eSPC, an Online Data Analysis Platform for Molecular Biophysics

KinGenie 1.0
User Documentation

THIS MANUAL IS STILL UNDER DEVELOPMENT

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1. Surface-based binding

1.1. Import data

1.1.1. Input file

KinGenie can parse files generated by Octet (Sartorius) and Gator instruments, and common comma-separated-values (CSV) files.

A) Octet Biolayer interferometry (BLI)

Import the files with extension *.frd*. There should be one file per sensor. Data from multiple sensors can be imported simultaneously, if all of them share the same metadata.

B) Gator Biolayer interferometry

To import the results from a Gator BLI experiment, many files need to be imported together: the comma-separated-values (csv) file with the traces data, that contain the words 'Assay' and 'Channel'; the files describing the metadata, named *Settings.ini* and *ExperimentStep.ini*.

C) CSV file

For multi-cycle kinetics, the CSV file should have the following columns:

- *Time*: time data in seconds
- *Signal*: signal values at each time point
- *Smax*: integer, useful to identify if the Smax parameter should be fitted globally or not
- *Analyte_concentration_micromolar_constant*: analyte concentration in micromolar units

For single-cycle kinetics, the CSV file should have one extra column:

- Cycle: cycle number for single cycle kinetics

Note: CSV example files can be exported from the Simulation panel.

1.1.2. Processing

Given a certain experiment, the sensor traces can be processed in the following ways:

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- Alignment of the association phase: For each step, labelled in the metadata as a step where the association occurs, the mean of the last ten points from the previous step (should be the baseline) is calculated and subtracted from the association step and all subsequent steps.

- Baseline subtraction: Given a group of sensors and a reference sensor, the signal from the reference sensor is subtracted from the signal of the other sensors.

- Average: Given a group of sensors, a new sensor is created with the average signal.

- Inter-step correction (Alignment of the dissociation phase): For each step, labelled in the metadata as a step where the dissociation occurs, the difference between the mean of the first *n* points and the mean of the last *n* points from the previous step (should be the association) is calculated and subtracted from the dissociation step. The value of n is selected by the user.

- Merge steps by index: Given the index of a certain reference step, the next or previous step inherits the step type. For example, if step 5 (dissociation) is selected as reference step, and step 6 (baseline) as “step to merge”, both steps will be labelled as dissociation.

- Merge by step name: Given a step type, such as association or dissociation, all steps coming a step of that type, will inherit the selected step type.

Given two experiments, the sensor traces from one experiment can be processed in the following way:

- Subtract experiment: Given two different experiments with an equal number of sensors, equal number of steps and time step, the signal data from one experiment is subtracted from the signal data from the other experiment, sensor by sensor.

For a more precise analysis of surface-based experiments, it is recommended to perform double-reference. In other words, to subtract, from each sensorgram, the signal produced by a sensor with ligand and without analyte, and the signal produced by a sensor without ligand and with analyte (at the same concentration).

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1.2. Analysis

1.2.1. Creating a Dataset

Once the traces are imported and preprocessed, they can be combined for global analysis. Traces with the same sample ID will be fitted with global kinetic parameters, such as K_d and k_{off}. Additionally, traces with the same sample ID and same S_{max} ID will be fitted with a shared S_{max} parameter, if the option to fit the data linked by S_{max} is activated. The S_{max} parameter is related to the theoretical total binding capacity of the sensor. Usually, the S_{max} parameter should be shared only if the association and dissociation curves were performed with the same sensor, commonly referred to as single-cycle kinetics.

1.2.1. Region of interest

Surface-based binding experiments contain two types of steps where (un)binding takes place: association and dissociation. During the association phase, the concentration of the analyte is kept constant, while during the dissociation the concentration of the analyte drops to zero. Both regions can be fitted with shared kinetic parameters. Alternatively, it is possible to fit the association phase, or only the dissociation phase. When fitting the dissociation phase, the k_{off} is estimated, but not the K_d.

Lastly, if the end of the association phase reached equilibrium, because binding occurred too fast (due to very fast on-rates), and there is not enough curvature in the association phase, the steady-state signal versus ligand concentration curve can be fitted. Overall, the regions of interest for the fitting are:

- Association and dissociation (K_d and k_{off} is fitted)
- Association (K_d and k_{off} is fitted)
- Dissociation (k_{off} is fitted)
- Steady-state (K_d is fitted)

1.2.3. Models

In the models below, ***L* refers to the immobilized molecule (ligand)**, and ***A* to the** molecule flowing at constant concentration in-solution, also called **analyte**.

1.2.3.1. One-to-one (kinetics)

The system is described by the chemical equation:

$$A + L \rightleftharpoons AL$$

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The differential equations for each species concentration are:

$$\frac{d[AL]}{dt} = [A][L]k_{on} - [AL]k_{off}$$

$$\frac{d[A]}{dt} = [AL]k_{off} - [A][L]k_{on}$$

$$\frac{d[L]}{dt} = [AL]k_{off} - [A][L]k_{on}$$

where k_{on} is the association rate, and k_{off} is the dissociation rate. The equilibrium dissociation constant is related to k_{on} and k_{off} as follows:

$$K_d = \frac{k_{off}}{k_{on}}$$

The signal S evolves according to the ordinary differential equation (ODE):

$$\frac{dS}{dt} = \frac{k_{off}}{K_d}[A](S_{max} - S) - k_{off}S$$

where $\frac{dS}{dt}$ is the rate of change of signal over time and S_{max} is the total binding capacity. The analytical solution of the ODE is:

$$S(t, S_{eq}, S_0, \nu) = S_{eq} + (S_0 - S_{eq}) \cdot e^{-\nu \cdot t}$$

where

$$S_{eq}([A], K_d, S_{max}) = \frac{[A]}{[A]+K_d}S_{max}$$

$$\nu([A], K_d, k_{off}) = k_{off}\frac{[A]+K_d}{K_d}$$

Overall, there are three variables to be fitted: k_{on}, K_d, and S_{max}. S_0 corresponds to the initial signal.

Note: If the dissociation phase is analysed alone, excluding the association phase, then we fit the initial signal. On the other hand, the K_d parameter can not be fitted. The analytical solution for the dissociation phase is:

$$S(t, S_0, k_{off}) = S_0 \cdot e^{-k_{off} \cdot t}$$

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1.2.3.2. One-to-one (steady-state)

In this model, the association phase is assumed to have reached equilibrium, and the signal, at a given analyte concentration is described by the following equation:

$$S([A], K_d, S_{max}) = \frac{S_{max}[A]}{K_d + [A]}$$

where S is the signal at equilibrium, R_{max} is the maximum theoretical response that could be observed (at infinite analyte concentration), [A] is the analyte concentration, and K_d is the equilibrium dissociation constant.

Note: One important assumption of the steady-state model is that all sensors have the same binding capacity. Therefore, for a correct application of the steady-state model, it is important that all biosensors present the same amount of immobilized ligand.

1.2.3.3. One-to-one (mass transport limitation)

The system is described by the chemical equations:

$$A_{bulk} \rightleftharpoons A_{surface}$$

$$L + A_{surface} \rightleftharpoons LA$$

The equilibrium between A_{bulk} and $A_{surface}$ implies that the concentration of A at the surface of the biosensor, where the ligand is immobilized, is different from the concentration in the surrounding solution.

The differential equations for each species concentration are:

$$\frac{d[A_{bulk}]}{dt} = k_{tr}[A]_{surface} - k_{tr}[A_{bulk}]$$

$$\frac{d[L]}{dt} = [LA]k_{off} - [L][A_{surface}]k_{on}$$

$$\frac{d[A_{surface}]}{dt} = [LA]k_{off} + k_{tr}[B]_{bulk} - k_{tr}[A_{surface}] - [L][A_{surface}]k_{on}$$

$$\frac{d[LA]}{dt} = [L][A_{surface}]k_{on} - [LA]k_{off}$$

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where k_{tr} is the transport rate constant, k_{on} is the association rate, and k_{off} is the dissociation rate. Two non-redundant ODEs that provide a description of the system during the association phase are:

$$\frac{dS}{dt} = (k_{off}[A_{surface}] / K_d) \cdot (S_{max} - S) - k_{off}S$$

$$\frac{d[A_{surface}]}{dt} = k_{tr}([A]_0 - [A_{surface}]) - \frac{dS}{dt}$$

where $\frac{dS}{dt}$ corresponds the rate of change of signal over time and $\frac{d[A_{surface}]}{dt}$ corresponds to the rate of change of the concentration of $A_{surface}$ over time. $[A]_0$ is the total concentration of A.

Overall, there are four variables to be fitted: k_{off}, K_d, k_{tr} and S_{max}.

Moreover, during the dissociation phase, when the concentration of A is zero, the differential equation required to describe the evolution of the signal is:

$$\frac{dS}{dt} = (-k_{off}S) / (1 + (\frac{k_{on}}{k_{tr}})(S_{max} - S))$$

Note: This model can only be used for fitting if the association and dissociation phases are selected.

1.2.3.4. One-to-one (induced fit / two-step)

The system is described by the chemical equations:

$$L + A \rightleftharpoons LA_{int}$$

$$LA_{int} \rightleftharpoons LA$$

LA_{int} refers to an intermediate complex that changes to LA after an induced fit step. The differential equations for each species concentration are:

$$\frac{d[LA_{int}]}{dt} = [L][A]k_{on} + [LA]k_{rev} - [LA_{int}]k_c - [LA_{int}]k_{off}$$

$$\frac{d[L]}{dt} = [LA_{int}]k_{off} - [L][A]k_{on}$$

$$\frac{d[A]}{dt} = [LA_{int}]k_{off} - [L][A]k_{on}$$

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$$\frac{d[LA]}{dt} = [LA_{int}]k_c - [LA]k_{rev}$$

Two non-redundant ODEs that provide a description of the system during the association phase are:

$$\frac{dS_1}{dt} = (-k_{off} - k_{on}[A])S_1 - k_{off}S_2 + k_{off}S_{max}$$

$$\frac{dS_2}{dt} = -k_cS_1 + (-k_c - k_{rev})S_2 + k_cS_{max}$$

The total observed signal at any time is calculated as:

$$S_{total} = S_{max} - S_1$$

In multi-cycle kinetics, the initial conditions are:

$$S_1 = S_{max}$$

$$S_2 = 0$$

Overall, there are five variables to be fitted: k_{on}, k_{off}, k_c, k_{rev} and S_{max}.

Note: This model can only be fitted if the association and dissociation phases are selected. One important assumption of this model, often omitted, is that the signal generated by the intermediate and induced complex is the same.

1.2.3.5 One-to-one (conformational selection)

The system can be described by the chemical equations:

$$L_{inactive} \rightleftharpoons L_{active}$$

$$L_{active} + A \rightleftharpoons LA$$

L_{active} refers to the ligand in an active conformation ready for binding, while $L_{inactive}$ is not capable of binding. The differential equations for each species concentration are:

$$\frac{d[L_{inactive}]}{dt} = [L_{active}]k_{rev} - [L_{inactive}]k_c$$

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$$\frac{d[L_{active}]}{dt} = [L_{inactive}]k_c - [L_{active}]k_{rev} - [L_{active}][A]k_{on} + [LA]k_{off}$$

$$\frac{d[A]}{dt} = [LA]k_{off} - [L_{active}][A]k_{on}$$

$$\frac{d[LA]}{dt} = [L_{active}][A]k_{on} - [LA]k_{off}$$

Two non-redundant ordinary differential equations that provide a description of the system during the association phase are:

$$\frac{dS_1}{dt} = (-k_c - k_{rev})S_1 - k_{rev}S_2 + k_{rev}S_{max}$$

$$\frac{dS_2}{dt} = -k_{on}[A]S_1 + (-k_{on}[A] - k_{off})S_2 + k_{on}[A]S_{max}$$

In multi-cycle kinetics, the initial conditions are:

$$S_1 = S_{max} / (\frac{k_c}{k_{rev}} + 1)$$

$$S_2 = 0$$

The total observed signal at any time is S_2. Overall, the ODEs contain five variables to be fitted: k_{on}, k_{off}, k_c, k_{rev} and S_{max}.

Note: This model can be used for simulating curves, but it is not available for fitting. One important assumption is that L_{active} and $L_{inactive}$ are in equilibrium before the interaction with the analyte.

1.2.3.6. Heterogenous analyte

In this model, the system can be described by the chemical equation:

$$A_i + L \rightleftharpoons A_iL \quad \forall i \in [1, N]$$

where A_i corresponds to the i-th analyte. Briefly, a Langmuir 1:1 interaction between each analyte A_i and the ligand (L) is assumed. The set of ODEs is:

$$\frac{dR_i}{dt} = k_{on,i} \cdot F_i \cdot [A]_0 \cdot R_{max,i} \cdot \delta - k_{off,i}R_i$$

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$$R_{tot} = \sum_{i=1}^{N} R_i$$

where the subindex *i* refers to the i-th analyte. And δ is defined as:

$$\delta = 1 - \sum_{j=1}^{N} \frac{R_j}{R_{max,j}}$$

Note: This model can be used for simulating curves of a mixture of two analytes, but it is not available for fitting.

1.2.3.7 Summary

Model name	**Type of analysis**	**Parameters (excluding S_{max})**	**Simulation**	**Fitting**
One-to-one (steady-state)	Equilibrium	K_d, k_{off}	YES	YES
One-to-one (kinetics)	Kinetics	K_d	YES	YES
One-to-one (mass transport limitation)	Kinetics	K_d, k_{off}, k_{tr}	YES	YES
One-to-one (induced fit / two-step)	Kinetics	K_d, k_{off}, k_c, k_{rev}	YES	YES
One-to-one (conformational selection)	Kinetics	K_d, k_{off}, k_c, k_{rev}	YES	NO
Heterogenous analyte	Kinetics	$K_{d,1}$, $k_{off,1}$, $K_{d,2}$, $k_{off,2}$	YES	NO

1.2.4. Signal and fitting

The one-to-one (steady-state) and one-to-one (kinetics) signal is calculated using an analytical equation, while for the mass-transport-limitation and heterogeneous analyte models we solve the ODE using the *solve_ivp* function from *scipy*, that numerically integrates a system of ordinary differential equations given a set of initial conditions. Last, for the induced-fit and conformational-selection models, the ODEs are solved using the matrix exponential method.

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Regarding the fitting, all models are fitted by applying non-linear least squares, as implemented in the *curve_fit* function from *scipy*. To aid the fitting algorithm, each parameter is constrained between certain bounds. If the parameters are too close to the bounds, the fitting should be discarded.

The standard deviation of all fitted parameters is then computed using the square root of diagonal values from the fit parameter covariance matrix reported by *scipy.curve_fit* function. These values are an approximation (**underestimation**) of the real errors. Relative errors are calculated as $100 * (std(\hat{\theta}) / \hat{\theta})$ where $\hat{\theta}$ refers to the estimate of the parameter and $std(\hat{\theta})$ to the standard deviation.

In the case of the one-to-one (steady-state) and one-to-one (kinetics) models, we also provide the marginal asymmetric confidence interval. Briefly, the lower and upper bounds of the 95 % confidence interval are given by the values of K_d (or k_{off}) satisfying

$$RSS(K_d) = RSS_0(1 + \frac{criticalValue}{n-p})$$

where RSS_0 is the residual sum of squares using the best estimates for all the parameters, $RSS(K_d)$ is the residual sum of squares using a fixed value of K_d (fitting again the other parameters), n is the number of data points, p is the number of parameters, and *critcialValue* is the critical value of the Fisher-Snedecor distribution with n - p and 1 degrees of freedom and a confidence level of 95 %.

General note:

In the kinetics models, the S_{max} parameter can be fitted globally or locally. It is recommended to fit the S_{max} parameter globally only in the case of single-cycle kinetics where all the association and dissociation phases were performed with the same sensor.

1.3. Glossary (surface-based binding)

Analyte	Biomolecule that is kept at a fixed concentration and remains in-solution
Association phase	Phase of a surface-based experiment where the concentration of the analyte is kept constant and flows over the surface
Baseline	Phase of a surface-based experiment where the biosensors measure the signal of the buffer. A step of this type is required before the

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	association step.
Biosensor	Disposable reactive surface used to immobilize the ligand and detect binding
Dissociation phase	Phase of a surface-based experiment where the concentration of the analyte is dropped to zero and the signal diminishes due to the dissociation of the complex. It comes immediately after the association phase
Double-reference	Method of background correction where, for each biosensor, the signal of the reference sensor is subtracted together with the signal of a sensor without immobilized ligand and with analyte to correct for non-specific binding to the surface
K_d	Equilibrium dissociation constant ($K_d = \frac{k_{on}}{k_{on}}$)
k_{off}	Rate of dissociation of the complex in the reaction: $PL \rightarrow P + L$
k_{on}	Rate of formation of the complex in the reaction: $P + L \rightarrow PL$
Ligand	In a surface-based experiment, the ligand refers to the biomolecule that is fixed to the surface, and is usually a protein. On the other hand, in a solution-based experiment, the ligand moves freely in solution and could be a small molecule
Loading	Phase of a surface-based experiment where the ligand is flown at a fixed concentration to immobilize it
Reference sensor	Sensor with ligand and without analyte used to correct for buffer background and instrument drift
S_{max}	Maximum theoretical binding capacity of the sensor. Depends on the amount of loading and is usually unique for a given sensor and experiment
Steady-state	Condition of a chemical reaction where all state variables remain constant, such as the concentration of the biomolecules.

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2. Solution-based binding

2.1. Import data

2.1.1. Input file

A) A CSV file with the following columns:

- Time: time data in seconds
- Signal: signal values at each time point
- Protein_concentration_micromolar: protein concentration in micromolar units
- Ligand_concentration_micromolar: ligand concentration in micromolar units

2.1.2. Processing

Given a certain experiment, a time cutoff can be selected such that all traces will be truncated at the specified time point.

2.2. Analysis

2.2.1 Creating a Dataset

After the traces are imported, inside the '2. Analyse' tab, a dataset for analysis can be created. Curves with the same SampleID will be fitted with shared kinetic parameters.

2.2.2. Models

2.2.2.1. Single exponential

In this model, the signal *Y* of each curve is fitted using a single exponential function:

$$Y = a_0 + a_1 \cdot e^{-k_{obs} \cdot t}$$

where a_0 is the intercept term, a_1 is the pre-exponential term, k_{obs} is the observed rate constant, and *t* is the time in seconds.

Note: In this model, the k_{obs} values are fitted individually.

2.2.2.2. Double exponential

In this model, the signal *Y* of each curve is fitted using a double exponential function:

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$$Y = a_0 + a_1 \cdot e^{-k_{obs,1} \cdot t} + a_2 \cdot e^{-k_{obs,2} \cdot t}$$

where a_0 is the intercept term, a_1 and a_2 are the pre-exponential terms, $k_{obs,1}$ and $k_{obs,2}$ are the observed rate constants, and t is the time in seconds.

Note: In this model, the $k_{obs,1}$ and $k_{obs,2}$ values are fitted individually.

2.2.2.3. One binding site

The system is described by the chemical equation:

$$A + B \rightleftharpoons AB$$

The differential equations for each species concentration are:

$$\frac{d[AB]}{dt} = [A][B]k_{on} - [AB]k_{off}$$

$$\frac{d[A]}{dt} = [AB]k_{off} - [A][B]k_{on}$$

$$\frac{d[B]}{dt} = [AB]k_{off} - [A][B]k_{on}$$

where k_{on} is the association rate, and k_{off} is the dissociation rate. The equilibrium dissociation constant is related to k_{on} and k_{off} as follows:

$$K_d = \frac{k_{off}}{k_{on}}$$

One ODE is required to calculate the species concentrations:

$$\frac{d[AB]}{dt} = ([A_t] - [AB])([B_t] - [AB])\frac{k_{off}}{K_d} - [AB]k_{off}$$

where $[A_t]$ is the total concentration of A, and $[B_t]$ is the total concentration of B. The initial condition is that $[AB]$ equals zero at $t = t_0$. The parameter t_0 can be left at zero, or fitted. The total signal is obtained as follows:

$$Y(t) = Y_a([A_t] - [AB]) + Y_b([B_t] - [AB]) + Y_{ab}([AB])$$

where Y_a, Y_b and Y_{ab} is the signal produced by free (unbound) A, free B and the complex AB.

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Note: Y_a, Y_b and Y_{ab} can be fitted or left at zero.

2.2.2.4. One binding site (induced fit)

The system is described by the chemical equations:

$$A + B \rightleftharpoons AB_{int}$$

$$AB_{int} \rightleftharpoons AB$$

AB_{int} refers to an intermediate complex that changes to AB after an induced fit step. The differential equations for each species concentration are:

$$\frac{d[A]}{dt} = [AB_{int}]k_{off} - [B][A]k_{on}$$

$$\frac{d[B]}{dt} = [AB_{int}]k_{off} - [B][A]k_{on}$$

$$\frac{d[AB_{int}]}{dt} = [B][A]k_{on} + [AB]k_{rev} - [AB_{int}]k_c - [AB_{int}]k_{off}$$

$$\frac{d[AB]}{dt} = [AB_{int}]k_c - [AB]k_{rev}$$

The initial condition is that $[AB_{int}]$ and $[AB]$ equal zero at $t = 0$. By using the law of mass-conservation, only the two last ODE are required to obtain the concentration of all species:

$$[A] = [A_t] - [AB_{int}] - [AB]$$

$$[B] = [B_t] - [AB_{int}] - [AB]$$

The total signal is obtained as follows:

$$Y(t) = Y_a([A_t] - [AB]) + Y_b([B_t] - [AB]) + Y_{ab,int}([AB_{int}]) + Y_{ab}([AB])$$

where Y_a, Y_b, $Y_{ab,int}$ and Y_{ab} is the signal produced by free (unbound) A, free B and the complex AB_{int} and the induced-complex AB.

Note: Y_a, Y_b, $Y_{ab.int}$, Y_{ab} can be fitted or left at zero. $Y_{ab.int}$ can be assumed to be equal to Y_{ab}.

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2.2.2.5. One binding site (conformational selection)

The system can be described by the chemical equations:

$$A_{inactive} \rightleftharpoons A_{active}$$

$$A_{active} + B \rightleftharpoons AB$$

A_{active} refers to the molecule *A* in an active conformation ready for binding, while $A_{inactive}$ is not capable of binding. The differential equations for each species concentration are:

$$\frac{d[A_{inactive}]}{dt} = [A_{active}]k_{rev} - [A_{inactive}]k_c$$

$$\frac{d[B]}{dt} = [AB]k_{off} - [A_{active}][B]k_{on}$$

$$\frac{d[A_{active}]}{dt} = [A_{inactive}]k_c - [A_{active}]k_{rev} - [A_{active}][B]k_{on} + [AB]k_{off}$$

$$\frac{d[AB]}{dt} = [A_{active}][B]k_{on} - [AB]k_{off}$$

The initial condition is that at $t = 0$, $[A_{active}]$ and $[A_{inactive}]$ are in equilibrium, and $[AB]$ equals zero. By using the law of mass-conservation, only the two last ODE are required to obtain the concentration of all species:

$$[A_{inactive}] = [A_t] - [A_{active}] - [AB]$$

$$[B] = [B_t] - [AB]$$

The total signal is obtained as follows:

$$Y(t) = Y_a([A_{inactive}] + [A_{active}]) + Y_b([B]) + Y_{ab}([AB])$$

where Y_a, Y_b, $Y_{ab,int}$ and Y_{ab} is the signal produced by free *A* (active or inactive), free *B* and the complex *AB*.

Note: This model is still only available for simulation, but not for fitting.

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2.2.4. Signal and fitting

To calculate the signal of the different models, the ODEs are solved using the *solve_ivp* function from *scipy*, that numerically integrates a system of ordinary differential equations given initial conditions.

Regarding the fitting, all models are fitted by applying non-linear least squares, as implemented in the *curve_fit* function from *scipy*. To aid the fitting algorithm, each parameter is constrained between certain bounds. If the parameters are too close to the bounds, the data is automatically re-fitted with broader bounds.

Contact details

For further assistance, please contact us:

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