NOTE

The independent sites approach to design binding models with AFFINImeter

I. Describing an ITC experiment in AFFINImeter

The appropriate design and use of binding models in AFFINImeter passes through an understanding of the nomenclature that the software uses to describe a given experimental setup and the species that take part in the assay.

AFFINImeter contemplates the presence of up to three species participating in the experiment: 1) the titrant, or compound placed in the syringe; 2) the titrate, or compound in the calorimetric cell and 3) a co-solute, or third compound that can be in the syringe and/or in the cell. These species are labelled in the reaction builder as follows:

A = titrant (main compound in the syringe)

M = titrate (main compound in the cell)

B = co-solute (third compound participating in the interaction)

As shown in Fig. 1, **M** always refers to the compound placed in the calorimetric cell and **A** always refers to the compound in the syringe. **B** always refers to a third component that can be in the syringe (Fig 1b), in the cell (Fig 1c) or in both places at once (Fig 1d):

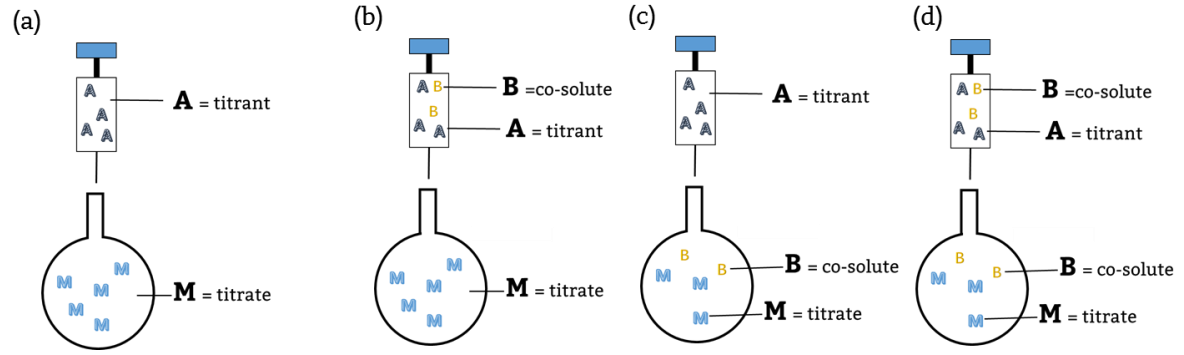


Fig.1 Representation of ITC experimental setups and nomenclature used in AFFINImeter.

II. Working with models based on an independent sites approach

To exemplify the use of the M-A-B nomenclature and the design of experiments based on independent sites[1], let´s start considering two cases: (a) one ligand binding to a multivalent receptor with 5 identical sites, independent from each other; (b) one ligand binding to a multivalent receptor with 5 non-identical sites, grouped in two sets having 2 and 3 identical sites, respectively.

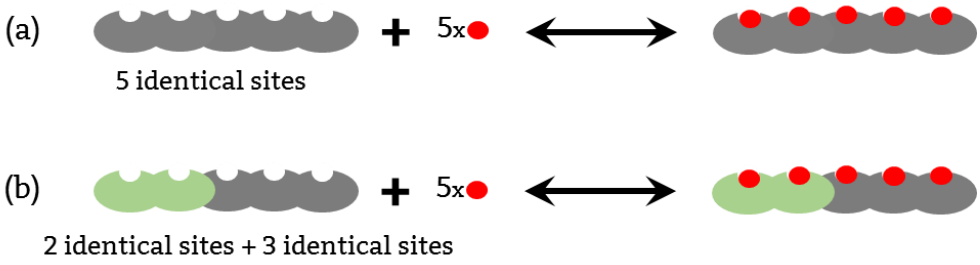
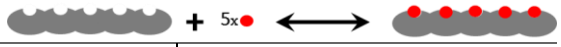
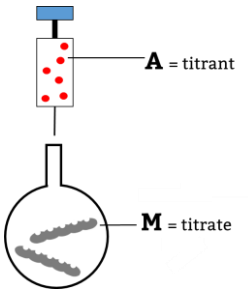


Fig.2 Examples of a ligand binding to a multivalent receptor with 5 independent sites.

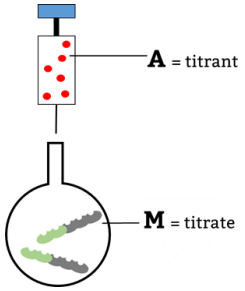
The thermodynamic profile of these interactions is usually determined through a direct titration where the multivalent receptor (typically a polymer or a biomacromolecule) is in the cell and the ligand is in the syringe. Table I illustrates the setup of both experiments, together with the nomenclature and the selection of the structural parameters characteristic of each case.

Table I.

REACTION: 

Experimental setup	Label	Structural parameters
	A = Ligand M = Receptor	Number of sets: 1 #Set | #Sites | Fit | Value/Eq. 1 | n(1,1) | ☐ | 5

REACTION SCHEME: 

Experimental setup	Label	Structural parameters
	A = Ligand M = Receptor	Number of sets: 2 #Set | #Sites | Fit | Value/Eq. 1 | n(1,1) | ☐ | 2 2 | n(1,2) | ☐ | 3

The only difference between both experiments resides in the settings of the reaction parameters that define the structural features of the interaction, that is the number of sets and sites of the titrant **M** (the multivalent receptor): equilibrium (a) is defined by 1 set of 5 equivalent sites while equilibrium (b) is defined by 2 sets of 2 and 3 equivalent sites, respectively. Note that, in both cases the number of sites per set is known and defined in the "Value/Eq" box of the reaction parameters. Moreover, the option "Fit" is unchecked to indicate that the parameter is constant throughout the fitting. If the number of sites per set is unknown, this will be set as a fitting parameter in a given range defined by the user during the analysis.

In a second example, let´s consider the presence of a third component in experiment (a), a second ligand that competes with the first ligand for binding to the multivalent receptor:

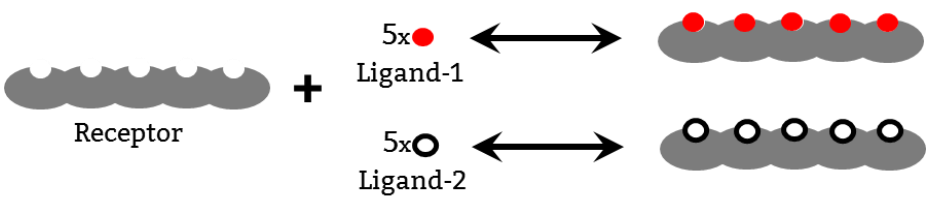
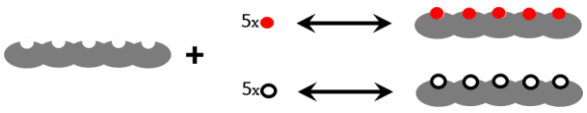


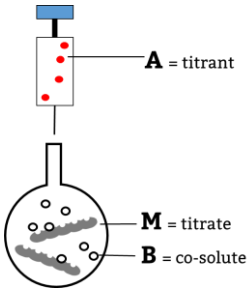
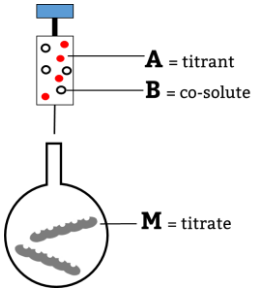
Fig.3 Example of two competing ligands binding to a multivalent receptor with 5 independent sites.

Introducing ligand-2 expands the possibilities of experimental design, i.e. Table II describes two different approaches in which ligand-2 acts as the co-solute **B**.

Table II

REACTION:



Experimental setup	Label	Structural parameters
	A = Ligand-1 M = Receptor B = Ligand-2	Number of sets: 1 #Set | #Sites | Fit | Value/Eq. 1 | n(1,1) | ☐ | 5
	A = Ligand-1 M = Receptor B = Ligand-2	Number of sets: 1 #Set | #Sites | Fit | Value/Eq. 1 | n(1,1) | ☐ | 5

The schemes of table II comprise the direct ITC titration of the receptor with ligand-1 in the presence of the co-solute (ligand-2) in the cell (first scheme) or in the syringe (second scheme). Both experimental setups are described by the same structural settings and the only difference resides in the location of the co-solute. The presence of the co-solute in the cell and/or in the syringe is stated in the settings of the corresponding dataseries (Fig. 4).

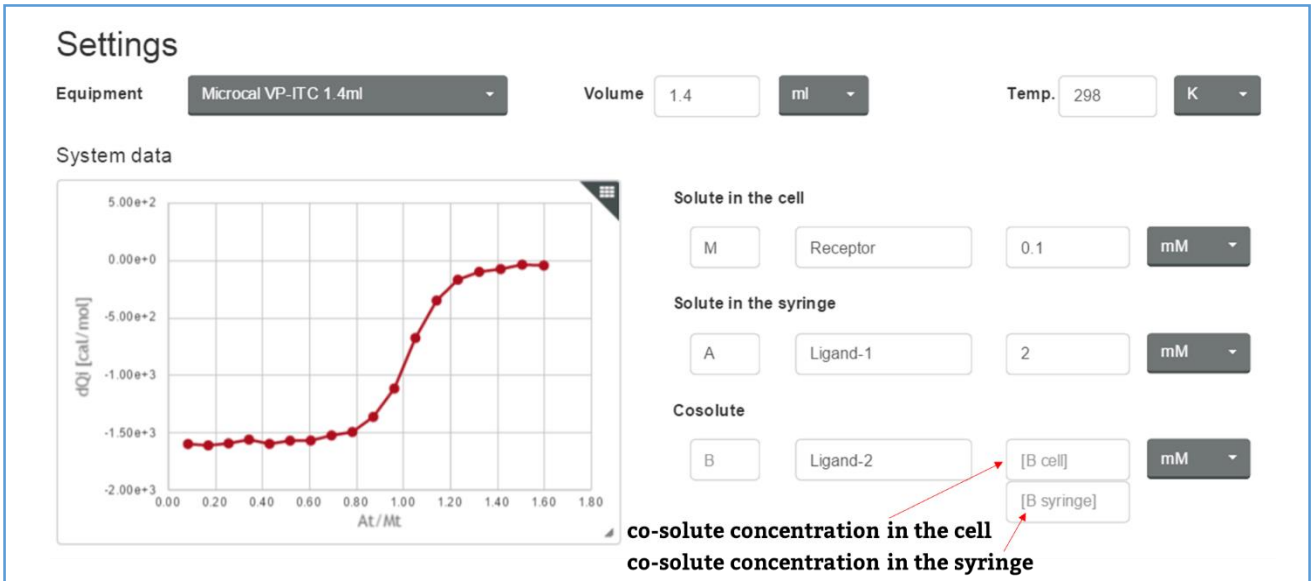


Fig.4 Example of dataserie settings to highlight the fields relative to the co-solute B.

References and comments

[1] For a detailed description of the independent sites approach, see our note "Stoichiometric and site binding constants: two approaches to analyze data with AFFINImeter".

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