Lectures 19:
Biological Membranes: Life in Two Dimensions (contd.)

Lecturer:
Brigita Urbanc
Office: 12-909
(*E-mail: *brigita@drexel.edu)

Course website:
www.physics.drexel.edu/~brigita/COURSES/BIOPHYS_2011-2012/

Vesicle formation/shedding is assisted by budding proteins: clathrin, COPI, COPII (vesicle *chaperones*)

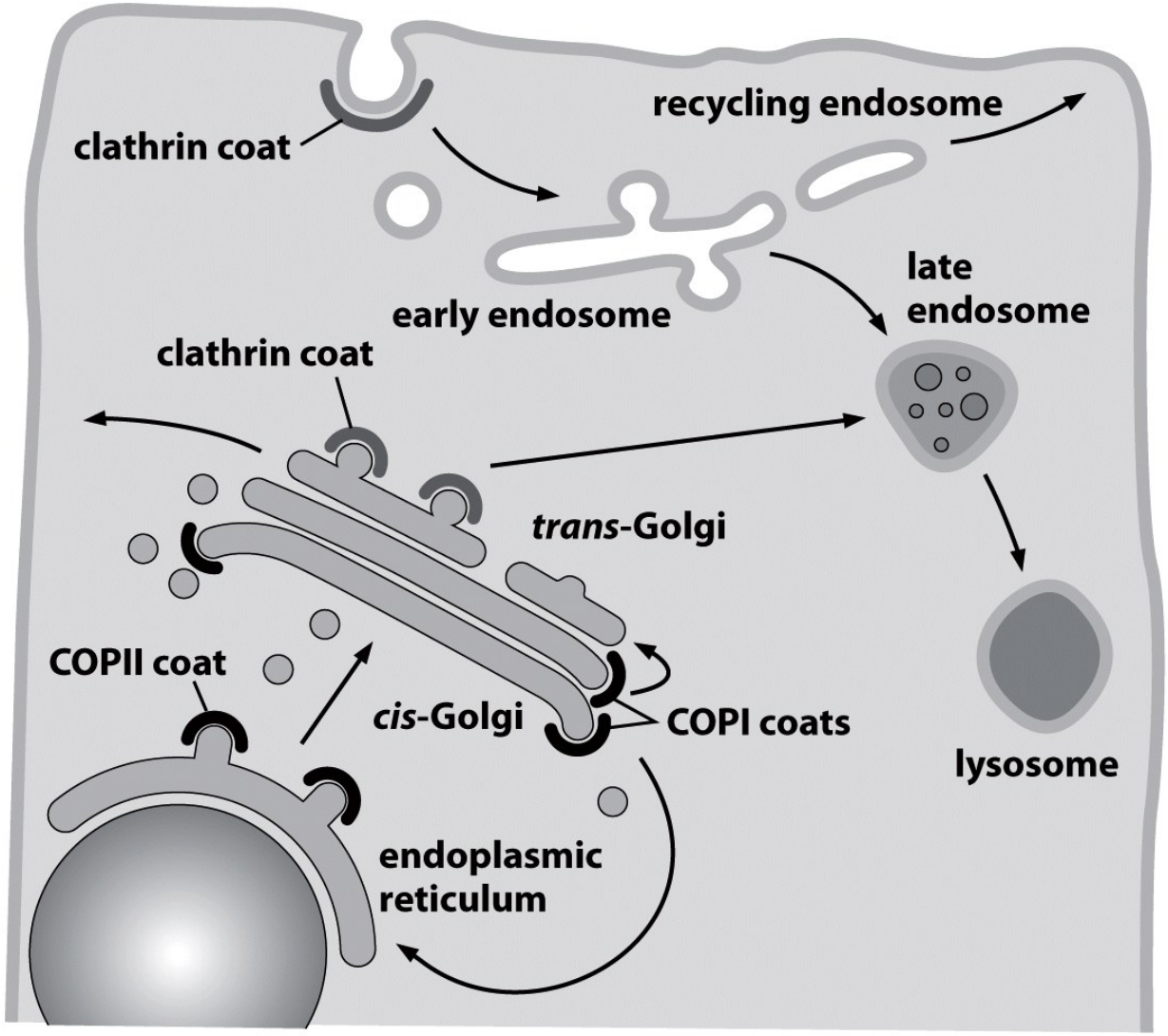


Figure 11.29 Physical Biology of the Cell (© Garland Science 2009)

Clathrin Structure: From Individual Triskelions to Closed Cages

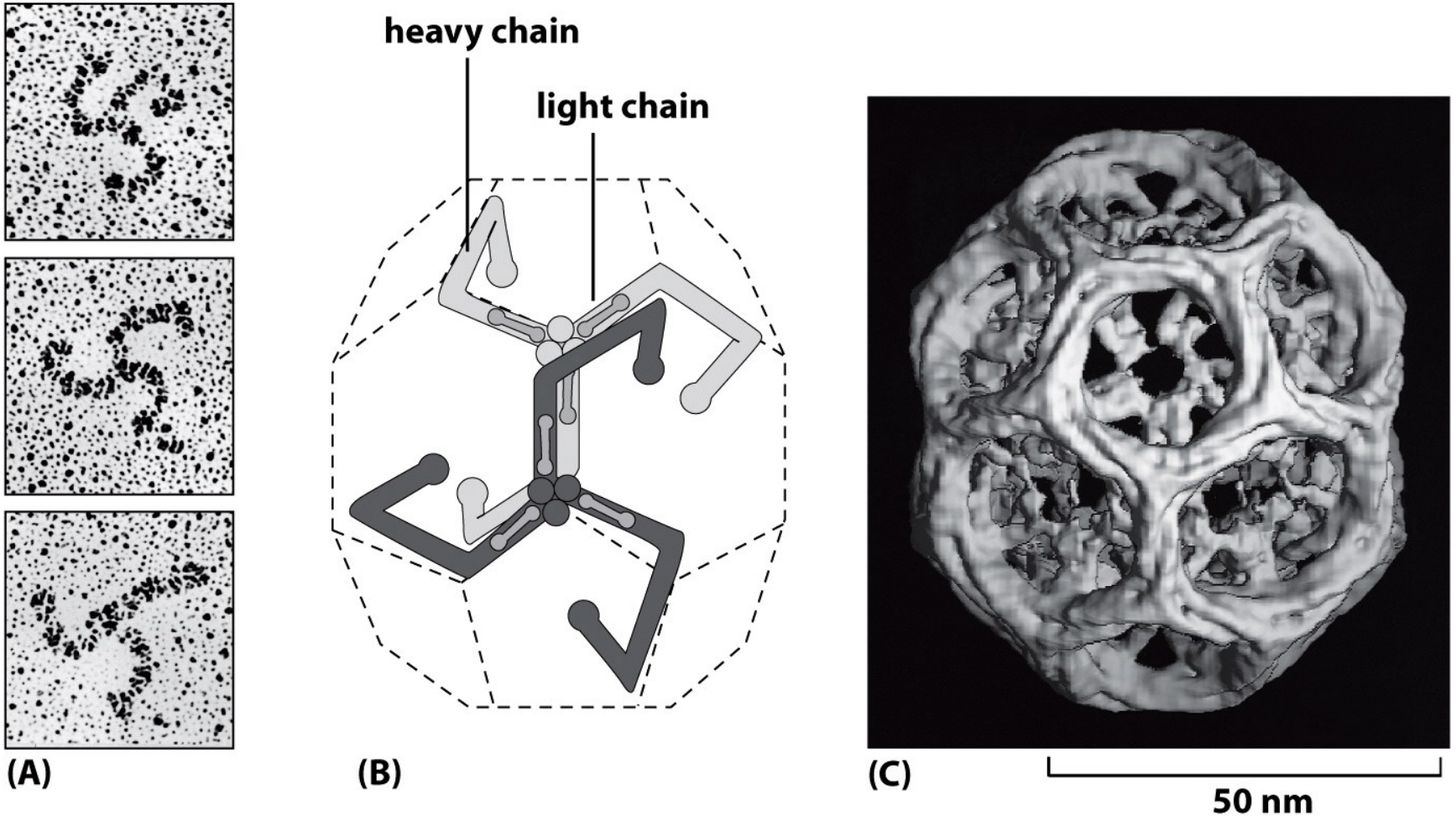


Figure 11.30 Physical Biology of the Cell (© Garland Science 2009)

Vesicle curvature induced by protein coating/self-assembly (energetically a *downhill reaction*)

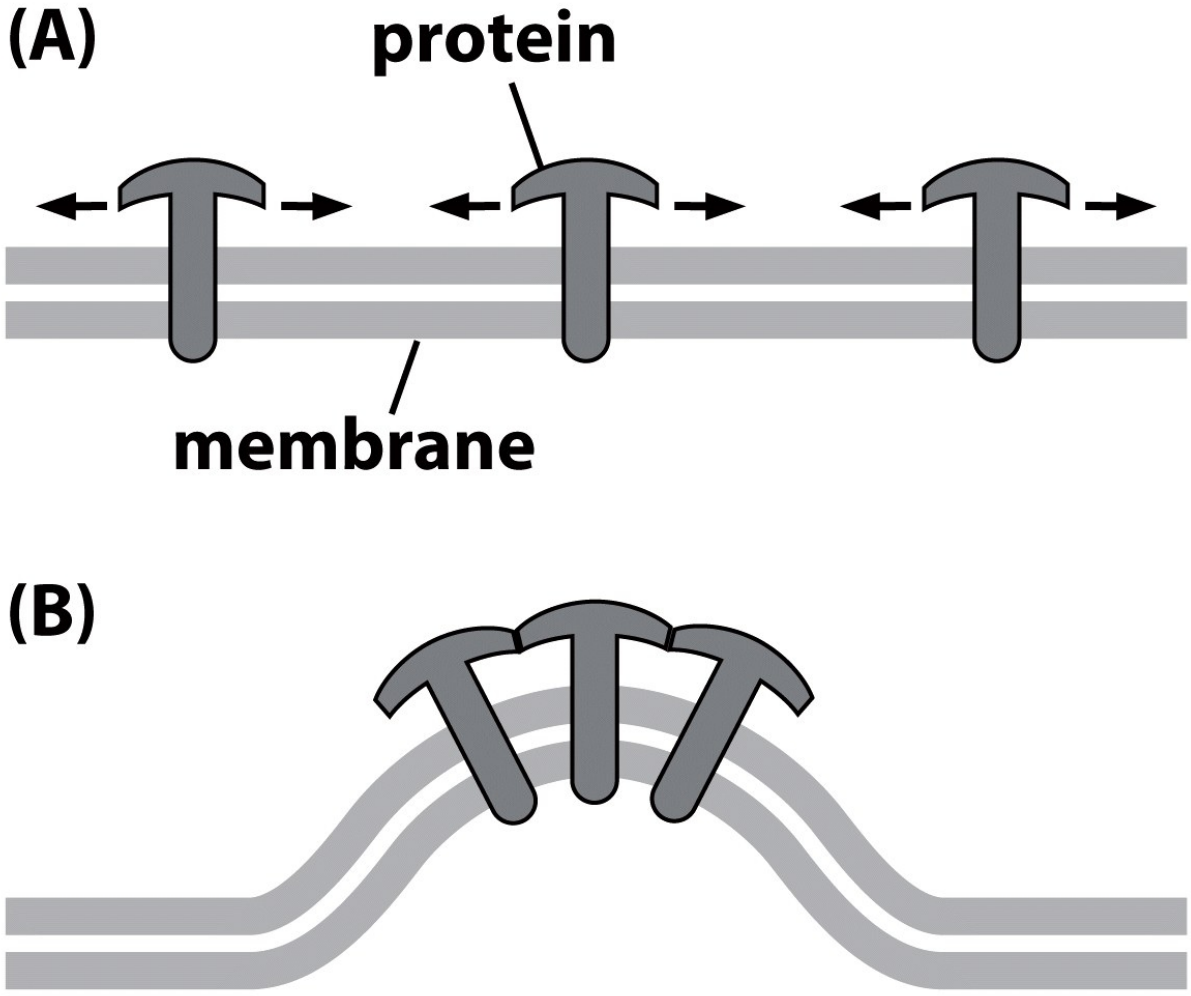


Figure 11.31 Physical Biology of the Cell (© Garland Science 2009)

Vesicle fusion catalyzed by proteins

A) specialized SNARE proteins help vesicles fuse with their target membrane

B) fusion of the influenza virus into the host cell via viral hemagglutinin protein undergoing a pH-induced change

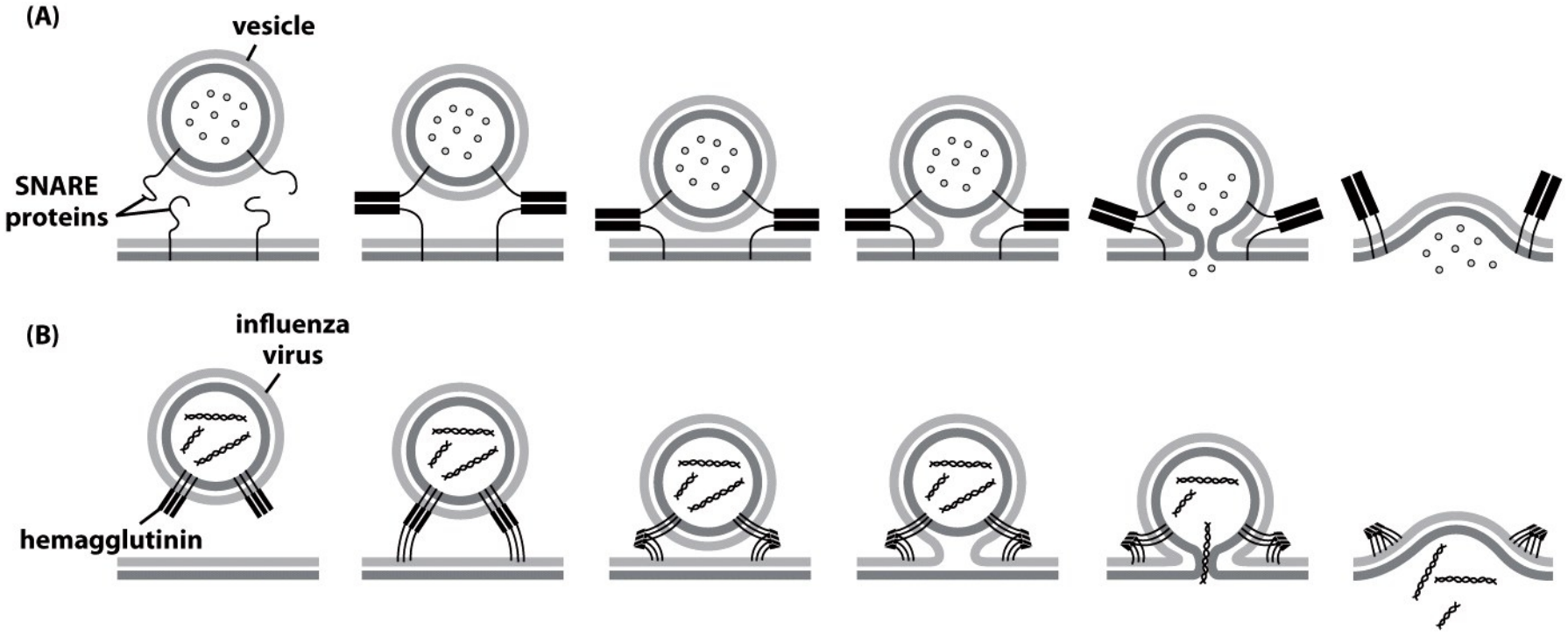
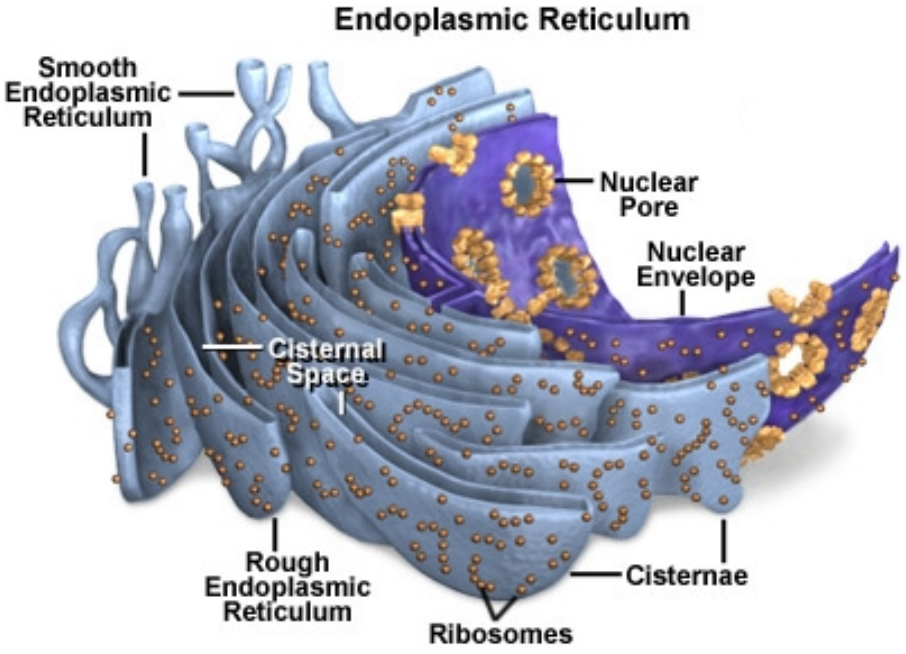
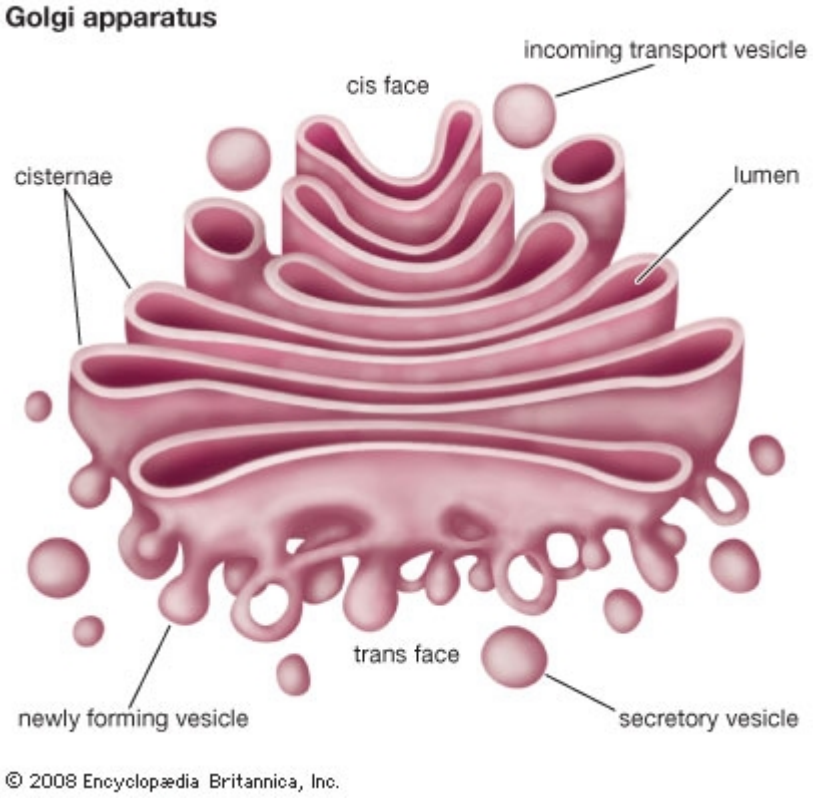


Figure 11.32 Physical Biology of the Cell (© Garland Science 2009)

Membranes and Shapes

Cell specialization resulted in diverse shapes of cells and their membranes: from spherical vesicles to tubular networks as in ER and pancake-like cisternae as in the Golgi apparatus.





Which biophysical interactions are responsible for the variety of shapes?

bacterium

Purkinje cell in mamallian cerebellum

magnetosomes

Parietal cells from the stomach

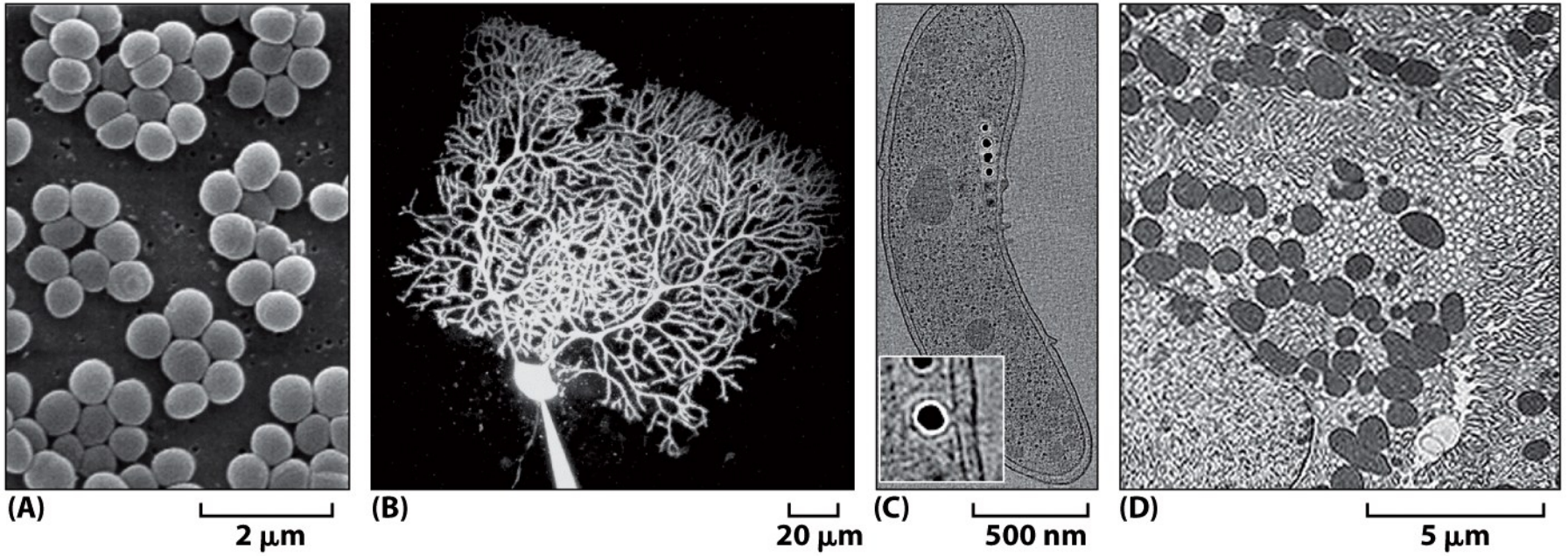


Figure 11.33 Physical Biology of the Cell (© Garland Science 2009)

Reconstruction of organelle shapes in a cultured pancreatic cell from serial thin section electron microscopy images

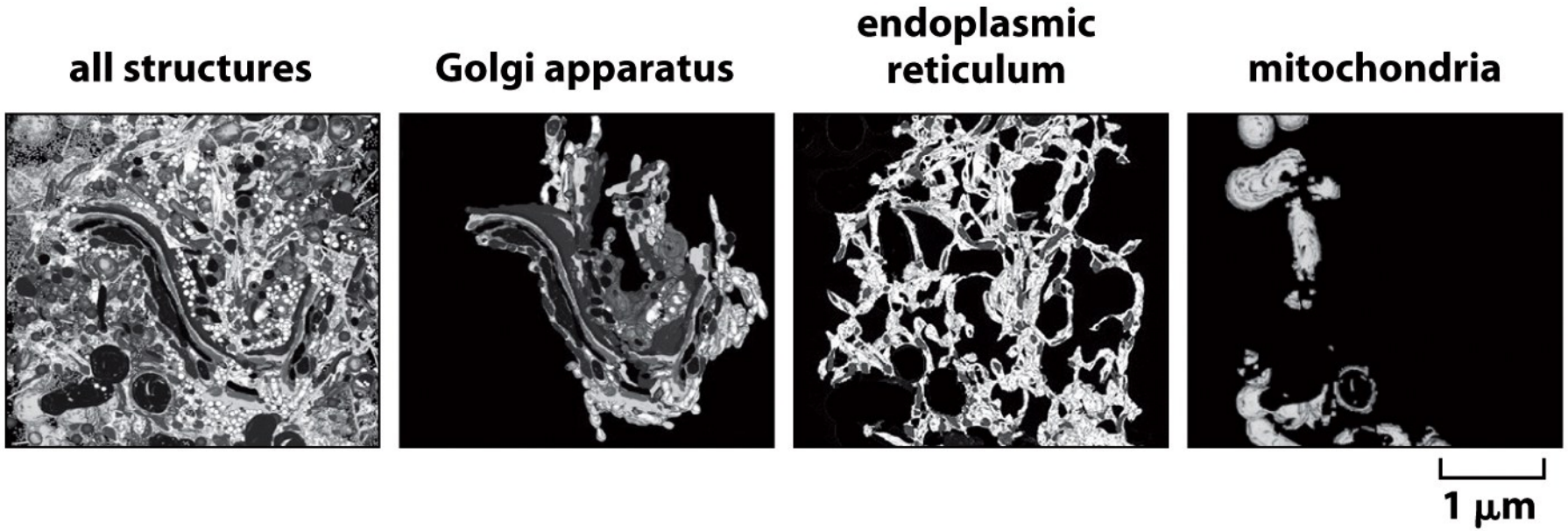


Figure 11.34 Physical Biology of the Cell (© Garland Science 2009)

Endoplasmic Reticulum

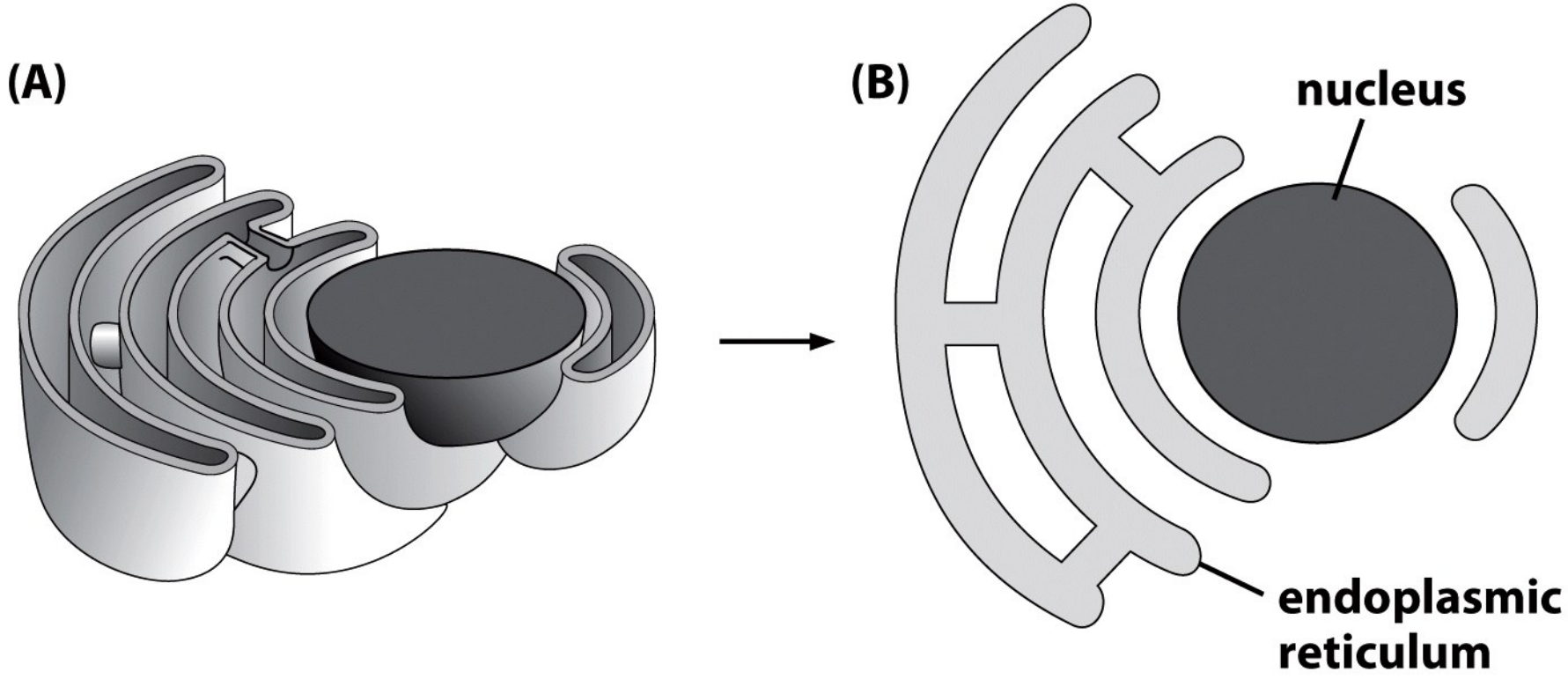


Figure 11.36 Physical Biology of the Cell (© Garland Science 2009)

Mitochondrion structure

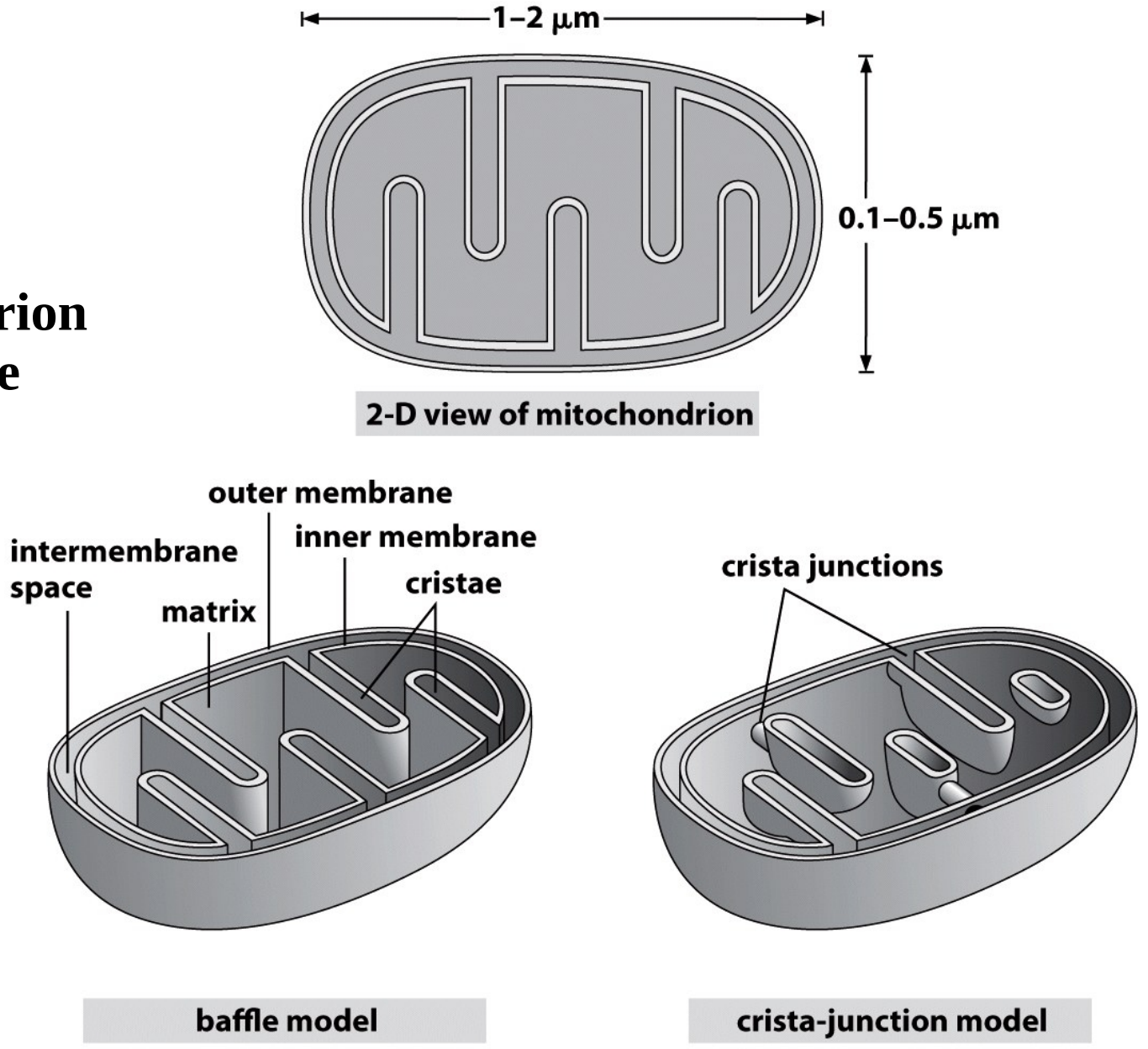


Figure 11.37 Physical Biology of the Cell (© Garland Science 2009)

Sizing up a mitochondrion:

- **shape: spherocylinder**
- **average length 1 mm**
- **average diameter 0.8 mm**
- **volume:**

$$\Omega_{\text{mito}} = \frac{4}{3}\pi r^3 + \pi r^2 h$$

$$\Omega_{\text{mito}} \approx 0.4\,\mu\text{m}^3$$

- **outer membrane area:**

$$A_{\text{mito}}^{\text{outer}} = 4\pi r^2 + 2\pi r h$$

$$A_{\text{mito}}^{\text{outer}} \approx 2.5\,\mu\text{m}^2$$

- **inner membrane area:**

$$A_{\text{mito}}^{\text{inner}} = A_{\text{mito}}^{\text{outer}} + 2N_{\text{disk}}A_{\text{disk}}$$

$$A_{\text{mito}}^{\text{inner}} \approx 20\,\mu\text{m}^2 \gg A_{\text{mito}}^{\text{outer}}$$

$$N_{\text{disk}} \approx 15$$

cryo-electron microscopy image

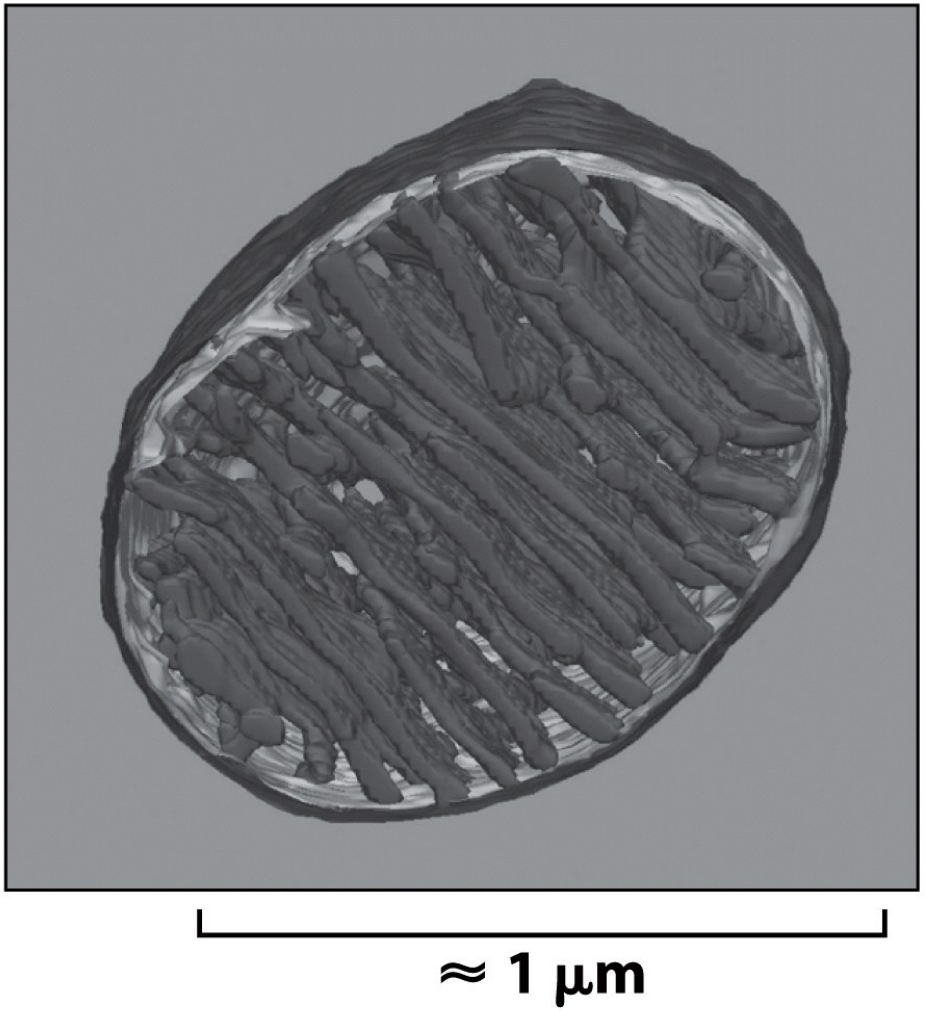


Figure 11.35 Physical Biology of the Cell (© Garland Science 2009)

Animal cells contain cytoskeletal network of filaments which provides the cell with a consistency to support shear stresses typical for solids (not liquids).

Example: a red blood cell that can adopt many different shapes.

How do we model a variety of red blood cell shapes?

- free energy of bending
- account for the fact than the inner and outer membrane differ in area
- include the presence of the under-lying cytoskeletal networks

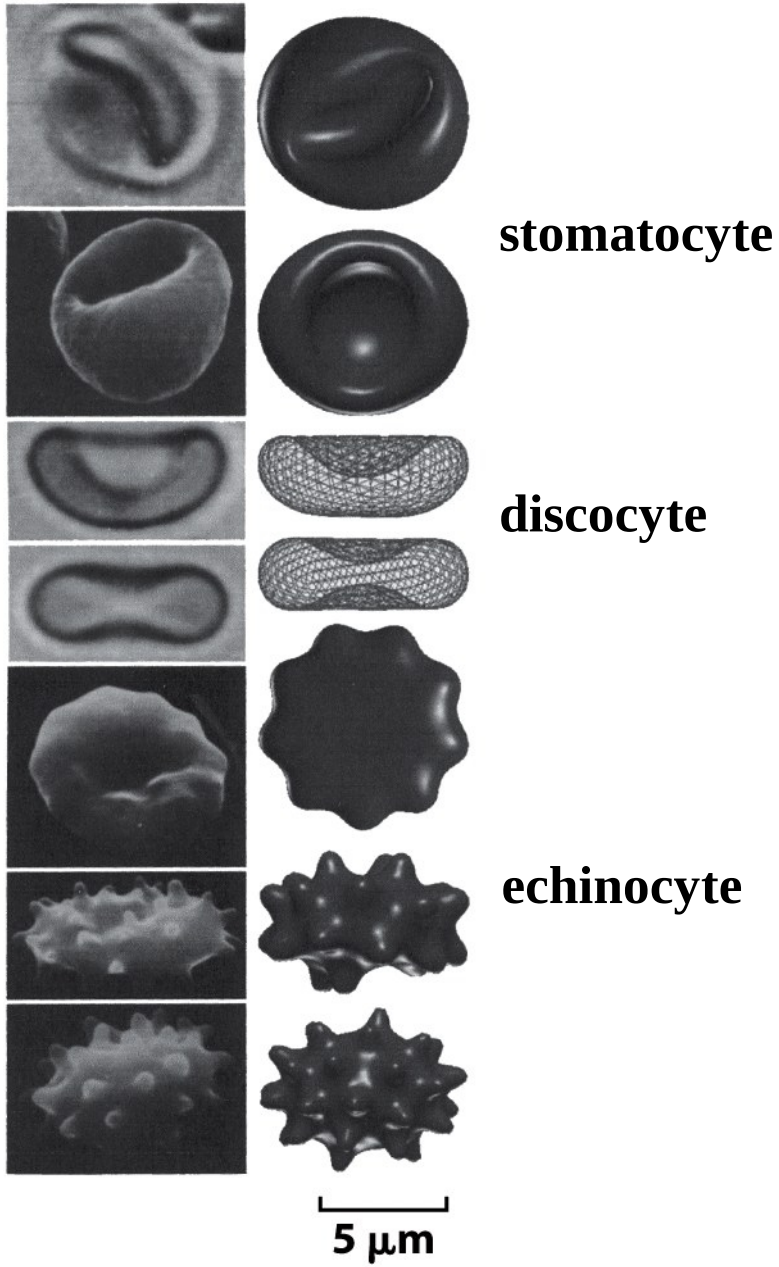


Figure 11.38 Physical Biology of the Cell (© Garland Science 2009)

The Active Membrane with Mechanosensitive Ion Channels

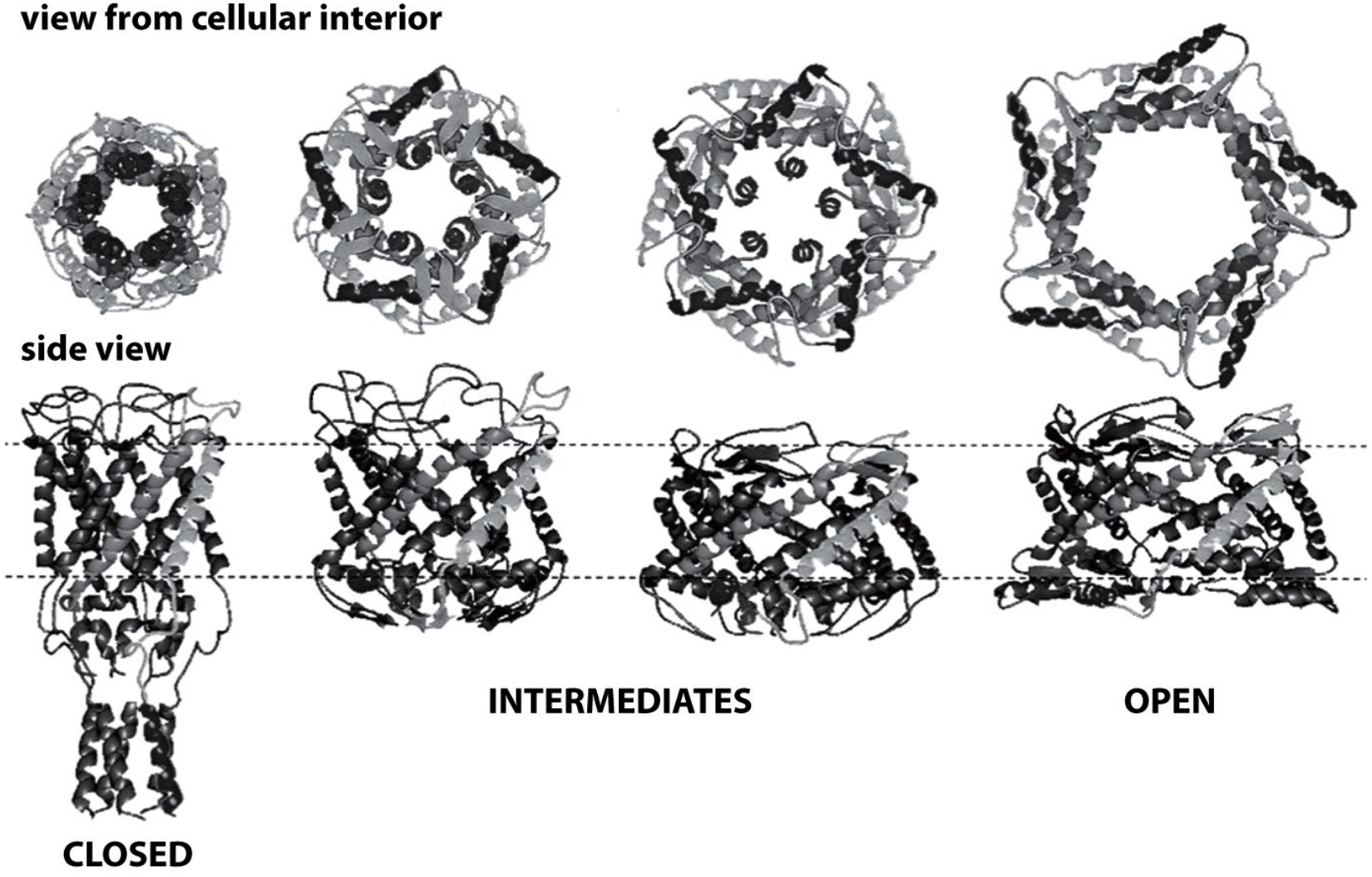


Figure 11.39 Physical Biology of the Cell (© Garland Science 2009)

What is a mechanosensitive ion channel?

- in bacteria, these channels respond by opening when osmotic pressure in a cell suddenly increases, thus protecting the cell from bursting and rupturing the membrane

- consider a model system consisting of the mechanosensitive channel of large conductance (MscL)

- examine the way gating is tied to membrane deformations and external tension

- key observable: the open probability as a function of the applied pressure

- note: the open probability depends upon the lengths of lipid tails of the membrane (membrane composition)!

Open probability for MscL embedded into different membranes

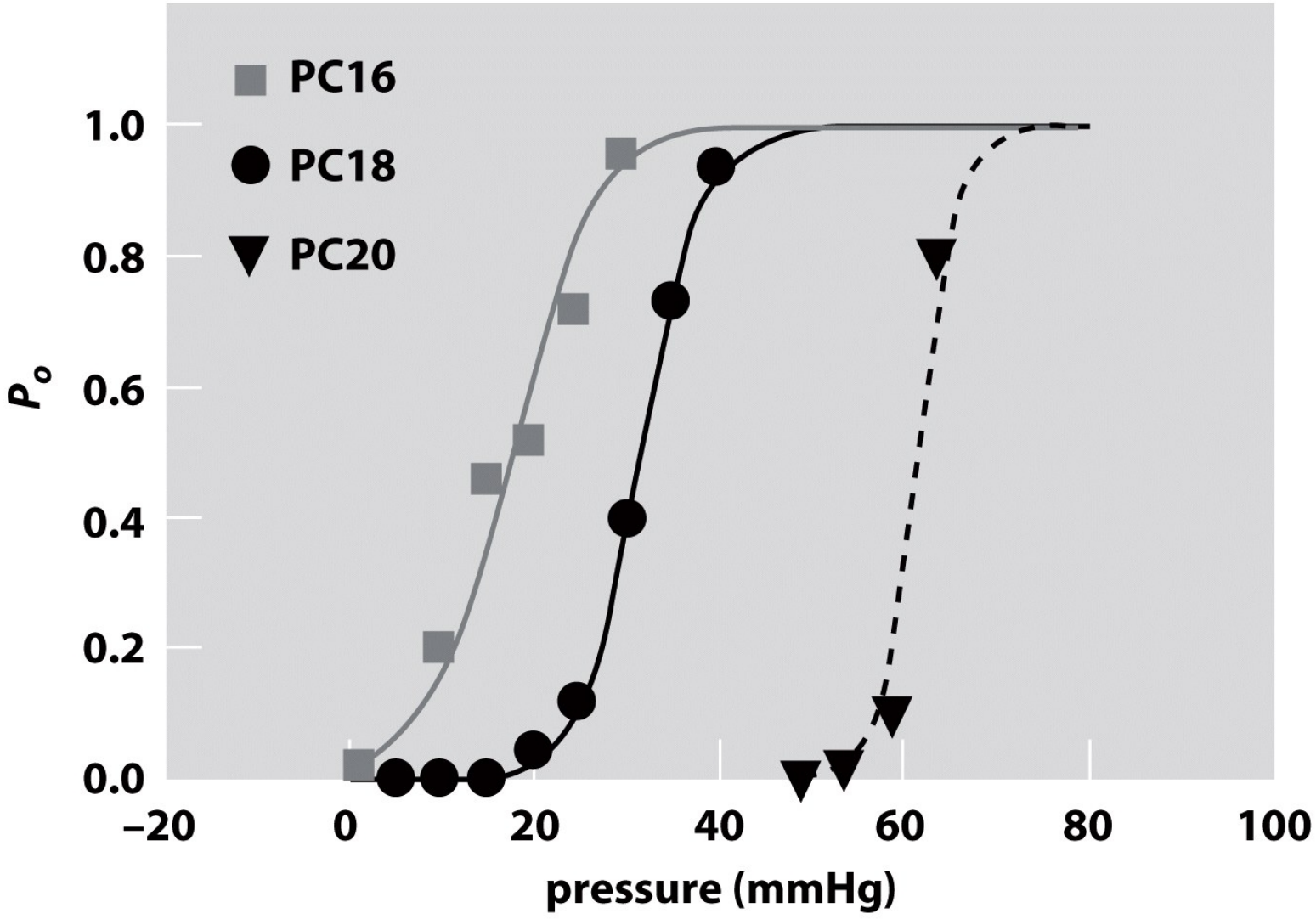


Figure 11.40 Physical Biology of the Cell (© Garland Science 2009)

Elastic Deformations of Membranes Induced by Proteins: Mismatch in the hydrophobic contact area

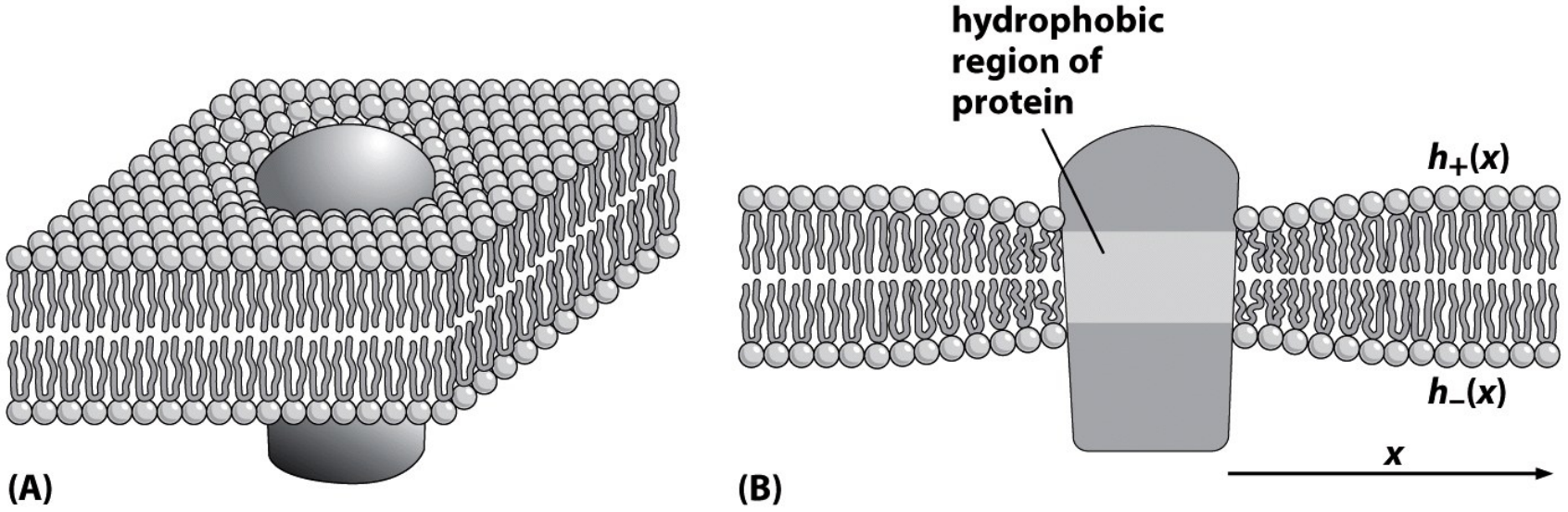


Figure 11.41 Physical Biology of the Cell (© Garland Science 2009)

Definitions of the midplane and half-width of the lipid bilayer:

$$h(x) = \frac{h_+(x) + h_-(x)}{2} \qquad w(x) = \frac{h_+(x) - h_-(x)}{2}$$

Calculation here is simplified to one dimension and excludes thermal fluctuations of the membrane-protein system.

The contribution of the bending to the free energy:

$$G_{bend}[h_+(x), h_-(x)] = \frac{K_b^{(1)}}{2} \int dx \left[\left(\frac{\partial^2 h_+(x)}{\partial x^2} \right)^2 + \left(\frac{\partial^2 h_-(x)}{\partial x^2} \right)^2 \right]$$

$K_b^{(1)} = K_b/2 \ldots$ bending modulus of individual leaflet

$$G_{bend}[h(x), w(x)] = \frac{K_b}{2} \int dx \left[\left(\frac{\partial^2 h(x)}{\partial x^2} \right)^2 + \left(\frac{\partial^2 w(x)}{\partial x^2} \right)^2 \right]$$

Plan of an approximate calculation:

- **the energy of a deformed membrane per unit length**
- **then the linear dimension is wrapped into a circle to find a result for a two dimensional situation**
- **correct way: use cylindrical coordinates**

Definitions: $w(x) = w_0 + u(x)$

$u(x)$... thickness variation

w_0... legth of the hydrophobic domain of bilayer

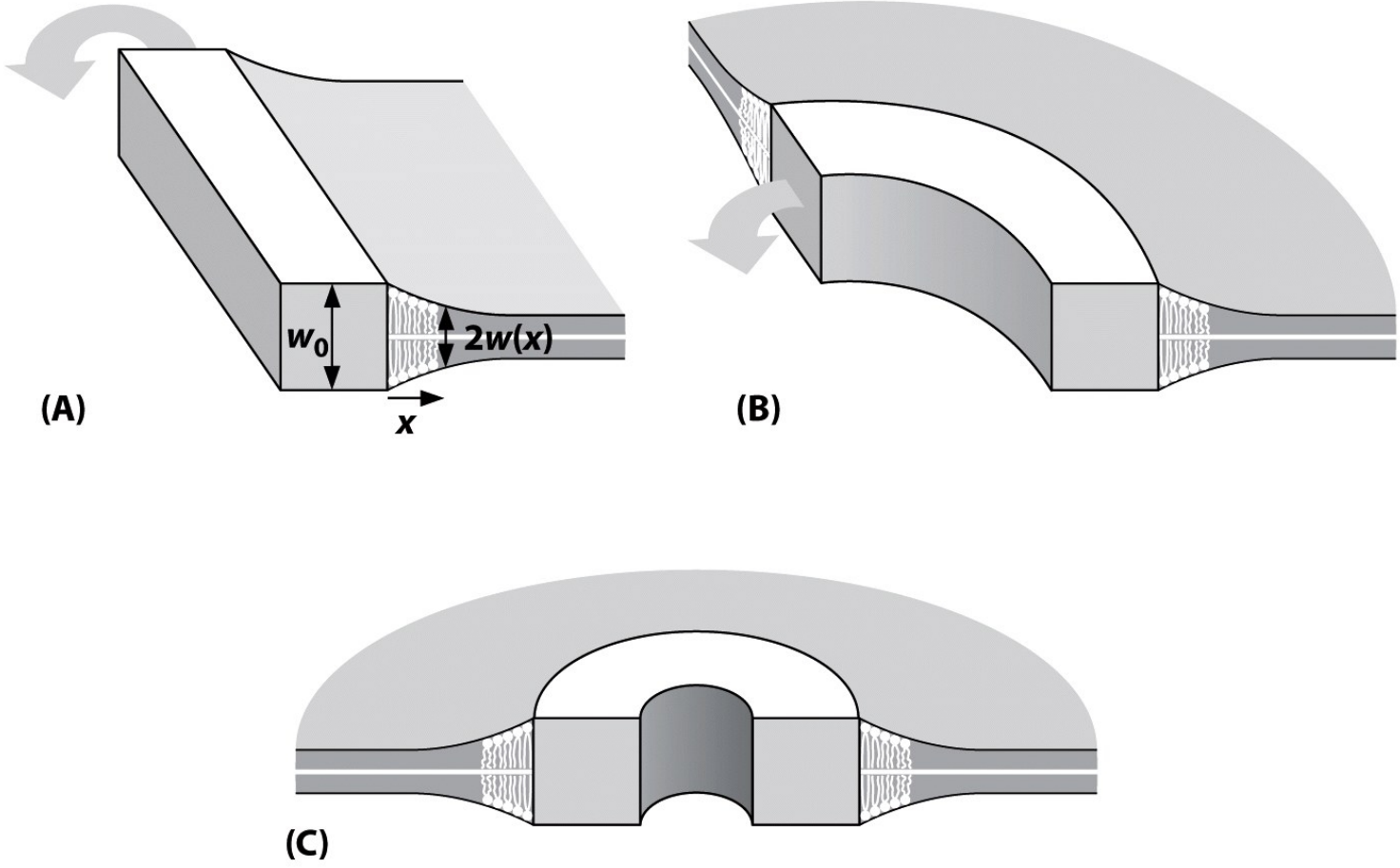


Figure 11.42 Physical Biology of the Cell (© Garland Science 2009)

Free energy due to

(a) the bending energy

(b) the hydrophobic mismatch $G_h[u(x)]$

$$G_h[u(x)] = \frac{K_b}{2}\int_R^\infty \left(\frac{d^2u}{dx^2}\right)^2 dx + \frac{K_t}{2w_0^2}\int_R^\infty u(x)^2 dx$$

We need to find the profile $u(x)$ **that minimizes the free energy.**

Boundary conditions:

at $x=R$ and at $x=\infty$

$$u(R) = \frac{W}{2} - w_0$$

W...length of the hydrophobic patch on a channel

$u'(R) = 0$

$u(\infty) = 0$

$u'(\infty) = 0$

Compute a functional derivative: $u(x) \rightarrow u(x) + \epsilon\,\eta(x)$

Minimize the free-energy functional: $\dfrac{\delta G_h}{\delta u(x)} = 0$

$$K_b \int_R^\infty u_{xx}\,\eta_{xx}\,dx + \frac{K_t}{w_0^2}\int_R^\infty u\,\eta\,dx = 0$$

$$u_{xx}(x) = \frac{\partial^2 u}{\partial x^2} \qquad \eta(R)=\eta(\infty)=\eta'(R)=\eta'(\infty)=0$$

First integration *per partes*: $\int u\,v'\,dx = u\,v - \int v\,u'\,dx$

$$K_b\,u_{xx}\,\eta_x\big|_R^\infty - K_b \int_R^\infty u_{xxx}\,\eta_x\,dx + \frac{K_t}{w_0^2}\int_R^\infty u\,\eta\,dx = 0$$

Second integration *per partes*:

$$-K_b\,u_{xxx}\,\eta\big|_R^\infty + K_b \int_R^\infty u_{xxxx}\,\eta\,dx + \frac{K_t}{w_0^2}\int_R^\infty u\,\eta\,dx = 0$$

$$\rightarrow \qquad K_b\frac{d^4u}{dx^4} + \frac{K_t}{w_0^2}u = 0$$

The trial solution for a linear differential equation with constant coefficients:

$$u = e^{\Lambda x}$$

Insert into the DE and find the equation for the parameter Λ :

$$\Lambda^4 + \frac{K_t}{K_b w_0^2} = 0$$

$$\Lambda = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4} \times (-1)^{1/4} = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4} \times (e^{i\pi})^{1/4} = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4} \times e^{i\pi/4}$$

Final solution can be expressed in terms of 4 different exponents:

$$\Lambda_{1,2,3,4} = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4} \left(\frac{\pm\sqrt{2}}{2} \mp i\frac{\sqrt{2}}{2}\right)$$

Only two of these 4 exponents satisfy the boundary conditions at ∞ :

$$\Lambda_2 = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4}\left(\frac{-\sqrt{2}}{2} + i\frac{\sqrt{2}}{2}\right) \qquad \Lambda_3 = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4}\left(\frac{-\sqrt{2}}{2} - i\frac{\sqrt{2}}{2}\right)$$

A general solution can finally be expressed:

$$u(x) = C_2 e^{\Lambda_2 x} + C_3 e^{\Lambda_3 x}$$

The boundary conditions at x = R result in:

$$C_2 e^{\Lambda_2 R} + C_3 e^{\Lambda_3 R} = W/2 - w_0$$

$$\Lambda_2 C_2 e^{\Lambda_2 R} + \Lambda_3 C_3 e^{\Lambda_3 R} = 0$$

Then the final solution for u(x) can be found:

$$u(x) = (W/2 - w_0)\, e^{-(\sqrt{(2)}/2)\lambda(x-R)}\left[\cos\frac{\sqrt{2}}{2}\lambda(x-R) + \sin\frac{\sqrt{2}}{2}\lambda(x-R)\right]$$

$$\lambda = \left(\frac{K_t}{K_b w_0^2}\right)^{1/4}$$

This solution can be inserted into the expression for the free energy to get:

$$G_h = G_h^1 + G_h^2$$

$$G_h^1 = \frac{K_b}{2}(W/2 - w_0)^2 \lambda^4 \int_R^\infty dx\, e^{-(\sqrt{(2)}/2)\lambda(x-R)} \left[1 - 2\sin\frac{\sqrt{2}}{2}\lambda(x-R)\cos\frac{\sqrt{2}}{2}\lambda(x-R)\right]$$

$$G_h^2 = \frac{K_t}{2w_0^2}(W/2 - w_0)^2 \lambda^4 \int_R^\infty dx\, e^{-(\sqrt{(2)}/2)\lambda(x-R)} \left[1 + 2\sin\frac{\sqrt{2}}{2}\lambda(x-R)\cos\frac{\sqrt{2}}{2}\lambda(x-R)\right]$$

$$G_h = \frac{K_t(W/2 - w_0)^2}{\sqrt{2}\lambda w_0^2} \ldots \text{energy per length}$$

Knowing the free energy due to a hydrophobic mismatch per length, we now generalize it for a cylindrically symmetric ion channel and add the free energy of the "loading device" introduced in Ch. 7:

$$G_{MscL} = G_h + G_{tension} = G_0 + \frac{K_t (W/2 - w_0)^2}{\sqrt{2}\lambda w_0^2} \times 2\pi R - \tau \pi R^2$$

The result in a more compact form:

$$G_{MscL} = G_0 + \frac{1}{2} K U^2 2\pi R - \tau \pi R^2$$

$$U = \frac{W}{2} - w_0$$

$$K = \sqrt{2}\left(\frac{K_t^3 K_b}{w_0^6}\right)^{1/4} \quad \text{... spring constant}$$

Observations:

- **a competition between minimizing hydrophobic mismatch (small R, closed channel) and minimizing the load term (large R, open channel)**

- **critical tension is the tension at which the free energies of the open and closed states are equal:**

$$G_{MscL}(R_c) = G_{MscL}(R_o) \quad \rightarrow$$

$$\frac{1}{2} K U^2 2\pi R_c - \tau_{crit} \pi R_c^2 = \frac{1}{2} K U^2 2\pi R_o - \tau_{crit} \pi R_o^2 \quad \rightarrow$$

$$\tau_{crit} = K U^2 \frac{1}{R_c + R_o}$$

- **the free energy difference between open and closed states at zero tension:**

$$\Delta G(\tau = 0) = G_{MscL}^{\tau=0}(R_o) - G_{MscL}^{\tau=0}(R_c) = \pi K U^2 (R_o - R_c)$$

Free energy of the membrane as a function of a channel radius for different values of applied tension

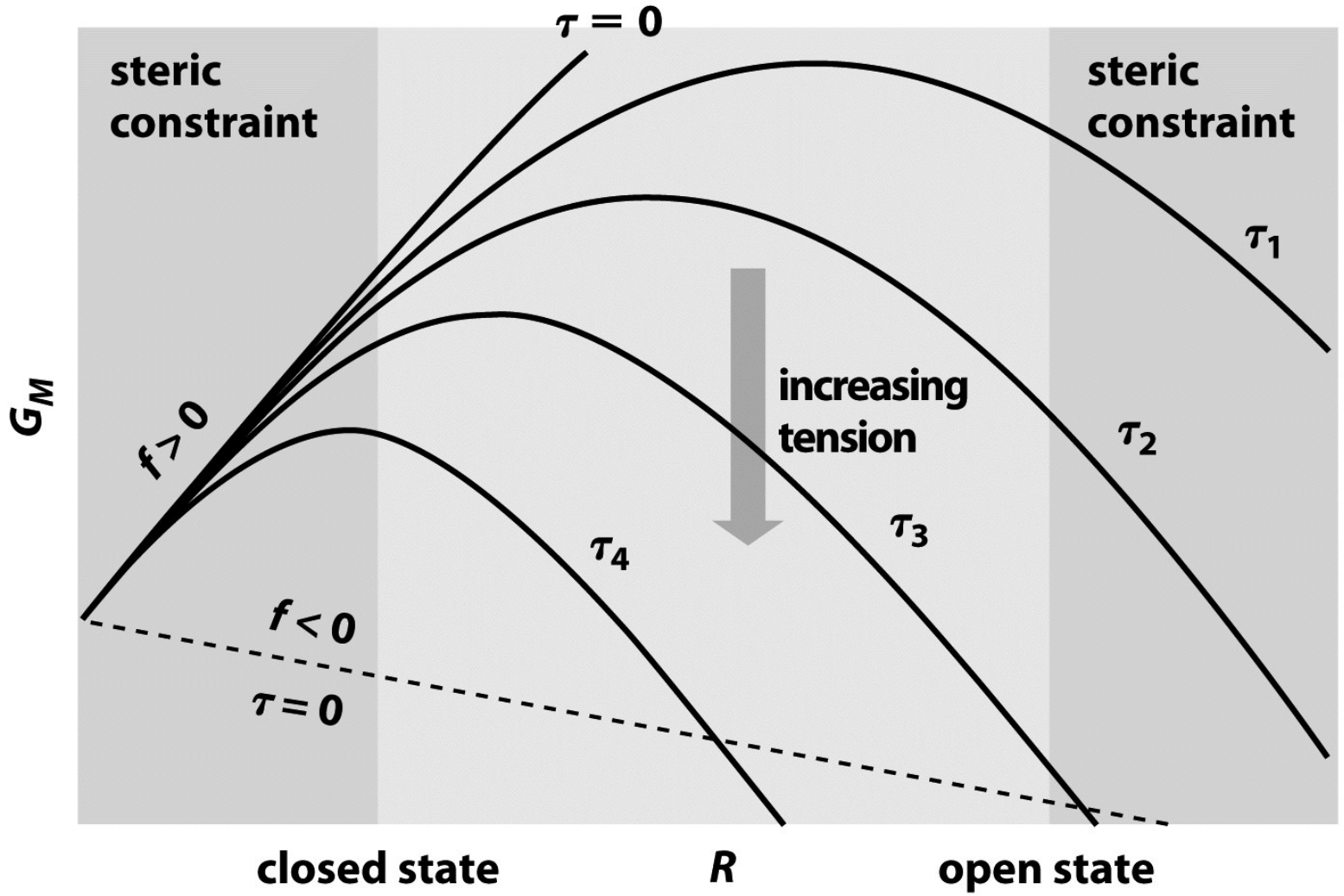


Figure 11.44 Physical Biology of the Cell (© Garland Science 2009)

Table 11.1 Comparison of theory and experiment for the mechanosensitive channel, MscL. Experiments measure the pressure difference at which the open probability is 1/2. Comparison between theory and experiment requires knowledge of the pipette radius to convert pressure into tension. (Data taken from E. Perozo et al., *Nat. Struc. Biol.* 9:696, 2002.)

	Theory		Experiment	
n	$\tau_{crit}\,(k_BT/\text{Å}^2)$	$\Delta G(\tau=0)\,(k_BT)$	$P_{1/2}$ (mmHg)	$\Delta G(\tau=0)\,(k_BT)$
16	$2.3\cdot10^{-3}$	5	24 ± 2	4
18	$5.2\cdot10^{-3}$	11.5	42 ± 5	9.4
20	$9.3\cdot10^{-3}$	20.4	72 ± 8	23.5

Table 11.1 Physical Biology of the Cell (© Garland Science 2009)