Electronic Supplementary Material (ESI) for Analyst.

Electronic Supplementary Information

Oxytocin detection at ppt level in human saliva by an extended-gate-type organic field-effect transistor

Kohei Ohshiro [a], Yui Sasaki [a], Qi Zhou [a], Xiaojun Lyu [a], Yusuke Yamanashi [b], Katsumasa Nakahara [b], Hirokazu Nagaoka [b], Tsuyoshi Minami [a,*]

[a] Institute of Industrial Science, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo, 153-8505, Japan

E-mail: tminami@iis.u-tokyo.ac.jp

[b] JNC Petrochemical Corp., 5-1, Goikaigan, Ichihara, Chiba, 290-8551, Japan

Contents

1. General

Materials

An organic semiconductor material, 3,9-dihexyldinaphtho[2,3-*b*:2,3-*d*]thiophene (C6-DNT-VW) was synthesized according to one of the co-author's previous report[1]. Other reagents and solvents were purchased from commercial suppliers and used without further purification. Dulbecco's phosphate-buffered saline and polystyrene (PS; M_w. 3000) were purchased from Sigma-Aldrich Co. LLC. *N*-hydroxysulufosuccinimide sodium salt, *N,N'*-diisopropylcarbodiimide, 2-aminoethanol, uric acid, (3-mercaptpropyl)triethoxysilane, triethoxy(pentafluorophenyl)silane, and pentafluorobenzenethiol were purchased from Tokyo Chemical Industry Co., Ltd. D-Glucose, L-lactic acid, streptavidin, potassium nitrate, human serum albumin, sodium chloride, sodium hydrogen carbonate, and sodium carbonate were purchased from FUJIFILM Wako Pure Chemical Industries, Ltd. 2-Morpholinoethanosulfonic acid was purchased from Dojindo Laboratories. Tween 20 and calcium nitrate were purchased from Kanto Chemical Co. Inc. Creatinine and vasopressin were purchased from Nakalai Tesque, Inc. and Peptide Institute, Inc., respectively. Biotinylated anti-oxytocin antibody (CYIQNCPLG) and oxytocin were purchased from Cosmo Bio Co., Ltd. Saliveht Aerosol was purchased from Teijin Pharma Ltd. Silicon wafers were purchased from SEIREN KST Corp. A gold (Au) particle for vacuum deposition to fabricate an OFET sensor was purchased from Tanaka Kikinzoku Kogyo Co., Ltd. The Ag/AgCl reference electrode (model: RE-1B) and Pt wire counter electrode were purchased from BAS Inc. A silver paste was purchased from FUJIKURA KASEI Co., LTD. Aqueous solutions for all experiments were prepared by using Milli-Q water (18.2 MΩ cm).

Measurements

Electrical characteristics of all OFET devices were measured using a semiconductor parameter analyzer (Agilent, 4156B). Photo-electron yield spectroscopy measurement was carried out using an AC-3 (Riken Keiki, Co.). The pH values of aqueous solutions were measured using a Seven Excellence pH meter from Mettler-Toledo, Ltd. Linear sweep voltammetry (LSV) was performed on an SP-300 potentiostat from Biologic.

2. Fabrication of the extended-gate-type OFET sensor

Fabrication scheme of the extended-gate-type OFET

A silicon substrate (*i.e.,* Si (n^{++})) as a gate electrode was oxidized to form a silicon dioxide layer (100 nm in thickness) as a dielectric. The oxidized substrate was ultrasonically treated with acetone and 2-propanol for 5 min in each process, followed by drying with N_2 gas. Subsequently, the substrate was cleaned by UV-O_3 treatment using a UV-O_3 cleaner (Filgen, UV253V8) for 30 min. Next, the substrate was immersed in a mixture of ethanol and water containing (3-mercaptpropyl)triethoxysilane (MPTES) (1vol.%) at room temperature for 1 h, followed by rinsing with ethanol. The treated substrate was baked at 120 °C for 10 min. The source and drain electrodes (Au, 30 nm in thickness) were deposited on the treated substrate by thermal deposition (ULVAC KIKO, Inc., VTS-350M/ERH) with a shadow mask, and subsequently cleaned by UV-O_3 treatment using the UV-O_3 cleaner for 30 min. The channel width and length of the device were 10,000 and 100 µm, respectively. The surface of the SiO_2 substrate was treated using triethoxy(pentafluorophenyl)silane (PFPTES) in methanol (0.1vol.%) at room temperature for 1 h, and the treated substrate was rinsed with ethanol and 2-propanol. After backing at 120 °C for 10 min, the gold electrodes were fully functionalized with pentafluorobenzenethiol (PFBT) (0.1vol.%) in methanol at room temperature for 1 h. The treated substrate was rinsed with ethanol and 2-propanol, and baked at 120 °C for 10 min. A mixture of C6-DNT-VW (2.0wt%) and polystyrene (M_w. 3000) (0.8wt%) was applied to obtain a reproducible uniform semiconductive layer. The mixture solution at 100 °C was slit-coated on the substrate at 80 °C using a robotic dispenser system (MUSASHI ENGINEERING, INC., IMAGE MASTER® 350). The semiconductive layer was partially wiped off using toluene. A fluorinated polymer material, CYTOP™ (5wt% CTL- 809A in CT-Solv. 180) was spin-coated onto the device, followed by baking at 120 °C for 1 h. Finally, the gate electrode was partially shaved to attach an Ag paste (Fig. S1).

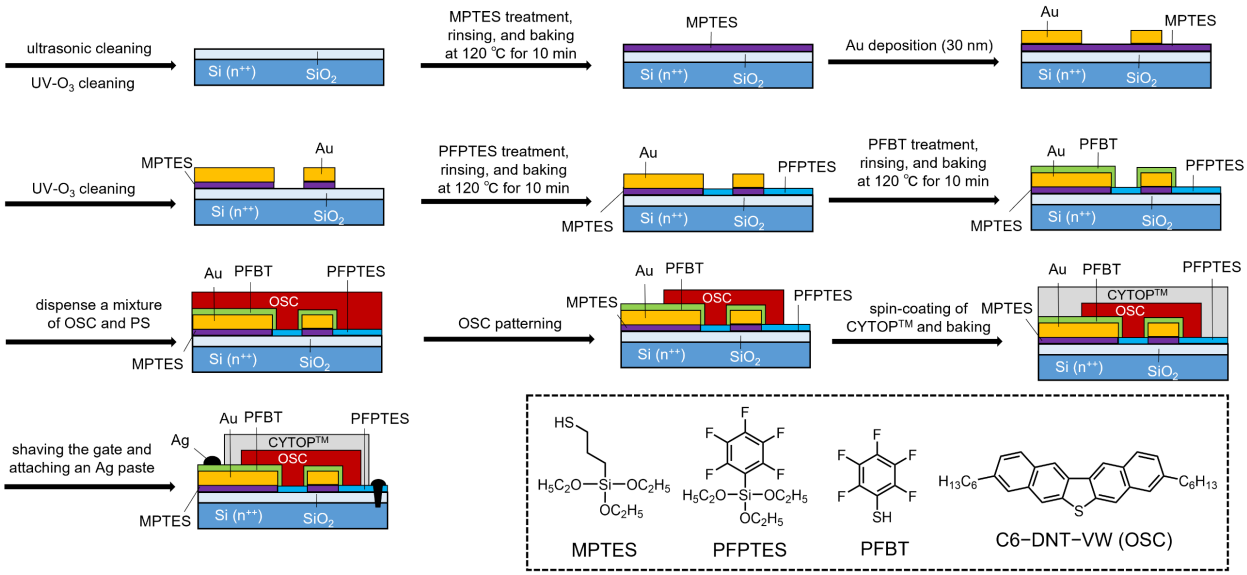

Fig. S1. Schematic illustration of the fabrication process for the OFET.

Fabrication scheme of the oxytocin detectable SAM on the extended-gate electrode

The extended-gate electrode with a sensing area of 15 mm^2 was fabricated through thermal vacuum deposition (Sanyu Electron Co., Ltd., SVC-700TMSGS) of gold (100 nm in thickness) on a polyethylene naphtalate (PEN) film (125 µm in thickness). The Au extended-gate electrode on the PEN film was immersed in an ethanol solution containing 3-mercaptopropionic acid (1 mM) at 25 °C for 1 h. The treated electrode was washed by ethanol and water, and then 10 µL of a 2-morpholinoethanesulfonic acid (MES) buffer solution (100 mM, pH 6.0) containing *N*-hydroxysulfosuccinimide (sulfo-NHS, 5 mM), *N,N'*-diisopropylcarbodiimide (*N,N'*-DIC, 40 mM), and sodium chloride (500 mM) were dropped onto the electrode. The coupling reaction time for the sulfo-NHS ester formation was set to be 30 min. After this period, the extended-gate electrode was washed by ethanol, and then 10 µL of a carbonate buffer solution (Na_2CO_3: 15 mM, $NaHCO_3$: 35 mM, pH 9.6) containing streptavidin (500 µg mL^{-1}) was dropped onto the treated electrode, followed by incubation for 2 h. To remove unreacted sulfo-NHS esters on the extended-gate electrode, 5 µL of Dulbecco's phosphate buffered saline (D-PBS) buffer solution (KCl: 2.7 mM, NaCl: 136 mM, KH_2PO_4: 1.5 mM, Na_2HPO_4: 8.1 mM) containing 2-aminoethanol (1 M) was dropped onto the electrode and left standing for 15 min. Subsequently, the electrode was immersed in D-PBS buffer solution containing Tween 20 (0.05wt%) and human serum albumin (HSA) (0.1wt%) for 15 min. Finally, the extended-gate modified with streptavidin was immersed in D-PBS solution containing the biotinylated anti-oxytocin antibody (30 µg mL^{-1}), Tween 20 (0.05wt%), and HSA (0.1wt%) at 25 °C for 30 min. The extended-gate electrode was washed by D-PBS buffer solution (Fig. S2).

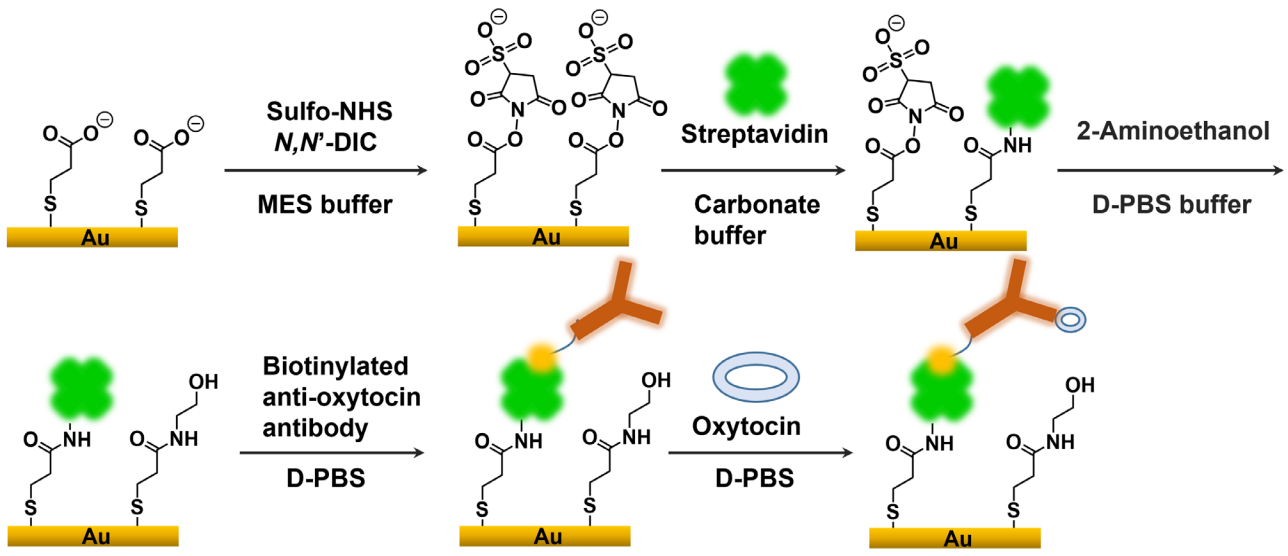

Fig. S2. Preparation scheme of the oxytocin detectable SAM on the extended-gate electrode.

3. Characterization of the extended-gate electrode

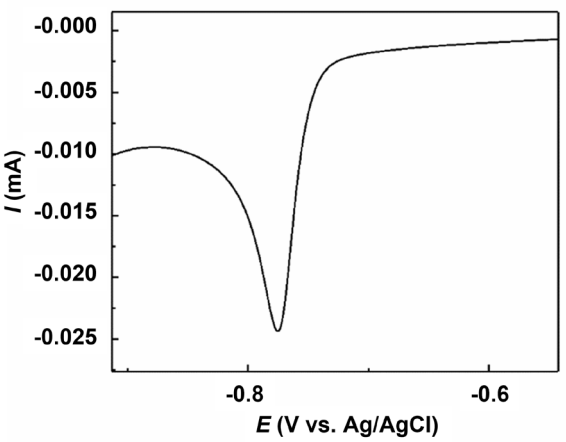

Fig. S3. Linear sweep voltammetry of the molecular density test in the KOH solution (0.1 M). Potential scans from 0 V to −1.6 V, and the scan rate was set to be 20 mV s^{-1}. The molecular density of 3-mercaptopropionic acid-based SAM on the Au electrode was calculated according to Faraday's raw with the integration of the peak area.

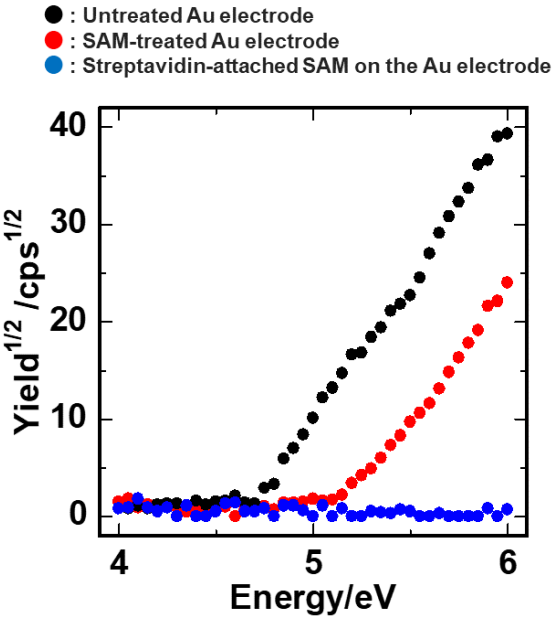

Fig. S4. Results of photo-electron yield spectroscopy measurements in air using the extended-gate electrode. Each plot represents untreated Au electrode (black circle), treated Au electrodes by 3-mercaptopropionic acid (red circle), and the streptavidin-attached SAM (blue circle)

Fig. S5. Results of wettability test using (A) the untreated Au electrode and the treated Au electrodes by (B) 3-mercaptopropionic acid and (C) the streptavidin-attached SAM.

4. Basic characteristics of the OFET

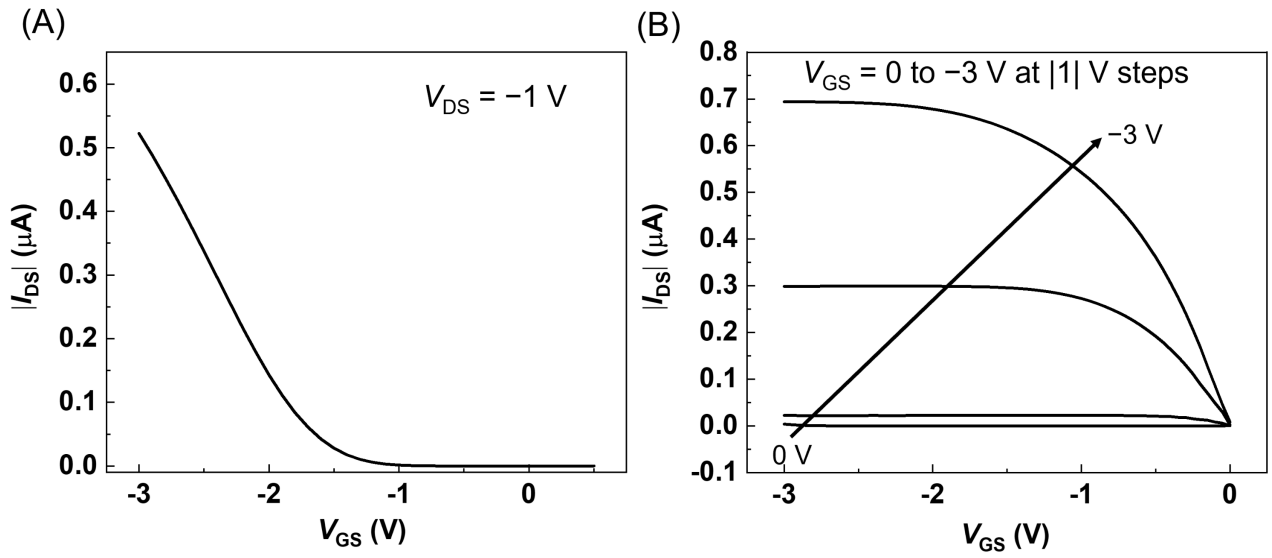

Fig. S6. (A) Transfer and (B) output characteristics of the fabricated OFET.

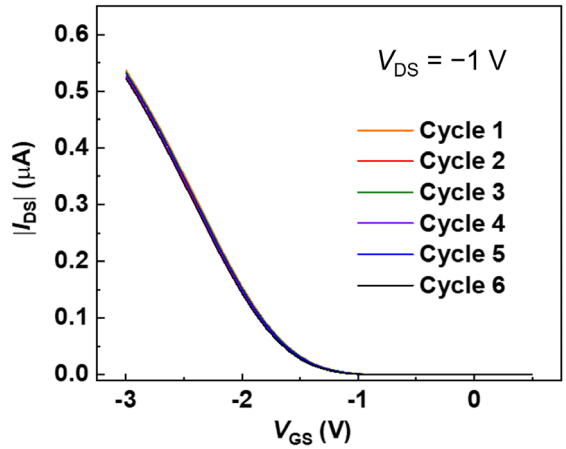

Fig. S7. Transfer characteristics of the OFET. The electrical measurements were repeated six times.

5. Electrical response to the biotinylated anti-oxytocin antibody

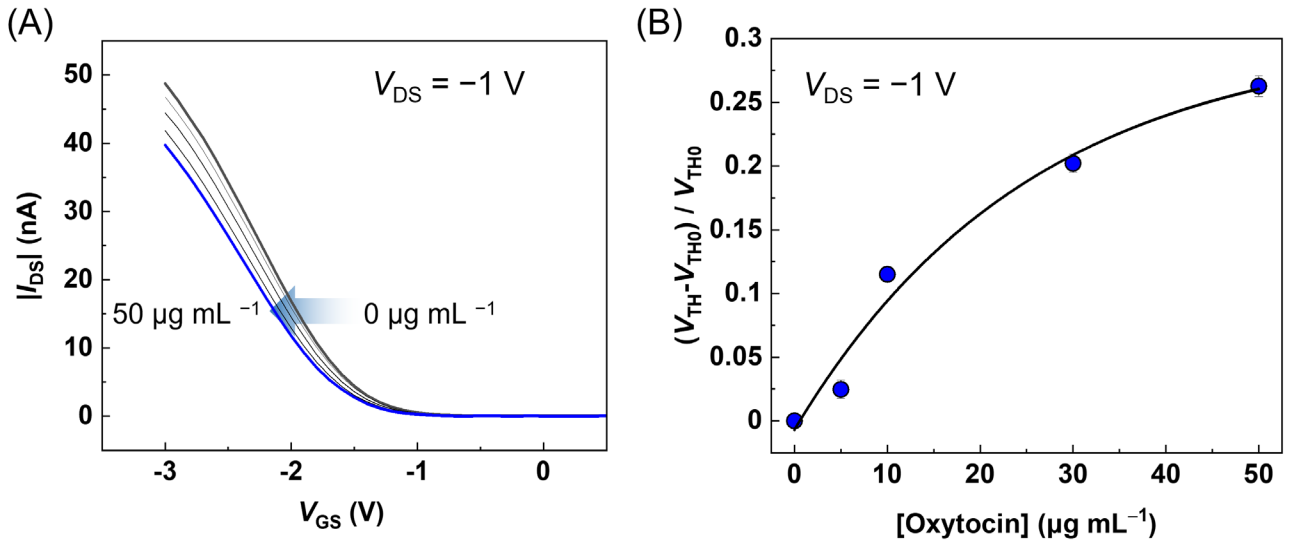

Fig. S8. (A) Transfer characteristics and (B) changes in threshold voltage (V_{TH}) of the OFET upon the addition of the biotinylated anti-oxytocin antibody in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%) at 37 °C. [Biotinylated anti-oxytocin antibody] = 0–50 μg mL^{-1}, incubation time: 30 min.

6. Limit of detection

Table S1. Comparison table for LoD values against oxytocin detection.

Methods for oxytocin detection	LoD (pg mL^{-1})	References
OFET-based immunosensor	0.57	This study
QCM(Molecularly imprinted polymer, MIP)	10	*Chem. Eur. J.*, 2003, **9**, 5107.
QCM (MIP)	30	*J. Pep. Sci.*, 2019; **25**, e3150.
Electrochemical (Nanocomposites)	230	*J. Mater. Sci: Mater. Electron.*, 2021, **32**, 25149.
Electrochemical (MIP)	6×10^{7}	*Biosens. Bioelectron.*, 2018, **100**, 251.
Electrochemical (Immunoassay)	10	BMEiCON-2018
Fluorescent (DNAzyme)	0.4	*Sensors*, 2020, **20**, 5956.
Fluorescent (DNAzyme)	2	*Sens. Actuators B Chem.*, 2018, **254**, 321.
Colorimetric (ELISA)	15	*Infant Behav. Dev.*, 2015, **41**, 26.
SPR (MIP)	3	*Sens. Actuators B Chem.*, 2015, **221**, 842.

7. Selectivity

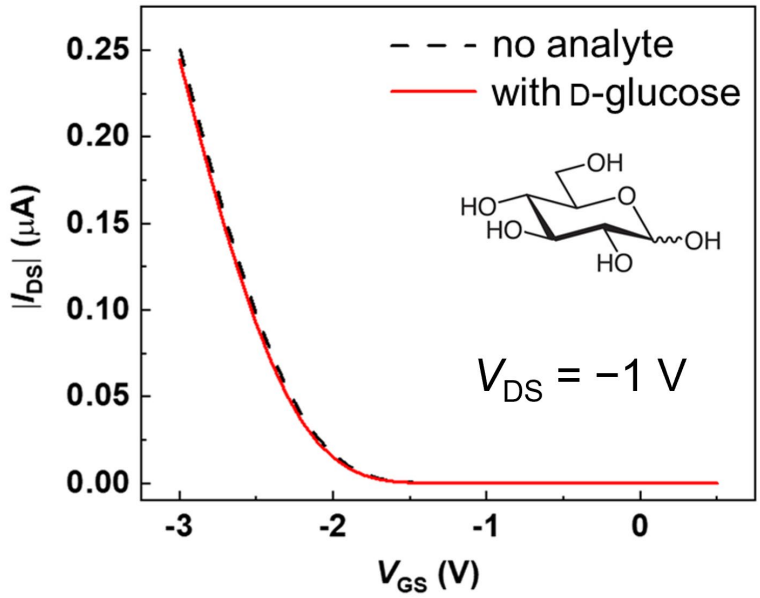

Fig. S9. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of D-glucose (10 μg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

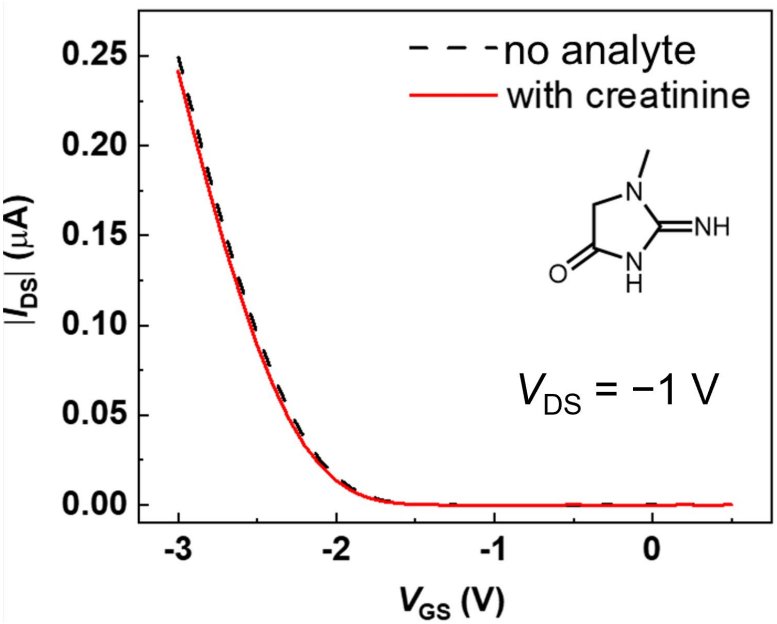

Fig. S10. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of creatinine (0.3 μg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

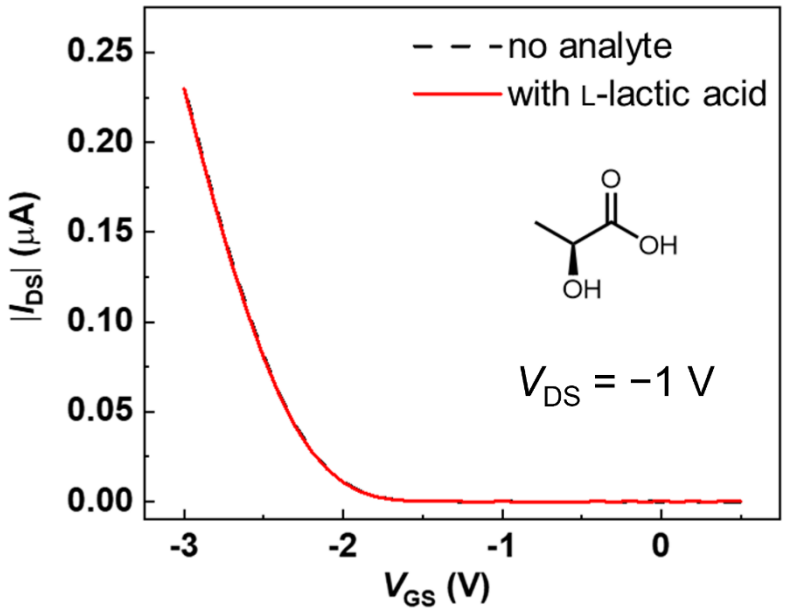

Fig. S11. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of L-lactic acid (18 μg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

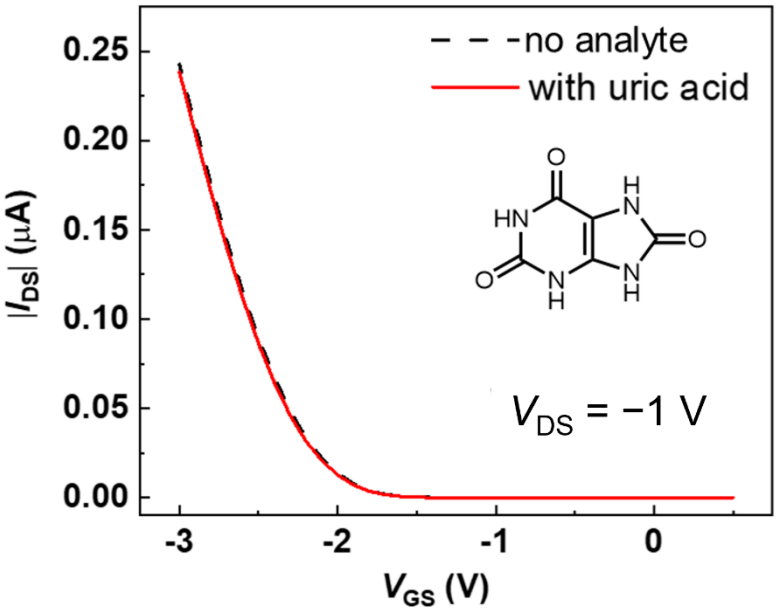

Fig. S12. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of uric acid (8 μg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

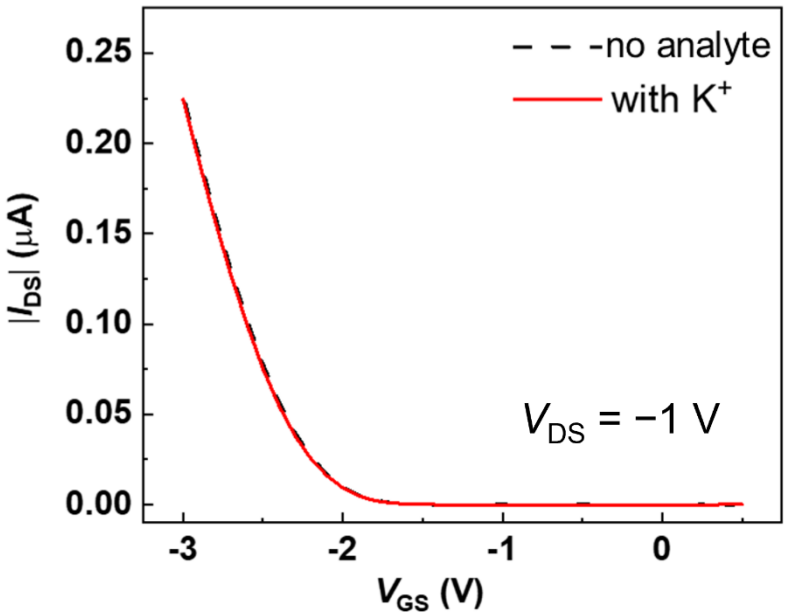

Fig. S13. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of potassium nitrate (547 μg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

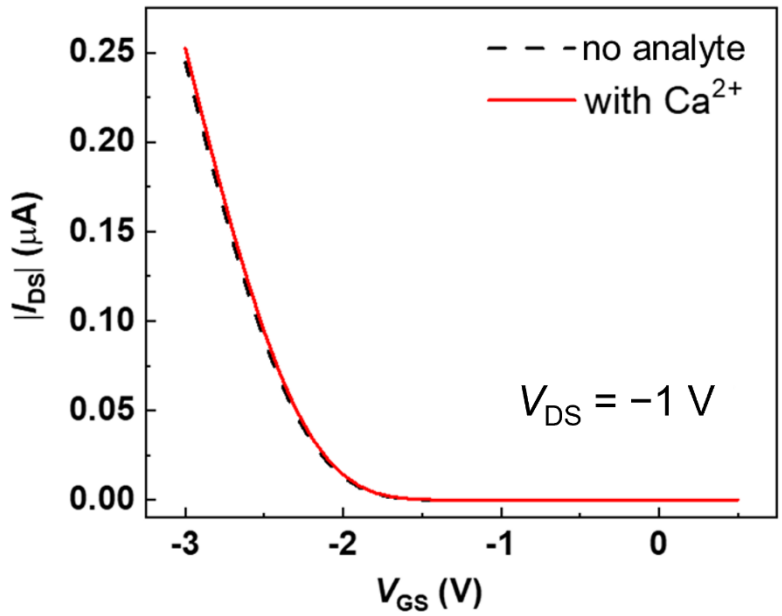

Fig. S14. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of calcium nitrate (48 μg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

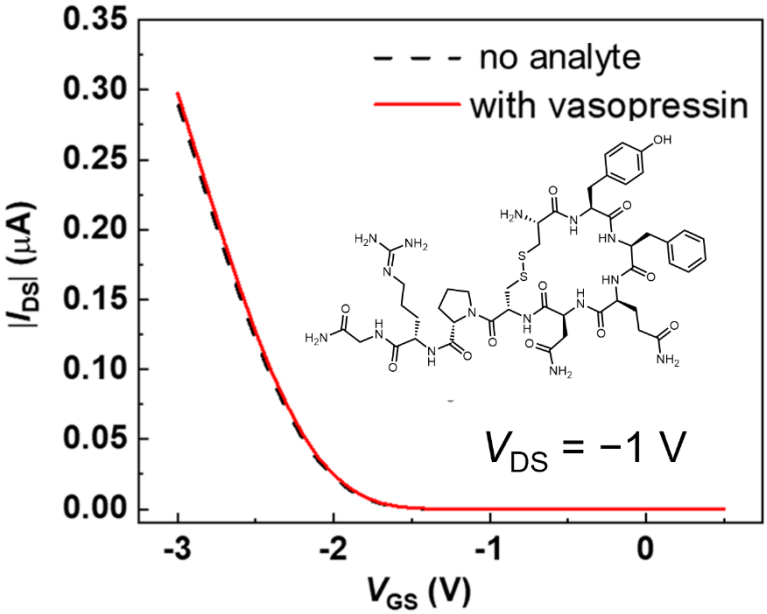

Fig. S15. Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of vasopressin (10 pg mL^{-1}) in D-PBS buffer solution containing Tween 20 (0.05wt%) and HSA (0.1wt%).

8. Electrical detection of oxytocin in artificial saliva

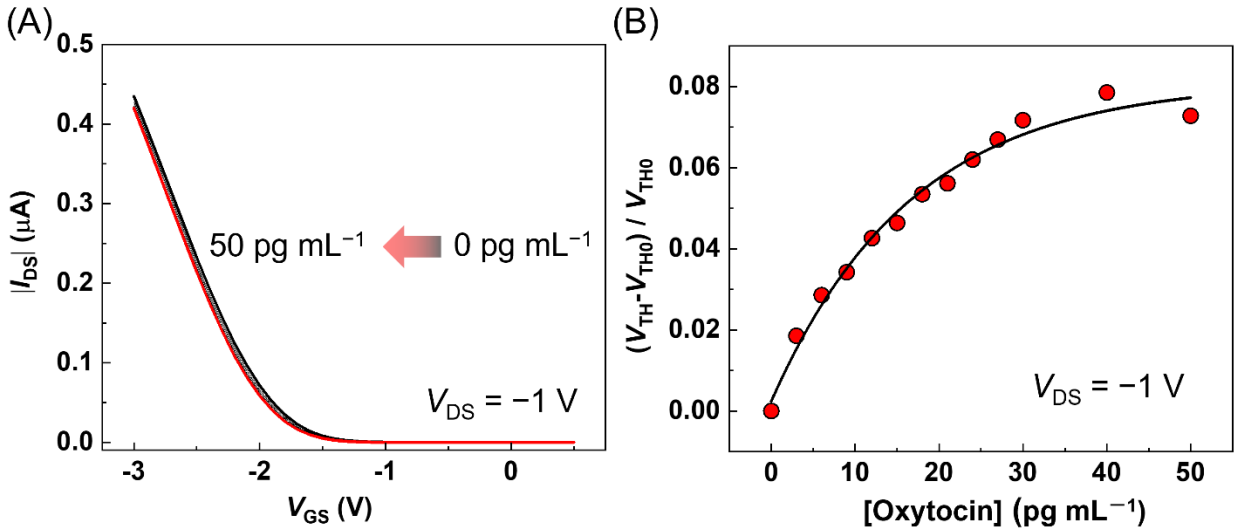

Fig. S16. (A) Transfer characteristics of the OFET functionalized with the biotinylated anti-oxytocin antibody upon the addition of oxytocin in artificial saliva. To prepare the artificial saliva, D-glucose (10 μg mL^{-1}), creatinine (0.3 μg mL^{-1}), L-lactic acid (18 μg mL^{-1}), uric acid (8 μg mL^{-1}), and human serum albumin (1.78 mg mL^{-1}) were additionally added into artificial medical saliva (Saliveht Aerosol). (B) Titration isotherm of the oxytocin detection. [Oxytocin] = 0–50 pg mL^{-1}.

9. Real sample analysis

The real sample analysis was carried out using human saliva sample taken from a healthy volunteer, which was authorized by the Ethics Committee of the University of Tokyo (authorization code: 20-108). As a pretreatment process, the collected human saliva was centrifuged at 6,000rpm for 6 min to remove precipitates in the sample. The obtained supernatant solution determined at pH 7.5 was applied to the chemical sensing by the OFET without further pretreatments.

Table S2. Result of the spike recovery test in human saliva.

	Added Oxytocin (pg mL^{-1})	Detected Oxytocin (pg mL^{-1})	Recovery Rate (%)
Human Saliva Sample	0	0.68 ± 3.41	-
	20	19.95 ± 2.69	96%
	25	26.29 ± 2.81	102%

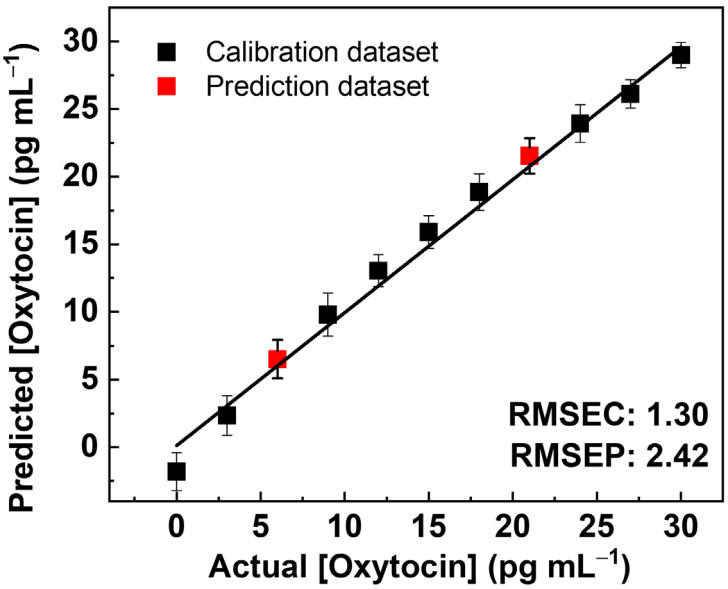

Fig. S17. Result of regression analysis for oxytocin in human saliva. The values of the root-mean-square errors of calibration (RMSEC) and prediction (RMSEP) represent the accuracy of the built calibration model and its predictive capacity.

Reference

1 T. Okamoto, C. Mitsui, M. Yamagishi, K. Nakahara, J. Soeda, Y. Hirose, K. Miwa, H. Sato, A. Yamano, T. Matsushita, T. Uemura, J. Takeya, Adv. Mater., 2013, **25**, 6392-6397.