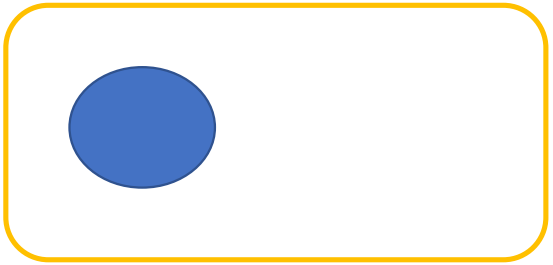
Molecular biology of the cell
BIO 207

Prof Wouter R. Karthaus PhD
EPFL-SV-ISREC

BIO207@EPFL.CH

Last week: Lipids in cell membranes

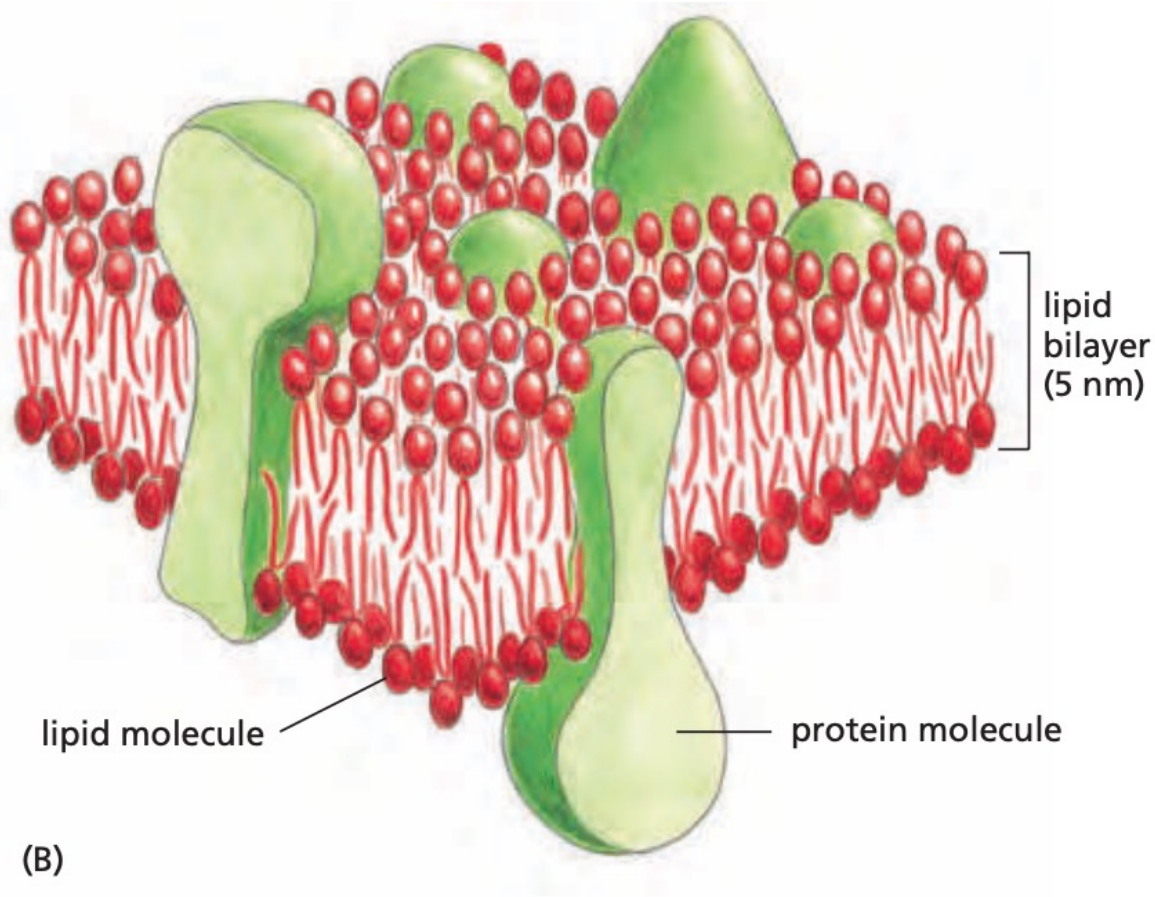


Functions of membrane lipids

Collective function:
impermeable barrier between the outside and inside of the cell - "solvent" for membrane proteins and other lipids

Semi-collective roles:
- Compartmentalization of 2D space into domains (lipid rafts) that may have specific functions (cellular interactions, signalling platforms, etc)

Individual roles
- Recognition point for other molecules (usually proteins). Ex: glycolipids towards the outside, Phosphatidylinositols towards the inside



Topic of the day

Cell membrane **<u>proteins</u>**

Plasma membrane (Outside of the cell)

Membranes of organelles
Golgi complex
Endoplasmic Reticulum
Nucleus
Mitochondrion

CHAPTER
10

Membrane Structure

Proteins in the cell membrane have many functions

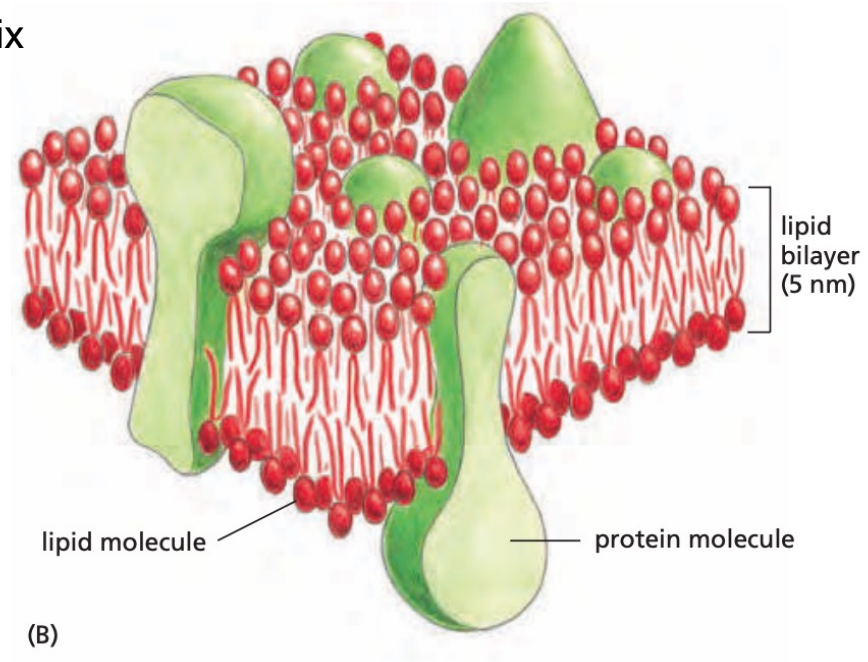
Including but not limited to:

- Transport of molecules across the cell membranes
- Adhesion. Either to neighbouring cells or to the extracellular matrix
- Communication with the outside milieu and other cells
    - Receptors for circulating chemical "signals"

  Growth factors, Insulin
- Creation of force
- Synthesis of ATP
- And many more!

Membrane prostate are a common target of drugs:
There's great interest in medicine and pharmacy

What are the structures and How do membrane proteins function?



The lipid bilayer, a recap

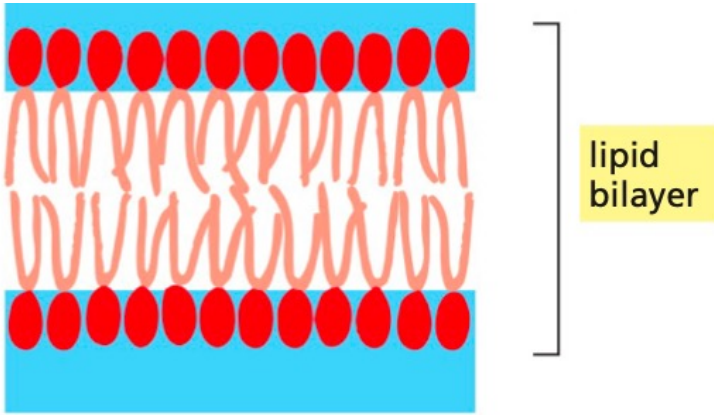
Outside of the cell

The plasma membrane
~ 5 nanometer across

2 types of molecules

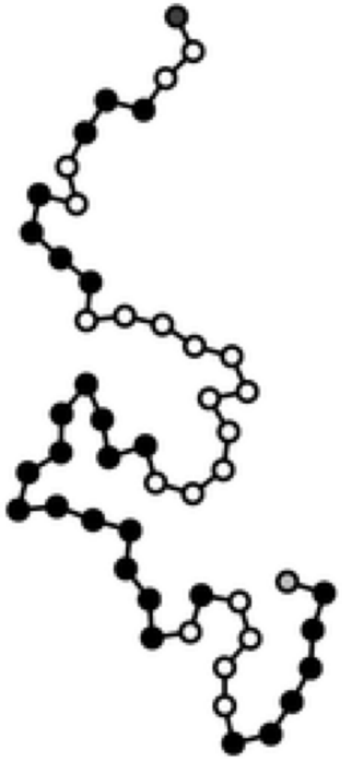
1: Lipids
2: Proteins

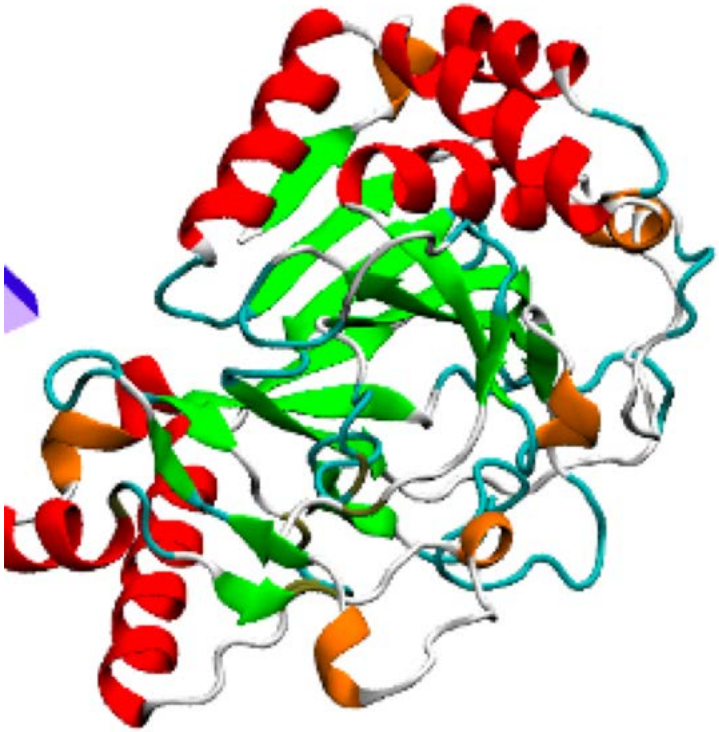
Inside of the cell



How do proteins interact with the cell membrane?

Back to the basics: Proteins are polymers (chains) of amino acids



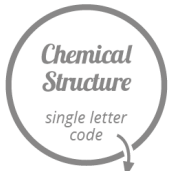


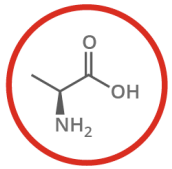
For a protein to function it needs to be folded correctly!

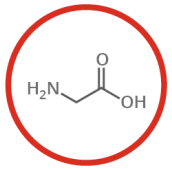
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

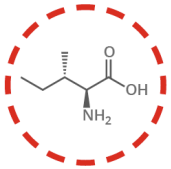
AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

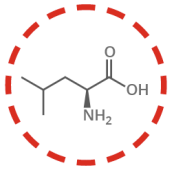
Chart Key: ALIPHATIC · AROMATIC · ACIDIC · BASIC · HYDROXYLIC · SULFUR-CONTAINING · AMIDIC · NON-ESSENTIAL · ESSENTIAL

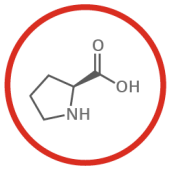


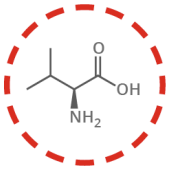


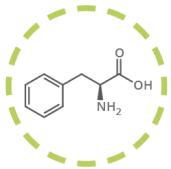


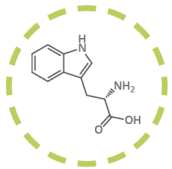


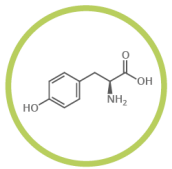


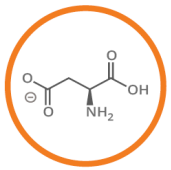


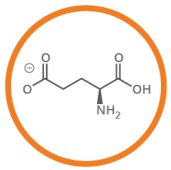


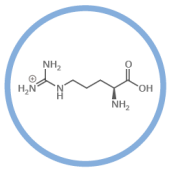


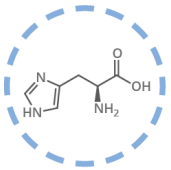


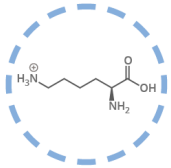


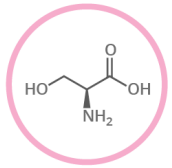


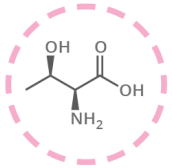


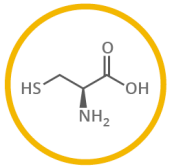


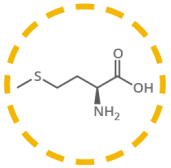


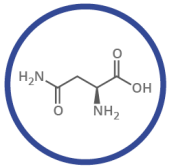


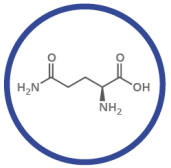








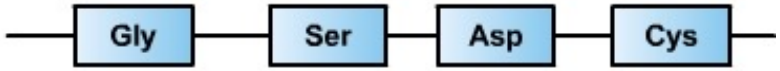




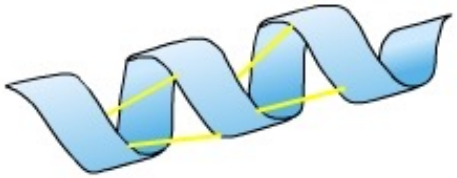
Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

Back to the basics: Proteins structure

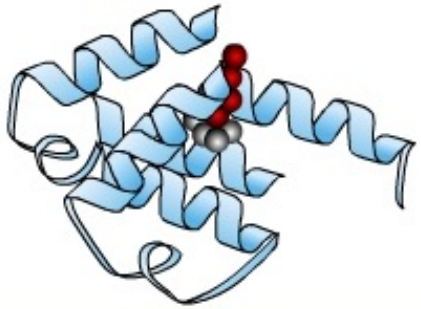
Primary Structure:



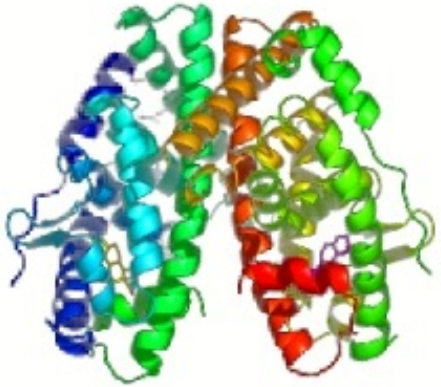
Secondary Structure:



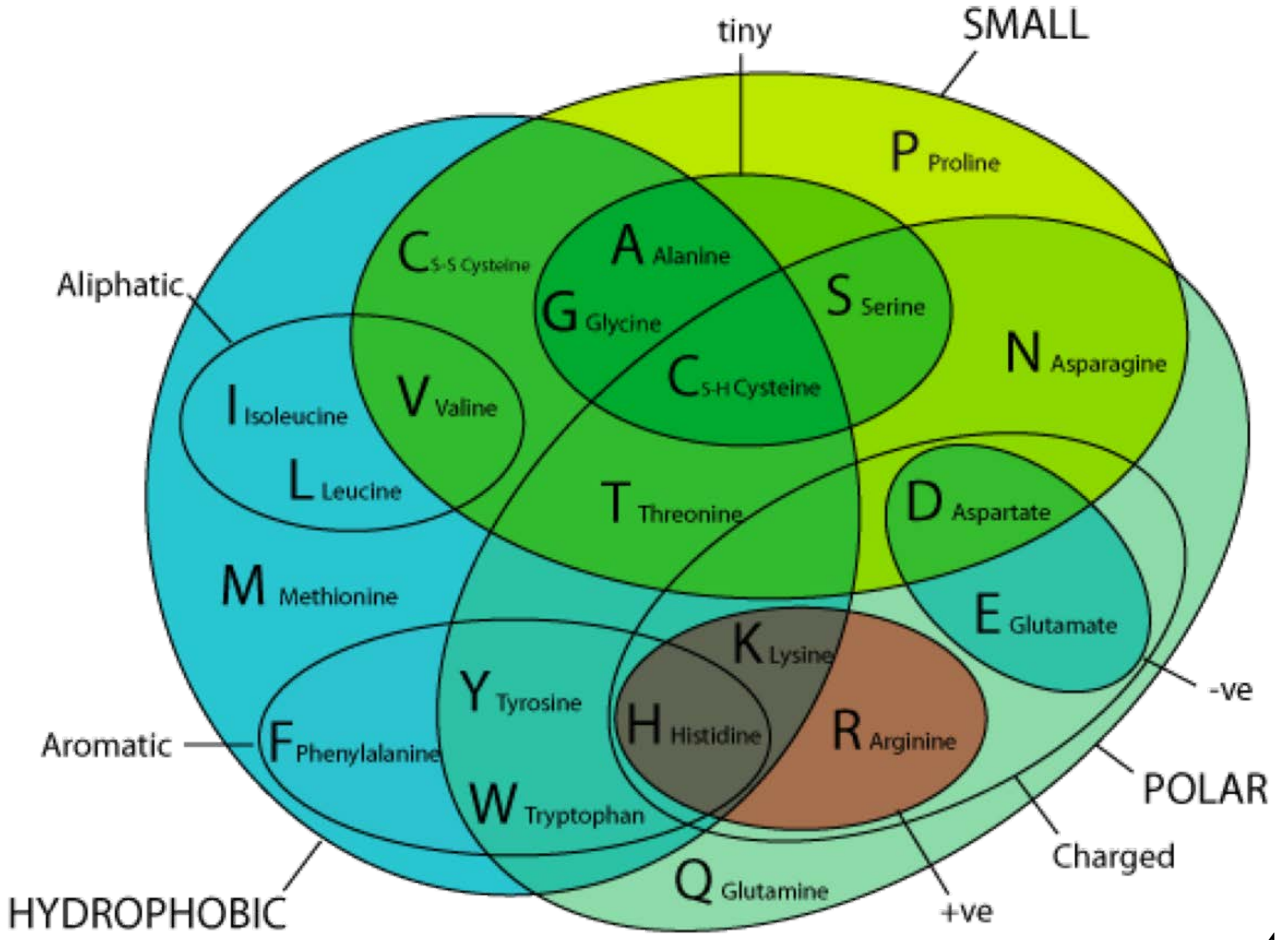
Tertiary Structure:



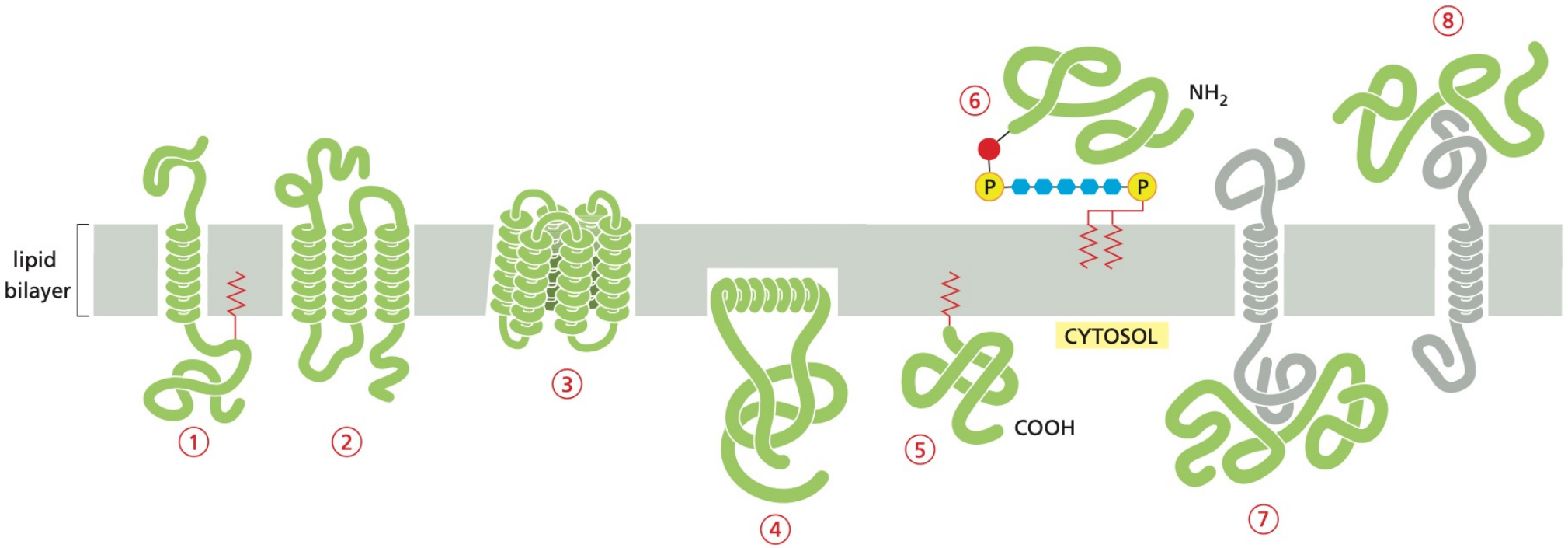
Quaternary Structure:



Amino Acids


tiny
SMALL
P Proline
C S-S Cysteine
A Alanine
S Serine
G Glycine
N Asparagine
C S-H Cysteine
Aliphatic
I Isoleucine
V Valine
L Leucine
T Threonine
D Aspartate
M Methionine
E Glutamate
K Lysine
-ve
Y Tyrosine
H Histidine
R Arginine
Aromatic
F Phenylalanine
POLAR
W Tryptophan
Charged
Q Glutamine
+ve
HYDROPHOBIC

Several ways a protein can interact with the membrane



1: Single pass alpha helix
2: Multipass alpha helix
3: Beta-Barrel
4: Alpha helix partioned in the cytosolic monolayer of the lipid bilayer
5: Covalently linked to a lipid
6: Anchored by GPI to the outside
7/8: non covalent binding to another protein

What are the molecular features of proteins crossing the cell membrane?

The amphipilic nature of the lipid bilayer

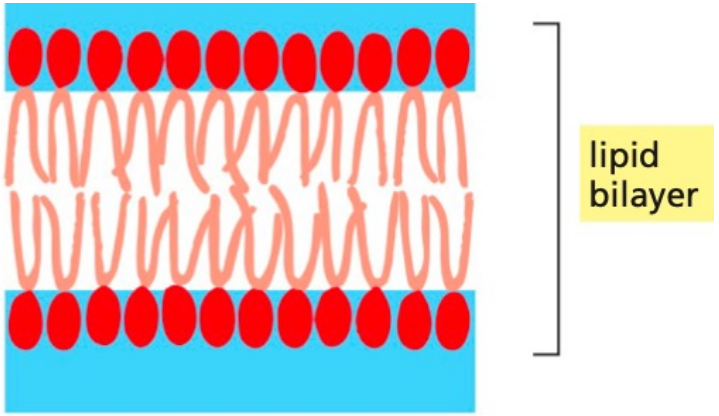
Outside of the cell

The plasma membrane
~ 5 nanometer across

Inside of the cell

2 types of molecules

1: Lipids
2: Proteins



How do proteins integrate into the mebrane?
What would be their features?

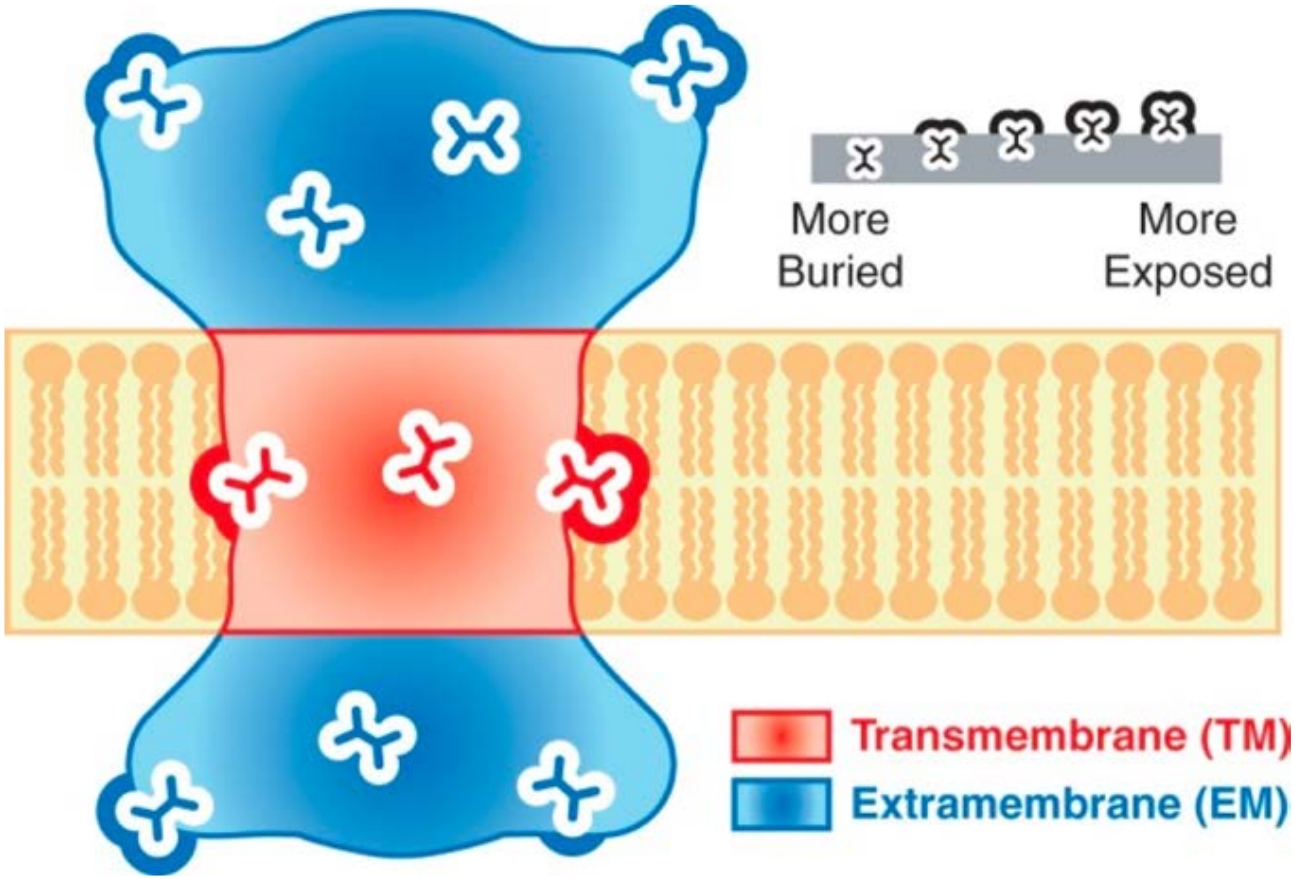
Proteins passing the cell membrane are amphipilic

There are specific **domains** in the protein that have differenct functions

Hydrophilic

Hydrophobic

Hydrophilic

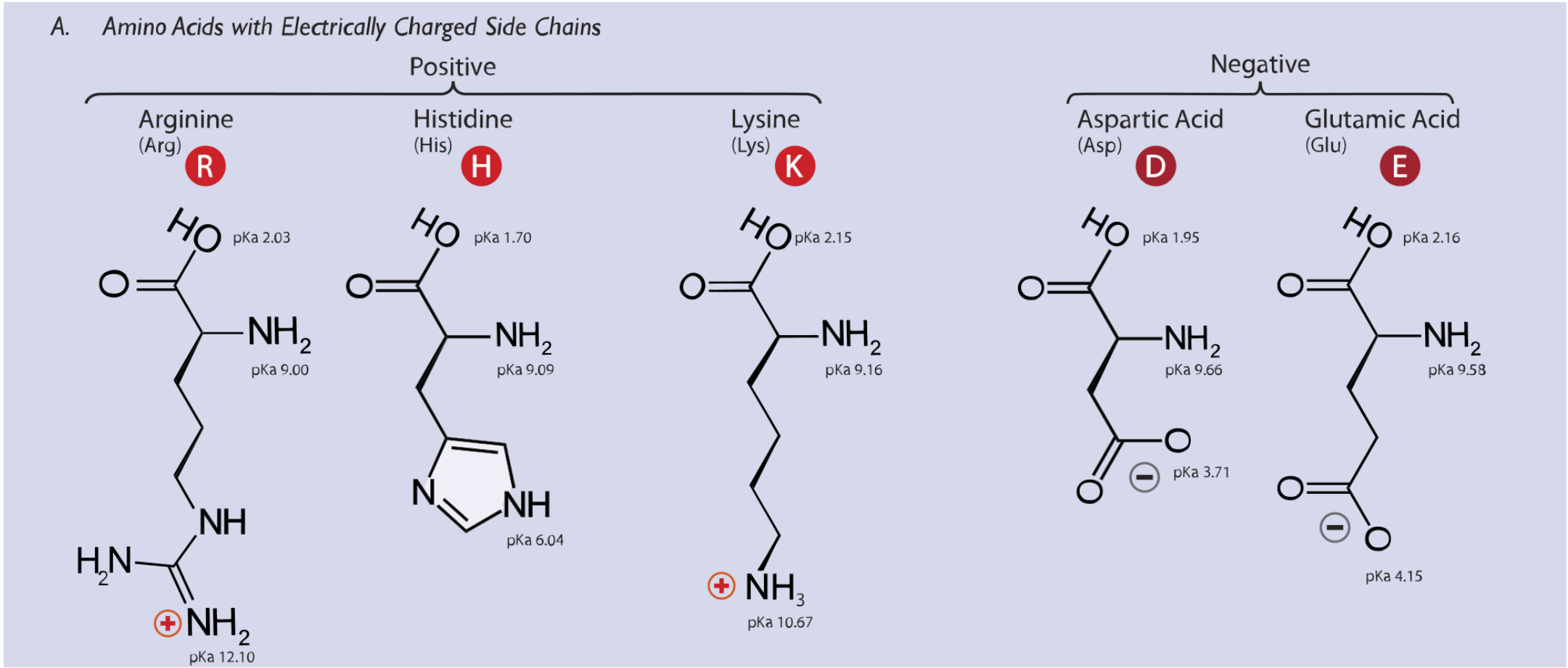


Hydrophobic

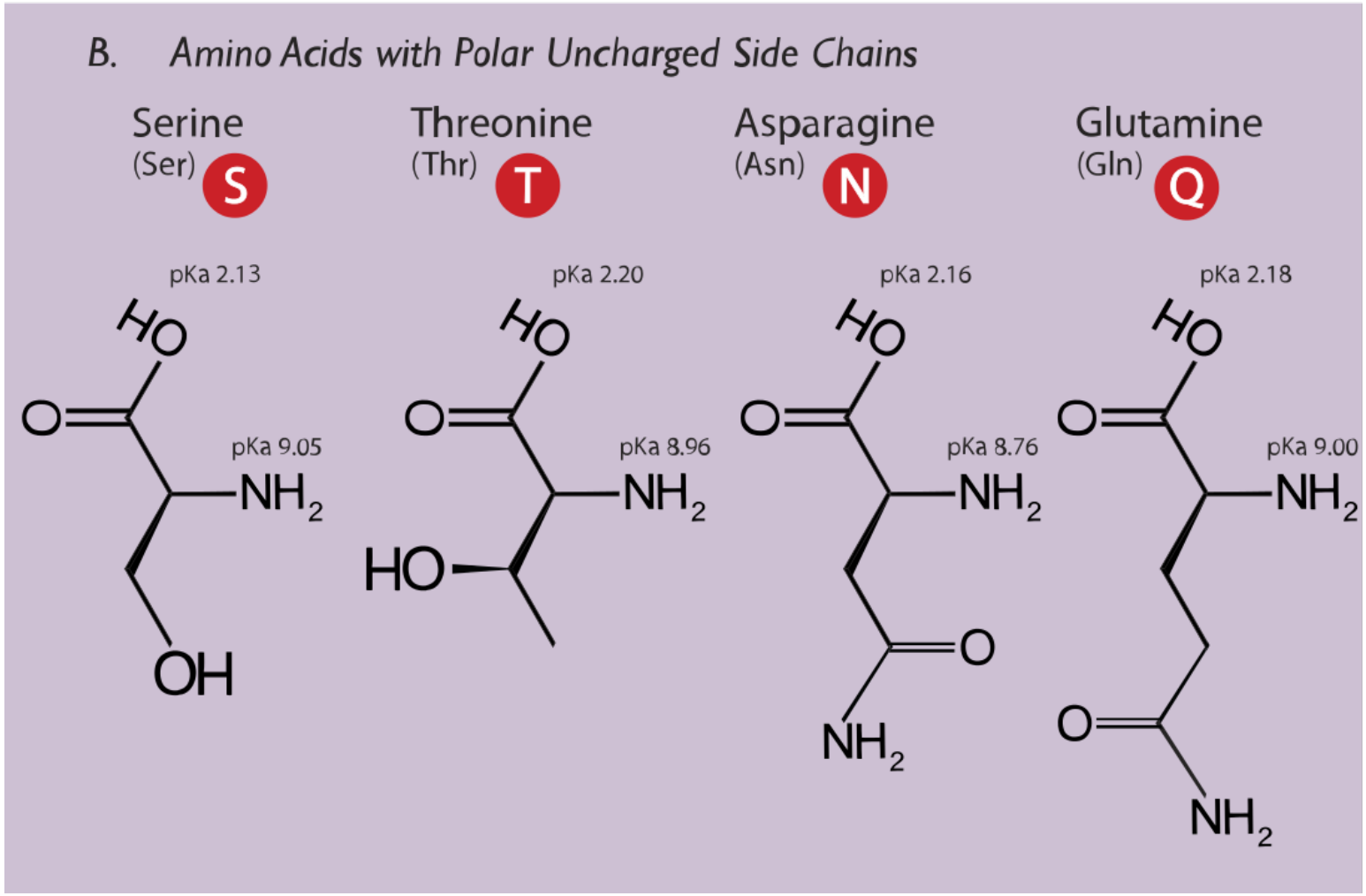
Hydrophilic

How do these membrane spanning proteins get their amphiphilic nature?

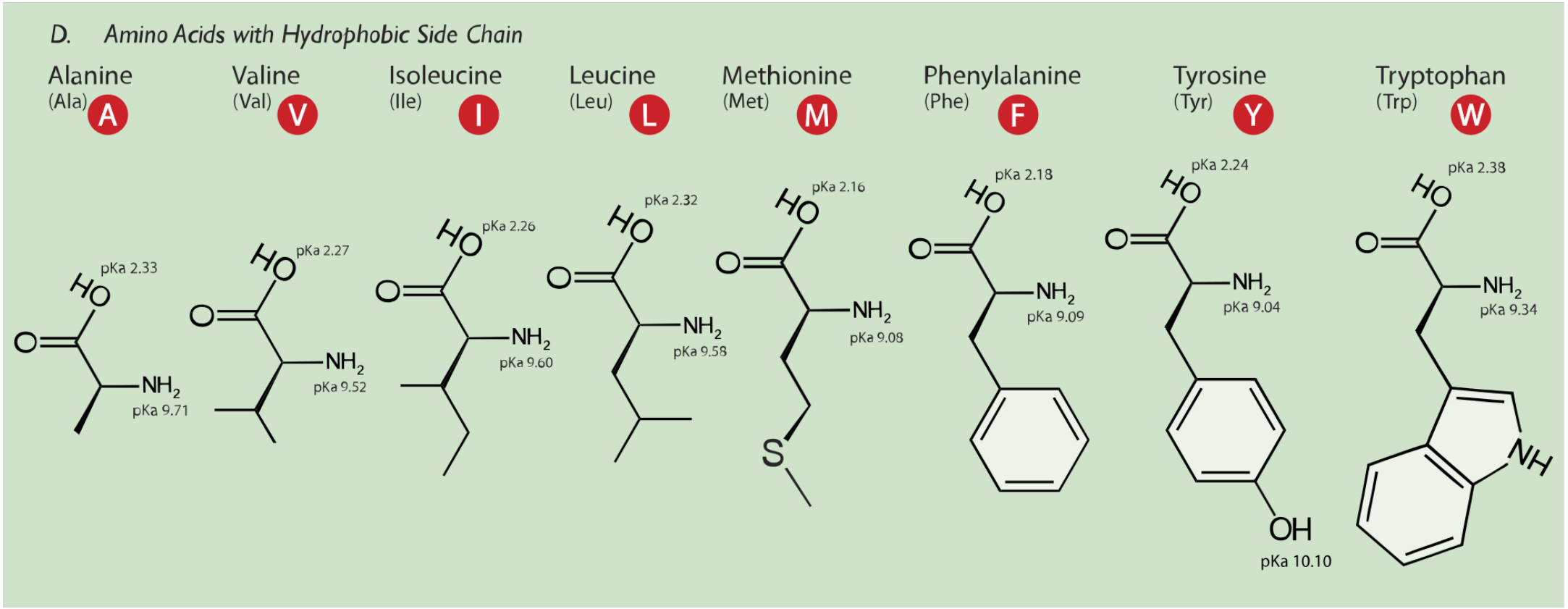
Amino acids with hydrophilic side chains



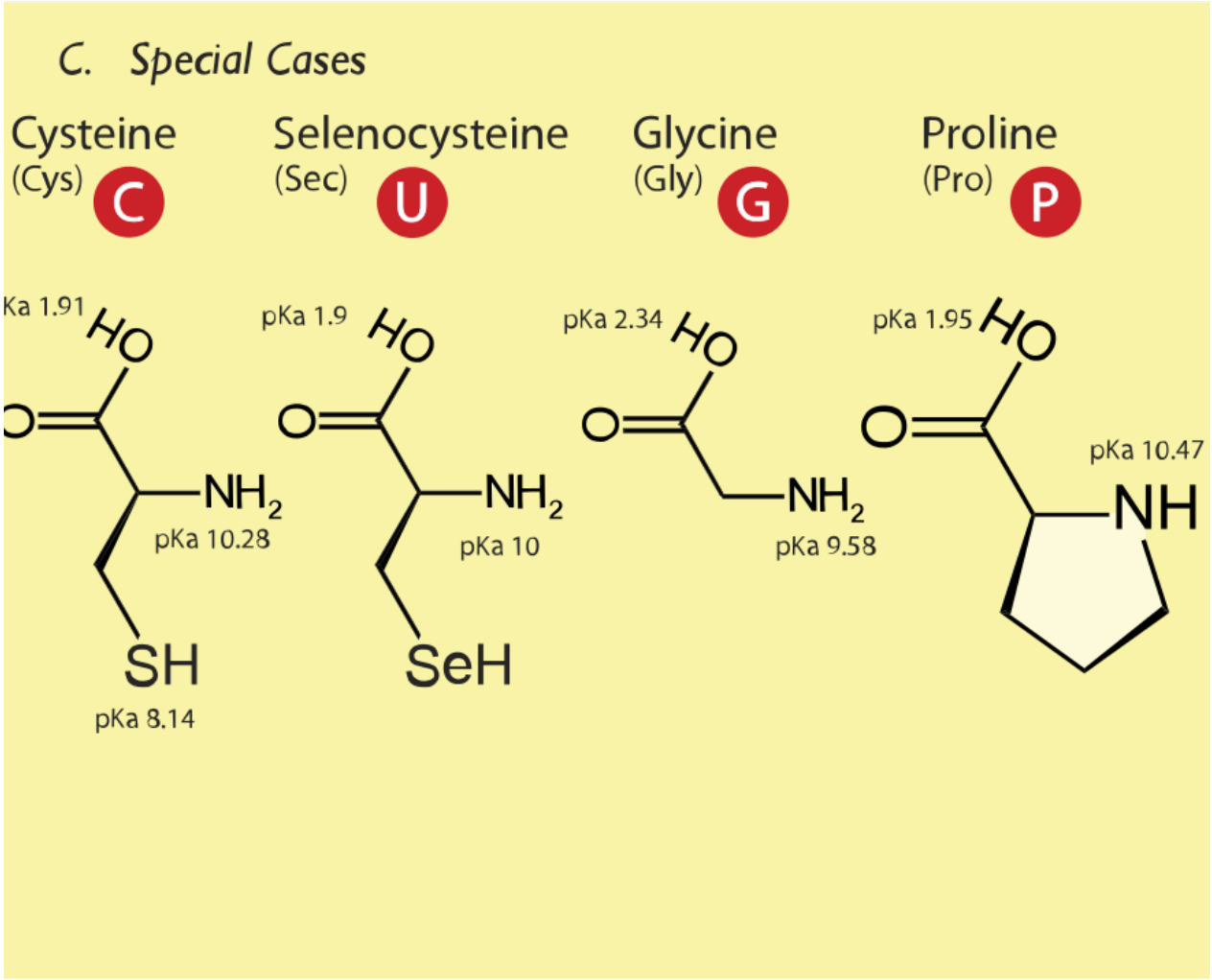
Amino acids with hydrophilic side chains



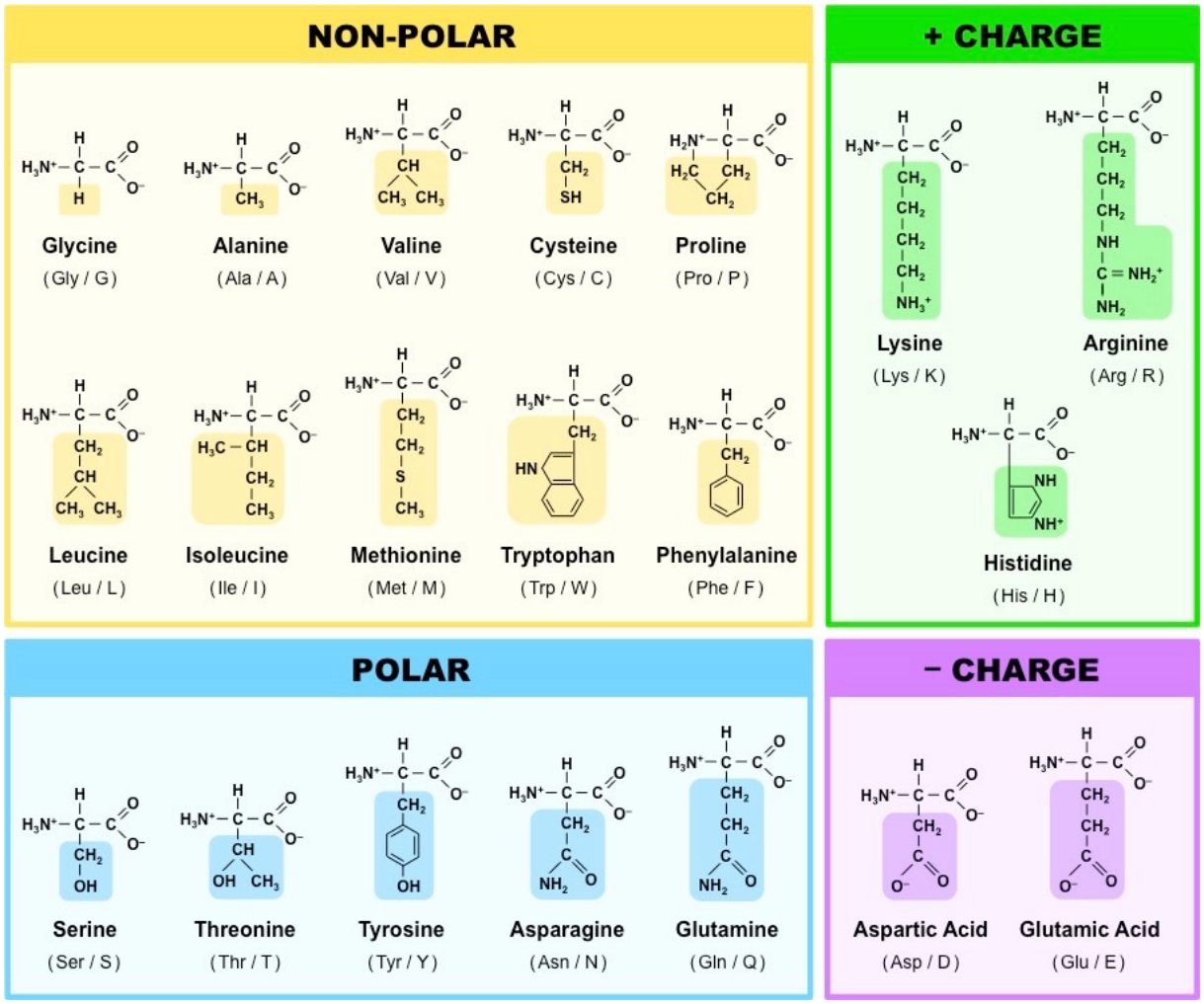
Amino acids with hydrophobic side chains

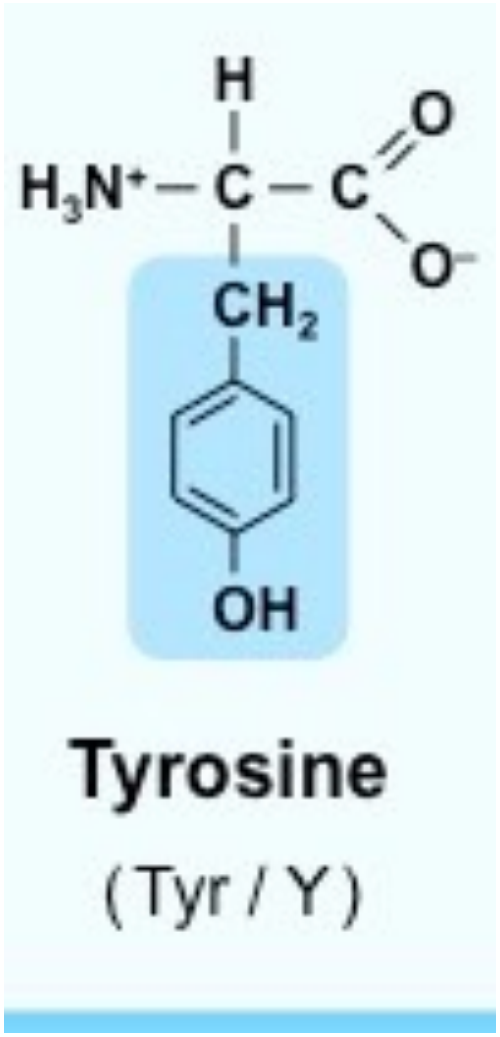


Amino acids with special case side chains



Polarity vs hydrophobicity





Benzene = hydrophobic

OH = Polar

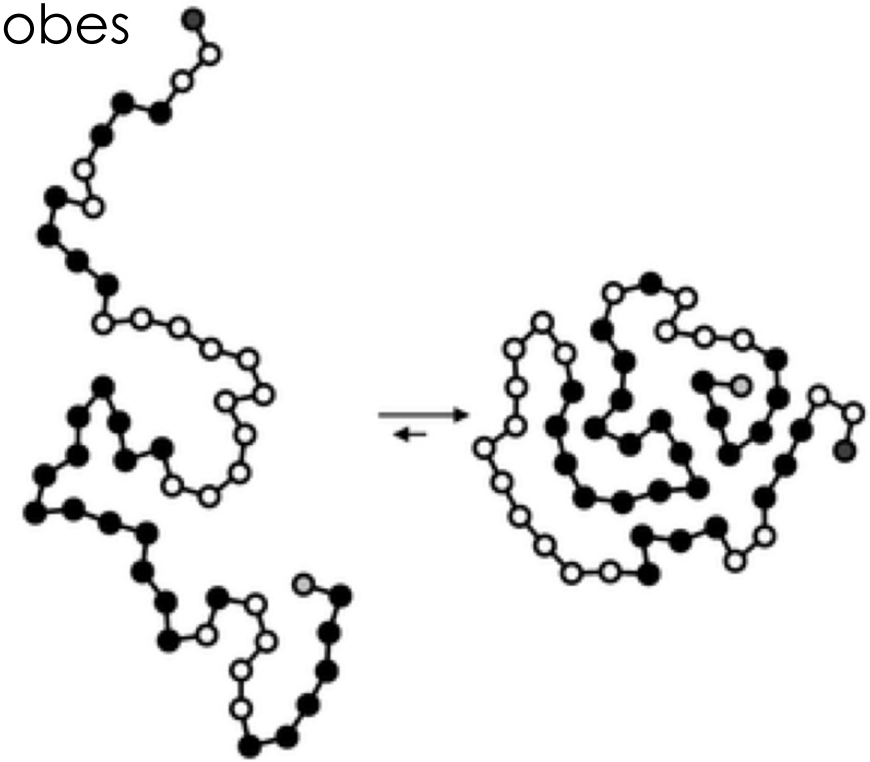
Based on their features AA get a hydrophobicity score

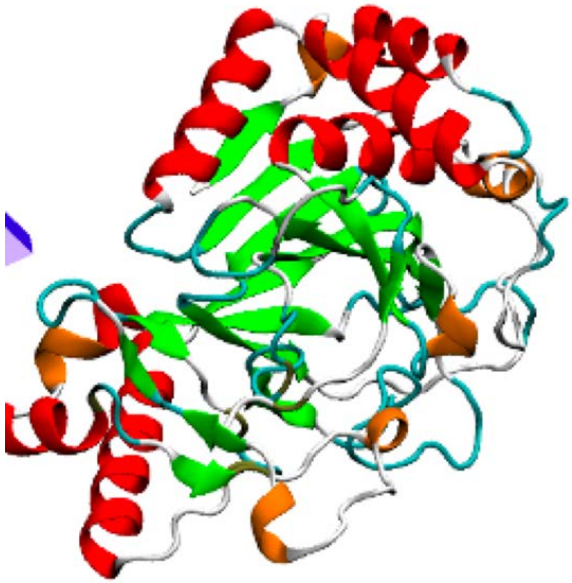
Partitioning between two non-miscible organic solvents, calculation of the transfer free energy

Amino Acid	3-Letter	1-Letter	Hydrophobicity / Hydropathy index[1]	polarity	acidity (pH)
Alanine	Ala	A	1.8	nonpolar	neutral
Arginine	Arg	R	-4.5	polar	basic (strongly)
Asparagine	Asn	N	-3.5	polar	neutral
Aspartate (aspartic acid)	Asp	D	-3.5	polar	acidic
Cysteine	Cys	C	2.5	polar	neutral
Glutamate (glutamic acid)	Glu	E	-3.5	polar	acidic
Glutamine	Gln	Q	-3.5	polar	neutral
Glycine	Gly	G	-0.4	nonpolar	neutral
Histidine	His	H	-3.2	polar	basic (weakly)
Isoleucine	Ile	I	4.5	nonpolar	neutral
Leucine	Leu	L	3.8	nonpolar	neutral
Lysine	Lys	K	-3.9	polar	basic
Methionine	Met	M	1.9	nonpolar	neutral
Phenylalanine	Phe	F	2.8	nonpolar	neutral
Proline	Pro	P	-1.6	nonpolar	neutral
Serine	Ser	S	-0.8	polar	neutral
Threonine	Thr	T	-0.7	polar	neutral
Tryptophan	Trp	W	-0.9	nonpolar	neutral
Tyrosine	Tyr	Y	-1.3	polar	neutral
Valine	Val	V	4.2	nonpolar	neutral

Hydrophobic non polair side chains move away from water

- hydrophobes

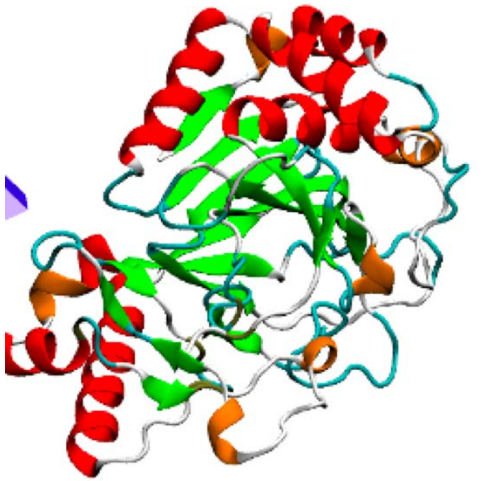




Two essential processes for correct folding of a protein soluble in water

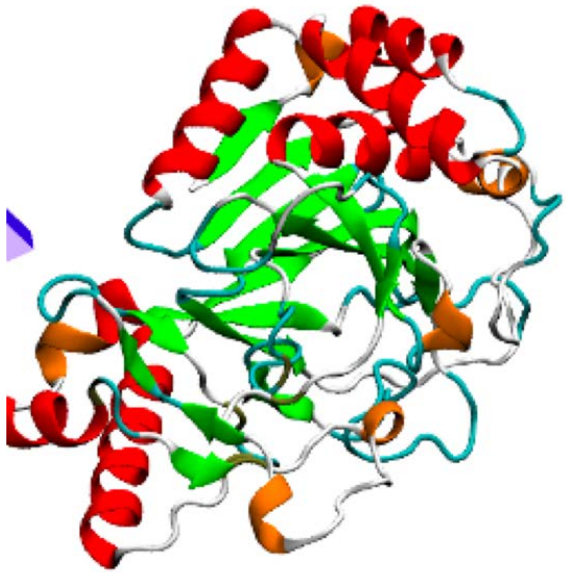
Establishment of secondary structures alpha helices and beta sheets

“Collapse” of the exposed hydrophobic surfaces

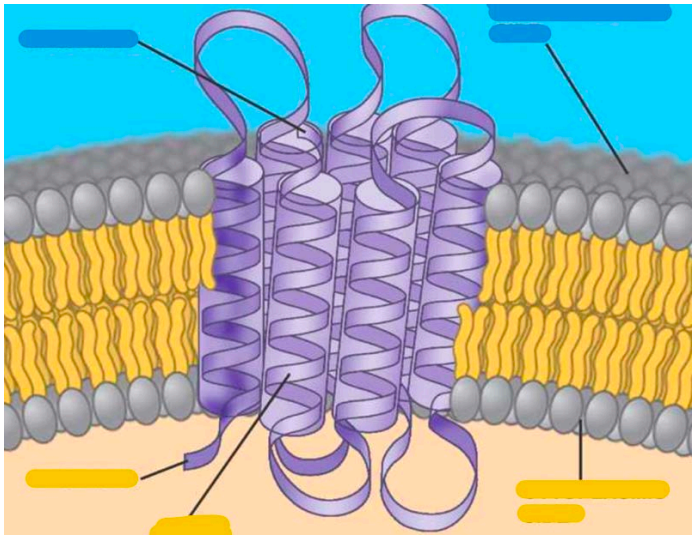


How do secondary protein structures interact with the cell membrane?

Transmembrane proteins



In a water soluble protein the outward facing surface is hydrophilic

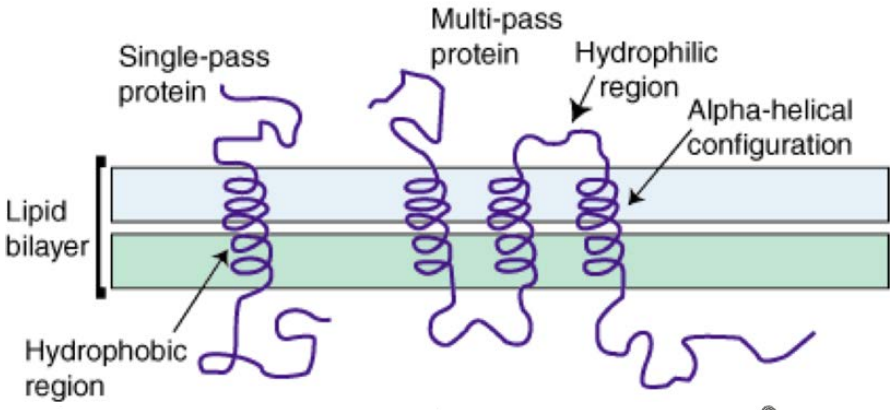


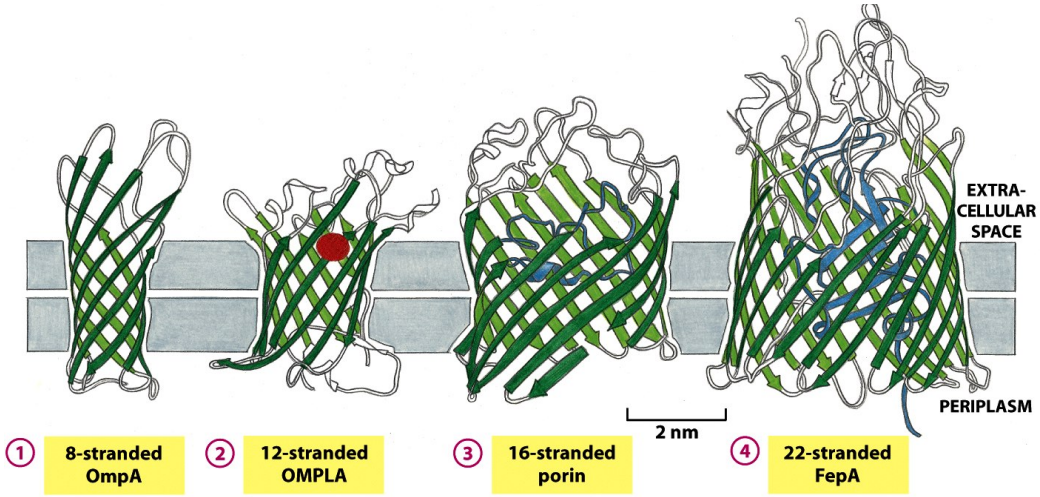
In a transmembrane protein the outward facing surface is hydrophobic, allowing interacting with the fatty acid tails of phospholipids

Transmembrane proteins

Two solutions: The transmembrane portion of the proteins is either formed by alpha helices or by ß-strands

There are no hybrid (alpha + B) solutions!



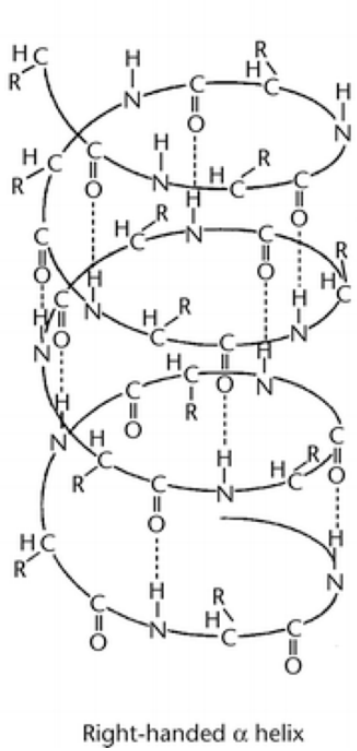


The number of alpha helices varies from 1 to ≈20

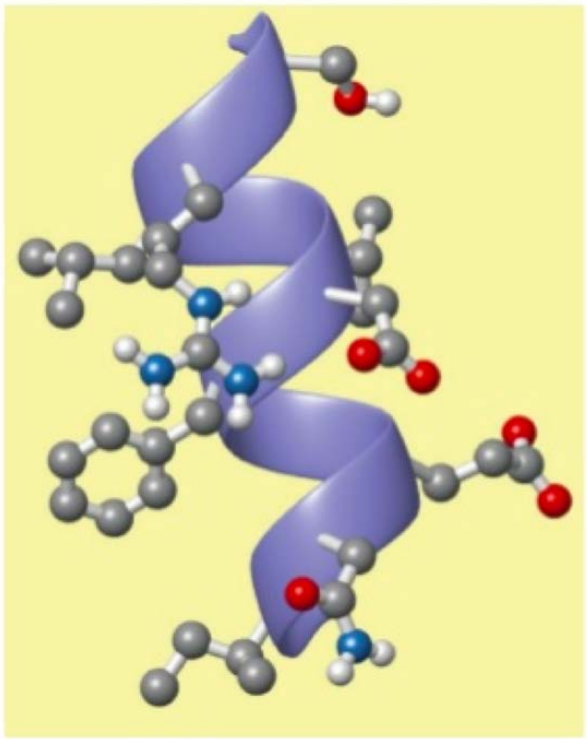
In mammals, the vast majority are alpha helices

The number of ß-strands in a **ß-barrel** can vary from 8 to ≈200

Transmembrane proteins: Alpha Helix

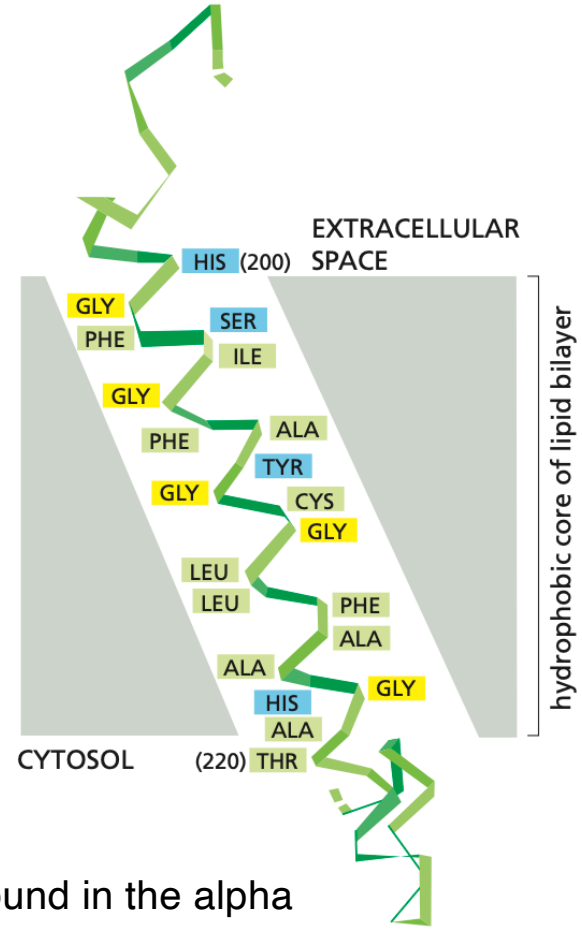


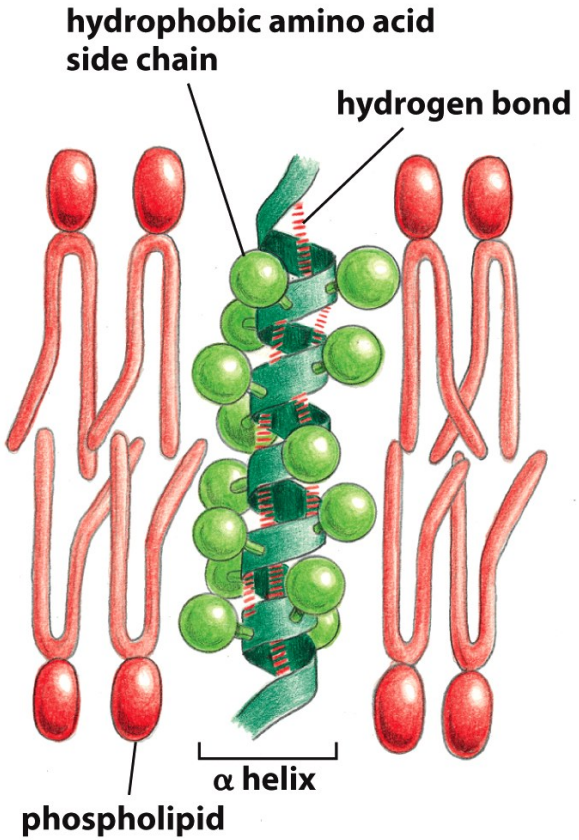
H-bonds within the alpha chain



The R-groups of the amino acids are exposed to the outside

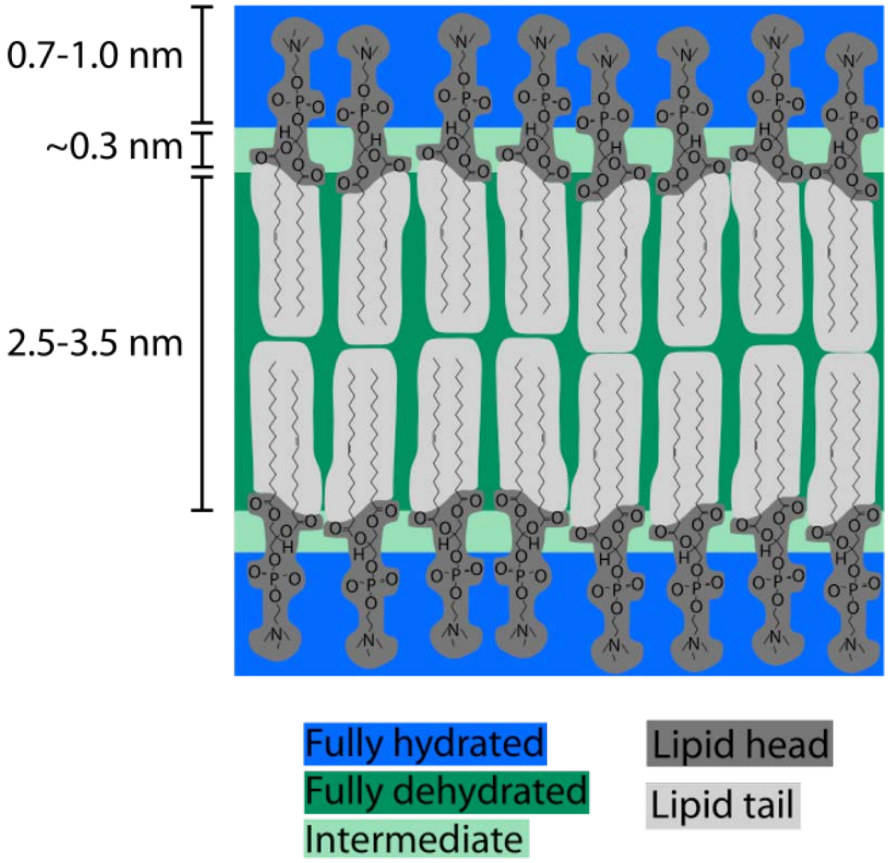
Transmembrane proteins: Alpha Helix



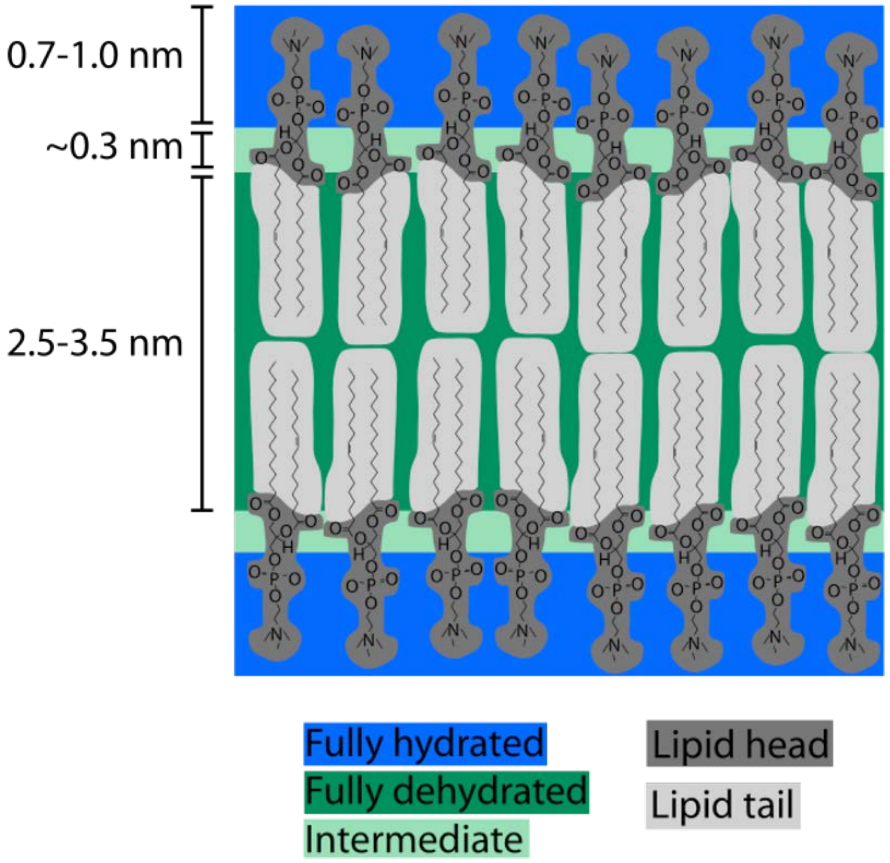


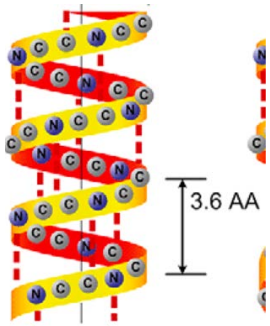
Majority of a.a. found in the alpha helix have hydrophobic side chains

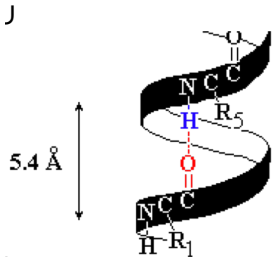
How long is an alpha helix spanning the membrane?



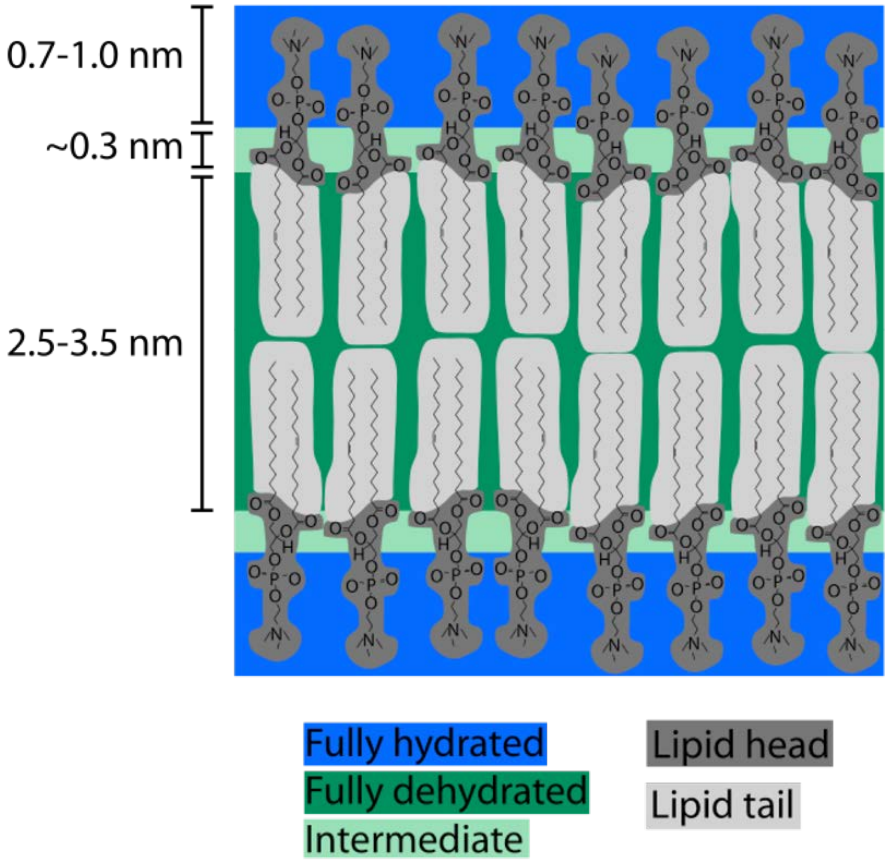
How long is an alpha helix spanning the membrane?

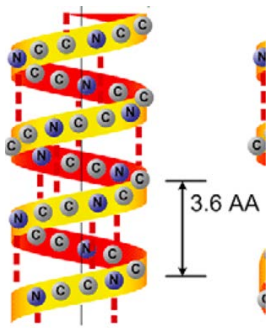


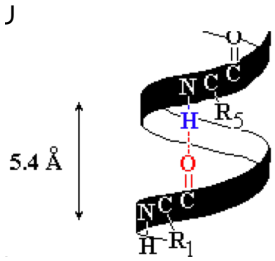




How long is an alpha helix spanning the membrane?





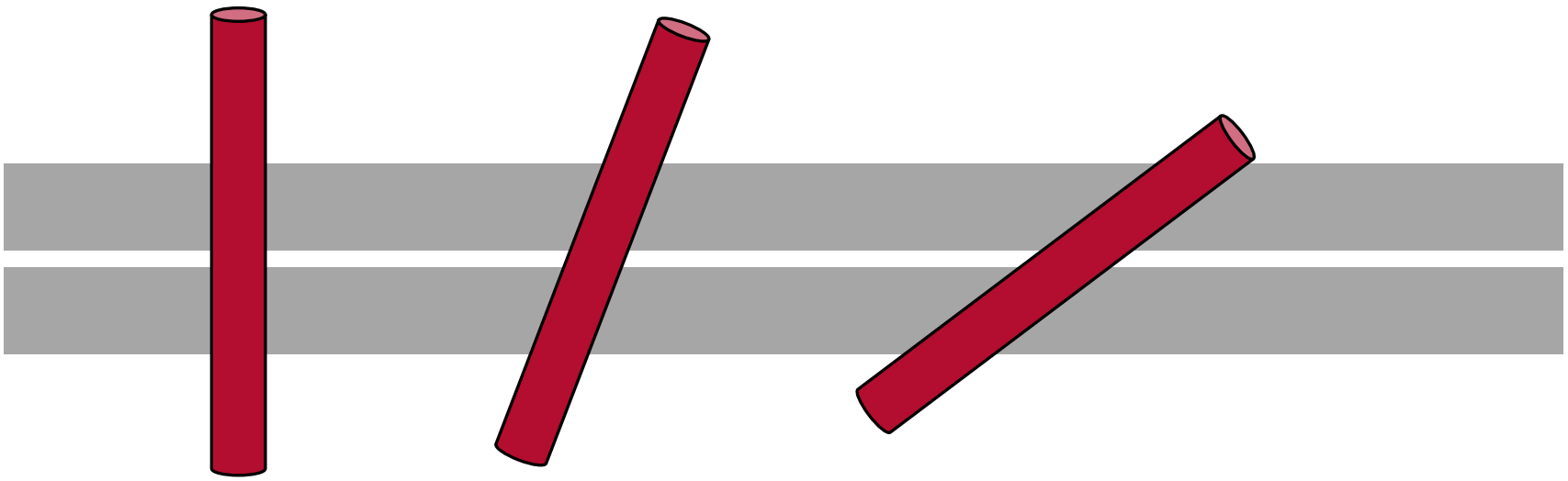


Alpha helix: 3.6 residues per turn 5.4 Å per turn

So displacement along the helix for 1 residue: 1.5 Å (0.15 nm) ≈ 20 a.a. to cross a membrane perpendicular to the membrane plane

But this will depend on the thickness of the membrane (which depends on its composition, temperature, ...), on the orientation of the helix

Varying lengths of the Alpha Helix



20 a.a. 26 a.a. 39 a.a.

Angle and membrane thickness

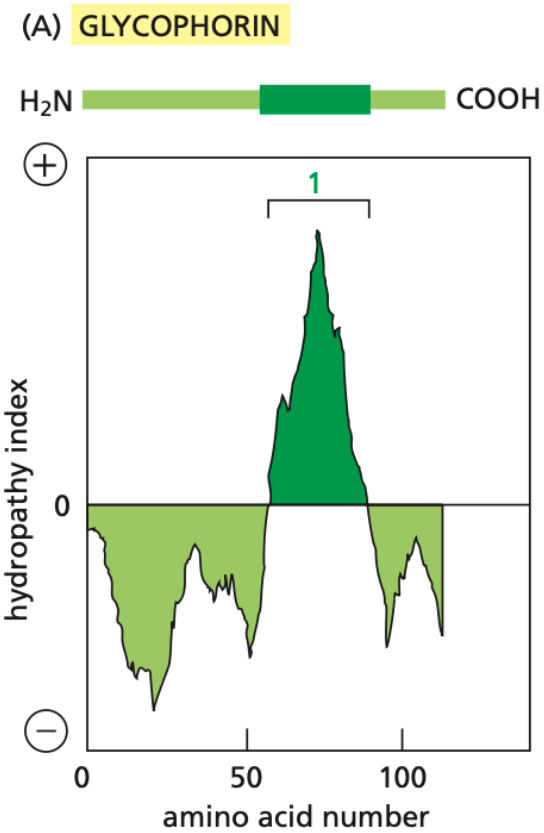
Based on the knowledge we have, can we predict of a protein is a transmembrane protein?

Using the hydropathy score

Use of bioinformatics to predict whether a protein is transmembrane and identify the sequence that is in the membrane

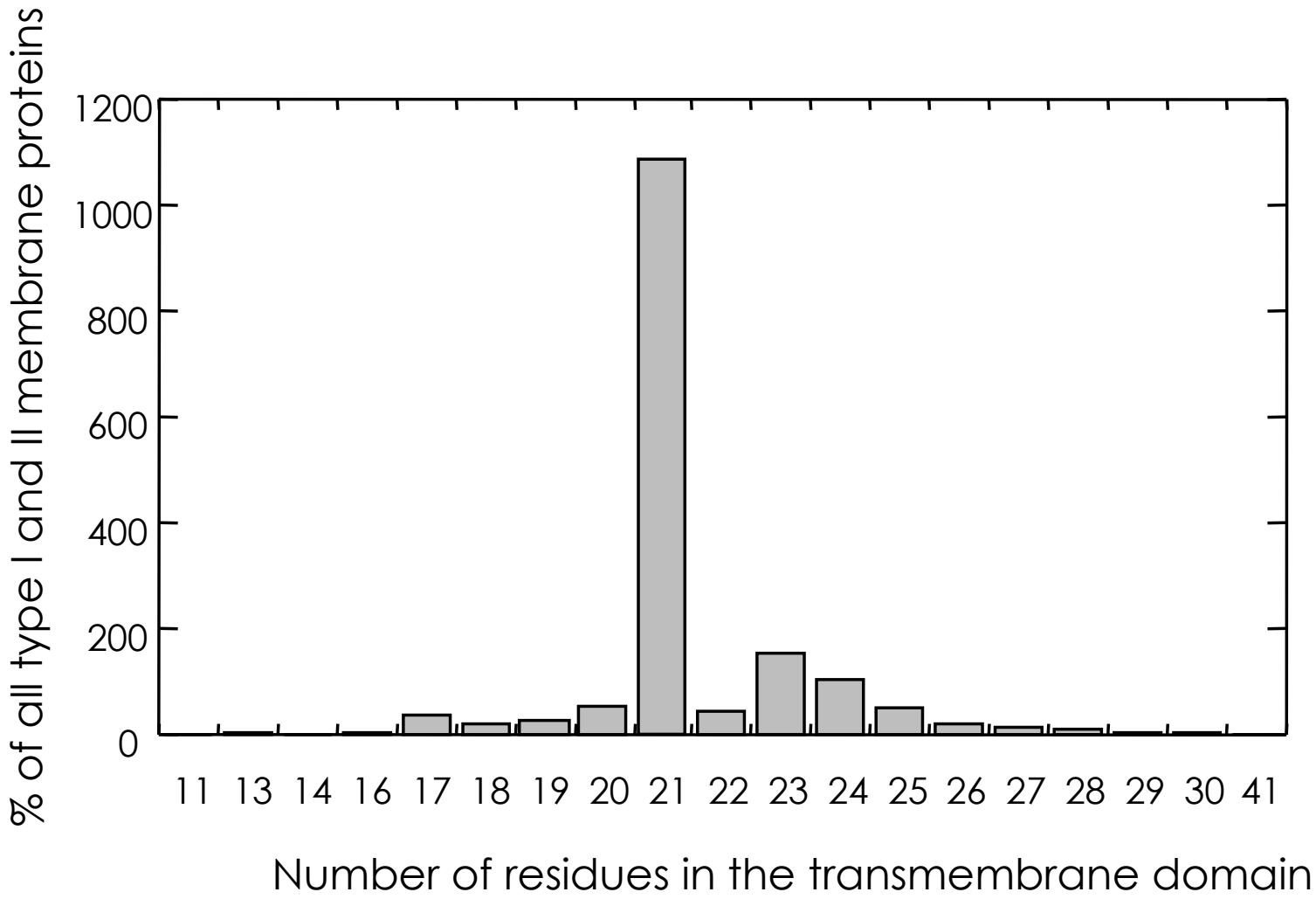
MVAERSPARS PGSWLFPGLW LLVLSGPGGL LRAQEQPSCR RAFDLYFVLD

MVAERSPARS PGSWLFPGLW LLVLSGPGGL LRAQEQPSCR RAFDLYFVLD



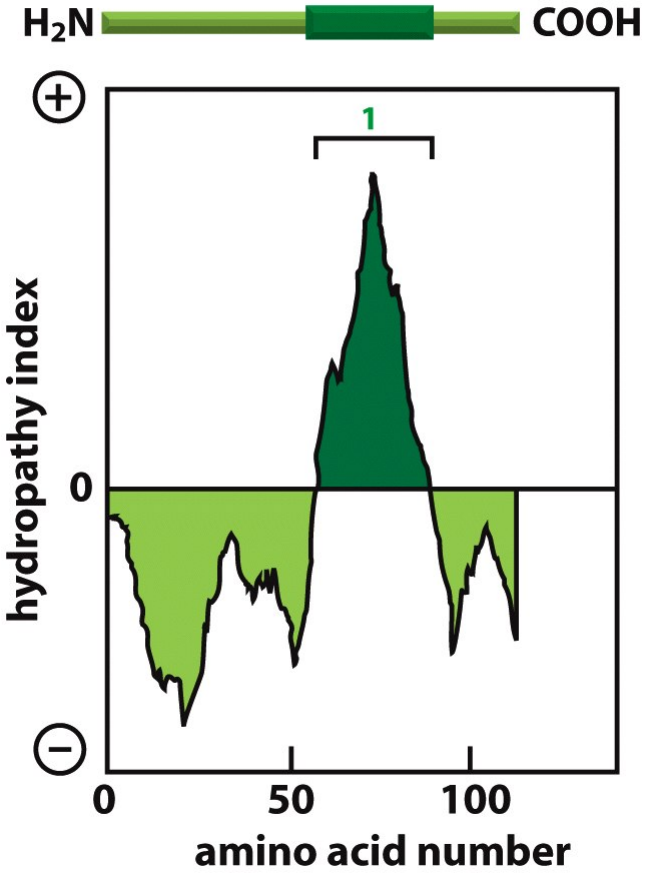
What would be a sensisble number to use as a window size?

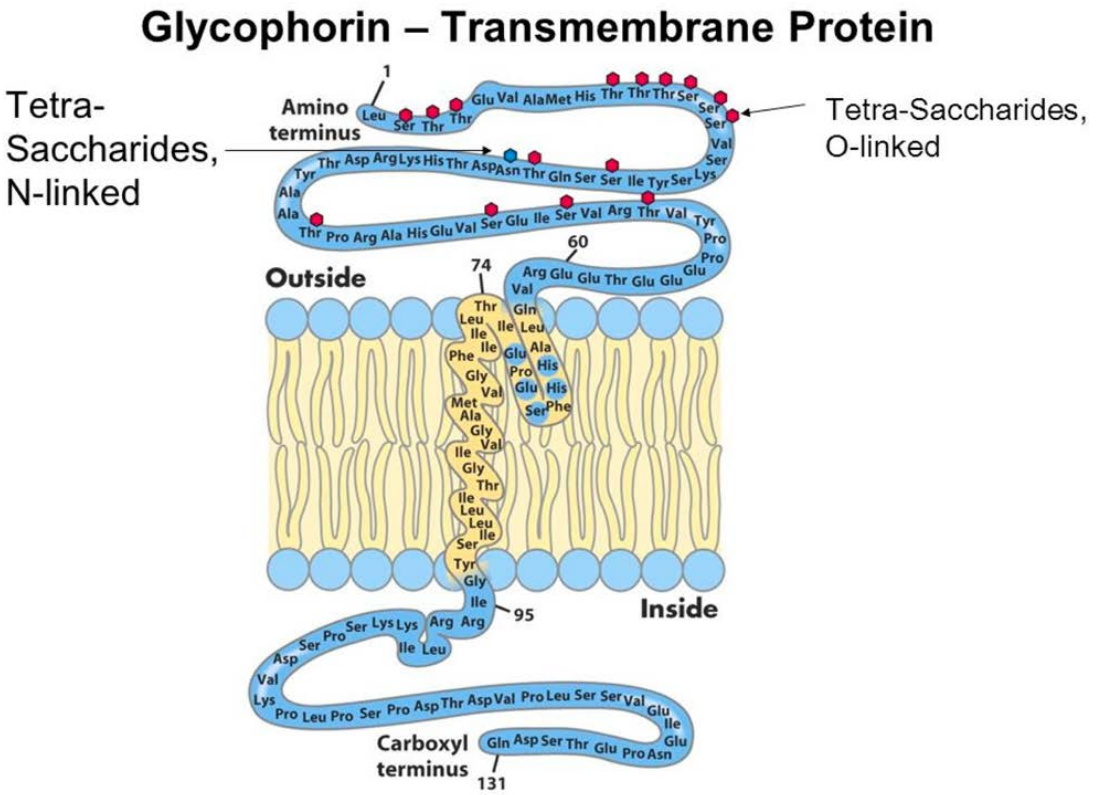
https://www.youtube.com/watch?v=frVoetPCMWY


% of all type I and II membrane proteins
1200
1000
800
600
400
200
0
11 13 14 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 41
Number of residues in the transmembrane domain

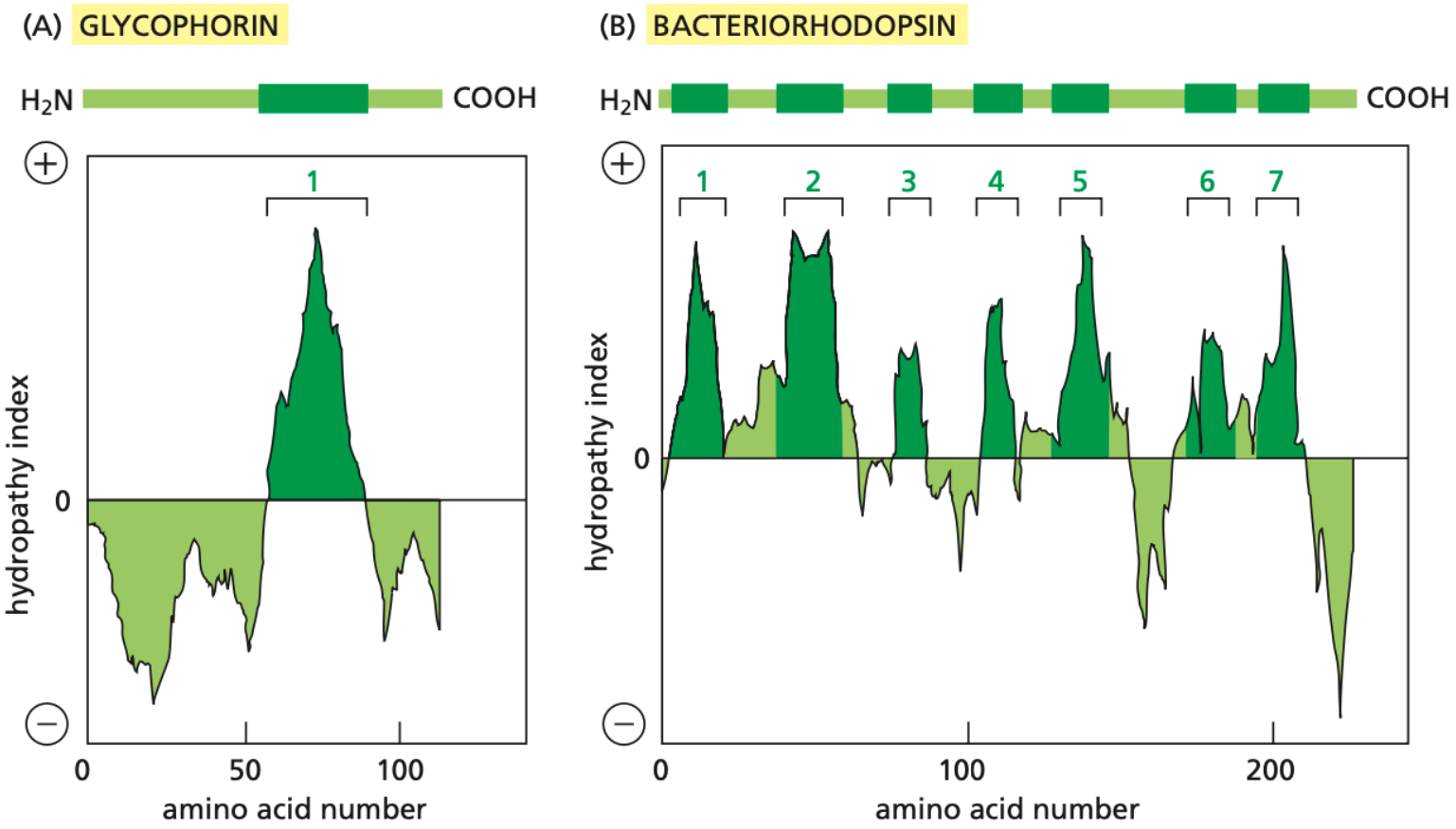
Prediction & Reality

GLYCOPHORIN

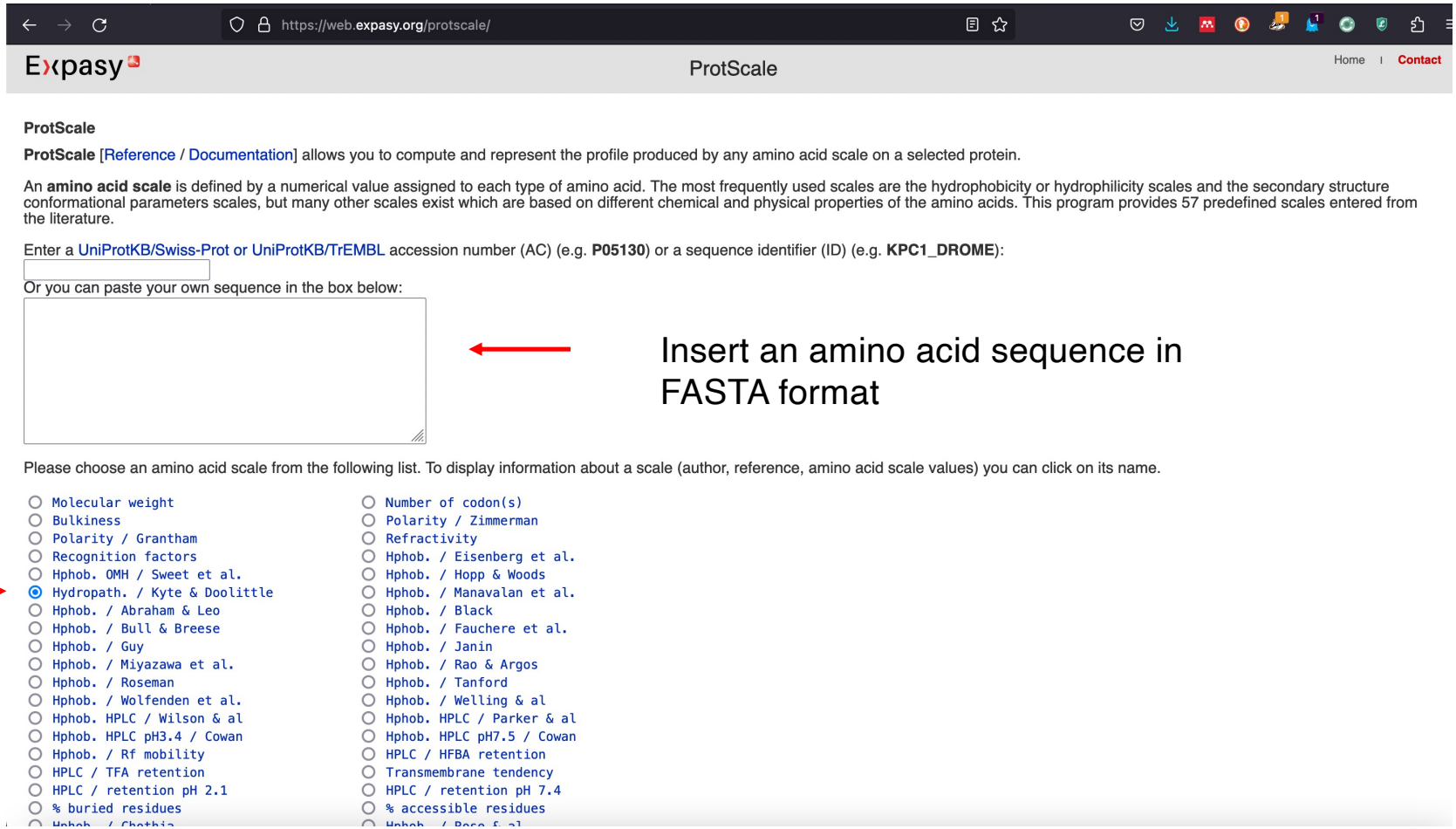




Single pass versus multipass membrane proteins



With a little help we can predict transmembrane proteins ourselves



membrane proteins across the tree of life

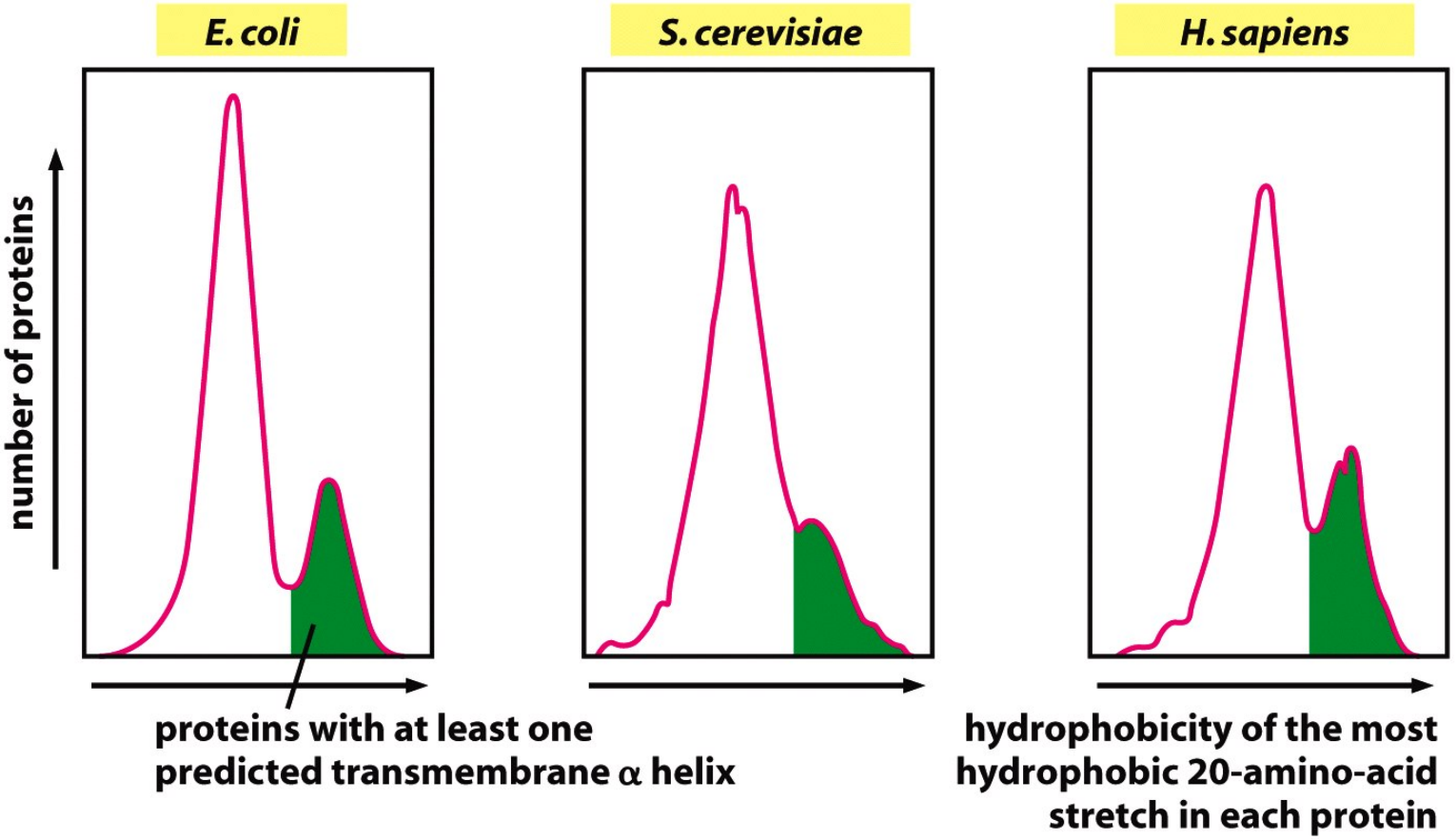
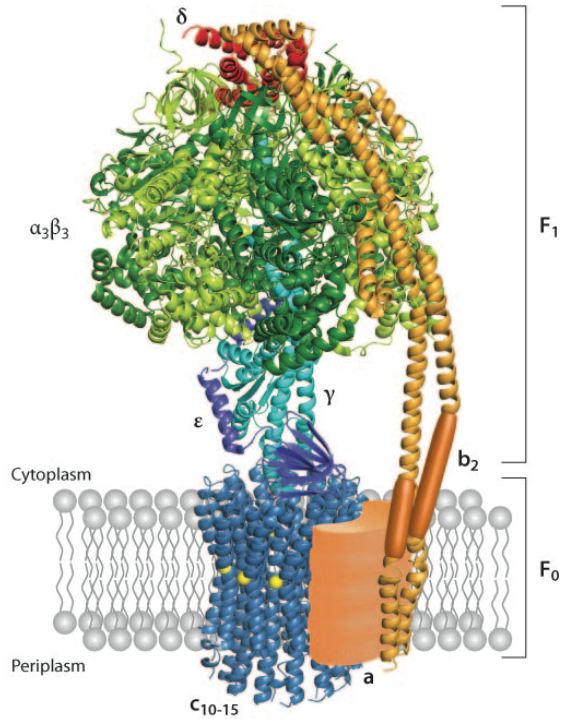


Figure 10-22c Molecular Biology of the Cell (© Garland Science 2008)

membrane proteins

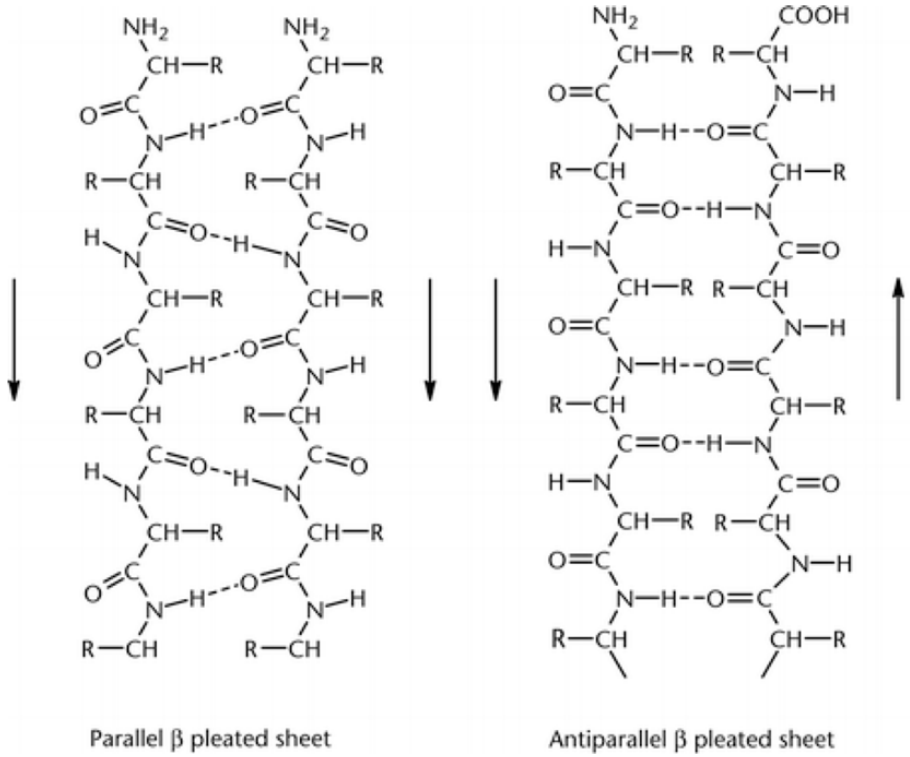


von Ballmoos C, et al. 2009.
Annu. Rev. Biochem. 78:649–72

Membrane proteins are asymmetric
There are always exceptions:
It is possible to have a charged a.i. in one transmembrane helix, forming for example an ionic interaction with another charged a.i. of another helix

How do ß-strands cross the membrane?

Transmembrane proteins: ß-Barrel



The first building blocks of a ß-Barrel: ß-Sheets, these are made from ß-Strands

The side chains of the amino acid face outwards

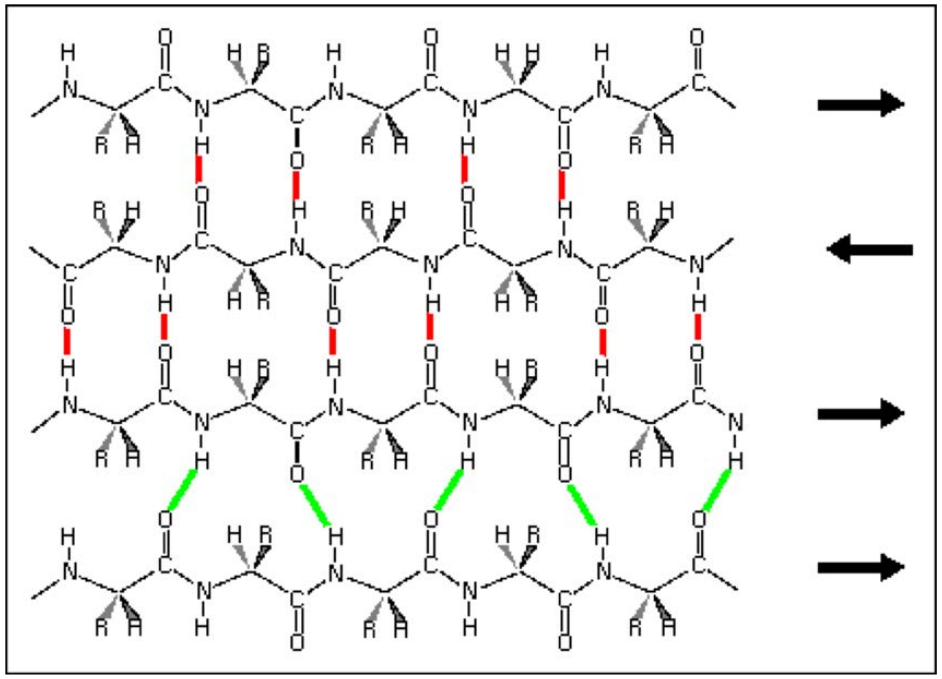
The ß-strands can form parallel or anti-parallel sheets due to the formation of hydrogen bonds between the alpha-chains
The side chains are not involved

Transmembrane proteins: ß-Barrel

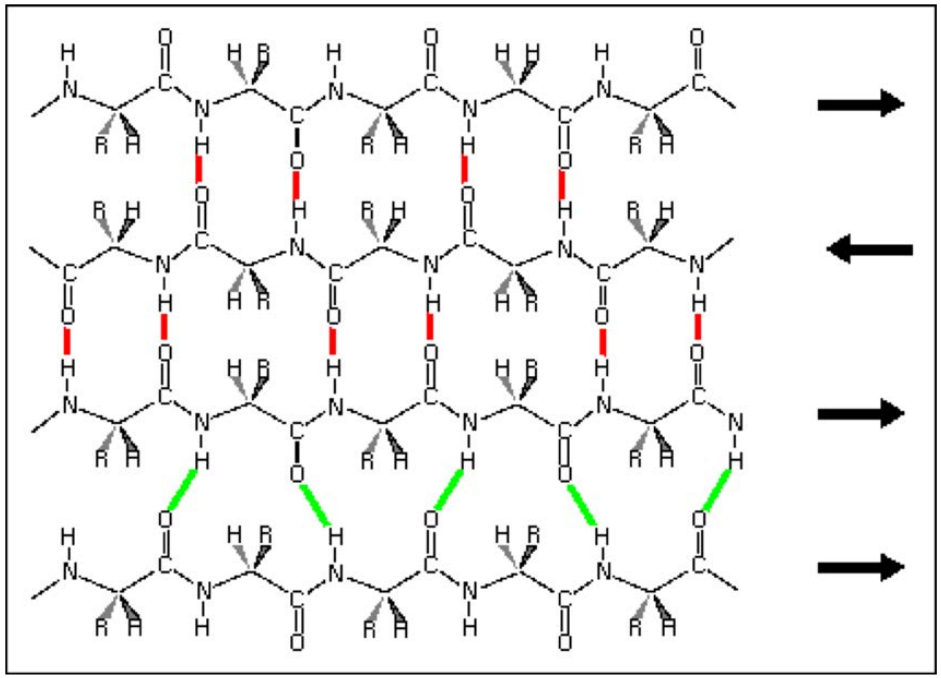
A ß-sheet is an "open" structure
It will always face the outside solvent (world)

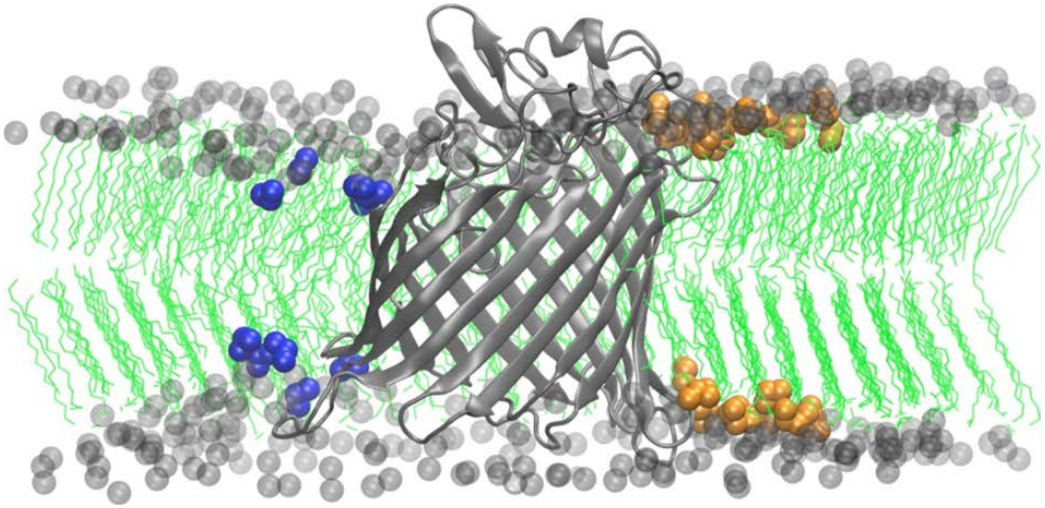
This is not a concern for a soluble protein that is in an aqueous medium

But what about the hydrophobic membrane?

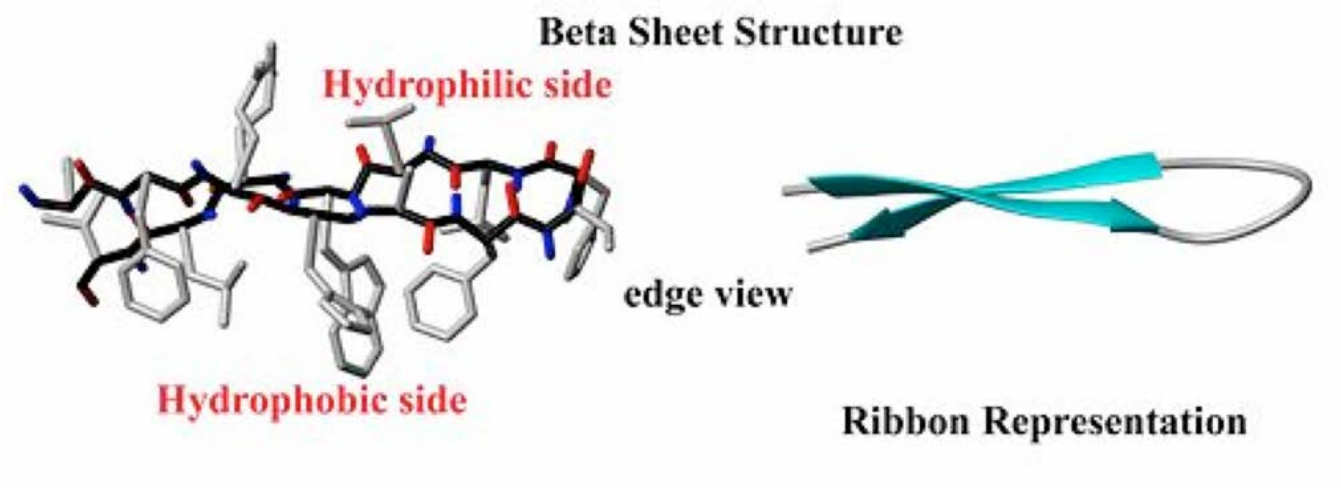


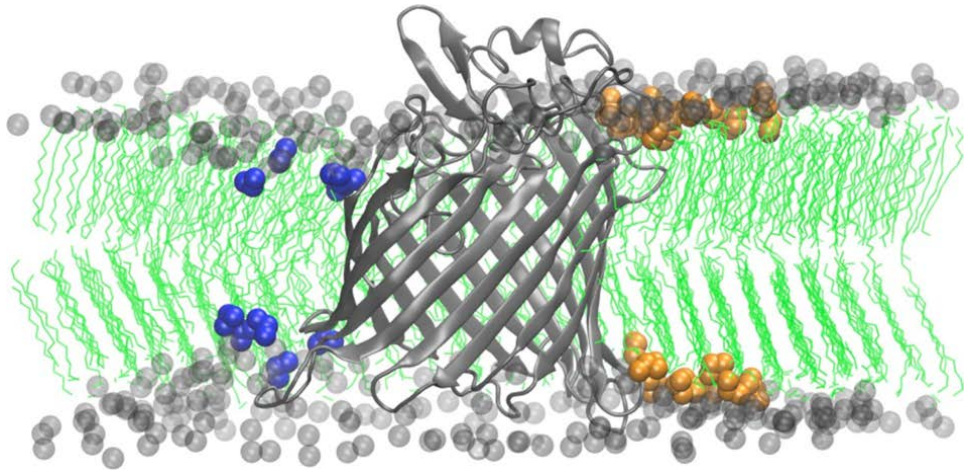
Transmembrane proteins: ß-Barrel





Transmembrane proteins: ß-Barrel

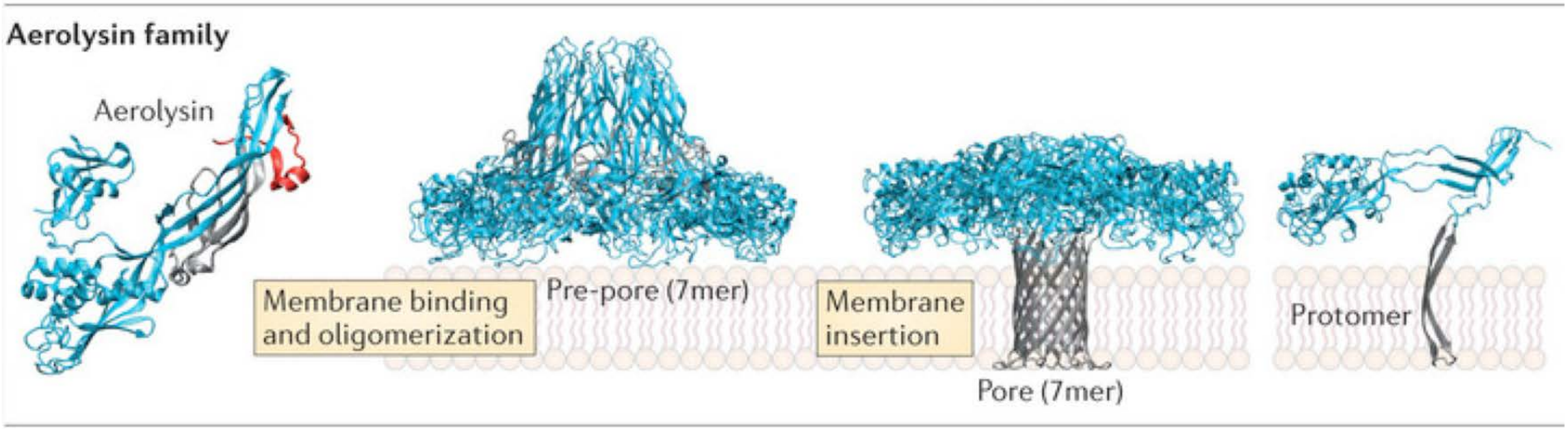




ß-strand form ß-sheets and those form a **ß-barrel** to have the hydrophic outer surface

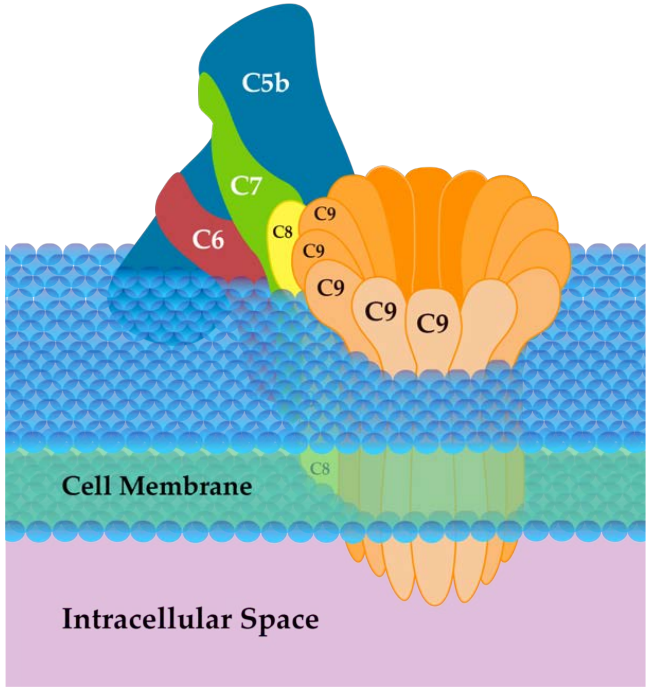
Transmembrane proteins: ß-Barrel

A bacterial ß-sheet transmembrane structure to form an "membrane attack complex”

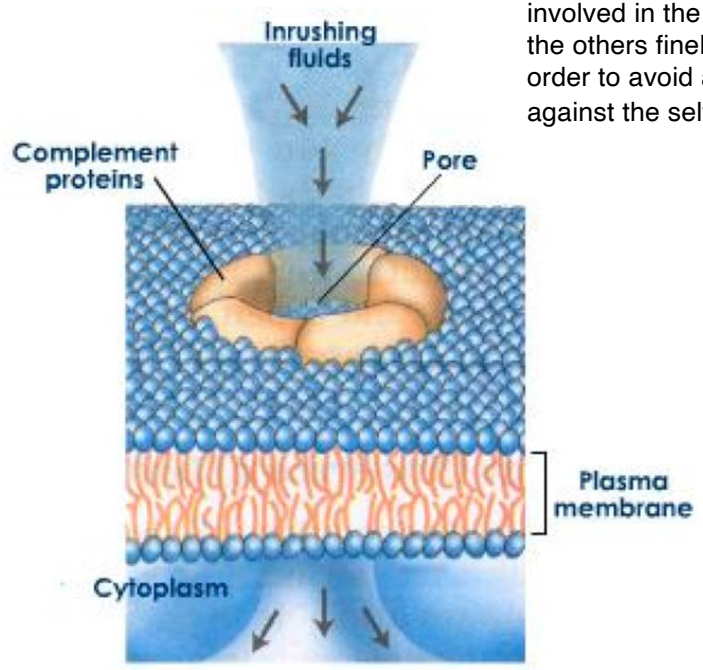


Transmembrane proteins: ß-Barrel

A rare case of ß-sheet transmembrane structure in animals: the complement cascade that culminates in the formation of the "membrane attack complex”

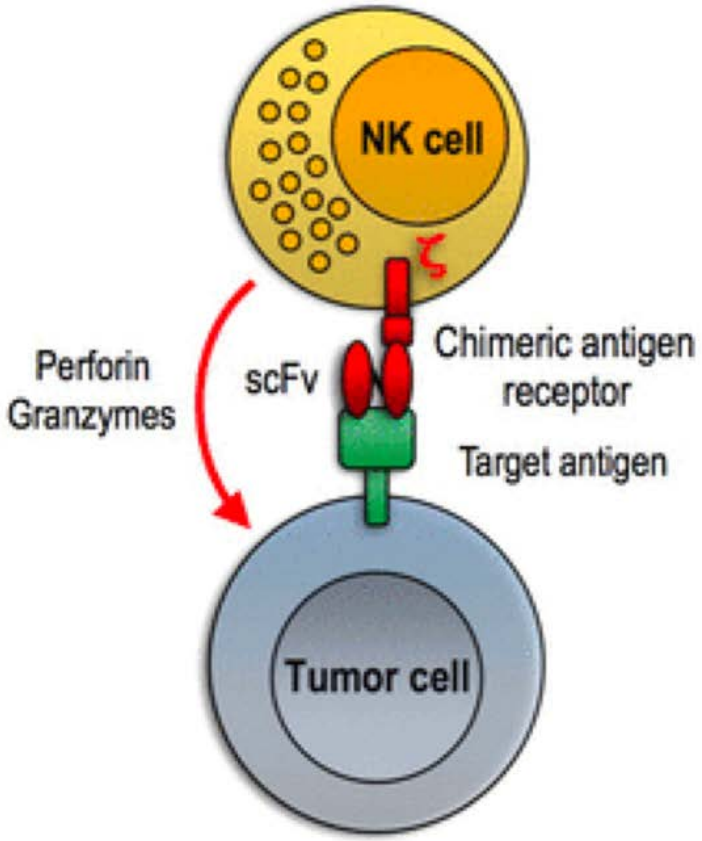


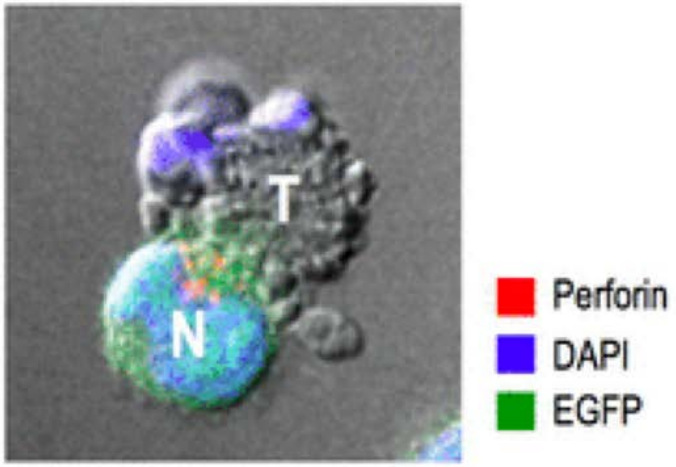
Wikipedia: The complement system is a group of 35 known serum proteins that are part of innate immunity. Twelve of these proteins are directly involved in the mechanisms of pathogen elimination, the others finely regulate the activity of the former in order to avoid an autoimmune reaction (reaction against the self).



Complement proteins creating a hole in the plasma membrane

Transmembrane proteins: ß-Barrel



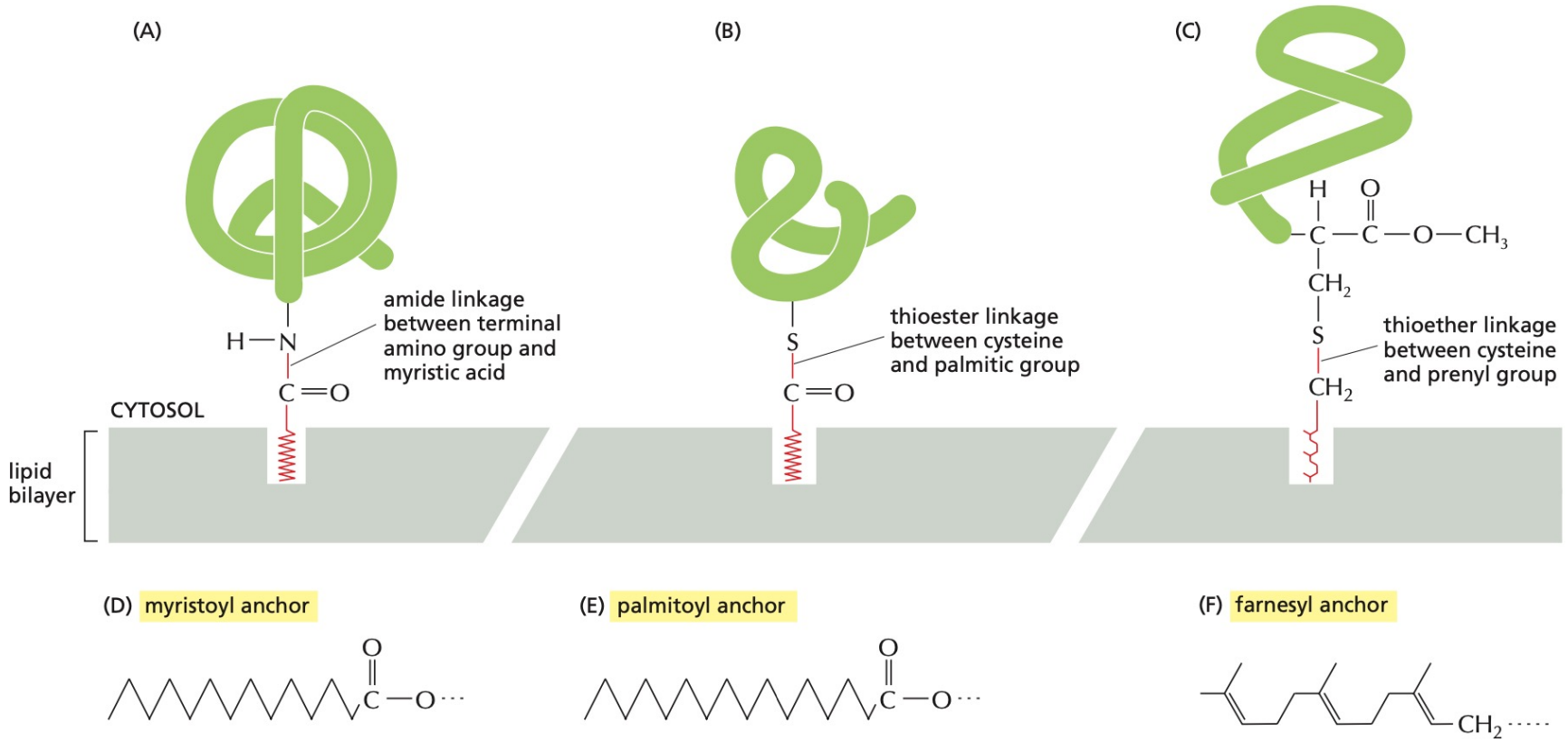


Another example: perforin released by NK cells of the immune system to kill tumor or virus-infected cells

Summary transmembrane proteins

- Two important secondary protein structures
  - Alpha Helices
  - ß-sheets
- Both interact with the cell membranes based on their hydrophobic and hydrophilic aspects
- These aspects are a consequence of the amino acid order and by the side chains of amino acids

A protein anchoring to the lipid membrane



The majority of these changes take place on the cytoplasmic side of the membranes

These modifications are generally irreversible

Prenyl group

A protein anchoring to the lipid membrane

```
        10         20         30         40         50
MTEYKLVVVG AGGVGKSALT IQLIQNHFVD EYDPTIEDSY RKQVVIDGET
        60         70         80         90        100
CLLDILDTAG QEEYSAMRDQ YMRTGEGFLC VFAINNTKSF EDIHHYREQI
       110        120        130        140        150
KRVKDSEDVP MVLVGNKCDL PSRTVDTKQA QDLARSYGIP FIETSAKTRQ
       160        170        180
RVEDAFYTLV REIRQYRLKK ISKEEKTPGC VKIKKCIIM
```

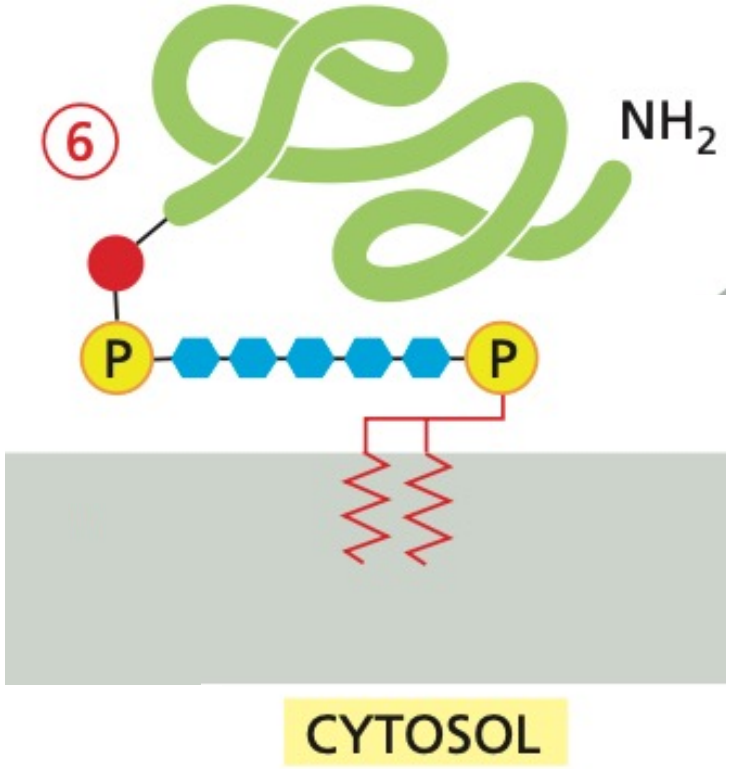
KRAS as an example

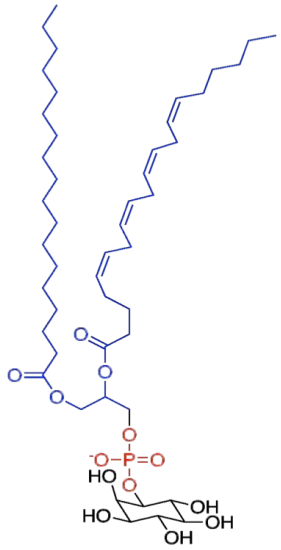
Palmitoylation (reversible modification)

Prenylation (irreversible modification)

Palmitoylation: only reversible lipidic modification

The GPI anchor

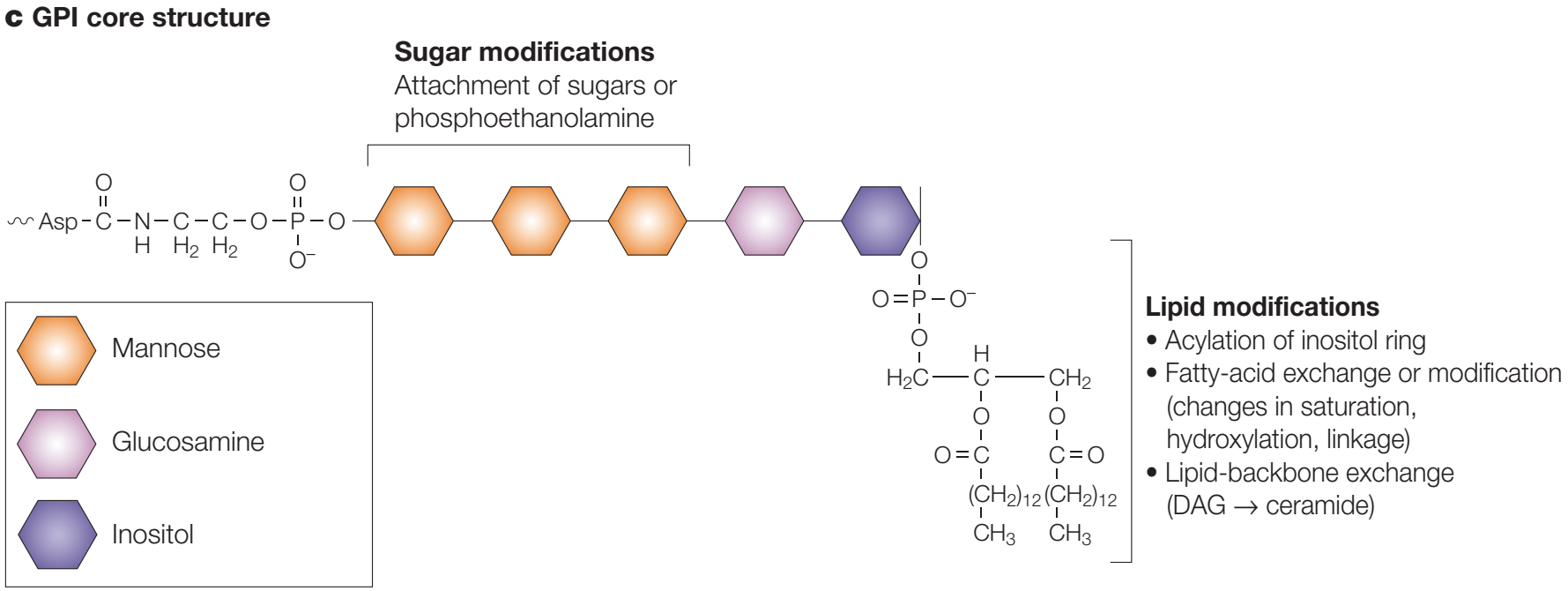




Phosphatidylinositols that becomes glycosylated

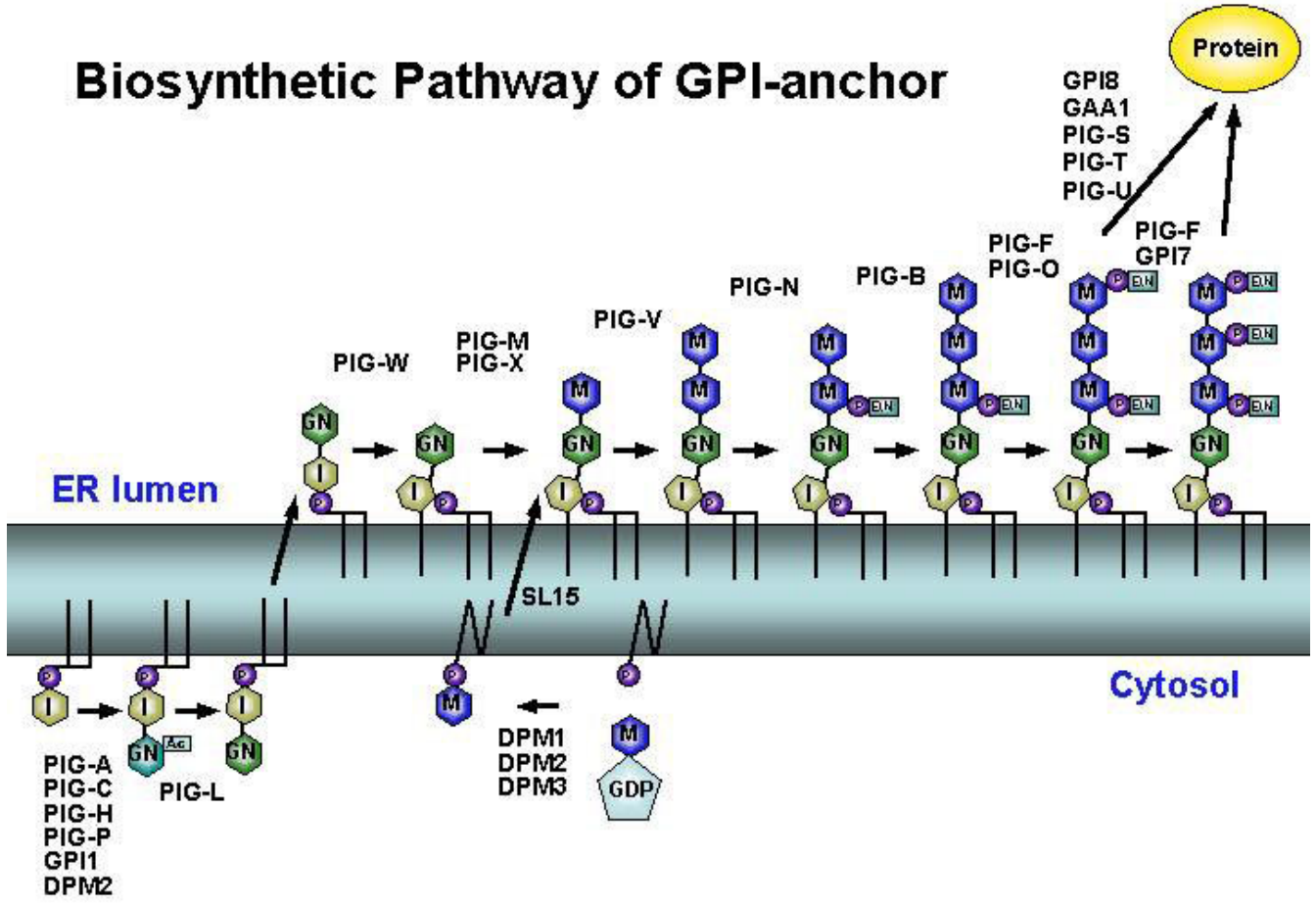
Glycosylphosphatidylinositol = GPI

The GPI anchor

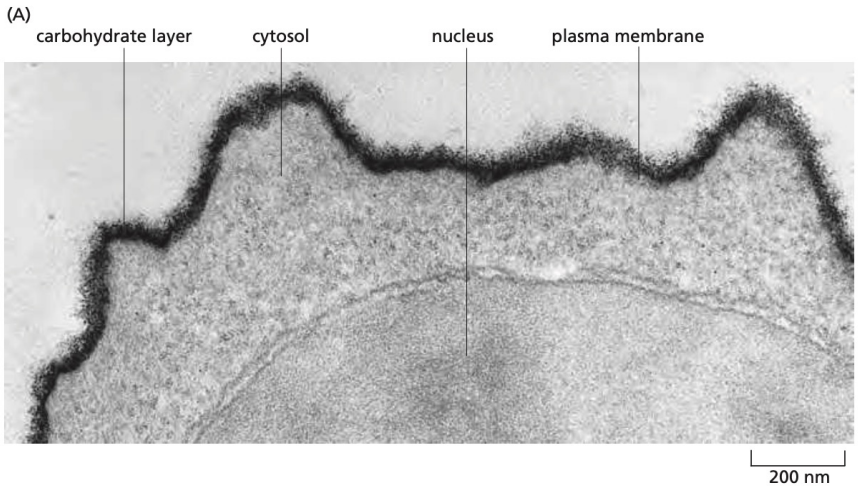


https://www.nature.com/articles/nrm1309

Generation of the GPI anchor



The carbohydrate layer on the cell surface



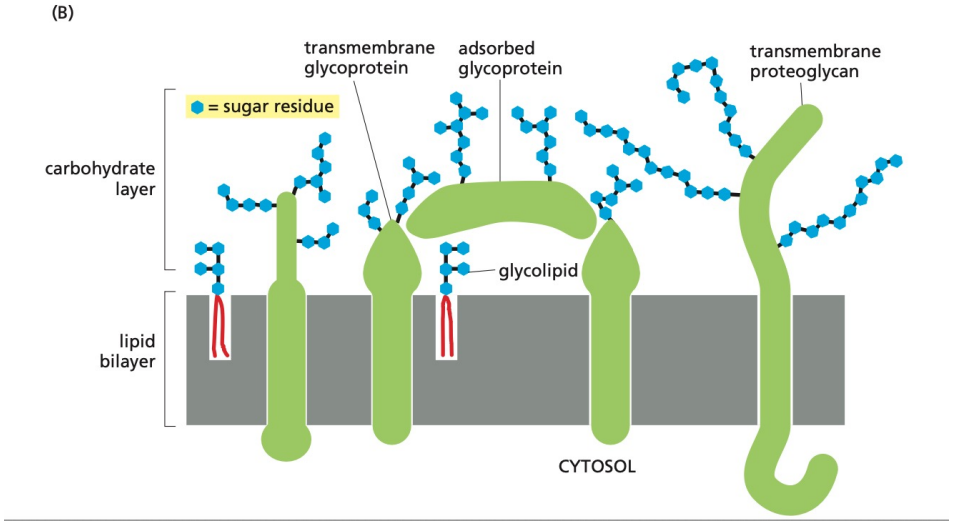
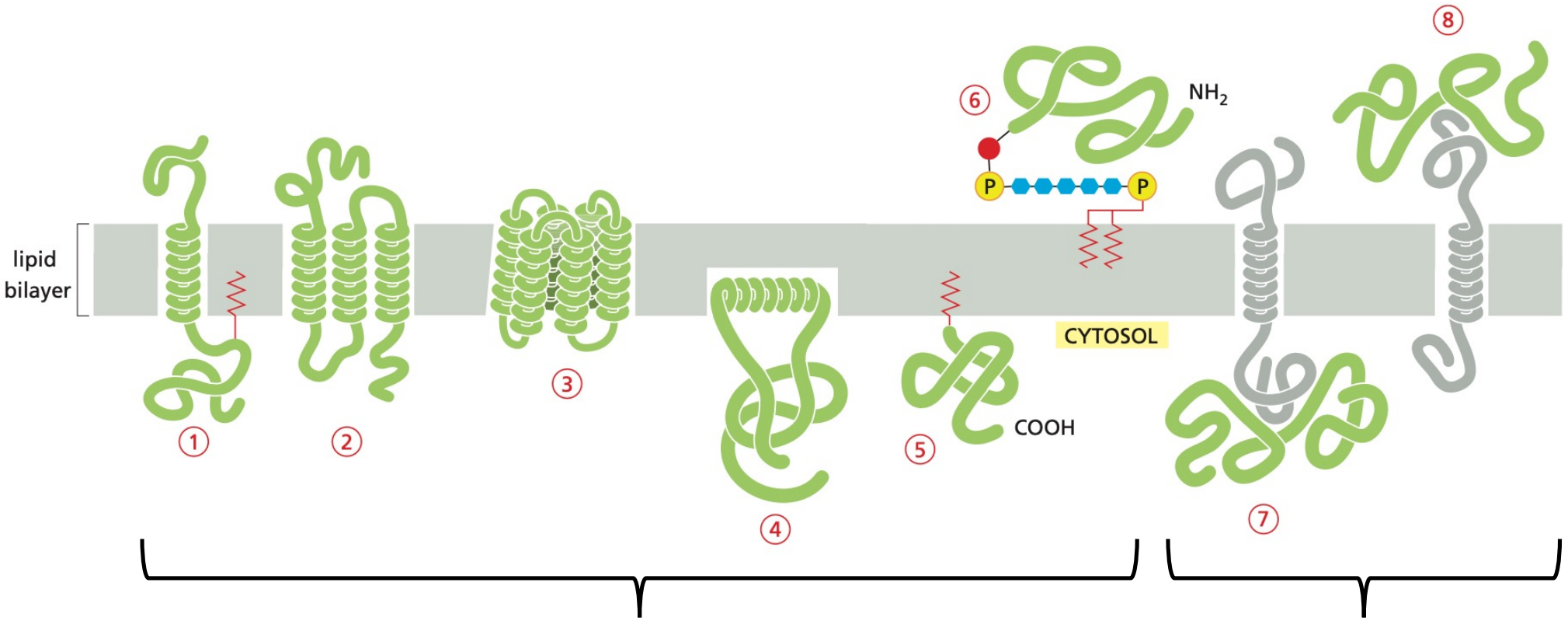


Figure 10–25 The carbohydrate layer on the cell surface. (A) This electron micrograph of the surface of a lymphocyte stained with ruthenium red emphasizes the thick carbohydrate-rich layer surrounding the cell. (B) The carbohydrate layer is made up of the oligosaccharide side chains of membrane glycolipids and membrane glycoproteins and the polysaccharide chains on membrane proteoglycans. In addition, adsorbed glycoproteins, and adsorbed proteoglycans (not shown), contribute to the carbohydrate layer in many cells. Note that all of the carbohydrate is on the extracellular surface of the membrane. (A, courtesy of Audrey M. Glauert and G.M.W. Cook.)

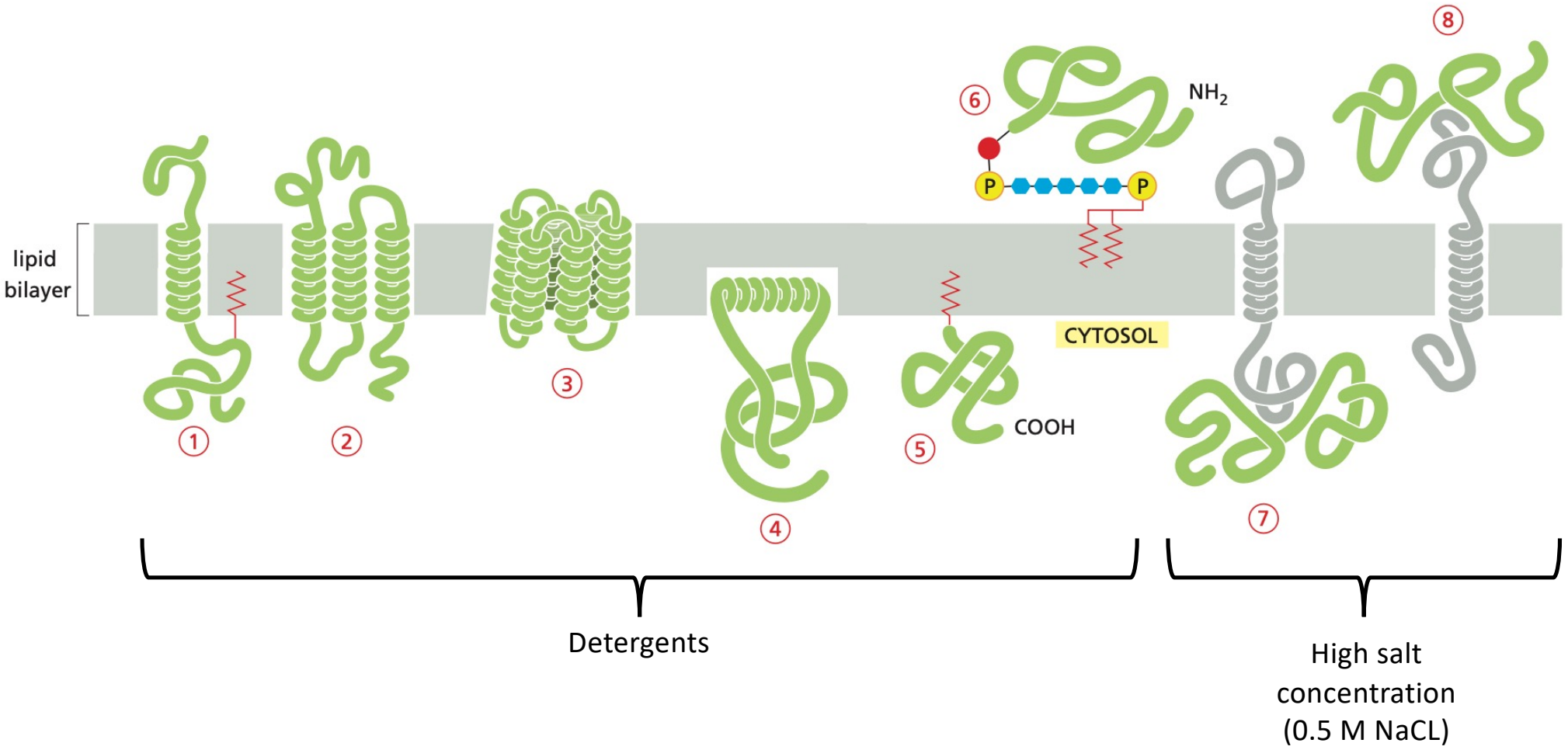
How can we isolate proteins from cell membrane?

Membrane protein isolation

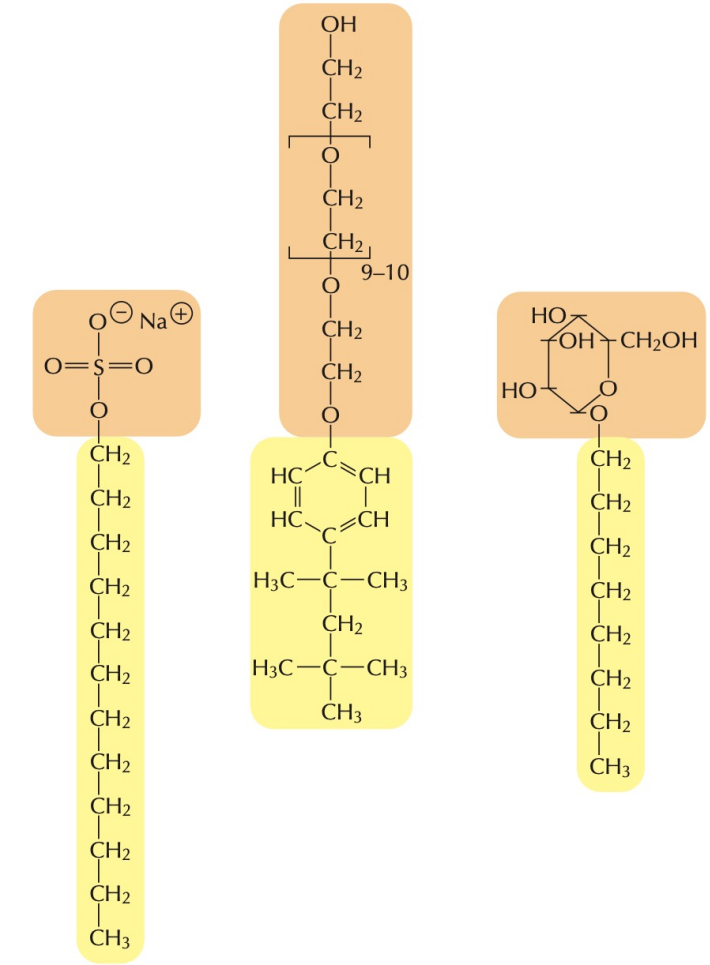


Any ideas?

Membrane protein isolation



The structure and function of detergents

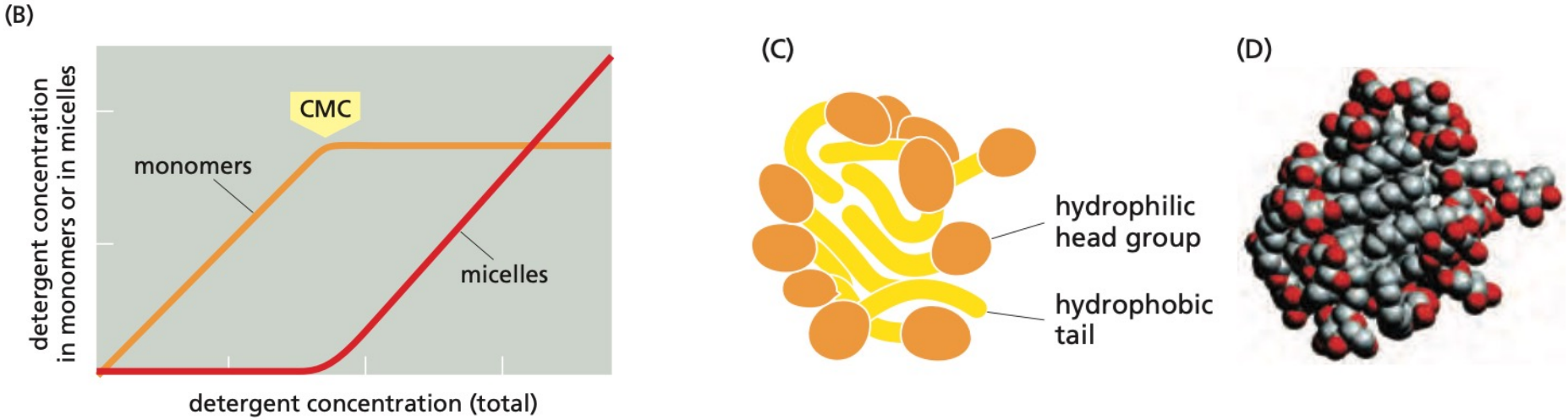


Where have we seen these amphiphilic features before?

Different detergents have different qualities

The negative charge of SDS also denatures proteins!

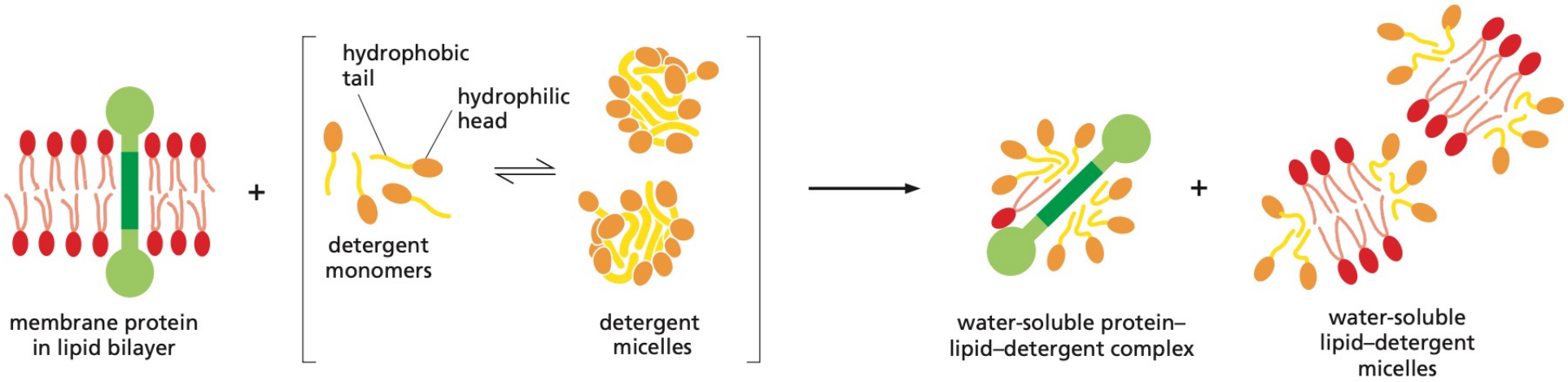
The structure and function of detergents



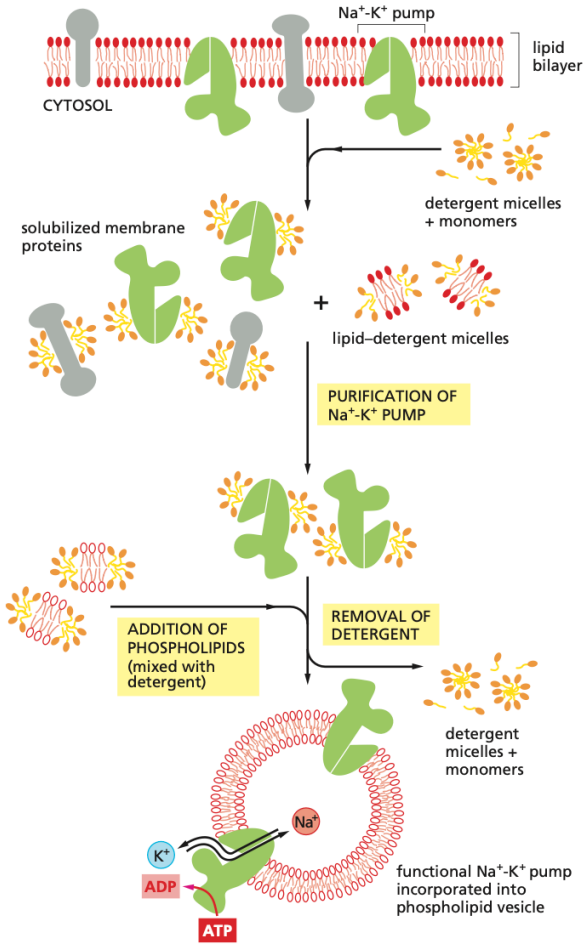
Detergents form micelles in water

Micelles structures are believed to be irregular in water due to packing constraints

A detergent in action



Why would we want to isolate membrane proteins?

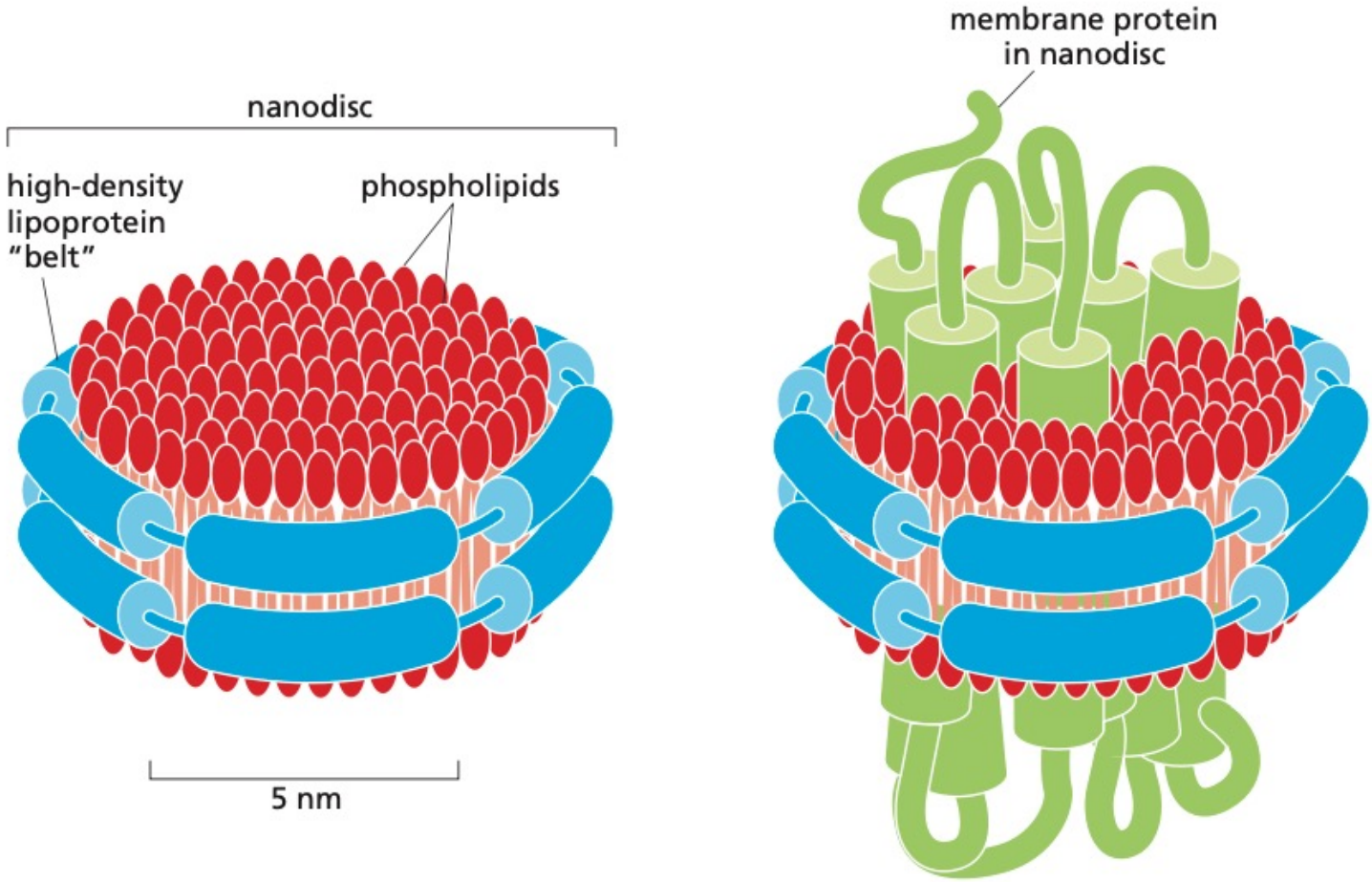


Soft" solubilization to preserve the structure and function of the protein

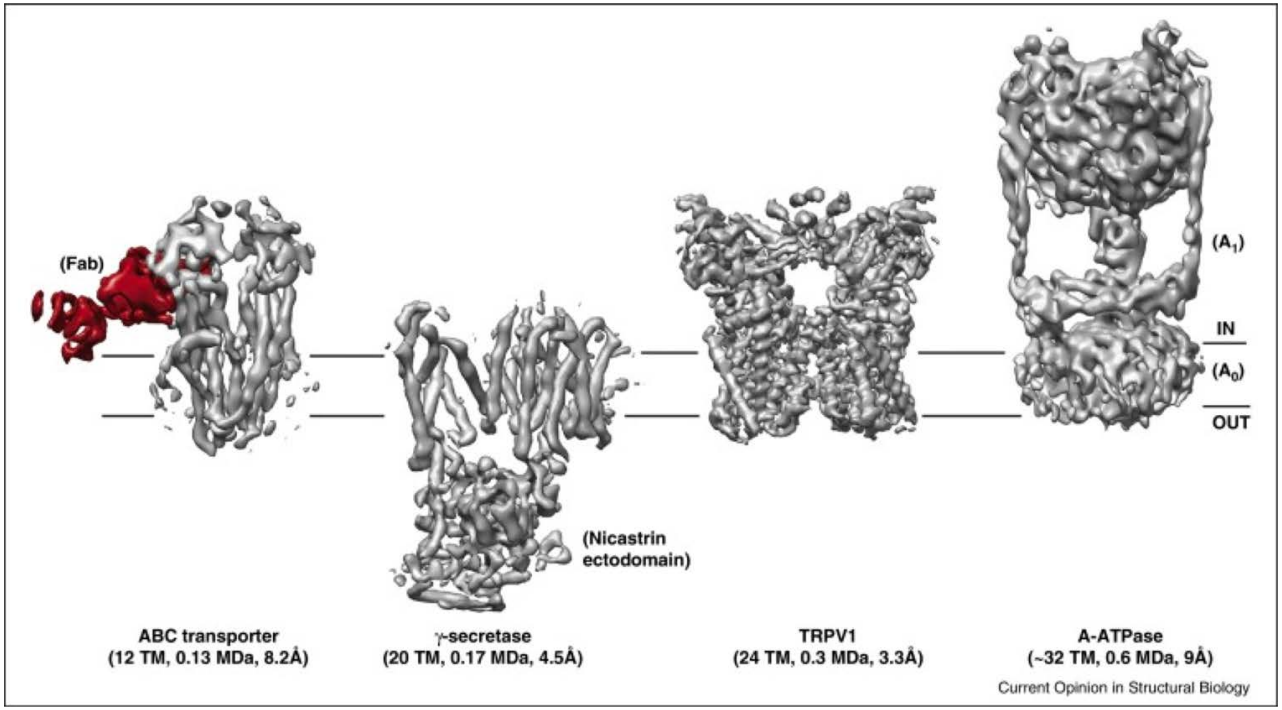
for the study if the shape (CryoEM, Crystallization etc)

Functional studies in isolation in the lab

Model of a membrane protein reconstituted into a nanodisc

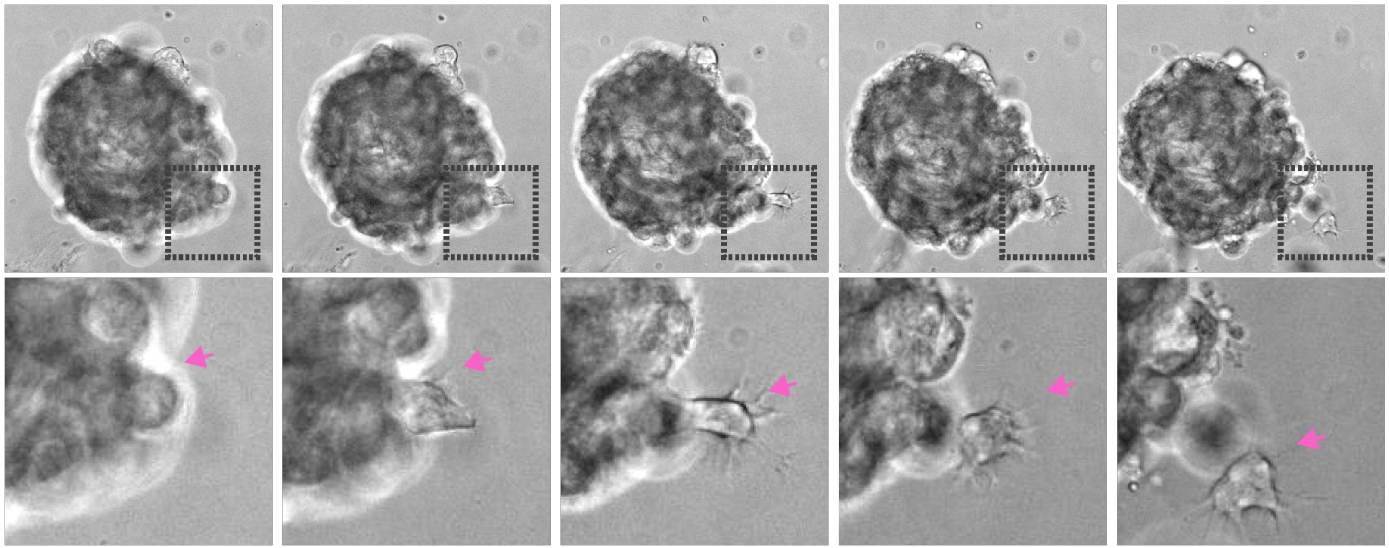


Examples of CryoEM images of membrane proteins

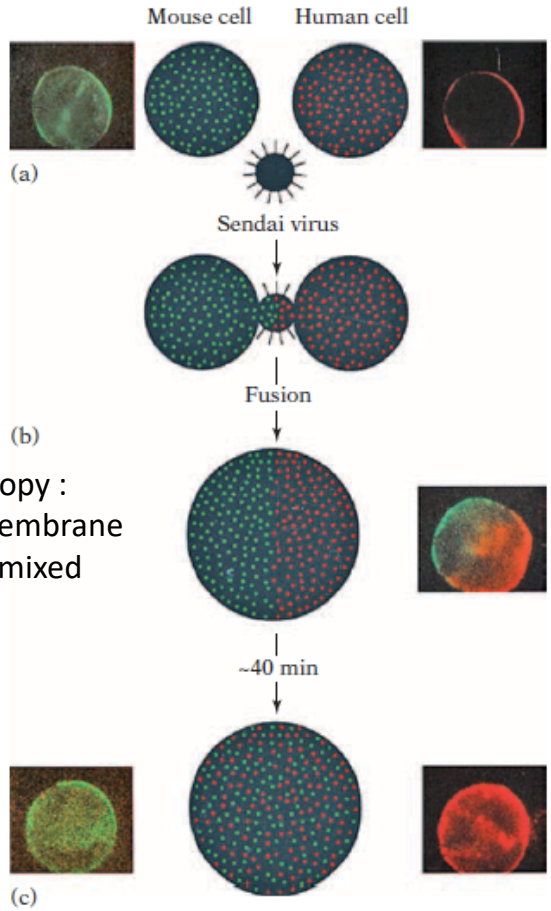


Membrane protein localization

The membrane is a fluid

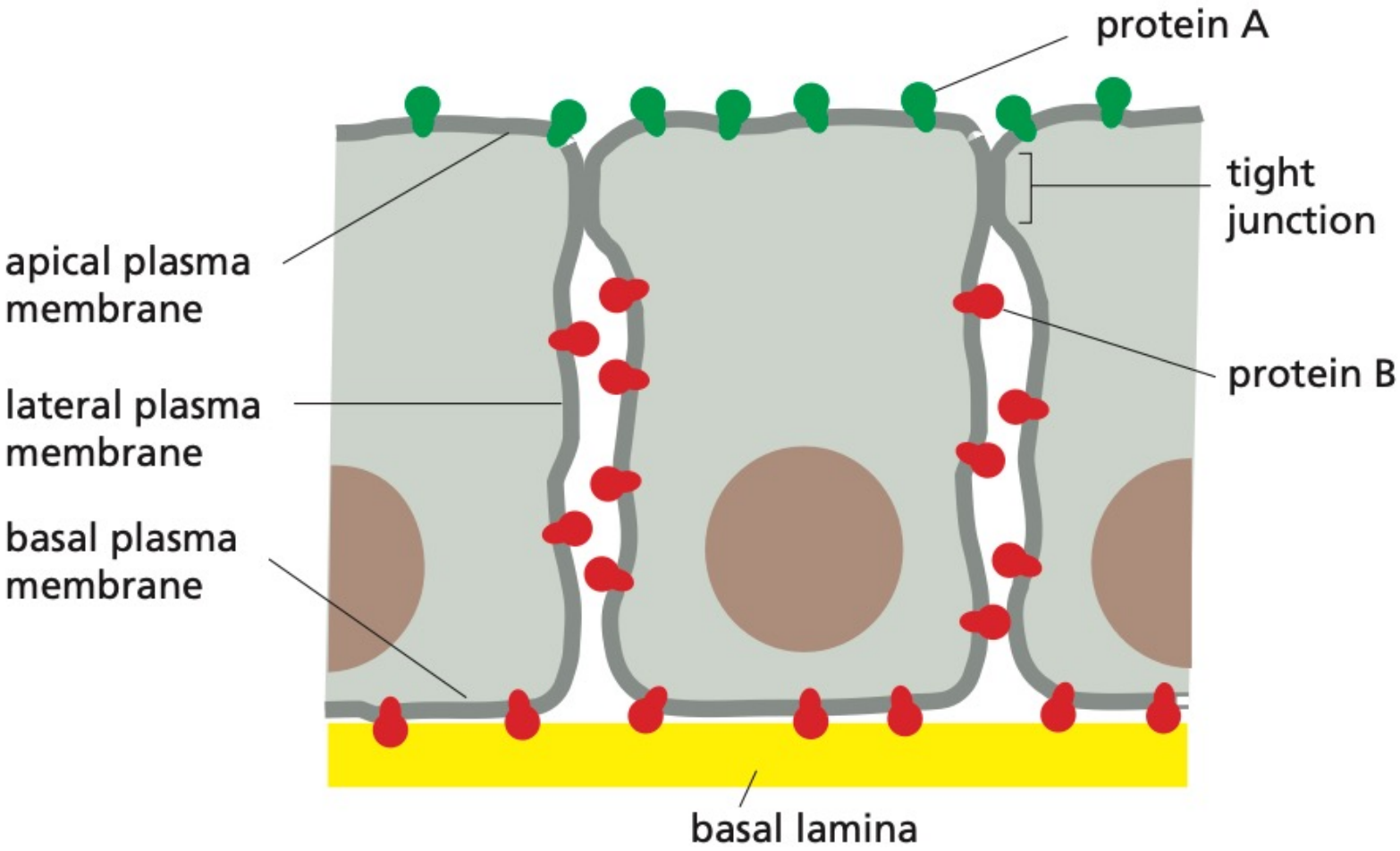


A demonstration of membrane fluidity

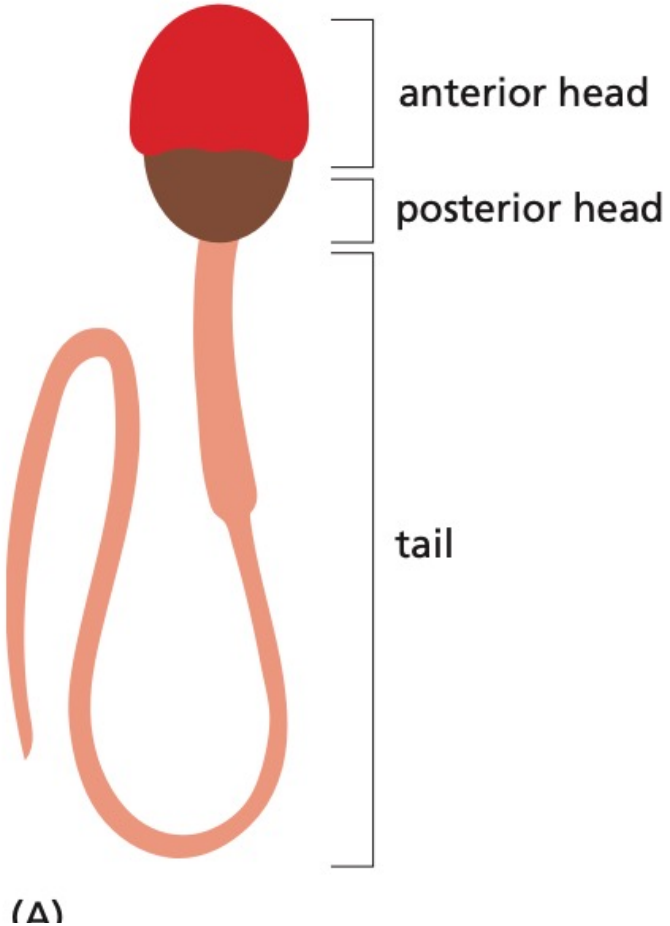


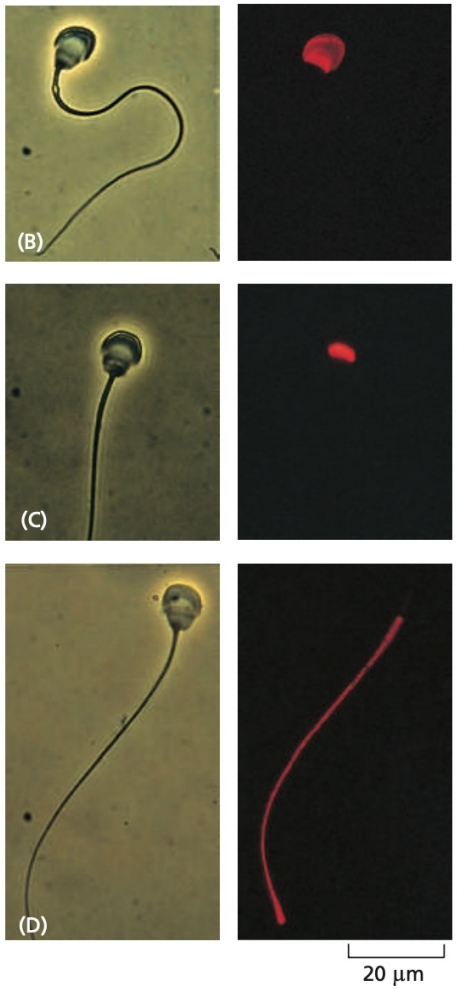
Fluorescence microscopy :
mouse and human membrane proteins are not intermixed

But proteins are found in distinct regions of the cell membrane



Three domains in the plasma membrane of a guinea pig sperm



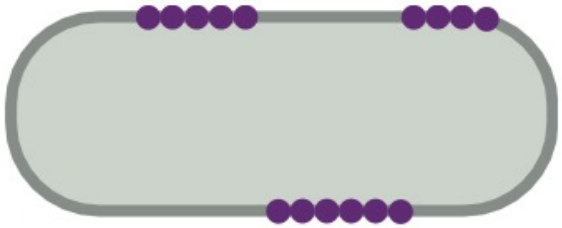


Restriction to a region of membrane proteins is essential for biological function!

How can cells restrict protein location on a cell membrane?

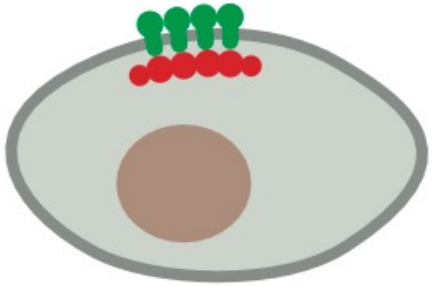
Four ways of restricting the lateral mobility of specific plasma membrane proteins

Self assembly into aggregates



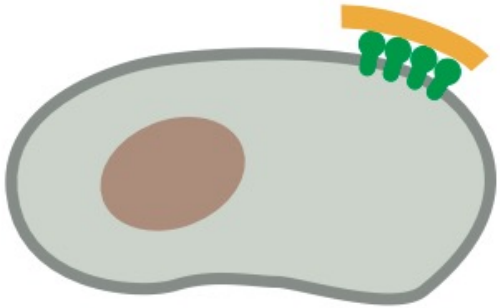
(A)

Interaction with inside



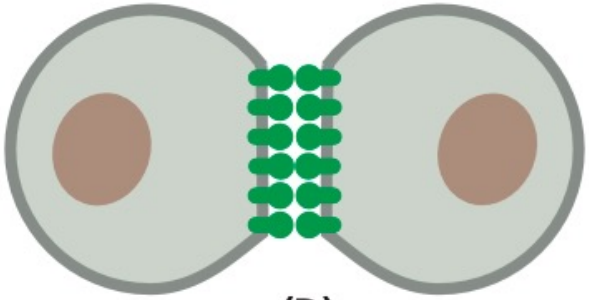
(C)

Interaction with outside



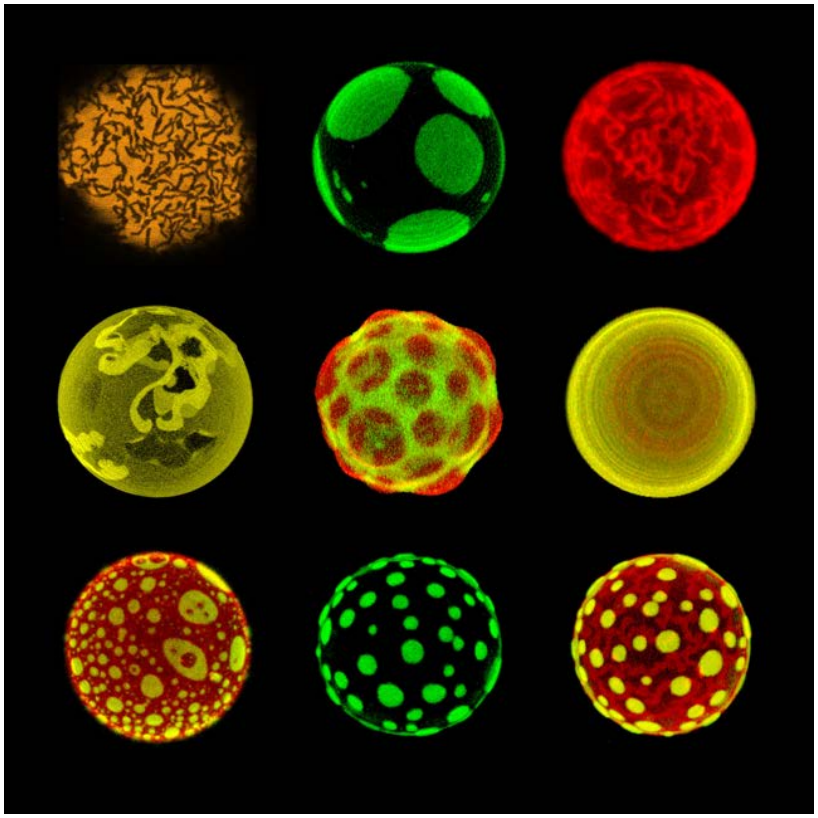
(B)

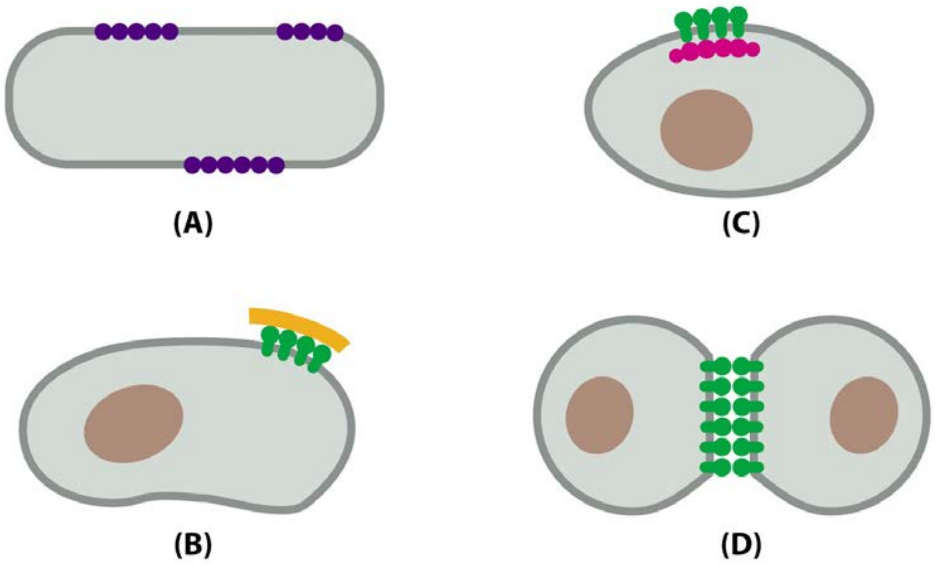
Cell-Cell Interaction



(D)

Lipids and proteins can form specific domains





Membranes are shaped by membrane-bending proteins

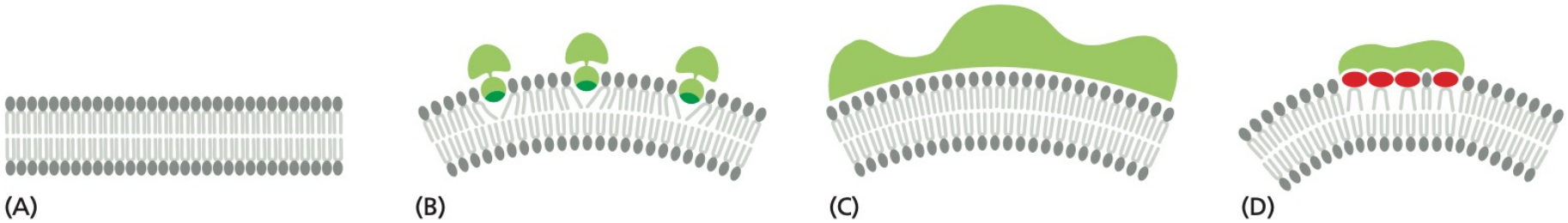
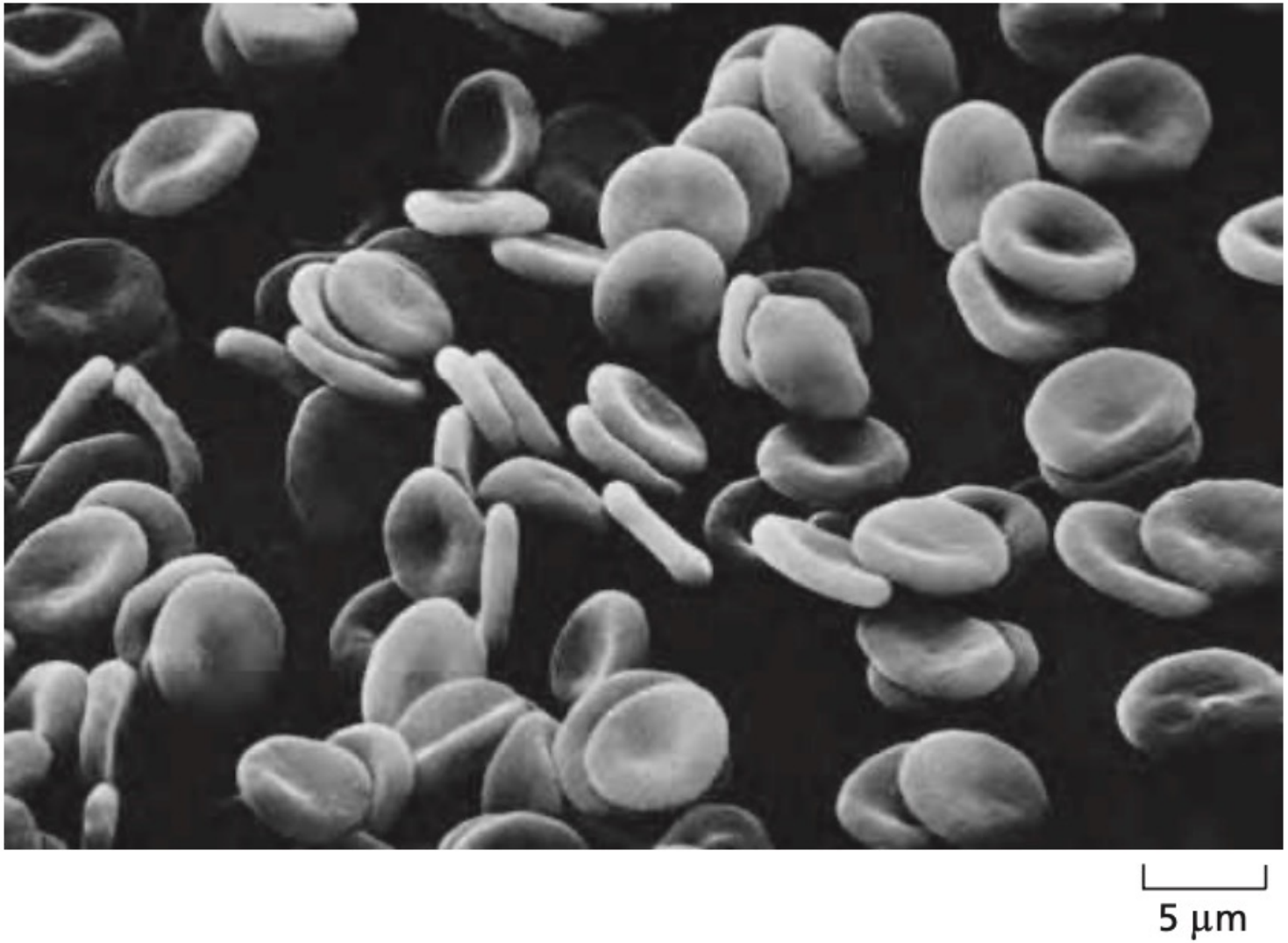
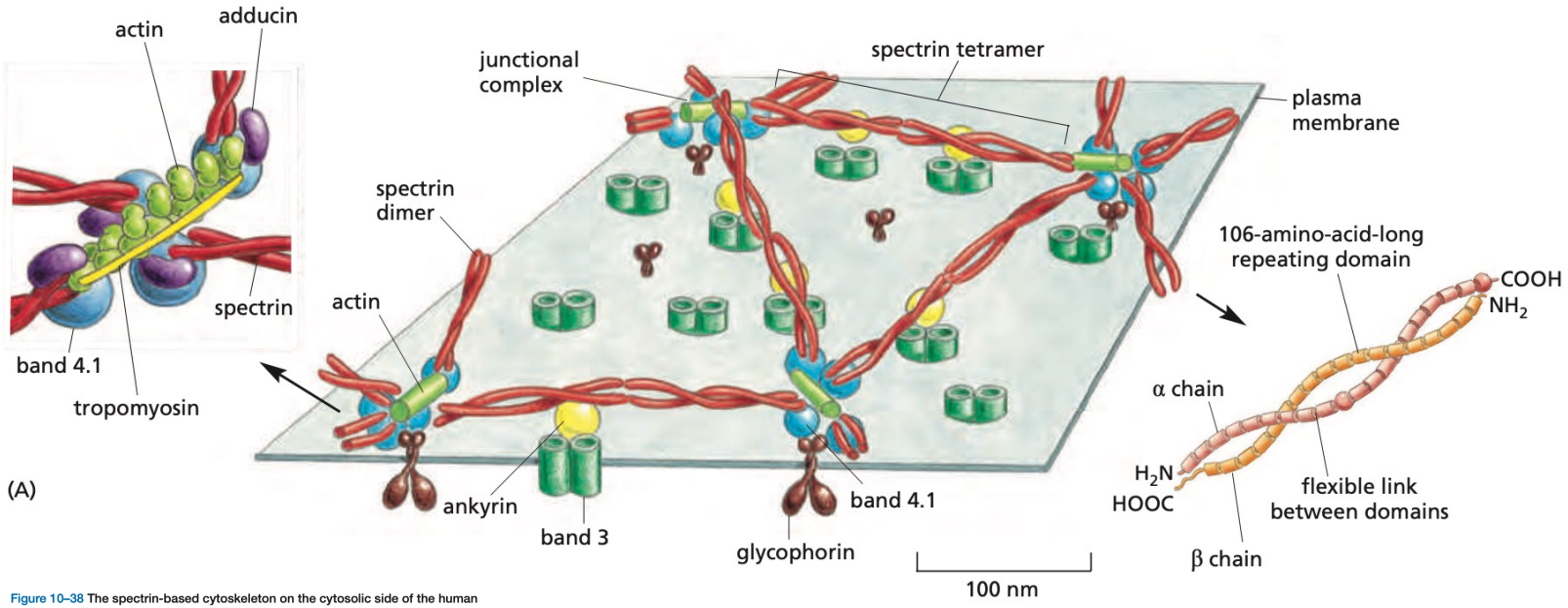


Figure 10–40 Three ways in which membrane-bending proteins shape membranes. Lipid bilayers are blue and proteins are green. (A) Bilayer without protein bound. (B) A hydrophobic region of the protein can insert as a wedge into one monolayer to pry lipid head groups apart. Such regions can either be amphiphilic helices as shown or hydrophobic hairpins. (C) The curved surface of the protein can bind to lipid head groups and deform the membrane or stabilize its curvature. (D) A protein can bind to and cluster lipids that have large head groups and thereby bend the membrane. (Adapted from W.A. Prinz and J.E. Hinshaw, *Crit. Rev. Biochem. Mol. Biol.* 44:278–291, 2009.)

Restriction by the cytoskeleton



The spectrin-based cytoskeleton on the cytosolic side of the human red blood cell plasma membrane



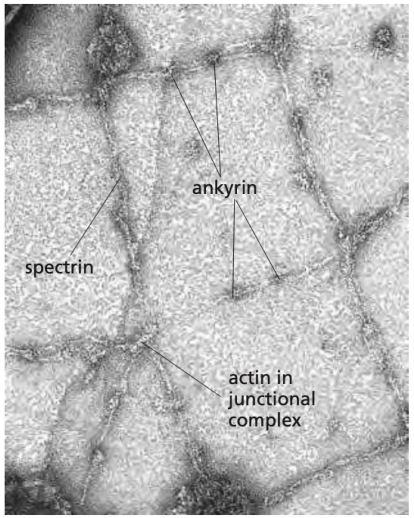
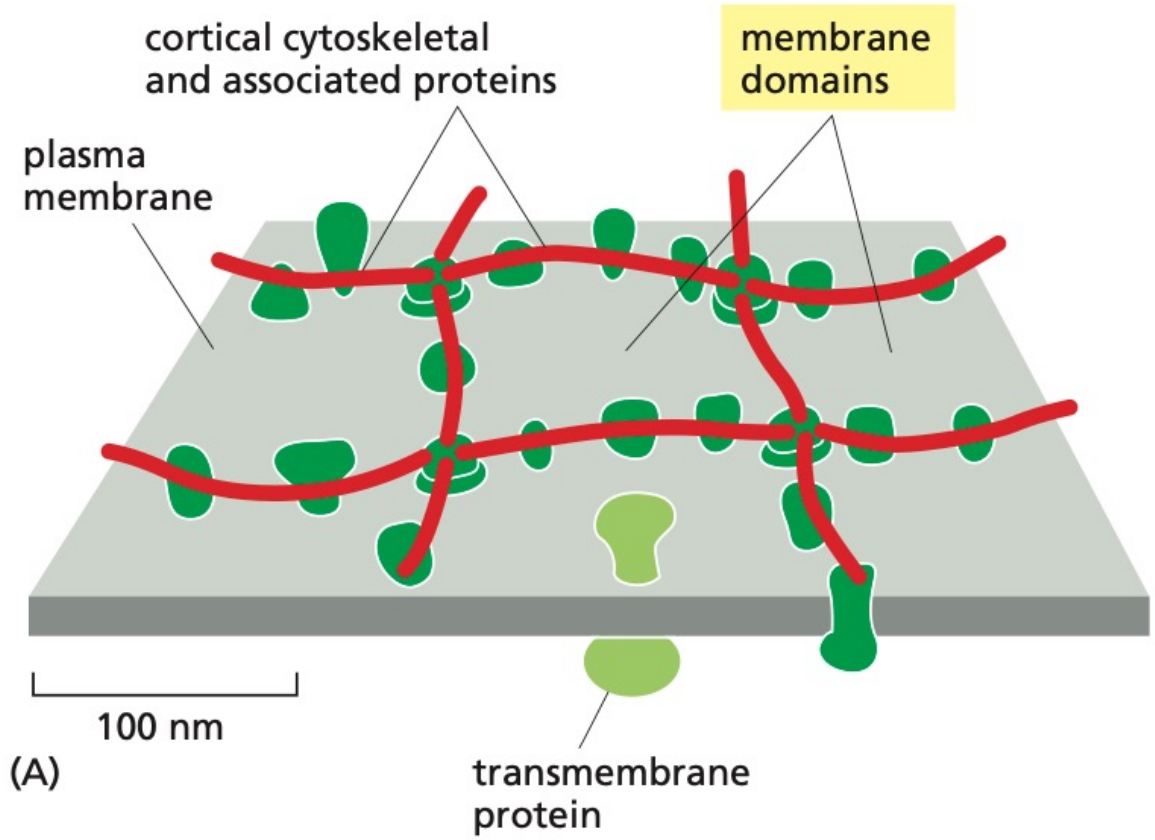


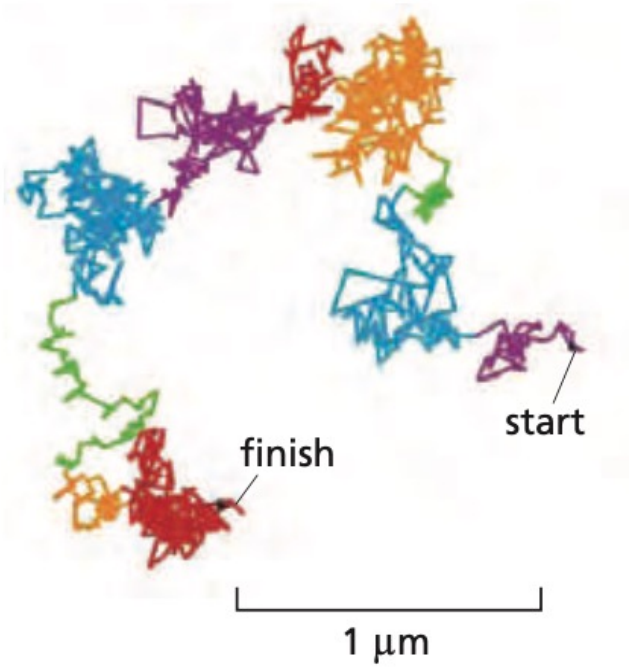
Figure 10–38 The spectrin-based cytoskeleton on the cytosolic side of the human red blood cell plasma membrane. (A) The arrangement shown in the drawing has been deduced mainly from studies on the interactions of purified proteins *in vitro*. Spectrin heterodimers (enlarged in the drawing on the *right*) are linked together into a netlike meshwork by "junctional complexes" (enlarged in the drawing on the *left*). Each spectrin heterodimer consists of two antiparallel, loosely intertwined, flexible polypeptide chains called α and β. The two spectrin chains are attached noncovalently to each other at multiple points, including at both ends. Both the α and β chains are composed largely of repeating domains. Two spectrin heterodimers join end-to-end to form tetramers.

The junctional complexes are composed of short actin filaments (containing 13 actin monomers) and these proteins—*band 4.1, adducin*, and a *tropomyosin* molecule that probably determines the length of the actin filaments. The cytoskeleton is linked to the membrane through two transmembrane proteins—a multipass protein called *band 3* and a single-pass protein called *glycophorin*. The spectrin tetramers bind to some band 3 proteins via *ankyrin* molecules, and to glycophorin and band 3 (not shown) via band 4.1 proteins.

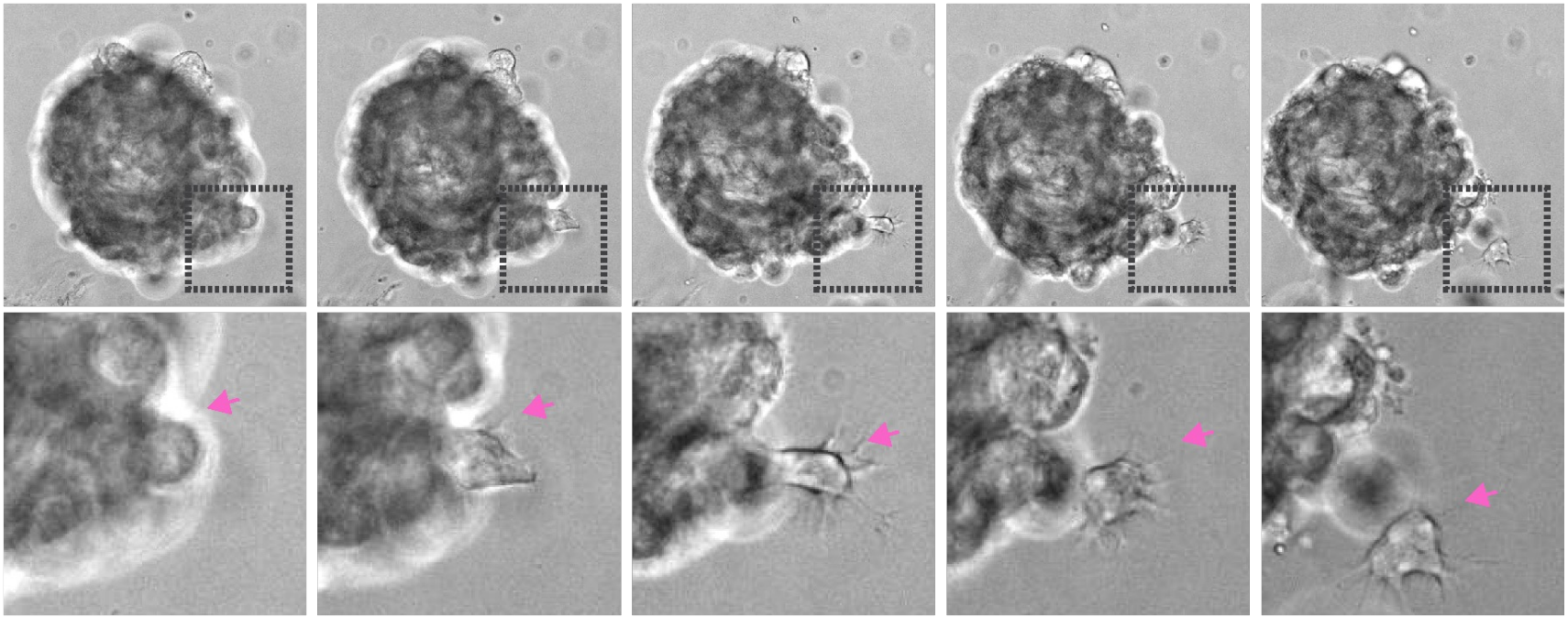
(B) The electron micrograph shows the cytoskeleton on the cytosolic side of a red blood cell membrane after fixation and negative staining. The spectrin meshwork has been purposely stretched out to allow the details of its structure to be seen. In a normal cell, the meshwork shown would be much more crowded and occupy only about one-tenth of this area. (B, courtesy of T. Byers and D. Branton, *Proc. Natl Acad. Sci. USA* 82:6153–6157, 1985. With permission from The National Academy of Sciences.)

Corralling plasma membrane proteins by cortical cytoskeletal filaments





Movement of this cell an interplay between the plasma membrane and the cytoskeleton



Summary membrane structure

- The transmembrane part of proteins is either formed by alpha helices or by ß strands, not by mixed structures
- Proteins can be post-translationally modified by acylated chains
- Transmembrane parts can be predicted
- Know how a detergent works
- Know that lipids and proteins are mobile in a membrane, know modes of immobilization and confinement
- Know the principle of FRAP