Methods 76 (2015) 137–148

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

SEDPHAT – A platform for global ITC analysis and global multi-method analysis of molecular interactions

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ARTICLE INFO

Article history:
Received 28 October 2014
Received in revised form 19 November 2014
Accepted 20 November 2014
Available online 2 December 2014

Keywords:
Isothermal titration calorimetry
Global analysis
Multi-method analysis
Protein interactions
Binding cooperativity
Linked binding

ABSTRACT

Isothermal titration calorimetry experiments can provide significantly more detailed information about molecular interactions when combined in global analysis. For example, global analysis can improve the precision of binding affinity and enthalpy, and of possible linkage parameters, even for simple bimolecular interactions, and greatly facilitate the study of multi-site and multi-component systems with competition or cooperativity. A pre-requisite for global analysis is the departure from the traditional binding model, including an '*n*'-value describing unphysical, non-integral numbers of sites. Instead, concentration correction factors can be introduced to account for either errors in the concentration determination or for the presence of inactive fractions of material. SEDPHAT is a computer program that embeds these ideas and provides a graphical user interface for the seamless combination of biophysical experiments to be globally modeled with a large number of different binding models. It offers statistical tools for the rigorous determination of parameter errors, correlations, as well as advanced statistical functions for global ITC (gITC) and global multi-method analysis (GMMA). SEDPHAT will also take full advantage of error bars of individual titration data points determined with the unbiased integration software NITPIC. The present communication reviews principles and strategies of global analysis for ITC and its extension to GMMA in SEDPHAT. We will also introduce a new graphical tool for aiding experimental design by surveying the concentration space and generating simulated data sets, which can be subsequently statistically examined for their information content. This procedure can replace the '*c*'-value as an experimental design parameter, which ceases to be helpful for multi-site systems and in the context of gITC.

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1. Introduction

Isothermal titration calorimetry (ITC) is a powerful, first-principles based technique to study molecular interactions label-free in solution with wide-spread applications in many scientific disciplines [1]. Unique among biophysical techniques, it observes directly the heat change ΔQ of a solution associated with a change of composition $\{\Delta c_i\}$ during the titration of a macromolecular component in the cell with increasing amounts of its binding partner(s) [2,3]. The differences in heat between chemical equilibria along discrete steps of the titration are measured as the integral of the differential power applied to keep a sample and reference solution at the same temperature. As has been reviewed elsewhere in detail [2–6], the shape and midpoint of the resulting titration isotherm yields information on the association constant K_A, and therefore the free energy of binding, as well as information on the binding stoichiometry. The amplitude of such titration isotherms contains information on the molar enthalpy changes ΔH of molecular complex formation, which in turn may be interpreted in the context of structural thermodynamics. For systems with multi-site binding, ITC can provide information on stoichiometry and cooperativity of molecular complexes [7–14].

Unfortunately, similar to most binding saturation curves, individual titration isotherms usually have relatively low intrinsic information content, due to the shallow concentration dependency of complex formation predicted by mass action law [15(2015)]. Furthermore, dependent on the interaction enthalpies, even for multi-site systems only a single transition may be observed. Thus, the number of binding parameters of an interacting system can quickly exceed what can be confidently determined from the given

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http://dx.doi.org/10.1016/j.ymeth.2014.11.012
1046-2023/Published by Elsevier Inc.

data. This can be true even for 'simple' bimolecular reactions with 1:1 stoichiometry, if constraints in the available concentration and amounts of material hinder the implementation of optimal experimental conditions, if limitations in the sensitivity of the calorimeter require application of suboptimal conditions (such as high '*c*-values' for high-affinity systems leading to essentially stoichiometric binding), or if uncertainties in the experimental concentrations exist. The relatively low information content of many ITC isotherms also often causes models with different binding mechanisms to describe the data equally well. In this case, independent knowledge (or assumptions) of the number and composition of complexes formed, their symmetry, and possible site-interactions is required for the justification of a particular model.

Strategies have been developed recently in several laboratories to enhance the information content of ITC, for example, related to experimental design [16,17], the improvement of the precision of isotherm data through advanced integration of the differential power trace [18,19], or the exploitation of kinetic data in the injection shapes [20]. Global strategies combine multiple experiments in global analyses of ITC (gITC) [9,21–28] and in global multi-method analysis (GMMA), the combination of ITC data with data from complementary biophysical disciplines [29–32,82]. Beyond merely organizing separate complementary experiments into a hierarchical, multi-stage interpretation, global analysis takes full advantage of all possible constraints of the model and the full statistics of the data by simultaneously and directly fitting all experimental data with one explicit global model. Natural applications of gITC analysis are multi-site binding processes of homo- and hetero-oligomerizing macromolecules [9–11,13–15(2015),23,33–37], displacement experiments [22,38,39], and protonation-linked and other linked binding analyses varying temperature to determine heat capacity changes or buffer composition to determine salt or other co-factor linkage [24,26,28].

SEDPHAT is a computational platform for global analysis of data from various biophysical techniques, including gITC, which has been widely adopted in ITC applications [11–14,15(2015), 17,19,24,33–62]. Distinguishing aspects of SEDPHAT in comparison with other gITC platforms include the absence of data-specific or model-specific programming by the user, allowing for seamless combination of titration experiments in a graphical user interface, and offering many different pre-programmed multi-site models for two- and three-component systems [9]. For fully exploiting the advantages of gITC, the concept of an empirical '*n*-value' as a combined parameter for concentration errors and numbers of sites [2] must be abandoned. In contrast, SEDPHAT exclusively allows for integral numbers of binding sites, and introduces explicit parameters accounting for errors in active concentrations, which may be shared among different experiments in the same global analysis [9].

The present communication has two goals: first, it reviews several extensions of SEDPHAT for the analysis of ITC data included since the original introduction [9]. Among those are a method for the unbiased, high-precision integration of the differential power trace [18], implemented in the companion program NITPIC [18,19(2015)] that we have developed to interface with SEDPHAT and automatically provide total heat changes including error estimates separately for each injection. Another companion program, GUSSI (by Dr. Chad Brautigam), can be spawned by SEDPHAT for improved presentation and publication quality graphs of the results of ITC and gITC. Further, greater flexibility in the treatment of concentration errors was introduced by distinguishing between inactive fractions and pure concentration errors. To allow more convenient statistical characterization of the analysis of ITC isotherms, automated methods to explore the error surface of the fit using *F*-statistics [63] were added. They include automated determination of confidence intervals of binding parameters (propagated from the error bars of the individual injection heats), the computation of error surface projections, as well as the display of two-dimensional error projections highlighting parameter correlations [30]. Finally, with the development of GMMA and the associated statistical tools in SEDPHAT, significant enhancement of ITC analyses became possible by incorporation of complementary data from orthogonal techniques [29–32].

A second goal of the present communication is to provide examples for gITC of single-site and multi-site interactions to demonstrate the potential of gITC. Since the design of experiments for multi-site systems is often non-trivial, we describe a new tool to simulate ITC and other types of data in SEDPHAT that will help to predict which experiments would be most useful to be combined in global analysis.

2. Methods

2.1. SEDPHAT basic principles and resources

The input for SEDPHAT consists of tables of integrated heats and injection schedules, after peak integration of the raw thermograms. The peak integration can be accomplished in the stand-alone software NITPIC, which creates or appends a pre-formed SEDPHAT configuration. Alternatively, the input data can be created manually by exporting integrated data tables from instrument-specific software. (Data from a two-part experiment where the syringe is reloaded in between can be 'stitched' together simply by combining data rows of the integrated heat tables.) From the table of integrated heats (saved as ''.dat''-file), SEDPHAT automatically creates a unified data file ('xp'-file) including all necessary ancillary parameters. Global analysis is achieved simply be loading more than one data set, each of the same kind or of different kinds, including different titration configurations or data collected by methods other than ITC. This may be achieved by drag-and-drop of 'xp'-files, by using the SEDPHAT loading menu functions, or by allowing NITPIC to extend an existing SEDPHAT configuration.

For a given interaction model and binding parameters, SEDPHAT calculates the populations of free and bound species on the basis of mass action law prior and after each injection, the finite differences between these equilibrium populations, and the associated changes in total heat content of the solution [9]. Differential equation based approaches [64–66] are not used due to the discrete nature of the injections. This affects the fit lines connecting the fitted values between each data point – in SEDPHAT these are linearly interpolated segments between the data points; this is a question solely of graphical representation without consequence for the data analysis and molecular binding parameters.

Independent of the interaction model, different approaches are available for the treatment of superimposed signals from heats of dilution (not including heats of pre-formed complexes, which are explicitly accounted for in the model): as default option, they are modeled as a constant baseline contribution to all isotherm points, to be refined in the fit. Alternatively, the baseline can be fixed, constrained to remain within a certain range of values (a new option added in SEDPHAT v.12.1) or eliminated and heats from a blank experiment with matching injections may be subtracted. (In addition, an option for a sloping baseline is available.)

To account for total concentration of components after each injection, values supplied from an instrument software, from NITPIC, or those calculated by SEDPHAT from the cell volume and injection schedule can be used, the latter assuming a full fixed-volume cell with either mixed or unmixed neck (which can accommodate different kinds of instruments, the default being the MicroCal configuration). Any configuration of initial concentrations in cell and syringe may be used (and different ones in different experi-