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Supplemental information for the article untitled "Underlying mechanisms in microbial solar cells: How modeling can help"

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1 Experimental information

1.1 Volume of algae and solution

The reaction rates that we estimate in this work are related to the specific experimental conditions the measurements were made in. For example k_{alg} scales with the number of algae actively performing photosynthesis. This reaction rate would therefore be reduced if the algal concentration was lower. The experimental concentration, 2×10^7 algae/mL is saturated from the biological point of view, but this means that overall the cumulated volumes of algae, called V_a in this document, represent only 1% of the solution (see Fig.1). It is important to take this into account since the diffusion of a few mediator molecules into algae will barely affect the solution concentration, but will drastically change the concentration inside V_a. We have to deal here with a major difference compared to classical chemical reactions where there is only one reaction compartment.

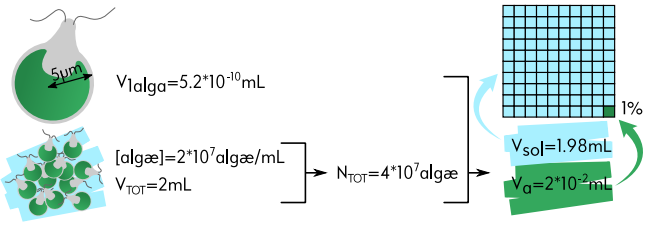
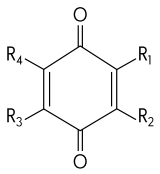


Fig. 1 Alga dimensions and concentration. The radius of a *Chlamydomonas reinhardtii* alga is about 5μm. Together with the algal concentration, this allows to deduce V_a, the cumulated volume of all algae contained in the 2mL algal suspension, which represents only 1% of the total volume.

1.2 Mediator lipophilicity

Table.1 presents calculated values of logP for some quinones (Q) and corresponding quinols (QH_2) using Advanced Chemistry Development (ACD/Labs) Software V11.02.



R_1	R_2	R_3	R_4	Q	QH_2
C_6H_5	H	H	H	1.95	2.39
CH_3	H	H	CH_3	1.22	1.39
Cl	H	H	Cl	1.73	2.26

Table 1 Calculated values of logP for quinones and quinols, depending on their substituents R_1, R_2, R_3 and R_4.

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2 Analytical description of the model

2.1 Mathematical formulation

We start by recalling the basis of the model described in the article and illustrated on the figure 1 of the main document. The volume of reaction consist of a volume V_s of solution in interaction with a volume V_a of algae. The following reactions represent the transfer of the molecules Q, and QH_2 between these two volumes:

$$\mathrm{Q} \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} \mathrm{Q}_a \tag{1}$$

$$\mathrm{QH_2} \underset{k_{out}}{\overset{k_{in}}{\rightleftharpoons}} \mathrm{QH_2}a \tag{2}$$

the subscript $_a$ being associated to molecules in the volume V_a. k are the first order kinetic reaction rates where subscript indicates the direction of diffusion (in or out) across the cell membrane.

In algae, the mediator can be reduced following:

$$\mathrm{Q}_a + 2H^+ + 2e^- \xrightarrow{k_{alg}} \mathrm{QH_2}a \tag{3}$$

, with the reaction rate k_{alg}.

In the solution (at the electrode) the mediator can be oxidized again, delivering current

$$\mathrm{QH_2} \xrightarrow{k_{el}} \mathrm{Q} + 2H^+ + 2e^- \tag{4}$$

, with the reaction rate k_{el}.

Combining balance equations 1, 2, 3 and 4, one can write deterministic equations for the concentrations of the 4 species, leading to the system of linear ordinary differential equations:

$$\begin{cases} \frac{d[\mathrm{Q}]}{dt} = -k_{in}[\mathrm{Q}] + \frac{V_a}{V}k_{out}[\mathrm{Q}_a] + k_{el}[\mathrm{QH_2}] \\ \frac{d[\mathrm{Q}_a]}{dt} = -k_{out}[\mathrm{Q}_a] + \frac{V}{V_a}k_{in}[\mathrm{Q}] - k_{alg}[\mathrm{Q}_a] \\ \frac{d[\mathrm{QH_2}]}{dt} = -k_{in}[\mathrm{QH_2}] + \frac{V_a}{V}k_{out}[\mathrm{QH_2}a] - k_{el}[\mathrm{QH_2}] \\ \frac{d[\mathrm{QH_2}a]}{dt} = -k_{out}[\mathrm{QH_2}a] + \frac{V}{V_a}k_{in}[\mathrm{QH_2}] + k_{alg}[\mathrm{Q}_a] \end{cases} \tag{5}$$

Because the kinetics equations are first written in terms of number of molecules, and then rewritten in terms of concentration, note that the volume ratios $\frac{V_a}{V}$ or $\frac{V}{V_a}$ appear only when there is a change of reference volume for the considered species.

The set of equations 5 can be written in the vectorial form:

$$\frac{d\overrightarrow{X}}{dt} = M\overrightarrow{X} \tag{6}$$

where $\overrightarrow{X}$ is the state vector of the system, describing the concentration of each species at any given time:

$$\overrightarrow{X} = \begin{pmatrix} [\mathrm{Q}] \\ [\mathrm{Q}_a] \\ [\mathrm{QH_2}] \\ [\mathrm{QH_2}a] \end{pmatrix} \tag{7}$$

and M is the rate matrix:

$$M = \begin{pmatrix} -k_{in} & \frac{V_a}{V}k_{out} & k_{el} & 0 \\ \frac{V}{V_a}k_{in} & -k_{out} - k_{alg} & 0 & 0 \\ 0 & 0 & -k_{in} - k_{el} & \frac{V_a}{V}k_{out} \\ 0 & k_{alg} & \frac{V}{V_a}k_{in} & -k_{out} \end{pmatrix} \tag{8}$$

The rates k_{in} and k_{out} are considered to be independent in general, because membrane effects could favour one way or another. Note that if the transfer across the membrane is purely diffusive, by assuming steady state, one can express the rates as a function of the surface of transfer S, the diffusion coefficient of the species D and the thickness of the diffusion boundary layer at interface δ :

$$k_{in}^{\text{purely diffusive}} = \frac{DS}{\delta V_s} \tag{9}$$

$$k_{out}^{\text{purely diffusive}} = \frac{DS}{\delta V_a} \tag{10}$$

In this hypothetical case, one would get the following relationship:

$$k_{out}^{\text{purely diffusive}} = \frac{V_s}{V_a}k_{in}^{\text{purely diffusive}} \tag{11}$$

2.2 Estimating the kinetic rates

2.2.1 Equilibrium between inwards and outwards diffusion across algae (Phase 2)

In the dark (at the begining of the experiment), the mediator is only in the oxidized state, and the dynamical system is reduced to the two first states. If

one define a subvector:

$$\overrightarrow{x} = \begin{pmatrix} [\mathrm{Q}] \\ [\mathrm{Q}_a] \end{pmatrix} \tag{12}$$

The dynamics reads:

$$\frac{d\overrightarrow{x}}{dt} = \begin{pmatrix} -k_{in} & \frac{V_a}{V} k_{out} \\ \frac{V}{V_a} k_{in} & -k_{out} \end{pmatrix} \overrightarrow{x} \tag{13}$$

The matrix associated has only one eigenvalue $\lambda = -(k_{in} + k_{out})$. We associate to this value the rate constant:

$$K = k_{in} + k_{out} \tag{14}$$

Taking into account initial conditions where all the mediator is out of algae ($[\mathrm{Q}](t=0) = [\mathrm{Q}^0]$), the time solution reads:

$$\begin{pmatrix} [\mathrm{Q}](t) \\ [\mathrm{Q}_a](t) \end{pmatrix} = \frac{[\mathrm{Q}]^0}{\frac{V_a}{V}(1+\frac{k_{in}}{k_{out}})} \begin{pmatrix} \frac{V_a}{V}\left(\frac{k_{out}}{k_{in}} + e^{-Kt}\right) \\ 1 - e^{-Kt} \end{pmatrix} \tag{15}$$

After equilibrium, at steady state the concentrations become:

$$\begin{pmatrix} [\mathrm{Q}]^\infty \\ [\mathrm{Q}_a]^\infty \end{pmatrix} = [\mathrm{Q}]^0 \begin{pmatrix} \frac{1}{\frac{k_{in}}{k_{out}}\left(1+\frac{k_{in}}{k_{out}}\right)} \\ \frac{1}{\frac{V_a}{V}\left(1+\frac{k_{in}}{k_{out}}\right)} \end{pmatrix} \tag{16}$$

2.2.1.1 Fitting the data Because NPQ is proportional to Q_a ($NPQ = \alpha\,[\mathrm{Q}_a]$), the second component of equation 15 gives the evolution of NPQ:

$$NPQ = \alpha\,[\mathrm{Q}_a]^\infty \left(1 - e^{-Kt}\right) \tag{17}$$

6 experiments have been fitted. The results of the fits are displayed for each experiment on figure 2. They are all plotted on figure 2, shifted on the x axis by their starting time and normalized by their amplitude on the y axis. We see that the data are not very sparsed. We find $K = 2.1 \times 10^{-2}$ s^{-1} with a standard error of 0.3×10^{-2} s^{-1}.

2.2.2 Reaction rate of re-oxidation at the electrode (Phase 5)

At the end of the experiment, when the photosynthesis is blocked by DCMU addition, $k_{alg} = 0$ and the two

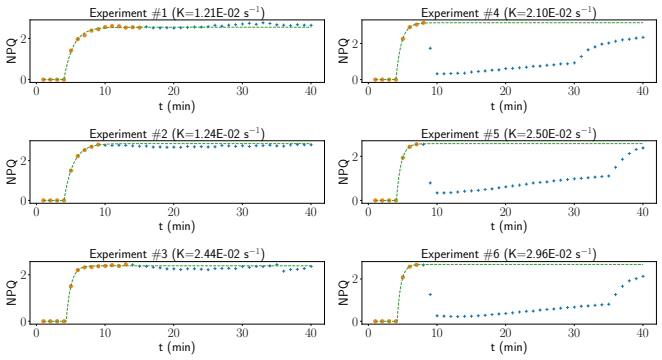

Fig. 2 Fit of the NPQ rise during Phase 2 for various experiments. The blue crosses indicate the raw data and the orange circles are the selection of the data coresponding to the rise of the NPQ. These data are fitted following equation 17 and the fit is displayed in green dashed lines.

concentrations QH_2 and $\mathrm{QH}_2 a$ become independant of the two other concentrations (see Matrix 8). We have again a simple system with only two species to solve. If one label $\overrightarrow{y}$ the vector state:

$$\overrightarrow{y} = \begin{pmatrix} [\mathrm{QH}_2] \\ [\mathrm{QH}_2 a] \end{pmatrix} \tag{18}$$

The dynamics reads:

$$\frac{d\overrightarrow{y}}{dt} = \begin{pmatrix} [2] - k_{in} - k_{el} & \frac{V_a}{V} k_{out} \\ \frac{V}{V_a} k_{in} & -k_{out} \end{pmatrix} \overrightarrow{y} \tag{19}$$

The coupling matrix of equation 19 has two eigen values:

$$\lambda_\pm = -\frac{k_{el} + k_{in} + k_{out}}{2} \left(1 \pm \sqrt{1 - \frac{k_{out}\,k_{el}}{\left(\frac{k_{el}+k_{in}+k_{out}}{2}\right)^2}}\right) \tag{20}$$

, which predicts a decrease of the current on the form of a sum of two exponentials with different rate constants $k_+ = -\lambda_+$ and $k_- = -\lambda_-$.

2.2.2.1 Data Figure 3 shows that this double exponential is observed in all experiments. Taking into account all fits, we get the following experimental values:

$$k_+ = 7.07 \times 10^{-2} \pm 2.91 \times 10^{-3}\,\mathrm{s}^{-1} \tag{21}$$

$$k_- = 5.58 \times 10^{-3} \pm 3.84 \times 10^{-4}\,\mathrm{s}^{-1} \tag{22}$$

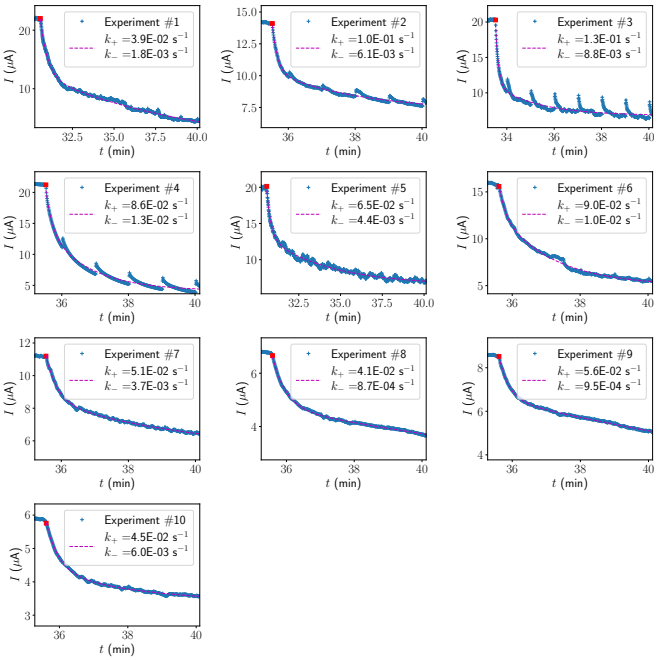

Fig. 3 Fit of the current decrease during Phase 5. The raw experimental data are indicated by blue crosses. The fit follows $I(t) = c_+ e^{-k_+ t} + c_- e^{-k_- t}$. c_+ and c_- are constants linked to the boundary conditions (How much QH_2 and QH_2a are present when the light is switched off). The rates k_+ and k_- are linked to the eigen values of equation 20: $k_\pm = -\lambda_\pm$.

We have 3 unknowns k_{in}, k_{out} and k_{el} for 3 equations :

$$\begin{cases} K &= k_{in} + k_{out} & \text{(23a)} \\ k_+ &= \frac{k_{el}+k_{in}+k_{out}}{2}\left(1+\sqrt{1-\frac{k_{out}\,k_{el}}{\left(\frac{k_{el}+k_{in}+k_{out}}{2}\right)^2}}\right) & \text{(23b)} \\ k_- &= \frac{k_{el}+k_{in}+k_{out}}{2}\left(1-\sqrt{1-\frac{k_{out}\,k_{el}}{\left(\frac{k_{el}+k_{in}+k_{out}}{2}\right)^2}}\right) & \text{(23c)} \end{cases}$$

By taking the values obtained in the last section for K, and the values obtained in this section for k_+ and k_-, one can solve numerically the equations 23. We get $k_{in} = 1.88 \times 10^{-2}\,s^{-1}$, $k_{out} = 2.17 \times 10^{-3}\,s^{-1}$ and $k_{el} = 2.53 \times 10^{-2}\,s^{-1}$. Be aware that these values are not based on one single experiment, but on the average. Therefore it only give the correct order of magnitude. See main text for the results obtained on each experiments. Our point here is that these values enable to do Taylor expansion of the square root in equation 20. Indeed the parameter

$$\xi = \frac{k_{out}\,k_{el}}{\left(\frac{k_{el}+k_{in}+k_{out}}{2}\right)^2} \qquad (24)$$

is small compared to 1 (numerically, we find $\xi \sim 0.1$).

Keeping only higher order terms in the Taylor expansion of equation 20 gives:

$$k_+ = -\lambda_+ \approx k_{in} + k_{out} + k_{el} \qquad (25)$$

$$k_- = -\lambda_- \approx k_{out} \qquad (26)$$

2.2.3 Full system (for description of Phase 3)

For the description of Phase 3, one needs to consider the full system described by the matrix 8. This matrix has three non null eigen values:

$$\begin{cases} \lambda &= -k_{in} - k_{out} & \text{(27a)} \\ \tilde{\lambda_+} &= -\frac{\tilde{K}}{2}\left(1+\sqrt{1-4\,\frac{k_{alg}k_{el}+k_{alg}k_{in}+k_{el}k_{out}}{\tilde{K}^2}}\right) & \text{(27b)} \\ \tilde{\lambda_-} &= -\frac{\tilde{K}}{2}\left(1-\sqrt{1-4\,\frac{k_{alg}k_{el}+k_{alg}k_{in}+k_{el}k_{out}}{\tilde{K}^2}}\right) & \text{(27c)} \end{cases}$$

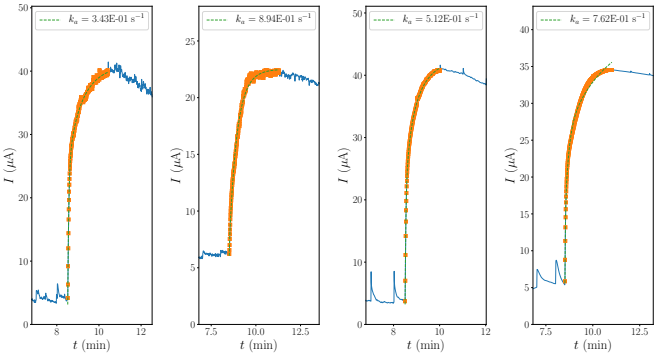

Fig. 4 Fit of the current rise during Phase 3. The raw experimental data are indicated by blue crosses. The fit follows $I(t) = c_0 + c_1\, e^{-k_1 t} + c_2\, e^{-k_2 t} + c_3\, e^{-k_3 t}$. The constants c are linked to the boundary conditions. The rates are linked to the eigen values of equation 27: $k_1 = -\lambda$, $k_2 = -\tilde{\lambda_+}$ and $k_3 = -\tilde{\lambda_-}$.

, where $\tilde{K} = k_{alg} + k_{el} + k_{in} + k_{out}$. One can perform the same kind of Taylor expansion that the one done in the previous section, to find a good approximation for the three time constants. The first one is the rate $k_{in} + k_{out}$ characteristic of the transfert rates inwards and outwards of the algae. The third one associated to $\tilde{\lambda_-}$ is going to be much slower than the two others. The second one (associated to $\tilde{\lambda_+}$) is the fastest : it is roughly the sum of all the rates:

$$-\tilde{\lambda_+} \approx \tilde{K} \tag{28}$$

Numerically, one do not need this approximation. As we know the numerical value of k_{in}, k_{out} and k_{el} we fit the experimental data with the exact equations 27 with only k_{alg} as a free parameter (and the relative amplitudes of the different terms).

The fits are shown figure 4. We find:

$$k_{alg} = 0.63 \pm 0.07\, s^{-1} \tag{29}$$

2.2.4 Methodology check

The fitting methodology has been numerically checked. We ran one simulation with known parameters for k_{in}, k_{out}, k_{el} and k_{alg} in order to obtain the simulated time evolution of intensity and NPQ. Then we used our methodology: We fit these two curves in order to extract values for k_{in}, k_{out}, k_{el} and k_{alg}. The relative error was always smaller than 2×10^{-6}.

2.3 Modeling toxicity

2.3.1 Screening effect

The light-screening effect is implemented by making k_{alg} vary over time. The effective k_{alg} depends on the light intensity perceived by photosynthetic chains, therefore it depends on the NPQ:

$$k_{alg}^{screening} = \frac{k_{alg}}{1 + NPQ} \tag{30}$$

, where k_{alg} is the reduction rate of Qa by illuminated algae in the absence of quenching ($NPQ = 0$), and $k_{alg}^{screening}$ its equivalent in the presence of quenching.

2.3.2 Different variations for modeling destructions of PC

In the main text we described how we modeled the destruction of PC by oxidative stress due to quinones with a second order kinetics. Here, we also explore two other possibility : first order kinetics, and Michael mechanism.

2.3.2.1 Mechanism deriving from oxidative stress : first order kinetics Although the destruction of PC is catalyzed by quinones, it is still possible to imagine a first order kinetics. In this hypothesis, the rate would be (instead of equation 13 of the main text) :

$$\frac{df_{PC}}{dt} = -k_{tox}^{1} f_{PC} \tag{31}$$

where k_{tox}^{1} is a first order kinetic rate.

2.3.2.2 Michael acceptor like mechanism In the case of the Michael mechanism, the rate is still of second order but the quinone would be degraded after destruction of a PC. This has been implemented in the simulation.

2.3.2.3 Comparison Figure 5 compiles all the possible mechanisms to see the effect of each assumption.

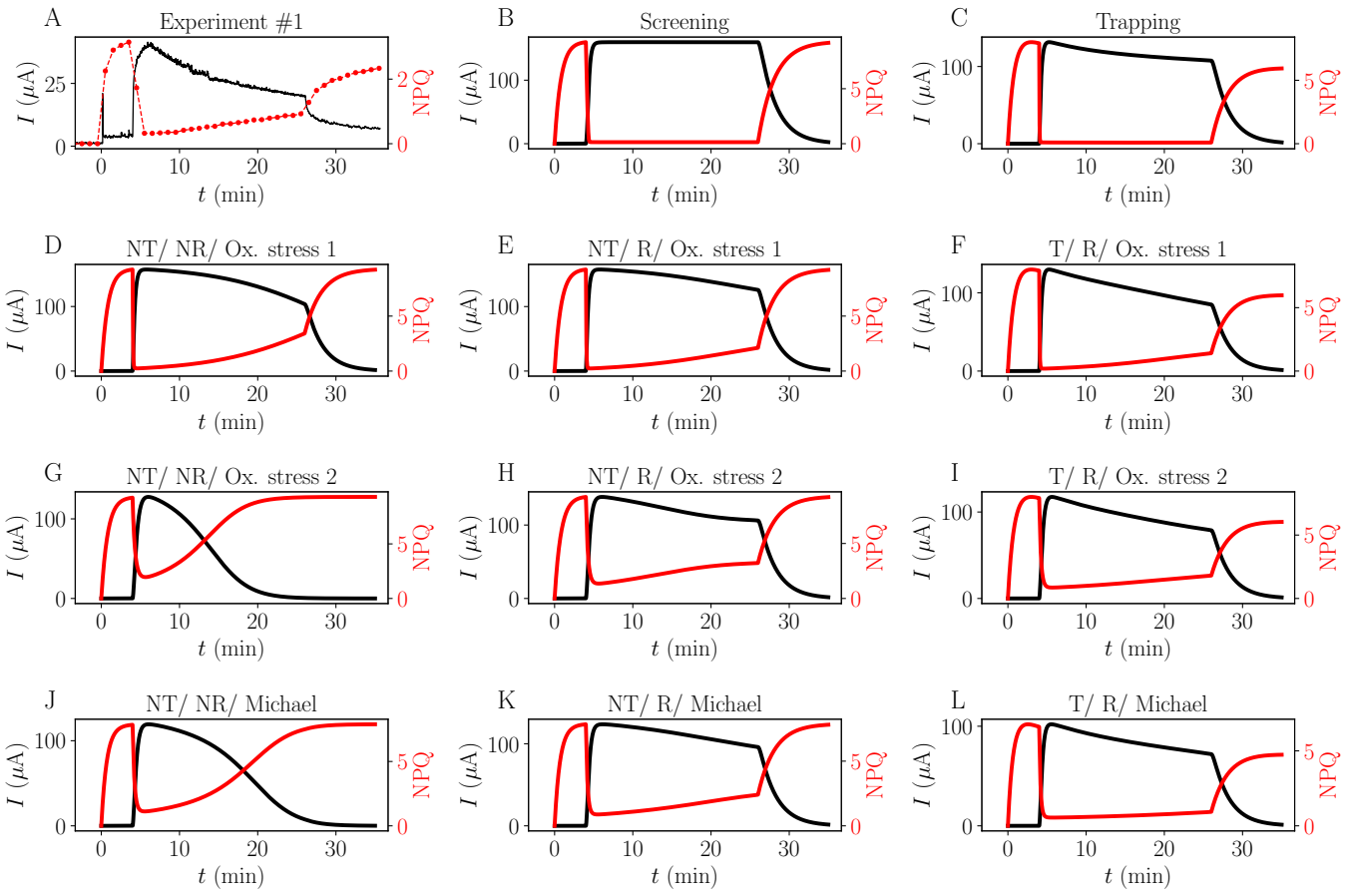

Fig. 5 Comparison of one typical experiment (A) with different hypotheses. All starting parameters are the same as figure 5 of the main text. (B) screening effect. (C) Trapping effect (same as main text). The row (G, H, I) corresponds to the second order kinetics already presented in figure 5 of the main text : T (respectively NT) stands for trapping with same parameters as (B) (respectively no trapping). R (respectively NR) stands for residual activity, ϕ_{ra} =2.3% (respectively ϕ_{ra} =0). The line (D, E, F) is analogous with a first order kinetics $k^1_{tox} = 2.5 \times 10^{-1}\text{s}^{-1}$. The line (J, K, L) corresponds to Michael mechanism.