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Thermal forces and diffusion

<u>Boltzmann</u>'s law states that in <u>thermal equilibrium</u>, the probability P_i of finding a particle in a state i with energy U_i is

$$P_i = \frac{1}{Z} e^{-\{U_i/kT\}}$$

$$Z = \sum_i e^{-\{U_i/kT\}}$$ partition function

kT : $\frac{1}{40}$ eV $\approx$ 25 meV
at room temp.
2.5 kJ/mol
0.6 kcal/mol

1 calorie = 4.1868

Boltzmann's law $\hat{=}$ each configuration of a close system is equally likely

Comparison of energies

kT	0.6 kcal/mol	1
green 500 nm photon	60 kcal/mol	100
ATP hydrolysis	15 kcal/mol	25
180 mV e^- potential	4.2[illegible] kcal/mol	7

Thermodyn. of Protein-DNA interaction (IA)

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Consider IA between protein P and DNA seg.

P : e.g. dimeric TF, activated by inducer

D : binding seg. of length L (core positions)
denoted by $\vec{S}$ $\{b_1, b_2 \dots b_L\}$

$$b \in \{A, C, G, T\}$$

thermal eq. between P and D in vitro

$$P + D \rightleftarrows PD$$

$$\frac{[P]\,[D]}{[PD]} = K(\vec{S})$$

$[P]$: free prot. conc.

$[PD]$: bound P-DNA

- K dissocation constant [concentration]

→ at $[P] = K$: $[PD] = [D]$ half occupation

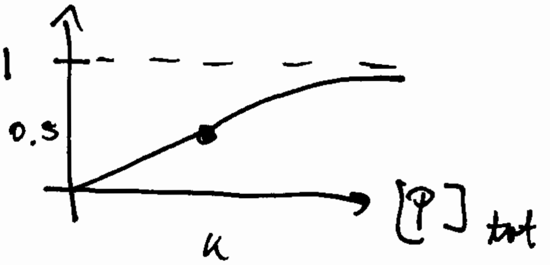
strong attraction = small K

K 1 nmol? 1 pmol ?

- fraction of DNA bound by P

$$f = \frac{[PD]}{[D]+[PD]} = \frac{[P][D]/K(\vec{S})}{D + [P][D]/K(S)} = \frac{[P]}{K+[P]}$$

$$= \frac{1}{1+\frac{K}{[P]}} \approx \frac{1}{1+\frac{K}{[P]_{tot}}}$$



$$\boxed{[P]_{tot} \approx [P]+[PD] \cong [P] \quad \text{if } [D]_{tot} \ll [P]}$$

We want : fractional occupation of target site in vivo

=> long DNA

sequences of length L

i.e. S_j with $j = 1, \dots, N-L$

and $K_j = \frac{[P][D_j]}{[PD_j]}$

target : $\vec{S}_0$

$$f(S_0) = \frac{1}{1 + K_0/[P]}$$

$[P]$: free conc.

different from $[P]_{tot}$

since much protein assoc. with DNA

- need to express $[P]$ in terms of $[P]_{tot}$

$$[P]_{tot} = [P] + \sum_j [PD_j] = [P]\left(1 + \sum_j \frac{[D_j]}{K_j}\right)$$

$[D_j] \sim$ 1 copy / cell , mostly unoccupied

$[D_j] \simeq [D] \sim 1\,nM$

$$\sum_j \frac{[D_j]}{K_j} \sim \sum_j \frac{1}{k_j} \qquad k_j \text{ in } nM$$

$$[P]_{tot} = [P]\left(1 + \sum_j k_j^{-1}\right) \simeq [P] \sum_j K_j^{-1}$$

(little free P : electrostatics !)

$$\boxed{f(S_0) = \frac{1}{1 + \frac{K_0}{[P]_{tot}}\left(\sum_j K_j^{-1}\right)} = \frac{1}{1 + \frac{\tilde{K}_0}{[P]_{tot}}}}$$

$\tilde{k}_0 = \sum_j K_0 / K_j$ in unit of nM

- effect of rest of genome is to shift K_0 by amount $\sum_j K_j^{-1}$

  many alternative binding opportunities each with worse affinity than $\vec{S}_0$

- magnitude of $\tilde{K}$?
  - difficult to measure directly
  - relate to free energy of binding

$$\tilde{K}(\bar{S}_0) = \cancel{\Sigma}\sum_j e^{-(\Delta G(\bar{S}_j) - \Delta G(\vec{S}_0))/kT}$$