Intrinsic Binding Parameters as a Necessity to Correlate Energetics with Structure

Vytautas Petrauskas, Asta Zubrienė, Egidijus Kazlauskas, Lina Baranauskienė, and Daumantas Matulis

Biothermodynamics and Drug Design Department, Vilnius University Institute of Biotechnology, Vilnius, Lithuania

vytautas.petrauskas@bti.vu.lt

Introduction

One of the major obstacles in the structure-based drug design field remains the poor correlation between structure and energy of lead compound binding. In order to draw a reasonable energy and structure correlation one has to determine the intrinsic binding thermodynamic parameters. The intrinsic binding parameters of inhibitor-protein complex usually differ from the observed parameters obtained directly by isothermal titration calorimetry (**ITC**) or other affinity experiments.
ITC is a well-established method for determining the association constant and other thermodynamic parameters (such as equilibrium binding enthalpy, entropy, and the Gibbs free energy) of intermolecular interactions in aqueous solutions. This method is also very powerful for determination of intrinsic binding parameters that could be used in structure-energy correlations. Moreover, the limitations of ITC when determining extremely weak and tight ligands binding can be partially overcome by supplementing the ITC results with data from thermal shift assay (**TSA**). Thus, TSA helps to determine or confirm affinities obtained by ITC.
Here we present the determination of the intrinsic binding parameters for two inhibitor-target protein systems: radicicol–Hsp90 and ethoxzolamide–carbonic anhydrase (CA II). Both proteins are widely used as drug targets and the improved inhibitors are being sought.

ITC and TSA raw data and ligand dosing curves

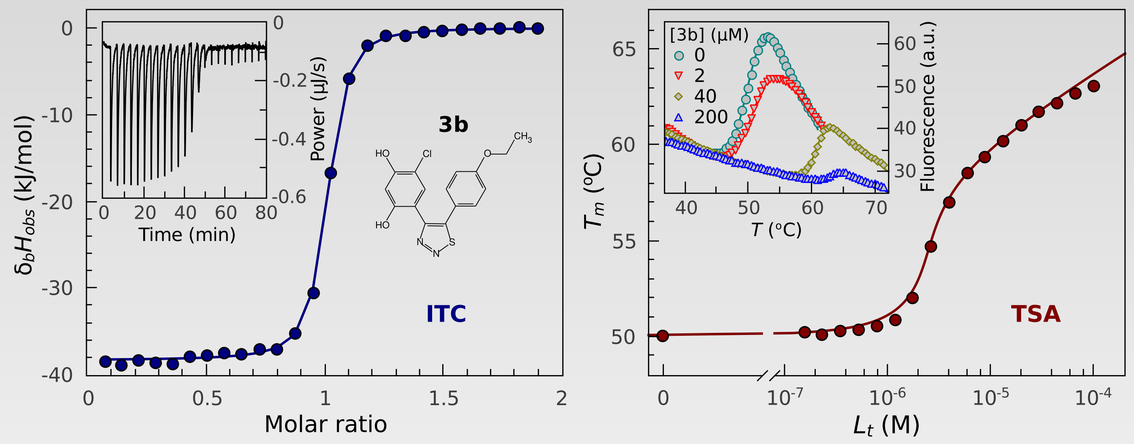

Figure: Ligand dosing curves obtained from ITC (left panel) and TSA (right panel) for lead compound **3b** binding to the N-terminal domain of Hsp90 target protein [1]. The left panel inset shows raw ITC data. The inset in the right panel shows raw TSA data curves obtained at four different ligand concentrations.

Determination of the intrinsic enthalpy

The relationship between observed ($\Delta_b H_{obs}$) and intrinsic ($\Delta_b H_{intr}$) enthalpies is

$$\Delta_b H_{obs} = \Delta_b H_{intr} + n\Delta_b H_{complex} + n\Delta_b H_{buffer},$$

where n is the number of linked protonation events and $\Delta_b H_{complex}$ is the enthalpy of protein and/or ligand binding-linked protonation.
Three steps are necessary to determine the intrinsic binding enthalpy [2]:

- the binding ITC experiment must be conducted in several buffers (left panel);
- if there is difference in observed enthalpies, then it is desirable to repeat ITC experiments in several buffers at multiple pHs (top right panel);
- the numbers of protons transferred are plotted as a function of pH, determining the pK_a of the linked protonation reaction (bottom right panel).

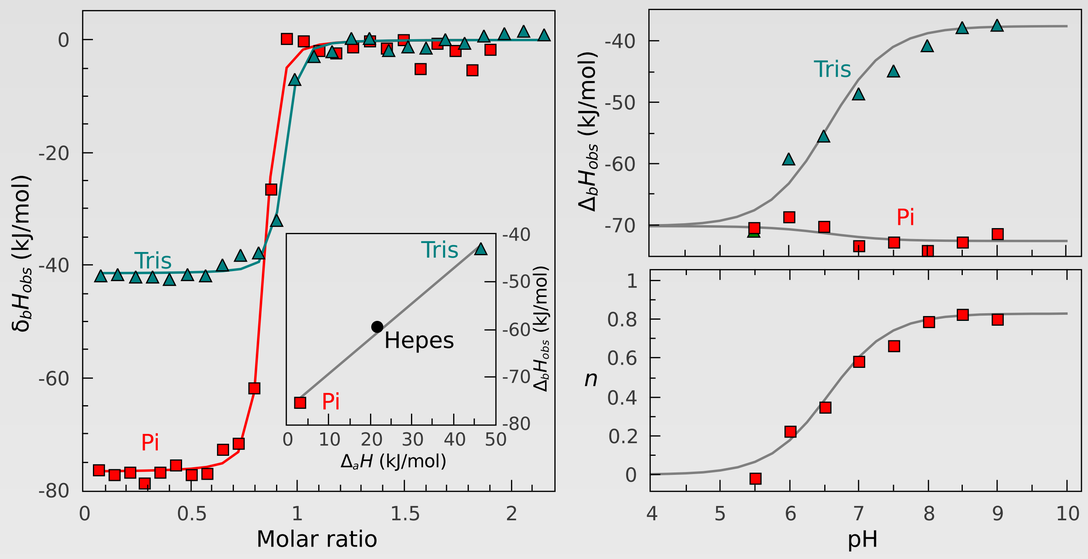

Figure: Determination of the intrinsic binding enthalpy for radicicol–Hsp90 system. [2]

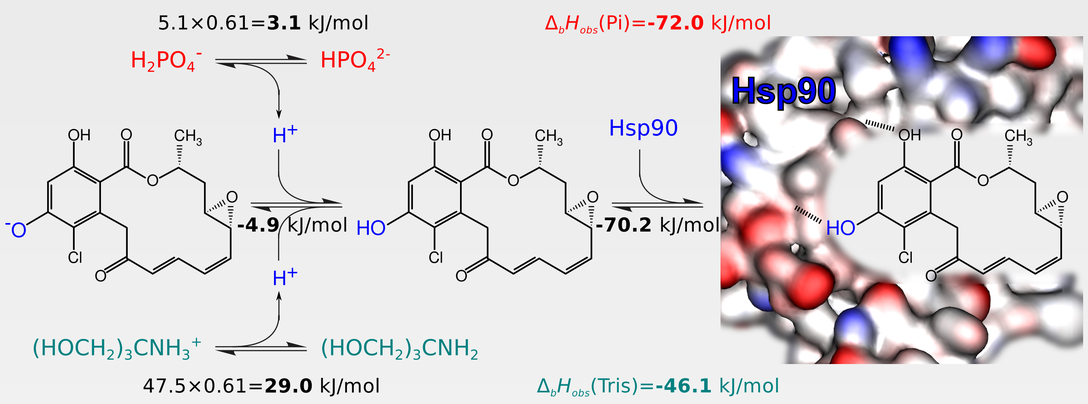

Figure: Quantitative depiction of linked protonation events occurring in aqueous buffer solution upon radicicol binding to Hsp90 at pH = 7.

Hsp90 protein structure

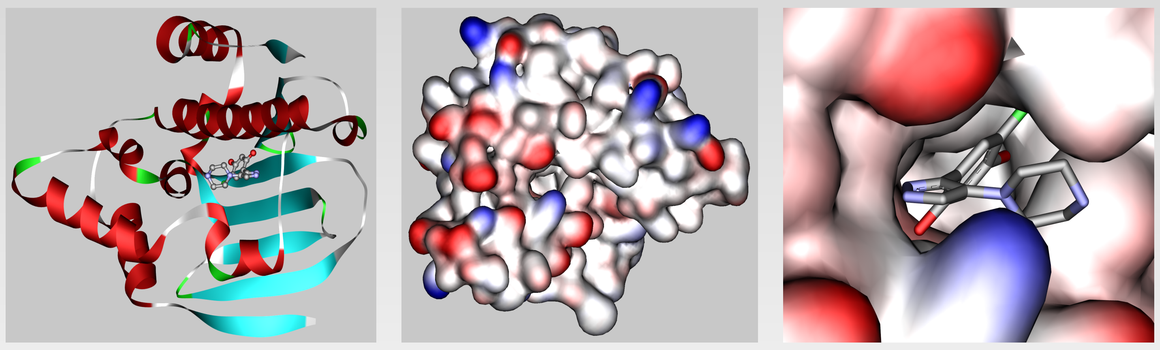

Figure: Hsp90N (236 a.a., PDB ID:2ccs) protein structure (left and middle panels) and active site of Hsp90N with bound inhibitor (right panel).

Determination of the intrinsic dissociation constant

If ligand binding is linked to the binding of a single proton, then the observed dissociation constant (K_{d_obs}) and the intrinsic dissociation constant to the protonated protein form (K_{d_intr}) are related by:

$$K_{d\_obs} = K_{d\_intr}\frac{1 + 10^{\mathrm{pH}-\mathrm{p}K_a^f}}{1 + 10^{\mathrm{pH}-\mathrm{p}K_a^b}},$$

where K_a^b and K_a^f are the proton dissociation constants from the liganded and unliganded protein, respectively.

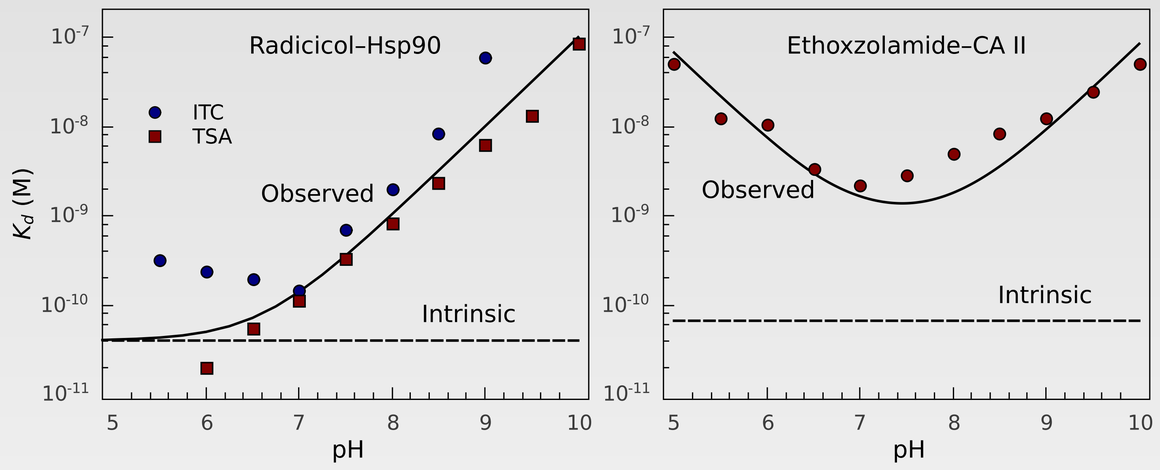

Figure: Observed and intrinsic K_ds are shown as a function of pH for radicicol–Hsp90 (left panel) and ethoxzolamide–carbonic anhydrase (right panel) systems. Experimental datapoints obtained by ITC and TSA are compared in left panel. Below pH = 7 the ITC reached its maximum K_d determination capability. TSA measurements at low pH are more accurate than ITC. [2–4]

Comparison of binding and inhibition constants

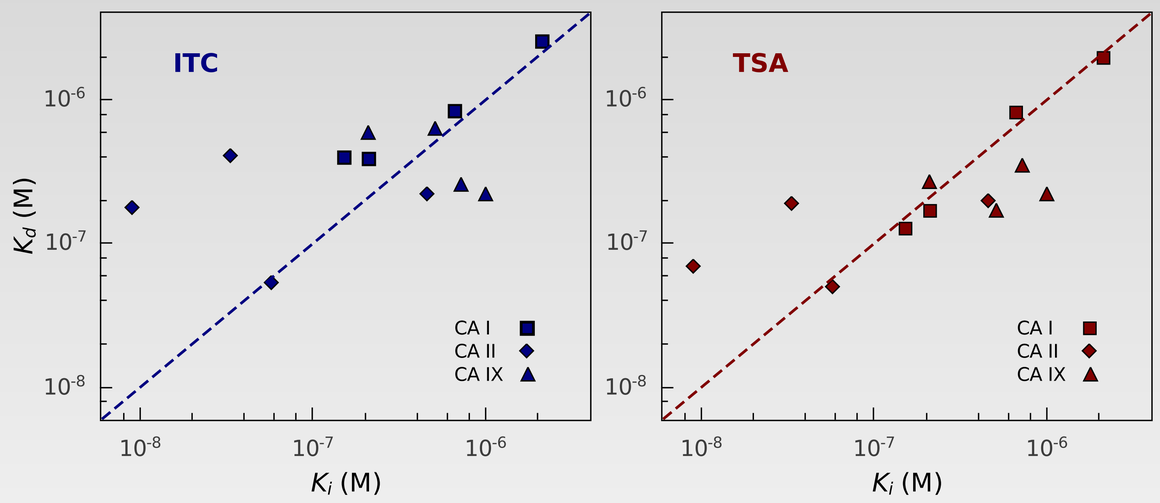

Figure: Comparison of the enzymatic activity inhibition constant K_i with K_ds determined from ITC (left panel) and TSA (right panel) data. [5]

References

[1] Cikotiene, I.; Kazlauskas, E.; Matuliene, J.; Michailoviene, V.; Torresan, J.; Jachno, J.; Matulis, D. *Bioorg Med Chem Lett* **2009**, *19*, 1089–1092.

[2] Zubriene, A.; Gutkowska, M.; Matuliene, J.; Chaleckis, R.; Michailoviene, V.; Voroncova, A.; Venclovas, C.; Zylicz, A.; Zylicz, M.; Matulis, D. *Biophys Chem* **2010**, *152*, 153–163.

[3] Krishnamurthy, V. M.; Kaufman, G. K.; Urbach, A. R.; Gitlin, I.; Gudiksen, K. L.; Weibel, D. B.; Whitesides, G. M. *Chem Rev* **2008**, *108*, 946–1051.

[4] Matulis, D.; Todd, M. J. In *Biocalorimetry 2*; Ladbury, J. E., Doyle, M. L., Eds.; Wiley, 2004; pp 107–132.

[5] Baranauskiene, L.; Hilvo, M.; Matuliene, J.; Golovenko, D.; Manakova, E.; Dudutiene, V.; Michailoviene, V.; Torresan, J.; Jachno, J.; Parkkila, S.; Maresca, A.; Supuran, C. T.; Grazulis, S.; Matulis, D. *J Enzyme Inhib Med Chem* **2010**, *25*, 863–870.

Acknowledgements

EU Structural Funds project “Postdoctoral Fellowship Implementation in Lithuania”.
Grant (No. LIG-16/2010) from the Research Council of Lithuania.