



MicroCal™

## Lead Optimization: Designing the Best Molecules to Move Forward

### Introducing Isothermal Titration Calorimetry into the Drug Discovery Process

#### Introduction

The process of advancing screening hits to leads and ultimately leads to clinical candidates requires extensive decision-making based on experimental data. Having access to the most informative data – data that enables the highest quality and most rapid decision-making — is critically important.

More and more, medicinal chemists, biologists and pharmacologists are using Isothermal Titration Calorimetry (ITC) to provide the information needed to make decisions about which molecules to modify to improve binding properties and which to abandon. Most currently used methods, including  $IC_{50}$ , fluorescence assays, scintillation proximity assays, or even surface plasmon resonance are effectively “affinity only” methods. These methods give little insight into the mechanism of binding which can lead to a large number of false positives. Only ITC provides the binding affinity, as well as the mechanism of binding, thus giving a true picture of the relationship between the lead and its target.

ITC is a unique technique that provides a complete picture of binding affinity, and most importantly, insights into the mechanism of binding, all from a single, easy-to-set-up experiment. ITC indicates whether a lead compound is binding to its target by specific polar interactions or perhaps more by hydrophobicity. As compounds are modified, ITC can rapidly measure the effect of the changes on the binding interaction.

#### Binding—Get the Whole Picture!

The binding affinity is recognized as an important parameter in evaluating the potential of a molecule to become an effective therapeutic. The observable and measurable binding affinity, however, is composed of multiple components, some of which favor the ability of a molecule to act as a potent and selective drug and some which do not. These individual components can be altered to maximize the overall binding affinity to favor drug-like characteristics, while minimizing those components that act against a molecule having desirable pharmaceutical characteristics.

A thorough understanding of all of the components of binding and their relationship to the binding affinity accelerates the development of hits to leads to drug candidates resulting in the achievement of extremely high affinity compounds with favorable drug-like properties. Only ITC measures the individual components of binding, allowing you to design the best candidates while reducing attrition rates and time. ITC can also be used to determine the affinity of very weakly binding fragments so you can learn the role of various functional groups or identify suitable fragments to combine to improve overall efficacy.

#### Designing the Best Molecules: Does the Drug Molecule have Favorable Interaction with the Target?

ITC directly measures the heat of the interaction of two molecules, the enthalpy, which reflects hydrogen bonding and other favorable

interactions between the surface groups on the drug molecule and those in the binding pocket within the target. The amount and type of hydrogen bonding is highly indicative of the specificity of the reaction—the better the bonding, the more specific the interaction. However, too many hydrogen donor groups on the drug molecule will cause low bioavailability due to poor cell membrane permeability. Conversely, too little hydrogen binding results in poor specificity. ITC data provides a way to optimize the number of hydrogen bonds with a minimum number of hydrogen bond donors and acceptors.

### **Is the Binding Due to a Hydrophobic Interaction?**

ITC can identify compounds that rely heavily on hydrophobic interactions for their affinity. ITC data provides the entropy of the reaction. Entropy is governed by hydrophobicity, water release and conformational change. These compounds are the most likely to bind other proteins, as well as the target, causing low therapeutic efficacy and side effects. Thus, drug molecules with strong hydrophobic interactions with the target are not a good starting point for a lead optimization program.

### **Does the Drug Molecule “Bind” with Conformational Change?**

ITC can suggest if large conformational changes occur upon binding, often inferring that there is some flexibility in the drug-target interaction. Flexibility is beneficial if binding to a class of proteins (i.e. more than one that are closely related) or to a protein that has naturally occurring mutations. For example, flexibility of interaction makes a good antiviral drug. Flexibility however is bad if the target is in an environment with related proteins that need to be avoided, i.e. when greater specificity is required.

Drug molecules can give the same affinity but differ widely in the mechanism of binding. Only ITC gives you all of this information so that the drug molecules being designed to move forward are the most likely to succeed!!

### **Plus!**

ITC measures all of these components in ONE experiment and guides you in developing a high affinity, selective lead candidate more quickly and efficiently!

And there is NO ASSAY DEVELOPMENT!

In addition, when synthesizing new compounds in the lead optimization process, many are produced in enantiomer and diastereomer forms. ITC can determine the binding affinity of both enantiomer and diastereomer forms of a molecule in one experiment without separation.

Start designing better molecules to move forward using ITC and get a better molecule faster!

### **For Further Reading**

Ruben, A. J., Kiso, Y., and Freire, E. (2006) Overcoming Roadblocks in Lead Optimization: A Thermodynamic Perspective. *Chem Biol Drug Des.* **67**, 2-4.

Frasca V. (2003) Isothermal Titration Calorimetry and Drug Design. MicroCal Application Review.

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