Quick guide

For Global fitting of ITC data with AFFINImeter

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I. The usefulness of global fitting

The global analysis approach consists in the simultaneous fitting of several isotherms where one or many fitting parameters are shared, while other parameters apply to each isotherm individually.

Global fitting is particularly useful for analysis of complex interactions (that involve the presence of more than one equilibrium) in which the registration of various dataseries under different experimental conditions is a requisite to get sufficient information to properly describe the interaction.

The main objective of performing global fitting is to achieve more robust analysis and reliable results.

II. Generating fitting projects for global analysis in AFFINImeter

The initial step for global analysis is the creation of one fit project with all the dataseries of interest. For this, use the option "new fit project" and choose between "stoichiometric approach" and "independent sites approach".

a) Global fitting based on a stoichiometric approach[1]

In the stoichiometric approach each isotherm has a binding model associated to it. This means that, each isotherm can be fitted to a different binding model.
Use the button "Add dataseries" to upload the first isotherm. Next, select the binding model that will be applied to that isotherm. Click on "Add dataseries" again to continue uploading more isotherms.

b) Global fitting based on an independent sites approach[2]

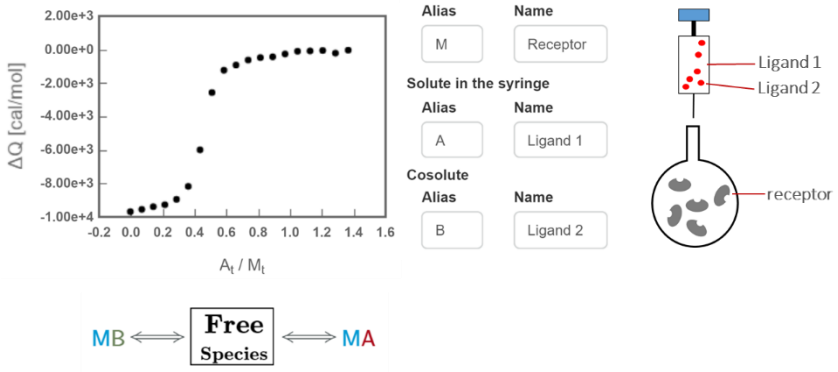
In the independent sites approach the isotherms are fitted to a multiple binding site approach where different number of sets and sites can be defined for each isotherm.

Once all the isotherms are uploaded into the fitting project the next step is to define which parameters are shared between isotherms.

III. How to share fitting parameters for global analysis

In this section we will explain how to share parameters in a global analysis; we will exemplify it using the case of an interacting system consisting of a mixture of ligands (L1 and L2) competing for the same monovalent receptor (R) and we will consider the global fitting of two isotherms collected under different experimental conditions[3]: Dataseries 1 is the direct titration of the mixture L1+L2 into R and Dataseries 2 is the reverse titration of R into the ligand mixture L1+L2 (figure 1 a) and b)):

a) Dataseries 1 (direct titration)



b) Dataseries 2 (reverse titration)

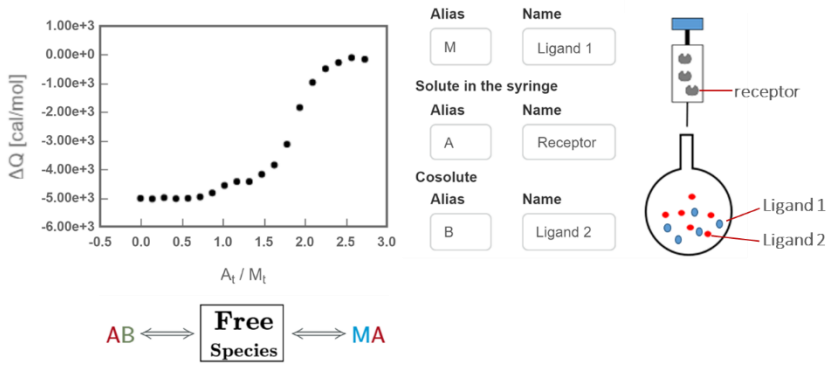


Fig.1

In the direct titration "M" represents the receptor, "A" represent Ligand 1 and "B" represents ligand 2; the binding model associated to this isotherm is a competitive model in which the complexes MA and MB are formed from free species. In the reverse titration "A" represents the receptor, "M" represent Ligand 1 and "B" represents ligand 2; the binding model associated to this isotherm is the reverse competitive model in which MA and AB are formed from free species.

Once we have uploaded the two files in the project the next step is to determine which parameters need to be shared between isotherms. By default, all the parameters are applied individually to each isotherm (Fig.2).

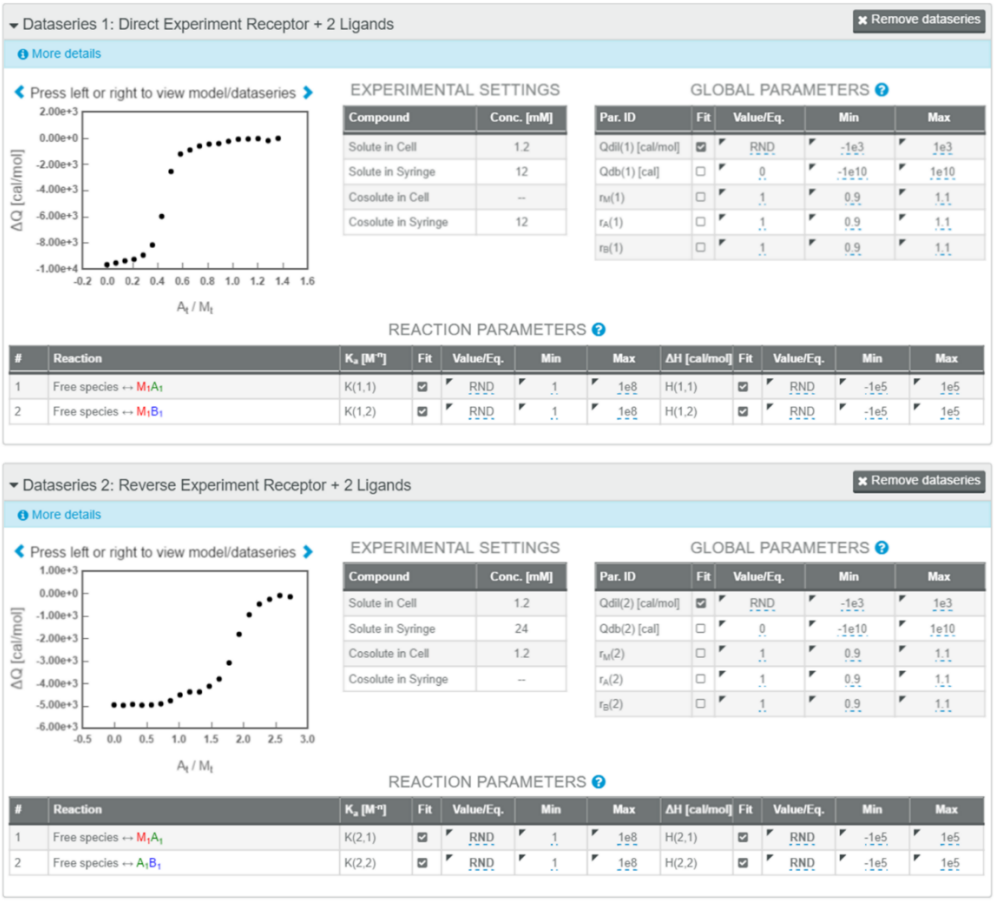


Fig.2

Note that the equilibrium **Free species→M_1A_1** in Dataseries 1 is equivalent to **Free species→M_1A_1** in Dataseries 2 as both represent the interaction of Ligand 1 with the receptor. Therefore, both equilibria share the same thermodynamic parameters (K and ΔH). Similarly, the equilibrium **Free species→M_1B_1** in Dataseries 1 is equivalent to **Free species→A_1B_1** in Dataseries 2 as both represent the interaction of Ligand 2 with the receptor. According with this information the following parameters will be linked between isotherms:

K(1,1) = K(2,1) and H(1,1)=H(2,1)

K(1,2) = K(2,2) and H(1,2)=H(2,2)

In order to link parameters in the fitting project click on the "Value/eq" box of the selected parameter (i.e K(2,1)) and type the name of the parameter that is equivalent (K(1,1)):

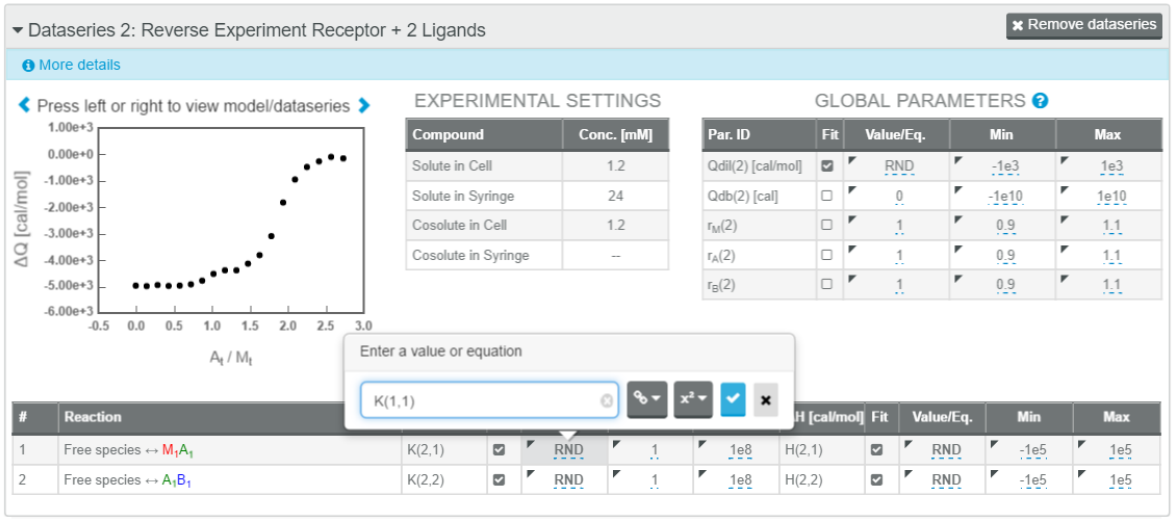


Fig.3

Figure 4 shows the project settings upon linking all shared parameters between isotherms. Here, the parameters Qdil and rM stay independent for each dataseries. Note that the fit box of the parameters that are linked to another appears unchecked.

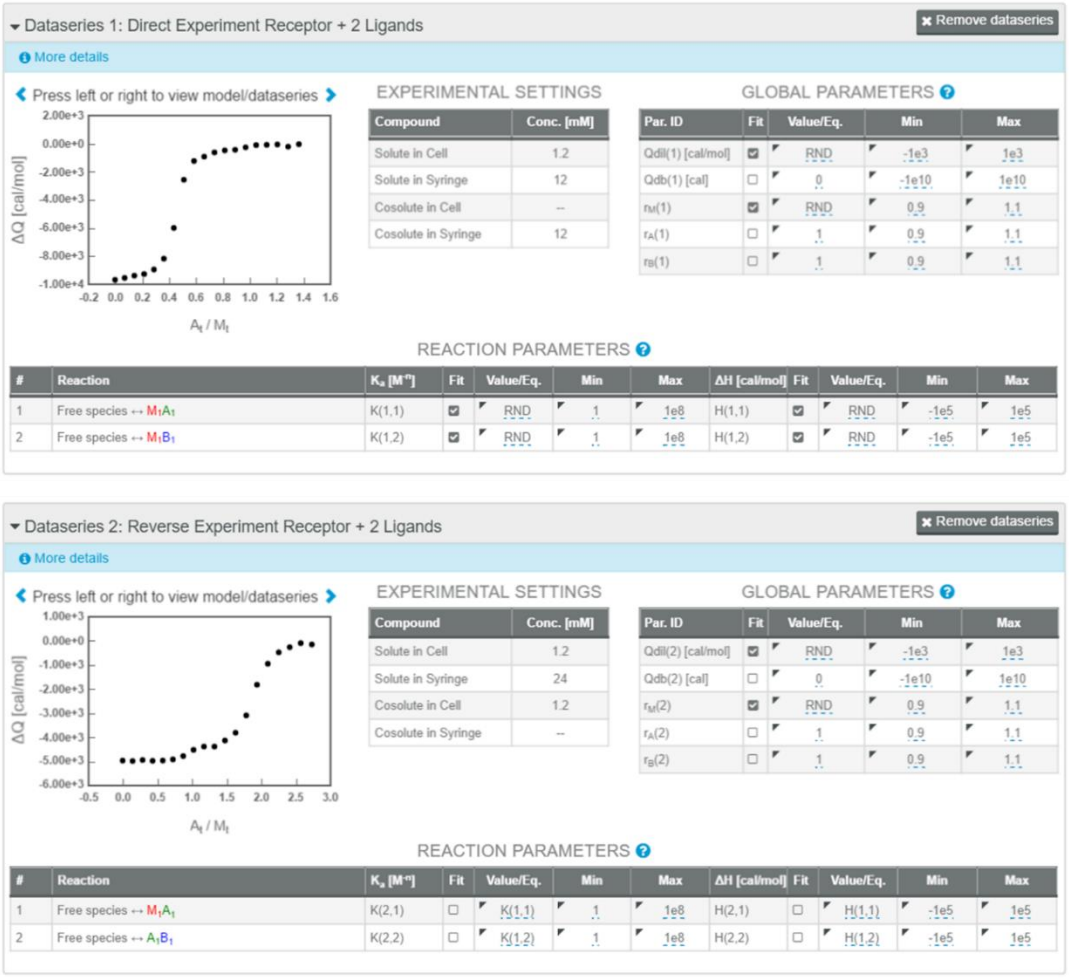


Fig. 4

Noteworthly, with AFFINImeter it is also possible to correlate parameters through mathematical equations. For instance, we can impose that the value of one association constant is two times the value of another association constant; this is exemplified in figure 5 where K(2,1) is two times K(1,1):

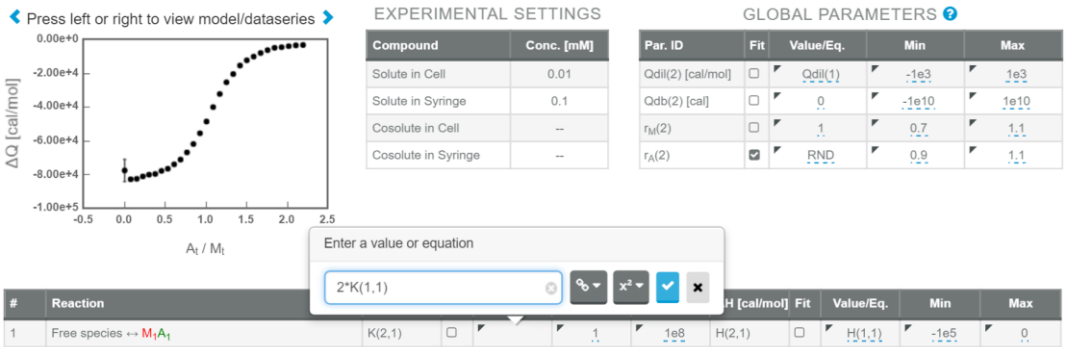


Fig.5

The use of mathematical equations to correlate fitting parameters in global analysis is a powerful tool; thus, it is of great utility in the evaluation of dependency/independency of sites and binding cooperativity in multivalent interactions where site and stoichiometric constants can be mathematically related. [4]

Find additional information of global fitting and related subjects here:

- AFFINImeter video tutorial. HOW to perform a global fitting. https://www.affinimeter.com/video_tutorials
- [1] Working with AFFINImeter models based on a stoichiometric equilibria approach. https://www.affinimeter.com/resources#miscellaneus
- [2] Working with AFFINImeter models based on an independent sites approach. https://www.affinimeter.com/resources#miscellaneus
- [3] Find this example of global fitting in your AFFINImeter account under the name "FIT --> Inverse + Reverse experiments simultaneously analyzed".
- [4] Stoichiometric and site constants: two approaches to analyze data with AFFINImeter. https://www.affinimeter.com/resources#miscellaneus

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