

# Chapter 11

## Biological Membranes and transport

Problems: 3, 4, 5, 8, 15, 18

Additional extra credit homework available

### 11.0 Introduction (figure 11.1)

membranes define boundary of cell and regulate transport across that membrane

also define internal compartments within eukaryotic cells

flexible self-sealing, selectively permeable to polar solutes

include an array of specialized proteins

catalyzing various cellular process

transporters for ions and organic molecules

receptors for external stimuli

contain triggers for cellular adhesion

inside cell

organize certain cellular processes including energy transduction

### 11.1 Composition & Architecture

Molecular Constituents

A. Each membrane has own characteristic lipids & proteins

table 11-1 & figure 11-2

reflects function of cell

Myelin sheath wrap around neuron as insulation - primarily lipids

PM of bacteria , mitochondria - lots to do so more protein

Each organelle of each tissue of each species has own unique composition of lipid

Protein composition are even more varied - much functional specialization

Some membrane proteins are glycoproteins

Carbohydrate portion plays role in protein stability and intra cellular destination

Some proteins covalently anchored to lipids

B. Shared Properties

impermeable to most polar are charged solutes

permeable to nonpolar species

5-8 nm thin (50-80A)

trilaminar in cross section Figure 11-1

Currently described with **fluid mosaic model** Figure 11-3

phospholipids and sterol form a lipid bilayer

Nonpolars face each other  
 Polar/charged stick out  
 Proteins are imbedded in the bilayer sheet at irregular intervals  
 Held in membrane by hydrophobic interaction  
 Some proteins protrude from one or both sides  
 Are oriented in membrane - have in side and out side  
 since no covalent bonds holding together, everything is free to move  
 around (fluid) laterally  
 Constantly moving and changing  
 Lets check the details

### C. Lipid Bilayer

glycerophospholipids, sphingolipids & sterols virtually insoluble in water  
 when mixed with water spontaneously form aggregates with separate  
 phases

see figure 11-4

Depending on physical nature of lipid and conditions get three major aggregates

**Micelles** spherical structure

10's-1000's of molecules

Hydrophobic core with no water

Charged/polar surface

Usually seen when cross-section of head is > cross-section  
of tail

So molecules is wedge shaped

This includes free fatty acids, lysophospholipids,  
detergents

**Bilayer** 2 lipid monolayers fat to fat against each other

Usually when head and tail about same cross-section

Glycerophospholipids, sphingolipids

Has hydrophobic edge so not stable

Tends to fold back on itself to make...

**Liposome** or vesicle

Lose edge, gain max stability, forms interior water  
compartment

Biological membranes

Typical 3 nm, just right for a lipid bilayer

Behave just like liposomes for transport of ions

Have electron density on periphery

Membrane lipids asymmetric distribution in bilayer figure 12-5

But not as absolute as proteins

Usually distribution changes but usually same lipid found on both faces

Note new terminology that is introduced

Leaflet refers to a single layer of the bilayer

Cytoplasmic leaflet is the inside layer

Extracellular leaflet is the outside layer

D. Three types of membrane proteins **Figure 11-6**

**Integral proteins**

Firmly associated with membrane

Removed only with agents that disrupt hydrophobic interaction with membrane

Detergents, Organic solvents, denaturants

**Peripheral Proteins**

Associated with membrane via electrostatic or H-bonding interaction between hydrophilic part of protein and polar head group of lipids

Released by interfering with H-bonding or electrostatics  
Carbonate at high pH

May serve to limit mobility of integral proteins by tethering to intercellular structures

**Amphitropic Proteins**

Both in cytosol and associated with membrane

Its placement is regulated by the cell

Has non-covalent interaction with membrane lipids or proteins

Reversible interactions take protein on and off membrane

E. Many membrane proteins span the lipid bilayer

Topology determined by reagents that react with proteins, but cannot pass through membrane

**Figure 11-7**

Glycophorin example (from red blood cells)

Amino terminal domain on outer surface

Also domain with carbohydrates

Tell this because can be cleaved with trypsin or carbohydrate removing enzymes when put into solution with whole RBC's

C-terminal end on inside of cell b/c does not react under same conditions

Segment in middle (residues 75-93) highly hydrophobic

Suggests that is buried in membrane

Asymmetric orientation is a general rule for all membrane bound proteins  
Carbohydrate on outside is also a general rule

F. Integral membranes held in membrane via hydrophobic interaction with lipids

Figure 11-8

Classified in 11-8

All integral membrane proteins

At least 1 highly hydrophobic region

Long enough to span membrane when in  $\alpha$  helix

Can be anywhere in overall sequence

Some protein have multiple sequences

For a long time could do X-ray crystallography to see actual structure

But past 10-20 years have figured out how

Fig 11-9 & 11-10

Photosynthetic center of purple bacteria (11-9)

Is inside out protein

Hydrophobics on outside

Hydrophilics on inside

Often can see lipids included in crystal structure 11-10

Often around outside, oriented just as you would expect

Called **annular lipids** because form a shell (annulus) around protein

Also sometimes find between monomers of multisubunit proteins

Here thought to be a grease-seal

G. Some features of topology can now be predicted by sequence

Can't predict exact structure

but unbroken sequence of 20 hydrophobic residues is strong indicator that a trans-membrane protein

This rule applied to genomic sequences

10-20% of all proteins are integral membrane proteins!

20-30 hydrophobics

Just enough to span 30Å membrane if  $\alpha$  helix

Use hydrophobicity (hydropathy) plots to locate membrane spanning region

Figure 11-11

Use statistics and chemical knowledge to come up with hydrophobicity index (don't look too hard, you might see Dr. Z's name in a reference)

Take average over several residues

Move window down and take next average

## Plot

Also see Tyr & Trp at interface between lipid & water **Figure 11-12**

Thought to anchor lipids because interact both with lipid and water  
 Also see lys, his, arg (positives) on cytoplasm surface  
 (Positive inside rule)

Some integral proteins use  $\beta$  barrels to span

**Figure 11-13**

Used in many porins

Because extended only takes 7-9 residues to span

Every-other residue is hydrophobic

Can't use hydrophobicity plot!

But some success if predict  $\beta$ -barrel motif first!

Need complete barrel. Sheet alone is not enough

H. Anchor by Covalent attachment of lipids

**Figure 11-14**

covalent attachment to fatty acids, isoprenoids, sterols, or GPI's  
 (glycosylated derivatives of phosphatidylinositol)

Single hydrocarbon chain barely enough

Either multiple chains

Or additional ionic interactions with membrane surface

Positive on protein with negative of phospholipid

Lipid attachment may serve to target protein to specific membrane location

GPI's exclusively outer face in specific regions

Myristoyl or Farnesyl or Geranylgeranyl on outside

## 11.2 Membrane Dynamics

Key feature Flexibility

can change shape without becoming leaky

Happens because individual lipids not covalently attached to can move

A. Groups in Bilayer ordered to varying degrees

depend on lipid composition and T

Low temp - semi-solid gel phase

Individual motion constrained

Little lateral motion or tail motion

'Paracrystalline'

High temp - liquid-disordered state - or fluid state

Hydrocarbon chains of fatty acids in constant motion

FA's in lateral motion

Interior or region more like fluid  
 Intermediate T - liquid-ordered state  
 Less motion of fatty acid tails  
 But still lots of lateral motion of FA's in plane

At physiological T (20-40C)

Long chain saturated FA (16:0 & 18:0)

Pack well - would go into semi-solid gel phase

Unsaturated FA's or short FA, make more fluid, more liquid orderd phase

Sterols tend to push toward semi-solid

Cells regulate composition to keep membrane fluidity constant

Table 11-2

B. Transbilayer movement of lipids requires catalyst

Flip-flop of lipids Figure 11-16 is negligible

Polar headgroup doesn't want to enter membrane

Family of enzymes do this function

Flippases - translocate aminophospholipids (phosphatidyl serine and phosphatidylethanolamine) from extracellular leaflet to cytosolic leaflet (outside layer to inside)

Creates asymmetric distribution

Phospho-ser and -ethanol on inside of membrane

Sphingolipids and phosphocholine on outside

Used ATP

Important because having phosphoser on outside is a signal to trigger apoptosis - programmed cell death

Floppases -translocate phospholipids from cytosolic leaflet to extracellular leaflet (inside layer to outside)

Also ATP dependent

Part of ABC transporter family (See next chapter)

Scramblases

Move any phospholipid across bilayer to follow concentration gradient

Does not need ATP

Phosphatidylinositol transfer proteins

Used in Lipid signaling and cell trafficking

### C. Lipids and proteins Diffuse laterally

lateral diffusion is fast **Figure 11-17**

can take only a second for lipid to circumnavigate cell

so composition of inner or outer surface quickly homogenizes self

Somewhat contradictory evidence if follow an individual lipid on a faster time scale

**Figure 11-18**

Tends to move, but stay in a region

Some kind for local corral?

Proteins vary

Some free to diffuse all over

Some tend to aggregate in patches

Some tethered to internal structures inside of cell

**Figure 11-19**

IS this the source of the lipid corrals?

### D. Sphingolipids and cholesterol cluster in membrane rafts

**Note this material is new this addition so is cutting edge new!**

Glycosphingolipids (cerebrosides & gangliosides) - generally contain long chain saturated FA's

form transient clusters in outer surface

**Figure 11-20**

Appear to include sterols

Excludes glycerophospholipids

That usually have 1 short and one unsaturated

Call these clusters 'cholesterol-sphingolipid microdomains'

Can even see with atomic force microscopy

Thicker and more ordered

Physically harder to dissolve

May even be associated with integral membrane proteins anchored to 2 long chain saturated FA's or GPI anchored proteins

Behave like a 'raft' drifting on surface

Depending on cell, rafts may be up to 50% of surface

may be a way to 'glue' together membrane proteins that have to associate for activity

### C. Caveolins define a special class of membrane rafts

Caveolins - integral membrane protein with 2 globular domains

Located on inner cell surface (cytosolic leaflet)

3 palmitic acids on carboxy terminal domain for more anchor

Binds cholesterol

Forces associated membrane to curve inward

Forms 'little caves' **caveolae** in cell surface

**Figure 11-21**

Lot of implications

D. Membrane curvature and Fusion are used in many Biological Processes

Ability of membranes to fuse without losing continuity is very important  
Cellular membranes, from nuclear, ER, Golgi, and various small vesicles  
are constantly reorganizing

Also exocytosis, endocytosis, cell division, fusion of egg and sperm

Most of the above start with increase of curvature of a local area of  
membrane

Three possible models shown **Figure 11-23**

Fusion of membranes requires the following events

1. Membranes recognize each other
2. Surfaces become closely opposed - water removed from interface
3. Local bilayer structure breaks down and outer leaflets fuse
4. Bilayers fuse

The above event should be triggered by specific signals or appropriate  
times

Proteins that do this called fusion proteins

Not to be confused with proteins from fused genes that are also  
called fusion proteins!

Will skip the SNARE example from text

E. Certain integral proteins mediate Cell-Cell interactions and adhesion

Several families of protein are used for specific attachment points

Either between cells

Or cells to extracellular matrix

**Integrins**- hetero dimeric proteins

Anchored to membrane by a single transmembrane helix /subunit  
 $\alpha\beta$  dimer site for extracellular proteins to attach  
(Collagen or fibronectin)

18 different  $\alpha$ 's

$\geq 8$  different  $\beta$ 's

Also used as receptors and signal transducers

Other proteins involved in surface adhesion

Cadherins (used to bind to identical protein on neighboring cell)

Selectins

Use  $Ca^{+2}$  to bind to specific polysaccharides on surface of  
adjacent cell

Part of blood clotting

### 11.3 Solute Transport across Membranes

need to get lots of things (small molecules) across membrane  
 sometime this is with a concentration gradient, sometimes against  
 almost always this is done by proteins

#### A. Passive transport

When two compartments separated by a permeable divider have difference concentrations solutes there is a concentration gradient or a chemical potential gradient, and the molecules will move by **simple diffusion** until concentrations are equal and the chemical potential gradient is zero

When ions of opposite charges are separated by a membrane there is an additional **membrane potential** ( $V_m$  that can be measured in V or mV) and ions will move across the membrane until the membrane potential is zero as well.

Together these two factors are called the **electrochemical gradient** or electrochemical potential

In cells we have a selectively permeable membrane, the lipid bilayer

To cross a polar substance must get rid of shell of hydration, then diffuse the 3 nm through the lipids where it really doesn't want to be  
 Then water molecules return to make it happy  
 Get energy profile like [figure 11-27](#)

Total E barrier so high that virtually no polar or charged gets through without help

A few gases can make it  $O_2$ ,  $N_2$ ,  $CH_4$  since are nonpolar

$H_2O$  can cross some membranes because of high conc., but where you want fast transport (kidneys) need help as well

Help for polar charged - Membrane proteins that lower activation E of transport. Call this process **facilitated diffusion of passive transport**

Not technically enzyme since no chemical reaction occurs  
 Protein involved called **transporters or permeases**

Few crystal structures, hard to isolate and hard to crystallize  
 Kinetic experiments lead us to think work like enzymes

Bind substrate stereo specifically by weak, noncovalent

interactions

Binding interactions replace interaction lost to water interaction and lipid interactions

Protein usually contain 1 or more membrane spanning regions

So may form a pore with hydrophilic on inside and hydrophobic on outside

#### B. Transporters grouped into superfamilies based on structure

Probably ~1000 different transporters in human genome

A few hundred from various species studies

3-D structure for a handful

So sequence similarities, hopefully, help us to organize and rationalize

#### Figure 11-28 on board

Transporters

Channels

Fast rate - approaching diffusion

Less stereospecificity

Usually not saturable

Carriers

Bind substrate with high specificity

Rate well below that of diffusion

Can be saturated

Subfamilies

Channels

Primary helical

Primarily beta barrel

### Carriers

- Facilitate diffusion down a concentration gradient
  - Usually uniporters
- Active transporters - move against conc gradient
  - Primary active transporters
    - Use E of a chemical reaction directly
  - Secondary active transporters
    - Couple a 'downhill' transport with an 'Uphill' transport

### Examples

#### C. Glucose Transporter in Red blood cells

Erythrocytes (like all cells) need E, use Glucose to get E. Glucose in blood is about 5mM  
get into cell via specific glucose transporter, about 50,000 faster than diffusion

Well studied transport is example 2

Transporter called GluT1

Type III integral protein

MW 45,000 12 hydrophobic regions - thought to be spanning helices

Most are amphipathic - both hydrophobic & hydrophilic

no X-ray structure

one model is side by side helices make transmembrane pore

Need 5-6 helices for a pore large enough for glucose

Can plot velocity of glucose transport vs external [Glucose]

see [figure 11-30](#)

Looks like an enzyme, same hyperbolic function as a saturating enzyme  
can derive rate constant just like an enzyme

Proposed mech see [figure 11-31](#)

Has a  $K_m$  of 1.5 mM for D-glucose

$K_m$  of 20 mM for mannose and 30 mM for galactose

So 10 fold selective for glucose

3000 mM for L-glucose to even more selective

Purely passive transport, glu going in and out of cell depending on concentration gradients

12 glucose transporters in human genome so far

Each unique kinetics, distribution and function ([table 11-3](#))

#### D. Chloride and Bicarbonate Cotransport

erythrocyte also used for transport of  $\text{CO}_2$  out of tissues

Pick up  $\text{CO}_2$  in peripheral tissues

in erythrocyte converted to  $\text{HCO}_3^-$  by carbonic anhydrase

$\text{HCO}_3^-$  goes back to plasma for transport to lungs In lungs then has to shuffle back to  $\text{CO}_2$  again

So need fast transport of  $\text{CO}_2$  and  $\text{HCO}_3^-$

$\text{CO}_2$  is nonpolar, so passes membrane all by itself with no help

$\text{HCO}_3^-$  an ions so it needs help

#### **Chloride-bicarbonate exchanger or anion exchange protein**

Increases permeability of  $\text{HCO}_3^-$  by  $>1,000,000$

Integral membrane protein

12 membrane spanning regions

Classed as a cotransport system

MUST transport a Cl in opposite direction of  $\text{HCO}_3^-$

Why would cell want to do this?

Ion gradient - if move - one way, get charge imbalance + fouled gradient up

Talk about this a bit

**Uniport** proteins carry 1 solute across membrane

**Cotransport** proteins, carry two solutes across membrane at 1 time

**Symport** both molecules move in same direction

**Antiport** solutes go in opposite directions

#### E. Active transport

movement against an electrochemical gradient

energetically unfavored, so must be coupled to exergonic process

#### **Primary active transport**

Transport coupled directly to a exergonic chemical reaction

#### **Secondary active transport**

A cotransport system where the transport of one molecule going with its gradient is used to push as second solute against its gradient

For Chemical equation have seen calculate E needed using equation:

$$\Delta G = \Delta G^{\circ} + RT \ln K$$

$$\Delta G = \Delta G^{\circ} + RT \ln P/R$$

by analogy, the E for a transport system where P is a concentration across a membrane and R is the concentration on this side of a

membrane

$$\Delta G = \Delta G^{\circ'} + RT \ln C_2/C_1$$

So if there is a 10 fold difference in concentration

$$\Delta G = 8.315 \text{ J/molK} \times 298\text{K} \times \ln(10/1) = 5,705 \text{ J or } 5.7 \text{ kJ}$$

This hold for UNCHARGED solutes

If an ion moving without its counterion, then you are also creating an electric field, and making electrical work. In this case the equation must be modified

$$\Delta G = \Delta G^{\circ'} + RT \ln C_2/C_1 + Z F \Delta V$$

F - charge on ion

F is Faraday's constant (96,480 J/Vmol)

$\Delta V$  is potential across membrane (in volts)

Eukaryotic Cells  $\Delta V$  is .05 to .2V, interior negative relative to outside thus can be significant contribution

Four types of ATP-dependent active transporters (Transport ATPases)

P-type: reversibly phosphorylated by ATP during transport process

Will show two examples

F-type : ATP synthases If no protons, simply hydrolyses ATP

If proton gradient, proton move down gradient to make ATP

V-type: V for vacuole ATP E is used to pump protons into a vacuole against its gradient

ABC transporters

F. P-type ATPases Undergo phosphorylation

Cation transporters, reversibly phosphorylated by ATP as part of transport

Similar sequences, especially near ASP that is phosphorylated

All sensitive to inhibition by Vanadate

(phosphorous analog - see top left hand column page 397)

Integral membrane protein, multiple membrane spanning regions

Also have a second subunit

Widely distributed

Na K ATPase Na K antiporter

Ca ATPase Ca uniporter

H/K ATPase H AND k TO ACIDIFY STOMACH

Best understood are the P-type  $\text{Ca}^{2+}$  pump

Sarcoplasmic and endoplasmic reticulum calcium (SERCA) pump

Two closely related pumps

Used to pump  $\text{Ca}^{2+}$  out of cytosol  
 Why? Cell filled with  $\text{P}_i$  and  $\text{PP}_i$  at mM conc  
 If Ca high, get ppt

The sarcoplasmic pump **Figure 11-35**

Pumps CA into sarcoplasmic reticulum  
 A specialized organ of muscle cell part of mechanism  
 for making muscle contract

80% of protein in sarcoplasm

110,000 Molar mass

10 membrane spanning regions

3 cytosolic domains made from long loops

N domain- binds ATP and  $\text{Mg}^{2+}$

P domain - has Asp that gets phosphorylated for  
 mech

A domain -actuator- interface between N & P

M domain- refers to transmembrane domain

Mechanism **Figure 11-36**

Walk through mech.

Using ATP to phosphorylate the P site causes a large  
 conformation change - key to mechanism

Second example  $\text{Na}^+\text{K}^+$  ATPase (**figure 11-37**)

in almost all animal cells

$\text{Na}^+$  lower inside than outside

$\text{K}^+$  Higher inside than outside

established by  $\text{Na}^+ \text{K}^+$  ATPase

1 ATP moves 3  $\text{Na}^+$  out and 2  $\text{K}^+$  in

Integral membrane protein

2 subunits 50,000 and 110,000

Both membrane spanners

Mech not certain, but current theory **figure 11-37 & 11-38**

Don't have as much structural detail so go with clamshell model

Enzyme<sub>i</sub> high affinity for Na, Binds Na from interior

ATP to ADP plus P-Enz<sub>ii</sub>

(i.e. get phosphorylated)

This form low affinity for Na, High affinity for K

Kicks Na to outside, grabs K

Now enzyme de-phosphorylated

Returns to original state and recycles

3+ out 2+ in

Electrogenic - Generates a charge imbalance across  
 membrane

-50 to -70 mV (inside neg relative to outside)

Keeping this imbalance is extremely important to cell

It is estimated that this reaction alone uses about 25% of bodies' E at rest

#### H. F Type ATPases **Figure 11-39**

Main role in mitochondria and chloroplast ATP synthesis

(F for energy coupling factors)

Use ATP to push  $H^+$  against a gradient

But actual role is reverse:  $H^+$  down gradient to make ATP

More appropriate name **ATP synthases**

$H^+$  gradient made by oxidation and light powered proton pumps

Multisubunit protein

$F_0$  a transmembrane pore (integral membrane protein)

$F_1$  a peripheral protein that does the ATP synthesis

#### I. V-type ATPases - Proton pumps **Fig 11-40**

Structurally and perhaps mechanistically similar to F-type

Used to acidify intracellular compartments of many cells

For instance vacuoles of plants and fungi pH 3-6

(V for vacuole)

Also lysosomes, endosomes, Golgi complex, secretory vesicles

Are NOT cyclically phosphorylated

Not inhibited by vanadate

All have similar complex structure (see figure)

All have membrane binding domain that serves as a proton channel

All have a peripheral domain that is ATP binding site and contains ATPase

#### J. ABC Transporters

ABC - ATP Binding Cassette

Large family of ATP dependent transporters that move 'stuff' out of cell

Amino acids, peptides, proteins, metal ions, lipids, bile salts, hydrophobic compounds

One called Multi-drug transporter (MDR1) responsible for drug resistance in tumor cells because pumps hydrophobic drugs out of cell!

All ABC's have

2 nucleotide binding domains (NBD's)

NBD's similar sequence  
 Presumably a conserved molecular motor  
 Use to transport other things  
 2 transmembrane domains  
 In some proteins all domains are part of a single polypeptide  
 Sometimes 2 subunits each with an NBD and a  
 transmembrane  
 Many found in plasma membrane, but some in ER, mitochondria or  
 lysosomes  
 Most are pumps, but some are ATP activated ion channels

Structure Fig 11-41

Transport a wide variety of things  
 Some are very specific  
 some are very promiscuous  
 At least 48 gene in human genome  
 Defect lead to a variety of diseases - See box 11-3

Seen in plants and microorganisms=

K. Ion gradients provide E for secondary active transport  
 Gradient made by  $\text{Na}^+$  or  $\text{H}^+$  then used as driving force for cotransport of  
 other solutes  
 a table of some of these cotransport system [table 11-4](#)

Example: E coli *lactose permease*

Usually high  $\text{H}^+$  in periplasmic space due to transport of H  
 Let protons back in cell and used to move other things  
 Lactose transporter Cal 100X higher in cell  
 (Transports lactose)  
 417 residues  
 Acts as monomer  
 Lets 1 proton in for each lactose

Member of MFS - Major Facilitator Superfamily

28 subfamilies

Most with 12 transmembrane helices

Little sequence homology

Thought to have similar topology/3D structure?

[Figure 11-43](#)

6 helices N terminal half

6 helices C-terminal half

Roughly 2 fold symmetry

In crystal form

See large aqueous cavity with substrate binding site

On cytosol side

No opening to outside

Propose transport involves rock motion of two domains

Coupled with substrate binding & proton movement

So clamshell model not all that far fetched!!

Example II Intestinal epithelial cells (figure 11-44)

Glucose and certain AA's

Have high Na in intestinal lumen

Let in with glu or AA

2 Na/1 glu

Using both the Na conc and cell electric potential

One in cell use a uniporter system to let into blood

Also have Na K pumping Na out of other side so Na doesn't get too big in cell

Since ion gradients are used in almost any cell for active transport and E synthesis

Any drug that collapses gradient is a poison

Valinomycin and monensin - antibiotics Figure 11-45

Are ionophores ion-bearers

L. Aquaporins form hydrophilic transmembrane channels

1 family of integral membrane proteins called aquaporins (AQP's)

Used for rapid movement of water

Used in erythrocytes so can swell and shrink depending on osmolarity of surrounding, particularly in kidney microtubules where ion concentration are used to first remove ions, then reabsorb water

also used in vacuoles of plants to open and close cell in response to changed in osmotic pressure

Used on water secreting exocrine glands that produce sweat, saliva or tears

All AQP's type III membrane proteins (several spanning regions, + hydrophilic regions both sides of membranes)

AQP-1 MW = 28,000 6 membrane spanning helices, in addition works as a tetramer to make hole in membranes Figure 11-46

Hole just large enough for single file of water molecules. Water can flow at rate of  $10^9$  molecule/sec (fastest enzyme  $4 \times 10^7$ /sec)

High rate suggests that is indeed just a hole in the cell, but so small does not allow passage of ions or other small solutes

While lets water through, will not let  $H_3O^+$  through.

Why is this important? (Would collapse proton gradient)

How does it do this?

Structure - 4 identical monomers (28,000 each)

Each monomer has 6 transmembrane helices and two shorter helices with  
Asn-Pro-Ala (NPA)

Located near middle of pore

The two short helices meet in the middle

Residues lining pore are generally hydrophobic but carbonyl O's are spaced out to allow water to make a single file

ASN in NPA spaces out water molecules so cannot do proton hopping

Also Arg & His residues repel + charges so proton further prevented

#### M. Ion selective Channels

First recognized in nerve cells

now know in all cells, as well as intracellular membrane of eukariotes

Ion channels coupled with ion pumps

Determines cells permeability to specific ions

Regulates cell's internal conc of given ions

In nerve cells very rapid changes in these levels

Used to send polarization wave down a nerve cell

Used to trigger muscle contraction

Used to trigger muscle contraction

Not same as ion transporter

Much faster  $10^7$  -  $10^8$  ions per channel/second

Almost the diffusion max

Cannot be saturated

Are 'Gated' turned on and off in response to event

Ligand-gated channels - binding of some allosteric effector turns on

Voltage-gated channels- Charge on protein domain moves due to membrane potential, causes channel to open or close

First recognized in neurons

Now observed in PM of all cells

In general very fast on in a fraction of a msec, and one for a few msec

N. Ion Channel function is measured electrically  
 Channel usually open for a millisecond  
 most chemical instruments can't go that fast  
 need to measure electrically  
 As voltage or current

Patch Clamp technique Neher & Sakmann 1976

Figure 11-47

Pick a few channels off a membrane and measure current flow with an electrode

Can measure  $10^4$  ions moving through a single channel in 1 ms  
 Can measure exact on/ off  
 Can measure that it takes 2 acetylcholine molecules to open a channel

O. Structure of  $K^+$  channel

1998 *Streptomyces lividans*

Sequence similar to all other known  $K^+$  channels  
 Including those in neurons

Figure 11-48

Sequences most similar in pore region

$K^+$   $r = 1.33 \text{ \AA}$  passes 10,000 faster than

$Na^+$   $r = .95 \text{ \AA}$

(For comparison Water O-H distance  $e .9 \text{ \AA}$ , O-H H bond  $1.8 \text{ \AA}$ )

K rate about  $10^8$  /sec, just about at diffusion

4 identical subunits that span the membrane  
 Each subunit membrane spanning helices  
 Makes a cone, with wide end toward extracellular space

At both side of pore are several neg charged residues, presumable to attract cations

On inner side, wide water filled cavity

About 2/3 through membrane channel narrow, so water must be stripped of ion

Water coordination replace by carbonyl O of protein

Figure 11-49

Just the right side fo K

But too far away for Na

A few well placed mutations can remove selectivity

K pass through single file

In crystal structure can se 2 K one at each end, about  $7.5 \text{ \AA}$  apart

Since may very similar channels getting this one right was a major breakthrough

#### Mammalian Voltage Gated $K^+$ Channel

Structurally more complex

**Figure 11-50**

Mech of channel similar, but add protein domains to sense membrane potential and trigger opening and closing of channel

one critical transmembrane helix contains 4 Arg's - thought to move helix up and down in membrane in response to membrane potential

Also have  $Na^+$  or  $Ca^{2+}$  channels that exclude  $K^+$

Cavity designed to fit the hydrated radius of ion

#### P. Gated channels central in neuron function

Must have high specificity and fast response

Neuronal  $Na^+$  Channel (**Voltage gated** ion channel)

$Na$  channels in nerve and muscles cells

highly selective for  $Na$  (100x or more)

High flux rates ( $>10^7$ )

Normally closed

Opened (activated) by change in membrane potential  
within millisecond closes back down

Basis for signals going down neuron

Acetylcholine receptor ( a **ligand gated** ion channel)

Passage of electrical signal from a neuron to a muscle cell

Nicotinic means sensitive to nicotine

Differs from a muscarinic acetylcholine receptor

Muscarin a mushroom inhibitor

Acetylcholine (**structure left hand column page 410**)

Released from motor neuron

Diffused a few  $\mu m$  to PM of muscle cell

Binds to receptor

Channel opens

$Na^+$ ,  $Ca^{2+}$ ,  $K^+$  pass easily

Other cations and anions blocked

$Na$  passage can't be saturated

$2 \times 10^7$  ions/sec

Channel then shuts off - hence the term 'gated'  
 Inward flow of + charge depolarizes myocyte (Cell potential to zero)  
 This triggers muscle contraction

Gating mechanism not know  
 Mech for this is not known

Ion channels that respond to  $\gamma$ -aminobutyric acid (GABA), glycine and serotonin appear to be in same superfamily, so probably work in same way

GABA and Glycine are  $\text{Cl}^-$  or  $\text{HCO}_3^-$  specific  
 Serotonin is cation specific

Another class of ligand gated ion channels respond to INTRACELLULAR

Ligands:

cGMP vertebrate eye  
 cGMP, cAMP olfactory nerves  
 ATP inositoltriphosphate in many cells

These types typically 6 membrane spanning helical domains  
 More detail chapter 13 (Not covered)

#### R. Physiological consequence of defective ion channels

Genetic defect in voltage gated NA channels of myocytes  
 Paralysis or stiffness

AA change in chloride channel causes cystic fibrosis  
 (Defect is not in Cl channel but in cell response to Cl)

#### Toxins

Tetrodotoxin (pufferfish)  
 Saxitoxin (red tide organism)  
 Bind to voltage gated Na Channels  
 Saxitoxin not poisonous to shellfish, but concentrate it to kill us!

Several other toxins mentioned including active toxin of curare and 2 snake venom but not much detail