

SUPPLEMENTARY INFORMATION

For the paper:

Computing steady-state metal flux at microorganism and bioanalytical sensor interfaces in multiligand systems. A reaction layer approximation and its comparison with the rigorous solution.

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APPENDIX A: NOTATION AND SYMBOLS

Latin symbols

| Symbol | Name | Units | Equation |
|--|--|---------------------------|------------|
| A_j | coefficients in the solution (related to formal concentrations at the surface) | none | (B-24) |
| A | coupled system matrix | none | (B-13) |
| B | constant in the solution of Laplace equation | mol s^{-1} | (6) |
| $c_0 \equiv [\text{M}]/[\text{M}]^*$ | normalised concentration of species M | none | (B-7) |
| $c_i \equiv \frac{D_{\text{ML}_i} K_i [\text{L}] [\text{M}^i\text{L}]}{D_{\text{M}} [\text{M}^i\text{L}]^*}$ | normalised concentration of species ML_i | none | (B-6) |
| D_{M} | diffusion coefficient of species M | m^2s^{-1} | (1) |
| $D_{\text{M}^i\text{L}_k}$ | diffusion coefficient of species M^iL_k | m^2s^{-1} | (11) |
| \dot{D}_i | average diffusion coefficient of all complexes containing ligand ^iL | m^2s^{-1} | (16), (13) |
| \bar{D}_i | weighted average diffusion coefficient for complexes with | m^2s^{-1} | (19), (20) |

| | | | |
|-------------------------------|---|--|-------------|
| | ligands n° 1 to i | | |
| F | total flow through a surface | mol s^{-1} | (4) |
| f_i | formal species | none | (B-11) |
| G | constant in the solution of Laplace equation | $\text{mol m}^{-1} \text{s}^{-1}$ | (6) |
| J | flux at a certain position (indicated by superscript or subscript) | $\text{mol m}^{-2} \text{s}^{-1}$ | (3), (4) |
| K_a | surface complexation constant | $\text{mol}^{-1} \text{m}^3$ | (3) |
| ${}^iK = {}^i k_a / {}^i k_d$ | stability constant of complex M^iL | $\text{mol}^{-1} \text{m}^3$ | (E-1) |
| K'_{equiv} | equivalent excess-ligand stability constant $K'_{\text{equiv}} = {}^1K[{}^1L] + {}^2K[{}^2L]$ | none | (E-4) |
| k and k' | number of ligands in the complex M^1L_k (where k spans from 1 to n_1) and in the complex $M^2L_{k'}$ (where k' spans from 1 to n_2) | none | (11), (17) |
| ${}^i k_a$ ${}^i k_d$ | association and dissociation rate constants of complex M^iL . Note: all complexes M^iL_k with $k > 1$ are supposed to be in equilibrium with M^iL . | $\text{mol}^{-1} \text{m}^3 \text{s}^{-1}$ and s^{-1} | (1) and (2) |
| k_{int} | interfacial rate constant (e.g. for internalisation) | s^{-1} | (3) |
| \mathcal{L} | lability index | none | (48) |
| iL | ligand of type i | none | (1) |

| | | | |
|------------|---|---------------------|------------------|
| m | Maximum number of different types of ligands (${}^1L, {}^2L.. {}^mL$) | none | (26) |
| $[M]^*$ | bulk free metal ion concentration | mol m^{-3} | (24) |
| $[M]_t$ | Total metal concentration | mol m^{-3} | (26), (28) |
| $[M^iL_k]$ | concentration of complex M^iL_k , formed by one metal ion and k ligands of type i | mol m^{-3} | (2) |
| $\{MR\}$ | surface concentration of occupied receptors | mol m^{-2} | (3) |
| n_j | number of successive complexes with ligand jL | none | (11), (16), (17) |
| $\{R\}$ | surface concentration of transport sites | mol m^{-2} | (3) |
| r | radial co-ordinate | m | (5) |
| r_0 | radius of the spherical active surface | m | (4) |
| T_j | constants in the solution of the uncoupled differential equations | none | (B-18), (B-19) |

Greek symbols

| Symbol | Name | Units | Equation |
|------------|---|-------|------------|
| α_i | Degree of complexation of M for the set of multicomplexes with ligands $n^\circ 1$ to i | none | (19), (22) |

| | | | |
|---------------------------------|---|-----------------------------------|-----------------------------|
| ${}^i\beta_k$ | cumulative stability constant of complex M^iL_k | $(\text{m}^3 \text{mol}^{-1})^k$ | (15), (23) |
| γ_i | global stability quotient for the set of multicomplexes with ligands iL . | dimensionless none | (13), (14), (18) |
| δ | diffusion layer thickness | m | (18), (19), (25) , fig 1 |
| ε | Relative difference between fluxes computed by RLA (reaction layer approach) and RS (rigorous solution) | none | (47) |
| $\kappa_{a,i}, \kappa_{d,i}$ | dimensionless association and dissociation rate constants of complex M^iL | none | (B-4), (B-5), (D-6) |
| λ_j | eigenvalues of matrix \mathbf{A} | none | (B-13), (B-16) |
| μ_i | reaction layer thickness of complex M^iL | m | (1), fig 1 |
| ξ_i | degree of lability of complex M^iL | none | (40) |
| $\rho \equiv r/r_0$ | dimensionless co-ordinate | none | (B-8) |
| $\tau (\tau_1, \tau_2, \tau_3)$ | total resistance (and components) to the flux | s m^{-1} | (29), (35) |
| χ | auxiliary variable | $\text{mol m}^{-1} \text{s}^{-1}$ | (5), (11) |

APPENDIX B. RIGOROUS SOLUTION FOR PARALLEL COMPLEXATION IN FINITE SPHERICAL DIFFUSION COUPLED WITH INTERNALISATION.

B.1.- Stating the problem

Consider a mixture arising from m parallel reactions between a metal M and m different ligands (all of them being in excess with respect to [M] so that each $[^iL]$ is a constant). Also note that successive complexes M^iL_k ($k > 1$) are not considered below.



We aim at rigorously solving - by extending previous methodology ^[20;22]- the steady-state reaction-diffusion problem for the particular case of finite spherical diffusion towards a consuming surface (of radius r_0) where only M is consumed with a first order internalisation process (i.e. not necessarily under maximum flux conditions).

The corresponding continuity equations are:

$$D_M \left(\frac{d^2[M]}{dr^2} + \frac{2}{r} \frac{d[M]}{dr} \right) + k_d[M^iL] - k_a[^iL][M] = 0 \quad (B-2)$$

and

$$D_{M^iL} \left(\frac{d^2[M^iL]}{dr^2} + \frac{2}{r} \frac{d[M^iL]}{dr} \right) - k_d[M^iL] + k_a[^iL][M] = 0 \quad i = 1..m \quad (B-3)$$

B.2.- Some definitions

We introduce the dimensionless association rate constant,

$$\kappa_{a,i} \equiv \frac{k_a[^iL]r_0^2}{D_M} \quad (B-4)$$

the dimensionless dissociation rate constant,

$$\kappa_{d,i} \equiv \frac{k_d^i r_0^2}{D_{M^iL}} \quad (\text{B-5})$$

the dimensionless complex concentration,

$$c_i \equiv \frac{D_{M^iL} K^i [L]}{D_M [M^iL]^*} \quad 0 < i \leq m \quad (\text{B-6})$$

the dimensionless metal ion concentration,

$$c_0 \equiv \frac{[M]}{[M]^*} \quad (\text{B-7})$$

and the normalised distance

$$\rho \equiv \frac{r}{r_0}. \quad (\text{B-8})$$

The previous eqns. (B-2) and (B-3) can now be expressed as:

$$\nabla^2 c_0 + \sum_{i=1}^m \kappa_{d,i} c_i - \left(\sum_{i=1}^m \kappa_{a,i} \right) c_0 = 0 \quad (\text{B-9})$$

And

$$\nabla^2 c_i - \kappa_{d,i} c_i + \kappa_{a,i} c_0 = 0 \quad 0 < i \leq m \quad (\text{B-10})$$

where now the spherical Laplacian operator is dimensionless.

B.3.-Recasting the differential equations

We combine the original concentrations in the reaction-diffusion equations so that they revert to one

Laplace equation

$$\nabla^2 f_0 = 0 \quad (\text{B-11})$$

(where f_0 represents the concentration of some formal species), and m Helmholtz equations:

$$\nabla^2 f_j = \lambda_j f_j \quad 0 < j \leq m \quad (\text{B-12})$$

where f_j represents the concentration of some formal species and λ_j is an eigenvalue of the matrix

associated to the system (B-9)-(B-10)

$$\mathbf{A} \equiv \begin{pmatrix} \left(\sum_{i=1}^m \kappa_{a,i} \right) & -\kappa_{d,1} & -\kappa_{d,2} & \cdots & -\kappa_{d,h} \\ -\kappa_{a,1} & \kappa_{d,1} & 0 & \cdots & 0 \\ -\kappa_{a,2} & 0 & \kappa_{d,2} & \cdots & 0 \\ \vdots & \vdots & \vdots & & \vdots \\ -\kappa_{a,h} & 0 & 0 & \cdots & \kappa_{d,h} \end{pmatrix} \quad (\text{B-13})$$

We stress that $\lambda_1, \lambda_2, \dots, \lambda_m$ are function of $\kappa_{a,i}$ and $\kappa_{d,i}$, but totally independent of the geometry and boundary conditions.

The sought combination (see eqns. (21), (30) and (32) in ref. ^[22]) can be expressed as

$$c_i = \sum_{j=0}^m s_{ij} f_j \quad (\text{B-14})$$

with

$$s_{0j} = 1 \quad 0 \leq j \leq m \quad (\text{B-15})$$

and

$$s_{ij} = \frac{\kappa_{a,i}}{\kappa_{d,i} - \lambda_j} \quad 0 < i \leq m \quad (\text{B-16})$$

B.4.- The particular case of finite spherical diffusion

We solve each of the eqns. (B-11)-(B-12) and impose the boundary condition of bulk values at a finite distance corresponding to

$$\rho_1 = \frac{r_0 + \delta}{r_0} \quad (\text{B-17})$$

leaving one constant in each solution to be determined later on from the surface boundary conditions.

The solution of eqn. (B-11) can be left in terms of one integration constant T_0

$$f_0 = 1 + T_0 \left(\frac{1}{\rho} - \frac{1}{\rho_1} \right) \quad (\text{B-18})$$

while the solution of eqns. (B-12) can be expressed as

$$f_j = \frac{T_j}{\rho} \left(e^{-\sqrt{\lambda_j} \rho} - e^{-\sqrt{\lambda_j} (\rho - 2\rho_1)} \right) \quad 0 < i \leq m \quad (\text{B-19})$$

Notice that the formal surface ($\rho=1$, denoted with superscript 0) concentrations for $j>0$ can be expressed

$$f_j^0 = T_j \left(e^{-\sqrt{\lambda_j}} - e^{-\sqrt{\lambda_j} (\rho - 2)} \right) \quad 0 < i \leq m \quad (\text{B-20})$$

We impose the surface boundary condition to c_0 (in terms of a certain c_0^0 yet to be known)

$$c_0^0 = \sum_{j=0}^m f_j^0 = 1 + T_0 \left(1 - \frac{1}{\rho_1} \right) + \sum_{j=1}^m T_j \left(e^{-\sqrt{\lambda_j}} - e^{-\sqrt{\lambda_j} (1 - 2\rho_1)} \right) \quad (\text{B-21})$$

For each complex, we can impose the null flux condition

$$0 = \left(\frac{dc_i}{d\rho} \right)_{\rho=1} = \sum_{j=0}^m s_{ij} \left(\frac{df_j}{d\rho} \right)_{\rho=1} \quad 0 < i \leq m \quad (\text{B-22})$$

from this expression, and after some algebra one obtains

$$1 - c_0^0 = \sum_{j=1}^m f_j^0 \left[\left(1 - \frac{1}{\rho_1} \right) \frac{\kappa_{d,i}}{\kappa_{d,i} - \lambda_j} \left(1 + \sqrt{\lambda_j} \coth[\sqrt{\lambda_j} (\rho_1 - 1)] \right) - 1 \right] \quad 0 < i \leq m \quad (\text{B-23})$$

Defining

$$A_j \equiv \frac{f_j^0}{1 - c_0^0} \quad (\text{B-24})$$

allows to determine the m coefficients A_j via the linear system

$$1 = \sum_{j=1}^m A_j \left[\left(1 - \frac{1}{\rho_1} \right) \frac{\kappa_{d,i}}{\kappa_{d,i} - \lambda_j} \left(1 + \sqrt{\lambda_j} \coth[\sqrt{\lambda_j} (\rho_1 - 1)] \right) - 1 \right] \quad i : 1..m \quad (\text{B-25})$$

B.5.- Computing the flux

The metal ion gradient can be expressed in terms of c_0^0 , from eqns. (B-7), (B-8), eqn. (35) in ref.

[22], (B-18), (B-21), (B-20) and (B-24) as

$$\left(\frac{d[M]}{dr} \right)_{r=r_0} = \frac{[M]^*}{r_0} \left(\frac{dc_i}{d\rho} \right)_{\rho=1} = \frac{[M]_r}{r_0} \left(\frac{df_0}{d\rho} \right)_{\rho=1} = -\frac{[M]_t}{r_0} T_0 = \frac{[M]_t}{r_0} \left(\frac{1 + \sum_{j=1}^m A_j}{1 - 1/\rho_1} \right) (1 - c_0^0) \quad (\text{B-26})$$

where $[M]_t$ is the total bulk metal concentration.

The value of c_0^0 can be isolated by imposing the equality of the incoming flux arriving by diffusion with the internalisation flux

$$J = D_M \left(\frac{d[M]}{dr} \right)_{r=r_0} = \frac{[M]_t}{r_0} \left(\frac{1 + \sum_{j=1}^m A_j}{1 - 1/\rho_1} \right) (1 - c_0^0) = k_{\text{int}} K_a \{R\} [M]_0 = k_{\text{int}} K_a \{R\} [M]^* c_0^0 \quad (\text{B-27})$$

so that

$$J = \frac{[M]^*}{r_0 (1 - 1/\rho_1)} + \frac{1}{D_M \frac{[M]_t}{[M]^*} \left(1 + \sum_{j=1}^m A_j \right) + k_{\text{int}} K_a \{R\}} \quad (\text{B-28})$$

APPENDIX C.- RIGOROUS SOLUTION FOR THE COMPUTATION OF SURFACE CONCENTRATIONS.

Combining (B-14), (B-15), (B-16), (B-20) and (B-24), it can be seen that

$$\frac{[M^iL]_0}{[M^iL]^*} = \frac{[M]_0}{[M]^*} + \left(1 - \frac{[M]_0}{[M]^*} \right) \sum_{j=1}^m \frac{A_j \lambda_j}{\kappa_{d,i} - \lambda_j} \quad i : 1..m \quad (\text{C-1})$$

But for its actual evaluation, we need to compute $[M]_0$ first. For instance, from (B-28) and (3),

$$[M]_0 = \frac{J}{k_{\text{int}} K_a \{R\}} = \frac{[M]^*}{1 + \frac{r_0 (1 - 1/\rho_1) k_{\text{int}} K_a \{R\}}{D_M \frac{[M]^*}{[M]_t} \left(1 + \sum_{j=1}^m A_j\right)}} \quad (\text{C-2})$$

As suggested in eqn (46) of ref. ^[22], an adequate definition of the lability degree is

$$\xi_i \equiv \left(1 - \frac{[M^iL]_0}{[M^iL]^*}\right) / \left(1 - \frac{[M]_0}{[M]^*}\right) \quad (\text{C-3})$$

because then we can express

$$J = \left(\frac{1}{r_0} + \frac{1}{\delta}\right) ([M]^* - [M]_0) \left(D_M + \sum_{i=1}^m D_{ML} K^i [L] \xi_i\right) \quad (\text{C-4})$$

Using this definition (C-3) and some algebra we obtain

$$\xi_i = 1 - \left(\sum_{j=1}^m \frac{A_j \lambda_j}{\kappa_{d,i} - \lambda_j}\right) \quad (\text{C-5})$$

which is similar to eqn. (57) in ref. ^[22].

APPENDIX D.- THE RIGOROUS SOLUTION: AVOIDING A SINGULAR MATRIX.

For the particular case of 2 complexes and 1 metal ($m=2$), the system of 3 differential equations :

$$\nabla^2 c_0 + \sum_{i=1}^2 \kappa_{d,i} c_i - \sum_{i=1}^2 \kappa_{a,i} c_0 = 0 \quad (\text{D-1})$$

$$\nabla^2 c_1 - \kappa_{d,1} c_1 + \kappa_{a,1} c_0 = 0 \quad (\text{D-2})$$

$$\nabla^2 c_2 - \kappa_{d,2} c_2 + \kappa_{a,2} c_0 = 0 \quad (\text{D-3})$$

with

$$\kappa_{d,1} = \kappa_{d,2} \quad (\text{D-4})$$

numerically yields a singular linear system for eqn. (B-25), as can be expected from the equality

$$\frac{\kappa_{d,1}}{\kappa_{d,1} - \lambda_j} = \frac{\kappa_{d,2}}{\kappa_{d,2} - \lambda_j} \text{ for any } j. \text{ Furthermore, there are analytical infinity entries due to one}$$

eigenvalue being equal to the common κ_d .

In this case ($\kappa_{d,1} = \kappa_{d,2}$), prior to the diagonalisation procedure detailed in section B.2, it is convenient to prepare an equivalent system (whose specific parameters will be labelled "equiv"). If we define an equivalent "total complex" concentration

$$c_{\text{equiv}} = c_1 + c_2 \quad (\text{D-5})$$

and an equivalent dimensionless association rate constant

$$\kappa_{a,\text{equiv}} \equiv \kappa_{a,1} + \kappa_{a,2} \quad (\text{D-6})$$

and add eqns. (D-2) and (D-3), the original system of 3 differential equations (with 3 unknowns) reverts to just a system of 2 eqns

$$\nabla^2 c_{\text{equiv}} - \kappa_{d,1} c_{\text{equiv}} + \kappa_{a,\text{equiv}} c_0 = 0 \quad (\text{D-7})$$

$$\nabla^2 c_0 + \kappa_{d,1} c_{\text{equiv}} - \kappa_{a,\text{equiv}} c_0 = 0 \quad (\text{D-8})$$

with 2 unknowns, totally parallel to the original system (D-1)-(D-3) (but with one equation less, i.e. $m=1$). In this way the "standard" procedure (section B.3) can be applied from this point onwards with an equivalent concentration and an equivalent dimensionless rate constant.

This strategy of a previous combination can be extended to the case of several complexes sharing common dimensionless dissociation constants. One can gather all the complexes in an equivalent one with expressions analogous to (D-5) and (D-6).

APPENDIX E: PHYSICAL INTERPRETATION OF THE NEGATIVE DIFFERENCE BETWEEN RS AND RLA.

We can analytically estimate the RLA-error due to the inadequacy of eqn. (1) in the particular case of complexes having the same lability ($\mathcal{L}_1 = \mathcal{L}_2$), the same diffusion layer thickness ($\mu_1 = \mu_2$) and the same mobility ($D_M = D_{M^1L} = D_{M^2L}$). From these particular conditions, one finds that ${}^1k_a[{}^1L] = {}^2k_a[{}^2L]$, ${}^1K[{}^1L] = {}^2K[{}^2L]$ and therefore ${}^1k_d = {}^2k_d$. In this case, the direct application of eqn. (29) for 2 ligands under diffusion limited conditions yields

$$\tau = \frac{r_0}{D_M} - \frac{r_0^2}{r_0 + \delta} \frac{1}{D_M (1 + 2 {}^1K[{}^1L])} + \frac{r_0^2}{r_0 + \mu_1} \left(\frac{1}{D_M (1 + 2 {}^1K[{}^1L])} - \frac{1}{D_M} \right) \quad (\text{E-1})$$

As shown by eqn. (D-7), the individual continuity equations for M^1L and M^2L revert to just one equation which is formally identical to the case of only one complex with an equivalent rate constant $k_{a,\text{equiv}}[L]_{\text{equiv}} = {}^1k_a[{}^1L] + {}^2k_a[{}^2L]$ and an equivalent complex concentration $[ML]_{\text{equiv}} = [M^1L] + [M^2L]$ leading to an equivalent stability constant $K'_{\text{equiv}} = {}^1K[{}^1L] + {}^2K[{}^2L]$ or $K'_{\text{equiv}} = 2 {}^1K[{}^1L]$ when ${}^1K[{}^1L] = {}^2K[{}^2L]$. One can compute the corresponding reaction layer thickness as:

$$\mu_{\text{equiv}} = \sqrt{\frac{D_M}{k_{a,\text{equiv}}[L]_{\text{equiv}}}} = \sqrt{\frac{D_M}{{}^1k_a[{}^1L] + {}^2k_a[{}^2L]}} \quad (\text{E-2})$$

or, when ${}^1k_a[{}^1L] = {}^2k_a[{}^2L]$:

$$\mu_{\text{equiv}} = \sqrt{\frac{D_M}{2 {}^1k_a[{}^1L]}} = \frac{\mu_1}{\sqrt{2}} \quad (\text{E-3})$$

The direct application of eqn. (29) for 1 (equivalent) ligand under diffusion limited conditions yields

$$\tau = \frac{r_0}{D_M} - \frac{r_0^2}{r_0 + \delta} \frac{1}{D_M (1 + K'_{\text{equiv}})} + \frac{r_0^2}{r_0 + \mu_{\text{equiv}}} \left(\frac{1}{D_M (1 + K'_{\text{equiv}})} - \frac{1}{D_M} \right) \quad (\text{E-4})$$

which coincides with (E-1) except in the value of the diffusion layer thickness. Comparison of eq. (E-1) and (E-4) shows the limitation of eqn (1) when the μ values are close to each other and allows to predict the sign and absolute value of the error. Indeed, μ_{equiv} is smaller than μ_1 , so (E-1) overestimates τ , and so, RLA, via eqn. (28), underestimates the flux.

The quantitative expression of ε can be obtained for the general case of a set of m ligands with identical labilities and reaction layer thicknesses. Then $K'_{\text{equiv}} = m {}^1K[\text{L}]$ and $\mu_{\text{equiv}} = \frac{\mu_1}{\sqrt{m}}$ which can be used in eqn. (E-4) to eventually compute the flux with (28). In the particular case of maximum flux (i.e. the surface acting as a perfect sink), the error in the flux (given by (47)) between the direct application of eq. (29) and the refined approach given by (E-4), becomes

$$\varepsilon = \frac{m {}^1K[\text{L}] r_0 \left(\frac{1}{r_0 + \mu_1} - \frac{\sqrt{m}}{r_0 \sqrt{m} + \mu_1} \right)}{1 + m {}^1K[\text{L}] - \frac{r_0}{r_0 + \delta} - \frac{r_0 m {}^1K[\text{L}]}{r_0 + \mu_1}} \quad (\text{E-5})$$

The validity of this expression is shown in Figs. 4 and 9.