Blosum matrices

What are they?

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BioSys, DTU

Outline

- Alignment scoring matrices
  - How are Blosum matrices constructed?
  - What is a BLOSUM50 matrix and how is it different from a BLOSUM80 matrix?
  - What is the difference between a Blosum scoring matrix and the Blosum frequency substitution matrix?

Sequence Alignment

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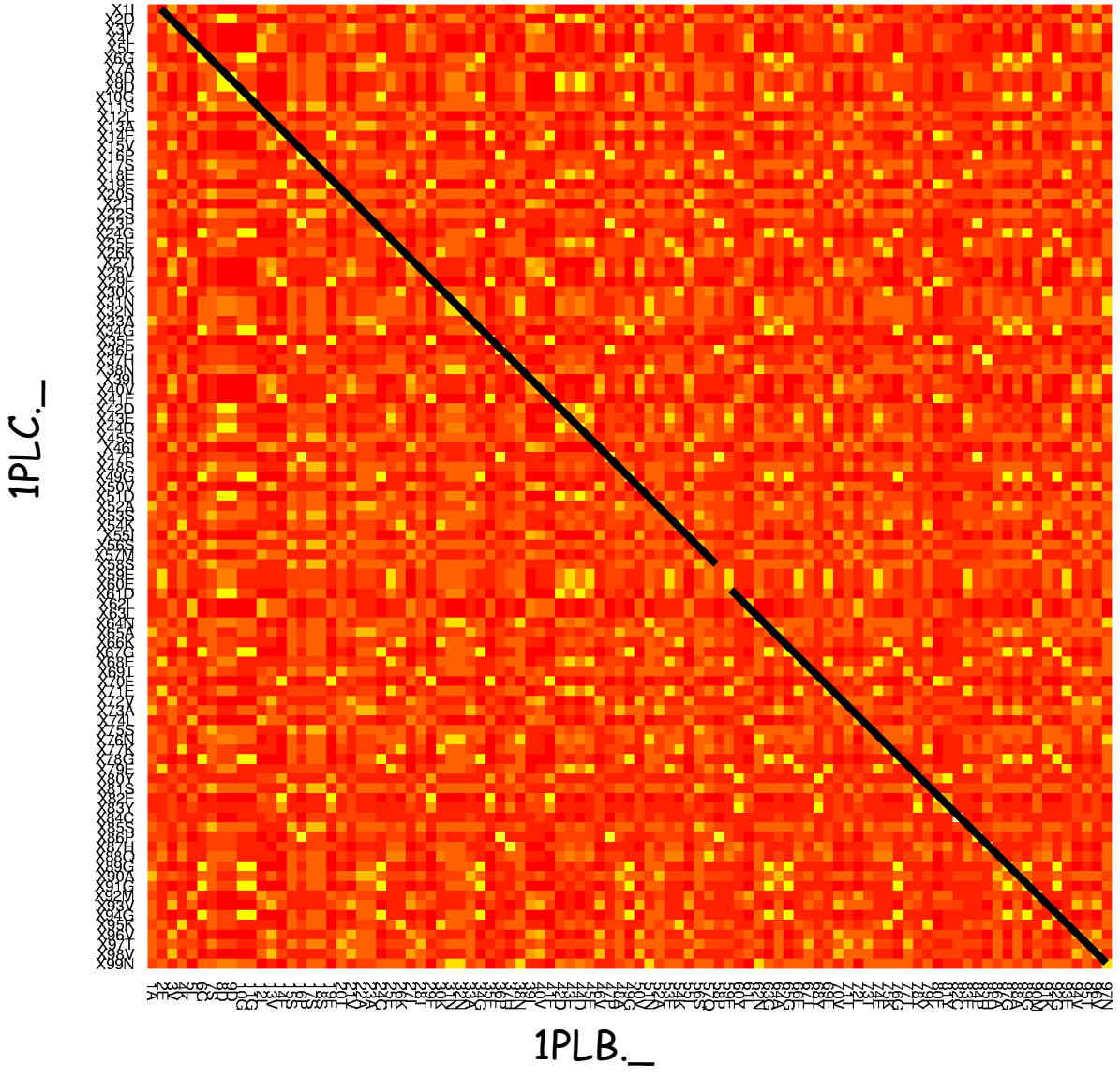

Where is the active site?

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Sequence alignment. Infer function (and functional residues) from one protein to another

```
1K7C.A TVYLAGDSTMAKNGGGSGTNGWGEYLASYLSATVVNDAVAGRSARSYTREGRFENIADVVTAGDYVIVEFGHNDGGSLSTDN
              S                                  G                                  N
1WAB._ EVVFIGDSLVQLMHQCE---IWRELFS---PLHALNFGIGGDSTQHVLW--RLENGELEHIRPKIVVVWVGTNNHG------

1K7C.A GRTDCSGTGAEVCYSVYDGVNETILTFPAYLENAAKLFTAK--GAKVILSSQTPNNPWETGTFVNSPTRFVEYAEL-AAEVA
1WAB._ ----------------------HTAEQVTGGIKAIVQLVNERQPQARVVVLGLLPRGQ-HPNPLREKNRRVNELVRAALAGHP

1K7C.A GVEYVDHWSYVDSIYETLGNATVNSYFPIDHTHTSPAGAEVVAEAFLKAVVCTGTSL
                                        H
1WAB._ RAHFLDADPG---FVHSDG--TISHHDMYDYLHLSRLGYTPVCRALHSLLLRL---L
```

Homology modeling and the human genome

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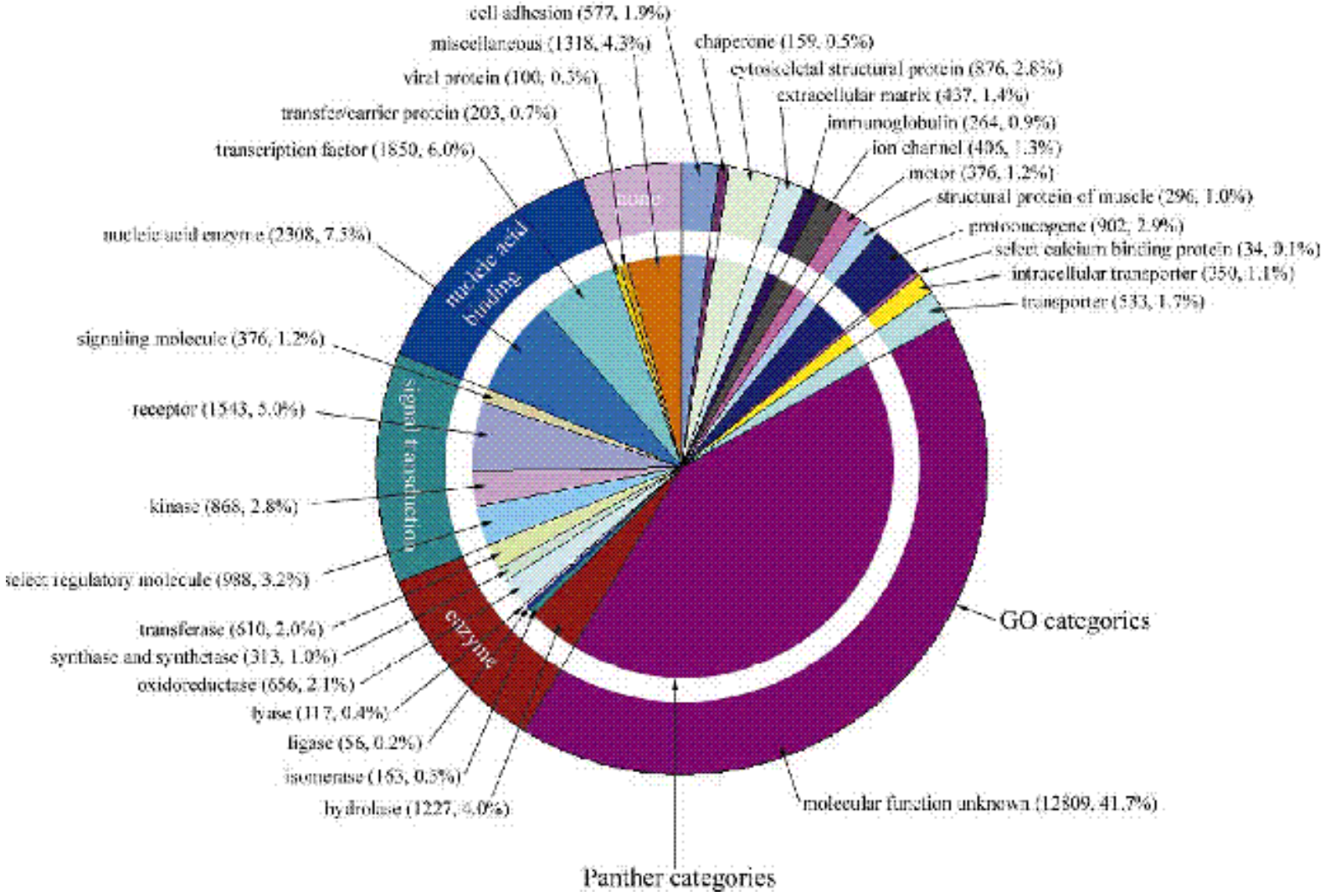

BLOSUM = BLOck SUbstitution Matrices

- Focus on conserved domains, MSA's (multiple sequence alignment) are ungapped blocks.
- Compute pairwise amino acid alignment counts
  - Count amino acid replacement frequencies directly from columns in blocks
  - Sample bias:
    - Cluster sequences that are x% similar.
    - Do not count amino acid pairs within a cluster.
    - Do count amino acid pairs across clusters, treating clusters as an "average sequence".
    - Normalize by the number of sequences in the cluster.
- BLOSUMXX matrices
  - Sequences that are xx% similar are clustered during the construction of the matrix.

Log-odds scores

- Log-odds scores are given by
  - Log(Observation/Expected)
- The log-odd score of matching amino acid j with amino acid i in an alignment is

$$\log(\frac{P_{ij}}{Q_i \cdot Q_j})$$

- where P_{ij} is the frequency of observing amino i aligned with j, and Q_i, Q_j are the frequencies of amino acids i and j in the data set.
- The log-odd score is (in half bit units)

$$S_{ij} = 2 \cdot \log_2(\frac{P_{ij}}{Q_i \cdot Q_j})$$

So what does this mean? An example

N_{AA}	= 14
N_{AD}	= 5
N_{AV}	= 5
N_{DA}	= 5
N_{DD}	= 8
N_{DV}	= 2
N_{VA}	= 5
N_{VD}	= 2
N_{VV}	= 2

P_{AA}	= 14/48
P_{AD}	= 5/48
P_{AV}	= 5/48
P_{DA}	= 5/48
P_{DD}	= 8/48
P_{DV}	= 2/48
P_{VA}	= 5/48
P_{VD}	= 2/48
P_{VV}	= 2/48

1: VVAD
2: AAAD
3: DVAD
4: DAAA

MSA

Q_A	= 8/16
Q_D	= 5/16
Q_V	= 3/16

So what does this mean?

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$P_{AA} = 0.29$	$Q_A Q_A = 0.25$
$P_{AD} = 0.10$	$Q_A Q_D = 0.16$
$P_{AV} = 0.10$	$Q_A Q_V = 0.09$
$P_{DA} = 0.10$	$Q_D Q_A = 0.16$
$P_{DD} = 0.17$	$Q_D Q_D = 0.10$
$P_{DV} = 0.04$	$Q_D Q_V = 0.06$
$P_{VA} = 0.10$	$Q_V Q_A = 0.09$
$P_{VD} = 0.04$	$Q_V Q_D = 0.06$
$P_{VV} = 0.04$	$Q_V Q_V = 0.03$

1: VVAD
2: AAAD
3: DVAD
4: DAAA

MSA

$Q_A = 0.50$
$Q_D = 0.31$
$Q_V = 0.19$

So what does this mean?

$P_{AA} = 0.29$	$Q_A Q_A = 0.25$	$S_{AA} = 0.44$
$P_{AD} = 0.10$	$Q_A Q_D = 0.16$	$S_{AD} = -1.17$
$P_{AV} = 0.10$	$Q_A Q_V = 0.09$	$S_{AV} = 0.30$
$P_{DA} = 0.10$	$Q_D Q_A = 0.16$	$S_{DA} = -1.17$
$P_{DD} = 0.17$	$Q_D Q_D = 0.10$	$S_{DD} = 1.54$
$P_{DV} = 0.04$	$Q_D Q_V = 0.06$	$S_{DV} = -0.98$
$P_{VA} = 0.10$	$Q_V Q_A = 0.09$	$S_{VA} = 0.30$
$P_{VD} = 0.04$	$Q_V Q_D = 0.06$	$S_{VD} = -0.98$
$P_{VV} = 0.04$	$Q_V Q_V = 0.03$	$S_{VV} = 0.49$

- BLOSUM is a log-likelihood matrix:

$$S_{ij} = 2\log_2(P_{ij}/(Q_i Q_j))$$

The Scoring matrix

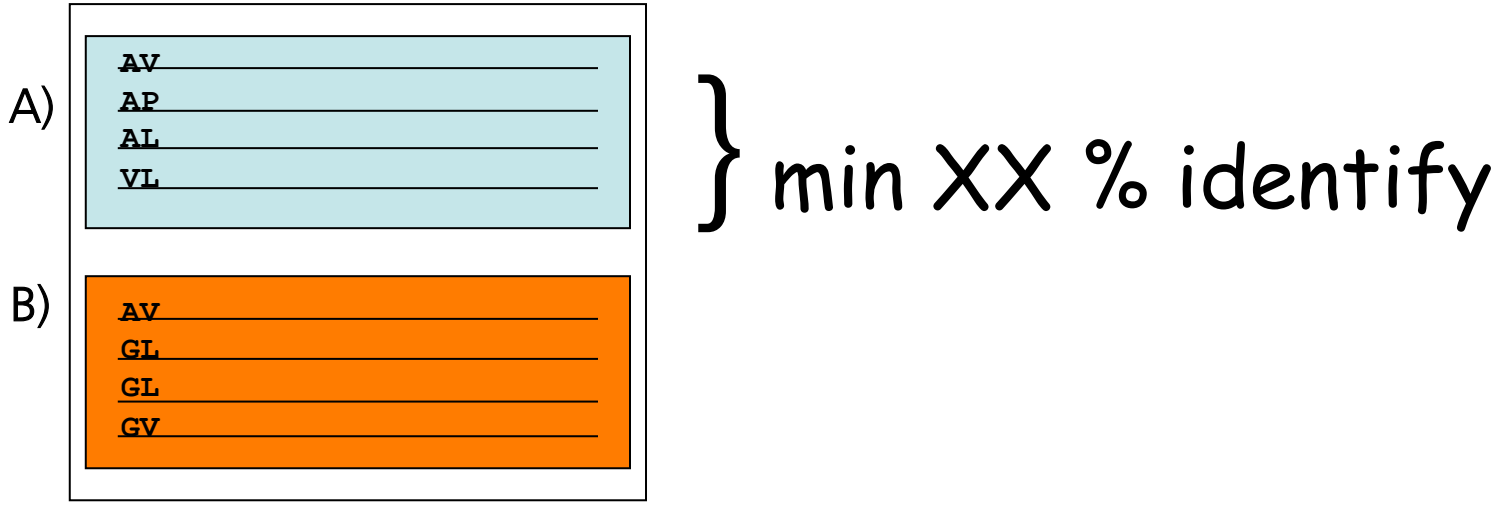
	A	D	V
A	0.44	-1.17	0.30
D	-1.17	1.54	-0.98
V	0.30	-0.98	0.49

```
1: VVAD
2: AAAD
3: DVAD
4: DAAA
```

MSA

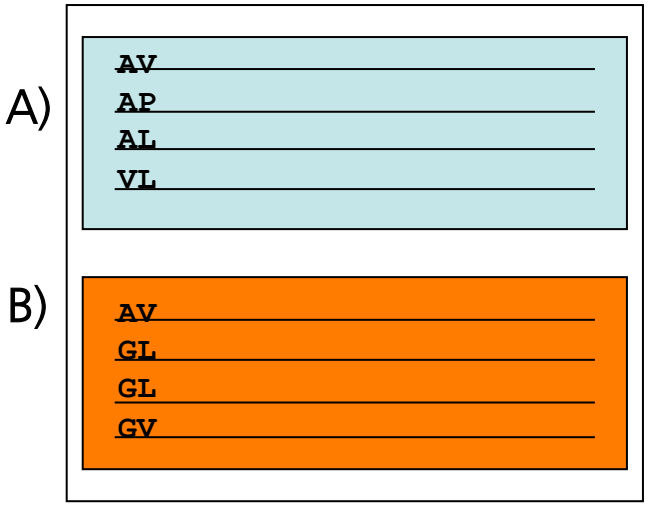
And what does the BLOSUMXX mean?

- Cluster sequence Blocks at XX% identity
- Do statistics only *<u>across</u>* clusters



- Normalize statistics according to cluster size

And what does the BLOSUMXX mean?



$$N_{AA} = \frac{3}{4} \cdot \frac{1}{4} = \frac{3}{16}$$

$$N_{AG} = \frac{3}{4} \cdot \frac{3}{4} = \frac{9}{16}$$

$$N_{VA} = \frac{1}{4} \cdot \frac{1}{4} = \frac{1}{16}$$

$$N_{VG} = \frac{1}{4} \cdot \frac{3}{4} = \frac{3}{16}$$

And what does the BLOSUMXX mean?

- High Blosum values mean *high similarity between* clusters
  - Conserved substitution dominate
- Low Blosum values mean *low similarity between* clusters
  - Less conserved substitutions dominate

BLOSUM80

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	7	-3	-3	-3	-1	-2	-2	0	-3	-3	-3	-1	-2	-4	-1	2	0	-5	-4	-1
R	-3	9	-1	-3	-6	1	-1	-4	0	-5	-4	3	-3	-5	-3	-2	-2	-5	-4	-4
N	-3	-1	9	2	-5	0	-1	-1	1	-6	-6	0	-4	-6	-4	1	0	-7	-4	-5
D	-3	-3	2	10	-7	-1	2	-3	-2	-7	-7	-2	-6	-6	-3	-1	-2	-8	-6	-6
C	-1	-6	-5	-7	13	-5	-7	-6	-7	-2	-3	-6	-3	-4	-6	-2	-2	-5	-5	-2
Q	-2	1	0	-1	-5	9	3	-4	1	-5	-4	2	-1	-5	-3	-1	-1	-4	-3	-4
E	-2	-1	-1	2	-7	3	8	-4	0	-6	-6	1	-4	-6	-2	-1	-2	-6	-5	-4
G	0	-4	-1	-3	-6	-4	-4	9	-4	-7	-7	-3	-5	-6	-5	-1	-3	-6	-6	-6
H	-3	0	1	-2	-7	1	0	-4	12	-6	-5	-1	-4	-2	-4	-2	-3	-4	3	-5
I	-3	-5	-6	-7	-2	-5	-6	-7	-6	7	2	-5	2	-1	-5	-4	-2	-5	-3	4
L	-3	-4	-6	-7	-3	-4	-6	-7	-5	2	6	-4	3	0	-5	-4	-3	-4	-2	1
K	-1	3	0	-2	-6	2	1	-3	-1	-5	-4	8	-3	-5	-2	-1	-1	-6	-4	-4
M	-2	-3	-4	-6	-3	-1	-4	-5	-4	2	3	-3	9	0	-4	-3	-1	-3	-3	1
F	-4	-5	-6	-6	-4	-5	-6	-6	-2	-1	0	-5	0	10	-6	-4	-4	0	4	-2
P	-1	-3	-4	-3	-6	-3	-2	-5	-4	-5	-5	-2	-4	-6	12	-2	-3	-7	-6	-4
S	2	-2	1	-1	-2	-1	-1	-1	-2	-4	-4	-1	-3	-4	-2	7	2	-6	-3	-3
T	0	-2	0	-2	-2	-1	-2	-3	-3	-2	-3	-1	-1	-4	-3	2	8	-5	-3	0
W	-5	-5	-7	-8	-5	-4	-6	-6	-4	-5	-4	-6	-3	0	-7	-6	-5	16	3	-5
Y	-4	-4	-4	-6	-5	-3	-5	-6	3	-3	-2	-4	-3	4	-6	-3	-3	3	11	-3
V	-1	-4	-5	-6	-2	-4	-4	-6	-5	4	1	-4	1	-2	-4	-3	0	-5	-3	7

$\langle S_{ii} \rangle = 9.4$

$\langle S_{ij} \rangle = -2.9$

BLOSUM30

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	0	0	-3	1	0	0	-2	0	-1	0	1	-2	-1	1	1	-5	-4	1
R	-1	8	-2	-1	-2	3	-1	-2	-1	-3	-2	1	0	-1	-1	-1	-3	0	0	-1
N	0	-2	8	1	-1	-1	-1	0	-1	0	-2	0	0	-1	-3	0	1	-7	-4	-2
D	0	-1	1	9	-3	-1	1	-1	-2	-4	-1	0	-3	-5	-1	0	-1	-4	-1	-2
C	-3	-2	-1	-3	17	-2	1	-4	-5	-2	0	-3	-2	-3	-3	-2	-2	-2	-6	-2
Q	1	3	-1	-1	-2	8	2	-2	0	-2	-2	0	-1	-3	0	-1	0	-1	-1	-3
E	0	-1	-1	1	1	2	6	-2	0	-3	-1	2	-1	-4	1	0	-2	-1	-2	-3
G	0	-2	0	-1	-4	-2	-2	8	-3	-1	-2	-1	-2	-3	-1	0	-2	1	-3	-3
H	-2	-1	-1	-2	-5	0	0	-3	14	-2	-1	-2	2	-3	1	-1	-2	-5	0	-3
I	0	-3	0	-4	-2	-2	-3	-1	-2	6	2	-2	1	0	-3	-1	0	-3	-1	4
L	-1	-2	-2	-1	0	-2	-1	-2	-1	2	4	-2	2	2	-3	-2	0	-2	3	1
K	0	1	0	0	-3	0	2	-1	-2	-2	-2	4	2	-1	1	0	-1	-2	-1	-2
M	1	0	0	-3	-2	-1	-1	-2	2	1	2	2	6	-2	-4	-2	0	-3	-1	0
F	-2	-1	-1	-5	-3	-3	-4	-3	-3	0	2	-1	-2	10	-4	-1	-2	1	3	1
P	-1	-1	-3	-1	-3	0	1	-1	1	-3	-3	1	-4	-4	11	-1	0	-3	-2	-4
S	1	-1	0	0	-2	-1	0	0	-1	-1	-2	0	-2	-1	-1	4	2	-3	-2	-1
T	1	-3	1	-1	-2	0	-2	-2	-2	0	0	-1	0	-2	0	2	5	-5	-1	1
W	-5	0	-7	-4	-2	-1	-1	1	-5	-3	-2	-2	-3	1	-3	-3	-5	20	5	-3
Y	-4	0	-4	-1	-6	-1	-2	-3	0	-1	3	-1	-1	3	-2	-2	-1	5	9	1
V	1	-1	-2	-2	-2	-3	-3	-3	-3	4	1	-2	0	1	-4	-1	1	-3	1	5

$\langle S_{ii} \rangle = 8.3$

$\langle S_{ij} \rangle = -1.16$

The different Blosum matrices

- The BLOSUM alignment scoring matrix is a log-likelihood matrix

$$S_{ij} = 2 \cdot \log_2\left(\frac{P_{ij}}{Q_i \cdot Q_j}\right)$$

- The Blosum frequency substitution matrix, is a conditional probability matrix of matching amino acids j given you have amino acid i

$$P(j \mid i) = \frac{P_{ij}}{Q_i}$$

- and

$$S_{ij} = 2 \cdot \log_2\left(\frac{P_{ij}}{Q_i \cdot Q_j}\right) = 2 \cdot \log_2\left(\frac{P(j \mid i)}{Q_j}\right)$$

The way from frequencies to log-odds

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	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	0.29	0.03	0.03	0.03	0.02	0.03	0.04	0.08	0.01	0.04	0.06	0.04	0.02	0.02	0.03	0.09	0.05	0.01	0.02	0.07
R	0.04	0.34	0.04	0.03	0.01	0.05	0.05	0.03	0.02	0.02	0.05	0.12	0.02	0.02	0.02	0.04	0.03	0.01	0.02	0.03
N	0.04	0.04	0.32	0.08	0.01	0.03	0.05	0.07	0.03	0.02	0.03	0.05	0.01	0.02	0.02	0.07	0.05	0.00	0.02	0.03
D	0.04	0.03	0.07	0.40	0.01	0.03	0.09	0.05	0.02	0.02	0.03	0.04	0.01	0.01	0.02	0.05	0.04	0.00	0.01	0.02
C	0.07	0.02	0.02	0.02	0.48	0.01	0.02	0.03	0.01	0.04	0.07	0.02	0.02	0.02	0.02	0.04	0.04	0.00	0.01	0.06
Q	0.06	0.07	0.04	0.05	0.01	0.21	0.10	0.04	0.03	0.03	0.05	0.09	0.02	0.01	0.02	0.06	0.04	0.01	0.02	0.04
E	0.06	0.05	0.04	0.09	0.01	0.06	0.30	0.04	0.03	0.02	0.04	0.08	0.01	0.02	0.03	0.06	0.04	0.01	0.02	0.03
G	0.08	0.02	0.04	0.03	0.01	0.02	0.03	0.51	0.01	0.02	0.03	0.03	0.01	0.02	0.02	0.05	0.03	0.01	0.01	0.02
H	0.04	0.05	0.05	0.04	0.01	0.04	0.05	0.04	0.35	0.02	0.04	0.05	0.02	0.03	0.02	0.04	0.03	0.01	0.06	0.02
I	0.05	0.02	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.27	0.17	0.02	0.04	0.04	0.01	0.03	0.04	0.01	0.02	0.18
L	0.04	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.01	0.12	0.38	0.03	0.05	0.05	0.01	0.02	0.03	0.01	0.02	0.10
K	0.06	0.11	0.04	0.04	0.01	0.05	0.07	0.04	0.02	0.03	0.04	0.28	0.02	0.02	0.03	0.05	0.04	0.01	0.02	0.03
M	0.05	0.03	0.02	0.02	0.02	0.03	0.03	0.03	0.02	0.10	0.20	0.04	0.16	0.05	0.02	0.04	0.04	0.01	0.02	0.09
F	0.03	0.02	0.02	0.02	0.01	0.01	0.02	0.03	0.02	0.06	0.11	0.02	0.03	0.39	0.01	0.03	0.03	0.02	0.09	0.06
P	0.06	0.03	0.02	0.03	0.01	0.02	0.04	0.04	0.01	0.03	0.04	0.04	0.01	0.01	0.49	0.04	0.04	0.00	0.01	0.03
S	0.11	0.04	0.05	0.05	0.02	0.03	0.05	0.07	0.02	0.03	0.04	0.05	0.02	0.02	0.03	0.22	0.08	0.01	0.02	0.04
T	0.07	0.04	0.04	0.04	0.02	0.03	0.04	0.04	0.01	0.05	0.07	0.05	0.02	0.02	0.03	0.09	0.25	0.01	0.02	0.07
W	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.03	0.05	0.02	0.02	0.06	0.01	0.02	0.02	0.49	0.07	0.03
Y	0.04	0.03	0.02	0.02	0.01	0.02	0.03	0.02	0.05	0.04	0.07	0.03	0.02	0.13	0.02	0.03	0.03	0.03	0.32	0.05
V	0.07	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.16	0.13	0.03	0.03	0.04	0.02	0.03	0.05	0.01	0.02	0.27

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

$$S_{AA} = 2 \cdot \log_2\left(\frac{P(A \mid A)}{Q_A}\right) = 2 \cdot \log_2\left(\frac{0.29}{0.074}\right) = 3.9$$

$$S_{AR} = 2 \cdot \log_2\left(\frac{P(R \mid A)}{Q_R}\right) = 2 \cdot \log_2\left(\frac{0.03}{0.052}\right) = -1.6$$