NOTE

The stoichiometric equilibria 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 illustrated 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):

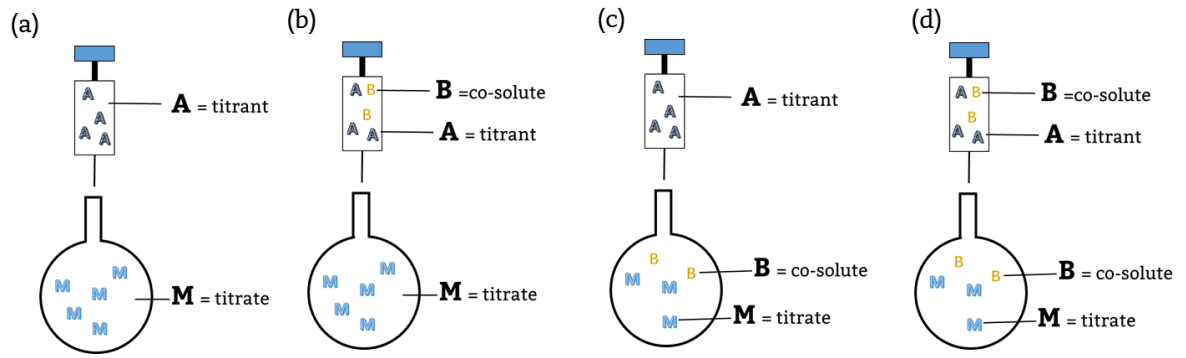


Fig.1 Representation of ITC experimental setups and nomenclature used in AFFINImeter.

II. Working with models based on a stoichiometric equilibria approach

To exemplify the use of the M-A-B nomenclature and the design of experiments based on a stoichiometric equilibria[1] approach, let´s first consider the simplest case of a ligand binding to a receptor to form a 1:1 complex (Fig. 2):

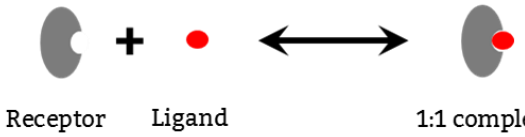
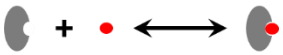
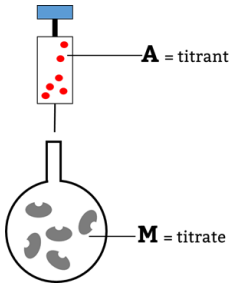
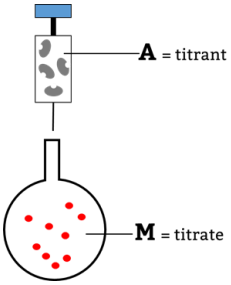


Fig.2 General scheme of a ligand binding to a receptor to form a 1:1 complex.

The thermodynamic profile of this interaction can be determined through a direct ITC titration where the receptor (typically a macromolecule) is placed in the cell and the ligand is in the syringe. Additionally, the reverse experiment can be performed where the ligand is in the cell and the receptor is in the syringe. Table I illustrates the setup of both experiments, together with the labelling and the corresponding binding model (previously designed with the reaction builder of AFFINImeter).

Table I.

REACTION: 		
Experimental setup	Label	Reaction scheme
Direct titration 	**A** = Ligand **M** = Receptor	**Free Species** ⟺* MA
Reverse titration 	**A** = Receptor **M** = Ligand	**Free Species** ⟺* MA

Note that the difference between direct and reverse experiments resides in the label assigned to the ligand and to the receptor that varies depending on their location (cell or syringe). Both experiments are described by the same binding model.

In a second example let´s consider the presence of a third component in the ITC experiment: the case of two ligands that compete for binding to the same receptor site to form a 1:1 complex (Fig. 3):

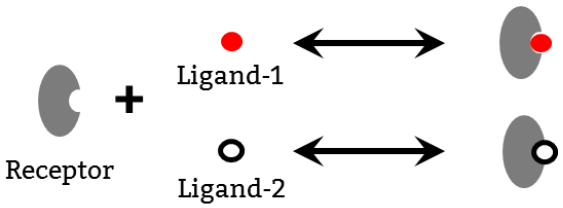
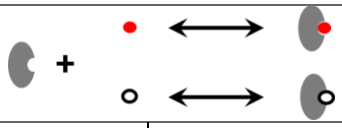
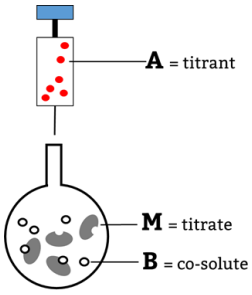
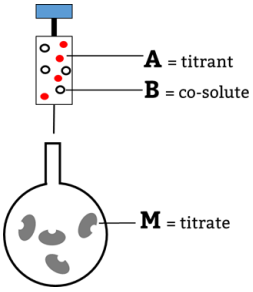
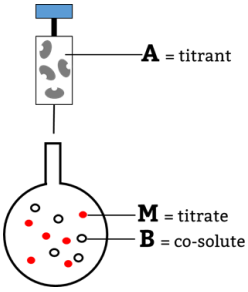


Fig.3 Example of two competing ligands binding to a monovalent receptor.

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

Table II.

REACTION: 

Experimental setup	Label	Reaction scheme
Direct titration 		
Direct titration 		
Reverse titration 		

The first and second setups of the table 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 binding model and the only difference resides in the location of the co-solute. The third scheme describes the reverse ITC titration in the presence of the co-solute (ligand-2) in the cell. Here, the receptor is the titrant and is labelled as **A**, ligand-1 is **M**, the main titrate, and ligand-2 is the co-solute **B**. The corresponding binding model describes the formation of the complexes **AM** (receptor – ligand-1) and **AB** (receptor – ligand-2).

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).

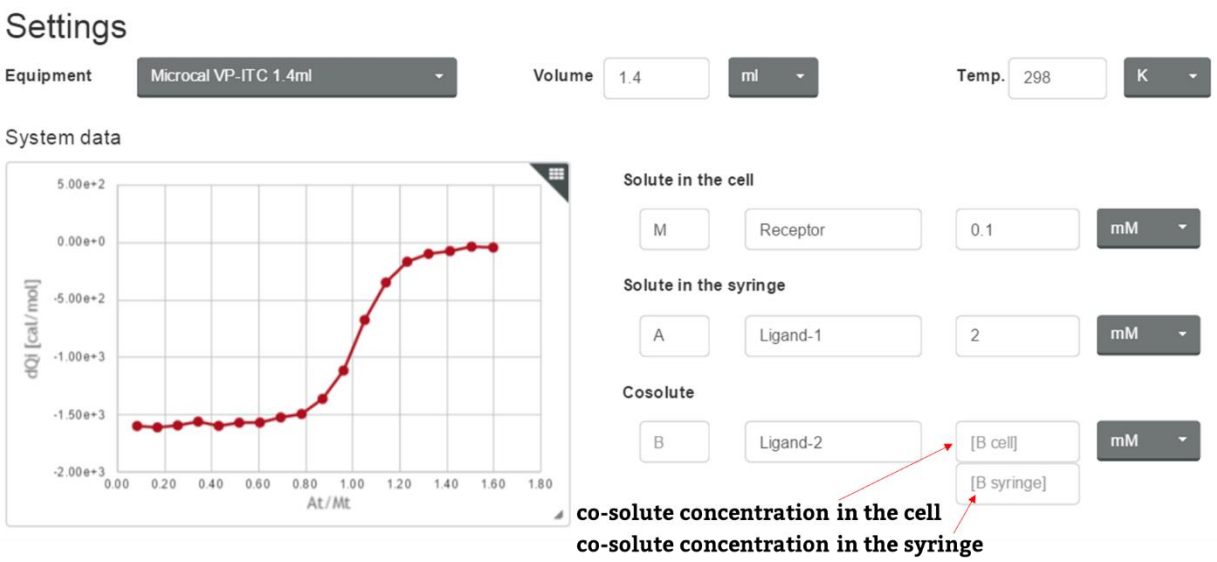


Fig.4 Example of dataserie settings, highlighting the fields relative to the location of co-solute B.

References and comments

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

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