

Decoding Non-Faradaic Signal Transduction in Organic Electrochemical Transistor Based Biosensors

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Summary and General Assessment

This manuscript presents a thorough mechanistic study of non-faradaic signal transduction in organic electrochemical transistor (OECT) biosensors. The authors systematically decouple and quantify the contributions of gate electrochemical potential and capacitance to device response, using model systems rather than biological samples. They develop a unified sensing model that connects electrochemical and transistor-level parameters, enabling rational optimization of OECT-based biosensors. The work concludes with design guidelines for selecting operating voltages, gate geometry, and channel materials.

Overall, this study represents an important advance toward a quantitative and predictive framework for non-faradaic OECT biosensing. The experiments are meticulously designed, the analysis is comprehensive, and the conclusions are well supported by data. The paper is of clear significance to researchers in bioelectronics, electrochemical sensing, and materials engineering.

1. Significance and Originality

The work provides a conceptual and methodological breakthrough in interpreting and designing non-faradaic OECT sensors. Previous studies have largely relied on empirical optimization; this paper introduces a unified, physics-based rationale comparable in impact to early models of faradaic electrochemical sensors.

2. Soundness of Conclusions and Evidence

The conclusions are well supported by:

- Systematic experimental design with clean model systems (well-justified substitution for biomolecular assays).
- Parallel electrochemical measurements (OCP, EIS) correlated to OECT transfer characteristics.
- Independent monitoring of electrode potentials via the EP-IV setup, which validates the mechanistic model.
- Application to real protein sensing, confirming predictive value.

Minor considerations:

- The conversion of LoD in millivolts or nanofarads to chemical LoD (LoD_{chem}) is discussed conceptually but not demonstrated experimentally. Including at least one worked example would strengthen the practical impact.

- While the assumption that target binding can be mimicked solely by changes in potential/capacitance is justified, an explicit comparison to an actual biomolecular interface (e.g., with a simple aptamer system) would reinforce external validity.

4. Methodological Evaluation

Points for improvement:

- The uncertainty analysis could be more explicit — e.g., propagation of measurement noise to calculated ΔV_{drop} or LoD_{mV}.

- Some equations used to derive LoD_{chem} are only referenced as “see Supplementary Discussion”; including at least one explicit derivation in the main text would enhance clarity for readers outside the immediate field.

5. Data Analysis and Interpretation

- Figure 5b and 6a present complex dependencies; including summary plots (e.g., response surfaces or heatmaps) might help visualize the interplay of ΔE , ΔC_{DL} , and V_{GS} .
- The authors could consider discussing potential sources of drift or hysteresis in long-term operation, which may affect reproducibility in real bioassays.

6. Transparency and Reproducibility

The methods are reported with commendable detail, sufficient for reproduction by other groups. The inclusion of all necessary materials, references to synthetic procedures, and explicit device geometry parameters meets expected field standards.

If available, sharing raw datasets and LabVIEW scripts (e.g., for EP–IV operation) as supplementary materials would further promote transparency.

7. Recommendations/Comments

1. The entire mechanistic framework is derived using model electrochemical gates (e.g., variable Ag/AgCl potentials, gold area-controlled capacitances), rather than true biofunctionalized electrodes.

2. Although the final SARS-CoV-2 experiment provides qualitative confirmation, it is not quantitatively linked to the model parameters (ΔE , ΔC_{DL} , ΔV_{drop}).

3. The authors should validate their framework using at least one real biomolecular interface (e.g., aptamer–protein or antibody–antigen system) where independent measurements of ΔE and ΔC_{DL} are possible.

- a. This would demonstrate that their model holds under realistic surface chemistry and ionic screening conditions.
- b. Otherwise, the model risks being conceptually elegant but empirically unverified.

4. Figures 3–6 present trends (e.g., normalized response, LoD_{mV} , ΔV_{drop}) but lack explicit error bars or confidence intervals on most quantitative data.

5. For a paper emphasizing quantitative modeling, there is no discussion of uncertainty propagation (from potential and capacitance measurements to current modulation).

6. Suggestion: include statistical analysis ($n \geq 3$ replicates) and error propagation for key derived parameters such as:

- a. ΔV_{drop} , LoD_{mV} , and LoD_{nF}
- b. $g_{m,eff}$ and NR_{max}
- c. ΔE – ΔI_{DS} correlations

This would establish the reliability and reproducibility of the quantitative framework.

7. The paper proposes four possible interference regimes (Fig. 6c-iii), but only one is experimentally shown ($+\Delta E / -\Delta C_{DL}$, constructive case).

8. Demonstrating at least one destructive-interference scenario (e.g., $+\Delta E$ and $+\Delta C_{DL}$) would critically strengthen the claim that both synergy and cancellation occur.

9. The framework's predictions depend on OMIEC capacitance and doping state, yet the manuscript reports results from only single-channel instances for each material.

10. The authors should show:

- a. Variability across multiple devices (≥ 3 per OMIEC).
- b. Any hysteresis or stability data (drift, bias stress, hydration).
- c. Discussion on whether OMIEC hydration or ion-trapping affects reproducibility of ΔE vs ΔC_{DL} coupling.

11. The conversion from LoD_{mV} or LoD_{nF} to chemical LoD (LoD_{chem}) is stated but not explicitly derived in the main text.

12. Similarly, the relationship between channel capacitance (C_{CH}) and gate polarization (V_{drop}) is described qualitatively.

13. The authors should provide explicit derivations or equations (not only “see Supplementary Discussion”) for:

- a. LoD_{chem} calculation, with sample numeric substitution.
- b. Expression linking ΔV_{drop} to ΔC_G and $C_{CH}(V_{GS})$.
- c. Analytical condition for maximum constructive interference ($\Delta E/\Delta C_{DL}$ ratio).

14. These additions would make the framework quantitatively verifiable and applicable by others.

15. All analyses use steady-state transfer curves, but in practical biosensing, signal transduction often occurs dynamically (e.g., impedance or pulsed biasing).

16. The authors should discuss whether their model remains valid under time-varying signals or AC operation — for instance, whether ΔC_{DL} –driven responses are frequency dependent.

17. A small dynamic validation (e.g., chronoamperometric or impedance-tracked response) would extend the model's relevance.

18. The framework assumes ideal interfaces; it does not consider:

- a. Ionic strength and Debye length variations in biofluids.
- b. Non-specific adsorption or dielectric drift.
- c. Temperature dependence of OECT response.

19. These should be briefly addressed to indicate the model's practical boundaries and guide readers applying it in complex biological matrices.

Comments from co-reviewer

The authors propose a mechanistic framework that identifies the contributions of changes in the gate electrochemical

potential and changes in gate/interface capacitance to the OECT electrical response. This work is potentially significant and represents original research, revealing one of the key challenges in implementing OECT-based sensors. While the work does not reveal any obvious errors, there are a number of methodological and formal shortcomings that require correction before publication. Conceptually, the method (separating the capacitance/potential contributions) is sound and necessary, but practical implementation may require refinement: more rigorous controls, statistics, and time-frequency measurements.

Comments and recommendations:

1. Could the authors please clarify their statement: "Unlike traditional electrochemical two- or three-electrode setups, OECTs operate without a reference electrode, facilitating miniaturization and improving biocompatibility." (lines 40 and 41)? First, in many OECT configurations, the gate electrode actually acts as a pseudo-reference electrode, although a separate reference electrode can be used in some studies for precise potential control. Second, could the authors please explain in more detail how the absence of a reference electrode directly contributes to improved biocompatibility? This connection is not immediately clear, and ref. [15] is not sufficiently useful to support this claim.
2. The terms "constructive and destructive interference" are typically used in wave physics, where they refer to superposition of oscillatory quantities with defined phase. That isn't what occurs in the reviewed article, and using this terminology may mislead readers. Therefore, I would recommend using different terminology to describe the effects in OECT output associated with combined changes in gate properties. For example, noting that the two terms can act in the same direction or oppose each other. Alternatively, if the authors wish to keep the analogy, they must provide a clear, explicit explanation to justify it.
3. Please be more precise in the reference selection in the Introduction. For example, in lines 49 and 50, the authors first in the text discuss the features of the non-Faradaic mode: "When target binding at the gate electrode occurs without an accompanying electrochemical reaction, the sensor operates in a non-faradaic mode.20–23". However, the term "non-Faradaic" is not mentioned at all in Refs. [20–23], and readers who follow these links hoping for more detailed explanations may become confused.
4. In the figures, for greater clarity and uniformity, I would recommend using the same colors (or some other method of highlighting) for all graphs to indicate the "blank" and "target" modes.
5. Regarding the separation of the two mechanisms contributing to the OECT response (changes in gate electrochemical potential and changes in gate capacitance), the manuscript provides a clear conceptual framework and dedicates distinct sections to each contribution. In practice, however, the setup configuration and material properties complexity may limit how cleanly these two effects can be disentangled. Given these challenges, could the authors clarify whether the proposed methodology could be further refined by explicitly outlining the limits of reliability, considering possible deviations from the used assumptions as well as experimental uncertainties?
6. The authors note (in the Introduction) that when the gate serves as the primary data interface, OECTs support reusability thanks to replaceable gate electrodes and a modular device architecture. This suggests the sensor is intended for repeated use. However, the question of reproducibility arises: since the drain current can both increase and decrease with repeated use, how do the authors ensure stable and reliable sensor responses over multiple cycles? Are there specific calibration strategies or methods implemented to maintain reproducibility despite potential changes in device characteristics with repeated use?
7. Although the authors mention OECT sensor optimization, an inherent challenge of OECT-based devices remains device-to-device variability due to manufacturing process inconsistencies. This variability can lead to differences in transistor characteristics even among nominally identical samples. Could the authors provide more details on the number of samples used for the experimental evaluation? Furthermore, it would be useful to understand how reproducible the obtained sensor responses are across devices. Furthermore, given the natural variability in device performance, could the authors comment on how the proposed optimization compares to this underlying variability? In particular, can the observed improvement in sensor performance due to optimization be clearly distinguished from fluctuations caused by manufacturing process differences? Quantitative assessments, for example based on drain current or sensitivity variations, would greatly help clarify this issue.
8. Judging by the OECT schematic diagrams, the source and drain electrodes were not insulated from the electrolyte. How could parasitic leakage currents between the source and gate, and between the source and drain, affect the experimental results and their interpretation? How might they affect the sensor's sensitivity?
9. Could the authors clarify what exactly is meant by "deformations" (line 449)?
10. The current definition of the analyzed capacitance remains ambiguous, and it is unclear which specific capacitance components are included in the model, which are neglected, and on what basis these choices are made. Because different capacitance contributions (e.g. interfacial, geometric, double-layer, parasitic, and etc.) exhibit distinct physical origins and frequency dependencies, a more explicit discussion is needed. Incorporating frequency-dependent measurements would strengthen the validity of the proposed capacitance model.

(Remarks to the Author)

The manuscript authored by Inal and co-workers provides a rigorous and much-needed mechanistic framework to dissect and quantify non-faradaic transduction pathways in OECT-based biosensors. By separating and characterizing the contributions from variations in the electrochemical potential of the gate (ΔE_G) and in the interfacial gate capacitance (ΔC_G), the authors present a unifying model that clarifies several inconsistencies in the current literature. The introduction of EP-IV mapping, the explicit bias design rules, and the adoption of the effective transconductance ($g_{m,eff}$) as a figure of merit make this work particularly relevant for advancing standardization in OECT biosensing. Overall, this is a conceptually strong and potentially field-defining study. I support the publication of this work. My detailed comments are outlined below.

- The analysis on p-type (PEDOT:PSS, p(g3C2T2-T)) and n-type (p(C6NDI-T)) channels highlights the impact of doping state and CCH(VGS) profile. To confirm generality, please add (i) CCH(V) maps for all OMIECs tested, and (ii) at least one test under different ionic strengths to assess how Debye length modulates ΔE_G and ΔC_G visibility.
- To support broader adoption of your results, please (i) normalize $g_{m,eff,max}$ by channel area/volume for cross-platform comparison and (ii) provide a summary table comparing IDS-, g_m -, and ΔV_{GS} -based sensing metrics under realistic noise and drift.
- Add a brief discussion to broad the applicability of the proposed approach including OECTs integrated with additive technologies, (see for example: 10.1002/advs.202508872, 10.1002/sml.202410499), which open opportunities for additive manufactured biosensors.
- Consider adding a short box in the main text explaining the distinction between “electrochemical potential” and “voltage” for clarity to non-electrochemical readers.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

Comments

Minor revision

This manuscript presents a thorough and mechanistic investigation into non-Faradaic signal transduction in Organic Electrochemical Transistor (OECT)-based biosensors. The authors successfully deconvolute the individual and combined contributions of gate electrochemical potential and capacitance changes to the OECT output. The establishment of a rational sensing model and the provided design rules for optimizing performance are significant contributions to the field. The work is compelling and well-supported; however, several points should be addressed to further strengthen the manuscript before publication.

1. In Figure 7, the authors demonstrated that the device response of n-type OECTs was exclusively generated by a change in gate potential, while both potential changes and capacitance contributed to the signals detected by p-type OECTs. The p-type OECTs are in “ON” state at the measured V_{gs} range with high channel capacitance, resulting in a low C_g/C_{ch} . This condition makes the device sensitive to changes in gate capacitance. In contrast, n-type OECTs are “OFF” with low channel capacitance and thus high C_g/C_{ch} . The high C_g/C_{ch} ratio favors efficient channel gating by the potential drop, resulting in the device predominantly sensitive to gate potential changes and masking the contribution of capacitance variations. Therefore, quantitatively analyze the relationship between the device response and the C_g/C_{ch} ratio for both channel types is suggested to strengthen authors’ conclusions.

2. To further decouple the effects of channel material properties from those of device geometry, I recommend a key control experiment. The authors should characterize the response of devices that share the same gate electrode but are fabricated with OECT channels of different geometries (different channel capacitance).

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Dear authors,

Your revision of the manuscript is extensive and most of the comments were addressed carefully.

Comments about AC operation and LoDchem practical demonstration were not fully addressed. But we appreciate your honest reply and understand that this experimental study would require a dedicated extensive study. So, we support removing of it from the discussion.

37 samples we find in a low range of the statistical confirmation, but this is extensive enough to make relevant statements. So, also, accepted.

Next, I would like to point on minor formatting issues:

missing symbols around ΔV drop normalization,

Sometimes Figure is referenced with parentheses, sometimes without - across entire manuscript.

Some claims remain stronger than the data strictly support:

“predictive framework”; “general design rules”; “reliable across diverse environments”

The work is now much closer to supporting these claims, but still mainly validated on PEDOT:PSS, limited OMIECs, a few protein systems. It is clear that is impossible to test everything, so I would suggest slightly tempering universal language.

Also, sentences are quite long - it is hard to read. I would recommend authors to split them and shorten. Do not rely on AI in this case, do it yourself.

Final recommendation of the Reviewer is to accept this publication after minor corrections listed above.

Reviewer #2

(Remarks to the Author)

The revised manuscript presents noteworthy results that are well contextualized within the existing literature, making a meaningful contribution to the field and related areas. The evidence convincingly supports the conclusions, the data analysis is robust, and the methodology meets expected standards and ensures reproducibility.

Reviewer #3

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Reviewer #4

(Remarks to the Author)

The revisions are satisfactory and respond well to my previous comments. I have no further issues and recommend the manuscript for publication.

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Reviewer 1 and co-Reviewer

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2. Soundness of Conclusions and Evidence

The conclusions are well supported by:

- Systematic experimental design with clean model systems (well-justified substitution for biomolecular assays).
- Parallel electrochemical measurements (OCP, EIS) correlated to OECT transfer characteristics.
- Independent monitoring of electrode potentials via the EP-IV setup, which validates the mechanistic model.
- Application to real protein sensing, confirming predictive value.

We thank the Reviewers for this summary and appreciating our work. Below, we will respond to each of their comments separately. However, some of their comments were conceptionally connected (and sometimes the same) so we combined them below and responded to all of them in one section. All the other comments were treated separately.

#6 – The authors could consider discussing potential sources of drift or hysteresis in long-term operation, which may affect reproducibility in real bioassays.

#11 – The framework's predictions depend on OMIEC capacitance and doping state, yet the manuscript reports results from only single-channel instances for each material. The authors

should show: a) Variability across multiple devices (≥ 3 per OMIEC); b) Any hysteresis or stability data (drift, bias stress, hydration); c) Discussion on whether OMIEC hydration or ion-trapping affects reproducibility of ΔE vs ΔC_{DL} coupling.

#14 – The framework assumes ideal interfaces; it does not consider: a) Ionic strength and Debye length variations in biofluids; b) Non-specific adsorption or dielectric drift; c) Temperature dependence of OECT response. These should be briefly addressed to indicate the model's practical boundaries and guide readers applying it in complex biological matrices.

#19 – Regarding the separation of the two mechanisms contributing to the OECT response (changes in gate electrochemical potential and changes in gate capacitance), the manuscript provides a clear conceptual framework and dedicates distinct sections to each contribution. In practice, however, the setup configuration and material properties complexity may limit how cleanly these two effects can be disentangled. Given these challenges, could the authors clarify whether the proposed methodology could be further refined by explicitly outlining the limits of reliability, considering possible deviations from the used assumptions as well as experimental uncertainties?

#20 – The authors note (in the Introduction) that when the gate serves as the primary data interface, OECTs support reusability thanks to replaceable gate electrodes and a modular device architecture. This suggests the sensor is intended for repeated use. However, the question of reproducibility arises: since the drain current can both increase and decrease with repeated use, how do the authors ensure stable and reliable sensor responses over multiple cycles? Are there specific calibration strategies or methods implemented to maintain reproducibility despite potential changes in device characteristics with repeated use?

#21 – Although the authors mention OECT sensor optimization, an inherent challenge of OECT-based devices remains device-to-device variability due to manufacturing process inconsistencies. This variability can lead to differences in transistor characteristics even among nominally identical samples. Could the authors provide more details on the number of samples used for the experimental evaluation?

Furthermore, it would be useful to understand how reproducible the obtained sensor responses are across devices.

Furthermore, given the natural variability in device performance, could the authors comment on how the proposed optimization compares to this underlying variability?

In particular, can the observed improvement in sensor performance due to optimization be clearly distinguished from fluctuations caused by manufacturing process differences?

Quantitative assessments, for example based on drain current or sensitivity variations, would greatly help clarify this issue.

We thank the Reviewer for their valuable input. In response to several comments—specifically comments #6, #11, #14, #19, #20, and #21 reported above, as well as [Reviewer 2 comments #26 and #28](#), we recognize the importance of explicitly discussing additional OECT-related limitations that may hinder application of the methodology proposed in this work and constrain sensing-

performance optimization. Accordingly, we added a new section entitled “Considerations for non-Faradaic signal transduction using OECTs”, and additional new data and Figures to address these points.

In this section, we discuss measurement drift, reproducibility, bias stress, Debye length, non-specific adsorption, temperature dependence, long-term operation, device-to-device variability, definition of a safe operational window, and validation/reliability protocols, supported by additional experiments and/or relevant literature.

Here, we present the new section and the associated Supplementary Information:

Considerations for non-Faradaic signal transduction using OECTs

The theoretical framework outlined above enables the rational optimization of OECT-based sensors only when sources of signal distortion are minimized. Specifically, the mechanism described relies on changes in gate electrochemical properties, induced by a sensing event, to modulate channel conductivity at different doping voltages. For this transduction pathway to remain valid, two conditions must be satisfied: (i) background parasitic effects must be minimized, and (ii) target binding must induce a measurable change in gate electrochemical properties that can be transduced and amplified by the device.

Background parasitic effects are often overlooked in OECT sensor development, despite their impact on signal fidelity. These effects arise primarily from an intrinsic reduction in channel material conductivity during device operation, measurement noise, device-to-device variability, and drift. Among these, loss of channel current is the most prevalent and often induced by inappropriate selection of operating voltages.[\https://pubs.acs.org/doi/10.1021/acsaelm.1c01171, <https://doi.org/10.1557/s43579-023-00511-6>] Operation-induced changes in I_{DS} can be misinterpreted as target-induced signals, leading to false positives or an erroneous calibration. To isolate such parasitic contributions, we performed control experiments using a stable gate electrode (Ag/AgCl in 3 M NaCl) while acquiring consecutive transfer curves under a sensor operation like protocol but in the absence of a target (i.e., 35 transfer curves including six hypothetical concentrations plus a blank; Fig. 8a-i). Despite the absence of any change in gate electrode properties, the device transfer characteristics reproduced the features of a conventional calibration curve, including a latent regime near the limit of detection, a quasi-linear region, and apparent saturation at higher “concentrations” (Fig. 8a-ii). This highlights the risk of misinterpreting stability-driven artifacts as genuine sensing signals.

To mitigate these artifacts, identification of a stable operating V_{GS} range is important. By repeatedly acquiring transfer curves using a stable, non-polarizable Ag/AgCl electrode within progressively expanded voltage ranges and monitoring I_{DS} drift, the onset of instability can be quantified. For PEDOT:PSS, a V_{GS} range of -0.6 to 0 V yielded stable output over 150 measurements, whereas extending the range in either direction introduced cumulative current loss (Fig. 8b-i, see Supplementary Fig. 19 for the corresponding transfer curves). Defining an acceptable degradation threshold (e.g., 5%) enables identification of a maximum safe operating window, which can be translated to other gate materials such as Au by accounting for their equilibrium potential offsets (Fig. 8b-ii). If the intrinsic potential of the gate material or the sensing induced ΔE_G lead to a biasing outside of the safety window, the operating voltages could be shifted accordingly. This approach provides a practical guideline for selecting operating conditions that preserve channel stability. Supplementary Discussion 10 and Supplementary Figs. 20-21 provide a protocol to verify channel stability and assess the reliability of sensing data.

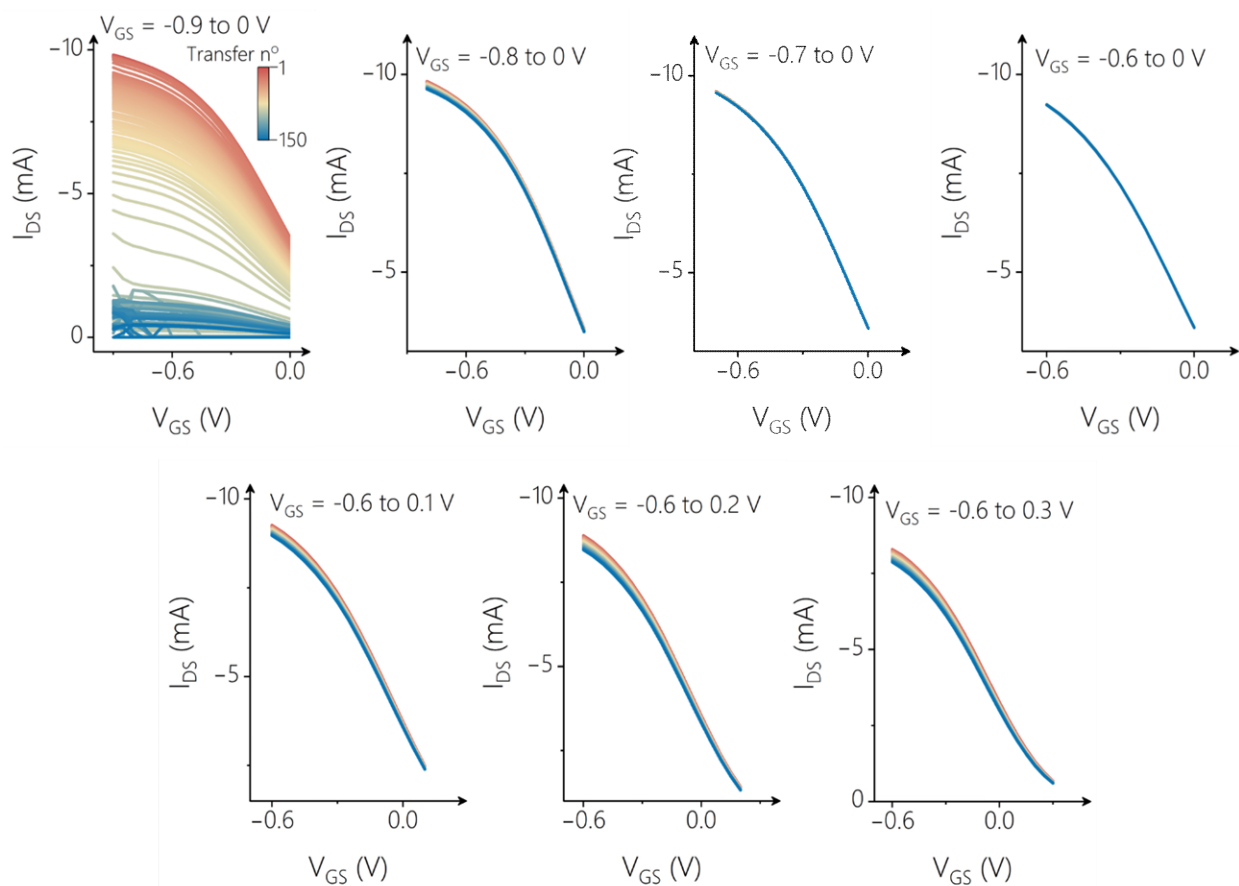
Although instability due to biasing can be minimized, it cannot be fully eliminated, particularly during long-term measurements. Its impact however depends on the temporal structure of the measurement protocol. When sensing relies on comparing closely spaced measurements (e.g., sequential blank-target acquisition), the artefact remains negligible. However, when signals are referenced to an initial baseline over extended acquisition sequences, cumulative loss of current introduces systematic errors. In our experiments (continuous operation within a stable V_{GS} window for 3 h with 1000 transfer curves), reliable measurements were maintained over ~ 100 consecutive transfer curves, beyond which deviations exceeded acceptable thresholds (Fig. 8c-i and Supplementary Fig. 22). This defines a practical limit for continuous operation, even under stable operating conditions. If the sensor operation requires continuous transfer curve acquisition, device architectures incorporating multiple OECT channels can be used (Fig. 8c-ii). This configuration allows replacement of degraded channels during operation while maintaining comparable device characteristics. We show that PEDOT:PSS OECT channels fabricated on the same and 4 other substrates exhibit low variability in key parameters such as $g_{m,eff,max}$ (Fig. 8c-iii), enabling reliable channel-to-channel substitution. Furthermore, selecting operating voltages near the OFF state of the device reduces I_{DS} variability arising from spin-coating,^[<https://doi.org/10.1002/aelm.202100891>] improving channel-channel reproducibility (Fig. 8c-iv).

Another common source of error is the drift of the input signal. The electrochemical potential and capacitance of an electrode can drift over time due to spontaneous parasitic reactions (e.g., O_2 reduction, water oxidation)^[<https://doi.org/10.1021/acs.analchem.7b01856>, <https://www.sciencedirect.com/science/article/pii/S2590137024000736>] or instability of the biofunctionalization layer.^[<https://doi.org/10.1021/acs.analchem.2c05398>] Such drift is difficult to distinguish from true sensing signals. Even when negligible over the timescale of a single data-point acquisition, cumulative drift over the course of a measurement protocol can lead to false results.^[<https://doi.org/10.1021/acs.analchem.7b01856>] To demonstrate this effect, we simulated a calibration protocol in which each hypothetical target concentration was measured by acquiring several consecutive transfer curves, followed by a resting period during which the device was not operated to mimic the incubation time required between successive concentrations (Fig. 8d). To isolate the contribution of input-signal drift, we kept the gate electrode material and size constant, using either a low-polarizable Au electrode or a PEDOT:PSS electrode, and we did not expose the gate to target bearing solutions. The two gate materials were selected to highlight differences in surface reactivity toward parasitic reactions. In both cases, executing the sensing protocol produced an apparent OECT response resembling a conventional calibration curve, despite any sensing event (Fig. 8d-i). When we simultaneously monitored the gate electrode potential, we observed an analogous trend, confirming a substantial drift of the input signal (Fig. 8d-ii). The transduction mechanism underlying this artifact is illustrated in Fig. 8d-iii. As the gate potential drifts from its initial value (e.g., Blank) to a later value (e.g., C_6), the effective gate bias shifts by an equivalent amount (step a). This change in effective gate bias alters the channel doping state and thus the measured I_{DS} (step b), generating an apparent “sensing” signal. Mitigation strategies include the use of chemically stable gate materials, surface passivation (e.g., self-assembled monolayers),^[<https://doi.org/10.1021/acsaelm.1c01171>, <https://doi.org/10.1021/acs.analchem.7b01856>] and differential measurement schemes employing reference channels to subtract drift contributions.^[<https://doi.org/10.1038/s41928-025-01465-4>, <https://doi.org/10.3390/molecules29071459>, <https://doi.org/10.1016/j.bios.2022.114691>] The

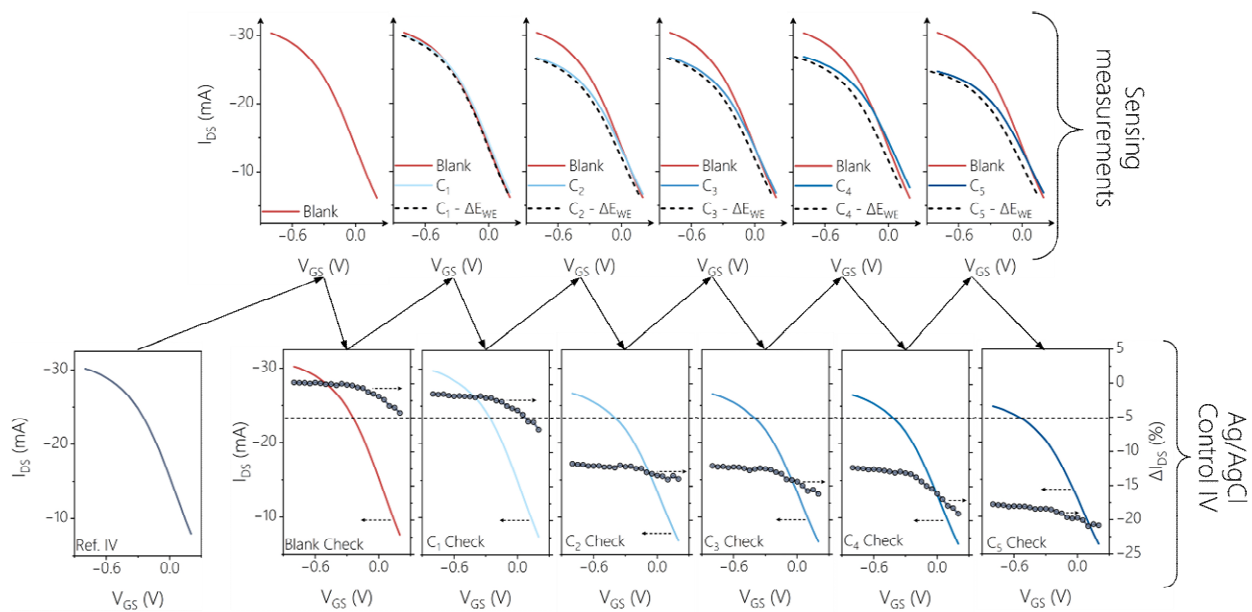
acquired under a sensing-like protocol in the absence of target binding and **(ii)** the corresponding calibration curve extracted at $V_{GS} = 0$ V, reproducing the characteristic features of a conventional sensor response. **b)** Identification of a stable V_{GS} operating window. **(i)** Evolution of I_{DS} loss during continuous operation. The OEET was characterized by acquiring n^0 transfer curves within progressively expanded V_{GS} ranges, either toward positive (red) or negative (blue) voltages, while monitoring I_{DS} at a fixed V_{GS} . **(ii)** Cumulative current loss at the 150th transfer curve as a function of V_{GS} . Knowledge of the reference gate (Ag/AgCl) and the sensing gate (Au), including its expected potential shift upon target binding, enables translation of the stability window between gate materials. **c)** Considerations for long-term sensing measurements. **(i)** One thousand consecutive transfer curves were acquired using an Ag/AgCl gate, and I_{DS} loss was calculated as the percent difference between the n^{th} and the $n^{th}-r$ transfer curve. The current loss, referenced to the initially stored blank value (r), was lower than 5% up to 100 consecutive transfer curves. **(ii)** Optical micrograph of a representative OEET chip comprising 16 channels ($100 \times 10 \mu\text{m}$, $W \times L$) and one planar gate (7 mm^2) on a $17 \times 17 \text{ mm}$ substrate. **(iii)** Mean transfer curve with standard deviation across four PEDOT:PSS OEET chips (37 channels tested in total; non-uniform channels excluded), demonstrating reproducible device characteristics. $g_{m,eff,max}$ is $3.3 \pm 3\% \text{ V}^{-1}$ ($3.3 \cdot 10^{-3} \text{ V}^{-1} \cdot \mu\text{m}^{-2}$, normalized by area). **(iv)** Relative standard deviation of I_{DS} as a function of V_{GS} , highlighting reduced variability near the OFF state. **d)** Gate-drift-induced OEET responses that mimic a sensing signal. **(i)** Apparent calibration curves obtained without target binding for different gate materials. **(ii)** Corresponding evolution of the gate electrochemical potential **(iii)** Schematic of the underlying artifact: (a) drift in gate potential shifts the effective gate bias, and (b) alters the measured I_{DS} .

Supplementary Discussion 10: Assessment of OEET stability during successive transfer curve acquisition

To ensure that the channel current is stable during a sensing protocol that involves successive transfer curve acquisition, a control measurement can be integrated. The OEET channel is first characterized using an Ag/AgCl gate within a previously identified safe voltage window; this serves as the reference measurement (Ref. IV, Supplementary Fig. 20). After each sensing measurement (e.g., each concentration used to build a calibration curve), the channel is re-characterized under identical conditions (C_n Check, Supplementary Fig. 20). Each control measurement is then compared to the initial reference to quantify the relative current loss, ΔI_{DS} (%), which should remain below a predefined threshold (e.g., <5%). Supplementary Fig. 20 shows an case of a progressive I_{DS} loss following successive sensing steps, with substantial degradation already evident at the third measurement (C_2). This is further confirmed by the failure of the EP-IV curve-shifting procedure to achieve overlap after compensating for the change in gate electrochemical potential (ΔE_{WE}), indicating that the observed response is dominated by parasitic effects. This protocol enables both assessment of OMIEC stability and selective exclusion of unreliable data. For instance, in Supplementary Fig. 20, only the initial experiments (Blank and C_1) satisfy the stability criterion ($\Delta I_{DS} < 5\%$), whereas subsequent measurements must be discarded. By contrast, Supplementary Fig. 21 presents a stable sensing experiment corresponding to Fig. 3a–c. In this case, Ag/AgCl control measurements consistently show ΔI_{DS} below 5%; and the EP-IV analysis yields near-perfect overlap of the transfer curves after compensating for the gate-potential variation. All sensing data points in this experiment can therefore be considered reliable.

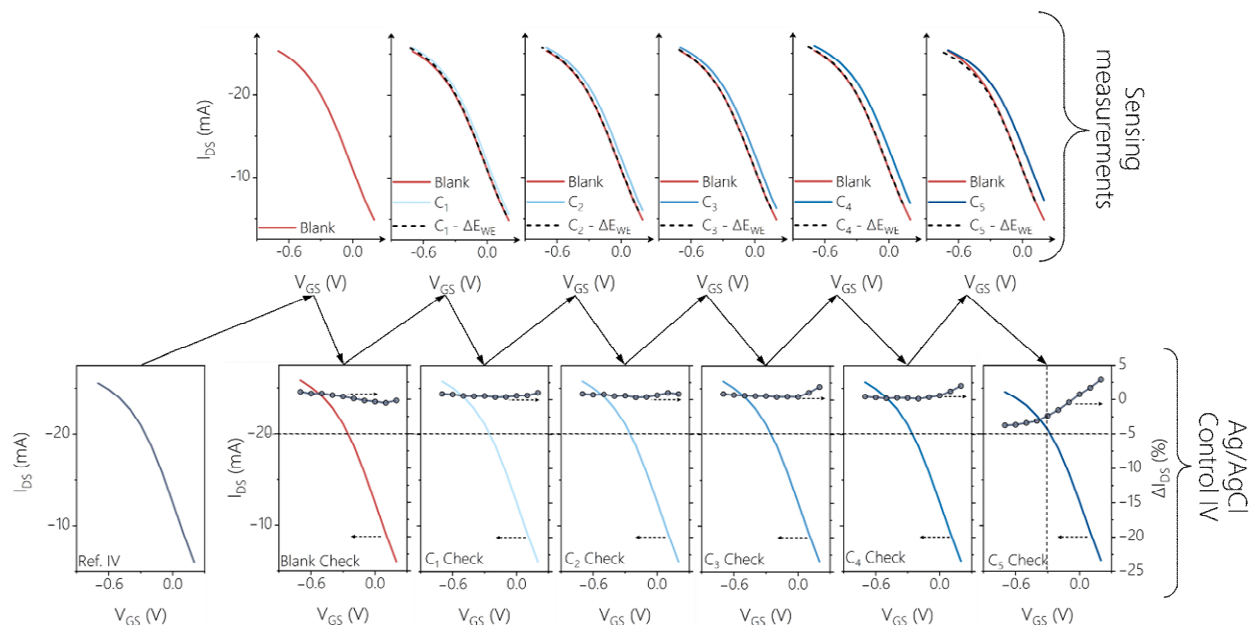


Supplementary Figure 19. Transfer curves acquired during the protocol used to identify the stable V_{GS} operating window reported in Fig. 8b.

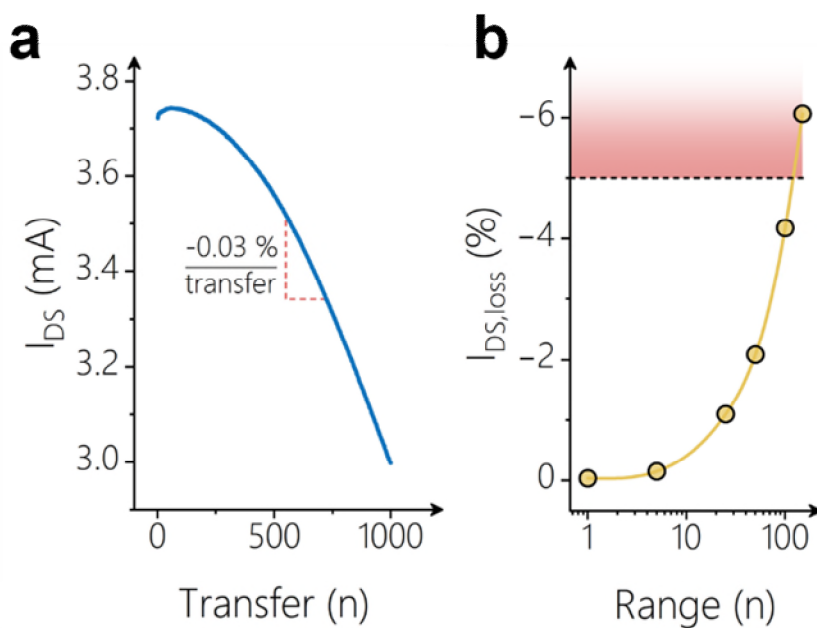


Supplementary Figure 20. OECT sensing measurements in which the gate electrochemical potential changes progressively (top row), together with control measurements with Ag/AgCl gate

performed in parallel to assess channel stability (bottom row). The arrows show the sequence of the measurements. The control measurements reveal a progressive loss of I_{DS} over the course of the experiments, which prevents the EP-IV analysis from providing a correct interpretation of the sensing mechanism.



Supplementary Figure 21. Experiment analogous to Supplementary Fig. 20 but performed under conditions where channel current is stable, allowing the EP-IV analysis to provide a correct interpretation of the sensing mechanism.



Supplementary Figure 22. OECT channel stability. (a) The decrease in I_{DS} (at a fixed V_{GS}) during 1000 consecutive transfer curves. (b) Corresponding relative I_{DS} loss during long-term operation as a function of the measurement-range protocol.

For clarity, we provide below separate responses to each of the comments listed above.

#6 – The authors could consider discussing potential sources of drift or hysteresis in long-term operation, which may affect reproducibility in real bioassays.

Long-term operation has been addressed in Fig. 8c and the corresponding Discussion.

Drift-related effects have been addressed in Fig. 8d and the corresponding Discussion.

#11 – The framework’s predictions depend on OMIEC capacitance and doping state, yet the manuscript reports results from only single-channel instances for each material. The authors should show: a) Variability across multiple devices (≥ 3 per OMIEC); b) Any hysteresis or stability data (drift, bias stress, hydration); c) Discussion on whether OMIEC hydration or ion-trapping affects reproducibility of ΔE vs ΔC_{DL} coupling.

a) Variability across multiple devices ($n = 37$), including both intra- and inter-array variability, was investigated in Fig. 8c-ii–iv and the corresponding Discussion.

b) Stability was investigated in Fig. 8a, Fig. 8b, and Fig. 8d and the corresponding Discussion.

c) Changes in gate electrochemical potential (ΔE_G) and capacitance (ΔC_{DL}) are intrinsic properties of the sensing electrode, governed by interactions between the electrode surface and surrounding electrolyte and its composition (e.g., water, ions, and the analyte). These quantities constitute the primary output signals in potentiometric and impedimetric sensing, whereas in OECT-based sensing they serve as intermediate variables that are transduced and amplified as the channel current changes.

#14 – The framework assumes ideal interfaces; it does not consider: a) Ionic strength and Debye length variations in biofluids; b) Non-specific adsorption or dielectric drift; c) Temperature dependence of OECT response. These should be briefly addressed to indicate the model’s practical boundaries and guide readers applying it in complex biological matrices.

It is correct that the framework assumes ideal interfaces, otherwise the sensors would not be accurate and worth to analyze. We do not claim to be generating a framework to correct the sensor output for the artefacts. Our intention, however, is not to provide a correction framework for such non-idealities, but rather to establish a mechanistic foundation for interpreting OECT responses under controlled conditions where these effects are minimized.

To address the reviewer’s concern, we have now included a dedicated section (“Considerations for non-Faradaic signal transduction using OECTs”) in which we explicitly discuss the practical boundaries of the framework. In this section, we systematically investigate and illustrate several key parasitic effects that can produce OECT responses indistinguishable from true sensing signals, including; (i) OMIEC instability leading to apparent calibration behavior (Fig. 8a), (ii) identification of stable operating voltage windows (Fig. 8b), (iii) the impact of instability on

sensing reliability as a function of the measurement protocol (Fig. 8c-i and Supplementary Fig. 22), (iv) a reliability-assessment protocol for monitoring device stability and selectively excluding compromised data (Supplementary Figs. 20 and 21), (v) the role of intrinsic measurement noise relative to input signals (Fig. 3d, 5c, 6a-ii and Supplementary Fig. 9), (vi) device-to-device variability (Fig. 8c-iii,iv), and (vii) drift in gate electrochemical properties producing apparent sensing responses (Fig. 8d). To the best of our knowledge, these aspects have not been systematically addressed in prior OECT sensing studies, and we therefore provide new experimental evidence to delineate these limitations.

Regarding some other possibly occurring points raised by the reviewer:

Debye screening and ionic strength: These effects have been extensively studied in electrolyte-gated transistors and OECTs. Prior work has shown that increasing the Debye length enhances the effective coupling between target binding and gate electrochemical properties, leading to larger transfer-curve shifts and improved sensitivity (e.g., [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387), and <https://doi.org/10.1021/ja205684a>). Strategies to mitigate screening, including electrolyte dilution and high-frequency gate modulation, are also well established (e.g., [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387), <https://doi.org/10.1021/nl203666a> and see <https://pubs.acs.org/doi/10.1021/acsnano.0c08622> for a review).

Non-specific adsorption and dielectric drift: These phenomena are likewise well documented and have been shown to alter gate electrochemical properties and OECT responses (e.g., <https://doi.org/10.1063/5.0029994>, <https://doi.org/10.1016/j.ccej.2024.148980>, <https://doi.org/10.1021/acssensors.4c03072>). Established mitigation strategies include surface functionalization and antifouling coatings.

Temperature dependence: Temperature-dependent effects on the performance of these sensors have been investigated for wearable formats (e.g., [10.1038/s41928-025-01465-4](https://doi.org/10.1038/s41928-025-01465-4))

Given that these effects have been thoroughly treated in prior literature and our aim here is not to investigate each of them, these are outside the scope of our study. Instead, we have now explicitly acknowledged these factors as boundary conditions of the framework, and we guide the reader to the relevant literature where they are addressed in detail. Overall, the revised manuscript now more clearly delineates the conditions under which the framework is applicable, while distinguishing between intrinsic device physics captured by the model and extrinsic artefacts that must be controlled experimentally.

#19 – Regarding the separation of the two mechanisms contributing to the OECT response (changes in gate electrochemical potential and changes in gate capacitance), the manuscript provides a clear conceptual framework and dedicates distinct sections to each contribution. In practice, however, the setup configuration and material properties complexity may limit how cleanly these two effects can be disentangled. Given these challenges, could the authors clarify whether the proposed methodology could be further refined by explicitly outlining the limits of reliability, considering possible deviations from the used assumptions as well as experimental uncertainties?

The limitations that may affect the applicability of the methodology presented in this work are discussed in the new section, “Considerations for non-Faradaic signal transduction using OECTs” which summarizes the main constraints and provides experimental demonstrations.

#20 – The authors note (in the Introduction) that when the gate serves as the primary data interface, OECTs support reusability thanks to replaceable gate electrodes and a modular device architecture. This suggests the sensor is intended for repeated use. However, the question of reproducibility arises: since the drain current can both increase and decrease with repeated use, how do the authors ensure stable and reliable sensor responses over multiple cycles? Are there specific calibration strategies or methods implemented to maintain reproducibility despite potential changes in device characteristics with repeated use?

Device repeated-use effects are addressed in Fig. 8c-i and the corresponding Discussion.

Device reproducibility is addressed in Fig. 8c-ii–iv and the corresponding Discussion.

Stability and identification of a safe operating window are addressed in Fig. 8b and the corresponding Discussion.

Calibration strategies and methods implemented to maintain reproducibility are described in Fig. 8b and the corresponding Discussion, and in Supplementary Discussion 10: Assessment of OECT stability during successive transfer curve acquisition.

#21 – Although the authors mention OECT sensor optimization, an inherent challenge of OECT-based devices remains device-to-device variability due to manufacturing process inconsistencies. This variability can lead to differences in transistor characteristics even among nominally identical samples. Could the authors provide more details on the number of samples used for the experimental evaluation?

Furthermore, it would be useful to understand how reproducible the obtained sensor responses are across devices.

Furthermore, given the natural variability in device performance, could the authors comment on how the proposed optimization compares to this underlying variability?

In particular, can the observed improvement in sensor performance due to optimization be clearly distinguished from fluctuations caused by manufacturing process differences?

Quantitative assessments, for example based on drain current or sensitivity variations, would greatly help clarify this issue.

Device-to-device variability and reproducibility are addressed in Fig. 8c-ii–iv and the corresponding Discussion. We characterized multiple devices ($n = 37$), obtaining an I_{DS} relative standard deviation below 6.5%. Notably, the variability decreases to <5%, a commonly adopted threshold in analytical chemistry, for $V_{GS} > -0.2$ V. Accordingly, all calibration points reported in this work were acquired at $V_{GS} > 0$ V to minimize uncertainty. Under these conditions, the <5% variability associated with the manufacturing process can be considered negligible.

We provide below our responses to the remaining comments.

#1 – The conversion of LoD in millivolts or nano farads to chemical LoD (LoD_{chem}) is discussed conceptually but not demonstrated experimentally. Including at least one worked example would strengthen the practical impact.

#4 – Some equations used to derive LoD_{chem} are only referenced as “see Supplementary Discussion”; including at least one explicit derivation in the main text would enhance clarity for readers outside the immediate field.

We address these two comments together, as they both relate to LoD_{chem} .

The conversion of LoD_{mV} or LoD_{nF} into LoD_{chem} relies on the following equations reported in the Supplementary Information:

$$\Delta\phi_G = \frac{nQ_{\text{tgt}}}{\epsilon_{\text{tgt}}\epsilon_0}d_{\text{tgt}}$$
$$\Delta C_G = \frac{\Delta\epsilon_r\epsilon_0 A}{\Delta d}$$

These expressions relate changes in electrode potential and capacitance to characteristic parameters of the target. We agree with the Reviewer that an experimental demonstration would be valuable. However, performing such a validation would require independently measuring, for a given target protein:

n – the surface density of bound protein. This could be quantified by quartz crystal microbalance with dissipation monitoring (QCM-D). Accurate conversion of frequency and dissipation shifts (across multiple overtones) into mass changes would require independent measurements of electrolyte density, viscosity, and temperature, as well as film density, thickness, and viscoelastic parameters (shear viscosity and shear modulus), and the use of an appropriate viscoelastic model for the adsorbed protein layer.

Q_{tgt} – the net charge of a single target molecule, which depends on isoelectric point, electrolyte pH, and ionic strength. Values provided by suppliers are typically computed from the unfolded protein sequence and functional groups and are therefore not directly applicable. This parameter would need to be experimentally determined, e.g., using zeta-potential measurements.

ϵ_{tgt} – the relative dielectric permittivity of the hydrated protein in the electrolyte environment, which would require dielectric spectroscopy measurements.

d_{tgt} – the effective thickness of the target region that electrostatically interacts with the conductive gate surface. This would require knowledge of the protein-layer thickness (e.g., liquid AFM) combined with surface-potential measurements acquired at different ionic strengths.

$\Delta\epsilon_r$ – the change in the gate effective dielectric permittivity, which would require electrochemical impedance spectroscopy (EIS) and, independent quantification of any thickness change (e.g., liquid AFM).

A – the gate electroactive area, which should be determined using conventional electrochemical methods (e.g., redox probe).

Each of these measurements can carry substantial uncertainty due to protocol-dependent artifacts, stochastic fluctuations, and protein instability. Given the number of independently required parameters, the resulting error propagation to LoD_{chem} —combined with OEECT-specific limitations discussed above (e.g., electrode drift, protein desorption, and OMIEC degradation)—would make

such a demonstration extremely challenging. Moreover, executing this validation rigorously would likely require a dedicated study in its own right.

Because the main goal of this work is to provide a simple and practical framework for optimizing OECT-based sensing, including this comparison would unnecessarily complicate the discussion without providing a directly actionable tool for most readers. We therefore removed this section and focused the text on (i) why these limits should be minimized and (ii) the advantage of determining them using the straightforward approach proposed here.

The main text has been revised as follows:

Establishing a direct relationship between the transfer-curve shift (ΔV_{GS}) and the corresponding input signal (ΔE_G) enabled us to quantify the minimum detectable change in gate potential ($LoD_{\Delta EG}$) that the OECT can resolve (Fig. 3d). This value corresponds to $|LoD_{mV}|$, defined as the smallest shift in V_{GS} required to move I_{DS} beyond the measurement-noise envelope ($I_{DS} \pm 3S_{blk}$), where S_{blk} is the standard deviation in I_{DS} measured using the blank gate electrode, see Supplementary Fig. 9). We found that $LoD_{\Delta EG}$ depends on the operating voltage, making, once more, biasing conditions a critical parameter in optimizing sensing performance. In this case, the lowest $LoD_{\Delta EG}$ was just 3 mV, and it could be obtained when the device was operated at the most positive V_{GS} , which also corresponds to the regime of highest NR. This value is consistent with those reported in non-faradaic PEDOT:PSS-based OECT sensing studies targeting biologically relevant analytes. Estimating $LoD_{\Delta EG}$ using this approach enables rapid and cost-effective optimization of OECT geometry, channel material, and operating conditions to lower the detectable input threshold before introducing biological targets.

...

We next calculated the minimum change in gate capacitance detectable by the OECT, i.e., $LoD_{\Delta CG}$. Given that $LoD_{\Delta CG}$ depends on both gate size and operational voltage, we considered operation at the most sensitive bias ($V_{GS} = +0.1$ V) and reported in Fig. 5c its dependency on the gate size. The values were obtained analogously to the $LoD_{\Delta EG}$, where the $|LoD_{mV}|$ was further converted into an equivalent capacitance value ($LoD_{\Delta CG}$) using the relationships reported in Supplementary Figs. 11a and 11c. As is the case with the device sensitivity, the $LoD_{\Delta CG}$ exponentially improved by decreasing the gate size, with a minimum detectable gate capacitance change calculated to be 2 nF.

#2 – While the assumption that target binding can be mimicked solely by changes in potential/capacitance is justified, an explicit comparison to an actual biomolecular interface (e.g., with a simple aptamer system) would reinforce external validity.

#24 – The current definition of the analyzed capacitance remains ambiguous, and it is unclear which specific capacitance components are included in the model, which are neglected, and on what basis these choices are made. Because different capacitance contributions (e.g. interfacial, geometric, double-layer, parasitic, and etc.) exhibit distinct physical origins and frequency dependencies, a more explicit discussion is needed.

Incorporating frequency-dependent measurements would strengthen the validity of the proposed capacitance model.

We address these two comments together, as they both relate to the variations of electrochemical properties induced by protein-electrode interactions.

We added “Supplementary Discussion 1: Effect of Target–Electrode Interactions on Gate Electrochemical Properties.” In this section, we explain that a sensing electrode, independent of its use in an OECT, is primarily characterized by three electrochemical properties that can change upon target binding: electrochemical potential, double layer capacitance, and charge-transfer resistance. Each parameter can be monitored independently as a sensing output, corresponding to potentiometric sensing, impedimetric sensing, and faradaic-based sensing, respectively. We experimentally verified these effects by characterizing the binding of SARS-CoV-2 spike protein to a nanobody-functionalized electrode (as one example), and we benchmarked the binding in parallel using quartz crystal microbalance measurements as an independent, non-electrochemical validation.

The main text has been revised as follows:

When a biomarker binds to a receptor-immobilized electrode, the electrode’s electrochemical properties, such as the electrochemical potential, double layer capacitance, and charge-transfer resistance, can change (see Supplementary Discussion 1 and Supplementary Fig. 1). Because changes in charge-transfer resistance are not relevant for non-faradaic sensing, we focus here on the electrochemical potential and double layer capacitance and analyze their individual contributions to the OECT response as well as their combined effect.

To isolate the contribution of electrochemical potential, we fabricated model gate electrodes with different equilibrium potentials yet constant capacitance. This was achieved by assembling Ag/AgCl reference electrodes with different Cl^- concentrations in the internal electrolyte (Supplementary Discussion 2).

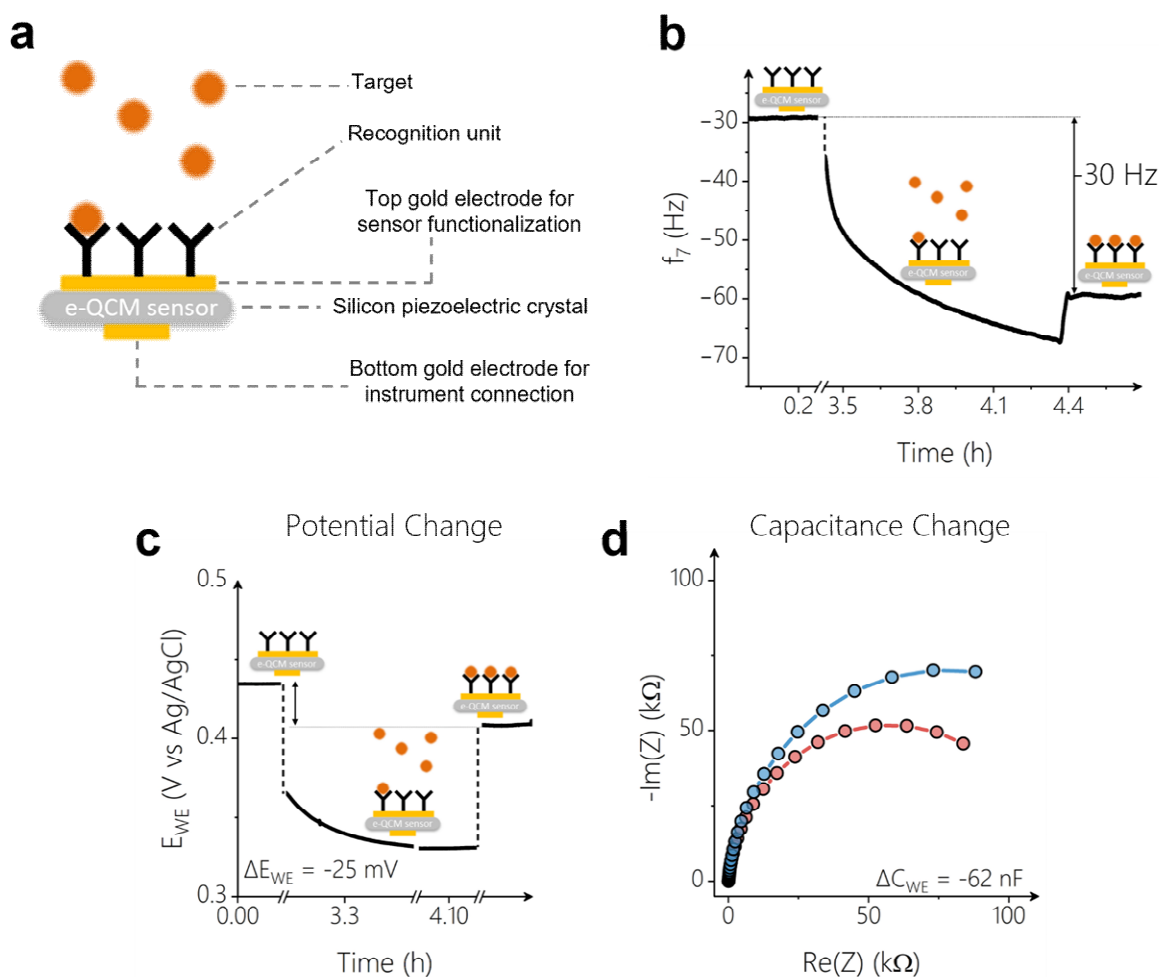
The supplementary material has been revised as follows:

Supplementary Discussion 1: Effect of Target-Electrode Interactions on Gate Electrochemical Properties

To verify how gate electrochemical properties change when a biomarker interacts with the electrode surface, we monitored the sensing interface using an electrochemical quartz crystal microbalance (e-QCM-D; Supplementary Fig. 1a).[<https://doi.org/10.1002/adma.202202972>, <https://doi.org/10.1021/acsami.2c20502>] The sensing model used in this experiment is a nanobody functionalized gold electrode for the detection of SARS-CoV-2 spike protein.[<https://doi.org/10.1038/s41551-021-00734-9>] This approach enables real-time monitoring of the resonance-frequency shift of a piezoelectric quartz–gold electrode on which the nanobody was immobilized to capture the target (Supplementary Fig. 1b). The e-QCM-D provides a benchmark validation of binding because its primary signal arises from piezoelectric mass loading rather than electrochemical transduction.[<https://doi.org/10.1063/1.4963312>, <https://doi.org/10.1002/cctc.201300900>] For a given excitation, the oscillation frequency is highly sensitive to changes in electrode mass, which can vary upon protein adsorption at the surface. The –30 Hz frequency shift observed here confirms protein binding to the recognition layer.

Protein binding can modify the electrode surface potential and dipole distribution through the introduction of additional charges, leading to polarization of the interfacial bilayer.[<https://doi.org/10.1039/C9MH01544B>, <https://doi.org/10.1016/j.bios.2004.06.022>, <https://doi.org/10.1021/acs.chemrev.1c00290>] Changes in surface potential (or electrochemical potential) can be measured directly using Kelvin probe force microscopy (KPFM),[<https://doi.org/10.1016/j.bios.2004.06.022>, <https://doi.org/10.1002/admi.202201829>] or open circuit potential (OCP) measurements,[<https://doi.org/10.1002/adfm.201904513>,

<https://doi.org/10.1016/j.electacta.2008.04.036>] or inferred indirectly from electrochemical impedance spectroscopy (EIS).[\[https://doi.org/10.1063/1.4986190\]](https://doi.org/10.1063/1.4986190) Here, we monitored the electrochemical potential via OCP and measured a -25 mV shift upon target binding (Supplementary Fig. 1c). The electrode double layer capacitance can also change, for example decrease due to formation of a more insulating protein layer or increase if binding disrupts packing within the bilayer and enhances ion accessibility at the interface.[\[https://doi.org/10.1021/ac1018233\]](https://doi.org/10.1021/ac1018233), <https://doi.org/10.1038/ncomms7010>] Such changes can be quantified using EIS or cyclic voltammetry (CV). In Supplementary Fig. 1d, we report a capacitance decrease of -62 nF extracted from EIS through Randles circuit fitting. The charge-transfer resistance (R_{CT}) may also vary depending on how target binding alters electrode accessibility to a redox probe in solution,[\[https://doi.org/10.1002/adhm.202303509\]](https://doi.org/10.1002/adhm.202303509) as reflected by the increase in semicircle diameter in the Nyquist plot in Supplementary Fig. 1d. We do not investigate the contribution of R_{CT} to the OECT response here our work focuses on non-redox based, non-Faradaic sensing that relies on changes in electrochemical potential and capacitance.[\[https://doi.org/10.1039/C9MH01544B\]](https://doi.org/10.1039/C9MH01544B), <https://doi.org/10.1039/C7RA09832D>, <https://doi.org/10.1002/adma.202515338>, [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387)]



Supplementary Figure 1. Effect of Target-Electrode Interactions on Gate Electrochemical Properties. **a)** Schematic of the e-QCM sensor architecture. **b)** Resonance-frequency shift (7th overtone) of the e-QCM crystal during binding of SARS-CoV-2 spike protein to the immobilized nanobody. The initial phase corresponds to the electrode in buffer (blank); the middle phase corresponds to exposure to buffer containing 1 nM target (incubation); and the final phase corresponds to rinsing with target-free buffer to remove unbound species, leaving only specifically bound protein (washing). **c)** Simultaneous measurement of the e-QCM-D electrode's open-circuit potential (electrochemical potential). **d)** Nyquist plot of the nanobody-functionalized electrode before and after target binding measured using electrochemical impedance spectroscopy (EIS). The electrode double layer capacitance was obtained by fitting the curve to the Randles equivalent circuit shown in the inset.

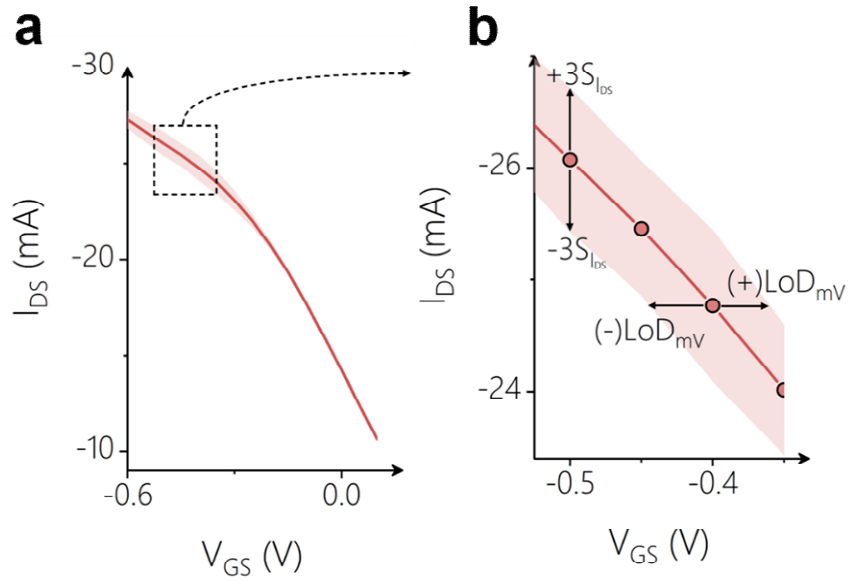
#3 – The uncertainty analysis could be more explicit — e.g., propagation of measurement noise to calculated ΔV_{drop} or LoD_{mV}

#9 – Figures 3–6 present trends (e.g., normalized response, LoD_{mV} , ΔV_{drop}) but lack explicit error bars or confidence intervals on most quantitative data. For a paper emphasizing quantitative modeling, there is no discussion of uncertainty propagation (from potential and capacitance measurements to current modulation). Suggestion: include statistical analysis ($n \geq 3$ replicates) and error propagation for key derived parameters such as: ΔV_{drop} , LoD_{mV} , and LoD_{nF} ; b. $g_{\text{m,eff}}$ and NR_{max} ; $\Delta E - \Delta I_{\text{DS}}$ correlations. This would establish the reliability and reproducibility of the quantitative framework.

We address these two comments together, as they both relate to the propagation of measurement noise.

Measurement noise was evaluated throughout the experiments; however, standard deviations were not reported in every figure because each plot was designed to emphasize specific aspects of the sensing mechanism, and uncertainty was explicitly discussed in Fig. 3d, Supplementary Fig. 9, and Fig. 5c. We agree that it is important for readers to assess whether the performance improvements reported here are significant relative to the intrinsic variability of the OECD output signal (I_{DS}). This comparison is necessary to determine whether the proposed optimization strategies provide meaningful gains beyond the device's inherent limitations. Accordingly, we have added error analysis to additional key figures.

To further clarify the procedure used to extract the minimum detectable change in the gate electrochemical properties, we revised Supplementary Fig. 9 by adding an additional panel that provides a step-by-step graphical explanation of the calculation of these values, and we included the relevant details in the figure caption:



Supplementary Figure 9. Noise associated with the PEDOT:PSS OECT transfer curve measurement. **a)** Representative transfer curve; the shaded region denotes $\pm 3\times$ the standard deviation (S_{blk}) of the measurement ($n = 3$). **b)** The $\pm 3S_{blk}$ band at each I_{DS} value defines the transfer-curve noise envelope. The corresponding width from the I_{DS} along the V_{GS} axis represents the minimum change in gate potential (ΔE_G) required to shift the transfer curve beyond the noise region. Since $(+)LoD_{mV}$ and $(-)LoD_{mV}$ are similar in absolute value, the limit of detection can be reported as their average magnitude, independent of the shift direction ($|LoD_{mV}|$). Because of the one-to-one correspondence between ΔE_G and ΔV_{GS} , $|LoD_{mV}| = LoD_{\Delta EG}$.

Fig.3d was also modified accordingly.

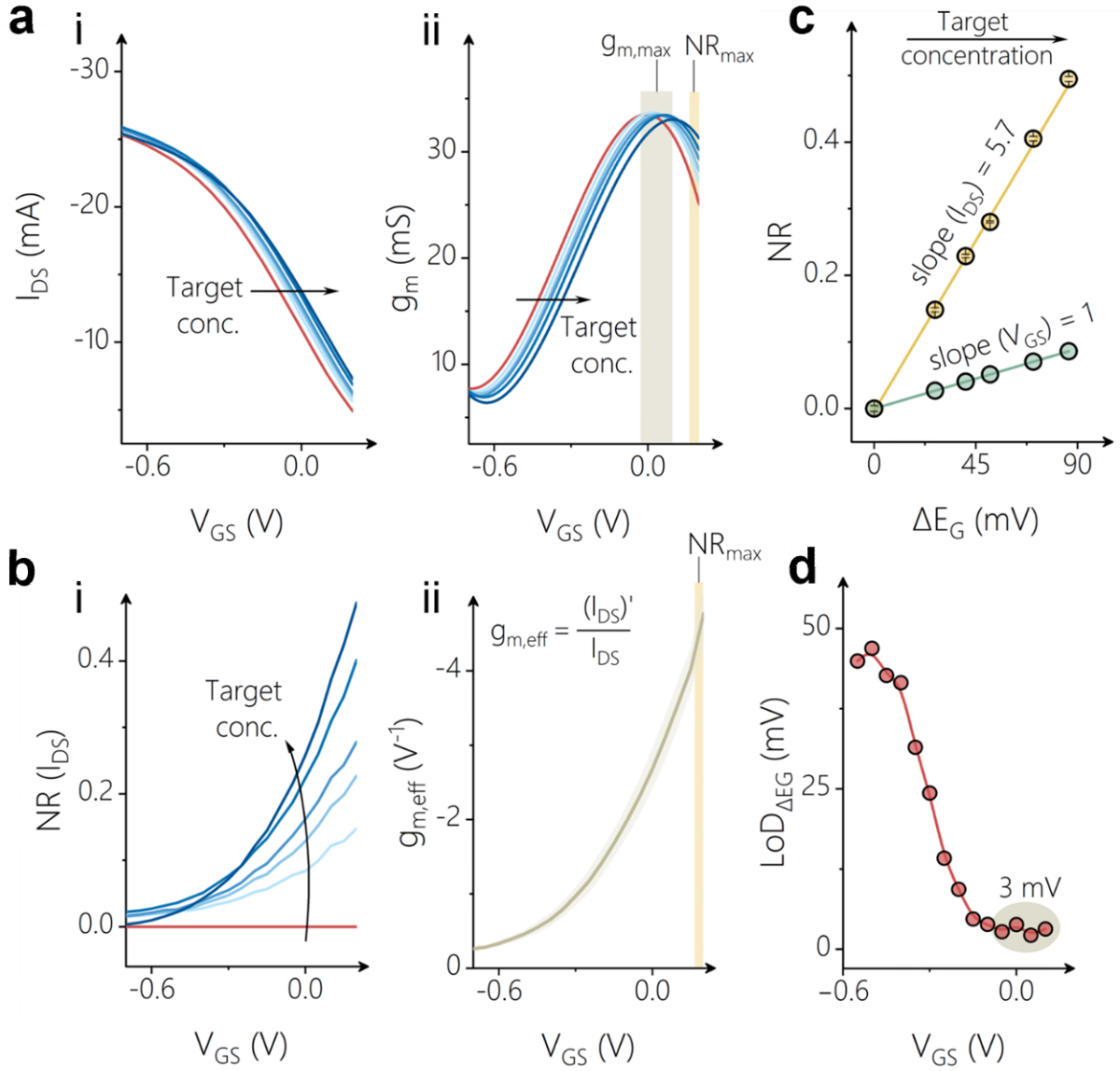


Figure 3. Optimal biasing conditions and output signal for highest OEET response. ... d) Calculated $\text{LoD}_{\Delta\text{EG}}$ as function of V_{GS} , considering the I_{DS} noise window defined by $3S_{\text{blk}}$.

More details were also included in the main text:

Establishing a direct relationship between the transfer-curve shift (ΔV_{GS}) and the corresponding input signal (ΔE_{G}) enabled us to quantify the minimum detectable change in gate potential ($\text{LoD}_{\Delta\text{EG}}$) that the OEET can resolve (Fig. 3d). This value corresponds to $|\text{LoD}_{\text{mV}}|$, defined as the smallest shift in V_{GS} required to move I_{DS} beyond the measurement-noise envelope ($I_{\text{DS}} \pm 3S_{\text{blk}}$), where S_{blk} is the standard deviation in I_{DS} measured using the blank gate electrode, see Supplementary Fig. 9). We found that $\text{LoD}_{\Delta\text{EG}}$ depends on the operating voltage, making, once more, biasing conditions a critical parameter in optimizing sensing performance. In this case, the lowest $\text{LoD}_{\Delta\text{EG}}$ was just 3 mV, and it could be obtained when the device was operated at the most positive V_{GS} , which also corresponds to the regime of highest NR. This value is consistent with

those reported in non-faradaic PEDOT:PSS-based OECT sensing studies targeting biologically relevant analytes.[<https://doi.org/10.1016/j.bios.2019.111668>, [10.1126/sciadv.abg8387](https://doi.org/10.1016/j.bios.2019.111668), <https://doi.org/10.1002/adma.201102017>, <https://doi.org/10.1039/C7RA09832D>] Estimating $LoD_{\Delta EG}$ using this approach enables rapid and cost-effective optimization of OECT geometry, channel material, and operating conditions to lower the detectable input threshold before introducing biological targets.

We also clarified the calculation of $LoD_{\Delta CG}$ as follows:

We next calculated the minimum change in gate capacitance detectable by the OECT, i.e., $LoD_{\Delta CG}$. Given that $LoD_{\Delta CG}$ depends on both gate size and operational voltage, we considered operation at the most sensitive bias ($V_{GS} = +0.1$ V) and reported in Fig. 5c its dependency on the gate size. The values were obtained analogously to the $LoD_{\Delta EG}$, where the $|LoD_{mV}|$ was further converted into an equivalent capacitance value ($LoD_{\Delta CG}$) using the relationships reported in Supplementary Figs. 11a and 11c. As is the case with the device sensitivity, the $LoD_{\Delta CG}$ exponentially improved by decreasing the gate size, with a minimum detectable gate capacitance change calculated to be 2 nF.

The values of $LoD_{\Delta EG}$ and $LoD_{\Delta CG}$ are already an expression of the uncertainty associated with I_{DS} stability. By analogy, when reporting a measurement as $\bar{x} = x \pm 3\sigma$, the term 3σ represents the measurement noise/uncertainty and is not itself assigned an additional error term. Here, σ corresponds to the current-domain noise, mapped back into an equivalent voltage-domain uncertainty—as the OECT operates as a voltage-to-current transducer. For the same reason, it was necessary to express the effect of gate capacitance (or its change) in voltage terms, such as V_{drop} or ΔV_{drop} .

The uncertainty associated with $g_{m,eff}$ is now reported in the new Fig. 8c-iii, based on characterization of 37 OECT channels, and is 3%.

Experimental error associated with electrochemical potential measurements (e.g., E_G or ΔE_G) of Ag/AgCl based electrodes can be considered negligible for two reasons: (i) these electrodes are functionally equivalent to commercial reference electrodes, and the Nernst equilibrium therefore stabilizes their potential (see Fig.2a); and (ii) their potential is measured against an equivalent electrode of the same type, such that the measurement is inherently relative rather than absolute, and variability attributable to the working electrode cannot be resolved independently. Similarly, the error associated with E_G or ΔE_G measurements using Au gates can be considered negligible because, prior to measurement, the gates were allowed to equilibrate in the electrolyte until their potential drift reached a stable value, analogous to a reference-like steady state (<20 $\mu V/s$). Together, these procedures minimize noise arising from OECT measurement. These details were added to the Methods as follows:

Gate electrodes with different electrochemical potential and constant capacitance were prepared by assembling custom made Ag/AgCl reference electrodes using the repair kit components purchased from ALS Japan. Solutions with different NaCl concentrations were introduced inside the sample holder along with an Ag wire previously kept in bleach for at least 15 minutes to induce the formation of an AgCl coating. The quality of the fabricated electrodes and any drift-related interference were assessed by measuring their electrochemical potential relative to a commercial Ag/AgCl reference electrode, confirming a drift rate below 20 $\mu V/s$. Gate electrodes with different capacitance and constant electrochemical potential were obtained using the same gold straight wire progressively immersed in the measurement solution using a Manual Micromanipulator (PreSens)

whose micrometric screws allowed to precisely increase the surface area exposed to the electrolyte. Gate electrodes with different capacitance and electrochemical potential were obtained using independent gold electrodes purchased from ALS Japan. Prior to the measurements, the Au electrodes were allowed to equilibrate in the electrolyte until their electrochemical potential stabilized, thereby minimizing drift-related interference.

Experimental error associated with the subsequent transduction into I_{DS} or ΔI_{DS} is determined by (i) the stability of the signal from an individual OECT channel and (ii) the reproducibility of that signal across channels. These aspects are addressed in the preceding part of this response (see Supplementary Fig. 9) and in the new section added to the main text (see Fig. 8c). Accordingly, the error associated with NR_{max} has been included in Fig.3c and Fig.5a-iii.

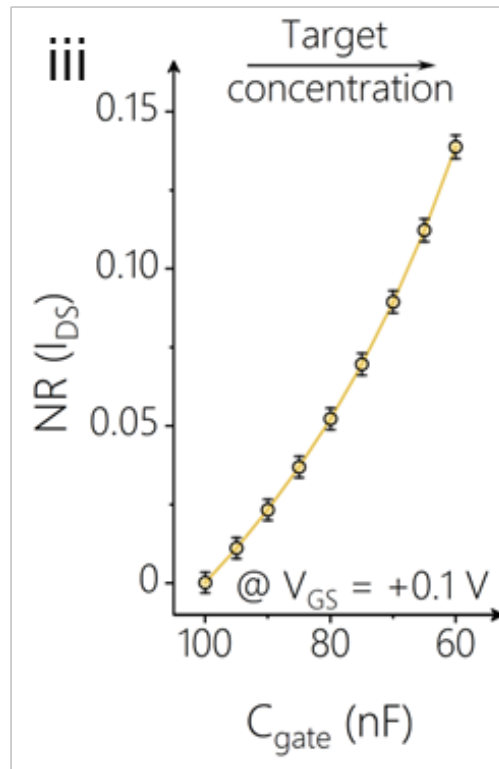


Figure 5. Optimal biasing conditions and gate capacitance for highest response. a) Transfer curves recorded with gate electrodes of decreasing capacitance (and constant electrochemical potential), simulating increasing target concentrations bound to the gate (i). The OECT NR as a function of V_{GS} (ii), and calibration curve obtained at $V_{GS} = +0.1$ V (iii).

The impact of the measurement noise on the OECT input signals (ΔE_G and ΔC_G) expressed in the voltage-domain terms relevant for OECT transduction (ΔE_G and ΔV_{drop}), is now included in Fig.6a-ii. In this figure, the individual contributions and their cumulative effect are compared with the intrinsic noise of the output signal, expressed as LoD_{mV} . This representation highlights the operational voltage regions in which each contribution, and their combination, produces a signal that exceeds the noise level.

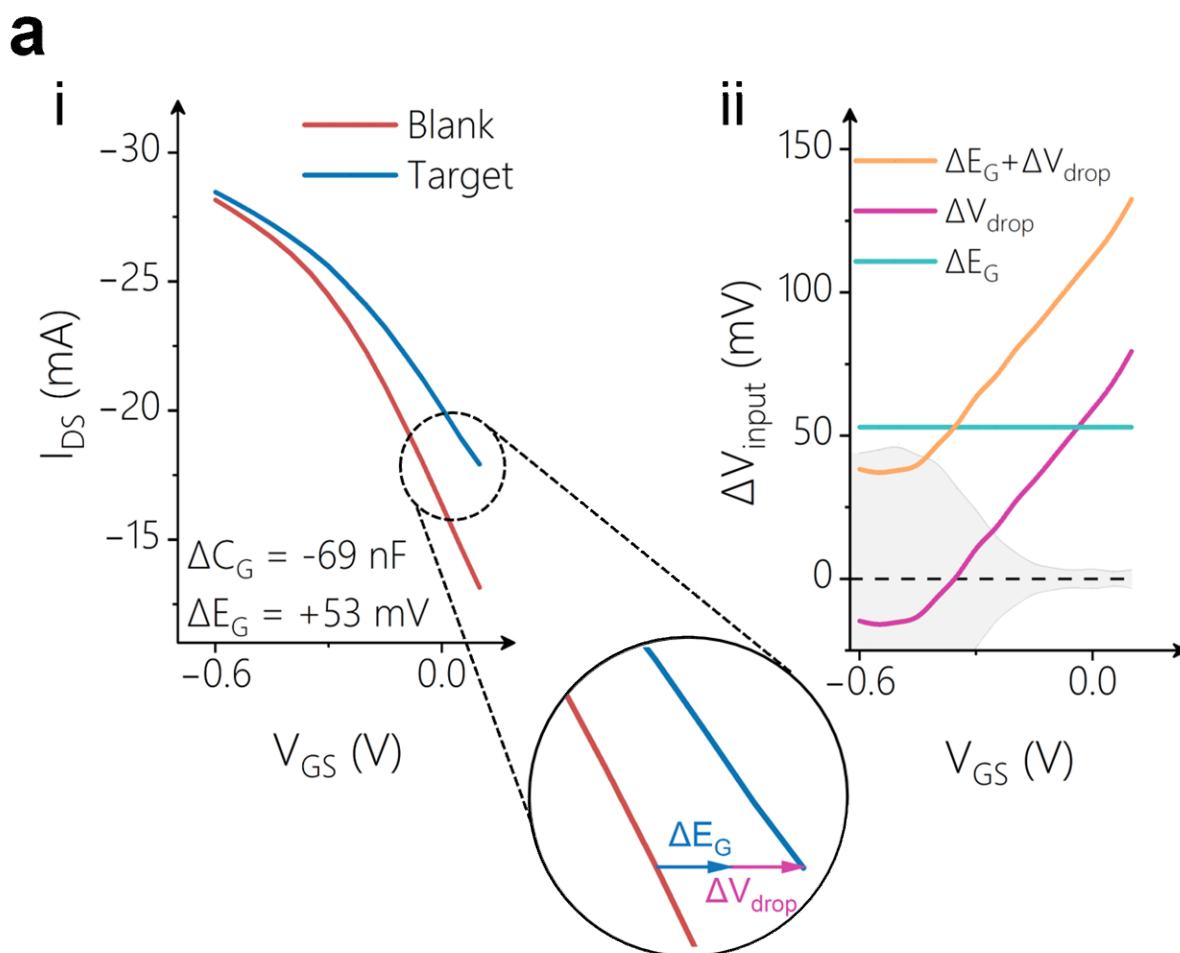


Figure 6. Combined effects of gate potential and capacitance changes on OECT output. a) OECT transfer characteristics when the gate undergoes a simultaneous change in electrochemical potential (+53 mV) and capacitance (-69 nF) upon target binding (i). Magnitude of the measurement noise (shaded area corresponding to LoD_{mV}) and OECT input signal (ΔV_{input}) at different operating voltages displayed as individual contributions from the gate potential shift (ΔE_G), the polarization change (ΔV_{drop}), and their combined effect ($\Delta E_G + \Delta V_{drop}$). The dashed line separates the regions in which ΔV_{input} shifts the transfer curve toward more positive or more negative V_{GS} (ii).

The corresponding main text was revised as follows:

The two corresponding sensing input signals (ΔE_G and ΔV_{drop}) are unified under the term ΔV_{input} , which we quantified at each operating voltage and compared with the intrinsic measurement noise (Fig. 6a-ii). Supplementary Discussion 9 provides a step-by-step guide to applying the EP-IV analysis to extract ΔV_{input} as a function of V_{GS} . While ΔE_G remained constant across the V_{GS} scan, ΔV_{drop} increased with more positive V_{GS} values due to higher C_{CH} that increased gate polarization. The combined contribution to the output signal exceeds the measurement noise (LoD_{mV}) primarily at operating biases closer to the device OFF state.

#5 – Figure 5b and 6a present complex dependencies; including summary plots (e.g., response surfaces or heatmaps) might help visualize the interplay of ΔE , ΔC_{DL} , and V_{GS} .

The variations ΔE_G , ΔC_G are intrinsic properties of the sensing electrode and, as such, are independent of the OECT as they arise upon target binding prior to the electrode use as the OECT gate. Accordingly, OECT-based sensing studies typically first characterize the sensing electrode (subsequently employed as the gate) using conventional electrochemical techniques to quantify changes in these input parameters (<https://doi.org/10.1002/adma.202515338>, [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387), <https://doi.org/10.1002/adma.202202972>).

Although there may be a correlation between how much the physical parameters governing changes in sensing electrode capacitance (ΔC_G) can interfere with the amount of electrochemical potential shift achievable (ΔE_G), and vice versa, investigating this interplay is beyond the scope of this work and would require an independent study, as it is relevant not only to OECT-based sensing but to electrochemical sensing in general (e.g., impedimetric and potentiometric sensing). For example, prior work has explored correlations between ΔE and ΔR_{CT} (<https://doi.org/10.1063/1.4986190>). Despite any possible interplay between ΔE_G and ΔC_G , the effect of V_{GS} on gate polarization is unchanged, because it is governed by the voltage distribution across two capacitors in series, the larger the capacitance of an element, the smaller the voltage drop across it (see Supplementary Discussions 6 and 8, and <https://doi.org/10.1002/aelm.202001173> for graphical illustrations, including surface plots).

Constructing a representation in which ΔE_G , ΔC_G , and V_{GS} are varied simultaneously while monitoring the output current I_{DS} would require a four-dimensional plot, likely resulting in a less interpretable visualization than those already included in the manuscript. Moreover, selectively and independently tuning ΔE_G and ΔC_G in an accurate manner over a broad parameter space is complex. While the gate potential can be systematically varied at constant capacitance by exploiting the Nernst equation (Supplementary Fig. 2), and capacitance can be tuned, by varying the area of a relatively inert material (Fig. 4a), tuning both simultaneously typically necessitates changes in both electrode area (to modulate capacitance) and material (to modulate potential). However, different materials can exhibit distinct redox activities towards the electrolyte and intrinsic redox states in the electrolyte, generating faradaic contributions that can add to I_{GS} and indirectly perturb I_{DS} . [<https://doi.org/10.1016/j.snr.2026.100464>] This can compromise interpretation of the EP-IV analysis, analogous to the interference observed under OMIEC I_{DS} loss conditions (Supplementary Fig. 20). We have therefore added this consideration at the end of Supplementary Discussion 9, as follows:

In this experiment, simultaneous changes in both parameters were realized using two gold electrodes of different areas. While capacitance can be conveniently tuned by adjusting gate size, the gate potential is hardly controllable even for electrodes made of the same material because it depends on multiple factors. [[10.1039/C5MH00160A](https://doi.org/10.1039/C5MH00160A), [https://doi.org/10.1016/0039-6028\(73\)90117-9](https://doi.org/10.1016/0039-6028(73)90117-9)] Consequently, systematically sampling the full space of possible ΔE_G and ΔC_G combinations is non-trivial. Nevertheless, this limitation does not preclude investigation of the OECT sensing transduction mechanism: representative single cases, such as the example reported above, are consistent with the trends established in the preceding analyses and support the conclusions drawn.

The figures highlighted by the Reviewer address concepts that are inherently complex, which is why we investigated them separately. Moreover, each figure conveys a distinct aspect of the

transduction mechanism, and we believe that presenting them independently supports a clearer, step-by-step understanding. Specifically:

- Fig. 5b-i and Supplementary Fig. 11a,b focus on selecting the optimal V_{GS} . They show that, irrespective of the absolute gate capacitance, both the magnitude of gate polarization and the change in polarizability upon target binding exhibit the same dependence on V_{GS} , which can be approximated as linear. This enables straightforward identification of the V_{GS} that maximizes the sensing input signal.
- Fig. 5b-ii focuses on selecting the optimal gate size, demonstrating that smaller electrodes yield a larger OECT response for a given change in electrode capacitance; Supplementary Discussion 8 further discusses the practical limits of this strategy.
- Fig. 6a and Supplementary Discussion 9 provide a step-by-step workflow for performing the EP-IV analysis in the general case where both gate electrochemical potential and capacitance vary simultaneously.

To the best of our ability, we also illustrated the simultaneous change in gate potential and capacitance, and its dependence on the applied V_{GS} , not only at the transfer-curve level but, importantly, within the EP-IV framework in Supplementary Fig. 15. Given the complexity of the full derivation, we tried to simplify it by focusing on the quantities most directly relevant to sensing experiments (i.e., ΔE_G and ΔV_{drop}), rather than employing a more theoretical approach and explicitly carrying all intermediate terms (i.e., ΔE_G , V_{drop}^B , V_{drop}^T , and ΔV_{drop}) as in the previous version. We believe that this choice made the analysis more accessible while preserving the underlying physical interpretation.

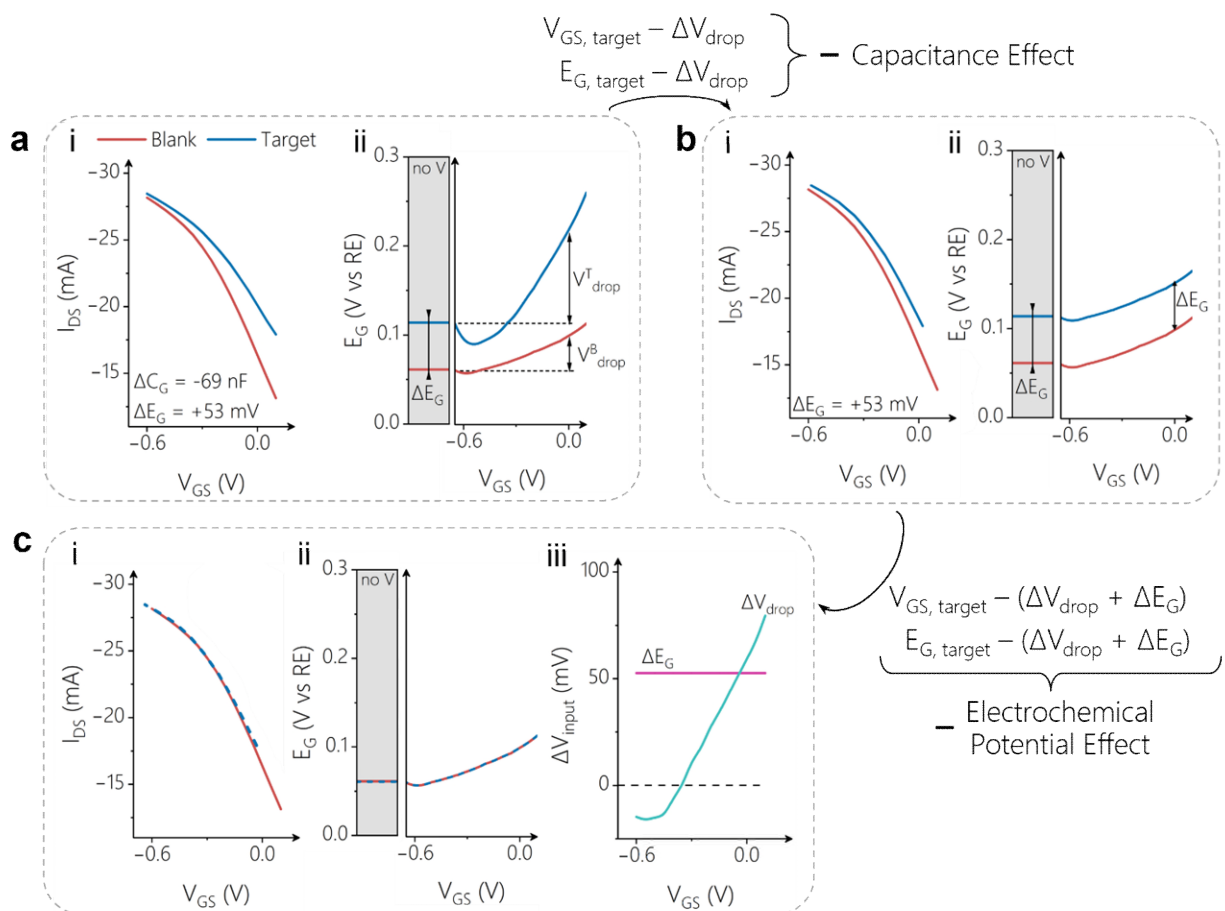
Supplementary Discussion 9 has been revised as follows:

Supplementary Discussion 9: Quantification of the Combined Electrochemical Potential and Capacitance Effect on the OECT Response

To investigate the combined contributions of changes in gate electrochemical potential and capacitance to the OECT response, we applied the EP-IV analysis under conditions in which the gate electrode undergoes both types of changes (Supplementary Fig. 15). In the example shown here, the gate potential increased by 53 mV and the capacitance decreased by 69 nF, consistent with realistic sensing conditions.[\[https://doi.org/10.1002/adma.202202972,](https://doi.org/10.1002/adma.202202972)
[https://doi.org/10.1002/adma.201102017\]](https://doi.org/10.1002/adma.201102017) The transfer curves measured using the Blank and Target gates are reported in Supplementary Fig. 15a-i, the corresponding changes in gate electrochemical properties are annotated within the plot to highlight the parameters driving the OECT response. The potentials of the two gate electrodes (Supplementary Fig.15a-ii) are shown under two conditions: (1) prior to applying any external bias (“no V”), and (2) during transfer-curve acquisition. The shaded area of the “no V” condition displays the variation of gate electrochemical potential (ΔE_G) upon target binding. The influence of the capacitance change, manifested as different degrees of electrode polarization at the OECT terminals, arises only when operating voltages are applied during transfer curve measurements. Under bias, the two gates polarize to different extents because of their different capacitances. These two polarization conditions, denoted V_{drop}^B and V_{drop}^T , are indicated, for example, at $V_{GS} = 0$ V. As expected, the higher capacitance of the *Blank* gate results in lower polarization ($V_{drop}^B < V_{drop}^T$). By calculating the difference between the two polarization values at each specific V_{GS} , ($\Delta V_{drop} = V_{drop}^T - V_{drop}^B$), we quantify the binding-induced change in gate polarization as a function of operating bias. Subtracting ΔV_{drop} from the Target traces ($V_{GS, target} - \Delta V_{drop}$ and $E_{G, target} - \Delta V_{drop}$) effectively

removes the contribution of the capacitance change. The resulting transfer curve retains only the effect of ΔE_G (Supplementary Fig. 15b-i), and the Target gate exhibits the same polarizability as the Blank gate (Supplementary Fig. 15b-ii). In this capacitance-compensated representation, the residual offset between $E_{G, \text{blank}}$ and $E_{G, \text{target}}$ equals the ΔE_G measured under “no V” conditions and remains constant across the full V_{GS} range, consistent with the case of isolated gate potential variations. If ΔE_G is also subtracted from the corrected Target traces ($V_{GS, \text{target}} - \Delta V_{\text{drop}} - \Delta E_G$ and $E_{G, \text{target}} - \Delta V_{\text{drop}} - \Delta E_G$), the Blank and Target traces overlap (Supplementary Figs. 15c-i, ii), indicating that both gate potential and capacitance contributions have been removed. This behavior mirrors what is observed in Figs. 2b-i and 4b and confirms that, once all changes in gate electrochemical properties (potential and capacitance) are accounted for, the OECT responses becomes identical. The input signals can be expressed under a unified voltage term, ΔV_{input} , since both manifest in the form of voltage, and can be reported as function of V_{GS} (Supplementary Fig. 15c-iii). This representation is consistent with the individual analyses of gate potential and capacitance effects (Fig. 2b-ii and Supplementary Fig. 11a, respectively).

In this experiment, simultaneous changes in both parameters were realized using two gold electrodes of different areas. While capacitance can be conveniently tuned by adjusting gate size, the gate potential is hardly controllable even for electrodes made of the same material because it depends on multiple factors.[[10.1039/C5MH00160A](https://doi.org/10.1039/C5MH00160A), [https://doi.org/10.1016/0039-6028\(73\)90117-9](https://doi.org/10.1016/0039-6028(73)90117-9)] Consequently, systematically sampling the full space of possible ΔE_G and ΔC_G combinations is non-trivial. Nevertheless, this limitation does not preclude investigation of the OECT sensing transduction mechanism: representative single cases, such as the example reported above, are consistent with the trends established in the preceding analyses and support the conclusions drawn.



Supplementary Figure 15. EP-IV analysis in which both gate electrochemical potential and capacitance change upon target binding. ... b) Data corrected by subtracting the measured gate polarization (V_{drop}^B and V_{drop}^T) from the transfer curves and the electrochemical potential profiles. **c)** Further correction of the *Target* data by subtracting the remaining electrochemical potential difference (ΔE_G) between *Blank* and *Target* conditions.

The reference to Supplementary Discussion 9 in the main text was revised as follows:

Supplementary Discussion 9 provides a step-by-step guide to applying the EP-IV analysis to extract ΔV_{input} as a function of V_{GS} .

#7 – The methods are reported with commendable detail, sufficient for reproduction by other groups. The inclusion of all necessary materials, references to synthetic procedures, and explicit device geometry parameters meets expected field standards.

If available, sharing raw datasets and LabVIEW scripts (e.g., for EP-IV operation) as supplementary materials would further promote transparency.

A Supplementary Data file containing the datasets for all figures in the manuscript will be provided at the end of the editorial process. No LabVIEW script was used to perform the EP-IV measurements, because the potentiostat used for the EP measurements and the SMU used for the

IV measurements were operated independently. The potentiostat was controlled using its native software, whereas the SMU was controlled via a LabVIEW script that performs only the IV acquisition. This script provides basic control of a dual-channel SMU, in which one channel is held at a constant voltage while the other performs a voltage scan. Equivalent IV measurements can be performed using any bipotentiostat or multichannel potentiostat, including the same potentiostat used for the EP measurements. Transfer curves acquired with an SMU yield I_{DS} values identical to those obtained using a potentiostat provided proper instrument calibration and that cable connections and polarity are correctly configured (see Figure R1 for reference).

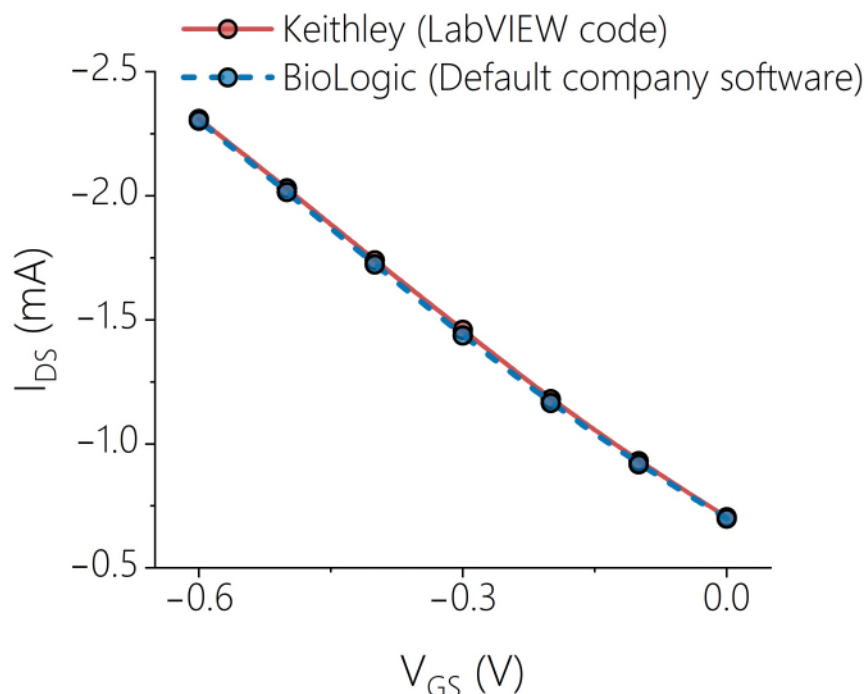


Figure R1. Comparison of PEDOT:PSS OECT transfer curves acquired using a Keithley SMU controlled by custom LabVIEW code and a BioLogic potentiostat operated with the manufacturer’s default software.

We chose to perform EP and IV measurements with separate instruments because simultaneously executing EP and IV measurements using a multichannel BioLogic potentiostat available in our laboratory led to internal interference and irreversible damage to the OECT. We only later identified the appropriate settings to prevent this interference, as described in detail in the following work (<https://doi.org/10.1038/s41467-024-51883-9>). Notably, this interference does not occur with other potentiostat platforms, such as PalmSens.

We included in the main text a specific statement indicating that the raw data are available:

Data Availability

All data generated in this study are provided within the main text and in the Supplementary Information. Source data are provided in this paper as an additional supplementary file.

#8 – Although the final SARS-CoV-2 experiment provides qualitative confirmation, it is not quantitatively linked to the model parameters (ΔE , ΔC_{DL} , ΔV_{drop}). The authors should

validate their framework using at least one real biomolecular interface (e.g., aptamer–protein or antibody–antigen system) where independent measurements of ΔE and ΔC_{DL} are possible. This would demonstrate that their model holds under realistic surface chemistry and ionic screening conditions. Otherwise, the model risks being conceptually elegant but empirically unverified.

#10 – The paper proposes four possible interference regimes (Fig. 6c-iii), but only one is experimentally shown ($+\Delta E$ / $-\Delta C_{DL}$, constructive case). Demonstrating at least one destructive-interference scenario (e.g., $+\Delta E$ and $+\Delta C_{DL}$) would critically strengthen the claim that both synergy and cancellation occur.

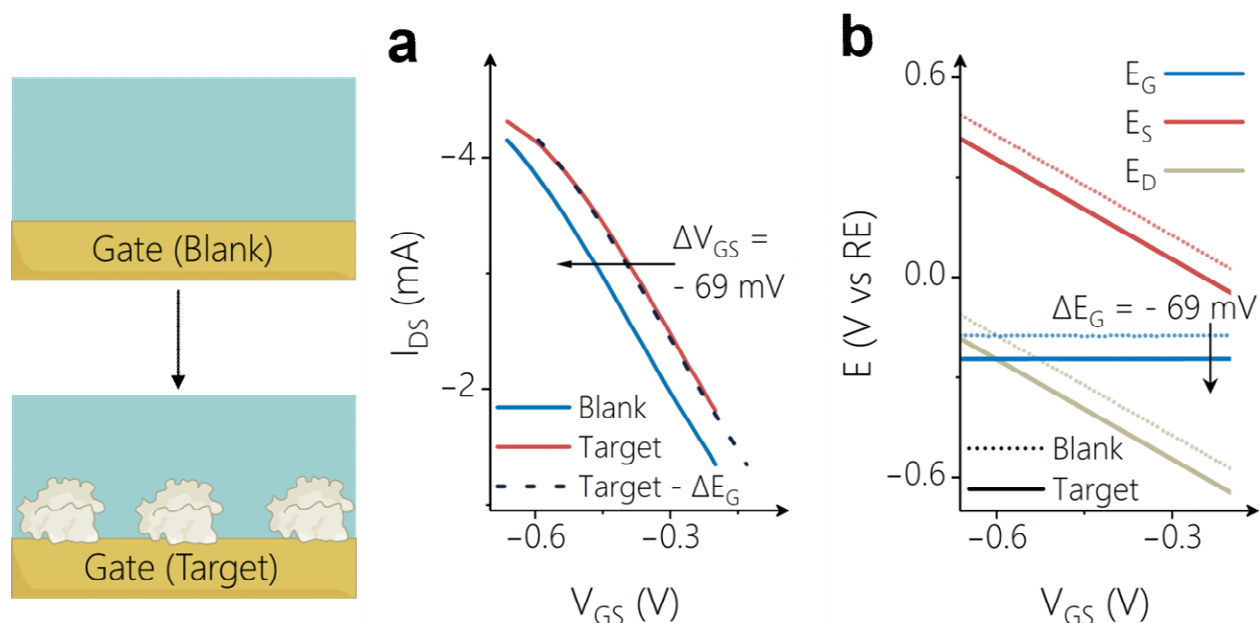
We address these two comments together, as the experiments described below answer both points: (i) using real biomolecules to correlate ΔE_G and ΔC_{DL} with the OECT output, and (ii) providing an experimental example of destructive interaction.

We included two additional experiments to illustrate the effects of protein–electrode interactions on both the gate electrochemical properties and the OECT response. ΔE_G and ΔC_{DL} were independently measured and correlated with the transistor output. Ionic screening was also considered by performing the measurements in diluted buffer to increase the Debye length. The first experiment isolates the contribution of gate potential changes by using a PEDOT-based gate with high capacitance and negligible polarization, whereas the second experiment also captures capacitance effects by using a small Au gate. Notably, the latter experiment was also used to provide an experimental demonstration of destructive interaction, as requested by the Reviewer.

The experiment illustrating the protein-induced OECT response arising from gate electrochemical potential change was incorporated into the manuscript as follows:

The positive shift in all the electrochemical potential of the OECT components leads to a more oxidized state for the OMIEC, which, in the case of p-type materials like PEDOT:PSS generates higher I_{DS} for the same applied V_{GS} . Accordingly, a higher V_{GS} is needed to de-dope the OMIEC to the same level, leading to the observed positive shift of the transfer curve.

To verify that the model described above based on model electrodes is translatable to real protein binding events, we designed an experiment where we examined the OECT response before and after the binding (adsorption) of bovine serum albumin (BSA) on its gate electrode. We selected BSA-electrode system as BSA is a relatively robust and widely used model protein for probing protein-electrode interactions, avoiding the need for extensive optimization of gate functionalization protocols.⁵ To render the Au gate non-polarizable, PEDOT:PSS was electrochemically deposited. To minimize Debye screening,²⁶ measurements were performed in a dilute buffer. Upon BSA binding at the gate electrode, the gate potential decreased by 69 mV, shifting the source and drain potentials by an equivalent amount. The transfer curve exhibited a corresponding shift toward more negative V_{GS} , in agreement with the results obtained with the model electrodes (Supplementary Fig. 5).



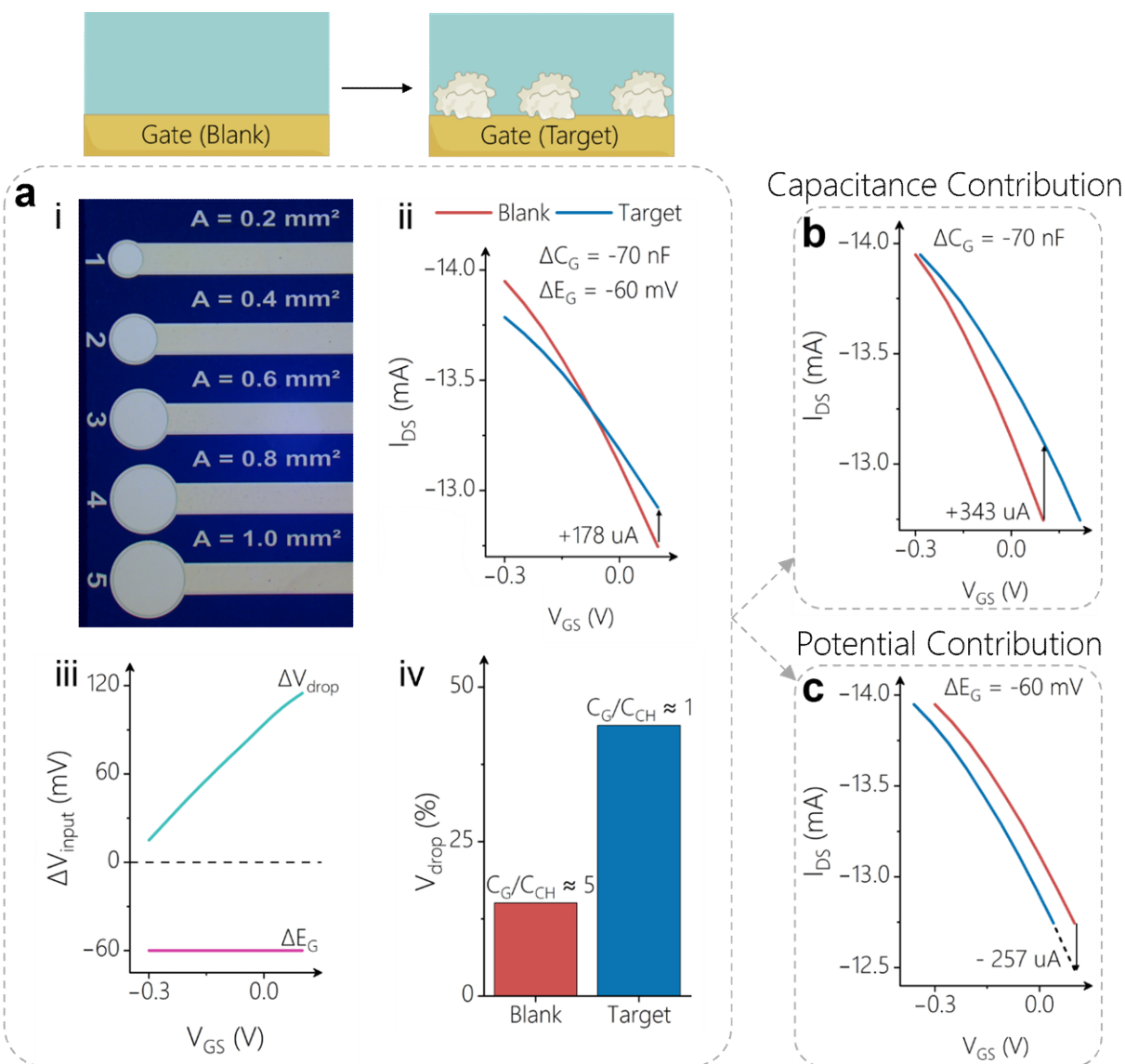
Supplementary Figure 5. Effect of protein-induced gate electrochemical potential changes on OECT output. PEDOT:PSS gate incubated with bovine serum albumin (BSA) and corresponding OECT measurement performed in 1 mM phosphate buffer. **a)** Transfer curves recorded before (blank) and after (target) BSA binding at the gate; the black dashed trace corresponds to the target-bound curve shifted horizontally by 69 mV. **b)** Corresponding electrochemical potentials of gate (E_G), source (E_S) and drain (E_D); dotted lines indicate the blank-gate condition and solid lines represent the potentials after BSA binding.

The experiment illustrating the protein-induced OECT response arising from gate electrochemical potential and capacitance changes was incorporated into the manuscript as follows:

To demonstrate that real protein-gate electrode interactions can induce simultaneous changes in gate potential and capacitance as we showed here with model electrodes, we performed a sensing experiment by examining BSA binding at a polarizable gate electrode. For the PEDOT:PSS OECT channel used here, an appropriate gate electrode area was first identified (Supplementary Fig. 16a-i) to obtain gate polarization during device operation (i.e., polarization is above 50 mV at the V_{GS} of interest). This geometry matching placed the device within the region of interest (ROI) for sensitivity to gate-capacitance, as described in Supplementary Discussion 8 and Supplementary Fig. 14; in this example, a C_G/C_{CH} of approximately 5 was selected. The sensing measurement was then performed by comparing the OECT response before and after BSA binding (Supplementary Fig. 16a-ii). The protein interacting with the electrode surface produced an I_{DS} increase of 178 μA at the optimal V_{GS} near the device OFF state. Using the protocol described in Supplementary Discussion 9, we extracted the corresponding ΔV_{input} (Supplementary Fig. 16a-iii) and found that protein binding caused a decrease in gate potential of 60 mV, accompanied by a simultaneous capacitance decrease of 70 nF. The two contributions, however, lie on opposite sides of the ΔV_{input} vs. V_{GS} plot: ΔE_G induced a negative V_{GS} shift of the transfer curve, whereas ΔC_G produced a positive V_{GS} shift. This corresponds to the $(-\Delta E_G; -\Delta C_G)$ case in Fig. 6c-iii, i.e., a destructive interaction.

The effect of gate-capacitance variation on the OECT response, and the validity of the model used here, particularly Equation S10, was further supported by quantifying the fraction of V_{GS} drop at the gate (V_{drop} (%)) before and after target binding and relating it to the C_G/C_{CH} (Supplementary Fig.16a-iv). In the blank condition, with $C_G/C_{CH} \approx 5$, we measured an average drop of 15% of the applied V_{GS} during the transfer curve, in close agreement with the theoretical value of 16.7% predicted by Equation S10. After target binding, C_G/C_{CH} decreased to approximately 1, yielding a measured drop of 44%, again consistent with the theoretical expectation of 50%.

The extracted ΔV_{input} values enable reconstruction of the OECT response associated with each electrochemical parameter, analogous to Fig. 6c-i,ii. As it was demonstrated in Fig. 4b, an isolated change in gate capacitance produces a tilt of the transfer curve, which in this case would correspond to an I_{DS} increase of 343 μA (Supplementary Fig.16b). Conversely, as it was shown in Supplementary Fig. 5, if the OECT was exclusively sensitive to a change in gate potential upon protein binding, a uniform shift of the transfer curve would be observed, which in this case would correspond to an I_{DS} decrease of 257 μA (Supplementary Fig.16c). These two contributions oppose each other: the capacitance decrease tends to turn the device ON, whereas the potential decrease tends to turn it OFF, illustrating a representative case of destructive interaction after protein binding at the gate electrode.



Supplementary Figure 16. Effect of protein-induced changes in gate electrochemical potential and capacitance on OECT output under destructive interaction. **a)** Gate substrates with varies sizes were used to identify, via EP-IV analysis, the gate area that, when interfaced with the OECT channel, yielded appreciable gate polarization (**i**). Transfer curves acquired in 1 mM phosphate buffer using a 1 mm² gold gate before and after incubation with BSA 0.5 wt% (**ii**). ΔV_{input} values extracted by EP-IV analysis following the protocol described in [Supplementary Discussion 9](#). The dashed line separates the regions in which ΔV_{input} shifts the transfer curve toward more positive or more negative V_{GS} (**iii**). Average percentage V_{GS} drop across the gate during the transfer-curve measurement, extracted using the EP-IV analysis and correlated with measured gate and channel capacitances to verify Equation S10 (**iv**). Based on the extracted ΔV_{input} values, calculated hypothetical target transfer curves for the cases of **b)** an exclusive gate-capacitance change and **c)** an exclusive gate-potential change.

The additional experimental information related to these experiments have been included in the methods section as follows:

Gold gate electrodes used for BSA sensing were either acquired from ALS Japan or made using KAUST cleanroom facilities as described above. The electrodes were sequentially cleaned in 2% (m/m) detergent solution, acetone, and isopropanol for 5 min each. The electrodes were then electrochemically cleaned by cyclic voltammetry in 500 mM sulfuric acid, from -0.2 to 1.5 V vs Ag/AgCl at 100 mV/s for 10 cycles. For the non-polarizable gate condition, PEDOT was electrochemically deposited using chronoamperometry from a 10 mM EDOT solution containing 0.1 M lithium perchlorate as the supporting electrolyte, at 1.1 V vs Ag/AgCl for 10 s. For the polarizable gate condition, bare Au electrode was used. The blank sensor response was obtained by immersing the gate electrode in 1 mM phosphate buffer together with the OECT. A 0.5% (m/m) BSA solution was prepared by dissolving solid BSA in 1 mM phosphate buffer (pH 7.2). The target electrode was prepared by incubating the gate in the BSA solution for 20 min, followed by rinsing with 1 mM phosphate buffer. The electrode was then used as the gate to perform the target measurement in 1 mM phosphate buffer. Gate electrochemical potential was monitored during the EP-IV analysis performed in parallel with the OECT operation, while the gate capacitance was measured by EIS after the OECT measurement.

For the target used in the experiments described above, we selected BSA because it enables us to focus specifically on protein–electrode interactions without requiring time-consuming optimization of gate functionalization. Achieving a measurable change in gate potential and/or capacitance upon binding to an electrode that is already modified with a passivation layer and a recognition element is, in itself, a challenging task that typically requires a dedicated study. In practice, it involves extensive optimization of electrode chemical and electrochemical cleaning,[<https://doi.org/10.1021/acsami.4c01159>, <https://doi.org/10.1002/adhm.202303509>] surface morphology,[<https://doi.org/10.1073/pnas.1315485111>, <https://doi.org/10.1039/C7RA09832D>, <https://doi.org/10.1021/acs.jpcc.6b05158>] passivation SAM concentration and composition (including mixed-SAM ratios),[<https://doi.org/10.1016/j.bios.2004.04.017>] incubation times, and environmental conditions (e.g., light exposure and applied bias) during SAM formation.[<https://doi.org/10.1021/jacs.2c06192>, <https://doi.org/10.1021/la9913046>, <https://doi.org/10.1002/cphc.202000988>, <https://doi.org/10.1002/celc.201600308>] Additional parameters include recognition-unit concentration and incubation time, storage conditions (buffer composition and light exposure), target buffer composition, target incubation time, washing procedures, and electrochemical pre-treatments before and after target exposure, among others.[<https://doi.org/10.1038/s41467-018-05235-z>, <https://doi.org/10.1002/admi.202201829>]

This level of optimization is a general requirement not only for OECT-based sensing, but also for conventional potentiometric and impedimetric sensing, which rely on the same underlying electrode–electrolyte interface and form the basis of the OECT sensing approaches discussed in this work. A comprehensive optimization of these parameters is beyond the scope of the present study. Nevertheless, multiple reports have demonstrated that, with appropriate electrode functionalization, changes in electrode potential and/or capacitance can be achieved upon protein binding.[<https://doi.org/10.1002/adma.202515338>, [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387)] This supports our use of model gate electrodes whose potential and capacitance changes fall within the ranges reported in the literature. Accordingly, we chose a simple protein target such as BSA, which is also widely used as a benchmark protein in fundamental studies of this type.[<https://doi.org/10.1021/ac1018233>]

#12 – The conversion from LoD_{mV} or LoD_{nF} to chemical LoD (LoD_{chem}) is stated but not explicitly derived in the main text. Similarly, the relationship between channel capacitance (C_{CH}) and gate polarization (V_{drop}) is described qualitatively. The authors should provide explicit derivations or equations (not only “see Supplementary Discussion”) for: a) LoD_{chem} calculation, with sample numeric substitution; b) Expression linking ΔV_{drop} to ΔC_{G} and $C_{\text{CH}}(V_{\text{GS}})$; c) Analytical condition for maximum constructive interference ($\Delta E/\Delta C_{\text{DL}}$ ratio). These additions would make the framework quantitatively verifiable and applicable by others.

Please see response to comment #1 and #4 for considerations regarding LoD_{chem} .

We included the equations correlating the gate capacitance (and as consequence the variation of gate capacitance, ΔC_{G}) and the output signal (i.e., I_{DS} or V_{drop}) and its variation (ΔI_{DS} or ΔV_{drop}), by including an additional supplementary discussion:

Supplementary Discussion 7: Interpolation of Expected Output Values

The data reported in Supplementary Fig. 11 provide an extensive OECT characterization correlating gate capacitance (C_{G}) to gate polarization (V_{drop}) at each applied V_{GS} . This calibration enables extraction of empirical relationships describing this dependence and, in turn, prediction of the expected OECT output current (I_{DS}) from the specific gate input parameters (C_{G} and E_{G}) and their variations.

To perform this analysis, the OECT response must first be calibrated using a reference gate electrode so that the OMIEC doping state can be expressed in thermodynamic units (i.e., electrochemical potentials). Accordingly, an initial characterization step was carried out using an Ag/AgCl pellet electrode. The electrochemical potential of this electrode is reproducible and can also be calculated via the Nernst equation based on the electrolyte composition. Under these conditions, the doping voltage can be reported as V_{GS} (V vs Ag/AgCl), emphasizing the thermodynamic relevance of the applied voltages (Supplementary Fig. 13a). This calibration allows voltages applied with a given gate to be converted into thermodynamic values that can be directly associated with the measured I_{DS} .

For a PEDOT:PSS OECT, the transfer curve can be empirically approximated by a quadratic expression:

$$I_{\text{DS}} = B_0 + B_1 \cdot V_{\text{GS},\text{eff}} + B_2 \cdot V_{\text{GS},\text{eff}}^2 \quad \text{Equation S11}$$

Where B_0 , B_1 and B_2 are the fitted coefficients ($-1.46\text{E-}2$ A, $3.84\text{E-}2$ A/V and 2.65 A/V², respectively), and $V_{\text{GS},\text{eff}}$ is the effective gate voltage which depends on the electrochemical properties of the gate.

The effective gate voltage is calculated by accounting for (i) the difference between the gate electrochemical potential and the reference condition of Ag/AgCl pellet (first term) and (ii) the additional shift in effective doping voltage induced by gate polarization (second term), according to:

$$V_{\text{GS},\text{eff}} = V_{\text{GS}} - (E_{\text{G}} - E_{\text{G},\text{ref}}) - (D_0 + D \cdot V_{\text{GS}}) \quad \text{Equation S12}$$

where V_{GS} is the instrumental gate bias applied using the gate electrode under test, E_{G} is the electrochemical potential of the gate, $E_{\text{G},\text{ref}}$ is the electrochemical potential of the reference gate (Ag/AgCl pellet, 50 mV vs Ag/AgCl 3M in 1x PBS), D_0 and D are coefficients from the linear relationship in Supplementary Fig. 11a that links V_{drop} , V_{GS} and gate capacitance.

Finally, the dependence of V_{drop} on gate capacitance is required. As shown in Supplementary Fig. 11c, the linear coefficients D_0 and D vary exponentially with $\log C_G$, following:

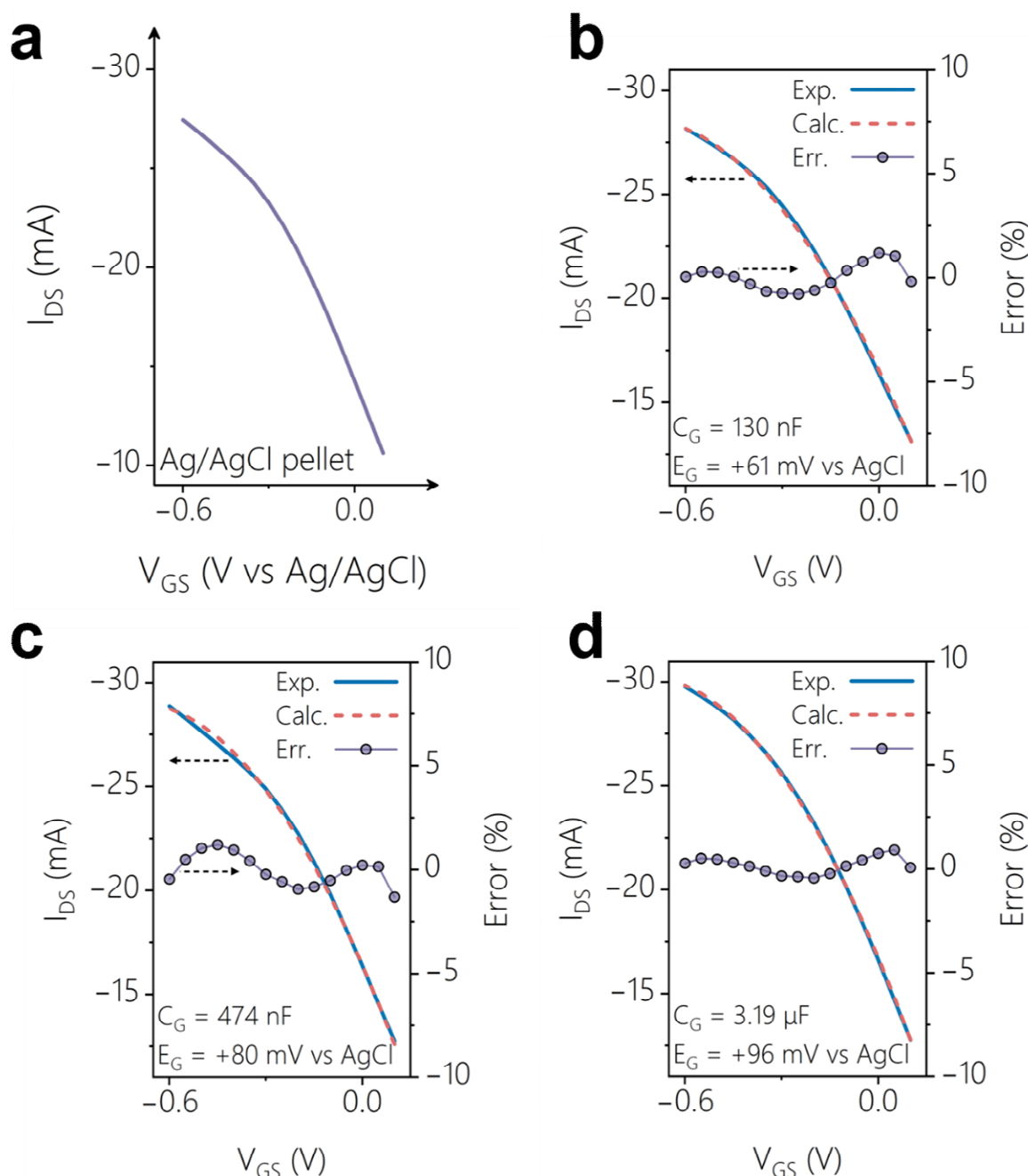
$$D_0 = y_i + A_i \exp - (\log C_G - x_i)/t_i \quad \text{Equation S13}$$

$$D = y_s + A_s \exp - (\log C_G - x_s)/t_s \quad \text{Equation S14}$$

where y_i , A_i , x_i and t_i are the fit parameters describing the intercept D_0 (2.43E-3 V, 9.688E-2 V, -7.2202 and 3.95E-1, respectively), and y_s , A_s , x_s and t_s are the fit parameters describing the slope D (5.38E-3 V, 2.512E-1 V, -7.2170 and 3.44E-1, respectively).

Together, these empirical relationships allow prediction of the expected OECT output for a gate electrode with defined electrochemical properties (E_G and C_G). As an illustrative example, we report three cases of gate electrodes with different properties, within the range of variations induced by target binding, and compare the measured transfer curves with the corresponding calculated curves, showing that the prediction error remains within an acceptable range lower than 5%, (Supplementary Fig. 13b-d).

These equations enable exploration of hypothetical scenarios involving variations in gate electrochemical properties, thereby facilitating sensing optimization, for example, with respect to bias selection for extracting I_{DS} , initial gate size, and the direction of electrochemical property changes. This approach is relevant when the resulting calibration can be transferred to a batch of devices with comparable performance, provided that the channels are identical. Importantly, this calibration is not intended to be performed on every OECT channel used for sensing, as it requires extensive device operation that may induce degradation.



Supplementary Fig. 13. Interpolation of expected output values. **a)** Transfer curve of a PEDOT:PSS OECT using a reference Ag/AgCl pellet gate electrode to estimate the output current (I_{DS}) at each thermodynamic voltage (electrochemical potential, V vs Ag/AgCl). **b-d)** Representative transfer curves measured using Au gate electrodes with different electrochemical potentials and capacitances, compared with the curves generated using Equation S11. The right y-axis reports the percent difference between the experimental and calculated values. The electrochemical potential and capacitance of each gate electrode are indicated in the corresponding panel.

This additional analysis was mentioned in the main text as follows:

The analysis described here required characterization of the OECT response over a broad range of gate capacitances and polarization values, across multiple doping biases. This dataset enabled extraction of an empirical relationship linking C_G , V_{drop} and I_{DS} . Supplementary Discussion 7 details the derivation of this correlation and shows how it can be used to predict the OECT response as a function of gate potential and capacitance.

We thank the Reviewer for pointing out that the conditions leading to maximum constructive interference were not clearly stated; this information has now been added to the main text. The conditions that maximize constructive interference between the contributions arising from changes in gate potential (ΔE_G) and gate capacitance (ΔC_G) are both governed by the same key figure of merit, the transconductance efficiency ($g_{m,\text{eff}}$). In the main section “Transducing gate electrochemical potential changes”, we originally highlighted this concept as follows:

“...we find that the highest NR (NR_{max}) occurs at the most positive V_{GS} ... We found that the critical figure of merit for sensing performance is the transconductance efficiency ($g_{m,\text{eff}}$, Fig.3b-ii) an optimal OECT should present its maximum gm as close as possible to its onset potential.”

While in the main section “Transducing gate capacitance changes”, we originally highlighted this concept as follows:

“The device showed the highest sensitivity to gate capacitance changes when operated at the most positive V_{GS} (Fig. 5a-ii)... not only because this is the voltage that maximized $g_{m,\text{eff}}$, but also because it corresponds to the voltage at which the gates undergone the strongest polarization (Supplementary Fig. 11a). ... The magnitude of ΔV_{drop} can thus be maximized by operating the device at voltages that lead to a high C_{CH} ...”

For a complete analysis, we have also added now the following statement to the main section of “Combined effects of gate potential and capacitance changes on the OECT output”:

Constructive **interaction** occurs in the ($+\Delta E_G; -\Delta C_G$) and ($-\Delta E_G; +\Delta C_G$) scenarios, where both contributions shift the transfer curve in the same direction, amplifying the sensor output. In particular, the most pronounced effect is observed at biases that combine high $g_{m,\text{eff}}$ with high gate polarization ($V_{\text{GS}} = +0.1 \text{ V}$). This demonstrates the value of using smaller gate electrodes to enhance sensitivity to capacitance changes upon target binding.

#13 – All analyses use steady-state transfer curves, but in practical biosensing, signal transduction often occurs dynamically (e.g., impedance or pulsed biasing). The authors should discuss whether their model remains valid under time-varying signals or AC operation — for instance, whether ΔC_{DL} –driven responses are frequency dependent. A small dynamic validation (e.g., chronoamperometric or impedance-tracked response) would extend the model’s relevance.

The majority of label-free, non-faradaic OECT-based sensors reported in the literature rely on steady-state measurements rather than transient approaches, as steady-state protocols are simpler and more user-friendly: they typically require only selection of an operating voltage window and direct readout of the drain current. [<https://doi.org/10.1016/j.bios.2019.111668>,
[10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387), <https://doi.org/10.3389/fbioe.2019.00354>,
<https://doi.org/10.1002/adma.202202972>, [10.1088/2058-8585/aad0cb](https://doi.org/10.1088/2058-8585/aad0cb),

<https://doi.org/10.1002/adma.202515338>, <https://doi.org/10.1002/adma.201102017>,
<https://doi.org/10.1038/s41551-021-00734-9>, <https://doi.org/10.1021/acsami.8b04970>,
<https://doi.org/10.1039/C7RA09832D>, <https://doi.org/10.1021/acsnano.0c09893>] For this reason,
we focused exclusively on steady-state operation in this work.

By contrast, transient methods may be more experimentally demanding, requiring instrumentation capable of high-frequency excitation and fast sampling. In addition, the data analysis is less straightforward, as it involves extracting derived parameters—such as phase shift, impedance, or transconductance in frequency-domain measurements—and time constants from exponential fits in pulsed measurements, typically using dedicated software or custom code. [<https://doi.org/10.1016/j.sna.2018.12.032>, <https://doi.org/10.1002/adhm.201300632>] These approaches are comparatively specialized and are most commonly applied to more niche purposes such as probing barrier tissue integrity of cell membranes or lipid bilayers.

Electrode polarization, which depends on the electrical double-layer capacitance (C_{DL}), arises from ion accumulation at the electrode–electrolyte interface. [<https://doi.org/10.1021/acsaelm.5c01861>]

Previous studies have shown that high-frequency operation can modify this behavior—for example, by reducing Debye screening—which has been exploited in electrolyte-gated transistor sensing. [<https://doi.org/10.1021/nl203666a>] Therefore, transient sensing conditions (e.g., high-frequency excitation and small V_{GS} amplitudes) are expected to affect the OECT output by altering the contribution of gate polarization. However, given the limited use of these methods in label-free, non-faradaic OECT sensing, a systematic investigation of such effects is beyond the scope of this work.

Nevertheless, we agree that clarifying the applicability of our approach is valuable. Accordingly, we add this clarification:

Here, we address these questions by systematically deconstructing the individual contributions of gate potential and capacitance changes to the OECT output in non-faradaic sensing configuration. Specifically, we focused on transfer-curve based evaluation of sensor output, as it is the most widely used technique.

#15 – Could the authors please clarify their statement: “Unlike traditional electrochemical two- or three-electrode setups, OECTs operate without a reference electrode, facilitating miniaturization and improving biocompatibility.” (lines 40 and 41)? First, in many OECT configurations, the gate electrode actually acts as a pseudo-reference electrode, although a separate reference electrode can be used in some studies for precise potential control.

Second, could the authors please explain in more detail how the absence of a reference electrode directly contributes to improved biocompatibility? This connection is not immediately clear, and ref. [15] is not sufficiently useful to support this claim.

We thank the Reviewer for raising this point, as citing only Ref. 15 may not be sufficient to substantiate the limitations of conventional reference electrodes in biological environments and their role in OECTs.

Common reference electrodes include:

- Standard hydrogen electrode (SHE): $2H^+ + 2e^- \rightleftharpoons H_2$
- Silver chloride electrode: $AgCl + e^- \rightleftharpoons Ag + Cl^-$
- Calomel electrode (SCE): $Hg_2Cl_2 + 2e^- \rightleftharpoons 2Hg + 2Cl^-$
- Mercury sulfate electrode: $Hg_2SO_4 + 2e^- \rightleftharpoons 2Hg + SO_4^{2-}$
- Mercury oxide electrode: $HgO + 2e^- + 2H^+ \rightleftharpoons Hg + H_2O$

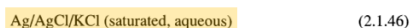
- Copper sulfate electrode: $\text{Cu}_2^+ + 2\text{e}^- \rightleftharpoons \text{Cu}$

Reference 15 is *Electrochemical Methods: Fundamentals and Applications* by Bard and Faulkner, a standard textbook widely used in undergraduate electrochemistry courses and beyond, which can help the reader understanding the role and importance of reference electrodes. The text describes multiple reference electrode designs and provides representative examples (see Fig. R2). As indicated by the list above and discussed in Ref. 15, most conventional reference electrodes rely on intrinsically toxic materials, which limits their biocompatibility. The main exception is the SHE; however, it is impractical for most experimental settings, as also noted in Ref. 15. In addition, leakage of the internal filling solution into the sample can occur, which is particularly problematic for biological measurements. To support this point, we added studies documenting electrolyte leakage from reference electrodes to the manuscript (<https://doi.org/10.1021/acs.analchem.9b00876>, <https://doi.org/10.2116/analsci.19P235>).

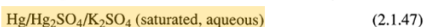
2.1.7 Reference Electrodes

Many reference electrodes other than the NHE and the SCE have been devised for electrochemical studies in aqueous and nonaqueous solvents. Several authors have provided discussions on the subject (16–18).

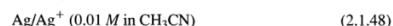
Usually there are experimental reasons for the choice of a reference electrode. For example, the system



has a smaller temperature coefficient of potential than an SCE and can be built more compactly. When chloride is not acceptable, the mercurous sulfate electrode may be used:



With a nonaqueous solvent, one may be concerned with the leakage of water from an aqueous reference electrode; hence a system like



might be preferred.

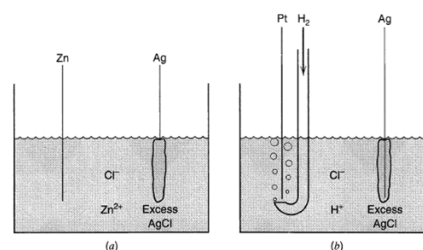
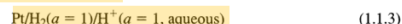


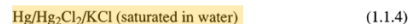
Figure 1.1.1 Typical electrochemical cells. (a) Zn metal and Ag wire covered with AgCl immersed in a ZnCl₂ solution. (b) Pt wire in a stream of H₂ and Ag wire covered with AgCl in HCl solution.

responds to the interfacial potential difference at the corresponding electrode. Most of the time, one is interested in only one of these reactions, and the electrode at which it occurs is called the *working* (or *indicator*) *electrode*. To focus on it, one standardizes the other half of the cell by using an electrode (called a *reference electrode*) made up of phases having essentially constant composition.

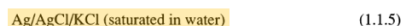
The internationally accepted primary reference is the *standard hydrogen electrode* (SHE), or *normal hydrogen electrode* (NHE), which has all components at unit activity:



Potentials are often measured and quoted with respect to reference electrodes other than the NHE, which is not very convenient from an experimental standpoint. A common reference is the *saturated calomel electrode* (SCE), which is



Its potential is 0.242 V vs. NHE. Another is the *silver–silver chloride electrode*,



with a potential of 0.197 V vs. NHE. It is common to see potentials identified in the literature as “vs. Ag/AgCl” when this electrode is used.

Figure R2. *Electrochemical Methods: Fundamentals and Applications* by Bard and Faulkner. Examples of reference electrodes-related content inside the book.

Conventional reference electrodes are routinely required in electrochemical sensing techniques such as chronoamperometry, cyclic voltammetry, differential pulse voltammetry, square-wave voltammetry, and electrochemical impedance spectroscopy. They are essential either to bias the working electrode at a defined electrochemical potential to drive a faradaic reaction linked (directly or indirectly) to analyte concentration, or to provide a stable reference against which the working-electrode potential, directly linked to the target concentration, can be measured. For example, in potentiometric sensing, a working (sensing) electrode is paired with a reference electrode to read its potential.

An analogous sensing principle can be implemented using OECTs. However, in this case, a conventional reference electrode is not strictly required: the working electrode can instead serve

as the OECT gate, and information about its potential is transduced into a specific doping state of the organic semiconductor and thus into the drain current, I_{DS} . Consequently, changes in gate potential directly modulate the semiconductor doping level and produce corresponding changes in I_{DS} , enabling potentiometric readout without a conventional reference electrode. This concept is illustrated in prior reports, which we have added to the manuscript (<https://doi.org/10.3389/fbioe.2019.00354>, <https://doi.org/10.1021/acsami.8b04970>).

A notable example of an OECT sensor that incorporates a reference electrode to improve sensing performance is the study in <https://doi.org/10.1038/s41467-023-37402-2>, which employs an Ag/AgCl-based electrode; this choice limits the device's biocompatibility.

The above literature has been cited in the main text as follows:

Unlike traditional electrochemical two- or three-electrode setups, OECTs operate without a reference electrode, <https://doi.org/10.3389/fbioe.2019.00354>, <https://doi.org/10.1021/acsami.8b04970> facilitating miniaturization and improving biocompatibility. [Electrochemical Methods, Allen Bard, <https://doi.org/10.1021/acs.analchem.9b00876>, <https://doi.org/10.2116/analsci.19P235>]

#16 – The terms "constructive and destructive interference" are typically used in wave physics, where they refer to superposition of oscillatory quantities with defined phase. That isn't what occurs in the reviewed article, and using this terminology may mislead readers. Therefore, I would recommend using different terminology to describe the effects in OECT output as associated with combined changes in gate properties. For example, noting that the two terms can act in the same direction or oppose each other. Alternatively, if the authors wish to keep the analogy, they must provide a clear, explicit explanation to justify it.

We thank the Reviewer for highlighting this point, which could potentially confuse readers. Accordingly, we replaced the term "interference" with "interaction" throughout the manuscript.

#17 – Please be more precise in the reference selection in the Introduction. For example, in lines 49 and 50, the authors first in the text discuss the features of the non-Faradaic mode: "When target binding at the gate electrode occurs without an accompanying electrochemical reaction, the sensor operates in a non-faradaic mode.20–23". However, the term "non-Faradaic" is not mentioned at all in Refs. [20–23], and readers who follow these links hoping for more detailed explanations may become confused.

We have now added in the list two studies that state the concept of non-faradaic sensing explicitly in the context of conventional electrochemical sensing:

<https://doi.org/10.1002/anse.202400037>: "An alternative strategy to overcome these drawbacks is the use of non-Faradaic impedance spectra, which are acquired in the absence of added redox-active markers."

<https://doi.org/10.26434/chemrxiv-2025-8cflf>: "...this is the first demonstration of a non-faradaic voltammetric and impedimetric F^- sensor..."

We then added a more theory-focused reference that explicitly distinguishes faradaic and non-faradaic processes at the gate electrode in the context of channel doping modulation:

<https://doi.org/10.1063/1.2190077>: "Two regimes of operation are identified. The first involves a conventional redox process where two Faradaic processes balance: oxidative doping of the

polymer to modulate conductivity is coupled to reduction at the gate electrode. The second involves a nonconventional hybrid mode of operation: doping is coupled to the charging of an ionic double layer at the gate electrode, which is a non-Faradaic process.”

We also retained the previously cited references, as they describe OECT-based sensing studies that operate in a non-faradaic regime even if this is not stated explicitly. We hope that the brief definition already included in the manuscript—“When target binding at the gate electrode occurs without an accompanying electrochemical reaction, the sensor operates in a non-faradaic mode.”—will further clarify these principles for the reader.

#18 – In the figures, for greater clarity and uniformity, I would recommend using the same colors (or some other method of highlighting) for all graphs to indicate the "blank" and "target" modes.

We tried to maintain a consistent color scheme, with red indicating the blank condition and a blue gradient indicating the target conditions. Because this convention is used from Fig. 2 onward, we consider the color coding in Fig. 1 acceptable; moreover, to avoid confusion, each plot of Fig. 1 includes a legend defining the corresponding transfer curves. At present, only Fig. 6c deviates from this scheme, as the blank is not shown in red and the target is not shown in blue; however, panels (i) and (ii) include internal legends. For panel (iii), where space constraints prevented inclusion of a legend, we clarified the meaning of each transfer curve in the caption as follows:

c) OECT response to positive (+) and negative (-) variations in gate potential **(i)** and positive and negative variations in gate capacitance caused by target binding **(ii)**. OECT response to simultaneous potential and capacitance variations at the gate electrode, illustrating the four possible combinations of signal **interaction** (constructive or destructive); **the dashed line denotes the blank condition, whereas solid lines denote the target condition (iii).**

#22 – Judging by the OECT schematic diagrams, the source and drain electrodes were not insulated from the electrolyte. How could parasitic leakage currents between the source and gate, and between the source and drain, affect the experimental results and their interpretation? How might they affect the sensor's sensitivity?

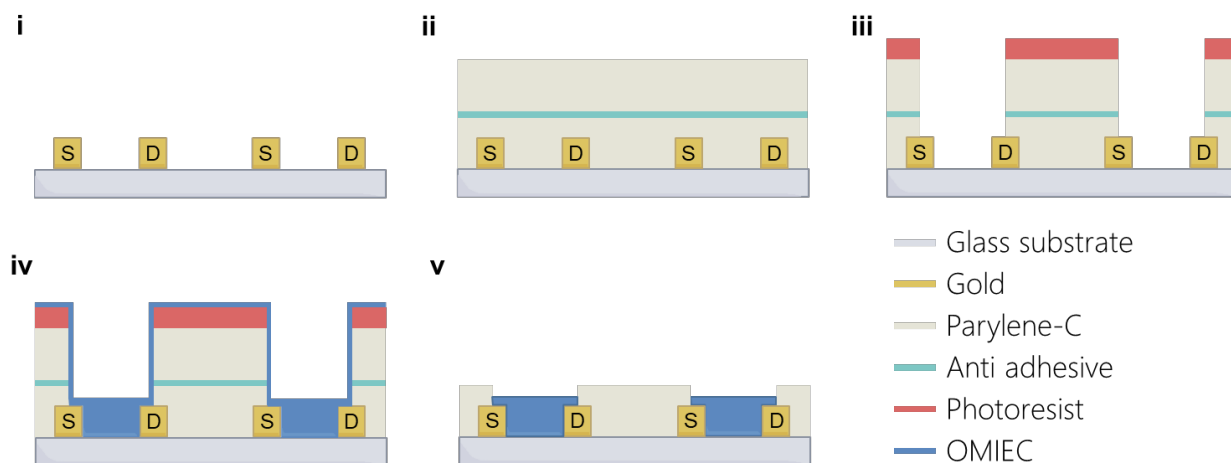
The OECT schematics are intended purely for illustrative purposes and therefore do not depict all device components (e.g., the insulating layer), the OMIEC–Au overlap, or the true relative dimensions of the features. We agree that omitting the insulating layer in the schematic could cause confusion, particularly because the Methods section previously cited prior literature without explicitly describing the fabrication protocol. Accordingly, we detailed OECT channel fabrication section to include a description of the full process.

The Methods section was revised as follows:

OECT Channel Fabrication

...Briefly, glass wafers were cleaned in piranha solution, after which a first photoresist layer was spin-coated and UV-exposed using a contact aligner to define the source and drain electrode patterns. Cr (10 nm) and Au (100 nm) were then sputtered, and a standard lift-off process was performed to remove excess metal, leaving the patterned contacts. A first parylene layer was deposited as an insulating coating to prevent parasitic effects arising from ion accumulation

(capacitive coupling) and undesired electrochemical reactions (faradaic processes) at the metal-electrolyte interface. An anti-adhesive layer was subsequently spin-coated, followed by deposition of a second parylene-C layer acting as a sacrificial film. A second photolithography step was used to define the contact-pad areas via photoresist deposition, UV patterning, and plasma etching.



Supplementary Figure 23. OECT fabrication protocol. Schematic of the fabrication workflow of two OECT channels, including source (S) and drain (D) patterning (i); deposition of two Parylene-C insulating layers separated by an anti-adhesive layer (ii); patterning of the insulating stack and definition of the channel area via photoresist lithography followed by plasma etching (iii); OMIEC spin-coating (iv); and peel-off of the sacrificial layer (v).

#23 – Could the authors clarify what exactly is meant by "deformations" (line 449)?

A change in gate electrochemical potential (ΔE_G) shifts the entire I_{DS} - V_{GS} transfer curve horizontally by an amount equal to ΔE_G (see Fig. 2b). In contrast, gate polarization (V_{drop})—and thus its sensing-induced change (ΔV_{drop})—depends on the gate-to-channel capacitance ratio C_G/C_{CH} , which varies with V_{GS} because the OMIEC capacitance changes with doping (see Supplementary Fig. 12a). Therefore, unlike ΔE_G , V_{drop} is not constant but depends on V_{GS} (see Supplementary Fig. 11a). As a result, the I_{DS} values are shifted by a *non-uniform amount* along the V_{GS} axis, causing an apparent deformation of the transfer curve.

We thank the Reviewer for noticing this typo. We replaced “deformed shift” with “non-uniform shift” to align the terminology with that used in the preceding sections. In addition, for clarity to readers with limited electrochemistry background, we added a supplementary note explaining how analogous effects arise in conventional two-electrode electrochemical configurations that do not involve OECTs, as also requested by Reviewer 2; [see response to comment #30 from Reviewer 2](#).

Reviewer 2

The manuscript authored by Inal and co-workers provides a rigorous and much-needed mechanistic framework to dissect and quantify non-faradaic transduction pathways in OECT-based biosensors. By separating and characterizing the contributions from variations in the electrochemical potential of the gate (ΔE_G) and in the interfacial gate capacitance (ΔC_G), the authors present a unifying model that clarifies several inconsistencies in the current literature. The introduction of EP–IV mapping, the explicit bias design rules, and the adoption of the effective transconductance ($g_{m,eff}$) as a figure of merit make this work particularly relevant for advancing standardization in OECT biosensing. Overall, this is a conceptually strong and potentially field-defining study. I support the publication of this work. My detailed comments are outlined below.

We thank the Reviewer for appreciating our work and the comments.

#25 – The analysis on p-type (PEDOT:PSS, p(g₃C₂T₂-T)) and n-type (p(C₆NDI-T)) channels highlights the impact of doping state and $C_{CH}(V_{GS})$ profile. To confirm generality, please add (i) $C_{CH}(V)$ maps for all OMIECs tested

The capacitance–doping potential trends for the two mentioned OMIECs are reported for the Reviewer in Fig. R3. Please see response to [comment #31 from Reviewer 4](#) for more details.

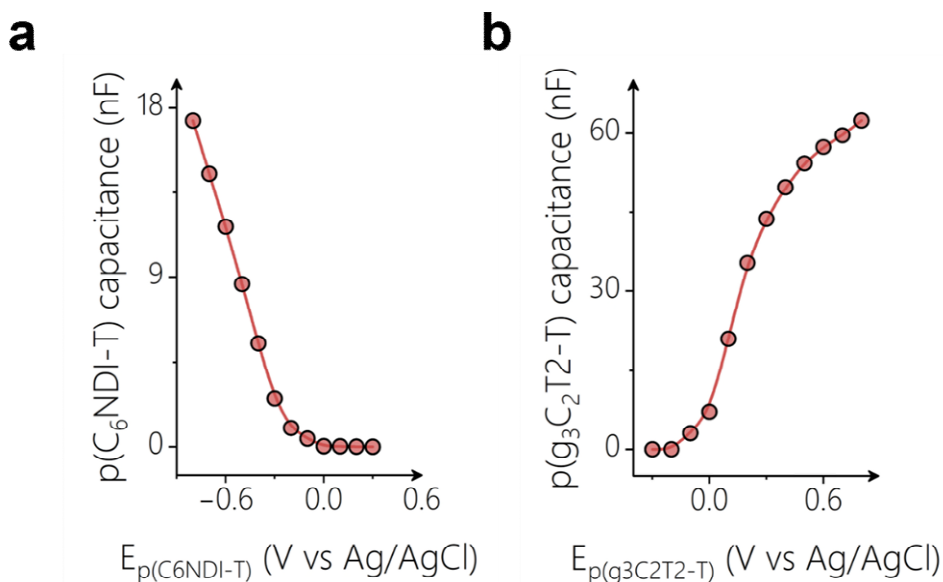
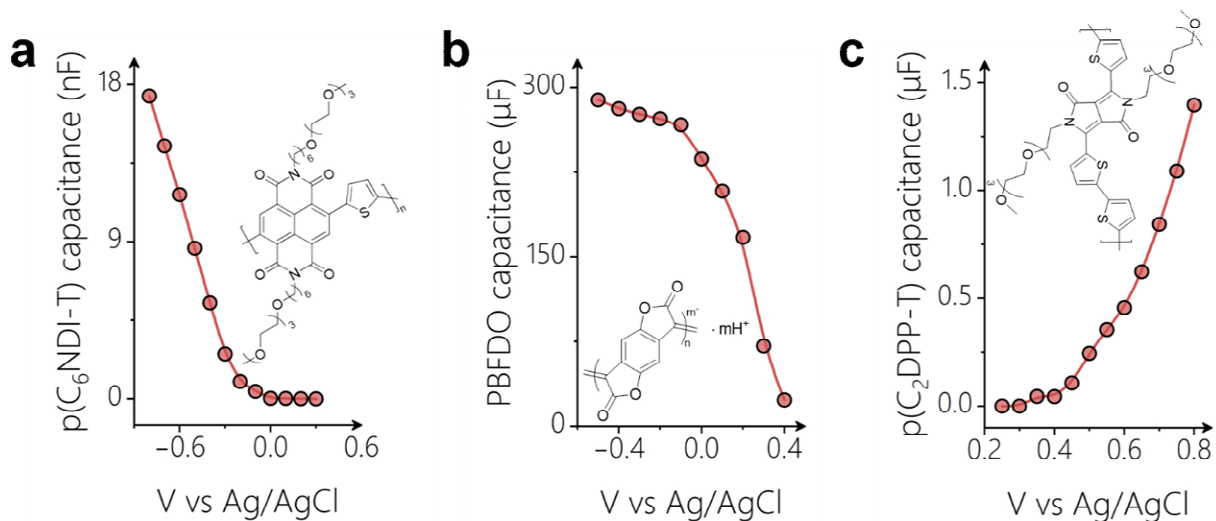


Figure R3. Capacitance of (a) p(C₆NDI-T) and (b) p(g₃C₂T₂-T) measured at different electrochemical potentials.

However, in light of Reviewer 1 comments #8 and #10 requesting more detailed protein-based experiments, the original version of Fig. 7 became redundant, as it also included two protein-based examples in addition to a preliminary OMIEC screening. To avoid this redundancy, we removed the original protein experiments associated with those OMIECs and refocused Fig. 7 on a more comprehensive OMIEC screening and on the effect of channel size, as also requested by Reviewer 4 in comment #32. Consequently, the measurements on p(g₃C₂T₂-T) were considered redundant (given the analogous behavior already captured by PEDOT:PSS) and were removed.

The capacitance–doping potential trends for the additional materials included in the revised Fig. 7 have now been added in Supplementary Fig. 18.



Supplementary Figure 18. OMIEC capacitance as function of doping potential. Capacitance of (a) p(C₆NDI-T), (b) PBFDO, and (c) p(C₂DPP-T) measured at different electrochemical potentials.

Please see response to comment #31 and #32 from Reviewer 4 for more details about this revised section.

#26 – and (ii) at least one test under different ionic strengths to assess how Debye length modulates ΔE_G and ΔC_G visibility.

We thank the Reviewer for highlighting this issue. As such effect lays under the category of parasitic effects that can compromise reliable non-faradaic OECT sensing and limit application of the methodology proposed here to maximize sensing performance, we now covered it within the discussion reported in the new section of the manuscript, similarly to temperature and other drift related issues requested by Reviewer 1.

To the best of our knowledge, other parasitic effects such as OMIEC degradation leading to analogous OECT responses as sensing calibration, (Fig.8a), identification of stable operational biases, (Fig.8b), evaluation of the impact of OMIEC degradation on the sensing performance based on the sensing protocol, (Fig.8c-i and Supplementary Fig. 22), development of a reliability-assessment protocol to monitor OMIEC degradation during sensing and the quality of the acquired data (Supplementary Figs. 20 and 21), intrinsic measurement noise compared to the sensing input signals and not only output, (Fig.3d, 5c, 6a-ii and Supplementary Fig.9), relevance of OECT fabrication and performance reproducibility with respect to operational conditions and sensing relevant parameters, (Fig.8c-iii,iv), background drift leading to analogous OECT responses as sensing calibration (Fig.8d), have not been sufficiently reported in the current literature, and for this reason we performed an additional set of experiments reported in Figure 8 for briefly illustrating them. On the other hand, Debye length related issues and their impact on variation of sensing electrode potential and capacitance have been substantially covered in the description of

their source mechanism and impact on electrolyte gated transistors, for example as in [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387), and <https://doi.org/10.1021/ja205684a>. In the first case (Fig. 3 of the cited reference), a progressive increase of the V_{GS} shift of the transfer curve was accurately correlated to the increase of Debye length which led to the inclusion of larger portions of the target within the Debye layer, the more the electrolyte was diluted, and an higher impact on the electrochemical properties that function as input in the OECT sensing. Similarly, in the second study, although performed with chronoamperometry, an analogous effect is observed, with increased impact on I_{DS} as the electrolyte is diluted to include larger portions of the protein layer within the Debye length.

Moreover, this issue has not only been extensively described, but also solutions for addressing it have been proposed such as in [10.1126/sciadv.abg8387](https://doi.org/10.1126/sciadv.abg8387), operating the device in diluted electrolyte to improve the sensitivity and limit of detection, or in <https://doi.org/10.1021/nl203666a>, with the use of high-frequency gate stimulations for preventing the formation of the screening layer, and more options as described in this brief review, <https://pubs.acs.org/doi/10.1021/acsnano.0c08622>. For these reasons, we considered it more appropriate to discuss this limitation by referring to studies in which it is treated in greater detail as a central focus. Accordingly, we added a brief discussion of this topic in the newly introduced section and cited the relevant literature.

Accordingly, the main text was modified as follows:

Considerations for non-Faradaic signal transduction using OECTs

... Finally, successful signal transduction requires that target binding produces a measurable change in gate electrochemical properties (ΔE_G and/or ΔC_G). In biological environments, this process is often limited by electrostatic screening governed by the Debye length.[\[https://doi.org/10.1021/acsnano.0c08622\]](https://doi.org/10.1021/acsnano.0c08622) If the Debye length is shorter than the distance from the electrode surface at which the target binds, changes in the gate electrochemical properties of interest may be effectively screened and thus become negligible. Strategies to overcome this limitation include reducing ionic strength,[\[https://doi.org/10.1021/acsnano.0c08622\]](https://doi.org/10.1021/acsnano.0c08622), <https://doi.org/10.1021/ja205684a> using high-frequency modulation of the V_{GS} to disrupt the formation of screening layers,[\[https://doi.org/10.1021/nl203666a\]](https://doi.org/10.1021/nl203666a) and engineering surface and recognition-unit chemistries that position recognition events within the electrochemically visible region.[\[https://doi.org/10.1073/pnas.1315485111\]](https://doi.org/10.1073/pnas.1315485111), <https://doi.org/10.1021/acs.nanolett.5b00133>, [10.1126/science.aao6750](https://doi.org/10.1126/science.aao6750), [https://doi.org/10.1002/adfm.202527955\]](https://doi.org/10.1002/adfm.202527955) ... Together, these considerations define the practical boundaries within which the proposed non-Faradaic transduction framework can be reliably applied. Control of operating conditions, device stability, and interfacial processes is essential to ensure that the measured OECT response reflects true sensing events rather than artifacts arising from degradation or drift.

#27 – To support broader adoption of your results, please (i) normalize $g_{m,eff,max}$ by channel area/volume for cross-platform comparison

The area-normalized values of $g_{m,eff,max}$ have now been added to the original Discussion and to the newly introduced section, “Considerations for non-Faradaic signal transduction using OECTs”. Note that the two reported values differ because they were obtained from two different batches of OECT devices and from different bottles of PEDOT:PSS (Clevios PH1000).

Accordingly, the main text was revised as follows:

The $g_{m,eff}$ is calculated by dividing the derivative of the transistor output signal by the signal itself.^{47,48} The OECTs used in this experiment had a maximum $g_{m,eff}$ of -4.77 V^{-1} ($-4.77 \cdot 10^{-3}\text{ V}^{-1} \cdot \mu\text{m}^{-2}$, when normalized by the channel area).

and

We show that PEDOT:PSS OECT channels fabricated on the same and 4 other substrates exhibit low variability in key parameters such as $g_{m,eff,max}$ (Fig. 8c-iii, $-3.3 \pm 3\% \text{ V}^{-1}$, or $-3.3 \cdot 10^{-3} \text{ V}^{-1} \cdot \mu\text{m}^{-2}$ when normalized by the channel area), enabling reliable channel-to-channel substitution.

#28 – (ii) provide a summary table comparing I_{DS} -, g_m -, and ΔV_{GS} -based sensing metrics under realistic noise and drift

We thank the Reviewer for highlighting issues related to noise and drift, which prompted us to explicitly discuss additional OECT-related limitations that may hinder application of the methodology proposed in this work and constrain sensing-performance optimization. Accordingly, we added a new section entitled “Considerations for non-Faradaic signal transduction using OECTs”, in which drift is addressed, and we expanded and refined the discussion of intrinsic measurement noise.

The drift issue is addressed in the newly introduced section as follows:

Considerations for non-Faradaic signal transduction using OECTs

...

Another common source of error is the drift of the input signal. The electrochemical potential and capacitance of an electrode can drift over time due to spontaneous parasitic reactions (e.g., O_2 reduction, water oxidation)^{[<https://doi.org/10.1021/acs.analchem.7b01856>, <https://doi.org/10.1016/j.biosx.2024.100509>] or instability of the biofunctionalization layer.^[<https://doi.org/10.1021/acs.analchem.2c05398>] Such drift is difficult to distinguish from true sensing signals. Even when negligible over the timescale of a single data-point acquisition, cumulative drift over the course of a measurement protocol can lead to false results.^[<https://doi.org/10.1021/acs.analchem.7b01856>] To demonstrate this effect, we simulated a calibration protocol in which each hypothetical target concentration was measured by acquiring several consecutive transfer curves, followed by a resting period during which the device was not operated to mimic the incubation time required between successive concentrations (Fig. 8d). To isolate the contribution of input-signal drift, we kept the gate electrode material and size constant, using either a low-polarizable Au electrode or a PEDOT:PSS electrode, and we did not expose the gate to target bearing solutions. The two gate materials were selected to highlight differences in surface reactivity toward parasitic reactions. In both cases, executing the sensing protocol produced an apparent OECT response resembling a conventional calibration curve, despite any sensing event (Fig. 8d-i). When we simultaneously monitored the gate electrode potential, we observed an analogous trend, confirming a substantial drift of the input signal (Fig. 8d-ii). The transduction mechanism underlying this artifact is illustrated in Fig. 8d-iii. As the gate potential drifts from its initial value (e.g., Blank) to a later value (e.g., C_6), the effective gate bias shifts by an equivalent amount (step a). This change in effective gate bias alters the channel doping state and thus the measured I_{DS} (step b), generating an apparent “sensing” signal. Mitigation strategies include the use of chemically stable gate materials, surface passivation (e.g., self-assembled}

monolayers),[<https://doi.org/10.1021/acsaelm.1c01171>,
<https://doi.org/10.1021/acs.analchem.7b01856>] and differential measurement schemes employing reference channels to subtract drift contributions.[<https://doi.org/10.1038/s41928-025-01465-4>,
<https://doi.org/10.3390/molecules29071459>, <https://doi.org/10.1016/j.bios.2022.114691>] The latter approach can also compensate for artifacts induced by variations in temperature and mechanical strain.

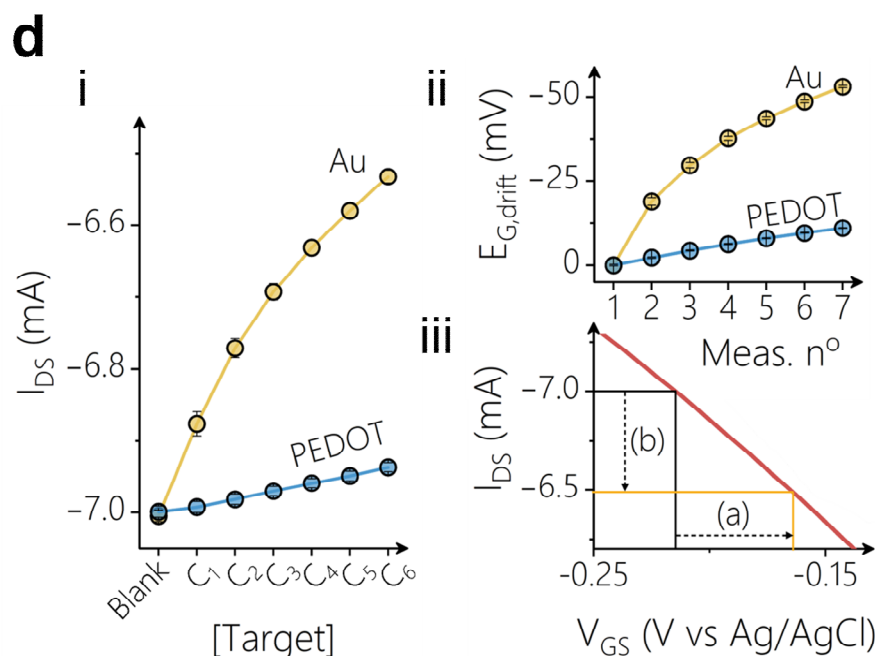


Figure 8. Additional considerations for the interpretation of OECT sensor output. ... d) Gate-drift-induced OECT responses that mimic a sensing signal. **(i)** Apparent calibration curves obtained without target binding for different gate materials. **(ii)** Corresponding evolution of the gate electrochemical potential **(iii)** Schematic of the underlying artifact: (a) drift in gate potential shifts the effective gate bias, and (b) alters the measured I_{DS} .

As for the intrinsic measurement noise, please see response to [comment #3 and #9 from Reviewer 1](#) for details.

We thank the Reviewer for drawing attention to the use of g_m as an analytical signal, which allowed us to clarify its role across different sensing scenarios. In our view, using g_m as the sensing metric, rather than I_{DS} or ΔV_{GS} , is generally discouraged. Whereas I_{DS} or V_{GS} are directly measured during the sensing experiment, g_m is calculated post hoc from the acquired I_{DS} - V_{GS} data. Given the intrinsic instrumental uncertainty in I_{DS} measurements and device-to-device variability in the output signal (see Supplementary Fig. 9 and Fig. 8c-iii), deriving an additional parameter from I_{DS} inevitably propagates and can amplify the error associated with the value. By contrast, g_m can be particularly useful in faradaic OECT sensing, where redox activity from the target and interfering species can modify the local slope of the I_{DS} - V_{GS} response. In this context, analyzing g_m can enhance discrimination and resolution of overlapping signals. [<https://doi.org/10.1038/srep35419>,
<https://doi.org/10.1016/j.snb.2023.134313>]

We incorporated this consideration into the main text as follows:

The importance of signal efficiency is a general principle that applies to any parameter used as analytical signal. For example, as shown in Supplementary Fig. 8, the same effect occurs if g_m is used as output value, occasionally employed as sensing signal.[<https://doi.org/10.1021/acsami.8b04970>, <https://doi.org/10.1021/acsami.3c12397>] The g_m calculation, however, involves differentiation of I_{DS} , which amplifies the noise propagation. Moreover, g_m is relevant as an analytical output in faradaic sensing, where it can improve resolution of multiple redox signals.[<https://doi.org/10.1016/j.snb.2023.134313>, <https://doi.org/10.1038/srep35419>]

#29 – Add a brief discussion to broad the applicability of the proposed approach including OECTs integrated with additive technologies, (see for example: 10.1002/advs.202508872,10.1002/sml.202410499), which open opportunities for additive manufactured biosensors.

We incorporated this discussion into the main text as follows:

In the absence of such understanding, OECT biosensor design remains empirical, hindering the rational development of a high-performance, reliable platform for broad adaptation. Coupling this understanding with cost-effective OECT fabrication methods, such as additive manufacturing, .[<https://doi.org/10.1002/advs.202508872>, <https://doi.org/10.1002/sml.202410499>, <https://doi.org/10.1038/s41528-020-0078-9>] can lay the foundation for scalable development of high-performance electronics platforms capable of reliable, ultrasensitive biomarker detection.

#30 – Consider adding a short box in the main text explaining the distinction between “electrochemical potential” and “voltage” for clarity to non-electrochemical readers

Given the length of the main text—particularly after adding the new section, “Considerations for non-Faradaic signal transduction using OECTs”—we chose to include this discussion in the Supplementary Information as Supplementary Discussion 5.

Supplementary Discussion 5: Distinguishing applied voltage from electrochemical potential

Supplementary Fig. 10a shows the EP-IV characterization obtained during a linear voltage scan of an Au electrode in a solution containing the redox probe $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$. The measurement was performed using the setup illustrated in Supplementary Fig. 10a-i, analogous to Supplementary Fig. 4, except that a two-electrode configuration was used. The experiment was repeated using two non-polarizable electrodes of different materials connected to the (–) lead (denoted (–)₁ and (–)₂), while the electrode connected to the (+) lead was kept constant.

During the voltage scan, the $\text{Ru(II)} \rightarrow \text{Ru(III)}$ oxidation occurs in both cases, but at different applied voltages (Supplementary Fig. 10a-ii), separated by 31 mV (ΔV_p). When the curve obtained with (–)₂ is shifted horizontally by ΔV_p , it overlaps with the (–)₁ curve ((–)₂ + ΔV_p , dashed line), analogous to the behavior observed in OECT transfer curves (Fig. 2b-i).

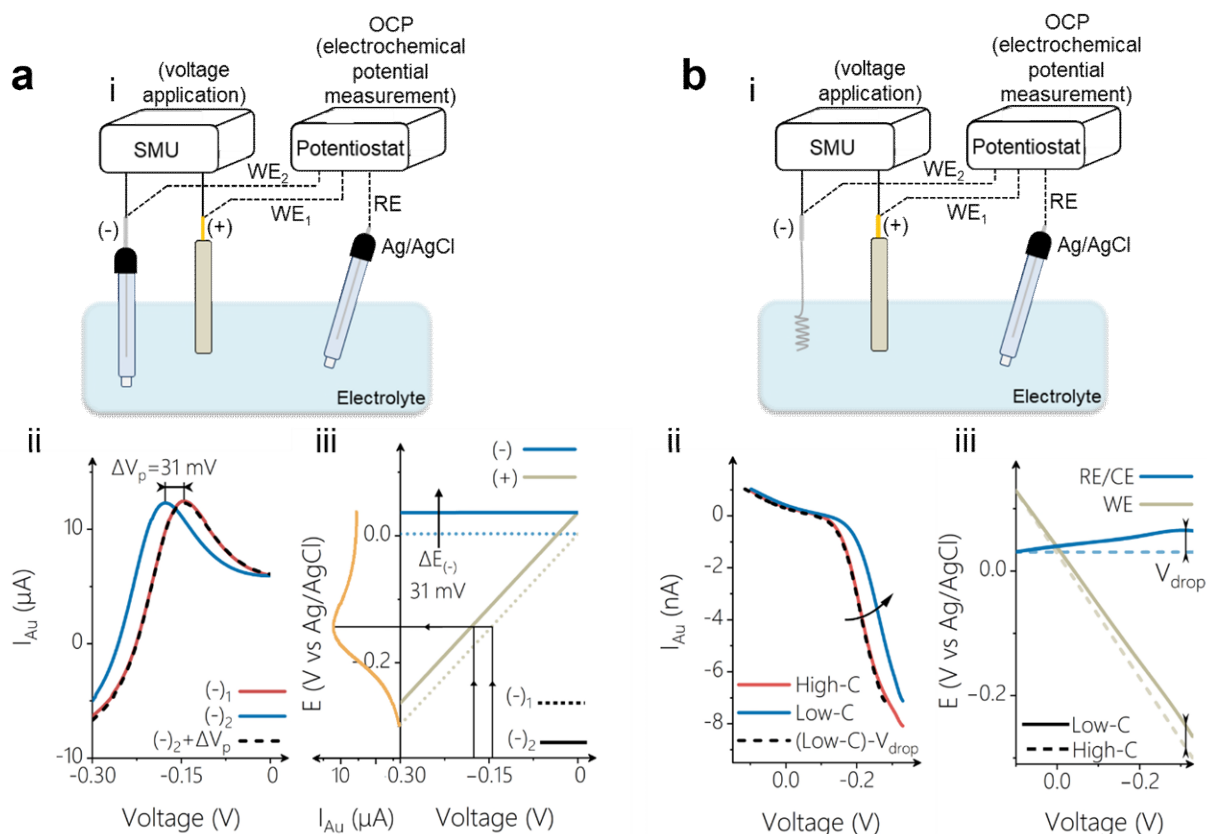
The origin of this effect becomes clear when the electrochemical potentials of the (+) and (–) electrodes are simultaneously measured versus a reference electrode (Ag/AgCl in 3 M NaCl) during the voltage scan (Supplementary Fig. 10a-iii). The potentials of the two (–) electrodes differ by a constant offset of 31 mV ($\Delta E_{(-)}$), matching the horizontal shift observed in the I_{Au} trace. Likewise, the (+) potential traces are vertically offset by the same amount, consistent with the behavior observed for the OECT (Fig. 2b-ii).

This analysis highlights the fundamental distinction between applied voltage and electrochemical potential. Drawing two vertical lines at the voltages corresponding to the Ru oxidation peak for the two (–) electrodes shows that both intersect the (+) electrochemical-potential trace at the same value (Supplementary Fig. 10a-iii). Thus, the voltage applied by the SMU represent only the electrical potential difference between two arbitrary points—here, the (+) and (–) electrodes—and, by itself, has no thermodynamic meaning. In contrast, the electrochemical potential has thermodynamic significance: in both (–) cases, the Ru oxidation peak occurs at the same electrochemical potential. Specifically, the electrochemical potential measured by the potentiostat corresponds to the potential difference between the electrode under investigation and a reference electrode whose potential is stable, well defined, and governed by the Nernst equation. As a result, the Ru oxidation peak may appear at different applied voltages depending on the specific two-electrode configuration, but it is thermodynamically associated with a unique electrochemical potential.

An analogous concept applies to OECT operation: while a given I_{DS} can be obtained at different applied V_{GS} , depending on gate properties (Fig. 3a-i), the same I_{DS} corresponds to a specific and unique OMIEC doping state.

A similar analogy explains the non-uniform transfer-curve shift observed upon variation of gate capacitance. In this case, an analogous experimental setup was employed, with the (+) electrode fixed and the capacitance of the (–) electrode varied (Supplementary Fig. 10b-i). Specifically, the (–) electrode capacitance decreased from a High-C condition (low-polarizability electrode) to a Low-C condition (high-polarizability electrode) by reducing the electrode area (Supplementary Fig. 10b-ii). Decreasing the (–) electrode capacitance produced a pronounced but non-uniform shift in the I_{Au} trace; consistent with the behavior observed in OECT transfer curves (Fig. 4b). Simultaneous measurements of electrode electrochemical potentials reveal that, under low-capacitance conditions, the (–) electrode no longer maintains a constant potential but instead exhibits a bias-dependent deviation due to polarization (Supplementary Fig. 10b-iii). This effect propagates to the (+) electrode, such that at a given applied voltage, different electrochemical potentials are reached, mirroring the behavior observed in OECTs (Fig. 4c).

The polarization of the low-capacitance (–) electrode can be quantified at each applied bias as V_{drop} . When the I_{Au} trace measured under Low-C conditions is corrected by subtracting the corresponding V_{drop} at each voltage, it collapses onto the High-C curve, demonstrating that the same electrochemical potential, or redox state, was reached despite different applied voltages.



Supplementary Figure 10. Distinguishing applied voltage from electrochemical potential. a) Linear voltage scan coupled with electrochemical potential measurements in a two-electrode configuration. The (-) electrodes used had different potentials while the (+) electrode was the same in all experiments. Schematic of the experimental setup (**i**), current recorded as a function of the applied voltage (**ii**), and electrochemical potentials of the (+) and (-) electrodes during the scan (**iii**). **b)** The same measurement, this time using (-) electrodes of different capacitances. Schematic of the experimental setup (**i**), current recorded as a function of the applied voltage (**ii**), and electrochemical potentials of the (+) and (-) electrodes during the scan (**iii**).

This discussion is referenced in the main text as follows:

This effect, and the analogous ΔE_G induced transfer-curve shift, are discussed in Supplementary Discussion 5 using a conventional two-electrode configuration, highlighting the parallels with the OECT output and clarifying the key distinction between voltage and electrochemical potential.

Reviewer 4

Minor revision. This manuscript presents a thorough and mechanistic investigation into non-Faradaic signal transduction in Organic Electrochemical Transistor (OECT)-based biosensors. The authors successfully deconvolute the individual and combined contributions of gate electrochemical potential and capacitance changes to the OECT output. The establishment of a rational sensing model and the provided design rules for optimizing performance are significant contributions to the field. The work is compelling and well-supported; however, several points should be addressed to further strengthen the manuscript before publication.

We thank the Reviewer for appreciating our work and the comments.

#31 – In Figure 7, the authors demonstrated that the device response of n-type OECTs was exclusively generated by a change in gate potential, while both potential changes and capacitance contributed to the signals detected by p-type OECTs.

The p-type OECTs are in “ON” state at the measured V_{gs} range with high channel capacitance, resulting in a low C_g/C_{ch} . This condition makes the device sensitive to changes in gate capacitance. In contrast, n-type OECTs are “OFF” with low channel capacitance and thus high C_g/C_{ch} . The high C_g/C_{ch} ratio favors efficient channel gating by the potential drop, resulting in the device predominantly sensitive to gate potential changes and masking the contribution of capacitance variations. Therefore, quantitatively analyze the relationship between the device response and the C_g/C_{ch} ratio for both channel types is suggested to strengthen authors’ conclusions.

The reviewer is correct in their conclusions. The C_g/C_{CH} ratio depends on the channel material’s intrinsic capacitance and makes one material less or more sensitive to capacitance changes at the gate electrode.

The capacitance–doping potential trends for the two mentioned OMIECs are reported for the Reviewer in Fig. R3. As expected, based on prior studies of analogous enhancement-mode materials (see, for example, the structurally related system to p(C₆NDI-T) reported in <https://doi.org/10.1002/adma.202313121>), enhancement-mode OMIECs exhibit a broad capacitance modulation range: capacitance is very low in the undoped regime and then increases sharply once the onset potential is reached.

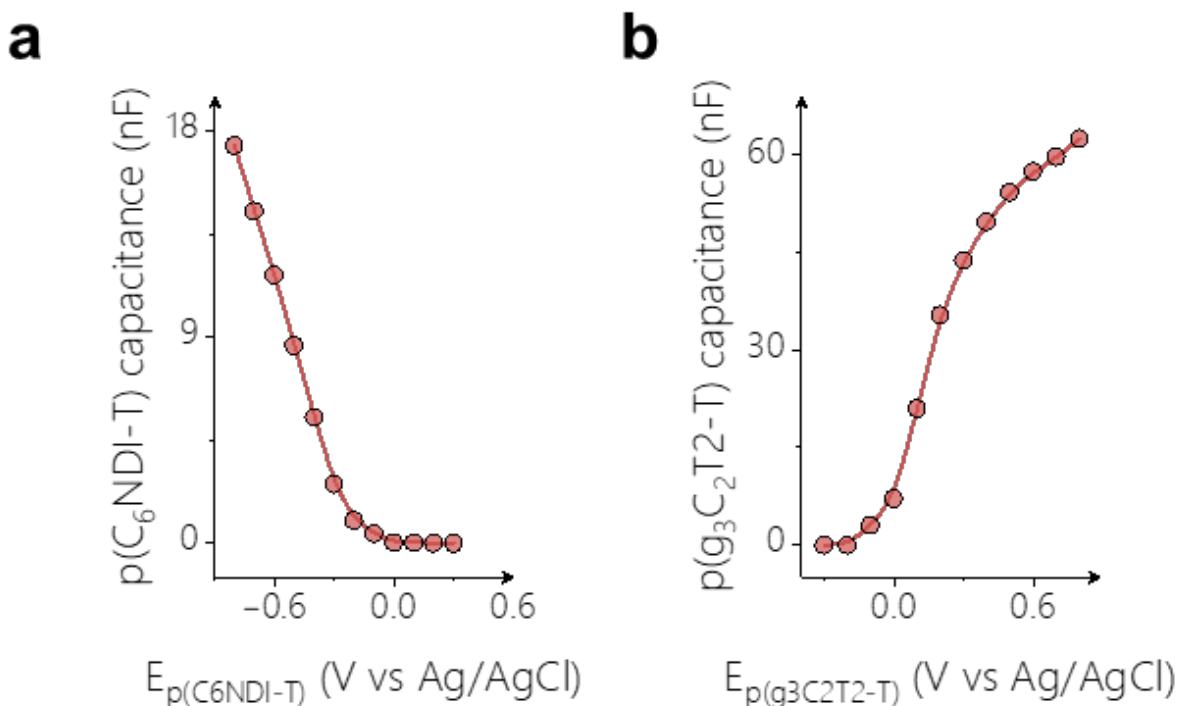


Figure R3. Capacitance of (a) p(C₆NDI-T) and (b) p(g₃C₂T₂-T) measured at different electrochemical potentials.

The capacitance of p(g₃C₂T₂-T) and p(C₆NDI-T) was measured using planar electrodes rather than OECT channels due to limitations in EIS fitting quality and the phenomenon described below. Because these OMIECs span a wide conductivity range and can reach a fully OFF state, directly measuring the channel capacitance as a function of gate bias is particularly challenging: the OMIEC regions not in direct contact with the source and drain electrodes cannot be efficiently biased to the intended potential. For this additional reason, it is not possible provide an accurate value of the channel capacitance for the p(g₃C₂T₂-T) and p(C₆NDI-T) OMIECs, and consequently, a reliable C_G/C_{CH} ratio from OECT channel measurements. Nevertheless, an indirect indication of the much lower capacitance of p(C₆NDI-T) compared to p(g₃C₂T₂-T) during the OECT operation can be obtained from capacitance measurements on planar electrodes (Figure R3) and by comparing the devices' I_{DS} levels (ex-Fig.7). While the p(g₃C₂T₂-T) OECT reaches drain currents in the mA range, the p(C₆NDI-T) device operates at currents approximately three orders of magnitude lower (μ A), which is consistent with the lower capacitance of the material measured on the planar electrodes. In any case, the physical principle governing the distribution of the doping voltage between the gate and the channel is the standard voltage division across two capacitors in series. This concept has been observed in this work and has also been demonstrated in several prior studies through detailed modelling and experimental validation (e.g., <https://doi.org/10.1002/aelm.202001173>, <https://doi.org/10.1002/adfm.202302205>, and <https://doi.org/10.1021/acsaelm.5c01861>). Here, we focus on how this effect manifests in the transfer-curve shape. These physical principles do not change when switching OMIEC type, as the device remains an OECT with a semiconductor channel, albeit operating at different voltages and current levels. Rather, what changes is simply the range of C_{CH} values accessible for a given voltage, leading to a different partitioning of V_{GS} .

For the Reviewer's clarity, we provide an additional experiment illustrating the origin of this issue. In Figure R4 we measured the electrochemical potentials at the source and drain of a p(C₆NDI-T) coated OECT channel while modulating V_{GS} . When no V_{GS} is applied (yellow region), the enhancement-mode OMIEC relaxes to its equilibrium potential, corresponding to a fully undoped state with negligible conductivity (see new Fig. 7b-x). Under these conditions, the OMIEC over the source and drain contacts is effectively insulating, and the measured signal is dominated by noise, comparable to an open-circuit potential measurement with the working-electrode leads disconnected. When $V_{GS} = 0$ V is applied, only the source reaches the intended potential, whereas the drain side remains insensitive to the applied bias. This indicates that, in an EIS measurement under these conditions, only the OMIEC region above the source contact, plus an undefined fraction extending into the channel on the glass substrate, would contribute to the extracted capacitance. By contrast, at a more doping bias ($V_{GS} = -0.35$ V), the electric field and associated doping extend across the full channel, enabling the drain to reach the same potential as the source. Only in this regime can the entire OMIEC channel be considered uniformly biased and contributing to the measurement. In this experiment V_{DS} was not applied in order to isolate the magnitude of this parasitic effect.

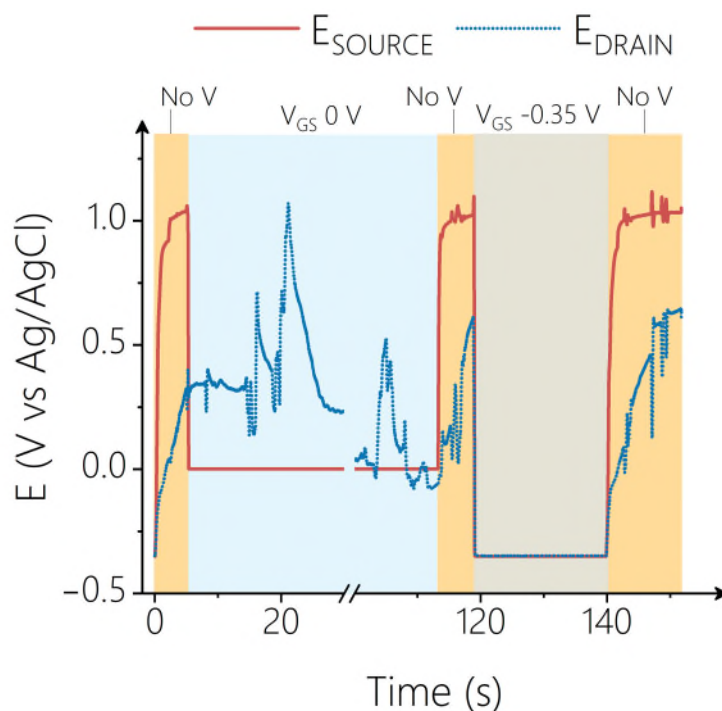


Figure R4. Electrochemical potentials at the source and drain of a p(C₆NDI-T) OECT channel (100x10 μ m) monitored while applying different V_{GS} values. The brown region corresponds to $V_{GS} = -0.35$ V, the yellow region to the resting condition with no applied V_{GS} , and the light-blue region to $V_{GS} = 0$ V.

The Methods section has been revised accordingly to describe how the capacitance of the OMIECs was determined:

OMIEC Capacitance measurements

The capacitance of PEDOT:PSS OECT channels was measured by shorting the source and drain electrodes to form the working electrode and performing EIS at different electrochemical potentials, using the settings described above. In contrast, the capacitance of p(C₆NDI-T), PBFDO and p(C₂DPP-T) OECT channels cannot be measured reliably using this approach due to poor EIS fit quality and their low conductivity and uniform doping across the channel. Their capacitance of was instead measured using Au electrodes (500x500 μm) coated with the corresponding OMIEC via cyclic voltammetry and the integration of the doping scan.

In light of Reviewer 1 comments #8 and #10 requesting more detailed protein-based experiments, the original version of Fig. 7 became redundant, as it also included two protein-based examples in addition to a preliminary OMIEC screening. To avoid this redundancy, we removed the original protein experiments associated with those OMIECs and refocused Fig. 7 on a more comprehensive OMIEC screening and on the effect of channel size, as also requested by Reviewer 4 in comment #32. Consequently, the measurements on p(g₃C₂T2-T) were considered redundant (given the analogous behavior already captured by PEDOT:PSS) and were removed.

The section was restructured as follows:

Tuning OECT sensing via channel geometry and OMIEC selection

In the preceding sections, we focused primarily on the gate electrode as a tunable component for optimizing OECT sensors. The channel properties, specifically channel capacitance/geometry and the type of OMIEC, can also affect sensor performance....

The framework established above can also provide guidelines for selecting channel materials beyond PEDOT:PSS to enhance sensing performance without modifying device geometry. Given the growing library of OMIECs,[\[https://doi.org/10.1038/s41578-024-00652-7\]](https://doi.org/10.1038/s41578-024-00652-7),[\[https://doi.org/10.1016/j.matt.2023.05.001\]](https://doi.org/10.1016/j.matt.2023.05.001), [\[https://doi.org/10.1021/accountsr.4c00124\]](https://doi.org/10.1021/accountsr.4c00124) OECTs can be realized across a broad range of material-property combinations, including p-type and n-type as well as intrinsically doped and intrinsically undoped. In [Fig. 7b](#), we illustrate one representative example for each case, showing how appropriate OMIEC selection can be used to control the direction of the I_{DS} response (i.e., turn ON or turn OFF) when sensing-induced changes in gate electrochemical potential and capacitance occur. The doped p-type OMIEC we used is PEDOT:PSS ([Fig. 7b-i](#)), the doped n-type OMIEC is poly(benzodifurandione) (PBFDO) ([Fig. 7b-v](#)), the undoped p-type OMIEC is p(C₂DPP-T) ([Fig. 7b-ix](#)), and the undoped n-type OMIEC is p(C₆NDI-T) ([Fig. 7b-xiii](#)). The capacitance of these materials at different doping potentials is reported in Supplementary Fig. 18.

The intrinsic doping state of the OMIEC is a key parameter to assess when selecting the channel material because it determines the direction of the OECT response to changes in gate capacitance. This assessment can be performed by comparing a transfer curve acquired with a non-polarizable gate (e.g., Ag/AgCl) to a measurement acquired without a gate electrode. The former approximates the response obtained with a sensing gate of very high capacitance, whereas the latter approximates the response corresponding to a gate of very low capacitance. All sensing responses arising from changes in gate capacitance lie between these two conditions. Increasing gate capacitance enables stronger modulation of I_{DS} (approaching the Ag/AgCl-gated response), whereas decreasing gate capacitance reduces I_{DS} modulation and shifts the response toward the OMIEC's native doping state (approaching the “no-gate” condition). Intrinsically doped OMIECs, regardless of their type (n- or p-), retained high I_{DS} values upon gate removal ([Fig. 7b-ii and vi](#)), and a turn ON response to a decrease in gate capacitance ([Fig. 7b-iii and vii](#)). In contrast, intrinsically undoped OMIECs

exhibited complete suppression of I_{DS} upon gate removal ([Fig. 7b-x and xiv](#)), resulting in a device turn OFF response when the gate experiences a decrease in its capacitance ([Fig. 7b-xi and xv](#)). As for the changes in gate electrochemical potential, we observed a consistent direction of transfer-curve shift that is independent of the OMIEC type or intrinsic doping state. A positive ΔE_G produced a positive shift, and a negative ΔE_G produced a negative shift. Accordingly, an increase in gate electrochemical potential led to a consistent turn ON in p-type OMIECs ([Fig. 7b-iv and xii](#)) and a consistent turn OFF in n-type OMIECs ([Fig. 7b-viii and xvi](#)). These conclusions are summarized in Table 1.

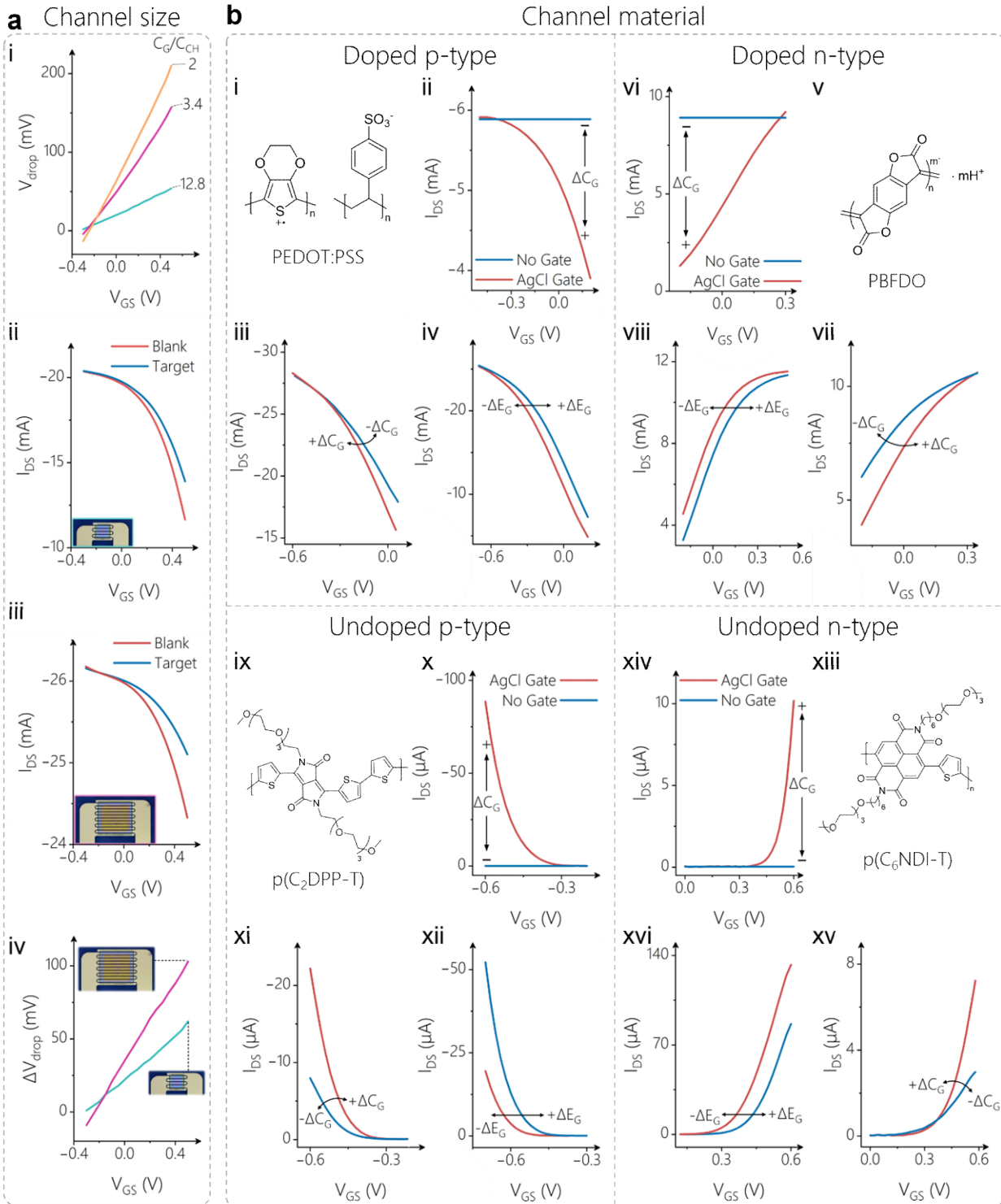
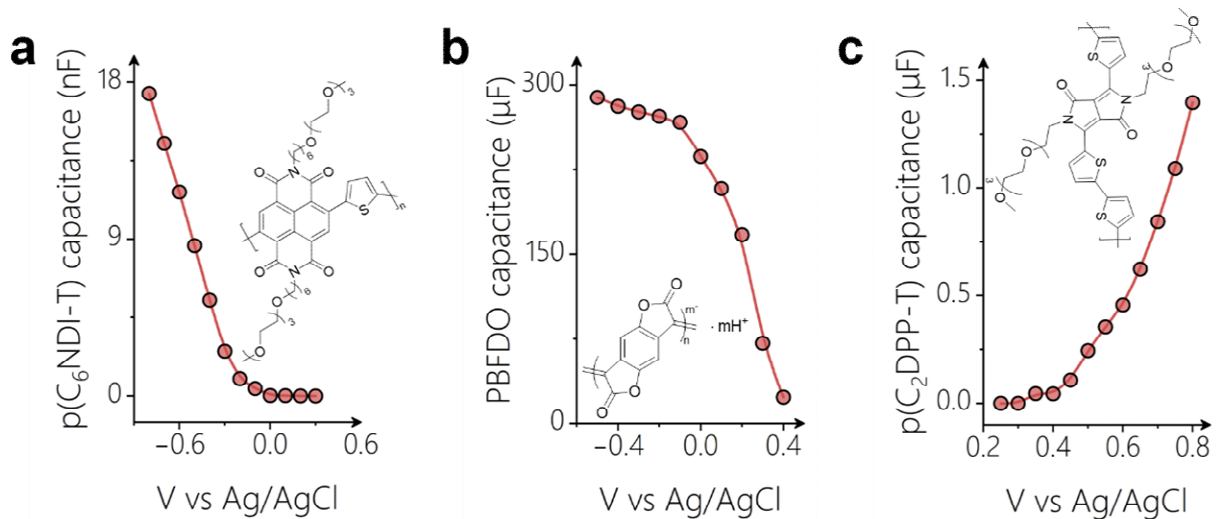


Figure 7. The effect of channel size and OMIEC type on OECT sensor response a) Effect of channel capacitance on the OECT response to gate-capacitance variations. V_{drop} during transfer-curve acquisition for PEDOT:PSS OECTs measured with the same gate (Au, 5 mm diameter) and increasing channel areas of $200 \times 200 \mu\text{m}$, $450 \times 450 \mu\text{m}$, and $625 \times 625 \mu\text{m}$, corresponding to C_G/C_{CH} ratios of 12.8, 3.4, and 2, respectively (i). OECT response to an exclusive gate capacitance change for initial C_G/C_{CH} values of 12.8 (ii) and 3.4 (iii), and the corresponding sensing input, ΔV_{drop} (iv).

ΔV_{drop} (iv). **b)** OECTs comprising different OMIECs in the channel and their responses to variations in gate electrochemical properties assessed using model electrodes. Chemical structures of the OMIECs (**i**, **v**, **ix** and **xiii**). Assessment of the intrinsic OMIEC conductivity by acquiring transfer curves with and without a non-polarizable gate (**ii**, **vi**, **x** and **xiv**). Device response to changes in gate capacitance (**iii**, **vii**, **xi** and **xv**). Device response to changes in gate electrochemical potential (**iv**, **viii**, **xii** and **xvi**).



Supplementary Figure 18. OMIEC capacitance as function of doping potential. Capacitance of (a) p(C₆NDI-T), (b) PBFDO, and (c) p(C₂DPP-T) measured at different electrochemical potentials.

We agree with the Reviewer that a further analysis of the relationship between C_G/C_{CH} and device response would strengthen our conclusions. Accordingly, we incorporated this correlation analysis earlier in the manuscript, specifically within the newly added experiments introduced in response to Reviewer 1 comments #8 and #10, and within the additional experiment introduced in response to Reviewer 4 comment #32.

In Supplementary Fig. 16 we monitored changes in C_G/C_{CH} during protein sensing, calculated the expected V_{drop} using Equation S10 ($V_{\text{drop}} = V_{\text{GS}} (C_{CH}/(C_{CH}+C_G))$), and compared it with the experimentally measured V_{drop} , finding close agreement with the model (see our response to Reviewer 1 comments #8 and #10 for details).

In Fig.7a and Supplementary Fig. 17 we further show that C_G/C_{CH} can also be tuned by varying the channel size (and thus C_{CH}) using OECT channels with increasing dimensions. The corresponding C_G/C_{CH} values were measured and correlated with V_{drop} , confirming that gate polarization increases as the capacitance ratio decreases, as predicted by the model. Moreover, ΔV_{drop} , the key input parameter for capacitance-based sensing, also increases as C_G/C_{CH} decreases. For additional details, please see our response to Reviewer 4 comment #32.

#32 – To further decouple the effects of channel material properties from those of device geometry, I recommend a key control experiment. The authors should characterize the

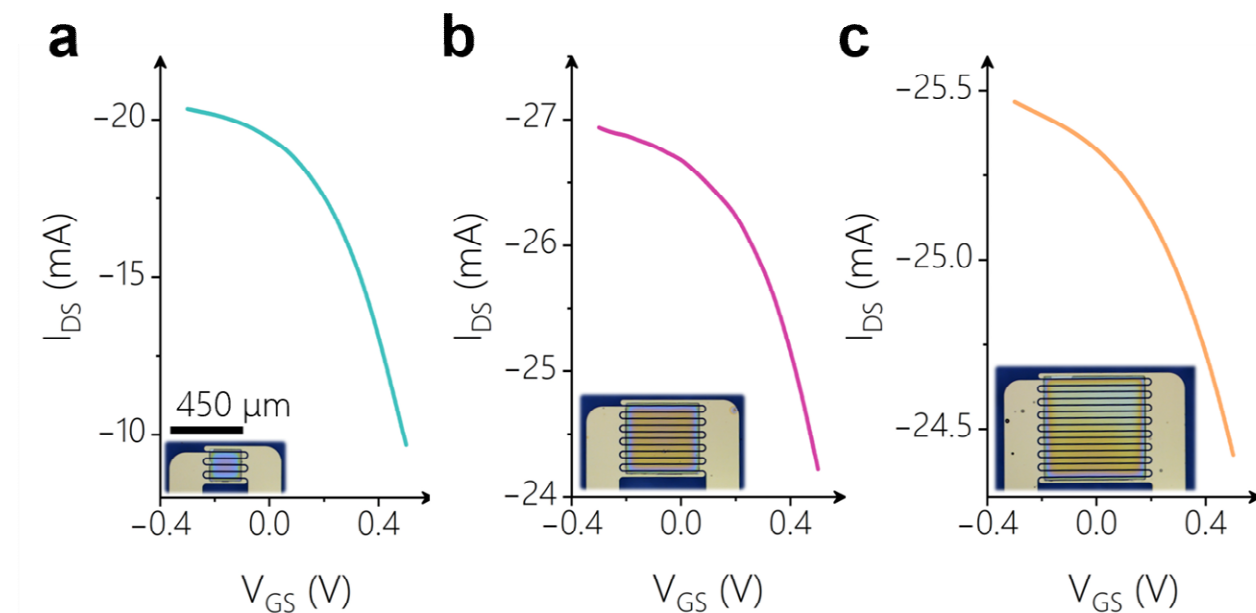
response of devices that share the same gate electrode but are fabricated with OECT channels of different geometries (different channel capacitance).

We thank the Reviewer for this comment, which prompted us to improve the section discussing OECT channel materials by broadening it to provide a more comprehensive overview of channel properties, including both different OMIECs and variations in channel geometry/size.

The section was revised as follows -please see the new **Figure 7** above:

Tuning OECT sensing via channel geometry and OMIEC selection

In the preceding sections, we focused primarily on the gate electrode as a tunable component for optimizing OECT sensors. The channel properties, specifically channel capacitance/geometry and the type of OMIEC, can also affect sensor performance. The C_G/C_{CH} ratio, and thus gate polarization, which governs OECT sensitivity to capacitance variations, can be adjusted by changing the channel-size. When a polarizable gate electrode of fixed dimensions (e.g., a 5 mm-diameter Au gate, as in this example) was interfaced with PEDOT:PSS channels of increasing sizes ($200 \times 200 \mu\text{m}$, $450 \times 450 \mu\text{m}$, and $625 \times 625 \mu\text{m}$; Supplementary Fig. 17), C_G/C_{CH} decreased from 12.8 to 3.4 and 2, respectively. The reduction in C_G/C_{CH} produced a progressive increase in V_{drop} during transfer-curve characterization, as shown in Fig. 7a-i. When gate capacitance was reduced by target binding, here simulated by using a smaller gate electrode (i.e., 3 mm-diameter), the OECT exhibited a turn-ON response (Fig. 7a-ii,iii). Notably, with a larger channel and correspondingly smaller C_G/C_{CH} ratio, the ΔV_{drop} was higher (Fig. 7a-iv), demonstrating that channel geometry provides an additional degree of freedom for optimizing sensing performance.



Supplementary Figure 17. Transfer curves of PEDOT:PSS OECTs with different channel dimensions gated by the same polarizable Au electrode (5 mm diameter). Channel dimensions: $200 \times 200 \mu\text{m}$ (a), $450 \times 450 \mu\text{m}$ (b) and $625 \times 625 \mu\text{m}$ (c). The corresponding C_G/C_{CH} ratios are 12.8, 3.4, and 2, respectively. The micrographs in each panel show the OECT channels.