

S1. Determination of the OECT characteristic parameters from continuous scanning

In **Figure S1A**, the transfer characteristic curve of a PEDOT-PAH-based OECT, obtained by continuously sweeping the gate potential at 10 mV s^{-1} and measuring the drain-source current at a fixed drain-source voltage, $V_{DS} = -50 \text{ mV}$, is shown. The threshold voltage, V_{TH} , that represents the gate potential required to initiate polymer oxidation was obtained by fitting the linear region of the I_{DS} vs. V_G profile and extrapolating the line to the value where $I_{DS} = 0 \text{ mA}$. In addition, the transconductance, computed as $g = dI_{DS}/dV_G$, is shown as a function of V_G , where the procedure for obtaining V_{Gmax} is represented.

Once V_{Gmax} was determined, I_{DSmax} was computed as the I_{DS} value at $V_G = V_{Gmax}$. For obtaining the real-time monitoring of the enzyme adsorption, the procedure was as follows. The transfer curves were registered in KCl solution by continuously sweeping the potential to reversibly oxidize and reduce the polymer, yielding a baseline signal. Then, the urease solution was injected without interrupting data acquisition, and after 30 min, the cell was rinsed with KCl.

For data treatment, the transfer curves were separated into “off” and “on” curves, corresponding to the reduction and oxidation processes, respectively. Finally, one I_{DSmax} value was determined from each “off” curve and the I_{DSmax} values of successive transfer curves were plotted as a function of time for reconstructing the current-time curves analogous to traditional measurements in chronoamperometric mode (as that shown in **Figure 1E**). The same procedure was performed for the monitoring of the LbL construction and for the urea sensing measurements. **Figure S1B** shows the raw data obtained by this method for urea sensing with a urease/PEDOT-PAH transistor.

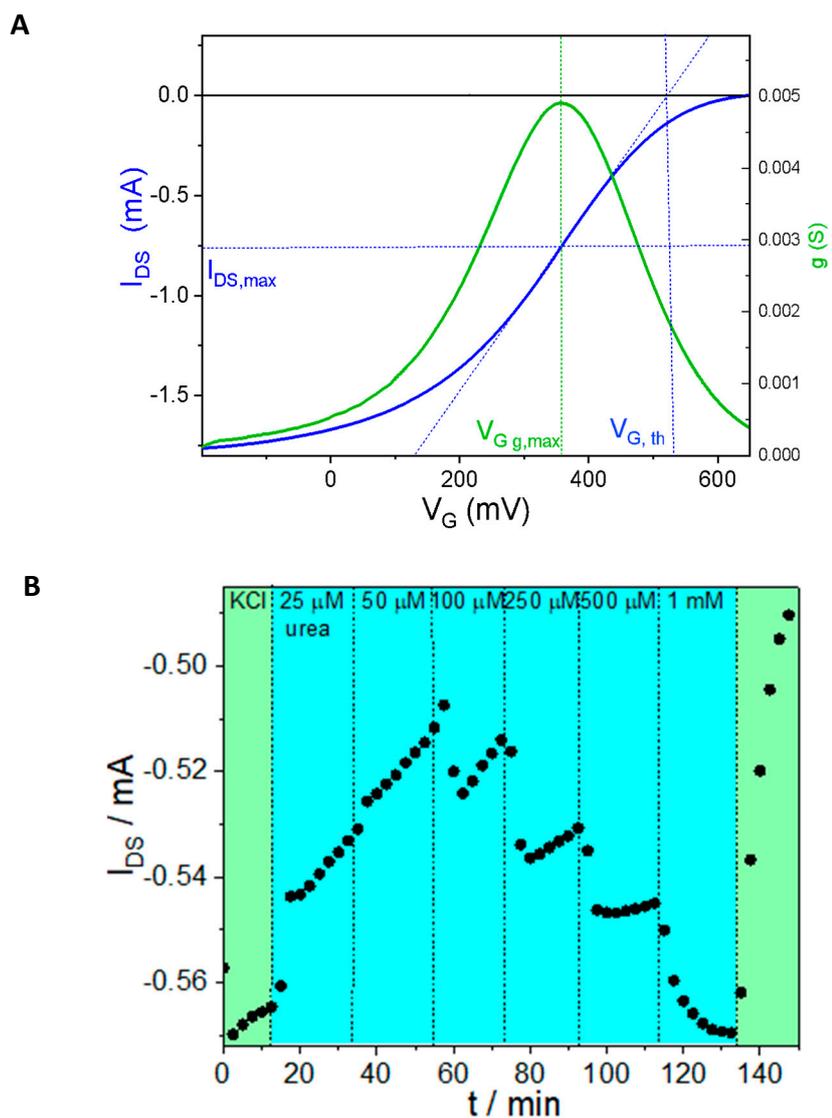


Figure S1. Determination of the OECT characteristic parameters from continuous scanning. (A) Procedure for obtaining the OECT characteristic parameters, V_{TH} , V_{Gmax} and I_{DSmax} , from the transfer curve (blue) and the transconductance vs. V_G profile (green) of a PEDOT-PAH film. (B) Chronoamperometric-like response for urea sensing experiments with a urease/PEDOT-PAH OECT obtained by analyzing each transfer curve of continuous scanning, as shown above. In this case, I_{DS} is determined at the maximum transconductance gate potential.

S2. Urea sensing reproducibility with urease/PEDOT-PAH-based OECTs

Urea biosensing was performed by employing two urease-modified OECTs, as shown in **Figure S2**.

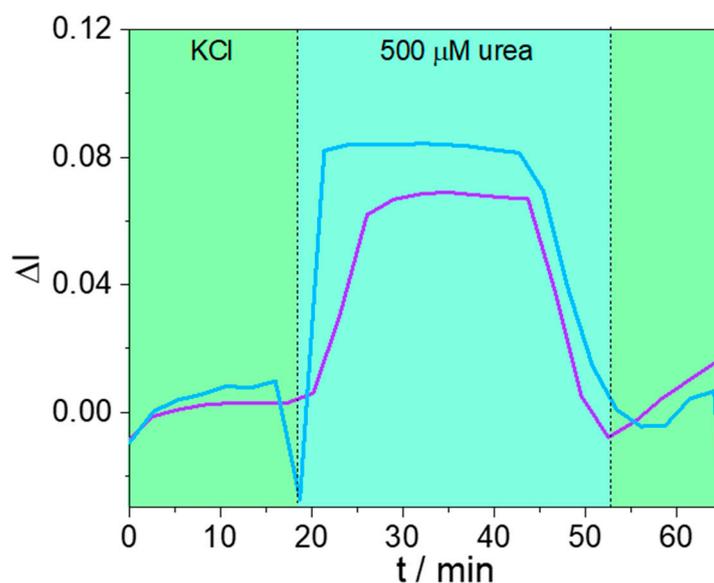


Figure S2. Urea sensing reproducibility with urease/PEDOT-PAH-based OECTs. Baseline-corrected time evolution of the urea sensing response with urease/PEDOT-PAH OECTs for one single injection of 500 μM urea in 10 mM KCl for two different sensors.

S3. Urease-free control experiment

To ensure the response to urea comes from the enzymatic activity, a control experiment was performed as follows. Firstly, the OECT response to the injection of urea increasing concentrations was monitored using an unmodified PEDOT-PAH OECT. Then, the same sensor was modified by urease electrostatic adsorption and its response to the same urea concentrations was measured. The results in **Figure S3** clearly indicate that the response to the presence of urea comes from the presence of the enzyme on the conducting channels of the OECT.

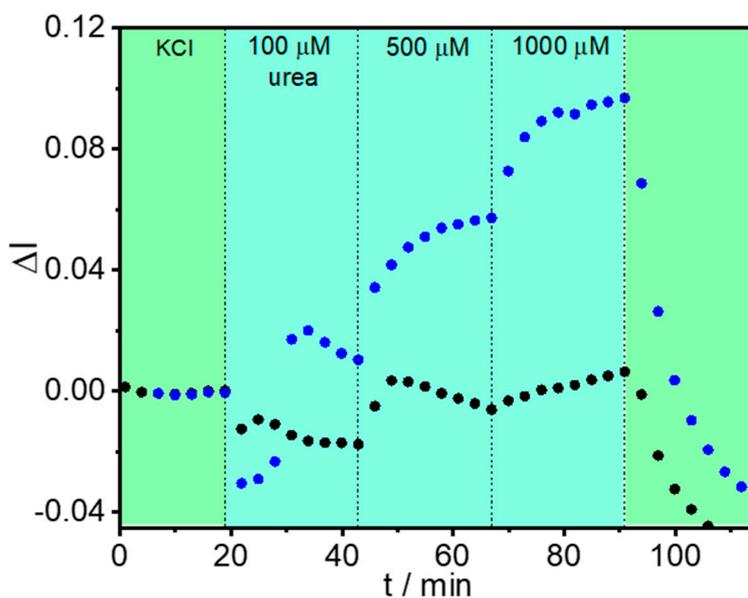


Figure S3. Urease-free control experiment. Response of OECT to the presence of increasing concentrations of urea in 10 mM KCl before (black) and after (blue) urease adsorption.

S4. Reproducibility of the performance of the urease/PEI-modified OECTs

The reproducibility of the performance of the urease/PEI-functionalized PEDOT-PAH OECTs for sensing urea in the 100–1000 μM range was studied by comparing the results of five different OECTs. **Figure S4** illustrates the reproducibility of this linear trends in terms of the relative current change.

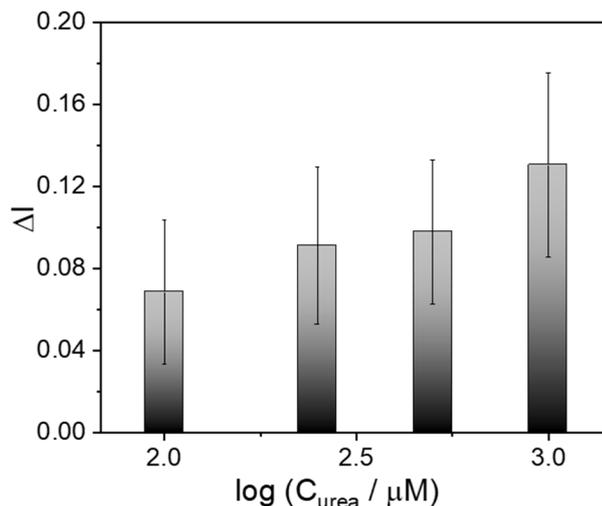


Figure S4. Reproducibility of the performance of the urease/PEI-modified OECTs. Average relative change in I_{DSmax} as a function of logarithm of urea concentration for different transistors. Bars correspond to the SD ($n = 5$).

S5. Effect of the addition of two urease/PEI bilayers on the sensing response

The I_{DS} vs. V_{G} curves of a urease/PEI-modified OECT were registered while urea solutions of increasing concentrations were injected to the cell (**Figure S5**). A shift in the curves to higher gate values can be appreciated. In addition, the initial profile is recovered when the transistor is rinsed with KCl.

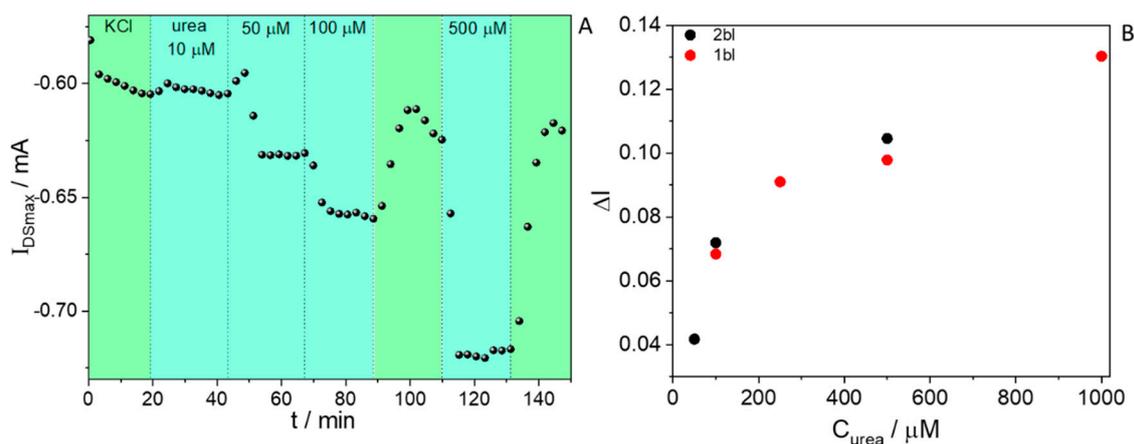


Figure S5. Effect of the addition of two urease/PEI bilayers on the sensing response. Time evolution of the I_{DSmax} during successive injections of increasing concentrations of urea for the two urease/PEI bilayer systems (A). Relative change in I_{DSmax} as a function of urea concentration for assemblies with one and two bilayers (B).

S6. Stability of the OECT response to urea

To evaluate the stability of the sensors based on the electrostatic integration of urease and PEI for urea sensing, we performed successive injections of solutions with the same concentration (500 μM) alternating with blank solutions (10 mM KCl). The results shown in **Figure S6** demonstrate good reproducibility of the response in quantitative terms and, in turn, good stability of the device.

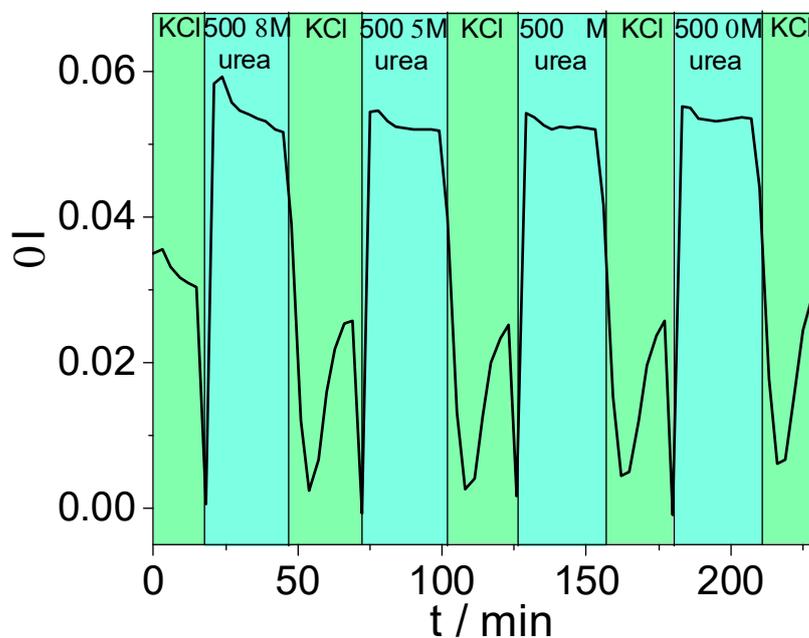


Figure S6. Stability of the sensing response of the urease/PEI-modified OECTs. Time evolution of the relative changes in $I_{D_{S_{max}}}$ during successive alternating injections of 500 μM urea and 10 mM KCl solutions.