Calorimetry (ITC/Thermodynamics and drug design)

Lecture 7
MEDCH 528
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Molecular Analysis Facility/Analytical Biopharmacy Core

Isothermal Titration Calorimetry

$$Q_i = n \cdot M_t \cdot \Delta H_B \cdot V_0 \cdot \Theta$$

Ligand/macromolecule in the cell - titrant/analyte in the syringe

Macromolecule (protein, mAb, liposome, etc) in the calorimetric cell is titrated with a binding partner at constant temperature.

Protein Macromolecule (Ligand) In the cell

Small molecule Protein (Analyte) In the syringe

ITC monitors:

- Changes in conformational states
- Polar and non-polar interactions in the active site
- Proton transfer upon binding
- Changes in hydration and hydrogen bonding

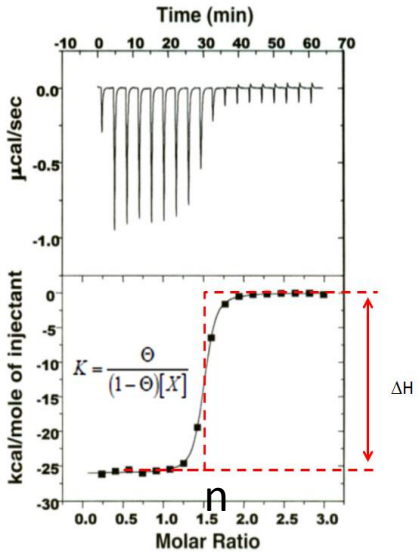
Characterizing the type of binding = Primary objective of an ITC experiment

Ligand/macromolecule in the cell
Titrant/analyte in the syringe

Macromolecule (protein, mAb, liposome, etc) in the calorimetric cell is titrated with a binding partner at constant temperature.

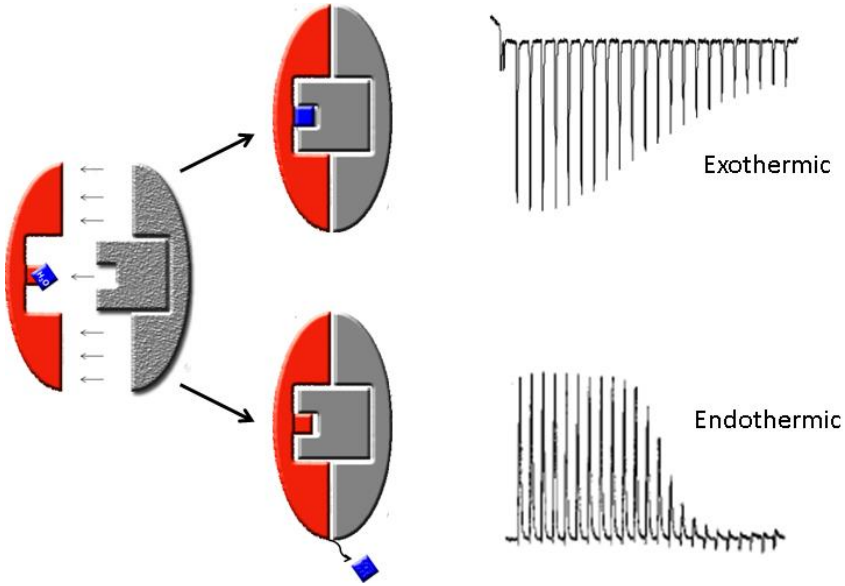
Pure sample – SEC, Ion exchange, mixed phase, GPC.
Good sample preparation:

- Dialysis of ligand and analyte in the same buffer
- Accurate concentration measurement
  - Spectroscopic – Abs at 280nm
  - Refractometry
  - Least accurate are colorimetric assays.

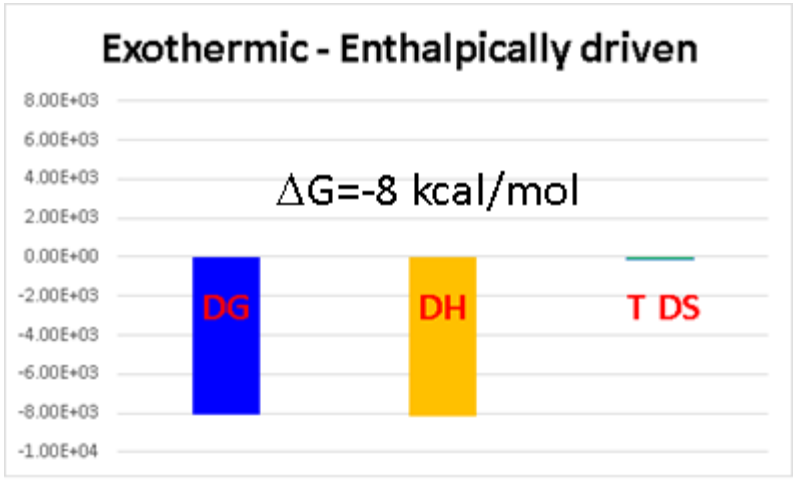


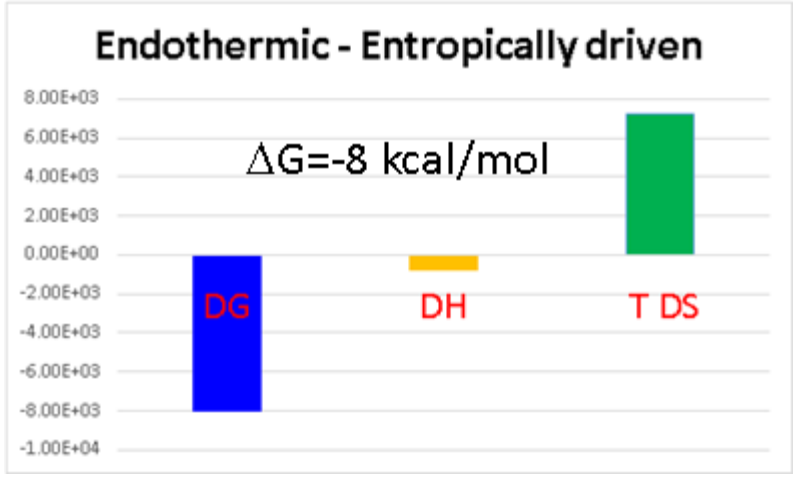
Characterizing the type of binding

$$\Delta G = \Delta H - T\,\Delta S$$

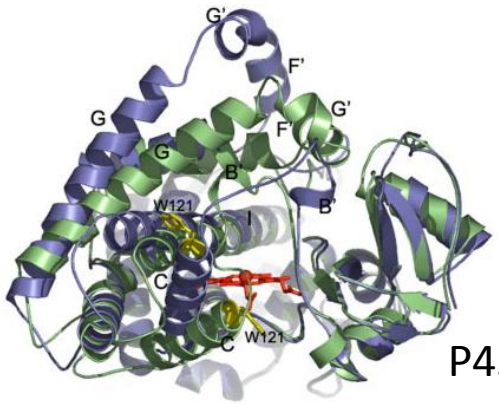


$$\Delta C_p \gg \Delta S$$
$$\Delta C_p \neq 0$$

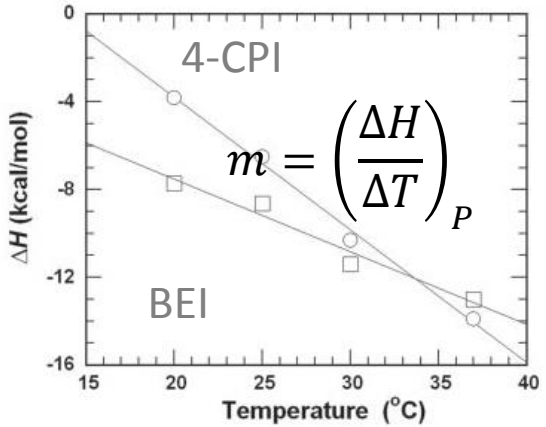




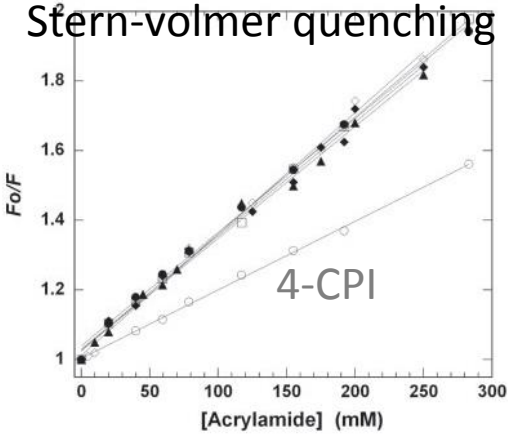
Entropy-enthalpy compensation



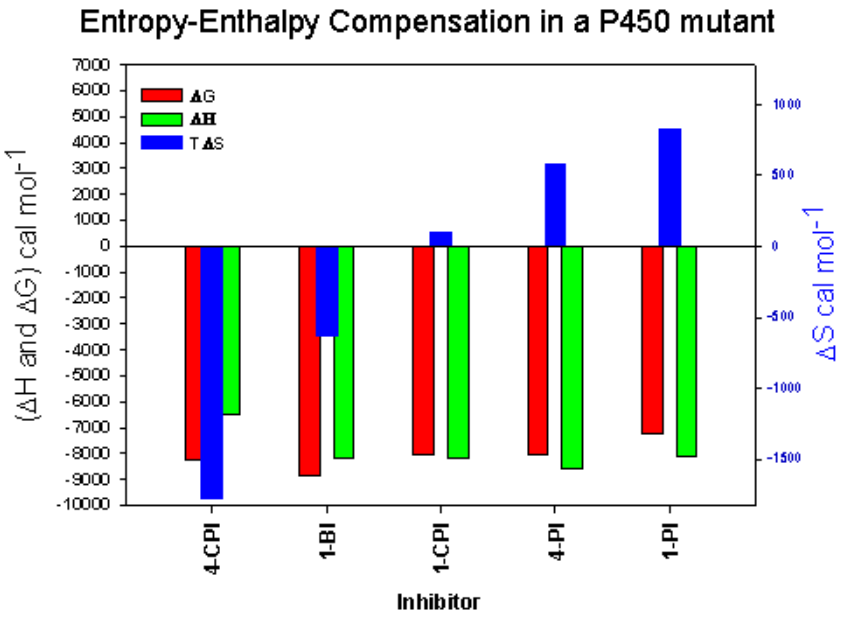
P450 2B4



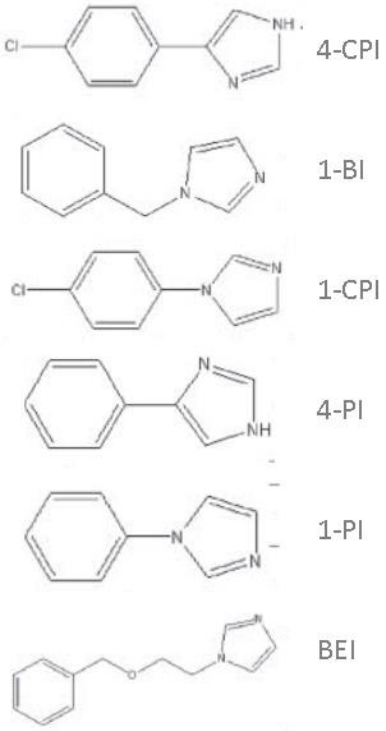
Stern-volmer quenching



$65nM = K_D^{1\text{-BI}} < K_D^{4\text{-CPI}} < K_D^{1\text{-CPI}} < K_D^{4\text{-PI}} < K_D^{4\text{-PI}} < K_D^{BEI} < K_D^{1\text{-PI}} = 486nM$

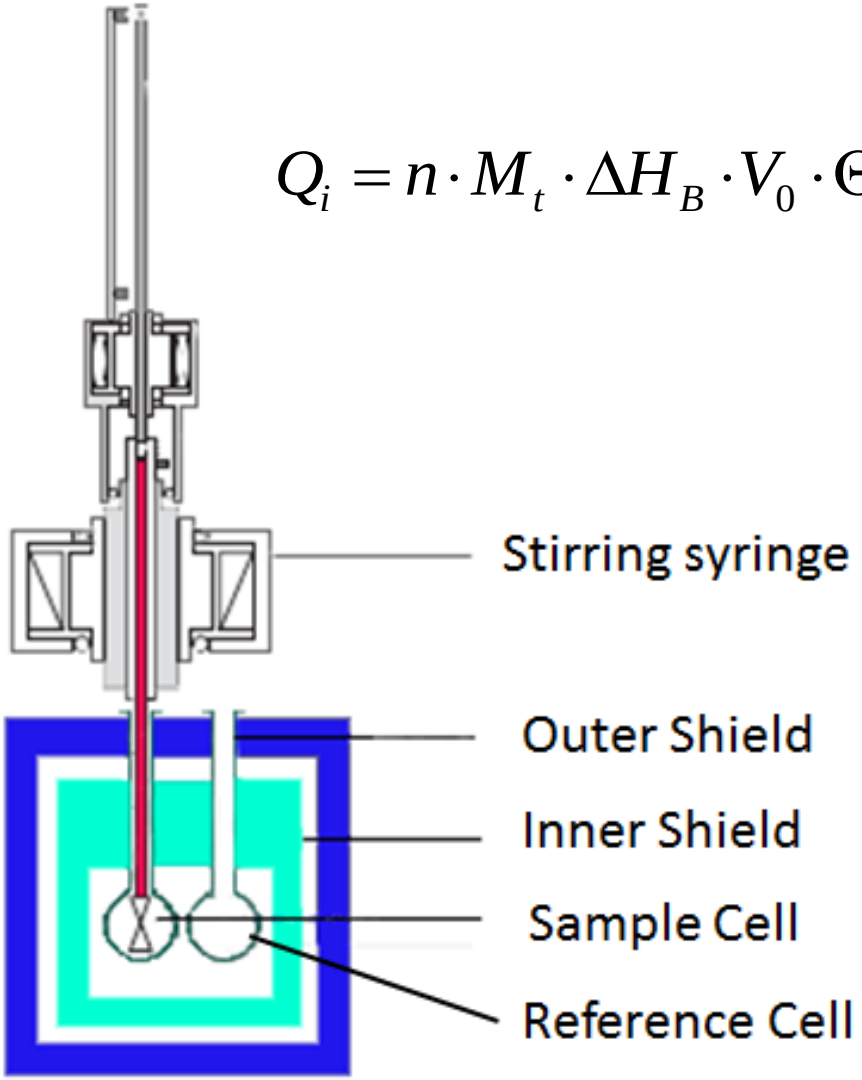


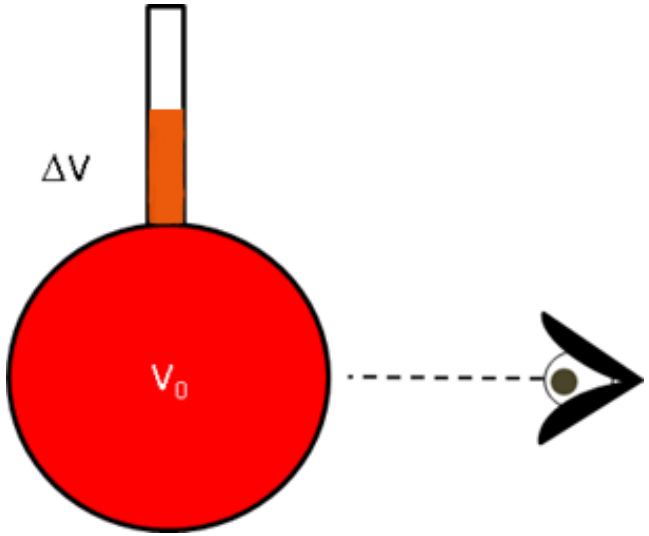
Muralidhara et. al. JBC 2006



The instrument

$$Q_i = n \cdot M_t \cdot \Delta H_B \cdot V_0 \cdot \Theta$$





$$M_t^{\,0} \cdot V^0 = M_t V^0 + \frac{1}{2} \cdot \left[M_t + M_t^{\,0} \right] \cdot \Delta V$$

$$X_t^{\,0} \cdot V^0 = X_t V^0 + \frac{1}{2} \cdot \left[X_t \right] \cdot \Delta V$$

$$Q_{(i-1)} \cdot V^0 = Q_i \cdot V^0 + \frac{1}{2} \cdot \left[Q_i + Q_{(i-1)} \right] \cdot \Delta V$$

$M_t^{\,0}$ = Initial Ligand concentration (macromolecule)

V^0 = Working Volume

Q_i = heat of binding after the i^{th} injection

Θ = is the fraction of binding sites occupied by analyte

The Analysis

$$Q_i = n \cdot M_t \cdot \Delta H_B \cdot V_0 \cdot \Theta$$

$M_t^{\,0}$ = Initial Ligand concentration (macromolecule)

V^0 = Working Volume

Q_i = heat of binding after the i^{th} injection

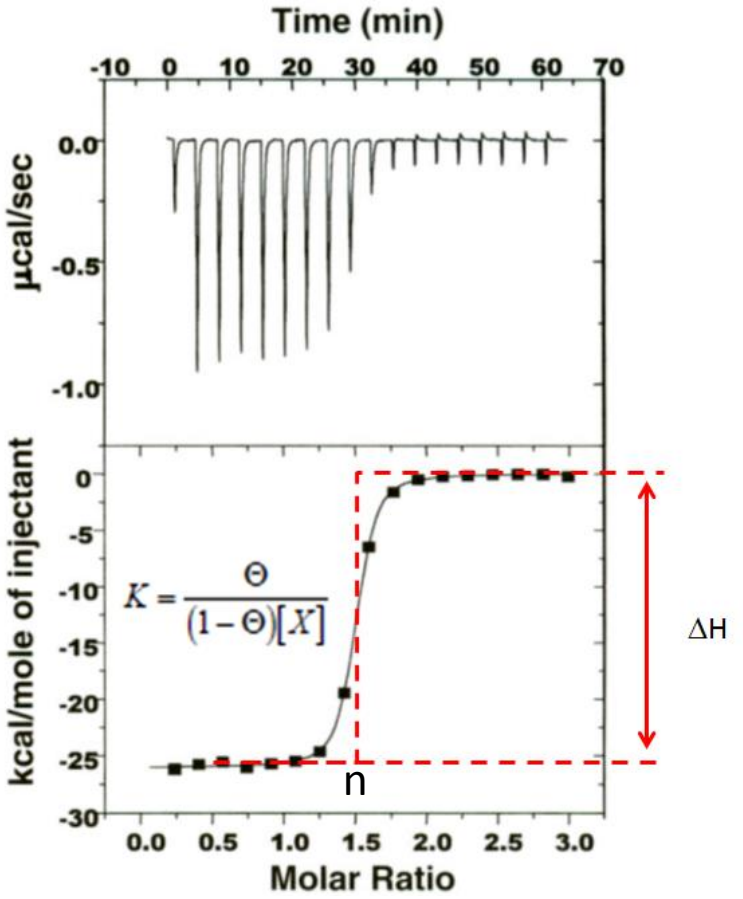
Θ = is the fraction of binding sites occupied by analyte

$$M_t = M_t^{\,0}\left[\frac{1-\frac{\Delta V}{2\cdot V_0}}{1+\frac{\Delta V}{2\cdot V_0}}\right]$$

$$X_t = X_{free} + n\cdot\Theta\cdot M_t$$

$$K_a = \frac{\Theta}{(1-\Theta)\cdot X_{free}}$$

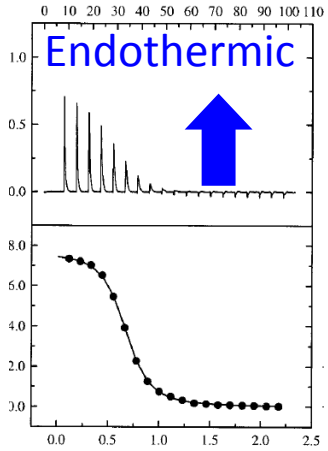
$$X_t = X_t^{\,0}\left[1-\frac{\Delta V}{2\cdot V_0}\right]$$



$$0 = \Theta^2 + \Theta\cdot\left[1+\frac{X_t}{n\cdot M_t}+\frac{1}{n\cdot K_a\cdot M_t}\right]+\frac{X_t}{n\cdot M_t}$$

Quadratic equation is solved for Θ

High Affinity interaction by competition assay



Low affinity interaction

P

B

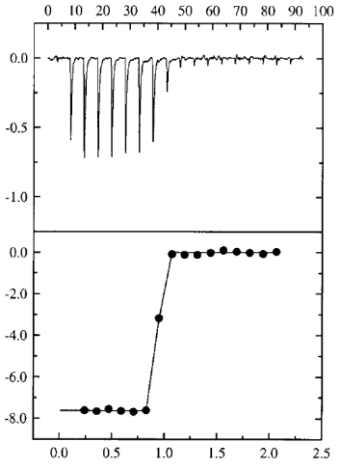
$$K_b = \frac{[PB]}{[P]\ [B]}$$

PB

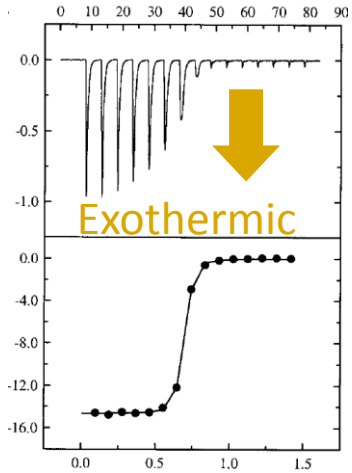
A

PA

$$K_a = \frac{[PA]}{[P]\ [A]}$$



$$K_{app} = \frac{K_a}{1 + [B]\ K_b}$$



Displacement of B with A.

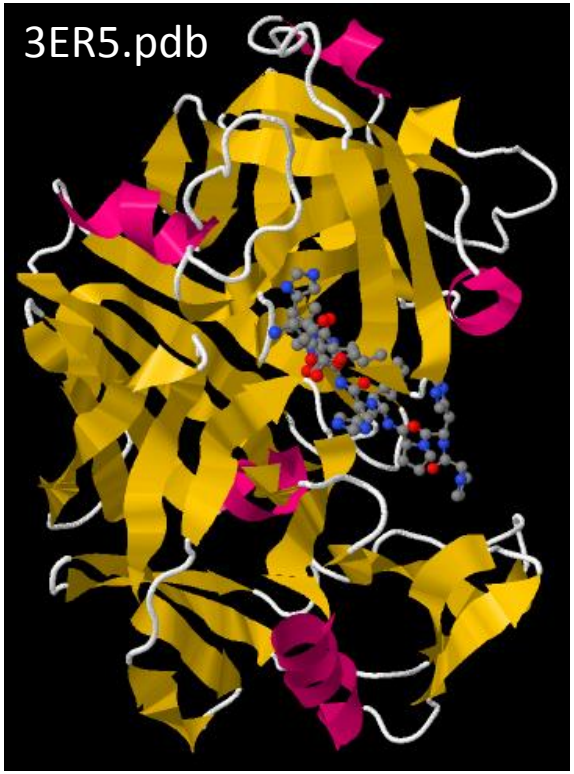
$$\Delta H_{app} = (\Delta H_a - \Delta H_b)\frac{[B]\ K_b}{1 + [B]\ K_b}$$

Sigurskjold, et. al. Analytical Biochem. 2000, vol. 277, pp.260-266

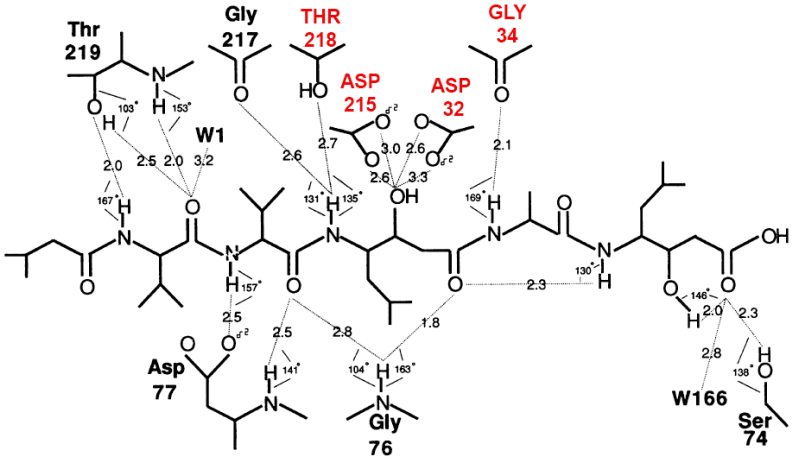
Wang, et. al. FEBS Lett. 1995, vol.360, pp.111-114

Study of Aspartyl protease

MW=35086.76



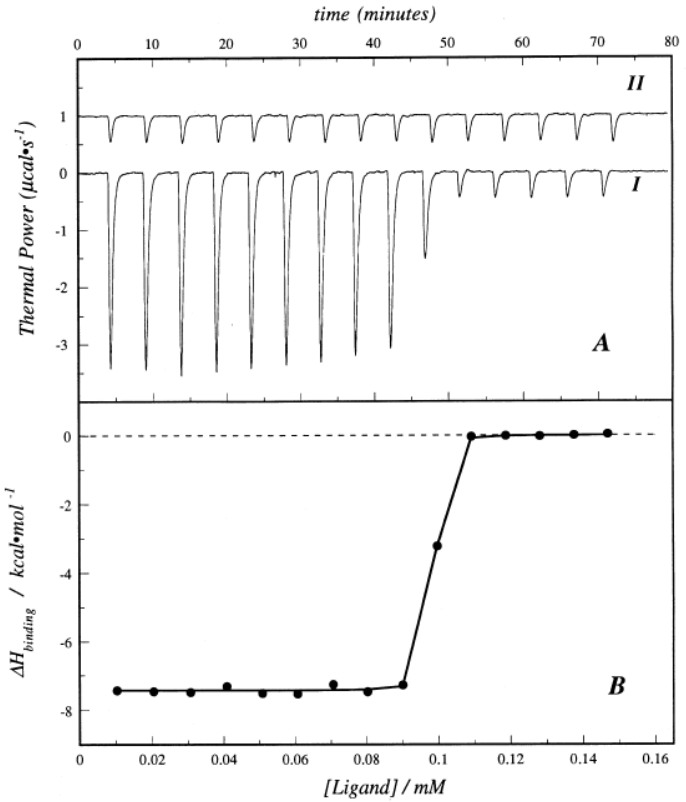
Bailey et. al. Biochem J. 1993 289, 363-71.



pH = 4.6

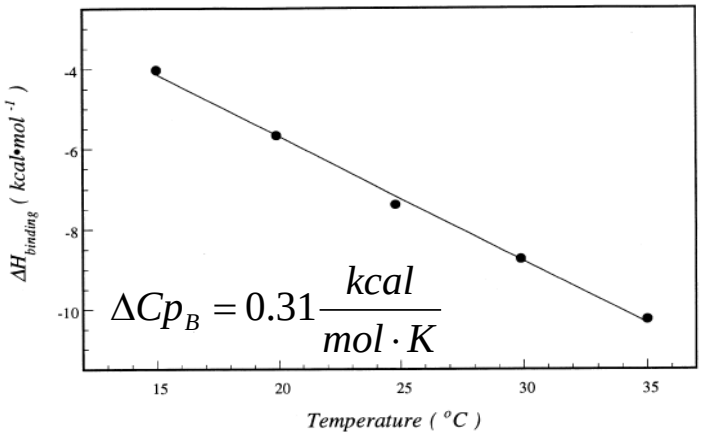
T = 25°C

ΔH_B = -7.38 kcal/mol

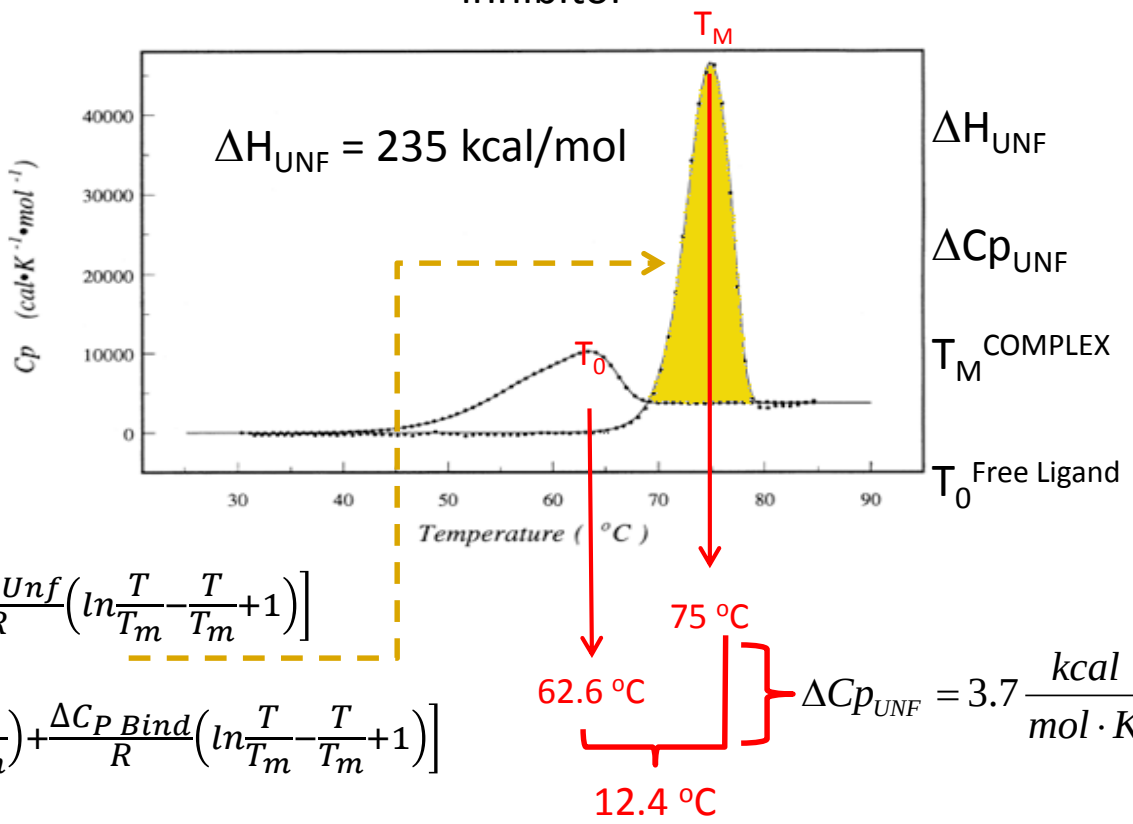


ITC/DSC for Ultra-tight binding

Temperature dependence of ΔH_B



DSC endotherms for Endothiapepsin ± inhibitor



$$K_{Bind}(T_m) = e^{-\left[\frac{\Delta H(T_0)Unf}{R}\left(\frac{1}{T}-\frac{1}{T_m}\right)+\frac{\Delta C_{P\,Unf}}{R}\left(ln\frac{T}{T_m}-\frac{T}{T_m}+1\right)\right]}$$

$$K_{Bind}(T) = K_{Bind}(T_m)e^{-\left[\frac{\Delta H_{Bind}}{R}\left(\frac{1}{T}-\frac{1}{T_m}\right)+\frac{\Delta C_{P\,Bind}}{R}\left(ln\frac{T}{T_m}-\frac{T}{T_m}+1\right)\right]}$$

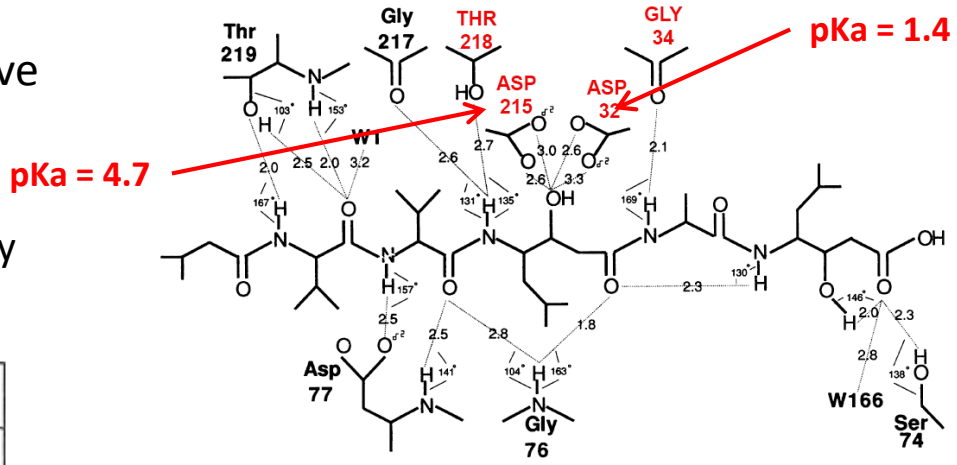
$$K_{Bind}(27^oC) = 51\text{pM}$$

Ligand stabilization of the thermal transition

Gomez, Javier; Freire, Ernesto; "Thermodynamic Mapping of the Inhibitor Site of the Aspartic Protease Endothiapepsin", J. Mol. Bio. , 1995, vol.252, pp337-350

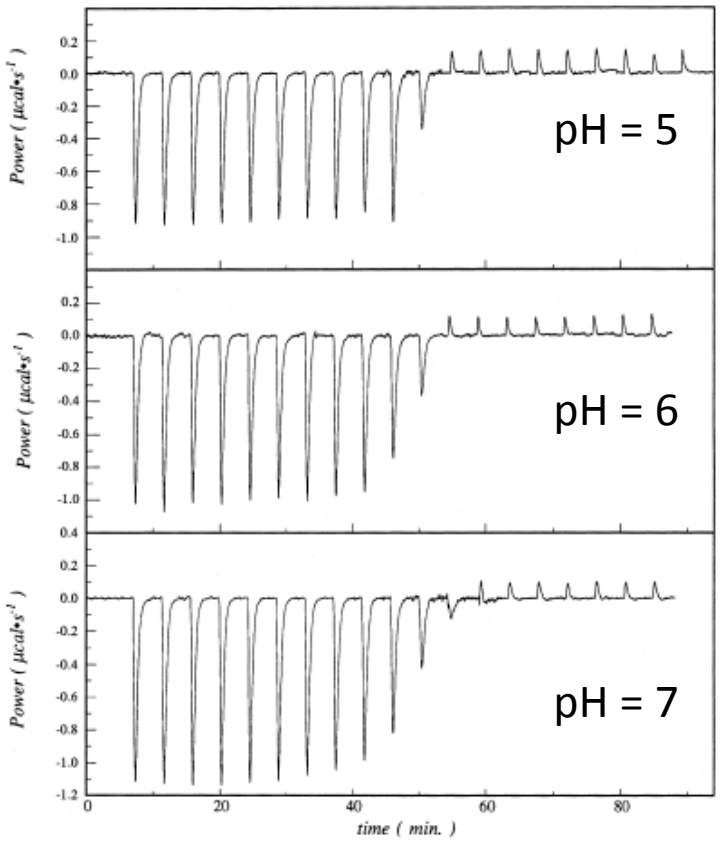
Brandts, John F; Lin, Lung-Nan, "Study of Strong to Ultratight Protein Interactions Using Differential Scanning Calorimetry", Biochemistry, 1990, vol.29, pp6927-6940

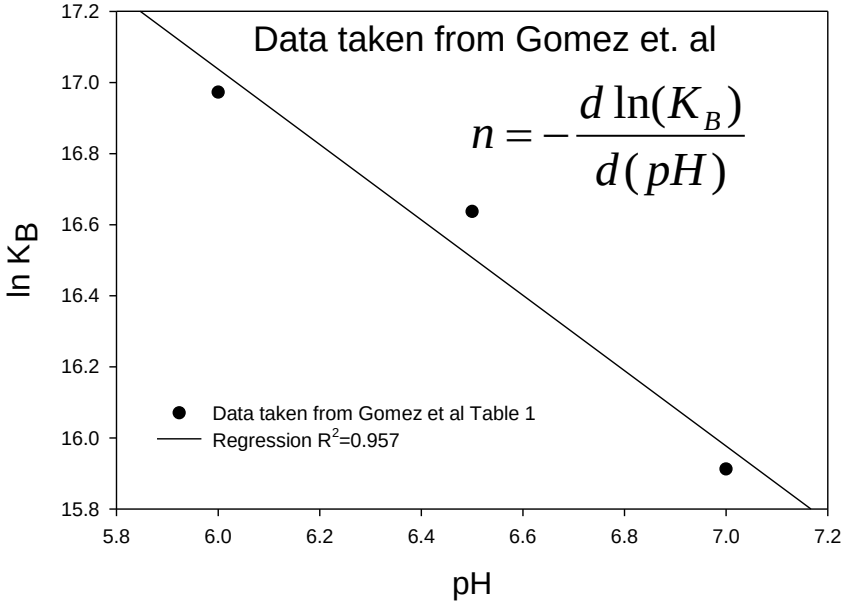
Measuring proton exchange at the active site



Tang, JBC, vol.246 1971 pp. 4510-4517
Fruton, Bergmann, Science vol.87 1938 p.557

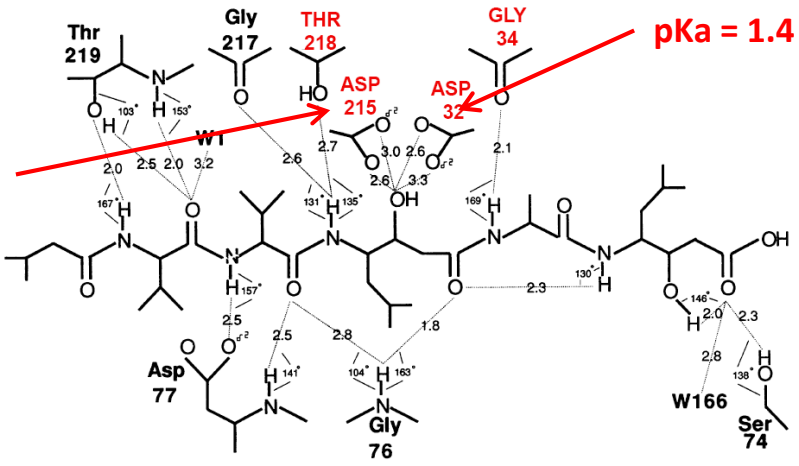
Increasing the pH reduces the affinity for peptatin A



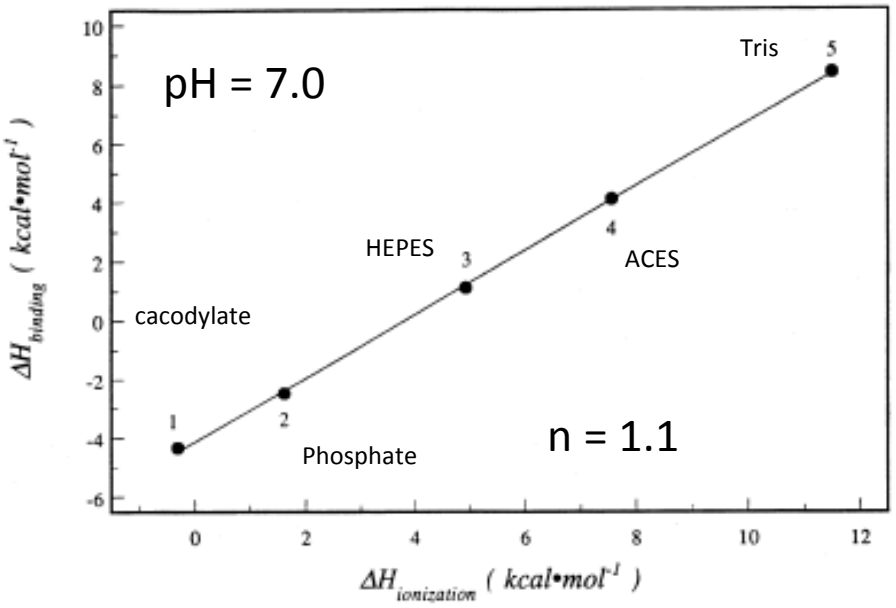


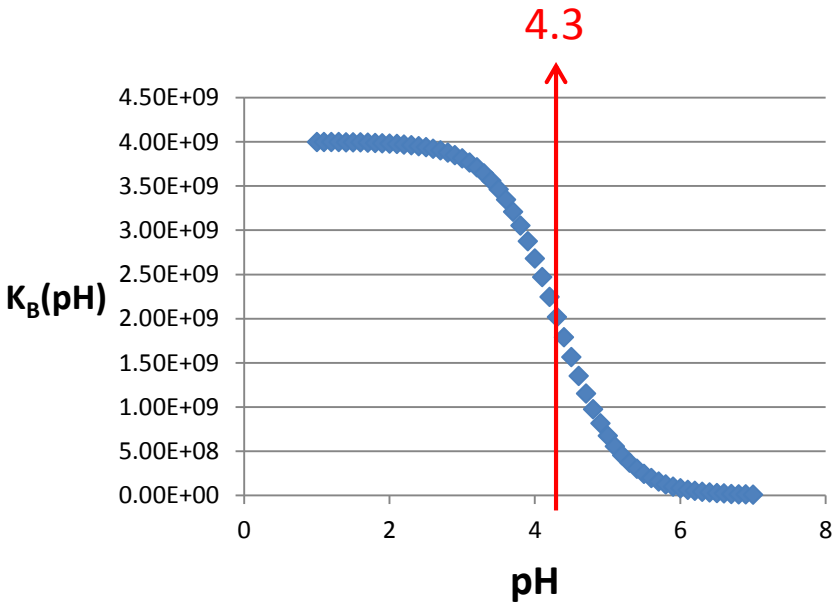
Gomez, Javier; Freire, Ernesto; "Thermodynamic Mapping of the Inhibitor Site of the Aspartic Protease Endothiapepsin", J. Mol. Bio. , 1995, vol.252, pp337-350

Characterizing the protonation state at the active site



Enthalpy of binding as a function of the ionization enthalpy



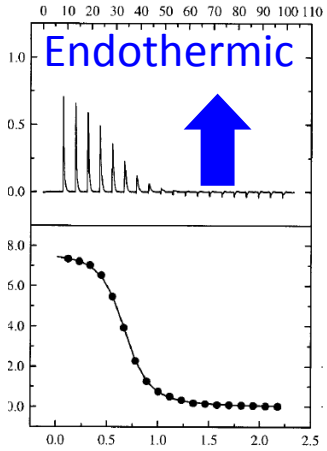


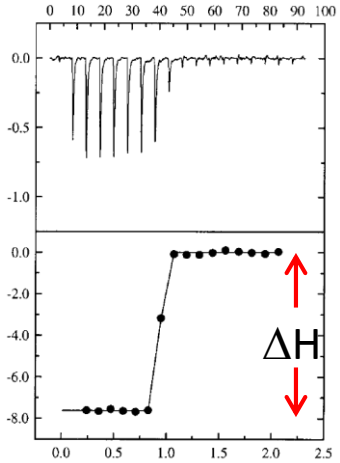
$$\Delta H_{Binding} = \Delta H_{rxn} + n\Delta H_{Ionization}$$

$$K_B(pH) = K_B(initial) \cdot \frac{10^{(pKa-pH)}}{1+10^{(pKa-pH)}}$$

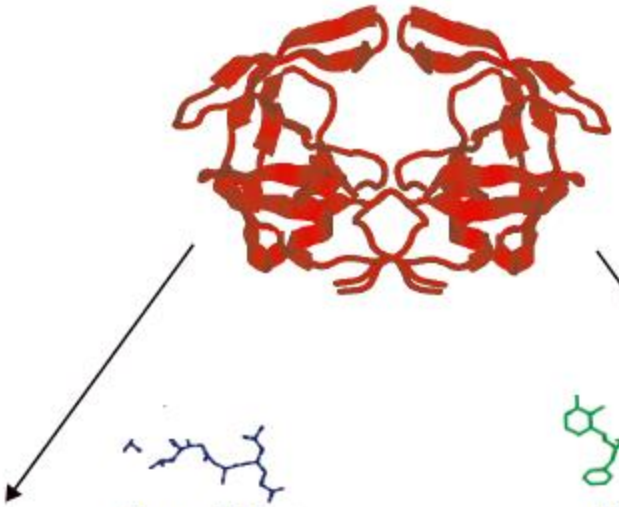
Gomez, Javier; Freire, Ernesto; JMB. , 1995, vol.252, pp337-350
Marciniszyn, J. Jr., Hartsuck JA, Tang JJN, JBC 1976 vol.251, pp7088-7094

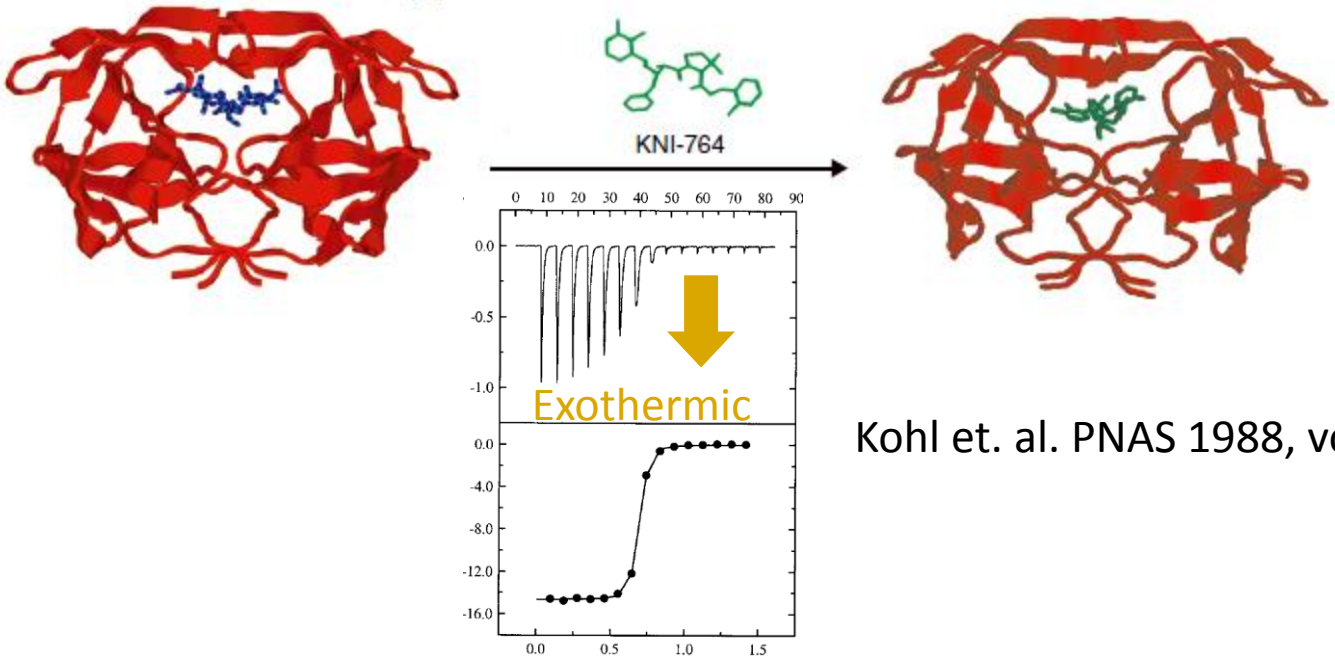
Displacement isothermal titration calorimetry of HIV-1 protease inhibitors affinity





$\Delta G = -RT\ln K_a$

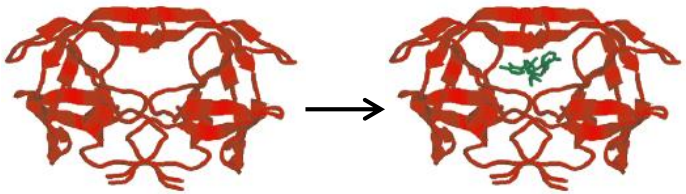




Kohl et. al. PNAS 1988, vol.88, p.4686

Exothermic versus Endothermic Binding: polar vs non-polar interactions

Glu-Asp-Leu +



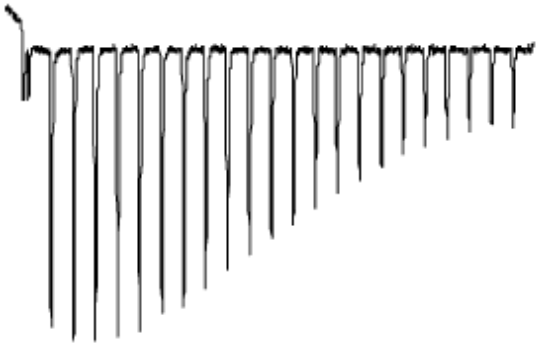
$\Delta G = -6.0$ kcal/mol

$\Delta C_P = -60$ cal/mol-K

Polar interactions in the binding site

$\Delta G_B = \Delta H_B - T\,\Delta S_B$

$$-R \cdot T \ln(K_B) = \Delta G_B$$

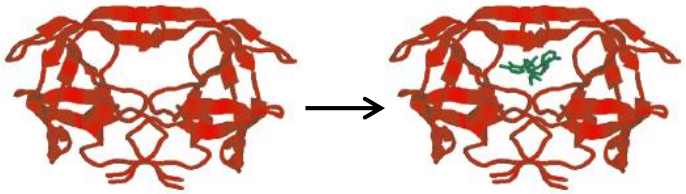


$\Delta H = -3.6$ kcal/mol

Non Polar : Polar

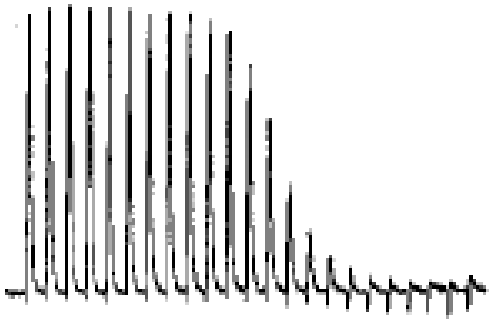
400 Å^2 : 380 Å^2

Acetyl-Pepstatin +



$\Delta G = -8$ kcal/mol

$\Delta C_P = -452$ cal/mol-K



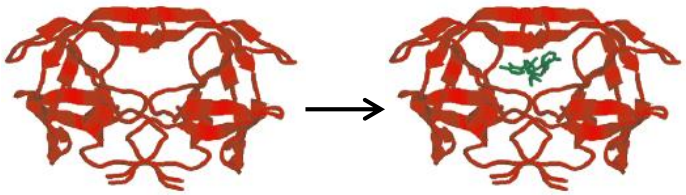
$\Delta H = 7.0$ kcal/mol

Non Polar : Polar

854 Å^2 : 450 Å^2

Exothermic versus Endothermic Binding: solvation and conformational entropy

Glu-Asp-Leu +



ΔG = -6.0 kcal/mol

ΔC_P = -60 cal/mol-K

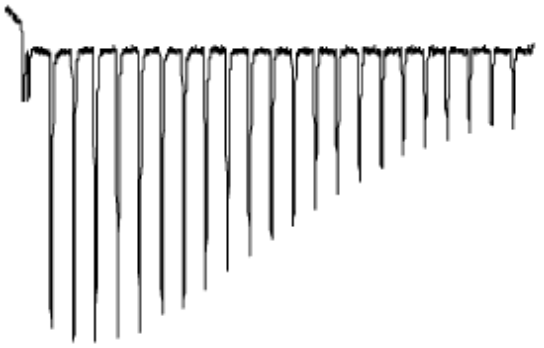
ΔS = 9.0 cal/mol-K

Polar interactions in the binding site

$$\Delta G_B = \Delta H_B - T\,\Delta S_B$$

Solvation

Conformational



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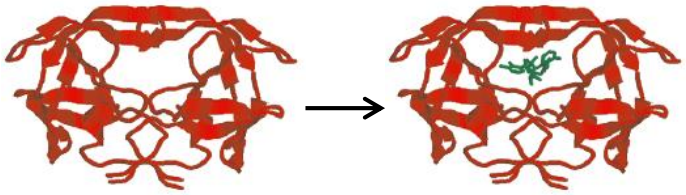
Non Polar : Polar

400 Å^2 : 380 Å^2

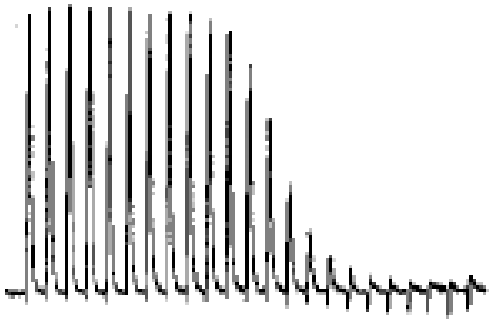
Acetyl-Pepstatin +



ΔG = -8 kcal/mol

ΔC_P = -452 cal/mol-K

ΔS = 50.0 cal/mol-K



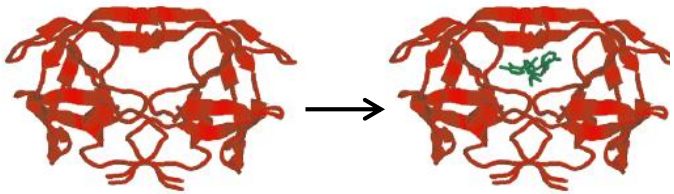
ΔH = 7.0 kcal/mol

Non Polar : Polar

854 Å^2 : 450 Å^2

Exothermic versus Endothermic: temperature dependence of binding

Glu-Asp-Leu +

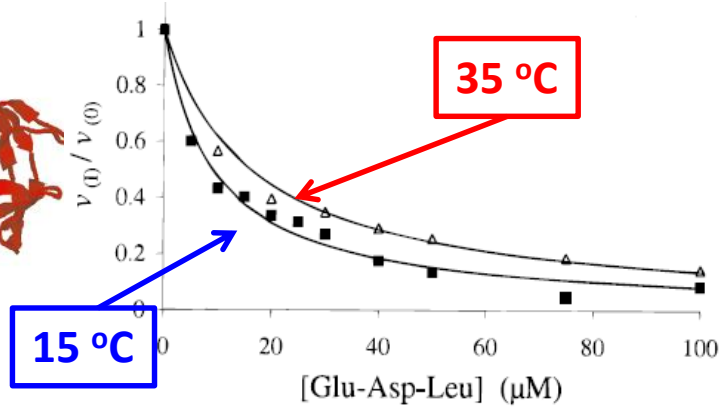


ΔG = -6.0 kcal/mol

ΔC_P = -60 cal/mol-K

ΔS = 9.0 cal/mol-K

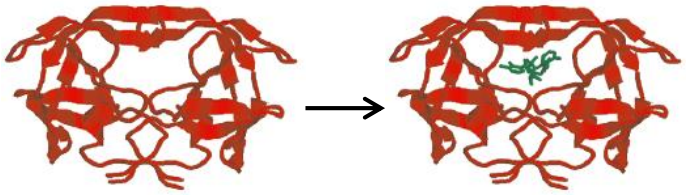
$\Delta G = \Delta H - T\,\Delta S$



ΔH = -3.6 kcal/mol

Exothermic: affinity decreases with increasing temperature

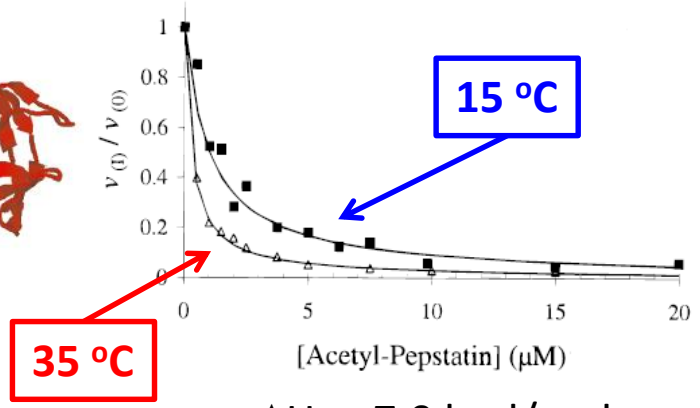
Acetyl-Pepstatin +



ΔG = -8 kcal/mol

ΔC_P = -452 cal/mol-K

ΔS = 50.0 cal/mol-K

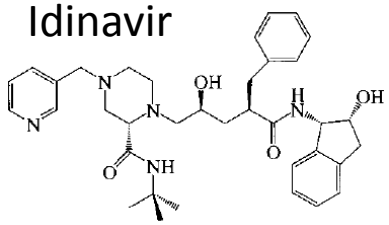


ΔH = 7.0 kcal/mol

Endothermic: affinity increases with increasing temperature

Binding of NNRTs to WT HIV-1 protease

Idinavir



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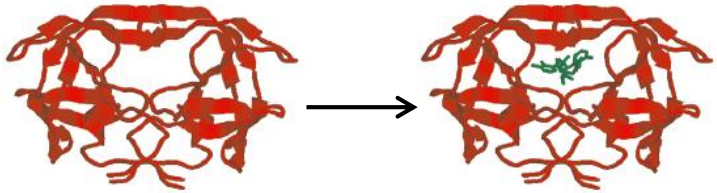


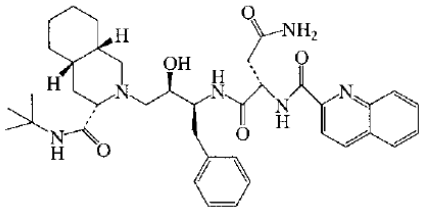
ΔH = 3.9 kcal/mol K_i = 2.0 nM

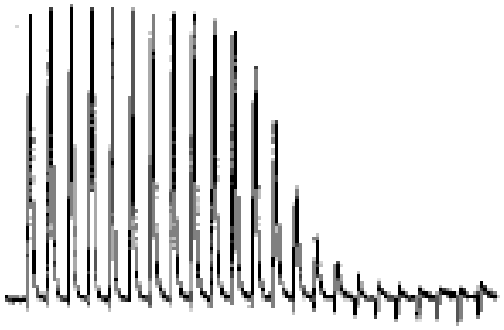
$-T\Delta S$ = -15.7 kcal/mol

ΔC_P = -0.450 kcal/mol-K

Non-pol:pol =3.3

Saquinavir





Velazquez-Campoy, et. al. Biochemistry, 2000, vol. 39, pp. 2201-2207

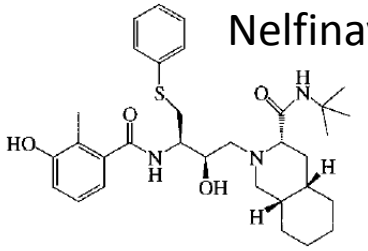
ΔH = 2.2 kcal/mol K_i = 4.0 nM

$-T\Delta S$ = -14.0 kcal/mol

ΔC_P = -0.340 kcal/mol-K

Non-pol:pol =2.8

Nelfinavir



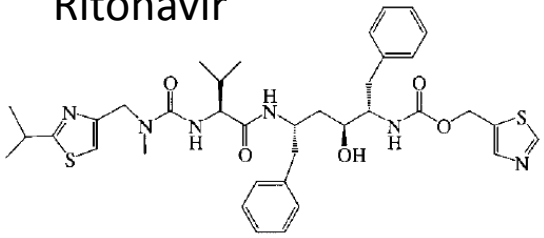
ΔH = 2.8 kcal K_i = 2.0 nM

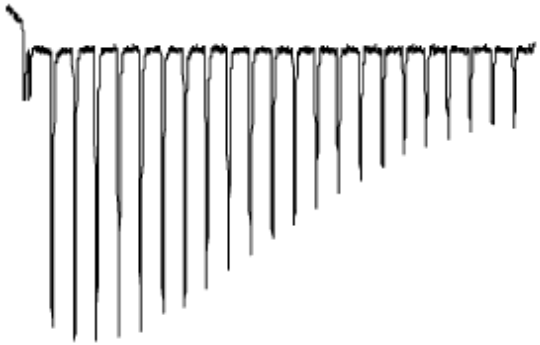
$-T\Delta S$ = -14.2 kcal/mol

ΔC_P = -0.400 kcal/mol-K

Non-pol:pol =3.2

Ritonavir





Schon et. al. Biophys. Chem. 2003, vol.105, pp. 221-230

ΔH = -2.3 kcal K_i = 0.3 nM

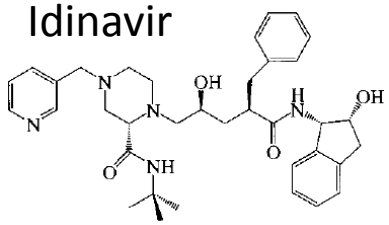
$-T\Delta S$ = -11.2 kcal/mol

ΔC_P = -0.380 kcal/mol-K

Non-pol:pol =2.3

Binding of NNRTs to WT HIV-1 protease

Idinavir



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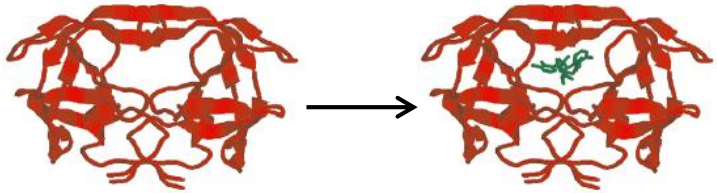
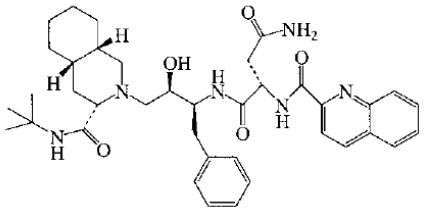
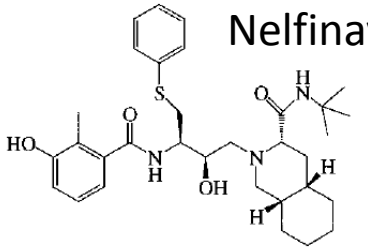


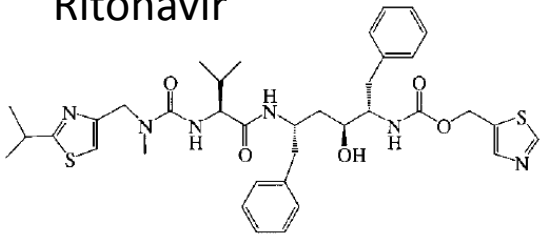
Saquinavir

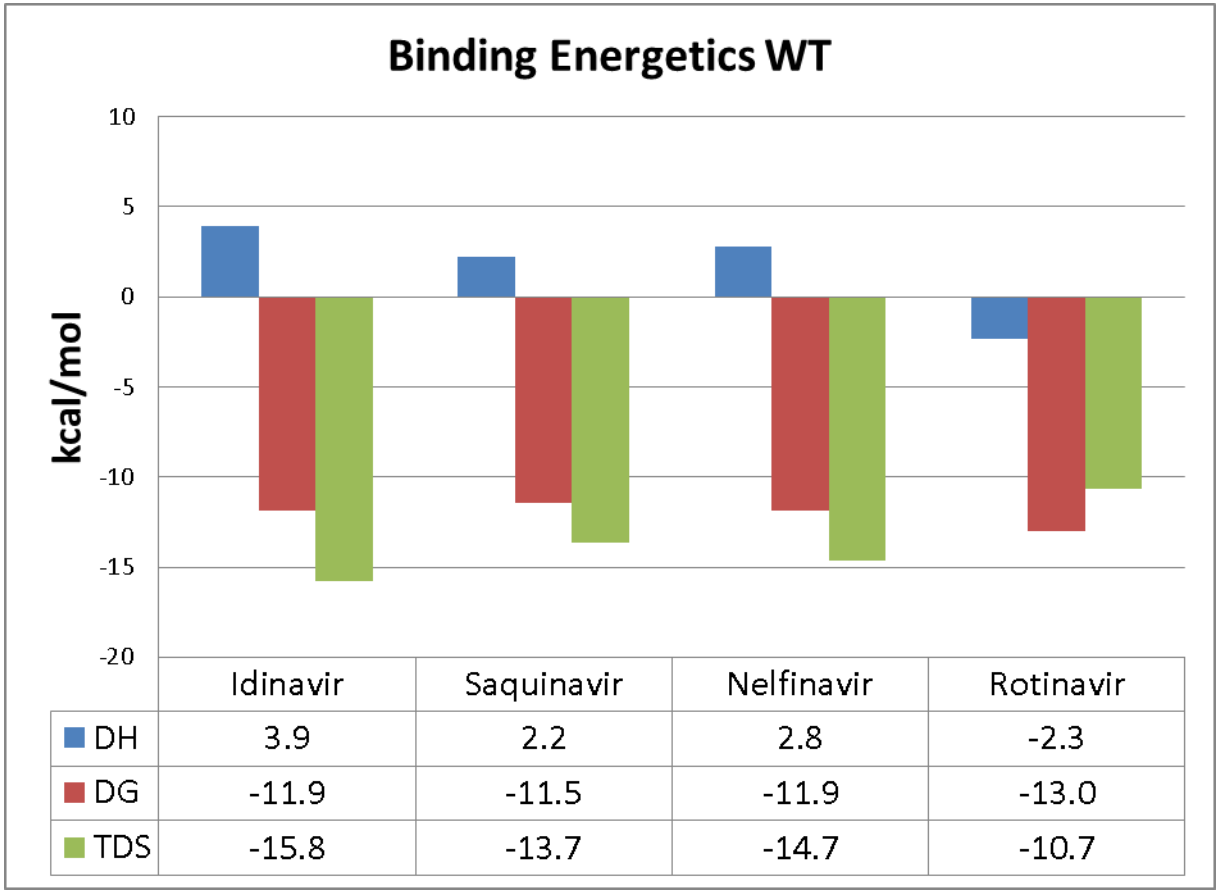


Nelfinavir



Ritonavir

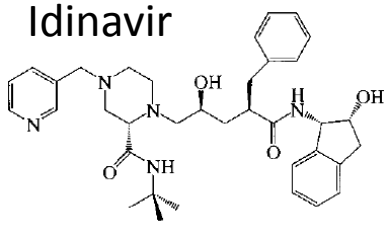




	Idinavir	Saquinavir	Nelfinavir	Rotinavir
DH	3.9	2.2	2.8	-2.3
DG	-11.9	-11.5	-11.9	-13.0
TDS	-15.8	-13.7	-14.7	-10.7

Binding of NNRTs to and active site mutant HIV-1 protease V82F/I84V

Idinavir



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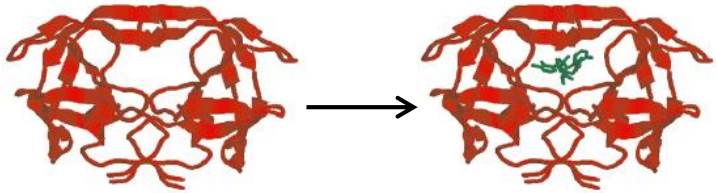
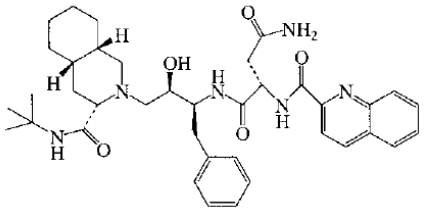
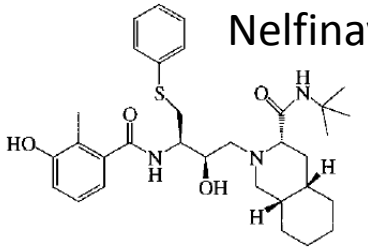


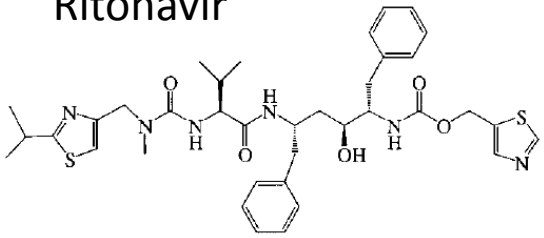
Saquinavir

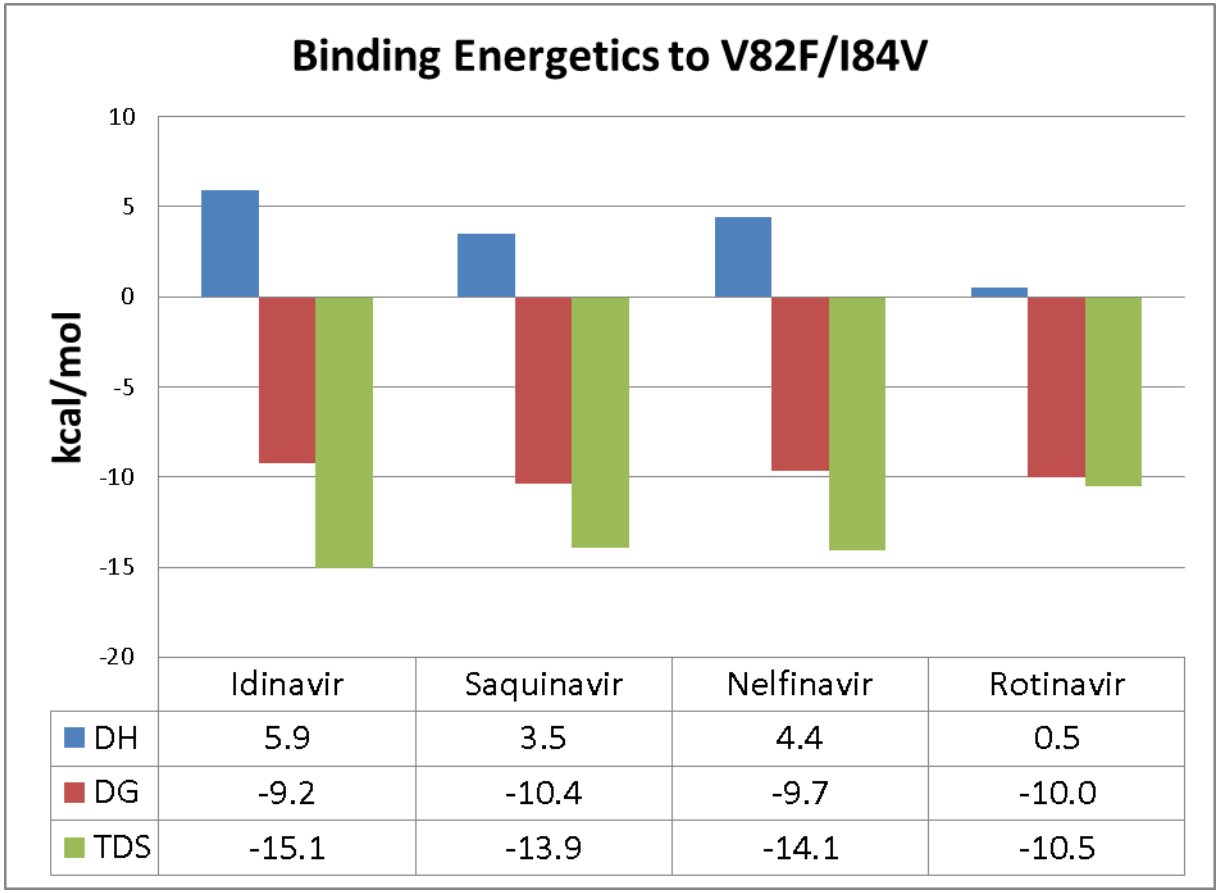


Nelfinavir



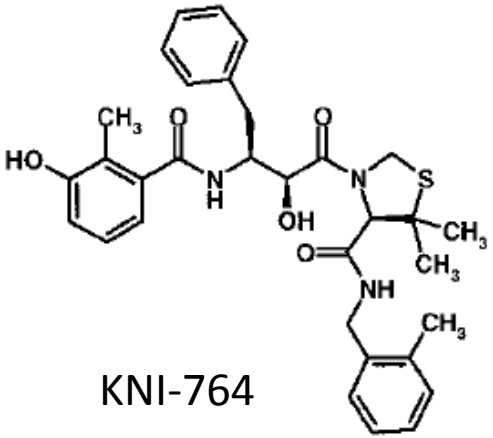
Ritonavir



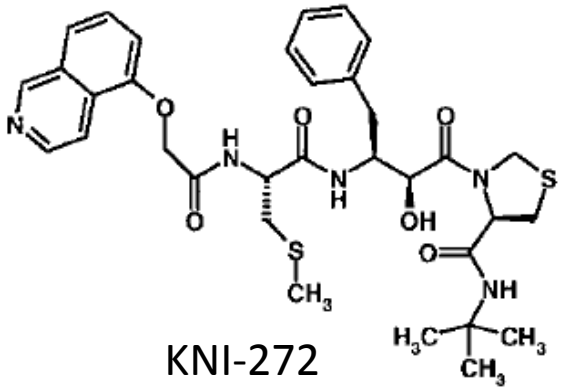


	Idinavir	Saquinavir	Nelfinavir	Rotinavir
DH	5.9	3.5	4.4	0.5
DG	-9.2	-10.4	-9.7	-10.0
TDS	-15.1	-13.9	-14.1	-10.5

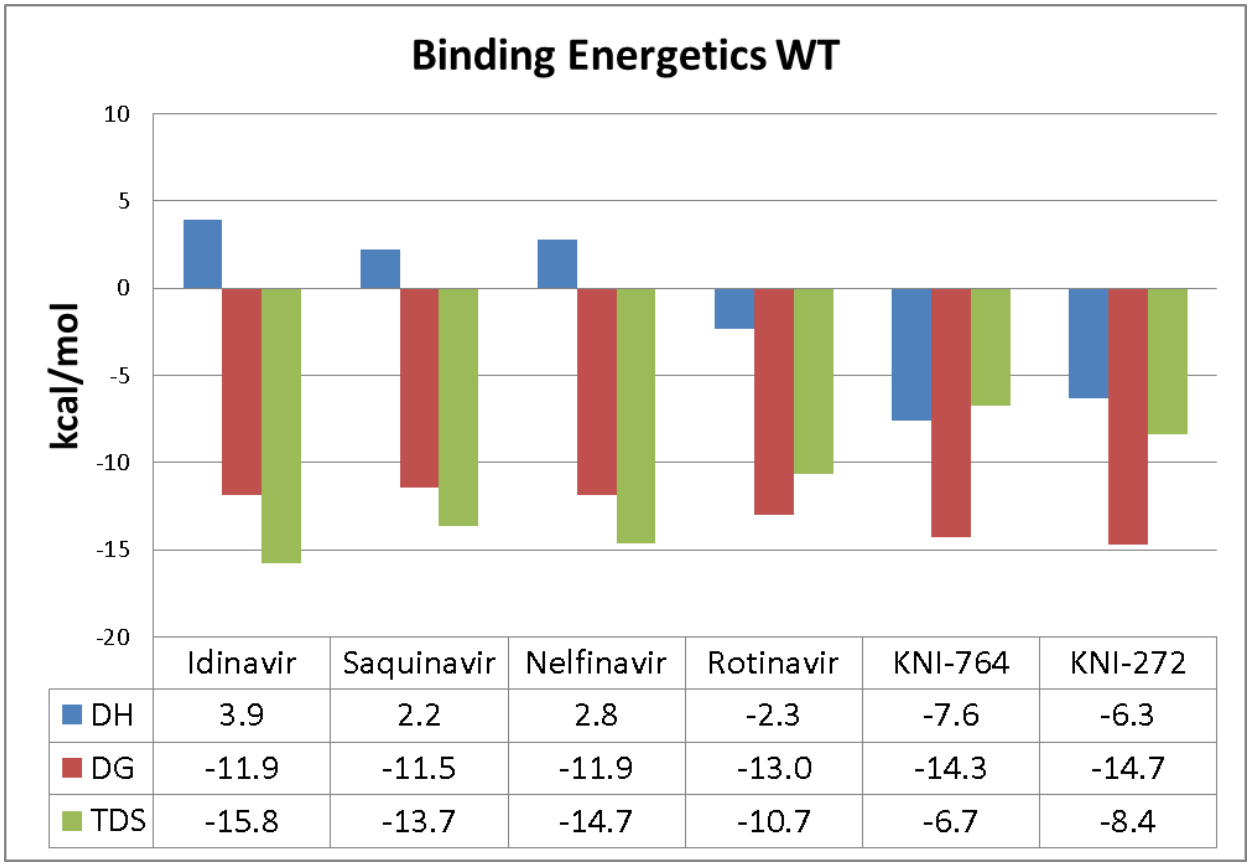
2nd generation protease inhibitors: WT protease



KNI-764



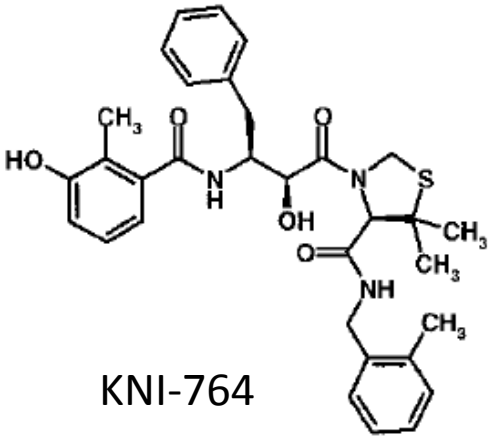
KNI-272



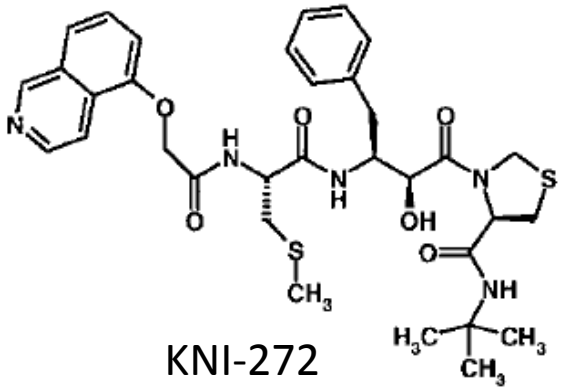
	Idinavir	Saquinavir	Nelfinavir	Rotinavir	KNI-764	KNI-272
DH	3.9	2.2	2.8	-2.3	-7.6	-6.3
DG	-11.9	-11.5	-11.9	-13.0	-14.3	-14.7
TDS	-15.8	-13.7	-14.7	-10.7	-6.7	-8.4

Yoshiumura et. al. PNAS , 1999, vol.96, pp. 8675-8680

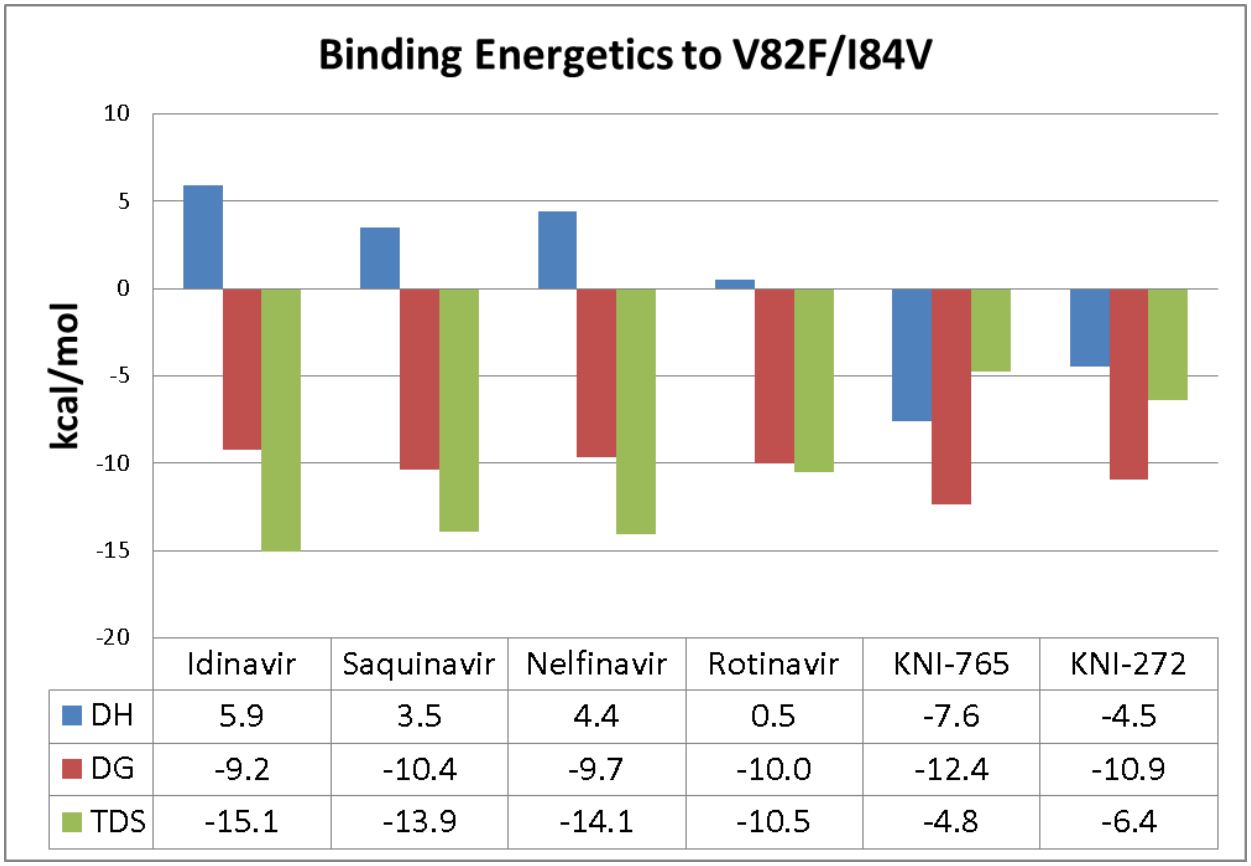
2nd generation protease inhibitors: V82F/I84V binding



KNI-764



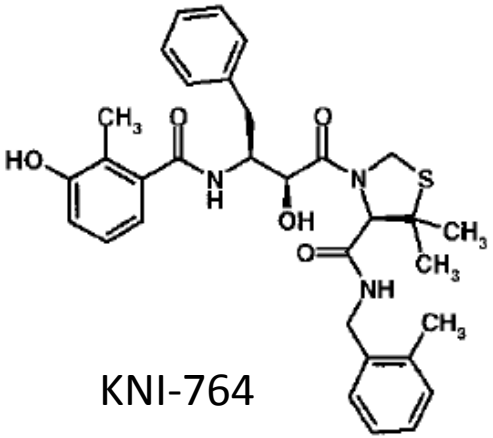
KNI-272



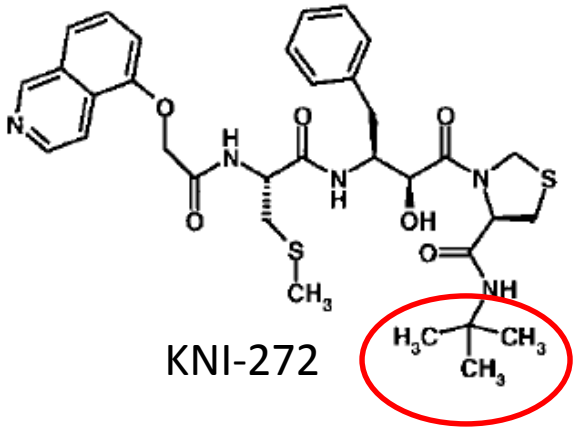
	Idinavir	Saquinavir	Nelfinavir	Rotinavir	KNI-765	KNI-272
DH	5.9	3.5	4.4	0.5	-7.6	-4.5
DG	-9.2	-10.4	-9.7	-10.0	-12.4	-10.9
TDS	-15.1	-13.9	-14.1	-10.5	-4.8	-6.4

Yoshiumura et. al. PNAS , 1999, vol.96, pp. 8675-8680
Luque et. al. Biochemistry, 1998, vol.37, pp. 5791-5797
Velazquez-Campoy et. al. Protein Science, 2000, vol. 9 pp.1801-1809
Velazquez-Campoy et. al. Archives Biochem Biophys., 2001, vol. 390, pp. 169-175
Levitt, et. al. Curr. Op. Struct. Bio. 2001, vol.11, pp.560-566

2nd generation protease inhibitors: V82F/I84V binding

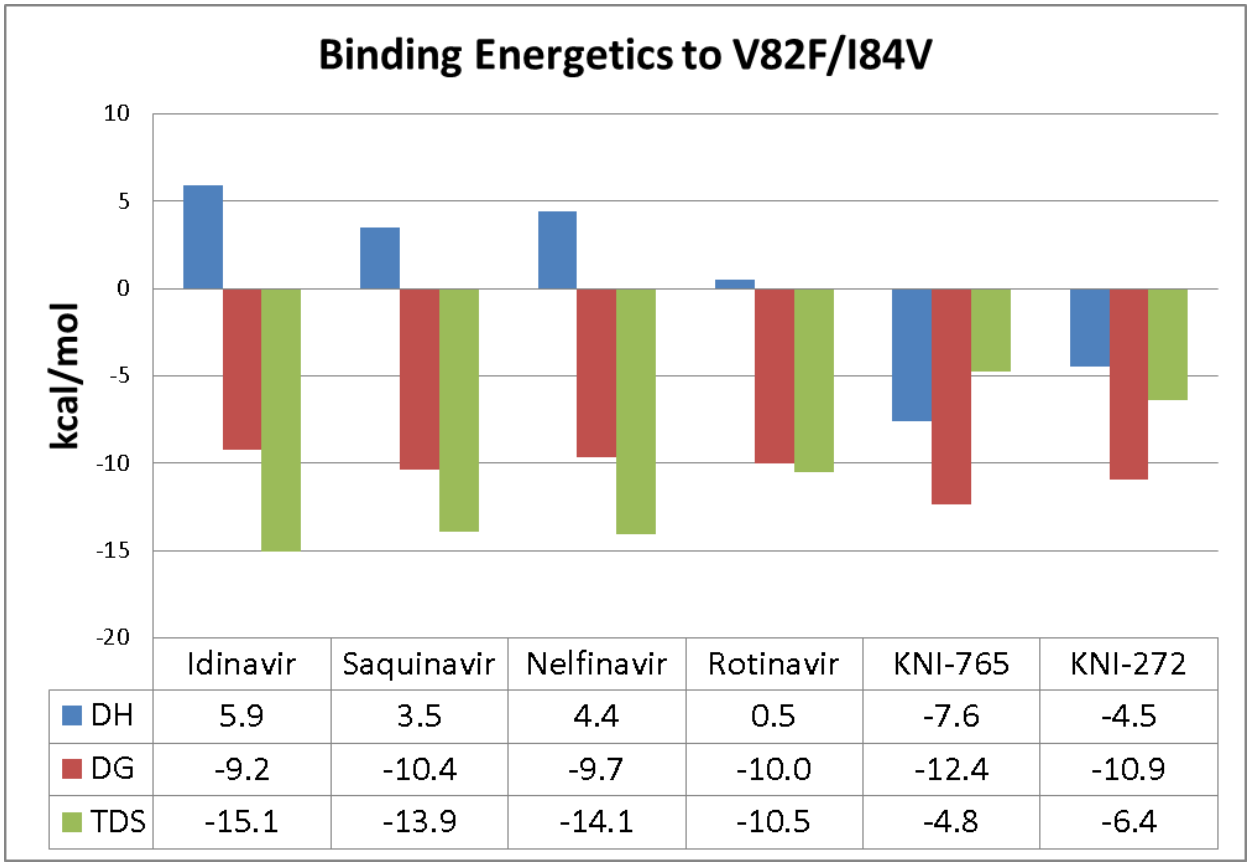


KNI-764



KNI-272

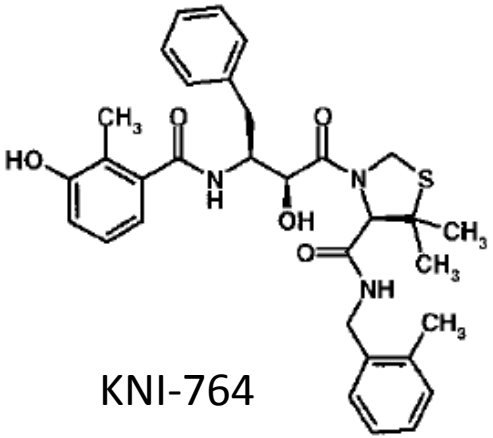
Structurally constrained



	Idinavir	Saquinavir	Nelfinavir	Rotinavir	KNI-765	KNI-272
DH	5.9	3.5	4.4	0.5	-7.6	-4.5
DG	-9.2	-10.4	-9.7	-10.0	-12.4	-10.9
TDS	-15.1	-13.9	-14.1	-10.5	-4.8	-6.4

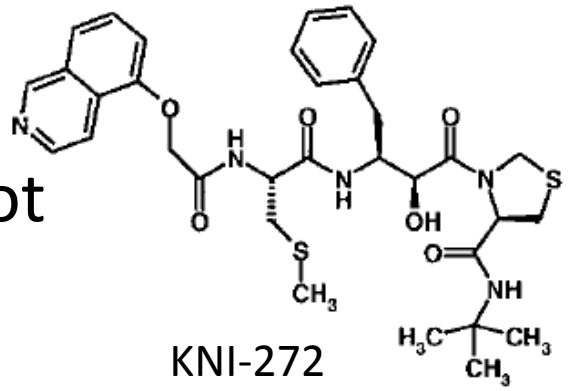
Yoshiumura et. al. PNAS , 1999, vol.96, pp. 8675-8680
Luque et. al. Biochemistry, 1998, vol.37, pp. 5791-5797
Velazquez-Campoy et. al. Protein Science, 2000, vol. 9 pp.1801-1809
Velazquez-Campoy et. al. Archives Biochem Biophys., 2001, vol. 390, pp. 169-175
Levitt, et. al. Curr. Op. Struct. Bio. 2001, vol.11, pp.560-566

Thermodynamic design parameters in drug design



KNI-764

High affinity is not sufficient



KNI-272

- High affinity – $K_D \approx 10^{-11}$
- Conformationally flexible
- Exothermic interactions – increased hydrophilic character
  - In the examples here the ability to trap water in the active site resulted in the increased exothermic nature of the binding

More soluble conformationally flexible molecules not more hydrophobic constrained molecules