Supplementary Information

Anti-Drug Antibody Detection with Label-Free Electrolyte-Gated Organic Field-Effect Transistors

Matteo Sensi[1], Marcello Berto[1], Sara Gentile[1], Marcello Pinti[1], Andrea Conti[2], Giovanni Pellacani[2], Carlo Salvarani[3], Andrea Cossarizza[4], Carlo Augusto Bortolotti[1], Fabio Biscarini[1,5]*

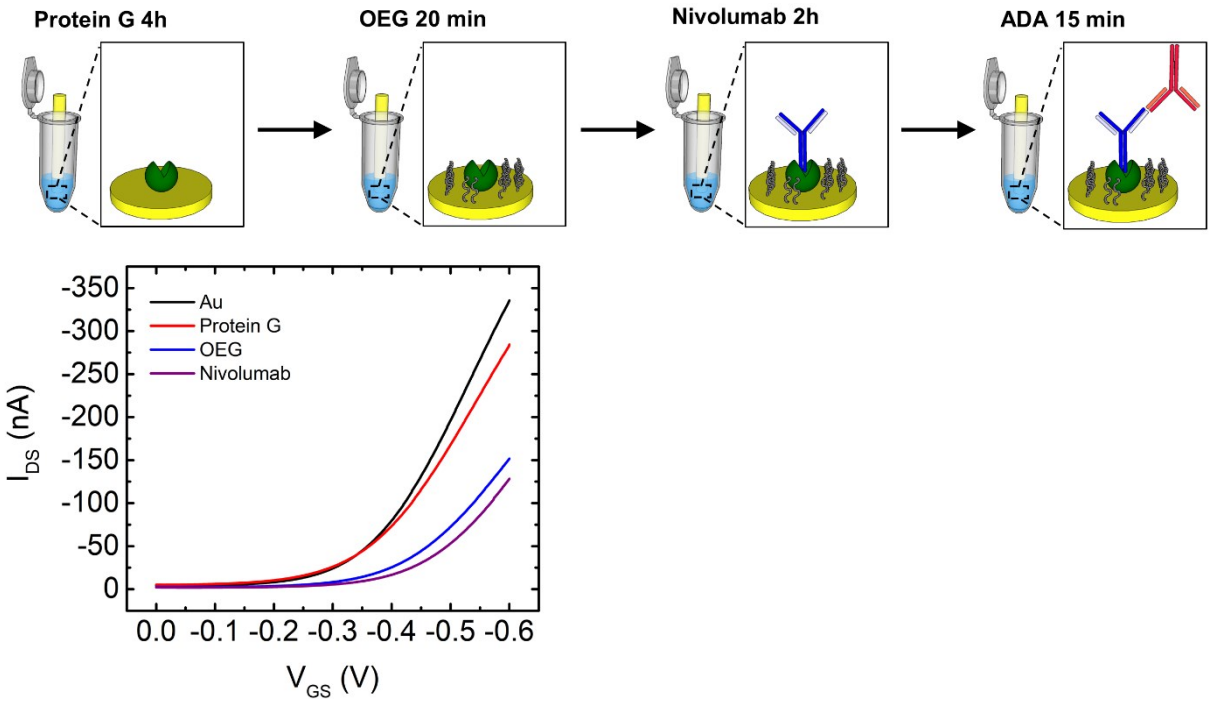
[1] Dipartimento di Scienze della Vita - Università di Modena e Reggio Emilia, Via Campi 103, 41125 Modena, Italy

[2] Dipartimento Medicine Specialistiche, S.C. Dermatologia, Azienda Ospedaliero Universitaria di Modena, Via Del Pozzo 71, 41121 Modena, Italy

[3] Unità Reumatologica, Università di Modena e Reggio Emilia, Modena, and Unità di Reumatologia, Azienda USL-IRCCS di Reggio Emilia

[4] Dipartimento di Scienze Mediche e Chirurgiche Materno-Infantili e dell'Adulto, Università di Modena e Reggio Emilia, Via Campi 287, 41125 Modena, Italy

[5] Center for Translational Neurophysiology - Istituto Italiano di Tecnologia, Via Fossato di Mortara 17-19, 44100 Ferrara, Italy



1. Gate biofunctionalization procedure

Figure S1. *(above) The different steps of the biofunctionalization of the Au wire that acts as the gate electrode in the EGOFET sensor are shown. The gate is incubated ex situ in a 1.5 µl tube containing a specific functionalization solution (Protein G; OEG; Nivolumab), then is incubated with the solution containing the Antidrug antibody (ADA) at a certain concentration; (below) After each incubation step, the wire was rinsed with PBS, and used as the gate for the measurement of transfer curves, to assess the result of the functionalization for the first three steps, and for dosing the ADA content of the solution (see figure 2 in main text).*

2. ADA non-specific binding on Au electrodes

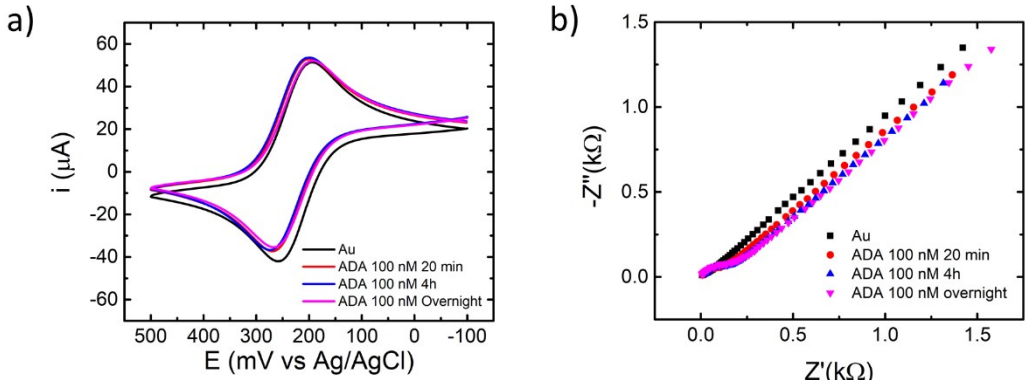


Figure S2. *Electrochemical characterization of ADA nonspecific binding on gold electrodes. a) Cyclic voltammetry b) and Electrochemical impedance spectroscopy of gold wire exposed to ADA. The electrochemical parameters are the same reported in the materials and methods section of the main text.*

The marginal adsorption of ADAs on bare Au electrode is assessed by means of cyclovoltammetry and electrochemical impedance spectroscopy. The small variation is further depleted by using OEG-coated Au gate.

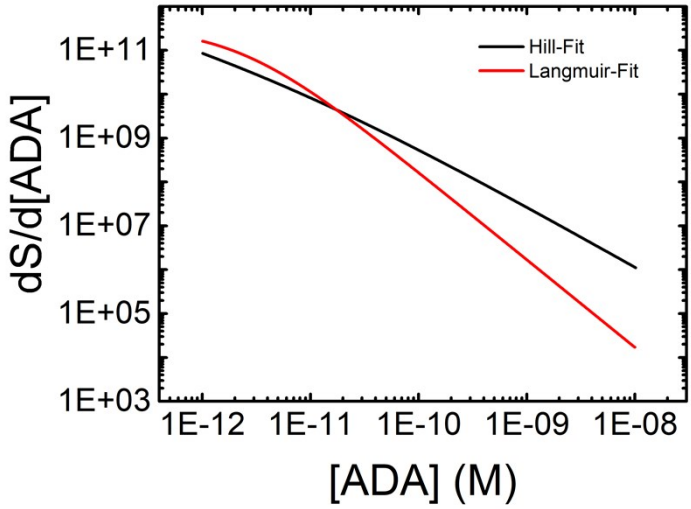


Figure S3. *Sensitivity of the device. Numerical derivative dS/d[ADA] plotted as a function of [ADA]. The line is the derivative of the best Hill-fit and Langmuir-fit obtained from figure 3b in the main text. It should be noticed that in both cases the device is ultrasensitive at the lowest concentrations, whereas the Hill-type trend is more sensitive above nM concentration.*

3. Sensitivity of the immunosensor

4. First and second order derivative of transfer curves

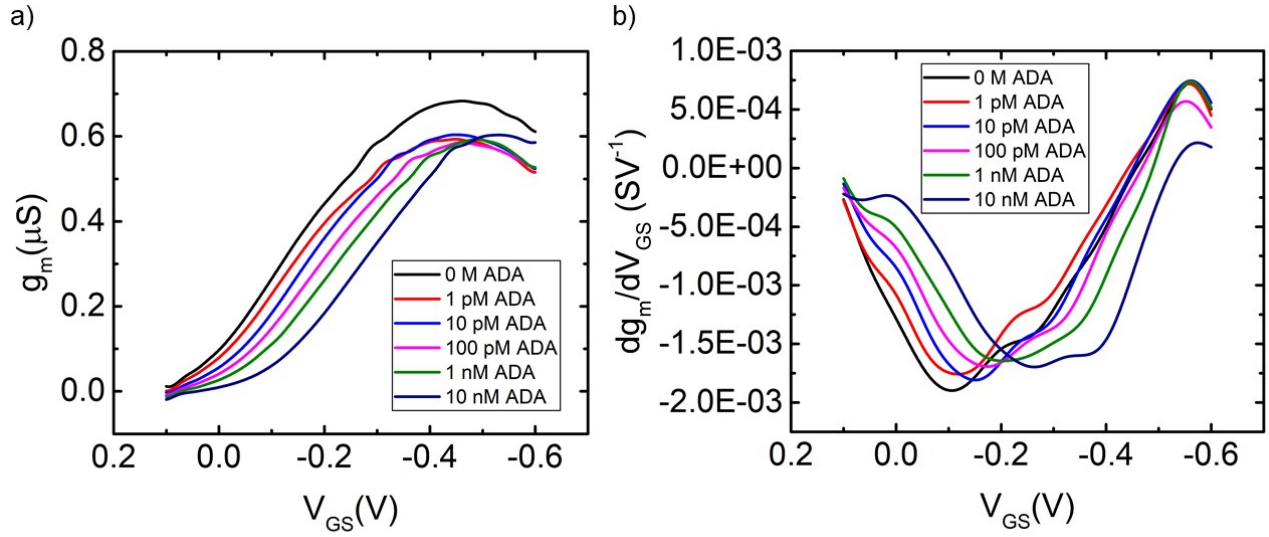


Figure S4. *First order (g_m) and second order derivative of the transfer curves. a) Transconductance of the transfer curves shown in figure 3 in the main text, determined as the first order derivative of I_{DS} vs V_{GS}. The maxima of curves are equal to the g_m values calculated in the main text by linear fit. b) Second order derivative*

of the transfer curves shown in figure 3. The minima of the curves correspond to the threshold voltages. The curves have been smoothed (FFT filter, 5 window points).

5. Stability of the device during the incubation procedure

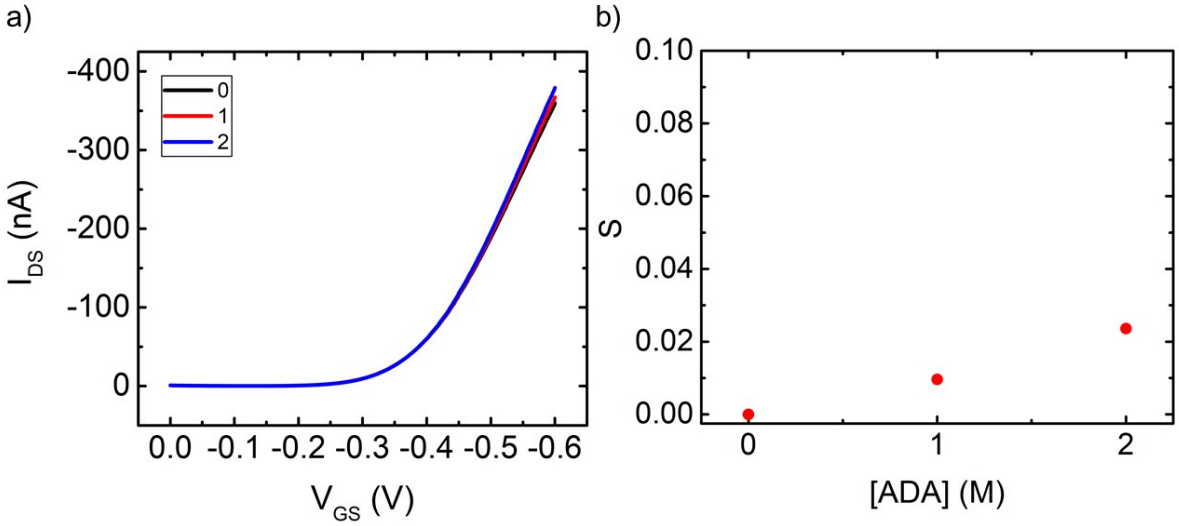


Figure S5. *Stability of the EGOFET during the incubation procedure. a) Transfer curves and b) signal generated by incubating the gate, functionalized as in figure S1, in PBS instead of ADAs. V_{DS} = -0.2 V, PBS 50 mM, pH 7.4.*

The signal generated by the PBS incubation is negligible compared to the ones observed upon incubation in ADAs.

6. Transfer curves and EIS spectra of the device exposed to anti-Infliximab ADAs

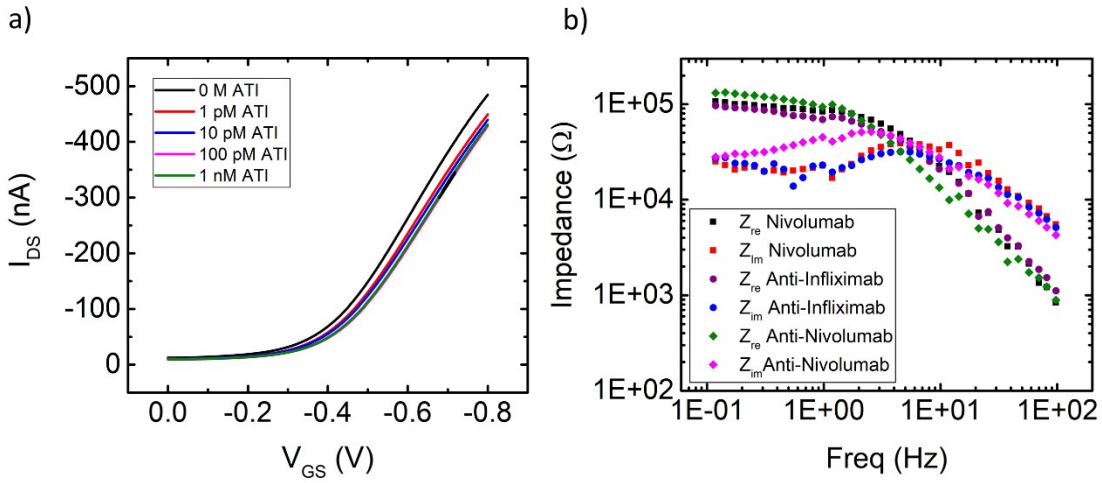


Figure S6. *Nivolumab probe exposed to anti-Infliximab ADAs (ATIs). The gate has been functionalized with the protocol showed in figure S1, have been incubated in ATIs solutions of increasing concentration. a) The reported transfer curves are one of the datasets used to calculate the signal reported in figure 4a in the main text. V_{DS} = -0.2 V, PBS 50 mM, pH 7.4. b) Plot of impedance components as a function of frequency.*

7. Infliximab probe exposed to anti-Nivolumab ADAs

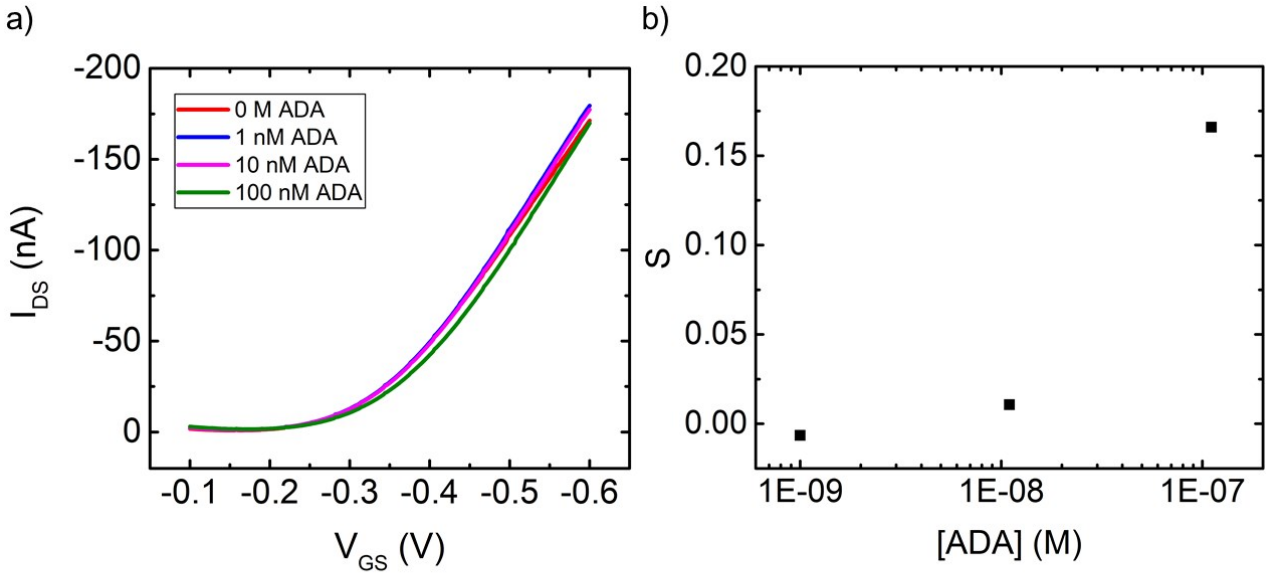


Figure S7. *Infliximab probe exposed to anti-Nivolumb ADAs. The gate of the EGOFET has been functionalized with the protocol showed in figure S1 but replacing Nivolumab with Infliximab. The gate has been incubated in ATIs solutions of increasing concentration. The reported transfer curves are one of the datasets used to calculate the signal reported in figure 4a in the main text.* V_{DS} *= -0.2 V, PBS 50 mM, pH 7.4.*

The anti-Nivolumab ADAs are highly selective for Nivolumab, as demonstrated by the low nonspecific signal in figure S7b.

8. Examples of ADAs label free biosensors

Drug	Method	ADAs Range	Reference
Adalimumab	SPR	5-200 µg/ml Diluted serum	F. Real-Fernández et al., Anal. Bioanal. Chem., 2015, 407, 7477–7485.
Panitumumab	Biacore SPR	0.65 to 15 µg/ml Diluted serum	J. A. Lofgren, et al., J. Immunol., 2007, 178, 7467–7472.
Infliximab	SPR	5–40 µg/mL Undiluted serum	M. Beeg, A. et al., Sci. Rep., 2019, 9, 1–9.

9. Threshold Voltage and Transconductance of the biosensor

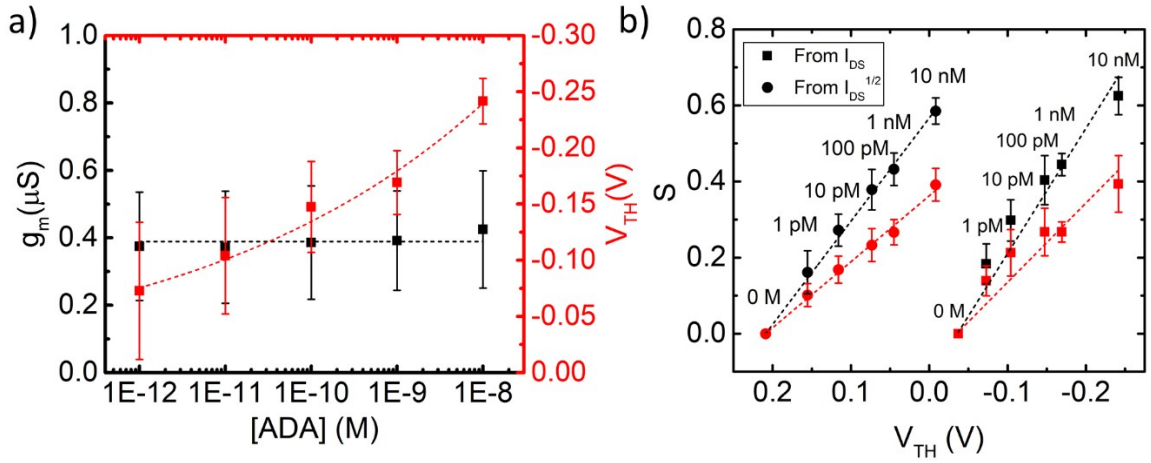


Figure S8. Figures of merit of the biosensor. a) Transconductance (g_m) and threshold voltage (V_{TH}) vs. [ADA] in the semilog plot. The values are the average of 4 different devices at each concentration, with standard deviation error bars. The dashed red lines in panels (c) and (d) are a guide for the eyes. (d) Signal as a function of threshold voltage, both calculated from the linear fit of I_{DS} (circles) or $I_{DS}^{1/2}$ (squares). The V_{GS} are -0.1 V (black) and -0.3 V (red) for the data extracted from I_{DS}, -0.3 V (black) and -0.5 V (red) for the data extracted from $I_{DS}^{1/2}$.

Materials and methods

Device fabrication

The Test Patterns(TPs) characterized by 4 interdigitated electrodes with channel L = 10 μm and width W = 5 mm (W/L = 500) patterned by photolithography and lift-off (1 cm2 total area) were purchased from "Fondazione Bruno Kessler" (FBK, Trento, Italy). The electrodes are made of 50 nm thick gold, attached by a few nm chromium adhesive layer to a quartz substrate. TPs were cleaned following the procedure: (i) a rinse with acetone (10 ml) to remove the photoresist layer, (ii) drying with nitrogen flow, (iii) washing again in hot acetone for 10 min, (iv) drying with nitrogen flow, (v) washing in hot piranha solution (2 ml H_2O_2, 2 ml H_2SO_4), (vi) rinsing with H_2O_d, (vii) drying with nitrogen flow. An 80 μl drop of Tips-Pentacene 1% in weight, in 80:20 Toluene:Hexane, was spin-coated on the TPs and successively cured at 60°C in oven for 30 minutes.

Drug and antibodies

Nivolumab (Opdivo©) and Infliximab (Remicade©) were provided by Dr. Andrea Conti, Section of Dermatology, in normal saline solution. Human Anti-Nivolumab antibodies in Fab monovalent format were purchased from BioRad (AbD30255, HuCal Fab monovalent), resuspended in PBS 50 mM pH 7.4 and stored at -20°C.

Human Anti-Infliximab monoclonal antibodies were purchased from BioRad (AbD19370_hIgG1), resuspended in PBS 50 mM pH 7.4 and stored at -20°C.

Gate functionalization

The polycrystalline Au wire gate electrode was cleaned as follows: flaming it in oxidizing conditions, immersion in hot KOH for 4 h, rinse with abundant water and immersion in hot concentrated H_2SO_4 for 2 h. The electrode was then cycled 20 times between +1.5 and −0.25 V at 0.1 V s^{-1} in 1 M H_2SO_4. The GE was functionalized according to the following protocol: (i) a first incubation in a phosphate buffer saline (PBS 50 mM, pH 7.4) of Cys-Protein G (2 mg/ml) for 4 h at room temperature (RT), (ii) a rinse with PBS, (iii) immersion in OEG thiol 100 μM (in PBS 50 mM, pH 7.4) for 20 min (iv) a rinse with PBS, (v) incubation with Nivolumab solution (1 mg/ml in PBS 50 mM, pH 7.4) for 2 h at RT and (vi) a final rinse with PBS.

Electrochemical characterization

Cyclic voltammetry (CV) measurements were performed to estimate the electrode active area and the coverage after cys-Protein G immobilization, OEG SAM formation and Nivolumab immobilization by means of the Randles–Sevçik equation (Fig. 2a). The method consists of monitoring the cathodic current, corresponding to the $[Fe(CN)_6]^{3-}$ reduction, as a function of the incubation time of the working electrode. Protein G binding the gold surface, causes an increase of the passivation of the electrode, seen by the decrease of the faradaic current. We then use the Randles–Sevcik equation to

extract variation in the electrode active area. The measurements were performed in 1 M KCl and 5 mM $K_3[Fe(CN)_6]$ at 50 mV s^{-1}. The Impedance spectra shown in Fig. 2b and 5b, were performed in 5 mM $K_3[Fe(CN)_6]$, 1M KCl at an initial potential of 0.22 V and in a frequency range from 0.1 Hz to 10 KHz. The gate was functionalized as previously described. A CH Instrument potentiostat 760c model was used for the cyclic voltammetry (CV) experiments and the Electrochemical Impedance Spectroscopy (EIS), which were carried out using a three-electrode cell. The gate electrode was used as a working electrode (WE), whereas a Pt wire and an Ag/AgCl electrode (Elbatech, Livorno Italy) were chosen as counter electrode (CE) and reference electrode (RE), respectively. The gold WE was cleaned as described above in the text.

Electrical characterization

In all measurements source, drain, and gate electrodes were connected to an Agilent B2902A Source Measure Unit. Two voltages were applied between Source and Drain (V_{DS}) and Source and Gate (V_{GS}). The source is connected to earth and the current is measured both at the gate electrode (I_{GS}) and at the drain electrode (I_{DS}). The transfer characteristics were recorded by sweeping the gate-source voltage (V_{GS}) from +0.1 to −0.6 V while leaving the drain-source voltage (V_{DS}) constant at −0.2 V (linear regime), in line with previous works from our group. All measurements were carried out at room temperature in a buffer solution (PBS 50 mM, pH 7.4).

The gate electrode was incubated ex situ in solutions containing increasing [ADA] before electrical measurements for 15 min. The gate electrode area was kept constant by means of a passivation layer.

We calculated the theoretical limit of detection (LOD) as the mean value of the blank +3 times its corresponding standard deviation, using the responses of the negative controls as blank responses and extracting the correspoding concentration on the fittd curve (A. Gustavo González, M. Ángeles Herrador, *TrAC - Trends Anal. Chem.* **2007**, *26*, 227–238).

Acknowledgments

This work was funded by EuroNanoMed III project “AMI” , by the University of Modena and Reggio Emilia through Projects “FAR2015” and “FAR2017”, and Fondazione di Vignola.