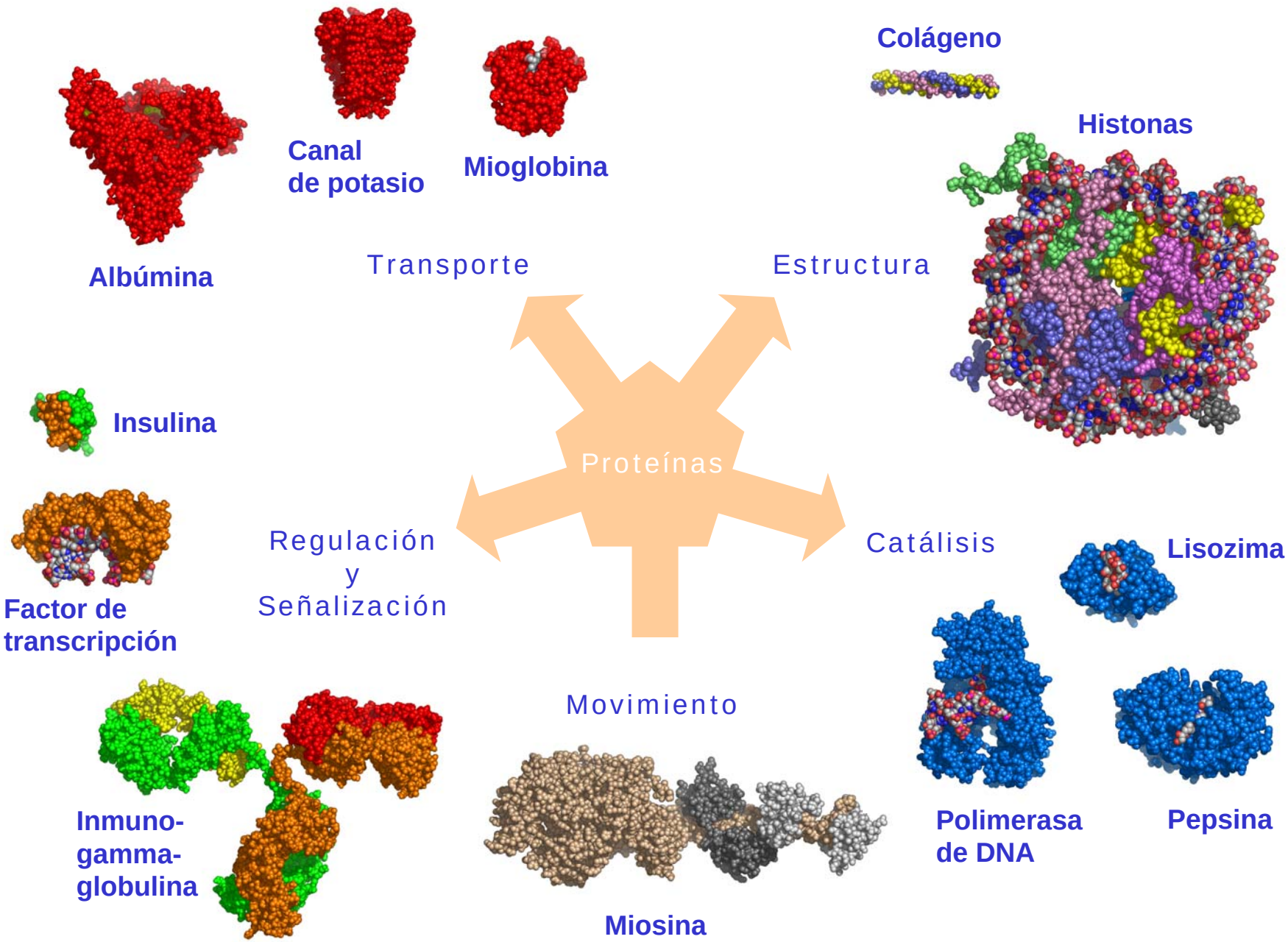
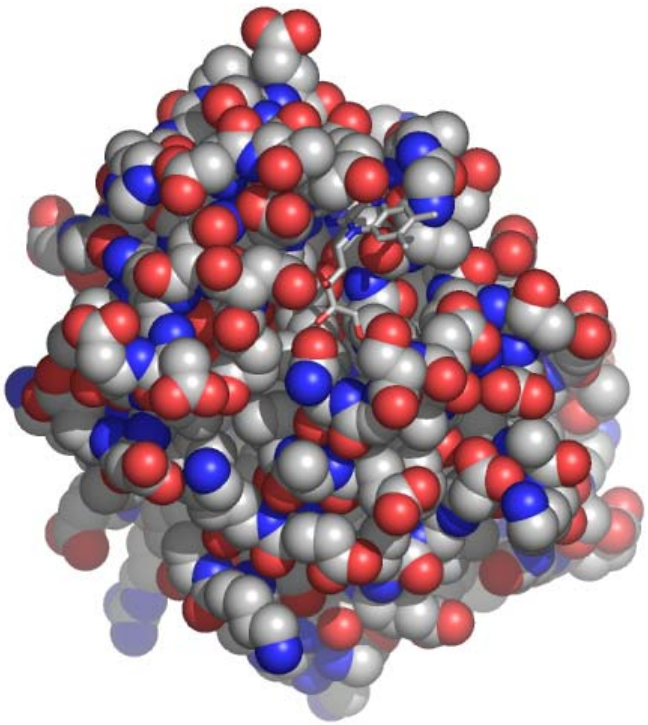
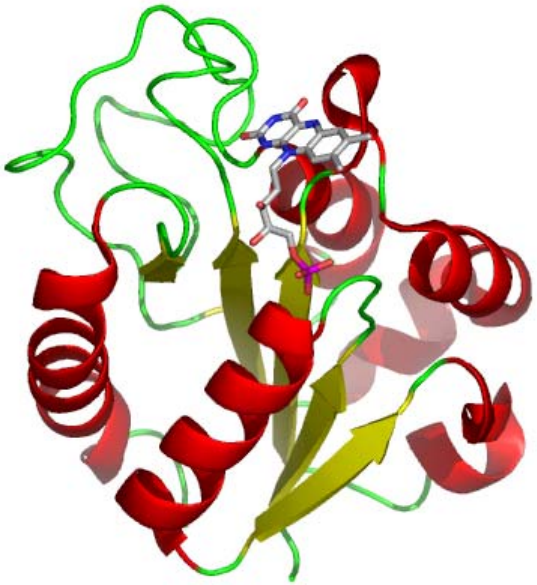
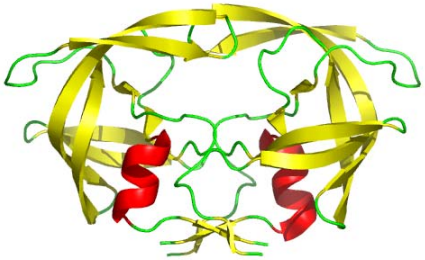

Albúmina
Canal de potasio
Mioglobina
Colágeno
Histonas
Transporte
Estructura
Proteínas
Insulina
Factor de transcripción
Regulación y Señalización
Catálisis
Lisozima
Polimerasa de DNA
Pepsina
Inmuno-gamma-globulina
Movimiento
Miosina

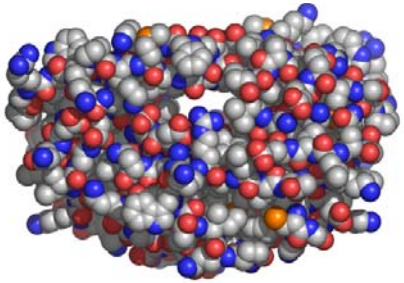


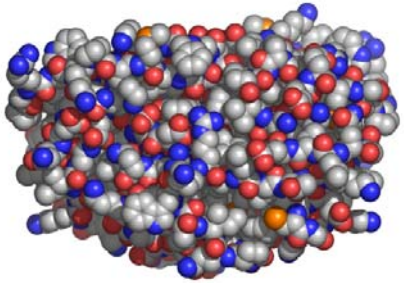


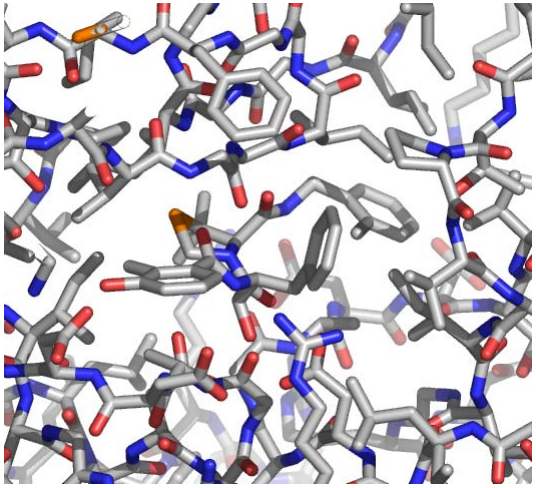
Red de interacciones
- intramoleculares
- intermoleculares

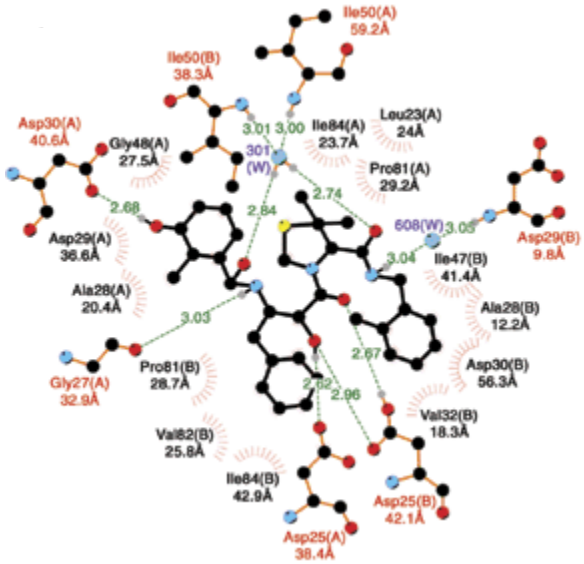
Enlaces de hidrógeno
Interacciones de van der Waals
Interacciones electrostáticas
Interacciones hidrofóbicas







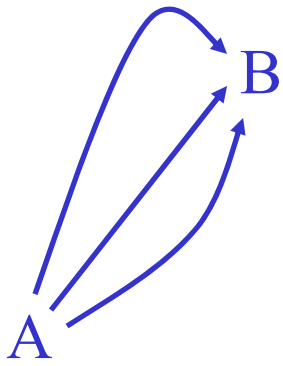



Ile50(A)
59.2Å
Ile50(B)
38.3Å
Asp30(A)
40.6Å
Gly48(A)
27.5Å
3.01
3.00
Ile84(A)
23.7Å
Leu23(A)
24Å
301
(W)
Pro81(A)
29.2Å
2.68
2.84
2.74
Asp29(A)
36.6Å
608(W)
3.05
Asp29(B)
9.8Å
3.04
Ile47(B)
41.4Å
Ala28(A)
20.4Å
3.03
Ala28(B)
12.2Å
2.67
Gly27(A)
32.9Å
Pro81(B)
28.7Å
Asp30(B)
56.3Å
2.82
2.96
Val32(B)
18.3Å
Val82(B)
25.8Å
Ile84(B)
42.9Å
Asp25(B)
42.1Å
Asp25(A)
38.4Å

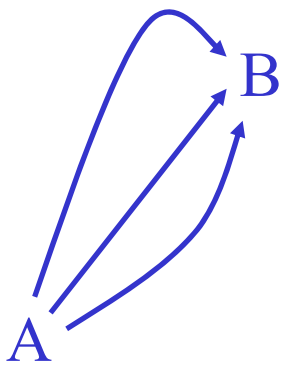
$$E = E(S, V, N_i)$$
$$H = H(S, P, N_i) = E + PV$$
$$G = G(T, P, N_i) = H - TS = E - TS + PV$$

$$dE = TdS - PdV + \sum_i \mu_i dN_i$$
$$dH = TdS + VdP + \sum_i \mu_i dN_i$$
$$dG = -SdT + VdP + \sum_i \mu_i dN_i$$

$$\Delta G = \Delta H - T\Delta S$$
$$\Delta H = \Delta H(T_0) + \Delta C_P (T - T_0)$$
$$\Delta S = \Delta S(T_0) + \Delta C_P \ln\left(\frac{T}{T_0}\right)$$



$$\Delta C_P = \left(\frac{\partial \Delta H(T)}{\partial T}\right)_P = T\left(\frac{\partial \Delta S(T)}{\partial T}\right)_P = -T\left(\frac{\partial^2 \Delta G(T)}{\partial T^2}\right)_P$$



1ª Ley de la Termodinámica — Balance energético

$$\Delta E = Q - W$$

$$dE = \bar{d}Q - \bar{d}W = TdS - PdV + \sum_i \mu_i dN_i$$

reversible $\quad \bar{d}Q = TdS \quad \bar{d}W = PdV - \sum_i \mu_i dN_i$

irreversible $\quad \bar{d}Q < TdS \quad \bar{d}W < PdV - \sum_i \mu_i dN_i$

2ª Ley de la Termodinámica — Direccionalidad o viabilidad

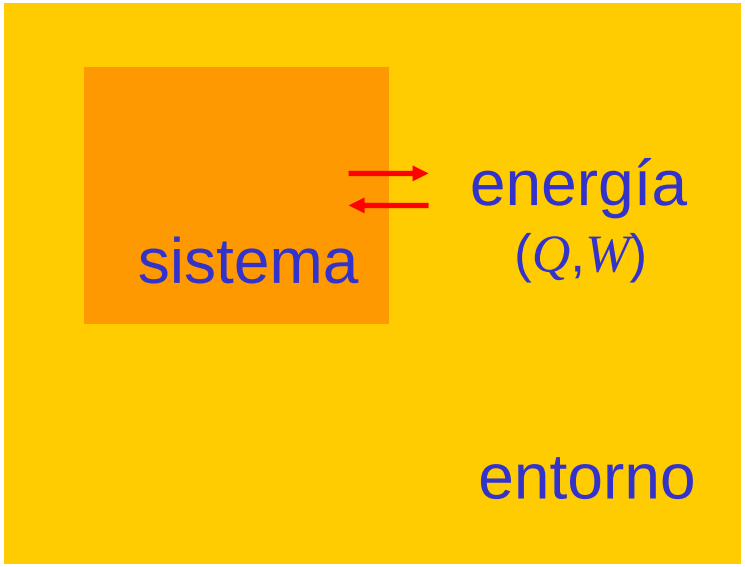
$\Delta S \geq 0$ en un sistema aislado

$dS \geq 0$

reversible $\quad dS = 0$

irreversible $\quad dS > 0$

imposible $\quad dS < 0$



Supongamos que el sistema no es aislado, pero es cerrado

$$dE = dE_s + dE_e = 0$$

$$dS = dS_s + dS_e \geq 0$$

$$dS = dS_s + \frac{\bar{d}Q_e}{T} = dS_s - \frac{\bar{d}Q_s}{T} \geq 0$$

$$TdS_s - \bar{d}Q_s \geq 0$$

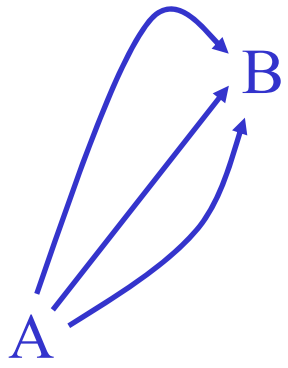
$TdS_s - dE_s \geq 0 \quad dE_s - TdS_s = dF_s \leq 0$ si V constante

$TdS_s - dH_s \geq 0 \quad dH_s - TdS_s = dG_s \leq 0$ si P constante

E, V constantes $\quad dS_s \geq 0$

T, V constantes $\quad dF_s \leq 0$

T, P constantes $\quad dG_s \leq 0$



$G = G(T, P, N)$ energía libre de Gibbs

$G = E - TS + PV = H - TS = F + PV$

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

$$dG = dE - TdS - SdT + VdP + PdV = -SdT + VdP + \sum_i \mu_i dN_i$$

T, P constantes

$dG > 0$ proceso no favorable $\mathrm{A} \not\rightarrow \mathrm{B}$

$dG = 0$ equilibrio $\mathrm{A} \leftrightarrow \mathrm{B}$

$dG < 0$ proceso favorable $\mathrm{A} \rightarrow \mathrm{B}$

$$A \leftrightarrow B$$

$$K_{eq} = e^{-\left(\Delta G^0 / RT\right)} = e^{-\Delta H^0 / RT} e^{\Delta S^0 / R}$$

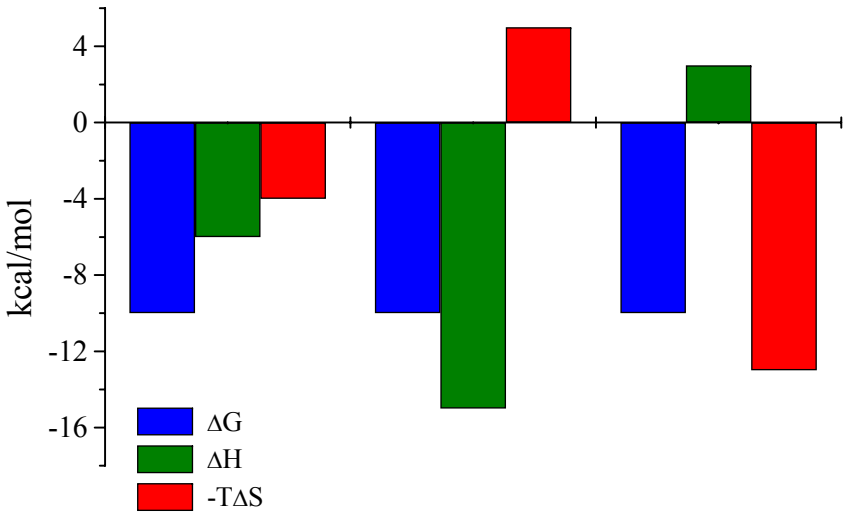
$$\Delta G = \Delta H - T\Delta S$$

$\Delta H > 0$ endotérmica

$\Delta H < 0$ exotérmica

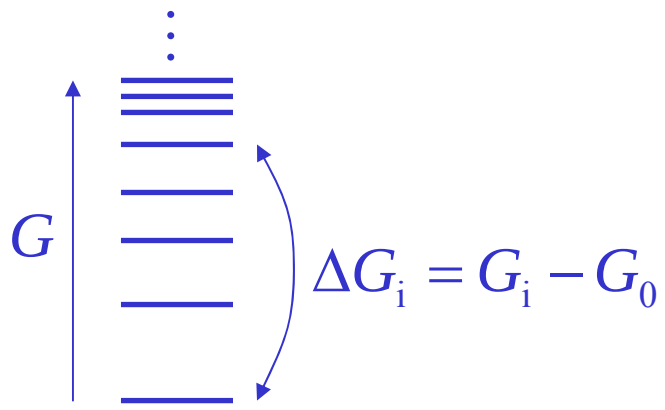
$\Delta G > 0$ endergónica

$\Delta G < 0$ exergónica



ΔH cambio de energía almacenada en enlaces e interacciones
refleja número, tipo y calidad de enlaces
$\Delta H < 0 \Rightarrow$ si $T\uparrow$, entonces $K_{eq}\downarrow$
$\Delta H > 0 \Rightarrow$ si $T\uparrow$, entonces $K_{eq}\uparrow$

ΔS medida de desorden
refleja orden-desorden en enlaces, flexibilidad conformacional y solvatación



$$i = 0, 1, \ldots, n \quad \text{estados} \quad \Rightarrow \quad P_i$$

$$T, P \ \text{ cte} \quad \Rightarrow \quad P_i \propto \exp\left(-\Delta G_i / RT\right)$$

$$\sum_{i=0}^{i=n} P_i = 1$$

$$P_i = \frac{\exp(-\Delta G_i / RT)}{Q}$$

$$Q = \sum_{i=0}^{i=n} \exp(-\Delta G_i / RT)$$

función de partición

i	G	ΔG	$\exp(-\Delta G/RT)$
0	G_0	0	1
⋮	⋮	⋮	⋮
i	G_i	ΔG_i	$\exp(-\Delta G_i/RT)$
⋮	⋮	⋮	⋮
n	G_n	ΔG_n	$\exp(-\Delta G_n/RT)$

A propiedad observable del sistema $\Rightarrow$ ¿ medida de A ?

$$\langle A \rangle = \sum_{i=0}^{i=n} P_i A_i$$ promedio de colectividad

$$\overline{A} = \lim_{t \to +\infty} \frac{1}{t} \int_0^t A(t')dt'$$ promedio temporal

Sistema ergódico $\Rightarrow$ $\langle A \rangle = \overline{A}$

En general, $\langle A \rangle \neq A_i$, $\forall i$

$$Q = \sum_i \omega_i \exp\left(-\Delta H_i / RT\right) = \sum_i \exp\left(-\Delta G_i / RT\right)$$

$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$

i	ΔG	ΔH	ΔS
0	0	0	0
⋮	⋮	⋮	⋮
i	ΔG_i	ΔH_i	ΔS_i
⋮	⋮	⋮	⋮
n	ΔG_n	ΔH_n	ΔS_n

$$\langle \Delta G \rangle = -RT \ln Q$$

$$\langle \Delta H \rangle = RT^2 \frac{\partial \ln Q}{\partial T} = -T^2 \frac{\partial\left(\langle \Delta G \rangle / T\right)}{\partial T}$$

$$\langle \Delta S \rangle = R\left(\ln Q + T \frac{\partial \ln Q}{\partial T}\right) = -\frac{\partial \langle \Delta G \rangle}{\partial T}$$

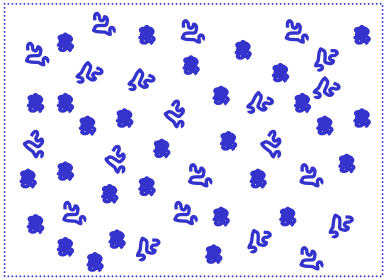
$$Q = \sum_i \exp\left(-\Delta G_i / RT\right)$$

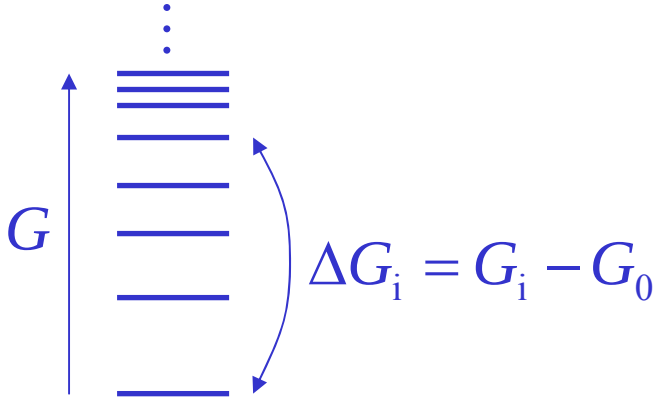
$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$

$$\left.\begin{aligned} N_i &= N P_i = N \frac{\exp\left(-\Delta G_i / RT\right)}{Q} \\ N_0 &= N P_0 = N \frac{1}{Q} \end{aligned}\right\} \quad \frac{N_i}{N_0} = \exp\left(-\Delta G_i / RT\right)$$

$$Q = \sum_i \frac{N_i}{N_0} = \sum_i \frac{C_i}{C_0}$$

Equilibrio Conformacional





$$i = 0, 1, \ldots, n \quad \text{estados} \quad \Rightarrow \quad P_i$$

$$T, P \ \text{cte} \quad \Rightarrow \quad P_i \propto \exp\left(-\Delta G_i / RT\right)$$

$$\sum_{i=0}^{i=n} P_i = 1$$

$$\Delta G_{\mathrm{i}} = \Delta G_{\mathrm{i}}(T, P, pH, \mu, [D], [L], \ldots)$$

$$\Delta G_{\mathrm{i}}([D]) = \Delta G_{\mathrm{i}}^{0} - m[D]$$

$$\Delta G_{\mathrm{i}}(pH) = \Delta G_{\mathrm{i}}^{0} + \sum_{\mathrm{j}=1} n_{\mathrm{j}} RT \ln\left(\frac{1+10^{pKa_{j,1}-pH}}{1+10^{pKa_{j,i}-pH}}\right)$$

$$\Delta G_{\mathrm{i}}(T) = \Delta H_{\mathrm{i}}(T_{\mathrm{m,i}}) + \Delta C_{\mathrm{P,i}}(T - T_{\mathrm{m,i}}) - T\left(\Delta S_{\mathrm{i}}(T_{\mathrm{m,i}}) + \Delta C_{\mathrm{P,i}} \ln(T / T_{\mathrm{m,i}})\right)$$

$$\Delta G_{\mathrm{i}}([L]) = \Delta G_{\mathrm{i}}^{0} + RT \ln\left(\frac{1+K_{\mathrm{B,1}}[L]}{1+K_{\mathrm{B,i}}[L]}\right)$$

Estudio de estabilidad de proteínas

- Bioquímica y Biofísica
  - Interacciones intramoleculares
  - Plegamiento de proteínas
- Biotecnología
  - Ingeniería de proteínas
  - Sensores moleculares
- Biomedicina
  - Enfermedades conformacionales
- Farmacología
  - Formulación de fármacos
  - Control de calidad

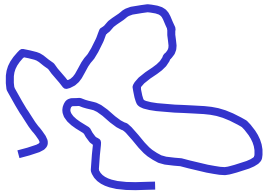
En un contexto dado, la estructura primaria de una proteína determina su estructura tridimensional, que corresponde a un mínimo energético

Conformación nativa ≡ conformación de mínima energía estructurada, estable, funcional, activa

- temperatura moderada
- ausencia de agentes denaturalizantes
- pH moderado

Pérdida de la Conformación Nativa

- pH ácido
- temperatura extrema
- agentes desnaturalizantes
- hidrólisis
- mutaciones



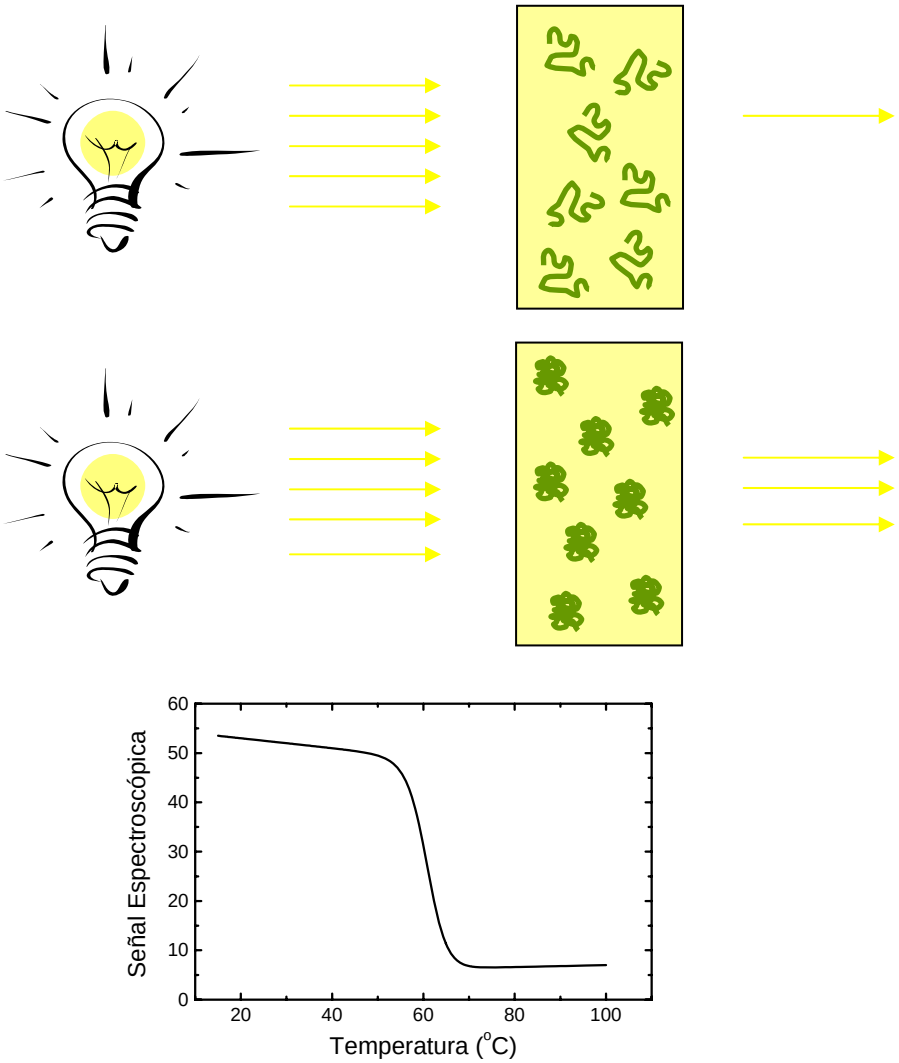
Cuantificación de la estabilidad → Energía requerida para desestabilizar la estructura tridimensional molecular

Técnicas Experimentales:

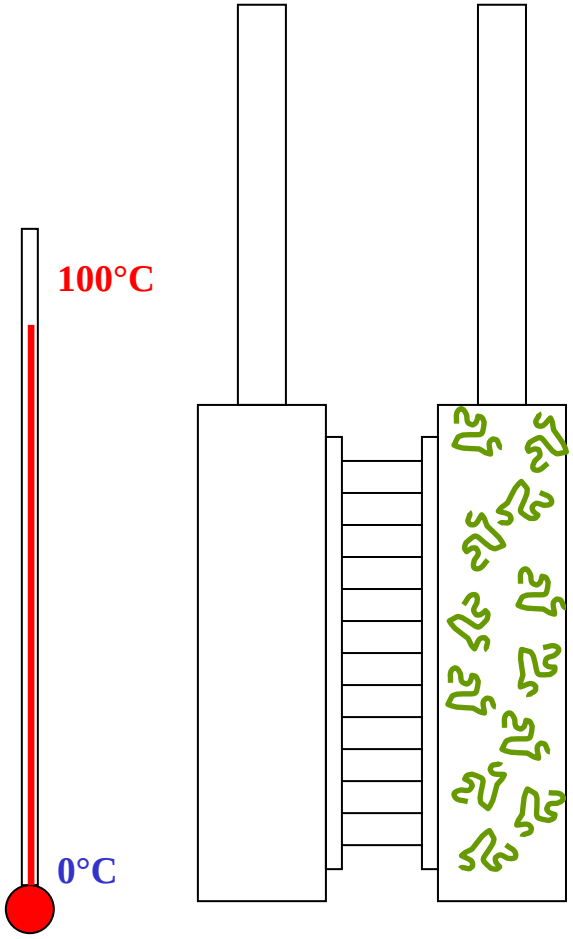
- Espectroscopía (UV, F, CD, NMR, FTIR)
- Calorimetría (DSC)

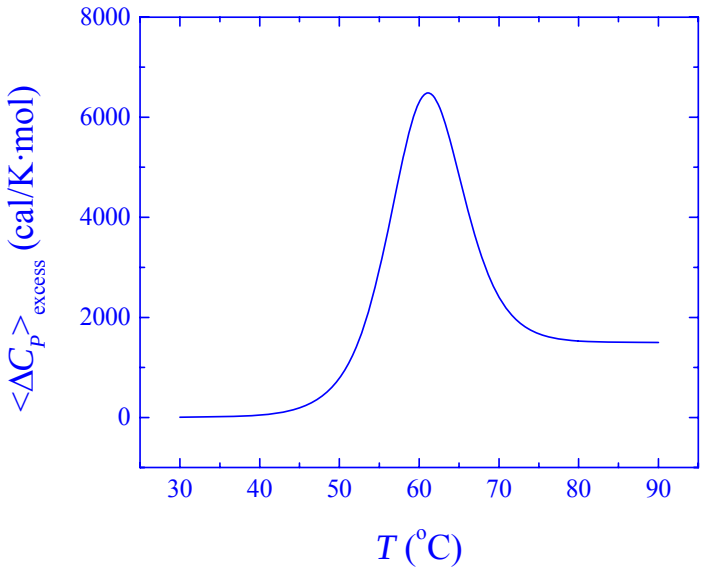
- Propiedad con diferentes valores para cada estado
- Señal medida proporcional al avance del proceso de desplegamiento
- Información global o local

Espectroscopía

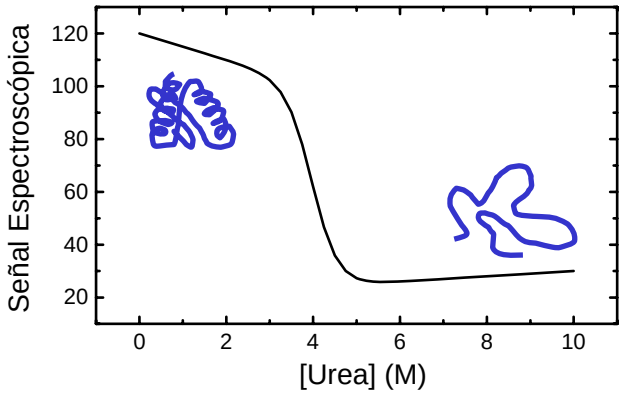


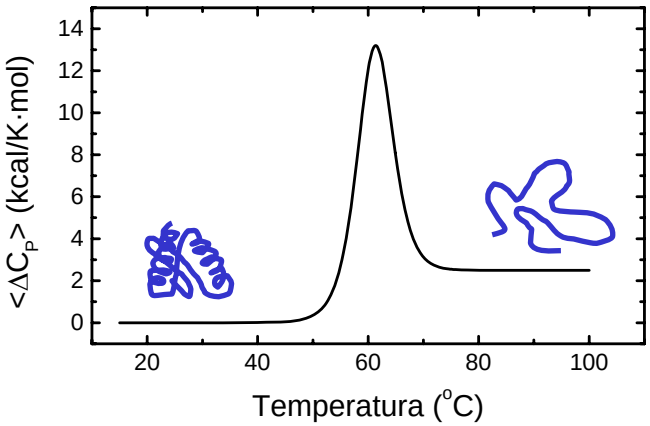
Calorimetría Diferencial de Barrido



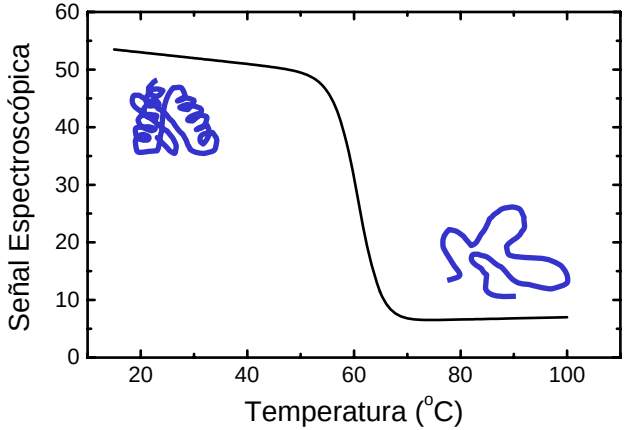


Medida de la capacidad calorífica de una disolución de macromolécula en función de la temperatura





?



Desplegamiento Cooperativo → Reducción del número de estados accesibles

Estados parcialmente plegados no son significativamente poblados

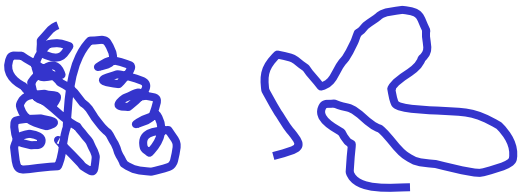
Estabilidad $\longrightarrow$ ΔG

Energía de Gibbs de estabilización o desplegamiento

Diferencia de energía de Gibbs entre el estado nativo y el estado desplegado

$$\Delta G = G_{\mathrm{U}} - G_{\mathrm{N}}$$

$$N \leftrightarrow U$$



$$\Delta G = -RT \ln K$$

$$K = \frac{[U]}{[N]}$$

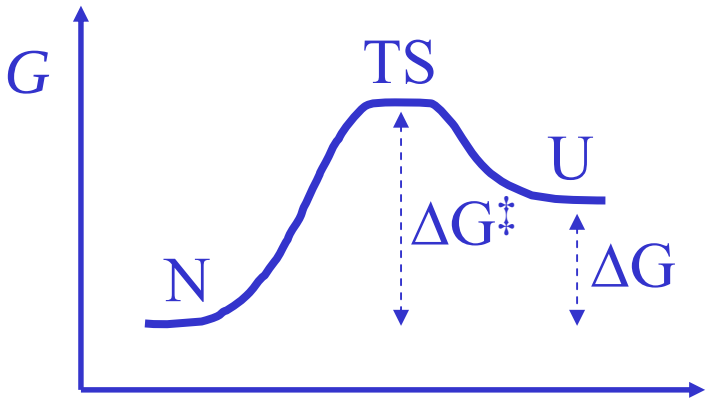
Estable $\Rightarrow$ $\begin{cases} \Delta G > 0 \\ K < 1 \end{cases}$

Estabilidad $\uparrow$ $\Rightarrow$ $\begin{cases} \Delta G \uparrow \\ K \downarrow \end{cases}$

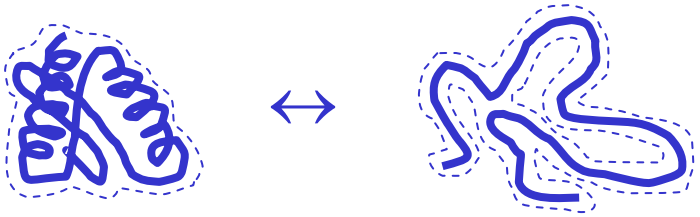
Equilibrio de desplegamiento o desnaturalización

↓

No se obtiene información cinética
No se consideran procesos irreversibles



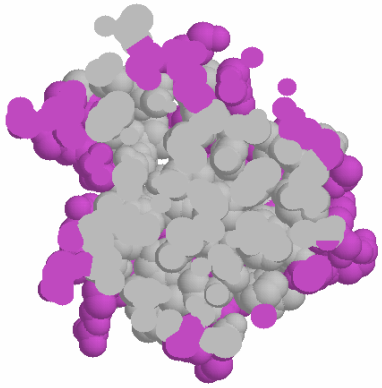
$$N \leftrightarrow U$$



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

Balance entre estado nativo y desplegado, tomando como referencia la interacción con el solvente

- Enlaces de hidrógeno
- Interacciones de van der Waals
- Interacciones electrostáticas
- Interacciones hidrofóbicas
- Entalpía de solvatación
- Entropía de solvatación
- Entropía conformacional



Hydrophobic
Polar/Charged

El papel del agua es extremadamente importante

Desplegamiento de dos estados

$$N \leftrightarrow U$$

i	G	ΔG	$\exp(-\Delta G/RT)$
	G_N	0	1
	G_U	ΔG	K

$$Q = 1 + K$$

$$\begin{cases} P_N = \dfrac{1}{1+K} \\ P_U = \dfrac{K}{1+K} \end{cases}$$

$$\Delta G = \Delta G(T, P, pH, \mu, [D], [L], \ldots)$$

$$\Delta G([D]) = \Delta G^0 - m[D]$$

$$\Delta G(pH) = \Delta G^0 + \sum_{\mathrm{j=1}}^{\mathrm{j=m}} n_{\mathrm{j}} RT \ln\left(\frac{1+10^{pKa_{\mathrm{j,N}} - pH}}{1+10^{pKa_{\mathrm{j,U}} - pH}}\right)$$

$$\Delta G(T) = \Delta H(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}}(T - T_{\mathrm{m}}) - T\left(\Delta S(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}} \ln(T / T_{\mathrm{m}})\right)$$

$$\Delta G([L]) = \Delta G^0 + RT \ln\left(\frac{1+K_{\mathrm{B,N}}[L]}{1+K_{\mathrm{B,U}}[L]}\right)$$

Desnaturalización Térmica

$$\Delta G(T) = \Delta H(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}}(T - T_{\mathrm{m}}) - T\left(\Delta S(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}} \ln(T / T_{\mathrm{m}})\right)$$

$$\Delta H(T_{\mathrm{m}}) = \Delta H(T = T_{\mathrm{m}})$$

$$\Delta S(T_{\mathrm{m}}) = \Delta S(T = T_{\mathrm{m}})$$

$$\Delta C_{\mathrm{P}} = \left(\frac{\partial \Delta H}{\partial T}\right)_{\mathrm{P}} = T\left(\frac{\partial \Delta S}{\partial T}\right)_{\mathrm{P}} = -T\left(\frac{\partial^2 \Delta G}{\partial T^2}\right)_{\mathrm{P}}$$

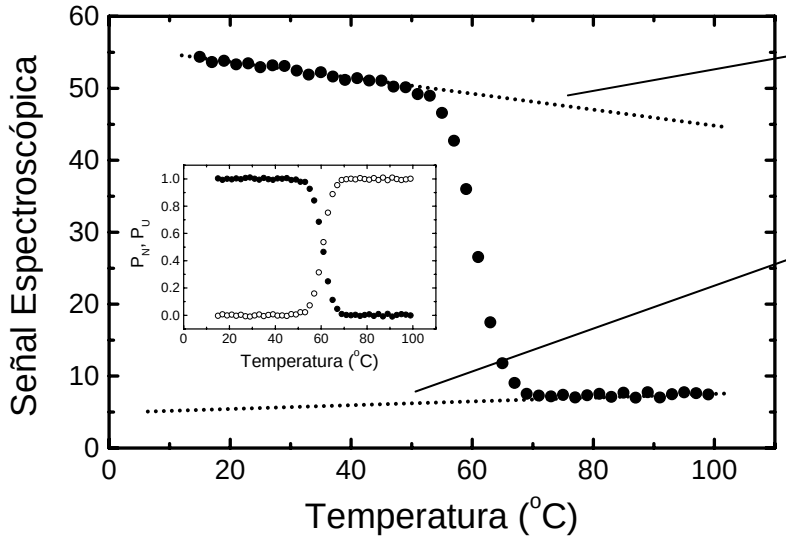
$$\Delta G(T) = -RT \ln K$$

$$\Delta G(T_{\mathrm{m}}) = 0 \quad \Rightarrow \quad \begin{cases} K = 1 \\ P_{\mathrm{N}} = P_{\mathrm{U}} = \dfrac{1}{2} \\ \Delta S(T_{\mathrm{m}}) = \dfrac{\Delta H(T_{\mathrm{m}})}{T_{\mathrm{m}}} \end{cases}$$

$\Delta H(T_m) > 0$	Ruptura de interacciones de van der Waals Ruptura de enlaces de hidrógeno Solvatación de grupos polares y apolares
$\Delta S(T_m) > 0$	Libertad conformacional Solvatación de grupos polares y apolares
$\Delta C_P > 0$	Solvatación de grupos polares y apolares

$\Delta C_P = \text{cte}$

$\Delta C_P = a + bT + cT^2$

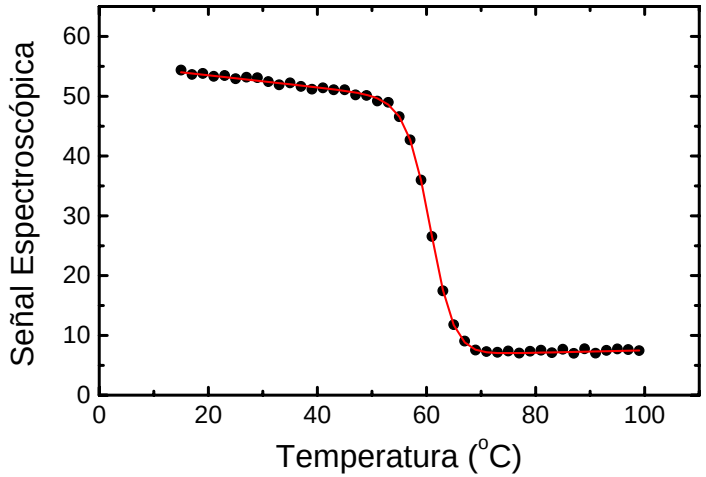


$$A_N = (a_N + b_N T)$$

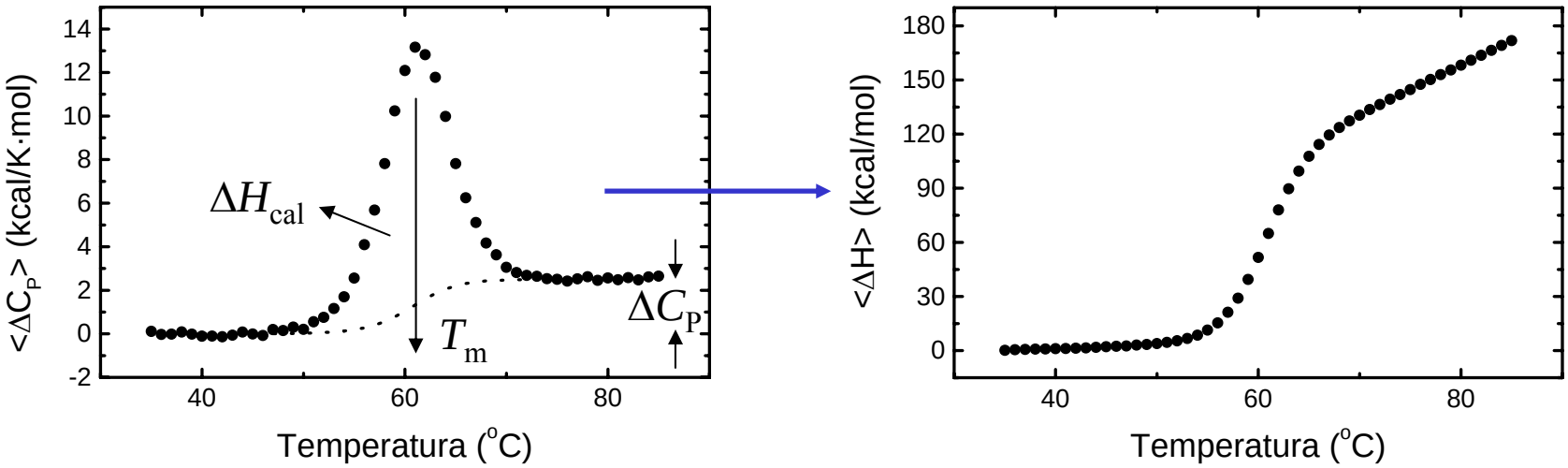
$$A_U = P_U (a_U + b_U T)$$

$$\langle A \rangle = P_N A_N + P_U A_U$$

$$\langle A \rangle = \frac{(a_N + b_N T) + e^{-(\Delta H(T_m) + \Delta C_P (T - T_m) - T(\Delta S(T_m) + \Delta C_P \ln(T/T_m)))/RT} (a_U + b_U T)}{1 + e^{-(\Delta H(T_m) + \Delta C_P (T - T_m) - T(\Delta S(T_m) + \Delta C_P \ln(T/T_m)))/RT}}$$



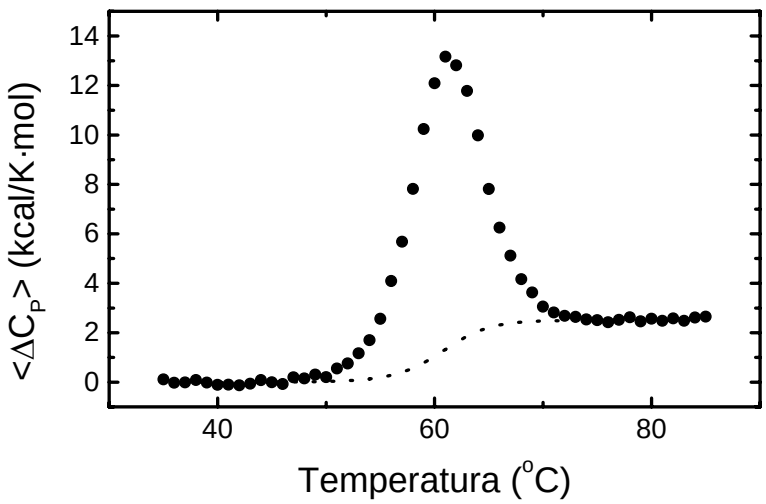
$\Delta H(T_m)$	101 ± 2 kcal/mol
T_m	60.69 ± 0.04 °C
ΔC_P	2.2 ± 0.7 kcal/K·mol



$$\Delta H(T_{\mathrm{m}}) = \int_{T_0}^{T_1} \langle \Delta C_{\mathrm{P}} \rangle dT$$

$$\Delta S(T_{\mathrm{m}}) = \int_{T_0}^{T_1} \frac{\langle \Delta C_{\mathrm{P}} \rangle}{T} dT$$

$$\langle \Delta H \rangle = \int_{T_0}^{T} \langle \Delta C_{\mathrm{P}} \rangle dT$$



$$\Delta H(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}}(T - T_{\mathrm{m}})$$

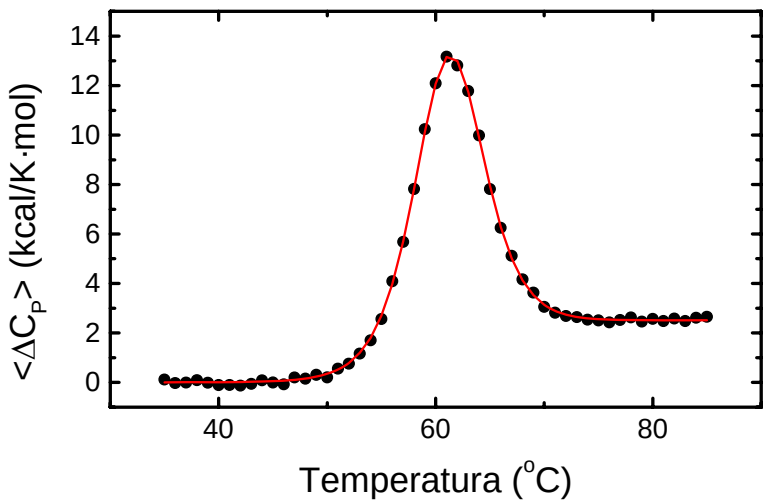
$$\langle \Delta H \rangle = P_{\mathrm{N}} \Delta H_{\mathrm{N}} + P_{\mathrm{U}} \Delta H_{\mathrm{U}}$$
$$= P_{\mathrm{U}} \Delta H$$

$$\Delta G(T) = \Delta H(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}}(T - T_{\mathrm{m}})$$
$$- T\left(\Delta S(T_{\mathrm{m}}) + \Delta C_{\mathrm{P}} \ln(T / T_{\mathrm{m}})\right)$$

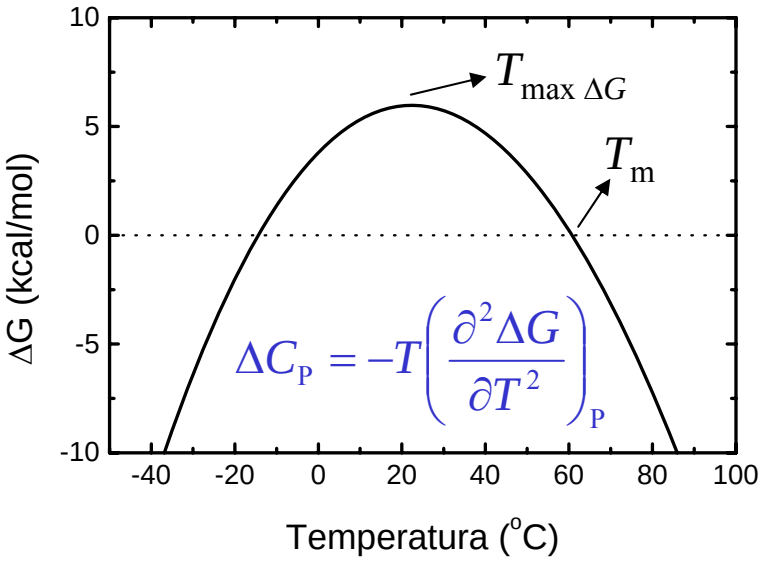
$$Q = 1 + \exp\left(-\Delta G / RT\right)$$

$$\langle \Delta H \rangle = RT^2 \frac{\partial \ln Q}{\partial T}$$

$$\langle \Delta C_{\mathrm{P}} \rangle = \left(\frac{\partial \langle \Delta H \rangle}{\partial T} \right)_{\mathrm{P}} = \frac{K}{(1+K)^2} \frac{\Delta H^2}{RT^2} + \frac{K}{1+K} \Delta C_{\mathrm{P}}$$



T_m	60.75 ± 0.03 °C
$\Delta H(T_m)$	102 ± 0.3 kcal/mol
ΔC_P	2.51 ± 0.02 kcal/K·mol

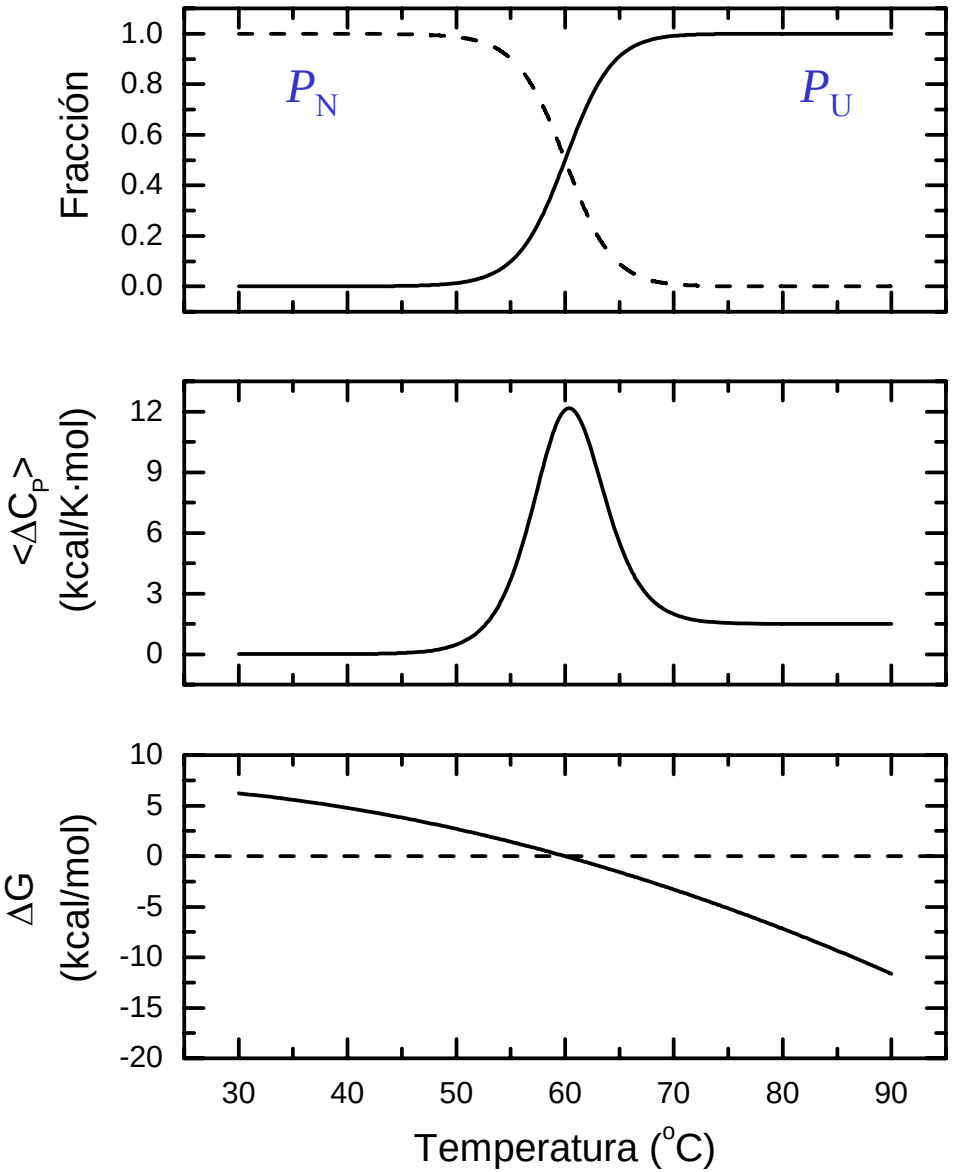


$$\left(\frac{\partial \ln K}{\partial T}\right)_P = \frac{\Delta H}{RT^2}$$

$$\left(\frac{\partial \Delta G}{\partial T}\right)_P = -\Delta S \qquad \Delta S = 0 \quad \Rightarrow \quad \max_T \Delta G$$

$$T_{\max_T \Delta G} = T_S = T_m \exp\left(-\frac{\Delta H(T_m)}{\Delta C_P T_m}\right)$$

$$\max_T \Delta G = \Delta H(T_m) - \Delta C_P T_m \left(1 - \exp\left(-\frac{\Delta H(T_m)}{\Delta C_P T_m}\right)\right)$$

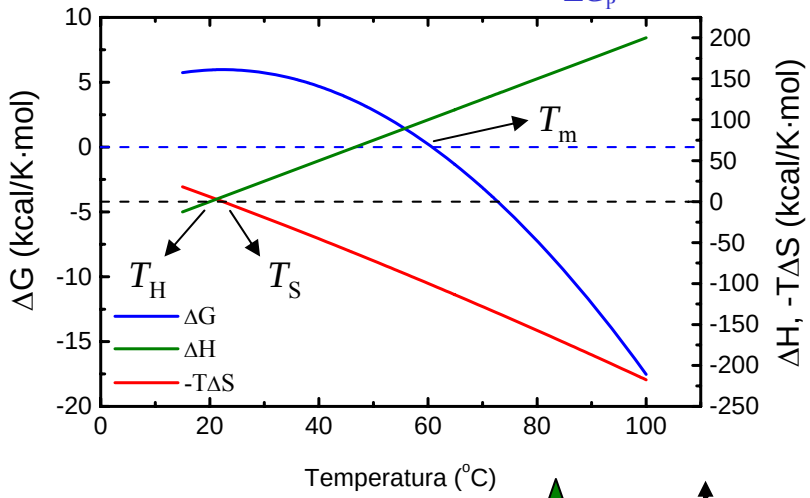

Fracción
1.0
0.8
0.6
0.4
0.2
0.0
P_N
P_U
<ΔC_P>
(kcal/K·mol)
12
9
6
3
0
ΔG
(kcal/mol)
10
5
0
-5
-10
-15
-20
30
40
50
60
70
80
90
Temperatura (°C)

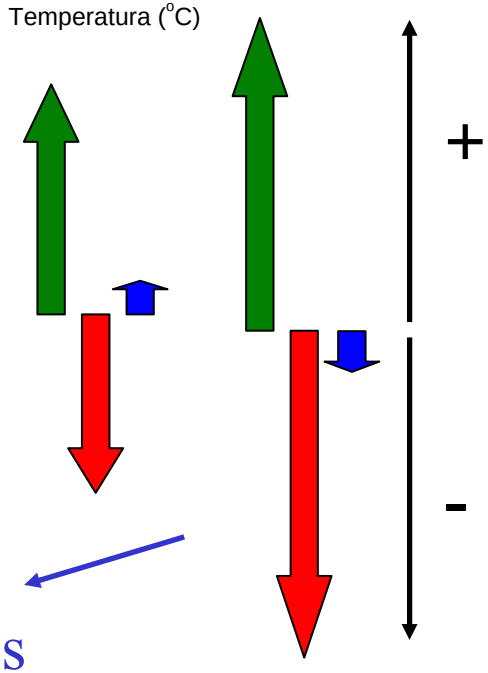
$$\Delta G = \Delta H - T\Delta S$$

Enlaces de hidrógeno
Interacciones de van der Waals
Interacciones electrostáticas
Interacciones hidrofóbicas

$$\Delta S(T_S) = 0 \quad \Rightarrow \quad T_S = T_{\max_T \Delta G}$$

$$\Delta H(T_H) = 0 \quad \Rightarrow \quad T_H = T_m - \frac{\Delta H(T_m)}{\Delta C_P}$$





estabilizado entálpicamente

estabilizado entrópicamente

Estabilidad “marginal”
Dificultad para cálculos predictivos

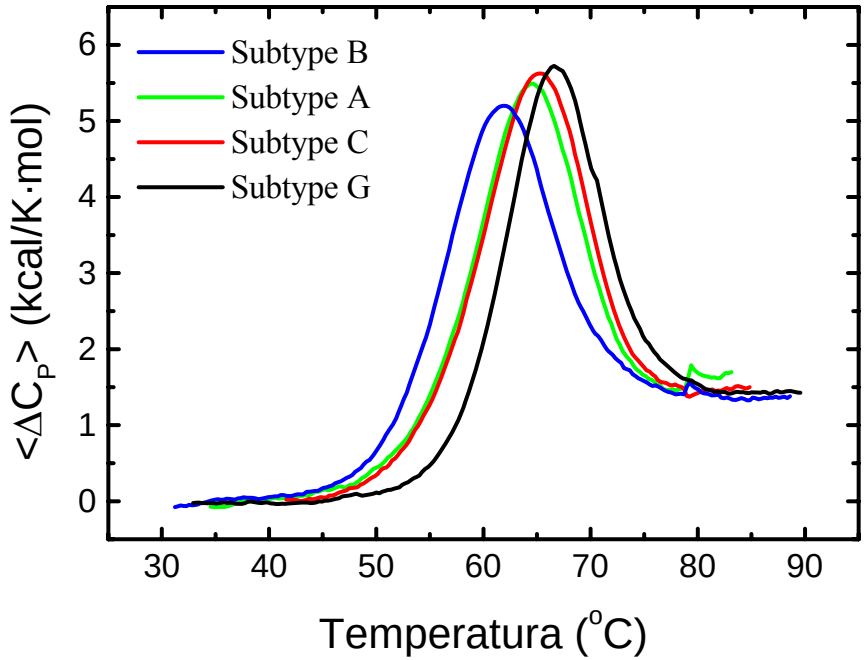
Posible justificación de una estabilidad no muy elevada:

- Permitir cierta flexibilidad y dinámica relacionadas con la función molecular
- Posibilitar la degradación mediante proteasas
- Impedir cinética de plegamiento lenta con intermedios de plegamiento muy estables
- Evitar la aparición de estructuras incorrectamente plegadas estables (trampas cinéticas)

Razones para una estabilidad no muy baja:

- Estados parcial- o totalmente desplegados significativamente poblados (inactivos y susceptibles de degradación)

Diferencias de estabilidad



Problema: $\Delta G = \Delta G(T)$

↓

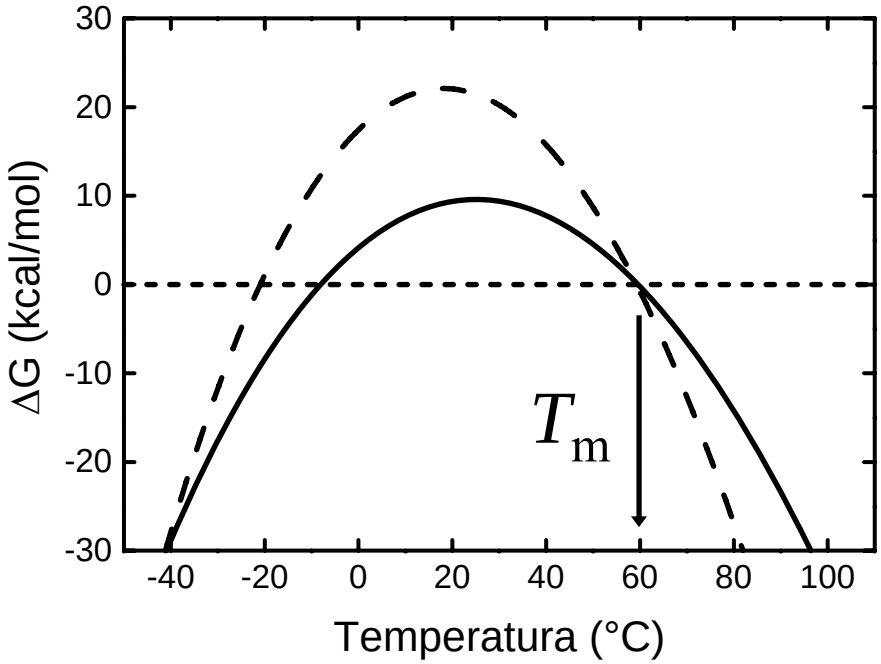
Utilizar una temperatura común

$$\Delta\Delta G(T_m^{WT}) = \Delta G^{MUT}(T_m^{WT}) - \Delta G^{WT}(T_m^{WT})$$

$$\Delta C_P^{MUT} \approx \Delta C_P^{WT} \longrightarrow \Delta\Delta G(T_m^{WT}) \approx \Delta S(T_m^{WT})\Delta T_m$$

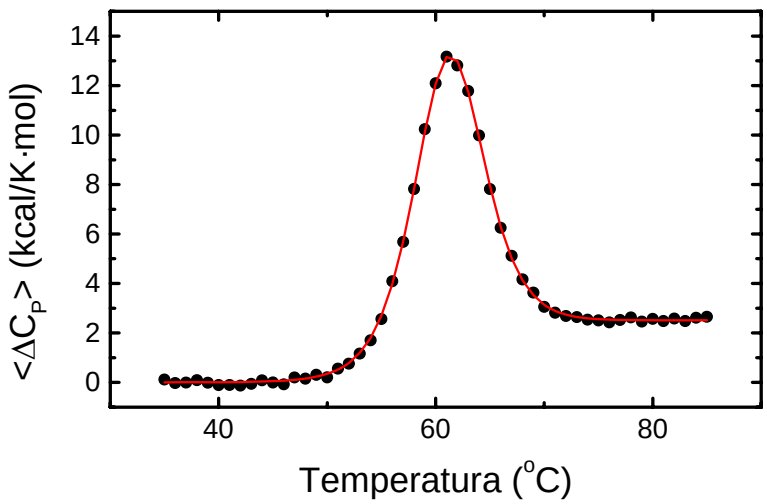
$$\Delta\Delta G \approx -\left(\frac{\partial \Delta G}{\partial X}\right)_{WT} \Delta X_m = -\left(\frac{\partial \Delta G}{\partial X}\right)_{WT} \left(X_m^{MUT} - X_m^{WT}\right)$$

Estabilidad a elevada y baja temperatura

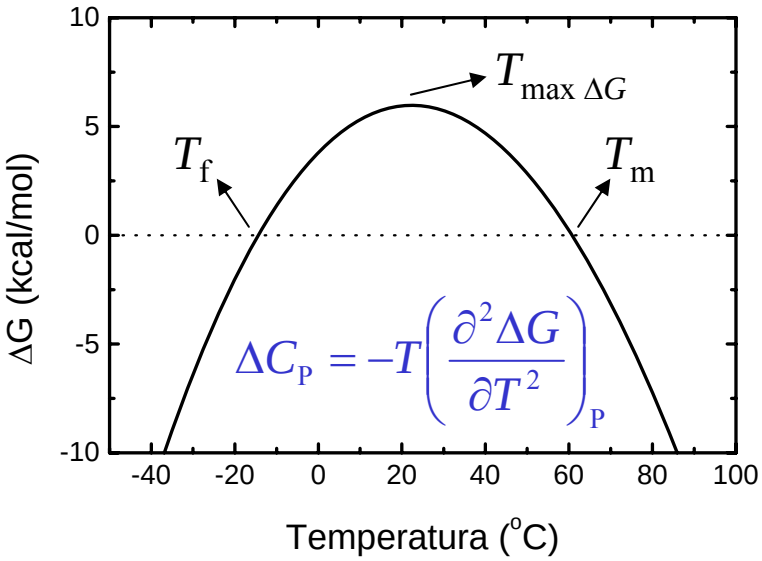


$\Delta H(T_m)$	180 kcal/mol
$\Delta S\ (T_m)$	0.54 kcal/(K·mol)
ΔC_P	5.0 kcal/(K·mol)
$\Delta H\ (T_m)$	350 kcal/mol
$\Delta S\ (T_m)$	1.05 kcal/(K·mol)
ΔC_p	8.0 kcal/(K·mol).

¿ Qué proteína es más estable ?



T_m	60.75 ± 0.03 °C
$\Delta H(T_m)$	102 ± 0.3 kcal/mol
ΔC_P	2.51 ± 0.02 kcal/K·mol



$$T_f \approx \frac{T_m^2}{3T_m - 2T_H} \qquad T_f \approx 2T_S - T_m$$

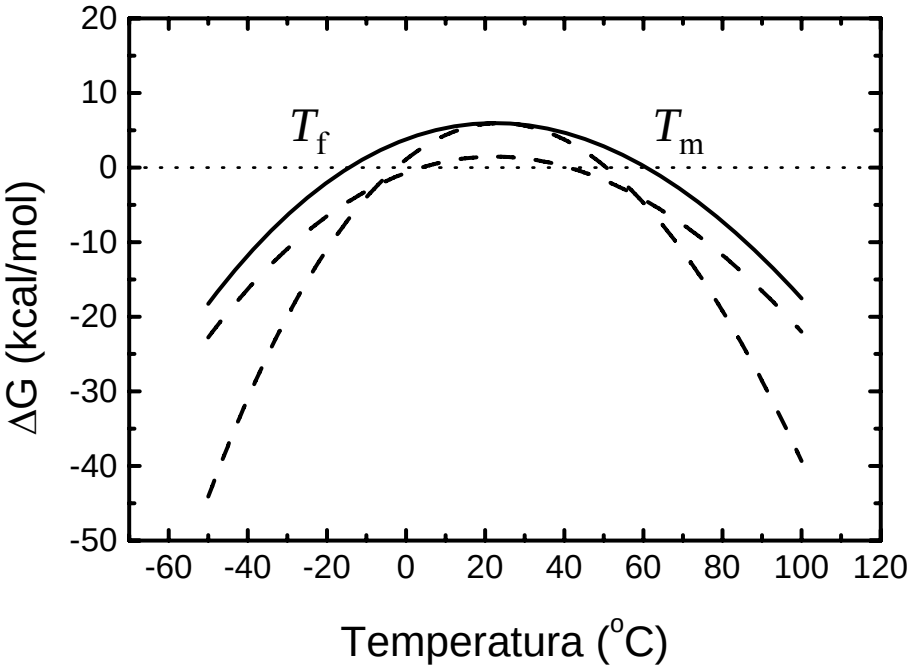
$$T_{\max_T \Delta G} = T_S = T_m \exp\left(-\frac{\Delta H(T_m)}{\Delta C_P T_m}\right)$$

$$\max_T \Delta G = \Delta H(T_m) - \Delta C_P T_m \left(1 - \exp\left(-\frac{\Delta H(T_m)}{\Delta C_P T_m}\right)\right)$$

$$\Delta C_P = -T\left(\frac{\partial^2 \Delta G}{\partial T^2}\right)_P$$

$$\Delta G(T_m) = \Delta G(T_f) = 0$$

Desnaturalización Fría



Para una proteína globular típica:

T_m	~ 50-70 °C
$\Delta H(T_m)$	~ 80-120 kcal/mol
ΔC_P	~ 2-3 kcal/K·mol
$\Delta G(25\ °C)$	~ 5-10 kcal/mol
$T_{max\Delta G}$	~ 20-30 °C
T_f	< 0 °C

Desplegamiento de tres estados

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG_1	K_1
	ΔG_2	K_2
	$\Delta G_1+\Delta G_2$	K_1K_2

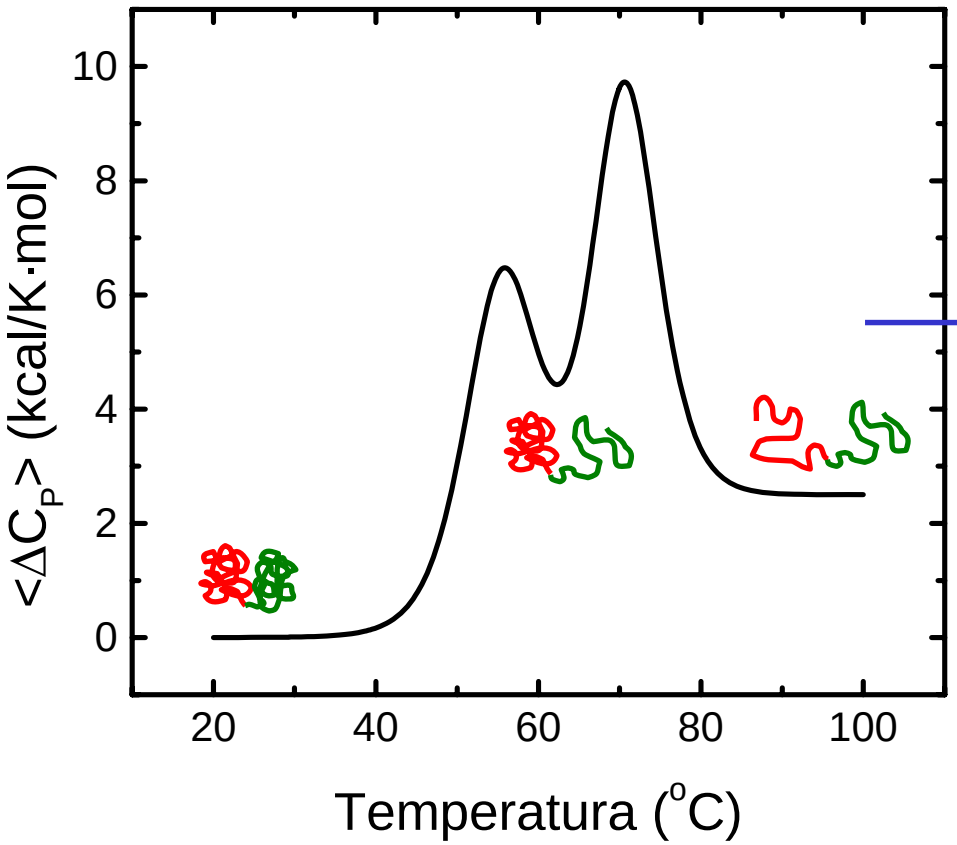
$$Q = 1 + K_1 + K_2 + K_1K_2 = (1 + K_1)\,(1 + K_2)$$

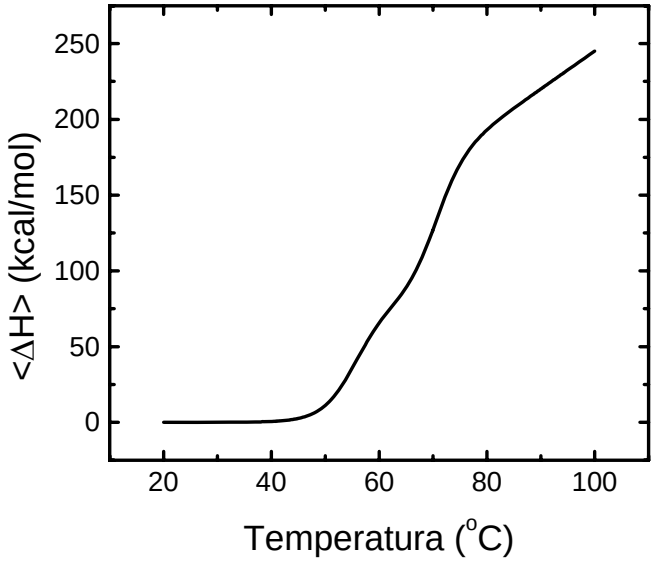
$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG_1	K_1
	$\Delta G_1+\Delta G_2$	K_1K_2

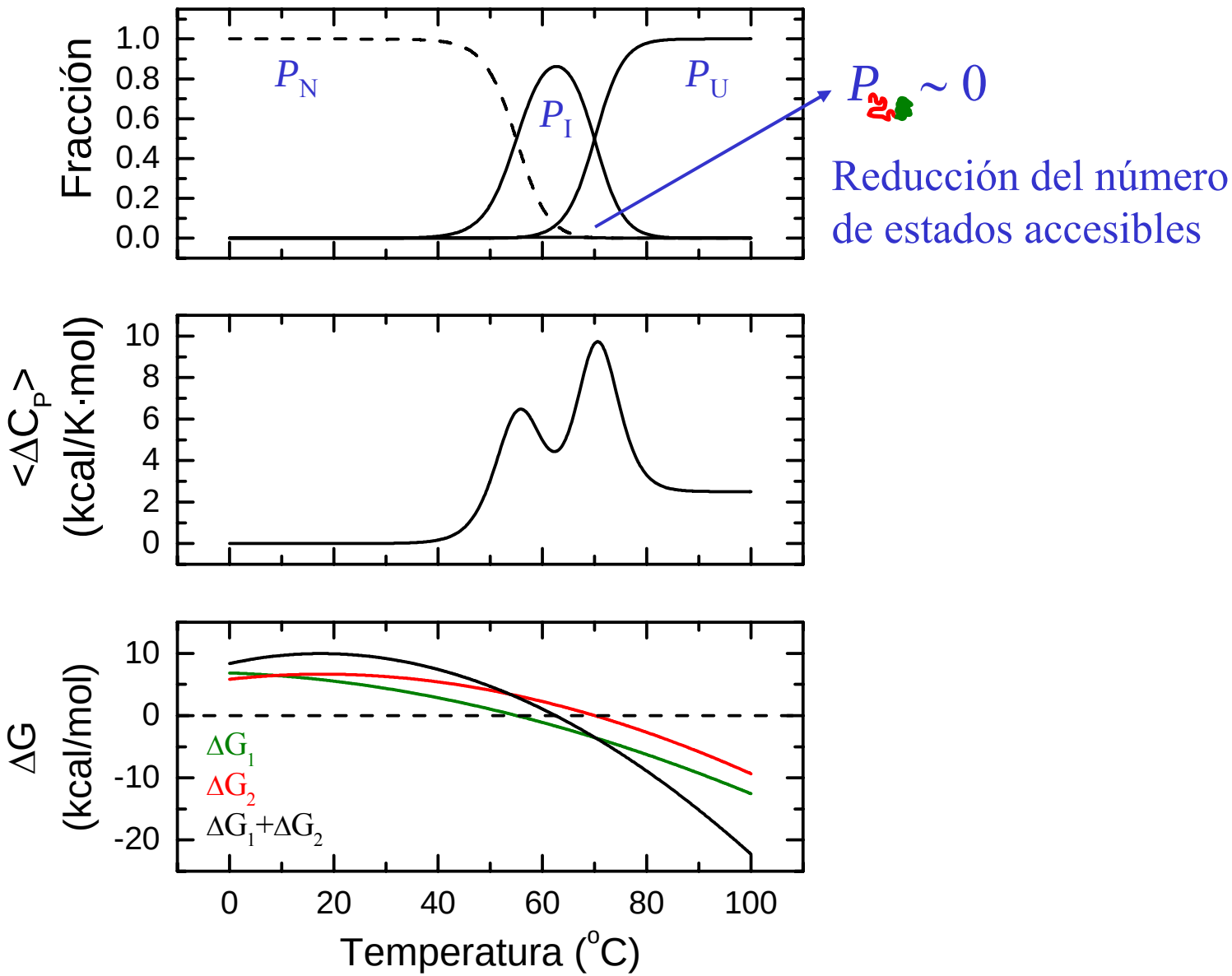
$$Q = 1 + K_1 + K_1K_2 \neq (1 + K_1)\,(1 + K_2)$$

$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$

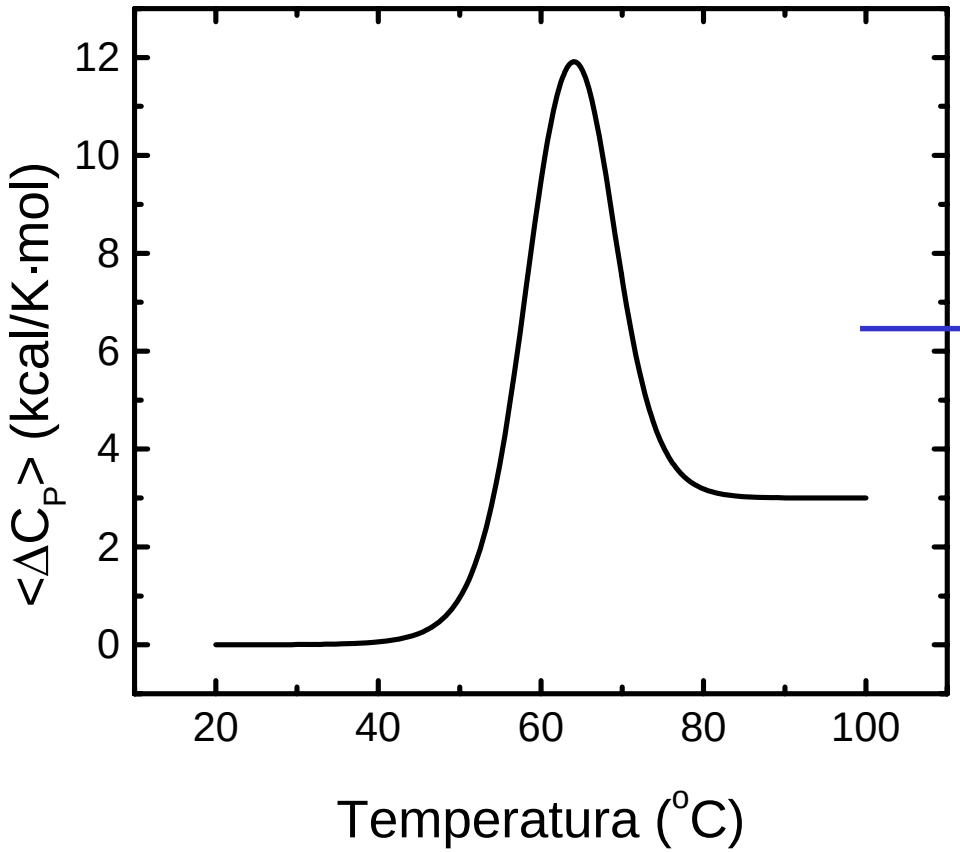


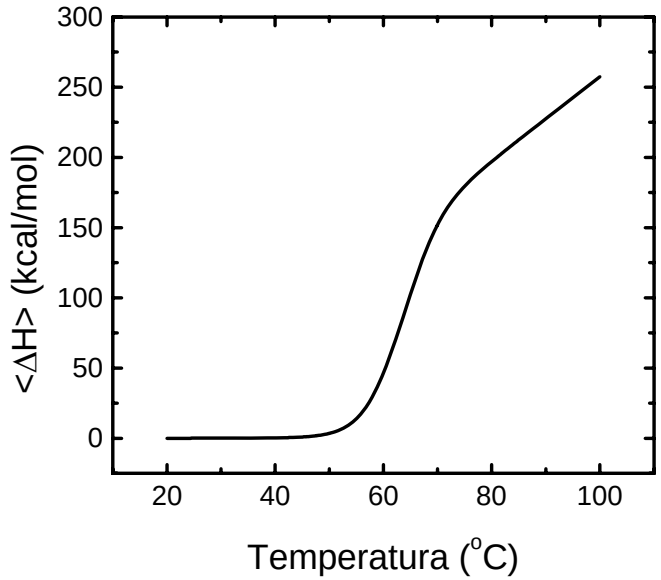


$$\langle \Delta H \rangle = \int_{T_0}^{T} \langle \Delta C_{\mathrm{P}} \rangle dT$$


Fracción
1.0
0.8
0.6
0.4
0.2
0.0
P_N
P_I
P_U
$P_{2_1} \sim 0$
Reducción del número
de estados accesibles
<ΔC_P>
(kcal/K·mol)
10
8
6
4
2
0
ΔG
(kcal/mol)
10
0
-10
-20
ΔG_1
ΔG_2
ΔG_1+ΔG_2
0
20
40
60
80
100
Temperatura (°C)

¿Transición de dos estados ?





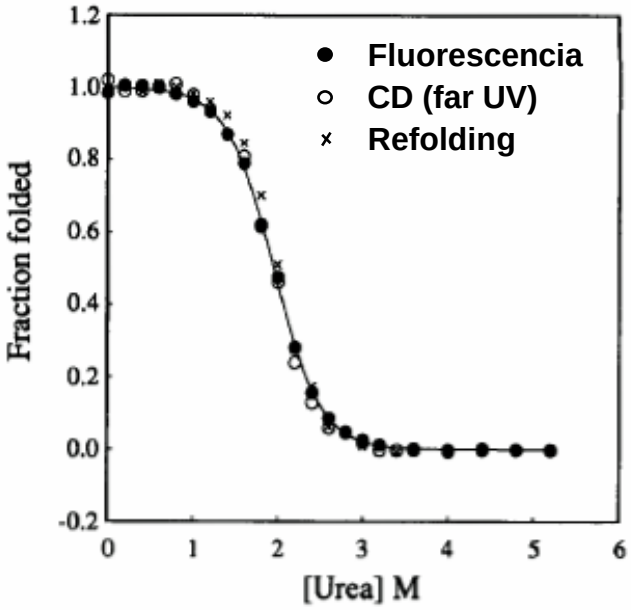
$$\langle \Delta H \rangle = \int_{T_0}^{T} \langle \Delta C_{\mathrm{P}} \rangle dT$$

Tests de dos estados

- Superposición de las curvas de desplegamiento obtenidas con diversas técnicas (espectroscopía)

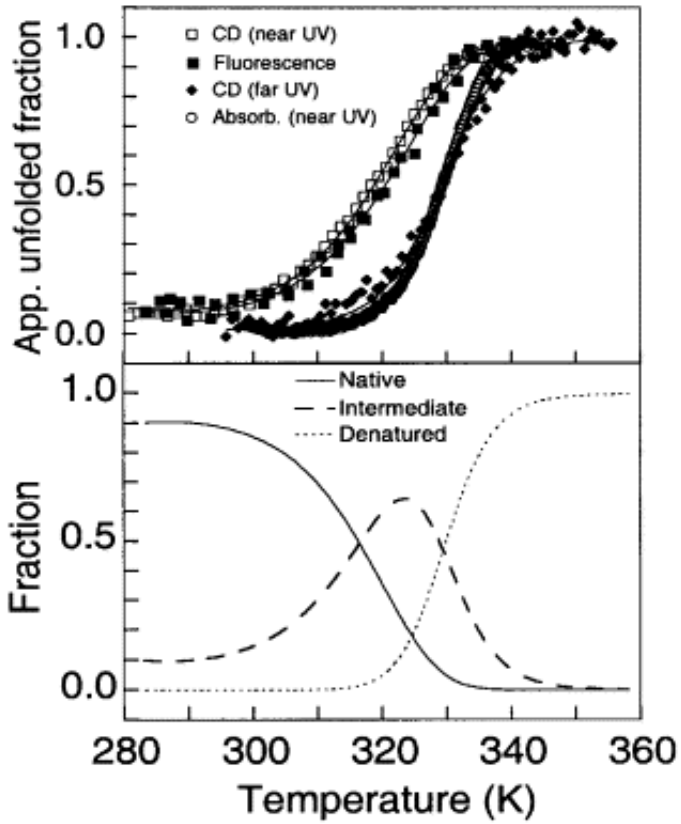
- Valor del cociente $\frac{\Delta H_{\mathrm{vH}}}{\Delta H_{\mathrm{cal}}}$ (calorimetría)

Dos estados



Genzor et al. *Protein Science* (1996) 5, 1376-1388

Tres estados



Irún et al. *J. Mol. Biol.* (2001) 306, 877-888

¡ Cuidado !

- Superposición de curvas obtenidas con distintas técnicas es condición necesaria pero no suficiente
- Espectroscopía nos informa de comportamiento global y/o local
- Transición de dos estados implica total cooperatividad (ausencia de otros estados parcialmente plegados)
- Errores y ruido en las medidas pueden enmascarar algunos detalles y fenómenos

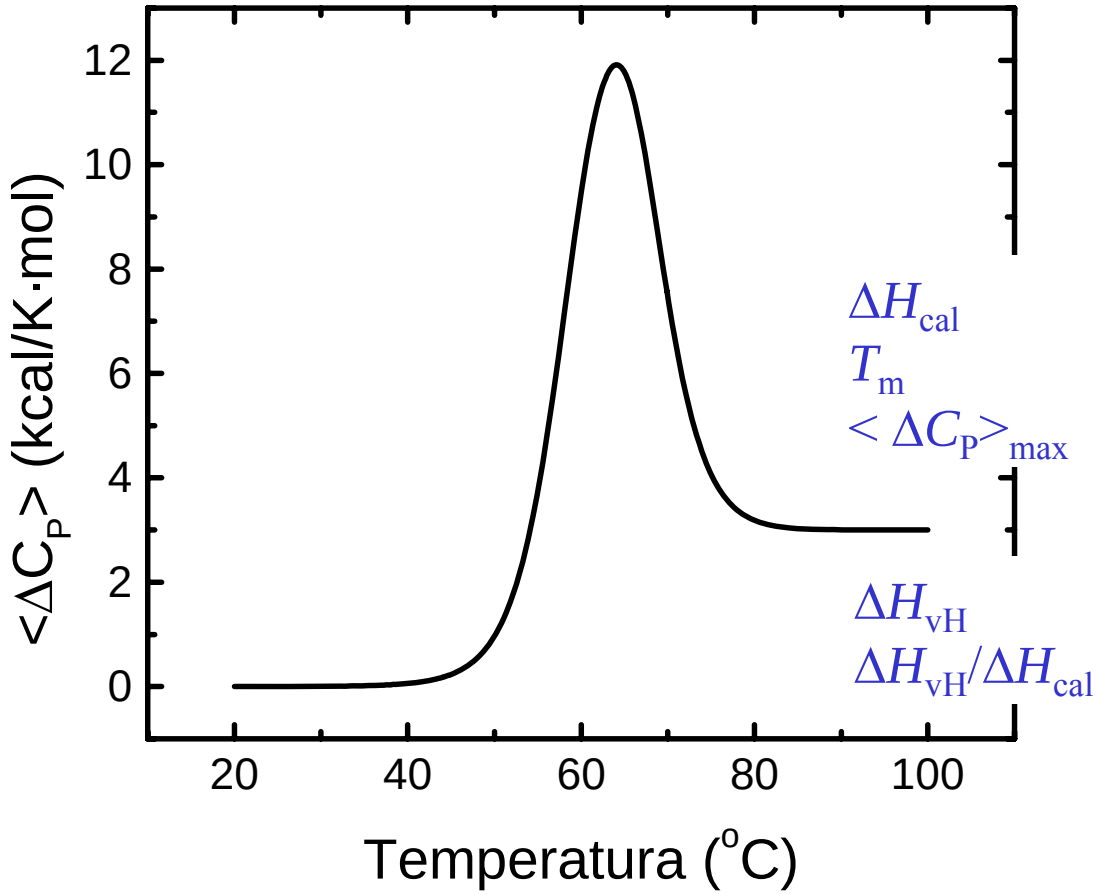
$$\frac{\partial \ln K}{\partial T} = \frac{\Delta H}{RT^2}$$ van't Hoff enthalpy

$$\Delta H_{\mathrm{vH}} = \frac{4RT_{\mathrm{m}}^2 < \Delta C_{\mathrm{P}} >_{\mathrm{max}}}{\Delta H_{\mathrm{cal}}}$$

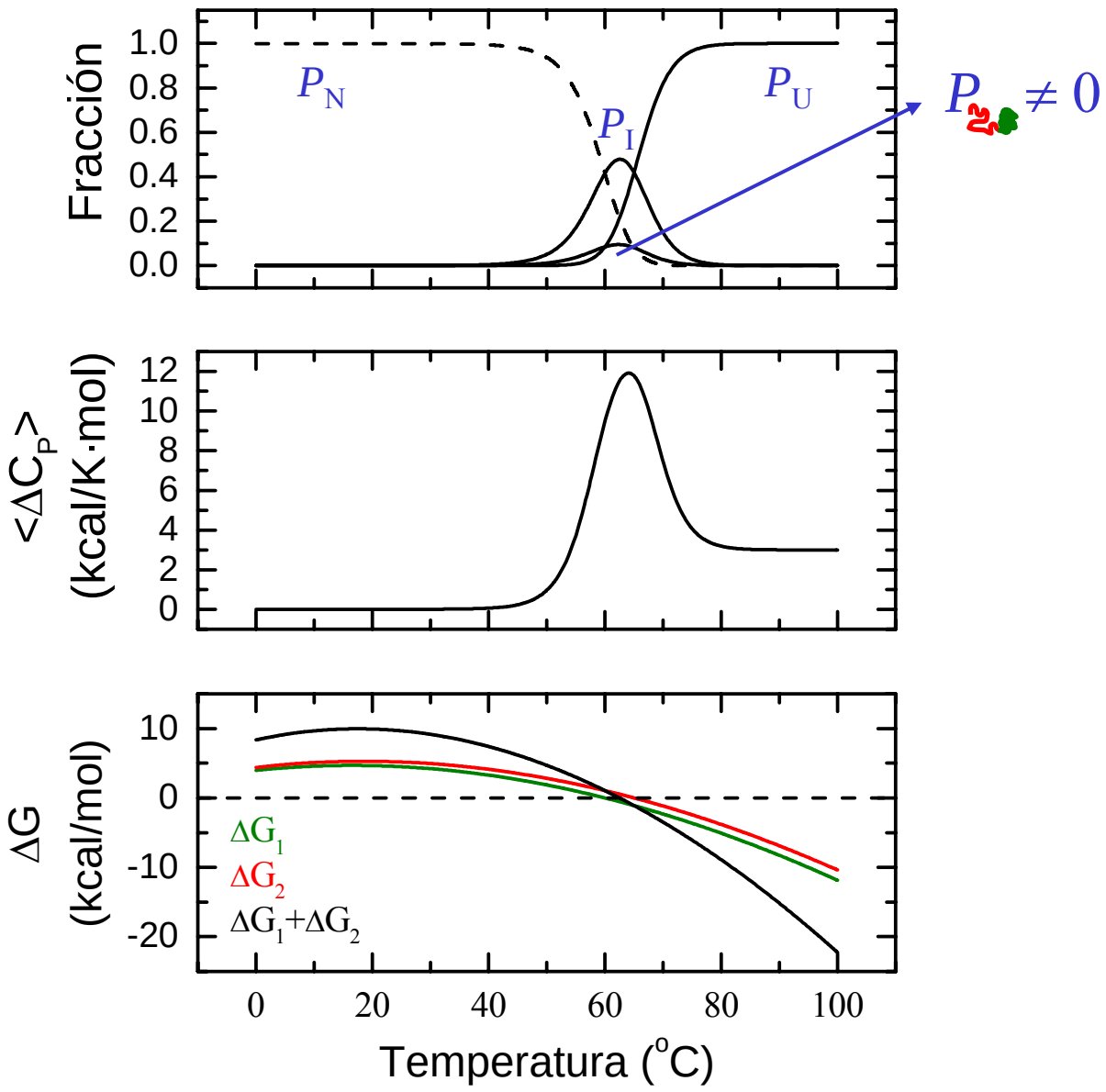
$\frac{\Delta H_{\mathrm{vH}}}{\Delta H_{\mathrm{cal}}} = 1$ unidad cooperativa ≡ molécula (dos estados)

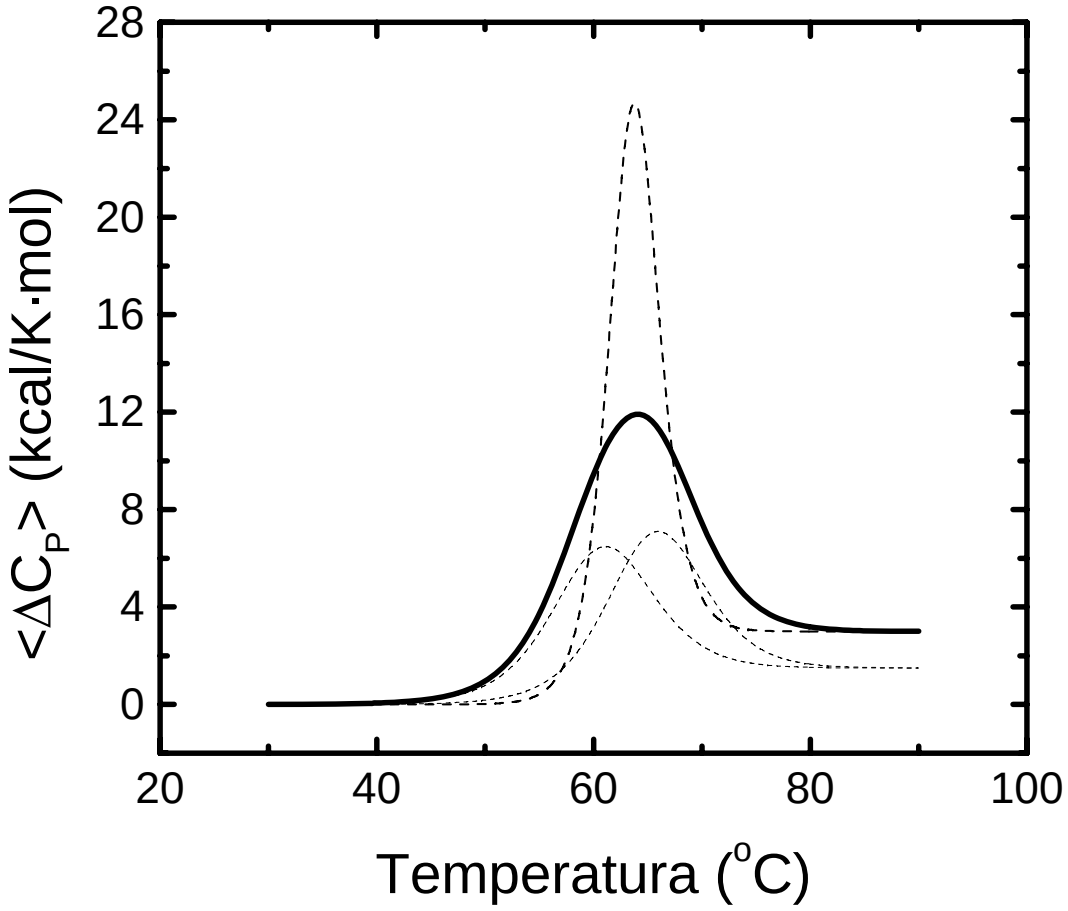
$\frac{\Delta H_{\mathrm{vH}}}{\Delta H_{\mathrm{cal}}} < 1$ unidad cooperativa < molécula (estados intermedios)

$\frac{\Delta H_{\mathrm{vH}}}{\Delta H_{\mathrm{cal}}} > 1$ unidad cooperativa > molécula (nativo está asociado)

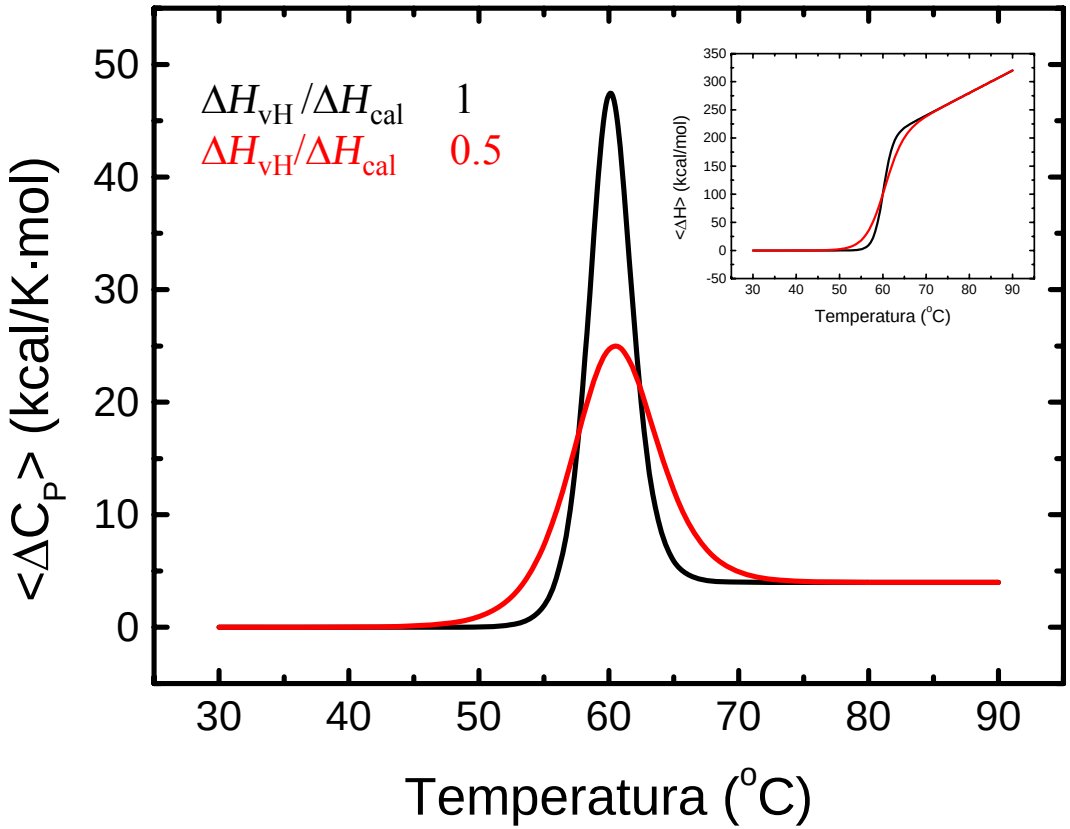


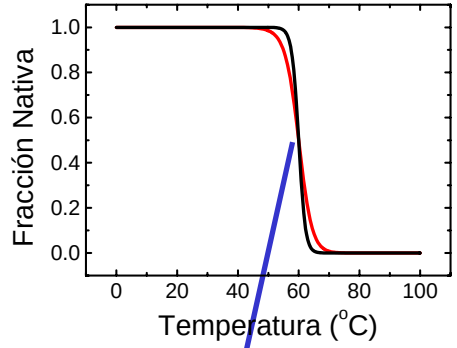
ΔH_{cal}	144.3 kcal/mol
T_m	63.5 ºC
$\langle \Delta C_P \rangle_{max}$	10.2 kcal/K·mol
ΔH_{vH}	63.4 kcal/mol
$\Delta H_{vH}/\Delta H_{cal}$	0.44


Fracción
1.0
0.8
0.6
0.4
0.2
0.0
P_N
P_I
P_U
$P_{2I} \neq 0$
<ΔC_P>
(kcal/K·mol)
12
10
8
6
4
2
0
ΔG
(kcal/mol)
10
0
-10
-20
ΔG_1
ΔG_2
ΔG_1+ΔG_2
0
20
40
60
80
100
Temperatura (°C)

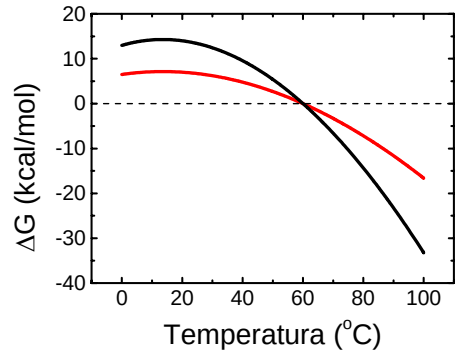

28
24
20
16
12
8
4
0
20
40
60
80
100
<ΔC_P> (kcal/K·mol)
Temperatura (°C)

T_m	60ºC	60ºC
$\Delta H(T_m)$	200 kcal/mol	2 × 100 kcal/mol
ΔC_P	4 kcal/K·mol	2 × 2 kcal/K·mol





$$\left.\frac{\partial P_N}{\partial T}\right|_{T=T_m} = -\frac{\Delta H_{vH}}{4RT_m^2}$$

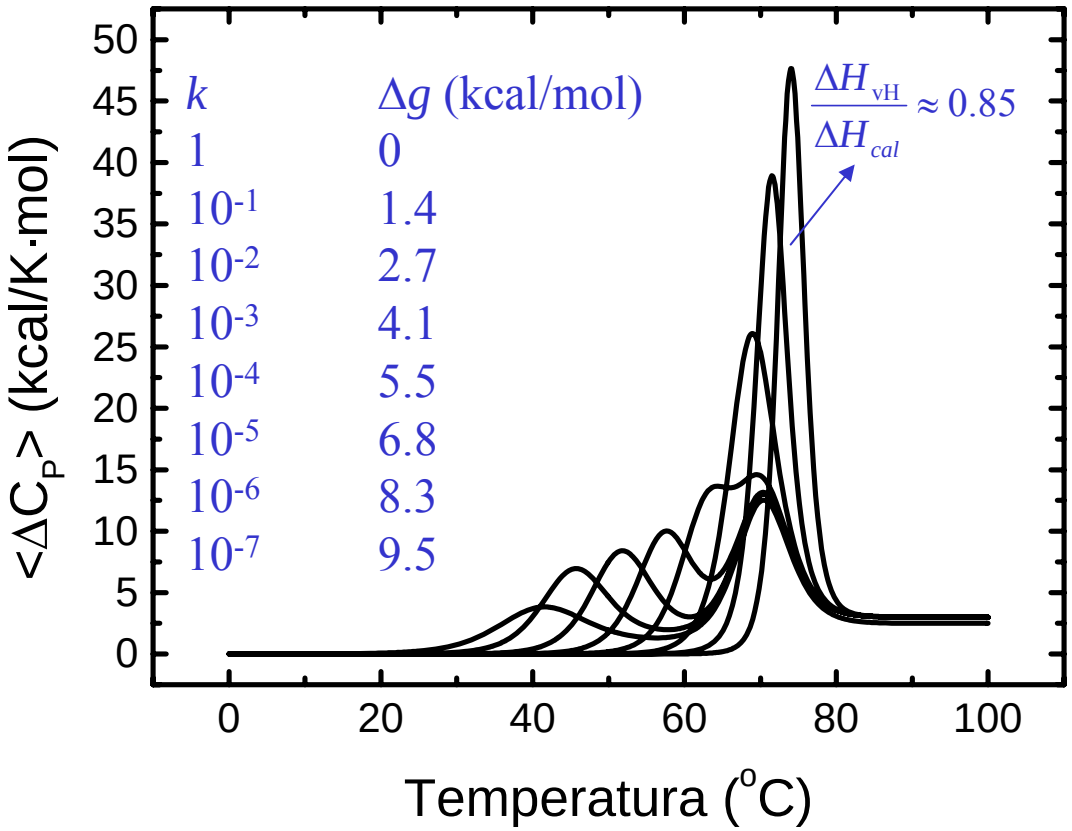


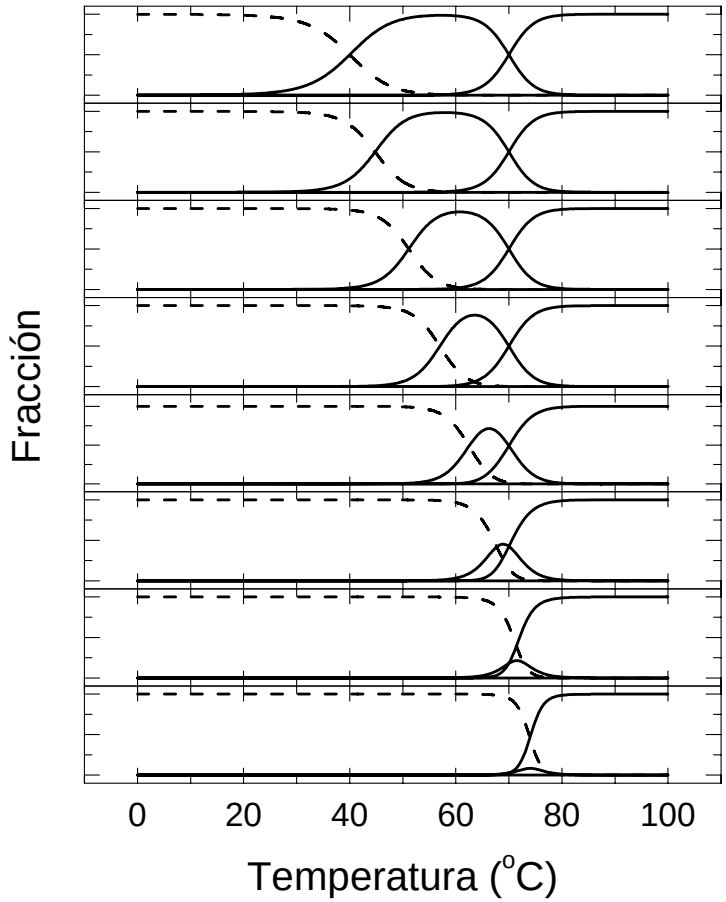
Dos dominios interaccionantes

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	$\Delta G_1+\Delta g$	$K_1 k$
	$\Delta G_2+\Delta g$	$K_2 k$
	$\Delta G_1+\Delta G_2+\Delta g$	$K_1 K_2 k$

$$Q = 1 + kK_1 + kK_2 + kK_1K_2 \neq (1 + kK_1)\,(1 + kK_2)$$

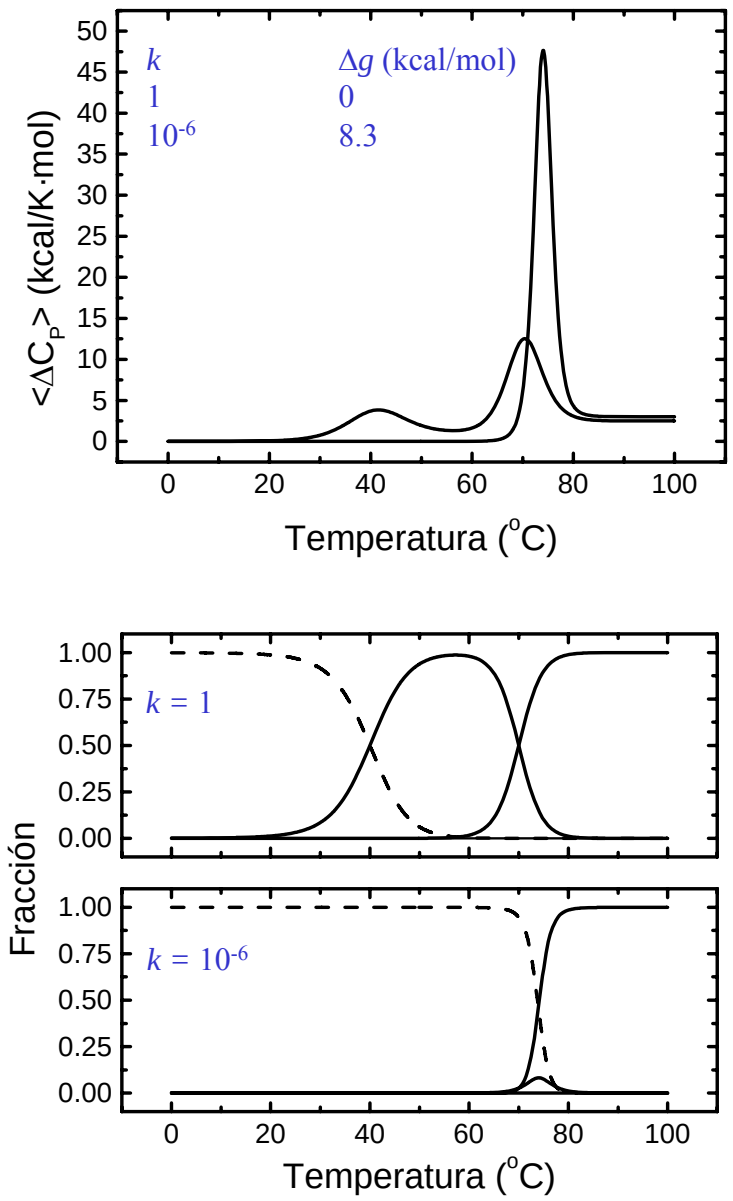
$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$

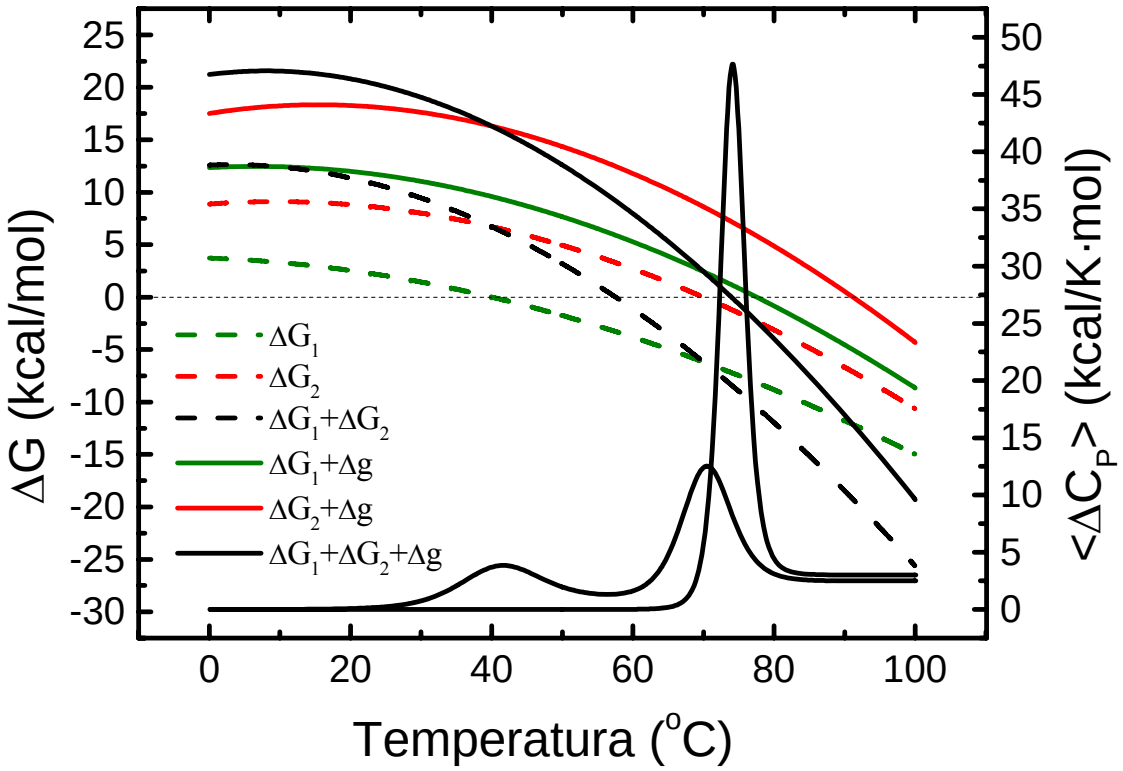




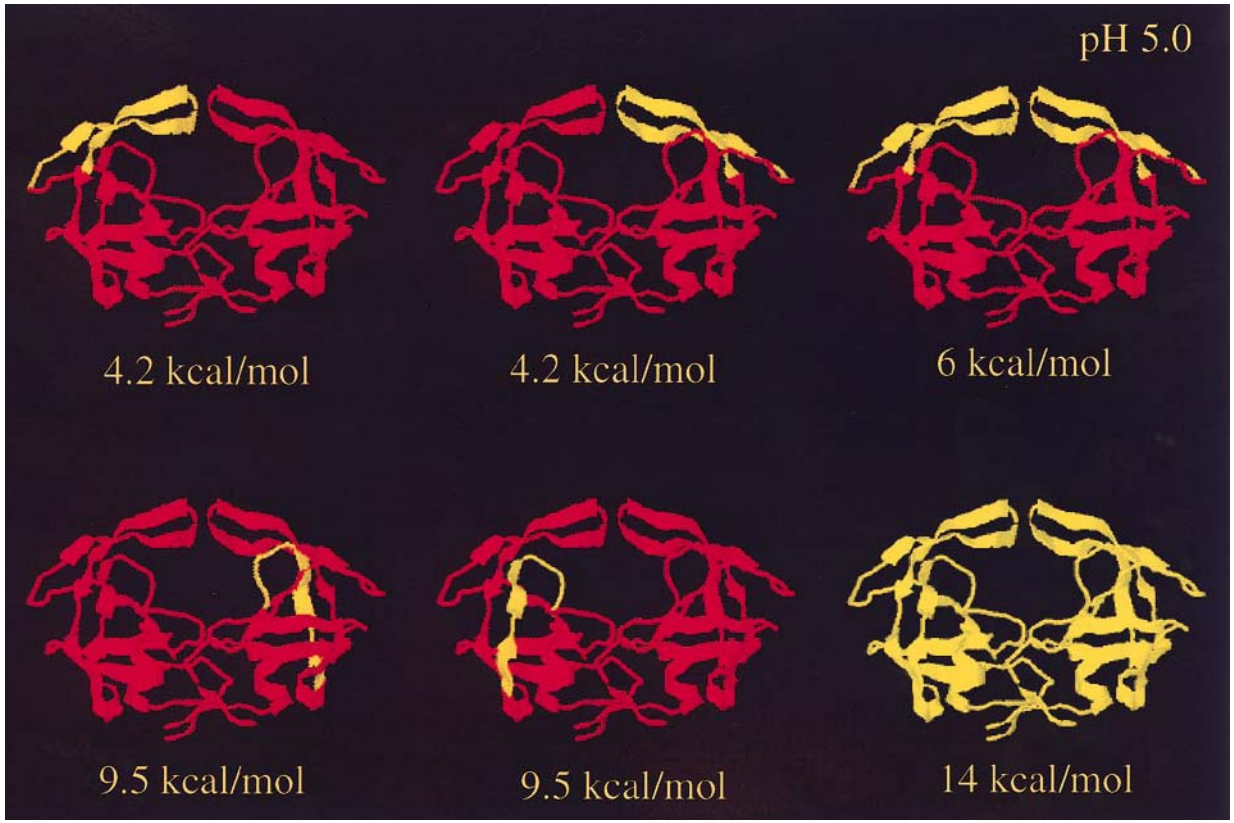
$k = 1 \quad \Delta g = 0$ **independencia**

$k \neq 1 \quad \Delta g \neq 0$ **cooperatividad**





Reducción del número de estados accesibles



Cooperatividad ⇒ No aditividad

Todd et al. *J. Mol. Biol*. (1998) 283, 475-88

Unión de Ligandos
y
Estabilidad de Proteínas

$$N \underset{K^0}{\leftrightarrow} U$$

$$K^0 = \frac{[U]}{[N]}$$

$$\Delta G^0 = -RT \ln K^0$$

$$P_{\mathrm{N}} = \frac{[N]}{[P]_{\mathrm{T}}} = \frac{1}{1 + K^0}$$

$$P_{\mathrm{U}} = \frac{[U]}{[P]_{\mathrm{T}}} = \frac{K^0}{1 + K^0}$$

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG^0	K^0

$$Q = 1 + K^0$$

$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$

$$
\begin{array}{ccc}
N & \overset{K^0}{\leftrightarrow} & U \\
+ & & \\
L & & \\
\updownarrow K_a & & \\
NL & &
\end{array}
$$

$$K = \frac{[U]}{[N]+[NL]} = \frac{[U]}{[N]}\frac{1}{1+K_a[L]} = \frac{K^0}{1+K_a[L]}$$

$$\Delta G = \Delta G^0 + RT\ln\left(1+K_a[L]\right)$$

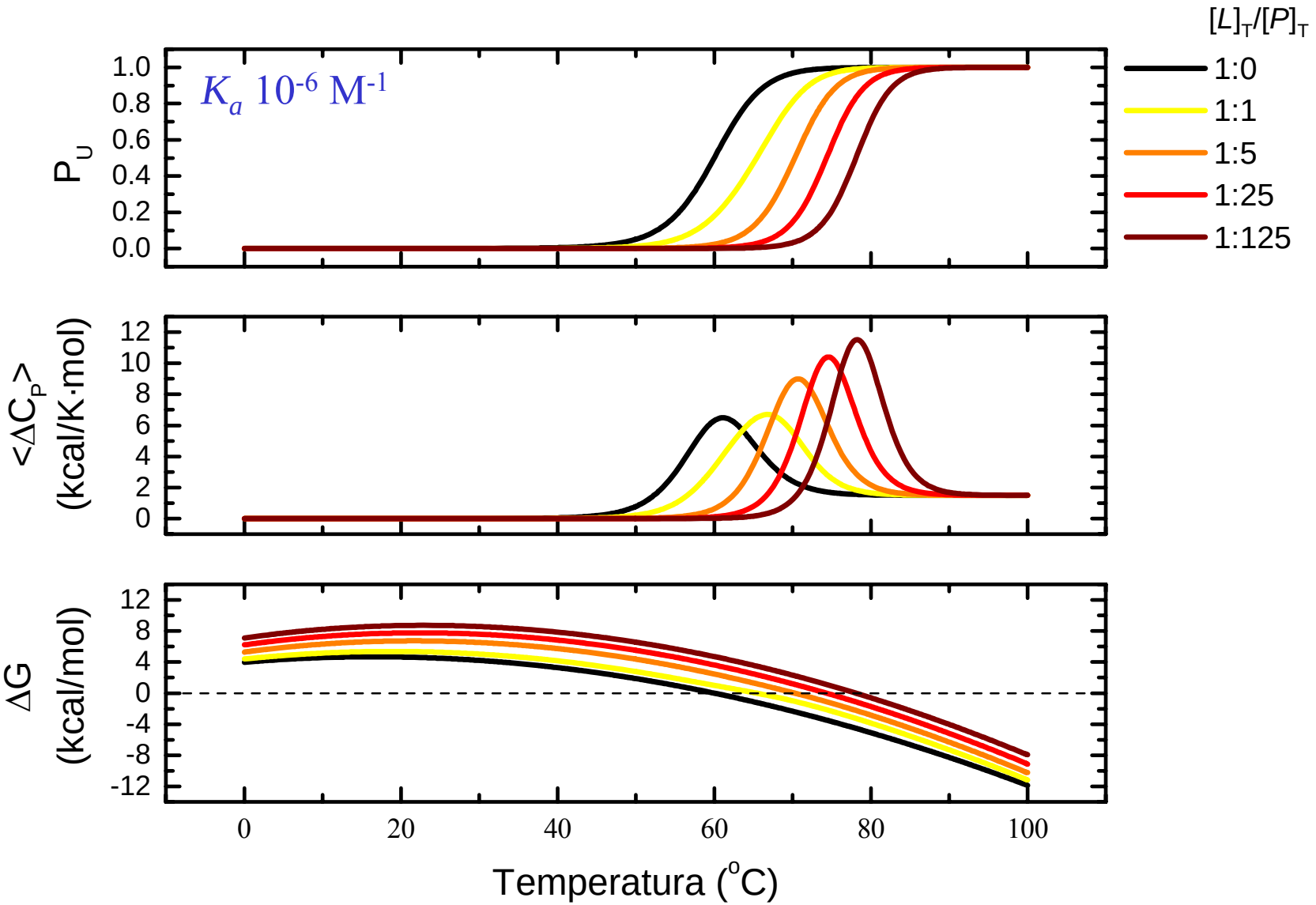
$$P_{\mathrm{N}} = \frac{[N]+[NL]}{[P]_{\mathrm{T}}} = \frac{1}{1+K} = \frac{1+K_a[L]}{1+K_a[L]+K^0}$$

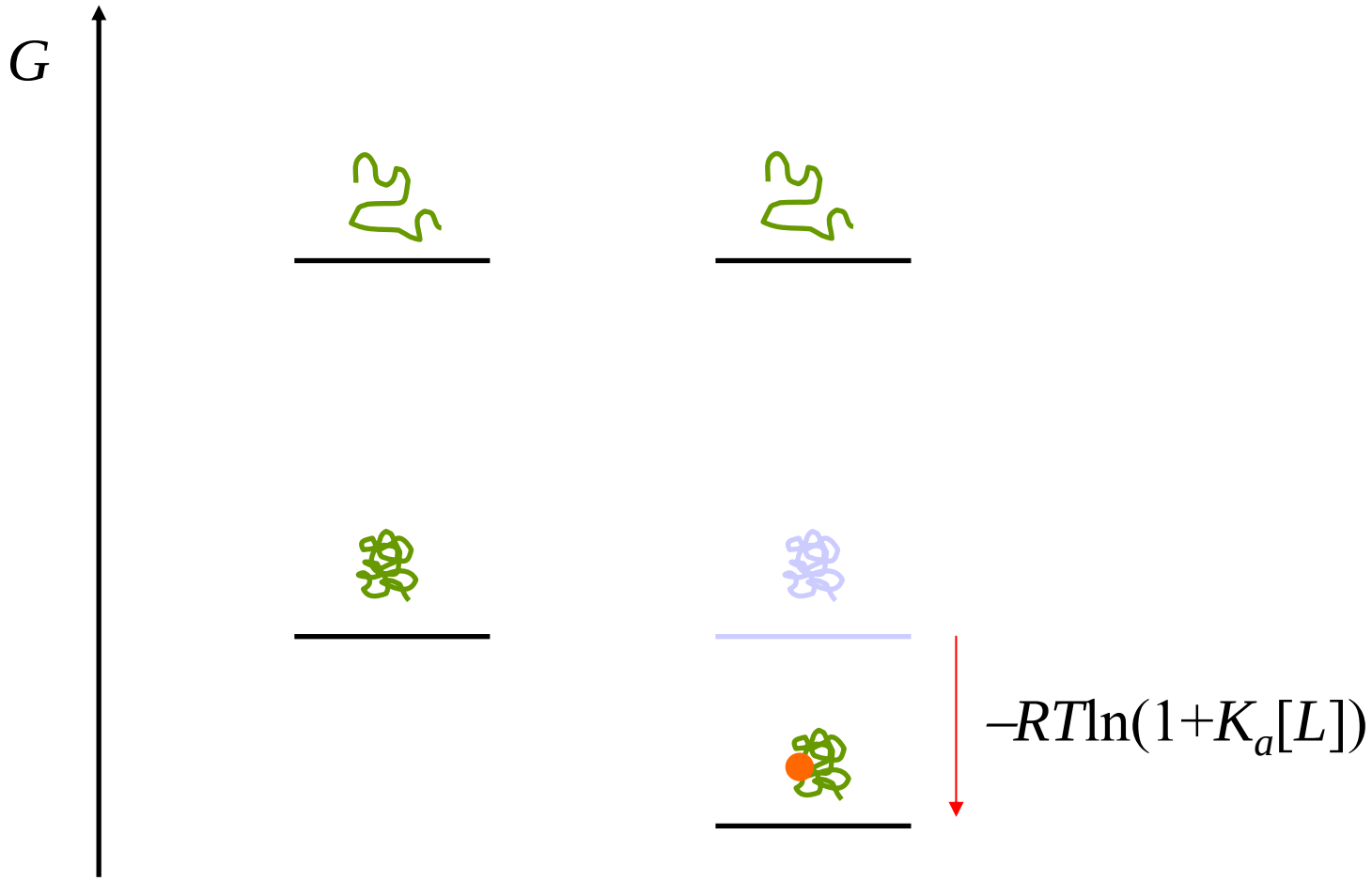
$$P_{\mathrm{U}} = \frac{[U]}{[P]_{\mathrm{T}}} = \frac{K}{1+K} = \frac{K^0}{1+K_a[L]+K^0}$$

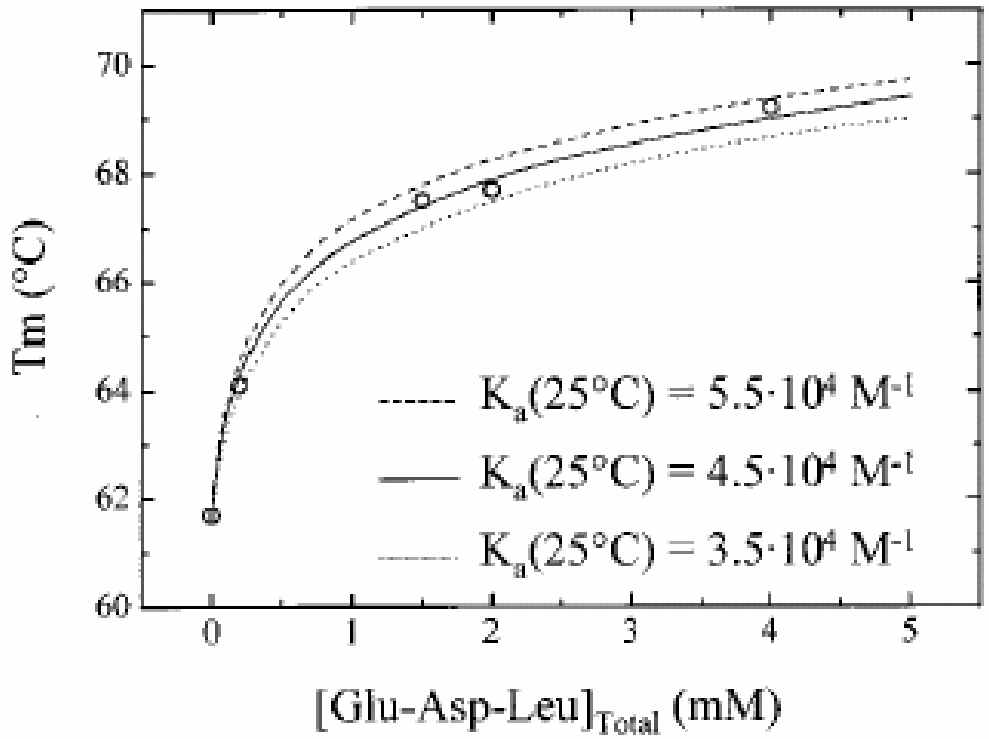
i	ΔG_{i}	$\exp(-\Delta G_{\mathrm{i}}/RT)$
	0	1
	ΔG_{bind}	$K_a[L]$
	ΔG^0	K^0

$$Q = 1 + K_a[L] + K^0$$

$$P_{\mathrm{i}} = \frac{\exp\left(-\Delta G_{\mathrm{i}} / RT\right)}{Q}$$


K_a 10^{-6} M^{-1}
P_U
1.0
0.8
0.6
0.4
0.2
0.0
$[L]_T/[P]_T$
1:0
1:1
1:5
1:25
1:125
$<\Delta C_P>$ (kcal/K·mol)
12
10
8
6
4
2
0
ΔG (kcal/mol)
12
8
4
0
-4
-8
-12
0
20
40
60
80
100
Temperatura (°C)


G
–RTln(1+Ka[L])


Tm (°C)
70
68
66
64
62
60
0
1
2
3
4
5
[Glu-Asp-Leu]Total (mM)
Ka(25°C) = 5.5·10⁴ M⁻¹
Ka(25°C) = 4.5·10⁴ M⁻¹
Ka(25°C) = 3.5·10⁴ M⁻¹

$$
\begin{array}{ccc}
N & \leftrightarrow & U \\
 & K^0 & + \\
 & & L \\
 & K_a \updownarrow & \\
 & & UL
\end{array}
$$

$$K = \frac{[U]+[UL]}{[N]} = \frac{[U]}{[N]}\left(1+K_a[L]\right) = K^0\left(1+K_a[L]\right)$$

$$\Delta G = \Delta G^0 - RT\ln\left(1+K_a[L]\right)$$

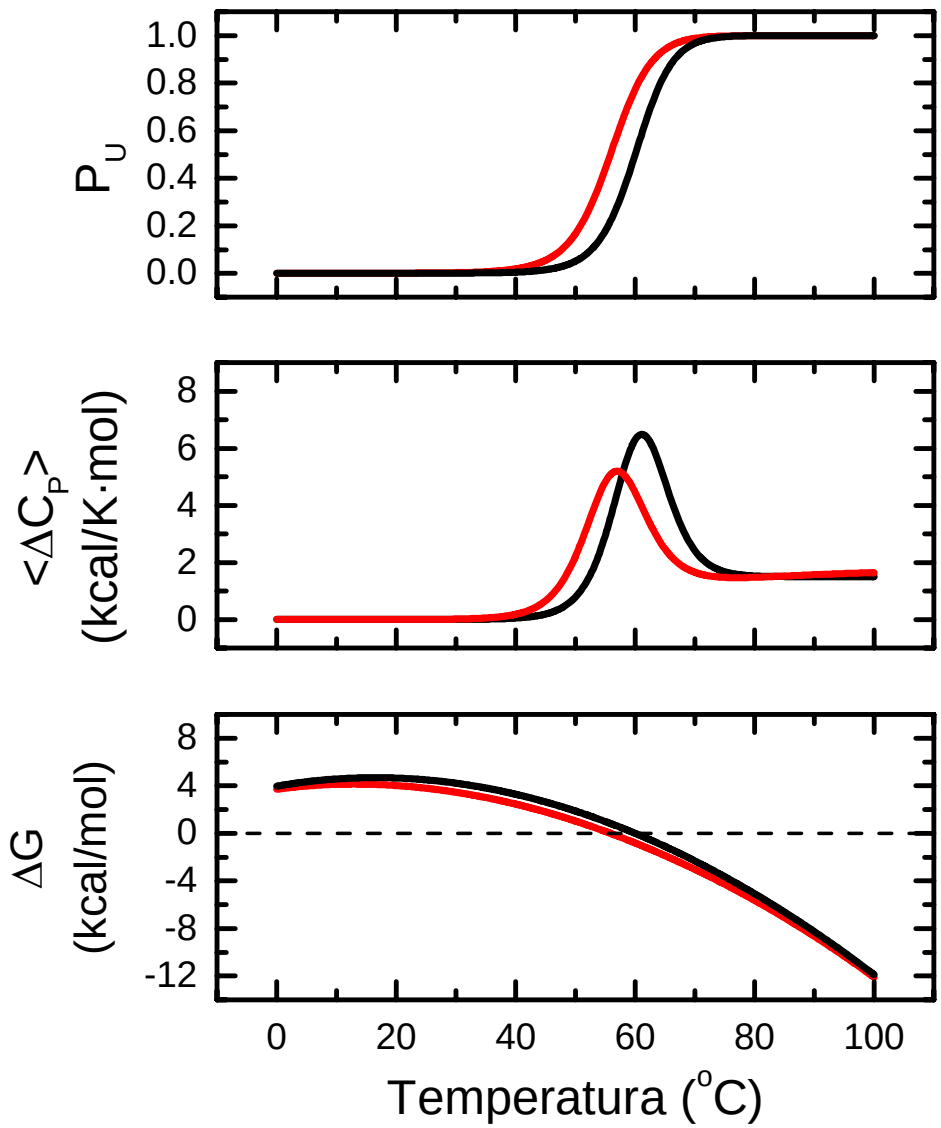
$$P_{\mathrm{N}} = \frac{[N]}{[P]_{\mathrm{T}}} = \frac{1}{1+K} = \frac{1}{1+K^0\left(1+K_a[L]\right)}$$

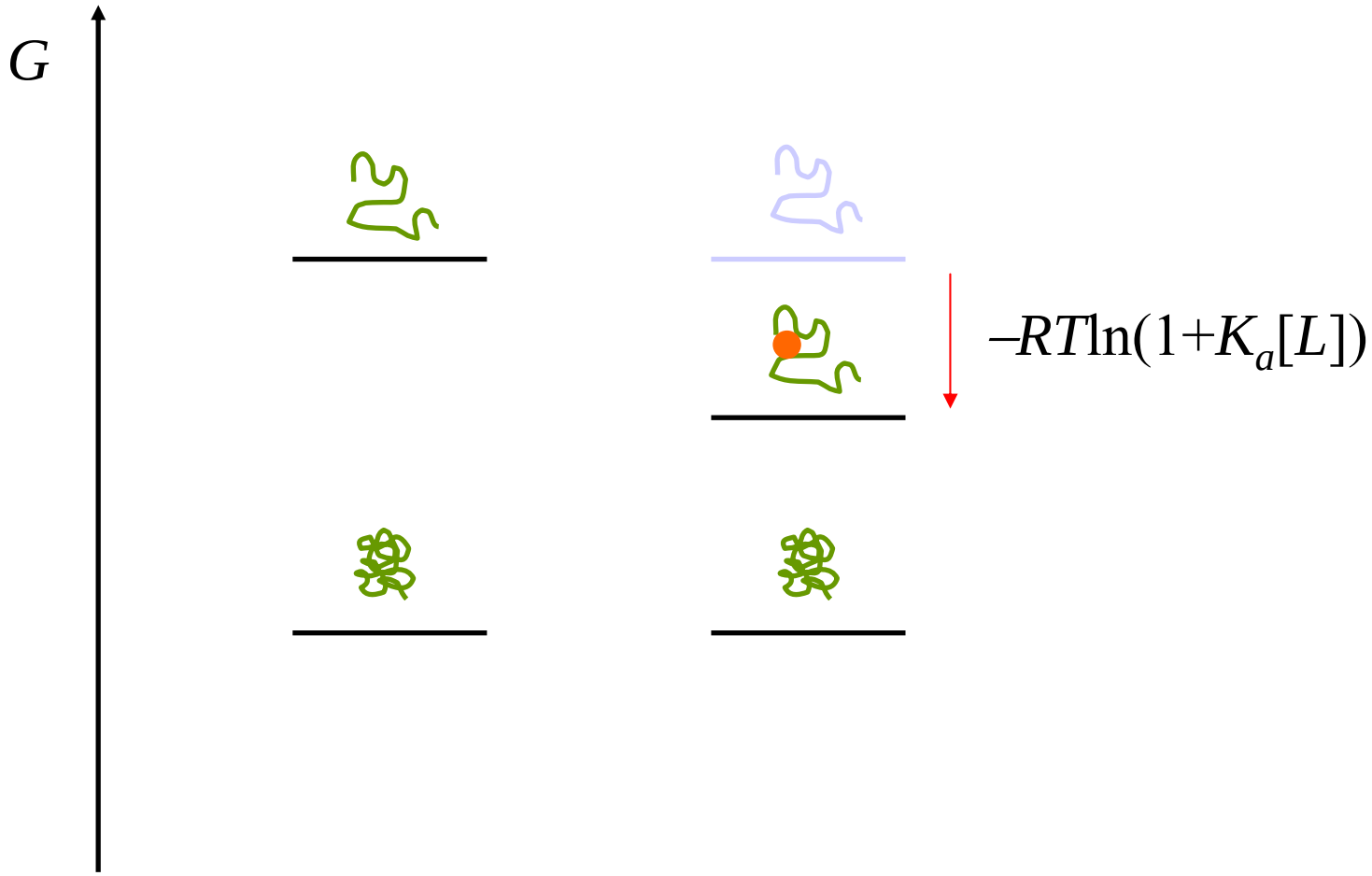
$$P_{\mathrm{U}} = \frac{[U]+[UL]}{[P]_{\mathrm{T}}} = \frac{K}{1+K} = \frac{K^0\left(1+K_a[L]\right)}{1+K^0\left(1+K_a[L]\right)}$$

i	ΔG_i	$\exp(-\Delta G_i/RT)$
	0	1
	ΔG^0	K^0
	$\Delta G^0+\Delta G_{\text{bind}}$	$K^0K_a[L]$

$$Q = 1 + K^0(1+K_a[L])$$

$$P_i = \frac{\exp\left(-\Delta G_i / RT\right)}{Q}$$


1.0
0.8
0.6
0.4
0.2
0.0
P_U
8
6
4
2
0
$<\Delta C_p>$
(kcal/K·mol)
8
4
0
-4
-8
-12
ΔG
(kcal/mol)
0
20
40
60
80
100
Temperatura (^oC)


G
–RTln(1+Ka[L])

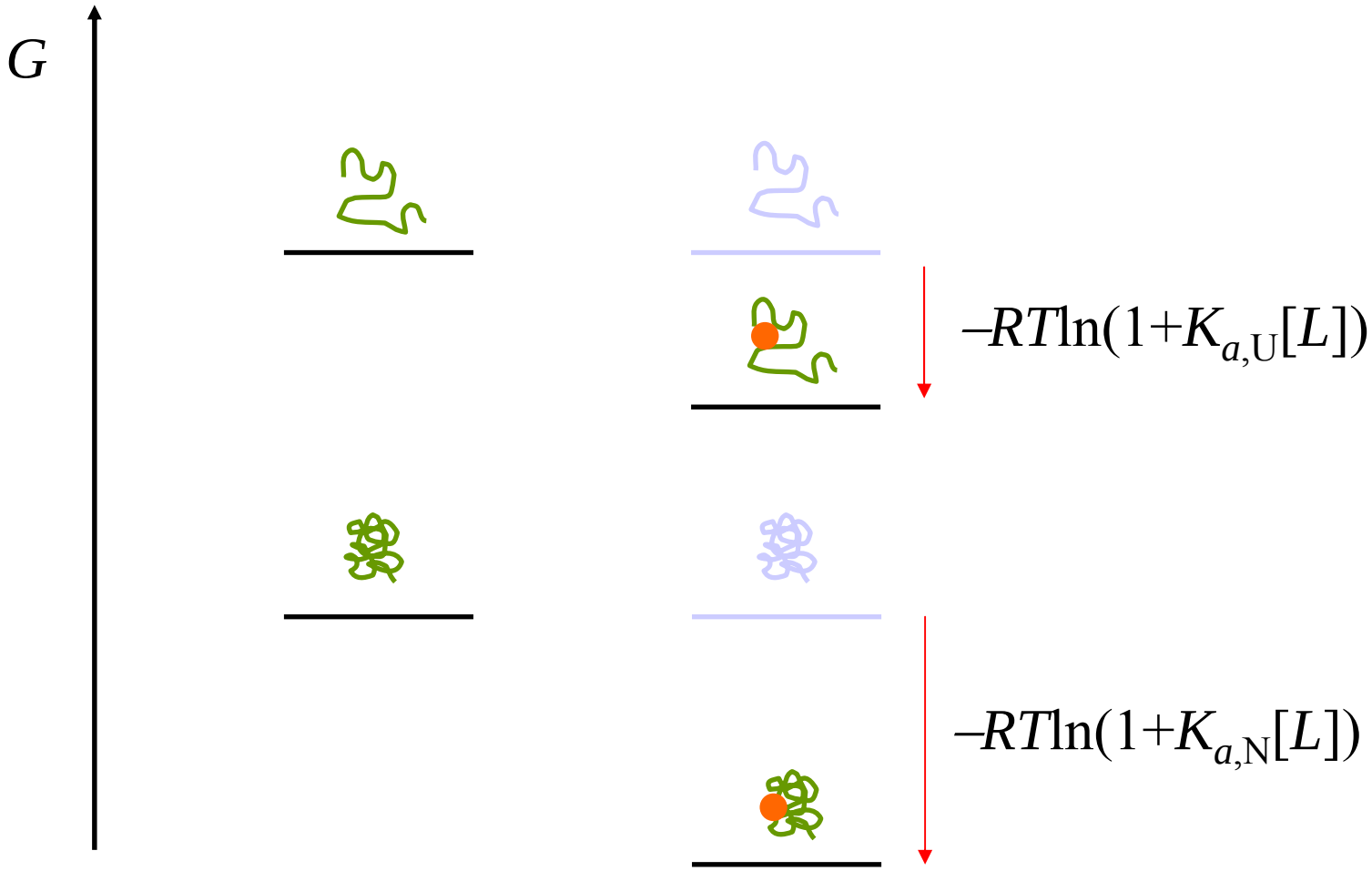
$$
\begin{array}{ccc}
N & \xleftrightarrow{K^0} & U \\
+ & & + \\
L & & L \\
K_{a,\mathrm{N}} \updownarrow & & \updownarrow K_{a,\mathrm{U}} \\
NL & & UL
\end{array}
$$

$$K = \frac{[U]+[UL]}{[N]+[NL]} = \frac{[U]}{[N]}\frac{1+K_{a,\mathrm{U}}[L]}{1+K_{a,\mathrm{N}}[L]} = K^0\,\frac{1+K_{a,\mathrm{U}}[L]}{1+K_{a,\mathrm{N}}[L]}$$

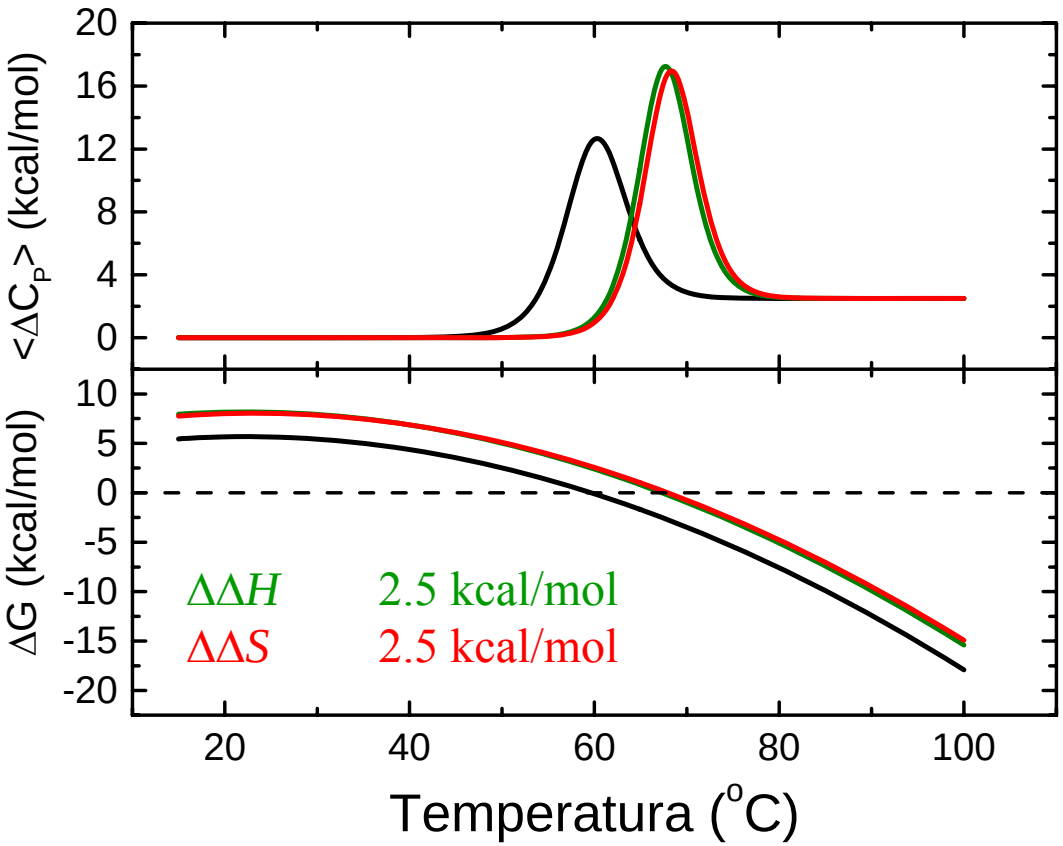
$$\Delta G = \Delta G^0 + RT\ln\left(\frac{1+K_{a,\mathrm{N}}[L]}{1+K_{a,\mathrm{U}}[L]}\right)$$

$$P_{\mathrm{N}} = \frac{[N]+[NL]}{[P]_{\mathrm{T}}} = \frac{1}{1+K} = \frac{1+K_{a,\mathrm{N}}[L]}{1+K_{a,\mathrm{N}}[L]+K^0\left(1+K_{a,\mathrm{U}}[L]\right)}$$

$$P_{\mathrm{U}} = \frac{[U]+[UL]}{[P]_{\mathrm{T}}} = \frac{K}{1+K} = \frac{K^0\left(1+K_{a,\mathrm{U}}[L]\right)}{1+K_{a,\mathrm{N}}[L]+K^0\left(1+K_{a,\mathrm{U}}[L]\right)}$$


G
–RTln(1+Ka,U[L])
–RTln(1+Ka,N[L])

Estabilización de proteínas



$$\Delta\Delta G(T_{\mathrm{m}}^{\mathrm{WT}}) \approx \Delta S(T_{\mathrm{m}}^{\mathrm{WT}})\Delta T_{\mathrm{m}}$$

Estabilización de proteínas

$$\Delta\Delta H, \Delta\Delta S \;\Rightarrow\; \Delta\Delta G > 0$$

- Enlace disulfuro intramolecular
- Interacción catión-π
- Resíduo con mayor propensión helicoidal
- Carga positiva en extremo C de α-hélice
- Carga negativa en extremo N de α-hélice
- Rellenado de cavidad intramolecular
- Optimización de carga electrostática
- Neutralización de enlaces de hidrógeno superficiales

Estudio de unión de ligandos

- Bioquímica y Biofísica
  - Interacciones intramoleculares
  - Plegamiento de proteínas

- Biotecnología
  - Ingeniería de proteínas
  - Motores moleculares

- Biomedicina
  - Inhibidores y activadores
  - Enfermedades conformacionales

- Farmacología
  - Control de calidad

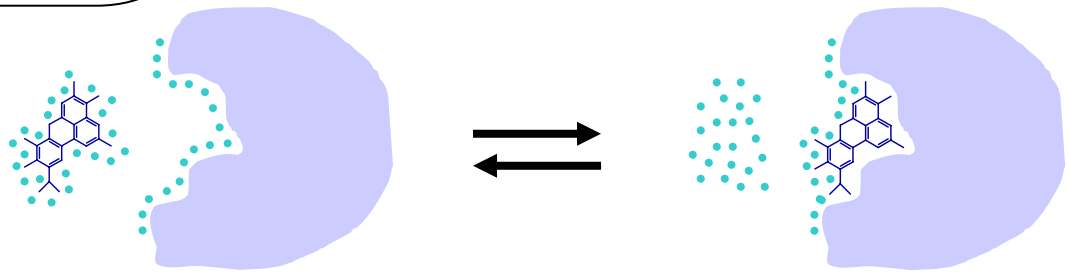
$$\Delta G = \Delta H - T\Delta S_{conf\text{-}PR} - T\Delta S_{conf\text{-}Inh} - T\Delta S_{solv}$$

Interacciones M-L
- van der Waals
- enlaces de H
- de/protonación

Desolvatación

Pérdida de entropía debido a una pérdida de grados de libertad

Ganancia de entropía debido a la desolvatación



- Cómo interaccionan M y L entre sí?
- Cómo interaccionan M y L con el disolvente?

Tipos de interacciones:

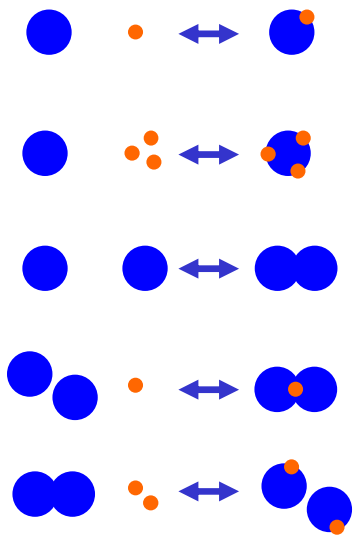
$$M + L \leftrightarrow ML$$

$$M + \mathrm{n}L \leftrightarrow ML_{\mathrm{n}}$$

$$\mathrm{m}M \leftrightarrow M_{\mathrm{m}}$$

$$\mathrm{m}M + \mathrm{L} \leftrightarrow M_{\mathrm{m}}\mathrm{L}$$

$$M_{\mathrm{m}} + \mathrm{L} \leftrightarrow \mathrm{m}ML$$



Técnicas experimentales

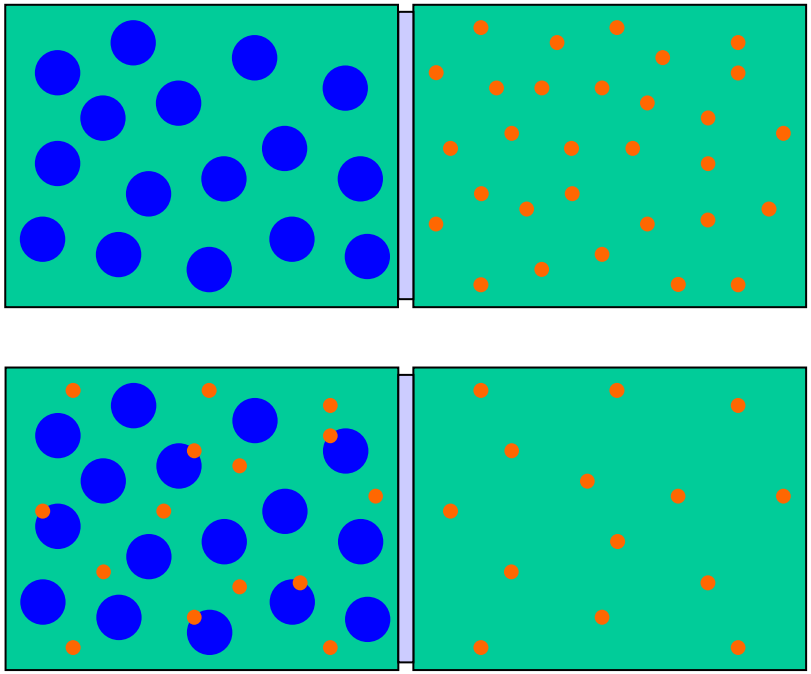
Método 1

- Diálisis
- Ultracentrifugación analítica

Método 2

- Espectroscopía
- Calorimetría

Diálisis



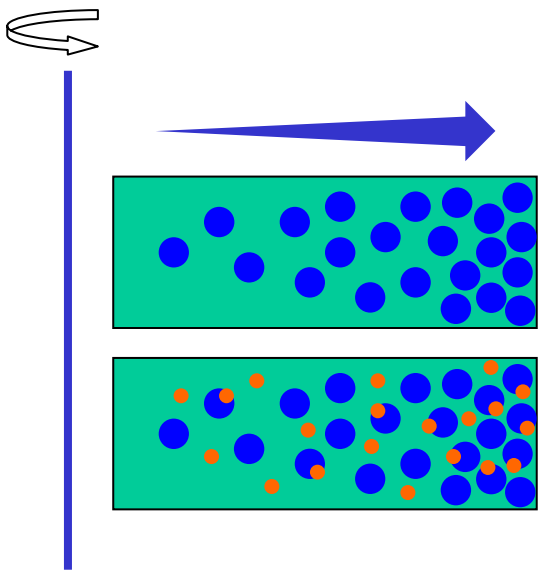
$$[L]_{T1} = [L]_1 + [ML]$$

$$[L]_{T2} = [L]_2$$

$$\mu_{L1} = \mu_2 \quad \Rightarrow \quad [L]_1 = [L]_2$$

$$[ML] = [L]_{T1} - [L]_1 = [L]_{T1} - [L]_2 \quad \Rightarrow$$ concentraciones en equilibrio

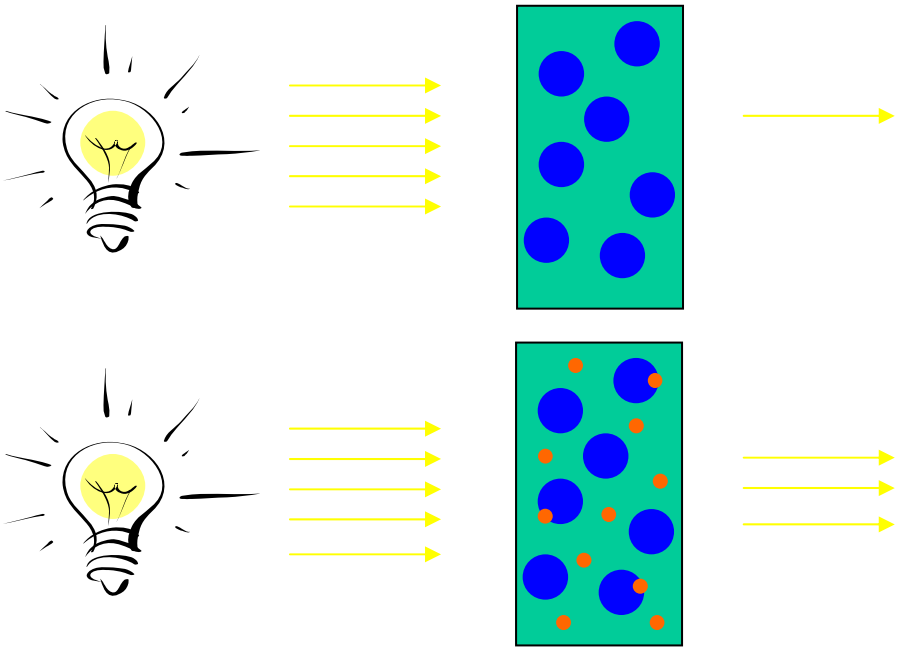
Ultracentrifugación

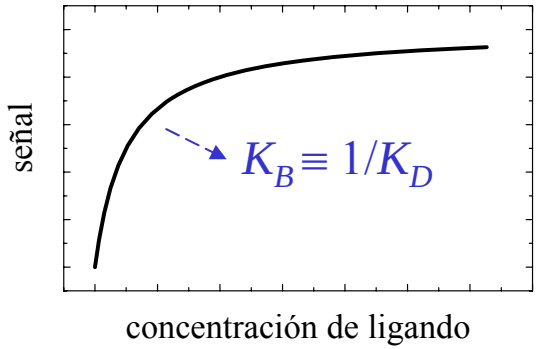


$$C(r) = C_0 e^{\beta M_W} \quad \Rightarrow \quad \text{peso molecular}$$

Distribución de pesos moleculares en disolución $\Rightarrow$ concentraciones en equilibrio

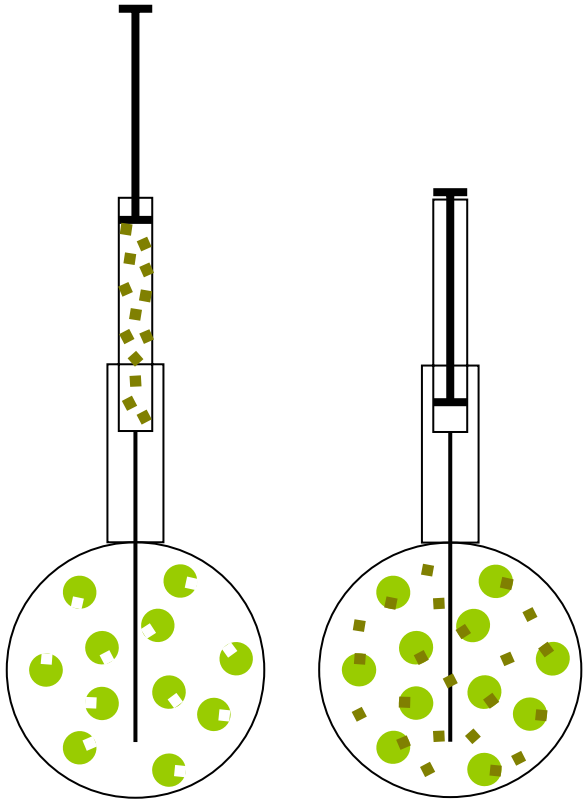
Espectroscopía



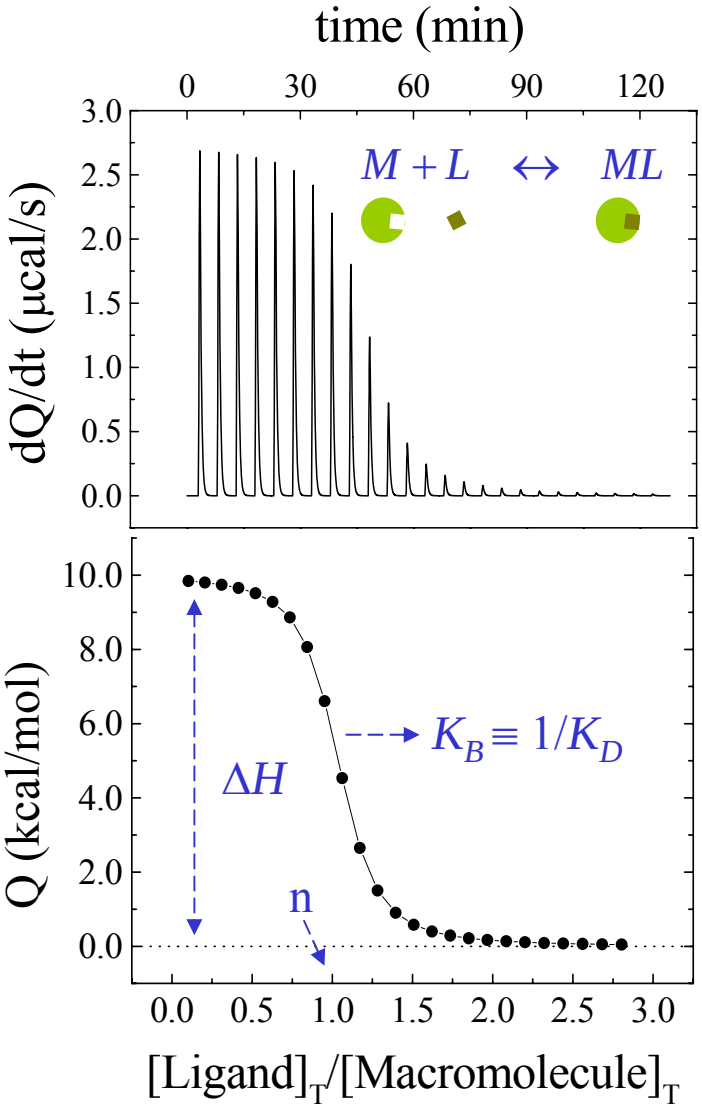


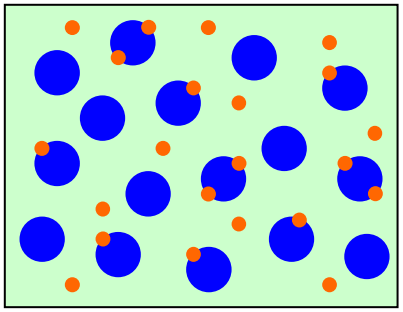
$$\frac{\partial \ln K_B}{\partial T} = \frac{\Delta H}{RT^2}$$

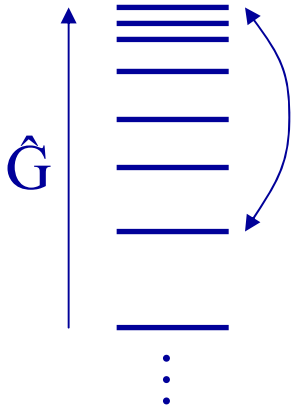
Calorimetría



$$q_i = V\Delta H\left([ML]_i - [ML]_{i-1}\right)$$







$$\Delta\hat{G}_i = \hat{G}_i - \hat{G}_0 = -RT\ln\frac{[ML_i]}{[ML]}$$

$$i = 0, 1, \ldots, n \quad \text{states} \quad \Rightarrow \quad P_i$$

$$T, P \ \text{constant} \quad \Rightarrow \quad P_i \propto \omega_i \exp\left(-\Delta\hat{H}_i/RT\right) = \exp\left(-\Delta\hat{G}_i/RT\right)$$

$$\sum_{i=0}^{n} P_i = 1$$

$$P_i = \frac{\exp\left(-\Delta\hat{G}_i/RT\right)}{Z} \qquad Z = \sum_{i=0}^{n} \exp\left(-\Delta\hat{G}_i/RT\right)$$

partition function or binding polynomial

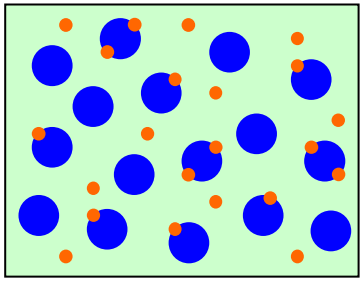
i	$\hat{G}$	$\Delta\hat{G}$	$\exp(-\Delta\hat{G}/RT)$
0	$\hat{G}_0$	0	1
⋮	⋮	⋮	⋮
I	$\hat{G}_i$	$\Delta\hat{G}_i$	$\exp(-\Delta\hat{G}_i/RT)$
⋮	⋮	⋮	⋮
n	$\hat{G}_n$	$\Delta\hat{G}_n$	$\exp(-\Delta\hat{G}_n/RT)$

$$\Delta\hat{G}_i = \Delta\hat{H}_i - T\Delta\hat{S}_i = f_i(T, pH, \ldots)$$

Binding and Linkage: Functional Chemistry of Biological Macromolecules. J. Wyman and S. Gill. Ed. University Science Books

A observable property of the system $\rightarrow$ measurement of A?

$$\langle A \rangle = \sum_{i=0} P_i A_i$$ ensemble average



$$\overline{A} = \lim_{t \to +\infty} \frac{1}{t} \int_0^t A(t')dt'$$ time average

ergodic system $\rightarrow$ $\langle A \rangle = \overline{A}$

observed value $= V[M]_T \langle A \rangle$

observed value $= [M]_T \langle A \rangle$

$$Z = \sum_i \exp\left(-\Delta\hat{G}_i/RT\right)$$

$$P_i = \frac{\exp\left(-\Delta\hat{G}_i/RT\right)}{Z}$$

$$\langle \Delta G \rangle = -RT\ln Z$$

$$\langle \Delta H \rangle = RT^2 \frac{\partial \ln Z}{\partial T} = -T^2 \frac{\partial\left(\langle \Delta G \rangle/T\right)}{\partial T}$$

$$\langle \Delta S \rangle = R\left(\ln Z + T\frac{\partial \ln Z}{\partial T}\right) = -\frac{\partial \langle \Delta G \rangle}{\partial T}$$

$$Z = \sum_i \exp\left(-\Delta\hat{G}_i/RT\right)$$

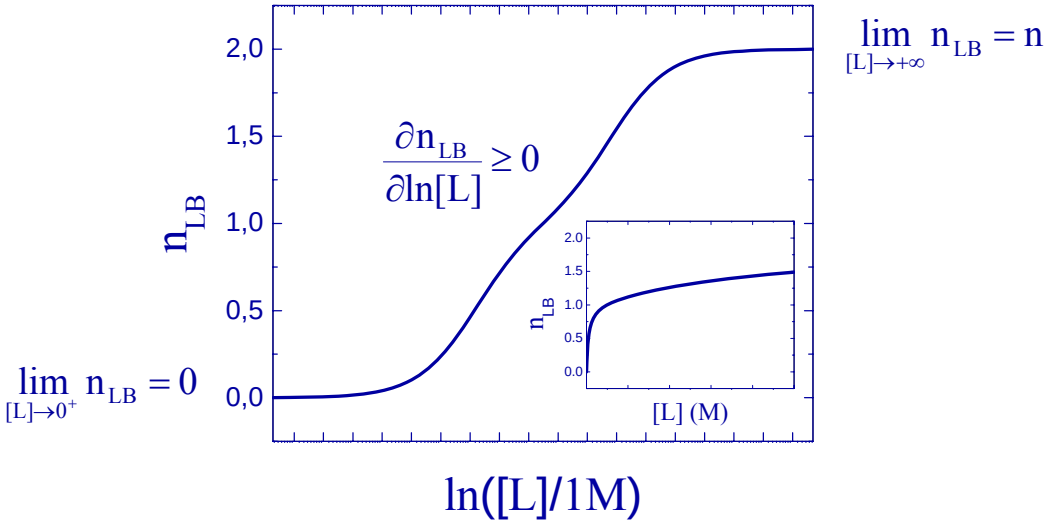
$$P_i = \frac{\exp\left(-\Delta\hat{G}_i/RT\right)}{Z}$$

$$\left.\begin{aligned} N_i &= NP_i = N\frac{\exp\left(-\Delta\hat{G}_i/RT\right)}{Z} \\ N_0 &= NP_0 = N\frac{1}{Z} \end{aligned}\right\} \quad \frac{N_i}{N_0} = \exp\left(-\Delta\hat{G}_i/RT\right)$$

$$Z = \sum_i \frac{N_i}{N_0} = \sum_i \frac{C_i}{C_0} = \sum_i \frac{[ML_i]}{[M]}$$

Number of ligand molecules bound per macromolecule

$$n_{LB} = \frac{[L]_B}{[M]_T} = \frac{\sum_{i=0}^{n} i[ML_i]}{\sum_{i=0}^{n} [ML_i]} \qquad F_B = \frac{n_{LB}}{n}$$



Binding and Linkage: Functional Chemistry of Biological Macromolecules. J. Wyman and S. Gill. Ed. University Science Books

$$\beta_i = \frac{[ML_i]}{[M][L]^i} = \exp\left(-\Delta G_i/RT\right)$$ overall association constants

$$K_i^* = \frac{[ML_i]}{[ML_{i-1}][L]}$$ step-wise association constants

$$\beta_i = \prod_{j=1}^{i} K_j^* \qquad K_i^* = \frac{\beta_i}{\beta_{i-1}} \qquad \beta_i[L]^i = \frac{[ML_i]}{[ML]} = \exp\left(-\Delta\hat{G}_i/RT\right)$$

$$\Delta\hat{G}_i = \Delta G_i - iRT\ln[L]$$

$$Z = \sum_{i=0}^{n} \beta_i[L]^i = 1 + \beta_1[L] + \beta_2[L]^2 + \ldots$$

$$= \sum_{i=0}^{n} \left(\prod_{j=1}^{i} K_j^* \right)[L]^i = 1 + K_1^*[L] + K_1^* K_2^*[L]^2 + \ldots$$

$$Z = \sum_{i=0}^{n} \frac{[ML_i]}{[M]} = \sum_{i=0}^{n} \beta_i [L]^i$$

$$n_{LB} = \frac{\sum_{i=0}^{n} i[ML_i]}{\sum_{i=0}^{n} [ML_i]} = \frac{\sum_{i=0}^{n} i\beta_i [L]^i}{\sum_{i=0}^{n} \beta_i [L]^i}$$

$$n_{LB} = \left(\frac{\partial \ln Z}{\partial \ln [L]} \right)_{P,T} = \frac{\sum_{i=0}^{n} \beta_i [L]^i i}{Z} = \sum_{i=0}^{n} P_i \, i = \langle i \rangle$$

$$\langle \Delta H \rangle = RT^2 \left(\frac{\partial \ln Z}{\partial T} \right)_{P,[L]} = \frac{\sum_{i=0}^{n} \beta_i [L]^i \Delta H_i}{Z} = \sum_{i=0}^{n} P_i \Delta H_i$$

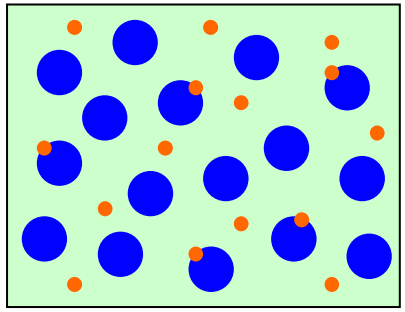
Measurement of thermodynamic binding parameters: ΔG, K_A, ΔH, ΔS

- Direct measurement of equilibrium concentrations (method 1)

$$[L],[M],[ML] \rightarrow K_A \rightarrow \Delta G$$

$$\frac{\partial \ln K_A}{\partial T} = \frac{\Delta H}{RT^2}$$

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



- Measurement of signal proportional to advance of reaction (method 2)

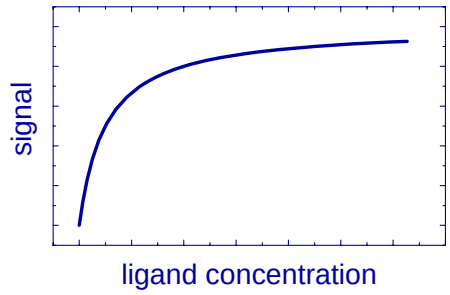
$$\text{signal} \propto F_B$$

$$n_{LB} = \frac{[L]_B}{[M]_T} = nF_B$$

$$\text{model} \rightarrow K_A, \Delta H \rightarrow \Delta G$$

$$\frac{\partial \ln K_A}{\partial T} = \frac{\Delta H}{RT^2}$$

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



$$n_{LB} = \frac{\partial \ln Z}{\partial \ln [L]}$$

$$[L]_T = [L] + n_{LB}[M]_T$$ conservation or balance equation

$$[L] + n_{LB}[M]_T - [L]_T = 0 \quad \rightarrow \quad [L] \quad \rightarrow \quad P_i \quad \rightarrow \quad [ML_i] = P_i[M]_T$$

observed value $= V[M]_T \langle A \rangle = V[M]_T \sum_{i=0} P_i A_i = V \sum_{i=0} [ML_i] A_i$

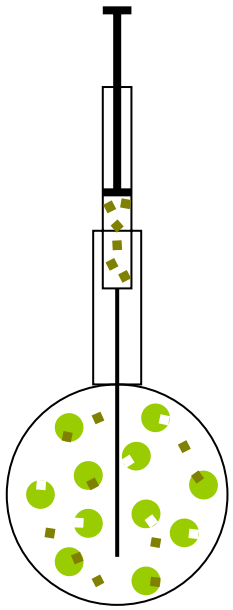
observed value $= [M]_T \langle A \rangle = [M]_T \sum_{i=0} P_i A_i = \sum_{i=0} [ML_i] A_i$

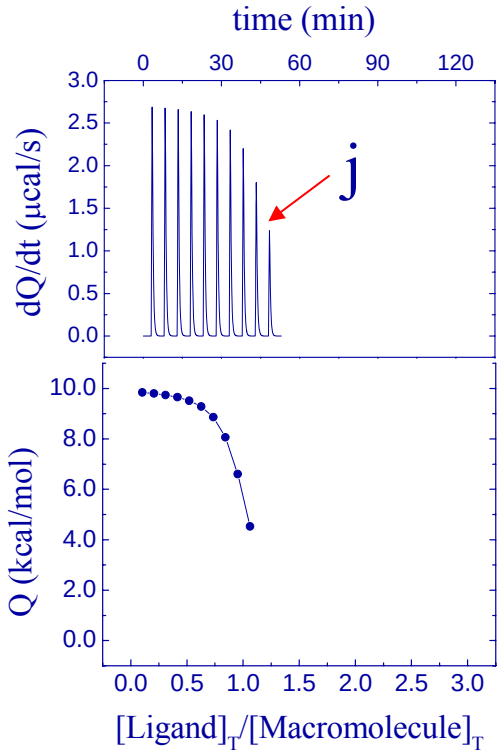
Note: The free species concentrations are unknown; we only manage total concentrations

$$Q_{T,j} = V[M]_{T,j}\langle \Delta H \rangle_j = V[M]_{T,j}\sum_{i=0} P_{i,j}\Delta H_i = V\sum_{i=0}[ML_i]_j \Delta H_i$$

$$Q_j = Q_{T,j} - Q_{T,j\text{-}1}\left(1-\frac{v}{V}\right) = V[M]_{T,j}\sum_{i=0}\left(P_{i,j} - P_{i,j\text{-}1}\left(1-\frac{v}{V}\right)\right)\Delta H_i$$

$$= V\sum_{i=0}\left([ML_i]_j - [ML_i]_{j\text{-}1}\left(1-\frac{v}{V}\right)\right)\Delta H_i$$





$$[L]_j + n_{LB\,j}[M]_{T,j} - [L]_{T,j} = 0$$

$$[M]_{T,j} = [M]_0\left(1-\frac{v}{V}\right)^j$$

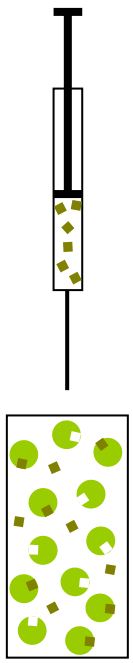
$$[L]_{T,j} = [L]_0\left(1-\left(1-\frac{v}{V}\right)^j\right)$$

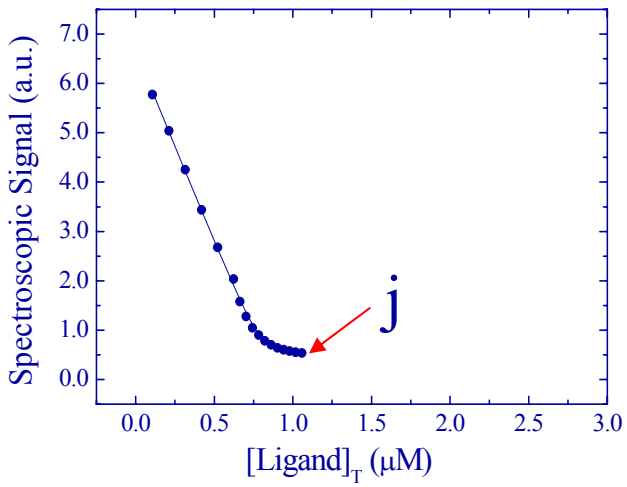
$$A_j = [M]_{T,j}\langle A\rangle = [M]_{T,j}\sum_{i=0} P_{i,j}A_i = \sum_{i=0}[ML_i]_j A_i$$

$$= [M]_{T,j}A_0 + \sum_{i=0} P_{i,j}[M]_{T,j}\left(A_i - A_0\right)$$

$$= [M]_{T,j}A_0 + \sum_{i=0}[ML_i]_j\left(A_i - A_0\right)$$

$$[L]_j + n_{LB\,j}[M]_{T,j} - [L]_{T,j} = 0$$





$$[M]_{T,j} = [M]_0\left(1 - \frac{v}{V}\right)^j$$

$$[L]_{T,j} = [L]_0\left(1 - \left(1 - \frac{v}{V}\right)^j\right)$$

$$[M]_{T,j} = [M]_0\frac{V}{V + jv}$$

$$[L]_{T,j} = [L]_0\frac{jv}{V + jv}$$

Ligand binding to a simple protein

A single binding site

Binding affinity $\longrightarrow$ ΔG Gibbs energy for complex formation

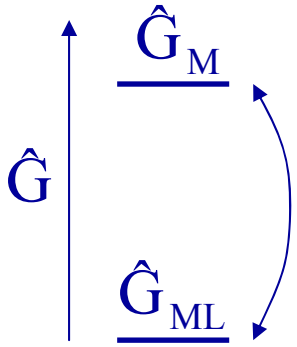
Gibbs energy difference between free and bound states

$$M + L \leftrightarrow ML$$

$$\Delta \hat{G} = \hat{G}_{ML} - \hat{G}_{M}$$

$$\Delta G = -RT\ln K_A$$

$$K_A = \frac{[ML]}{[M][L]}$$



Binding $\Rightarrow$ $\begin{cases} \Delta G < 0 \\ K > 1 \end{cases}$

Affinity $\uparrow$ $\Rightarrow$ $\begin{cases} \Delta G \downarrow \\ K \uparrow \end{cases}$

$$M + L \leftrightarrow ML$$

i	$\Delta\hat{G}_i$	$\exp(-\Delta\hat{G}_i/RT)$
	0	1
	$\Delta G - RT\ln[L]$	$K[L]$

$$Z = 1 + K[L]$$

$$n_{LB} = \frac{K[L]}{1 + K[L]}$$

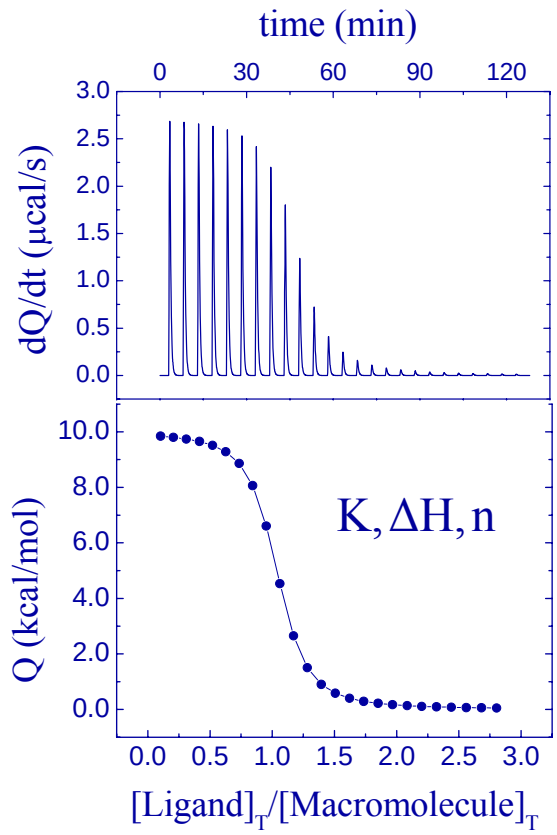
$$Z = 1 + K[L]$$

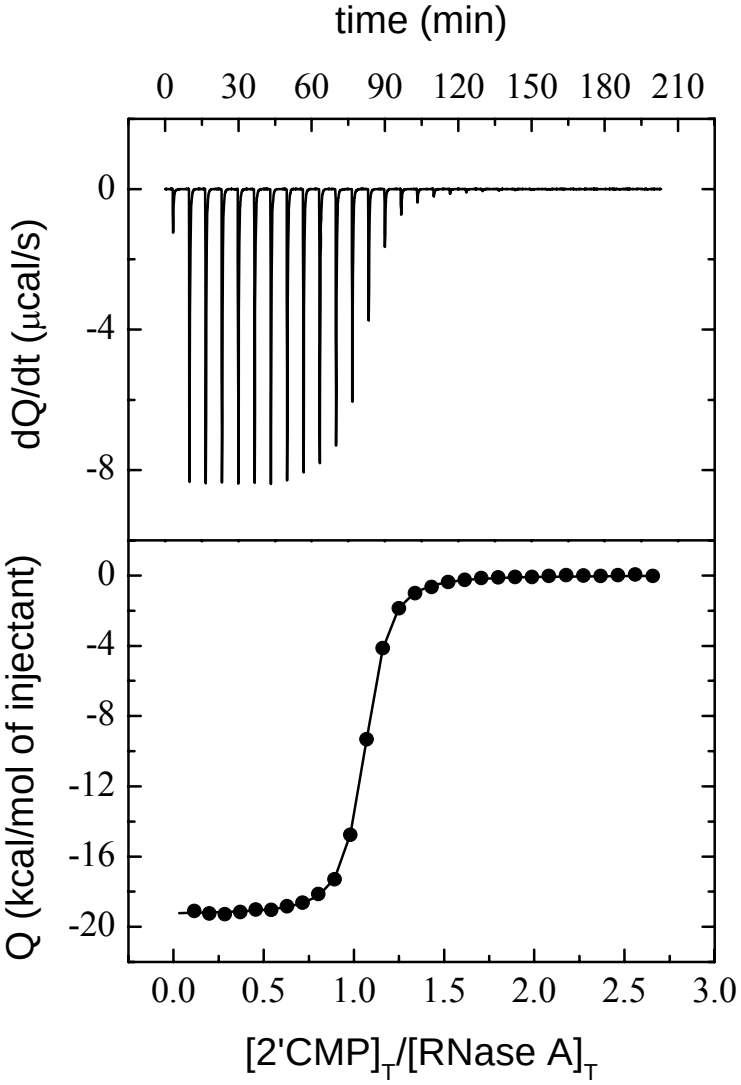
$$n_{LB} = \frac{K[L]}{1 + K[L]}$$

$$[L] + n_{LB}[M]_T - [L]_T = 0$$

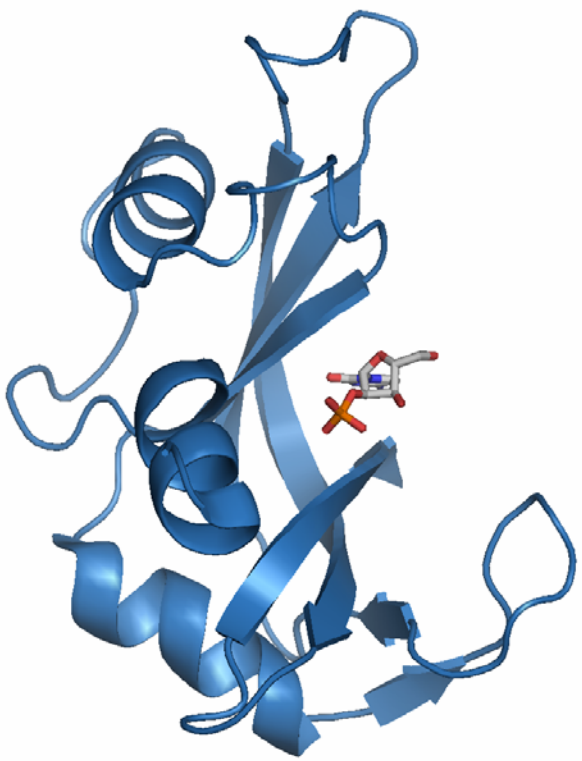
$$[M] = \frac{1}{1 + K[L]}[M]_T \qquad [ML] = \frac{K[L]}{1 + K[L]}[M]_T$$

$$Q_j = V\left([ML]_j - [ML]_{j-1}\left(1 - \frac{v}{V}\right)\right)\Delta H$$

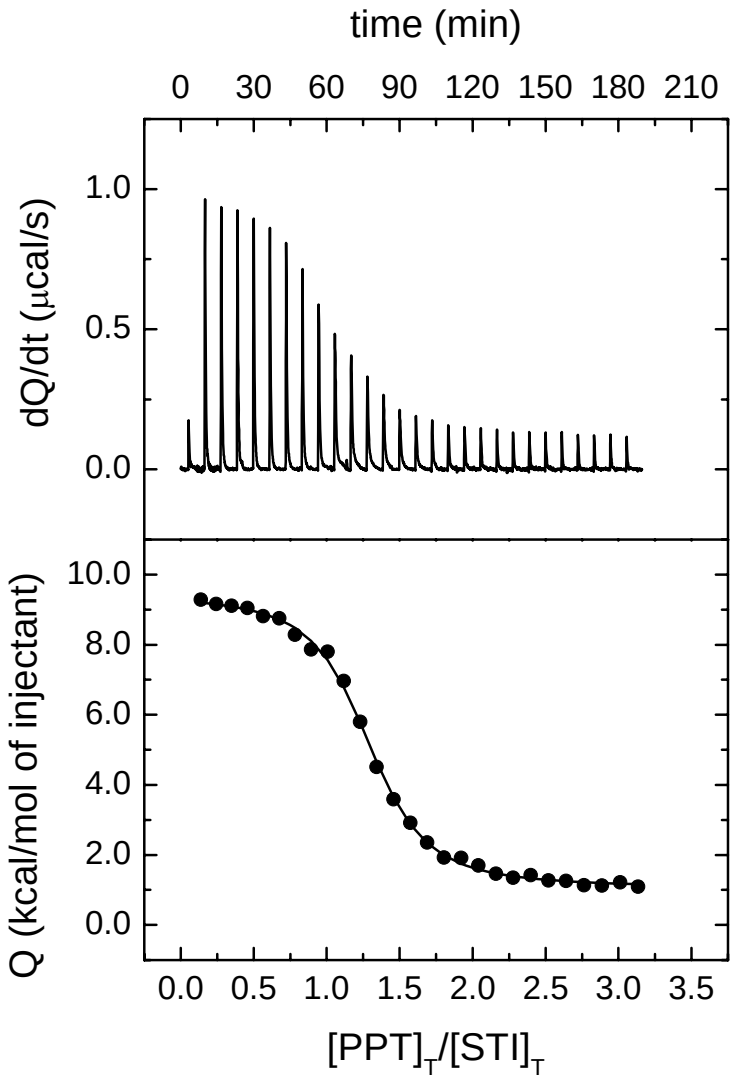




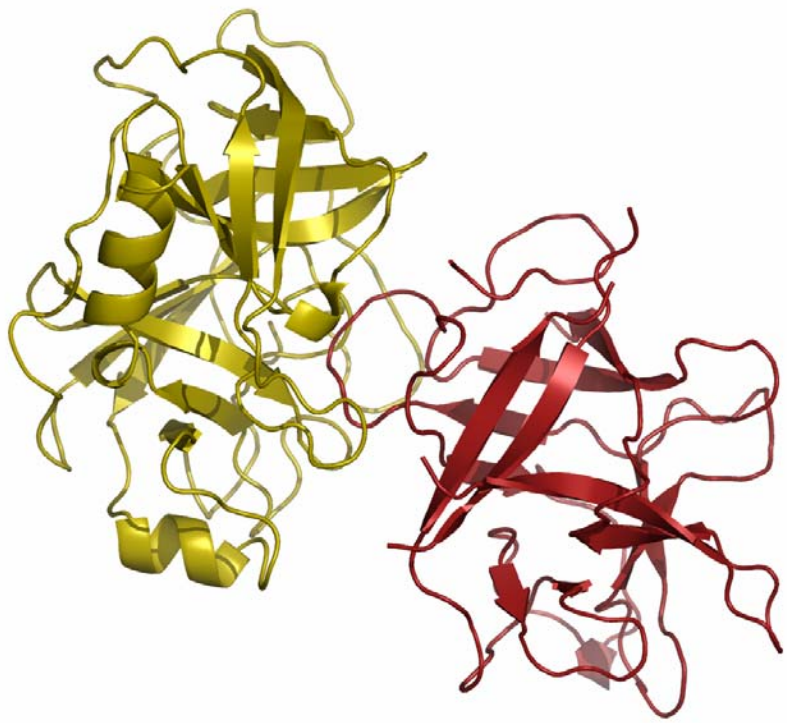
Bovine Pancreatic Ribonuclease A
2'CMP



K_A	$2.9 \cdot 10^6\ M^{-1}$
ΔH	-19.3 kcal/mol
n	1.02



Soybean Trypsin Inhibitor
Pancreatic Porcine Trypsin



K_A	$1.5 \cdot 10^6\ M^{-1}$
ΔH	8.4 kcal/mol
n	1.2

Ligand binding to a “complex” protein

Two binding sites

$$M + 2L \quad \leftrightarrow \quad ML_2$$

i	$\Delta\hat{G}_i$	$\exp(-\Delta\hat{G}_i/RT)$
	0	1
	$\Delta G_1 - RT\ln[L]$	$\beta_1[L]$
		
	$\Delta G_2 - 2RT\ln[L]$	$\beta_2[L]^2$

$$Z = 1 + \beta_1[L] + \beta_2[L]^2$$

$$n_{LB} = \frac{\beta_1[L] + 2\beta_2[L]^2}{1 + \beta_1[L] + \beta_2[L]^2}$$

$$Z = 1 + \beta_1[L] + \beta_2[L]^2$$

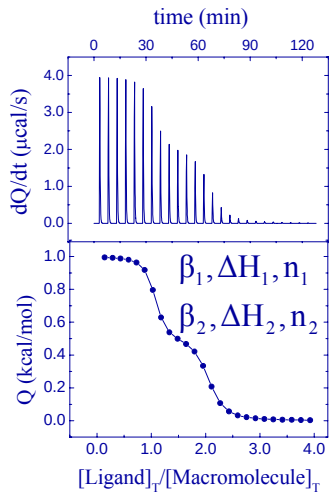
$$n_{LB} = \frac{\beta_1[L] + 2\beta_2[L]^2}{1 + \beta_1[L] + \beta_2[L]^2}$$

$$[L] + n_{LB}[M]_T - [L]_T = 0$$

$$[M] = \frac{1}{1 + \beta_1[L] + \beta_2[L]^2}[M]_T \qquad [ML] = \frac{\beta_1[L]}{1 + \beta_1[L] + \beta_2[L]^2}[M]_T$$

$$[ML_2] = \frac{\beta_2[L]^2}{1 + \beta_1[L] + \beta_2[L]^2}[M]_T$$

$$Q_j = V\left(\left([ML]_j - [ML]_{j-1}\left(1 - \frac{v}{V}\right)\right)\Delta H_1 + \left([ML_2]_j - [ML_2]_{j-1}\left(1 - \frac{v}{V}\right)\right)\Delta H_2\right)$$



$\frac{4\beta_2}{\beta_1^2} = 1$ Identical and independent binding sites

$\frac{4\beta_2}{\beta_1^2} < 1$ Non-identical and independent binding sites, or negative cooperativity

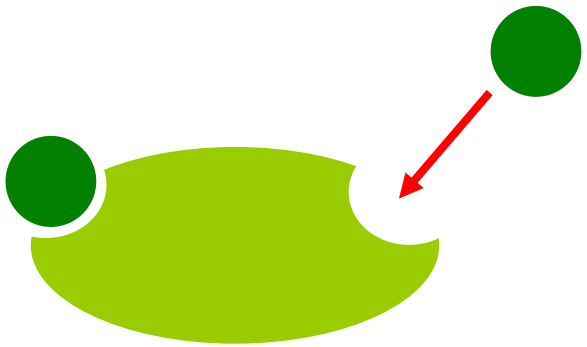
$\frac{4\beta_2}{\beta_1^2} > 1$ Positive cooperativity

First, perform data analysis with model-free general formalism.
Then, once the system is classified, then, apply site-specific models.

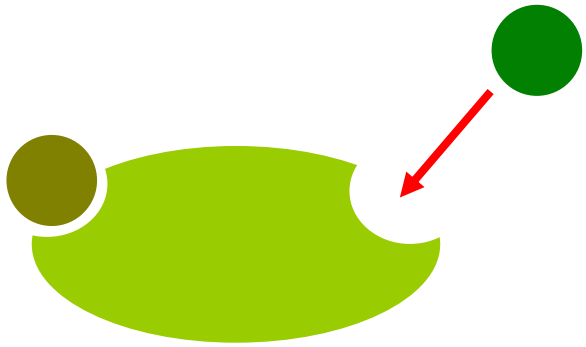
What if the binding sites are different, but they display cooperativity?

Extra-thermodynamic considerations (e.g. structural features) may help in deciding which model applies.

Binding Cooperativity in Proteins



Homotropic Interaction



Heterotropic Interaction

[M], [A], [MA], [MA_2]

[M], [A], [B], [MA], [MB], [MAB]

Quantifying binding cooperativity

$$n_{Hill} = \left.\frac{\partial \log\left(\frac{n_{LB}}{n - n_{LB}}\right)}{\partial \log[L]}\right|_{n_{LB}=\frac{n}{2}}$$

Hill coefficient

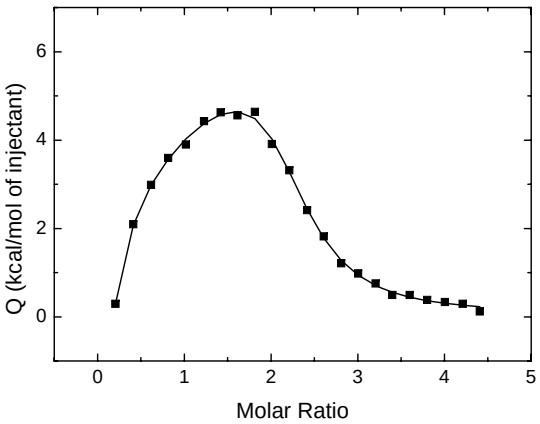
$$\left.\frac{\partial n_{LB}}{\partial \log[L]}\right|_{n_{LB}=\frac{n}{2}} = n_{Hill}\frac{n}{4}$$

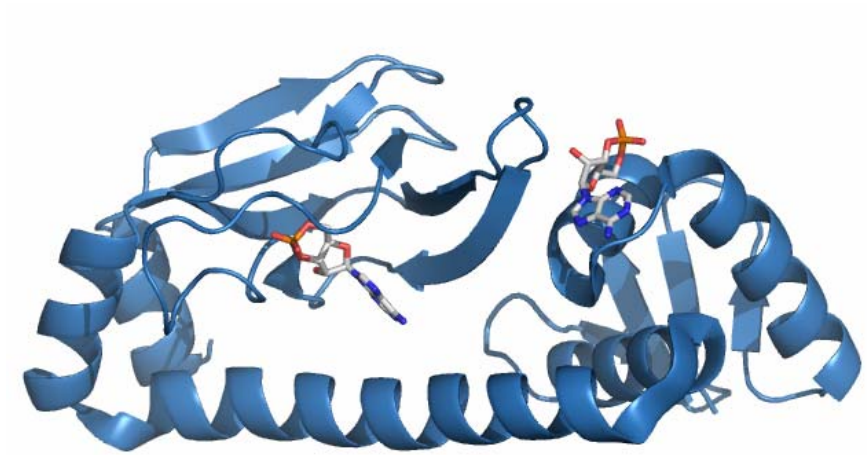
Binding capacity

$0 \le n_{Hill} < 1$	negative cooperativity
$n_{Hill} = 1$	independent binding
$1 < n_{Hill} \le n$	positive cooperativity

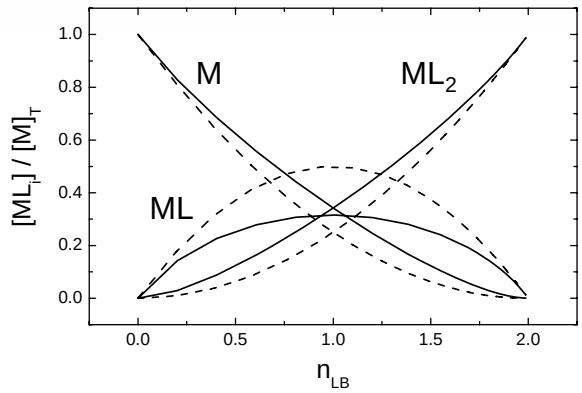
- Measure of the ability for accepting or delivering large quantities of ligand for relatively small changes in ligand concentration
- Efficiency of biochemical signal transduction (response to changes in ligand concentration)

cAMP Receptor Protein + cAMP



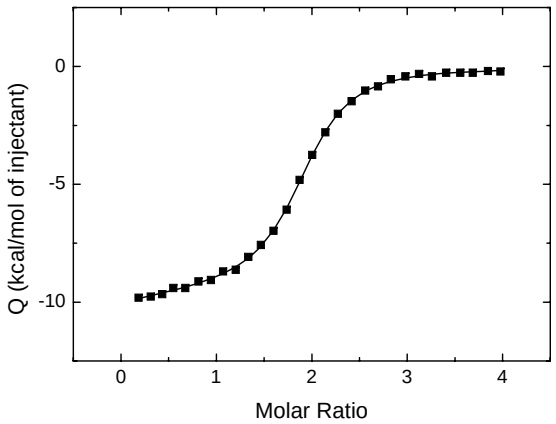


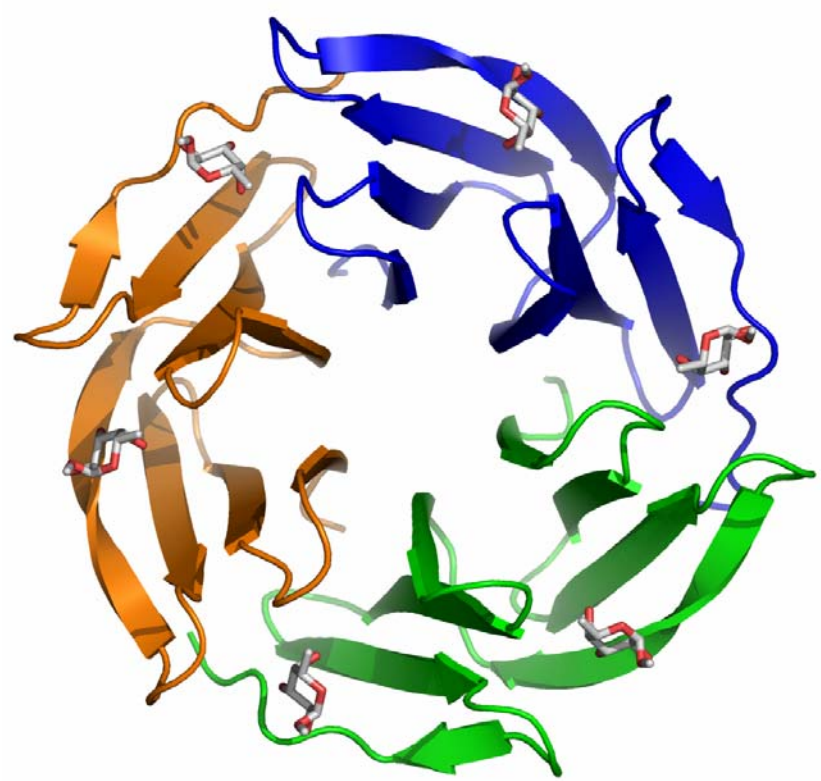
β_1	$5.5 \cdot 10^4\ M^{-1}$
ΔH_1	-1.9 kcal/mol
β_2	$4.2 \cdot 10^9\ M^{-2}$
ΔH_2	9.9 kcal/mol
$4\beta_2/\beta_1^2$	5.4
n_{Hill}	1.40



Gorshkova et al. (1995). *Journal of Biological Chemistry* **270** 21679-21683

R. solanacearum Lectin + α-Methyl-Fucoside

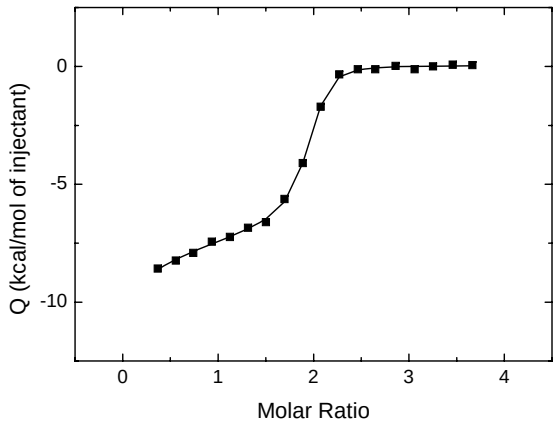


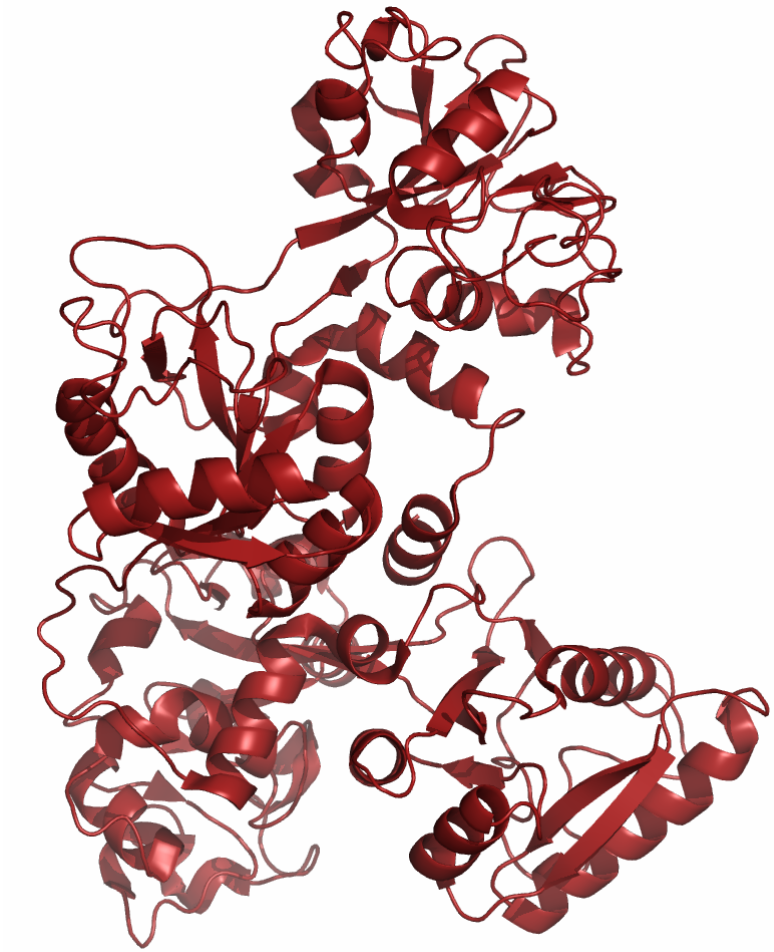


β_1	$3.0 \cdot 10^6\ M^{-1}$
ΔH_1	-10.1 kcal/mol
β_2	$2.4 \cdot 10^{12}\ M^{-2}$
ΔH_2	-19.1 kcal/mol
$4\beta_2/\beta_1^2$	1.1
n_{Hill}	1.02

Kostlanova et al. (2005). *Journal of Biological Chemistry* **280** 27839-27849

Human Transferrin + Fe^{3+}

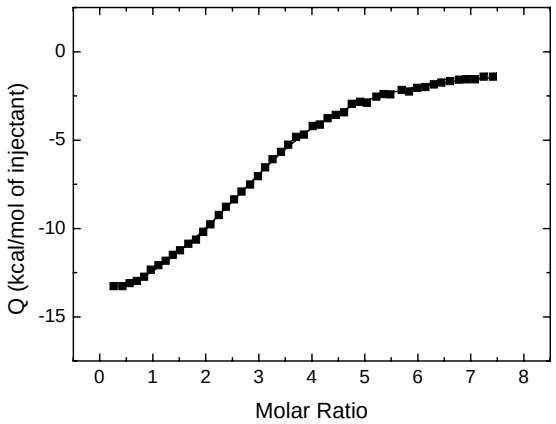


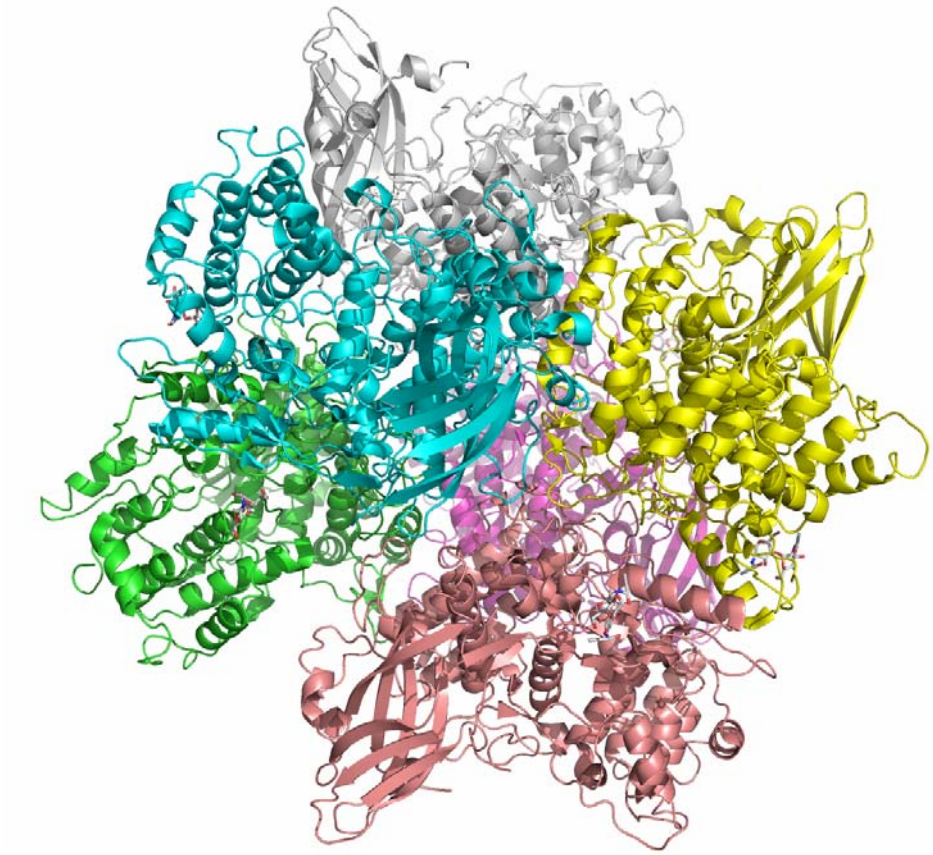


β_1	$1.0 \cdot 10^7\ M^{-1}$
ΔH_1	-9.5 kcal/mol
β_2	$5.4 \cdot 10^{13}\ M^{-2}$
ΔH_2	-15.0 kcal/mol
$4\beta_2/\beta_1^2$	2.2
n_{Hill}	1.19

Lin et al. (1993). *Biochemistry* **32** 9398-9406

H. vulgaris Hemocyanin + Caffeine

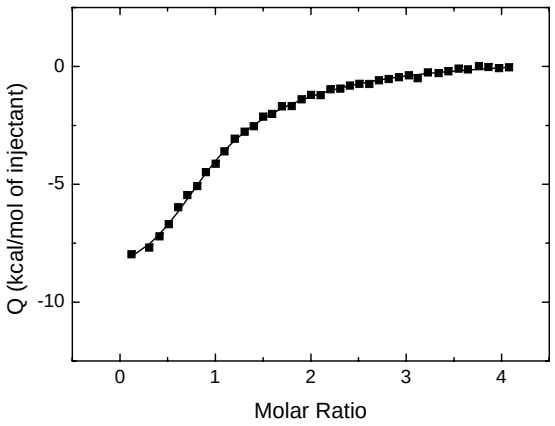


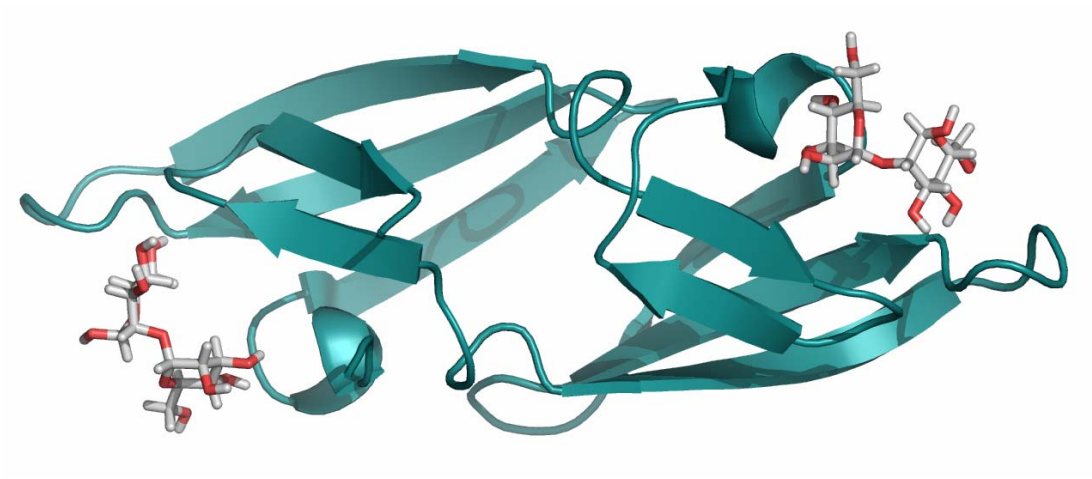


β_1	$3.3 \cdot 10^4\ M^{-1}$
ΔH_1	-23.8 kcal/mol
β_2	$2.6 \cdot 10^9\ M^{-2}$
ΔH_2	-33.2 kcal/mol
$4\beta_2/\beta_1^2$	9.6
n_{Hill}	1.51

Menze et al. (2001). *Journal of Experimental Biology* **204** 1033-1038

Cyanovirin + Manα1$\rightarrow$2Man

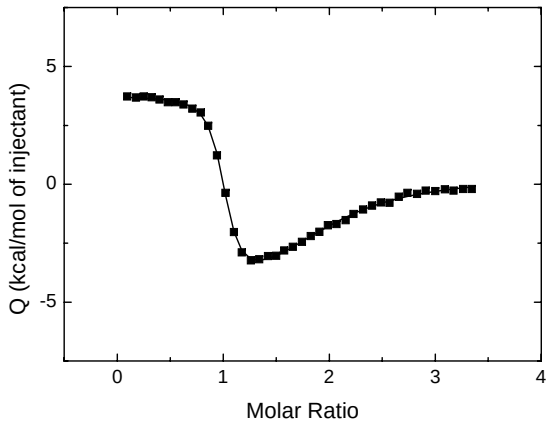


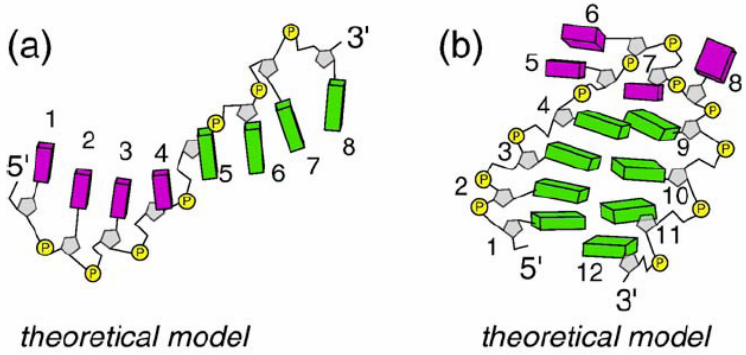


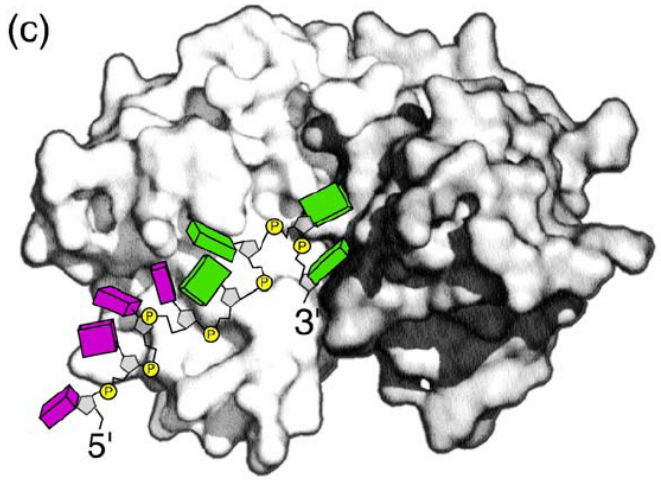
β_1	$4.6 \cdot 10^5$ M^{-1}
ΔH_1	-9.5 kcal/mol
β_2	$1.4 \cdot 10^{10}$ M^{-2}
ΔH_2	-18.1 kcal/mol
$4\beta_2/\beta_1^2$	0.27
n_{Hill}	0.68

Bewley et al. (2001). *Journal of the American Chemical Society* **123** 3892-3902

O. nova d($T_4G_4T_4G_4$) + Telomere Binding Protein α Subunit N-domain



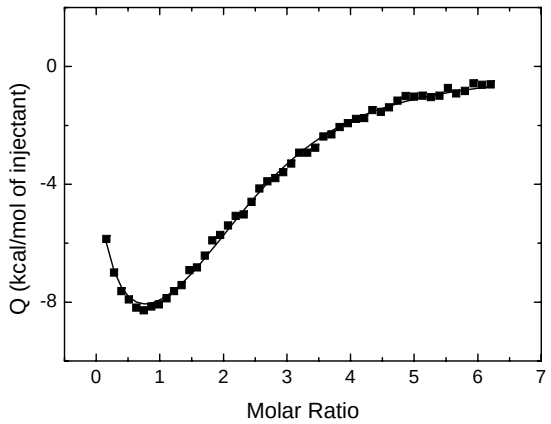


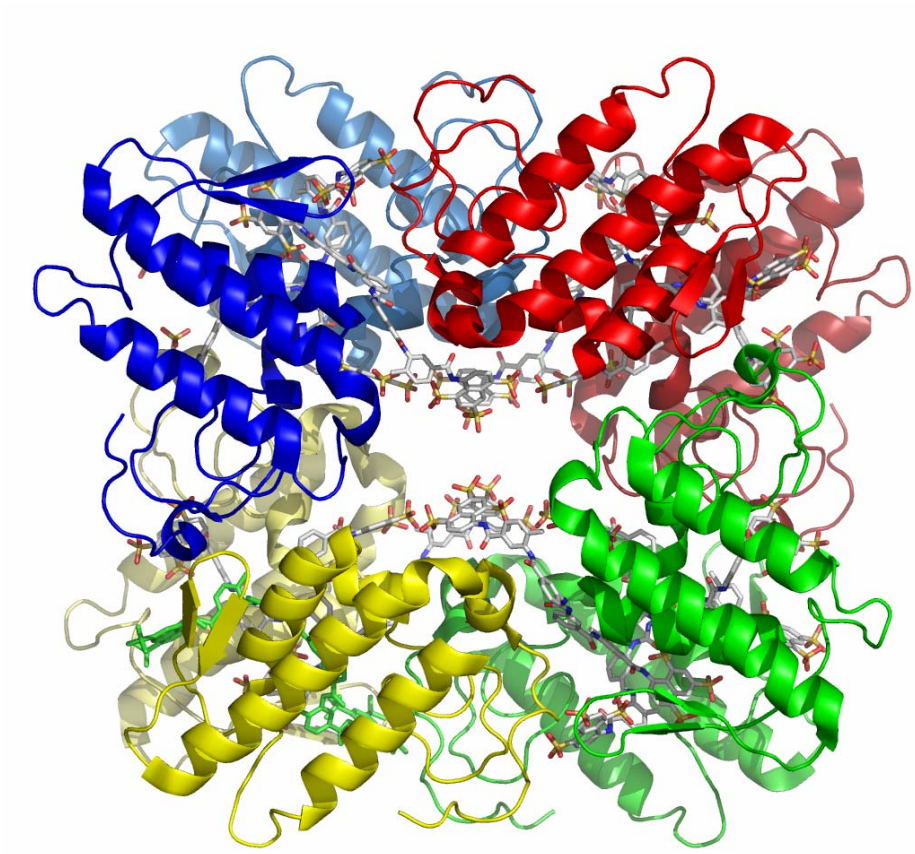


β_1	$2.5 \cdot 10^7$ M^{-1}
ΔH_1	3.4 kcal/mol
β_2	$3.3 \cdot 10^{12}$ M^{-2}
ΔH_2	-2.5 kcal/mol
$4\beta_2/\beta_1^2$	0.022
n_{Hill}	0.26

Buczek and Horvath. (2006). *Journal of Molecular Biology* **359** 1217-1234

E. carinatus Ecarpholin S + Suramin





β_1	$3.1 \cdot 10^4\ M^{-1}$
ΔH_1	-21.9 kcal/mol
β_2	$3.3 \cdot 10^9\ M^{-2}$
ΔH_2	-45.3 kcal/mol
$4\beta_2/\beta_1^2$	14.1
n_{Hill}	1.56

Zhou et al. (2008). *Biophysical Journal* **95** 3366-3380

Ligand binding to a “complex” protein

Two nonidentical and independent binding sites

$$M + 2L \quad \leftrightarrow \quad ML_2$$

i	$\Delta\hat{G}_i$	$\exp(-\Delta\hat{G}_i/RT)$
	0	1
	$\Delta G_1 - RT\ln[L]$	$K_1[L]$
	$\Delta G_2 - RT\ln[L]$	$K_2[L]$
	$\Delta G_1 + \Delta G_2 - 2RT\ln[L]$	$K_1K_2[L]^2$

$$Z = 1 + (K_1 + K_2)[L] + K_1K_2[L]^2 = (1 + K_1[L])(1 + K_2[L])$$

$$n_{LB} = \frac{(K_1 + K_2)[L] + 2K_1K_2[L]^2}{1 + (K_1 + K_2)[L] + K_1K_2[L]^2} = \frac{K_1[L]}{1 + K_1[L]} + \frac{K_2[L]}{1 + K_2[L]}$$

$$Z = 1 + (K_1 + K_2)[L] + K_1K_2[L]^2$$

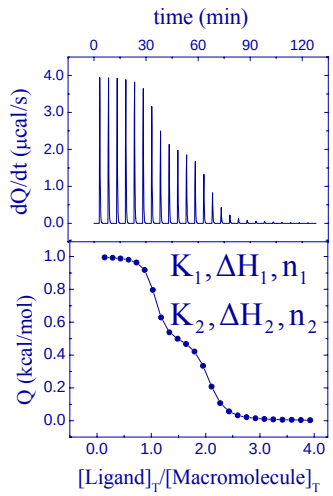
$$n_{LB} = \frac{(K_1 + K_2)[L] + 2K_1K_2[L]^2}{1 + (K_1 + K_2)[L] + K_1K_2[L]^2}$$

$$[L] + n_{LB}[M]_T - [L]_T = 0$$

$$[M] = \frac{1}{1 + (K_1 + K_2)[L] + K_1K_2[L]^2}[M]_T \qquad [ML] = \frac{K_1[L]}{1 + (K_1 + K_2)[L] + K_1K_2[L]^2}[M]_T$$

$$[LM] = \frac{K_2[L]}{1 + (K_1 + K_2)[L] + K_1K_2[L]^2}[M]_T \qquad [LML] = \frac{K_1K_2[L]^2}{1 + (K_1 + K_2)[L] + K_1K_2[L]^2}[M]_T$$

$$Q_j = V\left(\left([ML]_j - [ML]_{j-1}\left(1 - \frac{v}{V}\right)\right)\Delta H_1 + \left([LM]_j - [LM]_{j-1}\left(1 - \frac{v}{V}\right)\right)\Delta H_2 + \left([LML]_j - [LML]_{j-1}\left(1 - \frac{v}{V}\right)\right)(\Delta H_1 + \Delta H_2)\right)$$



Ligand binding to a “complex” protein

Two identical and independent binding sites

$$M + 2L \quad \leftrightarrow \quad ML_2$$

i	$\Delta\hat{G}_i$	$\exp(-\Delta\hat{G}_i/RT)$
	0	1
	$\Delta G - RT\ln[L]$	$K[L]$
	$\Delta G - RT\ln[L]$	$K[L]$
	$2\Delta G - 2RT\ln[L]$	$K^2[L]^2$

$$Z = 1 + 2K[L] + K^2[L]^2 = \left(1 + K[L]\right)^2$$

$$n_{LB} = \frac{2K[L] + 2K^2[L]^2}{1 + 2K[L] + K^2[L]^2} = \frac{2K[L]}{1 + K[L]}$$

$$Z = 1 + 2K[L] + K^2[L]^2$$

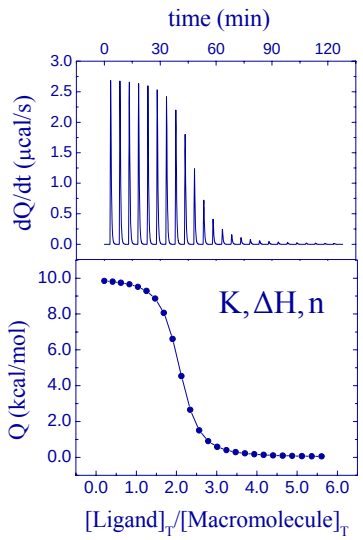
$$n_{LB} = \frac{2K[L] + 2K^2[L]^2}{1 + 2K[L] + K^2[L]^2}$$

$$[L] + n_{LB}[M]_T - [L]_T = 0$$

$$[M] = \frac{1}{1 + 2K[L] + K^2[L]^2}[M]_T \qquad [ML] = \frac{2K[L]}{1 + 2K[L] + K^2[L]^2}[M]_T$$

$$[ML_2] = \frac{K^2[L]^2}{1 + 2K[L] + K^2[L]^2}[M]_T$$

$$Q_j = V\left(\left([ML]_j - [ML]_{j-1}\left(1 - \frac{v}{V}\right)\right)\Delta H + \left([ML_2]_j - [ML_2]_{j-1}\left(1 - \frac{v}{V}\right)\right)2\Delta H\right)$$



Ligand binding to a “complex” protein

Two identical and cooperative binding sites

$$M + 2L \quad \leftrightarrow \quad ML_2$$

i	$\Delta\hat{G}_i$	$\exp(-\Delta\hat{G}_i/RT)$
	0	1
	$\Delta G - RT\ln[L]$	$K[L]$
	$\Delta G - RT\ln[L]$	$K[L]$
	$2\Delta G + \Delta g - 2RT\ln[L]$	$\alpha K^2[L]^2$

$$Z = 1 + 2K[L] + \alpha K^2[L]^2$$

$$n_{LB} = \frac{2K[L] + 2\alpha K^2[L]^2}{1 + 2K[L] + \alpha K^2[L]^2}$$

$$\frac{4\beta_2}{\beta_1^2} = \alpha$$

if $\alpha > 1$:

$$Z = 1 + 2K[L] + \alpha K^2[L]^2 \neq (1 + K_1[L])(1 + K_2[L])$$

$$n_{LB} = \frac{2K[L] + 2\alpha K^2[L]^2}{1 + 2K[L] + \alpha K^2[L]^2} \neq \frac{K_1[L]}{1 + K_1[L]} + \frac{K_2[L]}{1 + K_2[L]}$$

if $\alpha < 1$:

$$Z = 1 + 2K[L] + \alpha K^2[L]^2 = (1 + K_1[L])(1 + K_2[L])$$

$$n_{LB} = \frac{2K[L] + 2\alpha K^2[L]^2}{1 + 2K[L] + \alpha K^2[L]^2} = \frac{K_1[L]}{1 + K_1[L]} + \frac{K_2[L]}{1 + K_2[L]}$$

nonidentical independent binding sites are equivalent to identical binding sites with negative cooperativity

$$Z = 1 + 2K[L] + \alpha K^2[L]^2$$

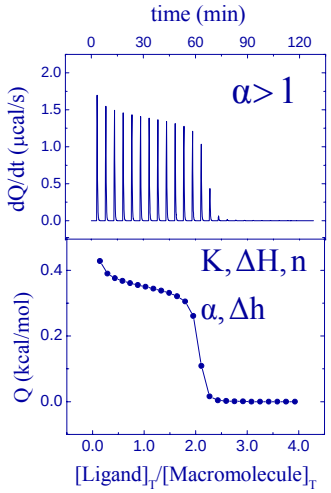
$$n_{LB} = \frac{2K[L] + 2\alpha K^2[L]^2}{1 + 2K[L] + \alpha K^2[L]^2}$$

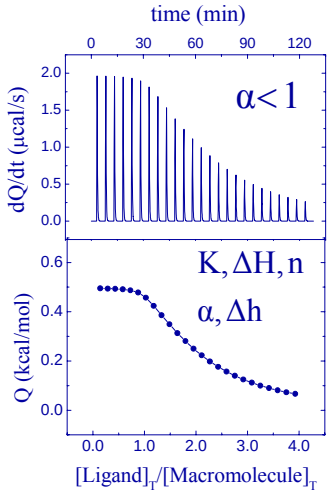
$$[L] + n_{LB}[M]_T - [L]_T = 0$$

$$[M] = \frac{1}{1 + 2K[L] + \alpha K^2[L]^2}[M]_T \qquad [ML] = \frac{2K[L]}{1 + 2K[L] + \alpha K^2[L]^2}[M]_T$$

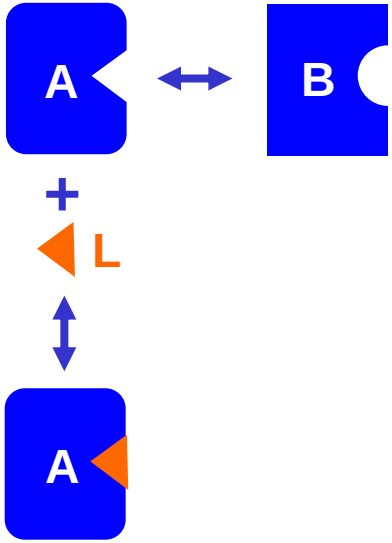
$$[ML_2] = \frac{\alpha K^2[L]^2}{1 + 2K[L] + \alpha K^2[L]^2}[M]_T$$

$$Q_j = V\left(\left([ML]_j - [ML]_{j\text{-}1}\left(1 - \frac{v}{V}\right)\right)\Delta H + \left([ML_2]_j - [ML_2]_{j\text{-}1}\left(1 - \frac{v}{V}\right)\right)(2\Delta H + \Delta h)\right)$$





Equilibrio conformacional modulado por unión de ligando



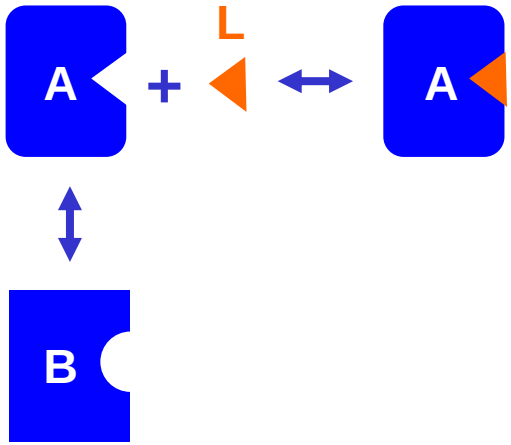
$$K = \frac{[B]}{[A]}$$

$$K^L = \frac{[B]}{[A]+[AL]} = \frac{[B]}{[A]+K_A[A][L]} = \frac{[B]}{[A]}\frac{1}{1+K_A[L]}$$

$$K^L = K\frac{1}{1+K_A[L]}$$

$$\Delta G^L = \Delta G + RT\ln\left(1+K_A[L]\right)$$

Unión de ligando modulada por equilibrio conformacional



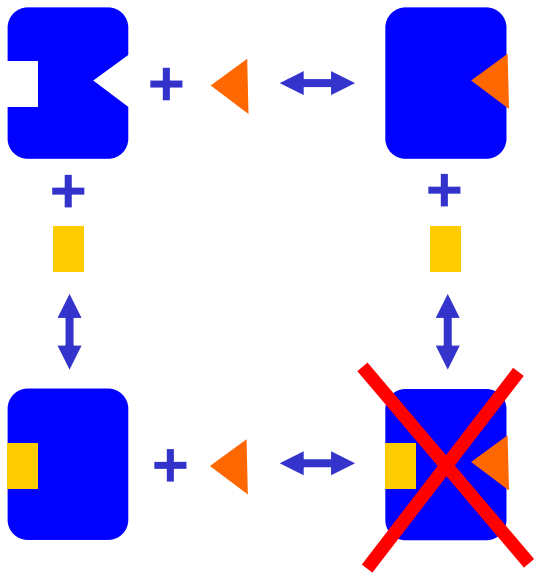
$$F_{BL} = \frac{[AL]}{[A]+[AL]} = \frac{K_L[L]}{1+K_L[L]}$$

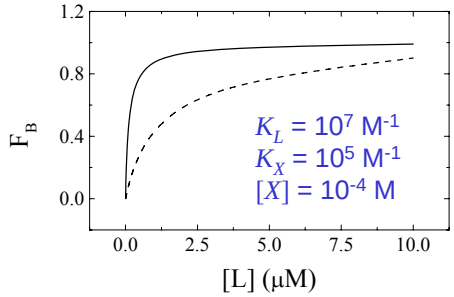
$$F_{BL}^{B} = \frac{[AL]}{[A]+[AL]+[B]} = \frac{K_L[L]}{1+K_L[L]+K} = \frac{\frac{K_L}{1+K}[L]}{1+\frac{K_L}{1+K}[L]}$$

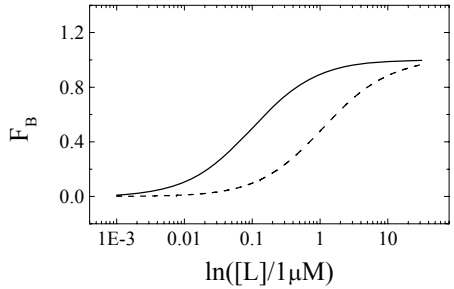
$$K_L^B = \frac{K_L}{1+K}$$

$$\Delta G_L^B = \Delta G_L + RT\ln(1+K)$$

Equilibrio de unión modulado por la unión de otro ligando







$$F_B = \frac{[ML]}{[M]+[ML]} = \frac{K_L[L]}{1+K_L[L]}$$

$$F_B^X = \frac{[ML]}{[M]+[ML]+[MX]} = \frac{K_L[L]}{1+K_L[L]+K_X[X]} = \frac{\dfrac{K_L}{1+K_X[X]}[L]}{1+\dfrac{K_L}{1+K_X[X]}[L]}$$

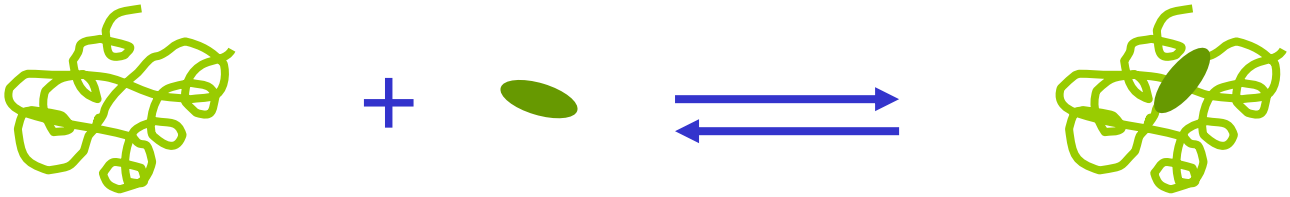
$$K_L^X = \frac{K_L}{1+K_X[X]}$$

$$\Delta G_L^X = \Delta G_L + RT\ln\left(1+K_X[X]\right)$$

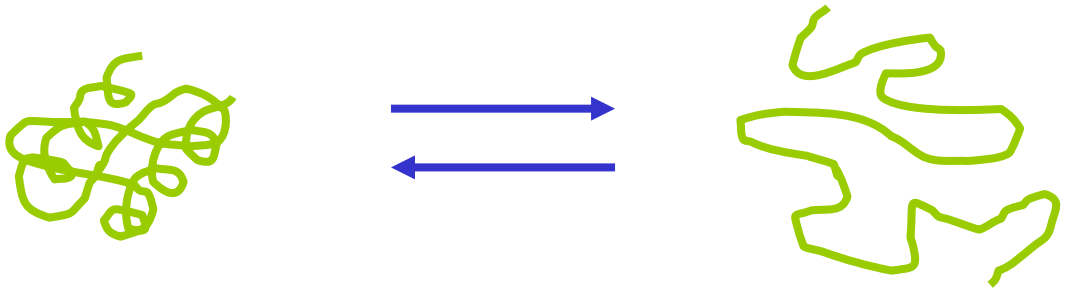
Isothermal Titration Calorimetry
&
Differential Scanning Calorimetry

Adrián Velázquez Campoy

Binding Equilibrium



Conformational Equilibrium



Every biological process can be decomposed in a sequence of sequential and/or simultaneous elemental processes

$$A \rightleftharpoons B$$

Gibbs Energy $\Delta G = G_B - G_A$ $\begin{cases} > 0 \text{ unfavorable} \\ < 0 \text{ favorable} \end{cases}$

$$\Delta G, K_{eq}, \Delta H, \Delta S, \Delta C_P$$

Gibbs energy
Equilibrium constant
Enthalpy
Entropy
Heat Capacity

$$\mathrm{A} \rightleftharpoons \mathrm{B}$$

Gibbs Energy $\quad \Delta G = G_{\mathrm{B}} - G_{\mathrm{A}} \quad \begin{cases} > 0 \text{ unfavorable} \\ < 0 \text{ favorable} \end{cases}$

$$\Delta G,\ K_{eq},\ \Delta H,\ \Delta S,\ \Delta C_P$$

$$\Delta G = -RT \ln K_{eq} \qquad \Delta C_P = \left(\frac{\partial \Delta H}{\partial T} \right)_P$$

$$\Delta G = \Delta H - T\Delta S \qquad \Delta C_P = T\left(\frac{\partial \Delta S}{\partial T} \right)_P$$

$$\Delta G(T) = \Delta H(T_0) + \Delta C_P (T - T_0) - T\left(\Delta S(T_0) + \Delta C_P \ln \frac{T}{T_0} \right)$$

ΔG, K_{eq}

↓

ΔH, ΔS

↓

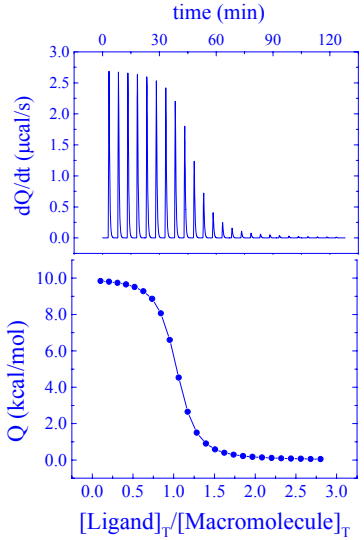
environmental variables
T, pH, ionic strength, solutes

↓

information about the inter- and intramolecular
interactions involved in the process

Two calorimetric techniques can be used to perform an exhaustive thermodynamic characterization of binding and conformational equilibrium processes:

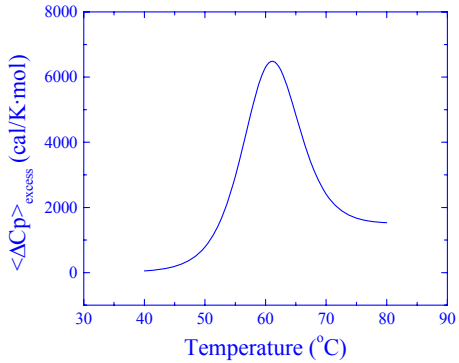
ITC Determination of Enthalpy, Affinity, Entropy and of Binding.



→ ΔH, K_a, n, ΔG, ΔS, ΔC_P

$$M + L \leftrightarrow ML$$

DSC Characterization of Thermodynamic Stability.

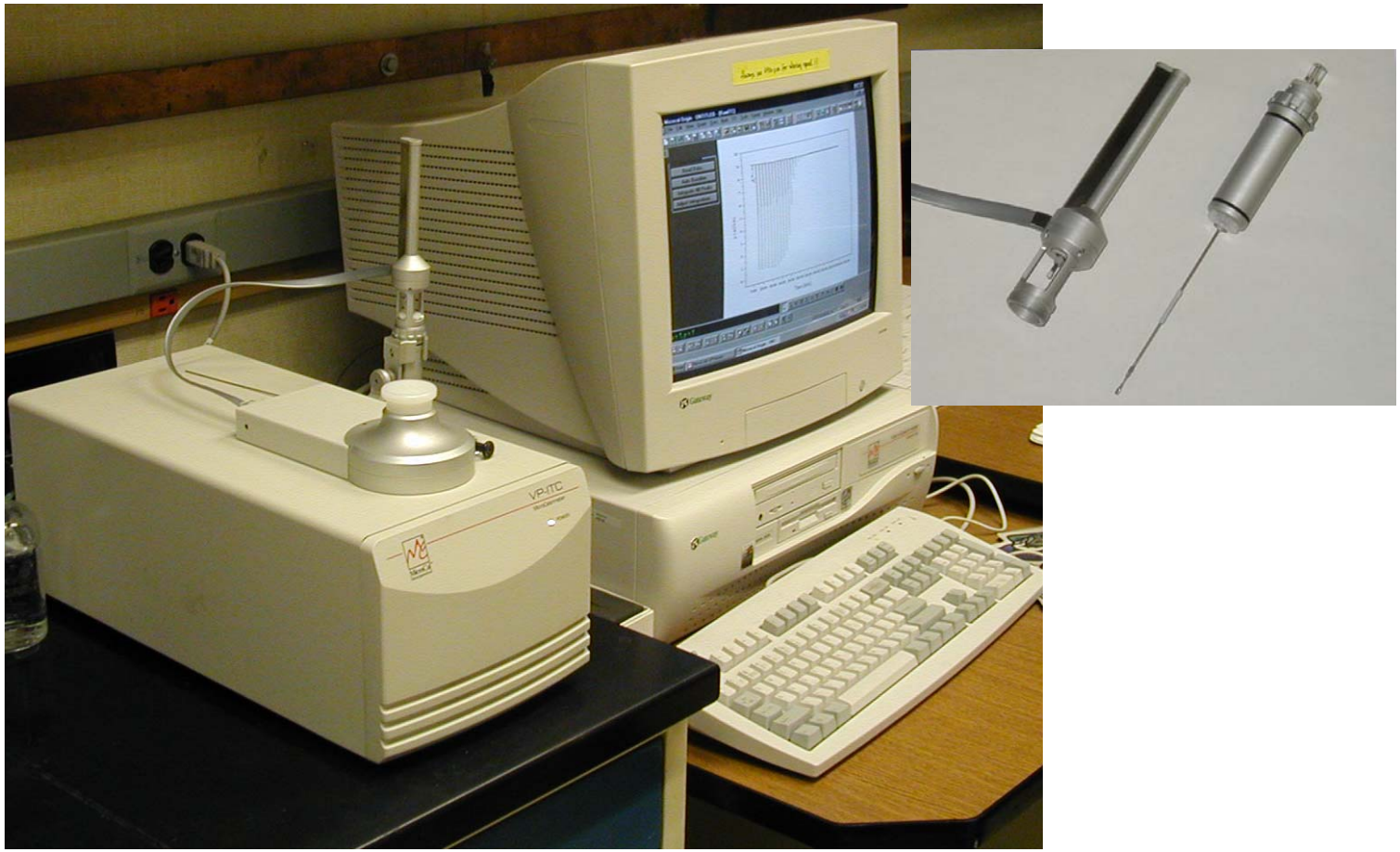


→ ΔH, T_m, ΔC_P, ΔS, ΔG, K

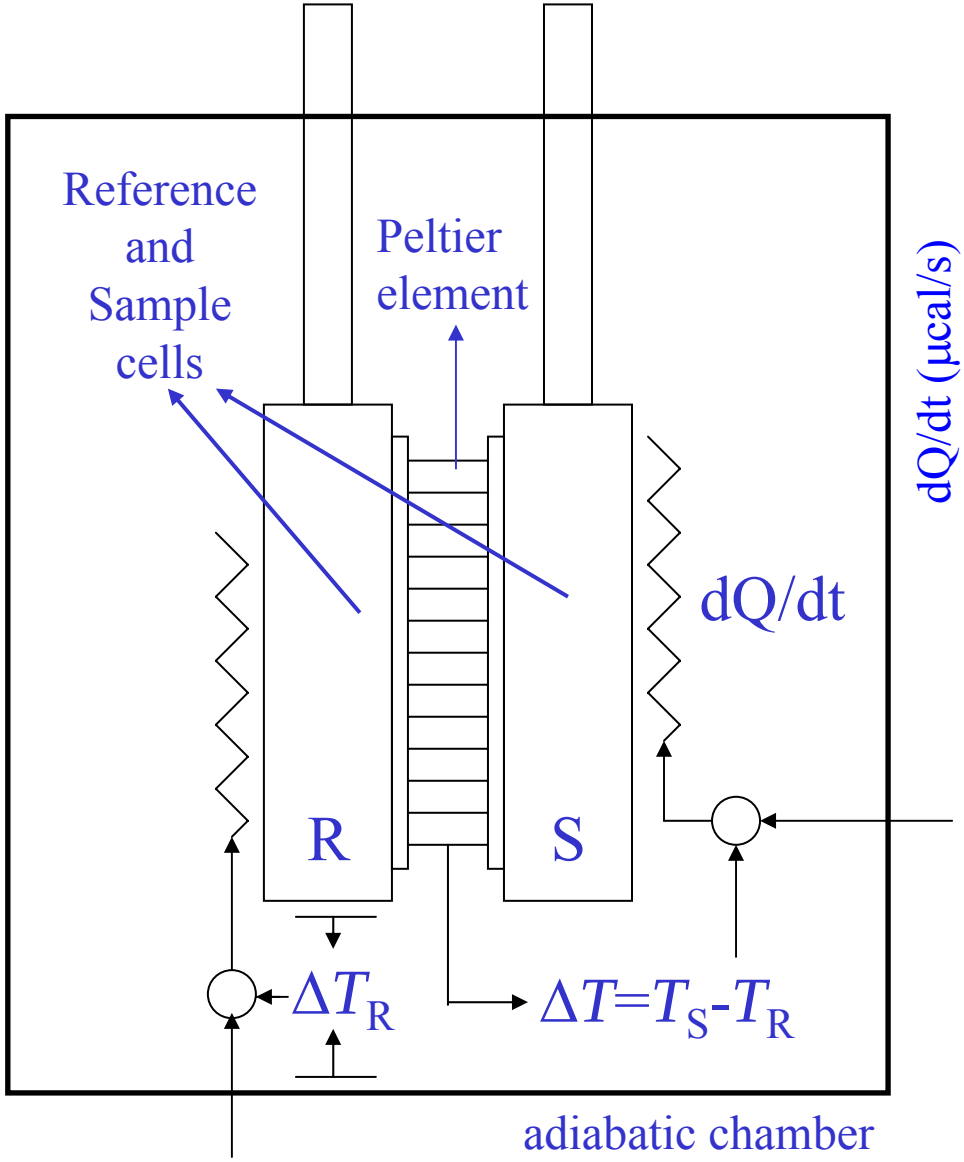
Native ↔ *Unfolded*

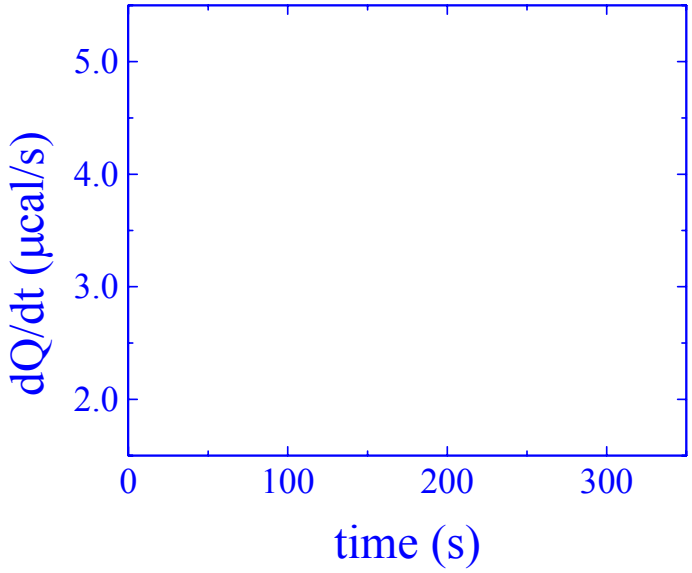
Isothermal Titration Calorimetry

Isothermal Titration Calorimetry



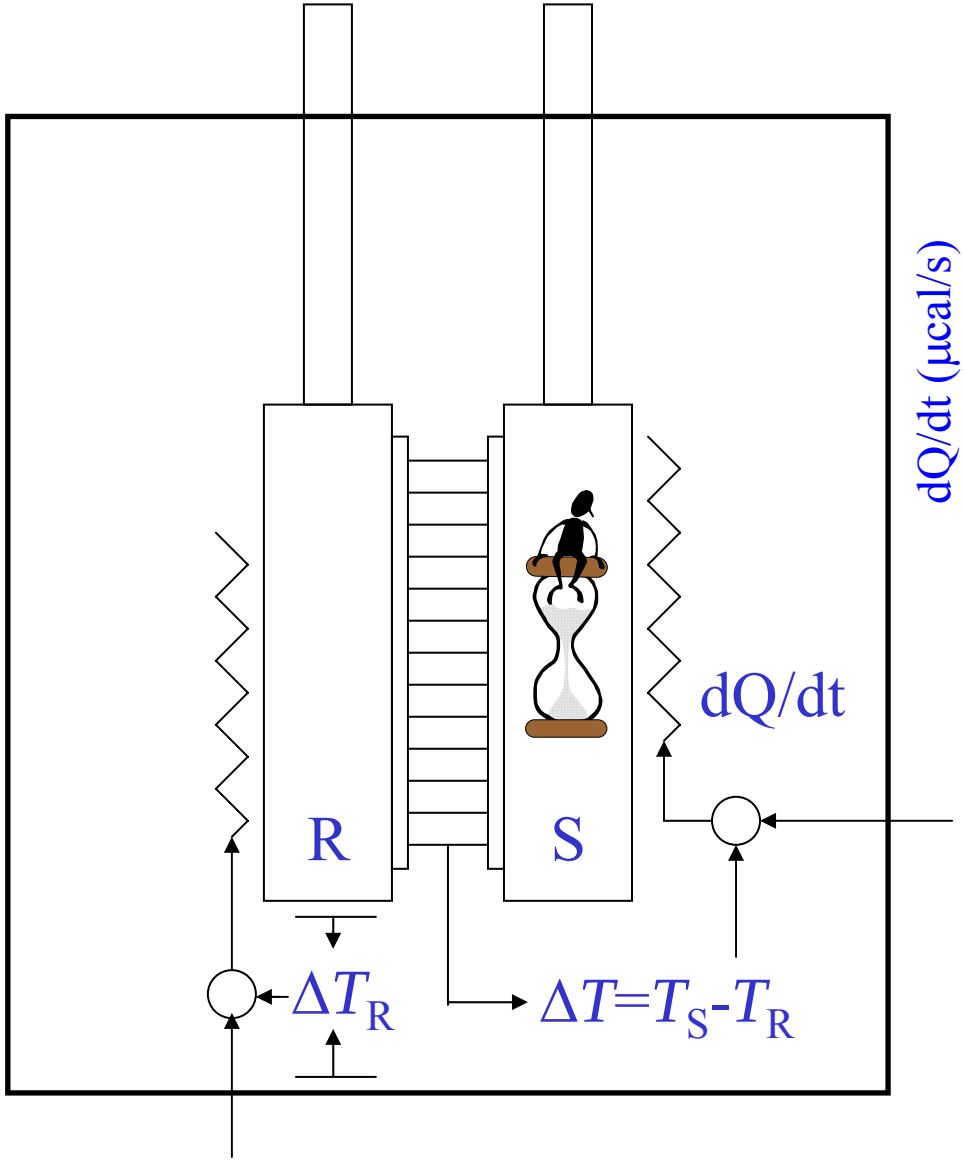
Isothermal Titration Calorimetry

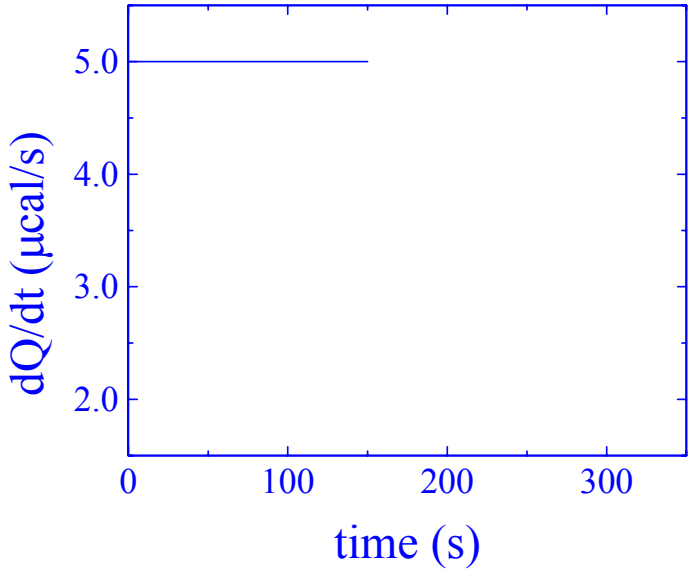




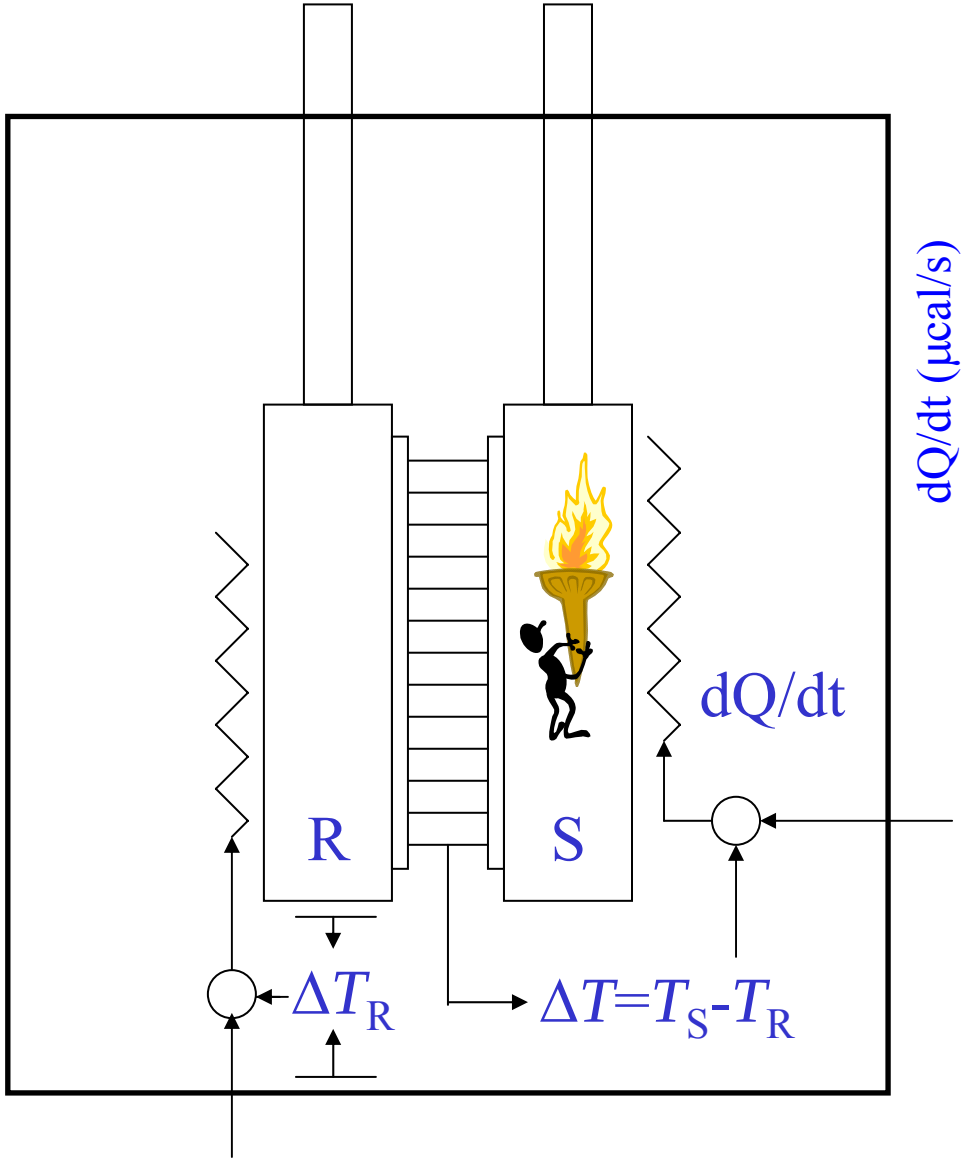
The measured signal is the amount of heat per time unit, dQ/dt, that must be provided or removed in order to keep ΔT null.

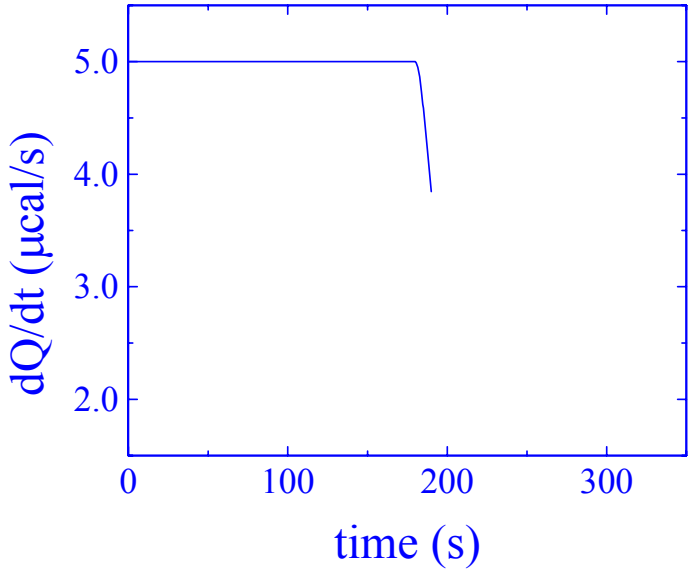
Isothermal Titration Calorimetry



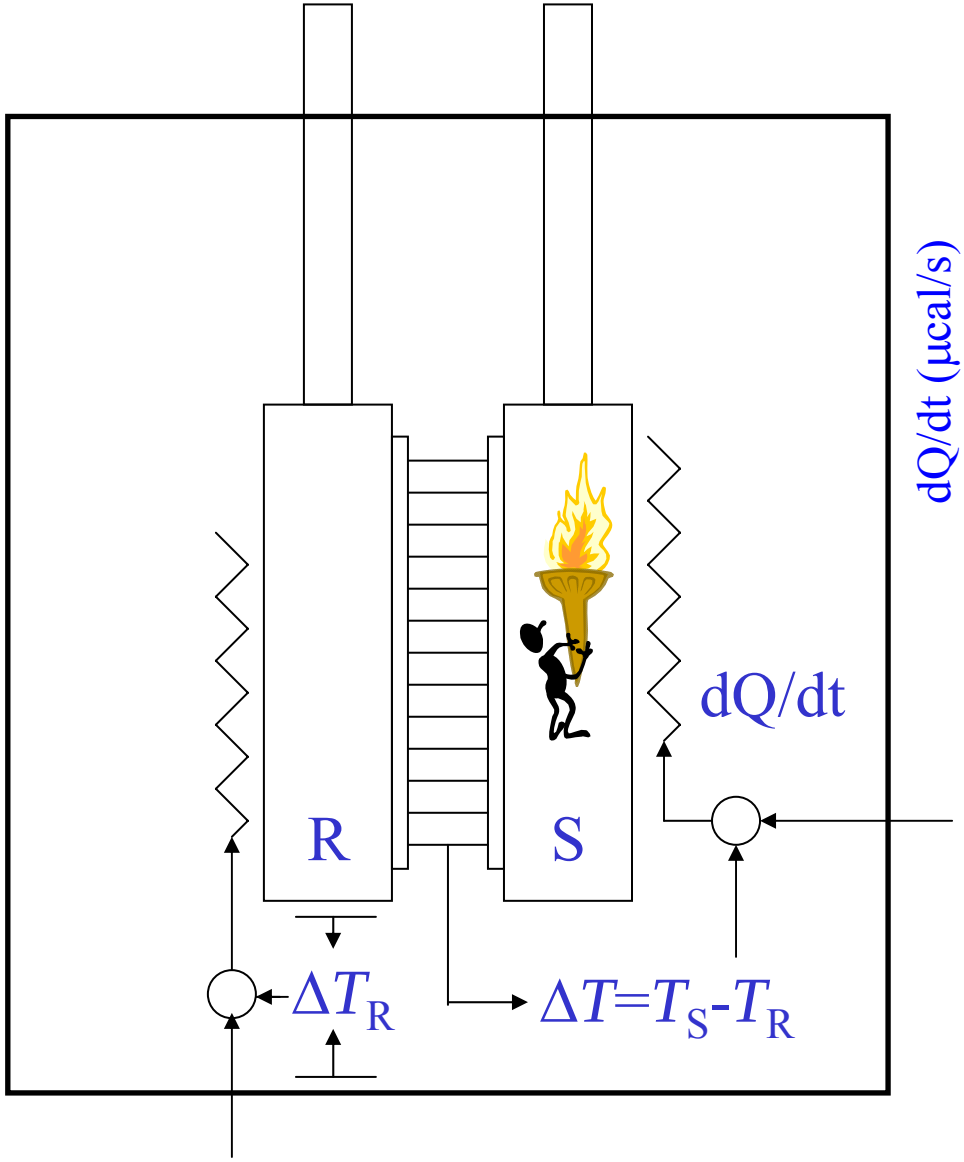


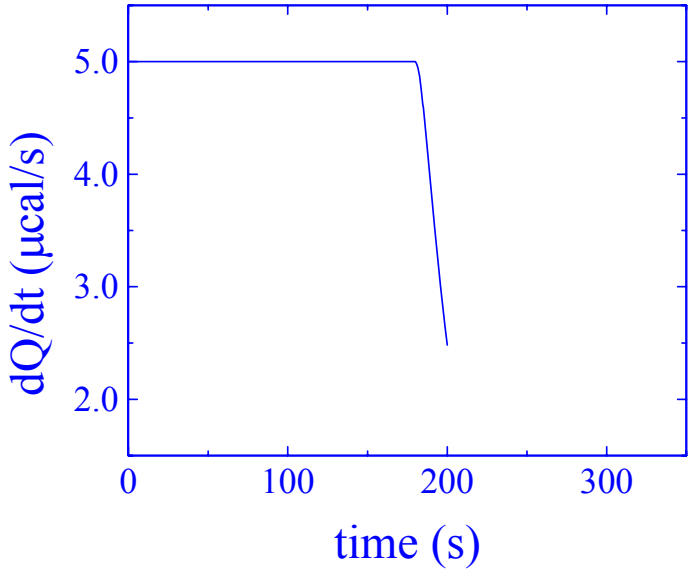
Isothermal Titration Calorimetry



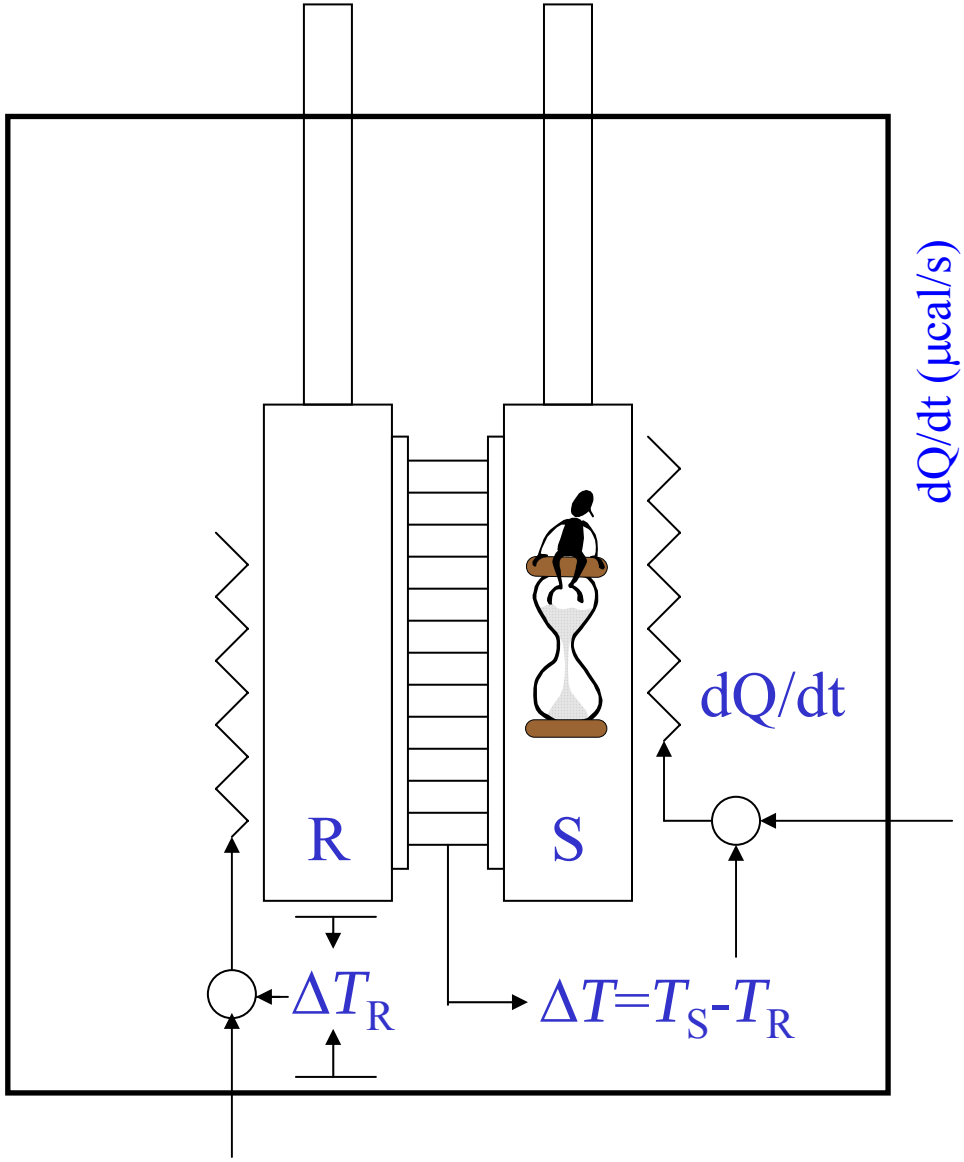


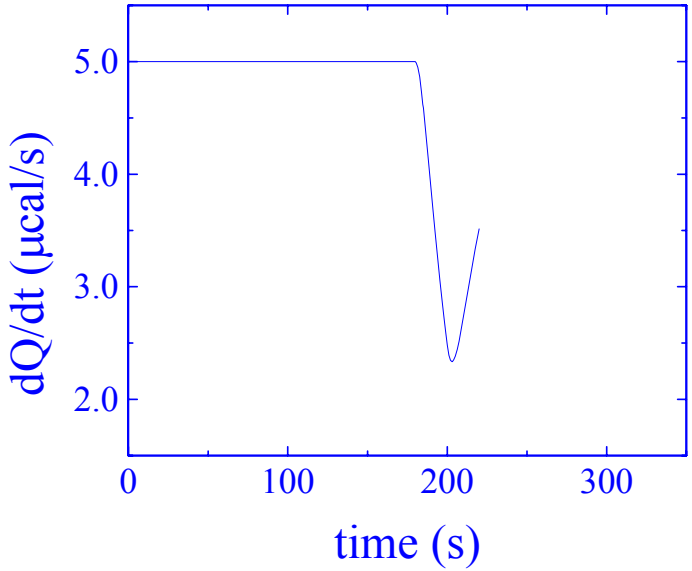
Isothermal Titration Calorimetry



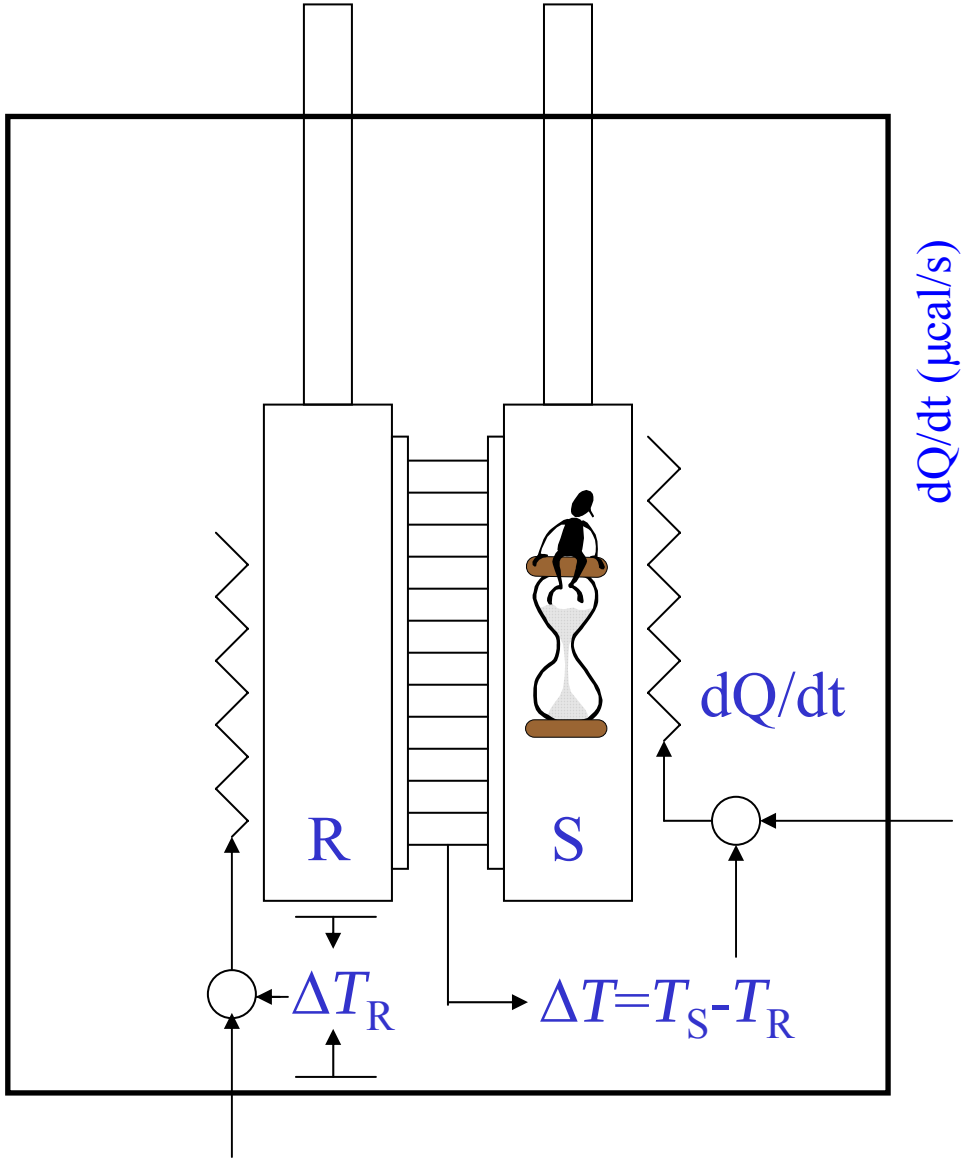


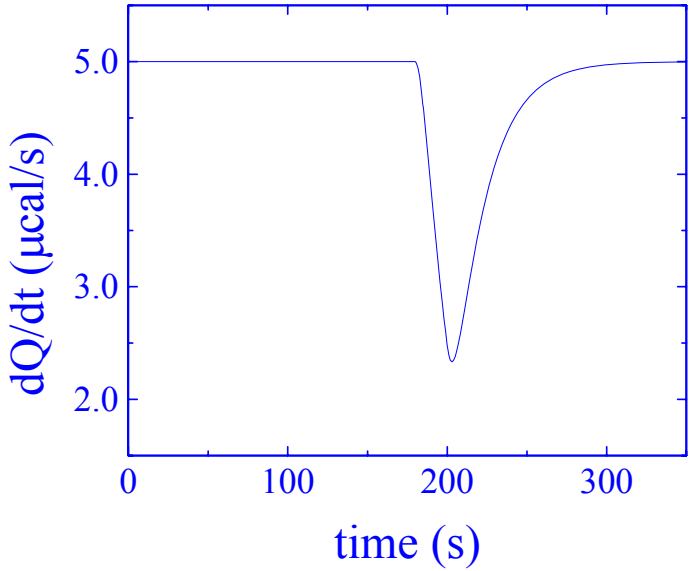
Isothermal Titration Calorimetry



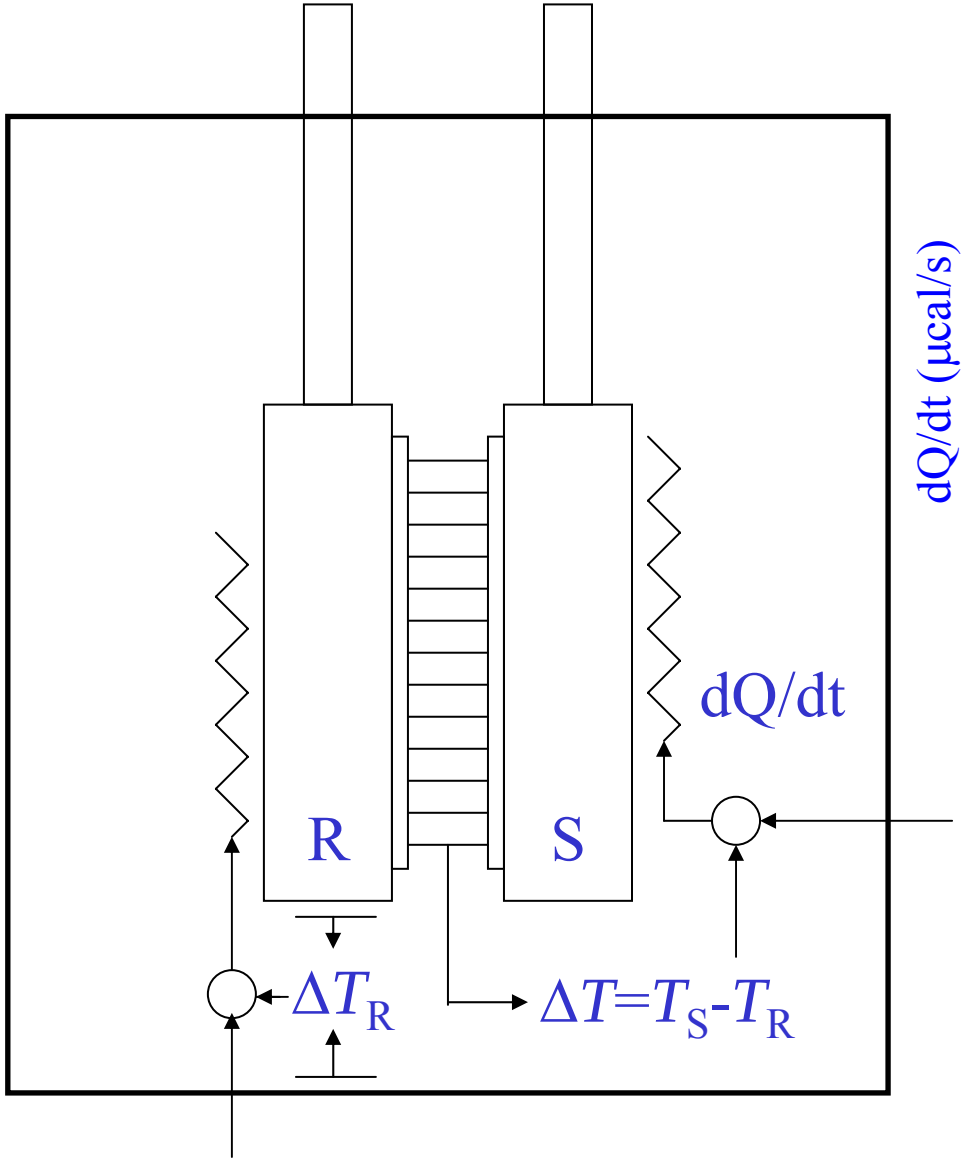


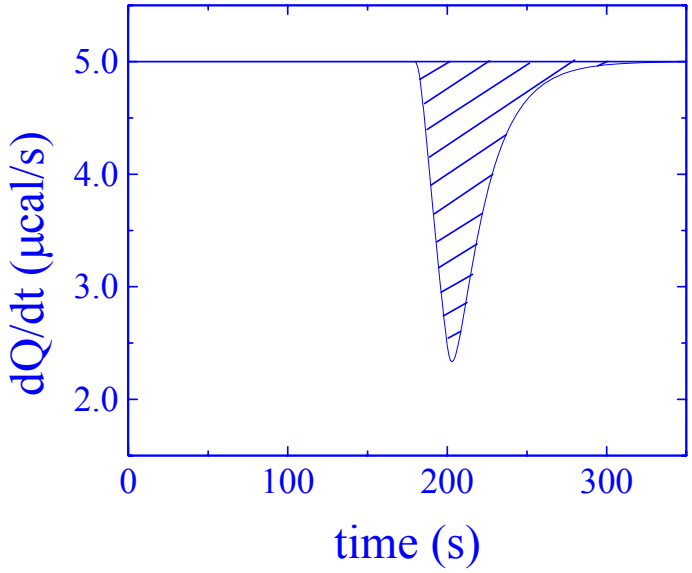
Isothermal Titration Calorimetry





Isothermal Titration Calorimetry

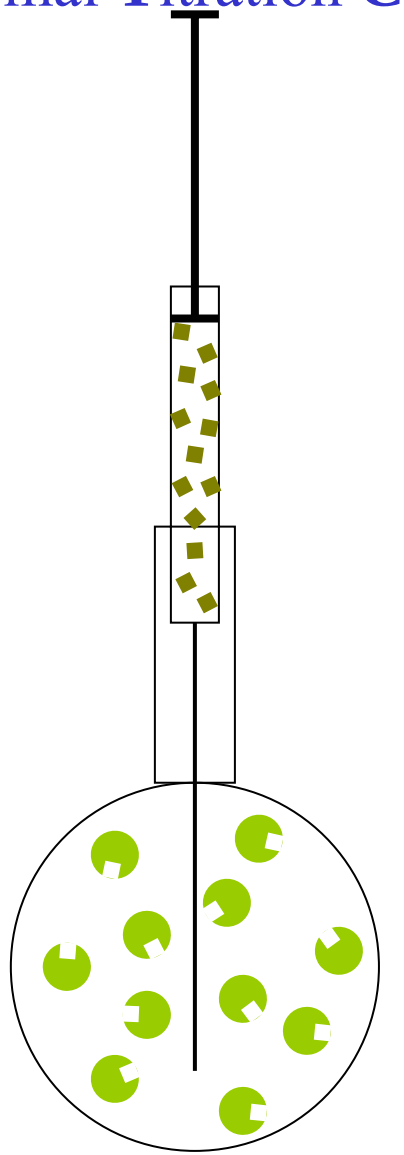


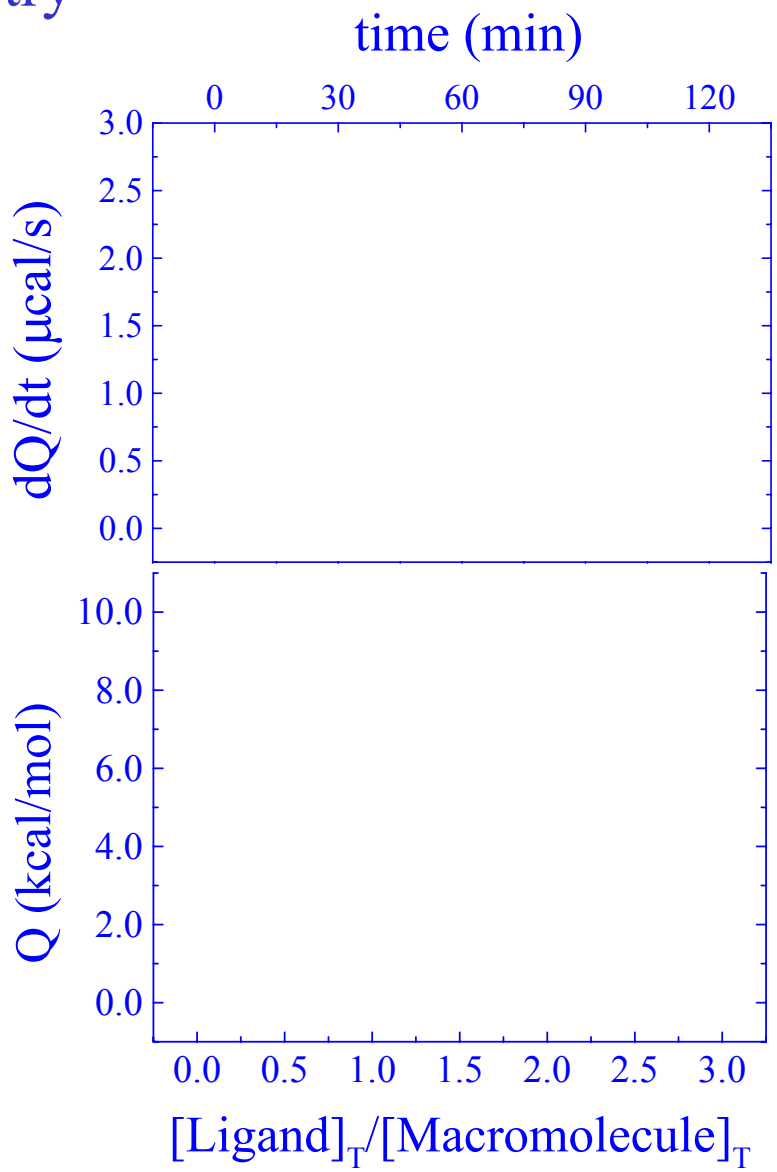


$$Q = \int_{t_o}^{t_f} \frac{dQ}{dt}\, dt$$

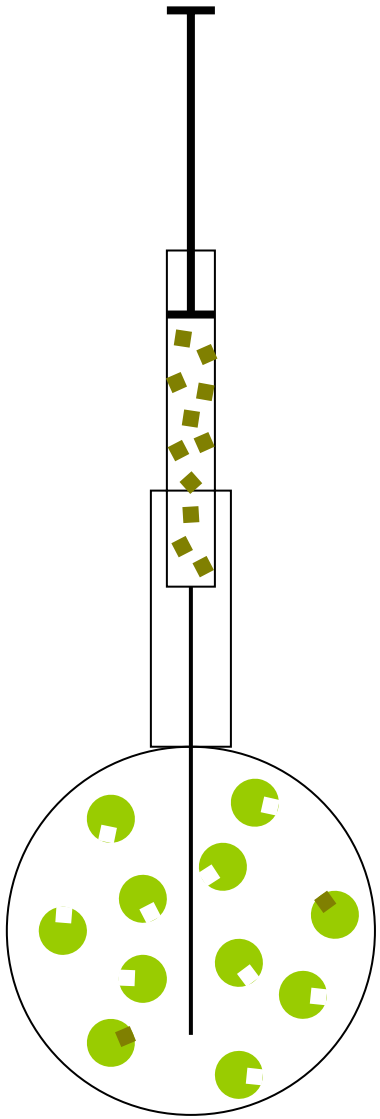
Total heat released or absorbed during the thermal event

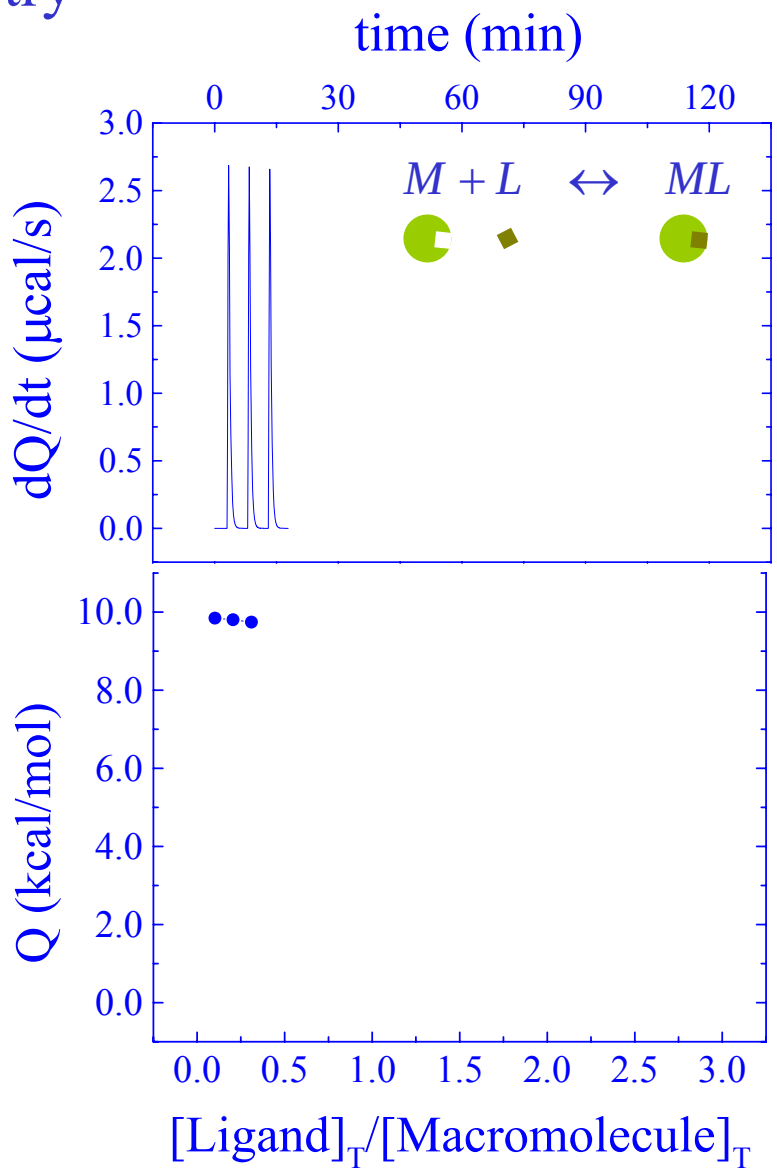
Isothermal Titration Calorimetry



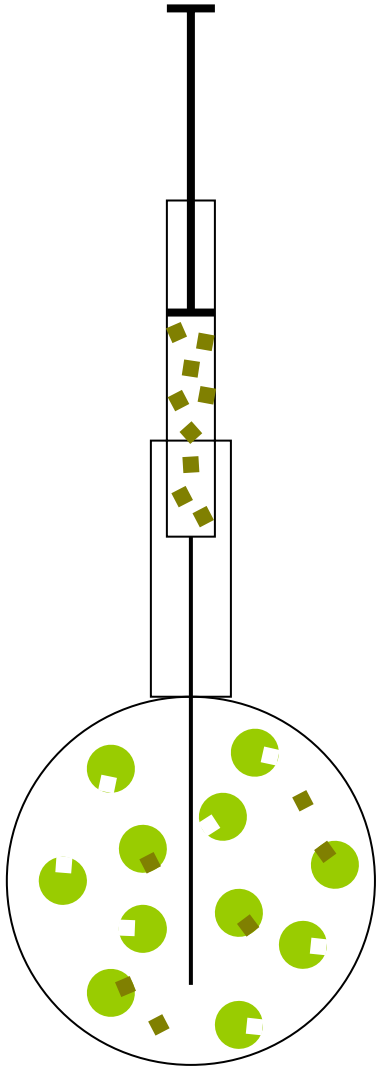


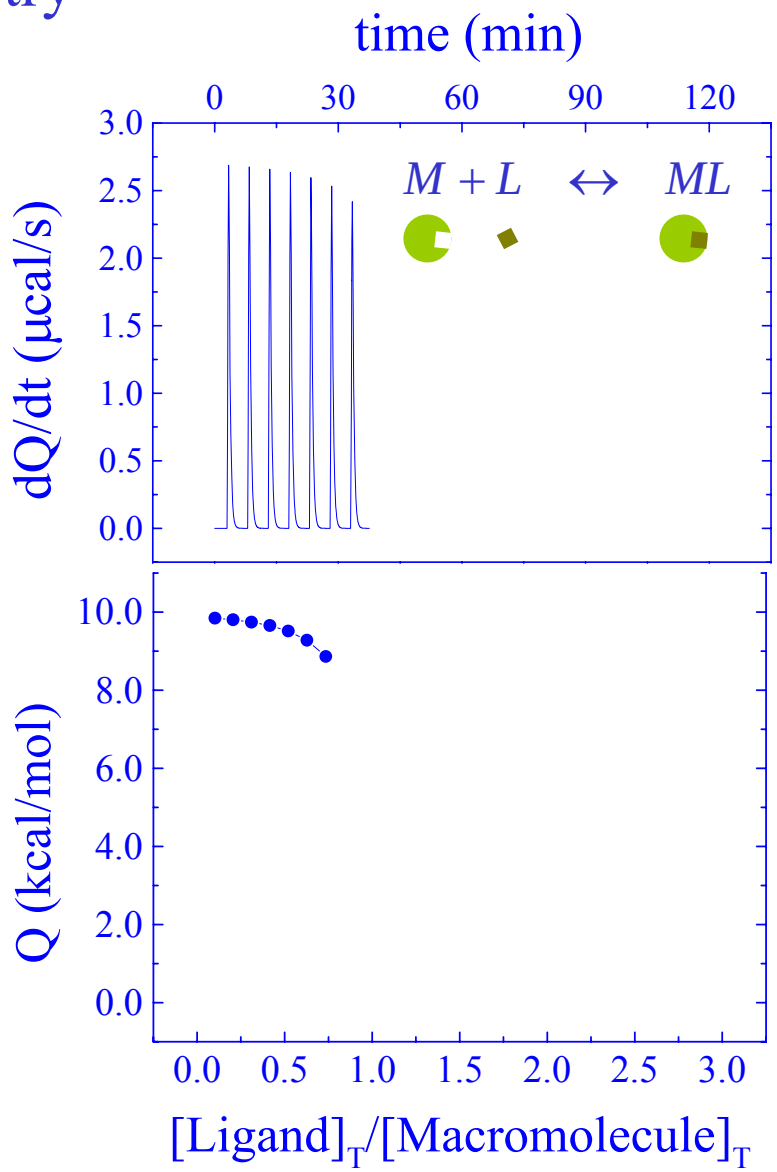
Isothermal Titration Calorimetry



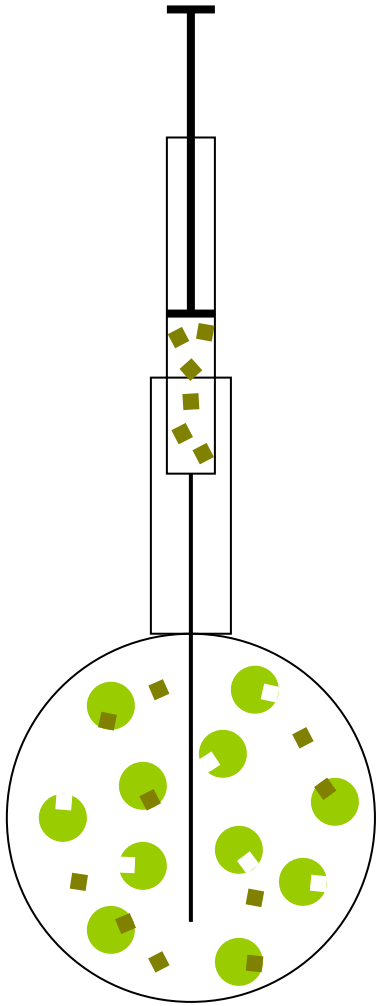


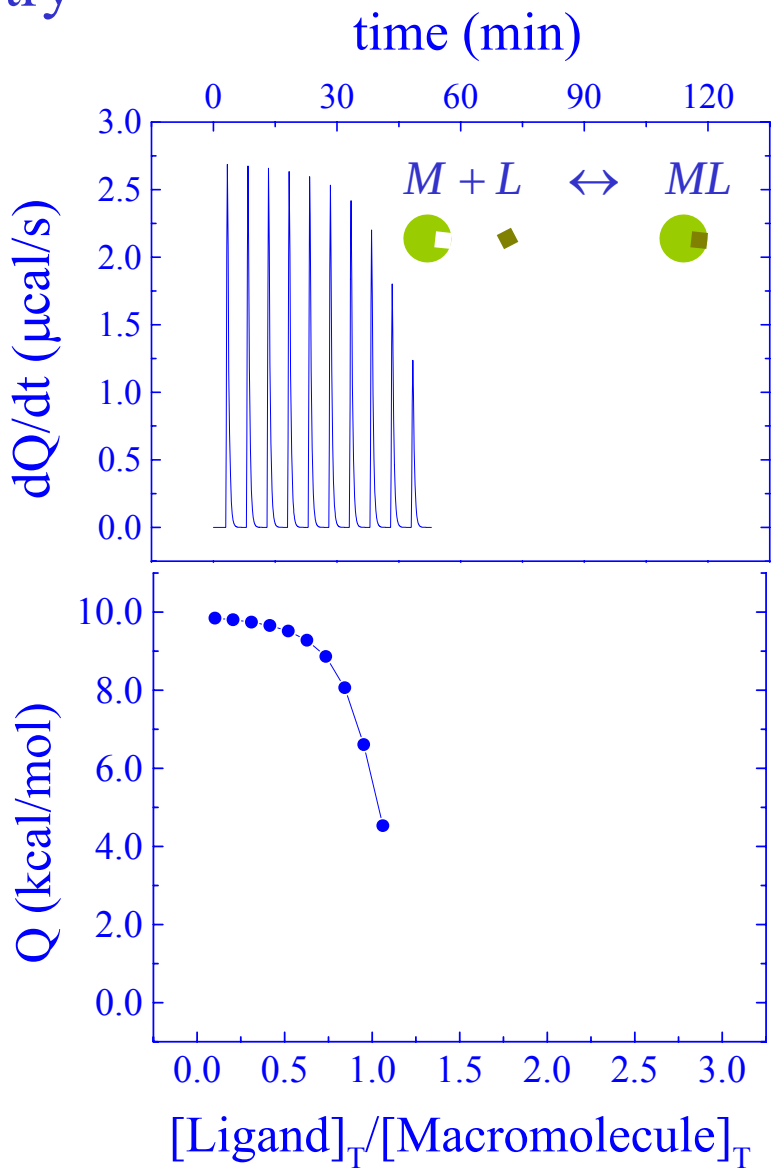
Isothermal Titration Calorimetry



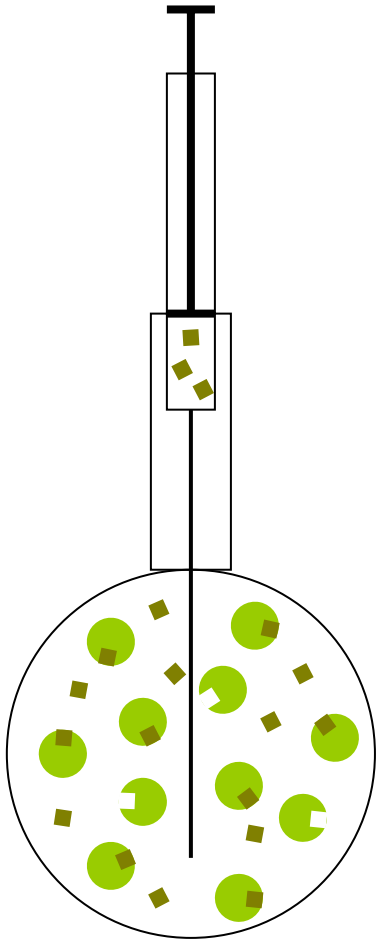


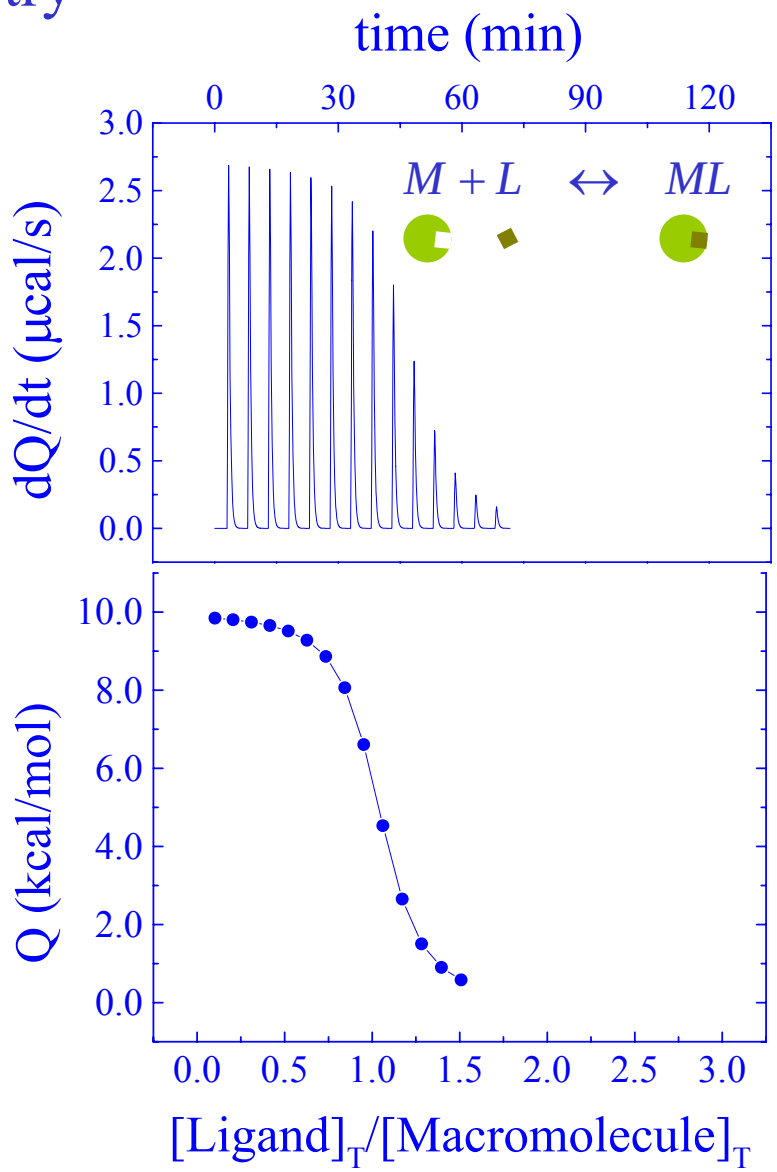
Isothermal Titration Calorimetry



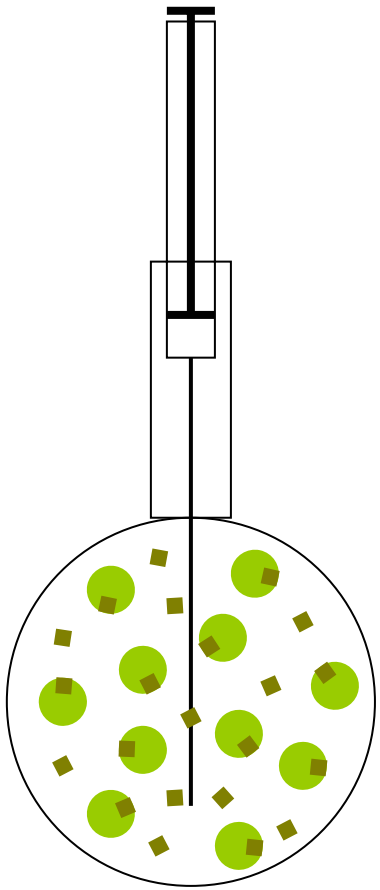


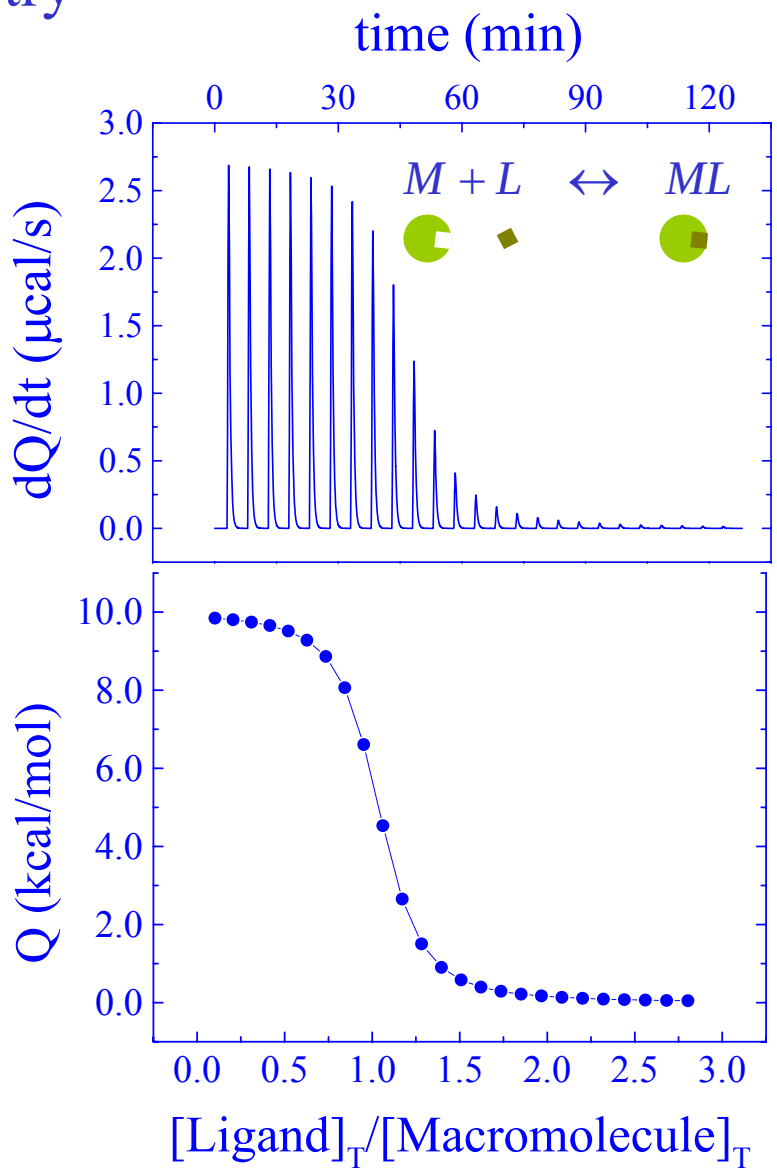
Isothermal Titration Calorimetry



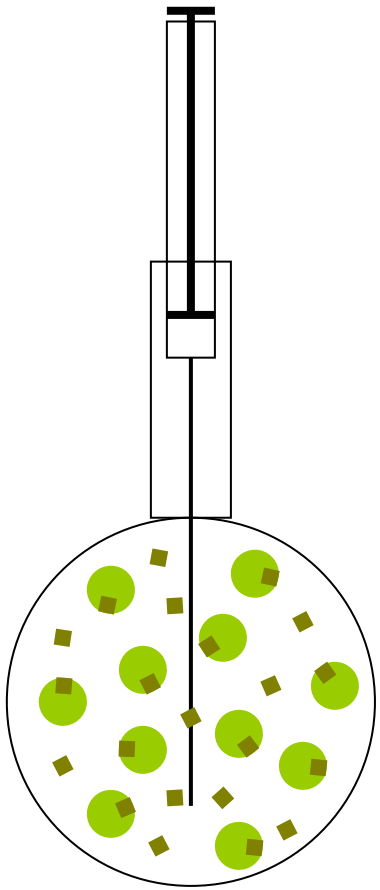


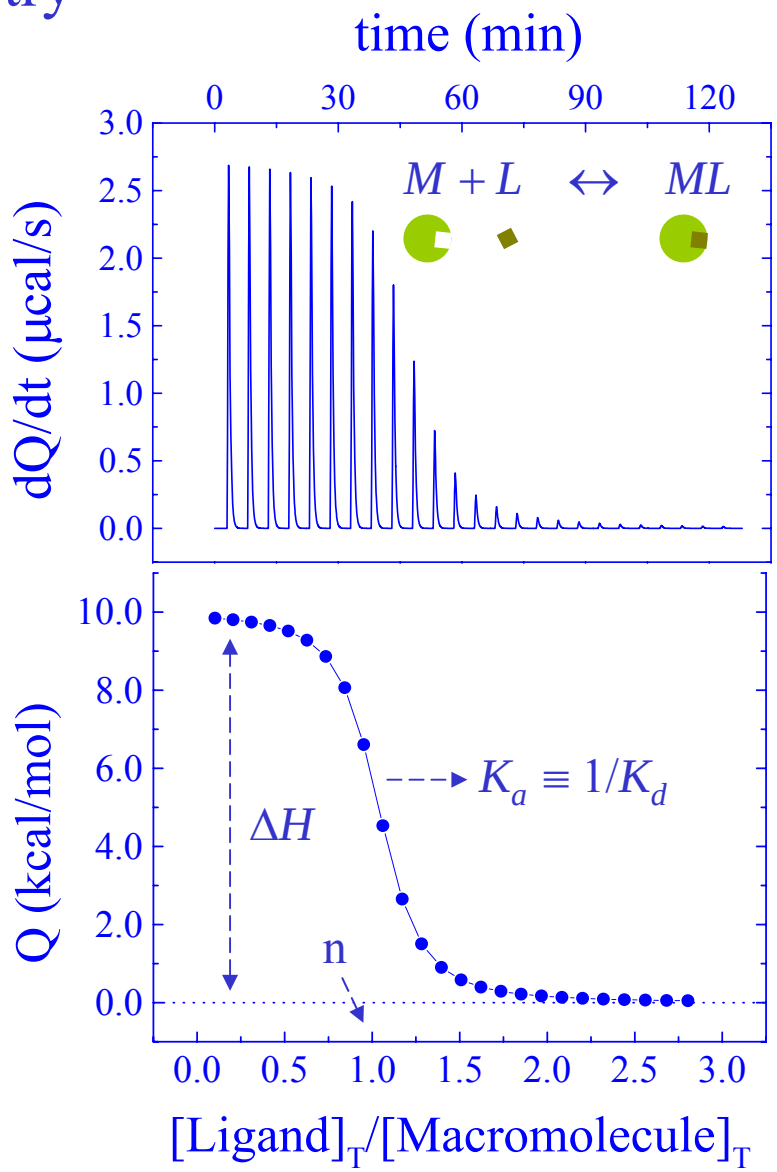
Isothermal Titration Calorimetry





Isothermal Titration Calorimetry





ITC: Advantages

Complete thermodynamic charaterization: ΔH, K_a, n, ΔG and ΔS

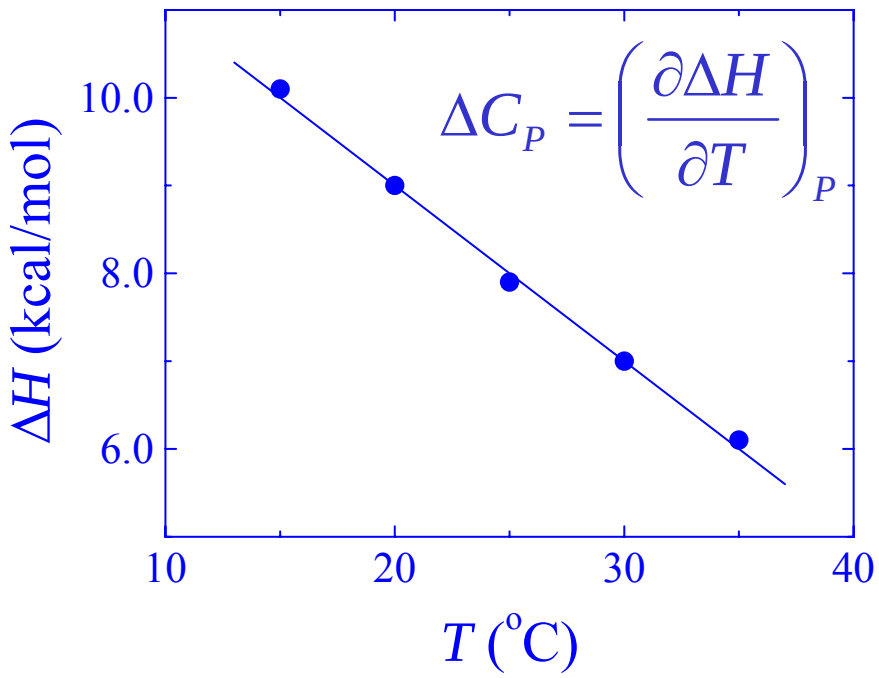
Direct determination of the binding enthalpy (with no additional assumptions)

Heat is a universal signal

Absence of reporter labels

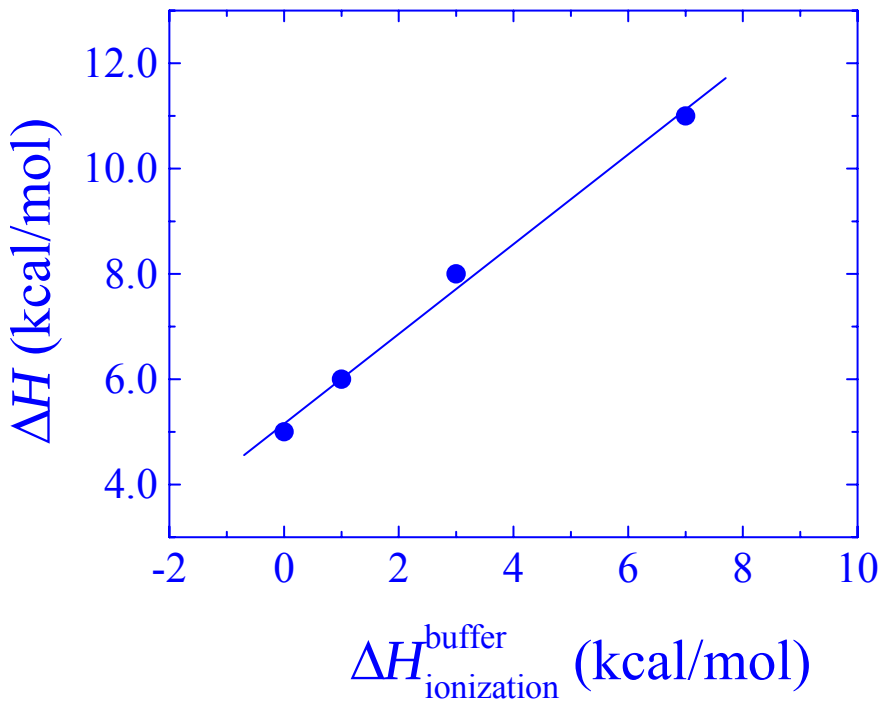
Non-destructive technique

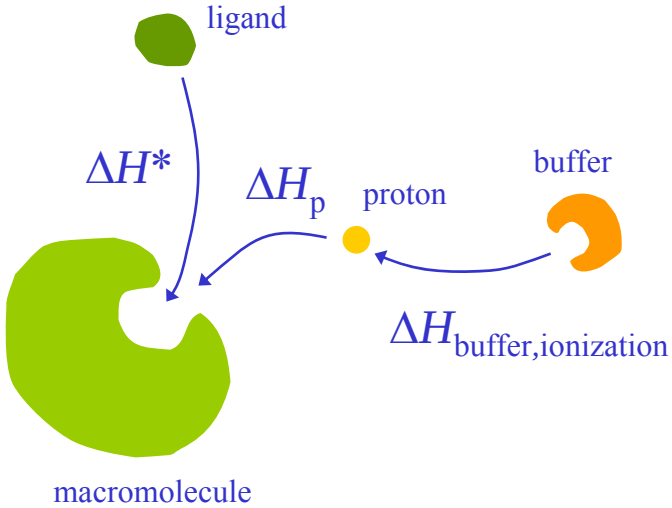
Determination of the Binding Heat Capacity



$$\Delta H(T) = \Delta H(T_0) + \Delta C_P (T - T_0)$$

Determination of Proton Transfer Processes Coupled to Ligand Binding



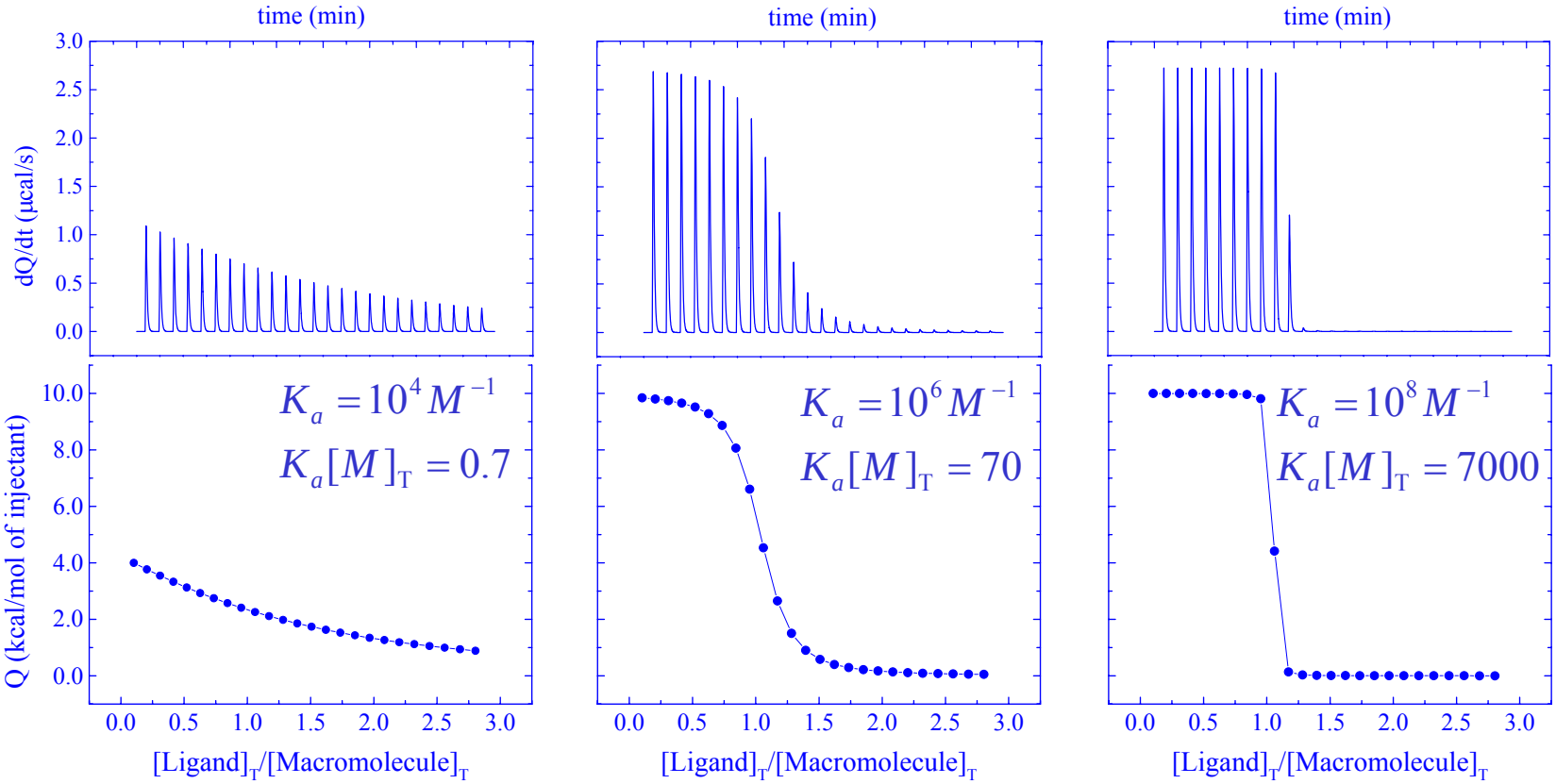


$$\Delta H = \Delta H^{0} + n_{H^{+}} \Delta H^{\text{buffer}}_{\text{ionization}}$$

ΔH^0 binding enthalpy independent of the buffer

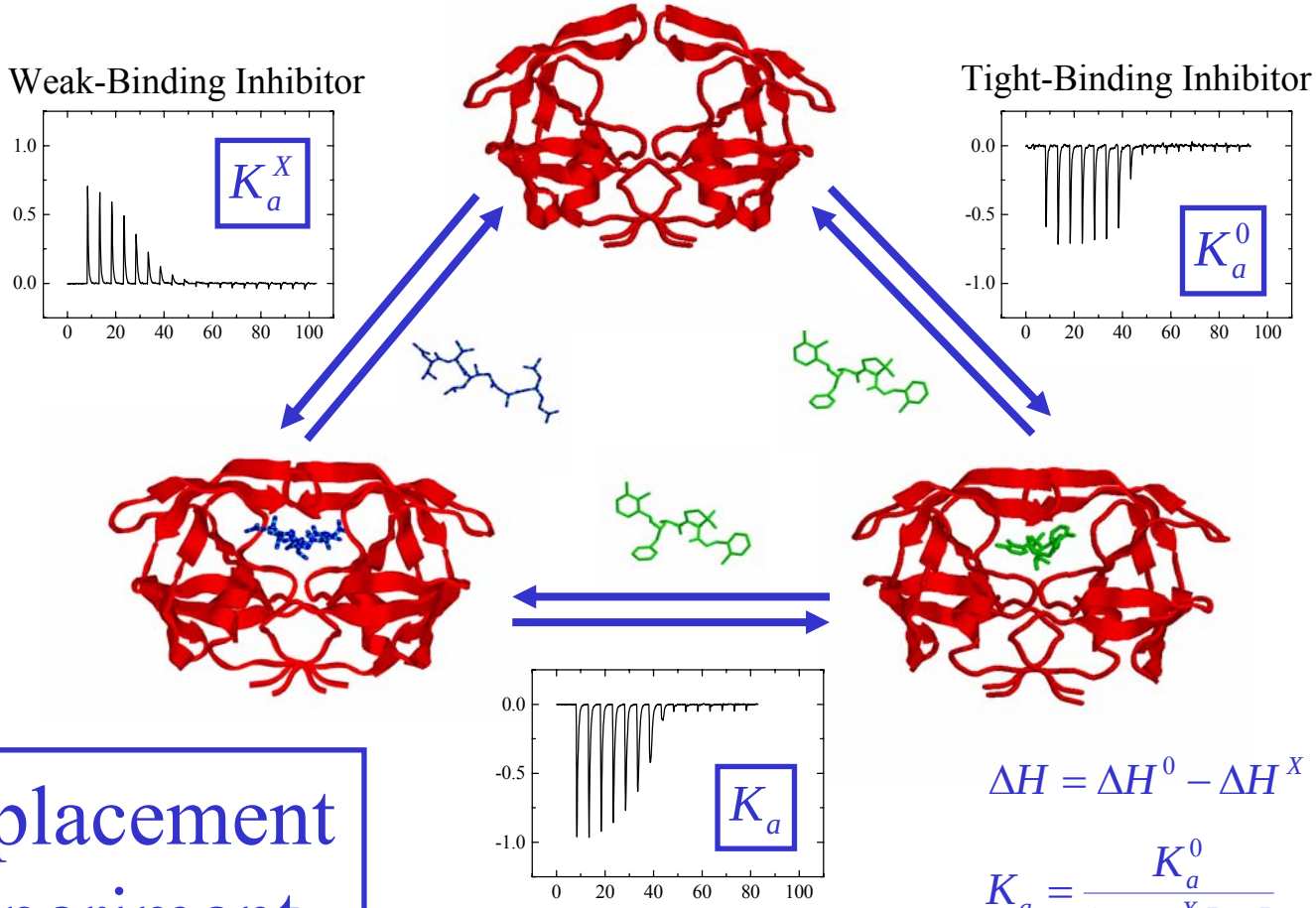
n_{H+} number of protons exchanged between M-L complex and solution

ITC: Considerations



- $10^4 M^{-1} \leq K_a \leq 10^8 M^{-1}$
- $\Delta H \neq 0$
- amount of sample (~ 1 mg)
- fast analytic technique (< 2 hours/experiment)

Extension of the Practical Limits for Reliable Affinity Determination

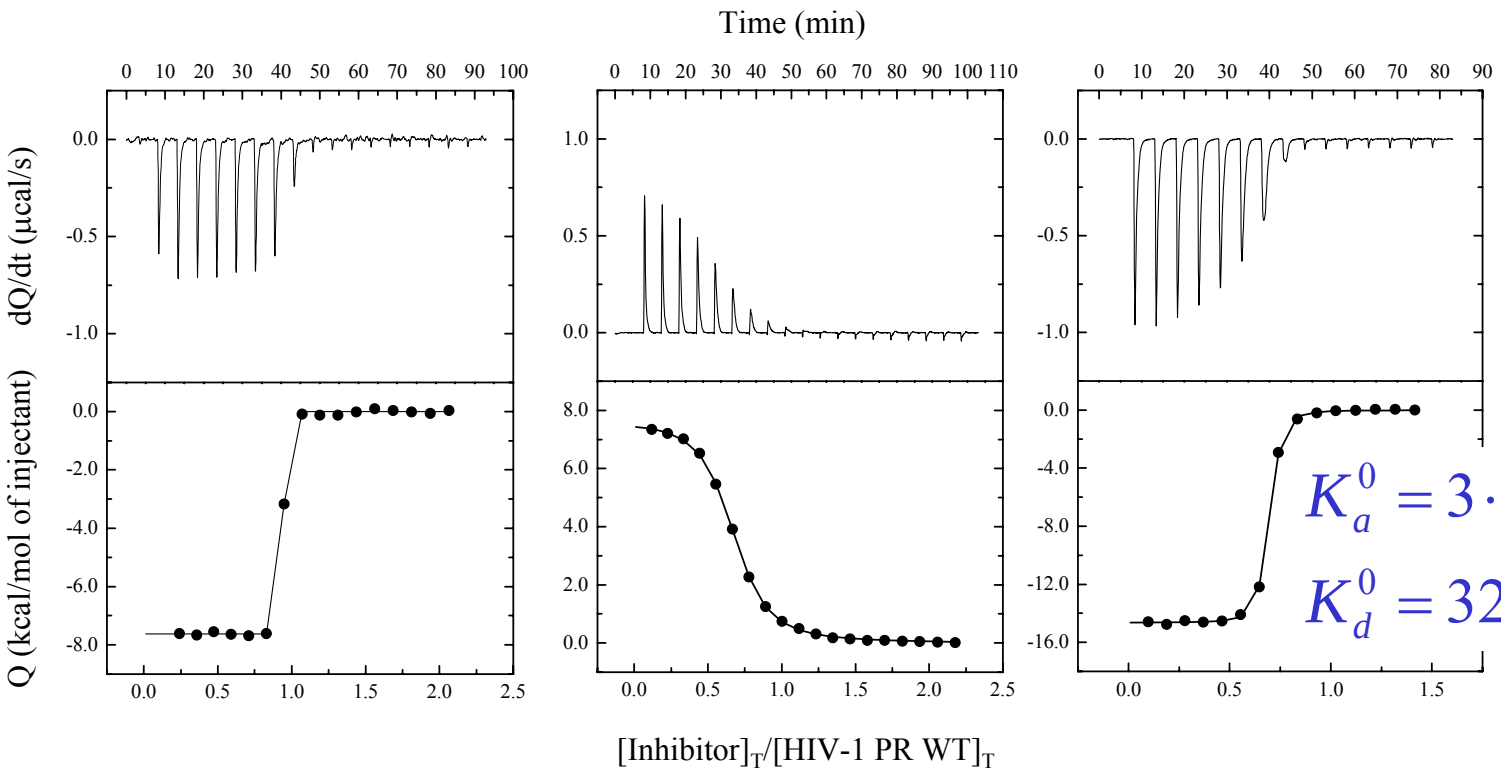


$$\Delta H = \Delta H^0 - \Delta H^X \frac{K_a^X [X]}{1 + K_a^X [X]}$$

$$K_a = \frac{K_a^0}{1 + K_a^X [X]}$$

Displacement Experiment Scheme

Measuring High Binding Affinity



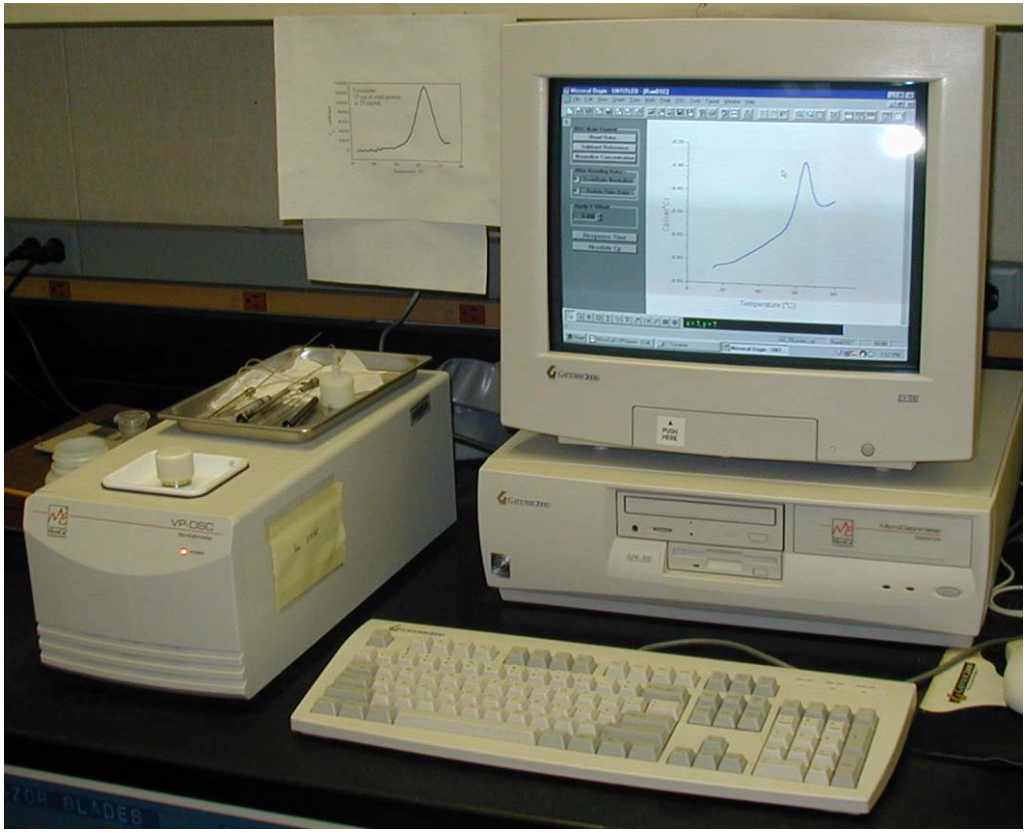
$$K_a^0 = 3 \cdot 10^{10}\,\mathrm{M}^{-1}$$

$$K_d^0 = 32\ \mathrm{pM}$$

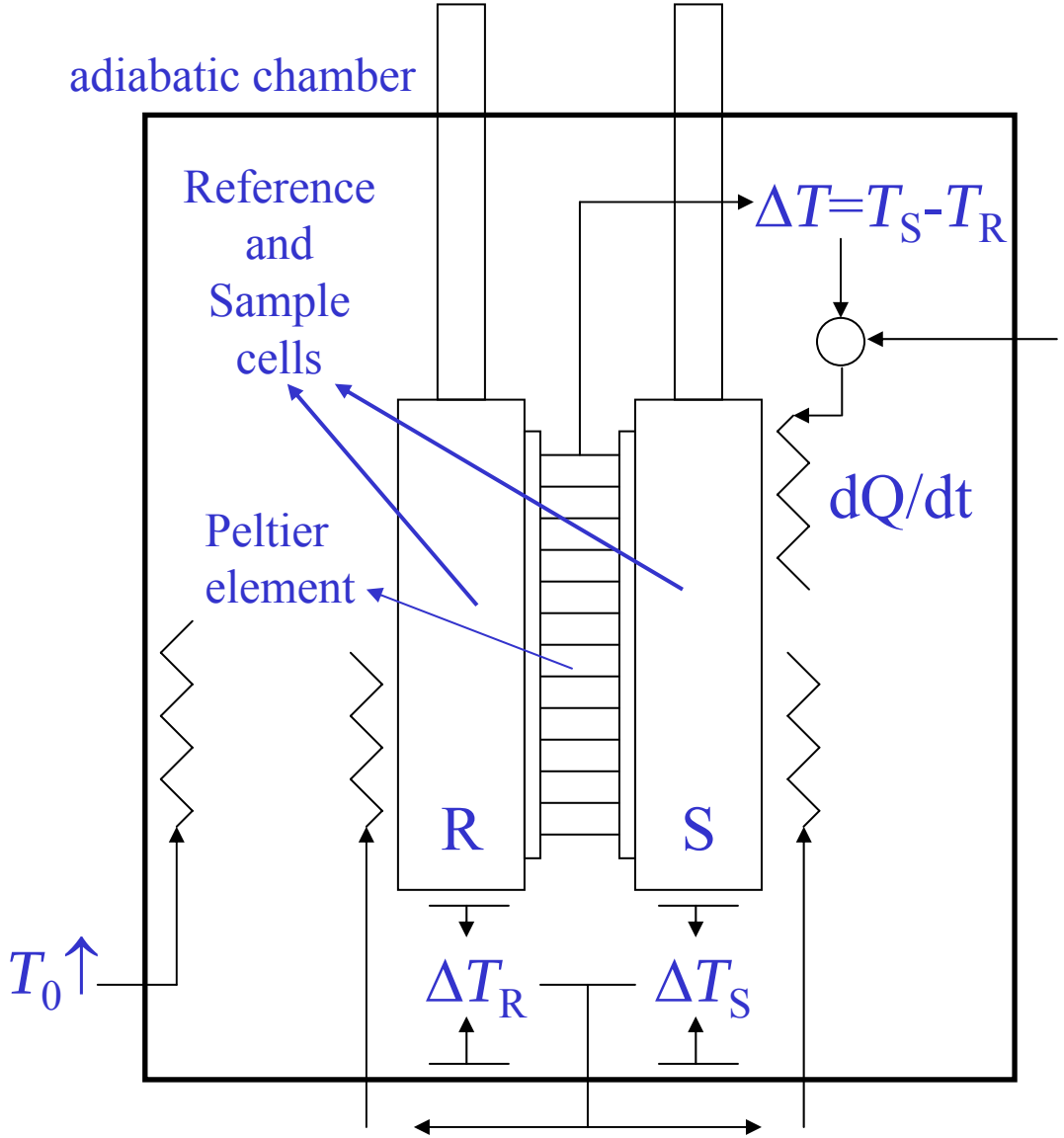
KNI-764	Ac-Pepstatin	KNI-764
⇩	⇩	⇩
Protease	Protease	Protease + Ac-Pepstatin
K_a^0	K_a^X	$K_a = \dfrac{K_a^0}{1 + K_a^X [X]}$

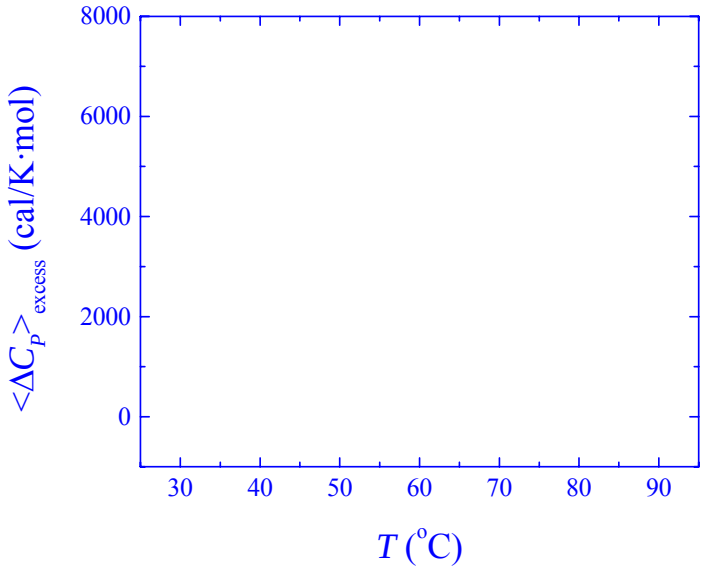
Differential Scanning Calorimetry

Differential Scanning Calorimetry



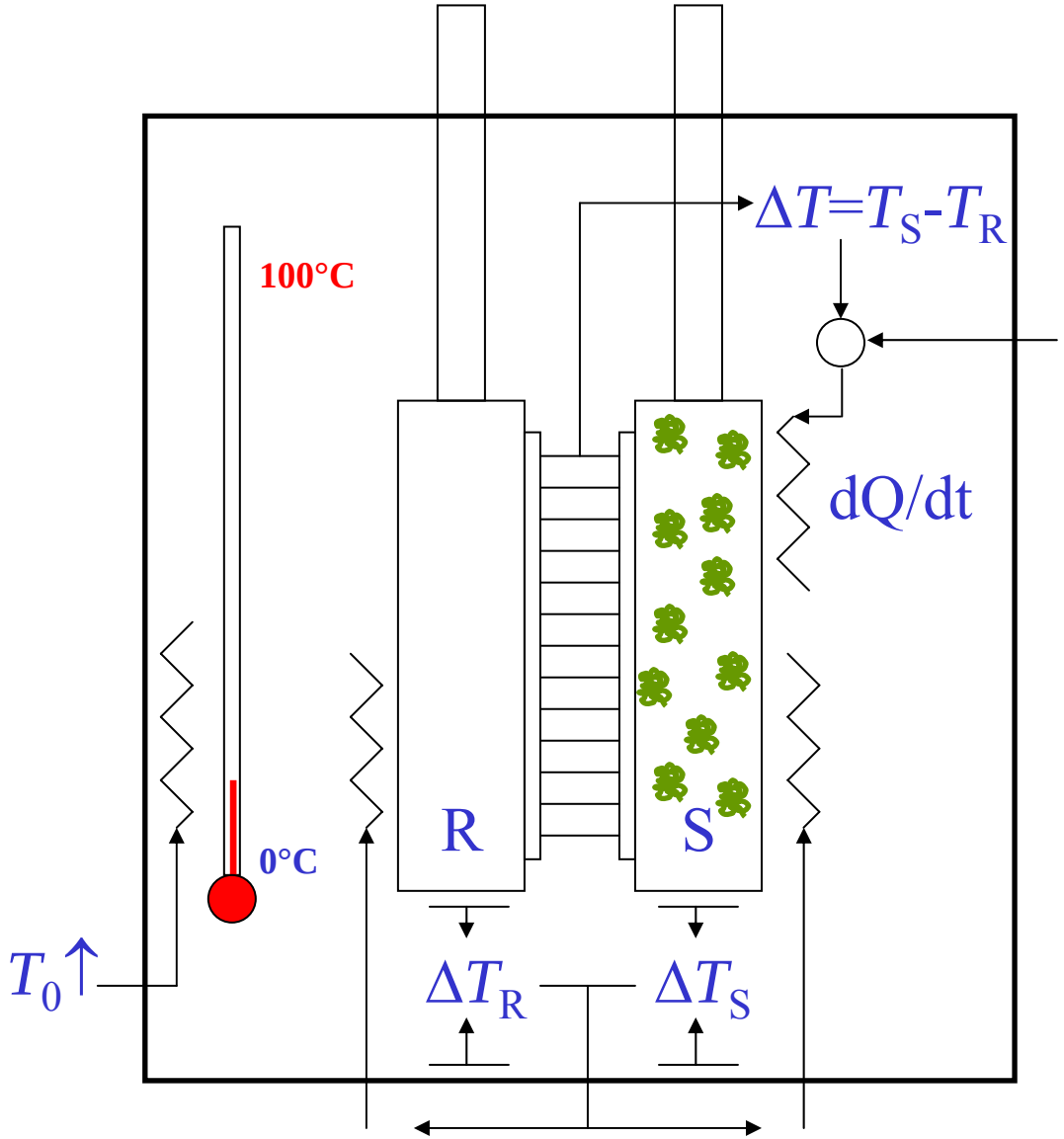
Differential Scanning Calorimetry

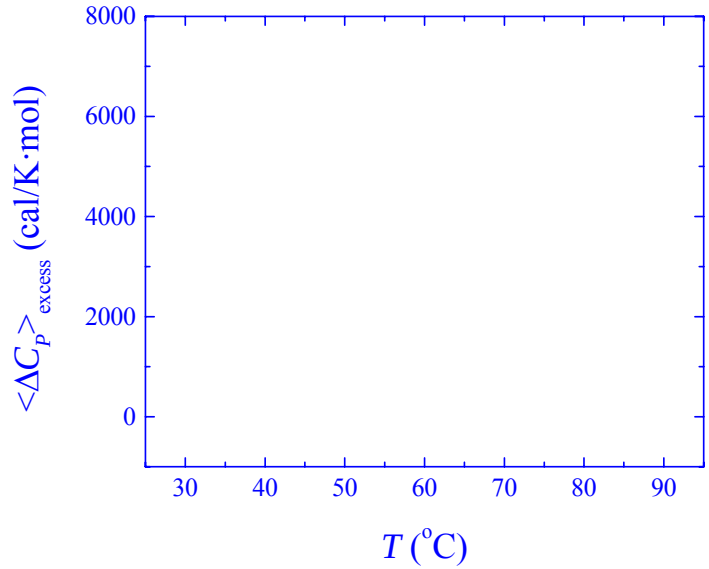




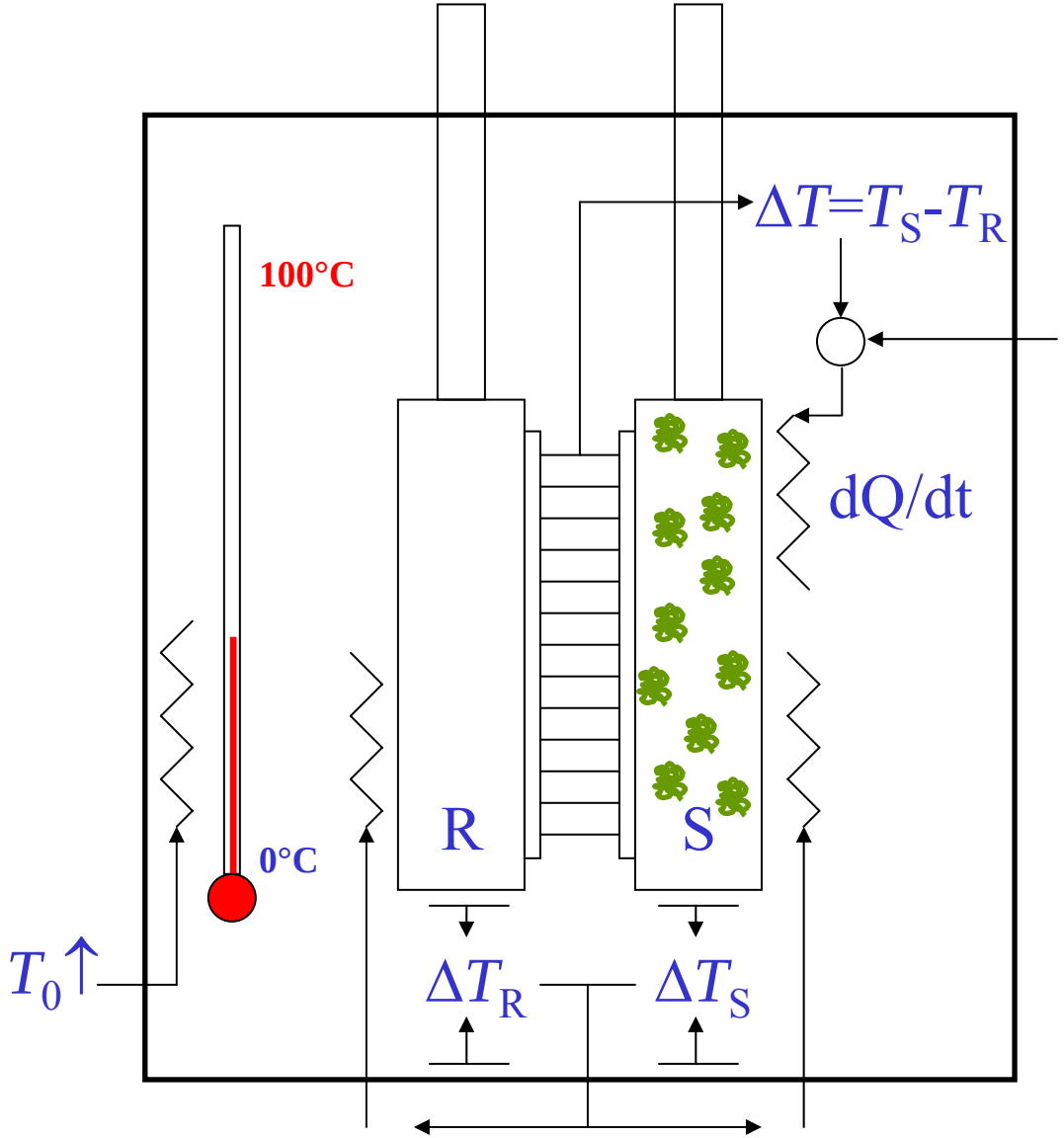
The measured signal is the amount of heat per time unit, dQ/dt, that must be provided or in order to keep ΔT null. Later, thermal power is converted to heat capacity.

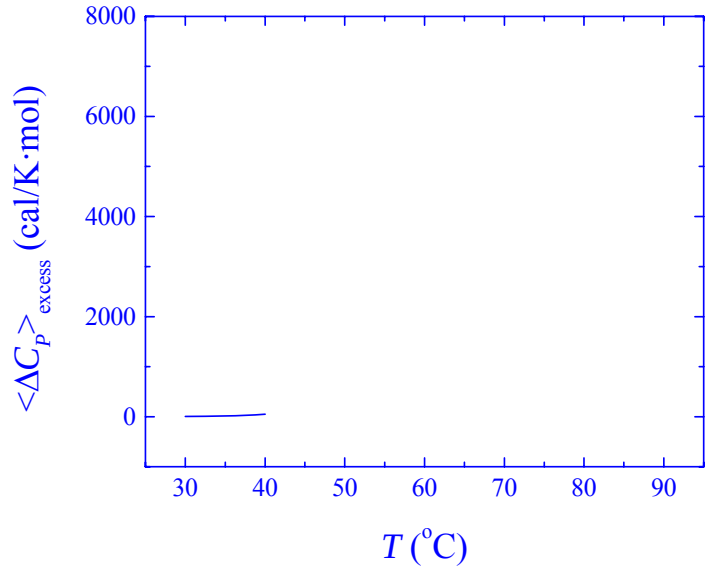
Differential Scanning Calorimetry



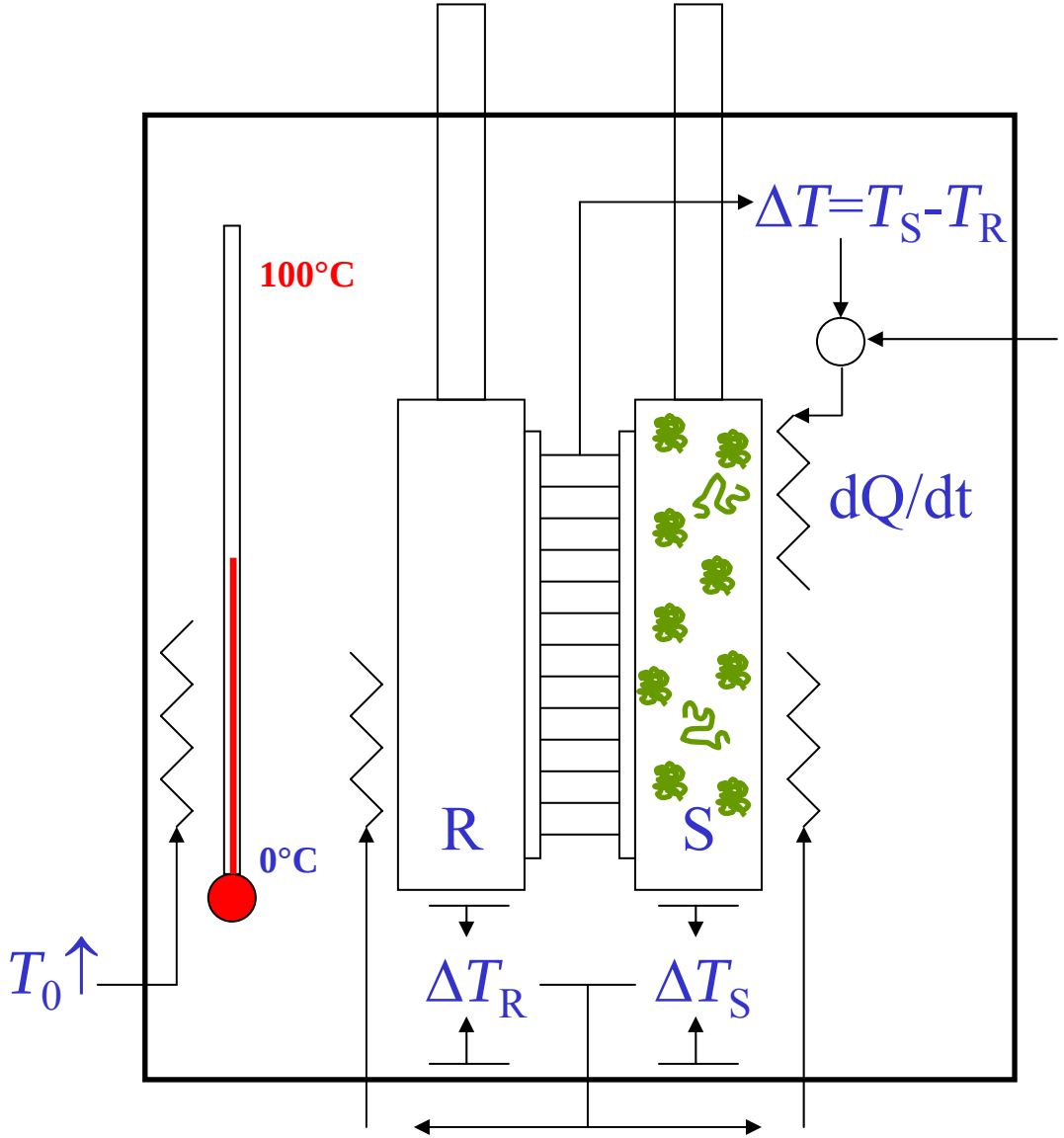


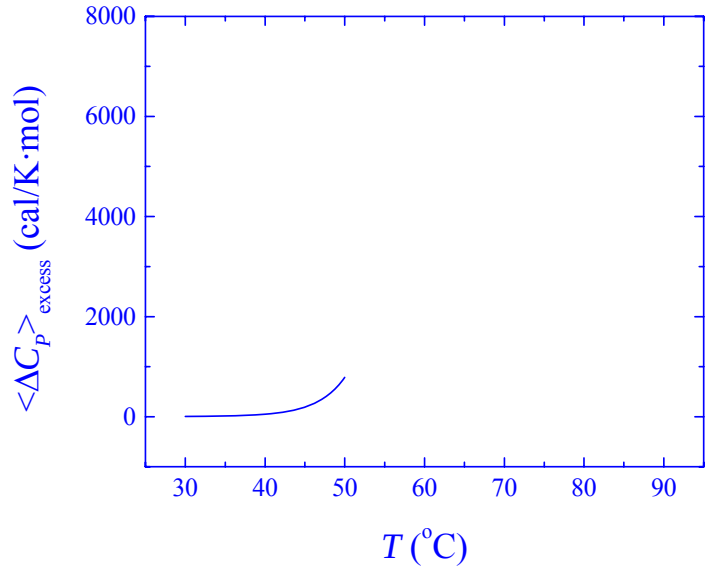
Differential Scanning Calorimetry



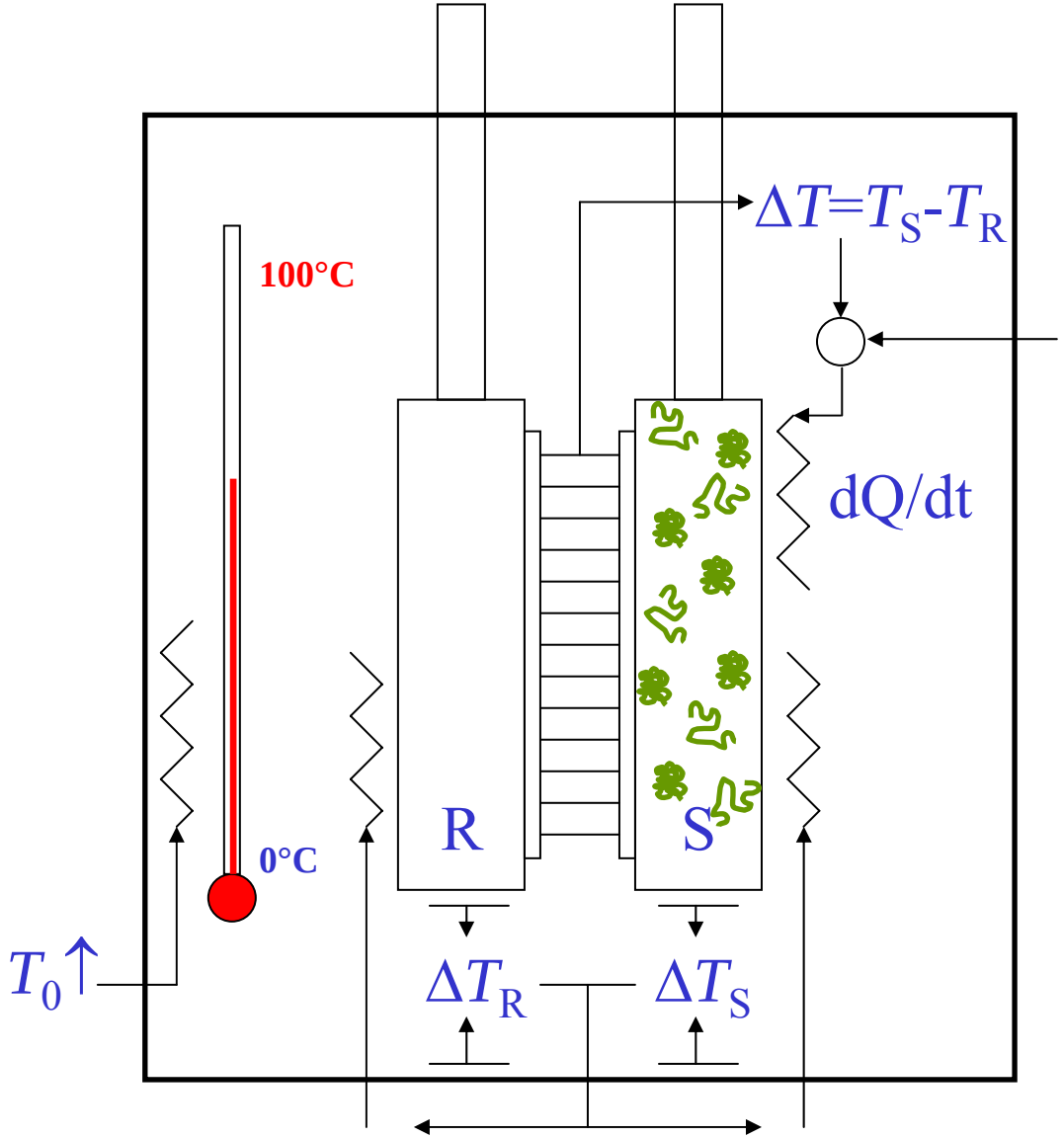


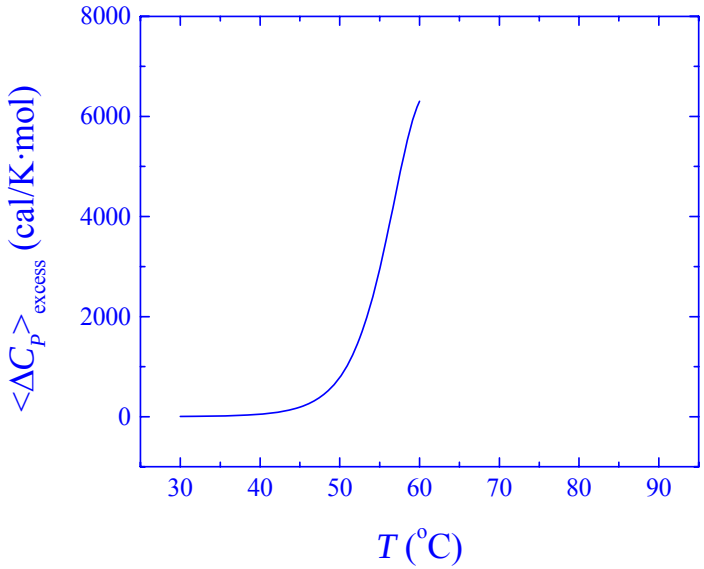
Differential Scanning Calorimetry



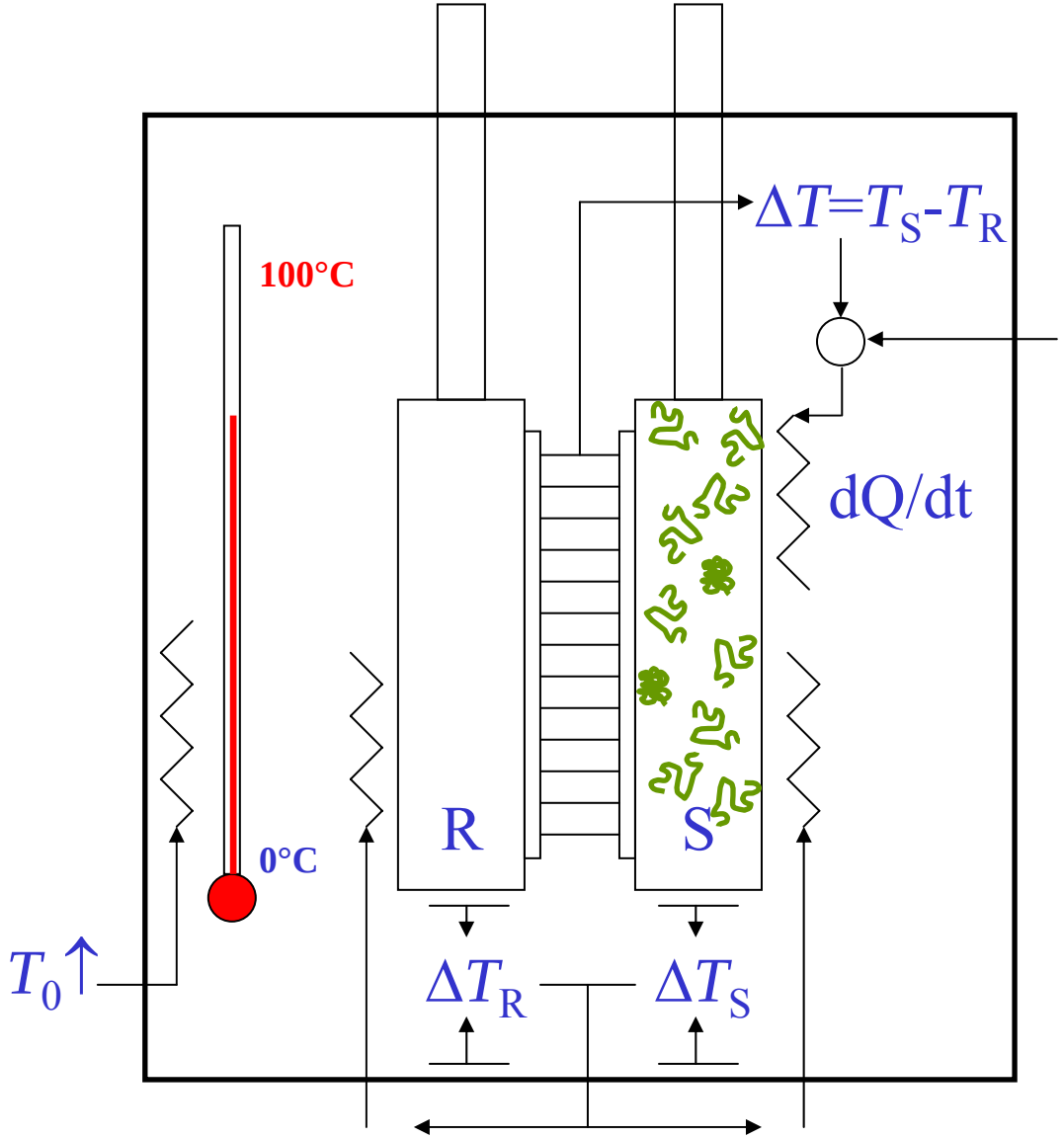


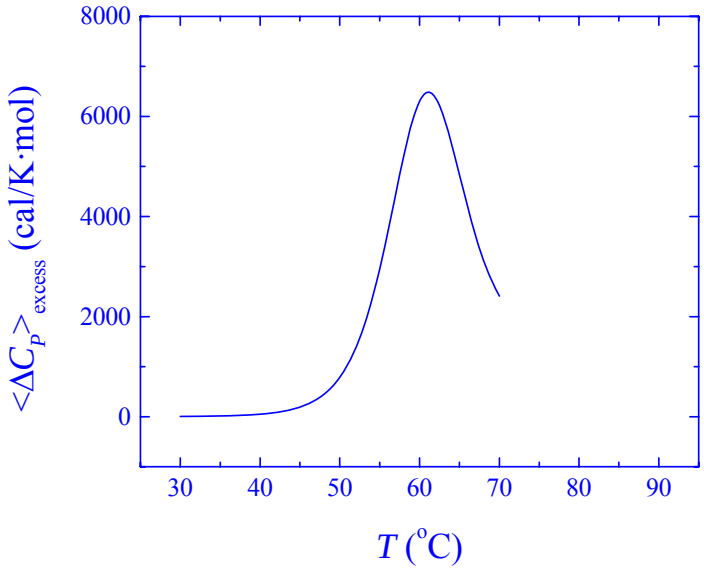
Differential Scanning Calorimetry



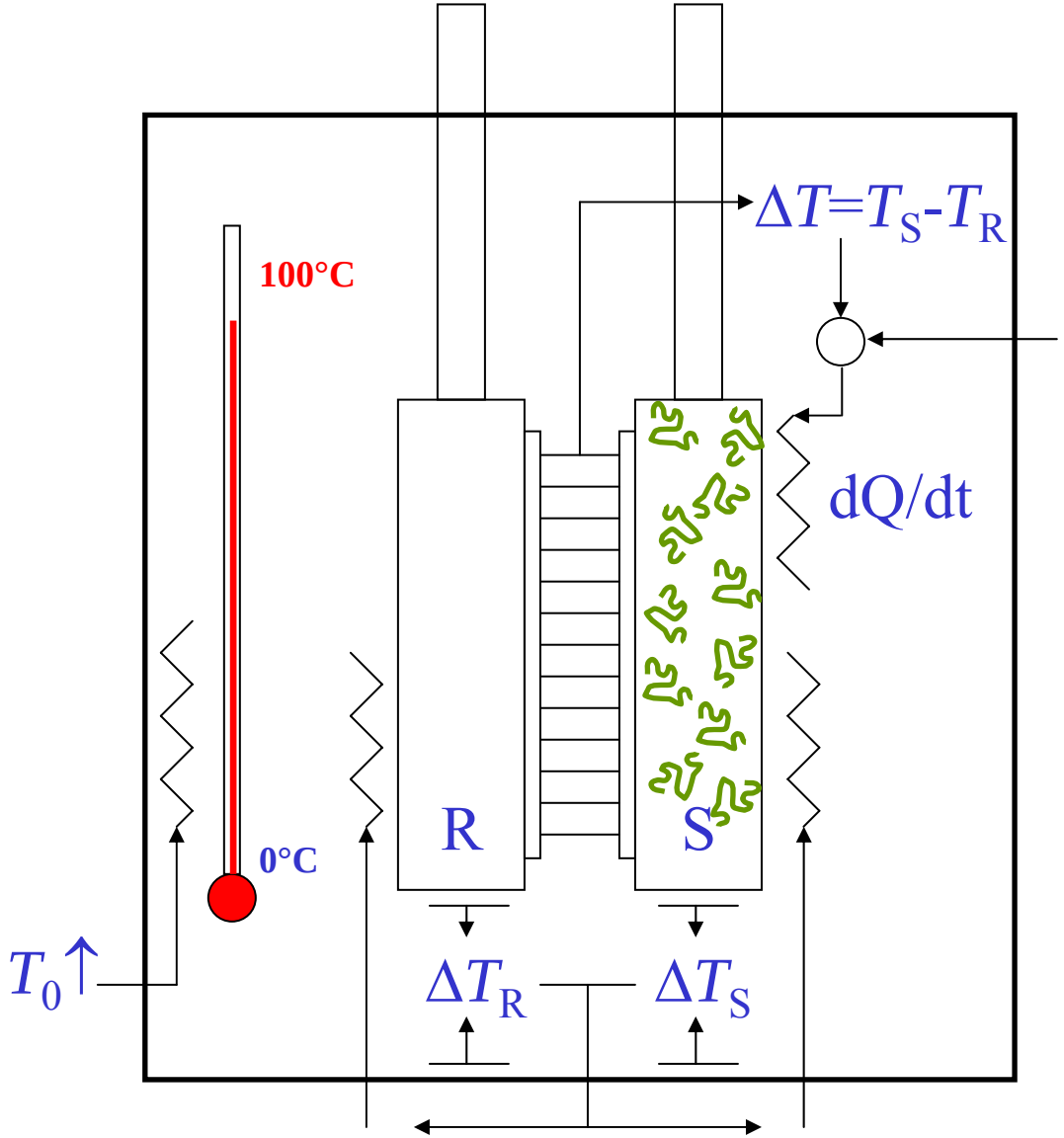


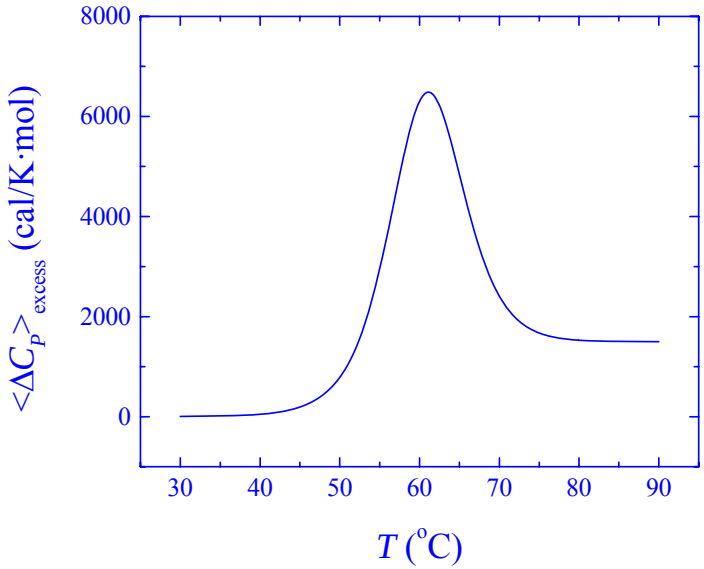
Differential Scanning Calorimetry



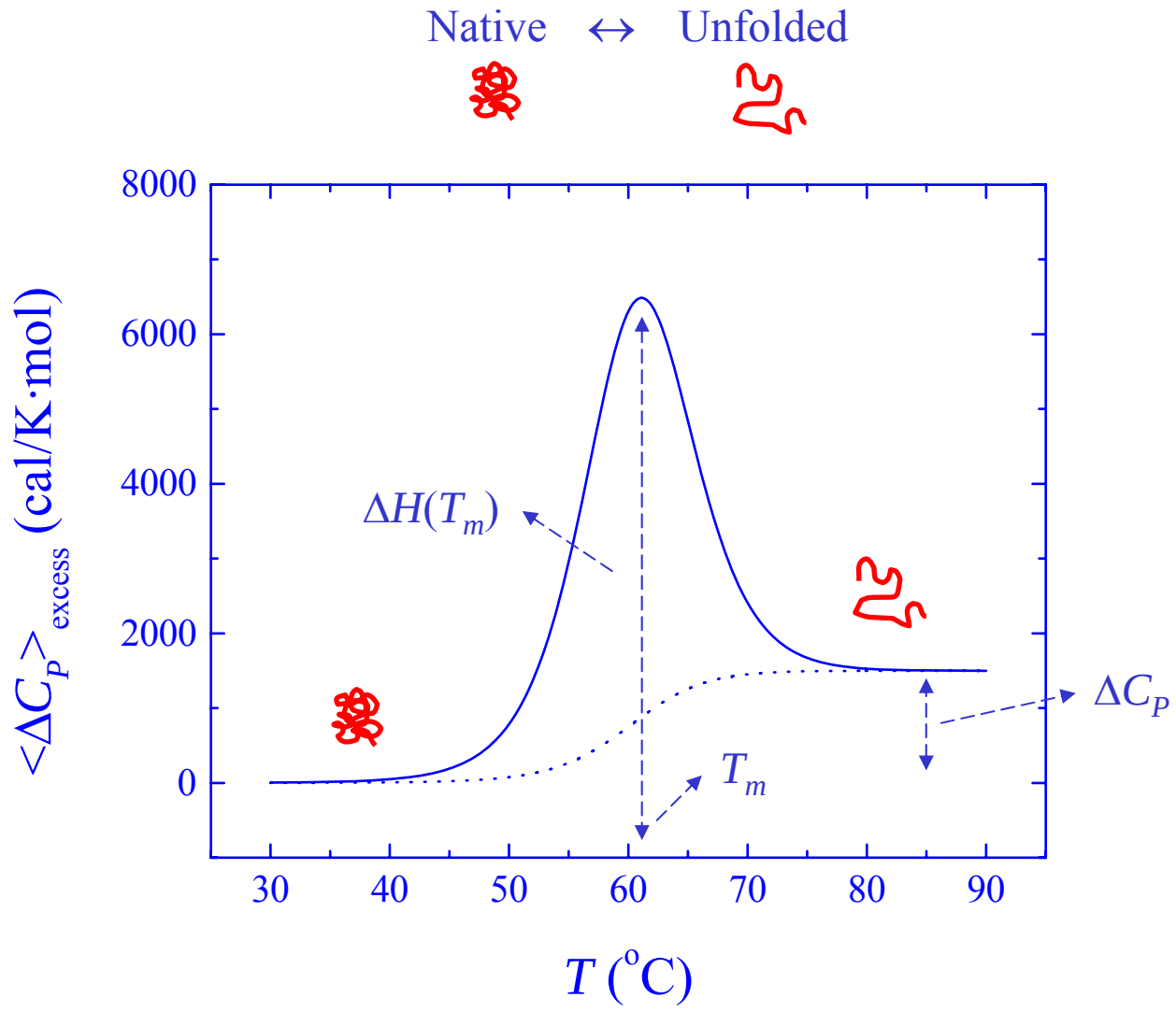


Differential Scanning Calorimetry



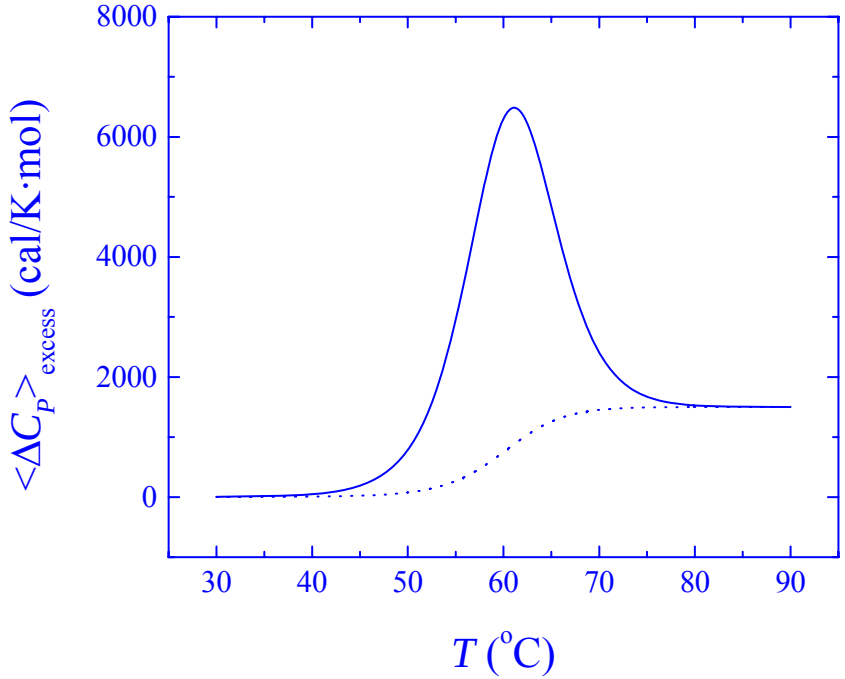


Differential Scanning Calorimetry



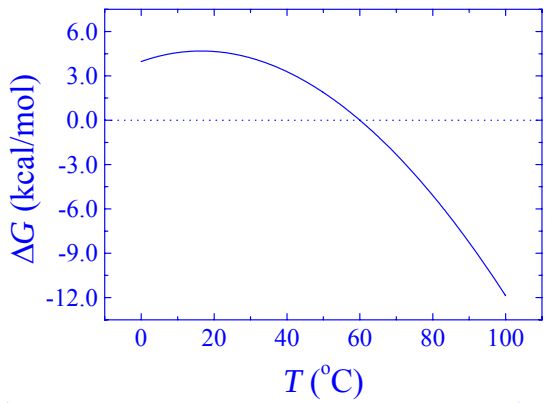
Differential Scanning Calorimetry

Native ↔ Unfolded



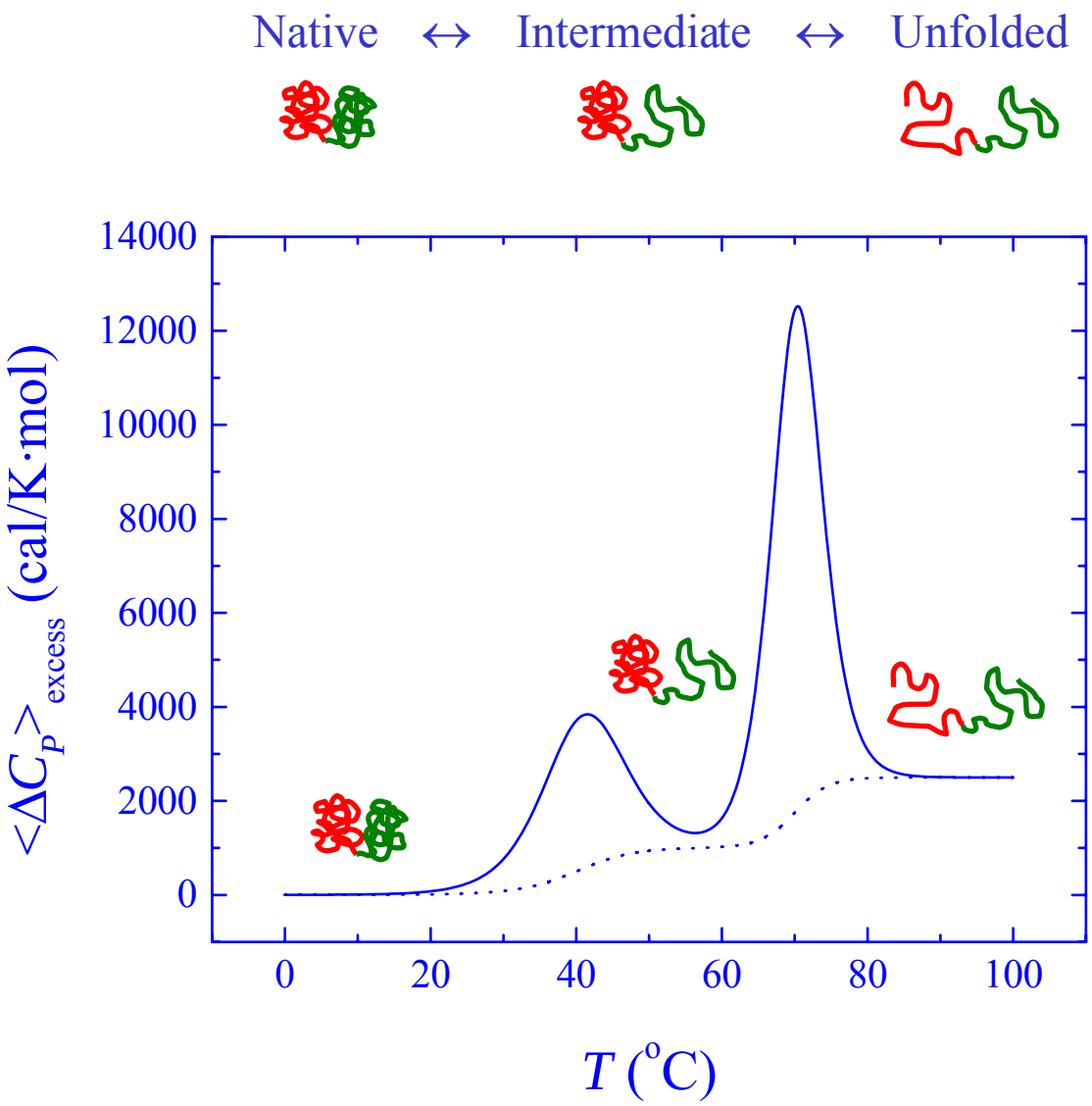
$$\Delta H(T_m) = \int_{T_o}^{T_f} <\Delta C_P>_{excess} \, dT$$

$$\Delta S(T_m) = \int_{T_o}^{T_f} \frac{<\Delta C_P>_{excess}}{T} \, dT$$



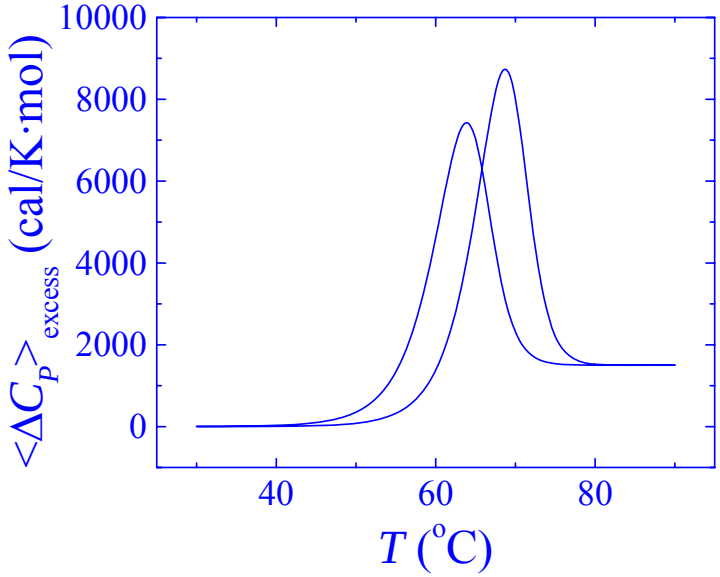
$$\Delta G(T) = \Delta H(T_m) + \Delta C_P (T - T_m) - T\left(\Delta S(T_m) + \Delta C_P \ln \frac{T}{T_m} \right)$$

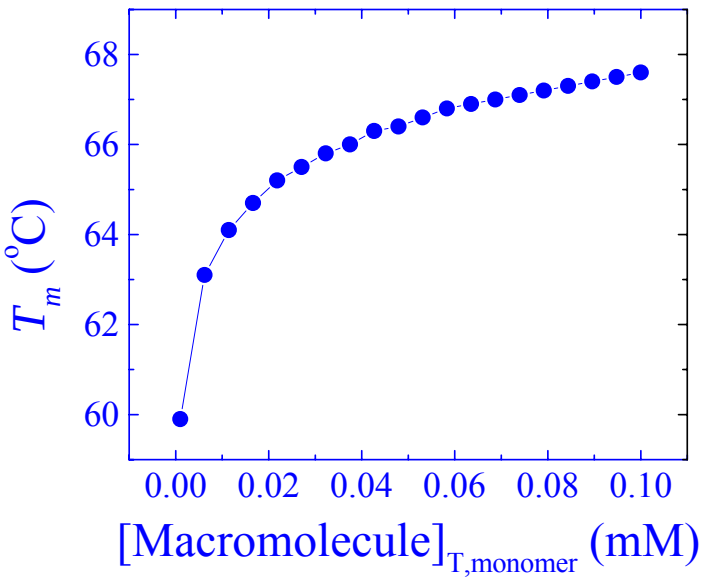
Differential Scanning Calorimetry



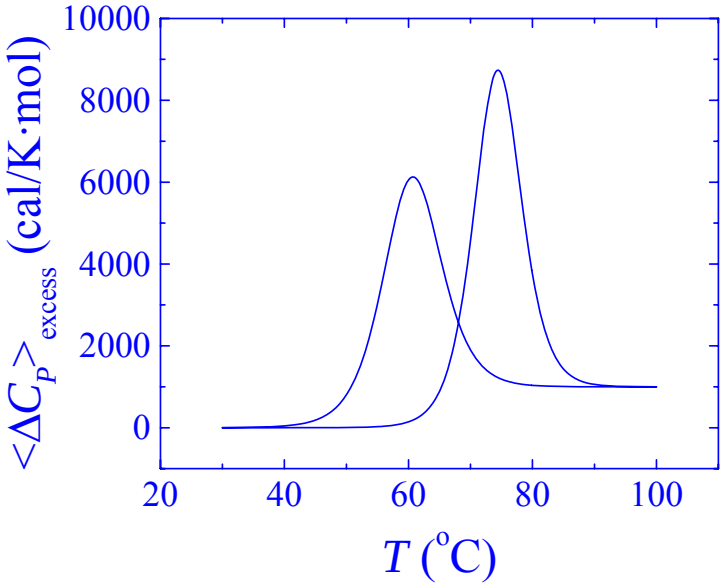
Differential Scanning Calorimetry

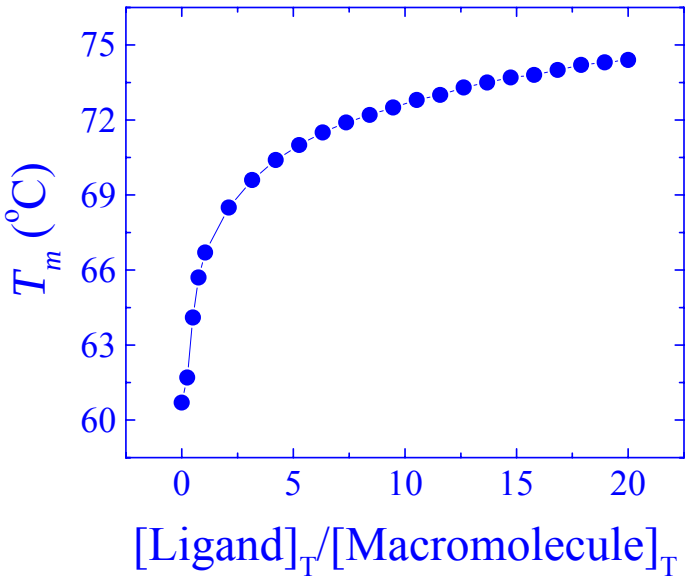
$\text{Native}_2 \leftrightarrow 2\ \text{Unfolded}$





Determination of Binding Affinity: Effect of Ligand Binding on the Stability of the Macromolecule





$$\Delta G = \Delta G^0 + RT \ln\left(1 + K_a[L]\right)$$