Applied Biophysics for Drug Discovery
Applied Biophysics for Drug Discovery

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Over the last two decades, biophysics has reemerged as a core discipline in drug discovery. Many may argue that biophysical methods never truly left discovery, but all will note the renewed present importance and central role of such methods. This reemergence is driven by three primary forces: the birth of fragment-based drug discovery schemes, the recognition of and desire to mitigate artifacts in traditional biochemical screening, and a desire to accelerate the transition from first-in-class to best-in-class molecules by focusing on hit and lead kinetics. Each of these strategies or goals requires various information-rich biophysical methods to experimentally execute. This text aims to summarize some of the key methods emerging from these three broad enterprises. First, though, it will map the contours of these three drivers of biophysics’ reemergence and link them to the chapters that follow.

Fragment-based drug discovery and fragment-based lead discovery are slightly different names for the same discovery approach: using a library of relatively small compounds to probe the surface of a target protein for binding sites. Fragment-based discovery approaches are animated by the information theory-based idea that relatively simple, small compounds sample chemical space more effectively than larger, more complex molecules [1, 2]. In practice, this approach drives one to develop low complexity screening libraries [3, 4]; consequently, the binding interactions with target proteins are generally very weak. Weak interactions require sensitive methods to unambiguously detect the binding event [5]. In simple bimolecular binding, the concentration of the complex is driven by the concentration of the ligand; this drives many scientists to screen their fragment libraries at relatively high concentrations. Effective screening methods must both be able to detect relatively weak interactions in the context of relatively high compound concentrations; several biophysical methods are well suited for this demanding screening campaign [6]. Various NMR approaches have been successfully applied to identify and characterize weak small molecule–protein interactions [7]. This text explores both traditional protein-detected NMR [8] approaches in Chapters 9 and 10.
Introduction

and nontraditional NMR [9, 10] approaches in Chapter 8. Both approaches have merit and are usefully applicable in partially overlapping circumstances. Surface plasmon resonance (SPR) [11, 12] and microscale thermophoresis (MST) [13] have also been successfully deployed in fragment screening campaigns to detect weak interactions. Chapters 5 and 6 explore applications of MST and SPR beyond fragment-based discovery, respectively.

A second force driving the reemergence of biophysical methods in drug discovery has been the desire to identify and eliminate high-throughput screening hits that operate through uninteresting nuisance mechanisms. Brian Schoichet recognized and characterized some commonly observed nuisance phenomena; many of these nuisance mechanism enzymatic assay hits had weak micromolar activities and showed either a flat or highly irregular SAR [14]. Schoichet’s team determined that the aberrant behavior in biochemical screening assays was driven by poor solubility resulting in compound aggregate formation. These compound aggregates, present in extremely low concentration, serve as protein sinks, adsorbing most of the target protein, yielding what appeared to be detectable but weak inhibition [15]. His team demonstrated that many of these aggregation-based inhibitors could be culled from screening hits by comparing activity in an assay with no or very low detergent to a high detergent assay condition. Compounds that lose activity in the high detergent assay were likely to be uninteresting nuisance hits.

Several biophysical methods complement the differential detergent biochemical assay [16]. In the biochemical assay approach, the presence of aggregates is inferred, whereas in the biophysical approaches, the aggregates are directly detected. SPR is uniquely suited such direct detection of nuisance behavior in a buffer matched to the original biochemical screening buffer [17]. Aggregated compounds generate complex binding responses that are not simple 1:1 interactions but rather reflect the partitioning of the aggregated compound between the free buffer and the protein captured on the sensor chip. Aggregated compounds also show complex binding to the sensor surface with no target protein captured, providing a simple, parallel means to detect nonideal interactions in real time during library screening. Hit validation workflows now commonly employ SPR, mass spectrometry, and other biophysical methods to remove nuisance mechanism hits [18].

A third trend driving the reemergence of biophysics in drug discovery is the desire to optimize kinetic or thermodynamic properties with an aim to rapidly progress from a first-in-class compound to a best-in-class compound. When comparing a first-in-class compound to a best-in-class compound, the best-in-class molecule generally has high selectivity for the pharmacologic target and consequently a lengthy residence time with that target [19]. Detailed understanding of compound binding kinetics [20] and inhibitory mechanism leads to better candidates with properties more like an ideal best-in-class compound [21]. SPR allows real-time analysis of binding kinetics [22]; streamlined experimental approaches allow rapid compound sorting based on kinetic parameters [23]. Combining thermodynamic data with affinity and kinetic data further characterizes the intermolecular interactions, enabling detailed SAR and further compound optimization [24]. This idea is explored and different methods applied inform interaction quality in Chapters 2, 4, 7, and 11.

The text concludes with a case study in Chapter 14 that joins many of the methods and concepts discussed in earlier chapters. The Pfizer research team used a combination of traditional biochemical analysis, focused structural information derived from NMR,
References


2

Thermodynamics in Drug Discovery

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2.1 Introduction

For the drug discovery scientist, the term “thermodynamics” refers to the study of the heat change that occurs when biomolecules interact. It can be measured either directly by isothermal titration calorimetry (ITC) or indirectly by using any technique that can be used to determine an affinity over a range of temperatures such as surface plasmon resonance (SPR) or fluorescence.

The change in temperature that occurs when molecules interact is, for all practical purposes, a universal phenomenon and has led to the use of ITC to study a wide variety of biomolecular interactions; these include, but are not limited to, protein–small molecule, protein–protein, protein–nucleic acid, protein–metal ion, protein–carbohydrate, nucleic acid–nucleic acid, and ion–ion interactions. The broad applicability of ITC and the exceptionally low errors in affinity determination typically observed using the technique have made it the gold standard for measuring $K_D$ [1].

In addition to being a convenient label‐free probe for studying interactions, the heat change is related to the binding enthalpy ($\Delta H$) of the interaction and, taken together with the affinity $K_D$, can be used to calculate the change in entropy of the process. This thermodynamic data gives insight into the non-covalent forces responsible for driving binding and recognition. It can be used to direct SAR programs and help reveal the energetic “hot spots” that are key for molecular recognition and that need to be retained throughout lead optimization.

In this chapter we present an overview of the current use of thermodynamics in the drug discovery process. This includes a brief outline of the techniques employed to generate thermodynamic data as well as more detailed discussion of the complexities surrounding the data interpretation. In addition, the utility of enthalpy as a probe for binding in fragment-based drug discovery programs and for understanding complex interactions will be highlighted.
2.2 Methods for Measuring Thermodynamics of Biomolecular Interactions

Thermodynamic data can be obtained either directly by ITC or indirectly by any method that can be used to determine a $K_D$ as a function of temperature such as SPR or fluorescence.

2.2.1 Direct Method: Isothermal Titration Calorimetry

Isothermal titration calorimeters measure the heat change that occurs when two molecules interact. Heat is liberated or absorbed as a result of the redistribution of non-covalent bonds when the interacting molecules go from the free to the bound state. ITC monitors these heat changes by measuring the differential power required to maintain zero temperature difference between a reference and a sample cell as the binding partners are mixed.

The reference cell usually contains water or buffer, while the sample cell contains one of the binding partners and a stirring syringe that holds the other binding partner (the ligand). The ligand is injected into the sample cell, typically in 0.5–2 µl aliquots, until the ligand concentration is two- to threefold greater than the sample. Each ligand injection results in a heat pulse that is integrated with respect to time and normalized for concentration to generate a titration curve of kcal/mol versus molar ratio (ligand/sample). A binding model is fitted to the resulting isotherm (data) to obtain the affinity ($K_D$), stoichiometry ($N$), and enthalpy of interaction ($\Delta H$). The Gibbs free energy ($\Delta G$) and the change in the entropy ($\Delta S$) upon binding can then be calculated using the relationship

$$\Delta G = RT \ln K_D = \Delta H - T \Delta S$$

(2.1)

where $R$ is the gas constant and $T$ is the absolute temperature in Kelvin. In addition to these parameters, it is possible to determine the change in heat capacity of an interaction ($\Delta C_p$) by determining the change in enthalpy at different temperatures ($T$) and using the relationship

$$\Delta C_p = \frac{\partial \Delta H}{\partial T}$$

(2.2)

2.2.2 Indirect Methods: van’t Hoff Analysis

2.2.2.1 Enthalpy Measurement Using van’t Hoff Analysis

It is possible to access enthalpy and entropy values without the need for calorimetric experiments. These thermodynamic parameters may be estimated using indirect methods, which make use of the temperature dependence of the binding affinity, by employing the van’t Hoff equation. This allows estimates of entropy and enthalpy to be made using any technique that allows the determination of the binding affinity at a range of temperatures. Equation 2.3 is an integrated form of the van’t Hoff equation, and it is clear from inspection that the enthalpy can be derived from changes in binding affinity as long as the constant pressure heat capacity change upon ligand binding ($\Delta C_p$) is known or can be fitted. The binding entropy can then be determined from the Gibbs–Helmholtz equation.

$$\Delta G = RT \ln K_D = \Delta H - T \Delta S$$

(2.3)
2.3 Thermodynamic-Driven Lead Optimization

The observation by Ernesto Freire [2] that for two drug classes, the HIV protease inhibitors and the statins, the “best-in-class” drugs have the most favorable binding enthalpy has driven many drug discovery laboratories to include thermodynamic data in their decision-making processes.

It has also been suggested that thermodynamic profiles could be used to identify inhibitors that were optimized for a number of properties including flexibility, to minimize drug resistance caused by rapid mutation of the target binding site [3]; specificity, to reduce side effects caused by nonspecific binding [4–6]; and solubility in water, to maximize the ligand efficiency of polar interactions [7, 8].

2.3.1 The Thermodynamic Rules of Thumb

In the last 10 years or so, a series of guidelines have emerged that have been broadly used to interpret thermodynamic data and have been proposed as key drivers for lead optimization programs [9, 10]. At the simplest level they can be summarized as:

- Hydrogen bonds have a favorable enthalpy.
- Hydrophobic interactions have a favorable entropy.
- Conformational changes are entropically unfavorable.

By applying these guidelines the medicinal chemist can, in theory, test the success or failure of their optimization strategies. For example, if an effective hydrogen bond was
successfully introduced, then one would expect to see an increase in the affinity of the interaction and a more negative enthalpy. If so, further iterations could be tested, and if not, determination of the complex structure may reveal some interesting and unexpected SAR. Equally, the success or failure of strategies to rigidify a ligand scaffold can be assessed by monitoring any reduction in unfavorable entropy of an interaction.

A good example of this type of approach, and the use of these rules of thumb, is the interpretation of the thermodynamic data for the interaction of a parent inhibitor (KNI-10026) and two derivatives (KNI-10007 and KNI-10006) with plasmspsin II, an antimalarial target [11] (see Figure 2.1).

The introduction of a hydroxyl group to the parent compound resulted in an increase in the favorable enthalpy of binding from −1.2 to −6.0 kcal/mol that is consistent with the introduction of an additional hydrogen bond. However there was a concomitant reduction in the affinity from 16 to 76 nM due to the greater entropy loss. This enthalpy–entropy compensation (EEC) is common in lead optimization and will be described in more detail elsewhere in this chapter. By changing the stereochemistry of the hydroxyl group in the second inhibitor, the affinity of the interaction was increased to 0.5 nM. In this case the enthalpic advantage of the additional hydrogen bond was maintained while minimizing the entropy loss. The differences in the change in entropy of this interaction were attributed to the additional burial of hydrophobic groups in the binding pocket for the tighter binder KNI-10006.

Either coincidentally or because of the emergence of ITC as a convenient assay to determine the quality of a hydrogen bond, there have been a number of articles promoting enthalpy-driven lead optimization strategies [4, 7]. It is clearly an attractive prospect to be able to quickly develop a drug with high efficacy using a combination of ITC, X-ray crystallography, molecular modeling, and medicinal chemistry. However, more recently, and perhaps not surprisingly, examples have emerged [1] suggesting that thermodynamic lead optimization is more complex than originally thought. Here we outline a number of additional factors that need to be considered when attempting thermodynamic lead optimization.

2.3.2 Enthalpy–Entropy Compensation

EEC is a phenomenon that has been discussed in the scientific literature over many years. EEC appears to be a real and demonstrable effect that many groups have experienced, but the cause may be due to more than one effect occurring across and within the experimental measurements [9]. The basic proposal is quite simple. Consider complex formation between a target protein and a ligand. This binding event is the result of the disruption of interactions of each free partner with the solvent, forming new interactions with each other in the complex. During optimization, the structure of the ligand is modified in order to produce increased bonding interactions with the protein binding site. This will tend (generally) to make $\Delta H^\circ$ more negative. However, by introducing further points of interaction, there tends to be an increased order in the complex as a result of the modification, producing a more unfavourable contribution to $\Delta S^\circ$. Often, these two opposing contributions to $\Delta G^\circ$ tend to be of similar magnitude in many studies on biological systems. Hence, the traditional medicinal chemistry approach of building new chemical functionality into a molecule to improve the interaction with the binding site (favorable enthalpy) tends to introduce constraints to movement of the molecule