GEM^4 – August 2006 – MIT – Cell and molecular mechanics in biomedicine

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

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- Basic considerations

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

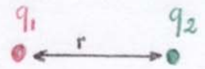
and use U as potential energy (for E)

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

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

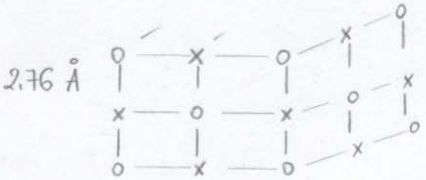


Space, time and energy scales in mechanobiology - 2.

ex: sodium chloride NaCl

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

$U_{NaCl} = 120$ kcal / mol



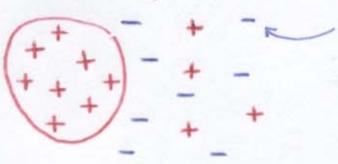
thermal energy as energy ruler

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

a salt NaCl crystal is stable at room temperature $\begin{cases} \text{at } T = 300\text{ K}, \quad k_B T \simeq 0.59 \text{ kcal / mol} \\ E \gg k_B T \text{ is unlikely while } E \lesssim \text{ is probable} \end{cases}$

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 $i = 1, 2, 3, \dots$ $\begin{cases} \text{of bulk concentration } \rho_{\infty i} \\ \text{of valency } z_i \ (\text{with } z_{Na} = +1 \end{cases}$

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

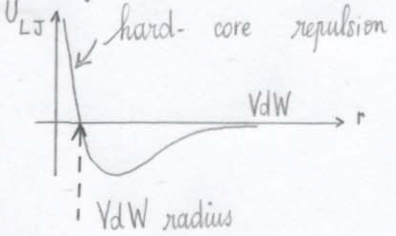
1 M $\quad l_D = 3$ Å $\quad l_D = 960$ nm $\sim 1\ \mu$m

② Van der Waals / steric interactions

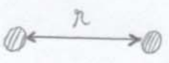
Lennard Jones potential $U_{LJ}(r) = A\left[\underset{\star}{\left(\frac{\sigma}{r}\right)^{12}} - \underset{\bullet}{\left(\frac{\sigma}{r}\right)^{6}}\right]$

★ steric : strong, short-ranged.

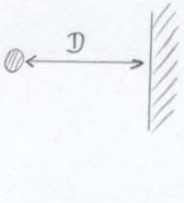
● VdW



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



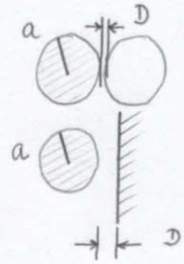
VdW $\sim \frac{1}{D^3}$



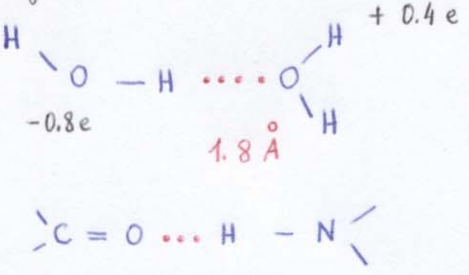
Space, time, and energy scales in mechanobiology - 3.

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

VdW can matter at macroscopic scales, by walls.



③ Hydrogen bonds

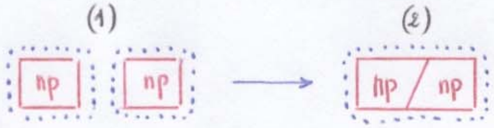


$\begin{cases} \text{short - ranged} \\ \text{directional} \end{cases}$

accounts for specificity of protein structure

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

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



(2) entropically preferable because less order

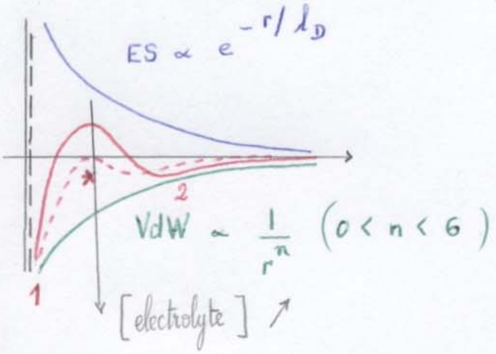
hydrophobic interaction is attractive
entropic
long - ranged (~ 10 nm)

it is also $\begin{cases} \text{proportional to solvent - accessible surface area of nonpolar groups} \\ = \text{surface - tension driven } \gamma \end{cases}$

$\gamma \sim 72 \text{ mJ / m}^2$ (air) or 50 mJ / m^2 (H - carbons), relative to water
$\sim 80 \text{ cal / mol. Å}^2$ 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 Lai

- add random variables :

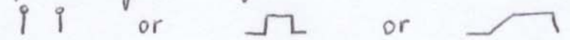
$$Y = X_1 + X_2 + .. + X_N$$
$$\bar{Y} = \langle Y \rangle \equiv E[Y] = E[X_1] + E[X_2] + .. + E[X_N]$$
$$\text{var}[Y] \equiv \langle (Y - \langle Y \rangle)^2 \rangle = \text{var}[X_1] + \ldots + \text{var}[X_N]$$
$$\shortparallel$$
$$E[(Y - E[Y])^2] \qquad = N \text{ var}[X]$$

from linearity of operator :
average becomes more deterministic

$$y = \frac{Y}{N}, \quad E[y] = \frac{E[Y]}{N} = E[X]$$
$$\text{var}[y] = \frac{\text{var}[Y]}{N^2} = \frac{N \text{ var}[X]}{N^2} \xrightarrow{N \to \infty} 0$$

as more samples

- X may be sampled by probability density



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 $\Rightarrow$ 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 ± a

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

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

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

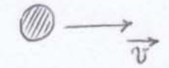
but spread now $\text{var}[x(t)] = \frac{t}{\Delta t} \text{var}[\Delta x^2] = \nu t a^2$ with $\frac{1}{\Delta t} = \nu$

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

parabolic kinetics

Green's function solution to diffusion equation.

- Brownian motion :



$$F_{\text{drag}} = -6\pi r \eta v = -\lambda v = m \dot{v}$$

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

problematic !

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

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

$m \dot{v} = F_{diss} + F_{stim.}$ = 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

uncorrelated fluct.

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

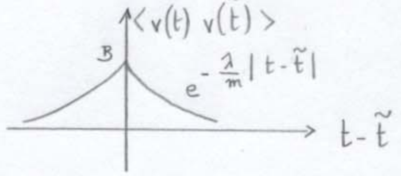
convolution = solution of Langevin equation with noise

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 ($\propto T$)

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



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

$$\left.\begin{aligned} \langle x^2(t) \rangle &= 2 \mathcal{D} t \\ \frac{d}{dt} \langle x(t)\, x(t) \rangle &= 2\mathcal{D} = 2 \langle x(t) \, v(t) \rangle \end{aligned}\right\}$$

or

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

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)

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.

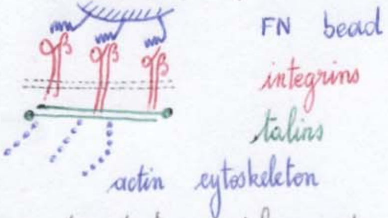
Cellular sensing of force and geometry in rigidity responses: protein unfolding by force

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 ∝ +/+ with fibronectin
reinforcement response mediated by integrins
intracellular / extracellular communication ⇒ feedback and integrated reponse after a few cycle

- early response ? and its role ?
  - spatial ditribution 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 ∝ 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)