

Supporting Information to:

**The use of potentiometric sensors to study (bio)molecular interactions.**

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## Abstract

This Supporting Information contains a step by step description of all the “how to do it” procedures which the authors found most practical. The steps are demonstrated for the basic drug lidocaïne. The association constant  $K_{\text{ass}}$  between the drug and the rubber-phase membrane is calculated by the Linearization method. More intermediate results are shown as compared to the examples given in the article, and more graphs are given. The experimental conditions are the same as those used in the manuscript for promazine under section 2.1. Completely comparable data have been obtained in analogous conditions for other cationic- (Noscapine and Ritodrine) and anionic (malonic- and maleic acid) organic compounds, but these results will be published later together with other data.

## Supporting information

Sensorgrams as shown in figure S-1 were observed when different concentrations of lidocaine were injected as a square concentration pulse in a FIA system (see the manuscripts' Experimental section). The "association", or "on" phase, the plateau region ( $R_{\max}$ ), and the "dissociation" or "off" phase are clearly visible in figure S-1, left graph. If we plot all the  $R_{\max}$  values (responses measured in the plateau region) as a function of the logarithm of the concentration, the typical Nicolskii-Eisenman curve was obtained as shown in fig. S-1, right graph. The green curve is the result of a "solver" (Excel) non-linear least squares minimization curve fitting to a Nicolskii-Eisenman function of the type  $E = E^0 + S \log(c + Cst)$ : see eqn. 3 in the article. From this plot, Solver calculated values of  $E^0$ ,  $S$  and  $Cst$  as 326mV, 59.1mV and 0.0000030 respectively.

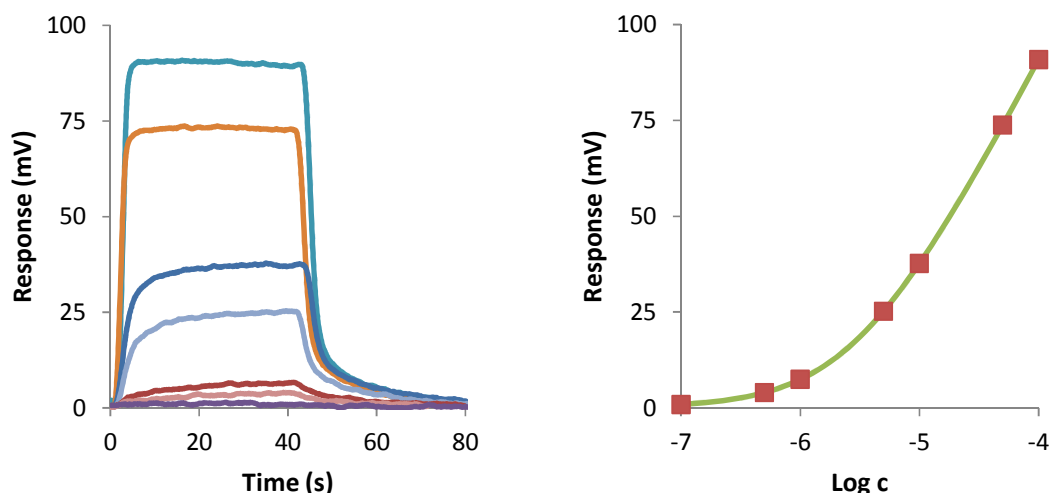


Figure S-1: Potentiometric responses (mV) of  $10^{-4}$  to  $10^{-7}$ M lidocaine injections in FIA. The responses (in mV) are shown as a function of time (left) and as a function of the logarithm of the concentration (right).

Instead of a response in mV, a  $R = 10^{mV/S} - 1$  conversion was then plotted. This is a transpose of the Nicolskii-Eisenman equation (see eqn.4 of the article), which is linearly related to the analyte concentration: see figure S-2.

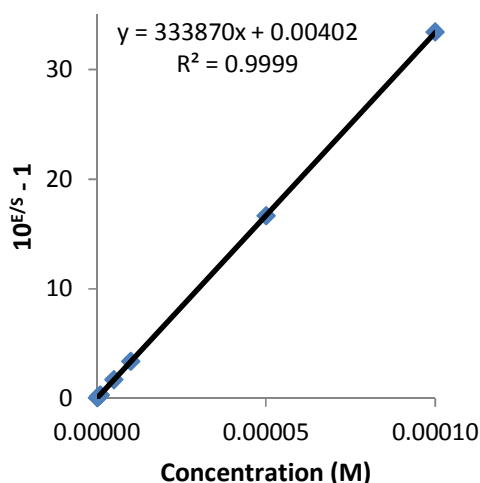


Figure S-2: Potentiometric responses of  $10^{-4}$  to  $10^{-7}$ M lidocaine injections in FIA after transformation to a concentration related signal.

The experimental data of the “association” phase were fit by a non-linear least squares method to a function of the form of  $R(t) = R_{max}(1 - e^{-k_{obs}t})$  by Solver (fig. S-3). When transformed to  $\ln\left(1 - \frac{R(t)}{R_{max}}\right) = -k_{obs} \cdot t$ , plots of  $\ln\left(1 - \frac{R(t)}{R_{max}}\right)$  versus  $t$  for the experimentally obtained data yielded straight lines. When applied to the “on” phase data of the  $10^{-5}\text{M}$  lidocaine sensorgram (figure S-3 left),  $R^2 = 0.999$  was calculated by Excel’s linear regression. All other curves at different concentrations yielded comparable results.

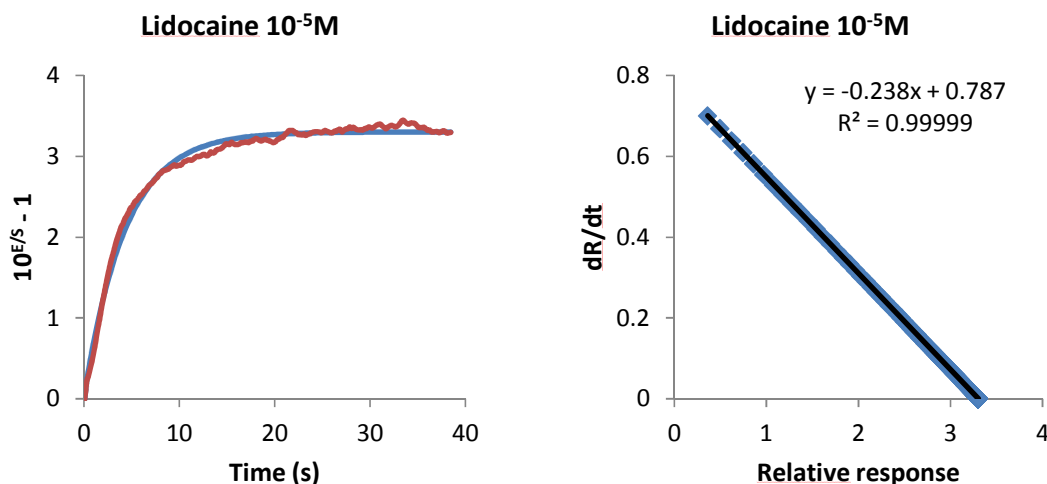


Figure S-3: Experimental data of the “association” or “on” curve of  $10^{-5}\text{M}$  lidocaine. The  $R(t) = R_{max}(1 - e^{-k_{obs}t})$  function was checked with Solver (left graph). The right part is a graphical representation of eqn. 11 of the manuscript.

From the  $R = 10^{\frac{mV}{s}} - 1$  (y-axis) versus  $t$  (x-axis) “on” parts of the sensorgram plots, we then derived  $\frac{dR}{dt}$  versus  $R$  graphs. These should yield straight lines as predicted by eqn.11 of the manuscript:  $\frac{dR}{dt} = k_{on}c_{analyte}R_{max} - (k_{on}c_{analyte} + k_{off})R$ . Good linearity was observed over the whole concentration range, with  $R^2$  values as calculated by Excel to exceed 0.99. The slope of this line (“Slope” in the graphs) equals  $-(k_{on}c_{analyte} + k_{off})$ .

Finally, a plot of  $-(k_{on} \cdot c_{analyte} + k_{off})$  versus  $c_{analyte}$ , yielded a straight line of the form:

Slope ( $s^{-1}$ ) =  $-1.97 \times 10^4 c_{analyte} - 0.0716$ , with  $R^2 = 0.998$  (fig. S-4).

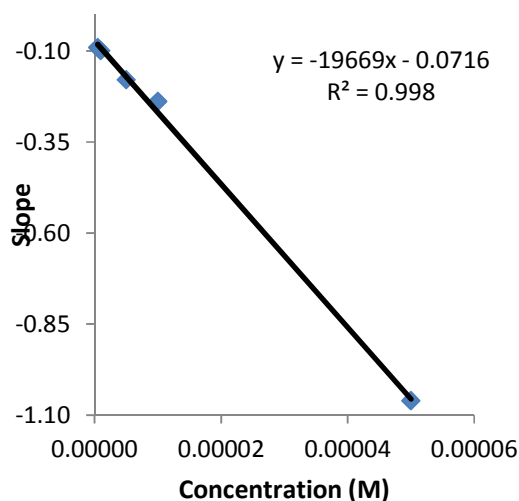


Figure S-4: Representation of the slope ( $= k_{on} \cdot C_{analyte} + k_{off}$ ) as a function of the concentration.

From this equation,  $k_{on}$  and  $k_{off}$  can be obtained as:

$$k_{on} = 1.18 \times 10^6 \text{ min}^{-1} \text{ M}^{-1}, \quad k_{off} = 4.30 \text{ min}^{-1}.$$

$$\text{Calculating } K_{ass} = \frac{k_{on}}{k_{off}} = 2.75 \times 10^5 \text{ M}^{-1}.$$

This corresponds to a  $\Delta G$  value of interaction of lidocaine with the sensor surface material of  $7.29 \text{ kcal mol}^{-1}$ .

The experimental data of the “dissociation” or “off” phase were fit by a non-linear least squares method to a function of the form of  $R(t) = R_{max}(e^{-k_{obs}t})$  and fit with Solver (fig. S-5).

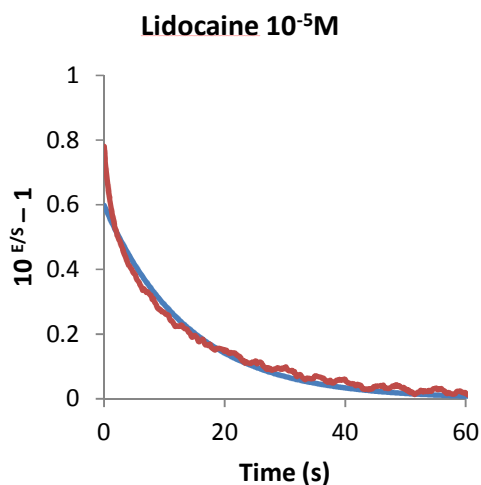


Figure S-5: Experimental data of the “off” curve of  $10^{-5} \text{ M}$  lidocaine. The  $R(t) = R_{max}(e^{-k_{obs}t})$  function (red) was fitted by Solver (blue).

The  $k_{obs}$  value obtained for lidocaine at a  $10^{-5} \text{ M}$  concentration from these data was  $0.0721 \text{ s}^{-1}$  or  $4.33 \text{ min}^{-1}$ . This perfectly fits the value as calculated above from the “on” phase kinetics

1 (0.0716 s<sup>-1</sup>). All other curves at different concentrations yielded comparable results with an  
2 average value of  $k_{\text{off}}$  equal to 4.49 min<sup>-1</sup> (St. Dev.: 0.526 min<sup>-1</sup>).

3 The sensors are continuously regenerated as the eluent is running at a 1 mL min<sup>-1</sup> flow-rate.  
4 We never had to use a regeneration buffer to have the baseline return to its original position.  
5 Occasionally, slight irreversible phenomena occurred as indicated by an increased baseline  
6 after the desorption step. It occurred to us that this occasional phenomenon was due to a badly  
7 prepared coating. The phenomenon was observed more frequently with the gelatin-type  
8 coatings than with the rubber-type coatings. The rubber-type coatings are very robust with  
9 lifetimes exceeding several months. The gelatin type coatings are at this moment still more  
10 fragile and could be used for about one week only.

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