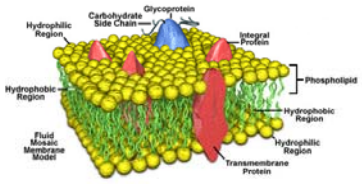
Biochemistry 660

Methods in Membrane Biochemistry



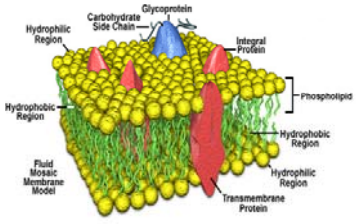
Greg Kabachinski (Martin Lab)
Kabachinski@wisc.edu

Biochemistry 660

Date	Speaker	Title	Host
Sep. 14	Donald M. Engelman Yale University	pH Dependent Insertion of a Transmembrane Helix: Mechanism and Applications in Cancer and Drug Delivery	Contemporary Biochemistry
Sep. 21	Josep Rizo UT Southwestern Medical Center at Dallas	Structural Insights into the Mechanism of Neurotransmitter Release	Contemporary Biochemistry
Sep. 28	John H. Bushweller University of Virginia	NMR Solution Structure and Function Studies of the Integral Membrane Enzyme DsbB	Contemporary Biochemistry
Oct. 5	Brian Crane Cornell University	Structure and Reactivity of the Receptor Kinase Array that Mediates Bacterial Chemotaxis	Contemporary Biochemistry
Oct. 12	Brian Kobilka Stanford California	Structural Insights into G Protein coupled Receptor Activation	Contemporary Biochemistry
Oct. 13	Erik Sontheimer Northwestern University	TBA	RNA Mini Group
Oct. 26	Kerwyn Casey Huang Stanford University	Curvature-mediated Localization of Cardiolipin in Bacteria	Contemporary Biochemistry
Nov. 2	William Dowhan University of Texas-Houston Medical School	How do Lipids Organize Membrane Proteins	Contemporary Biochemistry
Nov. 9	James U. Bowie UCLA	What Drives Membrane Protein Folding?	Contemporary Biochemistry
Nov. 16	Anne Kenworthy Vanderbilt School of Medicine, Nashville TN	Lipid Raft Organization and Dynamics in Cell Membranes	Contemporary Biochemistry

http://www.biochem.wisc.edu/seminars/

Processes Involving Biological Membranes



Separation/Segregation

- protein specificity
- ion gradients

Exo/Endocytosis

Cell Division

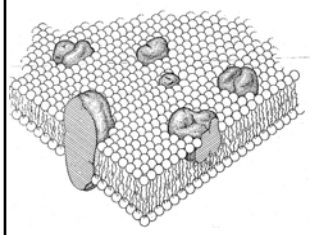
Signal Transduction

Cell Motility and Cell Shape

http://www.molecularexpressions.com/cells/plasmamembrane/plasmamembrane.html

Fluid Mosaic Model

1972 Singer & Nicholson



Lipids -serve as an impermeable barrier

Proteins - most other functions

Cholesterol - enhances barrier

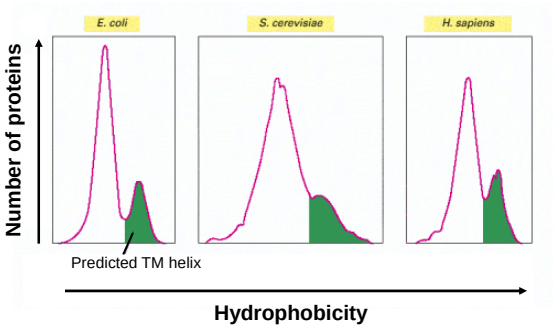
Carbohydrates - glycolipids and glycoproteins

Proteins vs Lipids

	Protein	Lipid
Plasma Membrane	50%	50%
Axon	20%	80%
Mitochondria	75%	25%

% is given by weight

Membrane Proteins in the Genome



Alberts Molecular Biology of the Cell 2002 Garland Science

Hydropathy Plot

Kyte and Doolittle

Identifies hydrophobic regions

Hydrophobicity of each amino acid (y-axis) versus location in protein(x-axis)

Hydropathy Plot

Amino acid residue	Transfer free energy kcal mol^{-1} (kJ mol^{-1})
Phe	3.7 (15.5)
Met	3.4 (14.3)
Ile	3.1 (13.0)
Leu	2.8 (11.8)
Val	2.6 (10.9)
Cys	2.0 (8.4)
Trp	1.9 (8.0)
Ala	1.6 (6.7)
Thr	1.2 (5.0)
Gly	1.0 (4.2)
Ser	0.6 (2.5)
Pro	−0.2 (−0.8)
Tyr	−0.7 (−2.9)
His	−3.0 (−12.6)
Gln	−4.1 (−17.2)
Asn	−4.8 (−20.2)
Glu	−8.2 (−34.4)
Lys	−8.8 (−37.0)
Asp	−9.2 (−38.6)
Arg	−12.3 (−51.7)

Free-energy changes for the transfer from hydrophobic to an aqueous environment

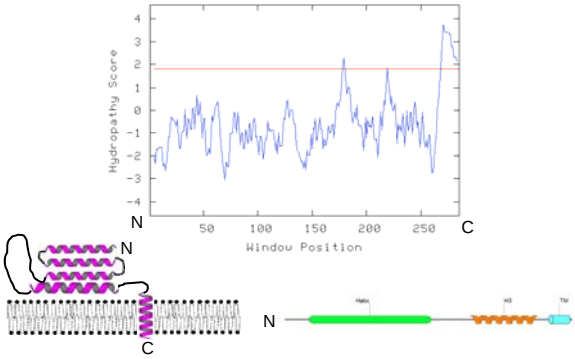
Hydrophilic and charged amino acids have values at or below 0

Hydrophobic amino acids have values above positive values

Do this for all amino acids in a protein and look for hydrophobic sections of about 20 amino acids!

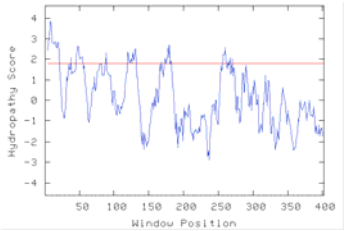
Stryer Biochemistry 2001 WH Freeman

Kyte-Doolittle Hydropathy plot



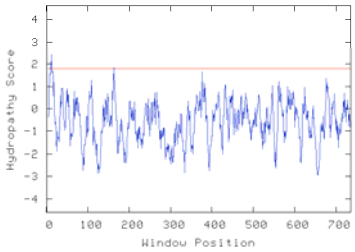
Kyte-Doolittle Hydropathy plot

How many transmembrane domains would you predict this protein to have?



Kyte-Doolittle Hydropathy plot

Is this a membrane protein?



Membranes Found in the Cell

You have identified a possible transmembrane protein, does that necessarily mean it is on the plasma membrane?

Membranes Found in the Cell

Types of membranes in the cell

Membranes Found in the Cell

You have identified a possible transmembrane protein, does that necessarily mean it is on the plasma membrane?

NO! Plasma membrane makes up only a small fraction of total cell membrane

ER membrane has 40 X surface area compared to the plasma membrane

Other membrane bound organelles

Organelle Membrane Determination

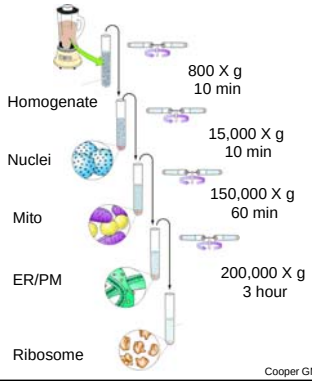
How to determine where your membrane can be found?

Look for a signal sequence

Use microscopy

Subcellular fractionation

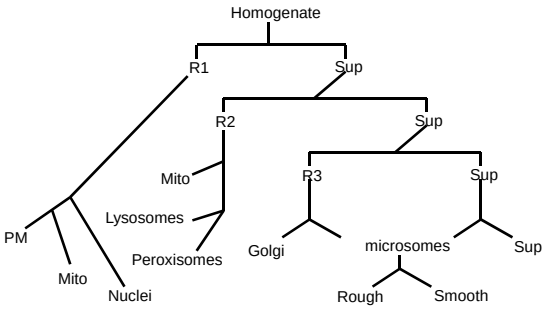
Subcelluar Fractionation



Cooper GM The Cell A Molecular Approach

Subcelluar Fractionation

Fleischer S, Kervina M. **Subcellular fractionation of rat liver.** *Methods Enzymol.* 1974;31(Pt A):6-41.



Subcellular Fractionation

Things to be thinking about :

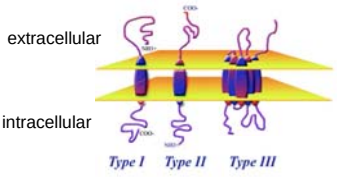
Track your protein through the fractions by western blotting

Identify the fraction with antibodies or enzyme assays

PURITY OF FRACTION

For example; Does your "mitochodrial fraction" contain only proteins found in the mitochondria?

Membrane Protein Topology



Type I - single TM domain with N-terminus exterior side

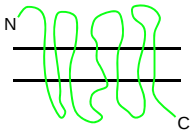
Type II - single TM domain with C-terminus exterior side

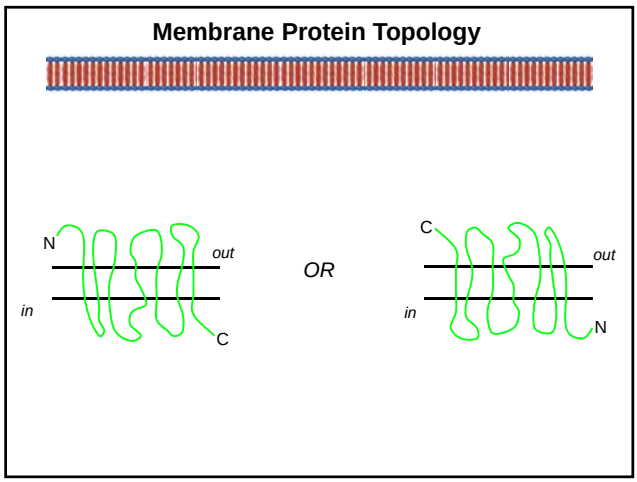
Type III - Multiple spanning TM domains

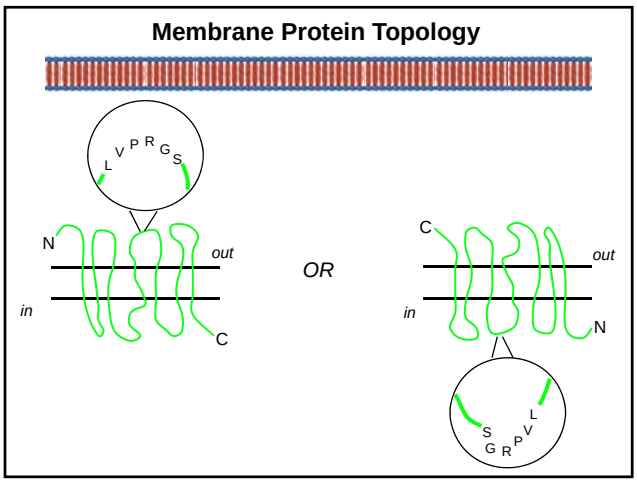
Nature, Vol. 434, 234-238, 10 March 2005

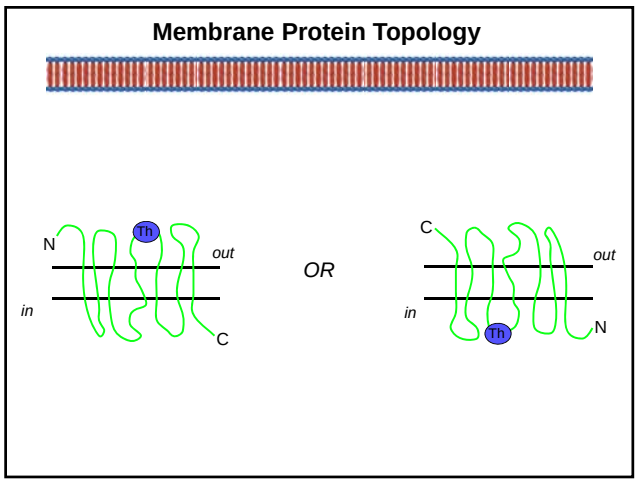
Membrane Protein Topology

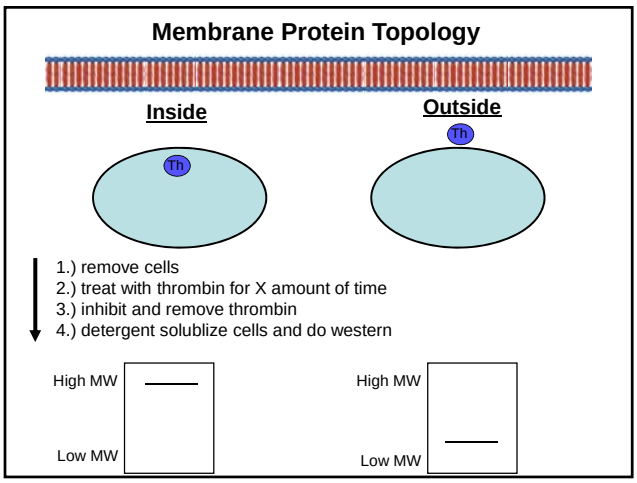
Prerequisite in elucidating protein function is to determine its topology in the membrane



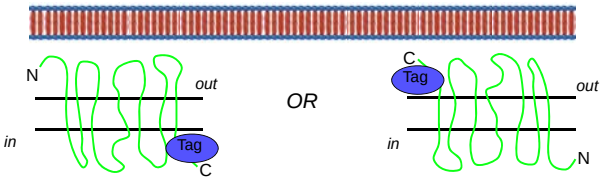

Membrane Protein Topology
N
out
in
C
OR
C
out
in
N


Membrane Protein Topology
L V P R G S
N
out
in
C
OR
C
out
in
N
S G R P V L


Membrane Protein Topology
N
Th
out
in
C
OR
C
out
in
Th
N


Membrane Protein Topology
Inside
Outside
Th
Th
1.) remove cells
2.) treat with thrombin for X amount of time
3.) inhibit and remove thrombin
4.) detergent solublize cells and do western
High MW
Low MW
High MW
Low MW

Membrane Protein Topology



Examples of Tags:

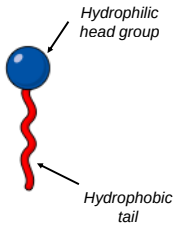
Glycosylation sites
Cys residues
Antibody epitopes
Proteolytic sites
Enzymes
- Alkaline phosphatase
- β-galactosidease
- β-lactamase

Detergents

What are some applications detergents are used in?

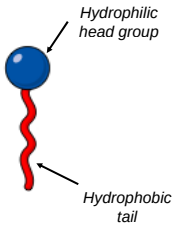
Detergents

Detergents - amphiphilic compounds, segregated polar and apolar domains



Detergents

Detergents - amphiphilic compounds, segregated polar and apolar domains



- measurable water solubility as monomers and aggregates
- belong to a class of compounds known as surfactants
- **Surfactants** - molecules that reduce interfacial surface tension in mixtures by absorbing to interfaces

Detergents

Detergents can fall into one of three categorizes

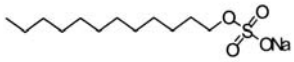
Category depends on the hydrophilic head group of the detergent

Detergents

Detergents can fall into one of three categorizes

1. **Ionic -** cationic or anionic *example*: SDS

Extracting proteins from membranes
Harsh and denaturing

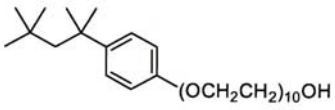


Detergents

Detergents can fall into one of three categorizes

2. **Nonionic -** *example*: Triton X-100

Mild and non denaturing
Commonly used in crystallography

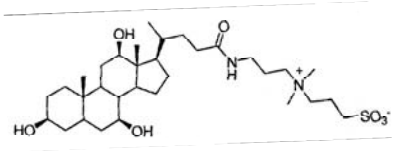


Detergents

Detergents can fall into one of three categorizes

3. **Zwitterionic -** *example*: CHAPS

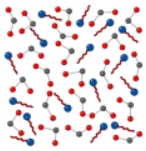
Contain a positive and negative charge
Not as harsh as ionic detergents
Widely used in NMR structural studies



Micellization

Detergent monomers in an aqueous solution can undergo a phase transition

Detergent monomers —*Self-association*→ **Micelle**

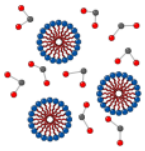


Driven by the hydrophobic effect

As more detergent is added, H-bonding network is disrupted and water molecules must be rearranged

Results in unfavorable entropy

Detergent molecules will self - associate to decrease water accessible surface



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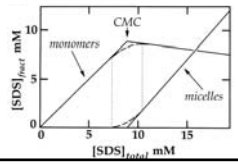
CMC

CMC - Critical Micelle Concentration

The concentration of detergent above which monomers will self assemble into micelles

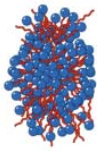
CMC is different and specific for each detergent

When working with membrane proteins a general rule of thumb is to use at least 2X the concentration of the CMC



Garavito RM *et. al.* JBC 2001

The Micelle



Asymmetrical and have rough surfaces where alkyl tails are disorganized

Few nm in diameter

Have a molecular weight of less then 100 kDa

Dynamic structures - micelles are in rapid exchange with free monomers

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Detergent Removal

1. Dialysis - detergent solutions can be diluted below the CMC so that micelles will disintegrate into monomers

Good for detergents with high CMCs

2. Absorption with hydrophobic beads

Good for detergents with low CMCs

3. Column chromatography

Commonly Used Detergents

Detergent	FW	Type	CMC mM	Aggregation #
Brij-35	1198	N	0.092	40
CHAPS	614.9	Z	8	10
Deoxycholic Acid	414.6	A	6	22
NP-40	603	N	50	100-155
Octyl-glucoside	292.4	N	18	
SDS	288.38	A	2.6	3
Triton X-100	647	N	0.23	75-165
Tween 20	1228	N	0.059	

Summary of Detergents

There is **NOT** a set of "golden rules" for the use of detergents for membrane proteins

Understanding of physical-chemical properties is useful in deciding which detergent to use

- Type of detergent
- CMC
- Aggregation number

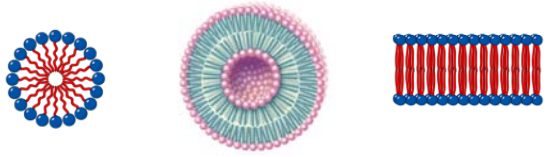
Detergents are expensive $$$$$$$$

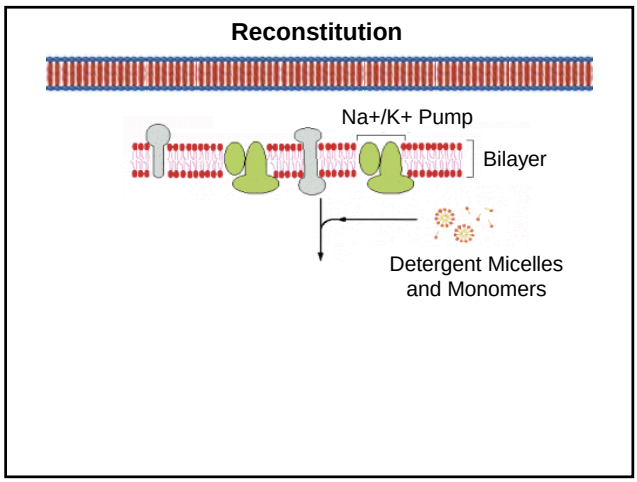
Reconstitution

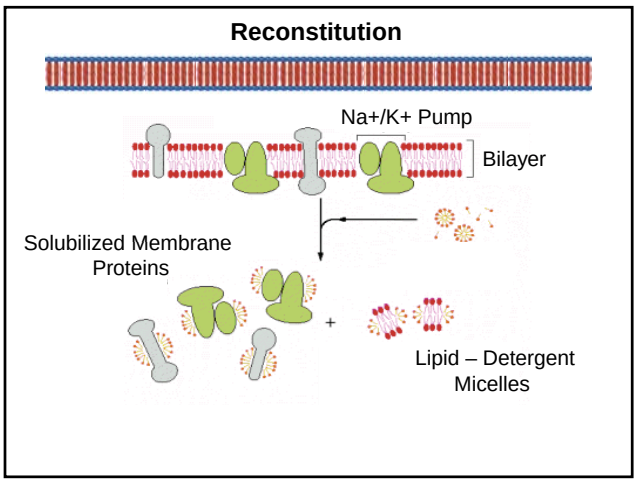
To study membrane protein function in more physiological environment

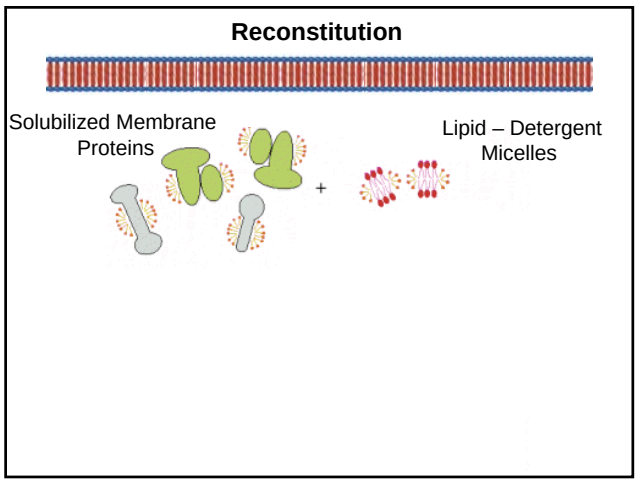
Higher Lipid Structure

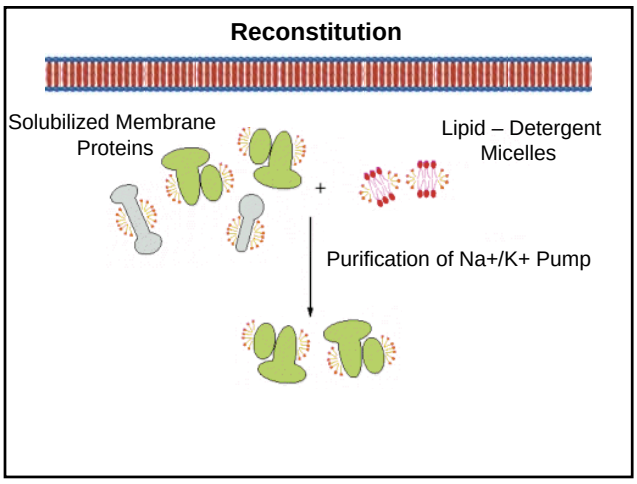
Micelle Liposome Bilayer




Reconstitution
Na+/K+ Pump
Bilayer
Detergent Micelles
and Monomers

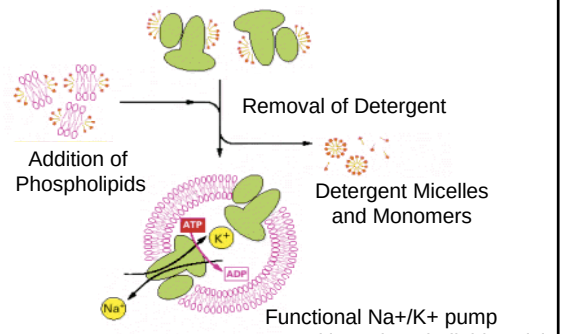

Reconstitution
Na+/K+ Pump
Bilayer
Solubilized Membrane
Proteins
+
Lipid – Detergent
Micelles


Reconstitution
Solubilized Membrane
Proteins
+
Lipid – Detergent
Micelles


Reconstitution
Solubilized Membrane
Proteins
+
Lipid – Detergent
Micelles
Purification of Na+/K+ Pump

Reconstitution

Reconstitution



Other Methods

Electrophysiology -

- Patch Clamping to measure current and voltage changes
- Capacitance - measure addition or subtraction of membranes

Microscopy -

- EM - freeze fracture
- AFM (Atomic Force Microscopy)
- FRAP (Fluorescence Recovery After Photobleaching)

Helpful References

Singer SJ Nicolson GL **The fluid mosaic model of the structure of cell membranes.** *Science* 1972; 175 (23):720-731

Kyte J Doolittle RF **A simple method for displaying the hydropathic character of protein** *J Mol Biol* 1982; 157(1):105-132

Fleischer S, Kervina M. **Subcellular fractionation of rat liver.** *Methods Enzymol.* 1974;31(Pt A):6-41.

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