Counting Molecules with Receptors

Berg and Purcell, 1977

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Physics 141
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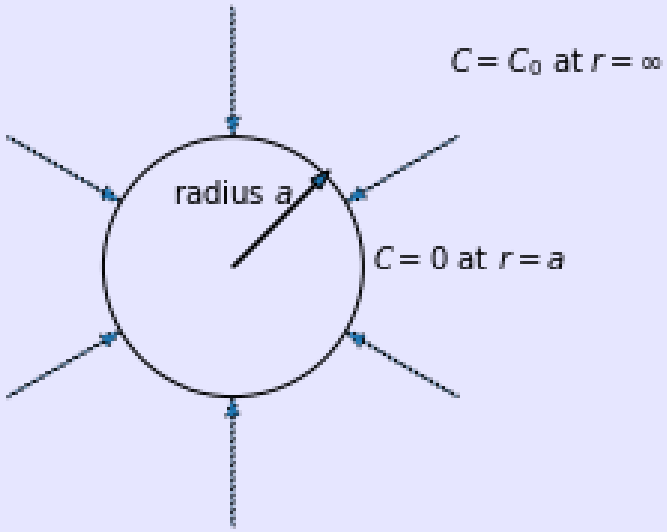
Week 6

Counting molecules by capture

Solving the steady-state diffusion equation



- Consider a spherical cell of radius a immersed in an unbounded medium.
- The medium contains in low concentration some molecules with diffusion constant D.
- The local concentration, $c(\boldsymbol{x}, t)$ will be expressed in molecules per unit volume.

The spatial and temporal variation of $c(\boldsymbol{x}, t)$ is governed by the diffusion equation

$$D\nabla^2 c = \frac{\partial c}{\partial t} \qquad (1)$$

In steady state, the current I of molecules to the cell, in molecules per second, is given by the product of surface area $4\pi a^2$ and the flux, J, from Fick's First Law:

$$I = 4\pi a D c_\infty \qquad (2)$$

where c_∞ is the concentration far from the cell.

Electrostatics analogy

Electrostatics

Laplace's Equation for charge-free space

$$\nabla^2 \phi = 0$$

Electric field vector

$$\boldsymbol{E} = \boldsymbol{\nabla} \phi$$

Total electric charge Q on a surface:

$$Q = \frac{1}{4\pi} \int_S E \cdot d\boldsymbol{s}$$

Diffusion

Time-independent diffusion equation:

$$\nabla^2 c = 0$$

Diffusive current density:

$$\boldsymbol{J} = -D \boldsymbol{\nabla} c$$

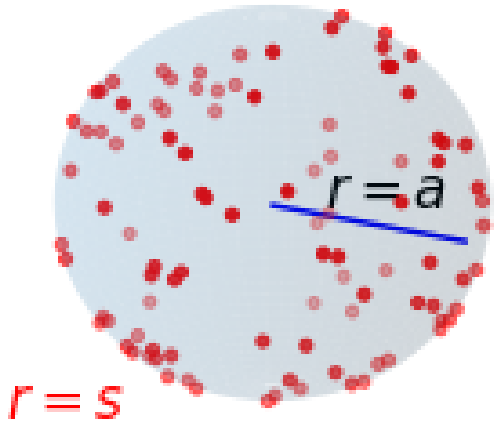
Total diffusive current entering closed surface:

$$I = \int_S \boldsymbol{J} \cdot d\boldsymbol{s}$$

$$I = 4\pi C D c_\infty$$

where C is the electrical capacitance of an object in cgs units

Counting molecules using a patchy sphere



Capacitance of a sphere $r = a$:

$$C = a$$

Total flux:

$$I = 4\pi D a c_\infty$$

Capacitance of a thin disk $r = s$:

$$C = 2s/\pi$$

Total flux:

$$I = 8 D s c_\infty$$

Capacitance of an insulating sphere, $r = a$, with N conducting patches of radius s:

$$C = \frac{Nsa}{Ns + \pi a}$$

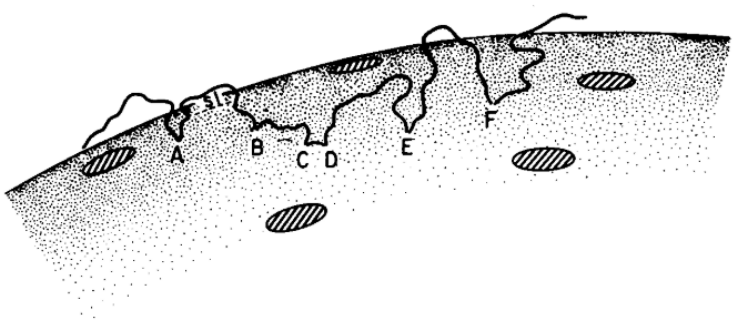
Flux to the patchy sphere:

$$I = 4\pi D c_\infty \frac{Nsa}{Ns + \pi a}$$

$$= I_{max} \frac{Ns}{Ns + \pi a}$$

$$I\left(N = \tfrac{\pi a}{s}\right) = \tfrac{1}{2}$$

Patchy receptors



- The path of a diffusing molecule that has touched the surface of a cell of radius a at a sequence of points A, B, . . . F.
- The receptor patches, shown shaded, are of radius s.

The probability that a molecule at $r = s + a$ from the sphere of radius a will hit the surface before escaping:

$$P_s = \frac{a}{a+s}$$

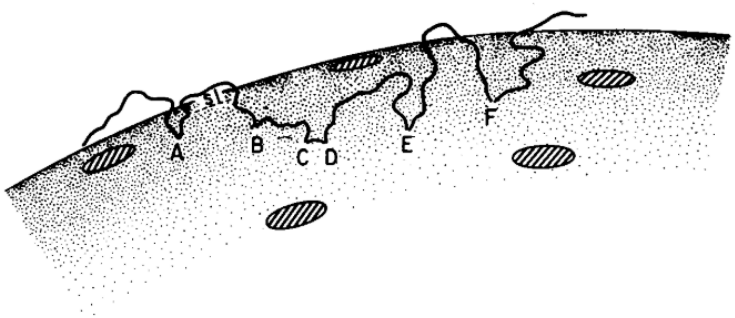
The probability that a molecule at $r = a + s$ executes n trips to the surface, separated by reappearances at $r = a + s$ before escaping:

$$P_s^n(1 - P_s)$$

The average number of surface trips is:

$$\begin{aligned}\bar{n} &= \sum_{n=0}^{\infty} nP_s^n(1 - P_s) \\ &= \frac{P_s}{1 - P_s} \\ &= \frac{a}{s}\end{aligned}$$

Patchy receptor, cont'd



- The probability of not hitting a receptor patch in a single random encounter is $\beta = 1 - (Ns^2/4a^2)$.

- If each contact is an independent attempt, the probability that a molecule starting at $r = a + s$ survives all contacts until it escapes to infinity is:

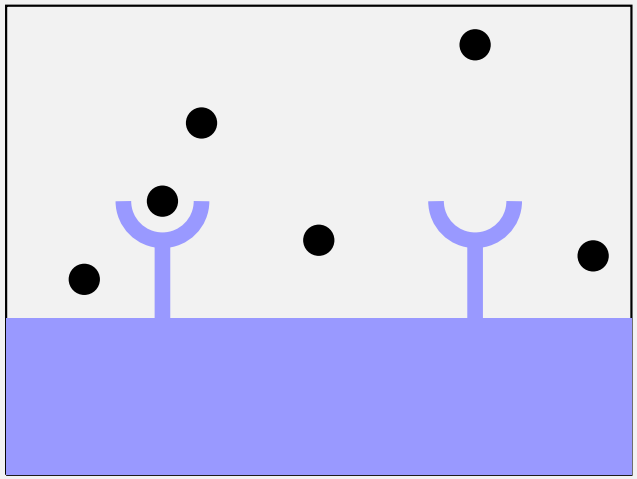
$$P_{esc} = \sum_{n=0}^{\infty} \beta^n n P_s^n (1 - P_s) = \frac{1 - P_s}{1 - \beta P_s} = \frac{4a}{4a + N_s}$$

- Since $1 - P_{esc}$ is the fraction of all arriving molecules that ultimately are captured, the resulting current is:

$$\frac{J}{J_{max}} = \frac{Ns}{4a + Ns}$$

Measuring concentration with receptors

Mean receptor occupancy



- Time-averaged occupancy of a receptor is given by a single dissociation constant, K
- In equilibrium at concentration c, the expected average occupancy is:

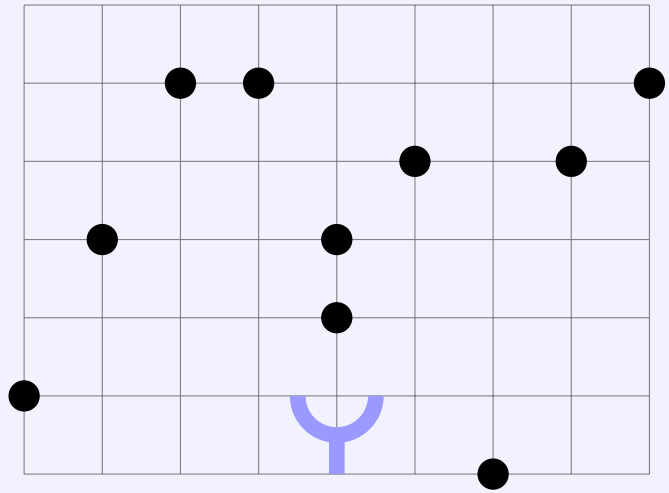
$$\bar{p} = \frac{c}{c + c_{1/2}}$$

Law of Mass Action

$$R + L \leftrightarrow R \cdot L$$

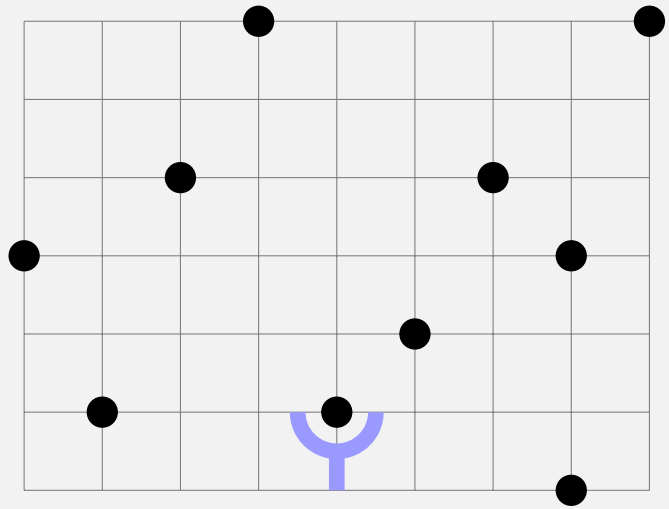
$$\frac{d[R \cdot L]}{dt} = k_{on}[R][L] - k_{off}[R \cdot L]$$

$$\bar{p} = \frac{[R \cdot L]}{[R][L] + [R \cdot L]} = \frac{[L]}{[L] + k_{off}/k_{on}}$$



Physics of the unbound state

- Energy $= L\epsilon_{sol}$
- Multiplicity $= \frac{\Omega!}{L!(\Omega-L)!} \approx \frac{\Omega^L}{L!}$
- Weight $= \frac{\Omega^L}{L!} e^{-\beta L \epsilon_{sol}}$



Physics of the bound state

- Energy $= (L-1)\epsilon_{sol} + \epsilon_b$
- Multiplicity $= \frac{\Omega!}{(L-1)!(\Omega-L+1)!} \approx \frac{\Omega^{L-1}}{(L-1)!}$
- Weight $= \frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\epsilon_{sol}+\epsilon_b]}$

An entropic view of receptor occupancy, cont'd

The probability of binding is the weight of the bound states divided by the sum of the weights of the bound and unbound states:

$$\bar{p} = \frac{\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\epsilon_{sol}+\epsilon_b]}}{\frac{\Omega^{L-1}}{(L-1)!} e^{-\beta[(L-1)\epsilon_{sol}+\epsilon_b]} + \frac{\Omega^L}{L!} e^{-\beta L\epsilon_{sol}}}$$

Multiply top and bottom by $(L!/\Omega^L)e^{\beta L\epsilon_{sol}}$:

$$\bar{p} = \frac{(L/\Omega)e^{-\beta\Delta\epsilon}}{1 + (L/\Omega)e^{-\beta\Delta\epsilon}}$$

where $\Delta\epsilon = \epsilon_b - \epsilon_{sol}$.

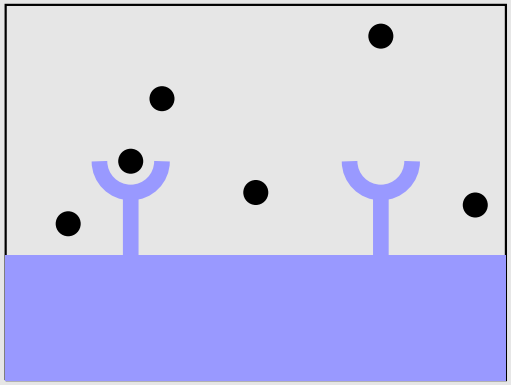
The overall volume of the box is V_{box}, so we can write ligand concentration as $c = L/\Omega V_{box}$. This results in:

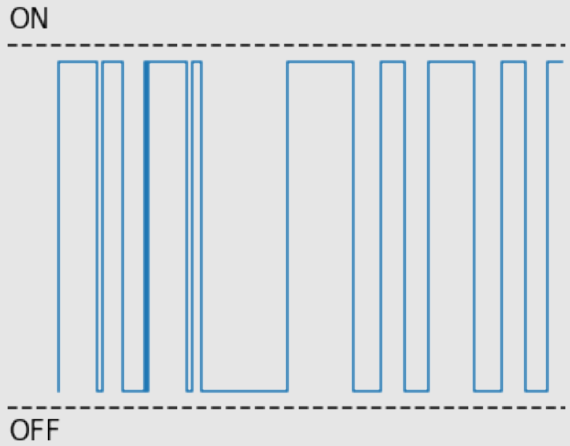
$$\bar{p} = \frac{(c/c_0)e^{-\beta\Delta\epsilon}}{1 + (c/c_0)e^{-\beta\Delta\epsilon}}$$

where $c_0 = 1/V_{box}$.

Calculating concentration from receptor occupancy

Temporal dynamics of receptor occupancy





Receptor occupancy over time

- An attached molecule has a constant probability per unit time of detaching. If the probability of detachment in dt is dt/τ_b, the mean occupancy time is τ_b
- If an empty receptor is an adsorber, the current to it is $4Dsc$. The probability that a vacant patch becomes occupied during dt is thus $4Dscdt$.

At steady state:

$$\bar{p}/\tau_b = 4(1-\bar{p})Dsc$$

Since $\bar{p} = 1/2$ when $c = c_{1/2}$

$$\tau_b = (4Dsc_{1/2})^{-1}$$

Temporal dynamics of receptor occupancy cont'd

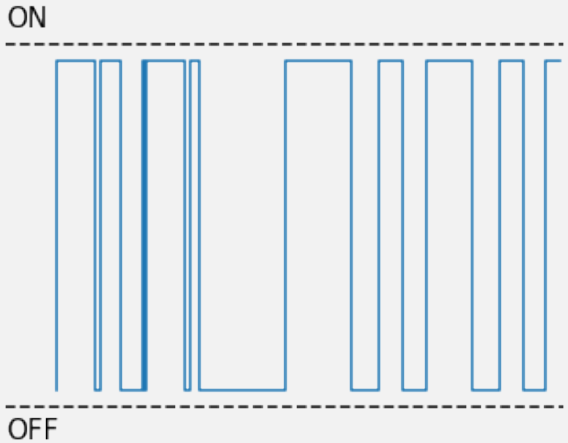


Figure: Receptor occupancy over time

If the only information about c is the function $p(t)$ for one receptor recorded for a time T, the cell can extract this information by forming its average:

$$p_T = \frac{1}{T}\int_{t_1}^{t_1+T} p(t)dt$$

If p_T is taken as the $\bar{p}$, it can be used to derive c:

$$c/c_{1/2} = p_T/(1-p_T)$$

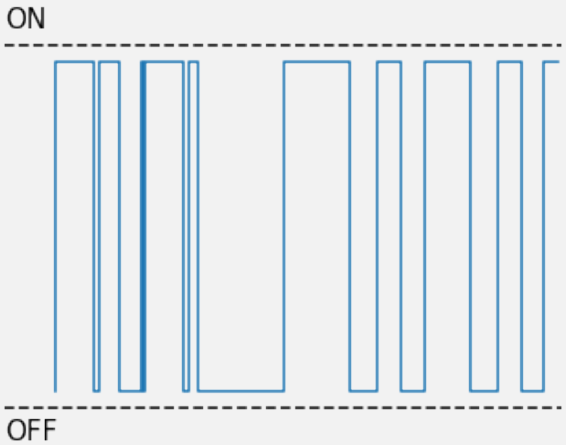
Fractional error in measuring concentration is related to mean and variance in receptor occupancy:

$$\frac{\delta c}{c} = \frac{\delta \bar{p}}{\bar{p}(1-\bar{p})}$$

Variance in measurements of $\bar{p}$ can be computed from the autocorrelation function of $p(t)$

$$G(\tau) = \langle p(t)p(t+\tau)\rangle$$

The autocorrelation function of receptor occupancy



Receptor occupancy over time

- p_T is the average of $p(t)$ over the time of observation:

$$p_T = \frac{1}{T}\int_{t_1}^{t_1+T} p(t)dt$$

- A useful tool is the autocorrelation function of $p(t)$:

$$G(\tau) = \langle p(t)p(t+\tau)\rangle$$

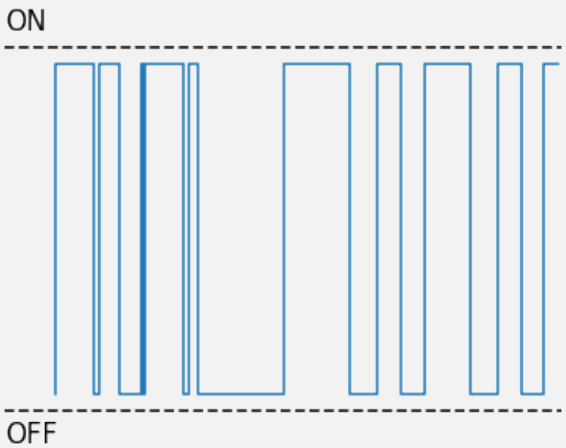
- $G(\tau)$ is an even function of τ: $G(\tau) = G(-\tau)$
- From the definition of p_T

$$p_T^2 = \frac{1}{T^2}\int_{t_1}^{t_1+T} dt' \int_{t_1}^{t_1+T} p(t)p(t')dt$$

- Introducing the autocorrelation function, we obtain:

$$\langle p_T^2\rangle = \frac{1}{T^2}\int_{0}^{T} dt' \int_{0}^{T} G(t'-t)dt$$

The autocorrelation function of receptor occupancy



Receptor occupancy over time

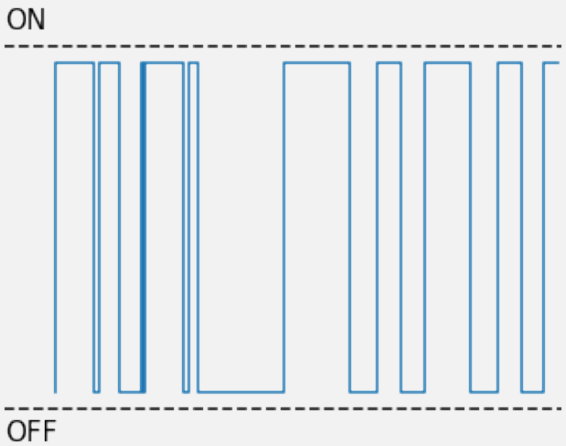
- Consider a large number n pairs of observations, one at t the other at $t + \tau$.
- Segregate the pairs in which the first observation is bound, i.e., $p(t) = 1$.
  - If n is large, there will be about $n\bar{p}$ pairs
  - The number with $p(t + \tau) = 1$ will be $nG(\tau)$
  - These "1,1" pairs are the only ones for which $p(t)p(t + \tau) \neq 0$
- Shift the time of the second observation from $t + \tau$ to $t + \tau + d\tau$
  - Some of the "1,1" pairs will become "1,0" pairs. This number is

$$nGd\tau/\tau_b$$

  - Some of the "1,0" pairs will become "1,1" pairs, this number is

$$n(\bar{p} - G)[\bar{p}/(1 - \bar{p})]d\tau/\tau_b$$

The autocorrelation function of receptor occupancy



Receptor occupancy over time

Fractional error in measuring concentration is from the counting statistics of the total number of measured molecules!

- We should now have $nG(\tau + d\tau)$ "1,1" pairs, which requires:

$$dG = -Gd\tau/\tau_b + (\bar{p} - G)[\bar{p}/(1-\bar{p})]d\tau/\tau_b$$

- Integrating and requiring that $G(0) = \bar{p}$

$$G(\tau) = \bar{p}^2 + \bar{p}(1-\bar{p}) \exp\left[-\frac{|\tau|}{(1-\bar{p})\tau_b}\right]$$

- Assuming $T \gg \tau_b$:

$$\langle p_T^2 \rangle - \langle p_T \rangle^2 = \frac{2\bar{p}(1-\bar{p})^2\tau_b}{T}$$

- For the rms error in $\bar{c}$ we get

$$\frac{\Delta c_{rms}}{\bar{c}} = \sqrt{\frac{2\tau_b}{T\bar{p}}} = \sqrt{\frac{2}{\nu}}$$

where $\nu = 4Ds\bar{c}(1-\bar{p})T$