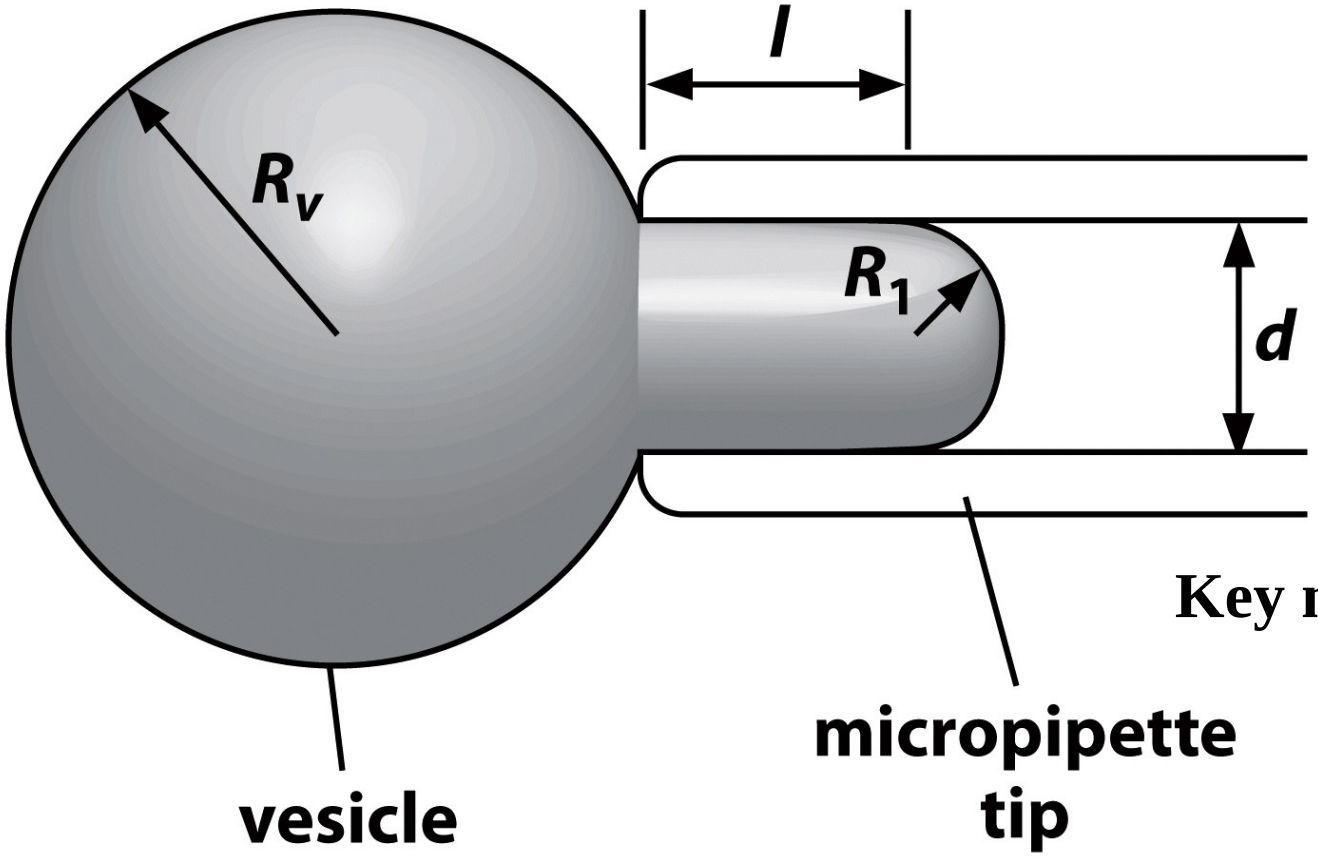
Lectures 18:
Biological Membranes: Life in Two Dimensions (contd.)

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

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

Structure, Energetics, and Function of Vesicles

Energetics assessed by micropipette aspiration experiment



Key measurements:

R_v, R_1, l

Figure 11.22 Physical Biology of the Cell (© Garland Science 2009)

Micropipette aspiration experiment (fluorescent images)

Membrane doped with a small fluorescent molecule rhodamine.

Measured tensions:

1.1 mN/m (top)
3.2 mN/m (middle)
7.4 mN/m (bottom)

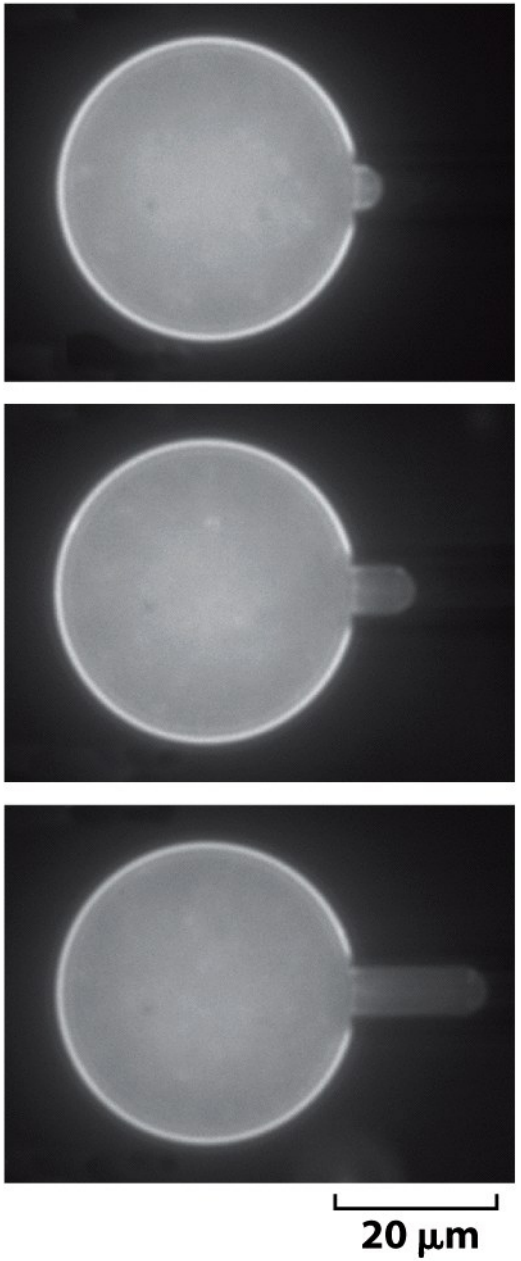


Figure 11.23 Physical Biology of the Cell (© Garland Science 2009)

By measuring the pressure difference and geometric parameters, membrane tension, bending and area stretch moduli can be found:

$$\tau, K_b, K_a$$

The pressure difference between the interior of the vesicle and the surrounding medium (Laplace-Young relation): $\Delta p_{outside} = \frac{2\tau}{R_v}$

The pressure difference between the interior of the vesicle and the inside of the micropipette: $\Delta p_{inside} = \frac{2\tau}{R_1}$

The pressure difference between the inside of the micropipette and the surrounding media, which is controlled experimentally, is:

$$\Delta p = \Delta p_{inside} - \Delta p_{outside}$$

so that finally the tension can be expressed as:

$$\tau = \frac{\Delta p}{2} \frac{R_1}{1-(R_1/R_v)}$$

Determining the area stretch modulus: K_a

$$\tau = K_a \frac{\Delta a}{a_0}$$

Δa ... the change of membrane area

a_0 ... the area of the reference state

Calculate the change in the are due to micropipette suction:

$$\Delta a = 2\pi R_1 l + 2\pi R_1^2$$

$$\frac{\Delta a}{a_0} = \frac{R_1^2(1+l/R_1)}{2R_v^2}$$

τ versus $\frac{R_1^2(1+l/R_1)}{2R_v^2}$ gives K_a as a slope

A series of tensions applied by controlling the pipette pressure; areas were measured for each pressure

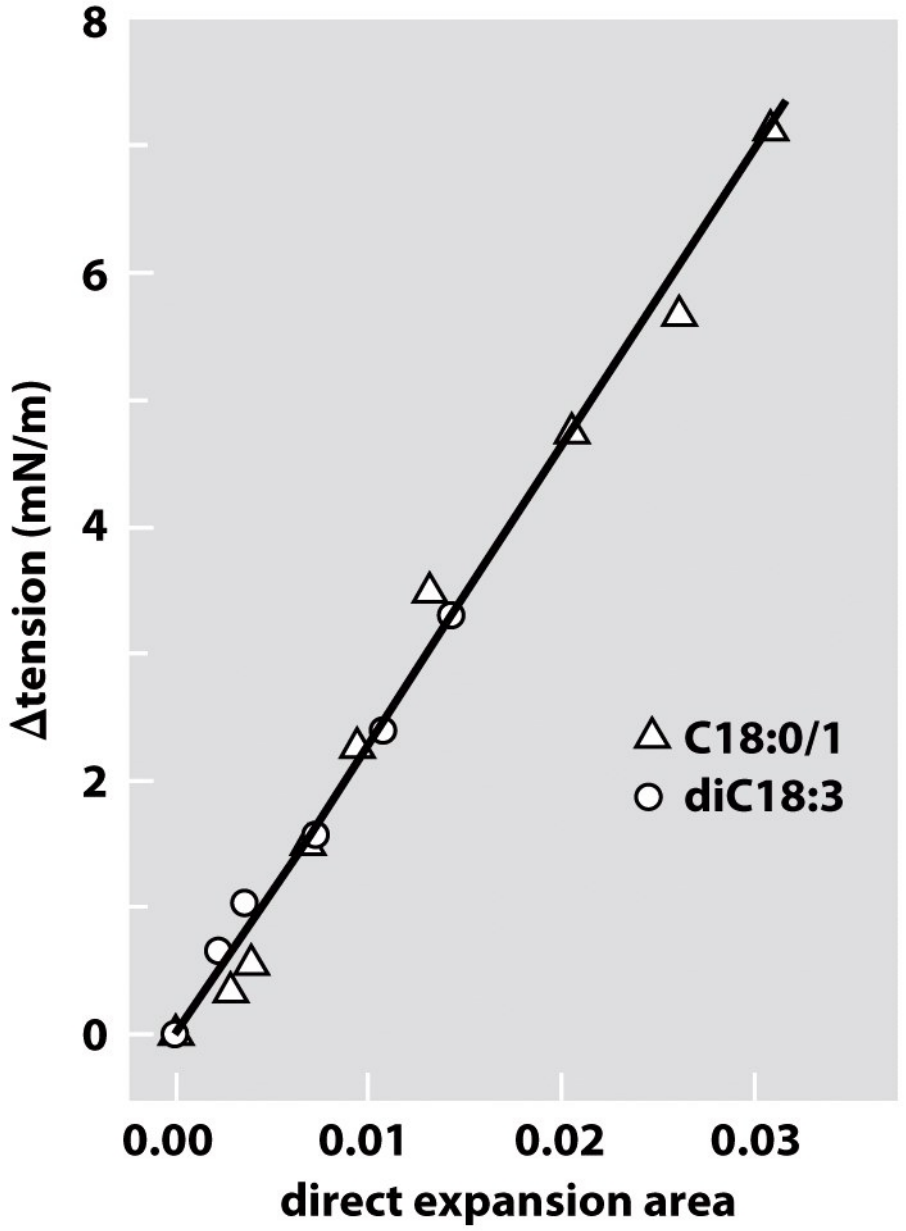


Figure 11.24a Physical Biology of the Cell (© Garland Science 2009)

Measured values of the area stretch modulus for different lipids.

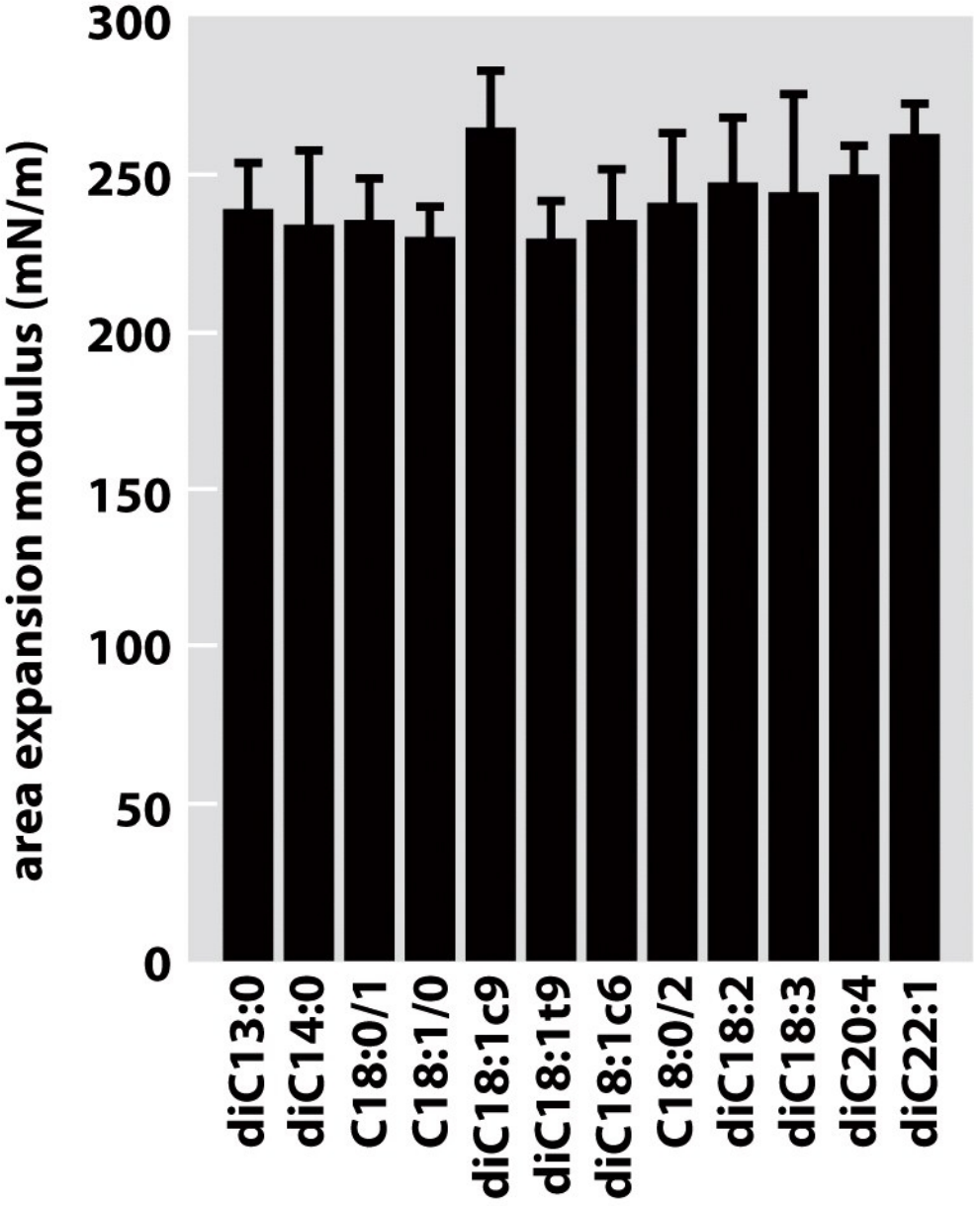


Figure 11.24b Physical Biology of the Cell (© Garland Science 2009)

***In vivo* membranes include:**

- **endoplasmic reticulum**
- **trans-Golgi network**

tubular and recticular structure

Biological tubules are hypothesized to form by membrane-attached motor proteins: Tubules are pulled out of a membrane (right).

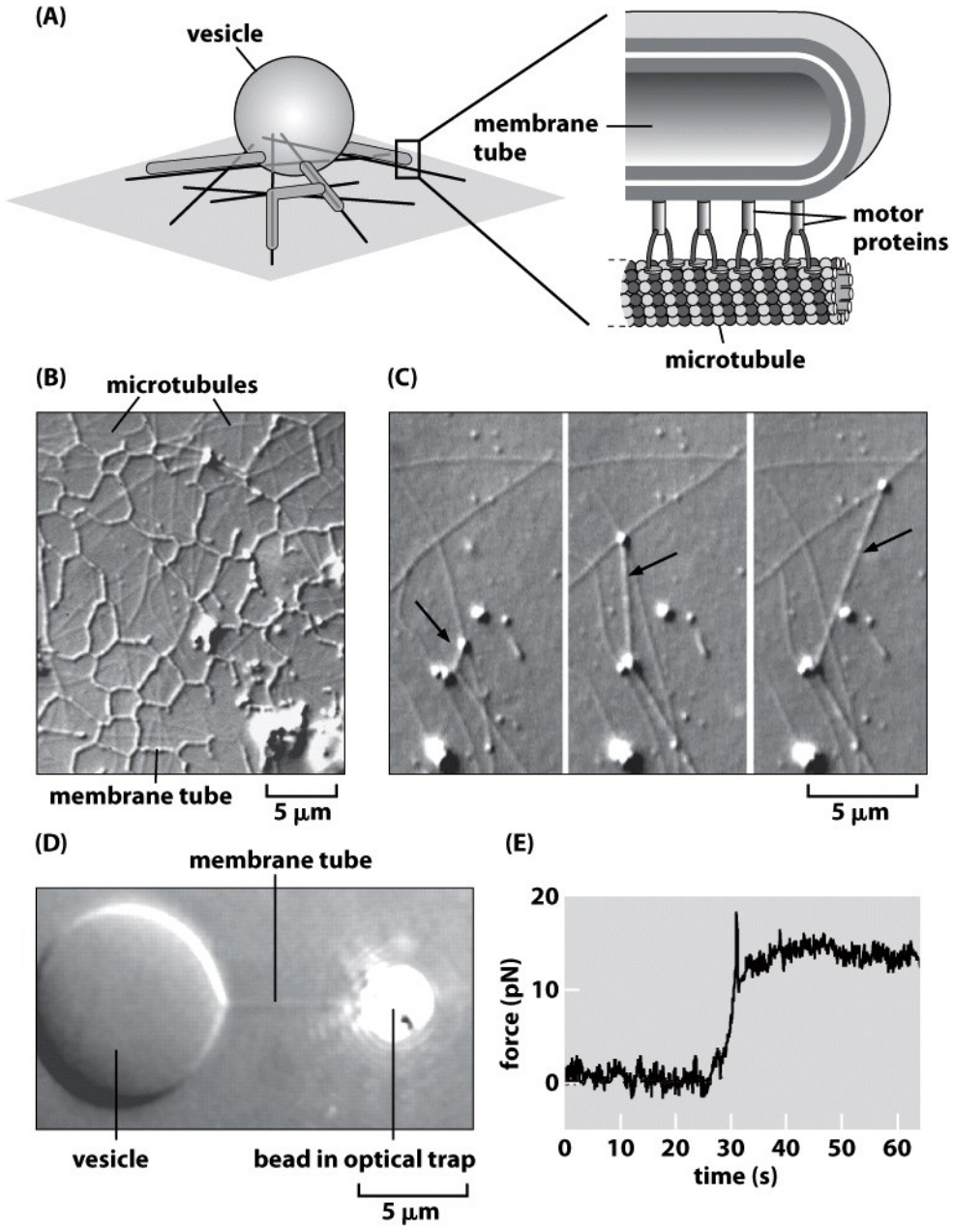


Figure 11.25 Physical Biology of the Cell (© Garland Science 2009)

Groups of motor proteins can help pull membrane tubes out of a spherical vesicles

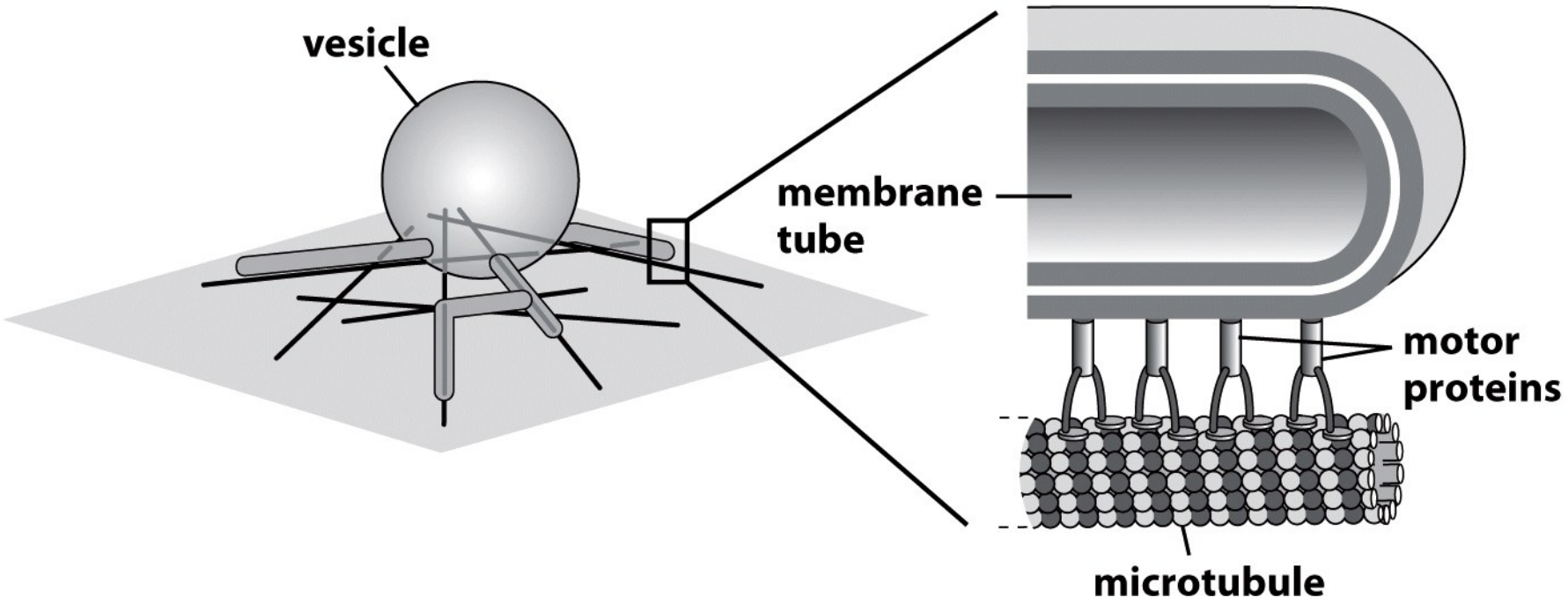


Figure 11.25a Physical Biology of the Cell (© Garland Science 2009)

Differential interference contrast microscopy on an *in vitro* experiment:

microtubules with *Xenopus* egg cytosolic proteins helps form an elaborate web of rat liver ER membrane

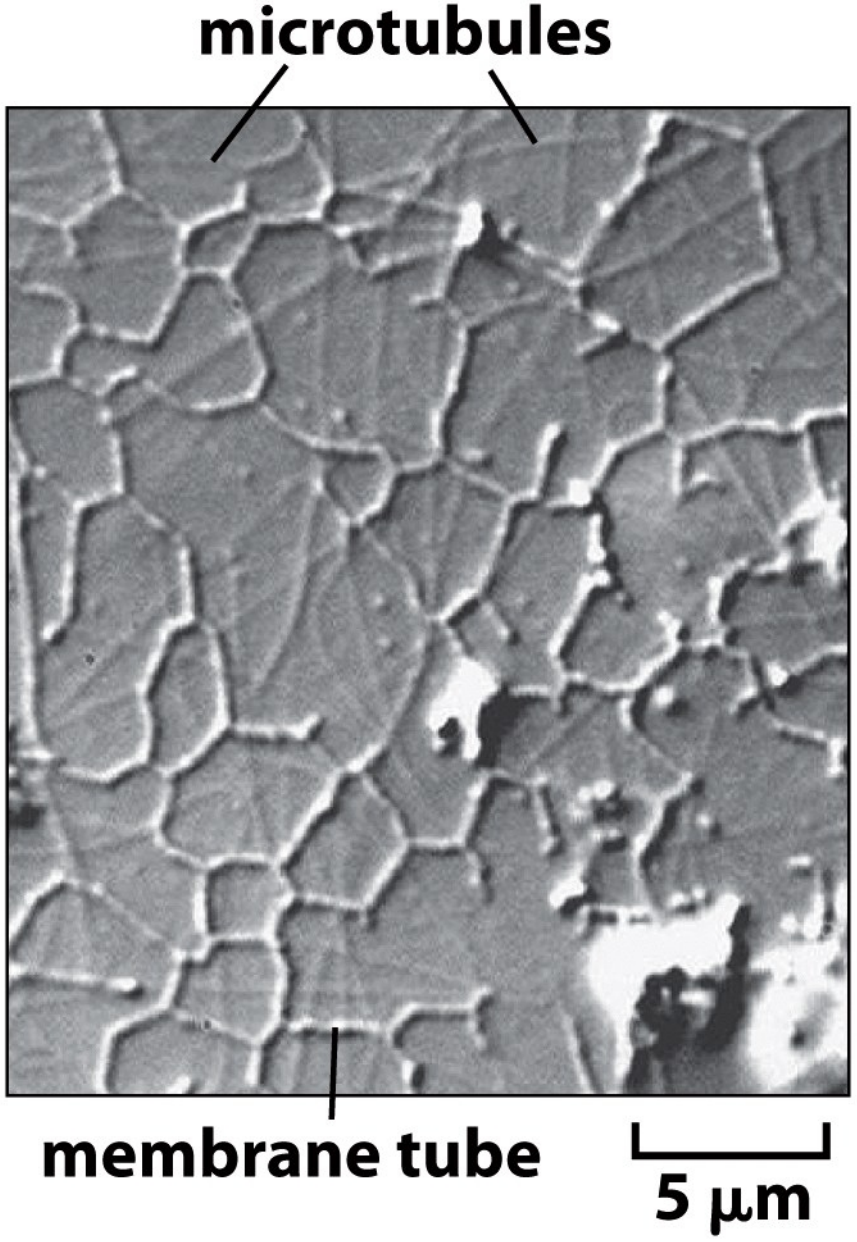


Figure 11.25b Physical Biology of the Cell (© Garland Science 2009)

A single tubule pulled out and switches microtubule tracks: 11 second experiment

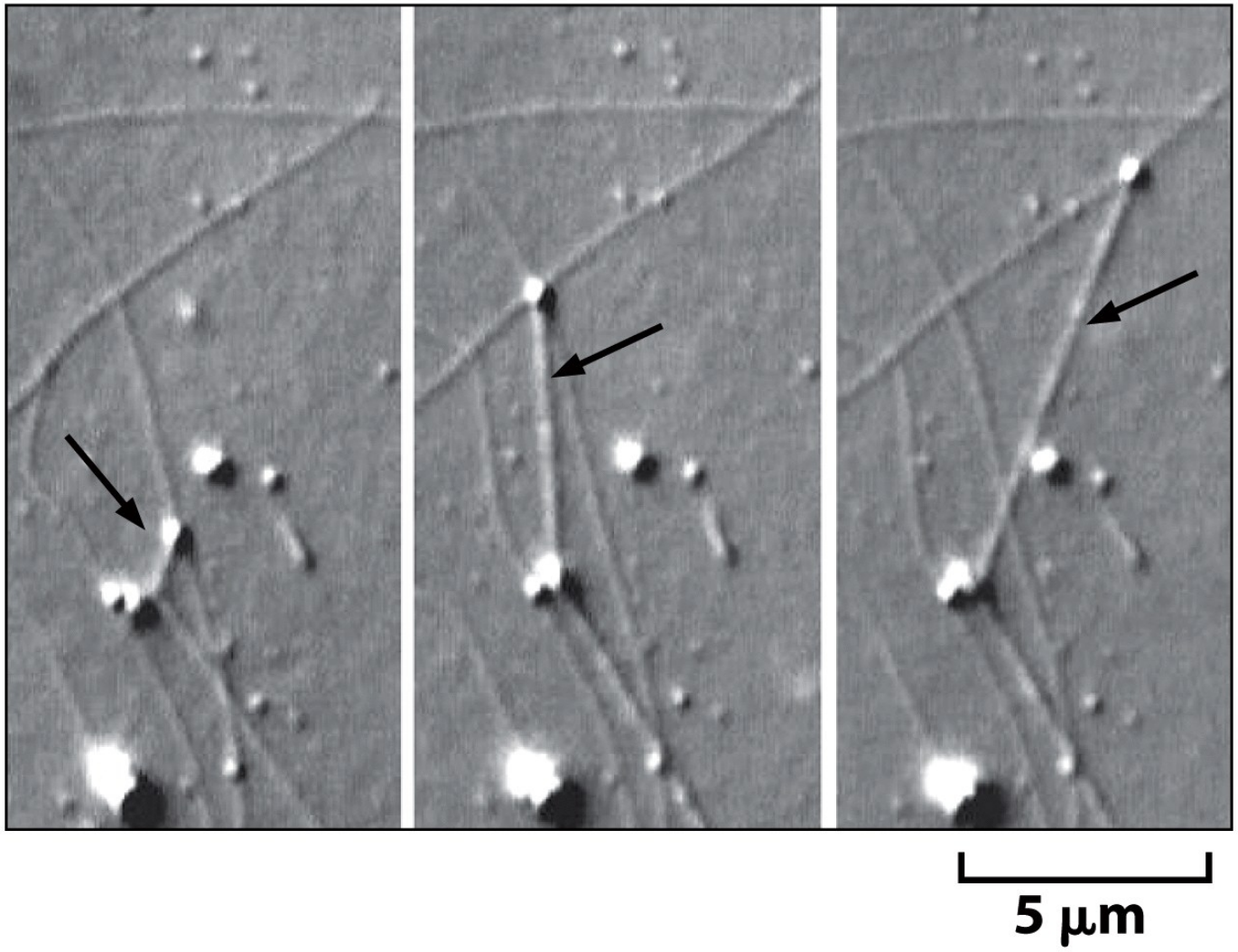


Figure 11.25c Physical Biology of the Cell (© Garland Science 2009)

Measuring the force associated with a tether formation

Using optical trap with a bead attached to a vesicle and pulled out

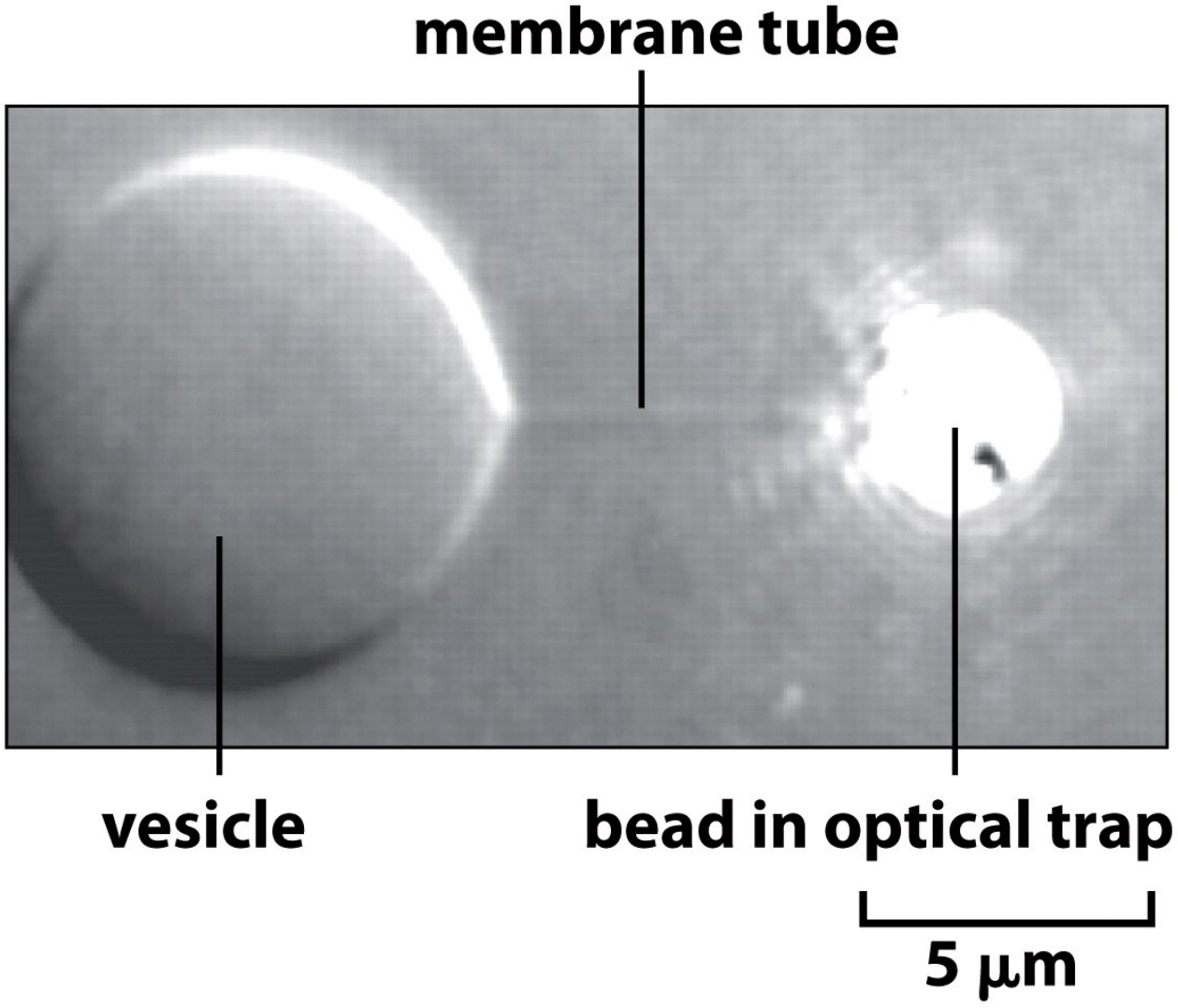


Figure 11.25d Physical Biology of the Cell (© Garland Science 2009)

When a tether is formed, the force on the bead increases abruptly

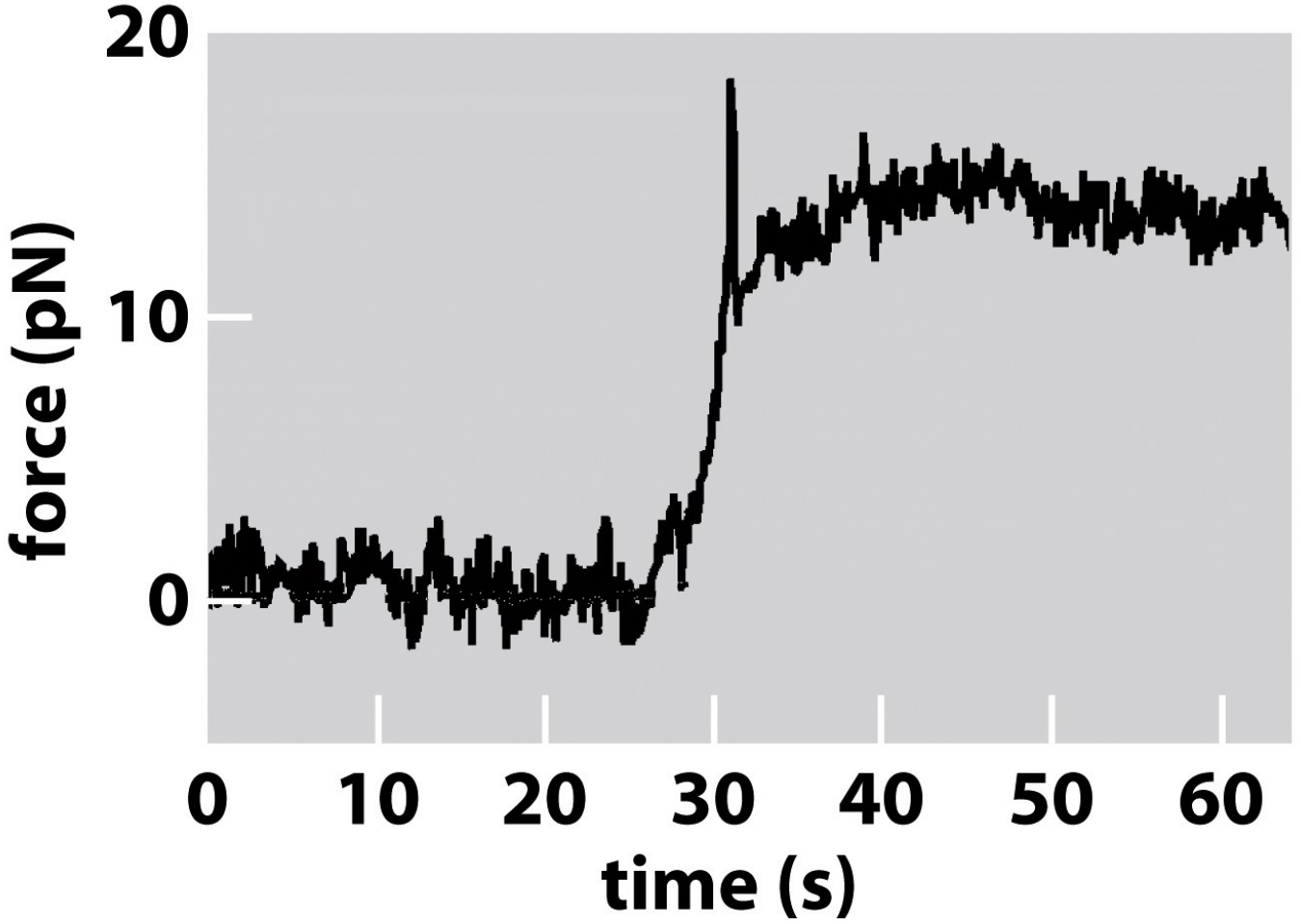


Figure 11.25e Physical Biology of the Cell (© Garland Science 2009)

Calculation of a force needed to pull out a tether

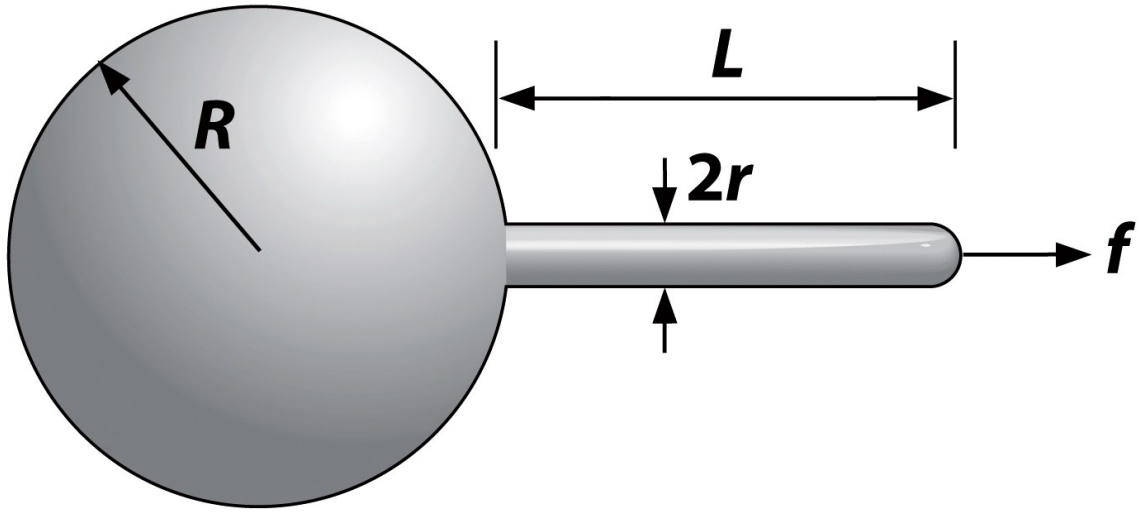


Figure 11.26 Physical Biology of the Cell (© Garland Science 2009)

Free energy associated with bending and stretching of the vesicle + tether system

$$G_{bend} = \frac{1}{2} K_b \left(\frac{2}{R}\right)^2 4\pi R^2 + \frac{1}{2} K_b \left(\frac{1}{r}\right)^2 2\pi r L + \frac{1}{2} K_b \left(\frac{2}{r}\right)^2 \frac{1}{2} 4\pi r^2$$

$$G_{bend} = 12\pi K_b + \pi K_b \frac{L}{r}$$

$$G_{stretch} = \frac{K_a}{2}\frac{(a-a_0)^2}{a_0}$$

$$a_0 = 4\pi R^2 \qquad a = a_0 + 2\pi r L \qquad r \ll L, R$$

Two additional terms contribute to the free energy:

- **work against the pressure difference between the inside and outside of the vesicle:**

$$G_{pV} = -\Delta p\left(\frac{4}{3}\pi R^3 + r^2 \pi L\right)$$

- **work done by the pulling force:** $G_{load} = -fL$

The total free energy of the vesicle + tether system is:

$$G_{tot} = 12\pi K_b + \pi K_b \frac{L}{r} + G_{stretch} - \Delta p\left(\frac{4}{3}\pi R^3 + \pi r^2 L\right) - fL$$

The equilibrium shape of a vesicle and a tether is the one that minimizes the total free energy with respect to R, r, & L:

$$\frac{\partial G_{tot}}{\partial R} = 0 \qquad \frac{\partial G_{tot}}{\partial r} = 0 \qquad \frac{\partial G_{tot}}{\partial L} = 0$$

$$\frac{\partial G_{stretch}}{\partial R} = K_a \frac{a - a_0}{a_0} \frac{\partial a}{\partial R} = \tau 8\pi R$$

$$\frac{\partial G_{stretch}}{\partial r} = \tau 2\pi L \qquad \frac{\partial G_{stretch}}{\partial L} = \tau 2\pi r$$

$$\text{Eq.}(1): \; 8\pi\tau R - 4\pi\Delta p R^2 = 0$$

$$\text{Eq.}(2): \; -\pi K_b \frac{L}{r^2} + 2\pi\tau L - 2\pi\Delta p r L = 0$$

$$\text{Eq.}(3): \; \pi K_b \frac{1}{r} + 2\pi\tau r - \pi\Delta p r^2 - f = 0$$

Solution:

$$\text{Eq.}(1) \;\rightarrow\; \Delta p = \frac{2\tau}{R} \qquad \text{the Laplace} - \text{Young relation}$$

$$\text{Eq}(2): \; -\pi K_b \frac{L}{r^2} + 2\pi\tau L - 4\pi\tau L \frac{r}{R} = 0 \;\rightarrow\; r \approx \sqrt{\frac{K_b}{2\tau}}$$

$$\text{Eq}(3): \;\rightarrow\; f \approx 2\pi\sqrt{2K_b\tau} \quad \text{force} - \text{tension relation}$$

Measured forces for pulling tethers from the ER and Golgi are ~ 10 pN, so tension can be estimated to be:

$$\tau = \frac{f^2}{8\pi^2 K_b} \approx 0.015 \text{ pN/nm} \qquad r \approx 50 \text{ nm}$$

$$K_b \approx 20\, k_B T \approx 20 \times 4 \text{pN nm} = 80 \text{pN nm}$$

Vesicles in Cells

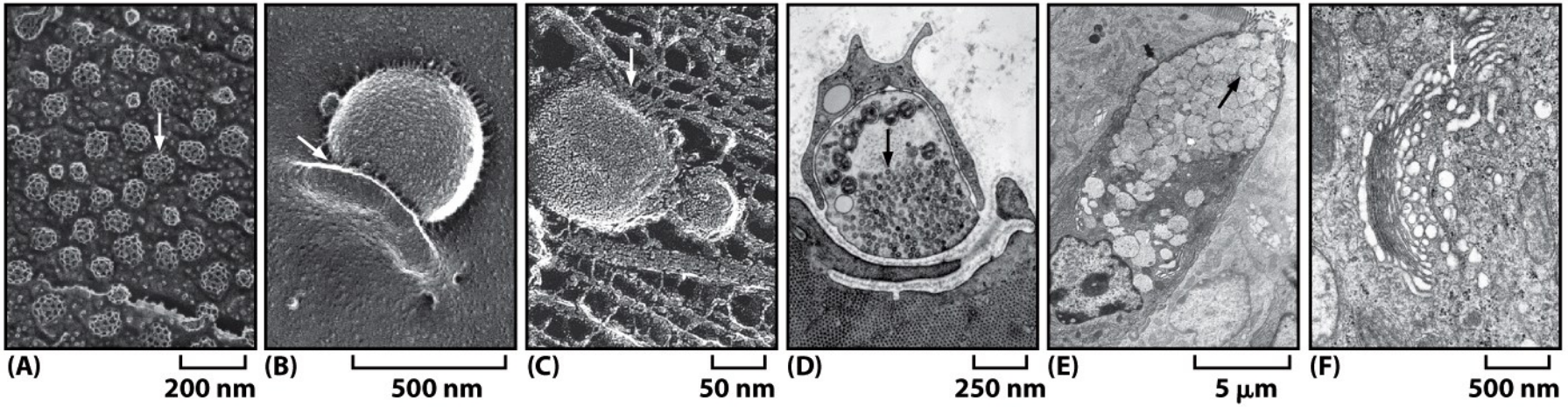


Figure 11.27 Physical Biology of the Cell (© Garland Science 2009)

A) *clathrin* coated pits on a cell membrane before *endocytosis*
B) membrane wrapping around a large particle (*phagocytosis*)
C) a transport vesicle within the axon of a neuron
D) thin section through *a synapse* of a junction between a muscle cell and nerve cell packed with synaptic vesicles
E) *goblet cell* in intestine dumping giant mucus-containing vesicles
F) thin section of a *Golgi apparatus* with small transport vesicles that contain proteins to be transported

Function of vesicles in the cell:

(1) uptake of the matter from extracellular space into the cell: *endocytosis*

(2) delivering matter to the outside: *exocytosis*

(3) moving matter (proteins, lipids) inside the cell: *intracellular transport*

Example of endocytosis: A)

-uptake of cholesterol containing particles (LDL) from the bloodstream; cholesterol from diet is packaged in liver; LDL particles about 20 nm long

-LDL bind to the receptor on the cell membrane

-bound receptors cluster together because the clathrin molecules attached to the receptor on the intracellular side self-assemble into pits

Example of exocytosis: synaptic vesicles deliver neurotransmitter molecules into extracellular space to be taken up by a muscle cell

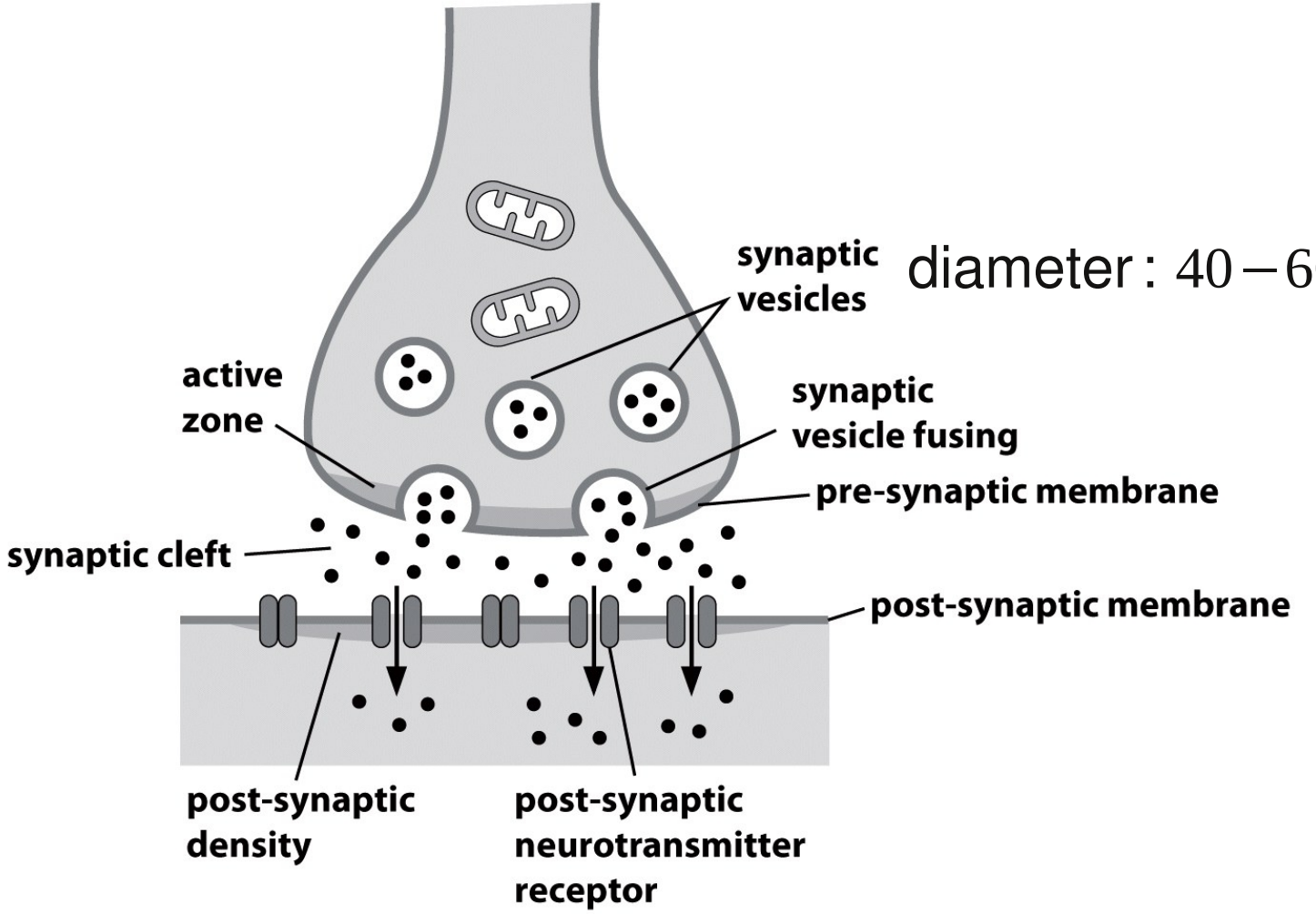


Figure 11.28 Physical Biology of the Cell (© Garland Science 2009)

Free energy needed to form a vesicle is independent of the vesicle size:

$$G_{vesicle} = \frac{K_b}{2} \int_{\partial \Omega} \left(\frac{2}{R} \right)^2 da$$

$$G_{vesicle} = \frac{K_b}{2} \left(\frac{2}{R} \right)^2 4\pi R^2 = 8\pi K_b$$

$$K_b \approx 10-20\ k_B T \ \rightarrow \ 250-500\, k_B T \ \text{energy for a vesicle}$$

How can this energy be provided?

- incorporate proteins that favor curved state
- incorporate curvature-inducing lipids

Lipid census for synaptic vesicles:

diameter $D \approx 60\,\text{nm}$ volume $V \approx 10^5\,\text{nm}^3$

each acetylcholine molecule $(450\,\text{Da})$ takes: $1\,\text{nm}^3$ volume

each vesicle contains: 10^5 neurotransmitter molecules

A rule of thumb for a cell such as fibroblast:
The flux of vesicles is such that the area taken up by vesicle in 1 hour is equal to the entire plasma membrane area.

$$a_{\text{fibroblast}} \approx 2000\,\text{nm}^2$$
$$a_{\text{vesicle}} \approx 3\times 10^4\,\text{nm}^2$$

$60{,}000$ vesicles per hour $\approx 1{,}000$ vesicles per minute

$$500\ k_B T \times 1{,}000 \text{ per minute} \approx 25{,}000\ \text{ATP/min}$$