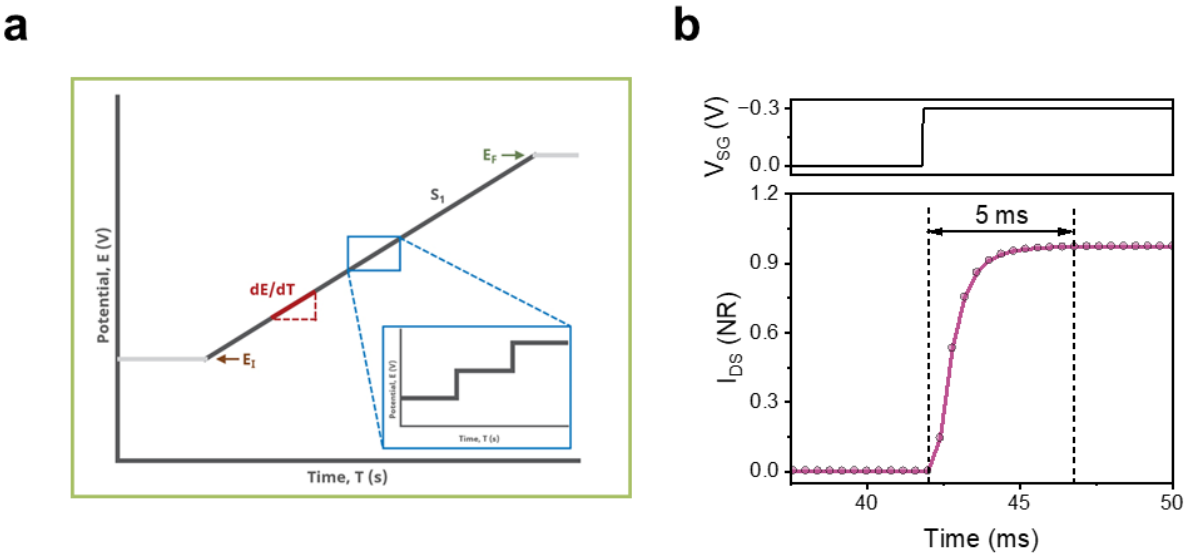


Figure R1. a) Typical waveform of a linear scan of voltage. **b)** Normalized response of the PEDOT:PSS OECT current upon a V_{GS} pulse. The OMIEC reaches a stable response after 5 ms .

As per the reviewer's request, we modified Figure S13, as follows:

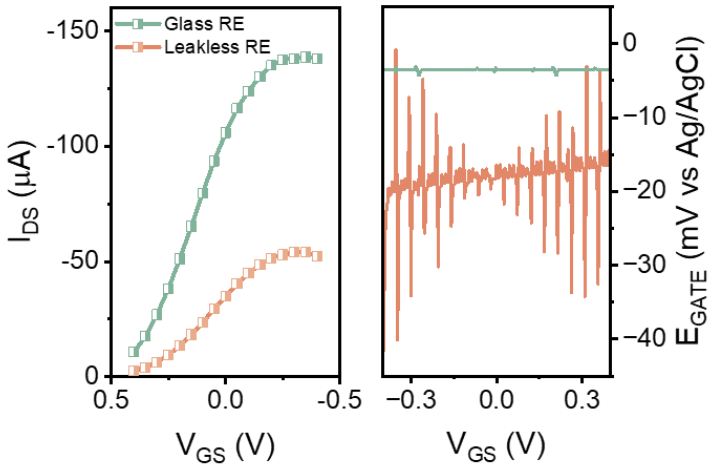


Figure S13. Left: transfer curve of a PEDOT:PSS OECT when the G is an Ag/AgCl with either a glass porous membrane or a leakless membrane for the ionic connection with the electrolyte. Right: the electrochemical potential of the two G terminals during the transfer curve.

6. In Supplementary Discussion 9, the authors mention that all electrodes will vary their potential over time, yet no evidence is provided to support this claim.

This potential variation is a well-known electrochemical phenomenon (*Principles of Instrumental Analysis*, 7th edition, from Skoog, Holler and Crouch, Chapter 23). We showed it in our work with one electrode material (Pt, Figure S12a). An electrode forms an electrochemical system called a "Redox indicator" in the conditions shown in the experiment in Figure S12a (a conductive surface directly exposed to the electrolyte). In these conditions, the electrode surface serves as a pure indicator of the redox state of the electrolyte. The electrode works as a source or sink of electrons

for the redox active species in the solution, allowing electron transfer and the establishment of a surface potential directly correlated with the electrolyte composition. This phenomenon is, however, not always reproducible due to the involvement of electrochemical reactions from other electroactive species present in the solution, some with slow kinetics. This remarks the importance of separating the G_S from the main electrolyte when experiments like the one reported in Figure 7 are performed.

7. Additionally, there is a lack of information explaining why the pOECT exhibits negligible change when exposed to both fresh and old media. It is unclear if measurements were taken following a 3-day incubation in cell media.

With old media, we refer to the media that cells grew in for 3 days. This is because we grew cells for 3 days on top of transistor channels. For the particular experiment that the reviewer refers to, we take the media from the cells grown on a petri dish for 3 days and investigate how the potential gate electrodes would react to it.

A leakless reference electrode is intrinsically isolated from the electrolyte. Hence, any variation of electrolyte composition will not affect the surface potential of this electrode. Therefore, pOECTs gated with a leakless reference electrode as G_S will have stable electrode potential with no effect from cell media. We expect the devices measured in fresh cell media or old (after 3-day incubation) to have no difference in channel currents if the channel has no specific interactions with the media components. In our case, we see no significant difference, which means that the OMIEC is stable. It is important to remember that there is a change in the electrolyte composition after three days of cell exposure. This is confirmed by the experiment in Figure S12a, where the Pt WE is used as a redox indicator for the solution.

To make the experimental conditions clearer, we modified the Supplementary Discussion 7 as follows:

"In Fig.S14, a pOECT is gated by a leakless RE as G_S, and a Pt wire is used as G_G and is operated in two different electrolytes, a fresh cell media and the cell media that the cells produced after 3 days of culture. In these conditions, the response of the pOECT is identical, regardless of the freshness of the media, also showing that the OMIEC behavior is not significantly affected by the different composition of the electrolyte."

We added the following information to the description of Figure S12:

"In this evaluation, we used a fresh cell culture media and a conditioned cell culture media, which is a medium whose chemical composition has changed since it was left in contact with the cell culture for 3 days and contains components secreted by the cells."

8. The authors assert that the pOECT has size efficiency advantages over conventional OECT due to the lack of need for size control of the gate. However, I disagree with this perspective because the size of the Ag/AgCl pellet is not necessarily smaller compared to an Au electrode.

We understand that our previous schematics about the pOECT structure might have been confusing, which we believe is resolved by the modifications we made. Please see the responses above, also to the first and last comment of Reviewer 1 and the changes we made to illustrate that there is no Ag/AgCl pellet necessary in the pOECT.

Additionally, the size of the electrode could be minimized by selecting alternative gate materials.

From the theoretical point of view, it is true that the size of the gate electrode for an OECT could be minimized by selecting alternative gate materials with higher capacitance in order to reduce its polarizability. However, as evidenced in Figure S8, even when we chose an alternative material with extremely high capacitance, this is not true when we use OMIECs in the channel. Moreover, most functionalization strategies involve the formation of self-assembled monolayers on top of gold surfaces due to the straightforward formation of Au-S bonds. With OECT-based sensing, we are very much restricted in the size and type of the components we choose for the gate. The device geometry and component optimization take very long when we want to build sensors. With the pOECT, none of this is necessary as we can practically use any type and size of channel and gate. This further demonstrates the superiority of the pOECT configuration, which is much more versatile and can produce efficient potentiometric sensing with a much wider range of materials (even with a high impedance, glass-like sensing surface).

We also have described above how we can miniaturize the pOECT and the additional electrode (G_G) does not impose any size limitations. In fact, we also show that the OECT may need larger sizes to operate fully, whereas the pOECT does not (Figure S8).

9. However, as the authors acknowledge, miniaturizing OECT is a critical goal, yet adding another gate electrode could increase the device's dimensions. Moreover, despite the claim that OECT is a robust sensing platform capable of attomole-femtomole scale detection, the presented data only pertains to the micromole scale. Consequently, I believe that the paper's concept and ideas may not align with the high standards of Nature Communications.

Hopefully, with the modifications we made to the text and the corrections to the figures, it is now clear that pOECT has no geometrical constraints and we prove that it can be easily miniaturized, as much as the OECT can be. As for the low LoD detection, we clarified why we used reliable electrodes and sensing models to show the superiorities of this novel configuration. We believe that through the revision of our work, we have efficiently demonstrated that:

(i) The pOECT can achieve high accuracy in potentiometric sensing and much larger device performance with similar or even a smaller device size than the OECT; please see the response to Reviewer 1.

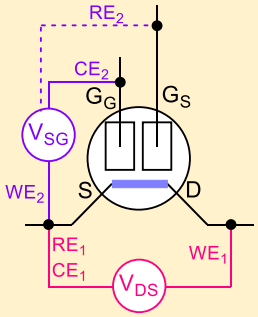
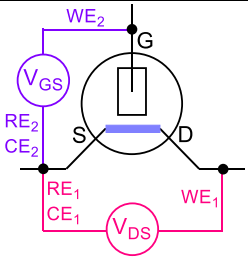
(ii) Presenting attomole-femtomole sensing would require a sensing demonstration using an antibody functionalization for a protein target detection. However, this type of sensing is not efficiently described by a thermodynamic equation and this would not allow us to evaluate the accuracy improvement of the pOECT over the OECT, which is the main goal of this work. Moreover, these attomole-femtomole concentrations would provide an input signal of the same mV range as the ion sensing proposed in this work, thus, without changing the outcome and meaning of the study; please see the response to comment 3.

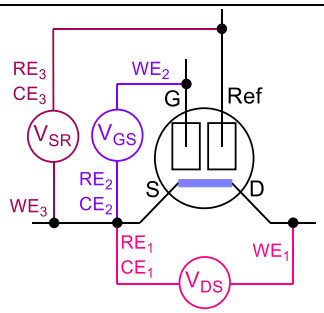
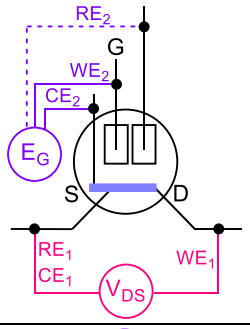
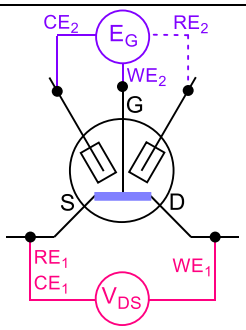
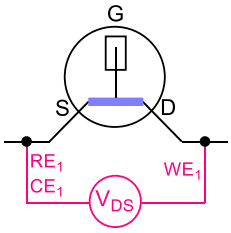
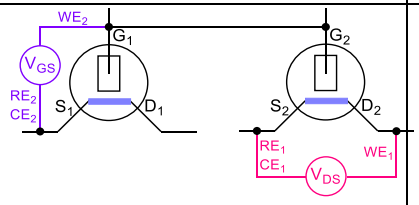
The main purpose of the pOECT is the removal of the leakage current (I_{GS}) from the sensing interface to fully achieve the required conditions of potentiometric sensing, removing any artifact from the sensor output, leading to the maximum theoretical accuracy and, at the same time being a non-destructive/non-harmful technique for the biolayer employed as a sensing interface.

The sensing interface polarization is a major issue in OECT potentiometric sensing, which has never been openly recognized and which could lead to significant artifacts and data misinterpretations such as false positives or false negatives, lowering the reliability of the measurements. Despite the several OECT variations proposed in the literature, see Table R1 for a detailed list, none is able to provide the fundamental advantage of the setup that we developed. The pOECT is the only and first example reported in the literature of OECT-based sensor that is able to maintain the sensing electrode in an open circuit condition, the fundamental requirement of potentiometric sensing. Most biomarkers are not redox active; hence, their electrochemical label-free detection necessitates a potentiometric measurement. Considering the importance of these biomarkers, any effort in improving the accuracy of the devices for their detection, especially if the achieved accuracy is the theoretical maximum, should be valued with high recognition.

Note that while developing a setup whose main purpose is to improve the accuracy of transistor-based potentiometric sensing, we obtained a platform with several more advantages. For the first time, it is possible to use sensing interfaces/materials of high impedance, increasing the library of possible applications, and for the first time, we provide a completely non-destructive platform for the sensing interface, all while keeping the device size to a practical and portable scale.

Table R1. Most relevant OECT configurations reported in the literature, with their specific schematics and the type of sensing they have been developed for. For the configurations capable of performing potentiometric sensing, the shortcomings are summarized compared to the pOECT setup.

Name	Schematics	Type of sensing	Disadvantages compared to pOECT	Ref.
pOECT		**(i)** Potentiometric		
OECT		**(i)** Faradaic **(ii)** Impedimetric **(iii)** Potentiometric	**(i)** Current and voltage directly applied to the sensing electrode **(ii)** Thermodynamic equilibrium disturbed **(iii)** Electrode degradation due to gate current	[1] [2]

Dual gate for high potential faradaic reactions		**(i)** Faradaic		[3]
Referenced-OECT		**(i)** Faradaic		[4]
First published OECT		**(i)** Impedimetric on the channel		[5]
Wrighton configuration		**(i)** Potentiometric	**(i)** Sensing electrode polarized **(ii)** Parasitic reactions on the G **(iii)** Necessity to optimize the size of the G **(iv)** Working only at an equivalent of V_{GS} = 0 V	[6]
Dual gate OECT for drift reduction		**(i)** Faradaic **(ii)** Impedimetric **(iii)** Potentiometric	**(i)** Current and voltage directly applied to the sensing electrode **(ii)** Thermodynamic equilibrium disturbed **(iii)** Electrode degradation due to gate current	[7]

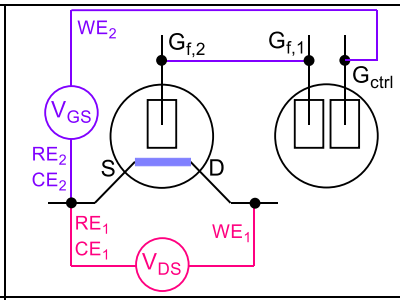
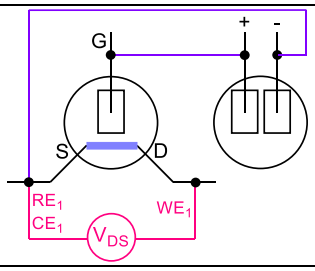
Floating gate		**(i)** Impedimetric **(ii)** Potentiometric	**(i)** Current and voltage directly applied to the sensing electrode **(ii)** Thermodynamic equilibrium disturbed **(iii)** Electrode degradation due to gate current	[8]
Reaction cell		**(i)** Spontaneous faradaic reactions		[9]

Table References

[1] Salvigni, L., Mariani, F., Gualandi, I., Decataldo, F., Tessarolo, M., Tonelli, D., Fraboni, B. and Scavetta, E., 2023. Selective detection of liposoluble vitamins using an organic electrochemical transistor. Sensors and Actuators B: Chemical, 393, p.134313.

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[4] Ji, X., Lin, X. and Rivnay, J., 2023. Organic electrochemical transistors as on-site signal amplifiers for electrochemical aptamer-based sensing. Nature Communications, 14(1), p.1665.

[5] White, H.S., Kittlesen, G.P. and Wrighton, M.S., 1984. Chemical derivatization of an array of three gold microelectrodes with polypyrrole: fabrication of a molecule-based transistor. Journal of the American Chemical Society, 106(18), pp.5375-5377.

[6] Gualandi, I., Tessarolo, M., Mariani, F., Tonelli, D., Fraboni, B. and Scavetta, E., 2019. Organic electrochemical transistors as versatile analytical potentiometric sensors. Frontiers in Bioengineering and Biotechnology, 7, p.354.

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[8] White, S.P., Sreevatsan, S., Frisbie, C.D. and Dorfman, K.D., 2016. Rapid, selective, label-free aptameric capture and detection of ricin in potable liquids using a printed floating gate transistor. ACS Sensors, 1(10), pp.1213-1216.

[9] Tan, S.T.M., Keene, S., Giovannitti, A., Melianas, A., Moser, M., McCulloch, I. and Salleo, A., 2021. Operation mechanism of organic electrochemical transistors as redox chemical transducers. Journal of Materials Chemistry C, 9(36), pp.12148-12158.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

I am satisfied that all of my comments have been addressed.

Reviewer #2 (Remarks to the Author):

I am generally satisfied with the authors' responses, which seems to meet the standards of Nature Communications. However, I remain skeptical about their claims concerning miniaturization. In Fig. S8, the authors demonstrate that six Ag/AgCl electrodes are necessary to achieve a current level equivalent to that of the pOECT, intending to illustrate the miniaturization effect of the new sensing configuration. Yet, a single Ag/AgCl electrode appears to perform similarly to the pOECT. In such cases, the purported advantage of the pOECT configuration seems diminished. Additionally, to truly claim miniaturization, the overall device structure should be reduced, akin to advancements seen in transistor technology. However, the demonstrated new configuration requires an additional gate area regardless of its size or fabricated dimensions (coplanar or pellet). Therefore, I suggest that the authors highlight the enhanced stability and accuracy of the sensing platform, rather than focusing on miniaturization.

Reviewer #3 (Remarks to the Author):

The manuscript reports on how the addition of an additional gate electrode improves the performance of usually three terminal OECT devices in electrochemical sensing applications. Different experiments involving pH-sensing, ion sensing and cell-impedance sensing are presented to support this claim. The impact of different OECT channel materials on sensor performance is also investigated. All methods are well described and the work is adequately structured and all critical information is included. However, I agree with the general critiques of the other reviewers of the manuscript that the novelty is limited: the idea to substitute the single gate electrode of an OECT by a gate reference and a gate counter electrode is not novel. Such an approach simply implements the standard procedure of electrochemistry to separate the potential sensing electrode (reference) form the current emitting electrode (counter electrode) to avoid polarization effects and other non-linear and hysteric responses as explained correctly in the manuscript. It is the basic reason why a potentiostat is the fundamental measurement instrument in electrochemistry. Accordingly, such separation of the gate electrode has already been presented to investigate transport in OMIEC materials and generally transport phenomena at semiconductor-electrochemistry interfaces in different works. Also research on transport in conducting polymers started with this approach before the more simplistic OECT design was introduced. Finding now a paper that proposes this kind of step-back as a significant novelty is a bit confusing, even though the reported findings are all correct and helpful to improve the performance of some potentiometric sensing applications.
In addition, I also agree with the other reviewers that some of the crucial statements in the manuscript (for example in the conclusions "… to overcome the severe limitations of the classical OECT when employed for potentiometric sensing") should be revised. OECTs have demonstrated quite reliable performance as sensor devices and "severe limitations" seems not adequate. Also, for OECTs there are other sensing mechanisms that operate in an amperomentric manner relying on the existence of a gate current flow.
To conclude, I would suggest a more specialized journal to publish this report due to the missing novelty. At the same time, I acknowledge the usefulness of the work. Many groups are active in the field of OECT sensors, I am conviced that the manuscript will find a wide readership. It will be a good support for researchers with less background in electrochemistry and I leave the decision about the relevance of the manuscript at the discretion of the editor. Below I list also some more technical points that should be addressed prior to a publication.
- Figure 6: in the schematic of the setup the sensing gate Gs is indicated, but in the 3D scheme it is called floating gate. The scheme should also include how the Pani-Gs is connected.
- Figure 7: the cutoff frequency should be reported in units of Hz and not in normalized units.
- Personally, I find the intermediate structure introduced as gate referenced-OECT quite useless and confusing. Also, in a three electrode geometry it should be obvious that only the potential differences between the electrodes determine the device response and the position of the "zero" on the source or on the gate does not have an impact.
- The supplementary methods should contain a more detailed description about how the

potentiostat was operated. In simple laboratory potentiostats the WE is at virtual ground and many of the connection schemes shown in the paper could not be realized as they require a floating potentiostat circuit.
- The manuscript should clearly reference that using RE and CE electrode as “gate” is standard in the characterization of transport phenomena in semiconductor electrochemistry.

Peer review file

Manuscript: Reconfiguration of organic electrochemical transistors for high-accuracy potentiometric sensing, NCOMMS-24-09539A-Z

We thank the reviewers for their valuable comments and for giving us the opportunity to improve this work and communicate our results more effectively. We are excited to address their concerns and revise our manuscript based on the questions. We have addressed each reviewer's comments (in bold) in a point-by-point response, as detailed below. All modifications to the text and figures are highlighted in yellow here and in our manuscript.

On behalf of all authors,

Sahika Inal

Reviewer #1 (Remarks to the Author):

I am satisfied that all of my comments have been addressed.

Thank you for the endorsement.

Reviewer #2 (Remarks to the Author):

I am generally satisfied with the authors' responses, which seems to meet the standards of Nature Communications. However, I remain skeptical about their claims concerning miniaturization. In Fig. S8, the authors demonstrate that six Ag/AgCl electrodes are necessary to achieve a current level equivalent to that of the pOECT, intending to illustrate the miniaturization effect of the new sensing configuration. Yet, a single Ag/AgCl electrode appears to perform similarly to the pOECT. In such cases, the purported advantage of the pOECT configuration seems diminished. Additionally, to truly claim miniaturization, the overall device structure should be reduced, akin to advancements seen in transistor technology. However, the demonstrated new configuration requires an additional gate area regardless of its size or fabricated dimensions (coplanar or pellet).

We thank the Reviewer for the positive feedback about the revisions made. We also thank them for this final comment that has encouraged us to perform experiments that delve further into the miniaturization point. We agree with the Reviewer that the OECT performance with a single AgCl is close to the pOECT. However, the measurement with single or multiple Ag/AgCl electrodes as G/G_S should not be considered as an option for operating the OECT/pOECT in potentiometric sensing due to functionalization limitations and non-polarizability of the AgCl electrode, which would prevent the potentiometric analytical signal to arise. We reported the operation with Ag/AgCl to represent the OECT operation in conditions of similar performance as the pOECT; conditions that otherwise are difficult to achieve through a gold electrode (the capacitance of an Au electrode of equivalent area is much smaller than an Ag/AgCl electrode). We understand that using an electrode (Ag/AgCl) that cannot be employed in OECT-based potentiometric sensing

instead of the more common gold can lead to confusion. For this reason, we decided to remove the experiments reported in the original **Figure S8** and perform an analogous experiment with gold G and G_S instead. These experiments are now reported in the new version of **Supplementary Figure 8** (page 20).

In this experiment, we show a pOECT operated with a gold G_S of very small size (diameter 170 µm) along with a G_G of 1.6 mm in diameter. In these conditions, the G_S polarization is minimal (1 mV). The experiment demonstrates that if we want to obtain an analogous small polarization with the OECT (necessary to claim an accurate potentiometric measurement), we need to use a gate electrode of 4 cm diameter. The gate of this OECT is significantly larger than the G_S of the pOECT (55-thousand times larger). Even when considering the sum of the G_G and G_S areas, we note that the pOECT components occupy a much smaller area (625 times) than the OECT. We report in **Figure R1** a visual representation, in scale, of the corresponding gating electrode sizes.

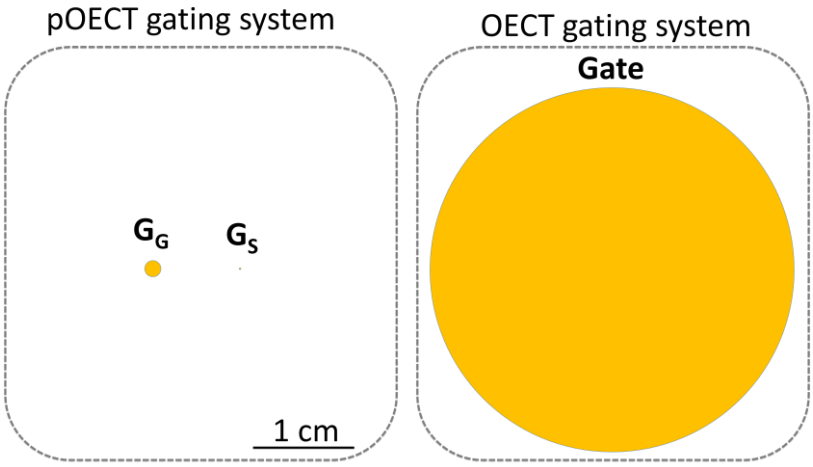


Figure R1. Comparison of the gating electrode sizes of OECT and pOECT necessary to obtain a sensing interface polarization not larger than 1 mV during device operation.

The main text (page 13, lines 6 to 15) and **Figure 4** (page 14) have been modified to report these findings:

"To construct an OECT with comparable accuracy as the pOECT, the G area could be increased, to reduce its polarization. However, the geometry required to attain the same pOECT performance exceeds any reasonable size for a small, portable device. In **Figure 4c**, we report how the G_S size can be reduced to a diameter as small as 170 µm while keeping the sensing electrode polarization to a very low value (1 mV). With an OECT, it is necessary to use a G with 4 cm diameter to minimize the electrode polarization to the same level as the pOECT, requiring a sensing electrode area of 55,000 times larger (**Supplementary Discussion 4**). This would be necessary to claim an accurate OECT potentiometric measurement. The pOECT allows for the use of smaller sensing interfaces, a significant advantage over the OECT in terms of device miniaturization while increasing device performance and ensuring accuracy."

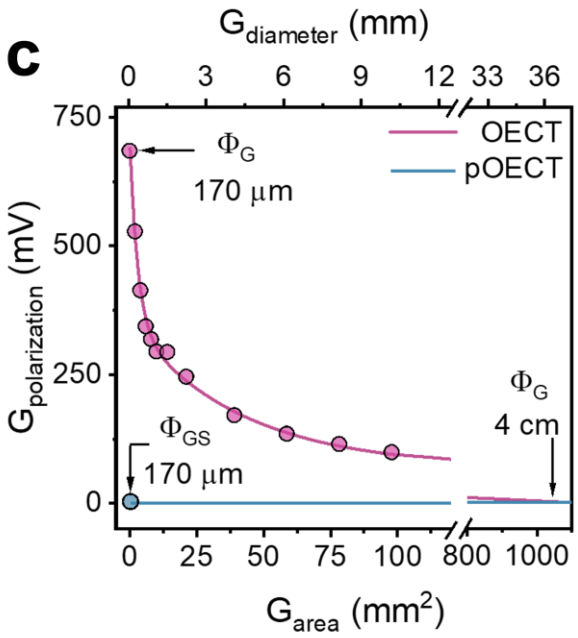


Figure 4. Performance comparison between pOECT and OECT … c) Relationship between the sensing interface (G and G_S) polarization and the electrode diameter/area in a pOECT and OECT bearing the same n-type channel. G and G_S are Au electrodes with varying diameters (Φ) and G_G is an Au electrode (1.6 mm diameter). …

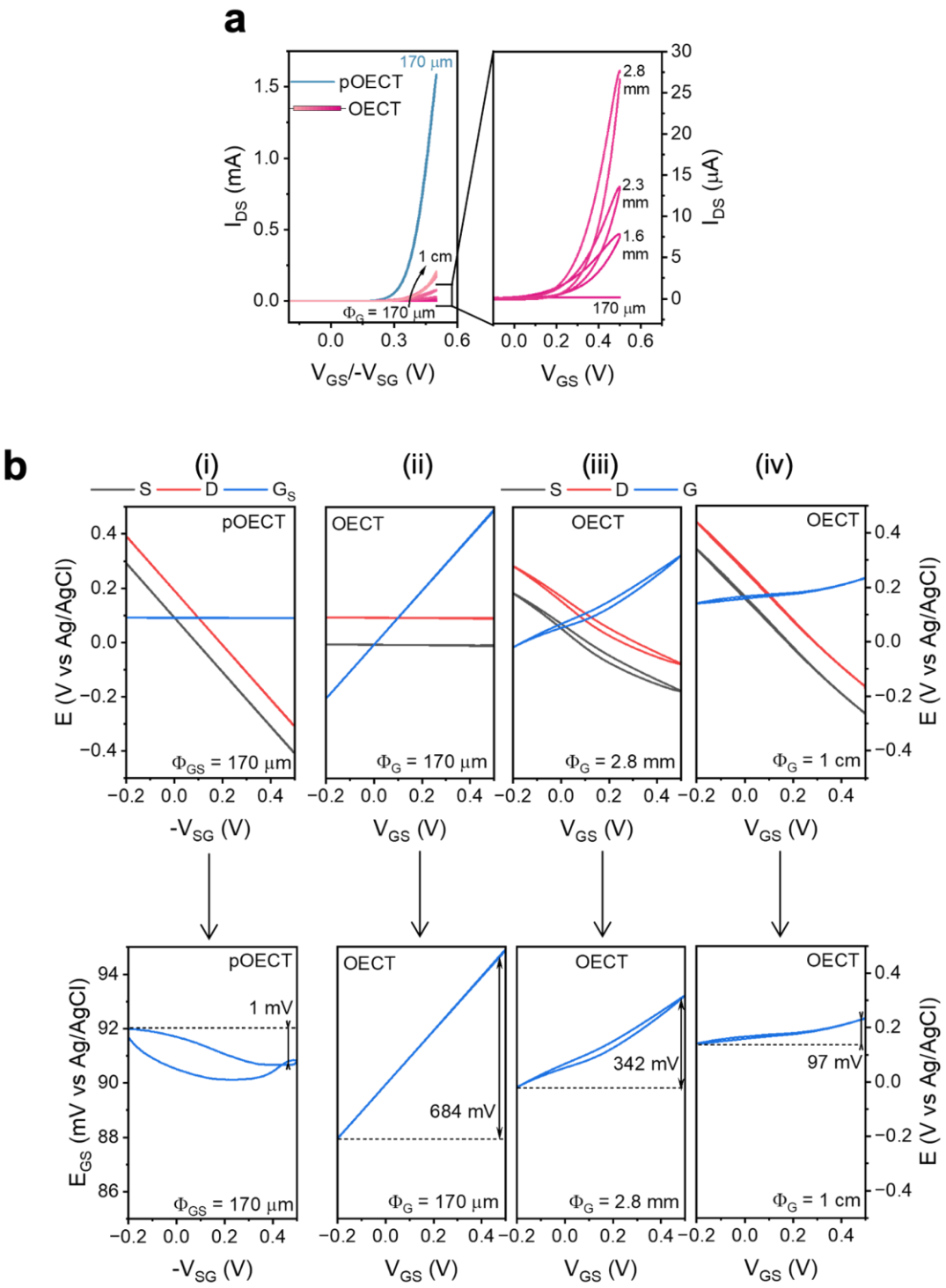
Supplementary Figure 8 (page 20) and the corresponding **Supplementary Discussion 4** (from page 18, line 19 to page 19, line 14) have been modified as follows:

"… For example, **Supplementary Figure 8a** shows the transfer curves of a pOECT in which the G_S is a very small Au electrode (170 µm in diameter) and an OECT in which the G is an Au electrode, the size of which is progressively increased from a diameter of 170 µm to 1 cm. The pOECT shows a full I_{DS} modulation up to 1.5 mA, with a minimal G_S polarization of 1 mV as reported in **Supplementary Figure 8b-i**. Note that, as already demonstrated in **Figure 2c**, the size optimization of the G_G is not crucial for determining the pOECT performance. Hence, we used an Au electrode of 1.6 mm diameter as GG in this experiment. When the same small G_S is used as G in an OECT (**Supplementary Figure 8a, right**), the I_{DS} modulation is absent because the applied V_{GS} drops almost entirely on the G itself (684 mV polarization), as demonstrated in **Supplementary Figure 8b-ii.** If we want to bring the I_{DS} modulation of the OECT to the same level as the pOECT, we need to increase the size of the G. By progressively increasing the G diameter from 170 µm up to 1 cm, we observe a gradual increase in I_{DS} modulation, **Supplementary Figure 8a-right**. The increase in G size gradually reduces the G polarization, as shown in **Supplementary Figure 8b-ii-iv**. However, even with a G diameter of 1 cm, which corresponds to an area 4200 times larger than the G_S of the pOECT, the G is still polarized (97 mV), **Supplementary Figure 8b-iv**, leading to a lower I_{DS} modulation compared to that of a pOECT operated with a much smaller sensing interface.

If we correlate the measured G polarization with the G diameter or area, we obtain the trend reported in **Figure 4c**. We observe an exponential decrease of the G polarization with size, but the G polarization is comparable to the G_S polarization only when the G reaches a diameter of 4 cm. This demonstrates that an OECT with an equivalent accuracy as the pOECT (minimal sensing interface polarization) requires the G to be 55-thousand times larger. Even when we consider the

total gating area of the pOECT (i.e., the size of the G_G plus that of the G_s), the pOECT gating system area is 625 times smaller than that of the OECT ($A_G/(A_{GS}+A_{GG})$).

We can conclude that an OECT with comparable performance as a pOECT requires a gate electrode of unpractical size. Hence, the pOECT, despite the integration of a second electrode, remains smaller than an OECT designed for potentiometric sensing applications."

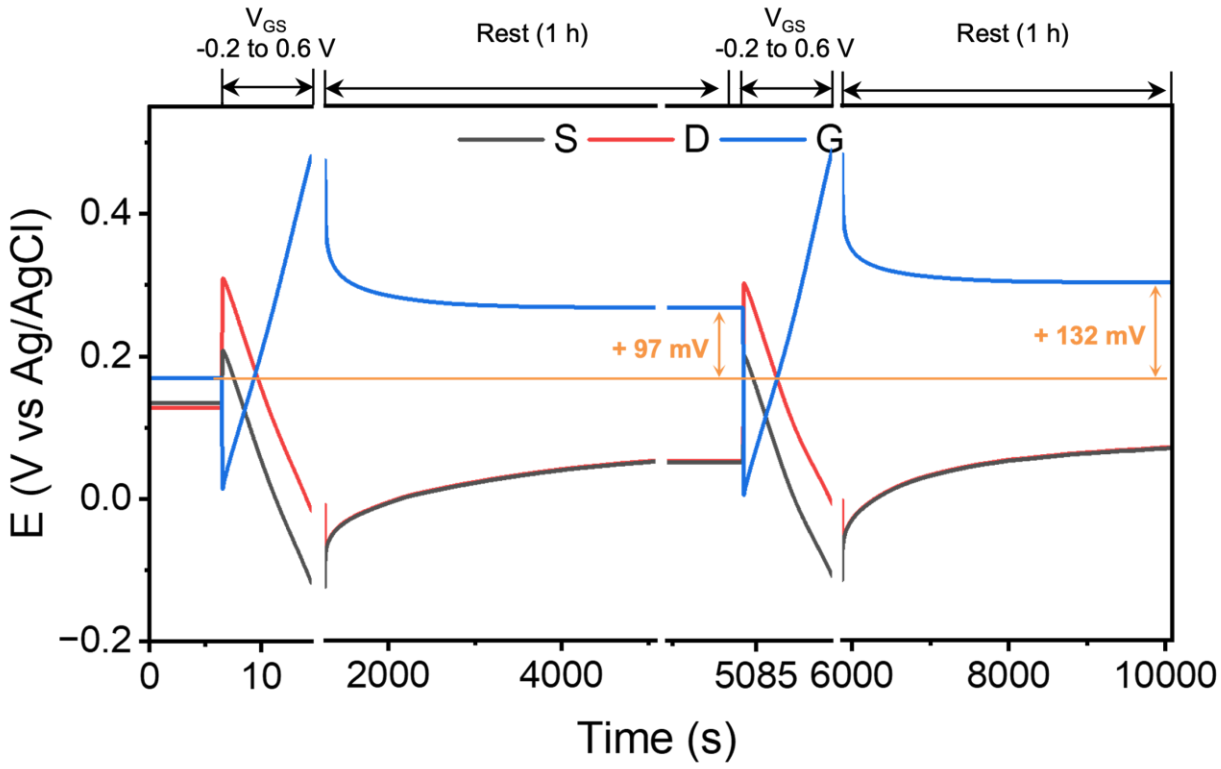


Supplementary Figure 8. Sensing electrode polarization vs. size in OECT and pOECT. a) Left: Transfer curves of a pOECT (blue; operated with G_S of Au- 170 µm of diameter and G_G of Au- 1.6 mm of diameter) and an OECT (pink; operated with G electrodes with a diameter ranging from 170 µm to 1 cm. Right: zoomed-in OECT transfer curves. **b)** Electrochemical potential changes of the OECT/pOECT components during the transfer curves, for a specific G/G_S diameter (Φ). The bottom panel shows the electrochemical potentials exclusively for the G/G_S, reporting the total electrode polarization.

We hope that these experiments are sufficient to demonstrate that the pOECT can be designed at a significantly smaller size than the OECT when both can perform accurate potentiometric sensing.

Therefore, I suggest that the authors highlight the enhanced stability and accuracy of the sensing platform, rather than focusing on miniaturization.

We thank the Reviewer for this comment. We performed a new set of experiments to investigate the device stability. These experiments are shown in **Supplementary Figure 9** (page 22) and **Figure 4-d,e** (page 14).



Supplementary Figure 9. Permanent alteration of the sensing electrode potential upon OECT operation. Electrochemical potential vs time of the n-type OECT components during two consecutive sets of 50 transfer curves each, separated by a resting period. No voltage is applied until the 5th second, followed by the first transfer curve of the first set (V_{GS} -0.2 to 0.6 V); the subsequent 49 cycles are not displayed. At the end of the first set, there is a one-hour resting period during which no voltage is applied. This period shows a new equilibrium potential of the G due to the I_{GS}, leading to a permanent increase in the electrode surface potential of 97 mV. This

increase represents the permanent change of the electrode properties, i.e., a new potential and loss of analytical information. This also causes a different output current in the second set of transfer curves (after 5000 s) and a further change of the equilibrium surface potential of the sensing electrode (+132 mV).

The corresponding transfer curves of this measurement are shown in the new **Figure 4 d-e** (page 14) and prove the higher stability of the pOECT. We also removed the stability measurements in **Supplementary Figure S7** to avoid repetition.

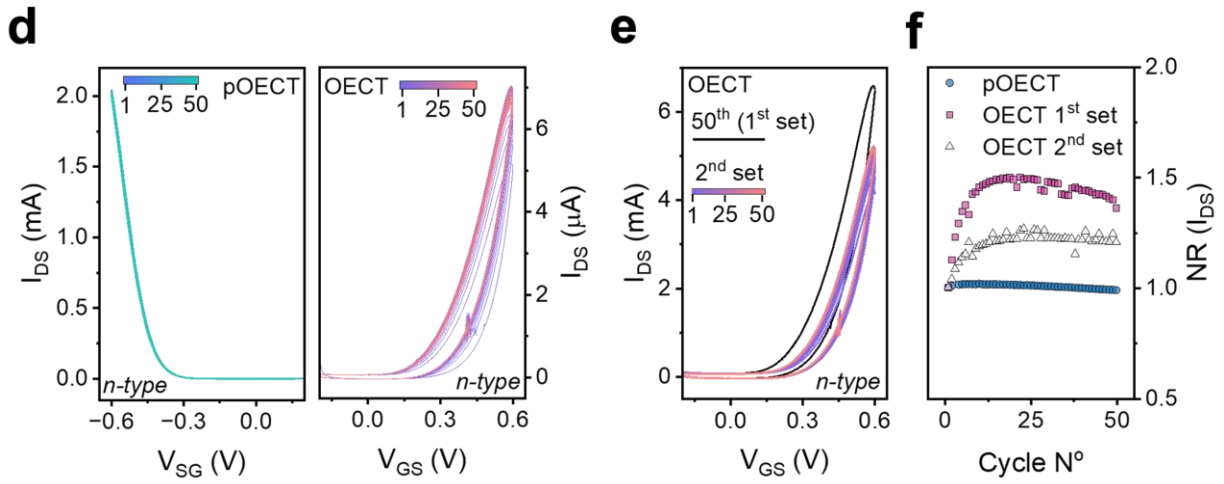


Figure 4. Performance comparison between pOECT and OECT … d) 50 consecutive transfer curves with pOECT and OECT, where the G/G_S is a 3 mm-diameter and G_G is 1.6 mm-diameter Au electrode. **e)** The 50th (final) transfer curve of the first cycling set (black) and the transfer curves recorded during the second set of cycling tests for the OECT. **f)** Normalized response at V_{SG} = -V_{GS} = -0.55 V of the pOECT and OECT during one set of transfer curves and two consecutive sets of transfer curves respectively. …

We now changed the title of the previous subsection “Thermodynamic stability of the pOECT - Comparison with the OECT” as “The performance comparison of the pOECT versus the conventional OECT” (page 12, line 11) to summarize all the advantages compared to the OECT in one single section. The text reads as follows (page 13, lines 16 to 23):

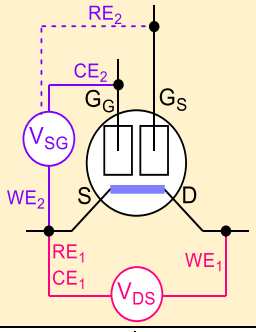
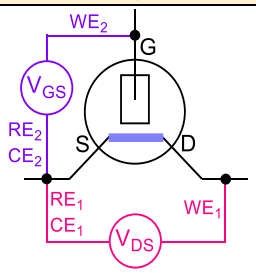
“Since the G_S is not polarized, the pOECT output response remains constant upon consecutive biasing cycles. In contrast, the OECT shows deviations with a continuous drift of the transfer curve (Fig. 4d). Fig. 4e shows the OECT output during a second set of biasing cycles. The second set of transfer curves differs from the first one due to the leakage current (IGS), which permanently alters the electrochemical potential of the gate (+97 mV, Supplementary Fig. 9). In Fig. 4f, we plot the OECT and pOECT I_{DS} at constant operating voltages during the entire duration of these biasing experiments, evidencing the unstable OECT response compared to stable pOECT channel current.”

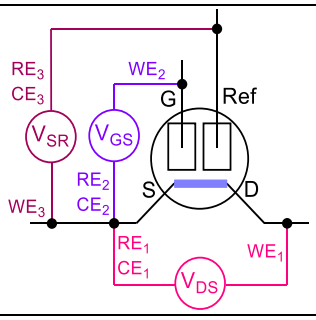
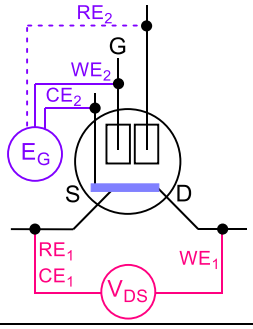
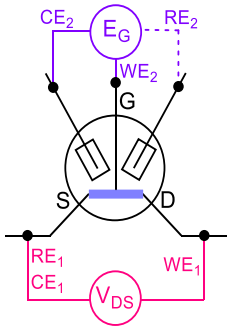
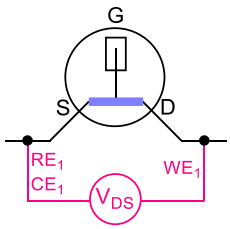
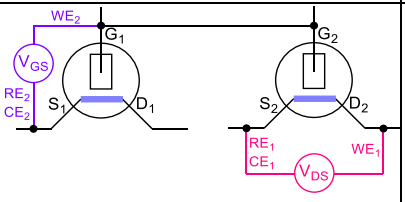
Reviewer #3 (Remarks to the Author):

The manuscript reports on how the addition of an additional gate electrode improves the performance of usually three terminal OECT devices in electrochemical sensing applications. Different experiments involving pH-sensing, ion sensing and cell-impedance sensing are presented to support this claim. The impact of different OECT channel materials on sensor performance is also investigated. All methods are well described and the work is adequately structured and all critical information is included. However, I agree with the general critiques of the other reviewers of the manuscript that the novelty is limited: the idea to substitute the single gate electrode of an OECT by a gate reference and a gate counter electrode is not novel.

To the best of our knowledge, there is no OECT-based publication for potentiometric sensing that uses the configuration reported in this work. In **Table R1**, provided in the previous response letter, we summarized other alternative OECT sensing setups as well as the first published OECT configuration. In none of these, a sensing electrode is connected to the RE terminal of the gating system, along with an auxiliary electrode as CE to provide the doping current, for a total of four electrodes involved in the device operation.

Table R1. Most relevant OECT configurations reported in the literature, with their specific schematics and the type of sensing they have been developed for. For the configurations capable of performing potentiometric sensing, the shortcomings are summarized compared to the pOECT setup.

Name	Schematics	Type of sensing	Disadvantages compared to pOECT	Ref.
pOECT		**(i)** Potentiometric		
OECT		**(i)** Faradaic **(ii)** Impedimetric **(iii)** Potentiometric	**(i)** Current and voltage directly applied to the sensing electrode **(ii)** Thermodynamic equilibrium disturbed **(iii)** Electrode degradation due to gate current	[1] [2]

Dual gate for high potential faradaic reactions		**(i)** Faradaic		[3]
Referenced-OECT		**(i)** Faradaic		[4]
First published OECT		**(i)** Channel material characterization		[5]
Wrighton configuration		**(i)** Potentiometric	**(i)** Sensing electrode polarized **(ii)** Parasitic reactions on the G **(iii)** Necessity to optimize the size of the G **(iv)** Working only at an equivalent of V_{GS} = 0 V	[6]
Dual gate OECT for drift reduction		**(i)** Faradaic **(ii)** Impedimetric **(iii)** Potentiometric	**(i)** Current and voltage directly applied to the sensing electrode **(ii)** Thermodynamic equilibrium disturbed **(iii)** Electrode degradation due to gate current	[7]

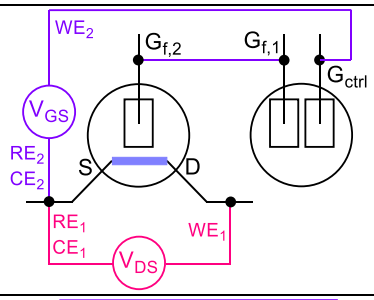
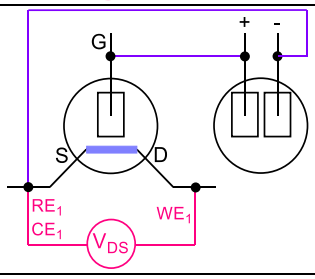
Floating gate		**(i)** Impedimetric **(ii)** Potentiometric	**(i)** Current and voltage directly applied to the sensing electrode **(ii)** Thermodynamic equilibrium disturbed **(iii)** Electrode degradation due to gate current	[8]
Reaction cell		**(i)** Spontaneous faradaic reactions		[9]

Table References

[1] Salvigni, L., Mariani, F., Gualandi, I., Decataldo, F., Tessarolo, M., Tonelli, D., Fraboni, B. and Scavetta, E., 2023. Selective detection of liposoluble vitamins using an organic electrochemical transistor. Sensors and Actuators B: Chemical, 393, p.134313.

[2] Liu, H., Yang, A., Song, J., Wang, N., Lam, P., Li, Y., Law, H.K.W. and Yan, F., 2021. Ultrafast, sensitive, and portable detection of COVID-19 IgG using flexible organic electrochemical transistors. Science advances, 7(38), p.eabg8387.

[3] Wu, S.E., Yao, L., Shiller, A., Barnard, A.H., Azoulay, J.D. and Ng, T.N., 2021. Dual-Gate Organic Electrochemical Transistors for Marine Sensing. Advanced Electronic Materials, 7(6), p.2100223.

[4] Ji, X., Lin, X. and Rivnay, J., 2023. Organic electrochemical transistors as on-site signal amplifiers for electrochemical aptamer-based sensing. Nature Communications, 14(1), p.1665.

[5] White, H.S., Kittlesen, G.P. and Wrighton, M.S., 1984. Chemical derivatization of an array of three gold microelectrodes with polypyrrole: fabrication of a molecule-based transistor. Journal of the American Chemical Society, 106(18), pp.5375-5377.

[6] Gualandi, I., Tessarolo, M., Mariani, F., Tonelli, D., Fraboni, B. and Scavetta, E., 2019. Organic electrochemical transistors as versatile analytical potentiometric sensors. Frontiers in Bioengineering and Biotechnology, 7, p.354.

[7] Song, Y., Zhang, H., Mukhopadhyaya, T., Hall, A.S. and Katz, H.E., 2022. Sensitive organic electrochemical transistor biosensors: Comparing single and dual gate functionalization and different COOH-functionalized bioreceptor layers. Biosensors and Bioelectronics, 216, p.114691.

[8] White, S.P., Sreevatsan, S., Frisbie, C.D. and Dorfman, K.D., 2016. Rapid, selective, label-free aptameric capture and detection of ricin in potable liquids using a printed floating gate transistor. ACS Sensors, 1(10), pp.1213-1216.

[9] Tan, S.T.M., Keene, S., Giovannitti, A., Melianas, A., Moser, M., McCulloch, I. and Salleo, A., 2021. Operation mechanism of organic electrochemical transistors as redox chemical transducers. Journal of Materials Chemistry C, 9(36), pp.12148-12158.

Such an approach simply implements the standard procedure of electrochemistry to separate the potential sensing electrode (reference) form the current emitting electrode (counter electrode) to avoid polarization effects and other non-linear and hysteric responses as explained correctly in the manuscript. It is the basic reason why a potentiostat is the fundamental measurement instrument in electrochemistry. Accordingly, such separation of the gate electrode has already been presented to investigate transport in OMIEC materials and generally transport phenomena at semiconductor-electrochemistry interfaces in different works. Also research on transport in conducting polymers started with this approach before the more simplistic OECT design was introduced. Finding now a paper that proposes this kind of step-back as a significant novelty is a bit confusing, even though the reported findings are all correct and helpful to improve the performance of some potentiometric sensing applications.

We agree with the Reviewer that the development of the pOECT follows the principles of the basic working mechanism of a potentiostat because the pOECT is operated with a potentiostat. Despite the claim that this configuration is so trivial, we could not find any publication in which an OECT is operated in the way we reported, specifically for potentiometric sensing. The Reviewer states that a configuration in which RE and CE are separated and used for modulating the OMIEC doping state while measuring its conductivity has already been presented in studies investigating transport in OMIECs. We think the reviewer refers to the configuration shown here in **Figure R2-a-d** (literature examples) and **Figure R2-g** (general schematic for comparison with the OECT and pOECT setup). We define this configuration “Bipotentiostat-OECT” because these measurements were generally performed by using a bipotentiostat. In a bipotentiostat, the two channels operating WE_1 and WE_2 share the same RE and CE. The same happens in a Bipotentiostat-OECT in which the potentials of S and D are modulated by using the same RE and the same CE. However, in a pOECT RE_1 and RE_2 are two distinct electrodes as well as CE_1 and CE_2. In particular, in a pOECT RE_1 = CE_1= Source, RE_2 = Conventional working electrode/sensing electrode and CE_2 = Conventional counter electrode. On the other hand, in a Bipotentiostat-OECT for OMIECs characterization RE_1 = RE_2 = RE = Conventional reference electrode, and CE_1 = CE_2 = CE = Conventional counter electrode. We summarized this in **Table R2**, where it is evident that the only common element between the pOECT and the Bipotentiostat-OECT configuration is the use of a conventional counter electrode as CE_2. In the Bipotentiostat-OECT, any polarization issue is removed. But although the pOECT and Bipotentiostat-OECT can bias the transistor channel at the same electrochemical potentials (**Figure R3b**), these configurations are not identical also in terms of output signal regardless the use of a p-type or n-type channel (**Figure R3-a,c**). The Bipotentiostat-OECT is able to provide the correct I_{DS} value (analytical signal in the OECT-based potentiometric sensing) only if the approximation $\Delta i_F << i_p$ is true (see **Figure R2-b**), leading to a not always accurate value, as highlighted in **Figure R3-a,c**. On the other hand, the pOECT is always able to provide the real and most accurate I_{DS} value, since the instrument is actively

applying a voltage difference between the S and D (V_{DS}) and directly measuring the current (I_{DS}). The Bipotentiostat-OECT also does not provide directly the analytical signal (I_{DS}) which instead has to be calculated after the measurement from the real device output signals, I_{WE1} and I_{WE2}, (**Figure R3-d**), further complicating the data analysis process and propagating the error associated to the I_{WE} measurement.

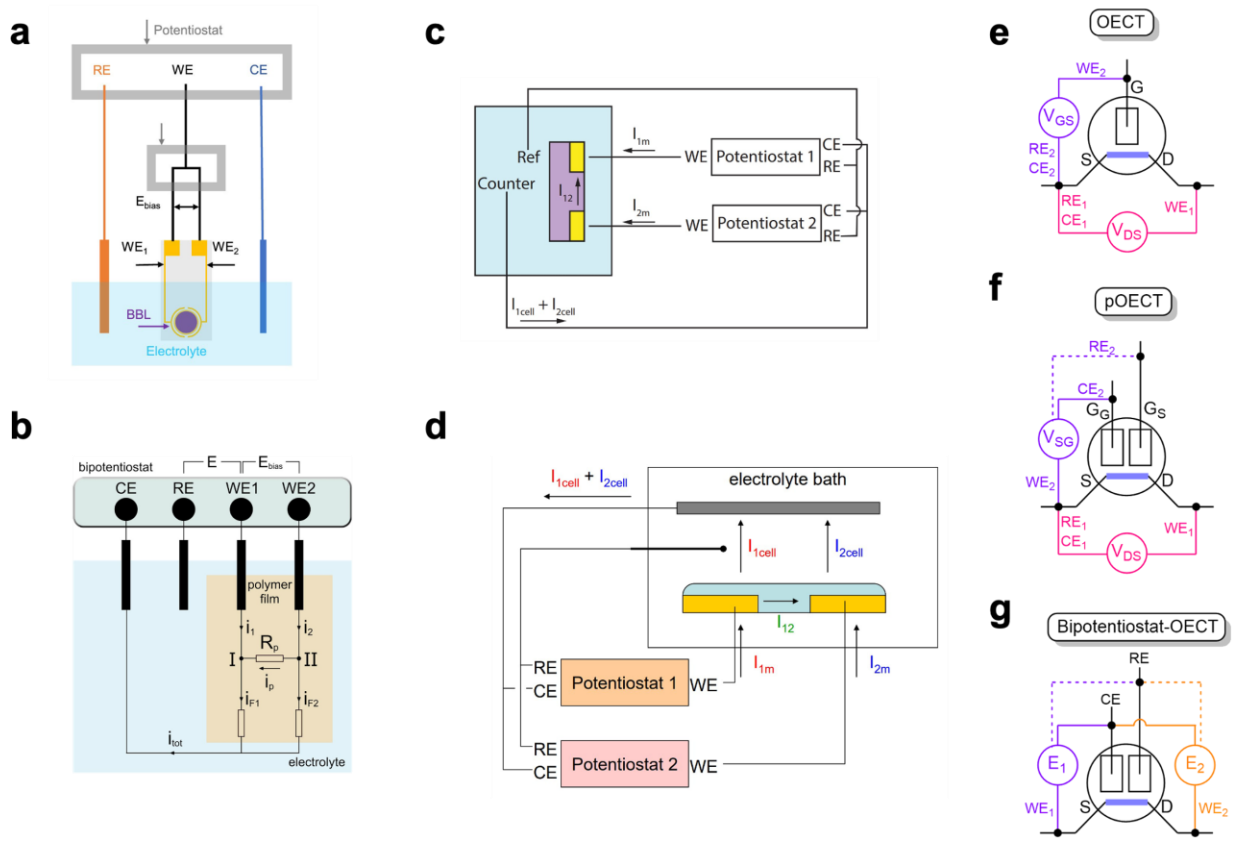


Figure R2. Examples of Bipotentiostat-OECT setups reported in literature: a) T. Ma, et al., *Understanding the mechanism of a conjugated ladder polymer as a stable anode for acidic polymer-air batteries*, 2023, Joule, b) C. Karlsson, et al., *Ion- and Electron Transport in Pyrrole/Quinone Conducting Redox Polymers Investigated by In Situ Conductivity Methods*, 2015, Electrochim. Acta, c) G. LeCroy, et al., *Role of aggregates and microstructure of mixed-ionic–electronic-conductors on charge transport in electrochemical transistors*, 2023, Mater. Horiz., d) Nathan Alan Vandesteeg, *Synthesis and characterization of conducting polymer actuators*, 2007, Thesis. Schematic of e) OECT and f) pOECT setups used for potentiometric sensing. g) Schematic of a bipotentiostat setup used to investigate conductivity and transport phenomena in OMIECs.

Table R2. Details on the electrical connections in a pOECT and a Bipotentiostat-OECT

	Bipotentiostat-OECT	pOECT
WE_1	Source	Drain
RE_1	Conventional reference electrode	Source
CE_1	Conventional counter electrode	Source
WE_2	Drain	Source
RE_2	Conventional reference electrode	Conventional working electrode
CE_2	Conventional counter electrode	Conventional counter electrode

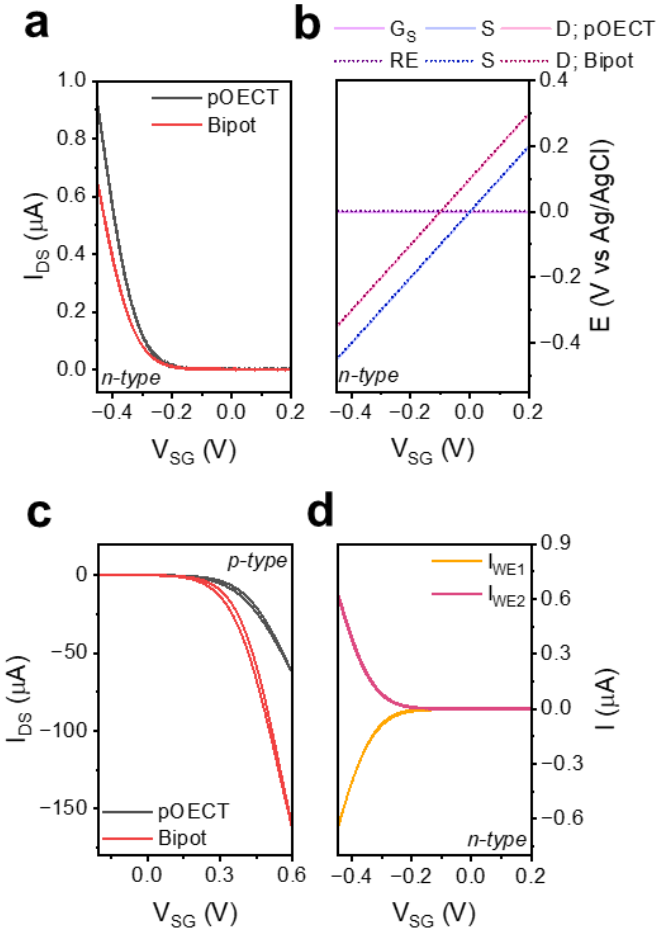


Figure R3. a) Transfer curves of an n-type OMIEC channel in either the pOECT or Bipotentiostat-OECT configurations. **b)** Electrochemical potentials of the n-type pOECT and Bipotentiostat-OECT components during the transfer curve. **c)** Transfer curves of a p-type OMIEC channel in either the pOECT or Bipotentiostat-OECT configurations. An Ag/AgCl was used as G_S in the pOECT and as RE in the Bipotentiostat-OECT, a Pt wire was used as G_G in the pOECT and as CE in the Bipotentiostat-OECT. The I_{DS} of the Bipotentiostat-OECT was calculated as $(I_{WE2}-I_{WE1})/2$, as reported in the literature (C. Karlsson, et al., *Ion- and Electron Transport in Pyrrole/Quinone Conducting Redox Polymers Investigated by In Situ Conductivity Methods*, 2015, Electrochim. Acta). **d)** Output signals of the Bipotentiostat-OECT from which the approximated I_{DS} value is calculated.

Even if other publications, which we were not able to find, use the exact electrical connections of the pOECT for investigating the transport phenomena of OMIECs, this still does not compromise the novelty of this work. In fact, in studies where OMIECs properties are investigated, the electrode connected to the RE_2 terminal should be a conventional reference electrode whose potential remains constant for the whole experiment. On the other hand, in this work, we approached the pOECT configuration in a non-conventional way, by using what is normally employed as a working electrode (sensing electrode) as a reference electrode for the doping voltage application. In our setup, the electrochemical potential of the RE_2 is no longer constant as in the transport phenomena studies, but continuously changes depending on the concentration of the target of interest. The application of the configuration is also different (biosensing vs. transport phenomena studies). Therefore, we believe our work is novel, and the configuration is not obvious especially since the issue of gate/sensing interface polarization is often not discussed in the field of OECT-based sensing (see **Supplementary Discussion 5: Nernstian and Super Nernstian response of pOECT and OECT**, page 23, for more evidence).

To avoid any conflict with possible previous publications, we modified the abstract (page 1, line 21), introduction (page 4, line 8,9) and conclusions (page 24, line 15), removing the adjective "new" and specifying that this configuration is introduced in the field of OECT-sensing:

"In this work, we introduce an organic electrochemical transistor sensing configuration, called potentiometric OECT (pOECT)…"

"To overcome these problems and use the OECTs at their maximum performance for biosensing, we introduced an OECT sensing configuration called potentiometric-OECT (pOECT)."

"We have introduced an OECT sensing configuration, called the pOECT…"

In addition, I also agree with the other reviewers that some of the crucial statements in the manuscript (for example in the conclusions "… to overcome the severe limitations of the classical OECT when employed for potentiometric sensing") should be revised. OECTs have demonstrated quite reliable performance as sensor devices and "severe limitations" seems not adequate.

We modified the manuscript accordingly (page 4, line 1 and page 24, line 15,16):

"In this case, the OECT input signal ~~becomes~~ can become unreliable, making the device output inaccurate."

"We have introduced an OECT sensing configuration, called the pOECT, to overcome ~~the severe~~ some possible limitations of the classical OECT when employed for potentiometric sensing…"

Also, for OECTs there are other sensing mechanisms that operate in an amperometric manner relying on the existence of a gate current flow.

We agree with the Reviewer that there are other sensing mechanisms that rely on the existence of the gate current flow as an input analytical signal, which is then amplified as an output signal in I_{DS}. This type of sensing is faradaic sensing, where the oxidation or reduction of the analyte on the gate electrode provides an extra gate current used to modulate further the doping state of the

OMIEC (L. Salvigni, et al., *Selective detection of liposoluble vitamins using an organic electrochemical transistor*, 2023, Sens. Actuators B Chem). However, faradaic sensing differs from potentiometric sensing, in which no redox reaction occurs and no current flows. This is why, in the conclusions, we specified that the limitations illustrated in this work regarding the OECT are only for potentiometric sensing: "…to overcome some possible limitations of the classical OECT when employed for potentiometric sensing…".

To conclude, I would suggest a more specialized journal to publish this report due to the missing novelty. At the same time, I acknowledge the usefulness of the work. Many groups are active in the field of OECT sensors, I am conviced that the manuscript will find a wide readership. It will be a good support for researchers with less background in electrochemistry and I leave the decision about the relevance of the manuscript at the discretion of the editor.

We thank the reviewer for the support.

Below I list also some more technical points that should be addressed prior to a publication.

- Figure 6: in the schematic of the setup the sensing gate Gs is indicated, but in the 3D scheme it is called floating gate. The scheme should also include how the Pani-Gs is connected.

In the 3D scheme of **Figure 6-top**, the floating gate is the system composed of two vertical electrodes and labelled as Ag/AgCl floating gates. This element is not necessary, for example, if a microfluidic channel is employed for the ionic connection between the two electrolyte chambers or if the measurement can be carried out in a single electrolyte, as specified in the main text. For this reason, the floating gate is not reported in the schematic of **Figure 6-bottom**, which illustrates the general configuration and not the specific case in which the measurement has to be carried out in two separate electrolytes.

In the 3D scheme of **Figure 6-top**, the G_S label was reported as "PANI-G_S" since the G_S is made of PANI, and it is reported next to the planar electrode used as G_S. For clarity, we rotated the PANI-G_S of 180° to have the electrical connection of this electrode located right in front of its corresponding label.

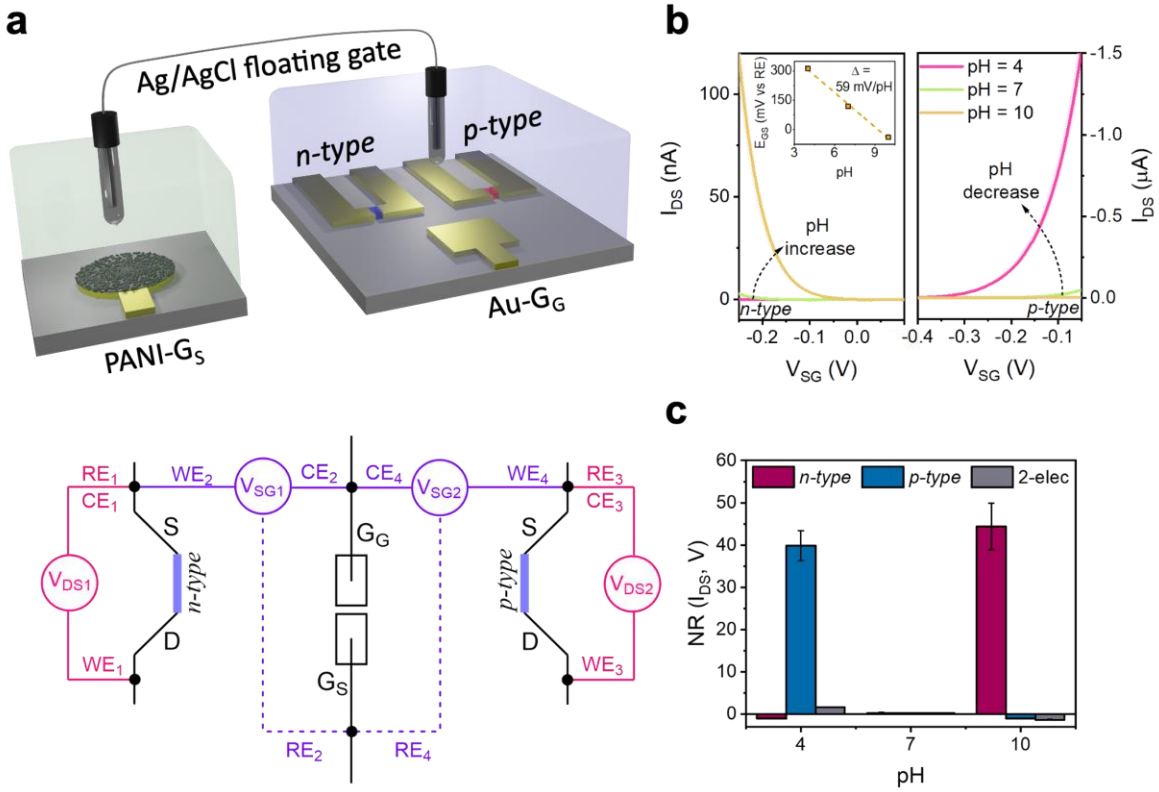


Figure 6*. Complementary channel pOECT for exclusive turn-ON operation **….** Bottom: a schematic of the setup where each channel is operated using the same G_S and G_G and has its independent drain voltage (V_{DS1}, V_{DS2}) and gate voltage (V_{SG1}, V_{SG2}). The dashed lines represent the high-impedance connections of the potentiostat. The floating gate is not represented. …*

The PANI-G_S of **Figure 6-top** corresponds to the G_S of **Figure 6-bottom**, where it is represented how the electrode is connected to the instrument, hence, through the RE connector. We prefer not to report in the 3D scheme how the PANI-G_S is connected because (i) this would be redundant and (ii) we should then also report how all the other electrodes are connected, making the whole figure very crowded. For more clarity, we specified in the caption of **Figure 6** that the floating gate is not represented in the schematic (**Figure 6a-bottom**); see previous image.

- Figure 7: the cutoff frequency should be reported in units of Hz and not in normalized units.

Many authors do normalization when reporting bandwidth curves for this type of sensing (some examples the literature are: Scherrine A. Tria, et al., 2014, Adv. Healthc. Mater., Magali P. Ferro, et al., 2018, Adv. Biosys., M. Ramuz, et al., 2014, Adv. Mat.). This is because the main sensor output they look for is the response time change, not the actual value. The value of interest is not the absolute frequency but its dynamic change. The reference value we use in the normalization is the response time of the OECT where the cells are completely detached from the channel. Hence, the device shows the fastest response time (highest frequency). The experiment aims to

monitor the dynamic evolution of the channel response from the conditions of the slowest response (cell-containing), which can be labeled as 0, and the condition of the fastest response (no cells), which can be labeled as 1. The normalization also helps to compensate the not excellent reproducibility of the spin coating process used to deposit the OMIEC on the transistor channels. The accuracy of this approach is further confirmed by the small standard deviation (**Figure 7c-bottom**) and the similarity in the device response when the signal is normalized and the diffusion delay of each device is subtracted from the output (**Supplementary Figure 17**). The non-normalized values would look like this while we prefer to present the normalized form:

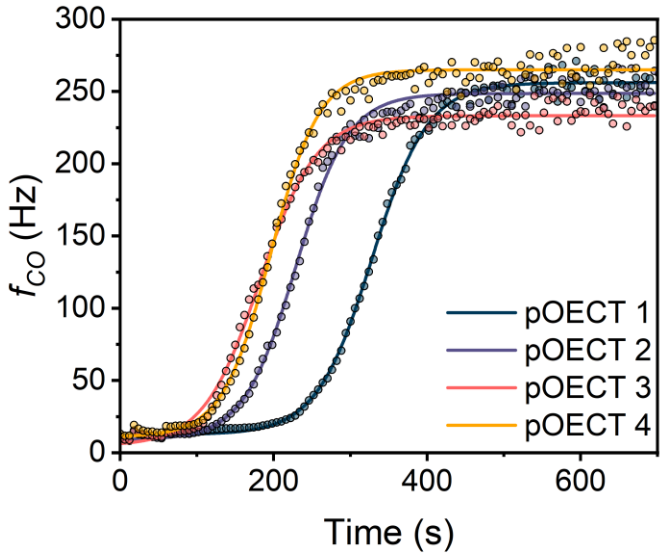


Figure R4. Change in the cut-off frequency during time of four pOECT channels triggered by the trypsin addition.

- Personally, I find the intermediate structure introduced as gate referenced-OECT quite useless and confusing. Also, in a three electrode geometry it should be obvious that only the potential differences between the electrodes determine the device response and the position of the "zero" on the source or on the gate does not have an impact.

We believe that the intermediate structure allows the reader to understand how the OECT configuration is gradually converted to the pOECT configuration. This intermediate configuration might look unnecessary for someone with a background in electrochemistry and understanding for how a potentiostat works. However, since the OECT sensors are studied by scientists with different backgrounds, we believe it is useful to keep this intermediate configuration in the illustration.

- The supplementary methods should contain a more detailed description about how the potentiostat was operated. In simple laboratory potentiostats the WE is at virtual ground and many of the connection schemes shown in the paper could not be realized as they require a floating potentiostat circuit.

We thank the Reviewer for this comment. We added more details in the supplementary methods (page 3, lines 14 to 21) on how to operate the OECT, gate referenced-OECT and pOECT configurations with either a multichannel potentiostat or a combination of an SMU and a potentiostat, as follows:

"Device operation, characterization, and sensing measurements: All electrochemical measurements were performed with a BioLogic VSP-300 multichannel potentiostat. The transfer curves of the OECT/pOECT were recorded by connecting the devices according to the schematics reported in **Figure S1**. Specifically, two channels of the multichannel potentiostat were used, the first one for the application of V_{DS} and the second one for the application of V_{GS} or V_{SG}. The instrument can be operated in either floating or grounded mode. The same device operation can be obtained also by combining a source measure unit for the application of V_{DS} and a single channel potentiostat for the application of V_{GS} or V_{SG}."

We also modified the initial part of Results and Discussion – The pOECT configuration section (page 5, line 1,2) as follows:

*"*The top panel in **Fig.1a** presents the electrical connections of a conventional OECT, as an electrochemical cell*."*

As per reviewer's interest, we operated the pOECT in floating or grounded mode and found no significant difference in both I_{DS} and electrochemical potentials of the device components. For this reason, we reported in the supplementary methods that the instrument can be operated in either floating or grounded mode. Please see **Figure R5.**

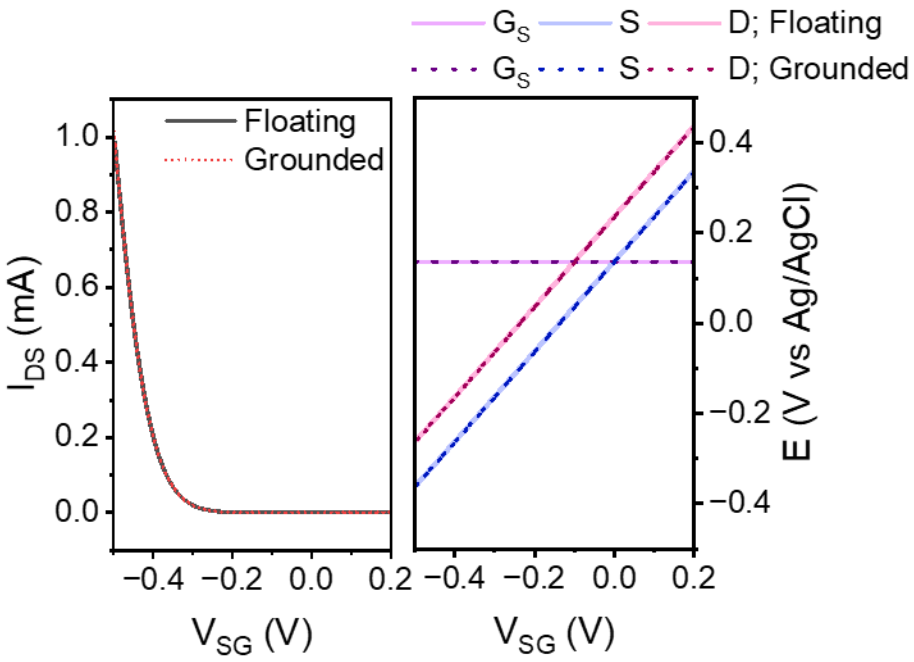


Figure R5. pOECT transfer curve (left) and electrochemical potentials of the pOECT components (right) when the instrument is operated either in floating or grounded mode.

- The manuscript should clearly reference that using RE and CE electrode as “gate” is standard in the characterization of transport phenomena in semiconductor electrochemistry.

Separating RE and CE is in fact a standard method in electrochemistry, and has not only been used for the characterization of transport phenomena of semiconductors. Secondly, as described above, the transport studies that used a potentiostat to characterize OECTs/FETs have not used the configuration we introduced here. We, therefore, do not think that we should particularly highlight the use of an electrochemical cell for transport studies (since we neither focus on OMIEC characterization here nor the method that we use is the same as the previous works). The main message of this work is that OECT-based biosensors are also electrochemical systems and should be treated as such. They should also be configured in a way that they satisfy the main requirements of an electrochemical cell, to prevent artifacts, degradation, and loss of accuracy but also improve current response. This is a very important consideration that is currently missing in biosensor literature.

We modified the introduction of the manuscript as follows (page 4, line 11,12):

"… In this configuration, we maintain the sensing electrode in OCP conditions by minimizing any current flow/voltage bias at this interface, bringing the OECT as close as possible to the ideal conditions of an electrochemical cell."

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

I am satisfied with the responses from the authors.

Reviewer #3 (Remarks to the Author):

The authors provide reasonable answers to the critical points raised by the reviewers. The argumentation about the novelty of the pOECT configuration in sensing experiments is now convincing and the manuscript is almost ready for publication. I would only ask the authors to introduce references to earlier articles where the same configuration was used for different purposes or general materials characterization. For example the pOECT configuration is described in the chapter "4.5 Surface Conductivity Measurements" of the book "Semiconductor Electrochemistry" by Rudiger Memming. In this text it is well explained that a potentiostat is used to apply a bias on a semiconducting thin film (such as the OECT channel) with reference to a sensing electrode (RE). Current is delivered through the CE electrode. The conductivity of the semiconducting material is investigated with a second voltage source and an ammeter. In the chapter several works are cited that use such configuration. In this context also the configuration called "First published OECT" by the authors differs from the pOECT configuration only in an almost insignificant detail. For measurements in the linear regime, IV characteristics would be identical to the pOECT configuration measurements. A more recent work that uses the pOECT configuration for the characterization of transport properties is also found in Bonafe et al. "Charge Carrier Mobility in Organic Mixed Ionic–Electronic Conductors by the Electrolyte-Gated van der Pauw Method" Adv. Elect. Mat. 2021.

Peer review file

Manuscript: Reconfiguration of organic electrochemical transistors for high-accuracy potentiometric sensing, NCOMMS-24-09539B

We thank the reviewers for their valuable comments and for giving us the opportunity to improve this work and communicate our results more effectively. We have addressed each reviewer's comments (in bold) in a point-by-point response, as detailed below. All modifications to the text and figures are highlighted in yellow here and in our manuscript.

On behalf of all authors,

Sahika Inal

Reviewer #2 (Remarks to the Author):

I am satisfied with the responses from the authors.

We thank the reviewer for the endorsement.

Reviewer #3 (Remarks to the Author):

- **The authors provide reasonable answers to the critical points raised by the reviewers. The argumentation about the novelty of the pOECT configuration in sensing experiments is now convincing and the manuscript is almost ready for publication.**

We thank the Reviewer for their positive feedback.

- **I would only ask the authors to introduce references to earlier articles where the same configuration was used for different purposes or general materials characterization.**

Based on the following comments, we agree with the Reviewer on acknowledging the existence of other OECT-derived setups in which the gating system is constituted of two different components to improve the performance of the measurement. These setups are not the same as the pOECT, considering their different application, electrical connections or electrode properties, however, we agree that they are worth to mention, and we thank the Reviewer for pointing this out. The main text has been modified as follows (page 5, line 30):

"A few other OECTs have used an electrochemical cell-type gating system to control the doping state of the channel (Bonafè, F., et al, Charge Carrier Mobility in Organic Mixed Ionic–Electronic Conductors by the Electrolyte-Gated van der Pauw Method. Adv Electron Mater 7, (2021), Memming, R. Semiconductor Electrochemistry. (Wiley, 2015), Ji, X., et al, Organic

electrochemical transistors as on-site signal amplifiers for electrochemical aptamer-based sensing. Nat Commun 14, 1665 (2023)), yet these setups have entirely different purposes and working mechanisms compared to the pOECT.'

- **For example the pOECT configuration is described in the chapter "4.5 Surface Conductivity Measurements" of the book "Semiconductor Electrochemistry" by Rudiger Memming.**

We thank the reviewer for pointing us to the chapter for a configuration that may be similar to ours. We quote here one part that we think that the Reviewer refers to:

"*4.5*

Surface Conductivity Measurement

… There are two ways of measuring the conductivity and its changes. In the first method, a second ohmic contact has to be produced at the backside of the semiconductor species. In order to measure conductivity changes, a voltage has to be applied across the two ohmic contacts. When varying the potential across the interface, the conductivity may change. … The two ohmic contacts should be symmetrical to the semiconductor–liquid junction [32]."

The text does not report any schematic clarifying how the device is connected for such measurement hence, it is difficult to clarify if the same configuration as the pOECT is described. For example, the text does not specify if the potential across the semiconductor interface is varied, i.e., either using an SMU, analogously to an OECT, where only one doping electrode works as a combined RE/CE, or using a potentiostat, analogously to a pOECT, where the doping system is separated into two independent RE and CE. In the case of simple OMIEC characterizations, a non-polarizable electrode such as an Ag/AgCl pellet is used as a gate in OECT, and a conventional reference electrode such as Ag/AgCl would be used with a potentiostat, leading to identical device responses. On the other hand, for sensing applications, pOECT and OECT have significantly different performances. However, even if a potentiostat is used for performing the measurement, we still cannot say that the configuration is identical to the pOECT (see next comment).

- **In this text it is well explained that a potentiostat is used to apply a bias on a semiconducting thin film (such as the OECT channel) with reference to a sensing electrode (RE). Current is delivered through the CE electrode. The conductivity of the semiconducting material is investigated with a second voltage source and an ammeter.**

In chapter 4.2.1 of the referred text (quoted below), it is explained that the bias applied to the semiconducting thin film is referenced to a reference electrode and not a sensing electrode, as mentioned by the Reviewer. This is a substantial difference with respect to the pOECT setup, which changes the device working mechanism.

" 4.2.1

Voltammetry

Current–potential measurements at semiconductor electrodes are usually performed in a cell with three electrodes under potentiostatic conditions. The cell … consists of the working electrode (WE, a semiconductor electrode …), a large counter electrode (CE, usually a Pt electrode), and a reference electrode (RE). … There are several choices for an RE such as a normal hydrogen electrode (NHE), a saturated calomel electrode (SCE), or a silver/silver chloride electrode (Ag/AgCl).”

This means that a conventional reference electrode is used in the setup described by the book, such as NHE, SCE and Ag/AgCl. In this setup, the potential of this electrode is always constant, and the device response is only modulated by the applied doping voltage. In the pOECT described in our work, no conventional reference electrode is used, and the bias applied to the semiconducting thin film is referenced to a sensing electrode, whose potential continuously changes depending on the target concentration. In this setup, the device response is modulated not only by the applied doping voltage (as in the book setup) but also by the variation of the sensing electrode potential. In short, while in the book, a conventional reference electrode is connected to the RE cable of the doping circuit, in a pOECT, instead, a conventional working electrode (sensing electrode) is connected to the RE cable of the doping circuit. Despite the fact both configurations can be operated by the same instrument through analogous cable connections, the electrochemical properties of the electrodes employed are significantly different. Hence, their working mechanism is different.

In the first version of the manuscript, we initially introduced the pOECT with a schematic that showed an AgCl pellet/reference electrode connected to the G_S terminal, which, however, was never employed in any of the sensing experiments reported later but was mentioned only as a conceptual exercise. This led to confusion for the reviewers to the point that the manuscript was rejected. Once we modified the text, exclusively describing the pOECT setup in its real components, both Reviewers found no more confusion in the working mechanism of the pOECT and approved the manuscript for further evaluation.

- **In the chapter several works are cited that use such configuration.**

The book chapter mentioned by the Reviewer (4.5 Surface Conductivity Measurements) reports one literature reference (number 33). References 33 and 34 reported in the same chapter refer to a different technique that involves the use of microwaves. From reference number 33, we tried to identify the schematic connection of the setup. However, the manuscript is in German. Regardless of this inconvenience, the electrochemical schematic reported in the referred manuscript (please see Figure R1) does not suggest configurations similar to pOECT.

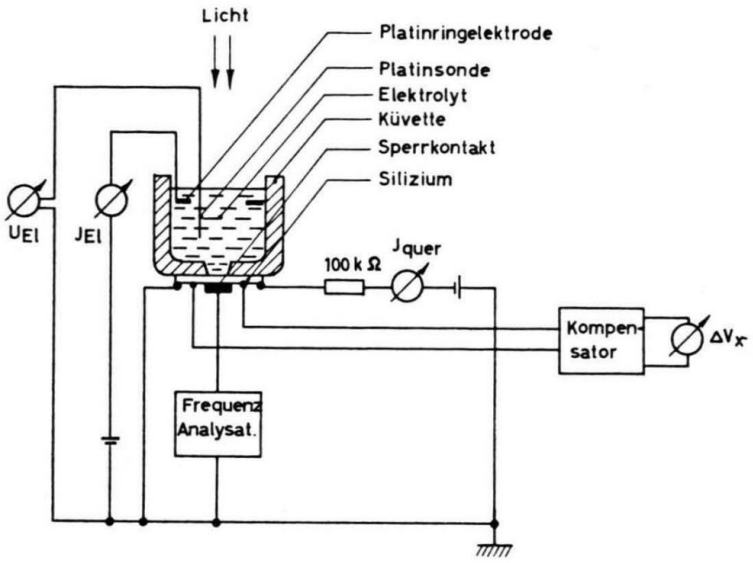


Figure R1. Electrochemical schematic reported in Von H. U. Harten, Oberflächenleitung und Oberflächenrekombination an der Grenze Silicium-Elektrolyt, 1961, 16a, 1401, Z. Naturforsch.

- **In this context also the configuration called "First published OECT" by the authors differs from the pOECT configuration only in an almost insignificant detail. For measurements in the linear regime, IV characteristics would be identical to the pOECT configuration measurements.**

As explained before, electrode properties and application are different, leading to a different working mechanism, and the same transistor channel would respond differently in the two cases.

- **A more recent work that uses the pOECT configuration for the characterization of transport properties is also found in Bonafe et al. "Charge Carrier Mobility in Organic Mixed Ionic–Electronic Conductors by the Electrolyte-Gated van der Pauw Method" Adv. Elect. Mat. 2021.**

The configuration referred to by the Reviewer (Figure R2) cannot be considered equivalent to the pOECT configuration despite splitting the gating system into two independent RE and CE. It is a great work that analyzes the carrier mobility of a mixed conductor by use of a potentiostat to apply the gating voltage, and a 4-point probe setup to monitor the conductivity. In this configuration, the authors use an AgCl pellet, which can be considered a conventional reference electrode since the measurement is performed in 0.1 M PBS. Similarly, as explained before, this leads to a working mechanism completely different than the pOECT where instead, the RE has a continuously variable electrochemical potential. Moreover,, the setup has two additional electrodes compared to the pOECT, for a total of four electrodes in contact with the OMIEC and 6 electrodes for the whole setup. While in a pOECT the V_{DS} is applied to the same electrode at which the V_{SG} is applied (source), in the setup of Figure R3, the doping voltage is not applied to the electrode 1 or 2 (where V_{DS} is applied), but to the additional electrode (3). Moreover, not only is current applied to create a gradient along two electrodes connected by the OMIEC (1 and 2),

but also the formation of a spontaneous voltage is measured between the other two additional electrodes (3 and 4), a component not present in the pOECT. The final difference between this configuration and the pOECT is that it can be used exclusively with PEDOT-like OMIECs. If enhancement mode OMIECs used in our work are employed in the setup of Figure R3, no electrical current would be measured between electrodes 1 and 2 unless an overestimated doping potential is applied to electrode 3. Since these materials are intrinsically non-conductive, the whole OMIEC region between electrodes 3,4 and 1,2 (probed region) would remain non-conductive, leading to a non-conductive channel between 1 and 2, even if the OMIEC has already reached a significant conductivity between 3 and 4. This would require a significant overpotential (potential larger than the intrinsic onset potential of the OMIEC) applied to electrode 3 in order to measure a current between 1 and 2, leading to the wrong estimation of the material properties. On the other hand, the pOECT configuration can be used with any type of OMIEC.

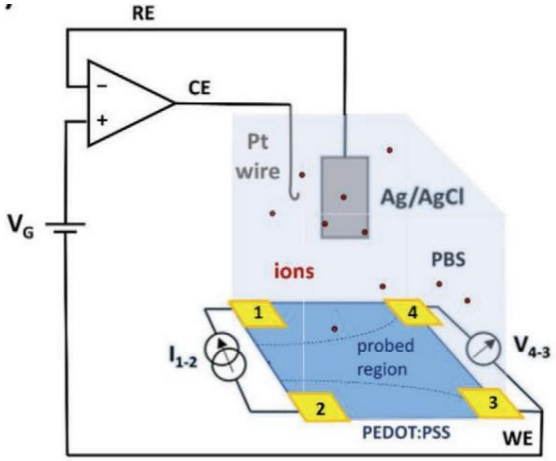


Figure R2. Electrochemical configuration reported in Bonafe et al. “Charge Carrier Mobility in Organic Mixed Ionic–Electronic Conductors by the Electrolyte-Gated van der Pauw Method” Adv. Elect. Mat. 2021.

On the approach of splitting the OECT gating system into two independent RE and CE connections, we can also consider another unconventional OECT configuration, the Ref-OECT (Figure R3, from Xudong et al., “Organic electrochemical transistors as on-site signal amplifiers for electrochemical aptamer-based sensing”, Nat. Comm. 2023). In this case, the split is introduced to improve the OECT performance in faradaic sensing. Splitting the gating system into a 3-electrode configuration, instead of the traditional 2-electrode of conventional OECTs, represents a common characteristic between the pOECT, Ref-OECT, Figure R2-setup, the setup described in “Semiconductor Electrochemistry” book. However, how the potentiostat cables are connected to the device or the electrochemical properties of the electrodes used in the device significantly change the transistor response, the working mechanism, and the application. Overall, we cannot define any of these configurations as equivalent, but we agree with the Reviewer that the advantages of the general practice of not combining RE and CE should be mentioned in the manuscript.

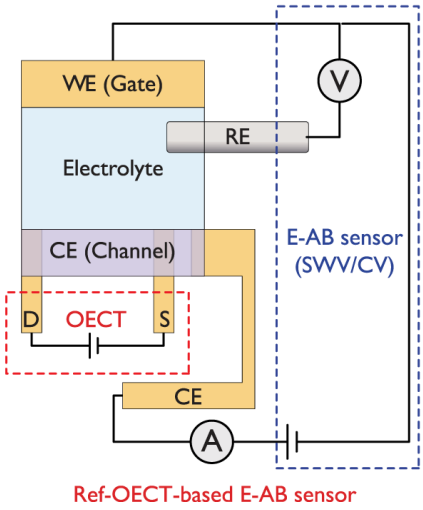


Figure R3. Ref-OECT configuration, reported in X. Ji, X. Lin & J. Rivnay, Organic electrochemical transistors as on-site signal amplifiers for electrochemical aptamer-based sensing, 14, 1665 (2023), Nat. Comm.