Classifying and comparing proteins

Plan

- why ?
- domains
- protein space – does it exist ? Meaning ?
- classifications
  - hierarchical vs pragmatic / empirical
  - continuous or clustered ?
- sequence similarity vs structure similarity
- example classifications
- comparison measures

Andrew Torda, Wintersemester 2014 / 2015, GST…

Why ?

Background – details later

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

- function prediction / annotation
- interpretation
- structure prediction

  - examples

Function prediction (annotation)

Most common question

- gene (sequence) associated with disease – no idea of chemistry
- look for related sequences with known (annotated) function

No answer ?

- structure available ? yes ..
- look for related structures in protein data bank with known function

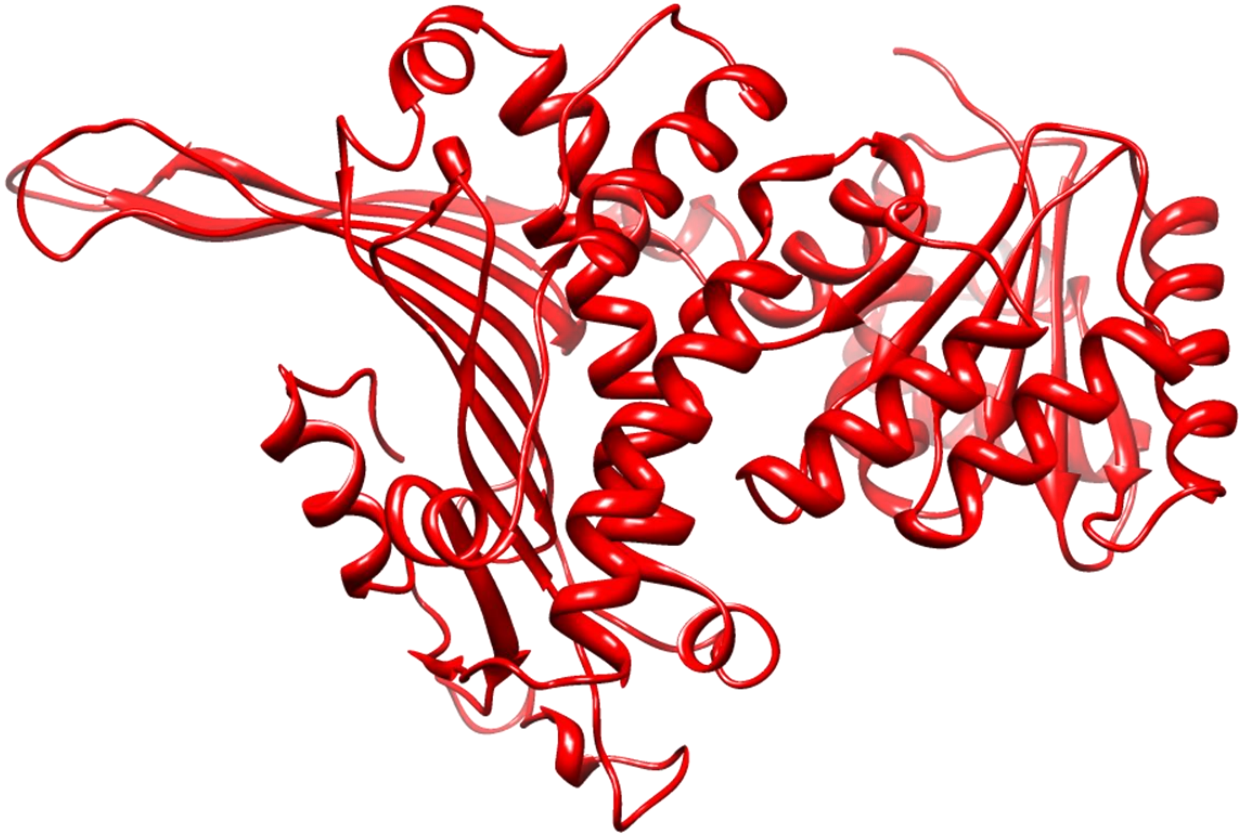
Interpretation of structures

- you know what your protein does
- you cannot crystallise it with reactants (substrates)
- you cannot see which residues are essential to function

- find a related structure which crystallises with its (maybe different) reactants
- example
  - 1dpg – oxidoreductase
  - acts on sugar, no idea where sugar binds

where does sugar bind in 1dpg ?

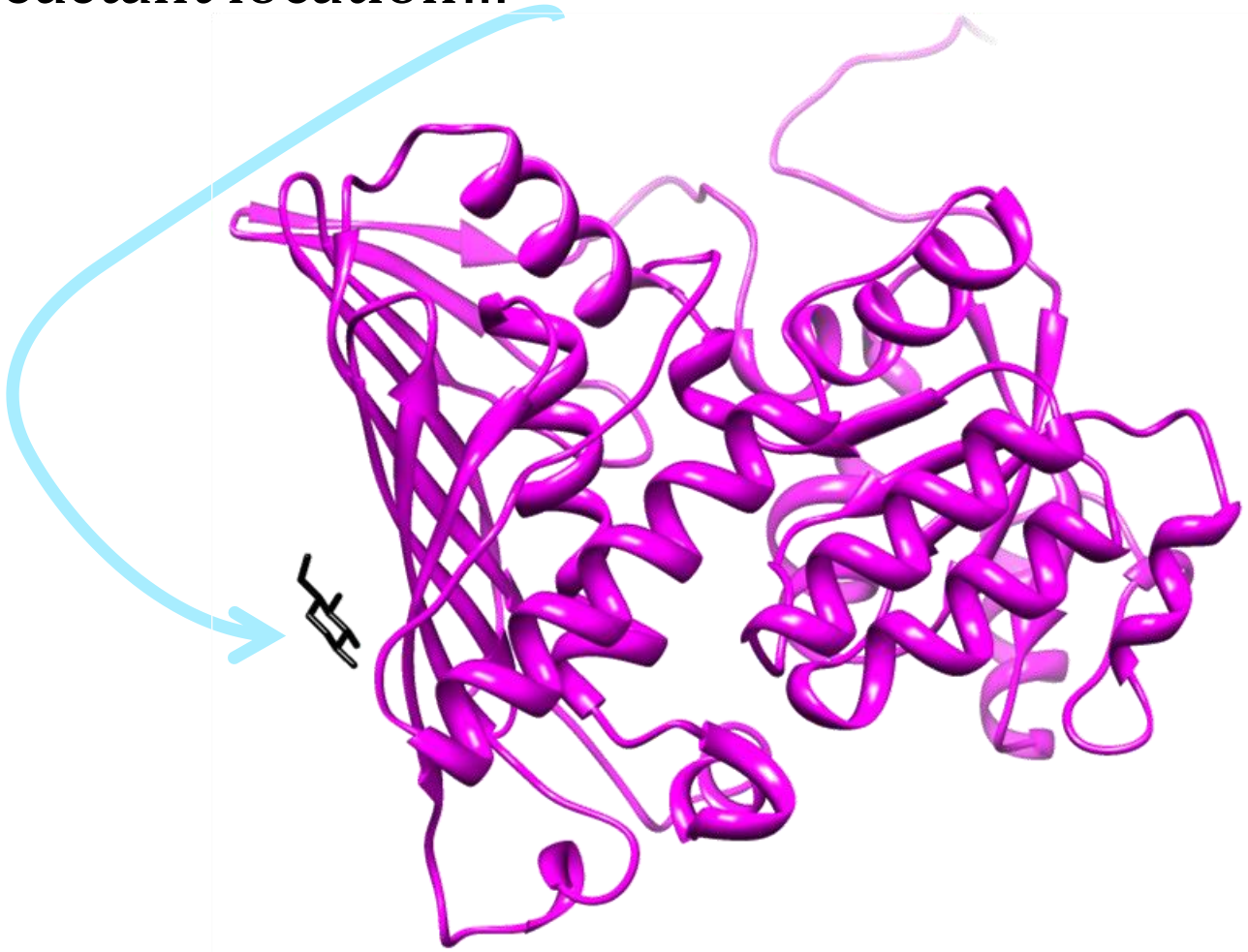
- 1dpg – glucose-6-phosphate d.h. (one chain)
- no idea where sugar binds / which residues are important
- well studied – never crystallised with sugar



1ryd – glucose fructose oxidoreductase

- special – managed to crystallise with sugar

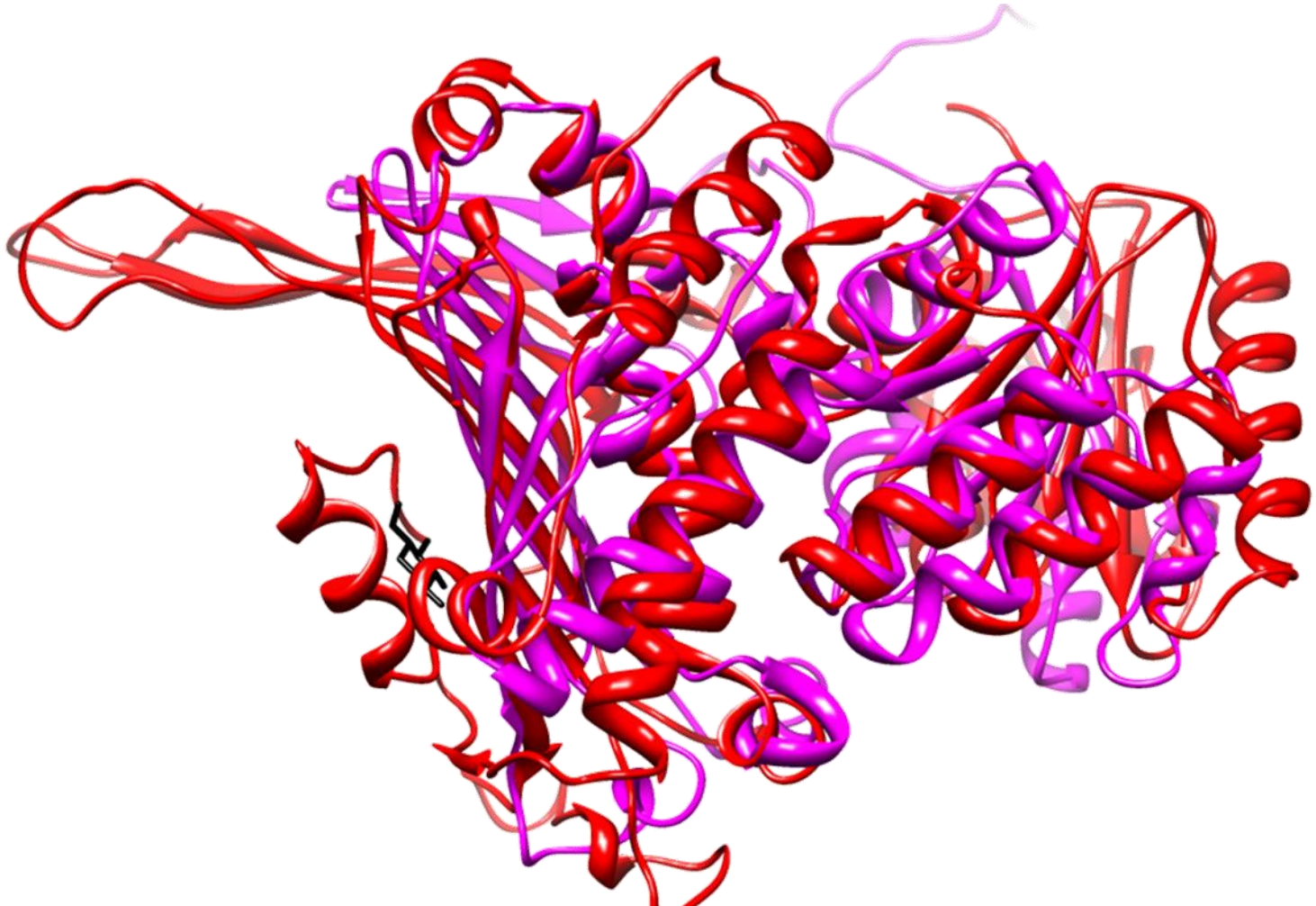
Transfer the reactant location…



1dpg & 1ryd

Claim

- from structural similarity one knows which residues in 1dpg are important



Classification and structure prediction

How many possible protein structures are there ?

- astronomical

How many 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

Sequence vs structure similarity

- Protein Databank ≈ 10^5
- 90 % sequence similarity ≈ 3.3×10^4 classes
- different shapes 2 to 5×10^3

- fewer classes when structure-based
- structure-based classes are larger

- speculations and explanations later
- now
  - domains
  - sequence space
  - hierarchical and non-hierarchical

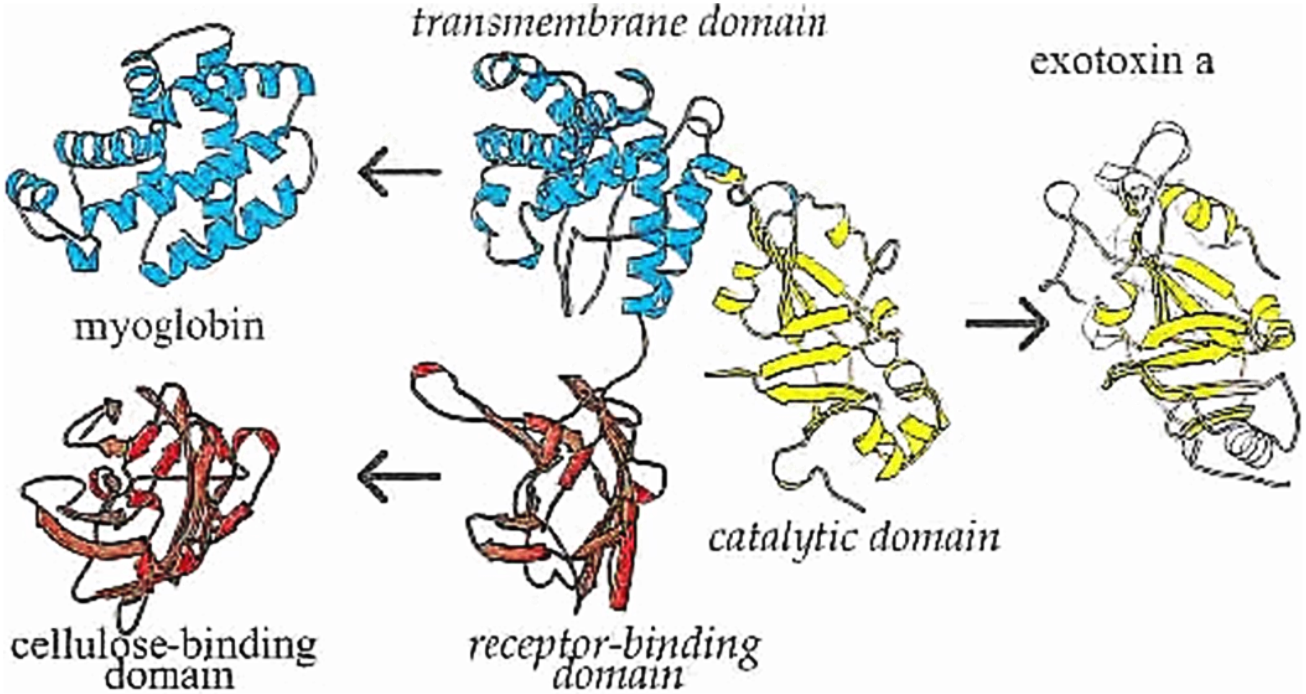
Domains

Why mention ?

- many groups work on domains, not whole proteins

Reasons

- many structures are labelled "a domain of protein X"
- evolution – convincing picture (diptheria toxin)



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

Domains – evolutionary viewpoint

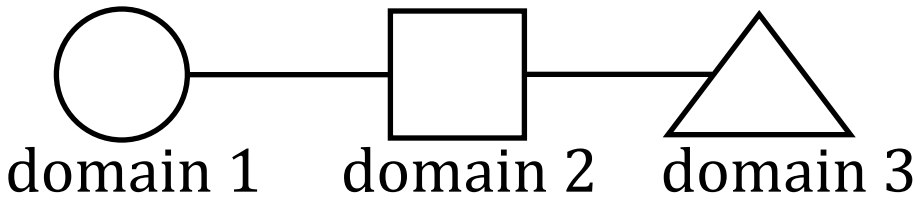
Idealised view..

Claim / belief

- evolution goes faster by mixing / swapping domains between proteins

Do we all agree on domains ?

- 3 viewpoints



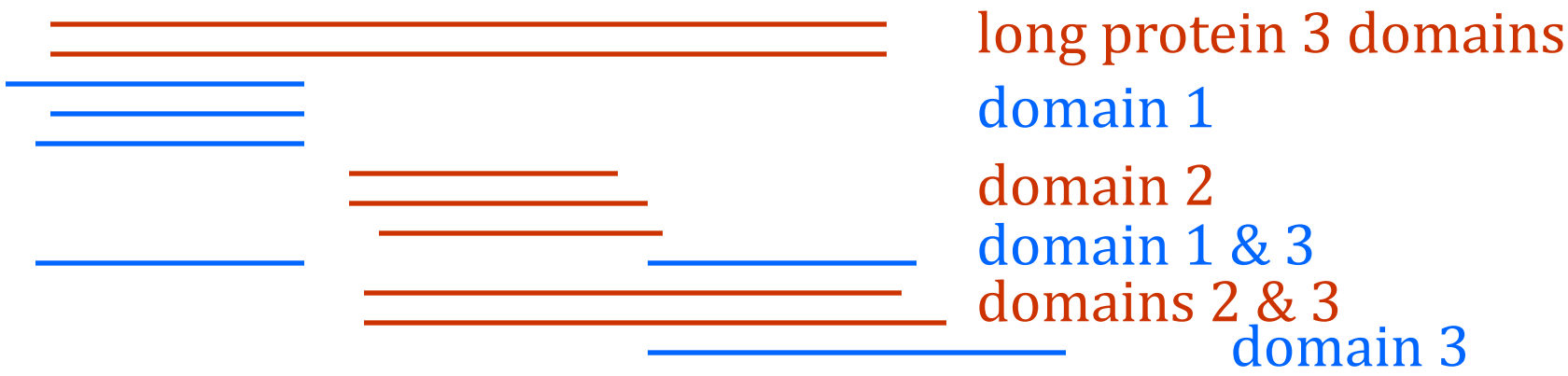
Domains in Biochemistry (view 1)

Domains = parts of a protein with different functions

- catalytic domain, regulator-binding domain…

Sequence level domains (view 2)

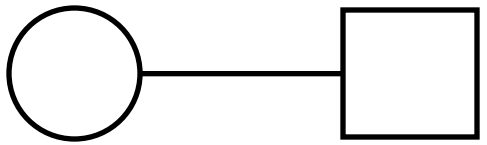
Align a group of sequences



- appears to have 3 domains
- no reference to structures or function

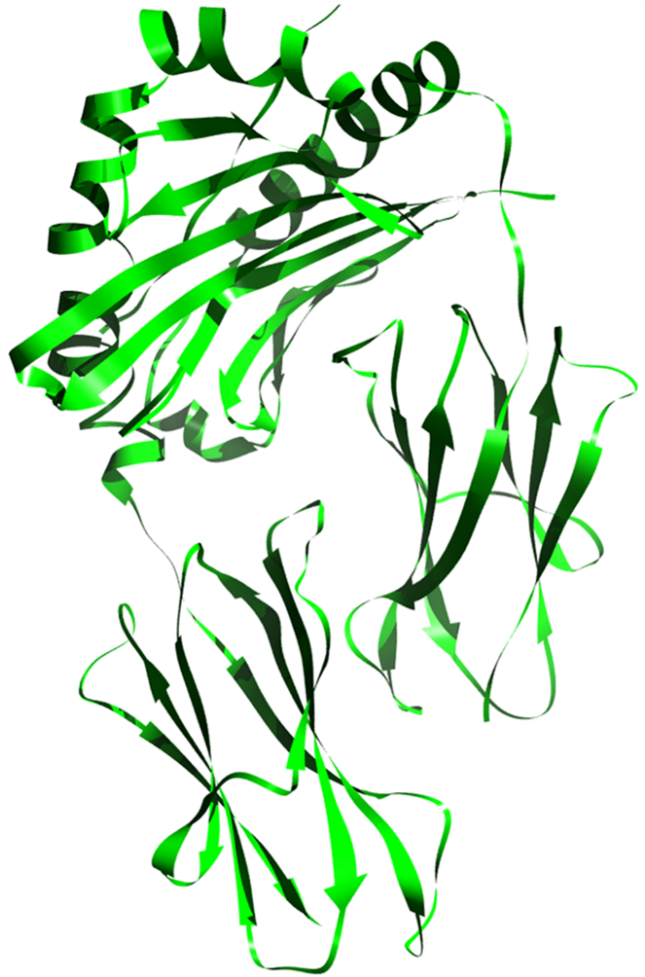
Domains in Structures (view 3)

Many structures solved look like…



example: histocompatibility module (1iak)

- 3 domains



Domains for these lectures

Usually structure based

- compact units
- stable in solution (usually)

Can we really expect to classify proteins ?

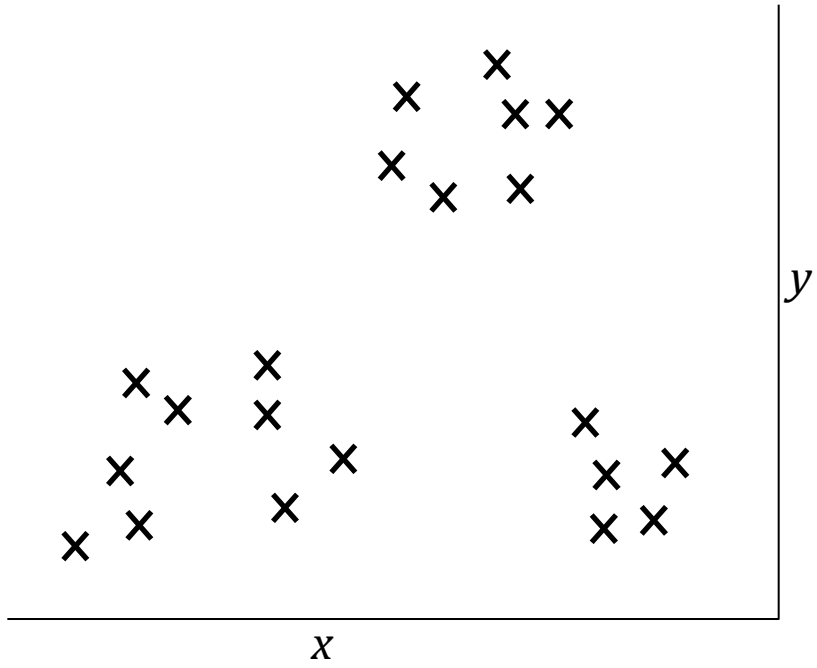
Next set of slides - two questions

- are there protein classes / families ?
- what is "protein space" or what would it mean ?

Protein classes / families

Questions

- what do classes mean ?
- do you expect them ?



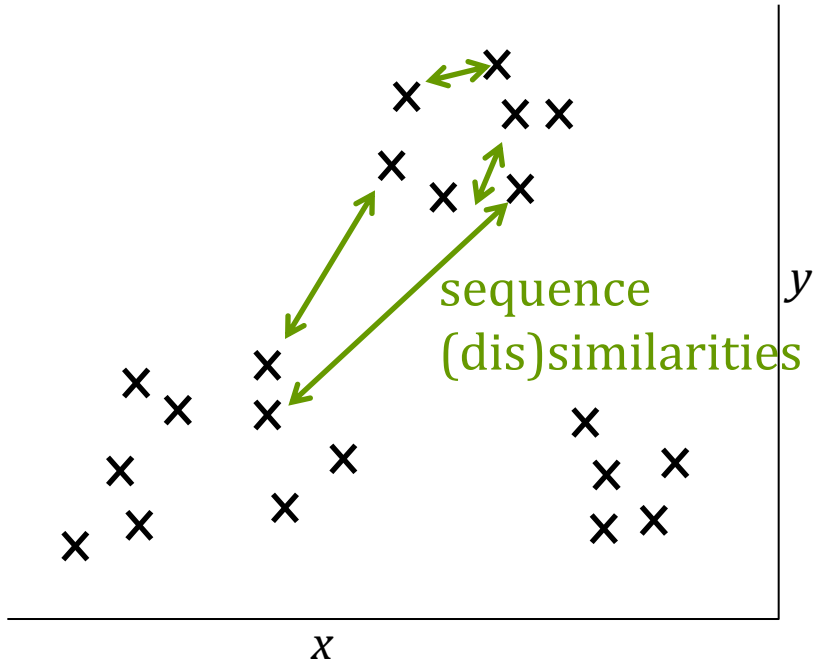
Meaning…

- each cross is a protein
  - what are *x* and *y* ?
  - two ways to answer
    - generic *n*-dimensional distances
    - example from sequence space

Spaces for proteins

Sequence example

- we can compare any two sequences
- measure (dis)similarity
  - matches, similarity score, …



I have a matrix of $n(n-1)$ distances

- how would I go to x, y ?
- how many dimensions ?

If I have similarities between objects

- there is some implied $(n-1)$ dimensional space

- a different way to have a sequence space

distance geometry
high dimensional

Sequence Space

- convenient way to explain ideas of sequence similarity

Conventional spaces

- 1D (x), 2D (x, y), 3D (x, y, z), 4D (x, y, z, w), …
- let us estimate how big a space or problem is
- some problem – discrete space
  - how many variables do I have ? (a, b, c, …)
  - how many values can each variable have ?
    - a 3 values, b 4 values, c 5
    - number of points in space = $3 \times 4 \times 5$

Protein sequences

- each position can have 1 of 20 values
- total number of sequences $20 \times 20 \times .. = 20^{N_{res}}$
- like a space of N_{res} dimensions

Representing a Sequence

Protein sequence and structural coordinates

	1	2	3	4	5	6	7	…	N_{res}
x	1.2	2.3	…						10.3
y	2.4	3.5	…						11.1
z	1.7	2.9	…						15.5
seq	W	A	C	A	A	…			D

Consider the first three residues

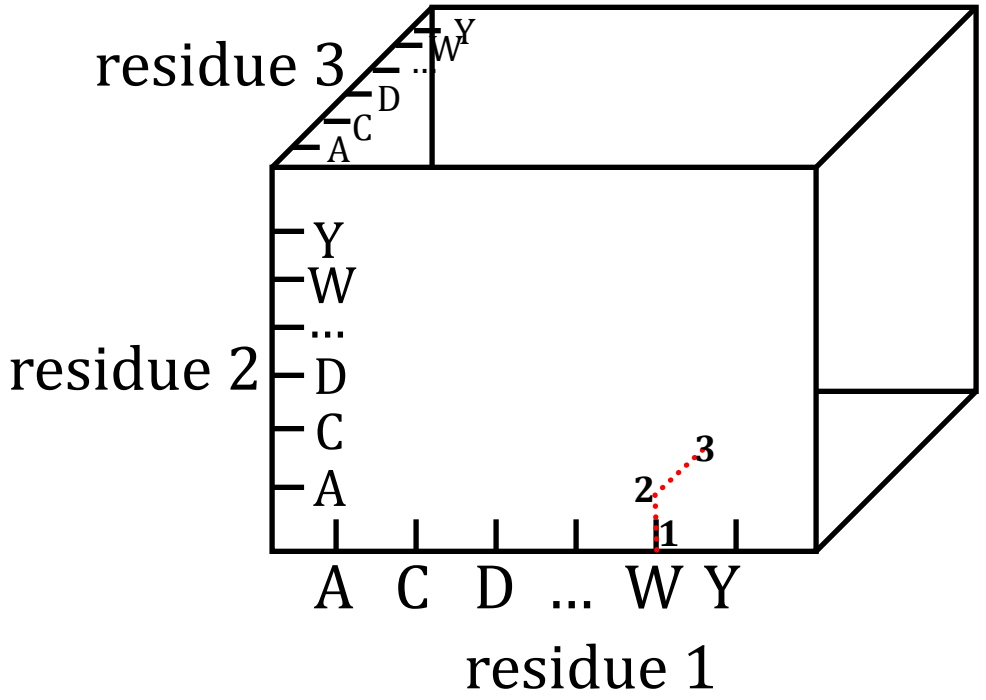
WAC (for pictures only)

Finding a Sequence in This Space

Real diagram is a box of N_{res} dimensions

- this one 3 dimensions

	1	2	3	4	5	6	7	...	N_{res}
x	1.2	2.3	...						10.3
y	2.4	3.5	...						11.1
z	1.7	2.9	...						15.5
seq	W	A	C	A	A	...			D



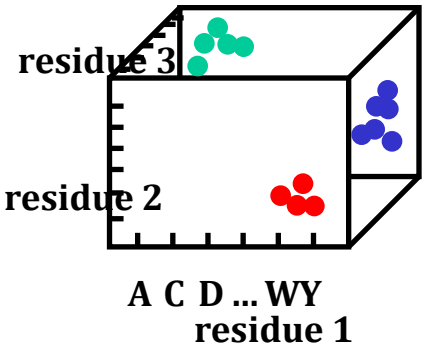
- looking for sequences…

Families in Sequence Space

Similar sequences should land near each other

How realistic ?

- picture is a simplification
- only works for $N_{seq1} = N_{seq2}$
- very useful
  - distances between sequences



Will return next semester

Summarise spaces

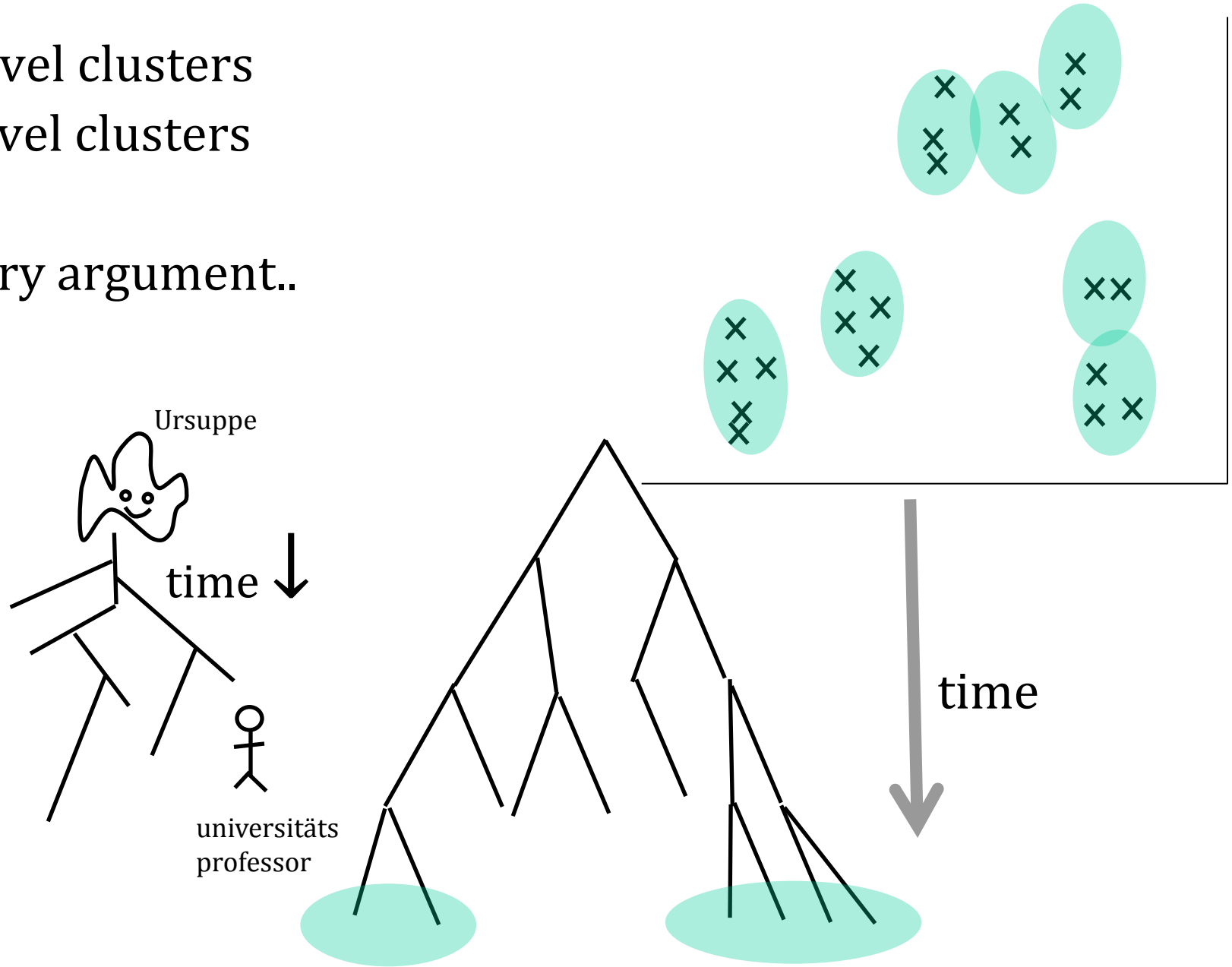
We can imagine a space of proteins

1. from similarities between points $(n-1)$ dimensional
2. sequence space N_{res} dimensional

Should we expect a hierarchy ?

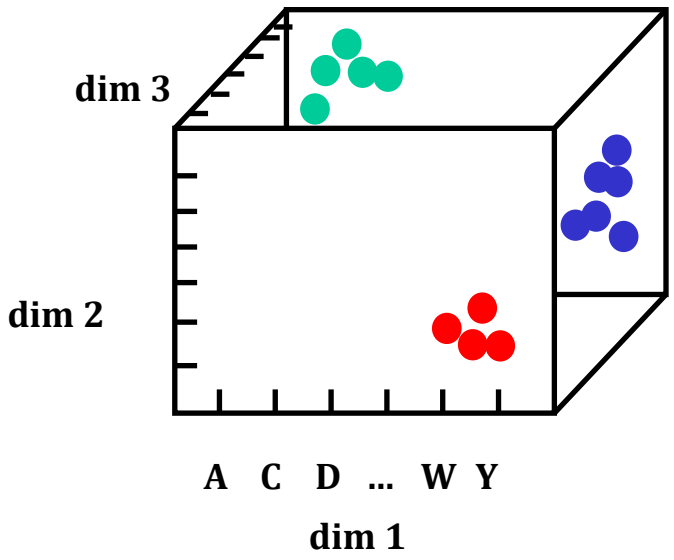
- 7 lowest level clusters
- 3 higher level clusters

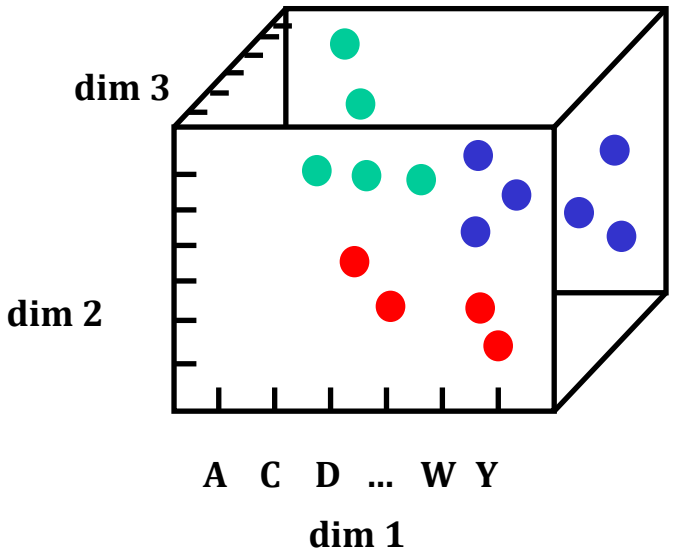
- evolutionary argument..



Do we expect protein families ?

- No real answer
- we have an idea of spaces – sequence or structure based
- how are proteins distributed ?

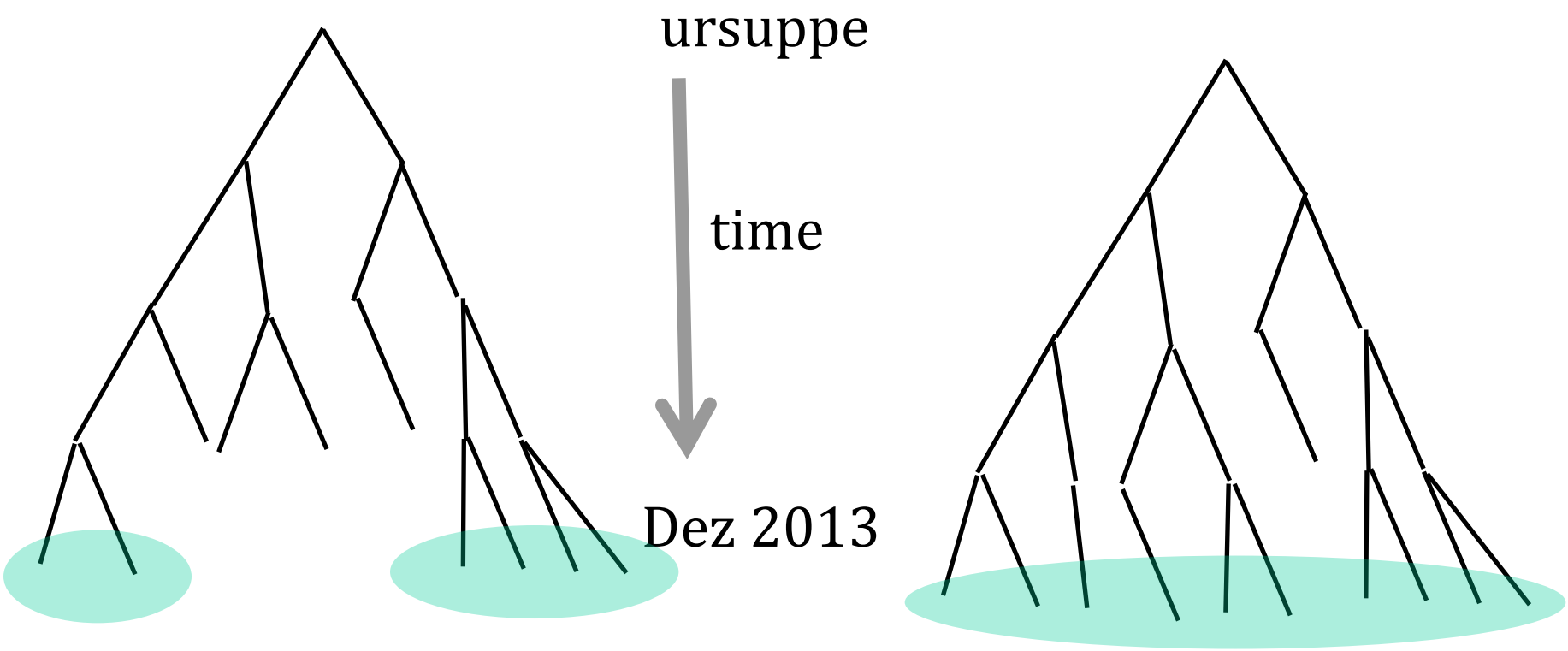




- should you expect clusters ?

Evolution and phylogeny

Shape / density of tree of life



clear
families

no families

Questions for fun

Do we expect hierarchy ?

- some people do

Do we expect clusters ?

- some people ..

Structure vs Sequence similarities

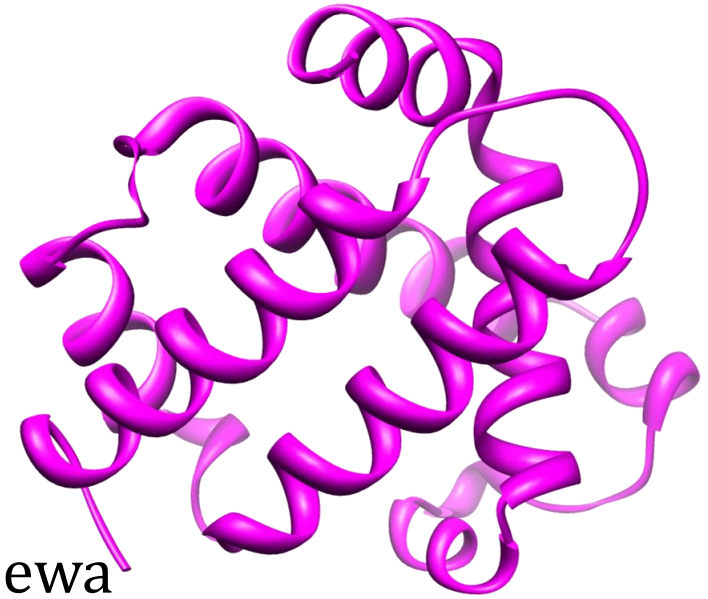
More different than you might expect

Similar sequences

- have not evolved for too long
- expect similar structures

Other way round ? Examples

1ecd
erythrocruorin

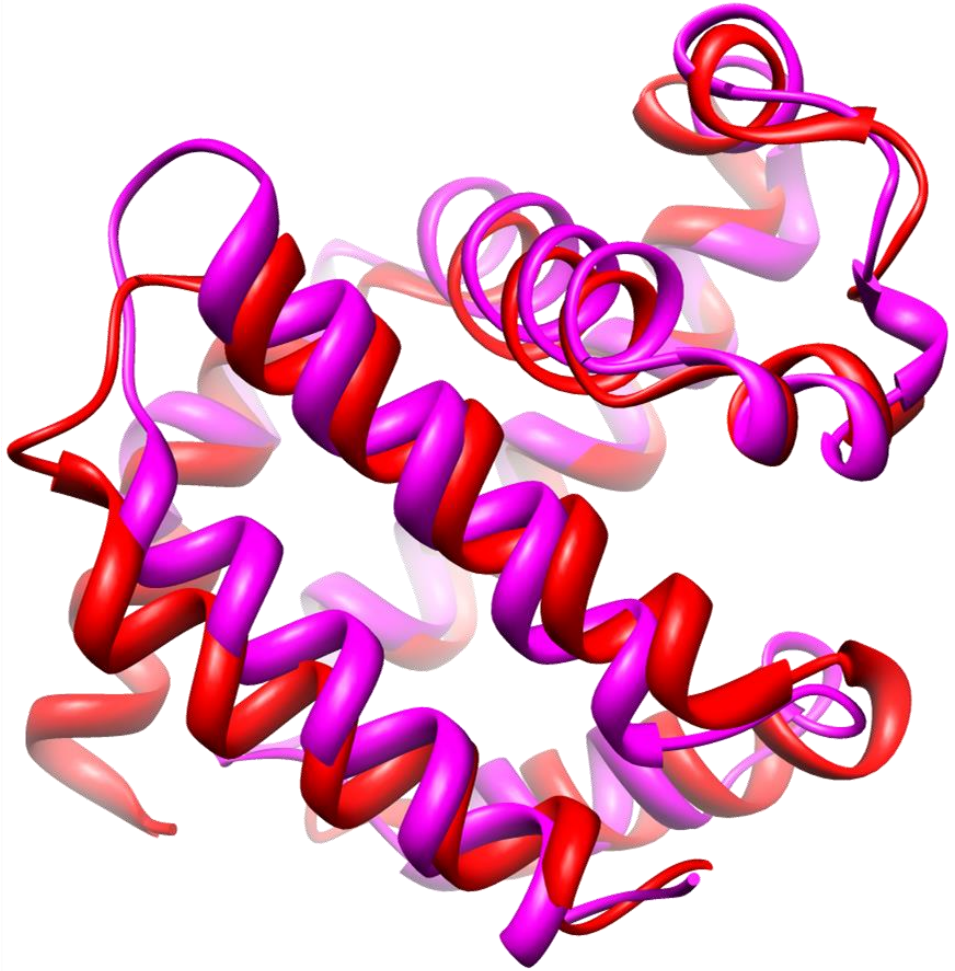


1ewa
dehaloperoxidase

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://public.zbh.uni-hamburg.de/salami/

Example family

Example, neighbours of 1cun chain A

- look at sequence identity (% id)
- alignment length (lali = number of residues)
- 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

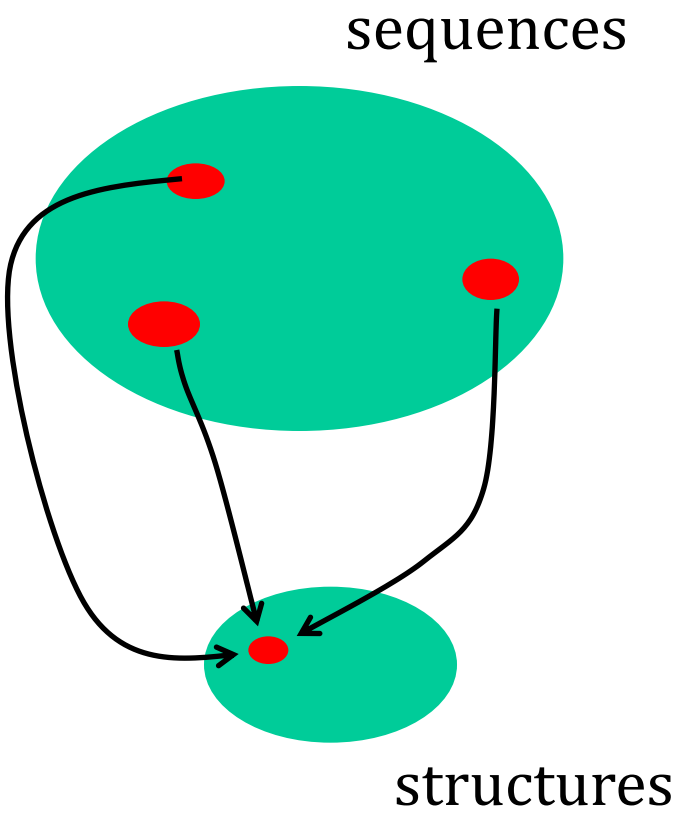
Structure vs Sequence

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 does determine structure
  - many sequences could fit structure (more next semester)

Surprising ?

- consider near universal proteins
  - 100's millions years evolution, function largely preserved

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* %
- result (jan 2014)

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

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

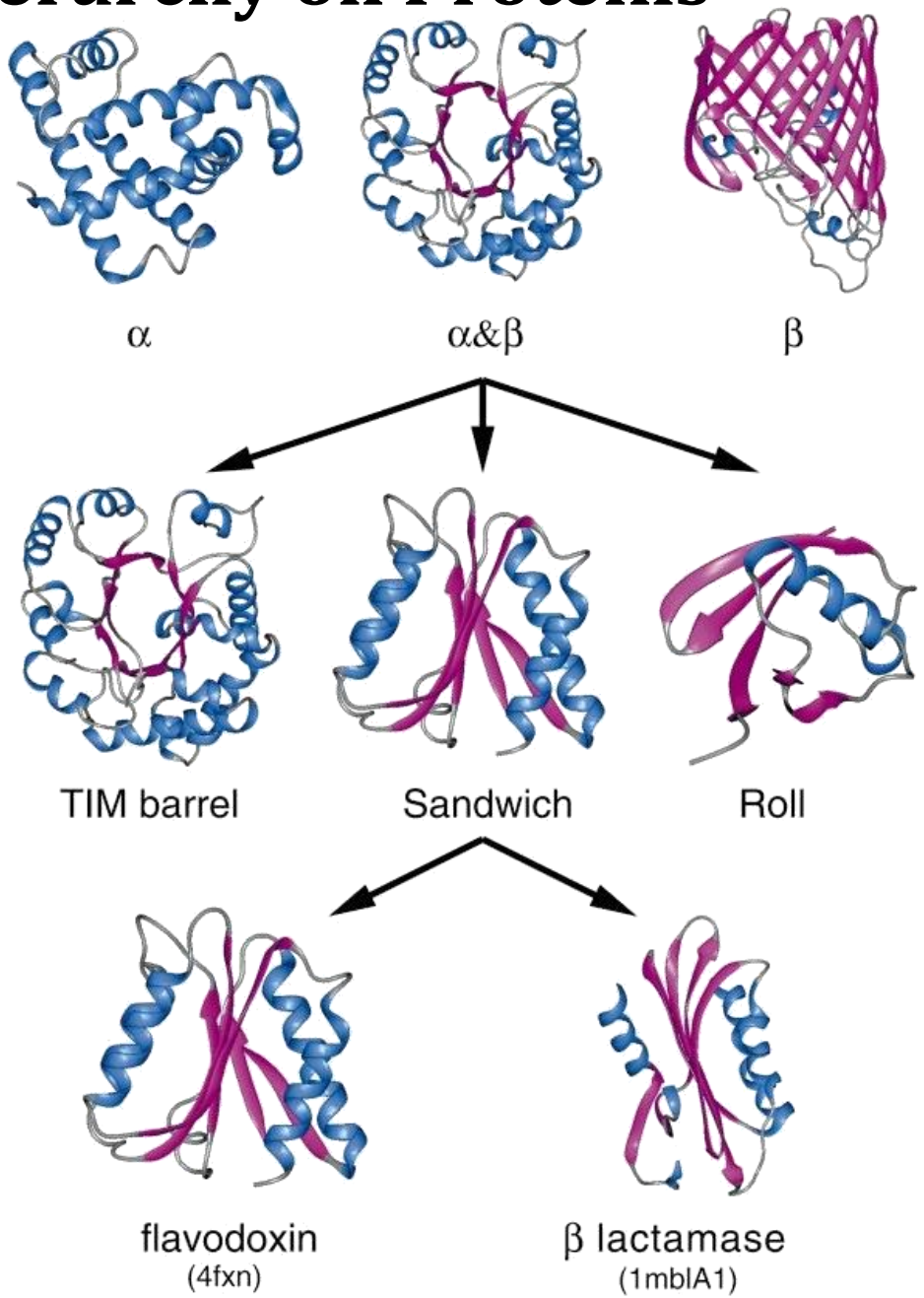
- now.. examples of structure based classifications

Imposing a Hierarchy on Proteins

- parts may correspond to evolution
- top level ?

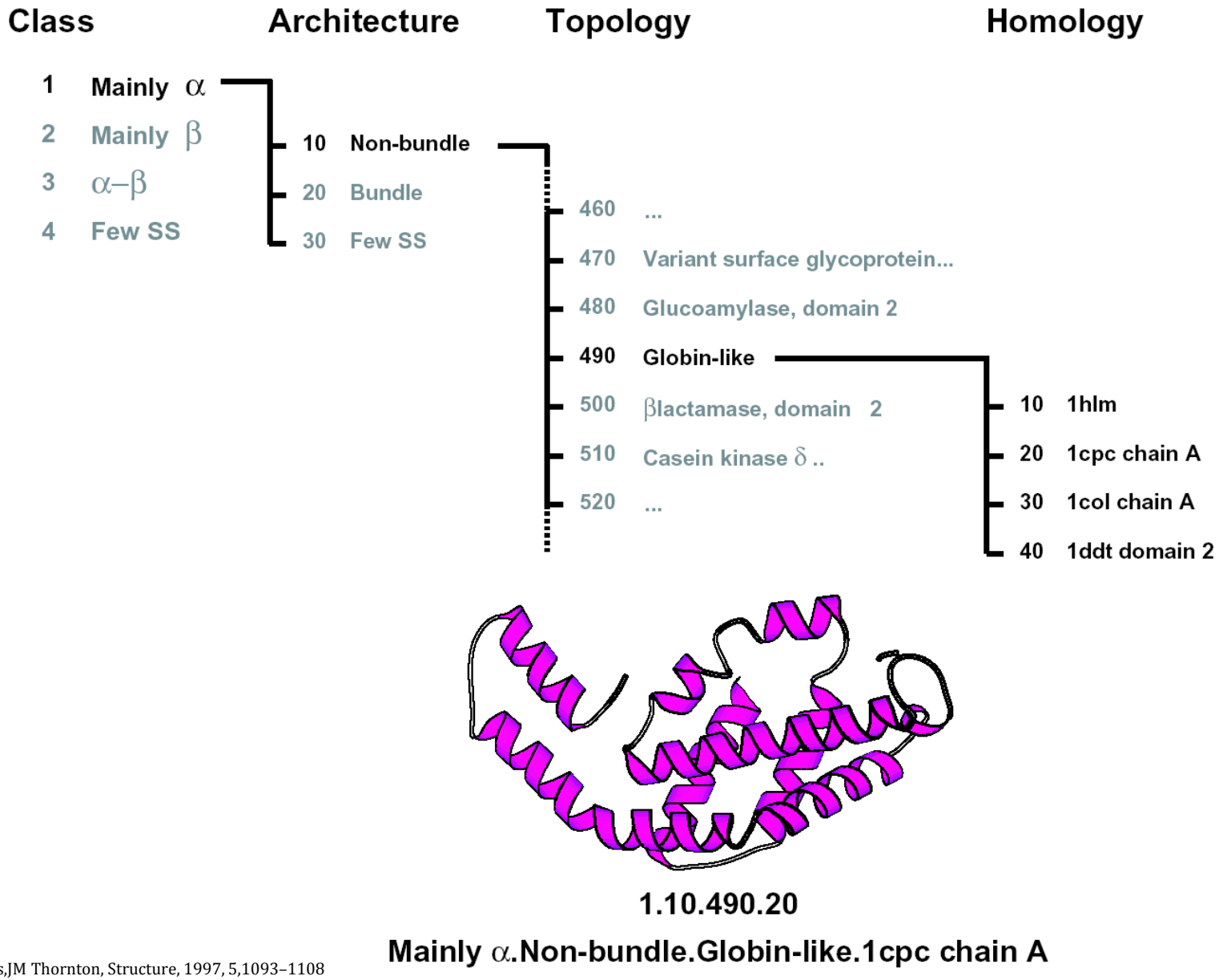
How useful and applicable ?

- examples



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

Example from "CATH"

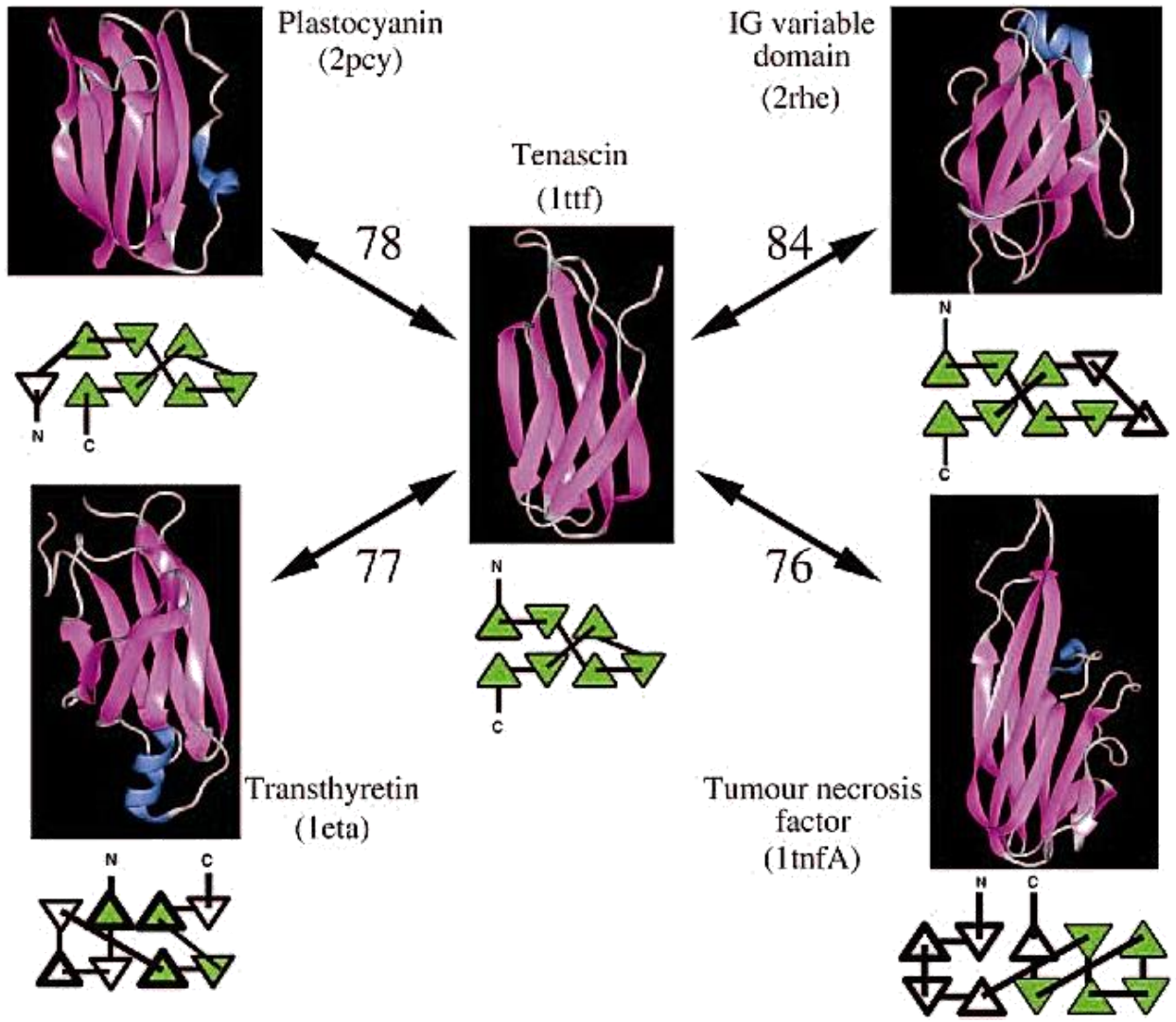


CA Orengo AD Michie, S Jones,DT Jones, MB Swindells,JM Thornton, Structure, 1997, 5,1093–1108

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

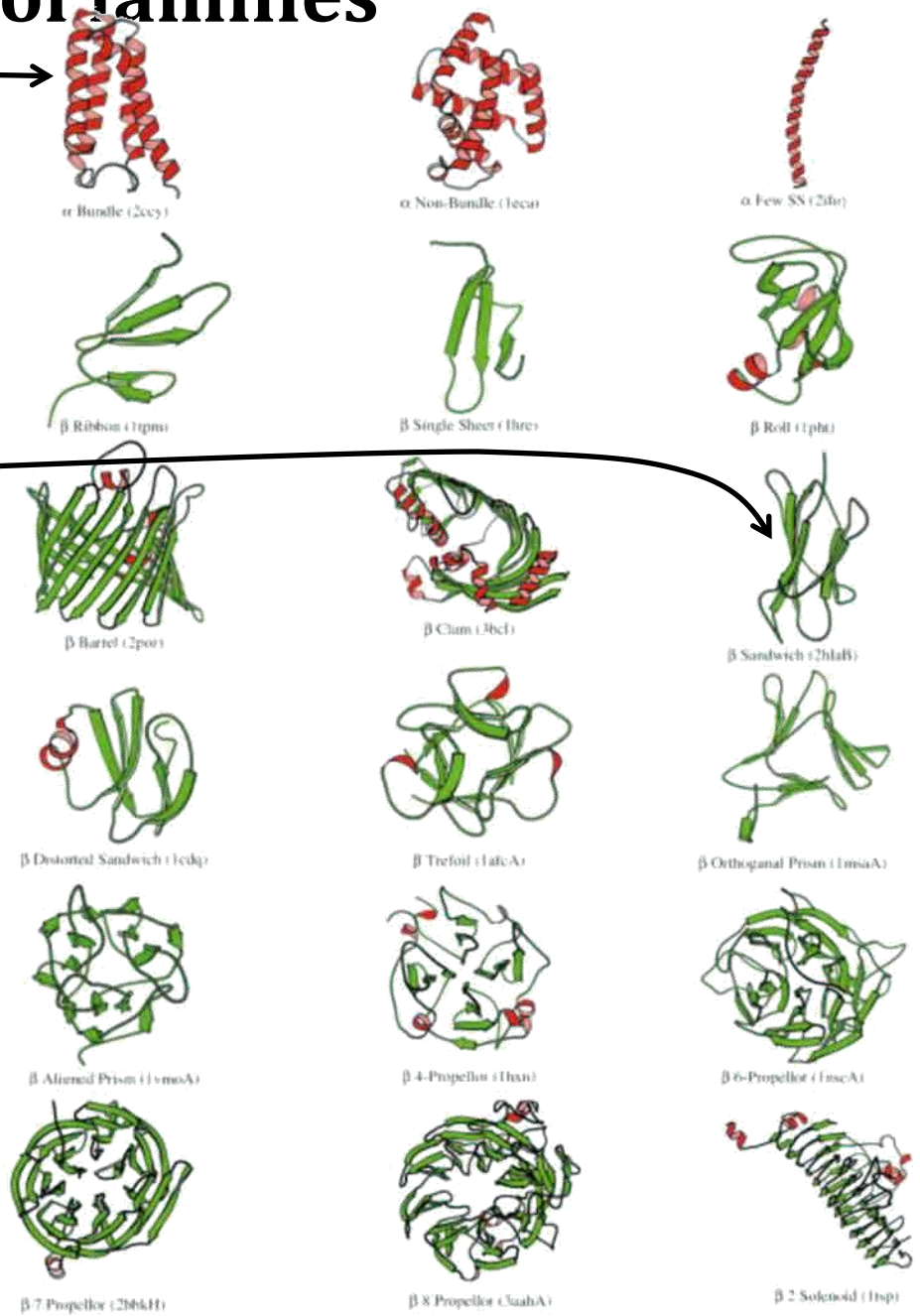
β-sandwich ≈1236 domains, 15 %

some families ?

- < 0.01 %

Interesting…

- some families very popular, some not



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

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 ?

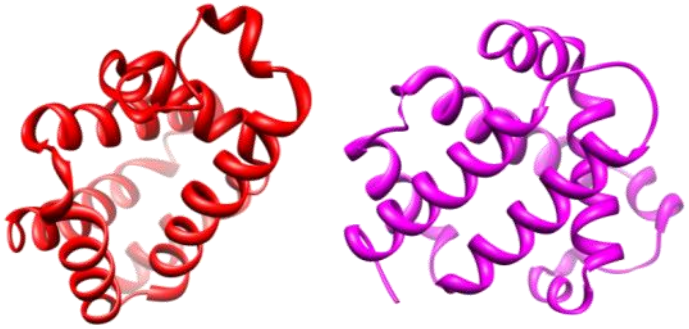
Biases ? PDB has

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

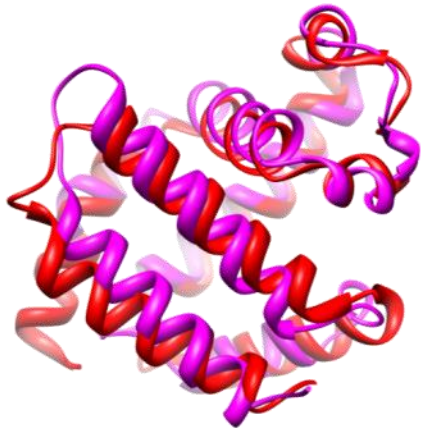
Forget Evolution

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…

Protein Structure Comparison / Numerical

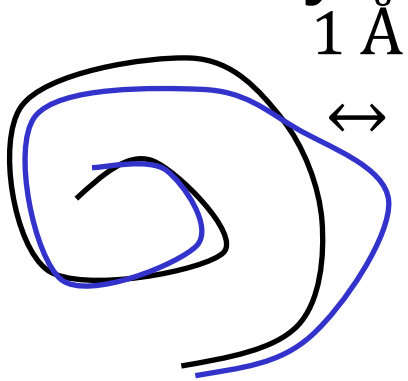
Most common protein structural question

- 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

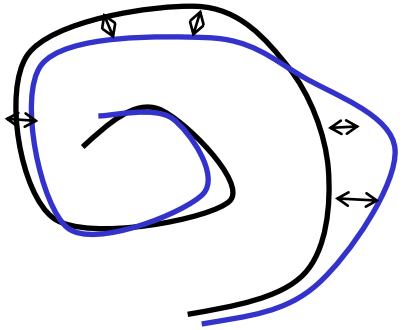
Numerical Comparison of Structures - Easy



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 $\sigma = \left(N^{-1} \sum_{i=1}^{N} (x_i - \bar{x})^2 \right)^{1/2}$

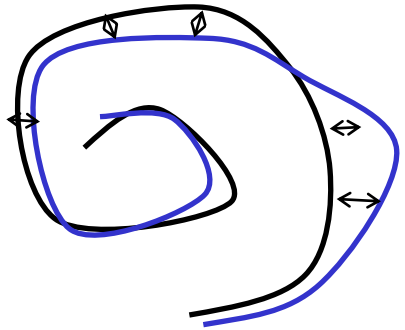
- apply this to coordinates of r and r'

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

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

Calculating rmsd

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

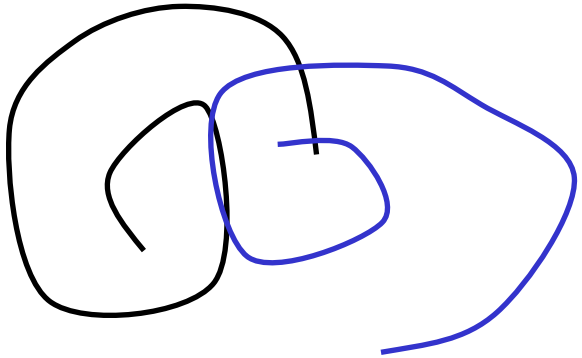


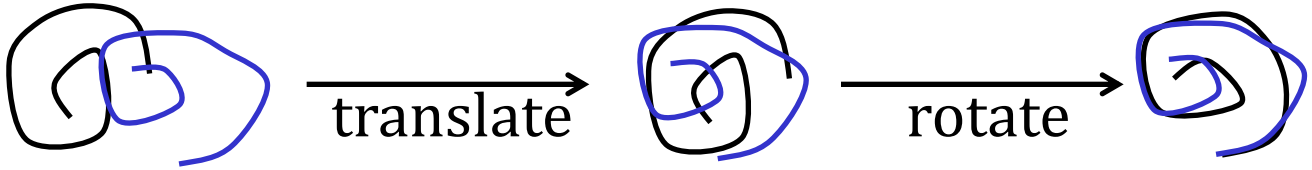
- start at one end
- difference between pairs of atoms

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

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

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

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

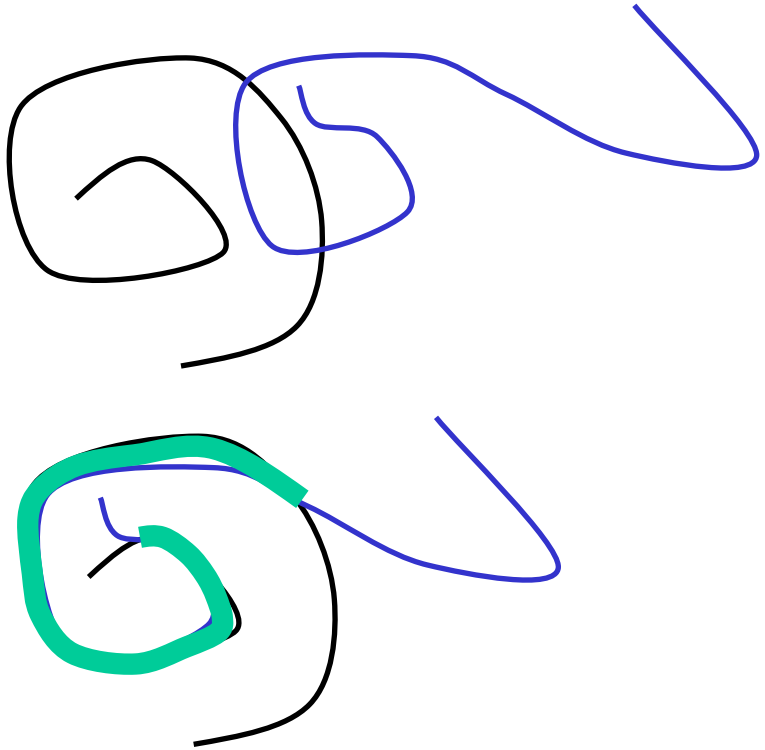
Still not finished with simple *rmsd*

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

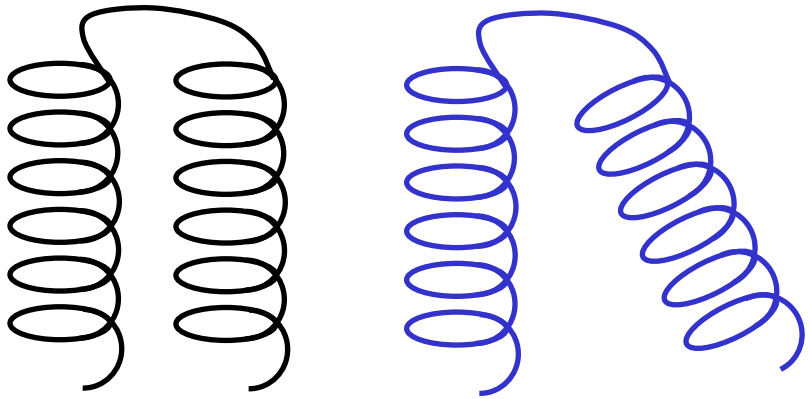
Implicit rule

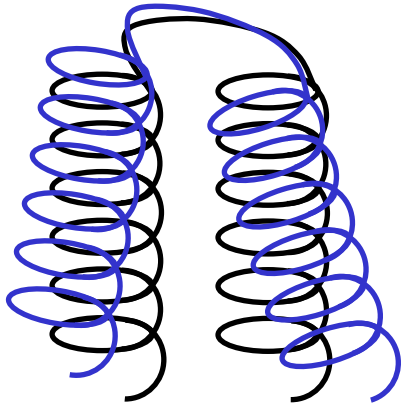
- as number of atoms ↓ calculated *rmsd* ↓

Why Not 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

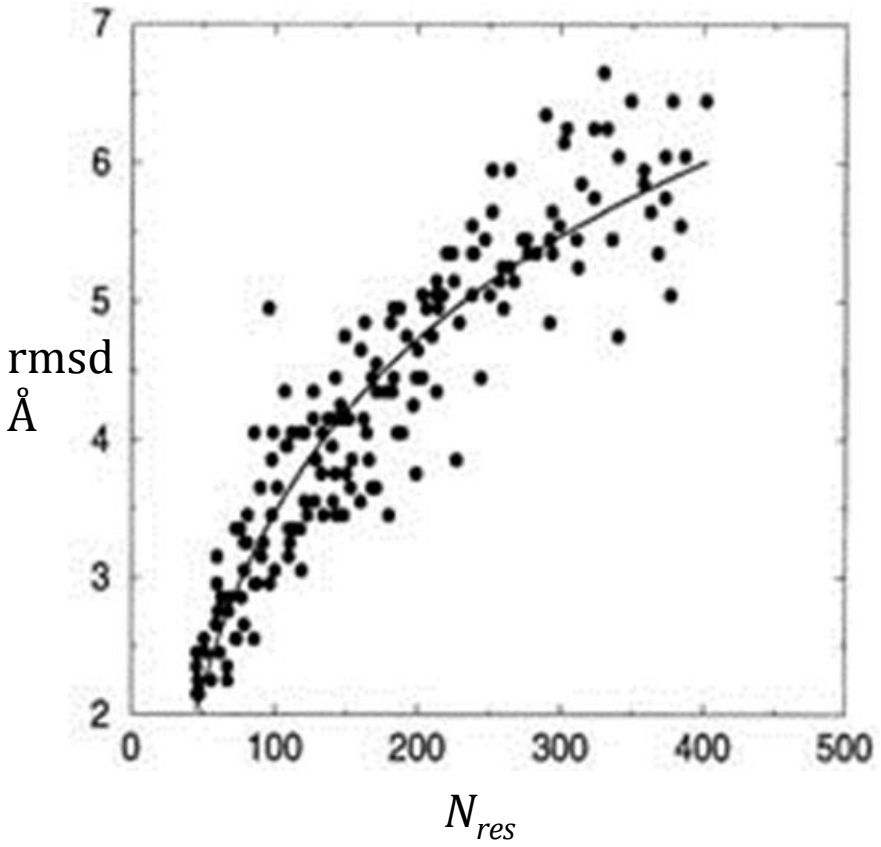
- ligands / medikamente…

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

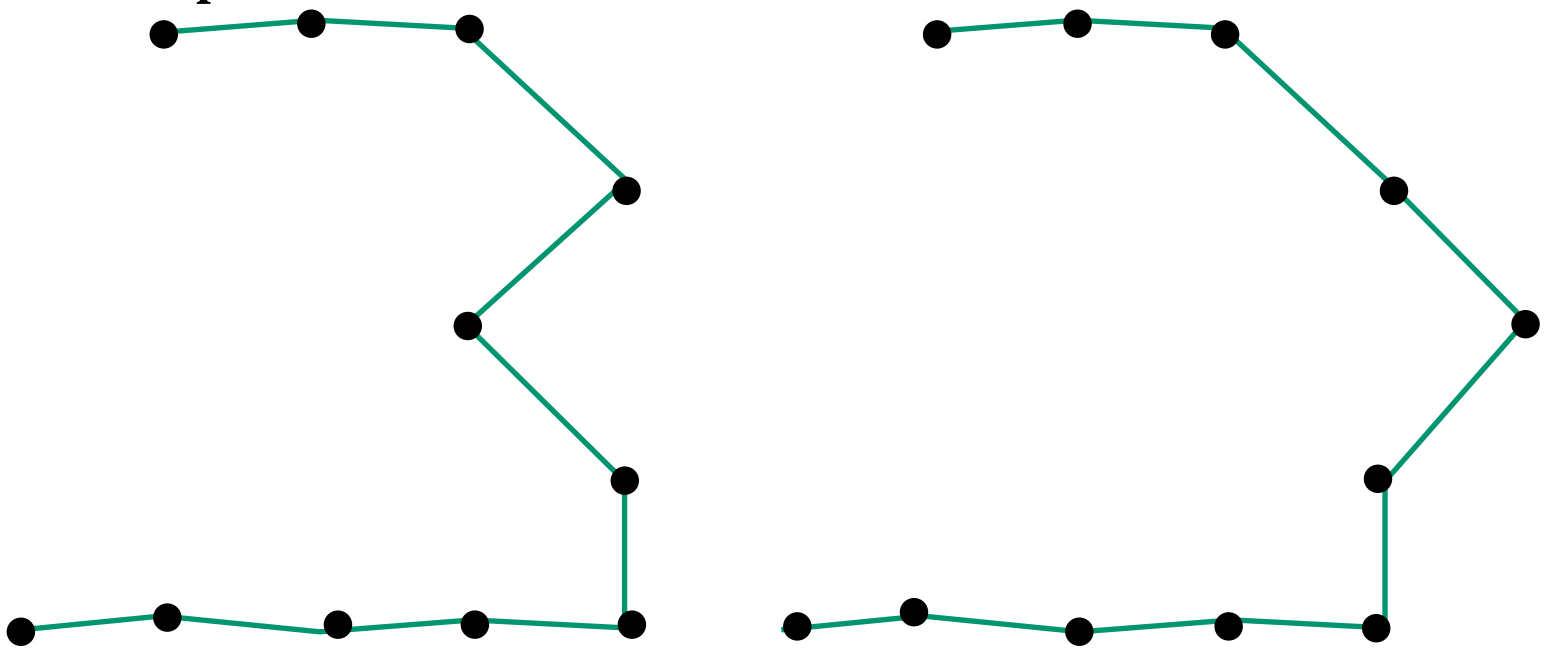
rmsd size dependence

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

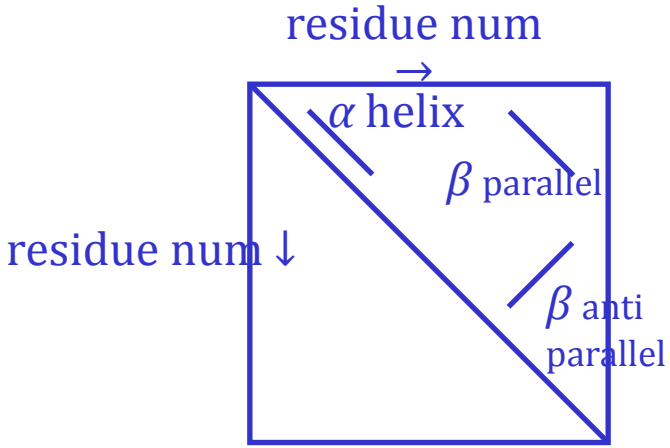
- C^{α} distance matrices
- simply measure the distance between C^{α} atoms

	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

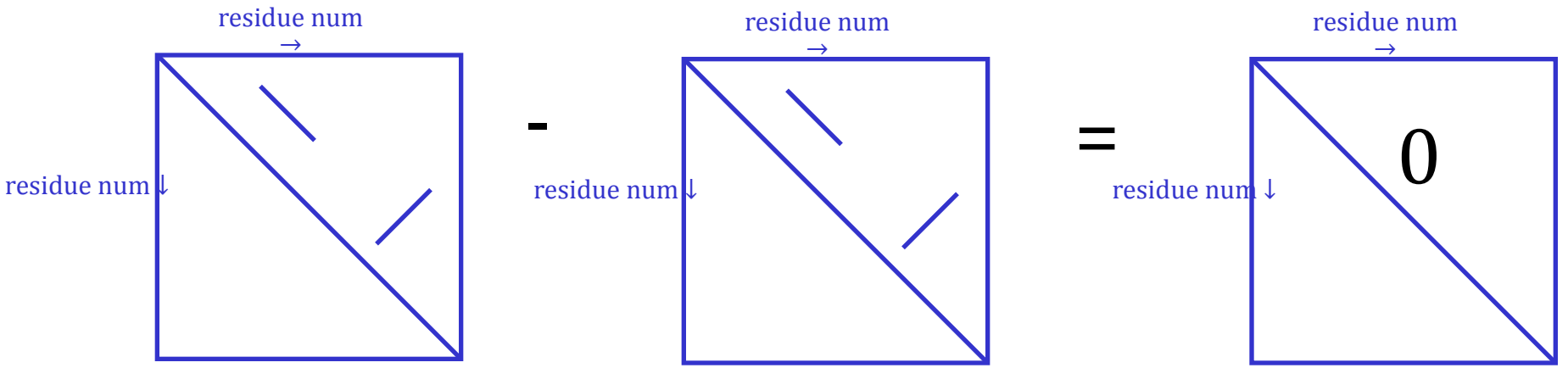
- plot C^{α} distance matrix, points below 4 Å
- can make α-helices and β-sheets clear



Distance matrix for comparing structures

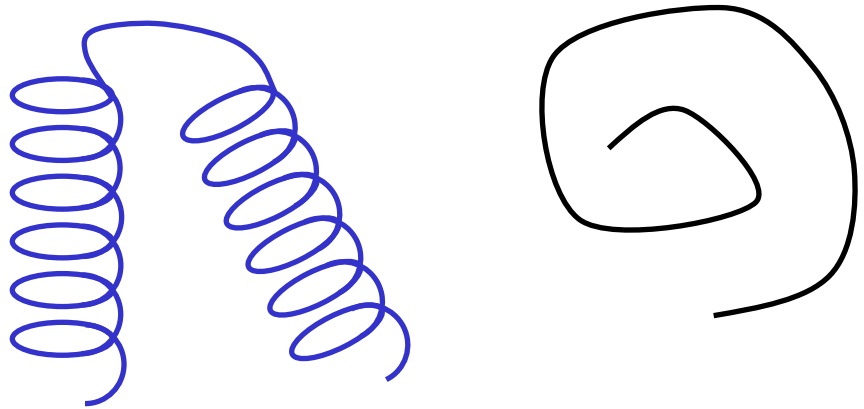
Take two similar proteins

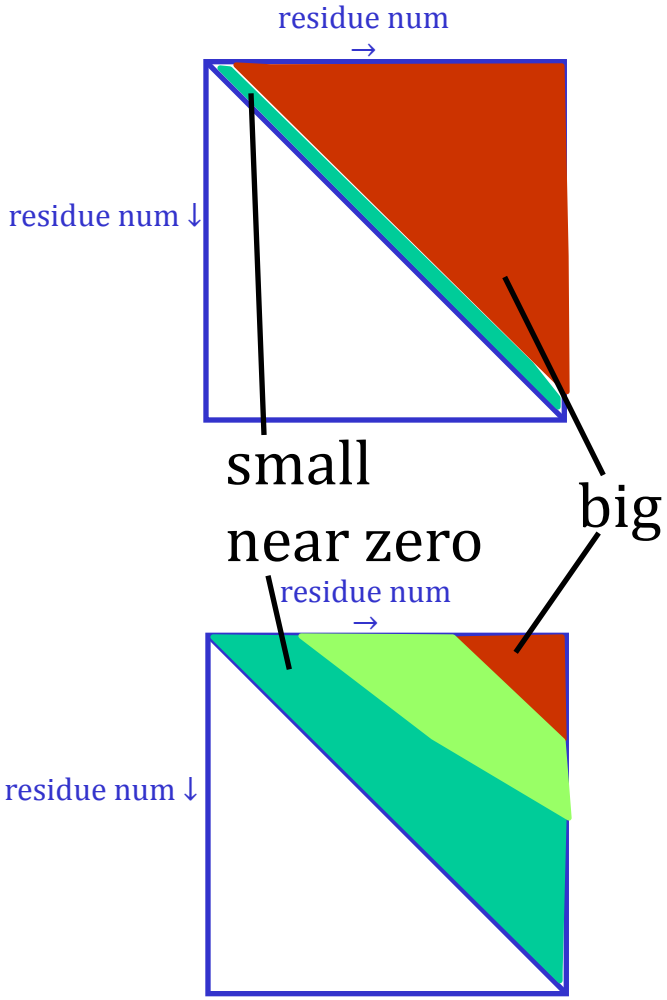
- look at the difference of distance matrices



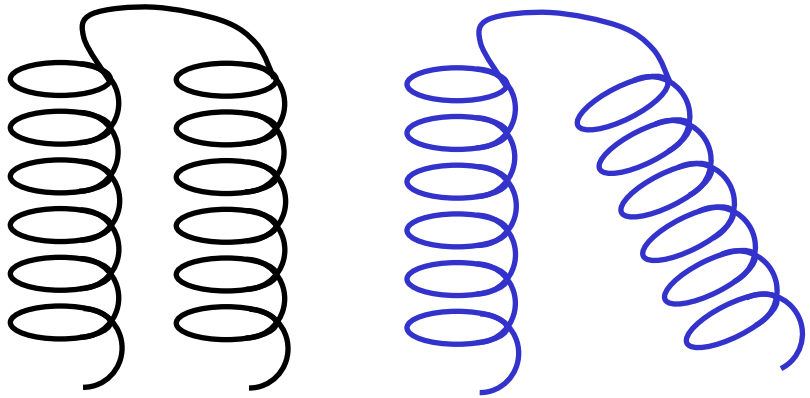
Comparing Distance Matrices

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^{α} atoms *i* and *j* $\quad d_{ij} = \left|\vec{r}_i - \vec{r}_j\right|$

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

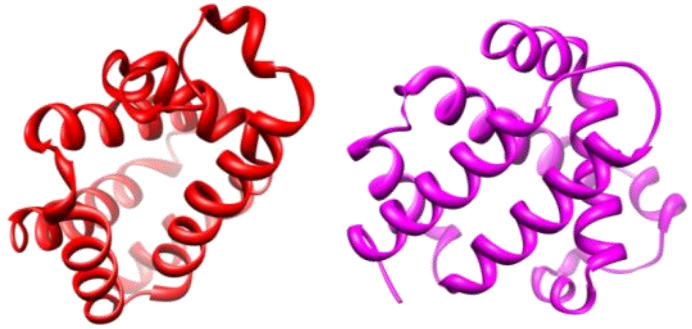
- normalised over top half of matrix

Summary – Comparing Models / Structures

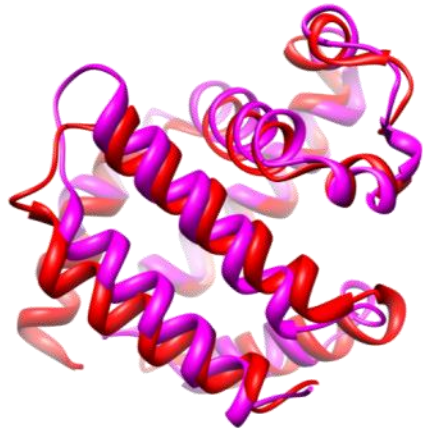
- *rmsd*
  - most popular
  - requires superposition (translate + rotate)
  - can be fooled by "hinge" movements
  - size dependent
- to look at the shape of a molecule use C^{α} or backbone atoms
- numbers in Å have a physical meaning
- to look for the common core of a structure, find a subset of backbone
- 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

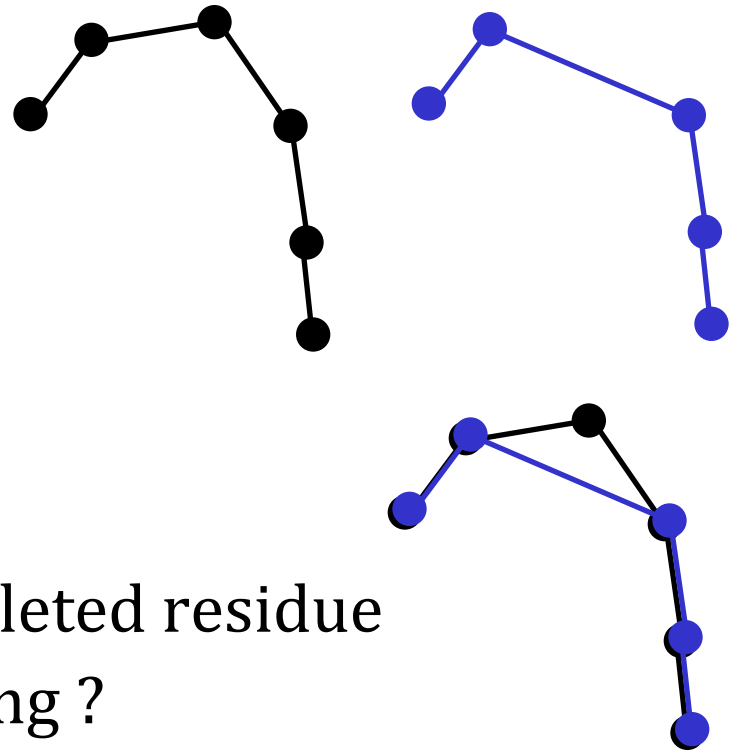
Comparing Very Different Proteins

Sequence alignment vs identity

- as identity ↓, errors ↑

Consequence

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



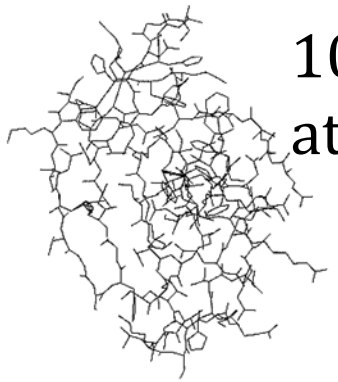
How difficult ?

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

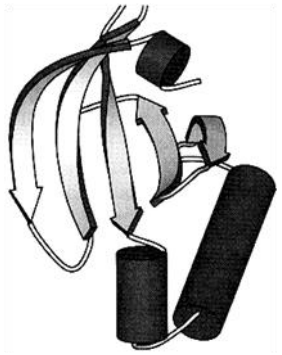
Strategies For Comparing Different Structures
1. use secondary structure

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*

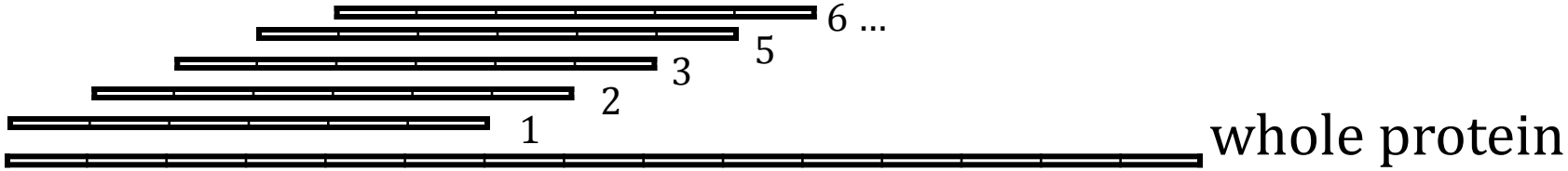
pictures from "Structural Bioinformatics", ed Bourne, PE and Weissig, H.

2. Peptide fragment strategy

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

Ingredients

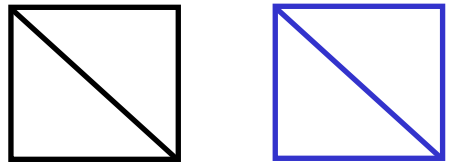
- break protein into overlapping fragments (length 6 or 8)
- protein is no longer a string of residues nor a whole structure



- 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 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

protein 1 fragments →

- 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

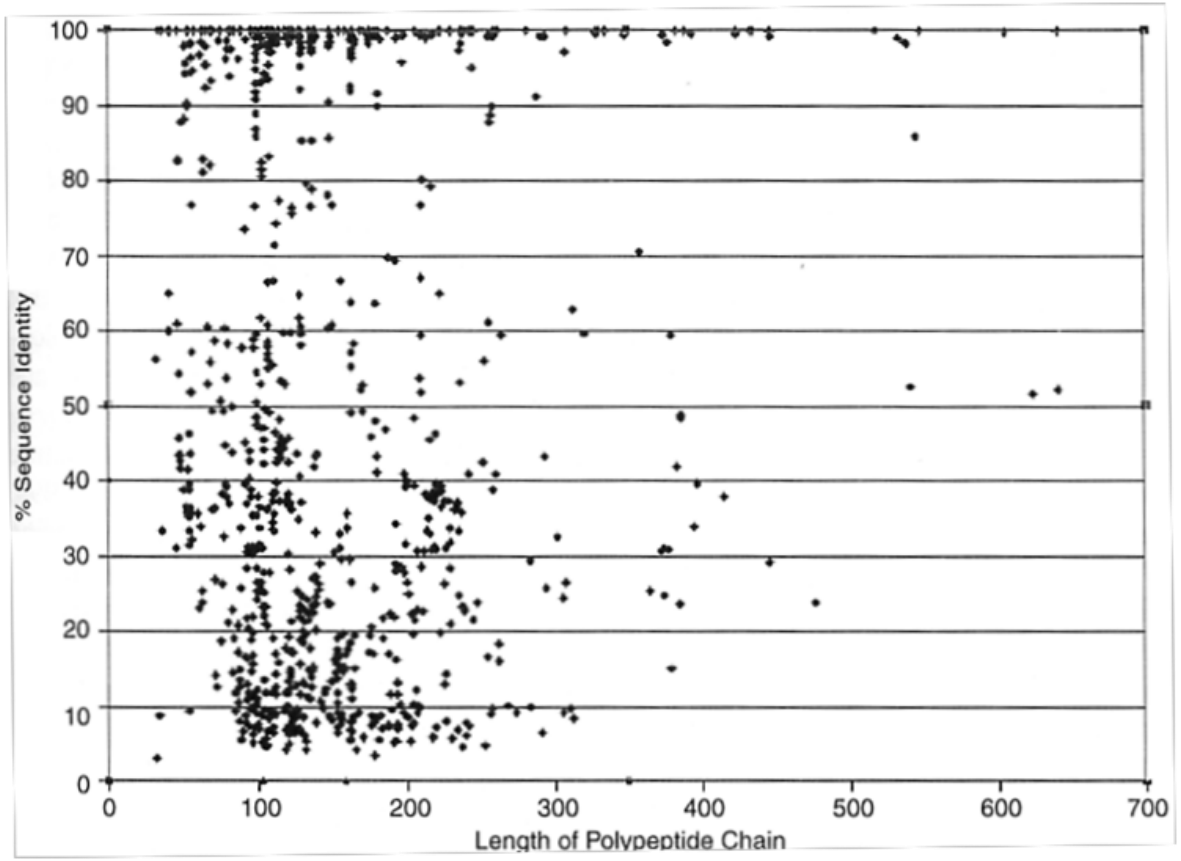
Comparing Different Size Protein Structures

- 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