

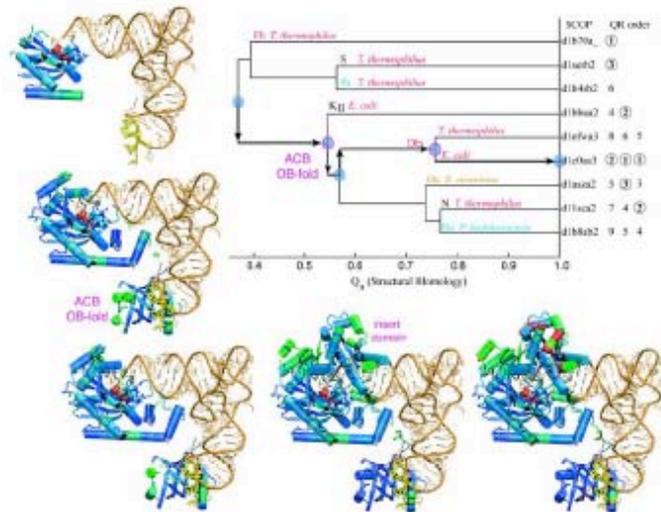
Part III - Bioinformatics Studies Using Multiseq in VMD

- Aminoacyl tRNA Synthetases
- Aquaporins

Lake Tahoe, 2005, Computational Biology Workshop

Evolution of Protein Structure

Aspartyl-tRNA Synthetase



VMD Developers:

Dan Wright

John Eargle

John Stone

Dr. Zan Luthey-Schulten

Brijeet Dhaliwal

Patrick O'Donoghue

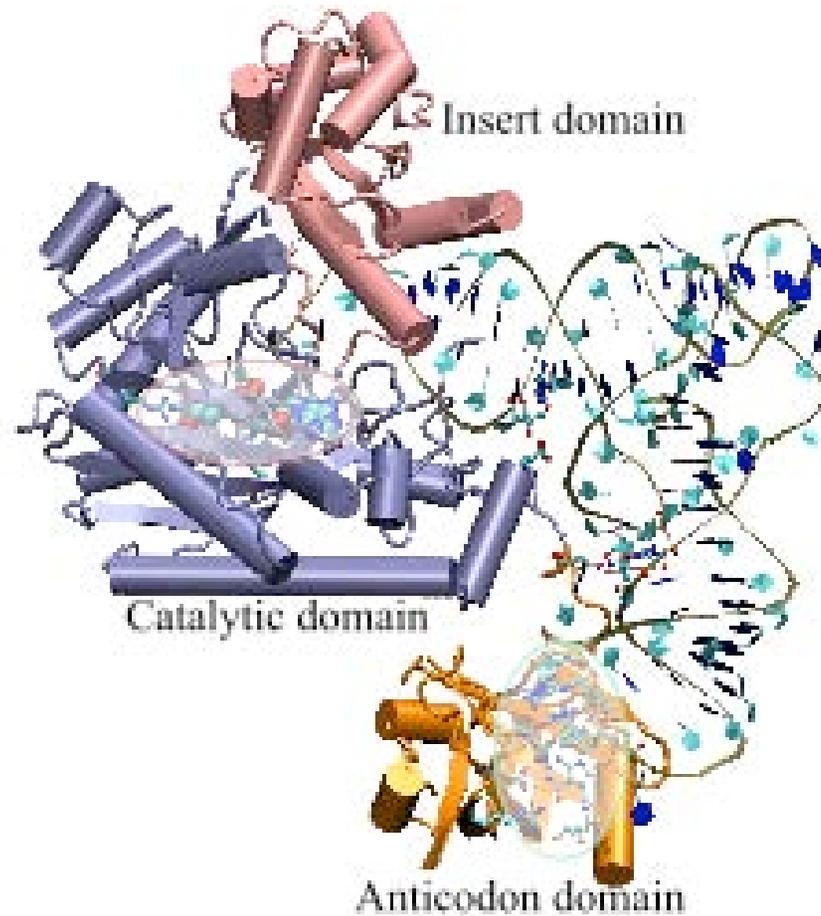
Rommie Amaro

April 2004.

Multiple Sequence Alignments

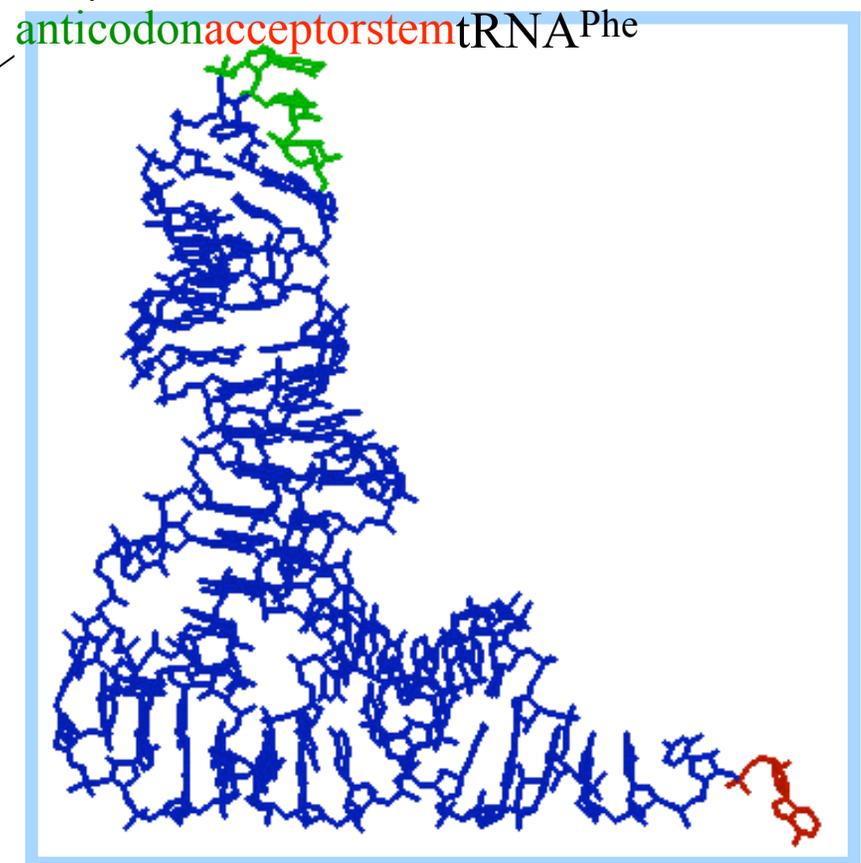
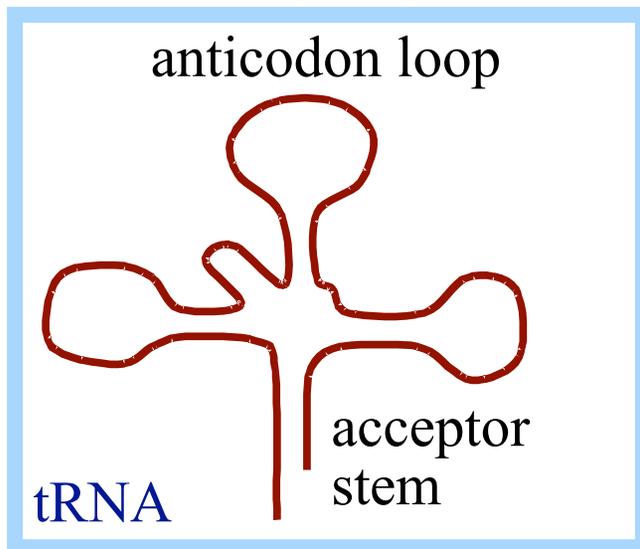
- “The aminoacyl-tRNA synthetases, perhaps better than any other molecules in the cell, optimize the current situation and help to understand (the effects) of HGT” Woese (PNAS, 2000; MMBR 2000)
- Carl Woese - Crafoord Prize 2003

Step 1: Explore active site in catalytic domain and anticodon domain.



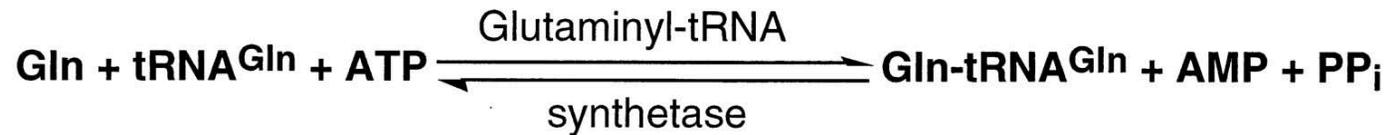
Standard Dogma Molecular Biology

- DNA → RNA → Proteins
- Role of AARS?
- Charging of t-RNA

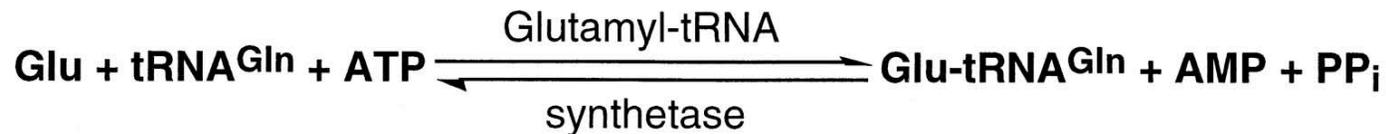


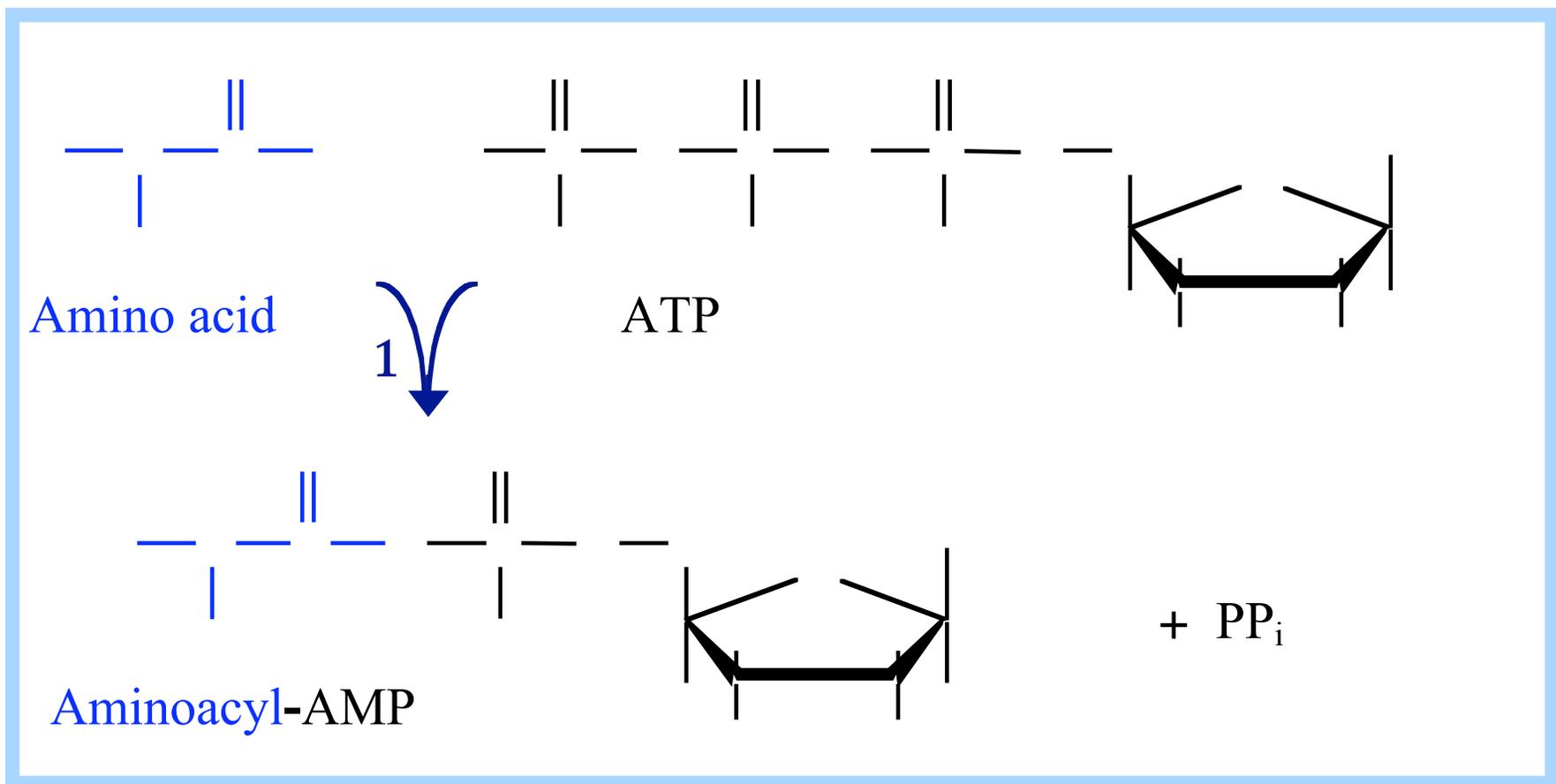
Charging the tRNA

Direct acylation



tRNA-dependent amino acid modification

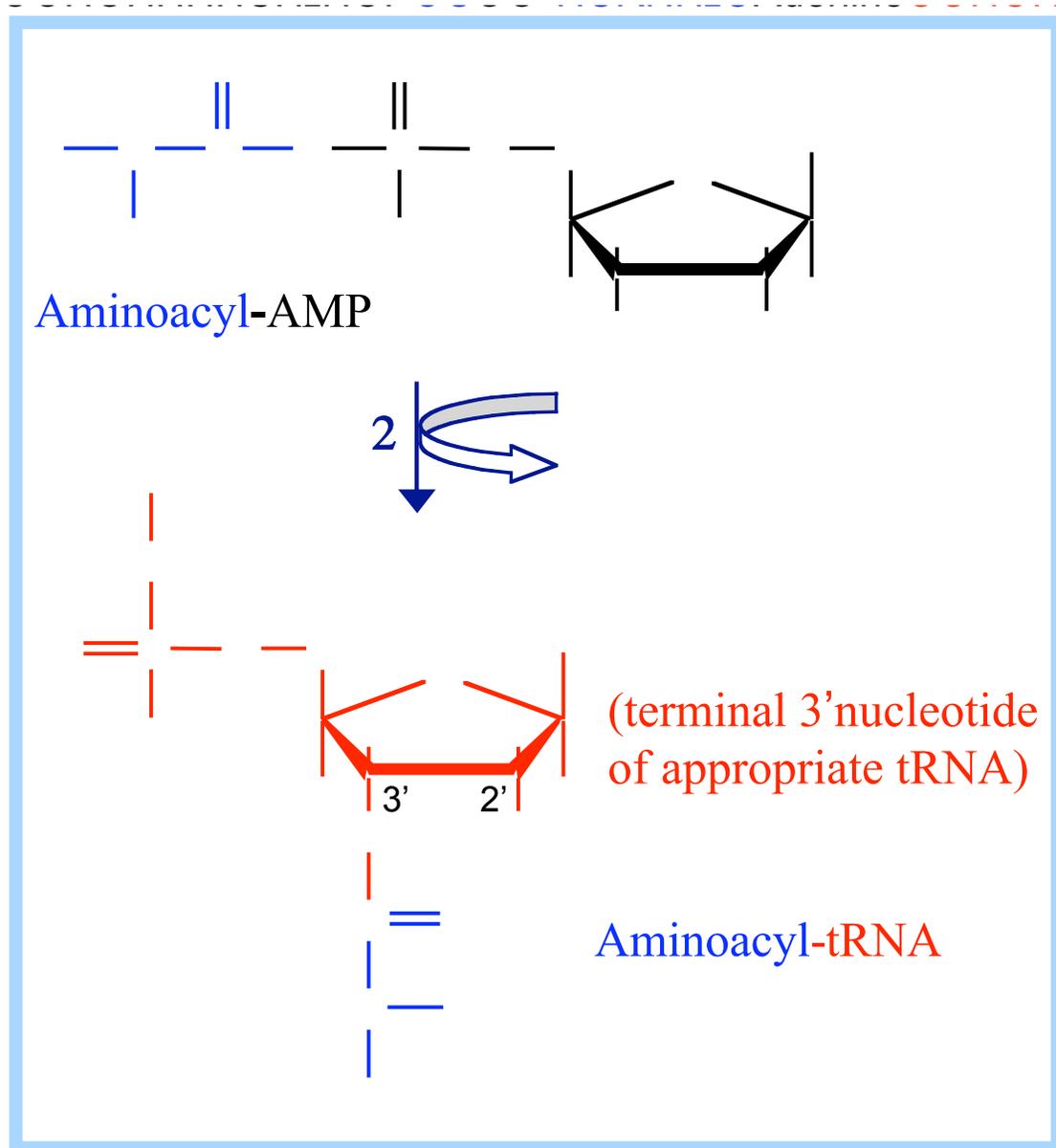




Aminoacyl-tRNA Synthetases catalyze linkage of the appropriate amino acid to each tRNA. The reaction occurs in two steps.

In **step 1**, an O atom of the amino acid α -carboxyl attacks the P atom of the alpha phosphate of ATP.

In **step 2**, the 2' or 3' OH of the terminal adenosine of tRNA attacks the amino acid carbonyl C atom.

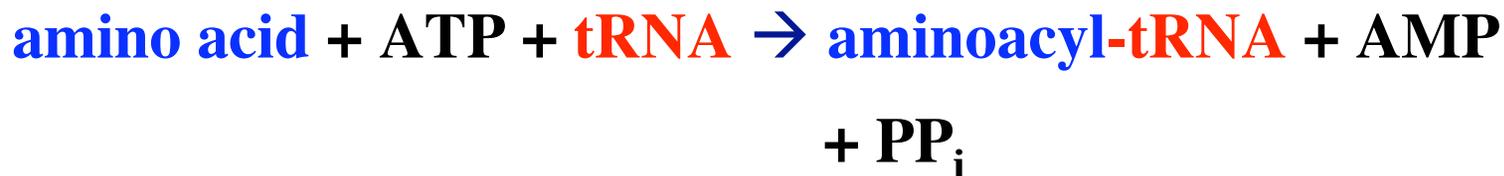


Aminoacyl-tRNA Synthetase

Summary of the 2-step reaction:

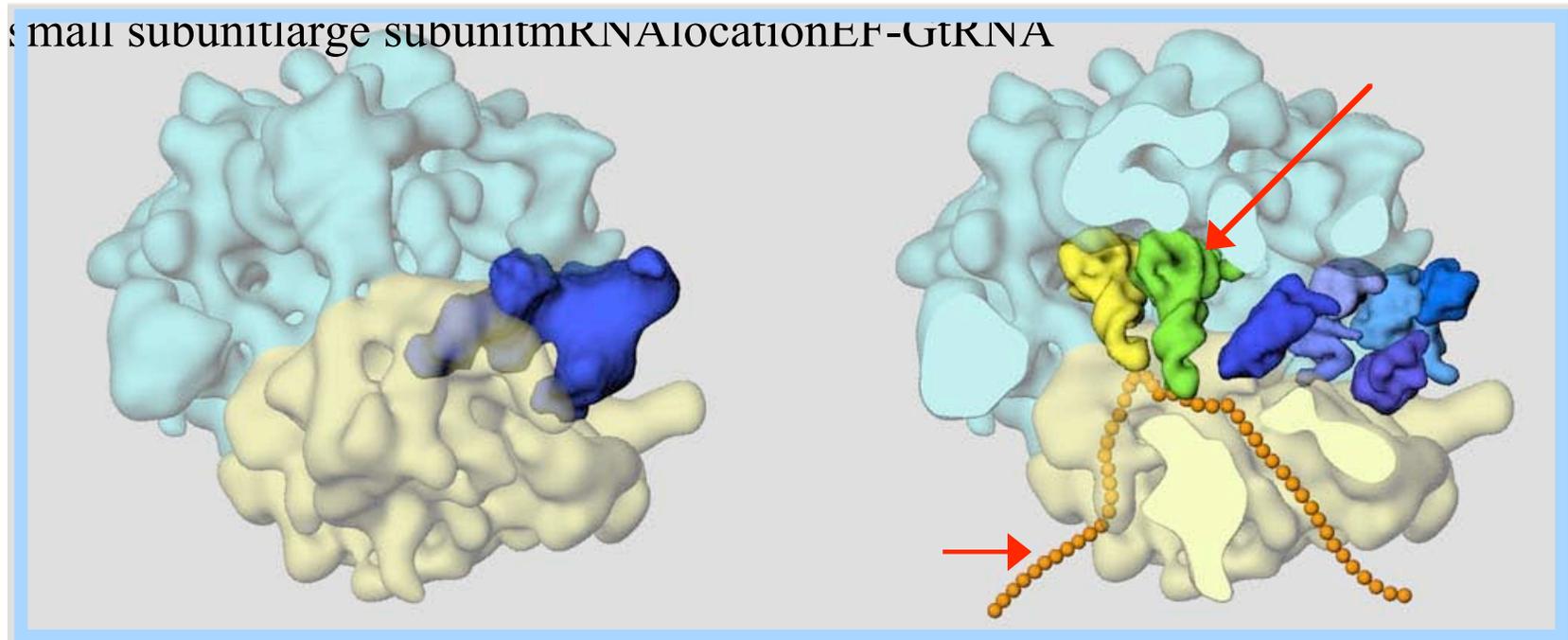


Overall Reaction:



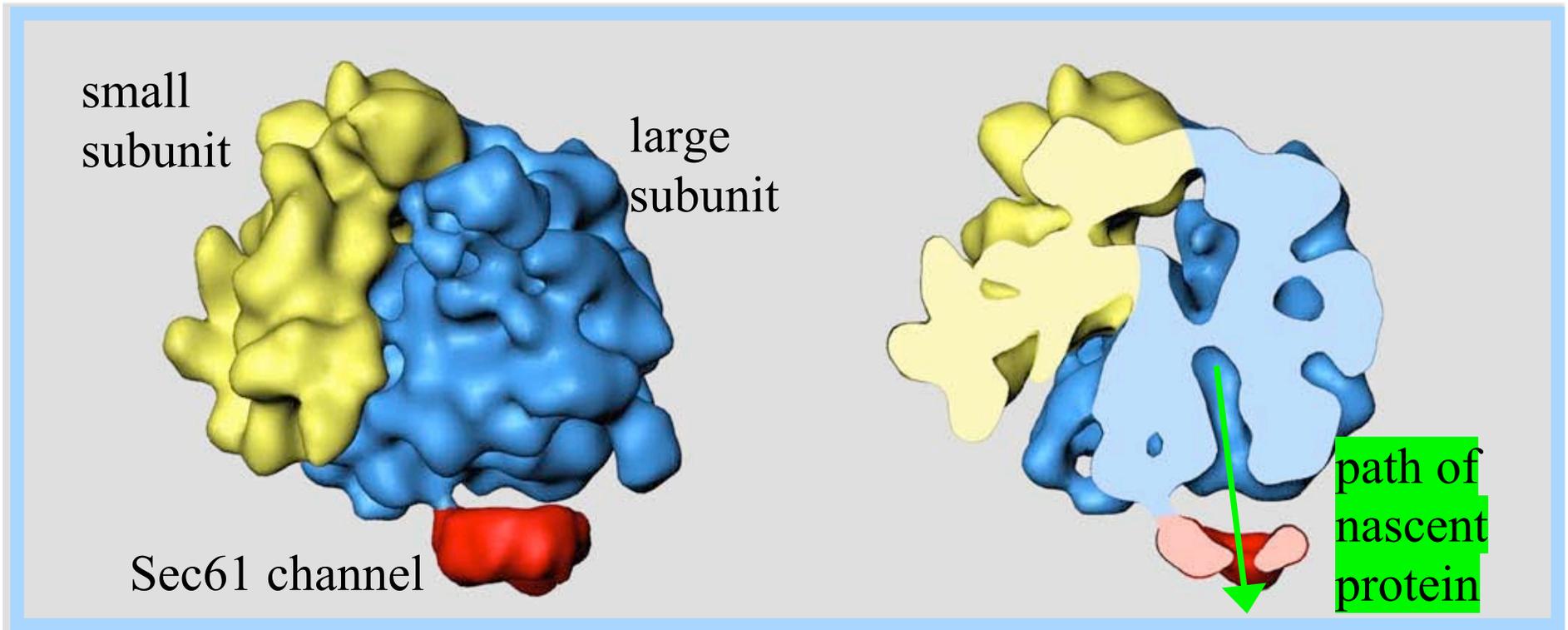
Next step: EF and Ribosome for Protein Synthesis

Structure of the *E. coli* Ribosome



The cutaway view at right shows positions of tRNA (P, E sites) & mRNA (as orange beads).

Figure: Laboratory of Joachim Frank, Wadsworth Center
cryo-EM and 3D image reconstruction



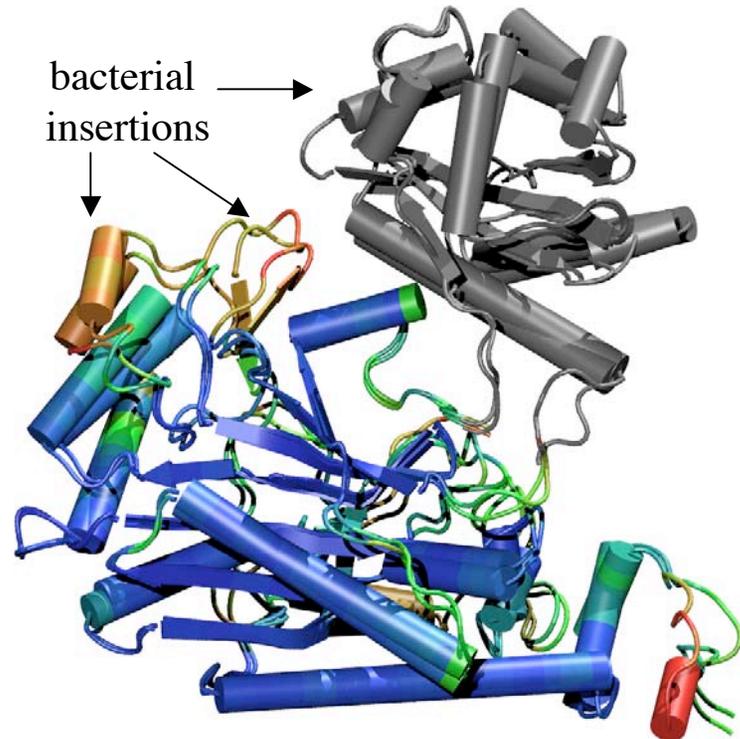
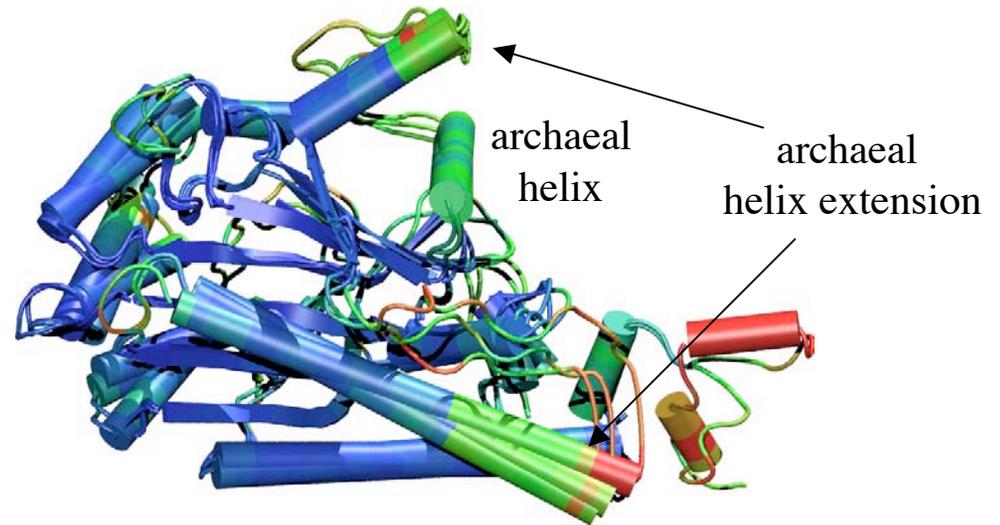
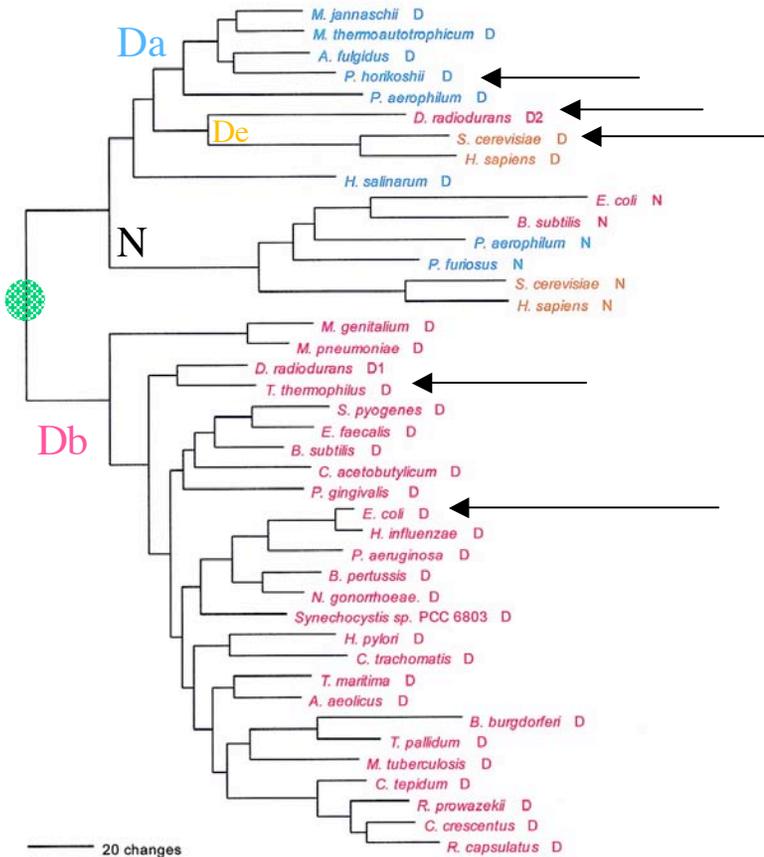
The cutaway view at right shows that the **tunnel** in the yeast large ribosome subunit, through which nascent polypeptides emerge from the ribosome, **lines up** with the lumen of the ER **Sec61 channel**.

Figure provided by Joachim Frank, whose lab carried out the cryo-EM & image reconstruction on which these images are based.

Horizontal Gene Transfer in Protein Structure

Sequence Phylogeny

AspRS-AsnRS Group

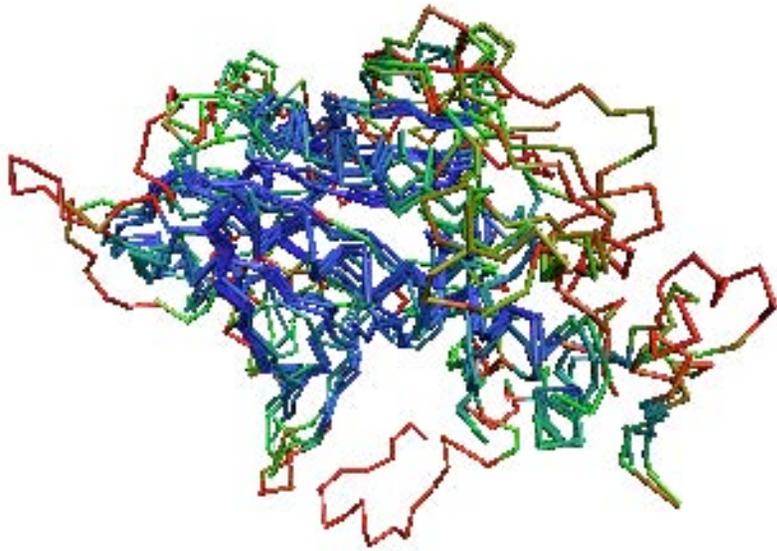


Multiseq extension in VMD

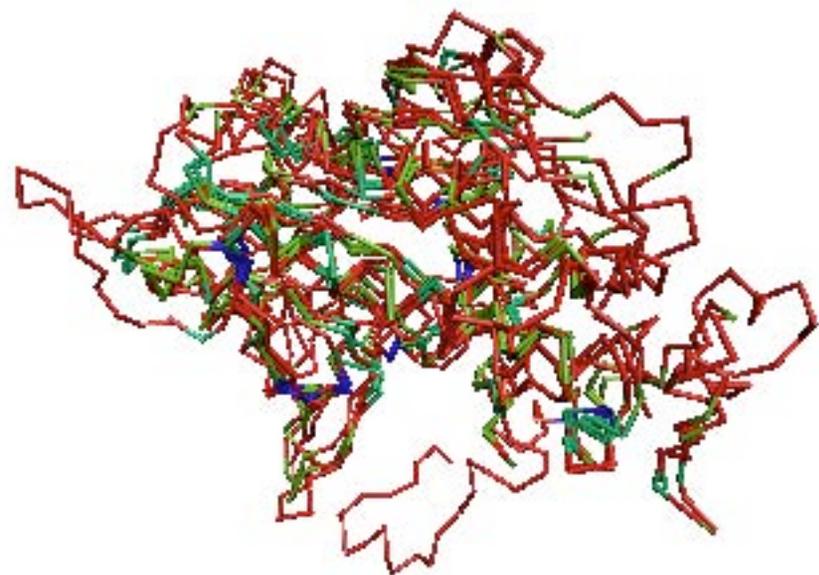
The screenshot shows the VMD 1.8.3a2 OpenGL Display interface. The main window displays a 3D ribbon representation of a protein structure, colored in blue, green, and red. A menu titled "Extensions" is open, listing various plugins: sequence, autoimd, apbsrun, imd, contactmap, pdbtool, ramaplot, rmsd, solvate, timeline, multiseq, tkcon, and vmdmovie. Below the main window, a "treeWindow" displays a phylogenetic tree with the following entries: d1efwa3.ent Thermus thermophilus B, d1c0aa3.ent Escherichia coli B, d1n9wb1.ent d1n9wb1.ent, d1asza2.ent Saccharomyces cerevisiae E, and d1b8aa2.ent Pyrococcus kodakaraensis A. A scale bar of 0.56 is shown below the tree. At the bottom, a "Sequence Display" window shows a sequence alignment for five entries: d1b8aa2.ent, d1asza2.ent, d1n9wb1.ent, d1c0aa3.ent, and d1efwa3.ent. The alignment is as follows:

```
d1b8aa2.ent  IDTEGERLLGKYM--MENENAPLYFLYQYPS-----EAKPFYIMKYDN-----K--PEICRAFDLEYRGI
d1asza2.ent  LSTENEKFLGKLV--RDKYDQDFYILDKFP-----EIRPFYTMPDPA-----N--PKYSNSYDFFMRGEI
d1n9wb1.ent  LSEEAERLLGEYA--KERWGSDFWLVTRYPR-----SVRPFYTYTYP-EE-----DGTTRSFDLLFRGL
d1c0aa3.ent  ---GSD-KP-DLRDE---SKWAPLWVIDFPMFE--DDGEGGLTAMHHPFTSPK-DMTAAELKAAPENAVANAYDMVINGY
d1efwa3.ent  ---GSD-KP-DL-RR---EGFRFLWVVDFFLLEWDEEEEAWTYMHHHPFTSPHPED-LPLLEKDPGRVRLAYDLVLNGVI
```

Conservation

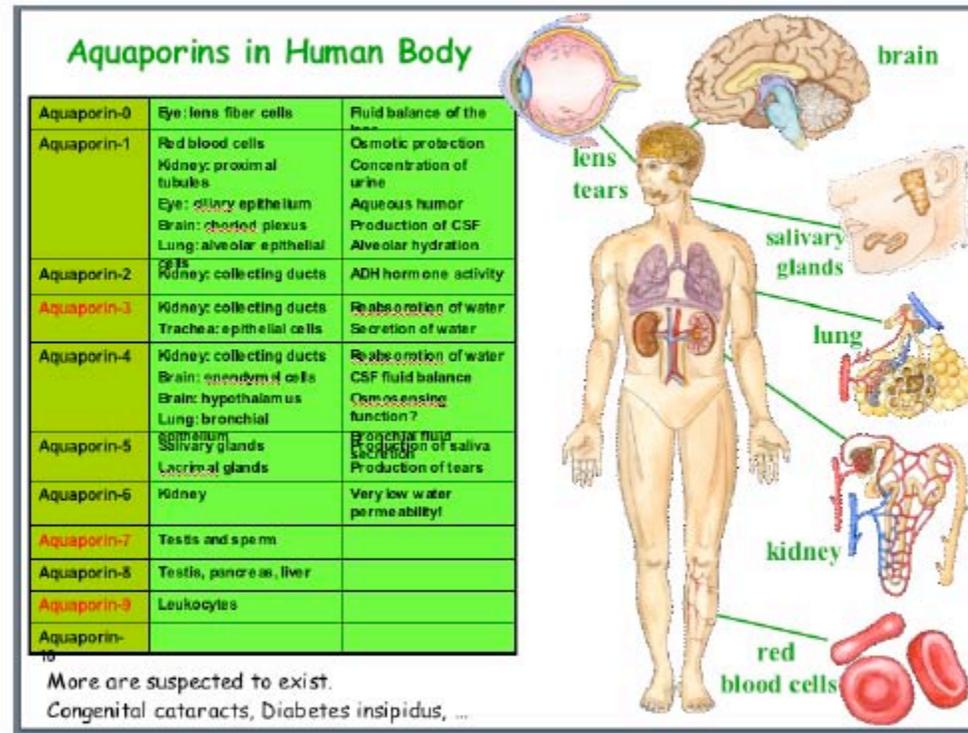


Core Structure Conserved



Sequence Identity of Core
Less than 15%

Aquaporins



VMD Developers:

John Stone

Dan Wright

John Eargle

Fatemeh Khalili

Elizabeth Villa

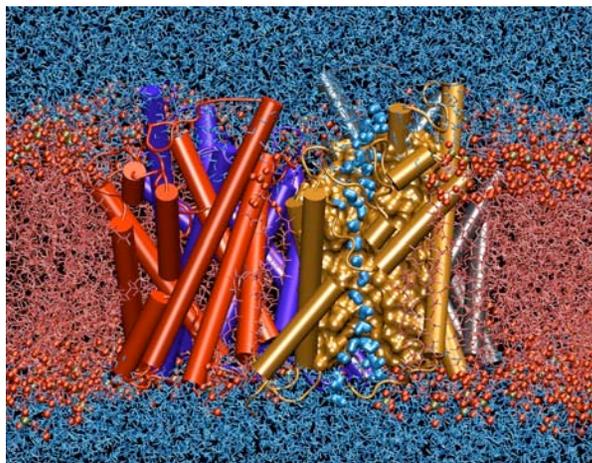
Emad Tajkhorshid

Brijeet Dhaliwal

Zan Luthey-Schulten

Towards Understanding Membrane Channels

The versatile, highly selective and efficient aquaporin



GlpF Structure (Stroud et al)

NAMD with full electrostatics

Periodic boundary conditions

NpT ensemble at 310 K

1ns equilibration

Protein: ~ 15,000 atoms

Lipids: ~ 40,000 atoms

Water: ~ 51,000 atoms

Total: ~ 106,000 atoms

4 hrs / ns – 1024 TSC CPUs

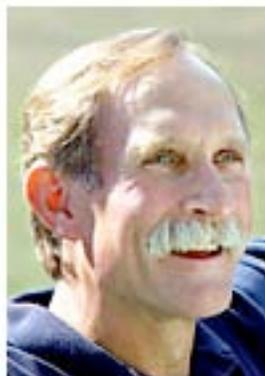


The Nobel Prize in Chemistry 2003

"for discoveries concerning channels in cell membranes"

"for the discovery of water channels"

"for structural and mechanistic studies of ion channels"



Peter Agre

🕒 1/2 of the prize

USA

Johns Hopkins University School of Medicine
Baltimore, MD, USA

b. 1949



Roderick MacKinnon

🕒 1/2 of the prize

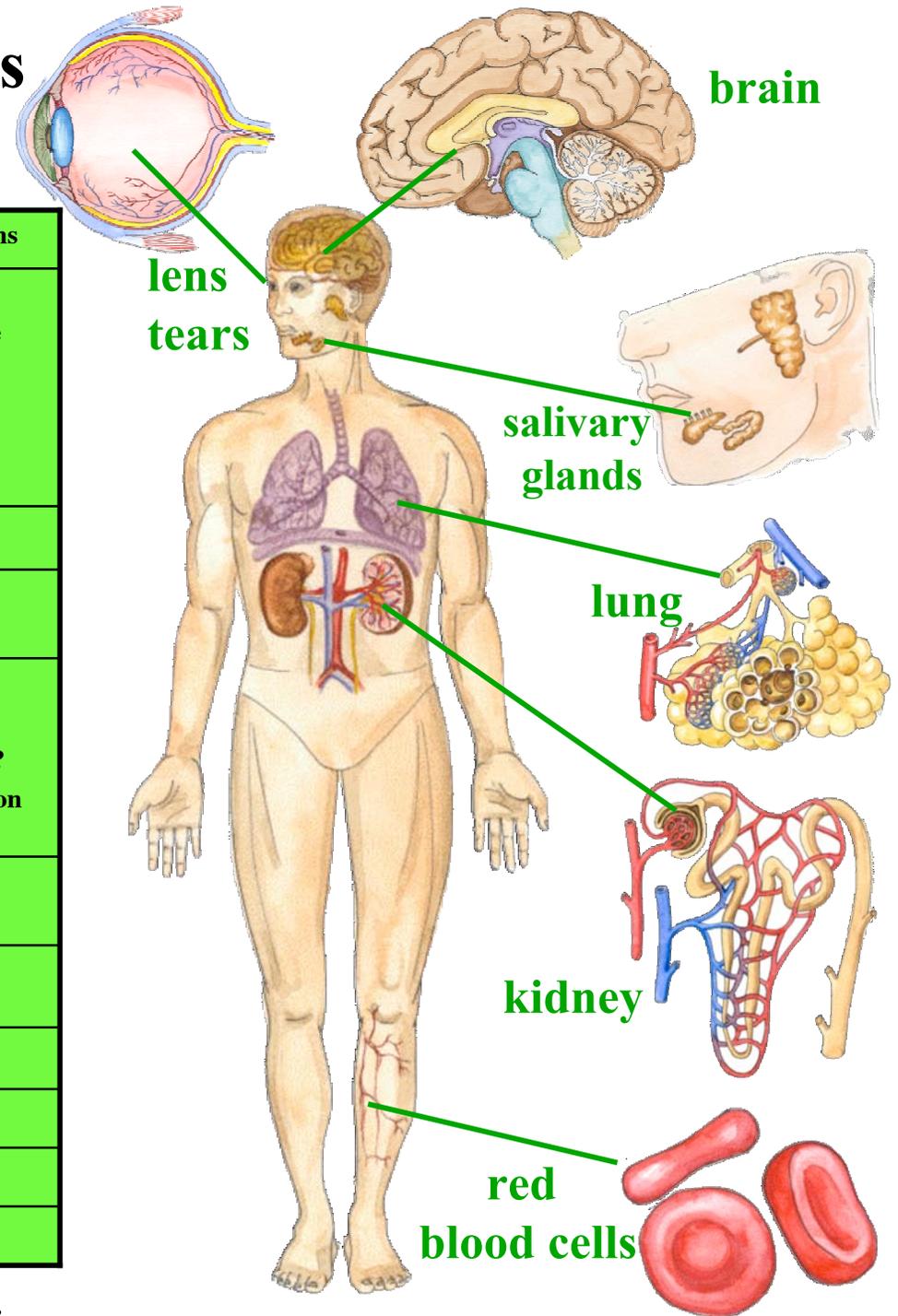
USA

Rockefeller University, Howard Hughes Medical Institute
New York, NY, USA

b. 1956

Water and **Glycerol** Channels in the 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.

Functionally Important Features of Aquaporins

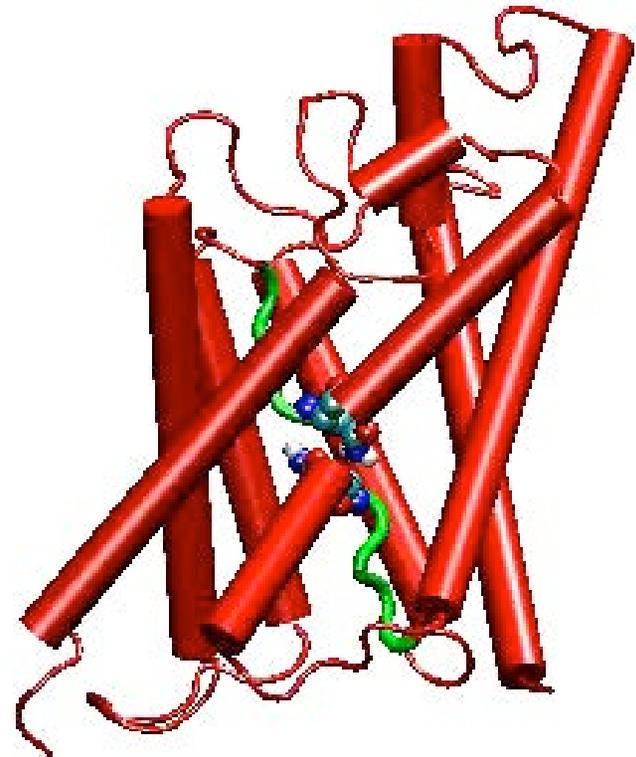
- Water and glycerol transport
- Exclusion of ions and protons
- Tetrameric arrangement in membrane

Aquaporins of known structure:

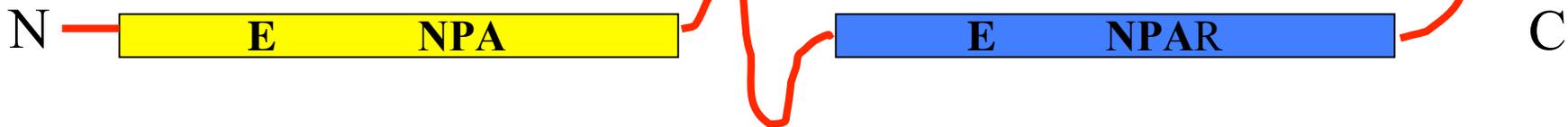
GlpF – E. coli glycerol channel (aquaglyceroporin)

– Fu, et al., Science (2000)

AQP1 – Mammalian aquaporin-1 (pure water channel) -Sui et al, Nature (2001)



~100% conserved -NPA- signature sequence



Load Aquaporin 1J4N into VMD

The image shows the VMD (Visual Molecular Dynamics) interface. The main window, titled "VMD Main", contains a menu bar (File, Molecule, Graphics, Display, Mouse, Extensions, Help) and a table of loaded molecules:

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1	T	A	D	F	1J4N	2029	1	0

Below the table are playback controls including a timeline, zoom, loop, step, and speed buttons.

The "Graphical Representations" window is open, showing the configuration for the selected molecule "1: 1J4N". It includes buttons for "Create Rep" and "Delete Rep", and a table for defining representations:

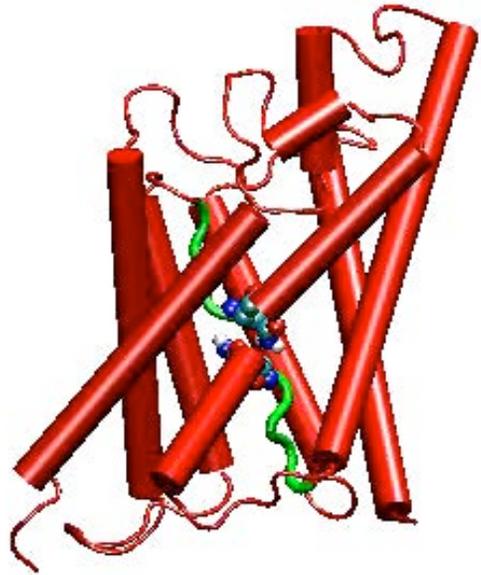
Style	Color	Selection
Tube	Name	all

Below this table, the "Selected Atoms" field is set to "all". The "Draw style" tab is active, showing "Coloring Method" set to "Name" and "Material" set to "Opaque". The "Drawing Method" is set to "Tube". At the bottom, the "Radius" is set to 0.5 and "Resolution" is set to 11. There are "Apply Changes Automatically" and "Apply" buttons.

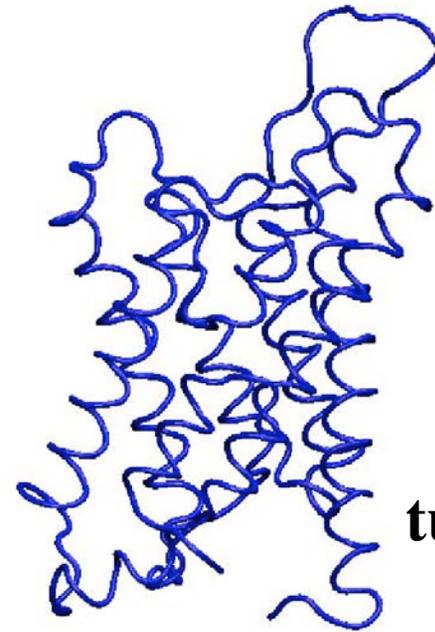
The "VMD 1.8.2b7 OpenGL Display" window shows a 3D rendering of the Aquaporin protein structure as a cyan tube model, oriented vertically.

VMD Permits Different Rendering Styles

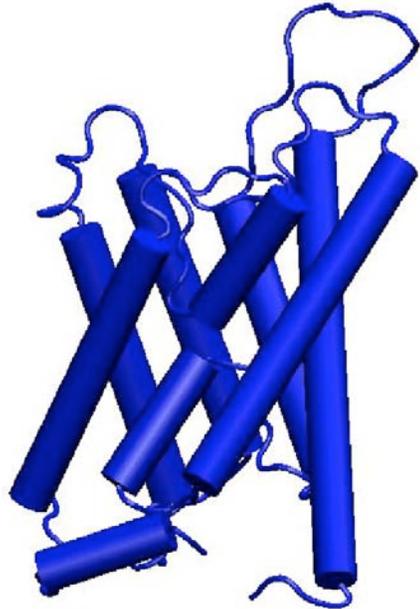
movie



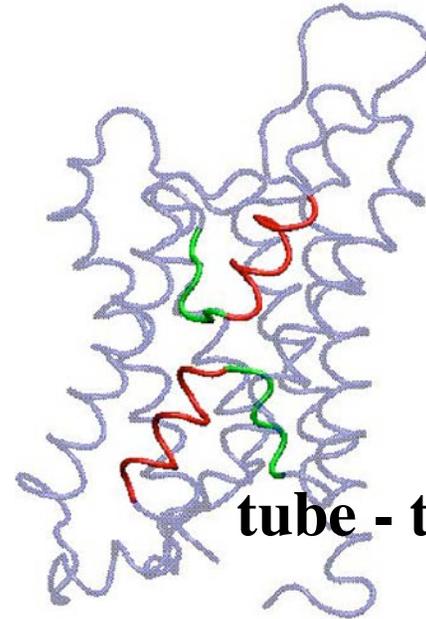
tube



cartoon



tube - transparent



Load Aquaporins 1j4n, 1fqy, 1lda, 1rc2 into VMD

The image shows the VMD (Visual Molecular Dynamics) software interface. The main window displays a list of loaded molecules:

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1		A	D		1J4N	2029	1	0
2		A	D		1FQY	1661	1	0
3		A	D		1lda	1997	1	0
5	T	A	D		1rc2	3530	1	0

The Graphical Representations window shows the selected molecule (5: 1rc2) with the following settings:

- Style: Tube
- Color: ColorID 3
- Selection: chain A
- Coloring Method: ColorID
- Material: Opaque
- Drawing Method: Tube
- Radius: 0.5
- Resolution: 11

The Multiple Sequence Alignment window displays the following alignment:

PDB code	Description
1j4n	Bovine AQP1
1fqy	Human AQP1
1lda	E. coli Glycerol Facilitator (GlpF)
1r2c	E. coli AqpZ

```

d1fqya_.ent  KLPWRVAVAEFLATTLFVFIISIGSALGFKYPVGNWQTAVQDNVKSLSLAFGLSIATLA
d1j4na_.ent  HASEFKKKLFWRAVVAEFLAHILFIFISIGSALGFHYPIKSNQTTGAVQDNVKSLSLA
d1lda_.ent   TLFGQCIAEFLGTGLLIFFGVGCVAALKVAGASFGQWEISVINGLGVAMATYLTAGV
d1rc2a_.ent  HFRKLAEECFGTFWLVFGGCSAVLAAGFPELIGFAGVALAFGLTLVLTMAFVGH
  
```

Aligning Structures and Sequences

The image displays the VMD (Visual Molecular Dynamics) software interface, illustrating the process of aligning protein structures and sequences. The main window, titled "VMD 1.8.2b7 OpenGL Display", shows a 3D ribbon representation of a protein structure, colored by residue type (blue for helix, red for sheet, yellow for loop). The "VMD Main" window provides a table of loaded molecules and playback controls. The "Graphical Representations" window shows the configuration for the selected molecule (5: 1rc2), including style (Tube), color (ColorID 3), and selection (chain A). The "Multiple Sequence Alignment" window displays a sequence alignment of four proteins: 1fgy, 1j4n, 1lda, and 1rc2.

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1		A	D	F	1J4N	2029	1	0
2		A	D	F	1FQY	1661	1	0
3		A	D	F	1lda	1997	1	0
5	T	A	D	F	1rc2	3530	1	0

Selected Molecule: 5: 1rc2

Style: Tube, Color: ColorID 3, Selection: chain A

Selected Atoms: chain A

Coloring Method: ColorID 3, Material: Opaque

Drawing Method: Tube

Radius: 0.5, Resolution: 11

Apply Changes Automatically: Apply

```
1fgy -----KLFWRVAVAEFLATTLFVFFISIGSAL-GF-KY---PVGNNQTAVDNPKVSLAPGLSIATLAQS-VGHISGAHLNPAVTLGILLSCQISIF-RAI
1j4n MASEFKKKLFWRAVVAEFLAMILFIFISIGSAL-GF-HYPIKSNQT-TGAVQDNVKVSLAPGLSIATLAQSVGH-ISGAHLNPAVTLGILLSCQ-ISVLRAI
1lda -----TLKGQCIAEFLGTGLLIFPGVGCVA-ALKVA-----G-A-SFGQWEISVIWGLGVAMATYLTG-VVSGAHLNPAVTIALWLFA-CFDKRVV
1rc2 -----MFRKLAECFPTFWLVFGCGSAVLA-AG-----FPE-LGIGFAGVALAPGLTVLTMFAVAG-HISGGHFNPAVTIGLWAGG-RFPAREVV
```

Comparing Structures by Similarity - Q Value

The image displays the VMD (Visual Molecular Dynamics) software interface, illustrating the process of comparing protein structures by similarity using the Q Value.

VMD Main Window: Shows a list of loaded molecules:

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1	A	D			1J4N	2029	1	0
2	A	D			1FQY	1661	1	0
3	A	D			1lda	1997	1	0
5	T	A	D		1rc2	3530	1	0

Graphical Representations Window: Shows the configuration for the selected molecule (5: 1rc2):

- Selected Molecule:** 5: 1rc2
- Style:** Tube
- Color:** ColorID 3
- Selection:** chain A
- Coloring Method:** ColorID
- Material:** Opaque
- Drawing Method:** Tube
- Radius:** 0.5
- Resolution:** 11

VMD 1.8.2b7 OpenGL Display Window: Shows a 3D ribbon representation of the protein structure (1rc2) colored by residue.

Multiple Sequence Alignment Window: Shows a sequence alignment of four proteins (1f4y, 1j4n, 1lda, 1rc2) with a context menu open over the alignment. The menu options include:

- RMSD Per Residue
- Tree
- STAMP Parameters
- Bulk Residue Selection
- Molecule Coloring
 - Q per residue (checked)
 - Sequence Identity per residue
- Highlight Style

The alignment shows the following sequences:

```

1f4y  ...KLFWAQVADPLATLLEVFSTIGSAL-GF-FY---PVDNRQTAVDNKKVSLAFGLSATLAQS-VGHIISGAHLNFAVTLGLLLSQGISIF-RAI
1j4n  MASEFKKLFWRARVVAEFLAMILFIFISIGSAL-GF-HYPIKSNQT-TCAVQDNVKSVAFLGLSIATLAQSVGH-ISGAHLNFAVTLGLLLSQGI-SVLRRAI
1lda  -----TLRGQCIAEPLGTGLLIPFGVGCVA-ALKVA-----G-A-SFGQWEISVIWGLGVAMAIIYDTA-GVSGAHLNFAVTLALWLFA-CFDKRRV
1rc2  -----MFRKLAACEPGTFLVLFVGGCGSAVLA-AG-----FPE-LGIGFAGVALAPGLTTLTMAFAVG-HISGGHPNFAVTIGLRWAGG-RFPAKEV
    
```

Comparing Structures by Similarity - Q Value

The image displays the VMD (Visual Molecular Dynamics) software interface, illustrating the process of comparing protein structures by similarity using the Q Value method.

VMD Main Window: Shows a table of loaded molecules and playback controls.

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1	A	D			1J4N	2029	1	0
2	A	D			1FQY	1661	1	0
3	A	D			1lda	1997	1	0
5	T	A	D		1rc2	3530	1	0

Graphical Representations Window: Shows settings for the selected molecule (5: 1rc2). The "Style" is set to "Tube", "Color" is "ColorID 3", and "Selection" is "chain A". The "Drawing Method" is also set to "Tube".

VMD 1.8.2b7 OpenGL Display Window: Displays a 3D ribbon representation of the protein structure, colored by residue type (green, blue, red).

Multiple Sequence Alignment Window: Shows a sequence alignment of four proteins: 1f4y, 1j4n, 1lda, and 1rc2. A context menu is open over the alignment, with "Molecule Coloring" selected, and "Q per residue" checked. Other options include "RMSD Per Residue", "Tree", "STAMP Parameters", "Bulk Residue Selection", "Highlight Style", "Sequence Identity per residue", "Align Molecules...", "FASTA", "Highlight PDB", and "Distance RMSD".

Exhibiting Sequence Identity - Side View

The image displays the VMD (Visual Molecular Dynamics) software interface, illustrating how to exhibit sequence identity in a side view. The main window, titled "VMD 1.8.2b7 OpenGL Display", shows a 3D ribbon representation of a protein structure, colored by sequence identity. The structure is composed of multiple chains, with colors ranging from red to blue, indicating different levels of identity. The "VMD Main" window shows a table of loaded molecules:

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1		A	D	F	1J4N	2029	1	0
2		A	D	F	1FQY	1661	1	0
3		A	D	F	1lda	1997	1	0
5	T	A	D	F	1rc2	3530	1	0

The "Graphical Representations" window shows the selected molecule "5: 1rc2" and its representation settings:

- Selected Molecule: 5: 1rc2
- Style: Tube
- Color: ColorID 3
- Selection: chain A
- Coloring Method: ColorID
- Material: Opaque
- Drawing Method: Tube
- Radius: 0.5
- Resolution: 11

The "Multiple Sequence Alignment" window shows a sequence alignment of four proteins: 1fqy, 1j4n, 1lda, and 1rc2. The alignment is displayed in a side view, with columns of identical residues highlighted in yellow. The alignment is as follows:

```
1fqy  -----KLFWRVVAEFLATTLFVFISISGAL-GF-KY---FVGNQTAVDNWKVSLAFGLSIATLAQS-VGHISGAEINPAVTLGLLLSCOISIF-RV
1j4n  MASEFKKLLFWRVVAEFLAMILFIFISISGAL-GF-HYPINSNQ-TGAVQDNVKVSLAFGLSIATLAQSVGH-ISGAELNPAVTLGLLLSCO-ISVLRV
1lda  -----TLRGQCIAEFLGTGLLFFGVGVVA-ALKVA-----G-A-SFGQWEISVIWGLGVMAIYLTA-GVSGAEINPAVTIALWLFV-CFDKRV
1rc2  -----MFRKLAECFQTFWLVFGCCSAVLA-AG-----FPE-LGIGFAGVALAFGLTVLTMFAVVG-HISGGHFNPAVTIGLWAGG-RFPAREV
```

Exhibiting Sequence Identity - Top View

The image displays the VMD (Visual Molecular Dynamics) software interface. The main window, titled "VMD 1.8.2b7 OpenGL Display", shows a top-down view of a protein structure represented as a multi-colored tube. The structure is composed of several chains, with colors corresponding to different sequence identities. The "VMD Main" window shows a table of loaded molecules:

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1		A	D	F	1J4N	2029	1	0
2		A	D	F	1FQY	1661	1	0
3		A	D	F	1lda	1997	1	0
5	T	A	D	F	1rc2	3530	1	0

The "Graphical Representations" window shows the selected molecule "5: 1rc2" with a "Tube" style and "ColorID 3" color. The "Multiple Sequence Alignment" window displays the following alignment:

```
1fqy  -----KLFWRVVAEFLATTLFVFIISIGSAL-GF-KY---FVGNQTAVDNWKVSLAFGLSIATLAQS-VGHSAGHLNPAVTLGLLSCOISIF-RV
1j4n  MASEFKKLLFWRVVAEFLAMILFIFISIGSAL-GF-HYPIKSNQ-TGAVQDNVKVSLAFGLSIATLAQSVGH-ISGAGHLNPAVTLGLLSCO-ISVLRV
1lda  -----TLRGQCIAEFLGTGLLEFFGVGVVA-ALKVA-----G-A-SFGQWEISVIWGLGVMAIYLTA-GVSGAGHLNPAVTIALWLFV-CFDKRV
1rc2  -----MFRKLAECFQTFWLVFGCCSAVLA-AG-----FPE-LGIGPAGVALAFGLTVLTMFAVVG-HISGGHFNPAVTIGLWAGG-RFPAREV
```

Showing Conserved Residues - Monomer

The image displays the VMD (Visual Molecular Dynamics) software interface, illustrating how to show conserved residues in a monomer. The interface is divided into several windows:

- VMD Main:** Contains a menu bar (File, Molecule, Graphics, Display, Mouse, Extensions, Help) and a table of loaded molecules.
- Graphical Representations:** Shows the selected molecule (5: 1rc2) and its representation settings. The 'Style' is set to 'Tube', 'Color' to 'ColorID 3', and 'Selection' to 'chain A'. The 'Drawing Method' is also set to 'Tube'.
- VMD 1.8.2b7 OpenGL Display:** Displays a 3D ribbon representation of the protein structure, colored by chain (red, green, yellow).
- Multiple Sequence Alignment:** Shows a sequence alignment of four proteins: 1fqy, 1j4n, 1lda, and 1rc2. Conserved residues are highlighted in yellow.

ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1	A	D			1J4N	2029	1	0
2	A	D			1FQY	1661	1	0
3	A	D			1lda	1997	1	0
5	T	A	D		1rc2	3530	1	0

```

1fqy  -----KLFWRVVAEFLATTLFVFISISGAL-GF-KY---PVGNNQTAVDNWKVSLAFGLSIATLAQS-VGHISGAEINPAVTLGLLSCOISIF-RV
1j4n  MASEFKKLFWRVVAEFLAMILFIFISISGAL-GF-HYPIKSNQ-TGAVQDNVKVSLAFGLSIATLAQSVGH-ISGAELNPAVTLGLLSCO-ISVLRV
1lda  -----TLRGQCIAEFLGTGLLEFFGVGVVA-ALKVA-----G-A-SFGQWEISVIWGLGVMAIYLTA-GVSGAEINPAVTLALWLFV-CFDKRV
1rc2  -----MFRKLAEECPGTFWLVFGCCSAVLA-AG-----FPE-LGIGFAGVALAFGLTVLTMFAVVG-HISGGHFNPAVTIGLWAGG-RFPAREV
  
```

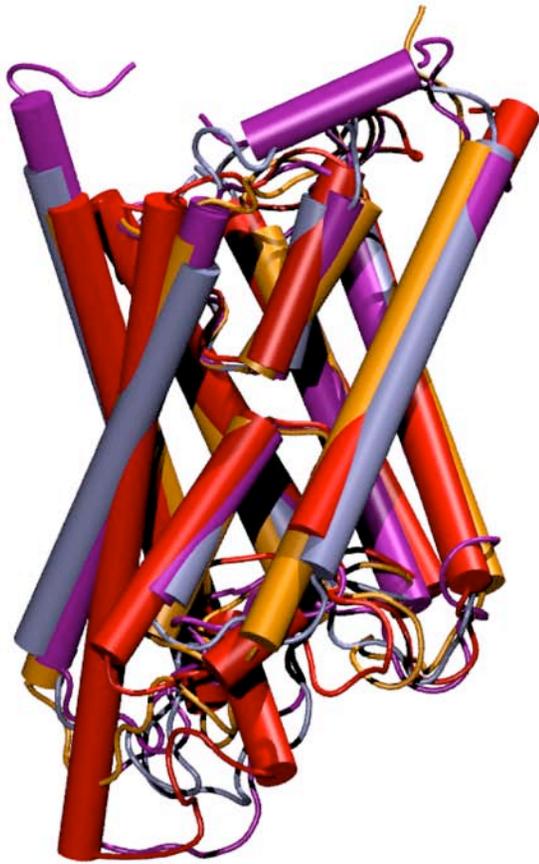
Showing Conserved Residues - Tetramer

The image displays the VMD (Visual Molecular Dynamics) interface. The main window, titled "VMD Main", shows a table of loaded molecules and a playback control. The "Graphical Representations" window is open, showing settings for the selected molecule "5: 1rc2". The "Selected Molecule" dropdown is set to "5: 1rc2". The "Create Rep" button is visible. The "Style" is set to "Tube", "Color" is "ColorID 3", and "Selection" is "chain A". The "Selected Atoms" field contains "chain A". The "Draw style" is "Tube", "Coloring Method" is "ColorID", "Material" is "Opaque", and "Drawing Method" is "Tube". The "Radius" is set to 0.5 and "Resolution" is set to 11. The "Apply Changes Automatically" checkbox is checked, and the "Apply" button is visible.

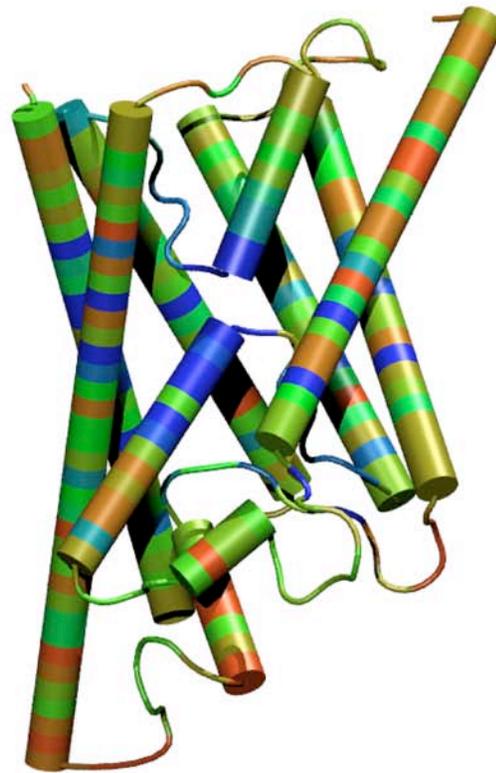
ID	T	A	D	F	Molecule	Atoms	Frames	Vol
1	A	D			1J4N	2029	1	0
2	A	D			1FQY	1661	1	0
3	A	D			1lda	1997	1	0
5	T	A	D		1rc2	3530	1	0

The "VMD 1.8.2b7 OpenGL Display" window shows a 3D ribbon representation of a tetramer protein structure. The structure is composed of four subunits, each represented by a different color: red, blue, orange, and grey. The conserved residues are highlighted in yellow. The structure is shown in a top-down view, with the subunits arranged in a circular pattern. Below the ribbon representation, a blue surface representation of the same structure is shown, with the conserved residues highlighted in yellow. The surface representation shows the overall shape and topology of the protein, with the conserved residues appearing as small yellow spheres on the surface.

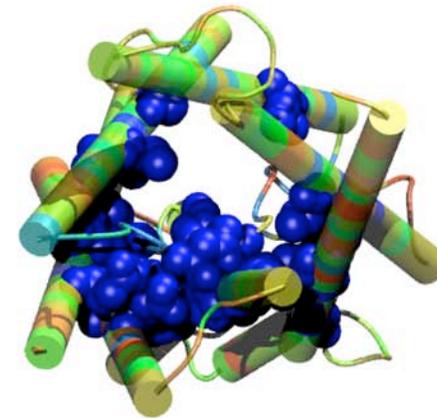
Structure and Sequence Comparisons Water/Glycerol Channels



2 AQP1, GLPF, AQPZ
from animal and bacteria



GLPF Sequence Conservation



Top view

Acknowledgements - Tutorials

Seq Alignment

- Rommie Amaro
- Felix Autenrieth
- Brijeeet Dhaliwal
- Barry Isralewitz
- Taras Pogorelov
- Anurag Sethi

Evolution AARS

- Rommie Amaro
- Patrick O'Donoghue
- Brijeeet Dhaliwa

Bioinformatics

Aquaporins

- Fateme Araghi
- Brijeeet Dhaliwal
- Elizabeth Villa

VMD Developers: Dan Wright, John Eargle, John Stone