Classifying and comparing proteins

Plan

- why ?
- domains
- classifications
  - hierarchical vs pragmatic / empirical
  - continuous or clustered ?
- sequence similarity vs structure similarity
- example classifications
- comparison measures

Andrew Torda, Wintersemester 2015 / 2016, GST…

Why ?

Background – details later

- evolutionarily close proteins - similar structures
- evolutionarily remote proteins - may have similar structures

- function prediction / annotation
- interpretation
- structure prediction – can I predict this sequence fits to that structural class ?

Examples..

Transfer of properties

Arguments as with homology

- Homology modelling
  - can I find a related protein ?
  - can I say my protein has similar function / structure ?
- Classifications of proteins
  - I have classes of proteins – some members are well characterised
  - If you can say your protein is in class X,
    - probably has similar function to other members

Structure prediction

How many possible protein structures are there ?

- astronomical

How many interesting / different protein structures actually occur on earth ?

- 2×10^3 to 5×10^3

de novo / *ab initio* prediction ?

- search in giant space

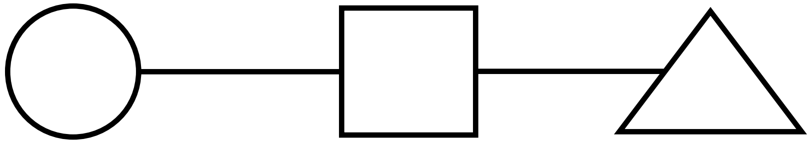
Find most likely protein fold ?

- search amongst 10^3 to 10^4 structures
- find the class of your protein - crude structure prediction

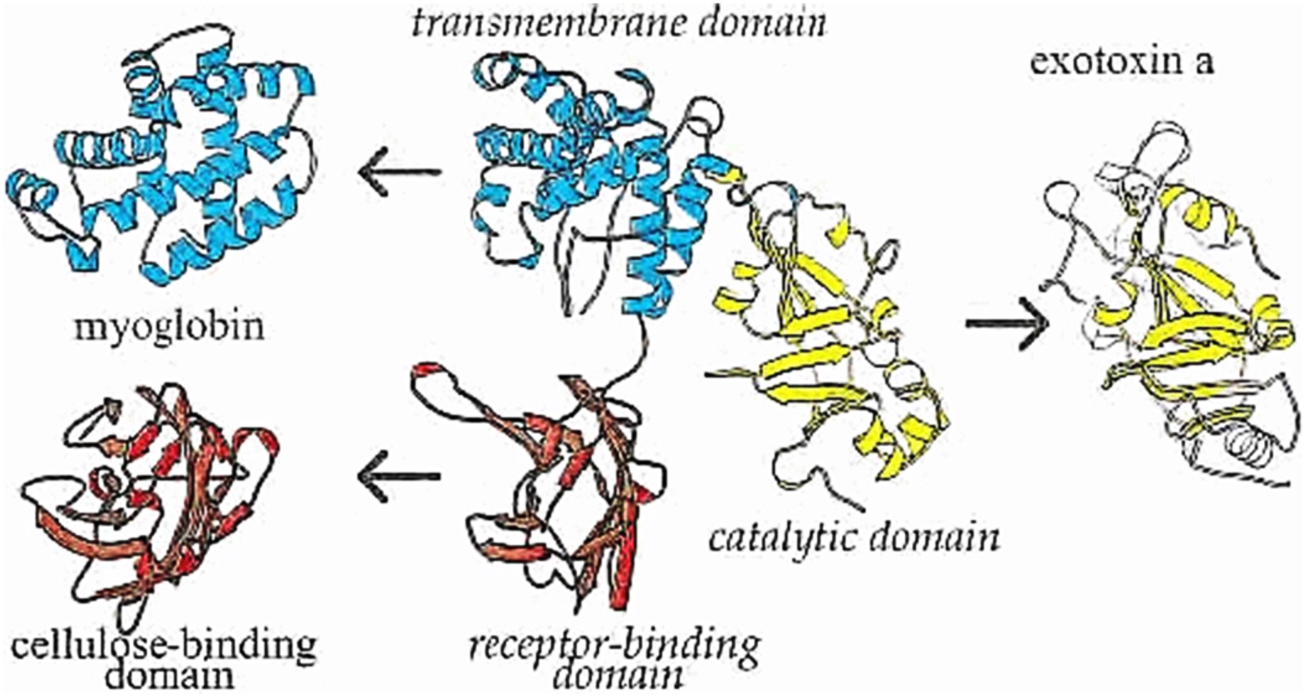
Domains

We will usually talk about protein protein domains (not whole proteins)

- association of domains with function and evolution..



- most literature classifications work with domains



from Holm, L & Sander, C. Proteins, 33, 88-96 (1998) Dictionary of recurrent domains in protein structures

Domains for these lectures

Usually structure based

- compact units

In these lectures

- no functional domains
- no sequence-based

Should we classify by structure or sequence ?

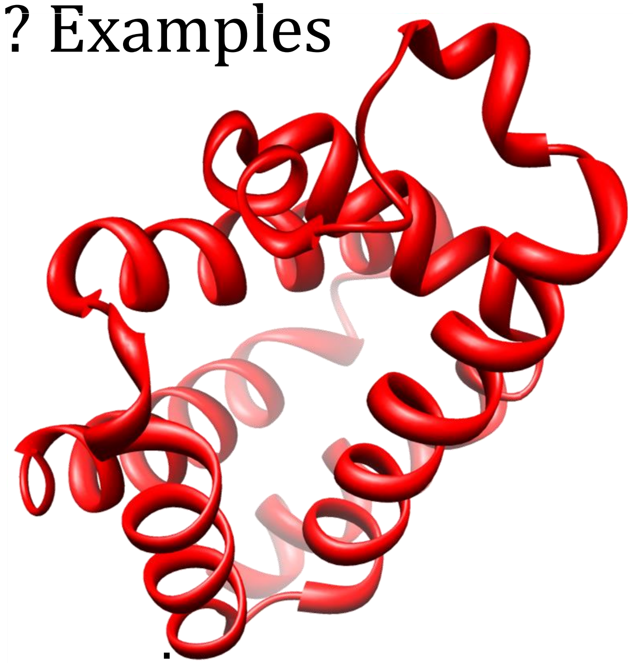
Structure vs Sequence similarities

More different than you might expect

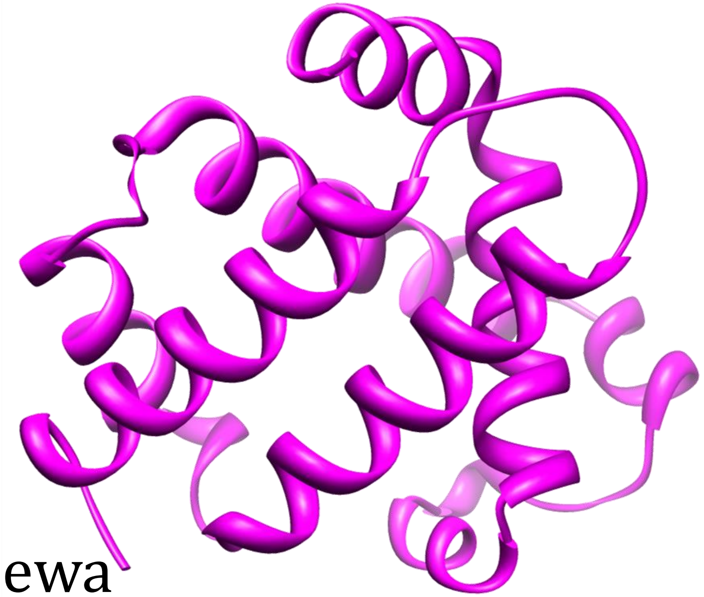
Similar sequences

- have not evolved for too long
- expect similar structures

Other way round ? Examples



1ecd
erythrocruorin



1ewa
dehaloperoxidase

very different sequences

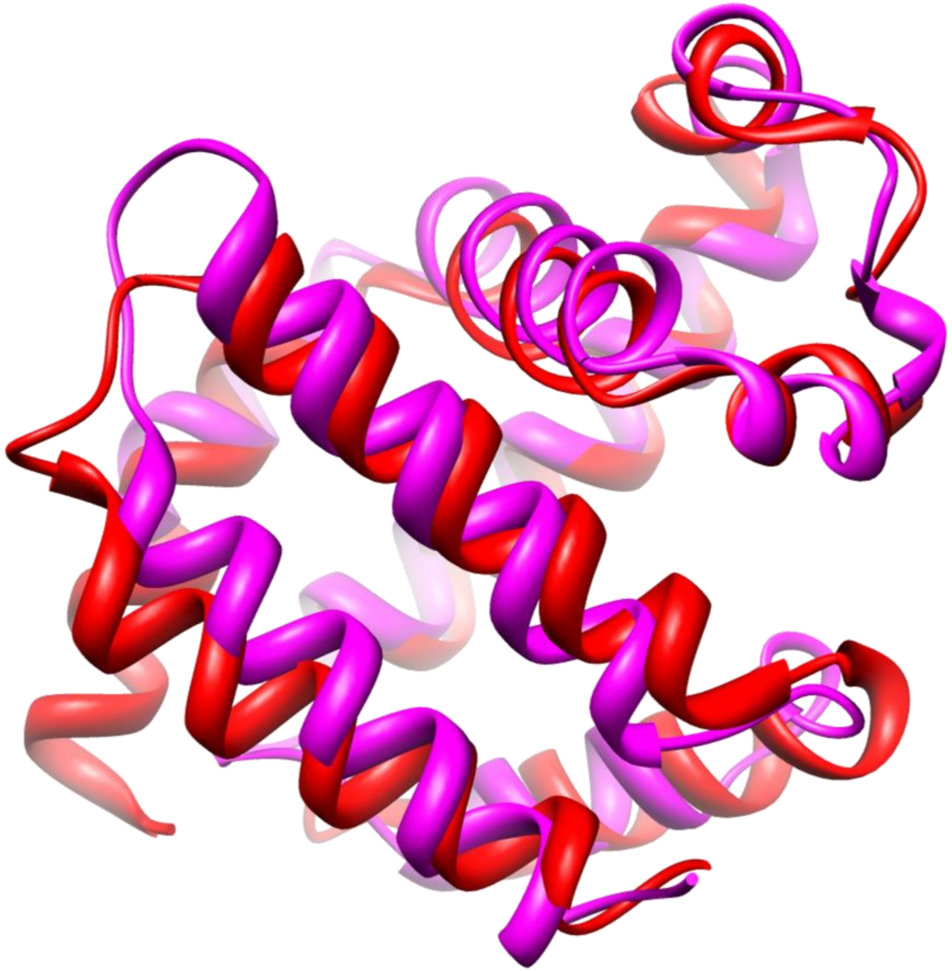
1ecd & 1ewa

- 17% sequence identity (very low)
- structures almost identical

Is this an exception ?

- 100's of examples
- totally normal

- play with our server



http://flensburg.zbh.uni-hamburg.de/~wurst/salami/

Example family

Example, neighbours of 1cun chain A

- look at sequence identity (% id)

root mean square diff in Å

```
No Chain    %id lali rmsd  Description
 1 1cunA    100  213  0.0  ALPHA SPECTRIN
 2 1hciA     24  111  1.6  ALPHA-ACTININ 2
 3 1ek8A     12  106  4.4  RIBOSOME RECYCLING FACTOR
 4 1oxzA      9   91  2.5  ADP-RIBOSYLATION FACTOR BINDING PROTEIN GGA1
 5 1eh1A      8  102  4.6  RIBOSOME RECYCLING FACTOR
 6 1hx1B      5  105  3.1  HEAT SHOCK COGNATE 71 KDA
 7 1dd5A      8  103  4.7  RIBOSOME RECYCLING FACTOR
 8 1lvfA      9   98  2.6  SYNTAXIN 6
 9 1bg1A      9   99  2.3  STAT3B
10 1hg5A      5   98  3.0  CLATHRIN ASSEMBLY PROTEIN SHORT FORM
11 1hs7A     14   92  2.5  SYNTAXIN VAM3
12 1dn1B     10  101  2.7  SYNTAXIN BINDING PROTEIN 1
13 1ge9A      6  108  4.6  RIBOSOME RECYCLING FACTOR
14 1fewA      8  125  3.5  SECOND MITOCHONDRIA-DERIVED ACTIVATOR OF
15 1qsdA      4   90  2.4  BETA-TUBULIN BINDING POST-CHAPERONIN COFACTOR
16 1e2aA      6   95  2.8  ENZYME IIA
17 1i1iP      7   95  3.3  NEUROLYSIN
18 1fioA      8  100  2.6  SSO1 PROTEIN
19 1m62A      8   81  2.8  BAG-FAMILY MOLECULAR CHAPERONE REGULATOR-4
20 1k4tA      6  147 25.8  DNA TOPOISOMERASE I
```

alignment length

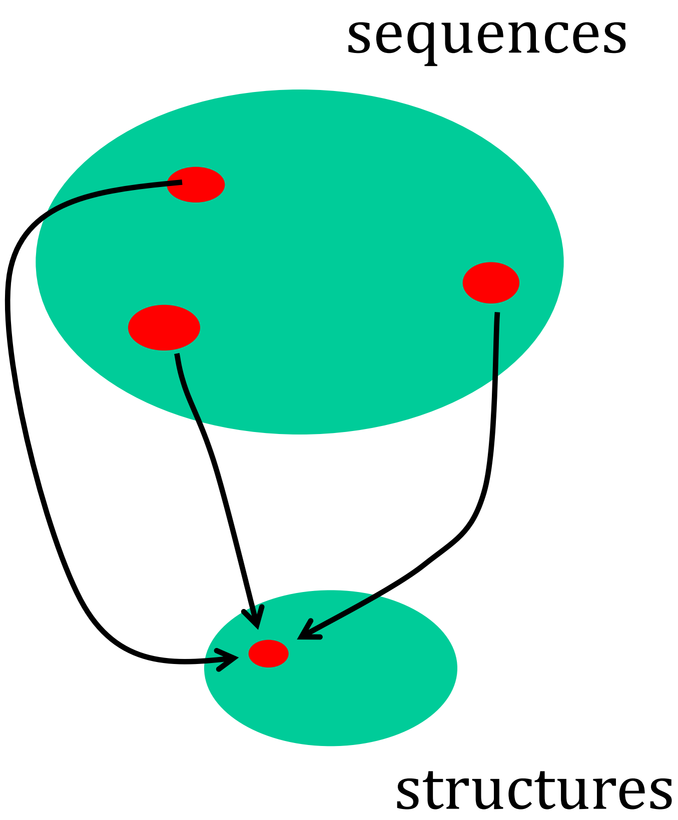
Structure vs Sequence

There are 1000's of such families

Summarise

- similar sequences
  - similar structures
- very different sequences
  - similar or different structures

why ?



Structures < Sequences… Why ?

Evolution

- many small changes
- if structure changes, function breaks, you die
- sequences change as much as possible within this constraint

Chemistry

- sequence determines structure
  - many sequences could fit structure (more next semester)

Surprising ?

- consider near universal proteins
  - 100's millions years evolution, function largely preserved
  - sequence has changed radically

Classifying by sequence

Forget hierarchy (for now)

- tools - any alignment program (blast, fasta, clustal, …)
- method
  - survey all proteins in the protein databank
  - collect all pairs > *x* %

similarity	num clusters
90 %	30 321
70%	26 171
50%	22 050

- result (jan 2014)

- how many structure classes ? 2 to 5 × 10^3 ?
- some sequence classes are not really different from each other

Now.. examples of structure based classifications

Clusters and hierarchies

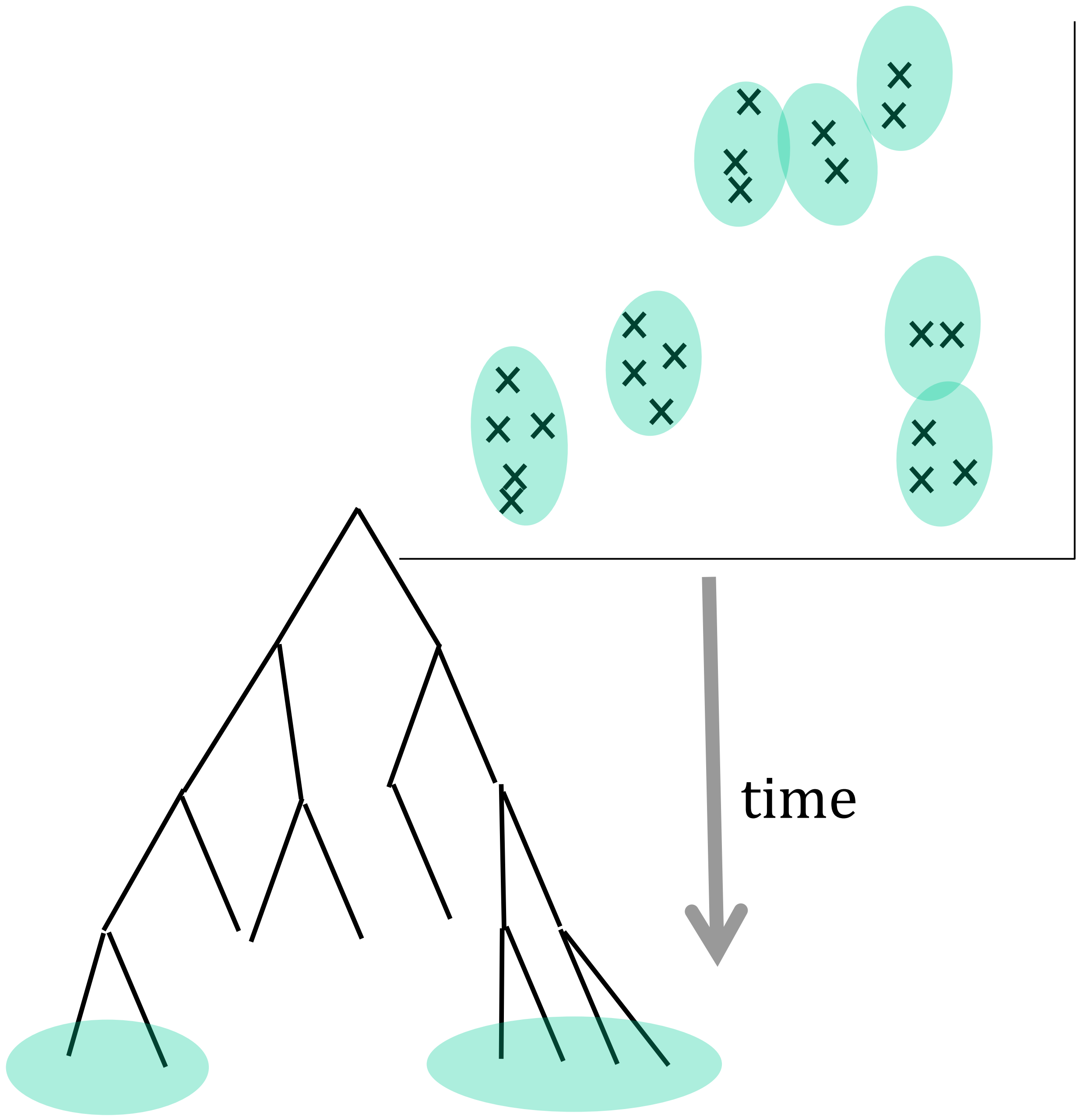
Are there clusters ? Yes

- Sequence-based ? Do a sequence search for a haemoglobin or profilin
  - find 10^3 to 10^4 homologues – this is some kind of cluster
- Structure-based ?
  - search for haemoglobins (or your favourite protein)
  - find 10^2 – 10^3 similar structures – a cluster

Are they hierarchical ? No idea

- what is the question ? (reminder from last lecture)

Maybe there should be protein clusters



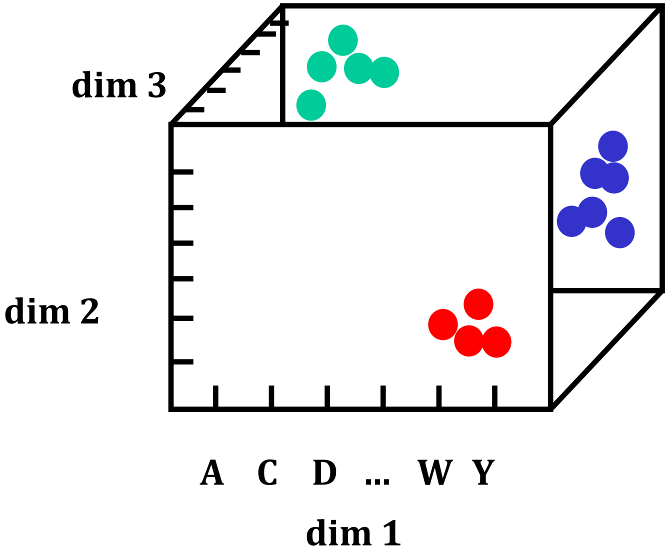
space of proteins

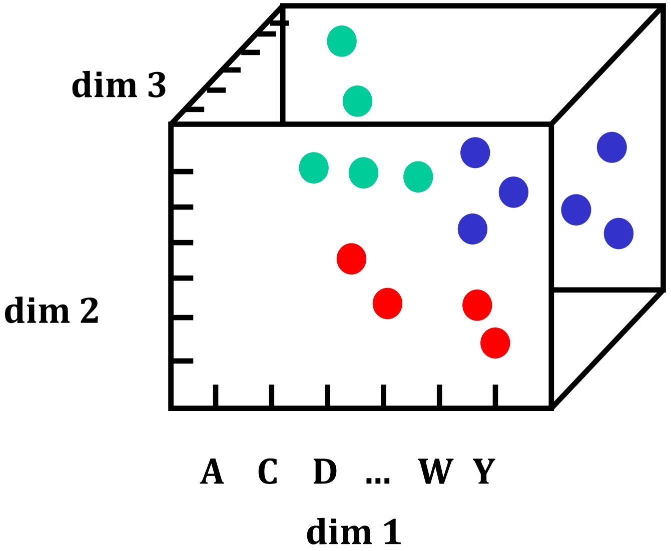
time

OR..

If we knew every protein that every existed anywhere

- would we be able to connect the clusters ?





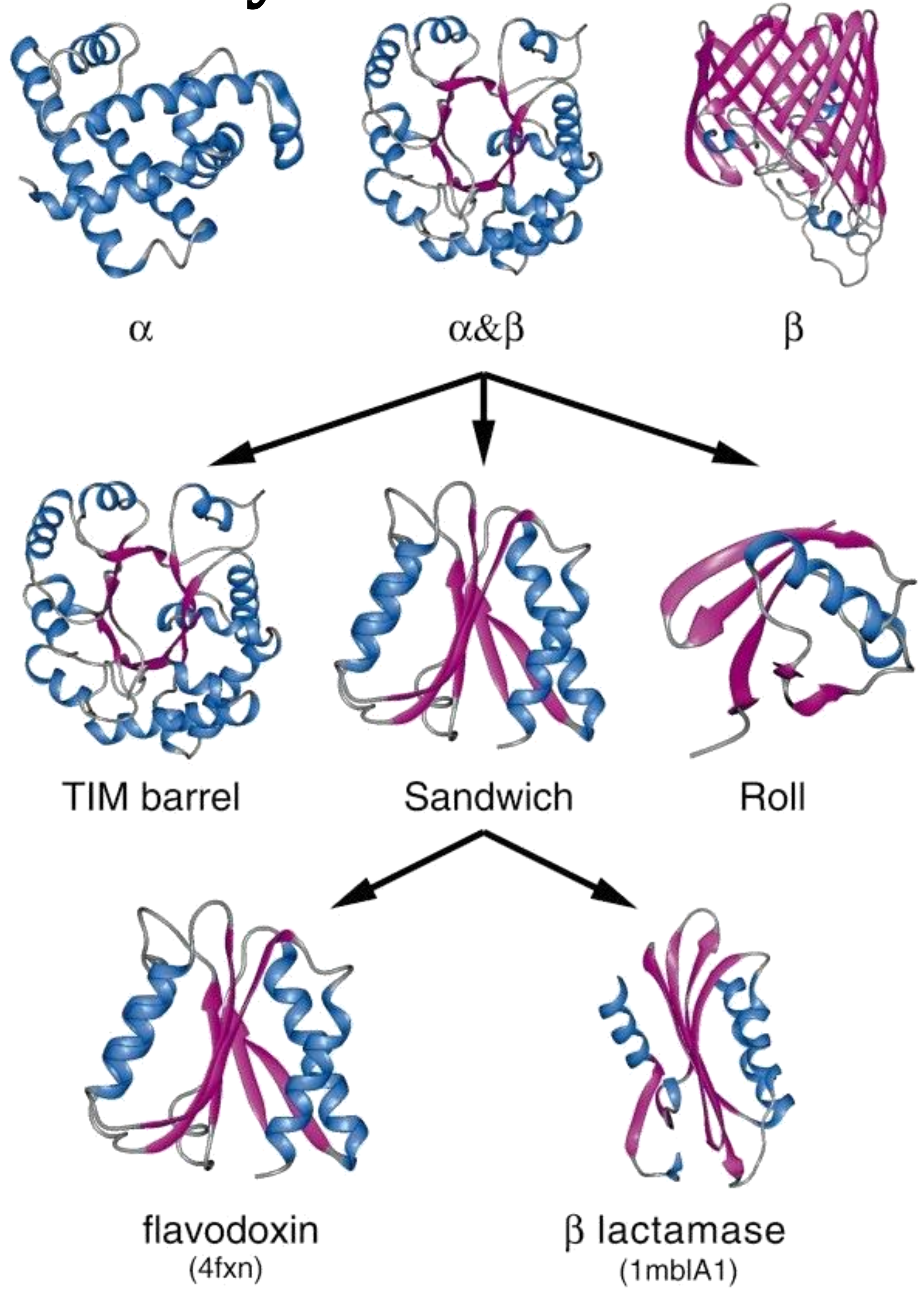
- An example of a hierarchical classification

Imposing a Hierarchy on Proteins

- parts may correspond to evolution
- top level ?

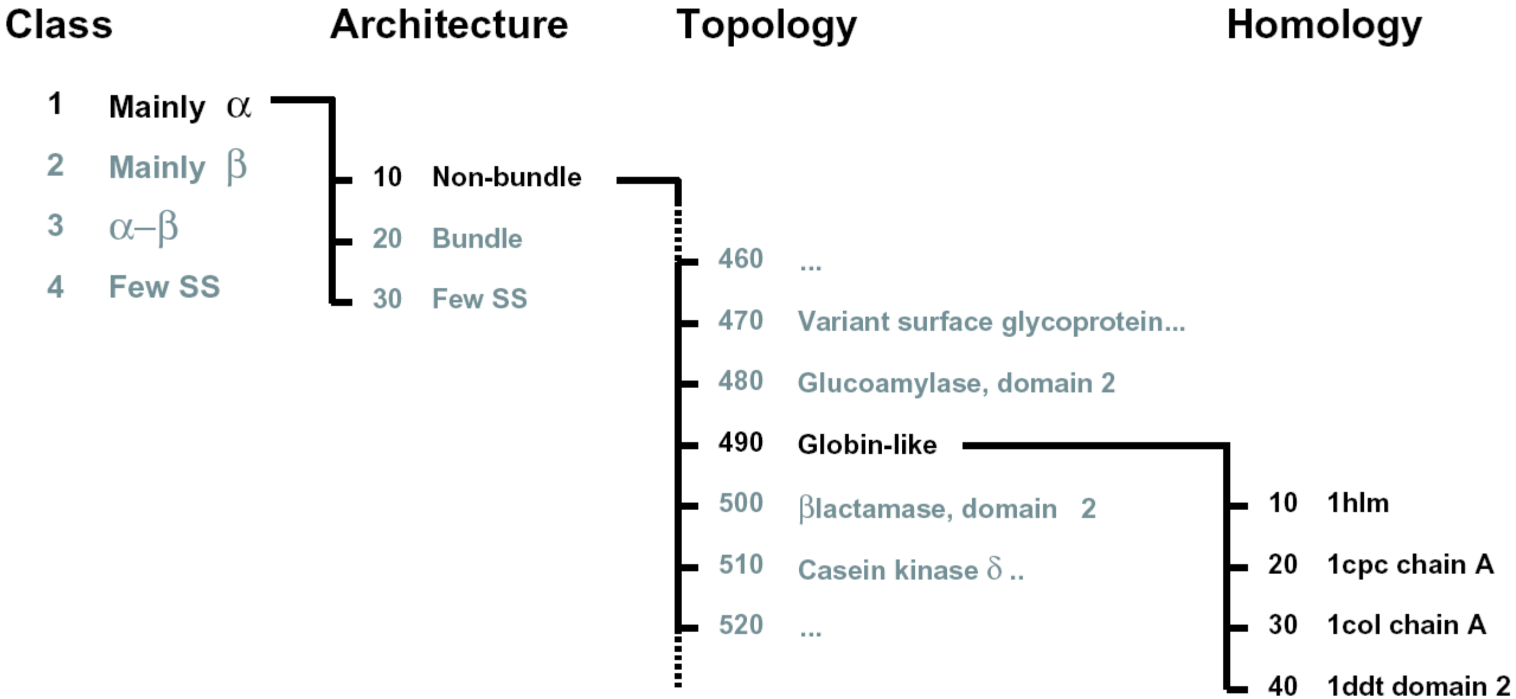
How useful and applicable ?

- examples



CA Orengo AD Michie, S Jones,DT Jones, MB Swindells,JM Thornton, Structure, 1997, 5,1093–1108

Example from "CATH"



Class

1 Mainly α

2 Mainly β

3 α–β

4 Few SS

Architecture

10 Non-bundle

20 Bundle

30 Few SS

Topology

460 ...

470 Variant surface glycoprotein...

480 Glucoamylase, domain 2

490 Globin-like

500 βlactamase, domain 2

510 Casein kinase δ ..

520 ...

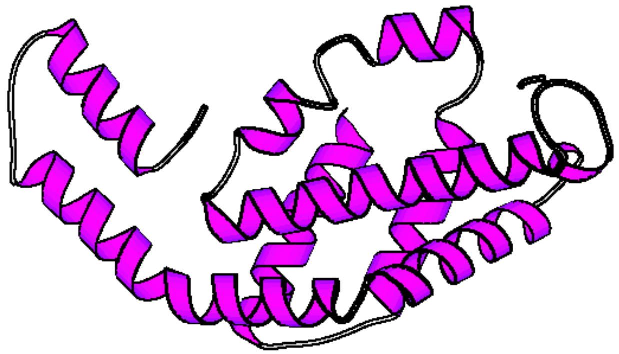
Homology

10 1hlm

20 1cpc chain A

30 1col chain A

40 1ddt domain 2



1.10.490.20

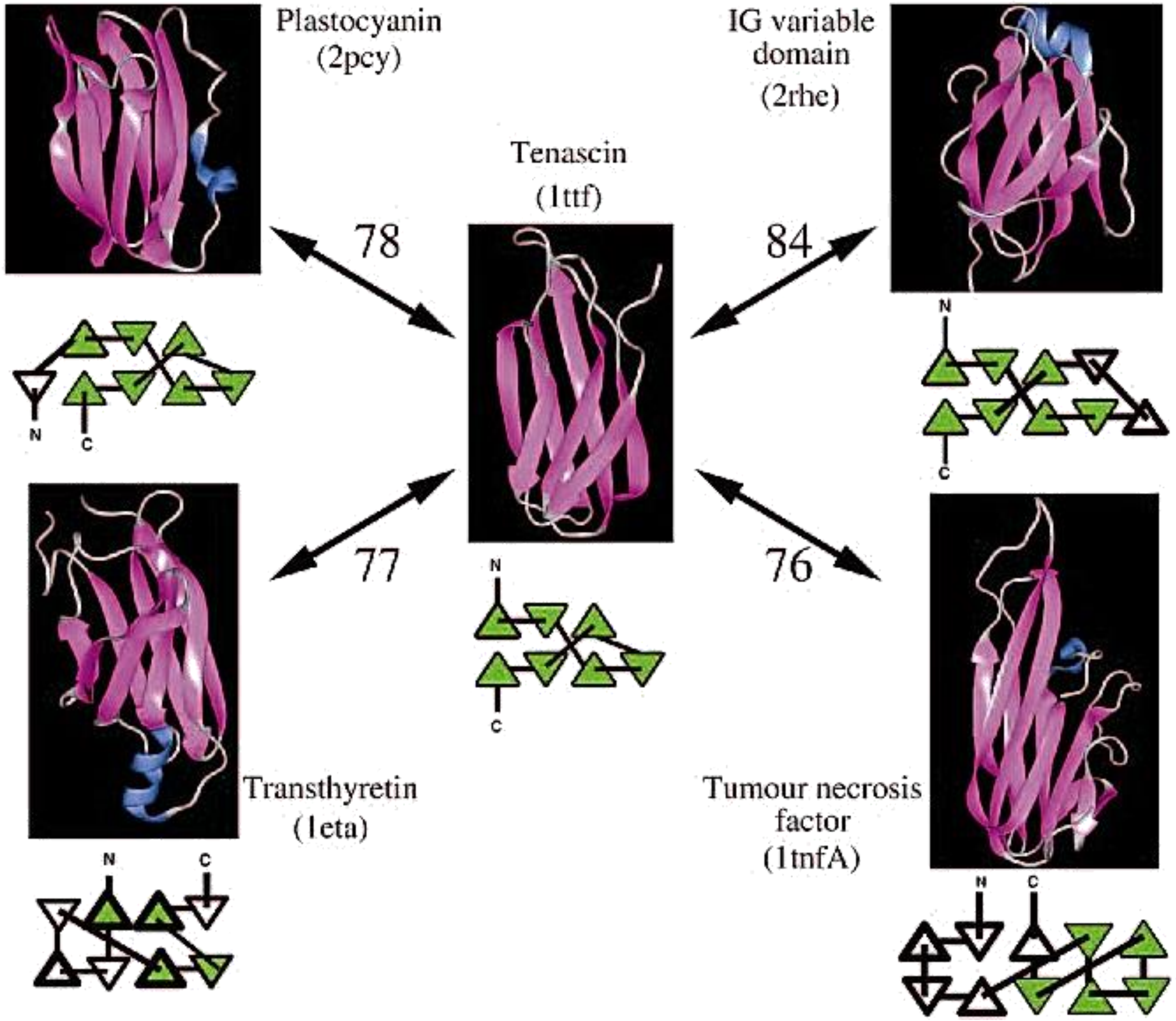
Mainly α.Non-bundle.Globin-like.1cpc chain A

CA Orengo AD Michie, S Jones,DT Jones, MB Swindells,JM Thornton, Structure, 1997, 5,1093–1108

Evolution and Classification

Can we interpret structures in evolutionary terms ?

- sometimes
- for more remote proteins
  – not really possible
- some typical figures from a literature classification



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Lots of families

α-helix bundles ?

- ≈226 domains,
- 3 % surveyed structures

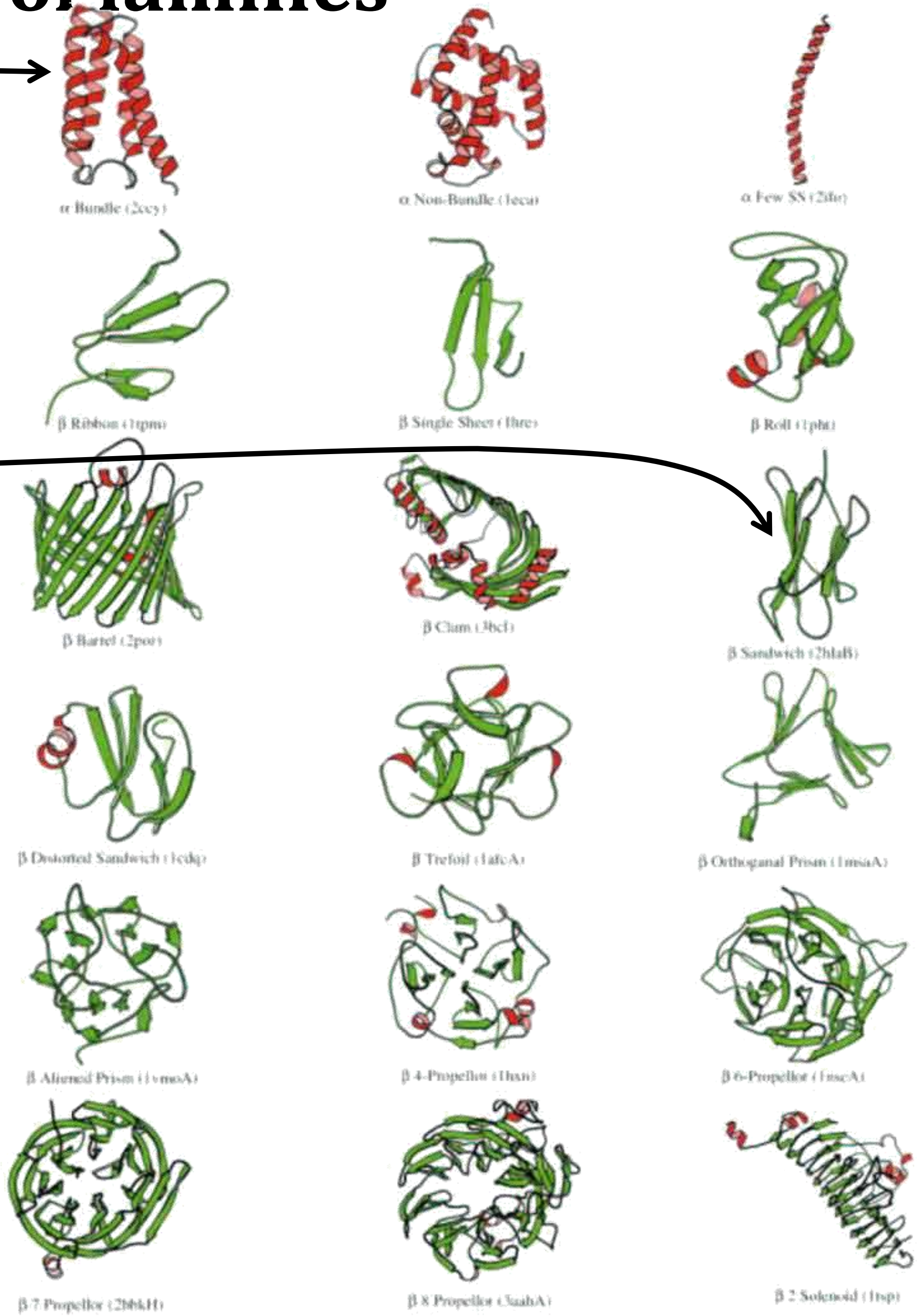
β-sandwich ≈1236 domains, 15 %

some families ?

- < 0.01 %

Interesting…

- some families very popular, some not



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Some families populated more than others ?

Are some structures more stable ? physics ?

Can some "accommodate" more sequences / tolerate more mutations ?

- next semester

Are some older in evolutionary terms ?

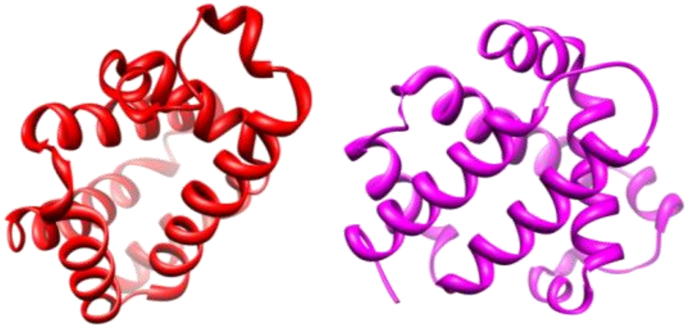
Biases ? PDB has

- mainly soluble, globular proteins which crystallised
- few membrane-bound proteins

Hierarchy ?

Is the hierarchy really justified ?

- at low levels maybe
- at higher levels ? (α,α/β, ..)



Better to discover relationships automatically

Imagine I can compare arbitrary proteins

- have some measure of similarity
- use this to classify

Huge problem

- proteins are different sizes and shapes
- how to compare ?

Summary

- Classification would be useful
- Given a distance (dissimilarity) one can invent a space for sequences or structures
- not known if it
  - exists
  - is hierarchical
- sequence vs structure similarity
  - different sequences can fold to same structure
- imposing a hierarchy on protein structures – very *ad hoc*
- one can forget hierarchy – simply use a clustering method
  - one will need a measure of similarities
  - big topic…

FORGET HIERARCHIES

- forget evolution
- forget hierarchies
- just look for similarities

Protein Structure Comparison / Numerical

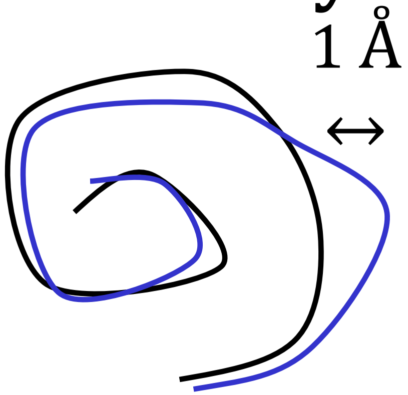
Most common protein structural question

- how much has my protein moved over a simulation ?
- how similar are these NMR models for a structure ?
- how close is my model to the correct answer ?

- more difficult
  - how similar is rat to human haemoglobin ?

- two cases
  1. same protein, same number of atoms
  2. different proteins
- first
  - measures for easy cases

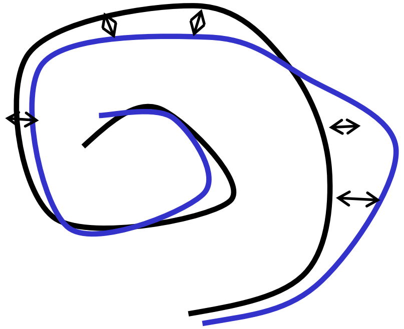
Numerical Comparison of Structures - Easy



What units would we like ?

- scale of similarity (0 to 1.0) ?
- comparison of angles
- distance / Å ? most common / easy to interpret

- looks a bit like the average difference between coordinates
- consider analogy with standard deviation / variance



From Standard Deviation to RMSD

Analogy with comparing a set of numbers

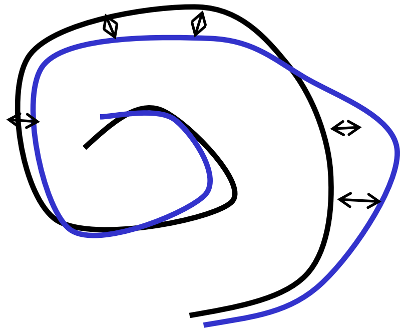
- get average (mean) $\bar{x} = N^{-1} \sum_{i=1}^{N} x_i$
- standard deviation $\sigma = \left(N^{-1} \sum_{i=1}^{N} (x_i - \bar{x})^2\right)^{1/2}$
- apply this to coordinates of r and r'

$$rmsd = \left(\frac{1}{N} \sum_{i=1}^{N} |\vec{r}_i - \vec{r}_i'|^2\right)^{1/2}$$

- rms / *rmsd* / RMSD = root mean square difference

Calculating rmsd

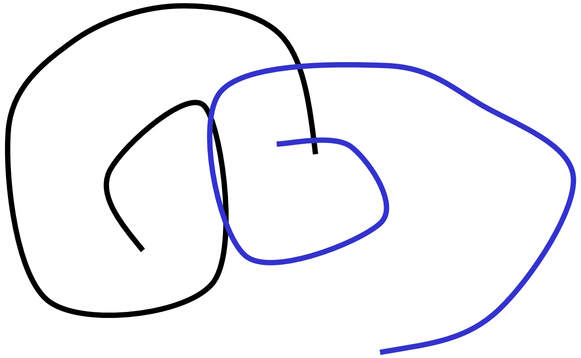
$$rmsd = \left(\frac{1}{N}\sum_{i=1}^{N}|\vec{r}_i - \vec{r}_i'|^2\right)^{1/2}$$



start at one end

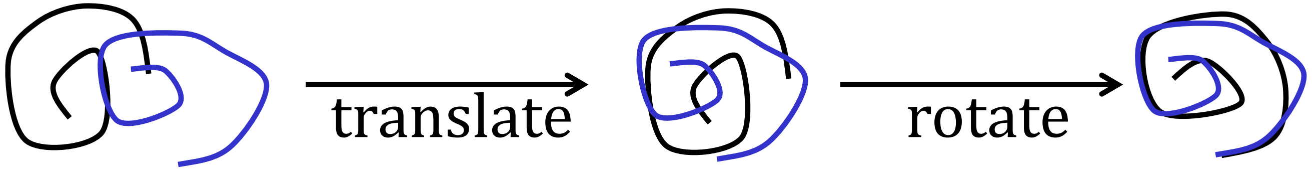
- difference between pairs of atoms

$$|\vec{r}_i - \vec{r}_i'|^2 = (x_i - x_i')^2 + (y_i - y_i')^2 + (z_i - z_i')^2$$



Problem..

- coordinates are normally…
  - what to do ?



Translation and Rotation

translation

- c.o.m. = centre of mass $\vec{r}_{com} = \left(\sum_{i=1}^{N} m_i\right)^{-1} \sum_{i=1}^{N} \vec{r}_i m_i$
- subtract difference vector $\vec{r}_{diff} = \vec{r}_{com} - \vec{r}'_{com}$

rotation

- rotation matrix to minimise

$$rmsd = \left(\frac{1}{N}\sum_{i=1}^{N}|\vec{r}_i - \vec{r}_i'|^2\right)^{1/2}$$

summary

- translate
- rotate
- apply formula

Still not finished

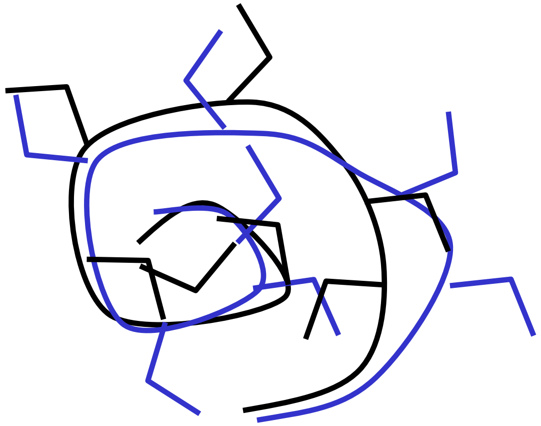
Which Atoms ?

What tells me the shape of a protein ?

- backbone trace

What happens if you include all atoms ?

- bigger *rmsd*
- normal choice
  - C^{α}
- sometimes
  - N, C^{α}, C
- all atoms ?
  - when a model is very close



Still not finished with simple *rmsd*

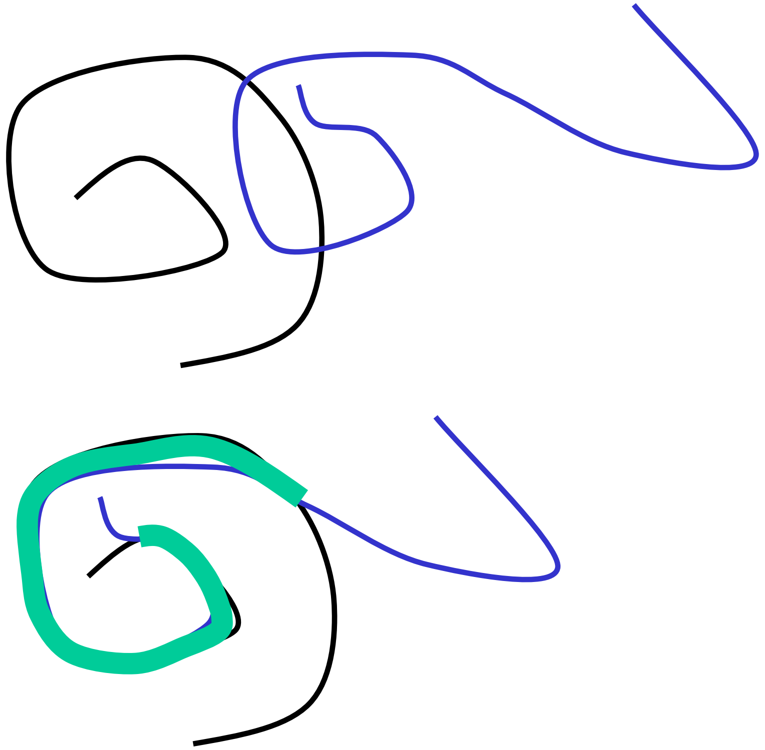
Parts Of Proteins

Two models of a molecule

- mostly very similar
- is *rmsd* a good measure ?

Identify similar parts

(method used in chimera)



define

```
superimpose ({r},{r'}, {d}) {
        translate ({r'},{r'}, {d})
        rotate ({r},{r'}, {d})
}
```

where **{d}** is some subset of sites

Selection of Interesting Atoms

Define a threshold like **`thresh`** =2 Å

```
{d}={|$r_i$-$r'_i$|} i=1..N
sort {d}

diff= rmsd ({$r_i$},{$r_i'$})
while (diff > thresh) {
  remove largest d
  superimpose ({$r$},{$r'$}, {d})
  recalculate distances
  diff = rmsd ({$r$},{$r'$}, {d})
}
if (diff < thresh)
  return {d}, diff
else
  return broken
```

Result ? a subset of interesting atoms

Subsets of Atoms

Originally, quantify structural differences as Å *rmsd*

Alternative quantity implied

- number of residues used for *rmsd* below threshold

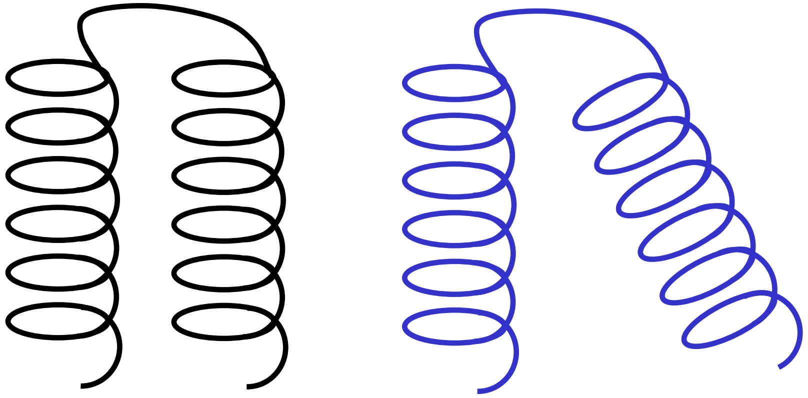
Implicit rule

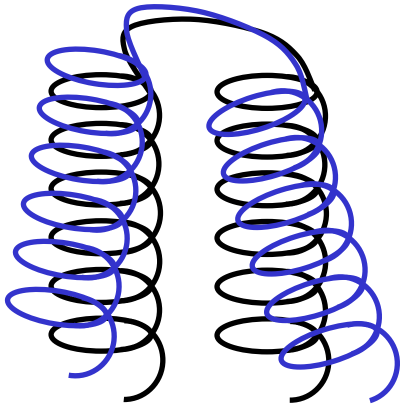
- as number of atoms ↓ calculated *rmsd* ↓

Why not to use *rmsd*

Helices identical, fold identical

- *rmsd* ?





- superposition requires rotation, affects all atoms

- big *rmsd*, but structure has hardly changed
- do not see that helices are identical
- more problems

Size dependence

Two proteins with 5 Å *rmsd* – similar or not ?

Consider proteins of different sizes

- maximum difference with N_{res}= 50 or N_{res}= 100 ?
- consider random structures with N_{res}= 50 or N_{res}= 100

- for small proteins 5 Å *rmsd* may be bad
- for large proteins 5 Å *rmsd* may be almost identical

extends to comparisons of small molecules

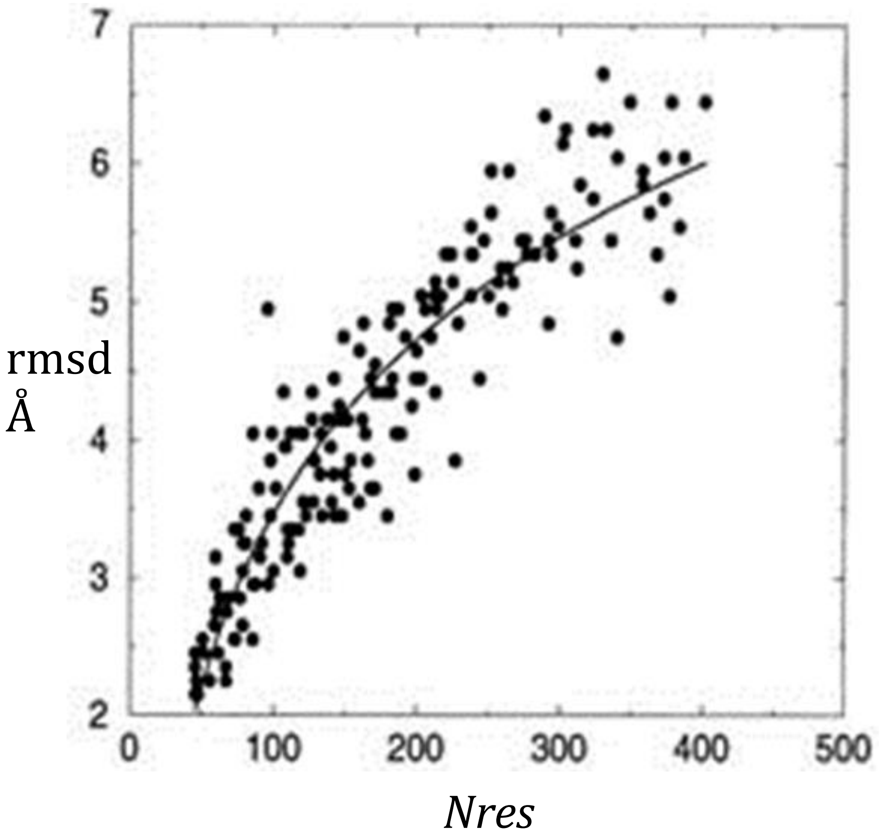
- ligands / medikamente…

What would one expect for random structures ?...

Size dependence

Empirical

- survey of random protein comparisons



Theoretical

- can find result from compact polymer theory (Florey)

  not in these lectures

Carugo, O. & Pongor, S., Protein Sci. 10: 1470–1473, 2001

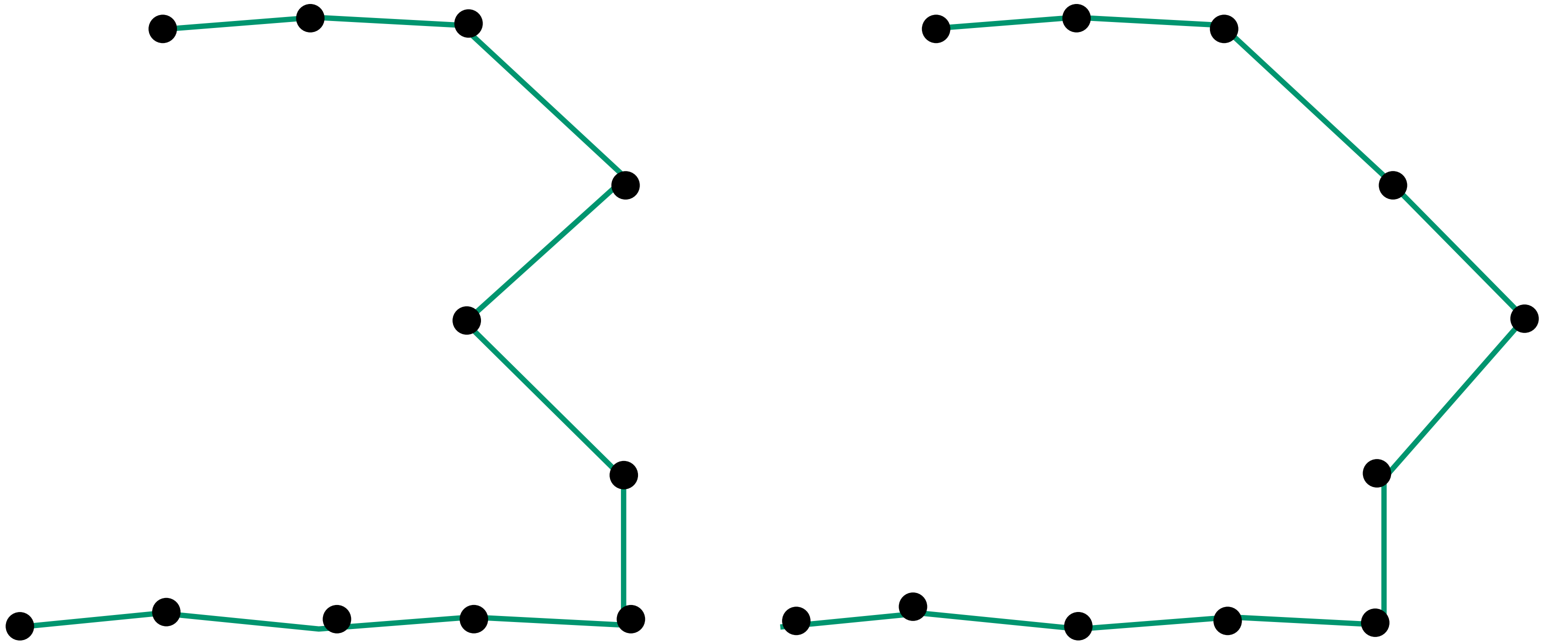
rmsd size dependence

good rule

- $rmsd_{interesting} = a + b(N_{res})^{1/3}$ for some constants a, b

problems with *rmsd* measure – alternatives

- angles ? OK – angles compensate for another



- distance matrices …

Distance Matrices With Numbers

Another characteristic of structures

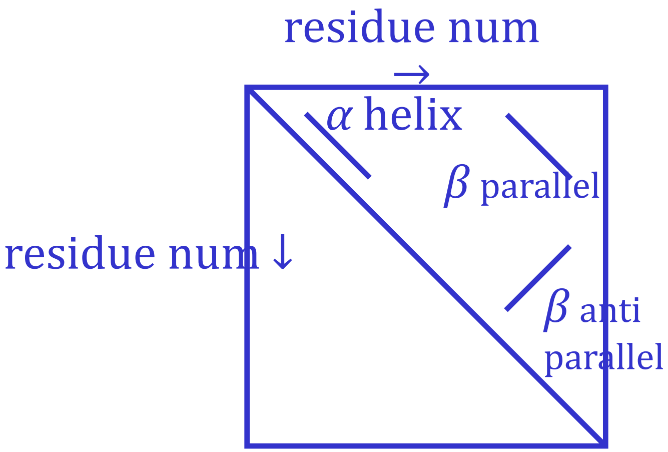
- C^{α} distance matrices
- measure the distance between C^{α} atoms

	1	2	3	4	5	6	7	…		N
1	0	3.8	6	7	…					
2		0	3.8	5	…					
3			0	3.8	4.5	…				
4				0	3.8					
5					0	3.8				
6						0	3.8			
7							0	3.8		
…								0	3.8	
									0	3.8
N										0

Distance Matrix for Recognising Structure

One way to summarise a structure

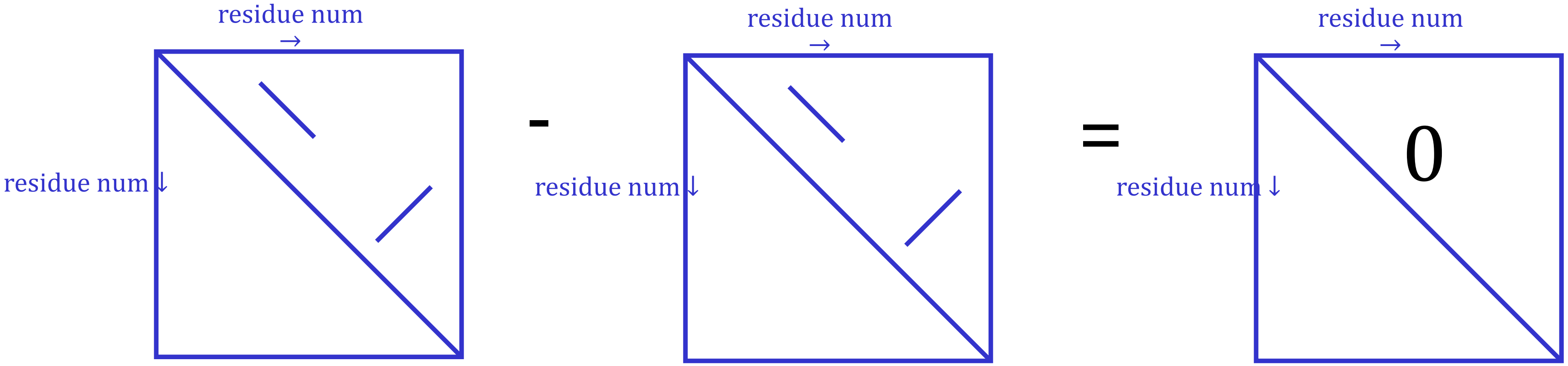
- plot C^{α} distance matrix, points below 4 Å
- can make α-helices and β-sheets clear



Distance matrix for comparing structures

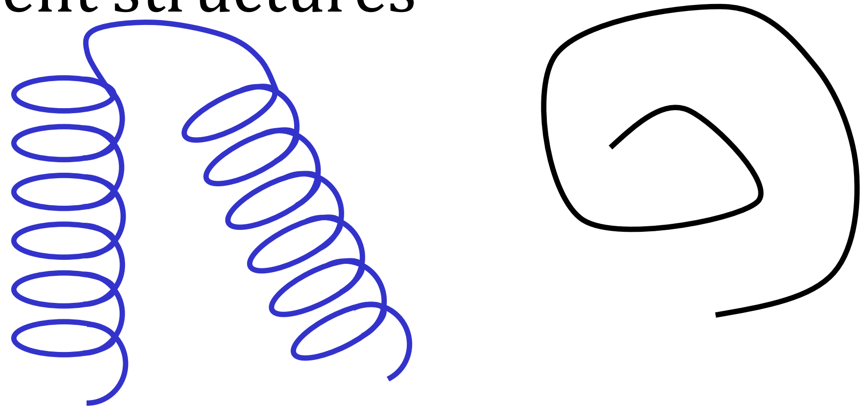
Take two similar proteins

- look at the difference of distance matrices

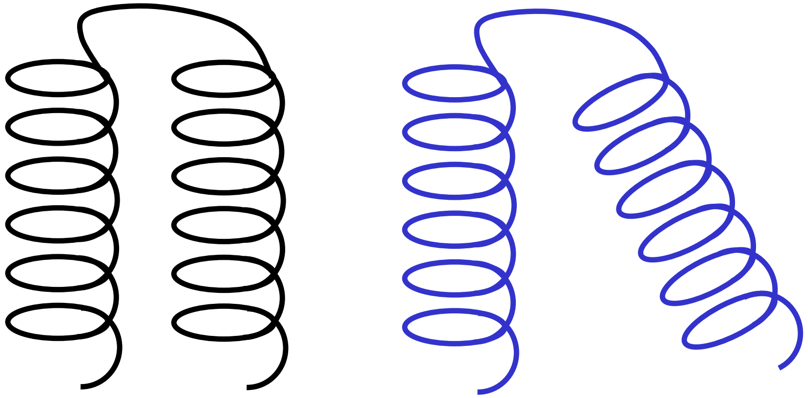


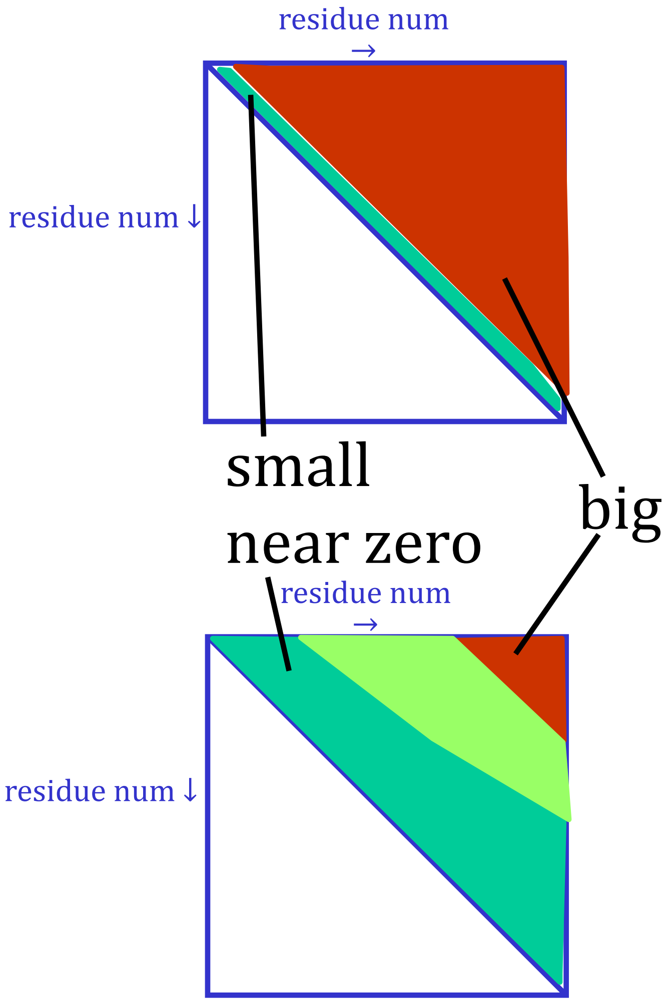
Comparing Distance Matrices

two very different structures



two related structures





pictures are better than any single measure, but…

From Distance Matrices to Single Number

For lots of comparisons, single number is more convenient

Root mean square (*rms*) difference of distance matrices

- distance between C$^\alpha$ atoms *i* and *j* $d_{ij} = \left|\vec{r}_i - \vec{r}_j\right|$

rms of distance matrices measure is

$$rms = \left(\frac{2}{N(N-1)} \sum_{i=1}^{N} \sum_{j>i}^{N} \left(d'_{ij} - d_{ij} \right)^2 \right)^{1/2}$$

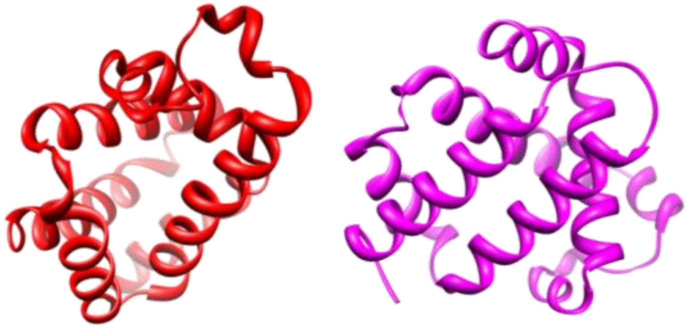
Like all other *rms* quantities

- normalised over top half of matrix

Summary – Comparing Models / Structures

- *rmsd*
  - most popular
  - requires superposition (translate + rotate)
  - can be fooled by "hinge" movements
  - size dependent
- to look at the shape of a molecule use C^{α} or backbone atoms
- numbers in Å have a physical meaning
- to look for the common core of a structure, find a subset of backbone
- other measures may be better than *rmsd*
- weakness of all measures
  - a single number can never capture all information

Comparing Proteins – different sizes



- compare red and blue proteins
- if we know which residues match
  - easy (use any *rms* formula)
- which residues match ?
  - sequence alignment ?

protein 1	A	C	D	W	Y	T	R	P	K	L	H	G	F	D	S	A	C	V	N
protein 2	A	C	D	W	W	T	-	P	K	V	H	G	Y	D	S	A	C	V	N

- green residues – mismatches (no problem)
- pink residues – ignore
- is this useful for similar proteins ? very (rat vs human haemoglobin)
- for very different proteins ? no

Comparing Very Different Proteins

Sequence alignment vs identity

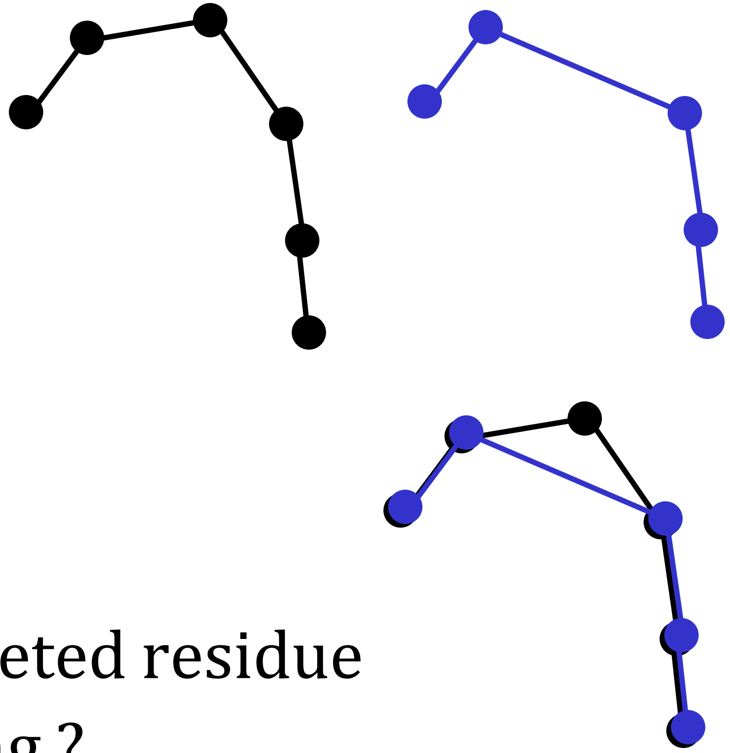
- as identity ↓, errors ↑

Consequence

- methods needed
  - operate on C^{α}
  - do not require sequence

How difficult ?

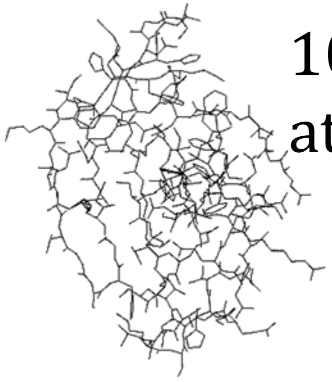
- superposition requires recognising the deleted residue
- can we use standard dynamic programming ?
  - no
- gap/insertion at any position, any length
  - combinatorial explosion



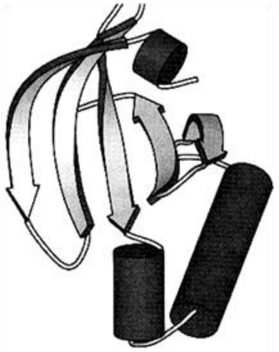
Strategies For Comparing Different Structures
1. use secondary structure

Combinatorial explosion is the problem

- reduce size of problem
- use elementsof secondary structure



10^2-10^3 atoms



about 8 units

- define secondary structure
- search for superposition
- for each residue
    - find closest C^{α} in partner structure
    - use the set of matching residues to calculate *rmsd*

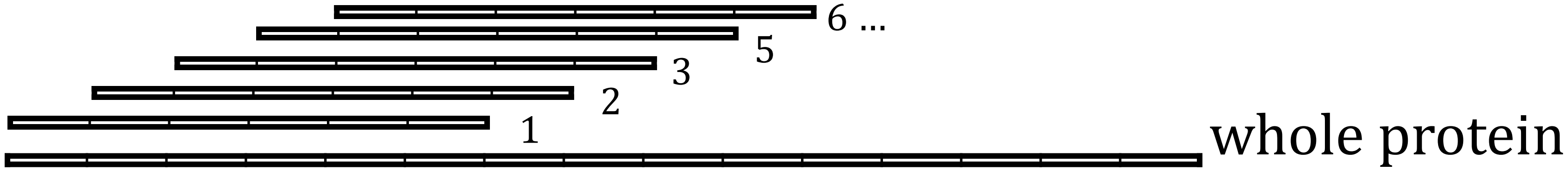
pictures from "Structural Bioinformatics", ed Bourne, PE and Weissig, H.

2. Peptide fragment strategy

- more general version of idea on previous page
- basis of most popular methods

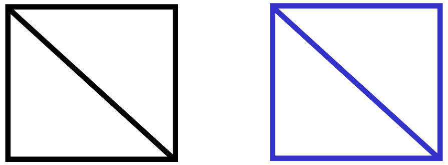
Ingredients

- break protein into overlapping fragments of structure (length 6 or 8)
- protein is no longer a string of residues nor a whole structure



- each fragment is a little distance matrix

Fragment Based Comparison



- any two distance matrices can be compared
- two proteins length *N* and *M* can now be compared…

protein 1 fragments →

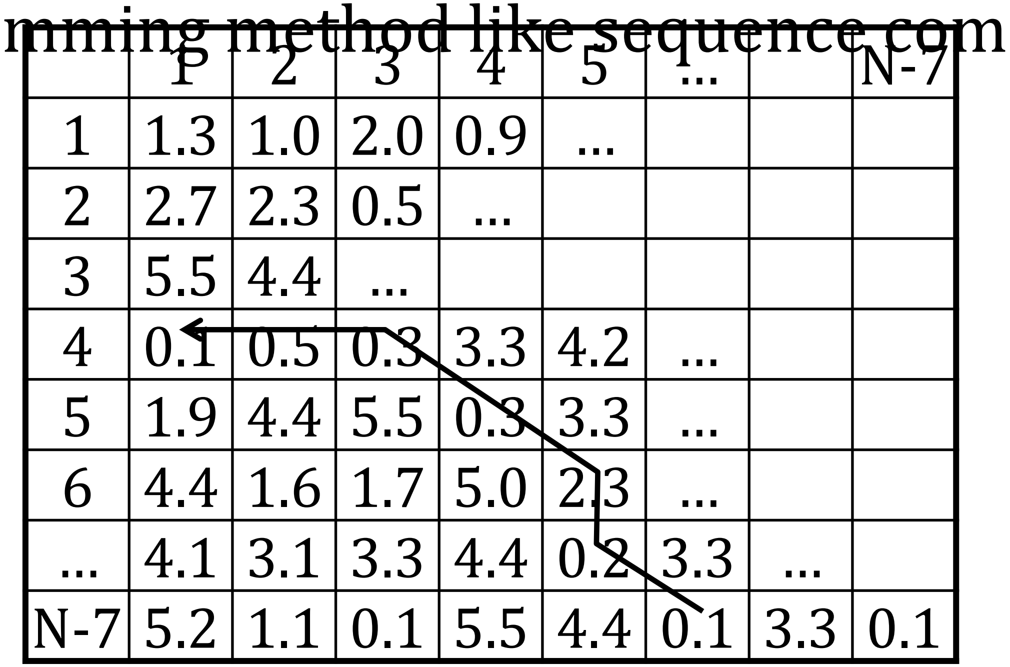
protein 2 fragments ↓

	1	2	3	4	5	…		*N*-7
1	1.3	1.0	2.0	0.9	…			
2	2.7	2.3	0.5	…				
3	5.5	4.4	…					
4	0.1	0.5	0.3	3.3	4.2	…		
5	1.9	4.4	5.5	0.3	3.3	…		
6	4.4	1.6	1.7	5.0	2.3	…		
…	4.1	3.1	3.3	4.4	0.2	3.3	…	
M-7	5.2	1.1	0.1	5.5	4.4	0.1	3.3	0.1

- imagine *rmsd*
- this is now like a sequence comparison problem

Finding Equivalent Fragments

- find optimal path through matrix
- classic dynamic programming method like sequence comparison



	1	2	3	4	5	...		N-7
1	1.3	1.0	2.0	0.9	...			
2	2.7	2.3	0.5	...				
3	5.5	4.4	...					
4	0.1	0.5	0.3	3.3	4.2	...		
5	1.9	4.4	5.5	0.3	3.3	...		
6	4.4	1.6	1.7	5.0	2.3	...		
...	4.1	3.1	3.3	4.4	0.2	3.3	...	
N-7	5.2	1.1	0.1	5.5	4.4	0.1	3.3	0.1

Like sequence comparison

- find optimal path through matrix
- classic dynamic programming method (N & W, S & W)
- uses gap penalties

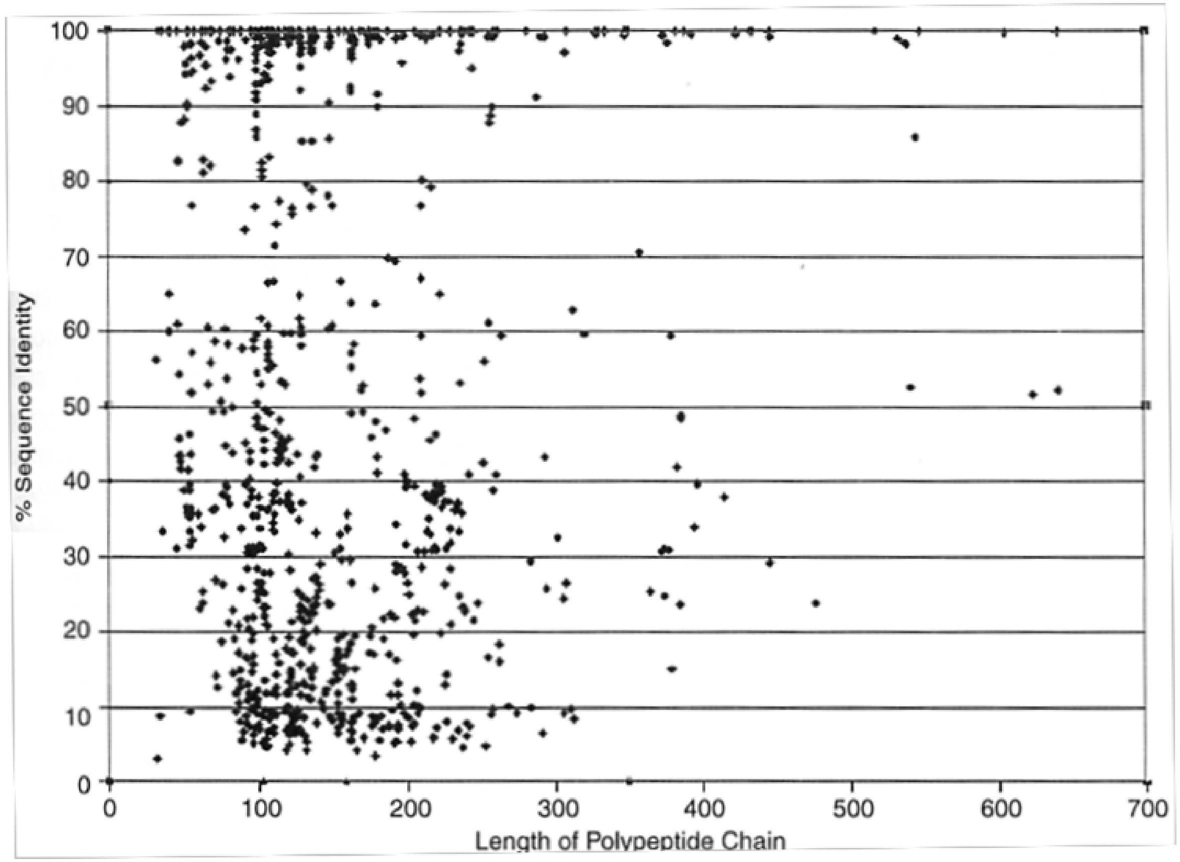
Comparing Different Size Protein Structures

- Break protein into overlapping fragments
- fragments can be compared to each other via distance matrices
- align like sequences
- from aligned fragments, get list of aligned residues
- using aligned residues, calculate *rmsd*, *rms* of overall distance matrices

How Important Are These Similarities ?

- survey 1 000 proteins
- find structurally similar pairs
- plot sequence identity

may not be found by sequence methods {



pictures from "Structural Bioinformatics", ed Bourne, PE and Weissig, H.

Summary of All Protein Comparisons

Classification of proteins

- could be done by sequence, better by structure

Structure comparison

- for one protein
  - selection of atoms
- for different proteins
  - requires list of matching atoms
- for similar proteins
  - can use pairs from sequence alignment
- for often dissimilar proteins
  - pure structure based method

Summary of everything

- classification is appealing
- very different answers using sequence or structure
- even if we believe in evolution
    - complete hierarchical scheme may be artificial