Can you please describe the 1:1 binding model used in kinetics studies on the Octet systems? How many parameters are being fitted?

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The simplest model used to describe the interaction between two biomolecules is represented by the equation below:

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

B represents the ligand molecule immobilized on the surface of the biosensor, and A is the analyte in solution. AB is the complex formed by binding A to B. This binding model assumes a simple 1:1 interaction, where one ligand molecule interacts with one analyte molecule, and binding is independent and of equal strength for all binding sites. Complex formation in this case follows pseudo-first-order kinetics. k_a is the association rate constant (also called k_{on}) and k_d is dissociation rate constant (also called k_{off}).

In a 1:1 bimolecular interaction, both the association and dissociation phases display time-resolved signals that are described by single exponential functions. In the association phase, biosensors immobilized with ligand molecules are dipped into a solution containing the ligand's binding partner, the

analyte, and binding interaction of the analyte to the immobilized ligand is measured. Analyte molecules bind at the same rate to every ligand binding site. The association curve follows a characteristic exponential association profile, with an exponential increase in signal followed by a leveling off to plateau as the binding reaches equilibrium. In the dissociation phase, the biosensor is dipped into a buffer solution free of any analyte molecules, and the bound analyte in the formed complex is allowed to come off the ligand. The dissociation curve follows a single exponential decay with signal eventually returning to baseline.

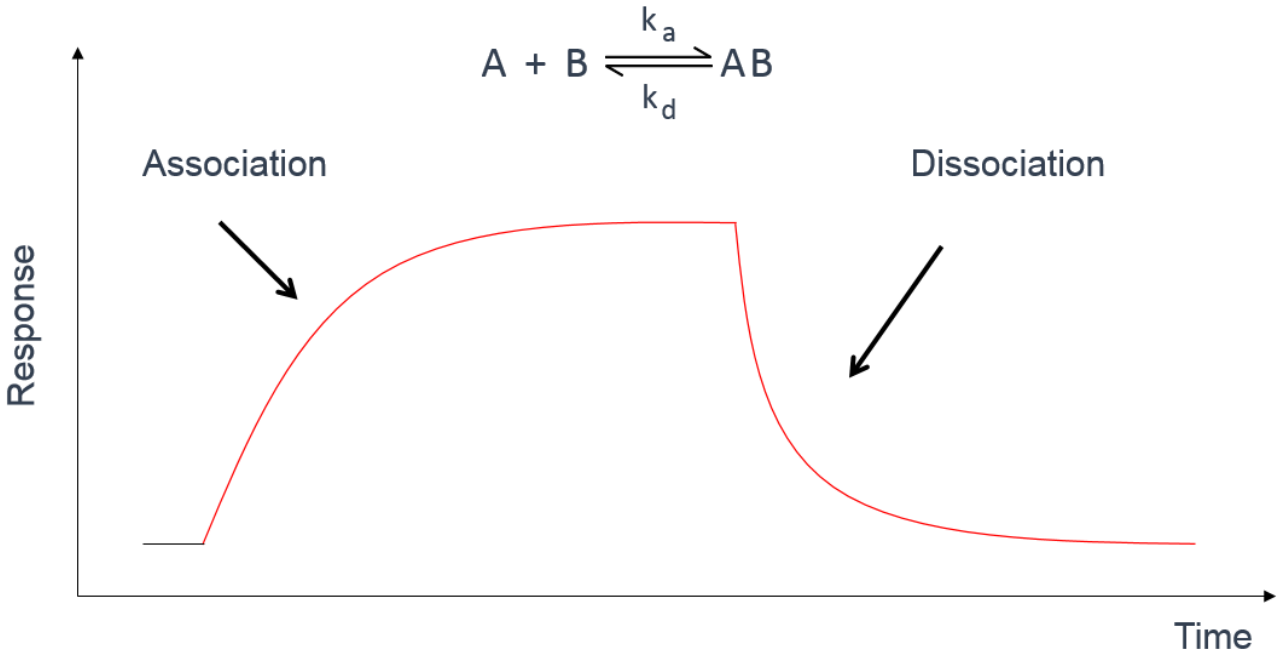

Figure 1: Illustration of an ideal sensorgram in a simple 1:1 binding model, where the association and dissociation phases are described by single exponential functions.

The equilibrium dissociation constant (or affinity constant) K_D can be calculated as the ratio of k_d to k_a.

$$K_D = \frac{[A] \bullet [B]}{[AB]} = \frac{k_d}{k_a}$$

The K_D corresponds to the concentration of analyte at which 50% of ligand binding sites are occupied at

equilibrium, or the concentration at which the number of ligand molecules with bound analyte equals the number of ligand molecules without bound analyte. There is an inverse relationship between K_D and affinity — a smaller affinity constant indicates a tighter interaction, or greater affinity of analyte to ligand.

The association and dissociation curves can be analyzed by fitting the data to one of the available curve fitting models in Octet Data Analysis or Data Analysis HT software. Curve fitting is a process of optimization by the software to construct a curve that has the best fit to a series of data points based upon the mathematical functions and constraints. Below we describe the curve fitting equations of partial and full fitting solutions for the 1:1 binding model, and the relationship between partial and full fitting.

1.Partial fitting solution.

In partial fitting, the equations for association (equation 1) and for dissociation (equation 2) are two separate and independent algorithms. Equation 1 only fits the association phase data without consideration of the dissociation data. Equation 2 only fits the dissociation data without considering the association data.

Partial association phase:

$$Y = Y_0 + R_{eq}(1 - e^{-k_{obs}*t})$$

(Equation 1)

Partial dissociation phase:

$$Y = Y_e + Y_\Delta e^{-k_d*t}$$

(Equation 2)

where

$$R_{eq} = R_{max}\frac{k_a*[Analyte]}{k_a*[Analyte] + k_d} = R_{max}\frac{[Analyte]}{[Analyte] + K_D}$$

(Equation 4)

and

$$k_{obs} = k_a*[Analyte] + k_d$$

(Equation 5)

Here:

- Y is the BLI signal in nm, which indicates the level of binding as a nm shift.
- t is time in seconds.
- Y_0 (in association phase, equation 1) is the fitted nm shift from zero (the initial Y value) assimilated to noise, or the initial Y binding level of the fitted association curve.
- k_a is the association rate constant.
- k_d is the dissociation rate constant.
- k_{obs} is the observed rate constant reflecting the overall rate of the combined association and dissociation of the two binding partners.
- [Analyte] refers to the provided concentration of the analyte in solution.
- R_{eq} (R equilibrium) is the fitted binding response value (nm shift) when the binding interaction reaches equilibrium between association and dissociation for a given analyte concentration.
- R_{max} represents the calculated maximum achievable binding for an analyte to a given level of immobilized ligand on the biosensor surface.
- Y_e (in dissociation phase, equation 2) is the fitted value that the exponential decay curve will eventually approach to.
- $Y_?$ (in dissociation phase, equation 2) is the nm shift difference between the first data point of the fitted dissociation curve and Y_e .

The association algorithm in equation 1 fits (or is optimized for) for R_{eq} and k_{obs}. The dissociation algorithm in equation 2 fits k_d. The software then calculates the results of R_{max}, k_a, and K_D accordingly based upon R_{eq}, k_{obs}, k_d if the analyte concentration is given, using equation 5 for k_a and equations 4 and 5 for R_{max} calculation. Note that R_{max}, k_a, and K_D values are not obtained directly from this partial fitting process, but simply the calculated results of fitted parameters (R_{eq}, k_{obs}, k_d) and analyte concentration. There are four points worth noting:

1. Both k_a and k_d are chemical properties of a given pair of interaction and they are not concentration-dependent.
2. The dissociation process is concentration-independent and Octet software generates the k_d value result using the dissociation data *without* the analyte concentration. The association process is k_a, k_d and concentration-dependent (equation 1 and equation 5), and Octet software includes both association and dissociation data and the analyte concentration to generate the k_a value result.
3. The rate constant fitted from equation 1 (association phase) is k_{obs}, not k_a. k_{obs} is the combined effect of k_a, analyte concentration, and k_d (equation 5). A bigger k_{obs} value does not necessarily indicate a bigger k_a value, since the impact of analyte concentration and k_d must be considered as well. Thus, one cannot simply compare the k_{obs} rate constants fitted from association curves only to compare the k_a differences.
4. Users should evaluate the biological relevance of the k_a and k_d obtained from the partial fitting algorithms. k_{obs} or k_d values are fitted independently from the association or dissociation data, without considering the data from the other. Therefore, their fitting results may not represent the "best fit" when considering association and dissociation data together as a whole. k_a is a calculated value from k_d , k_{obs} , and analyte concentration in the partial fitting:

$$k_a = \frac{k_{obs} - k_d}{[Analyte]}$$

In rare cases, when k_d is bigger than k_{obs}, the calculated k_a will be negative, which obviously deviates from the biological reality and therefore result should not be used. In such a scenario, use the full fitting algorithm (described in the next section) to fit both association and dissociation data together to obtain reliable k_a and k_d values.

2. Full fitting solution for a 1:1 binding.

The full 1:1 binding model with three unknowns (k_a , k_d , R_{max}) fits the data from both

association and dissociation together, and the fitting is described in the following three equations:

Association phase:

$$Y = R_{max}\frac{1}{1+\frac{k_d}{k_a*[Analyte]}}(1-e^{-\left(k_a*[Analyte]+k_d\right)t})$$

(Equation 6)

Dissociation phase:

$$Y = Y_A e^{-k_d(t-t_A)}$$

(Equation 7)

$$Y_A = R_{max}\frac{1}{1+\frac{k_d}{k_a*[Analyte]}}(1-e^{-\left(k_a*[Analyte]+k_d\right)t_A})$$

(Equation 8)

Here:

- Y is the BLI signal in nm, which indicates the level of binding as a nm shift.
- t is time in seconds.
- k_a is the association rate constant.
- k_d is the dissociation rate constant.
- [Analyte] refers to the provided concentration of the analyte in solution.
- R_{max} represents the fitted maximum achievable binding for an analyte to a given level of immobilized ligand on the biosensor surface.
- t_A represents the time at the end of association, which is also the time at the beginning of dissociation.
- Y_A represents the calculated nm shift at the end of association (when time is at t_A).

When $t = t_A$ and is plugged into equation 6, Y becomes Y_A and equation 6 becomes equation 8. Thus, the calculated binding level (Y_A) at the last experimental time point in the association phase at time t_A becomes the calculated binding level at the beginning time point of the dissociation phase, since the dissociation always starts from the maximal binding achieved at the end association. While equations 7 and 8 describe the fitting equations for the dissociation phase, the analyte concentration in equation 8 refers to the analyte concentration in the association phase, as in dissociation the biosensors are dipped into a buffer solution without any analyte.

The full fitting model fits for (or optimizes for) R_{max}, k_a, k_d (three parameters), if the analyte concentration is given, using equations 6, 7 and 8. The software then calculates k_{obs}, R_{eq} and K_D values based upon the results of R_{max}, k_a, k_d and the analyte concentration using equation 5 for k_{obs} and equation 4 for R_{eq} calculation. Note that the values of k_{obs}, R_{eq} and K_D are not obtained directly from the curve fitting process, but simply the calculation results of the fitted values (R_{max} , k_a , k_d) and analyte concentration.

For more information on k_{obs}, R_{max} and R_{eq}, refer to What are Rmax and Req, and what is their relationship? and What are the formulas for Req and kobs?

3. Relating full and partial fitting 1:1 models.

Although the full fitting and partial fitting models appear to be totally different, one may wonder if there is any relationship between these two models. Rearrangement of 1:1 full fitting equations yields equations like the partial fitting equations, allowing a better understanding of the relationship between full and partial fitting 1:1 models.

Recall full fitting association phase in equation 6,

$$Y = R_{max}\frac{1}{1+\frac{k_d}{k_a*[Analyte]}}(1-e^{-\left(k_a*[Analyte]+k_d\right)t})$$

(Equation 6)

Rearrange it to get

$$Y = R_{max}\frac{k_a*[Analyte]}{k_a*[Analyte]+k_d}(1-e^{-\left(k_a*[Analyte]+k_d\right)t})$$

Plugging the values of R_{eq} and k_{obs} according to

$$R_{eq} = R_{max}\frac{k_a*[Analyte]}{k_a*[Analyte]+k_d}$$

(Equation 4)

and

$$k_{obs} = k_a * [Analyte] + k_d$$

(Equation 5)

Equation 6 becomes

$$Y = R_{eq}(1 - e^{-k_{obs} * t})$$

(Equation 9)

Similarly, recall equation 8 of Y_A (dissociation phase),

$$Y_A = R_{max} \frac{1}{1 + \frac{k_d}{k_a * [Analyte]}} (1 - e^{-\left(k_a * [Analyte] + k_d\right) t_A})$$

Rearrange it to get

$$Y_A = R_{max} \frac{k_a * [Analyte]}{k_a * [Analyte] + k_d} (1 - e^{-\left(k_a * [Analyte] + k_d\right) t_A})$$

Plug R_{eq} and k_{obs} according equations 4 and 5, equation 8 becomes

$$Y_A = R_{eq}(1 - e^{-k_{obs}*t_A})$$

(Equation 10)

Equation 10 can also be obtained when plugging (t_A, Y_A) to equation 9.

Thus the full fitting model can be simply re-writen as:

Association phase:

$$Y = R_{eq}(1 - e^{-k_{obs}*t})$$

(Equation 9)

Dissociation phase:

$$Y = Y_A e^{-k_d(t - t_A)}$$

(Equation 7)

$$Y_A = R_{eq}(1 - e^{-k_{obs}*t_A})$$

(Equation 10)

Where

$$R_{eq} = R_{max} \frac{k_a*[Analyte]}{k_a*[Analyte]+k_d}$$

(Equation 4)

$$\text{and } k_{obs} = k_a * [Analyte] + k_d$$

(Equation 5)

This format of full fitting equations is writen in a way that is similar to the expression of partial fitting equations, and can therefore be used to better understand the similarity and difference between full fitting and partial fitting of the 1:1 software model.

The side by side comparison between the full fitting and partial fitting equations are shown in Table 1.

	Partial fitting	Full fitting rearranged	Full fitting
Association	$Y = Y_0 + R_{eq}(1 - e^{-k_{obs}*t})$	$Y = R_{eq}(1 - e^{-k_{obs}*t})$	$Y = R_{max}\frac{1}{1+\frac{k_d}{k_a*[Analyte]}}(1 - e^{-(k_a*[Analyte]+k_d)t})$
Dissociation	$Y = Y_e + Y_\Delta e^{-k_d*t}$	$Y = Y_A e^{-k_d(t-t_A)}$	$Y = Y_A e^{-k_d(t-t_A)}$
		$Y_A = R_{eq}(1 - e^{-k_{obs}*t_A})$	$Y_A = R_{max}\frac{1}{1+\frac{k_d}{k_a*[Analyte]}}(1 - e^{-(k_a*[Analyte]+k_d)t_A})$
Notes	Association and dissociation are independent algorithms	Rearange to show similarity of full fitting vs partial fitting equations	One single algorithm
	Software model fits (optimizes for) for R_{eq} and k_{obs} in association, and fits k_d in dissociation	$R_{eq} = R_{max}\frac{k_a*[Analyte]}{k_a*[Analyte]+k_d} = R_{max}\frac{[Analyte]}{[Analyte]+K_D}$	Software model fits (optimizes for) k_a, k_d, and R_{max} with all equations together
	Software calculates R_{max}, k_a and K_D.	$k_{obs} = k_a*[Analyte]+k_d$	Software calculates k_{obs}, R_{eq} and K_D

Y is the BLI signal in nm, which indicates the level of binding as a nm shift.

Y_0 (partial fitting, association) is the fitted nm shift from zero (the initial Y value) assimilated to noise, or the initial Y binding level of the fitted association curve.

Y_e (partial fitting, dissociation) is the fitted value that the exponential decay curve will eventually approach to.

Y_Δ (partial fitting, dissociation) is the nm shift difference between the first data point of the fitted dissociation curve and Y_e.

Y_A (full fitting) represents the calculated nm shift at the end of association (when time is at t_A).

t is time in seconds.

t_A (full fitting) represents the time at the end of association, which is also the time at the beginning of dissociation.

R_{max} represents the calculated (in partial fitting) or fitted (in full fitting) maximum achievable binding for an analyte to a given level of immobilized ligand on the biosensor surface.

R_{eq} (R equilibrium) is the fitted (in partial association fitting) or calculated (in full fitting) binding response value (nm shift) when the binding interaction reaches equilibrium between association and dissociation for a given analyte concentration.

k_{obs} is the observed rate constant reflecting the overall rate of the combined association and dissociation, of the two binding partners.

k_a is the association rate constant.

k_d is the dissociation rate constant.

[Analyte] refers to the provided concentration of the analyte.

Table 1: Comparison of the equations used in the full fitting 1:1 model with the partial fitting 1: 1 model. Click table for zoomed view.

The full fitting and partial fitting algorithm have similarities in which the association curves are described by exponential association functions related to k_a , k_d and analyte concentration, and dissociation curves are described by exponential decay equations that are concentration-independent. However, significant differences exist for these two mathematic models:

1. The full fitting option assumes that an interaction is fully reversible, so that as the dissociation step time approaches infinity, all of the analyte bound to the ligand will dissociate. Since the dissociation curve will eventually reach the pre-association baseline, the rate of dissociation is extrapolated until it reaches zero signal on the Y-axis. The partial dissociation model assumes the interaction is not fully reversible and therefore the dissociation will not reach pre-association baseline. Only a portion of the analyte bound will dissociate even as the step time approaches infinity, and the rate of dissociation is fitted to the measured data only. Thus, in the partial fitting model for the dissociation phase, the baseline offset (Y_e) is an extrapolated value that the curve will eventually end. In the regular partial fitting dissociation model, the software does not

force Y_e to be the zero value, and optimizes its value based upon the curve shape while optimizing k_d values. Partial dissociation can be used to fit portions of curves in data sets with significant biphasic dissociation, however partial fitting may tend to give higher k_d values. Partial fitting with the "Dissoc. Approaches Zero" option forces Y_e to zero, while optimizing k_d. Note that in partial fitting, the association and dissociation equations are independent from each other, and therefore the value of Y_e is not related to association.

2. The full fitting option also introduces a restriction between the binding levels of the association and dissociation, where the model assumes the calculated binding level at the last experimental time point of association is the same as the calculated binding level at the beginning time point of dissociation. However, there is no such restriction for partial fitting, as the association and dissociation phases are fitted independently. In partial fitting, while R_{eq} can be extrapolated from the association phase data, R_{eq} has nothing to do with dissociation phase fitting.

For more information on full vs. partial fitting, refer to What is the difference between the local full fit vs. partial fit? How do I choose which one to use?

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