



## Calorimetry, Binding, and Thermodynamics in the Drug Discovery Arena

Synopsis of a presentation from the 2007 Trends in Microcalorimetry Conference  
presented by:

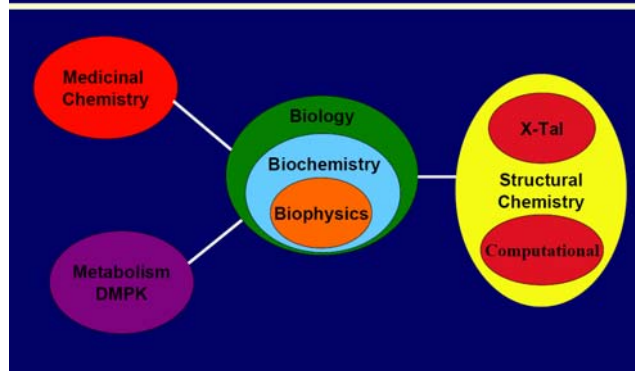
**Stephanie Leavitt, PhD.**  
**Gilead Sciences**  
**Protein Chemistry**  
**Foster City, CA**

In this presentation Dr Leavitt discussed how calorimetry is used throughout the drug discovery and development process at Gilead from target validation, to verification of screening and other bio-assay data, to structure-activity-relationship (SAR) generation and verification, and to elucidating and compensating for drug resistance mechanisms.

### How is Calorimetry used?

- **Target Validation**
  - Positive-Known target
  - Negative-Possible target?
- **Validation across assays**
  - Biosensor-SPR
  - IC50 assay
- **Enhancing drug discovery**
  - Moiety substitutions
  - Linker substitutions
- **Resistance mechanisms**
  - HCV Protease
  - HIV Protease

### Biophysics Interfaces with many other Groups



Calorimetry data in conjunction with data from other technologies, such as biosensor and crystallography, is often used to validate and enhance each other, and it also has the potential to influence and drive drug discovery efforts at multiple stages. This presentation illustrated examples where calorimetry in conjunction with other technologies has been used successfully.

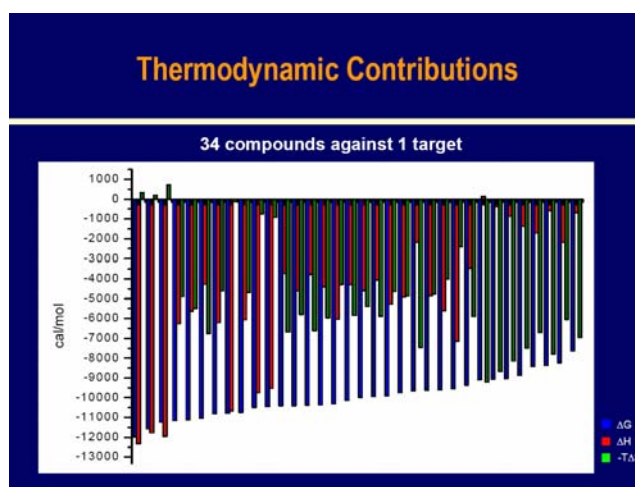
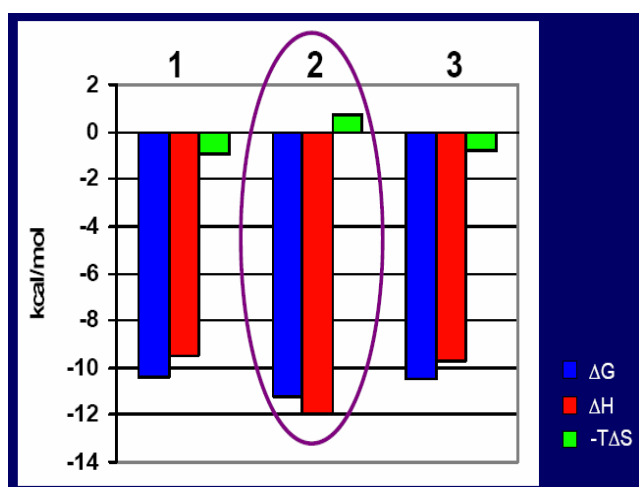
Calorimetry uniquely answers a variety of questions and, when used together with other technologies, the calorimetry data can be enlightening. The relevant questions can range from as simple as: “does this small molecule bind to protein X?; through to more complex, like mapping out important interactions in the binding site; all the way to detailed studies looking at resistance mechanisms.

The overriding objective is, of course, to make better drugs. This is achieved by utilizing the insights gained from thermodynamic parameters ( $\Delta G$ ,  $\Delta H$ ,  $-T\Delta S_{\text{binding}}$ ,  $-T\Delta S_{\text{solvation}}$ ,  $\Delta C_p$  and  $K_d$ ) in combination with the other

available kinetic and structural data. As a consequence, the drug discovery process is enhanced and better information is available during the critical decision-making stages of the discovery and development process.

Calorimetry, specifically isothermal titration calorimetry (ITC), measures the heat released or absorbed during a bio-molecular interaction event. It is the only technique which allows simultaneous determination of all binding parameters ( $n$ ,  $K$ ,  $\Delta H$  and  $\Delta S$ ) in a single experiment. The ITC technique provides this unique capability in an experiment that is completely label-free, in-solution and requires no immobilization of either the target macromolecule or ligand.

The parameters  $n$  (stoichiometry),  $K$  (binding constant) and  $\Delta H$  (enthalpy) are the independent variables of thermodynamic interest and are determined directly. The parameter  $\Delta G$  (Gibbs free energy) is calculated from the relationship  $\Delta G = -nRT \ln K$ ; and  $\Delta S$  (entropy) is determined from the relationship  $\Delta G = \Delta H - T\Delta S$ . A complete thermodynamic profile for multiple compounds is typically displayed as shown below.

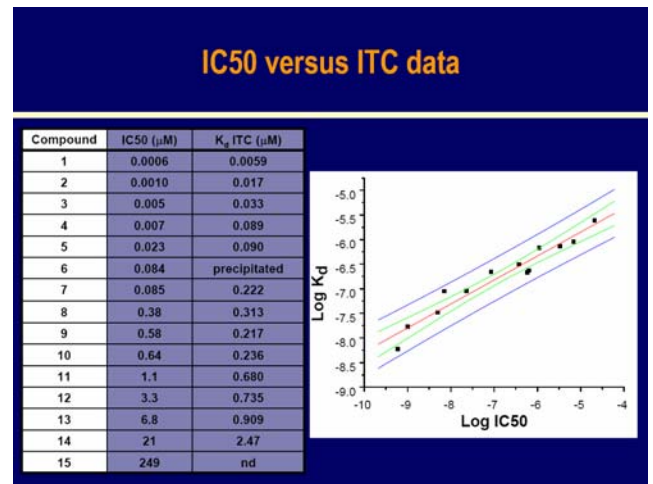
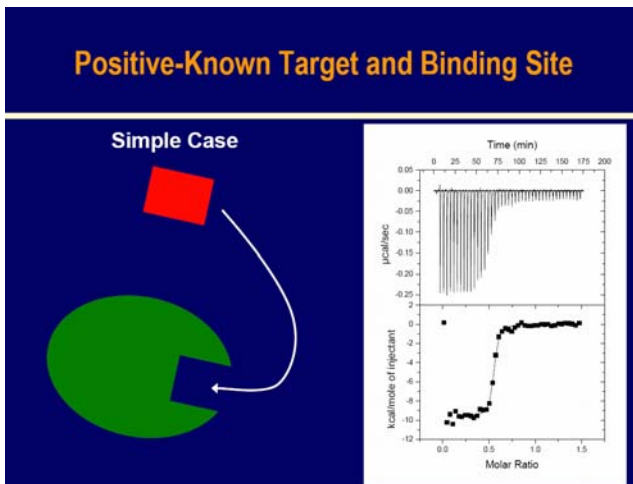


During SAR optimization, the thermodynamic profiles resulting from the binding of many different ligands to a target can be compared. In most cases, the most favorable binding situation results when  $\Delta G$  is most favorable with the most favorable  $\Delta H$  contribution.

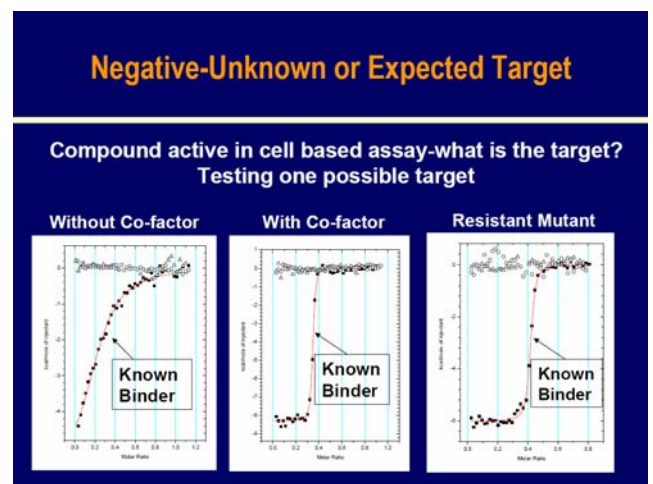
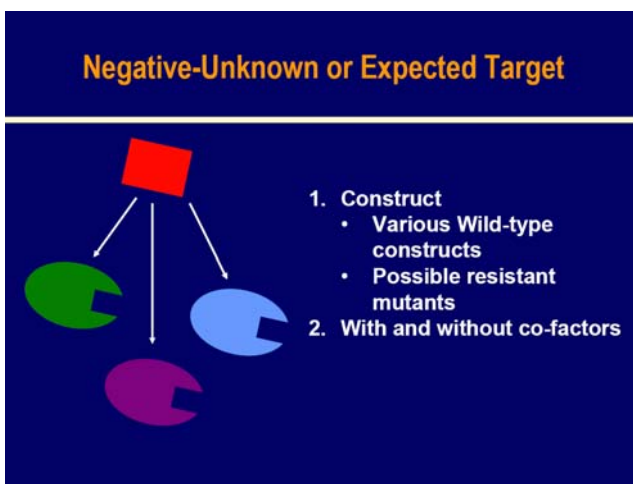
## Target Validation and Bioassay Validation

In the early stages of the drug discovery process, interest in a compound or a series of compounds is the result of those compounds being identified as "hits" in a high throughput screening (HTS) assay. The specific biomolecular target responsible for the biological activity in the HTS assay may or may not be known.

Calorimetry provides a means to both correlate binding data (Log  $K_d$  values) with biological activity data (Log  $IC_{50}$  values), and confirm (or deconvolute) the actual binding mechanism (e.g. the expected target versus an unsuspected target and the expected versus unsuspected binding site).



In the example shown here, binding of the ligand to a known target is confirmed from the ITC data, and an excellent correlation of the Log  $K_d$  values to the Log  $IC_{50}$  values is demonstrated

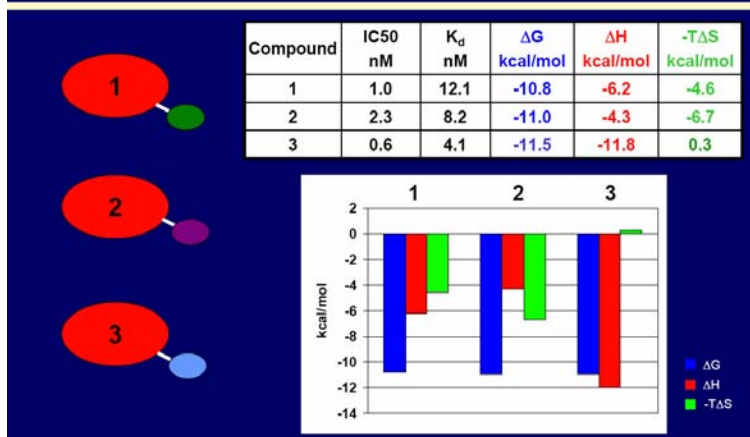


In this example, where the target is unknown (or the binding results for the expected target are negative), binding to alternative potential targets and target forms can be explored by ITC.

## Enhancing Drug Discovery

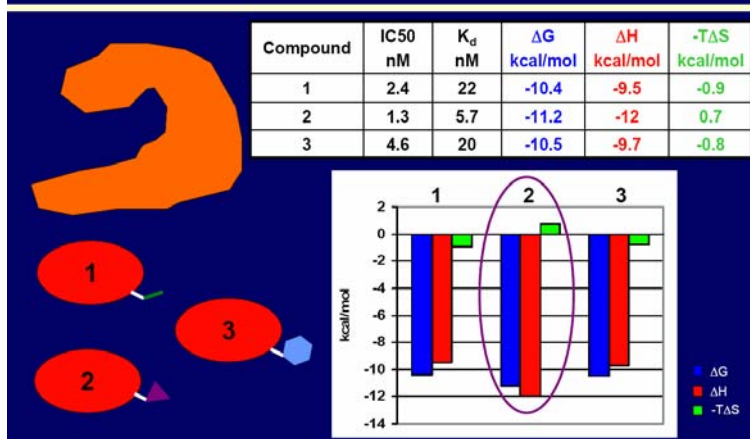
Thermodynamic profiles provide a detailed picture of the changes in binding dynamics throughout the discovery and development process. Each change in size, shape, rigidity and charge distribution can be evaluated for its effect on both the  $K_d$  as well as the enthalpy and entropy contributions to the binding.

### Differences in Chemical Isosteres



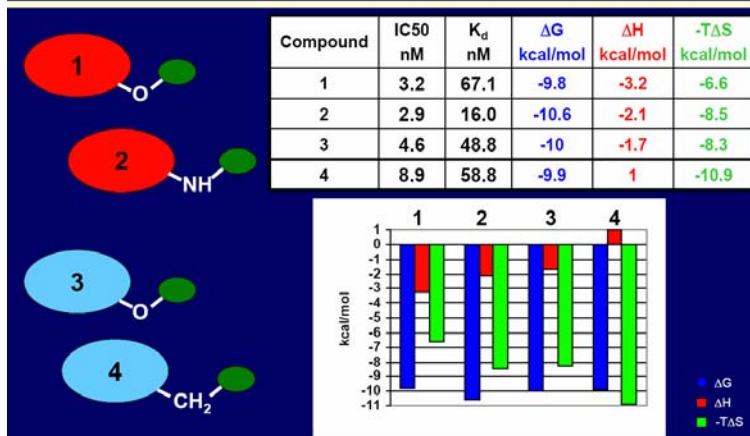
Thermodynamic profiles resulting from ITC data for a series of chemical isosteres provides an important component of the SAR lead optimization.

### Filling in a Pocket



Similar to the thermodynamic profiles generated for the isosteres series, the impact on binding strength and thermodynamics resulting from molecular modifications to the size and shape of the ligand can be quickly evaluated.

### Changing Rotational Freedom



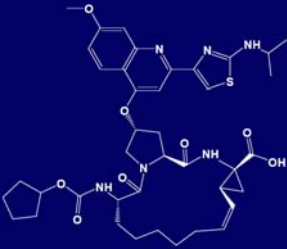
The impact on binding strength and thermodynamics resulting from molecular modifications that alter the rigidity of the ligand are clearly evident from the thermodynamic profile results.

## Assessing Resistance Profiles

Thermodynamic profiles also provide a direct means to assess the likelihood of resistance and to identify likely mutation sources of resistance. In a reversal of a typical experiment, a single ligand can be assessed for binding activity against a wild type and potentially resistant mutants.

### BILN – 2061 Compound

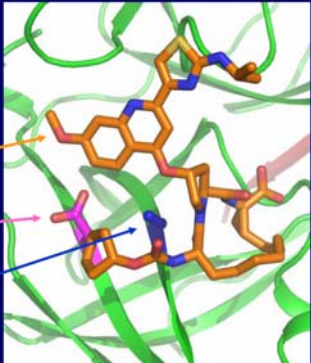
- Product based competitive inhibitor of NS3 protease
- A156T and D168V cause a 357-fold and a 144-fold increase in IC50, respectively



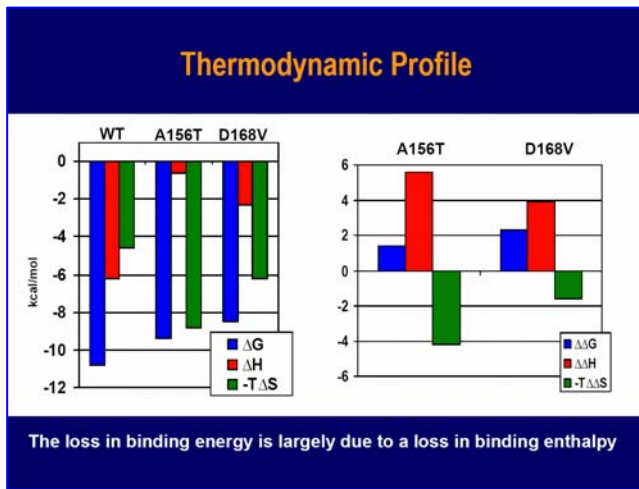
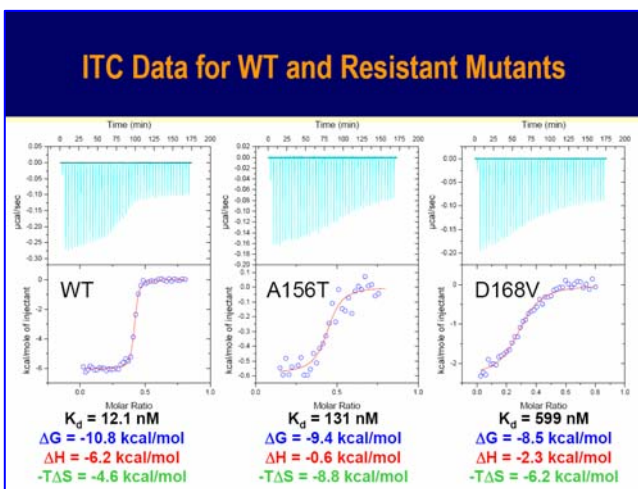
### Drug Resistant Mutants

Mutations introduced into the NS3\_4a fusion protein

- BILN-2061
- D168V
- A156T



Starting with a ligand of interest, wild type target and potentially resistant mutants, the change in binding profile for the mutants versus the wild type can be easily assessed.

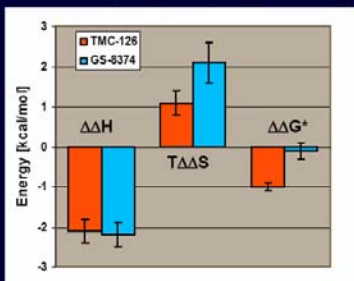


The data shown here suggest that resistance was developed by a loss of binding enthalpy suggesting a reduction in the number of polar contacts to the mutants.

Using ITC allows the key parameters of enthalpy and binding energy to be determined, with the resultant entropy also known. Understanding the enthalpy – entropy compensation can help identify the best compounds to move forward in a discovery program.

## Entropic Compensation As the Mechanism for Improved Resistance Profile

\*  $\Delta\Delta G = \Delta G(\text{mut}) - \Delta G(\text{WT})$  [kcal/mole]



Loss of the enthalpic contribution of GS-8374 binding due to resistance mutations is effectively compensated by an increase in the entropic contribution

*The entropic compensation of GS-8374 compared to TMC-126 permits a much lower reduction in binding energy hence an improved resistance profile*

### Discussion and Summary

This presentation illustrated how calorimetrically determined thermodynamic binding profiles can be applied in multiple stages of the drug discovery and development process to enhance:

- Target discovery and validation
- Hit selection and SAR lead optimization
- Confirmation of other assay data and data from complimentary biophysical techniques
- Insights into specificity, selectivity and resistance

Thermodynamic profiles provide a guiding tool for the development of new therapeutics, and allow a complete view of the relationship between the thermodynamic binding forces (enthalpy and entropy) and structure to be developed.

**For more information about the use of ultrasensitive microcalorimetry in the drug discovery and development processes, copies of review articles on lead optimization or other reviews from the 2007 Current Trends in Microcalorimetry Conference, please contact MicroCal.**

MicroCal, LLC  
www.microcal.com  
Email: info@microcal.com  
Toll-Free: 800.633.3115  
Tel: 413 586 7720