Introduction to evolutionary concepts and VMD/MultiSeq - Part I

Characterizing your systems

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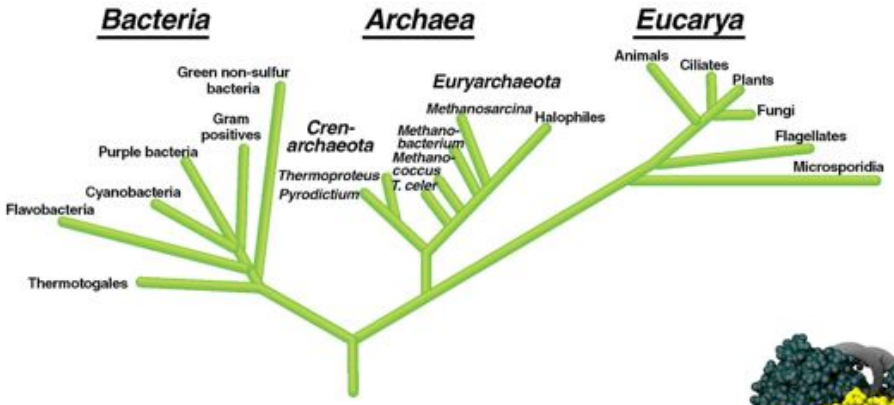
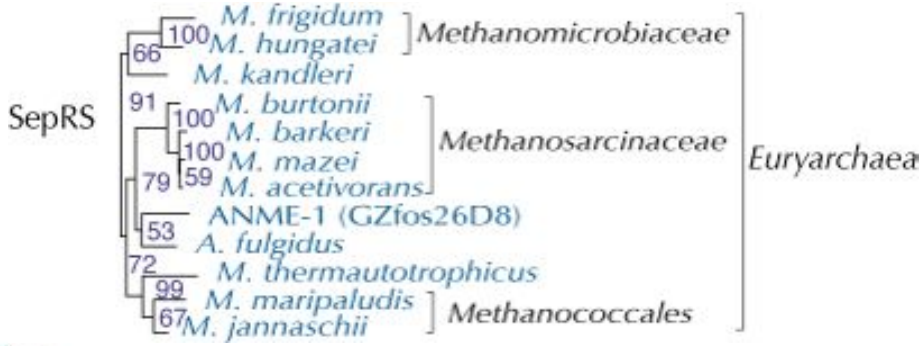
VMD/MultiSeq - "A Tool to Think"

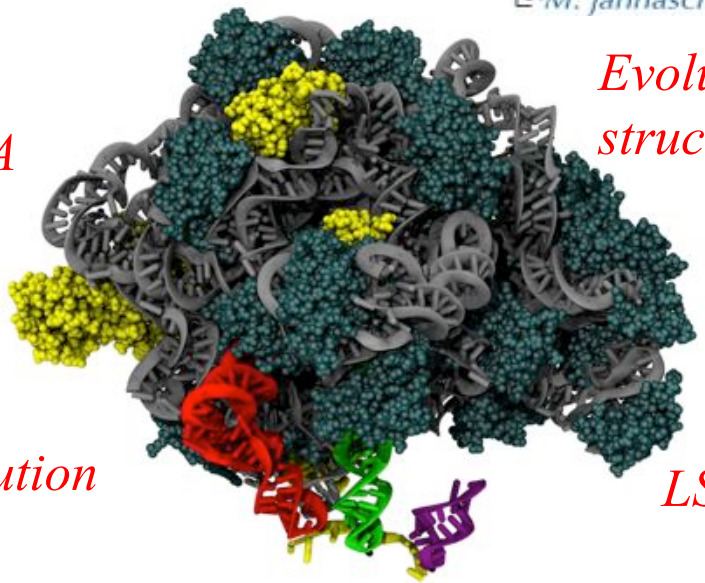
Carl Woese - *"VMD is far from a simple visualization tool for a biologist, it is a true thinking tool. Without it a whole class of biological hypotheses would simply not exist."*





UPT - Woese 16S rRNA

Evolutionary profiles for protein structure & function prediction

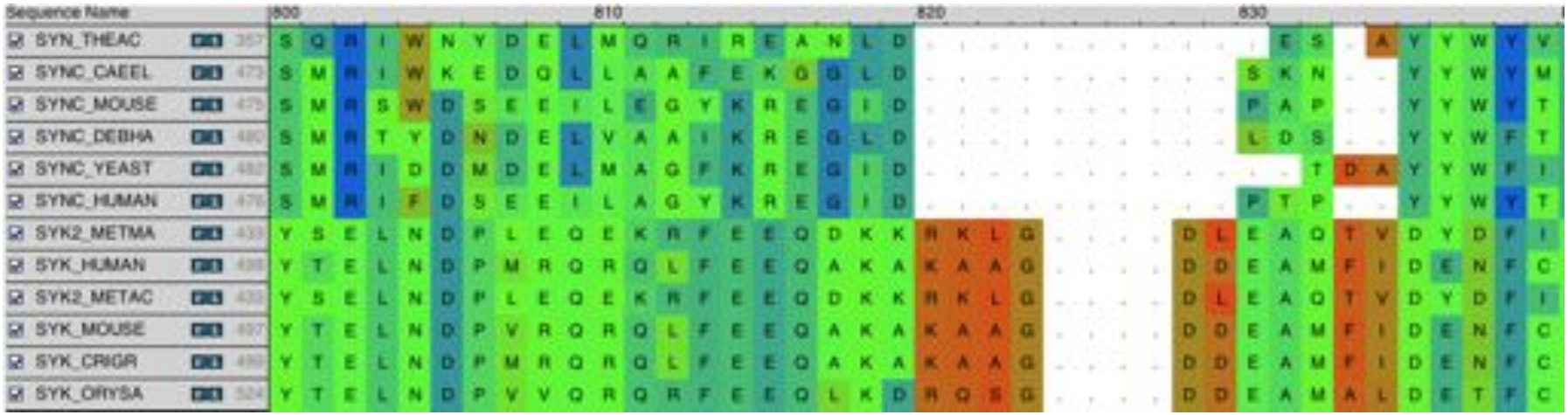


Signatures ribosomal evolution

LSU (23S rRNA + rproteins)

Why Look at More Than One Sequence?

1. Multiple Sequence Alignment shows patterns of conservation



2. Are these positions functionally important? Active sites, folding,..

3. What and how many sequences should be included?

4. Where do I find the sequences and structures for MS alignment?

5. How to generate pairwise and multiple sequence alignments?

New Tools in VMD/MultiSeq

Protein / RNA Sequence Data

SwissProt DB (400K), Greengenes RNA (100K)

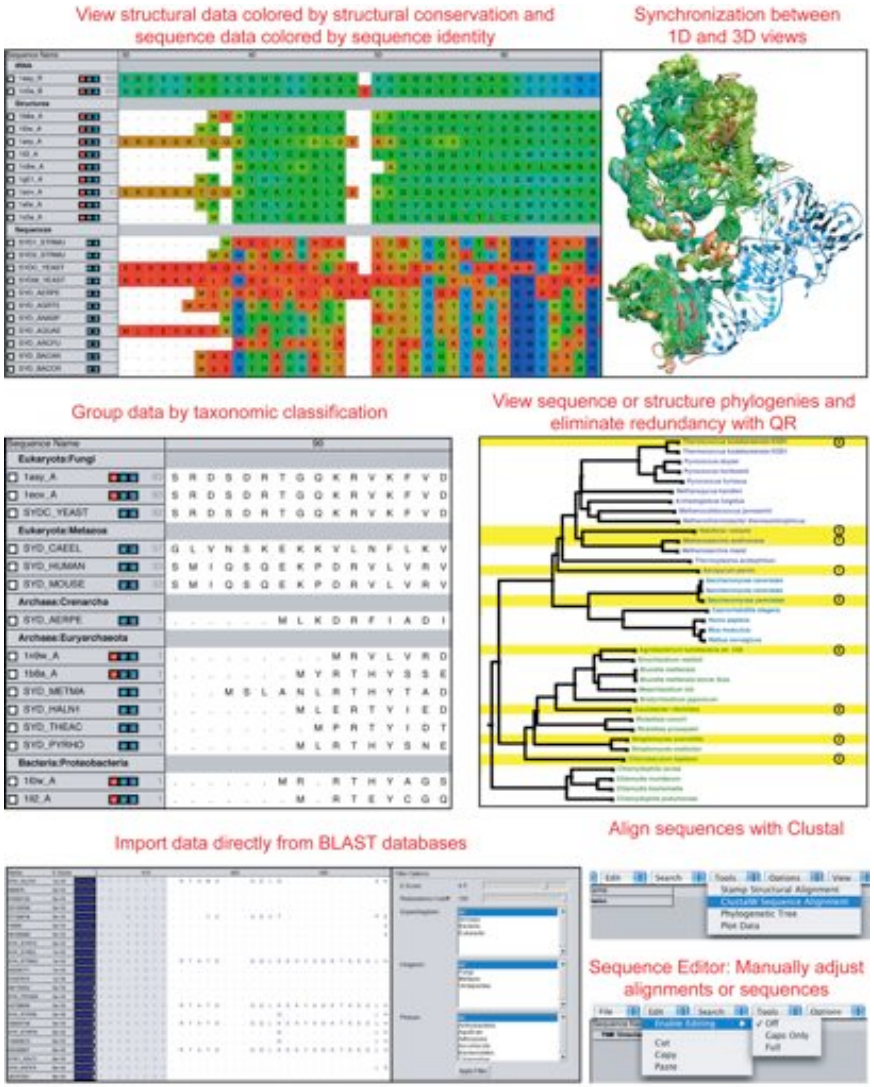
Signatures, Zoom

Metadata Information, Clustal, MAFFT & Phylogenetic Trees

RAXml Trees, Genomic Content, Temperature DB

Blast & PsiBlast

Sequence Editor



Sequence /Structure Alignment

Protein & RNA secondary structure

QR non-redundant seq / str sets

Cluster analysis / Bioinformatics scripting

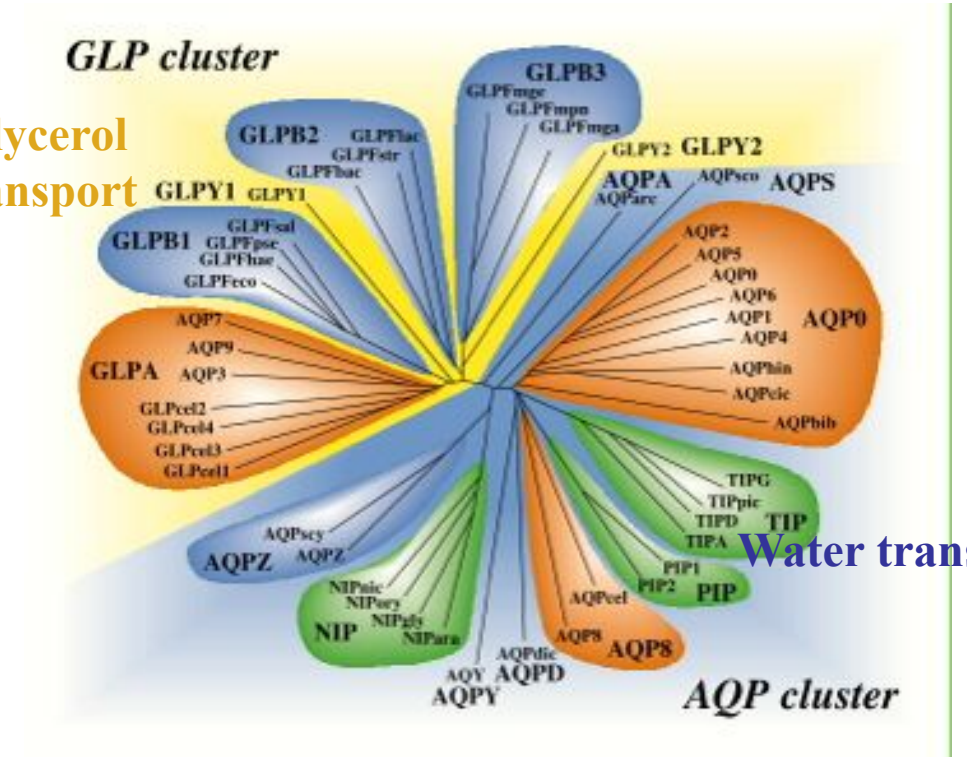
Tutorials MultiSeq/ AARS

EF-Tu/Ribosome

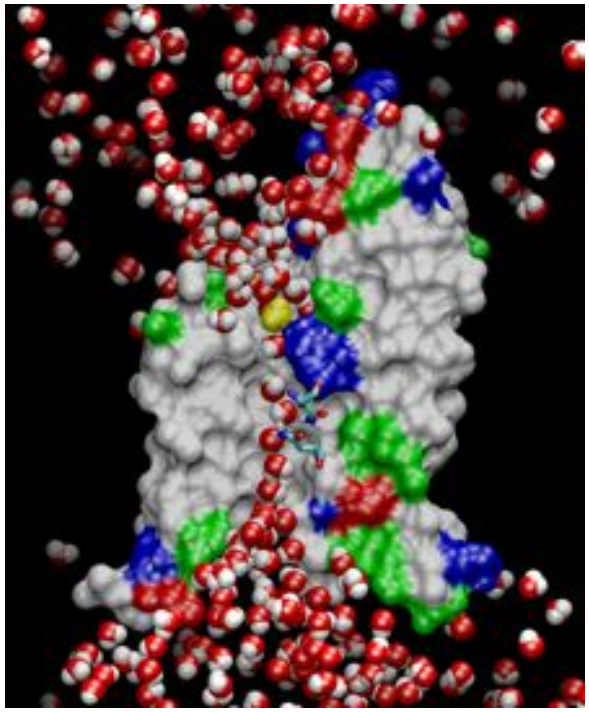
J. Eargle, D. Wright, Z. Luthey-Schulten, *Bioinformatics,* 22:504 (2006)

E. Roberts, J. Eargle, D. Wright, Z. Luthey-Schulten, *BMC Bioinformatics,* 7:382 (2006)

Aquaporin Superfamily: Bacterial & Eucaryal



Water transport



Heymann and Engel *News Physiol. Sci.* (1999)

Archaeal AqpM *M. Marburgensis*, *JBC* 2003, *PNAS* 2005

```
AQP0_HUMAN ---LNTLHPAVSVGQATTVEIFLTLQFVLCIFATYDE-RRNGQLGSVALAVGFSLALGHLFGMYYTGAGM  183
AQP1_HUMAN ---RNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDR-RRRDLGGSAPLAIGLSVALGHLLAIDYTGCGI  191
AQP2_HUMAN ---VNALSNSTTAGQAVTVELFLTLQLVLCIFASTDE-RRGENPGTPALSIGFSVALGHLLGIHYTGCSM  183
AQP3_HUMAN GIFATYPSGHLDMINGFFDQFIGTASLIVCVLAIVDPYNNPVPRGLEAFTVGLVVLVIGTSMGFNSGYAV  214
AQP4_HUMAN ---VTMVHGNLTAGHGLLVELIITFQLVFTIFASCDS-KRTDVTGSIALAIGFSVAIGHLFAINYTGASM  212
AQP5_HUMAN ---VNALNNNTTQGQAMVVELILTFQLALCIFASTDS-RRTSPVGSPALSIGLSVTLGHLVGIYFTGCSM  184
AQP6_HUMAN ---INVVRNSVSTGQAVAVELLLTLQLVLCVFASTDS-RQTS--GSPATMIGISWALGHLIGILFTGCSM  195
AQP7_HUMAN GIFATYLPDHMTLWRGFLNEAWLTGMLQLCLFAITDQENNPALPGTEALVIGILVVIIGVSLGMNTGYAI  225
AQP8_HUMAN -AAFVTVQEQGQVAGALVAEIILTTLLALAVCMGAIN--EKTKGPLAPFSIGFAVTVDILAGGPVSGGCM  209
AQP9_HUMAN HIFATYPAPYLSLANAFADQVVATMILLIIVFAIFDSRNLGAPRGLEPIAIGLLIIVIASSLGLNSGCAM  215
GLPF_ECOLI GTFSTYPNPHINFVQAFAVEMVITAILMGLILALTDDGNGVPRGPLAPLLIGLLIAVIGASMGPLTGFAM  202
     ruler ...180.......190.......200.......210.......220.......230.......240....
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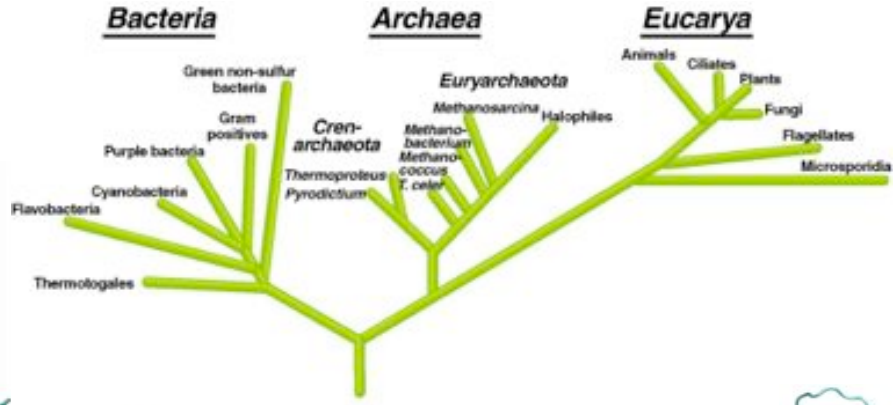
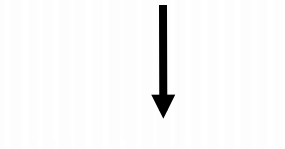
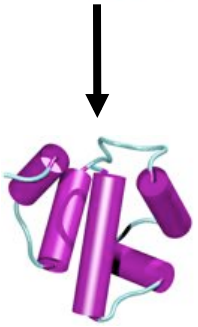
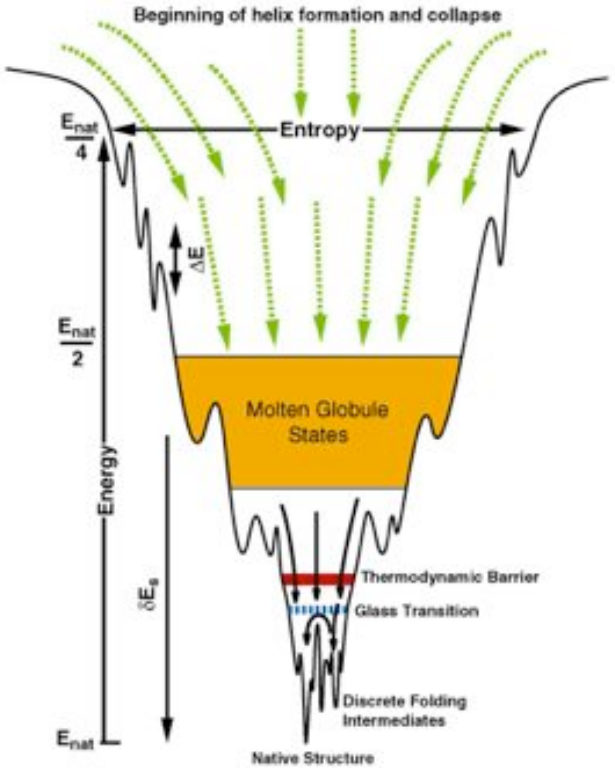
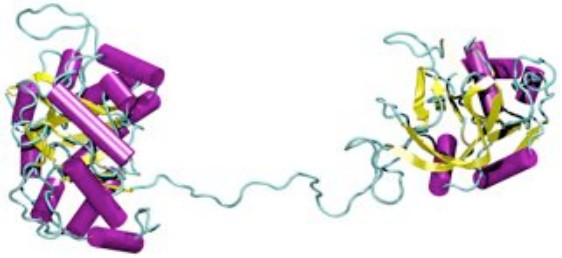
Protein (RNA) Folding, Structure, & Function





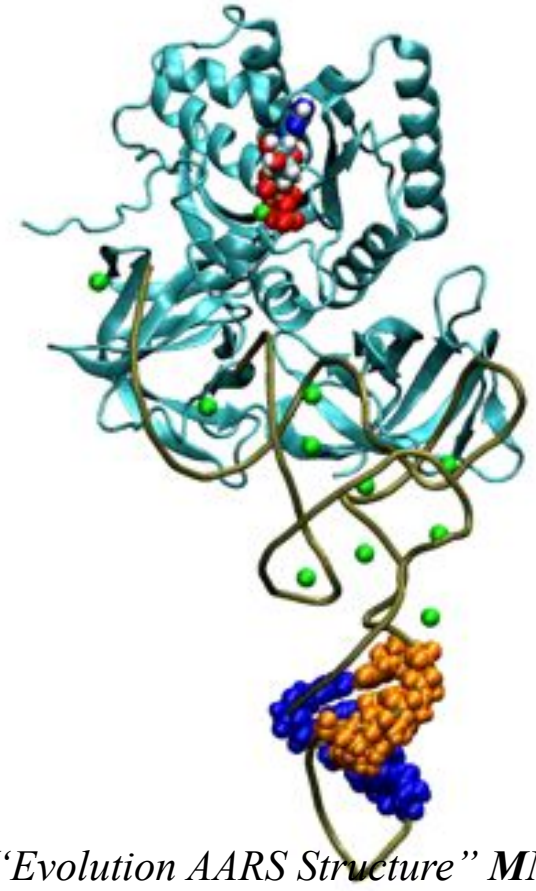


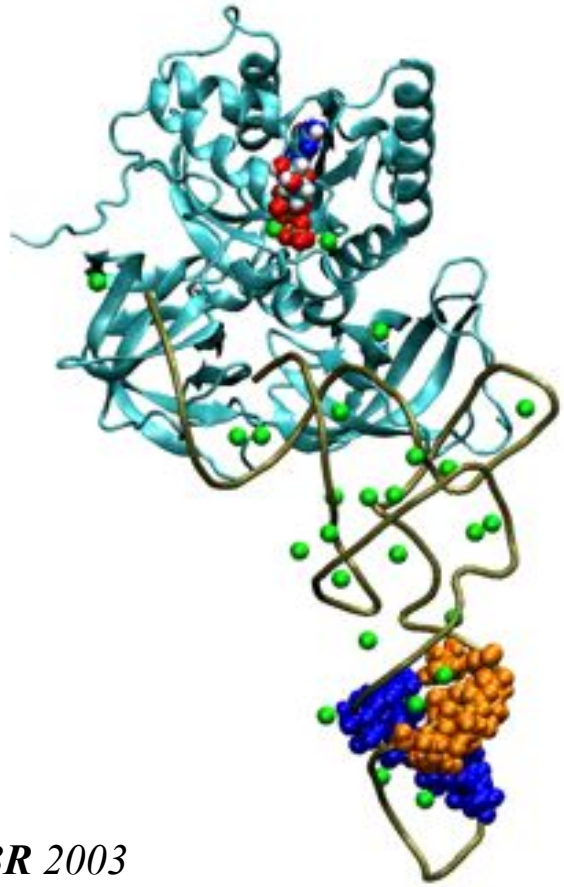


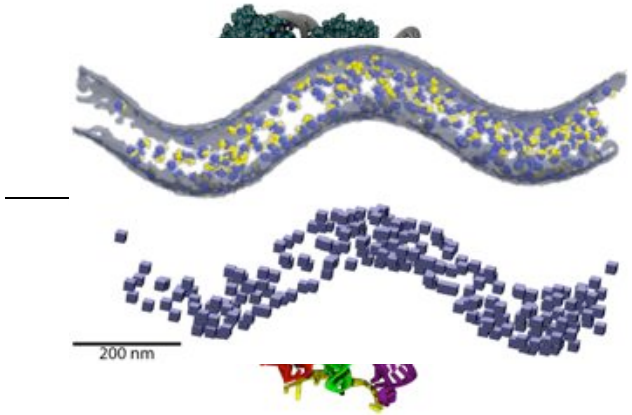


Protein:RNA Complexes in Translation

Evolution, Dynamics, Analysis







Proteins/RNA
Polyribosomes
Ribosome

"Signatures ribosomal evolution"
***PNAS** 2008, **BMC** 2009, **BJ** 2010*
*"Motion L1 Stalk:tRNA" **JMB 2010***
"Whole cell simulations on GPUs"
***IEEE** 2009,**Plos CB** 2011,**PRL**2011*

*"Evolution AARS Structure" **MMBR** 2003*
*"Evol. Profiles Class I&II AARS" **JMB** 2005*
*"Evolution SepRS/CysRS" **PNAS** 2005*
*"Dynamic Signaling Network" **PNAS** 2009*
*"Exit Strategy Charged tRNA" **JMB** 2010*
*"Mistranslation in Mycoplasma" **PNAS** 2011*

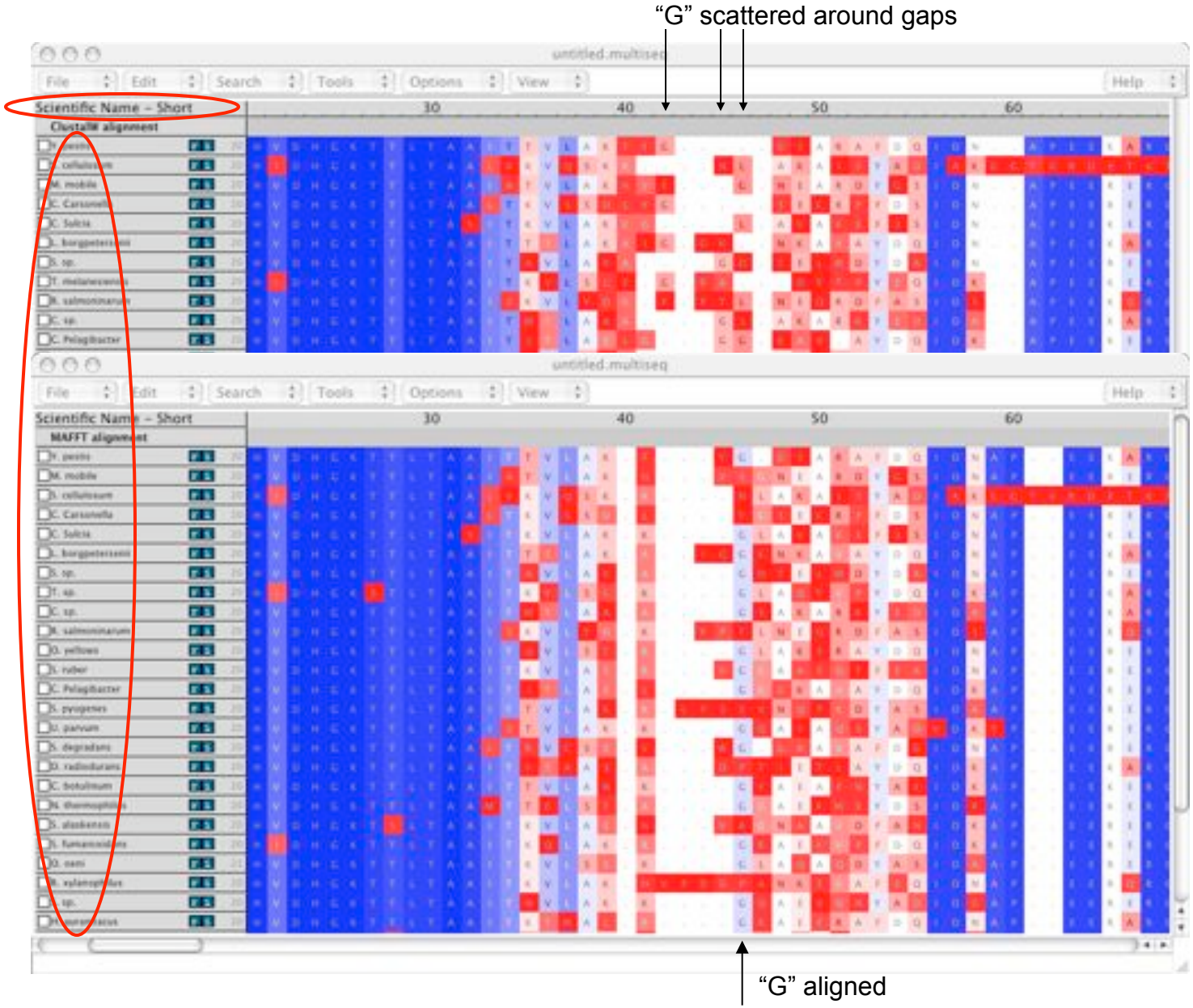
*"Dynamical Recognition Novel Amino Acids" **JMB** 2008*
*"tRNA Dynamics" **FEBS** 2010*

Basic principles of evolutionary analysis for proteins & RNAs

- Comparative analysis of sequences and structures
- Multiple sequence alignments (gaps and editing)
- Sequence and structure phylogenetic trees*
- Reference to 16S rRNA tree
- Horizontal or lateral gene transfer events
- Genomic context
- Evolutionary profiles representing diversity
- Conservation analysis of evolutionary profiles

*Various models of evolutionary change

Alignment of ~200 EF-Tu sequences in VMD/MultiSeq



ClustalW alignment

~ 5 minutes

MAFFT* alignment

~ 30 seconds

More sequences!

* "Mafft" Katoh, Misawa, Kuma,Miyata, NAR 2002, 2005

STAMP - Multiple Structural Alignments

1. Initial Alignment Inputs

- Multiple Sequence alignment
- Ridged Body "Scan"
- Pairwise Alignments and Hierarchical Clustering

2. Refine Initial Alignment & Produce Multiple Structural Alignment

$$P_{ij} = \left\{ e^{-d_{ij}^2/2E_1} \right\} \left\{ e^{-s_{ij}^2/2E_2} \right\}$$

P_{ij} — probability that residue i on structure A is equivalent to residue j on structure B.

d_{ij} — distance between i & j

S_{ij} — conformational similarity; function of rms between i-1, i, i+1 and j-1, j, j+1.

- Dynamic Programming (Smith-Waterman) through P matrix gives optimal set of equivalent residues.
- This set is used to re-superpose the two chains. Then iterate until alignment score is unchanged.
- This procedure is performed for all pairs with no gap penalty

R. Russell, G. Barton (1992) *Proteins* **14:** 309.

Multiple Structural Alignments

STAMP – cont'd

2. Refine Initial Alignment & Produce Multiple Structural Alignment

Alignment score:

$$S_c = \frac{S_p}{L_p} \frac{L_p - i_A}{L_A} \frac{L_p - i_B}{L_B}$$

$$S_p = \sum_{aln.path} P_{ij}$$

L_p, L_A, L_B — length of alignment, sequence A, sequence B

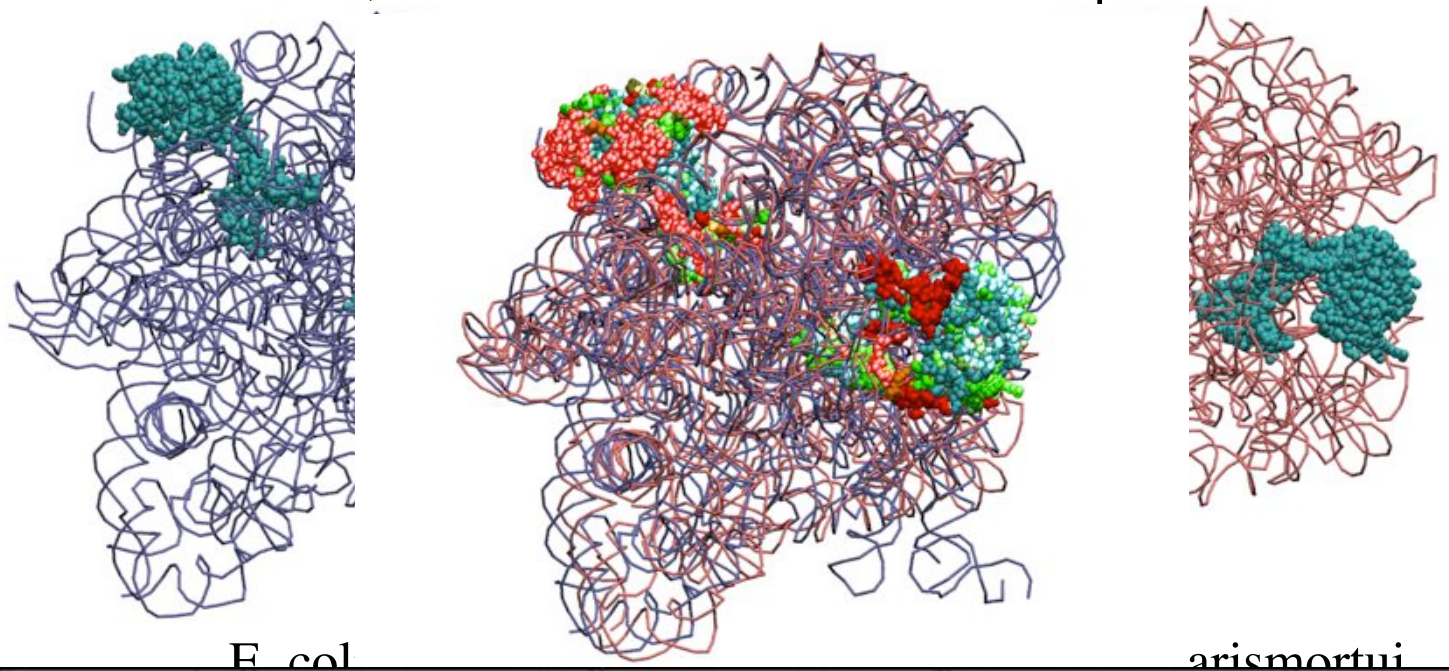
i_A, i_B — length of gaps in A and B.

Multiple Alignment:

- Create a dendrogram using the alignment score.
- Successively align groups of proteins (from branch tips to root).
- When 2 or more sequences are in a group, then average coordinates are used.

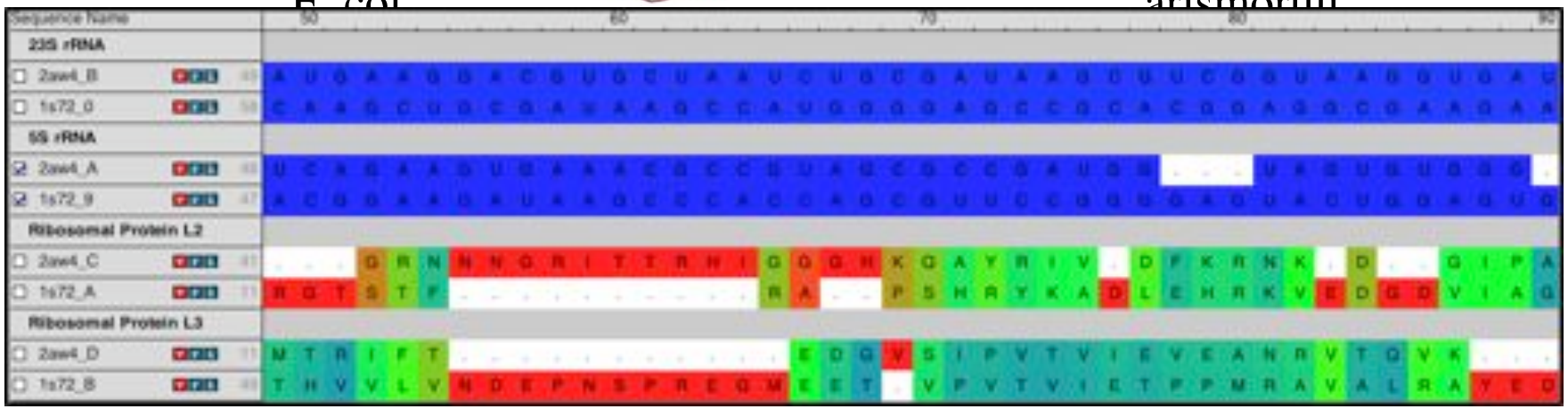
Structural Overlaps - STAMP

Ribosome large subunit showing ribosomal proteins L2 and L3
180,000 atoms in 4 rRNAs and 58 proteins



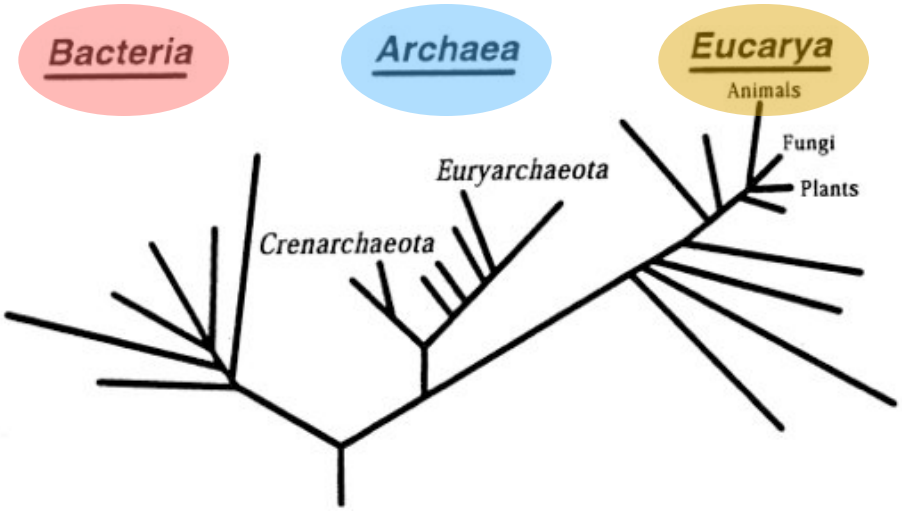
E. coli

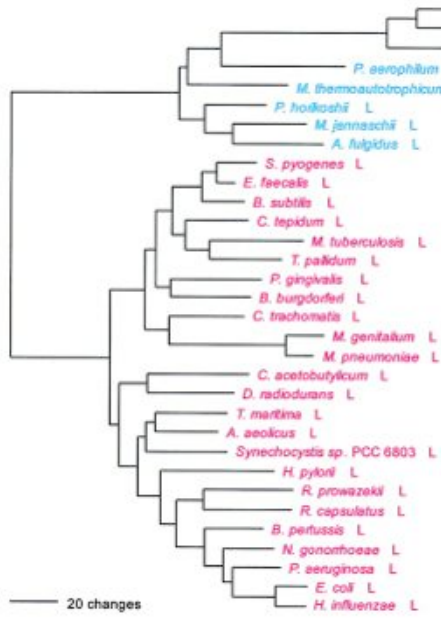
arismortui



Universal Phylogenetic Tree

3 domains of life





Eucarya

Archaea

Bacteria

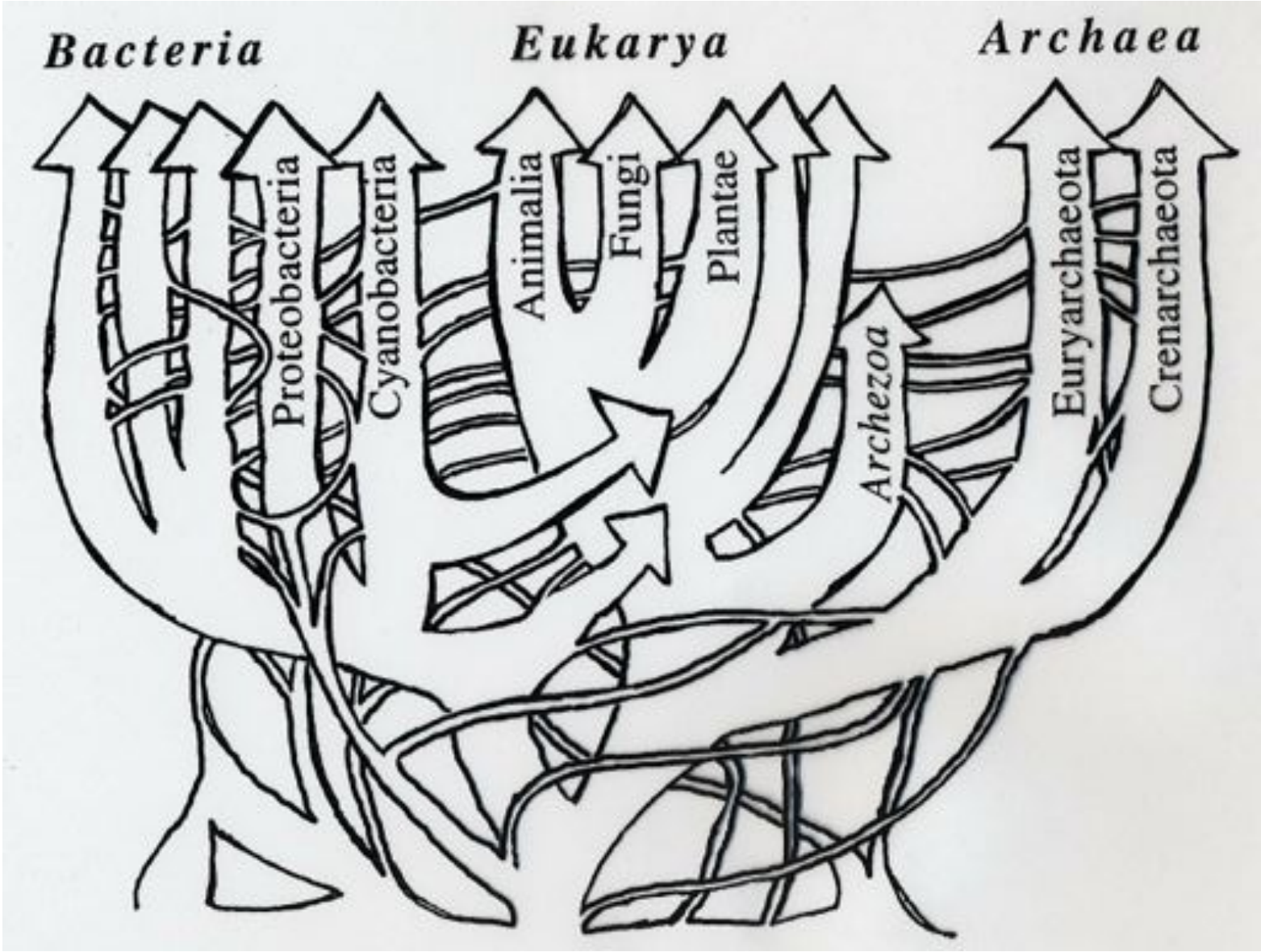
Reference 16S rRNA tree

Leucyl-tRNA synthetase displays the full canonical phylogenetic distribution.

For review see Woese *PNAS* 2000

Woese, Olsen, Ibba, Soll *MMBR* 2000

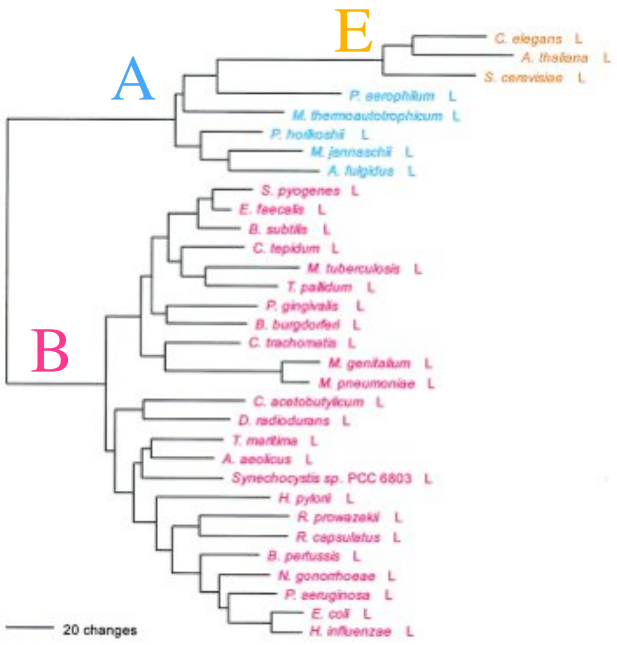
Look for horizontal gene transfer events



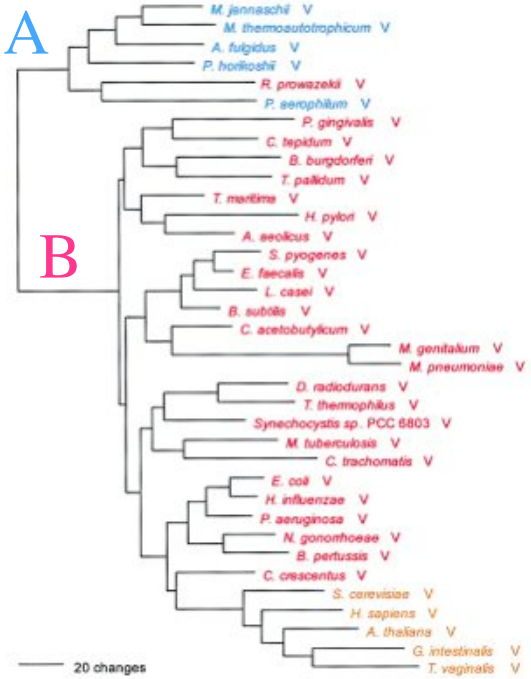
After W. Doolittle, modified by G. Olsen

Phylogenetic Distributions

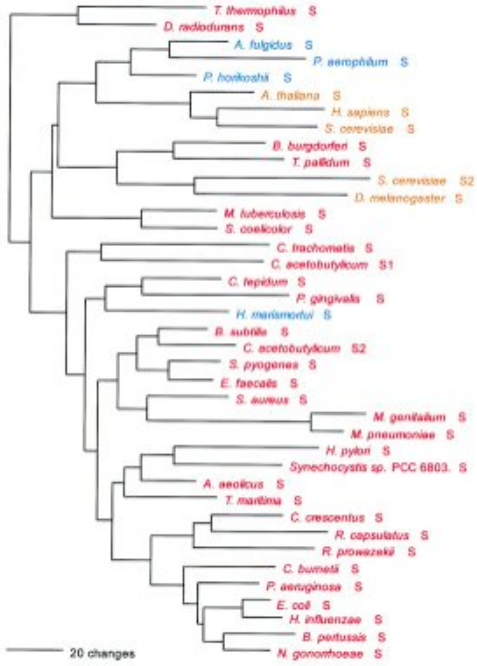
Full Canonical



Basal Canonical



Non-canonical



increasing inter-domain of life Horizontal Gene Transfer

"HGT erodes the historical trace, but does not completely erase it…." G. Olsen

Woese, Olsen, Ibba, Soll *MMBR* 2000

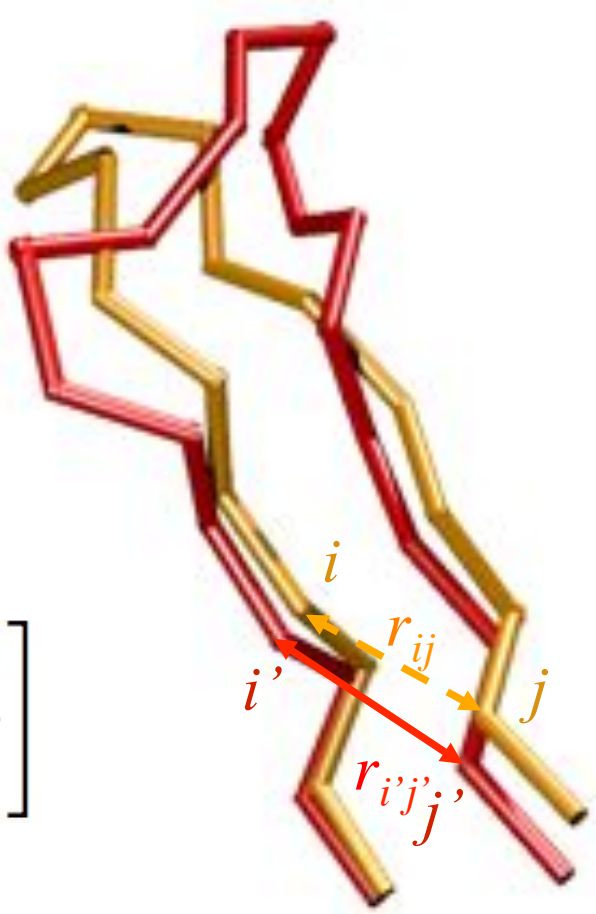
Protein Structure Similarity Measure

Q_H Structural Homology

fraction of native contacts for aligned residues + presence and perturbation of gaps

$$Q_H = \aleph\left[q_{aln} + q_{gap}\right]$$

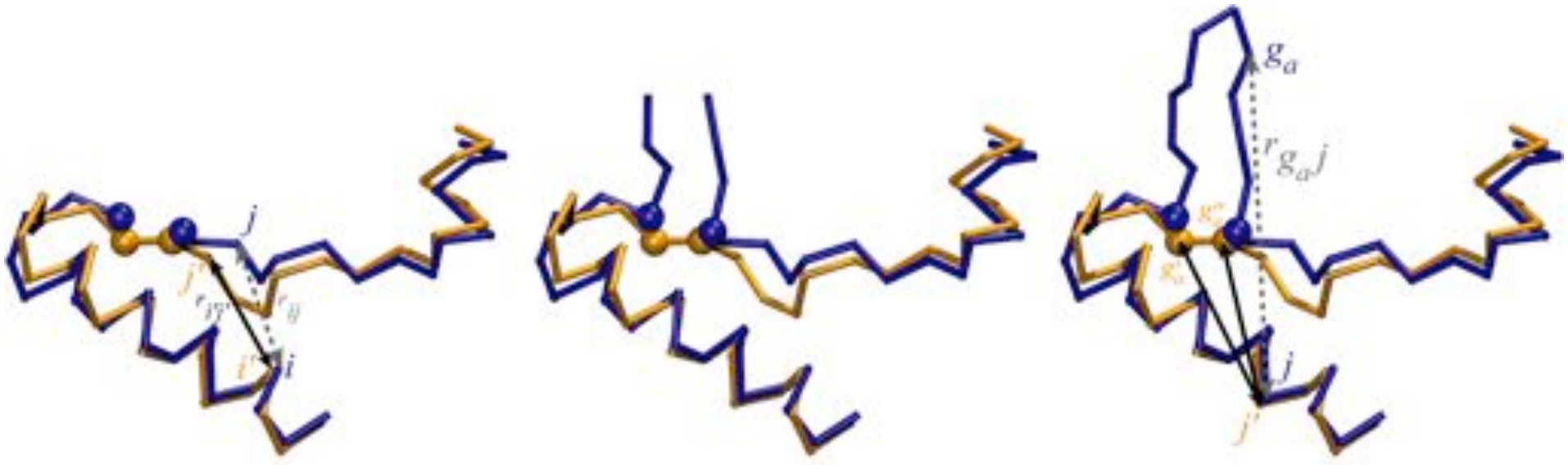
$$q_{aln} = \sum_{i<j-2} \exp\left[-\frac{(r_{ij} - r_{i'j'})^2}{2\sigma_{ij}^2}\right]$$

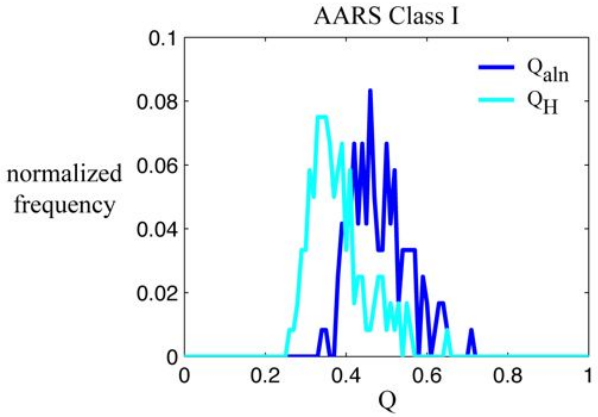


O'Donoghue & Luthey-Schulten *MMBR* 2003.

Structural Similarity Measure: The effect of insertions

"Gaps should count as a character but not dominate" C. Woese



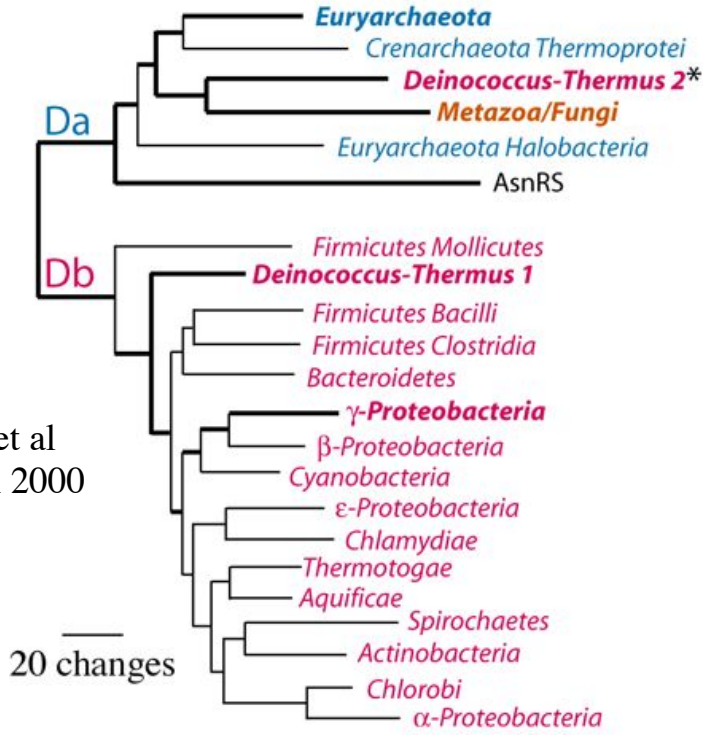


$$q_{gap} = \sum_{g_a} \sum_{j}^{N_{aln}} \max \left\{ \exp\left[-\frac{\left(r_{g_a j} - r_{g'_a j'}\right)^2}{2\sigma^2_{g_a j}} \right], \exp\left[-\frac{\left(r_{g_a j} - r_{g''_a j'}\right)^2}{2\sigma^2_{g_a j}} \right] \right\}$$

$$+ \sum_{g_b} \sum_{j}^{N_{aln}} \max \left\{ \exp\left[-\frac{\left(r_{g_b j} - r_{g'_b j'}\right)^2}{2\sigma^2_{g_b j}} \right], \exp\left[-\frac{\left(r_{g_b j} - r_{g''_b j'}\right)^2}{2\sigma^2_{g_b j}} \right] \right\}$$

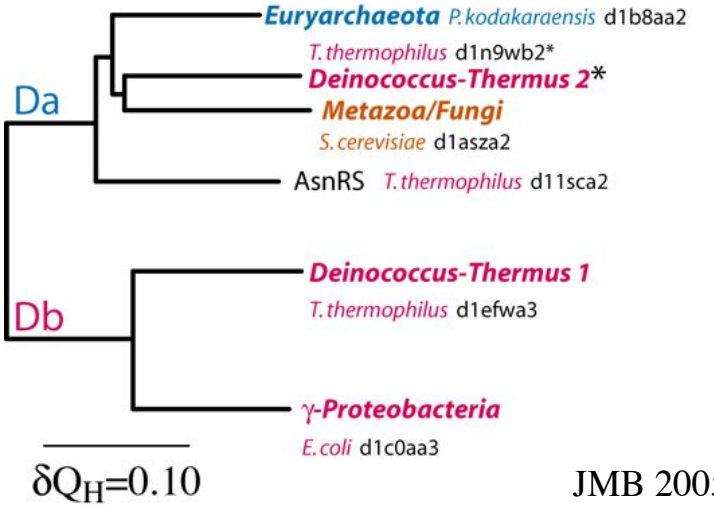
Structure encodes evolutionary information!

sequence-based phylogeny

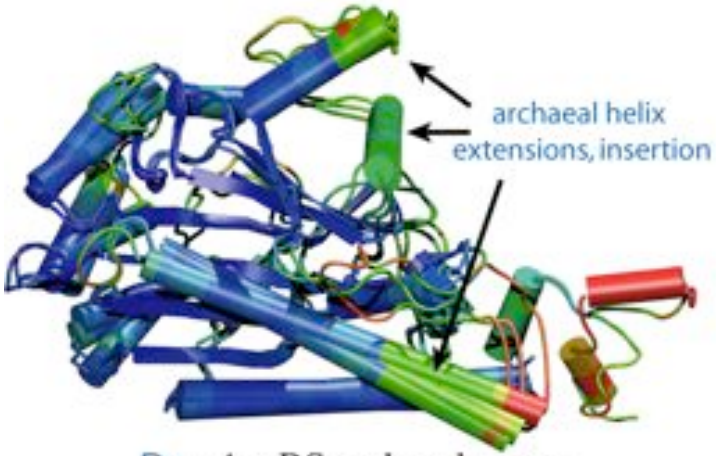


Woese et al
MMBR 2000

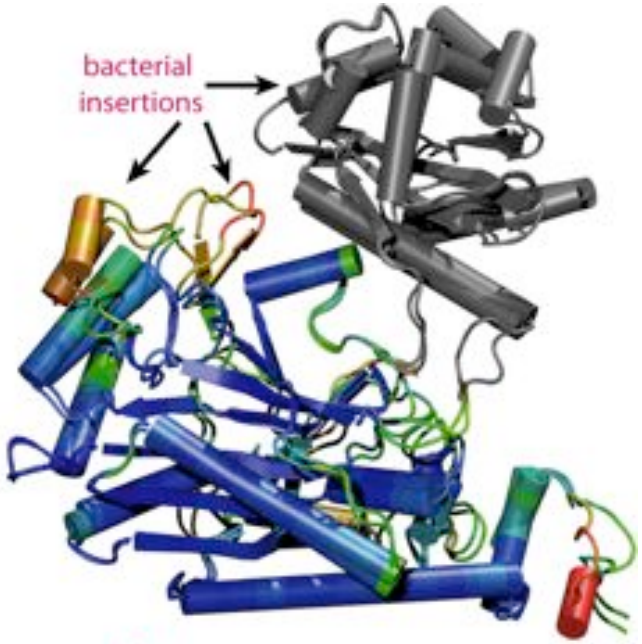
structure-based phylogeny



JMB 2005
MMBR 2003



Da - AspRS archaeal genre



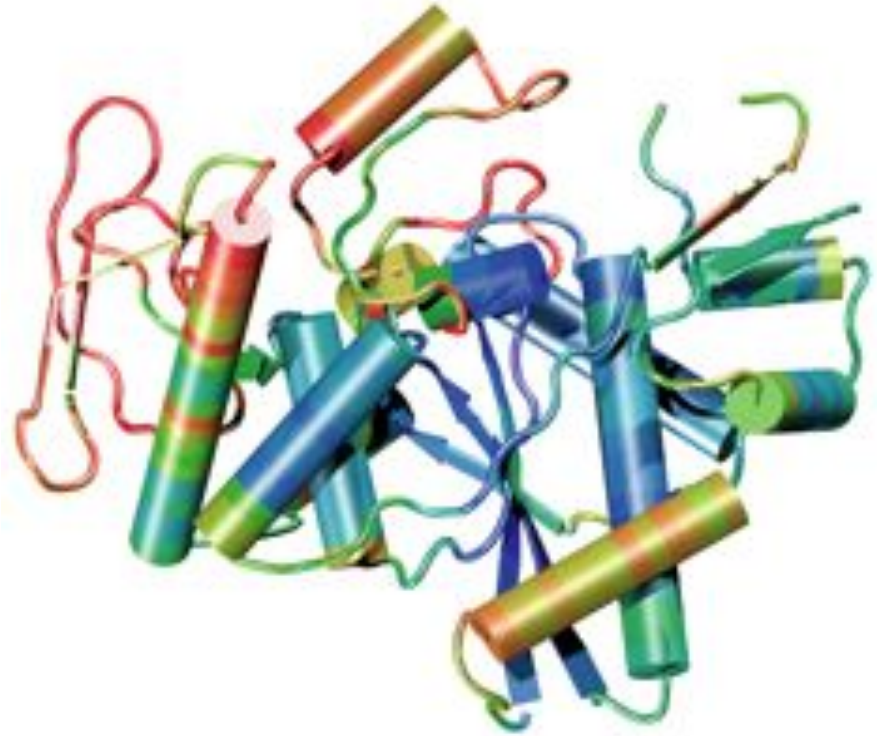
Db - AspRS bacterial genre

Structure reveals distant evolutionary events

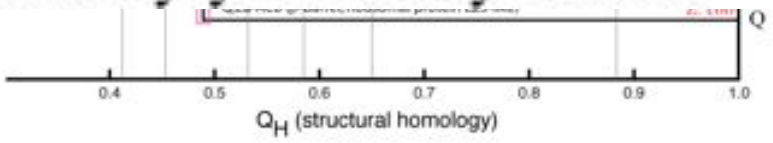
Class I AARSs

structure-based phylogenetics

sequence-structure overlap



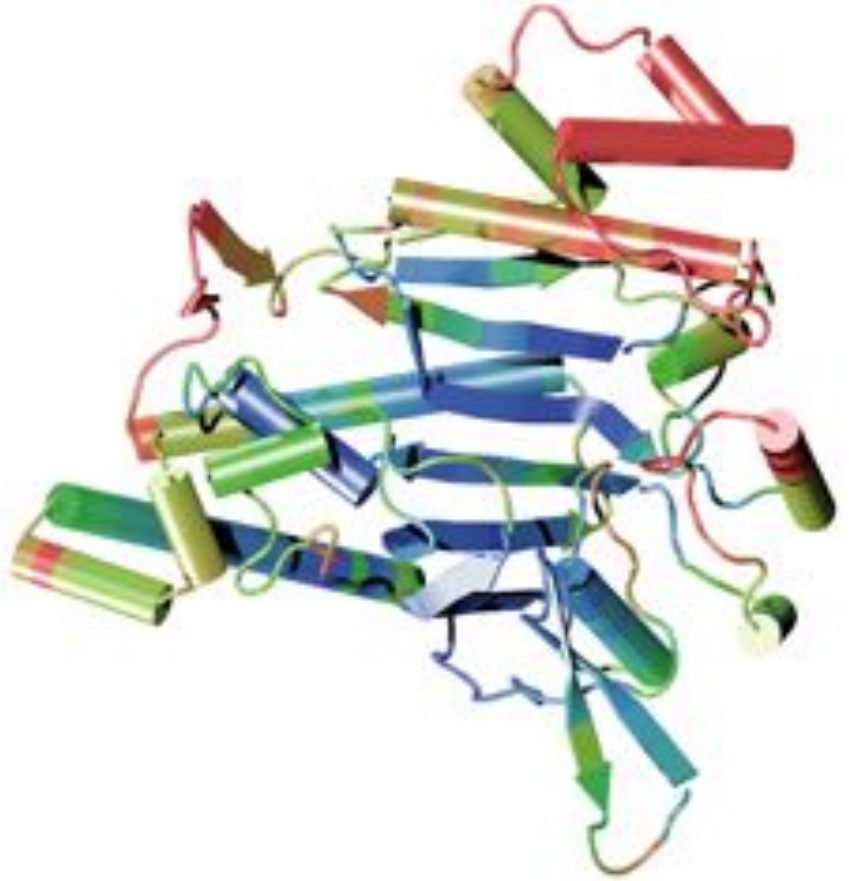
Class I Lysyl-tRNA Synthetase



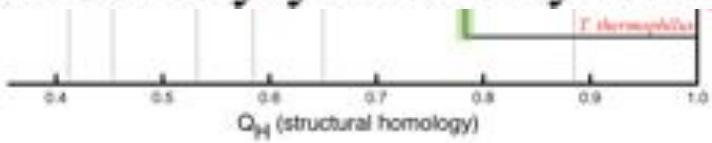
Class II AARSs

structure-based phylogenetics

sequence-structure overlap



Class II Lysyl-tRNA Synthetase



Sequences define more recent evolutionary events

Conformational changes in the same protein.

ThrRS
T-AMP analog, 1.55 A.
T, 2.00 A.

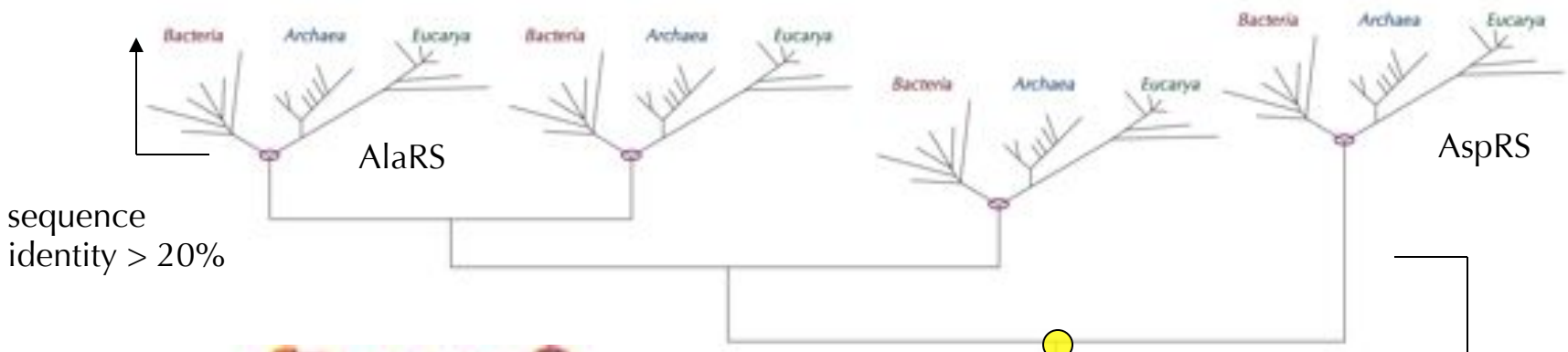
$Q_H = 0.80$
Sequence identity = 1.00

Structures for two different species.

ProRS
M. jannaschii, 2.55 A.
M. thermoautotrophicus, 3.20 A.

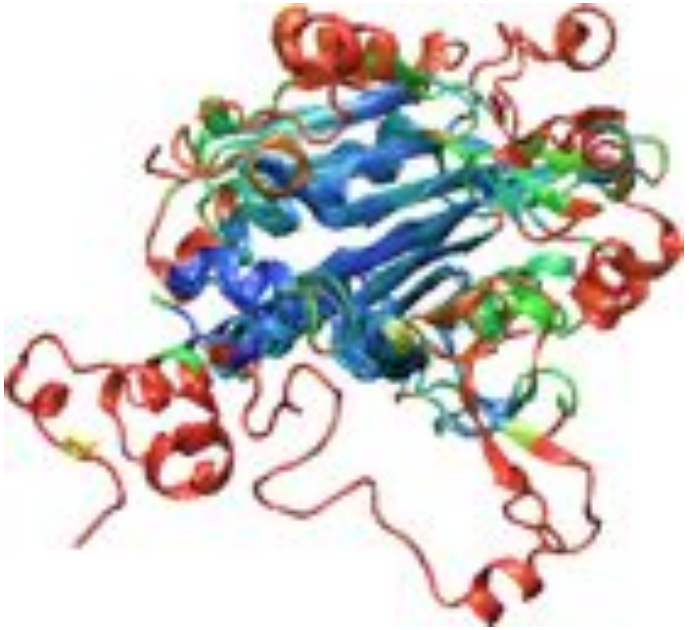
$Q_H = 0.89$
Sequence identity = 0.69

Relationship Between Sequence & Structure



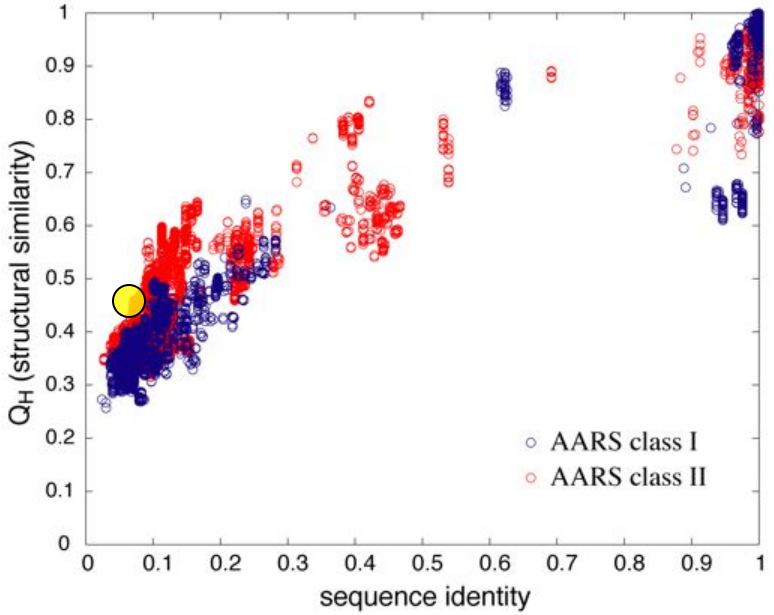
sequence identity > 20%

The sequence signal degrades rapidly.
sequence identity < 10%



Structural superposition of AlaRS & AspRS.

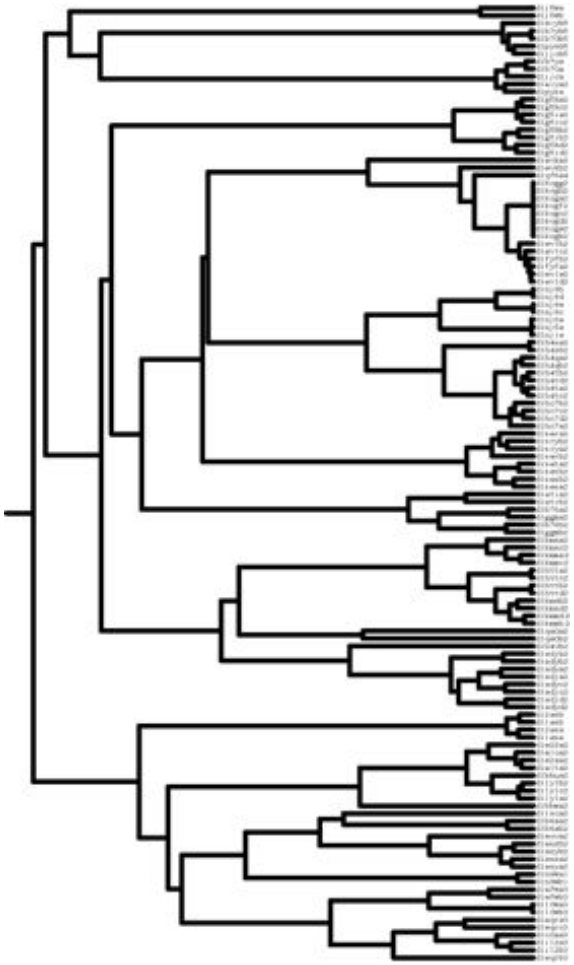
Sequence id = 0.055, Q_H= 0.48



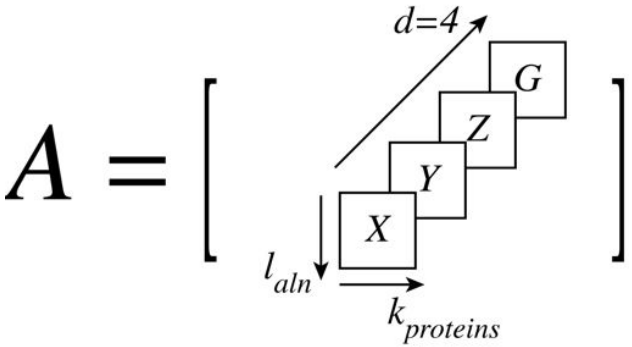
O'Donoghue & Luthey-Schulten (2003) *MMBR* 67: 550–73.
Structural alignment & visualization software MultiSeq/VMD

Non-redundant Representative Profiles

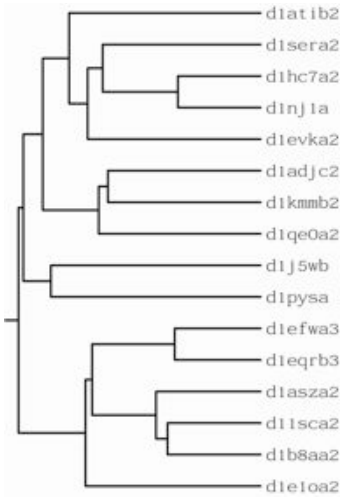
Too much information
129 Structures

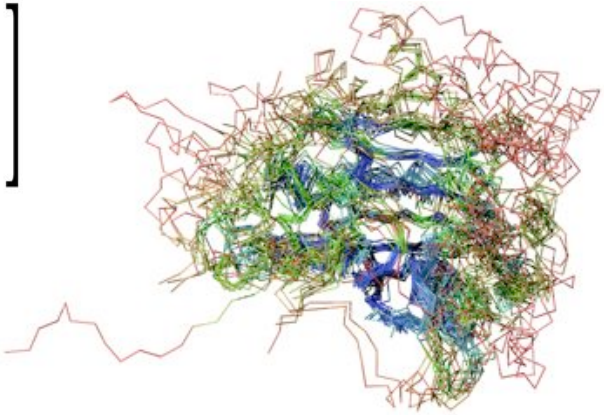


Multidimensional QR factorization of alignment matrix, *A*.



Economy of information
16 representatives





QR computes a set of maximal linearly independent structures.

P. O'Donoghue and Z. Luthey-Schulten (2003) *MMBR* **67**:550-571.

P. O'Donoghue and Z. Luthey-Schulten (2005) *J. Mol. Biol.*, **346**, 875-894.

Numerical Encoding of Proteins in a Multiple Alignment

Encoding Structure

Rotated Cartesian + Gap = 4-space

Aligned position $(x_{C_\alpha}, y_{C_\alpha}, z_{C_\alpha}, 0)$

Gapped position $(0, 0, 0, g)$

Gap Scaling $g = \gamma \dfrac{\|X\|_{F_4} + \|Y\|_{F_4} + \|Z\|_{F_4}}{\|G\|_{F_4}}$

adjustable parameter

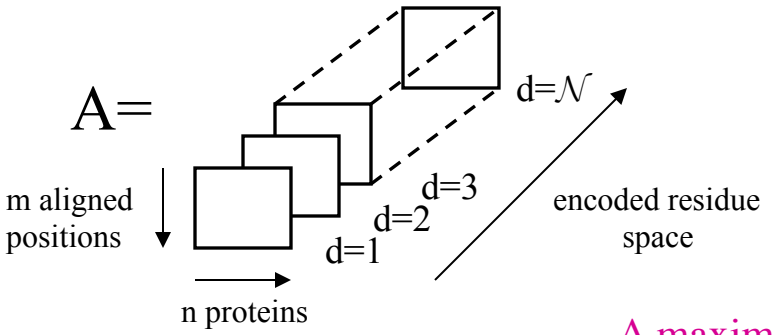
Sequence Space

Orthogonal Encoding = 24-space

23 amino acids (20 + B, X, Z) + gap

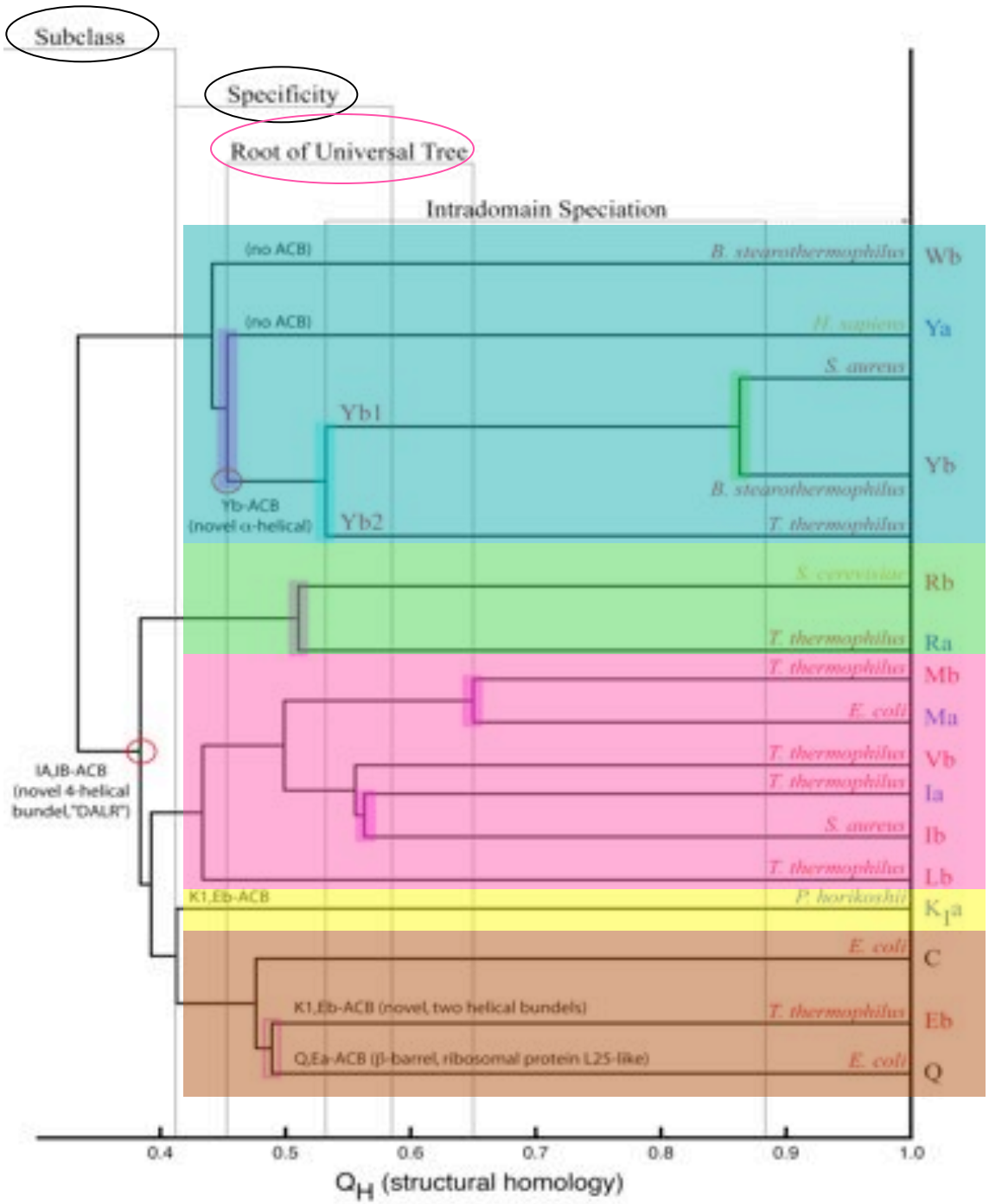
A = (1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
B = (0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
C = (0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
…
GAP = (0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1)

Alignment is a Matrix with Linearly Dependent Columns



$$Q_{(d)}^T A_{(d)} P = Q_{(d)}^T \begin{bmatrix} & & & G \\ & & & Z \\ & & & Y \\ & & & X \\ m_{aln} & & & n_{proteins} \end{bmatrix} P = \tilde{R}_{(d)}$$

A maximal linearly independent subset can be determined with respect to a threshold, e.g., similarity measure threshold.



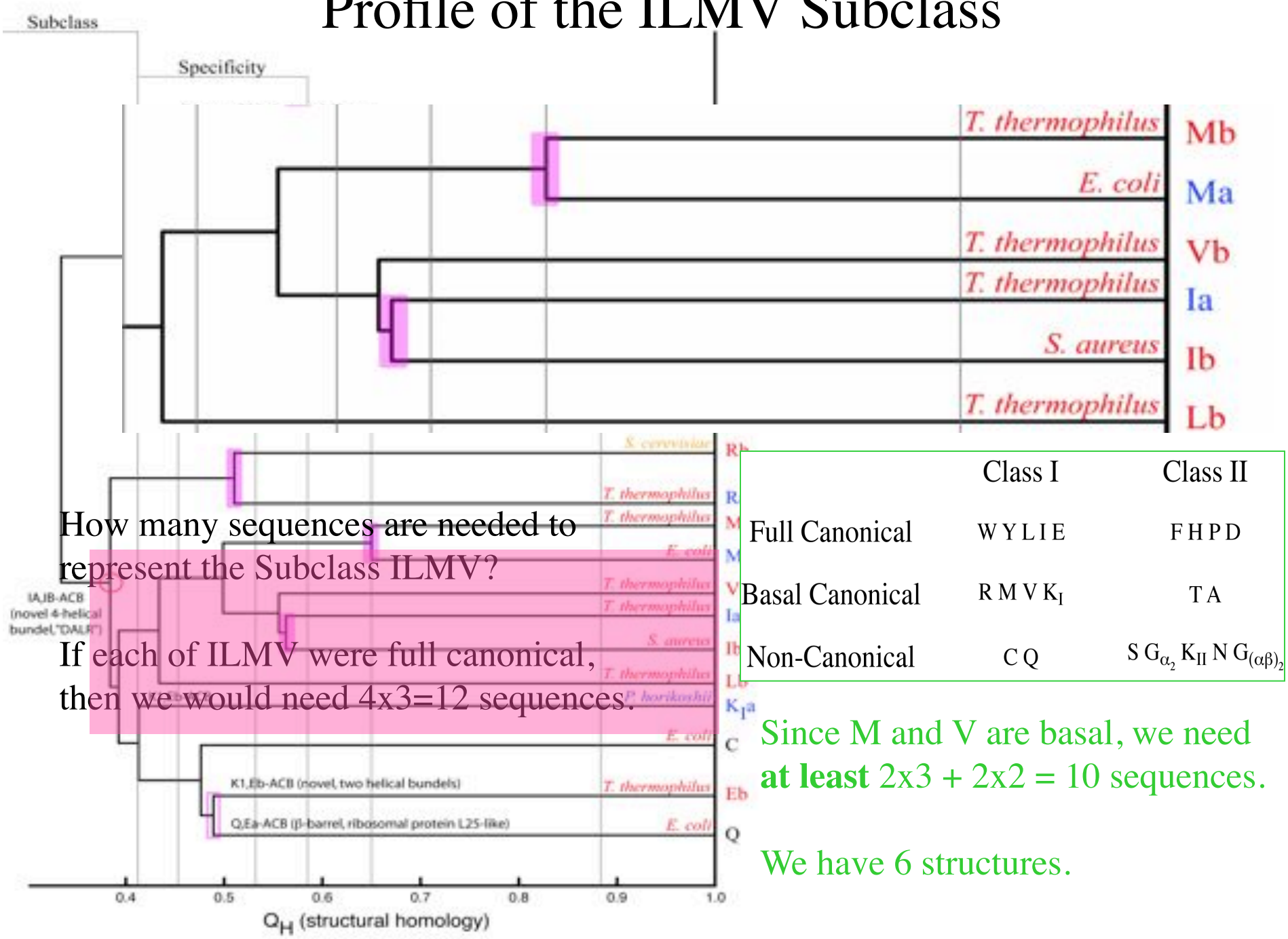
Class I AARSs evolutionary events

5 Subclasses

Specificity – 11 Amino acids

Domain of life A,B,E

Profile of the ILMV Subclass



How many sequences are needed to represent the Subclass ILMV?

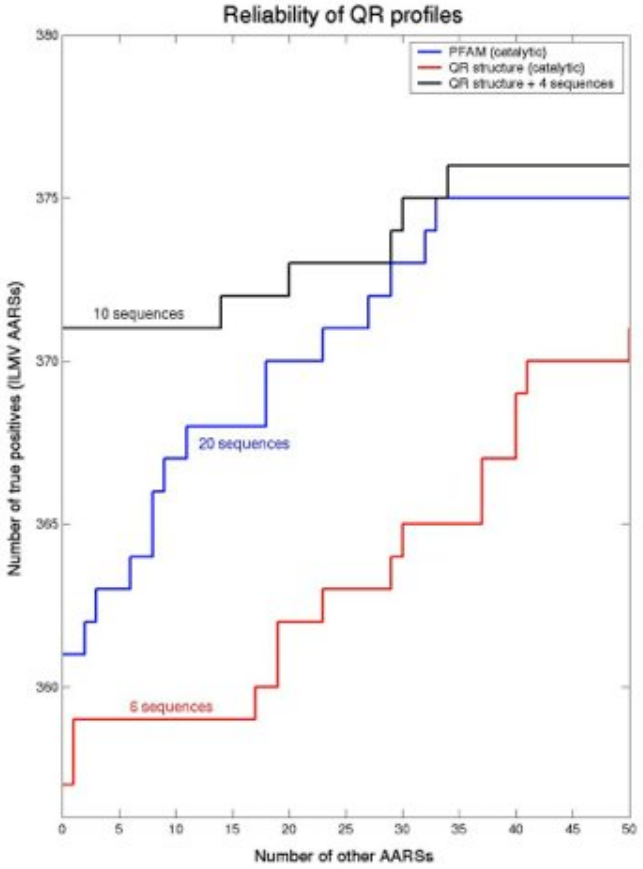
If each of ILMV were full canonical, then we would need 4x3=12 sequences.

	Class I	Class II
Full Canonical	W Y L I E	F H P D
Basal Canonical	R M V K_I	T A
Non-Canonical	C Q	S G_{α_2} K_{II} N $G_{(\alpha\beta)_2}$

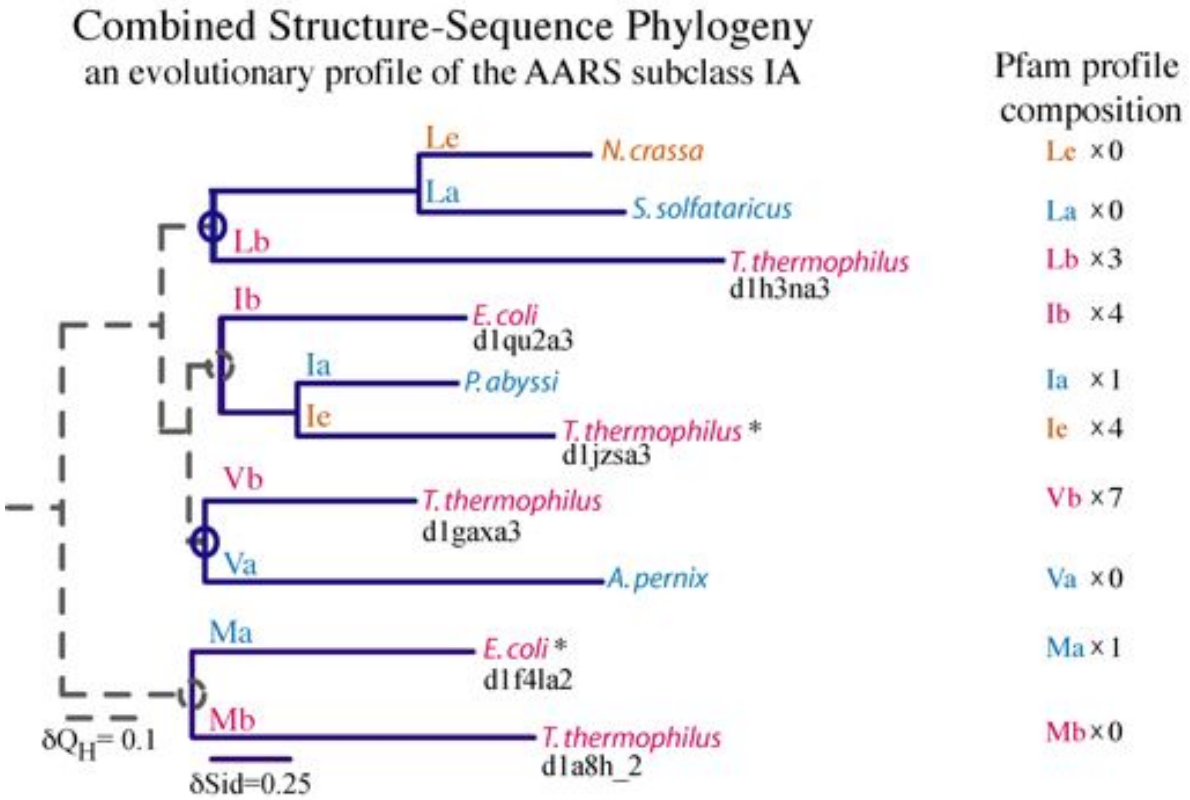
Since M and V are basal, we need **at least** 2x3 + 2x2 = 10 sequences.

We have 6 structures.

Evolutionary Profiles for Homology Recognition
AARS Subclass ILMV



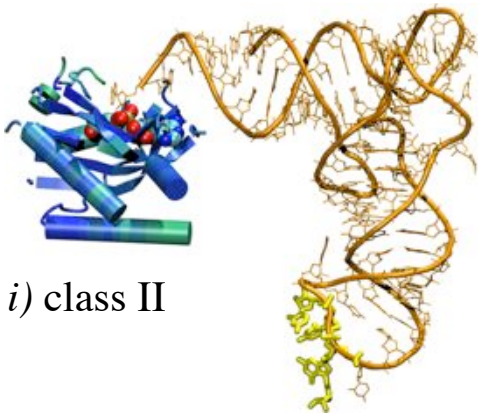
false positives



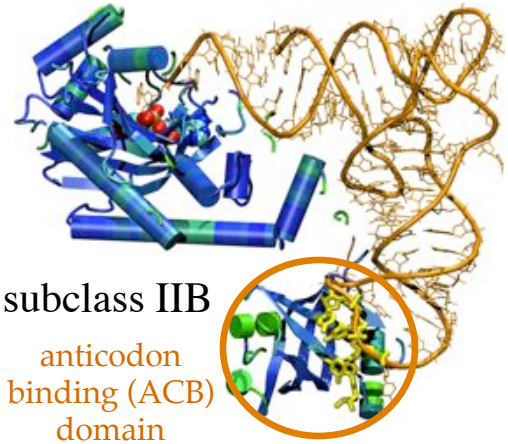
The composition of the profile matters.
Choosing the right 10 sequence makes all the difference.

A. Sethi, P. O'Donoghue, Z. Luthey-Schulten (2005) *JMB, PNAS*

Design - Evolution of Structure and Function in Class II

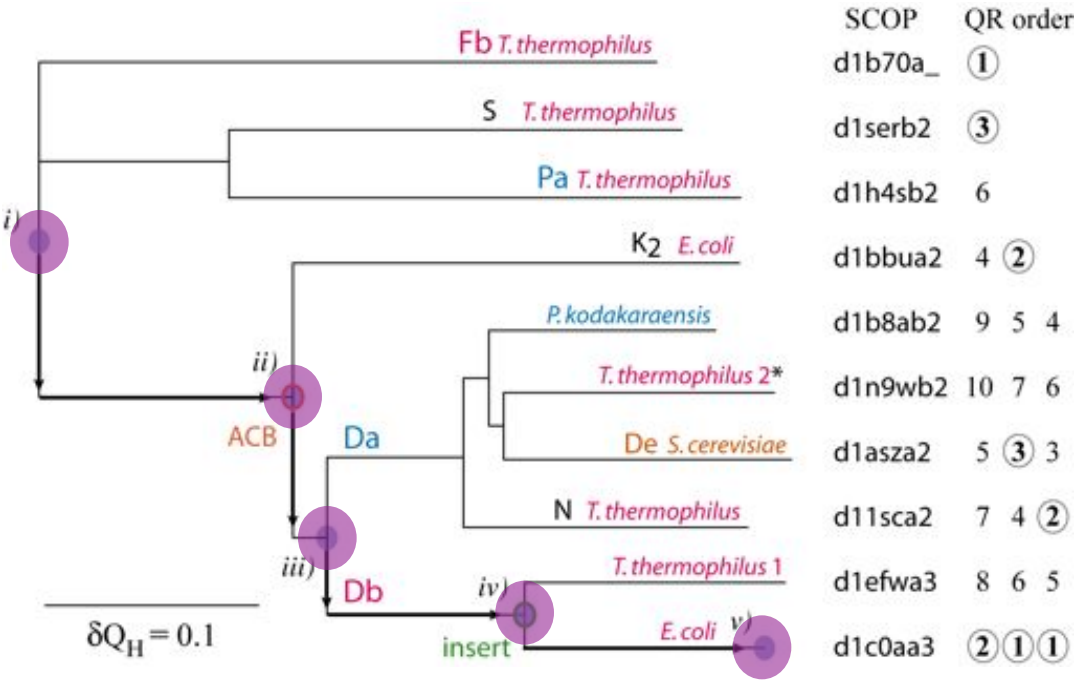


i) class II

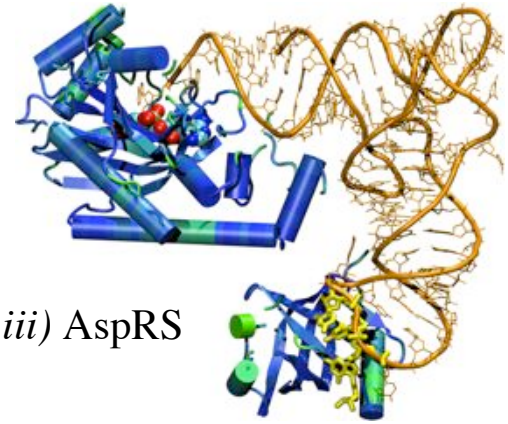


ii) subclass IIB

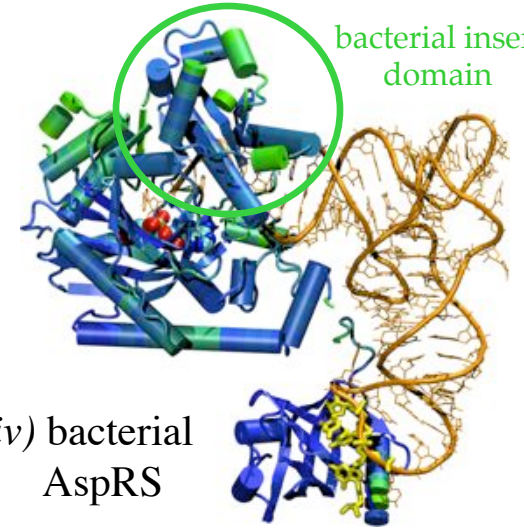
anticodon binding (ACB) domain



SCOP	QR order
d1b70a_	(1)
d1serb2	(3)
d1h4sb2	6
d1bbua2	4 (2)
d1b8ab2	9 5 4
d1n9wb2	10 7 6
d1asza2	5 (3) 3
d11sca2	7 4 (2)
d1efwa3	8 6 5
d1c0aa3	(2) (1) (1)

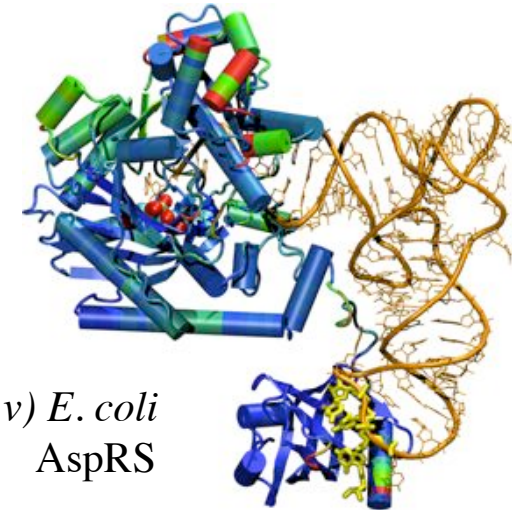


iii) AspRS



bacterial insert domain

iv) bacterial AspRS



v) E. coli AspRS

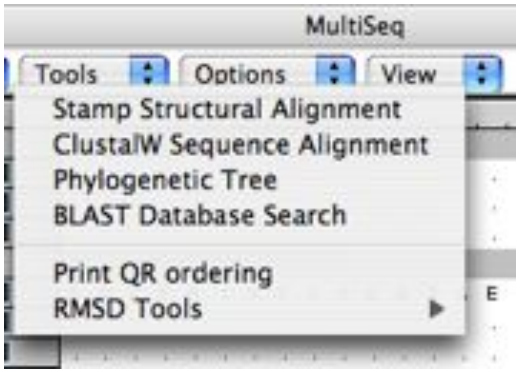
JMB 2005

Summary Structural Profiles

1. Structures often more conserved than sequences!! Similar structures at the Family and Superfamily levels.
   Add more structural information to identify core and variable regions
2. Which structures and sequences to include? Use evolution and eliminate redundancy with QR factorization

What is MultiSeq?

- MultiSeq is an extension to VMD that provides an environment to combine sequence and structure data
- A platform for performing bioinformatics analyses within the framework of evolution
- Provides software for improving the signal-to-noise ratio in an evolutionary analysis by eliminating redundancy (StructQR, SeqQR, Evolutionary Profiles "EP")
- Visualizes computationally derived metrics (Q_{res}, Q_H,..) or imported experimental properties



- Integrates popular bioinformatics tools along with new algorithms (ClustalW, MAFFT, BLAST, STAMP, Signatures, Mutual information, QR, PT,….)

Choose MAFFT to perform multiple sequence alignment

New Tools in VMD/MultiSeq

Protein / RNA Sequence Data

SwissProt DB (400K), Greengenes RNA (100K)

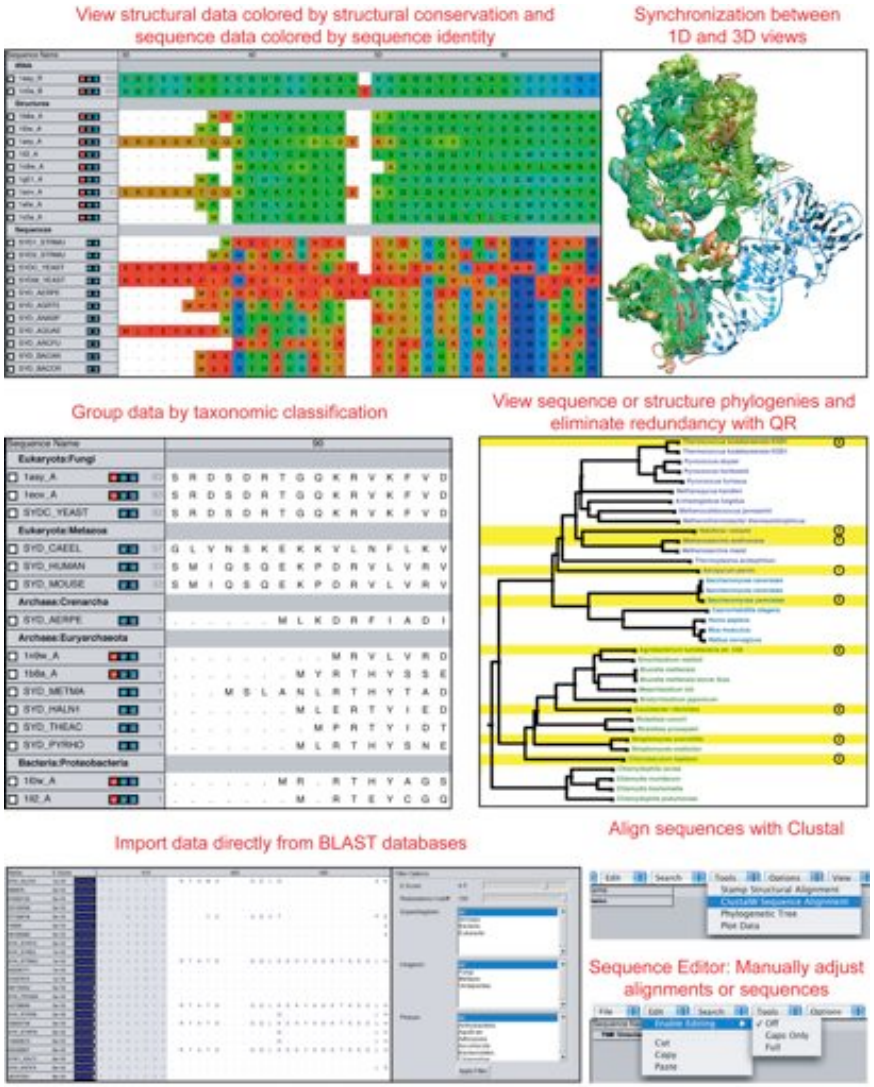
Signatures, Zoom

Metadata Information, Clustal & Phylogenetic Trees

RAXml Trees, Genomic Content, Temperature DB

Blast & PsiBlast

Sequence Editor



Sequence /Structure Alignment

Protein & RNA secondary structure

QR non-redundant seq / str sets

Cluster analysis / Bioinformatics scripting

Tutorials MultiSeq/ AARS

EF-Tu/Ribosome

J. Eargle, D. Wright, Z. Luthey-Schulten, *Bioinformatics,* 22:504 (2006)

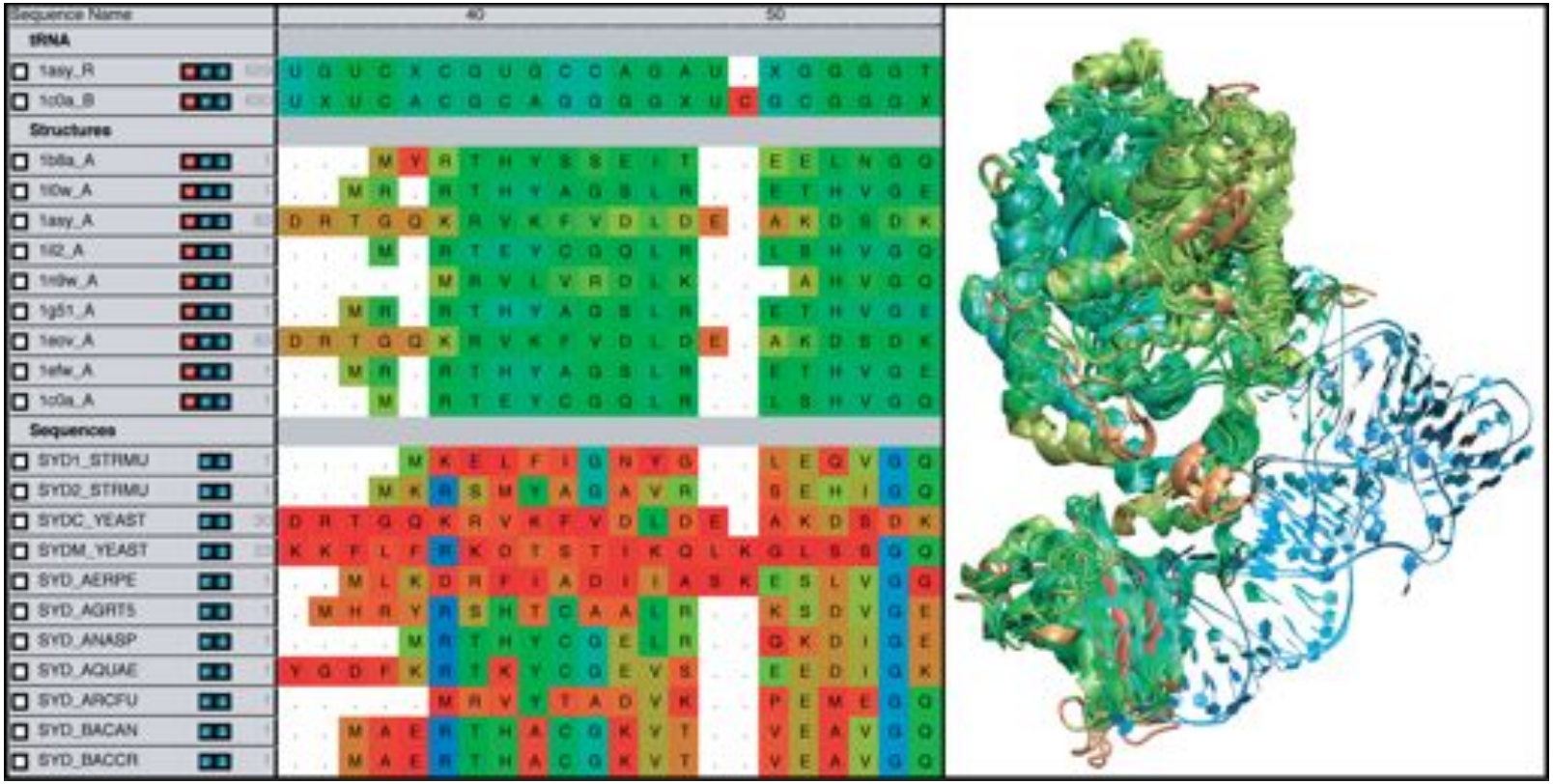
E. Roberts, J. Eargle, D. Wright, Z. Luthey-Schulten, *BMC Bioinformatics,* 7:382 (2006)

MultiSeq Combines Sequence and Structure

- Align sequences or structures; manually edit alignments
- View data colored by numerous metrics including structural conservation and sequence similarity
- Synchronized coloring between 1D and 3D views

Variation in structures

Variation in sequences

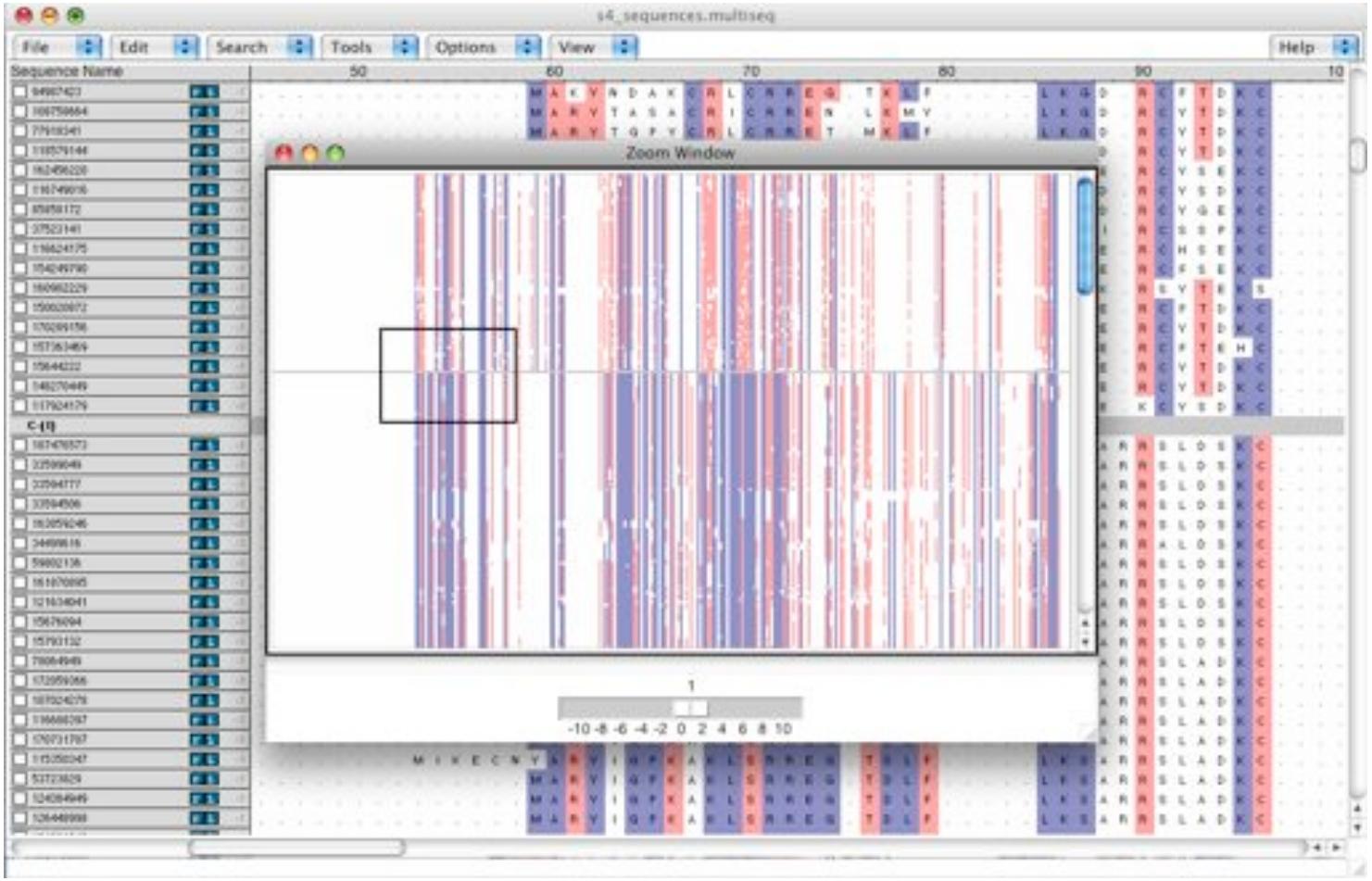


Load large sequence sets

Swiss-Prot (Proteins)	Greengenes (RNA)*
Curated sequences	Environmental 16S rRNA
392,667 sequences	90,654 entries
Unaligned	Aligned (7682 positions)
177 MB on disk	670 MB on disk
2 minutes to load	2.5 minutes to load *
2.4 GB memory used	4.0 GB memory used*

Sequence editor

- New sequence API allows editing of large alignments. Align closely related sequences by group, combine groups, and then manually correct.
- Zoom window gives an overview of the alignment, quickly move the editing window to any part of the alignment.

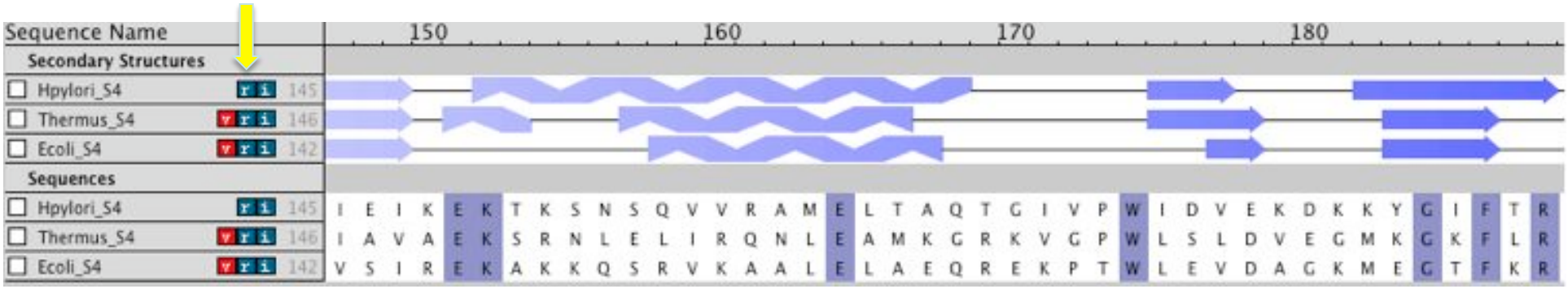


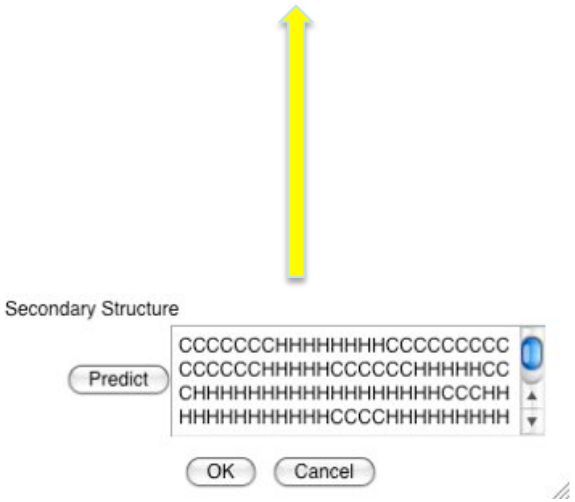
660 sequences of ribosomal protein S4 from all complete bacterial genomes*.

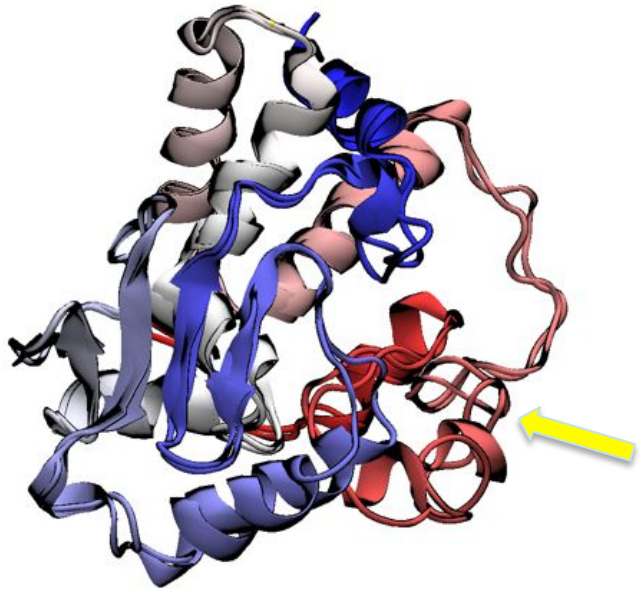
* K. Chen, E. Roberts, Z Luthey-Schulten (2009) BMC Bioinformatics

Secondary structure prediction

- Integration with PSIPRED* to predict secondary structure of sequences.
- Compare to VMD STRIDE predictions from structures.







Modeling of *Helicobacter pylori* ribosomal protein S4 using two known bacterial structures from *Thermus thermophilus* and *Escherichia coli*.

Zinc-binding site replaced by salt bridge in *H. pylori*.

* D. Jones (1999) *J Mol Biol*

PSIPRED installation

- PSIPRED is not included with VMD, must be installed locally.
- Configured in the MultiSeq software preferences dialog (File->Preferences).

Requires a sequence database filtered for problematic regions. Here using Swiss-Prot for relatively fast predictions.

Export Modeller compatible alignments

- MultiSeq can automatically export SIF alignment files compatible with Modeller.

```
>P1; Hpylori_S4
sequence:Hpylori_S4:::::::0.00:0.00
MARYRGAVERLERRFGVSLALKGE-RRLSGKSALDKRAYGPGQHGQR-RAKTSDYGLQLK
EKQKAKMMYGISEKQFRSIFVEANRLDGNTGENLIRLIERRLDNVVYRMGFATTRSSARQ
LVTHGHVLVDGKRLDIPSYFVRSGQKIEIKEKTKSNSQVVRAMELTAQTGIVPWIDVEKD
KKYGIFTRYPEREEVVVPIEERLIVELYSK*

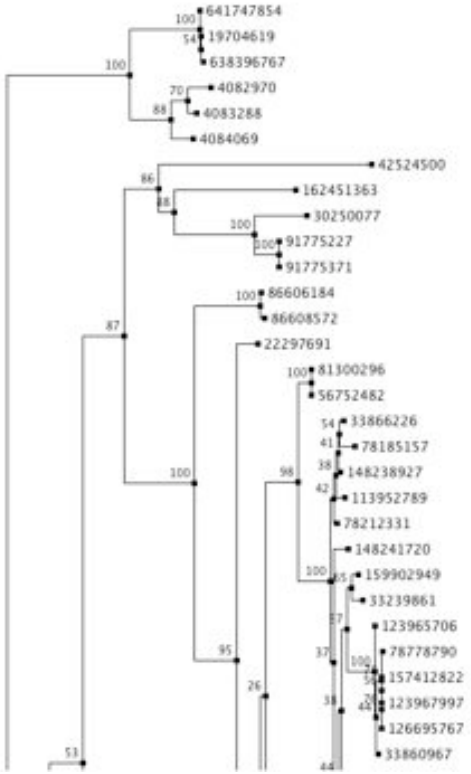
>P1; Thermus_S4
structureX:Thermus_S4:2:D:209:D:::-1.00:-1.00
-GRYIGPVCRLCRREGVKLYLKGE-RCYSPKCAMERRPYPPGQHGQKRARRPSDYAVRLR
EKQKLRRIYGISERQFRNLFEEASKKKGVTGSVFLGLLESRLDNVVYRLGFAVSRRQARQ
LVRHGHITVNGRRVDLPSYRVRPGDEIAVAEKSRNLELIRQNLEAMKGRKVGPWLSLDVE
GMKGKFLRLPDREDLALPVNEQLVIEFYSR*

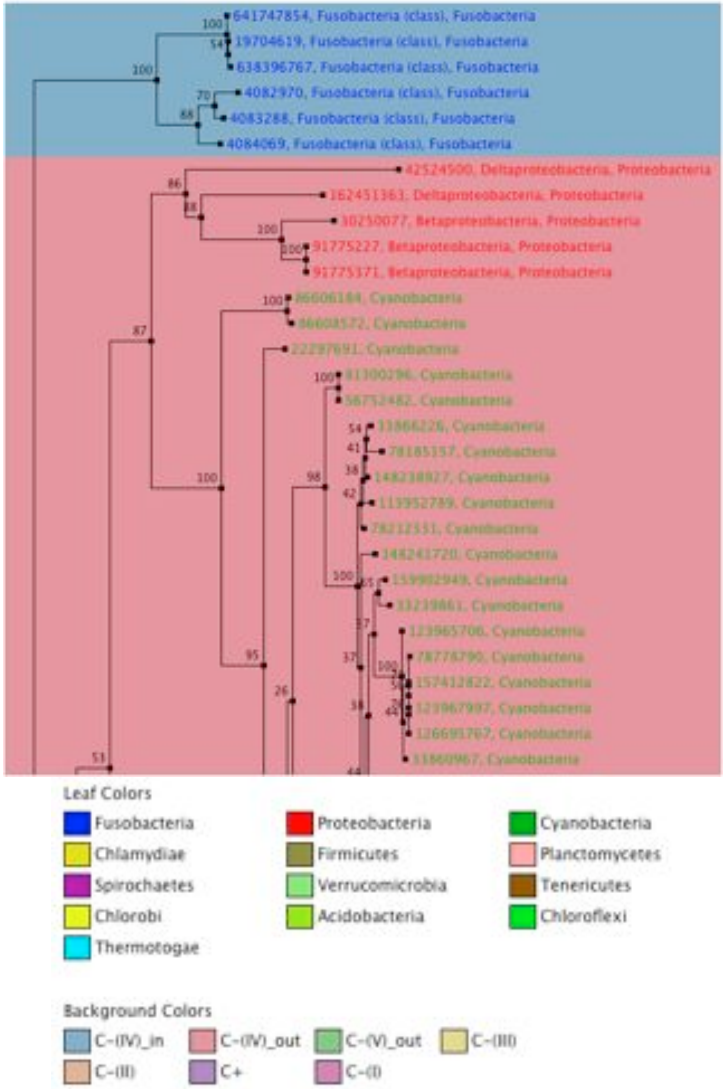
>P1; Ecoli_S4
structureX:Ecoli_S4:1:D:205:D:::-1.00:-1.00
-ARYLGPKLKLSRREGTDLFLKSGVRAIDTKCKIE---QAPGQHGAR-KPRLSDYGVQLR
EKQKVRRIYGVLERQFRNYYKEAARLKGNTGENLLALLEGRLDNVVYRMGFGATRAEARQ
LVSHKAIMVNGRVVNIASYQVSPNDVVSIREKAKKQSRVKAALELAEQREKPTWLEVDAG
KMEGTFKRKPERSDLSADINEHLIVELYSK*
```

```
a = mymodel(env, alnfile='alignment.ali', knowns=('Ecoli_S4','Thermus_S4'), sequence='Hpylori_S4')
a.starting_model = 1
a.ending_model = 20
a.make()
```

Phylogenetic tree editor

- Automatically add annotations and colors to phylogenetic trees based on taxonomy, enzyme, temperature class, and/or MultiSeq groupings.





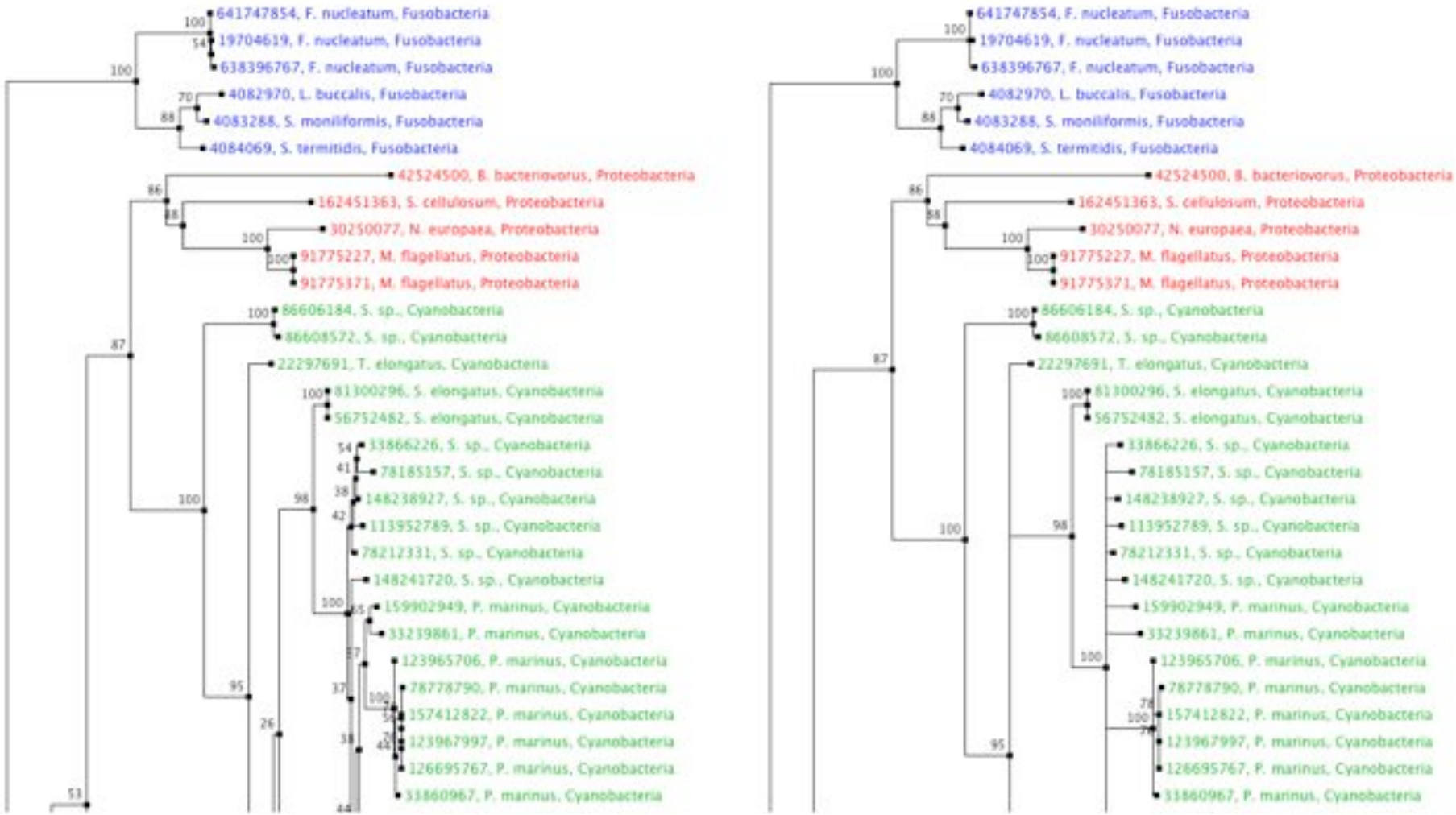
Maximum likelihood tree of 660 S4 sequences reconstructed using RAxML.

A cluster of five proteobacterial sequences branch near the cyanobacterial sequences. These are cases of horizontal gene transfer.

Elijah Roberts 2009

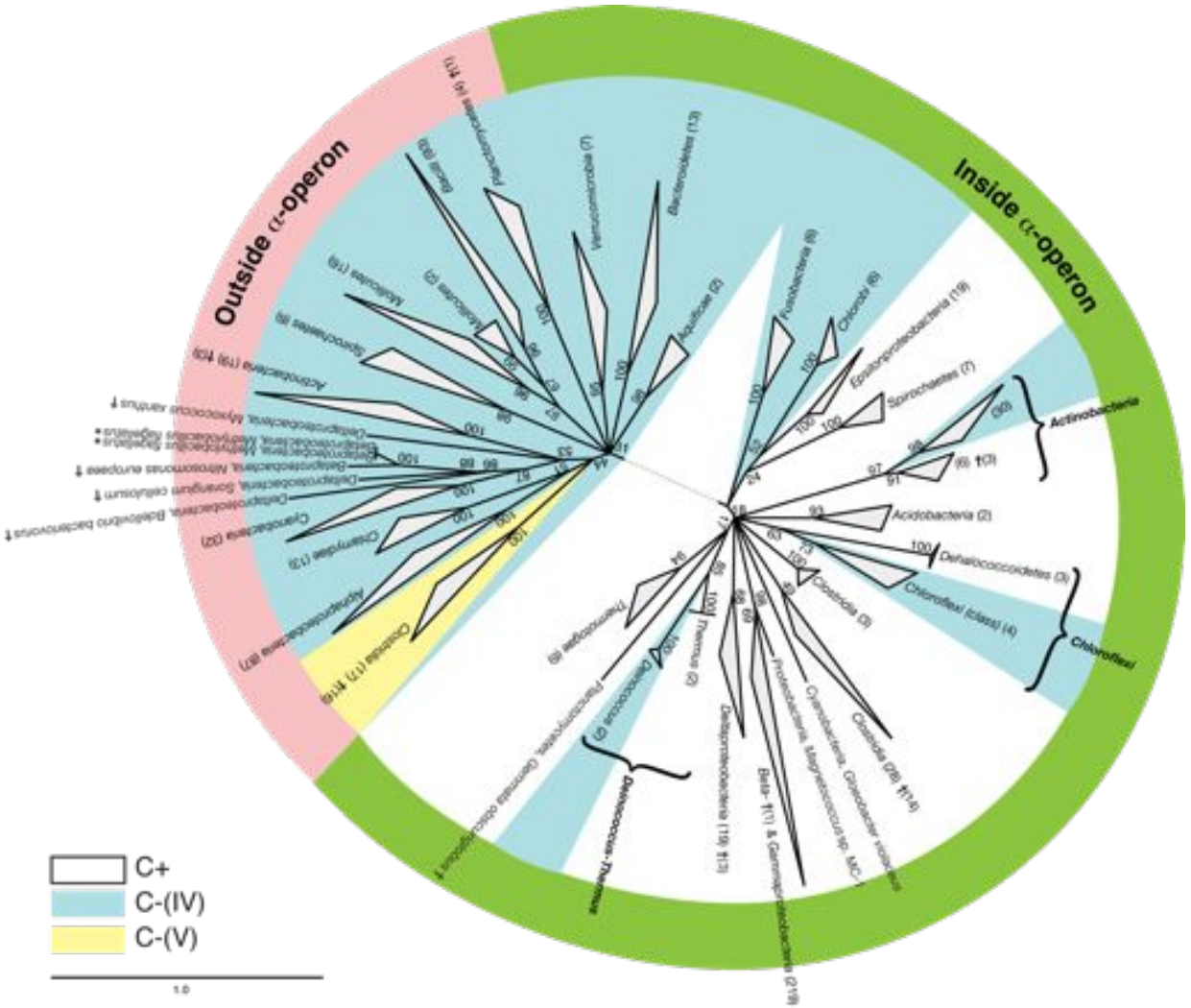
Edit the physical layout of the tree

- Nodes with low support can be removed.
- Nodes can be rotated for easier reading.



Manipulate branches to simplify the tree

- Manually collapse by node.
- Automatically collapse clades that are alike according to taxonomy, enzyme, temperature class, and/or MultiSeq grouping.
- Set the root of the tree manually, if known from external sources.



Combined phylogenetic tree and genome content analysis of ribosomal protein S4 for all complete bacterial genomes.

Roberts, Chen, ZLS, **BMC Evol. Bio**. 2009

Phylogenetic tree generation

- Generate distance based trees only over well-aligned columns (no indels).
- Export alignments in Phylip format (PHY) compatible with RAxML for maximum likelihood reconstructions.
- Import Newick trees from phylogenetic reconstruction programs (including RAxML).

Scripting MultiSeq

- All MultiSeq functions can now be scripted.
- Scripting an analysis provides benefits:
  - It can be checked for correctness.
  - It can be quickly repeated by anyone.
  - It can be modified later with new functionality.
  - It can be run on a cluster in VMD text mode. (if it can be easily broken into independent chunks)
- Many functions are too user specific and/or too complex to be turned into a GUI.
- Some examples of MultiSeq scripts…

Genome content

- When using sequence from fully sequenced genomes, additional information is available in the genome content.
- Conservation of gene ordering, neighbors, or intergenic regions can provide additional evolutionary information not contained in the sequence.
- Gene names and ordering can be obtained from the genome PTT files, want to organize the information in an evolutionarily meaningful manner.

Location	Strand	Length	PID	Gene	Synonym	Code	COG	Product
3437638..3438021	-	127	16131173	rplQ	b3294	-	COG0203J	50S ribosomal subunit protein L17
3438062..3439051	-	329	16131174	rpoA	b3295	-	COG0202K	RNA polymerase, alpha subunit
3439077..3439697	-	206	16131175	rpsD	b3296	-	COG0522J	30S ribosomal subunit protein S4
3439731..3440120	-	129	16131176	rpsK	b3297	-	COG0100J	30S ribosomal subunit protein S11
3440137..3440493	-	118	16131177	rpsM	b3298	-	COG0099J	30S ribosomal subunit protein S13
3440640..3440756	-	38	16131178	rpmJ	b3299	-	COG0257J	50S ribosomal subunit protein L36
3440788..3442119	-	443	16131179	secY	b3300	-	COG0201U	preprotein translocase membrane subunit
3442127..3442561	-	144	16131180	rplO	b3301	-	COG0200J	50S ribosomal subunit protein L15
3442565..3442744	-	59	16131181	rpmD	b3302	-	COG1841J	50S ribosomal subunit protein L30
3442748..3443251	-	167	16131182	rpsE	b3303	-	COG0098J	30S ribosomal subunit protein S5

Combined genomic context/phylogenetic tree

- Use a script to walk through a phylogenetic tree, find the genome content near the source gene, create a graphical representation of the combined data.

```
proc draw_genome_context_of_phylogeny {args} {

    # Load the sequences.
    set alignment [::SeqData::Fasta::loadSequences $alignmentFilename]

    # Load the tree
    set tree [::PhyloTree::Newick::loadTreeFile $treeFilename]

    # Reorder the alignment by the tree.
    set treeAlignment {}
    set leafNodes [::PhyloTree::Data::getLeafNodes $tree]
    foreach node $leafNodes {
        set foundNode 0
        set nodeName [::PhyloTree::Data::getNodeName $tree $node]
        foreach sequence $alignment {
            if {$nodeName == [::SeqData::getName $sequence]} {
                lappend treeAlignment $sequence
                set foundNode 1
                break
            }
        }
    }

    # Draw the genomic context.
    drawGenomicContextOfAlignment $outputFilename $treeAlignment $contextDistance $scaling $genomeDirectory
}
```

Combined genomic context/phylogenetic tree

```
proc drawGenomicContextOfAlignment {outputFilename alignment contextDistance scaling genomeDirectory} {

    foreach sequence $alignment {

        # Make sure we have the GI number for this sequence.
        set giNumber [::SeqData::getSourceData $sequence "gi"]

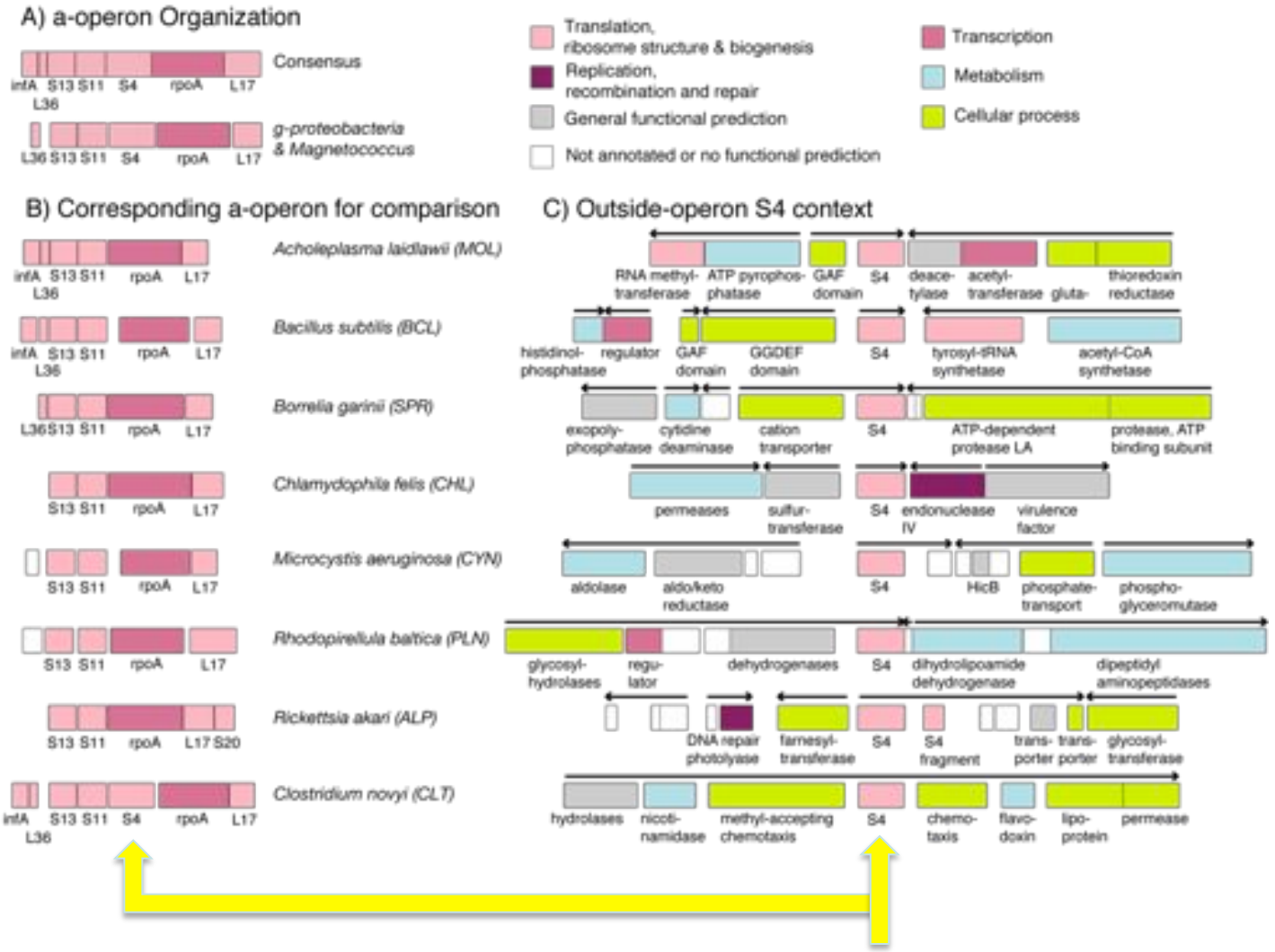
        # Make sure we can tell which genome this sequence is from.
        set taxonomy [join [::SeqData::getLineage $sequence 1 0 1] ","]
        if {![info exists genomeTaxonomyMap($taxonomy)]} {
            error "ERROR) Unknown genome for sequence [::SeqData::getName $sequence]: $taxonomy"
        }

        # Go through each of the genome context files for the genome.
        set foundGene 0
        foreach genomeName $genomeTaxonomyMap($taxonomy) {
            ...
        }
    }

    # Draw the genomic context.
    drawMultipleGenomicContext $outputFilename $alignment $geneFiles $genePositions $geneStrands $contextDistance
}
```

Genome content future directions

- Genome content still a work in progress.
- Good candidate for a GUI: combined phylogenetic tree/ genome content viewer.
- Can also use COG codes to color by gene function.
- Still need API for manipulating PTT files.



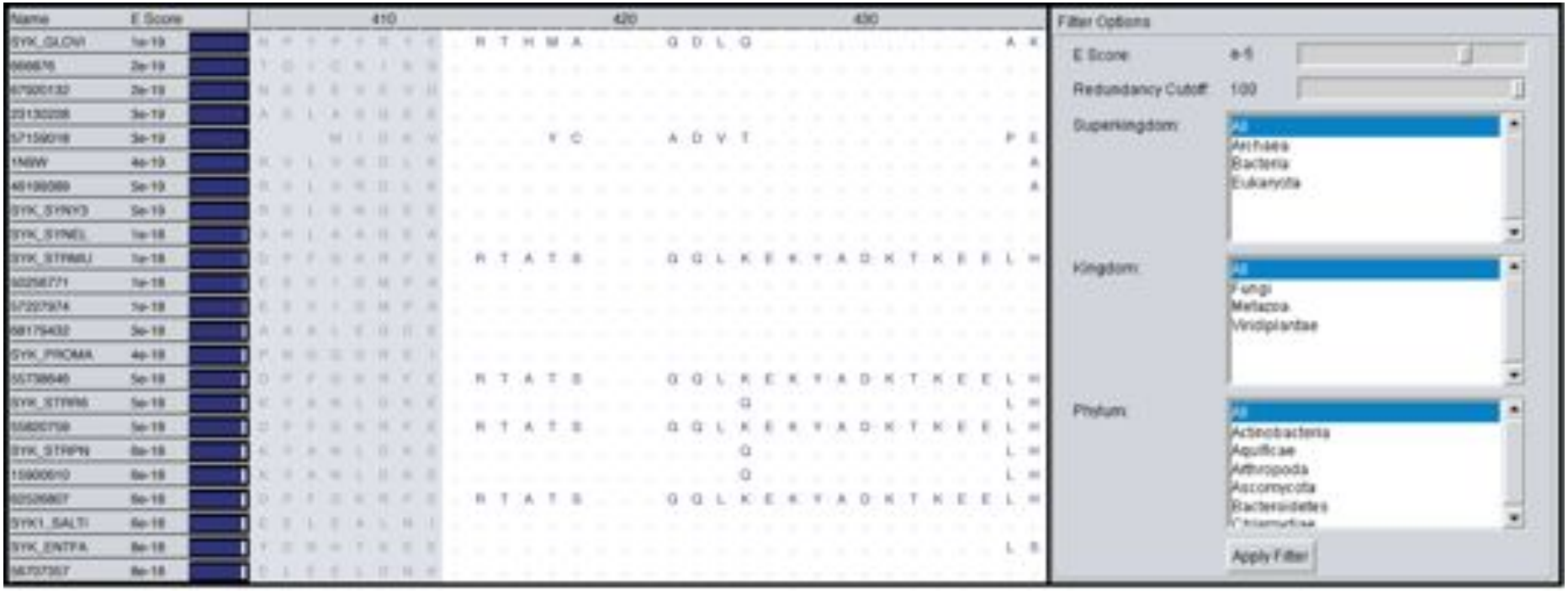
Genome content of ribosomal protein S4 by occurrence of the gene in the alpha operon.

Fifteen Clostridia genomes contain two copies of S4: one zinc-binding and one zinc-free.

Roberts, Chen, ZLS, **BMC Evol. Bio**. 2009

BLAST DB Searching

- Import sequence data directly from BLAST databases
- Search using a single sequence or an EP profile
- Filter results based on taxonomy or redundancy (QR)



Protein sequence alignment

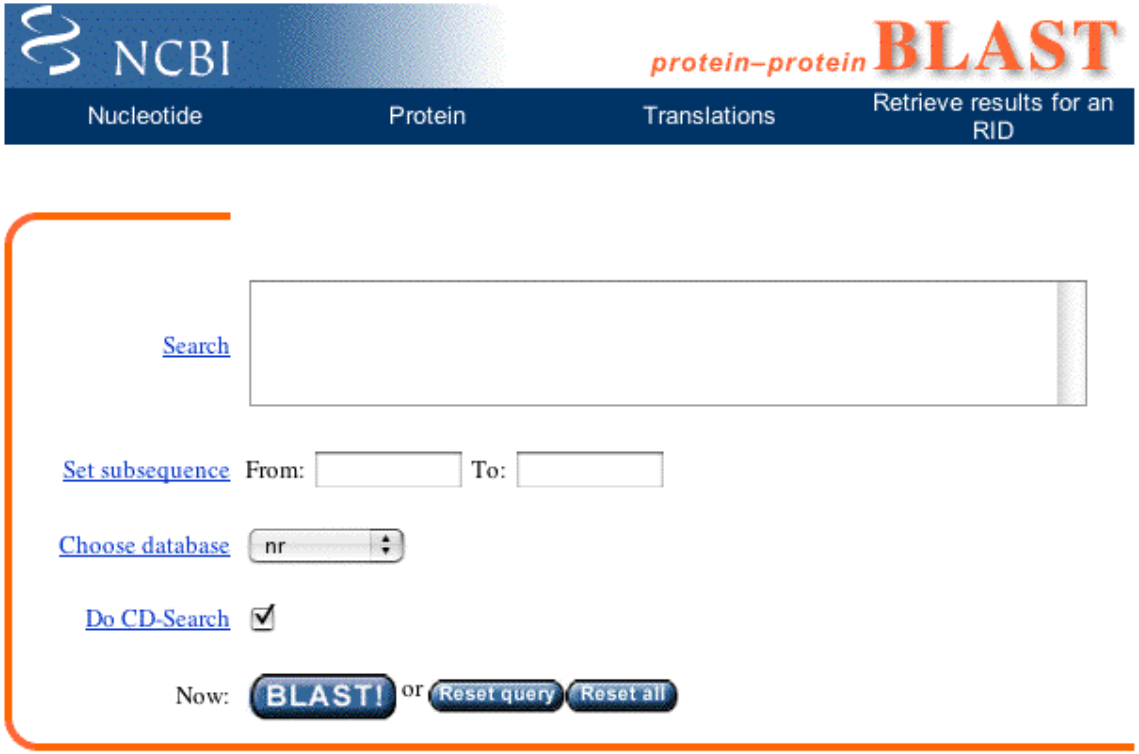
How do I align two similar, but different sequences ?

Sequence 1: $a_1\ a_2\ a_3$ - - $a_4\ a_5 \ldots a_n$

Sequence 2: c_1 - $c_2\ c_3\ c_4\ c_5$ - $\ldots c_m$

There exist fast web tools, e.g., BLAST search: **http://www.ncbi.nlm.nih.gov/**

See also Blastn, Psi-Blast, ….



Sequences from Swiss-Prot, NCBI, JGI, ….

Structures from PDB, CATH, SCOP, …..

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Search Swiss-Prot/TrEMBL for aqp Go Clear

NiceProt View of Swiss-Prot: P47865

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[Entry info] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information	
Entry name	**AQP1_BOVIN**
Primary accession number	**P47865**
Secondary accession numbers	None
Entered in Swiss-Prot in	Release 33, February 1996
Sequence was last modified in	Release 44, July 2004
Annotations were last modified in	Release 45, October 2004
Name and origin of the protein	
Protein name	**Aquaporin-CHIP**
Synonyms	**Water channel protein for red blood cells and kidney proximal tubule** **Aquaporin 1** **Water channel protein CHIP29**
Gene name	**Name: AQP1**
From	Bos taurus (Bovine) [TaxID: 9913]
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Cetartiodactyla; Ruminantia; Pecora; Bovidae; Bovinae; Bos.
References	
[1]	SEQUENCE FROM NUCLEIC ACID. **TISSUE**=Ocular ciliary epithelium;

Final Blast Result: Sequence Alignment

>gi|46395801|sp|Q88F17|AQPZ_PSEPK Aquaporin Z

```
          Length = 230

 Score =  119 bits (299), Expect = 6e-27
 Identities = 70/186 (37%), Positives = 105/186 (56%), Gaps = 12/186 (6%)

Query: 53  VSLAFGLSIATLAQSVGHISGAHLNPAVTLGLLLSCQISVLRAIMYIIAQCVGAIVATAI 112
           V+ AFGL++ T+A ++GHISG HLNPAV+ GL++  +      + Y+IAQ +GAI+A  +
Sbjct: 40  VAFAFGLTVLTMAFAIGHISGCHLNPAVSFGLVVGGRFPAKELLPYVIAQVIGAILAAGV 99

Query: 113 LSGITSSLP--DNSLGL--NALAP----GVNSGQGLGIEIIGTLQLVLCVLATTDRRRRD 164
           +  I S     + S GL  N  A     G   G G   E++ T   ++ ++  TD R
Sbjct: 100 IYLIASGKAGFELSAGLASNGYADHSPGGYTLGAGFVSEVVMTAMFLVVIMGATDARAP- 158

Query: 165 LGGSGPLAIGFSVALGHLLAIDYTGCGINPARSFGSSVITHNF--QDHWIFWVGPFIGAA 222
             G  P+AIG ++ L HL++I  T   +NPARS G ++    +  Q  W+FWV P IGAA
Sbjct: 159 -AGFAPIAIGLALTLIHLISIPVTNTSVNPARSTGPALFVGGWALQQLWLFWVAPLIGAA 217

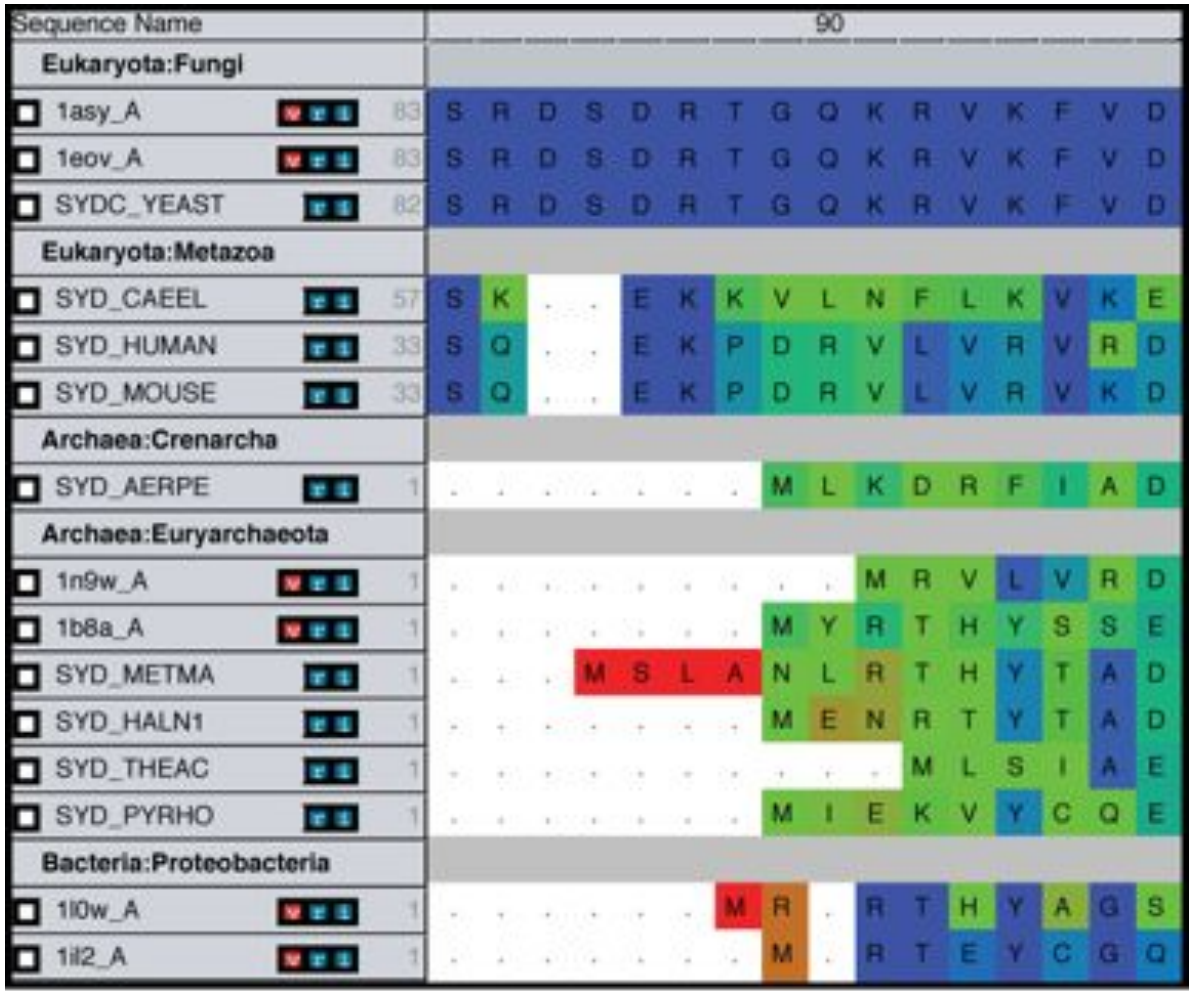
Query: 223 LAVLIY 228
           +   +Y
Sbjct: 218 IGGALY 223
```

Search returns approximate alignments - needing refinement!

Clustal, Muscle, MAFT, Tcoffee, pileup, Smith-Waterman, and manual editing in sequence editor

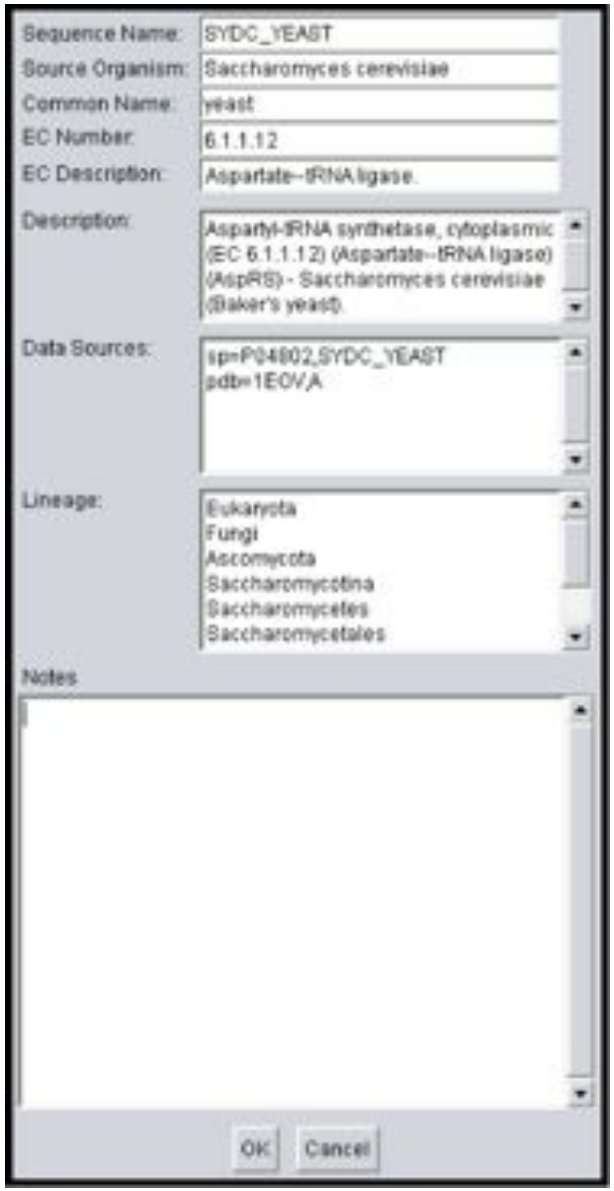
Flexible Grouping of Data

- Automatically group data by taxonomic classification to assist in evolutionary analysis (HGT) or create custom groups
- Apply metrics to groups independently, e.g bacterial signal



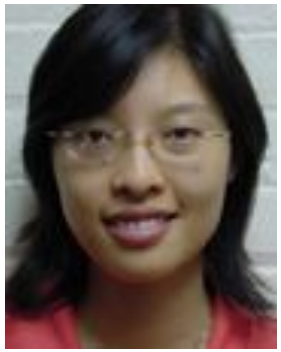
MultiSeq: Display and Edit Metadata

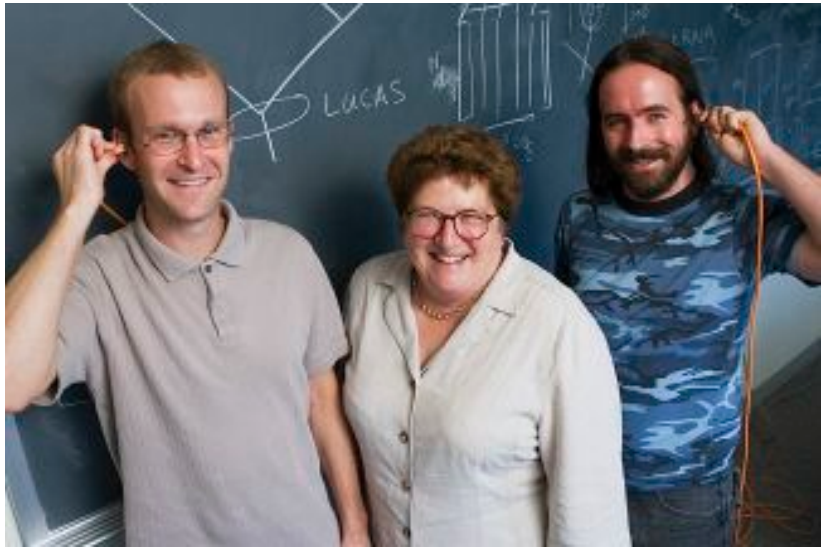
- External databases are cross-referenced to display metadata such as taxonomic information and enzymatic function
- Changes to metadata are preserved for future sessions
- Electronic Notebook: Notes and annotations about a specific sequence or structure can be added



Acknowledgements

- Elijah Roberts
- John Eargle
- Ke Chen
- Tyler Harpole
- Kirby Vandivort
- John Stone





NIH Center for Macromolecular Modeling and Bioinformatics

VMD/MultiSeq Tutorials

1. Evolution of Translation: AARS:tRNA
2. Evolution of Translation: EF-Tu:tRNA
3. Evolution of Translation: Ribosome
4. Dynamical Network Analysis - new