Part III - Bioinformatics Studies Using Multiseq in VMD

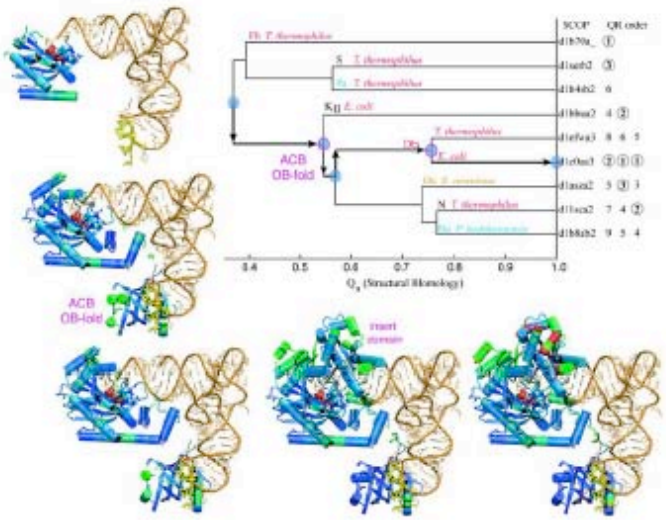
- Aminoacyl tRNA Synthetases
- Aquaporins

San Francisco, 2005, Computational Biology Workshop

University of Illinois at Urbana-Champaign
NIH Resource for Macromolecular Modeling and Bioinformatics
School of Chemical Sciences and Beckman Institute

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

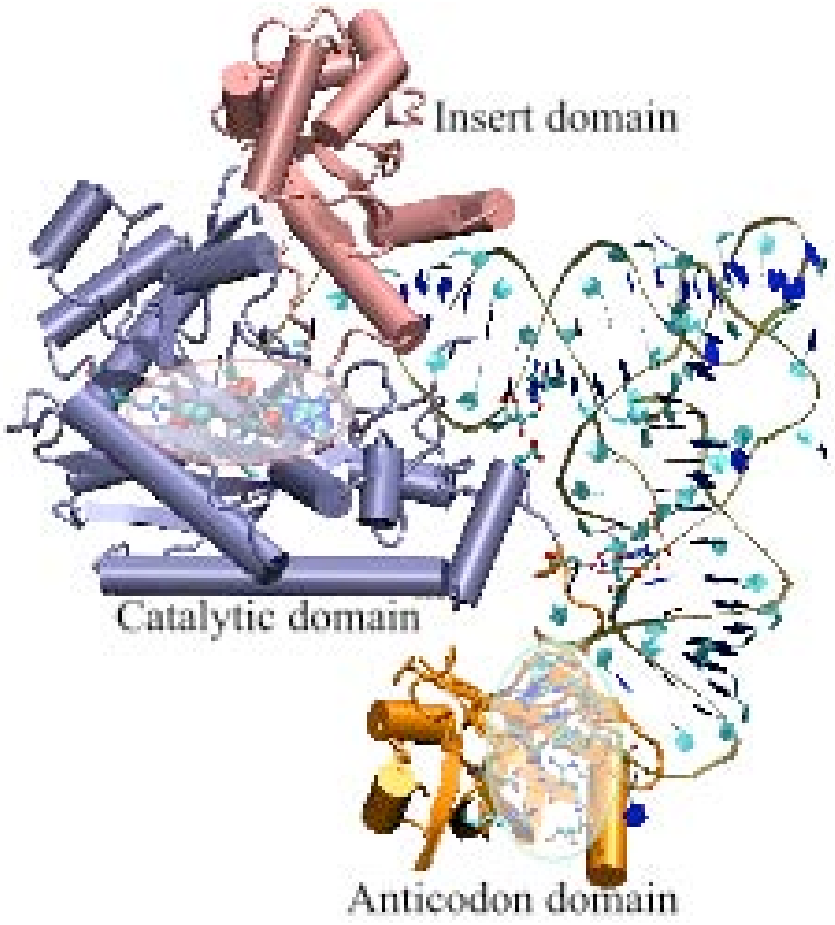
April 2004.

Multiple Sequence Alignments

- “The aminoacyl-tRNA synthetases, perhaps better than any other molecules in the cell, eptiomize the current situation and help to under standard (the effects) of HGT” Woese (PNAS, 2000; MMBR 2000)

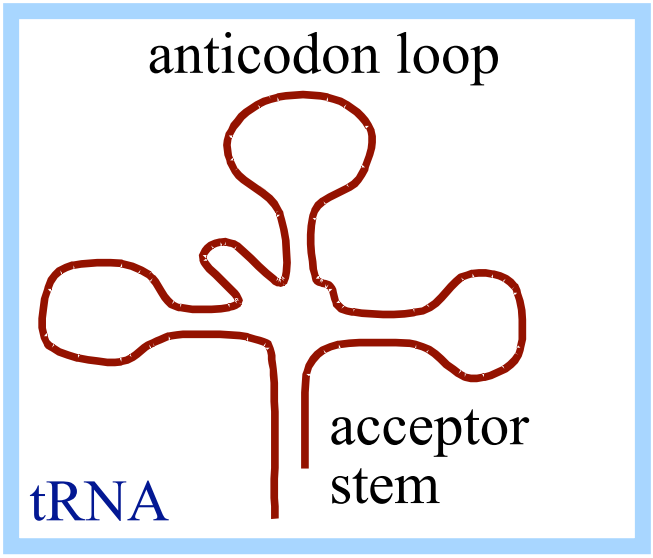
- Carl Woese - Crafoord Prize 2003

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

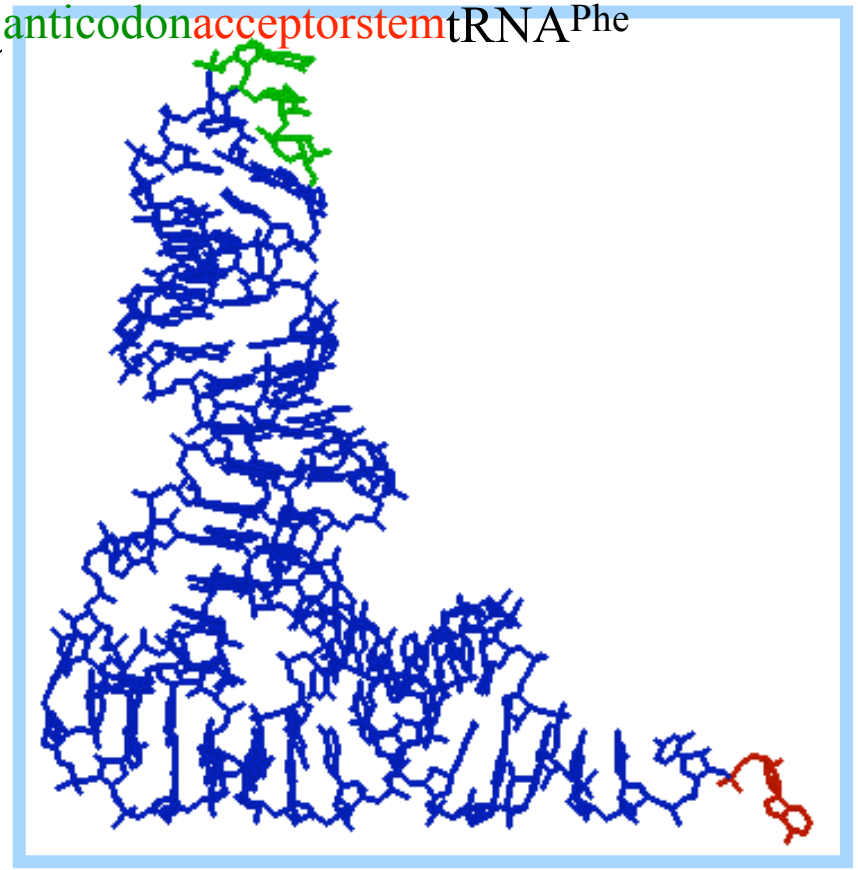


Standard Dogma Molecular Biology

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

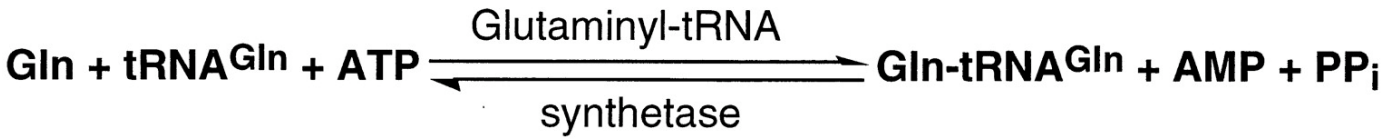


www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/25-trna.ppt

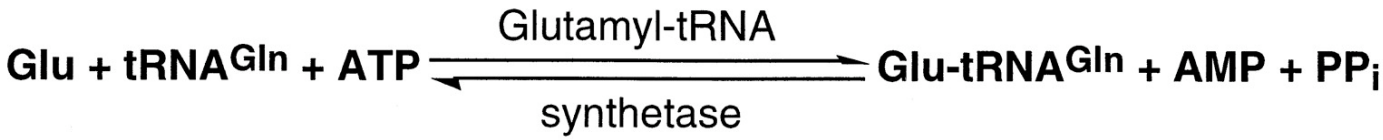


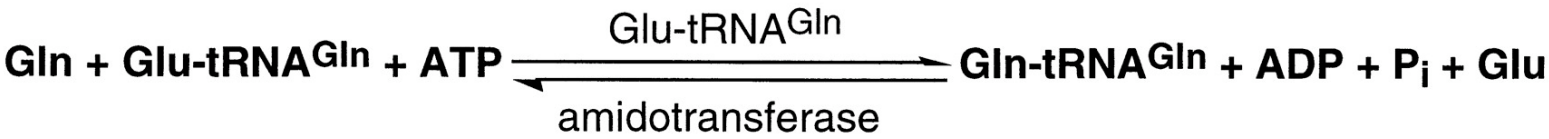
Charging the tRNA

Direct acylation

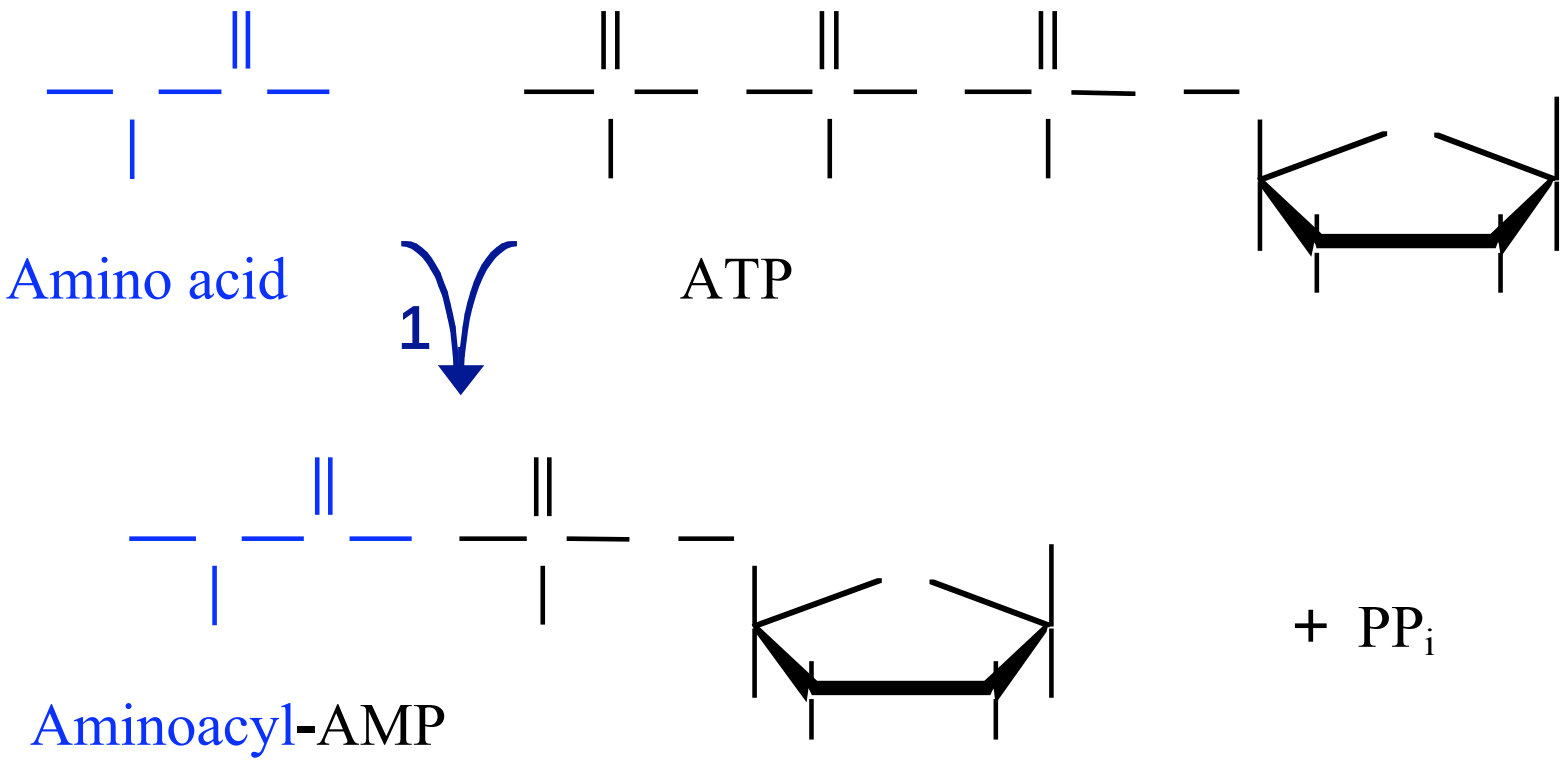


tRNA-dependent amino acid modification





Woese, Olsen (UIUC), Ibba (Panum Inst.), Soll (Yale) *Micro. Mol. Biol. Rev.* March 2000..

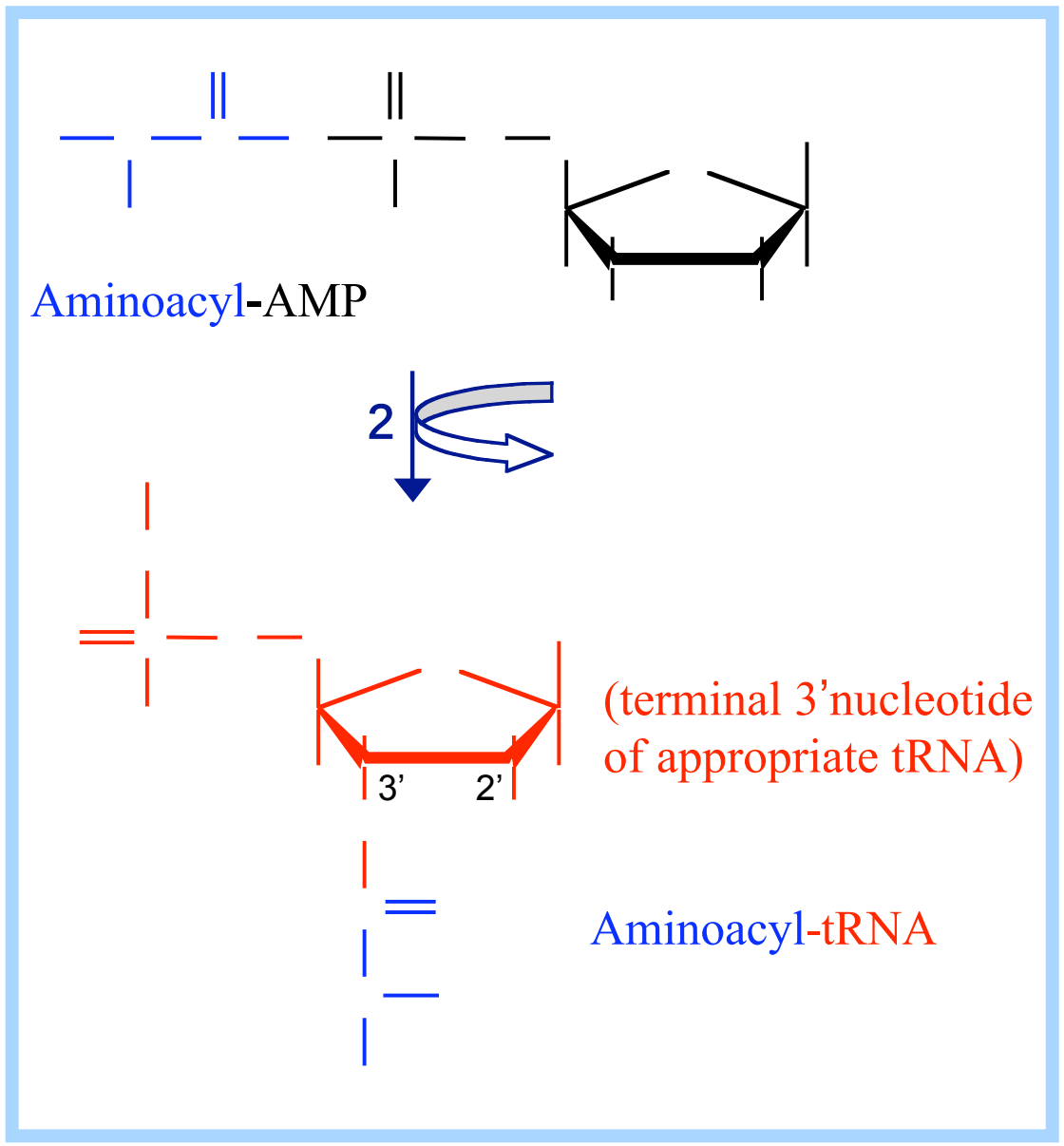


Aminoacyl-tRNA Synthetases catalyze linkage of the appropriate amino acid to each tRNA. The reaction occurs in two steps.

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

www.rpi.edu/dept/bcbp/molbioch**em**/MBWeb/mb2/part1/25-trna.ppt

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:

1. **amino acid + ATP → aminoacyl-AMP + PP_i**

2. **aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP**

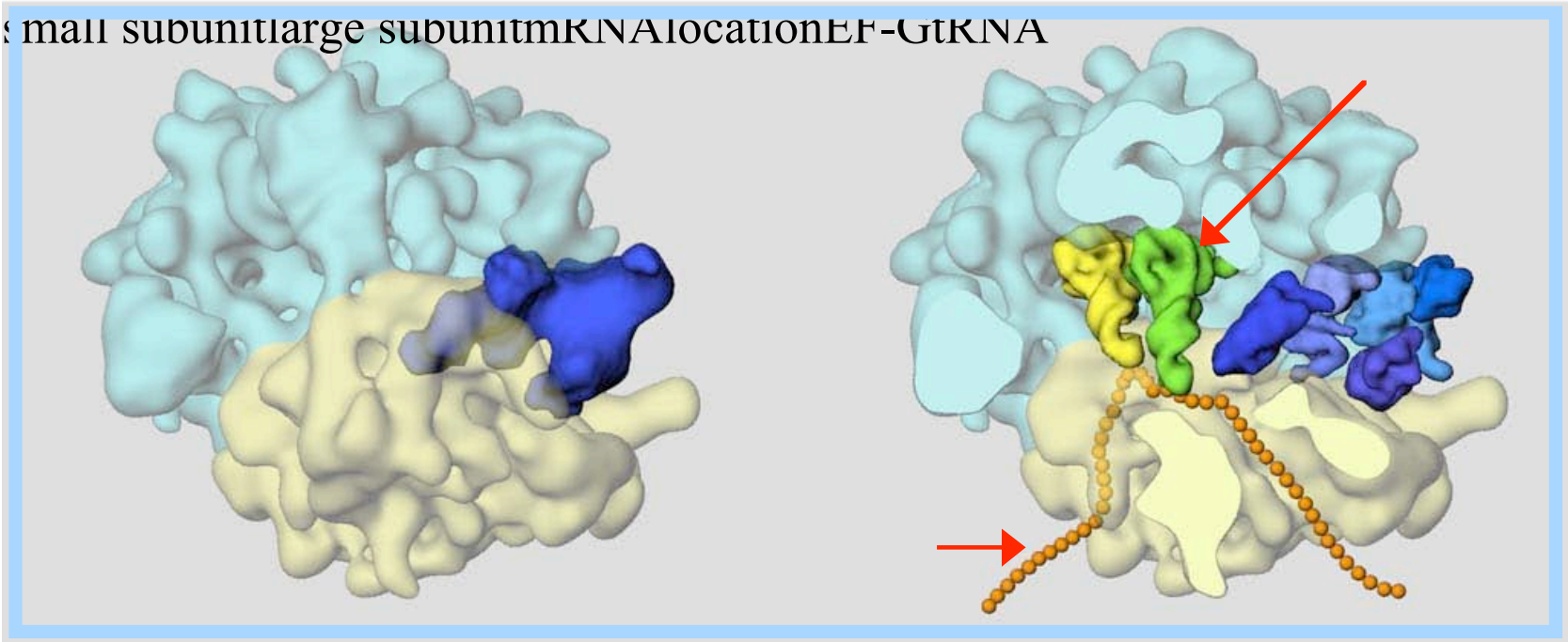
Overall Reaction:

amino acid + ATP + tRNA → aminoacyl-tRNA + AMP

+ PP_i

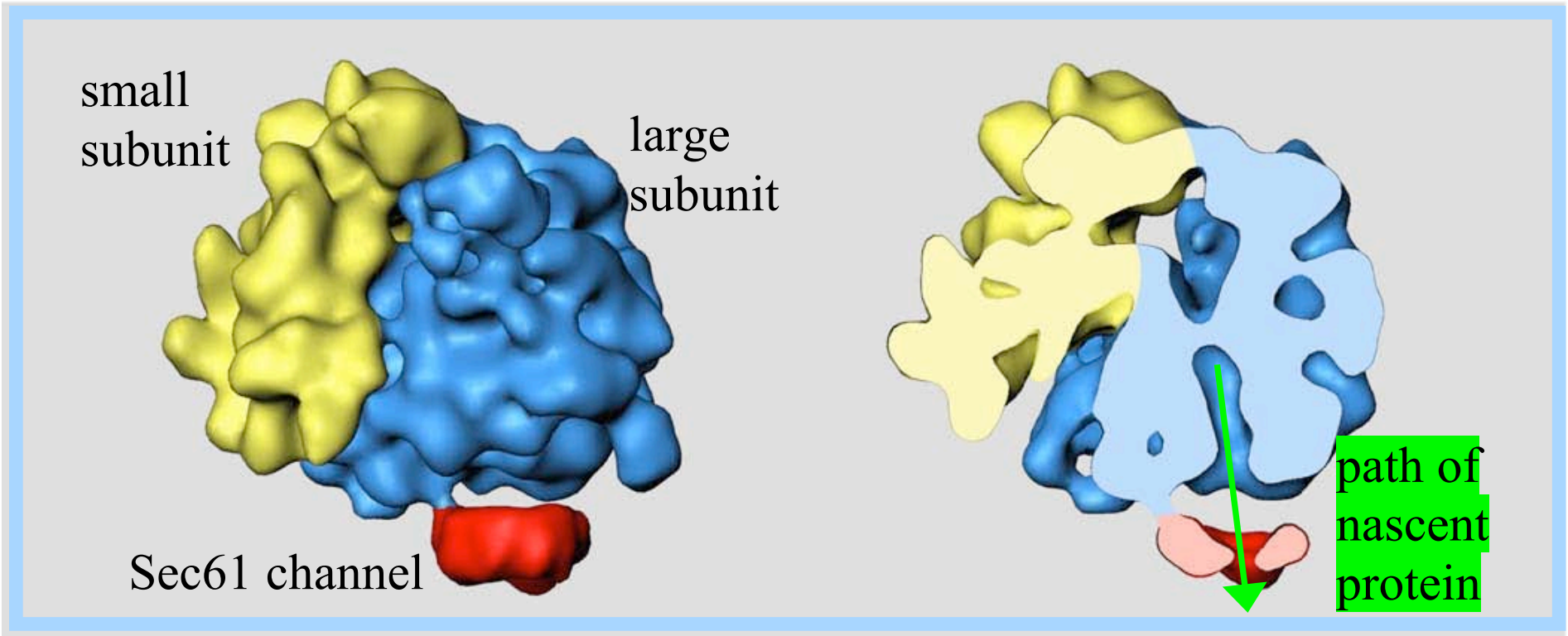
Next step: EF and Ribosome for Protein Synthesis

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



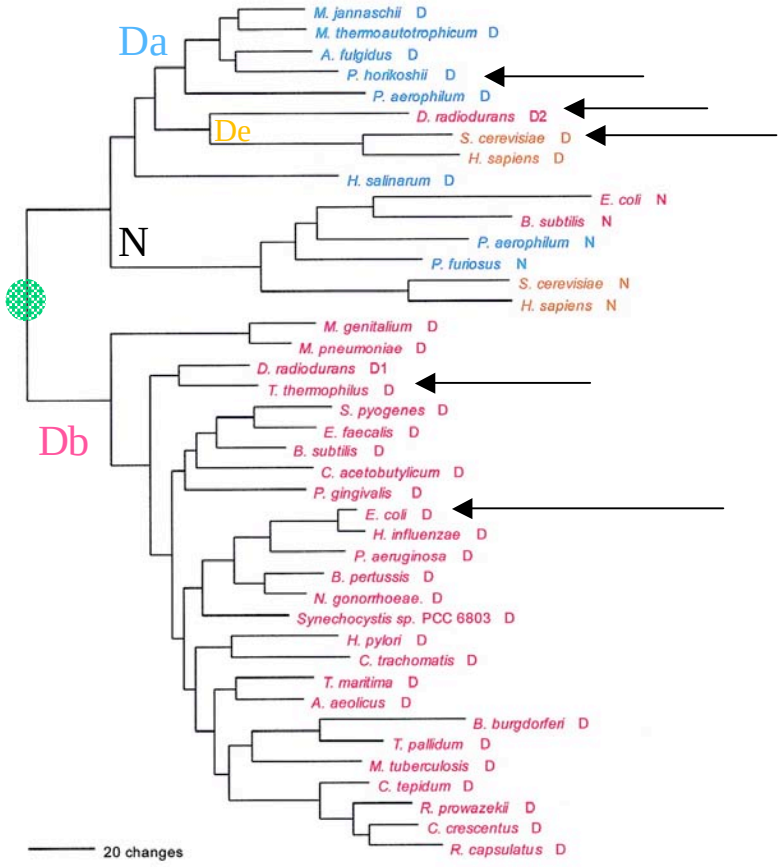
The cutaway view at right shows that the **tunnel** in the yeast large ribosome subunit, through which nascent polypeptides emerge from the ribosome, **lines up** with the lumen of the ER **Sec61 channel**.

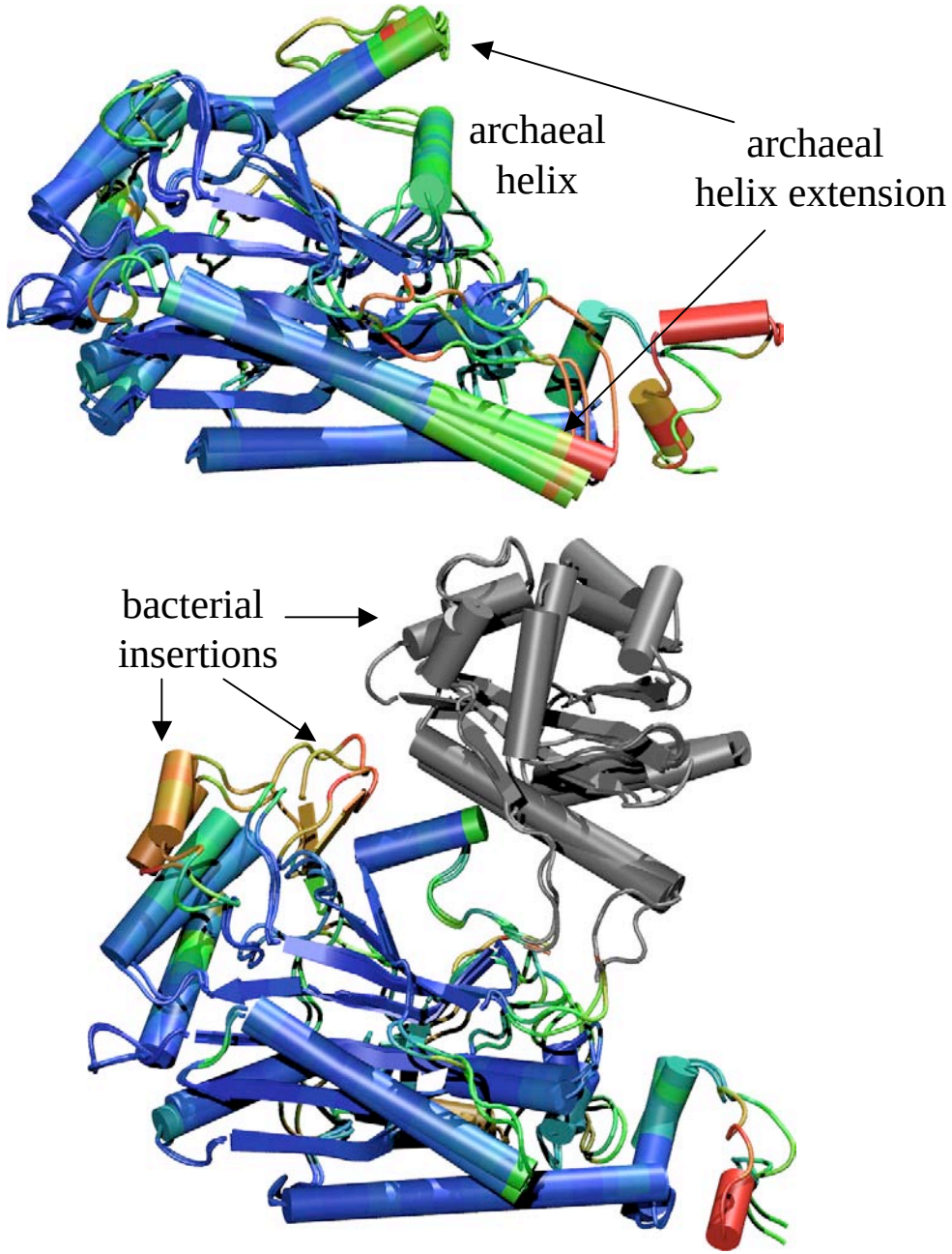
Figure provided by Joachim Frank, whose lab carried out the cryo-EM & image reconstruction on which these images are based.

Horizontal Gene Transfer in Protein Structure

Sequence Phylogeny

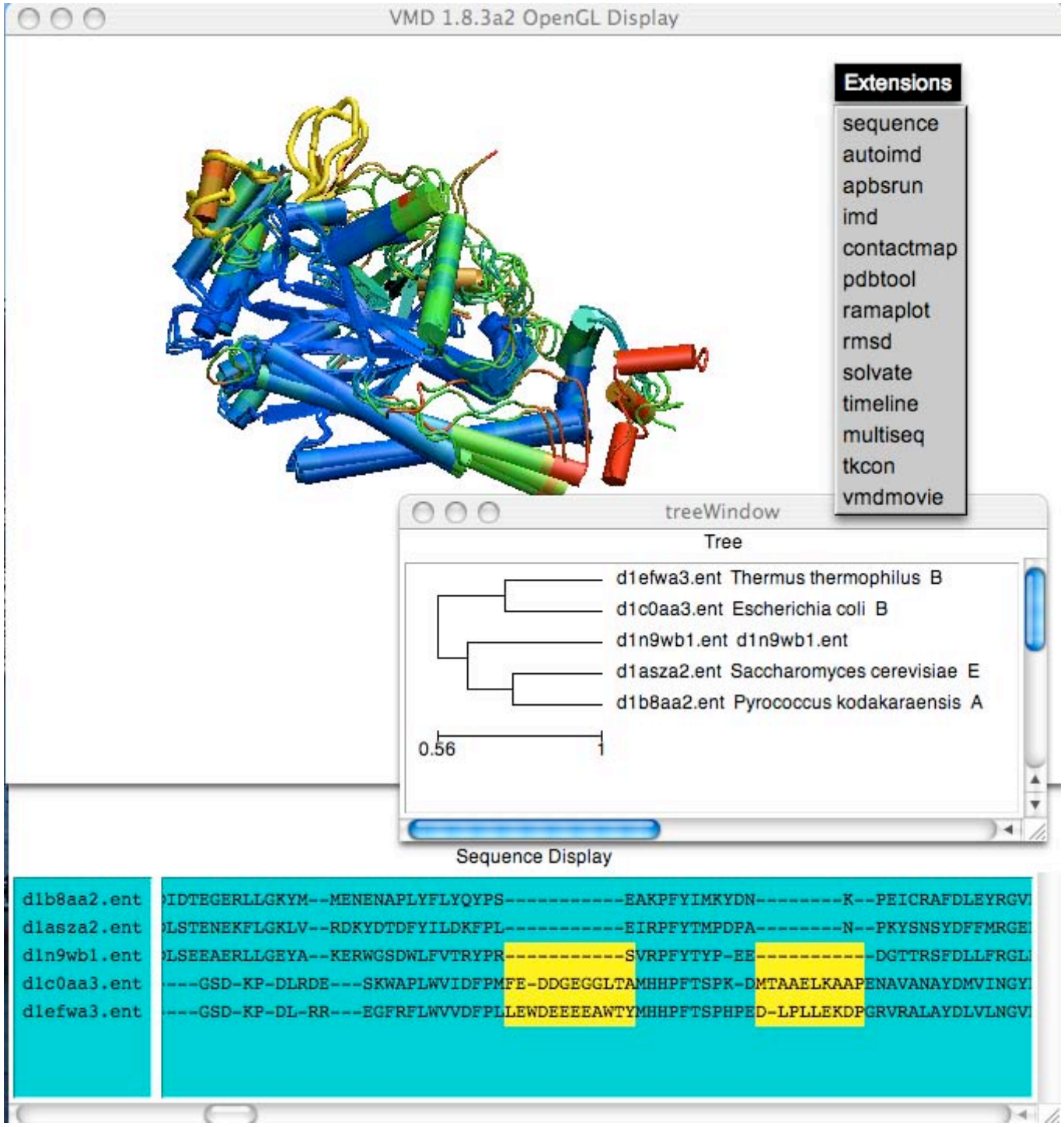
AspRS-AsnRS Group



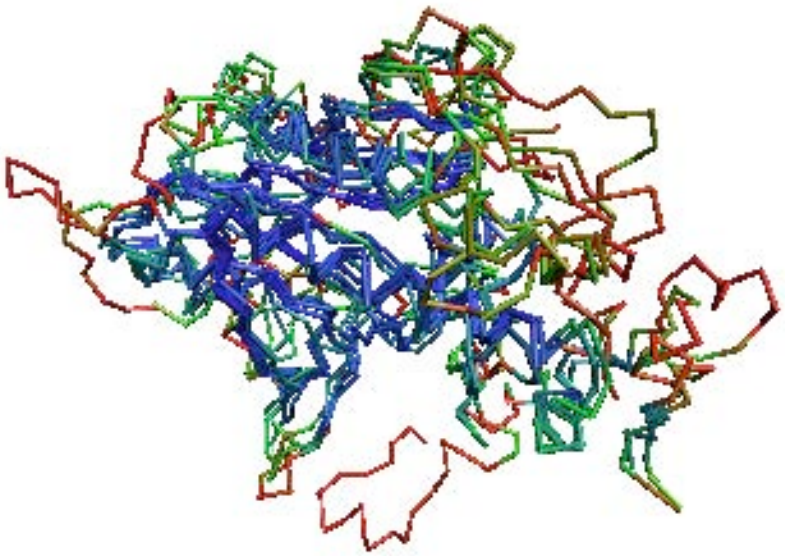


Multiseq

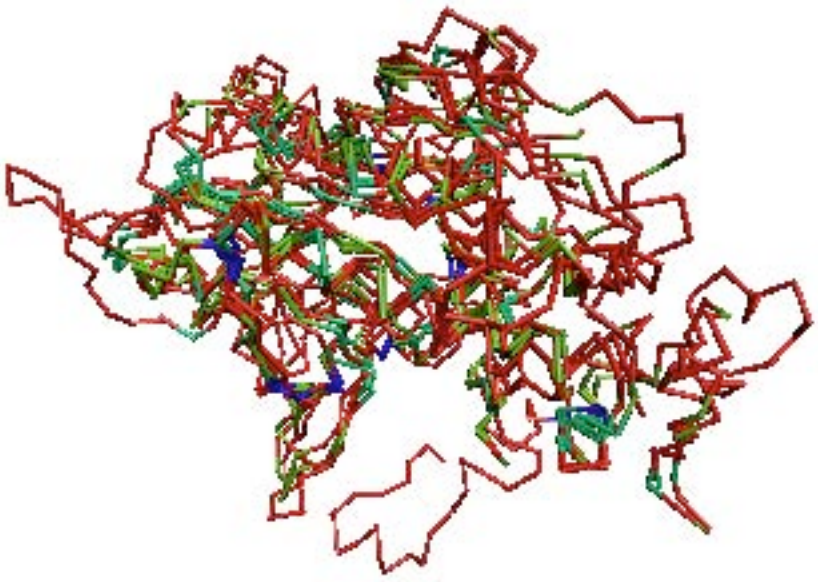
extension in VMD



Conservation



Core Structure Conserved



Sequence Identity of Core
Less than 15%

University of Illinois at Urbana-Champaign
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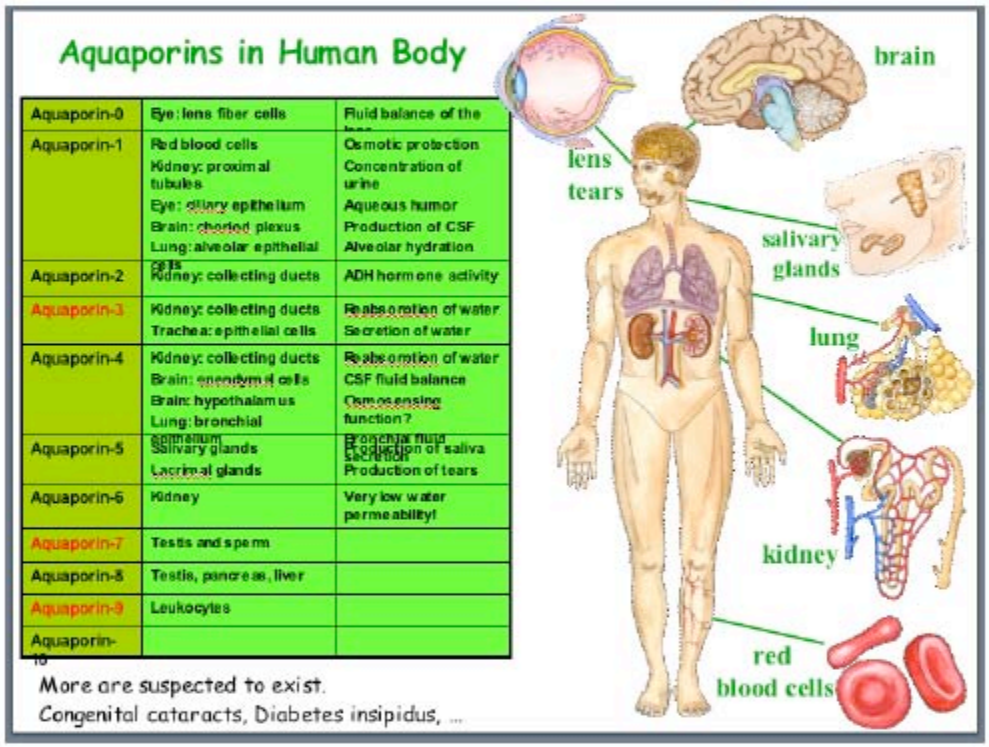
Aquaporins



VMD Developers:

John Stone

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Fatemeh Khalili

Elizabeth Villa

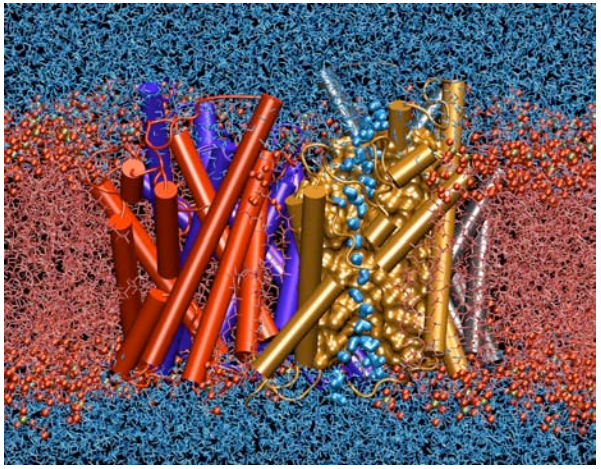
Emad Tajkhorshid

Brijeet Dhaliwal

Zan Luthey-Schulten

Towards Understanding Membrane Channels

The versaltile, highly selective and efficent 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

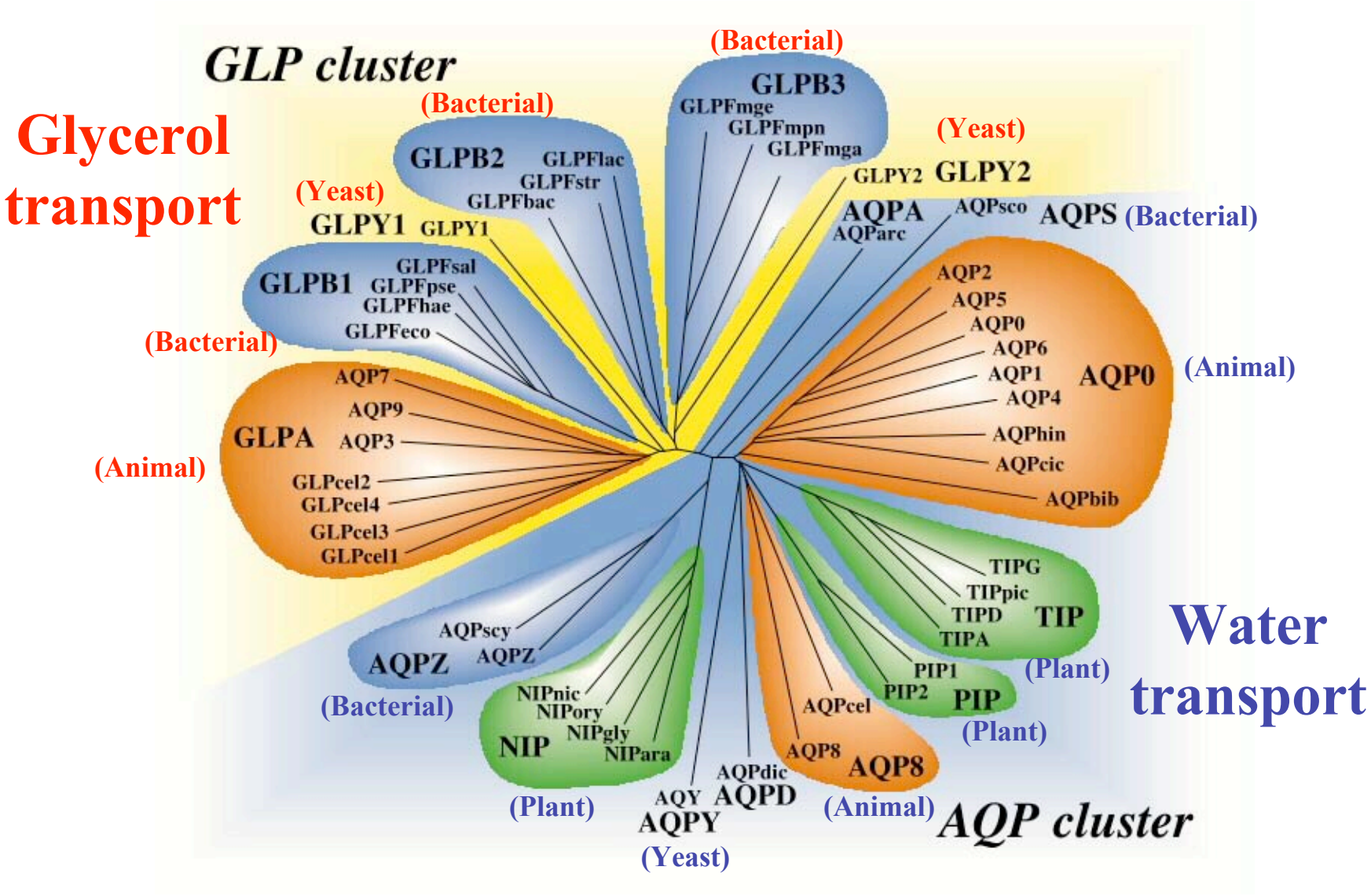
1/2 of the prize

USA

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

b. 1956

The Aquaporin Superfamily

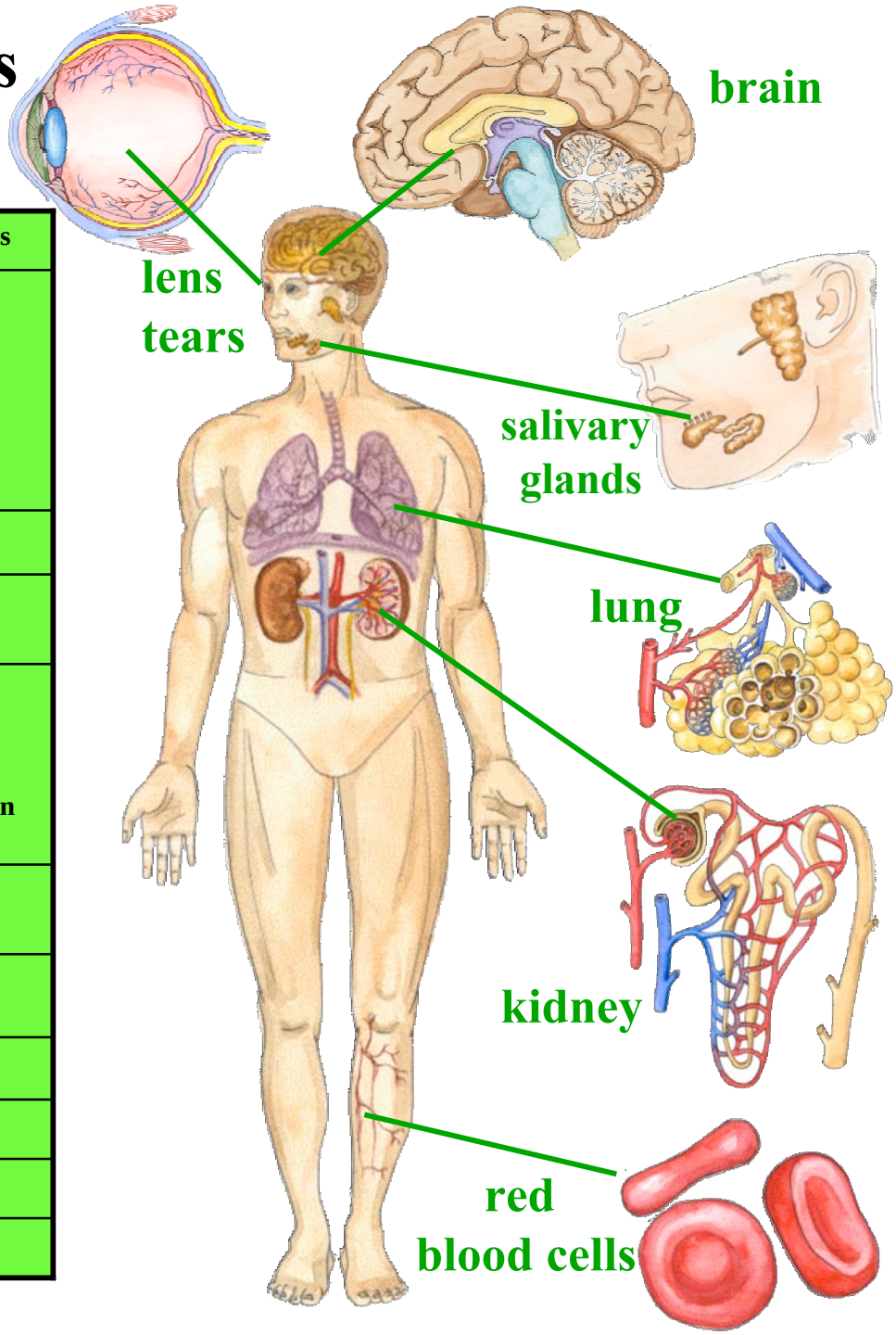


Heymann and Engel *News Physiol. Sci.* **14**, 187 (1999)

Water and Glycerol Channels in the Human Body

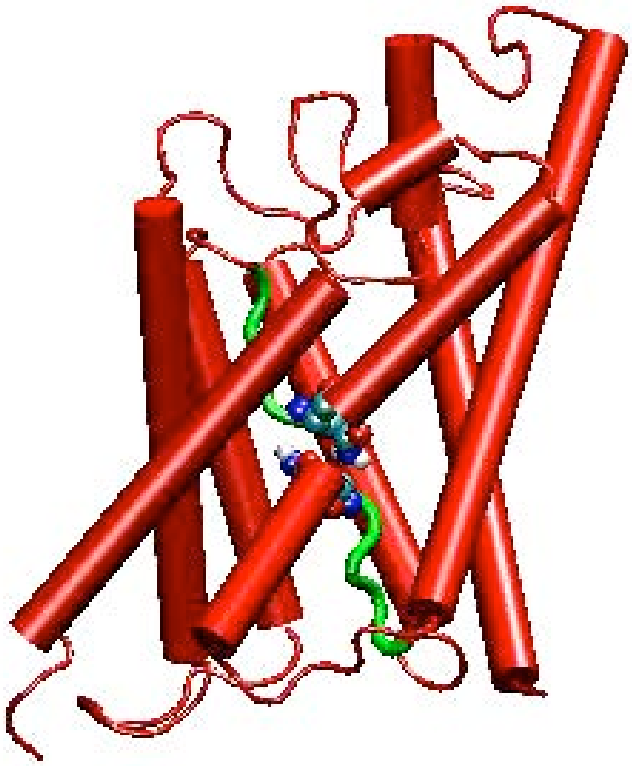
Aquaporin-0	Eye: lens fiber cells	Fluid balance of the lens
Aquaporin-1	Red blood cells Kidney: proximal tubules Eye: ciliary epithelium Brain: choriod plexus Lung: alveolar epithelial cells	Osmotic protection Concentration of urine Aqueous humor Production of CSF Alveolar hydration
Aquaporin-2	Kidney: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

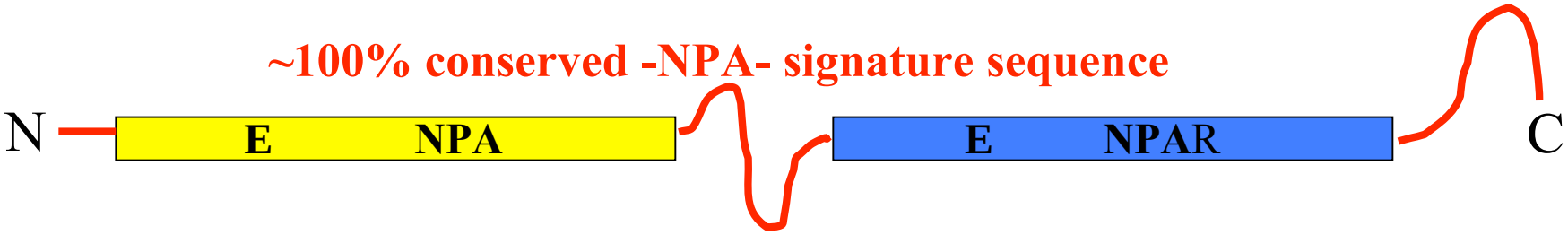
- Water and glycerol transport
- Exclusion of ions and protons
- Tetrameric arrangement in membrane



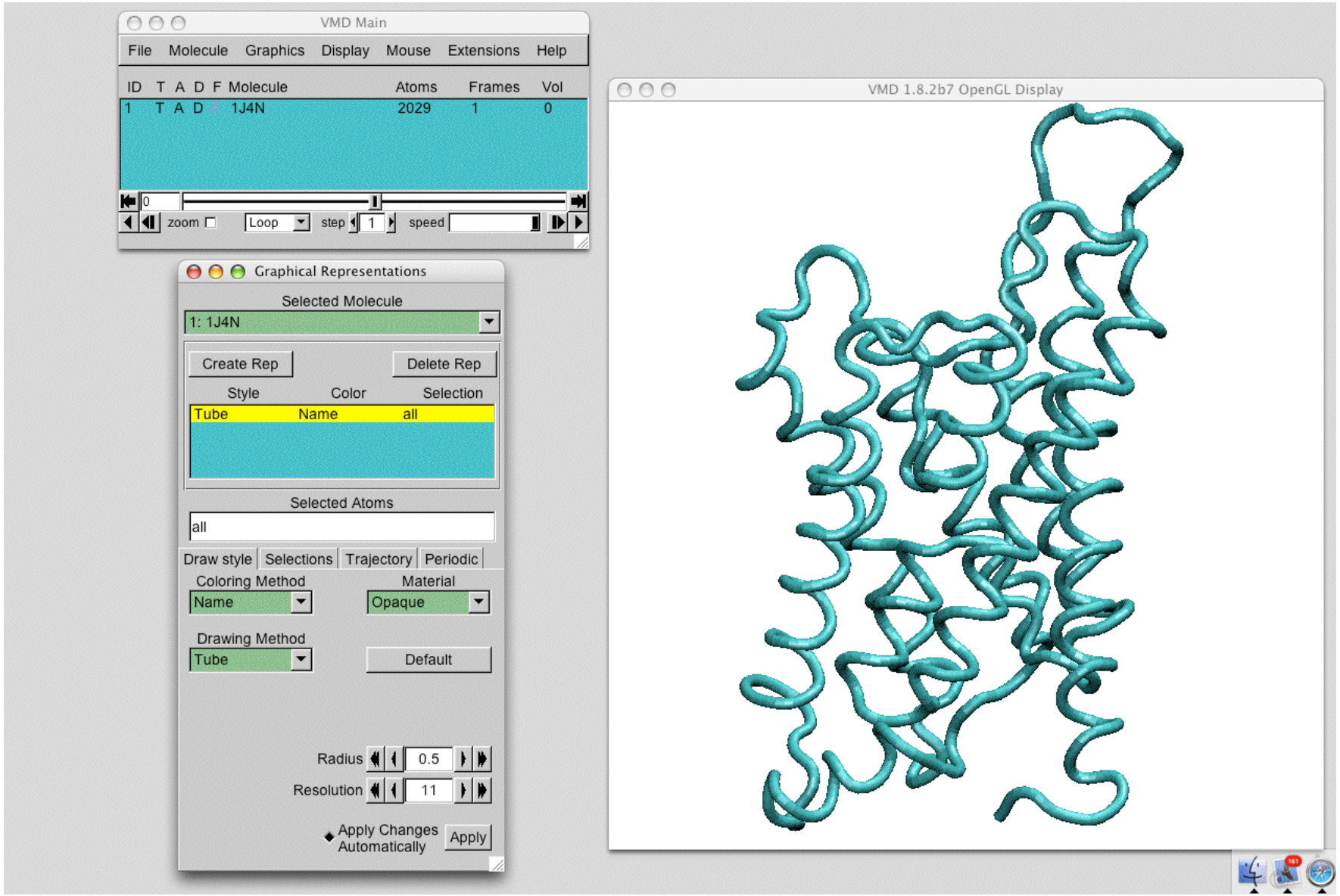
Aquaporins of known structure:

GlpF – E. coli glycerol channel (aquaglyceroporin) – Fu, et al., Science (2000)

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

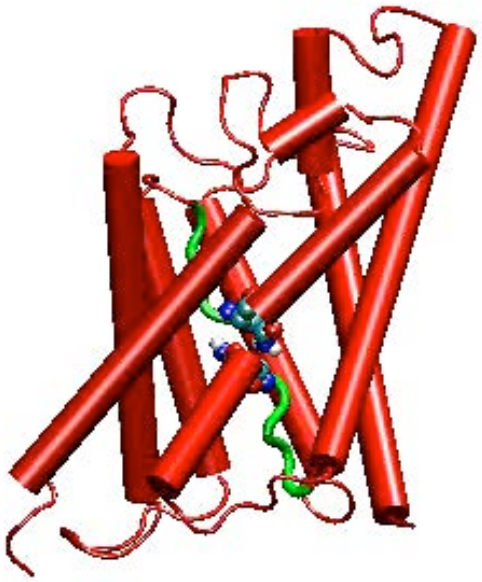


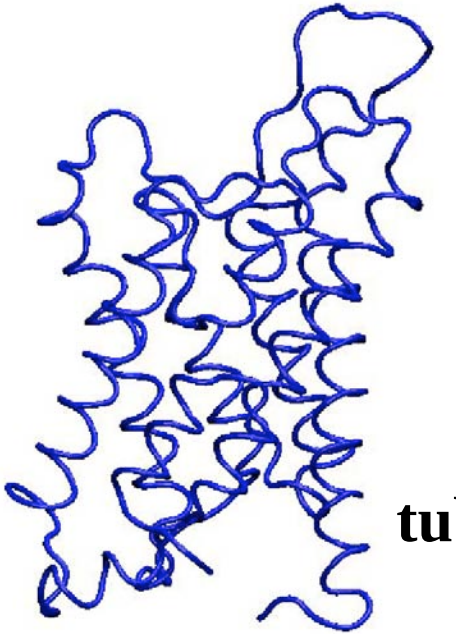
Load Aquaporin 1J4N into VMD



VMD Permits Different Rendering Styles

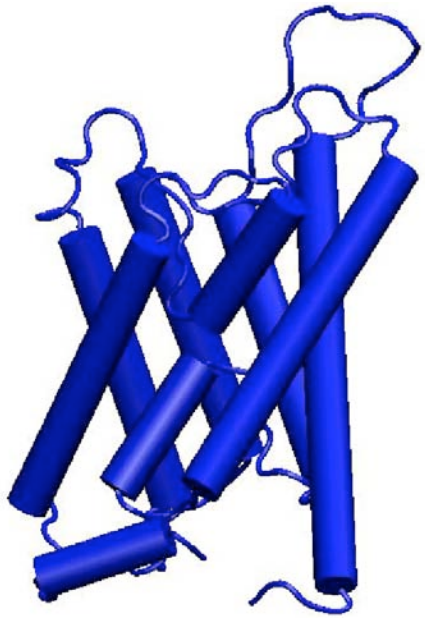
movie

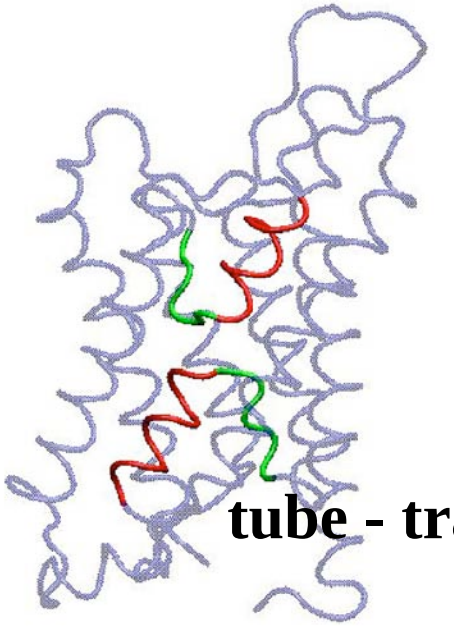




tube

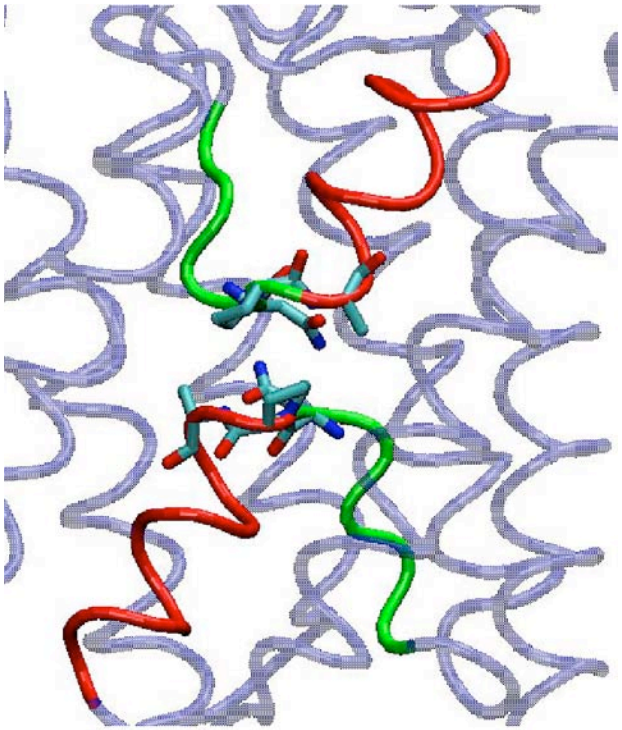
cartoon

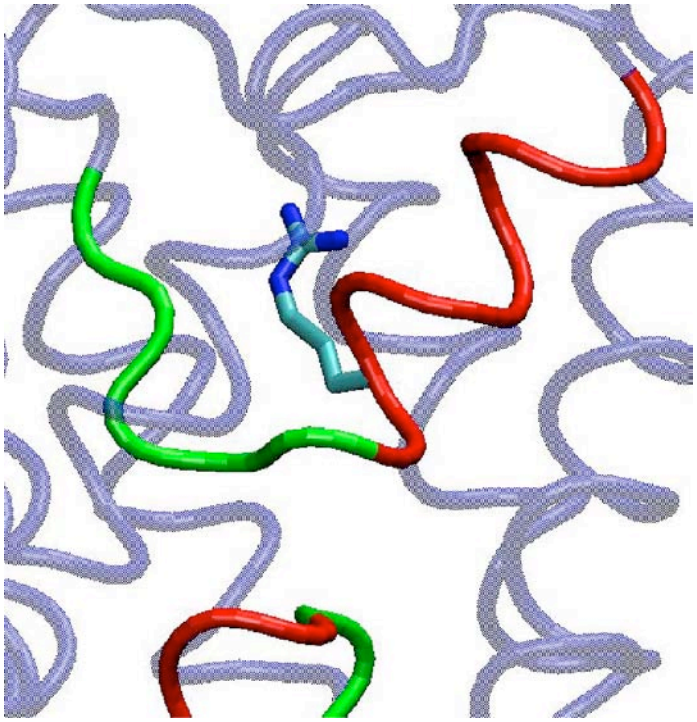




tube - transparent

Highlighting Key Conserved Residues





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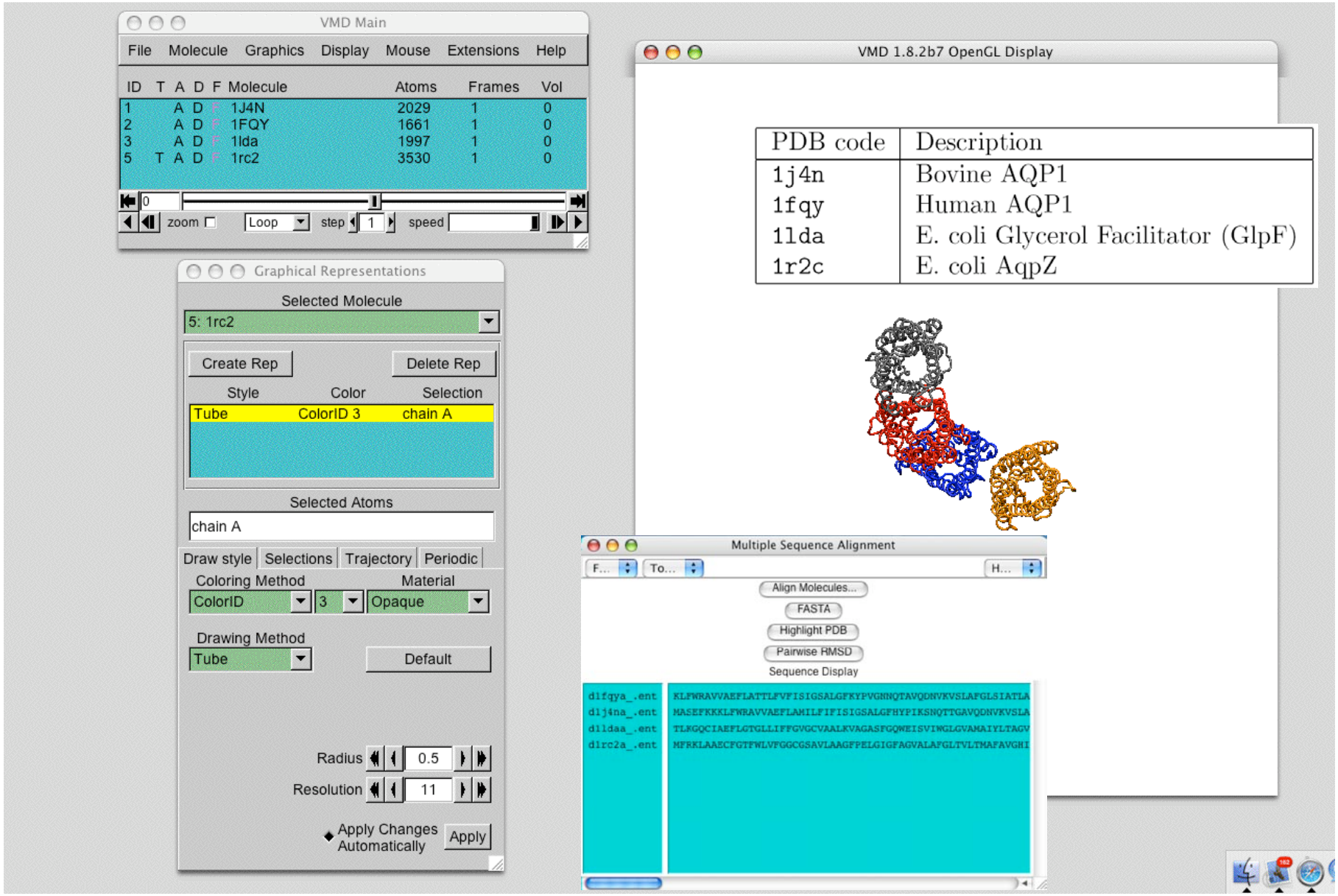
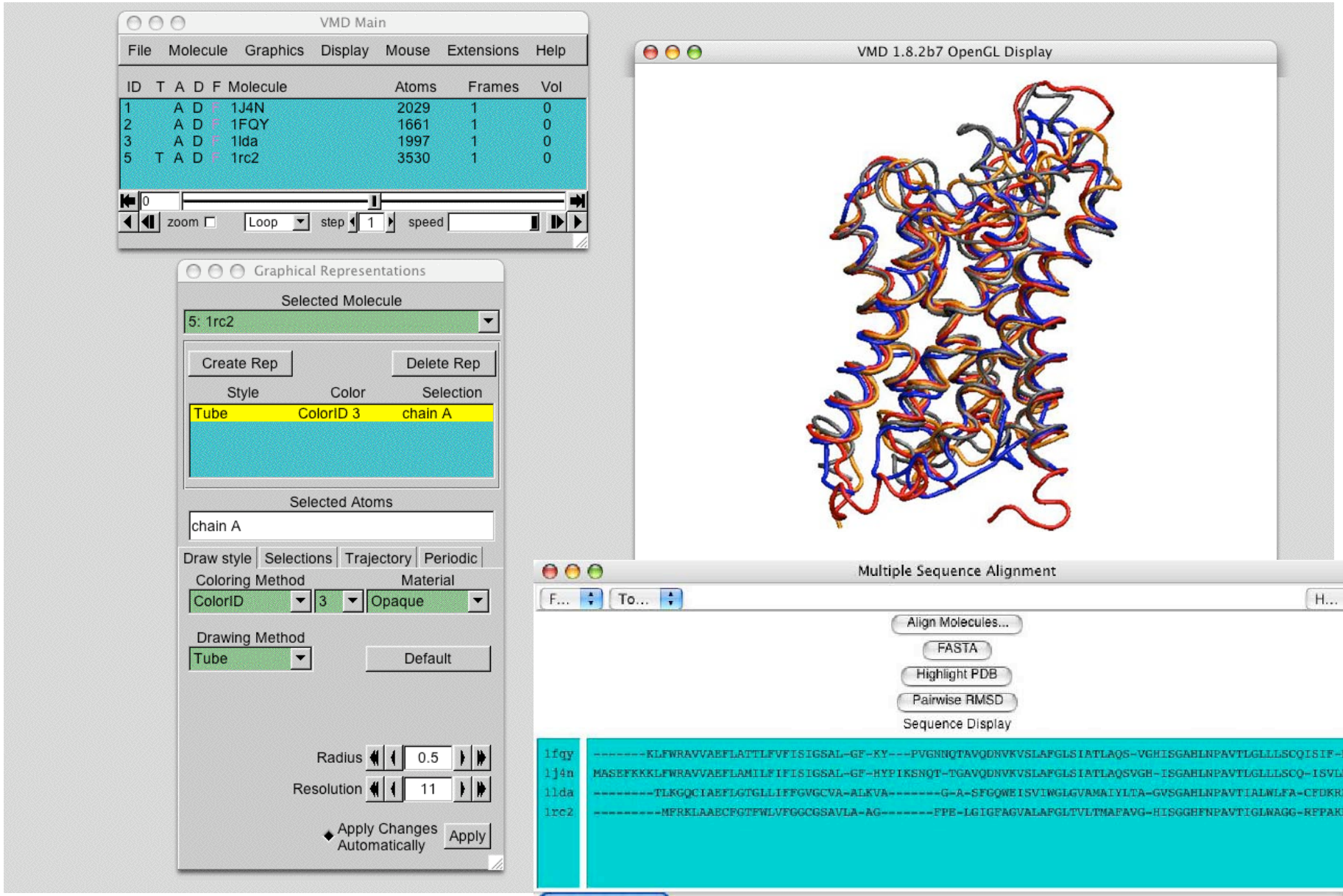
                 **:*  :  .  :.                        :   ..*:: ..          .:
AQP0_HUMAN       NPARSFAPAILTGNFT-------------NHWVYWVGPIIGGGLGSLLYDFLLFP-    225
AQP1_HUMAN       NPARSFGSAVITHNFS-------------NHWIFWVGPFIGGALAVLIYDFILAP-    233
AQP2_HUMAN       NPARSLAPAVVTGKFD-------------DHWVFWIGPLVGAILGSLLYNYVLFP-    225
AQP3_HUMAN       NPARDFGPRLFTALAGWGSAVFTTGQ--HWWWVPIVSPLLGSIAGVFVYQLMIGC-    267
AQP4_HUMAN       NPARSFGPAVIMGNWE-------------NHWIYWVGPIIGAVLAGGLYEYVFCPD    255
AQP5_HUMAN       NPARSFGPAVVMNRFSP------------AHWVFWVGPIVGAVLAAILYFYLLFP-    227
AQP6_HUMAN       NPARSFGPAIIIGKFT-------------VHWVFWVGPLMGALLASLIYNFVLFP-    237
AQP7_HUMAN       NPSRDLPPRIFTFIAGWGKQVFSNGE--NWWWVPVVAPLLGAYLGGIIYLVFIGS-    278
AQP8_HUMAN       NPARAFGPAVVANHWN-------------FHWIYWLGPLLAGLLVGLLIRCFIGD-    251
AQP9_HUMAN       NPARDLSPRLFTALAGWGFEVFRAGN--NFWWIPVVGPLVGAVIGGLIYVLVIEI-    268
GLPF_ECOLI       NPARDFGPKVFAWLAGWGNVAFTGGRDIPYFLVPLFGPIVGAIVGAFAYRKLIGR-    257
      ruler      ...250.......260.......270.......280.......290.......300
```

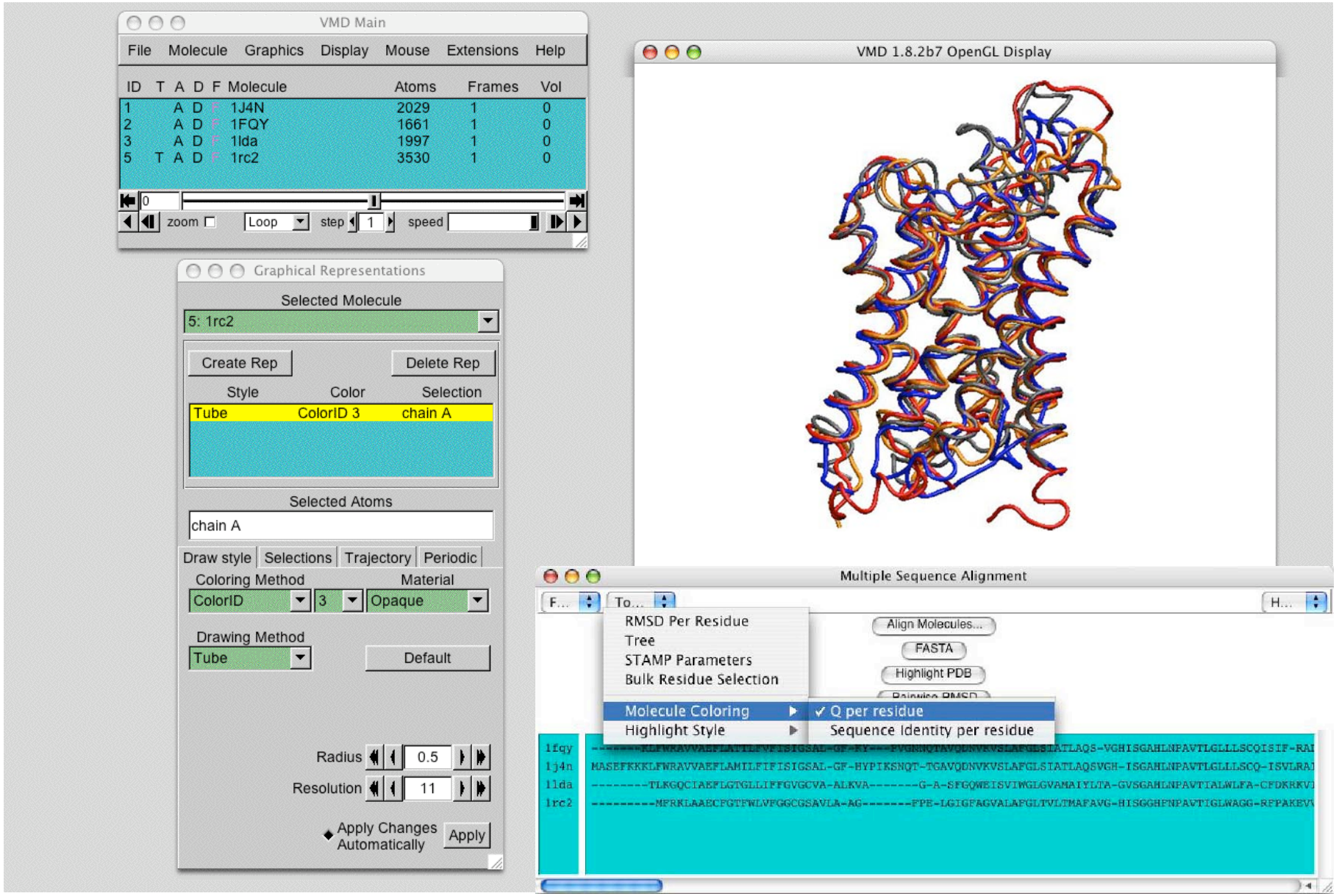
Load Aquaporins 1j4n, 1fqy, 1lda, 1rc2 into VMD



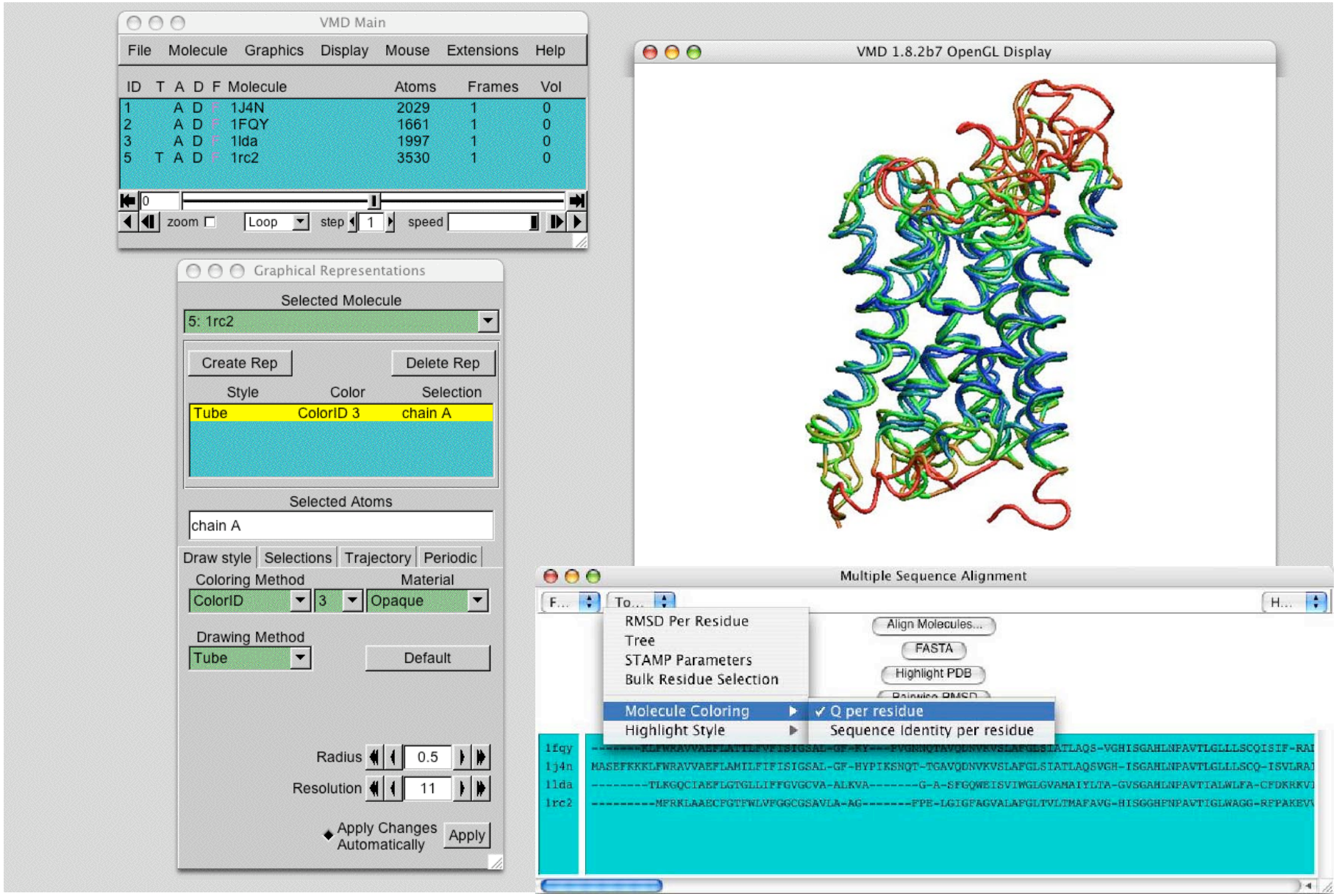
Aligning Structures and Sequences



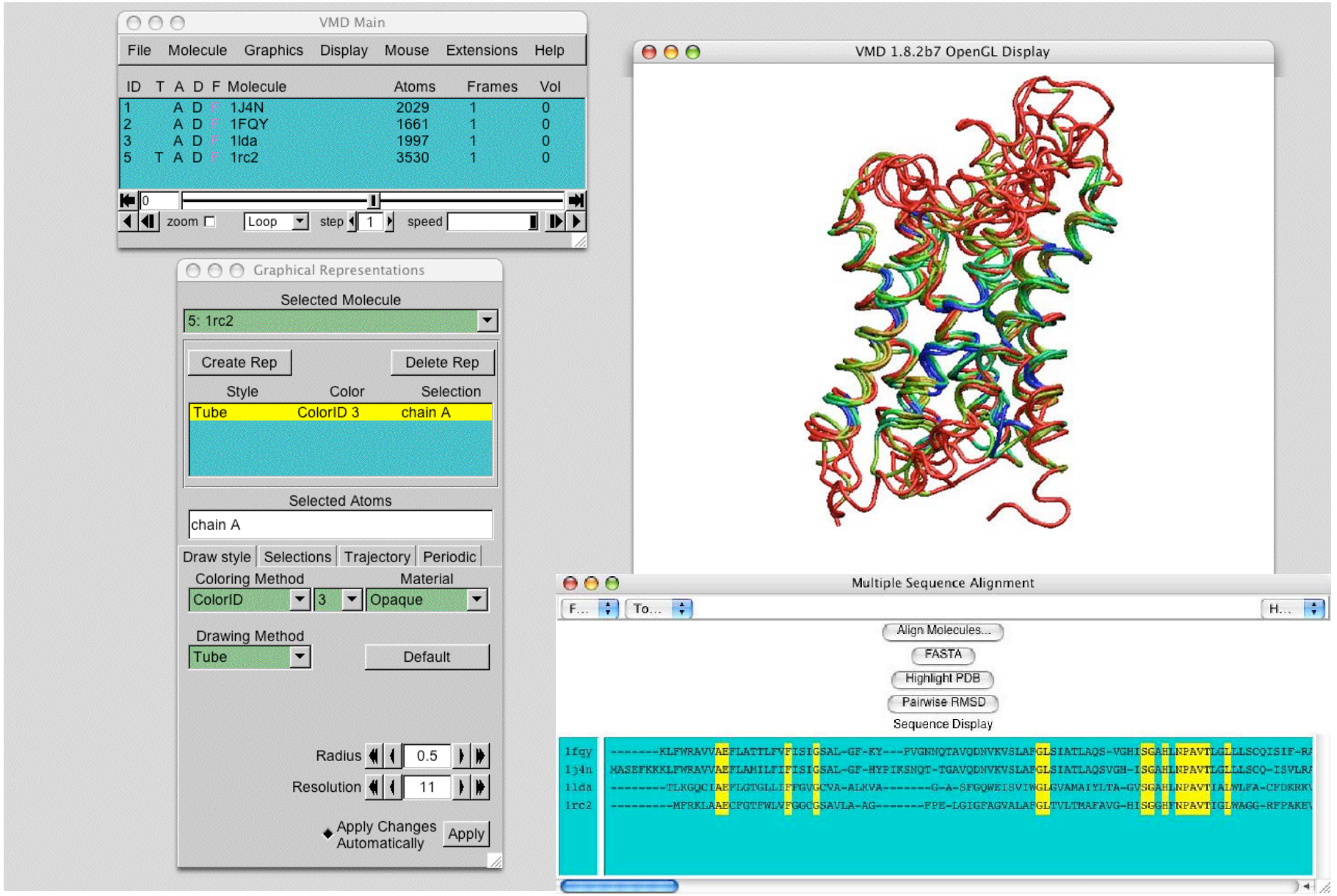
Comparing Structures by Similarity - Q Value



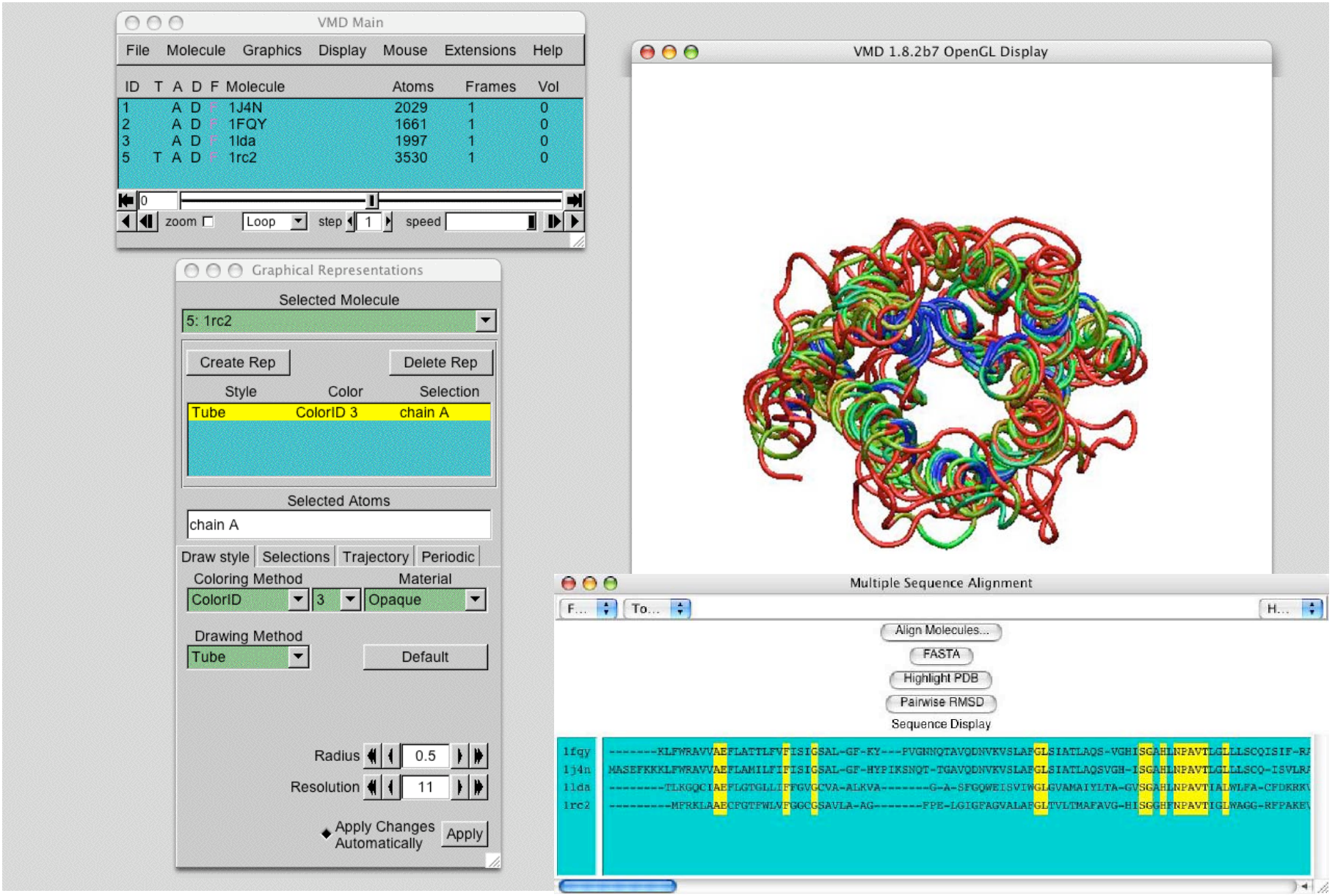
Comparing Structures by Similarity - Q Value



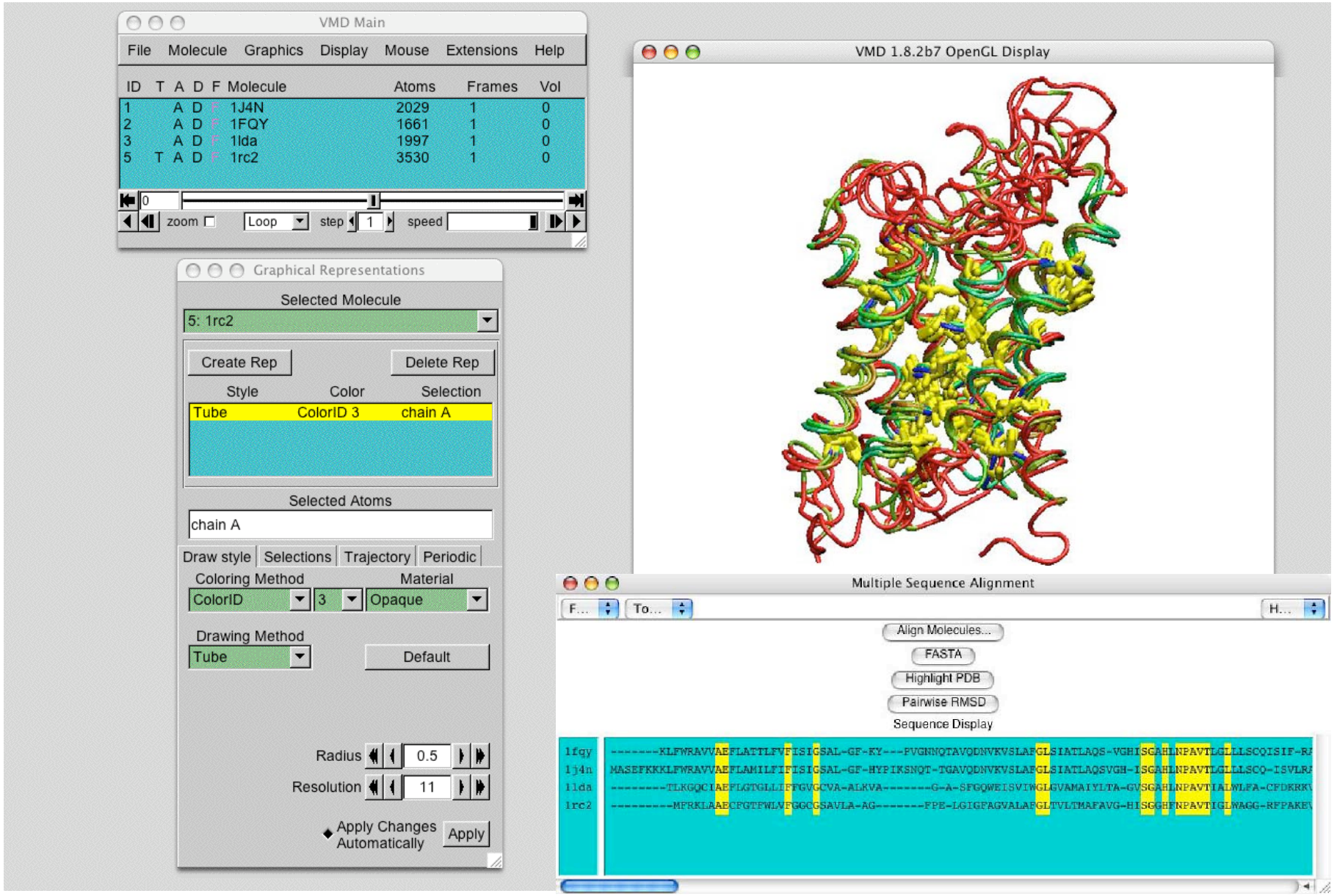
Exhibiting Sequence Identity - Side View



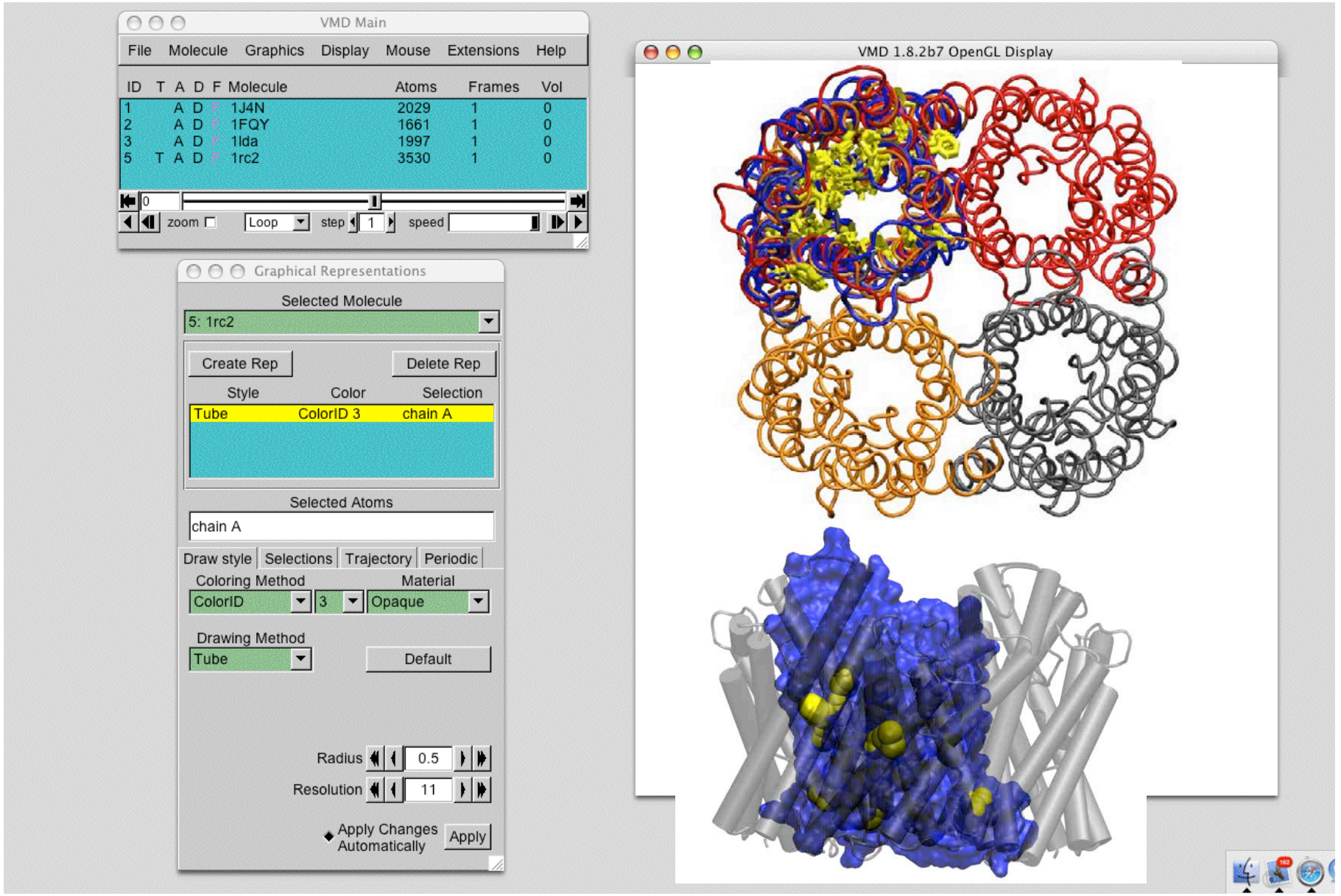
Exhibiting Sequence Identity - Top View



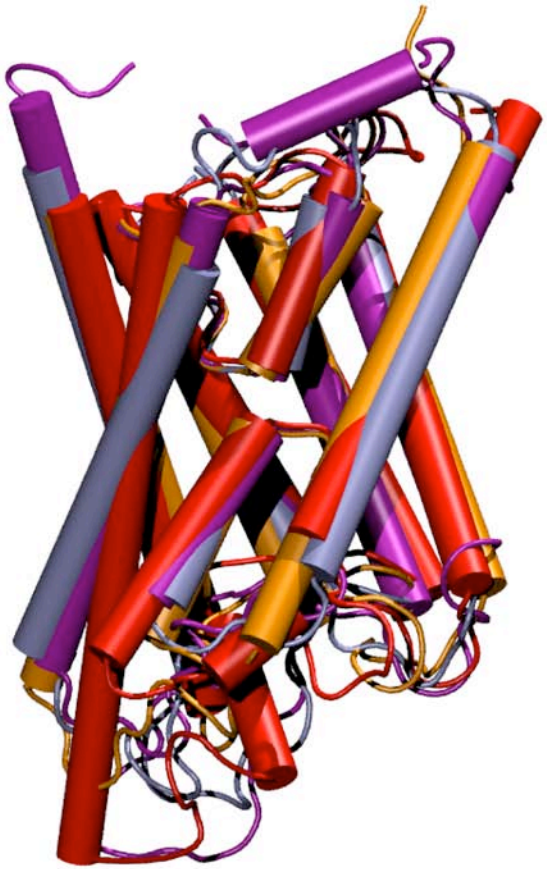
Showing Conserved Residues - Monomer



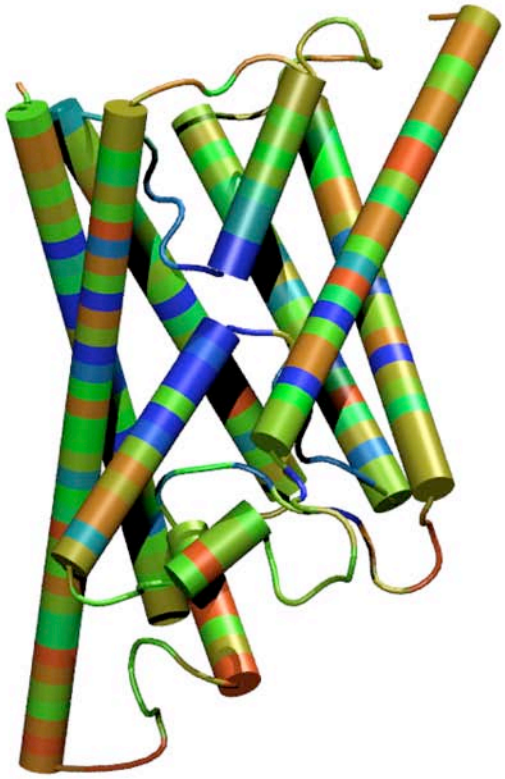
Showing Conserved Residues - Tetramer



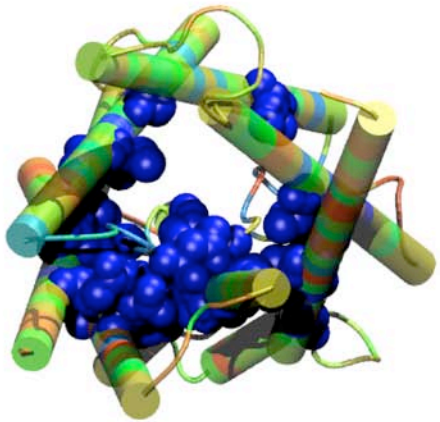
Structure and Sequence Comparisons
Water/Glycerol Channels



2 AQP1, GLPF, AQPZ
from animal and bacteria



GLPF Sequence Conservation



Top view

Acknowledgements - Tutorials

Seq Alignment

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

Evolution AARS

- Rommie Amaro
- Patrick O'Donoghue
- Brijeet Dhaliwa

Bioinformatics Aquaporins

- Fatemeh Araghi
- Brijeet Dhaliwal
- Elizabeth Villa

VMD Developers: Dan Wright, John Eargle, John Stone