General tutorial session #5: SPACE, TIME, AND ENERGY LANDSCAPE IN MECHANOBIOLOGY

08 / 11 / 2006

Molecular forces

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but first ☺

Funding opportunities at National Science Foundation

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new opportunities . nano- and bio- mechanics and materials
submission windows 09/01 - 10/01/2006 and 02/15 - 03/15/07

newer opportunities . partnerships for international research & education
due 10/30/2006 to support 14-17 projects, $ 2.5 M each.

newest opportunities . East Asia and Pacific summer institutes for U.S. citizens
for students in Australia, China, Japan, Korea, Taiwan.
. emerging frontiers in research and innovation (EFRI)
crosscutting & disciplinary areas.
2006 was the program's first year : 16 proposals
topics for 2007 solicitation : (1) autonomously reconfigurable engineered systems enabled by Cyberinfrastructure, (2) cellular and biomolecular engineering

- Basic considerations

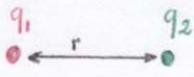
Generalized force as free energy gradient $f = -\frac{\partial E}{\partial x}$ or, in vector form $\vec{F} = -\vec{\nabla} E$
and use U as potential energy (for E)

1. what is the radial dependence of $\vec{f}$, U?
2. what is the interaction range of $\vec{F}$?
3. " " " energy implied by $\vec{F}$?

Types of intermolecular interactions : ① electrostatic (ES), ② Van der Waals / steric
③ hydrogen bonding, ④ hydrophobic

① electrostatic $U_{ES}(r) = \frac{1}{4\pi\varepsilon_0 \varepsilon} \cdot \frac{q_1 q_2}{r}$ with $\varepsilon_0 = 8.89 \times 10^{-12}\ C^2/J.m$

$\varepsilon = \begin{cases} 1 & \text{(air)} \\ 80 & \text{(water)} \end{cases}$ dielectric constant

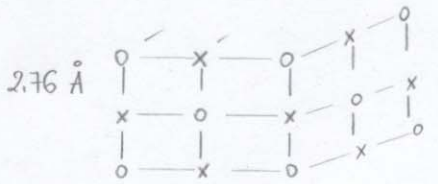


Space, time and energy scales in mechanobiology - 2.

ex: sodium chloride NaCl

$q_{Na} = -q_{Cl} = e = 1.6 \times 10^{-19}$ C

$U_{NaCl} = 120$ kcal / mol



thermal energy as energy ruler

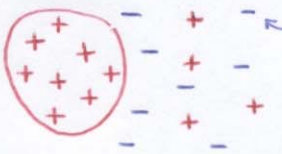
$p(E) \propto \exp\left(\frac{-E}{k_B T}\right)$

a salt NaCl crystal is stable at room temperature

- at $T = 300$ K, $k_B T \simeq 0.59$ kcal / mol
- $E \gg k_B T$ is unlikely while $E \lesssim$ is probable

but in water $U_{NaCl} = \frac{120}{80} = 1.5$ kcal / mol $> k_B T \Rightarrow$ salt dissolves

electrostatic screening: in an electrolyte solution where a charged group is buried



counterions imply screening

$V(r) = V_0 \, e^{-r/l_D}$

electrolyte $i = 1, 2, 3, \ldots$

- of bulk concentration $\rho_{\infty i}$
- of valency z_i (with $z_{Na} = +1$)

the Debye length is $l_D = \frac{1}{\sqrt{\sum_i \rho_{\infty i} \frac{z_i^2 e^2}{\varepsilon_0 \varepsilon k_B T}}}$

(no screening at high T from agitation!)

ex: [NaCl] = 1 mM $l_D = 9.6$ nm and in pure water @ pH = 7 (10^{-7} M)

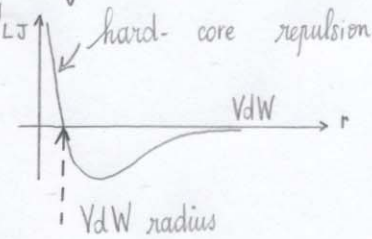
1 M $l_D = 3$ Å $l_D = 960$ nm ~ 1 µm

② Van der Waals / steric interactions

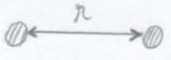
Lennard Jones potential $U_{LJ}(r) = A\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$

* steric : strong, short-ranged.

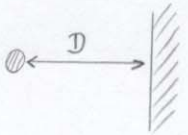
• VdW



VdW with surfaces : $VdW \sim \frac{1}{r^6}$

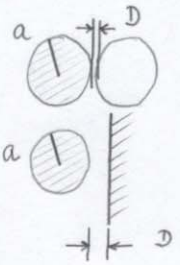


$VdW \sim \frac{1}{D^3}$



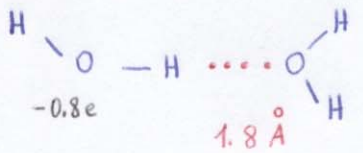
Space, time, and energy scales in mechanobiology - 3.

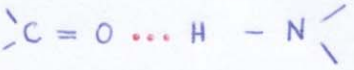
VdW with spheres $VdW \sim \frac{1}{D^3}$ if $a \gg D$



VdW can matter at macroscopic scales, by walls.

③ Hydrogen bonds

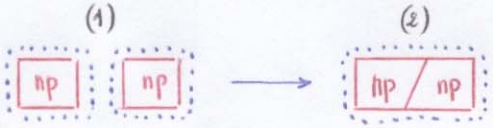




{ short - ranged
directional
accounts for specificity of protein structure

Compare energies
- $U_{H\text{-}bond} \sim$ 3 - 9 kcal / mol
- $U_{VdW} \sim$ 0.24
- $U_{ATP\ hydrolysis} \sim$ 14
- $U_{covalent\ bond} >$ 100

④ hydrophobicity : water around nonpolar molecules forms a network of hydrogen bonds = " clatrate "



(2) entropically preferable because less order

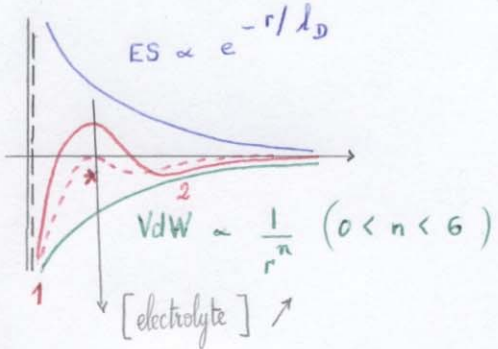
Hydrophobic interaction is attractive
entropic
long - ranged (~ 10 nm)

it is also { proportional to solvent - accessible surface area of nonpolar groups
= surface - tension driven γ

$\gamma \sim$ 72 mJ / m² (air) or 50 mJ / m² (H - carbons), relative to water
~ 80 cal / mol. Å² geometry - dependent

DLVO

most interactions above are attractive, insensitive to electrolytes : VdW, H - bonds
but ES interaction is repulsive, and sensitive to electrolytes.



* critical coagulation concentration → aggregation
1, 2 primary & secondary minima

Thermal forces and Brownian motion

Ju Li

- add random variables: $Y = X_1 + X_2 + .. + X_N$

$\bar{Y} = \langle Y \rangle \equiv E[Y] = E[X_1] + E[X_2] + .. + E[X_N]$

$\mathrm{var}[Y] \equiv \langle (Y - \langle Y \rangle)^2 \rangle = \mathrm{var}[X_1] + \ldots + \mathrm{var}[X_N]$

$\| \quad E[(Y - E[Y])^2] = N \, \mathrm{var}[X]$

from linearity of operator: $y = \frac{Y}{N}$, $E[y] = \frac{E[Y]}{N} = E[X]$

average becomes more deterministic

$\mathrm{var}[y] = \frac{\mathrm{var}[Y]}{N^2} = \frac{N \, \mathrm{var}[X]}{N^2} \xrightarrow{N \to \infty} 0$ as more samples

- X may be sampled by probability density ⫯ ⫯ or ⎍ or ◠

the central limit theorem states that, irrespective of the shape of X, Y is Gaussian

$$\rho(Y) \longrightarrow \frac{1}{\sqrt{2\pi N \sigma_x^2}} \exp\left(\frac{(Y - N E[Y])^2}{2 N \sigma_x^2} \right)$$

if you convolute 2 Gaussians, you get Gaussian ⟹ attractor shape

- diffusion equation (1D): $\partial_t \rho = -\partial_x (-\mathcal{D} \partial_x \rho) = \mathcal{D} \partial_x^2 \rho$

random walk motion of step $\pm a$

at $t = 0$ initial conditions known ⟹ position = delta function δ

$x(t) = 0 + \Delta x_1 + \ldots + \Delta x_{t/\Delta t}$

$E[x(t)] = 0$ on average

but spread now $\mathrm{var}[x(t)] = \frac{t}{\Delta t} \mathrm{var}[\Delta x^2] = \nu t \, a^2$ with $\frac{1}{\Delta t} = \nu$

$$\rho(x(t)) = \frac{1}{\sqrt{2\pi \; 2\mathcal{D}t}} \cdot \exp\left(-\frac{x^2}{2 \; 2\mathcal{D}t} \right)$$ by identifying $\mathcal{D} \equiv \frac{\nu a^2}{2}$

parabolic kinetics

Green's function solution to diffusion equation.

- Brownian motion: ◍ $\xrightarrow{v}$ $F_{drag} = -6\pi r \eta v = -\lambda v = m \dot{v}$

if $v(0) = v_0$, $v(t) = v_0 e^{-\frac{\lambda t}{m}} \xrightarrow[t \to +\infty]{} 0$ problematic!

contradicts equipartition theorem $\left\langle \frac{m v^2}{2} \right\rangle = \frac{k_B T}{2}$

Einstein : there is not only a dissipative force (drag) but also stimulative force (at microscopic scale).

$m\dot{v} = F_{diss} + F_{stim.} = \text{fluctuation} = -\lambda v + F_{fluct.}(t)$

$\langle F_{fluct}(t) \rangle = 0$

$\langle F_{fluct.}(t)\ F_{fluct.}(t') \rangle = b(t-t') = B\,\delta(t-t')$ white noise

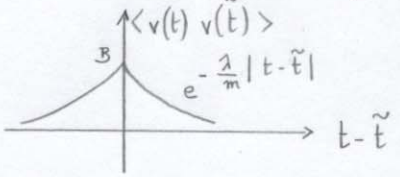
$v(t) = \frac{1}{m}\int_{-\infty}^{t} dt'\ F_{fluct}(t') \exp\left[\frac{-\lambda}{m}(t-t')\right]$ uncorrelated fluct.

convolution = solution of Langevin equation with noise

velocity correlation even if white noise : $\langle v(t)\,\tilde{v}(\tilde{t}) \rangle = (B/2m\lambda)\, e^{-\lambda/m(t-\tilde{t}}$

$[B] = \text{force}^2$

the ratio between square of stimulative force and dissipative force is fixed ($\propto T$)



$\frac{B}{2\lambda} = k_B T$ = Einstein's relation

- How do <u>diffusion and Langevin equations</u> match ?

$\langle x^2(t) \rangle = 2\mathcal{D}t$

$\frac{d}{dt}\langle x(t)\,x(t) \rangle = 2\mathcal{D} = 2\langle x(t)\,v(t) \rangle$

or $\mathcal{D} = \int_0^t g(t')\,dt'$: <u>fluctuation - dissipation theorem</u>

valid as $t \to +\infty$ observation time

$t \gg \frac{m}{\lambda}$ larger than molecular time scale (or macroscopic times)

or $\mathcal{D} = \langle x(t)\ v(t) \rangle$

$= \left\langle \int_0^t v(t')\,dt' \cdot v(t) \right\rangle$

$= \int_0^t \langle v(t)\,v(t')\,dt'$

$= \int_0^t \underbrace{\langle v(t')\,v(0) \rangle\,dt'}_{\equiv g(t')}$

This fluctuation - dissipation theorem (Green - Kubo formula) has many implications :

- thermal conductivity
- semiconductor's electrical conductivity
- shear viscosity

} <u>transport properties</u> <u>equilibrium fluct</u>

- $\mathcal{D} = \lambda k_B T$ Einstein's relation (no mass)

Space, time, and energy scales in mechanobiology - 6

"Reactions", energy landscapes and kinetics — Pat Doyle

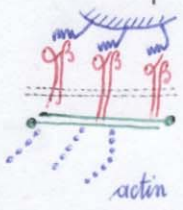
see Pat Doyle's handwritten notes.

Cellular sensing of force and geometry in rigidity responses: protein unfolding by force — Michael Sheetz

- reverse system engineering approach: what components are responsible for cellular responses?
capability to sense form, rigidity and respond is critical to biology
rapid neuronal sensing is through ion channels, but longer-term responses are cytoskeleton-based
transformation is described as the ability to respond to rigidity and grow

- sensing (geometry & rigidity / force) → transduction → response (Sheetz - Vogel Nat. Rev '06)
(ms - s) (s) (min, cycles)
force causes GFP-paxillin accumulation in RPTP α +/+ with fibronectin
reinforcement response mediated by integrins
intracellular / extracellular communication ⇒ feedback and integrated reponse after a few cycle

- early response ? and its role ?
. spatial distribution of integrins is important for talin binding
trimers of integrins ⇒ fibronectin strong attachment
2pN slip bond between integrins and cytoskeleton (through talins)
slip bonds ≠ catch bonds



. force-dependent reinforcement, where are involved C-Src, vinculin, RPTP α et al.
GFP-paxillin stretch-dependent binding to identify molecules necessary for reinforcement.
can the plasma membrane be removed and the cytoskeleton (CSK) alone show same ? yes!
CSK-bound proteins after stretch: { FAK, p130 Cas, PKB / Akt, paxillin
not vinculin (same in vivo & in vitro)
reversible, ATP-independent, mechanical

. measure signaling downstream : - { G protein activation by CSK
Rap 1, not Ras, is activated by stretch
- Src family kinase phosphorylation of p130 Cas if stretch

Cas : force transducer since Ⓟ Cas correlates with stretch application
protein unfolding or protein distortion responsible for this transduction ?

. stretch Cas SD itself ? pull a latex surface to uniformly stretch the molecule
Cas SD phosphorylation requires stretch for c-Src, Fyn, and c-Abl (not Csk) ph.
unfolding of Cas SD appears to be sufficient and necessary for its phosphorylation
(SD = substrate domain of Cas)
unfolded & phosphorylated Cas is at the periphery of cells (as assessed by Ab stain)

. titin domains unfold as expected for a force sensor (~ 200 pN per unf. event)
hydrogen bonds critically important to dictate unfolding forces & properties
→ graded force sensor in one single molecule : domains structure is key concept
(same with FN - Viola Vogel's work)

"Reactions", Energy Landscapes & Kinetics

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GEM4 Summer School 2006
Cell and Molecular Mechanics in BioMedicine
August 7–18, 2006, MIT, Cambridge, MA, USA

Key References

- *Mechanics of Motor Proteins & the Cytoskeleton*, J. Howard

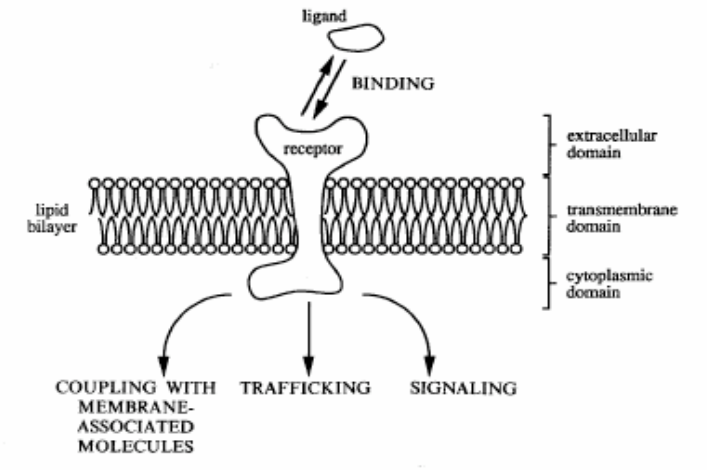
- *Biophysical Chemistry*, C. Cantor and P. Schimmel

- *Mechanical Processes in Biochemistry*, Bustamante *et al*., Ann. Rev. Biochem., **73**, 705-748, 2004

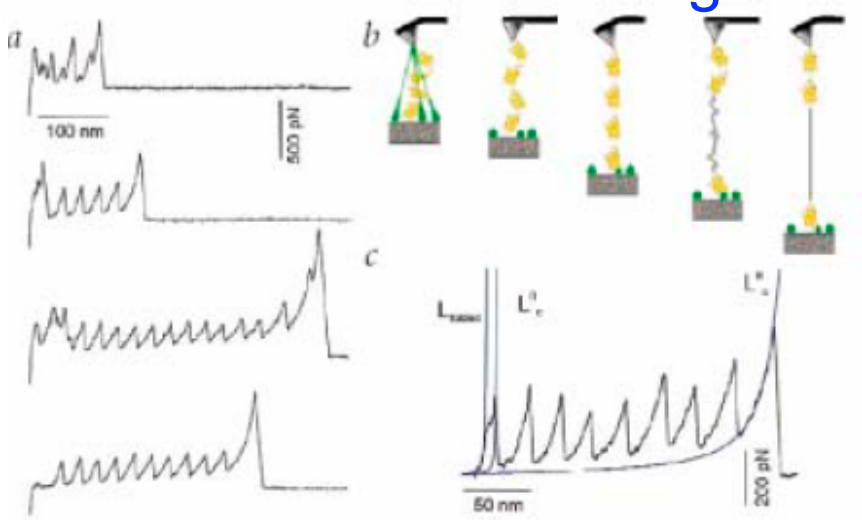
- *Brownian Motion in a Field of Force and the Diffusion Model of Chemical Reactions*, H.A. Kramers, Physica, **4**, 284-304, 1940

"Reactions"

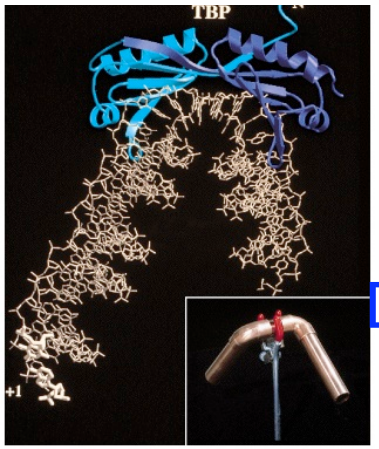
Receptor-ligand



Titin unfolding



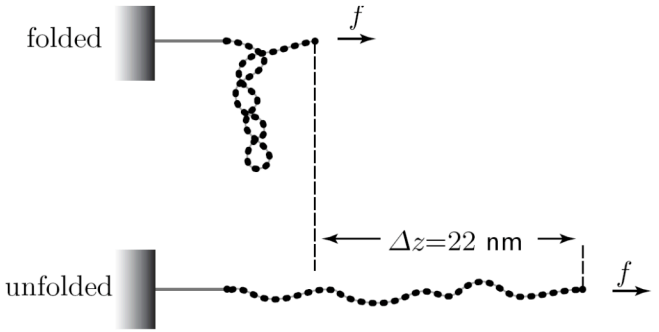
Fisher, Marszalek & Fernandez, *Nature* 2000



DNA-protein

Binding of TAT box-binding protein

RNA unfolding



Bustamante et al. 1997

Diffusion Limited Reactions

e.g. $A + B \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} AB$ → maybe limited be A "finding" B in solution?

how: diffusion!

$$\frac{d[B]}{dt} = -k_{on}[A][B] + k_{off}[AB]$$

$\frac{d[B]}{dt}$: $\frac{1}{s}\frac{mol}{\ell}$; k_{on}: $\frac{1}{s}\frac{\ell}{mol}$

reformulate

⇒

B "fixed"

assume:

- steady-state
- $C_A^{\infty} = C_A(r \to \infty)$
- $C_A(R) = 0$ ("fast rxn")

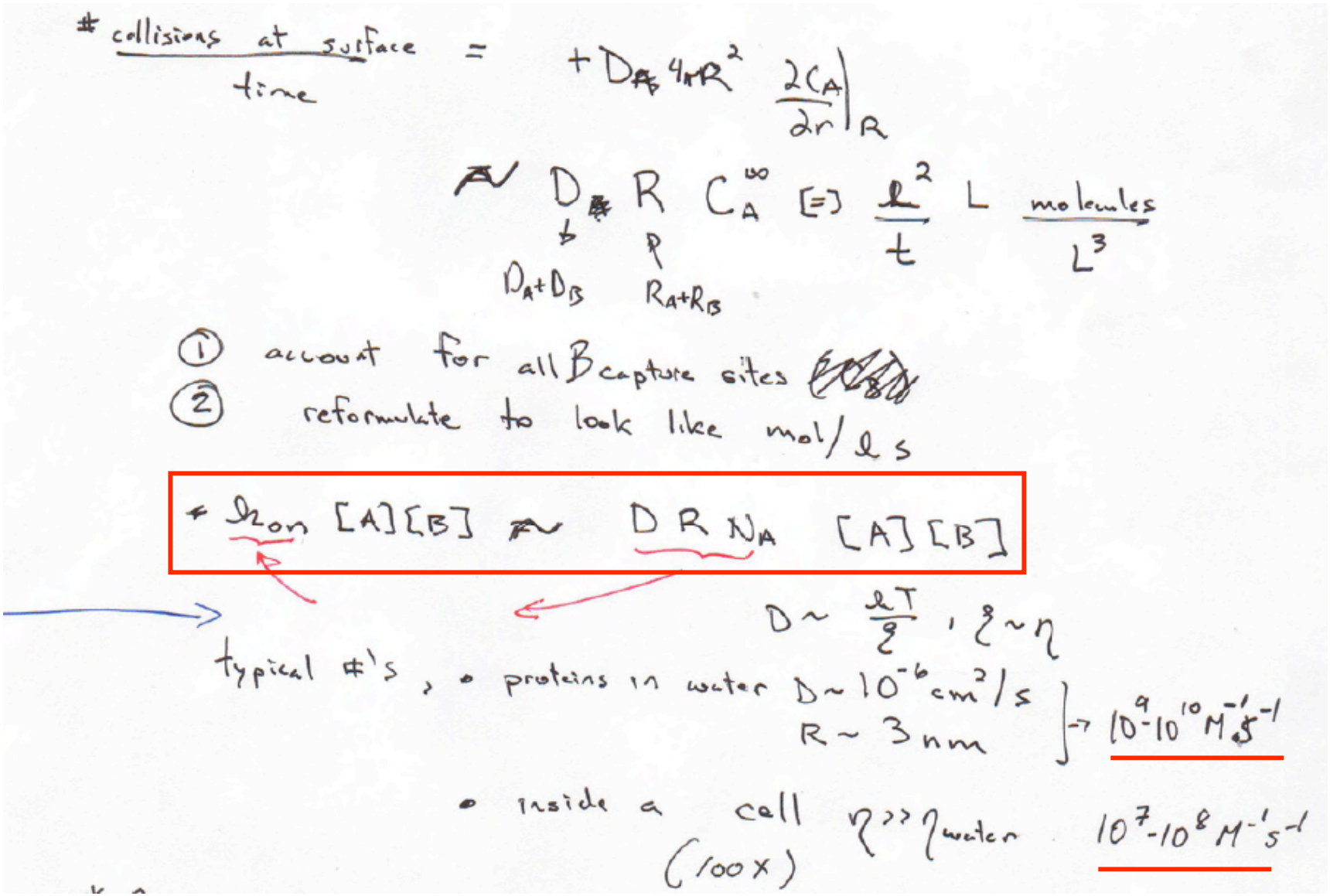
$$\nabla^2 C = 0$$

$$C_A = C_A^{\infty}\left(1 - \frac{R}{r}\right)$$

molecules

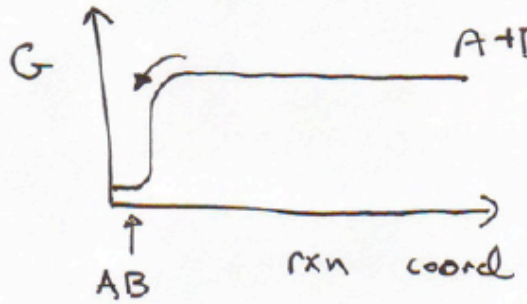
$$J_A = -D_A \frac{\partial C_A}{\partial r} \quad [=] \quad \frac{\text{molecules}}{\text{area time}}$$

Some details….



Geometry matters…

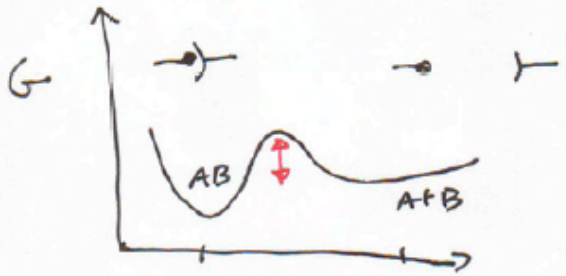
Energy Landscapes (1-D)



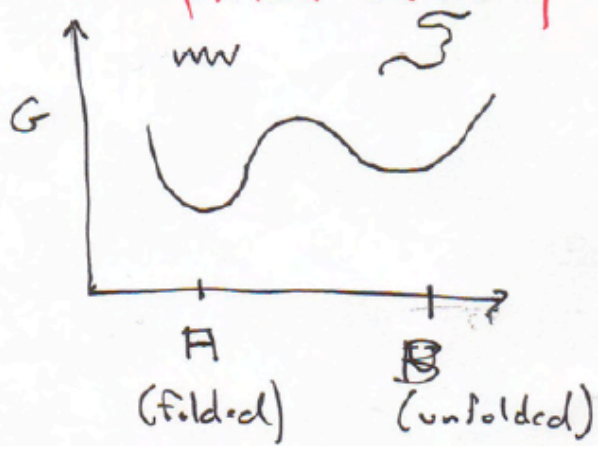
rxn limited

often barriers (several $k_B T$) exist

protein-ligand



protein-unfolding



Consider:

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \qquad \frac{d[A]}{dt} = -k_1[A] + k_{-1}[B]$$

equilibrium: detailed balance

$$\left.\frac{[B]}{[A]}\right|_{equil} = \frac{k_1}{k_{-1}} \equiv K_{eq}$$

eq. const.

Boltzmann

$$\frac{P_B}{P_A} = \frac{\exp(-G_B/k_B T)}{\exp(-G_A/k_B T)} = \exp\left(-\frac{\Delta G}{k_B T}\right)$$

(note G versus U $\Rightarrow$ ensemble of states)

NOTE: equilibrium doesn't care about the barriers!

Kinetics $\rightarrow$ very sensitive to barriers.

example: suppose $P_A(t=0) = 1$

$$P_A = \frac{1}{1+K_{eq}} \left\{1 - \exp\left[-(k_1 + k_{-1})\, t\right]\right\}$$

temporal dependence!

$$P_B = \frac{1}{1+K_{eq}} \left[K_{eq} + \exp\left[-(k_1 + k_{-1})\, t\right]\right\}$$

$$t \to \infty \qquad \frac{P_B}{P_A} = K_{eq}$$

Transition State Theory (Eyring ~1930)

... result, TS ⇒ unstable, short-lived state

$$\alpha_0 = k_{+1} = \frac{1}{\tau} \exp\left(-\Delta G_u^{\ddagger} / k_B T\right)$$

$$\beta_0 = k_{-1} = \frac{1}{\tau} \exp\left(-\Delta G_f / k_B T\right)$$

τ [=] time, $\quad \frac{\alpha_0}{\beta_0} = \exp\left(-\frac{\Delta G}{kT}\right)$ ✓

① ⚠ rates depend exponentially on barrier heights

② role of T

③ prefactor $\left(\frac{1}{\tau}\right)$ → Eyring ~ cov. bonds ⇒ Q.Mech. ⇒ vib. freq $k_B T/h \sim 6 \times 10^{12}\ s^{-1}$

→ Arrhenius

→ Kramers ~ "diffusion" in damped environment

④ any thing that affects energy landscape can change kinetics (possibly equil. too)

Fokker - Planck eqn (1-D)

probability flux

$$j(x,t) = -D\,\frac{dP(x,t)}{dx} + \frac{F(x)}{\xi}\,P(x,t) \qquad (1)$$

diffusion (Fick's law)

force

drag coeff.

F.-P. eqn

$$\frac{\partial P}{\partial t} = D\,\frac{\partial^2 P}{\partial x^2} - \frac{\partial}{\partial x}\left[\frac{F(x)}{\xi}\,P(x)\right] \qquad (2)$$

First Passage Time (simple approach)

$$D = \frac{kT}{\xi}$$

$$F = -\frac{dU}{dx}$$

- assume steady state flux (not equilibrium!)

j_0 [=] $\frac{1}{\text{time}}$

$t_0 \equiv \frac{1}{j_0} \equiv$ mean 1st passage time

1) $\frac{\partial P}{\partial t} = 0$ (s.s.)

2) $P(x_0) = 0$ (absorbing wall)

3) $\int_0^{x_0} P(x)\,dx = 1$ cons. probability

$$k_1 \approx \frac{1}{t_0} = j_0$$ rate const.

General Solution to Problem Posed

- if $j = \text{const.} \equiv j_o$, can rewrite (1) as:

$$\frac{d}{dx}\left\{ P(x) \exp\left[\frac{U(x)}{D\Sigma}\right]\right\} = -\frac{j_o}{D} \exp\left[\frac{U(x)}{D\Sigma}\right]$$

$D\Sigma \to kT$ (both terms)

- integrate from x to x_o

$$P(x_o) \exp\left[\frac{U(x_o)}{kT}\right] - P(x) \exp\left[\frac{U(x)}{kT}\right] = -\frac{j_o}{D} \int_x^{x_o} \exp\left[\frac{U(y)}{kT}\right] dy$$

$P(x_o) \to 0$

- multiply by $-\exp\left[\frac{-U(x)}{kT}\right]$, integrate From $0 \to x_o$

$$\int_0^{x_o} P(x)\, dx = \frac{j_o}{D} \int_0^{x_o} \exp\left[-\frac{U(x)}{kT}\right] \left\{ \int_x^{x_o} \exp\left[\frac{U(y)}{kT}\right] dy \right\} dx$$

$\int_0^{x_o} P(x)\, dx = 1$

First Passage Time

- re arrange

$$\underbrace{1/j_0}_{t_0} = \frac{1}{D}\int_0^{x_0} \exp\left[\frac{-U(x)}{k_B T}\right]\left\{\int_x^{x_0} \exp\left[\frac{U(y)}{k_B T}\right] dy\right\} dx$$

Need to input U(x) to solve.....

* Case 1 : suppose $U(x) = 0$

$$t_0 = \frac{x_0^2}{2D}$$

(1-D simple diffusion!)

Exponential Effect of Barrier Height

* Case 2: harmonic well: $U = \frac{1}{2} k x^2$

$$U_0 = U(x_0) \gg k_B T$$

(Kramers 1940)

$$t_0 \approx \frac{1}{D} \sqrt{\frac{\pi a T}{2K}} \sqrt{\frac{k_B T}{K x_0}} \exp\left[\frac{U_0}{k_B T}\right]$$

$\frac{1}{D}$: general; $\sqrt{\frac{\pi a T}{2K}} \sqrt{\frac{k_B T}{K x_0}}$: details of shape of well; $\exp\left[\frac{U_0}{k_B T}\right]$: general

$$t \approx \tau \sqrt{\frac{\pi}{4}} \sqrt{\frac{a T}{U_0}} \exp\left[\frac{U_0}{k_B T}\right]$$

where $\tau = \xi / K$

Relaxation time of a damped spring

Details of Derivation…

Solution For Case #2

$$U = \frac{1}{2} k x^2$$

$$t_o = \frac{1}{D} \int_0^{x_o} \exp\left[\frac{-U(x)}{kT}\right] \left\{ \int_x^{x_o} \exp\left[\frac{U(y)}{kT}\right] dy \right\} dx$$

$$\int_0^{x_o} \exp\left[\frac{U(y)}{kT}\right] dy - \int_0^{x} \exp\left[\frac{U(y)}{kT}\right] dy$$

dominant since $U_o >> kT$

$$t_o \approx \frac{1}{D} \int_0^{x_o} \exp\left[\frac{-kx^2}{2kT}\right] \left\{ \int_0^{x_o} \exp\left[\frac{ky^2}{2kT}\right] dy \right\} dx$$

$$t_o \simeq \frac{1}{D} \left\{ \int_0^{\infty} \exp\left[\frac{-kx^2}{2kT}\right] \right\} \left\{ \int_0^{x_o} \exp\left[\frac{ky^2}{2kT}\right] dy \right\}$$

$$t_o \simeq \frac{1}{D} \left\{ \sqrt{\frac{\pi kT}{2k}} \right\} \left\{ \frac{kT}{kx_o} \exp\left[\frac{kx_o^2}{2kT}\right] \right\}$$

Things that Affect Energy Landscapes

- Force (pulling/pushing, rate)
- Enzymes (catalysts)
- Salt, pH
- "additives" (protein drug stabilization)
- ....

Two State Example: Protein Unfolding

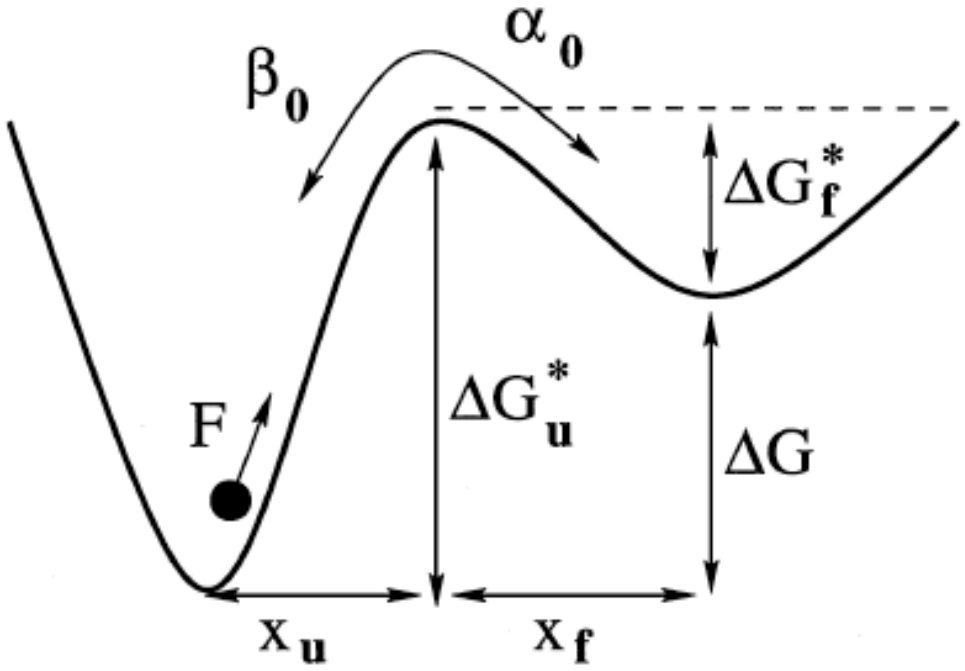
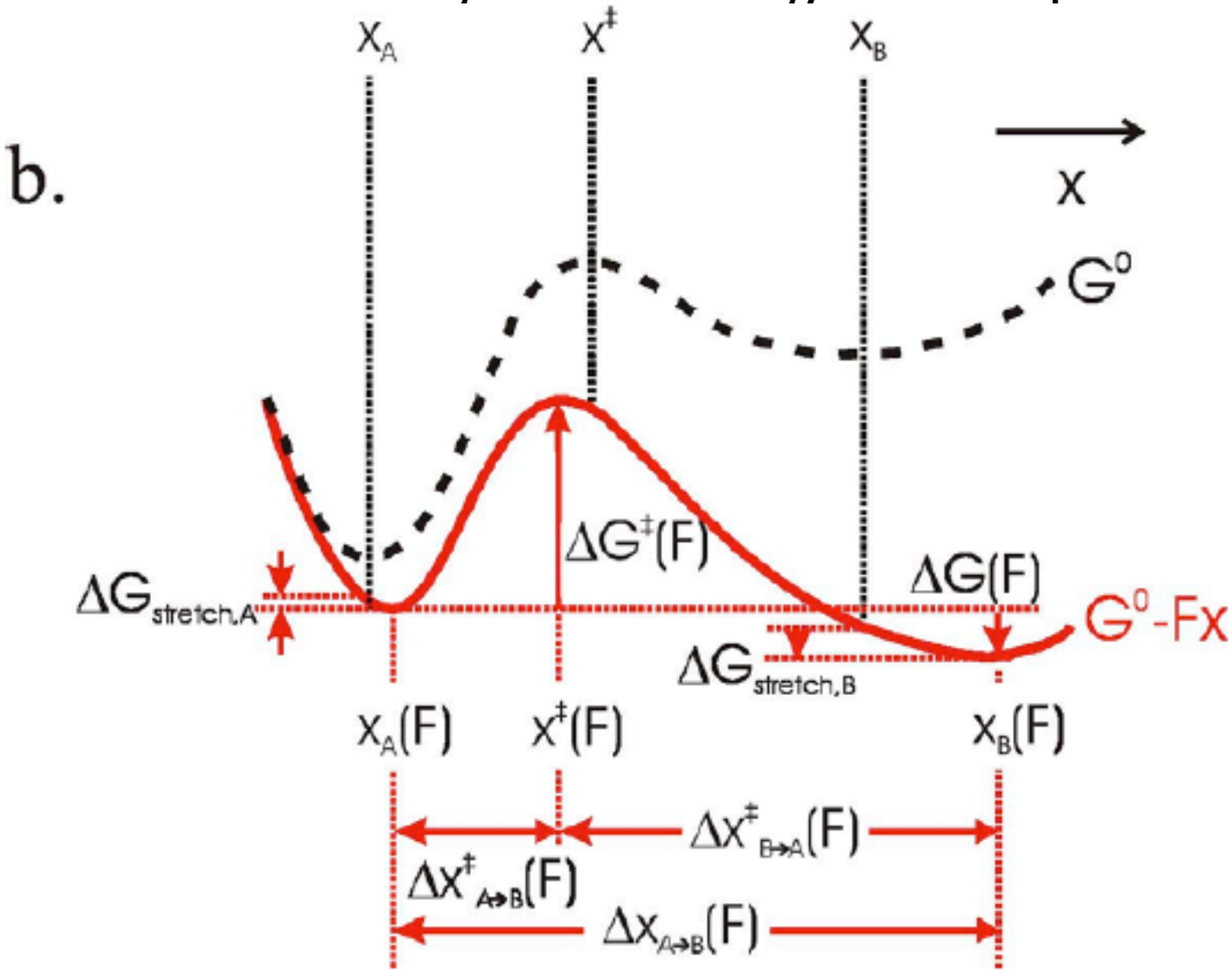


Figure 11 Two-level model for the unfolding of a protein domain (53). The domain in its native state (on the left) unfolds at a rate α_0 by hopping over the barrier (of energy difference ΔG_u^*) to unfolding. In the denatured state (on the right) the refolding rate β_0 is controlled by the energy difference with the transition state ΔG_f^*. When a force F is applied to the system, the free-energy difference ΔG between native and unfolded state is skewed toward unfolding by the work performed by the force: $F\ (x_u + x_f)$. (Courtesy of H. Gaub)

Strick, Allemand, Bensimon, Croquette, Ann. Rev. Biophys. 2000

Force Changes the Energy Landscape



Bustamante et al., Ann. Rev. Biochem. 2004