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Communications

Highly Sensitive Detection of Protein Biomarkers with Organic Electrochemical Transistors

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Abstract

The analysis of protein biomarkers is of great importance in the diagnosis of diseases. Although many convenient and low-cost electrochemical approaches have been extensively investigated, they are not sensitive enough in the detection of protein biomarkers with low concentrations in physiological environments. Here, we report a novel organic electrochemical transistor based biosensor that can successfully detect cancer protein biomarkers with ultrahigh sensitivity. The devices are operated by detecting electrochemical activity on gate electrodes, which is dependent on the concentrations of proteins labelled with catalytic nanoprobes. The protein sensors can specifically detect a cancer biomarker, human epidermal growth factor receptor 2, down to the concentration of 10^{-14} g/mL, which is several orders of magnitude lower than the detection limits of previously reported electrochemical approaches. Moreover, the devices can successfully differentiate breast cancer cells from normal cells at various concentrations. The ultrahigh sensitivity of the proein sensors is attributed to the inherent amplification function of the organic electrochemical transistors. This work paves a way for developing highly sensitive and low-cost biosensors for the detection of various protein biomarkers in clinical analysis in the future.

Cancer biomarker analysis plays an important role in the prognosis, diagnosis and therapy of cancers.[1] Within numerus biomarkers, protein cancer biomarkers can be found in serum, tissue, or body fluids such as saliva, and thus can be conveniently assessed even non-invasively and serially, or can be tissue-derived for either biopsy or special imaging.[2,3] To date, proteins are the most frequently used cancer biomarkers in oncology, including risk assessment, screening, differential diagnosis, determination of prognosis, prediction of responses to treatments, and monitoring of cancer progression. Many molecular biology methods have been developed for the analysis of protein cancer biomarkers, including western blotting,[4] gel electrophoresis,[5] mass spectrometry and enzyme linked immunosorbent assay,[6,7] which are normally time-consuming and laborious. Compared to the above methods, electrochemical immunosensors can provide convenient and cost-effective determination of protein cancer biomarkers and have been successfully used in detecting many types of protein cancer biomarkers.[8,9] However, with the increasing demands for assays on low-abundance protein cancer biomarkers, it is a grand challenge to utilize conventional electrochemical immunosensors in many practical applications due to their low sensitivity. Therefore, novel electrochemical approaches with high sensitivity and selectivity are urgently needed in the detection of cancer biomarkers.

Organic thin film transistors, including organic electrochemical transistors (OECTs) and organic field-effect transistors, have been successfully used as various types of biosensors,[10-16] in which OECTs are particularly suitable for biological sensing applications because the devices can operate in aqueous solutions. As evidenced by our previous works,[17,18] OECT-based biosensors have shown higher sensitivity than conventional electrochemical approaches due to their inherent amplification function. OECTs have been used for sensing proteins based on the electrostatic interactions between proteins and organic semiconductor channels. For example, Kanungo *et al* successfully fabricated OECT-based immunosensors using the

biospecific binding pair of goat antirabbit IgG and rabbit IgG.[19] Kim *et al* also demonstrated a functionalized OECT immunosensor for sensing prostate specific antigen/α1-antichymotrypsin complex.[20] However, these protein sensors are not sensitive enough for the detection of some specific biomarkers with very low concentration in physiological environments because the interactions between protein and organic semiconductor are normally very weak.

Here, we present OECT-based biosensors for the detection of protein biomarkers with ultrahigh sensitivity based on a novel mechanism. Human epidermal growth factor receptor 2 (HER2), a key prognostic protein biomarkers for the therapeutic treatment of breast cancer patients, was chosen as a target to be detected in our experiments.[21] HER2 was captured on the surface of the gate electrode of an OECT by an antibody and then specifically modified with catalytic nanoprobes. The sensing mechanism of the device is attributed to the electrochemical reaction catalyzed by the nanoprobes on the gate. We found that a weak electrochemical reaction on the gate electrode can result in an obvious change in the effective gate voltage of the transistor and thus lead to a pronounced response of channel current. The device could specifically detect HER2 down to the level of 10^{-14} g/mL (10^{-16} M), which is several orders of magnitude lower than the values obtained from conventional electrochemical approaches.[22] The protein sensors are able to differentiate breast cancer cells from normal cells by measuring both cell lysates and living cells. Therefore, the OECT-based protein sensors can serve as a versatile platform for highly sensitive biomarker assay in the future.

Figure 1a shows the design of the gate electrode of an OECT-based protein sensor. The Au gate electrode is firstly modified with a specific HER2 antibody ($Ab_{Capture}$) that is used to capture HER2 proteins in solutions. In our experiments, we used the monoclonal anti-HER2 antibody as $Ab_{Capture}$ for binding with HER2 with high affinity and selectivity.[23] With this

capture component, the gate electrode could be specifically modified with HER2 even in the present of some interferences. In the next step, the captured HER2 will be modified with catalytic nanoprobes because HER2 proteins are not electrochemically active. The nanoprobes are Au nanoparticles with the diameter of ~10nm (see supporting information, Figure S1), which are modified with a HER2 detection antibody ($Ab_{Detection}$) and an electrochemically active enzyme horseradish peroxidase (HRP).[24, 25] $Ab_{Detecion}$ we used is polyclonal Anti-HER2 antibody, which could selectively bind with HER2 without affecting the binding between HER2 and $Ab_{Capture}$.[24] Consequently, more HER2 protein captured on the gate electrode can lead to more HRP enzyme on the gate.

Figure 1b shows the operation mechanism of the OECT-based protein sensor. Because HRP enzyme can catalyze the electrochemical reaction of H_2O_2, the amount of HRP on the gate electrode can be characterized by adding H_2O_2 in a phosphate buffered saline (PBS) solution. The following reaction is catalyzed by the HRP enzyme:[25]

$$H_2O_2 + 2e^- + 2H^+ \overset{HRP}{\Longleftrightarrow} 2H_2O$$

Under a bias voltage, the redox current on the electrode should be proportional to the amount of HRP on the electrode surface. Assuming that the redox current is very low and not limited by the mass transfer of H_2O_2, the electrode current density i is given by the equation:[26]

$$i = W_{HRP} i_0 e^{\alpha\eta}, \quad (1)$$

where i_0 is the exchange current for per unit amount of HRP, η the applied overpotential, α constant and W_{HRP} is the amount of HRP modified on the gate. Since OECT is an potentiometric transducer, the redox current on the gate electrode is very low and limited by the leakage across the interface between the electrolyte and the PEDOT:PSS active layer. Consequently, the potential change on the gate surface induced by the reaction of H_2O_2 is given by:

$$\Delta V_G \propto -\frac{1}{\alpha} Log[W_{HRP}] \quad . \tag{2}$$

According to the device physics of OECTs,[27-29] the change of the effect gate voltage V_G^{eff} of the transistor is thus given by:

$$\Delta V_G^{eff} \propto \left(1 + \frac{C_{EC}}{C_{GE}}\right) \frac{1}{\alpha} Log[W_{HRP}], \tag{3}$$

where C_{GE} and C_{EC} are the capacitances of the two electric double layers (EDL) on the gate and the channel, respectively. Therefore, in the characterization of the protein sensors, we will use one solution containing target proteins to modify the gate electrode and another standard PBS solution with an addition of H_2O_2 for measuring the concentration of HRP enzyme.

To demonstrate the effect of HRP enzyme on the performance of OECTs, we firstly modified the Au gate electrodes of OECTs with HRP.[30] As shown in **Figure 1**a, an Au electrode is modified with mercaptoacetic acid (MAA) and HRP sequentially to get a HRP/MAA/Au electrode. We treated the Au gate electrodes in HRP solutions with different concentrations (10^{-7} to 1 g/mL) for 5 hours. It is reasonable to expect that the amount of HRP on an Au gate electrode increases with the increase of HRP concentration in the solution for modification. Then the OECTs were characterized in PBS solutions with the addition of H_2O_2 at the concentration of 10^{-4} M. **Figure 2**a shows the responses of the devices to the addition of H_2O_2 measured at the same operation voltages (V_{DS} = 0.05V and V_G = 0.6V). It is notable that the current response increases with the increase of HRP concentration. According to the channel current change and the transfer curve of each device, the change of the effective gate voltage of the device induced by the reaction of H_2O_2 is calculated and presented in **Figure 2**b. We can find that the effective gate voltage change ΔV_G^{eff} increases with the increase of HRP concentration and the detection limit (signal/noise ratio ≥3) of the device to the concentration of HRP in a solution is about 10^{-7} g/mL.[31]

In comparison, the HRP-modified Au electrodes were then characterized with the conventional cyclic voltammogram (C-V) method, as shown in **Figure 2**c. A current peak can be observed at the bias voltage of about -0.4V and the peak current i_p increases with the increase of HRP concentration, as shown in **Figure 2**d. It is notable that the minimum concentration of HRP in solutions that can be detected by measuring C-V curves is about 10^{-5} g/mL, indicating that OECT-based protein sensors are much more sensitive than typical C-V measurements. Considering that the redox peak current i_p is proportional to the density of HRP (W_{HRP}) modified on the gate electrode according to Equation (1), **Figure 2**d also presents the relationship between the density of HRP on the gate and the concentration of HRP in PBS solution for electrode modification. Based on this result, the change of the effective gate voltage ΔV_G^{eff} as a function of [HRP] amount can be schematically presented by plotting ΔV_G^{eff} against i_p (See supporting information, Figure S2). It can be found that ΔV_G^{eff} is proportional to $Log(i_p)$ in a certain region, which is consistent with Equation (3). The slope is smaller in the low concentration region of HRP, indicating that the device is more sensitive at low concentrations. This result is similar to the cases for many other OECT-based biosensors reported before, such as glucose,[32] dopamine[33] and uric acid[34] sensors. The performance of the HRP sensor lays the foundation for our devices to detect HER2 protein in the following experiments.

The OECTs with the design of gate electrodes shown in **Figure 1**a were used to detect the concentration of protein cancer biomarker HER2. The gate electrodes with the sandwiched structure of nanoprobes/HER2/$Ab_{Capture}$/MAA/Au were modified with HER2 protein and electrochemically active nanoprobes. Since a significant number of protein biomarkers in physiological environments have normal levels in ng mL^{-1} or even pg mL^{-1} range,[35] the OECT protein sensors should demonstrate a detection limit lower than this range to accurately differentiate the normal levels of cancer-free patients and the elevated levels indicative of

cancer. HER2 protein solutions with the concentrations varied from 10^{-6} to 10^{-15} g/mL were tested by using the devices. **Figure 3**a shows the channel current responses of the devices to additions of 10^{-4} M H_2O_2 in PBS solutions. The lower the concentration of protein solution used for gate modification, the lower the channel current change (ΔI_{DS}) is observed, indicating that the electrochemical activity on the gate electrode is dependent on the protein concentration monotonically. As shown in the supporting information in Figure S3, the channel current change increases with the increase of protein level and demonstrates a good linear response (R=0.9898) in a wide concentration range (10^{-7} g/mL to 10^{-14} g/mL). According to the channel current response, the change of effective gate voltage ΔV_G^{eff} of the device is calculated, which also demonstrates a linear relationship with the protein concentration on logarithmic axis, as shown in **Figure 3**b. The detection limit of the device is about 10^{-14} g/mL at the condition of signal to noise ratio higher than 3.[31] Therefore, the OECT-based protein sensors would be sensitive enough to detect the HER2 expression levels both in normal and cancer patients even using a small amount of samples.[36] The ultra-low detection limit of the protein sensor could be attributed to the inherent amplification function of the OECTs. In comparison, the HER2 modified gate electrodes were used as working electrodes in electrochemical C-V measurements (**Figure 3**c). The detection limit to HER2 protein in the C-V measurements is only about 10^{-8} g/mL, which is nearly 6 orders poorer than that of the OECT-based HER2 protein sensor. More importantly, we also notice that the OECT-based HER2 sensors are much more sensitive than the reported electrochemical approaches in literature, which normally exhibited the detection limit higher than 10^{-13} g/mL.[21]

To validate the effect of labelling nanoprobes, devices with or without the modification of the nanoprobes were characterized in PBS solutions. The preparation conditions of the two devices at other steps were controlled to be identical. Both devices were modified with HER2

protein by incubating HER2 PBS solutions (10^{-10} g/mL) on the Au gate electrodes for 2 hours. We found that the device labeled with nanoprobes demonstrated a significant decrease in channel current when 10^{-4} M H_2O_2 was added, while the device without nanoprobes showed no obvious response upon the addition of H_2O_2 (see supporting information, Figure S4). The results clearly confirm that the nanoprobes on the gate electrodes of OECTs play a key role on the device response to the addition of H_2O_2. Since the concentration of the nanoprobes is proportional to the concentration of HER2 proteins captured on the gate electrodes of OECTs, the channel current responses can be used to identify the concentrations of HER2 proteins in tested solutions after calibration.

Selectivity is a significant consideration for cancer cell biomarker sensors.[16] Human serum is a complex medium consisting of a myriad of biological elements and chemicals. Devices lack of selectivity would not be able to accurately and specifically differentiate the expression levels of HER2 protein in cancer cells and normal cells. To elucidate the selectivity of the functionalized OECT protein sensor, HER2 was replaced with other members of the HER family HER3 and HER4 with the same concentration in PBS solution in the procedure of device modifications while other steps and conditions are kept unchanged.[37] **Figure 3**d demonstrates the channel current responses of the sensors treated with HER2, HER3 and HER4 protein solutions. The OECT treated with HER2 protein solution displays an obvious current drop upon the H_2O_2 addition, while the control devices demonstrate no detectable response. These results indicate that HER2 protein serves as the specific bridge to covalently connect the $Ab_{Capture}$ layer and the nanoprobe outer layer in the sandwiched modification technique of the gate electrode. Without the HER2 layer, the multifunctional nanoprobes used in the next step would be easily rinsed away during the gate preparation procedure. As discussed above, the absence of nanoprobes on gate electrodes would not induced any response in channel currents when H_2O_2 was added. Therefore, the OECT-based protein

sensor is highly specific for the HER2 biomarker detection. This device also shows good stability during measurements in PBS (See supporting information, Figure S5).

Next, the OECT protein sensors are used to differentiate breast cancer cells from normal cells to demonstrate the accuracy of the devices in real biomedical analysis. We tested the lysates of two types of cells, including MCF-7 and NIH/3T3. MCF-7 cell is a breast cancer cell line primarily used in breast cancer research,[38] while NIH/3T3 cell (mouse embryonic fibroblast cell) is the standard normal cell line used for control experiments.[39] It has been reported that the expression levels of HER2 are higher in cancer cells than in normal cells.[40] In the gate modification procedure shown in **Figure 1**, cell lysate solutions were used to replace the HER2 protein solution while other steps are unchanged. Different densities of cells from 10 to 10^3 cells/μL were tested in the experiments. **Figure 4**a demonstrates the channel current responses of the OECTs modified with the MCF-7 cell lysate to the additions of 10^{-4} M H_2O_2 in PBS solutions. We can find that the OCETs modified with the breast cancer cell lysates show significant responses to the addition of H_2O_2 and the channel current change increases with the increase of cell density. **Figure 4**b shows the response of the control group modified with the normal NIH/3T3 cell lysate. The responses induced by H_2O_2 addition are relatively low in comparison with devices modified with the cancer cell lysates. Even when the NIH/3T3 cell lysate of 10^3 cells/μL solution was used in device modification, the channel current response is much lower than that observed in devices for sensing cancer cells, indicating the lower expression level of HER2 in NIH/3T3 cell than in cancer cells.

The protein sensors were used to directly test living cells in the next step. Because cancer cells have more HER2 proteins on their membranes than normal cells, cancer cells have a higher possibility to be captured by the HER2 antibody on the Au gate (See supporting information, Figure S6 & S7). On the other hand, higher density of HER2 protein on cancer

cells will enable the labelling of the nanoprobes with a density higher than that on normal cells. As show in **Figure 4**c, the sensors modified with MCF-7 cancer cells demonstrate obvious responses in channel currents upon H_2O_2 addition and the current change increases with the increase of cell density in the tested cell solutions. In contrast, the devices modified with NIH/3T3 normal living cells demonstrate negligible response to H_2O_2 addition even for a high concentration cell solution (10^3 cells/μL) because normal cells are difficult to be captured by the antibody $Ab_{Capture}$ (See supporting information, Figure S8). Therefore, the OECTs could differentiate the breast cancer cells from normal cells in the tests.

According to the channel current responses, the changes of effect gate voltage ΔV_G^{eff} induced by the reaction of H_2O_2 on the gates can be calculated. **Figure 4**d shows ΔV_G^{eff} of the devices modified with different cell lysates or cells. It is obvious that the responses of the devices treated with cancer cells are much larger than that of the device modified with normal cell lysate. Therefore, the functionalized OECTs can serve as high-performance protein biomarker sensors for highly sensitive and selective detections of HER2 from cancer cells. It is notable that ΔV_G^{eff} of the devices modified with living cells is smaller than that of the devices modified with cell lysates prepared from the same concentration cells, which can be attributed to the fact that HER2 protein inside living cells cannot be detected in the former case. For living cancer cells, only a limited proportion of HER2 protein located on the surface of cell membrane could react with the antibody and serve as the bridge to capture the catalytic nanoprobes. Therefore, the devices modified with cell lysates are able to load a higher amount of nanoprobes on the gate electrode and lead to larger responses.

In summary, OECTs are successfully used as highly sensitive protein sensors for the detection of protein cancer biomarkers. The gate electrodes of the devices are modified with an antibody that can selectively capture the target protein. By further modifying the protein on

the gate electrodes with catalytic nanoprobes, the devices show obvious current responses to additions of H_2O_2 and the magnitude of current response increases with the increase of protein concentration in the detected solution. The devices can specifically detect a cancer biomarker HER2 down to the level of 10^{-14} g/mL, which is several orders of magnitude lower than those of conventional electrochemical approaches. The OECT protein sensors demonstrate responses to a wide range of HER2 protein levels, from 10^{-14} g/mL to 10^{-7}g/mL, which is sensitive enough to detect the trace amount of HER2 level both in the breast cancer cells and normal cells. Based on this strategy, the protein sensors are successfully used to differentiate cancer cells from normal cells with excellent selectivity in testing either cell lysate or living cells. The sensing mechanism of the protein sensor is attributed to the monotonically increased electrochemical activity on the gate electrode with the increase of protein concentration. This work demonstrates that OECTs are a versatile platform for disposable, flexible and highly sensitive biosensors.

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Figures:

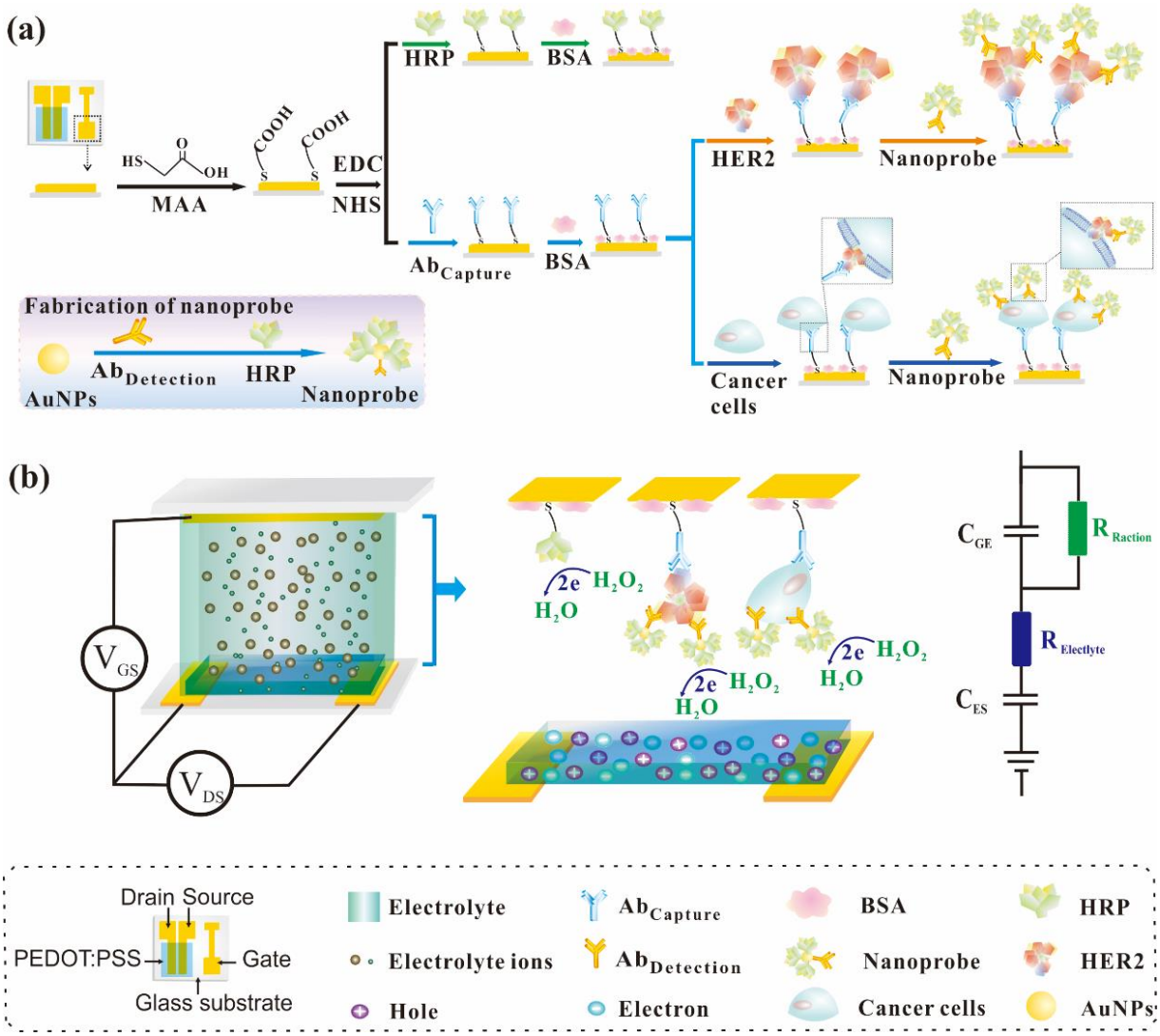

Figure 1. Scheme of the OECT-based biosensor for the detection of a cancer cell protein biomarker HER2. (**a**) The gate modification processes for the detection of HER2 protein biomarker and cancer cells. Below: the fabrication process of the multifunctional nanoprobe with high electrochemical activity. (**b**) Left: OECTs with functionalized gates characterized in liquid electrolytes. Middle: Three types of gates modified with HRP or HER2 protein or cancer cells for OECTs. Right: The equivalent circuit between the gate and the channel of an OECT in an electrolyte. C_{GE} and C_{EC} correspond to the capacitances of the two electric double layers at the gate/electrolyte interface and the electrolyte/channel interface, respectively.

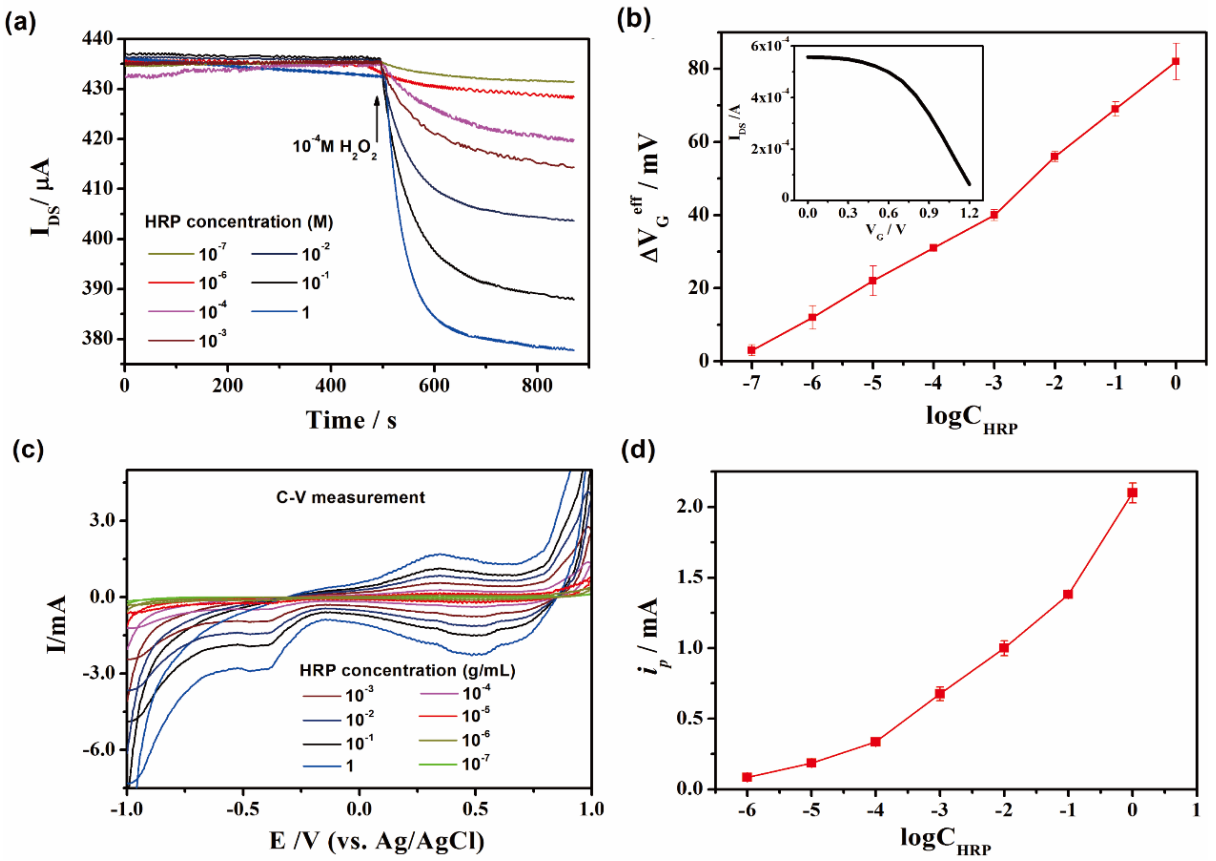

Figure 2. The influence of HRP enzyme on the electrochemical activity and the performance of OECTs with HRP/MAA/Au gate electrodes. (**a**) Channel current (I_{DS}) responses of OECTs to the addition of 10^{-4} M H_2O_2 in PBS solution. The gate electrodes of the OECTs are functionalized in HRP solutions with the concentrations changed from 10^{-7} to 1 g/mL. (**b**) The change of the effective gate voltage ΔV_G^{eff} as a function of HRP concentration during gate modification. (**c**) C-V measurement of the Au gates modified with HRP characterized in 10^{-4} M H_2O_2 PBS solutions. (**d**) Redox peak current as a function of HRP concentration during the modification of Au electrodes.

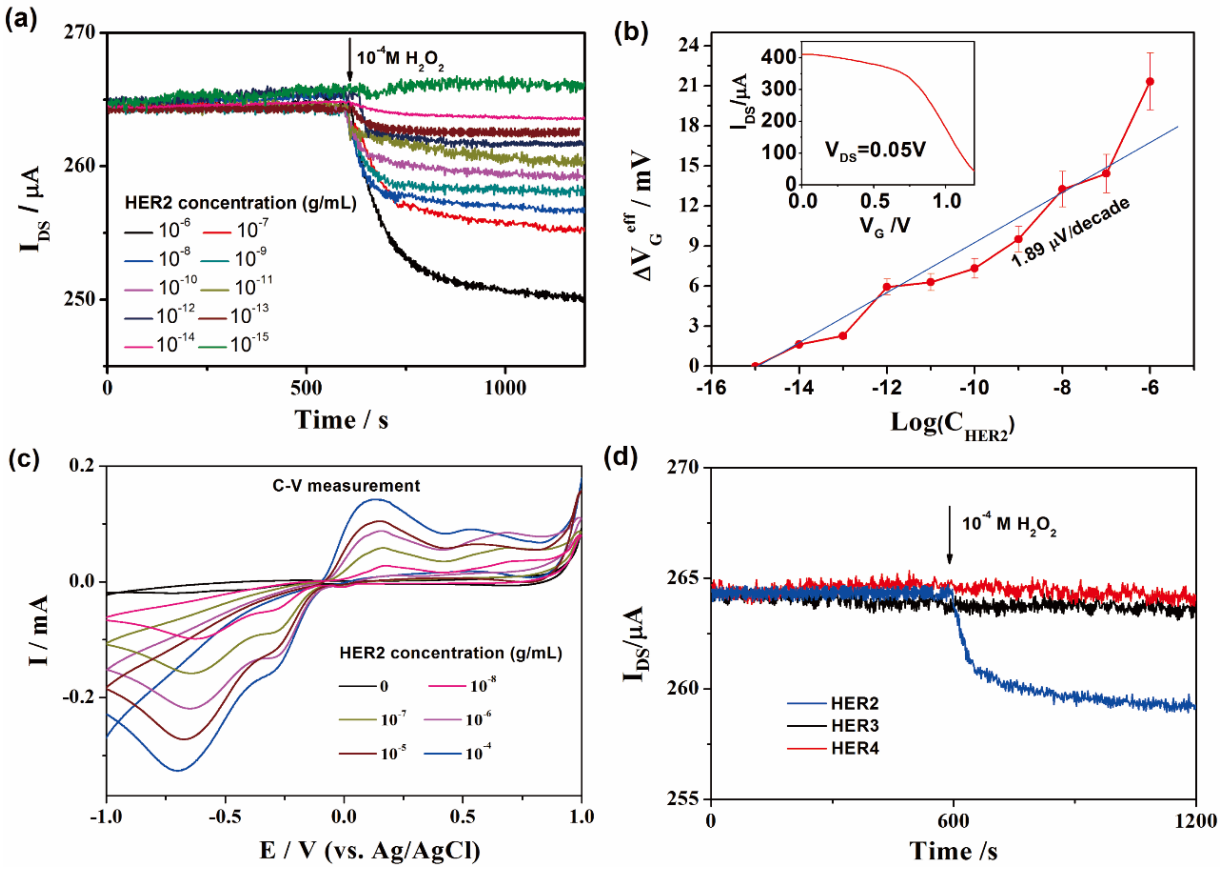

Figure 3. OECT-based protein sensors with nanoprobes/HER2/$Ab_{Capture}$/MAA/Au gate electrodes for the detection of cancer cell biomarker HER2. **(a)** The channel current responses of the devices to the addition of H_2O_2 at the operational voltages of V_G=0.6V and V_{DS}=0.05V. (**b)** The change of effective gate voltage (ΔV_G^{eff}) induced by the reaction of H_2O_2 on the gate electrodes. **(c)** C-V curves of the Au gates of the devices characterized in 10^{-4} M H_2O_2 PBS solutions (**d)** Current response of the OECT-based biomarker sensors modified with different proteins, including HER2(blue), HER3 (Black) and HER4 (Red).

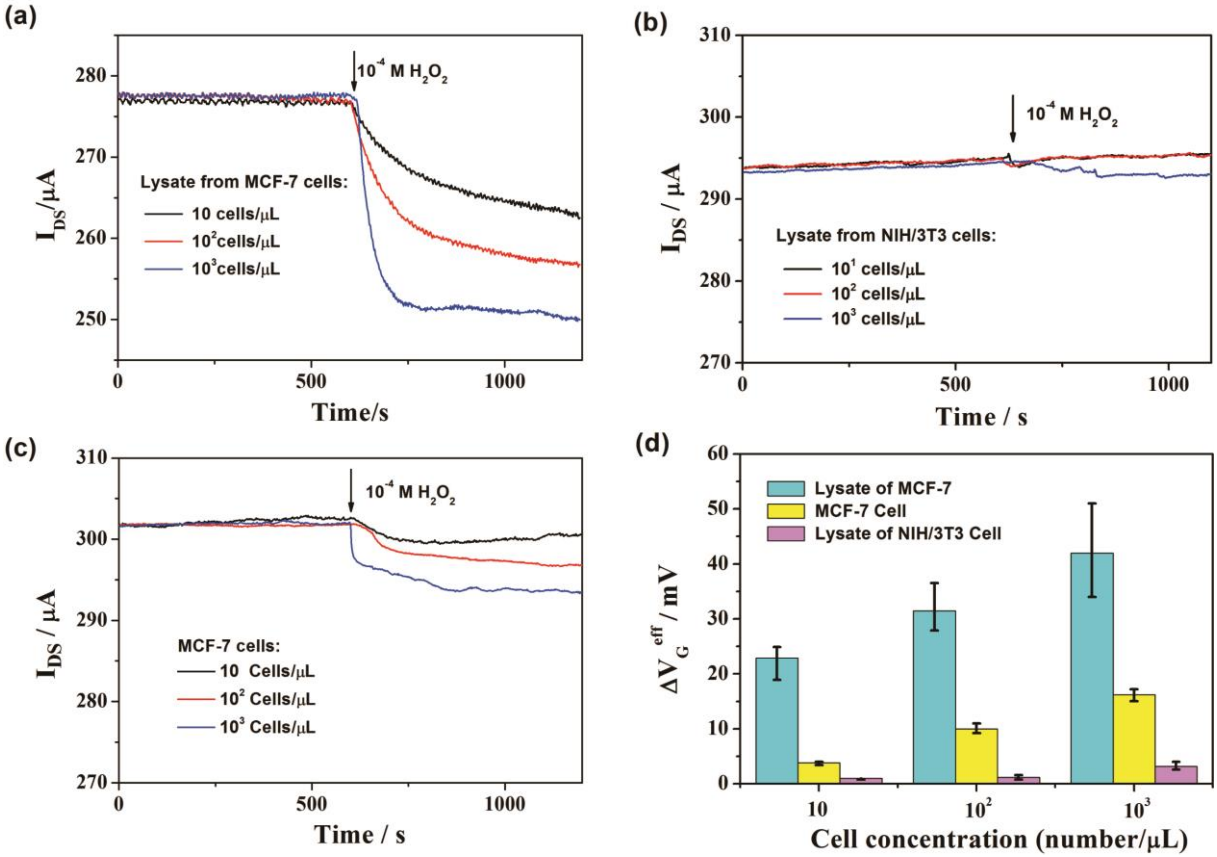

Figure 4. OECT-based protein sensors for the detection of cancer cells. **(a)** Current responses of OECTs modified with the lysate of MCF-7 cancer cells to the additions of 10^{-4} M H_2O_2. V_G=0.6V and V_{DS}=0.05V. **(b)** Current responses of OECTs modified with the lysate of normal cells NIH/3T3 (control devices). **(c)** Current responses of OECTs modified with MCF-7 cells on the gates. **(d)** The change of the effective gate voltage ΔV_G^{eff} for the devices modified with different cell lysates or whole cells. Error bars are calculated from at least 3 devices.

The table of contents entry should be 50–60 words long, and the first phrase should be bold. The entry should be written in the present tense and impersonal style. The text should be different from the abstract text.

We report novel organic electrochemical transistor based protein sensors that can specifically detect a cancer biomarker human epidermal growth factor receptor 2 down to the concentration of 10^{-14} g/mL (10^{-16} M) and differentiate breast cancer cells from normal cells at various concentrations. This work paves a way for developing ultrasensitive low-cost biosensors for the detection of biomarkers in clinical analysis.

Keyword: protein biomarker, Organic electrochemical transistor, biosensor

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Highly Sensitive Detection of Protein Biomarkers with Organic Electrochemical Transistors

ToC figure ((Please choose one size: 55 mm broad × 50 mm high **or** 110 mm broad × 20 mm high. Please do not use any other dimensions))