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

$$dn/dt = +k_{on}\,[M] \qquad \text{(capture)} \qquad (1)$$

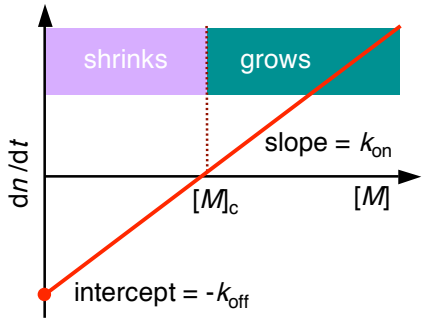
- k_{on} = capture rate constant, with units of $[concentration \cdot time]^{-1}$
- release rate does not depend on $[M]$

$$dn/dt = -k_{off} \qquad \text{(release)} \qquad (2)$$

- k_{off} has units of $[time]^{-1}$
- net change of filament size is

$$dn/dt = +k_{on}\,[M] - k_{off} \qquad (3)$$

- obtain k_{on} and k_{off} from a plot of dn/dt against $[M]$

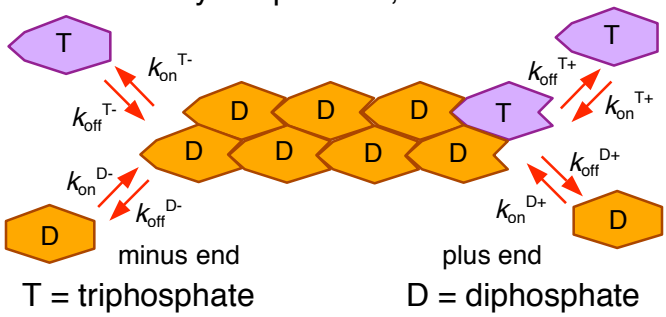


- $dn/dt < 0$ ---> filament is shrinking
- minimum concentration for filament growth (often called the *critical concentration*) occurs at $dn/dt = 0$, where

$$[M]_c = k_{off} / k_{on} \qquad (4)$$

Effects of hydrolysis

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



- 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 $(\mu M \cdot sec)^{-1}$ for k_{on}, sec^{-1} for k_{off}, and μM for $[M]_c$

monomer in solution	k_{on}^+	k_{off}^+ (*plus end*)	k_{on}^-	k_{off}^- (*minus end*)	$[M]_c^+$	$[M]_c^-$
actin						
ATP-actin	11.6±1.2	1.4±0.8	1.3±0.2	0.8±0.3	0.12±0.07	0.6±0.17
ADP-actin	3.8	7.2	0.16	0.27	1.9	1.7
microtubules						
growing (GTP)	8.9±0.3	44±14	4.3±0.3	23±9	4.9±1.6	5.3±2.1
rapid disassembly	0	733±23	0	915±72	not applicable	

- 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)

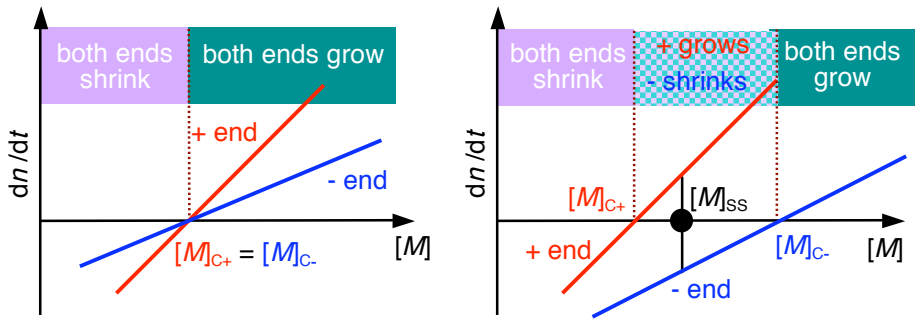
- simplest example: 2 x 2 inequivalent rate contants (+/- refer to the filament end)

$$dn^+/dt = k_{on}^+ [M] - k_{off}^+ \quad (5a)$$

$$dn^-/dt = k_{on}^- [M] - k_{off}^- \quad (5b)$$

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

$$[M]_c^+ = k_{off}^+ / k_{on}^+ \qquad [M]_c^- = k_{off}^- / k_{on}^- \quad (6)$$



Left side: if $[M]_c^+ = [M]_c^-$ (as in tubulin) both ends grow or both ends shrink simultaneously

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

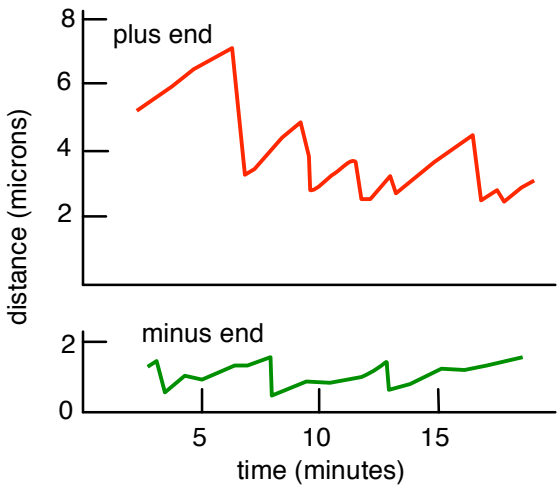
$$[M]_{ss} = (k_{off}^+ + k_{off}^-) / (k_{on}^+ + k_{on}^-) \qquad (7)$$

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

Dynamic instability of microtubules

- growth rate: say we use $k_{on} = 10\ (\mu M \cdot s)^{-1}$ $[M] = 10\ \mu M$ $k_{off} = 40\ s^{-1}$
  --> $dn/dt = 10 \cdot 10 - 40 = 60\ s^{-1}$
  --> change in length = 60 • (8 nm/dimer) / 13 protofilaments = 37 nm/s
  = 0.04 μm/s

- shrinkage rate: if $k_{off} \sim 800\ s^{-1}$
  --> change in length = 800 • (8 nm/dimer) / 13 protofilaments = 490 nm/s
  = 0.5 μm/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_{off}

- disassembly is sequential if a monomer diffuses away before the next monomer is released
- for diffusion in three dimensions
  $\langle x^2 \rangle = 6Dt$ (D is diffusion constant)

- choose $D \sim 10^{-12}$ m^2/s, as described in our first lecture on mobility
- if $\langle x^2 \rangle^{1/2}$ = 25 nm diameter of a microtubule, $t = (2.5 \times 10^{-8})^2 / 6 \cdot 10^{-12} \sim 10^{-4}$ seconds
- corresponds to $k_{off} \sim 10{,}000$ 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_{on}

- consider the free-monomer concentration profile near the filament end
- integrate monomer flux to get capture rate
  $k_{on} = 4\pi D (R_A + R_B)$

- example. say: $R_A + R_B = 12 + 6 = 18$ nm $D = 10^{-12}$ m^2/s
  ---> $k_{on} = 4\pi \cdot 10^{-12} \cdot 18 \times 10^{-9} = 2.3 \times 10^{-19}$ m^3/s

- unit conversion: 1 μM = $10^{-6} \times 6 \times 10^{23}$ / litre
  $= 10^{-6} \times 6 \times 10^{23} \times 10^3$ / m^3 = 6×10^{20} / m^3

  ---> $k_{on} = 2.3 \times 10^{-19} \cdot 6 \times 10^{20}$ (μM•m^3)$^{-1}$ m^3/s = 138 (μM•s)$^{-1}$ 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