

POLYMERIZATION OF ACTIN AND TUBULIN*(Mechanics of the Cell, Sec. 9.2)*

To be covered:

- rates of actin polymerization; treadmilling
- rates of microtubule growth and retreat
- diffusion-based expectations for rate constants

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

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

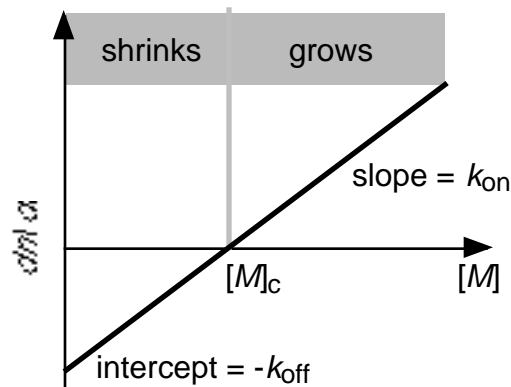
- k_{on} = capture rate constant, with units of $[\text{concentration} \cdot \text{time}]^{-1}$
- release rate does not depend on $[M]$

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

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

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

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

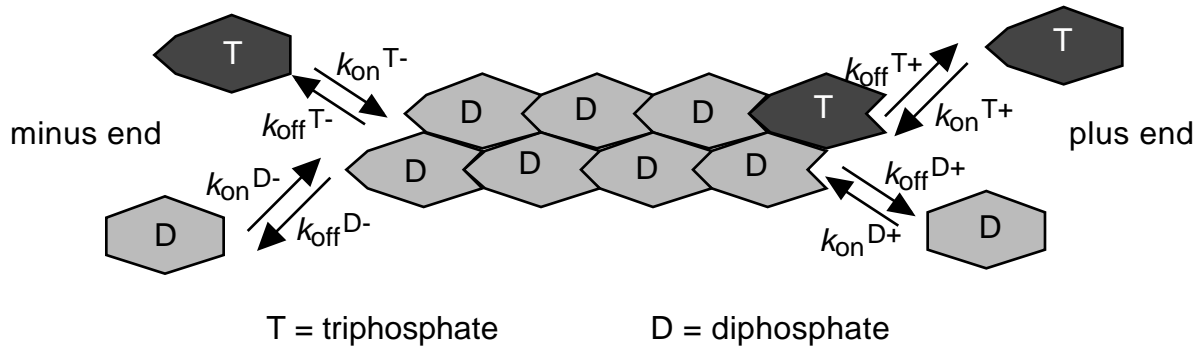


- $dn/dt < 0$ ---> filament is shrinking
- minimum concentration for filament growth (often called the *critical concentration*) occurs at $dn/dt = 0$, where

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

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

<i>monomer in solution</i>	k_{on}^+ (plus end)	k_{off}^+	k_{on}^- (minus end)	k_{off}^-	$[M]_c^+$	$[M]_c^-$
<i>actin</i>						
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
<i>microtubules</i>						
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
- $k_{\text{off}}/k_{\text{on}}$ gives the critical concentration $[M]_c$

Treadmilling (Wegner, 1976)

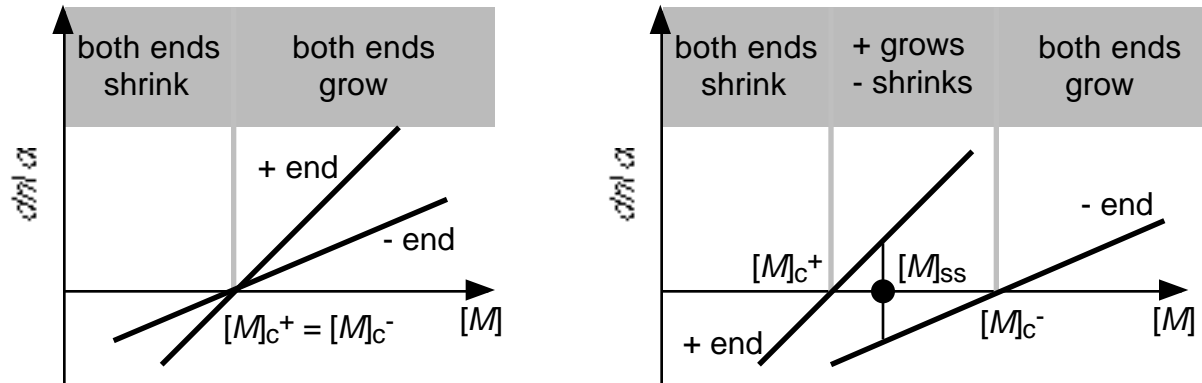
- simplest example: 2 x 2 inequivalent rate constants (+/- refer to the filament end)

$$dn^+/dt = k_{\text{on}}^+ [M] - k_{\text{off}}^+ \tag{5a}$$

$$dn^-/dt = k_{\text{on}}^- [M] - k_{\text{off}}^- \tag{5b}$$

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

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



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

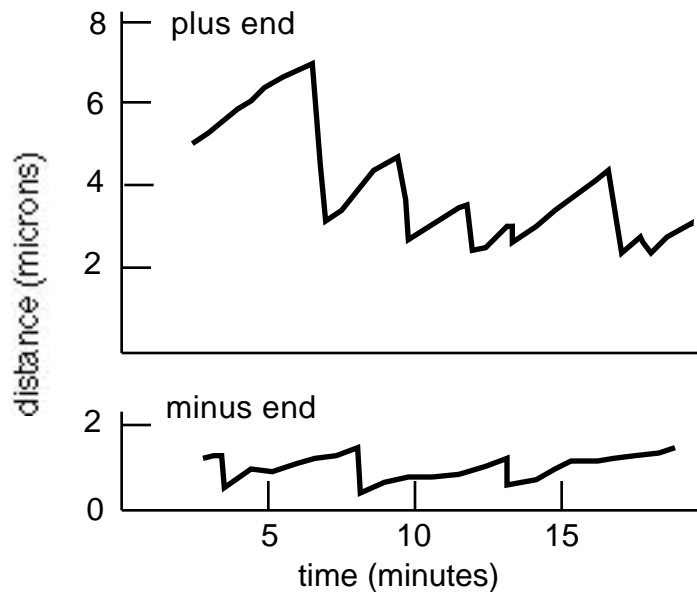
Right side: special case occurs when $dn^+ / dt = -dn^- / dt$: one end of the filament grows at the same rate as the other shrinks; this occurs at steady state value $[M]_{ss}$

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

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

Dynamic instability of microtubules

- growth rate: say we use $k_{on} = 10 (\mu\text{M}\cdot\text{s})^{-1}$ $[M] = 10 \mu\text{M}$ $k_{off} = 40 \text{ s}^{-1}$
 --> $dn / dt = 10 \cdot 10 - 40 = 60 \text{ s}^{-1}$
 --> change in length = $60 \cdot (8 \text{ nm/dimer}) / 13 \text{ protofilaments} = 37 \text{ nm/s}$
 = $0.04 \mu\text{m/s}$
- shrinkage rate: if $k_{off} \sim 800 \text{ s}^{-1}$
 --> change in length = $800 \cdot (8 \text{ nm/dimer}) / 13 \text{ protofilaments} = 490 \text{ nm/s}$
 = $0.5 \mu\text{m/s}$
- microtubules display dramatic changes in length (from Horio and Hotani, 1986)



- frequency of advance/retreat phases is given in the text

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

- consider the free-monomer concentration profile near the filament end
- integrate monomer flux to get capture rate
 $k_{on} = 4 D (R_A + R_B)$
- example. say: $R_A + R_B = 12 + 6 = 18 \text{ nm}$ $D = 10^{-12} (\text{m}^2 \cdot \text{s})^{-1}$
 $\rightarrow k_{on} = 4 \cdot 10^{-12} \cdot 18 \times 10^{-9} = 2.3 \times 10^{-19} \text{ m}^3/\text{s}$
 $= 2.3 \times 10^{-19} \cdot (6 \times 10^{20} \mu\text{M} / \text{m}^3) \text{ m}^3/\text{s} = 138 (\mu\text{M} \cdot \text{s})^{-1}$ 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
- unit conversion: micromolar = $10^{-6} \times 6 \times 10^{23} / \text{litre}$
 $= 10^{-6} \times 6 \times 10^{23} \times 10^3 / \text{m}^3 = 6 \times 10^{20} / \text{m}^3$