Amphipol Workshop
Engelman

Membrane Structural Principles

Paris
8 March 2010

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This lecture

1. Principles of bilayer formation
2. Properties of bilayers
3. Principles of protein-bilayer interaction
4. Principles of helical membrane protein structure
5. Formation of non-TM regions
6. Comparative stability in bilayers vs micelles

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1. Principles of bilayer formation

Lipids (Gr: lipos=fat)

- soluble in organic solvents
- sparingly soluble in water
- fatty acids are carboxylic acids
- more than half have have unsaturated bonds
- many are polyunsaturated
- nomenclature:

  #carbons : #double bonds (e. g. 18:1)

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amphiphiles

COO-

Hydrophobic

Hydrophilic

Amphiphilic = amphipathic

philos — loving

phobos — fear

pathos -- suffering

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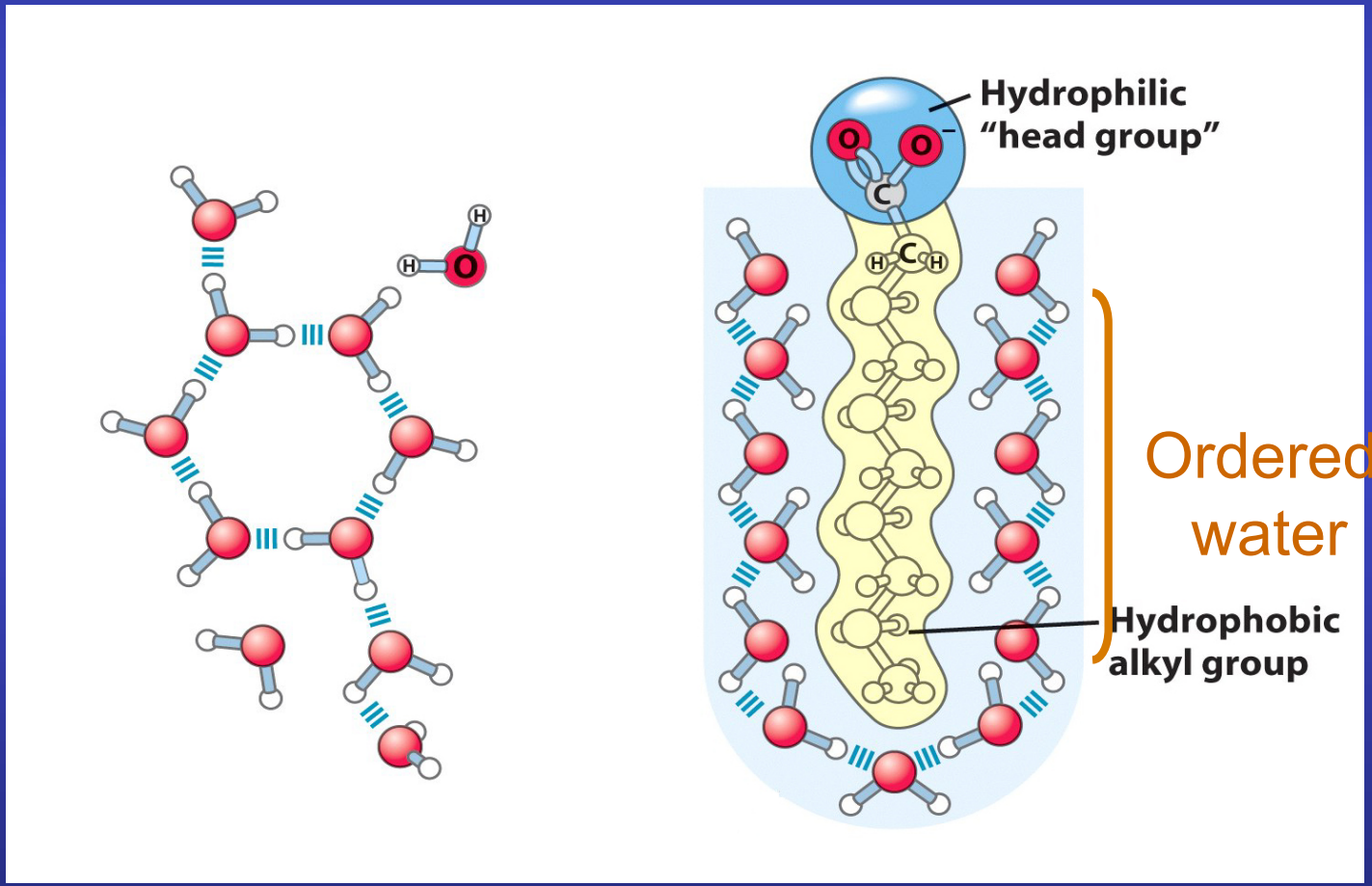
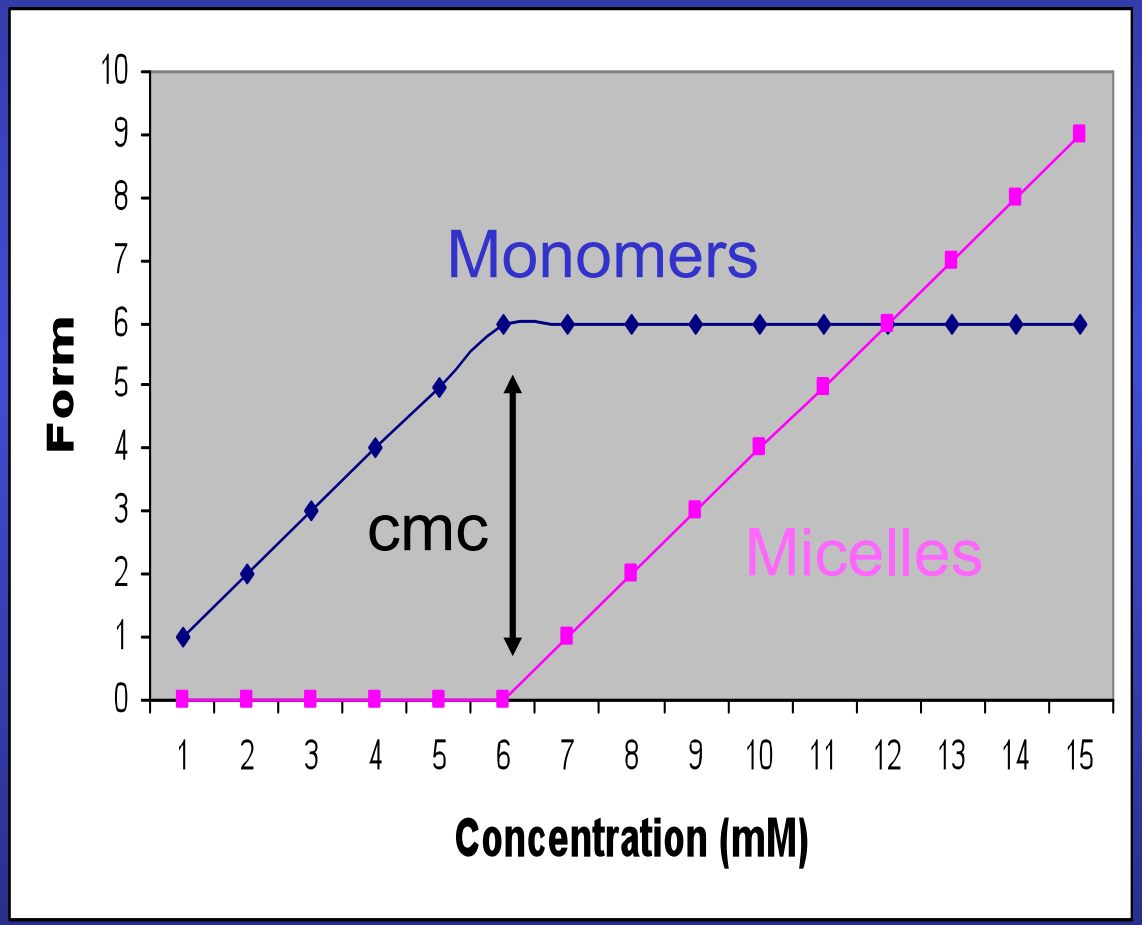
Amphiphilic molecules in aqueous solution

nonpolar regions of compounds order their surrounding water molecules and decrease entropy:



Amphiphiles form assemblies

As concentration is raised, solubility limit of monomer is reached—the critical micelle concentration (cmc), above which a mixture of monomers and micelles is present



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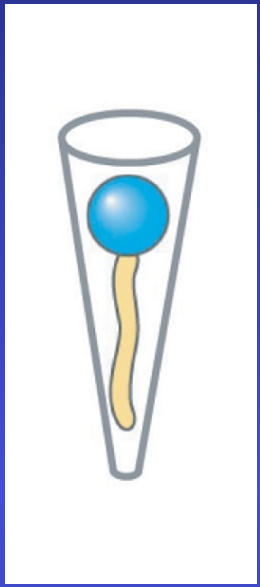
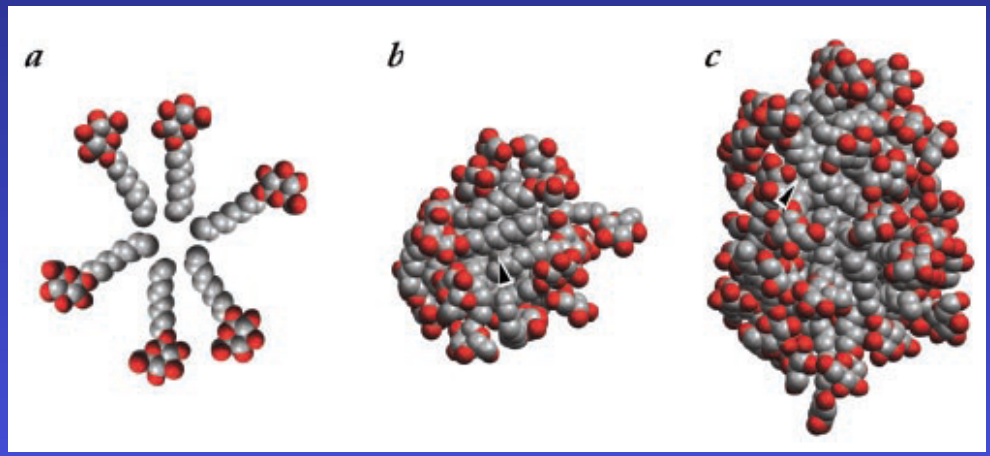
Single chain amphiphiles, such as fatty acids or detergents, form compact micelles



Conical shape allows micelle curvature



a. A textbook view—actual micelles have order gradient, center more ordered than periphery

b., c. Molecular dynamics simulations of two differently sized micelles of dodecylphosphocholine in water

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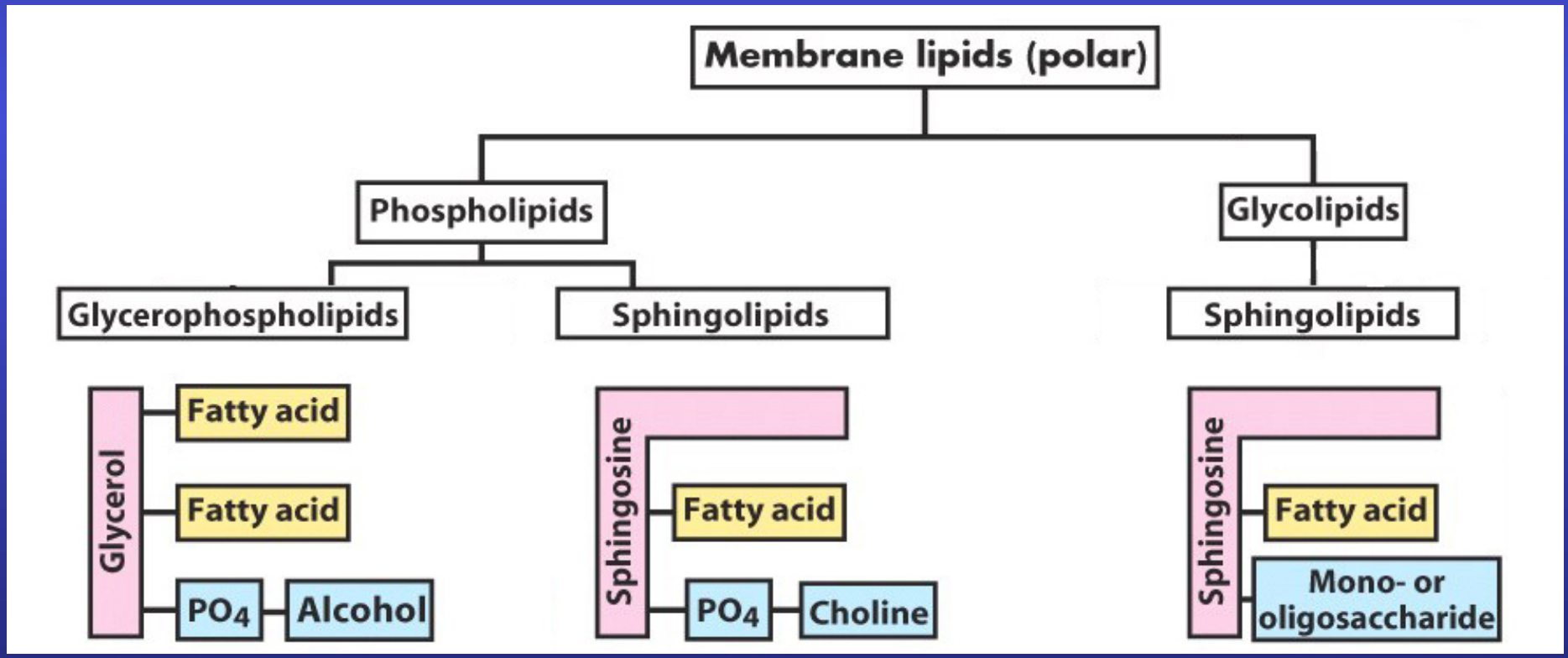
The three main classes of membrane lipids in animals: phospholipids, glycolipids, sterols

- in *phospholipids*, polar head group is an alcohol, linked through a phosphodiester
- in *glycolipids*, head group is one or more sugars, linked through glycosidic bond



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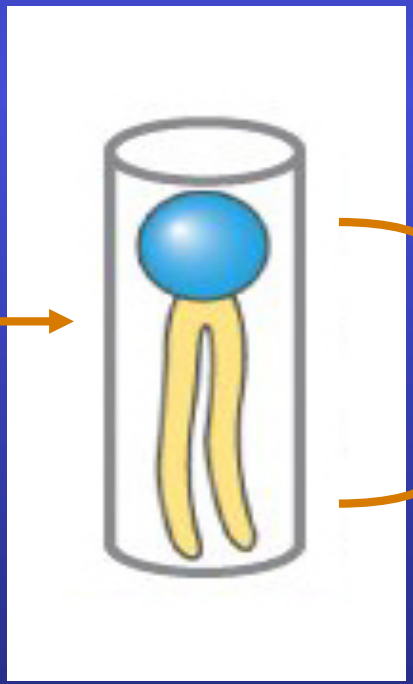
Constraints give average phospholipids "cylindrical shape"

Covalent tethering of chains constrains surface disorder: surface more ordered than interior



Cross section of headgroup ≈ cross section of tails

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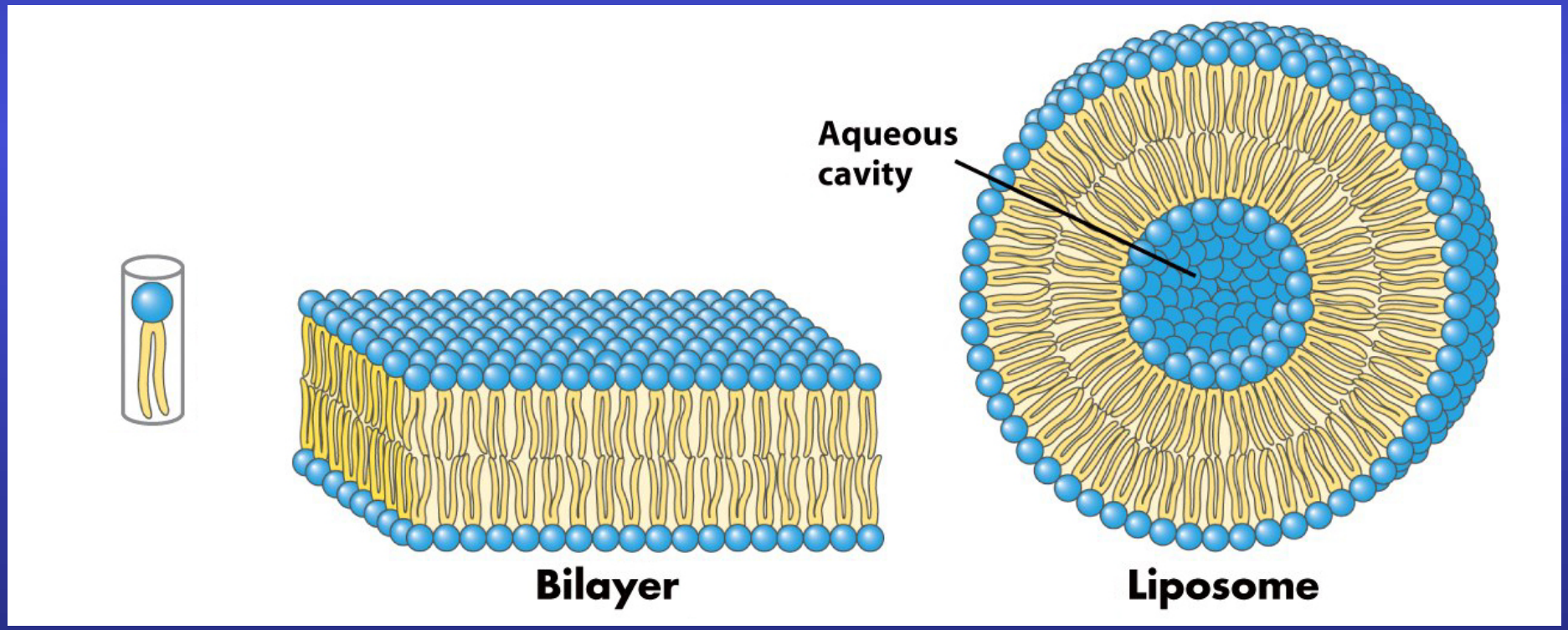
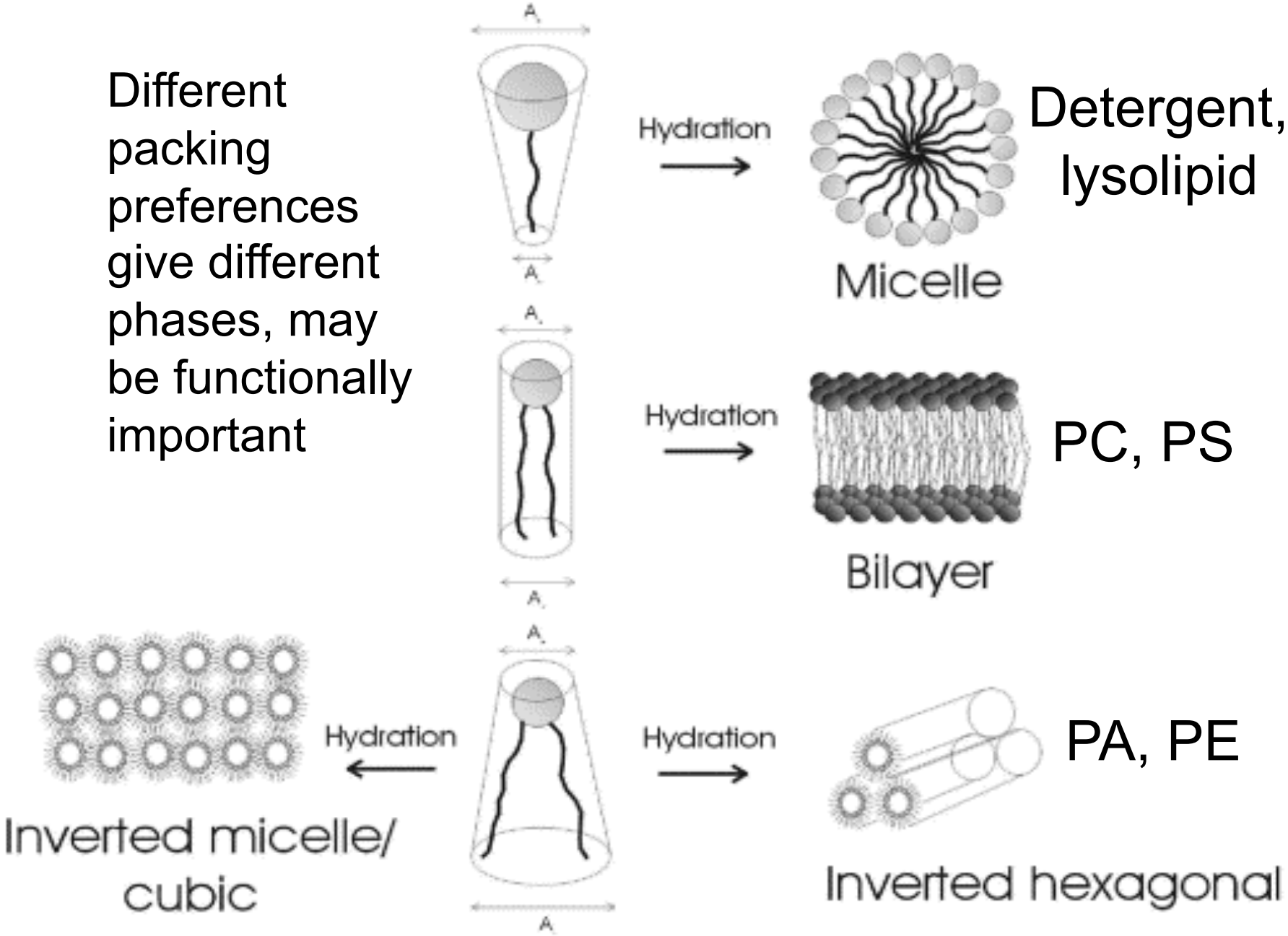
Assembly of amphiphilic lipids into membranes: structural lipids build bilayers

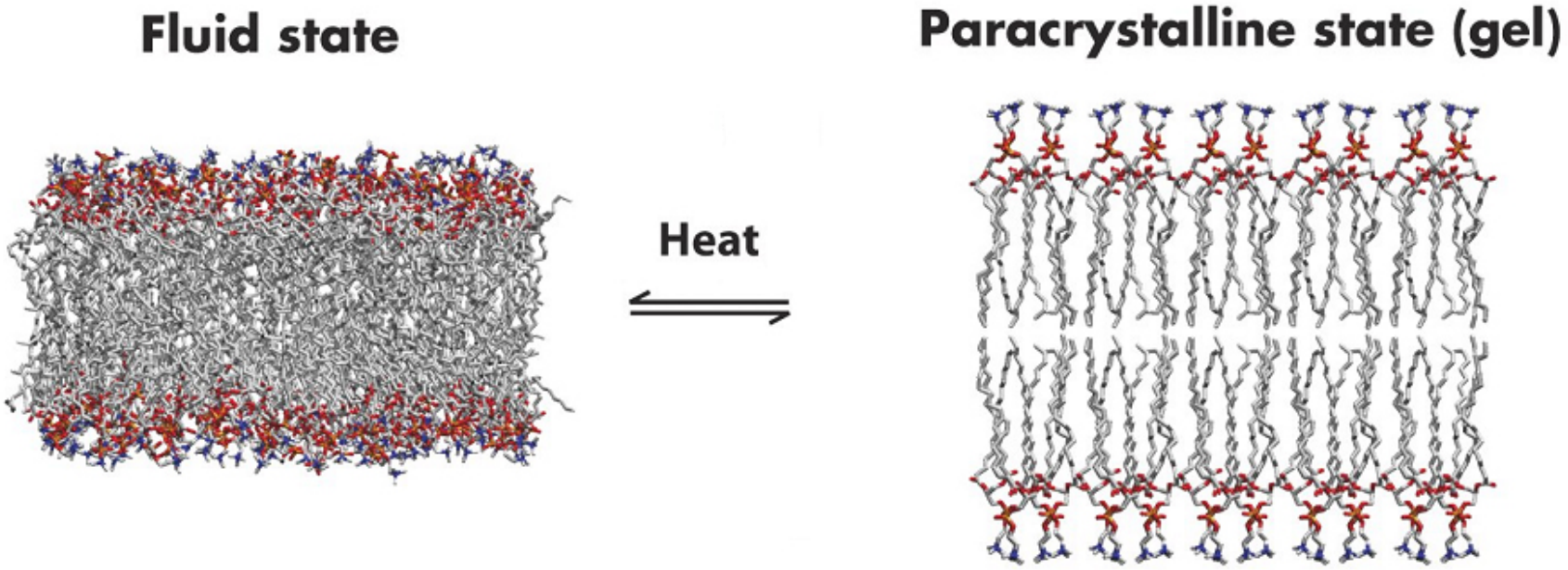
Cylindrical polar lipids: self-assembly into bilayers and vesicles



Different packing preferences give different phases, may be functionally important



Phase transitions of pure lipid bilayers



- heat increases thermal motion:
- no regular array of lipids as their acyl chains become less ordered, bilayer attains fluid-state properties
- fluidity is necessary for biological membranes to function

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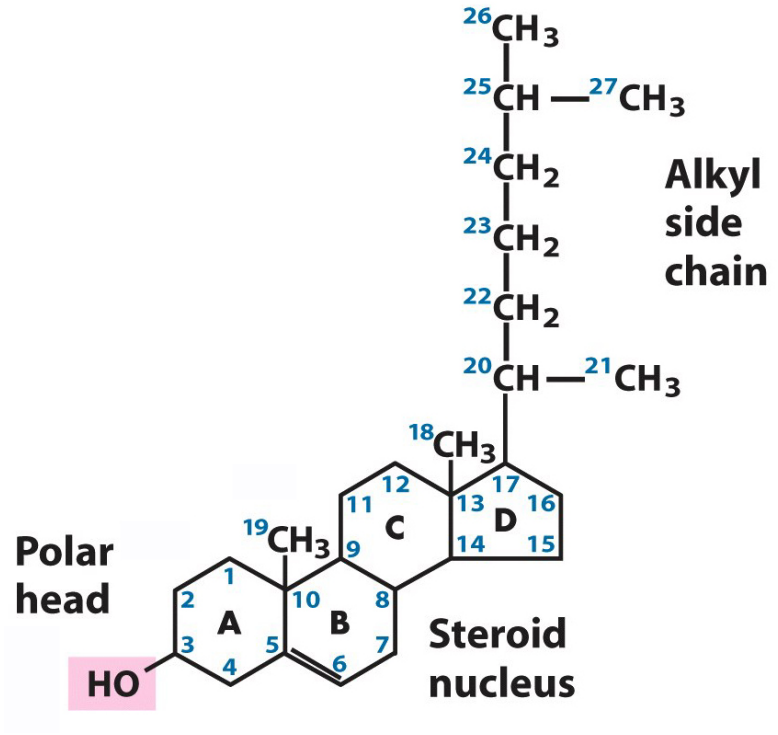
Cholesterol

small polar head group: C-3 hydroxyl

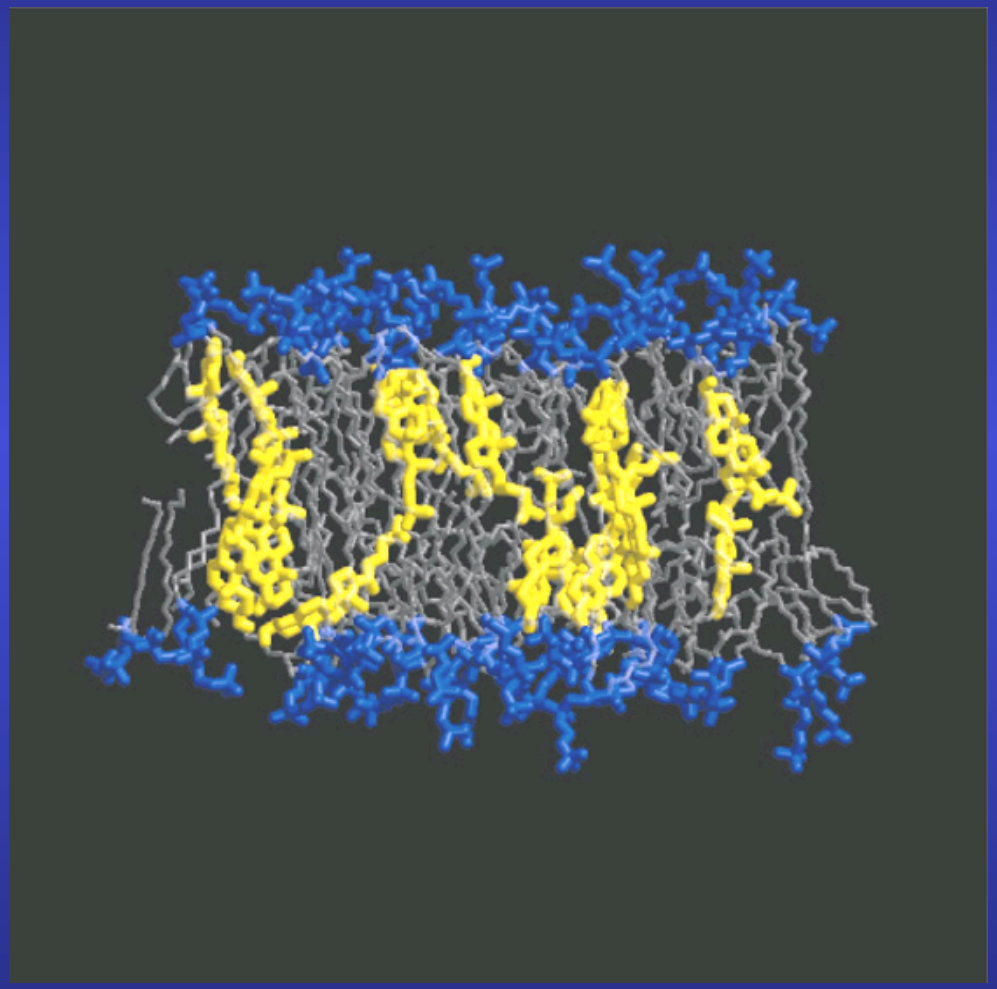


Structural function of cholesterol

Cholesterol modulates lipid membranes:

breaks up Van der Waals interactions and close packing of the phospholipid acyl chains

maintains lipid bilayer in fluid phase



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Bilayer design permits variety

- Eukarya: e.g. rat liver plasma membrane—CL, PC, PI, PS, PA, SM, Chol; 14:0, 15:0, 16:0, 16:1, 18:0, 18:1, 18:2..
- Archae (*H. salinarium*)—PGP, glycolipids, neutral lipids, ether-linked, single fatty chain type (branched, no double bonds)
- Prokarya (*E. coli*)—PE, PG, CL, lipid A; 16:0, 16:1, 18:1, cyclopropyl groups

So—many solutions given the principles of two-chain architecture, amphiphilicity

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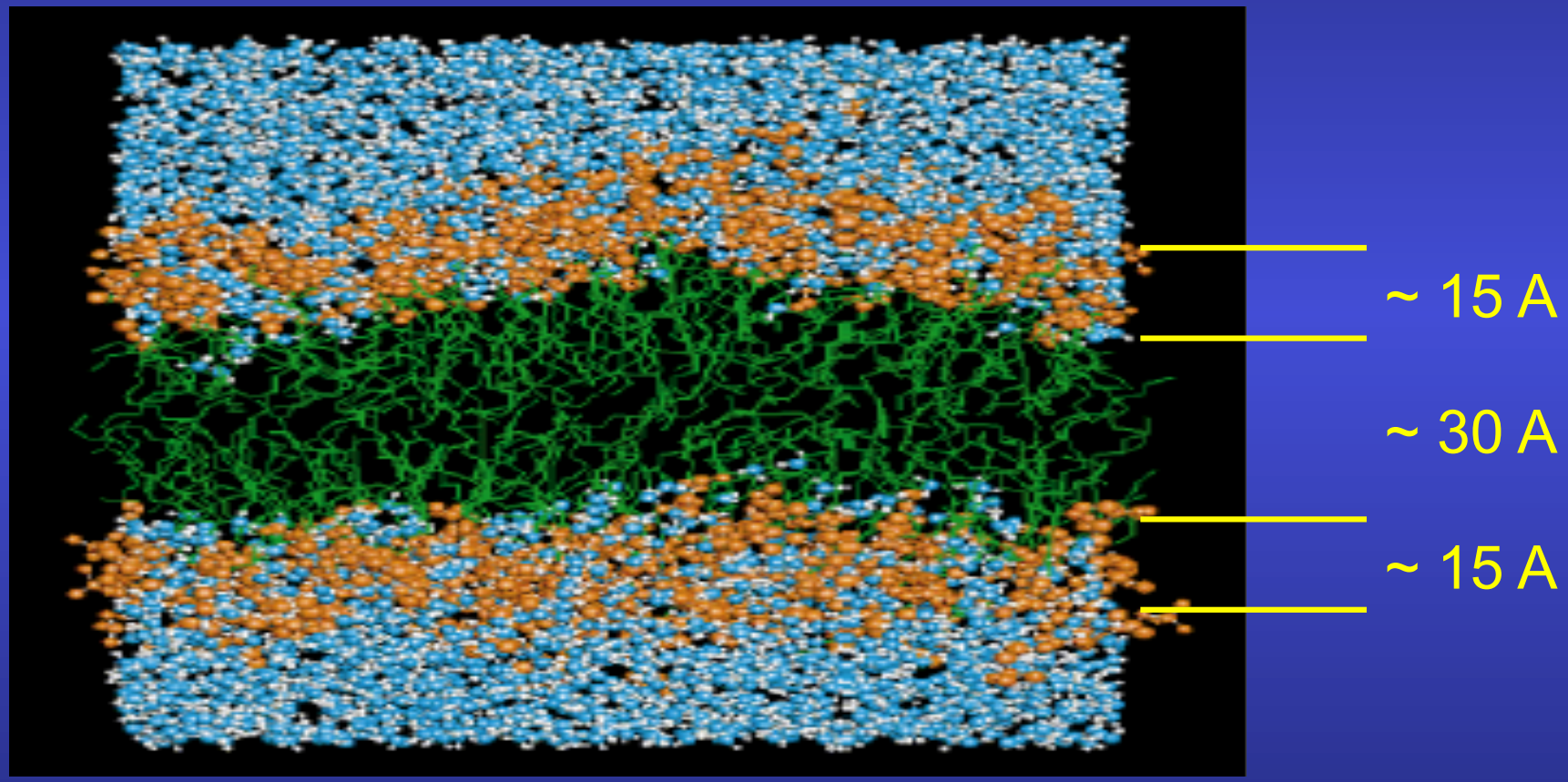
2. Properties of bilayers

MD simulation; note low conc of water in headgroup layer, relative dimensions of heads and tails



Concentration of headgroups in headgroup layers ~500-700mg/ml for PC!

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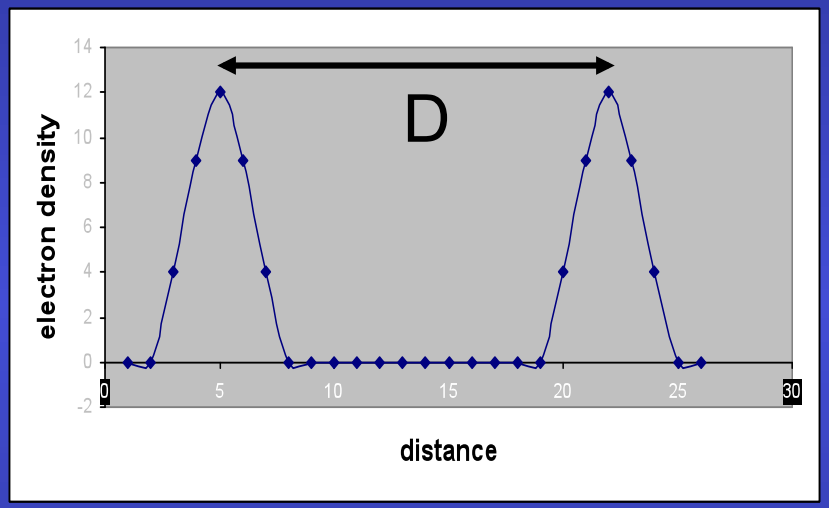
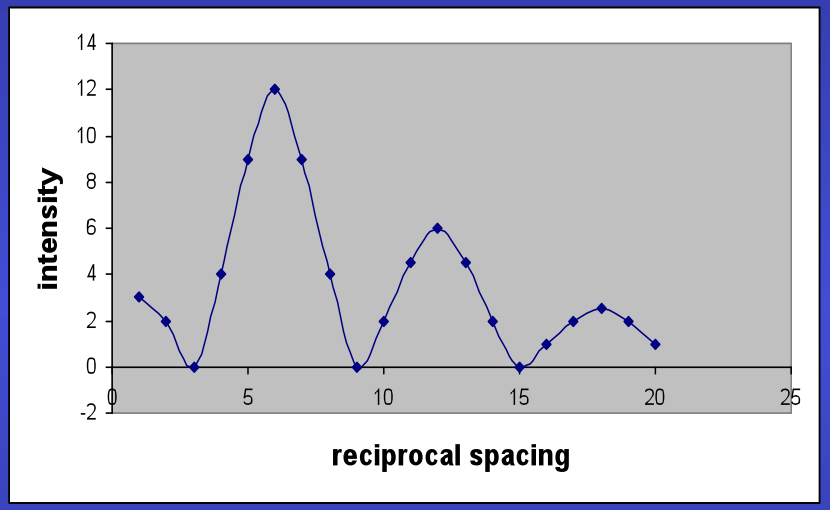
Measure fluid bilayer thickness by diffraction, vary chain length

(Lewis et al J Mol Biol 1983; 166:211)



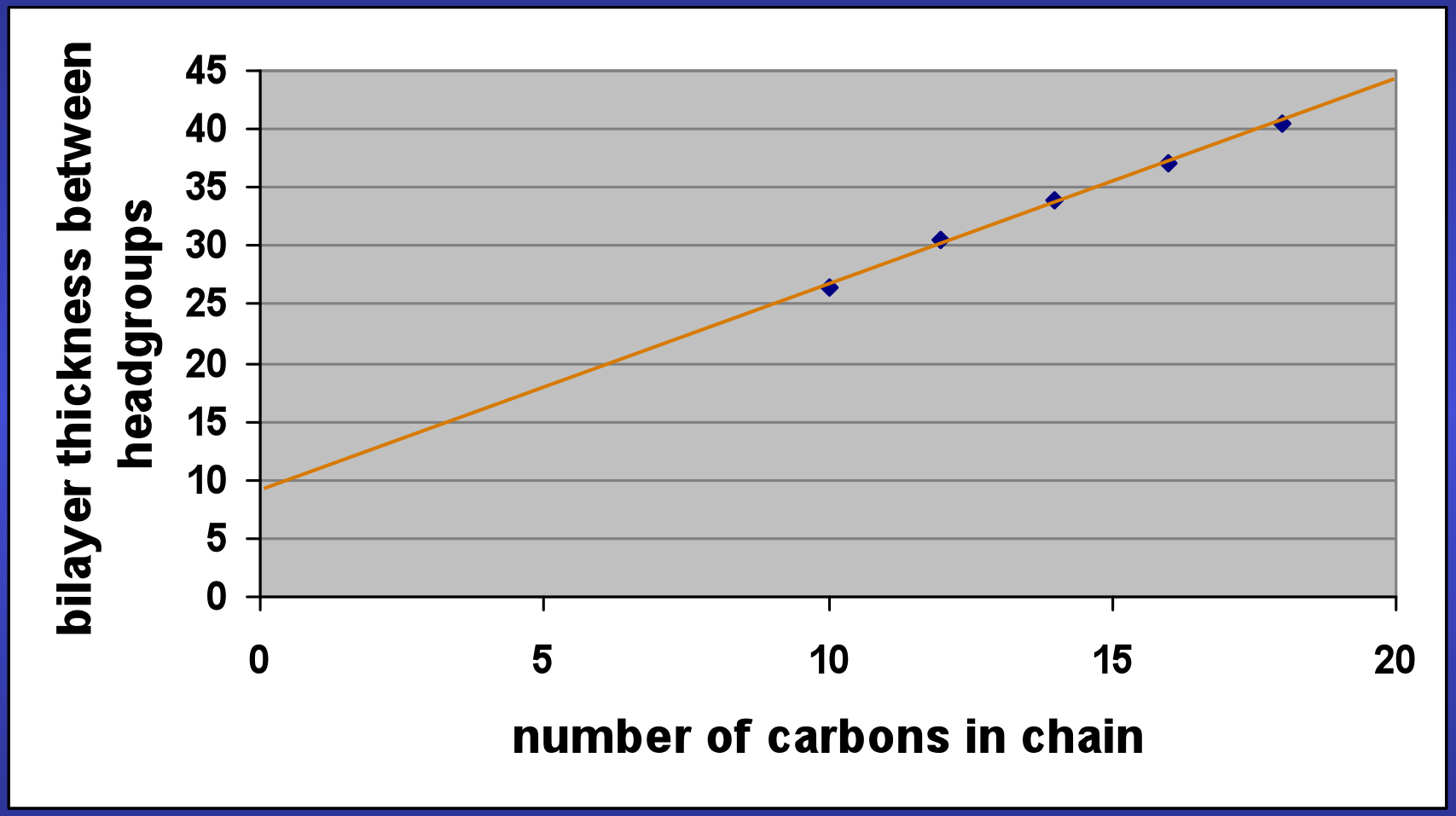


Electron density of bilayer profile, headgroup peaks at distance D

Q: why are there peaks?

Series of diffraction peaks at 1/D, 2/D…

Measure bilayer thickness by diffraction, vary chain length



Linearity implies constant area per lipid (~70A^2)

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Constant area/lipid arguments

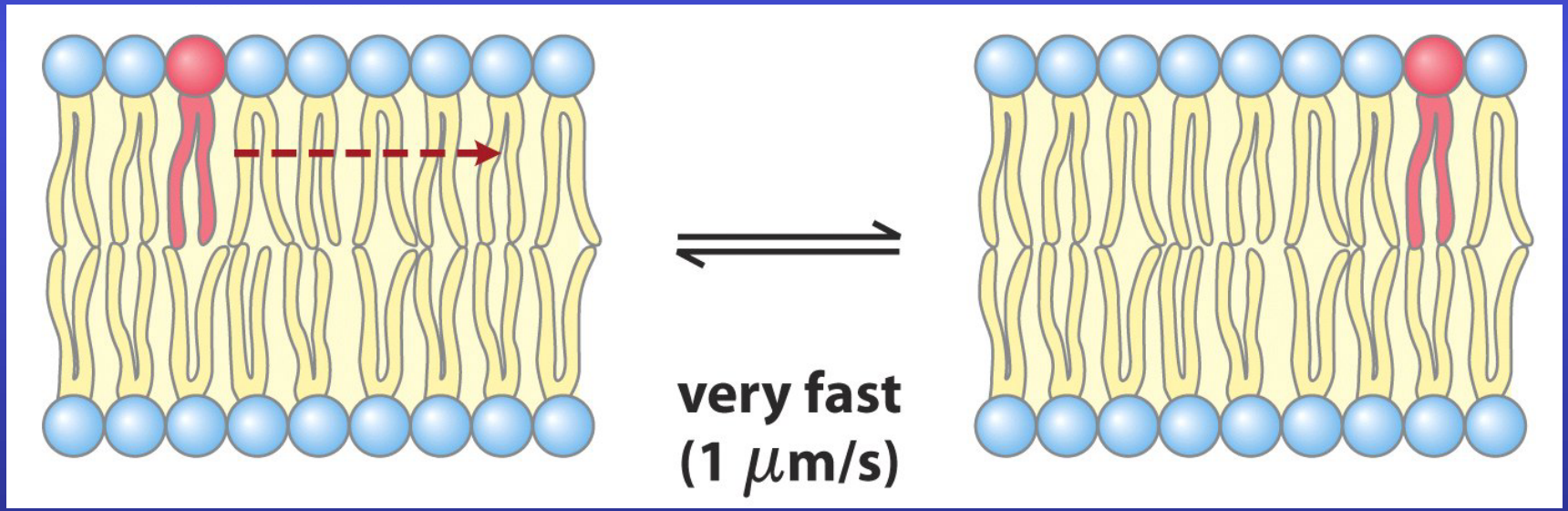
- Bilayer vesicles that are subjected to osmotic stress rupture and reseal to relieve the stress, rather than thinning to increase area by >5%

- Small increases (~ 1%) in the area of one monolayer, which are achieved by the addition of amphiphiles, cause gross shape changes in a bilayer.

- The conserved area arises from the balance of three regions of interaction: forces between head groups (acting to increase area per lipid), aqueous exposure of hydrophobic areas of chains (acting to decrease area) and lateral pressure from chain disorder (acting to increase area)

Membrane dynamics: lateral diffusion of lipids is common and rapid

example erythrocyte:
a lipid molecule can diffuse around the cell within seconds



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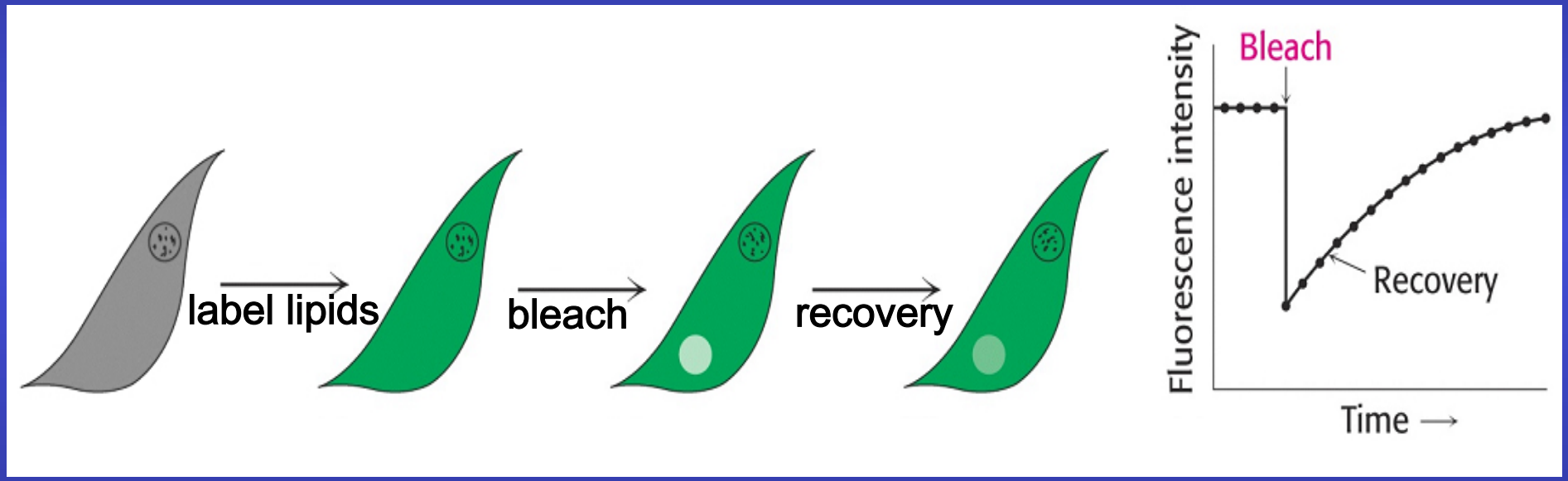
Probing lipid diffusion with labeled lipids: FRAP

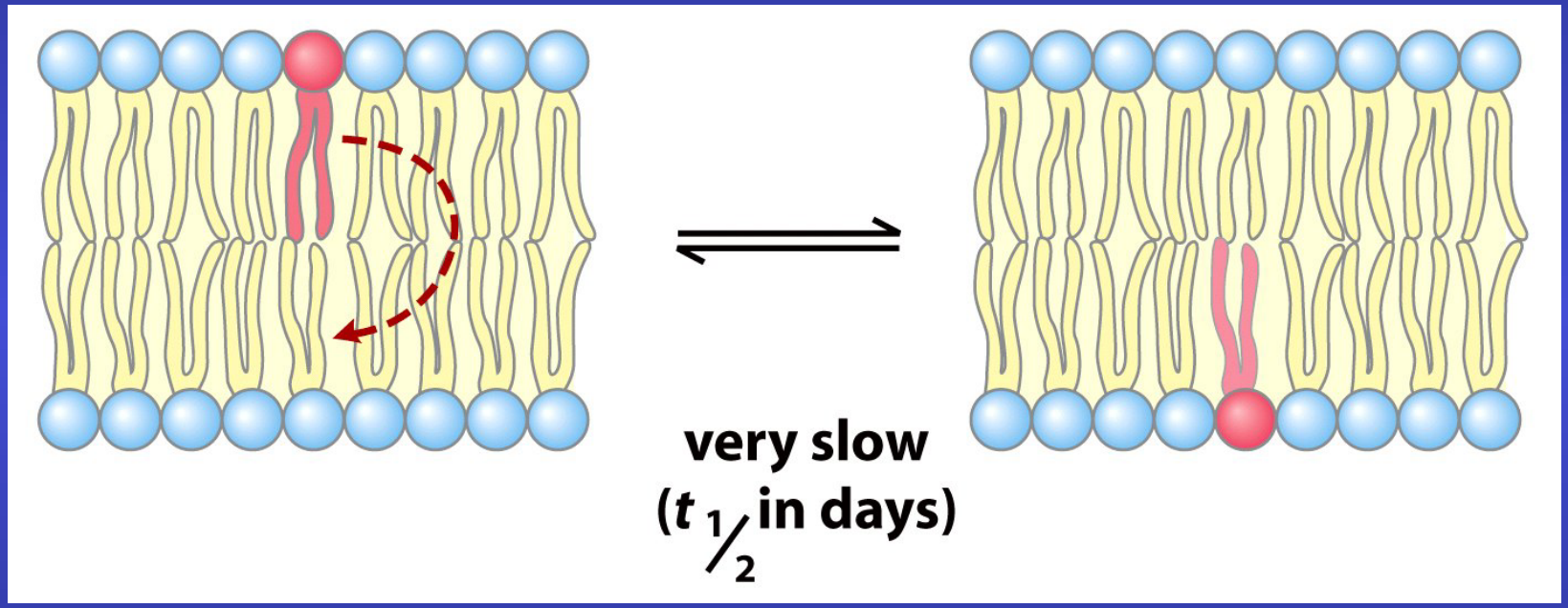
experimental approach using live cells:
FRAP (fluorescence recovery after photobleaching)



- bleach labeled lipids in plasma membrane with laser
- follow lipid diffusion as recovery of fluorescence in the bleached spot

Spontaneous flip-flop of phospholipids is a rare event

Why?



Flipping of lipid molecules can be catalyzed by flippases

Cholesterol can flip (Why?)

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Phospholipid properties promote regionalism

- Failure to flip allows bilayer asymmetry
- Variation in side to side lipid—lipid (and lipid—protein and protein—protein) interaction energies gives planar segregation

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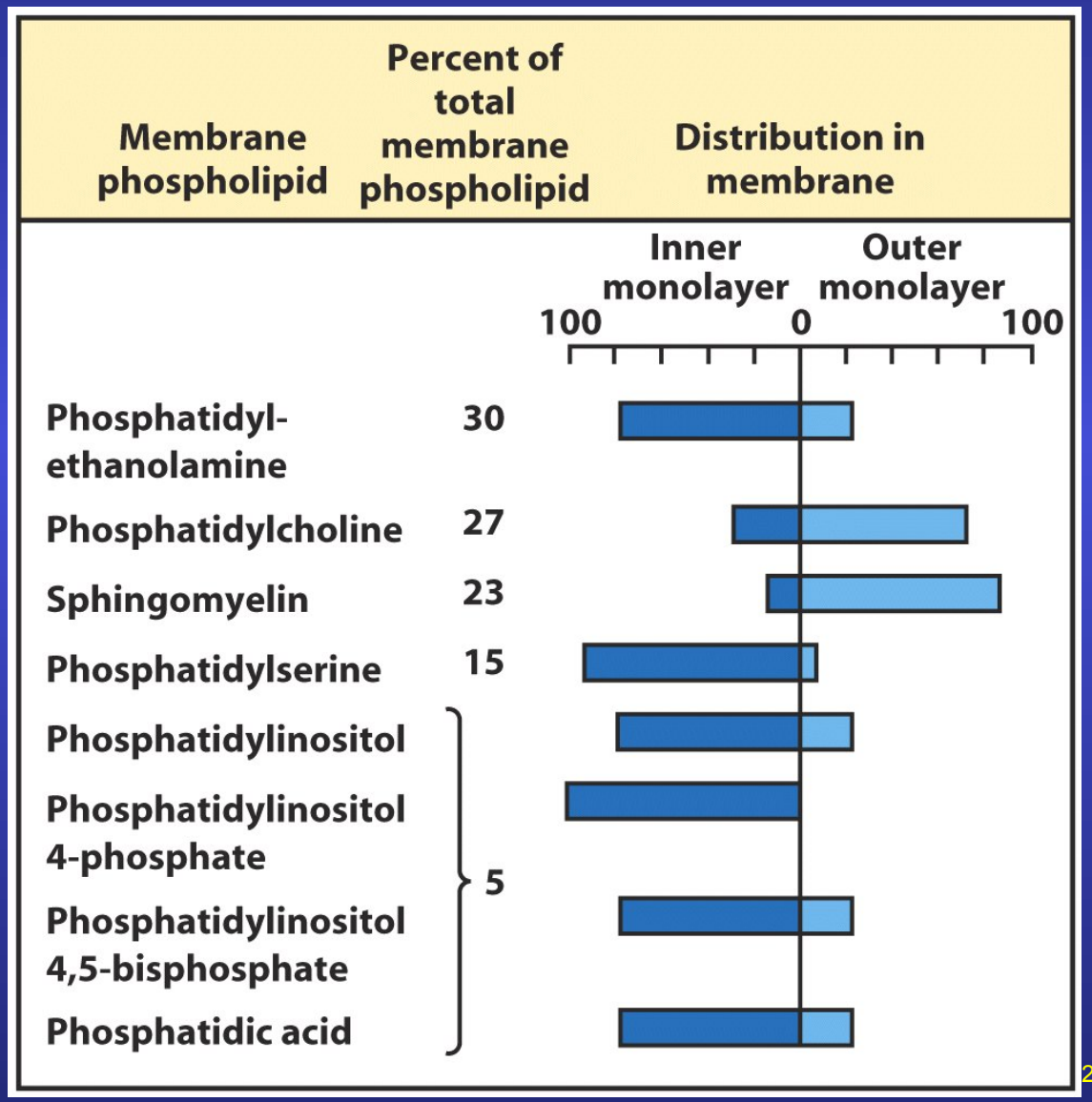
Phospholipids are asymmetrically distributed in membranes

example:
erythrocyte
Membrane

How would
this happen?



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Planar segregation

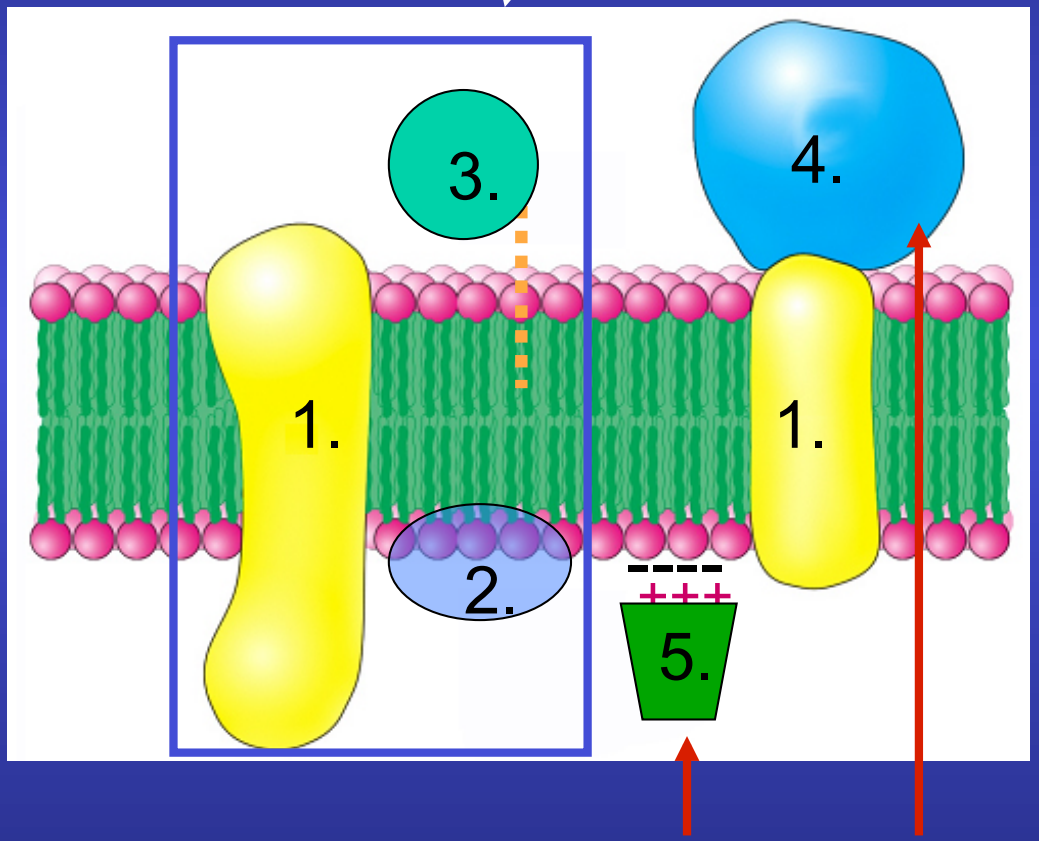
A natural consequence of heterogeneous composition:

--consider a planar liquid of n components.
--n(n-1) / 2 different kinds of pairwise interactions
--for the planar distribution to be random, these interactions must all be within $\Delta\Delta G < kT$
--coli membrane: n~1000, ~500,000 interactions
--impossible, so regions of different composition must arise.

--example: SM has NH, can H-bond to Chol, ⟶ "Rafts"

3. Principles of protein-bilayer interaction

1. hydrophobic effect across bilayer (many)
2. hydrophobic effect at bilayer surface (few)
3. covalent lipid anchors
4. via interaction with transmembrane (TM) proteins
5. via ionic interaction with lipid surface



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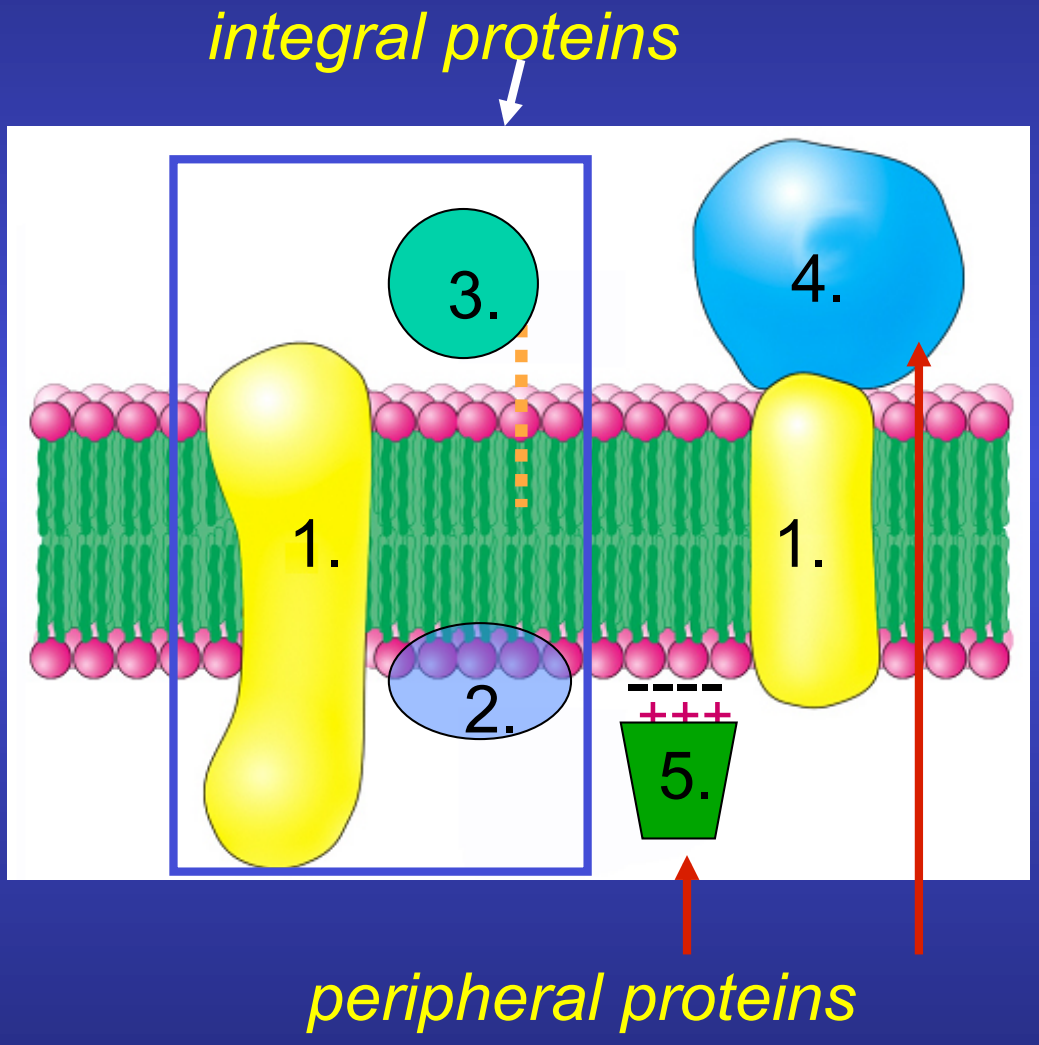
What is wrong with this picture?

1. Bilayer ordered
2. Headgroups too small
3. Proteins monomeric
4. Proteins too dilute
5. Bilayer thickness
6. constant
7. Etc.

Don't take schematics too seriously!



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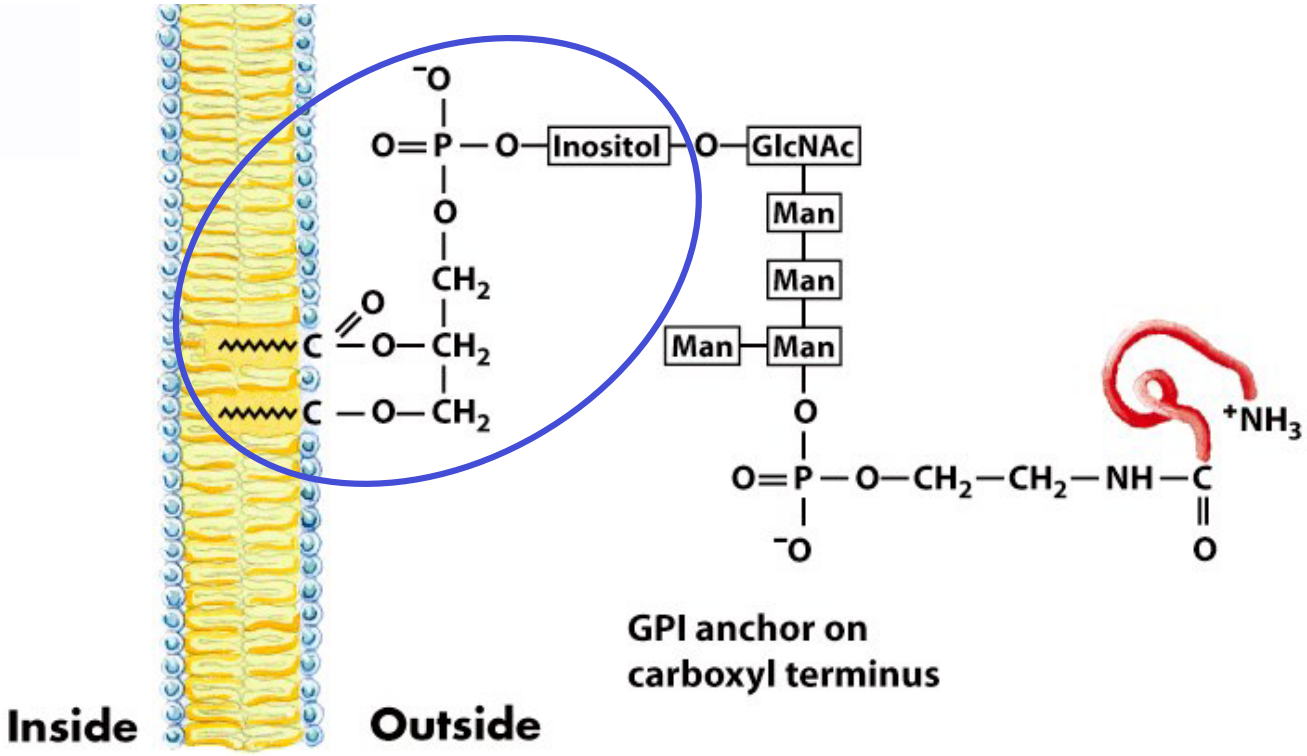
Lipids as protein anchors: GPI

glycosylphosphatidylinositol (GPI): anchors to protein C-terminus

link via phosphatidylinositol:

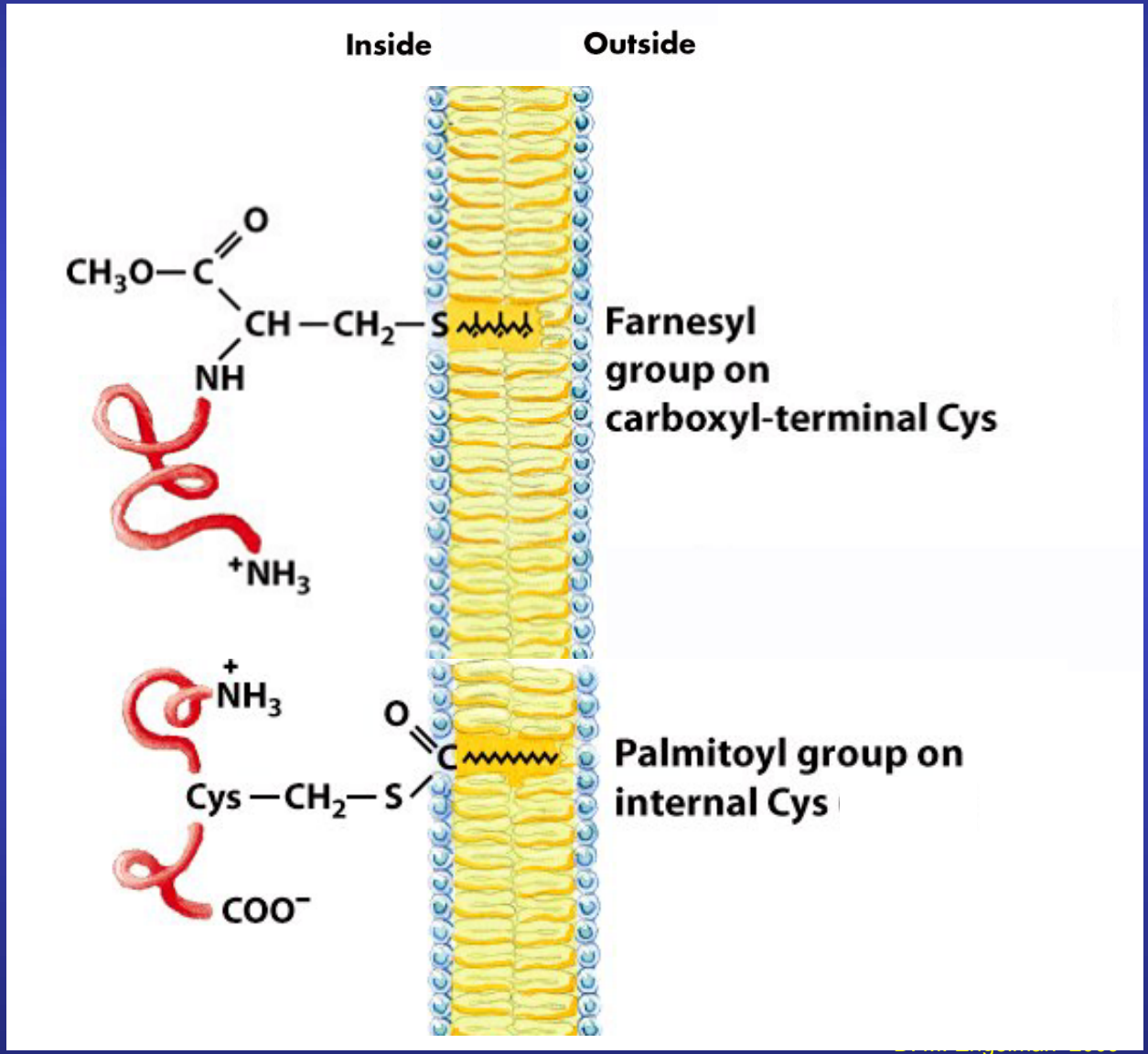


Other lipid anchors exist only on the inner leaflet of the plasma membrane

isoprenylation of a C-terminal Cys:

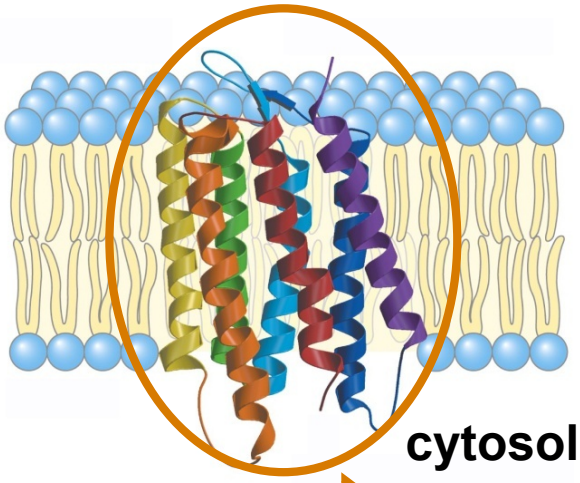
fatty acylation of an internal Cys:

Why do This?

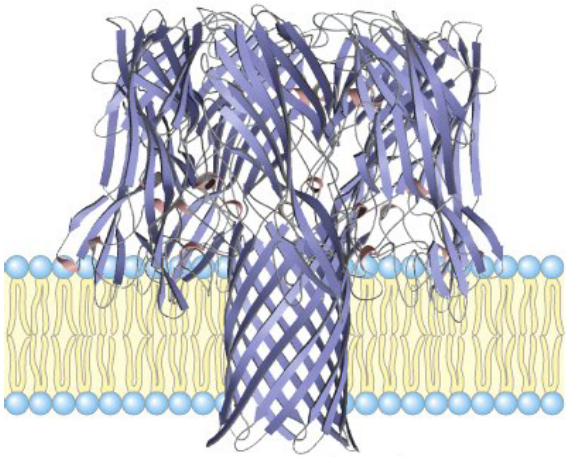


Introduction to integral membrane protein structures

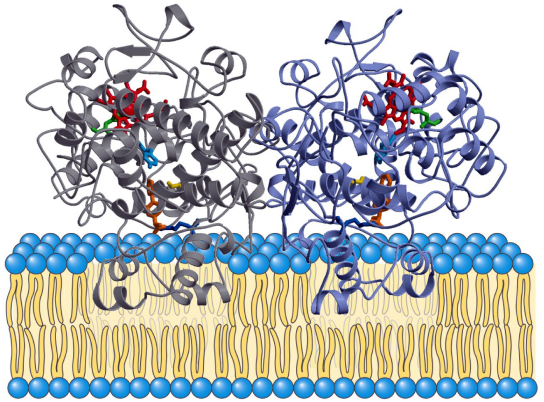
helix bundles (most common):



β barrel (somewhat rare):



partial membrane insertion (rare):



Our focus

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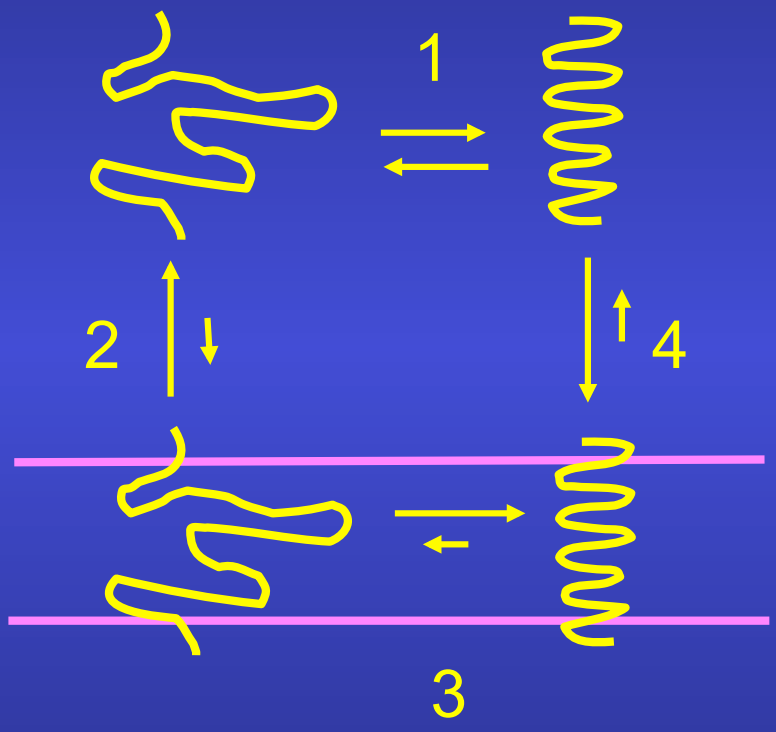
4. Principles of helical membrane protein structure



1. Helix formation in solution is near equilibrium
2. Moving unfolded peptide into bilayer is resisted by polar backbone H-bonds removed from water
3. Forming H-bonds strongly favors regular structure
4. Hydrophobic side chains partition out of water

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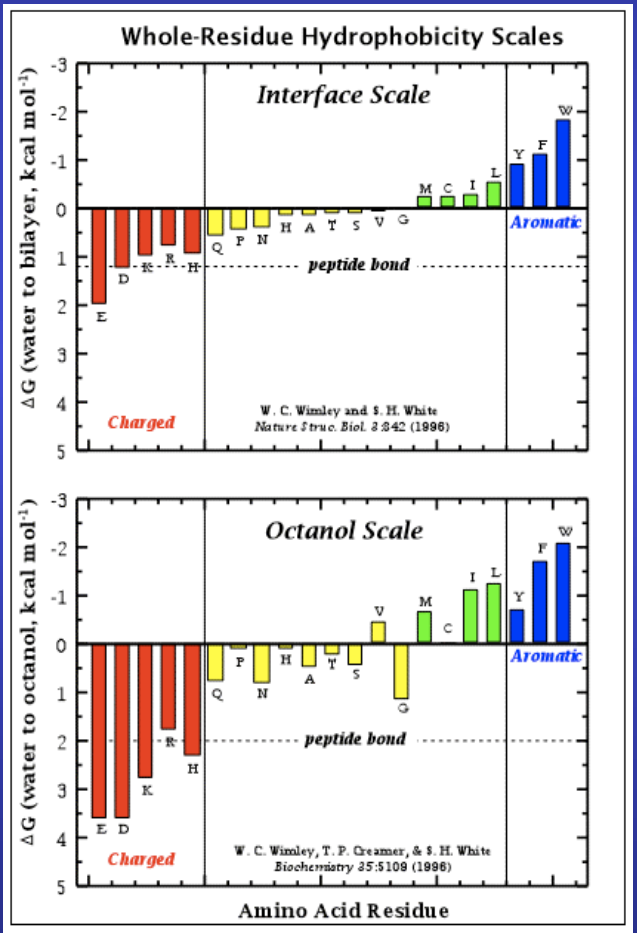
Prediction of transmembrane helices

- amino acids are assigned a hydrophobicity value based on the partitioning of peptides between (e.g.) octanol and water:
- $\Delta G^{o} = -RT\ln K = - RT\ln([\text{octanol}]/[\text{water}])$ for each amino acid
- free energy is summed over a defined length
- a hydrophobic sequence > 20 amino acids is a potential α-helical transmembrane region (Why?)

Improved hydrophobicity scales are being developed



Improved hydrophobicity scales are being developed. This scale was developed on the basis of octonol/water partition coefficients, and it includes the backbone

S H White J.Mol.Biol. 312(2001)927

Polar residues	
Asn	0.85
Gln	0.77
Thr	0.25
Pro	0.14
Ser	0.46
Acidic and basic residues	
Arg^{+}	1.81
Asp^{-}	3.64
Asp^{0}	0.43
Glu^{-}	3.63
Glu^{0}	0.11
His^{+}	2.33
His^{0}	0.11
Lys^{+}	2.80
Salt-bridges	
$Arg^{+}\cdots Asp^{-}$	−0.95
$Arg^{+}\cdots Glu^{-}$	−0.96
$His^{+}\cdots Asp^{-}$	−0.43
$His^{+}\cdots Glu^{-}$	−0.44
$Lys^{+}\cdots Asp^{-}$	0.04
$Lys^{+}\cdots Glu^{-}$	0.03

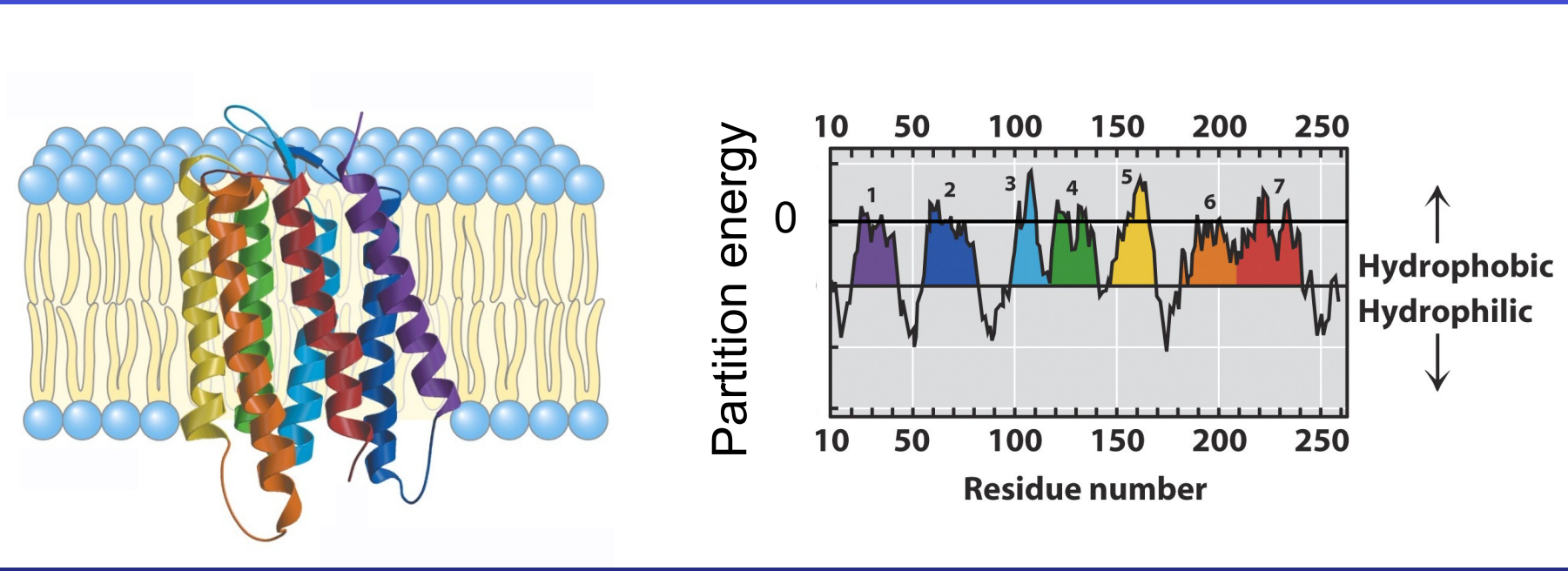
Some surprises Here—R+, for Example. Note the ion pair data

S H White J.Mol.Biol. 312(2001)927

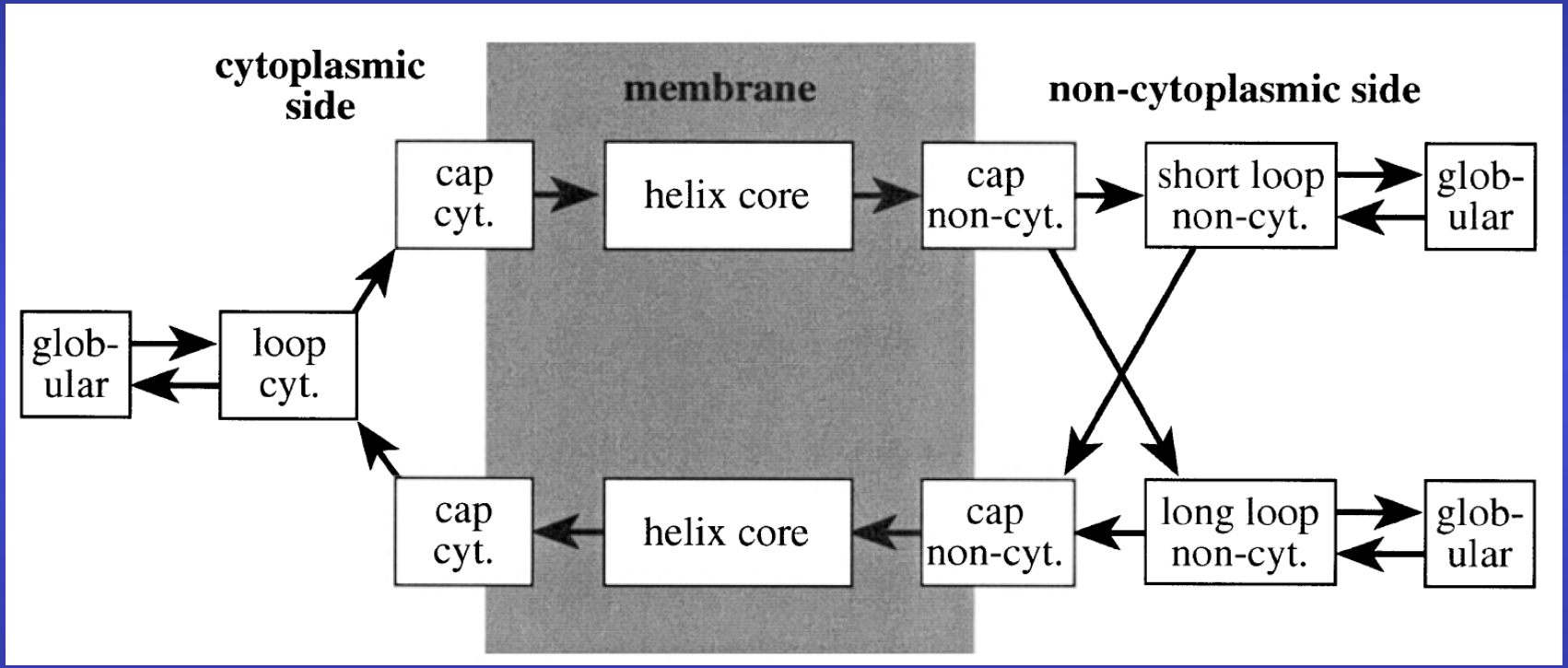
Prediction of transmembrane helices

Taking into account the polar character of the backbone shifts the zero point by ~1 kcal/mol for each aa

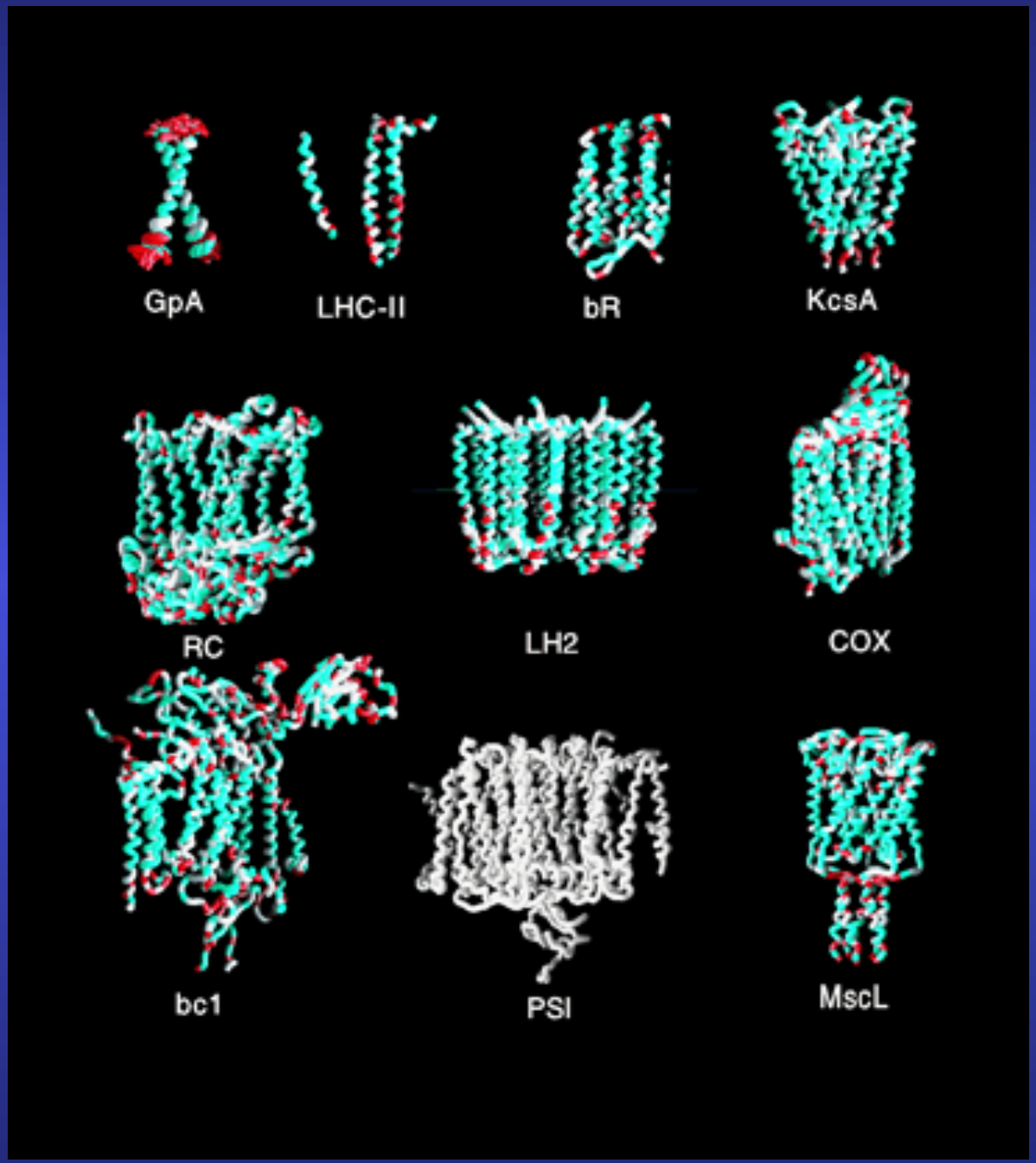
Prediction for bacteriorhodopsin:



A Different Approach: Hidden Markov Model



G von Heijne, J.Mol.Biol. 305(2001)567



Helical Membrane Proteins (1999)
Cyan = hydrophobic:

Helices are well-predicted as if they were separately inserted across bilayer

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So…helices are well predicted
as if they are immersed in
a lipid environment…

Yet, they are more in contact
with other helices than with lipid

Thus, the idea of helix formation
may be separable from helix interaction
to form higher order structure

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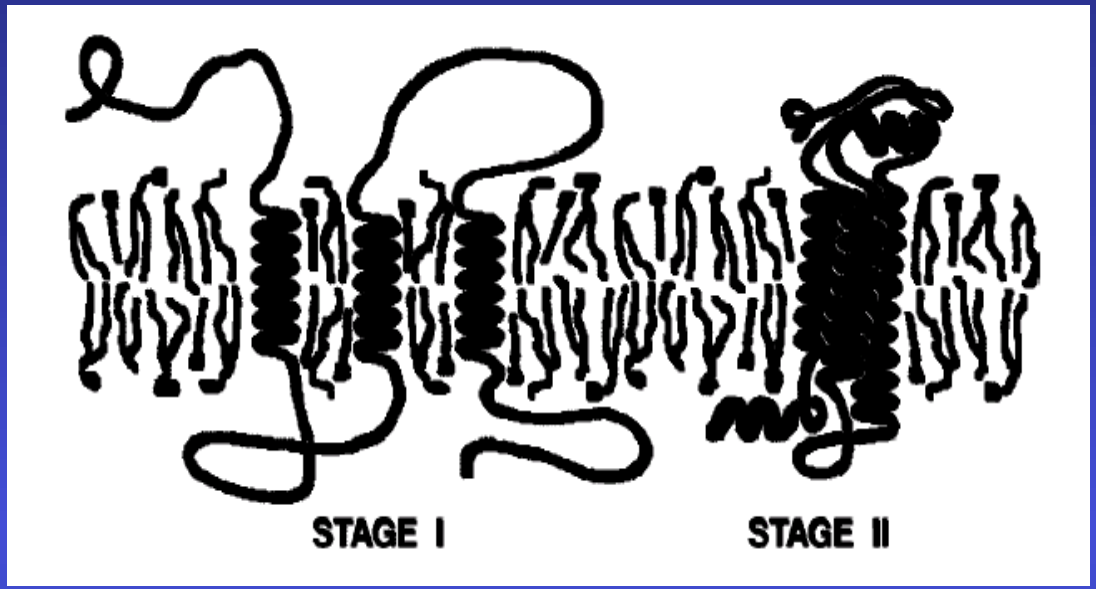
The Two Stage Model
(Popot et. al. 1990 Biochemistry)

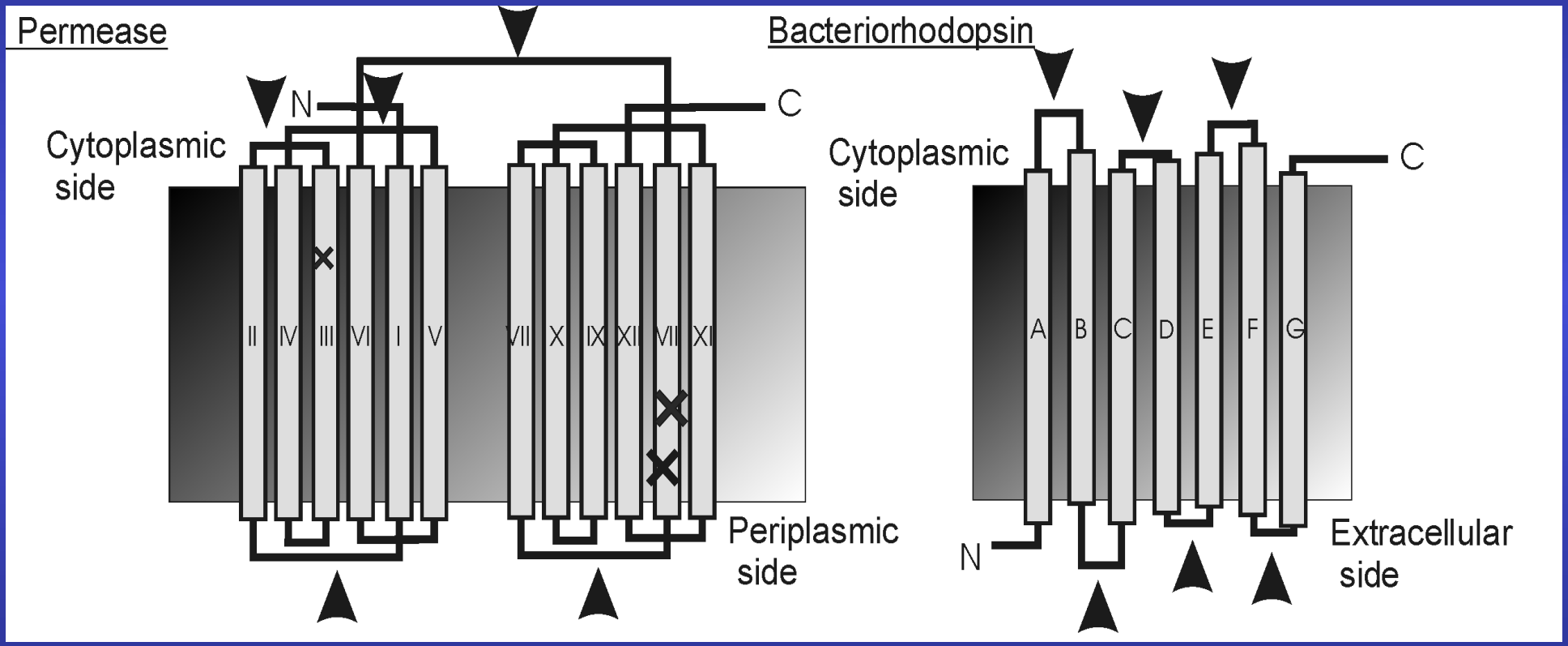


Stage I: Hydrophobic effect and main chain H-bonds stabilize individual helices across bilayer

Stage II: side-to-side association of helices driven by H-bonds and packing gives structure

Split Proteins Refold

Splits are shown for two proteins that recover function when fragments are mixed biochemically or genetically



▲ = functional split, X = non-functional

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TM formation uses the hydrophobic effect and main chain H-bonding. So, what holds helices together?

- Detailed packing
- H-bonding

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Motifs can give clues for TM interactions-- how to find them?

Informatic approach:

Study pair wise correlations of amino acids in TMs using databases

Such as SWISSPROT

Many Amino acid pairs are highly over- or under-represented:

Over	Under
GG4	II2
II4	GI4
GA4	IL1
IG1	FL1
IG2	FI4
VG2	IG4
(etc)	(etc)

Important chemical clues for further understanding

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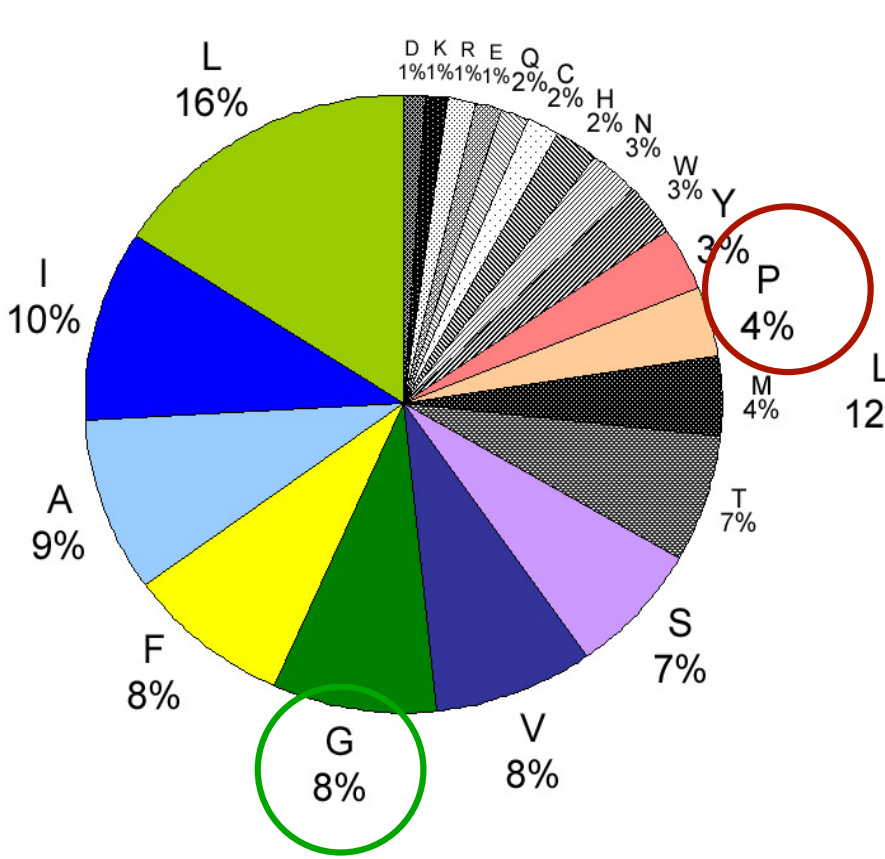
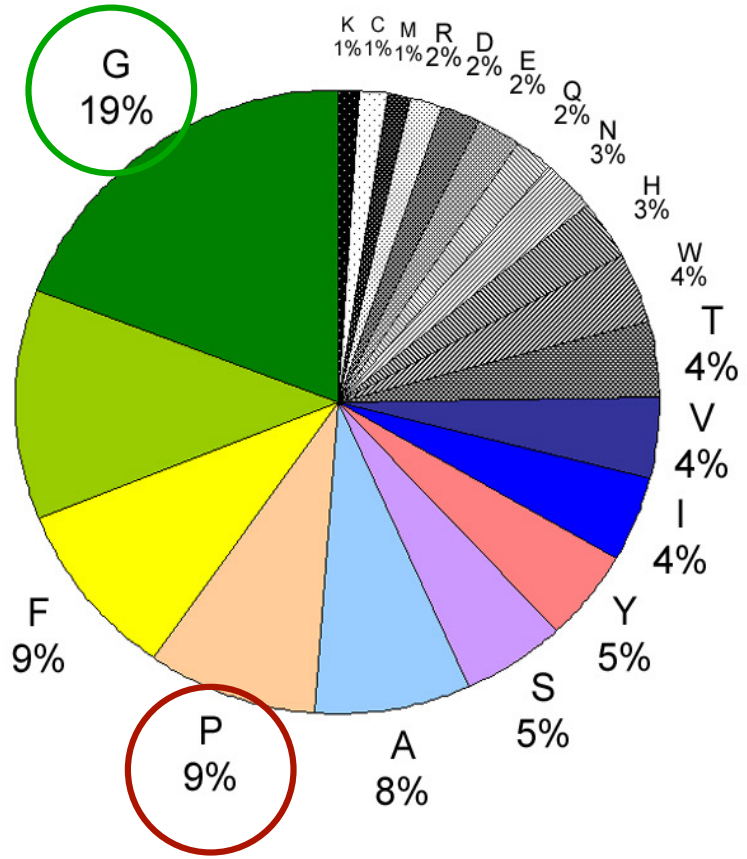
A larger scale study of families finds similar motifs, Also compositional biases



Amino acid composition of all residues in TM-helices of Pfam-A families



Amino acid composition of positionally conserved residues in TM-helices of Pfam-A families

Average composition

Conserved

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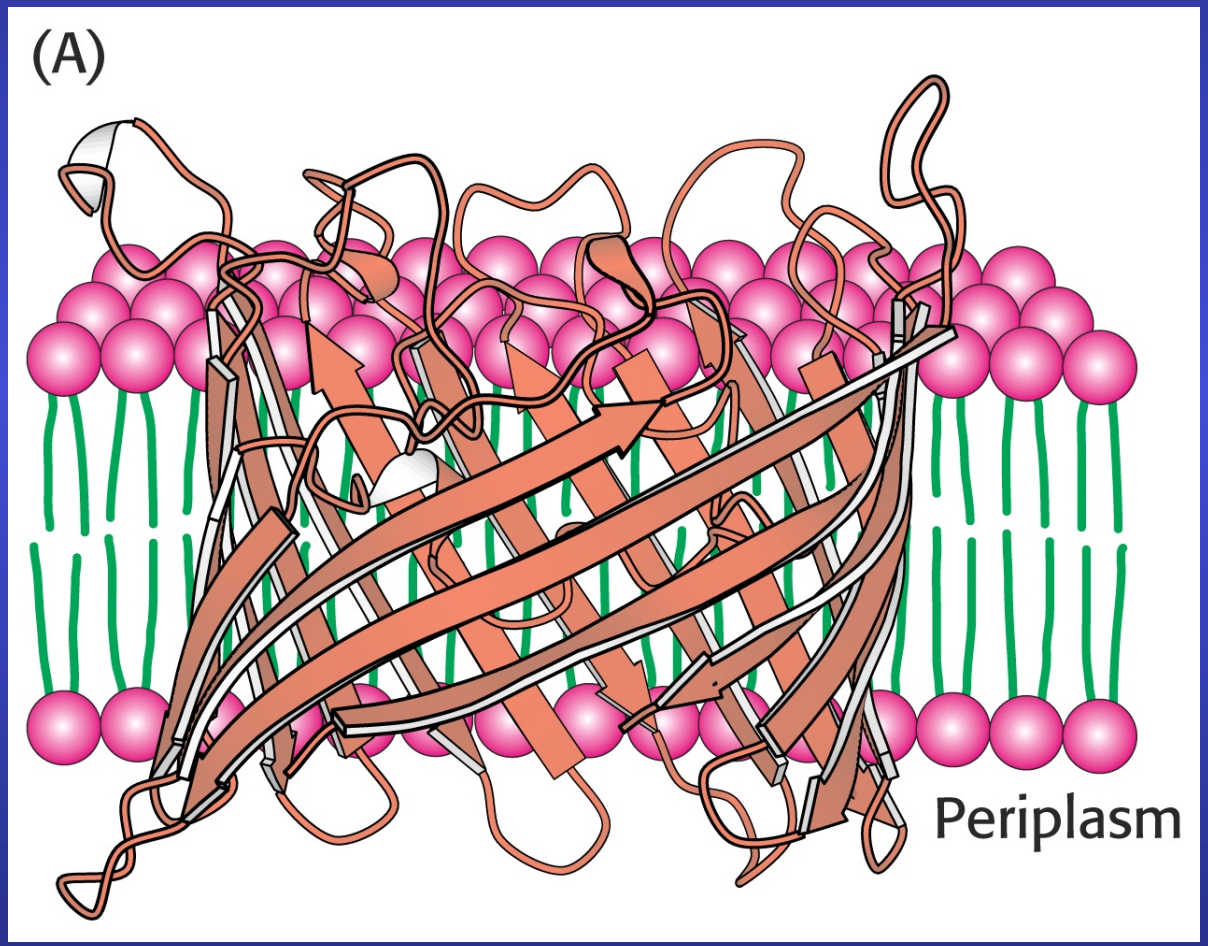
So far

- TMs predicted
- Interaction principles tested
- Motifs found

- Are TMs the only feature of membrane proteins inside bilayer?

β – barrels are another way to satisfy H-bonds and match hydrophobicity to the bilayer



Proteins of this type are found in bacterial, chloroplast, and mitochondrial outer membranes

A bacterial porin

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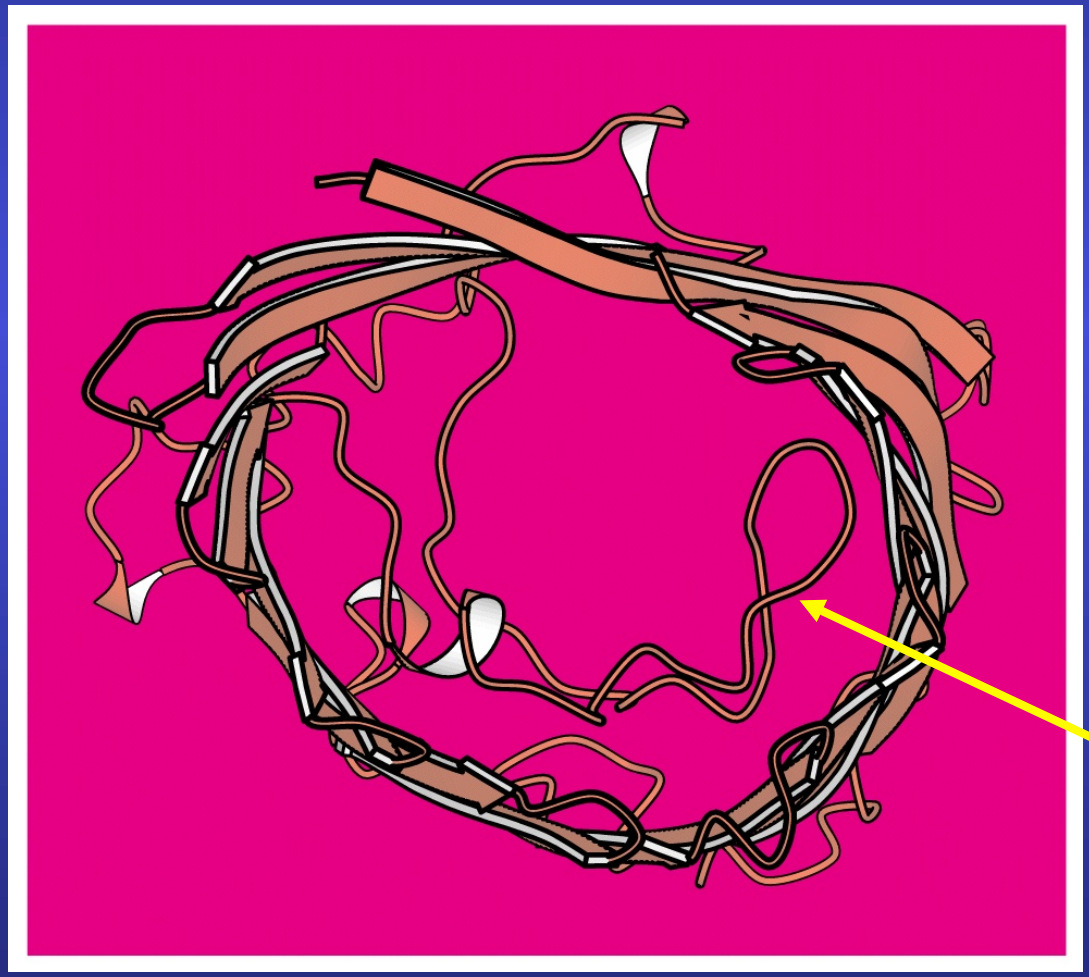
Porins present nonpolar aas to the lipid, but can have polar interiors



Other structures are permitted by sequestering them from the bilayer.

Note the internal loop

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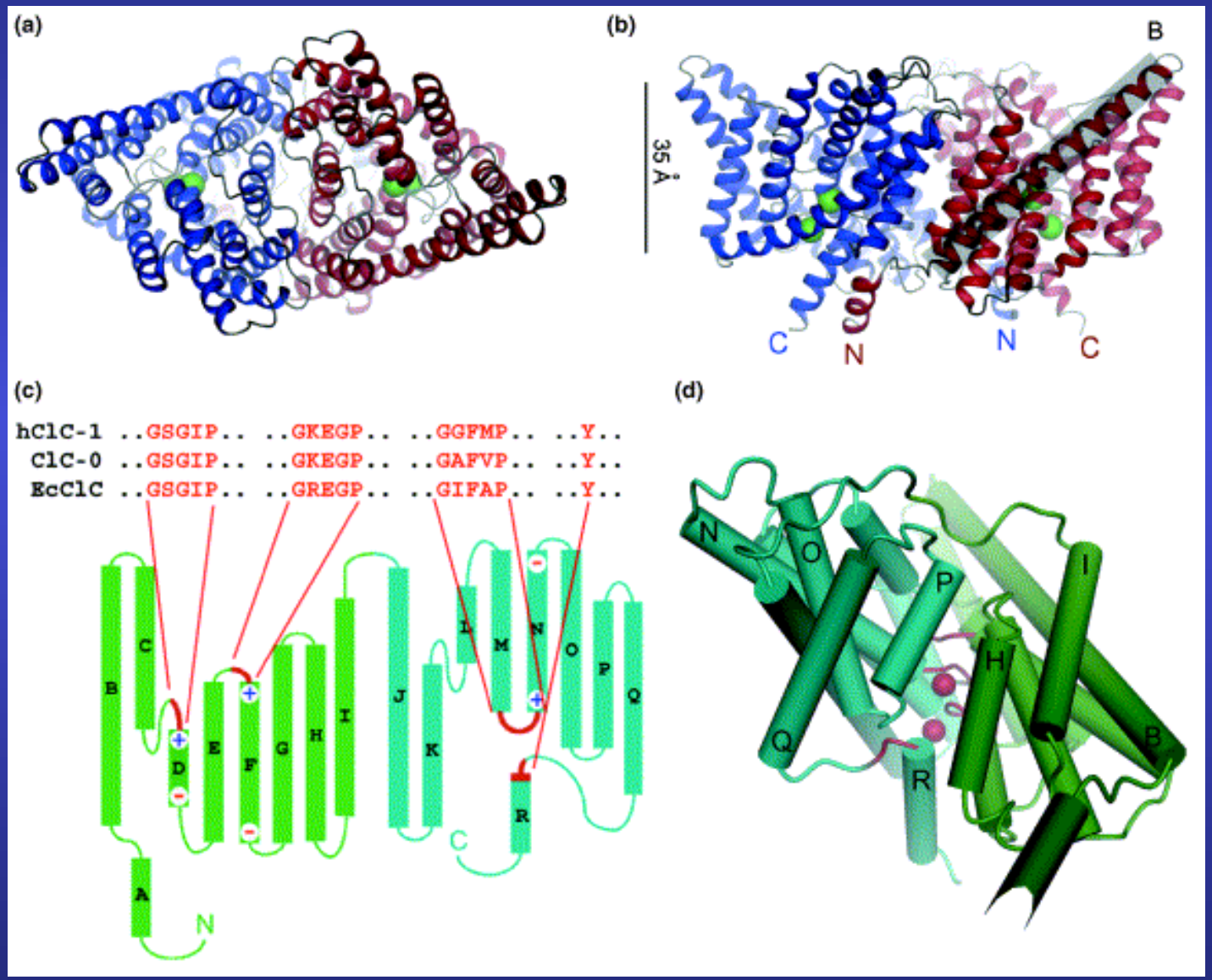
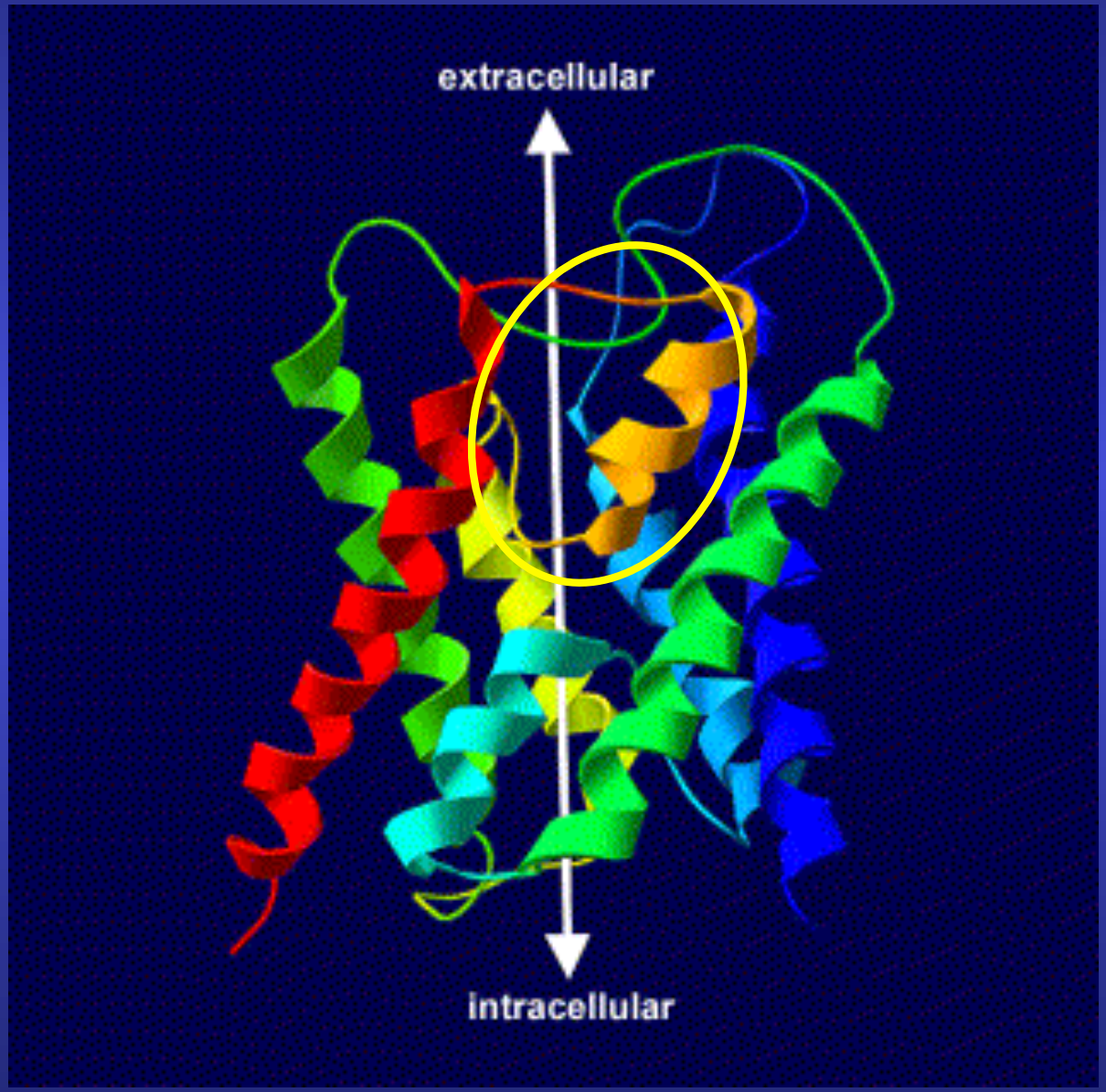
5. What of non-TM parts in known structures of helical proteins? e.g. Chloride channel



Several short helices and loops seen inside bilayer region

Sequestered from lipid by TM's

Another example: Aquaporin



Internal oligomeric interfaces have non-TM structure

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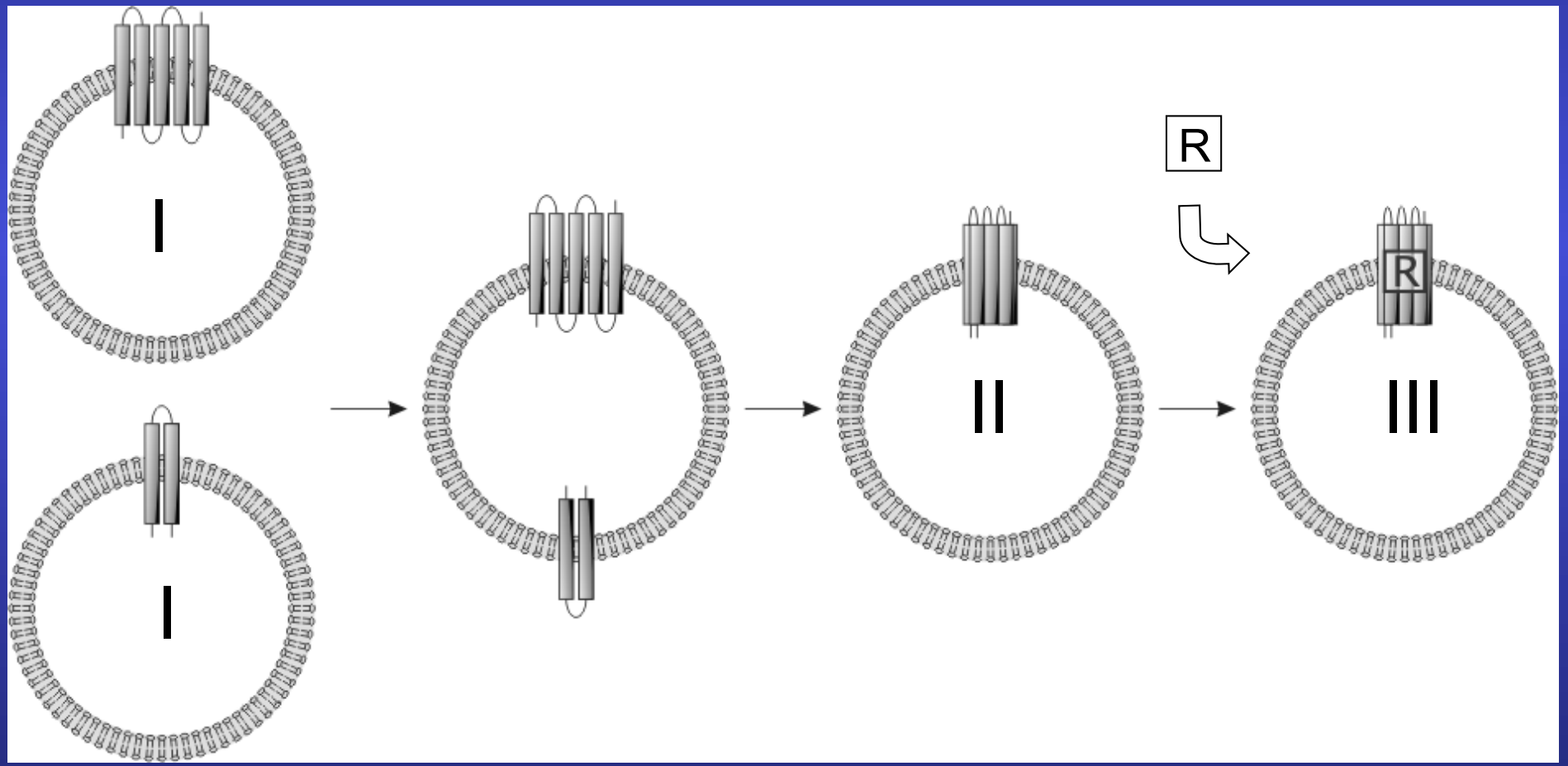
Stages seen in Split Bacteriorhodopsin Experiment:

Fragments Form Helices In Vesicles

Vesicle Fusion

Fragments Interact

Retinal Binds,Get Active BR

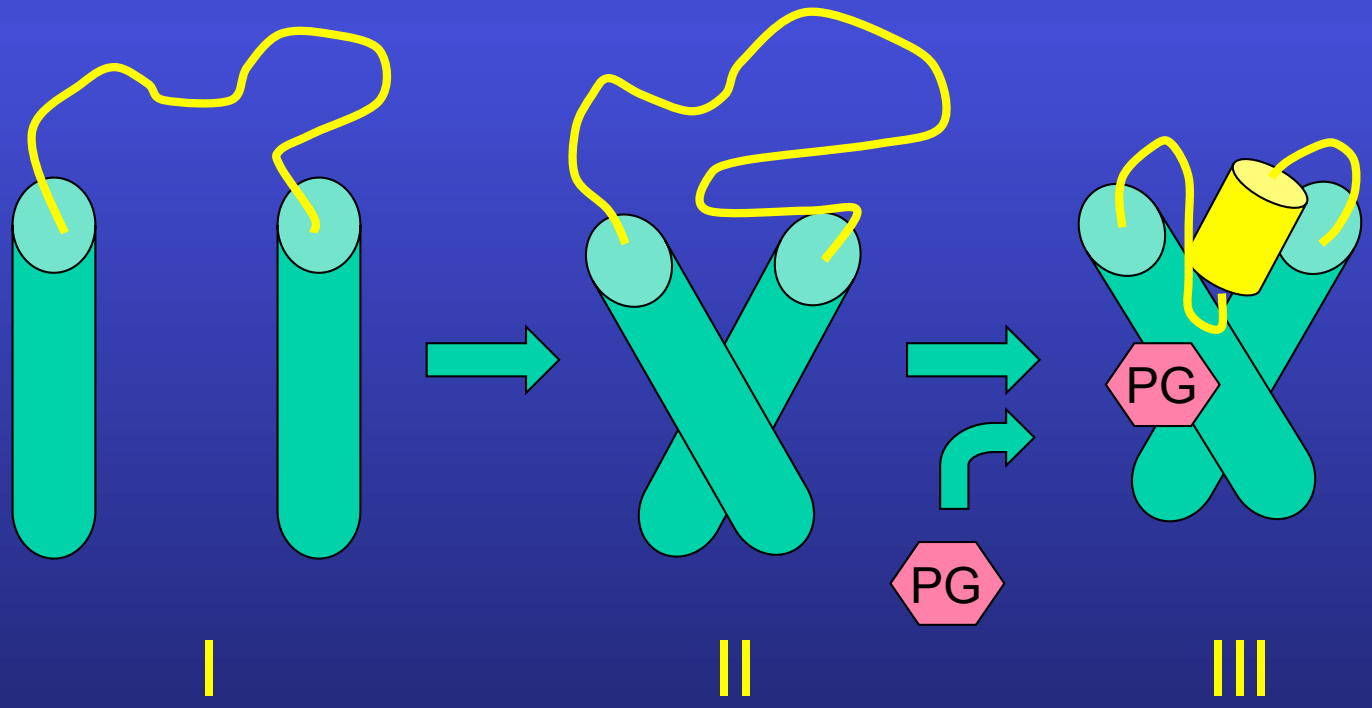


Popot et al, 1987

D. M. Engelman 2009

Third stage of 2 stage model?

Third Stage: Form sequestered space,
Re-entry of polypeptide,
Prosthetic groups bind



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6. Why might stability be higher in lipids than in detergents? Mismatch of properties.

- Bilayer lipid shapes match protein
- Hydrophobic dimensions
- Order profile
- Helix-helix interaction constraints
- Altered helix stability

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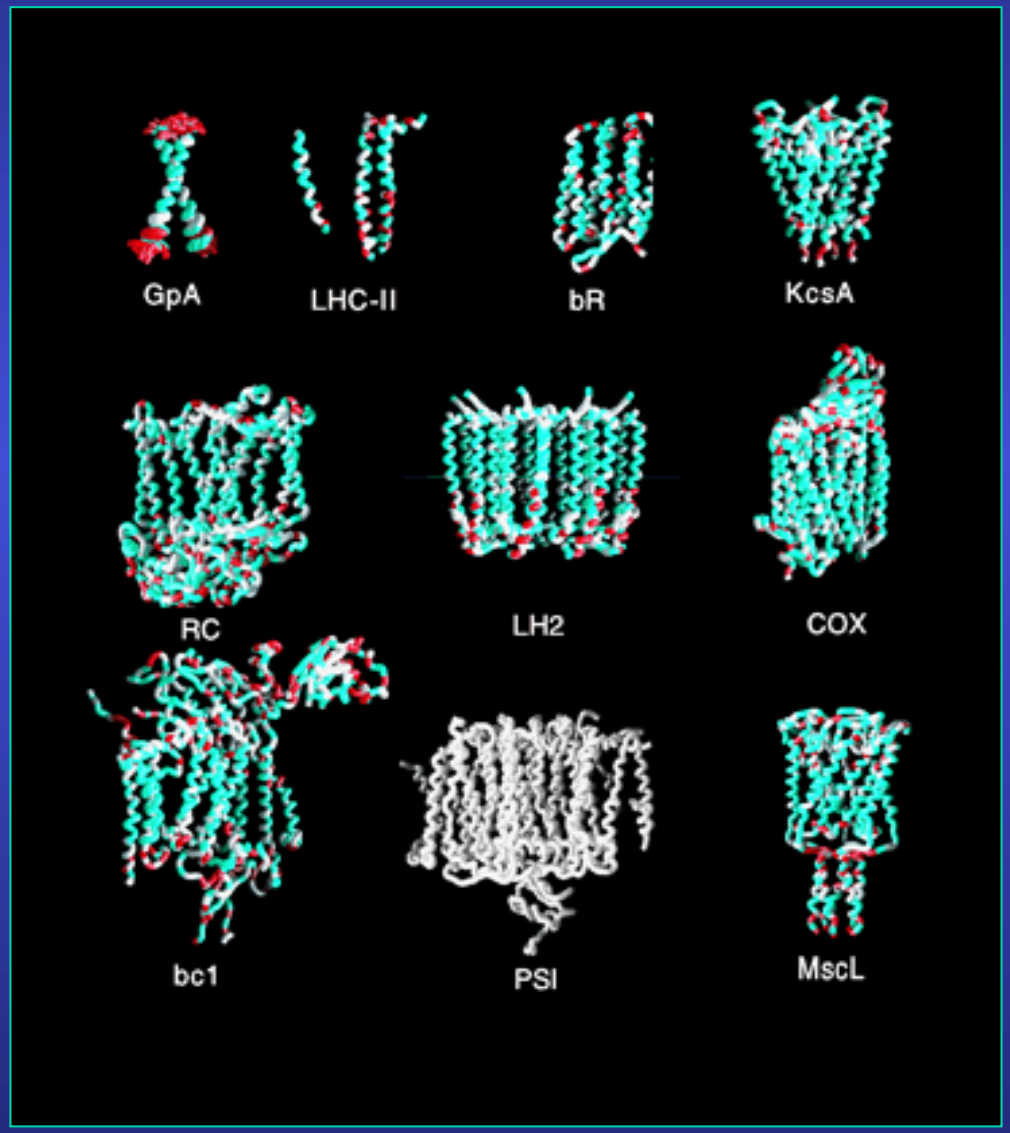
Bilayer lipid shapes match protein



Bilayer lipids are cylindrical,
So are proteins (mostly)

Detergent micelles have more spherical geometry

Mismatch

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Hydrophobic dimensions do not match (usually)

Detergent micelles cannot be larger than two of their chains, extended.
Typically 8-12 carbons, ~~16-24 A.

Bilayers & proteins have hydrophobic dimensions of 30+ A, typically

Distortion energy from perturbation of detergent tendencies

D. M. Engelman 2009

Order profile

Bilayer chains are most ordered near the aqueous interface, and disordered near the bilayer center.

Detergent chains are most ordered at the micelle center and disordered toward the aqueous interface

Not clear how this affects stability, but a different pressure gradient would exist

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Helix-helix interaction constraints

The interaction of helices is constrained by a bilayer environment since they have limited tilt Away from the bilayer normal

Micelles impose no such constraint, creating a larger scope for misfolding interactions

Altered helix stability

Since the dimensions and order profiles of micelles differ from bilayers, individual helices may have Altered stability, for example their ends may tend to unravel

Summary

- Lipid design: forms fluid bilayers
- Segregated regions expected
- Hydrophobic TM helices are individually stable
- Helices interact by packing and H-bonds
- Non TM structures accommodated by using TMs to segregate from lipid
- A number of factors may destabilize proteins in micelles