Part III - Bioinformatics Studies Using Multiseq in VMD

- Aminoacyl tRNA Synthetases
- Aquaporins

Chicago, 2005, Computational Biology Workshop
Evolution of Protein Structure
Aspartyl-tRNA Synthetase

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Multiple Sequence Alignments

• “The aminoacyl-tRNA synthetases, perhaps better than any other molecules in the cell, epitomize 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 $\rightarrow$ RNA $\rightarrow$ 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

\[
\begin{align*}
\text{Gln} + \text{tRNA}^{\text{Gln}} + \text{ATP} & \quad \text{Glutaminyl-tRNA synthetase} \quad \text{Gln-tRNA}^{\text{Gln}} + \text{AMP} + \text{PP}_i \\
\end{align*}
\]

tRNA-dependent amino acid modification

\[
\begin{align*}
\text{Glu} + \text{tRNA}^{\text{Gln}} + \text{ATP} & \quad \text{Glutamyl-tRNA synthetase} \quad \text{Glu-tRNA}^{\text{Gln}} + \text{AMP} + \text{PP}_i \\
\text{Gln} + \text{Glu-tRNA}^{\text{Gln}} + \text{ATP} & \quad \text{Glu-tRNA}^{\text{Gln}} \quad \text{amidotransferase} \quad \text{Gln-tRNA}^{\text{Gln}} + \text{ADP} + \text{P}_i + \text{Glu}
\end{align*}
\]

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 $\alpha$-carboxyl attacks the P atom of the alpha phosphate of ATP.

www.rpi.edu/dept/bcbp/molbiochem/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 $\rightarrow$ aminoacyl-AMP + PP$_i$
2. aminoacyl-AMP + tRNA $\rightarrow$ aminoacyl-tRNA + AMP

Overall Reaction:
\[
\text{amino acid} + \text{ATP} + \text{tRNA} \rightarrow \text{aminoacyl-tRNA} + \text{AMP} + \text{PP}_i
\]

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

Db

Da

N

20 changes
Multiseq extension in VMD
Conservation

Core Structure Conserved

Sequence Identity of Core
Less than 15%
Aquaporins

Aquaporins in Human Body

<table>
<thead>
<tr>
<th>Aquaporin</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>Aquaporin-0</td>
<td>Eye: lens fiber cells, Fluid balance of the eye</td>
</tr>
<tr>
<td>Aquaporin-2</td>
<td>Kidney: collecting ducts, ADH hormone activity</td>
</tr>
<tr>
<td>Aquaporin-3</td>
<td>Kidney: collecting ducts, Trachea: epithelial cells, Regulation of water and salt balance</td>
</tr>
<tr>
<td>Aquaporin-4</td>
<td>Kidney: collecting ducts, Brain: ependymal cells, Brain: hypothalamus, Lung: bronchial epithelium, Saliva, Production of tears</td>
</tr>
<tr>
<td>Aquaporin-5</td>
<td>Salivary glands, Lacrimal glands, Saliva, Production of tears</td>
</tr>
<tr>
<td>Aquaporin-6</td>
<td>Kidney, Very low water permeability</td>
</tr>
<tr>
<td>Aquaporin-7</td>
<td>Testes and sperm</td>
</tr>
<tr>
<td>Aquaporin-8</td>
<td>Testicle, pancreas, liver</td>
</tr>
<tr>
<td>Aquaporin-9</td>
<td>Leukocytes</td>
</tr>
<tr>
<td>Aquaporin-10</td>
<td>More are suspected to exist.</td>
</tr>
</tbody>
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Congenital cataracts, Diabetes insipidus...

VMD Developers:
John Stone
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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
1 ns 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

## Water and Glycerol Channels in the Human Body

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<td>Fluid balance of the lens</td>
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<td>Aquaporin-1</td>
<td>Red blood cells, Kidney: proximal tubules, Eye: ciliary epithelium, Brain: choroid plexus, Lung: alveolar epithelial cells</td>
<td>Osmotic protection, Concentration of urine, Aqueous humor, Production of CSF, Alveolar hydration</td>
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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:
- AQP1 – Mammalian aquaporin-1 (pure water channel) -Sui et al, Nature (2001)

~100% conserved -NPA- signature sequence
Load Aquaporin 1J4N into VMD
VMD Permits Different Rendering Styles

movie

tube

cartoon

tube - transparent
Highlighting Key Conserved Residues
Load Aquaporins 1j4n, 1fqu, 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
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Bioinformatics
Aquaporins
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