Electronic Supplementary Material (ESI) for Chemical Communications

INSEL: An *in silico* method for optimizing and exploring biorecognition assays

Miquel Avella-Oliver,[a] David Gimenez-Romero,[a] Sergi Morais,[a] Paulo Roberto Bueno,[b] Rosa Puchades[a] and Ángel Maquieira*[a]

[a] *IDM, Departamento de Química, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain. Fax: +34-96-3879349; Tel: +34-96-3877342; E-mail: amaquieira@qim.upv.es*
[b] *Instituto de Química, Departamento de Físico-Química, Universidade Estadual Paulista (UNESP), CP 355, 14800-900, Araraquara, São Paulo, Brazil. E-mail: prbueno@iq.unesp.br*

Electronic Supplementary Information (ESI)

Figure S1	Immobilization isotherm
Figure S2	Response curve for the competitive immunoassay model system.
Figure S3	Response curve for the non-competitive sandwich immunoassay model system.
Figure S4	Predicted and experimental dose-response curves for the competitive immunoassay model system
Figure S5	Predicted and experimental dose-response curves for the non-competitive sandwich immunoassay model system
Figure S6	Flux diagram of the proposed *in silico* approach
Figure S7	Titration and dose-response curves from classical optimization results
Materials and methods	Modelling, immunoassay, kinetic curves, immobilization isotherm, response curve and classical optimization

Supplementary Figures

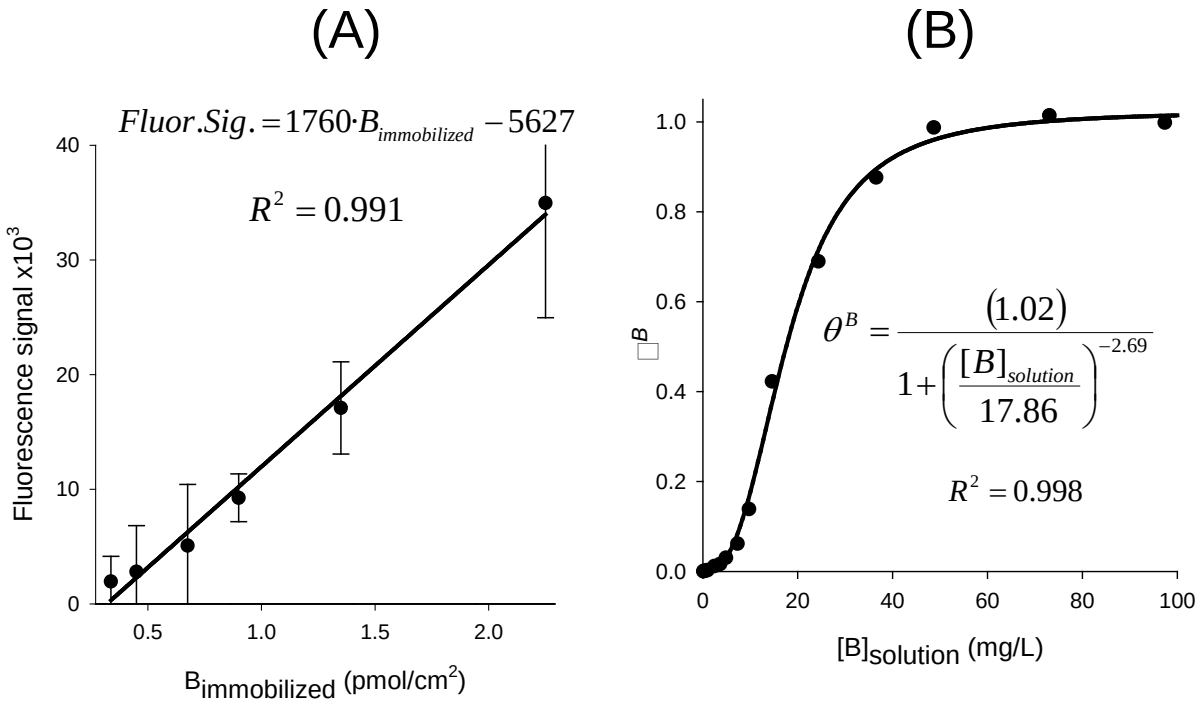

Fig. S1 Immobilization isotherm. (A) Calibration curve. (B) Immobilization isotherm. The regression equation allows to relate the probe solution concentration with the corresponding immobilized superficial concentration by means of its θ^B (ESI†, Materials and methods, Modelling). The experimental results fit the type V isotherm. From these data, an equation that relates θ^{RB} with the signal is established by adjusting the subsequent response curve (ESI†, Fig. S2-S3). Since probe molecules (BSA conjugate) are prolate ellipsoids, where the polar axis (140 Å) is larger than the equatorial axes (40 Å),[S1] the experimental superficial density obtained ($16 \cdot 10^{-15}$ mol/cm^2) and the values for the two limit orientations (equatorial $12 \cdot 10^{-15}$ mol/cm^2 and polar $43 \cdot 10^{-15}$ mol/cm^2) suggest that BSA is mainly physisorbed as an equatorially oriented monolayer.

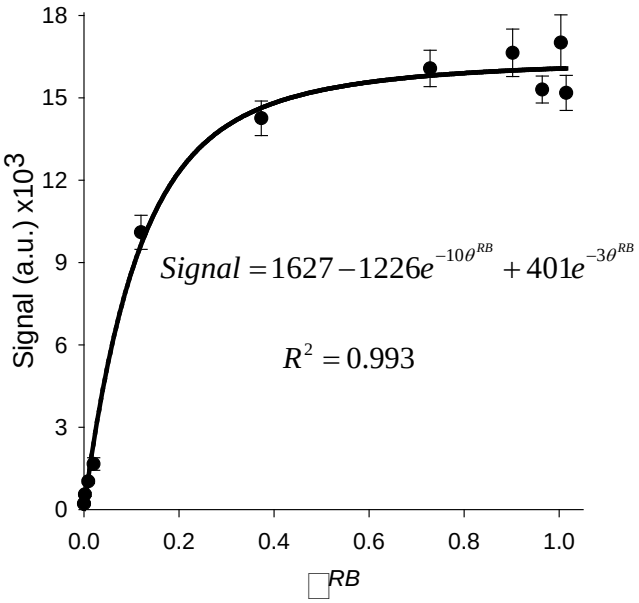

Fig. S2 Response curve for the competitive coating conjugate-based immunoassay model system. Experimental data fit to an exponential curve (rise to a maximum four parameters).

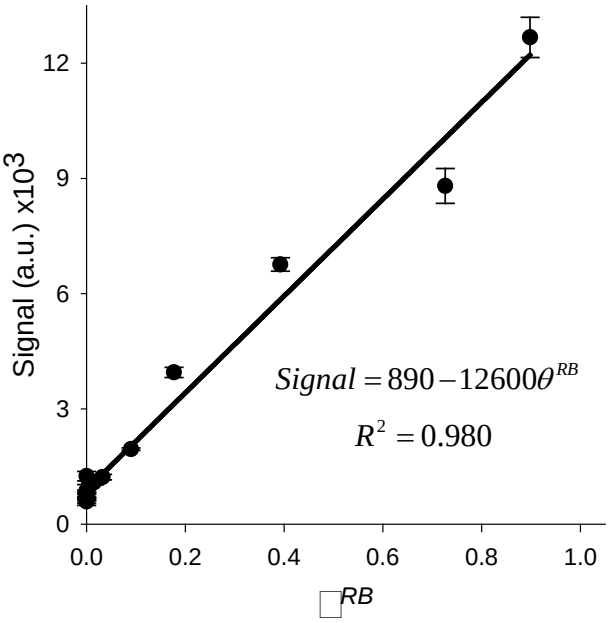

Fig. S3 Response curve for the non-competitive sandwich immunoassay model system. Experimental data fit to a linear equation.

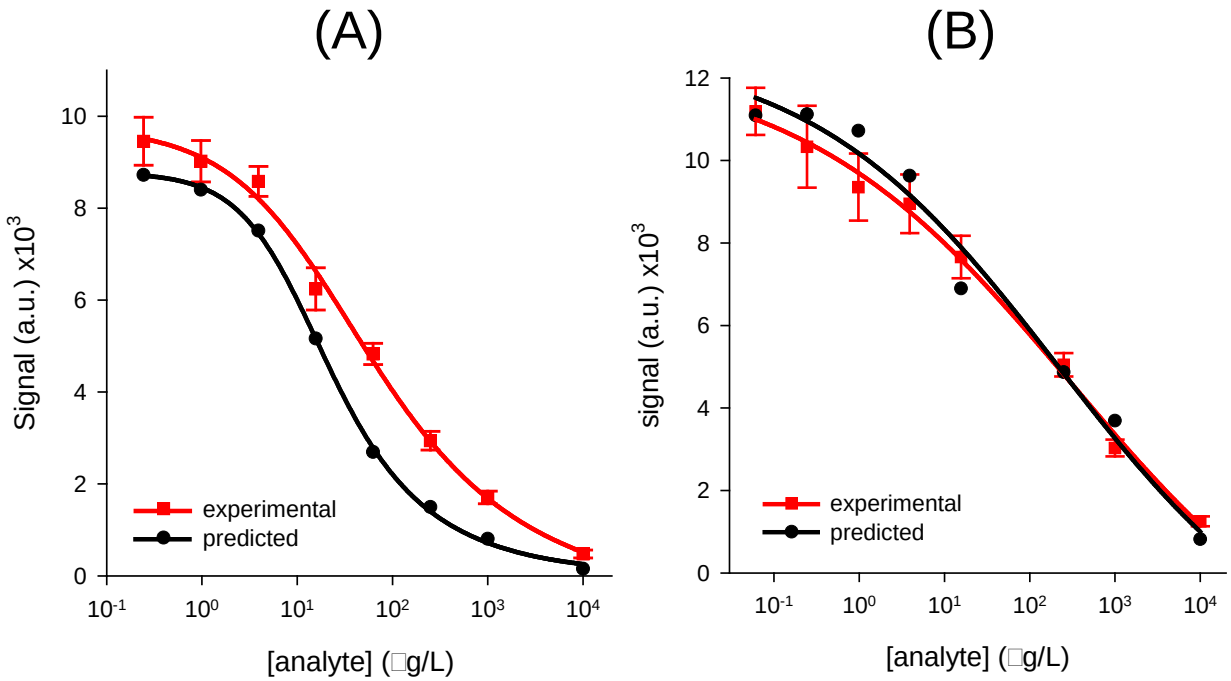

Fig. S4 Predicted and experimental dose-response curves for the competitive coating conjugate-based immunoassay model system. (A) The probe and receptor solution concentration are 11.90 mg/L and 0.34 mg/L, respectively, with an incubation time of 13 min (R^2 = 0.872). (B) The probe and receptor solution concentrations are 40.00 mg/L and 0.36 mg/L, respectively, with an incubation time of 26 min (R^2 = 0.952). It must be highlighted that predictive performance is enhanced by considering that the model is designed for only one epitope and that the biorecognition process is described by a first-order equation, whereas a polyclonal antibody is used as a receptor. Note: The predicted curves do not show errors bars because they are theoretical values.

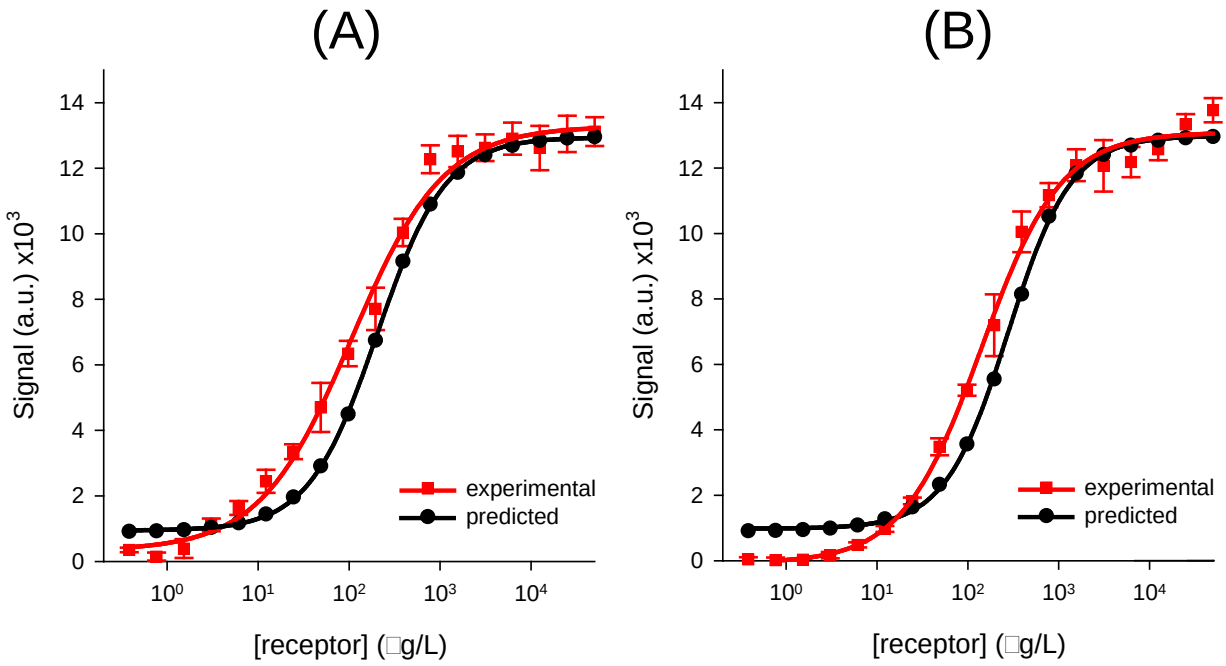

Fig. S5 Predicted and experimental dose-response curves for the non-competitive sandwich immunoassay model system. (A) Probe solution concentration 50 mg/L and incubation time 30 min (R^2 = 0.968). (B) Probe solution concentration 50. mg/L and 17 min incubation time (R^2 = 0.969). Note: The predicted curves do not show errors bars because they are theoretical values.

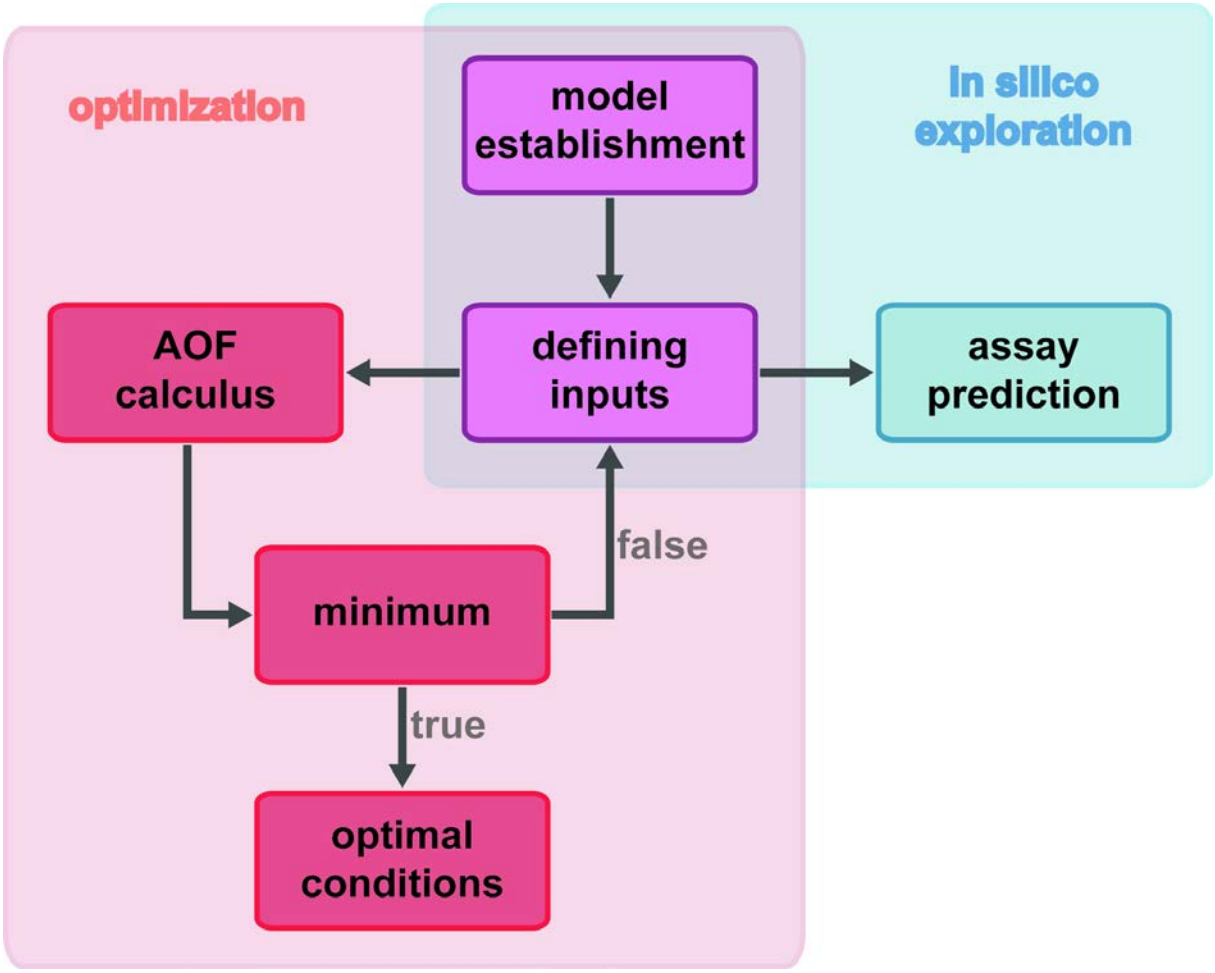

Fig. S6 Flux diagram of the proposed *in silico* approach.

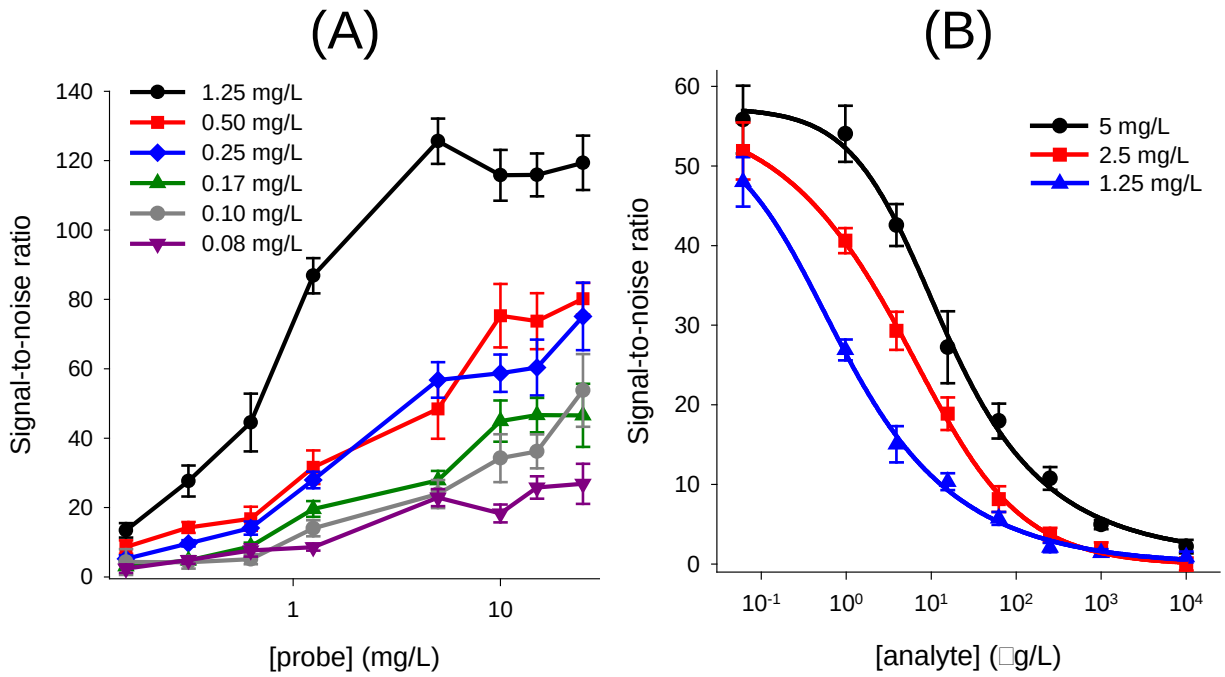

Fig. S7 Titration and dose-response curves from classical optimization. (A) Titration curves for different receptor concentrations. In this case, the receptor concentration of 0.25 mg/L and the probe concentration at around 2.5 mg/L were selected to be tested in the next step. (B) Dose-response curves at a fixed receptor concentration (0.25 mg/L) and three different probe concentrations (5, 2.5 and 1.25 mg/L). In this case, the receptor and probe concentrations of 0.25 and 5.0 mg/L, respectively, were selected as optimum. The incubation time in these experiments was 20 minutes.

Materials and methods

Modelling. Biointeraction assay modelling. The proposed biointeraction reaction scheme (Fig. 1 in manuscript) implies the ordinary differential equations system presented in Eq. (S1-S7). This system is numerically solved by the LSODA methodology and by considering the boundary conditions described in Eq. (S8) – (S11):

$$\frac{d[R_b(t)]}{dt} = -\,k_D^R\,([R_b(t)] - [R_i(t)]) - \,k_{on}^{RA}\cdot[A_b(t)]\cdot[R_b(t)] + \,k_{off}^{RA}\cdot\,[RA_b(t)] \qquad \text{Eq. (S1)}$$

$$\frac{d[A_b(t)]}{dt} = -\,k_D^A\,([A_b(t)] - [A_i(t)]) - \,k_{on}^{RA}\cdot[A_b(t)]\cdot[R_b(t)] + \,k_{off}^{RA}\cdot[RA_b(t)] \qquad \text{Eq. (S2)}$$

$$\frac{d[RA_b(t)]}{dt} = -\,k_D^{RA}\,([RA_b(t)] - [RA_i(t)]) + \,k_{on}^{RA}\cdot[A_b(t)]\cdot[R_b(t)] - k_{off}^{RA}\cdot[RA_b(t)] \qquad \text{Eq. (S3)}$$

$$\frac{d[R_i(t)]}{dt} = \,k_D^R\,([R_b(t)] - [R_i(t)]) + \,k_{off}^{RB}\cdot\theta^{RB}(t) - \,k_{on}^{RB}\cdot[R_i(t)]\cdot\theta^B(t) - \\ - k_{on}^{RA}\cdot[R_i(t)]\cdot[A_i(t)] + \,k_{off}^{RA}\cdot[RA_i(t)] \qquad \text{Eq. (S4)}$$

$$\frac{d[A_i(t)]}{dt} = k_D^A\,([A_b(t)] - [A_i(t)]) - \,k_{on}^{RA}\cdot[A_i(t)]\cdot[R_i(t)] + \,k_{off}^{RA}\cdot[RA_i(t)] \qquad \text{Eq. (S5)}$$

$$\frac{d[RA_i(t)]}{dt} = \,k_D^{RA}\,([RA_b(t)] - [RA_i(t)]) + \,k_{on}^{RA}\cdot[A_i(t)]\cdot[R_i(t)] - k_{off}^{RA}\cdot[RA_i(t)] \qquad \text{Eq. (S6)}$$

$$\frac{d\theta^{RB}(t)}{dt} = k_{on}^{RB}\cdot[R_i(t)]\cdot\theta^B(t) - \,k_{off}^{RB}\,\theta^{RB}(t) \qquad \text{Eq. (S7)}$$

$$\theta^{RB}(0) = \,[R_i(0)] = [A_i(0)] = [RA_i(0)] = [RA_b(0)] = 0 \qquad \text{Eq. (S8)}$$

$$\theta^B(0) = \theta_0^B \qquad \text{Eq. (S9)}$$

$$[R_b(0)] = [R]_0 \qquad \text{Eq. (S10)}$$

$$[A_b(0)] = [A]_0 \qquad \text{Eq. (S11)}$$

where R is receptor, A is analyte, B is probe, subscripts *b* and *i* refer to the bulk and interface respectively, $\boldsymbol{k_D^i}$ is the phenomenological mass transport constant of compound *i*, $\boldsymbol{k_{on}^i}$ and $\boldsymbol{k_{off}^i}$ are the formation and dissociation constants of *i*, θ^i is the coverage degree of *i* on the transducer surface and subscript 0 refers to the initial point. In this analytical system, kinetics is based on first-order equations and the diffusion of the species between the bulk and the interface is described by a phenomenological mass transport constant that accounts for diffusion, migration and convection.[S2]

Assay. Two different assays are employed as a model system for proving the concept in this study, a non-competitive sandwich immunoassay for immunoglobulins analysis and a competitive coating conjugate-based immunoassay for low molecular weight compounds determination. Given their comprehensiveness, these systems serve as a powerful proof-of-concept for the potential of the methodology here presented for generic biorecognition assays, specially the second one.

In this study, we used a microarray detection system based on the compact disc technology, as described elsewhere.[S3,S4]

The non-competitive model system studied is a sandwich immunoassay for rabbit antibodies determination. As this system does not involve competition, only the half reaction scheme (Fig. 1 in manuscript) concerning the processes between probe and receptor is required for the modelling. As noted in the scheme, in this system the probe is goat anti-rabbit polyclonal antibody and the receptor is polyclonal rabbit immunoglobulin. Although rabbit antibodies actually act as analyte in this assay, we denominate this element as receptor for keeping the generic notation of the presented reaction scheme.

The competitive model system studied is a coating conjugate-based immunoassay for atrazine (Atz) analysis. According to the reaction scheme (Fig. 1 in manuscript), in this system the analyte is Atz, the receptor is a polyclonal anti-atrazine antibody (pRAb-Atz) and the probe is a coating protein-hapten conjugate (BSA-2d)[S4]. This assay is based on the competition between the free analyte and the probe for the receptor binding.

Thus, the probe solution in carbonate buffer (0.1 M sodium carbonate, pH 9.6) is arrayed on the transducer surface by a non contact microprinter (25 nL/spot) and is left for 16 h at 4ºC for immobilization. Afterward, sensing surfaces are rinsed with PBS-T (8 mM sodium phosphate dibasic, 2 mM sodium phosphate monobasic, 137 mM sodium chloride, 3 mM potassium chloride, 0.05% Tween 20, pH 7.5), deionized water, and are dried by centrifugation. Then, receptor solutions in PBS-TT (PBS-T, 0.1 M taurine, pH 7.5) for the non-competitive system, or receptor and analyte mixture solutions in PBS-TT for the competitive system, are dispensed on the arrays. After the incubation time, sensing surfaces are rinsed again with PBS-T and deionized water, and are dried by centrifugation. Then, solutions of 5 nm colloidal gold-labelled goat anti-rabbit immunoglobulin in PBS-TT (1/30 dilution in the sandwich immunoassay and 1/100 dilution in the coating conjugate-based immunoassay) are dispensed on the arrays and allowed to react (20 min for the non-competitive system and 15 min for the competitive assay). Next, sensing surfaces are rinsed as before and dried by centrifugation. Finally, silver enhancer solution is dispensed and

distributed (1 mL per disk). After 8 min, the platform is rinsed with deionized water and dried again. Finally, the assay signal is measured.

Kinetic curves. To obtain the kinetic curves, an assay is performed within a range of incubations times. To characterize the interaction of the receptor with the probe and the analyte in competitive systems, this experiment includes the kinetic curve without analyte and the one with analyte (preferably an analyte concentration that is around the IC_{50} value) for a fixed probe and receptor concentrations. For non-competitive systems, only one kinetic curve for characterization was necessary.

Immobilization isotherm. To perform the immobilization isotherm experiment, a sensing strategy that allows to directly measuring the dispensed probe solutions is required to establish the corresponding calibration curve. In this case, we carry out these experiments by fluorescence measurements on polycarbonate chips. For this purpose, serial dilutions of BSA-Cy5 in carbonate buffer are arrayed on the chips by a non contact microprinter (25 nL/spot). After 16 h at 4ºC in the darkness, the chips are scanned using a fluorescence scanner (635 nm) and the subsequent data are employed as a calibration curve. Then, chips are rinsed with deionized water, dried by air flow and measured again. From these results, the immobilization isotherm is obtained.

The immobilization of the probe in this model system is based on passive physisorption. For assays involving different probe immobilization strategies analogue experiments must be performed, according to the corresponding immobilization methodologies.

Different assays utilizing similar probes, as well as different probes using the same carrier protein, will presumably show the same immobilization behaviour for a given transducer surface. Therefore, the immobilization isotherm experiment for characterizing the model should be avoided when prior knowledge on the system is available.

Response curve. To obtain the response curve, an assay with serial probe dilutions applying an excess of receptor (and without analyte in the competitive systems) must be performed. After considering the immobilization isotherm experiments and the response curve experimental data, the relationship between the analytical signal and θ^{RB} is obtained by adjusting the experimental results to an equation.

Classical optimization. The classical standard bi-parametrical optimization procedure is divided into two steps: titration and dose-response curve. To carry out titration experiments, the signal-to-noise ratio *vs.* probe concentration curves are performed for a range of receptor concentrations. From these data, the binary combinations that generate a signal-to-noise ratio of around 50 are selected in order to keep the whole linear range of the dose-response curve above the limit of quantification. The second step consists in building the dose-response curves of the binary conditions selected in the titration experiment. For this purpose, the signal-to-noise ratio *vs.* analyte concentration curves are carried out for each selected binary combination. From these results, the conditions that generate the lowest IC_{50} in the dose-response curves are selected as optimal conditions.

References

S1. J.L. Harris, E. Skaletsky and A. McPherson, *J. Mol. Biol*., 1998, **275**, 861.

S2. R.A. Vijayendran, F.S. Ligler and D.E. Leckband, *Anal. Chem.*,1999, **71**, 5405.

S3. S. Morais, L.A. Tortajada-Genaro, T. Arnandis-Chover, R. Puchades and A. Maquieira, *Anal. Chem.*, 2010, **82**,1954.

S4. J. Tamarit-Lopez, S. Morais, M. Bañuls, R. Puchades and A. Maquieira, *Anal. Chem.*, 2010, **82**,1954.