



Isothermal Titration Calorimetry

Fragments to Leads to Drug Candidates

Ultrasensitive Calorimetry for the Life Sciences™

Agenda

- Principles of microcalorimetry
- Factors involved in ligand/macromolecule binding
- Application examples of Calorimetry
 - Fragment binding
 - Structure Activity Relationship (SAR)
 - Enthalpy – Entropy Compensation (EEC)
- Summary & Instrument overview

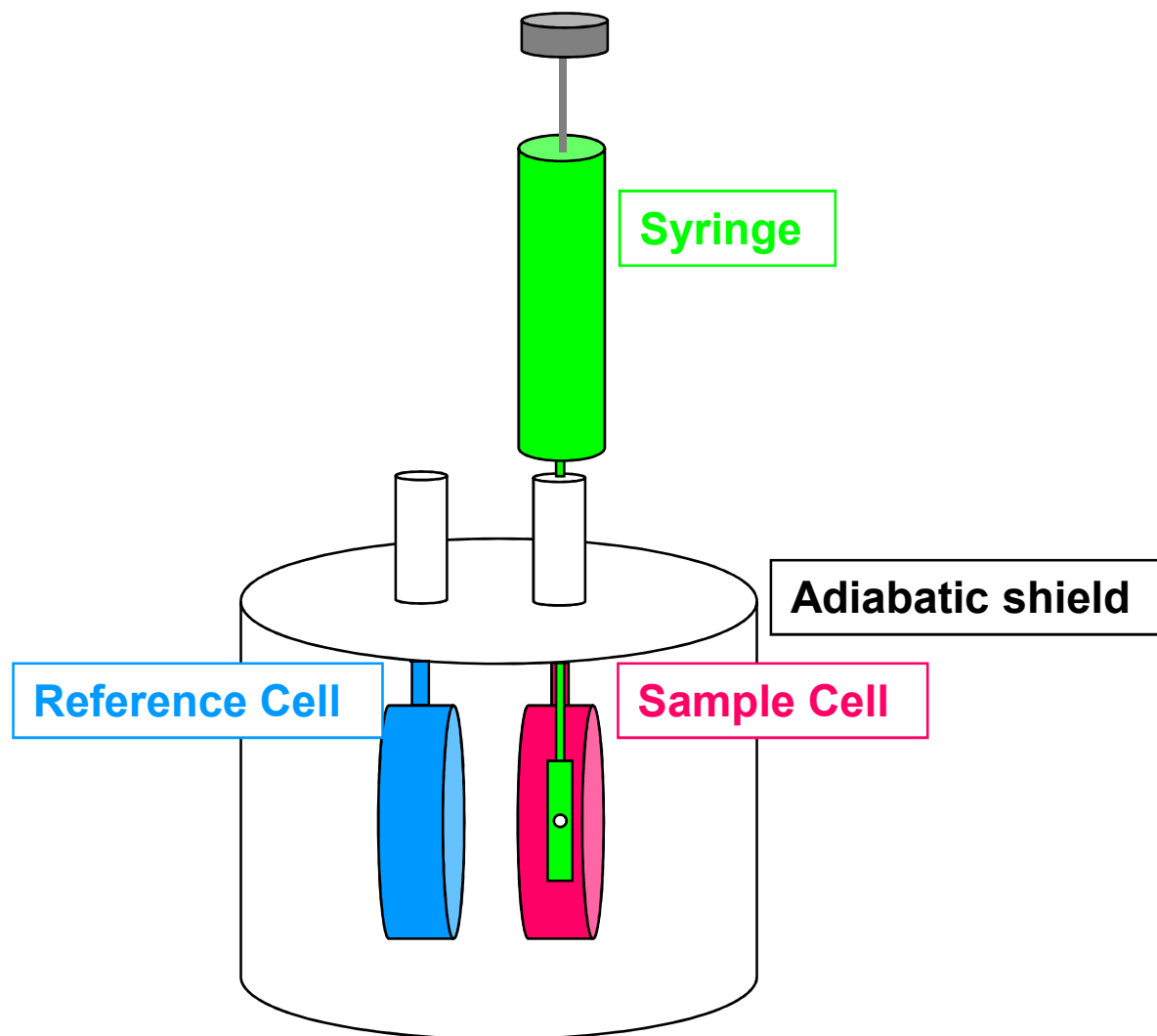
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Microcalorimetry Offers Enhanced Information Content

- Heat is our signal
 - Label-free
 - True in solution
 - No immobilisation requirement
 - No molecular weight limitations
 - Optical clarity unimportant
- Non-destructive
- Minimal to no assay development

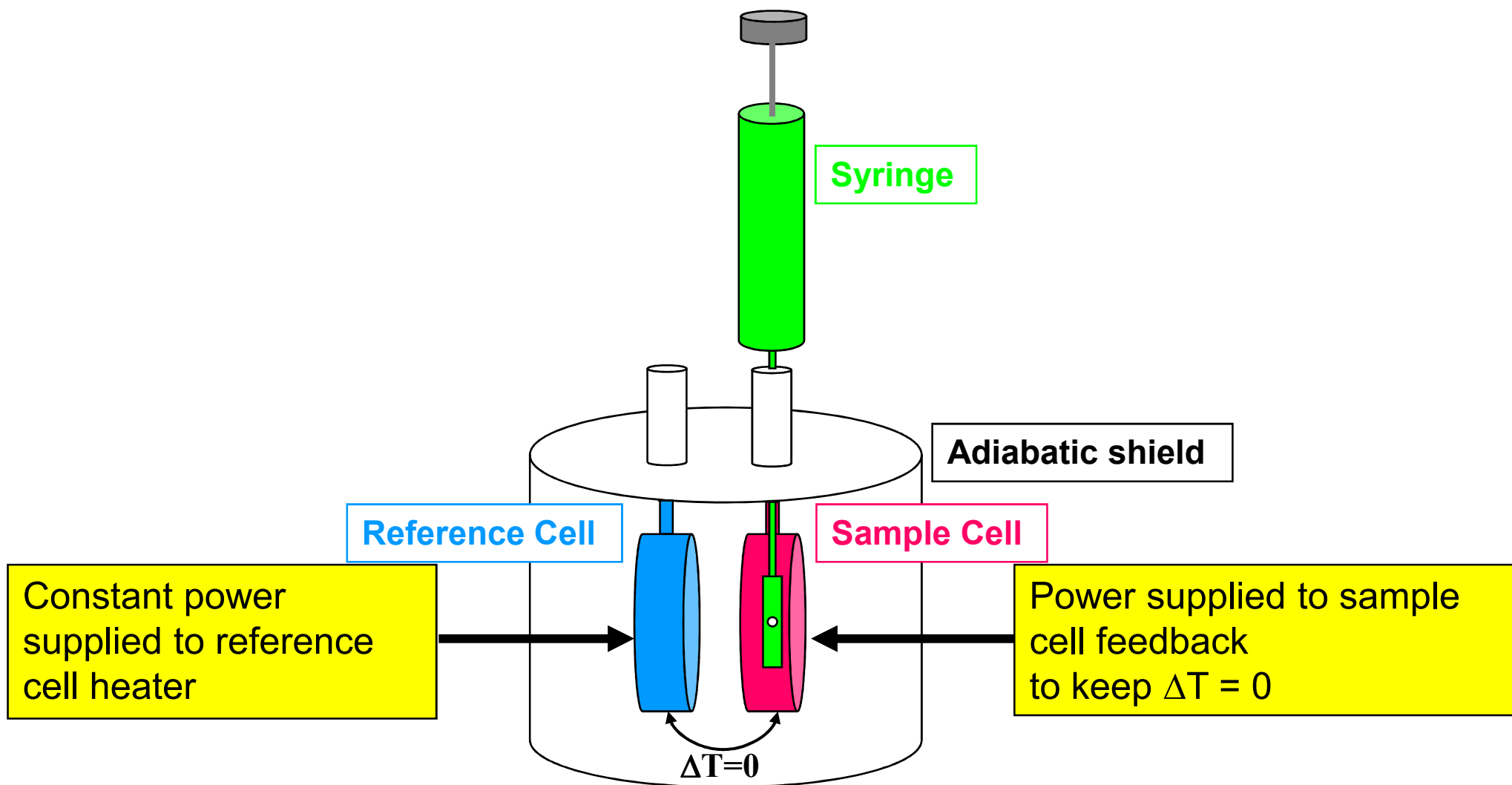
Schematic Representation of ITC



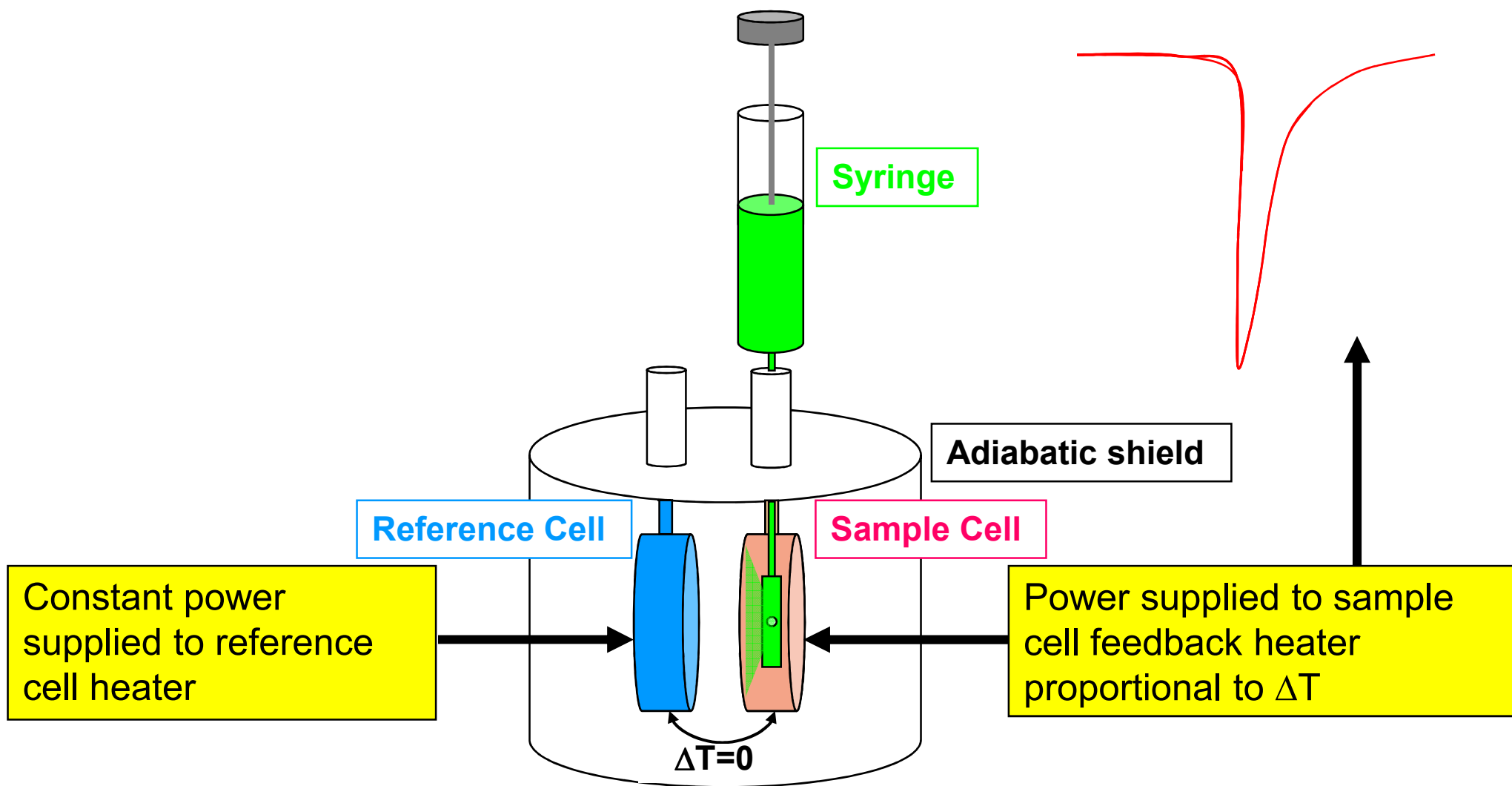
For more details see

T. Wiseman, S. Williston, J.F. Brandts and L.-N. Lin (1989) *Analytical Biochemistry* 179, 131-137 or
J.F. Brandts, L.-N. Lin, T. Wiseman, S. Williston and C.P. Yang (1990) *American Laboratory* 22, 30-41

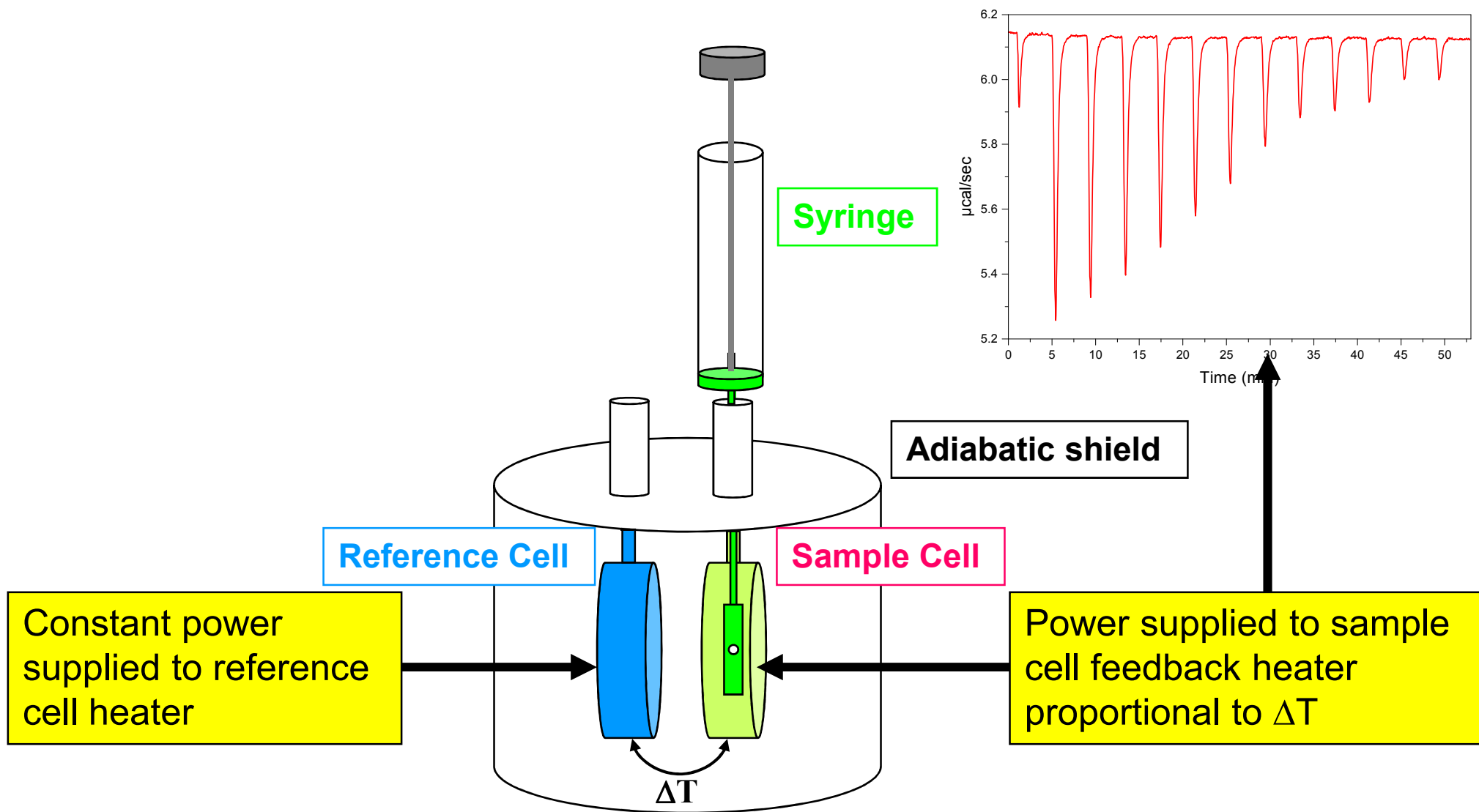
ITC Experiment



ITC Experiment



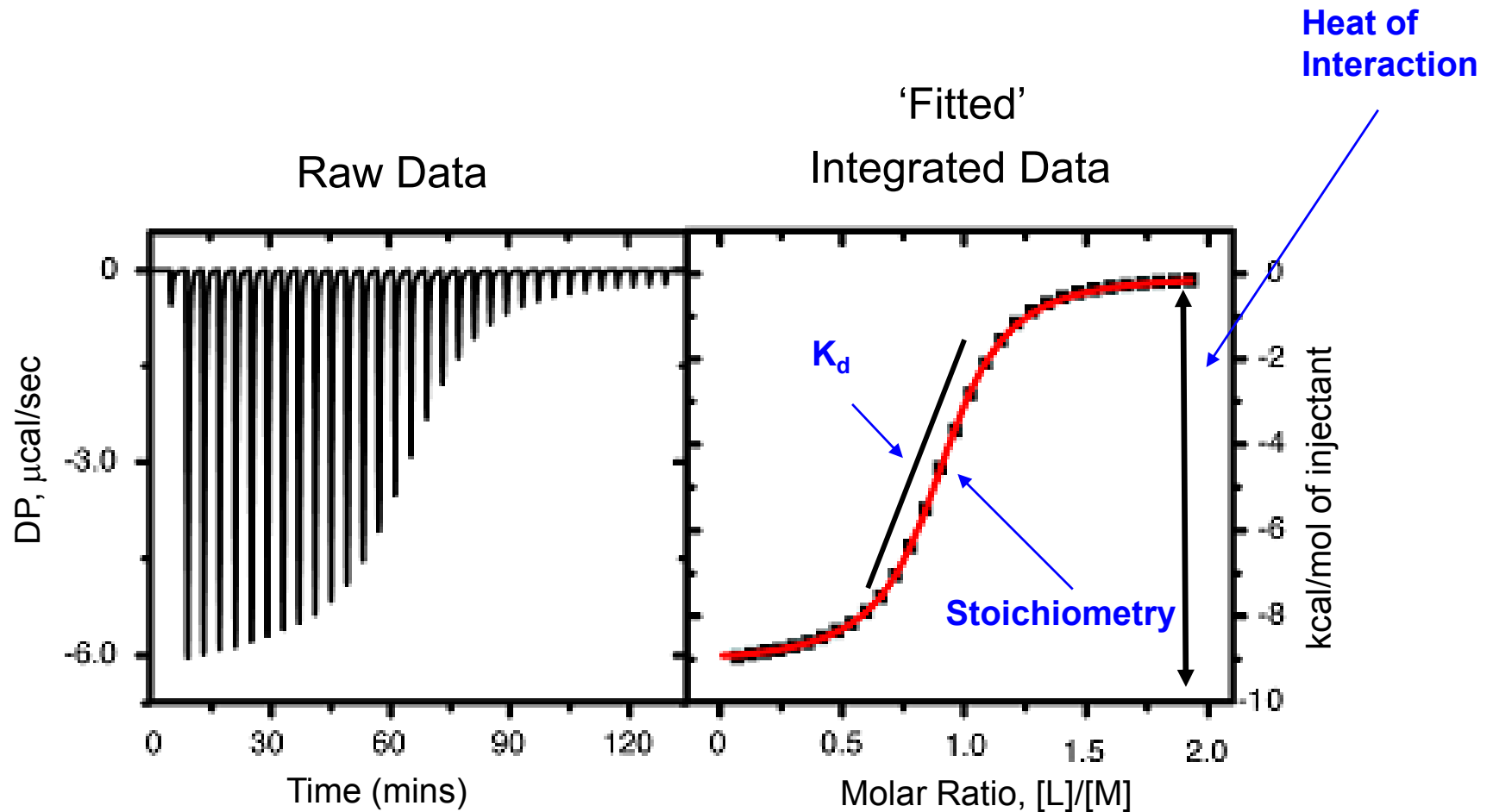
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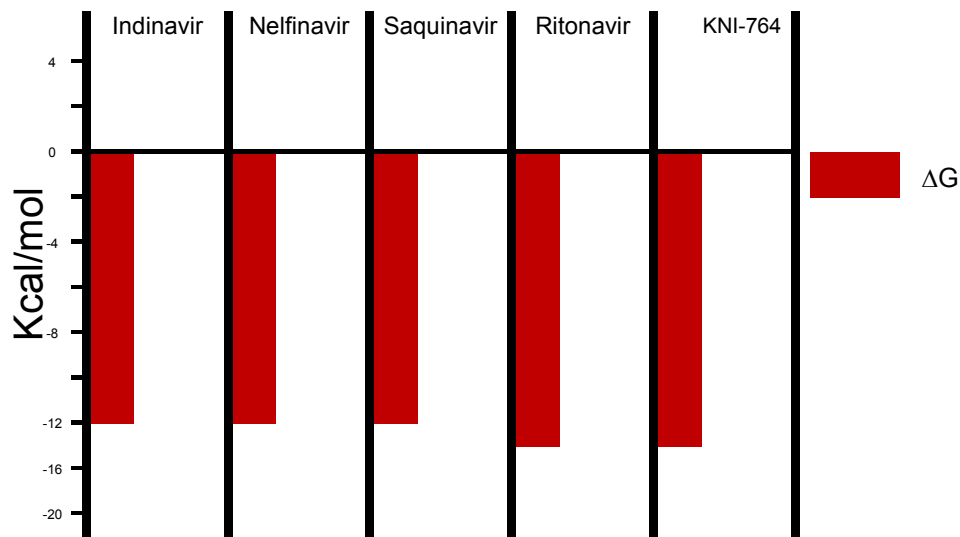
Typical Binding Data



Total Binding is Just Part of the Picture

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

Without ITC



Interaction of HIV protease with different inhibitors

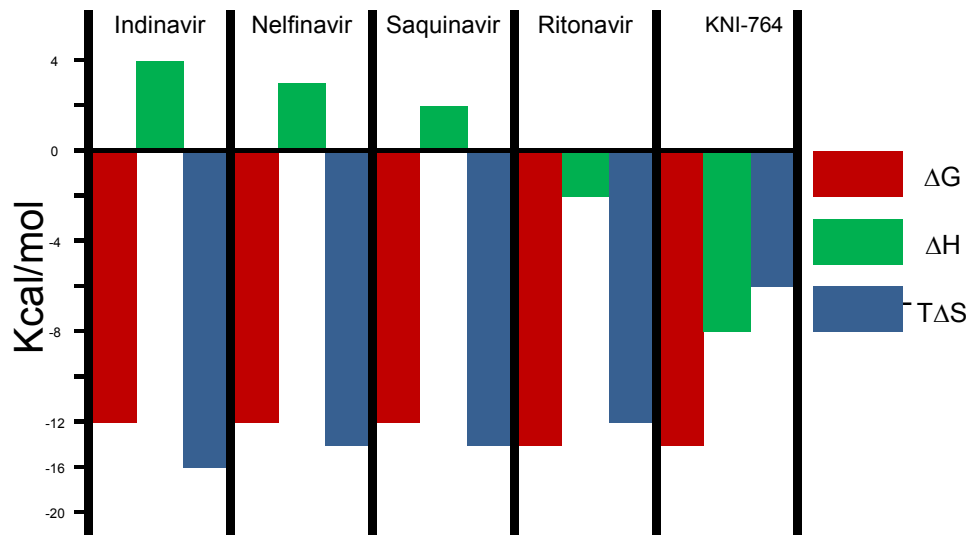
Contents of Binding Signature

- Tightness of binding

Enthalpy and Entropy Make up the Affinity

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

With ITC



Interaction of HIV protease with different inhibitors

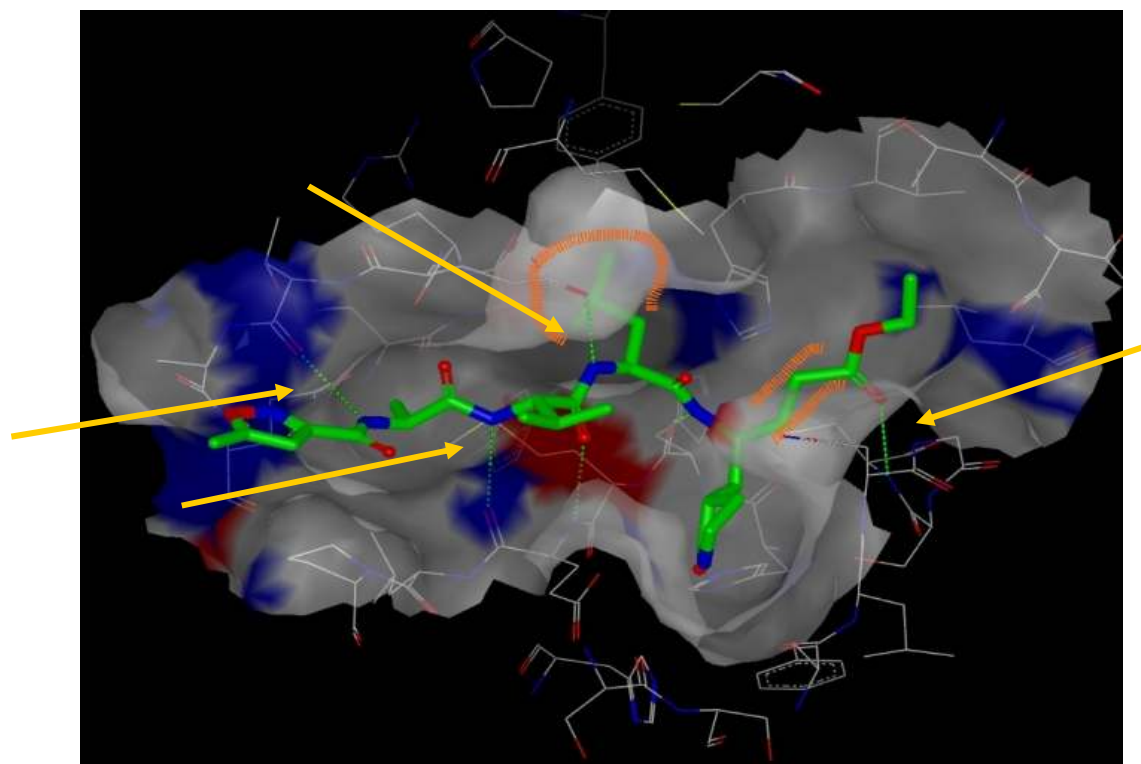
Contents of Binding Signature

- Tightness of binding
- Identifies binding mechanism
- Identifies opportunities to optimise selectivity

Energetic Signatures of the Binding Pocket: Enthalpy (ΔH)

- SARS Coronavirus main protease with an inhibitor docked into the binding pocket

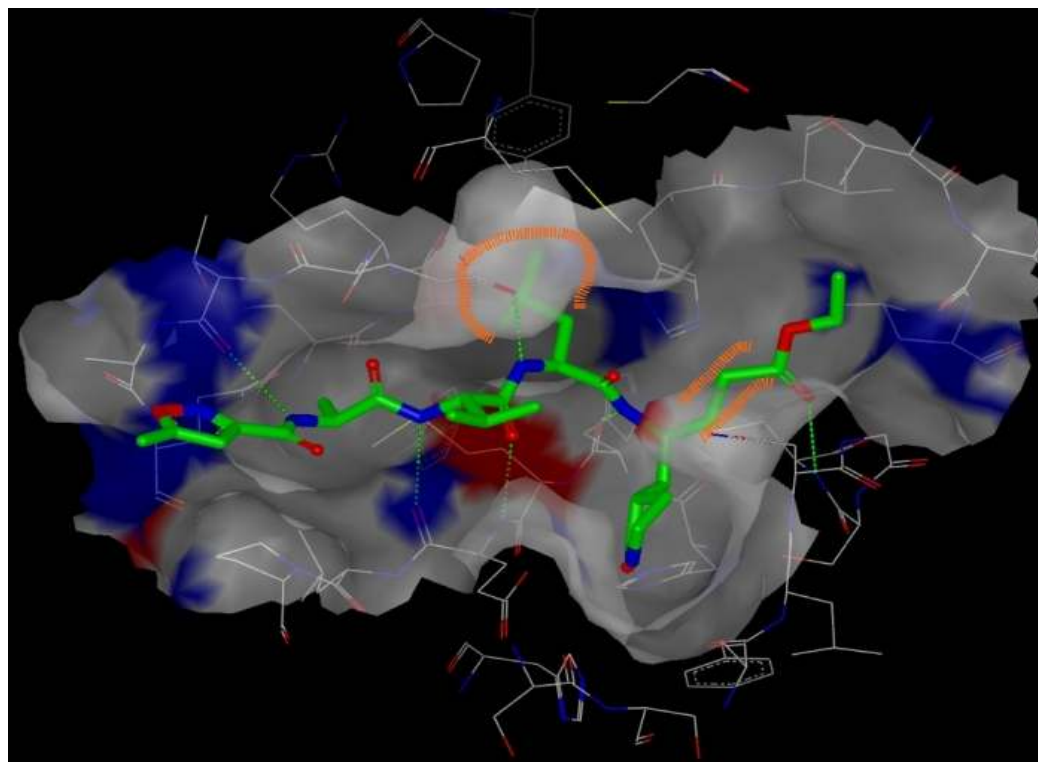
- Hydrogen bonds
- van der Waals interactions



These bonds are very specific to the binding pocket

Energetic Signatures of the Binding Pocket: Entropy (ΔS)

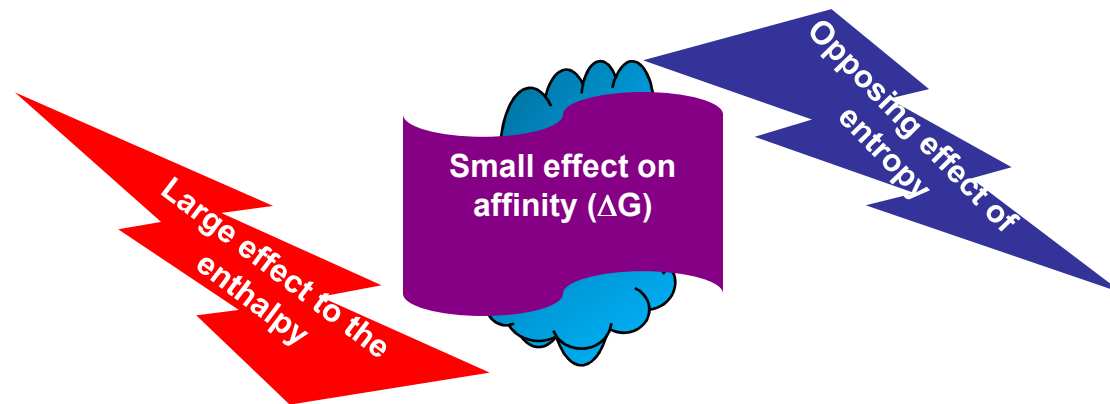
- SARS Coronavirus main protease with an inhibitor docked into the binding pocket
- hydrophobic interaction
- binding due to repulsion from the solvent rather than specific attraction to the macromolecule
- conformational changes



These bonds add to the affinity but are very non-specific to the binding pocket

Enthalpy – Entropy Compensation (EEC)

- Change in enthalpy followed by a change in entropy



- Linear correlation
- Challenge
 - Overcome EEC for a better affinity
- Benefit
 - Guides substitution with a little affinity penalty

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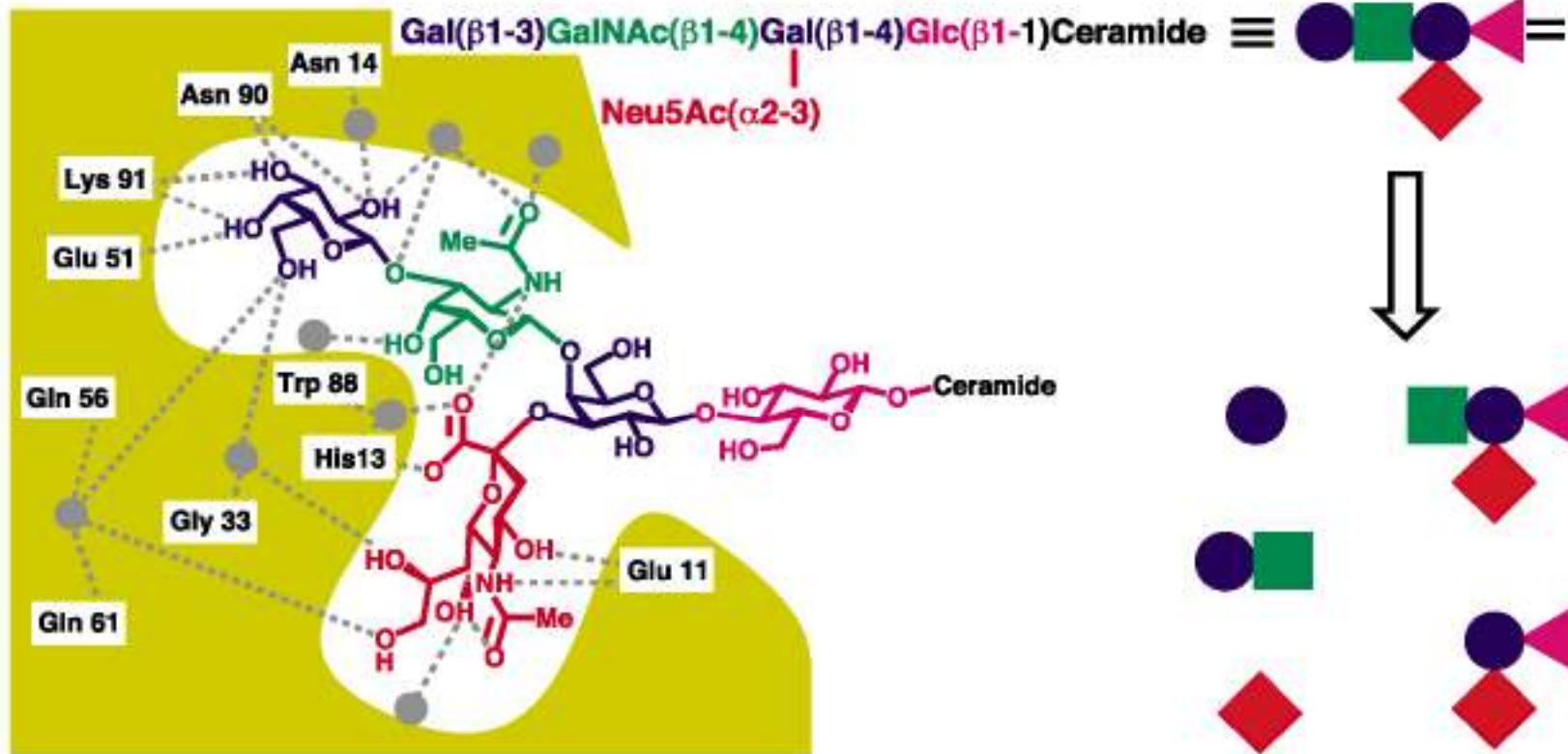
Fragment Based Drug Discovery

Studying Low Affinity Ligands By ITC

Fragment Based Approach

- Solve structure of target
- Design libraries using structural knowledge
- Screen with small library of low MW compounds (ITC, NMR, X-Ray)
- Identify most efficient ligand and build from there to form a high affinity compound with drug-like properties-ITC

Dissecting the GM1–CTB Interaction

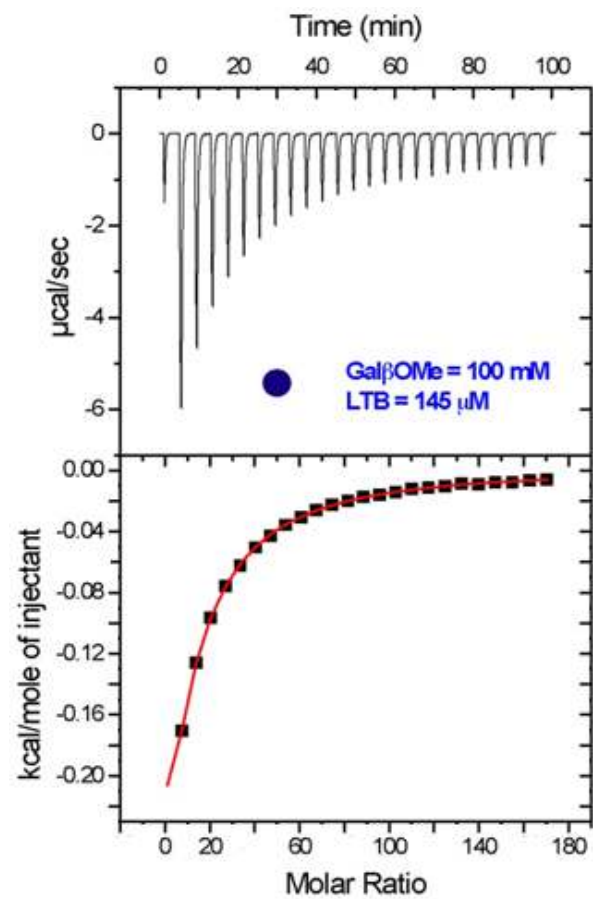


Evaluate the contribution that each monosaccharide makes to the CTB—GM1 interaction in solution

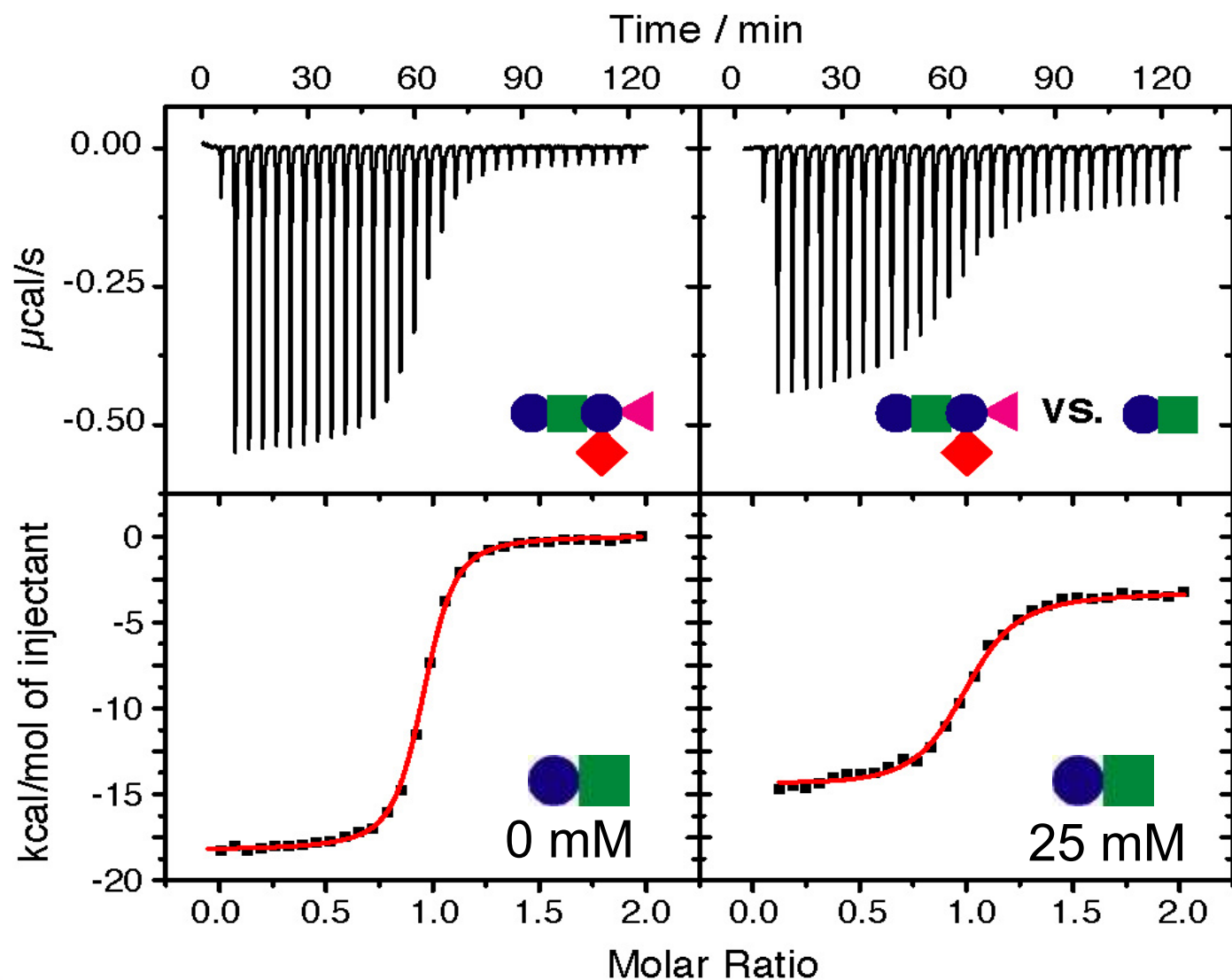
Weak Binding By ITC

- Low C value
 - Non Traditional ITC with low protein concentration and high concentration of ligand
 - Stoichiometry fixed
- Displacement/Competitive ITC
 - Strong binder displaces a weak binder
 - W. Sigurskjold, *Anal. Biochem.* 2000, 277, 260-266






Low C value



CTB Displacement Titrations



Summary of ITC Results

Ligand	K_d	ΔG , kcal/mol	ΔH , kcal/mol	$T\Delta S$, kcal/mol
GM1os 	43 ± 1.4 nM	-10.04 ± 0.02	-17.45 ± 0.03	-7.4 ± 50.03
Gal β OMe 	14.8 ± 1.6 mM	-2.5 ± 0.07	-9.0 ± 0.48	-6.53 ± 0.48
GM2os 	2.0 ± 0.2 mM	-3.67 ± 0.09	-4.35 ± 0.48	-0.69 ± 0.48
GalGalNAc 	7.6 ± 0.8 mM	-2.89 ± 0.08	-10.15 ± 0.43	-7.27 ± 0.45
Neu5Ac α OMe 	210 ± 100 mM	-0.92 ± 0.28	-10.7 ± 8.60	-9.77 ± 8.34

ITC in Fragment Based Drug Discovery

- Simple and accurate quantification of low affinity interactions
- Identify fragments suitable as scaffolds for lead optimisation
- The role of various functional groups of a compound to the overall binding can be determined
- Make use of the ΔH value

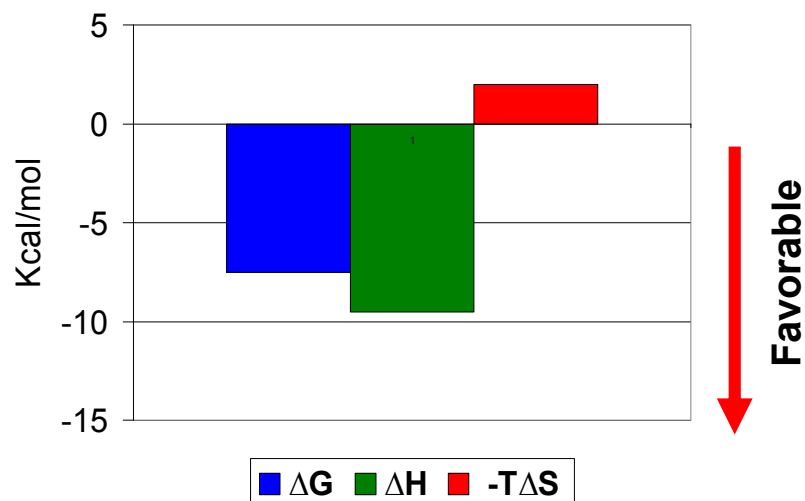
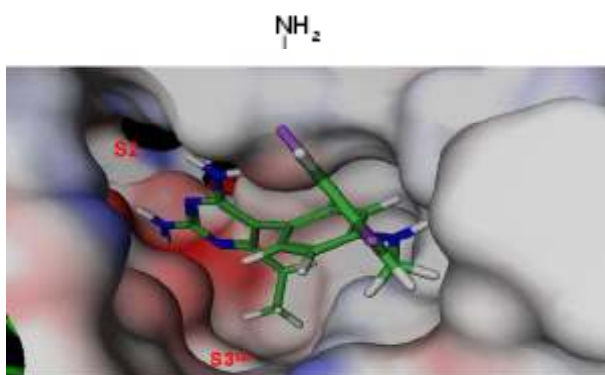
Optimising Diaminopyrimidine Renin Inhibitors

Aided by ITC and structural data

Abstracted from Sarver, et al, Anal. Biochem. 2007

Renin inhibitors ITC study

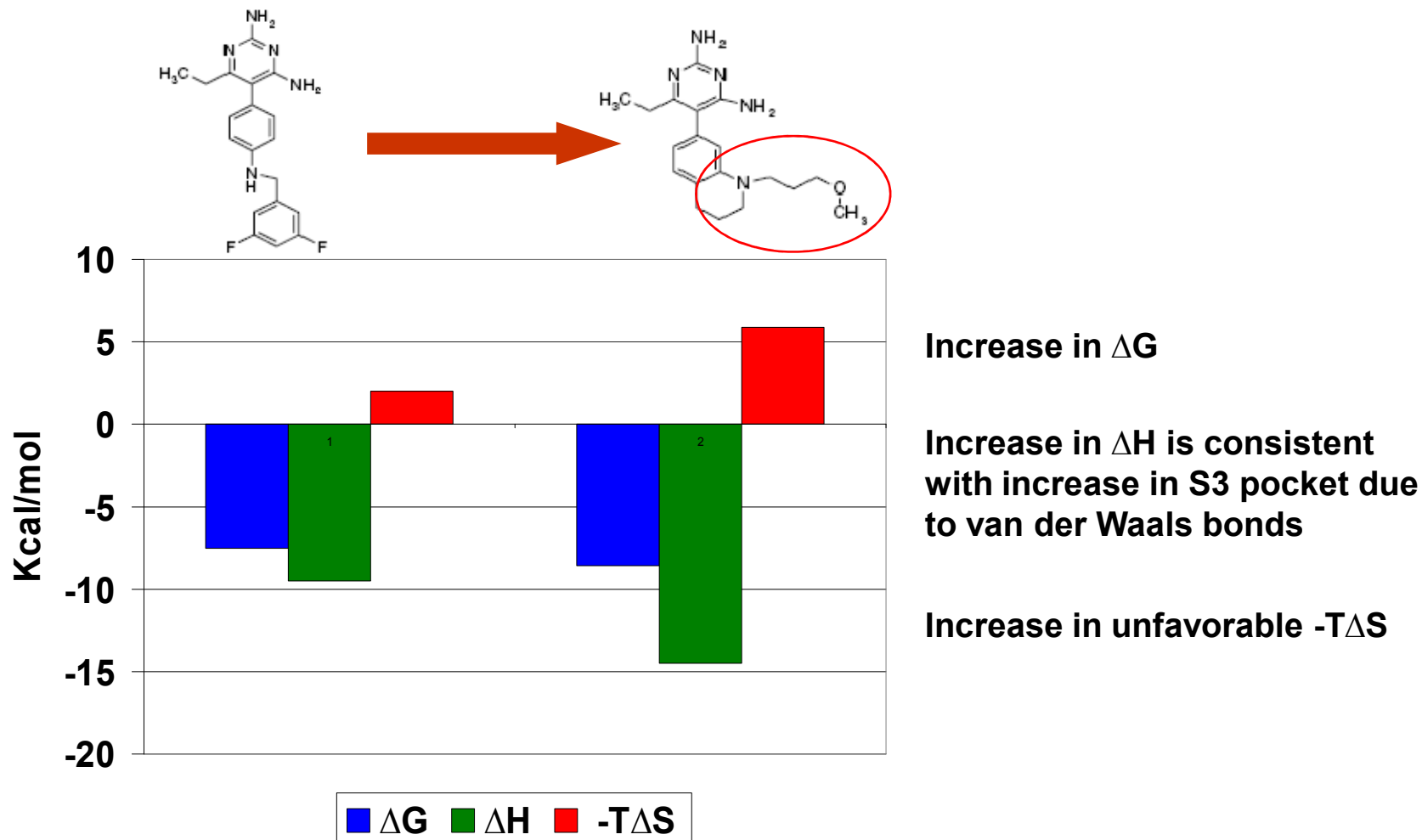
Thermodynamic and Structural data help design of better molecules



R. W. Sarver et al, *Anal. Biochem.* Binding Thermodynamics of substituted daminopyrimidine renin inhibitors (2007)

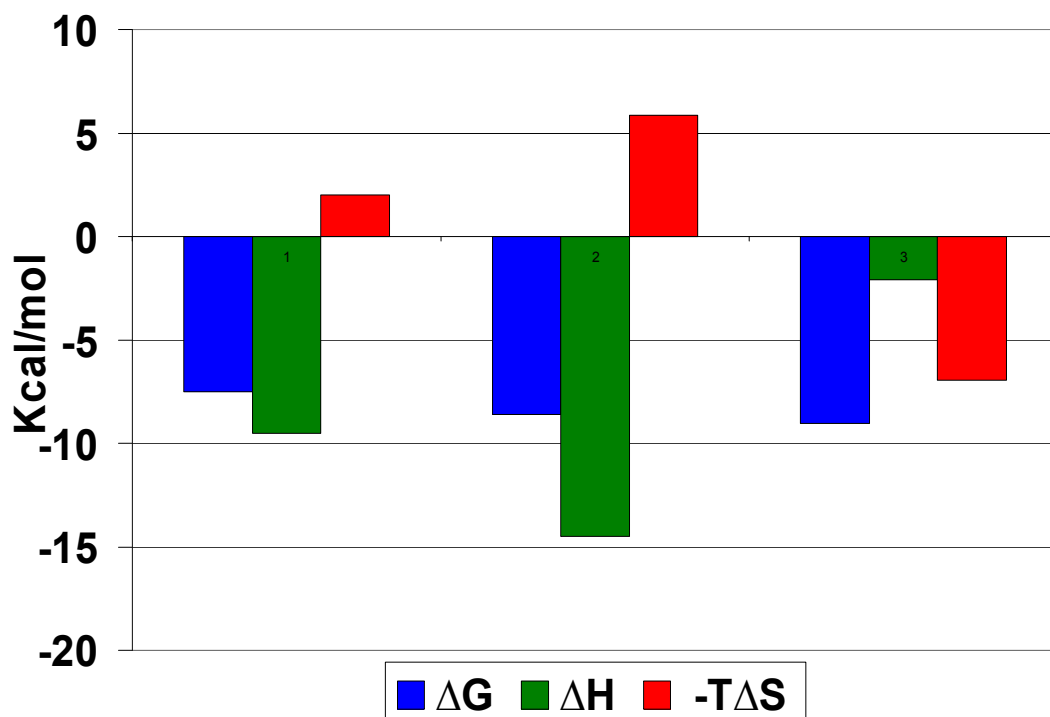
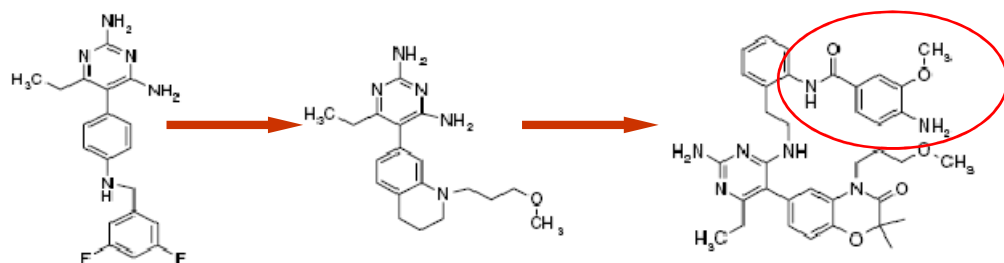
Renin inhibitors ITC study

Modeling suggested addition of ether would extend into S3 pocket



Renin inhibitors ITC study

Modeling suggest adding aryl- benamide to extend into S2 pocket



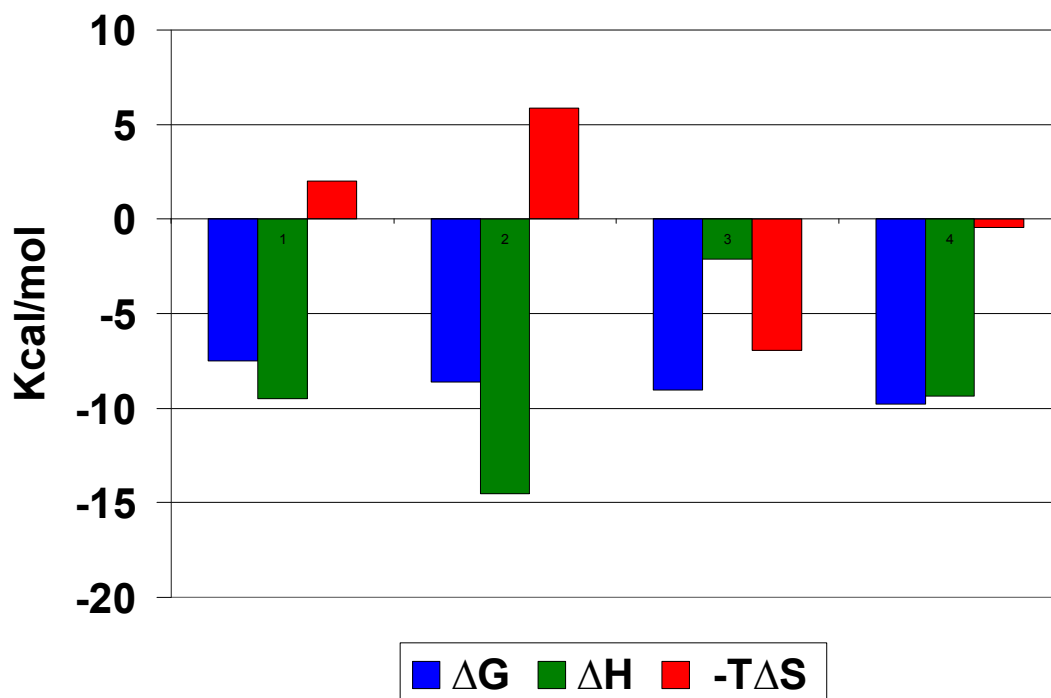
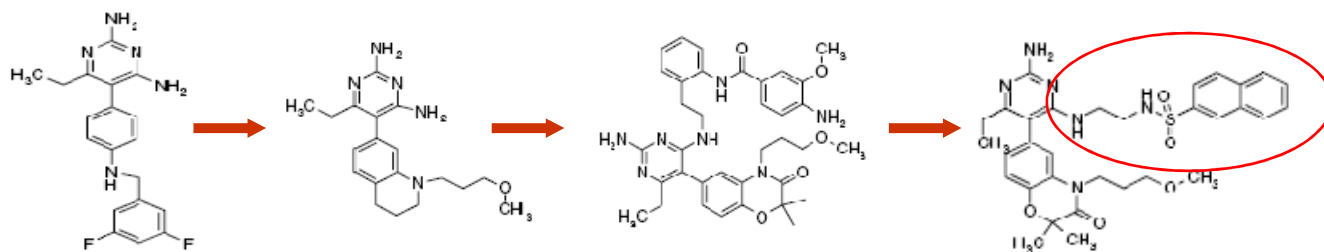
Slight increase in ΔG

Loss in ΔH indicating no significant hydrogen bonds were formed

Significant increase in $-T\Delta S$ due to hydrophobic binding in S2 pocket

Renin inhibitors ITC study

Modeling suggests substituting aryl-benamide with aryl-sulfonamide to improve H-bonds

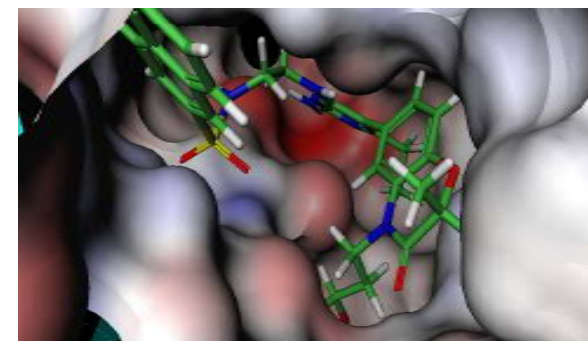
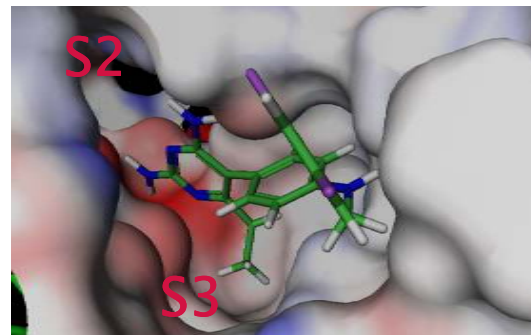
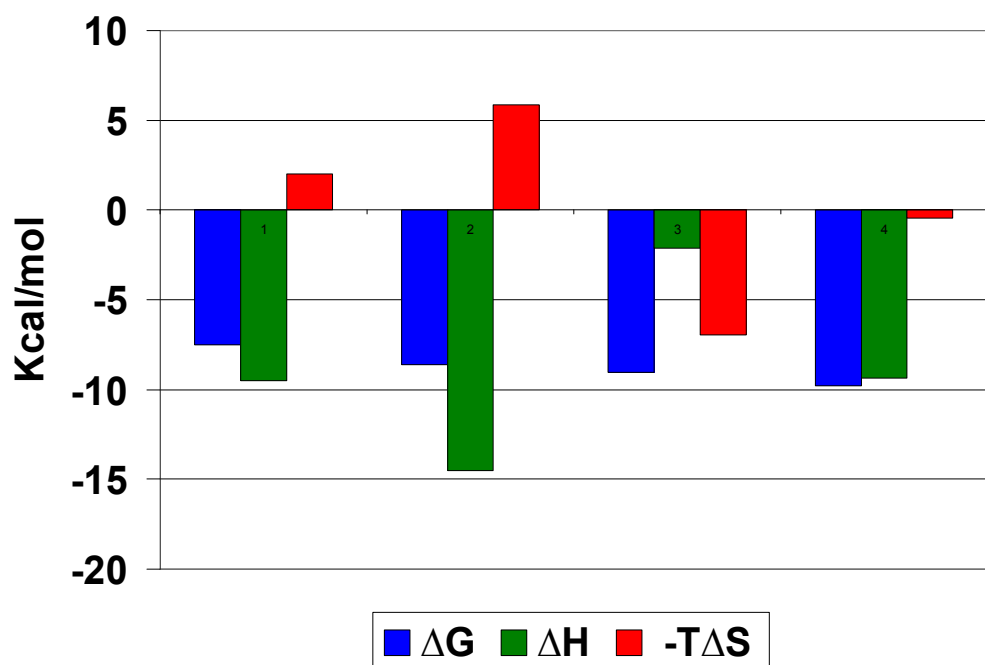


Increase in ΔG

Dramatic increase in ΔH is consistent with increase in S2 pocket H-bonds.

Decrease in $-T\Delta S$ due to conversion of hydrophobic binding in S2 pocket to H-bonds.

Renin inhibitors ITC study



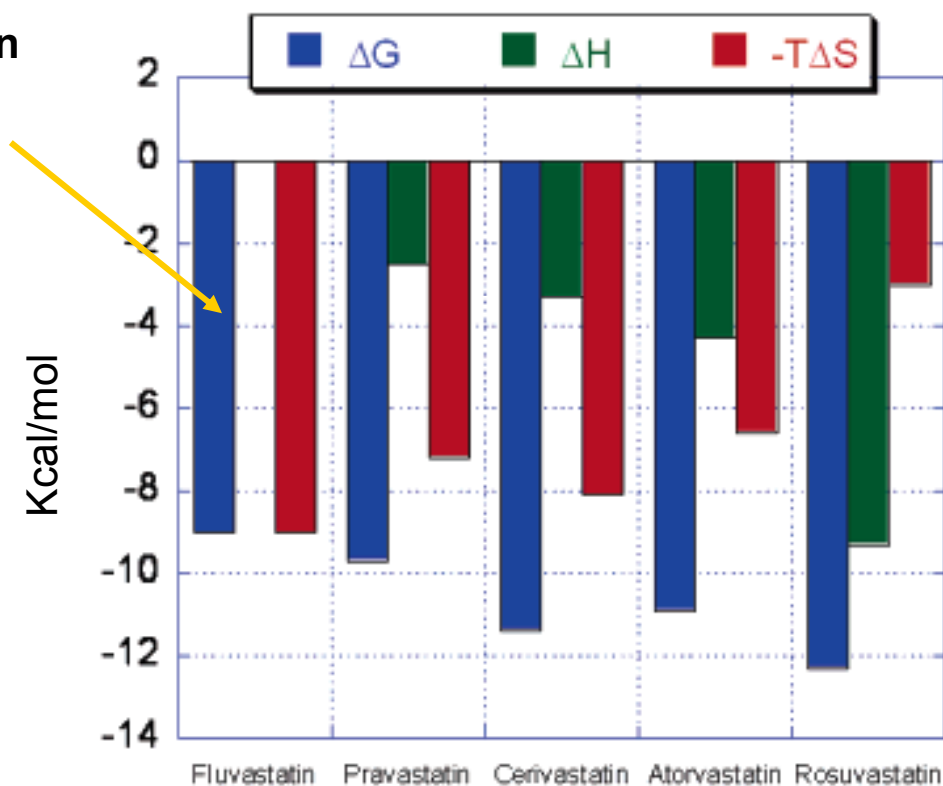
- Affinity improved 45X
- Small molecule, non peptidic renin inhibitor

ITC a step forward?

- Guide through the drug discovery process
- Better selectivity
- Reduce attrition rates in DMPK

Statins: Inhibitors of HMG CoA-Reductase

Clinically proven as least potent statin

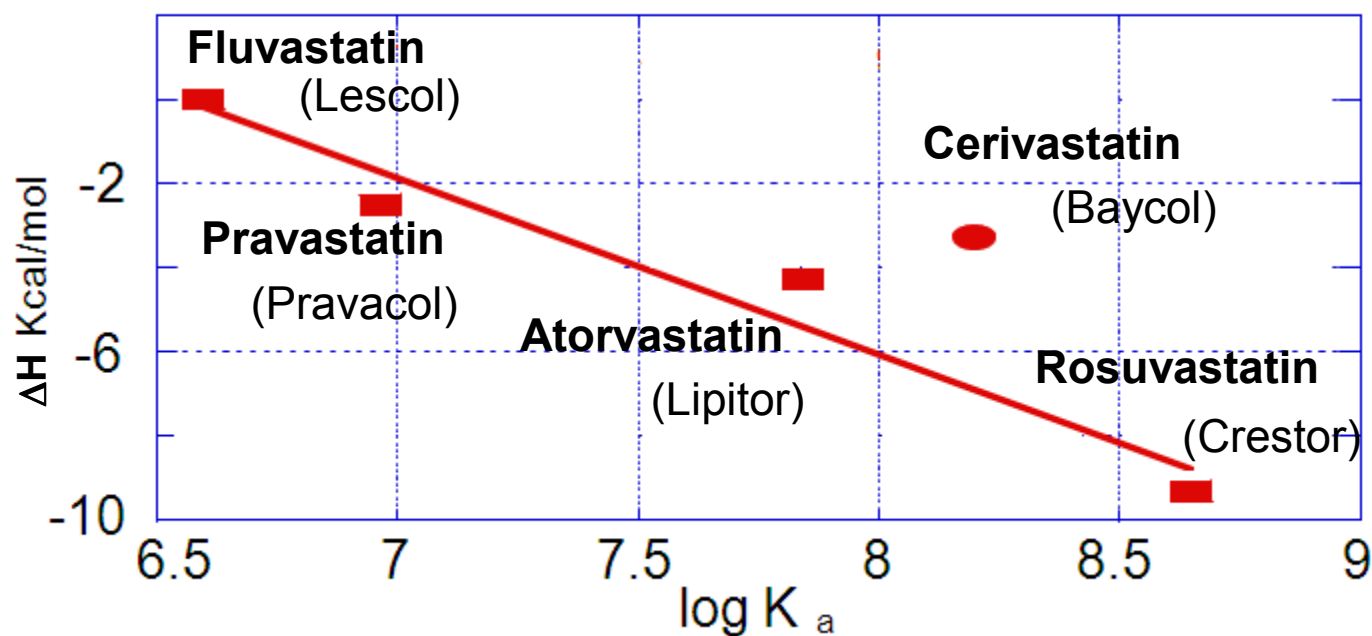


Dramatic increase in ΔH is consistent improved hydrogen and van der Waals bonds

Carbonell and Freire, *Binding thermodynamics of statins to HMG-CoA Reductase*, Biochem. 2005

Statins: Inhibitors of HMG CoA-Reductase

- Affinity increase of 125X
- High affinity correlates with high ΔH



Summary

- Measures heat changes associated with binding which relate to the mechanism of binding
- Reduce the time on structure determination
- Clear SAR that “affinity only” methods may miss
- Assess the potential for a compound to bind selectively
 - Less likely to cause side effects during DMPK studies

Isothermal Titration Calorimeters

VP-ITC



iTC₂₀₀



Auto-iTC₂₀₀



Sample Required: 50+ μg
Throughput: 4 - 5 per day
Ease of Use: Good

~5-10 μg
8 - 16 per day
Better

~5-10 μg
50 - 100/day
Best