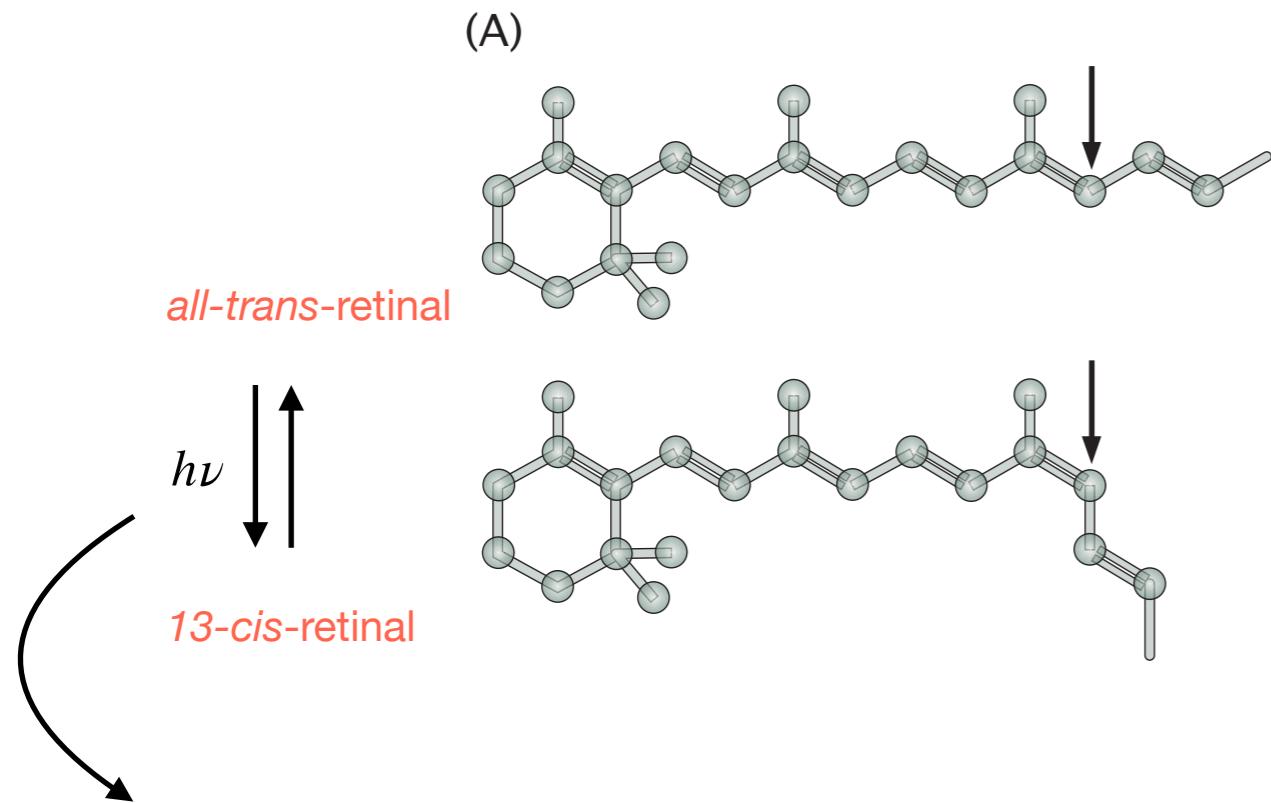


Quantitative rate theory for decay of macromolecules



This isomerization reaction is utilized by the transmembrane protein 'bacteriorhodopsin' in photosynthetic archaea for trans-membrane proton transport.

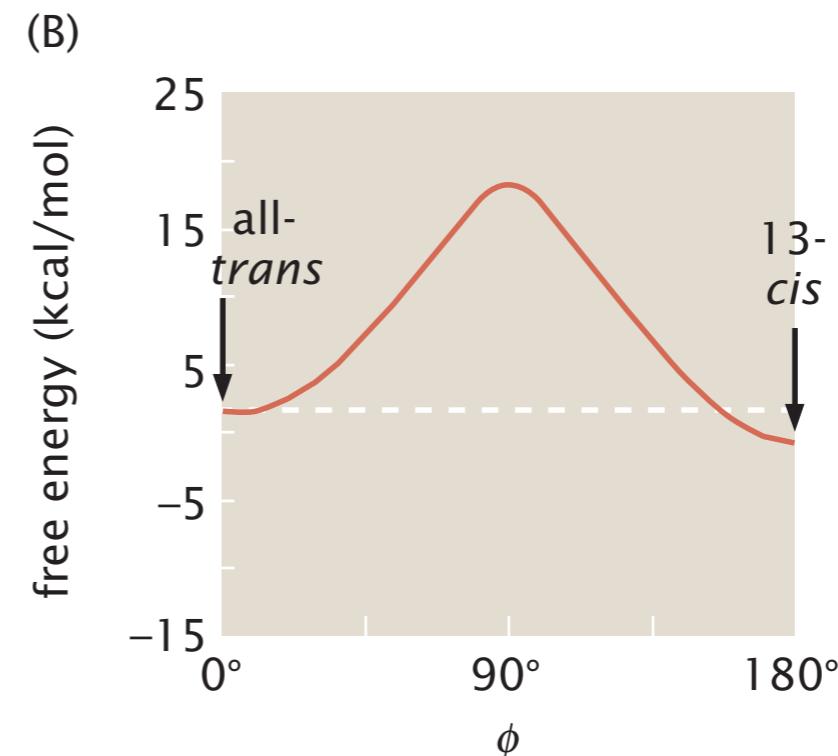
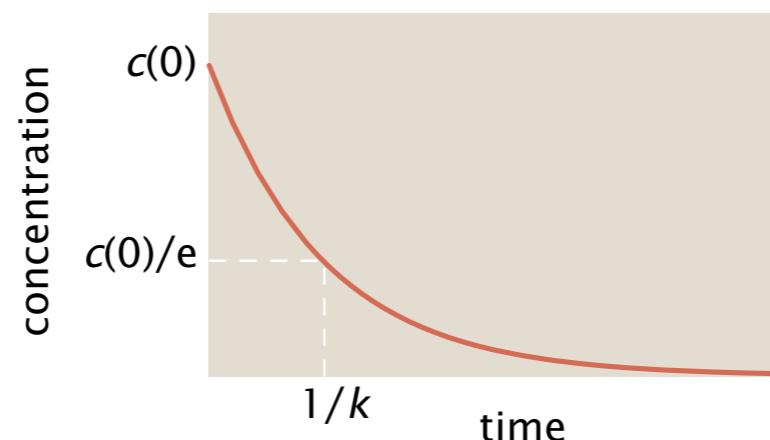
The rate equation takes the form:

$$\frac{dc(t)}{dt} = f(c; k) = -kc(t) \implies c(t) = c(0)e^{-kt}$$

k = rate of decay

Unit of k ? $1/\text{time}$

Concentration profile?



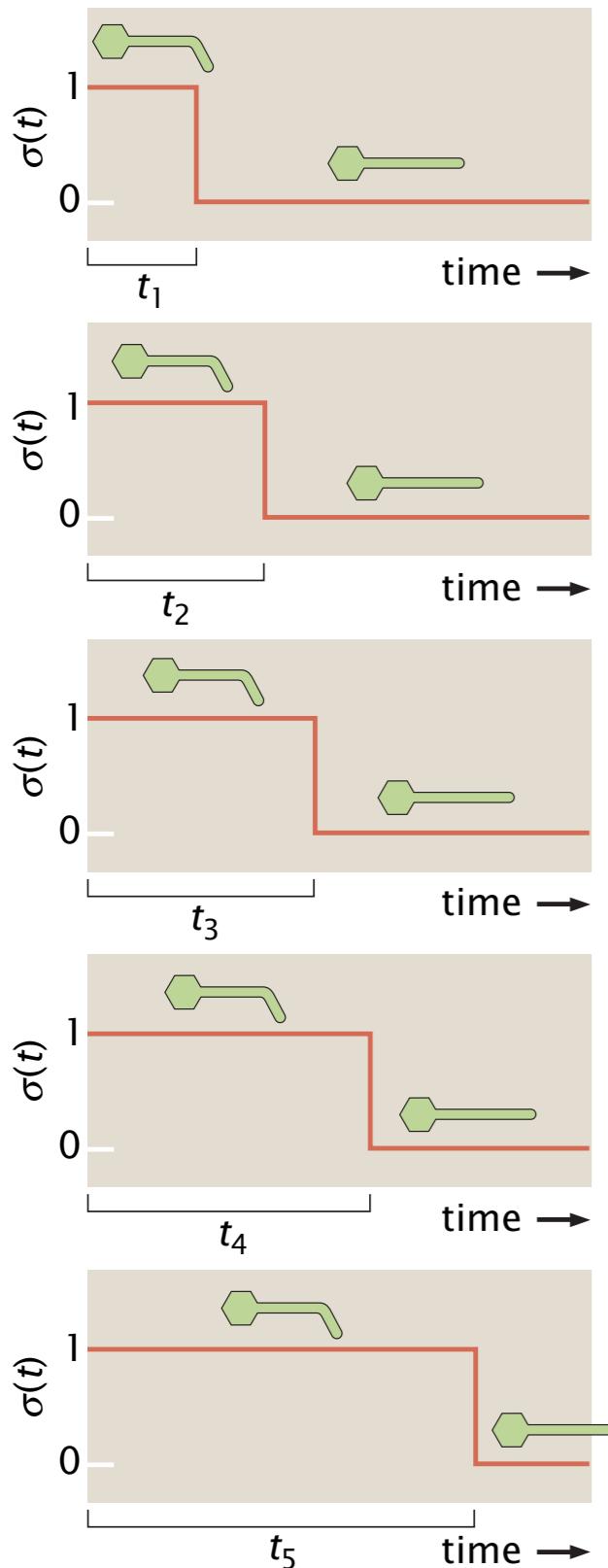
Angle of rotation around C atom at position 13

13-cis-retinal decays with time into all-trans-retinal following first-order kinetics

We can define a characteristic time

$$\tau = \frac{1}{k} \quad c(\tau) = \frac{c(0)}{e}$$

A single-molecule view of degradation



Microscopic trajectories of a system undergoing decay from state '1' to state '0'

$$\sigma_i(t) = \begin{cases} 1 & \text{if } t < t_i, \\ 0 & \text{otherwise} \end{cases}$$

where t_i = waiting time associated with the i -th trajectory

In this picture, rate constant k characterizes the average life-time of the macromolecules - this only works for a huge collection of molecules

Now let's analyze the trajectories

First, discretize time into steps of Δt , so any time $t = N\Delta t$

If a molecule survives till N steps and decay at the $N + 1$ -th time step, then probability of that process is

$$P(t) = \frac{(1 - k\Delta t) \times (1 - k\Delta t) \times (1 - k\Delta t) \times \dots \times (1 - k\Delta t) \times k\Delta t}{N \text{ time steps}}$$

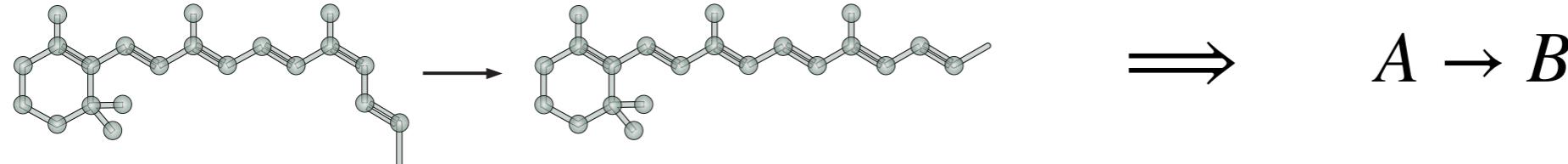
where $k\Delta t$ = probability of decay during a given time step

$(1 - k\Delta t)$ = probability of decay not happening during a given time step

$$\text{Rewriting, } P(t) = (1 - k\Delta t)^N k\Delta t = \left(1 - \frac{kt}{N}\right)^N k\Delta t \approx ke^{-kt} \Delta t$$

Therefore, the probability of decay in time interval between t & $t + \Delta t$ is: $P(t) = \frac{1}{\tau} e^{-t/\tau} \Delta t$

Decay of one species corresponds to growth in the number of a second species



Condition for mass conservation $\longrightarrow c_A(t) + c_B(t) = c_0$

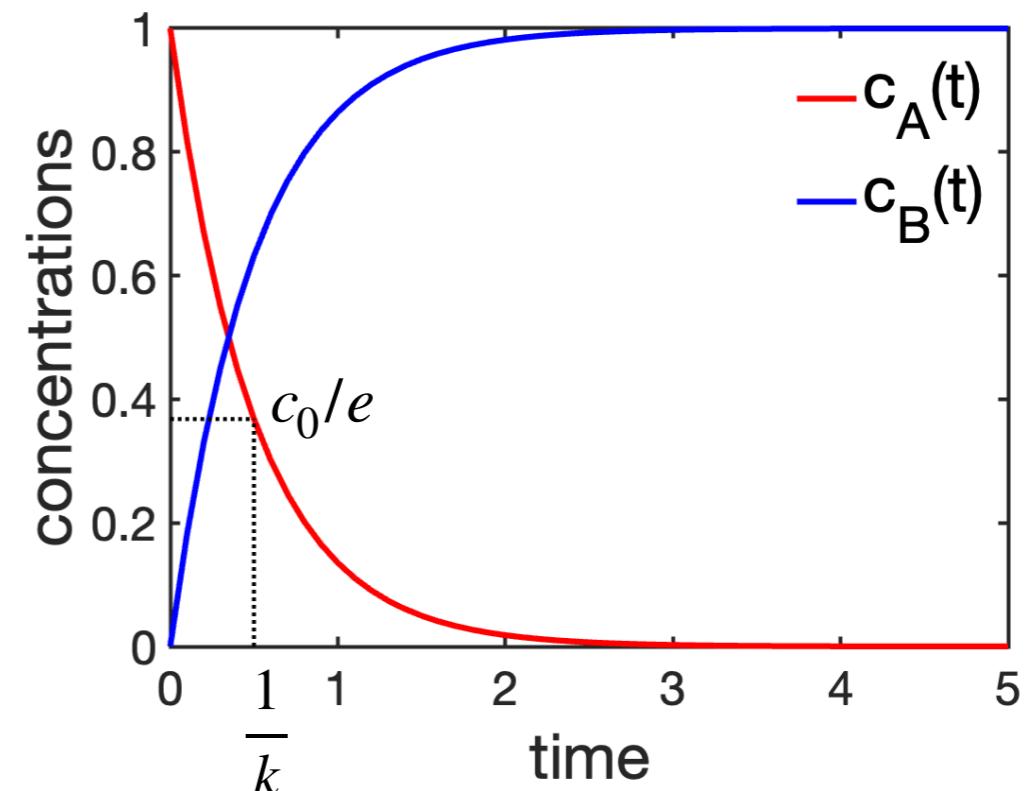
Taking a derivative, we get: $\frac{dc_A}{dt} = -\frac{dc_B}{dt} = -kc_A$ We can use this to estimate the form of $c_B(t)$

Let's start with an initial condition populated by only A molecules

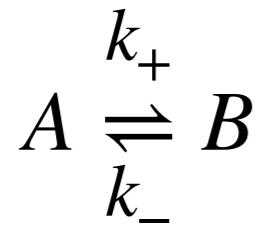
$$\frac{dc_B(t)}{dt} = kc_A(t) = k(c_0 - c_B(t))$$

$$\Rightarrow \frac{dc_B(t)}{c_0 - c_B(t)} = kdt$$

$$\Rightarrow c_B(t) = c_0(1 - e^{-kt})$$



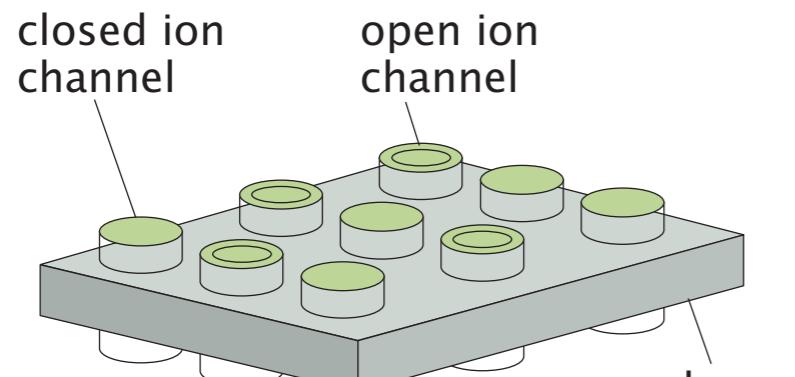
Reversible reaction



Here the rate equation becomes: $\frac{dc_A}{dt} = -k_+c_A(t) + k_-c_B(t)$

$$\text{&} \frac{dc_A}{dt} = -\frac{dc_B}{dt}$$

As an example we consider dynamics of an ion channel: $O \xrightleftharpoons[k_-]{k_+} C$ O = open state, C = closed state

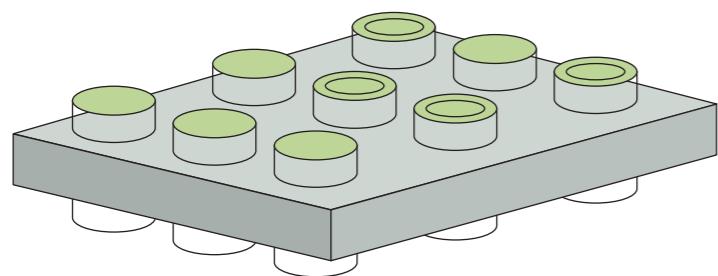


change in the number of open channels in a given time step

$$\Delta N_O = \underbrace{-k_+ N_O \Delta t}_{O \rightarrow C} + \underbrace{k_- N_C \Delta t}_{C \rightarrow O}.$$

$$\Rightarrow \frac{\Delta N_O}{\Delta t} = -k_+ N_O + k_- N_C,$$

Defining, $p_O = N_O/N$ and $p_C = N_C/N$.



For $\Delta t \rightarrow \infty$, we have $\frac{dp_O}{dt} = -k_+ p_O + k_- p_C$.

Ion channel dynamics...*contd*

$$\frac{dp_O}{dt} = -k_+ p_O + k_- p_C.$$

We can use $p_O + p_C = 1$, and write $\frac{dp_O}{dt} = -k_+ p_O + k_-(1 - p_O)$.

Solve this for p_O

Rewriting, $\frac{dp_O}{dt} = -(k_+ + k_-)p_O + k_-.$

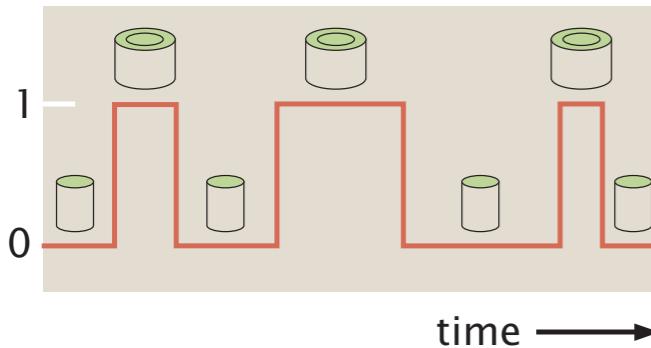
$$p_O(t) = \frac{k_-}{k_+ + k_-} + A e^{-(k_+ + k_-)t}.$$

Using, $p_O(0) = 1$ Final solution, $p_O(t) = \frac{k_-}{k_+ + k_-} + \frac{k_+}{k_+ + k_-} e^{-(k_+ + k_-)t}.$

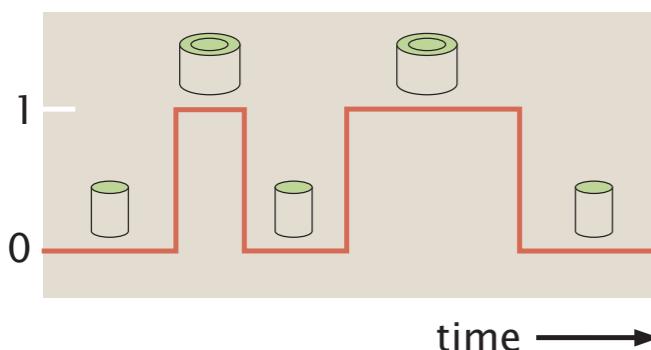
The dynamics of the channel is totally characterized using this probability

How can we estimate p_O independently?

Estimation of p_O from state trajectories

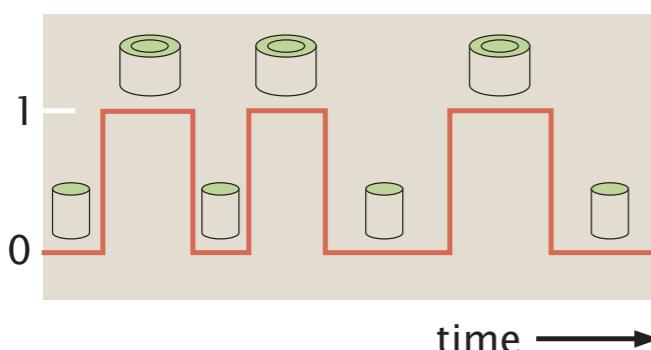


We can represent the opening-closing using a random signal $\sigma(t)$



What is the value of $\langle \sigma(t)\sigma(0) \rangle$? Between 0 and 1

This is precisely p_O that we want!



How can we calculate this average?

- Considering many many realizations of this trajectory - watch lots of ion channels!
- Watch a single ion channel for a long time and take a time average

— if its a stationary process



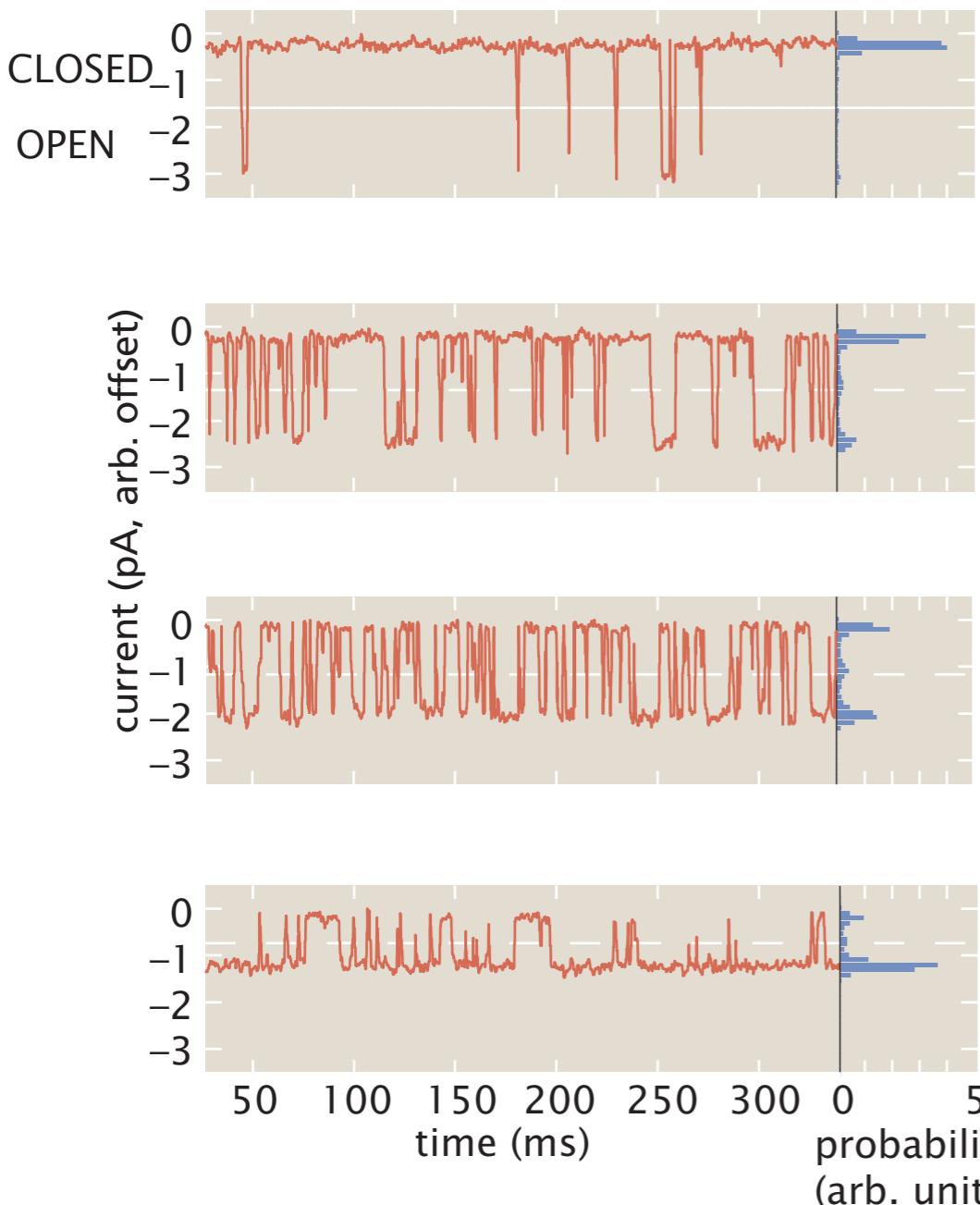
A process whose statistical properties don't change when shifted in time

Basically it looks the same in any time window you choose to look at!

The average $\langle \sigma(t)\sigma(0) \rangle$ is also known as the auto-correlation function of the random signal $\sigma(t)$

Example of ion channel dynamics

(B)



Sodium ion channel

applied
voltage
(mV)

-125

$\varepsilon_{\text{open}} - \varepsilon_{\text{closed}}$
($k_B T$)

3.24

CLOSED
OPEN

current (pA, arb. offset)

-105

1.14

-95

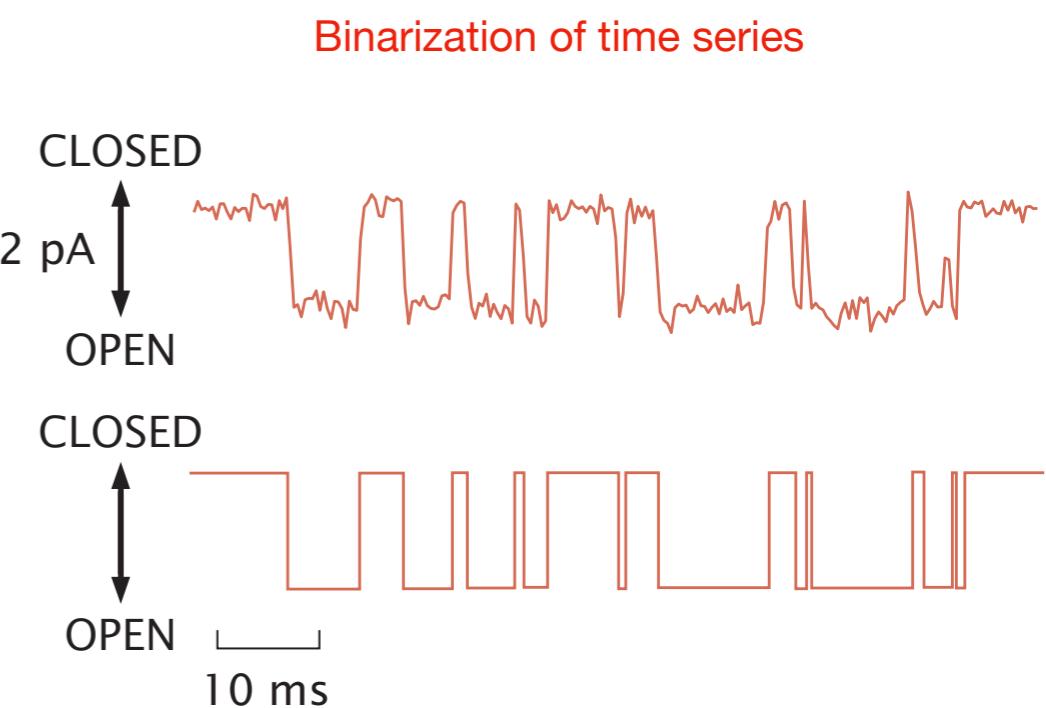
0.05

-85

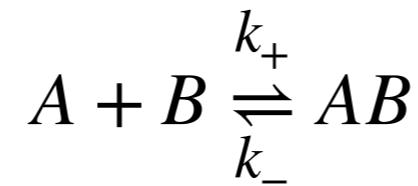
-1.27

probability
(arb. units)

time (ms)



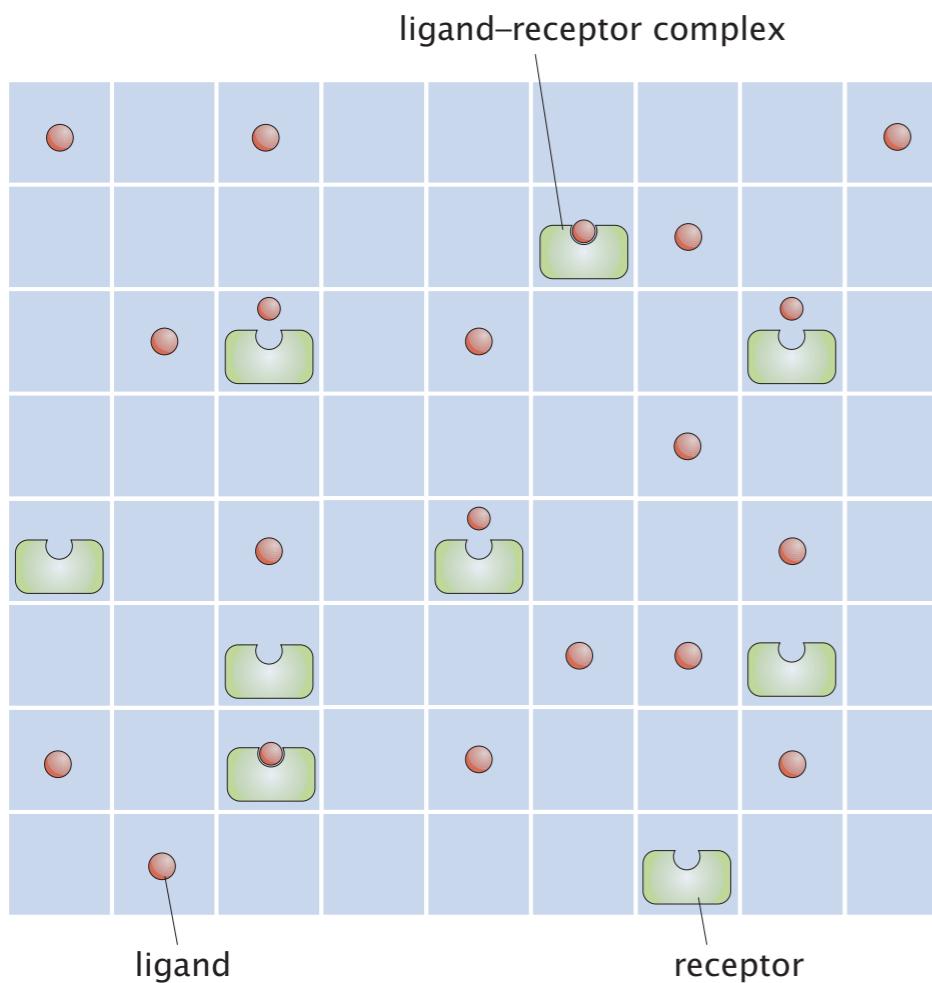
Bimolecular reactions



The rate equation: $\frac{dc_{AB}}{dt} = k_+ c_A(t) c_B(t) - k_- c_{AB}(t)$

Let's try to realize this through a toy model: ligand-receptor binding $L + R \rightleftharpoons LR$

We solve this using a lattice model



Assume there are Ω boxes each of volume v , L ligands and R receptors
Ligands and receptors need to be in the same box to form complex

Occupancy prob of ligand = L/Ω Occupancy prob of receptor = R/Ω

Rate of decay = k_{off} , and rate at which L and R are found in the same box = k'_on

In time Δt , the change in number of LR complex would be given by

$$\Delta N_{LR} = \frac{-(k_{off}\Delta t)N_{LR}}{\text{decay term}} + \left(\frac{\Omega}{\text{no. of boxes}} \times \frac{N_L N_R}{\frac{\Omega}{\Omega} \frac{\Omega}{\Omega}} \times (k'_on \Delta t) \right)$$

Can we recast this into more familiar forms?

Bimolecular reactions...*contd*

$$\Delta N_{LR} = \underbrace{-(k_{\text{off}} \Delta t) N_{LR}}_{\text{decay term}} + \left(\underbrace{\Omega}_{\text{no. of boxes}} \times \underbrace{\frac{N_L}{\Omega} \frac{N_R}{\Omega}}_{\text{box occupancy prob.}} \times (k'_{\text{on}} \Delta t) \right)$$

Rewrite into the following

$$\frac{d}{dt} \left(\frac{N_{LR}}{\Omega v} \right) = -k_{\text{off}} \left(\frac{N_{LR}}{\Omega v} \right) + \frac{\Omega}{\Omega v} \frac{N_L}{\Omega v} \frac{N_R}{\Omega v} v^2 k'_{\text{on}}$$

Can you see the concentration variables here?

Replacements: $[L] = N_L / \Omega v$, $[R] = N_R / \Omega v$, and $[LR] = N_{LR} / \Omega v$

and rewrite to get

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

Where $k_{\text{on}} = v k'_{\text{on}}$ The rescaled bimolecular on-rate

Dimensions? $M^{-1} s^{-1}$

Different from the first order rate constant!

We can get the dissociation constant from here

When forward and backward reaction rates are exactly balanced, we can write

$$d[LR]/dt = 0$$

Consequently, $-k_{\text{off}}[LR]_{\text{eq}} + k_{\text{on}}[L]_{\text{eq}}[R]_{\text{eq}} = 0$

where, $[L]_{\text{eq}}$, $[R]_{\text{eq}}$, $[LR]_{\text{eq}}$ are the equilibrium concentrations of the involved species

We can then define by rearranging,

$$K_d = \frac{[L]_{\text{eq}}[R]_{\text{eq}}}{[LR]_{\text{eq}}} = \frac{k_{\text{off}}}{k_{\text{on}}}$$

Law of mass action!