Membranes & Membrane Proteins

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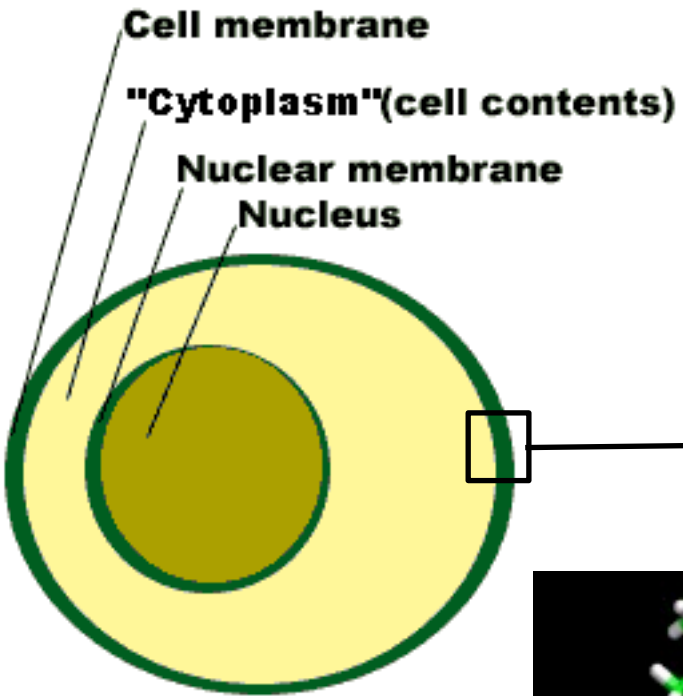
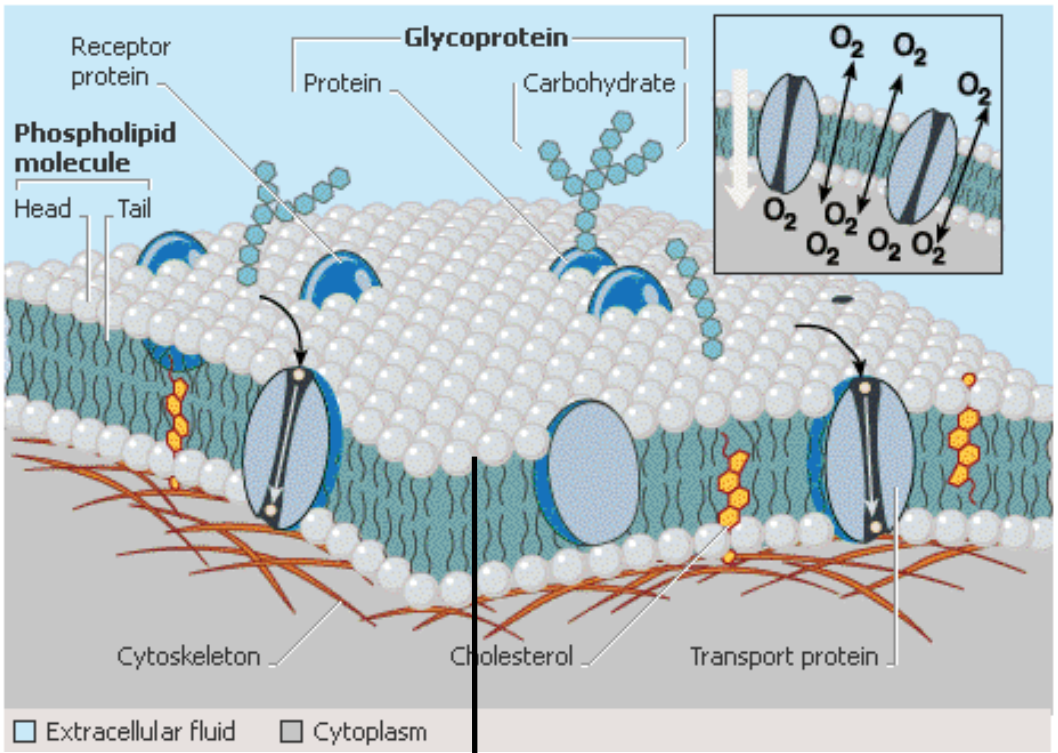
Chennai

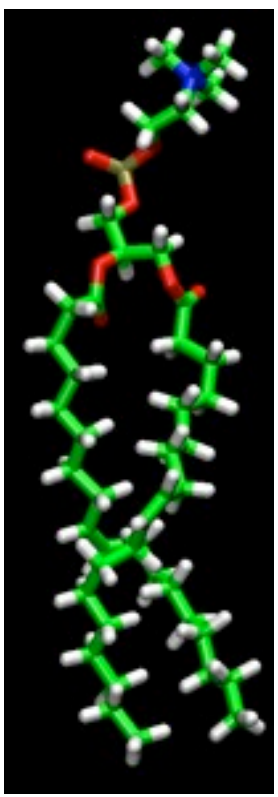
November 11 2010
IIT Kanpur

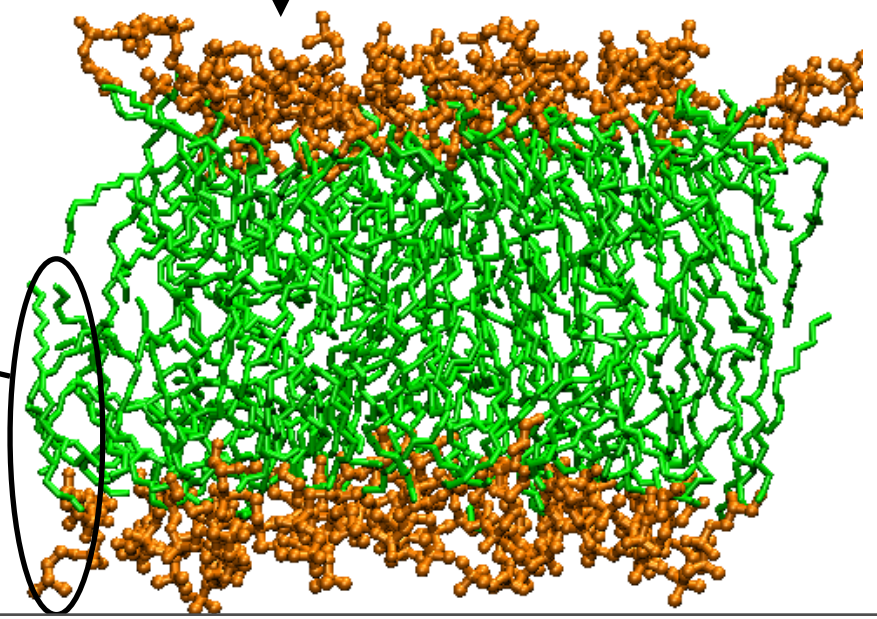
Perspective



Cell membrane



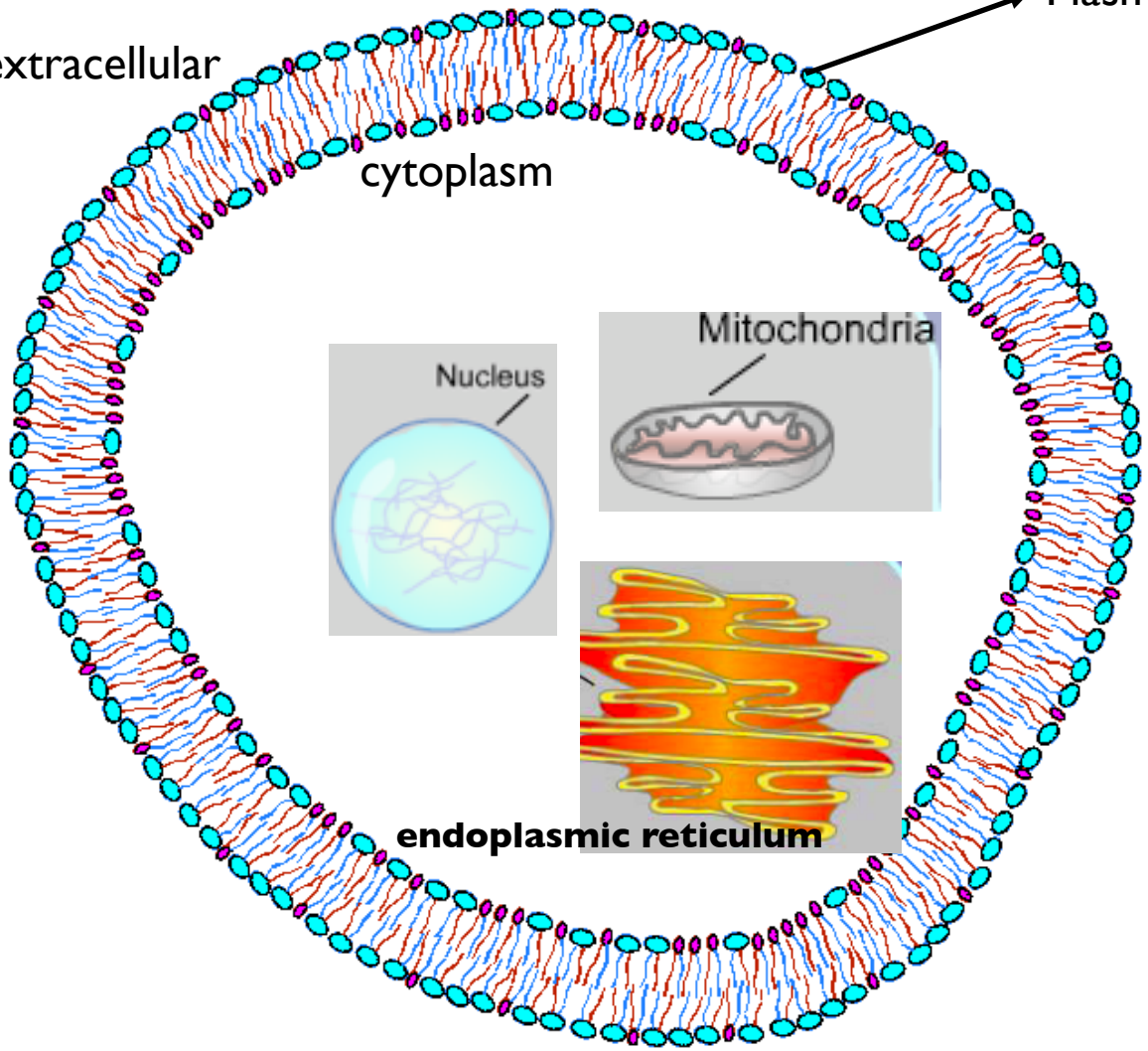




http://www.mcld.co.uk/hiv/images/aEukaryoticCell.gif

http://images.encarta.msn.com/xrefmedia/aencmed/targets/illus/ilt/T628876A.gif

Cellular Environment



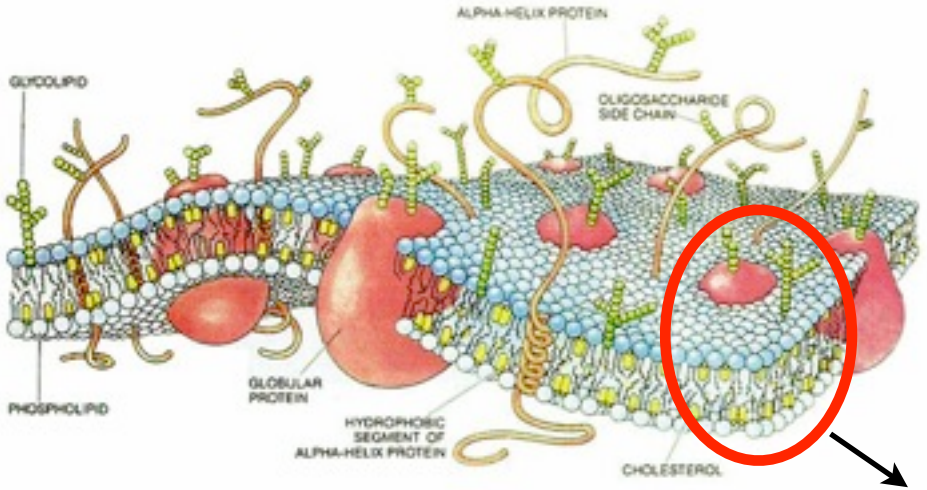
- acts as a compartment, only few molecules thick (60-100 Å)
- lipid molecules are added/removed, as the cell dimension changes
- can self-heal
- held together by hydrophobic/ noncovalent interactions
- has a fluid-like structure with rapid lipid diffusion
-electrical potential across membranes : -60 mV
- lipid molecules are synthesized in ER

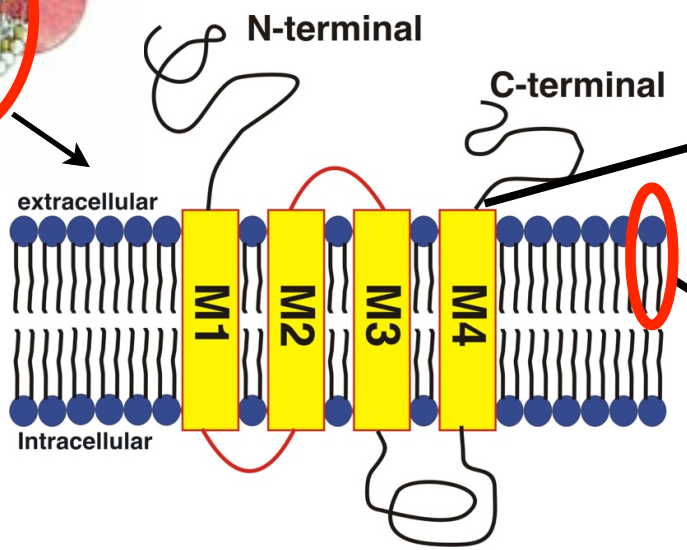
no membrane → no cell → no life

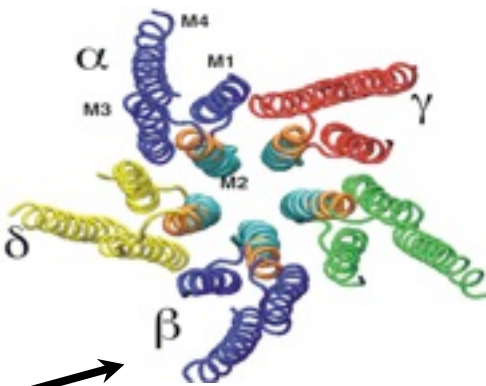
www.elsomresearch.com/.../nanosomes.htm

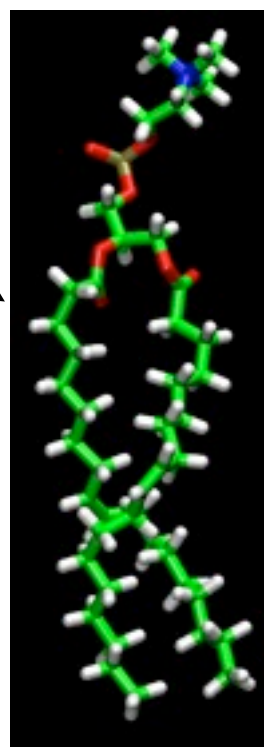
http://learn.genetics.utah.edu/units/basics/cell/

Membranes - Components







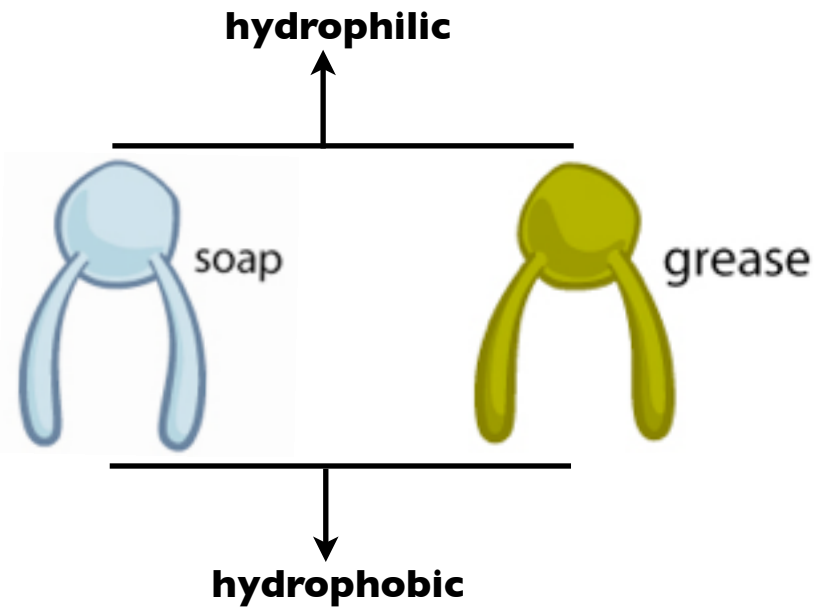


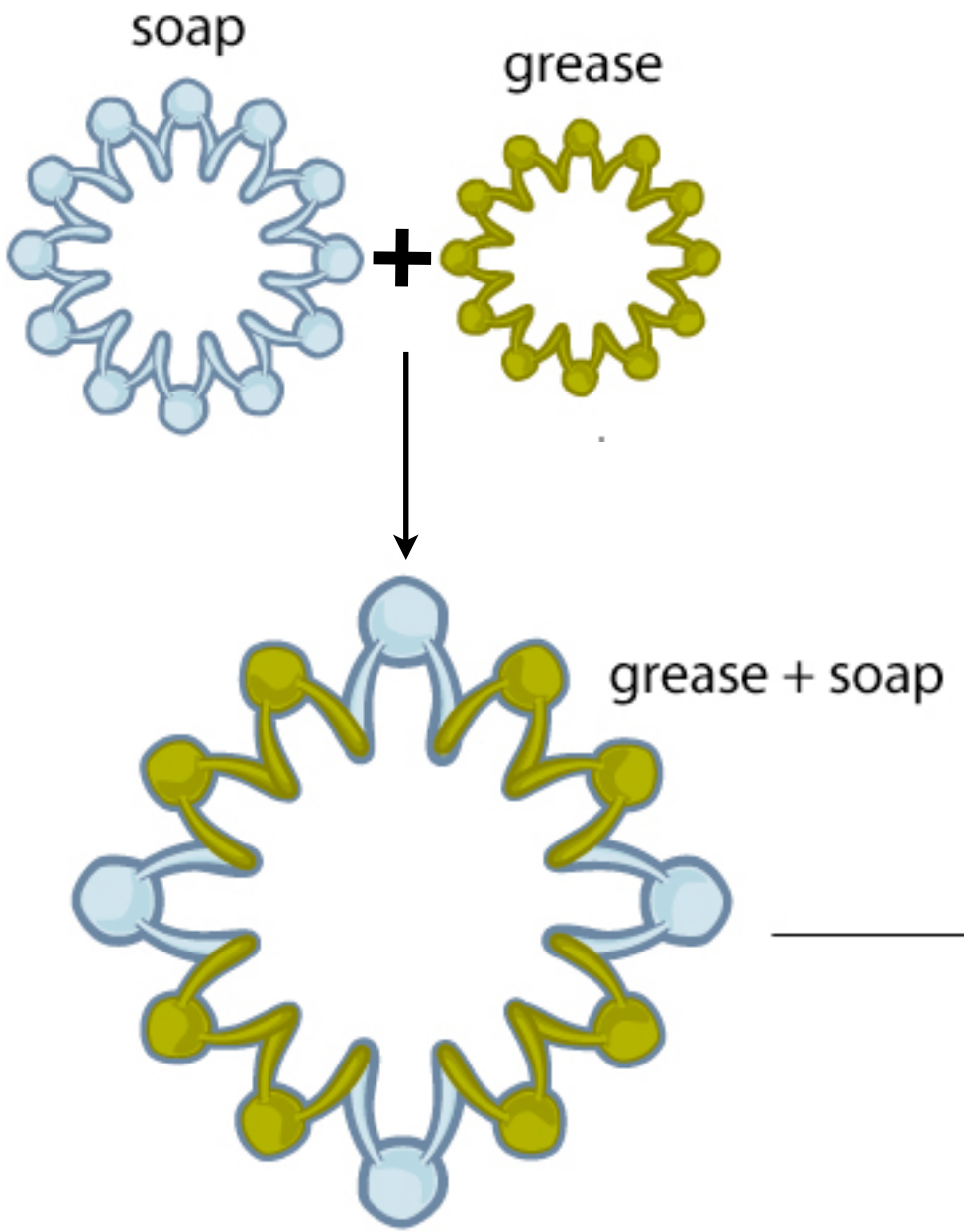
- membranes mostly contain lipids and proteins
- membrane lipids contain *hydrophobic* and *hydrophilic* moieties
- form barriers to free flow of charged species
- ~30% of proteins are membrane proteins
-~50% of current drug targets are membrane proteins

tosaweb.ncsd.k l 2.wy.us/.../diffusion_index.html

http://www.ncnr.nist.gov/programs/reflect/rp/biology/cell_membrane.html

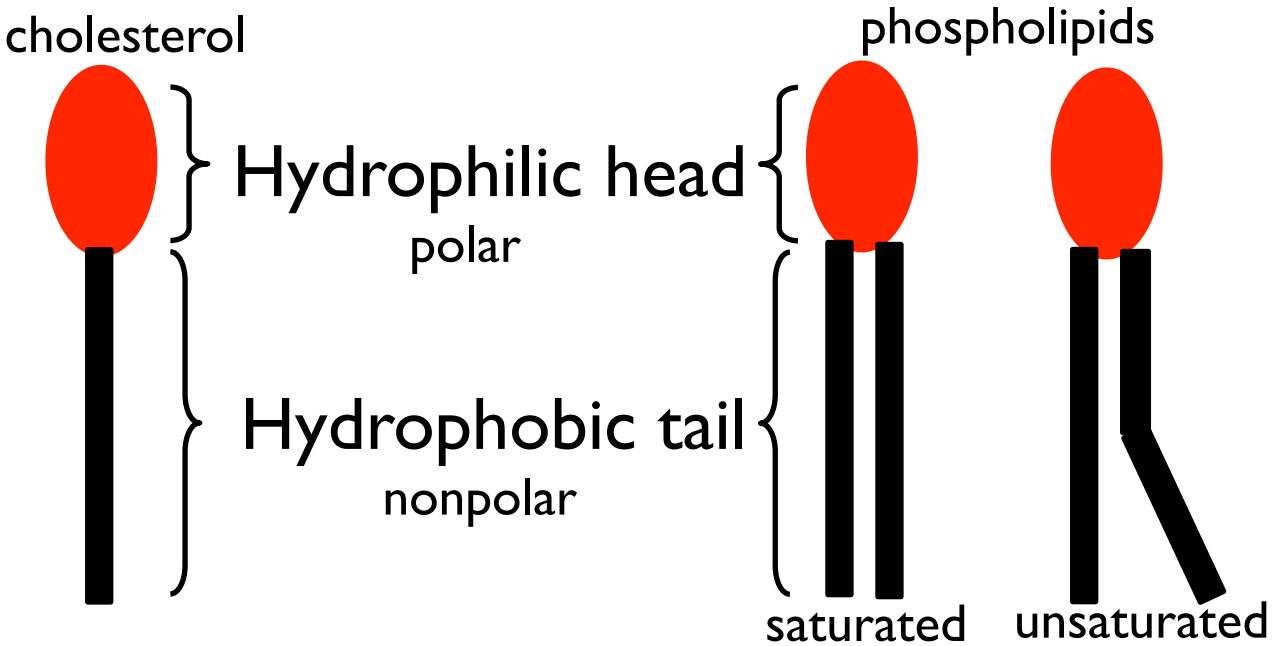
What is a lipid?

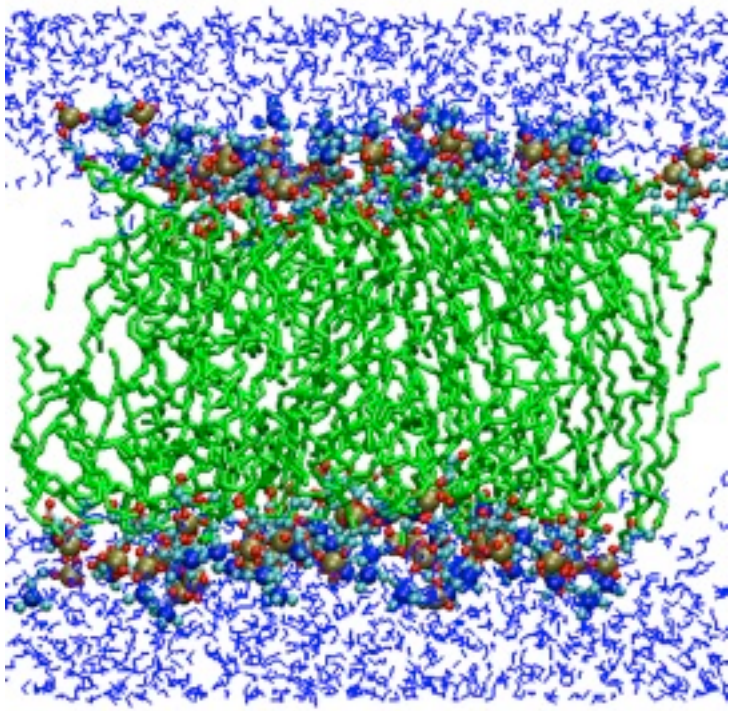




http://learn.genetics.utah.edu/content/labs/extraction/howto/detergent.html

Lipid Structure





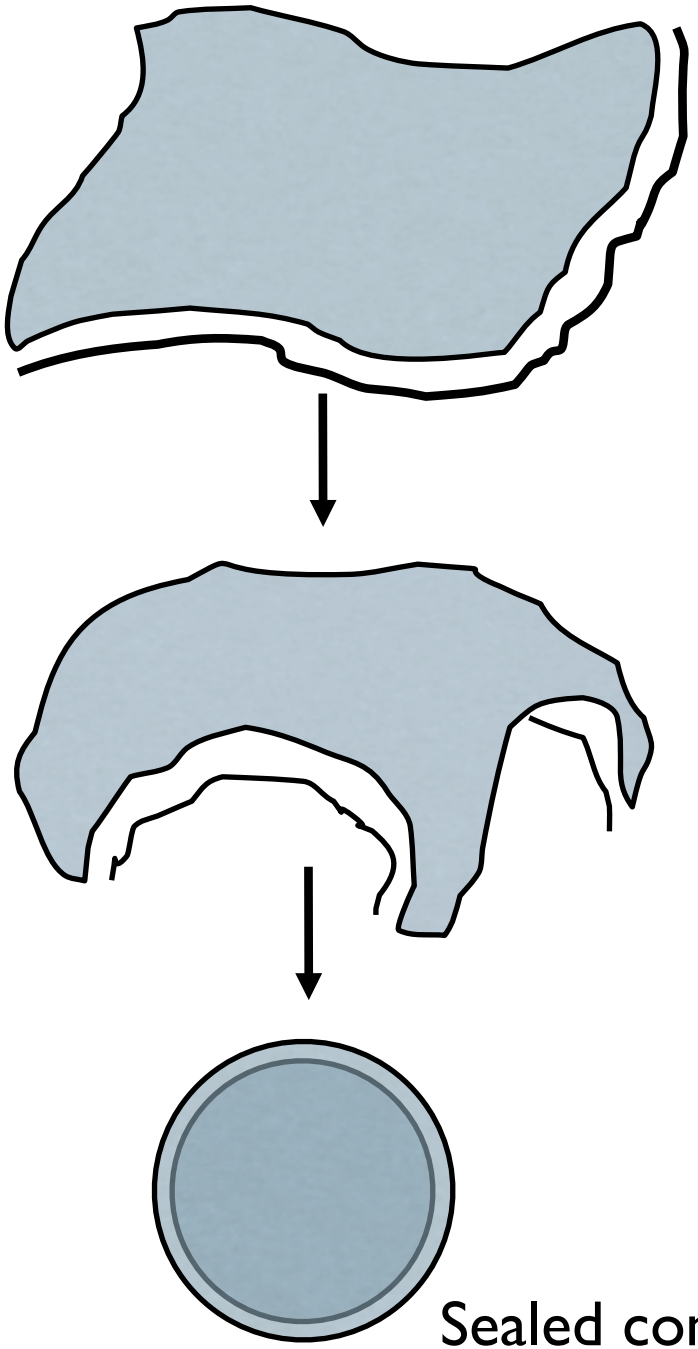
Lipid molecules are amphipathic

Hydrophilic molecules readily dissolve in water (forming favourable electrostatic / hydrogen bond interactions)

Hydrophobic molecules are insoluble in water. Energetic cost minimized if hydrophobic molecules cluster (e.g., oil coalesces to form a drop when dispersed in water)

Conflicting forces experienced by amphipathic molecules resolved in the formation of bilayer - energetically most favourable

Lipids to Cells

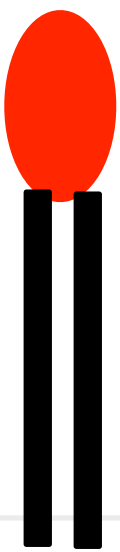


Free edges with exposed hydrophobic tails

- Self-healing property of lipids
- Free edges are energetically expensive

- overriding principle: free edges should be eliminated
- profound effect: formation of closed compartmental structures

- Amphipathic nature of lipids is fundamental

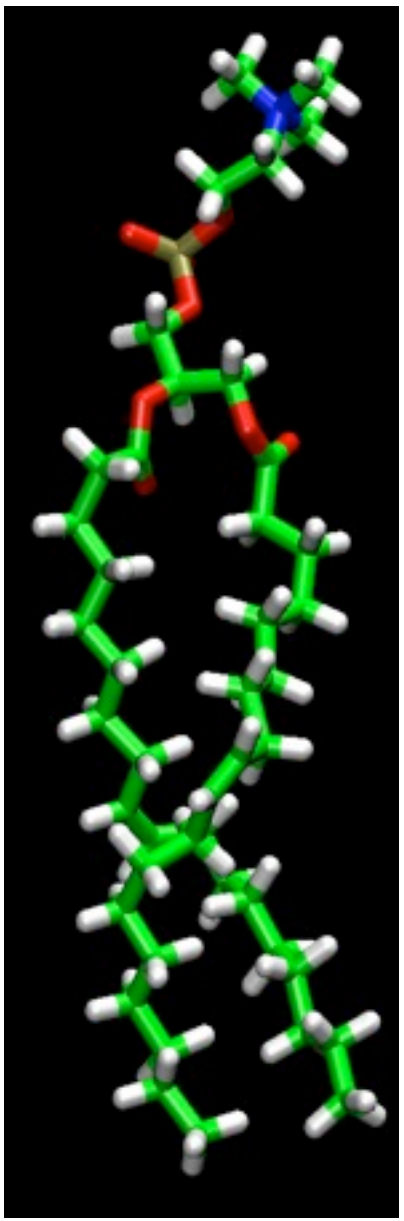


Sealed compartments

Phospholipid Structure

Polar

Non-polar



Head group

Phosphate

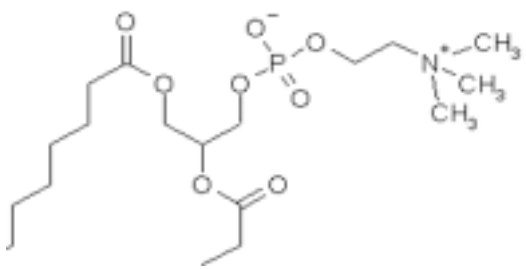
Glycerol

Fatty acid chain

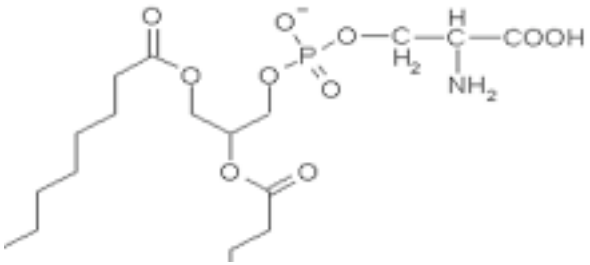
Fatty acid chain

zwitterionic

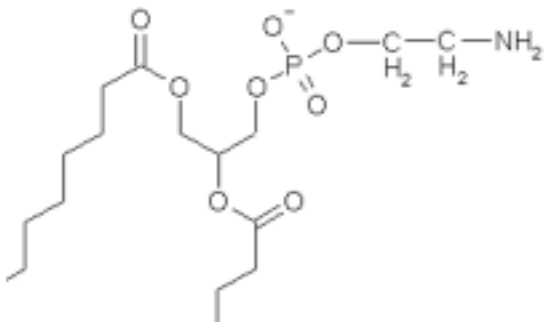
charged



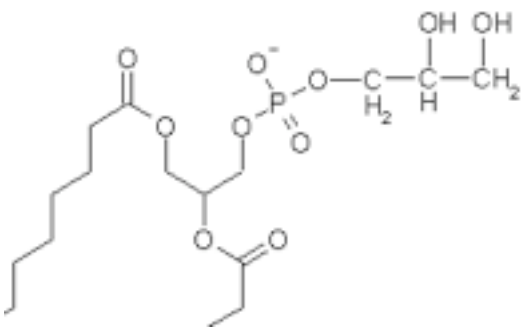
Choline - phosphatidyl choline. (PC)



Serine - phosphatidyl serine (PS)



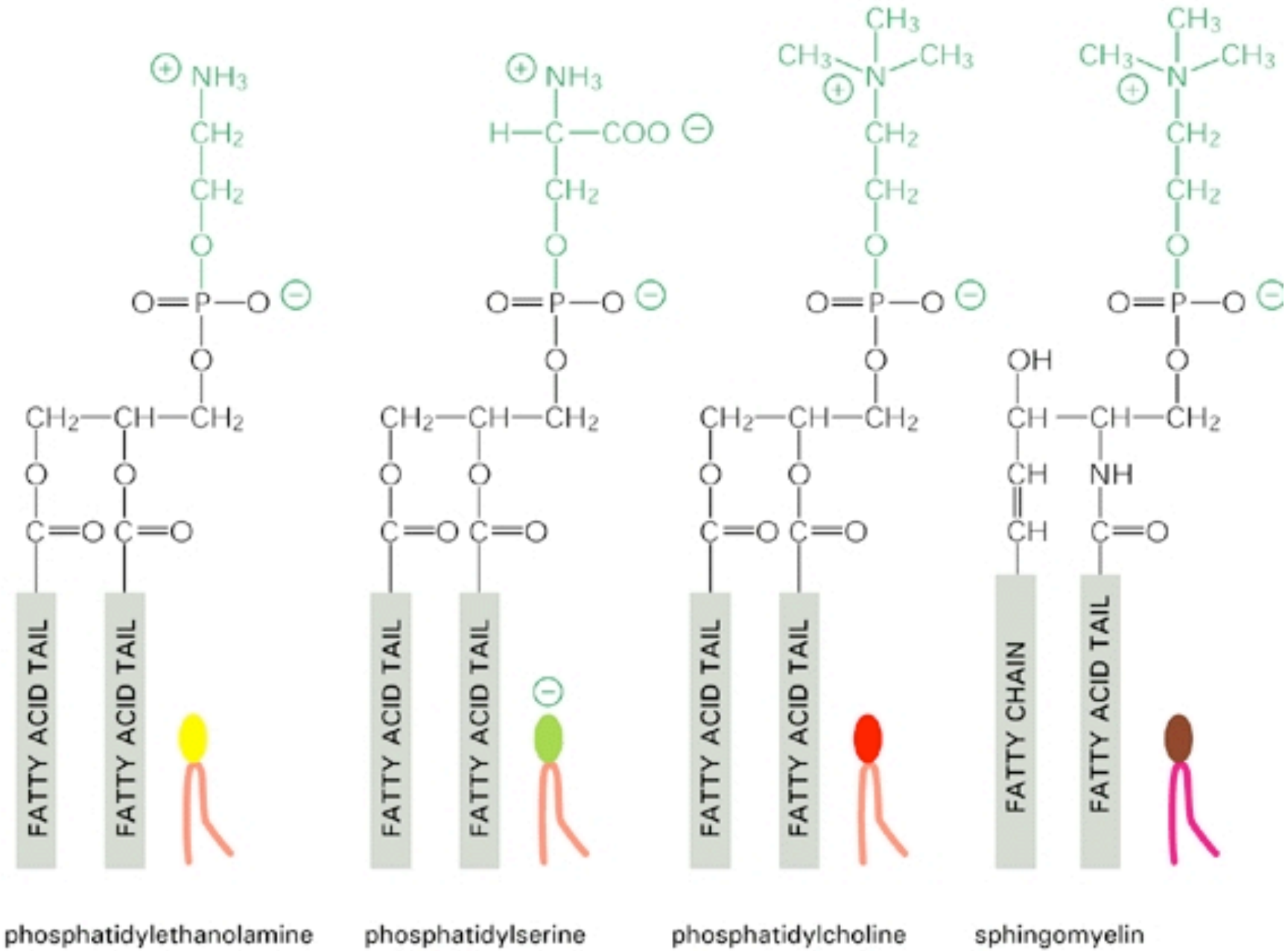
Ethanolamine - phosphatidyl ethanolamine. (PE)



Glycerol - phosphatidyl glycerol.(PG)

Charged lipids: ~ 10%

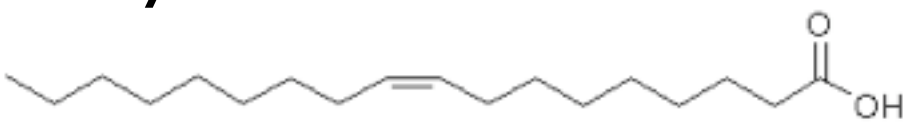
Lipid : Head groups



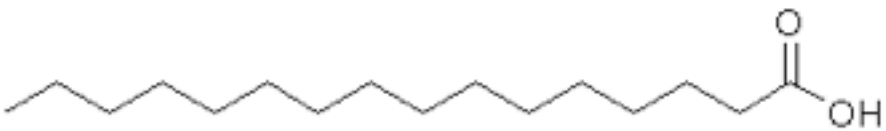
http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=cell&part=A2446

Lipid: Fatty Acid Chains

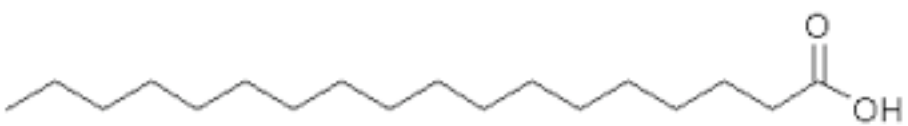
Fatty acid chains



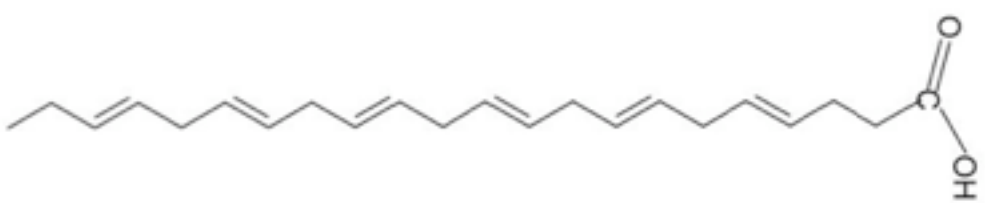
Oleic acid: monounsaturated C18 (18:1)



Palmitic acid: saturated C16 (16:0)



Stearic acid: saturated C18 (18:0)



Docasahexaenoic acid: saturated C22 (22:6)

Lipids can have two fatty acid chains of unequal length one or more chains can be unsaturated

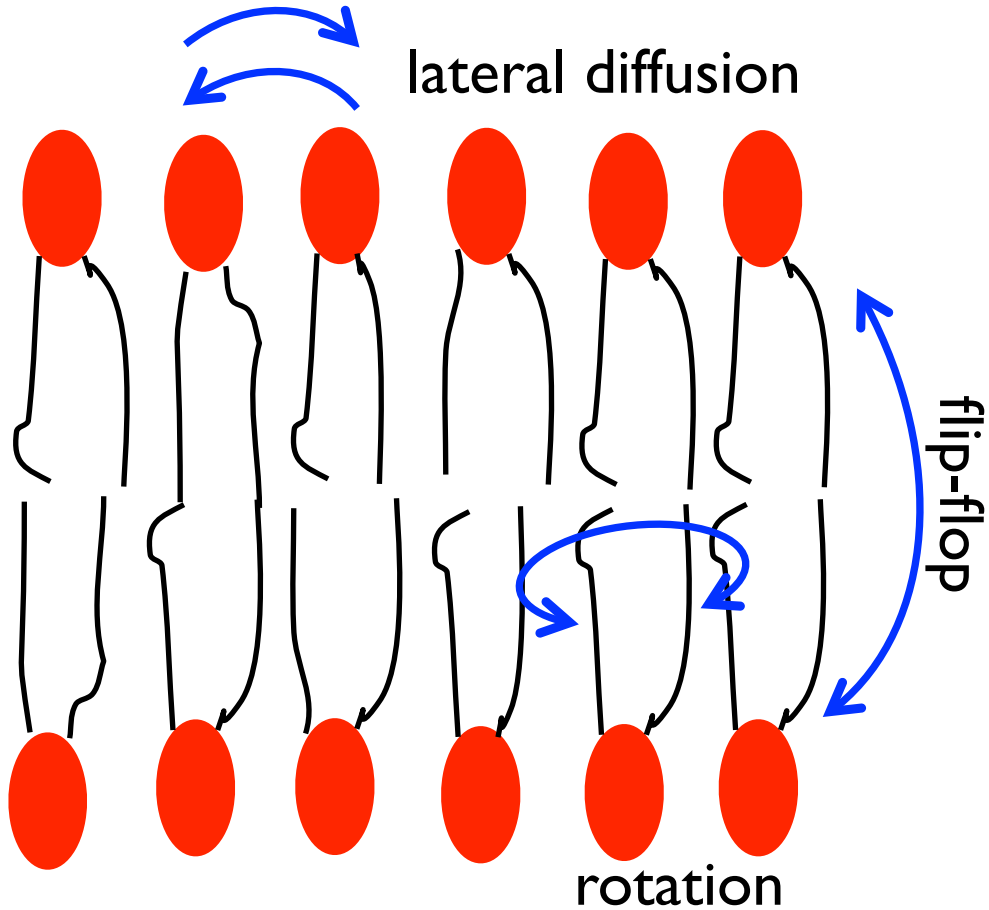
Lipids can have a rich variety by varying: fatty acid chain length, degree of saturation, polar head group etc.,

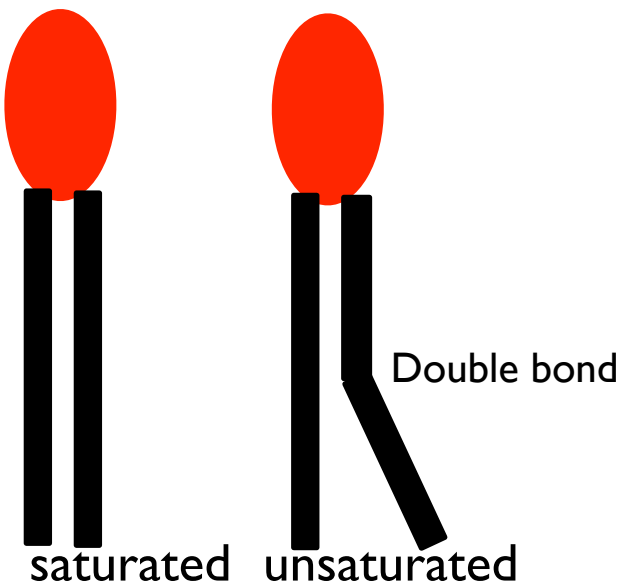
Membranes: Fluid Nature

Fluidity: ease with which lipid molecules move within plane of membrane

- depends on lipid composition, temperature

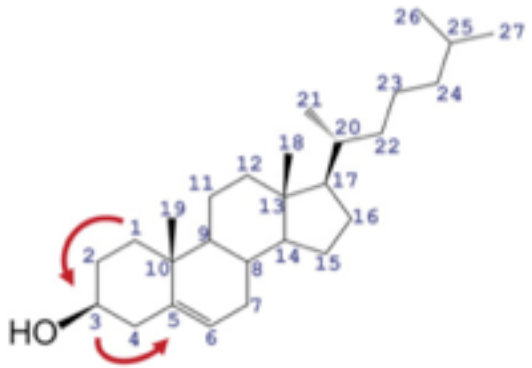
- regular packing leads to less fluidity; unsaturation increases fluidity

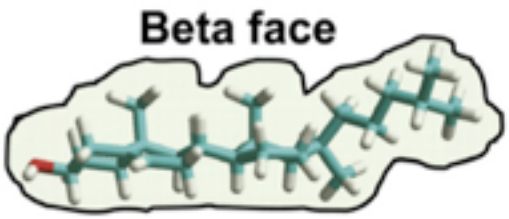


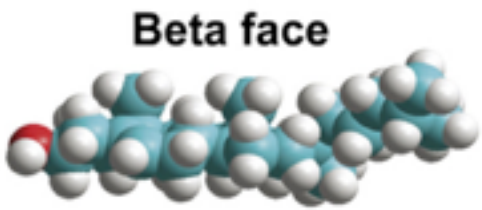


Membrane fluidity plays role in: cell signalling, movements of newly formed lipid molecules, cell division, cell fusion etc,

Cholesterol: a regulator lipid



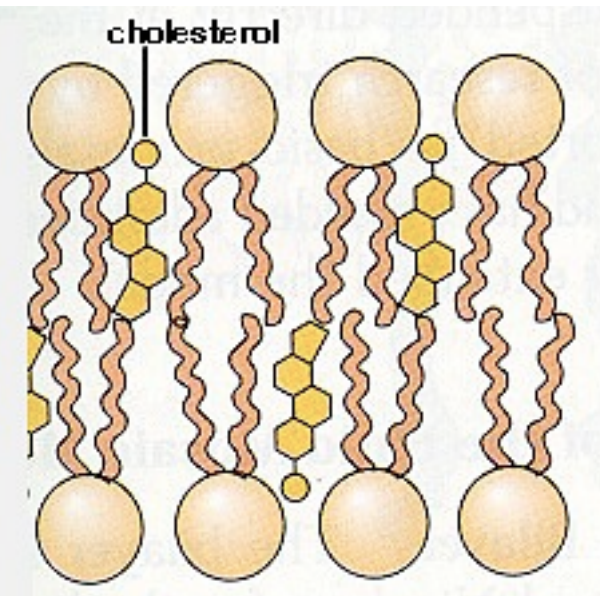




Cholesterol is one of the most abundant lipid molecules

most cells (animal) have ~ 20-50% cholesterol

Regulates the fluid-like nature of membranes, hence affects membrane dynamics. At low temperature, increases fluidity and high temperatures decreases fluidity



J. Fantini, F.J. Barrantes / Biochimica et Biophysica Acta 1788 (2009) 2345–2361

http://cellbio.utmb.edu/cellbio/membrane_intro.htm

Lipid Composition: Plasma membrane

Lipid	Percentage of Total Lipid by Weight					
	Liver Plasma Membrane	Erythrocyte Plasma Membrane	Myelin	Mitochondrion (inner and outer membranes)	Endoplasmic Reticulum	*E. coli*
Cholesterol	17	23	22	3	6	0
Phosphatidyl-ethanolamine	7	18	15	35	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidyl-choline	24	17	10	39	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	13	8	21	27	30

http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=cell&part=A2446

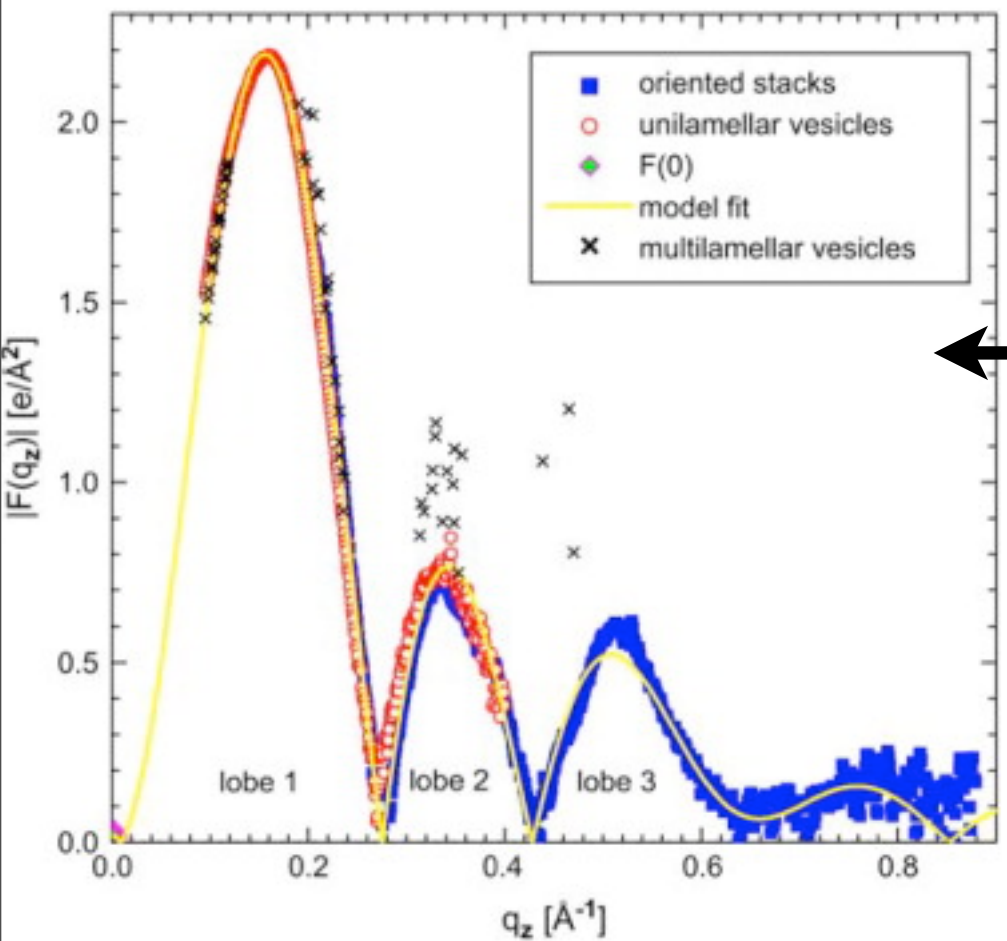
Membranes: what can be measured?

- average area/lipid
- membrane thickness
- electron density profile
- order parameters $S_{CD} = \left\langle \frac{3}{2}\cos^2\theta - \frac{1}{2} \right\rangle$
- tilt angle of hydrocarbon chains
- pressure profiles
- permeation of various small molecules across membranes
- diffusion of lipid molecules $\frac{1}{N_L}\sum_{\forall i}(x_i(t)-x_i(0))^2+(y_i(t)-y_i(0))^2$
- surface tension across membranes $\gamma = \frac{1}{2}\left\langle L_z\left(P_{zz} - \frac{1}{2}(P_{xx}+P_{yy})\right)\right\rangle$
- electrostatic potential change

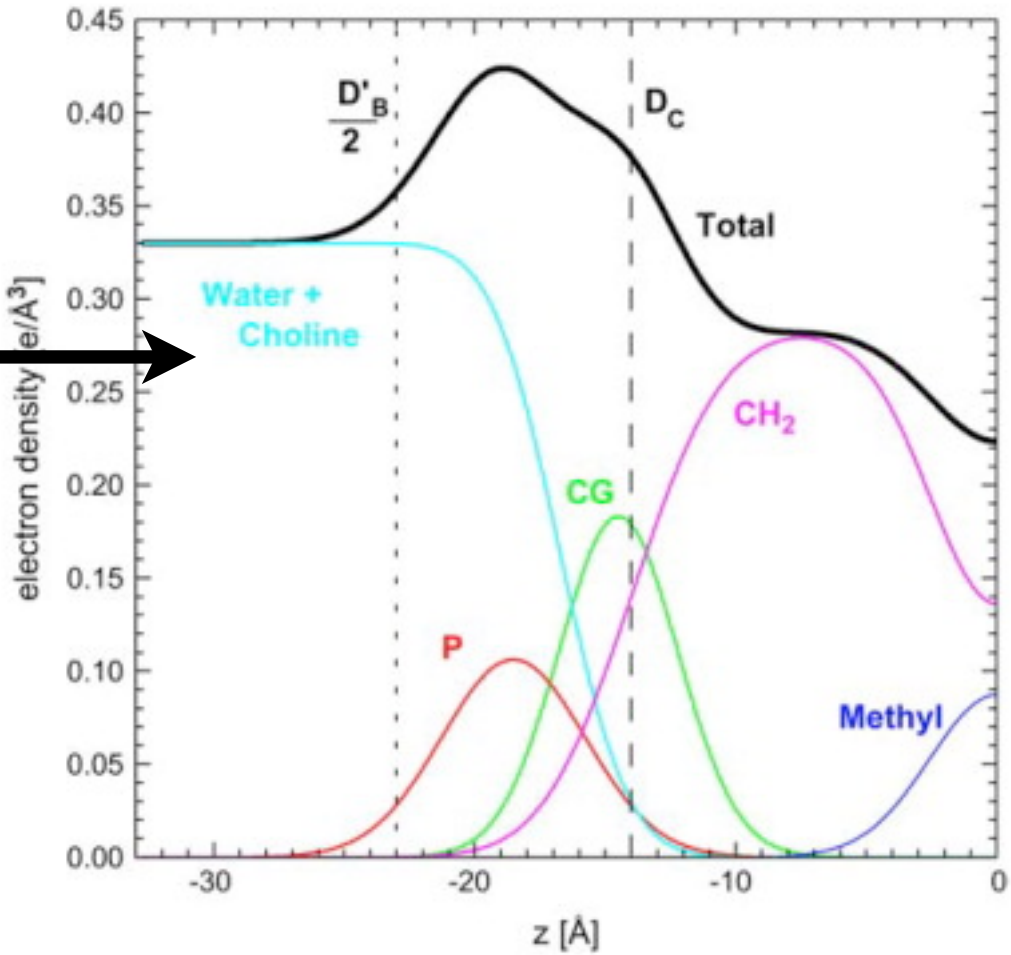
$$\Delta\Psi(z) = \Psi(z) - \Psi(\pm\infty) = -\frac{4\pi}{\varepsilon_0}\int_{\pm\infty}^{z} dz' \int_{\pm\infty}^{z'} \rho(z'')dz''$$

Membranes:Density profiles

Bilayer Form Factor for DPPC (50°C)



Density profile for DPPC (50°C)

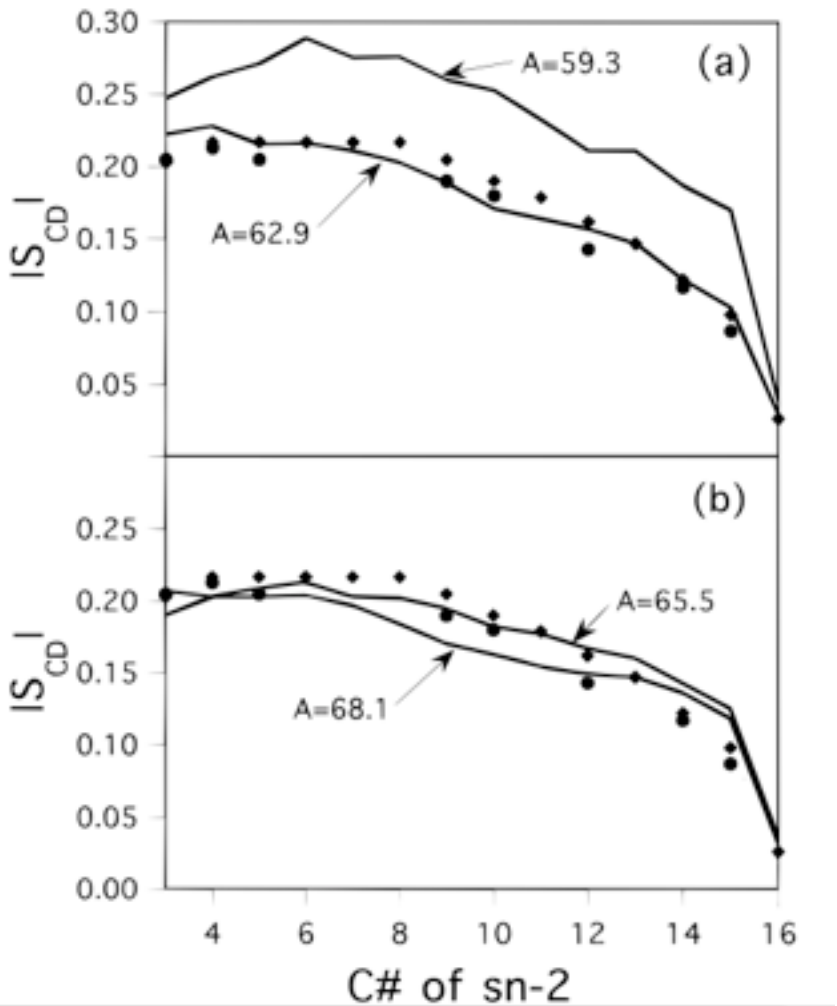


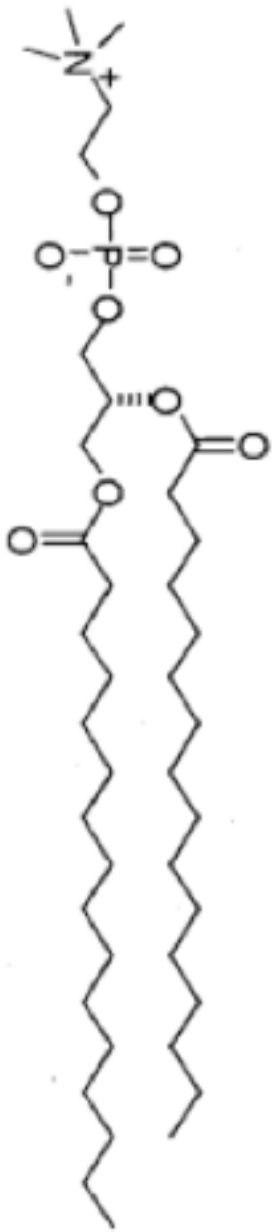
X-ray scattering

Biophysical Journal
Volume 90, Issue 11, 1 June 2006, Pages L83-L85

Membranes: Deuterium order parameter

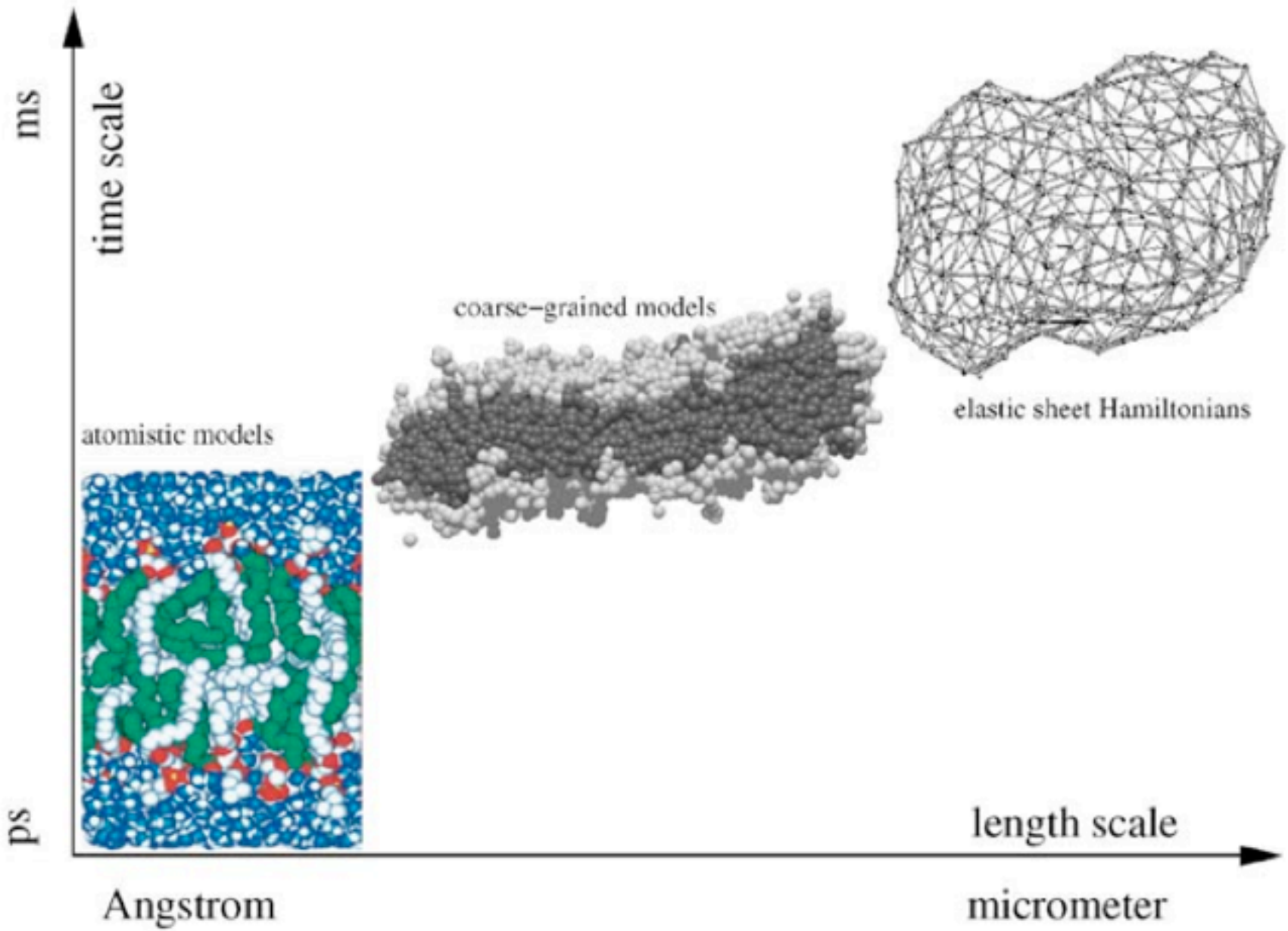
$$S_{CD} = \left\langle \frac{3}{2}\cos^2\theta - \frac{1}{2} \right\rangle$$





Langmuir 1997, *13*, 6555−6561

Membranes: length and time scales



Physics Reports 434 (2006) 113–176

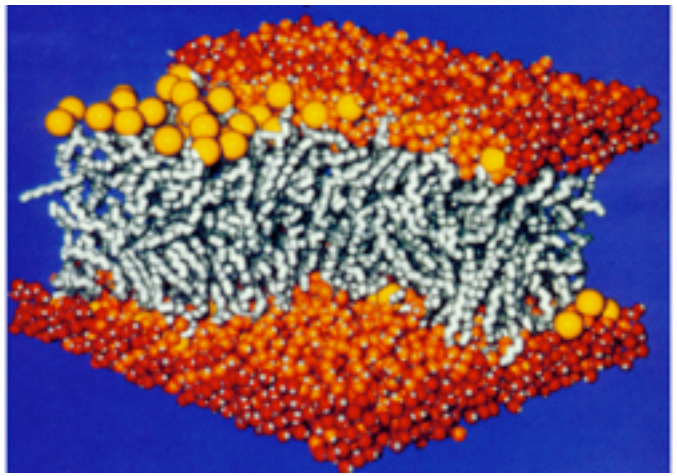
Lipid Simulations over time

Molecular Dynamics Simulation of a Bilayer of 200 Lipids in the Gel and in the Liquid-Crystal Phases

Helmut Heller,† Michael Schaefer,‡ and Klaus Schulten*

Beckman Institute and Department of Physics, University of Illinois, 405 North Mathews Avenue, Urbana, Illinois 61801

J. Phys. Chem. **1993,** *97,* 8343–8360



System: palmitoyloleoylphosphatidylcholine(POPC)

System size: 200 lipids
Simulation time: **263 ps**

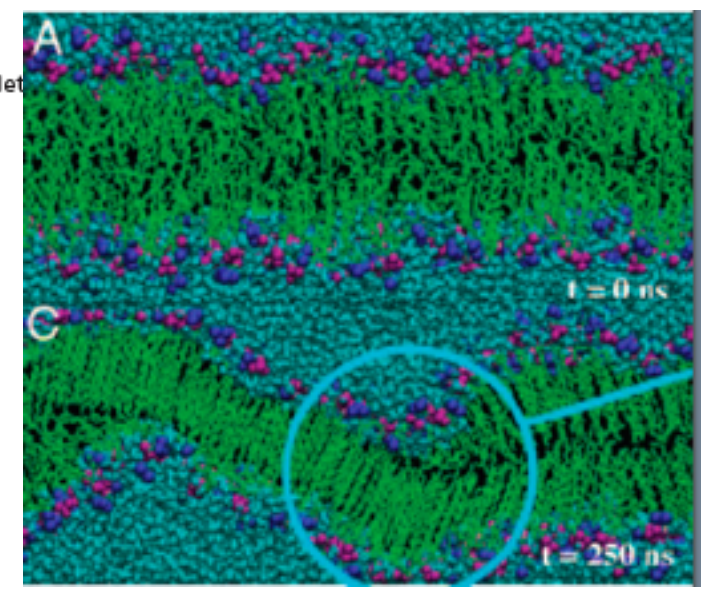
Program/force-field: EGO/CHARMM
Electrostatics: Long-range

Molecular structure of the lecithin ripple phase

Alex H. de Vries*, Serge Yefimov, Alan E. Mark, and Siewert J. Marrink

Molecular Dynamics Group, Department of Biophysical Chemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Net

5392–5396 | PNAS | April 12, 2005 | vol. 102 | no. 15



System: Dipalmitoylphosphatidylcholine(DPPC)

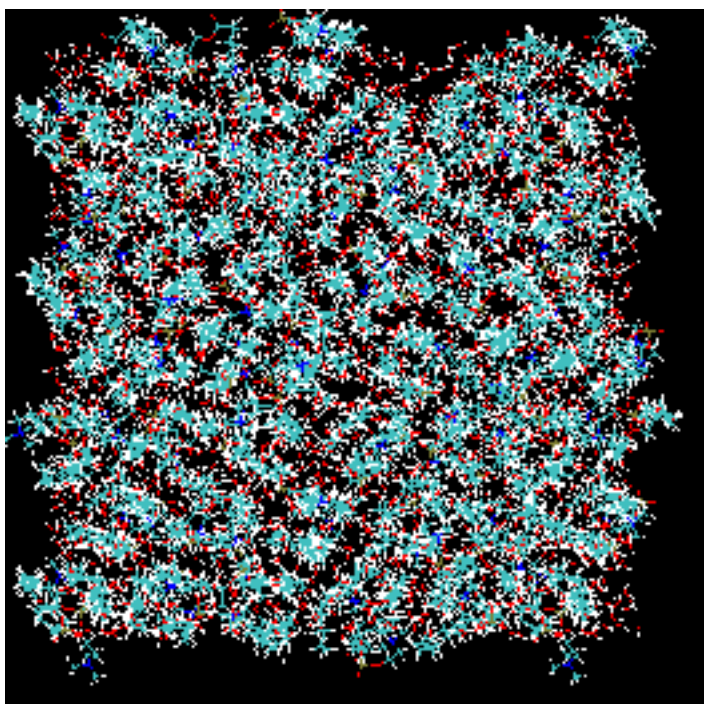
System size: 256 lipids
Simulation time: **250 ns**

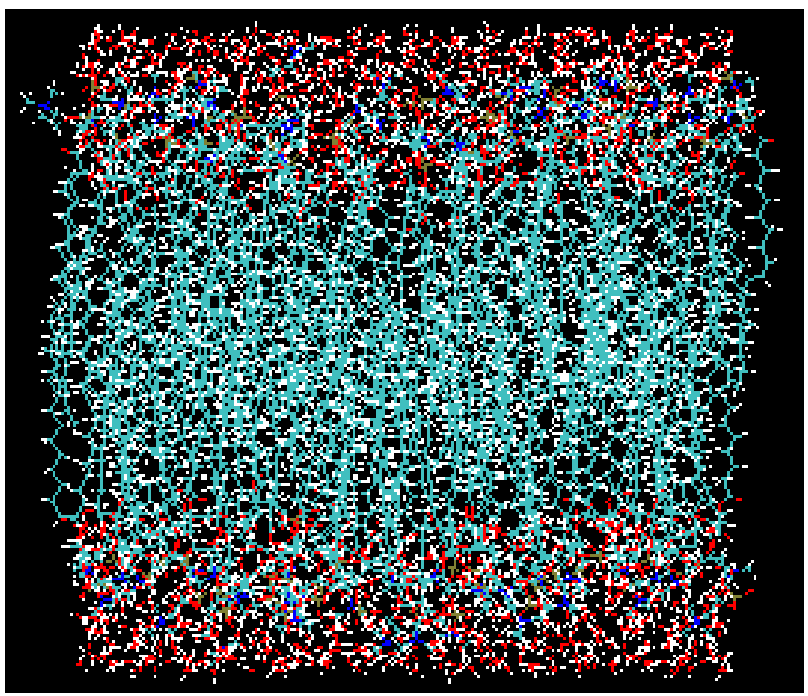
Program: GROMACS
Electrostatics: Long-range

Molecular Cell Biology, Fourth Edition W. H. FREEMAN, 2000

Membrane: Setup

- unlike proteins, no initial coordinates available
- make your own structures or look for existing bilayer patches
- hack into existing lipid topologies to build new ones
- CHARMM 27 force field has lipid topologies/parameter files
- VMD has membrane plugin (very limited)





Force Field

In the context of molecular mechanics, a **force field** (also called a forcefield) refers to the **functional form** **and** **parameter sets** used to describe the potential energy of a system of particles (typically but not necessarily atoms).

Many body potential

$$m_i \frac{d^2\mathbf{r}_i}{dt^2} = -\frac{\partial V\left(\mathbf{r}^N\right)}{\partial \mathbf{r}_i} \qquad (i = 1,\ldots,N)$$

$$V(\mathbf{r^N}) = \sum_b k_b(r - r_\mathrm{b})^2 + \sum_\theta k_\theta(\theta - \theta_\mathrm{o})^2 + \sum_\omega k_\omega(\omega - \omega_\mathrm{o})^2$$
$$+ \sum_\phi k_\phi(1 - \cos(n\phi - \delta)) + \sum_{i<j} 4\,\epsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r}\right)^{12} - \left(\frac{\sigma_{ij}}{r}\right)^{6}\right] + \sum_{i<j} \frac{q_i q_j}{r}$$

from Wiki

How to get parameters ?

Parameter set:

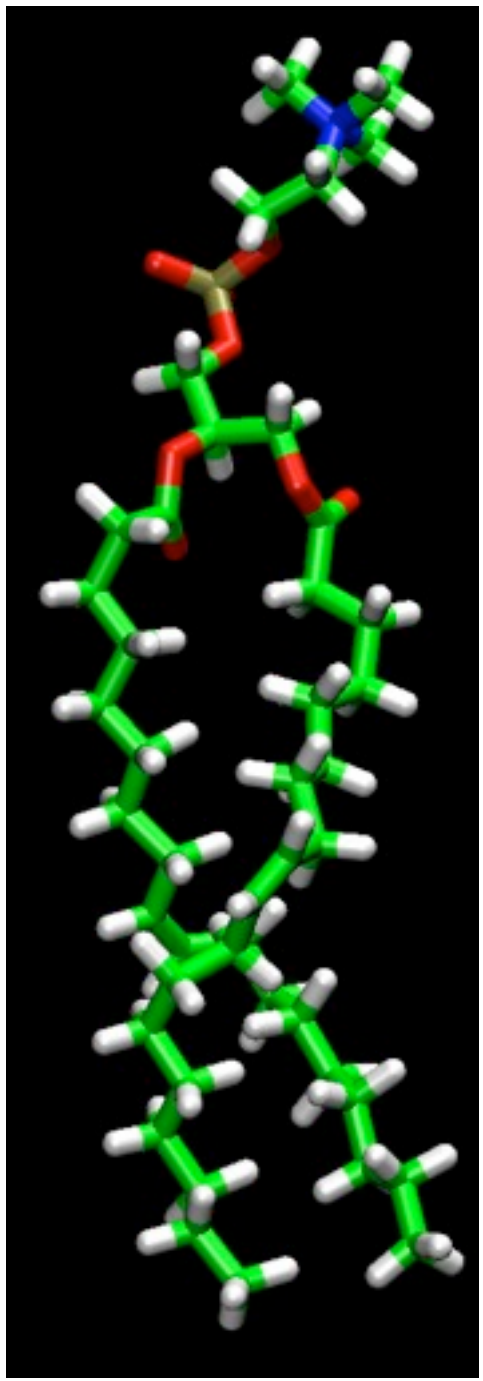
mass, van der waals radius, partial charges, equilibrium bond lengths, angles, dihedrals, force constants

Target data:

Experiments **&/** high-level quantum mechanics calculations like

IR, Raman, NMR, X-ray crystallography, microwave, heats of vaporization, enthalpies, *ab initio* calculations, normal mode analysis

Phospholipid Structure



Polar

Non-polar

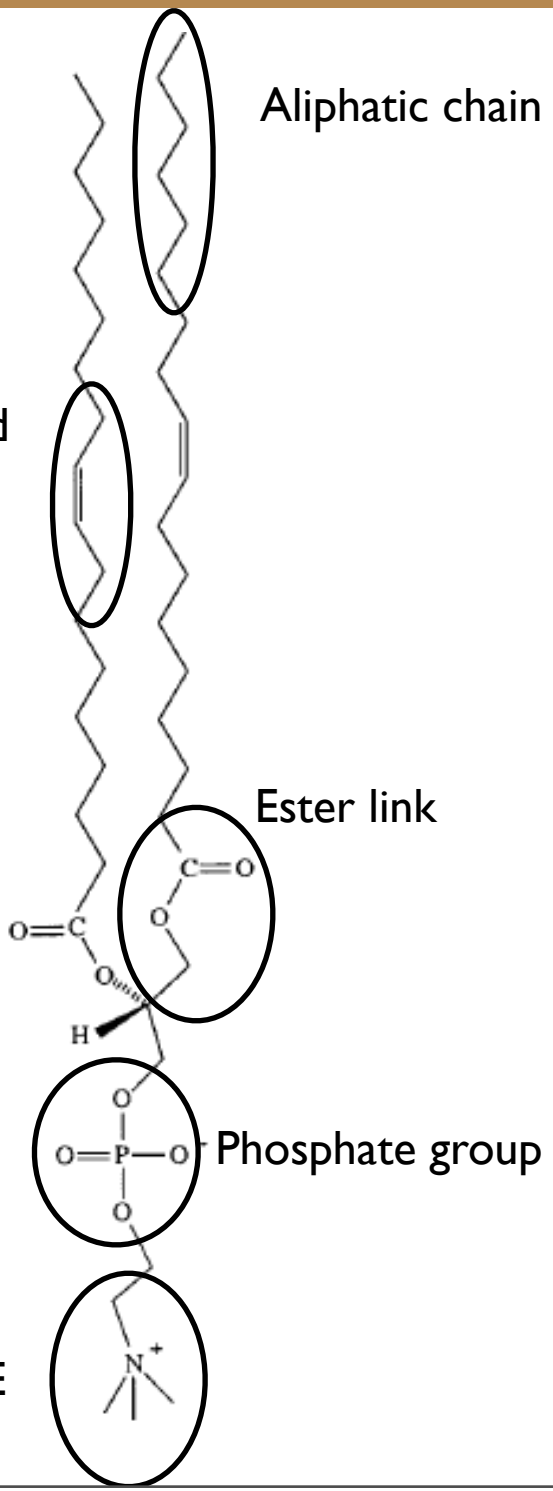
Head group

Phosphate

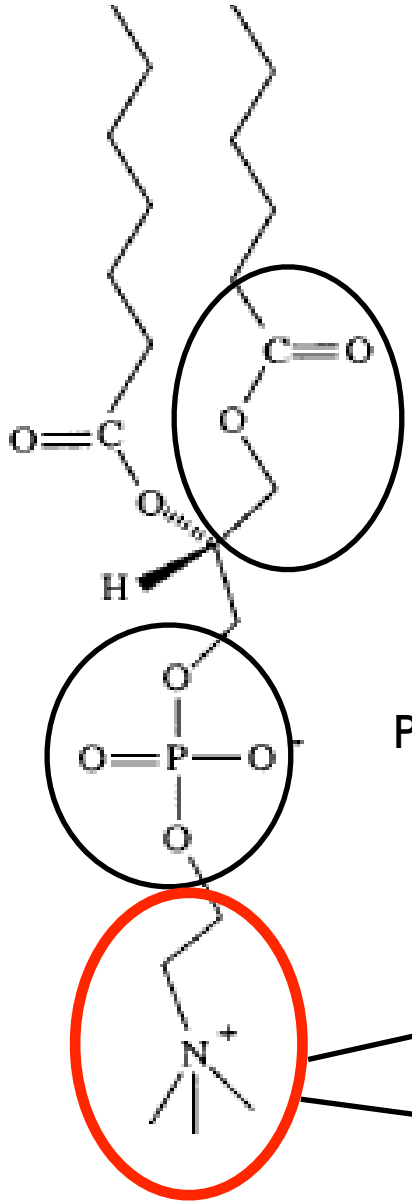
Glycerol

Fatty acid chain

Fatty acid chain

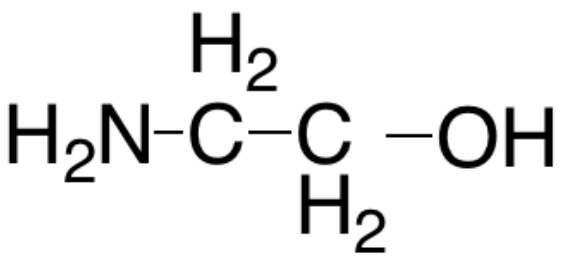


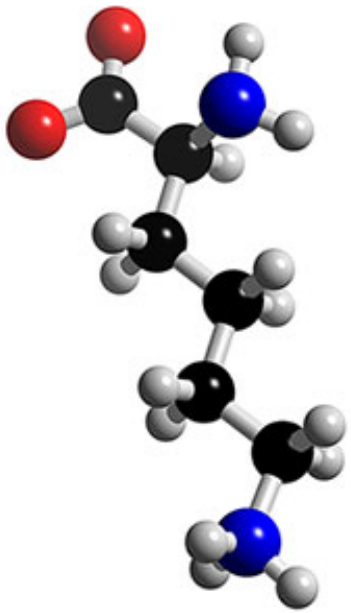
Head group parameters



Ester link

Ethanolamine

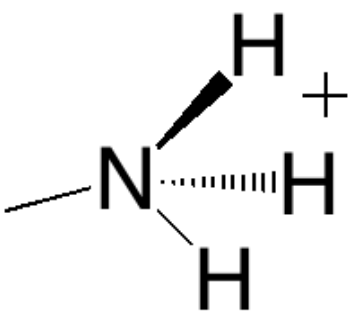




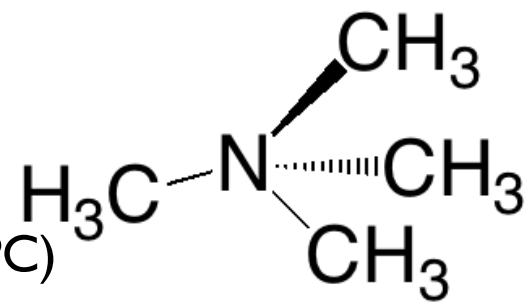
Lysine

Phosphate group

PC/PE

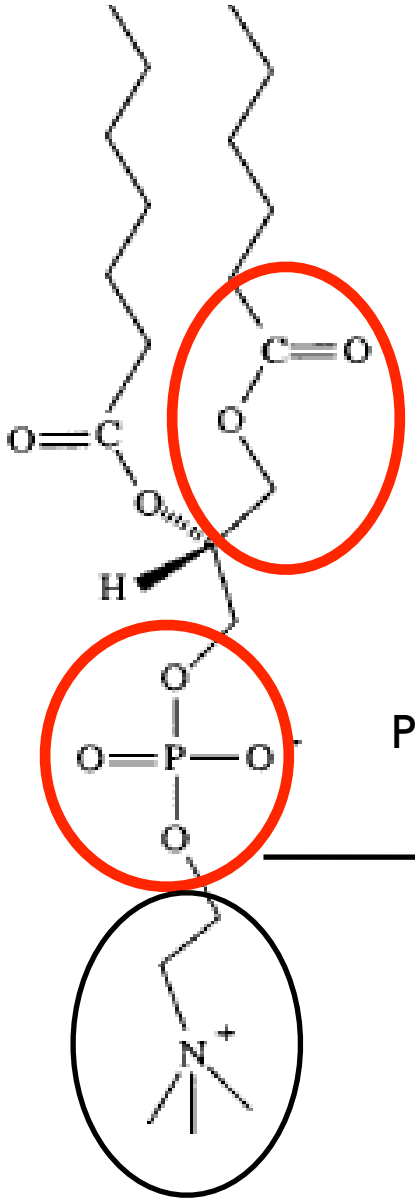


(PE)



Tetramethylammonium (PC)

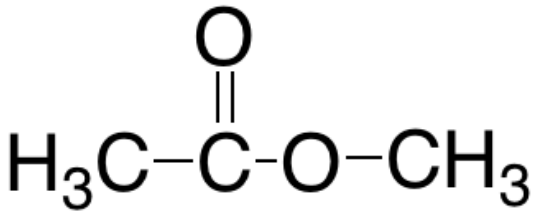
Phosphate/ester group parameters



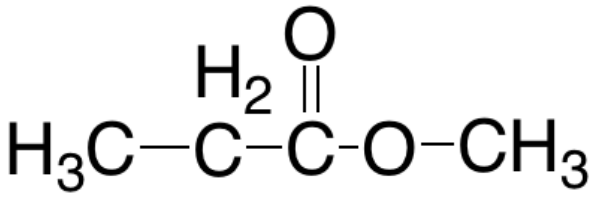
Ester link

PC/PE

Phosphate group

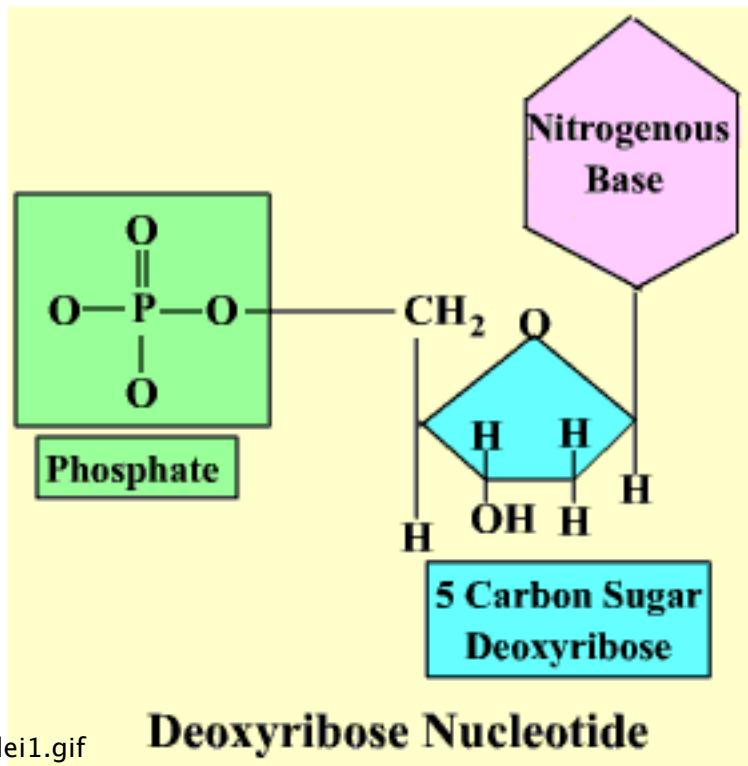


Methyl acetate



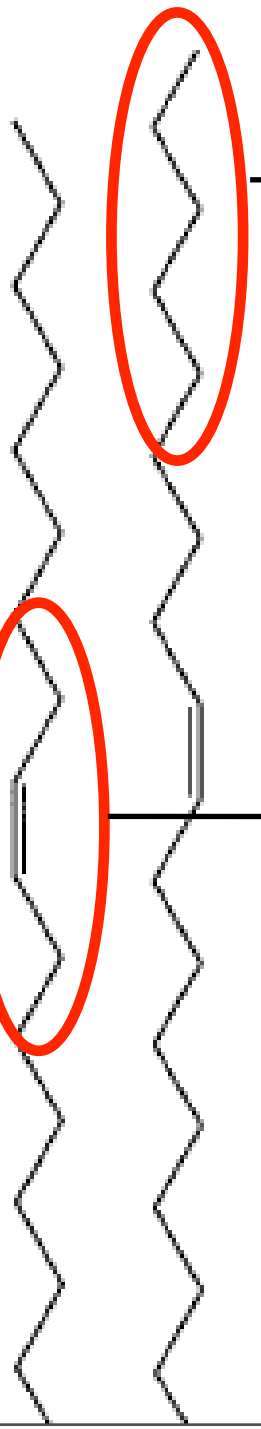
Methyl propionate

Phosphate group from nucleic acid parameters



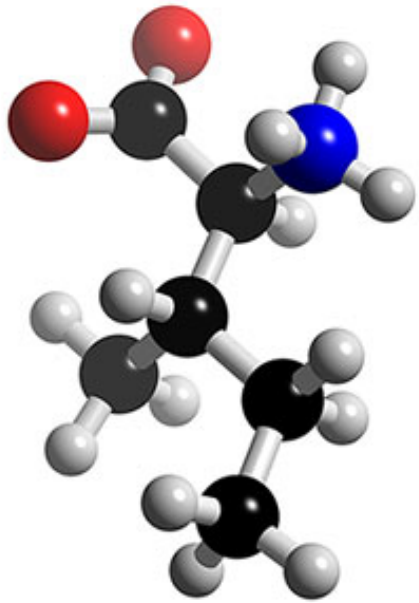
http://www.uic.edu/classes/bios/bios100/lecturesf04am/nuclei1.gif

Lipid chain parameters



From protein aliphatic side chain groups

isoleucine



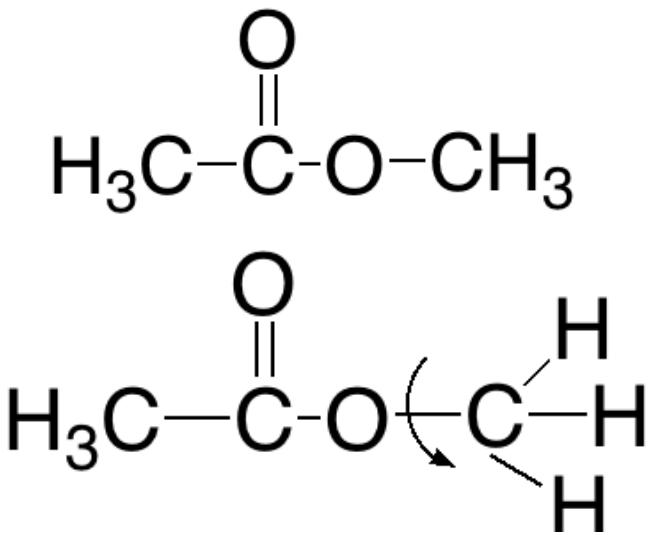
Parameters for unsaturated lipids

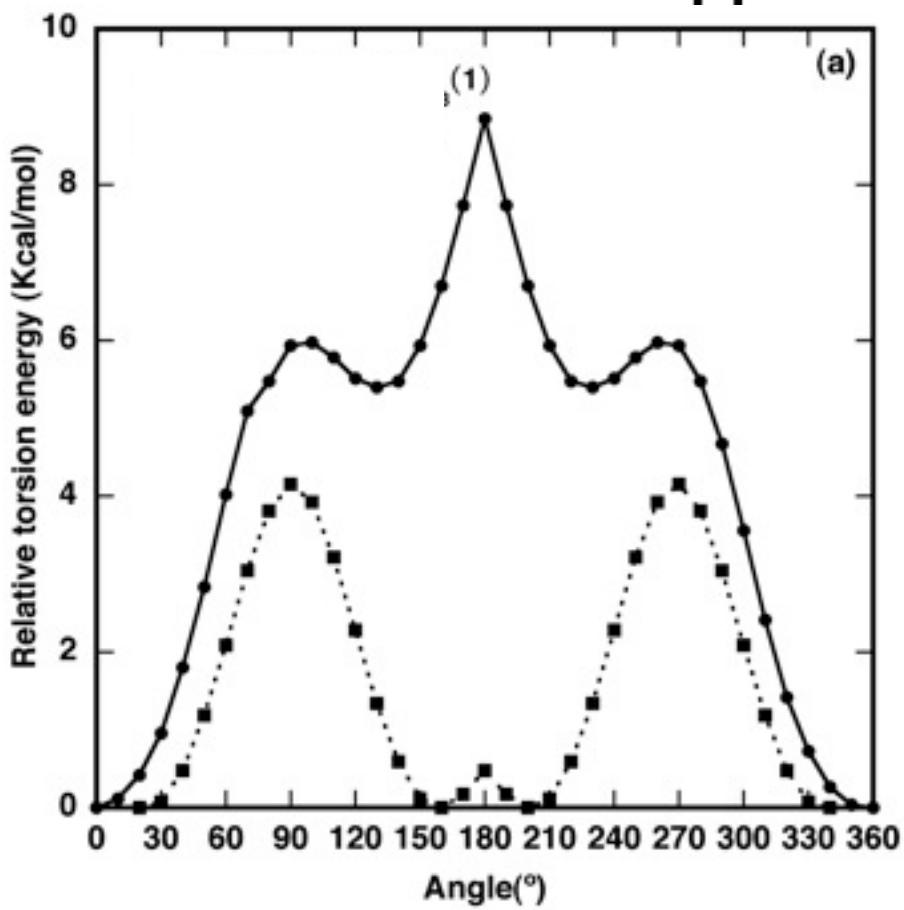
C=C–C–C

Biophysical Journal Volume 73 November 1997 2269–2279

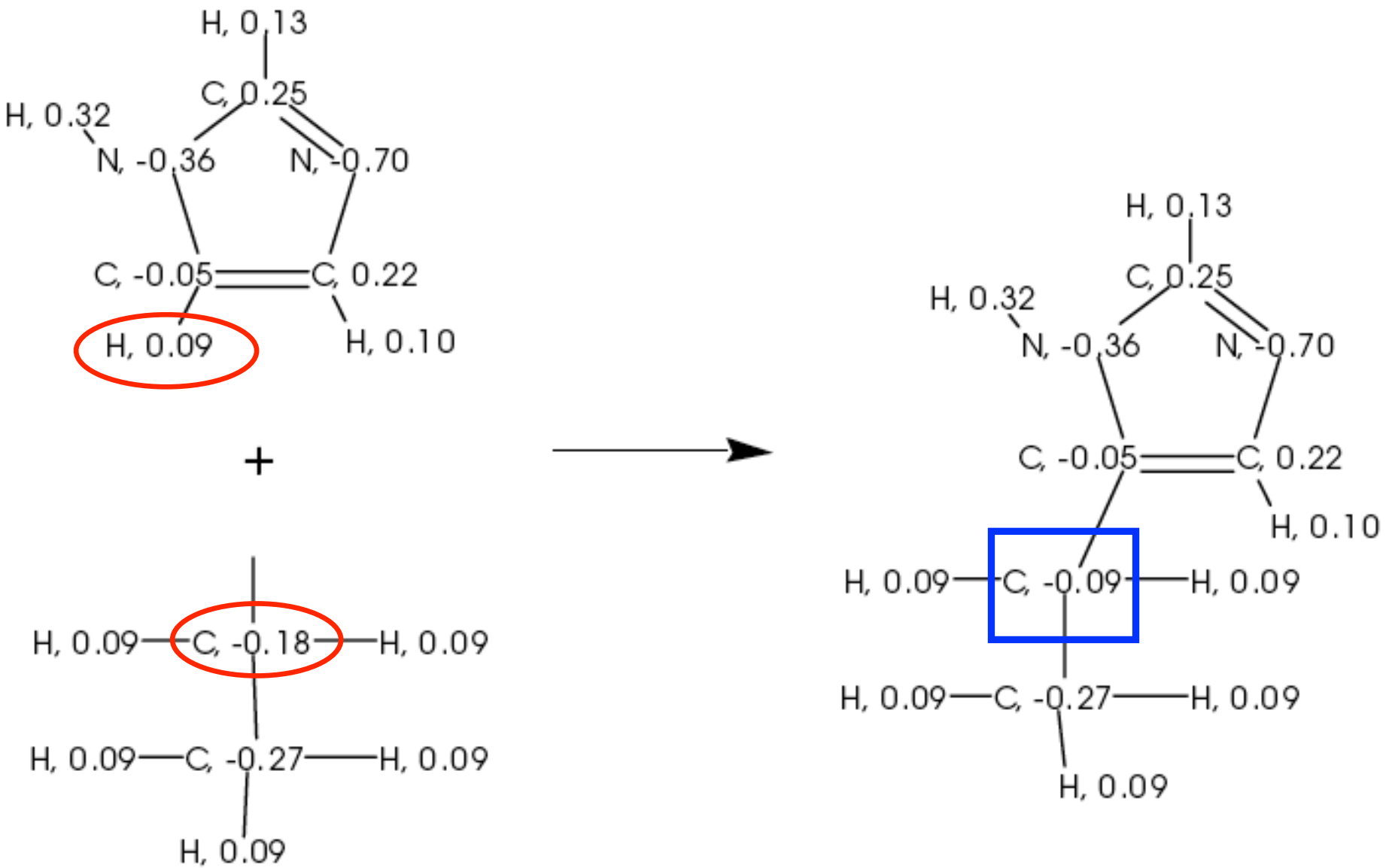
Parameterization

-breakdown into small model molecules
-intra equilibrium values from *ab initio* calculations(high-level of theory)
-intra force constants through 'frequency matching' methods (requires normal mode computation)
-partial charges by different schemes such as Mulliken/RESP (eg., Antechamber program in AMBER/ *ab initio* calculations)
-VDW parameters by analogy and further refined by using target data such as heat of vaporization, densities etc.,



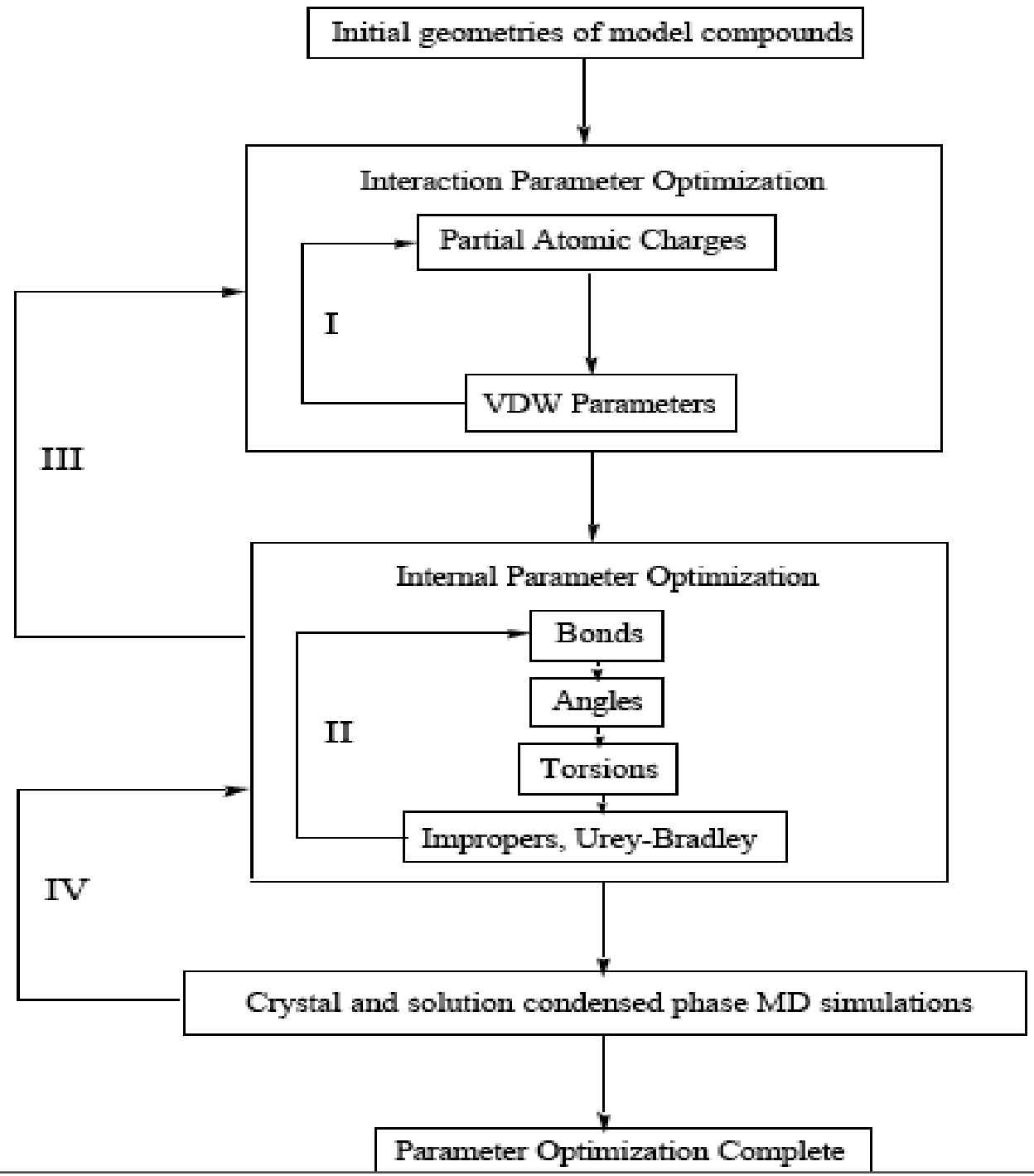


Stiching pieces (partial charges)

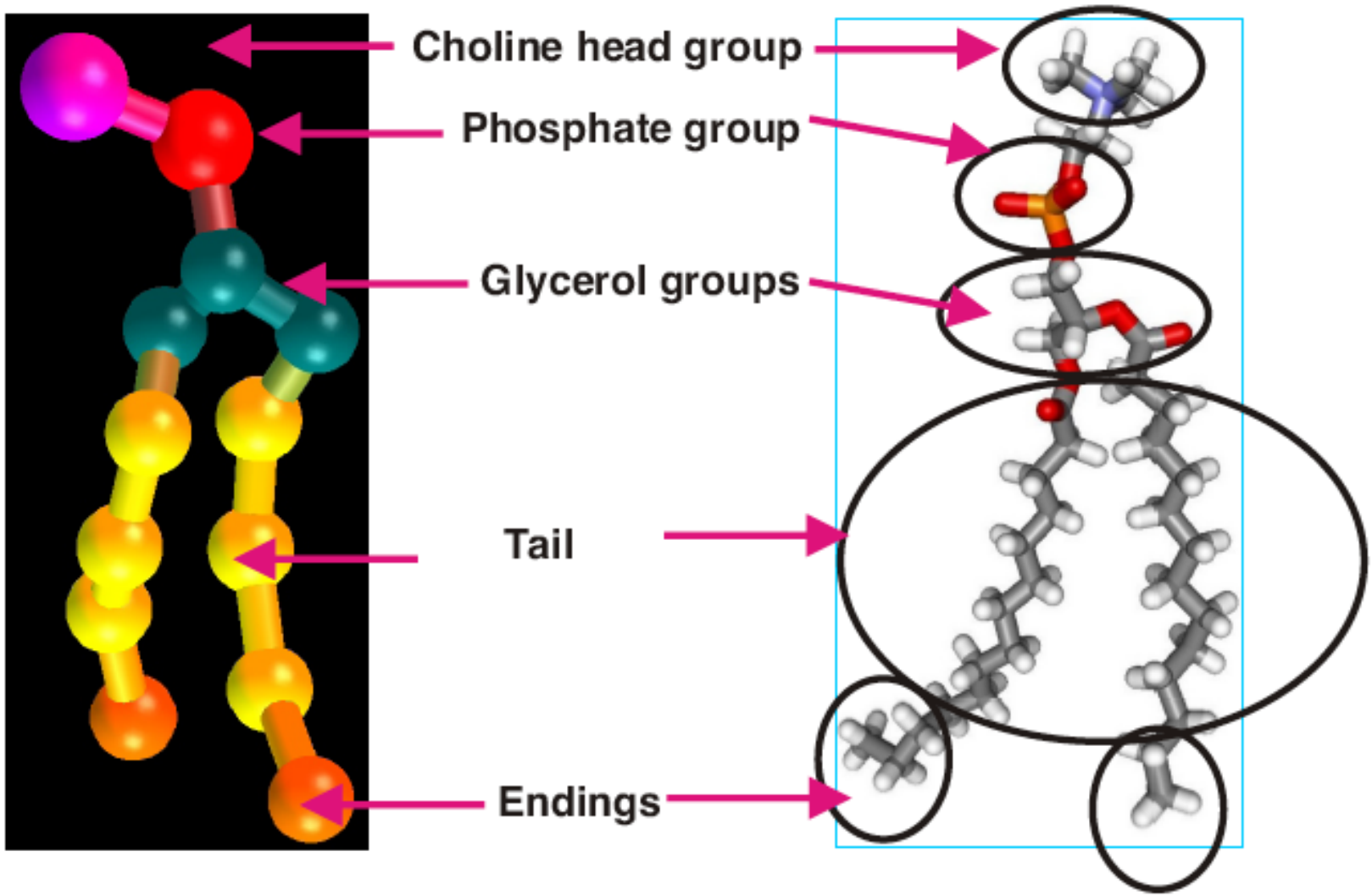


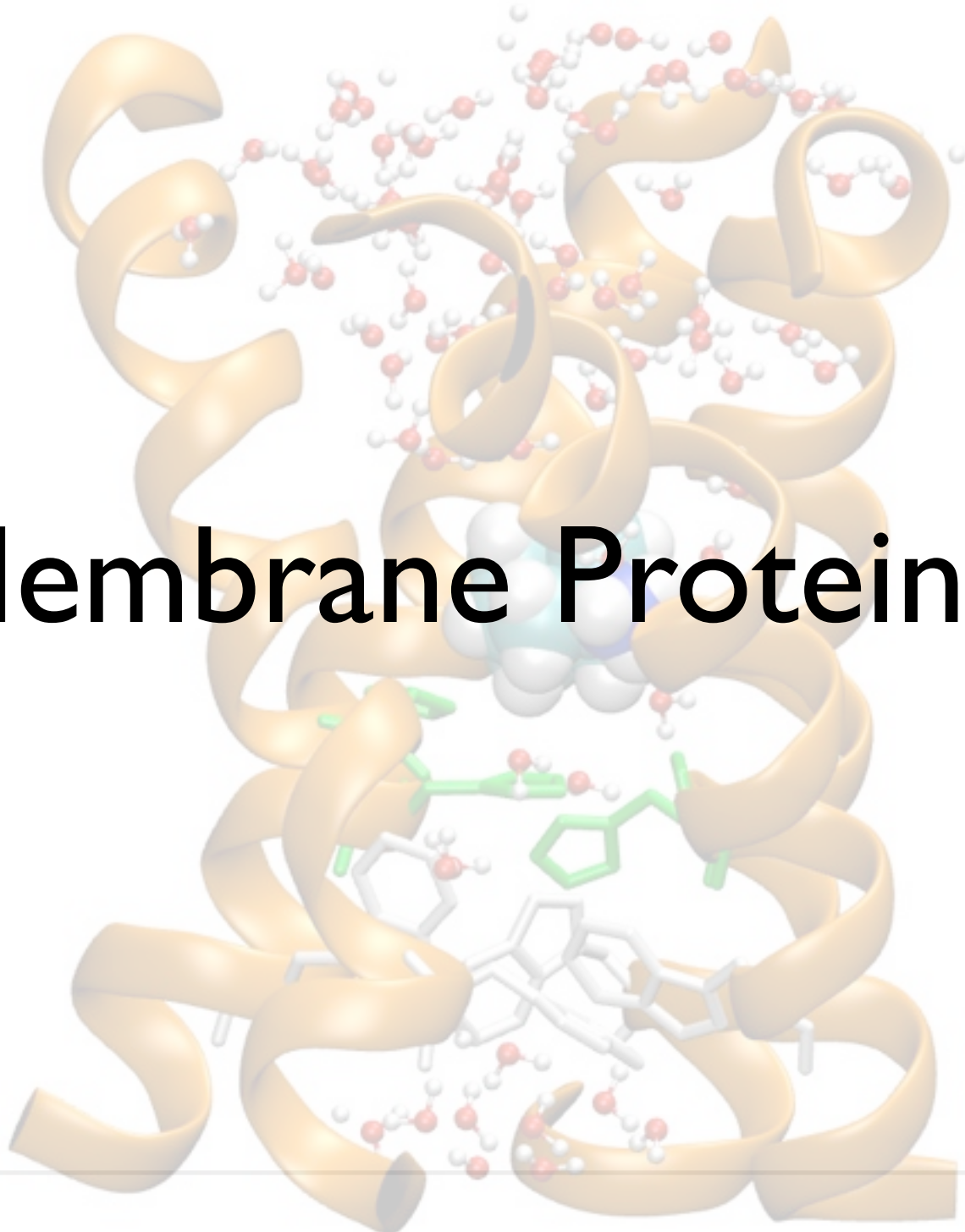
Parameter optimization strategy

Figure 1



Lipid: Coarse Graining





Membrane Proteins

Membranes: Permeability

Charged ions

Na^+, Cl^-

Large uncharged molecules

Amino acids

Small uncharged polar molecules

H_2O, ethanol

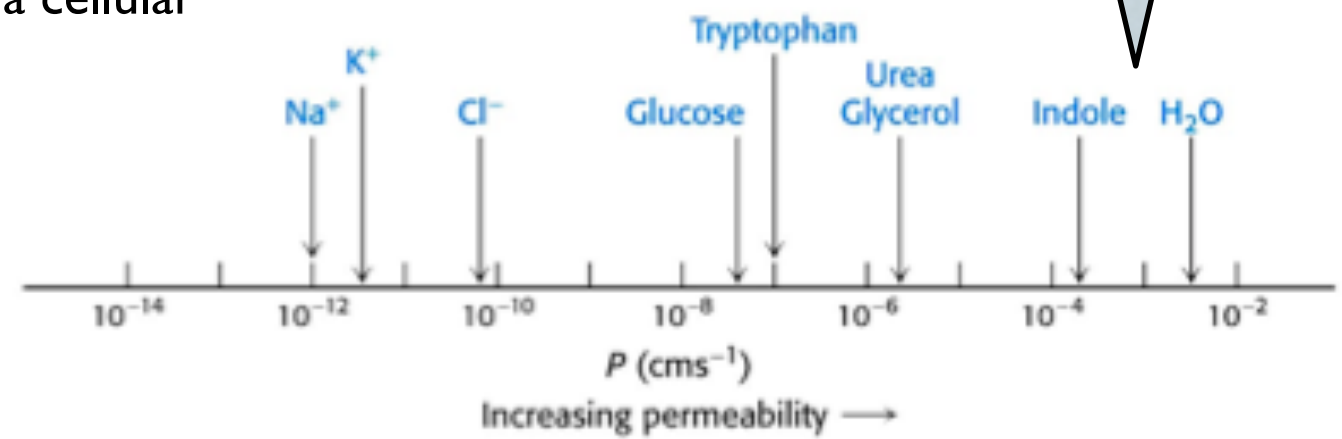
Small hydrophobic molecules

O_2, C_2, N_2, benzene

Extra cellular

Membrane

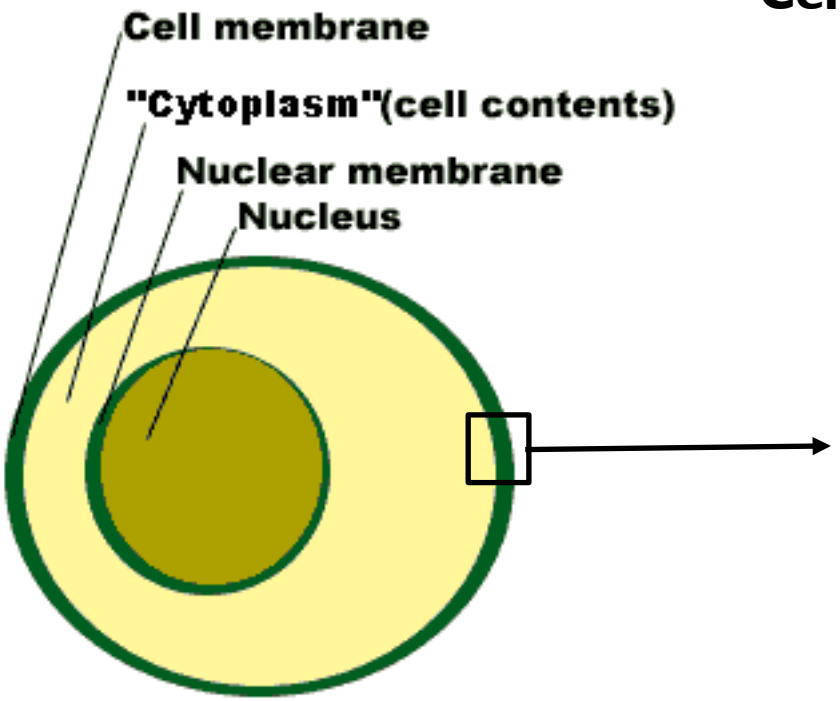
Intra cellular



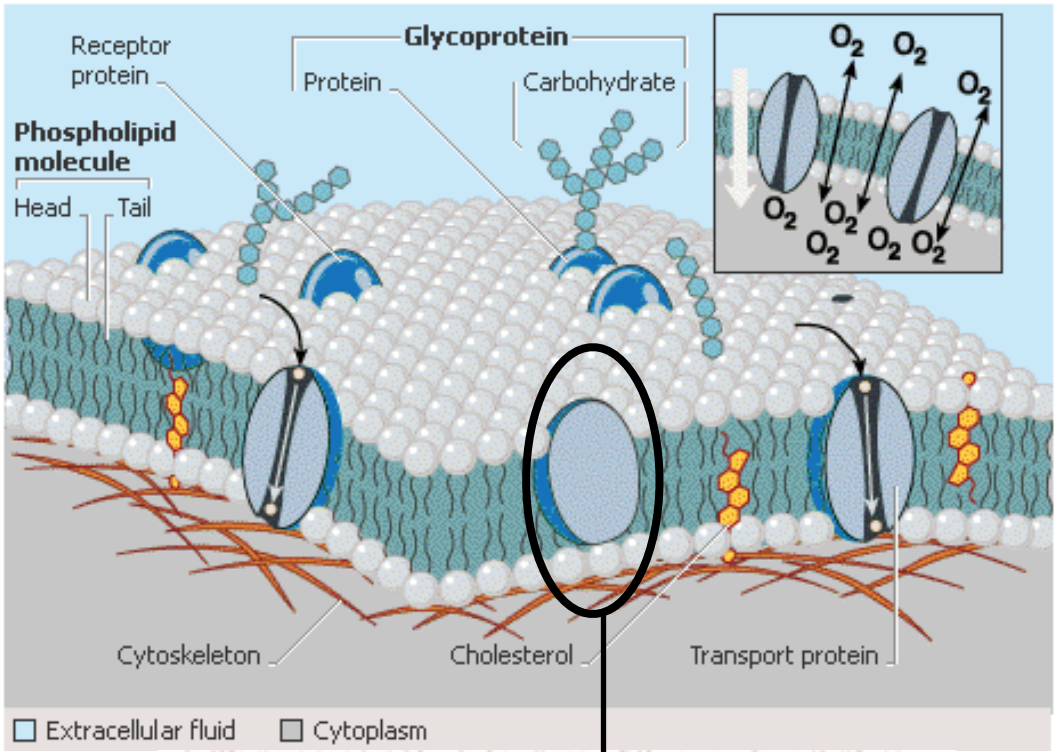
- movement of ions in and out of cells essential for life
- ion flows mediate processes such as signalling, electrical signals in nerves, muscles, pH balance etc.,
- ion currents cause changes in membrane potential

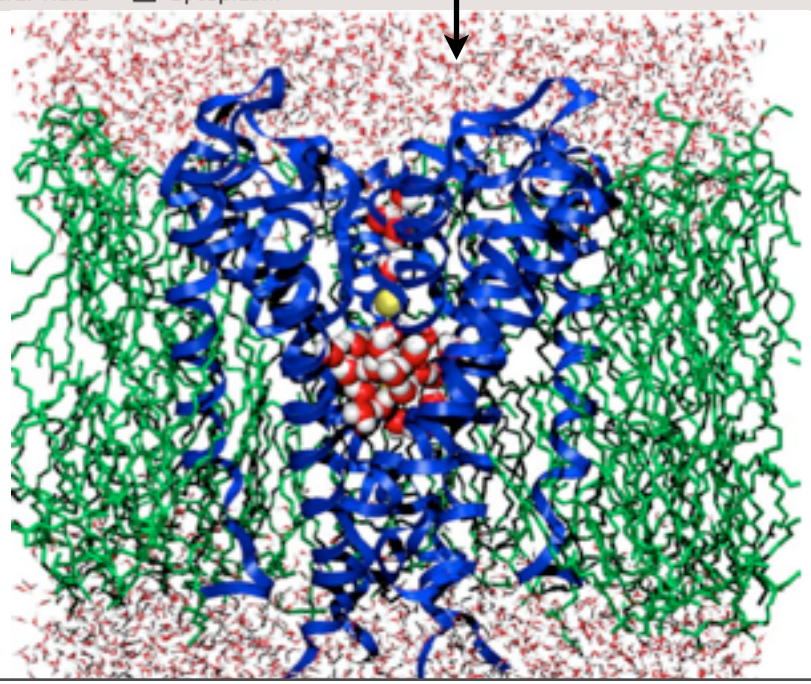
Specialized transport proteins are required.....

Perspective



Cell membrane

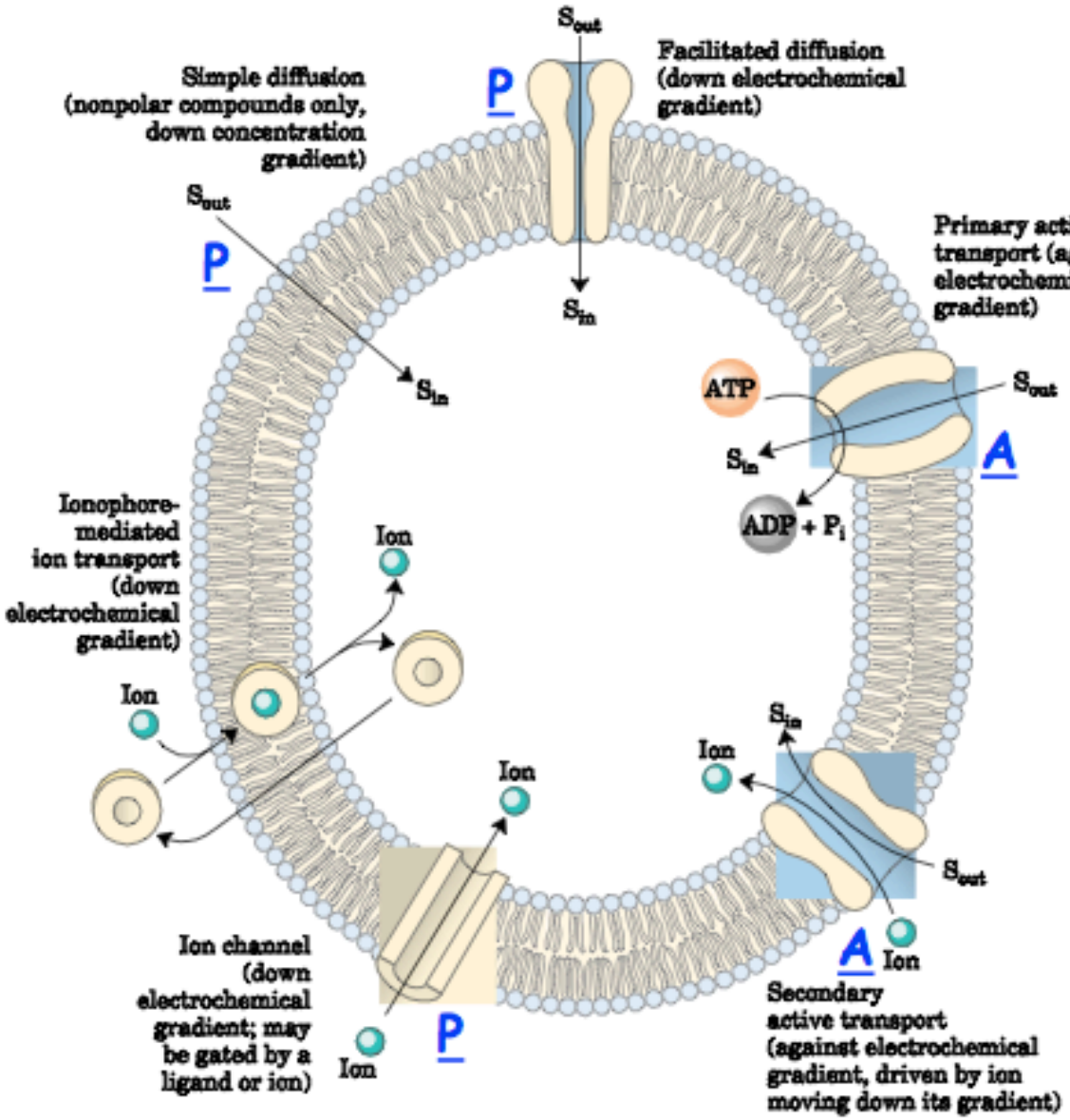




http://www.mcld.co.uk/hiv/images/aEukaryoticCell.gif

http://images.encarta.msn.com/xrefmedia/aencmed/targets/illus/ilt/T628876A.gif

Ion transport across membranes



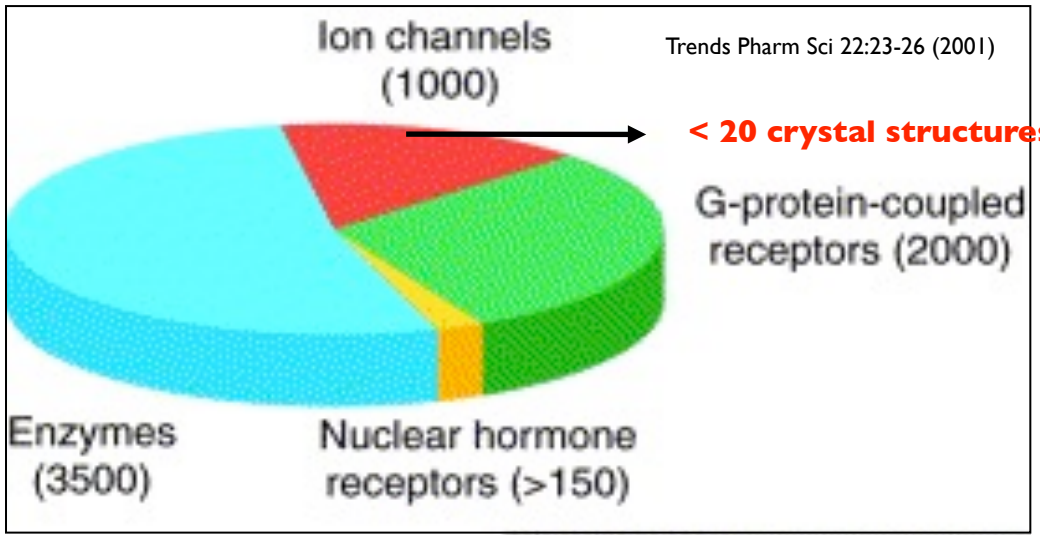
active: against concentration gradient, transporters

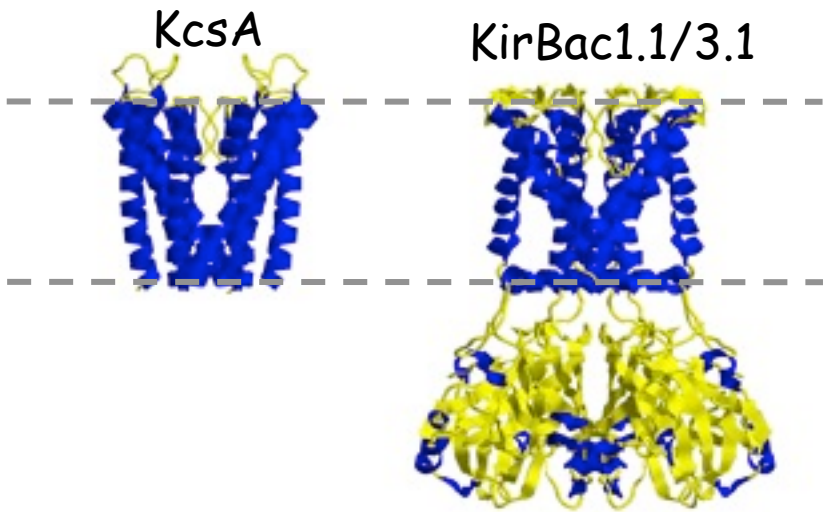
Passive & Active Transport Across Cell Membranes

passive: travel down the concentration gradient

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/Diffusion.html

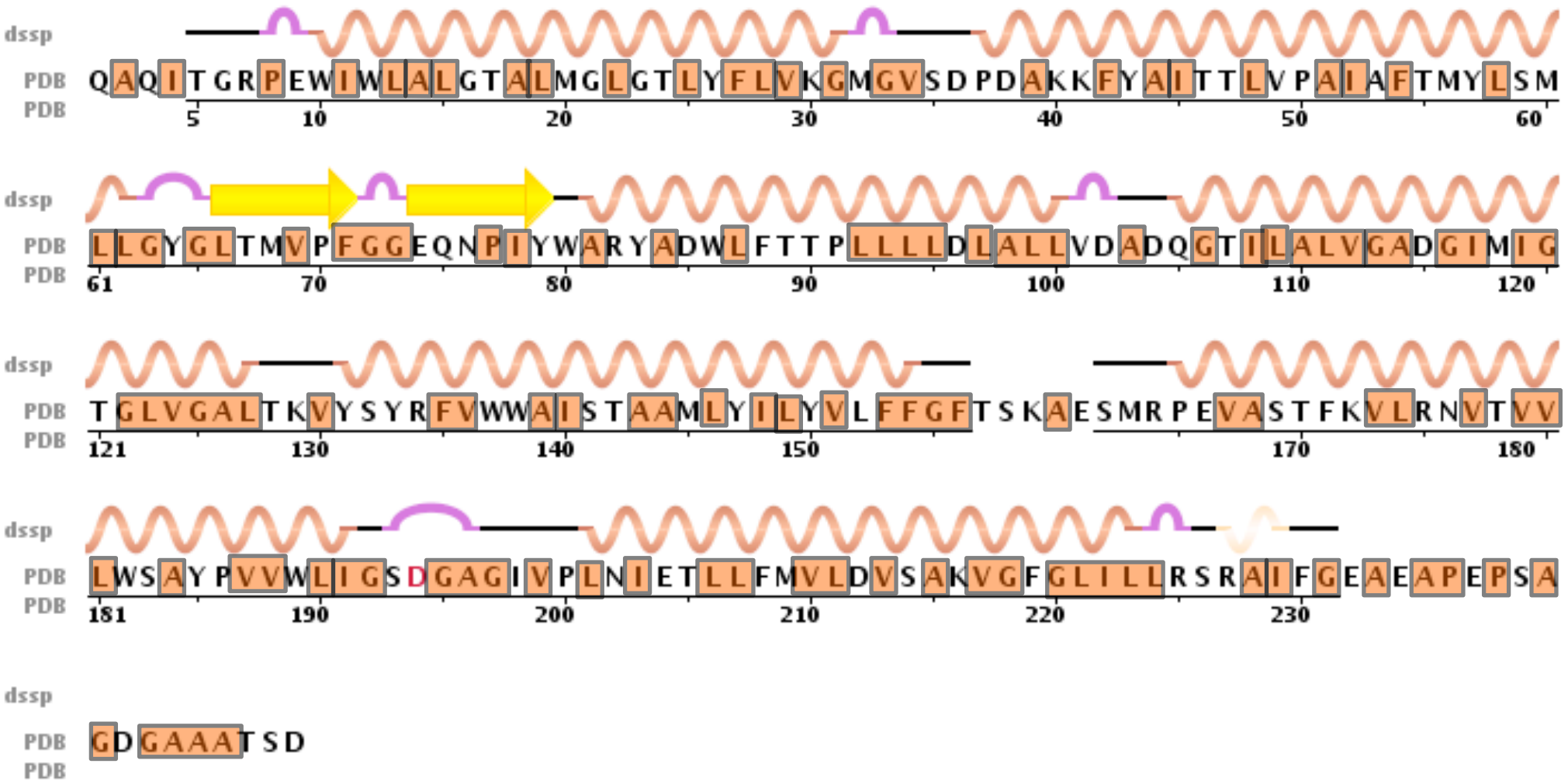
Membrane Proteins





- Genomics – Membrane Proteins constitute ca. 25% to 30% of all genes
- Membrane Proteins are implicated in many diseases: Diabetes, Parkinson's, drug resistance (tumours & bacteria) …
- Membrane Proteins are major drug targets
- ~50% of current drug targets are membrane proteins

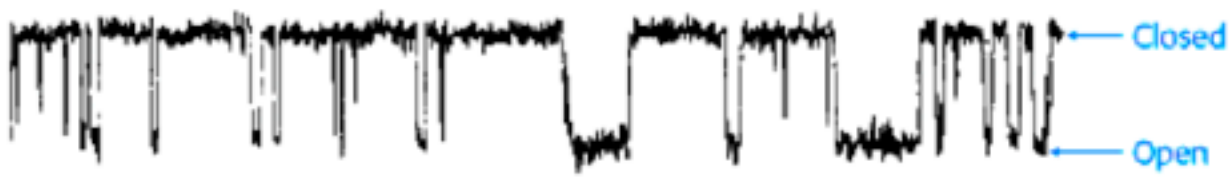
Rhodopsin


dssp
PDB
PDB
QAQITGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAKKFYAITTLVPAIAFTMYLSM
5
10
20
30
40
50
60
dssp
PDB
PDB
LLGYGLTMVPFGGEQNPIYWARYADWLFTTPLLLLDLALLVDADQGTILALVGADGIMIG
61
70
80
90
100
110
120
dssp
PDB
PDB
TGLVGALTKVYSYRFVWWAISTAAMLYILYVLFFGFTSKAESMRPEVASTFKVLRNVTVV
121
130
140
150
170
180
dssp
PDB
PDB
LWSAYPVVWLIGSDGAGIVPLNIETLLFMVLDVSAKVGFGLILLRSRAIFGEAEAPEPSA
181
190
200
210
220
230
dssp
PDB
PDB
GDGAAATSD

Nobel Prizes for ion channels:

- Alan Hodgkin and Andrew Huxley in (1963)
- Erwin Neher and Bert Sakmann(1991)
- Roderick MacKinnon (2003)

The Nobel Prize in Physiology or Medicine 1963 was awarded jointly to Sir John Carew Eccles, Alan Lloyd Hodgkin and Andrew Fielding Huxley "for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane".

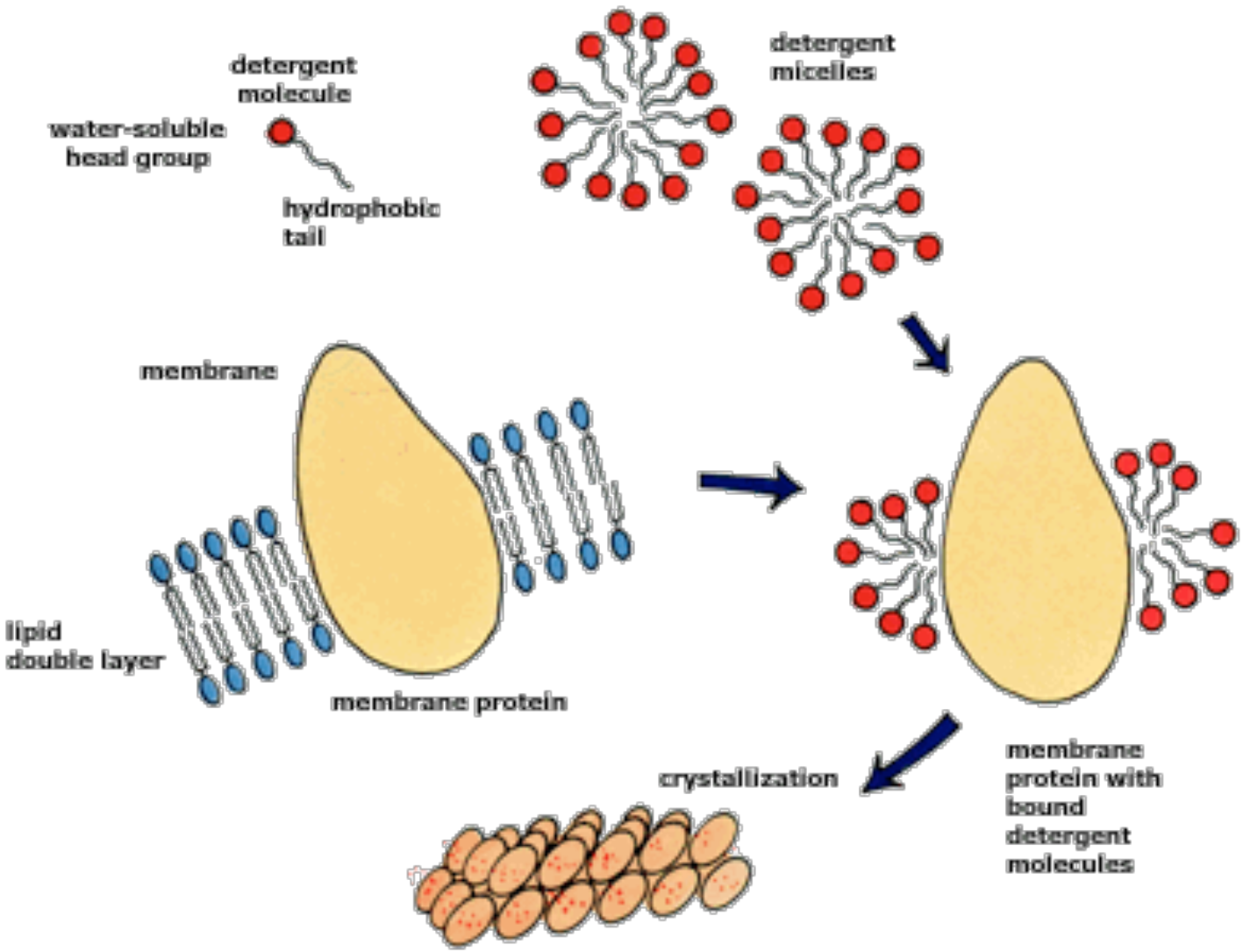


"This year's Laureates, Erwin Neher and Bert Sakmann, succeeded in making a conclusive demonstration that ion channels exist, by developing a technique by which the miniscule currents, flowing through a single ion channel molecule, could be measured."

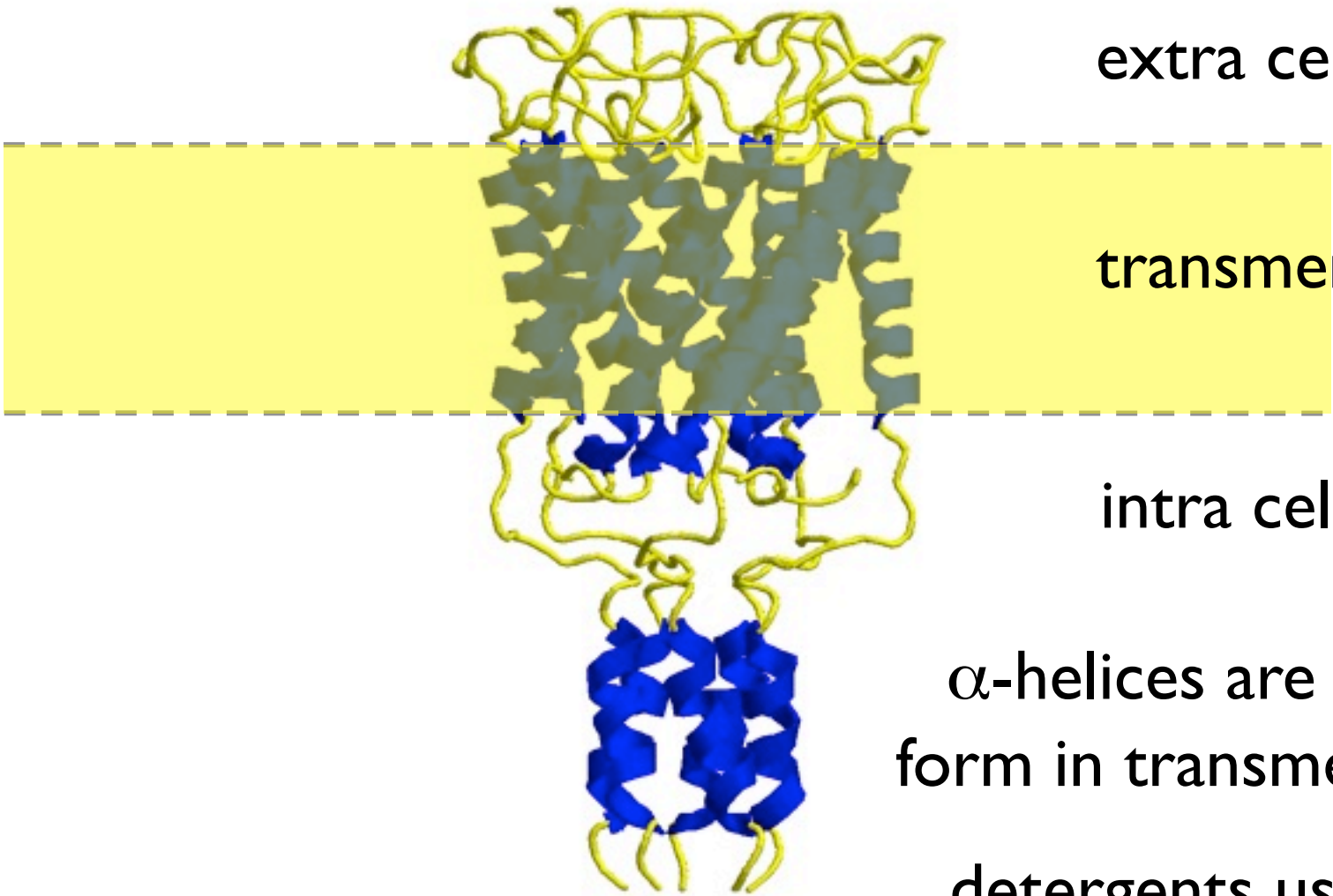
The Nobel Prize in Chemistry 2003 was awarded "for discoveries concerning channels in cell membranes" jointly with one half to Peter Agre "for the discovery of water channels" and with one half to Roderick MacKinnon "for structural and mechanistic studies of ion channels".

http://nobelprize.org/nobel_prizes/medicine/laureates/1991/sakmann-lecture.html
http://nobelprize.org/nobel_prizes/medicine/laureates/1991/neher-lecture.html
http://www.thenakedscientists.com/HTML/articles/article/the-ion-channel-through-the-keyhole/
http://nobelprize.org/nobel_prizes/medicine/laureates/1991/presentation-speech.html

Membrane crystallization



Membrane Proteins



extra cellular domain

transmembrane domain

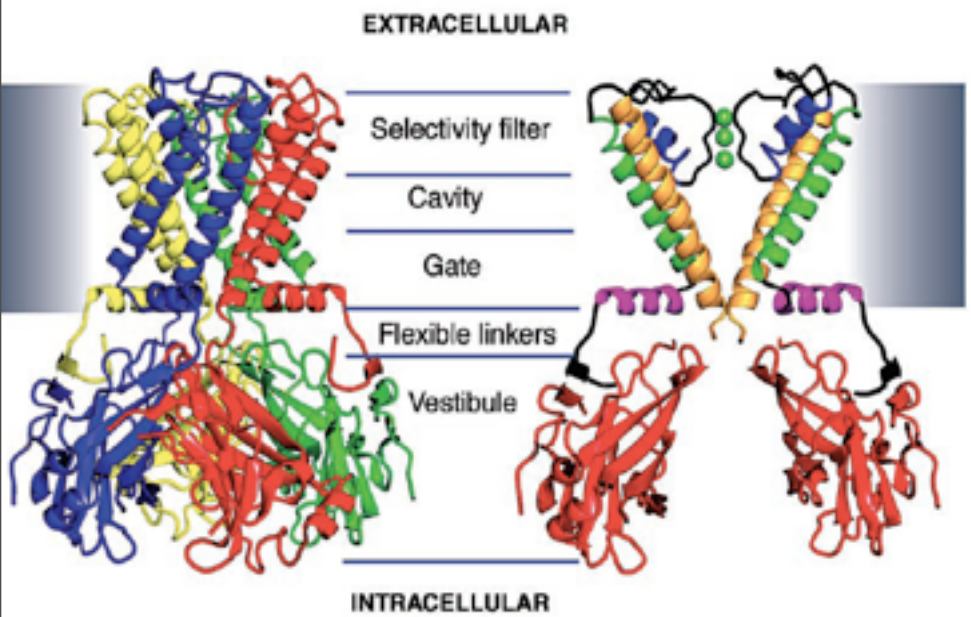
intra cellular domain

α-helices are most common form in transmembrane domain

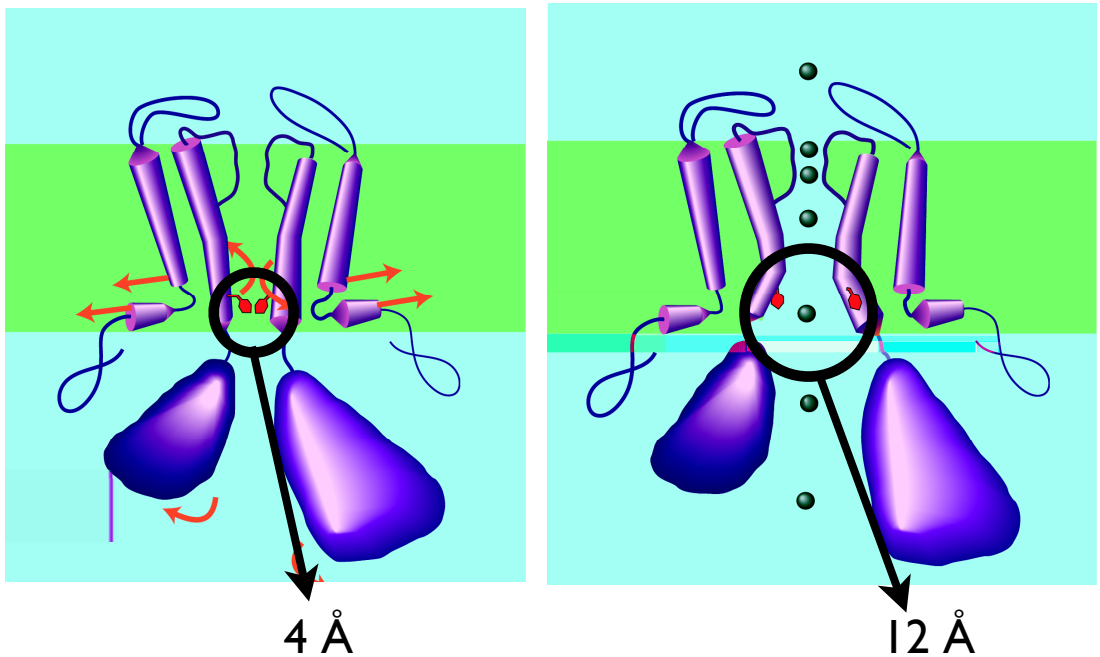
detergents used to separate membrane proteins from membrane

KirBac: Potassium channel

Close structure at 3.65 Å in 2003



Gating: 4 hydrophobic PHE residues



Ubiquitous -

Viruses, Bacteria, Yeast, Plants, Animals

Diverse Functions -

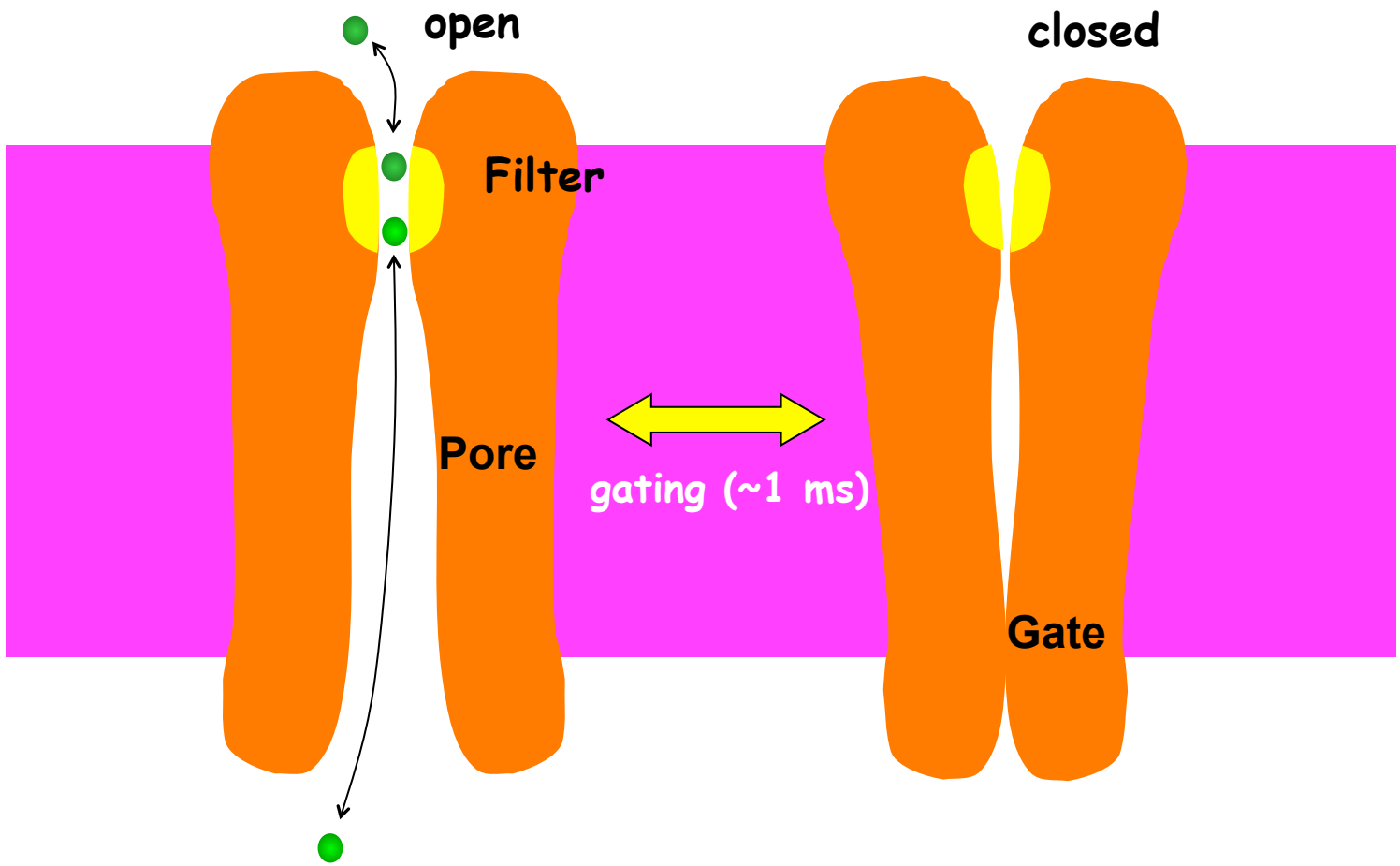
Electrical Excitability, Insulin Release, Cell Volume Control

Well Characterised -

Physiology, Molecular Biology, Structure

Kuo et al. Science 300,1922-1926, **2003**

Ion Channels: Gating

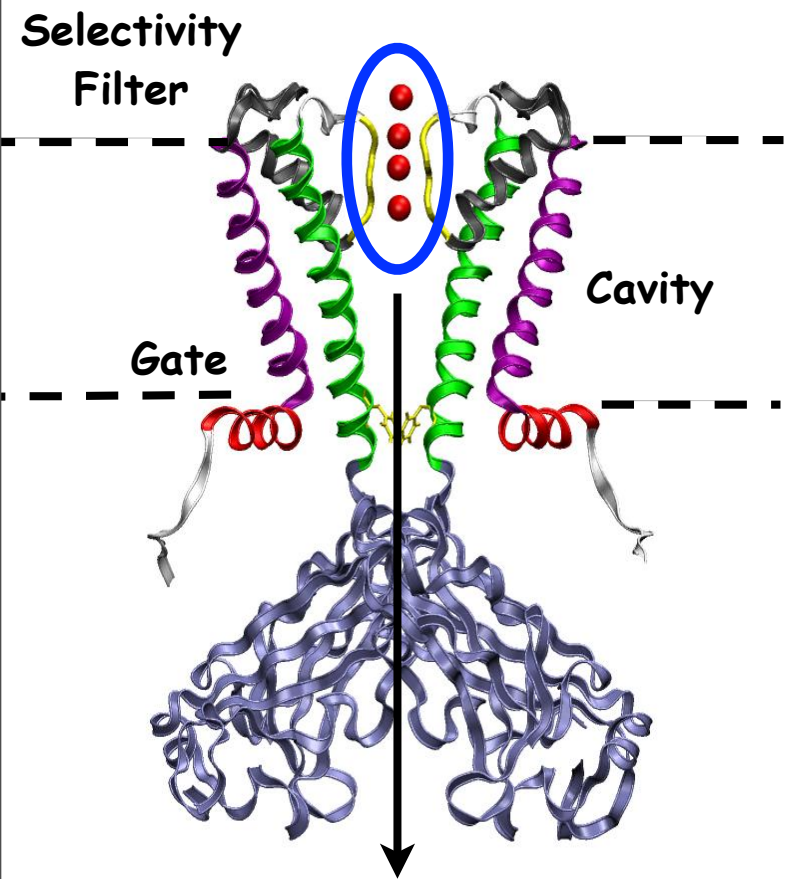


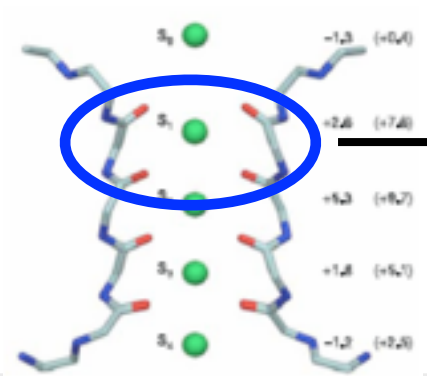
Gating : stimulus-triggered

Stimulus: ligand, voltage, stress

Ion Channels: Selectivity

Close structure at 3.65 Å in 2003

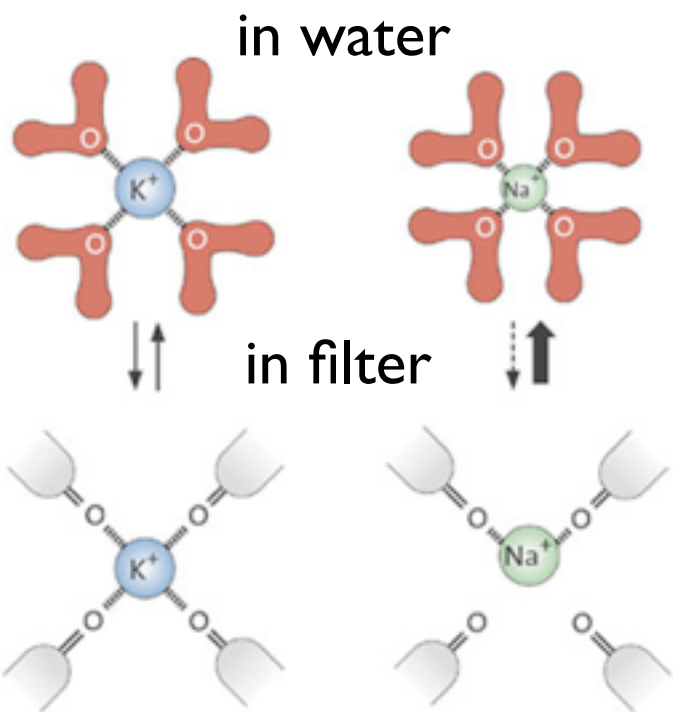




Potassium Selective:

K^+ radius = 1.33Å

Na^+ radius = 0.95Å



http://www.bio.miami.edu/~cmallery/150/memb/ion_channels.htm

Continuum Calculations

- Ion flux rate: ~10 million/second
- MD simulation time scale: ~ 100s of ns (10^{-15})
- Probability of permeation events very low
- Electrostatic free energy barrier measurement

Electrostatic free energy to transfer an ion from bulk solution to a point *r* in the pore of protein:

$$\Delta\Delta G(\mathbf{r}) = [\Delta G^{ic}(\mathbf{r}) - \Delta G^{c} - \Delta G^{i}]$$

$\phi(\mathbf{r}_i)$ is the electrostatic potential at the position of q_i.

$$\Delta G = \frac{1}{2}\sum_i q_i \phi(\mathbf{r}_i)$$

Finite difference Poisson Boltzmann equation:

$$\nabla\cdot(\epsilon\nabla\phi) = -\rho$$

Thank You