GEM4 Summer School OpenCourseWare

Space, Time, and Energy Landscapes Mechanobiology notes

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

08 / 11 / 2006

🟧 <u>Molecular forces</u>   <u>Wonmuk Hwang</u>

═══ but first ☺

🟧 <u>Funding opportunities at National Science Foundation</u>   <u>Jimmy Hsia</u>
                                                            jhsia @ nsf. gov

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

─ <u>Basic considerations</u>

Generalized force as free energy gradient $f = -\dfrac{\partial E}{\partial x}$ or, in vector form $\vec{F} = -\vec{\nabla} E$

1. what is the radial dependence of $\vec{f}$, U?   and use U as potential energy (for E)
2. what is the interaction range of $\vec{F}$?
3. "   "   " energy implied by $\vec{F}$?

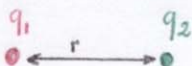Types of intermolecular interactions :   ① electrostatic (ES) , ② Van der Waals / steric
                                         ③ hydrogen bonding, ④ hydrophobic

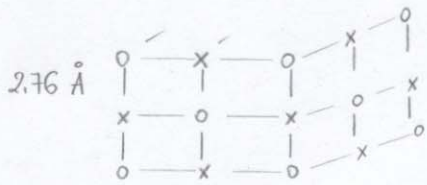① electrostatic $U_{ES}(r) = \dfrac{1}{4\pi \varepsilon_0 \varepsilon} \cdot \dfrac{q_1 q_2}{r}$ with $\varepsilon_0 = 8.89 \times 10^{-12} \ C^2/J \cdot m$

$q_1 \bullet \xleftarrow{\ r\ } \bullet q_2$ $\varepsilon = \begin{cases} 1 \ (air) \\ 80 \ (water) \end{cases}$ dielectric constant

ex: sodium chloride $Na\,Cl$

$2.76\,\text{Å}$

$q_{Na} = -q_{Cl} = e = 1.6 \times 10^{-19}\,C$
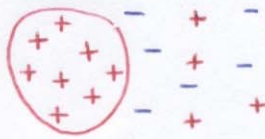
$U_{NaCl} = 120\ \text{kcal}\,/\,\text{mol}$

thermal energy as energy ruler

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

a salt $Na\,Cl$ crystal is stable at room temperature

$\begin{cases} \text{at } T = 300\ K, \quad k_B T \simeq 0.59\ \text{kcal}\,/\,\text{mol} \\ E \gg k_B T \text{ is unlikely while } E \lessdot \text{ is probable} \end{cases}$

but in water $U_{NaCl} = \dfrac{120}{80} = 1.5\ \text{kcal}\,/\,\text{mol} > k_B T \implies$ salt dissolves

<u>electrostatic screening</u>: in an electrolyte solution where a charged group is buried

counterions imply screening

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

electrolyte $i = 1, 2, 3, \dots$ $\begin{cases} \text{of bulk concentration } \rho_{\infty\,i} \\ \text{of valency } z_i \quad (\text{with } z_{Na} = +1 \end{cases}$
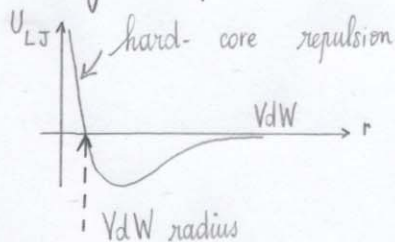
the Debye length is $l_D = \dfrac{1}{\sqrt{\sum_i \rho_{\infty\,i}\ \dfrac{z_i^2\, e^2}{\varepsilon_0\, \varepsilon\, k_B T}}}$

(no screening at high T from agitation!

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

$\qquad\qquad 1\ M \quad\ \ l_D = 3\ \text{Å} \qquad\qquad\qquad l_D = 960\ nm \sim 1\,\mu m$

② <u>Van der Waals / steric interactions</u>
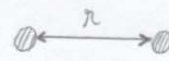
Lennard Jones potential $\quad U_{LJ}(r) = A\left[\left(\dfrac{\sigma}{r}\right)^{12} - \left(\dfrac{\sigma}{r}\right)^{6}\right]$
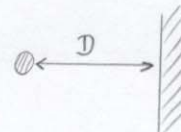
U_{LJ} — hard-core repulsion

VdW

r

VdW radius

* steric : strong, short-ranged.

• VdW

$\textcolor{red}{\star}$

$\textcolor{green}{\bullet}$

$Vd\,W$ with surfaces :

$VdW \sim \dfrac{1}{r^6}$ $\qquad r$
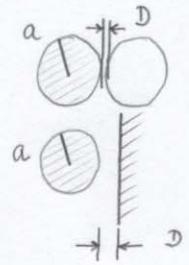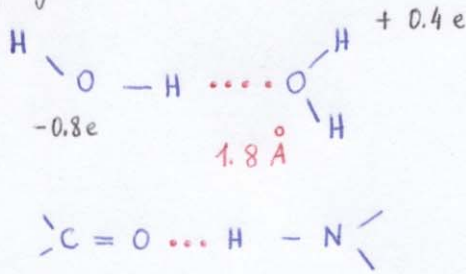
$VdW \sim \dfrac{1}{D^3}$ $\qquad D$

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

VdW with spheres   VdW $\sim \dfrac{1}{D^3}$ if $a \gg D$
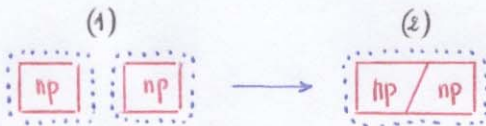
VdW can matter at macroscopic scales, by walls.

③ <u>Hydrogen bonds</u>   { short - ranged
directional
accounts for specificity of protein structure

H
O — H ···· O $+0.4e$
$-0.8e$ H
$1.8 \overset{\circ}{A}$ H

C = O ··· H — N

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

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

(1)   (2)
| np | | np | ⟶ | np / np |

(2) entropically preferable because less order

hydrophobic interaction is attractive
entropic
long-ranged ($\sim$ 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
$\sim$ 80 cal/mol·Å² geometry-dependent

<u>DLVO</u>

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

$ES \propto e^{-r/l_D}$

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

VdW $\sim \dfrac{1}{r^n}$ $(0 < n < 6)$

[electrolyte] ↗

Thermal forces and Brownian motion Ju Li

- add random variables:

$$Y = X_1 + X_2 + \ldots + X_N$$

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

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

$$\overset{\shortparallel}{E[(Y - E[Y])^2]} \qquad = N \; var[X]$$

from linearity of operator:
average becomes more deterministic

$$y = \frac{Y}{N}, \qquad E[y] = \frac{E[Y]}{N} = E[X]$$

$$var[y] = \frac{var[Y]}{N^2} = \frac{N \; var[X]}{N^2} \xrightarrow{N \to \infty} 0$$

as more samples

- X may be sampled by probability density

↑ ↑ or ⊓ or ⟋‾⟍

the <u>central limit theorem</u> states that, irrespective of the shape of X, Y is <u>Gaussian</u>

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

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

- <u>diffusion equation</u> (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

$$x(t) = 0 + \Delta x_1 + \ldots + \Delta x_{t/\Delta t}$$
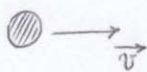
$$E[x(t)] = 0 \qquad \text{on average}$$

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

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

parabolic kinetics
<u>Green's function solution</u> to diffusion equation.

- Brownian motion :   ⊘ —→ v $F_{drag} = -6\pi r \eta v = -\lambda v = m\dot{v}$

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

problematic!

contradicts equipartition theorem $\langle \frac{mv^2}{2} \rangle = \frac{k_B T}{2}$

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

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

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

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

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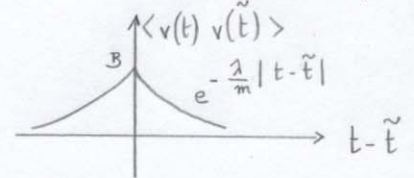
$$v(t) = \frac{1}{m} \int_{-\infty}^{t} dt' \, F_{fluct}(t') \, \exp\left[\frac{-\lambda}{m}(t-t')\right]$$

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 ($\propto T$)

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

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

$$\left. \begin{array}{l} \langle x^2(t) \rangle = 2 \mathcal{D} t \\[2mm] \dfrac{d}{dt}\langle x(t) \, x(t) \rangle = 2\mathcal{D} = 2 \langle x(t) \, v(t) \rangle \end{array} \right\} \text{or}$$

$$\text{or} \quad \mathcal{D} = \int_0^t g(t') \, dt' \quad : \quad \underline{\text{fluctuation - dissipation theorem}}$$

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

$$t \gg \frac{m}{\lambda} \quad \text{larger than molecular time scale}$$

(or macroscopic times)

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

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

· thermal conductivity
· semiconductor's electrical conductivity   }   <u>transport properties</u>   equilibrium fluct.
· shear viscosity

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

"Reactions", energy landscapes and kinetics   *Pat Doyle*

see Pat Doyle's handwritten notes.

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

- 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 <u>cytoskeleton-based</u>
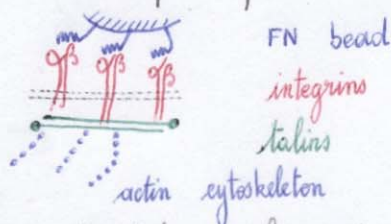 transformation is described as the ability to <u>respond to rigidity</u> and grow

- $\underline{\text{sensing}}$ (geometry & rigidity / force) $\rightarrow$ $\underline{\text{transduction}}$ $\rightarrow$ $\underline{\text{response}}$ $\begin{pmatrix} \text{Sheetz - Vogel} \\ \text{Nat. Rev '06} \end{pmatrix}$
   (ms - s) (s) (min, cycles)
  force causes GFP- paxillin accumulation in RPTP α + / + with fibronectin
  reinforcement response mediated by integrins
  intracellular / extracellular communication $\Rightarrow$ <u>feedback</u> and <u>integrated</u> response after a few cycle

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

 FN bead slip bonds $\neq$ catch bonds
integrins
talins
actin cytoskeleton

. 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 : $\begin{cases} \text{FAK, p130 Cas} , \text{ PKB / Akt, paxillin} \\ \text{not vinculin (same in vivo & in vitro)} \end{cases}$
reversible, ATP- independent, mechanical

. measure <u>signaling downstream</u> : $\Big\{$ G protein activation by csk

Rap 1, not Ras, is activated by stretch

- Src family kinase phosphorylation of p130 Cas if stretch

<u>Cas</u> : <u>force transducer</u> 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.

<u>unfolding of Cas SD</u> appears to be sufficient and necessary for its <u>phosphorylation</u>

(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

→ <u>graded force sensor in one single molecule</u> : domains structure is key concept

(same with FN - Viola Vogel's work)