AFFINIMETER

A new tool to analyze Isothermal Titration Calorimetry experiments

Juan Sabín,[1,2] Eva Muñoz[1] and Ángel Piñeiro[1,2]

[1]*Software 4 Science Developments, Ed. Emprendia s/n*

[2]*Dept. of Applied Physics, University of Santiago de Compostela.*

Campus Vida 15782, (A Coruña) Spain.

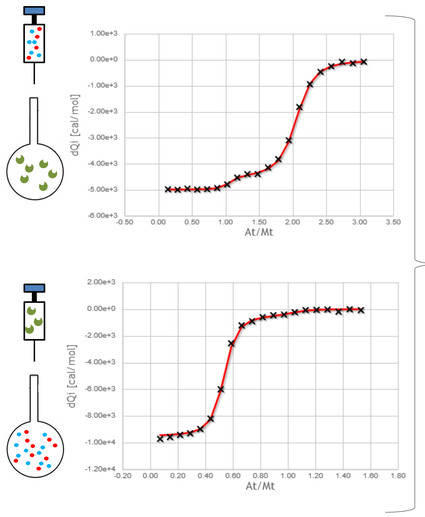
Website: www.afinimeter.com; E-mail: J.Sabin@affinimeter.com

Global analysis with reverse titration

During the hits-to-leads process in drug discovery programs, a significant number of compounds are synthesized as mixtures of isomers. ITC seems to be the only technique accurate enough to distinguish two different binding events occurring simultaneously in the same experiment.

However, there are circumstances in which isotherms can be easily misinterpreted. When $\Delta H_1 \approx \Delta H_2$ the titration of two ligands can give deceptively simple curves, hidden the competition binding process. In this case, data show a sigmoidal-like curve, similar to the one obtained with a simple 1:1 interaction.

Performing an additional reverse experiment (titrating the receptor into the ligands) and using AFFINImeter to globally fit both dataseries will allow researchers to clearly characterize both binding events.



M + B ⟷ MB
+
A
↕
MA

MB K_a= 1.2 ·10⁷ mM⁻¹
ΔH=-4.9 Kcal/mol

MB K_a= 5.3 ·10⁴ mM⁻¹
ΔH=-4.5 Kcal/mol

Ultra-high and ultra-low affinity interactions

High affinity interactions ($K_A \geq 10^9$ M^{-1}) yield square-shaped isotherms whose fitting yield accurate values of the binding enthalpy but only estimates of the association constant. Attempts to recover a sigmoidal shape requires the use of very low concentrations of the interactants. These experimental drawbacks can be circumvented by using the ITC **displacement method**. The receptor is titrated with a high affinity ligand, but in the presence of a weaker ligand in the sample cell that competes for the complexation with the receptor. With this experimental set up the apparent affinity of the strong ligand is "artificially" lowered, obtaining a sigmoidal isotherm that yields more accurate binding data

AFFINImeter can be used as an efficient tool in the analysis of ITC displacement assays as it offers the possibility of simultaneous, global fitting of various isotherms to different binding models and allow to "link" parameters of the fitting of different dataseries.

Ultra-high affinity interaction

M + A ⟷ MA

$K_1 > 10^9$ M^{-1} ??

Low affinity interaction

M + B ⟷ MB

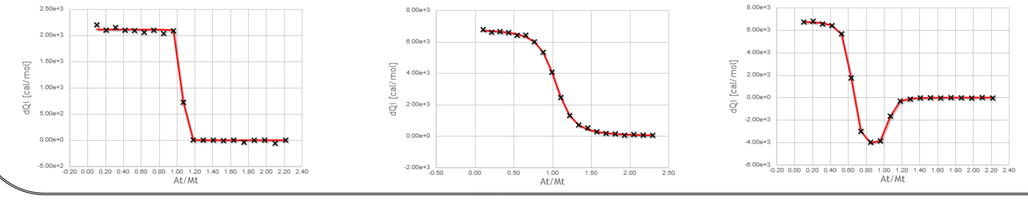
$K_2 = 4.1070\ 10^6$ M^{-1}

Displacement interaction

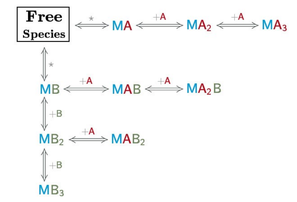
M + B ⟷ MB
+
A
↕
MA

$K_1 = 2.0211\ 10^9$ M^{-1}

$K_2 = 4.1070\ 10^6$ M^{-1}



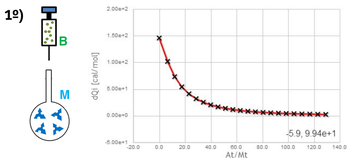
Competitive Model with complex interactions



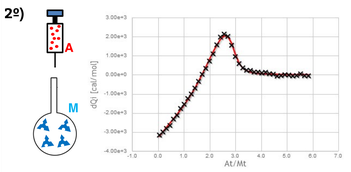
Study of the interaction of two different ions (Ca^{2+} and Mg^{2+}) with a protein that has three binding sites. Three independent experiments were performed: Mg titrated into protein, Ca titrated into protein and a competitive experiment where Ca was titrated into the cell containing protein and Mg.

Simultaneous fitting of several ITC curves to advanced models involves a significant number of thermodynamical parameters.
Local minima can drive the user to wrong results. Statistical analysis is required to guide the researcher in the selection of the most reasonable result.

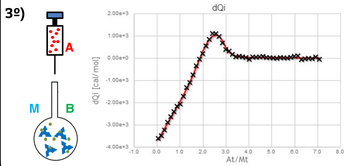
1º)



2º)



3º)



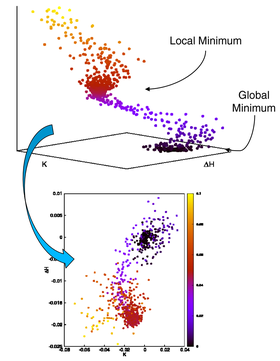
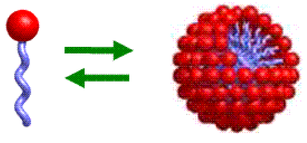


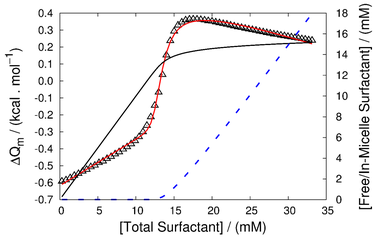
Fig. Projections in two and three dimensions of the screened vectors on the 14-dimensional geometrical space generated by the thermodynamical parameters

Aggregation and micellization studies



The determination of the aggregation numbers in micellization processes is usually studied trough fluorescent quenching, light scattering or small angle light scattering. Notably, dilution ITC experiments of surfactant solutions above the CMC have shown to be clearly sensitive to the structure of the molecules, and so to that of the corresponding aggregates. AFFINImeter offers a micellization model to determinate the aggregation numbers of surfactant micelles directly from single ITC experiments.

Additionally, the analysis provides a complete thermodynamic characterization of the demicellization processes, including the heat of dilution of surfactant monomers and micelles, as well as the enthalpy and the Gibbs energy changes for the molecular transfer from the solution to the aggregates.



△ Experimental Data
Best Fitting
Concentration of free surfactants
Concentration of micelles

$N_{aggregation}$= 30.4;
$\Delta H_{micellization}$= - 0.764 kcal/mol
$\Delta G_{micellization}$ = - 2.33 kcal/mol

Analytical discrimination of ligands in heterogeneous mixtures

AFFINImeter can successfully analyze ITC data of an interaction between a receptor with a heterogeneous mixture of ligands (isomers, heteropolymers, oligomers, etc..) even if the proportional concentrations of the ligands are unknown for the user.

AFFINImeter can quantitatively calculate concentrations, the species distribution and the thermodynamic parameters of the interaction for each complex.

In this example, two ligands at unknown concentrations compete for binding to a receptor with different K_a and ΔH.

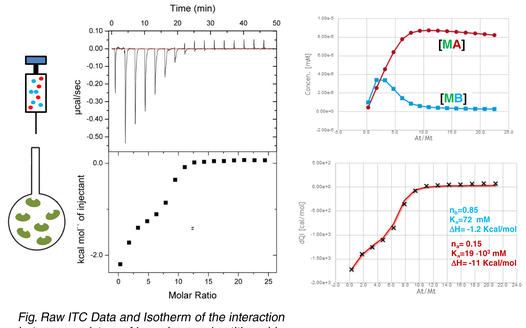
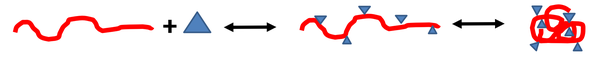


Fig. Raw ITC Data and Isotherm of the interaction between a mixture of heparines and antithrombin.

Ligand-induced conformational changes



Conformational changes induced by binding can be essential in biological processes; such as DNA/RNA condensation, induced-fit antigen binding in immunoglobulins, protein denaturation, polyplexes formation or enzyme catalysis.

The mayor handicap in the analysis of the ITC isotherm in these systems lays on the overlap of the endothermic/exothermic heat released in the binding and in the conformational changes.

AFFINImeter provides specific models to analysis conformational changes sequenced with binding interactions.

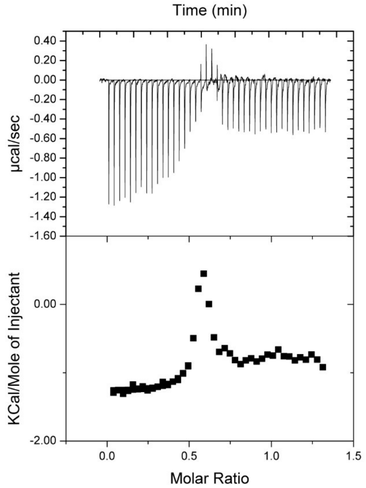


Fig. Raw ITC Data and Isotherm of the interaction between polyethylenimine and DNA