PHYS 4xx Polymerization of actin and tubulin

Simple polymerization

• definitions: ends of filament are equivalent; \( n \) = number of monomers in a single filament; \( t \) = time; \([M]\) = concentration of free monomer in solution

• capture rate of monomers by a single filament is proportional to the number of monomers available for capture
  \[
  \frac{dn}{dt} = +k_{on} [M]
  \]
  (capture) \hfill (1)

• \( k_{on} \) = capture rate constant, with units of \([\text{concentration} \cdot \text{time}]^{-1}\)

• release rate does not depend on \([M]\)
  \[
  \frac{dn}{dt} = -k_{off}
  \]
  (release) \hfill (2)

• \( k_{off} \) has units of \([\text{time}]^{-1}\)

• net change of filament size is
  \[
  \frac{dn}{dt} = +k_{on} [M] - k_{off}
  \]
  \hfill (3)

• obtain \( k_{on} \) and \( k_{off} \) from a plot of \( dn/dt \) against \([M]\)

• \( dn/dt < 0 \) --- filament is shrinking

• minimum concentration for filament growth (often called the critical concentration) occurs at \( dn/dt = 0 \), where
  \[
  [M]_c = k_{off} / k_{on}
  \]
  \hfill (4)

Effects of hydrolysis

• if filament ends are chemically inequivalent, the rate constants may be different

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• to measure rate constants, fix one end of the filament, or identify which end is which
• measured values depend on nature of solution (i.e. salt concentration)
• sample values for actin filaments (Pollard, 1986) and microtubules (Walker et al., 1988)
• units are (µM·sec)$^{-1}$ for $k_{on}$, sec$^{-1}$ for $k_{off}$, and µM for $[M]_c$

<table>
<thead>
<tr>
<th></th>
<th>$k_{on}^+$ (plus end)</th>
<th>$k_{off}^+$</th>
<th>$k_{on}^-$ (minus end)</th>
<th>$k_{off}^-$</th>
<th>$[M]_c^+$</th>
<th>$[M]_c^-$</th>
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<tbody>
<tr>
<td>actin</td>
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<tr>
<td>ATP-actin</td>
<td>11.6±1.2</td>
<td>1.4±0.8</td>
<td>1.3±0.2</td>
<td>0.8±0.3</td>
<td>0.12±0.07</td>
<td>0.6±0.17</td>
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<tr>
<td>ADP-actin</td>
<td>3.8</td>
<td>7.2</td>
<td>0.16</td>
<td>0.27</td>
<td>1.9</td>
<td>1.7</td>
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<tr>
<td>microtubules</td>
<td></td>
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<tr>
<td>growing (GTP)</td>
<td>8.9±0.3</td>
<td>44±14</td>
<td>4.3±0.3</td>
<td>23±9</td>
<td>4.9±1.6</td>
<td>5.3±2.1</td>
</tr>
<tr>
<td>rapid</td>
<td>0</td>
<td>733±23</td>
<td>0</td>
<td>915±72</td>
<td>not applicable</td>
<td></td>
</tr>
<tr>
<td>disassembly</td>
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• for both triphosphate and diphosphate monomer, the capture and release rates are almost always larger at the plus end than at the minus end
• the capture rates of the triphosphate are larger than the diphosphate at both ends
• $k_{off}/k_{on}$ gives the critical concentration $[M]_c$

**Treadmilling** (Wegner, 1976)

• simplest example: 2 x 2 inequivalent rate contants (+/- refer to the filament end)

\[
\begin{align*}
\frac{dn^+}{dt} &= k_{on}^+[M] - k_{off}^+ \\
\frac{dn^-}{dt} &= k_{on}^-[M] - k_{off}^- 
\end{align*}
\]

(5a) (5b)

• take $[M]$ to be the concentration of free triphosphate proteins
• may be different critical concentration at each end

\[
[M]_{c+} = k_{off}^+ / k_{on}^+ \quad [M]_{c-} = k_{off}^- / k_{on}^-
\]

(6)
Left side: if \([\mathcal{M}]_c^+ = [\mathcal{M}]_c^-\) (as in tubulin) both ends grow or both ends shrink simultaneously.

Right side: special case occurs when \(\frac{dn^+}{dt} = -\frac{dn^-}{dt}\): one end of the filament grows at the same rate as the other shrinks; this occurs at steady state value \([\mathcal{M}]_{ss}\):

\[\frac{[\mathcal{M}]_{ss}}{K_{off}^+ + K_{off}^-} = \frac{K_{on}^+ + K_{on}^-}{K_{on}^+ + K_{on}^-}\] (7)

- using data above for actin:
  \([\mathcal{M}]_{ss} = 0.17 \mu M\) and \(\frac{dn^+}{dt} = 0.6\)
- direct measure of \([\mathcal{M}]_{ss}\) under not dissimilar solution conditions yields 0.16 \(\mu M\) (Wegner, 1982)

Dynamic instability of microtubules:

- growth rate: say we use \(K_{on} = 10 \text{ (\mu M}\cdot\text{s}^{-1})\) \([\mathcal{M}] = 10 \mu M\) \(K_{off} = 40 \text{ s}^{-1}\)
  \(\Rightarrow\) \(\frac{dn}{dt} = 10 \cdot 10 - 40 = 60 \text{ s}^{-1}\)
  \(\Rightarrow\) change in length = 60 \(\cdot\) (8 nm/dimer) / 13 protofilaments = 37 nm/s = 0.04 \(\mu m/s\)

- shrinkage rate: if \(K_{off} \sim 800 \text{ s}^{-1}\)
  \(\Rightarrow\) change in length = 800 \(\cdot\) (8 nm/dimer) / 13 protofilaments = 490 nm/s = 0.5 \(\mu m/s\)

- microtubules display dramatic changes in length (from Horio and Hotani, 1986)

- frequency of advance/retreat phases is given in the text
Benchmark for $k_{\text{off}}$

- disassembly is sequential if a monomer diffuses away before the next monomer is released
- for diffusion in three dimensions
  \[ <x^2> = 6Dt \quad (D \text{ is diffusion constant}) \]
- choose $D \sim 10^{-12}$ m$^2$/s, as described in our first lecture on mobility
- if $<x^2>^{1/2} = 25$ nm diameter of a microtubule, $t = (2.5 \times 10^8)^2 / 6 \cdot 10^{-12} \sim 10^{-4} \text{ seconds}$
- corresponds to $k_{\text{off}} \sim 10,000 \text{ sec}^{-1}$
- perhaps an order of magnitude high for microtubules, and way too fast for actin, but shows that MT disassembly is not that far from being cooperative

Benchmark for $k_{\text{on}}$

- consider the free-monomer concentration profile near the filament end
- integrate monomer flux to get capture rate
  \[ k_{\text{on}} = 4\pi D (R_A + R_B) \]
- example. say: $R_A + R_B = 12 + 6 = 18$ nm \quad $D = 10^{-12}$ m$^2$/s
  \[ \implies k_{\text{on}} = 4\pi \cdot 10^{-12} \cdot 18 \times 10^{-9} = 2.3 \times 10^{-19} \text{ m}^3$/s
- unit conversion: $1 \mu\text{M} = 10^{-6} \times 6 \times 10^{23}$ / litre
  \[ = 10^{-6} \times 6 \times 10^{23} \times 10^3 / \text{m}^3 = 6 \times 10^{20} / \text{m}^3 \]
  \[ \implies k_{\text{on}} = 2.3 \times 10^{-19} \cdot 6 \times 10^{20} (\mu\text{M} \cdot \text{m}^3)^{-1} \text{ m}^3$/s = 138 (\mu\text{M} \cdot \text{s})^{-1} \text{ for microtubules} \]
- this is larger than the observed values by about an order of magnitude; does not include rotational diffusion for orientation of the incoming protein