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Review article

Signal and binding. II. Converting physico-chemical responses to macromolecule–ligand interactions into thermodynamic binding isotherms☆

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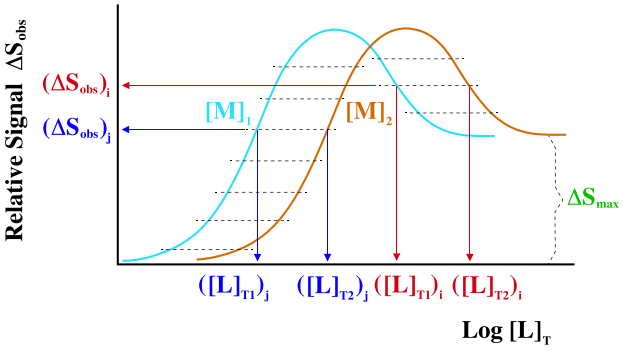
HIGHLIGHTS

- In general, there is no linear relationship between observed physico-chemical signals and the total average degree of binding.
- Signal and mass conservation relationships allow the construction of binding isotherms from physico-chemical titration curves.
- Ligand Binding Density Function (LBDF) Method allows the construction of binding isotherms using the ligand signal.
- The Empirical Function (EF) Method relates the observed signal to the total average degree of binding.

GRAPHICAL ABSTRACT



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ABSTRACT

Physico-chemical titration techniques are the most commonly used methods in characterizing molecular interactions. These methods are mainly based on spectroscopic, calorimetric, hydrodynamic, etc., measurements. However, truly quantitative physico-chemical methods are absolutely based on the determination of the relationship between the measured signal and the total average degree of binding in order to obtain meaningful interaction parameters. The relationship between the observed physico-chemical signal of whatever nature and the degree of binding must be determined and not assumed, based on some ad hoc intuitive relationship/model, leading to determination of the true binding isotherm. The quantitative methods reviewed and discussed here allow an experimenter to rigorously determine the degree of binding and the free ligand concentration, i.e., they lead to the construction of the thermodynamic binding isotherm in a model-independent fashion from physico-chemical titration curves.

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1. Introduction

Obtaining the equilibrium, binding isotherm is the primary method in analyses of ligand-macromolecule interactions. The equilibrium, binding isotherm is the functional dependence of the total average degree of binding (number of ligand molecules bound per macromolecule) upon the free ligand concentration [1–6]. True thermodynamic isotherm reflects only this functional dependence. In a practical experimental setup, it is the functional dependence of the total average degree of binding upon the total ligand concentration, although the free ligand concentration is still the independent variable (see below). In other words, the thermodynamic isotherm cannot be dependent upon any models/assumptions, concerning the relationships between the physico-chemical parameter used to monitor the binding and the total average degree of binding.

Nevertheless, the thermodynamic isotherm alone, obtained in model-independent fashion, provides the maximum stoichiometry and only approximates estimates of macroscopic affinities of the examined association reaction. The molecular aspects of the interactions, such as discrete character of binding sites, the overlap of potential binding sites, intrinsic binding constants, cooperativity parameters, allosteric equilibrium constants, etc., are obtained through the analysis of the constructed thermodynamic isotherm by statistical thermodynamic models [1–6]. In other words, *statistical thermodynamic models are not used to construct the binding isotherm but to analyze it*. Application of statistical thermodynamic models to examine the obtained thermodynamic binding isotherm is based on the knowledge about the studied systems, e.g., their structural characteristics, like lattice or subunit structure of the macromolecule, character of lattice monomers, geometrical arrangement of subunits, etc. This is an extra-thermodynamic knowledge, i.e., it reaches beyond the experimental, thermodynamic binding analysis.

Although the direct methods of studying the ligand–macromolecule interactions do provide the total average degree of binding and the corresponding free ligand concentration, they possess limitations like, e.g., application only for small ligand molecules (equilibrium dialysis), or may perturb the examined equilibrium (filter binding method, gel electrophoresis) [7–10,preceding paper]. On the other hand, the indirect methods, based on observing a physico-chemical signal, predominantly a spectroscopic signal reflecting saturation of the macromolecule or the ligand, require that the observed changes of the monitored physico-chemical parameter correlate with the total average degree of binding and the concentration of the free ligand [6,11–16,preceding paper].

In most interacting/binding systems subjected to the quantitative analysis, the functional relationship between the observed physico-chemical signal and the total average degree of binding is never a priori known. It has to be determined [6,11,12,16]. Very often, it is assumed that the observed change of the physico-chemical/spectroscopic signal is directly proportional to the degree of saturation of the macromolecule and/or the ligand, i.e., the signal is a linear function of the total average degree of binding. However, in such cases, the obtained interaction parameters are not more accurate than the applied assumption. This is not the problem in the case of single ligand binding processes where indeed the observed relative changes of the signal always reflect the saturation of the macromolecule or the ligand [1,6,16,17,preceding paper]. But it is already a lot to know about the examined system that only a single ligand molecule binds [17]. In more complex situations, even with binding reactions involving only two ligand molecules, the failure of the applied statistical thermodynamic model to "fit" the titration curve may be due either to the failure of the model or the failure of the assumption, on which the "binding isotherm" is based. Ignoring these facts will particularly be serious if the "isotherm" is the basis to decide, which alternative models actually describe the examined binding process [6,18,preceding paper].

Why would not the observed spectroscopic signal be a linear function of the total average degree of binding? For instance, the macromolecule may possess functionally different sites, each characterized by different spectroscopic properties, which are differently affected by the bound ligand [18]. If there are cooperative interactions, the physical state of the macromolecule and/or the ligand may change in different sites, as the saturation process progresses [18,19]. The number of cooperative contacts among binding sites and/or bound ligand molecules may not be a linear function of the total average degree of binding. Different binding modes of the ligand may be characterized by different responses of the physico-chemical/spectroscopic signal [12,20,21]. In more complex situations, combinations of all mentioned above cases may occur.

In this second part of our review, we address the fundamental problem of obtaining thermodynamic and physico-chemical/spectroscopic parameters free of assumptions about the relationship between the observed signal and the degree of ligand or macromolecule saturation. We will mainly address quantitative methods as applied to the use of the fluorescence intensity, which is the most often encountered spectroscopic technique in biochemical studies [6,11,16–19,20–30]. Nevertheless, we will also discuss the same analyses for other commonly applied physico-chemical signals (e.g., fluorescence anisotropy, polarization, calorimetry, sedimentation velocity). It should be stressed that the obtained relationships are general and applicable to any signal used to monitor interactions and proportional to the concentrations of different states of macromolecule or ligand (e.g., absorbance, circular dichroism, NMR line width, chemical shift, etc.).

Furthermore, we are concerned only with cases where the examined physico-chemical/spectroscopic signal originates only from the macromolecule or only from the ligand. This condition is easily experimentally realized and amounts to monitoring only the macromolecule or the ligand saturation process. Quantitative examination of one reaction is