Case study

The advantage of using AFFINImeter-spectroscopy for global analysis of binding curves

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The advantage of using AFFINImeter-spectroscopy for global analysis of binding curves

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AFFINImeter-spectroscopy is a new software for the analysis of saturation binding curves obtained from different biophysical techniques that has been designed following the philosophy of AFFINImeter, in which advanced tools of analysis are put to the service of researchers to get reliable analysis of binding data. Herein we show the usefulness of global analysis of binding curves for a more accurate determination of binding constants, exemplified with a 1:1 binding interaction monitored by 1D 1H-NMR chemical shift perturbation experiments.

Introduction

Titration experiments are central to the characterization of binding interactions where many different biophysical techniques can be employed to monitor how an observable (signal), sensitive to the complex formation, changes as a function of the titrant and titrate concentrations present in the sample. Examples of biophysical techniques used for such purpose are Nuclear Magnetic Resonance (NMR), Isothermal titration Calorimetry (ITC), Fluorescence, UV-Vis, Circular dichroism (CD), Micro Scale Thermophoresis (MST) and Surface Plasmon Resonance, to mention a few.

Spectroscopic techniques such as NMR typically offer, in a single titration, the possibility to monitor several signals assigned to different peaks of the titrate spectra, from which a set of binding curves can be built and analyzed. Assuming that all these signals are sensing the same binding event, analogous results should be obtained from individual analysis of the curves.

AFFINImeter-spectroscopy is a new software that has been designed for the advanced analysis of saturation binding curves from equilibrium binding titrations, regardless of the spectroscopic technique used. If offers the advanced tools of the software AFFINImeter (that was born as a software for the analysis of ITC data), such as model builder, global fitting and validation tools for a more robust and reliable data analysis.

Our aim in this case study is to show the relevance of performing global analysis of saturating binding curves instead of individual analysis. This is illustrated with a very simple example of a 1:1 binding interaction monitored by 1D 1H-NMR titration and analyzed with AFFINImeter-spectroscopy.

1D 1H-NMR titration of 1-phenyl-2,3,4,5-tetrahydro-1h-3-benzazepine-7,8-diol to β-cyclodextrin

The formation of the 1:1 complex between 1-phenyl-2,3,4,5-tetrahydro-1h-3-benzazepine-7,8-diol (S enantiomer, SFK) and β-cyclodextrin (BCD) can be followed by a 1D 1H-NMR titration experiment in D_2O; since the equilibrium between free species and the SFK–BCD complex is fast on the NMR time scale, monitoring the chemical shift perturbation ($\Delta\delta$) of the proton resonances sensitive to the binding event throughout the titration will provide the corresponding binding curves (Fig. 1).

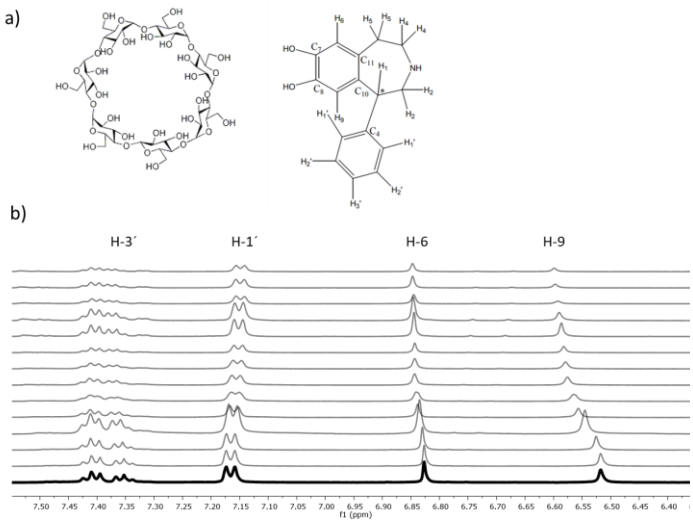

Fig 1. a) Chemical structure of BCD (left) and SFK (right). The chiral center of SFK is highlighted with an asterisk. In this study the S enantiomer was used; b) 1H-NMR spectra of titration of BCD into SFK (region between 6.3 and 7.5 ppm is shown). H-9, H-6, H-1´and H-3´resonances of SFK are highlighted.

In this study, $\Delta\delta$ observed of the SFK H-9, H-6, H-1´and H-3´resonances were used to generate the corresponding binding curves.[1] In AFFINImeter-spectroscopy curves are easily uploaded as text files containing columns of titrate concentration, titrant concentration and signal observed (and if desired, a column with error associated to the signal value).

Global *vs* individual analysis of the binding curves.

The four binding curves generated from the titration experiment were uploaded to the same AFFINImeter fitting project and analyzed using a 1:1 binding model (Fig. 2).

Curves were fitted in two ways: a) performing the individual analysis in which fitting of each curve involves two floating parameters: the binding association constant (K_A) and the maximum signal change at full saturation (Δs_{max}); b) global analysis in which all the curves share the same K_A while is Δs_{max} calculated individually for each curve. Generally, when different data sets have one or more parameters in common, incorporating restrictions that account for this relationship decreases the number of floating parameters per dataset, as compared to the individual analysis, and the accuracy of the parameter(s) calculated can be increased.

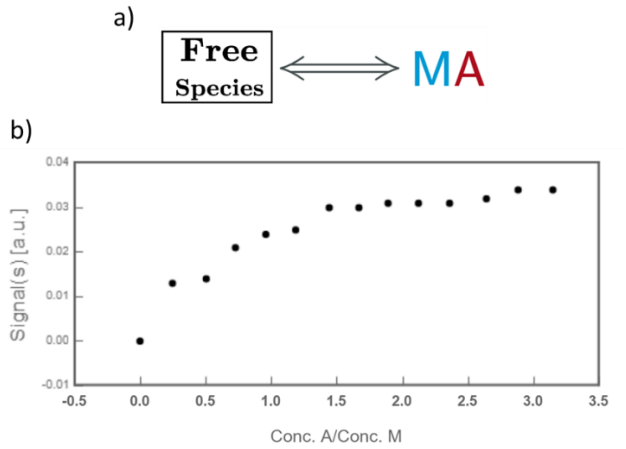

Fig 2. a) Schematic representation of the 1:1 binding model as shown in the software AFFINImeter where Free Species (M + A) are in equilibrium with the 1:1 complex MA; b) Binding curve uploaded into AFFINImeter-spectroscopy where "Signal" in this particular case represents $\Delta\delta$ (ppm), and ConcA/ConcM is the titrant to titrate molar ratio.

The results obtained using both approaches were compared, focusing not only in the K_A value obtained but also in the uncertainty associated to the measured values, which accounts for the accuracy of the result (Table I).

Table I.

Proton	K_A (M^{-1})	Relative error (%)
H-1´	330 ± 14	4
H-3´	1055 ± 63	6
H-6	1250 ± 62	5
H-9	1068 ± 31	3
Averaged	926 ± 43	4.6
Global analysis	1044 ± 26	2.5

From the individual analysis of each curve an averaged K_A was calculated; the corresponding propagated error was determined as the sum of each error divided by the number of curves. In the global analysis K_A and the error associated are unique values common to all the curves (Fig. 3). As can be seen from the results obtained the relative error (error divided by the corresponding K_A value) is almost 2 times lower when global analysis is applied, wich results in a calculation of the binding affinity with lower uncertainty (higher accuracy). It is

important to mention that the uncertainty reported of an averaged K_A calculated from individual analysis is also frequently given as the standard deviation of the mean, which can aggravate the situation producing an uncertainty unnecessarily large. In this example the standard deviation to the mean K_A yields a relative error of 44%, 17 times higher than the result obtained from global analysis.

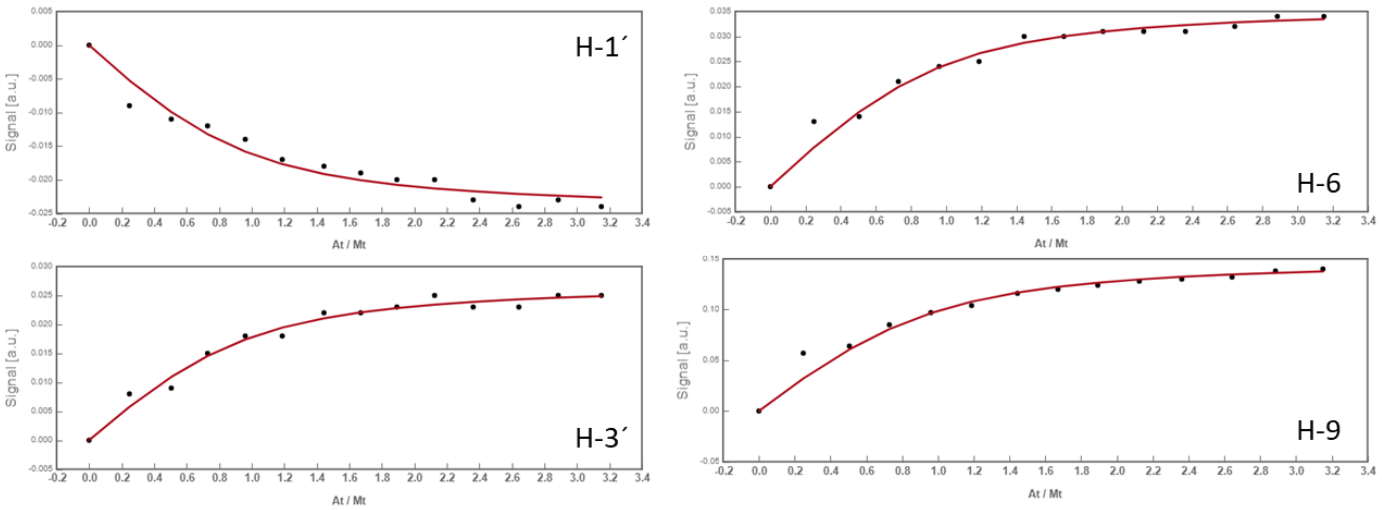

Fig 3. Global analysis of 1D 1H-NMR binding curves, performed with AFFINImeter-spectroscopy. A 1:1 binding model was employed and the association binding constant was calculated as a common parameter between curves.

Conclusions

The results described herein shows that even for the analysis of simple 1:1 binding data global analysis of binding curves is a better choice than individual analysis. It agrees with a more general statement that a global analysis consistently gives more reliable results than a more classical individual analysis. Furthermore, for more complex binding, the global analysis method gives much more robust results and avoids over-parameterized fittings.[2] Being aware of this, AFFINImeter-spectroscopy incorporates global analysis as a key tool for the robust analysis of binding curves coming from the same or different titration experiments, or even from different spectroscopic techniques.

References & Notes

[1] This experimental NMR titration data was part of the Master´s thesis of Marino Vega and was kindly provided by his supervisor Dr. Manuel Martín Pastor, head of the NMR core facility of the University of Santiago de Compostela.

[2] A J. Lowea, F M. Pfeffera, P. Thordarsonb, Determining binding constants from 1H NMR titration data using global and local methods: a case study using [n]polynorbornane-based anion hosts. Supramolecular Chemistry. **2012**, 24(8), pp 585–594.

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