

Administration

Sprache ?

- zu verhandeln

Selection of topics

- Proteins / DNA / RNA

Two themes (Semesterhälfte), 13 Termine

- Torda: larger molecules, proteins (6 Vorlesungen)
- Rarey: Chemoinformatics, Wirkstoffentwurf (6)
- Klausur

Administration

Who are we ? (Torda parts, 1st half of semester)

- Andrew Torda
- + Björn Hansen, Iryna Bondarenko, Florian Lauck
- administrative helper
Annette Schade (schade@zbh.uni-hamburg.de)

Where am I

- 42838 7331
- ZBH 1st floor (Bundesstr. 43)

Background

- numerical simulations

Übungen

- Mo 14:15
- Mo 16:15
- Fr 16:15

- wenig Flexibilität mit den Gruppen
- Sie können sich untereinander austauschen

- Konten für unseren Rechnerpool (ZBH)

Prüfungen

Schriftliche Klausur

- 90 Minuten
- Letzter Freitag des Semesters
(wegen eines Biologie-Praktikums)

Bücher

- nicht nötig
- "Understanding Bioinformatics"
Zvelebil, M. & Baum, J.O.
in Bibliothek – leider auf Englisch

Goals, why are we here ?

Overall

- Given some sequence data, what can we find out about it ?
- Some references to structures

Situations – you have

- DNA sequence data (very common)
- corresponding protein sequence (rather common)
 - splice variants, pre/pro-versionen
- structure of a protein (much, much less often)
- If you have the structure of a protein, you know the sequence

The Plan

- Sequence alignments
 - detour protein modelling
- Multiple sequence alignments

My examples

Usually well-studied proteins

- myoglobin, haemoglobin, DNA gyrase, metabolic enzymes

In my examples

- you have sequence and structure
- pretend you are trying to work something out from the sequence

In real world

- you may have only sequence information

Data

Structures

- protein data bank (10^5 files)

Sequences

- genbank, NCBI (4×10^7 sequences)

Freely available

- downloadable
- searchable via web
- requirement for publication (often)

Not much private/secret data

Predictions

- what shape is this molecule ?
- will this small molecule inhibit some enzyme ?
- will this molecule be broken down in the body quickly ?
- ...

Predictions – different approaches

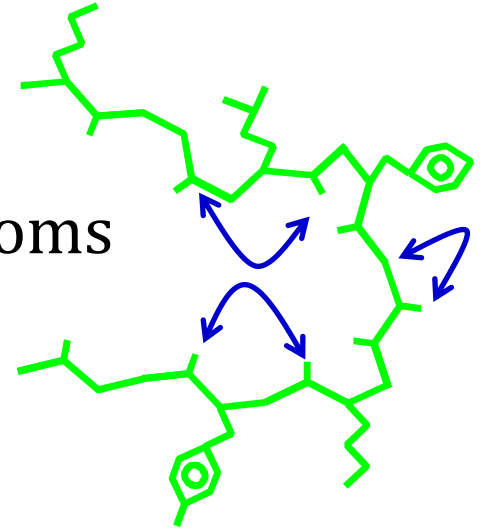
- First principles (physics, chemistry)
- Finding patterns (underlying principles not known)
- Similarity

... explanation

First principles prediction

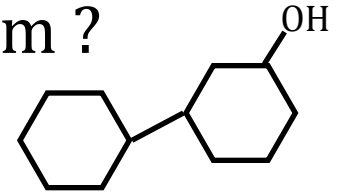
Protein structure example

- a protein molecule = set of atoms in space
- I know all the interactions between the atoms
- should be able to predict the 3D structure



Quantum chemistry

- I have a model for electron wave functions
- can I predict electron density around each atom ?
- predict pK_a for this molecule ?
- ...



Maybe best method

- elegant, expensive, needs good models

Finding patterns

Take known data – collect properties, look for correlations

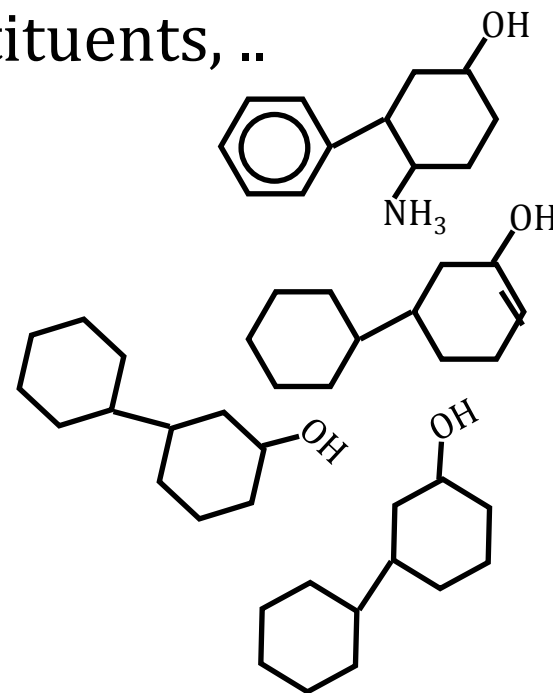
- look at mol wt, aromatic/aliphatic, substituents, ..
- for each molecule collect pK_a
- hope patterns can be found

Gene regulator recognition example

- take known examples
 - look at GC content
 - proximity to protein
 - sizes ...

Field of "data mining", machine learning

- little understanding of problem / chemistry
- often works



Similarity – answer many questions

DNA

- is this region coding ?
- where does the reading frame start ?
- is this region involved in regulator binding ?

Protein sequence

- can one guess the structure ?
- is this membrane bound ?
- does it have a certain activity (kinase, transferase, ..) ?

Protein structure

- what is a likely function ?

Prediction by similarity

Need databases

- DNA sequences, protein sequences
- therapeutics with known properties (2nd half of semester)

For some queries / your sequence.. Is your

- protein sequence similar to a known structure ?
- DNA sequence similar to a known regulatory region ?
- RNA similar to some RNase ?

Experiments are slow, expensive, dangerous, smelly

Similarity in sequences

Protein / nucleotide

- same ideas, differences later

Questions

- are two sequences similar ?
- suspected similarity
 - how reliable is it ?
- detailed alignments (modelling, important residues, ..)

Plan

- generalities
- alignment methods
- DNA versions
- Protein versions
- differences

Alignments and Similarities

Problem

.	.	.	A	C	A	C	T	G	A	C	T	A	.	.	.
.	.	.	.	.	A	T	T	G	A	G	T	A	.	.	.
.	.	.	.	.	1	0	1	1	1	0	1	1	.	.	.

- 6 of 8 positions match

Implicitly

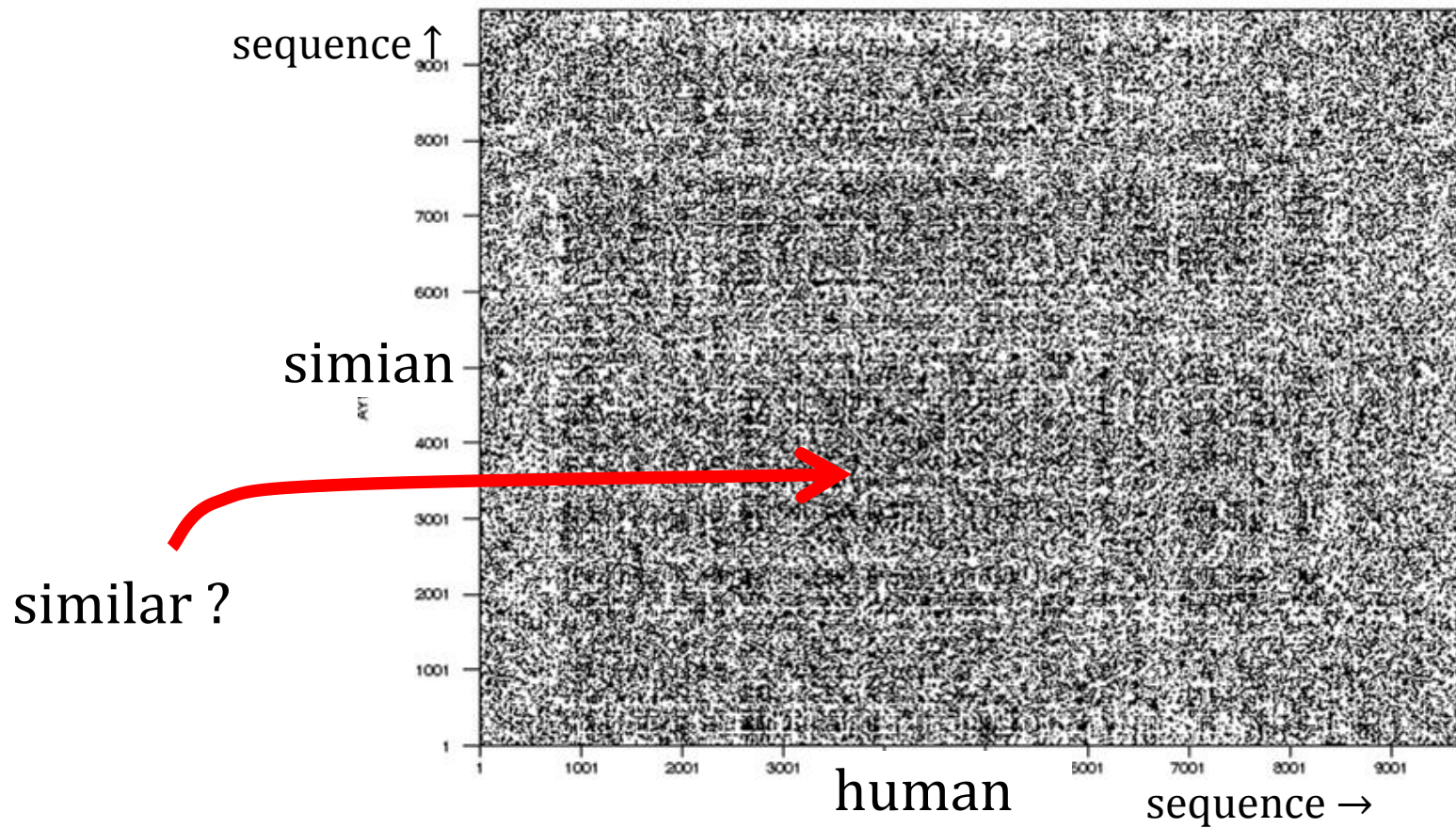
- I have already moved second sequence over the first
- gaps

.	.	.	A	C	A	C	T	T	G	A	C	T	A	.	.	.
.	.	.	.	.	A	T	T	-	G	A	G	T	A	.	.	.
.	.	.	.	.	1	0	1	0	1	1	0	1	.	.	.	

- alignment not so obvious (gaps anywhere)
 - quick look

dot plot

Human and simian HIV



dot plot filtered

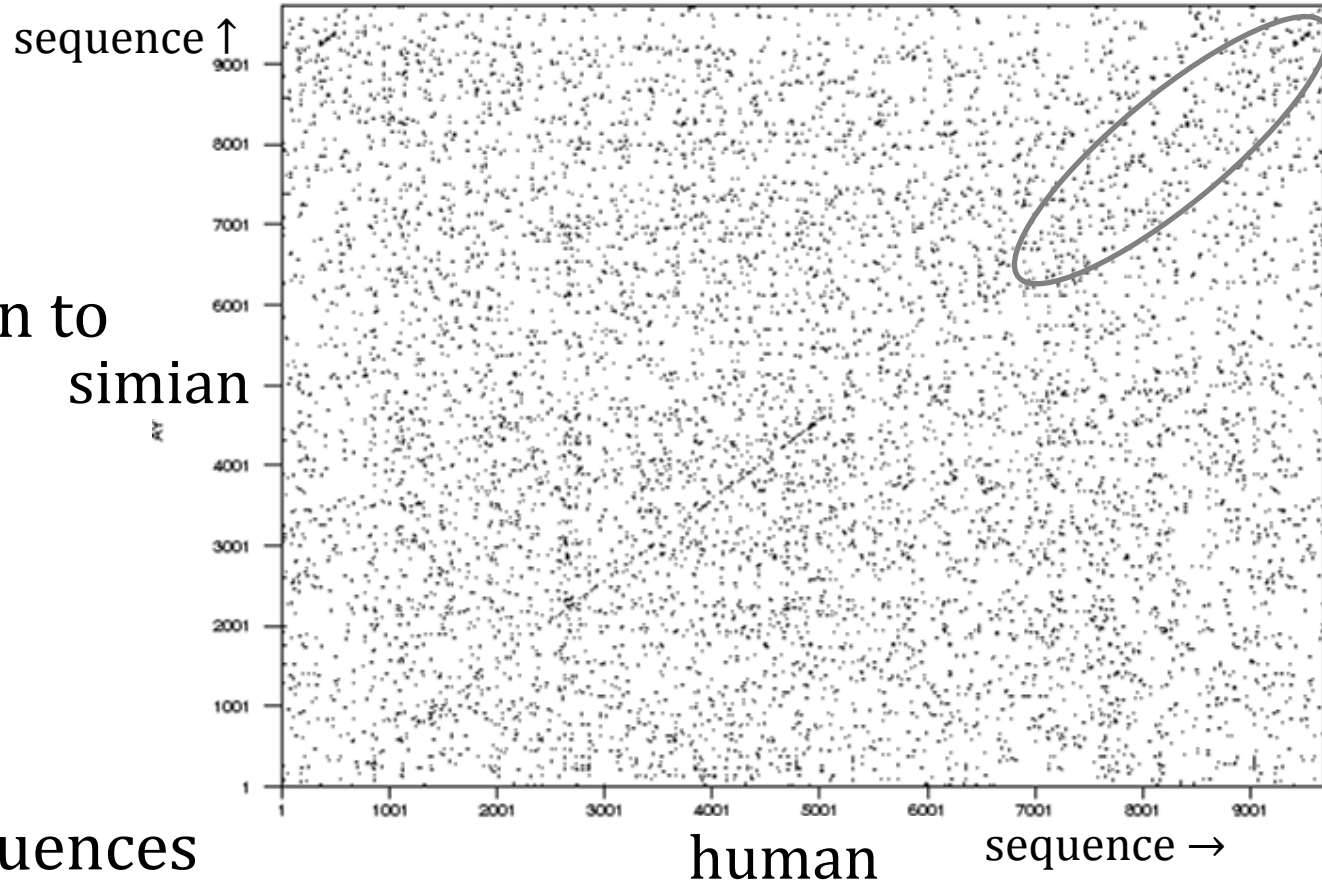
Similarity up to about 5200

Circled region ?

- not so clear
- easy for a human to recognise
- not so easy to automate

Worse case ...

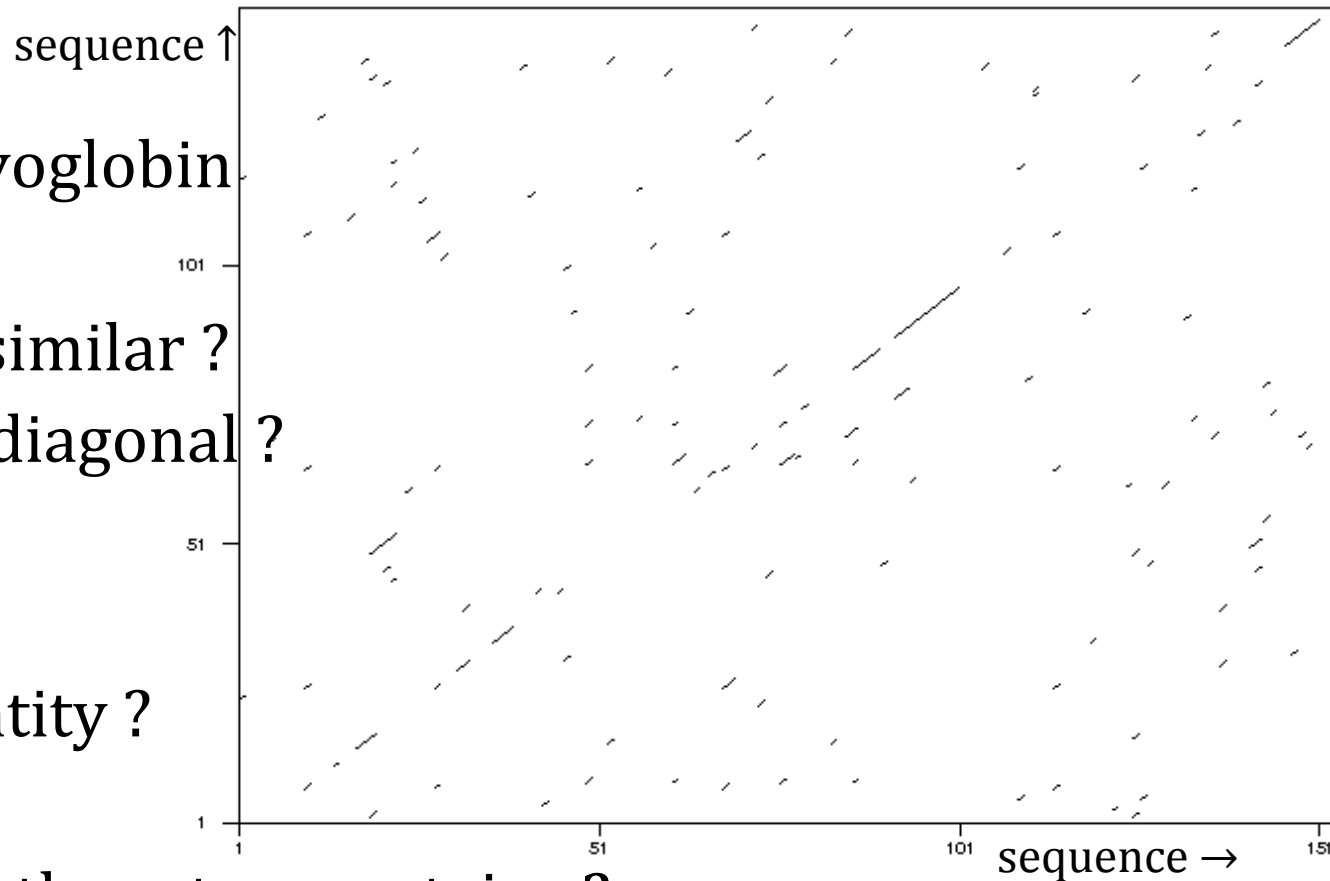
- two protein sequences



protein dot plot

2 proteins

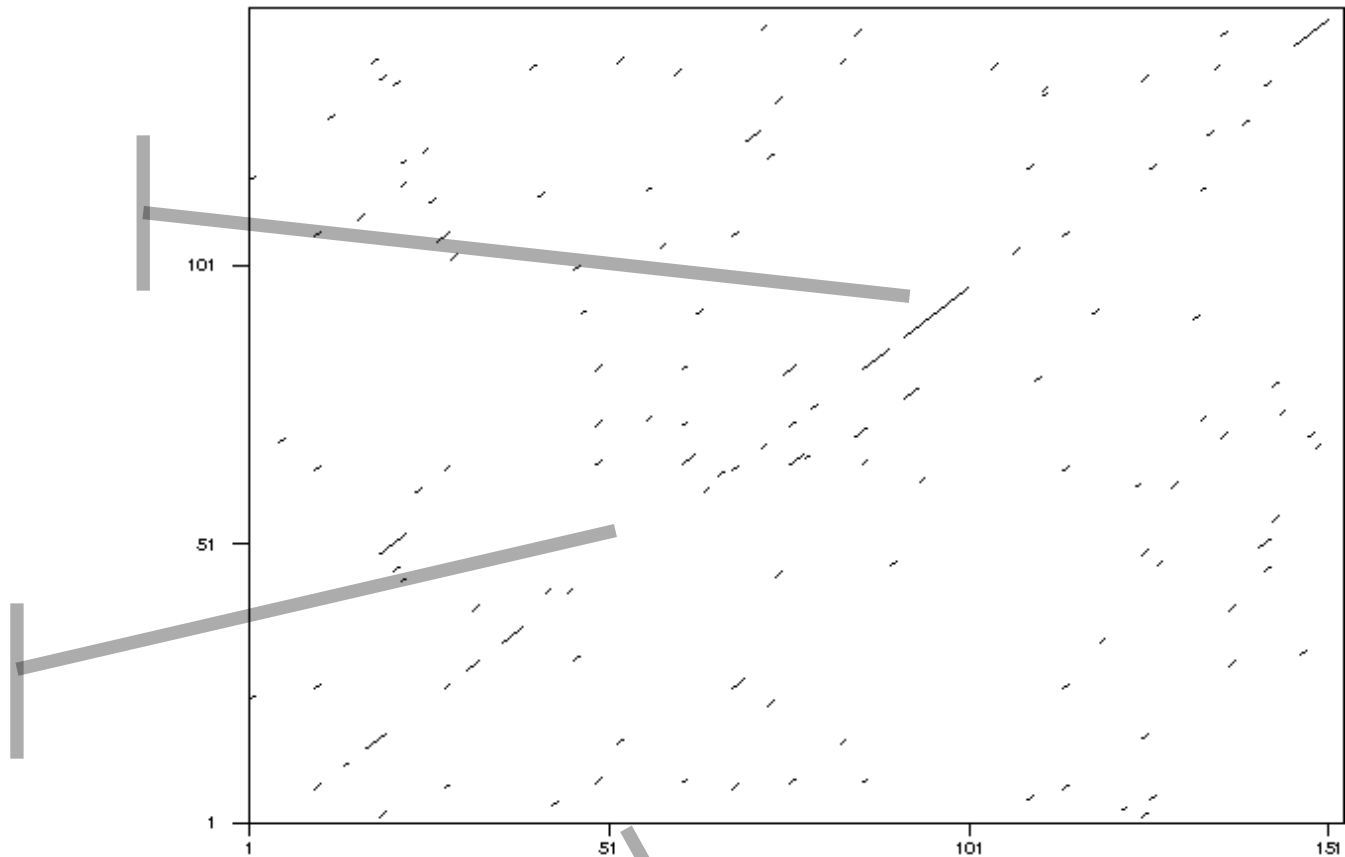
- 2nrl, 2o58
- tuna / horse myoglobin
- are they really similar ?
- how real is the diagonal ?
- what is the identity ?
 - $\approx 45\%$
- how similar are these two proteins ?
- is there a "correct alignment" ? Physical interpretation ?



Properties of alignment ?

Is the alignment
above diagonal ?

What is
happening here ?



Here we know the answer

- look at structures

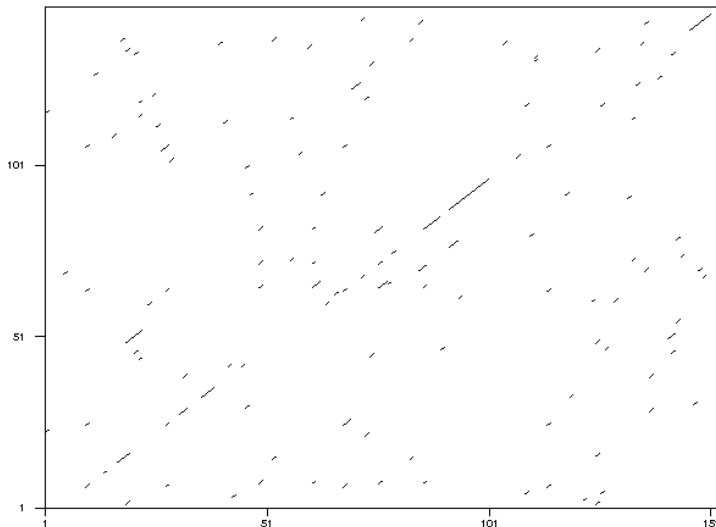
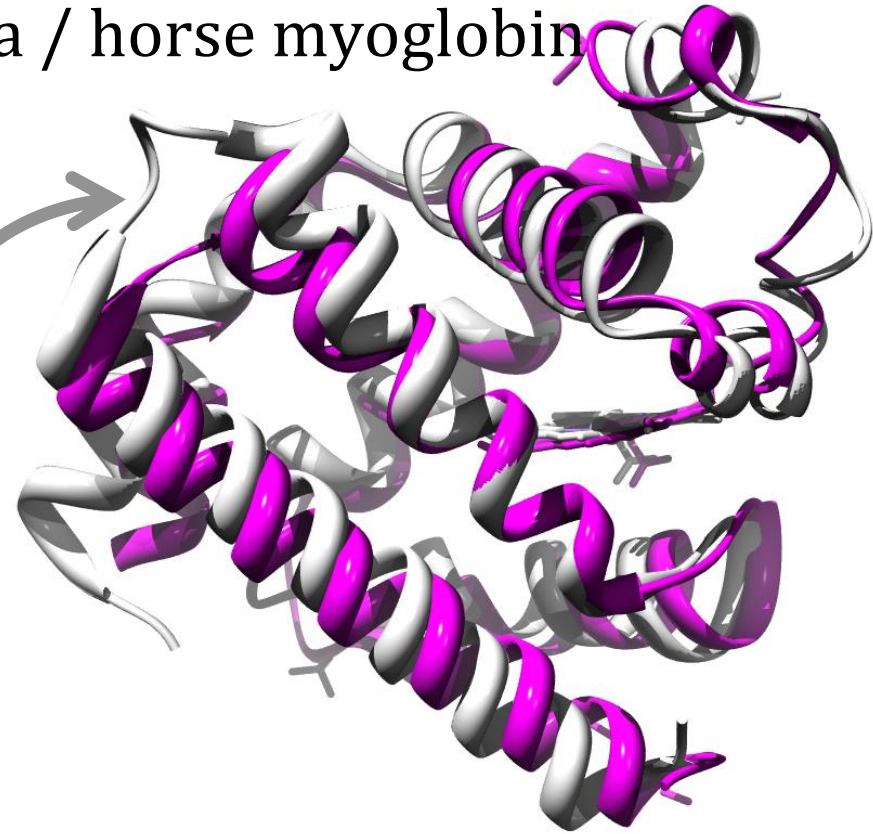
What is aligned to
residue 51 ?

If one knew the structure

The same proteins as before: tuna / horse myoglobin

There are no holes ?

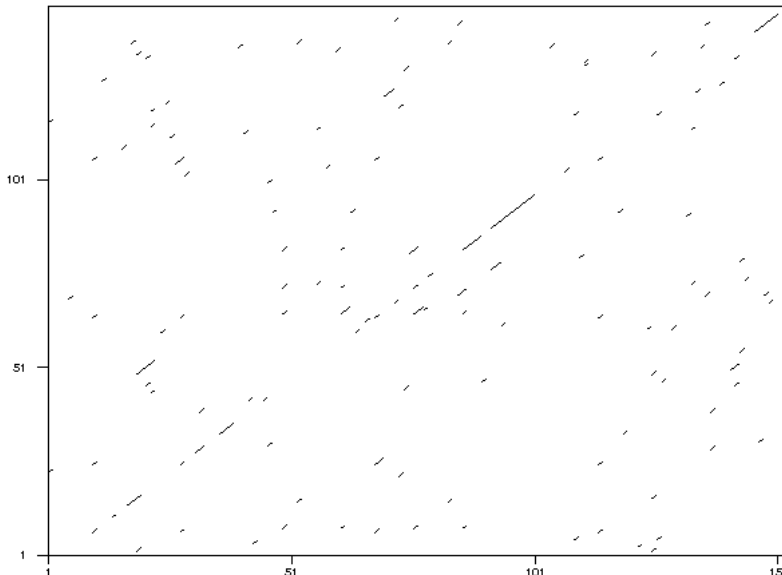
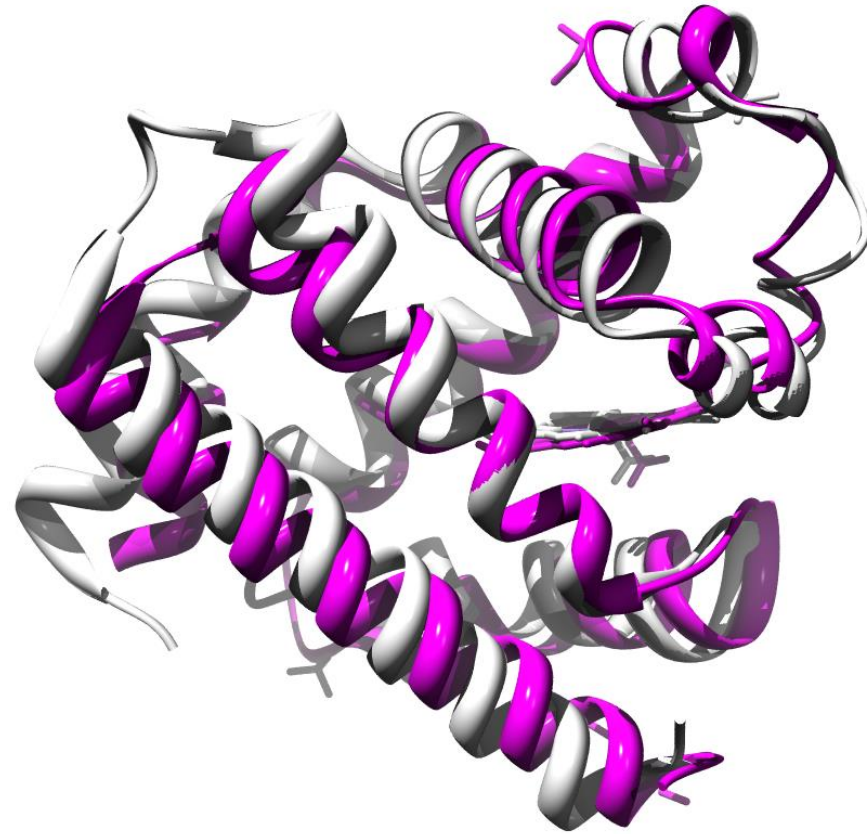
- there are some differences
 - some bits are longer



for almost every pink residue, there is a corresponding grey residue

If one knew the structure..

Would you have recognised this from dotplot ?



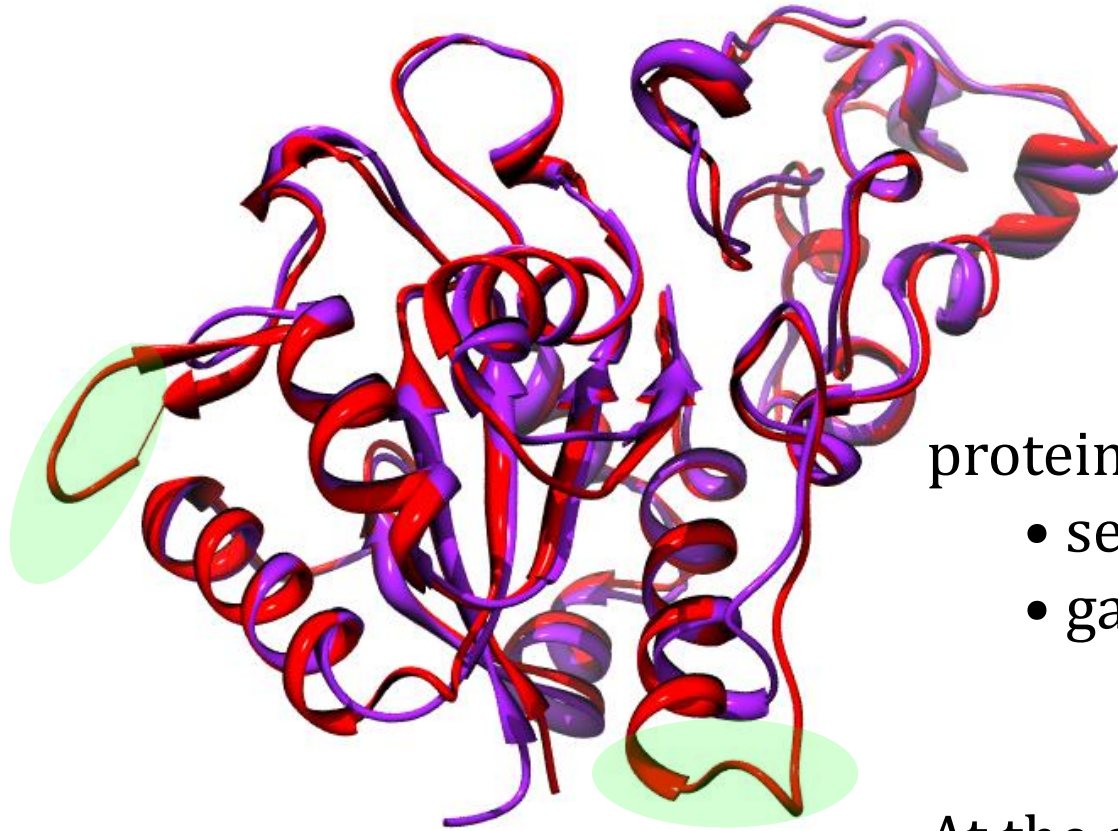
Look at residue 51 in dot plot

- aligned residue not clear

Look in structure

- aligned residues clear

Clearer Example



hydrogenases

- 40 % sequence identity
- 2frvG & 1cc1S

proteins – obviously similar

- sequence identity OK
- gaps and insertions

At the sequence level ?

Seq ID 40.6 % (103 / 254) in 280 total including gaps

:	1	:	2	:	3	:	4	:	5	:	6
:	0	:	0	:	0	:	0	:	0	:	0

kkapviwvqggctgcsvsllnavhprikeilldvislefthptvmasegemalahmyeia
krpsvvyllhnaectgcsesvlrtvdpvdelildvismdyhetlmagaghaveea-1-he

:	1	:	2	:	3	:	4	:	5	:
:	0	:	0	:	0	:	0	:	0	:

:	0	:	0	:	0	:	1	:	1	:	1
:	7	:	8	:	9	:	0	:	1	:	2
:	0	:	0	:	0	:	0	:	0	:	0

ekfngnffllvegaiptakegrycivgeakahhhevtmmelirdlapkslatvavgtcsa
aikg-dfvcvieggipmgdggwygk-----vggrnmydicaevapakaviaigtcat

0	:	0	:	0	:	0	:	1	:	1
6	:	7	:	8	:	9	:	0	:	1
0	:	0	:	0	:	0	:	0	:	0

:	1	:	1	:	1	:	1	:	1	:	1
:	3	:	4	:	5	:	6	:	7	:	8
:	0	:	0	:	0	:	0	:	0	:	0

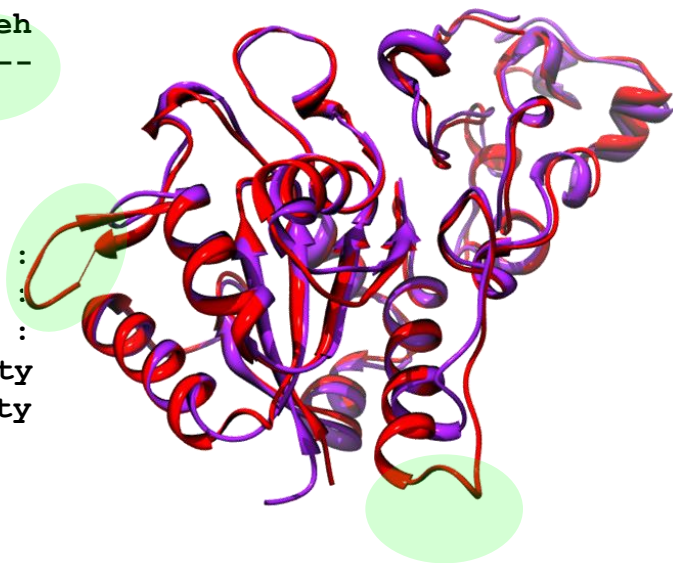
yggipaaegnvtgsksvrddffadekiekllvnvpgcphpdwmvgtlvaawshvlnpteh
yggvqaakpnptgtvgvnealgklgvkai--niagcppnmpnfvgtv--vhlltk-----

:	1	:	1	:	1	:	1	:	1	:
:	2	:	3	:	4	:	5	:	6	:
:	0	:	0	:	0	:	0	:	0	:

:	1	:	2	:	2	:	2	:	2	:
:	9	:	0	:	1	:	2	:	3	:
:	0	:	0	:	0	:	0	:	0	:

plpeldddgrplllffgdniencpyldkydnsefaetftkpg-----ckaelgckgksty
gmpeldkqgrpvmffgetvhdncprlkhfeagefatsfgspeakkgyclyelgckgpdty

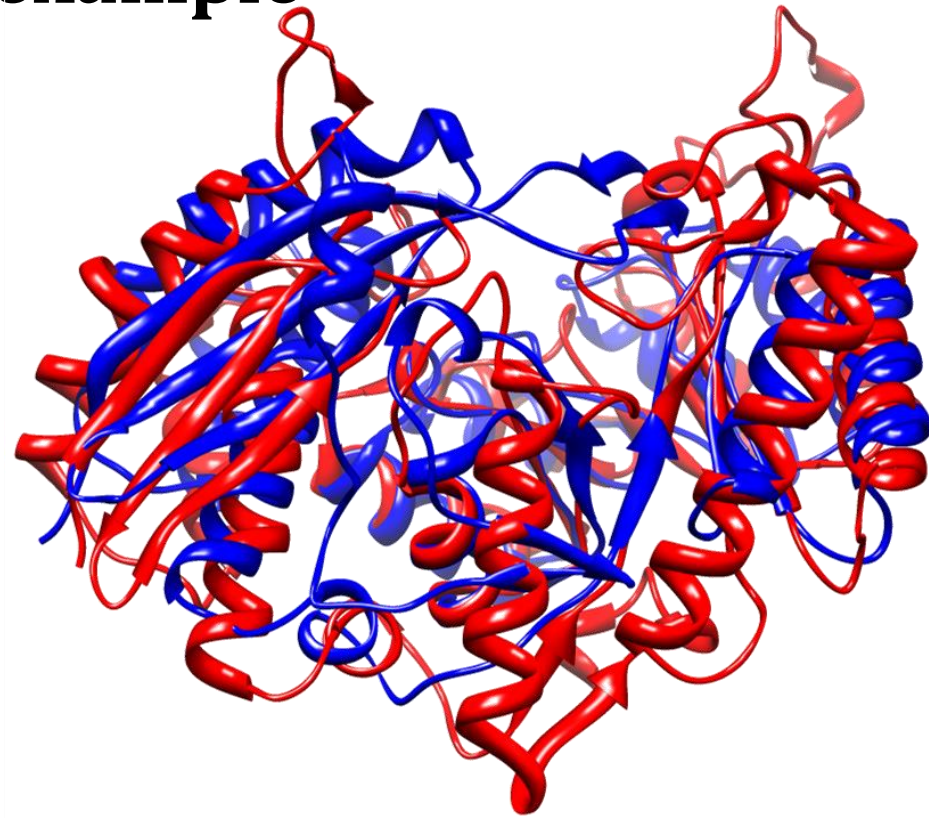
:	1	:	1	:	1	:	2	:	2	:	2
:	7	:	8	:	9	:	0	:	1	:	2
:	0	:	0	:	0	:	0	:	0	:	0



Another example

Structures known

- can easily get sequence alignment



2mnr mandelate racemase
4enl enolase

Seq ID 25.1 % (81 / 323) in 373 total including gaps

```

      1       2       3       4       5
      :       :       :       :       :
      :       :       :       :       :
sktyavlglgngghafaaylalkgqsv--lawdidaqr-----ikeiqdrgaiiaegpg
svehimrdv-nggwa-mryihangaslflavyyihifrglyygsykapreitwivgmviy
0       :       :       :       :       :       :       :
8       :       9       :       1       :       2       :       3       :
0       :       0       :       0       :       0       :       0       :

      :       :       :       :       :       :       :
      :       6       :       7       :       8       :       9       :
      :       0       :       0       :       0       :       0       :
la--gtahpdltsdiglavkdadvilivvpaihhasiaaniaisyiseqgli---ilnpg
llmmgtafmggyvlpwgqmsfwgatvitglfgaipg--igpsiqawllggpavdnatlnrf
1       :       1       :       1       :       1       :       1       :
4       :       5       :       6       :       7       :       8       :
0       :       0       :       0       :       0       :       0       :

      1       :       1       :       1       :       1       :       1       :
      1       :       2       :       3       :       4       :       5       :
      0       :       0       :       0       :       0       :       0       :
atggalefrkilrengapevtigetssmlftcrserpgqvtvnaikgamdfaclpaakag
fslhyllpf-viaalvaihiwafhttggnnptgvevrrtskadaekdtlpfwpvfvikdl
      :       2       :       2       :       2       :       2       :       2       :
      :       0       :       1       :       2       :       3       :       4       :
      :       0       :       0       :       0       :       0       :       0       :

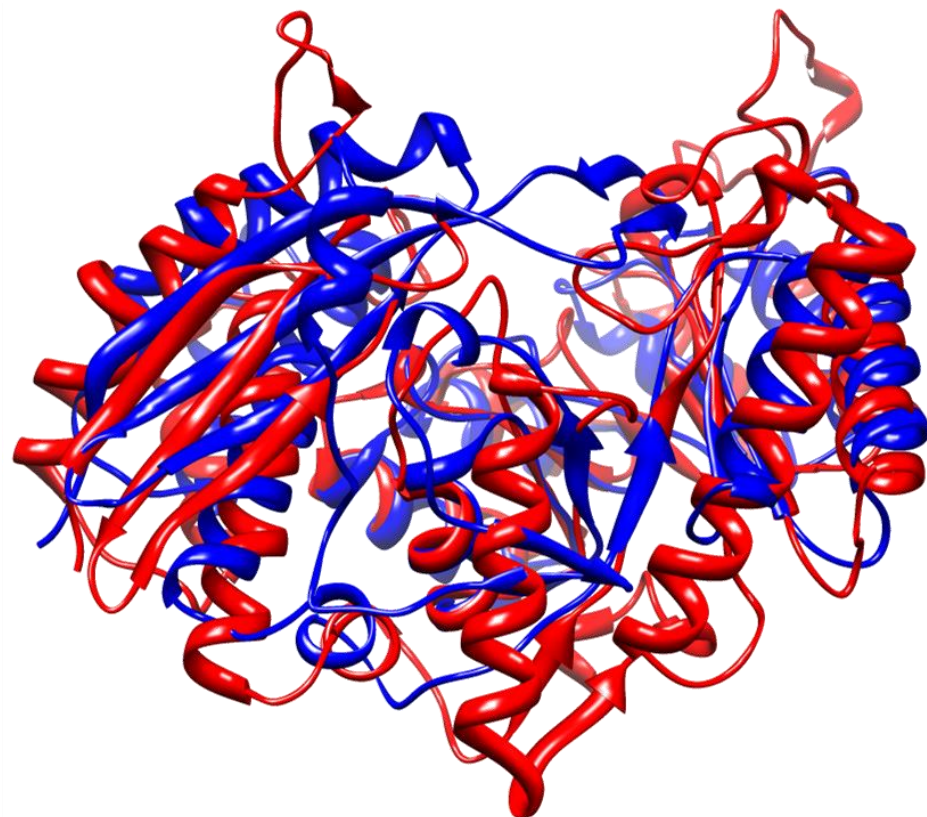
      1       :       1       :       1       :       2       :       2       :
      7       :       8       :       9       :       0       :       1       :
      0       :       0       :       0       :       0       :       0       :
waleqigsvlpqyvavenvlhtsltnv-navm-hplptllnaarcesgtpf----qyyl-
fala-l--vllgffavvaympnylghpdnyvqanplstpahivpewyflpfyailrafaa
      :       2       :       2       :       2       :       2       :       3       :
      :       6       :       7       :       8       :       9       :       0       :
      :       0       :       0       :       0       :       0       :       0       :

      :       2       :       2       :       2       :       2       :       2       :
      :       3       :       4       :       5       :       6       :       7       :
      :       0       :       0       :       0       :       0       :       0       :
-egitpsv-gslaekvdaeriaiakafdlnvpsvcewypatiyeavqgnpayrgiagpin
dvwvvilvdgltfgivdakffgviamfga-i-avmalapw-ldtskvrsgayr---pkf
3       :       3       :       3       :       3       :       3       :
1       :       2       :       3       :       4       :       5       :
0       :       0       :       0       :       0       :       0       :

      2       :       2       :       3       :       3       :       3       :
      8       :       9       :       0       :       1       :       2       :
      0       :       0       :       0       :       0       :       0       :
lntryffedvstglvplselgravnvptplidavldlisslidtdfrkegrtleklglsg
---rmwfwflvldfvlvtwvg-a--m--pt-eypydwis-liastywfay-flvilpllg
      :       3       :       3       :       3       :       4       :       4       :
      :       7       :       8       :       9       :       0       :       1       :
      :       0       :       0       :       0       :       0       :       0       :

3       :
4       :
0       :
ltaag--irsave
atekpepipasie
      :       4
      :       2
      :       0

```



Getting alignments

Do we always know the structure ?

- if so, we would not do these lectures
- sequences are cheap
- structures are expensive

Usually one only has the sequence

Mission for today ?

- how does one find the best alignment based on sequence ?

Alignment methods

Best alignment not obvious

.	.	.	.	.	.	.	C	C	A	T	C	C	G	C	.	.	
.	.	.	C	G	A	T	C	C	–	T	C	C	T	C	.	.	.

6 matches or

.	.	.	.	.	.	.	C	C	A	T	C	C	G	C	.	.	.	.	
.	.	.	.	.	.	.	C	G	A	T	C	C	T	C	C	T	C	.	.

also 6 matches

Can we invent some rules to say which is best ?

How many alignments ?

For two sequences of length 10, how many alignments could I generate ?

. A B C D E F G H I J

Q R S T U V W X Y Z

. Q R S T U V W X Y Z + more

with gaps

Q R S T U V W X Y - Z

Q R S T U V W X - Y Z then with

gap 2

Q R S T U V W X Y - - Z

. . .

- then with multiple gaps ... combinatorial explosion
- do not tackle the problem directly

Mission

For DNA, protein, RNA

- develop some scoring scheme
- maximize matches and similarities

Algorithm

- allow some gaps, not too many
- must be much faster than brute force
- these methods apply to proteins and nucleotides

What is coming

- simple scoring – DNA
- full alignment algorithm (Needleman and Wunsch)
- better scoring – proteins

Scoring for DNA

Sensible scheme

- matched pairs 2
- mismatch -3
- gaps -2

A	C	T	G	-	A	T	T	C	G	A
A	C	-	G	C	A	-	T	C	T	A
2	2	-2	2	-2	2	-2	2	2	-3	2

more sophisticated..

- gap opening costs - 2
- gap widening costs - 1
- so $cost = cost_{open} + (n_{gap} - 1)cost_{widen}$

Representing alignments

- sequences GATTCAGGTTA and GGATCGA

		g	g	a	t	c	g	a	
g									
a									
t									
t									
c									
a									
g									
g									
t									
t									
a									

- would mean
GGAT-CGA-----
-GATTC-AGGTTA
- notes...

Representing alignments

GGAT-CGA-----
 -GATTC-AGGTTA

		g	g	a	t	c	g	a	
g									
a									
t									
t									
c									
a									
g									
g									
t									
t									
a									

- alignment does not have to go to first / last row or column
- which is x and y is arbitrary
- gaps = row or column is skipped
- work ↘ or ↙ does not matter
- direction must be consistent
 - we only go → ↓

- make sure this is clear

Representing alignments with mismatches

- sequences GCTTCAGGTTA and GGATCGA

		g	g	a	t	c	g	a	
g									
c									
t									
t									
c									
a									
g									
g									
t									
t									
a									

- would mean
GGAT-CGA-----
-GCTTC-AGGTTA

Calculating alignment - steps

Needleman and Wunsch algorithm

1. fill score matrix
2. find best score possible in each cell
3. traceback

fill score matrix

- For convenience, add some zeroes to the ends

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0								0
a	0								0
t	0								0
t	0								0
c	0								0
a	0								0
g	0								0
g	0								0
t	0								0
t	0								0
a	0								0
	0	0	0	0	0	0	0	0	0

Mission

- find path through this matrix with best score
- account for gaps

fill score matrix

- For convenience, add some zeroes to the ends
- Add in match, mismatch scores

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-3	2	-3	-3	-3	2	0
t	0	-3	-3	-3	2	-3	-3	-3	0
t	0	-3	-3	-3	2	-3	-3	-3	0
c	0	-3	-3	-3	-3	2	-3	-3	0
a	0	-3	-3	2	-3	-3	2	2	0
g	0	2	2	-3	-3	-3	2	-3	0
g	0	2	2	-3	-3	-3	2	-3	0
t	0	-3	-3	-3	2	-3	-3	2	0
t	0	-3	-3	-3	2	-3	-3	-3	0
a	0	-3	-3	2	-3	-3	-3	2	0
	0	0	0	0	0	0	0	0	0

Mission

- find path through this matrix with best score
- account for gaps

Summing the elements

- start at top left
- move right, then next line
- at each cell
 - find best score it could possibly have

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0	-3	-4	-4	4	3	1	0	2
c	0	-3	-5	-5	-2	6	0	-2	1
a	0	-3	-5	-6	-3	0	3	6	3
g	0	2	0	-6	-4	-1	6	0	6
g	0	2	4	-3	-4	-2	5	3	4
t	0	-3	-1	1	4	-2	-1	2	3
t	0	-3	-3	-1	3	1	-1	0	2
a	0	-3	-4	3	-4	0	-2	4	0
	0	0	-2	0	3	1	0	1	4

Diagonal (no gaps)

for each cell, 3 possible scores

1. **diagonal (no gap)**
2. best from preceding column
3. best from preceding row

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0	-3	-4	-4	4	3	1	0	2
c	0	-3	-5	-5	-2	6	0	-2	1
a	0	-3	-5	-6	-3	0	3	6	3
g	0	2	0	-6	-4	-1	6	0	6
g	0	2	4	-3	-4	-2	5	3	4
t	0	-3	-1	1	4	-2	-1	2	3
t	0	-3	-3	-1	3	1	-1	0	2
a	0	-3	-4	3	-4	0	-2	4	0
	0	0	-2	0	3	1	0	1	4

GAT

GAT

GG

GG

preceding row (gap)

for each cell, 3 possible scores

1. diagonal (no gap)

2. **best from preceding row**

3. best from preceding column

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0	-3	-4	-4	4	3	1	0	2
c	0	-3	-5	-5	-2	6	0	-2	1
a	0	-3	-5	-6	-3	0	3	6	3
g	0	2	0	-6	-4	-1	6	0	6
g	0	2	4	-3	-4	-2	5	3	4
t	0	-3	-1	1	4	-2	-1	2	3
t	0	-3	-3	-1	3	1	-1	0	2
a	0	-3	-4	3	-4	0	-2	4	0
	0	0	-2	0	3	1	0	1	4

GAT
G-T

preceding column (gap)

for each cell, 3 possible scores

1. diagonal (no gap)
2. best from preceding row
3. **best from preceding column**

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0	-3	-4	-4	4	3	1	0	2
c	0	-3	-5	-5	-2	6	0	-2	1
a	0	-3	-5	-6	-3	0	3	6	3
g	0	2	0	-6	-4	-1	6	0	6
g	0	2	4	-3	-4	-2	5	3	4
t	0	-3	-1	1	4	-2	-1	2	3
t	0	-3	-3	-1	3	1	-1	0	2
a	0	-3	-4	3	-4	0	-2	4	0
	0	0	-2	0	3	1	0	1	4

T-C
TTC

The order of cells

- start at top left
- every cell has best score considering all possible routes
- at end, highest score is best path

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0								
c	0								
a	0								
g	0								
g	0								
t	0								
t	0								
a	0								
	0								

- would also work if we went left and up

Reading the alignment

- find highest scoring cell (last row or column)
- how did we reach this cell ?
 - how did we reach preceding cell ?
 - ...

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0	-3	-4	-4	4	3	1	0	2
c	0	-3	-5	-5	-2	6	0	-2	1
a	0	-3	-5	-6	-3	0	3	6	3
g	0	2	0	-6	-4	-1	6	0	6
g	0	2	4	-3	-4	-2	5	3	4
t	0	-3	-1	1	4	-2	-1	2	3
t	0	-3	-3	-1	3	1	-1	0	2
a	0	-3	-4	3	-4	0	-2	4	0
	0	0	-2	0	3	1	0	1	4

GGAT-CGA

-GATTC-AGGTTA

Trick with traceback

For each cell

- how did we reach it ? What was the preceding cell ?

		g	g	a	t	c	g	a	
	0	0	0	0	0	0	0	0	0
g	0	2	2	-3	-3	-3	2	-3	0
a	0	-3	-1	4	-3	-4	-5	4	0
t	0	-3	-3	-3	6	-1	-2	-3	4
t	0	-3	-4	-4	4	3	1	0	2
c	0	-3	-5	-5	-2	6	0	-2	1
a	0	-3	-5	-6	-3	0	3	6	3
g	0	2	0	-6	-4	-1	6	0	6
g	0	2	4	-3	-4	-2	5	3	4
t	0	-3	-1	1	4	-2	-1	2	3
t	0	-3	-3	-1	3	1	-1	0	2
a	0	-3	-4	3	-4	0	-2	4	0
	0	0	-2	0	3	1	0	1	4

GGAT-CGA

-GATTC-AGGTTA

Summary (Needleman and Wunsch)

- Alignments are paths through the matrix
- There is an astronomical number of possibilities (with gaps)
- This algorithm has visited all of them and found best
- allows for gap costs of form

$$cost = cost_{open} + (n_{gap} - 1)cost_{widen}$$

- best or only method ? wait..

Cost

- say both sequences are length n
- we have to visit n^2 cells in matrix
 - each time we have to look at a row or column of length $\approx n$
- total cost n^3 or worst cost $O(n^3)$
 - remember this for later

Smith and Waterman version

So far: global alignments

- best match, covers as much as possible

Imagine proteins with 3 domains

ABCDEABCDEABCDE

QRSTUVWXYZBCDEQRSTU

Want to see ...

ABCDEABCDEABCDE

||||

QRSTUVWXYZBCDEQRSTU

not worth trying to align

everything

Use “Smith and Waterman” method

- scoring scheme: matches positive, mismatches negative
- during traceback
 - do not just look for max score
 - start with positive score
 - stop if score goes negative
- result: “local alignments” – often most useful

Other alignment algorithms

Needleman and Wunsch / Smith Waterman

- for given problem – optimal results
- allow fancy gap penalties
- cost $O(n^3)$

Other methods

- $O(n^2)$ – very small limitation on gaps

Faster

- ...

Faster Seeded Methods (blast, fasta..)

Seeded

- idea: use seeds / fragments of length k
 - 11 - 28 for DNA
 - 2 - 3 for protein
- look for exact matches of query words in database
- extend if found
- time depends mainly on length $O(n)$
 - most of the time no matches
- slow extension when a match is found

Seed size

- very small = lots of unimportant matches (slow)
- too big – may miss a match if there are too many changes

Fast versus slow

2 sequences (protein or DNA)

- time not an issue
- 1 000 alignments ? Time still not an issue
- $10^3 \times 10^3$ alignments ? Your decision

Databases

- non-redundant protein sequence database
 - $\approx 3 \times 10^6$ sequences
 - $\approx 10^{10}$ residues
- must be fast
- maybe occasionally miss a word
- alignments may not be optimal

Problems so far

- We can align DNA sequences – maybe proteins
- how biological are the alignments, gaps and costs ?
- Coding versus non-coding DNA
 - 3 base pairs → 1 residue
ACAG lots ... CGA...
 - AC-G lots ... CGA ... one base deletion
 - 100's bases are shifted – amino acids in protein all wrong
 - non-coding region (binding / regulation / tRNA / rRNA..)
 - may not be so bad
- General problem – degeneracy ..

Degeneracy and Scoring

CCU, CCC, CCA, CCG are all proline (3rd position degenerate)

CCC→CCA no problem

CCC→ACC pro → ala (you die)

exactly the same mutation at DNA level (C→A)

- our scoring scheme does not know about this

rule

- some mutations will have no effect
- some are drastic
- usually the third base in each codon is least important

Can one do better ?

Scoring protein alignments

Two aspects

- forget DNA
- account for amino acid similarity

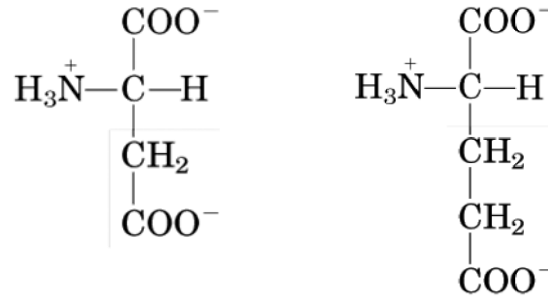
Instead of DNA – work directly with protein sequences

If our DNA is coding – easy to say

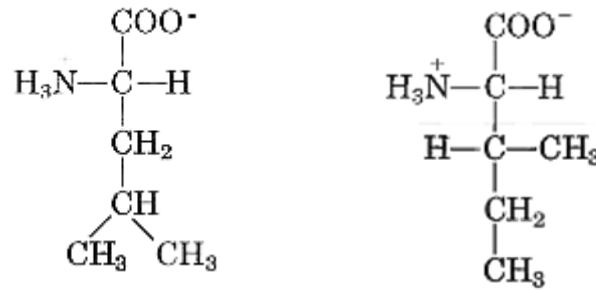
- CCUUCUUAU.. is pro-ser-tyr...
- immediate gain
 - CCC→CCA or similar will not be seen / affect alignment
- more subtle gain...

Amino acid similarities

asp and glu



leu and ile



- many more similar amino acids
- glu → asp mutation, does it matter ? sometimes not
- trp → asp, big hydrophobic to small polar ? usually bad news
- relevance to alignments

Why we need better protein scoring

ANDREWANDRWANDRWW aligned to QNDRDW

ANDREWANDRWANDRWW

QNDRDW-----

ANDREWANDR-WANDRWW

-----QNDRDW-----

ANDREWANDRWANDRWW

-----QNDRDW

- one of which is biologically more likely ($E \rightarrow D$)
- how would we do it numerically ?

Substitution matrices

Earlier in DNA

- match = 2
- mismatch = -3

	A	C	G	T
A	2	-3	-3	-3
C	-3	2	-3	-3
G	-3	-3	2	-3
T	-3	-3	-3	2

We want a matrix that says

	D	E	W	...
D	10	5	-5	
E	5	10	-5	
W	-5	-5	15	
...				

A full matrix..

A serious protein similarity matrix

blosum62:

	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

some features

- diagonal
- similar
- different

Using the score matrix

Algorithm (global alignment, local, fast, ...)

- unchanged
- only scoring changes

If possible use the protein sequence rather than DNA

- not all DNA codes for proteins
- regulators, tRNA, catalytic RNA, sRNA, ..
- not possible for genomic comparisons

Automatically includes codons, amino acid similarity, ..

Where does this kind of matrix come from ?

Substitution Matrices

Many

- PAM point accepted mutations
- BLOSUM blocks substitution matrix

Philosophy

- if two amino acids are similar, we will see mutations often

To quantify this..

- Take some very similar proteins (lots)

parts of some haemoglobins

HAHKLRVGPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLSTLAVHLPNDFTPAVHASLDKFLSSVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDAVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSK
HAHKLRVDPVNFKLLSHCLLSTLAVHLPNDFTPAVHASLDKFLSSVSTVLTSK
HAHKLRVDPVNFKLLSHCLLSTLAVHLPNDFTPAVHASLDKFLSSVSTVLTSK
HAHKLRVDPVNFKLLSHCLLSTLAVHLPNDFTPAVHASLDKFLSSVSTVLTSK
HAHKLRVDPVNFKLLSHCLLSTLAVHLPNDFTPAVHASLDKFLSSVSTVLTSK
HAHKLRVDPVNFKLLSHCLLVTLAAHHPDDFNPSVHASLDKFLANVSTVLTSK
HAHKLRVNPVNFKLLSHSLLVTLASHLPTNFTPAVHANLNKFLANDSTVLTSK
HAYKLRVDPVNFKLLSHCLLVTLACHHPTEFTPAVHASLDKFFTAVSTVLTSK
HAQKLRVDPVNFKFLGHCFLVVVAIHHPALTAEVHASLDKFLCAVGTVLTAK
HAQKLRVDPVNFKFLGHCFLVVVAIHHPALTAEVHASLDKFLCAVGTVLTAK
HAQKLRVDPVNFKFLGHCFLVVVAIHHPALTAEVHASLDKFLCAVGTVLTAK
HAQKLRVDPVNFKLLGQCFLVVVAIHNPSALTPEAHASLDKFLCAVGLVLTAK
HAYNLRVDPVNFKLLSQCIQVVLAVHMGKDYTPEVHAAFDKFLSAVSAVLAEK
HAYNLRVDPVNFKLLSHCFQVVLGAHLGREYTPQVQVAYDKFLAAVSAVLAEK
HAYILRVDPVNFKLLSHCLLVTLAARFPADFTAEAHAAWDKFLSVVSSVLTEK

parts of some haemoglobins

HAHKLRVGPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLSTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDAVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVDPVNFKLLSHCLLVTLA
 HAHKLRVNPVNFKLLSHSLLVTLA
 HAYKLRVDPVNFKLLSHCLLVTLA
 HAQKLRVDPVNFKFLGHCFLVVVA
 HAQKLRVDPVNFKFLGHCFLVVVA
 HAQKLRVDPVNFKFLGHCFLVVVA
 HAQKLRVDPVNFKLLGQCFLVVVA
 HAYNLRVDPVNFKLLSQCIQVVLAVHMGKDYTPPEVHAAFDKFLSAVSAVLAEK
 HAYNLRVDPVNFKLLSHCFQVVLGAHLGREYTPQVQVAYDKFLAAVSAVLAEK
 HAYILRVDPVNFKLLSHCLLVTLAARFPADFTAEAHAAWDKFLSVVSSVLTEK

- consider an example column
 - how many pairs do we have ?
1-2, 1-3, ... 2-3, 2-4, ... get n_{total}
 - count n_{HH} , n_{HY} , ..
 - $p_{HH} = n_{HH} / n_{total}$ would be probability that H is conserved (or another amino acid)
 - $p_{AB} = n_{AB} / n_{total}$ would be probability that A and B mutate to another

Calculating a substitution matrix

- We have all the probabilities p_{AB} and p_{AA}
- matrix element AB is $\log_2(p_{AB})$ why $\log_2$?
- is my example enough ?
 - needs much more data so as to get good probabilities

Different matrices

- Lots of details PAM vs BLOSUM vs ... (not important)
- Degree of homology
 - if two sequences are very similar most residues not changed
 - longer evolutionary time – many things change

Longer evolutionary times

So far: probability of one mutation $A \rightarrow B$

- in longer evolutionary time
- $D \rightarrow E \rightarrow D \rightarrow W \rightarrow D \dots$
 - multiple mutations
 - probability of conservation is lower (diagonal elements)
 - all off-diagonal elements will be bigger
- more formally - long time p is $p \times p \times p \times \dots$

How to count for this ?

- take matrix (like blosum) and do matrix multiplication
M M M ...
- result: a set of matrices
 - PAM10, PAM20, ...
 - Blosum62, blosum80, ...

Are these different matrices useful ?

In principle, yes

- looking for similar proteins – use blosum80
- more remote ? – use blosum62
- ...
- in practice ?
- better way to find remote homologues (soon)

Now

- should you believe any of this ?

Are alignments correct ?

Is there a correct alignment ?

Are alignments correct ?

1. algorithmically
2. mathematically
3. is my model sufficient ?

Is there a correct alignment ?

1. structurally
2. biologically / evolutionarily

Details..

Are alignments correct ? (algorithm)

Yes

(Needleman and Wunsch, Gotoh, Smith-Waterman)

Nearly

(seeded methods – blast)

- We have a scoring scheme
(identity, blosum similarity matrix)
- We find the alignment which maximises the score

Are alignments correct ? (compare with nature)

not necessarily

- blosum matrix comes from counting mutations
 - we find the most likely substitutions

Align ANDREW and ANDRWQANDRKWSANDRWWC

ANDR-WQANDRKWSANDRWWC

ANDREW----- guess 1 [includes gap

-----ANDREW----- guess 2

-----ANDREW- guess 3

Claim – we can find the most likely

- often correct, sometimes wrong

Are alignments correct ? (numerical)

How good is "most likely" ?

- closely related sequences – very good
- distant relatives (tuna / horse myoglobin) – will have mistakes
- repetitive sequences

Are alignments correct ? (model)

my model for evolution

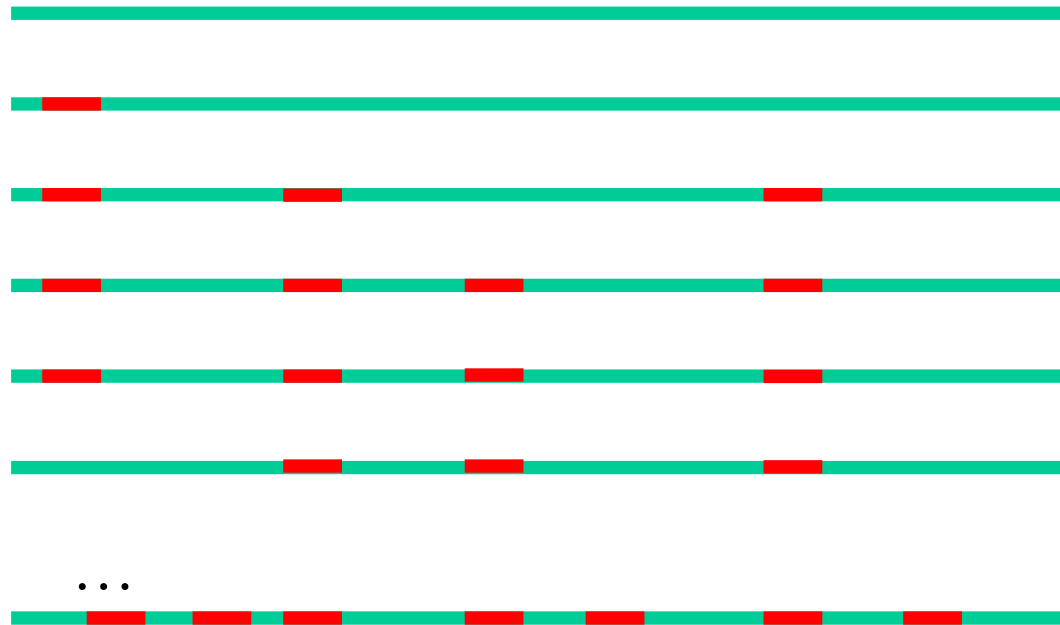
- mostly point mutations (DNA level)
- rarely a deletion/insertion

Is this enough ?

- not always
 - fusion, insertion of random DNA, splicing, duplications..

Is there a correct alignment ? evolutionary

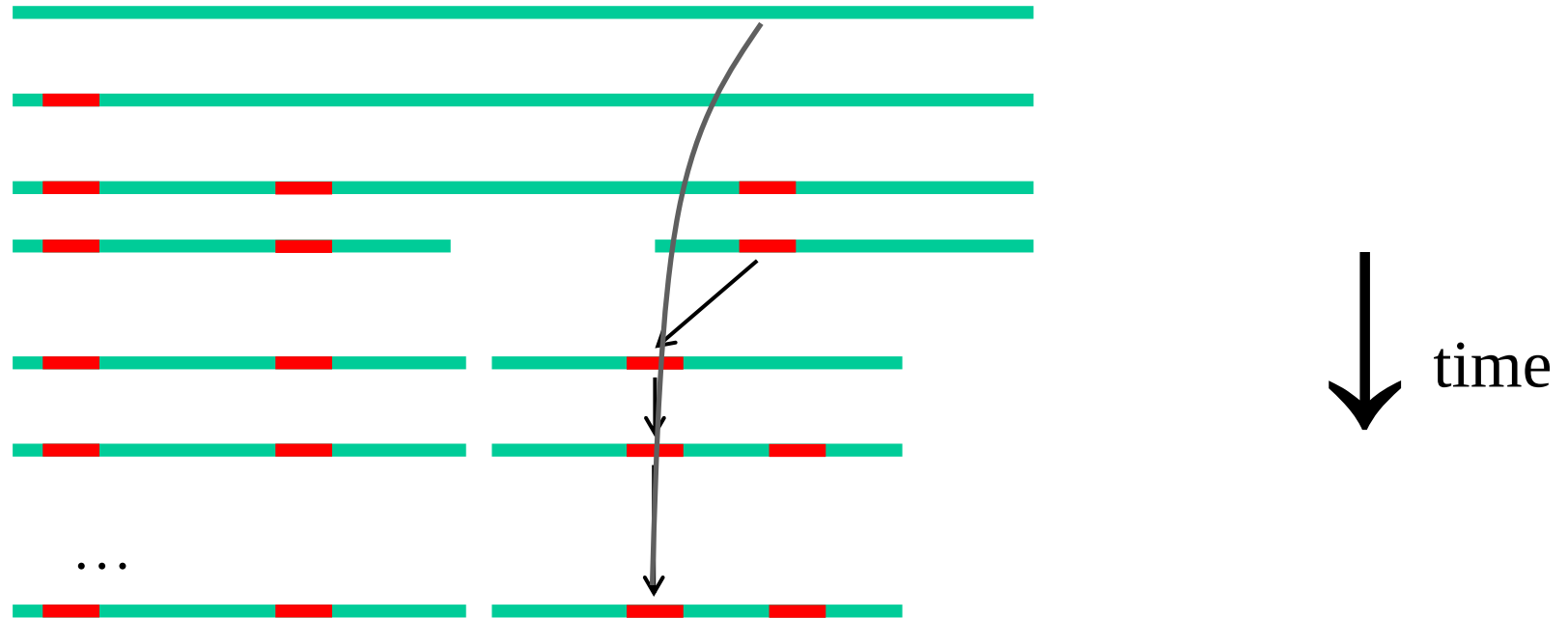
Yes



There is a correct sequence alignment

- with gaps ?

Is there a correct alignment ? evolutionary



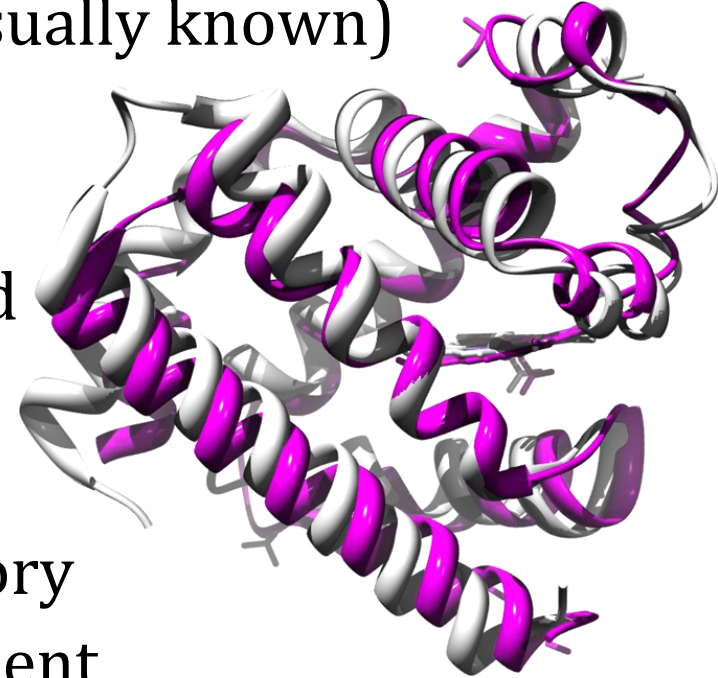
There is a correct alignment

- the evolutionary history of each residue / base

Is there a correct alignment ? (structure)

Yes

- every protein has a structure (not usually known)
- structures can be aligned
- corresponding residues can be found



This alignment may

- not correspond to evolutionary history
- not agree with best sequence alignment
(imagine deleting some residues on solvent exposed helix)

Alignments and searches

The practical goals - different problems

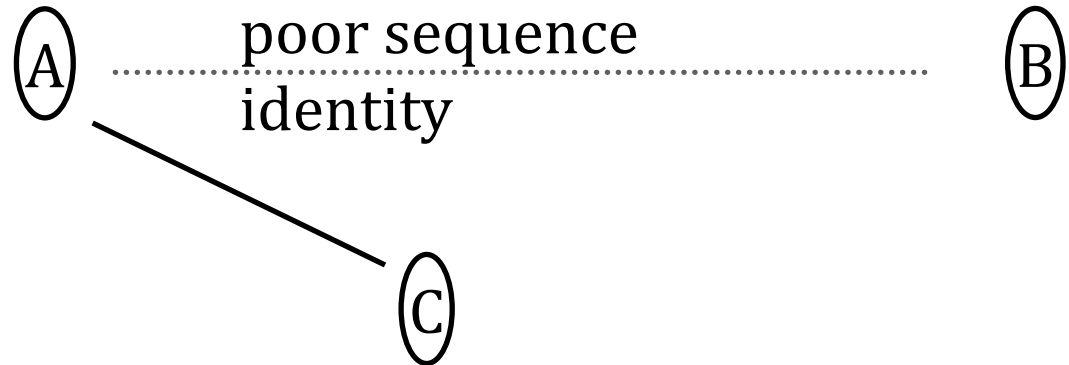
1. find the best alignment (modelling, predictions of important residues)
 - we care about alignment methods
2. find related known proteins (what kind of protein do I have ?)
 - we need sensitive search methods

iterated searches (psi-blast)

Search with protein A and find a very remote protein B

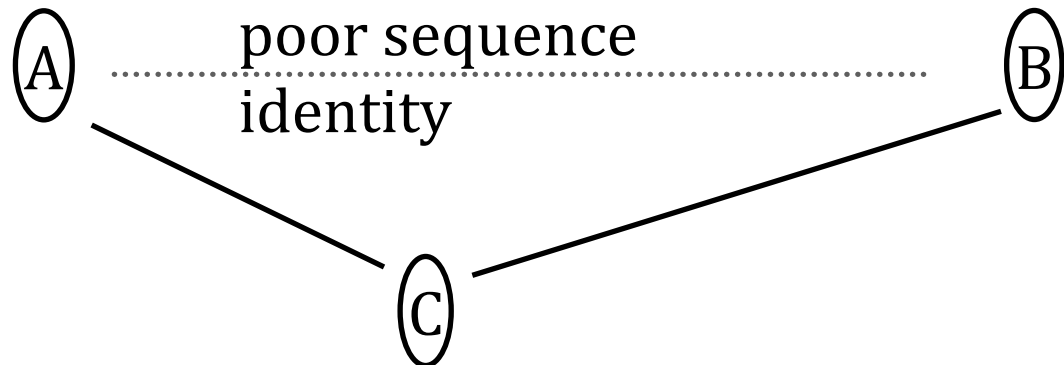


but there another protein C



search with C finds B

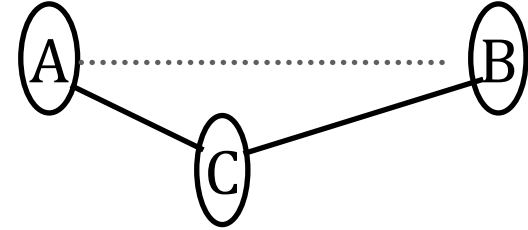
- the original AB relation is believable
- how to automate this ?



iterated searches (psi-blast)

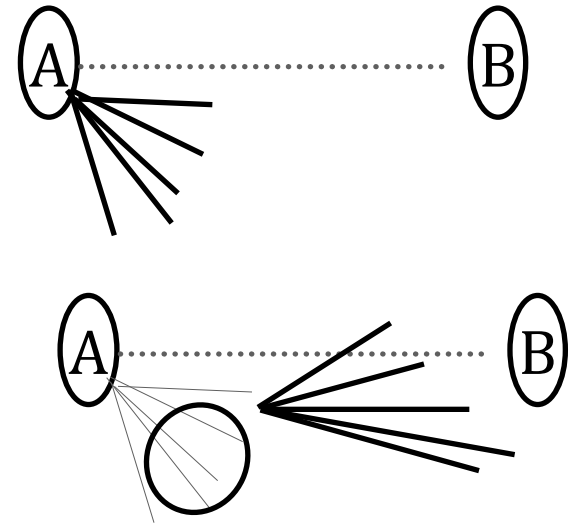
Searching with "A" finds lots of homologues

- cannot start a search with each



Alternative

- find all the homologues to A
- build an average sequence (profile)
- from this profile – repeat search
- build new average / repeat



Result

- at each step
- include reliable homologues
- eventually $A \rightarrow B$ may be found

iterated searches (psi-blast)

In practice

- really only one program (+ web page) NCBI blast / psiblast
- most significant advance in finding remote homologues in a decade

sequence identity / similarity / significance

Significance

- I find a homologue – is it evolutionarily related or just noise ?
 - probability estimations later
- how important is 10% sequence identity ? 90 % ?
- is 25 % identity in DNA as useful as in a protein ?

First principles DNA

- what would you expect by chance ?
GGATCGA
GATTCAGGTTA
 - At each position $\frac{1}{4}$ chance of a match
 - average 25 % sequence identity with random DNA
- ... very wrong

Naïve identity expectation – base usage

Two problems

1. uneven character frequency
2. gaps

Character frequency – what if I have a two letter alphabet ?

- a world with two bases

GCGGCGCGCCGCGCGCGCGCGC

average sequence identity 50 %

- a world with usually two bases - sometimes A or T

GCGACGCGTCGCGCGTTTCGCGC

average sequence identity : a bit less than 50 %

- a four letter alphabet

GCGACACGTCGTGAGTTCTTGC nearly 25 %

Naïve identity expectation – base usage

One extreme

- two types of base – 50 % sequence identity expected

Other extreme

- Random sequences – 25 % identity

Our world – somewhere between

How significant ?

- malaria is about $\frac{1}{3}$ GC (not $\frac{1}{2}$)
- *Streptomyces coelicolor* is 72 % GC
- GC differs between organisms, coding/non-coding regions

Consequence

- even randomly sampled sequences, will have > 25% sequence identity

Naïve identity expectation - gaps

- **ungapped: 2 matches from 9 aligned (22 %)**
GGATCGCAC
GACTGAGGTTA
- **one gap: 3 matches 8 aligned (38 %)**
GGATCGCAC
GACT-GAGGTTA
- **more gaps: 4 matches from 6 positions (50 %)**
GGATCGCAC
GACT-G-AGGTTA
- **more gaps: 5 matches from 6 positions (83 %)**
GGATC-GCAC
G-A-CTG-AGGTTA
- the more gaps one allows - the higher the identity
- One can make score arbitrarily good

Protein – random matches

20 amino acids

- naïve expectation – 5 %

%

ala 8.4

leu 8.3

gly 7.8

trp 1.5

cys 1.7

Proteins are not like a 20 character alphabet:

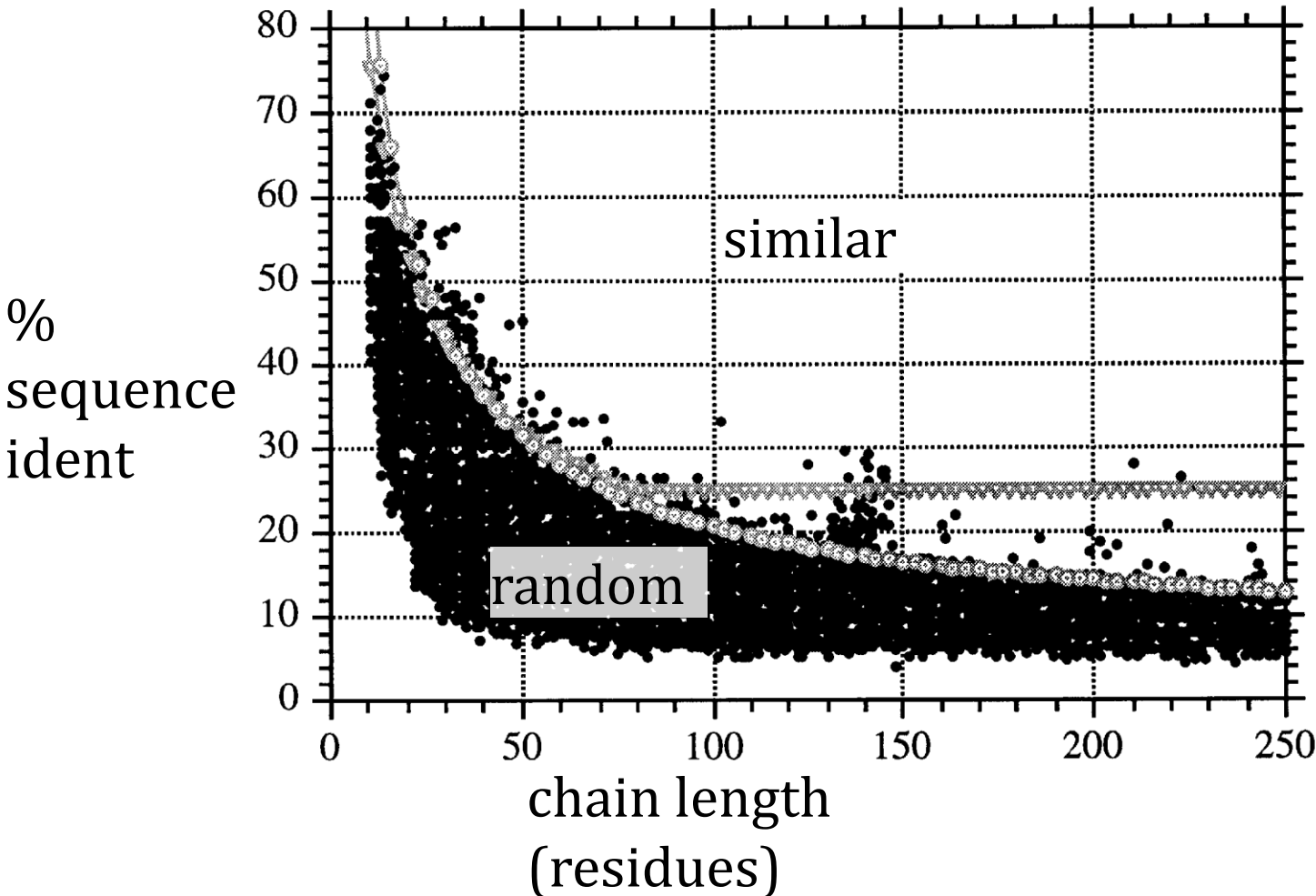
- varies between organisms
- varies between cell compartments, soluble, membrane bound...

Practical result - random sequences, realistic gaps

- 20 to 25 % identity by chance
- depends on length..

protein size and identity

- small proteins – need 30 % to believe they are related
- big proteins < 20 % , almost certainly related



Summarise problem and steps

Mission

- you have a protein sequence
 - no structure
 - maybe no biochemistry (substrates, binding targets, ..)
- find what you can
 - related proteins of known structure
 - related proteins with known function
- Is there
 - an answer ?
 - one set of steps ?

easy	98 % similar to protein of known function and structure
↕	
hard	weak possible similarity to a poorly characterised family

General Idea

Try easy steps first

- simple searches first
- see if enough information is found
- gradually go to more sensitive methods (slightly more error prone)

Use the “least speculative” methods first

- accurate alignments – not seeded
- simple blast searches before iterated ones

What are the expectations ?

For easy sequences

- very good molecular models
- no doubt about function

Middle difficult

- reasonable models
 - enough to guide mutagenesis (which residues can be mutated safely)

Very difficult

- not even sure what class of proteins or what function
- may be able to suggest experiments most likely to be useful

Protein Modelling

- Where has all this been leading to ?
- Why worry about similarity ?

Mission

- You have a protein sequence
 - no structure known
- You would like to build a model for the atomic coordinates

Why do protein modelling ?

- real structures (crystallography, NMR) are better
- crystallography
 - cost, crystallisation, phasing
 - think of membrane proteins
- NMR
 - limited in size, solubility
- what are the most important therapeutic targets ?
 - enzymes
 - receptors (where are they ?)
- crude models often used for crystallographic phasing

Overall scheme

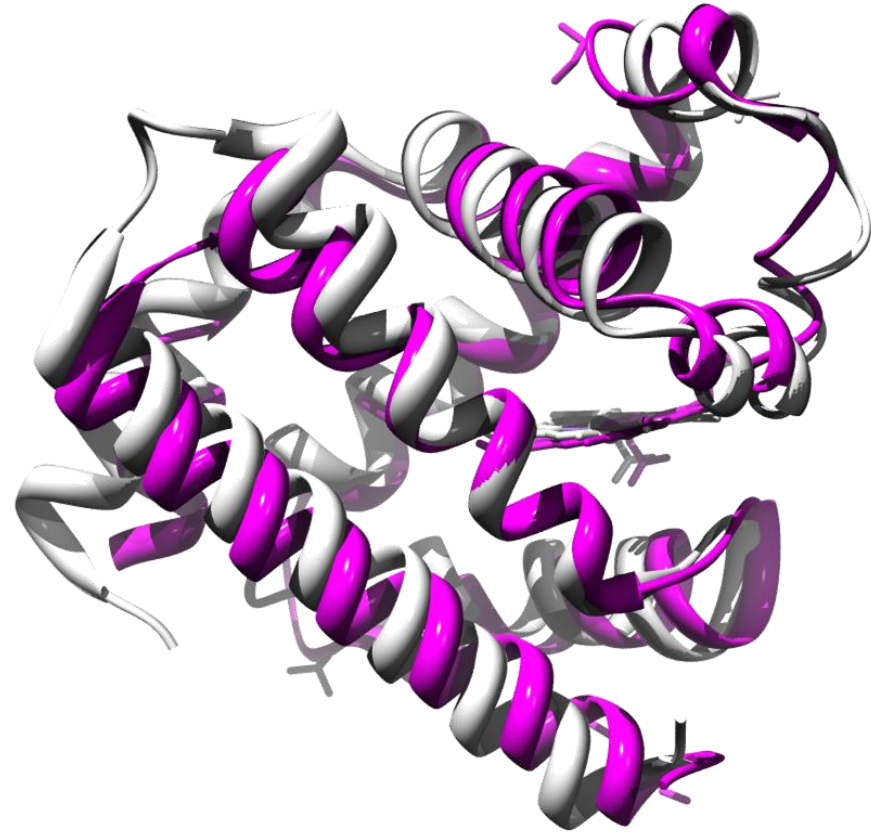
- for your sequence
 - find related proteins of known structure
 - gives you "template" structure
- sequence alignment
 - your sequence and sequence from template structure
- replace residues
 - where the residues are the same do not do much
 - where they differ, put your residues in place
- fix gaps, insertions
- fix side chains

will this work ?

What accuracy ? Examples

Tuna / horse myoglobin

- imagine you know the structure of tuna Mb
- align the sequences
- put residues from horse myoglobin onto tuna
- would make a good guess
- most atoms within 2 – 3 Å
- nasty case...



Accuracy – difficult example

Align sequences

seq id = 11 %

What would a model
look like ?

Alignment to 1v93A

Seq ID 11 % (25 / 227) in 268 total including gaps

```
: 5 : 6 : 7 : 8 : 9 :  
: 0 : 0 : 0 : 0 : 0 :  
afvsitygam-gstrersvawa-----qriqslglnplahlrtvaggsrkevaevlhrfv  
rrpsvvyhlhnaectgcseavlrafepyidtlildtllsldyhetimaaagdaaeaaaleqav  
: 1 : 2 : 3 : 4 : 5 : 6  
: 0 : 0 : 0 : 0 : 0 : 0
```

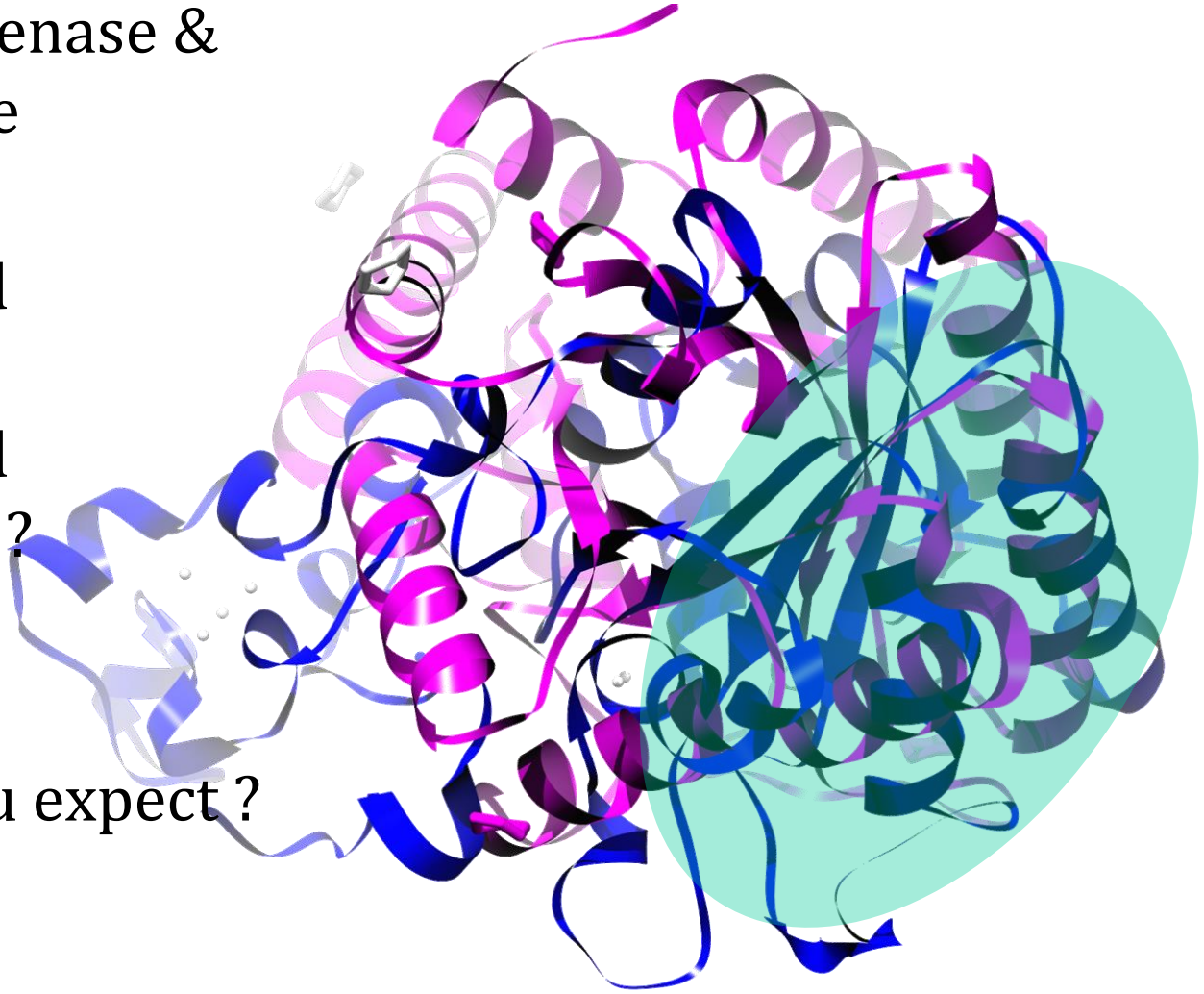
```
1 : 1 : 1 : 1 : 1 : 1 :  
0 : 1 : 2 : 3 : 4 : 5 :  
0 : 0 : 0 : 0 : 0 : 0 :  
esgvenllalrgdpprgervfrphpegfryaaelvalirerygdrsvsggaaype-ghpe  
nsphgfiavveggiptaangiygkvanh-tmldicsrilpka--qaviaygtcatfggvq  
: 0 : 0 : 0 : 1 : 1 : 1 :  
: 7 : 8 : 9 : 0 : 1 : 2 :  
: 0 : 0 : 0 : 0 : 0 : 0 :
```

```
1 : 1 : 1 : 1 : 2 :  
6 : 7 : 8 : 9 : 0 :  
0 : 0 : 0 : 0 : 0 :  
sesleadlr--hfkakveagldfa-itqlffnnahyfgflerarragigipil-----p  
aakpnptgakgvndalkhlgvkainiagcppnpynlvgtivvylnkaapeldslnrptm  
: 1 : 1 : 1 : 1 : 1 : 1 :  
: 3 : 4 : 5 : 6 : 7 : 8 :  
: 0 : 0 : 0 : 0 : 0 : 0 :
```

```
2 : 2 : 2 : 2 : 2 : 2 :  
1 : 2 : 3 : 4 : 5 : 6 :  
0 : 0 : 0 : 0 : 0 : 0 :  
gimpvtsyrqlrrftevcgasipgpllaklerhqddpkavleigvehavrqvaelleagv  
ffgqtvheqcprlphfdagefa-----psfeseeark-----gwclyelgc  
: 1 : 2 : 2 : 2 :  
: 9 : 0 : 1 : 2 :  
: 0 : 0 : 0 : 0 :
```


Accuracy – difficult example

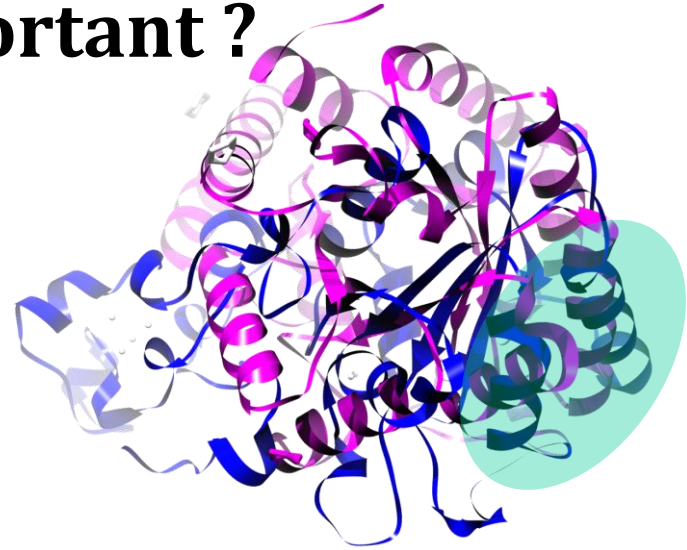
- 1ubr & 1v93
- Fe / Ni hydrogenase & oxidoreductase
- would you find this template ?
- would you find this alignment ?
- what could you expect ?



model quality – important ?

Do I need a good model ?

- design binding molecules ? – yes
- phasing in crystallography – no



Important question

- is this residue near the active site ? (chemical modification)
- accuracy not necessary
 - enzyme immobilization

Site directed mutagenesis + selection

- which are the critical residues to target ?

Expectations

	easy		hard
sequence identity	80-90 %		< 15 %
template	no problem	no problem	sometimes wrong
alignment	no errors	some parts wrong	some parts cannot be aligned
gaps / loops	very few		terrible
uses	designing ligands		predicting active sites
			mutagenesis

Relate to previous lectures

For your sequence – find a template

- if you cannot find it with blast / fasta – will be difficult

For many sequences – many templates equally good

Why all the talk about psi-blast / related sequences ?

- your protein may not have any close homologues

Template found - what next ?

alignment for modelling

Easy cases (sequence homologous to template)

- blast alignment OK
- any alignment OK

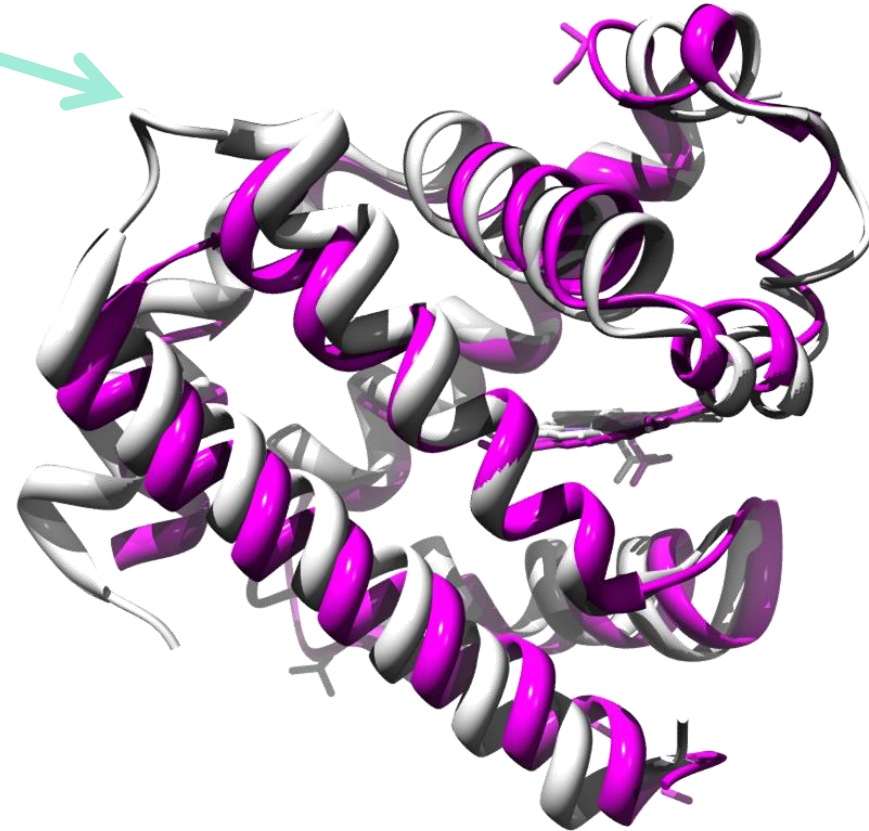
Harder cases

- use the best (slowest) alignment program
 - will not do any harm
- costs human time (computer time is insignificant)

insertions and gaps

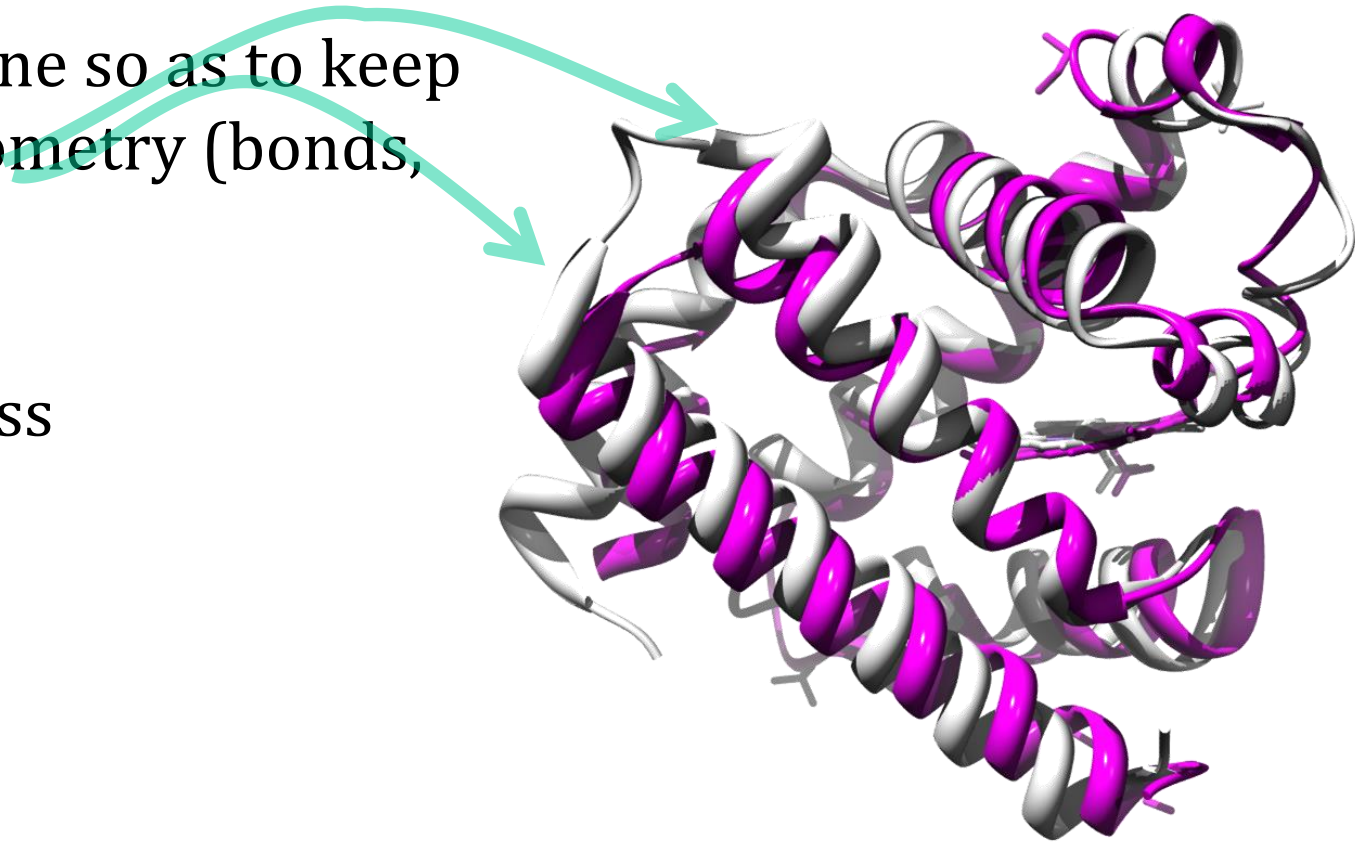
- dogma – gaps and insertions are less likely in regular secondary structure (α -helices, β -strands)
- more likely in "loops"

:	5	:	6	:	7	:	8	:	9	:
:	0	:	0	:	0	:	0	:	0	:
afvsitygam-gstrersvawa-----qriqslglnplahltvaggsrkevaevlhrfv										
rrpsvylhnaectgcsesvlrafepyyidtlildtllsldyhetimaaagdaaeaaleqav										
:	1	:	2	:	3	:	4	:	5	:
:	0	:	0	:	0	:	0	:	0	:
1	:	1	:	1	:	1	:	1	:	:
0	:	1	:	2	:	3	:	4	:	5
0	:	0	:	0	:	0	:	0	:	0
esgvenllalrgdpprgervfrphpegfryaaelvalirerygdrvsvggaaype-ghpe										
nspghfiaavveggiptaangiygkvanh-tmldicsrilpka--qaviaygtcatfggvq										
:	0	:	0	:	0	:	1	:	1	:
:	7	:	8	:	9	:	0	:	1	:
:	0	:	0	:	0	:	0	:	0	:
1	:	1	:	1	:	1	:	2	:	:
6	:	7	:	8	:	9	:	0	:	:
0	:	0	:	0	:	0	:	0	:	:
sesleadlr--hfkakveagldfa-itqlffnnahyfgflerarragigipil-----p										
aakpnptgakgvndalkhlgvkainiagcppnpynlvgtivyylnknaapeldslnrptm										
:	1	:	1	:	1	:	1	:	1	:
:	3	:	4	:	5	:	6	:	7	:
:	0	:	0	:	0	:	0	:	0	:



insertions and gaps

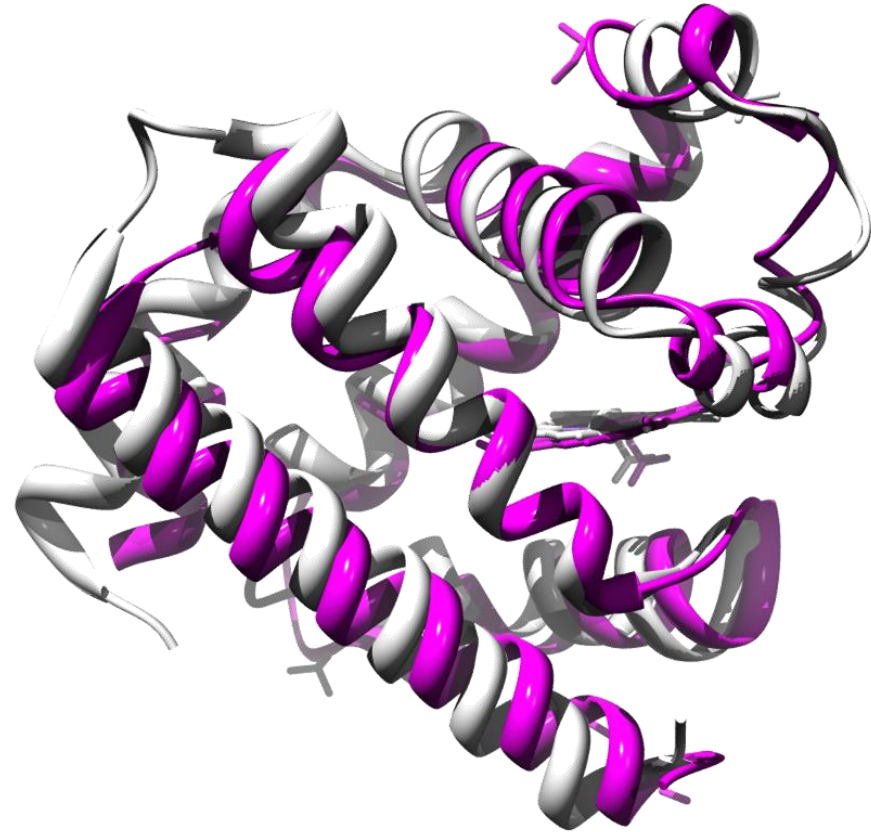
- imagine white is unknown, but pink is template
- where to put white loop residues ?
- fix end points
- join up backbone so as to keep reasonable geometry (bonds, angles)
- OK ? Just a guess
- Better ?



insertions and gaps

Generate many (10^2 or 10^3) guesses for loop

Calculate energy of each guess



Sidechains

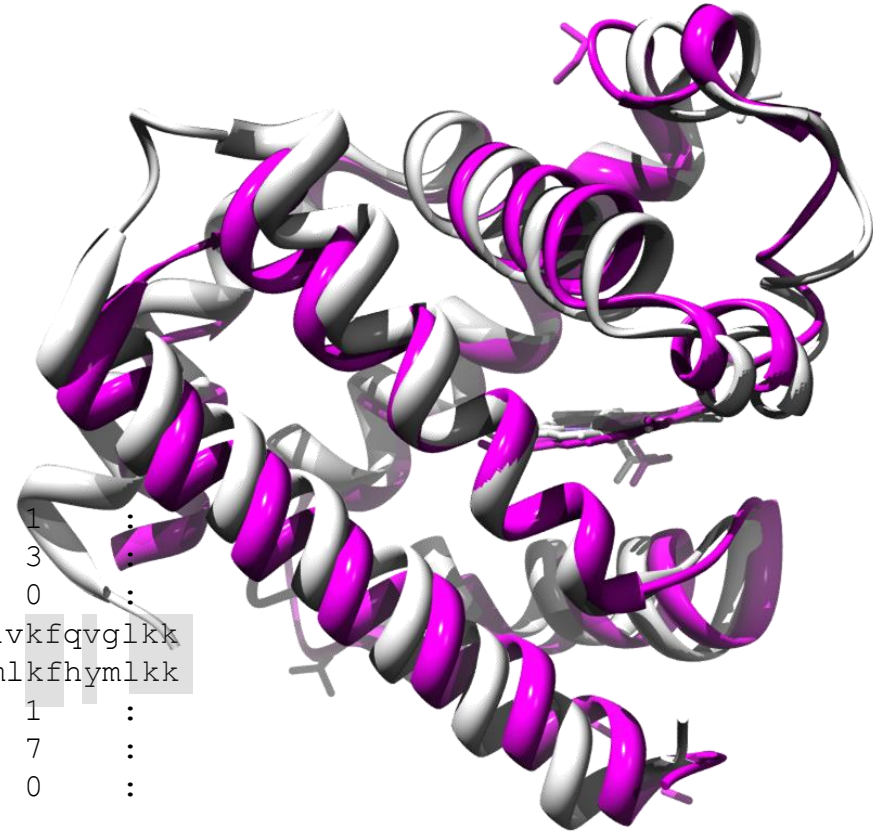
If my white one is the model

- where do we put sidechain atoms ?

Good strategy

- look at alignment
- find unchanged residues
- take sidechain coordinates
- rotate other sidechains to fit

0	:	0	:	1	:	1	:	1	:	1	:
8	:	9	:	0	:	1	:	2	:	3	:
0	:	0	:	0	:	0	:	0	:	0	:
lkssaieii	mlrs	nqsf	sled	mswsc	ggpdf	kycin	dvtka	ghtle	llepl	vkfqv	glkk
lkgaafel	cqlrf	ntvf	naet	gtwecg	---	rlsyc	ledta	ggfqq	llepm	lkfhym	lkk
:	1	:	1	:	1	:	1	:	1	:	:
:	3	:	4	:	5	:	6	:	7	:	:
:	0	:	0	:	0	:	0	:	0	:	:



Summarise protein modelling

finding a template

wrong template – rest of procedure is wrong

alignments

usually some residues are not perfect

fixing gaps and insertions

really a guess as to coordinates

placing sidechains

wirkstoff Entwurf – vital

rough guide to essential residues – may not matter