Part III - Bioinformatics Studies Using Multiseq in VMD

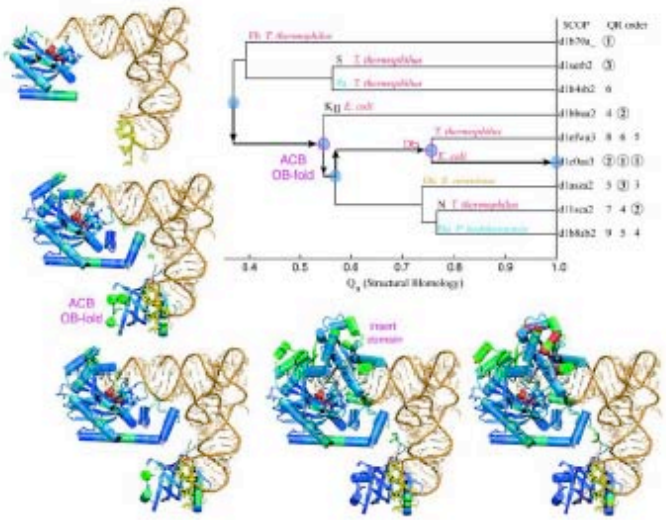
- Aminoacyl tRNA Synthetases
- Aquaporins

Lake Tahoe, 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

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Patrick O'Donoghue

Rommie Amaro

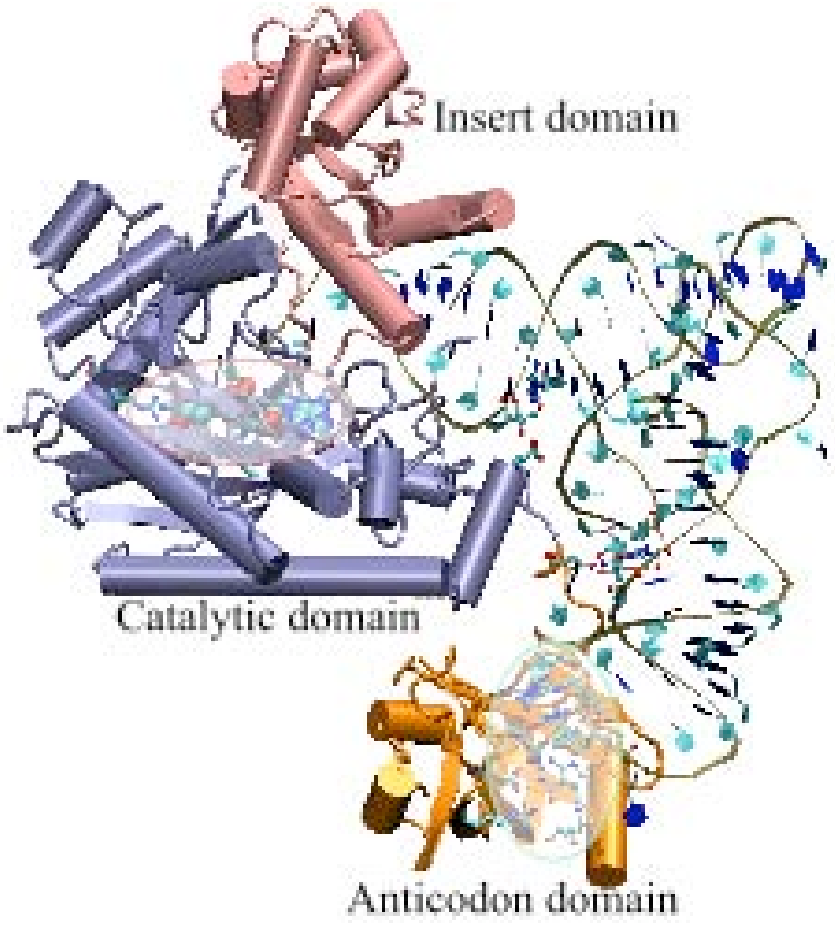
April 2004.

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)

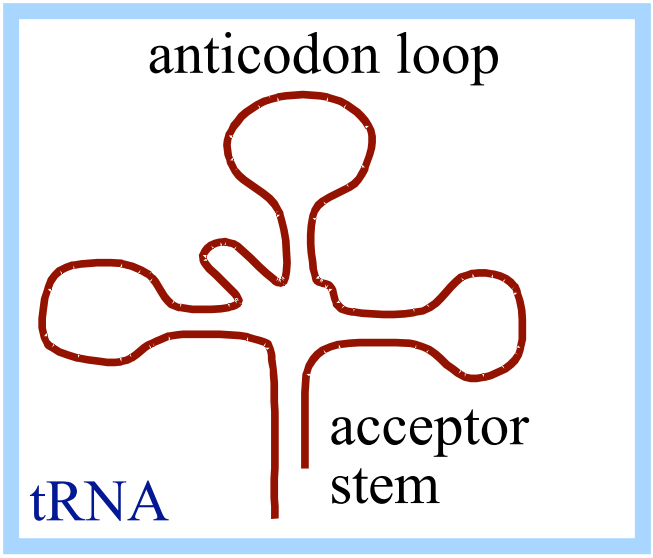
- Carl Woese - Crafoord Prize 2003

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

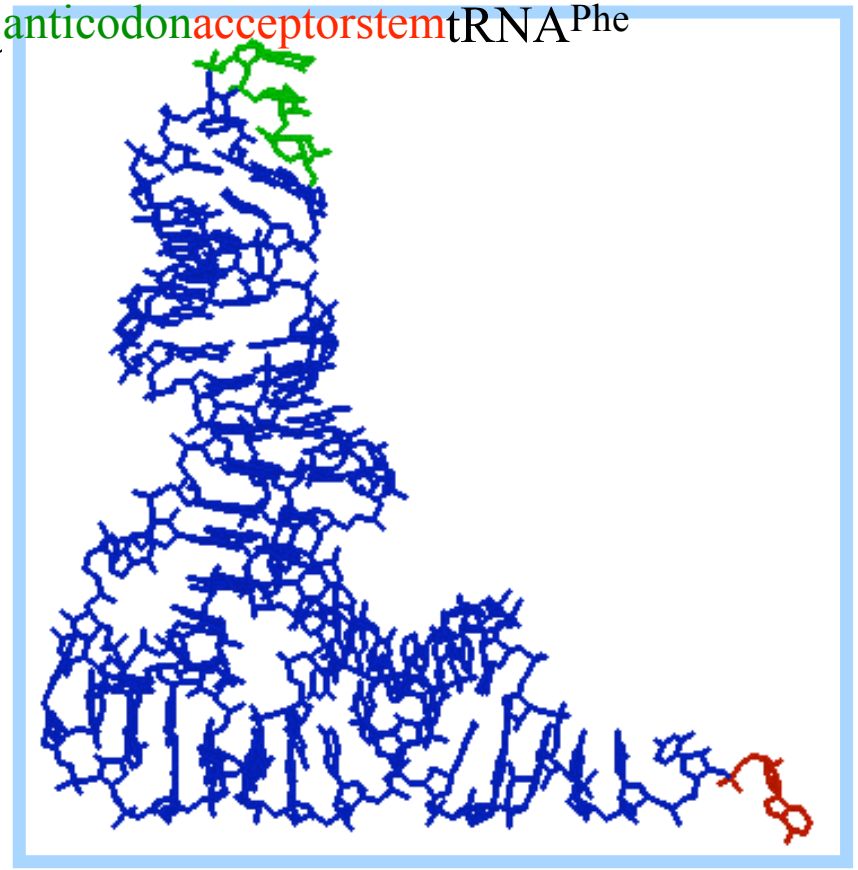


Standard Dogma Molecular Biology

- 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

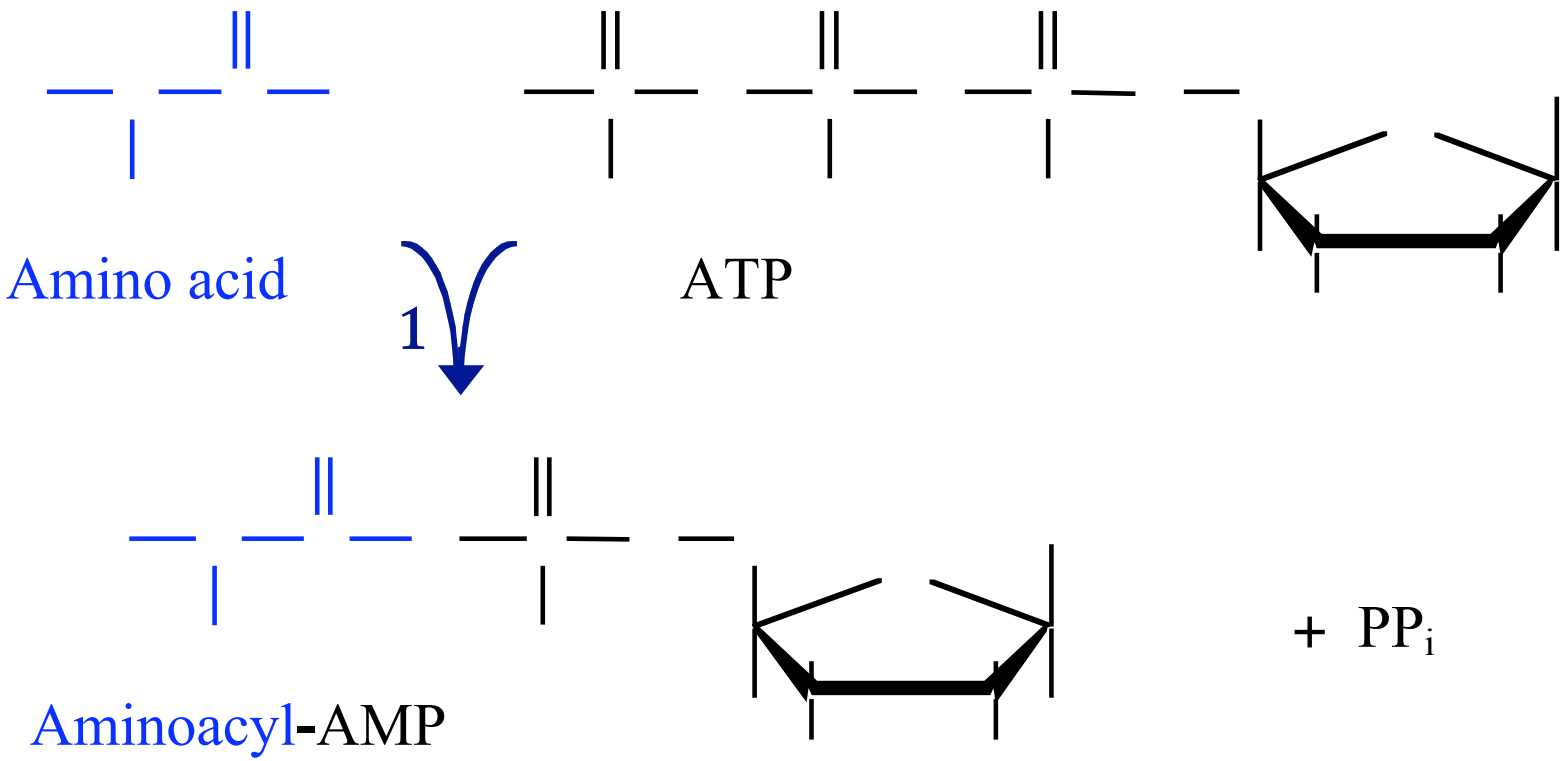
$$\mathbf{Gln + tRNA^{Gln} + ATP} \underset{\text{synthetase}}{\overset{\text{Glutaminyl-tRNA}}{\rightleftharpoons}} \mathbf{Gln\text{-}tRNA^{Gln} + AMP + PP_i}$$

tRNA-dependent amino acid modification

$$\mathbf{Glu + tRNA^{Gln} + ATP} \underset{\text{synthetase}}{\overset{\text{Glutamyl-tRNA}}{\rightleftharpoons}} \mathbf{Glu\text{-}tRNA^{Gln} + AMP + PP_i}$$

$$\mathbf{Gln + Glu\text{-}tRNA^{Gln} + ATP} \underset{\text{amidotransferase}}{\overset{\text{Glu-tRNA}^{\text{Gln}}}{\rightleftharpoons}} \mathbf{Gln\text{-}tRNA^{Gln} + ADP + P_i + Glu}$$

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

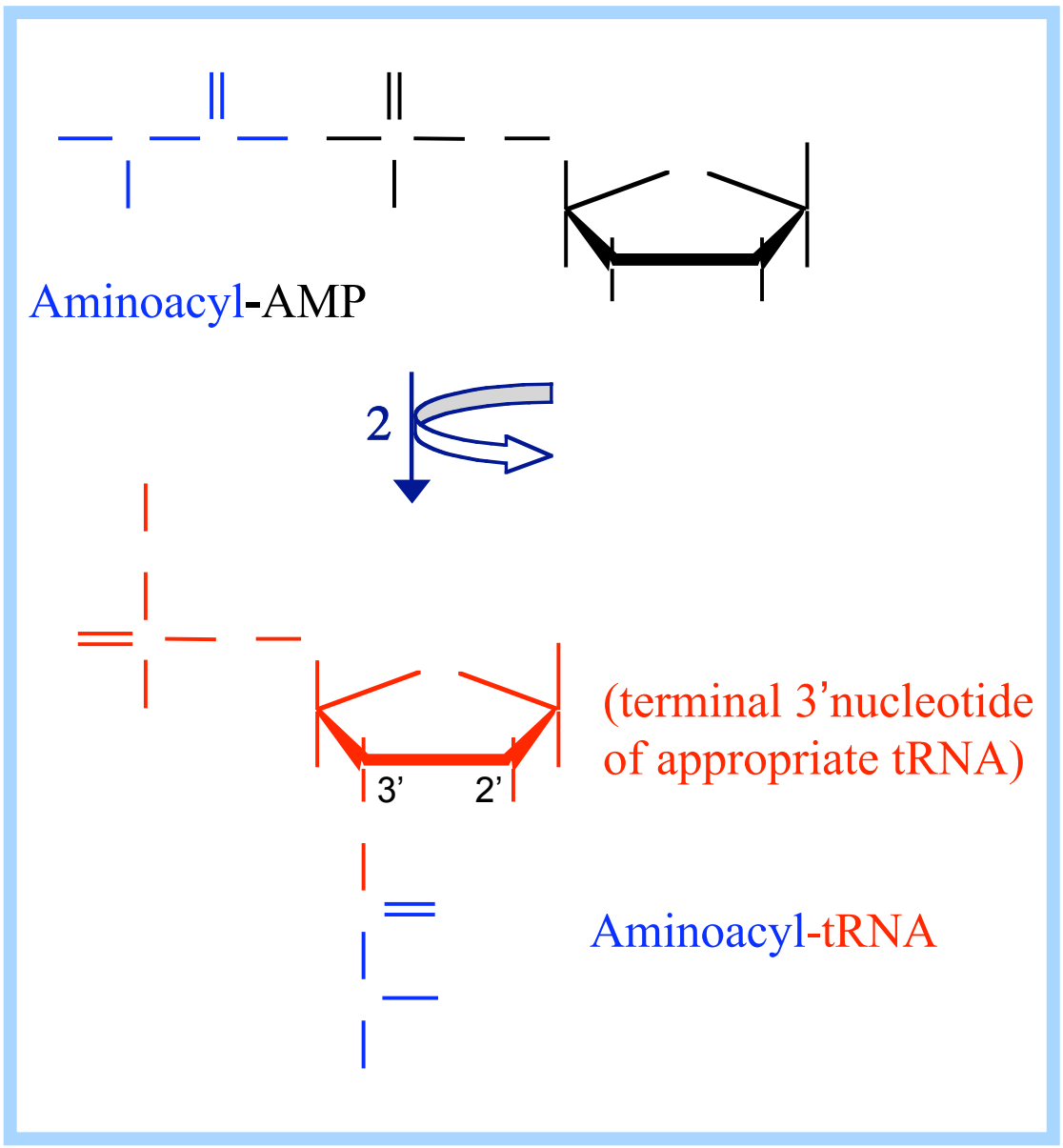


Aminoacyl-tRNA Synthetases catalyze linkage of the appropriate amino acid to each tRNA. The reaction occurs in two steps.

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**

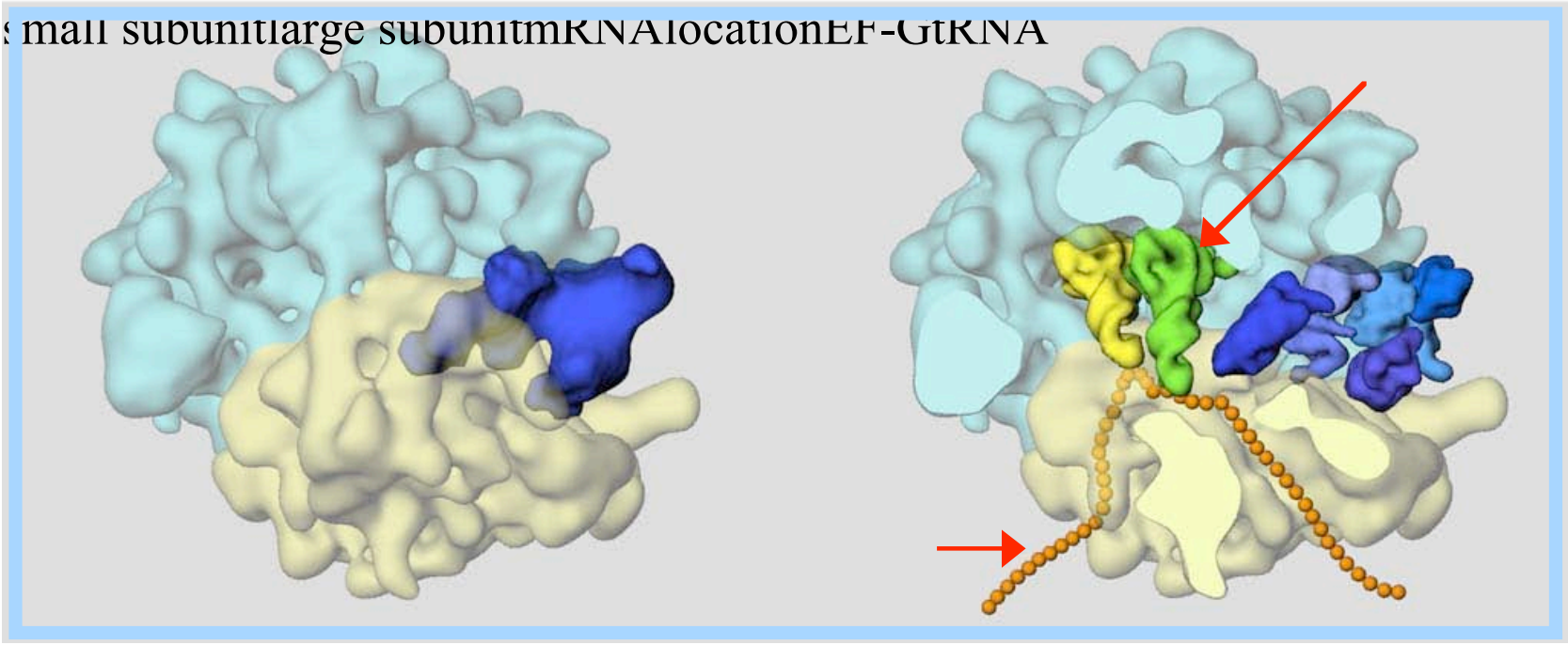
Overall Reaction:

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

+ PP_i

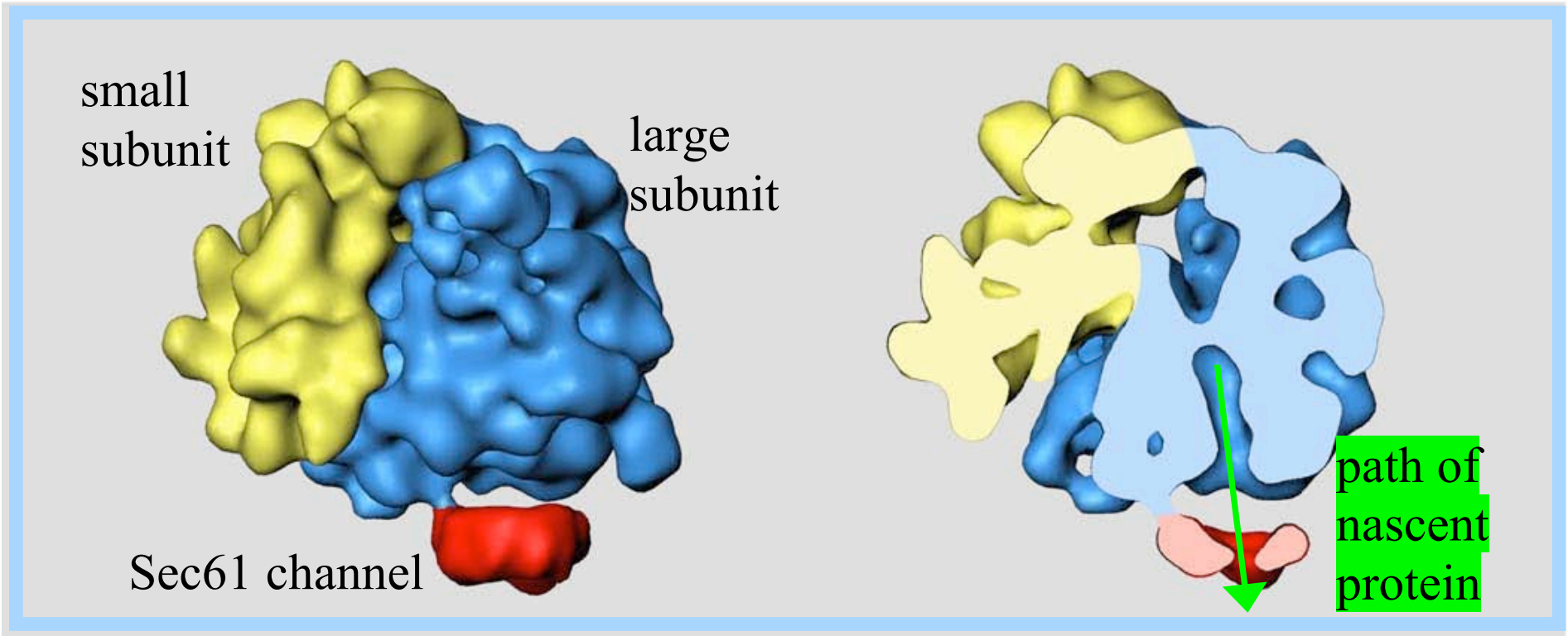
Next step: EF and Ribosome for Protein Synthesis

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



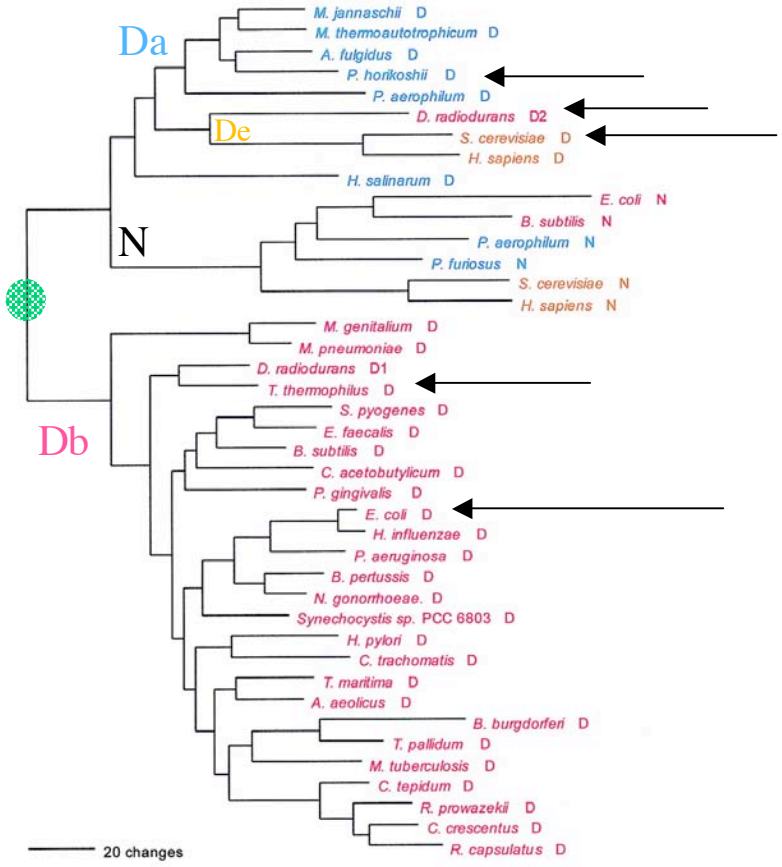
The cutaway view at right shows that the **tunnel** in the yeast large ribosome subunit, through which nascent polypeptides emerge from the ribosome, **lines up** with the lumen of the ER **Sec61 channel**.

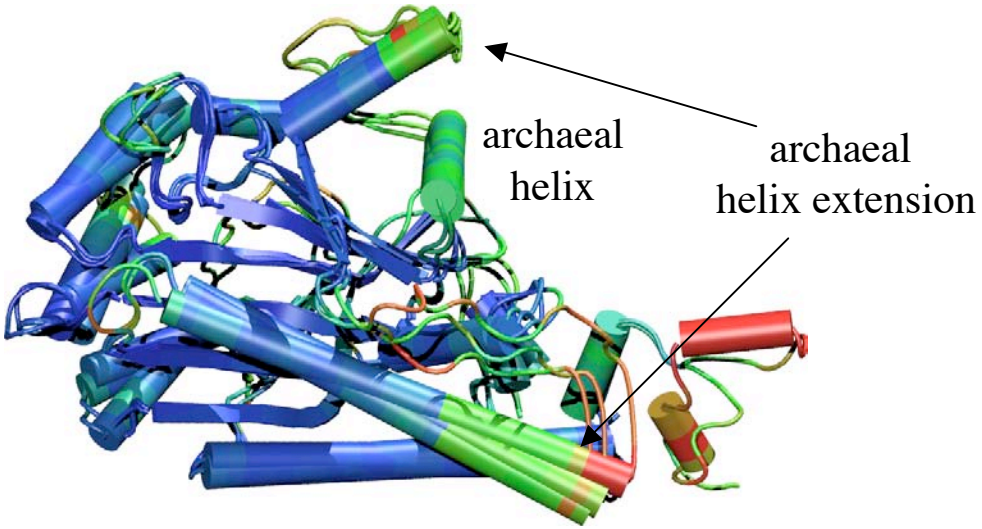
Figure provided by Joachim Frank, whose lab carried out the cryo-EM & image reconstruction on which these images are based.

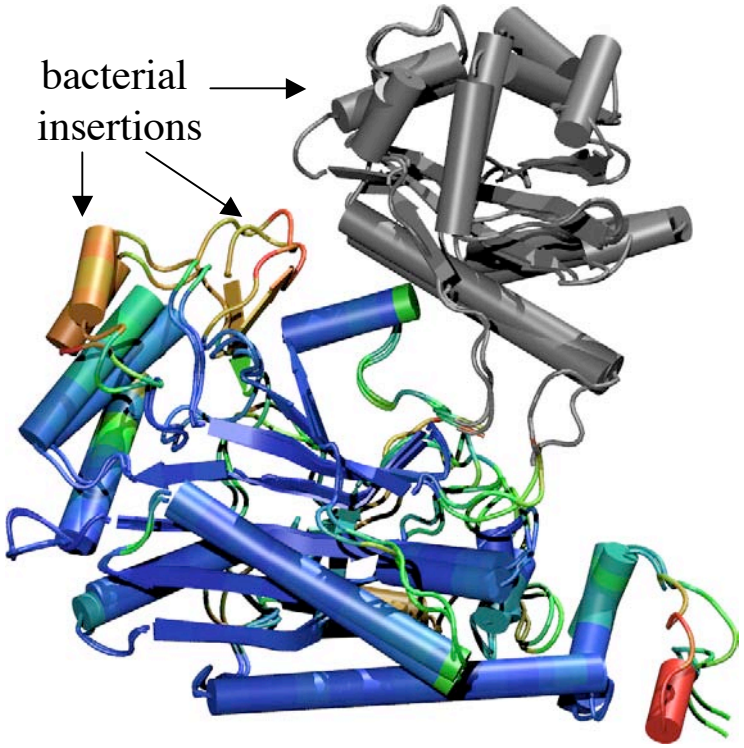
Horizontal Gene Transfer in Protein Structure

Sequence Phylogeny

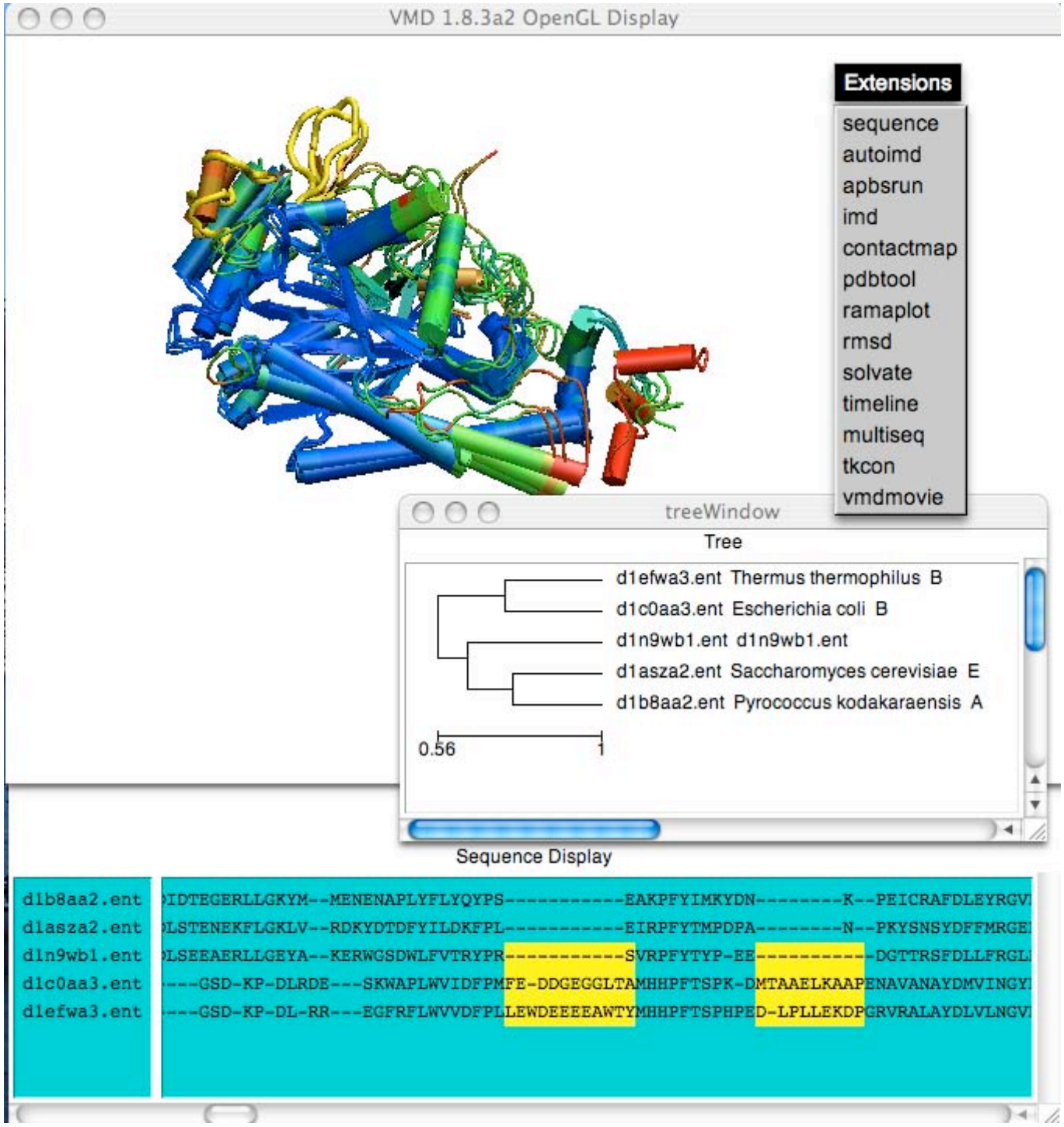
AspRS-AsnRS Group



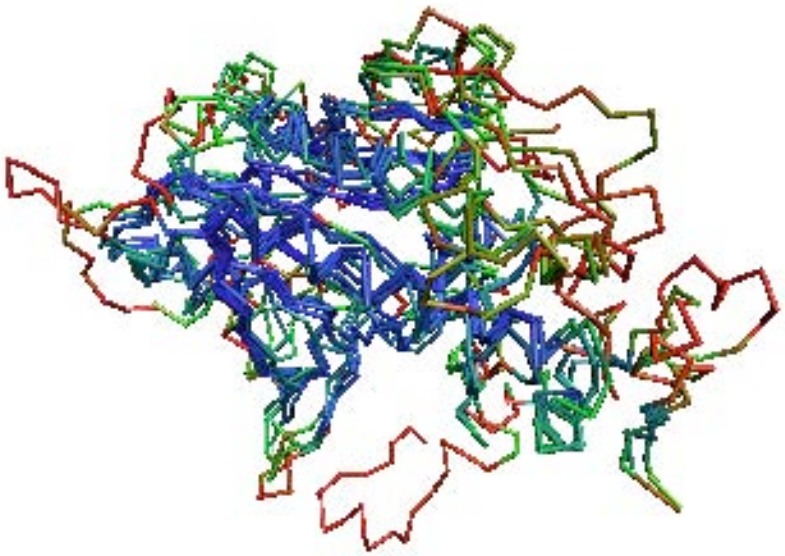




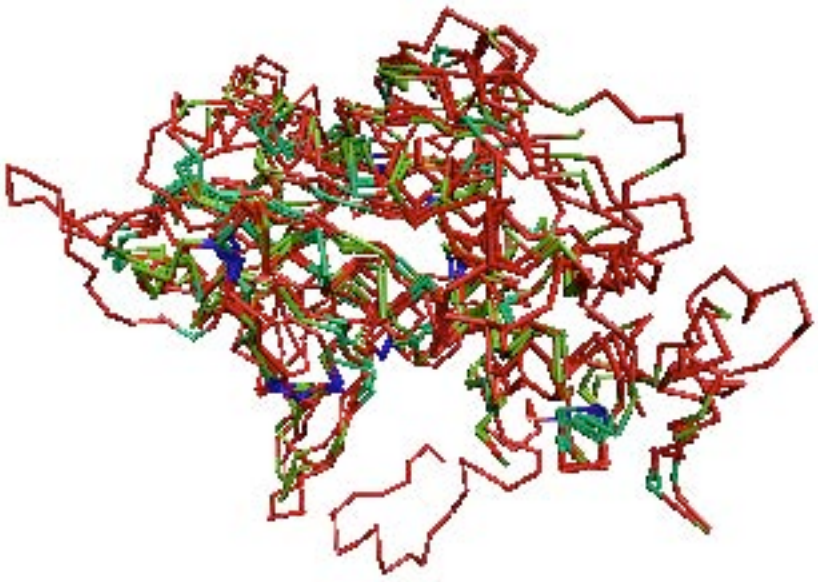
Multiseq
extension in VMD



Conservation



Core Structure Conserved



Sequence Identity of Core
Less than 15%

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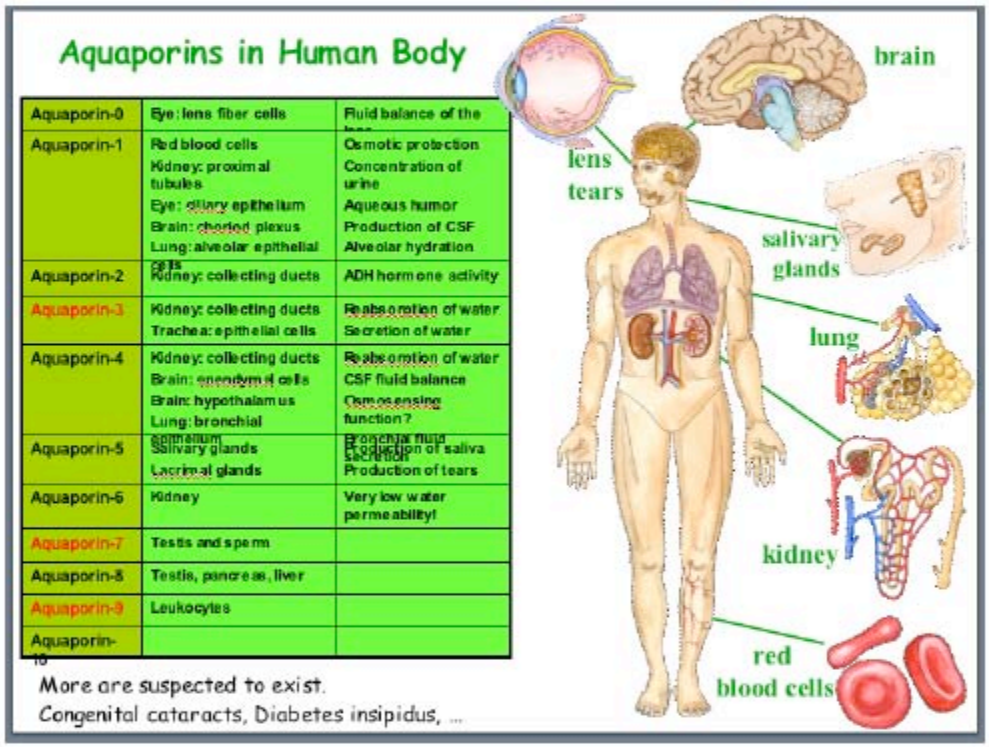
Aquaporins



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Emad Tajkhorshid

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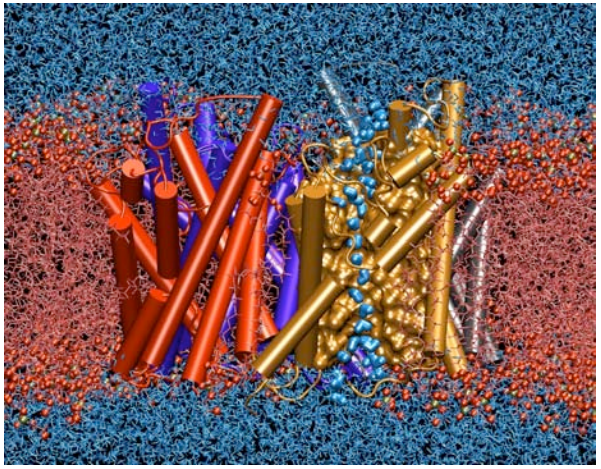
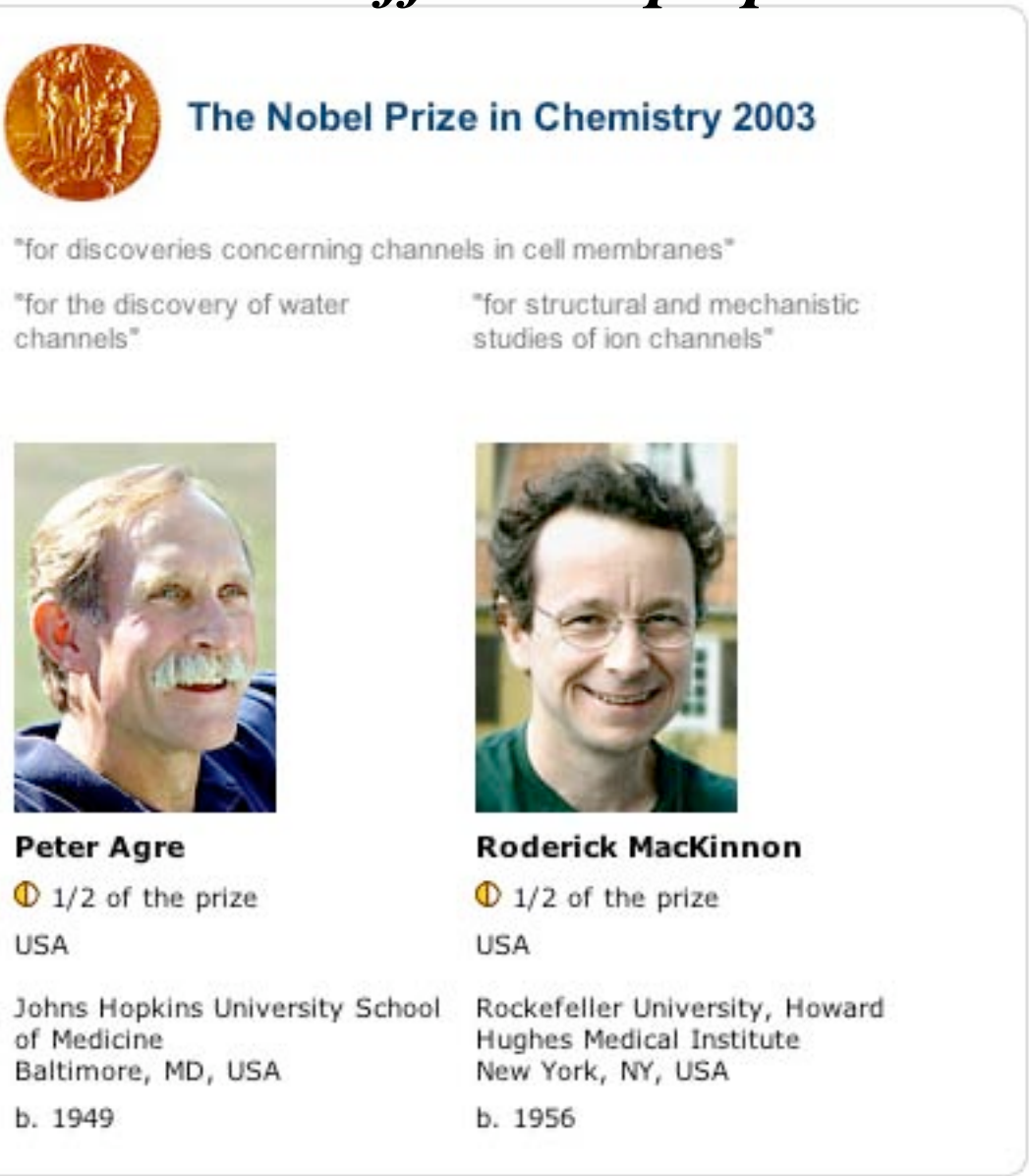
Towards Understanding Membrane Channels

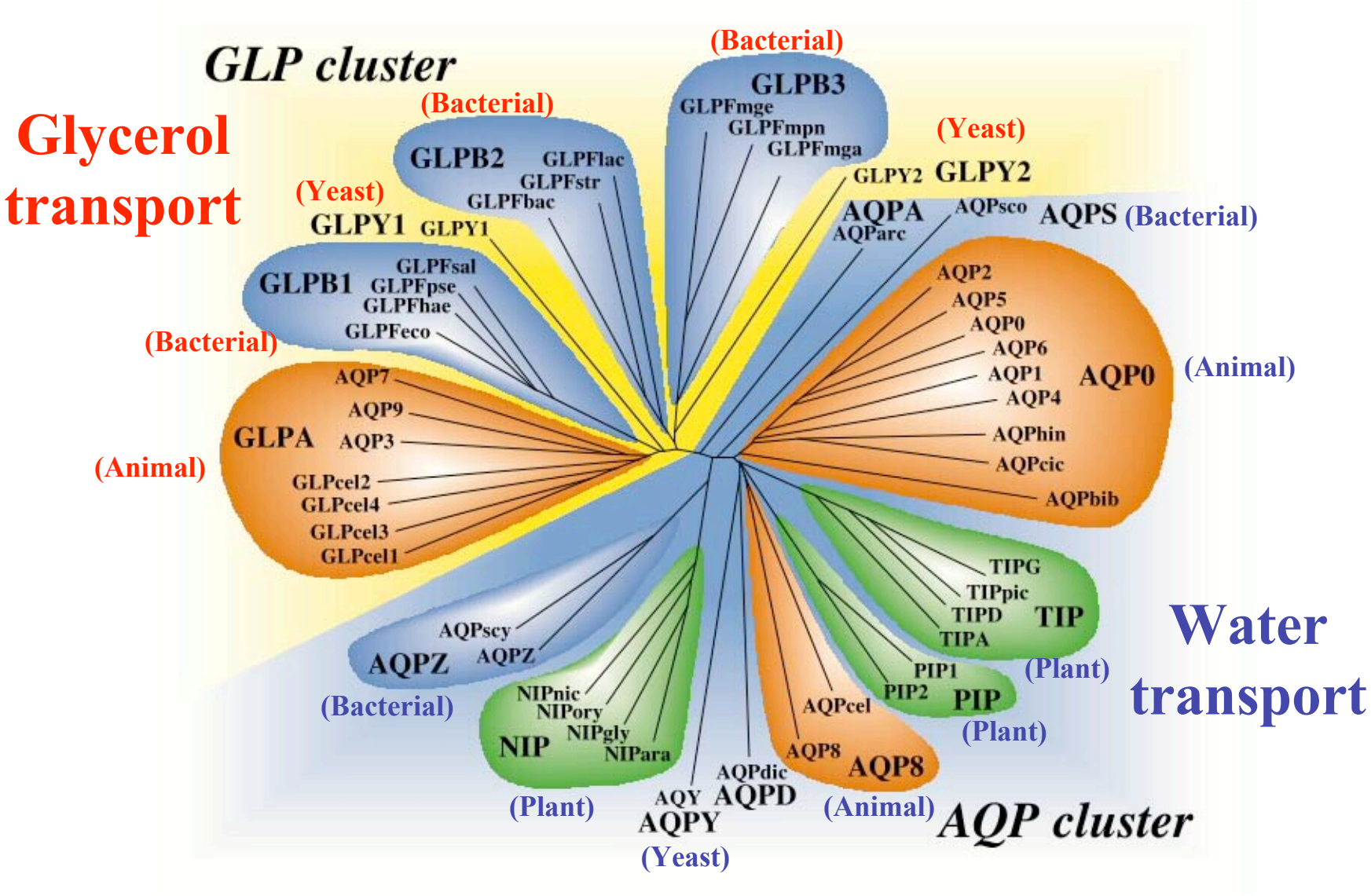
The versaltile, highly selective and efficent aquaporin



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 Aquaporin Superfamily

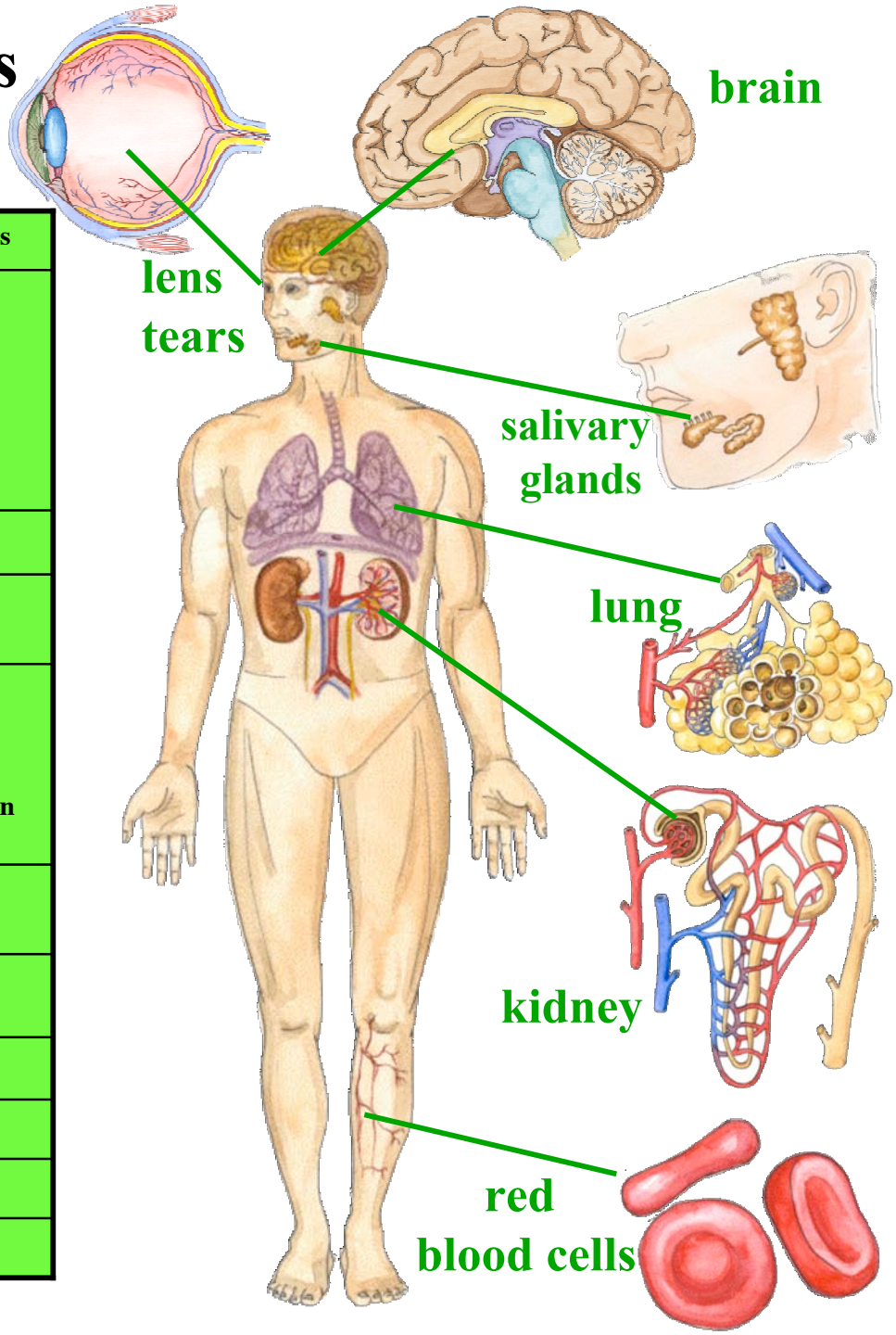


Heymann and Engel *News Physiol. Sci.* **14**, 187 (1999)

Water and Glycerol Channels in the Human Body

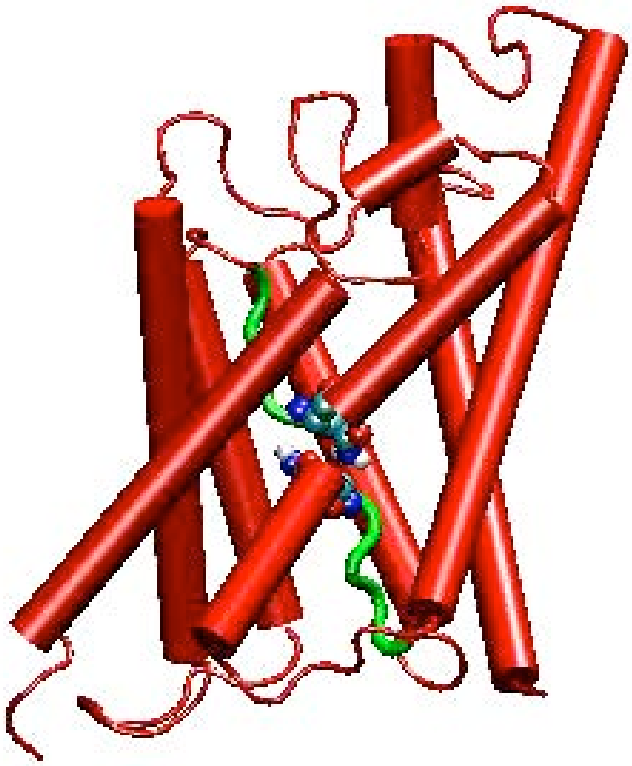
Aquaporin-0	Eye: lens fiber cells	Fluid balance of the lens
Aquaporin-1	Red blood cells Kidney: proximal tubules Eye: ciliary epithelium Brain: choriod plexus Lung: alveolar epithelial cells	Osmotic protection Concentration of urine Aqueous humor Production of CSF Alveolar hydration
Aquaporin-2	Kidney: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

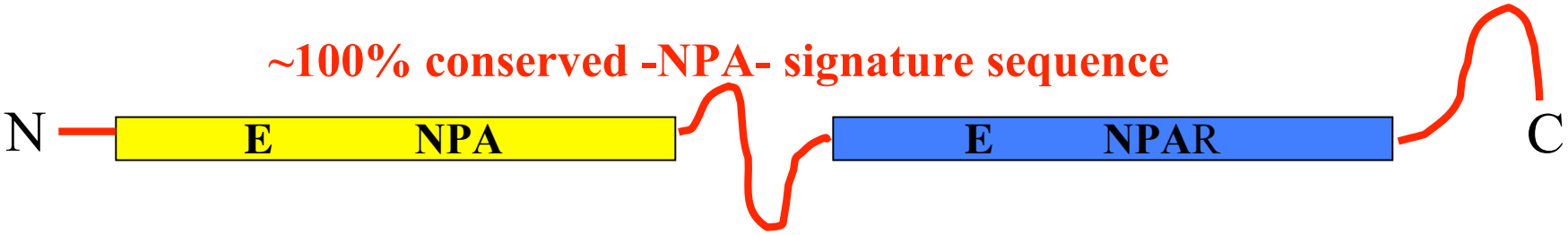
- Water and glycerol transport
- Exclusion of ions and protons
- Tetrameric arrangement in membrane



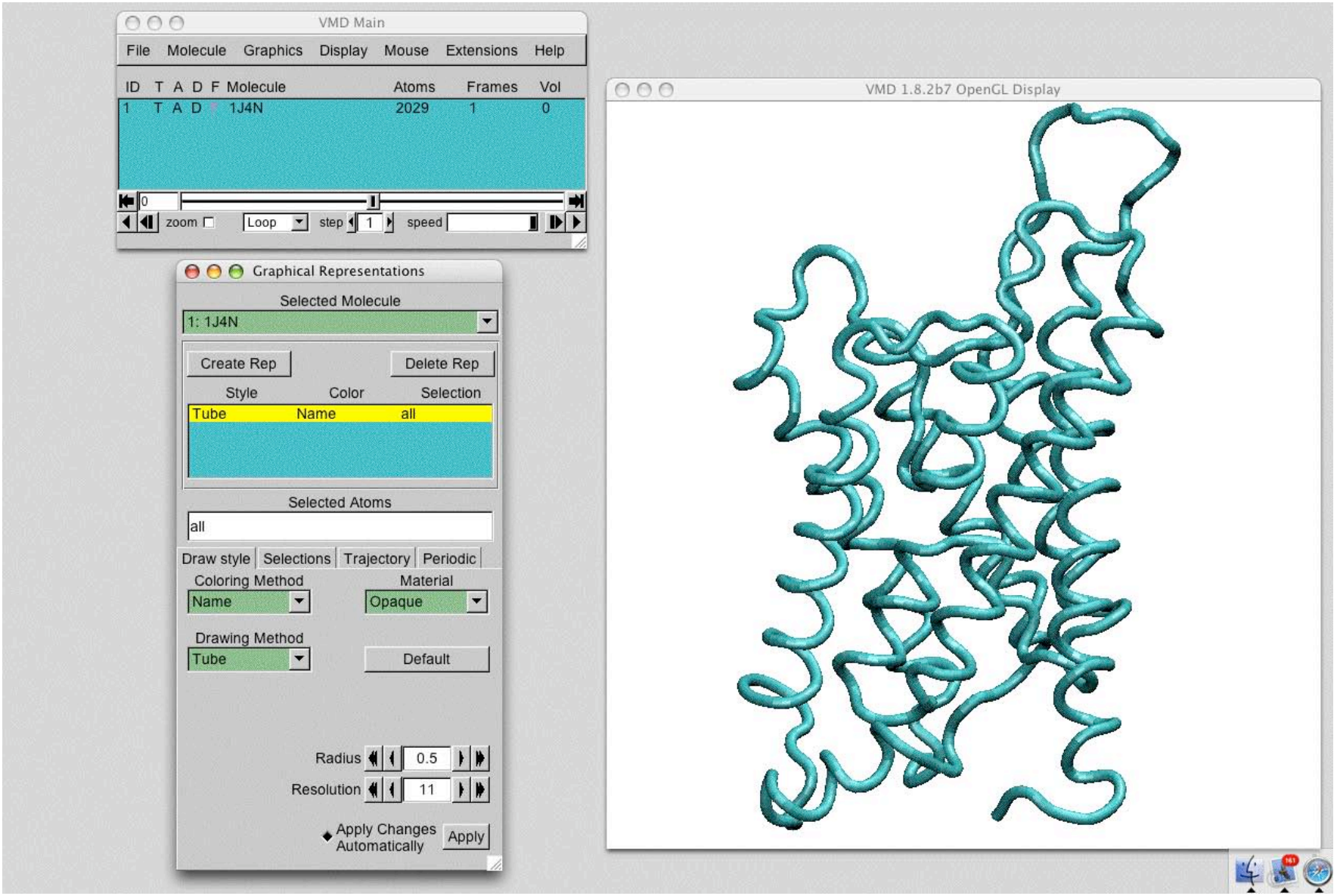
Aquaporins of known structure:

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

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

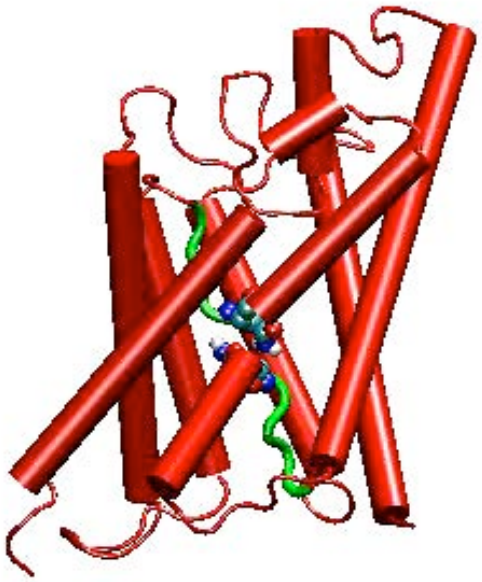


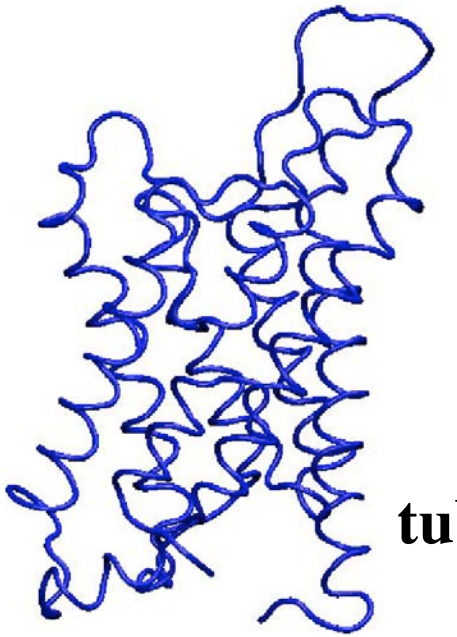
Load Aquaporin 1J4N into VMD



VMD Permits Different Rendering Styles

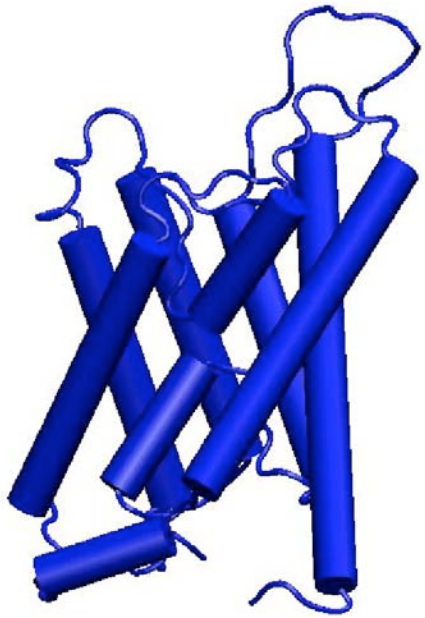
movie

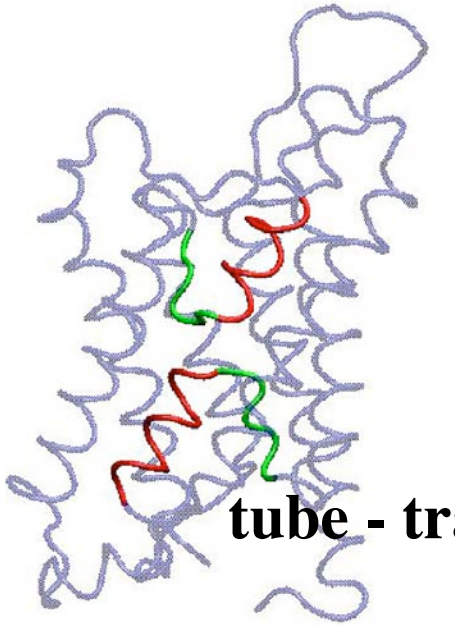




tube

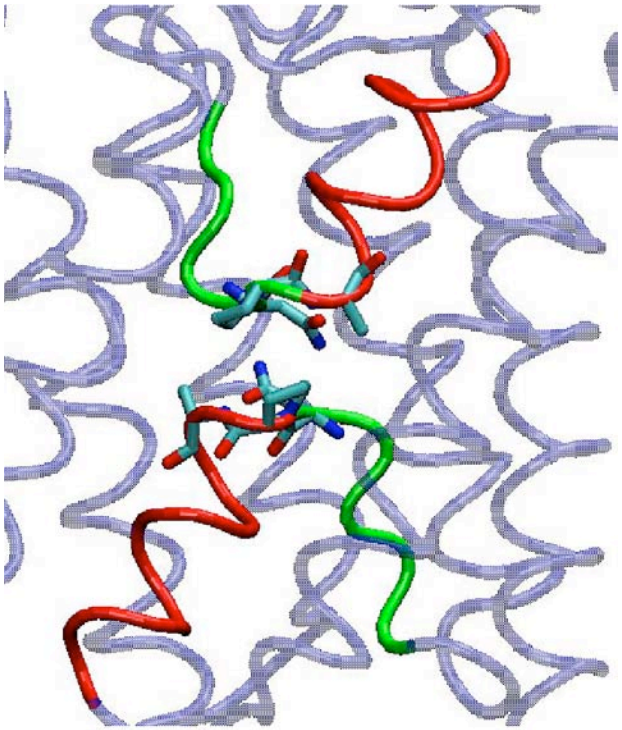
cartoon

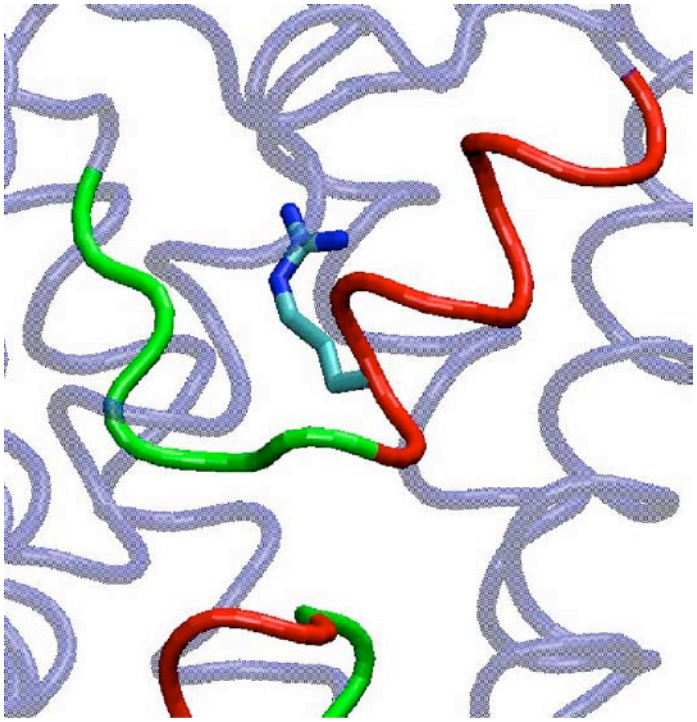




tube - transparent

Highlighting Key Conserved Residues





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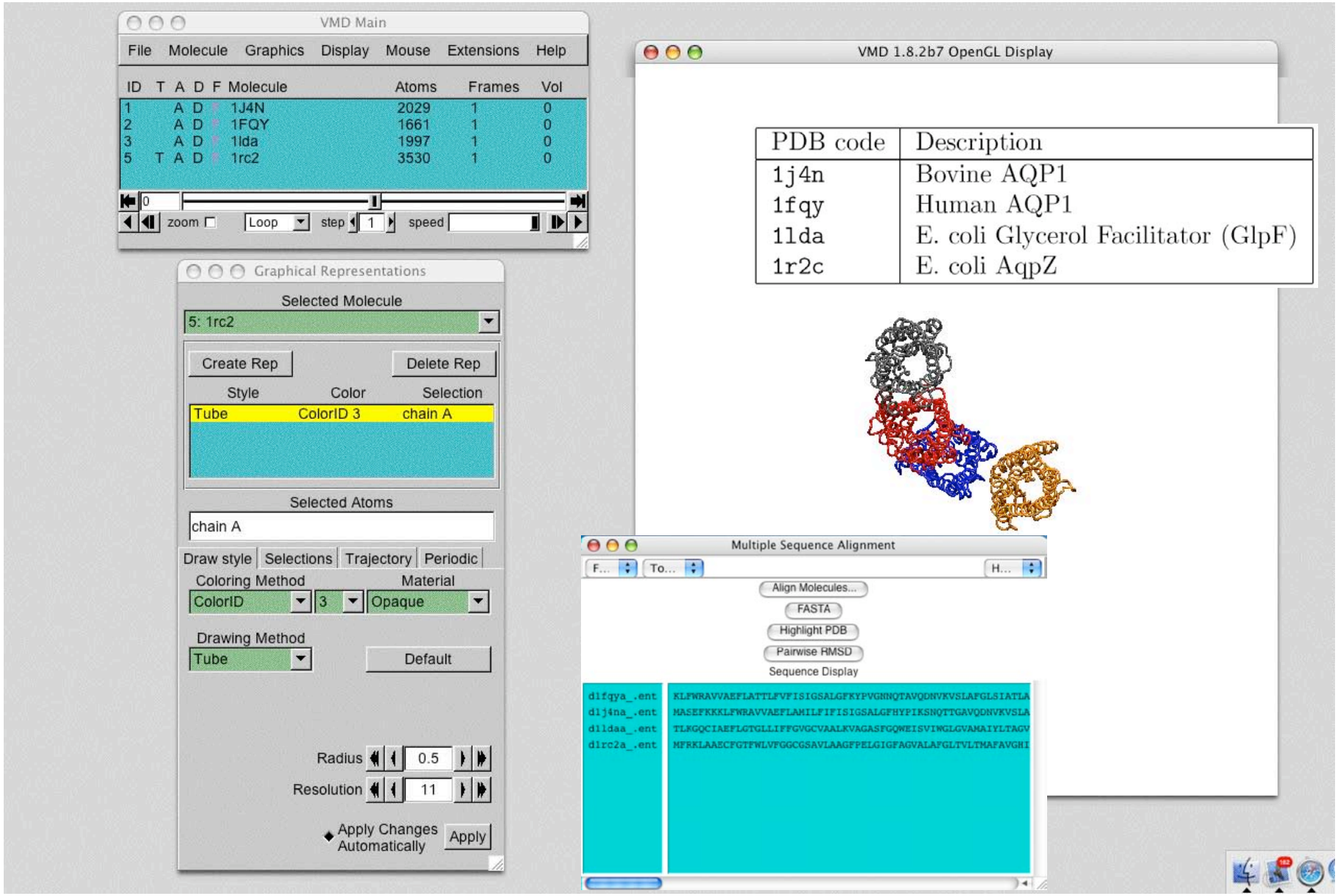
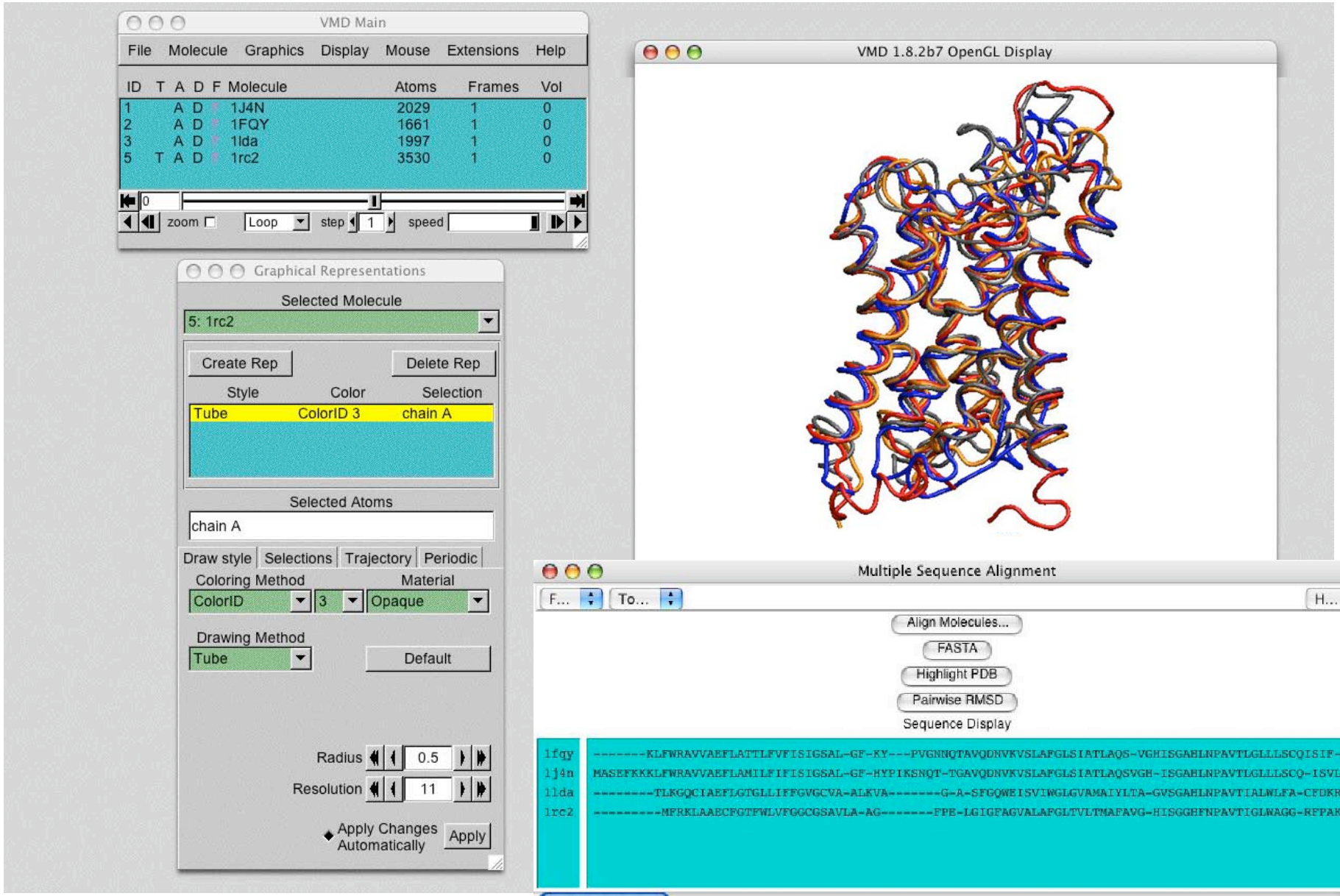
                 **:*  :  .  :.                        :   ..*::..          .:
AQP0_HUMAN  NPARSFAPAILTGNFT-------------NHWVYWVGPIIGGGLGSLLYDFLLFP-    225
AQP1_HUMAN  NPARSFGSAVITHNFS-------------NHWIFWVGPFIGGALAVLIYDFILAP-    233
AQP2_HUMAN  NPARSLAPAVVTGKFD-------------DHWVFWIGPLVGAILGSLLYNYVLFP-    225
AQP3_HUMAN  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
```

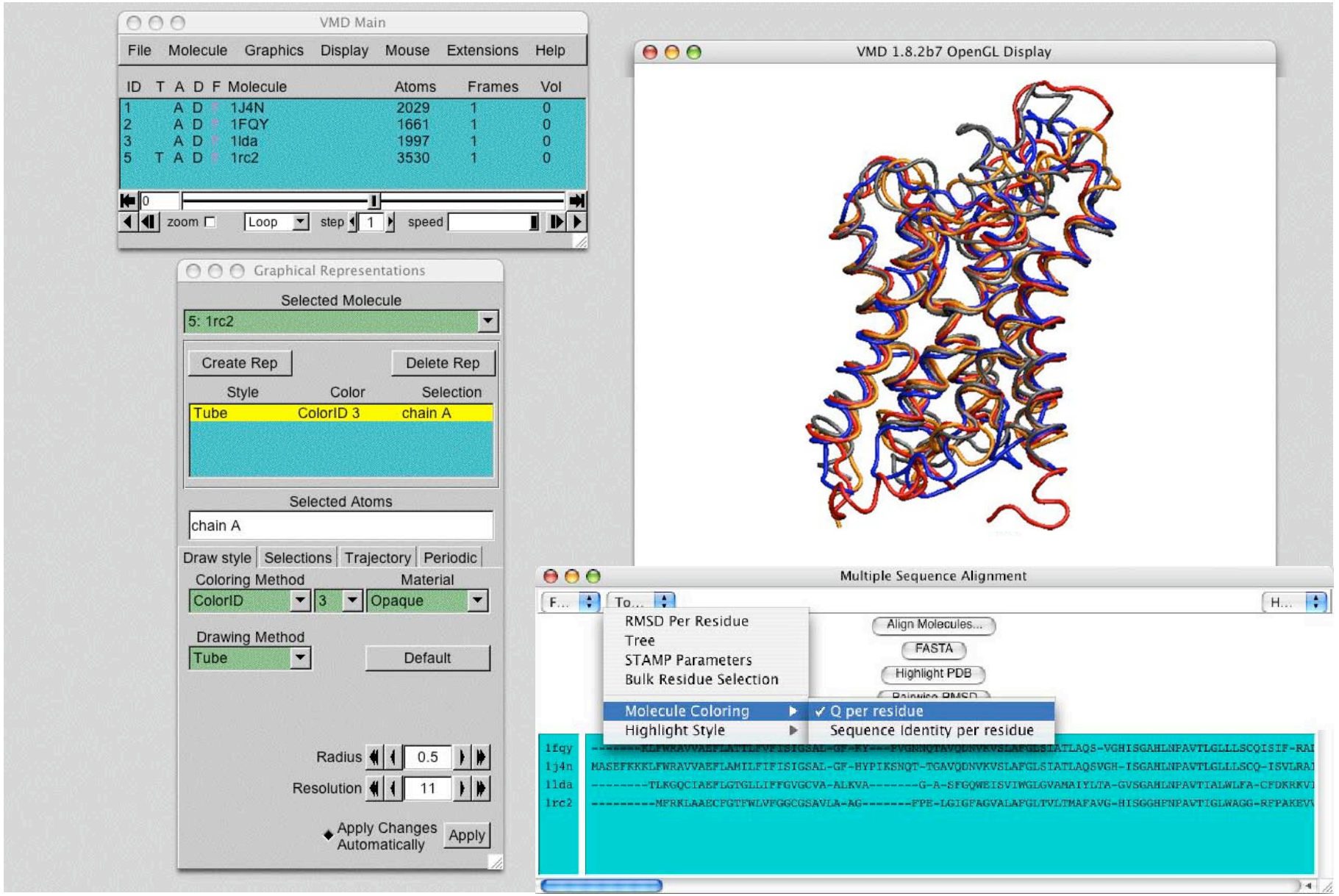
Load Aquaporins 1j4n, 1fqy, 1lda, 1rc2 into VMD



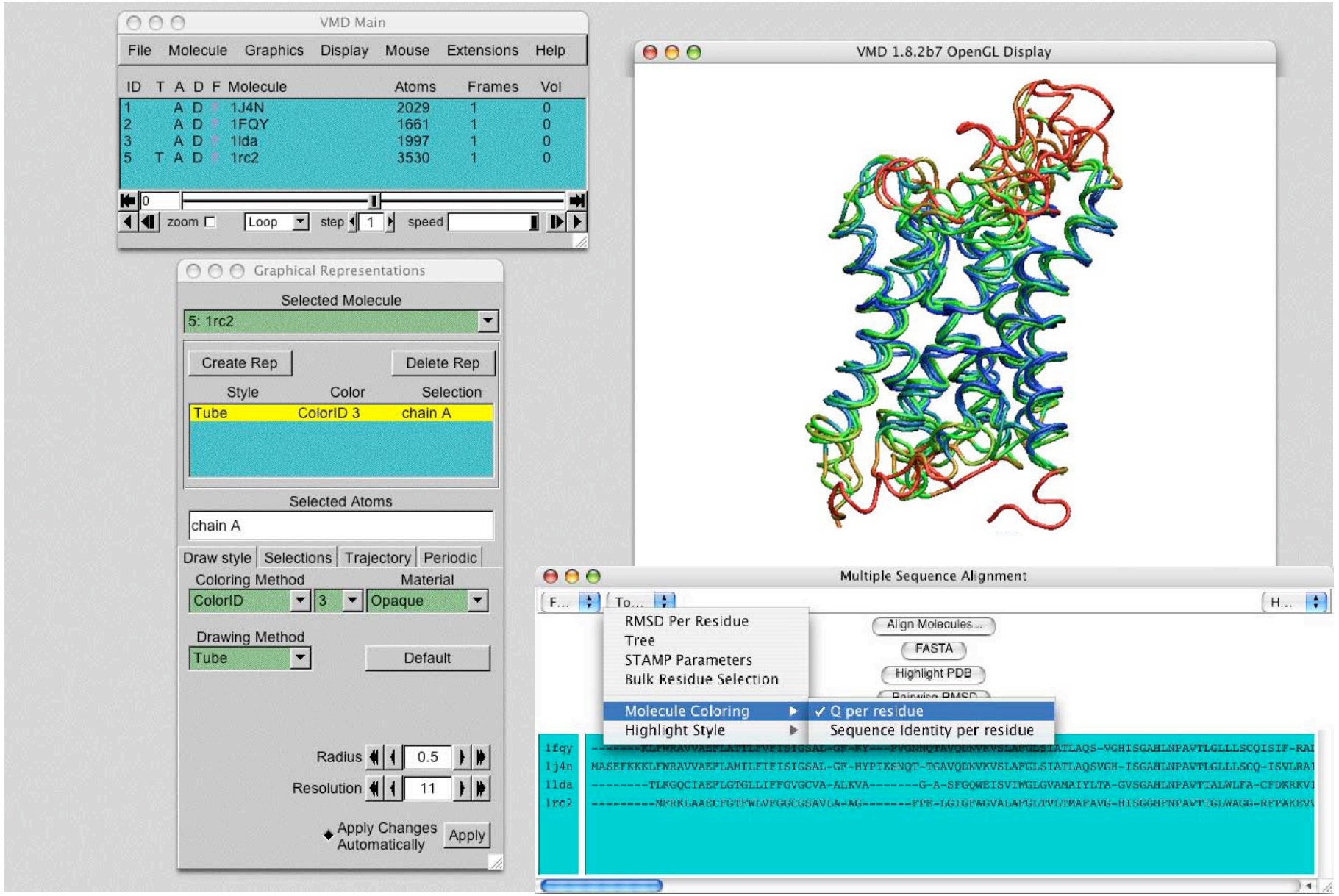
Aligning Structures and Sequences



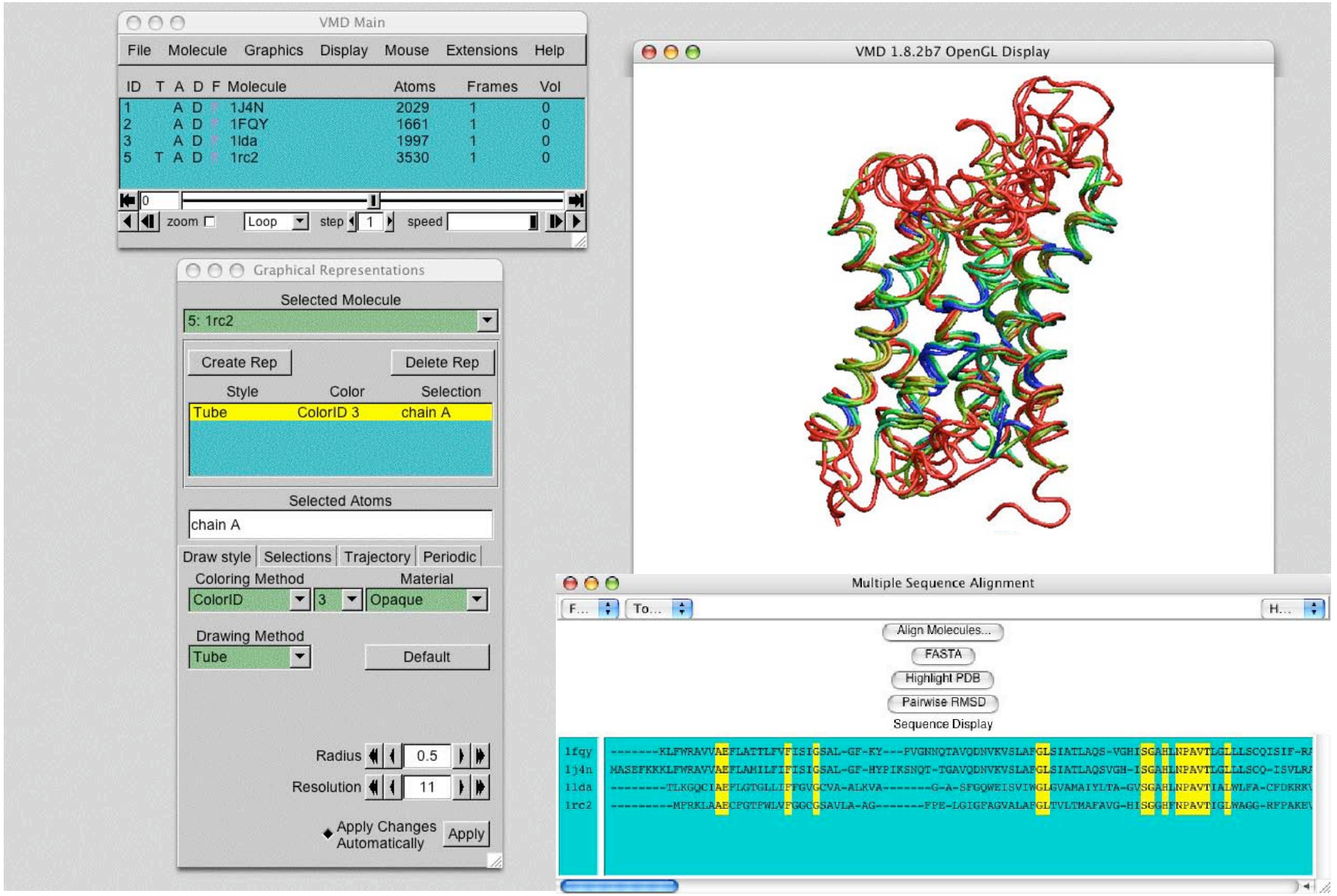
Comparing Structures by Similarity - Q Value



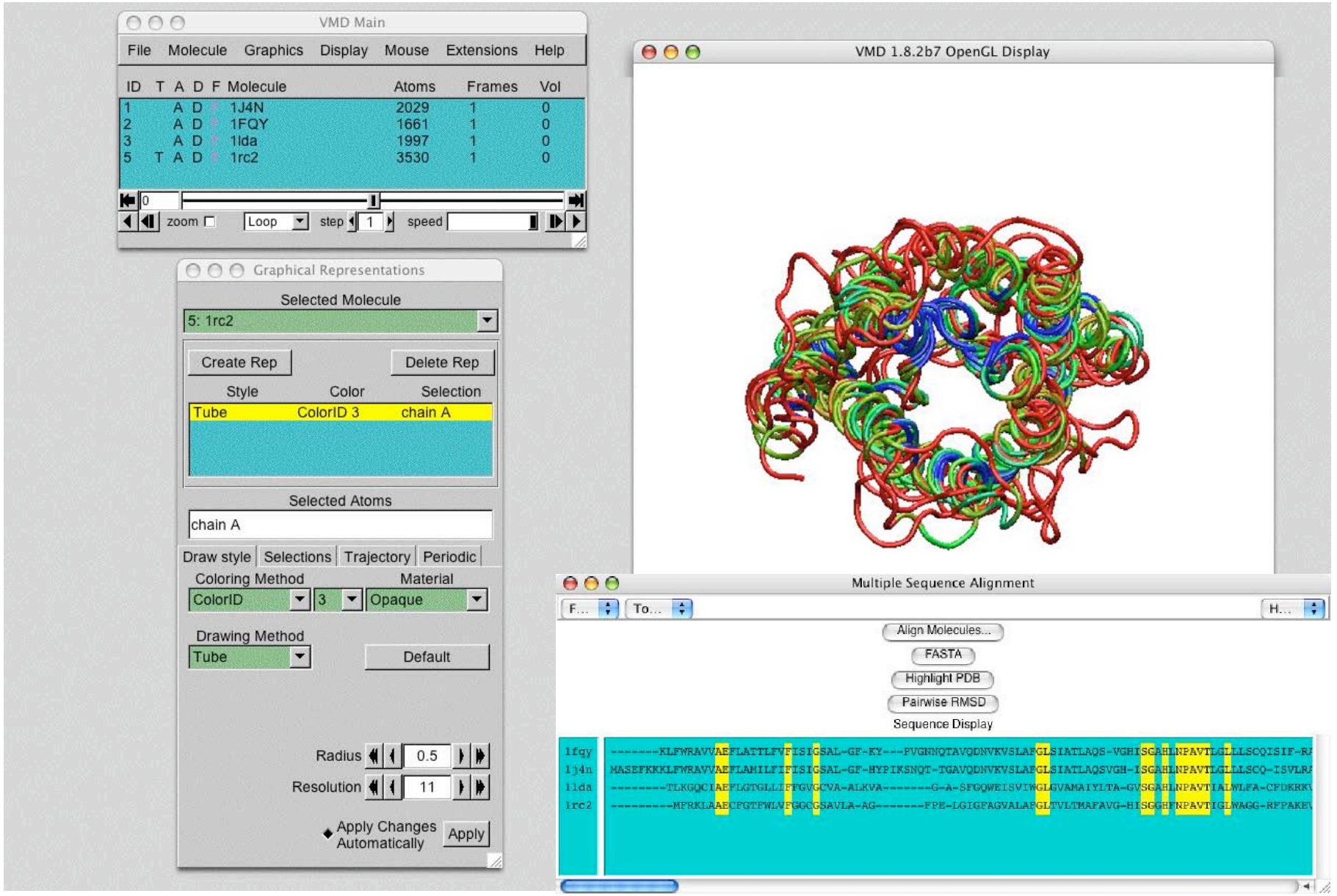
Comparing Structures by Similarity - Q Value



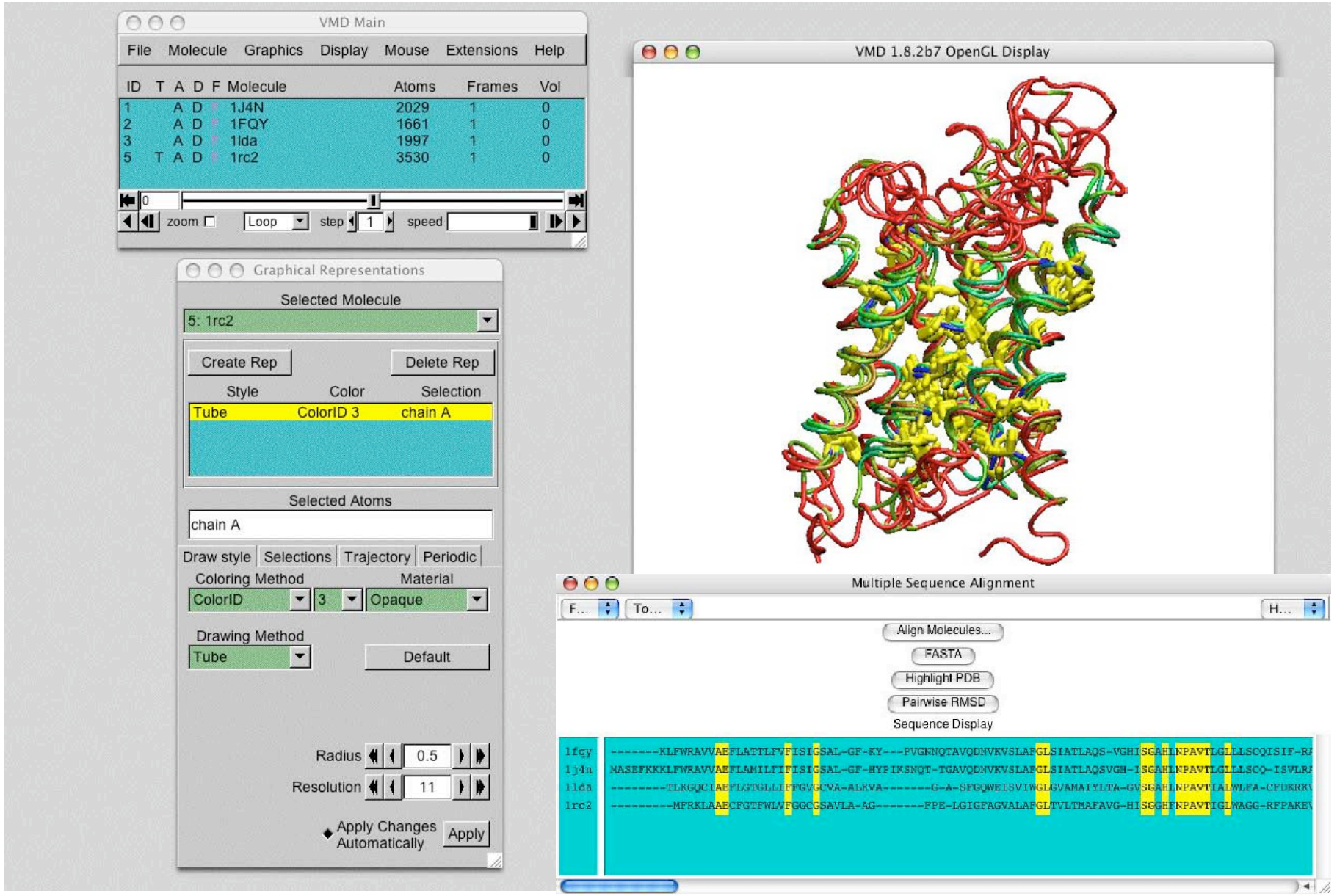
Exhibiting Sequence Identity - Side View



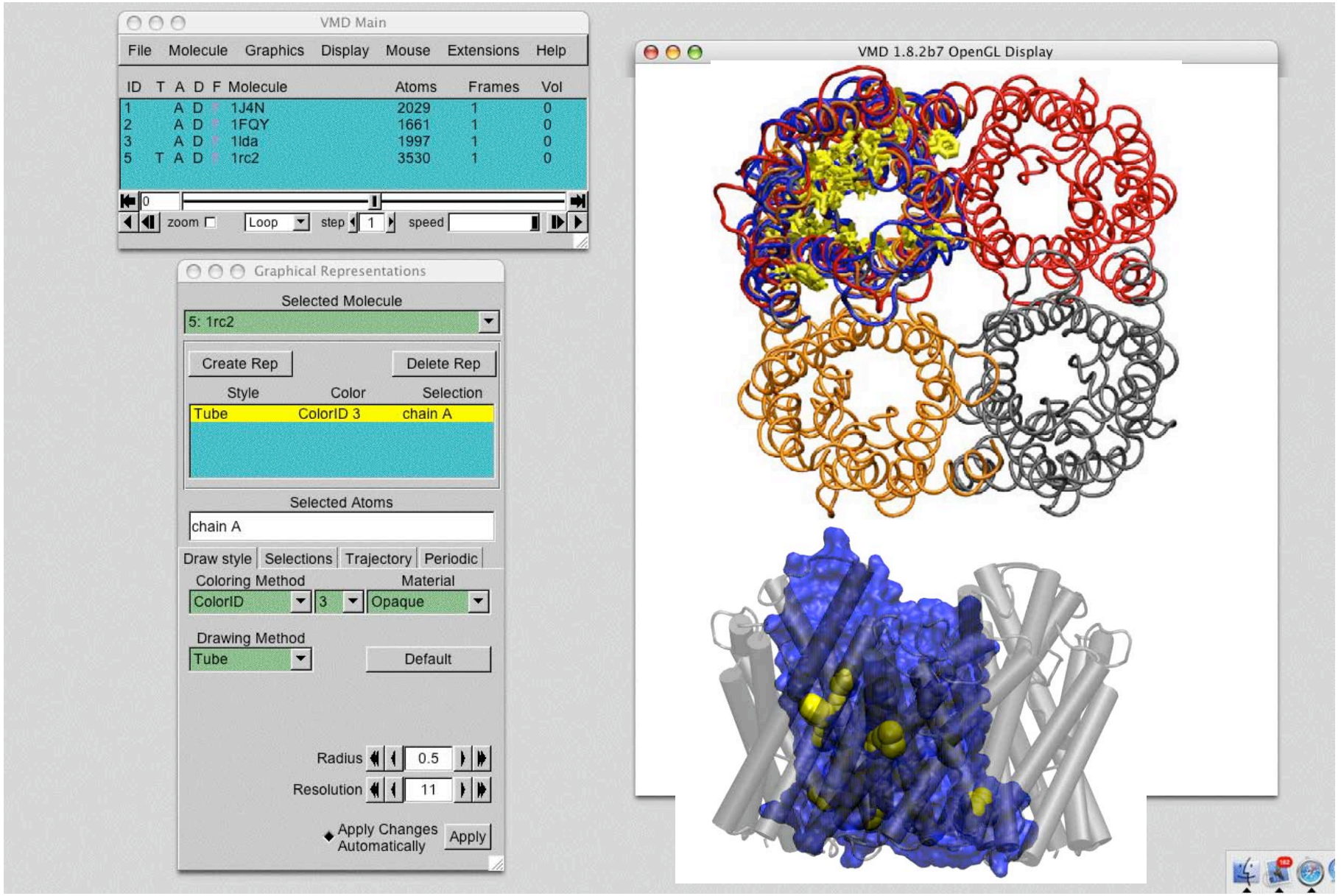
Exhibiting Sequence Identity - Top View



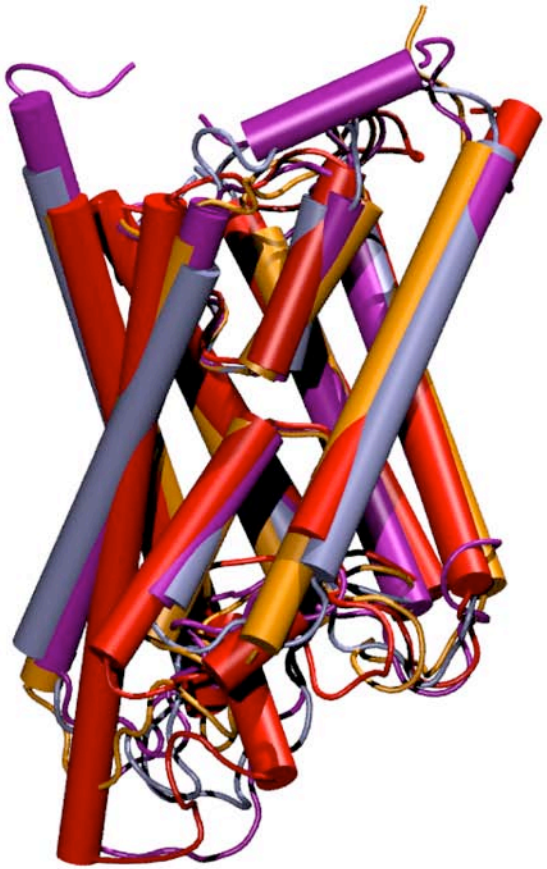
Showing Conserved Residues - Monomer



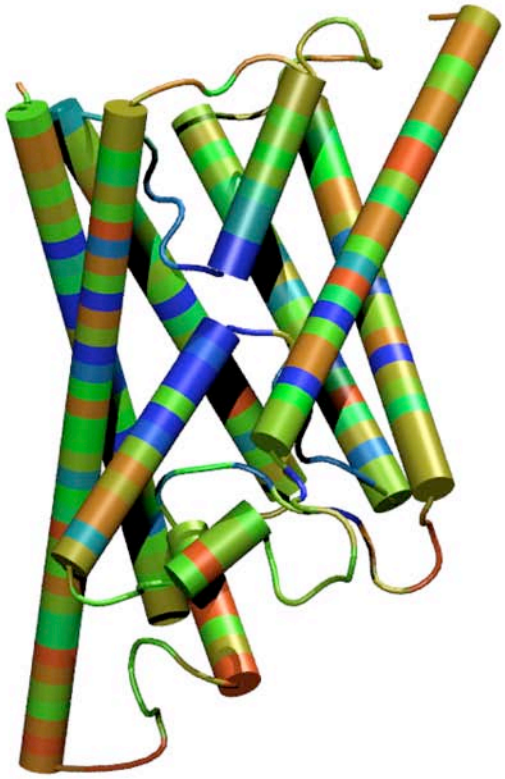
Showing Conserved Residues - Tetramer



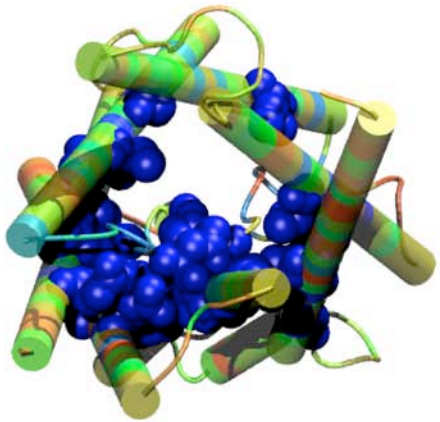
Structure and Sequence Comparisons
Water/Glycerol Channels



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