Multiple sequence alignments
similarity without sequence similarity

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Bis Jetzt

- Man hat eine Sequenz (Protein oder Nukleotid)
- Man will so viel wie möglich finden, um
  - Struktur vorherzusagen
  - Funktion vorherzusagen
- Jetzt Alignments, Evolution & Funktion

Multiple alignments

- mostly for proteins

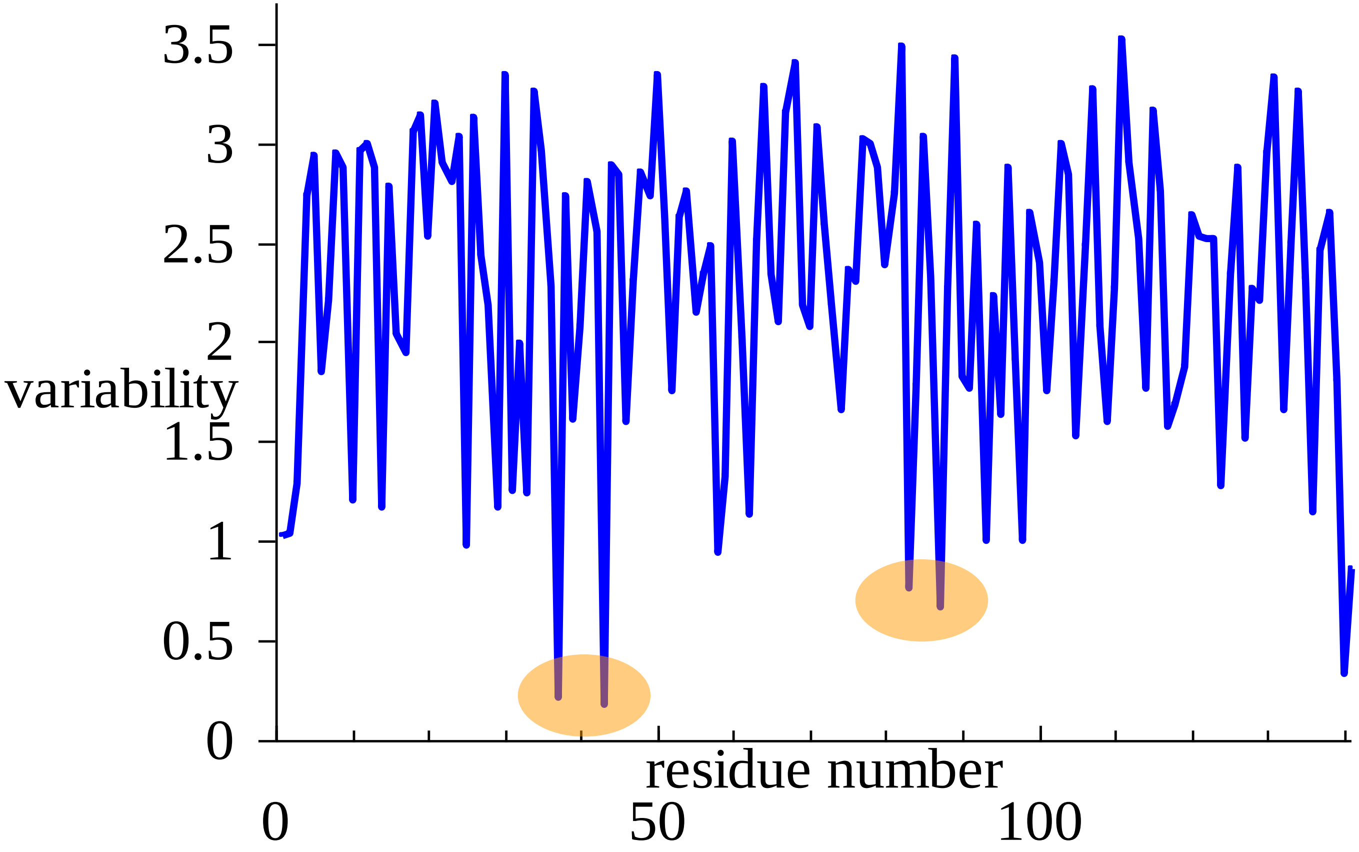
- what does a set of sequences look like ?

- data for a haemoglobin
- summarise this data

```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALEKMFLSFPTTKTYFPHFDLSHGSAQVKGHG
 LSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGDYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTHVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEAWERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEAWERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSAADKTNVKAAWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHFDLSHGSAQVKAHG
VLSAADKTNVKAAWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHFDLSHGSAQVKAHG
VLSADDKANIKAAWGKIGGHGAEYGAEALERMFCSFPTTKTYFPHFDVSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEAFERMFLSFPTTKTYFPHFDLSHGSAQVKGQG
VLSPADKTNVKAAWGKVGAHAGEYGAEAFERMFLSFPTTKTYFPHFDLSHGSAQVKGQA
VLSAADKSNVKAAWGKVGGNAGAYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKSNVKATWDKIGSHAGEYGGEALERTFASFPTTKTYFPHFDLSPGSAQVKAHG
VLSPADKSNVKAAWGKVGGHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTGTYFPHFDLSHGSAQVKGHG
VLSSADKNNVKACWGKIGSHAGEYGAEALERTFCSFPTTKTYFPHFDLSHGSAQVQAHG
VLSAADKSNVKAAWGKVGGNAGAYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VLSANDKSNVKAAWGKVGNHAPEYGAEALERMFLSFPTTKTYFPHFDLSHGSSQVKAHG
VLSPADKSNVKAAWGKVGGHAGDYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
    ...     ...      ... ...
```

Conservation / variability

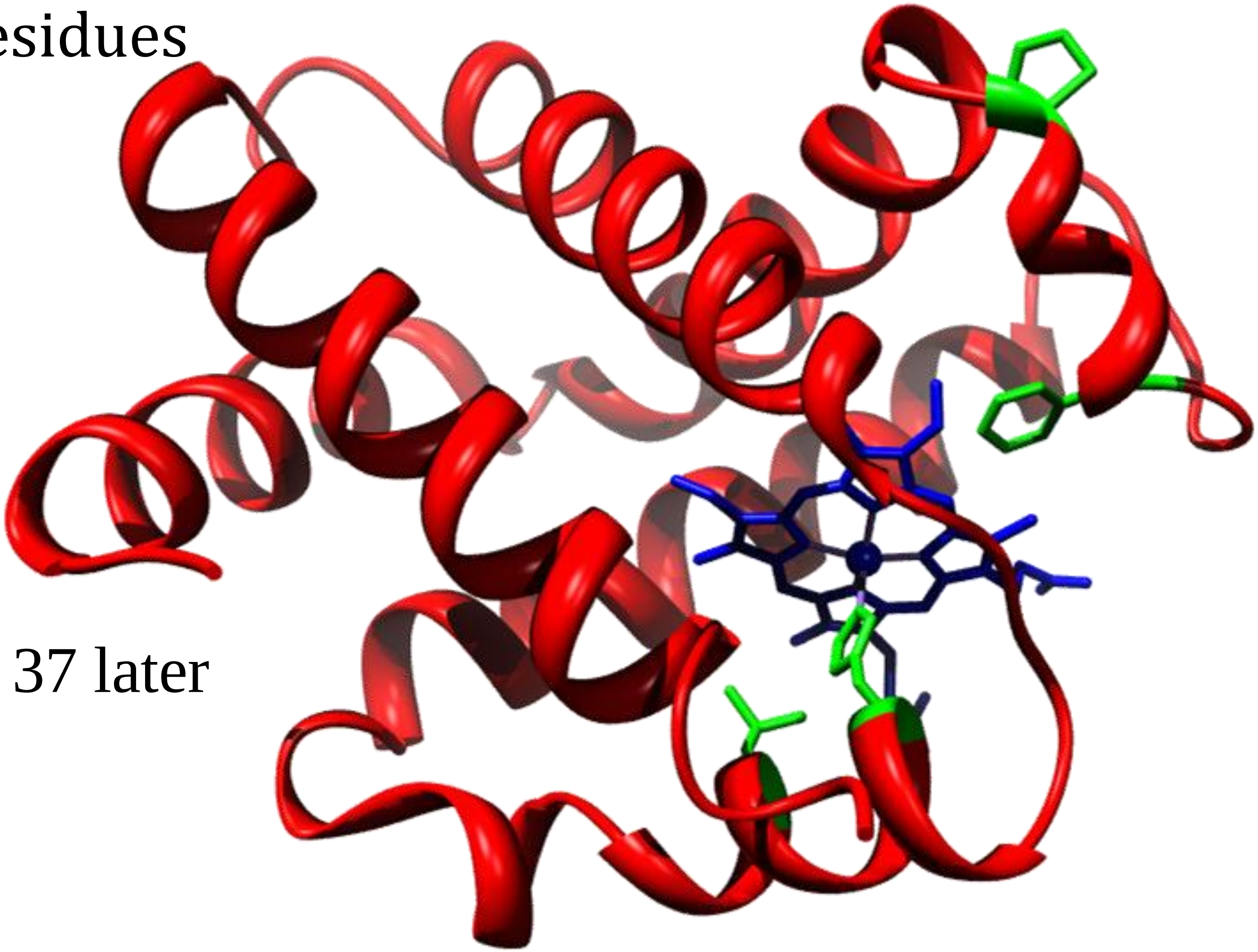
- look at residues 37, 43, 83 and 87



- how do we get these and what does it mean ?
- what does it mean for this protein ?

Conserved residues

- proximity to haem group
  - green residues



- more on pro 37 later

Beliefs in multiple sequence alignments

Similar proteins found in many organisms

- rarely identical
- where they are conserved will be connected with function
- how much they vary will reflect evolution (phylogeny)

How many homologues might you have ?

- many
  - some DNA replication proteins – almost every form of life
  - some glycolysis proteins – from bacteria to man
  - ..
- few
  - some exotic viral proteins
  - some messengers exclusively in human biochemistry
  - …

Many sequences - rigorous alignment

- two sequence alignment
  - optimal path through $n \times m$ matrix
- three sequence alignment
  - optimal path through $n \times m \times p$ matrix
- four sequence alignment
  - …
- m sequence alignment of n residues…. $O(n^m)$

- excuse to use lots of approximations
  - no guarantee of perfect answer

- reasonable starting point
  - begin with pairs of proteins

Scoring schemes

$$S_{a,b} = \sum_{i=1}^{N_{res}} match(s_{a,i}, s_{b,i})$$

- In pairwise problem

```
VLSPADKSNVKAGWGQVGAHAGDYGAEAIERMYLSFPSTKTYFPHTDISHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

  - Sum over where N_{res} is sequence length
  - *match($s_{a,i}$,$s_{b,i}$)* is the match/mismatch score of sequence *a* and *b* at position *i*
- invent a distance between two sequences like

$$d_{a,b} = 1 - \frac{S_{a,b}}{100 \cdot N_{res}} \qquad \text{or} \qquad d_{a,b} = \frac{1}{S_{a,b}}$$

- distance measure – mainly to see which sequences are most similar to each other

Scoring schemes for a multiple alignment

In the best alignment

- 1 is aligned to 2, 3, ..
- 2 to 3,4, …

```
1 VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
2 VITP-EQSNVKAAWGKVGAHAGEYGAEALEQMFLSYPTTKTYFP-FDLSHGSAQIKGHG
3 MLSPGDKTQVQAGFGRVGAHAG--GAEALDRMFLSFPTTKSFFPYFELTHGSAQVKGHG
4 VLSPAEKTNIKAAWGKVGAHAGEYGAEALEKMF-SYPSTKTYFPHFDISHATAQ-KGHG
5 –VTPGDKTNLQAGW-KIGAHAGEYGAEALDRMFLSFPTTK-YFPHYNLSHGSAQVKGHG
6 VLSPAEKTNVKAAWGRVGAHAGDYGAEALERMFLSFPSTQTYFPHFDLS-GSAQVQAHA
7 VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

Mission: for N_{seq} sequences

- S_{ab} : alignment score sequences a and b

$$score = \sum_{b \neq a}^{N_{seq}} \sum_{a=1}^{N_{seq}} S_{a,b}$$

- not quite possible
  - if I move sequences 4 and 5, may make a mess of 5 and 2

Aligning average sequences

```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VITPAEKTNVKAAWGKVGAHAGEYGAEALEQMFLSYPTTKTYFPHFDLSHGSAQIKGHG
```

and

```
IITPGDKTNVKAAFGKVGAHGGEYGAEALDRMFISFPSTKTYYPHFDLSHASAQVKAHG
VITPAEQTNIKGAWGQIGAHAGDYAADALEQMFLSYPTSKTYFPYFDLTHGSAQIKGHG
VITPAEKTQVKAAWGKVGGHAGEYGAEAIEQMFLTYPTTQTYFPHFELSHGTAQIKGHG
```

- at each position
  - use some kind of average in scoring
  - if a column has 2×D and 1×E score
    - score as 2/3 D + 1/3 E
- later.. call the average of S1 and S2: av(S1, S2)

Summarise ingredients

- pairwise scores + distances
- ability to align little groups of sequences

Progressive alignments

- known as guide tree / progressive method
- steps
  - build a distance matrix
  - build a guide tree
  - build up overall alignment in pieces

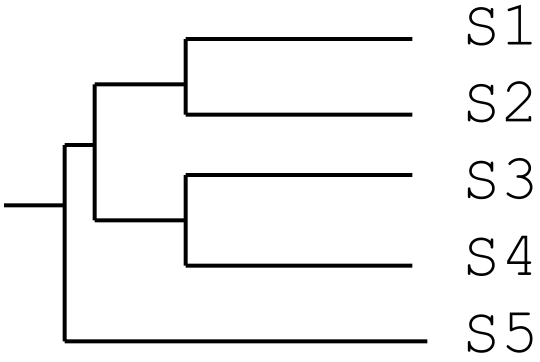
Progressive alignment - tree

S1	ATCTCGAGA
S2	ATCCGAGA
S3	ATGTCGACGA
S4	ATGTCGACAGA
S5	ATTCAACGA

Compute pairwise alignments, calculate the distance matrix

S1	-				
S2	.11	-			
S3	.20	.30	-		
S4	.27	.36	.09	-	
S5	.30	.33	.23	.27	-
	S1	S2	S3	S4	S5

calculate guide tree



Multiple alignment from guide tree

- gaps at early stages remain
- problems..
- S1/S2 and S3/S4 good
  - no guarantee of S1/S4 or S2/S3

- av(S1,S2) is average of S1 and S2

align S1 with S2

```
S1      ATCTCGAGA
S2      ATC-CGAGA
```

align S3 with S4

```
S3      ATGTCGAC-GA
S4      ATGTCGACAGA
```

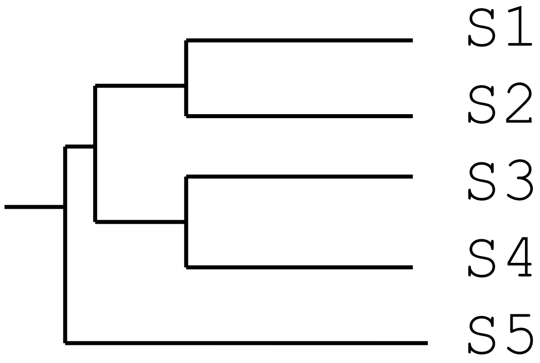
align av(S1,S2) with av(S3,S4)

```
S1      ATCTCGA--GA
S2      ATC-CGA--GA
S3      ATGTCGAC-GA
S4      ATGTCGACAGA
```

align av(S1,S2,S3,S4) with S5

```
S1      ATCTCGA--GA
S2      ATC-CGA--GA
S3      ATGTCGAC-GA
S4      ATGTCGACAGA
S5      AT-TCAAC-GA
```

Problems and variations

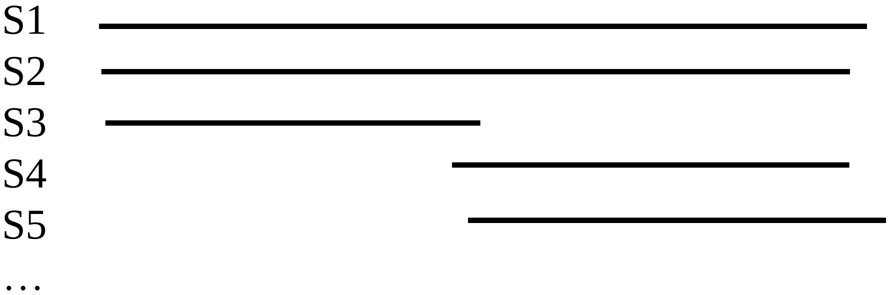


	S1	S2	S3	S4	S5
S1	-				
S2	.11	-			
S3	.20	.30	-		
S4	.27	.36	.09	-	
S5	.30	.33	.23	.27	-

What order should we join ?

- pairs are easy (S1+S2) and (S3+S4)
- which next ?

Real breakdown



- S1 and S2 are multi-domain proteins
  - S3 is not really related to S4 or S5
  - distance matrix elements are rubbish

Given an alignment

How reliable / believable ?

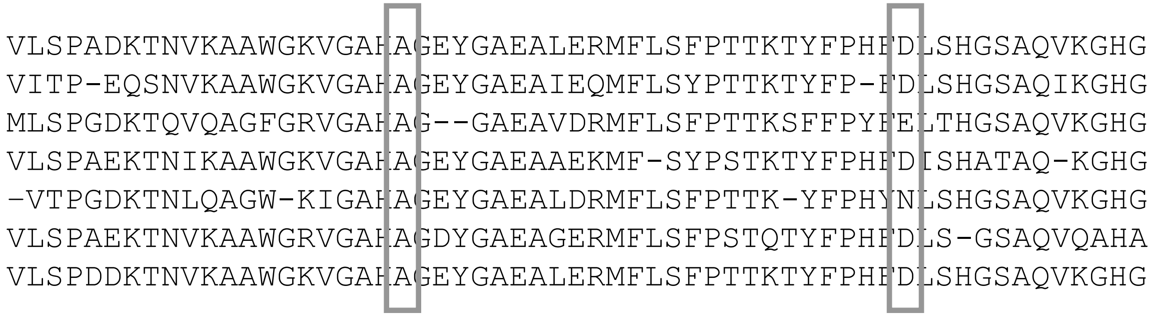
- set of very related proteins (an enzyme from 100 mammals)
    - no problem
- diverse proteins (an enzyme 100 organisms, bacteria to man)
    - maybe lots of little errors
- can break completely (domain example)

Is the tree a "phylogeny" ? A reflection of evolution ?

- more later

Measuring conservation / entropy

- Gibbs entropy
  - how much disorder do I have ? $S = -k \sum_{i=1}^{N_{states}} p_i \ln p_i$
  - in how many states may I find the system ?
- Our question
  - look at a column – how much disorder is there ?



```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VITP-EQSNVKAAWGKVGAHAGEYGAEAIEQMFLSYPTTKTYFP-FDLSHGSAQIKGHG
MLSPGDKTQVQAGFGRVGAHAG--GAEAVDRMFLSFPTTKSFFPYFELTHGSAQVKGHG
VLSPAEKTNIKAAWGKVGAHAGEYGAEAAEKMF-SYPSTKTYFPHFDISHATAQ-KGHG
-VTPGDKTNLQAGW-KIGAHAGEYGAEALDRMFLSFPTTK-YFPHYNLSHGSAQVKGHG
VLSPAEKTNVKAAWGRVGAHAGDYGAEAGERMFLSFPSTQTYFPHFDLS-GSAQVQAHA
VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

no
disorder

much
disorder

- Calculate an "entropy" for each column

Entropy

$$S = -\sum_{i=1}^{N_{states}} p_i \ln p_i$$

- We can forget *k* (Boltzmann – just scaling)
- We have a protein
  - 20 possible states
- What if a residue is always conserved ?
  - S = ln (1) = 0 (no entropy)
- What if all residues are equally likely ?
- p_i=1/20

$$S = -\sum_{i=1}^{20} \frac{1}{20} \ln \frac{1}{20} = -20 \cdot \frac{1}{20} \ln \frac{1}{20}$$

$$\approx 3$$

- my toy alignment…

Entropy

- first column is boring
- second
  - $p_{\mathrm{D}} = 5/7$
  - $p_{\mathrm{E}} = 1/7$
  - $p_{\mathrm{N}} = 1/7$

```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VITP-EQSNVKAAWGKVGAHAGEYGAEAIEQMFLSYPTTKTYFP-FDLSHGSAQIKGHG
MLSPGDKTQVQAGFGRVGAHAG--GAEAVDRMFLSFPTTKSFFPYFELTHGSAQVKGHG
VLSPAEKTNIKAAWGKVGAHAGEYGAEAAEKMF-SYPSTKTYFPHFDISHATAQ-KGHG
-VTPGDKTNLQAGW-KIGAHAGEYGAEALDRMFLSFPTTK-YFPHYNLSHGSAQVKGHG
VLSPAEKTNVKAAWGRVGAHAGDYGAEAGERMFLSFPSTQTYFPHFDLS-GSAQVQAHA
VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

$$S = -\left(\frac{5}{7}\ln\frac{5}{7} + \frac{1}{7}\ln\frac{1}{7} + \frac{1}{7}\ln\frac{1}{7}\right)$$

$$\approx 0.8$$

- example from start of this topic

Entropy from DNA

- exactly as for proteins
- will numbers be larger or smaller ?

  - max possible entropy

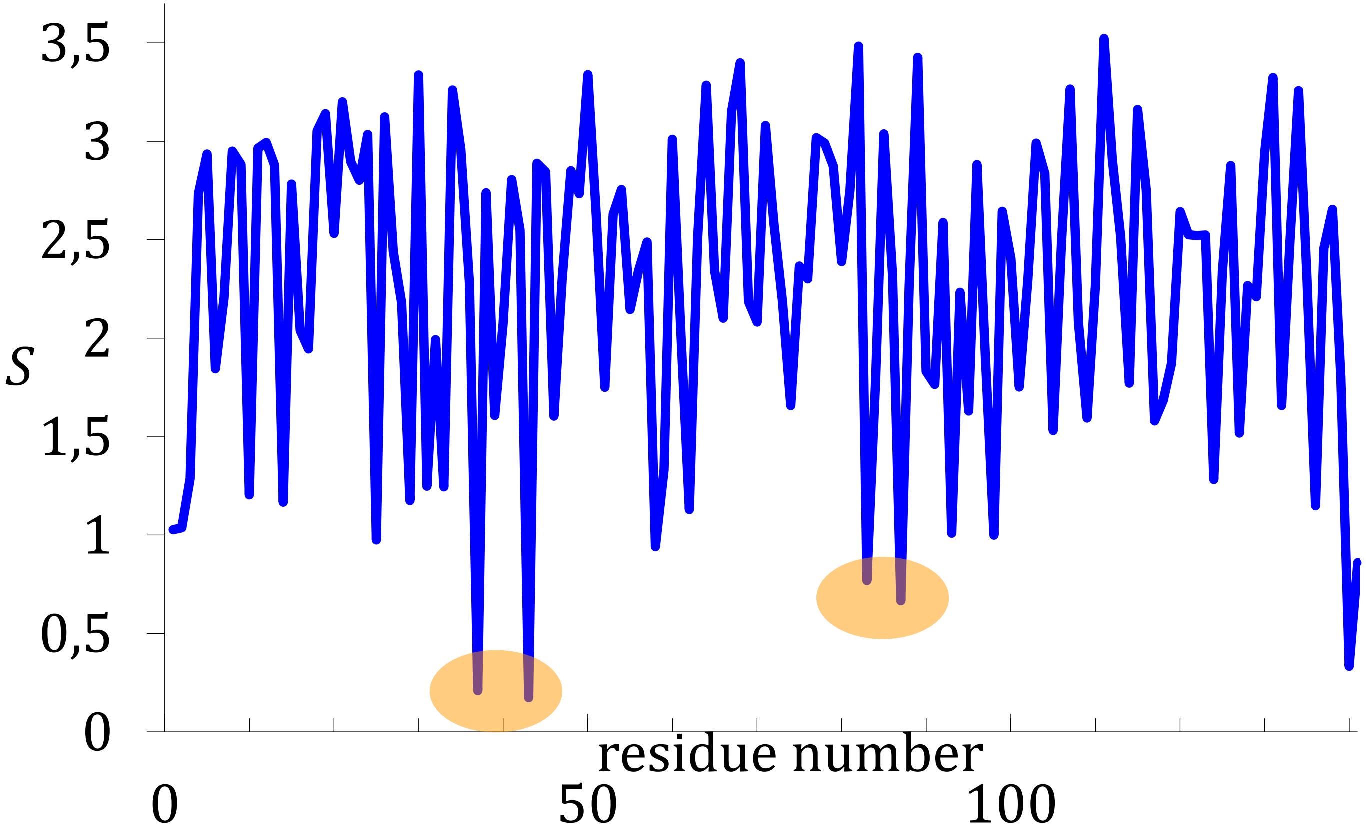
$$S = -4\left(\frac{1}{4}\ln\frac{1}{4}\right)$$

$$= -\ln\frac{1}{4}$$

$$\approx 1.4$$

Haemoglobin conservation

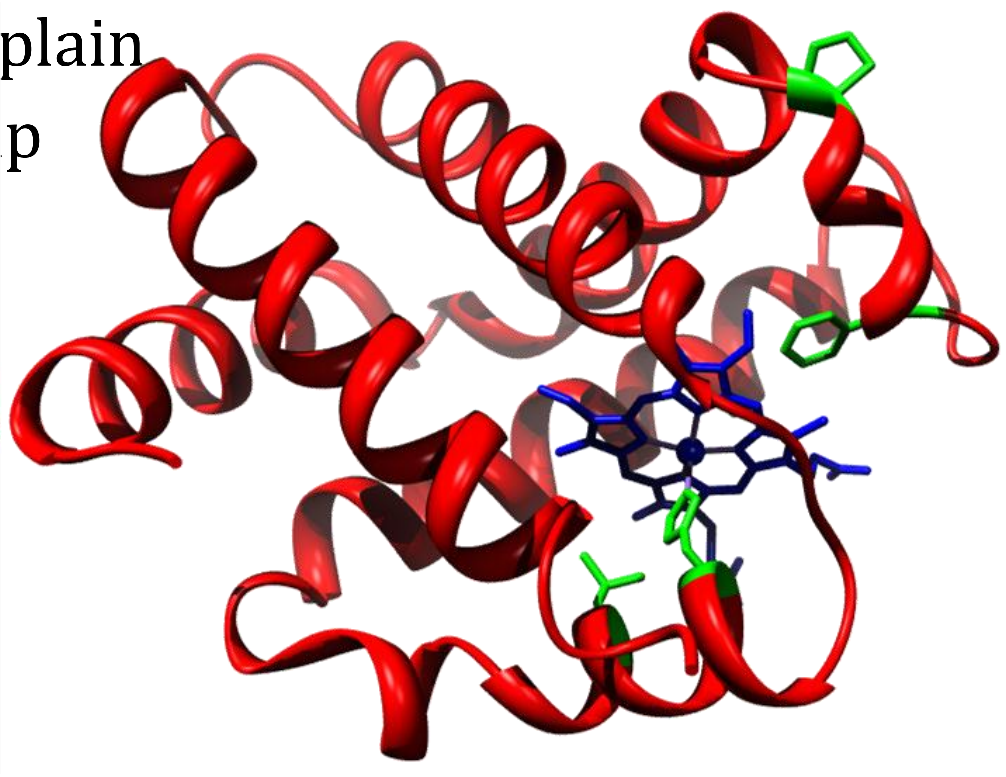
- look at residues 37, 43, 83 and 87



- 4 residues (maybe more) stand out as conserved
    - why ?

Conserved residues in haemoglobin

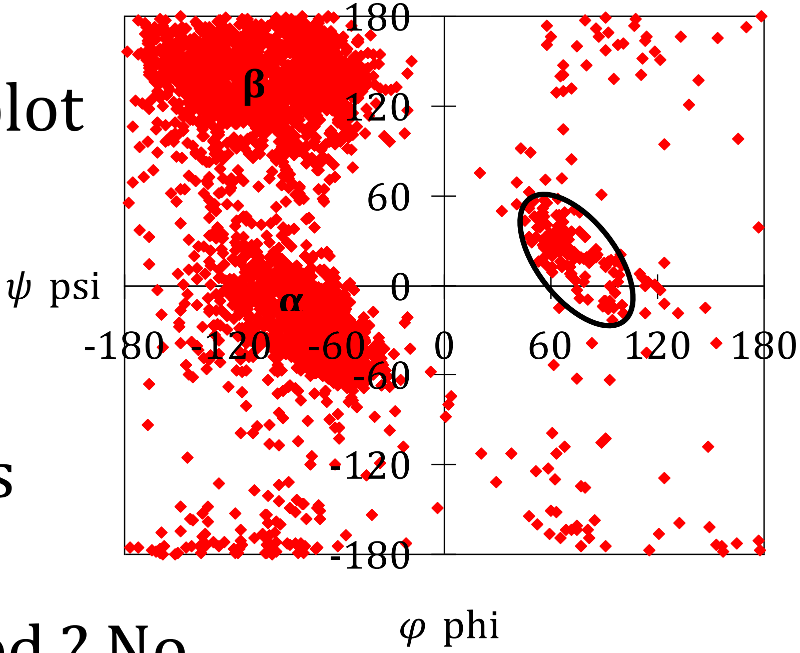
- 3 of the sites are easy to explain
  - interact with haem group
- Look at fourth site
  - proline
  - end of a helix



- what is special about proline ?
  - no Hbond donor
- here – if it mutates, maybe haemoglobin does not fold

Conservation for structure

- some residues have very special structural roles
  - proline – not an H-bond donor
    - often end of a helix
  - glycine – can visit part of φ ψ plot
    - found in some turns



- are all gly residues so important ?
  - NO – they occur in many places sometimes in turns
- are all pro residues very conserved ? No

Conservation for function

- in a serine protease
    - always a "catalytic serine"
    - can it mutate ? Not often
- in haemoglobin – residues necessary for binding haem
    - can they mutate ? rarely
    - changes properties of haemoglobin (bad news)
- dogma
    - residues in active site will be more conserved than other sites

Important summary

- conservation may reflect
  - important function
  - structural role
- mutagenesis / chemistry
  - what residue may I change to allow binding to a solid substrate ? (for biosensor/immobilized enzyme ?)
  - I want to try error prone PCR to select for new enzyme activity – which sites might I start with (active site) ?
- drug design example
  - target is an essential protein (basic metabolism, DNA synthesis, protein synthesis..)
  - is there some set of sequence features common to pathogen, different to mammalian protein ?

Evolution – do not trust conservation

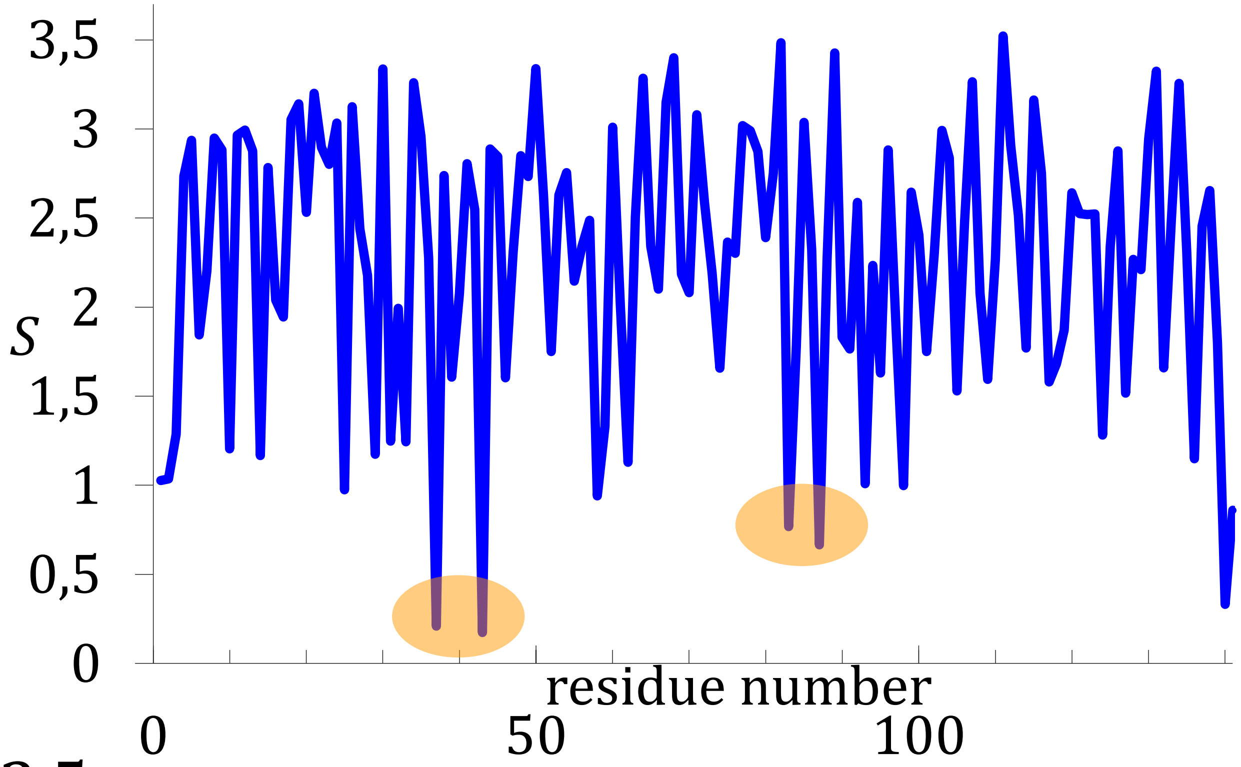
Imagine: two possible systems for some important enzyme

1. active site fits to essential biochemistry
   - any mutation – you lose
   - you see active site residues as conserved in a conservation plot
2. maybe enzyme is not absolutely perfect
   - some mutations kill you
   - some mutations OK
   - site does not appear perfectly conserved

If you have the choice, where would you evolve to ?

1. very fragile
2. likely to survive mutations

Conservation – how meaningful ?



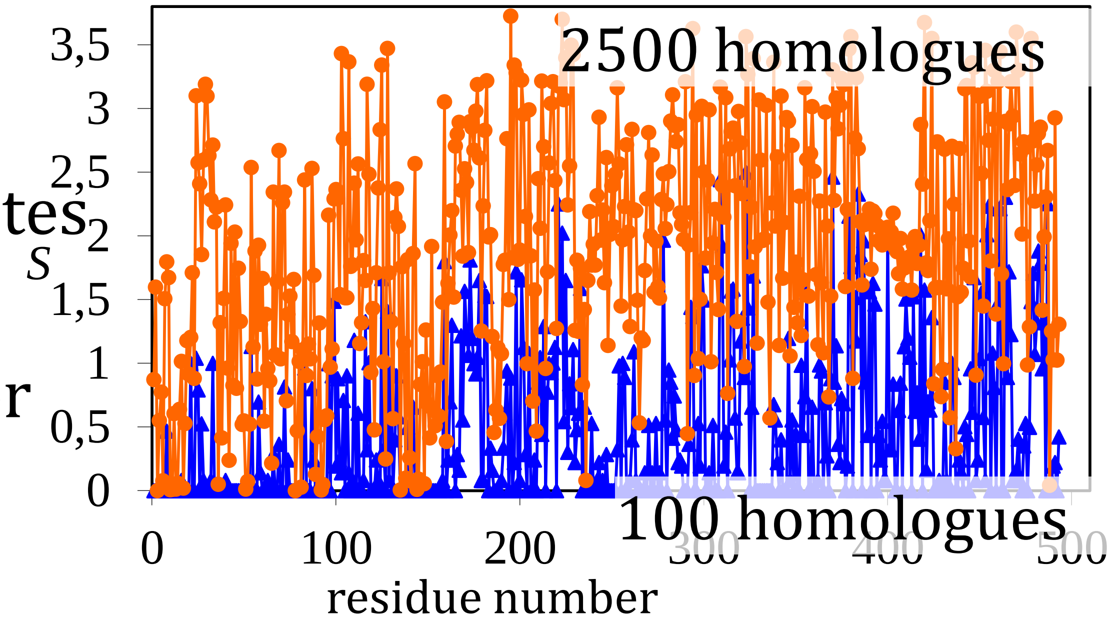
- Earlier Folien…
- values from 0 to 3.5

- what if I used more homologues ?

Conservation – how meaningful ?

- example sequence (1ab4, DNA gyrase)
- find 100 close homologues (mostly > 80% similarity) – calculate conservation
- find 2 500 close homologues (mostly > 50 % similarity) calculate conservation

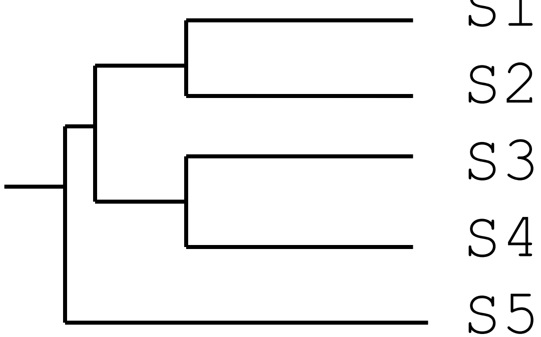
- fewer sequences
  - lots of conserved sites
- you can get the answer you want



Phylogeny / Evolution

Purely academic ? For fun ? Not always

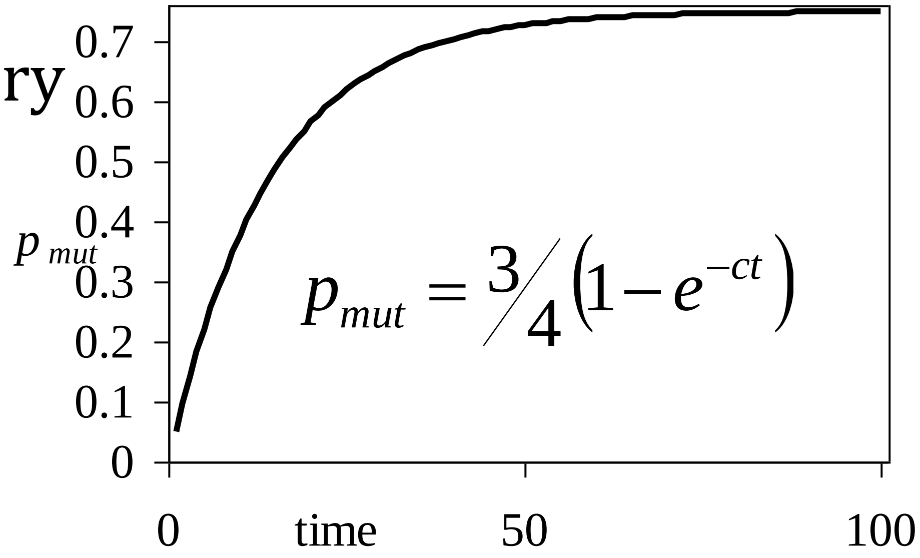
- possibly useful in explaining disease propagation
  - where did HIV come from ?
  - where did the flu pandemics come from ?
  - virus infects banana crop – where did it come from ?
- previously we had a "guide tree"
  - did (S1,S2) and (S3,S4) share an ancestor but not S5 ?
  - not so good
- branch lengths do not reflect evolutionary time
- there may be other similar trees which could be evolutionary paths



Evolutionary time

- compare two DNA sequences see
  - 1 mutation (represents time t)
  - 2 mutations (time $2t$)
  - 3 mutations (time $3t$)…
  - No !
- After some evolution
  - A → C → G two events (although looks like A→G)
  - A → C → G → C → A looks like zero mutations
- If I have infinite time
  - all bases / residues equally likely
  - p_{mut} =3/4 = 0.75 (DNA) or p_{mut}=19/20 (protein)

Mutation probability



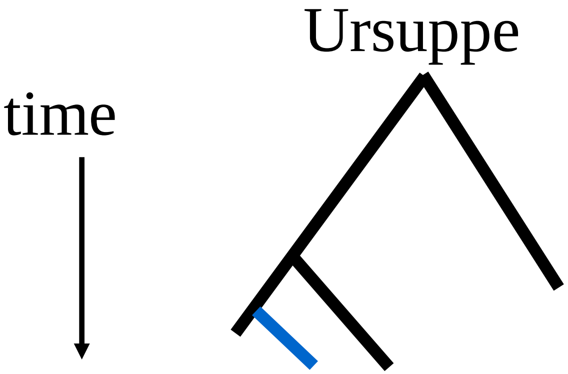
$$p_{mut} = \tfrac{3}{4}\left(1 - e^{-ct}\right)$$

- time units are rather arbitrary
- how would I estimate time ? (for DNA)
- $t \propto -\ln\left(1 - \frac{4}{3} p_{mut}\right)$
- p_{mut} ? count $\frac{n_{mut}}{n_{res}}$
- scaling of *t* not so important (relative time)

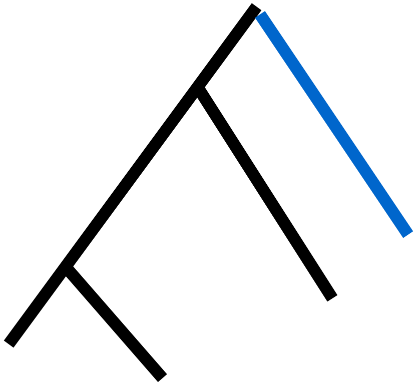
- for short times, p_{mut} changes fast
  - for small *t*, distances will be more reliable
    - as will be alignments
- is this enough for phylogeny ?
  - what about reliability ?

Problems in phylogeny

- not all sites mutate equally quickly
- not all species mutate equally quickly



but blue species (protein) mutates quickly



- blue appears to have branched off earlier

Problems estimating time

1. mutation rates vary wildly
   - changing environments – pH, temperature,..

2. imagine time t is such that p_{mut}= 0.25
   - we have random events
   - sometimes you see 23% mutation, sometimes 28%

- time estimates will never be accurate
- maybe we cannot find the correct tree
  - can we roughly estimate reliability ?

Reliability

- think of first alignment

```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VITP-EQSNVKAAWGKVGAHAGEYGAEAIEQMFLSYPTTKTYFP-FDLSHGSAQIKGHG
MLSPGDKTQVQAGFGRVGAHAG--GAEAVDRMFLSFPTTKSFFPYFELTHGSAQVKGHG
VLSPAEKTNIKAAWGKVGAHAGEYGAEAAEKMF-SYPSTKTYFPHFDISHATAQ-KGHG
-VTPGDKTNLQAGW-KIGAHAGEYGAEALDRMFLSFPTTK-YFPHYNLSHGSAQVKGHG
VLSPAEKTNVKAAWGRVGAHAGDYGAEAGERMFLSFPSTQTYFPHFDLS-GSAQVQAHA
VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

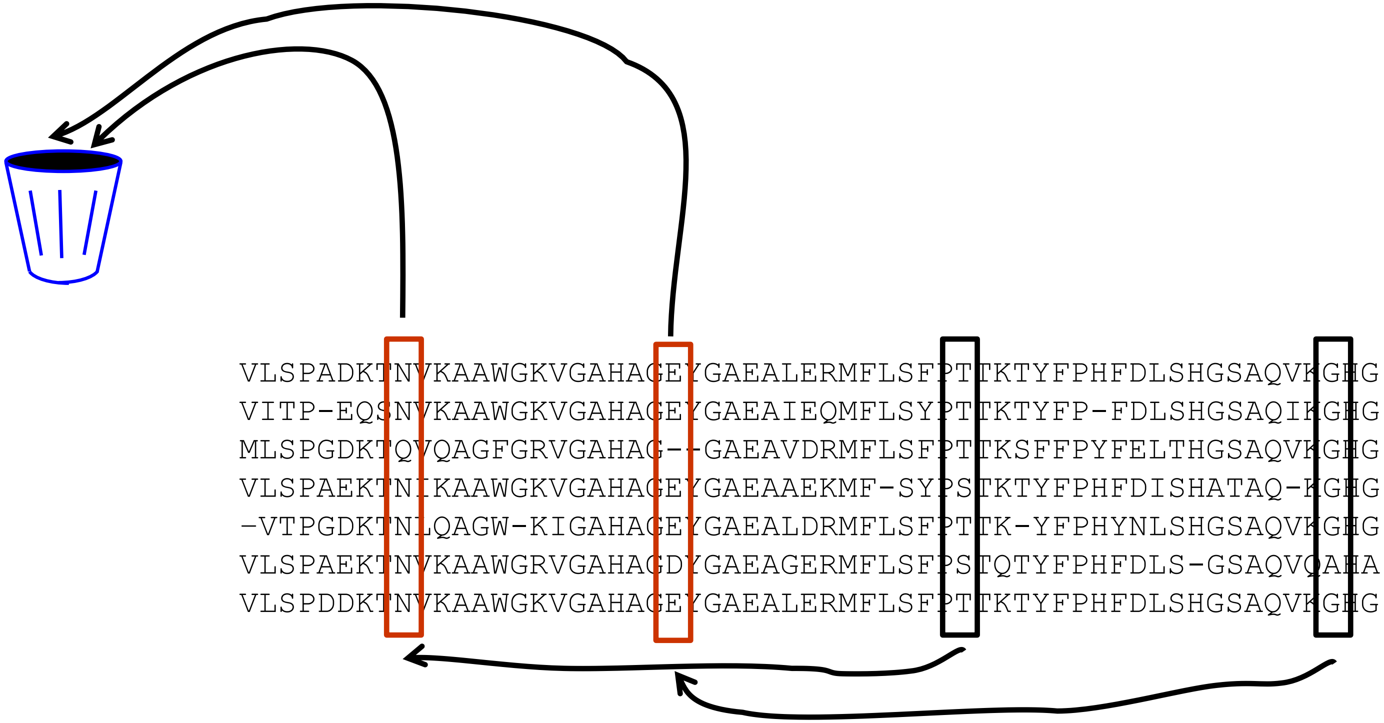
- what would happen if you deleted a column ?

- if the data is robust /reliable
    - not much
- if the tree is very fragile /sensitive
    - tree will change

- better…

Reliability

- repeat 10^2 to 10^3 times
  - delete 5 to 10 % of columns
  - copy random columns so as to have original size
  - recalculate tree
- how often did you see each branch



```
VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
VITP-EQSNVKAAWGKVGAHAGEYGAEAIEQMFLSYPTTKTYFP-FDLSHGSAQIKGHG
MLSPGDKTQVQAGFGRVGAHAG--GAEAVDRMFLSFPTTKSFFPYFELTHGSAQVKGHG
VLSPAEKTNIKAAWGKVGAHAGEYGAEAAEKMF-SYPSTKTYFPHFDISHATAQ-KGHG
-VTPGDKTNLQAGW-KIGAHAGEYGAEALDRMFLSFPTTK-YFPHYNLSHGSAQVKGHG
VLSPAEKTNVKAAWGRVGAHAGDYGAEAGERMFLSFPSTQTYFPHFDLS-GSAQVQAHA
VLSPDDKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

Monster example

- generate lots of trees
- for each subtree
    - see how often it is present

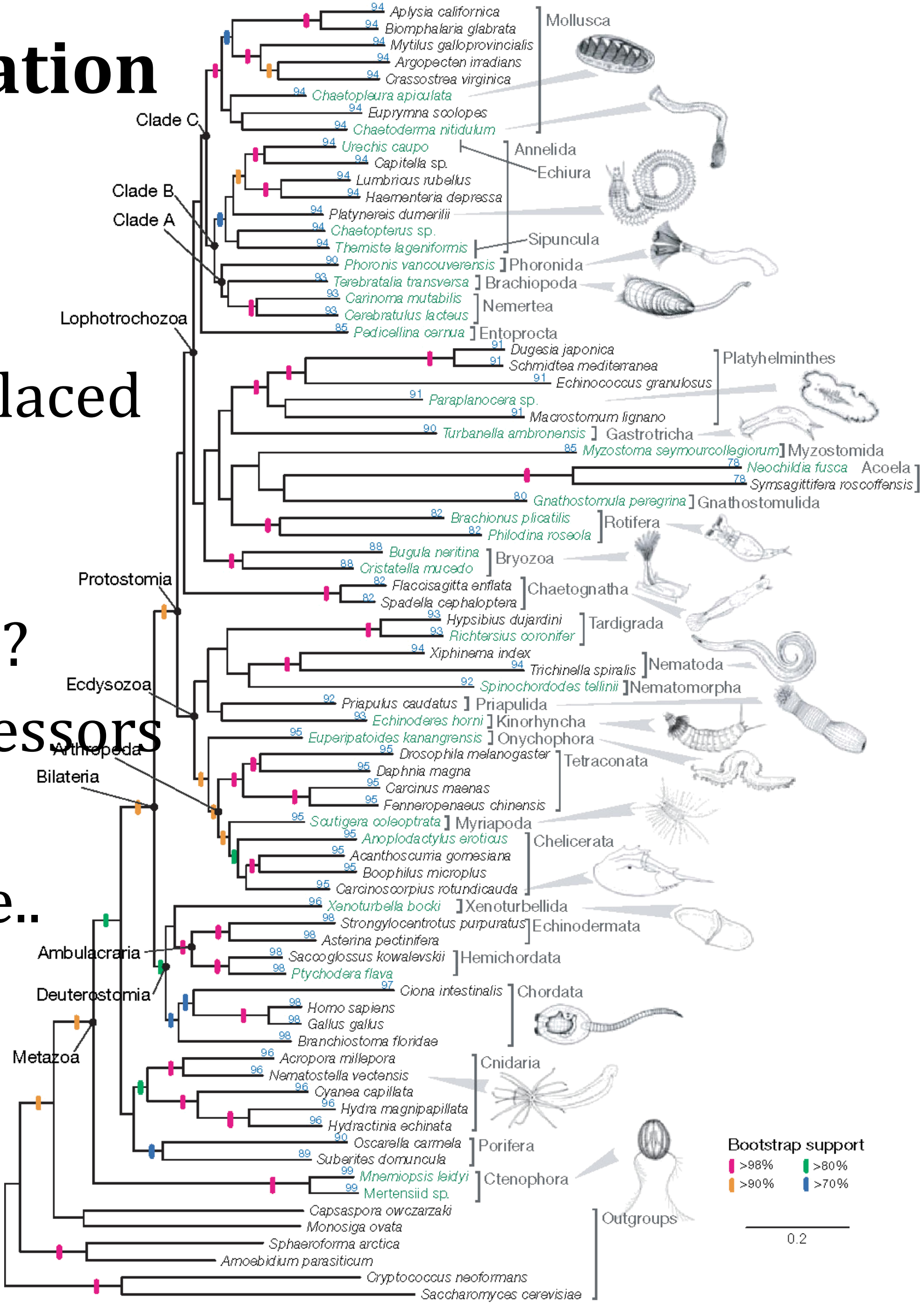
- example from cover of nature

Monster calculation

- we are usually placed near Hühne
- we are not so reliably placed with little worms

- how long does this take?
    - months on 120 processors

- a more applied example..



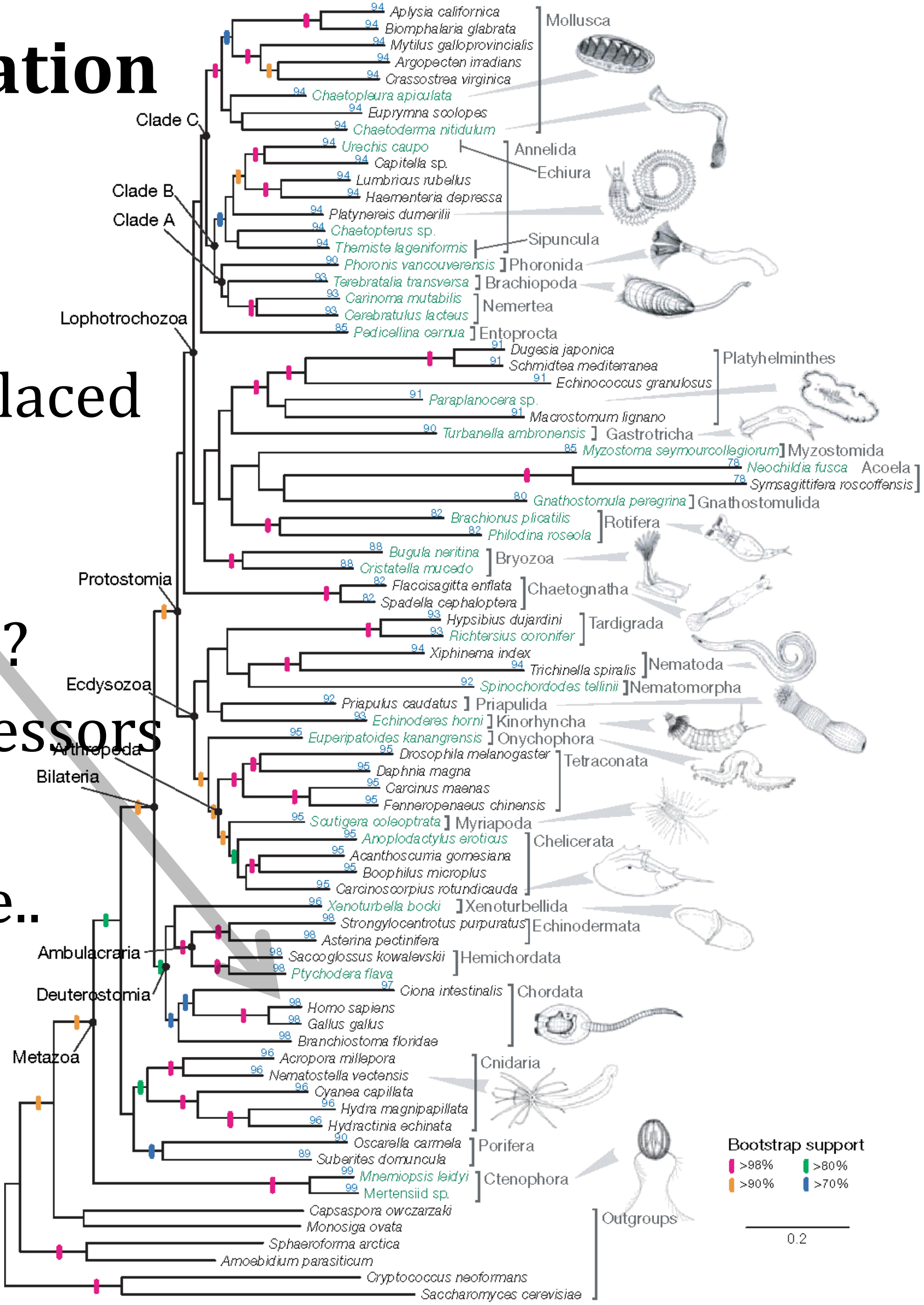
Dunn, CW et al, Nature, 402, 745-750 (2008)

Monster calculation

- we are usually placed near Hühne
- we are not so reliably placed with little worms

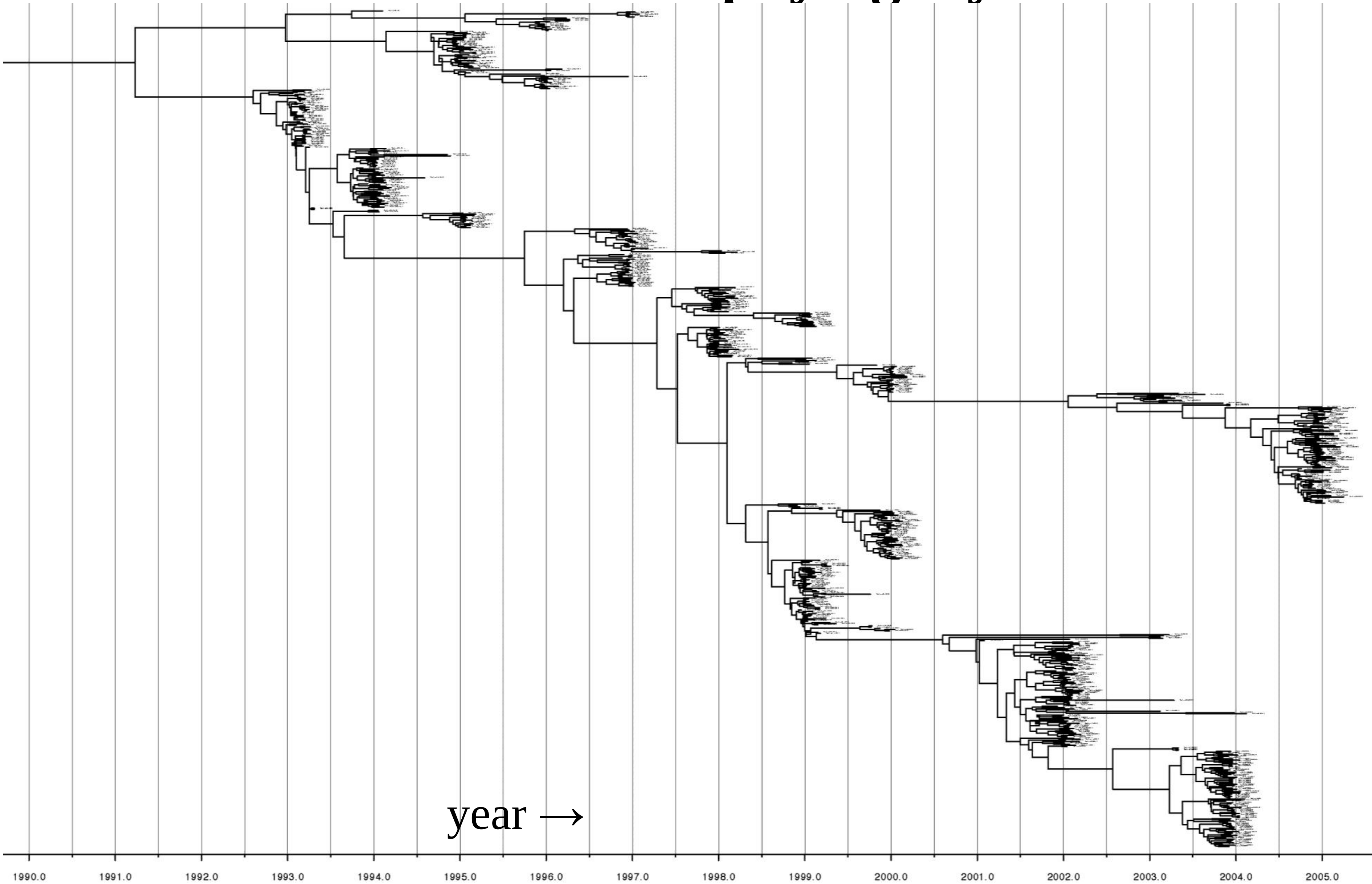
- how long does this take?
    - months on 120 processors

- a more applied example..



Dunn, CW et al, Nature, 402, 745-750 (2008)

Influenza virus phylogeny



Rambaut, A., .. Holmes, C. The genomic.. influenza A virus, Nature 452, 1-6, 2008

Summary

- multiple sequence alignment – conservation
  - find important residues (function or structure)
  - can quantify conservation
- relations between most similar proteins are most reliable
- best tree is never found
  - too difficult algorithmically
  - lots of errors – evolution is a random process
- rough idea of reliability
- quick tree – possible for hundreds of sequences
- more complicated methods – only practical for smaller numbers of sequences