Competition Experiments

- What are they?

- When would you use them?

Competition Experiments

Extend the affinity range that ITC can be used

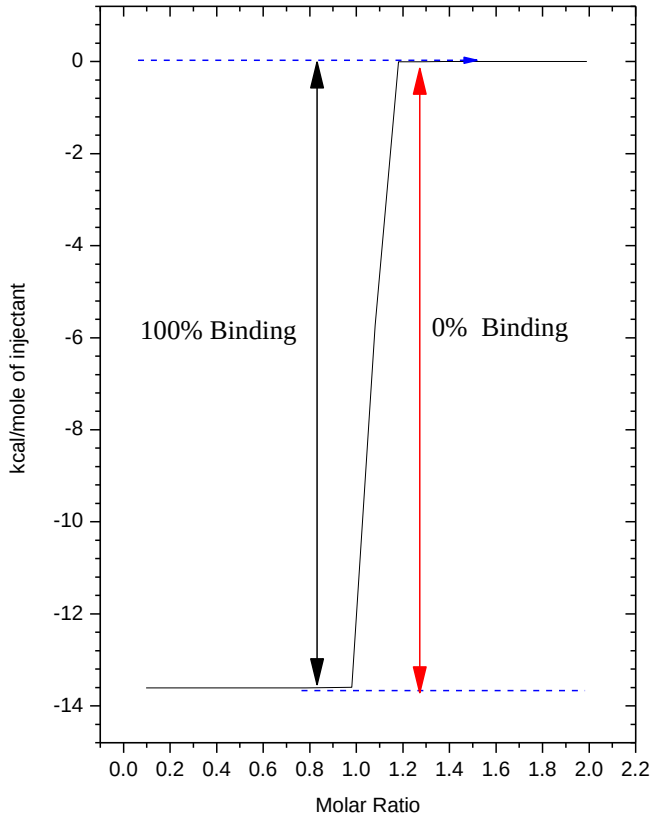
- Submillimolar (10^{-2}) to picomolar (10^{-12})

- Save Protein

- Extend the applications

Competition Experiments

- High C Experiment
- Poor affinity estimates



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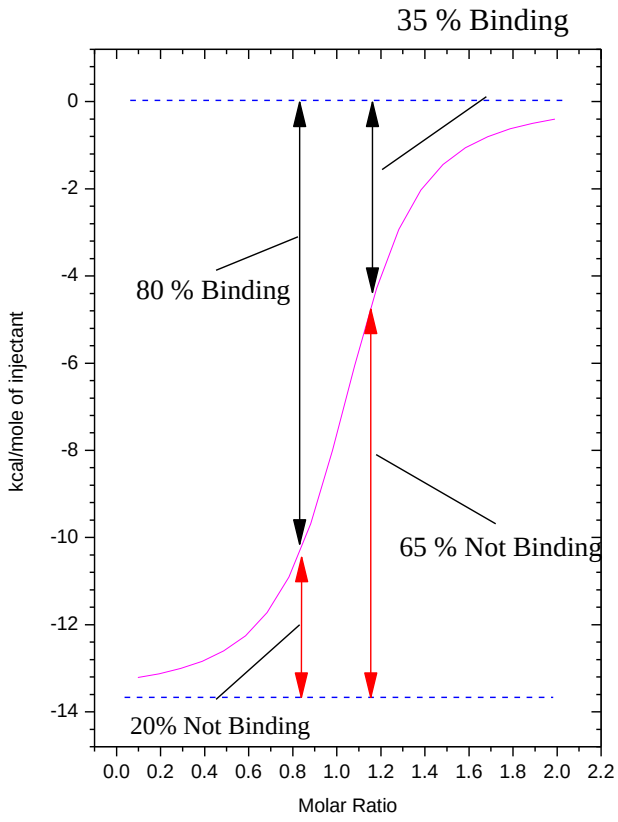
Competition Experiments

- Each injection results in some of the ligand binding and some remaining free- This is a good experiment.



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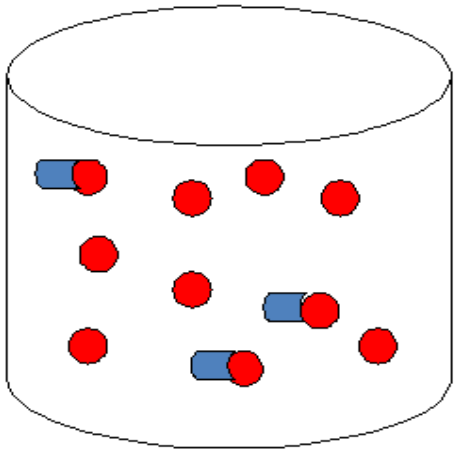
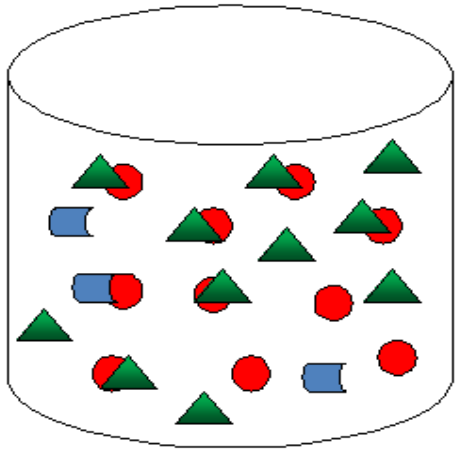
Tight Binders

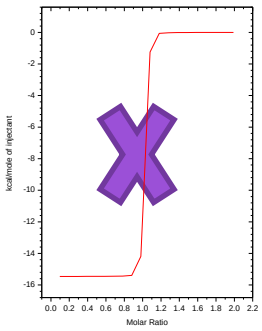


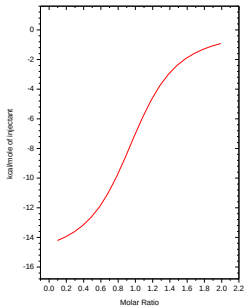


Protein

Tight Ligand

Competitor





Our Software
can ‘pull out’ the Kd
of the tight one

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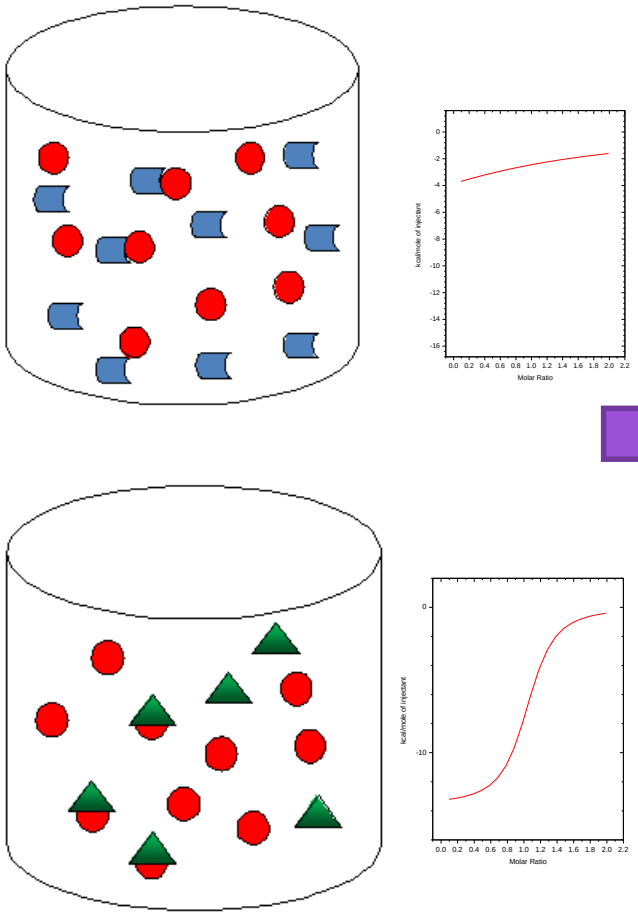
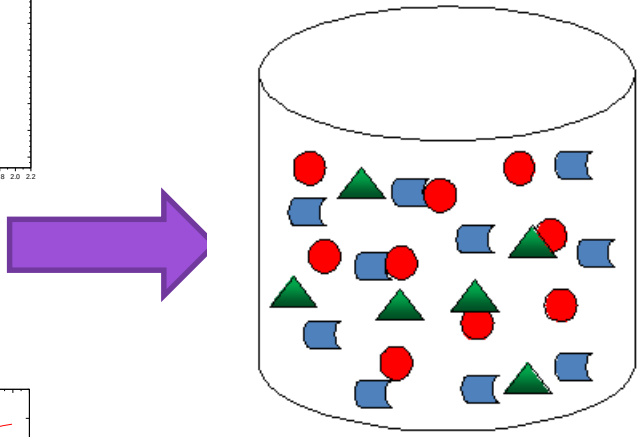
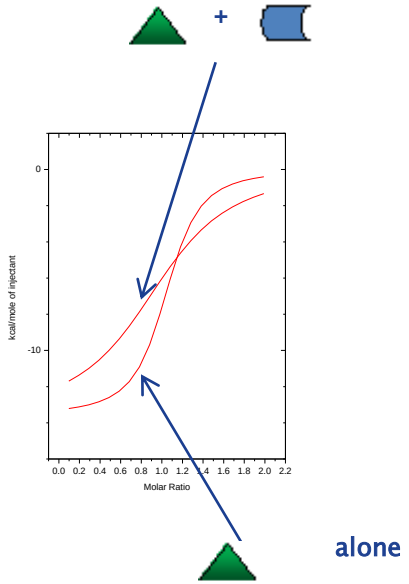
Weak Binders







Our software can pull the Weak Kd out

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Competition Experimental Design

$$C = [cell]/K_{D,app}$$

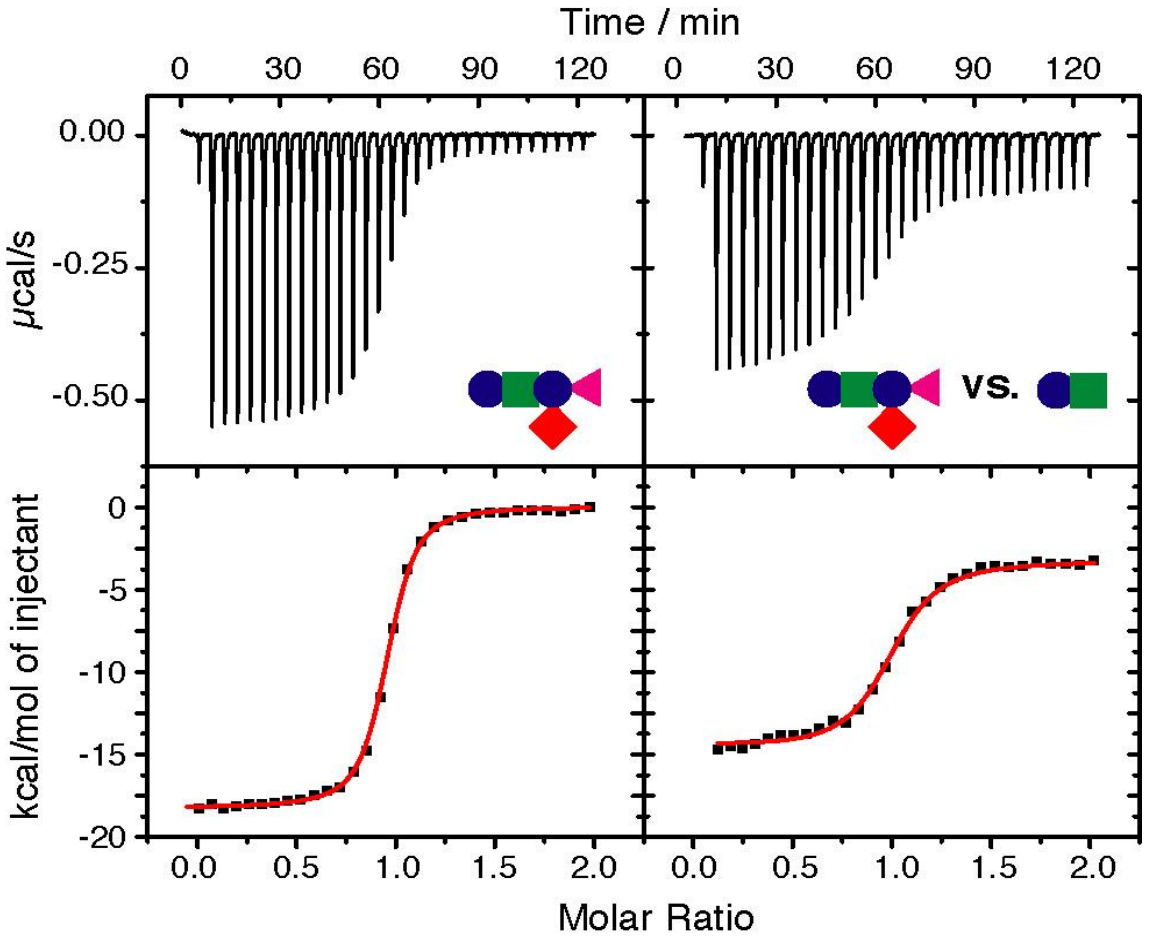
$$K_{D,app} = (1/K_{D,S})/(1+1/K_{D,W}[W])$$

Where $K_{D,S}$ and $K_{D,W}$ are the affinity of the strong and weak binders respectively and W is the concentration of the weak binder

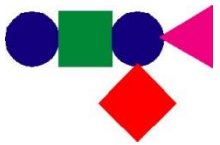
Competition Experimental Design

- Question 1- What will the effect be on $K_{D,app}$ if the [weak inhibitor, W] that is premixed with the protein is at $K_{D,W}$?
- Question 2- What effect will it have on the C value?
- Question 3-What concentration of [W], relative to K_D, should I use to determine $K_{D,S}$?

Application Note Example



10 µM CTB



110 µM

0 or 25 mM

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The Software

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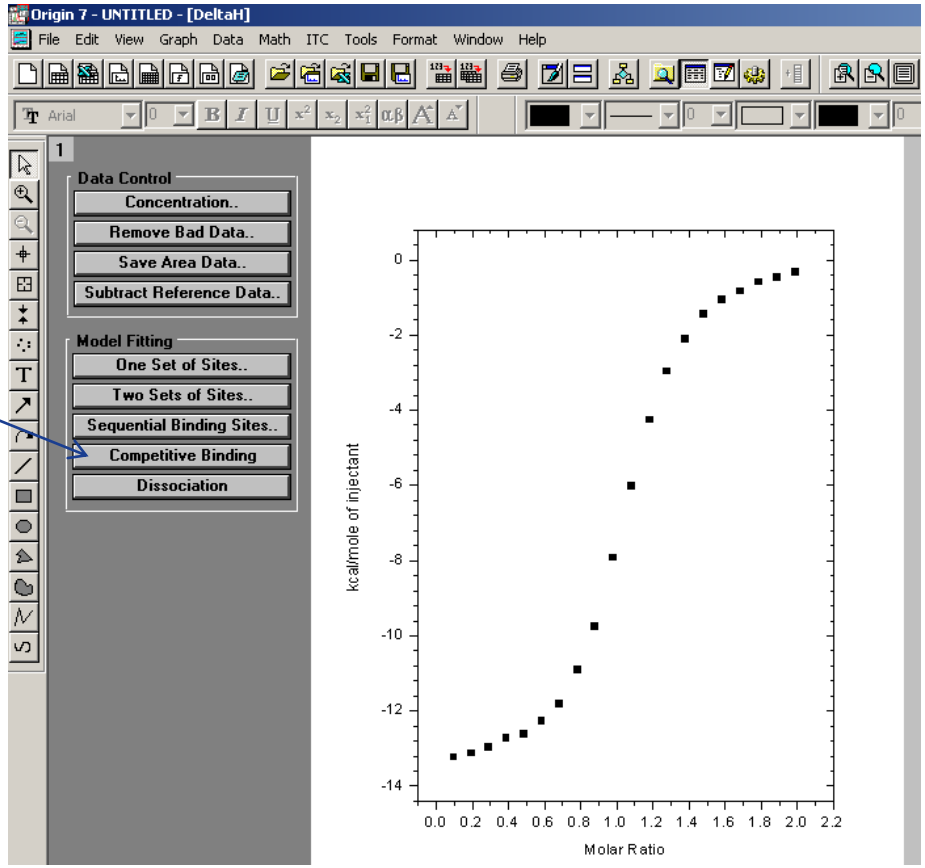
Competition Experiments

Competitive Binding



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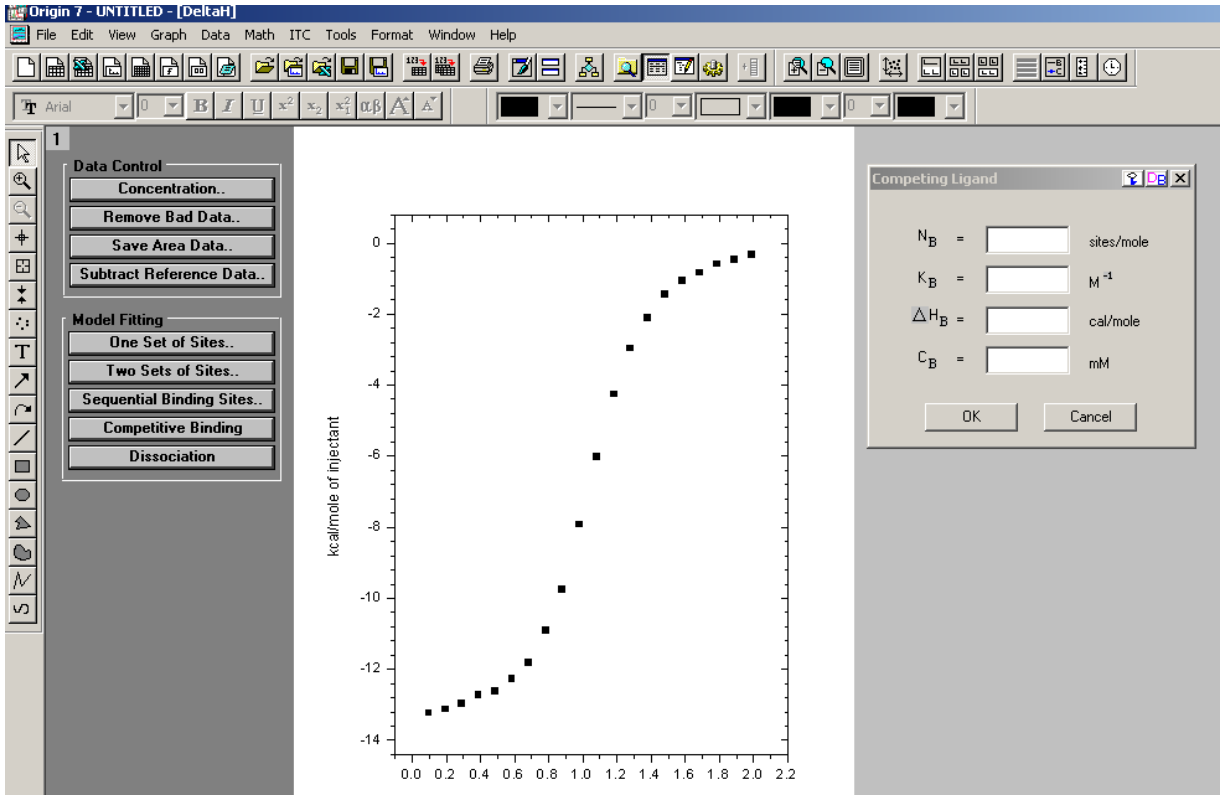
Competition Experiments



Competitor's Parameters

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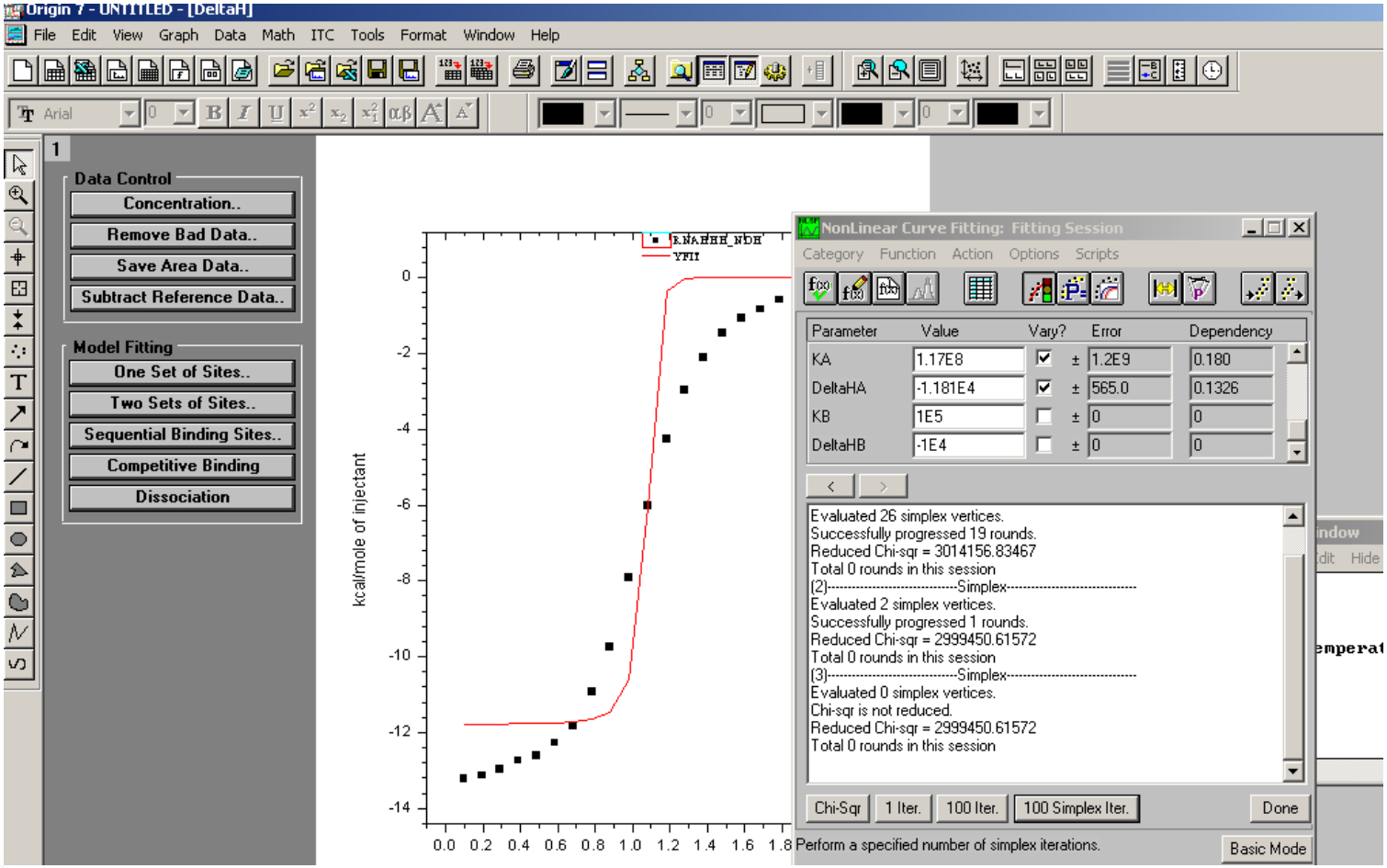
Competition Experiments



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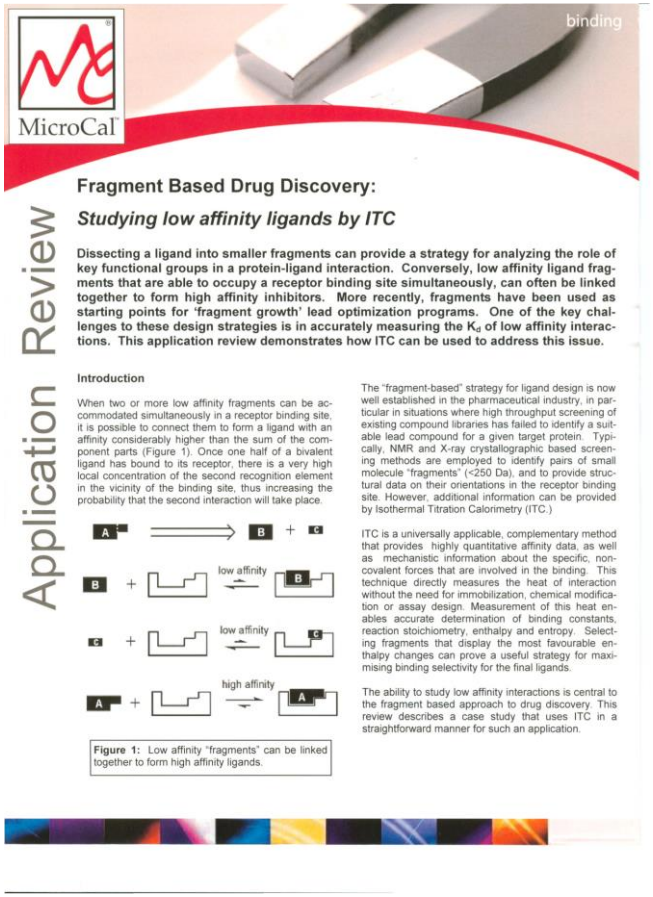
Helpful ‘Experimental Design’ Application Notes



MicroCal

binding

Application Review

Fragment Based Drug Discovery:

Studying low affinity ligands by ITC

Dissecting a ligand into smaller fragments can provide a strategy for analyzing the role of key functional groups in a protein-ligand interaction. Conversely, low affinity ligand fragments that are able to occupy a receptor binding site simultaneously, can often be linked together to form high affinity inhibitors. More recently, fragments have been used as starting points for ‘fragment growth’ lead optimization programs. One of the key challenges to these design strategies is in accurately measuring the K_d of low affinity interactions. This application review demonstrates how ITC can be used to address this issue.

Introduction

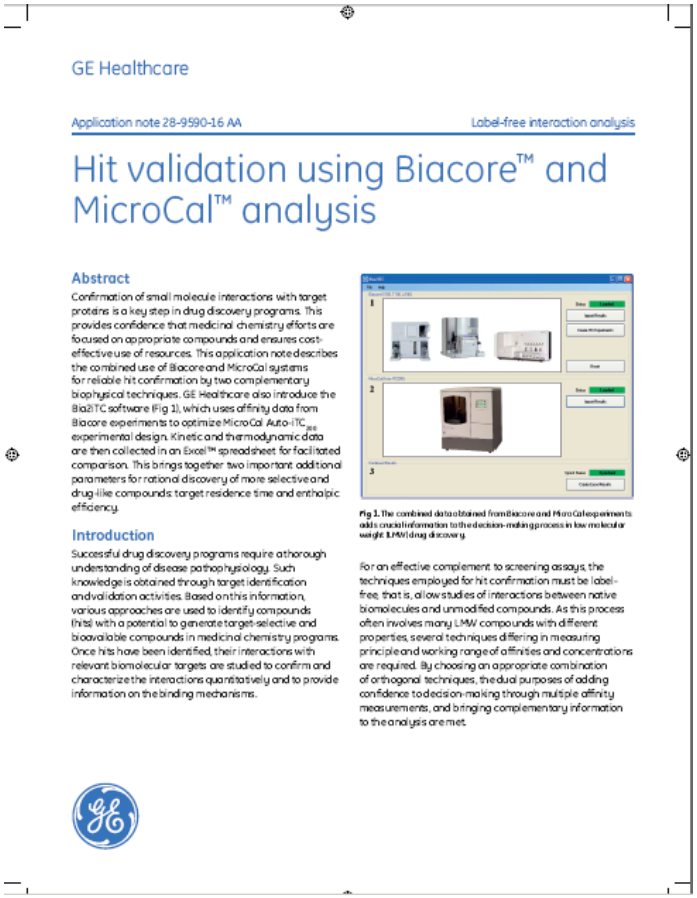
When two or more low affinity fragments can be accommodated simultaneously in a receptor binding site, it is possible to connect them to form a ligand with an affinity considerably higher than the sum of the component parts (Figure 1). Once one half of a bivalent ligand has bound to its receptor, there is a very high local concentration of the second recognition element in the vicinity of the binding site, thus increasing the probability that the second interaction will take place.

Figure 1: Low affinity “fragments” can be linked together to form high affinity ligands.

The “fragment-based” strategy for ligand design is now well established in the pharmaceutical industry, in particular in situations where high throughput screening of existing compound libraries has failed to identify a suitable lead compound for a given target protein. Typically, NMR and X-ray crystallographic based screening methods are employed to identify pairs of small molecule “fragments” (<250 Da), and to provide structural data on their orientations in the receptor binding site. However, additional information can be provided by Isothermal Titration Calorimetry (ITC.)

ITC is a universally applicable, complementary method that provides highly quantitative affinity data, as well as mechanistic information about the specific, non-covalent forces that are involved in the binding. This technique directly measures the heat of interaction without the need for immobilization, chemical modification or assay design. Measurement of this heat enables accurate determination of binding constants, reaction stoichiometry, enthalpy and entropy. Selecting fragments that display the most favourable enthalpy changes can prove a useful strategy for maximising binding selectivity for the final ligands.

The ability to study low affinity interactions is central to the fragment based approach to drug discovery. This review describes a case study that uses ITC in a straightforward manner for such an application.



GE Healthcare

Application note 28-9590-16 AA — Label-free interaction analysis

Hit validation using Biacore™ and MicroCal™ analysis

Abstract

Confirmation of small molecule interactions with target proteins is a key step in drug discovery programs. This provides confidence that medicinal chemistry efforts are focused on appropriate compounds and ensures cost-effective use of resources. This application note describes the combined use of Biacore and MicroCal systems for reliable hit confirmation by two complementary biophysical techniques. GE Healthcare also introduce the BiaJTC software (Fig 1), which uses affinity data from Biacore experiments to optimize MicroCal Auto-ITC$_{200}$ experimental design. Kinetic and thermodynamic data are then collected in an Excel™ spreadsheet for facilitated comparison. This brings together two important additional parameters for rational discovery of more selective and drug-like compounds: target residence time and enthalpic efficiency.

Introduction

Successful drug discovery programs require a thorough understanding of disease pathophysiology. Such knowledge is obtained through target identification and validation activities. Based on this information, various approaches are used to identify compounds (hits) with a potential to generate target-selective and bioavailable compounds in medicinal chemistry programs. Once hits have been identified, their interactions with relevant biomolecular targets are studied to confirm and characterize the interactions quantitatively and to provide information on the binding mechanisms.

Fig 1. The combined data obtained from Biacore and MicroCal experiments adds crucial information to the decision-making process in low molecular weight (LMW) drug discovery.

For an effective complement to screening assays, the techniques employed for hit confirmation must be label-free, that is, allow studies of interactions between native biomolecules and unmodified compounds. As this process often involves many LMW compounds with different properties, several techniques differing in measuring principle and working range of affinities and concentrations are required. By choosing an appropriate combination of orthogonal techniques, the dual purposes of adding confidence to decision-making through multiple affinity measurements, and bringing complementary information to the analysis are met.

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