nature portfolio

Peer Review File

On-site biosignal amplification using a single high-spin conjugated polymer

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

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)
The manuscript by Gaoyang Ge et al. presented a new molecular design concept for ambipolar OECT materials and successfully demonstrated their applications in on-site biosignal amplification. Although narrow-bandgap conjugated polymers could show good ambipolar properties in OFETs, their performances in OECTs remain limited. The authors proved that open-shell or high-spin conjugated polymers could be a solution. The concept is quite intriguing and might inspire more high-performance ambipolar materials. More importantly, the authors have taken a key step forward in fabricating single polymer-based logic circuits and amplifiers. The performance of the devices is also impressive. In addition, they provided a good theoretical analysis of their design strategy, which deepened the molecular level understanding. After the modifications made in our last submission to Nature Electronics, I believe the authored have resolved my issue. I highly appreciate this study and in principle agrees with the publication.

Reviewer #3

(Remarks to the Author)
I appreciate the authors' clarification of the relationship between open-shell high-spin electronic structure and ambipolar character. Figure R2, along with the comparison of six identified key parameters, provides a more coherent framework for interpreting the current results. However, I find the discussion about DC or quasi-static gains unconvincing.
As the authors noted, DC gains depend heavily on the input voltage sweep speed, with Table R1 showing that extremely slow sweeping (i.e., small voltage steps) yields an impressive DC gain of 809. However, in practical biosignal amplification applications, gains are much smaller and comparable to other reported OECT-based technologies. The argument that quasi-static gain measurements are useful for amplifying small biosignals is flawed, as small biosignals, particularly those that vary rapidly, would not be effectively detected or amplified if the inverter's response speed is too slow. Thus, even if the device shows high gains in quasi-static mode, it may underperform dynamically and fail to capture or amplify fast-changing signals accurately.

1) To provide a clearer comparison, I recommend that the authors report how their device's gain changes with varying voltage step sizes and sweep speeds. This would offer readers a more accurate assessment relative to existing technologies in the literature.

2) Additionally, I question the amplifier biasing in Figure 5d. In Figure 5f, a negative Vss appears to be used for DC biasing, but this is not explained in the manuscript. Clarification on the biasing approach is essential for interpreting the results accurately.

Point-to-Point Response

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Our response: We are deeply grateful for your recognition of this work. We truly appreciate the time and effort you have dedicated to this review, which has significantly improved the overall quality of the manuscript.

Reviewer #3:

I appreciate the authors' clarification of the relationship between open-shell high-spin electronic structure and ambipolar character. Figure R2, along with the comparison of six identified key parameters, provides a more coherent framework for interpreting the current results. However, I find the discussion about DC or quasi-static gains unconvincing.

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Our response: We are grateful for your recognition of our work in clarifying the relationship between open-shell high-spin electronic structure and ambipolar characteristics. Regarding the high gain observed in quasi-static mode, we acknowledge that the assumption of high quasi-static gain being universally beneficial for amplifying small biological signals could be controversial or flawed. We agree that the devices have limitations in their application scenarios and may only be suitable for detecting and amplifying certain biological signals with low frequencies. We have therefore toned down our claims regarding the usefulness of the quasi-static gain for small signal amplification in the manuscript. Additionally, by providing data on gain variations under different voltage step sizes and sweep speeds, we hope to further explore the applicable boundaries of this device. This also represents a crucial but often overlooked issue in the current field of biological signal amplification, which we plan to investigate in more depth in our future work. Nevertheless, we respectfully disagree with your statement that "in practical biosignal amplification applications, gains are much smaller and comparable to other reported OECT-based technologies".

First, the reduction in amplification factor from quasi-static measurements to practical applications is a common phenomenon, since gain could be influenced by many factors, including

signal frequency and solution environment. For instance, a quasi-static gain exceeding 700 V/V can amplify ECG signals by 50 times (*Adv. Funct. Mater.* 32, 2205129 (2021)). As a reference, we achieved a 75-fold amplification with a quasi-static gain of 809 V/V. Furthermore, as observed from the gain-V_{in} curve, even a minor voltage offset of just 0.0002 V can cause the gain to decrease from over 800 to around 100. Achieving such precise potential alignment during manual sensor testing, as in this manuscript, is highly challenging; however, with current automatic control technology, this alignment is not overly difficult. By implementing a program to automatically align the potential signal, maintaining the operating potential at 1/2 V_{DD}, the amplification factor could be further improved. Although these enhancements are not demonstrated in this manuscript, they should not be dismissed as lacking potential. In our lab, after optimizing the device structure (materials and device configuration), we could realize significantly higher practical biosignal amplification (> 100 V/V) with a high bandwidth (>1 kHz).

Second, we maintain that our polymer design strategy and devices are more advanced than other OECT-based technologies. In ECG signal amplification, Rashid et al. reported a quasi-static gain of 28 V/V achieving a 10-fold ECG signal amplification *(Sci. Adv.* 7, eabh1055 (2021)), which represents the state-of-the-art for OECT inverters used in ECG signal amplification. In contrast, we achieved a 73-fold ECG signal amplification with a quasi-static gain of 809 V/V. Although these quasi-static gains were measured under different voltage step sizes, the seven-fold higher ECG signal amplification clearly demonstrates the advantage of our polymer design strategy and device.

Based on your suggestions, we have toned down the discussions on inverter gains and made modifications to the manuscript and Supplementary Information. We believe that, after these revisions, the quality and clarity of the manuscript have been substantially improved, and we sincerely hope that you will support its publication.

***Q*1:** To provide a clearer comparison, I recommend that the authors report how their device's gain changes with varying voltage step sizes and sweep speeds. This would offer readers a more accurate assessment relative to existing technologies in the literature.

Our response: Thank you for your comment. We appreciate your suggestions and have provided

the relevant data. Fig. R1 clearly shows that as the voltage step size decreases, there is a significant improvement in gain. Notably, the theoretical maximum gains measurable at 10 mV, 1 mV, and 0.1 mV are 40 V/V, 400 V/V, and 4000 V/V, respectively. It can be observed that the experimental gain values obtained under the testing conditions of 10 mV and 1 mV closely approach these theoretical values. Additionally, the gains at different sweep speeds are also shown. As depicted in Fig. R2, a continuous increase in frequency diminishes the gain of the inverter. Even so, the devices still maintain a tenfold gain at sweep speeds of 100 Hz or a voltage step size of 10 mV, demonstrating outstanding voltage amplification capabilities and suitability for on-site sensing and amplification of electrophysiological signals.

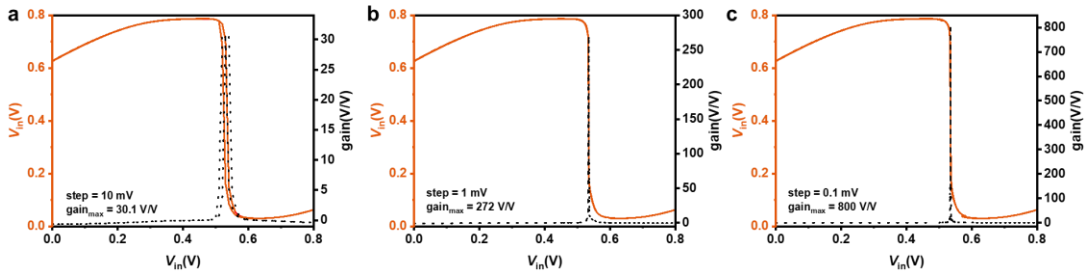

Fig R1 The variation of inverter gain with voltage step size based on the polymer P(TII-2FT). a 10 mV, **b** 1 mV, **c** 0.1 mV.

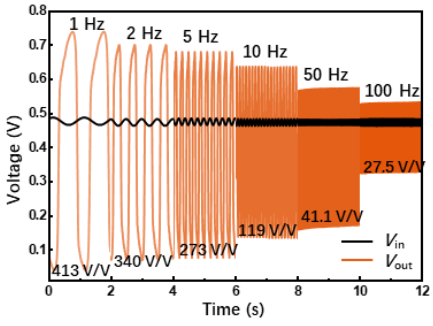

Fig R2 Dynamic response and corresponding gains of the amplifier using small sinusoidal signals at different frequencies.

We have supplemented the above results and discussions in the revised manuscript and

Supplementary Information. Changes have been made as follows:

In the revised manuscript:

(1) on p. 13

Information for more details). The vOECT inverters based on P(TII-2FT) demonstrated an ultra-high gain ($\partial V_{out}/\partial V_{in}$) of 809 V/V at a voltage step size of 0.1 mV with a simple device fabrication process (Supplementary Fig. 47 and Supplementary Fig. 48). This gain, obtained under a low supply voltage (V_{DD}) of 0.8 V, is significantly higher than that of previously reported inverters based on OECTs and OFETs (Fig. 4h). Interestingly, we observed reduced inverter gains with larger voltage step sizes (Supplementary Fig. 49), showing a gain of 272 V/V at a 1 mV step and 30.1 V/V at a 10 mV step. NAND and NOR gates were also fabricated

(2) on p. 14

low cytotoxicity and good biocompatibility of our polymers (Fig. 5a, b). The high gain of P(TII-2FT)-based inverters decreases significantly as the frequency increases (Fig. 5c), similar to other CMOS-like inverters (see below Supplementary Fig. 54 for more details). At a high frequency of 100 Hz, the gain still maintains a value of 27.5 V/V, demonstrating outstanding voltage amplification capabilities and suitability for on-site sensing and amplification of electrophysiological signals. Subsequently, flexible amplifiers based on P(TII-2FT) were

(3) on p. 15

This unexpectedly low amplification factor might be due to the high signal frequency and complex solution environment within the brain tissue fluid (Supplementary Fig. 54). However, this does not mean that the quasi-static gain is insignificant for practical application. First, the

(3) on p. 16

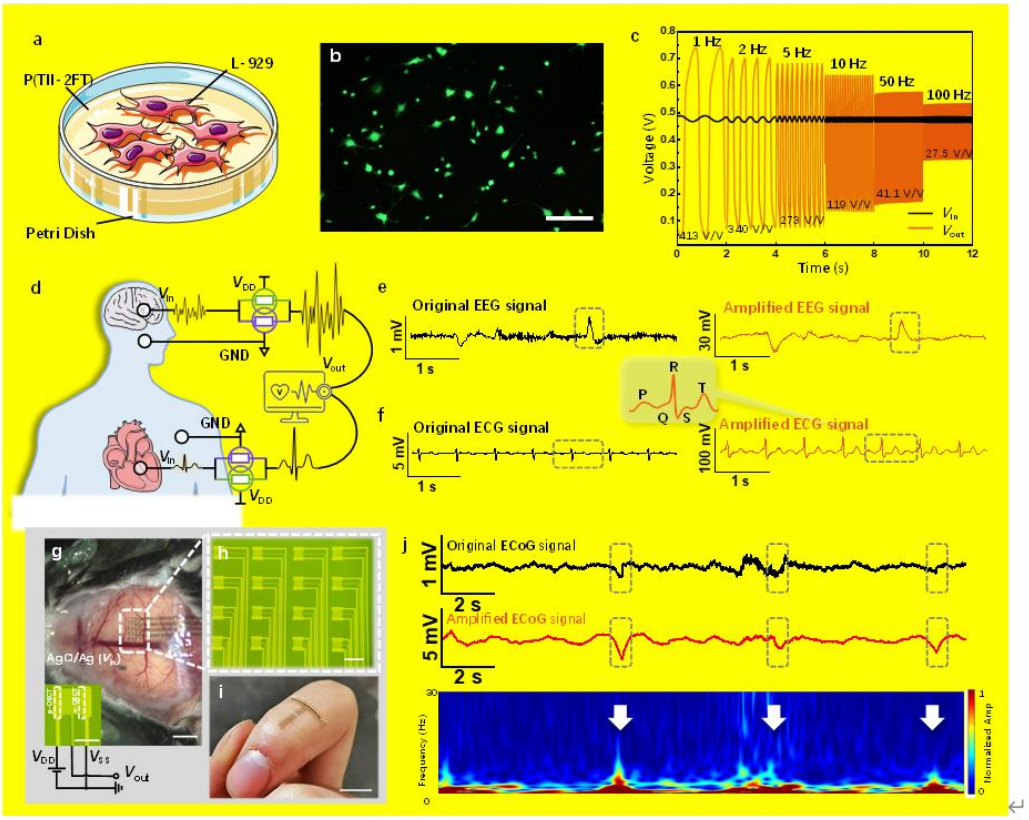

Fig. 5| Biosignal amplification using P(TII-2FT)-based amplifiers. a Schematic illustration of cell viability tests on P(TII-2FT) film. **b** Live (green fluorescence)/dead (red fluorescence) staining of mouse fibroblasts (L929) on P(TII-2FT) film (scale bar: 100 μm). **c** Dynamic response of the amplifier using small sinusoidal signals at different frequencies. The corresponding gains at different frequencies are also displayed. **d** Schematic illustration of the vOECT amplifier for recording **e** EEG and **f** ECG signals. **g** Photograph of in vivo ECoG

In the Supplementary Information: Supplementary Fig. 49.

***Q2*:** Additionally, I question the amplifier biasing in Figure 5d. In Figure 5f, a negative Vss appears to be used for DC biasing, but this is not explained in the manuscript. Clarification on the biasing approach is essential for interpreting the results accurately.

Our response: Thank you for your comment. In Figure 5f, we indeed used a negative V_{SS} while simultaneously adjusting V_{DD} to maintain $V_{DD} - V_{SS} = 0.8$ V. This testing method was employed in our on-site measurements to shift the position of maximum gain to 0 V. The 0 V gate bias eliminates the need to link the SMU separately, which not only reduces the complexity of the

external circuit but also minimizes the 50 Hz noise signal introduced by the SMU itself. Thus, this approach reduces interference from gate biasing during the acquisition of in vivo biosignals, enhancing the validity of our collected data. We appreciate you pointing out this omission in our work. We have supplemented the above discussions in the Supplementary Information. Changes have been made as follows:

In the Supplementary Information (the Signal acquisition and processing section):

Signal acquisition and processing

A function generator, Keithley 3390, was used to bias the inverter (V_{DD}). A PDA Source Meter was utilized to continuously record the output voltage of the amplifiers and the voltage from the AgCl/Ag or Au electrode at a sampling rate of 1 kHz, and exert a negative V_{SS} ($V_{DD} - V_{SS} = 0.8$ V). MATLAB