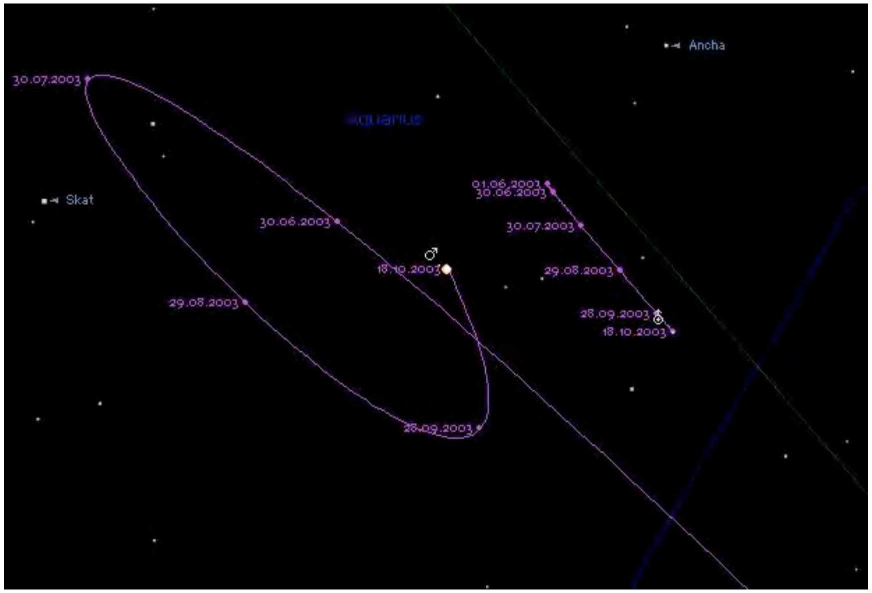
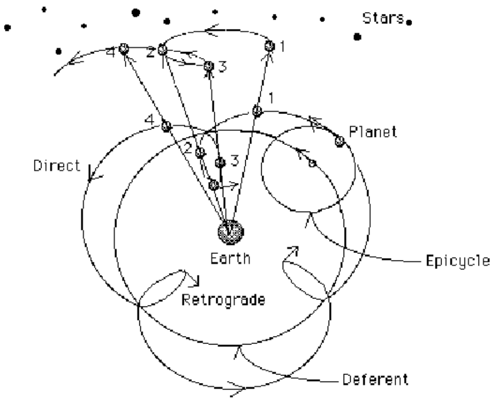
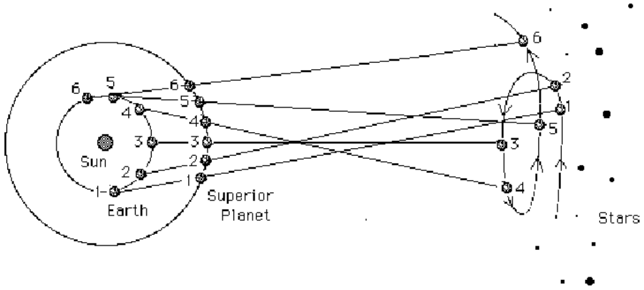
The “Biology is too complicated” Defense Rejected

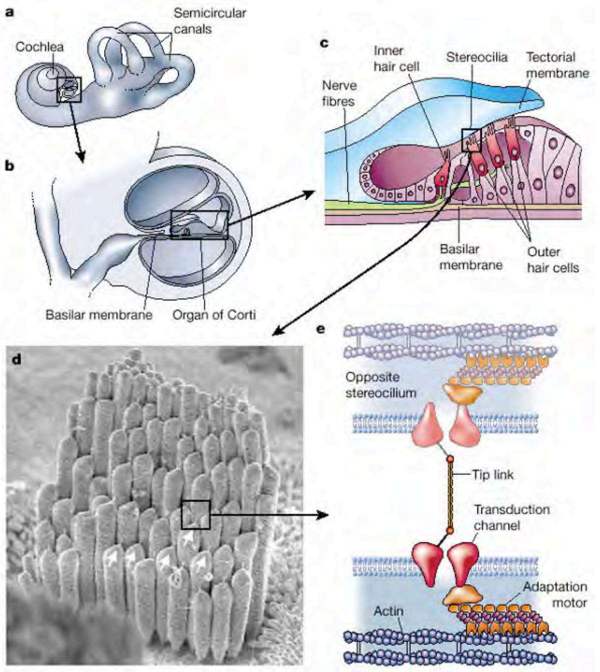


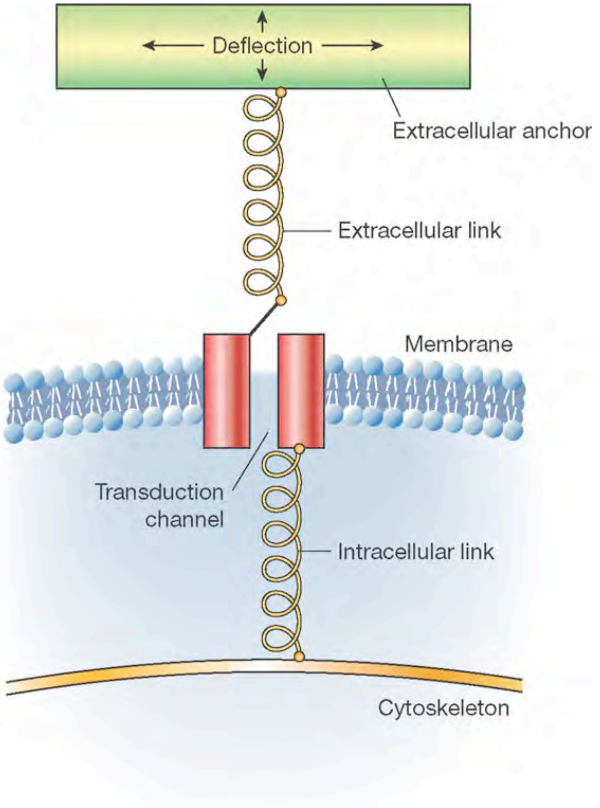




“The obvious is that which is never seen until someone expresses

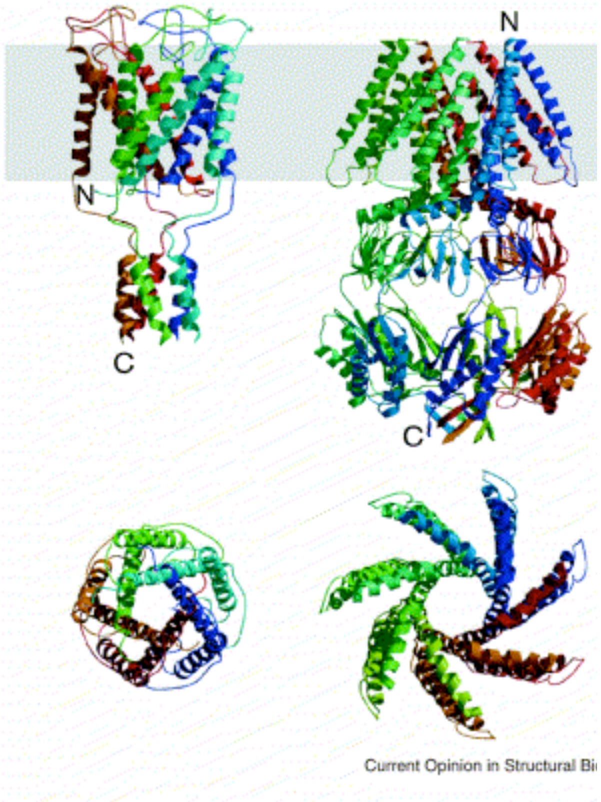
Ion Channels: Models of Gating





(Gillespie and Walker)

(Perozo and

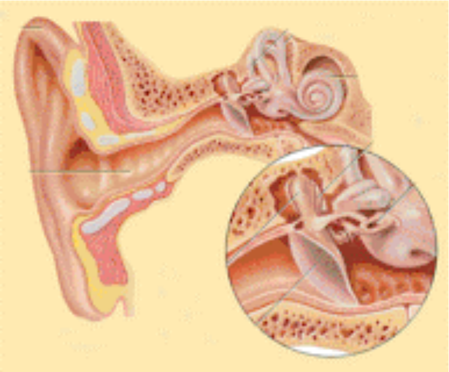


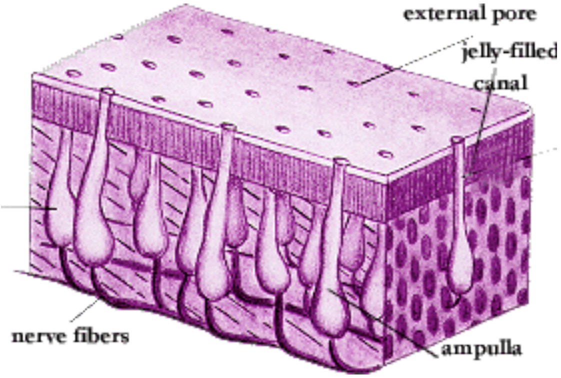
Rob Phillips

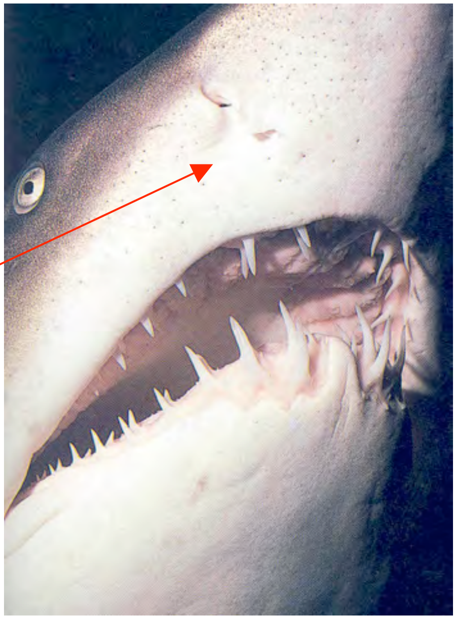
California Institute of Technology

Life and the Senses

- ***Living organisms are full of sensors, some of which we are conscious of, others of which we are not.***
- ***Obvious examples – touch, hearing, vision, taste, smell***
- ***Less obvious – sharks and the ampullae of Lorenzini – electrical detection.***
- ***Sensors from pH to temperature to sugar.***







Reminder on Ion Distribution and Transport in Cells

- *Cells divided into a number of membrane-bound compartments.*
- *Concentrations in different compartments can be orders of magnitude different.*
- *Proteins (ion channels, transporters) mediate these concentration gradients.*
- *Membrane proteins central to huge range of processes – cell signaling, nerve impulses, nutrient transport, etc.*

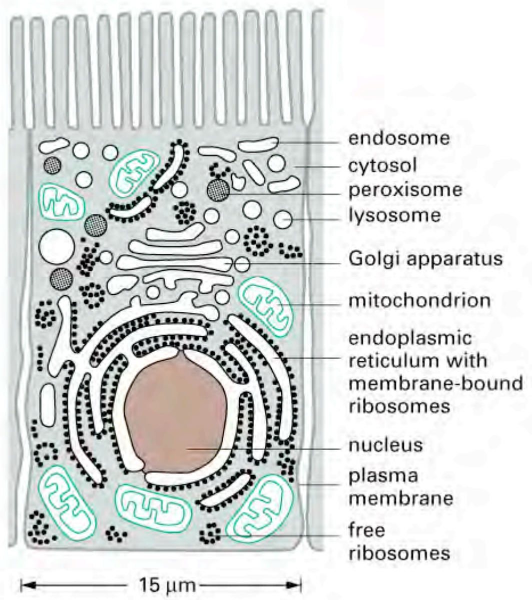
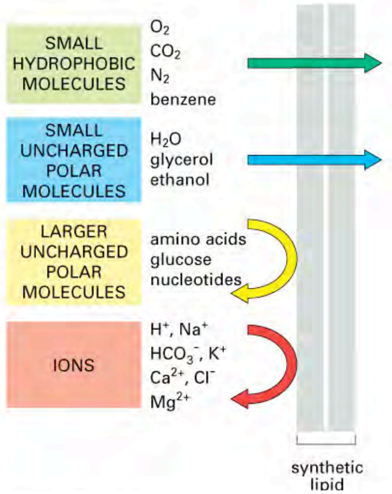


Figure 15-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

$$Ca^{2+}_{in} \approx 10^{-4} mM \quad Ca^{2+}_{out} \approx 1 mM$$

$$K^{+}_{in} \approx 140 mM \quad K^{+}_{out} \approx 5 mM$$



Crossing the Membrane

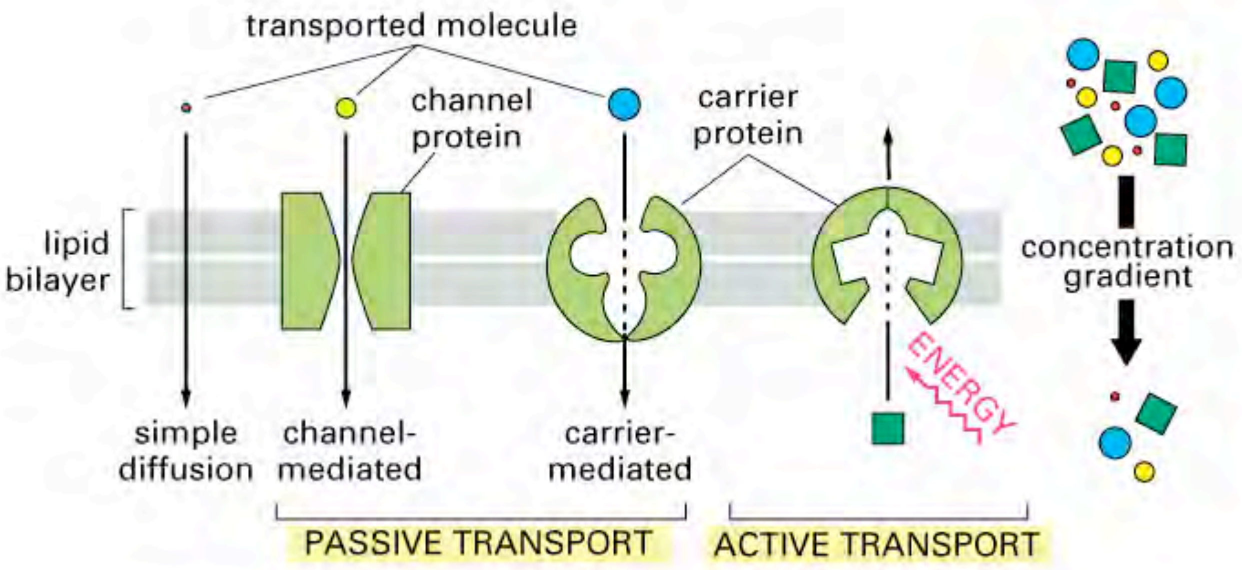


Figure 12-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Ion Channels and Transient Permeability

- *Channels open in response to a variety of different stimuli.*
- *Key mechanisms are voltage gating, ligand binding-induced gating and mechanical tension in the membrane.*

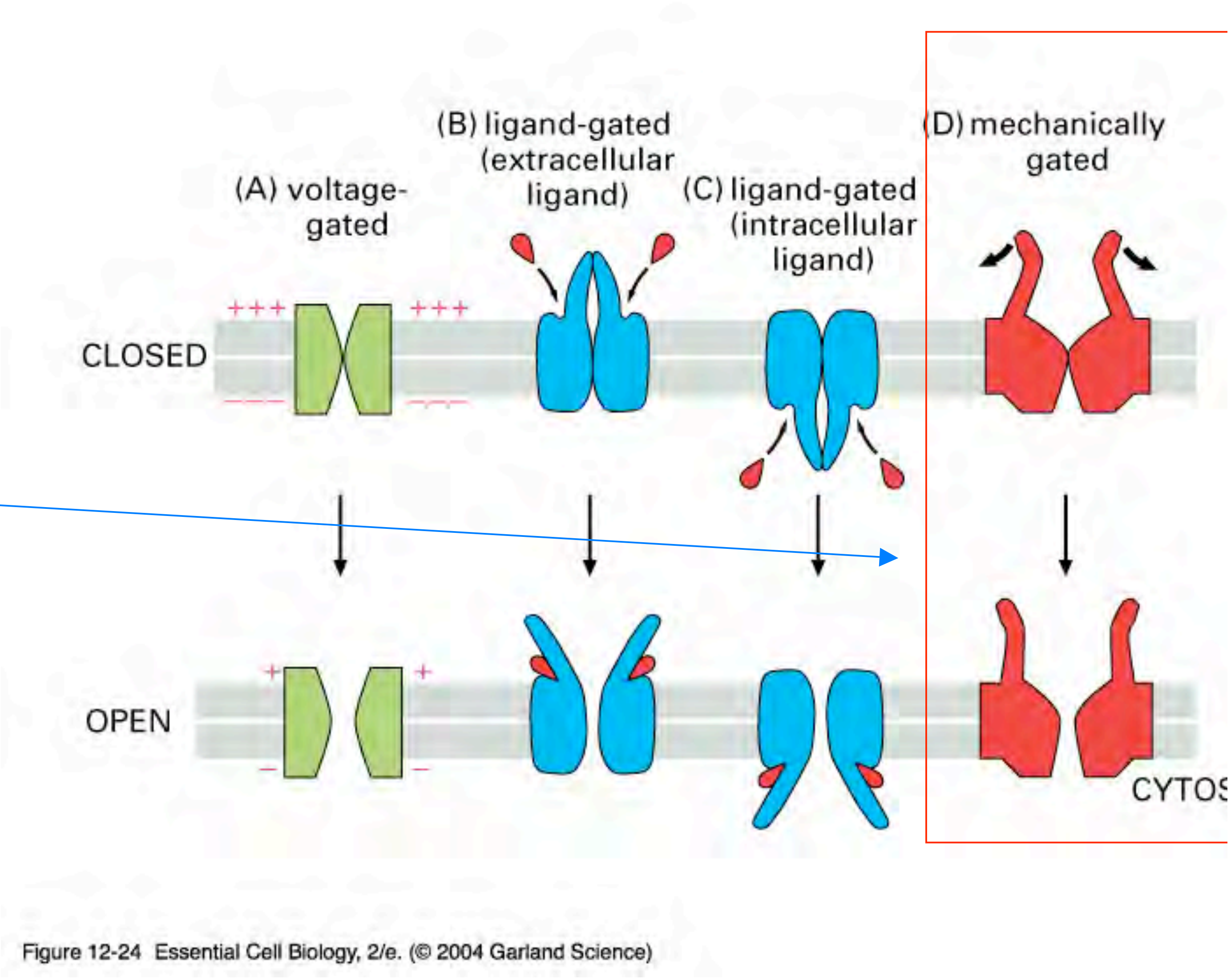


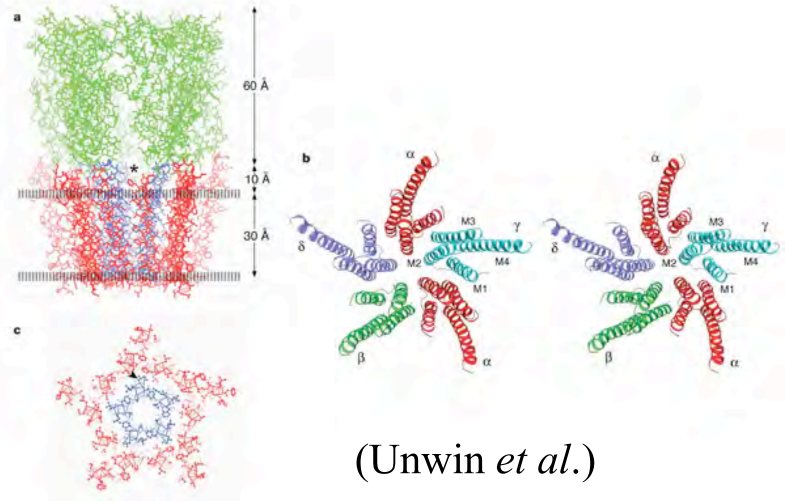
Figure 12-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

How We Know: Structural Biology

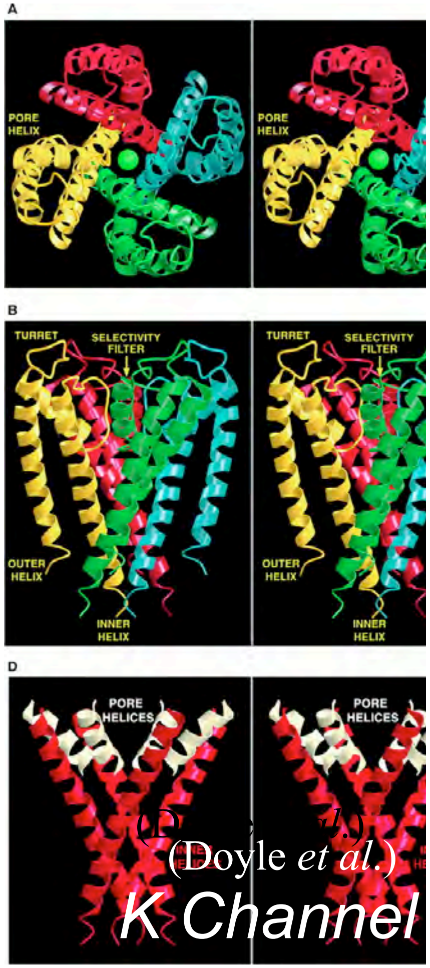
- ***Some famous examples of ion channels studied by structural biologists.***

Nicotinic acetylcholine receptor

EM & X Ray structures



(Unwin *et al.*)



(Doyle *et al.*)

K Channel

How We Know: Patch Clamping

- ***The idea: grab a patch of membrane and apply a potential difference to measure the currents.***
- ***Fraction of time spent open depends upon magnitude of driving force.***

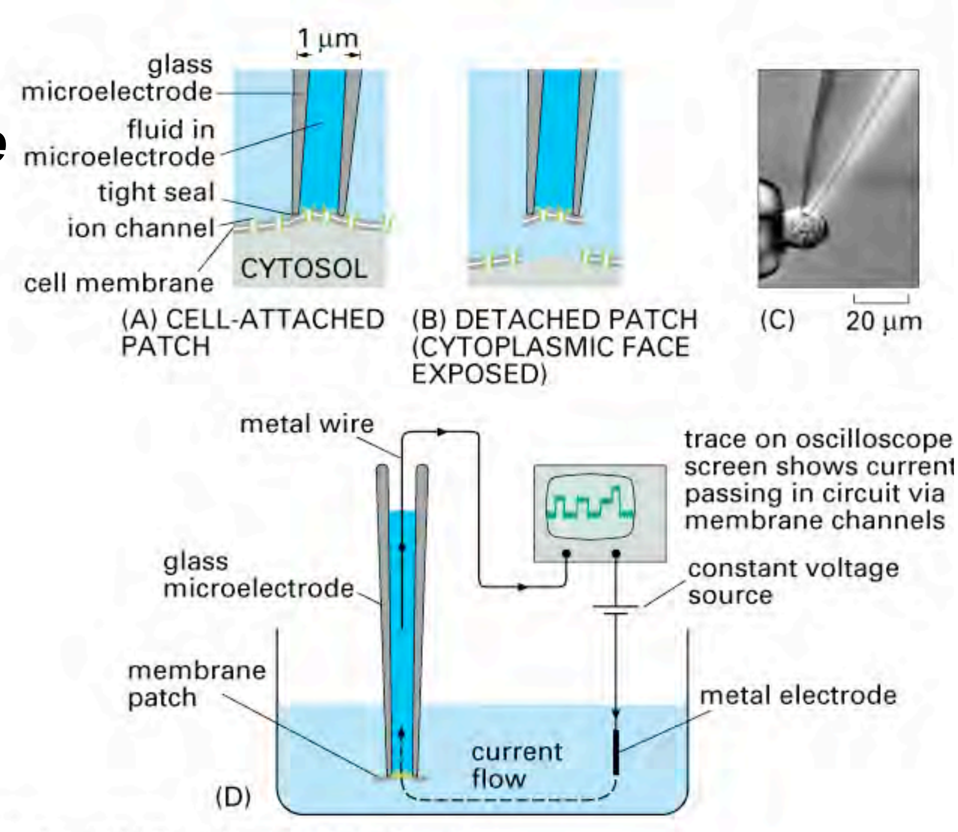
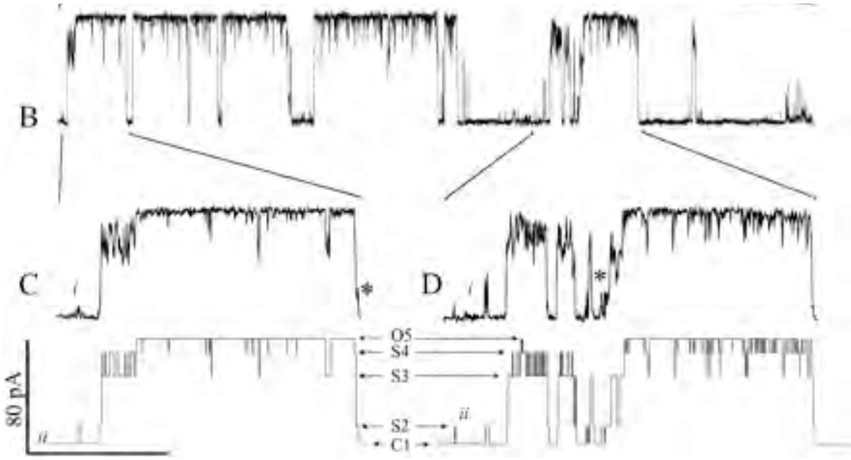


Figure 12-22 Essential Cell Biology, 2/e. (© 2004 Garland Science)

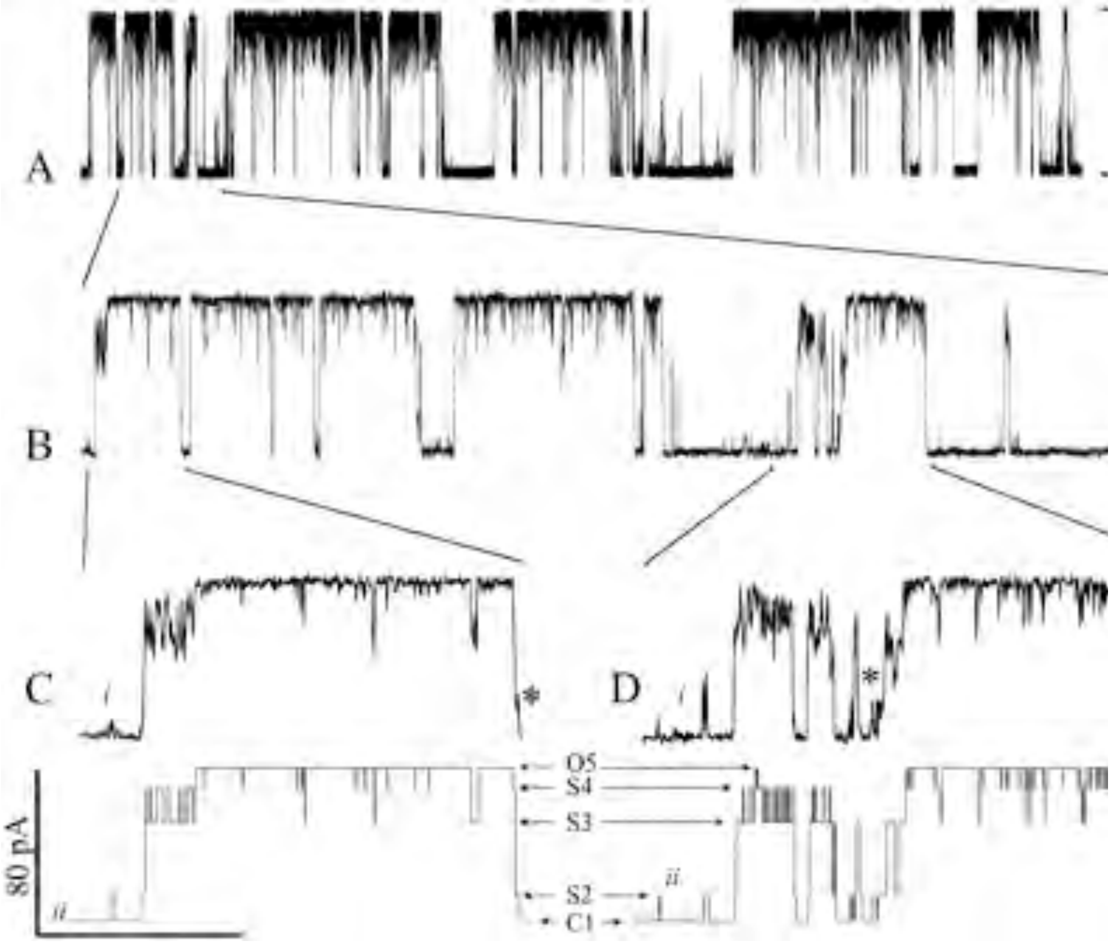
(Sukharev *et al.*)



pA currents lasting several milliseconds.

Conductance of MscL Under Tension

- ***Electrophysiology measurements (patch clamping) lead to current vs membrane tension.***
- ***Measurements reveal five distinct conductance substates.***



(Sukharev *et al.*)

Consequences of Ion Channel Gating: The Action Potential

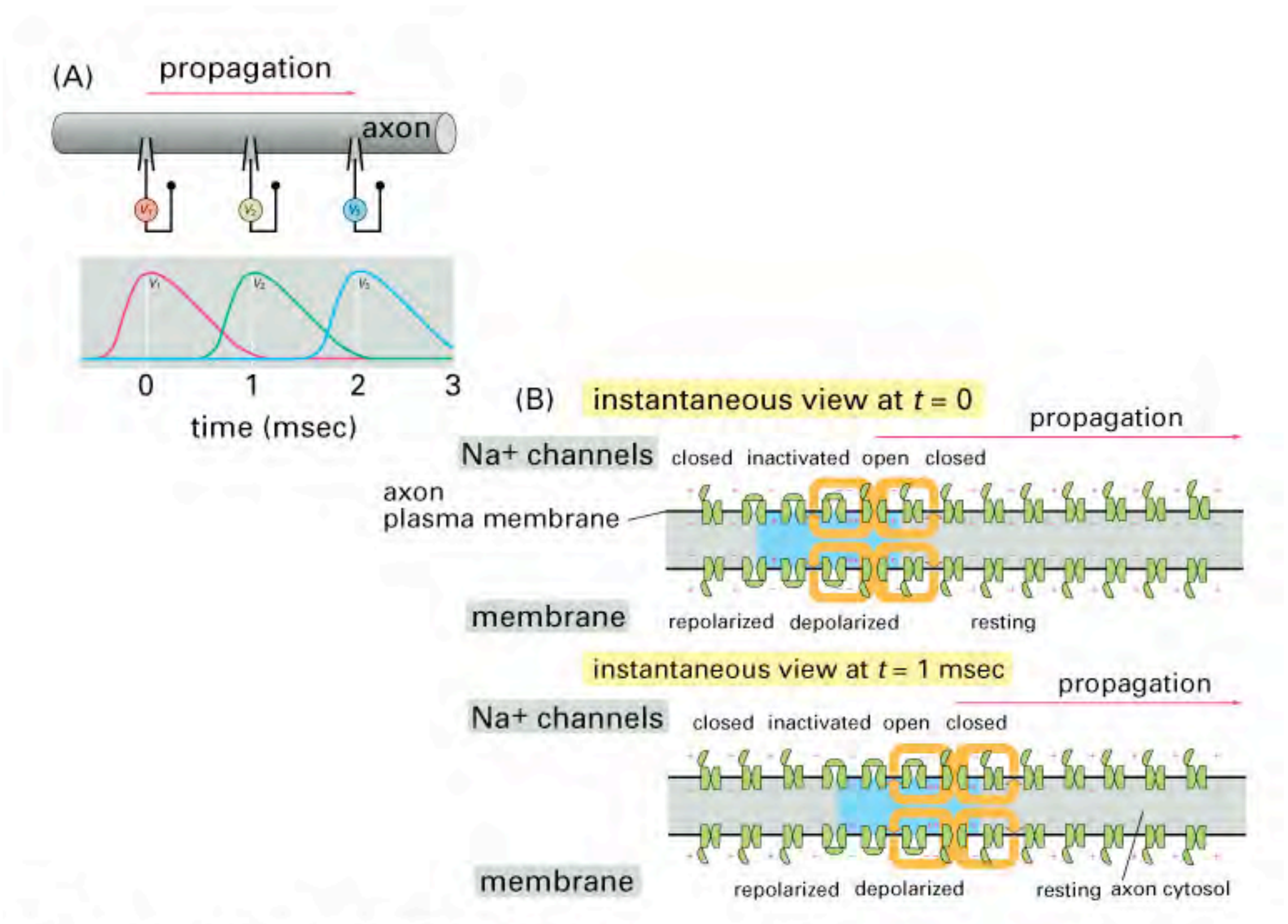
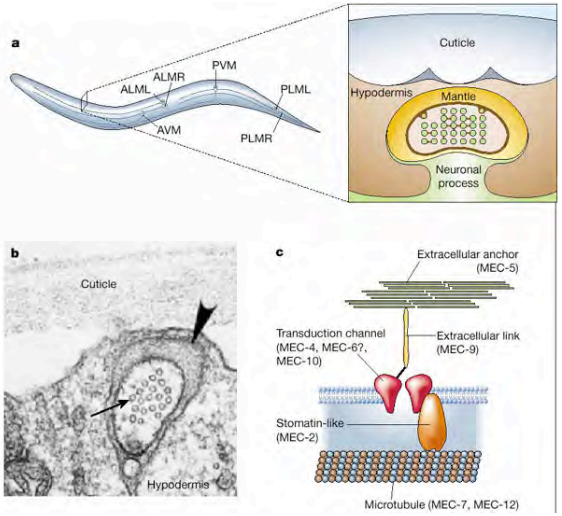


Figure 12-38 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Ubiquitous Phenomenon of Mechanosensation

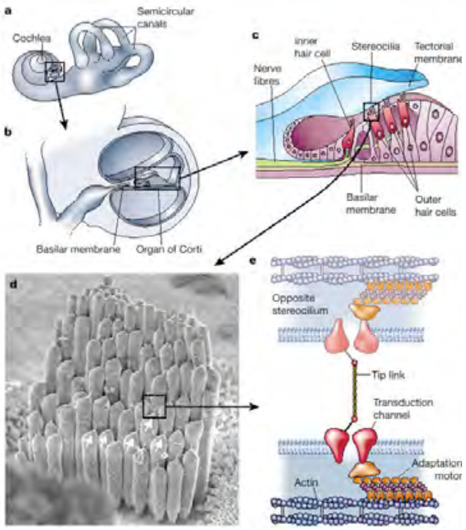
- ***The main point: mechanosensation is everywhere.***
- ***Informational currency is electrical – detection is mechanical.***
- ***Repetition of same motif – mechanical excitation results in transient flow of ions.***



Touch sensation in worm

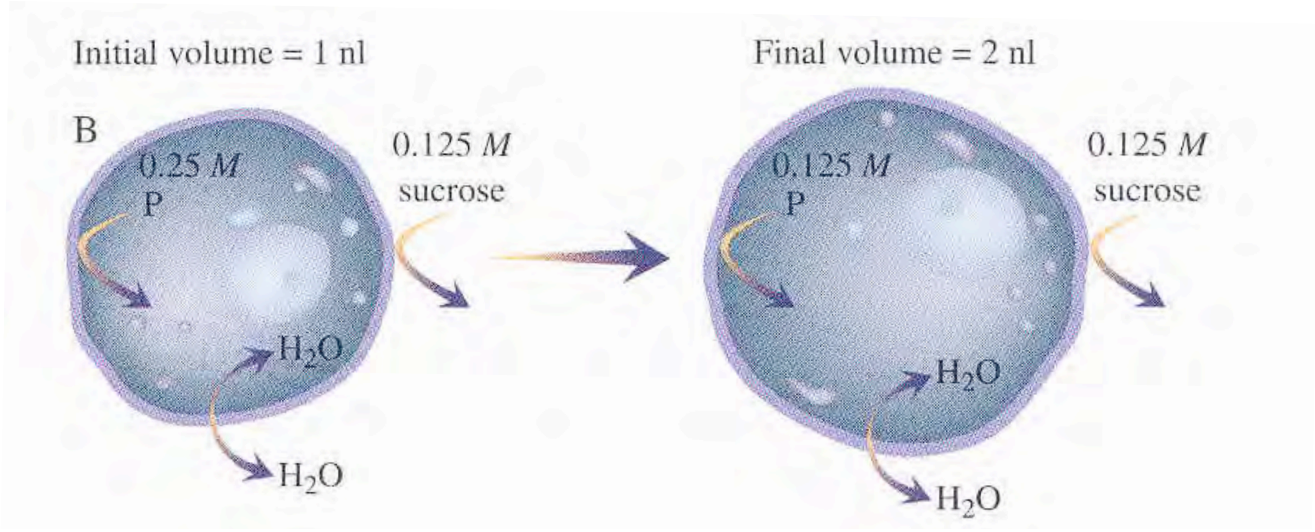
(Gillespie and Walker)

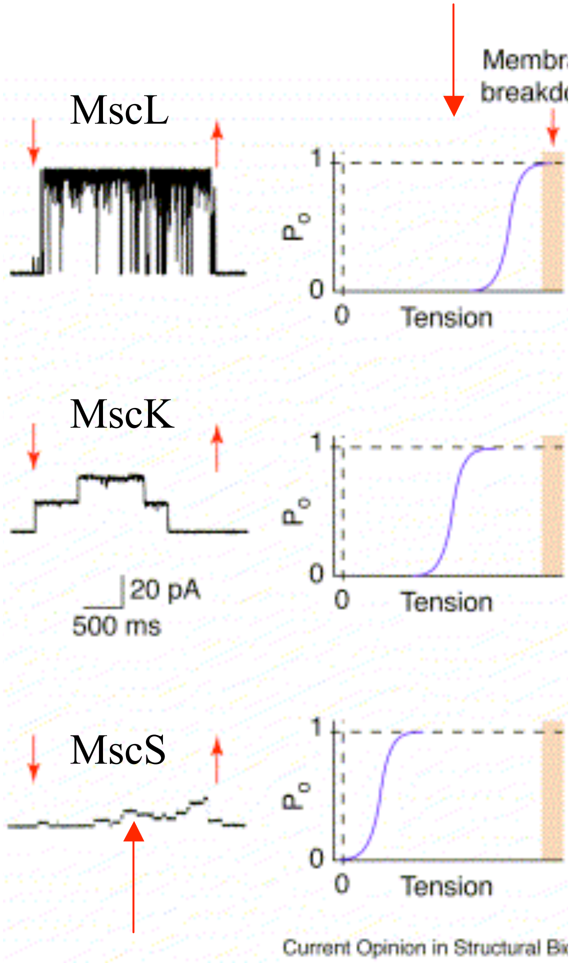
Mechanical response of hair cells



Mechanosensitive Channels as Osmotic Pressure Relief Valves

- ***Hierarchy of mechanically-gated channels.***
- ***Properties of channel have been investigated using electrophysiology.***
- ***Gating tension of MscL serves to avoid membrane rupture.***





(Perozo and Rees)

More on Osmotic Shock

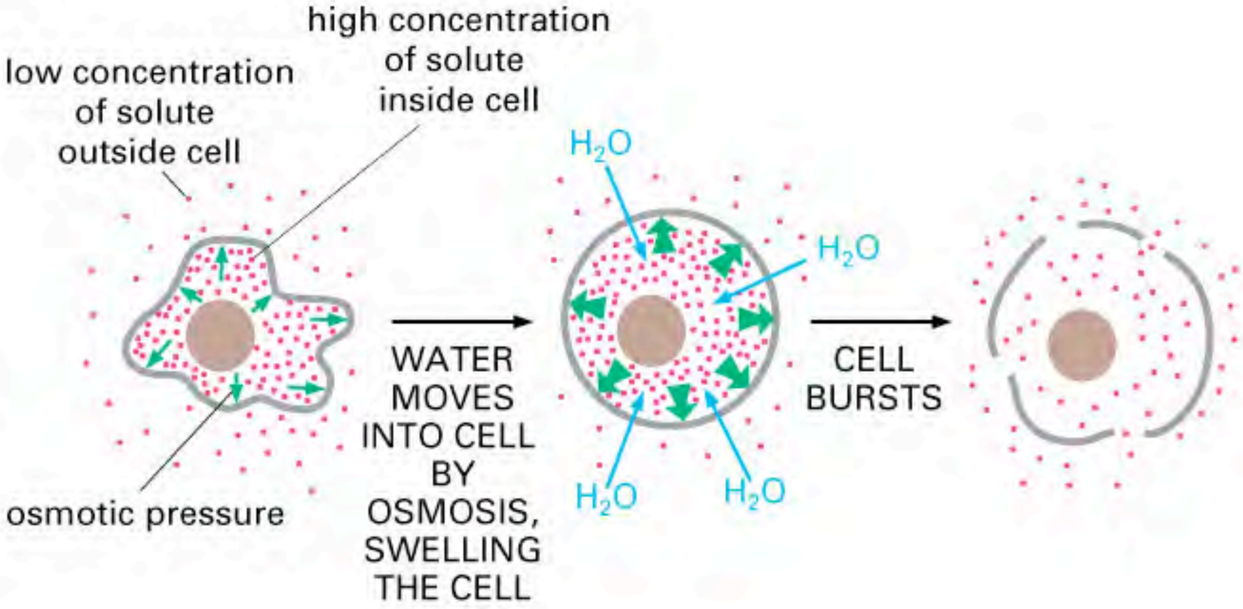
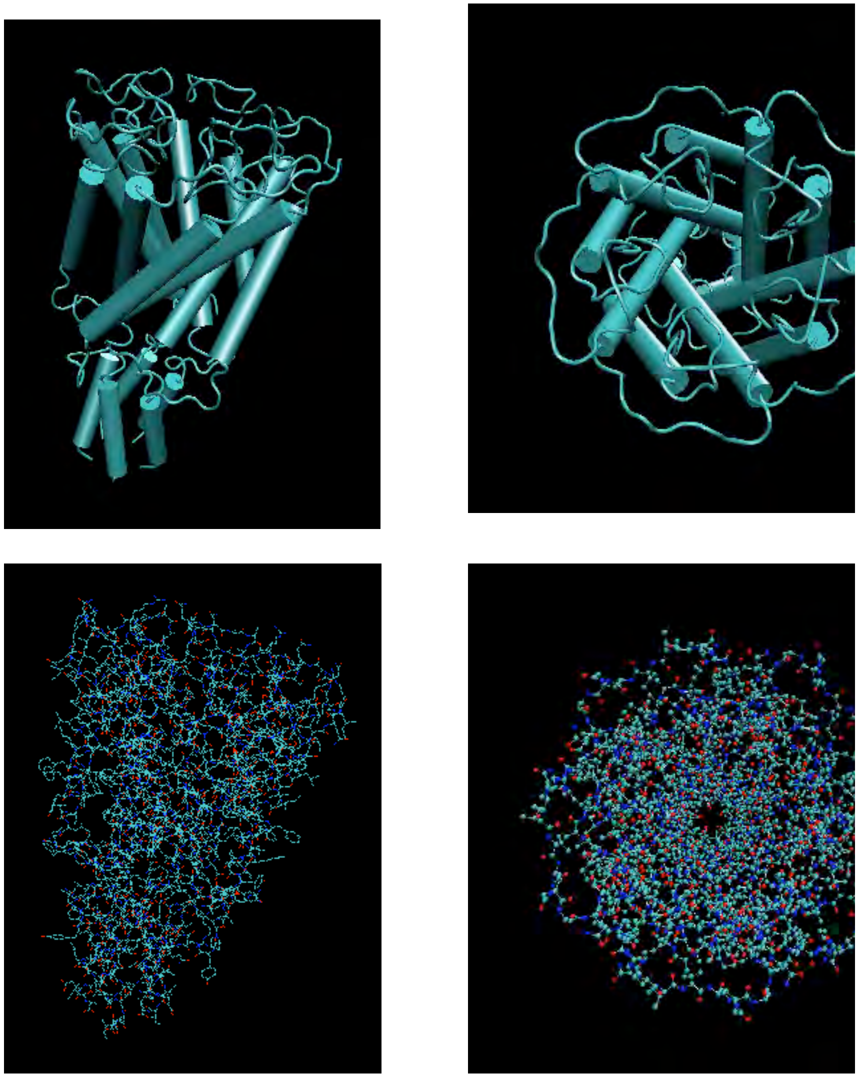
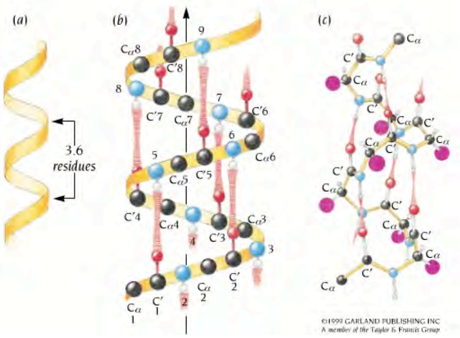


Figure 12-16 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Coarse-Grained Descriptions of Macromolecular Structure

- ***Description of biological structures can be undertaken from a variety of different perspectives.***
- ***Two key ways of viewing structure are ribbon diagrams and all-atom descriptions.***





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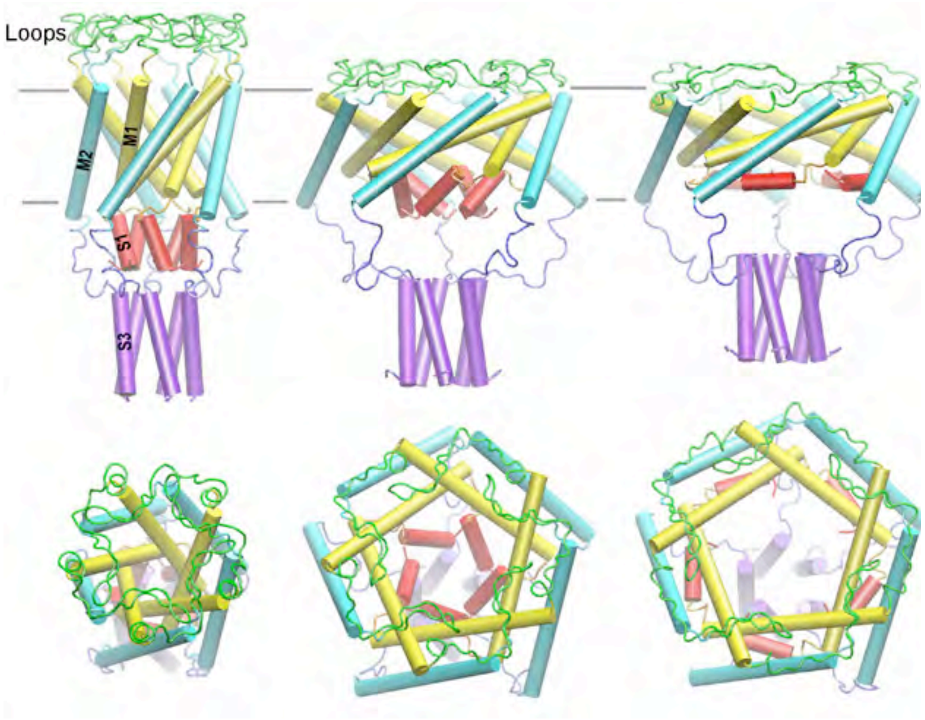
Conformational Change During Gating

- ***Hypothesized structural pathway for opening the channel. Tilting of alpha helices and corresponding opening of the pore.***
- ***Key Question: How does mechanical tension couple to the conformational change?***
- ***What are the energetic consequences to the surrounding membrane as a result of channel opening?***



(Sukharev *et al.*)

Lipid Bilayers (In Vitro)

- ***Hydrophobic tails and polar head groups.***
- ***Favorable for lipids to spontaneously assemble to form bilayers.***

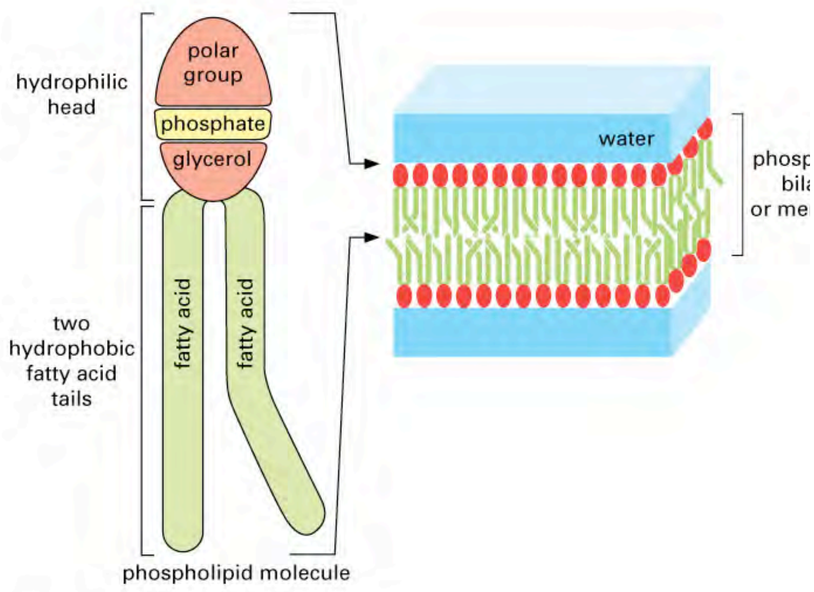
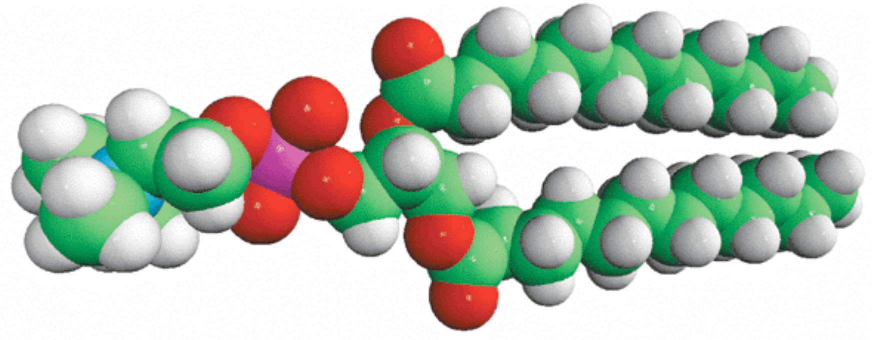
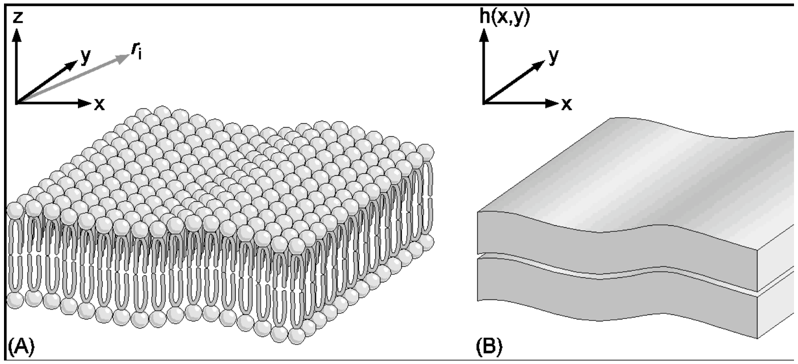


Figure 2-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)



(Avanti Polar Lipids)

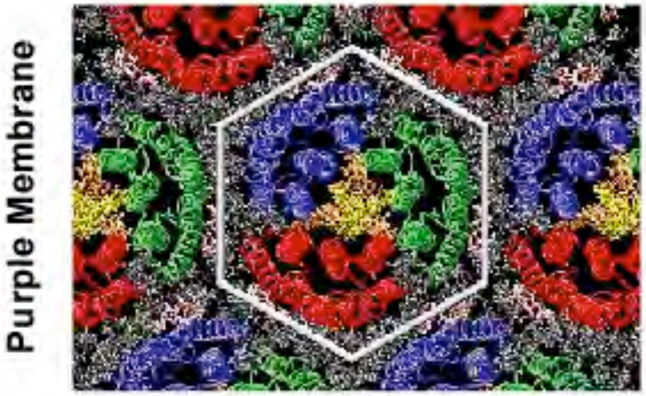


Molecular ***Continuu***

Membranes In Vivo

- ***Real biological membranes contain many different lipids & transmembrane proteins!***

	Purple Membrane	**Human**
M_L/M_P	**0.2**	**3-4**



Biophysics Group UIUC

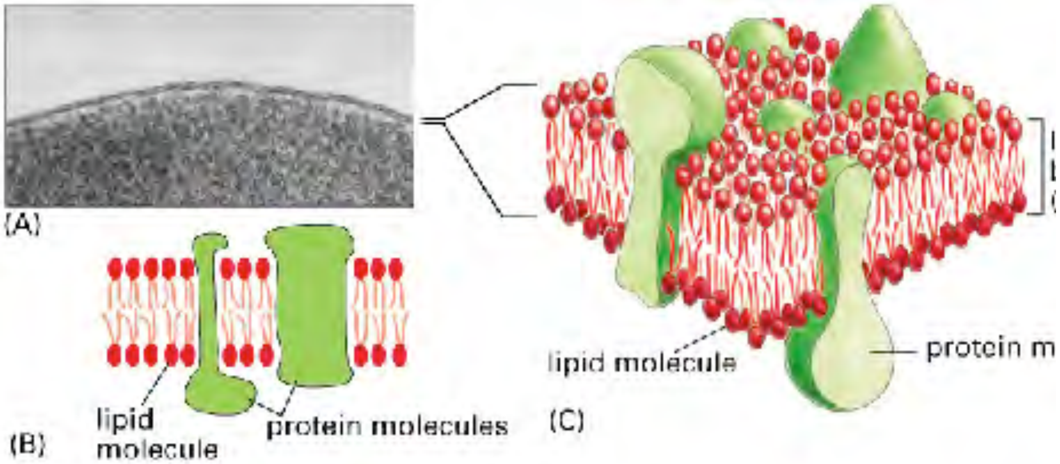
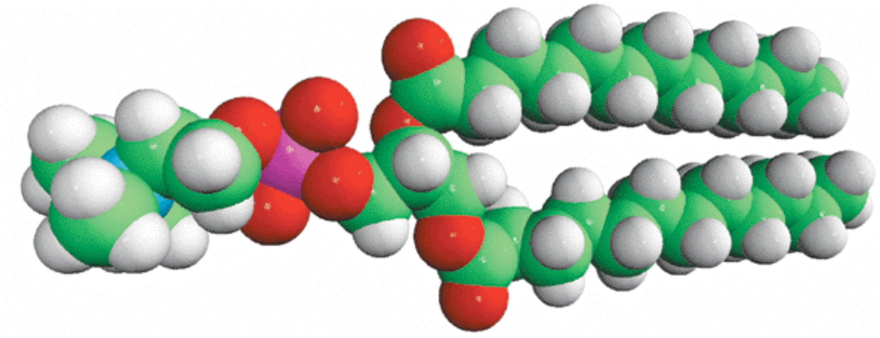


Figure 10–1. Molecular Biology of the Cell, 4th Edition.

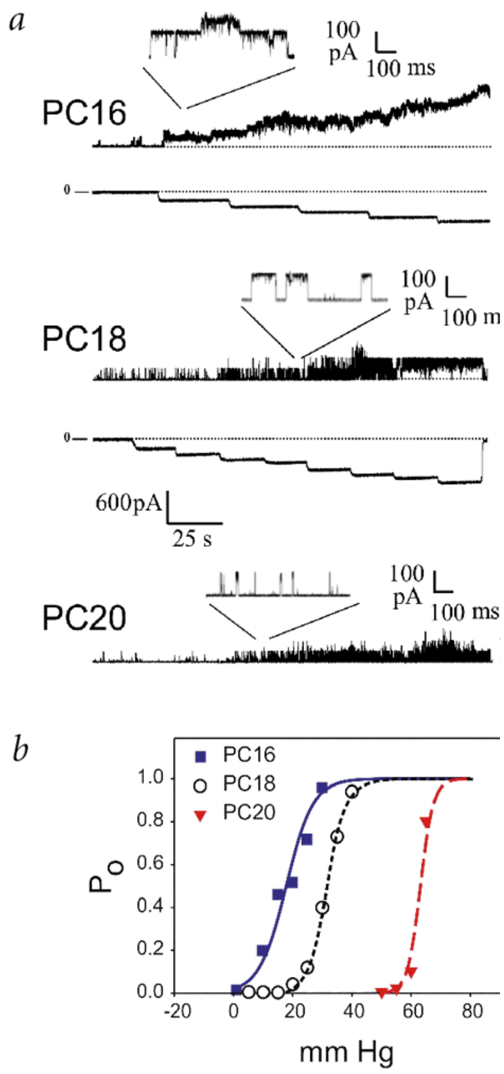
Experimental Challenges for Model: Lipid Tail Length

- ***Gating tension depends upon the length of the lipid tails.***



(Avanti Polar Lipids)

- ***Free energy cost associated with mismatch between thickness of protein and lipids.***



(Perozo *et al*.)

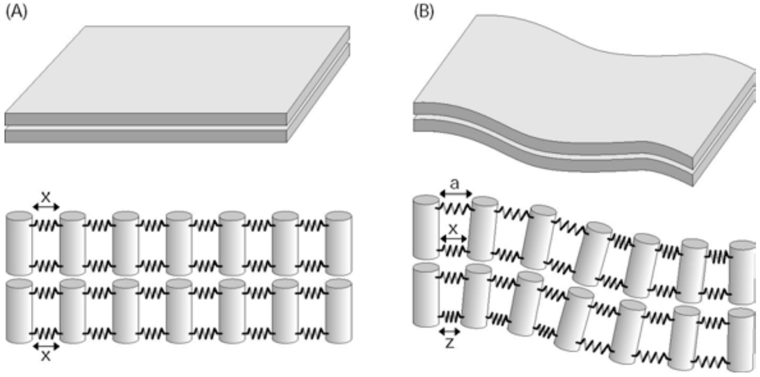
The Membrane Free Energy

- ***The idea: solve boundary problem for protein embedded in membrane (Huang, Andersen and others).***

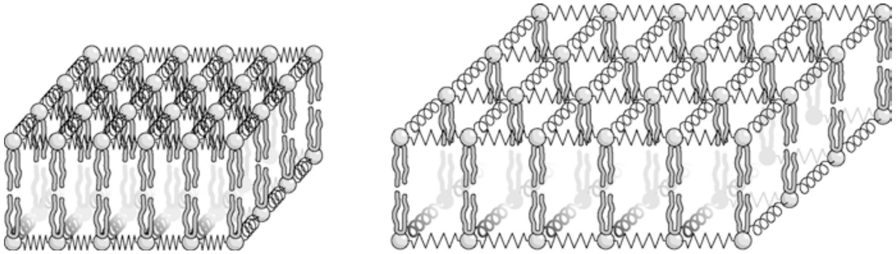
- ***We use elasticity theory and can thereby compute the energy as function of protein shape.***

Bending:

$$E = \int_{\mathcal{M}} d^2\sigma \; \left(\tfrac{1}{2} K_C \left[S - C_0 \right]^2 + K_G G \right.$$

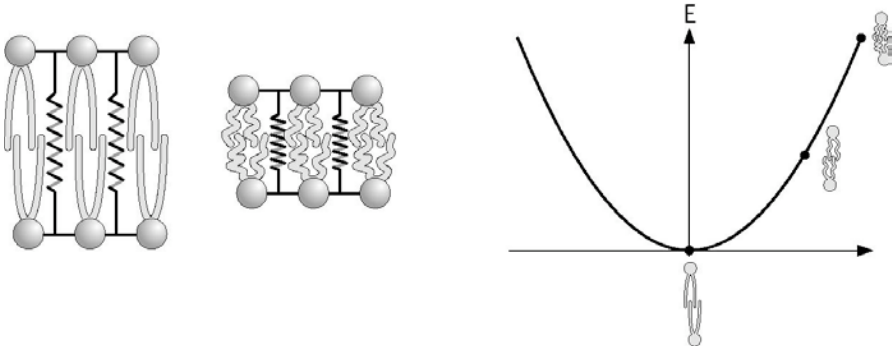


The Membrane Free Energy: Part 2



Tension (in plane Stretch):

$$E = \int_{\mathcal{M}} d^2\sigma\, \alpha$$



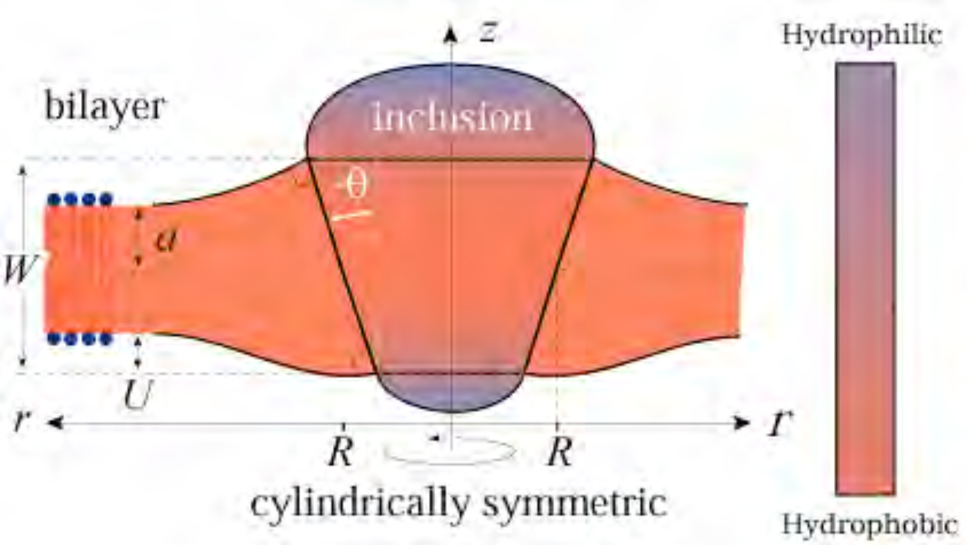
Stretch (out of plane):

$$E = \int_{\mathcal{M}} d^2\sigma\, \frac{1}{2} K_A \left(\frac{u}{a}\right)^2$$

Protein Boundary Value Problem

- ***Minimize free energy – Euler-Lagrange equations for midplane position (h) and thickness (2u).***
- ***Solve equations, match BC's, & compute deformation energy***

$$[K_B\nabla^4 - \alpha\nabla^2 + \frac{K_A}{a^2}]u = 0 \qquad [K_B\nabla^2 - \alpha]h = 0$$

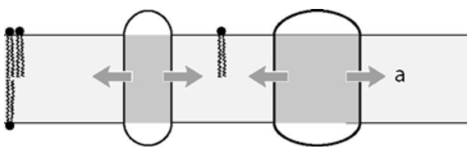


Bilayer Parameters:
$2a$ = Thickness
K_B = Bending Modulus
K_A = Thickness Deformation Modulus
C = Spontaneous Curvature

Inclusion geometry:
R = Radius
W = Thickness
θ = Interface Angle

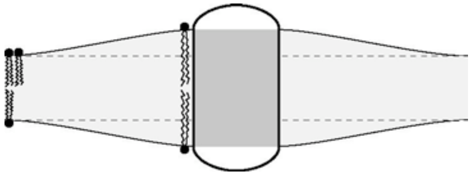
Dissecting the Free Energy

Applied Tension



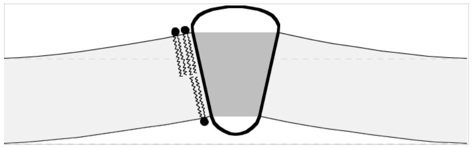
$$G_A = -\alpha A$$

Hydrophobic mismatch



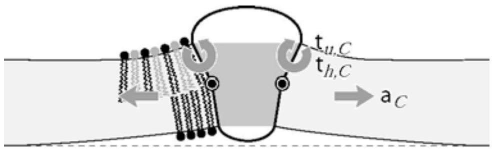
$$G_u = \frac{1}{2}K_{eff}U^2C$$

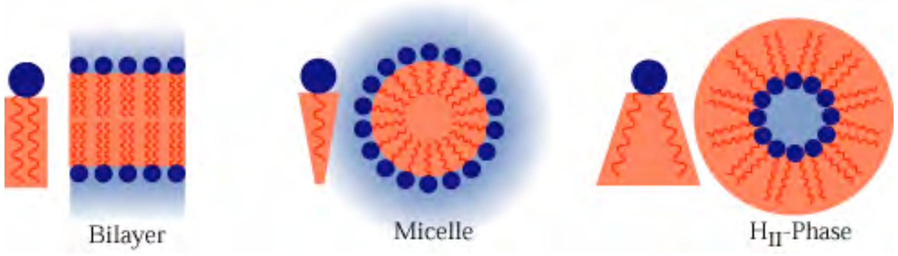
Midplane Bendi



$$G_H = \frac{1}{2}\sqrt{\alpha K_B}H$$

Spontaneous Curvature





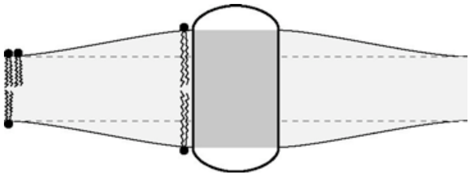
$$G_{C_0} = K_B(C_0H' + \bar{C}_0U')C$$

Conclusion: Competition between terms with different radial charac

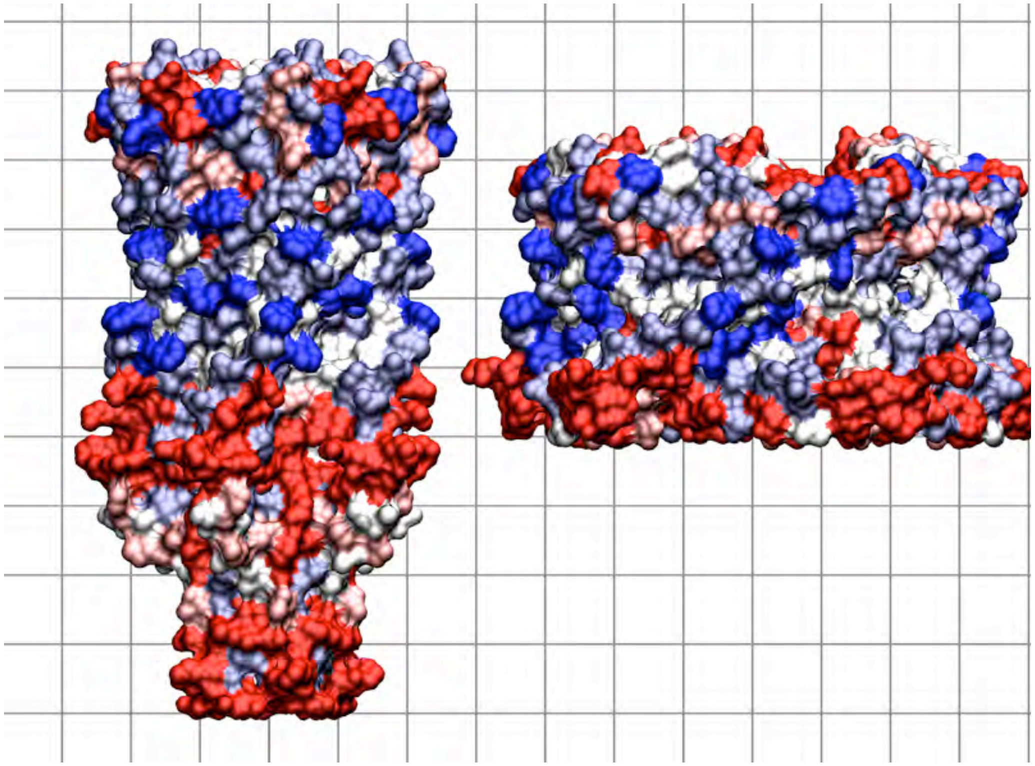
Line Tension & Applied Tension

Dissecting the Free Energy: Hydrophobic Mismatch

Hydrophobic mismatch



$$G_u = \frac{1}{2}K_{eff}U^2C$$



- ***Can tune the hydrophobic mismatch two ways: change the lipid or mutate the protein.***

An Effective Potential For Channel Opening

- ***Elastic deformation of the membrane is induced by channel.***
- ***Thickness mismatch leads to a line tension which works agains applied tension***
- ***Effective potential analogous to a nucleation problem.***

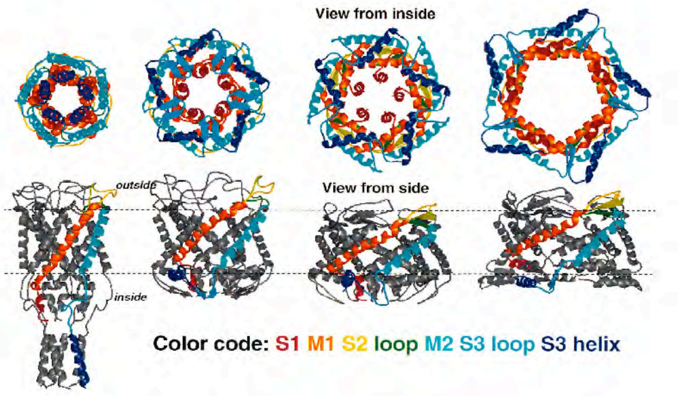
Effective potential for channel radius

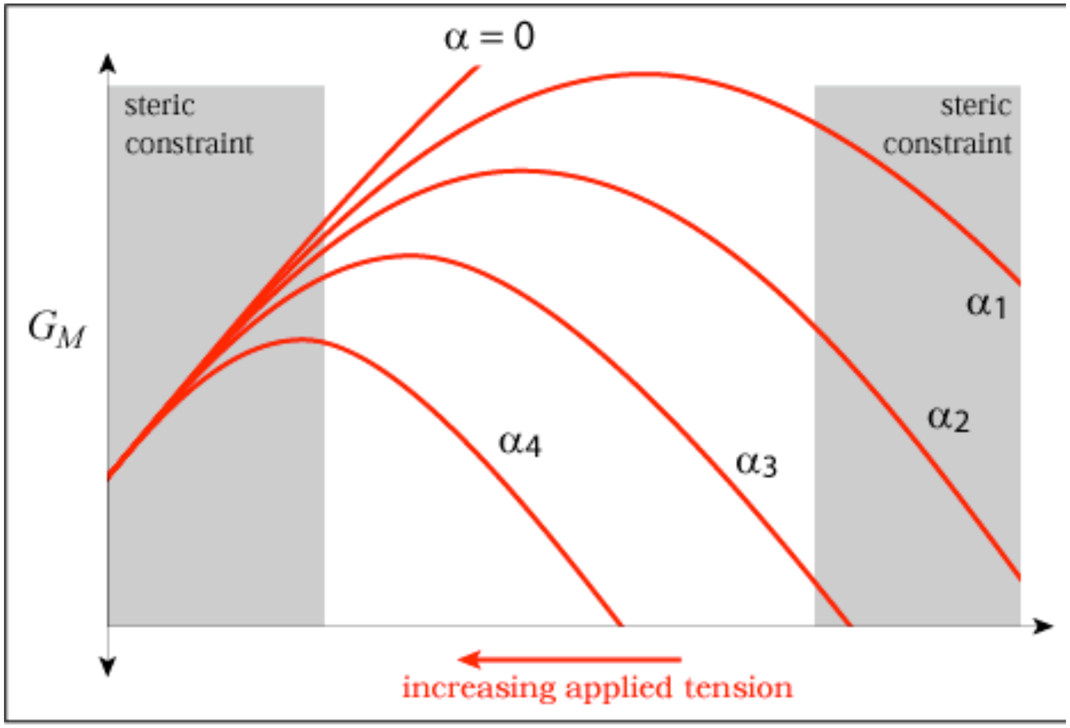
$$G_M = f\,2\pi R - \alpha\pi R^2$$

f = line tension

α = applied tension

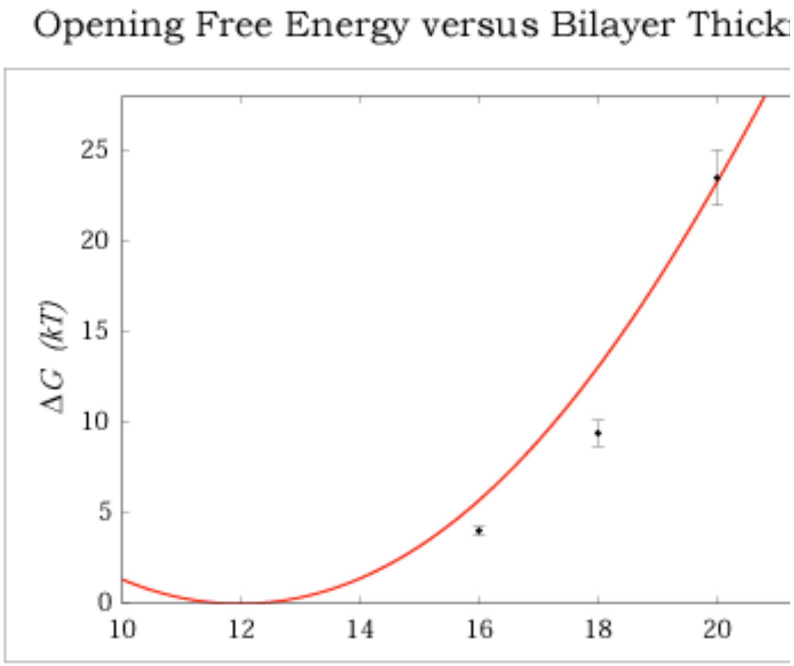
$$f > 0$$

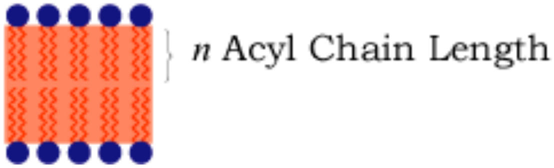




Experimental Predictions

- ***Critical tension depends upon lipid length.***
- ***Curvature inducing lipids can change the sign of the effective line tension – stabilizing open state.***
- ***Amino acid substitutions that tune the hydrophobic width of the channel alter gating tension in a systematic fashion.***

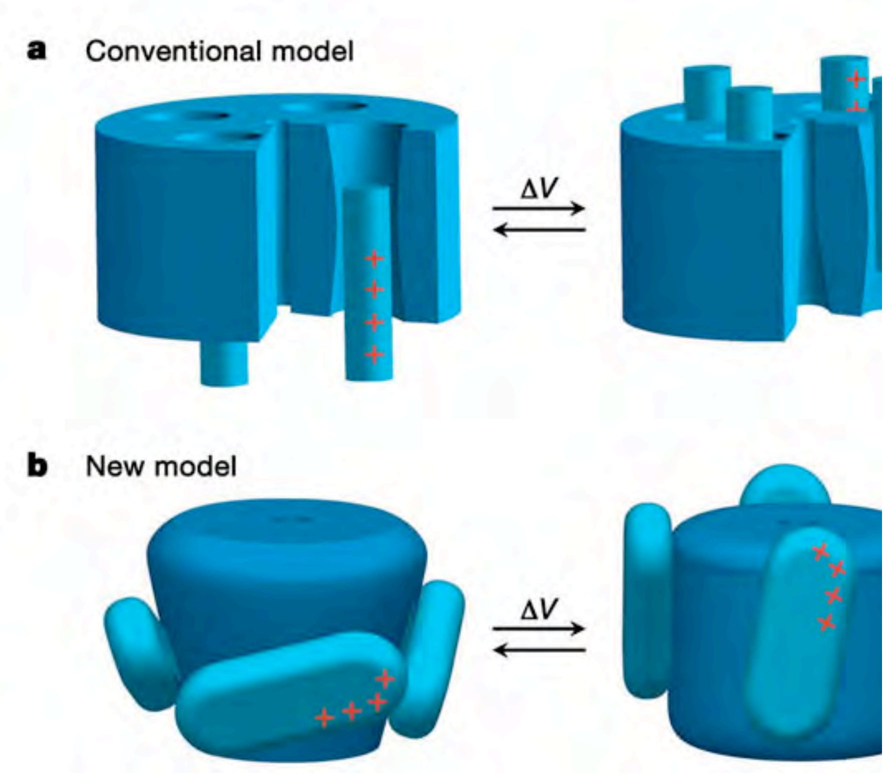

Opening Free Energy versus Bilayer Thick

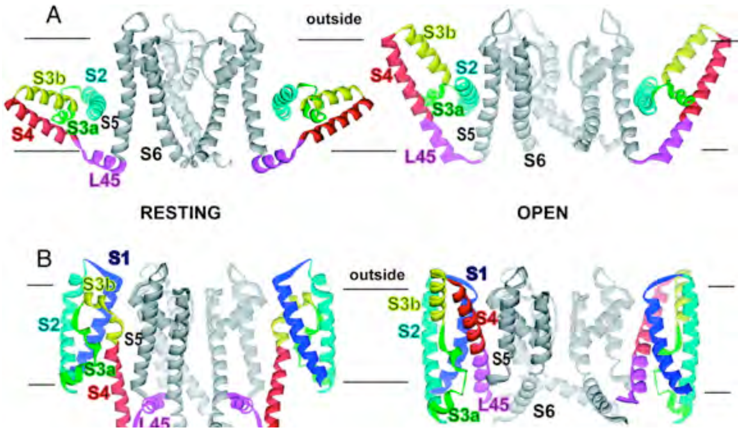


The Curious Case of Voltage Gating

- *The idea: ion channels (such as for K) are gated by voltage.*
- *Structural biologists have made huge progress, but their successes have left a wake of paradoxes.*
- *RP opinion: careless in treatment of membrane! Membrane mechanics distinguishes them.*

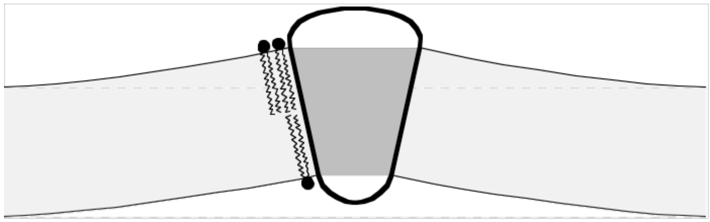
(Mackinnon *et al.*)



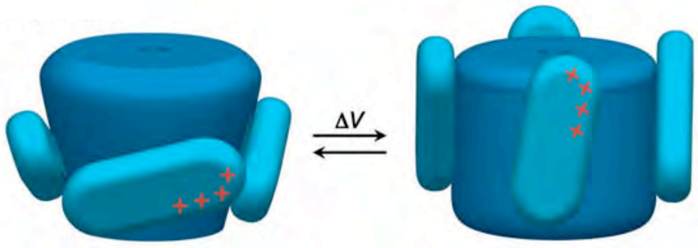


Flirting with a Simple Model of Voltage Gating

- ***Same logic – write free energy which reflects response of channel AND surrounding membrane.***



$$G_{membrane}[h(x)] = \underbrace{\frac{K_b}{2}\int \frac{h''^2}{(1+h'^2)^3}dx}_{\text{bending energy}} + \underbrace{\alpha \int (\sqrt{1+h'^2} - 1}_{\text{tension}}$$

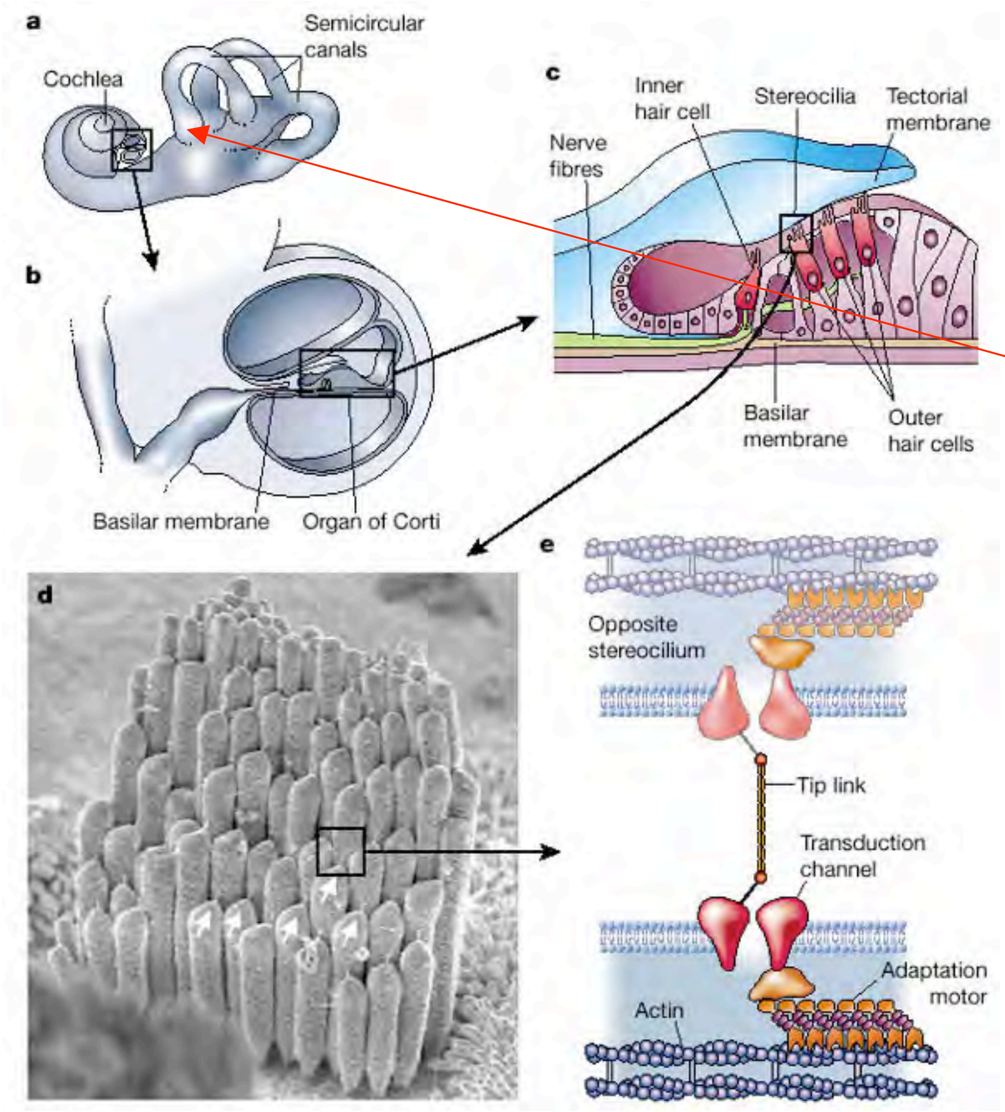


$$h(x) = \underbrace{\frac{tan\theta}{\lambda}e^{-\lambda x}}_{\text{trial solution}}$$

$$G_{protein} = pEcos\theta$$

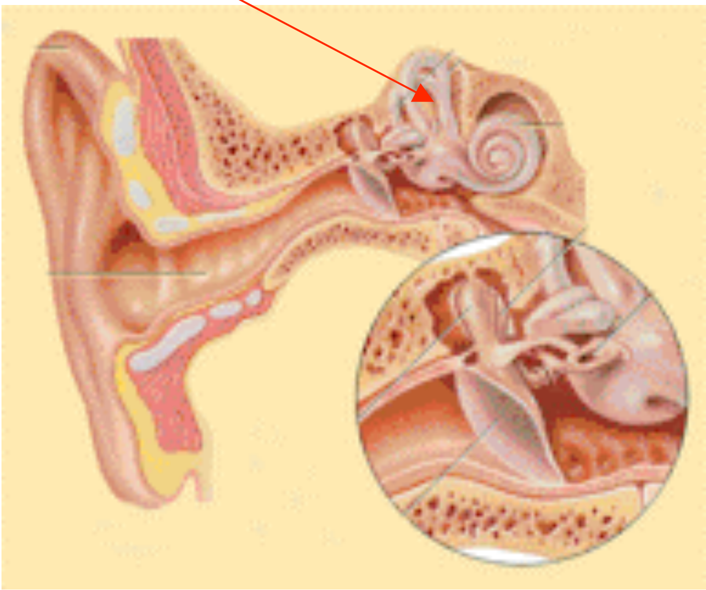
How gating depends upon voltage, tension (!), lipid character, etc… Testable – SMB bring it on! Two models have different consequences

Ear Structure and Function: Ion Channel Gating



- ***Collective respons multiple detectors driving multiple channels.***

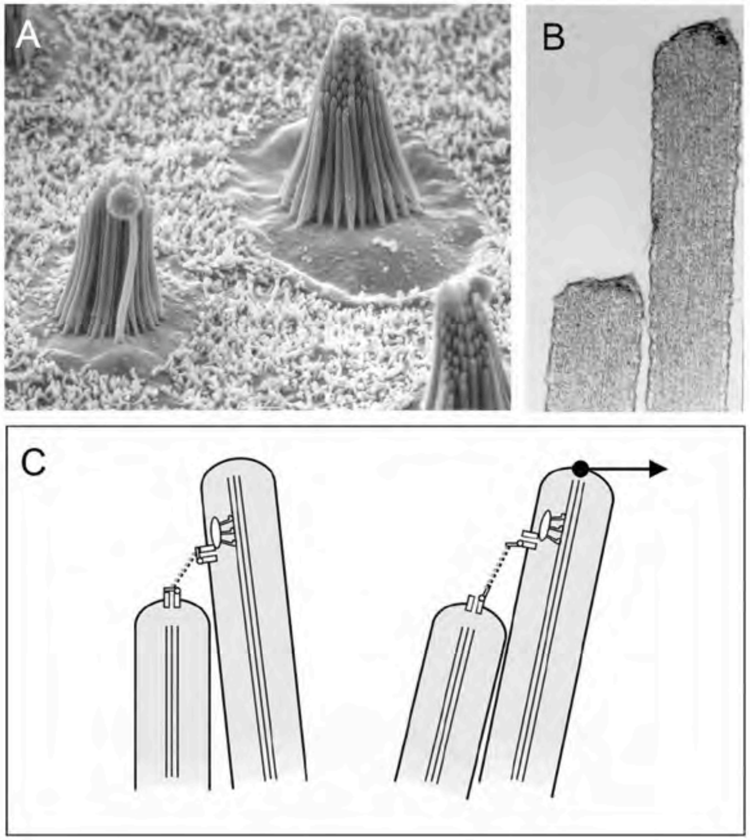
(Cochlear function.)



Richness of Dynamics: Adaptation

- ***Hair cells exhibit nonlinear response – they adapt to stimulus.***
- ***Relevant molecular participants are as yet unknown.***

(Sukharev *et al.*)



(Muller and Littlewood-Ev