Chapter 15
Rate Equations and Dynamics in the Cell
A Chemical Picture of Biological Dynamics

• A cell is a metropolis of chemical reactions.

• Chemical reactions come to an equilibrium by the competition between entropic and energetic terms, as we have learned in Chapter 6.

• Equilibrium state gives the final concentrations of the reactants and products.

• In this chapter, we will learn about the kinetics of reacting species.

• We are interested in how fast the system comes to an equilibrium.

• We will follow the average (expected) behavior of the system.

• In the context of living cells, we should not forget that the number of reacting molecules may be very small, may be localized or trapped in specific locations, and may not uniformly distributed.

• In such cases, we consider the stochastic behavior of individual molecules.
Isomerization or Molecular Decay

- Macromolecules e.g. mRNA are short-lived and they decay stochastically.
- (Right) Retinal is a light sensitive molecule which undergoes a conformational change upon photon absorption.
- $trans$-retinal is the lower energy form and $cis$-retinal is the high energy form.
- $trans$ to $cis$ isomerization requires $180^\circ$ rotation of a C-C bond.
- Ratios of $trans$ and $cis$ form are

\[
\frac{p_{trans}}{p_{cis}} = e^{-\left(G_{trans} - G_{cis}\right)/kT}
\]

$G_{trans} < G_{cis}$  $p_{trans} > p_{cis}$
The rate \( k \) in which trans to cis isomerization will happen depends on the energy barrier:

\[
k_{tc} \propto e^{-\Delta G_{tc}^*/kT}
\]

As you can see, cis to trans reaction will occur faster:

\[
k_{ct} \propto e^{-\Delta G_{ct}^*/kT}
\]

And, trans to cis ratio:

\[
\frac{p_{\text{trans}}}{p_{\text{cis}}} = \frac{k_{ct}}{k_{tc}} = \frac{e^{-\Delta G_{ct}^*/kT}}{e^{-\Delta G_{tc}^*/kT}} = e^{-(G_{\text{trans}} - G_{\text{cis}})/kT}
\]
1. Simple Reaction Scheme

\[ R_{\text{cis}} \rightarrow R_{\text{trans}} \]

- Assumption: The reaction is irreversible
- Number of molecules do not change
- Rate of change of concentration of \( \text{cis} \) form depends on the reaction rate and the concentration itself.

\[ c_{\text{cis}} + c_{\text{trans}} = c_0 \]

\[ c_{\text{cis}}(0) = c_0 \quad c_{\text{trans}}(0) = 0 \]

\[ \frac{dc_{\text{cis}}}{dt} = - \frac{dc_{\text{trans}}}{dt} \]

\[ \frac{dc_{\text{cis}}}{dt} = -kc_{\text{cis}}(t) \]

\[ c_{\text{cis}}(t) = c_o e^{-kt} = c_o e^{-t/\tau} \quad \text{where} \quad \tau = 1/k \]

\[ c_{\text{trans}}(t) = c_o (1 - e^{-kt}) \]

- Reaction rate \( (k) \) has units of \( \text{s}^{-1} \)
- Lifetime \( (\tau) \) has units of \( \text{s} \).
2. Reversible Reactions: Ion Channel Opening/Closing

\[ O \leftrightarrow C \]

\[ c_0 + c_c = c_{in} \]

\[ \frac{dc_0}{dt} = -\frac{dc_c}{dt} \]

for a single channel, probability can be substituted with the concentration.

\[ \frac{dp_o}{dt} = -k_+p_o + k_-p_c \]

since \( p_o + p_c = 1 \)

\[ \frac{dp_o}{dt} = -(k_+ + k_-)p_o + k_- \]

\[ p_o(t) = \frac{k_-}{k_+ + k_-} + Ae^{-(k_+ + k_-)t} \]

if we say \( p_o(0) = 1 \)

\[ A = \frac{k_+}{k_+ + k_-} \]

at \( t = \infty \)

\[ \frac{p_c}{p_o} = \frac{k_+}{k_-} = K_{eq} \]
3. Biochemical Reactions: Ligand-Receptor Binding

\[ L + R \rightleftharpoons_{k_{on}}^{k_{off}} LR \]

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

at equilibrium \(- k_{off} [LR]_{eq} + k_{on} [L]_{eq} [R]_{eq} = 0\)

\[ K_{eq} = \frac{[LR]_{eq}}{[L]_{eq} [R]_{eq}} = \frac{k_{on}}{k_{off}} \]

\[ K_d = \frac{1}{K_{eq}} \text{ for a single ligand binding to a receptor} \]
Quasi Steady State Equilibrium

\[ A \xrightleftharpoons[k_+]{k_-} B \rightarrow C \]

\[ k_+ \gg r \]

\[ \frac{dA}{dt} = -k_+A + k_-B \]

\[ \frac{dB}{dt} = k_+A - k_-B - rB \]

\[ \frac{dC}{dt} = rB \]

\[ \tau = k_-t, \quad k = \frac{k_+}{k_-} \quad \text{and} \quad \varepsilon = \frac{r}{k_-} \]

\[ \frac{dA}{d\tau} = -kA + B \]

\[ \frac{dB}{d\tau} = kA - (1 + \varepsilon)B \]

\[ \frac{dC}{d\tau} = \varepsilon B \]

\[ \left( \frac{A}{B} \right)(\tau) = \left( \frac{1}{k/(k+1)} \right) e^{-\left(k\varepsilon/(k+1)\right)\tau} + \left( \frac{k/(k+1)}{-k/(k+1)} \right) e^{-(k+1)\tau} \]
The reaction starts with A only.
A and B quickly reach to an equilibrium state, and B slowly decays to C.
The system never reaches to a true equilibrium state, unless all A and B turn into C.
Michaelis-Menten Enzyme Kinetics

\[ E + S \rightleftharpoons \frac{k_+}{k_-} ES \rightarrow E + P \]

\[
\frac{d[E]}{dt} = -k_+[E][S] + k_- [ES] + k_{cat}[ES]
\]

\[
\frac{d[S]}{dt} = -k_+[E][S] + k_- [ES]
\]

\[
\frac{d[ES]}{dt} = k_+[E][S] - k_- [ES] - k_{cat}[ES]
\]

\[
\frac{dP}{dt} = k_{cat}[ES]
\]

A choice of initial conditions that shows the essence of dynamics is

\[ [E]_0 \ll [S]_0 \text{ and } [ES]_0 = [P]_0 = 0 \text{ (no intermediates)} \]

enzyme concentration decreases first but ultimately increases once all of the substrate is depleted

Quasi-Steady State Assumption

one can assume that \( \frac{d[ES]}{dt} = 0 \text{ when } S \gg E \text{ and each enzyme is working at a constant rate.} \)

\[
\frac{d[ES]}{dt} = k_+[E][S] - k_- [ES] - k_{cat}[ES] = 0
\]

\[
\frac{[E][S]}{[ES]} = \frac{k_- + k_{cat}}{k_+} = K_M
\]
\[ K_M = \frac{k_- + k_{cat}}{k_+} \]

\( K_M \) Michaelis – Menten constant is the concentration of substrate, where the product is formed at half the maximum possible rate

\[ \text{if } k_{cat} \ll k_- \quad K_M = \frac{k_-}{k_+} = K_d \]

which means only half of the enzyme is filled up with the substrate.

\[ \frac{dP}{dt} = k_{cat} [ES] \quad \text{and} \quad [ES] = \frac{[E][S]}{K_M} \]

\[ V = \frac{dP}{dt} = k_{cat} \frac{[E][S]}{K_M} \]

\( V_{max} = k_{cat} [E_{tot}] \) all enzymes are bound to substrate, which occurs when \([S] \gg K_M\)

\[ V = \frac{V_{max} [E][S]}{[E_{tot}] K_M} = V_{max} \frac{[E][S]/K_M}{[E_{tot}]} \]

\[ [E_{tot}] = [E] + [ES] \quad \text{and} \quad [ES] = \frac{[E][S]}{K_M} \]

\[ V = V_{max} \frac{[E][S]/K_M}{([E] + [E][S]/K_M)} = V_{max} \frac{[S]/K_M}{(1 + [S]/K_M)} \]
- Enzyme concentration initially decreases, but then recovers.
- \([ES]\) remains nearly constant over the reaction after the rapid-equilibrium state is reached.
- Substrate degradation and product formation is nearly linear over the reaction period.
- At later time points \((t > 100 \text{ min})\), the linearity is lost, because the substrate is being depleted and \([S]\) is no longer significantly higher than \([E]\).
Conditions for Michaelis-Menten Modelling

• In order to model an enzymatic reaction, some conditions must be maintained:
  – Temperature, ionic strength, pH, and other physical conditions that might affect the rate must remain constant
  – Each enzyme can act on only one molecule at a time
  – The enzyme must remain unchanged during the course of the reaction.
  – The concentration of substrate must be much higher than the concentration of enzyme

• The model does not work so well, if
  – the substrate concentration is low compared to the enzyme
  – enzyme-substrate complex proceeds through multiple intermediate steps
  – enzyme uses multiple substrate molecules, which may bind to the active site cooperatively.
  – enzymes are allosterically activated by regulators that bind at locations other than the active site.
  – there are inhibitory molecules that interact with enzyme or enzyme-substrate complex directly.
Case Study: Cytoskeletal Dynamics

- Actin
- Microtubule
- Chromosomes

- Microtubule network plays a role in chromosome segregation, cargo transportation, motility, flagellar/ciliary sensory functions, motor neuron development…
- Actin network play a role in cytokinesis, muscle contraction, cargo transport, endocytosis, cell crawling, cell shape…
- Cytoskeleton is a dynamic structure that is always under construction.

http://cryoem.berkeley.edu/microtubules
http://www.youtube.com/watch?v=aDAw2Zg4IgE
Bacterial Cytoskeleton

(A) ADP-ParM

(B) ATP-ParM

(C) In vitro ParM polymerization
ParM mediates plasmid segregation

http://jcb.rupress.org/content/suppl/2007/11/26/jcb.200708206.DC1/JCB_200708206_V9.mov

http://mullinslab.ucsf.edu/animations.html
Cytoskeletal Polymerization

- Actin monomers spontaneously aggregate to form filaments in solution.
- Stable polymerization of actin requires generation of actin oligomers, nucleation step.
- After nucleation, actin rapidly polymerizes, growth phase.
- As filaments form, monomer concentration decreases. Finally, monomers reach the critical concentration, in which polymerization and depolymerization rates become equal.
- The system reaches the equilibrium state.
- Addition of nucleated actin, by using actin capping proteins that inhibit monomer dissociation, leads to rapid polymerization.
Models for Cytoskeletal Polymerization

- Polymerization with same on and off rates on both ends (e.g. ParM)

- Polymerization with distinct on and off rates on both ends, due to structural asymmetry of the filament (e.g. actin and microtubule). Filament polymerizes rapidly in the plus end.

- Polymerization with unequal rates and nucleotide hydrolysis reaction (T and D refer to NTP and NDP state of the monomer) at filament termini (actin and microtubule).

- In addition to polymerization with unequal rates plus nucleotide hydrolysis, monomers can be added or subtracted on any place within the filament.
1. Polymerization with Same on and off Rates on both Ends

\[ P_n + P_1 \rightleftharpoons P_{n+1} \]

at equilibrium \( \frac{dP_n}{dt} = 0 \) where \( P_n \) is the length of a polymer with \( n \) monomers

\[ K_d = \frac{[P_n][P_1]}{[P_{n+1}]} = \frac{k_{off}}{k_{on}} = \frac{1}{10 \text{ \mu M}^{-1} \text{s}^{-1}} \approx 0.1 \text{ \mu M} \text{ for actin} \]

when \( n = 1 \), \( K_d = \frac{[P_1][P_1]}{[P_2]} \Rightarrow [P_2] = \frac{[P_1]^2}{K_d} \)

when \( n = 2 \), \( K_d = \frac{[P_2][P_1]}{[P_3]} \Rightarrow [P_3] = \frac{[P_1]}{K_d} \left( \frac{[P_1]}{K_d} \right) = \frac{[P_1]^3}{K_d^2} \)

\[ [P_n] = \frac{[P_1]^n}{K_d^{n-1}} = K_d e^{n \ln([P_1]/K_d)} = K_d e^{-\alpha n} \text{ where } \alpha = -\ln([P_1]/K_d) \]

if \( [P_1] < K_d \), the distribution is the decreasing function of filament length.

the average length \( \langle n \rangle = \frac{\int_0^\infty nK_d e^{-\alpha n} dn}{\int_0^\infty K_d e^{-\alpha n} dn} = \frac{1}{\alpha} = -\frac{1}{\ln([P_1]/K_d)} = \frac{1}{\ln(K_d/[P_1])} \)

- We make the assumption that the only way a given filament can change its length is by either the addition or loss of a single monomer.
- We reject the process in which 4mer and 5mer filaments fuse together to make a 9mer.
Polymer Length Fluctuates in Time at Equilibrium

• Question: A system in equilibrium does not mean that it is static. How does the length of a polymer vary in time as a result of the shrinkage and growth?

the probability of growth by length $a$ in time $\Delta t$ \[ P(a) = k_{on} c_0 \Delta t \]

the probability of shrinkage by length $a$ in time $\Delta t$ \[ P(-a) = k_{off} \Delta t \]

the probability of nothing happening is \[ P(0) = 1 - P(a) - P(-a) \]

\[ \langle x \rangle = aP(a) - aP(-a) \Rightarrow \frac{d \langle x \rangle}{dt} = a(k_{on} c_0 - k_{off}) \]

the system is at equilibrium when $c_0 = c^*$ \[ P(a) = P(-a) \] and $c^* = k_{off}/k_{on}$

\[ \langle x \rangle = aP(a) - aP(-a) = 0 \text{ no change in mean length} \]

\[ \langle x^2 \rangle = a^2 P(a) + (-a)^2 P(-a) = 2a^2 k_{off} \Delta t \]

accumulated variance over $N$ steps \[ \langle L^2 \rangle = 2Na^2 k_{off} \Delta t \]

\[ N = \frac{t}{\Delta t} \text{ so } \langle L^2 \rangle = 2a^2 k_{off} t \]
2. Polymerization with Different on and off Rates on Two Ends

- The rates on two ends of the polymer are different due to structural asymmetry.
- However, ratios of the rates on two ends must equal each other, because same free energy is associated with the contacts.

\[ \Delta G^+ = \Delta G^- \implies \frac{k_{\text{off}}^+}{k_{\text{on}}^+} = \frac{k_{\text{off}}^-}{k_{\text{on}}^-} = K_d = \frac{1}{V} e^{\Delta G / kT} \]

\[ \frac{dn_+}{dt} = k_{\text{on}}^+ c_0 - k_{\text{off}}^+ \]
\[ \frac{dn_-}{dt} = k_{\text{on}}^- c_0 - k_{\text{off}}^- \]

- Both ends suffer the same fate (have the same critical concentration)
- Slopes of growth and shrinkage curves are determined by the ratios of $k_{\text{on}}$ that one end has faster dynamics than the other.
Nucleotide Hydrolysis and Polymerization

- Actin monomer can bind either ATP or ADP, and tubulin monomers can bind either GTP or GDP.
- Monomers undergo conformational changes upon nucleotide hydrolysis and adjust the rates of binding and unbinding.
- There are different on and off rates for ATP- and ADP-bound monomers.
- ASSUMPTION: Only ATP-bound monomers are coming on and off in the plus end (fast dynamics) and only ADP-bound monomers are coming on and off in the minus end (slow dynamics).
- In this case, the rate equations we derived are the same without the restriction of equality of the ratios of on and off rates.
- The two ends of the filament do not necessarily have the same fate, one can grow and other can shrink.
Filament Treadmilling

- In treadmilling, one end grows and the other end shrinks at the same rate.
- For a critical concentration $c_{TM}$, the plus end grows at the same rate the minus end shrinks.

\[
\frac{dn_+}{dt} = - \frac{dn_-}{dt}
\]

\[
k^+_on c_{TM} - k^+_off = -(k^-_on c_{TM} - k^-_off)
\]

\[
c_{TM} = \frac{k^+_off + k^-_off}{k^+_on + k^-_on}
\]
Dynamic Instability of Microtubules

- Dynamics of microtubules are punctuated by rapid shrinkage events, termed as catastrophe.
- Dynamic instability allows microtubule to rapidly grow and shrink, which may be useful in finding the targets during chromosome segregation.
- Dynamic instability can be modeled by an assumption that the growing filament has a cap of GTP bound monomers.
- The length of the cap is determined by a competition between monomer addition and hydrolysis rates.
- When the cap disappears, the filaments undergo catastrophe.
- NOTE: actin and microtubule dynamics are also modulated by nucleators, end capping proteins, filament depolymerizing enzymes, drugs, inhibitors etc.

http://www.youtube.com/watch?v=E1XczyCkN20&feature=results_main&playnext=1&list=PLB710ADD69770D527
Rate Equation Description of Filament Polymerization

- Assume each filament is identical.
- $M$ is the number of filaments
- $n$ is the number of subunits per filament
- $V$ is the volume of solution.

\[
\frac{dn}{dt} = k_{on}c(t) - k_{off}
\]

\[
\frac{dn}{dt} = k_{on}\left(c_0 - n(t)\frac{M}{V}\right) - k_{off} \quad \text{and} \quad n(0) = 0
\]

\[
n(t) = \frac{V}{Mk_{on}}(k_{on}c_0 - k_{off})e^{-k_{on}Mt/V}
\]
A Model for Dynamic Instability

- The monomers in the cap is not yet hydrolyzed.
- Hydrolysis front moves at a constant velocity, depending on the GTP hydrolysis rate.
- Growing tip moves at a different velocity, depending on the on rate and concentration.
- The catastrophe occurs when the hydrolysis front catches up the growing tip (e.g. the cap disappears).
- At a critical length of the polymer ($x_{tip}$), velocity of growth of the leading tip becomes equal to the speed of hydrolysis front.

\[
\frac{dx_{tip}}{dt} = \frac{a}{\tau}
\]

- $a$ is monomer length, $\tau$ is lifetime of hydrolysis.
\[
\frac{dx_{tip}}{dt} = a \frac{dn}{dt} = a \left[ k_{on} \left( c_0 - \frac{x_{tip} M}{a V} \right) - k_{off} \right]
\]

\[
x_{tip}(t) = \frac{aV}{M k_{on}} (k_{on} c_0 - k_{off}) e^{-k_{on} M t / V}
\]

At a critical length

\[
\frac{dx_{tip}}{dt} = \frac{a}{\tau}
\]

\[
a \left[ k_{on} \left( c_0 - \frac{x_{tip} M}{a V} \right) - k_{off} \right] = \frac{a}{\tau}
\]

\[
x_{tip-crit} = \frac{aV}{M k_{on}} \left( k_{on} - k_{off} - \frac{1}{\tau} \right)
\]

To find waiting time between catastrophes

\[
x_{tip-crit} = x_{tip}(t)
\]

\[
t = \frac{V}{M k_{on}} \ln(\tau(k_{on} c_0 - k_{off}))
\]