Electronic Cortisol Detection Using Antibody-Embedded Polymer Coupled to a Field-Effect Transistor

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Experimental Section

1. Antibody functionalization

ITO/PET substrates purchased from Sigma Aldrich were used. The substrates were sliced into 1.5 cm × 2 cm for the remote electrode. The substrates were cleaned with isopropanol for 20 min under ultrasonication, then rinsed with distilled water for 20 min, and dried under nitrogen gas. 10 mg/ml PSMA (678240, Sigma Aldrich) was added in a mixed solvent composed of 0.5 ml dichloromethane (DCM), 0.5 ml N,N-dimethylformamide (DMF), and 40 mg/ml of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). DMF was chosen among the organic solvents because high antibody activity was observed over an extended period in this solvent[1]. After an hour, 15 mg/ml N-hydroxysuccinimide (NHS) was added to the mixture. EDC/NHS was added to activate the carboxylic acid groups of PSMA. After spin-coating PSMA on ITO/PET substrate, 100 µl of 1 mg/ml antibody (CORT-2, Abcam) was immobilized on the surface by drop-coating for 6 hours at room temperature. Also, the anti-cortisol antibody we used has 100% reactivity to cortisol and 0% reactivity to 17-α-HPG according to the data sheet provided by Abcam. This reactivity is reflected in our data.

For anti-em PSMA, 100 µl of 1 mg/ml anti-cortisol was added 20 min after addition of NHS and stirred for 4 hours. The mixture was constantly stirred at 180 rpm with a tris bar at room temperature during the functionalization procedure. The resulting mixture was spin-coated on the freshly cleaned ITO/PET substrate, first at 500 rpm for 10 seconds, then at 3000 rpm for 120 seconds. The thickness of anti-em PSMA was about 300 nm measured by light amplification by stimulated emission of radiation (LASER) microscopy. O-ring with diameter of 4.5 mm and height of 1.78 mm was attached by epoxy glue to form a chamber.

2. Electrical measurement

The platform was divided into two parts: a commercial metal-oxide-semiconductor field-effect transistor (CD4007UB) and remote polymer sensing gate. Sensing membranes completed by following procedures above were connected to the gate of commercial FETs (Fig. 1a). An Ag/AgCl reference electrode was used to apply gate bias for all measurements. All transfer curves were measured using a Keithley semiconductor analyzer, at a drain voltage of 50 mV in gate voltage double sweep mode. All transfer curves were measured with a double sweep method to check for hysteresis in the transfer curves. PSMA membrane containing EDC and NHS were used for pH sensitivity and hysteresis experiments (in the absence of cortisol). For cortisol tests in both anti-em and anti-suf PSMA membranes, cortisol (C-106, Cerilliant) dissolved in methanol at concentration 1 mg/ml was diluted in 1× and 0.05× PBS to concentrations of 1 fg/ml to 10 ng/ml. For the specificity test, 17-α-HPG (H5752, Sigma Aldrich) dissolved in methanol at concentration 1 mg/ml was diluted in 1× and 0.05× PBS to the same concentrations as cortisol samples. For sweat tests, two types of artificial perspiration with different pH were purchased from Pickering Laboratories (Catalog number: 1700-0521 for pH 7.4 and 1700-0024 for pH4.5). 10 % of 1× PBS was added in the artificial perspiration as a buffer against non-ideal factors such as shifting background pH values. This mixture between artificial sweat and 1× PBS was used to dilute 1 mg/ml cortisol. Before evaluating drift or quantifying cortisol, all PSMA membranes were washed three times by PBS before they were stabilized for 30 - 40 minutes in PBS solution (Fig. S12). Transfer curves for each cortisol concentration were measured after 5 min of incubation with the cortisol solution, following initial stabilization of the membrane, using 20 µl of sample solution. The drift was evaluated by measuring transfer curve switching voltages with

the same frequency after stabilization of the membrane as in the cortisol sensing tests over 50 minutes in 1× and 0.05× PBS.

3. ELISA

Two different types of samples were prepared for fluorescence scan: surface functionalized PSMA samples and antibody embedded PSMA samples. Following the antibody functionalization methods discussed above, an ethanolamine treatment was applied prior to the ELISA FI measurement. Without ethanolamine, additional non-specific binding occurs by the detection antibody (Fig. S13). Therefore, ethanolamine was added to deactivate COOH groups on the surface to minimize non-specific binding. Ethanolamine (pH 13) was brought to pH 8.5 by adding hydrochloric acid.

For ELISA on the surface functionalized membrane, 10 mg/ml PSMA in the same organic solution as before was spin-coated on ITO/PET substrate with surface sensing area of 0.5 cm x 0.5 cm. 25 µL of 1mg/ml of the primary antibody (anti-cortisol) was then drop-coated and left at room temperature for 6 hours. After 6 hours, the surface was washed 5 times using 1× PBS. Ethanolamine solution was used to cover the functional surface and reacted for 20 minutes at room temperature. After 20 minutes, the surface was washed 5 times using 1× PBS. 100 µL of 100 µg/mL cortisol solution was then dropped on the surface and left at room temperature for reaction with the surface functionalized primary antibody. After 30 minutes of reaction time, the surface was washed 5 times using 1× PBS. Finally, 80 µL of 200 ng/ml FITC conjugated cortisol antibody (LS-C305750, LifeSpan BioSciences) was dropped on the washed surface and left at room temperature for reaction with the cortisol-antibody complex immobilized on the surface. After 30 minutes of reaction time, the surface was washed 5 times using 1× PBS. Fluorescence

scans were conducted on the samples via a plate reader. For antibody-embedded samples, 10 mg/ml PSMA in DCM/DMF and EDC/NHS samples were prepared as above. The primary antibody was injected into the PSMA solution 20 minutes after adding NHS. The PSMA solution was stirred at room temperature for 4 hours. The same procedures as surface functionalized samples were repeated to measure the FI.

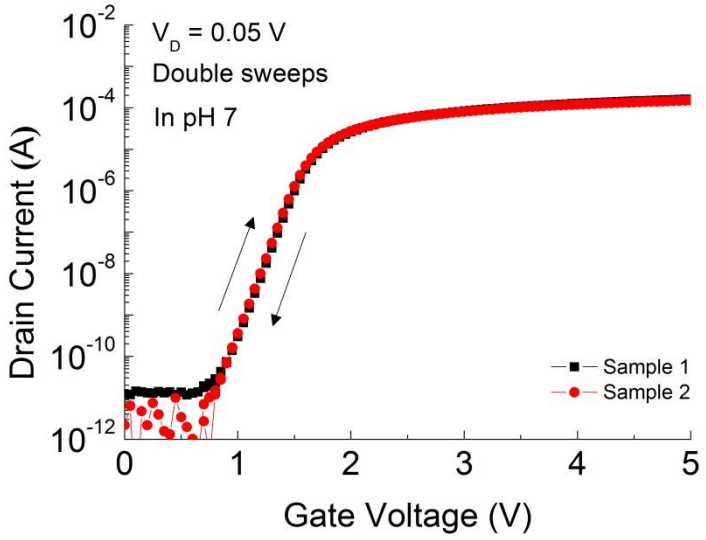

Figure S1. Two transfer curves measured in pH7 simultaneously. Each PSMA membrane is independently connected to the individual FET as shown in Fig. 1a. There is insignificant difference between two curves and no hysteresis was observed in double sweep.

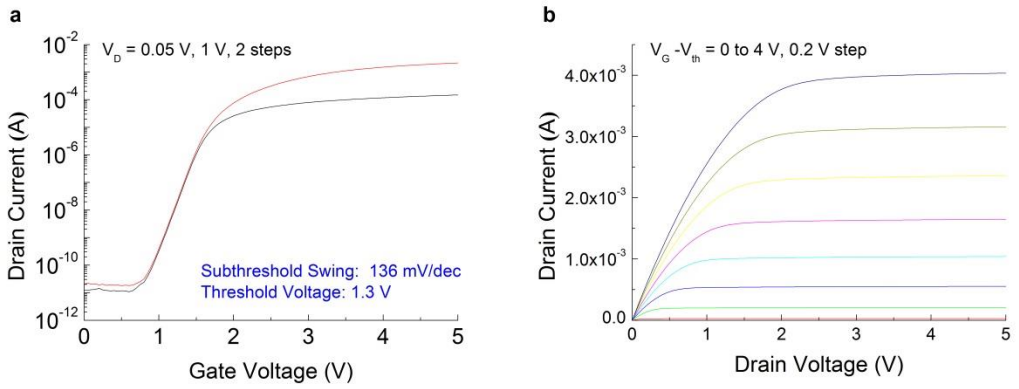

Figure S2. Representative transfer curve (a) and output characteristic (b) of commercial FET, CD4007UB. Subthreshold swing of 136 mV/dec and threshold voltage of 1.3 V were observed in the commercial FET.

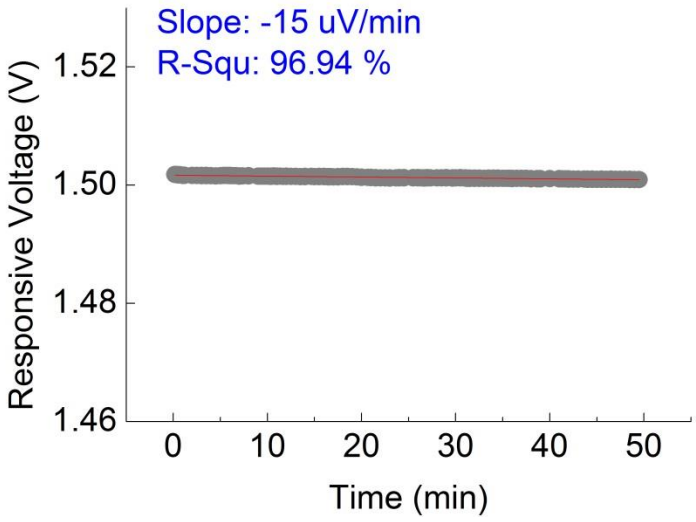

Figure S3. Drift property from intrinsic commercial FET. Transfer curves with gate voltage sweep from 0 to 5 V were repeated for 50 min at drain voltage of 50 mV. Responsive voltages (V_R) were extracted using gate voltages corresponding to a drain current of 1 μA. Drift occurs by a negative slope of -15 μA/min in the V_R vs time plot.

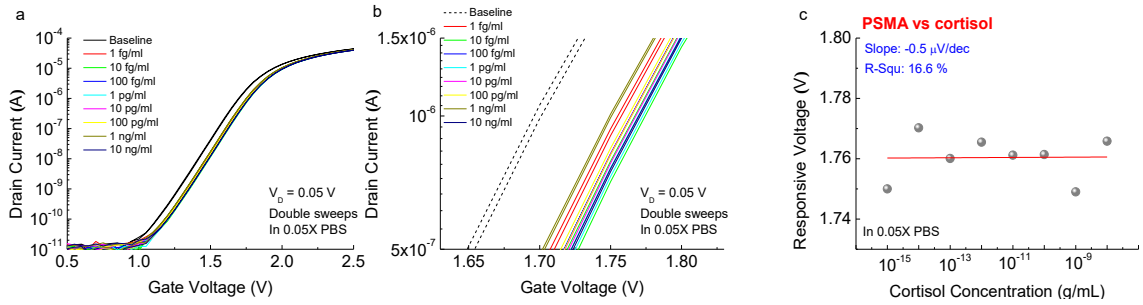

Figure S4a, Representative transfer curves of bare PSMA vs. cortisol concentration in 0.05× PBS. **S4b,** Close-up transfer curves of Figure **S4a**. **S4c,** V_R corresponding to reference current of 1 µA vs. different concentrations of cortisol in 0.05× PBS solution. Random signal was observed.

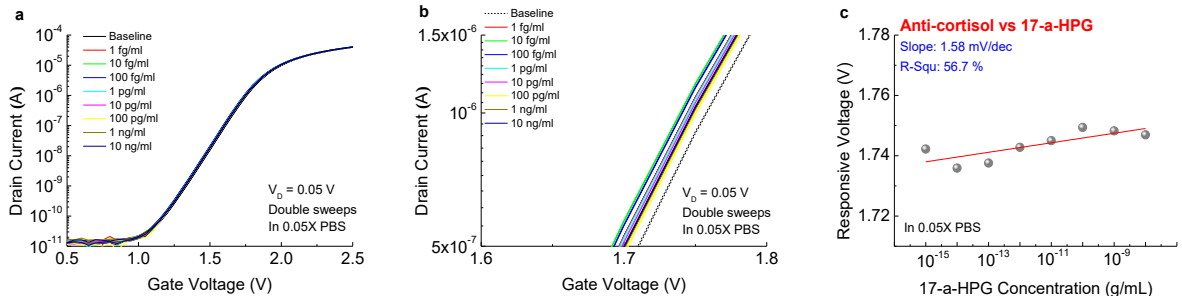

Figure S5a, Representative transfer curves from surface-functionalized PSMA by anti-cortisol vs. different 17-a-HPG concentration in 0.05× PBS. **S5b,** Close-up transfer curves of Figure **S5a**. **S5c,** V_R corresponding to reference current of 1 µA vs. different concentrations of 17-a-HPG in 0.05× PBS solution. Random signal was observed.

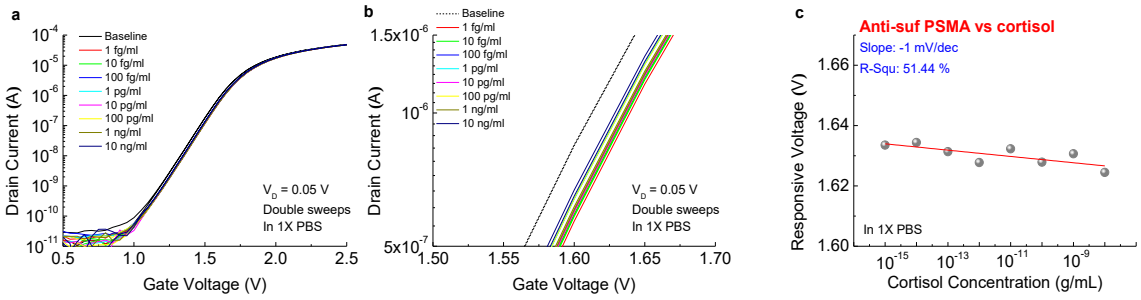

Figure S6a, Representative transfer curves of anti-suf PSMA vs. cortisol concentration in 1× PBS. **S6b,** Close-up transfer curves of Figure **S6a**. **S6c,** V_R corresponding to reference current of 1 µA vs. different concentrations of cortisol in 1× PBS solution. Random signal was observed.

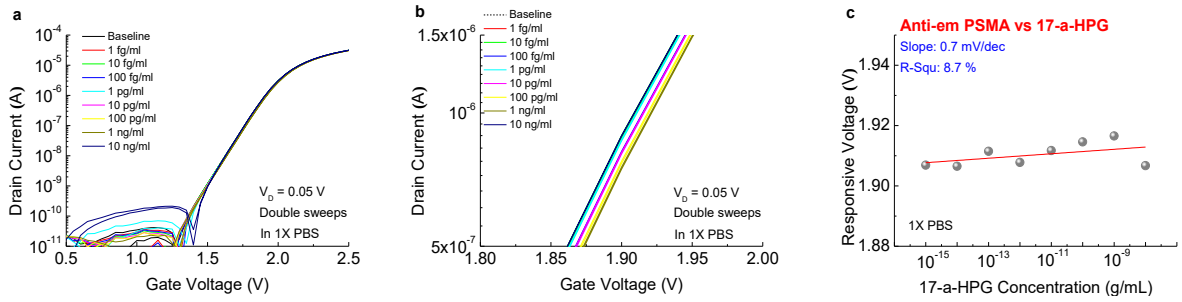

Figure S7a, Representative transfer curves of anti-em PSMA vs. different 17-a-HPG concentration in 1× PBS. **S7b,** Close-up transfer curves of Figure **S7a**. **S7c,** V_R corresponding to reference current of 1 µA vs. different concentrations of 17-a-HPG in 1× PBS solution. Random signal was observed.

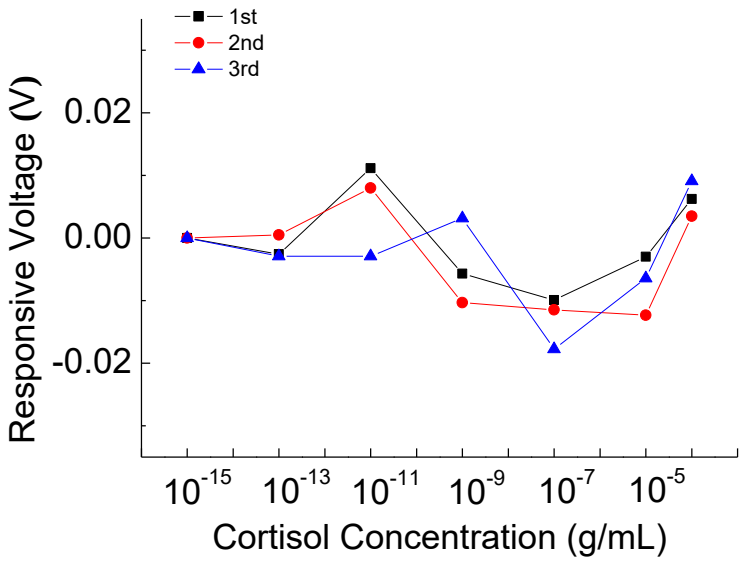

Figure S8, V_R vs. different concentrations of cortisol in artificial sweat with pH 7.4 showing random signals from 3 samples out of a total of 8. 10 % 1× PBS was added to sweat.

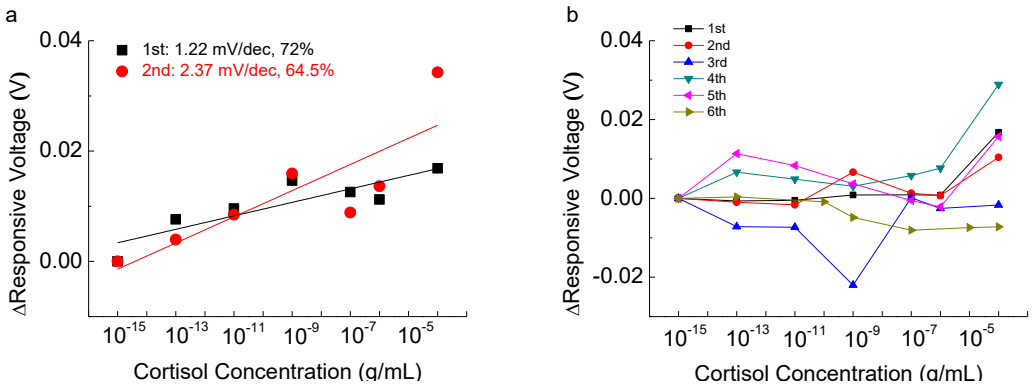

Figure S9a V_R vs. different concentrations of cortisol in 100 % artificial sweat with pH 7.4. Only 2 samples out of a total of 8 showed a linear response with less linearity for cortisol in 100 % artificial sweat with pH7.4. **S9b,** Random signals from 6 samples.

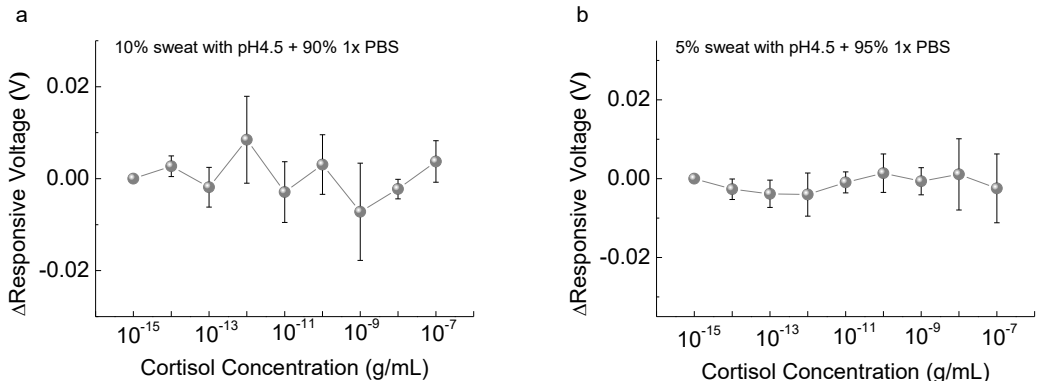

Figure S10a V_R vs. different concentrations of cortisol in 5 % artificial sweat and S10**b**, in 10 % artificial sweat with pH 4.5 diluted in 1× PBS. 6 samples out of a total of 6 showed random signals in 10 % artificial sweat. 4 samples out of a total of 4 showed random signals in 5 % artificial sweat.

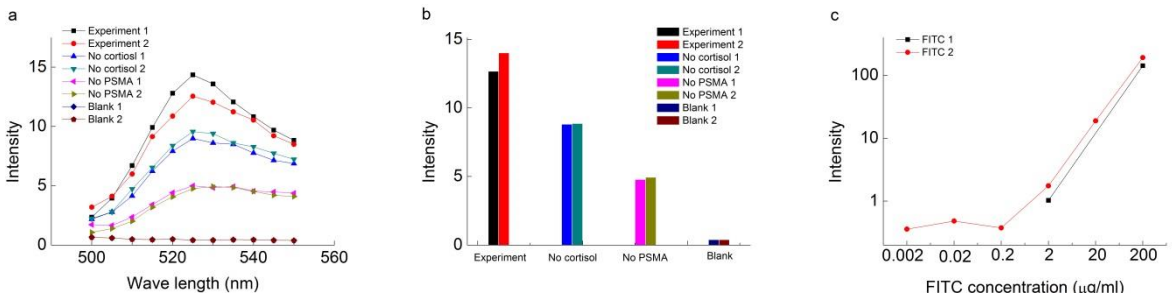

Figure. S11a, Fluorescence intensity regarding anti-em PSMA and the samples under different controls excluding cortisol or PSMA. 2 µg/ml cortisol was added for experimental samples. **S11b,** End points for each condition from Figure **S11a**. **S11c,** Calibration curve was drawn from the pure detection antibody including FITC. Intensity of fluorescence was saturated in concentration below 200 pg/ml, which is the detection limit of this ELISA kit. We approximated the concentration of the activated antibody through ELISA results with following steps:

1. Intensity from detection antibody with cortisol: 13.33 (average)
2. Nonspecific binding fluorescence intensity: 8.85 (average)
3. Fluorescence intensity from blank (system noise): 0.4 (average)
4. Fluorescence intensity from specific binding: $13.33 - 8.85 - 0.4 = 4.08$
5. Intensity of 4.08 is equivalent to 4.33 ug/mL on the calibration plot (Fig. S12c).
6. Multiply sample volume of detection antibody (30 uL) used in calculation plot by 4.33 ug/mL: 0.129 ug active capture antibody in the polymer (presumably corresponding to active antibody in anti-em PSMA)
7. Molecular weight of detection antibody: IgG (150 kg/mol) + FITC (389.382 g/mol). Approximate molecular weight of detection antibody is 150 kg/mol.
8. Mole of antibody would be $\frac{1.29 \times 10^{-6}\text{g}}{1.5 \times 10^{5}\text{g/mol}} = 8.66 \times 10^{-13}$ M

9. The number of antibody would be 8.66×10^{-13} M $\times 6.02 \times 10^{23}$ $\frac{\text{molecules}}{\text{M}} = 5.21 \times 10^{11}$ molecules in anti-em PSMA.
10. Sample size of anti-em PSMA is 0.5 cm by 0.5 cm. Thickness of the polymer is 300 nm. Volume of anti-em PSMA is 0.5 cm $\times$ 0.5 cm $\times 3 \times 10^{-5}$ nm $= 7.5 \times 10^{-3}$ cm^3.
11. Number density of antibodies is $\frac{5.21 \times 10^{11} \text{ molecules}}{7.5 \times 10^{-3} \text{ cm}^3} = 6.95 \times 10^{13}\ molecules/cm^3$
12. Mass concentration $= \frac{6.95 \times 10^{13}\ \frac{molecules}{cm^3} \times 1.5 \times 10^{5}\ \frac{g}{mol}}{6.02 \times 10^{23}\ \frac{molecules}{mol}} = 17.3\ \mu g/cm^3$
13. 90 ug/ml antibody mixed in PSMA solution (1100 uL).
14. 550 µL out of 1100µL solution spin-coated on PET/ITO substrate (1 x 1.5 cm)
15. Spin-coated film was divided into 6 films (0.5 x 0.5 cm), so 91.6 µL of solution per film
16. The amount of antibody used for each sample $90 \frac{\mu g}{mL} \times 91.6\ \mu L = 8.24$ ug
17. When we assume all antibodies immobilized on antibody-embedded PSMA.
18. About **1.53** % of this antibody was active in the final film.

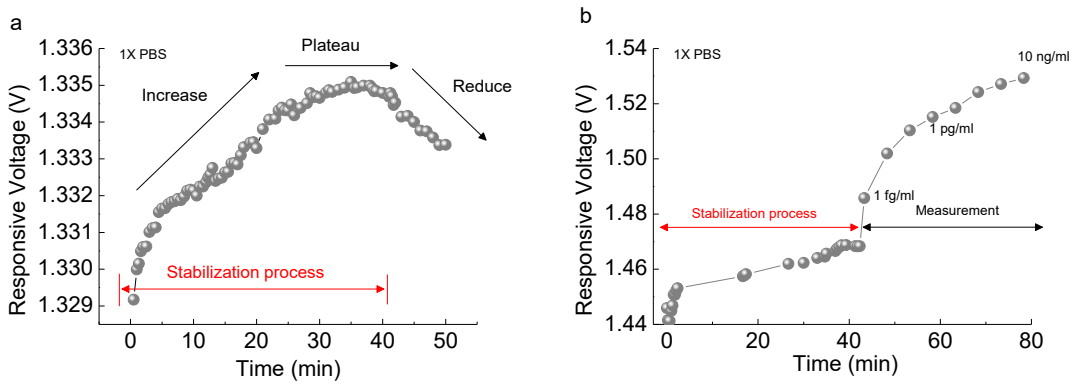

Figure S12a, Representative V_R vs. time plot typically observed in the stabilization process. V_R increases up to 30 to 40 min and then reaches a plateau where it is in equilibrium condition for measurement. If the transfer curve is repeated after the time in plateau, V_R decreased, which is shown as drift in Fig. 2f and Fig. 3d. Every sample was measured after reaching the stabilized states on the plateau. **S12b,** Representative V_R vs. time as a function of cortisol concentrations. After the stabilization process, cortisol concentrations increased V_R values, which are opposite trend to the curve after the plateau in **S12a.**

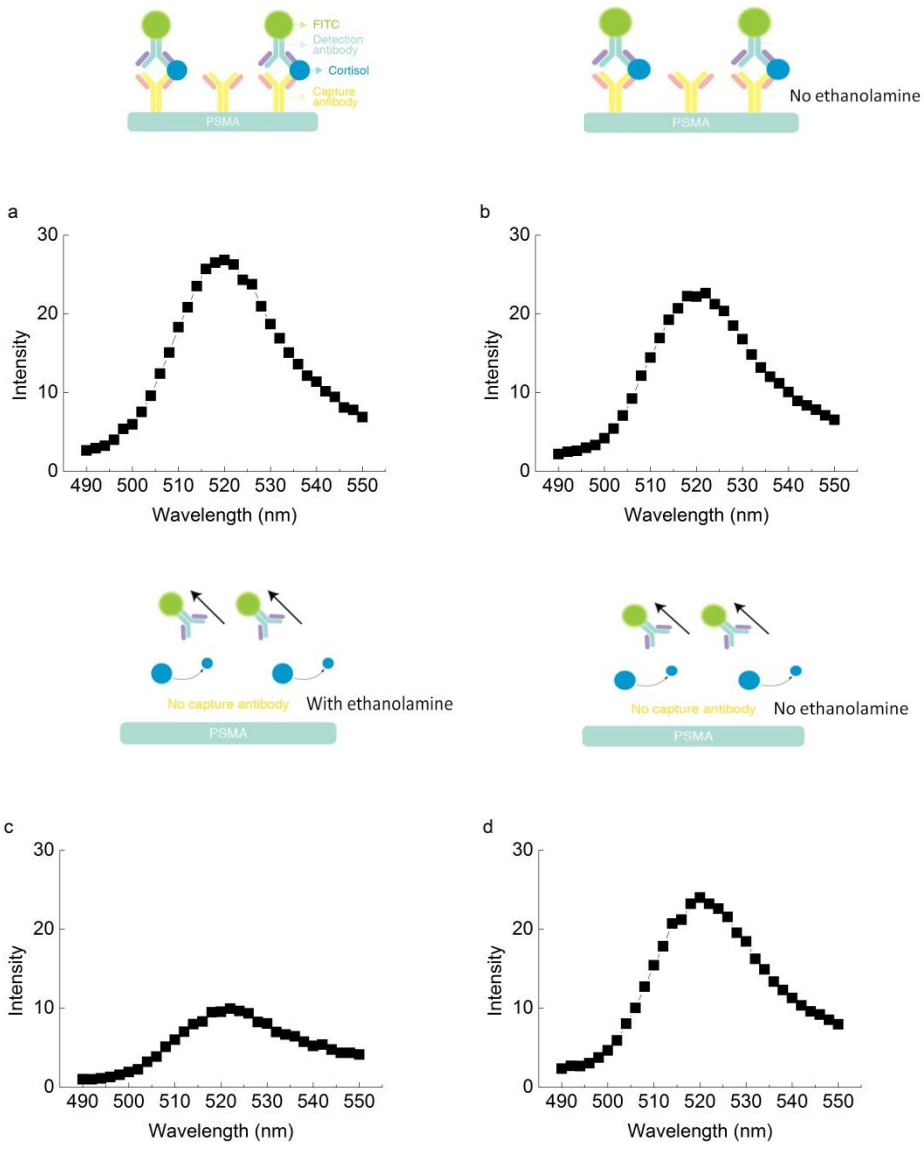

Figure S13a, Fluorescence intensity of FITC labeled anti-suf PSMA via standard ELISA. **S13b,** Fluorescence intensity excluding ethanolamine from procedures in step **S13a**. A similar intensity to that of Figure **S13a** was observed. **S13c,** Fluorescence intensity without primary antibody from procedures in step **S13a**. Reduced intensity was observed in **Fig. S13c** because ethanolamine reduced activated binding sites on the surface. **S13d,** Fluorescence intensity excluding primary antibody and ethanolamine in step **S13a**. Intensity of **Fig. S13d** is similar to that of **Fig. S13a** and **S13b** because of non-specific binding.

Reference for supporting material

1. Rehan, M.; Younus, H., Effect of organic solvents on the conformation and interaction of catalase and anticatalase antibodies. *Int. J. Biol. Macromol.* **2006,** *38*, 289-295.