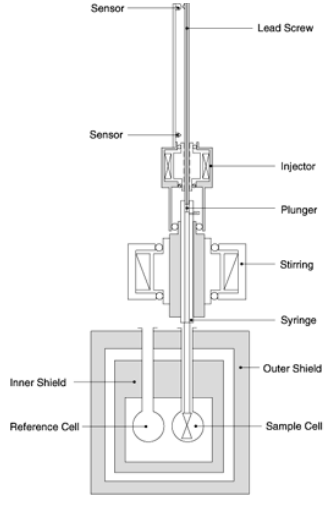
Izoterm titrációs kalorimetria

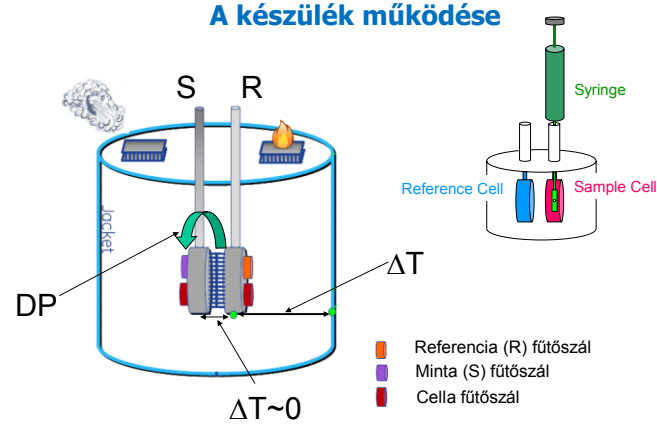
(isotherm titration calorimetry, ITC)

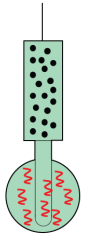
Két reaktáns összekeverésekor fellépő hőt méri közvetlenül valamely állandó hőmérsékleten. Az egyik reaktánst általában lépésenként adják hozzá a termosztált cellában lévő másikhoz.

A készülék felépítése

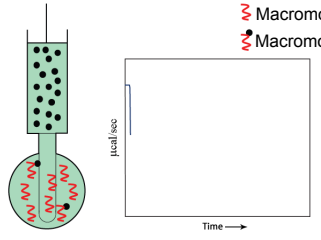


A készülék működése





- Ligand – in syringe
- Macromolecule in ITC cell



- Ligand in syringe
- Macromolecule in cell
- Macromolecule-ligand complex

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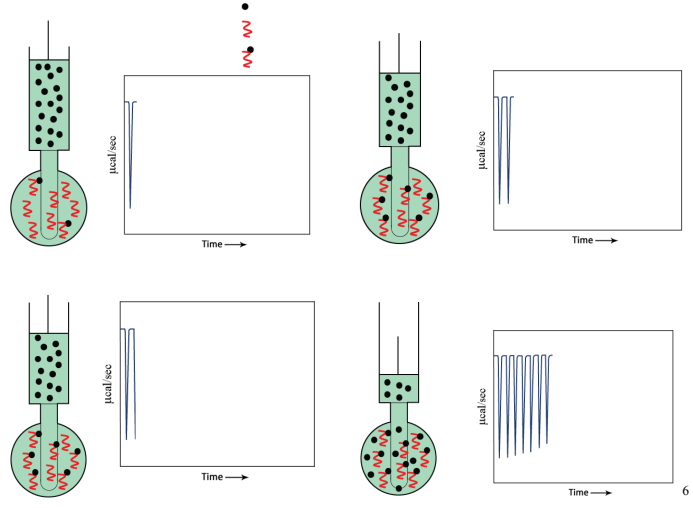

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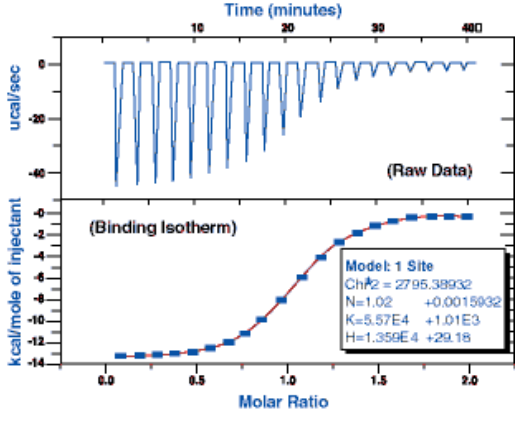
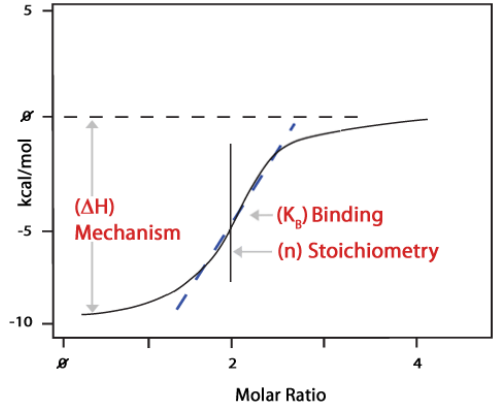
Tipikus kötődési izoterma



7



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Microcal VP-ITC készülék specifikációi

Performance Specifications (2 second filter)		
Noise Level (3 minute RMS windows averaged over 1 hour)	:	1 nanocal/sec (4 nanowatts)
Baseline Constancy (average one hour time intervals: 310 rpm stirring)	:	±5 nanocal/sec (±20 nanowatts)
Minimum Response Time	:	15 seconds
Operating Temperature Range	:	2° to 80° C

Kiértékelés

Modellek:

- egyféle kötőhely (single set of identical sites)
- kétféle kötőhely (two sets of independent sites)
- szekvenciális kötőhely sorozat (sequential binding sites)
- enzim/subsztrát/inhibitor (enzyme/substrate/inhibitor assay)
- dimer disszociáció (dimer dissociation model)
- versengő ligandok (competitive binding model)

Egyféle kötőhely

$$M + n \cdot X \leftrightarrow MX_n$$

$$K = \frac{\Theta}{(1-\Theta)[X]} \qquad X_t = [X] + n\Theta M_t$$

$$\Theta^2 - \Theta\left[1 + \frac{X_t}{nM_t} + \frac{1}{nKM_t}\right] + \frac{X_t}{nM_t} = 0 \rightarrow \Theta$$

$$Q = n\Theta M_t \Delta H V_o$$

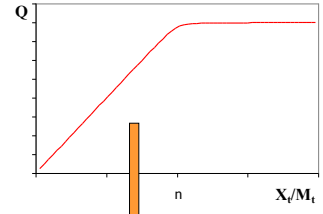
$$Q = \frac{nM_t\Delta HV_o}{2}\left[1 + \frac{X_t}{nM_t} + \cancel{\frac{1}{nKM_t}} - \sqrt{\left(1 + \frac{X_t}{nM_t} + \cancel{\frac{1}{nKM_t}}\right)^2 - \frac{4X_t}{nM_t}}\right]$$

$$c = n \cdot K \cdot M_t \rightarrow \infty$$

$$c = n \cdot K \cdot M_t \rightarrow \infty$$

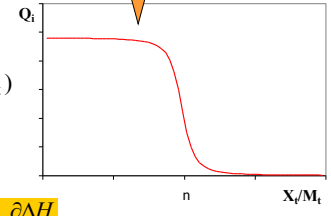
$$Q = M_t \Delta HV_0 \frac{X_t}{M_t} \quad \left(\frac{X_t}{M_t} \le n\right)$$

$$Q = nM_t \Delta HV_0 \quad \left(\frac{X_t}{M_t} > n\right)$$



$$X_t = i \cdot X_0$$

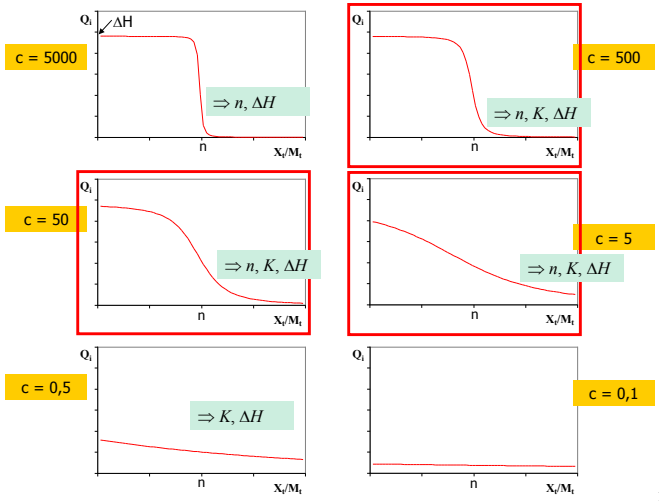
$$Q_i = Q(iX_0/M_t) - Q((i-1)X_0/M_t)$$



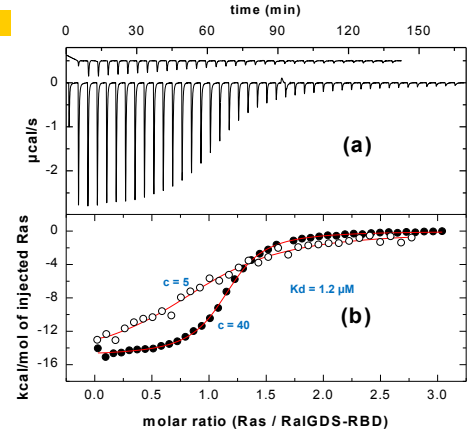
Illesztés $\Rightarrow n, K, \Delta H$

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

$$\Delta C_p = \frac{\partial \Delta H}{\partial T}$$



Egy példa:



Érzékeny tartomány: $c = n \cdot K \cdot M_t$

$1 \le c \le 1000$ elfogadható

$5 \le c \le 500$ jó

$10 \le c \le 100$ nagyon jó

Mérhető legkisebb hő:

$5\ \mu\text{cal} \le Q_i \cong \frac{Q}{10}\ (i = 10)$

$Q = nM_t \Delta H V_0$

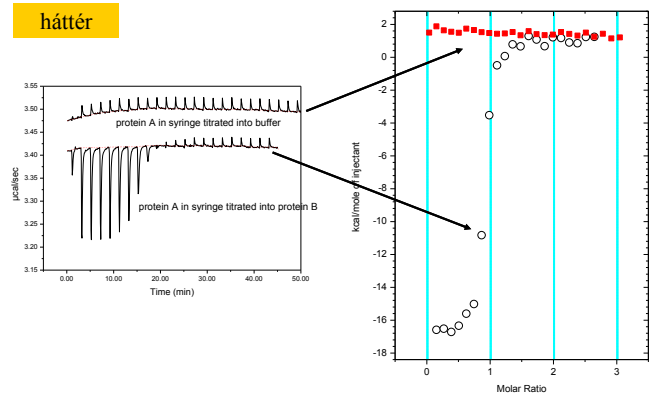
$\frac{0.0357}{n\Delta H} \le M_t \quad \left(\frac{\text{mol}}{\text{l}}\right)$

Pl.: $\Delta H = 10000$ cal/mol és $n = 1$

$4\ \mu\text{M} \le M_t$ $\Rightarrow$ $K \le \frac{1000}{n \cdot M_t} \le 3 \cdot 10^8\ \text{M}^{-1}$

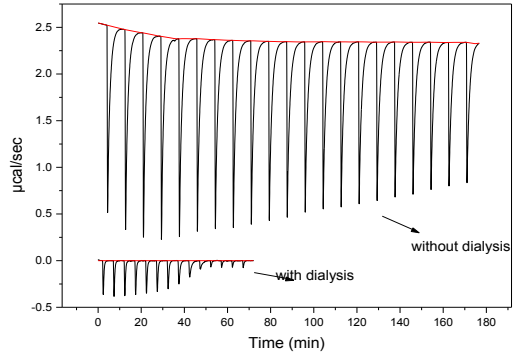
Gyakorlati megjegyzések

háttér



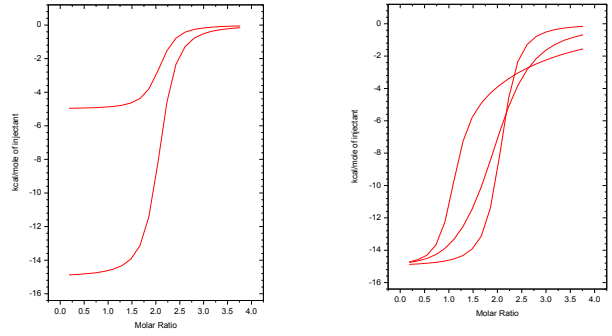
puffer

- referencia ≈ minta

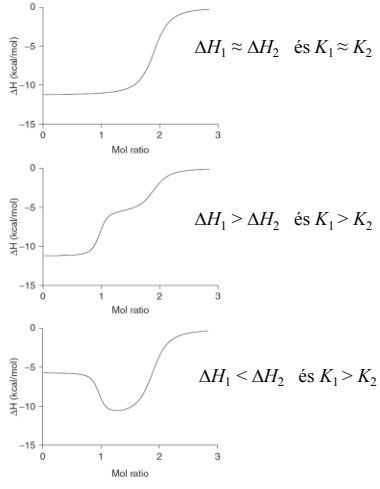


- $\Delta H_{ion} \approx 0$ (pl. foszfát, acetát, glicin, de nem Tris!)

modell



1M + 2X

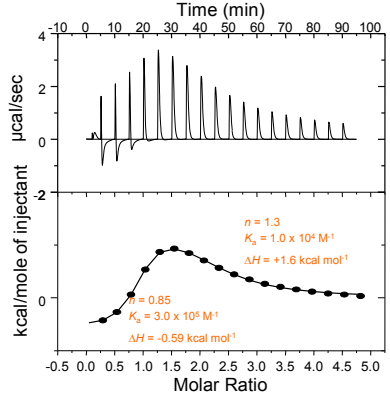


$\Delta H_1 \approx \Delta H_2$ és $K_1 \approx K_2$

$\Delta H_1 > \Delta H_2$ és $K_1 > K_2$

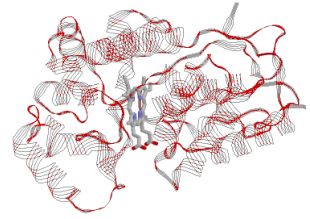
$\Delta H_1 < \Delta H_2$ és $K_1 > K_2$

Mn(II) ions + WT T5 5' nuclease

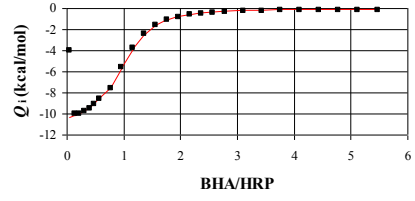


Alkalmazások

Tipikus alkalmazások: antigén-antitest
protein-ligandum
DNS-protein
receptor kötődés
gyógyszer-DNS
lipid-protein



Natív HRP + BHA szubsztrát kötődése



Modell:
egyféle kötőhely

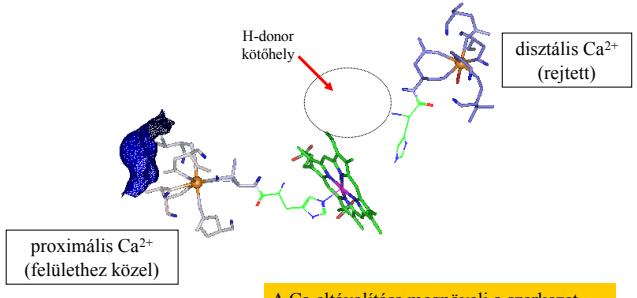
$n = 0.957$

$K = 1.68 \cdot 10^5 \text{ mol}^{-1}$

$\Delta H = -11.3 \frac{\text{kcal}}{\text{mol}}$

$\Delta S = -13.3 \frac{\text{cal}}{\text{mol} \cdot \text{K}}$

Ca mentes HRP + BHA szubsztrát kötődése



A Ca eltávolítása megnöveli a szerkezet flexibilitását, a fluktuációk erősségét, lecsökkenti a szubsztrát kötődés erősségét.

Referencia

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