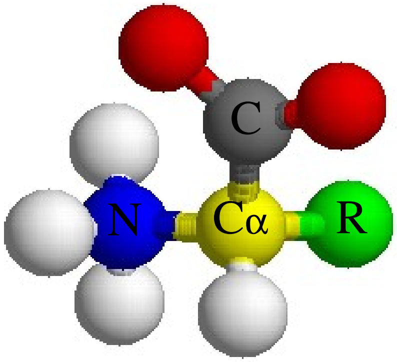
Physiochemical Properties of Amino Acids

Various Sources



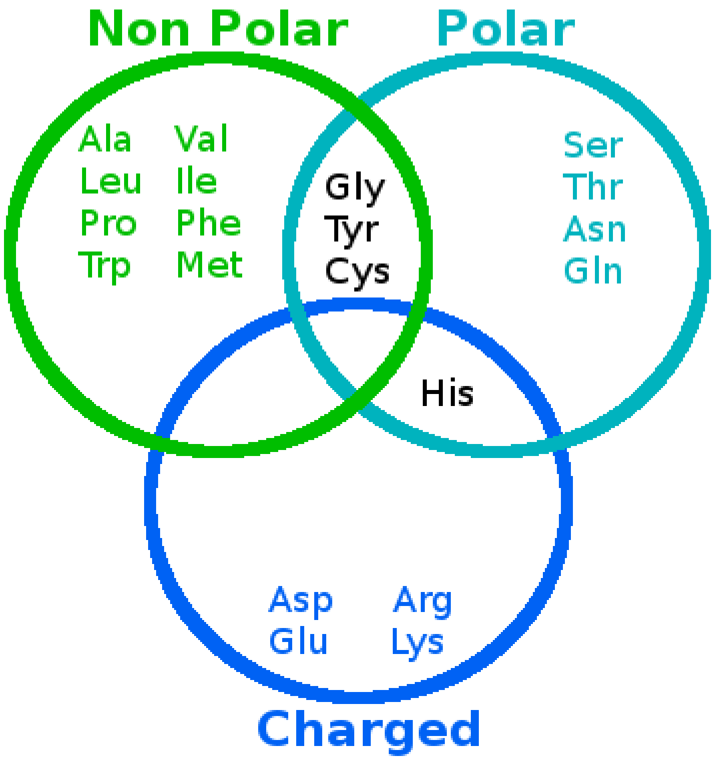
Review

Classification (Polarity)

- Amino Acids are classified according the physiochemical properties of their R-group
  - Common groupings are based upon polarity
- Polarity is defined as the magnitude of the dipole induced in the presence of an external electromagnetic field.

Amino Acids with 'intermediate' polarity

- Cys: polar when thiol (-SH) and non-polar when cystine (-S-S-)
- His: can be polar or polar charged near neutral pH
- Gly: proton is polar but R-group is small
- Tyr: polar -OH but R-group is large and aromatic



Amino acids grouped by Polarity (at neutral pH).

Classification (Hydropathy)

- Another common amino acid classification is based upon 'Hydrophobicity'
  - Hydrophobicity simply translates as 'water fearing' and is the opposite of Hydrophilicity
  - Hydrophobicity and Polarity are interrelated concepts – hydrophobic compounds are non-polar
- Hydrophobicity is a key component of the 'Hydrophobic Effect' in aqueous solution
  - Hydrophobic effect is the tendency of water to minimizes contact with hydrophobic molecules (compounds unable to effectively hydrogen bond with water exhibit large hydrophobic effects)

Superhydrophobic compounds (leaf surface) are virtually unwettable

Hydropathy Scales
(solute partitioning)

Quantifying the hydropathy of amino acids

Hydrophobicity is defined as the tendency not to dissolve in water

Experimentally, hydrophobicity values are derived from the partitioning of a solute between aqueous and non-polar solvents

- Typically add a small amount of solute to a separatory funnel containing aqueous and non-polar solvents.
- Mix, allow to equilibrate and quantify amount of solute in each solvent
- Quantified as an energy or K (partition coefficient)

$\Delta G = -RT \ln ([X]_{non\text{-}polar}/[X]_{aqueous})$ or

$K = [X]_{non\text{-}polar}/[X]_{aqueous}$

Hydropathy Scales

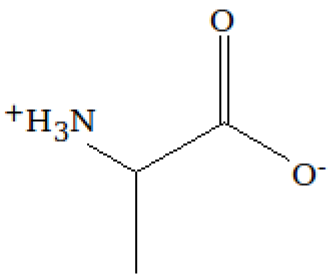
(solute partitioning)

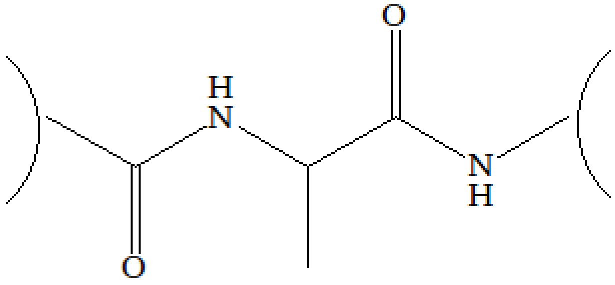
Experimentally, hydrophobicity values are derived from the partitioning of a solute between aqueous and non-polar solvents

Problem:

Hydrophobicity of a free amino acid is not the same as for the corresponding amino acid residue

Charges associated with main chain dramatically reduce hydrophobicity





Can we measure the hydrophobicity of an amino acid residue or its R-group?

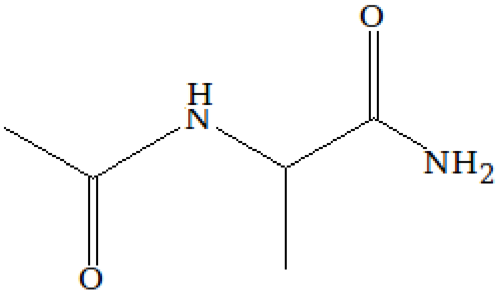
Hydropathy Scales
(solute partitioning)

Hydrophobicity of amino acid residues

Alternative model compounds:

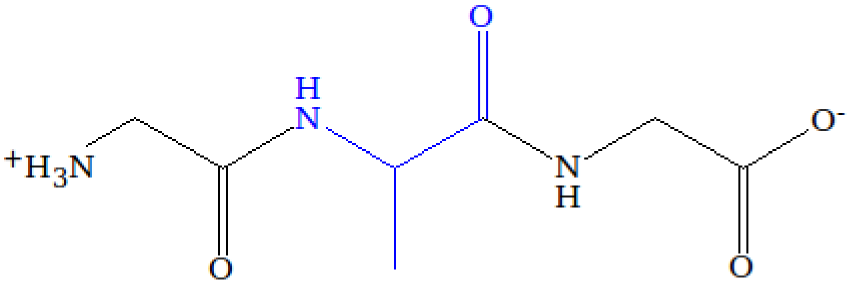
1 – Chemically synthesized R-group (ie. No main-chain atoms)

2 – Modified amino acids (acetylated and aminated main chain)



Charges associated with the main chain are neutralized by chemical modification

3 – Use tripeptides to minimize effect of main-chain charges



Hydropathy Scales

(structure-based accessibility)

Quantifying the hydropathy of amino acids

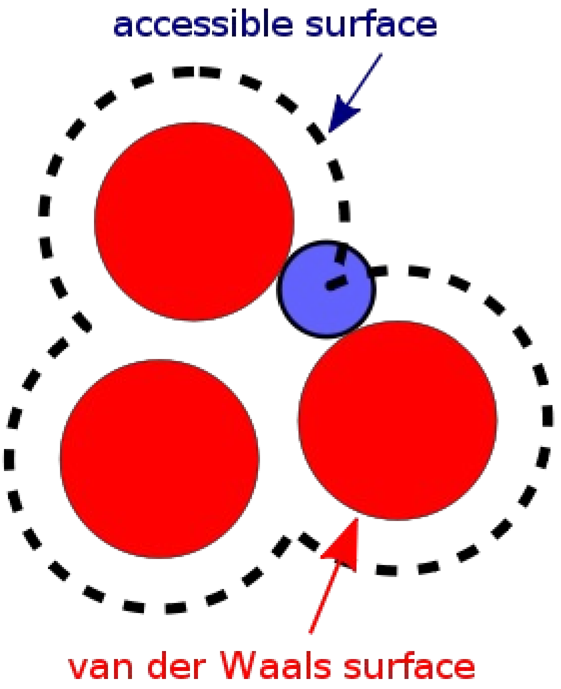
Hydrophobicity is defined as the tendency not to dissolve in water

Hydropathy values can also be derived from known protein structures

- Calculate the fraction of each residue type that is solvent accessible
- Assume solvent accessible residues are in an aqueous environment and inaccessible residues are in a non-polar environment
- Quantified using the same energy or K (partition coefficient) calculations

$$\Delta G = -RT \ln ([X]_{\text{non-polar}}/[X]_{\text{aqueous}}) \text{ or}$$

$$K = [X]_{\text{non-polar}}/[X]_{\text{aqueous}}$$



Hydropathy Scales
(structure-based accessibility)

Quantifying the hydropathy of amino acids

Hydropathy values can also be derived from known protein structures

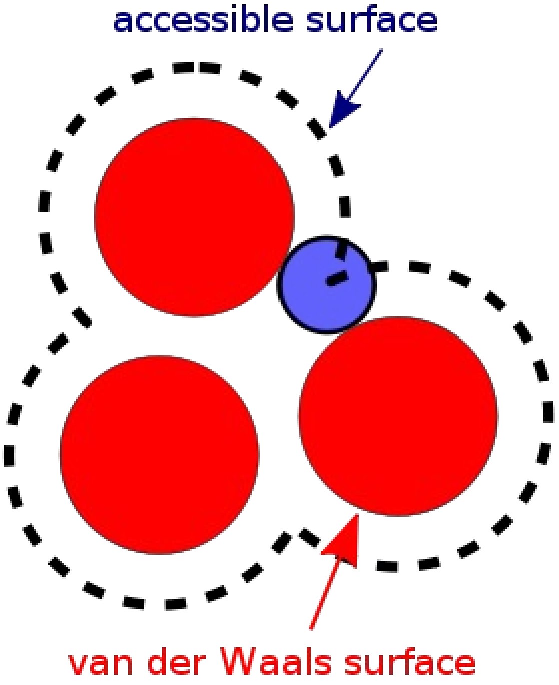
Problem:

1 – Protein structures are often determined in the presence of high concentrations of salts, glycols or organic solvents

- **How does this relate to hydrophobicity determined from partitioning experiments?**

2 – Structural constraints can bias derived hydrophobicities for residues with specific functions

- **eg. Pro is non-polar but it is preferrentially located on the surface of proteins**



Hydropathy Scales
(examples)

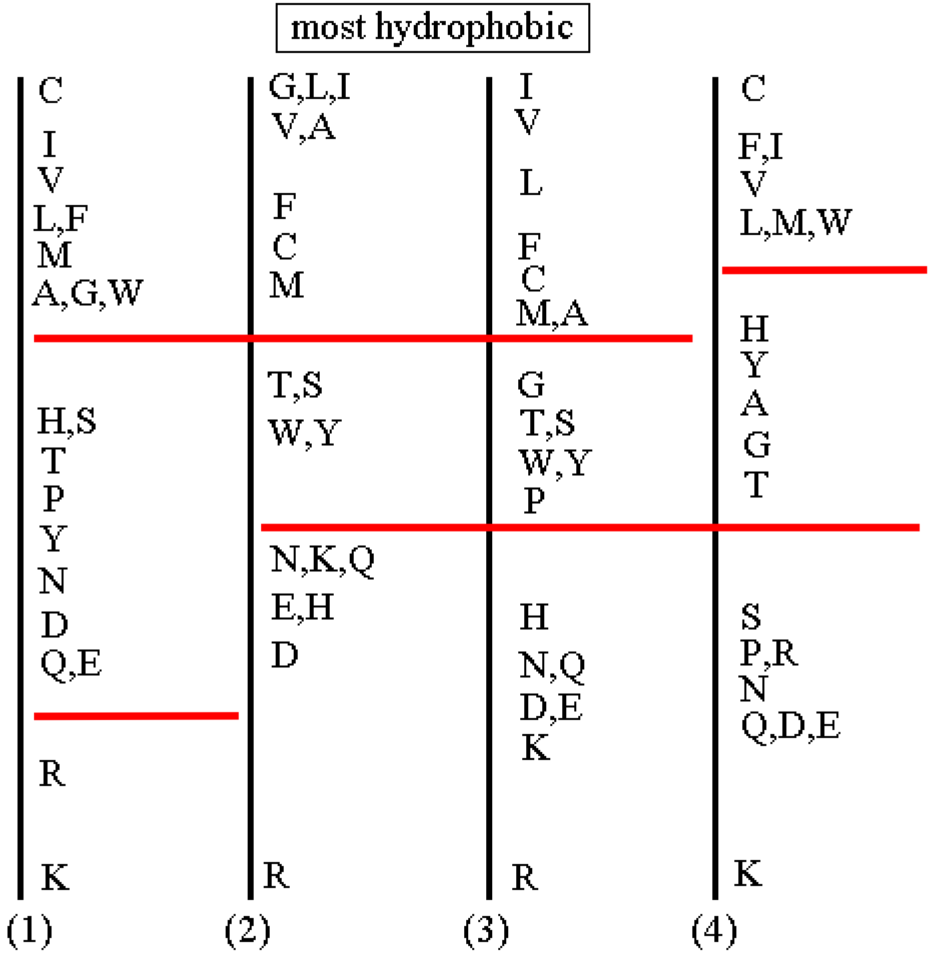
Four hydropathy scales

(2) and (3) were derived from solvent partitioning experiments using different model compounds

(1) and (4) were derived from protein structures using different criteria for accessibility

Scales are in general agreement and typically identify 3 clusters of amino acids with:

polar charged = least hydrophobic
polar = intermediate
non-polar = most hydrophobic


most hydrophobic

(1)
C
I
V
L,F
M
A,G,W
H,S
T
P
Y
N
D
Q,E
R
K

(2)
G,L,I
V,A
F
C
M
T,S
W,Y
N,K,Q
E,H
D
R

(3)
I
V
L
F
C
M,A
G
T,S
W,Y
P
H
N,Q
D,E
K
R

(4)
C
F,I
V
L,M,W
H
Y
A
G
T
S
P,R
N
Q,D,E
K

(1) J. Janin, Nature, 277(1979)
(2) R. Wolfenden, L. Andersson, P. Cullis and C. Southgate, Biochemistry 20(1981)
(3) J. Kyte and R. Doolite, J. Mol Biol. 157(1982)
(4) G. Rose, A. Geselowitz, G. Lesser, R. Lee and M. Zehfus, Science 229(1985).

Hydropathy Scales
(differences between methods)

Specific ranking within clusters vary considerably (primarily due to differences in experimental methods)

Examples:

Cysteine

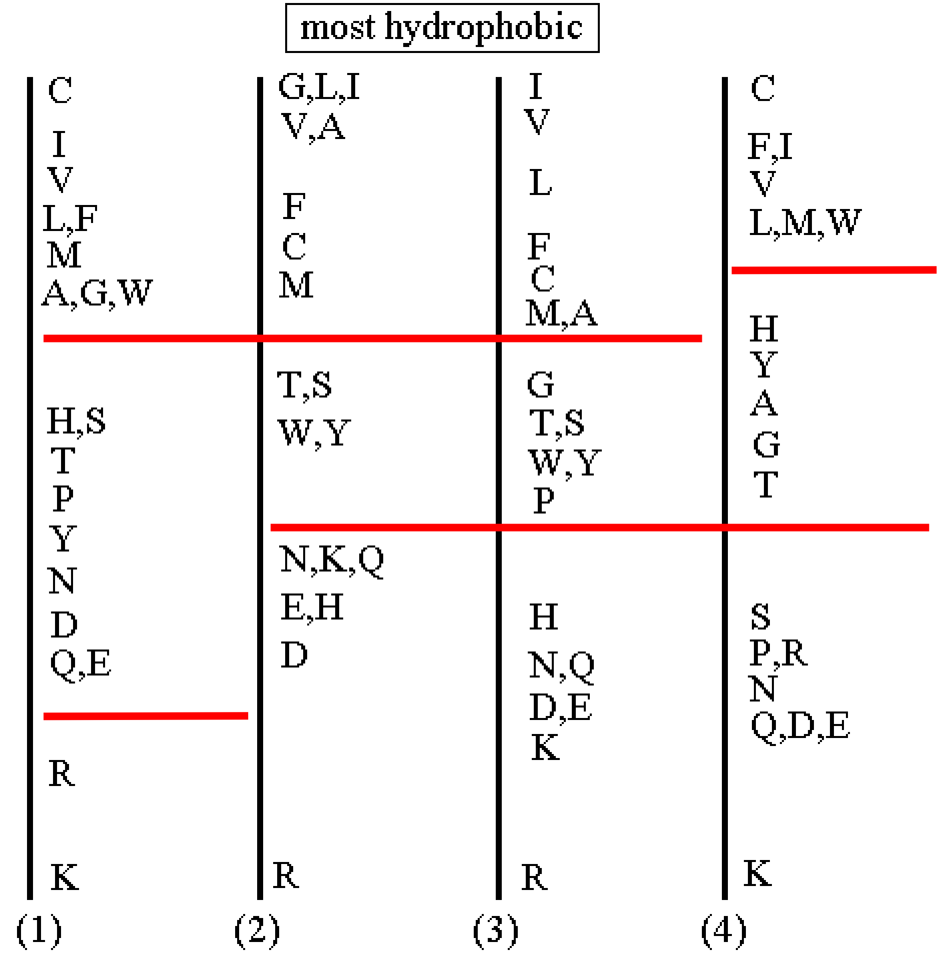
- most hydrophobic in structure-based methods
- due to the prevalence of very hydrophobic disulfides (cystine) in protein used in study

Proline

- Grouped with polar charged residues in method (4)
- Due to due structural roles (changing main-chain direction; capping helices)

Tryptophan

- Non-polar in structure-based methods
- Likely due to intermolecular interactions



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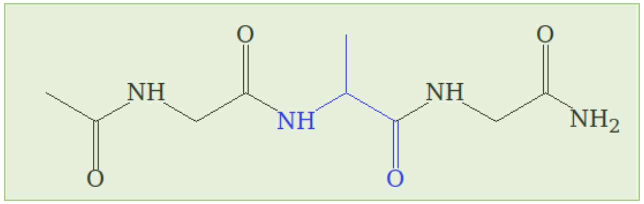
Hydropathy Scales
(example)



An example of calculated hydropathy values from (yet) another hydropathy scale (left)

- derived from tripeptide model compounds with main-chain charges neutralized

Log $K_{eq,tr}$ = 0
- indicates equal distribution between polar and non-polar phases

Example: energetic driving force
$Leu_{(water)} \leftrightarrow Leu_{(non\text{-}polar)}$

$\Delta G = - R\ T \ln K_{eq} \approx -20$ kJ/mol
(comparable to strong H-bond)

Note: cost of burying a charged residue (eg. Arg) is large and unfavorable (consistent with known structures)

	log $K_{eq,tr}$
Leucine	3.62
Isoleucine	3.62
Valine	2.97
Proline	2.51
Phenylalanine	2.19
Methionine	1.73
Tryptophan	1.71
Alanine	1.33
Cysteine	0.93
Glycine	0.69
Tyrosine	-0.10
Threonine	-1.89
Serine	-2.49
Histidine	-3.42
Glutamine	-4.07
Lysine	-4.08
Aparagine	-4.88
Glutamate	-5.00
Aspartate	-6.41
Arginine	-10.97

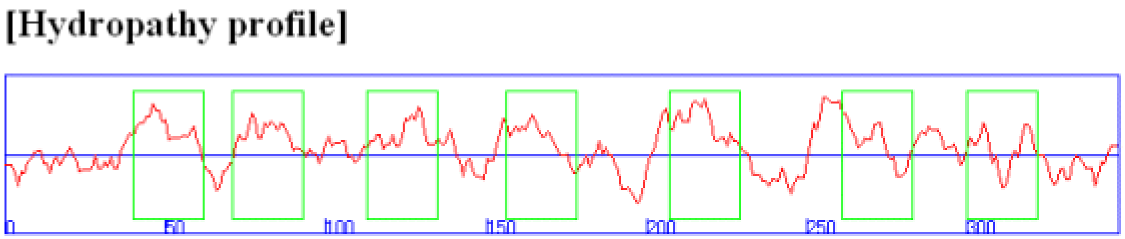
$K_{eq,tr} = [X]_{non\text{-}polar}/[X]_{aqueous}$

Hydropathy Scales
(uses)

Hydropathy parameters are often determined to characterize binding, catalysis or structure

QSAR (Quantitative Structure Activity Relationships)
- perform binding and catalysis studies using series of related compounds
- developed from initial work by Hammett describing how K_{eq} varies as a function of structure

Hydropathy plots (Kyte-Dolittle)
- identification of regions contributing to the hydrophobic core (or transmembrane helices)



- 7 transmembrane helices of rhodopsin on hydropathy plot (hydropathy value vs. primary sequence)

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Glutamate	-5.00
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$K_{eq,tr} = [X]_{non\text{-}polar}/[X]_{aqueous}$

Hydropathy Scales
(more physical methods)

Many indirect physical methods have been developed to measure hydropathy:

Reverse Phase Chromatography

Site-directed mutation and Protein thermal stability

Molar Heat Capacity

Transition temperature

Surface Tension

In broad terms, each of the methods yield similar hydropathy scales.

Specific differences between hydropathy scales are typically due to differences in experimental methods.

Hydropathy Scales
(uses)

Hydropathy parameters are often determined to characterize binding, catalysis or structure

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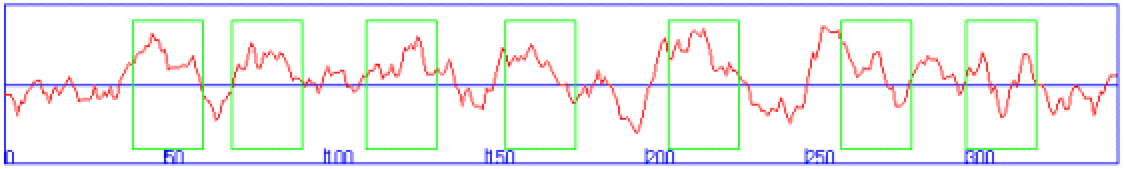
[Hydropathy profile]



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$K_{eq,tr} = [X]_{non\text{-}polar}/[X]_{aqueous}$

Sequence Similarity
(hydropathy and similarity)

All bioinformatic approaches require some quantitative measure to objectively evaluate agreement between a 'query' and a 'database' item

In the case of sequence searches, the quantitative measure is sequence similarity

Similarity (definition) – *The common physiochemical properties necessary to maintain the structural and functional properties of a biological macromolecule.*

Note: this implies that sequence searches are often detecting homology (eg. divergent evolution)

Example (simple)

Name									*Matches*	*Sub. C*	*Sub. NC*	*Gaps*
Query	A	G	T	R	V	L	Q	Q				
Target 1	A	G	T	R	-	L	Q	Q	7	0	0	1
Target 2	A	G	T	R	F	L	Q	Q	7	1	0	0
Target 3	A	G	S	K	E	E	Q	Q	4	2	2	0
Target 4	G	A	S	K	A	I	N	E	0	8	0	0

Sub. C is conservative substitutions and Sub. NC is non-conservative substitutions.

The query sequence is compared to a database of sequences and the above 4 matches are found -

Are each of these sequences similar to the query sequence?

Which target sequences are most and least similar to the query sequence?

To answer these fundamental questions we must quantify similarity.

Quantification of Similarity

Similarity is a non-SI unit that does not have a universally accepted quantitative definition.

- At least three quantitative (or semi-quantitative) methods have been utilized to quantify similarity
- (1) observed mutational frequencies in homologous proteins
- (2) amino acid hydropathy scales
- (3) accessible surface area using known protein structures

In each case, the quantitative method calculates a value that represents the similarity between any two amino acids

- For simplicity, the similarity values between residues is stored in a table (Similarity Table or Similarity Matrix)
- Using a Similarity Table, a similarity score can be calculated for any aligned sequences.

Example: Calculating similarity from mutational frequencies

Mutational frequencies are derived from aligned sequences of conserved protein families (eg. all globins or all cytochromes)

1) Each residue is mutated (to any other) at some frequency

2) Specific mutations (ie. Ala → Gly) also have an observed mutational frequency **Observed freq. (Ala → Gly) = (# of Ala → Gly) / (# of Ala mutations)**

3) Specific mutation rates are compared to expected rates based upon random mutation (ie. observed mutations are not randomly distributed) to quantify a 'SCORE'

SCORE = Observed freq. (Ala → Gly) / Expected$_{\text{random}}$ freq. (Ala → Gly)

Resulting SCORE can range over many orders of magnitude for all possible mutations

Typically express SCORE as the log (SCORE) to simplify representation

Note: Expressing the ratio as a log is an arbitrary choice that works reasonably well

Similarity Tables

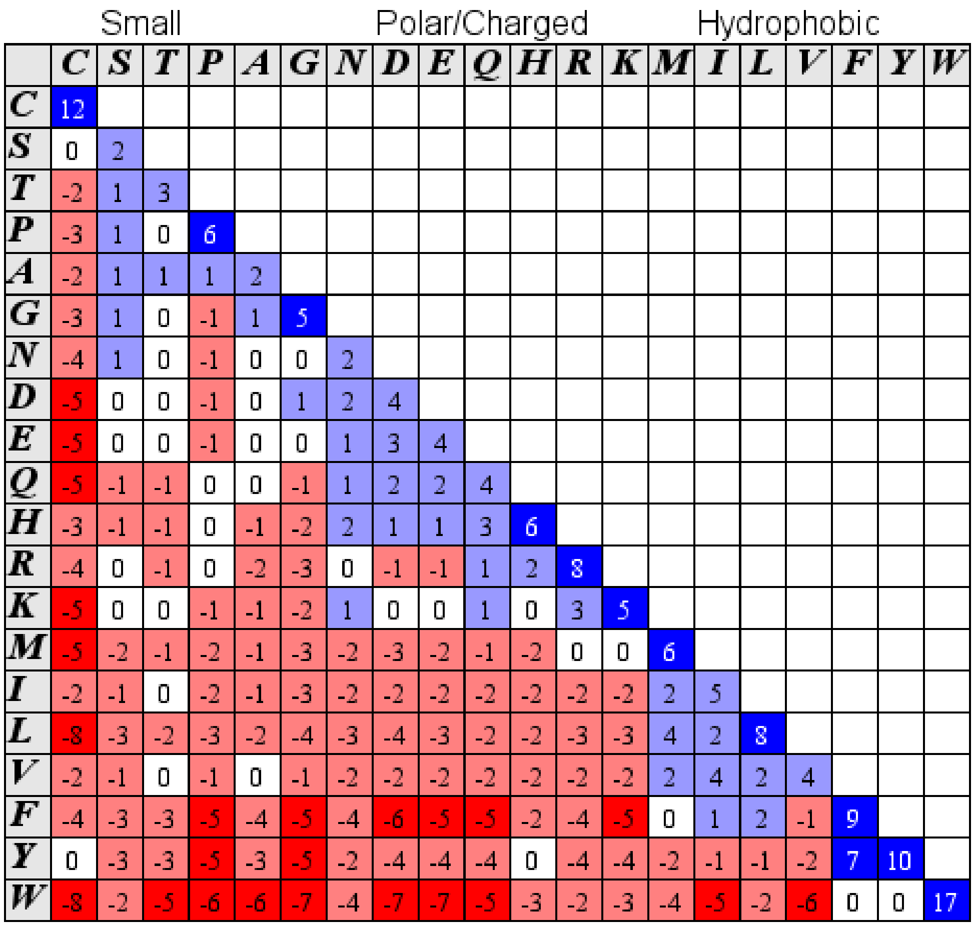
A similarity table based upon mutational frequencies (PAM250 = over a given evolutionary time scale)

Each cell in the table represents a 'similarity score' between any two residues

Positive values (blues) indicate the residues are similar

Negative values (reds) indicate the residues are dissimilar

Note: most residue pairs have negative values suggesting mutations are generally deleterious



Small Polar/Charged Hydrophobic

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	12																			
S	0	2																		
T	-2	1	3																	
P	-3	1	0	6																
A	-2	1	1	1	2															
G	-3	1	0	-1	1	5														
N	-4	1	0	-1	0	0	2													
D	-5	0	0	-1	0	1	2	4												
E	-5	0	0	-1	0	0	1	3	4											
Q	-5	-1	-1	0	0	-1	1	2	2	4										
H	-3	-1	-1	0	-1	-2	2	1	1	3	6									
R	-4	0	-1	0	-2	-3	0	-1	-1	1	2	8								
K	-5	0	0	-1	-1	-2	1	0	0	1	0	3	5							
M	-5	-2	-1	-2	-1	-3	-2	-3	-2	-1	-2	0	0	6						
I	-2	-1	0	-2	-1	-3	-2	-2	-2	-2	-2	-2	-2	2	5					
L	-8	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	8				
V	-2	-1	0	-1	0	-1	-2	-2	-2	-2	-2	-2	-2	2	4	2	4			
F	-4	-3	-3	-5	-4	-5	-4	-6	-5	-5	-2	-4	-5	0	1	2	-1	9		
Y	0	-3	-3	-5	-3	-5	-2	-4	-4	-4	0	-4	-4	-2	-1	-1	-2	7	10	
W	-8	-2	-5	-6	-6	-7	-4	-7	-7	-5	-3	-2	-3	-4	-5	-2	-6	0	0	17

PAM250

🟦	> 4	5.70%
🟦	1 to 4	19.50%
	0	16.20%
🟥	-1 to -4	47.20%
🟥	< -4	11.40%

Diagonal elements indicate likelyhood of conservation

More Similarity Tables

Evolutionary models for calculating sequence similarity typically outperform all other

- PAM and BLOSUM similarity tables are the most widely used

PAM or point accepted mutation (developed by Dayhoff) tables

- Utilize mutational frequencies within a small set of closely related proteins
- Consider all mutations and phylogenetic branches

BLOSUM or block summation tables are a slight improvement to PAM

- Does not consider all mutation or use phylogenetic branches
- Not all mutations are treated equally

In either case, the sequence identity within the set of closely related proteins used to calculate mutational frequencies is a variable.

- Sequence similarity searches always perform best when the sequence identity used to calculate mutational frequencies matches that of the target

Example: Similarity Scores

Name									*Matches*	*Sub. C*	*Sub. NC*	*Gaps*
Query	A	G	T	R	V	L	Q	Q				
Target 1	A	G	T	R	-	L	Q	Q	7	0	0	1
Query:Target1	2	5	3	8	-10	8	4	4	Total=24			
Target 2	A	G	T	R	F	L	Q	Q	7	1	0	0
Query:Target2	2	5	3	8	-1	8	4	4	Total=33			
Target 3	A	G	S	K	E	E	Q	Q	4	2	2	0
Query:Target3	2	5	1	3	-2	-3	4	4	Total=14			
Target 4	G	A	S	K	A	I	N	E	0	8	0	0
Query:Target4	1	1	1	3	0	4	1	2	Total=13			

The similarity score between any two sequences is simply :

Σ (similarity score for each residue pair) – Gap penalty

Using the PAM250 Similarity Table, we see Target 2 is most similar to the query sequence and Target 4 is least similar – IDENTITY IS THE MOST IMPORTANT FACTOR IN HIGH SCORES

Caveats: sequence length and composition influence the magnitude of the score

So ... are they homologs?

Can't answer this yet ... we need to know how similar two aligned sequences can be as a result of random chance

Calculating Similarity due to random chance:

Assumption 1: All residues occur at equal frequency in protein sequences

For sequences of equal length, the average sequence identity is 5% with 95% of alignments between 0-10%

Assumption 2: Protein sequences may have N- or C-terminal extensions

Average sequence identity is 8% with 95% of alignments between 4-12%

Assumption 1 (modified): Residues do not occur at equal frequency in proteins

Average sequence identity is ~10% with 95% of alignments between 5-15%

Assumption 2 (modified): Protein sequences may have inserted/deleted sequences

Average sequence identity is ~20% with 95% of alignments between 15-25%

Sequence identities up to 25% may be solely due to random chance

Where are we now?

Summary

(1) We have a method for calculating/quantifying residue similarity

Similarity tables calculated using mutation, physiochemical or structural properties of amino acids

(2) We have a method for calculating sequence similarity

Simply sum the similarity table scores for the aligned sequence including 'gap' and 'gap extension' penalties

(3) We have calculated the point at which sequence similarity is significant

While we used identities in the example calculation, sequences with aligned identities greater than 25% are likely homologs

Caveat: For very short sequences (<30 residues) the point at which sequence similarity is statistically significant rises sharply