Multiple sequence alignments similarity without sequence similarity

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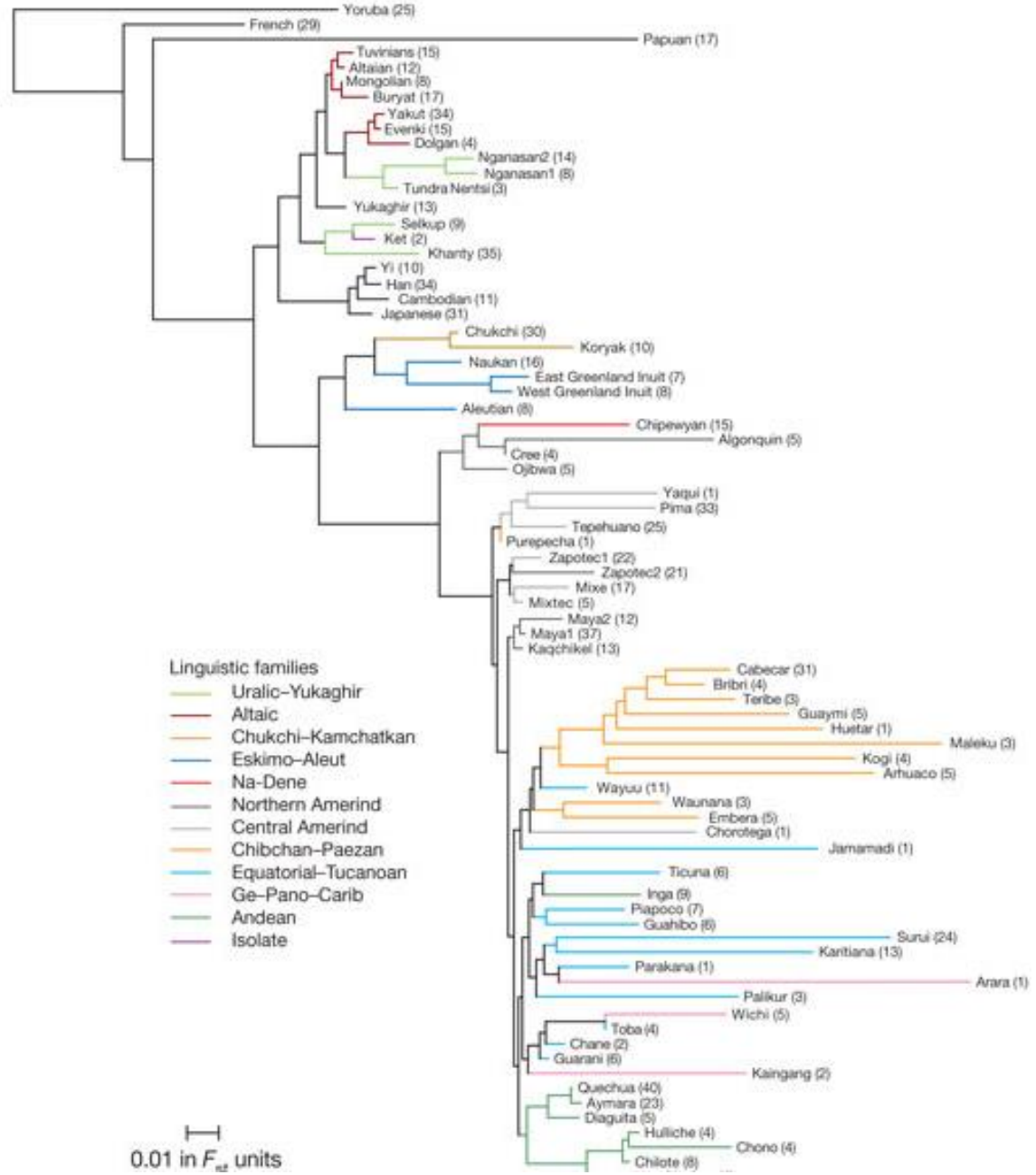
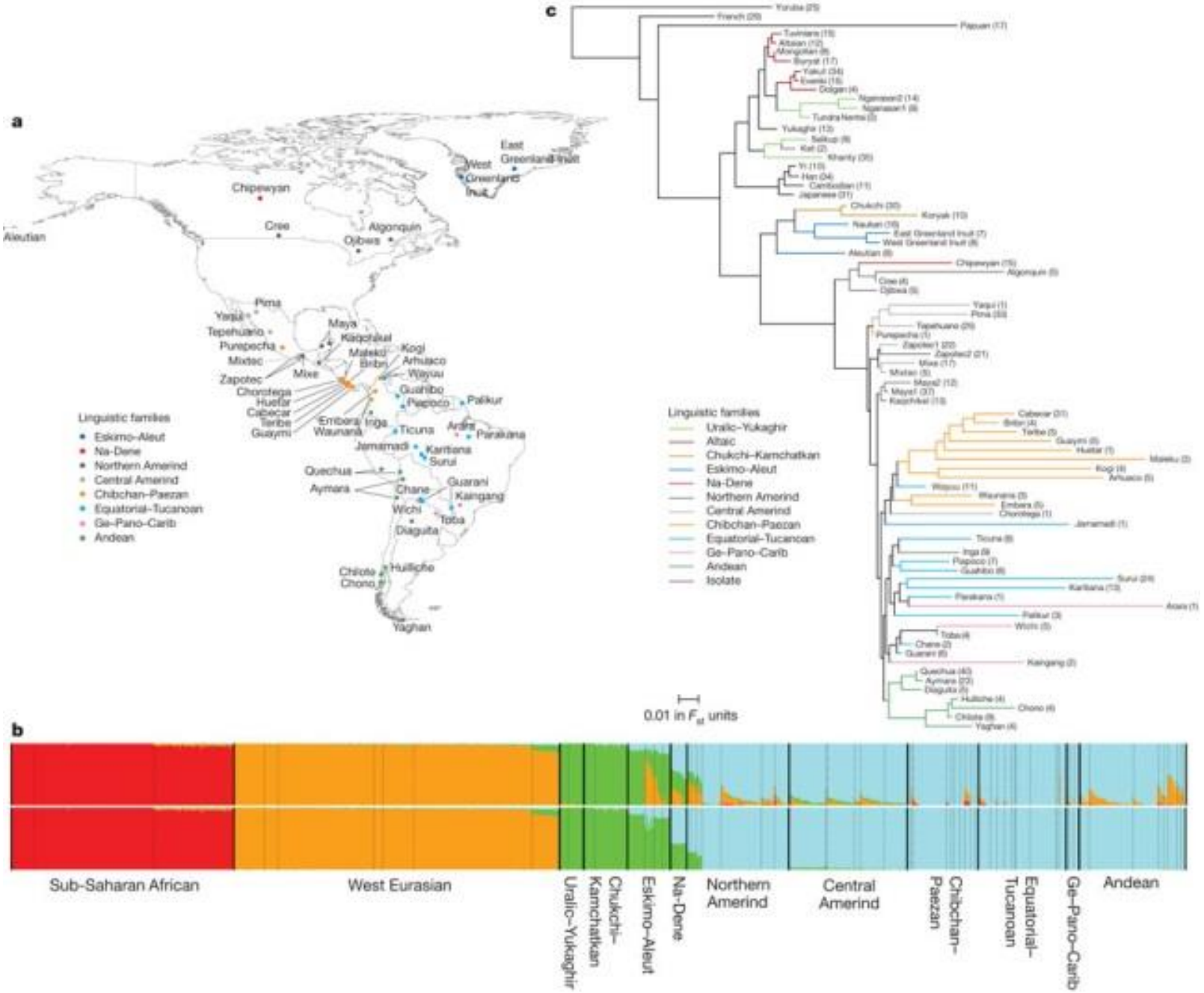
Bis Jetzt

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

52 Native American groups



Reich, D., …Ruiz-Linares, A., Nature, 488, 370 (2012), Reconstructing Native American .. History



Reich, D., …Ruiz-Linares, A., Nature, 488, 370 (2012), Reconstructing Native American .. History

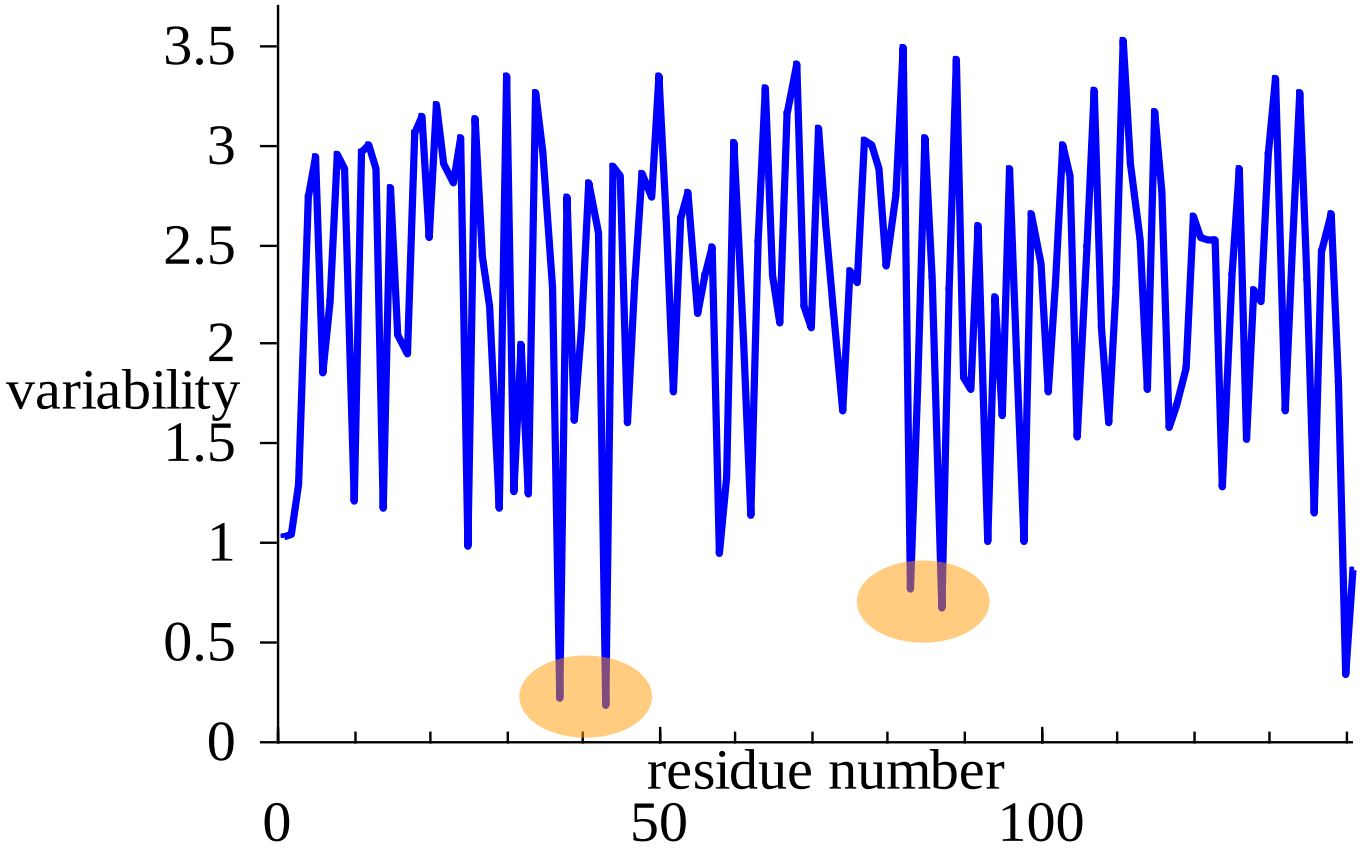
Multiple alignments

- 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

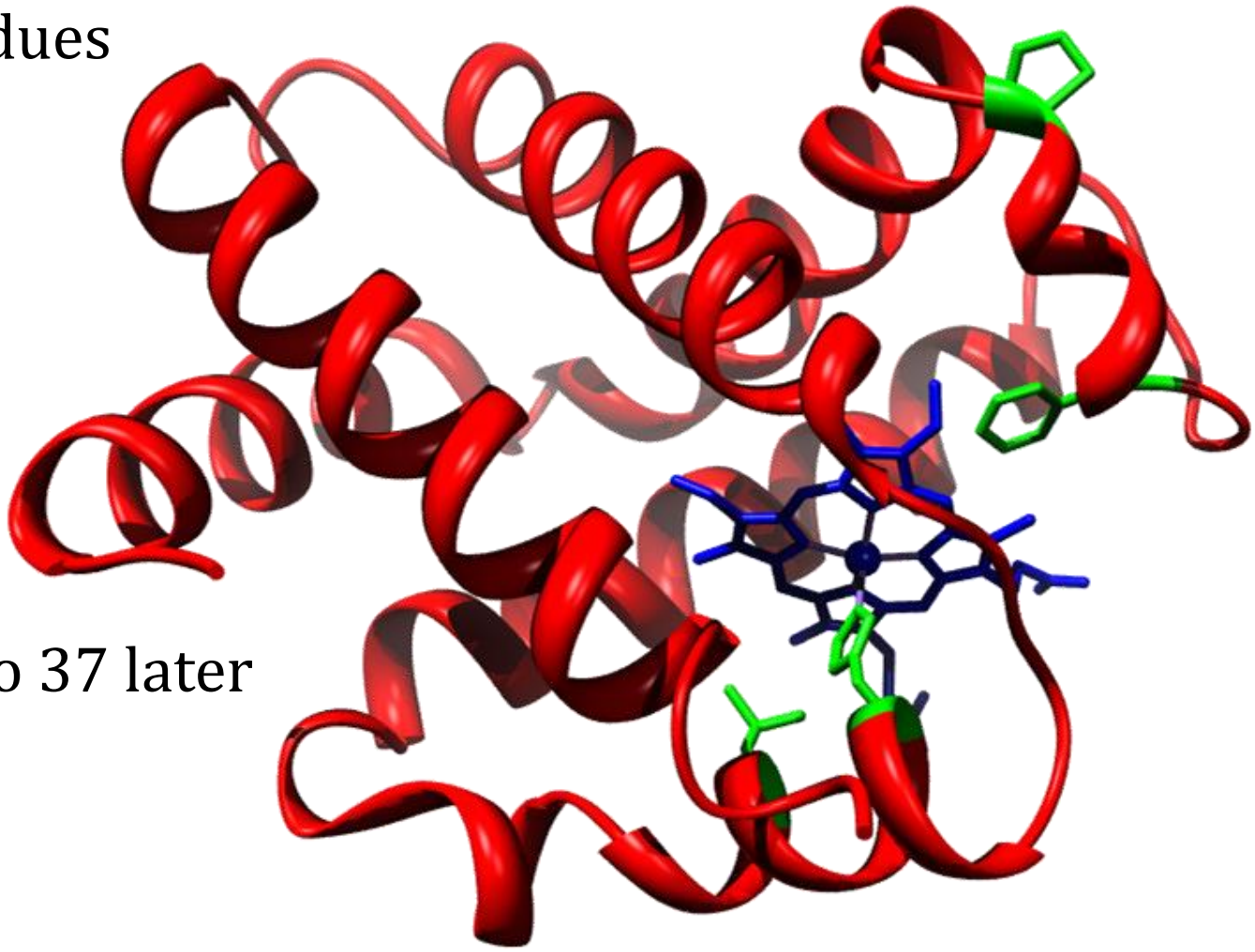


- how do we get these and what does it mean ?
- what does it mean for this protein ?

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

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

```
VLSPADKSNVKAGWGQVGAHAGDYGAEAIERMYLSFPSTKTYFPHTDISHGSAQVKGHG
MLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
```

In pairwise problem

- Sum over match()
  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

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

- distance measure –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 / neighbour joining method

Steps

- build a distance matrix
- build a guide tree
- build up overall alignment in pieces

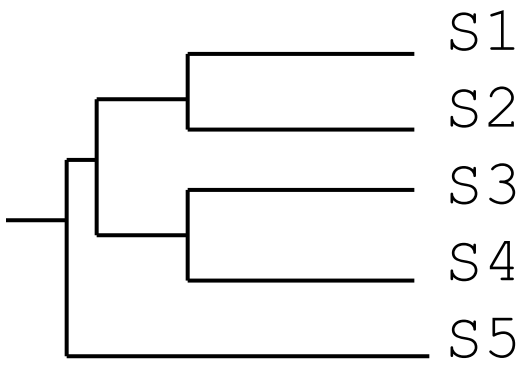
Progressive alignment - tree

```
S1    ATCTCGAGA
S2    ATCCGAGA
S3    ATGTCGACGA
S4    ATGTCGACAGA
S5    ATTCAACGA
```

Compute pairwise alignments, calculate the distance matrix

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

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
```

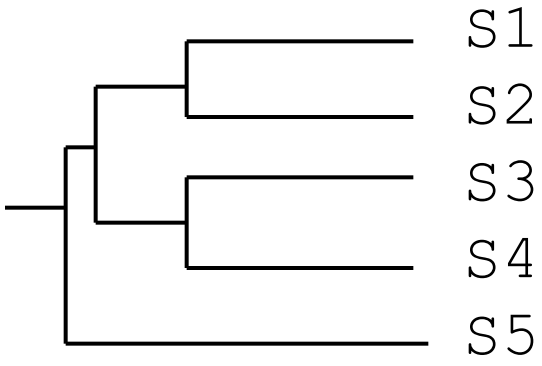
align av(S1,S2) with av(S3,S4)

```
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

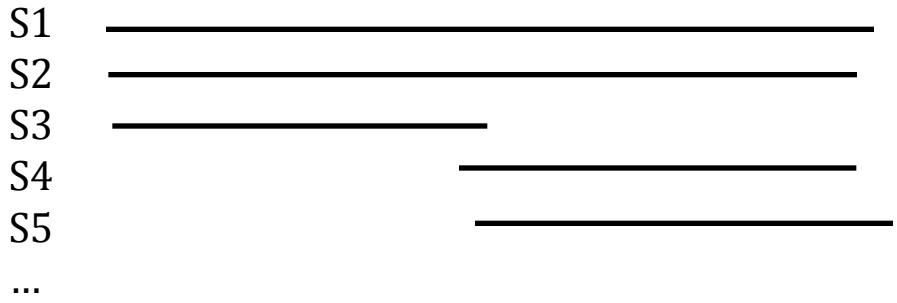


	S1	S2	S3	S4	S5
S1	-				
S2	.11	-			
S3	.20	.30	-		
S4	.27	.36	.09	-	
S5	.30	.33	.23	.27	-

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

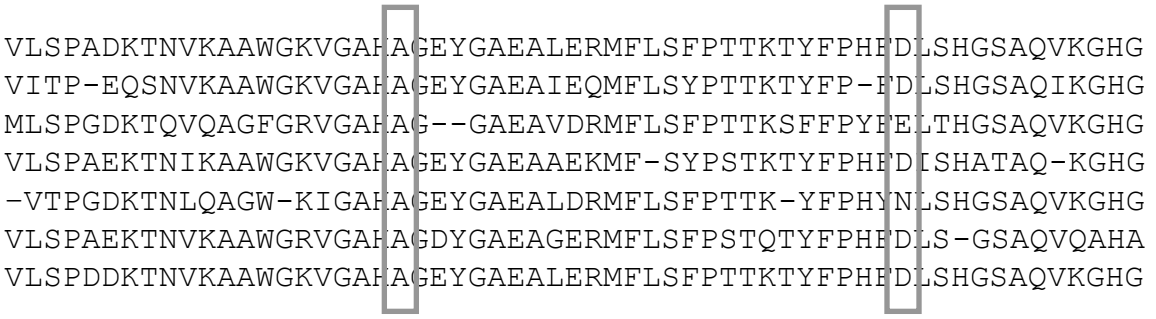
Measuring conservation / entropy

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 ?



no disorder

much disorder

Calculate an "entropy" for each column

Entropy

- forget k (Boltzmann – just scaling) $S = -\sum_{i=1}^{N_{states}} p_i \ln p_i$

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

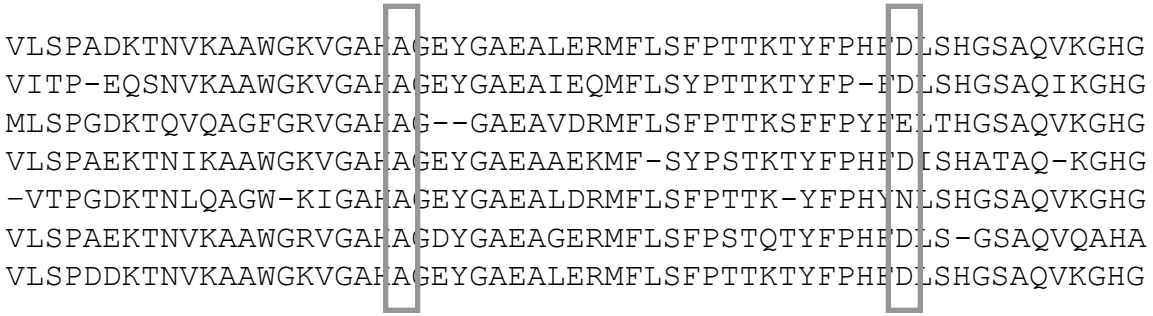
First column is boring

Second

$p_{\mathrm{D}} = {}^{5}/_{7}$

$p_{\mathrm{E}} = {}^{1}/_{7}$

$p_{\mathrm{N}} = {}^{1}/_{7}$



$$S = -\left(\frac{5}{7}\ln\frac{5}{7} + \frac{1}{7}\ln\frac{1}{7} + \frac{1}{7}\ln\frac{1}{7}\right)$$
$$\approx 0.8$$

- example from start of this topic

Entropy from DNA

Exactly as for proteins

Will numbers be larger or smaller ?

max possible entropy

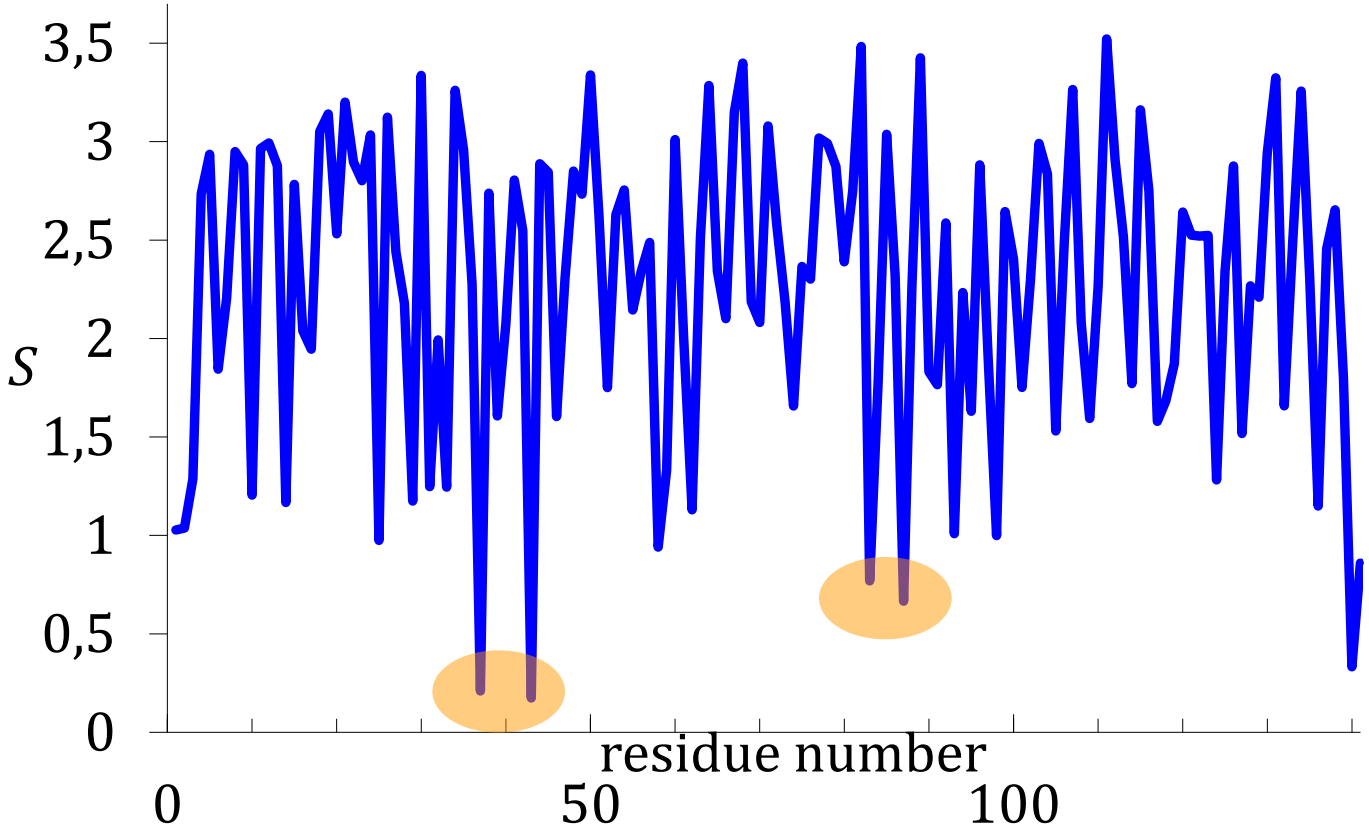
$$S = -4\left(\frac{1}{4}\ln\frac{1}{4}\right)$$

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

$$\approx 1.4$$

Haemoglobin conservation

Look at residues 37, 43, 83 and 87



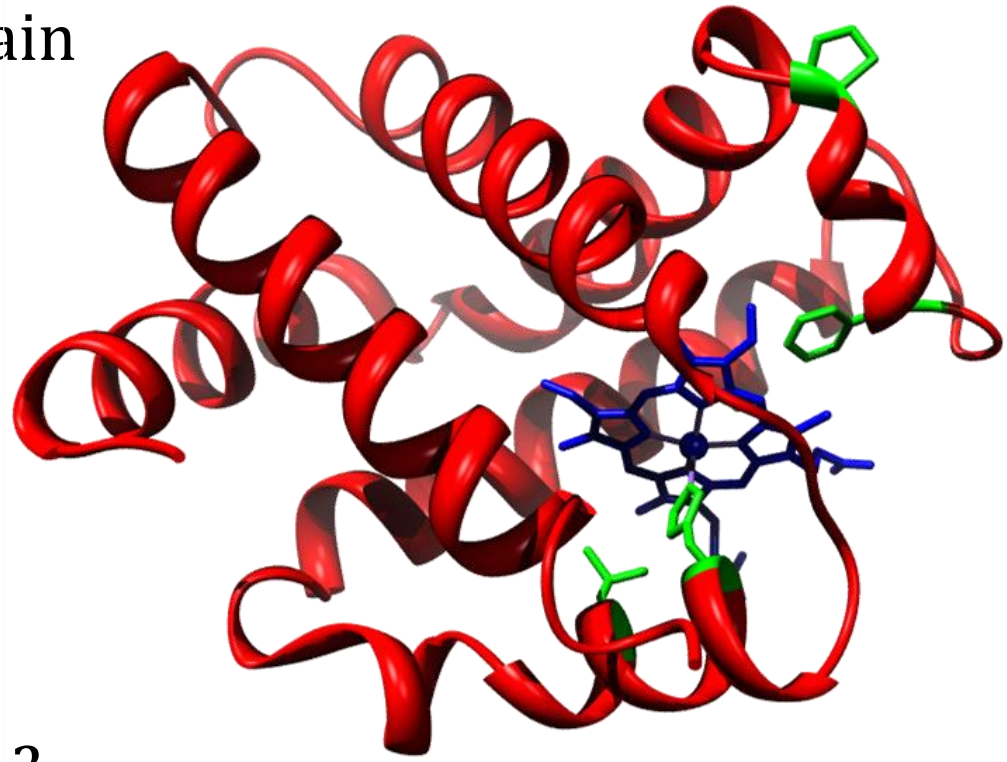
4 residues (maybe more) stand out as conserved

- 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

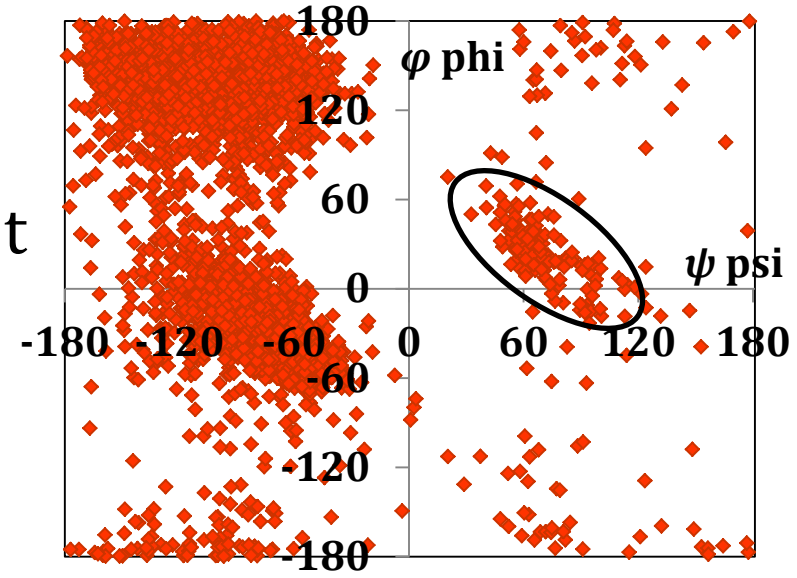
What is special about proline ?

- 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

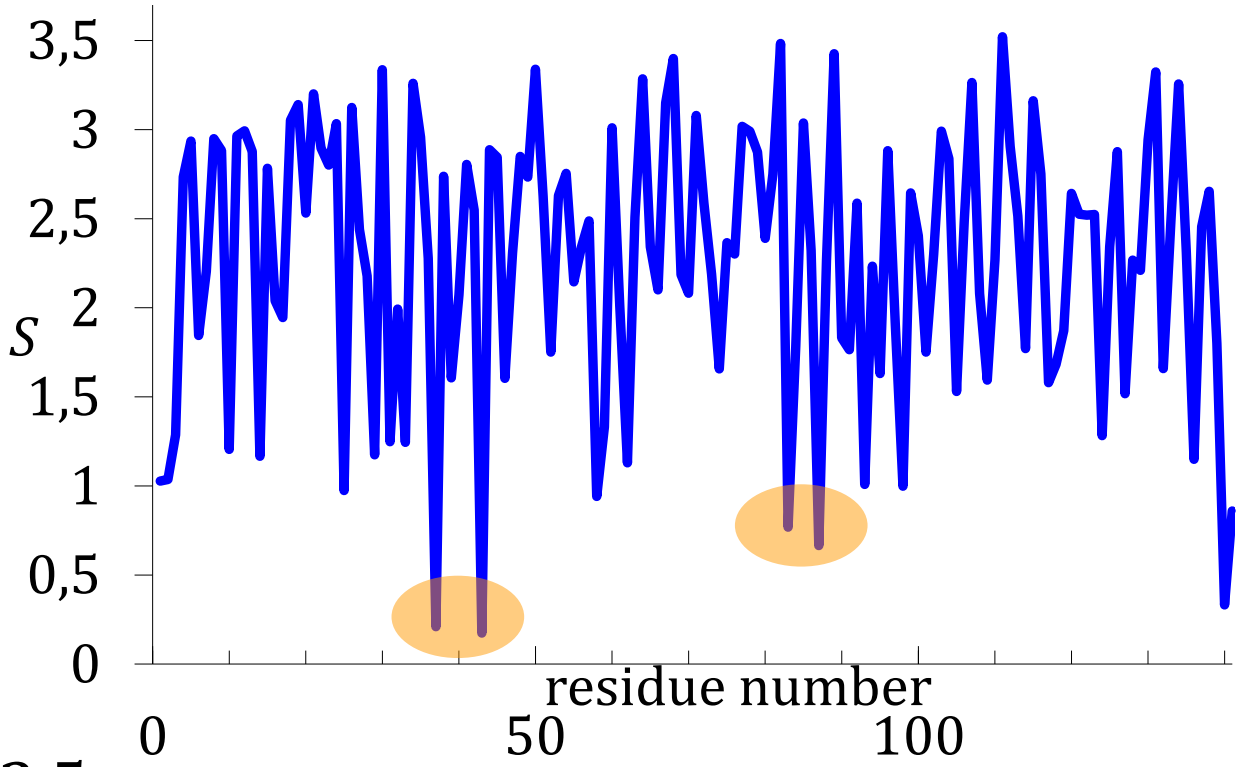
Imagine: two possible systems for some important enzyme

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

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 ?

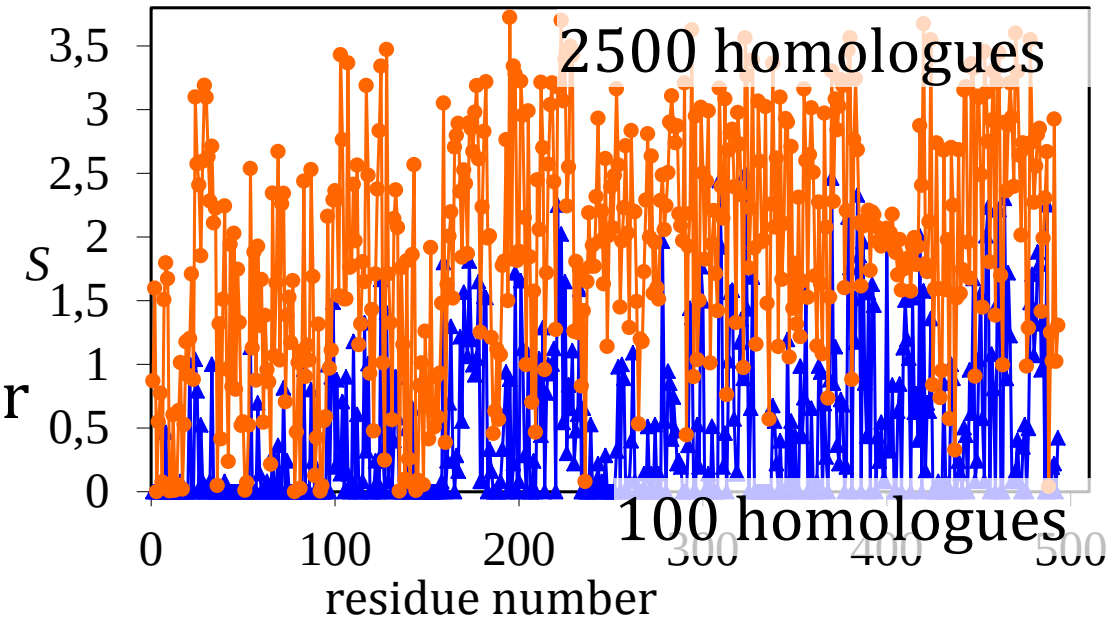
Conservation – how meaningful ?

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

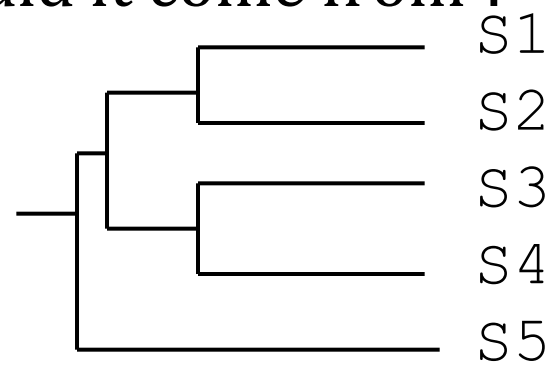
- lots of conserved sites
- you can get the answer you want



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)

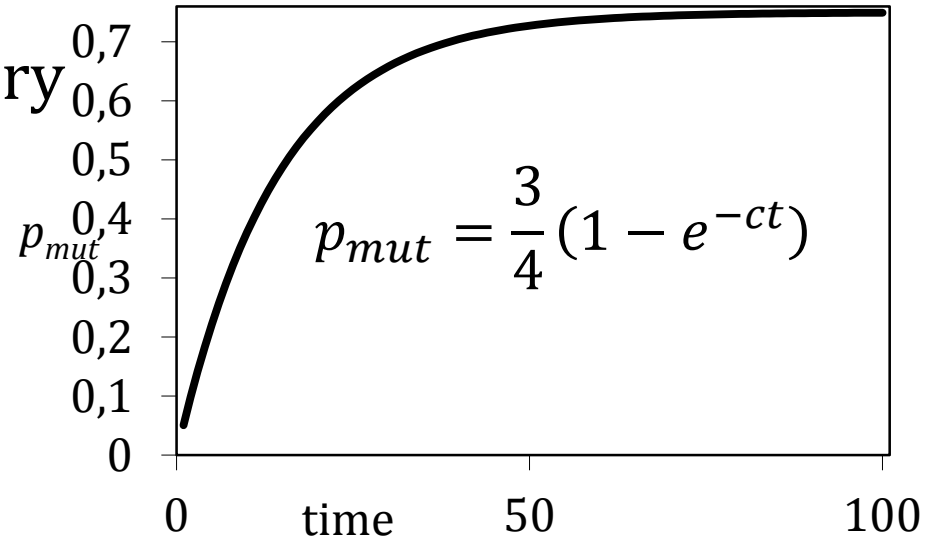
A → C → G → C → A looks like zero mutations

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

- 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

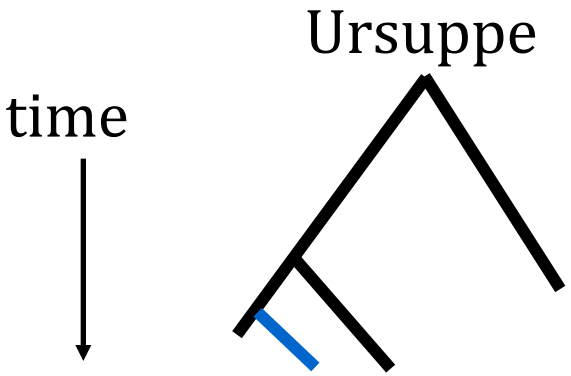
- for small t, distances will be more reliable
  - as will be alignments

Is this enough for phylogeny ?

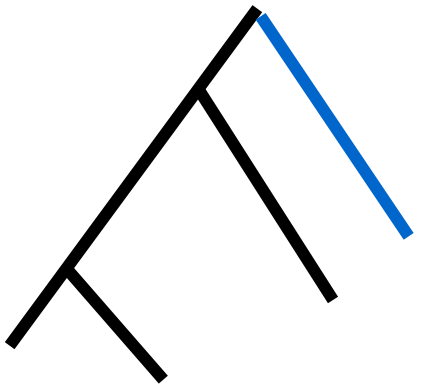
- what about reliability ?

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 ?

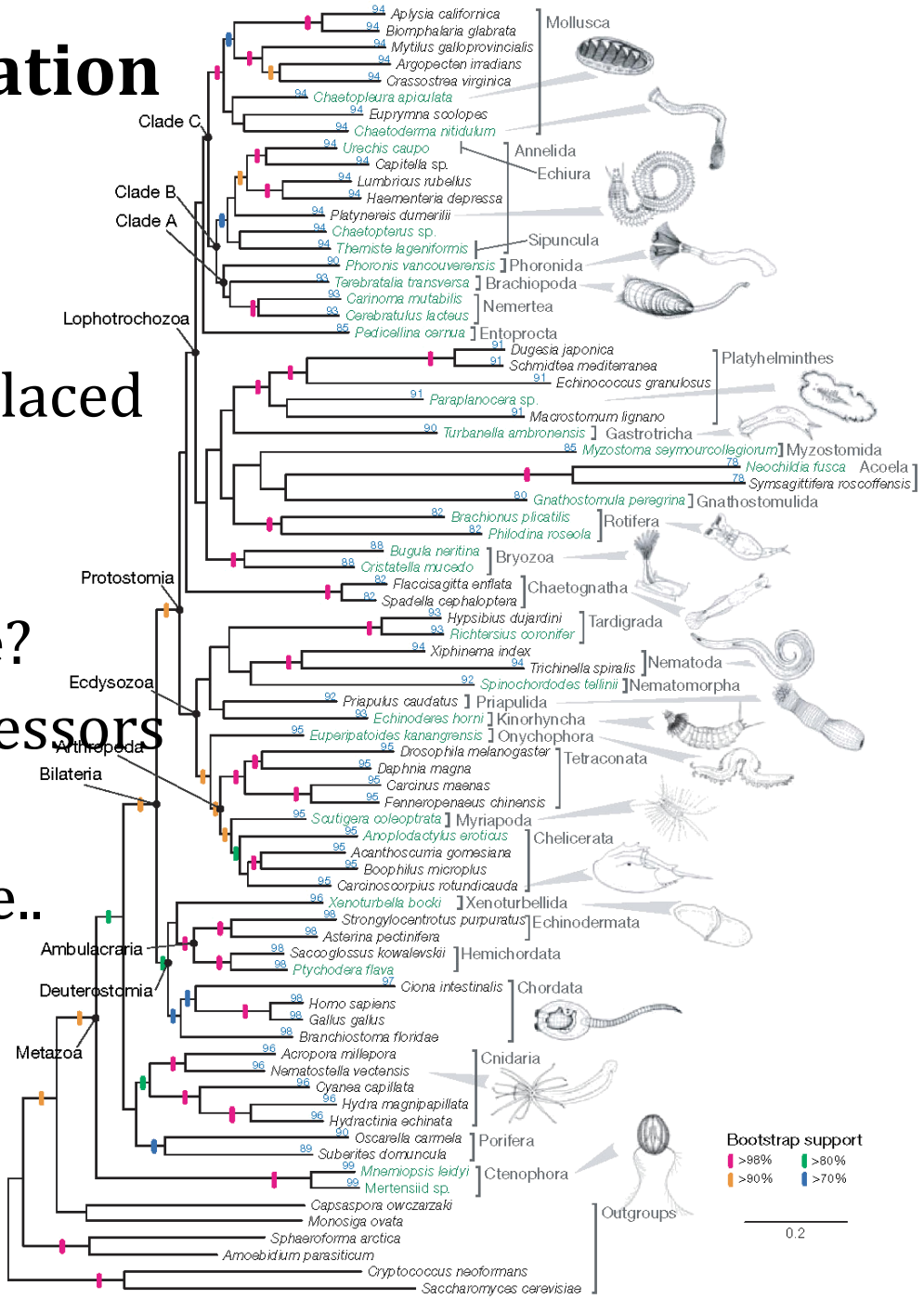
Monster example

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

- example from cover of nature

Monster calculation

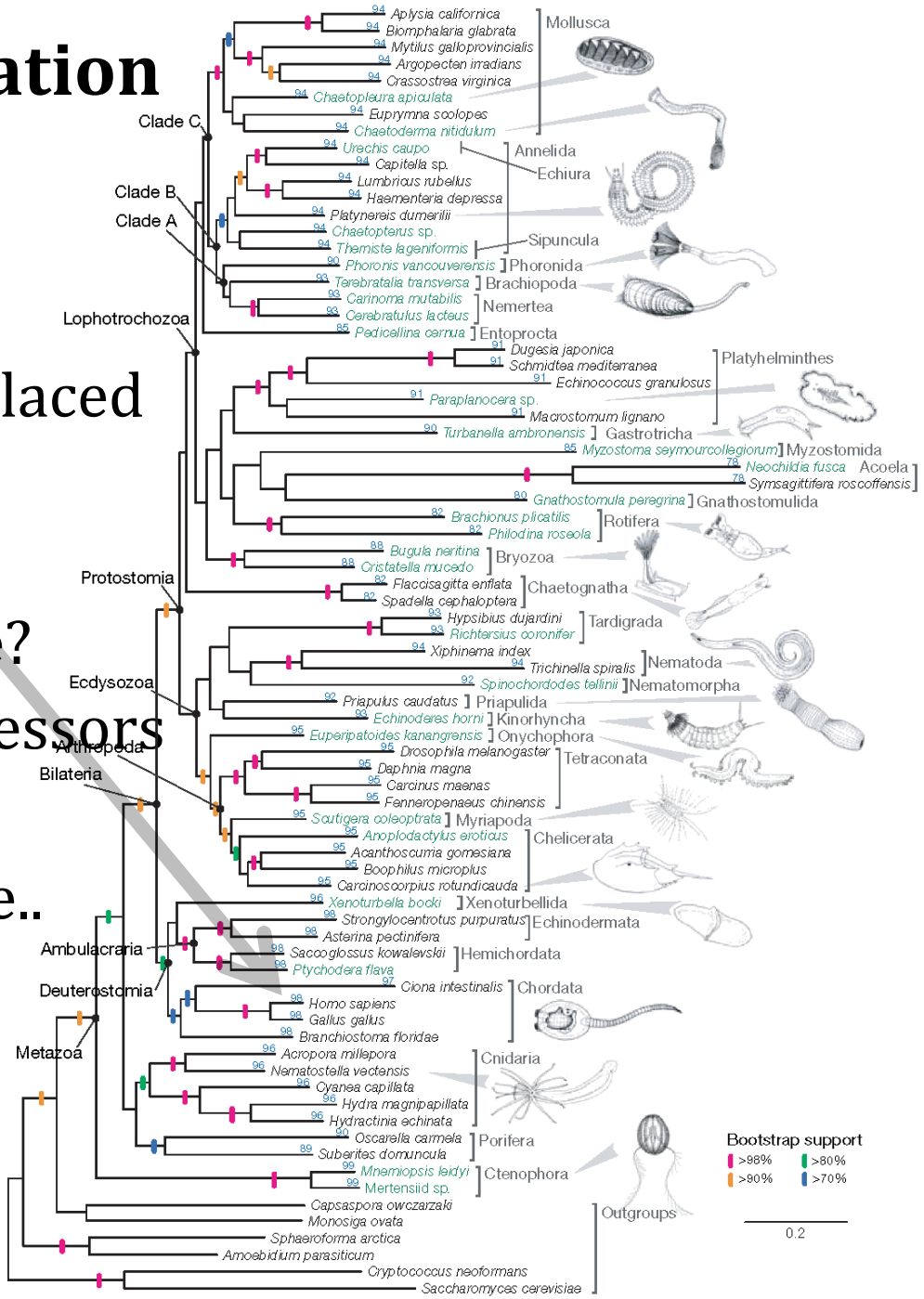
- We are usually placed near Hühne
- we are not so reliably placed with little worms
- how long does this take?
  - months on 120 processors
- a more applied example..



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

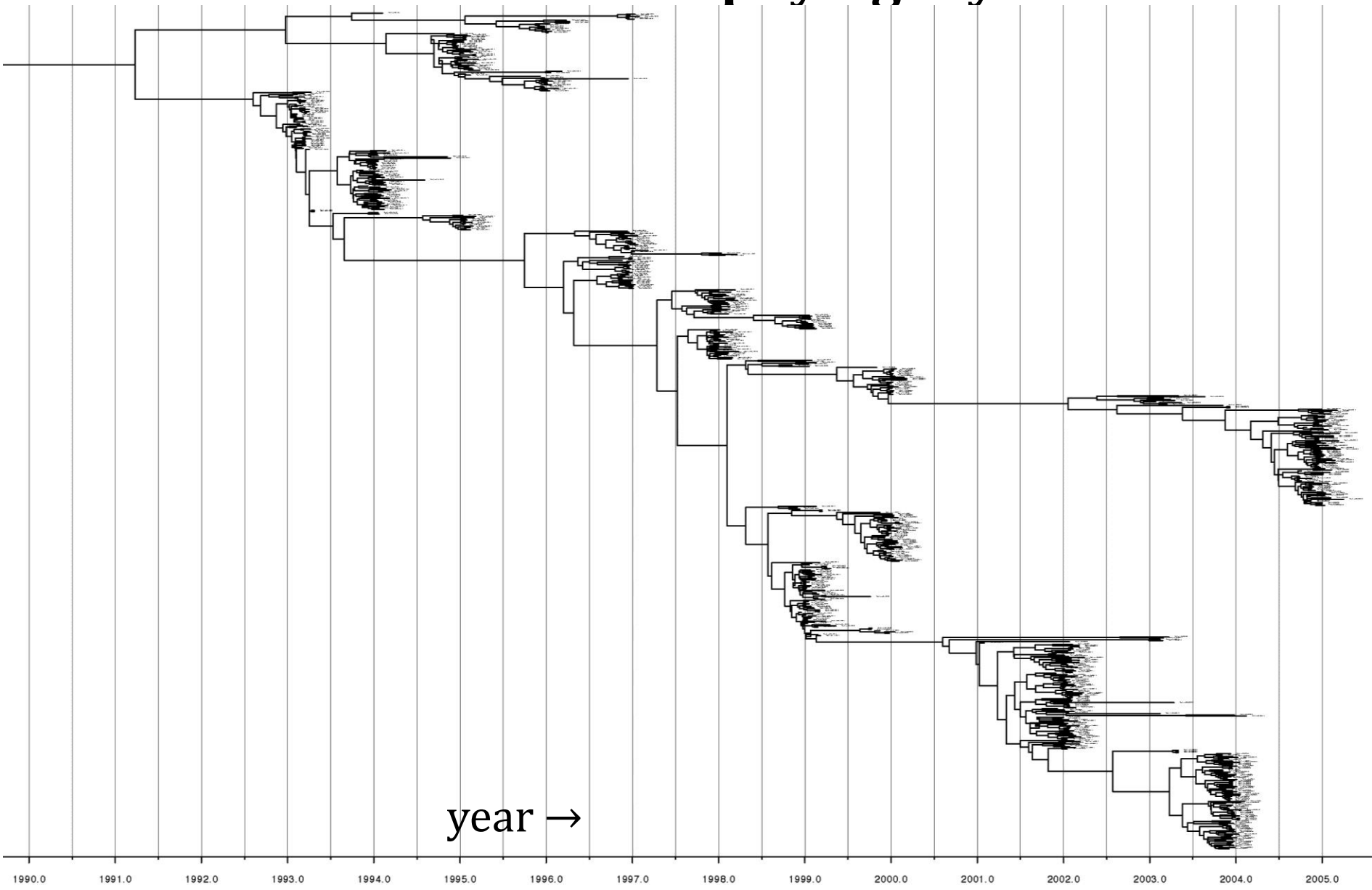
Monster calculation

- 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

DNA or protein sequences ?

- regulatory regions, RNA genes
- synonymous mutations
  - more common than ones that change amino acids
- non-synonymous mutations (amino acid changes)
  - more information D⇌E, I⇌L⇌V, ..
- frame shifts
  - destroy an amino acid sequence
  - small change at DNA level
    - how often do they occur ?
- alignment reliability
  - proteins
    - better, amino acid similarity
  - DNA
    - less information

DNA or protein sequences ?

	protein	DNA	time
synonymous changes	no	yes	short
a.a. changes	yes	no	longer
frame shifts	no	yes	
non-coding regions	no	yes	

Short time

- use DNA

Longer time

- use proteins

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

Protein structures and comparisons

Ultimate aim

- how to find out the most about a protein
- what you can get from sequence and structure information

On the way..

- remote similarities between proteins
- sequence versus structural similarity
- Detour
  - protein coordinates – representation, accuracy
- measures for similarity of coordinates

Sequence and structure similarity

Claim from before

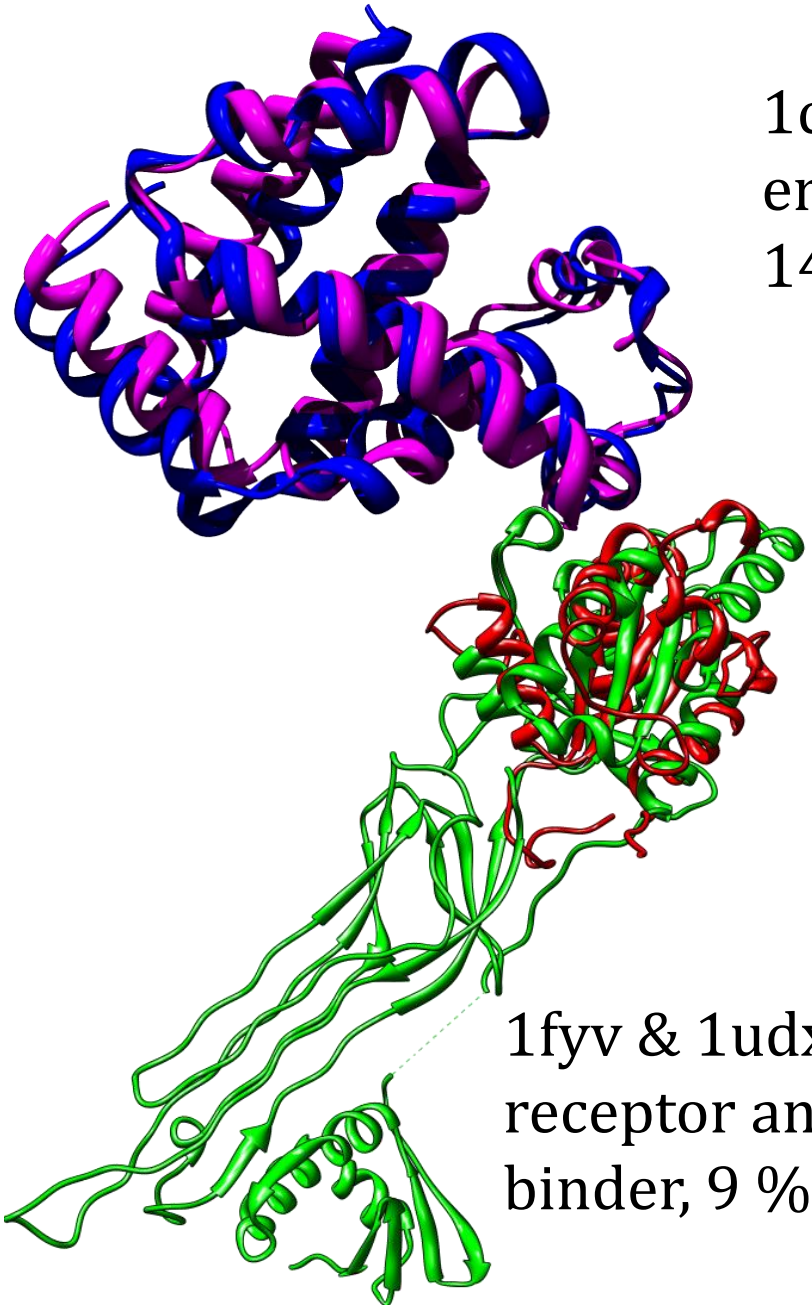
- if two sequences are similar – they are related – structures are similar

Question

- if two sequences are different

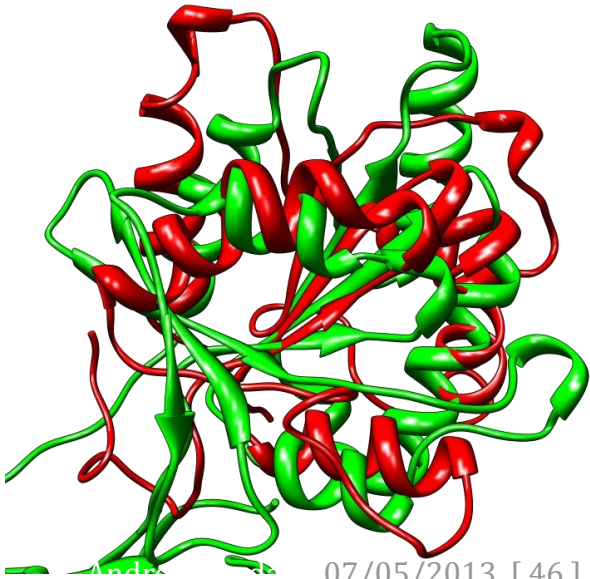
  are their structures different ?

Remote similarities



1cbl & 1eca (haemoglobin & erythrocruorin)
14 % sequence id

1fyv & 1udx, TLR receptor and nucleotide binder, 9 % sequence id



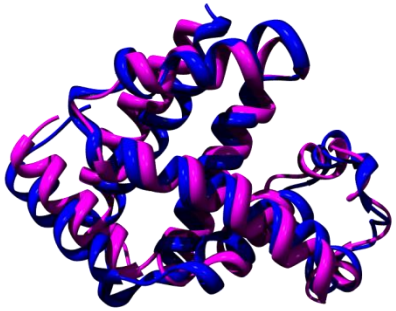
No sequence similarity – similar structures

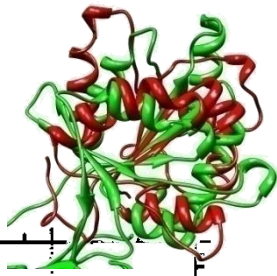
Are these rare ?

- easy to find 100s of examples

Does this agree with previous claims ?

- dot in diagram – two structures seem different



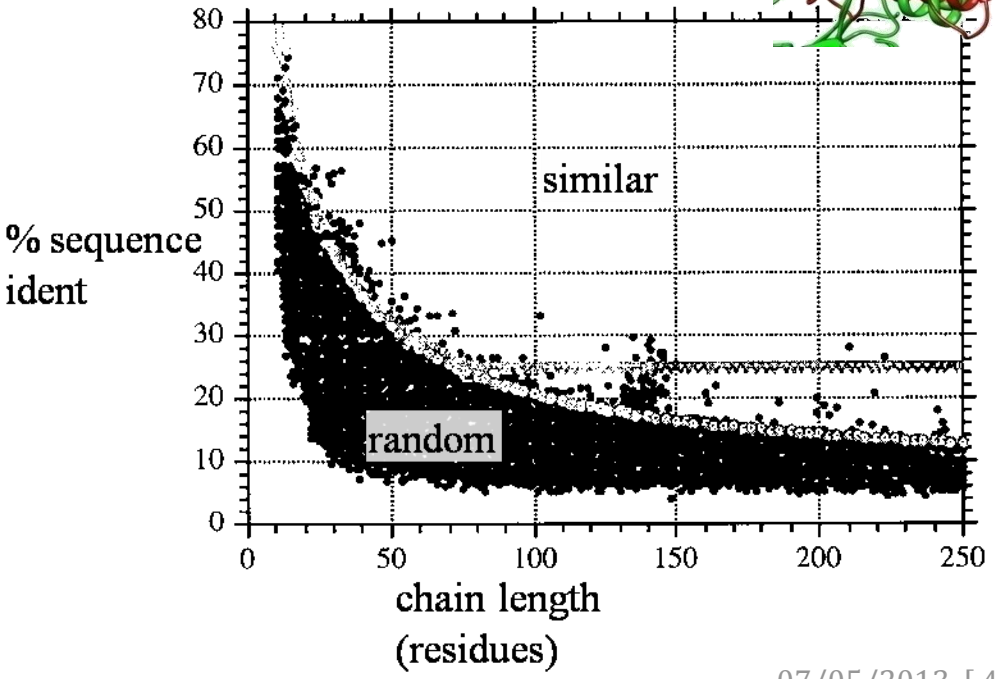


If sequences are similar

- structures will be similar

If sequences are different

- one does not know



Rost, B.Prot Eng, 12,85–94, 1999

Structure versus sequence similarity

Clear statement

- sequence changes faster than structure

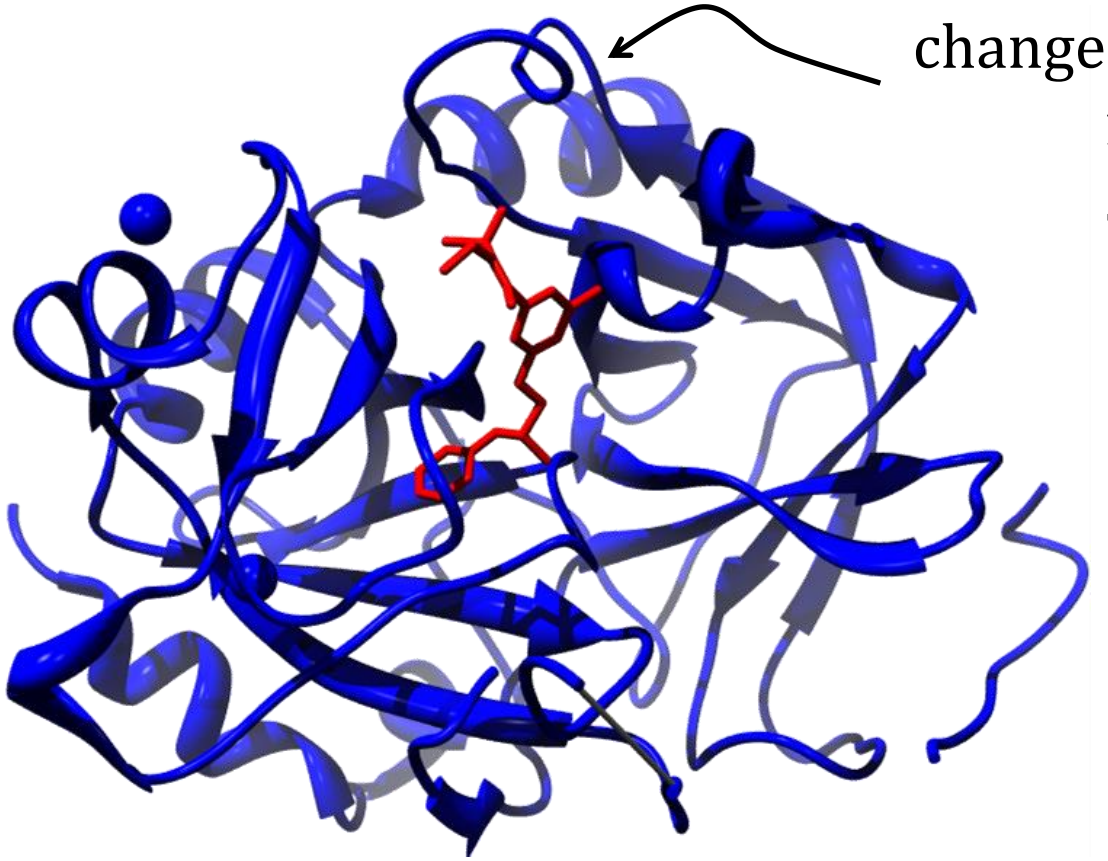
Reason ? Unclear

- possibility..

- protein function depends on having groups in orientation in space

Why can sequence change

View of molecular evolution…



change here

residue changes ? OK

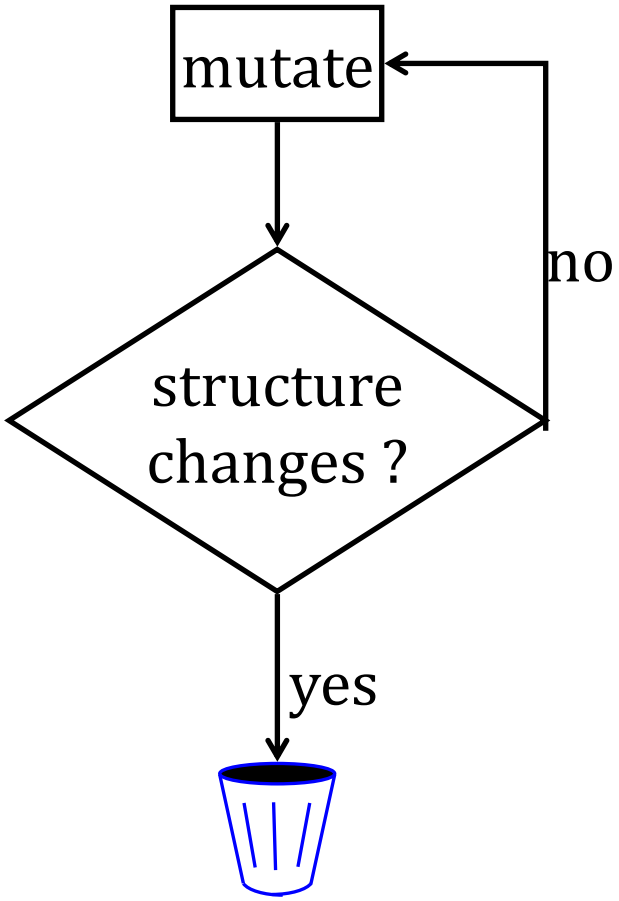
structure changes ? Bad

2j9m, 2cdk + aminopyridine

Simple view of molecular evolution

mutate continuously

- mutations which are not lethal
  - may be passed on (fixed)
- if structure changes
  - protein probably will not function
  - not passed on



Result

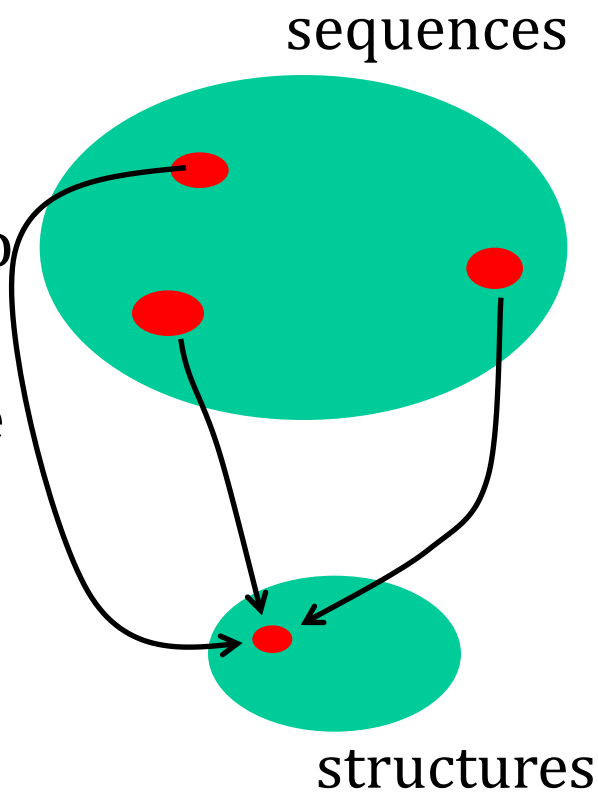
- evolution will find many sequences
  - compatible with structure
  - compatible with function
- how else would we see this ?

Sequence vs structure evolution

Sayings..

- Sequence and structure space
  - sequence space is larger
    - many different sequences map to similar structure
- sequence evolves faster than structure

- Truths…



Practical Consequences

Sequences of proteins are nearly always known

- similar sequence
  - usually similar structure, similar function
- sequences not (obviously) related
  - maybe similar structure
  - maybe similar function

What if structures are known ?

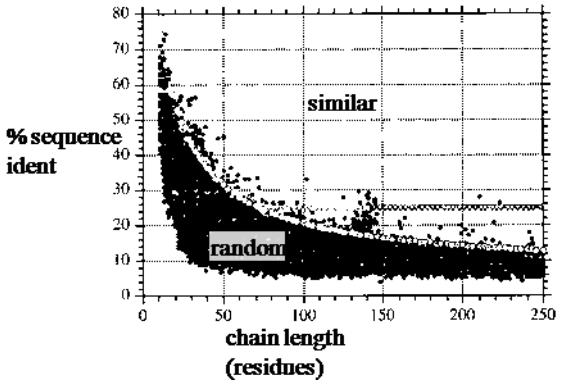
Sequence vs structure similarity

When comparing proteins

- more information is always better (sequence, structure,function)

Similar sequences

- structure and function will be similar
  - remember threshold graphs from earlier



Similar structures, different sequences

- evolutionary relationship implied but
  - bigger evolutionary distance
- not enough to be confident about function

- what do we mean by similar structures ?

Comparing protein structures

Comparing protein structures

- not an $O(n)$, $O(n^2)$, $O(n^3)$ problem
- requires approximations
  - optimal answer never guaranteed

Summarise and Stop

Multiple sequence alignments

- for conservation
- for phylogenies

Phylogenies

- not as reliable as the pictures imply

Structure vs sequence evolution

- sequence changes faster
- sequence similarity means a closer evolutionary relationship
  - functional similarity