

Brownian Dynamics Simulations

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<http://projects.villa-bosch.de/mcm/>



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\$\$\$: Klaus Tschira Foundation, EU, DAAD, AVH, BMBF, BIOMS, DFG


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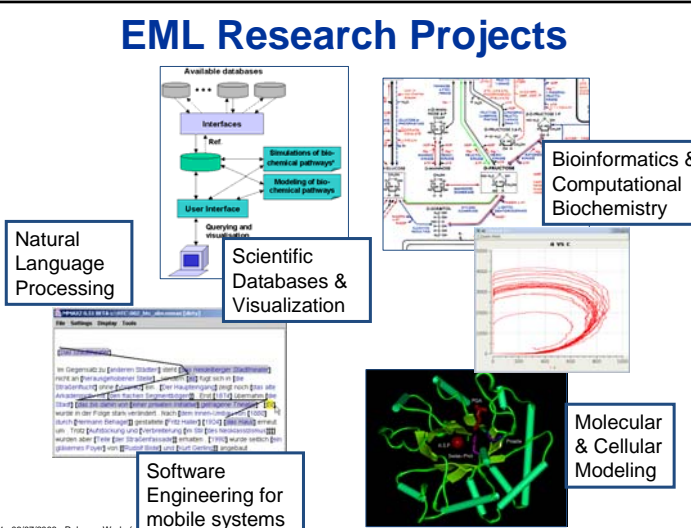
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EML Research Projects



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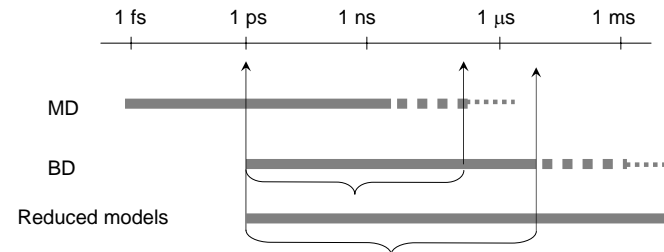
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Why Brownian dynamics?

- Diffusional processes are important in molecular biology
- Brownian dynamics provides a particulate (as opposed to continuum) description of diffusional processes
- Brownian dynamics can access longer timescales and spatial scales than molecular dynamics
- Applications of BD simulations
 - ◆ Protein Folding
 - ◆ DNA Bending and Supercoiling
 - ◆ Enzyme-Substrate Encounter
 - ◆ Protein-Protein Encounter
 - ◆ Diffusion in the Cell

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Timescales:



Molecular dynamics (MD) (Newtonian motions) – motion of all atoms,
 Brownian dynamics (BD) – motion of (all-atom detail) rigid proteins
 Reduced models – motion of a collection of interaction sites

MD and BD simulation results comparable in the time scale of 1-100 ns
 BD and Reduced model simulations compare in 1-10000 ns

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BD: Outlook

- BD is a useful computational tool for quantitative and qualitative calculation of molecular properties and for gaining mechanistic insights
- BD will find increasing application:
 - ◆ From the molecular to the cellular level +
- Multiscale simulations:
 - ◆ QM → MD → BD (at different levels of detail)
- Methodological improvements needed:
 - ◆ Molecular Flexibility
 - ◆ Force description (hydrodynamics, hydrophobicity...)

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Talk Outline

- | | |
|--|---|
| <ul style="list-style-type: none"> ▪ Theory <ul style="list-style-type: none"> ◆ Brownian Motion ◆ Brownian Dynamics Simulations ◆ Encounter Rate Computation ◆ Forces: <ul style="list-style-type: none"> ▫ Exclusion ▫ Electrostatics ▫ Hydrodynamic interactions ◆ Molecular Flexibility | <ul style="list-style-type: none"> ▪ Applications <ul style="list-style-type: none"> ◆ Protein Folding ◆ DNA Bending and Supercoiling ◆ Enzyme-Substrate Encounter ◆ Protein-Protein/NA Encounter ◆ Diffusion in the Cell ◆ Macromolecular crowding |
|--|---|


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Brownian Motion ⁽¹⁾

- 1827: Robert Brown observed pollen particles moving erratically in water in a zig-zag fashion, i.e. diffusing



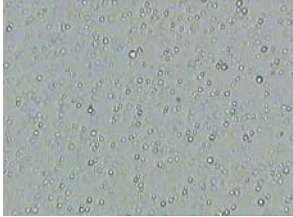
Small particles, moving with thermal motion, randomly collide with large particle → diffusive, random, motion of large particle in a fluid



See also: http://en.wikipedia.org/wiki/Brownian_motion

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Brownian Motion ⁽²⁾



Fat droplets suspended in milk.

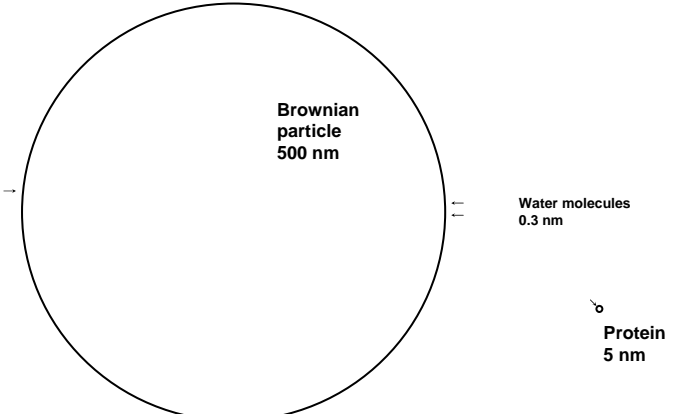
Smaller droplets jiggle more than the larger ones; some of them move out of the plane of focus and back again.

The milk was very dilute, just a needle wetted with milk, dipped and mixed into a drop of distilled water on the slide and covered with coverslip. The droplets range in size from about 0.5 to 3 μm

(Dave Walker, www.microscopy-uk.org.uk)

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Brownian motion ⁽³⁾



Brownian particle
500 nm

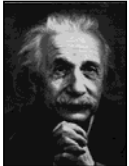


Water molecules
0.3 nm

Protein
5 nm

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Brownian Motion ⁽⁴⁾

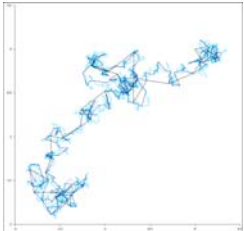
- 1905: Albert Einstein (Nobel Prize 1921) and Marian von Smoluchowski explained the phenomenon mathematically

- 1911: Jean Perrin, use of sedimentation equilibrium, showed discontinuity of matter, determined Avogadro's constant and validated theory (Nobel Prize 1926)

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Brownian Motion ⁽⁵⁾



Mean path of the particle is proportional to $\sqrt{(\text{time})}$

- Programs:
 - <http://www.openteach.com/products5.html>
 - Run
 - <http://www.phy.ntnu.edu.tw/java/gas2D/gas2D.html>
- Alter number of solvent molecules
- Alter solute:solvent mass ratio

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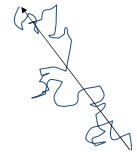
Brownian Motion ⁽⁶⁾

For n-D random walk:

$$\langle x^2 \rangle = 2nDt$$

D = diffusion coefficient
 $= k_B T / 6\pi\eta r = k_B T / \zeta$
 T = temperature
 η = solvent viscosity
 r = hydrodynamic radius of solute
 ζ = friction coefficient
(Stokes-Einstein relation for Brownian spheres)

Average displacement of a particle is proportional to $\sqrt{(\text{time})}$

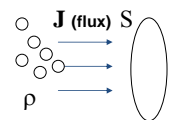


For water: at 293K: $\eta = 1.00\text{cP}$ (g/ms)
 at 298K: $\eta = 0.89\text{cP}$
 at 373K: $\eta = 0.13\text{cP}$
 Olive oil: at 293K: $\eta = 72\text{cP}$
 Peanut butter: at 293K: $\eta \sim 250000\text{cP}$
<http://xtronics.com/reference/viscosity.htm>

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Diffusion Equation

- Describes density fluctuations in a material undergoing diffusion
- Fick's First Law of diffusion : $\mathbf{J}(\mathbf{r}, t) = -D\nabla\rho(\mathbf{r}, t) + \frac{D\mathbf{F}\rho(\mathbf{r}, t)}{k_B T}$
- Fick's Second Law of diffusion : $\frac{\partial\rho(\mathbf{r}, t)}{\partial t} = -\nabla \cdot \mathbf{J}(\mathbf{r}, t)$



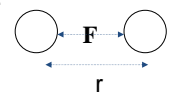
$$\frac{\partial\rho(\mathbf{r}, t)}{\partial t} = \nabla \cdot D\nabla\rho - \frac{\nabla \cdot D\mathbf{F}\rho(\mathbf{r}, t)}{k_B T}$$

- Reaction rate $k_{eff} = -\frac{1}{[A][B]} \frac{d[B]}{dt} = -\int_S \frac{\mathbf{J} \cdot d\mathbf{S}}{[B]}$

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Diffusion Equation - Analytic Solution

- 2 spheres: uniformly reactive surfaces, steady state: $\partial\rho/\partial t = 0$
- Smoluchowski: no forces : Laplace equation:

$$D\nabla^2\rho(\mathbf{r}) = 0 \quad \rho(\mathbf{r}_c) = 0 \quad \rho(\infty) = \rho_0 = [B]$$


$$k_{eff} = k_D = 4\pi r_c D$$

- Interparticle force $\mathbf{F} = -\nabla U(\mathbf{r})$

$$k_{eff} = k_D = 4\pi \left[\int_{r_c}^{\infty} \frac{\exp(U/k_B T)}{r^2 D(r)} dr \right]^{-1}$$

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Discrete and Continuum methods to model Diffusion

- Discrete: stochastic motions of individual particles
 - ◆ Monte Carlo
 - ◆ Langevin
 - ◆ Brownian Dynamics
- Continuum: concentration distribution probability
 - ◆ Finite Element
 - E.g. SMOL

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Brownian Motion

$$m \cdot \frac{d^2 \bar{\mathbf{R}}(t)}{dt^2} = -\gamma \cdot \frac{d\bar{\mathbf{R}}(t)}{dt} + \bar{\mathbf{F}} + \bar{\mathbf{S}}$$

- Langevin equation under high friction conditions

$$\frac{d\bar{\mathbf{R}}(t)}{dt} = \frac{1}{\gamma} \bar{\mathbf{F}} + \frac{1}{\gamma} \bar{\mathbf{S}}$$

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Langevin equation

$$m a = m dv/dt = F(x) - \zeta v + f(t)$$

- m = mass of diffusing particle
- dv/dt = acceleration of the particle
- $F(x)$ = systematic interaction force
- ζ = $k_B T/D$ = friction coefficient (0.4 ps⁻¹ for pure H₂O)
- v = velocity
- $f(t)$ = continuously fluctuating, stochastic force
 - independent of particle velocity;
 - fast variation compared to velocity variation
- → Langevin equation under high friction conditions

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Ermak-McCammon Brownian Dynamics

- Algorithm for simulating trajectories:

$$\mathbf{r}_i = \mathbf{r}_i^0 + \frac{1}{k_B T} (D_{ii} \mathbf{F}_i^0) + \mathbf{R}_i(\Delta t)$$

- r_i - coordinates of particle i before and after timestep Δt
- \mathbf{F} - Initial Forces
- D_{ii} - Diffusion constant $D_{ii} = k_B T / 6\pi\eta a$

- $\mathbf{R}_i(\Delta t)$ - Random stochastic displacement vector:
- $\langle \mathbf{R}_i \rangle = 0$ $\langle \mathbf{R}_i \mathbf{R}_i^T \rangle = 2D_{ii} \mathbf{I} \Delta t$
- Times \gg momentum relaxation time $\Delta t \gg m_i D_{ii}^0 / k_B T$

Ermak & McCammon JCP (1978) 69, 1352-60

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Hydrodynamic Interactions

HI between solute molecules results from induced fluid flow of solvent due to motion of solute molecules

Often neglected

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Ermak-McCammon Brownian Dynamics

- Algorithm for simulating trajectories:

$$\mathbf{r}_i = \mathbf{r}_i^0 + \frac{1}{k_B T} (D_{ii} \mathbf{F}_i^0 + \sum_{i \neq j} \mathbf{D}_{ij}^0 \cdot \mathbf{F}_j^0 \Delta t) + \mathbf{R}_i(\Delta t)$$
- r_i - coordinates of particle i before and after timestep Δt
- \mathbf{F} - Initial Forces
- D_{ii} - Diffusion constant $D_{ii} = k_B T / 6\pi\eta a$
- \mathbf{D}_{ij} - Diffusion tensor \rightarrow hydrodynamic interactions
- $\mathbf{R}_i(\Delta t)$ - Random stochastic displacement vector:

$$\langle \mathbf{R}_i \rangle = 0 \quad \langle \mathbf{R}_i \mathbf{R}_i^T \rangle = 2D_{ii} \mathbf{I} \Delta t \quad \langle \mathbf{R}_i \mathbf{R}_j^T \rangle = 2\mathbf{D}_{ij}^0 \Delta t$$
- Times $>$ momentum relaxation time $\Delta t \gg m_i D_{ii}^0 / k_B T$

Ermak & McCammon JCP (1978) 69, 1352-60

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Continuum Electrostatics

- Linearized Poisson-Boltzmann equation

$$-\epsilon_0 \nabla \cdot [\epsilon_r(\mathbf{r}) \nabla \phi(\mathbf{r})] = \rho^f(r) - \epsilon_0 \epsilon_r(r) \kappa^2(r) \phi(r)$$
- $$\kappa^2(r) = \frac{\beta}{\epsilon_0 \epsilon_r} \sum_1^N c_{i,bulk} q_i^2 = \frac{2e^2 N_A I}{\epsilon_0 \epsilon_r kT}$$

- Finite-difference
- Numerical solution

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Electrostatics: Effective (potential derived) Charges for Macromolecules in solvent


- Full electrostatic interaction:
- Test charge approximation:
- Effective charge approximation (incl. electrostatic desolvation):

Gabdouline & Wade, JPC, (1996) 100, 3868-78

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Electrostatic forces

- Approximation to PB solution:
 - Interaction ($\sum q_i \phi_j$) + desolvation terms



$$\Delta G_{desolvation} \approx \alpha \frac{\epsilon_s - \epsilon_p}{\epsilon_s (2\epsilon_s + \epsilon_p)} \sum_{ij} (1 + \kappa r_{ij})^2 \exp(-2\kappa r_{ij}) \frac{q_i^2 a_j^3}{r_{ij}^4}$$

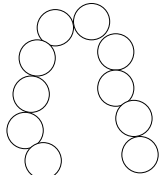
$$\kappa = \left(\frac{8\pi N_a e^2}{\epsilon kT} \right)^{1/2} I^{1/2}$$

Gabdoulline & Wade, JPC, (1996) 100, 3868-78
 Elcock et al. JMB (1999) 291, 149-162
 Gabdoulline & Wade, JMB (2001) 306, 1139-1155

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Flexible Molecules

- Chains or clusters of spheres
- Polymers: Gaussian chains
 - harmonic springs between beads
 - soft exponentials for volume exclusion
- Proteins: amino acid beads on a string
 - $E = E_{bond} + E_{angle} + E_{tors} + E_{exv} + E_{elec} + E_{si} + E_{helix}$
- Nucleic Acids: strings of beads or cylinders



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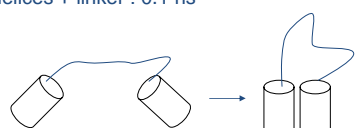
Talk Outline

<ul style="list-style-type: none"> Theory <ul style="list-style-type: none"> Brownian Motion Brownian Dynamics Simulations Encounter Rate Computation Forces: <ul style="list-style-type: none"> Exclusion Electrostatics Hydrodynamic interactions Molecular Flexibility 	<ul style="list-style-type: none"> Applications <ul style="list-style-type: none"> Protein Folding DNA Bending and Supercoiling Enzyme-Substrate Encounter Protein-Protein/NA Encounter Diffusion in the Cell Macromolecular crowding
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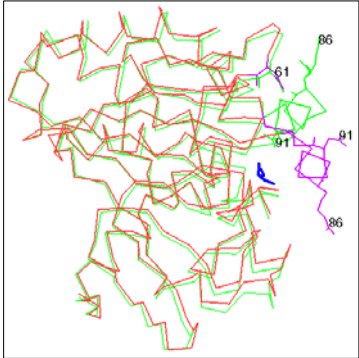
Protein Folding and Conformational Changes

- Secondary structure formation
 - helix-coil, β -sheet-coil
- Tertiary structure formation
 - Diffusion-Collision Model (Weaver & Karplus, 1976): microdomains (transiently structured) diffuse, collide, coalesce : viscosity dependent
 - Folding rates obtained
 - E.g. 2 helices + linker : 0.1 ns⁻¹



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Protein Loop motions : *Rhizomucor Miehei* lipase



- Interfacial activation → binding of non-polar ester substrate
- closed → open lid
- Open lid: ionic tethering: R86-D61
- MD & BD show lid opening facilitated as environment's dielectric constant is reduced
- Activity reduced by
 - Arg chem. modif
 - Guanidine

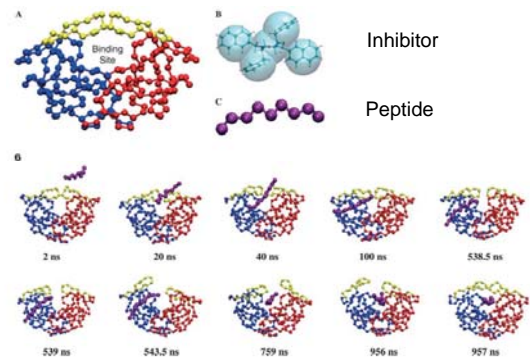
29 06/07/2008 - Rebecca Wade (c) Peters et al, *Biophys J.* (1996) 71, 119-129

Course Grain Simulations of Protein Dynamics

- Recently:
 - Many different, but similar FFs
 - Using reference experimental structure
 - Explore dynamics
 - Functional insights
 - E.g.
 - HIV Protease flaps and ligand binding
 - Ribosome
 - Acetylcholinesterase ligand gating

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Course Grain Simulations of Protein Dynamics – HIV Protease



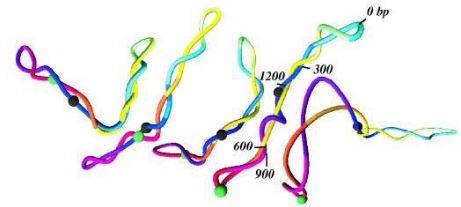
A Binding Site
B Inhibitor
C Peptide

D 2 ms, 20 ms, 40 ms, 100 ms, 538.5 ms, 539 ms, 543.5 ms, 759 ms, 956 ms, 957 ms

31 06/07/2008 - Rebecca Wade (c) Chang et al, *Chem Biol Drug Res* (2007) 69, 5-13

DNA Bending and Supercoiling

- Larger time, spatial scales than proteins
 - e.g. supercoiling ~100ms for 300-6000 bp supercoiled DNA (Schlick, Lanoowski)



<http://monod.biomath.nyu.edu/index/gallery.html>

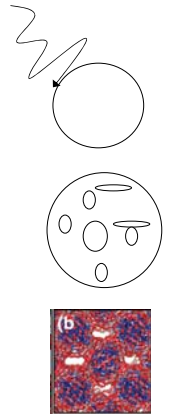
- Simulation unit < persistence length e.g. 500 Å in B-DNA
- Beads/stiff segments can represent 10s of base pairs

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Protein Diffusion Towards and in a Cell

- Towards a Cell
 - ◆ Small # independent receptors:

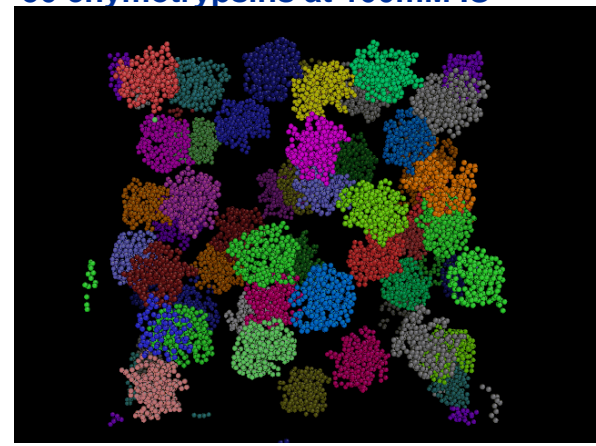
$$K_{\text{assoc}} = \sum K_{\text{assoc}}^i$$
 - ◆ Increased receptor density → saturation to Smoluchowski rate even when the surface not fully covered
- In a Cell
 - ◆ Macromolecular crowding
 - Solutions, crystals
 - ◆ Simple protein and nucleic acid models
 - ◆ Some simulations in atomic detail



Malek & Coppins, 2008, JPC, 112, 1549

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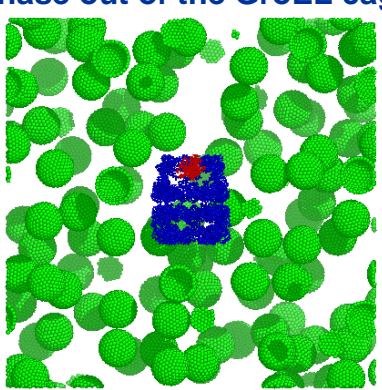
Diffusion of many proteins 50 chymotrypsins at 100mM IS



Adrian Elcock: <http://dadiddly.biochem.uiowa.edu/>

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Effect of crowding agents on activation energy for pulling rhodanase out of the GroEL cage



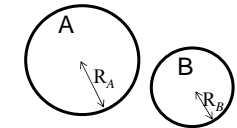
Elcock, PNAS (2003) 100, 2340-2344

Adrian Elcock: <http://dadiddly.biochem.uiowa.edu/>

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Bimolecular Diffusional Association Rate Constant

- The rate to form an Encounter Complex
- Smoluchowski rate (1917) for 2 spheres:



$$k_{on,sm} = 4\pi \cdot (D_A + D_B) \cdot (R_A + R_B) \sim 10^{10} \text{ M}^{-1}\text{s}^{-1}$$

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Bimolecular rate constant calculation from BD: NAM method

$$k_{on} = k_{b,sm} \cdot \frac{\beta}{1 - (1 - \beta)k_{b,sm} / k_{c,sm}}$$

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Association Rates

The rate to arrive here

When started here the probability to form encounter complex

Correction to take into account possible coming back when trajectory truncated here

$$k_{on} = k_{b,sm} \cdot \frac{\beta}{1 - (1 - \beta)k_{b,sm} / k_{c,sm}}$$

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Bimolecular rate constant calculation from BD: NAM method

- Start a large number of trajectories from b-surface
- Monitor reaction
- β - fraction of reactive trajectories

$$k_{on} = k_{b,sm} \cdot \frac{\beta}{1 - (1 - \beta)k_{b,sm} / k_{c,sm}}$$

- Rigid molecules
- Atomic detail
- No overlaps
- Electrostatic forces
- $\Delta r = (D\Delta t/kT)F + R$; etc
- $\langle R_i \rangle = 0$, $\langle R_i^2 \rangle = 2D\Delta t$ ($i = x, y, z$)
- Timestep $\Delta t \geq 1$ ps

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Interaction energy calculation

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Reaction conditions

- Monitoring formation of encounter complex according to number of native complex contacts
- find end point of diffusional association

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Diffusion-Controlled Catalysis

$$A + B \xrightleftharpoons[k_r]{k_D} A : B \xrightarrow{k_{cat}} products$$

- At steady state:

$$\frac{\partial [AB]}{\partial t} = 0 = k_D \cdot [A] \cdot [B] - (k_r + k_{cat}) \cdot [AB]$$

$$-\frac{\partial [B]}{\partial t} = k_D [A][B] - k_r [AB] = k_{eff} [A][B]$$

$$k_{eff} = \frac{k_D k_{cat}}{k_r + k_{cat}} \quad \text{If } k_{cat} \gg k_r, \text{ then } k_{eff} \approx k_D \approx 10^8 - 10^9 \text{ M}^{-1}\text{s}^{-1}$$

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Cu,Zn-Superoxide dismutase

- Diffusion-controlled
 - 4-9 x 10⁹ M⁻¹s⁻¹ at 20mM
- Many BD studies
- Two different dimer arrangements
 - Bovine
 - Photobacterium leigonahi
- Electrostatically enhanced association rates
 - Local +ve potential around binding sites

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Superoxide diffusion to superoxide dismutase and peroxidases

- SOD:**
 - K_{cat}/K_m ~ 10¹⁰ M⁻¹s⁻¹ (theoretical limit)
 - Rate enhanced by electrostatic interactions
 - Simulations: many; reproduce effects of charge mutations
- Peroxidases:**
 - Myeloperoxidase: rate (10⁷ M⁻¹s⁻¹) depressed by electrostatic interactions!

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Oscillations and cell function: e.g. activated neutrophils

NAD(P)H oscillation in activated neutrophils: Enhanced by melatonin in activated neutrophils
Olsen et al, BJ, 2003

Fluorescence Intensity

Structure-based molecular simulation using electrostatic interactions
Petty et al

©James A. Sullivan www.cellsalive.com

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Bridging from molecular simulation to biochemical networks:

Biochemical network simulation

$$\frac{d}{dt}[\text{col}] = k_1 \cdot [\text{H}_2\text{O}_2]_p [\text{per}3^+] - k_{-1}[\text{col}]$$

$$\frac{d}{dt}[\text{colIII}] = k_4 \cdot [\text{O}_2^-]_p [\text{per}3^+]$$

Simulations show oscillations

Observed oscillation of cellular metabolite concentrations

Structure-based molecular simulation using electrostatic interactions
per3

46 06/07/2008 - Rebecca Wade (c) *Stein, Gabdoulina, Wade Curr. Op. Struct. Biol. (2007) 17, 166-172*

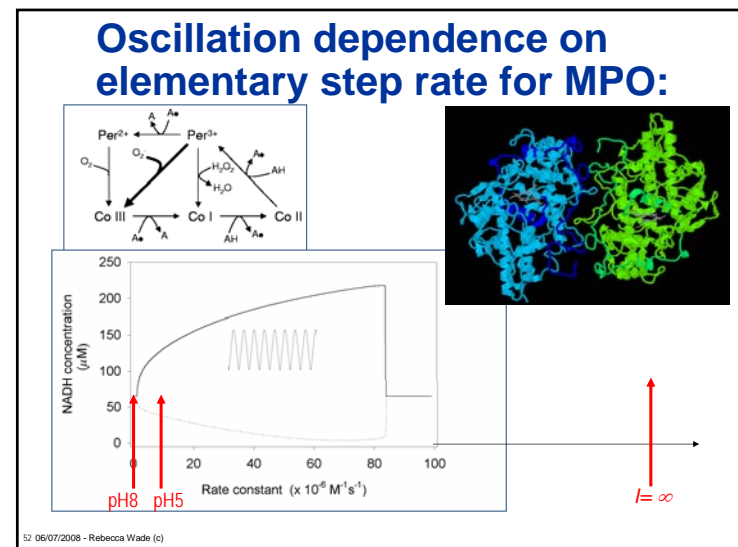
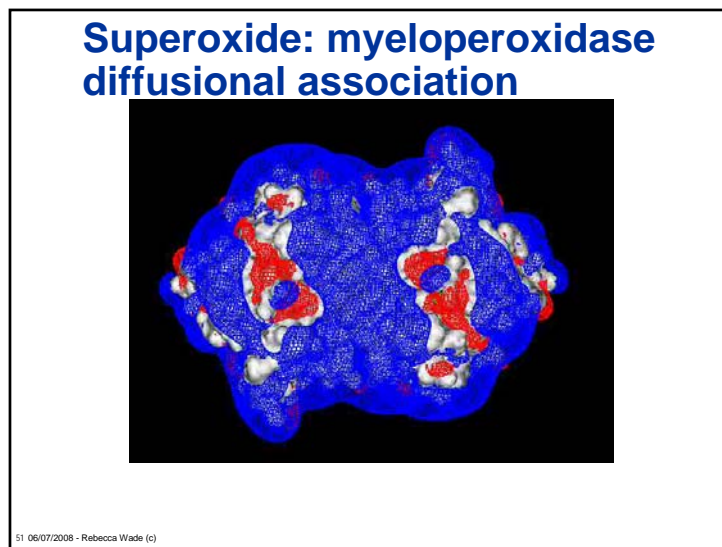
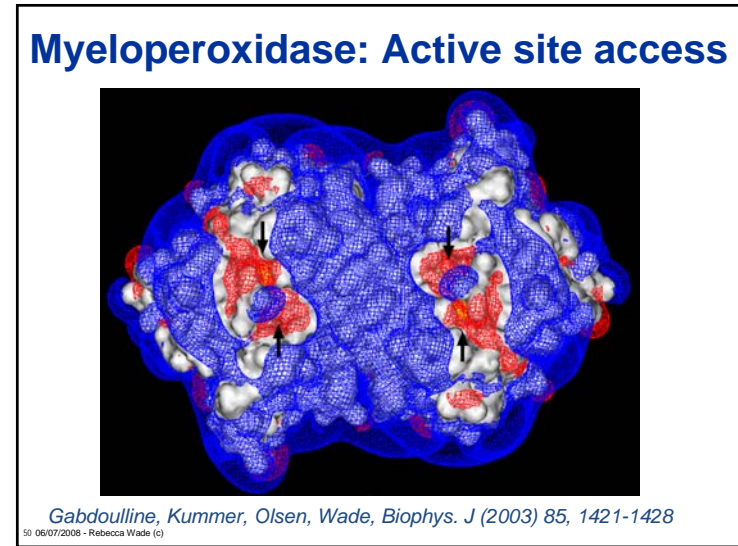
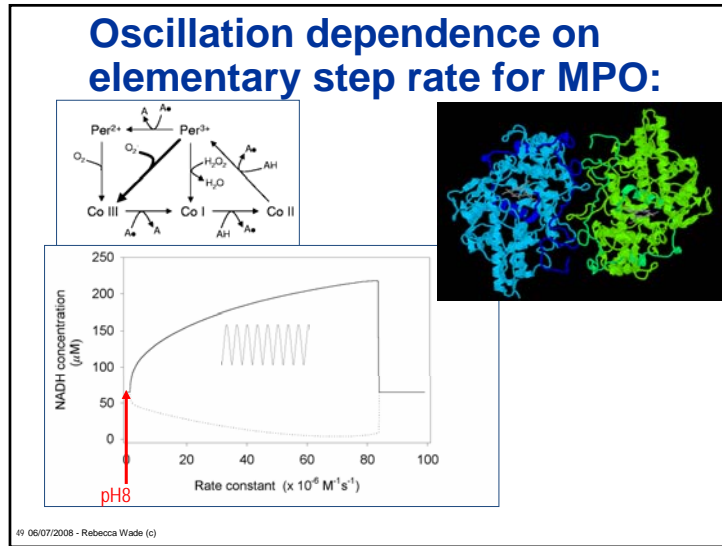
Myeloperoxidase PO Reaction

Reaction	Rate expression (R_i)	Rate constant
Reactions occurring in phagosome:		
1. $\text{H}_2\text{O}_2 + \text{per}^{3+} \xrightleftharpoons[k_{-1}]{k_1} \text{col}$	$k_1[\text{H}_2\text{O}_2]_p[\text{per}^{3+}]_p - k_{-1}[\text{col}]_p$	$k_1 = 5.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ $k_{-1} = 58 \text{ s}^{-1}$
2. $\text{col} + \text{MLTH} \xrightleftharpoons[k_{-2}]{k_2} \text{colI} + \text{MLT}$	$k_2[\text{col}]_p[\text{MLTH}]_p$	$k_2 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
3. $\text{colI} + \text{MLTH} \xrightleftharpoons[k_{-3}]{k_3} \text{per}^{3+} + \text{MLT}$	$k_3[\text{colI}]_p[\text{MLTH}]_p$	$k_3 = 4.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
4. $\text{per}^{3+} + \text{O}_2^- \xrightleftharpoons[k_{-4}]{k_4} \text{colII}$	$k_4[\text{per}^{3+}]_p[\text{O}_2^-]_p$	$k_4 = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
5. $2\text{H}^+ + 2\text{O}_2^- \xrightleftharpoons[k_{-5}]{k_5} \text{H}_2\text{O}_2 + \text{O}_2$	$k_5[\text{O}_2^-]_p^2$	$k_5 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
6. $\text{colIII} + \text{O}_2^- \xrightleftharpoons[k_{-6}]{k_6} \text{col} + \text{O}_2$	$k_6[\text{colIII}]_p[\text{O}_2^-]_p$	$k_6 = 1.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$
Reactions occurring in cytoplasm:		
7. $\text{NADPH} + \text{O}_2 \xrightleftharpoons[k_{-7}]{k_7} \text{NADP}^+ + \text{H}_2\text{O}_2$	$k_7[\text{NADPH}]_c[\text{O}_2]_c$	$k_7 = 1 \text{ M}^{-1} \text{ s}^{-1}$
8. $\text{NADP}^+ + \text{O}_2 \xrightleftharpoons[k_{-8}]{k_8} \text{NADP}^+ + \text{O}_2^-$	$k_8[\text{NADP}^+]_c[\text{O}_2]_c$	$k_8 = 5.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
9. $2\text{H}^+ + 2\text{O}_2^- \xrightleftharpoons[k_{-9}]{k_9} \text{H}_2\text{O}_2 + \text{O}_2$	$k_9[\text{O}_2^-]_c^2$	$k_9 = 5.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$
10. $\text{MLT} + \text{NADPH} \xrightleftharpoons[k_{-10}]{k_{10}} \text{MLTH} + \text{NADP}$	$k_{10}[\text{MLT}]_c[\text{NADPH}]_c$	$k_{10} = 3.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
11. $2\text{NADP} \xrightleftharpoons[k_{-11}]{k_{11}} \text{NADP}^2$	$k_{11}[\text{NADP}]_c^2$	$k_{11} = 6.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
12. $\text{NADPH} \xrightleftharpoons[k_{-12}]{k_{12}} \text{NADP}$	k_{12}	$k_{12} = 22-35 \mu\text{M s}^{-1}$
13. $\text{O}_2 \xrightleftharpoons[k_{-13}]{k_{13}} \text{O}_2^-$ (cytoplasm)	$k_{13} - k_{-13}[\text{O}_2]_c$	$k_{13} = 12.5 \mu\text{M s}^{-1}$ $k_{-13} = 4.5 \times 10^{-2} \text{ s}^{-1}$
Diffusion terms:		
14. O_2 (phagosome) $\xrightleftharpoons[k_{14}]{k_{14}} \text{O}_2$ (cytoplasm)	$k_{14}([\text{O}_2]_p - [\text{O}_2]_c)$	$k_{14} = 30 \text{ s}^{-1}$
15. H_2O_2 (phagosome) $\xrightleftharpoons[k_{15}]{k_{15}} \text{H}_2\text{O}_2$ (cytoplasm)	$k_{15}([\text{H}_2\text{O}_2]_p - [\text{H}_2\text{O}_2]_c)$	$k_{15} = 30 \text{ s}^{-1}$
16. MLTH (phagosome) $\xrightleftharpoons[k_{16}]{k_{16}} \text{MLTH}$ (cytoplasm)	$k_{16}([\text{MLTH}]_p - [\text{MLTH}]_c)$	$k_{16} = 10 \text{ s}^{-1}$
17. MLT (phagosome) $\xrightleftharpoons[k_{17}]{k_{17}} \text{MLT}$ (cytoplasm)	$k_{17}([\text{MLT}]_p - [\text{MLT}]_c)$	$k_{17} = 10 \text{ s}^{-1}$
18. O_2^- (phagosome) $\xrightleftharpoons[k_{18}]{k_{18}} \text{O}_2^-$ (cytoplasm)	$k_{18}([\text{O}_2^-]_p - [\text{O}_2^-]_c)$	$k_{18} = < 0.01 \text{ s}^{-1}$
NADPH oxidase:		
19. NADPH (cytoplasm) + 2O_2 (phagosome) $\xrightarrow[\text{NADPH}]{\text{NADPH oxidase}}$ NADP^+ (cytoplasm) + 2O_2^- (phagosome)	$\frac{V_{\text{NADPH}}([\text{O}_2]_p - [\text{O}_2]_c)}{K_{\text{NADPH}} + ([\text{O}_2]_p - [\text{O}_2]_c)}$	$V = 288 \mu\text{M s}^{-1}$ $K_{\text{NADPH}} = 150$ $K_{\text{O}_2} = 1.5 \mu\text{M}$ $K_{\text{NADPH}} = 60 \mu\text{M}$

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Myeloperoxidase PO Reaction

Reaction	Rate expression (R_i)	Rate constant
Reactions occurring in phagosome:		
1. $\text{H}_2\text{O}_2 + \text{per}^{3+} \xrightleftharpoons[k_{-1}]{k_1} \text{col}$	$k_1[\text{H}_2\text{O}_2]_p[\text{per}^{3+}]_p - k_{-1}[\text{col}]_p$	$k_1 = 5.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ $k_{-1} = 58 \text{ s}^{-1}$
2. $\text{col} + \text{MLTH} \xrightleftharpoons[k_{-2}]{k_2} \text{colI} + \text{MLT}$	$k_2[\text{col}]_p[\text{MLTH}]_p$	$k_2 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
3. $\text{colI} + \text{MLTH} \xrightleftharpoons[k_{-3}]{k_3} \text{per}^{3+} + \text{MLT}$	$k_3[\text{colI}]_p[\text{MLTH}]_p$	$k_3 = 4.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
4. $\text{per}^{3+} + \text{O}_2^- \xrightleftharpoons[k_{-4}]{k_4} \text{colII}$	$k_4[\text{per}^{3+}]_p[\text{O}_2^-]_p$	$k_4 = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
5. $2\text{H}^+ + 2\text{O}_2^- \xrightleftharpoons[k_{-5}]{k_5} \text{H}_2\text{O}_2 + \text{O}_2$	$k_5[\text{O}_2^-]_p^2$	$k_5 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
6. $\text{colIII} + \text{O}_2^- \xrightleftharpoons[k_{-6}]{k_6} \text{col} + \text{O}_2$	$k_6[\text{colIII}]_p[\text{O}_2^-]_p$	$k_6 = 1.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$
Reactions occurring in cytoplasm:		
7. $\text{NADPH} + \text{O}_2 \xrightleftharpoons[k_{-7}]{k_7} \text{NADP}^+ + \text{H}_2\text{O}_2$	$k_7[\text{NADPH}]_c[\text{O}_2]_c$	$k_7 = 1 \text{ M}^{-1} \text{ s}^{-1}$
8. $\text{NADP}^+ + \text{O}_2 \xrightleftharpoons[k_{-8}]{k_8} \text{NADP}^+ + \text{O}_2^-$	$k_8[\text{NADP}^+]_c[\text{O}_2]_c$	$k_8 = 5.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
9. $2\text{H}^+ + 2\text{O}_2^- \xrightleftharpoons[k_{-9}]{k_9} \text{H}_2\text{O}_2 + \text{O}_2$	$k_9[\text{O}_2^-]_c^2$	$k_9 = 5.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$
10. $\text{MLT} + \text{NADPH} \xrightleftharpoons[k_{-10}]{k_{10}} \text{MLTH} + \text{NADP}$	$k_{10}[\text{MLT}]_c[\text{NADPH}]_c$	$k_{10} = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
11. $2\text{NADP} \xrightleftharpoons[k_{-11}]{k_{11}} \text{NADP}^2$	$k_{11}[\text{NADP}]_c^2$	$k_{11} = 6.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
12. $\text{NADPH} \xrightleftharpoons[k_{-12}]{k_{12}} \text{NADP}$	k_{12}	$k_{12} = 22-35 \mu\text{M s}^{-1}$
13. $\text{O}_2 \xrightleftharpoons[k_{-13}]{k_{13}} \text{O}_2^-$ (cytoplasm)	$k_{13} - k_{-13}[\text{O}_2]_c$	$k_{13} = 12.5 \mu\text{M s}^{-1}$ $k_{-13} = 4.5 \times 10^{-2} \text{ s}^{-1}$
Diffusion terms:		
14. O_2 (phagosome) $\xrightleftharpoons[k_{14}]{k_{14}} \text{O}_2$ (cytoplasm)	$k_{14}([\text{O}_2]_p - [\text{O}_2]_c)$	$k_{14} = 30 \text{ s}^{-1}$
15. H_2O_2 (phagosome) $\xrightleftharpoons[k_{15}]{k_{15}} \text{H}_2\text{O}_2$ (cytoplasm)	$k_{15}([\text{H}_2\text{O}_2]_p - [\text{H}_2\text{O}_2]_c)$	$k_{15} = 30 \text{ s}^{-1}$



Electrostatics of 3 Peroxidases

myeloperoxidase pH 5

lactoperoxidase pH 5

myeloperoxidase pH 8

horseradish peroxidase pH 5

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From Molecular Structure to Cellular Oscillations?

- Combine Simulation Techniques:
 - Biochemical network simulation
 - Structure-based molecular simulation
- Molecular level insights
 - Association rate constant computed in range required for oscillatory behaviour
 - Electrostatic depression of binding rate constant
 - Structurally unrelated enzymes with same function have similar binding rate
 - Identify important regions of enzyme

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Protein-Protein/NA Association:

- Protein-protein:
 - Enzyme-inhibitor
 - Electron transfer
 - Antibody-antigen
- Protein-RNA:
 - Translation initiation factor -mRNA
- Protein-DNA:
 - Chromatin
 - Nuclear receptors

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Protein-Protein Association: Diffusional & non-diffusional steps

$A + B \rightleftharpoons A::B \rightleftharpoons AB$

Diffusional encounter

Translation and orientation by diffusion

Docking (non-diffusional)

Precise conformational matching

Diffusional association rate > association rate

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Association rates

$$\frac{d}{dt}[EI] = k_{on} \cdot [E][I] - k_{off} \cdot [EI]$$

Typical $K_{on} \sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$ means that

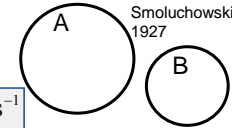
- at inhibitor concentration 1 micromolar
- if inhibitor does not unbind from enzyme
- at very low enzyme concentrations
- half of enzymes will bind substrate in 1 s

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Bimolecular Diffusional Association Rate

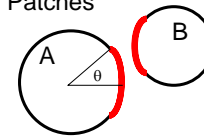
- The rate to form Encounter Complex
- Smoluchowski rate

$$k_{on,sm} = 4\pi \cdot (D_A + D_B) \cdot (R_A + R_B) \sim 10^{10} \text{ M}^{-1} \text{ s}^{-1}$$



Smoluchowski 1927

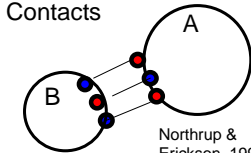
- Patches



$$k_a \gg k_{on,sm} \cdot f(\theta)$$

Solc & Stockmayer, 1973
H.-X. Zhou, 1993

- Contacts

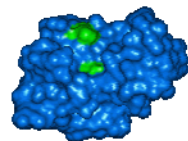


Northrup & Erickson, 1992

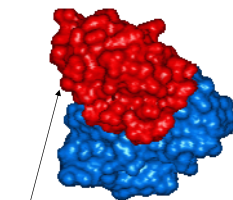
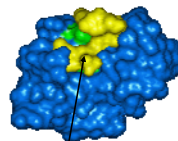
$$k_a \sim 10^6 \text{ M}^{-1} \text{ s}^{-1}$$

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Inhibition of barnase by barstar



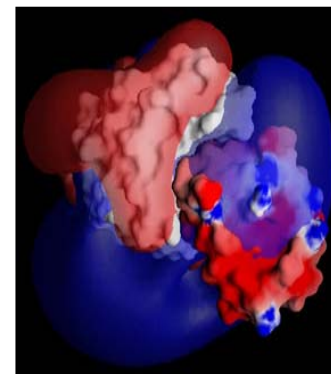
Barnase catalyses cleavage of single stranded RNA (outside the cell)



Barstar blocks the active site, and catalysis by barnase (in the cell)

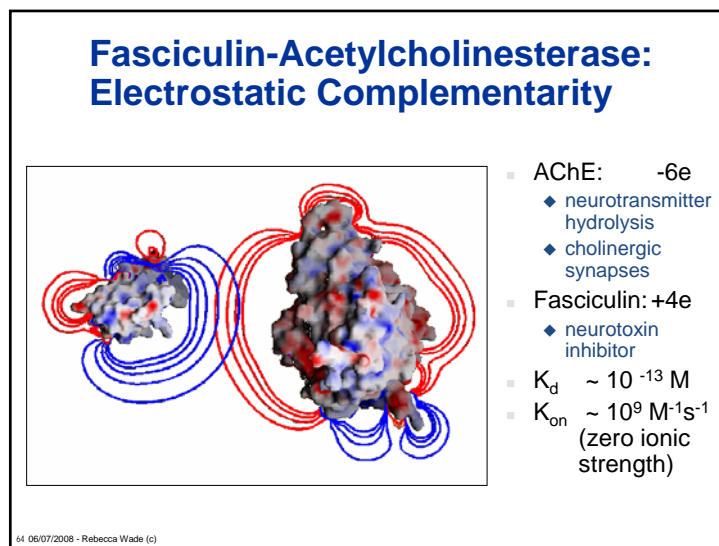
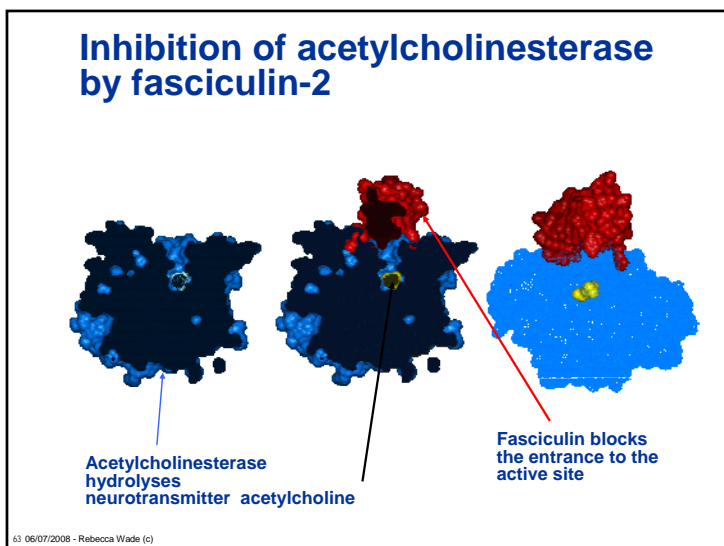
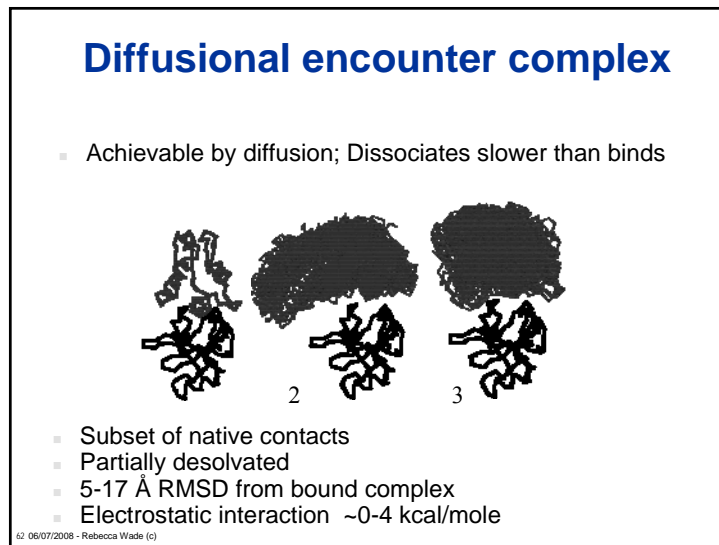
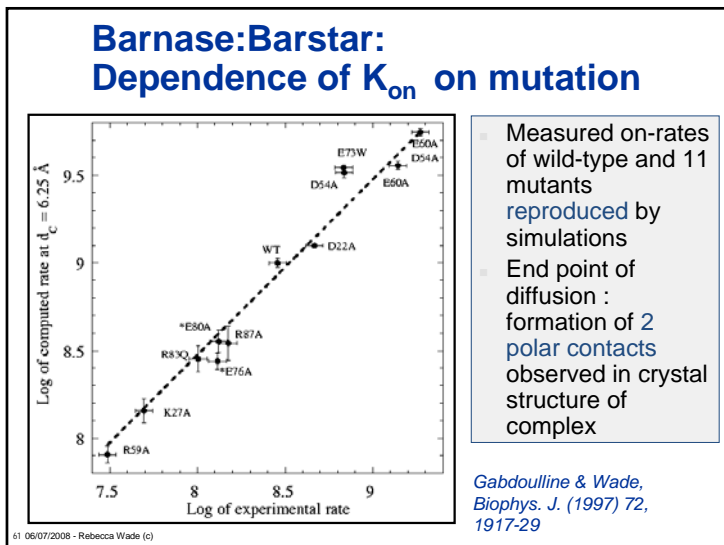
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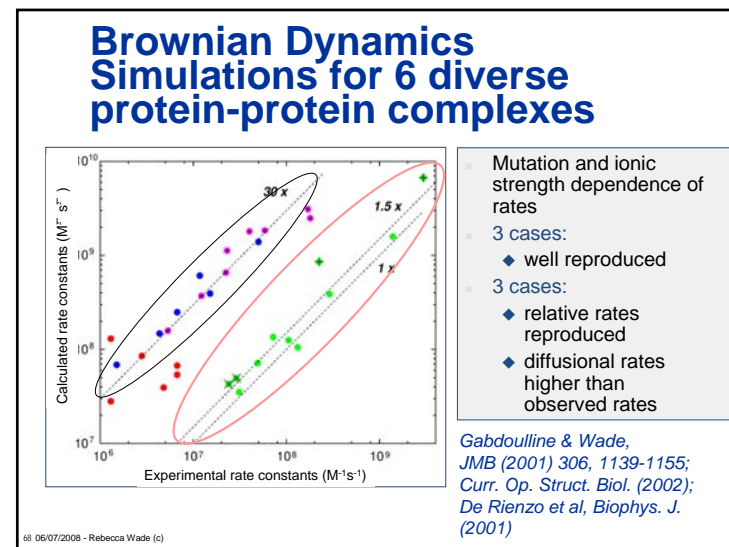
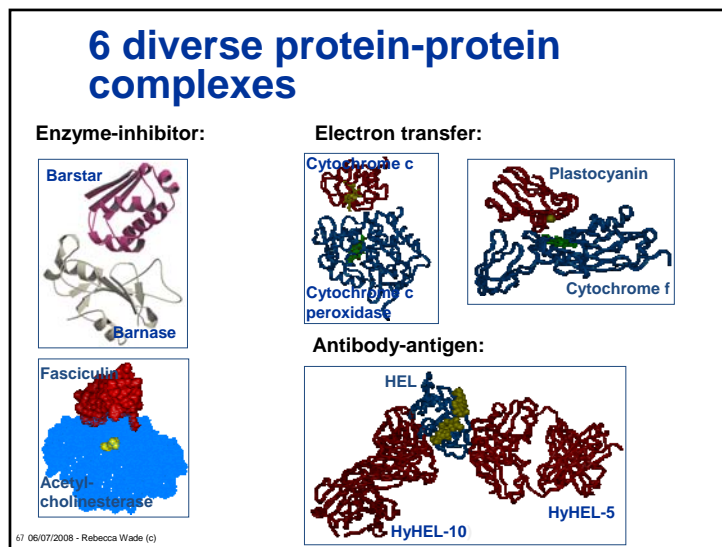
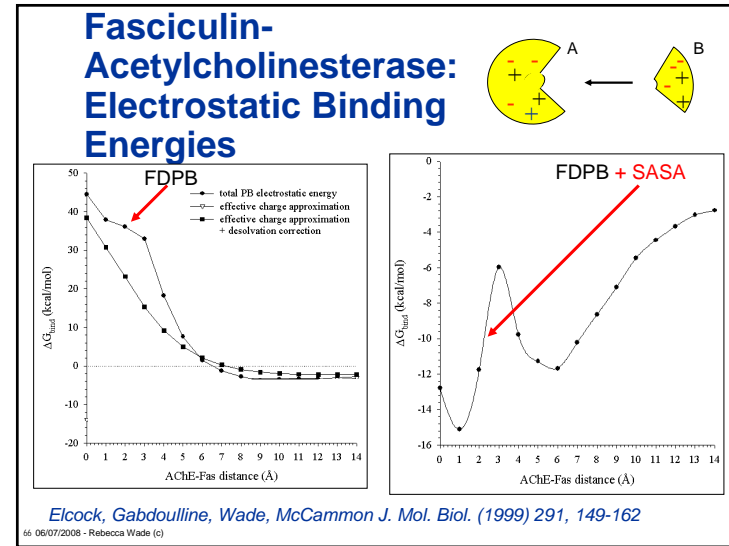
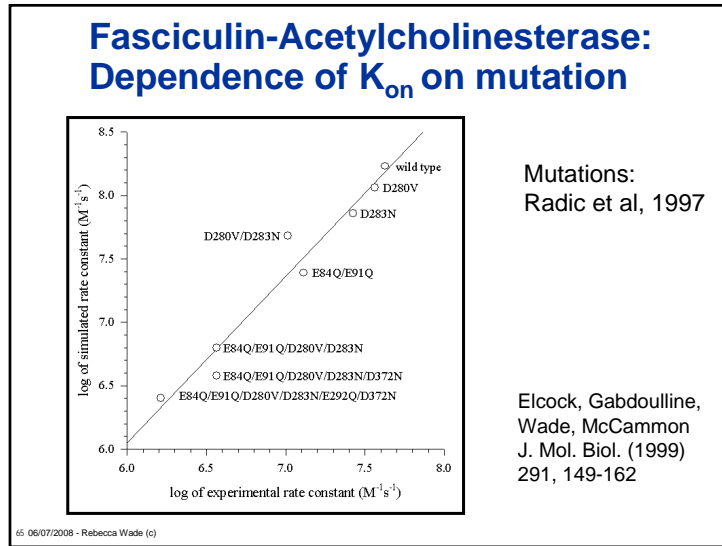
Barnase-Barstar: Electrostatic Complementarity



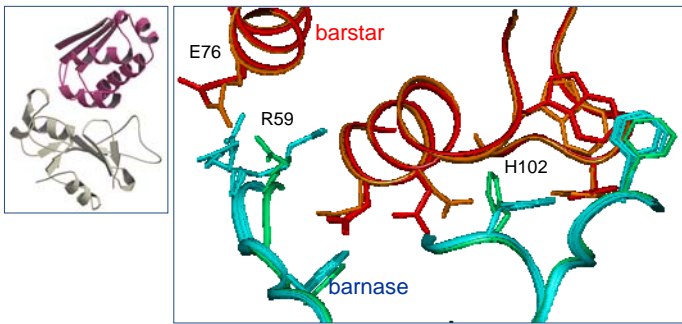
- Barnase : +2e
 - extracellular ribonuclease
 - Bacillus amyloliquefaciens
- Barstar : - 6e
 - intracellular inhibitor
- $K_d \sim 10^{-14} \text{ M}$
- $K_{on} \sim 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (zero ionic strength)

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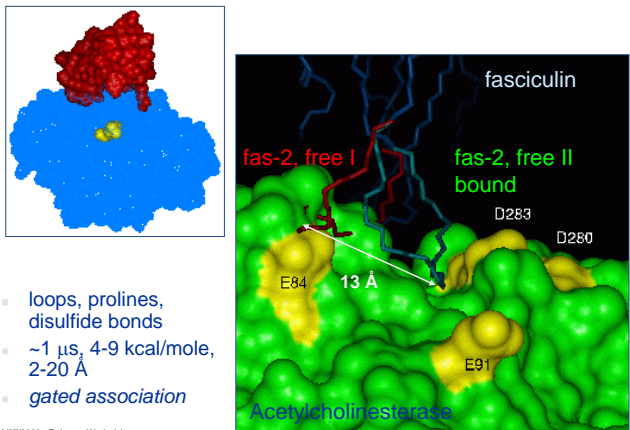
Flexibility - fast motions



- a few side-chain motions: ~100 ps, 0-3 kcal/mole, 2-5 Å
- may be neglected for computing association rates

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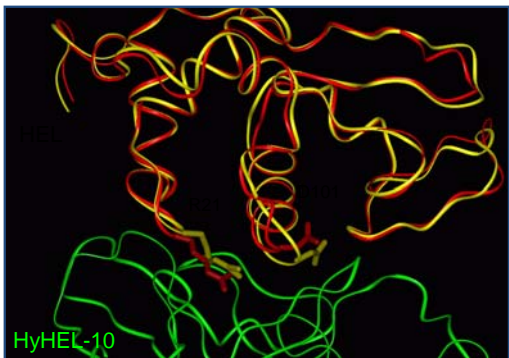
Flexibility- slow motions



- loops, prolines, disulfide bonds
- ~1 μs, 4-9 kcal/mole, 2-20 Å
- gated association

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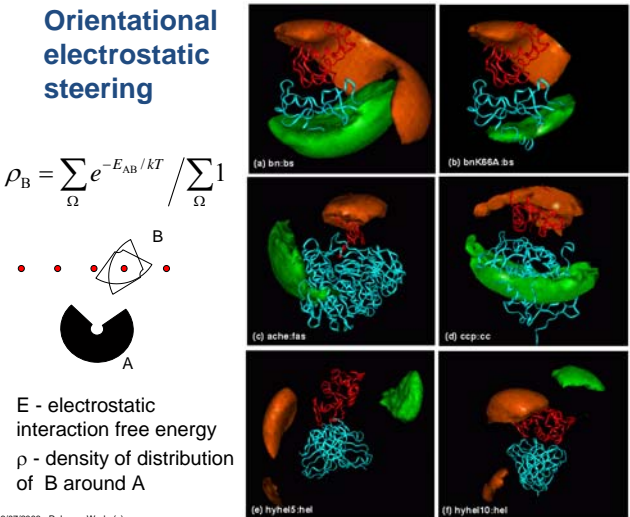
Flexibility - distortions



- HyHEL-10 (green)
- Bound HEL (red)
- Unbound HEL (yellow)
- Backbone distortion greater for D101 than R21

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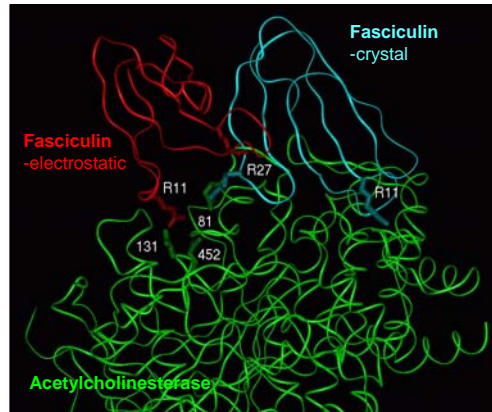
Oriental electrostatic steering

$$\rho_B = \frac{\sum_{\Omega} e^{-E_{AB}/kT}}{\sum_{\Omega} 1}$$


- E - electrostatic interaction free energy
- ρ - density of distribution of B around A

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Electrostatic steering of Acetylcholinesterase : Fasciculin association



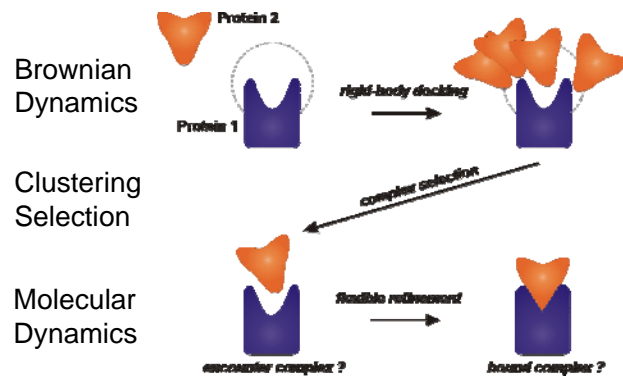
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Protein-protein binding: Association Rates

- Observed ■ $10^3 - 10^{10}$
- Diffusion limit (no orientational requirement) ■ 10^{10}
- Orientational restriction
 - reduces 1000 times ■ 10^7
- Electrostatic steering
 - may increase 1000 times ■ $10^7 - 10^{10}$
- Conformational gating
 - may reduce 100 times ■ $10^5 - 10^{10}$

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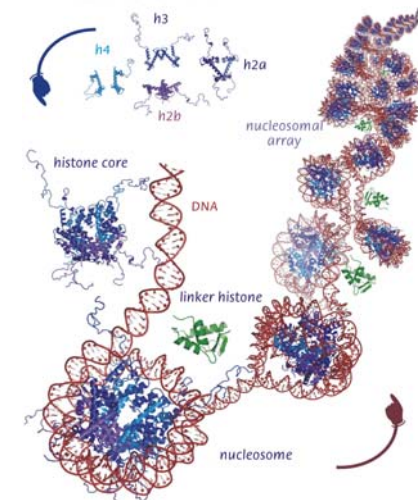
Protein-Protein Docking: Method overview



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Motiejunas et al, Proteins, 2008

Chromatin: Multiple scales



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Chromatin: Linker histone interactions

71 06/07/2008 - Rebecca Wade (c) Pachov, Gabdoulina, Wade NIC Proceedings (2007), 36, 69-74

Normal Mode Analysis

SDA Program: Simulation of Diffusional Association of Proteins

- All atom models of proteins
- Accurate modeling of electrostatic interactions
- Sophisticated encounter complex monitoring
- Tested in many protein-protein association cases
- <http://projects.villa-bosch.de/mcm/software/sda>
 - ◆ Version 5

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BD: Outlook

- BD is a useful computational tool for quantitative and qualitative calculation of molecular properties and for gaining mechanistic insights
- **BD will find increasing application:**
 - ◆ From the molecular to the cellular level +
- **Multiscale simulations:**
 - ◆ QM → MD → BD (at different levels of detail)
- **Methodological improvements needed:**
 - ◆ Molecular Flexibility
 - ◆ Force description (hydrodynamics, hydrophobicity...)

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Some Selected Literature:

- Madura, J.D., Briggs, J.M., Wade, R.C. and Gabdoulhine, R.R. Brownian Dynamics. In "Encyclopedia of Computational Chemistry", Eds. Schleyer, P.v.R., Allinger, N.L., Clark, T., Gasteiger, J., Kollman, P.A. and Schaefer, H.F., Schreiner, P.R. John Wiley & Sons: Chichester, UK, (1998) 1, 141-154.
- Gabdoulhine, R.R. and Wade, R.C. Simulation of the Diffusional Association of Barnase and Barstar. *Biophys. J.* (1997) 72, 1917-1929.
- Gabdoulhine, R.R. and Wade, R.C. Protein-protein Association: Investigation of Factors Influencing Association Rates by Brownian Dynamics Simulations. *J. Mol. Biol.* (2001) 306, 1139-1155.
- Gabdoulhine, R.R. and Wade, R.C. Biomolecular Diffusional Association. *Curr. Op. Struct. Biol.* (2002), 12, 204-213.
- Gabdoulhine, R.R., Kummer, U., Olsen, L.F. and Wade, R.C. Concerted Simulations Reveal How Peroxidase Compound III Formation Results in Cellular Oscillations. *Biophys. J.* (2003) 85, 1421-1428.

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DSMM: Database of Simulated Molecular Motions

The screenshot shows a web browser window displaying the DSMM website. The address bar shows the URL: <http://projects.villa-bosch.de/mcm/database/dsmm>. The page title is "DSMM - Database of Simulated Molecular Motions". The navigation menu includes "home", "browse", "search", "submit", and "links". The main content area features a graphic of a protein chain and a list of bullet points describing the database's purpose and contents. At the bottom, there is a footer with credits and contact information.

DSMM - Database of Simulated Molecular Motions

- The purpose of this database is to provide an easily-searchable source of information about movies showing biomolecular motions that have been generated by computer simulation. All of the movies are available through the internet.
- Molecules simulated include proteins, DNA, RNA, sugars and lipids. Simulation techniques include Molecular Dynamics, Brownian Dynamics and automated docking procedures.
- We welcome new submissions as well as feedback from your explorations of the DSMM.

Internet Explorer version 3.02 or higher or Netscape Communicator version 6 or higher is needed to browse, search, submit the movies.

Created by Giacomo Finocchiaro, Ting Wang, René Hoffmann, Aitor González and Rebecca Wade at the European Molecular Biology Laboratory and the Molecular and Cellular Modeling Group at the European Medical Laboratory in Heidelberg, Germany. All rights reserved.

If you have any problems, questions or comments please contact the [database administrator](#).

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