Biophysics of Membrane Proteins BIOPHYS 490M

WF 3:00-3:50

Office hours: Tue 2:00-3:00

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#3161, Beckman Institute
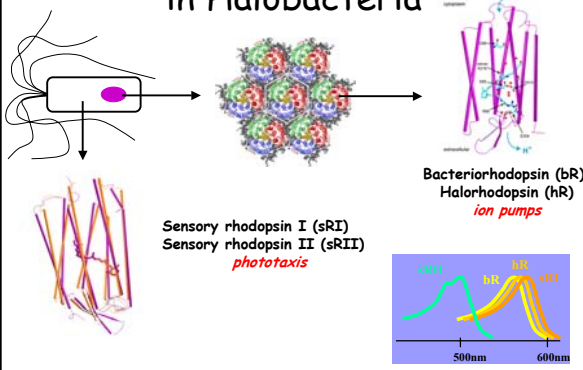
(217)-244-4361 emad@ks.uiuc.edu
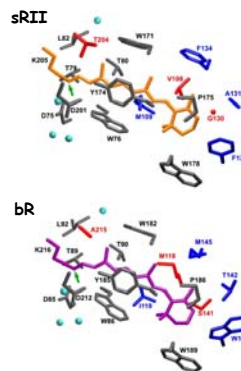
www.ks.uiuc.edu/~emad/BIOPHYS490M

Objectives of the Course

- General understanding of membrane proteins and membrane-protein interaction
- Examples of theoretical biophysical studies of membrane proteins
- General knowledge about methodologies in such studies
- What could be learnt from calculations and from careful examination of structure and dynamics of (membrane) proteins in terms of function
  structure-function relationship

- Experimenting with protein structures (term project – paper/presentation)

Bioenergetics and Phototaxis in Halobacteria

Bacteriorhodopsin (bR)
Halorhodopsin (hR)
ion pumps

Sensory rhodopsin I (sRI)
Sensory rhodopsin II (sRII)
phototaxis

Binding Sites of bR and sRII

sRII

bR

<u>Binding pocket structures</u>
- Aromatic residues.
- Hydrogen-bond network.
  (counter-ion asparatates, internal water molecules)

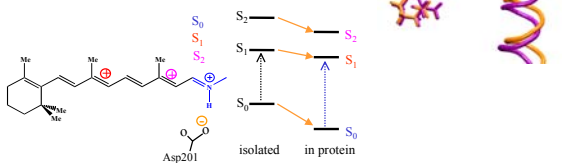Spectral shift cannot be reversed by mutagenesis substitutions

What is the main determinant of spectral tuning?

Structural Determinants of Spectral Tuning in retinal proteins

Orange: sensory rhodopsin II
Purple: bacteriorhodopsin

$N_{16} - C_\gamma$(Asp201: sRII) : 4.5 Å
$N_{16} - C_\gamma$(Asp212: bR)   : 5.2 Å

S_0
S_1
S_2

Asp201   isolated   in protein

References

General principles of membrane and membrane proteins structure
- Molecular Biology of the Cell - Alberts
- Biochemistry – Stryer
- Biochemistry – Voet & Voet

Methodologies/Structure
- Related web sites (will be introduced)

Case studies
- Research articles (files will be available in the web page of the course)
  www.ks.uiuc.edu/~emad/BIOPHYS490M

Systems Overview

- Biological Membranes
- Rhodopsin and GPCRs
- Sensory rhodopsin and cell signaling
- Bacteriorhodopsin and other bacterial rhodopsins
- Ion channels (K^+ / Cl^-)
- F_0-F_1-ATP-synthase
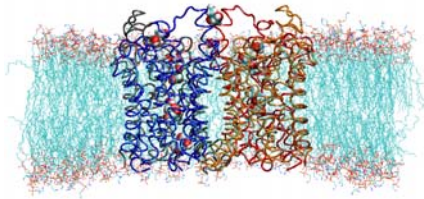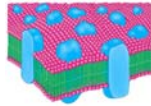- Water channels
- Mechanosensitive channels

Biological Membranes

- Structure
- Function
- Composition
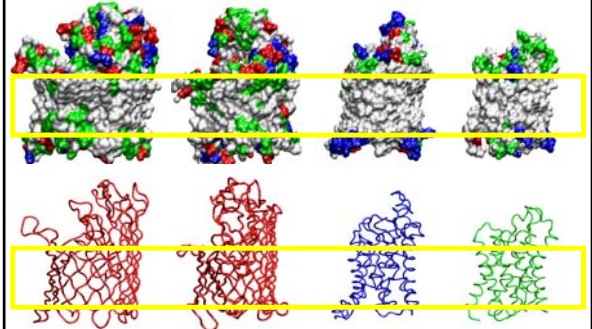- Physicochemical properties
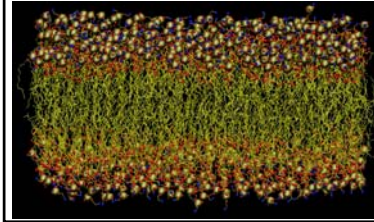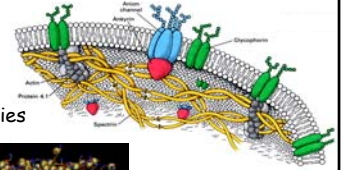
A patch of simulated POPE bilayer
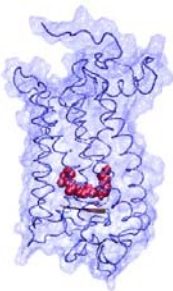
Protein-Membrane Interaction

Protein-Membrane Interaction

| OmpF | Maltoporin | GlpF | AQP1 |

Rhodopsins – Visual Receptors

- Vision
- G-protein coupled receptor
- Sensitivity (signaling cascade)
- Color vision (spectral tuning)
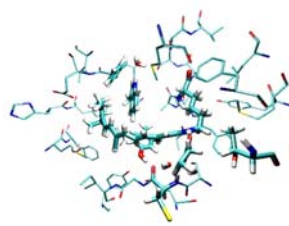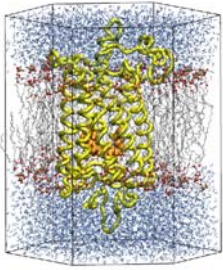- Phototaxis (Sensory rhodopsins)
- Light energy storage

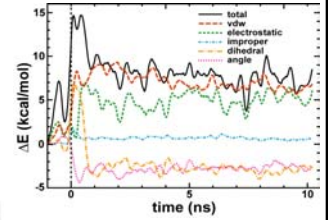## Exploring the process of vision   Light
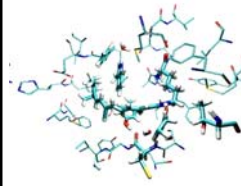

G-protein signaling pathway

Rod⁺   Cone

Rhodopsin

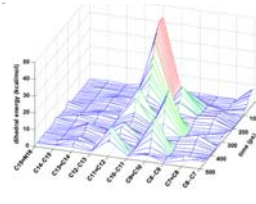## Rhodopsin and Signaling Process

Retinal isomerization induces conformational changes

Retinal Isomerization

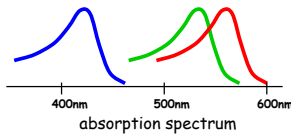Mechanism of storage of light energy

Twist Propagation

Color Vision

Color is sensed by red, green, and blue (rhodopsin) visual receptors.

400nm   500nm   600nm
absorption spectrum

Interesting point.
Their chromophores are the same!

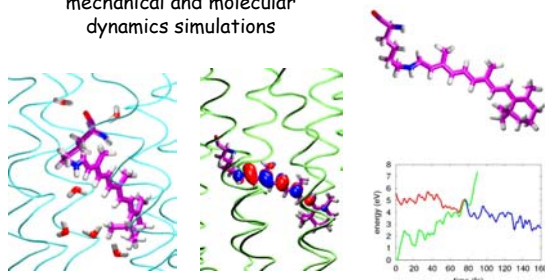**How does the protein tune its absorption spectrum?**

Bacterial rhodopsins

- Proton pump
- Oldest bacterial family
- The best studied membrane protein
- X-ray structures
- Other bacterial rhodopsins

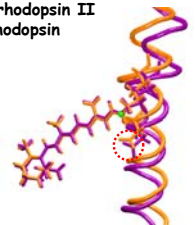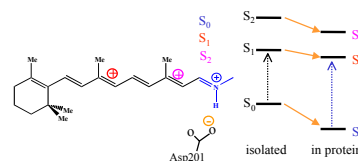## Coupling of photoabsorption and chromophore isomerization

Combined quantum mechanical and molecular dynamics simulations

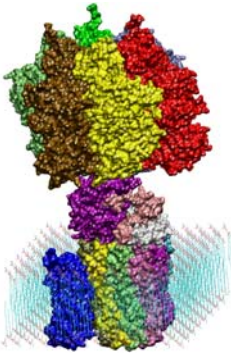## Structural Determinants of Spectral Tuning in retinal proteins

Orange: sensory rhodopsin II
Purple: bacteriorhodopsin

$N_{16} - C_{\gamma}(Asp201: sRII) : 4.5 \text{ Å}$
$N_{16} - C_{\gamma}(Asp212: bR) : 5.2 \text{ Å}$

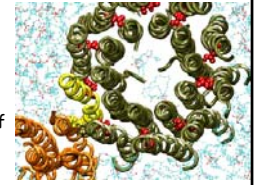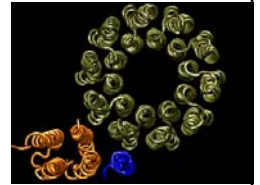S_0
S_1
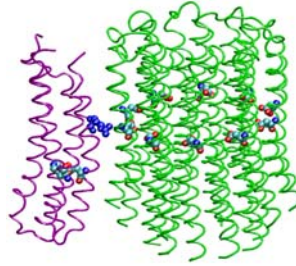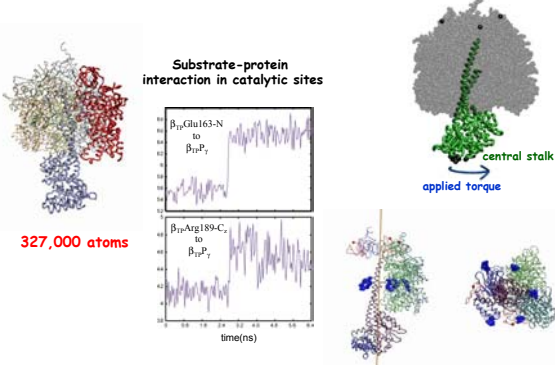S_2

Asp201

isolated   in protein

ATP-synthase

- Proton gradient
- ATP - energy of life
- Mechanical and chemical energy conversion
- Hydrolysis – synthesis

Rotary Motions of Membrane unit of ATP-synthase

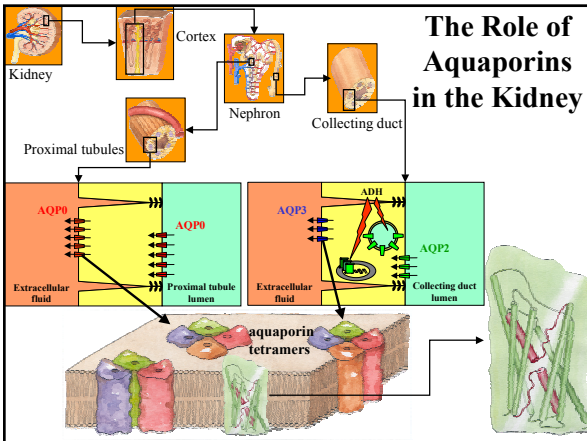Steered Molecular Dynamics simulation of single-helix rotation in the trans-membrane unit of ATP-synthase

Rotation of F_1-ATPase Central Stalk

Substrate-protein interaction in catalytic sites

β_{TP}Glu163-N to $\beta_{TP}P_T$

β_{TP}Arg189-C_z to $\beta_{TP}P_T$

time(ns)

327,000 atoms

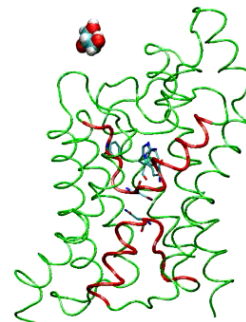central stalk

applied torque

Aquaporin Water Channels
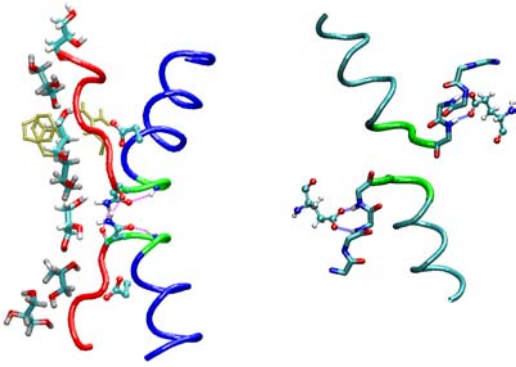

- Water transport
- Glycerol transport
- Permeation rate
- Substrate selectivity
- Stereoselectivity
- Filtering ions and protons

The Role of Aquaporins in the Kidney

Kidney

Cortex

Nephron

Proximal tubules

Collecting duct

AQP0

AQP0

AQP3

ADH

AQP2

Extracellular fluid

Proximal tubule lumen

Extracellular fluid

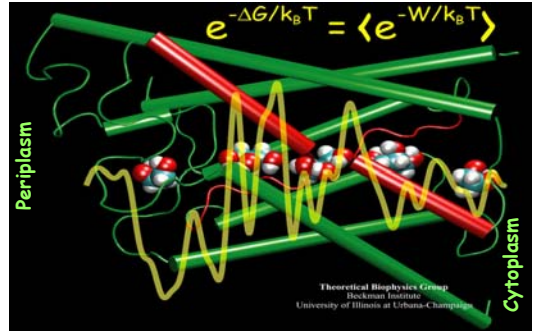Collecting duct lumen

aquaporin tetramers

Description of full conduction pathway

Role of Architecture in Function

Energetics of Conduction

$$e^{-\Delta G/k_B T} = \langle e^{-W/k_B T} \rangle$$

Periplasm

Cytoplasm

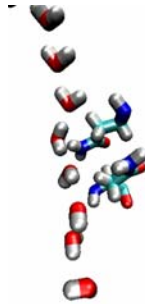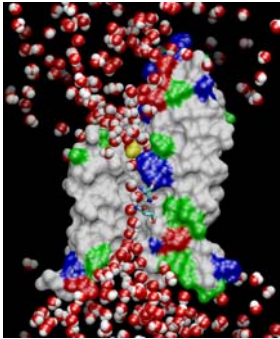Theoretical Biophysics Group
Beckman Institute
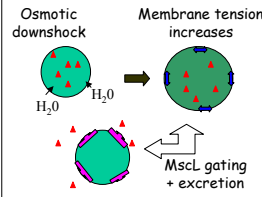University of Illinois at Urbana-Champaign

Asymmetric Profile in the Vestibules

Discovery of a Simple Mechanism for Proton Filtering

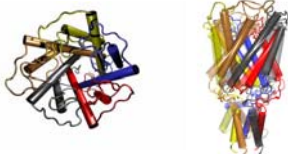## MscL – Mechanosensitive Channel

MscL is a bacterial safety valve

Osmotic downshock

Membrane tension increases

H_2O H_2O

MscL gating + excretion

Roles in Higher Organisms

hearing → ← balance

touch
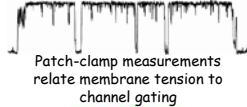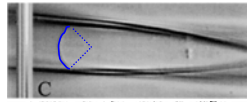
gravity

cardiovascular regulation
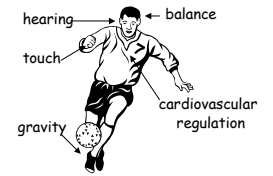
MscL - Mechanosensitive Channel

MscL gates by membrane tension

Pore expands to 30 Å as helices flatten out

C

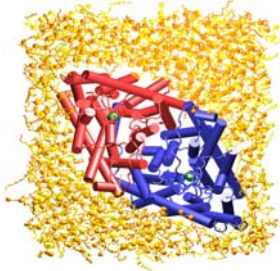Patch-clamp measurements relate membrane tension to channel gating

The protein is stiffest in the pinched gating region, in agreement with EPR measurements
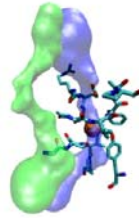
KcsA - Voltage-gated Potassium Channel

- Voltage regulation
- Action potential
- Selectivity
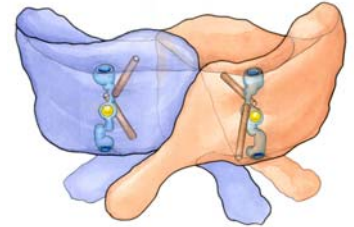- Efficiency

ClC Chloride Channel

- Dimer with separate pores
  α-helix dipoles coordinate
  ions
- very short pore region
- dangling termini
- voltage gates (mystery)

## ClC Chloride Channel

Characterization of
two pores

Unusual membrane spanning
regions

Methodological Discussions

- Modeling of proteins in membranes
- Molecular dynamics (MD) simulations
- Steered and interactive MD
- Exploring protein structures (PDB files)
- Visualization and Analysis of structures
  and dynamics trajectories
- Simulation of pressure gradient across the
  membrane

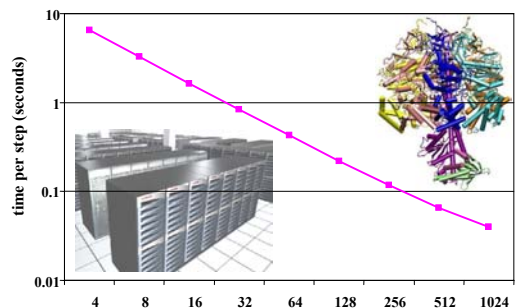## Modeling of proteins in membrane
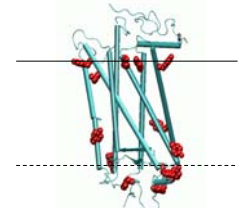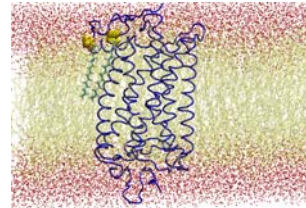
Embedding rhodopsin in a POPC bilayer
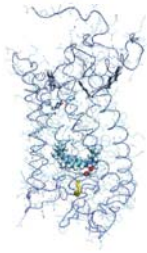
Modeling of proteins in membrane
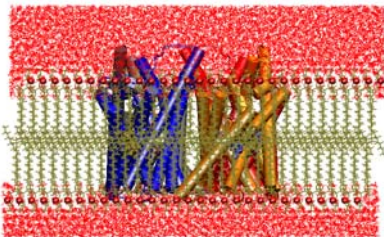
Embedding an aquaporin tetramer in a POPE bilayer

NAMD Molecular Dynamics

VMD: Molecular Visualization

On all platforms, e.g., Windows, Mac, Linux, Sun, SGI, …

Flexible Tcl scripting language

Stereoscopic 3D display

No limits on size of molecules

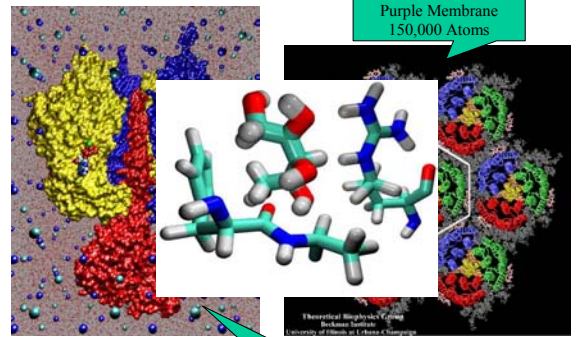Exports scenes to ray tracers for publication quality rendering.

Animates dynamics trajectories

Interactive Molecular Dynamics

VMD freely available, with source code from http://www.ks.uiuc.edu

VMD
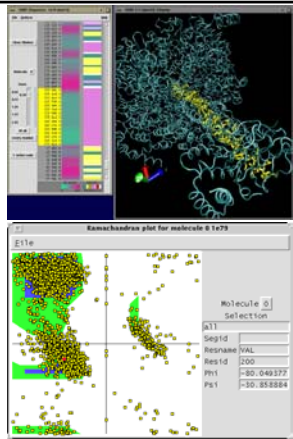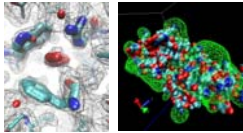Visual Molecular Dynamics

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VMD - www.ks.uiuc.edu/Research/vmd

Purple Membrane
150,000 Atoms

F1 ATPase
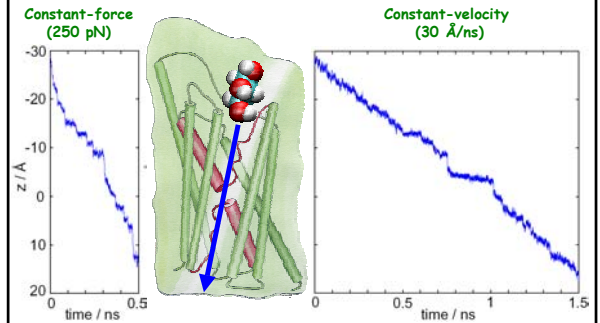327,000 Atoms

VMD
Visual Molecular Dynamics

Large Scale Visualization

Scripting interface
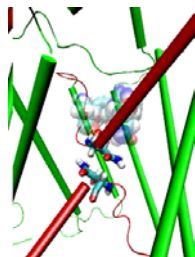3D/Stereo graphics

VMD
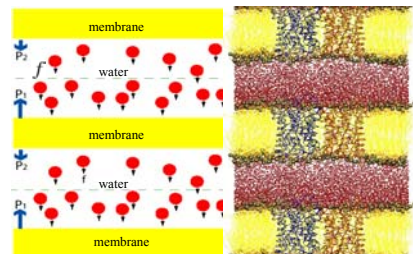Visual Molecular Dynamics

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Steered Molecular Dynamics

Constant-force
(250 pN)

Constant-velocity
(30 Å/ns)

z / Å

time / ns

time / ns

- Constant-force: $f(t) = C$
- Constant-velocity: $f(t) = k [vt - (x_t - x_0)]$

Interactive Molecular Dynamics

VMD ‹----------› NAMD

Inducing Hydrostatic Pressure Difference

membrane

P_2

f water

P_1

membrane

P_2

f water

P_1

membrane

Next session (Friday)

Basic principles of structure and
function of membranes and
Membrane-protein interactions
Biochemistry – Stryer, Chapter 12