Classifying and comparing proteins

Andrew Torda, Wintersemester 2010 / 2011, GST…

Plan

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

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

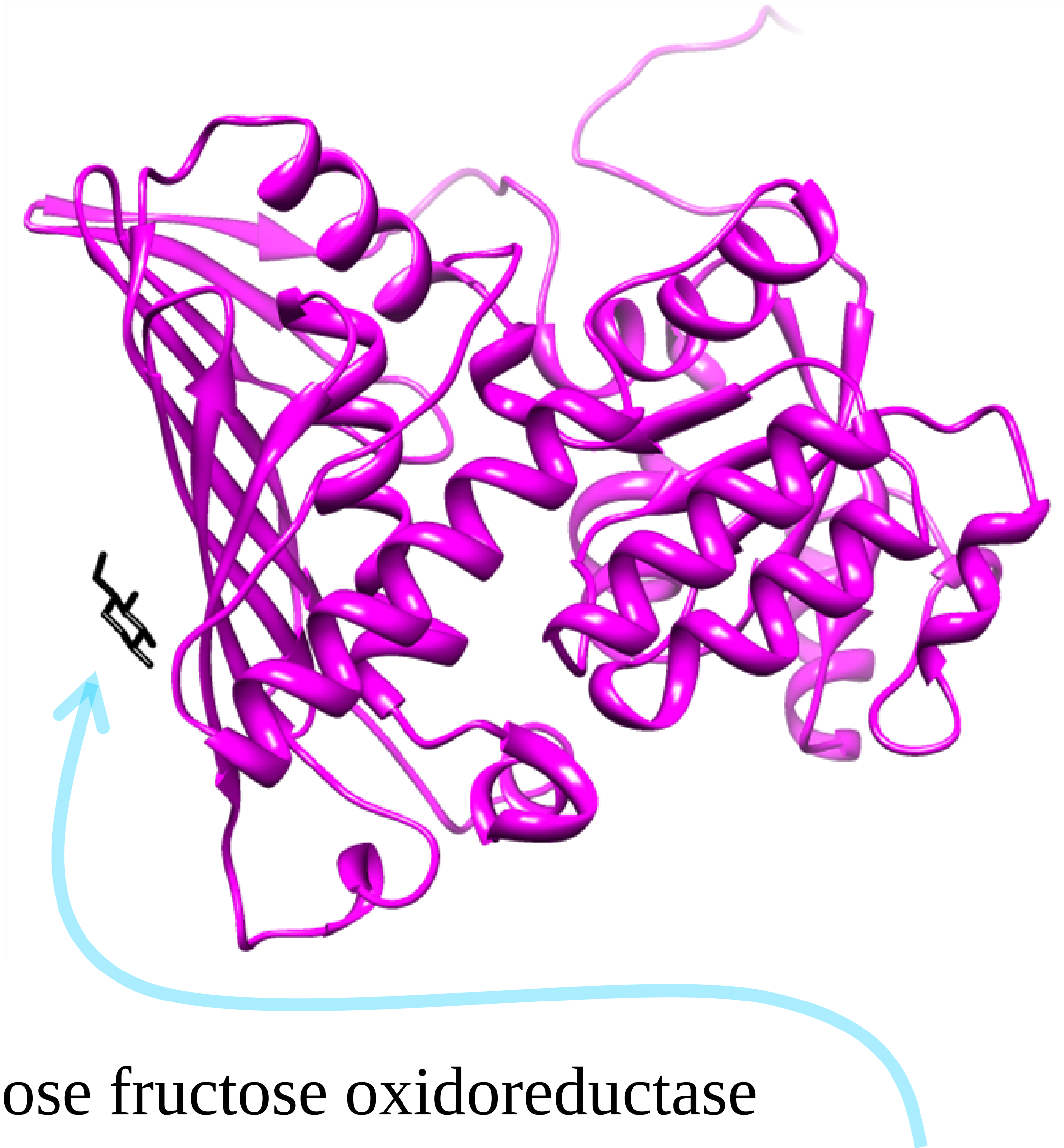
Interpretation of structures

- 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

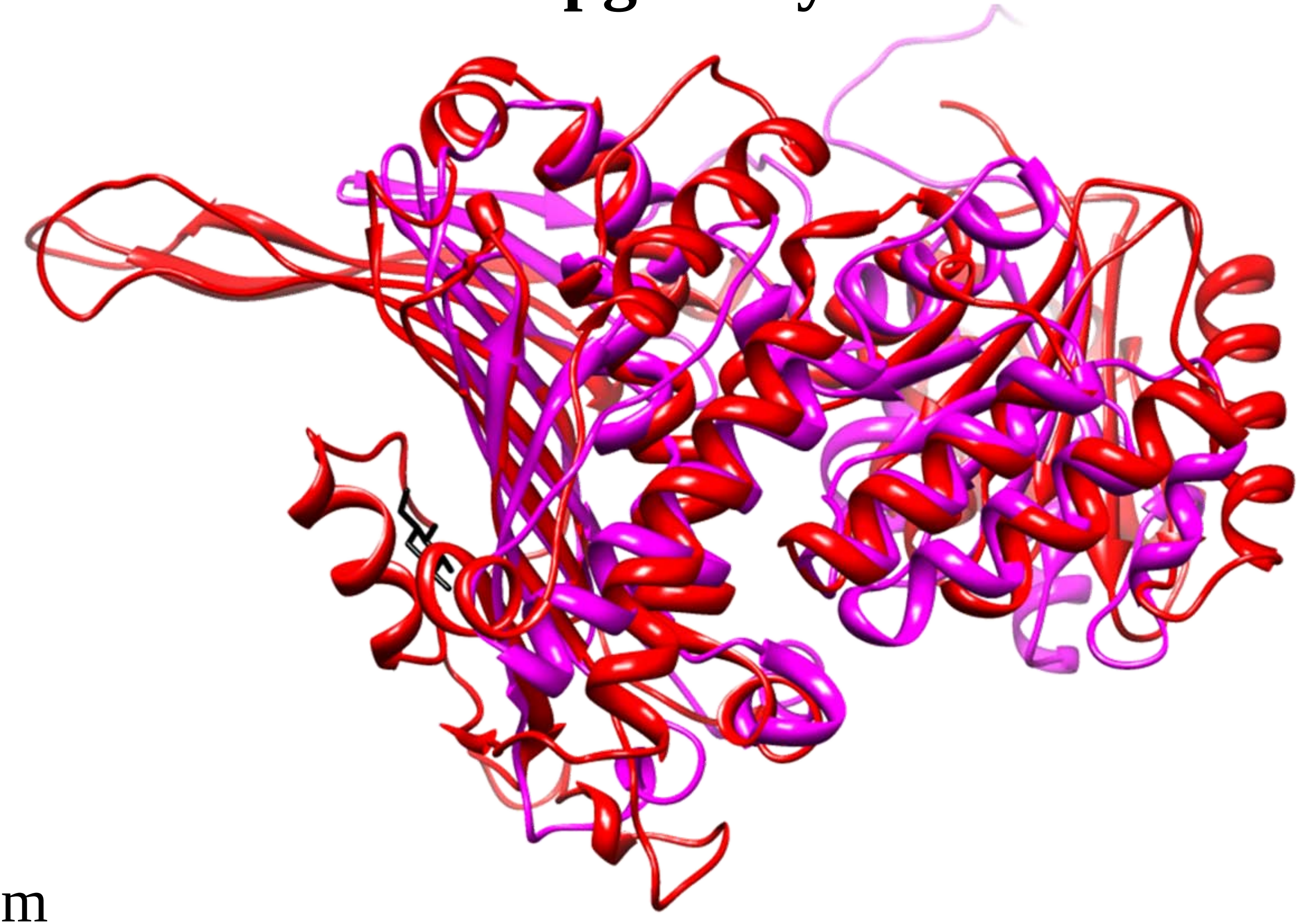
where does sugar bind in 1dpg ?

- 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

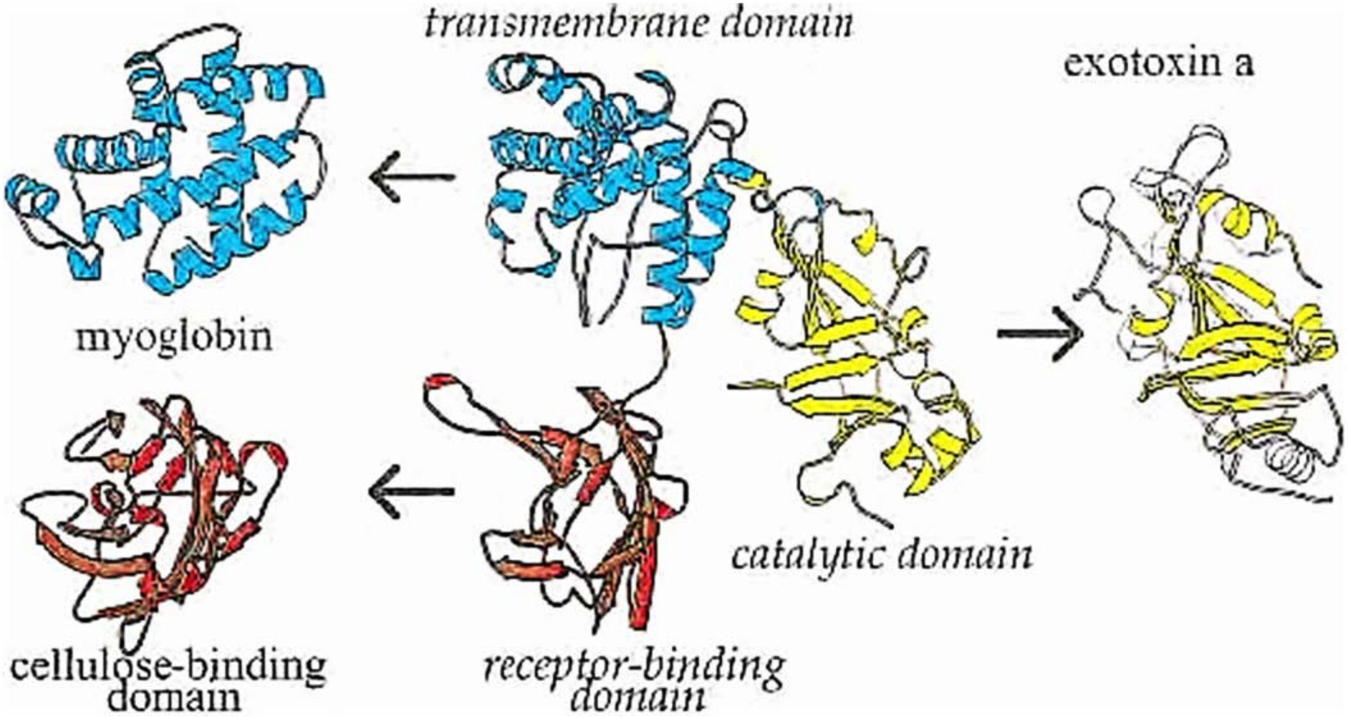
- Protein Databank ≈ 7.0×10^4
- 90 % sequence similarity ≈ 2.5×10^4 classes
- different shapes 2 to 5×10^3

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

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

Domains

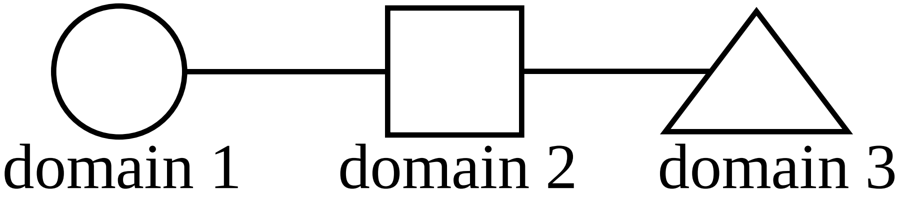
- Why mention ?
  - many groups work on domains, not whole proteins
- Reasons
  - many structures are labelled "a domain of protein X"
  - evolution – convincing picture (diptheria toxin)



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

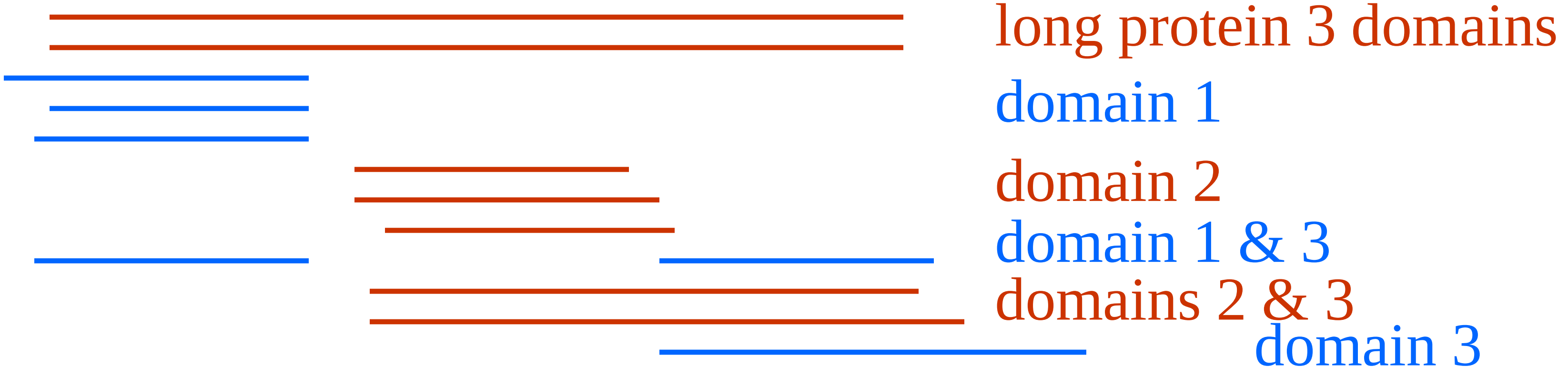
Domains in Biochemistry (view 1)

History / biochemistry / no structures

- invented story
- we have a big protein
  - catalyses A → B
  - C regulates it
- cleave protein (break with enzyme) to two parts
  - 1 still converts A →B
  - 2 binds C
  - interpretation
    - catalytic domain
    - C binding domain
- more generally
  - different pieces of protein, responsible for different functions

Sequence level domains (view 2)

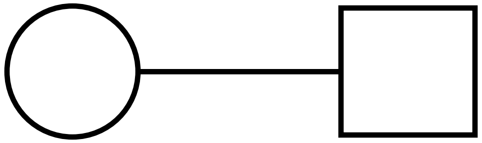
- Align a group of sequences



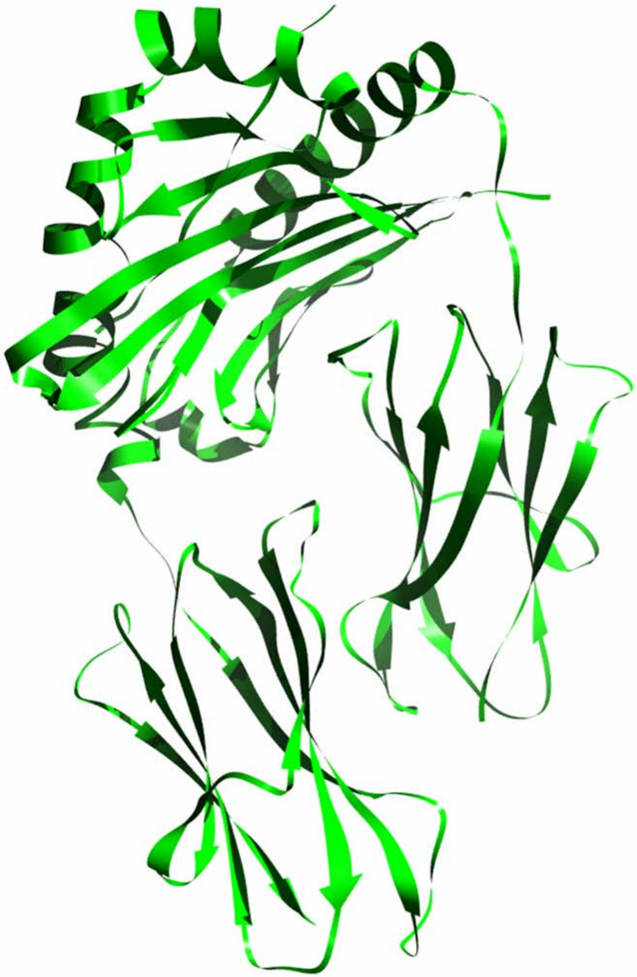
- appears to have 3 domains
- no reference to structures or function

Domains in Structures (view 3)

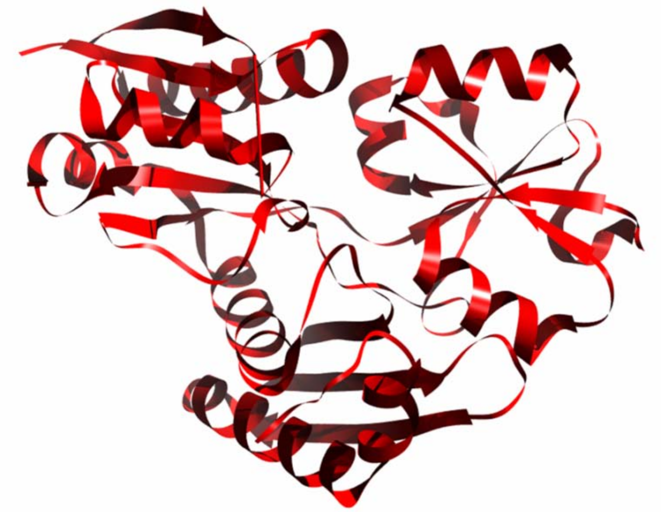
- Many structures solved look like…



- histocompatibility module (1iak)
  - 3 domains



- are they always so clear ?
- porphobilinogen deaminase (1gtk)



Domains for these lectures

- usually structure based
  - compact units
  - stable in solution (usually)

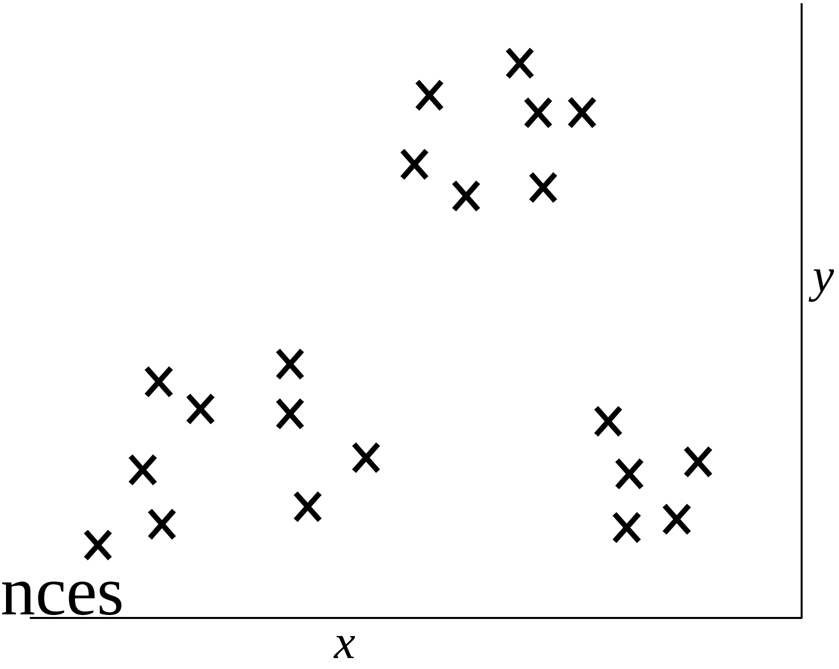
- generally ignore questions of domains being swapped

- can we really expect to classify proteins ?

Protein classes / families

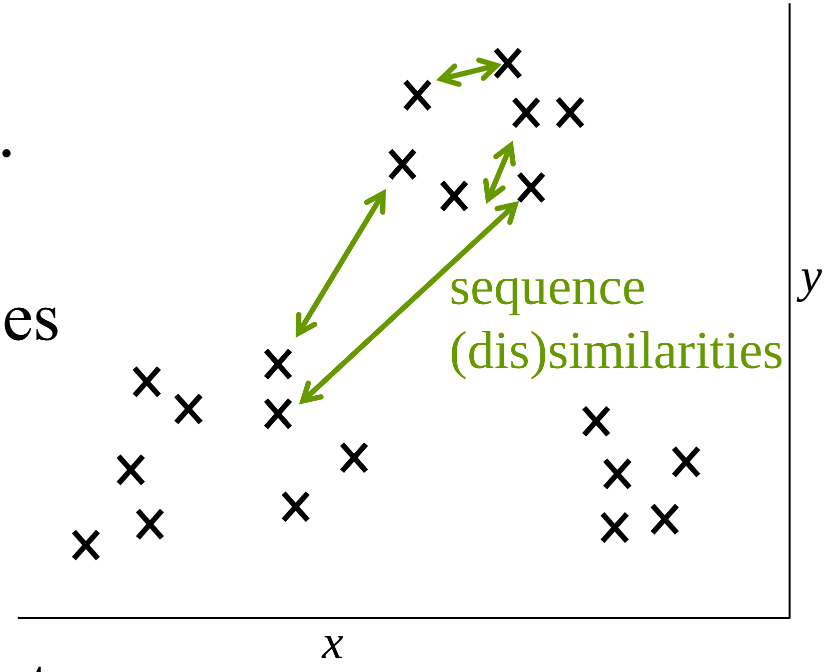
- questions
  - what do they mean ?
  - do you expect them ?

- 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
  - 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 × 4 × 5
- protein sequences
  - each position can have 1 of 20 values
  - total number of sequences = 20 × 20 ×…= 20^{Nres}
  - like a space of N_{res} dimensions

Representing a Sequence

- protein sequence and structural coordinates

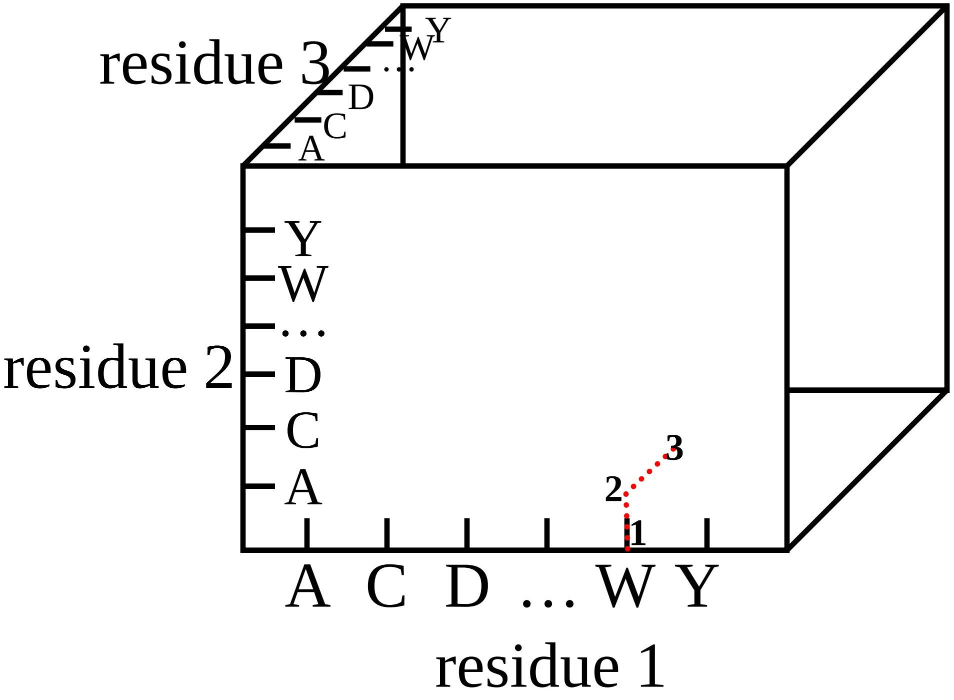
	1	2	3	4	5	6	7	…	N_{res}
x	1.2	2.3	…						10.3
y	2.4	3.5	…						11.1
z	1.7	2.9	…						15.5
seq	W	A	C	A	A	…			D

- 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**

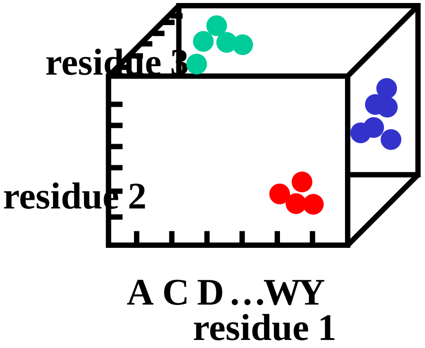


- looking for sequences…

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

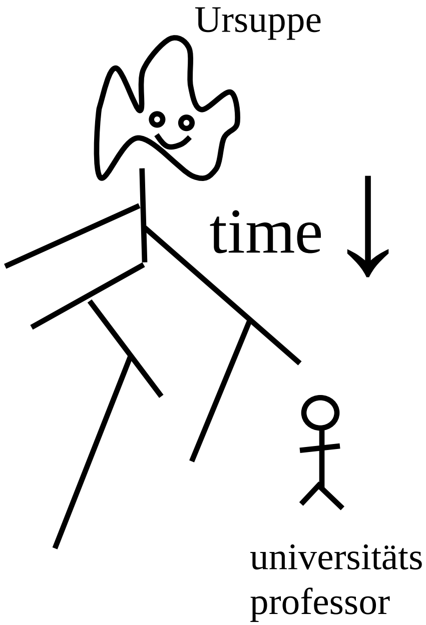


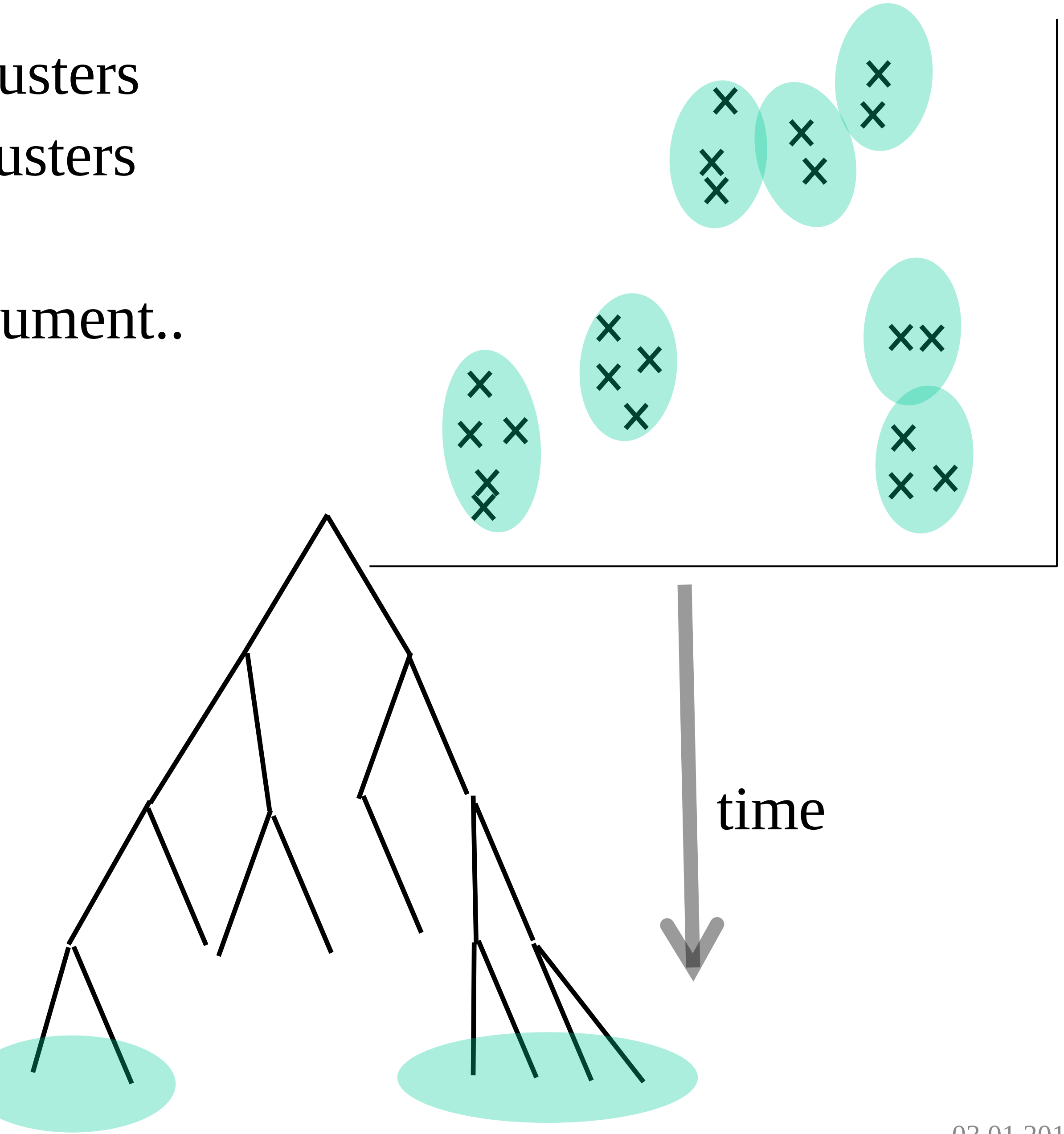
- Will return next semester

Should we expect a hierarchy ?

- 7 lowest level clusters
- 3 higher level clusters

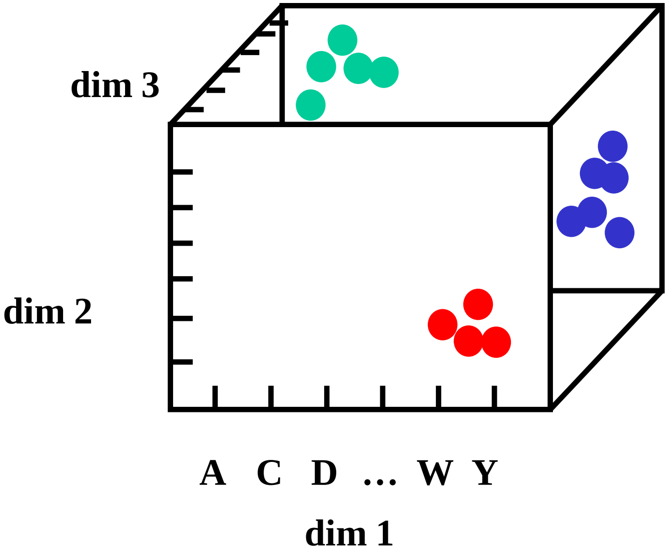
- evolutionary argument..

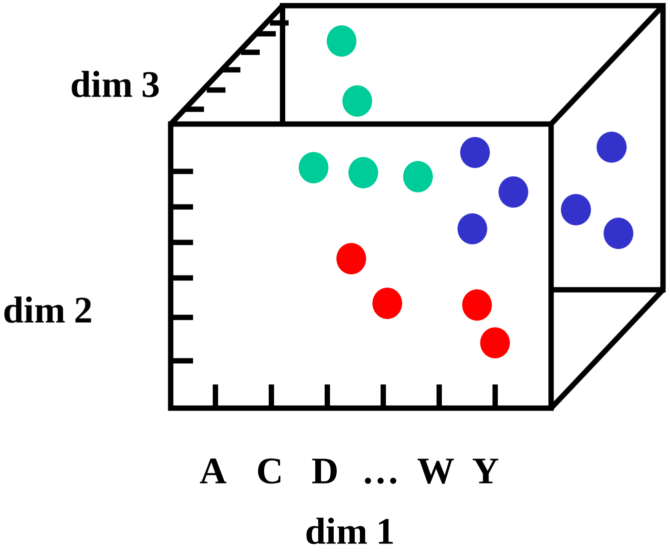




Do we expect protein families ?

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

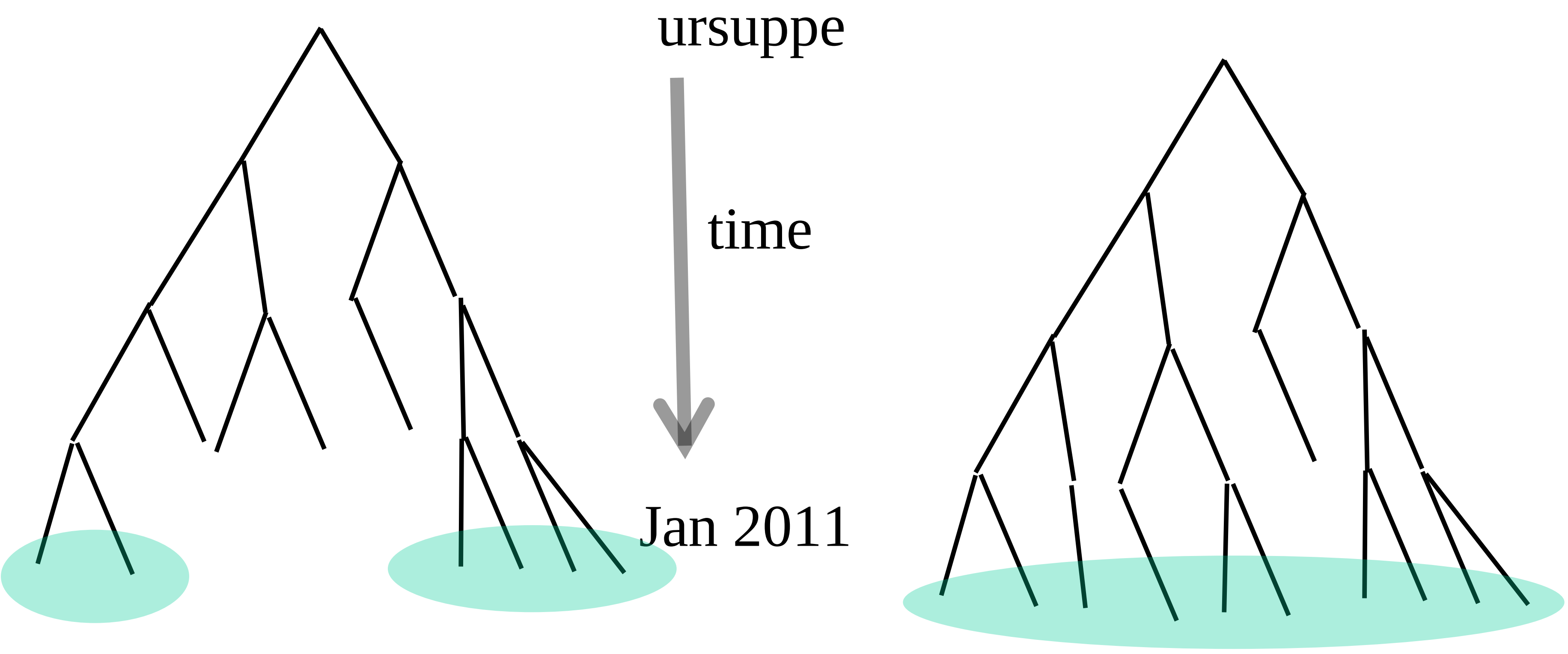




- should you expect clusters ?

Evolution and phylogeny

- shape / density of tree of life



ursuppe

time

Jan 2011

clear families

no families

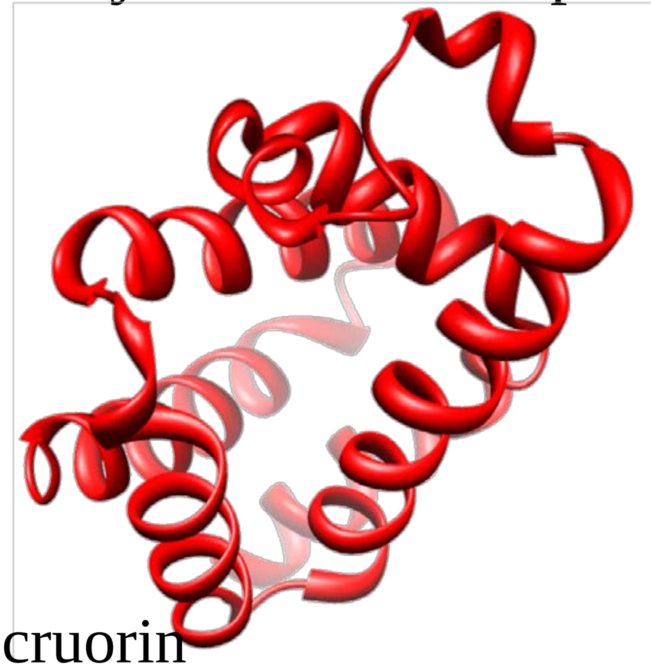
Questions for fun

- Do we expect hierarchy ?
    - some people do
- Do we expect clusters
    - some people ..

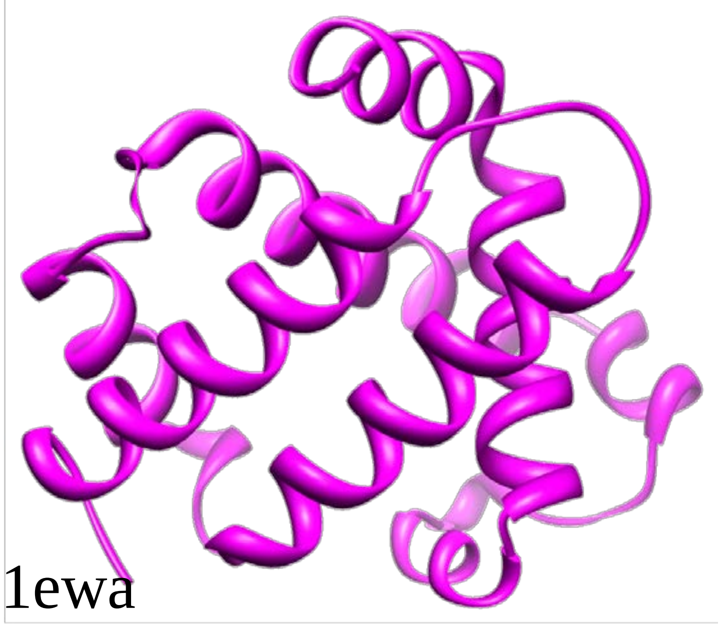
Structure vs Sequence similarities

- more different than you might expect

- similar sequences
  - have not evolved for too long
  - expect similar structures
- other way round ? Examples



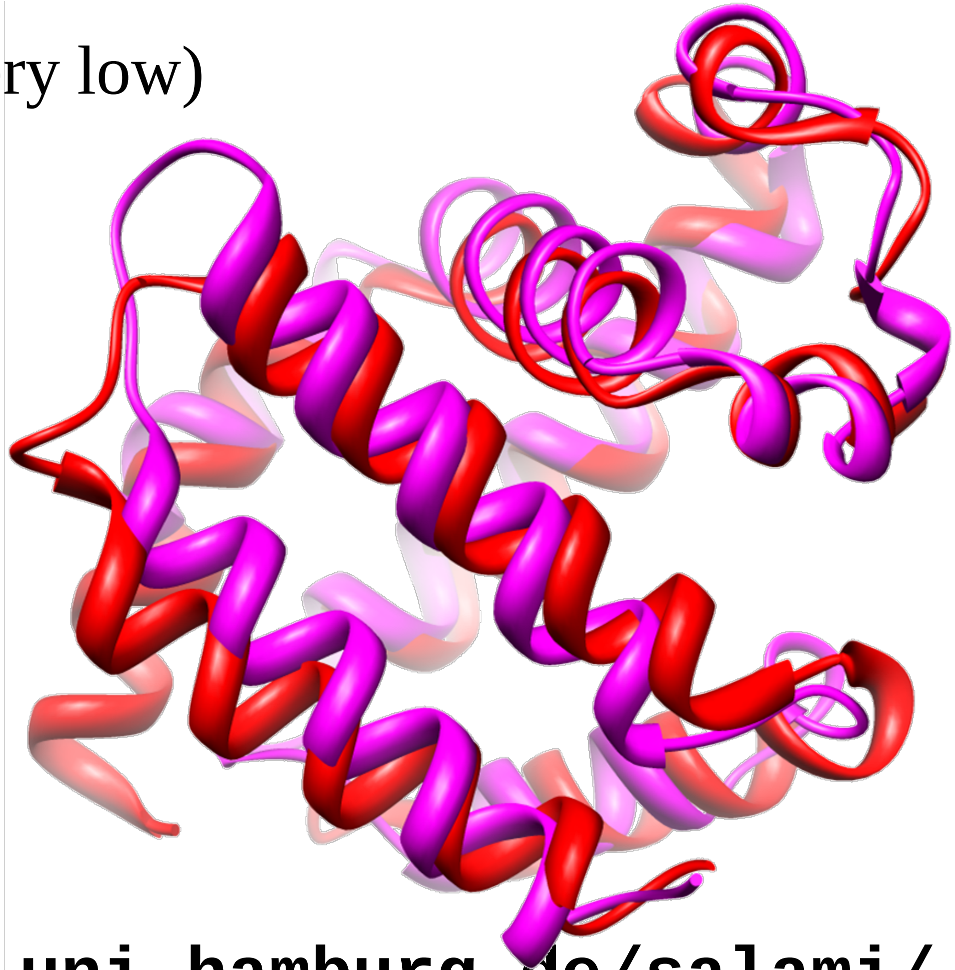
1ecd
erythrocruorin



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/`

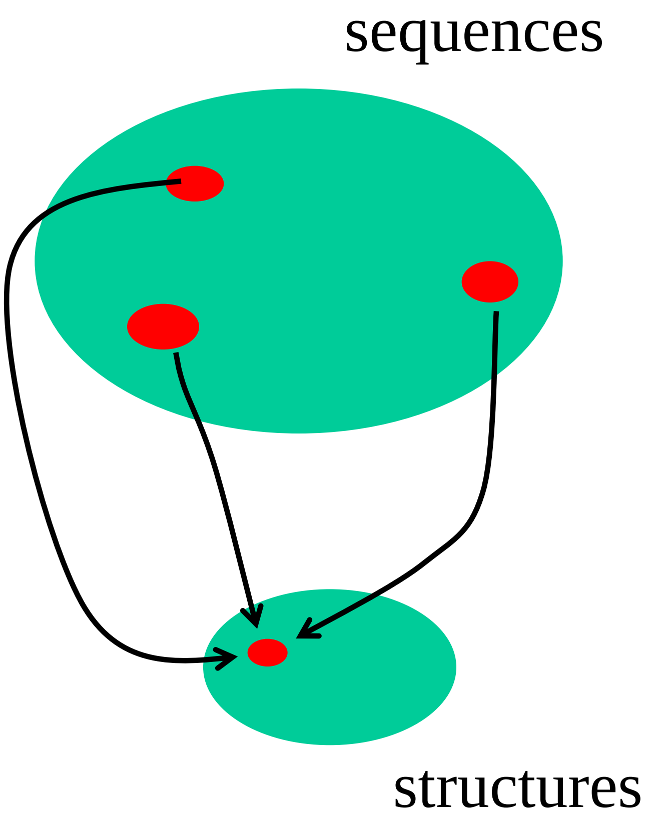
Example family

- 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 2011)

similarity	num clusters
90 %	25 304
70%	22 028
50%	18 711

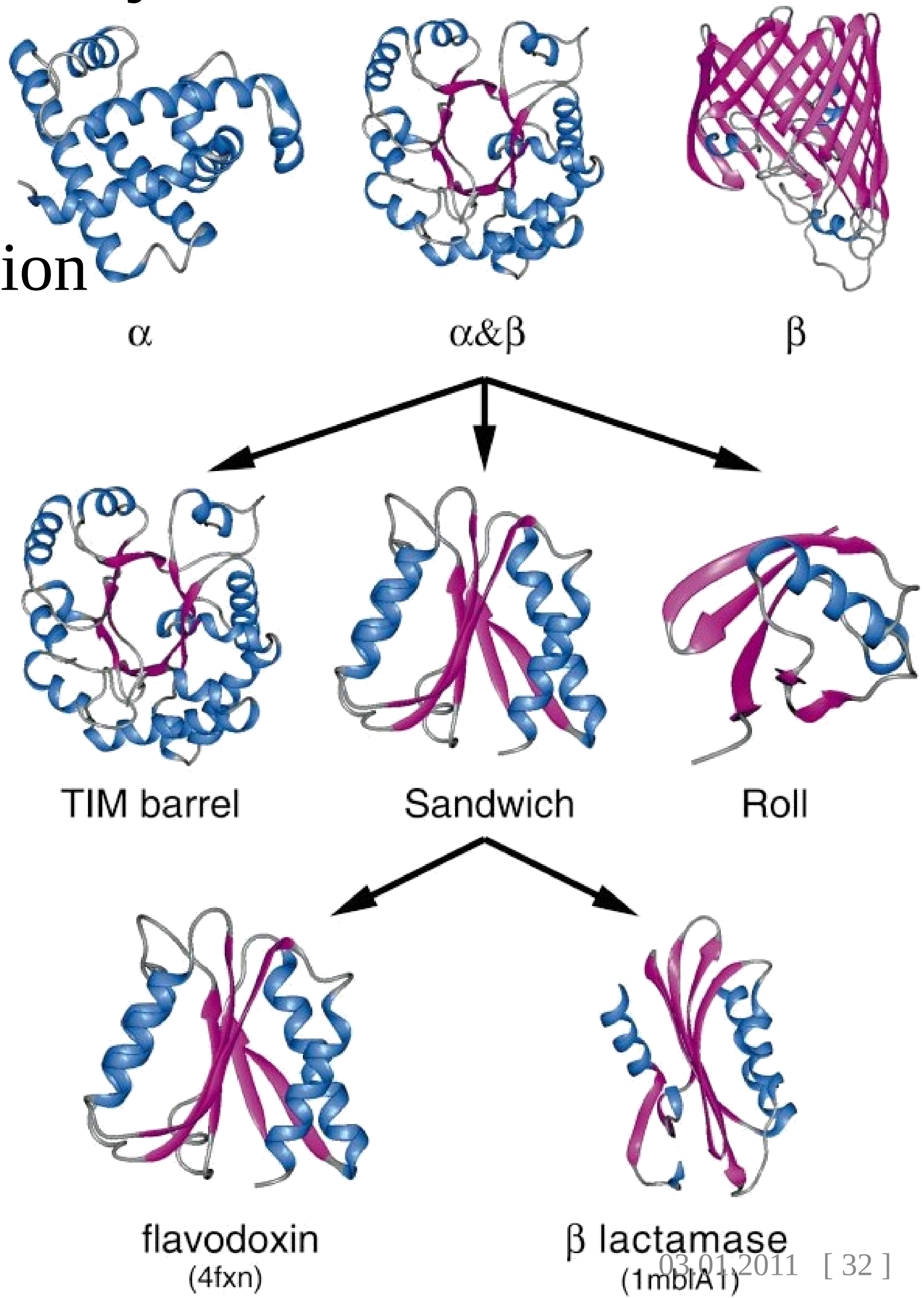
- how many structure classes ? 2 to 5 × 10^3 ?
- some sequence classes are not really different from each other

- now.. examples of structure based classifications

Imposing a Hierarchy on Proteins

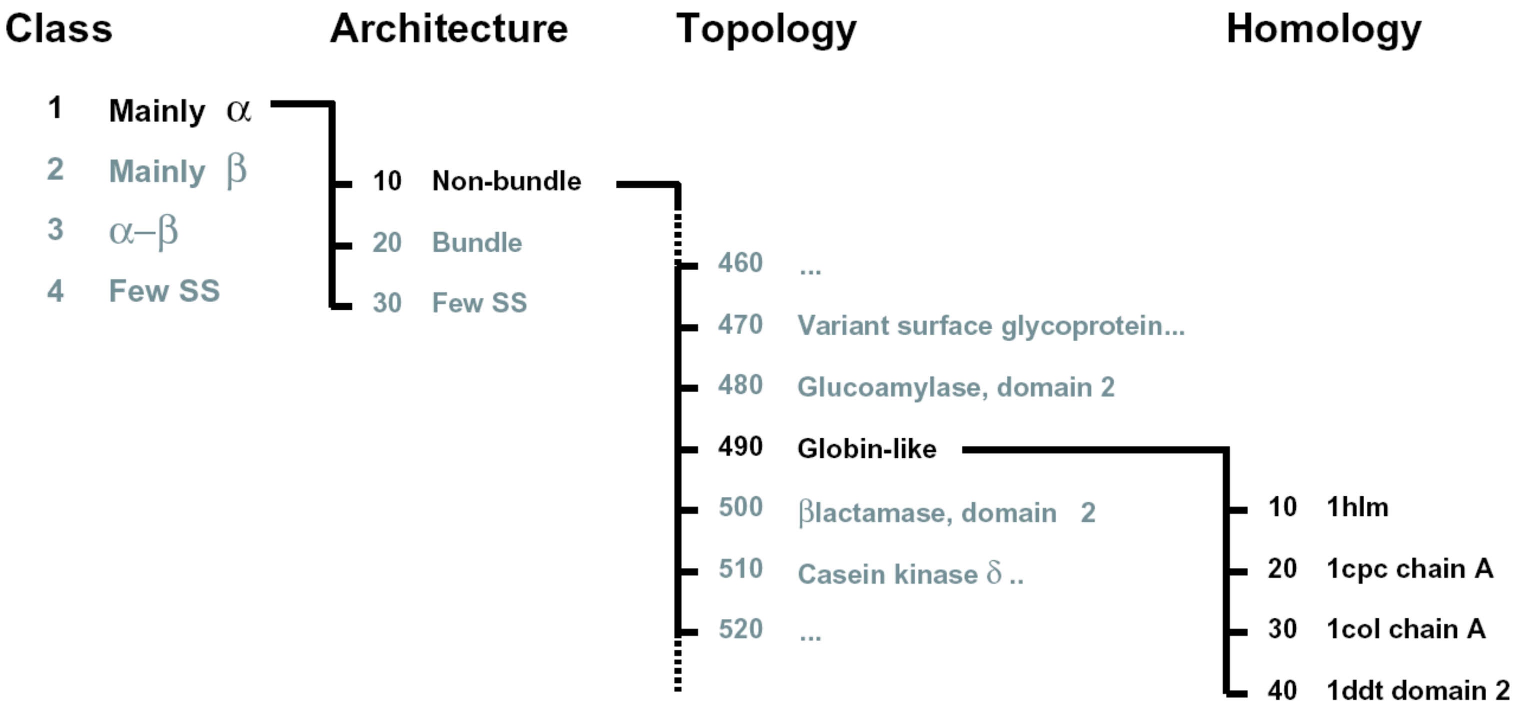
- parts may correspond to evolution
- top level ?

- 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"



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 ...

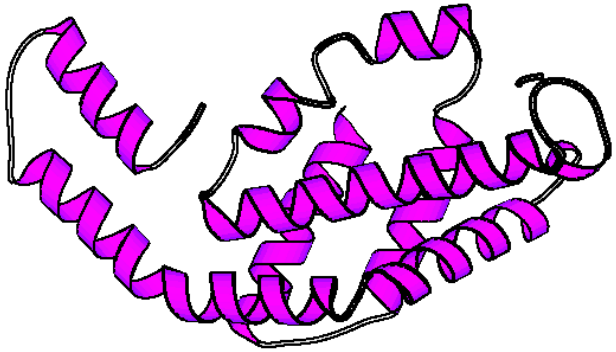
Homology

10 1hlm

20 1cpc chain A

30 1col chain A

40 1ddt domain 2



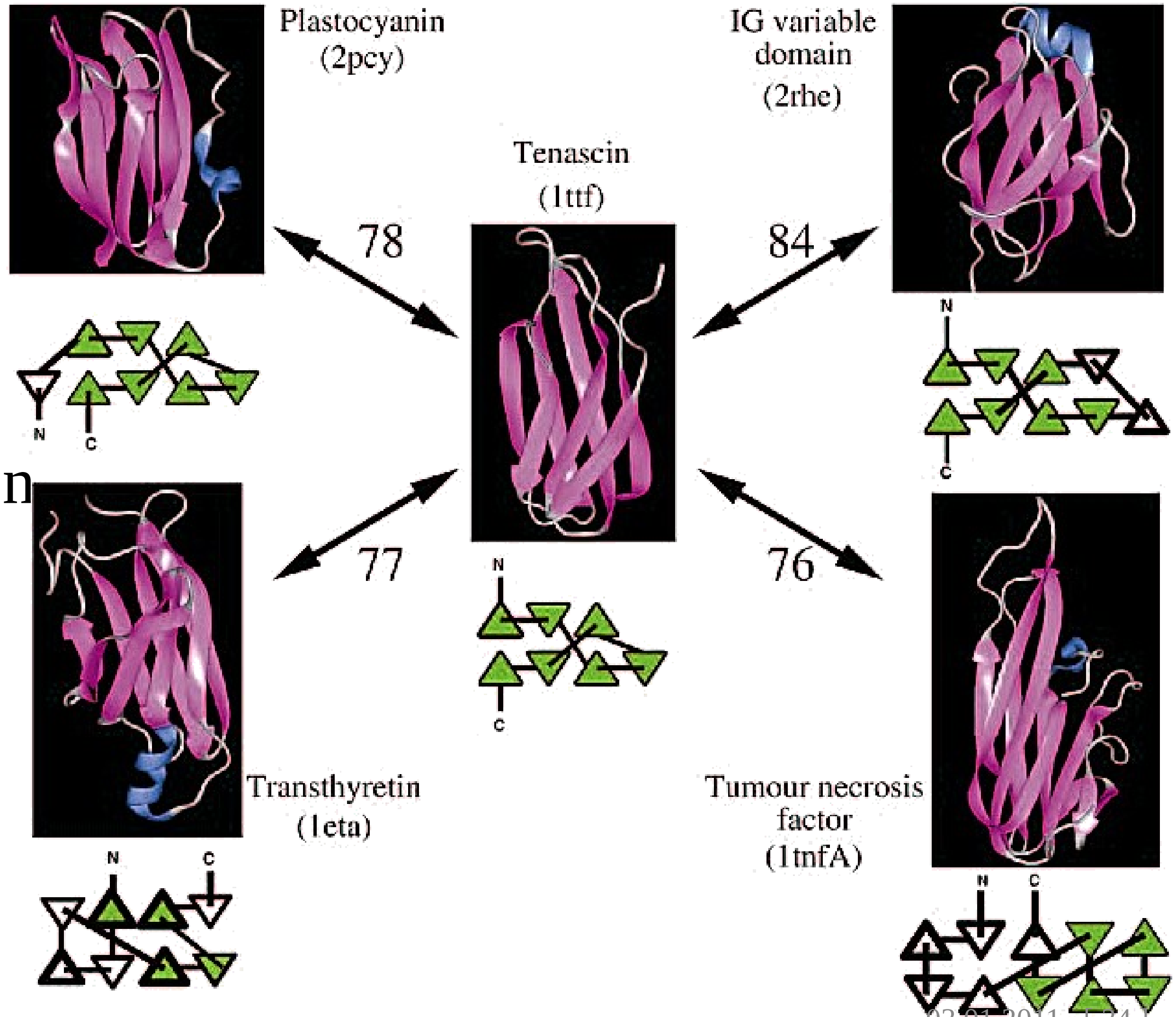
1.10.490.20

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
- given a classification some empiricism



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Lots of families

α-helix bundles ?

- ≈226 domains,
- 3 % surveyed structures

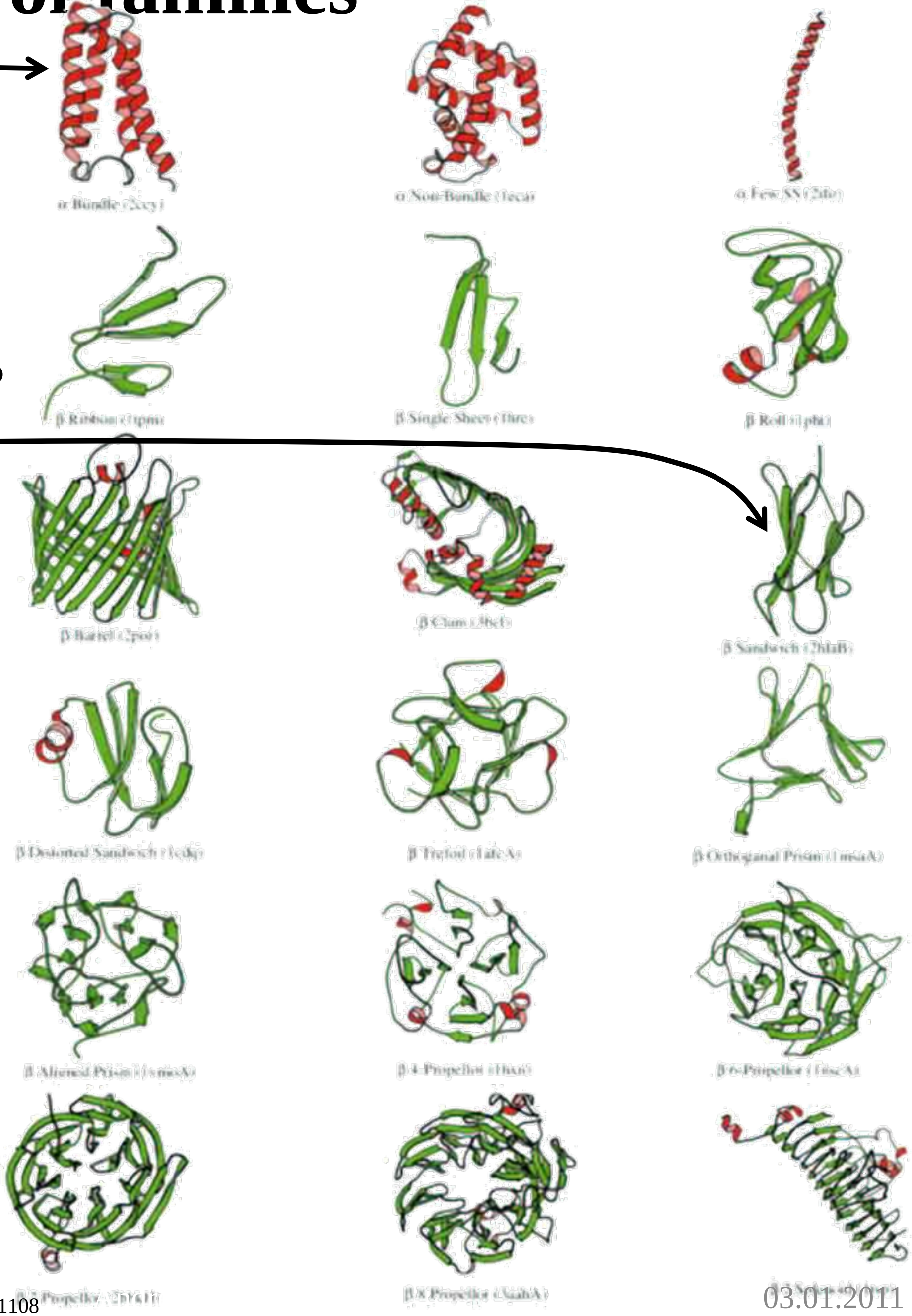
β-sandwich ≈1236 domains, 15 %

some families ?

- < 0.01 %

Interesting…

- some families very popular, some not



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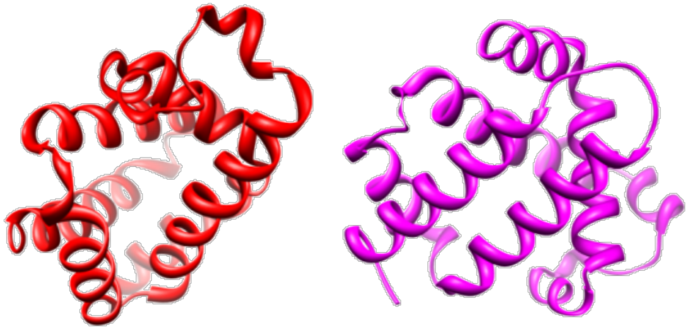
Why are some families populated more than others ?

- more next semester
- are some structures more stable ?
- are some older in evolutionary terms ?
- can some "accommodate" more sequences / tolerate more mutations
- reflection of physics ?

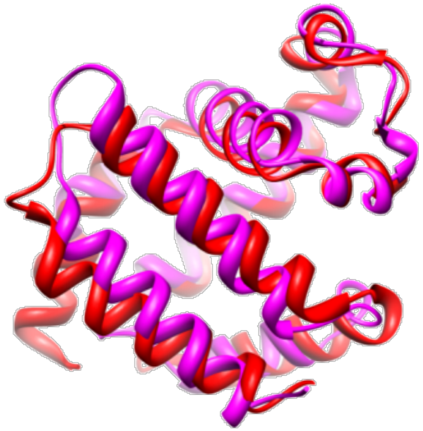
- biases ? PDB has
  - mainly soluble, globular proteins which crystallised
  - very few membrane-bound proteins

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

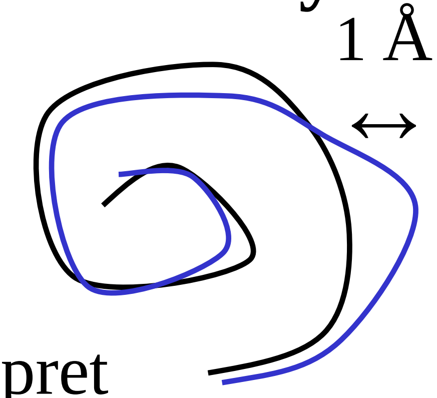
- how much has my protein moved over a simulation ?
- how similar are these NMR models for a structure ?
- how close is my model to the correct answer ?

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

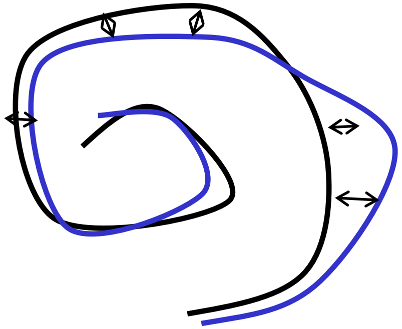
- two cases
  1. same protein, same number of atoms
  2. different proteins
- first
  - measures for easy cases

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) $$\bar{x} = N^{-1}\sum_{i=1}^{N} x_i$$

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

- variance σ^2 and standard deviation, σ
- apply this to coordinates of r and r'

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

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

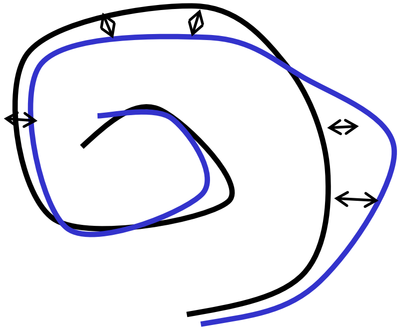
Vital

- formula above, names below
- rms = rmsd = RMSD = root mean square difference

Applying this…

Calculating rmsd

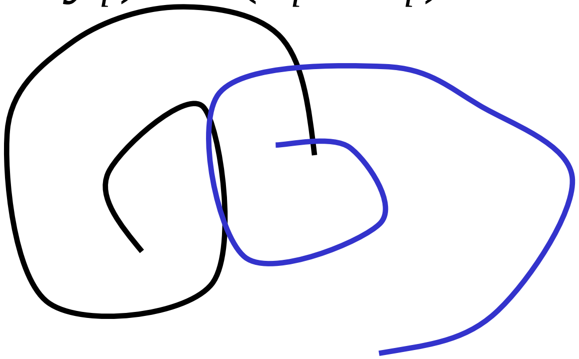
$$RMSD = \left(N^{-1} \sum_{i=1}^{N} \left| \vec{r}_i - \vec{r}_i' \right|^2 \right)^{1/2}$$

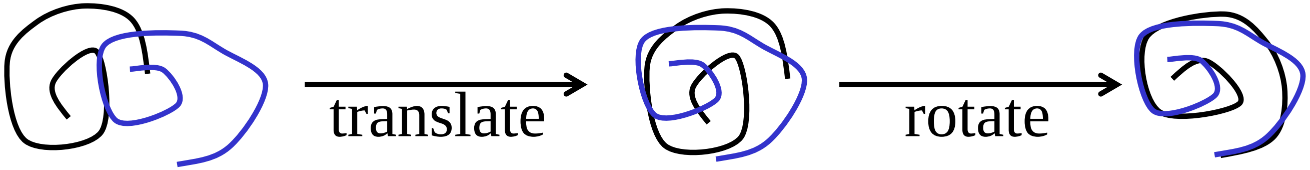


- start at one end
- difference between pairs of atoms

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

- huge problem..
  - coordinates are normally…
- what to do ?





translate

rotate

Translation and Rotation

translation

- c.o.m. = centre of mass
- subtract difference vector

$$\vec{r}^{\,c.o.m} = \left(\sum_{i=1}^{N} m_i \right)^{-1} \sum_{i=1}^{N} \vec{r}_i m_i$$

$$\vec{r}_{diff} = \vec{r}^{\,c.o.m.} - \vec{r}'^{\,c.o.m.}$$

- rotation
  - messier..
  - find rotation matrix to minimise

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

- summary
  - translate
  - rotate
  - apply formula
- still not finished

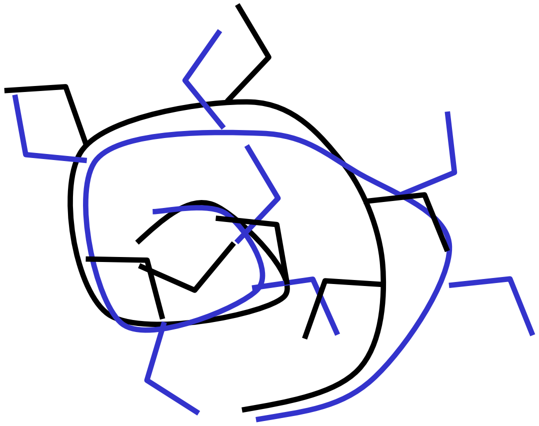
Which Atoms ?

What tells me the shape of a protein ?

- backbone trace

What happens if you include all atoms ?

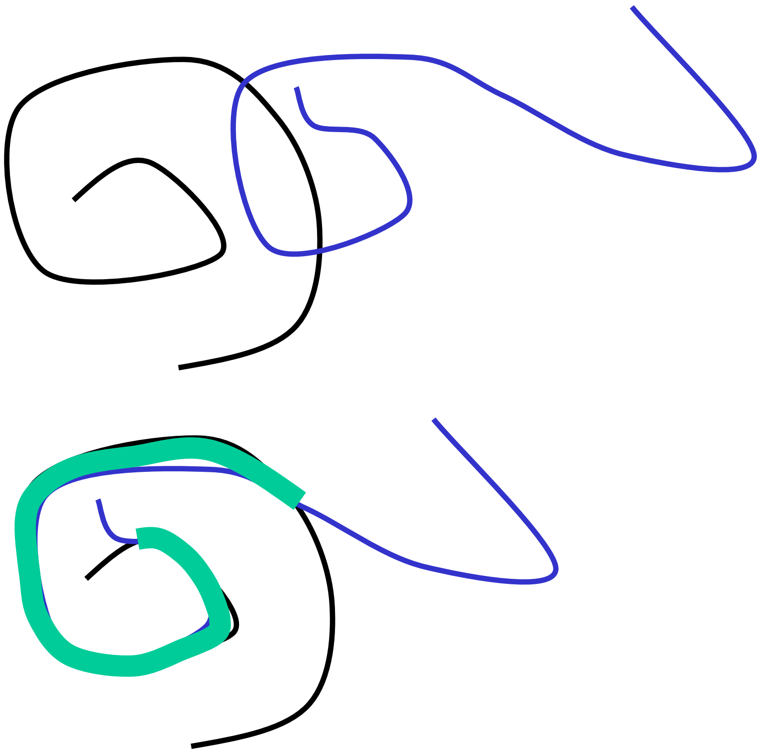
- bigger *rmsd*
- normal choice
  - C^{α}
- sometimes
  - N, C^{α}, C
- all atoms ?
  - when a model is very close



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
```

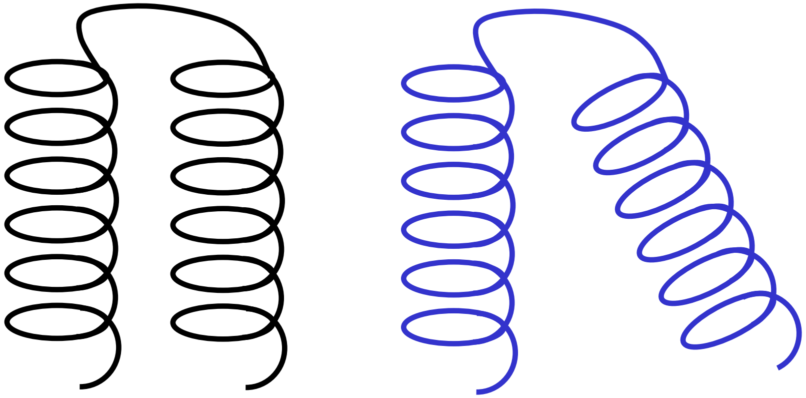
- result ? a subset of interesting atoms

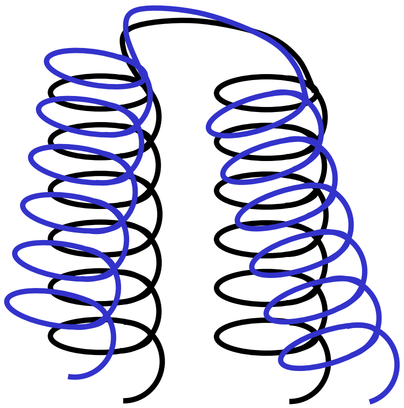
Subsets of Atoms

- 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
- solutions
  - use angles (other problems)
  - distance matrices

Distance Matrices With Numbers

Another characteristic of structures

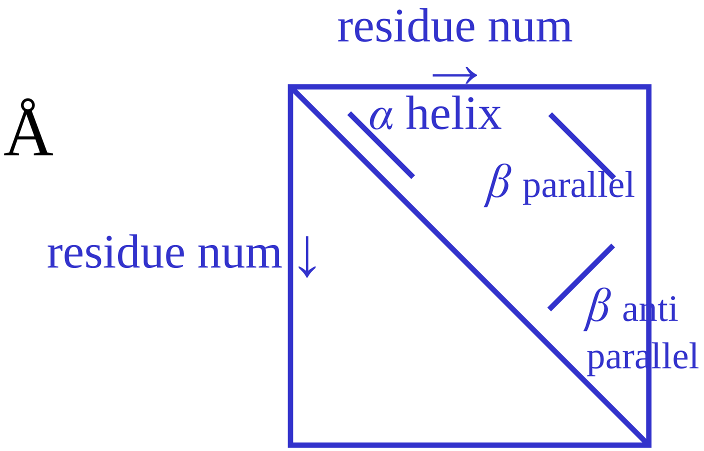
- C^{α} distance matrices
- simply measure the distance between C^{α} atoms

	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

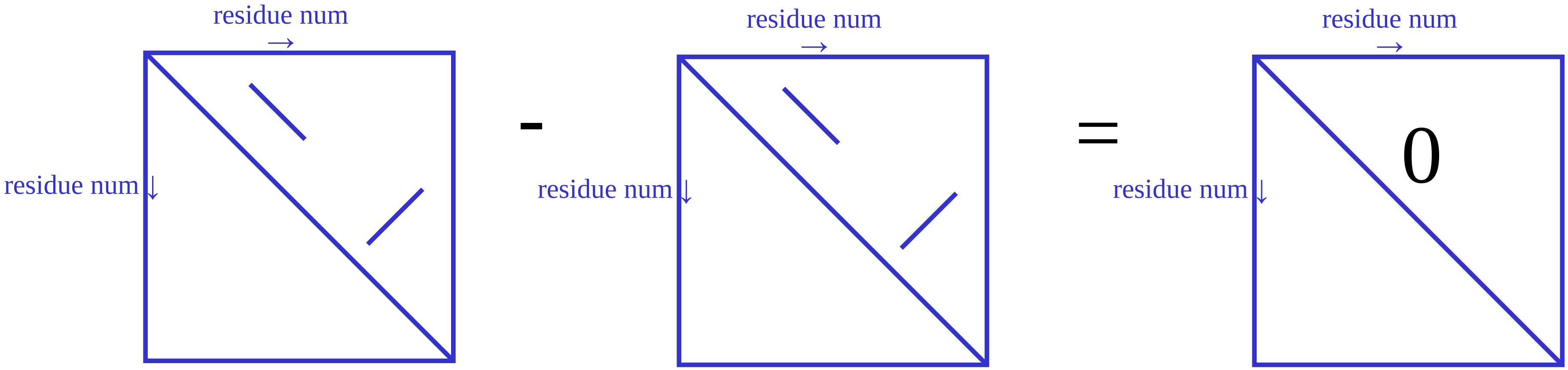
One way to summarise a structure

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



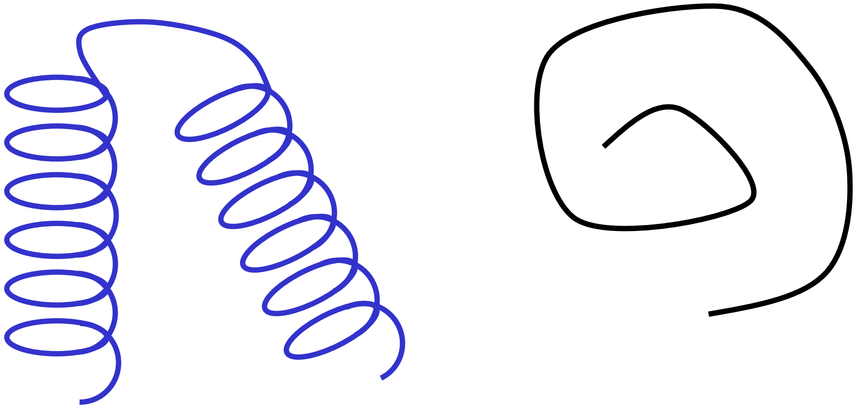
Distance matrix for comparing structures

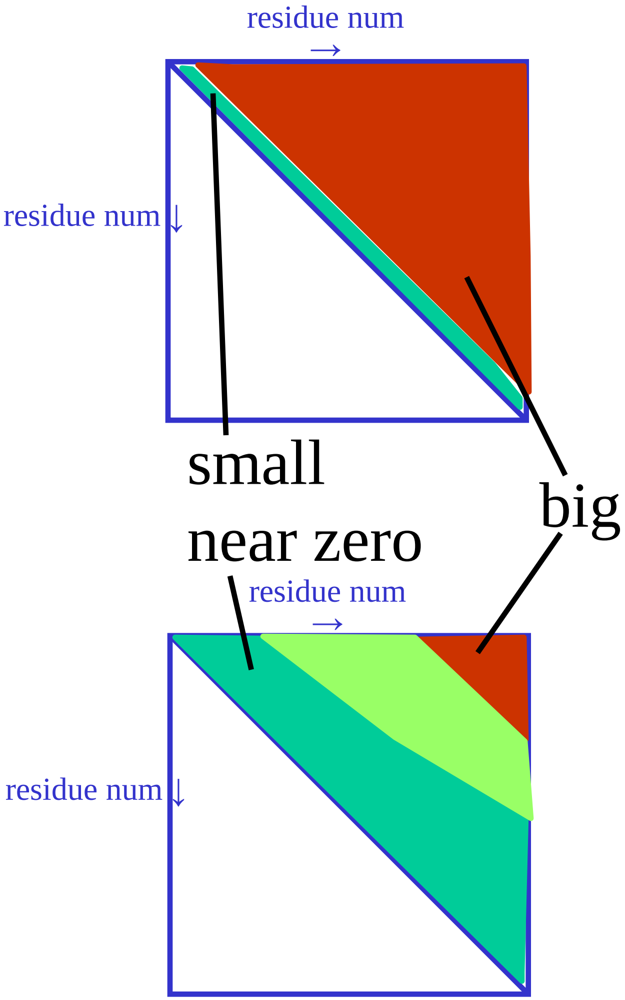
- take two similar proteins
  - look at the difference of distance matrices



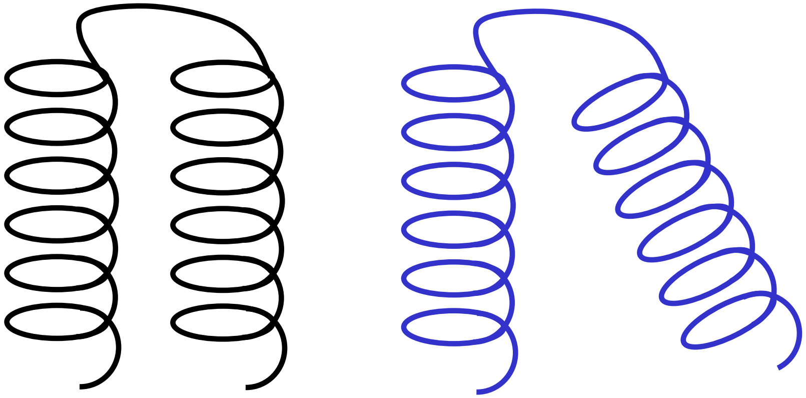
Comparing Distance Matrices

- consider 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
  - define distance between C^{α} atoms *i* and *j*

$$d_{ij} = \left| \vec{r}_i - \vec{r}_j \right|$$

- *rms* of distance matrices measure is

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

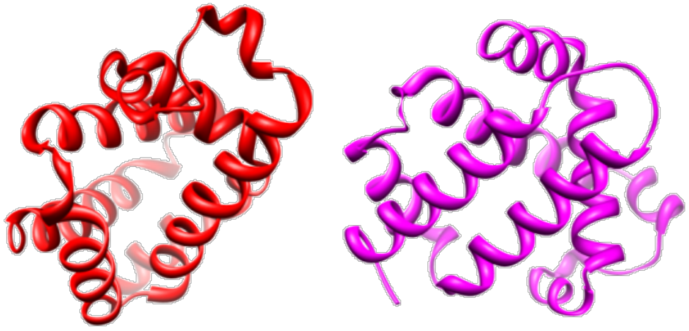
- just like all other *rms* quantities
  - normalised over top half of matrix

Summary – Comparing Models / Structures

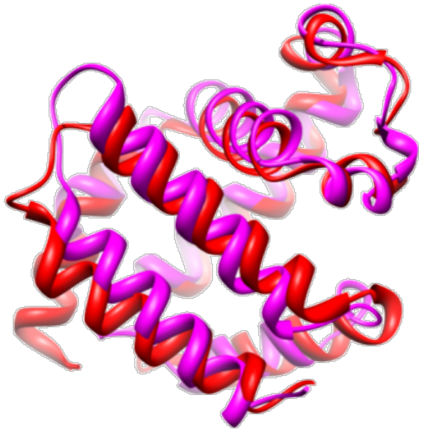
- *rmsd*
  - most popular
  - requires superposition (translate + rotate)
  - can be fooled by "hinge" movements
  - size dependent (not discussed here)
- to look at the shape of a molecule use C^{α} or backbone atoms
- numbers in Å have a physical meaning
- to look for the common core of a structure, 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 ?

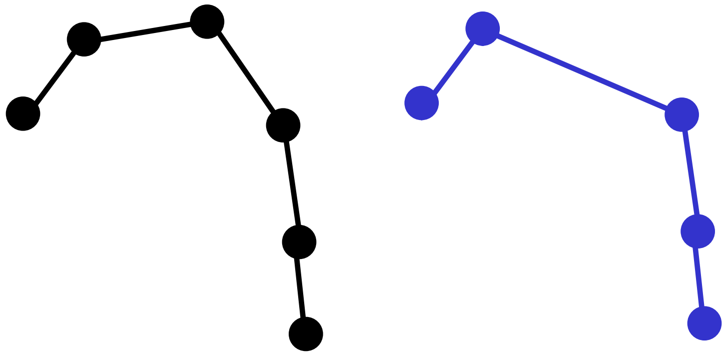


protein 1	A	C	D	W	Y	T	R	P	K	L	H	G	F	D	S	A	C	V	N
protein 2	A	C	D	W	W	T	-	P	K	V	H	G	Y	D	S	A	C	V	N

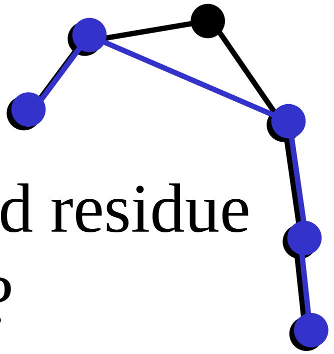


- green residues - backbone atoms
- pink residues – ignore
- is this useful for similar proteins ? very (rat vs human haemoglobin)
- for very different proteins ? no

Comparing Very Different Proteins

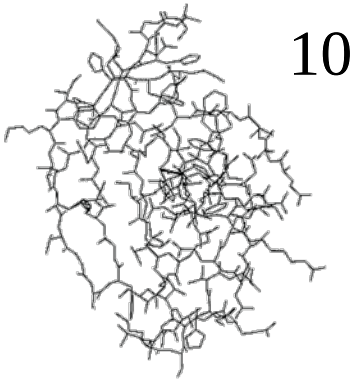


- sequence alignment vs identity
  - as identity ↓, errors ↑
- consequence
  - methods needed
    - operate on C^{α}
    - do not require sequence
- how difficult ?
  - superposition requires recognising the deleted residue
  - can we use standard dynamic programming ?
    - no
  - gap/insertion at any position, any length
    - combinatorial explosion

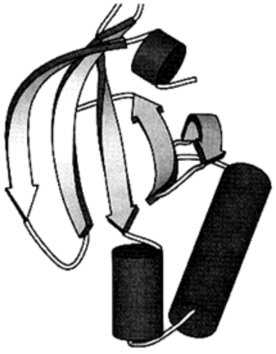


Strategies For Comparing Different Structures
1. use secondary structure

- Combinatorial explosion is the problem
  - reduce size of problem
  - use elements of secondary structure



10^2-10^3 atoms



about 8 units

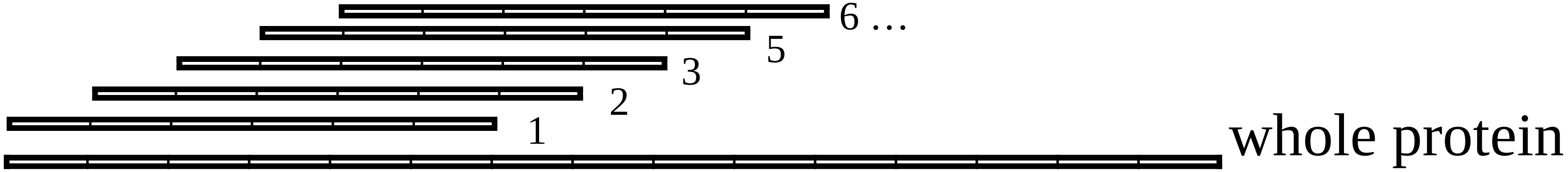
- define secondary structure
- search for superposition
- for each residue
  - find closest C^α in partner structure
  - use the set of matching residues to calculate *rmsd*

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

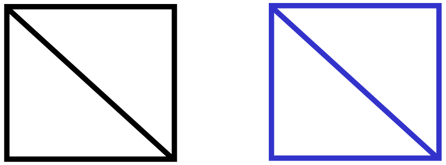
- Ingredients
  - break protein into overlapping fragments (length 6 or 8)
  - protein is no longer a string of residues nor a whole structure



- 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 1 fragments →

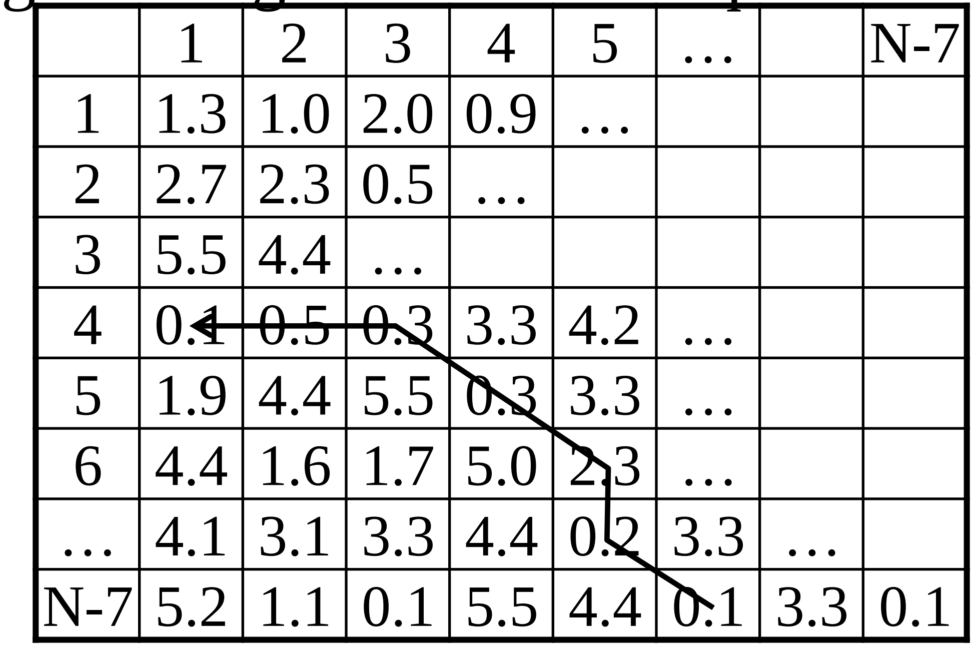
protein 2 fragments ↓

	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

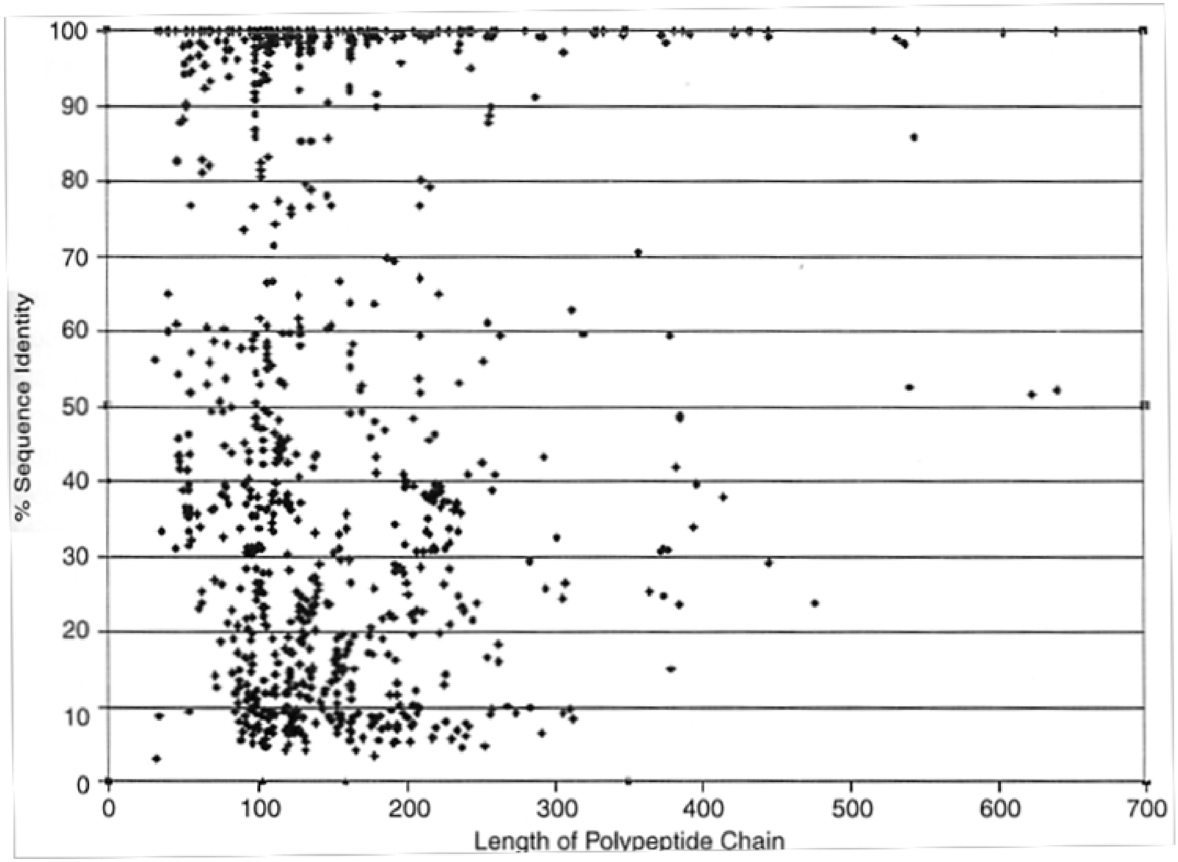
Comparing Different Size Protein Structures

- 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 1000 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