Statistical Biological Physics: from single molecule to cell

Focus: "Fluctuations and noise" in stochastic biological processes"

Here: Stochastic processes in living cells

Major challenge: highly noisy environment → operation of molecular components of a cell

- specific sources of noise in a cell?
- robustness to noise (attenuation of noise effects)
- stochastic genotype → reliable phenotype
- noisy crowded environment → diffusive transport
- molecular machines: conversion of chemical energy to work?
- physical limits of biochemical signalling (sensitivity of biochemical sensors to environmental sign.
- can cells exploit noise to enhance its performance or survival of host organism
- role of self-organization in the formation & maintenance of subcellular structures (e.g. cytoskeleton)

⇝ Topics:

- Diffusion in cells
- Stochastic ion channels
- Polymers and molecular motors
- Sensing the environment (Chemotaxis, Mechanotransduction, ..)
- Stochastic gene expression / regulatory networks
- Transport processes in cells
  anomalous diffusion, narrow escape problem, nanopores & channels, active transport, exclusion processes, random intermittent search processes
- Self-organisation in cells: active processes
  cellular length regulation, cell mitosis, cell motility
- Self-organization in cells: reaction-diffusion models
  Turing patterns, Min-Protein oscillations, cell polarization

Diffusion: $\langle \Delta x^2 \rangle = (2d) \cdot D \cdot \Delta t \quad (d = 1, 2, 3)$

3d: cell interior
2d: membrane
1d: axons

Sizes / times:

				small mol. $D \sim 100\,\mu m^2/sec$	protein $D \sim 10\,\mu m^2/sec$
Bacteria (E. coli)		$V \sim 1\,\mu m^3$	$L \sim 1\,\mu m$	$\tau \sim 1\,ms$	$\tau \sim 10\,ms$
Yeast		$V \sim 30\,\mu m^3$	$L \sim 3\,\mu m$	$\tau \sim 0.03\,s$	$\tau \sim 0.1\,sec$
Mamm. (HeLa)		$V \sim 3000\,\mu m^3$	$L \sim 10\,\mu m$	$\tau \sim 1\,sec$	$\tau \sim 10\,sec$

e.g. Ca^{2+}-diffusion: $D \sim 220\,\mu m^2/sec$

fast on microscopic length scales: $\Delta x \sim 1\,\mu m$ in $1\,ms$ $\quad \left(\sim \sqrt{6 \cdot 0.22\,\mu m^2}\right)$
slow on macroscopic —"— : $\Delta x \sim 1\,mm$ in $10^3\,sec \sim 1h$
$\Delta x \sim 1\,m$ in $10^9\,sec$ (years)

Protein / organelle diffusion
also slow on cellular scales ⟹ active transport!

Mathematical description: stochastic process of the particle's center of mass coordinate $X(t)$

Langevin-eq.: $$\boxed{\dot{X}(t) = \frac{1}{\gamma} F(X) + \sqrt{2D}\, \xi(t)} \quad (1), \qquad \langle \xi(t) \rangle, \quad \langle \xi(t)\xi(t') \rangle = \delta(t-t')$$

$\xi(t)$: Gaussian white noise

(or Ito stochastic differential eq.
$$\boxed{dX(t) = \frac{1}{\gamma} F(X) + \sqrt{2D}\, dw(t)} \quad (2), \qquad \langle dw(t) \rangle = 0, \quad \langle dw(t)\, dw(t') \rangle = \delta(t-t')\, dt\, dt'$$

γ = drag coeff.
F = force

n.b.: F = const: Brownian particle $\quad X(t) = \sqrt{2D} \int_0^t dw(t') + vt$
$v := F/\gamma$
$\rightsquigarrow \langle (X(t) - vt)^2 \rangle = 2Dt \qquad \langle X(t) \rangle = 0$

Note: $X(t)$ described by (1) or (2) is a *Markovian* stochastic process
i.e. it is fully characterized by $P(x,t \mid x_0 t_0)$, the prob. to find the particle at time t at position x if it was at t_0 at x_0

Markovian ⟹ Chapman-Kolmogorov eq. $$P(x,t \mid x_0, t_0) = \int_{-\infty}^{+\infty} dx'\, P(x,t \mid x',t')\, P(x',t' \mid x_0 t_0)$$

⟹ Fokker-Planck-eq. $$\boxed{\frac{\partial}{\partial t} P(x,t) = -\frac{1}{\gamma} \frac{\partial}{\partial x}\left[F(x)\, P(x,t)\right] + D \frac{\partial^2 P(x,t)}{\partial x^2}}$$

Derivation of the Fokker - Planck equation from the stochastic differential equation:

$$dx(t) = a(x,t)\,dt + b(x,t)\,dW_t\,, \qquad \langle dW_t\rangle = 0$$

$$\langle dW_t\, dW_{t'}\rangle = 0 \text{ f. } t\neq t'$$

$$\langle dW_t^2\rangle = dt$$

$$\left[W(t) = \int_0^t dt'\,\xi(t')\right]$$

$$\rightsquigarrow x(t) = x(t_0) + \int_{t_0}^{t} dt'\, a(x(t'),t') + \underbrace{\int_{t_0}^{t} dW(t')\, b(x(t'),t)}$$

Ito stochastic integral: Ito!

$$\int_{t_0}^{t} dW(t')\, G(t') = \lim_{n\to\infty} \sum_{i=1}^{n} G(t_{i-1})\left(W(t_i) - W(t_{i-1})\right)$$

Consider arbitrary function f of $x(t)$: $f[x(t)]$

$$\rightsquigarrow df[x(t)] = f[x(t)+dx(t)] - f[x(t)]$$

$$\stackrel{!}{=} f'[x(t)]\,dx(t) + \frac{1}{2} f''[x(t)]\,dx(t)^2 + \dots$$

$$\stackrel{!}{=} f'[x(t)]\left\{a(x(t),t)\,dt + b(x(t),t)\,dW(t)\right\} + \frac{1}{2} f''[x(t)]\, b^2(x(t),t)\,[dW(t)]^2 + \dots$$

$$\Rightarrow df[x(t)] = \left\{a(x(t),t))\, f'[x(t)] + \frac{1}{2}[b(x(t),t)]^2 f''[x(t)]\right\}dt + b[x(t),t]\, f'[x(t)]\, dW(t)$$

Ito's formula

$$\text{Now } \frac{d}{dt}\langle f[x(t),t]\rangle = \left\langle \frac{df[x(t),t]}{dt}\right\rangle = \frac{\langle df[x(t),t]\rangle}{dt}$$

$$= \left\langle a[x(t),t]\,\partial_x f + \frac{1}{2} b^2[x(t),t]\,\partial_x^2 f\right\rangle$$

(note $\langle dW(t)\rangle = 0$

On the other hand

$$\frac{d}{dt}\langle f[x(t),t]\rangle = \frac{d}{dt}\int dx\, f(x)\, p(x,t\,|\,x_0,t_0)$$

$$\stackrel{!}{=} \int dx\, f(x)\,\partial_t p(x,t\,|\,x_0,t_0)$$

$$\text{and } \frac{d}{dt}\langle f[x(t),t]\rangle = \int dx\left\{a(x,t)\,\partial_x f + \frac{1}{2} b^2(x,t)\,\partial_x^2 f\right\}\cdot p(x,t\,|\,x_0,t)$$

int. by parts

$$= \int dx\, f(x)\left\{-\partial_x\, a(x,t)\, p(x,t\,|\,x_0,t) + \frac{1}{2}\partial_x^2\, b^2(x,t)\, p(x,t\,|\,x_0,t_0)\right\}$$

Since f is arbitrary, it follows

Fokker-Planck eq.

$$\boxed{\partial_t p(x,t\,|\,x_0,t_0) = -\partial_x\left\{a(x,t)\, p(x,t\,|\,x_0,t_0)\right\} + \frac{1}{2}\partial_x^2\left\{b^2(x,t)\, p(x,t\,|\,x_0,t_0)\right\}}$$

Remarks:

- $F=0$ Diffusion eq. $\frac{\partial p}{\partial t} = D\frac{\partial^2 p}{\partial x^2}$
- $D=0$ deterministic, Liouville-eq. $p(x,t) = \int_{-\infty}^{+\infty} dx_0\, p(x_0)\, \delta(x - \phi(t,x_0))$ initial distr.   solution of $\dot{x} = F(x)/\gamma$
- equilibrium: Einstein relation $D\gamma = k_B T$
  $\Rightarrow$ temperature T $\rightsquigarrow$ stationary distribution $p(x) = \frac{1}{Z}e^{-u(x)/k_BT}$, $u' = F$
- FP written as continuity eq. $\frac{\partial p}{\partial t} = -\frac{\partial J}{\partial x}$ (conservation of probability)
  with probability flux $J = \frac{1}{\gamma}F(x)p(x,t) - D\frac{\partial p(x,t)}{\partial x}$
- spherical particle moving in a fluid w. viscosity η:
  Stokes relation: $\gamma = 6\pi\eta R$. E.g. $R = 10^{-9}$m, $\eta_{water} = 10^{-3}\frac{kg}{m\,sec}$
  $\rightsquigarrow D \approx 100\,\mu m^2/sec$
- 3d: $\frac{\partial p}{\partial t} = -\frac{1}{\gamma}\operatorname{div}(\underline{F}\cdot p) + D\Delta p$
- N non-interacting particle, $u(x,t) = N\cdot p(x,t)$ particle concentrations
  $\rightsquigarrow$ FP eq. leads to Smoluchowski eq. $\dot{u} = -\frac{1}{\gamma}\nabla \underline{F} u + D\Delta u$.
- boundary conditions for FP-eq: $p(\underline{x},t) = f(\underline{x},t)$ Dirichlet
  $J(\underline{x},t)\cdot\underline{n}(\underline{x}) = g(\underline{x},t)$ (v. Neumann.
  $f=0$: absorbing b.c., $g=0$ reflecting b.c.
- Ornstein-Uhlenbeck process: $dX = -kX\,dt + \sqrt{2D}\,dW(t)$
  $\rightsquigarrow \langle X(t)\rangle = X_0 e^{-kt}$, $\langle [X(t) - \langle X(t)\rangle]^2\rangle = \frac{D}{k}(1 - e^{-2kt}) \xrightarrow[k\to 0]{} 2Dt$
  und $\langle X(t)X(t+s)\rangle = \frac{1}{2k}e^{-ks}$ f. $X_0 = 0$
- Multiplicative noise: $dX(t) = A(X)\,dt + B(X)\,dW(t)$
  $\Rightarrow$ solution leads to stochastic integrals of the form $\int_0^t dW(t)\,A(X(t)) =: I$
  if X and W would be deterministic $\rightsquigarrow I = \lim_{N\to\infty}\sum_{j=0}^{N} A([1-\alpha]X_j + \alpha X_{j+1})\Delta W_j$
  $\Delta W_j = W((j+1)\Delta t) - W(j\Delta t)$, $X_j = X(j\Delta t)$
  $\rightsquigarrow$ only for $\alpha = 0$ is A statistically independent of α $\leftarrow$ Ito definition
  $\alpha = \frac{1}{2}$: Stratonovic definition
  $\rightsquigarrow$ FP eq for Ito: $\frac{\partial p}{\partial t} = -\frac{\partial(Ap)}{\partial x} + \frac{1}{2}\frac{\partial^2(B^2 p)}{\partial x^2}$
  — " — Stratonovic — " — $+ \frac{1}{2}\frac{\partial}{\partial x}B(x)\frac{\partial}{\partial x}[B(x)p(x,t)]$

Smoluchowski reaction rate for diffusion-limited reactions

Consider $A + B \rightarrow AB$, and assume that A and B react immediately upon encounter: then k in $\frac{d[AB]}{dt} = k[A]\cdot[B]$ is limited by their encounter via diffusion

$\rightsquigarrow$ First passage process

$\leftarrow$ target domain Ω

$\partial\Omega$ absorbing boundary

$c(\underline{x}, t)$ = concentration of B molecules

$$\frac{\partial c}{\partial t} = D\,\Delta c\,, \quad c(\underline{r} \in \partial\Omega, t) = 0\,, \qquad (*)$$
$$c(\underline{r}, 0) = c_0\,, \quad c(\underline{r} \to \infty, t) = c_0$$

Flux through target boundary: $J = D \int_{\partial\Omega} \underline{\nabla} c \cdot d\underline{f}$

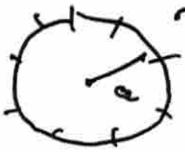
Solution of $(*)$ in 3d:

$$c(r,t) = c_0\left(1 - \frac{a}{r}\right) + \frac{a c_0}{r}\,\mathrm{erf}\left(\frac{r-a}{\sqrt{4Dt}}\right)$$

$$\rightsquigarrow J(t) = 4\pi a^2 D \left.\frac{\partial c}{\partial r}\right|_{r=a}$$
$$\overset{!}{=} 4\pi a D\left(1 + \frac{a}{\sqrt{\pi D t}}\right) \xrightarrow{t\to\infty} \underbrace{4\pi a D}_{=:k} c_0$$

$\Rightarrow$ Smoluchowski reaction rate: $k = 4\pi a D$, $[k] = \frac{m^3}{sec}$

Physics of chemoreception (Berg Purcell, 1977) ← read!



~ chemoreceptors on the surface of a bacterium

bacterium integrates signals over time τ_{avg}

for simplicity assume perfect absorber

⇝ # signaling mol. $N \sim a D c \tau_{avg}$ (Smoluchowski)
(bound receptors)

⇝ number fluctuations $\frac{\sqrt{N}}{N} \sim \frac{1}{\sqrt{a D c \tau_{avg}}}$

From the number of signaling molecules the cell estimates the ligand concentration c (note: $N = \frac{c}{c+K_d}$ in eq. ⇝ $c_* = \frac{N_*}{1-N_*} K_d$)

⇝ $\frac{\delta c}{c} \sim \frac{\delta N}{N} = \frac{1}{\sqrt{a D c \tau_{avg}}}$

c varies over distance Δl in a gradient $c \sim e^{-l/\lambda}$

$\Rightarrow \frac{\Delta c}{c}(l) \sim \Delta l / \lambda$

Condition for reliable signaling: $\frac{\delta c}{c} < \frac{\Delta c}{c}$

With $a \sim 1\,\mu m$, $D \sim 10^3\,\mu m^2/sec$, $c \sim 1\,mM = 6 \cdot 10^5/\mu m^3$, $\tau_{avg} \sim 1\,sec$
and $\lambda \sim 1\,cm = 10^4\,\mu m$:

$$\frac{\delta c}{c} = 10^{-4} < 10^{-4} \frac{\Delta l}{\mu m} \Rightarrow \Delta l = 1\,\mu m \text{ (length of E. coli)}$$
too small

if bacterium moves with $v \sim 10\,\mu m/sec$
and $\Delta t = \tau_{avg} \simeq 1\,sec \Rightarrow \Delta l = 10\,\mu m$
sufficient ✓

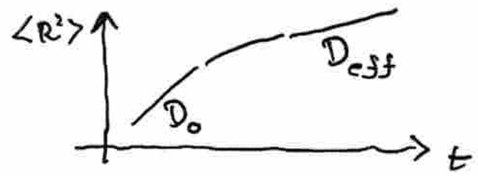
Anomalous diffusion

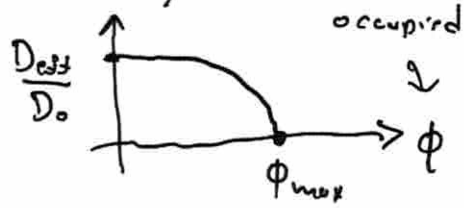
$\langle R^2 \rangle = 2d\, D\, t^{\alpha}$, $\alpha < 1$: subdiffusion
$\alpha > 1$: superdiffusion

With SPT methods subdiffusive behavior observed in cells / membranes

- molecular crowding (10-50% volume occupied)
- diffusion trapping ($\leftarrow$ multiple binding events)
- long time correlations (viscous environment $\to$ memory)

Diffusion in obstacle park:





Diffusion in dendrites $\to$ dendritic spines

simple 1d diffusion-trapping model of AMPA receptor trafficking:

$$\frac{\partial p}{\partial t} = D \frac{\partial^2 p}{\partial x^2} - \sum_{j=1}^{N} h \cdot (p_j - R_j / A)\, \delta(x - x_j)$$

$$\frac{\partial R_j}{\partial t} = \ell h\, (p_j - R_j / A)$$

R_j: prob. that receptor is trapped at j

$$\rightsquigarrow D_{eff} = \frac{D}{1 + A/\ell d}$$

ℓ dendrite circumference
A spine area
d inter-spine dist.
h effective hopping rate

Continuous-time random walk

$$R_n(\ell, t) = \sum_{\ell'} p(\ell - \ell') \int_0^t dt'\, \psi(t - t')\, R_{n-1}(\ell', t') \quad (*)$$

$\uparrow$ prob. to be at site ℓ at time t in n-th step

$p(\Delta\ell)$ = step-size distr.
$\psi(t)$ = waiting time distr.

$\psi(t) = \Lambda e^{-\Lambda t}$

$\Rightarrow$ normal diffusion

$$\frac{1}{\Lambda} \frac{dR_n}{dt} + R_n(\ell, t) \approx R_n(\ell, t + \epsilon) = \sum_{\ell'} p(\ell - \ell')\, R_{n-1}(\ell', t)$$

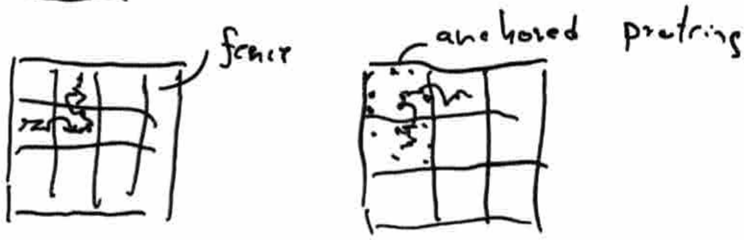
$\epsilon = \frac{1}{\Lambda} \ll 1$

general case: use Laplace trafo for $(*)$

$$\psi(t) \sim t^{-1-\beta} \Rightarrow \langle R^2(t) \rangle \sim t^{\beta}, \quad 0 < \beta < 1$$

Diffusion in the plasma membrane

picket-fence model:



Narrow escape problems

MFPT: $\tau(\underline{r})$ ← starting point

$$D\Delta\tau = -1 \qquad r \in \Omega$$

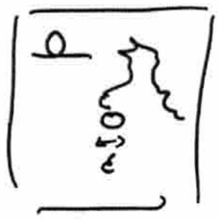
b.c.: $\tau(\underline{r}) = 0, \qquad \underline{r} \in \partial\Omega_{abs.}$

$\partial_n \tau(\underline{r}) = 0 \qquad \underline{r} \in \partial\Omega \setminus \partial\Omega_{abs}$

solution with Greens function method (↔ electrostatics)

$$\dots \rightsquigarrow \overline{\tau} = \frac{1}{D}\left\{ -\ln\varepsilon + \ln 2 + \frac{1}{8} \right\}$$ → Mangeat, HR

Diffusion to a small target

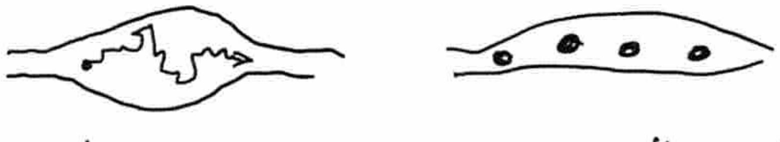


$$J(t) \simeq D|\Omega|\, \lambda_0 e^{-\lambda_0 D t}$$

$$\lambda_0 = \frac{2\pi\nu}{\Omega} + O(\nu^2)$$

$$\nu = -\frac{1}{\ln\varepsilon}$$ (time dependent reaction rate in 2d)

Membrane transport through nanopores & channels



- diffusion of ions or lipids through a narrow channel
- single-file diffusion (↔ ASEP)
- translocation of polymers through a pore

Run & tumble particle (simple model for active particle)

$$\partial_t P_+ = -v\partial_x P_+ + D\partial_x^2 P_+ + k(P_- - P_+)$$
$$\partial_t P_- = +v\partial_x P_- + D\partial_x^2 P_- + k(P_+ - P_-)$$

b.c.: $\mathcal{J}_\pm(-L,t) = \mathcal{J}_\pm(+L,t) = 0$, $\mathcal{J}_\pm = -DP_\pm' \pm vP_\pm$

stat. sol.: $DP_+'' - vP_+' + k(P_- - P_+) = 0$ $\Rightarrow DP_+' - vP_+\big|_{x=\pm L}$

$DP_-'' + vP_-' + k(P_+ - P_-) = 0$ $DP_-' + vP_-\big|_{x=\pm L}$

$$m = P_+ - P_-, \quad \rho = P_+ + P_-$$

$\rightsquigarrow$ $D\rho'' - vm' = 0$ (1)

$Dm'' - v\rho' - 2km = 0$ (2), b.c.: $D\rho' - vm|_{\pm L}$ bc1

$Dm' - v\rho|_{\pm L} = 0$

Int. (1): $D\rho' - vm = c_1 \underset{\text{bc1}}{\Rightarrow} c_1 = 0$

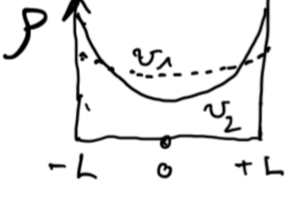
$\Rightarrow D\rho' = vm$

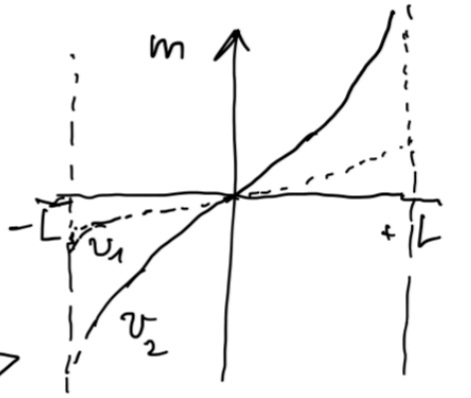
insert in (2): $Dm'' - \left(\frac{v^2}{D} + 2k\right)m = 0$

Solution: $m(x) = a e^{\mu x} + b e^{-\mu x}$, $\mu = \sqrt{\frac{v^2}{D^2} + \frac{2k}{D}}$

$\overset{\rightsquigarrow}{\rho' = \frac{v}{D}m}$ $\rho(x) = \frac{v}{D\mu}\left(ae^{\mu x} - be^{-\mu x}\right) + c_2$

.... $\rightsquigarrow$ $\rho(x) \propto \cosh(\mu x) + c$, $m(x) \propto \sinh(\mu x)$ [Malakar et al, JSTAT 043215 (2018)]





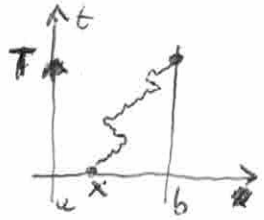
$v_2 > v_1$

wall accumulation

The same mechanism leads to wall and corner accumulation of particles in systems of active Brownian particles (ABP).

Also MIPS (motility induced phase separation)


First passage time (FPT) (example: homogeneous process, i.e. TTI)



$T =$ "exit time" or FPT

a, b absorbing boundaries, x = starting point at $t=0$

Distribution of exit times:

Prob. that at time t the particle is still in $[a,b]$:

$$G(x,t) = \int_a^b dx'\, p(x't \mid x,0) = \text{Prob}(T \geq t)$$

Backward FP eq: $\frac{\partial p(x,t \mid y t')}{\partial t'} = A(y,t) \frac{\partial p(x,t \mid y t')}{\partial y} - \frac{1}{2} B(y,t) \frac{\partial^2 p(x,t \mid y,t')}{\partial y^2}$

hom. process: $p(x',t \mid x,0) = p(x',0 \mid x,-t)$, A, B time indep.

$$\Rightarrow \frac{\partial}{\partial t} p(x't \mid x,0) = \frac{\partial}{\partial t} p(x',0 \mid x,-t) = -\frac{\partial}{\partial(-t)} p(x',0 \mid x,-t)$$

$$\underset{\text{BFP}}{=} A(x) \frac{\partial}{\partial x} p(x',t \mid x,-t) + \frac{1}{2} B(x) \frac{\partial^2 p(x't \mid x,-t)}{\partial^2 x}$$

$$\Rightarrow \boxed{\frac{\partial}{\partial t} G(x,t) = A(x) \frac{\partial}{\partial x} G(x,t) + \frac{1}{2} B(x) \frac{\partial^2}{\partial x^2} G(x,t)} \quad (3)$$

initial cond.: $p(x',0 \mid x,0) = \delta(x-x')$; boundary cond. $x=a$ or $b \rightsquigarrow$ absorb.

~~boundary condition~~ $G(x,0) = \begin{cases} 1 & a \leq x \leq b \\ 0 & \text{elsewhere} \end{cases}$ $\rightsquigarrow G(a,t) = G(b,t) = 0$

Moments of the exit time: $\langle f(T) \rangle =$

$$\text{Prob}(T \in [t, t+dt]) = \text{Prob}(T \geq t) - \text{Prob}(T \geq t+dt)$$

$$\overset{!}{=} -\{G(x,t+dt) - G(x,t)]$$

$$\overset{!}{=} -\partial_t G(x,t) \cdot dt$$

$$\Rightarrow \langle T \rangle \overset{\text{MFPT}}{=} T(x) = -\int_0^\infty t\, \partial_t G(x,t) = \int_0^\infty G(x,t)\, dt \quad (3a)$$

$$T_n(x) = \int_0^\infty t^{n-1} G(x,t)\, dt$$

Differential eq. for G: integrate (3) from 0 to ∞:

$$\int_0^\infty dt\, \partial_t G(x,t) = G(x,\infty) - G(x,0) = -1$$

$$\underset{(3)}{\Rightarrow} \boxed{-1 = A(x) \frac{\partial}{\partial x} T(x) + \frac{1}{2} B(x)\, \partial_x^2 T(x)} \quad \text{with (3a)}$$

Example: 1) pure diffusion: $A = 0$, $B = 2D$ $\rightsquigarrow$ $D T'' = -1$

$$\rightsquigarrow T(x) = \frac{x(L-x)}{2D}, \quad T\left(\frac{L}{2}\right) = \frac{L^2}{8D}$$

2) exiting at 0, reflecting at L:

$$T_0(x) = \frac{x(2L-x)}{2D} \underset{x=L}{=} \frac{L^2}{2D}$$

Master eq.

$$P(S\,t+dt\,|\,S_1 t_0) = P(S\,t\,|\,S_1 t_0) - \sum_{S'} \alpha(S'S)\,P(S\,t\,|\,S_1 t_0) + \sum_{S'} \alpha(S S')\,P(S'\,t\,|\,S_1 t_0)$$

$dt \to 0$:

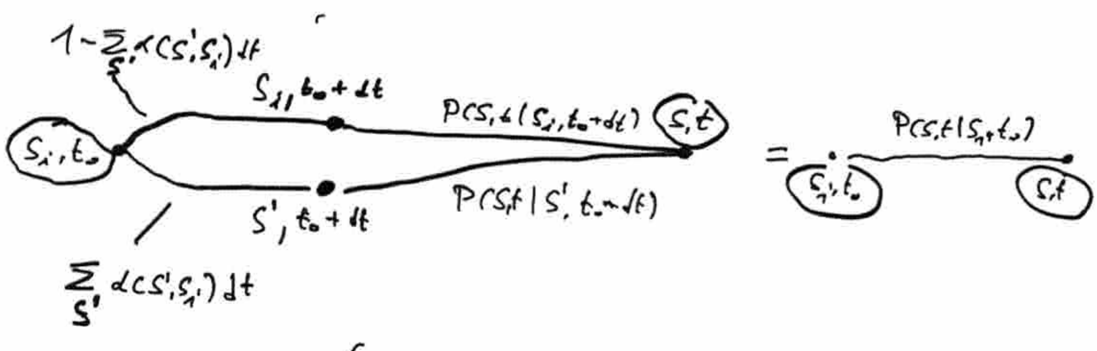
$$\boxed{\partial_t P(S\,t\,|\,S_1 t_0) = \sum_{S'} \left\{ \alpha(SS')\,P(S'\,t\,|\,S_1 t_0) - \alpha(S'S)\,P(S\,t\,|\,S_1 t_0) \right\}}$$

$$= \sum_{S'} M_f(SS')\,P(S'\,t\,|\,S_1 t_0)$$

w. $M_f(SS') = \alpha(S,S') - \delta_{SS'} \sum_{S''} \alpha(S''S)$

Def. $M_b(SS') := \alpha(S'S) - \delta_{SS'} \sum_{S''} \alpha(S''S)$

Backward Master eq



$\rightsquigarrow$ $P(S,t\,|\,S_1 t_0) = \left(1 - \sum_{S'} \alpha(S'_1 S_1)\,dt\right) P(S,t\,|\,S_1, t_0+dt) + \sum_{S'} \alpha(S'_1 S_1)\,dt\; P(S,t\,|\,S'_1 t_0+dt)$

$\rightsquigarrow$ $P(S,t\,|\,S_1 t_0) - P(S,t-dt\,|\,S_1 t_0)$

$$= -\sum_{S'} \alpha(S' S_1)\,dt\; P(S,t-dt\,|\,S_1 t_0) + \sum_{S'} \alpha(S' S_1)\,dt\; P(S\,t-dt\,|\,S_1 t_0)$$

$\rightsquigarrow$ $\partial_t P(S,t\,|\,S_1 t_0) = -\sum_{S'} \alpha(S'_1 S_1)\,P(S\,t\,|\,S_1 t_0) + \sum_{S'} \alpha(S' S_1)\,P(S\,t\,|\,S' t_0)$

$$S(S_F, t \mid S_i, t_0) = \sum_{S \neq S_F} P(S, t \mid S_i, t_0)$$

= Survival prob up to time t iff $\alpha(S, S_F) = 0 \quad \forall S \neq S_F$

$$\partial_t S(S_F t \mid S_i t_0) = \sum_{S \neq S_F} \partial_t P(S_F t \mid S_i t_0)$$

$$= \sum_{S \neq S_F} \sum_{S'} \{\alpha(S' S_i) P(S_F t \mid S' t_0) - \alpha(S' S_i) P(S t \mid S_i t_0)\}$$

$$= \sum_{S'} \{\alpha(S' S_i) S(S_F t \mid S' t_0) - \alpha(S' S_i) S(S_F t \mid S_i t_0)\}$$

$$FPT(S_F t \mid S_i t_0) = -\partial_t S(S_F t \mid S_i t_0)$$

$$\Rightarrow \partial_t FPT(S_F t \mid S_i t_0) = -\partial_t \{\partial_t S(S_F t \mid S_i t_0)\}$$

$$= \sum_{S'} \{\alpha(S' S_i) FPT(S_F t \mid S_i t_0) - \alpha(S S_i) FPT(S_F t \mid S' t_0)\}$$

$$= \sum_{S'} M_b(S_i, S') FPT(S_F t \mid S' t_0)$$

$$M_b(S S') = \alpha(S' S) - \delta_{SS'} \sum_{S''} \alpha(S'' S)$$

$$T(S_F \mid S_i) = \int_0^\infty d\tau\, \tau\, FPT(S_F \tau \mid S_i) \quad (t_0 = 0)$$

$$\Rightarrow \sum_{S'} M_b(S_i, S') T(S_F \mid S')$$

$$= \int_0^\infty d\tau\, \tau \sum_{S'} M_b(S_i, S') FPT(S_F \tau \mid S')$$

$$= \int_0^\infty d\tau\, \tau\, \partial_\tau FPT(S_F \tau \mid S_i)$$

$$= -\int_0^\infty d\tau\, FPT(S_F \tau \mid S_i)$$

$$= +\int_0^\infty d\tau\, \partial_\tau S(S_F \tau \mid S_i)$$

$$= \underbrace{S(S_F \infty \mid S_i)}_{=0} - \underbrace{S(S_F 0 \mid S_i)}_{=1}$$

$$= -1$$

$$\Rightarrow \underline{\underline{M}}_b \underline{T} = -\underline{1} \qquad T = (T(S_F \mid S_1'), \ldots, T(S_F \mid S_N'))^T$$

Example: Random walk

$$\dot{p}_n(t) = \frac{r}{2} p_{n-1}(t) + \frac{r}{2} p_{n+1}(t) - r\, p_n(t)$$

$$\rightsquigarrow \quad M_f(n,n') = \frac{r}{2}\delta_{n,n'+1} + \frac{r}{2}\delta_{n,n'-1} - r\,\delta_{n,n'}$$

absorbing at $n=0$ and $n=L$:

$$\text{b.c.} \quad p_0(t) = p_L(t) = 0$$

start at n: $\quad \text{MFPT}(n \to \text{exit}) =: \tau_n$

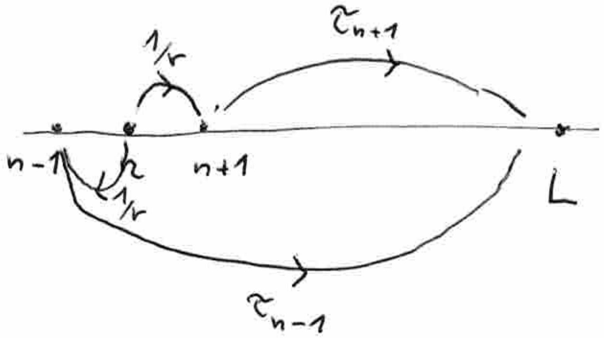
Backward eq: $\quad M_b = M_f^T = M_f$

$$\frac{r}{2}\tau_{n-1} + \frac{r}{2}\tau_{n+1} - r\tau_n = -1$$

$$\tau_0 = \tau_L = 0$$

Solution: $\quad \tau_n = \dfrac{n(L-n)}{r}$

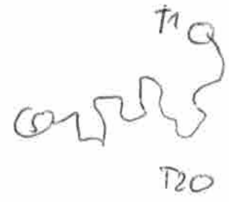
heuristics:



$$\tau_n = \frac{1}{2}\left(\frac{1}{r} + \tau_{n-1}\right) + \frac{1}{2}\left(\frac{1}{r} + \tau_{n+1}\right)$$

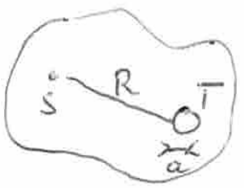
$$\Leftrightarrow \quad \frac{r}{2}\tau_{n-1} + \frac{r}{2}\tau_{n+1} - r\tau_n = -1$$

First passage problems:

 generic,  discrete,  two targets

MFPT of Brownian motion in confined geometries

first exit or narrow escape



1D $\langle T\rangle \sim \frac{L^2}{D}$

2D $\langle T\rangle \sim \frac{A}{2\pi D}\ln\frac{R}{a}$ Condamin et al PRL 2005

3D $\langle T\rangle \sim \frac{V}{4\pi D}\left(\frac{1}{a}-\frac{1}{R}\right)$

Note: Search time for Brownian motion in 2and 3d is always inv. prop to D and prop. to search area A, V

Accelerated search → "Enhanced reaction kinetics in biological cells" or "intermittent search"

Two mobility modes: 1) diffusive w. diff. const D

2) ballistic, constant velocity v in direction Ω

transition $1\to2$ w. rate $\frac{1}{\tau_1}$, $2\to1$ w. rate $\frac{1}{\tau_2}$

$P_0(\underline{r},t)$ = prob. f. particle in diffusive state at position $\underline{r}$ at time t

$P_\Omega(\underline{r},t)$ = —— " —— ballistic state w. direction Ω —— " ——

$$\rightsquigarrow \frac{\partial}{\partial t}P_0(\underline{r},t) = D\Delta P_0(\underline{r},t) - \frac{1}{\tau_1}P_0(\underline{r},t) + \frac{1}{\tau_2}\int d\Omega\, P_\Omega(\underline{r},t)$$

$$\frac{\partial}{\partial t}P_\Omega(\underline{r},t) = -\nabla(\underline{v}_\Omega P_\Omega(\underline{r},t)) + \frac{1}{\tau_1}\rho_\Omega(\underline{r})P_0(\underline{r},t) - \frac{1}{\tau_2}P_\Omega(\underline{r},t)$$

$\rho_\Omega(\underline{r})$ = prob. to move in direction Ω after $1\to2$ transition

$\hat{=}$ density of cytoskeleton filaments in direction Ω Schwarz, HR PRL 2016

fully disordered: $\rho_\Omega(\underline{r}) = \frac{1}{4\pi}$

Spherical target of radius a at position $\underline{r}'$

Def. $T_1(\underline{r})$ = MFPT starting in 1 at position $\underline{r}$

$T_2(\underline{r},v)$ = MFP starting in state 2 at pos. $\underline{r}$ w. veloc. $\underline{v}$

$\rightsquigarrow$ Backward eq. $D\Delta T_1 + \frac{1}{\tau_1}\int d\Omega\,\rho_\Omega(\underline{r})(T_2-T_1) = -1$

$$\underline{v}\cdot\nabla T_2 - \frac{1}{\tau_2}(T_2-T_1) = -1$$

w. absorb. b.c. on target surf

In 1d:
$$\frac{\partial}{\partial t} P_0(x,t) = D \frac{\partial^2}{\partial x^2} P_0(x,t) - \frac{1}{\tau_1} P_0(x,t) + \frac{1}{\tau_2} \left(P_+(x,t) + P_-(x,t) \right)$$
$$\frac{\partial}{\partial t} P_+(x,t) = -v \frac{\partial}{\partial x} P_+(x,t) + \frac{1}{2\tau_1} P_0(x,t) - \frac{1}{\tau_2} P_+(x,t)$$
$$\frac{\partial}{\partial t} P_-(x,t) = +v \frac{\partial}{\partial x} P_-(x,t) + \frac{1}{2\tau_1} P_0(x,t) - \frac{1}{\tau_2} P_-(x,t)$$

Backward eq. see Bénichou et al PRL 94, 198101 (2005)

Limits $L \gg v\tau_2, \sqrt{D\tau_1}, \sqrt{D\tau_1 / v\tau_2}$

$$\leadsto \langle T \rangle = \frac{L}{2\sqrt{D}} (\tau_1 + \tau_2) \frac{\tilde{\tau}/\tau_2^2 + 2/\tau_1}{\sqrt{\tilde{\tau}/\tau_2^2 + 4/\tau_1}} \quad \propto L \, !$$

$\tilde{\tau} = D/v^2$

In all dimensions pronounced minima, much smaller than $\langle T \rangle_0$ for purely diffusive search

Cell cytoskeleton is spatially inhomogeneous:

$$\leadsto \rho_\Omega(\underline{r}) = \begin{cases} p\, \delta_{\Omega,\Omega'(\underline{r})} + q\, \delta_{\Omega,\Omega'(-\underline{r})} & \text{in } C \\ 1/4\pi & \text{in } P(\Delta) \end{cases}$$

P = periphery (actin cortex) thickness Δ

C = center astral MT filaments

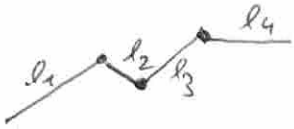
MFPT has pronounced minimum at $\frac{\Delta}{R} < 0.2$

$\leadsto$ <u>thin</u> actin cortex advantageous

(also for narrow escape problem <-> immunological synapse)

Schwarz, HR PRL 2016

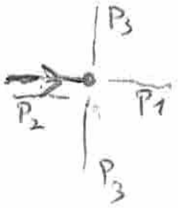
Hafner HR Biophys. J. 2018

Persistent random walk: 

exponential distribution of the length of ballistic excursions

$P(l) \sim e^{-\alpha l / l_p}$, l_p = persistence length.

⇝ lattice random walk with memory of the last step:



p_1 prob. to continue in the same direction $= p_3 + \epsilon$

p_2 prob. to go backwards $= p_3 - \delta$ $(\delta = 0)$

p_3 prob. to go orthogonal

Norm.: $2d\, p_3 + \epsilon - \delta = 1$

⇝ $P(l) = (1-p_1)\, p_1^{\,l-1}$

⇝ $l_p = \sum_{l=1}^{\infty} l\, P(l) = \frac{1}{1-p_1} = \frac{2d/(2d-2)}{1-\epsilon} \xrightarrow[\epsilon \to 1]{} \infty$

⇝ position process is non-Markovian (memory of the last direction)

but joint process of position and velocity of the searcher is Markovian!

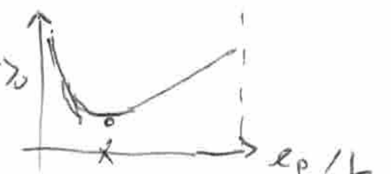
⇝ Master eq. (= FP on lattice) for $P(\underline{r}, \underline{e}_i, t)$

$$P(\underline{r}, \underline{e}_i, t+1) = p_1\, P(\underline{r}-\underline{e}_i, \underline{e}_i, t) + p_2\, P(\underline{r}+\underline{e}_i, -\underline{e}_i, t) + p_3 \sum_{\pm \underline{e}_j,\, j \neq i} P(\underline{r}-\underline{e}_j, \underline{e}_j, t)$$

⇝ Backward eq. for MFPT $T(\underline{r}, \underline{e}_i)$

$$T(\underline{r}, \underline{e}_i) = p_1\, T(\underline{r}+\underline{e}_i, \underline{e}_i) + p_2\, T(\underline{r}-\underline{e}_i, -\underline{e}_i) + p_3 \sum_{\pm \underline{e}_j,\, j \neq i} T(\underline{r}-\underline{e}_j, -\underline{e}_j) + 1$$

solvable via Fourier-trafo:

⇝ 

optimal persistence length.

[Tejedor et al, PRL 2012]

Random walk with n-step memory: $\langle T \rangle$ also computable

⇝ optimizable for $n = 1, 2, 3$ Meyer, HR PR 2022

Statphys of Ligand-receptor binding

L ligands, Ω lattice sites, 1 receptor

	energy	multiplicity	weight
unbound	$L\varepsilon_{sol}$	$\Omega! / L!(\Omega-L)! \approx \frac{\Omega^L}{L!}$	$\frac{\Omega^L}{L!} e^{-\beta L \varepsilon_{sol}}$
bound	$(L-1)\varepsilon_{sol} + \varepsilon_b$	$\Omega! / (L-1)!(\Omega-L+1)! \approx \frac{\Omega^{L-1}}{(L-1)!}$	$\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta\{(L-1)\varepsilon_{sol}+\varepsilon_b\}}$

$$\rightsquigarrow p_{bound} = \frac{e^{-\beta\varepsilon_b} \frac{\Omega^{L-1}}{(L-1)!} e^{-\beta(L-1)\varepsilon_{sol}}}{\frac{\Omega^L}{L!} e^{-\beta L\varepsilon_{sol}} + e^{-\beta\varepsilon_b}\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta(L-1)\varepsilon_{sol}}}$$

$$= \frac{\frac{L}{\Omega} e^{-\beta\Delta\varepsilon}}{1 + \frac{L}{\Omega} e^{-\beta\Delta\varepsilon}}, \quad \boxed{\Delta\varepsilon = \varepsilon_b - \varepsilon_{sol}}$$

Def: $c = L/V_{box}$, $c_0 = \Omega/V_{box}$ $\rightsquigarrow \frac{L}{\Omega} = \frac{c}{c_0}$

$$\rightsquigarrow p_{bound} = \frac{c/c_0\, e^{-\beta\Delta\varepsilon}}{1 + \frac{c}{c_0} e^{-\beta\Delta\varepsilon}} = \frac{c}{c + K_d}, \quad \boxed{K_d = c_0 e^{\beta\Delta\varepsilon}}$$

(typ. $c_0 = 1M$ if elementary voxel $\sim 1\,nm^3$)

Free energy: $F = E - TS$

$$\rightsquigarrow F(L) = L\varepsilon_{sol} - k_B T \ln \frac{\Omega!}{L!(\Omega-L)!}$$

$$\uparrow \approx \frac{\Omega^L}{L!}$$

chemical potential

$$\mu = F(L) - F(L-1)$$

$$= L\varepsilon_{sol} - k_BT\ln\frac{\Omega^L}{L!} - (L-1)\varepsilon_{sol} + k_BT\ln\frac{\Omega^{L-1}}{(L-1)!}$$

$$= \varepsilon_{sol} + k_BT\ln\frac{L}{\Omega}$$

$$= \varepsilon_{sol} + k_BT\ln\frac{c}{c_0} \rightsquigarrow e^{\beta\mu} = \frac{c}{c_0} e^{\beta\varepsilon_{sol}}$$

$$\Rightarrow p_{bound} = \frac{\frac{c}{c_0} e^{-\beta\Delta\varepsilon}}{1 + \frac{c}{c_0} e^{-\beta\Delta\varepsilon}} = \frac{e^{-\beta(\varepsilon_b-\mu)}}{1 + e^{-\beta(\varepsilon_b-\mu)}}$$

Simple MWC molecule: active / inactive state

	active energy	weight	inactive energy	weight
unbound	ϵ_A	$e^{-\beta\epsilon_A}$	ϵ_I	$e^{-\beta\epsilon_I}$
bound	$\epsilon_A+\epsilon_b^A-\mu$	$e^{-\beta\epsilon_A}\,e^{-\beta(\epsilon_b^A-\mu)}$	$\epsilon_I+\epsilon_b^I-\mu$	$e^{-\beta\epsilon_I}\,e^{-\beta(\epsilon_b^I-\mu)}$

$$\Rightarrow P_{active}(c) = P_{A,0}(c) + P_{A,b}(c) = \frac{e^{-\beta\epsilon_A}\left(1+\frac{c}{c_0}e^{-\beta\Delta\epsilon_b^A}\right)}{e^{-\beta\epsilon_A}\left(1+\frac{c}{c_0}e^{-\beta\Delta\epsilon_b^A}\right) + e^{-\beta\epsilon_I}\left(1+\frac{c}{c_0}e^{-\beta\Delta\epsilon_b^I}\right)}$$

$$\rightsquigarrow P_{active} = \frac{e^{-\beta\epsilon_A}(1+c/K_A)}{e^{-\beta\epsilon_A}\left(1+\frac{c}{K_A}\right) + e^{-\beta\epsilon_I}\left(1+\frac{c}{K_I}\right)}$$

Cooperativity in the MWC model → needs more than 1 binding site

	active	inactive
[ ]	$e^{-\beta\epsilon_A}$	$e^{-\beta\epsilon_I}$
[X]	$e^{-\beta\epsilon_A}\,e^{-\beta(\epsilon_b^A-\mu)}$	$e^{-\beta\epsilon_I}\,e^{-\beta(\epsilon_b^I-\mu)}$
[X]	— " —	— " —
[X X]	$e^{-\beta\epsilon_A}\,e^{-\beta(2\epsilon_b^A-2\mu)}$	$e^{-\beta\epsilon_I}\,e^{-\beta(2\epsilon_b^I-\mu)}$

$$Z_A = e^{-\beta\epsilon_A}\left(1+e^{-\beta(\epsilon_b^A-\mu)}\right)^2 = e^{-\beta\epsilon_A}(1+c/K_A)^2$$

$$Z_I = e^{-\beta\epsilon_I}\left(1+e^{-\beta(\epsilon_b^I-\mu)}\right)^2 = e^{-\beta\epsilon_I}(1+c/K_I)^2$$

with $e^{\beta\mu} = \frac{c}{c_0}e^{\beta\epsilon_{sol}}$, $K = c_0 e^{\beta\Delta\epsilon}$, $\Delta\epsilon = \epsilon_b - \epsilon_{sol}$

$$\rightsquigarrow P_{active} = \frac{Z_A}{Z_A+Z_I} = \frac{e^{-\beta\epsilon_A}\left(1+\frac{c}{K_A}\right)^2}{e^{-\beta\epsilon_A}\left(1+\frac{c}{K_A}\right)^2 + e^{-\beta\epsilon_I}\left(1+\frac{c}{K_I}\right)^2}$$

Generalization to n binding sites:

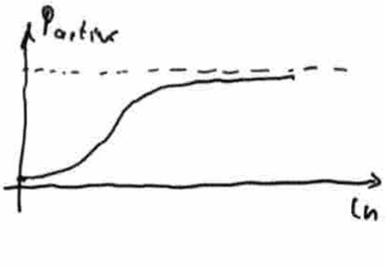
$$\boxed{P_{active}(c) = \frac{e^{-\beta\epsilon_A}\left(1+\frac{c}{K_A}\right)^n}{e^{-\beta\epsilon_A}\left(1+\frac{c}{K_A}\right)^n + e^{-\beta\epsilon_I}\left(1+\frac{c}{K_I}\right)^n}}$$

$$= \frac{(1+x)^n}{(1+x)^n + L(1+fx)^n} \qquad x = \frac{c}{K_A}, \quad L = e^{-\beta(\epsilon_I-\epsilon_A)}, \quad f = K_A/K_I$$

$$P_{active}(c=0) = \frac{1}{1+L} \quad \text{"leakiness"}$$

$$P_{active}(c\to\infty) = \frac{1}{1+f^n L} \quad \text{"saturation"}$$

e.g. $n=2$, $f=10^{-2}$, $L=10$



Law of mass action: $R + L \overset{K_d}{\rightleftarrows} LR$

$$\rightsquigarrow \quad \underset{\propto e^{-\beta\varepsilon_{sol}}}{[R][L]} = K_d \underset{\propto e^{-\beta\varepsilon_b}}{[LR]} \quad \text{or} \quad \frac{[LR]}{[L][R]} = \frac{1}{K_d}$$

receptors fixed

$[R] + [LR] = R_{tot}$

$$\rightsquigarrow K_d = e^{\beta(\varepsilon_b - \varepsilon_{sol})}$$

$$\rightsquigarrow \frac{[LR]}{(R_{tot} - [LR])\,[L]} = \frac{1}{K_d}$$

$$\rightsquigarrow \frac{[LR]}{[R_{tot}]} = \frac{[L]}{[L] + K_d}$$

MWC model f. $n = 2$:

$$\begin{tikzcd}
T_0 + L \arrow[r, leftrightarrow, "2\tilde{K}_T"] \arrow[d, leftrightarrow, "Y_0"] & T_1 + L \arrow[r, leftrightarrow, "\tilde{K}_T/2"] \arrow[d, leftrightarrow, "Y_1"] & T_2 \arrow[d, leftrightarrow, "Y_2"] \\
R_0 + L \arrow[r, leftrightarrow, "2\tilde{K}_R"] & R_1 + L \arrow[r, leftrightarrow, "\tilde{K}_R/2"] & R_2
\end{tikzcd}$$

$$\frac{[R_i]}{[T_i]} = Y_i$$

$$\frac{[T_1]}{[L][T_0]} = 2\tilde{K}_T, \quad \frac{[R_1]}{[L][R_0]} = 2\tilde{K}_R \qquad Y_0 = \frac{e^{-\beta\varepsilon_R}}{e^{-\beta\varepsilon_T}},$$

$$\frac{[T_2]}{[L][T_1]} = \tilde{K}_T/2, \quad \frac{[R_2]}{[L][R_1]} = \tilde{K}_R/2 \qquad \tilde{K}_T = \frac{1}{K_T}$$

$$\tilde{K}_R = \frac{1}{K_R}$$

$$\rightsquigarrow [T_1] = 2\tilde{K}_T [L][T_0]$$

$$[T_2] = \frac{\tilde{K}_T}{2}[L][T_1] = (\tilde{K}_T[L])^2 [T_0]$$

$$[R_1] = 2\tilde{K}_R [L][R_0]$$

$$[R_2] = (\tilde{K}_R[L])^2 [R_0]$$

$$p_{active} = \frac{[R_0] + [R_1] + [R_2]}{[R_0] + [R_1] + [R_2] + [T_0] + [T_1] + [T_2]}$$

$$= \frac{[R_0](1 + 2\tilde{K}_R[L] + (\tilde{K}_R[L])^2)}{[R_0](1 + 2\tilde{K}_R[L] + (\tilde{K}_R[L])^2) + [T_0](1 + 2\tilde{K}_T[L] + (\tilde{K}_T[L])^2)}$$

$$= \frac{Y_0(1 + \tilde{K}_R[L])^2}{Y_0(1 + \tilde{K}_R[L])^2 + (1 + \tilde{K}_T[L])^2}$$

$$= \frac{e^{-\beta\varepsilon_R}(1 + c/K_R)^2}{e^{-\beta\varepsilon_R}(1 + \frac{c}{K_R})^2 + e^{-\beta\varepsilon_T}(1 + c/K_T)^2}$$

Example for time-dependence

$$\frac{dP_0}{dt} = -kP_0 \quad \rightsquigarrow \quad P_0(t) = k e^{-kt}$$

$$\left(\int_0^\infty d\tau \, P_0(t) = 1 \right)$$

$$\rightsquigarrow \quad \langle t \rangle = 1/k$$

$$\langle t^2 \rangle = 2/k^2$$

$$\mu = 1/k$$

$$\sigma = \sqrt{\langle t^2 \rangle - \langle t \rangle^2} = 1/k$$

$$\rightsquigarrow \quad \sigma/\mu = 1$$

$$\frac{1}{k} \langle t \rangle = \int_0^\infty d\tau \, \tau e^{-k\tau}$$

$$= -\frac{1}{k} \tau e^{-kt} \Big|_0^\infty + \frac{1}{k} \int_0^\infty d\tau \, e^{-k\tau}$$

$$= -\frac{1}{k^2} e^{-kt} \Big|_0^\infty$$

$$= 1/k^2$$

$$\langle t^2 \rangle = k \int_0^\infty d\tau \, \tau^2 e^{-k\tau}$$

$$= k \frac{\partial^2}{\partial k^2} \underbrace{\int_0^\infty d\tau \, e^{-k\tau}}_{1/k}$$

$$= k \left[\frac{2}{k^3} \right] = 2/k^2$$

$$R_0 \xrightarrow{k_0} R_1 \xrightarrow{k_1} R_2 \longrightarrow \ldots R_{n-1} \xrightarrow{k_{n-1}} \text{reaction}$$

$$\dot{P}_0 = -k_0 P_0$$

$$\dot{P}_1 = k_0 P_0 - k_1 P_1$$

$$\dot{P}_2 = k_1 P_1 - k_2 P_2$$

$$\vdots$$

$$\tilde{P}_n(\omega) = \int_{-\infty}^{+\infty} dt \, e^{-i\omega t} P_n(t)$$

$$\int_{-\infty}^{+\infty} dt \, \dot{P}_n(t) e^{-i\omega t} = P_n(t) e^{-i\omega t} \Big|_{-\infty}^{+\infty} + \int_{-\infty}^{+\infty} dt \, i\omega P_n(t) e^{-i\omega t}$$

$$= i\omega \tilde{P}_n(\omega)$$

$$\tilde{P}_0(\omega) = \frac{1}{k_0 + i\omega} \quad \rightsquigarrow \quad P_0(t) = \int_{-\infty}^{+\infty} \frac{d\omega}{2\pi} \frac{e^{i\omega t}}{k_0 + i\omega}$$

$$k_0 + i\omega = 0 \quad \rightsquigarrow \quad \omega = ik_0$$

$$i\omega \tilde{P}_1(\omega) = \frac{k_0}{k_0 + i\omega} - k_1 \tilde{P}_1(\omega)$$

$$\tilde{P}_1(\omega) = \frac{k_0}{(k_0 + i\omega)(k_1 + i\omega)}$$

$$\vdots$$

$$P_{n-1}(\omega) = \frac{k_0 \cdot k_1 \cdot \ldots \cdot k_{n-2}}{(k_0 + i\omega)(k_1 + i\omega) \cdot \ldots \cdot (k_{n-1} + i\omega)}$$

$$\forall i: \; k_i = k$$

$$P_{n-1}(t) = \int_{-\infty}^{+\infty} \frac{d\omega}{2\pi} \frac{k^{n-1}}{(k + i\omega)^n} e^{i\omega t} = \frac{k^n}{(n-1)!} t^{n-1} e^{-kt}$$

$$\left(\int_0^\infty dt \, t^{n-1} e^{-kt} = \frac{(n-1)!}{k^n} \right)$$

$$\langle t \rangle_{n-1} = \frac{k^n}{(n-1)!} \int_0^\infty d\tau \, \tau \cdot \tau^{n-1} e^{-k\tau} = \frac{k^n}{(n-1)!} \cdot \frac{n!}{k^{n+1}} = \frac{n}{k}$$

$$\langle t^2 \rangle_{n-1} = \frac{k^n}{(n-1)!} \int_0^\infty d\tau \, \tau^{n+1} e^{-k\tau} = \frac{k^n}{(n-1)!} \cdot \frac{(n+1)!}{k^{n+2}} = \frac{n(n+1)}{k^2}$$

$$\sigma = \sqrt{\langle t^2 \rangle - \langle t \rangle^2} = \frac{\sqrt{n}}{k}$$

$$\rightsquigarrow \quad \frac{\sigma}{\mu} = \frac{1}{\sqrt{n}}$$

Kinetic proofreading

Major requirement: genetic code must be read with few mistakes during protein synthesis or DNA replication

Frequency ξ of errors: $\mathcal{O}(e^{-\Delta G_{CD}/k_B T})$

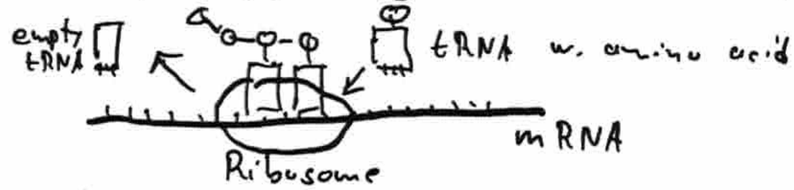
$\uparrow$ binding energy difference between correct (C) and incorrect (D) substrate

Problem: ΔG_{CD} cannot account for small error rates

typically $\xi \sim 10^{-4}$ in protein translation
10^{-3} in DNA transcription

$\rightsquigarrow$ Kinetic proof reading (Hopfield 1974, Ninio 1975) increases specificity by inclusion of intermediate steps

Protein synthesis:



Classical Michaelis-Menten scheme: (enzyme kinetics)

$$E + C \underset{k_{off}}{\overset{k_{on}[C]}{\rightleftarrows}} EC \xrightarrow{W} E + \text{correct amino acid inc.}$$

$$E + D \underset{k'_{off}}{\overset{k'_{on}[D]}{\rightleftarrows}} ED \xrightarrow{W} E + \text{incorrect} \quad \text{—— " ——}$$

W = catalytic rate

$$\rightsquigarrow \quad \frac{d[EC]}{dt} = k_{on}[E][C] - (k_{off} + W)[EC]$$

$$\frac{d[ED]}{dt} = k'_{on}[E][D] - (k'_{off} + W)[ED]$$

$$[E]_{total} = [EC] + [ED] + [E]$$

(*) steady state: $[EC] = [E]\dfrac{k_{on}[C]}{k_{off} + W}$; $[ED] = [E]\dfrac{k'_{on}[C]}{k'_{off} + W}$

$\rightsquigarrow$ translation rates: $R_{corr} = W[EC]$, $R_{incorr} = W[ED]$

$\rightsquigarrow$ error rate: $F_0 = \dfrac{R_{incorr}}{R_{corr}} = \left(\dfrac{k'_{on}[D]}{k'_{off} + W}\right) / \left(\dfrac{k_{on}[C]}{k_{off} + W}\right)$

typically: $k_{on} \approx k'_{on}$, $[D] \approx [C]$

$\rightsquigarrow$ F_0 is minimized for $W \ll k_{off}, k'_{off}$

$\rightsquigarrow$ $F_0(min) \approx \dfrac{k'_{on}}{k'_{off}} / \dfrac{k_{on}}{k_{off}} = \dfrac{K_C}{K_D} = e^{-\Delta G_{CD}/k_B T}$

This simple binding model neglects that upon binding to a codon
tRNA is chemically altered via the hydrolysis of GTP ($\leftrightarrow$ polymeris. of MTs)
! Transition to this new state is irreversible !
and — " — can also unbind from mRNA

$$\rightsquigarrow \quad E + C \underset{k_{off}}{\overset{k_{on}[C]}{\rightleftharpoons}} EC \xrightarrow{r} EC^* \xrightarrow{W} E + \text{correct amino acid inc.}$$

$$EC^* \underset{q_{on}}{\overset{q_{off}}{\rightleftharpoons}} E + C$$

$$E + D \underset{k'_{off}}{\overset{k'_{on}[D]}{\rightleftharpoons}} ED \xrightarrow{r} ED^* \xrightarrow{W} E + \text{incorrect} \text{ — " —}$$

$$ED^* \underset{q'_{on}}{\overset{q'_{off}}{\rightleftharpoons}} E + D$$

$[EC^*]$ is given by the balance of process w. r and w. q_{off}, neglect W

$$[EC^*] = r[EC]/q_{off}$$

$$\rightsquigarrow R_{correct} = W[EC^*] = Wr[EC]/q_{off} \overset{r \text{ small}}{\underset{\circledast}{\simeq}} Wr[E][C]\frac{k_{on}}{k_{off}}/q_{off}$$

$$\simeq Wr[E][C]/K_C\, q_{off}$$

$$\& \quad R_{incorr} = Wr[E][D]/K_D\, q'_{off}$$

$$\rightsquigarrow F = \frac{R_{incorr.}}{R_{corr}} = \frac{K_C}{K_D}\cdot\frac{q_{off}}{q'_{off}} \approx \frac{K_C}{K_D}\cdot\frac{K_C}{K_D}$$

$$q_{off}/q'_{off} \simeq k_{off}/k'_{off} \overset{k_{on}\simeq k'_{on}}{=} \frac{K_C}{K_D}$$

$$\rightsquigarrow F = F_0^2$$

$\rightsquigarrow$ The inclusion of an irreversible step $EA \rightarrow EA^*$,
which necessitates the expenditure of energy,
provides an additional opportunity for the incorrect
substrate to dissociate

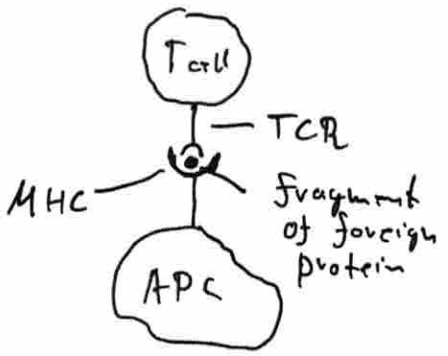
n irreversible proofreading stages:

$$E + C \underset{k_{off}}{\overset{k_{on}[C]}{\rightleftharpoons}} EC \xrightarrow{r_1} EC^* \xrightarrow{r_2} EC^{**} \cdots \rightarrow EC^{*\cdots*} \xrightarrow{W} E +$$

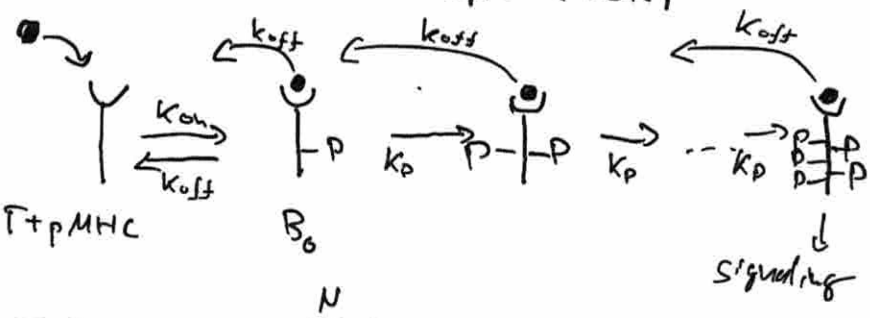
$$EC^* \rightarrow E, \quad EC^{**} \rightarrow E$$

$$\rightsquigarrow F = F_0^{n+1}$$

Kinetic proofreading in T-cell activation



Model: Ligand binding initiates modifications to the T cell receptor (TCR)



$$\frac{dT}{dt} = -k_{on}\, T \cdot P + k_{off} \sum_{i=1}^{N} B_i$$

$$\frac{dB_0}{dt} = k_{on}\, T \cdot P - k_{off} B_0 - k_p B_0$$

$$\frac{dB_i}{dt} = k_p \left(B_{i-1} - B_i\right) - k_{off} B_i$$

$$\frac{dB_N}{dt} = k_p B_{N-1} - k_{off} B_N$$

$\rightsquigarrow$ fraction of activated complexes: $\dfrac{B_N}{\sum_{i=1}^{N} B_i} = \left(\dfrac{k_p}{k_p + k_{off}}\right)^N$

exercise

probability that in any intermediate step i the T cell is modified before dissociation of pMHC

Note: only k_{off} can discriminate between antigens. Any small change in k_{off} gets highly amplified!

Simulation of chemical reactions: Gillespie algorithm

N reactive species $A_1, A_2, \dots, A_N$
with particle numbers $S_1, S_2, \dots, S_N$
M possible chemical reactions $R_1, R_2, \dots, R_M$

		# possible reactions h_μ
e.g.	$A_i \to A_j$	X_i
	$A_i + A_j \to A_k$	$X_i X_j$
	$2A_i \to A_j$	$X_i(X_i - 1)$ etc.

M reaction rates $r_1, r_2, \dots, r_M$
meaning: $r_\mu \, dt$ = prob. for our realization of reaction R_μ in time interval $[t, t+dt]$
$\leadsto$ $h_\mu r_\mu \, dt$ = prob. for reaction R_μ in dt

Master eq. $\frac{d}{dt} P(\underline{S}, t) = \sum_{\underline{S}'} w(\underline{S}' \to \underline{S}) P(\underline{S}', t) - \sum_{\underline{S}'} w(\underline{S} \to \underline{S}') P(\underline{S}, t)$

e.g. $A_1 + A_2 \to A_3$: $w\{(\overbrace{S_1, S_2, \dots}^{\underline{S}}) \to (\overbrace{S_1 - 1, S_2 - 1, S_3 + 1, \dots}^{\underline{S}'})\} = c_1 S_1 S_2$

$X_1^{max} \cdot X_2^{max} \cdot \ldots \cdot X_N^{max}$ coupled differential equations
usually impossible to solve.

$\leadsto$ <u>stochastic simulation</u>

given $S_1, \dots, S_N$ at time t
central quantity: $P(\tau, \mu)$ = probability density that <u>next</u> reaction happens at time t and is of type R_μ

One shows that $P(\tau, \mu) = h_\mu S_\mu \exp\left(-\sum_{\nu=1}^{M} h_\nu S_\nu \tau\right)$

Proof: $h_\mu S_\mu \, dt$ = prob. that reaction R_μ happens in time interval dt
Def.: $P_0(\tau)$ = prob. that in time interval $[t, t+\tau]$ <u>no</u> reaction occurs
$\leadsto P(\tau, \mu) = h_\mu S_\mu \cdot P_0(\tau)$
& $P_0(\tau) = \lim_{K \to \infty} \left(1 - \sum_{\nu=1}^{M} h_\nu S_\nu \frac{\tau}{K}\right)^K = \exp\left(-\sum_{\nu=1}^{M} h_\nu S_\nu \tau\right)$

note that $\int_0^\infty d\tau \sum_{\mu=1}^{M} P(\tau, \mu) = 1$.

Simulation of the stochastic process $\underline{S}(t)$:

<u>Direct method:</u> given $S_1(t), \dots, S_N(t)$

produce τ and μ via $P(\tau,\mu) = P_1(\tau)\, P_2(\mu|\tau)$

$$P_1(\tau) = \sum_{\mu=1}^{M} P(\tau,\mu) = a\, e^{-a\tau} \quad , \quad a = \sum_{\mu=1}^{M} h_\mu S_\mu$$

← uses 1 random number

$$\tau = -\frac{1}{a} \ln x \qquad x \in [0,1]$$

$$P_2(\mu|\tau) = \frac{a_\mu}{a} \quad , \quad a_\mu = h_\mu S_\mu$$

← uses M random numbers and tower sampling

<u>First reaction method:</u>

Generate M random times τ_μ according to

$$P_\mu(\tau) = a_\mu \exp(-a_\mu \tau)$$

Take the minimum of $\tau_1, \tau_2, \dots, \tau_M$, say τ_μ

then the <u>next</u> reaction is at τ_μ and it is of type R_μ

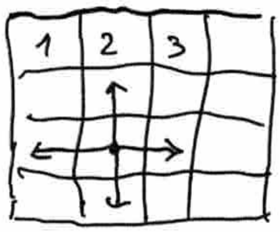
Proof: $\tilde{P}(\tau,\mu) = a_\mu \exp(-a_\mu \tau) \cdot \text{Prob}(\tau_\nu > \tau \mid \forall \nu \neq \mu)$

$$\text{prob}(\tau_\nu > \tau \mid \forall \nu \neq \mu) = \prod_{\nu \neq \mu} \underbrace{\int_\tau^\infty d\tau'\, a_\nu e^{-a_\nu \tau'}}_{= -e^{-a_\nu \tau'}\big|_\tau^\infty} = \prod_{\nu \neq \mu} e^{-a_\nu \tau}$$

$$\Rightarrow \tilde{P}(\tau,\mu) = a_\mu \exp\Big(-\sum_{\nu=1}^{M} a_\nu \tau\Big) = P(\tau,\mu) \quad \square$$

Advantage: needs only <u>one</u> random number (after initialization) for each next reaction. Sort times in a binary tree.

Inclusion of diffusion: <u>next-subvolume method</u>



divide volume in sub-volumes

diffusion is then an additional "reaction" in which particles hop to neighbouring subvolume.

→ Elf et al 2003, MesoRD package.