Analysis and comparison

Stories

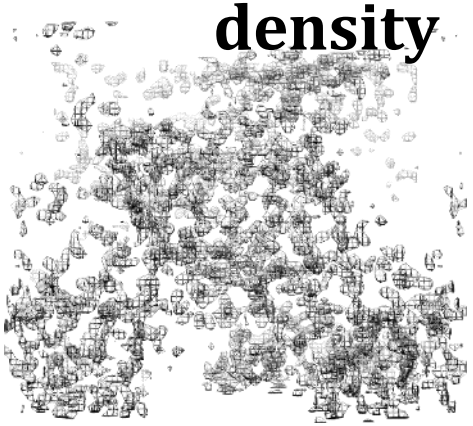
1. quality
2. surfaces
3. Comparing structures

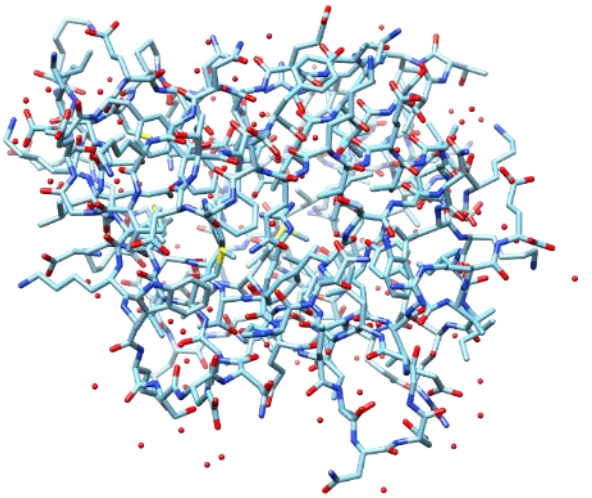
Quality

Meaning ?

- How good is the electron density ?
- How well are atoms placed ?

electron density

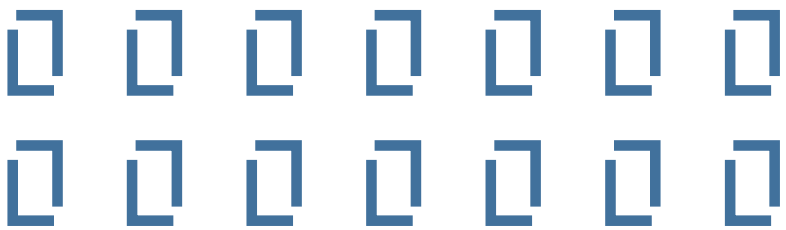


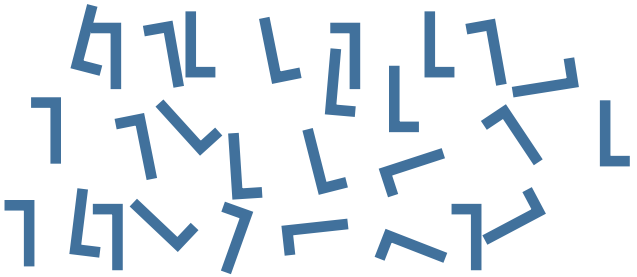


experimental issues

Crystal quality and size

- NaCl, sugar,.. crystallise in the kitchen
  - crystals large
  - soup → ordered state, ΔG is favourable
- proteins
  - not so regularly shaped
  - ordered arrangement may not be much better than random orientations
  - which has better free energy ? entropy ?

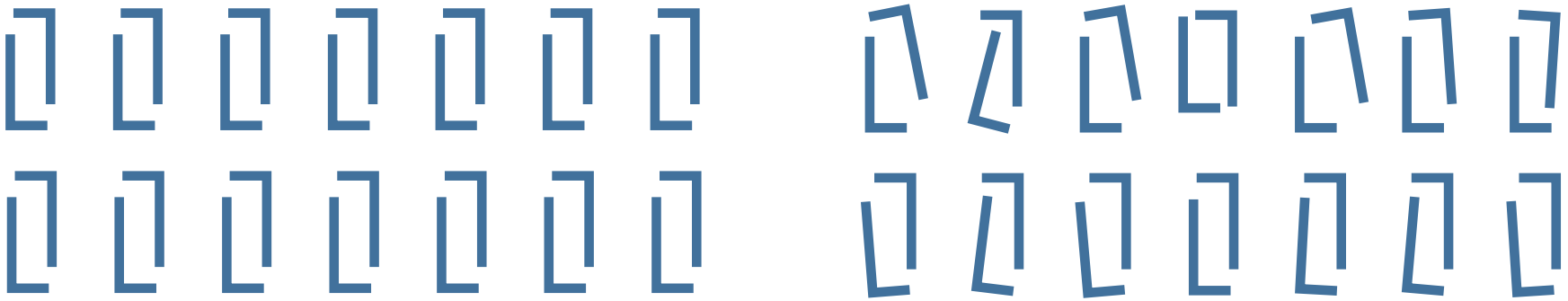




nice crystals / bad crystals

You get a crystal – some disorder

- you see the average
- if the position of atoms varies – the coordinates are
  - smeared – not well determined



Result

- resolution not so good
- atoms are put in wrong places
  - sidechains fit to noise, water, ..

Judging the structure

Two sides

1. fit to experiment (in Biophys lectures)
   R and R_{free}
2. how good is the structure itself ? (this topic)

What do people look at ?

- energies ?
- geometries properties

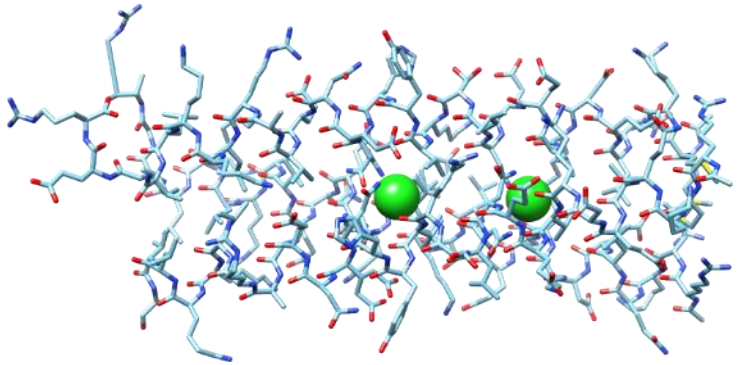
Why do we not use energies (so much) ?

Energies – not easy to use / assess

Two proteins with 100 residues

1. lots of big hydrophobic residues – lots of van der Waals
2. a long protein
   small core, interactions with water and ions

Difficult to compare energies



2wpz

You give me a protein and energy calculation

- can I judge the coordinates ? Not easy

What can one look at ?

- typical properties of proteins

Typical properties of proteins

- Ramachandran plots
- side-chain distributions
- clashes

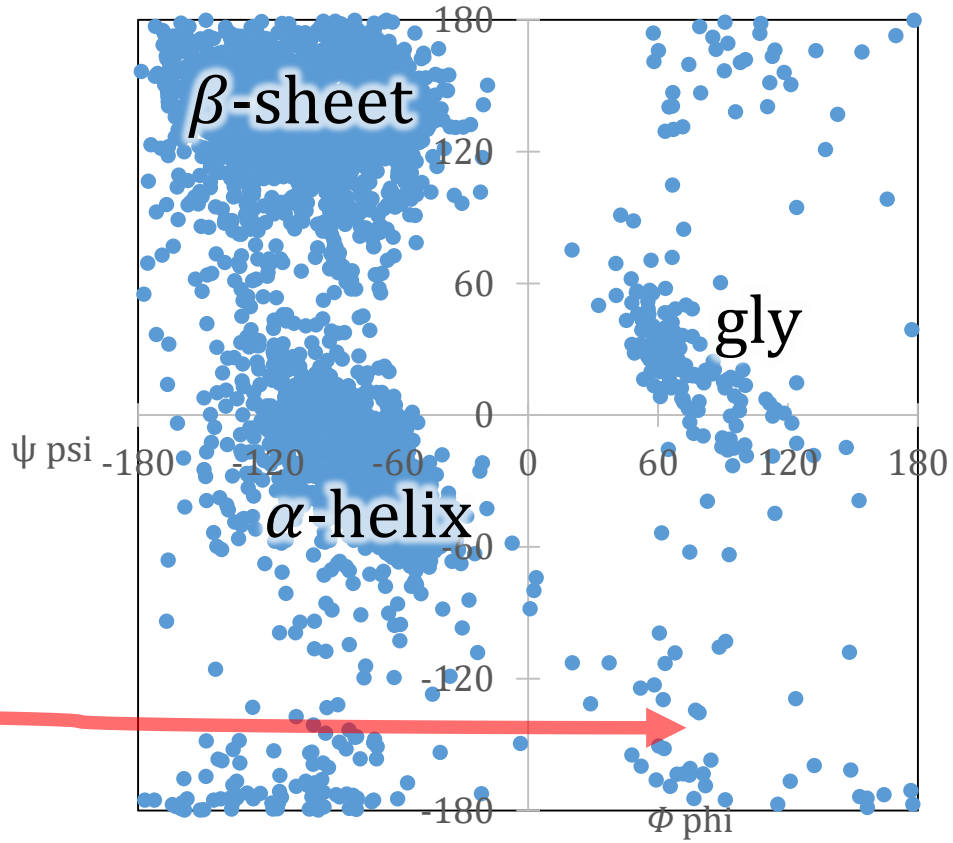
Ramachandran outliers

Random sampling of protein data bank

- which are bad and which are OK ?

- not every site is α-helix or β-sheet

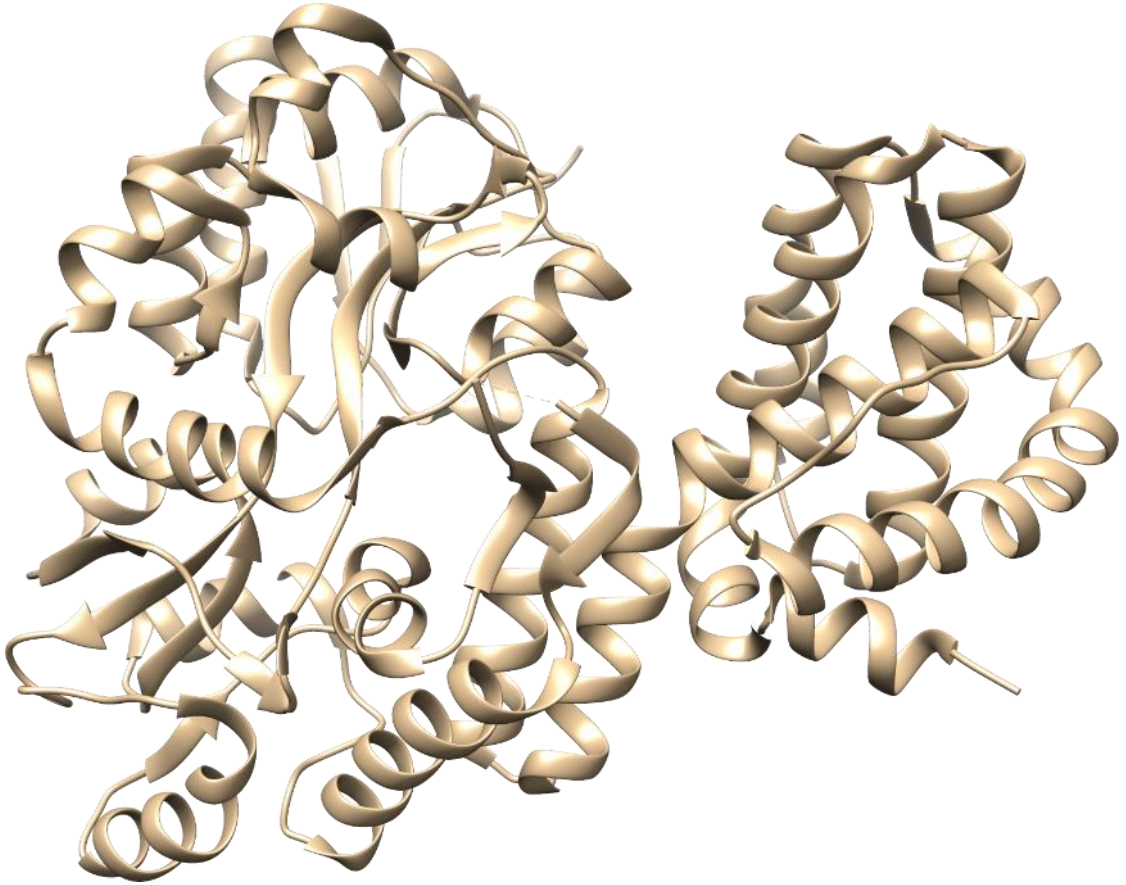
- some example proteins



not
all bad
some small residues

happy coordinates

Why do I know they are happy ?

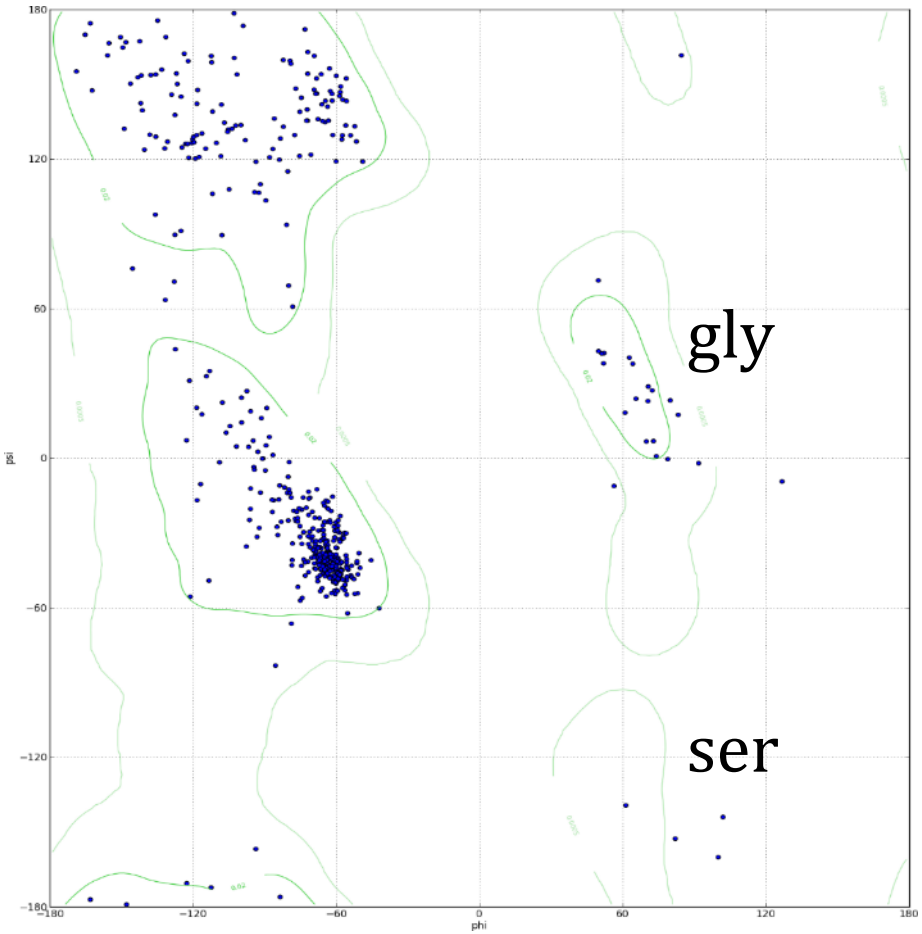


4wmx

happy Ramachandran plot

Each unusual residue was checked and OK

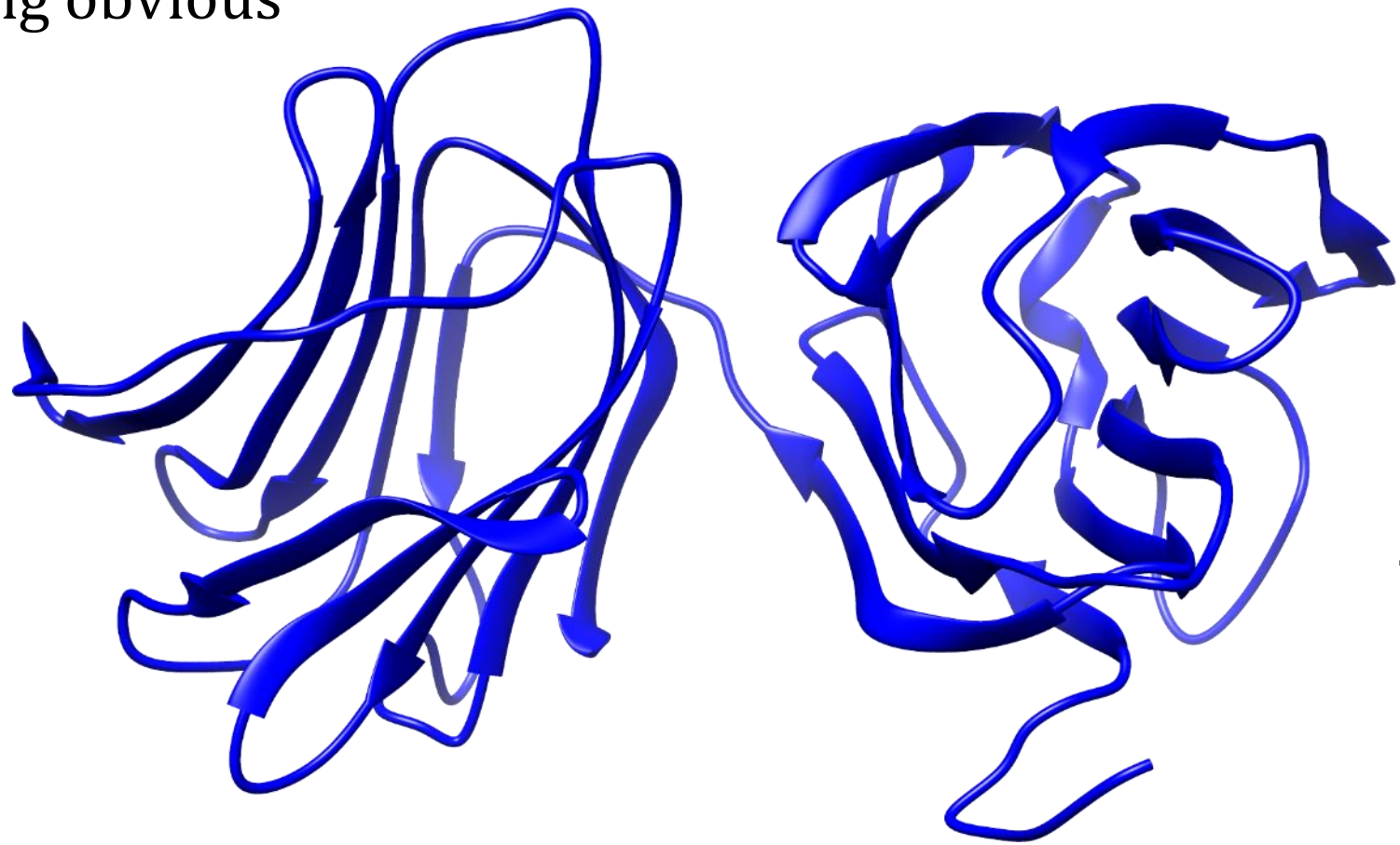
- gly and ser



4wmx

A bad structure

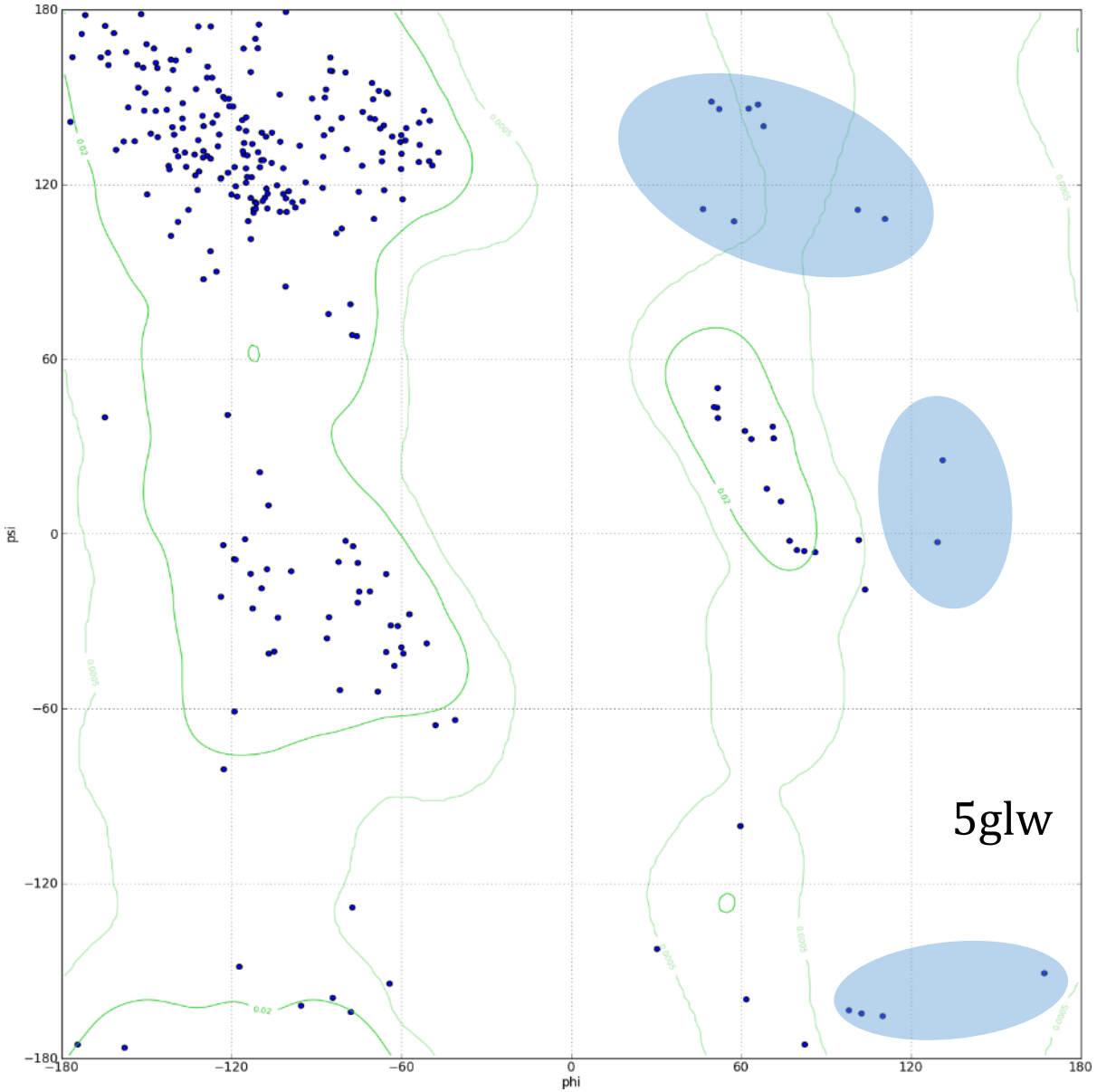
nothing obvious



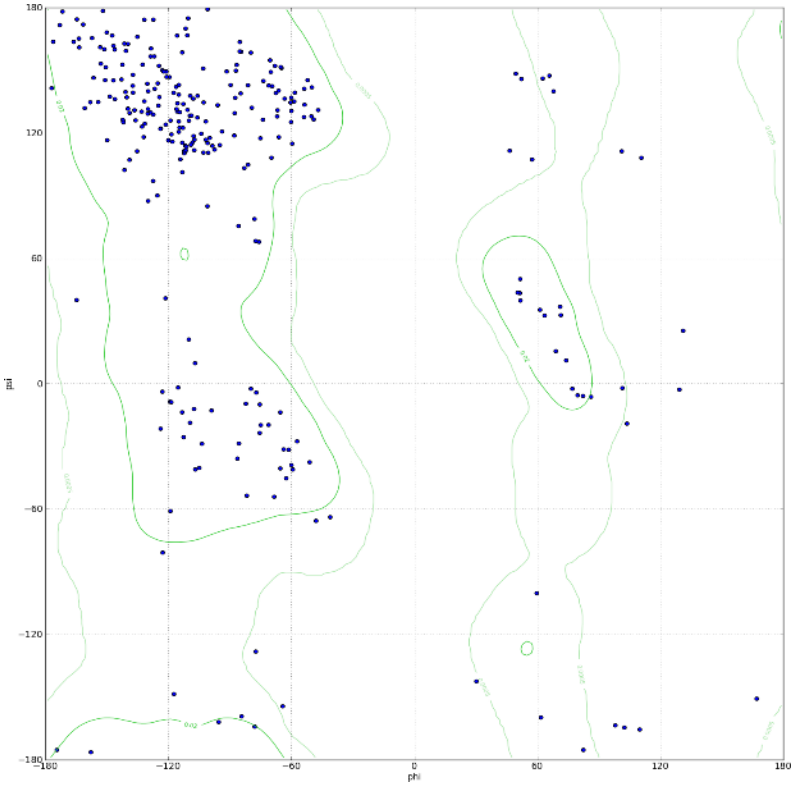
5glw

bad Ramachandran

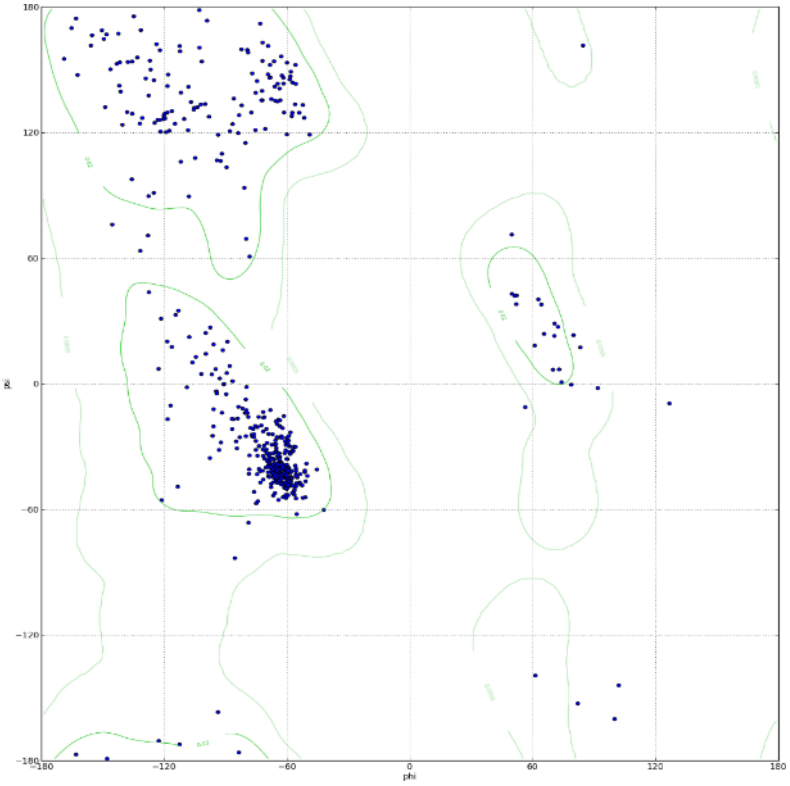
unlikely residues
cannot be explained



bad 5glw

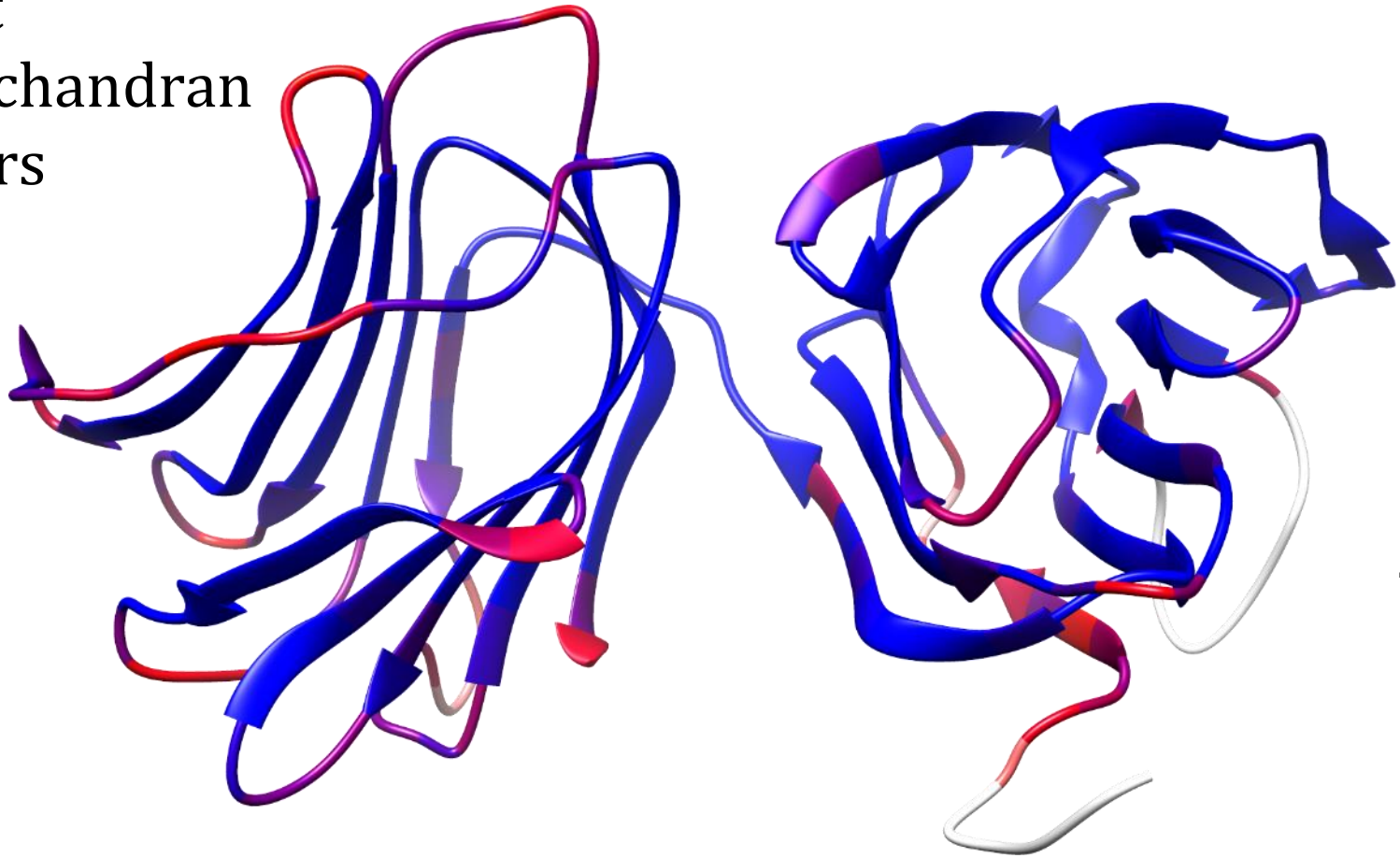


good 4wmx



Where are the problems in bad structure ?

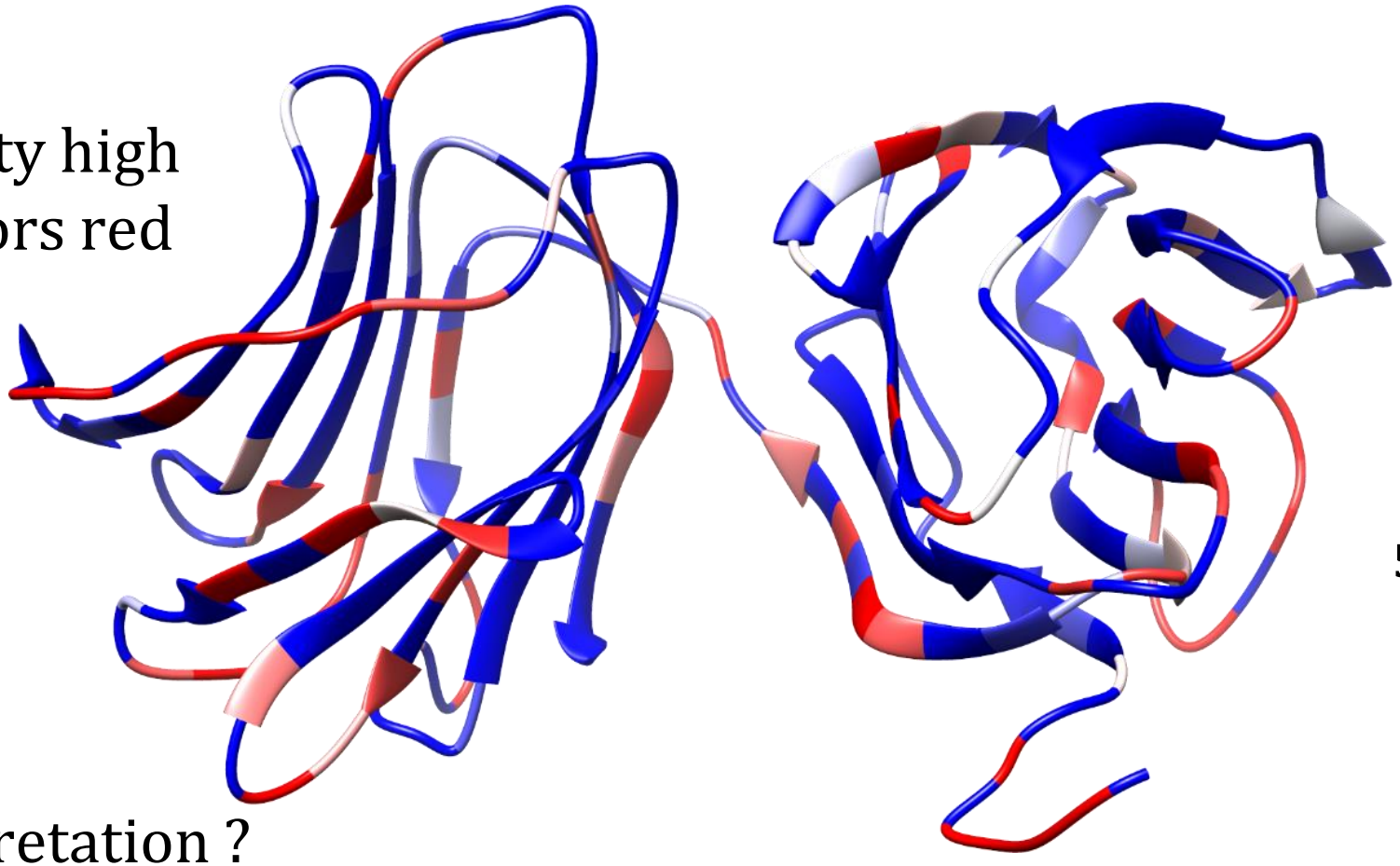
Worst Ramachandran outliers



5glw

Loops ?

Mobility high
B-factors red



5glw

Interpretation ?

- often did not know where atoms are
- placed them not in most likely positions

Clashes

Best method to assess ? energies

Fastest method

- for each atom i we have a radius r_i (textbook)
- for each pair of atoms calculate d_{ij}

  if $d_{ij} < r_i + r_j$

  complain

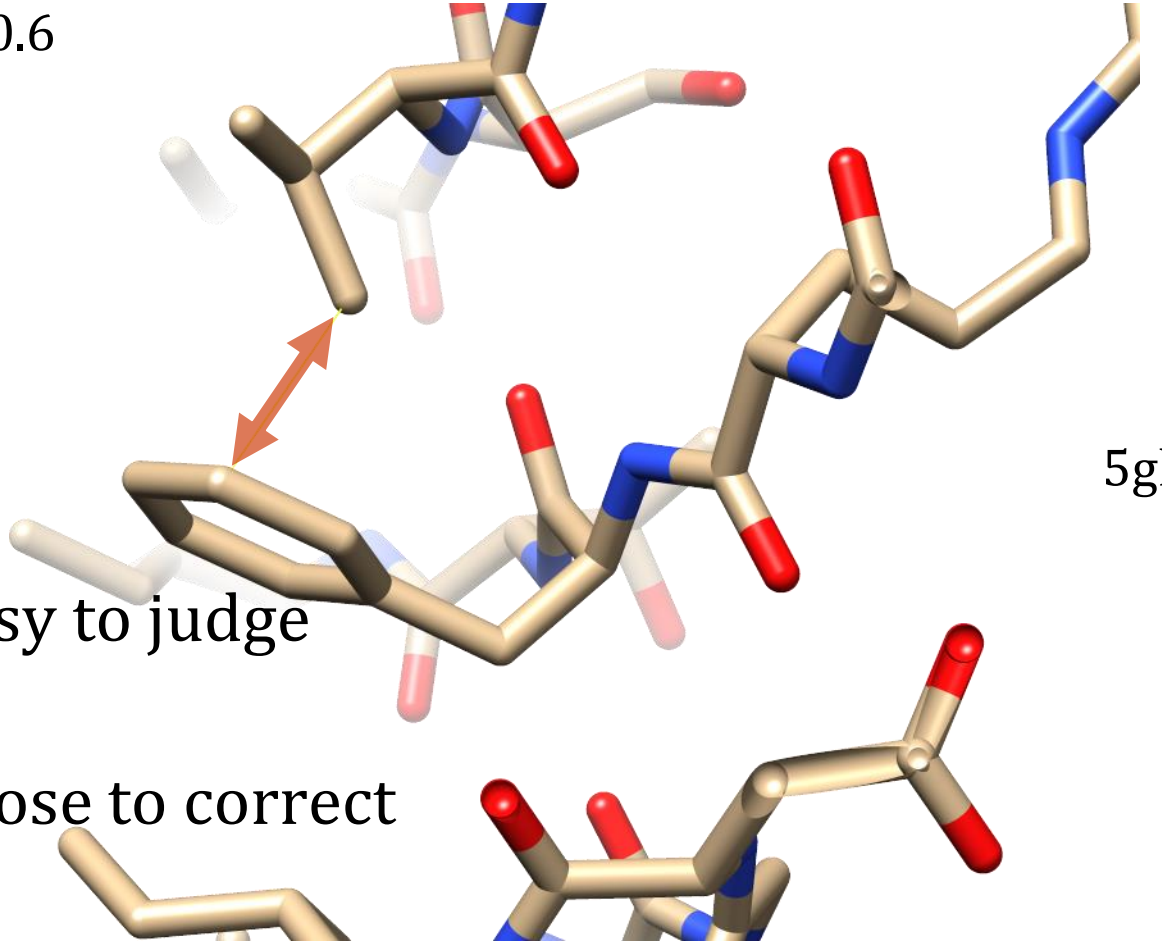
  - bad coordinates …

						too small (Å)
asn	44	N	his	43	ND1	0.8
lys	225	NZ	phe	197	CB	0.6
phe	38	CE1	val	60	CG1	0.6
glu	224	O	ile	227	CG2	0.6

a clash

- not so dramatic
- ½ Å, small but important in energy

phe 38 CE1 val 60 CG1 0.6



5glw

Clashes are not so easy to judge

- bad energy, but
- geometry is very close to correct

sidechain rotamers

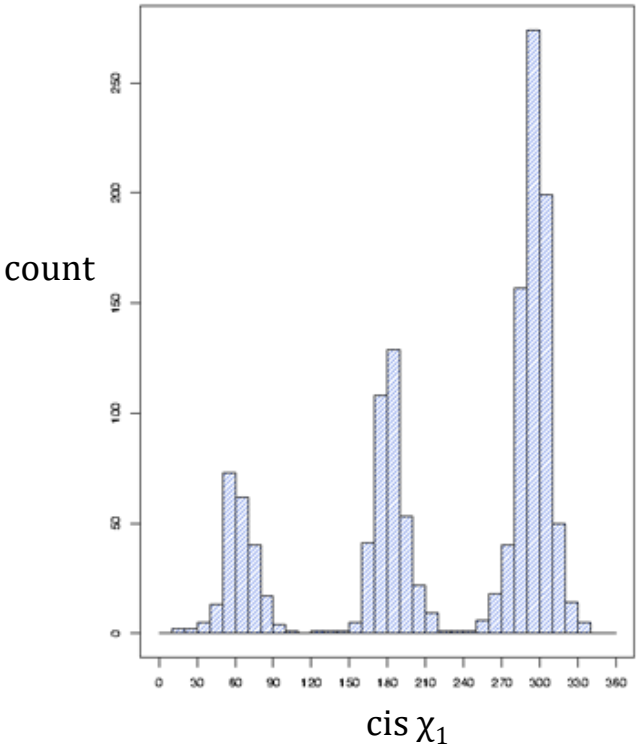
Torsion angle – energy model OK – not usually used

Empirical approach

- visit high-resolution structures in PDB
- collect data on each side-chain angle – make histograms

Look at coordinates

- for each sidechain angle decide on probability

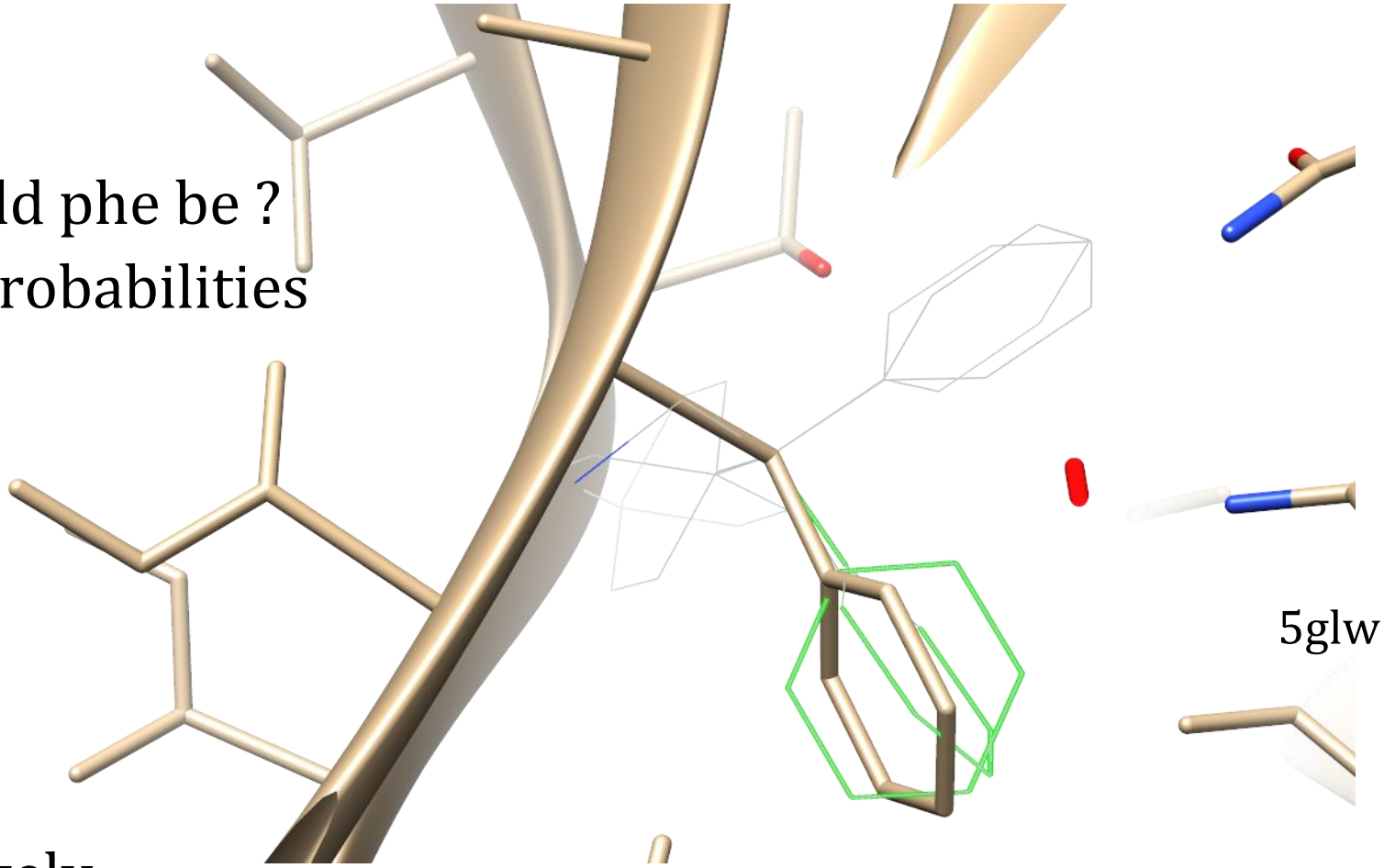


Dunbrack Jr, R.L., Curr. Opin. Struct. Biol. 12, 431-440 (2002)

rotamer modelling

Where could phe be ?

What are probabilities



5glw

Here

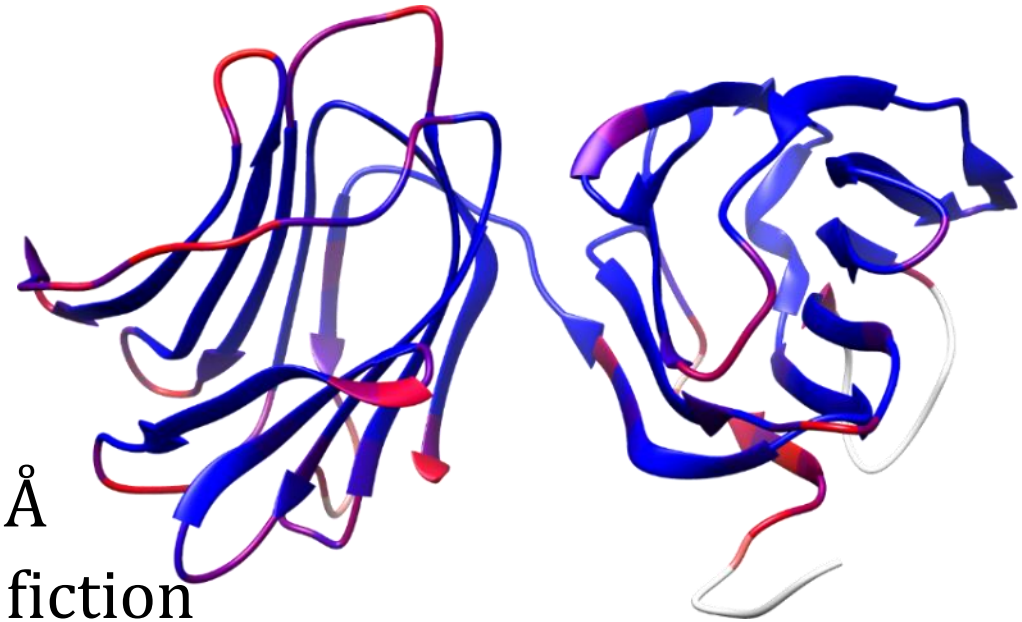
χ_1 likely

χ_2 less likely

Comparing good and bad examples

Both proteins (4wmx, 5glw)

- 2 Å resolution
- year 2017



Does it matter ?

- No. clash errors small ≈ ½ Å
- Yes. parts of backbone are fiction

Depends on application

- comparing with other proteins ? not important
- discussing ligand binding ? important

Where do problems come from ?

- Data – weak – where their software put atoms

summarise quality

Ramachandran plots

- physics – torsion angles, Lennard-Jones, electrostatics
- we look at frequencies in protein data bank

Clashes

- physics – Lennard-Jones and electrostatics
- we look at hard radii

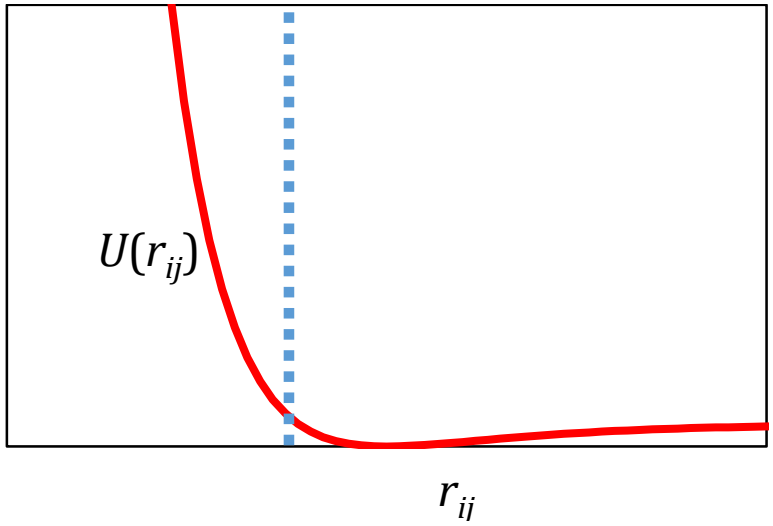
Rotamers

- physics - torsion angles, Lennard-Jones, electrostatics
- we use frequencies from protein data bank

Can we justify this ?

Good energy models or rough

Clashes ? rough approximation



Statistics / counting
(rotamers, backbone angles)

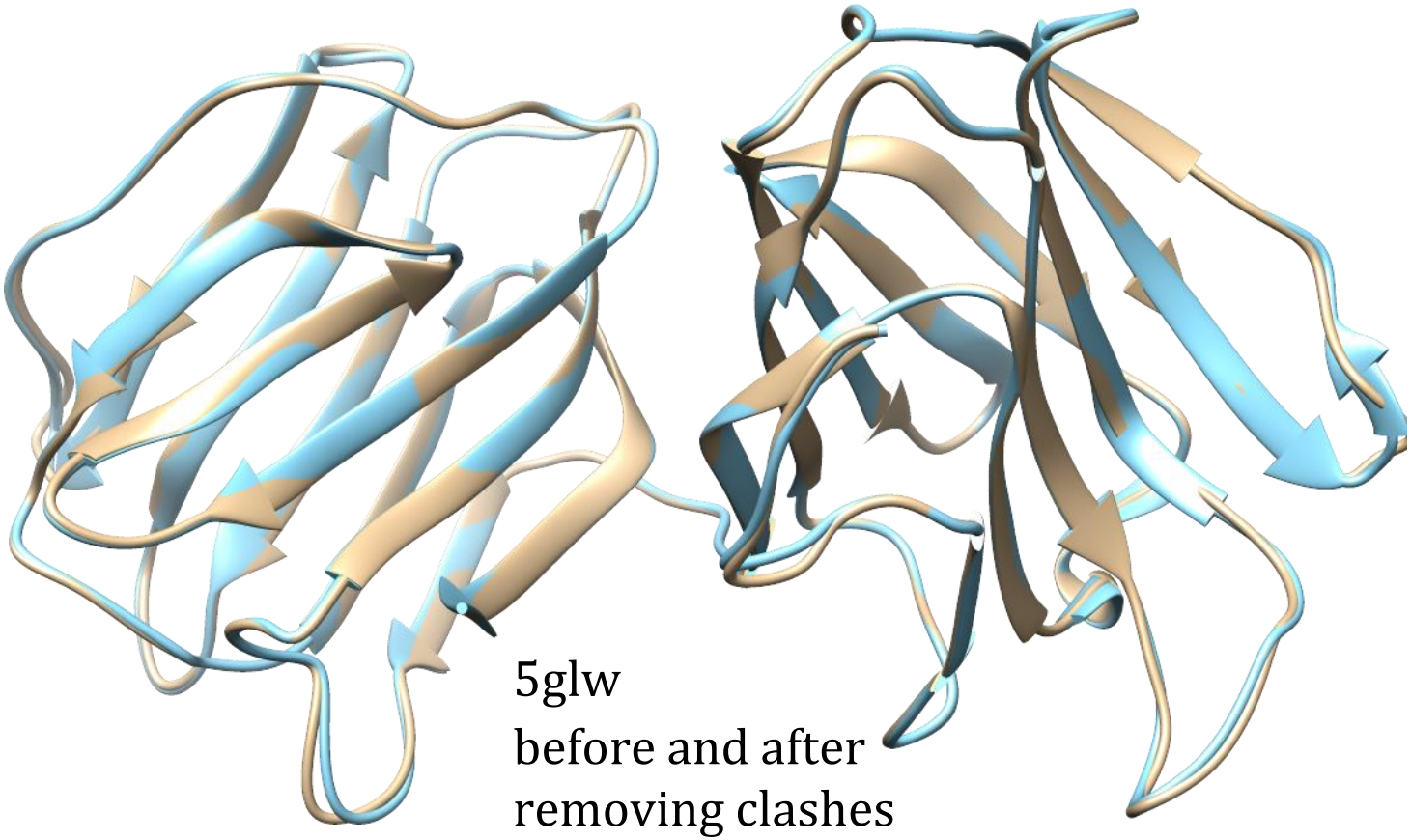
- what we see in the world reflects energies (Boltzmann relation)

$$p_i \propto \exp\left(\frac{-E_i}{kT}\right)$$

formula not for Klausur

What do we think of unhappy structures ?

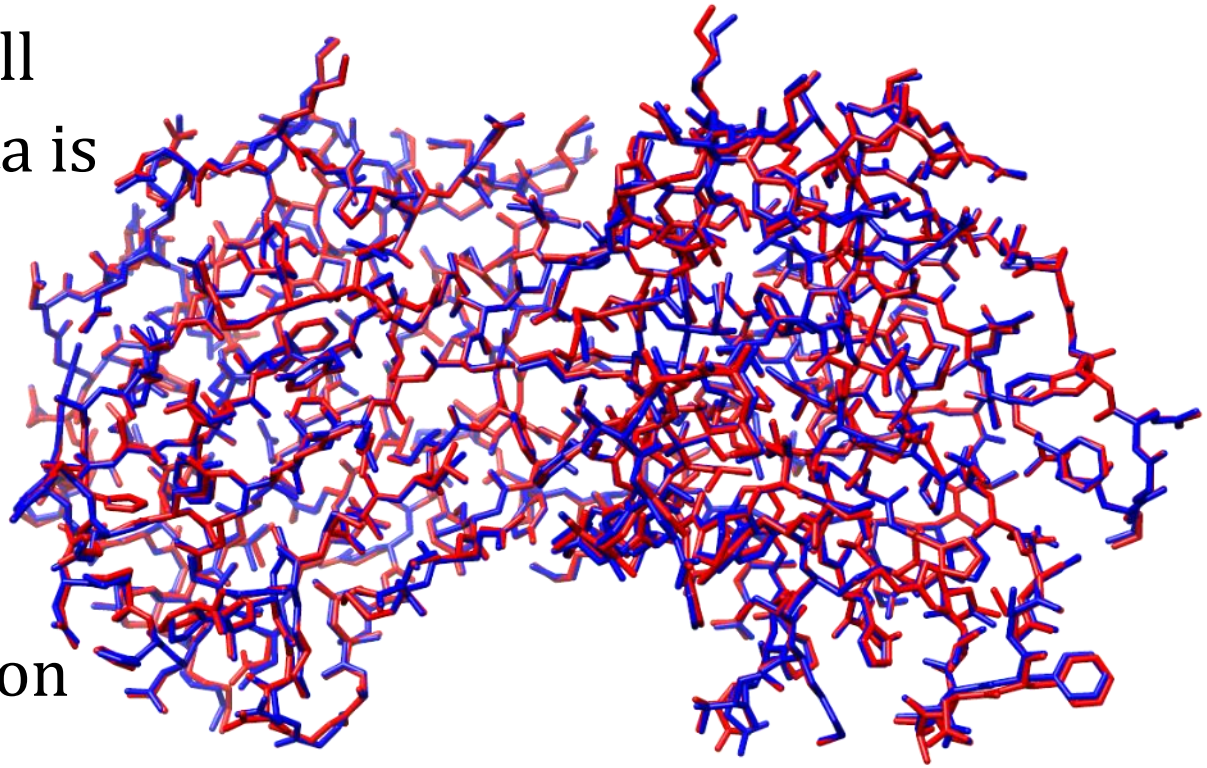
Are they necessarily wrong ? ask again in 3 minutes



5glw
before and after
removing clashes

- maybe the side-chains have moved ?

- differences are small
- agreement with data is no worse



- with a bit of effort authors could have avoided this attention

- there are some terrible structures in PDB search for obsolete PDB (just for fun)

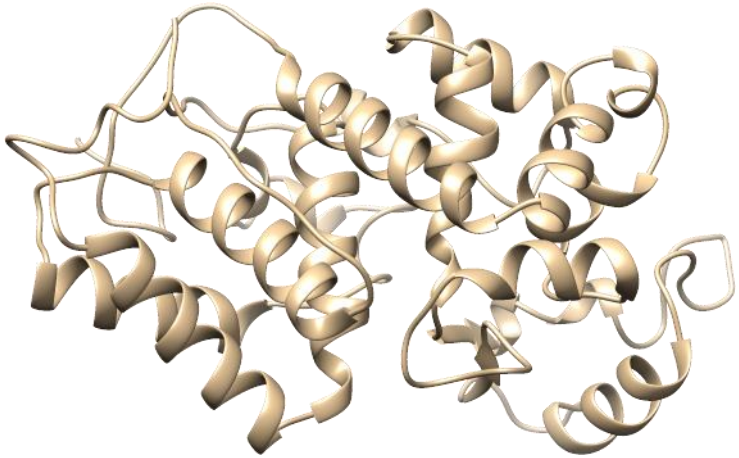
Surfaces

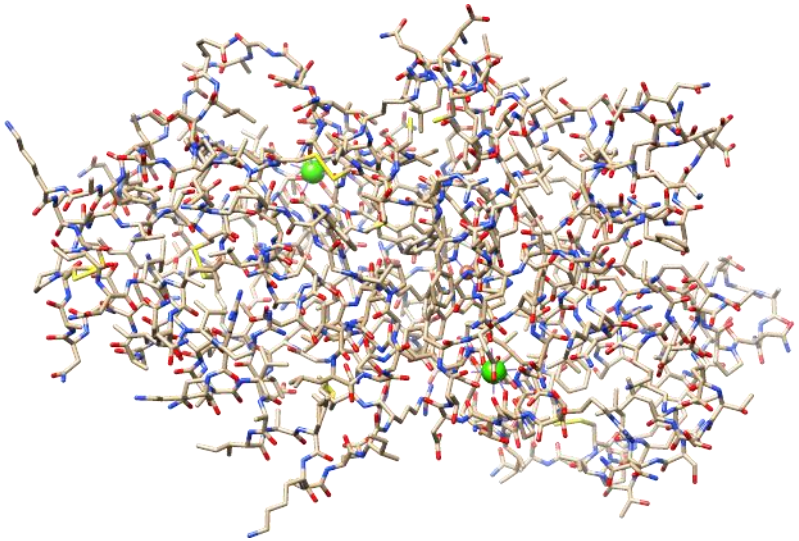
- not really a quality issue
- a property that quickly says if something is unusual

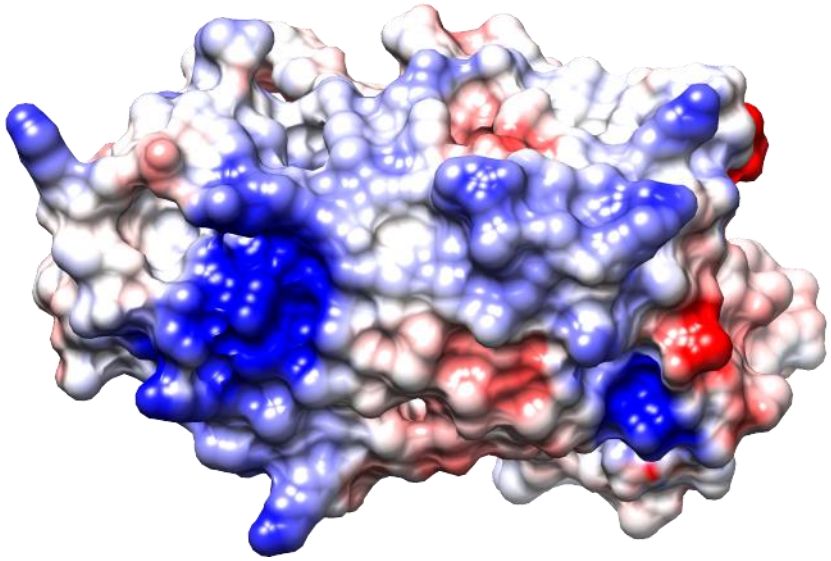
What do you expect ?

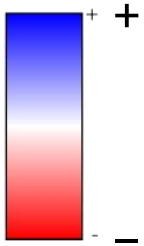
- surface must be more charged and polar than the middle
- lots of –ve or +ve charges ? not so common
  - acidic or basic proteins – do exist
- charged regions ? Interaction with substrates ?
- very neutral – will not be soluble

peroxidase









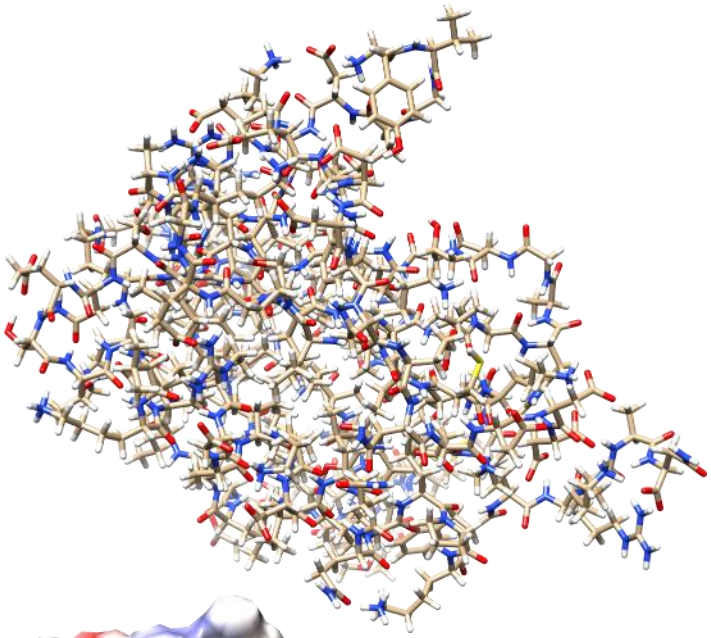
1qgj

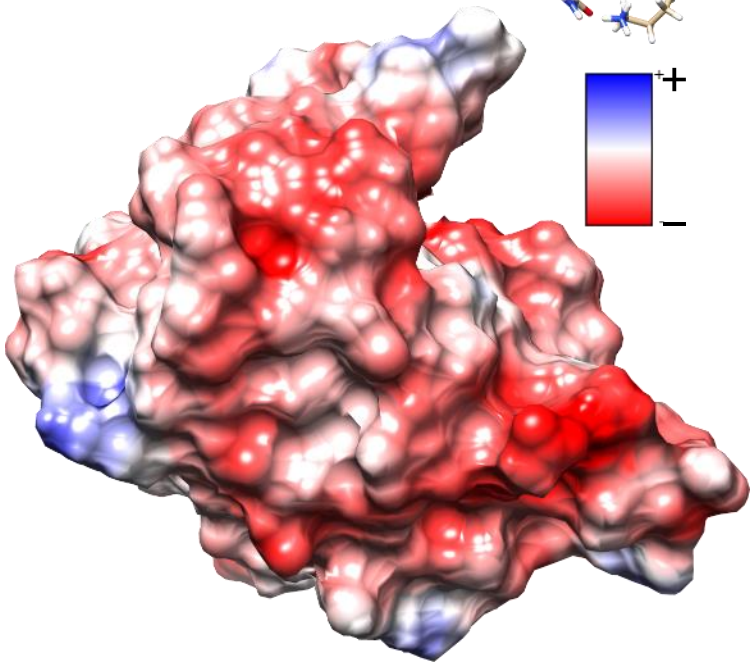
an acidic protein

Nothing wrong

- really an acidic protein

Might see with a simple pI calculation





5gow

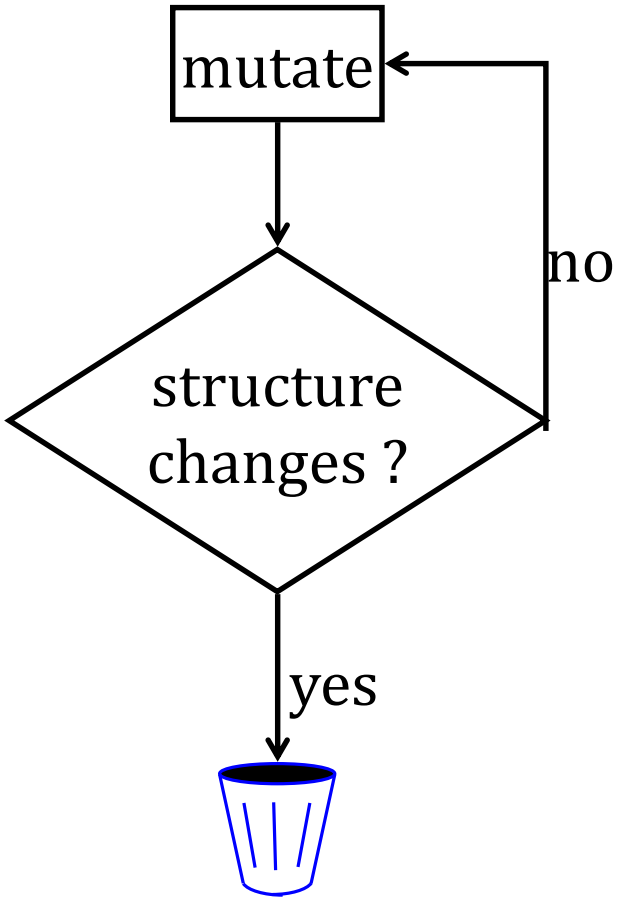
Structure comparisons

- Why ? Function prediction – evolution
- Sequence versus structure conservation..

Simple view of molecular evolution

mutate continuously

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



Result

- nature tests many sequences and keeps those that are compatible with structure

Structure determines function, but..

What is more informative

- sequence or structure similarity ?

	sequence similarity	structure similarity
closely related	yes	yes
less similar	no	yes
not related	no	no

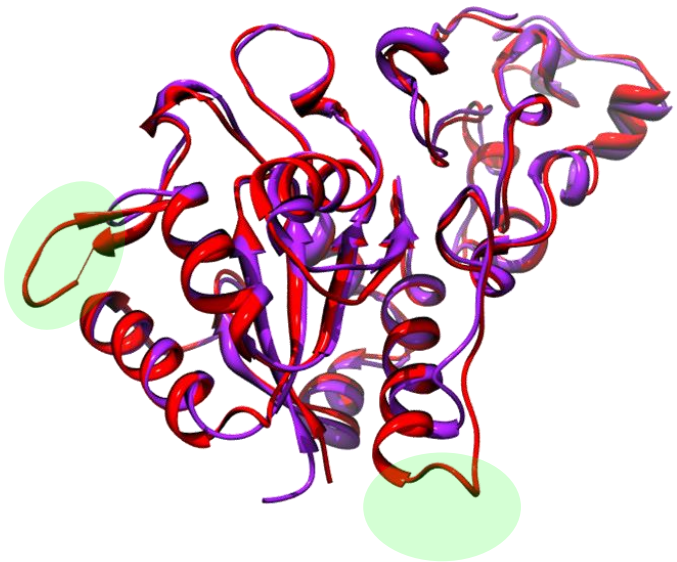
- look for sequence similarity – most helpful
- structure similarity 2nd choice
  - relationships that one would miss

Sequence versus structure alignment

- Aim: why can we not use sequence alignment methods
- Sequence alignment reminder (more in summersemester)
- reminder

sequence alignment

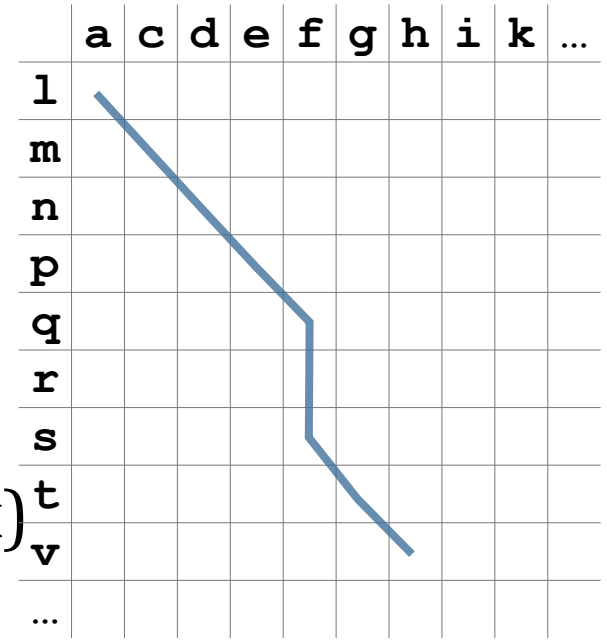
```
Seq ID 40.6 % (103 / 254) in 280 total including gaps
    :    1    :    2    :    3    :    4    :    5    :    6
    :    0    :    0    :    0    :    0    :    0    :    0
kkapviwvqgqggctgcsvsllnavhprikeilldvislefhptvmasegemalahmyeia
krpsvvylhnaectgcsesvlrtvdpyvdelildvismdyhetlmagaghaveea-l-he
   :    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
ekfngnfflvegaiptakegryci vgeakahhhevtmmelirdlapkslatvavgtcsa
aikg-dfvcvieggipmgdggywgk-------vggrnmydicaevapkakaviaigtcat
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
yggipaaegnvtgsksvrdffadekiekllvnvpgcpphpdwmvgtlvaawshvlnpteh
yggvqaakpnptgtvgvnealgklgvkai--niagcppnpmnfvgtv--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    :
plpeldddgrpllffgdnihencpyldkydnsefaetftkpg-----ckaelgckgpsty
gmpeldkqgrpvmffgetvhdncprlkhfeagefatsfgspeakkgyclyelgckgpdty
  :    1    :    1    :    1    :    2    :    2    :    2
  :    7    :    8    :    9    :    0    :    1    :    2
  :    0    :    0    :    0    :    0    :    0    :    0
```



Sequence alignment steps

steps

- similarity score
- sum up possible paths
- find optimal path



First step –similarity

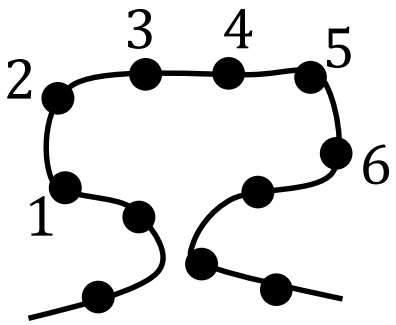
- look up in a table (blosum matrix) how similar is

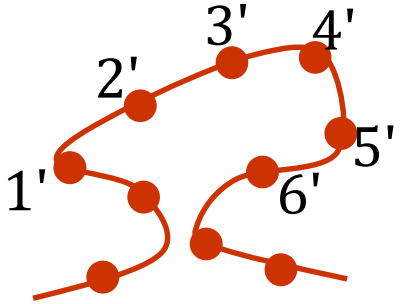
  a to **l**, **a** to **m**, **a** to **n**, .. **c** to **l**, ..

Can one do this with structures ?

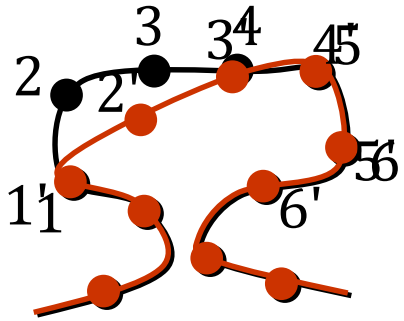
Difficulty with structure alignments

- to build a score matrix, must compare 1 to 1', 2', …
- 2' depends on 1', 3'
  - 1', 3' have not been aligned





- there is no obvious similarity measure comparing two sites in structure

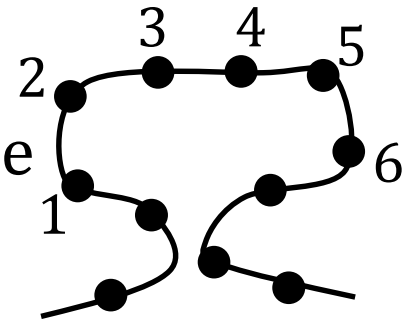


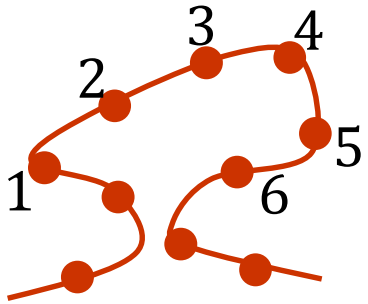
Time for guesses / approximations

Sequence philosophy – structure alignments

If each part of a structure has a label, can compare labels

- say 1 is α, 2 is α, 3 is γ
- similar labels in red structure





- can build a score matrix

	1	2	3	4	5	6	7	8	9	…
1										
2										
3										
4										
5										
6										
7										
8										
9										
…										

- fill with 1's and 0's

Could one use secondary structure ?

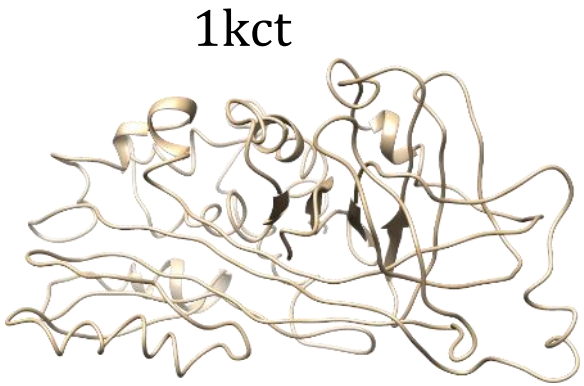
- would it work ?
  - not well

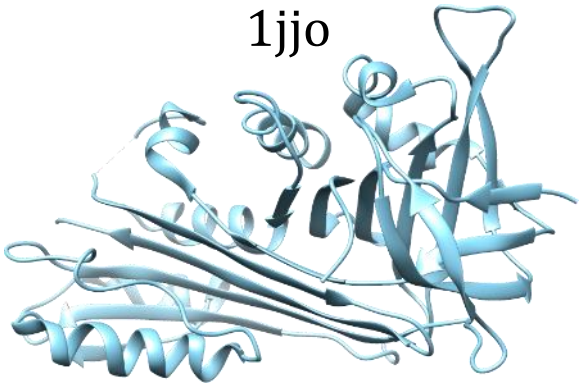
Alignments based on secondary structure

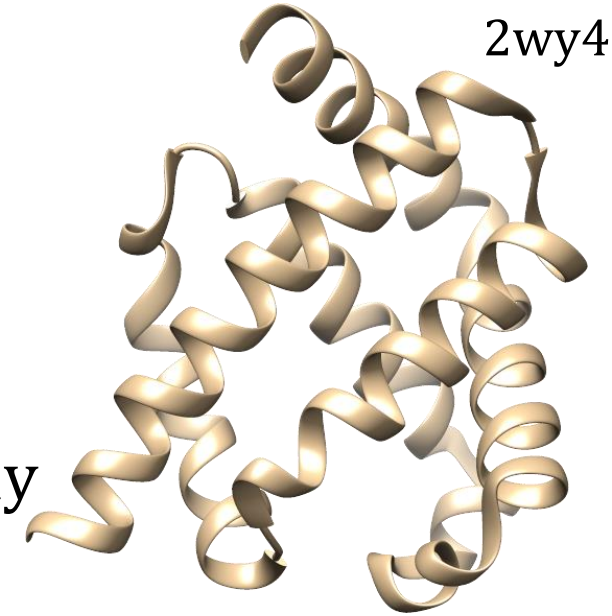
Problems

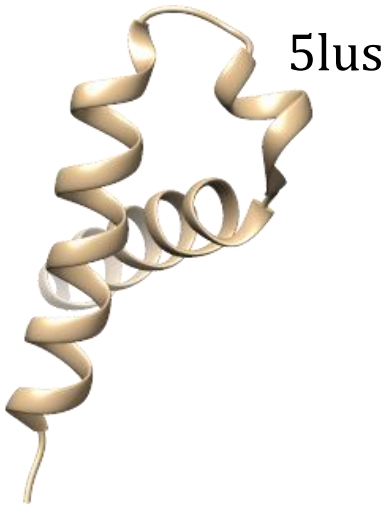
1. alphabet is too small
- does not capture similarity
  - lots of alternative alignments of nearly equal score

2. requires regular structure





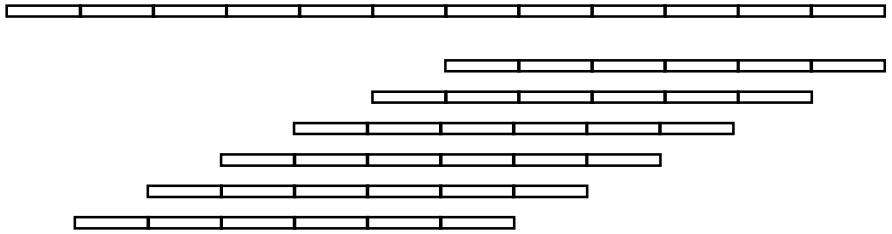


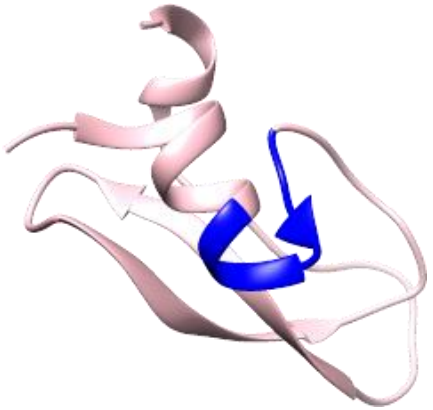


Labels on pieces of structure

Classic 2° not enough – better alphabet for structures

Break structure into fragments

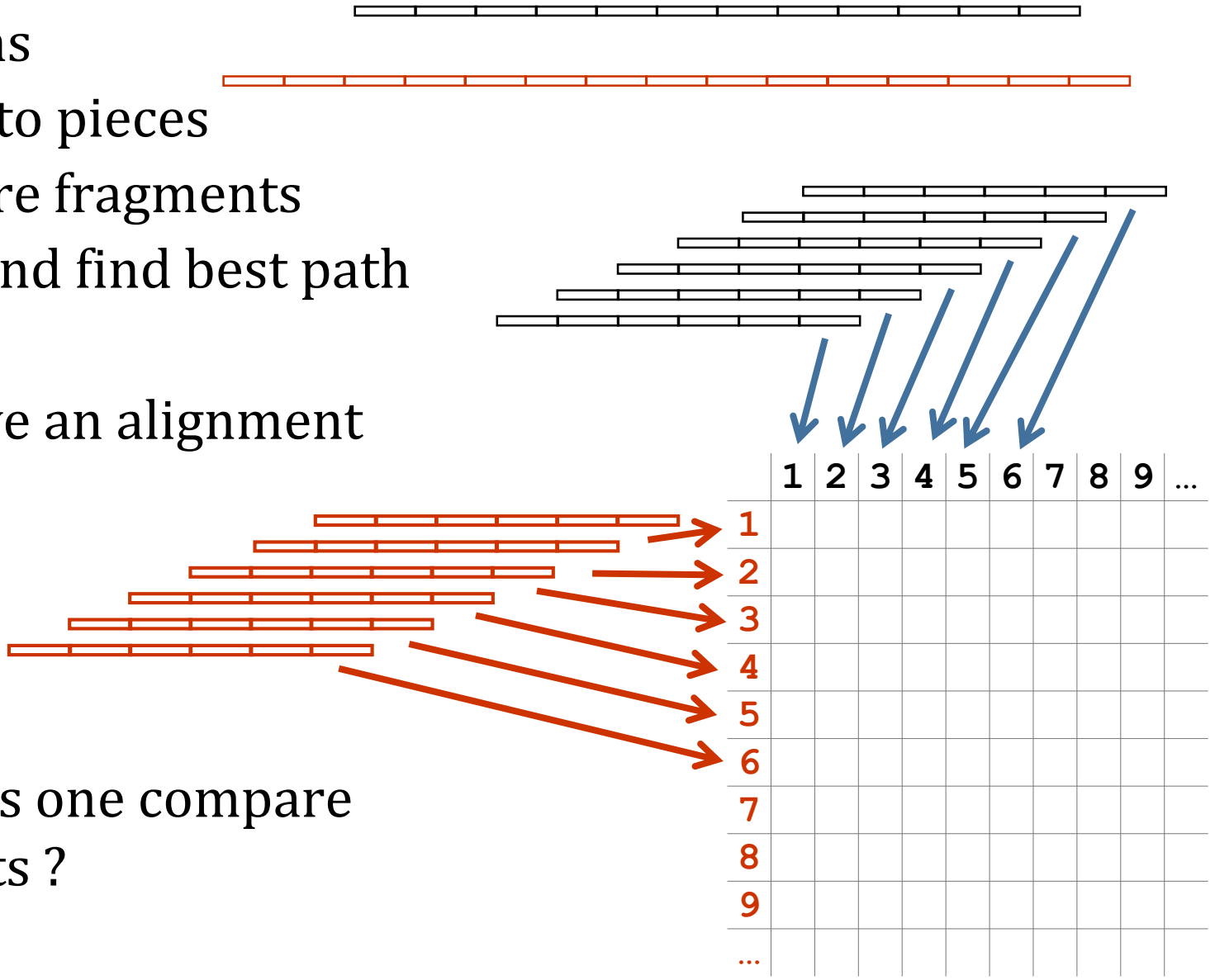




2 proteins

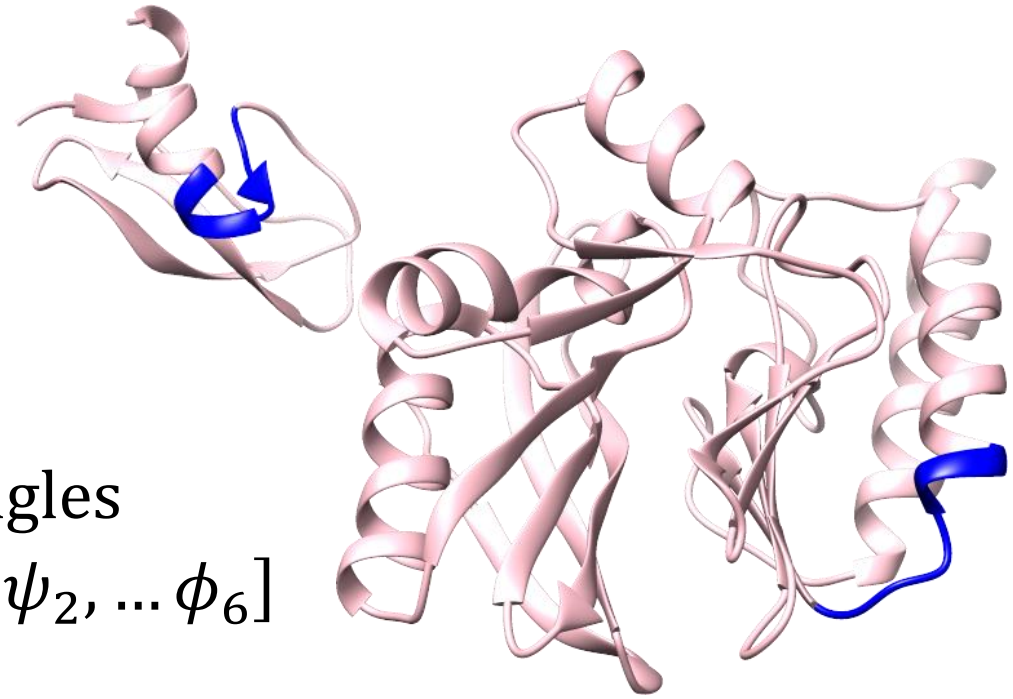
- split into pieces
- compare fragments
- score and find best path

- will give an alignment



How does one compare fragments ?

Comparing fragments - angles



Measure of similarity

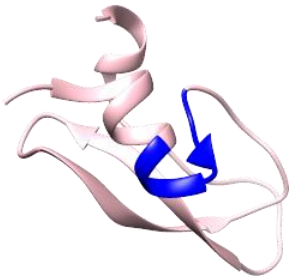
Example – angles

- turn into a list of ϕ, ψ angles
  - $\vec{p}_1$ is $[\phi_1, \phi_2, \dots \phi_6, \psi_1, \psi_2, \dots \phi_6]$
  - get $\vec{p}_2$ for protein 2
  - calculate $d = |\vec{p}_1 - \vec{p}_2|$
  - put $\frac{1}{d}$ into score matrix
    - if two fragments are similar, big positive value for similarity

Why is it nice ?

- works on regular structure or strange structure

Comparing fragments - distances



For each fragment

- look at C^{α} in middle
- get list of distances to C^{α}_{i-3}, C^{α}_{i-2}, …
- the fragment is set of distances

$$\vec{d}_1$$

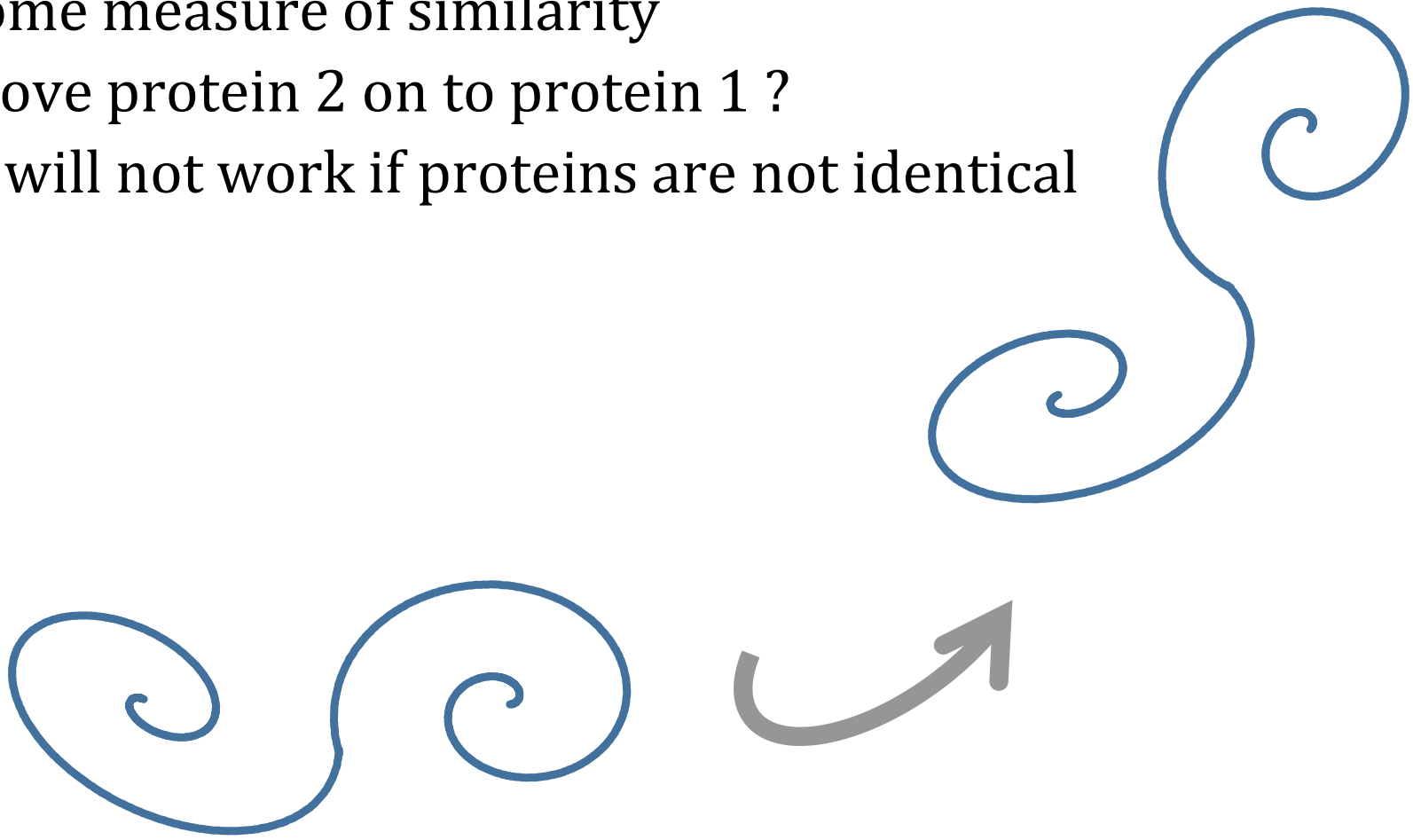
I can compare this vector of distances for different fragments

There will be a set of characteristic distances for

- α-helical fragments, β-sheet, common turns, anything

Optimal alignment

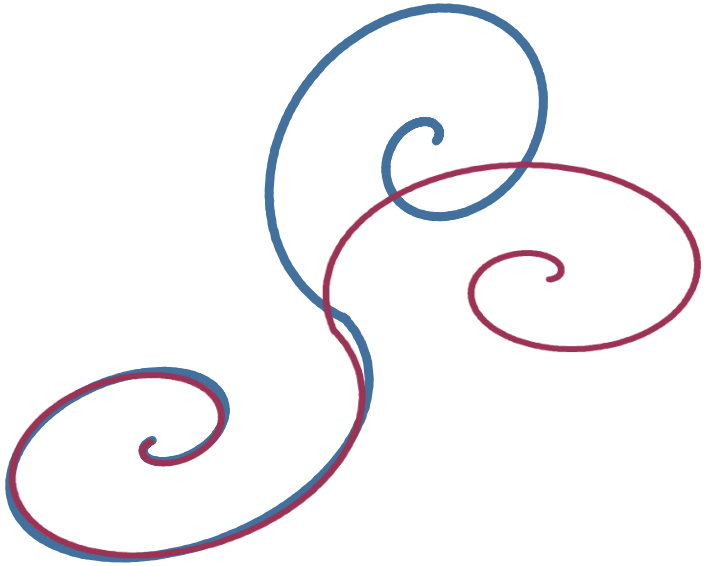
- some measure of similarity
- move protein 2 on to protein 1 ?
  - will not work if proteins are not identical



structure alignments – no correct answer

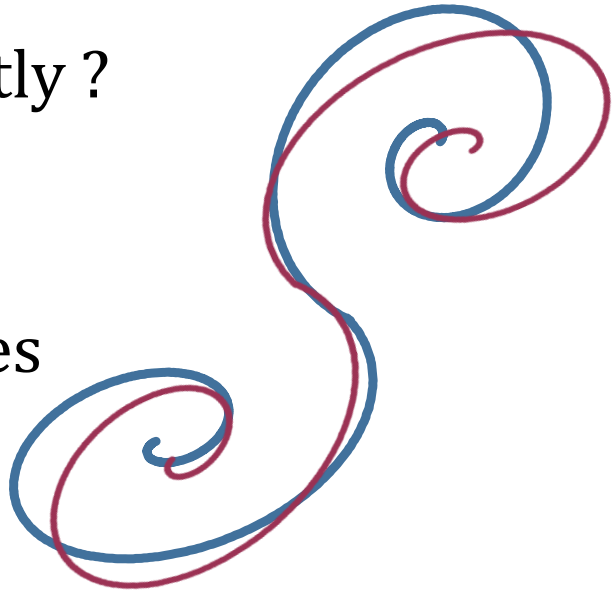
Two very similar proteins

- align parts perfectly



or

- align the whole proteins less exactly ?



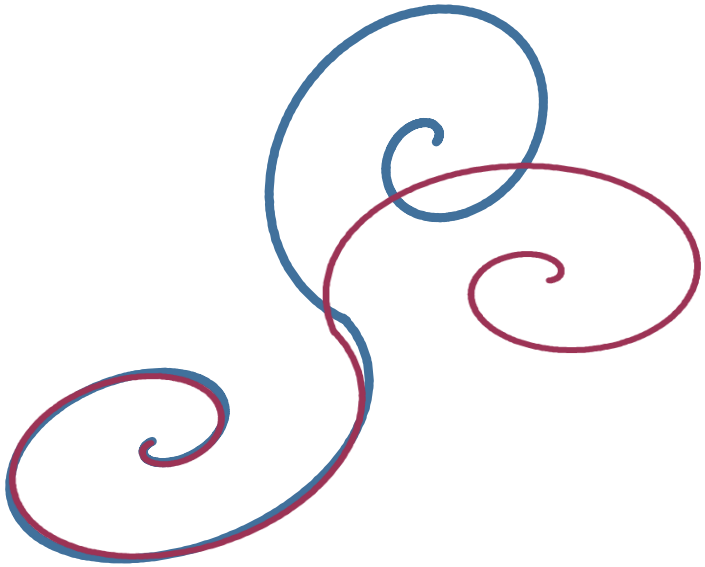
Arbitrary

- how many residues to align

Properties of structure alignments

- Much slower than sequence alignments
  - calculate fragments, angles, distances, ..
- no statistical basis (sequences use exchange frequencies)
- gap penalties – trial and error
- no definition of optimal

Quantifying similarity



Full information – the two proteins

- similar overall shape
- differ in the middle
- must be evolutionarily related
- probably same function

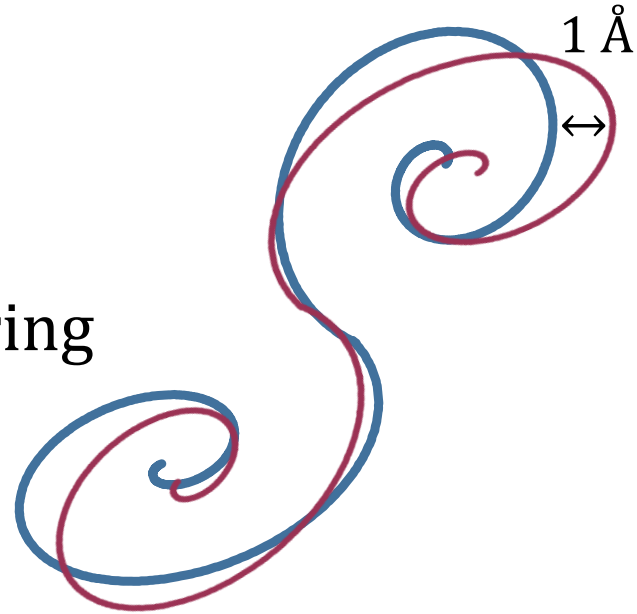
  - too hard

What we work with – one or two numbers

- give an idea of similarity

What do you want to tell me ?

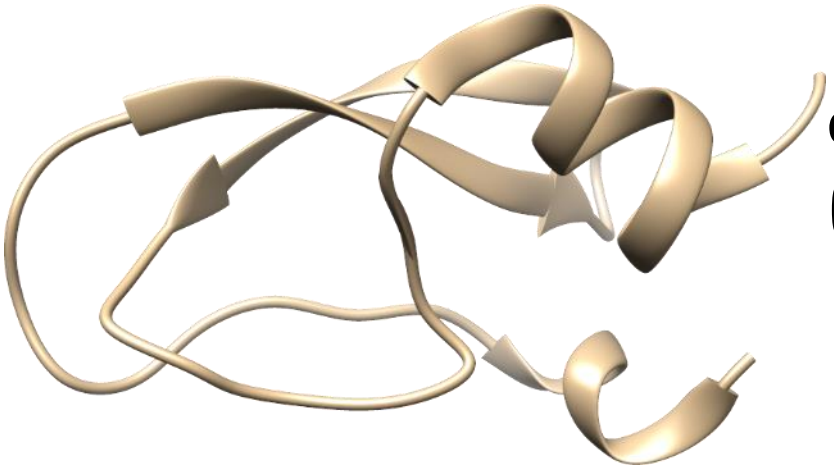
- typical distance between sites



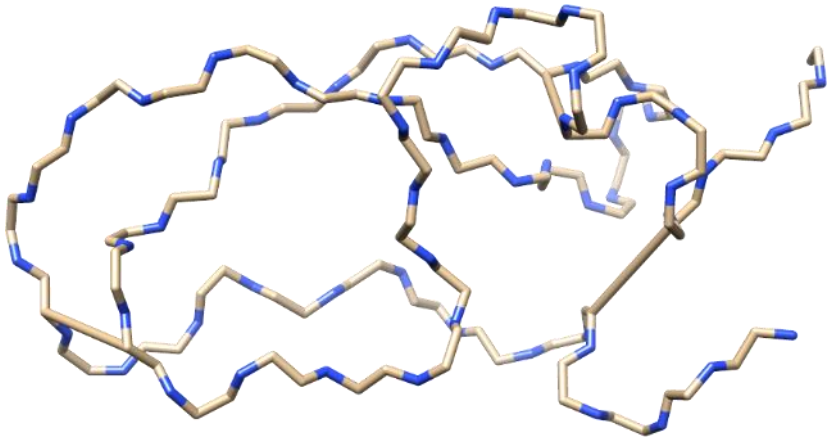
What sites ?

- Serious crystallographers comparing nearly identical structures
  - all atoms
- Most literature comparisons
  - much less …

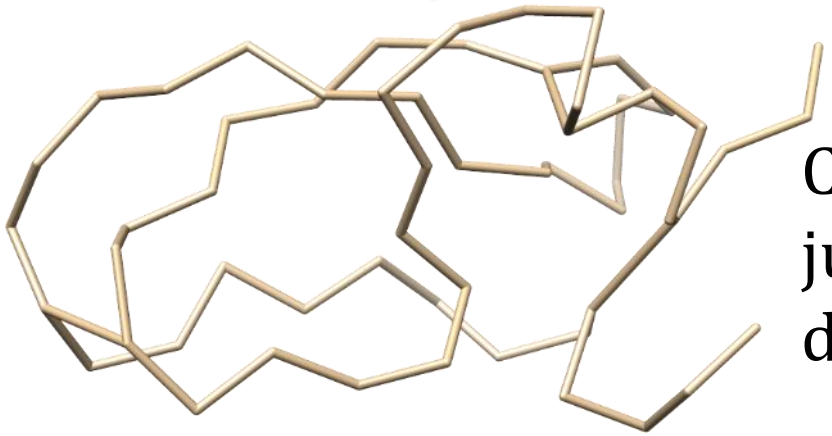
What atoms ?



clear 2° structure
(not a set of atoms)



backbone
N, C^{α}, C



C^{α}

just enough to
describe structure

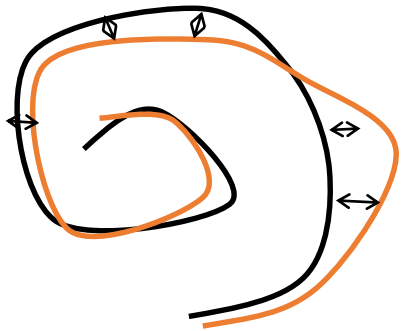
Atoms used

Most common choice

C^{α} atoms

1. present in every residue
2. a set of α carbons nearly defines the shape of a protein

How to get a single number for comparing structures



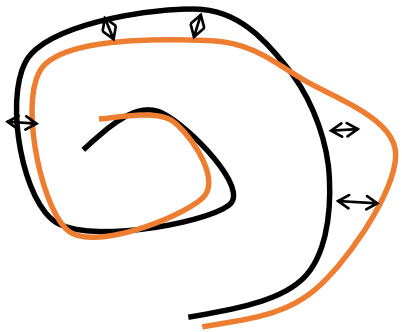
root mean square differences

To characterise the spread in a set of numbers

- standard deviation $\sigma = \left(\frac{1}{N-1}\Sigma_{i=1}^{N}(x_i - \bar{x})^2\right)^{1/2}$
  where $\bar{x}$ is mean

To characterise the structural differences

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

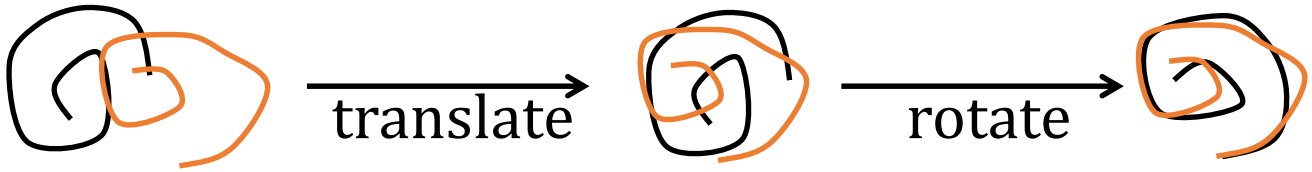


$\vec{r}_i$ is atom coordinates in first structure
$\vec{r}_i'$ coordinates in second structure

- rms / *rmsd* / RMSD / r_{rms}= root mean square difference

Some assumptions

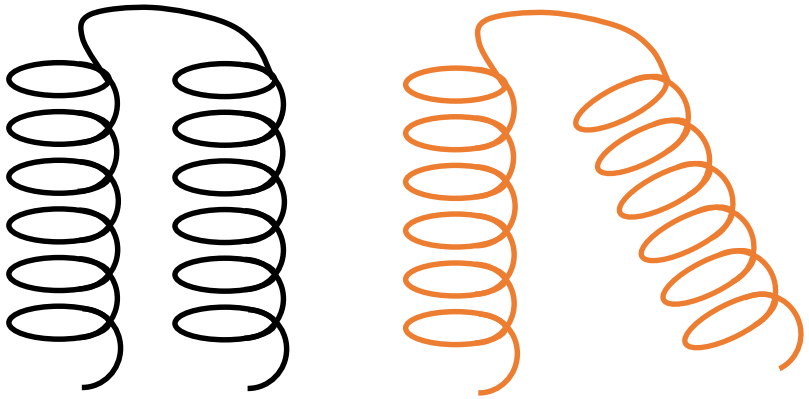
We have already done rotation and translation



We have a list of matching atoms
(from the alignment)

coordinate *rmsd* is evil

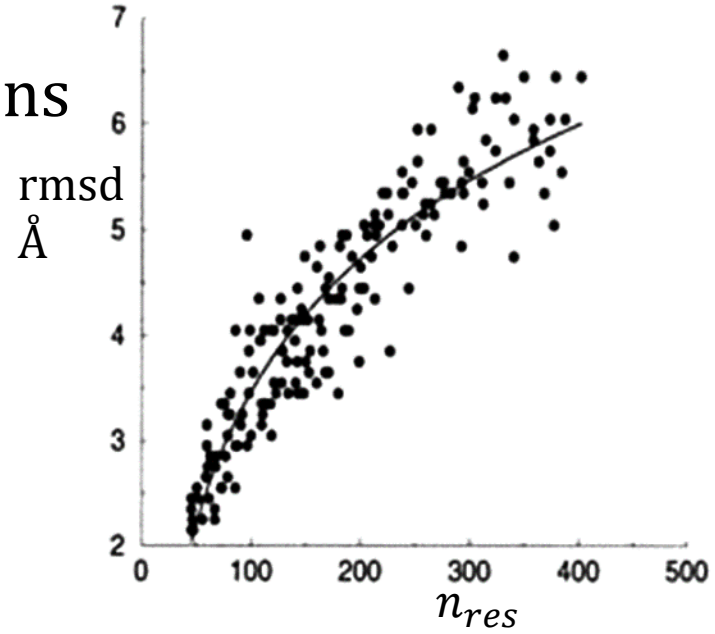
1. sensitive to small changes



2. size dependence - is 5 Å big ?

- for a small protein – yes
- for a big protein – no

- compare *rmsd* from random proteins
- roughly – *rmsd* grows with $n_{res}^{1/3}$



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

many alternatives to rmsd

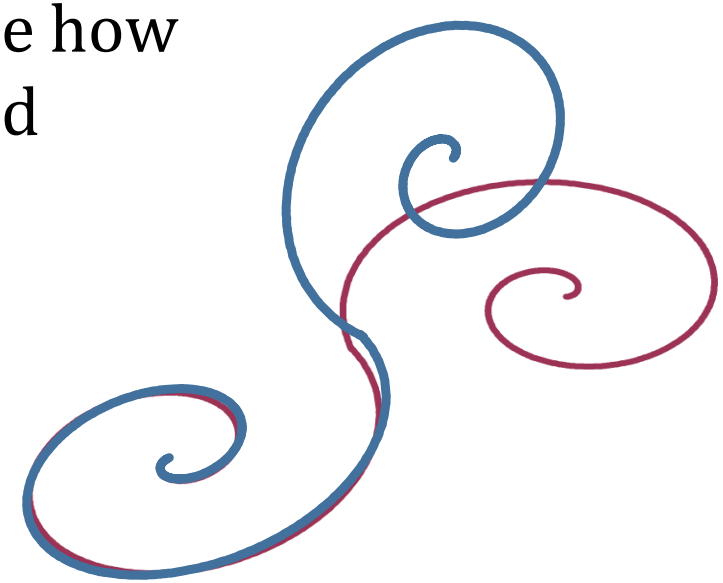
Sociologically important

1. TM-score (template modelling)

- scales distances depends on alignment length

2. GDT-score (global distance test)

- superimpose two structures and see how many residues can be superimposed with *rmsd* < 1 Å
- repeat with 2, 4, 8 Å
- get average



- what is the advantage ?

alternatives to rmsd - advantages

Why use these methods ?

- values from 0 (very different) to 1 (identical)
- less size-dependent

Why not use these methods

- very protein specific
  - assume residues / C^{α} sites
  - not even good for nucleotides
  - no help for small molecules

Summarise

- Why energies are not often used
- What properties does one look at ?
- Physics vs. statistics
- Why structure alignments are difficult
- Different ways to quantify similarity