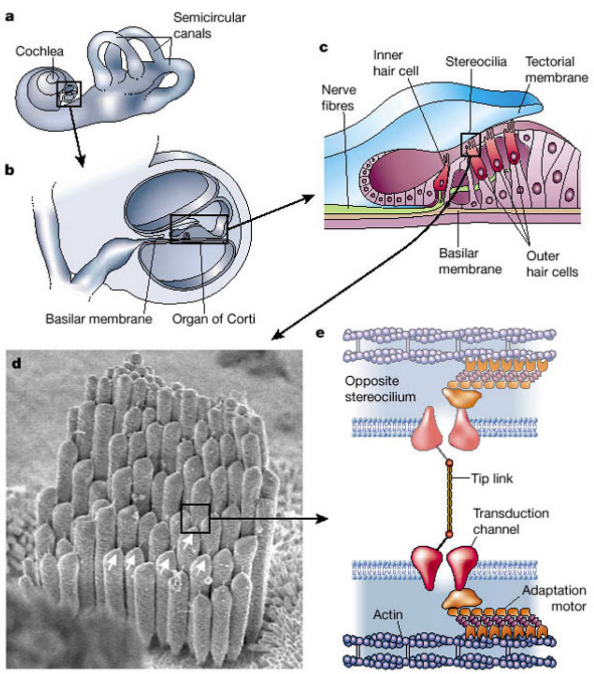
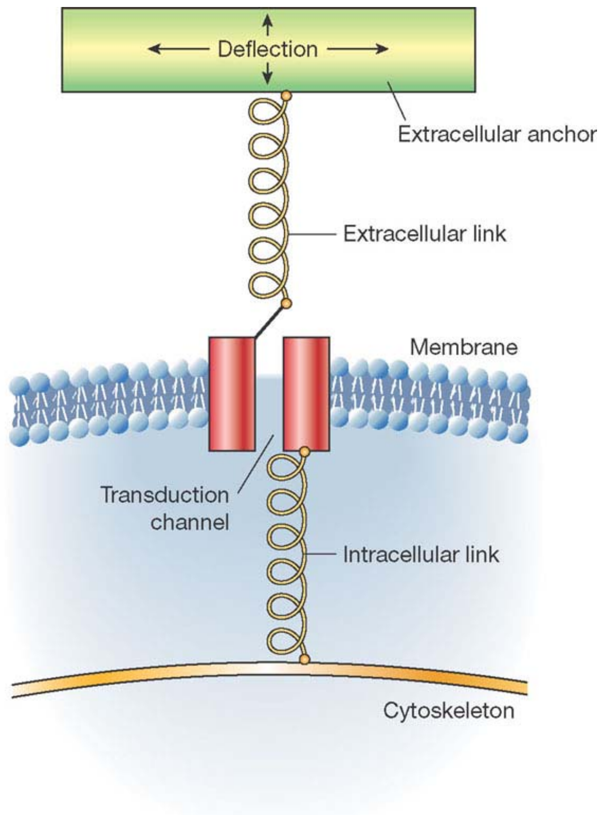
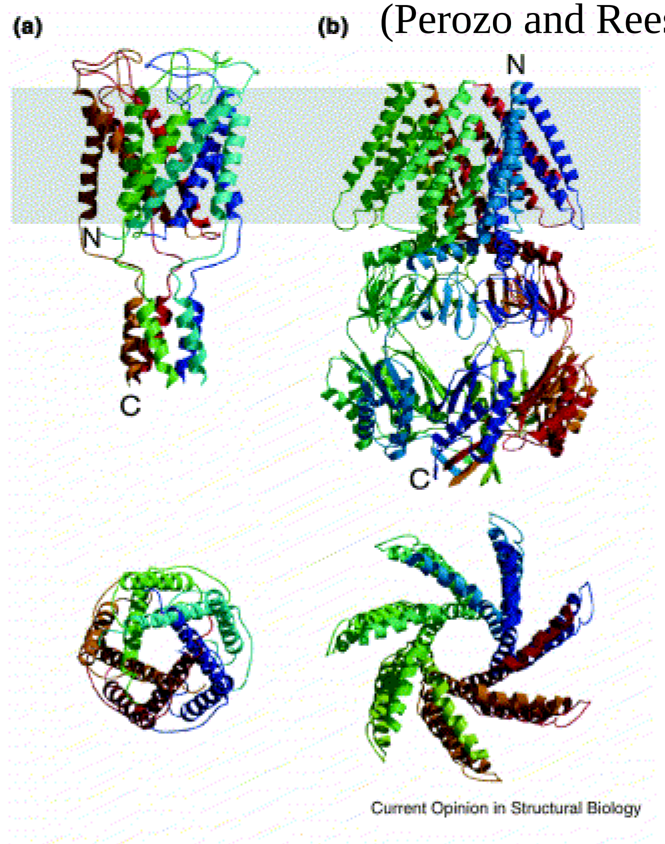
A Feeling for Mechanosensation: Gating Mechanosensitive Ion Channels





(Gillespie and Walker)

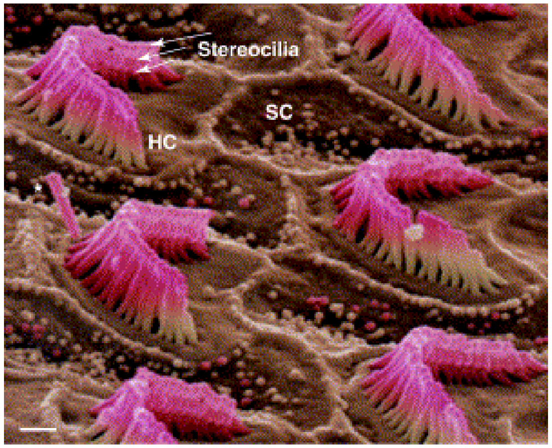


(Perozo and Rees)

Rob Phillips, Paul Wiggins, Corinne Ladous, Tristan Ursell and Doug Rees
California Institute of Technology

Overview

(Muller and Littlewood-Evans)



Background on The Senses

- Background on senses.
- Mechanosensation, in particular.
- Hypothesized mechanisms.

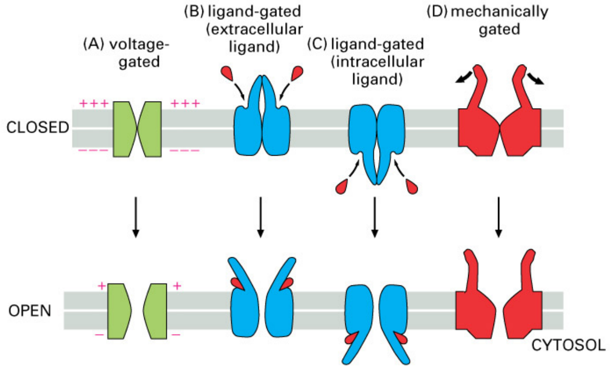
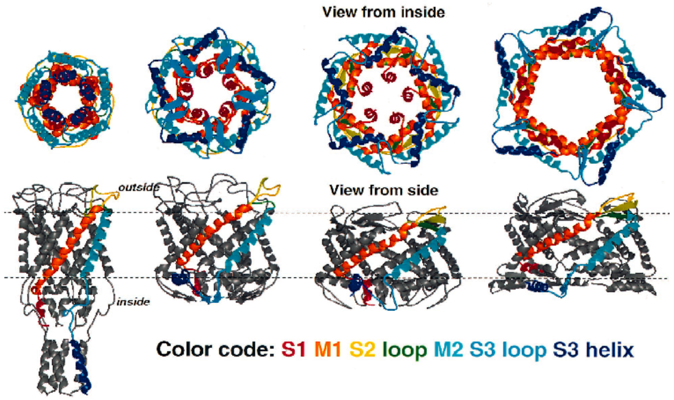


Figure 12-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Ion-Channels

- Ion distributions in cells.
- Transient ion permeability in cells.
- Ion channels.

(Sukharev *et al.*)

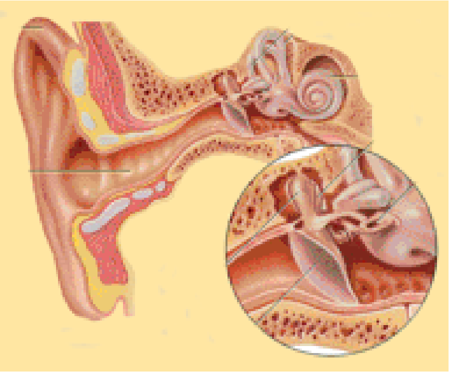


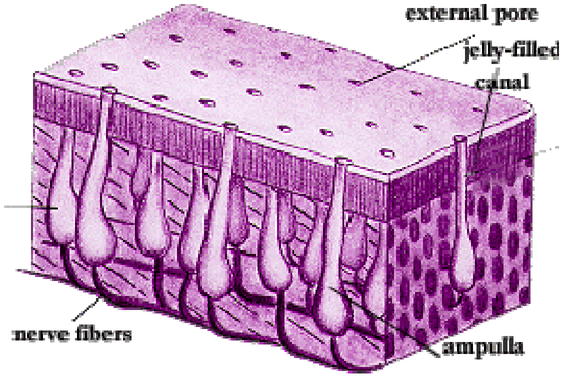
Mechanosensitive Channels

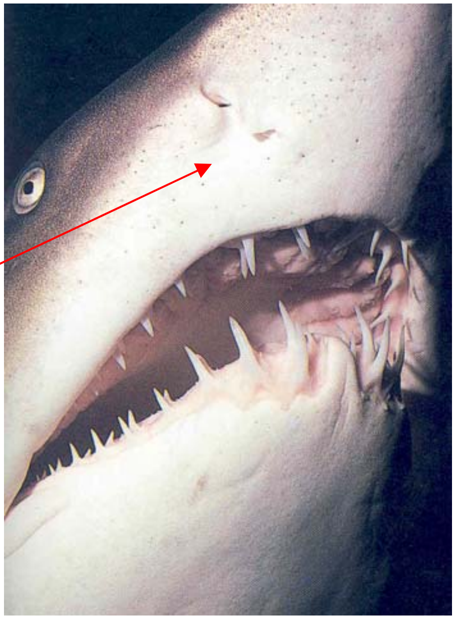
- Mechanosensation in bacteria.
- Experimental background: structural biology and electrophysiology.
- Elastic theory of tension gating.
- Contact with experiment!

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.***

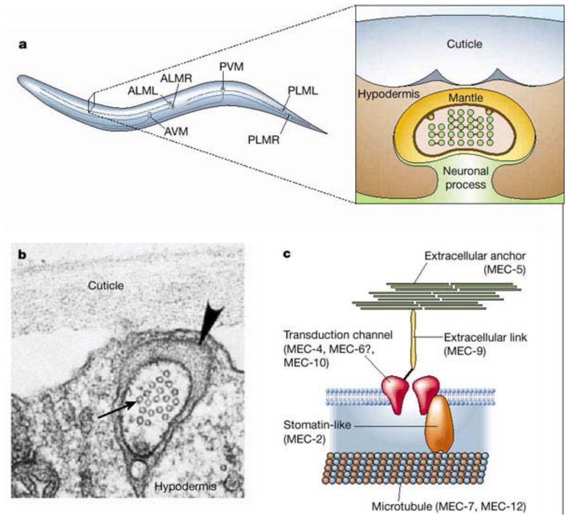






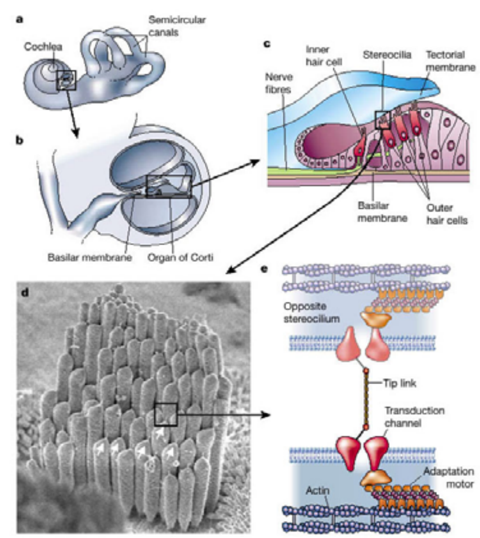
Ubiquitous Phenomenon of Mechanosensation

- ***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

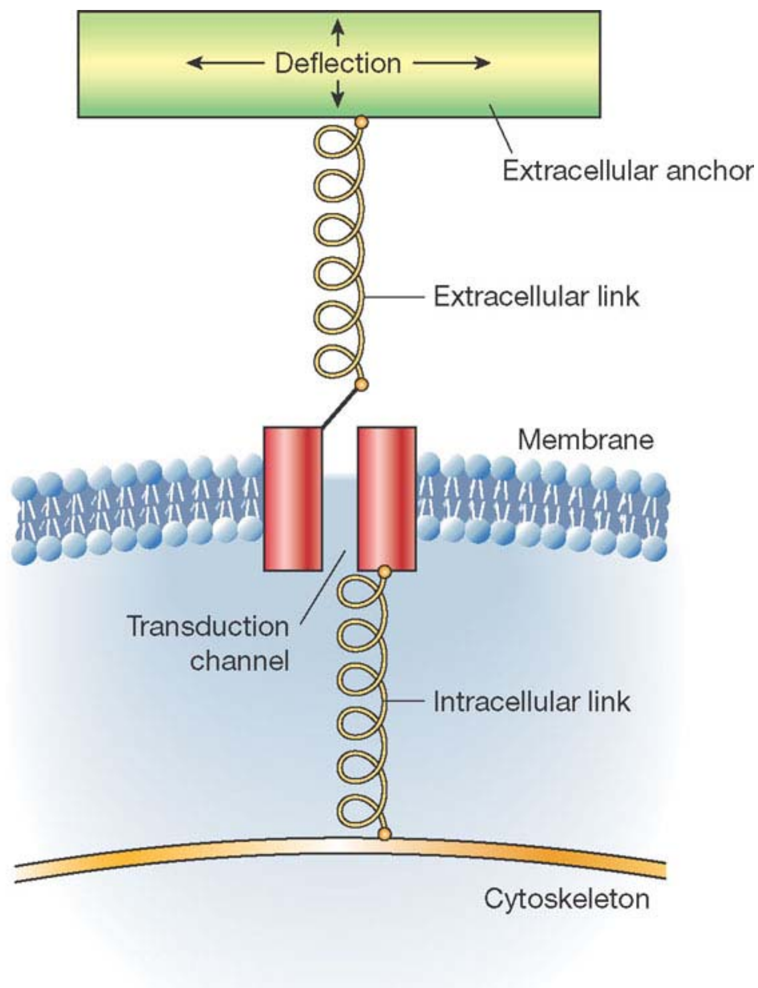
(Gillespie and Walker)



Mechanical response of hair cells

Generic Idea: Mechanical Excitation of Ion Channel Gating

- ***The idea is the coupling of the mechanical motions of a "detector" to the gating of an ion channel.***
- ***Ion channel – transmembrane protein that opens transiently to permit selective flow of ions. (more later)***
- ***Multiple examples in both eucaryotes and prokaryotes.***
- ***Molecular mechanisms in most cases are purely speculative!***
- ***This Talk: We exploit a knowledge of structure and electrophysiology for bacterial mechanosensitive channel to examine molecular mechanism of mechanosensation.***



(Gillespie and Walker)

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.*

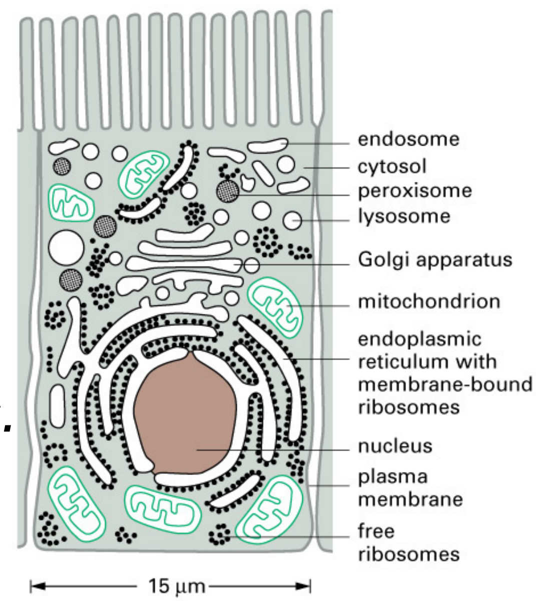
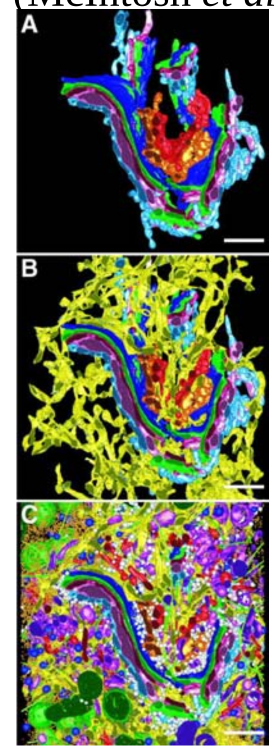


Figure 15-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

(McIntosh *et al.*)



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

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

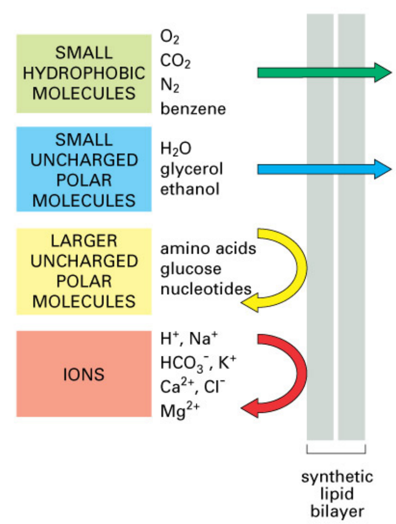
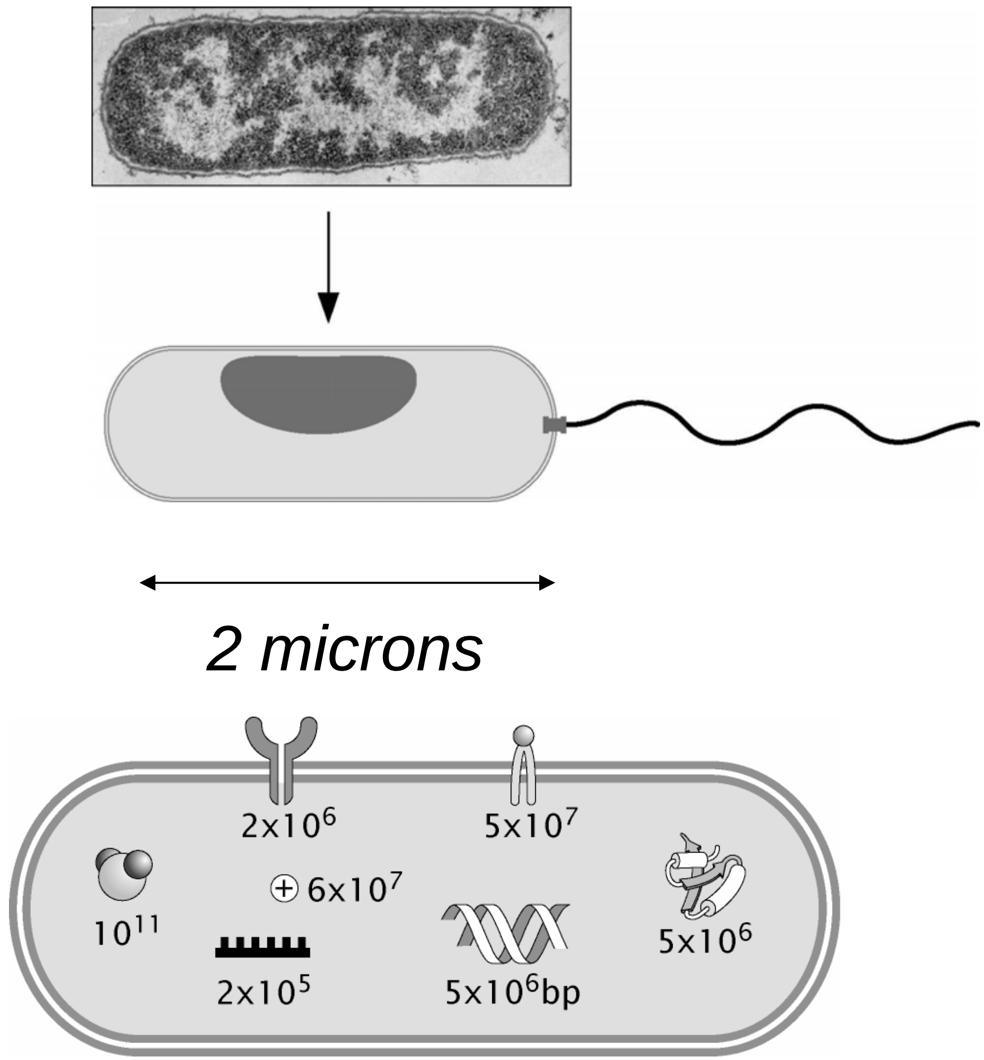


Figure 12-2 Essential Cell Biology, 2/e. (© 2004 Garland Science)

A Single Molecule Census of the Cell

- **The Standard Cell:** ***"Not everyone is mindful of it, but cell biologists have two cells of interest; the one they are studying and Escherichia coli." – Schaechter et al.***
- ***20-40% of the protein stockpile consists of integral membrane proteins. An estimate: roughly 500 copies each of 1000 different membrane proteins. ½ of the cell surface area is dedicated to these proteins.***



Not a full census: ignored lipopolysaccharides, peptidoglycan, etc.. – that is fun too!

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.*

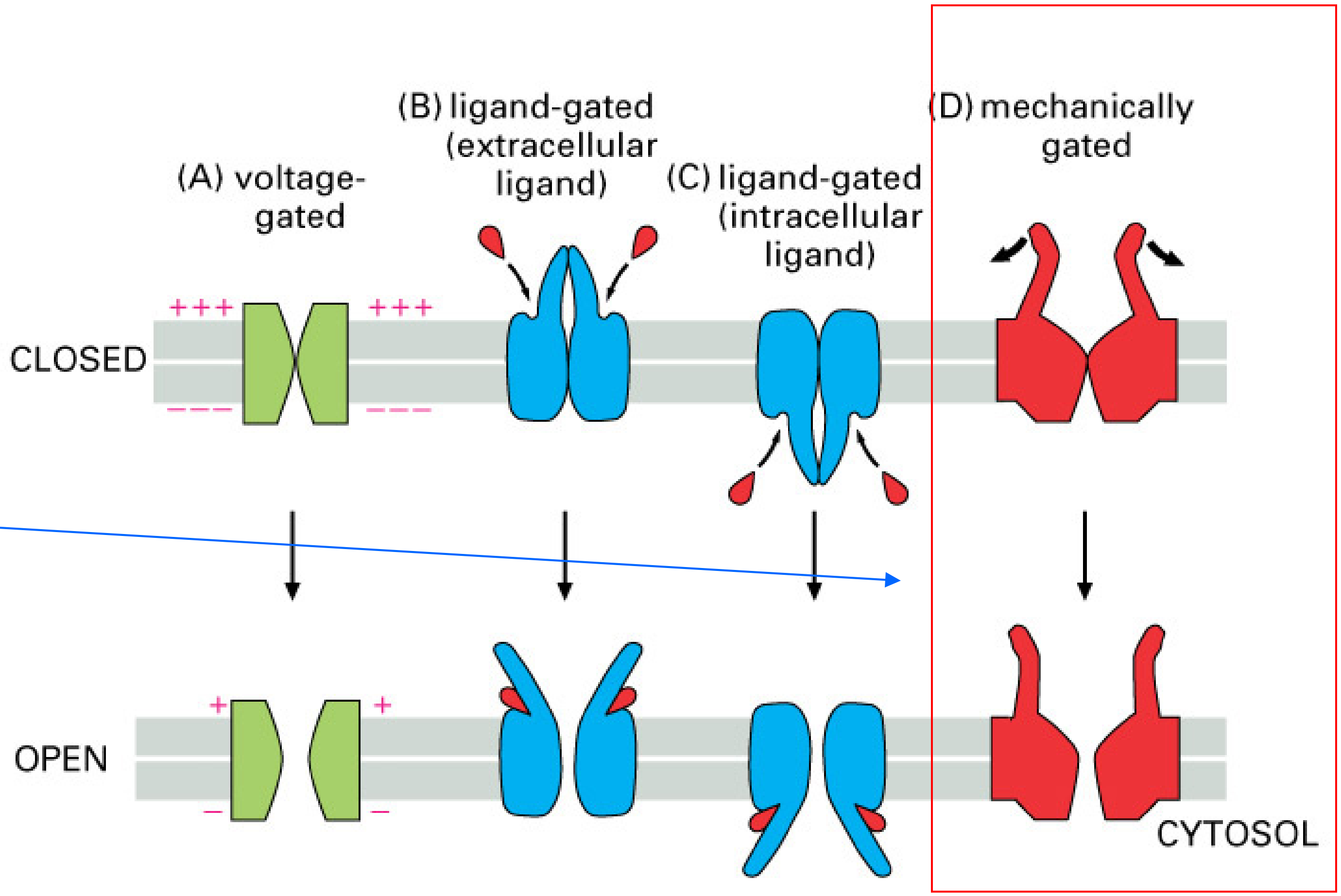
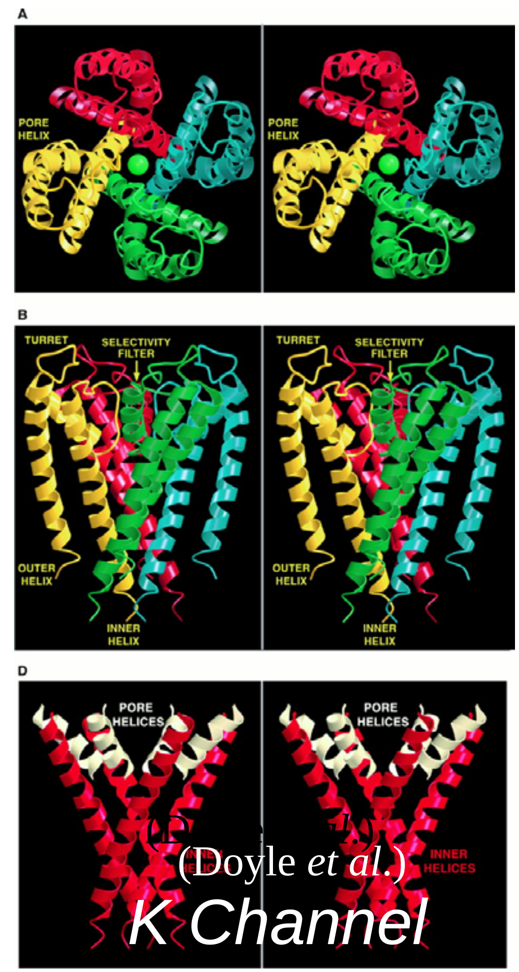


Figure 12-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

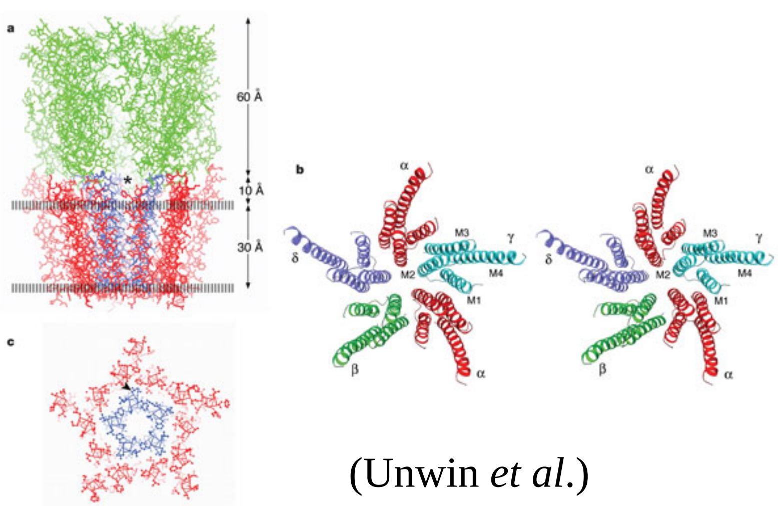
How We Know: Structural Biology

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

EM & X Ray structures



Nicotinic acetylcholine receptor



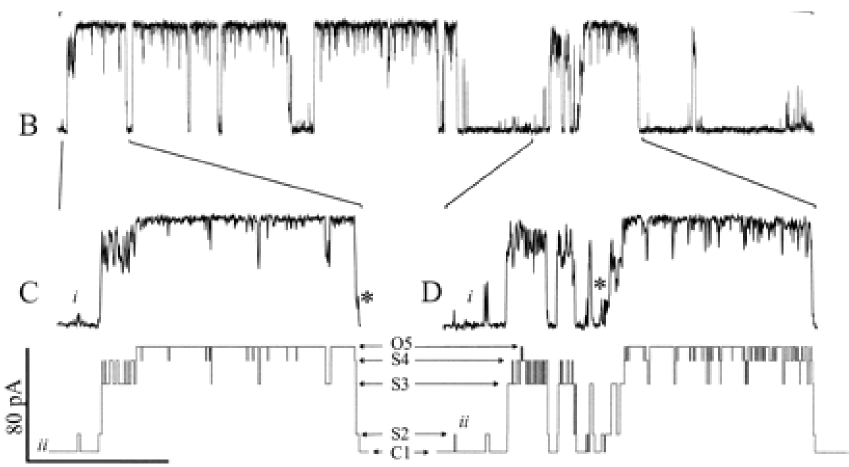
(Unwin *et al.*)

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.***

(Sukharev *et al.*)



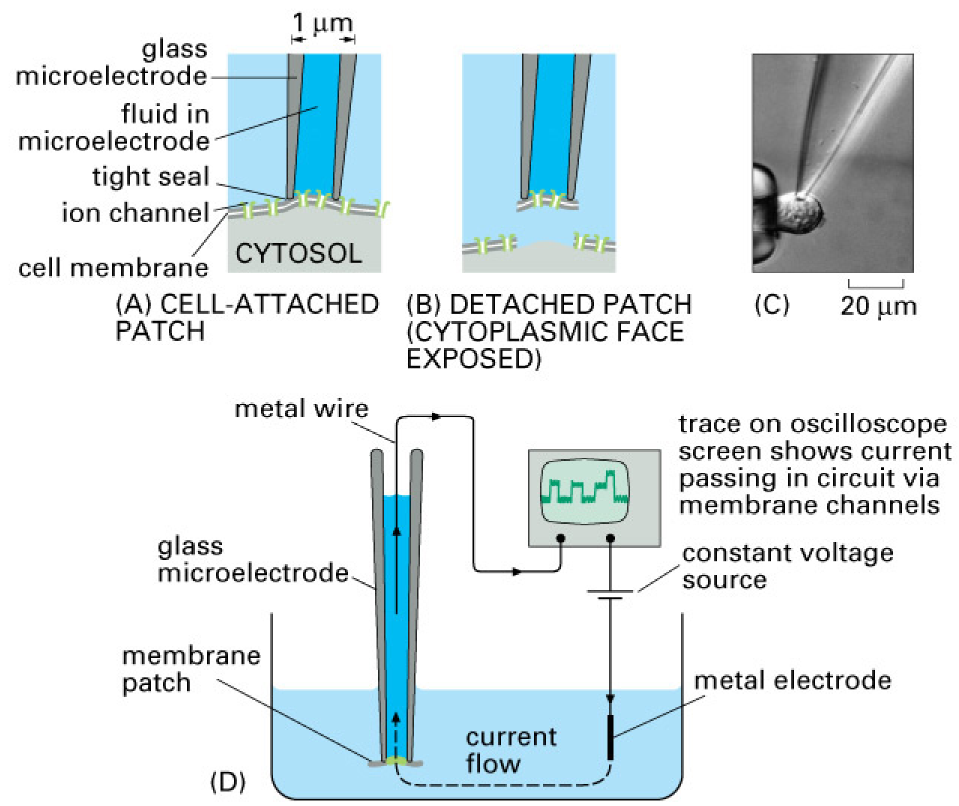
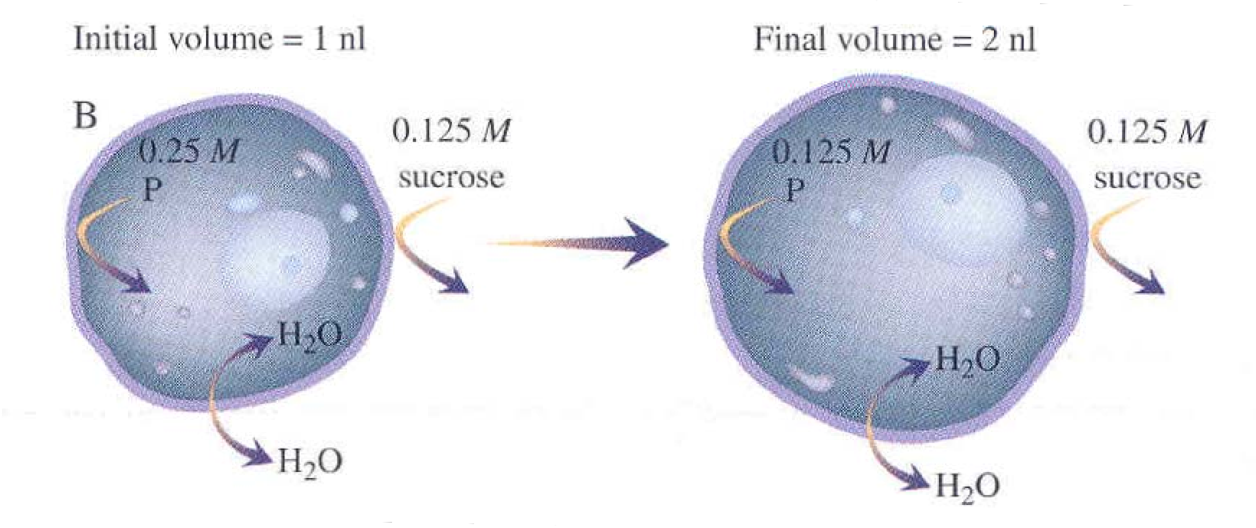


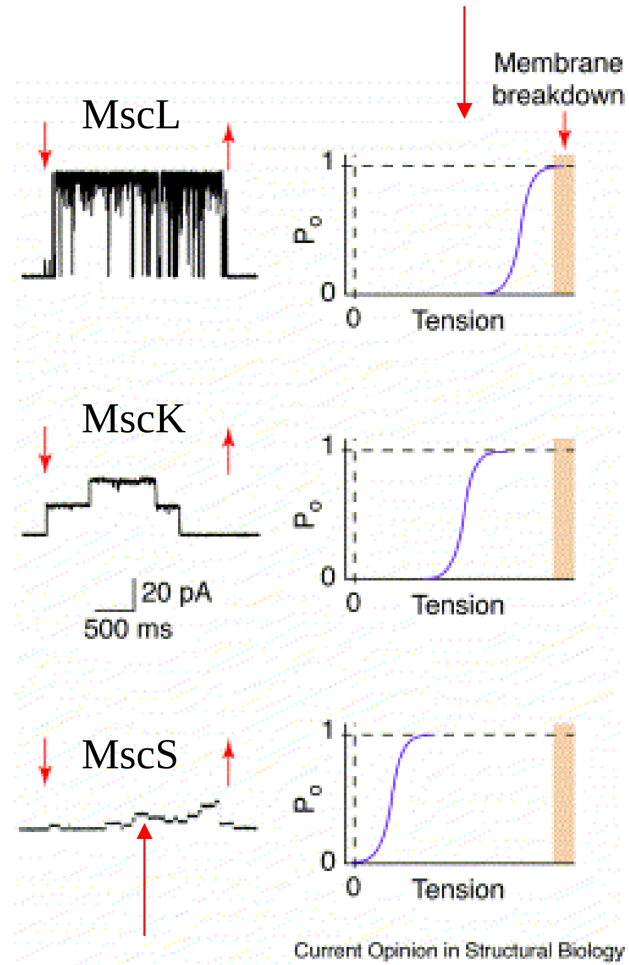
Figure 12-22 Essential Cell Biology, 2/e. (© 2004 Garland Science)

pA currents lasting several milliseconds.

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.***

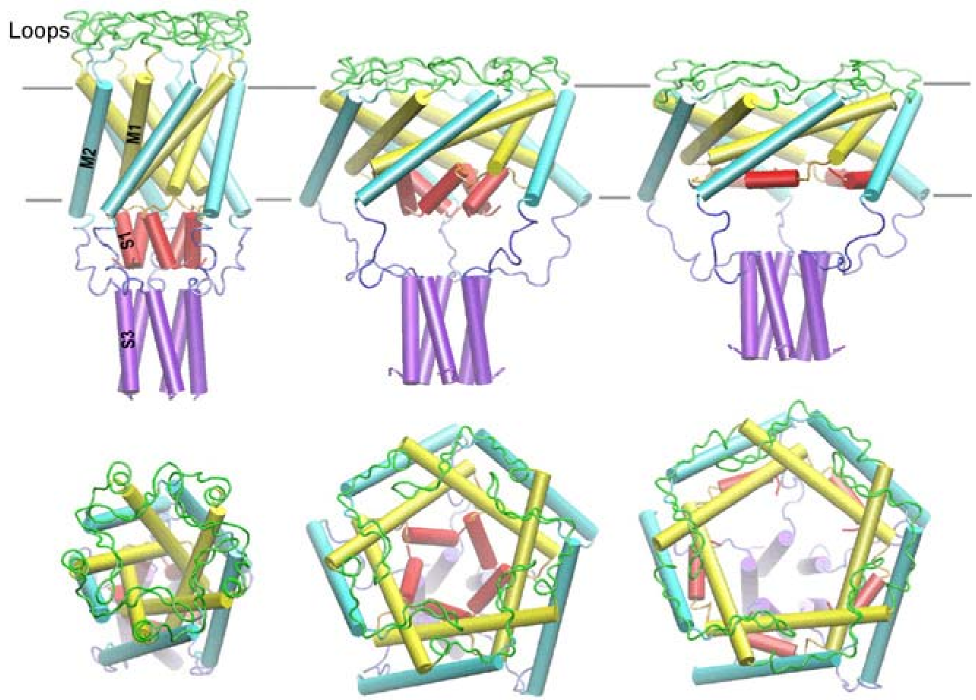




(Perozo and Rees)

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.***

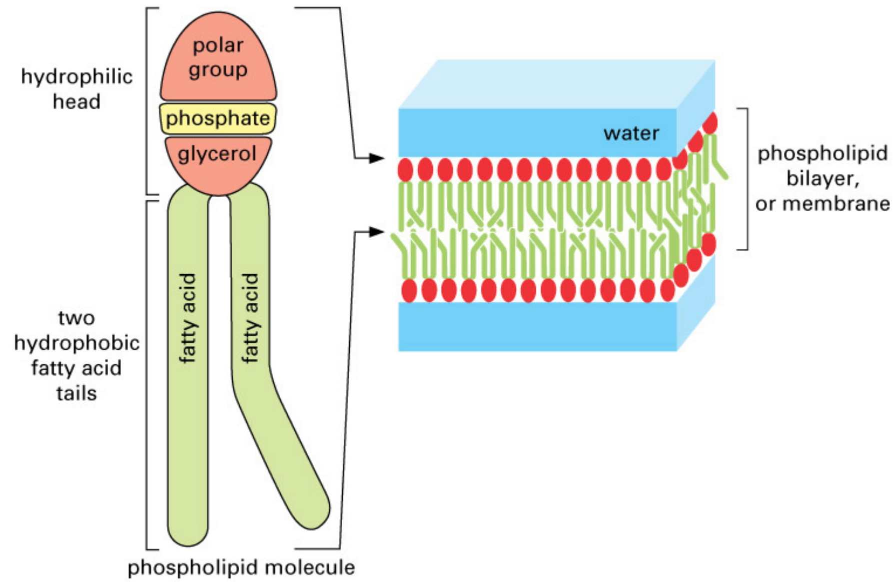
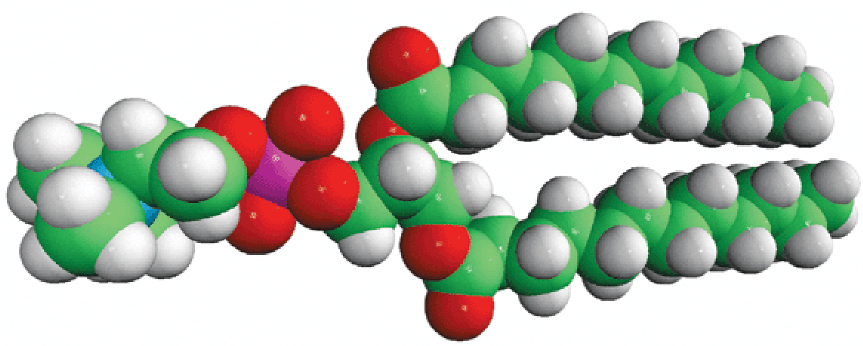
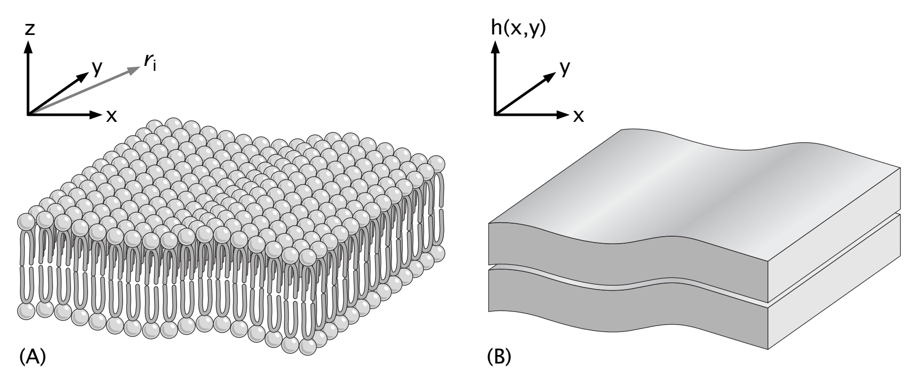


Figure 2-20 Essential Cell Biology, 2/e. (© 2004 Garland Science)



(Avanti Polar Lipids)

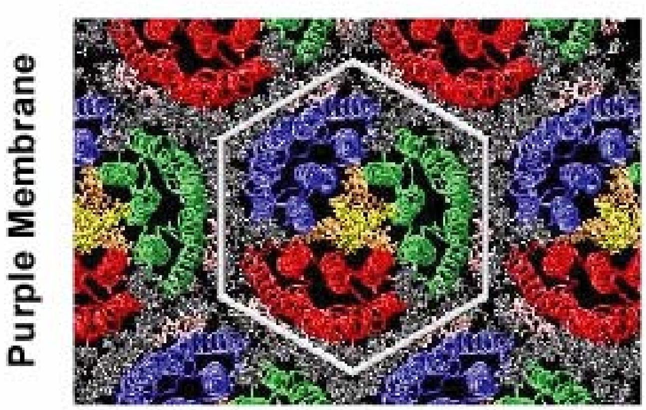


Molecular **Continuum**

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

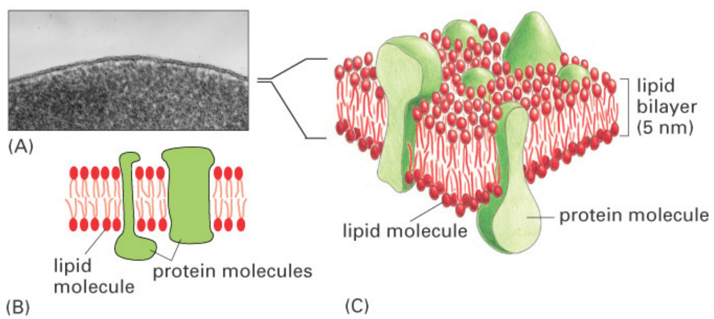
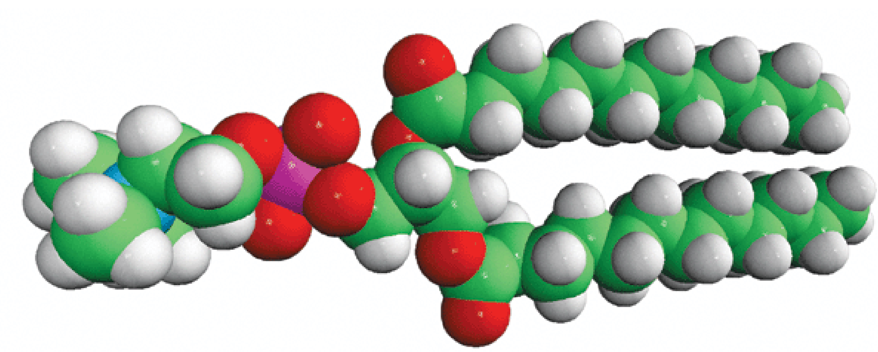


Figure 11-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

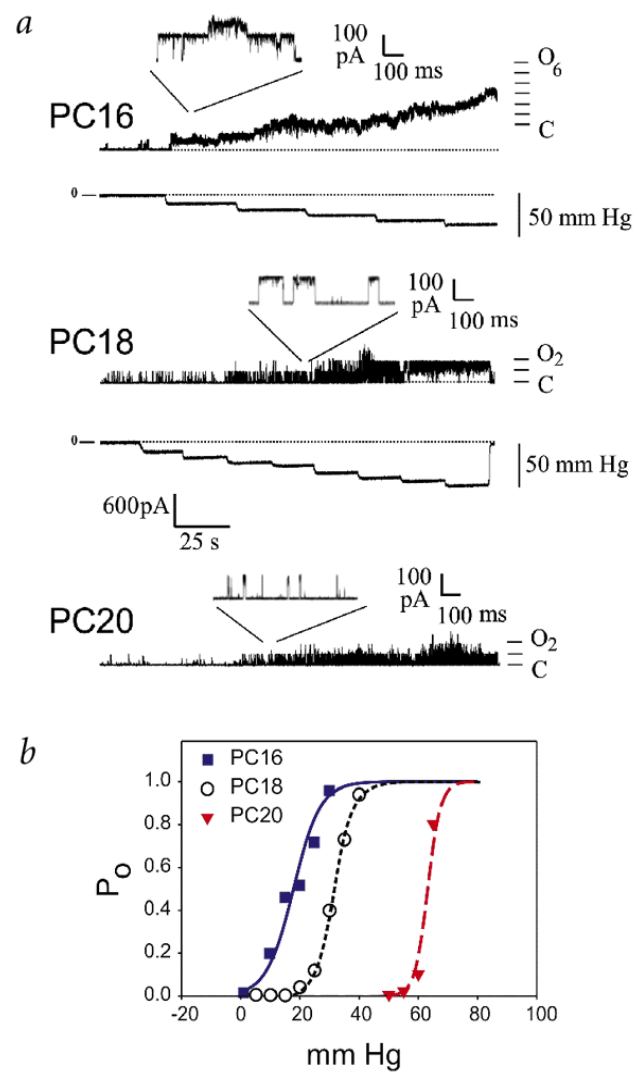
Experimental Challenges for Model: Lipid Tail Length

- ***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.***

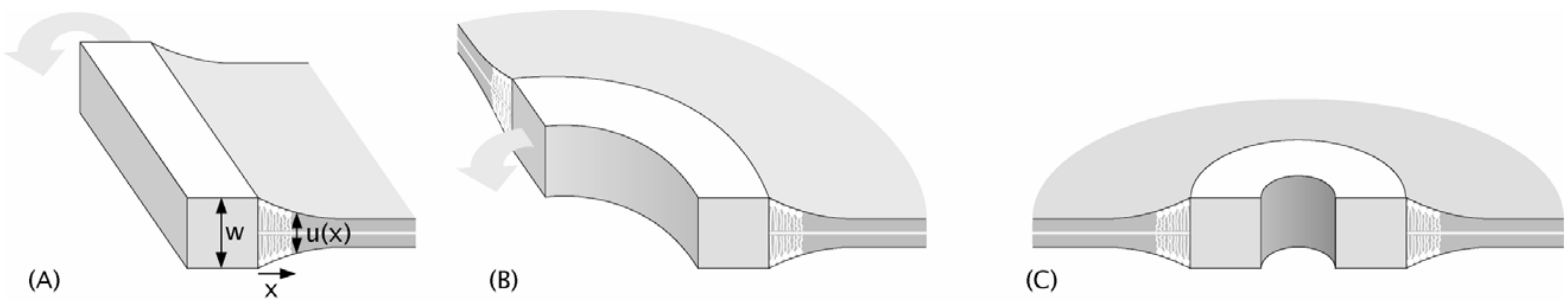


(Perozo *et al.*)

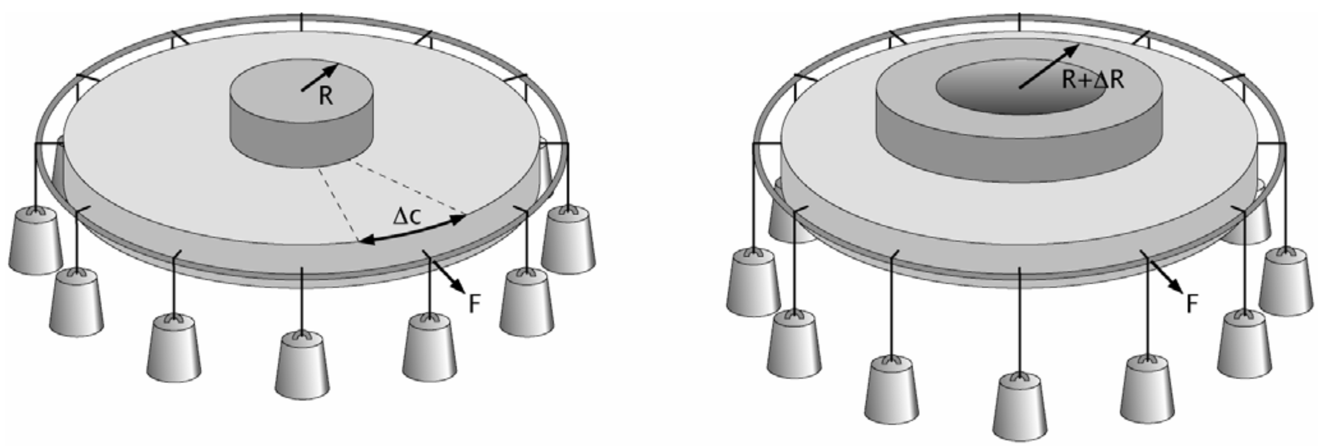
A Simple Elastic Model: Membrane-Induced Line Tension

- ***Presence of ion channel deforms the surrounding membrane – free energy cost.***
- ***Opening of channel leads to reduction in potential energy of loading device – that is an energy benefit.***

Channel gating and the membrane free energy.



Channel gating and the loading device.



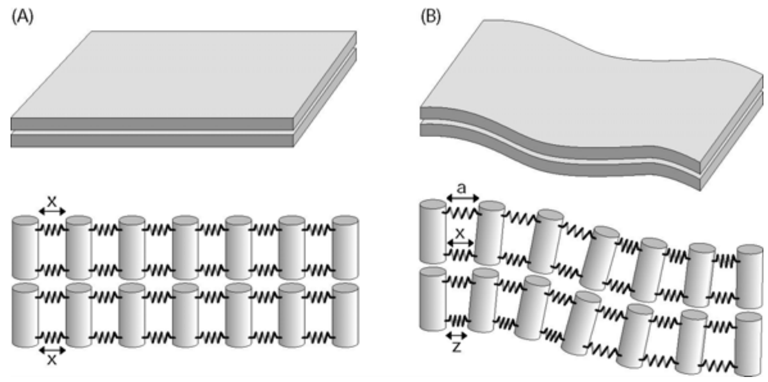
The Membrane Free Energy

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

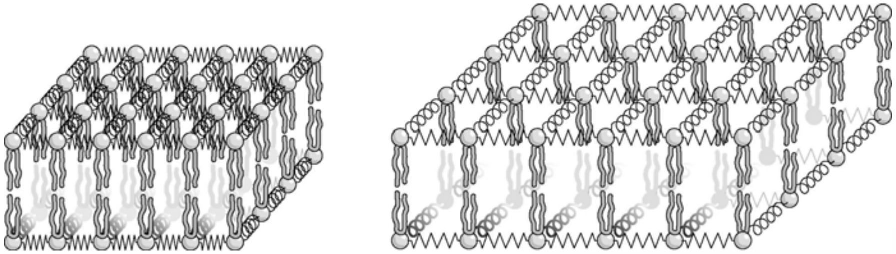
- ***We use elasticity theory and can thereby compute the energy as a function of protein shape.***

Bending:

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

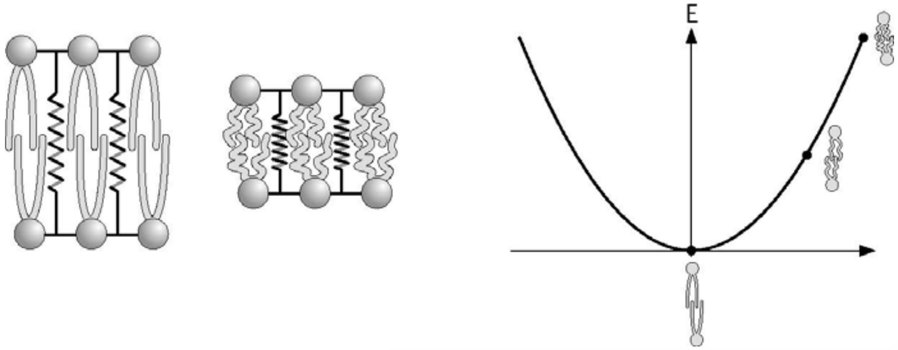


The Membrane Free Energy: Part 2



Tension (in plane Stretch):

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

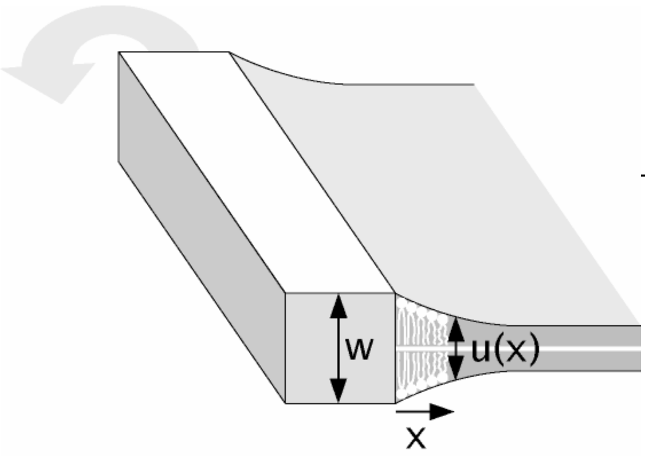


Stretch (out of plane):

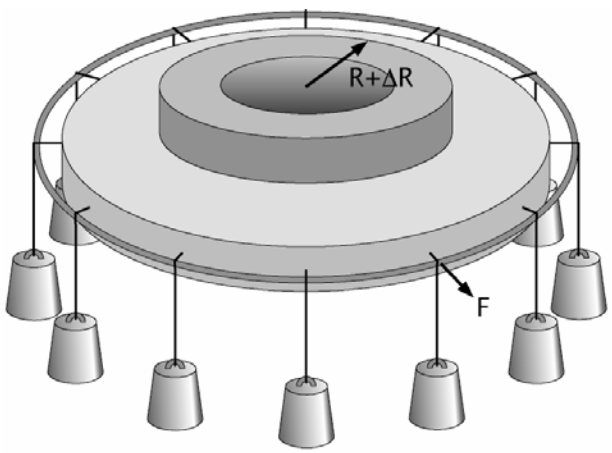
$$E = \int_{\mathcal{M}} d^2\sigma\; \tfrac{1}{2} K_A \left(\tfrac{u}{a}\right)^2$$

The Overall Free Energy

- ***Round up the usual suspects – minimization by Euler-Lagrange, find the profile, compute the energy.***



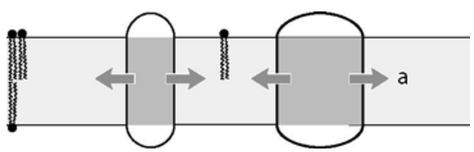
$$\rightarrow \quad G[u(x)] = \underbrace{\frac{K_b}{2}\int_R^\infty \left(\frac{d^2u}{dx^2}\right)^2 dx}_{\text{bending energy}} + \underbrace{\frac{K_a}{2a^2}\int_R^\infty u(x)^2 dx.}_{\text{thickness mismatch}}$$



$$\longrightarrow \qquad G_A = -\alpha\pi R^2$$

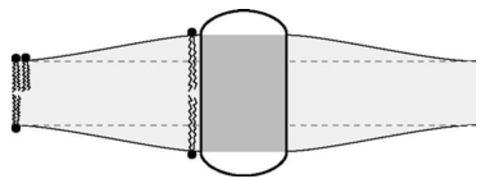
Dissecting the Free Energy

Applied Tension



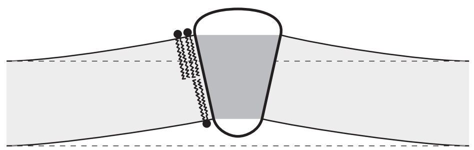
$$G_A = -\alpha A$$

Hydrophobic mismatch



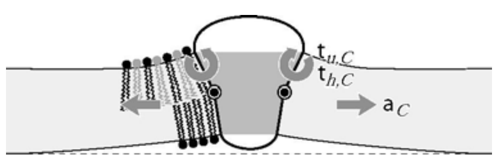
$$G_u = \frac{1}{2} K_{eff} U^2 C$$

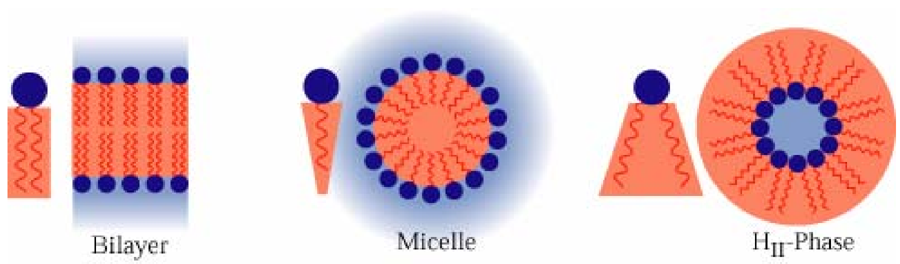
Midplane Bending



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

Spontaneous Curvature





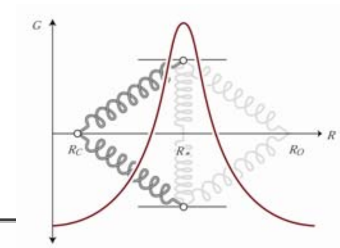
$$G_{C_0} = K_B(C_0 H' + \bar{C}_0 U')C$$

Conclusion: Competition between terms with different radial character!

Line Tension** & **Applied Tension

An Effective Potential For Channel Opening

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

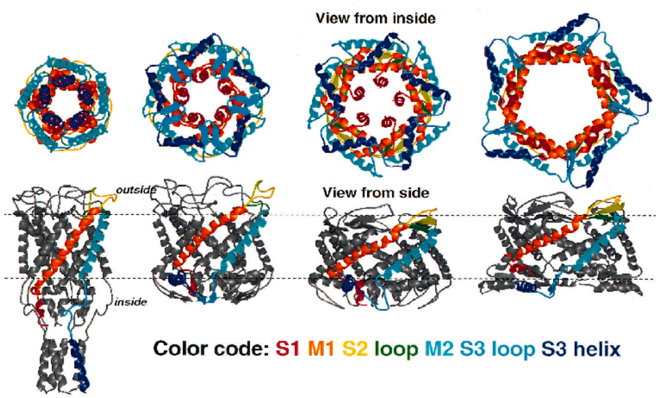


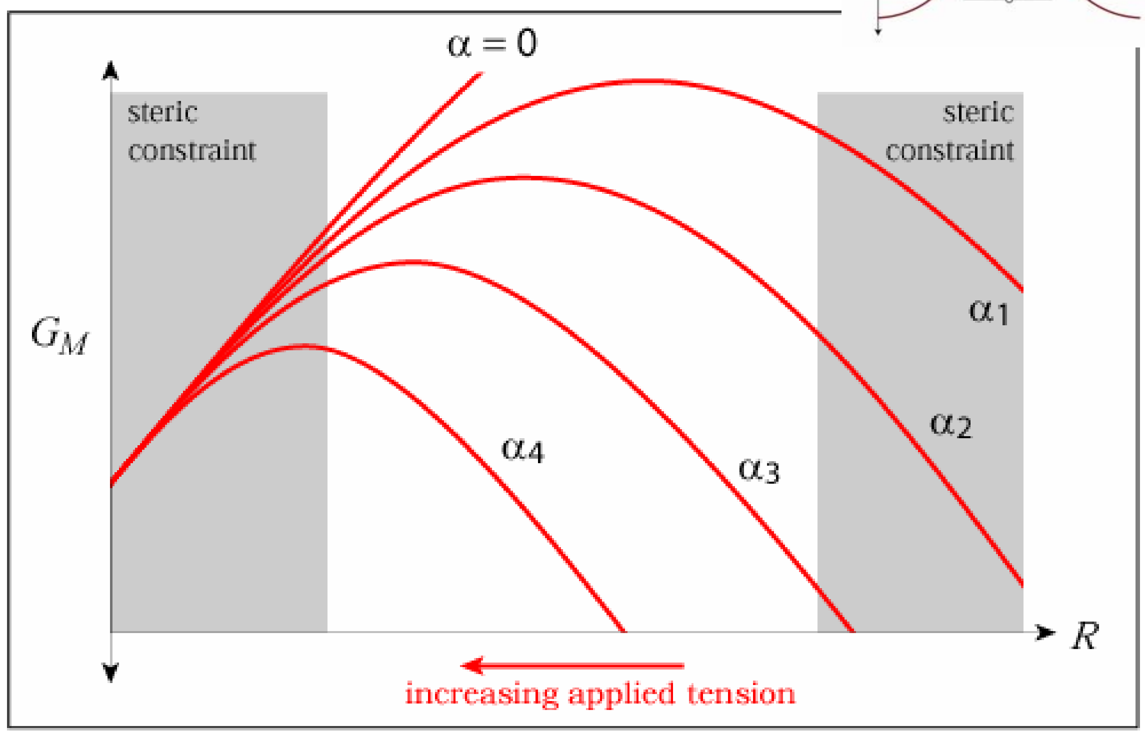
Effective potential for channel radius

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

f = line tension
α = applied tension

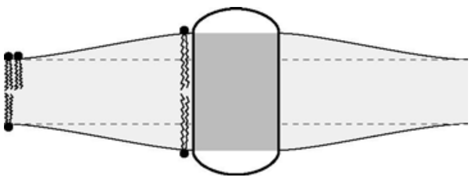
$$f > 0$$



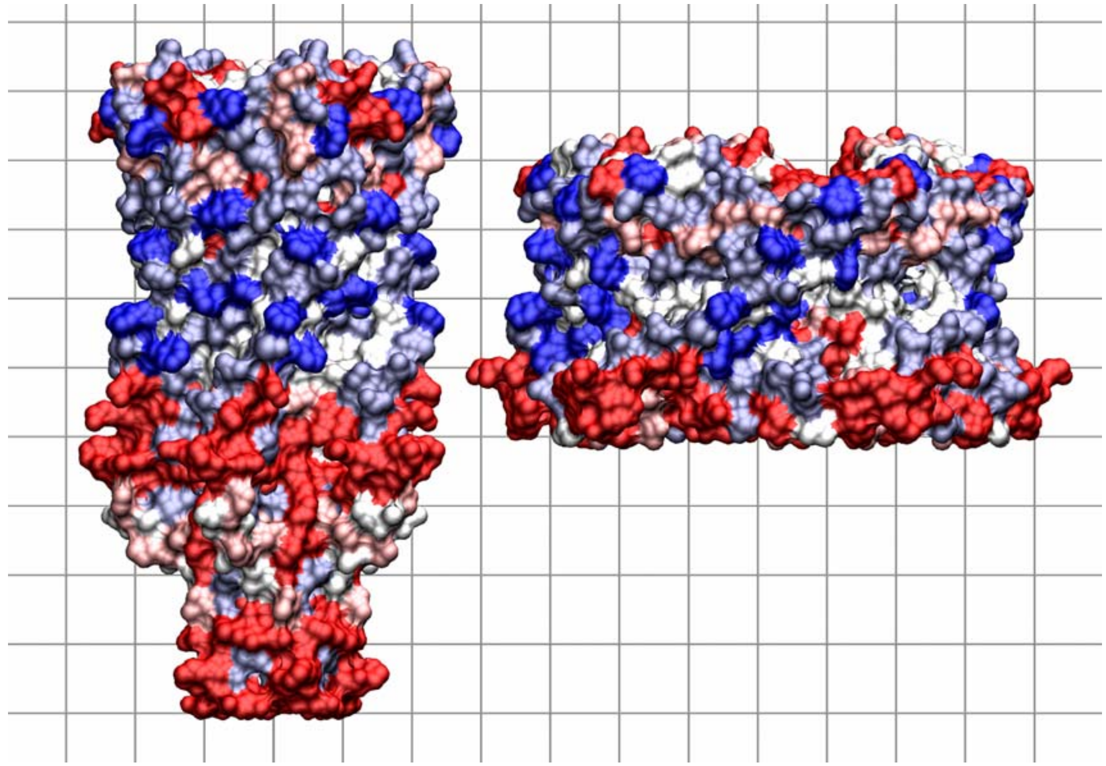


Dissecting the Free Energy: Hydrophobic Mismatch

Hydrophobic mismatch



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

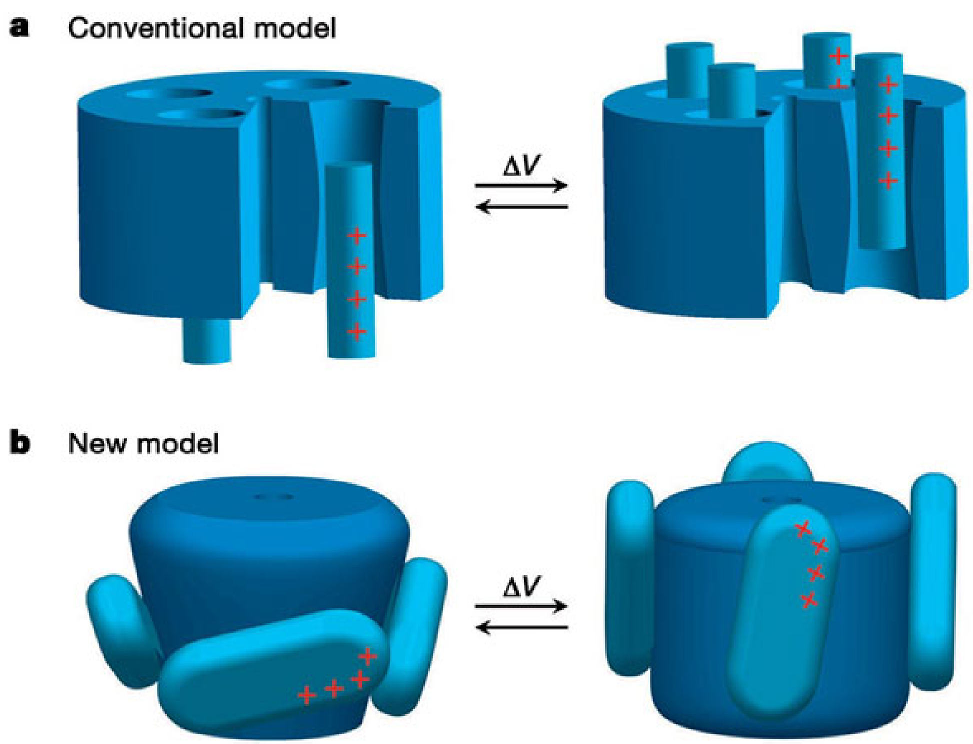


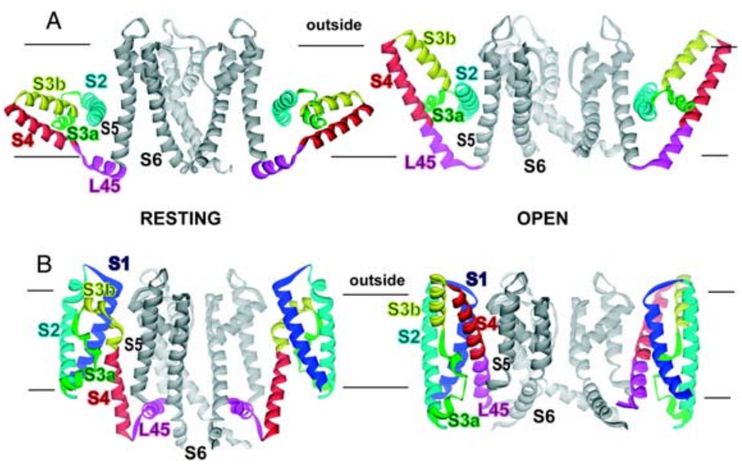
- ***Can tune the hydrophobic mismatch two ways: change the lipids or mutate the protein.***

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.***

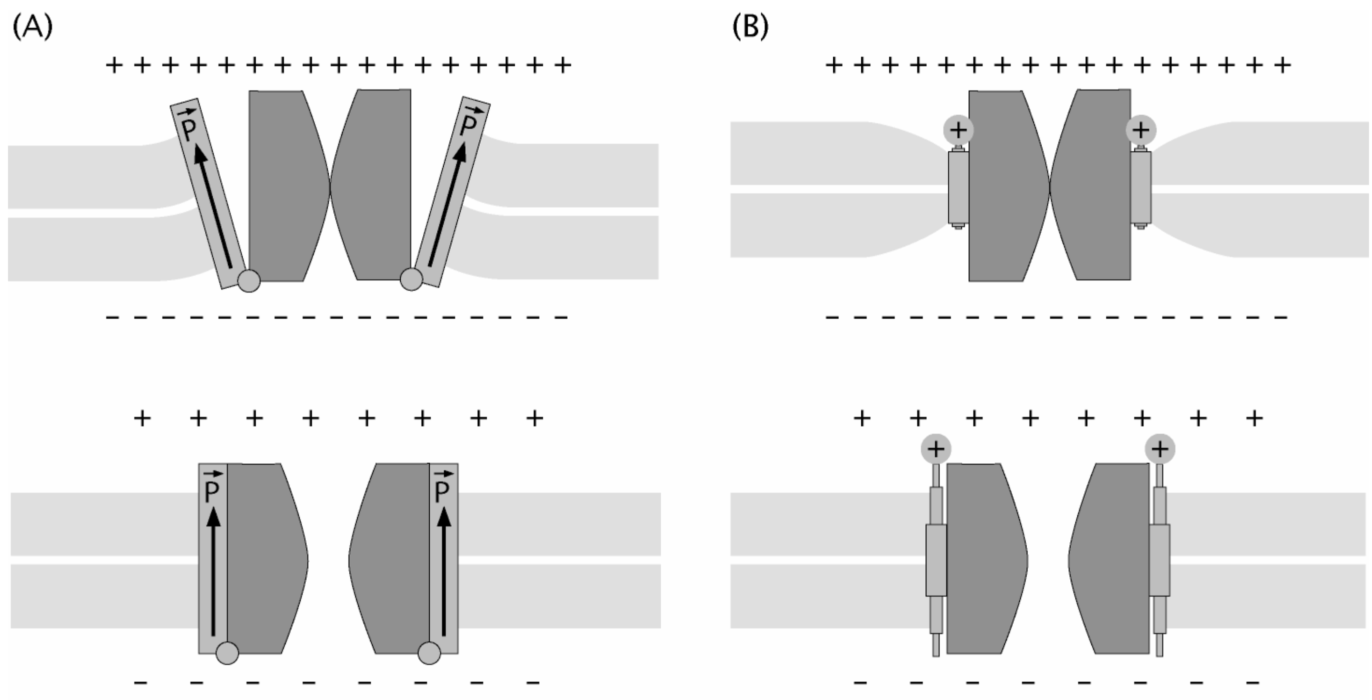
(Mackinnon *et al.*)





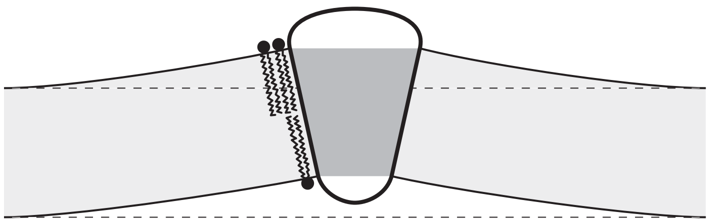
Mathematicizing the Models

- ***Different models have different consequences such as dependence on tension and lipid tail length.***

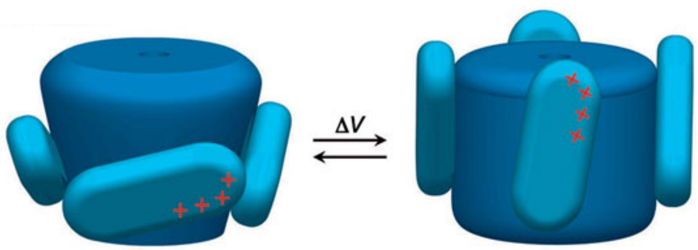


Flirting with a Simple Model of Voltage Gating

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



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



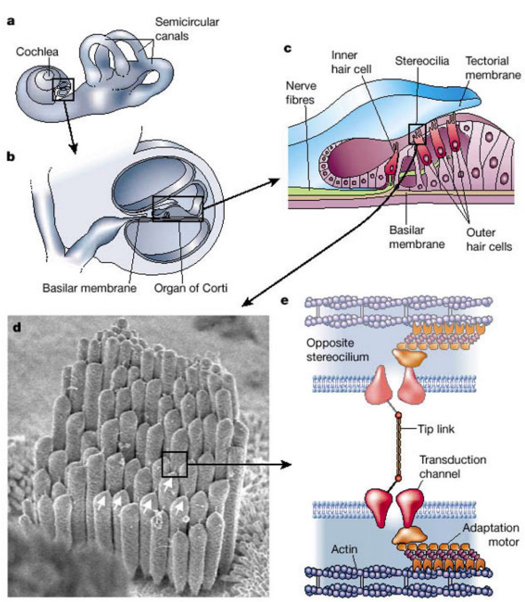
$$G_{protein} = pEcos\theta$$

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

How gating depends upon voltage, tension (!), lipid character, etc… Testable! Two models have different consequences.

Concluding Perspective

(Gillespie and Walker)



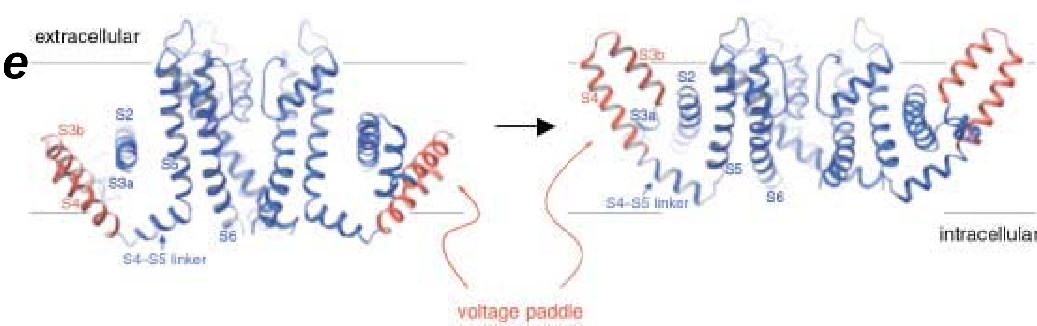
Mechanosensitive Channels

- ***Mechanosensation in bacteria a proving ground.***
- ***Membrane free energy a key player in dictating state of channel gating.***
- ***Experimental predictions: lipid length, spontaneous curvature, protein mutations, lifetimes, other channels.***

Membrane Proteins and Single Molecule Biophysics

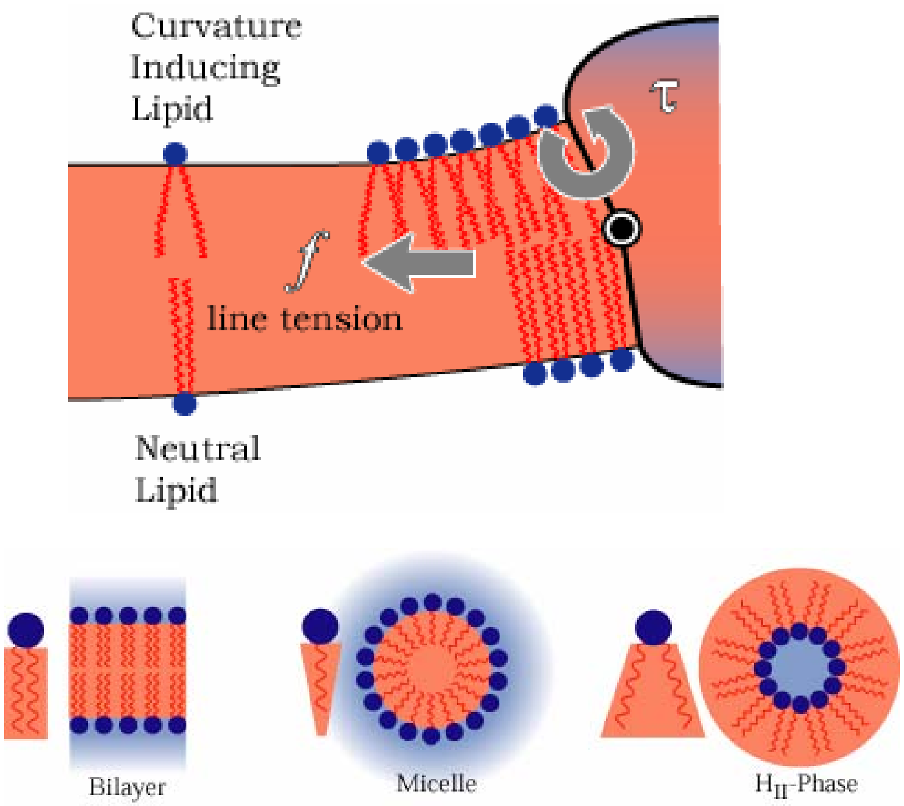
- ***Single molecule census – lots of membrane proteins.***
- ***Membrane proteins mediate the senses***

(Mackinnon *et al*.)

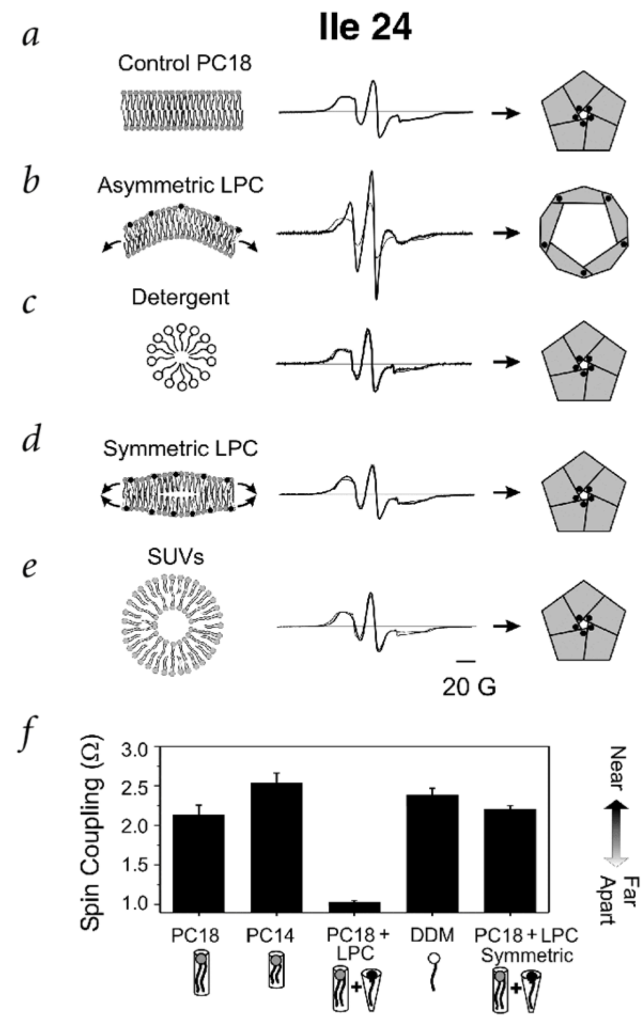


Experimental Consequences of Model: Spontaneous Curvature

- ***Gating tension depends upon concentration of curvature inducing lipids.***



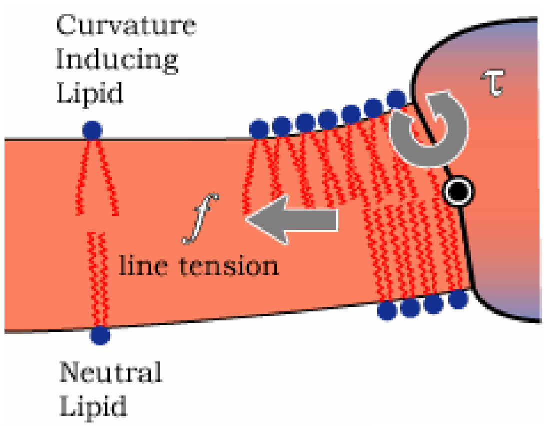
(Perozo *et al.*)



Model Predictions

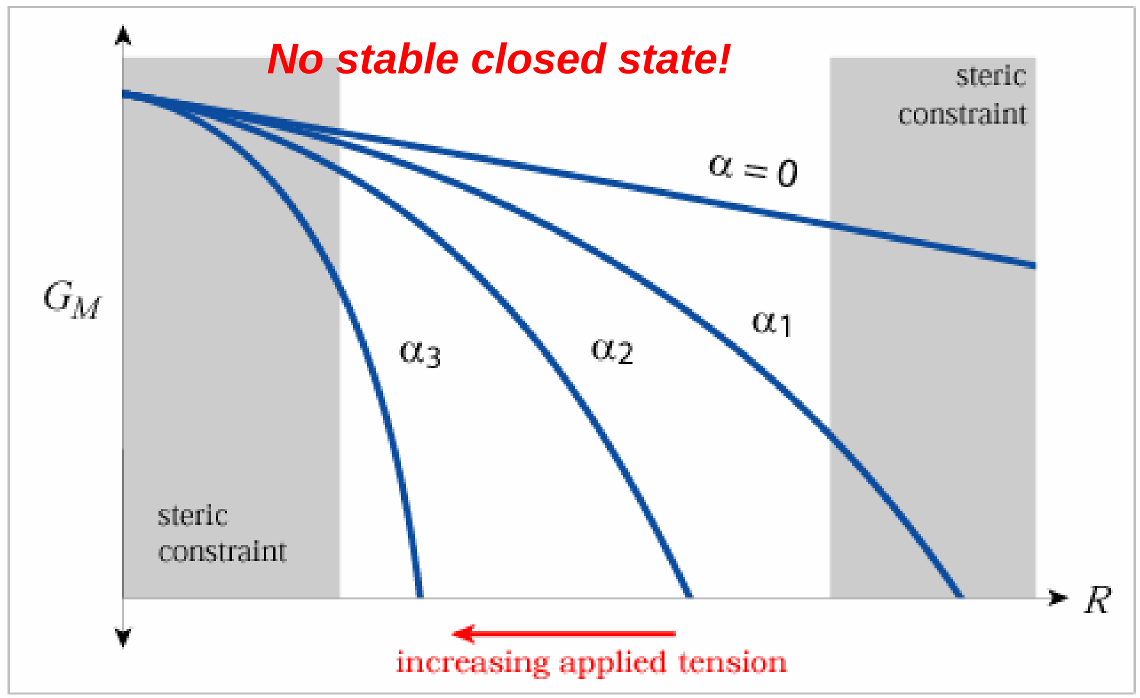
- ***Spontaneous curvature can induce negative line tension!***

Effective potential for channel radius



$$G_M = f\,2\pi R - \alpha\pi R^2 \qquad f < 0$$

f = line tension
α = applied tension



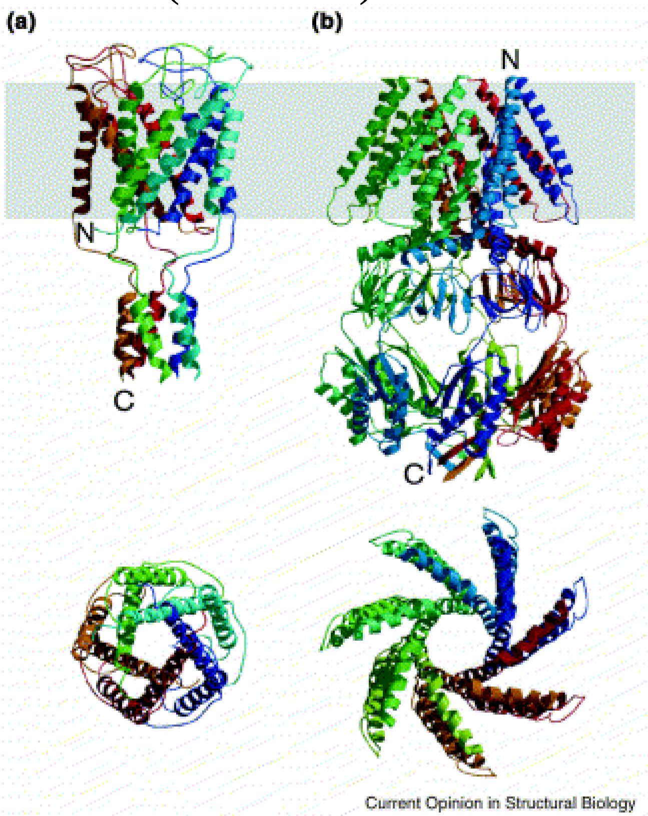
- ***Large spontaneous curvature can generically destabilize closed state.***

- ***Precisely this effect has been observed by Perozo et al.***

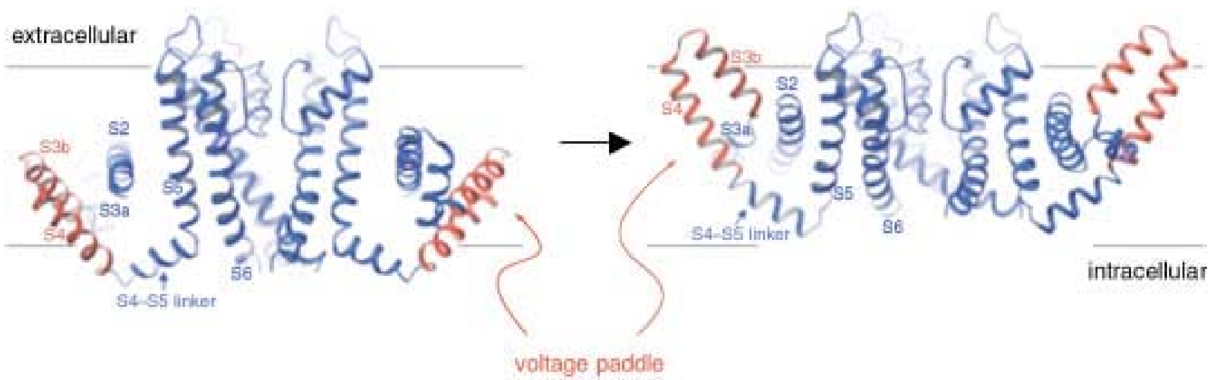
Other Consequences: Mechanosensitivity and Other Channels

- ***Conformational change in channel perturbs membrane.***

(Rees *et al.*)



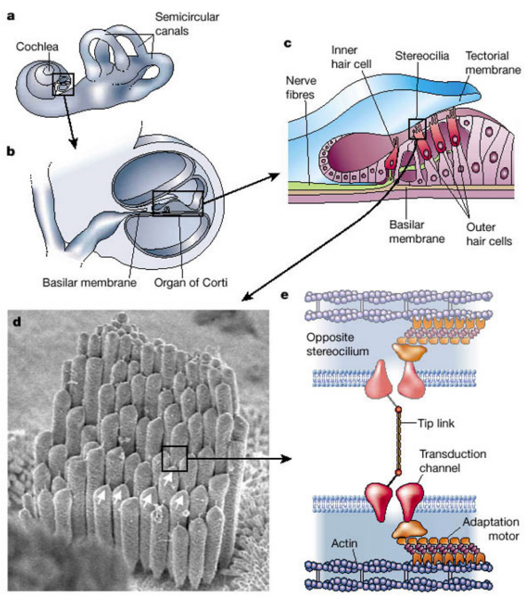
(Mackinnon *et al.*)



- ***MscS and KvAP: coupling of voltage sensitivity and mechanosensitivity.*** ***Speculative but very interesting/fun!***

Concluding Perspective

(Gillespie and Walker)



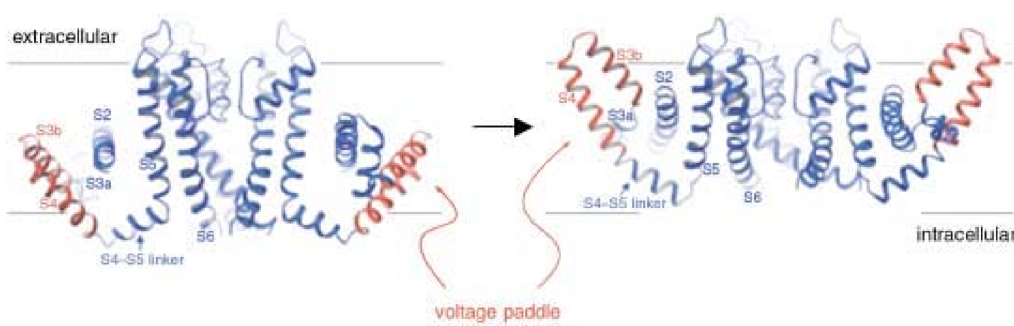
Mechanosensation

- ***Intriguing problems in biological physics having to do with mechanotransduction.***
- ***Coarse-grained mechanical description is needed.***

Mechanosensitive Channels

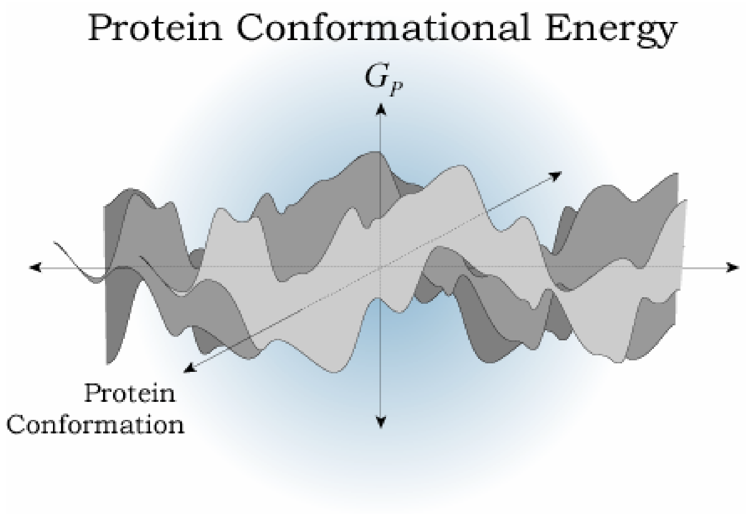
- ***Mechanosensation in bacteria a proving ground.***
- ***Membrane free energy a key player in dictating state of channel gating.***
- ***Experimental predictions: lipid length, spontaneous curvature, protein mutations, lifetimes, other channels.***
- ***Question of substates – need insights into channel free energy***

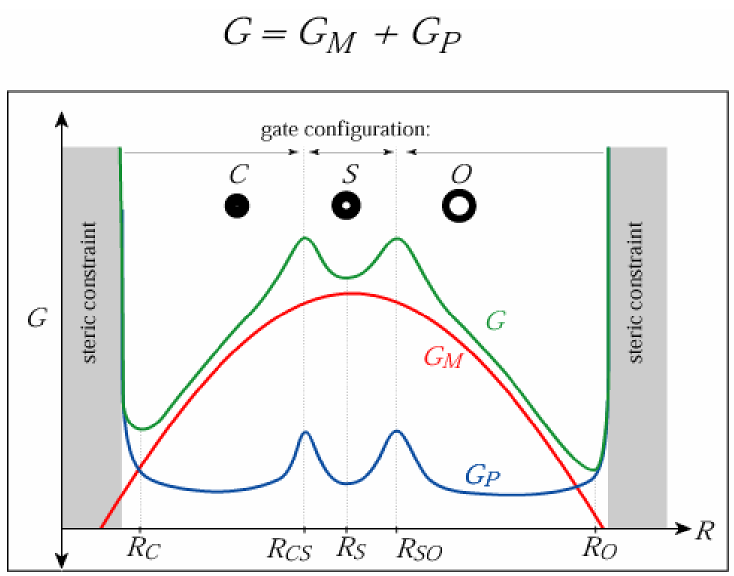
(Mackinnon *et al.*)



Physics of Mechanosensation

- ***Energy scale due to membrane deformations is comparable to measured free energy of gating.***
- ***We expect Protein Free energy to be very degenerate.***

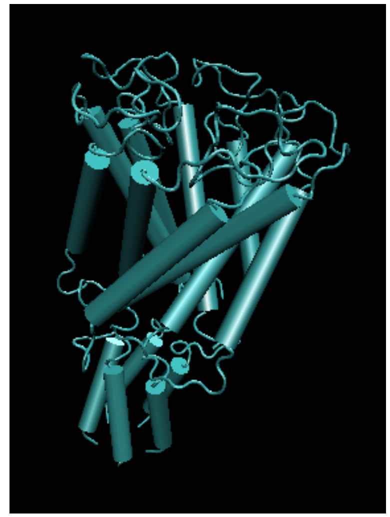
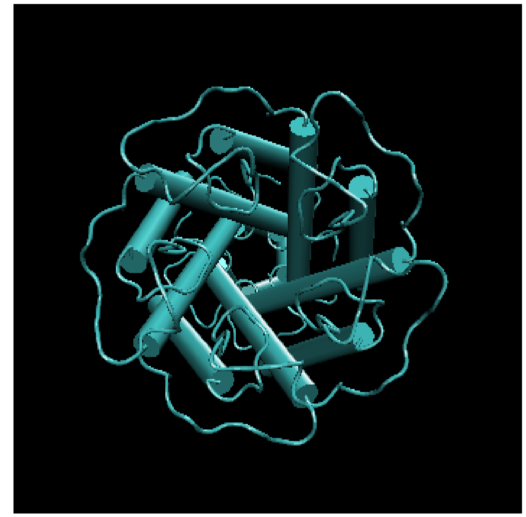
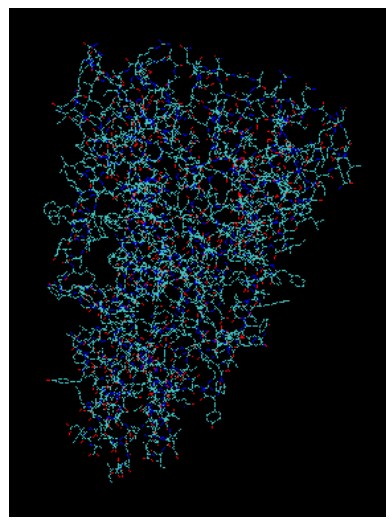
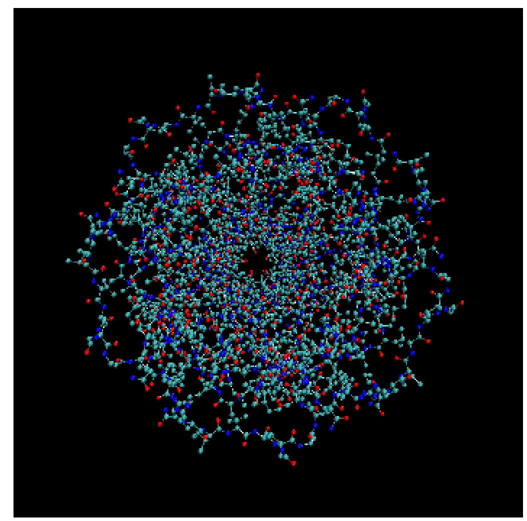


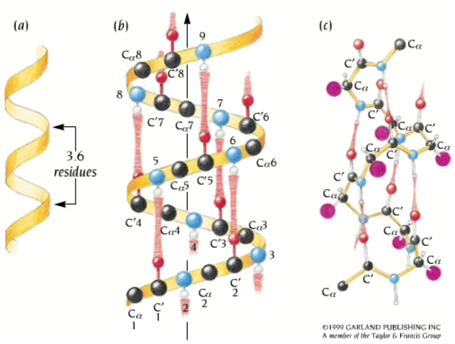


- ***Assumptions generically predict short lived substates.***
- ***Degeneracy is broken by the effective potential from membrane interaction.***
- ***Membrane is NOT a passive observer in the gating of channel.***

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.***

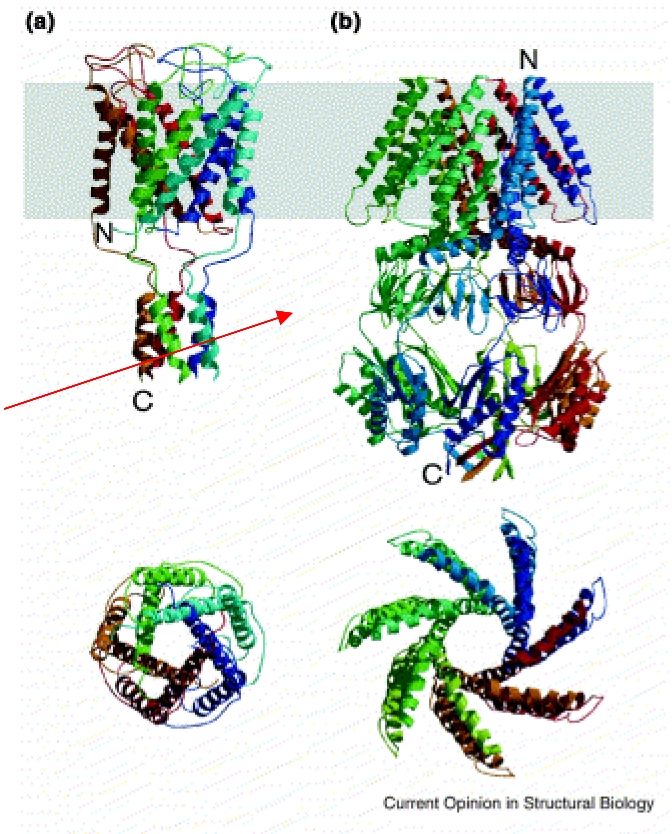


Structural Biology Sheds Light on MscL and MscS

- ***Structure of MscL captured in the closed state. (Homopentamer) Notice structural role of transmembrane alpha helices.***
- ***MscS captured in the open state.***

Cytoplasmic side

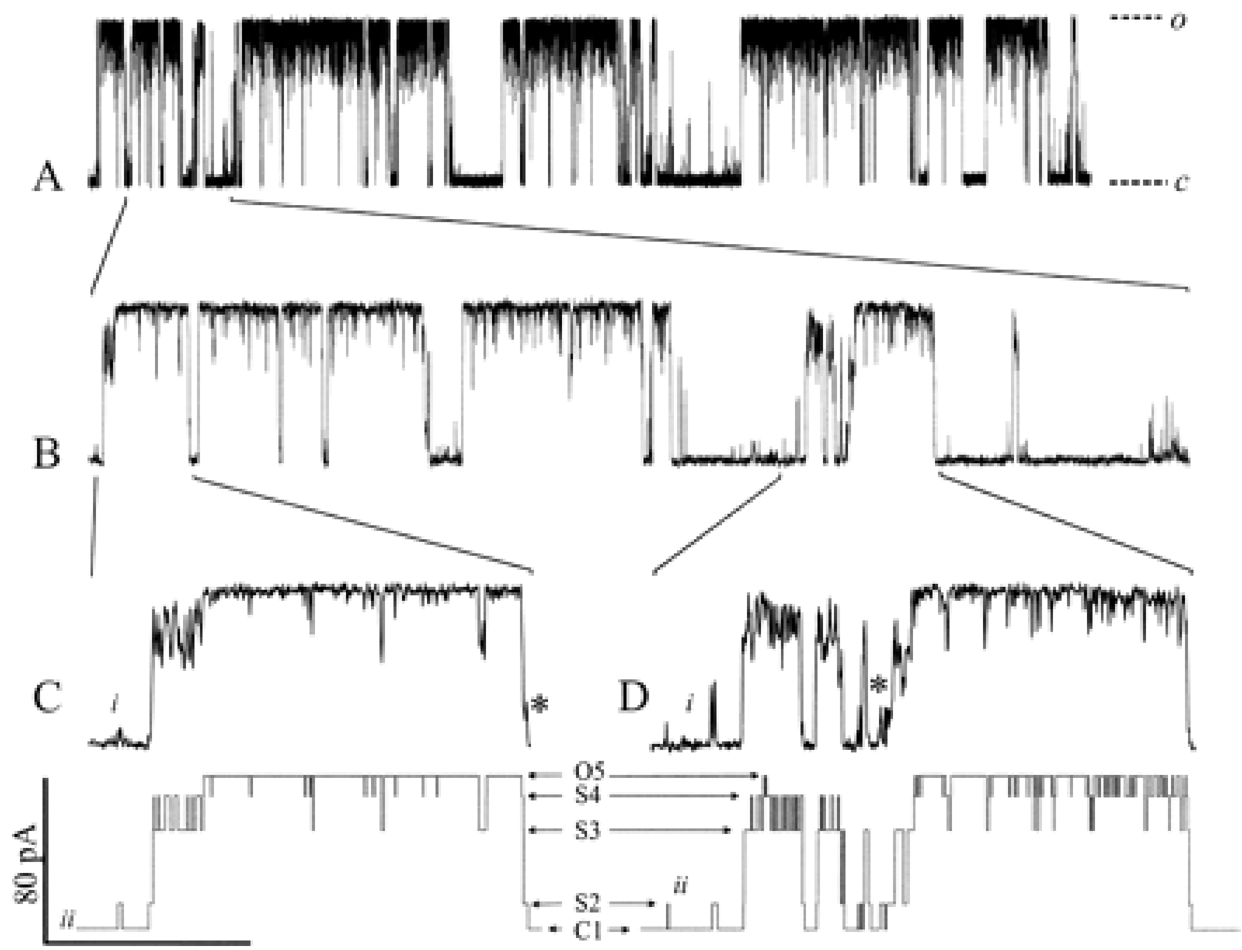
(Rees *et al.*)



MscL ***MscS***

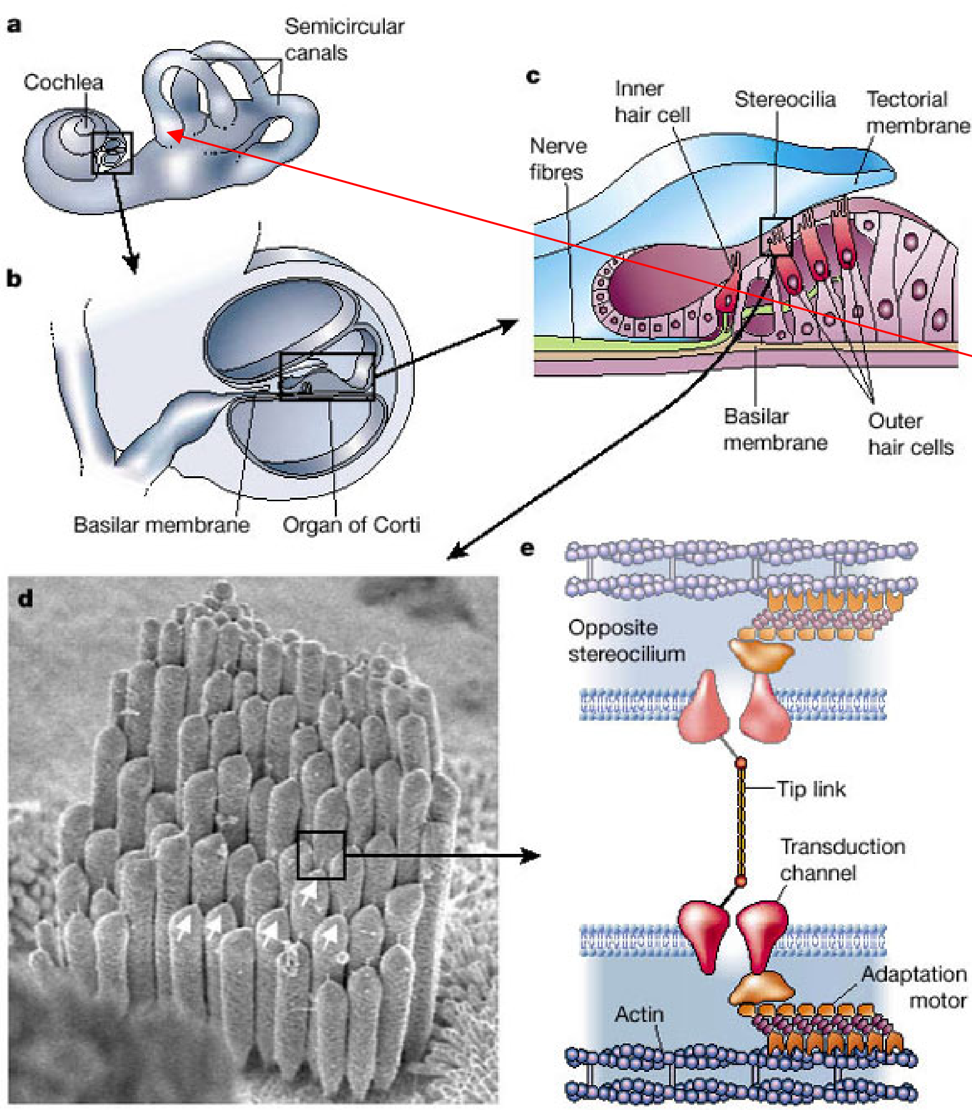
Conductance of MscL Under Tension

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



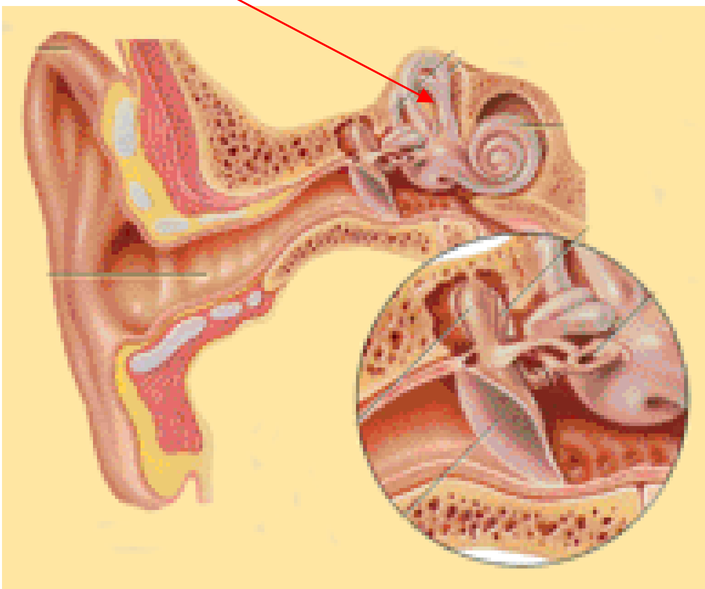
(Sukharev *et al.*)

Ear Structure and Function: Ion Channel Gating



- ***Collective response of multiple detectors driving multiple channels.***

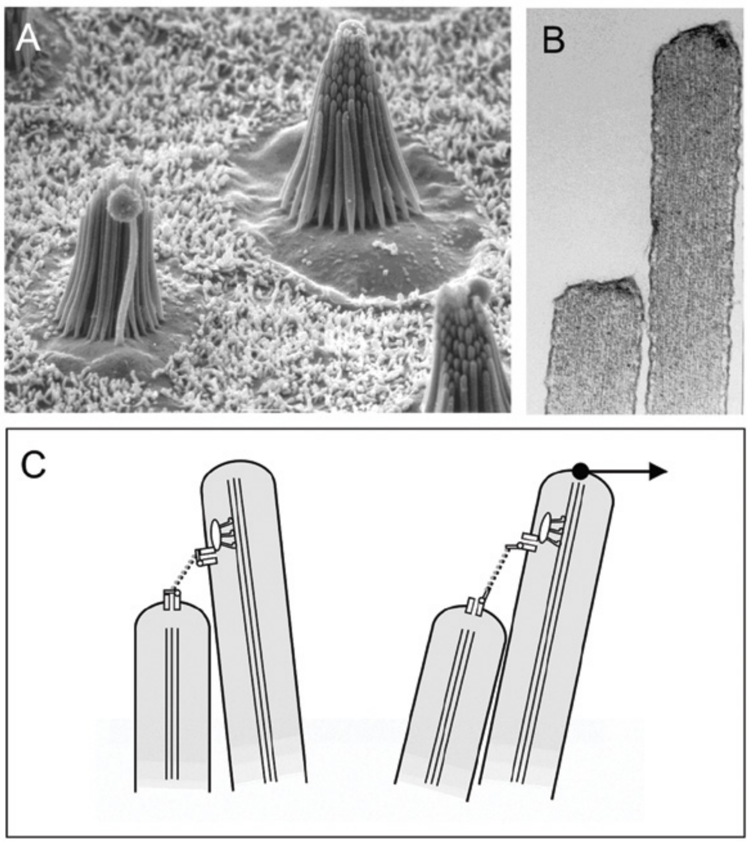
(Cochlear function.)



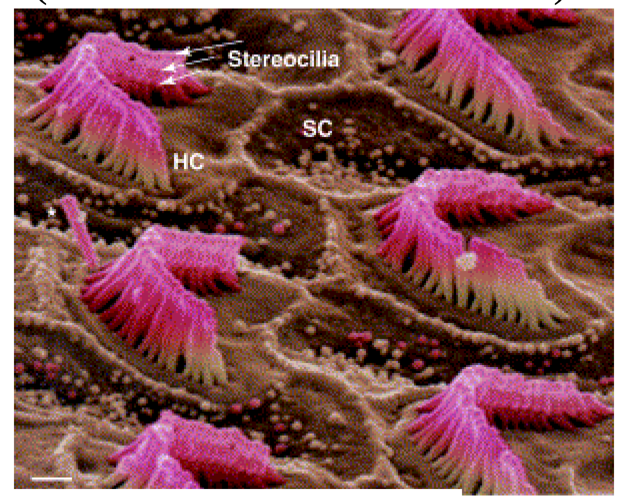
Richness of Dynamics: Adaptation

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

(Sukharev *et al.*)



(Muller and Littlewood-Evans)



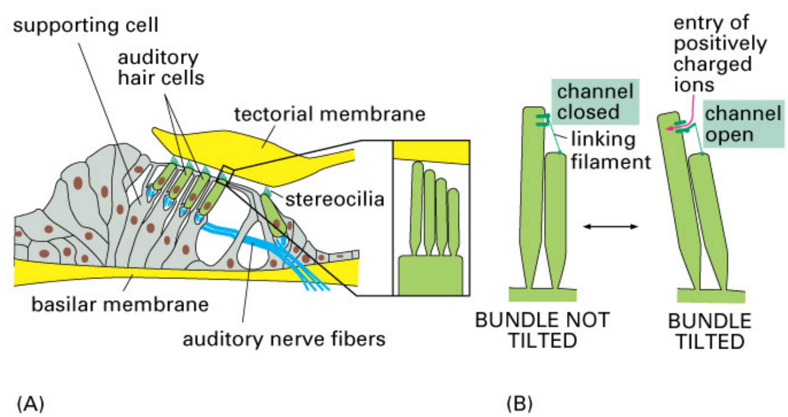
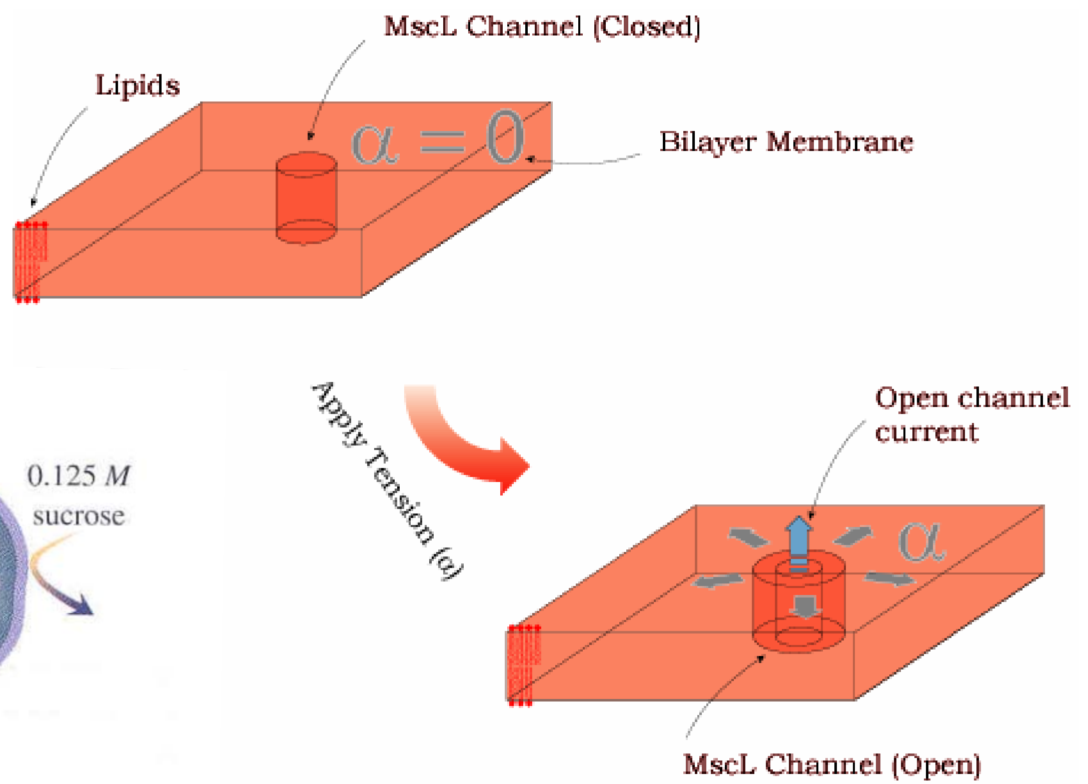
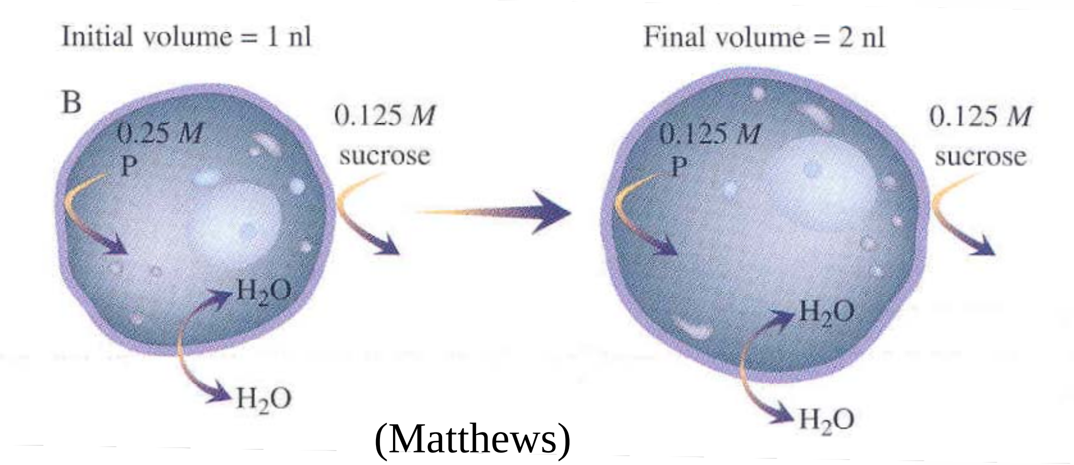


Figure 12-25 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Mechanosensitive Channel of Large Conductance (MscL)

- ***Bacterial channel serves as an emergency relief valve to respond to osmotic shock.***
- ***Structure of the channel is crystallography in the closed state (Doug Rees).***



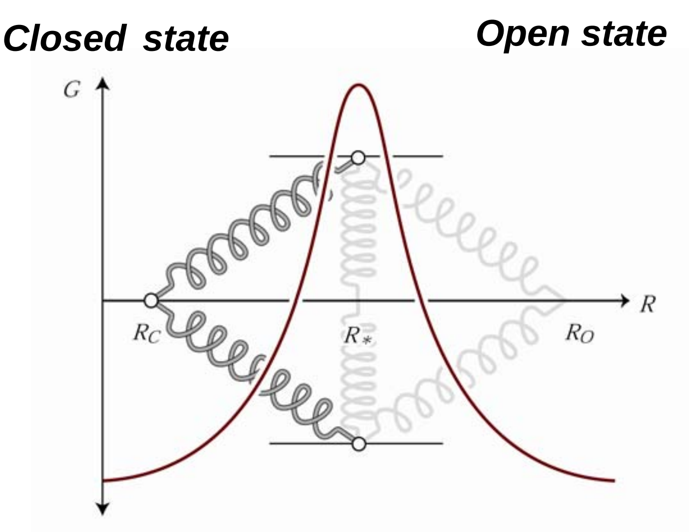


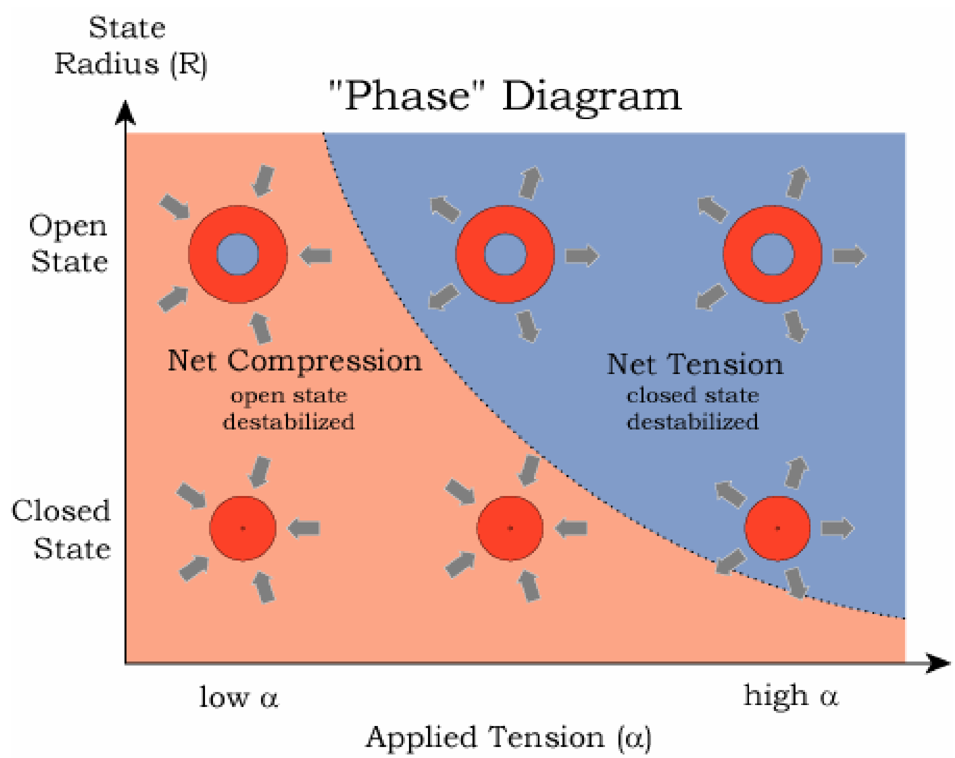
(Matthews)

Physics of Mechanotransduction

- ***Membrane-Protein interaction generically leads to a two-well potential!***
- ***Ion channel gating has the character of a bistable switch.***
- ***Depending upon the magnitude of the applied tension, either the closed or open state is stabilized.***
- ***Membrane is NOT a passive observer in the gating of channel.***

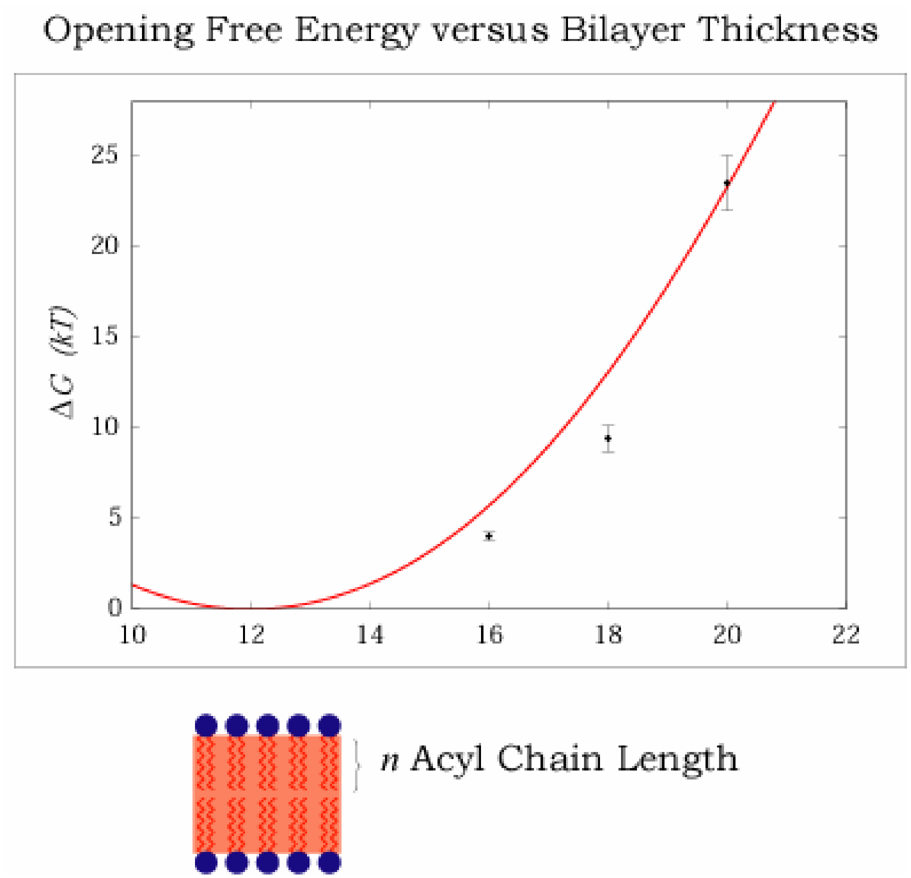
Bistable switch





Experimental Predictions

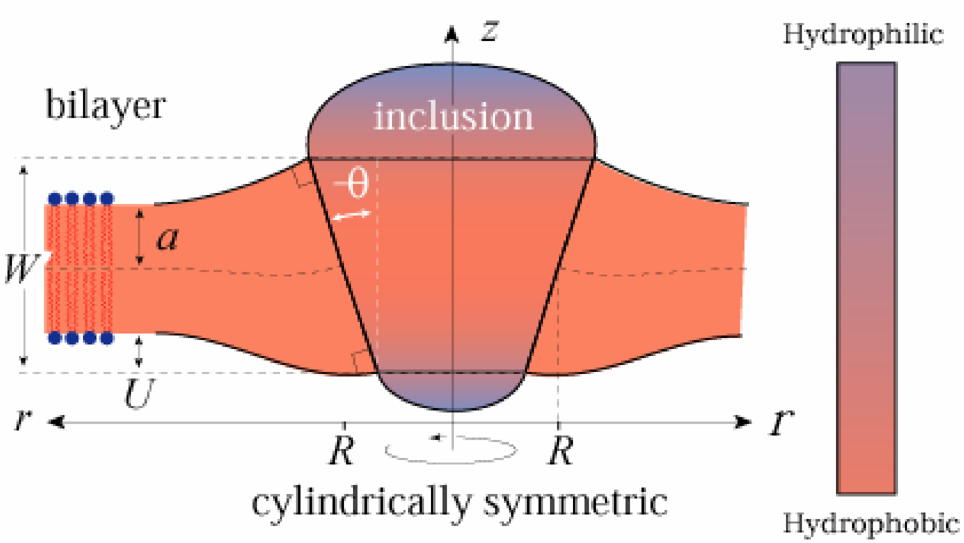
- ***Critical tension depends upon lipid length.***
- ***Curvature inducing lipids can change the sign of the effective line tension – stabilizing open state.***
- ***Amino acid substitutions that tune the hydrophobic width of the channel alter gating tension in a systematic fashion.***



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