Structure of a PDB file

index, name, resname, chain, resid, x, y, z, segname

```
ATOM     22  N   ALA B   3      -4.073  -7.587  -2.708  1.00  0.00      BH
ATOM     23  HN  ALA B   3      -3.813  -6.675  -3.125  1.00  0.00      BH
ATOM     24  CA  ALA B   3      -4.615  -7.557  -1.309  1.00  0.00      BH
ATOM     25  HA  ALA B   3      -4.323  -8.453  -0.704  1.00  0.00      BH
ATOM     26  CB  ALA B   3      -4.137  -6.277  -0.676  1.00  0.00      BH
ATOM     27  HB1 ALA B   3      -3.128  -5.950  -0.907  1.00  0.00      BH
ATOM     28  HB2 ALA B   3      -4.724  -5.439  -1.015  1.00  0.00      BH
ATOM     29  HB3 ALA B   3      -4.360  -6.338   0.393  1.00  0.00      BH
ATOM     30  C   ALA B   3      -6.187  -7.538  -1.357  1.00  0.00      BH
ATOM     31  O   ALA B   3      -6.854  -6.553  -1.264  1.00  0.00      BH
ATOM     32  N   ALA B   4      -6.697  -8.715  -1.643  1.00  0.00      BH
ATOM     33  HN  ALA B   4      -6.023  -9.463  -1.751  1.00  0.00      BH
ATOM     34  CA  ALA B   4      -8.105  -9.096  -1.934  1.00  0.00      BH
ATOM     35  HA  ALA B   4      -8.287  -8.878  -3.003  1.00  0.00      BH
ATOM     36  CB  ALA B   4      -8.214 -10.604  -1.704  1.00  0.00      BH
ATOM     37  HB1 ALA B   4      -7.493 -11.205  -2.379  1.00  0.00      BH
ATOM     38  HB2 ALA B   4      -8.016 -10.861  -0.665  1.00  0.00      BH
ATOM     39  HB3 ALA B   4      -9.245 -10.914  -1.986  1.00  0.00      BH
ATOM     40  C   ALA B   4      -9.226  -8.438  -1.091  1.00  0.00      BH
ATOM     41  O   ALA B   4     -10.207  -7.958  -1.667  1.00  0.00      BH
0000000000000000000000000000000000000000000000000000000000000000000000000000
         10        20        30        40        50        60        70
```

>>> It is an ascii, fixed-format file <<<

VMD Atom Selection Commands

index, name, resname, chain, resid, x, y, z, segname

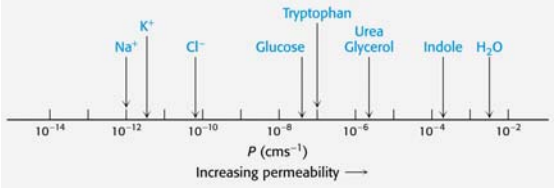
```
ATOM     22  N   ALA B   3      -4.073  -7.587  -2.708  1.00  0.00      BH
ATOM     23  HN  ALA B   3      -3.813  -6.675  -3.125  1.00  0.00      BH
```

```
(name CA CB) and (resid 1 to 4) and (segname BH)

protein and resname LYS ARG GLU ASP

water and within 5 of (protein and resid 62 and name CA)

water and within 3 of (protein and name O and z < 10)
```

We could have multiple representations with different atom selections, colors, and drawing methods

Bilayer Permeability

- Water is an exception: small size, lack of charge, and its high concentration



Water Transport across the Membrane

Always passive; bidirectional; osmosis-driven

1. Diffusion through lipid bilayers

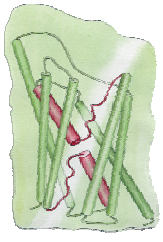
   slower, but enough for many purposes

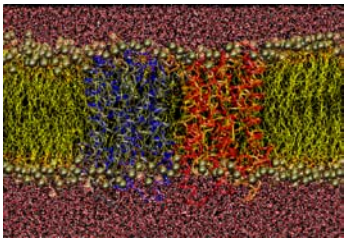
1. Channel-mediated

   Large volumes of water needed to be transported (kidneys).

   Fast adjustment of water concentration is necessary (RBC, brain, lung).

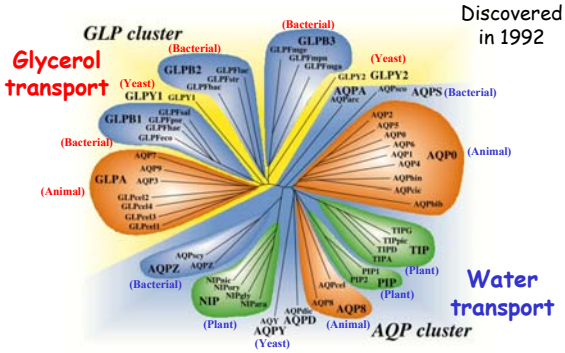
Aquaporins
Membrane water channels





The Aquaporin Superfamily

Discovered in 1992



Heymann and Engel *News Physiol. Sci.* **14**, 187 (1999)

Aquaporins in Human Body

Aquaporin-0	Eye: lens fiber cells	Fluid balance of the lens
Aquaporin-1	Red blood cells Kidney: proximal tubules Eye: ciliary epithelium Brain: choriod plexus Lung: alveolar epithelial cells	Osmotic protection Concentration of urine Aqueous humor Production of CSF Alveolar hydration
Aquaporin-2	Kidney: collecting ducts	ADH hormone activity
Aquaporin-3	Kidney: collecting ducts Trachea: epithelial cells	Reabsorption of water Secretion of water
Aquaporin-4	Kidney: collecting ducts Brain: ependymal cells Brain: hypothalamus Lung: bronchial epithelium	Reabsorption of water CSF fluid balance Osmosensing function? Bronchial fluid secretion
Aquaporin-5	Salivary glands Lacrimal glands	Production of saliva Production of tears
Aquaporin-6	Kidney	Very low water permeability!
Aquaporin-7	Testis and sperm	
Aquaporin-8	Testis, pancreas, liver	
Aquaporin-9	Leukocytes	
Aquaporin-10		

Additional members are suspected to exist.

Aquaporins and Diseases

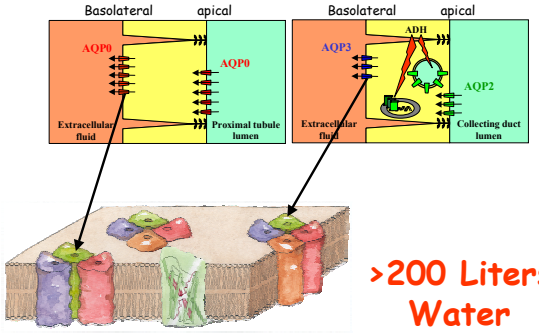
Aquaporin-0	Eye: lens fiber cells	Fluid balance of the lens
Aquaporin-1	Red blood cells Kidney: proximal tubules Eye: ciliary epithelium Brain: choriod plexus Lung: alveolar epithelial cells	Osmotic protection Concentration of urine Aqueous humor Production of CSF Alveolar hydration
Aquaporin-2	Kidney: collecting ducts	ADH hormone activity

two clear examples of disease have been identified as resulting from deficiency in aquaporins:

Mutations in the aquaporin-2 gene cause hereditary nephrogenic diabetes insipidus in humans.

Mice homozygous for inactivating mutations in the aquaporin-0 gene develop congenital cataracts.

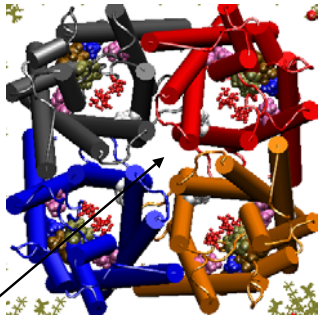
High Permeation to Water



Nephrogenic diabetes insipidus

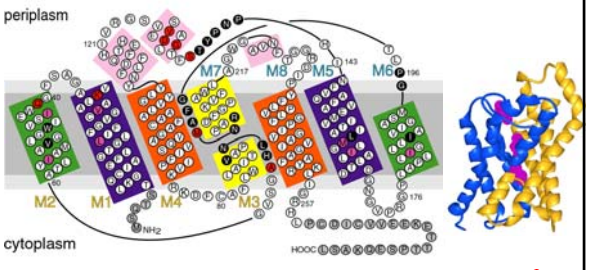
>200 Liters Water Everyday!

Tetrameric arrangement of Aquaglyceroporins in membrane



The central pore may be functional?

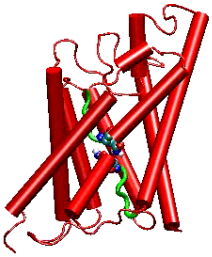
Architecture of the Channel



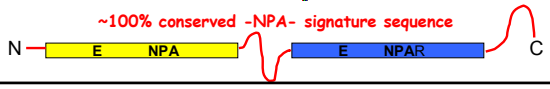
Fu, *et al*, *Science* **290**, 481 (2000)

Functionally Important Features

- Tetrameric architecture
- Amphipatic channel interior
- Water and glycerol transport
- Protons, and other ions are excluded
- Conserved asparagine-proline-alanine residues; NPA motif
- Characteristic half-membrane spanning structure

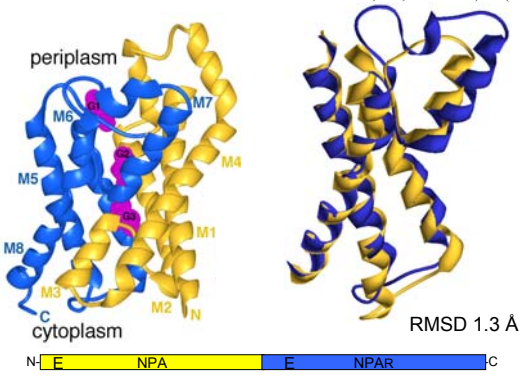


~100% conserved -NPA- signature sequence



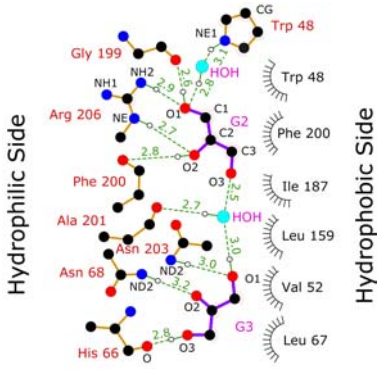
Channel Fold

Fu, *et al*, *Science* **290**, 481 (2000)



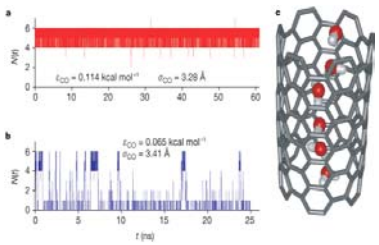
Internal gene duplication (20% conservation)

A Semi-hydrophobic channel



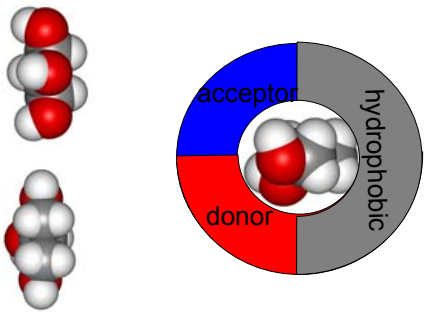
Fu, *et al*, *Science* **290**, 481 (2000)

Completely hydrophobic channels

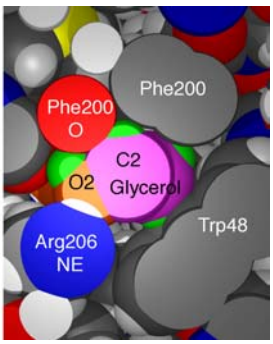


Hummer, et. al., Nature, 414: 188-190, 2001

Complementarity
glycerol molecule ↔ channel



Tight Packing in the Selectivity Filter



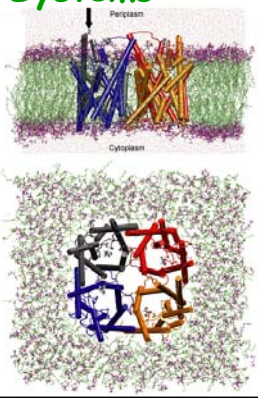
Simulated Systems

NAMD, CHARMM27
NpT ensemble at 310 or 298 K
1ns equilibration, 4ns production

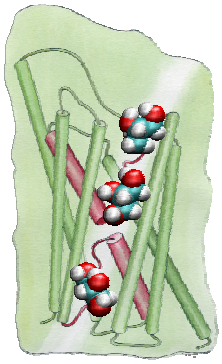
Protein:	~ 15,000 atoms
Lipids (POPE):	~ 40,000 atoms
Water:	~ 51,000 atoms
Total:	~ 106,000 atoms

3.5 days / ns - 128 O2000 CPUs
11 days / ns - 32 Linux CPUs

0.35 days / ns - 512 LeMieux CPUs

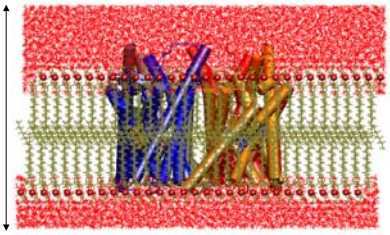


Glycerol-Saturated GlpF



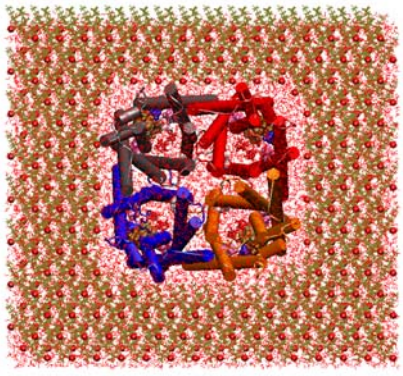
Embedding the protein in POPE lipid bilayer

77 A



Glpf: ~15,000 atoms Lipid: ~40,000 atoms (~320 POPE)
Cl: 4 ions Water: ~51,000 atoms (~17000 H2O)
Total: ~106,000 atoms

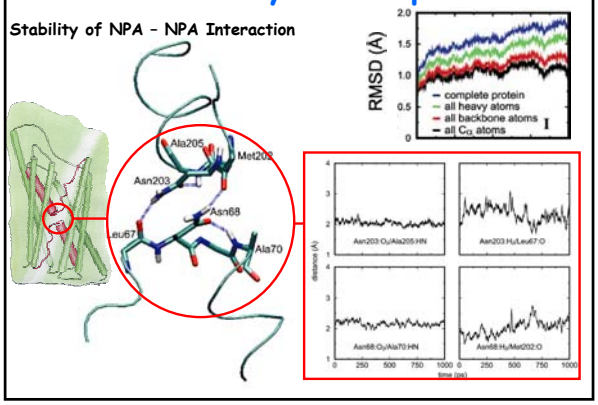
112 A



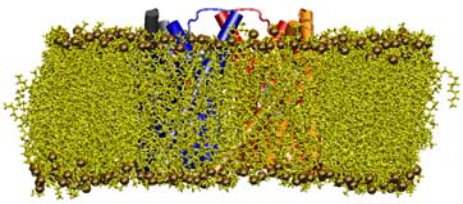
122 A

An extremely stable protein

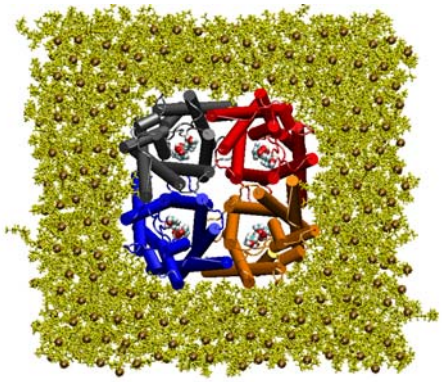
Stability of NPA - NPA Interaction



Lipid packing against the protein during NpT simulations

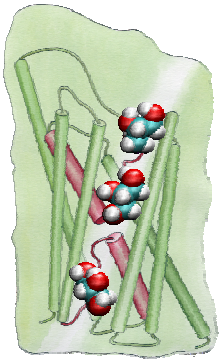


Lipid packing against the protein during NpT simulations

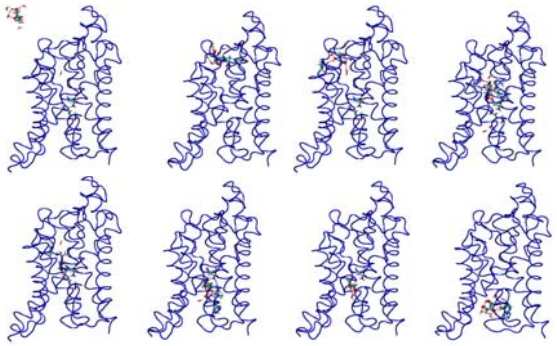


Glycerol Conduction

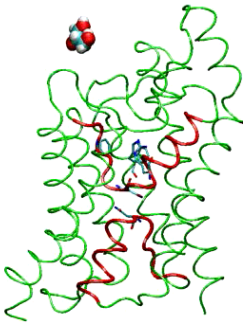
- Spontaneous glycerol conduction on ns time scale;
- Conduction occurs independently in each monomer;
- Exposed backbone carbonyl oxygen atoms dictate the glycerol and water pathway; this explains the non-helical secondary structure in the aquaporin family;
- Glycerol resides at the positions of conserved motif for the longest time during simulation = minimum energy sites;
- Water molecules are essential for the glycerol transport.



Description of full conduction pathway

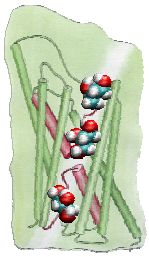


Description of full conduction pathway

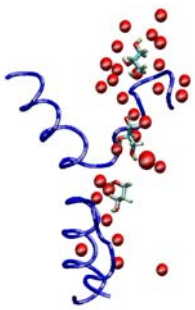


Channel Hydrogen Bonding Sites

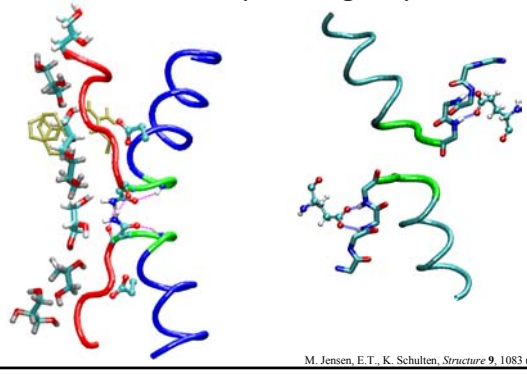
GLN	41	OE1 NE2	LEU	197	O
TRP	48	O NE1	THR	198	O
GLY	64	O	GLY	199	O
ALA	65	O	PHE	200	O
HIS	66	O ND1	ALA	201	O
LEU	67	O	ASN	203	ND2
ASN	68	ND2			
ASP	130	OD1	LYS	33	HZ1 HZ3
GLY	133	O	GLN	41	HE21
SER	136	O	TRP	48	HE1
TYR	138	O	HIS	66	HD1
PRO	139	O N	ASN	68	HD22
ASN	140	OD1 ND2	TYR	138	HN
HIS	142	ND1	ASN	140	HN HD21 HD22
THR	167	OG1	HIS	142	HD1
GLY	195	O	GLY	199	HN
PRO	196	O	ASN	203	HN HD21HD22
			ARG	206	HE HH21HH22



Glycerol pathway in Glpf



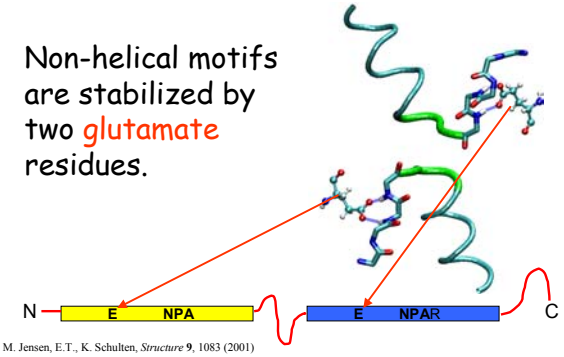
The Substrate Pathway is formed by C=O groups



M. Jensen, E.T., K. Schulten, *Structure* **9**, 1083 (2001)

The Substrate Pathway is formed by C=O groups

Non-helical motifs are stabilized by two glutamate residues.



M. Jensen, E.T., K. Schulten, *Structure* **9**, 1083 (2001)

Glycerol – water competition for hydrogen bonding sites