Protein Struktur

- Biologen und Chemiker dürfen mit Handys spielen (leise)
- go home, go to sleep

- wake up at slide 39

Andrew Torda, Wintersemester 2015/ 2016

Proteins - who cares ?

Most important molecules in life ? Ask the DNA / RNA people

- structural (keratin / hair)
- enzymes (catalysts)
- messengers (hormones)
- regulation (bind to other proteins, DNA, ..)
- industrial – biosensors to washing powder
- receptors
- transporters (O_2, sugars, fats)
- anti-freeze …

Proteins are easy

- data (protein data bank, www.rcsb.org)
  - $\approx 10^5$ files
- literature on function, interactions, structure
- software
  - viewers, molecular dynamics simulators, docking, ..
- nomenclature and rules

Proteins are not friendly

- one cannot take a sequence and predict structure/function
- data formats are full of surprises
- data contains error and mistakes

Protein Rules, Physics, Folklore

Physics / Chemistry

- protein + water = set of interacting atoms
  - can be calculated (not really)

Rules (not quantified)

- proteins unfold if you heat them (exceptions ?)
- many charged amino acids.. they are soluble
- if they are more than 300 residues, they have more than one domain,
- proteins fold to a unique structure (could you prove this ?)
  - lowest free energy structure

Protein chemistry

Chemists / biochemists

- sleep, go home
- one tiny surprise at the end of the lectures

Short version

- proteins are sets of building blocks (amino acids, residues, Reste)
- 20 types of residue
- chains of length few to 10^3 (100 or 200 typical)
- small ones (< ≈50 residues) are peptides
- they fold up to nice stable structures – why ?

Longer version..

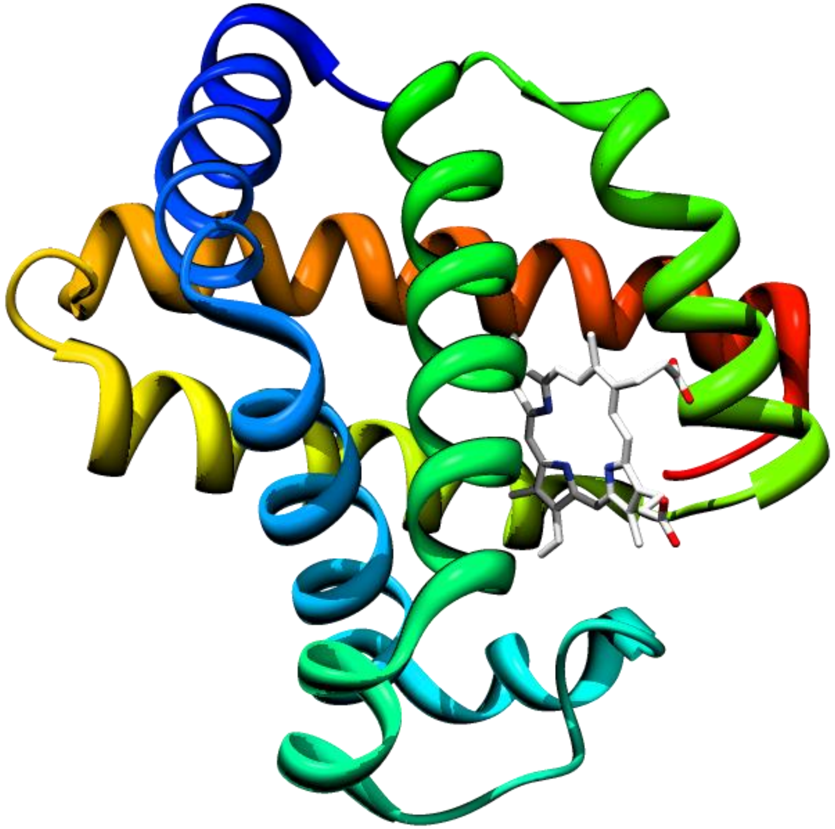
The Plan

- polymers
- different kinds of sidechain
- structure due to backbone (secondary strucure)
- properties of sidechains
- representation

Sizes

1 Å = 10^{-10} m or 0.1 nm

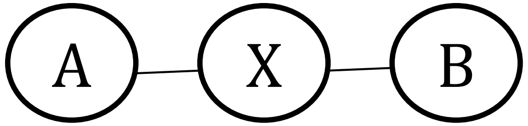
structure		size
bond	CH	1 Å
	CC	1.5 Å
protein radius		10 - 10^2 Å
α-helix spacing		5 ½ Å
C^{α}_{i} to C^{α}_{i+1}		3.8 Å



myoglobin picture 2w6w

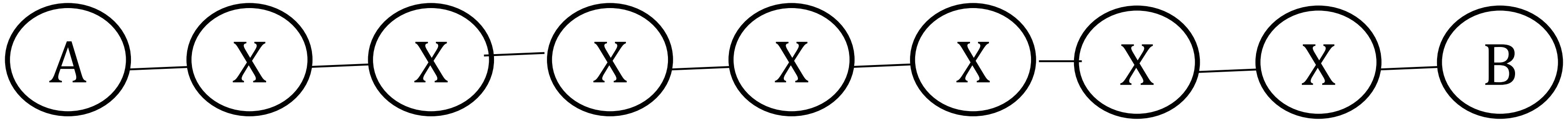
Proteins are polymers

- simple polymers



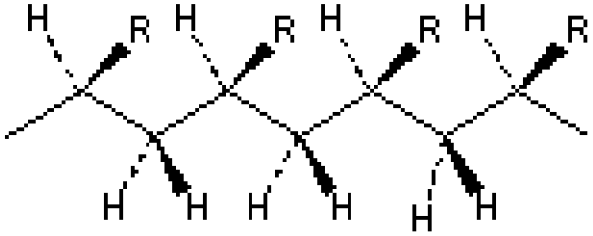
A X B

many times gives



A X X X X X X X B

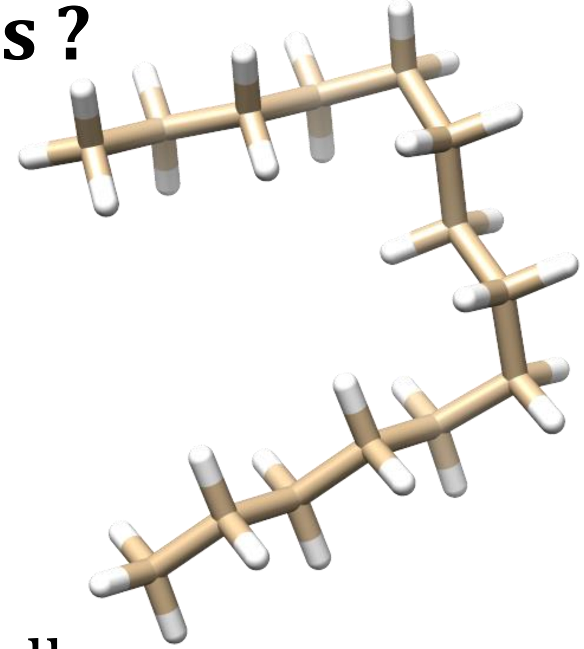
example



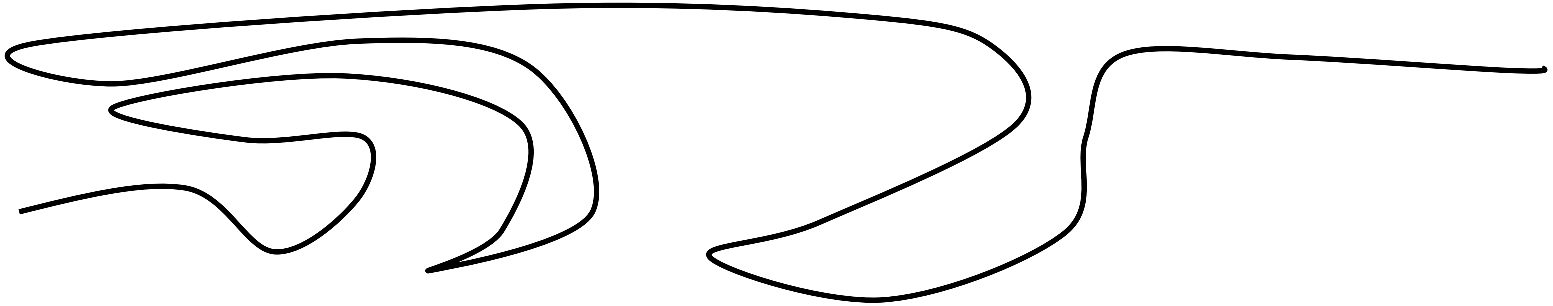
what kind of polymer would this give ?

Do you know what R is ?

Why are proteins interesting polymers ?

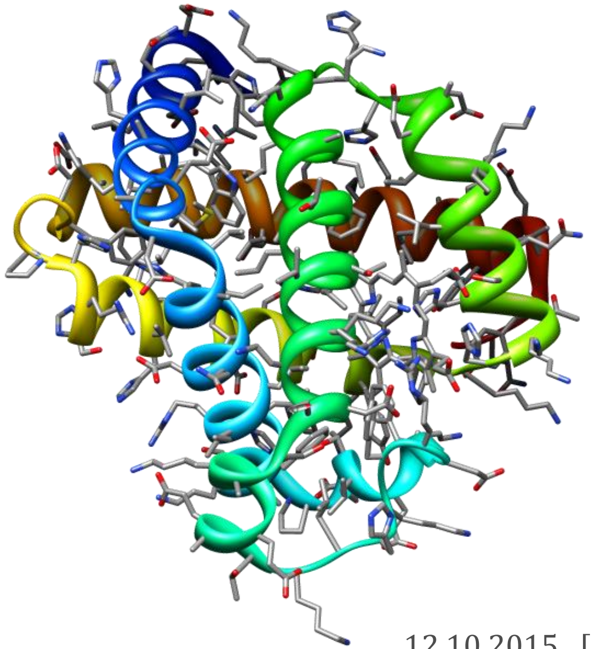


Boring polymer gives irregular structures



Each part of polymer wants to interact with all other parts equally

- no structural preferences
- plastic bags, Haushaltsfolie
- no regular structures

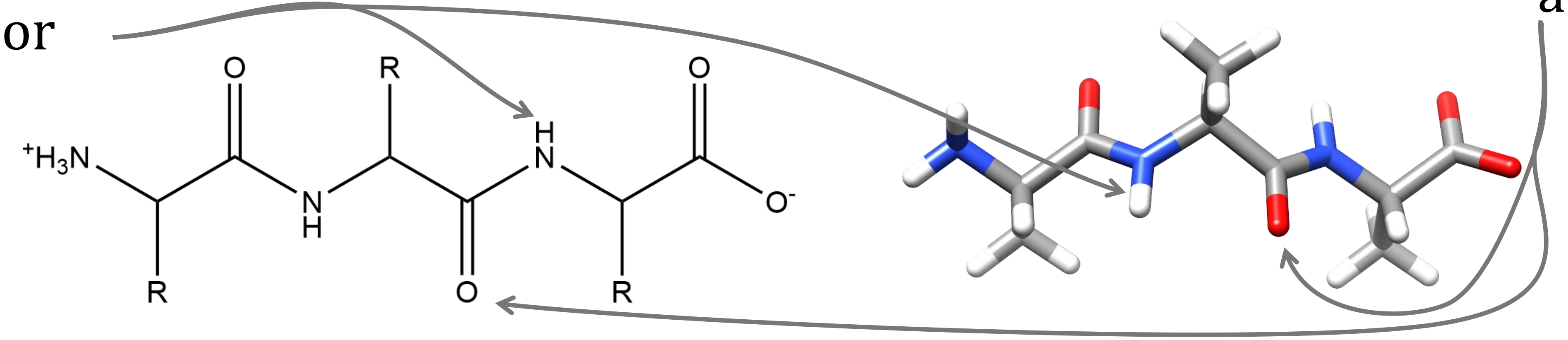


Properties that make proteins different from plastics ..

Giving proteins character 1

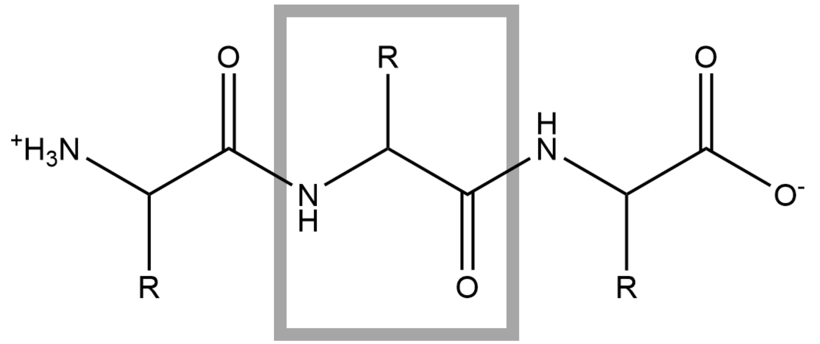
More complicated backbone with H-bond

donor



acceptor

- basis of standard regular structures in proteins (secondary structure)
- repeating polymer unit:

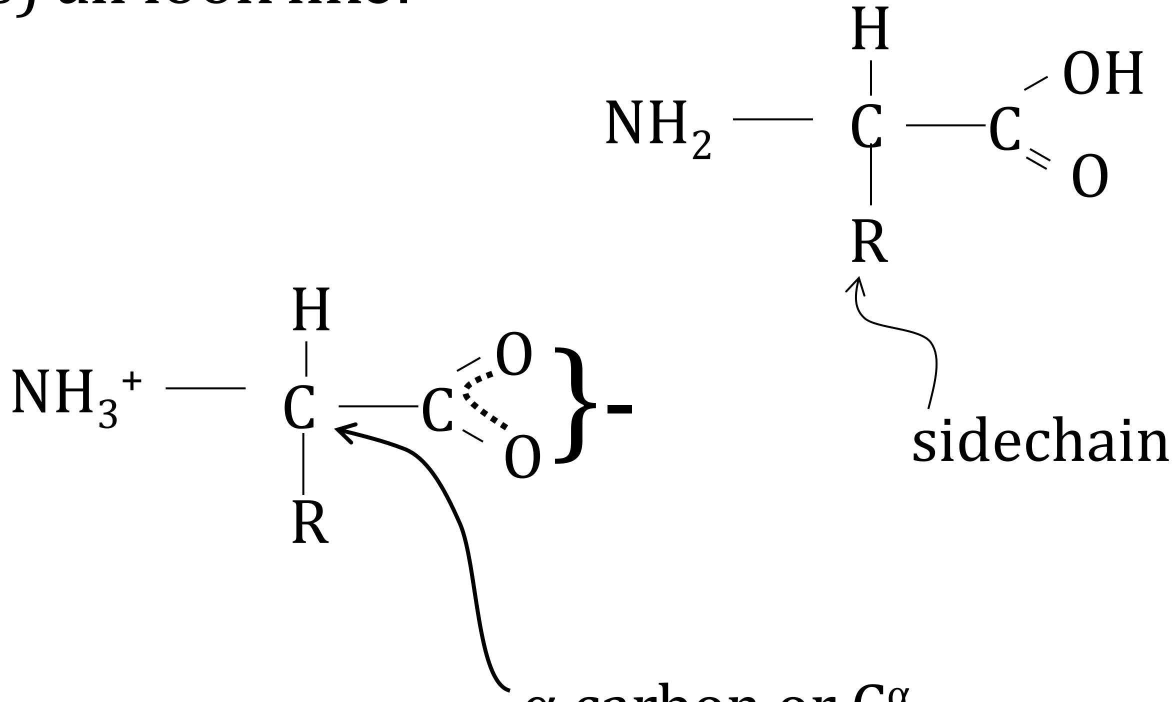


If this was all there was

- all proteins would be the same

protein chemistry

amino acids (monomers) all look like:



sidechain

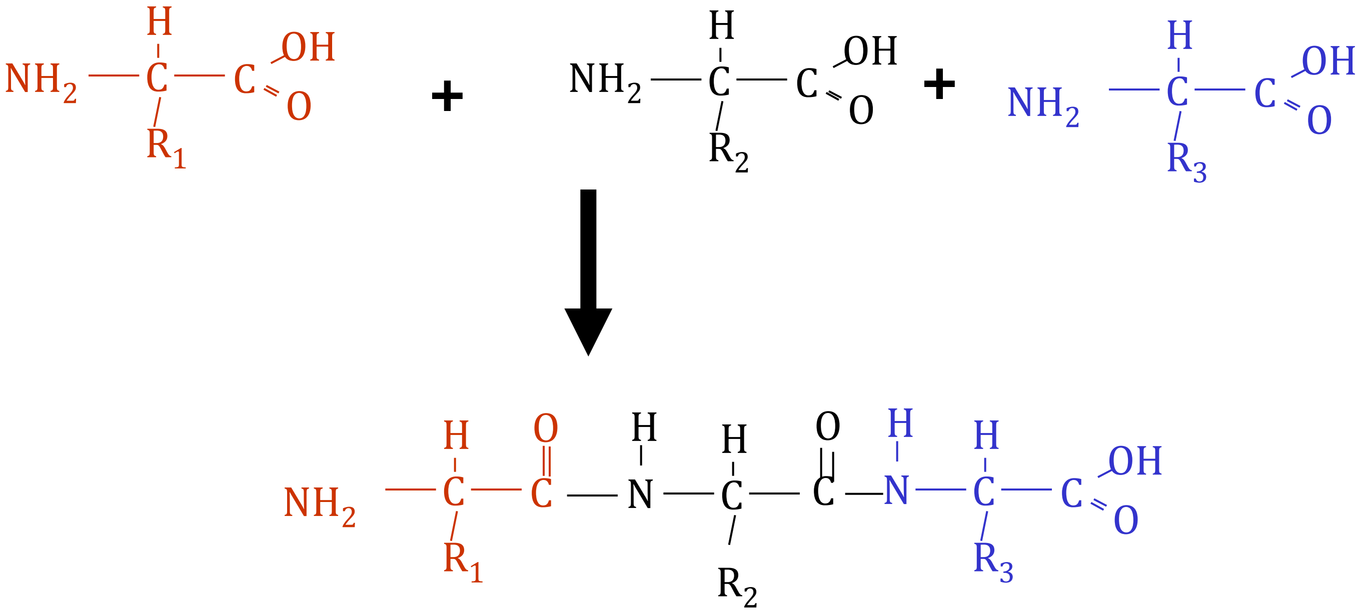
maybe

α carbon or C^{α}

How can we construct specific structures ?

- different kinds of "R" groups

Putting monomers together



- protein synthesis story (biochemistry lectures)
- peptides and proteins
  - < 30 or 40 residues = peptide
  - > 30 or 40 residues = protein

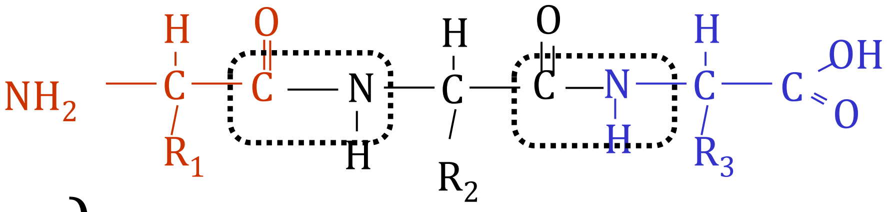
Backbone peptide bonds

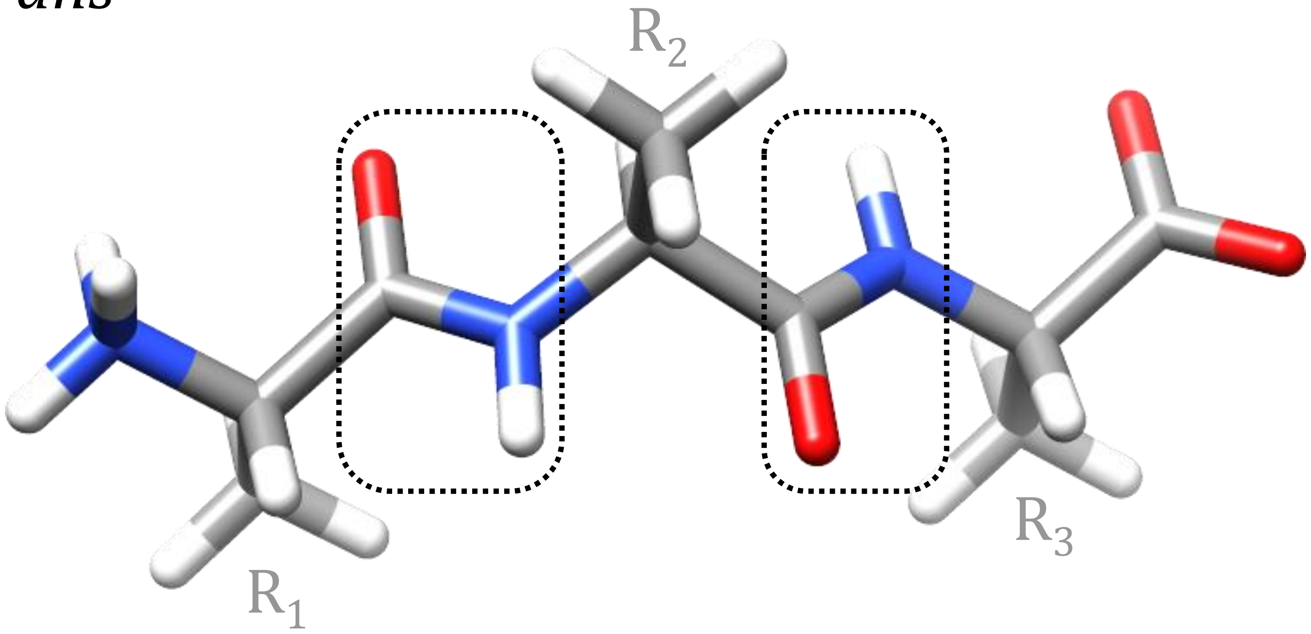
How many backbone angles ?

- 3 (ϕ, ψ, ω)

Peptide bond ω is planar

- partial double bond character (resonance forms)
- shorter than other C-N
- nearly always *trans*

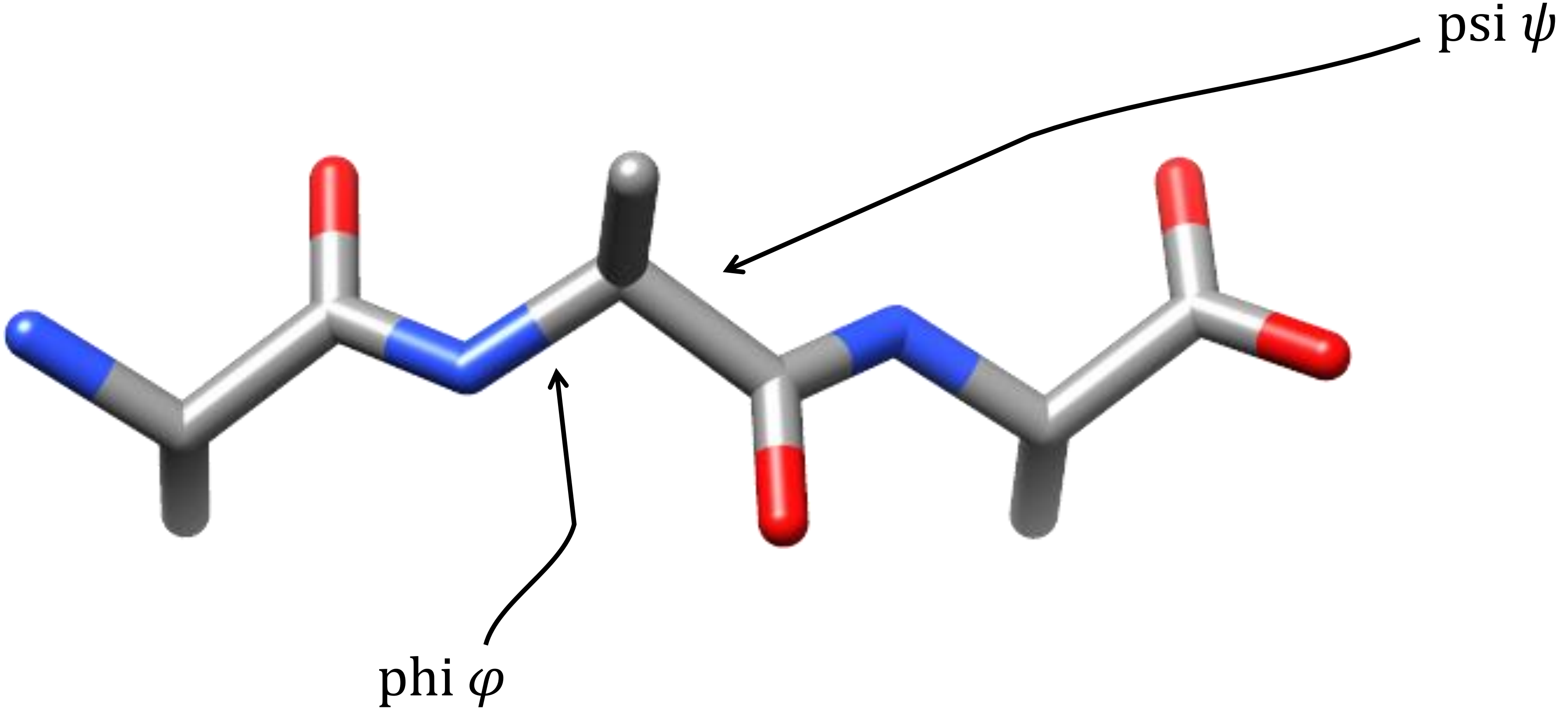




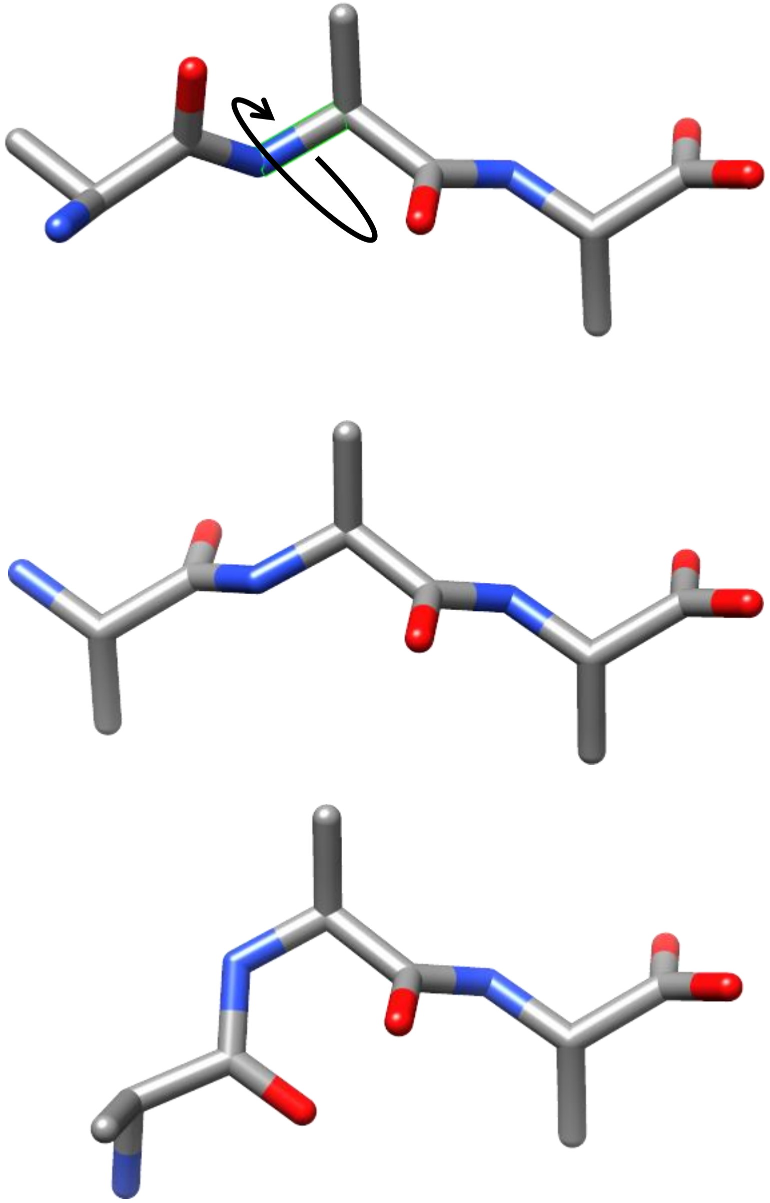
Note:
usually we
do not draw
H atoms

Backbone rotatable angles

Two rotatable angles ϕ, ψ



some ϕ rotations



can we rotate freely ?

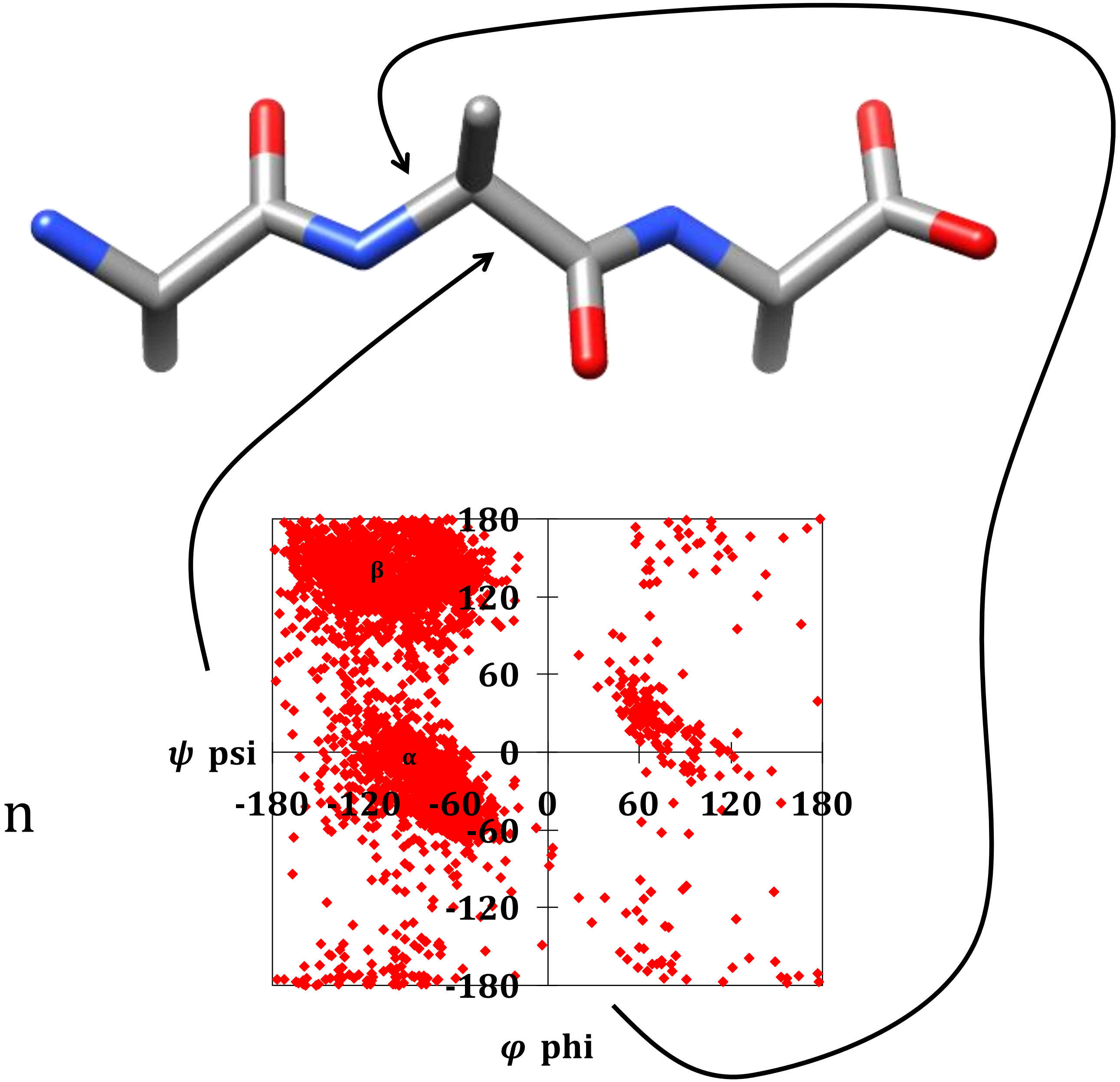
- no… steric hindrance

- look at bottom – two unhappy O atoms

ramachandran plot

can we rotate freely ?

- no… steric hindrance

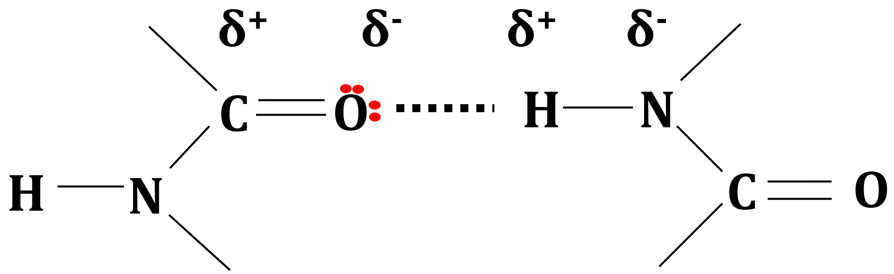


Ramachandran plot

will reappear very often

Backbone H bonds

- oxygen is slightly negative
- NH bond is polar



H-bonds

- can be near or far in sequence
- fairly stable at room temperature

Secondary structure

Regular structures using information so far

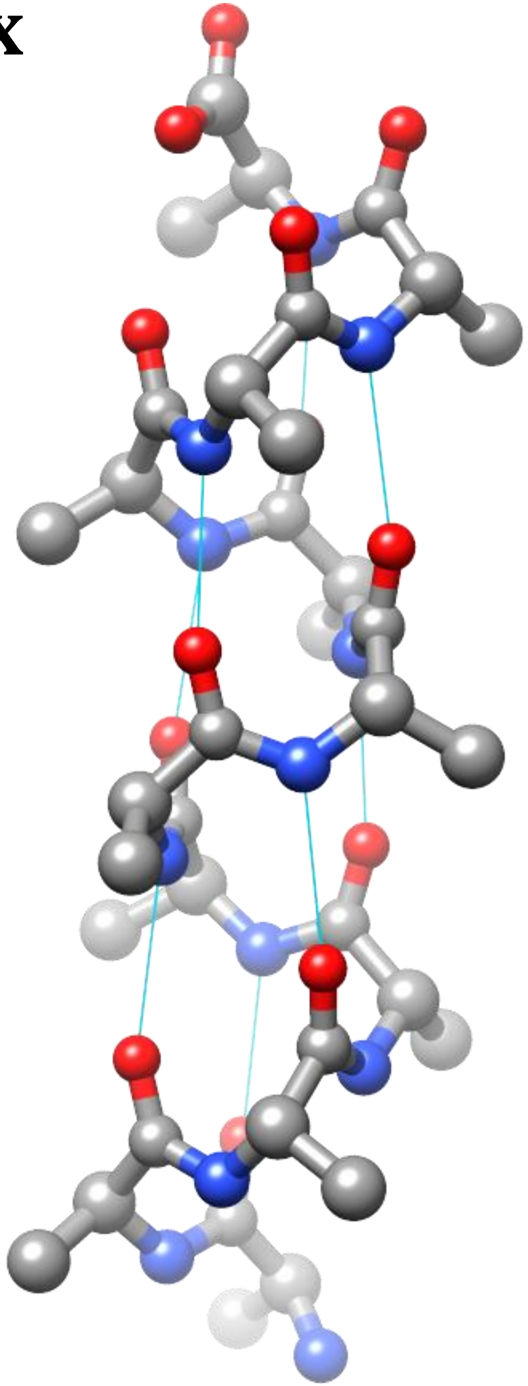
- rotate phi (φ), psi (ψ) angles so as to
    - form H-bonds where possible
    - do not force side chains to hit each other (steric clash)

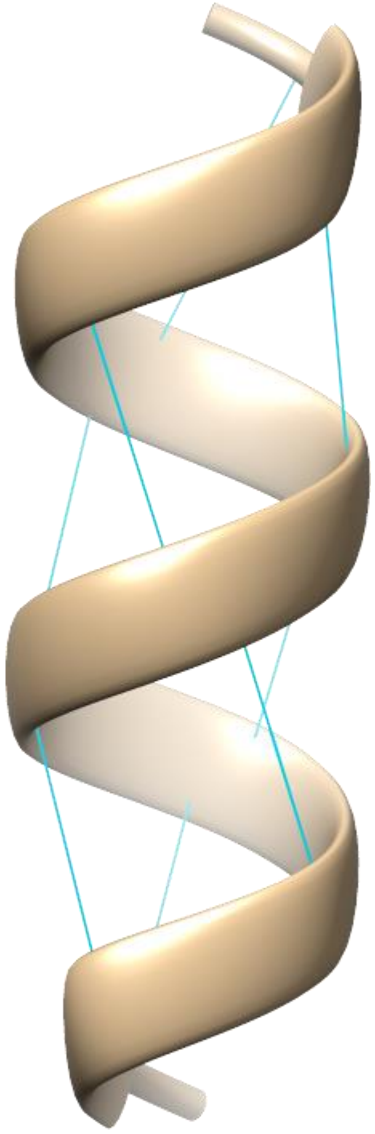
Two common structures

- α-helix
- β-strand / sheet

α helix

- each CO of residue *i* H-bonded to N of *i*+4
- 3.6 residues per turn
- 2 H-bonds per residue
- side chains well separated





β-sheet

β-strand

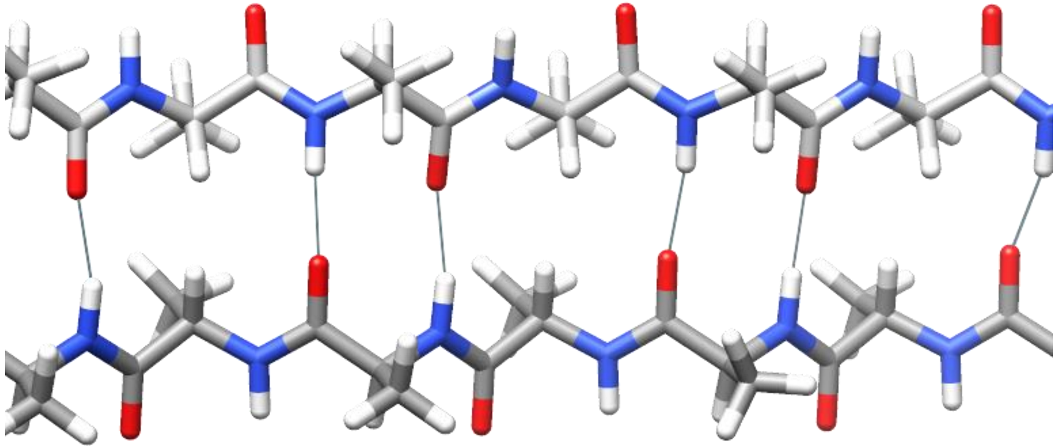
- stretch out backbone and make NH and CO groups point out

β-sheet

- join these strands together with H-bonds (2 H-bonds/residue)

anti-parallel

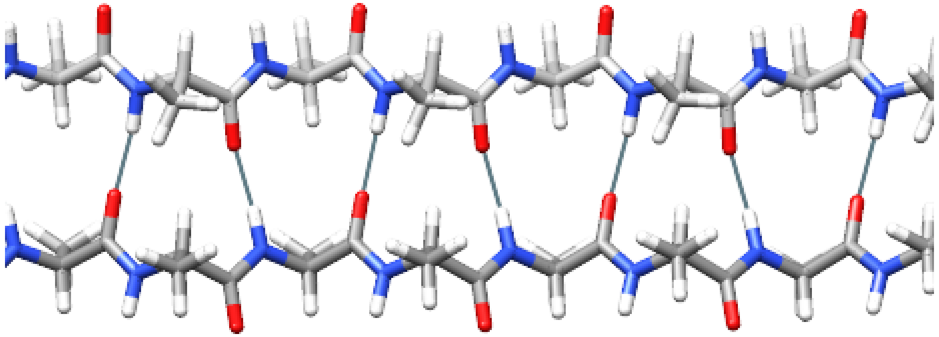
N⟶C



C⟵N

N⟶C

or parallel



N⟶C

After α-helix and β-sheet

Do helices and sheets explain everything ?   No

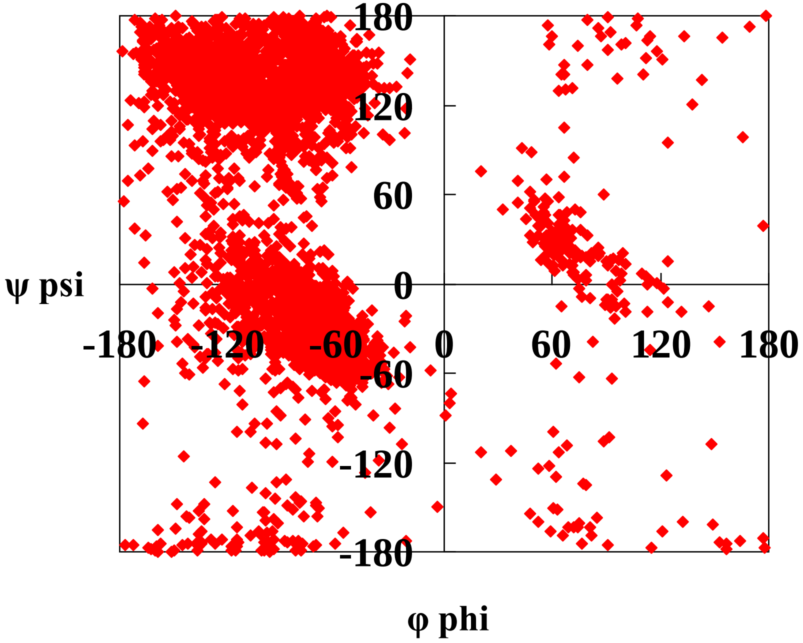
- there is flexibility in the angles (look at plot)
  - geometry is not perfectly defined
- there are local deviations and exceptions

Other common structures

- tighter helices
- some turns

Other structure

- coil, random, not named



What determines secondary structure ?

So far

- secondary structure pattern of H-bonding

Almost all residues have H-bond acceptor and donor

    - almost all could form α-helix or β-sheet

Difference ?

    - sequence of side-chains – overall folding

Why else are sidechains important

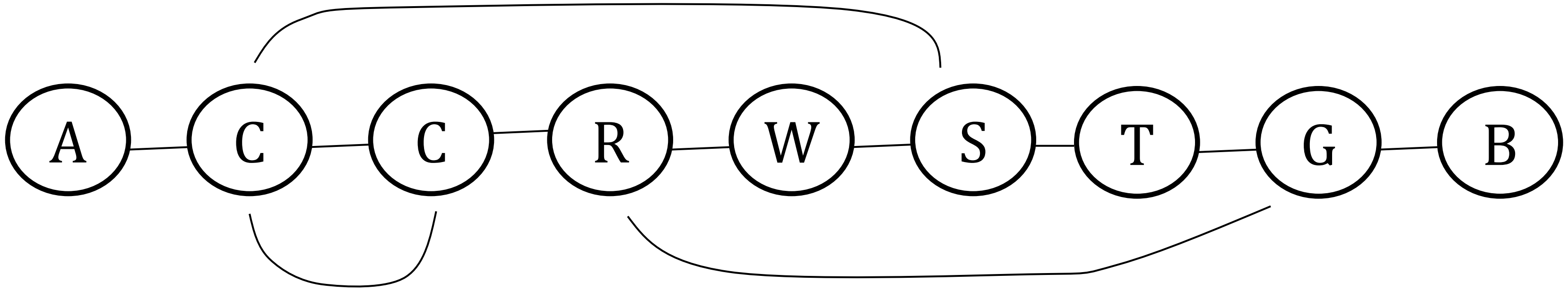
- chemistry of proteins (interactions, catalysis)

Fundamental dogma

- the sequence of sidechains determines the protein shape

side chain possibilities

- big / small
- charged +, charged -, polar
- hydrophobic (not water soluble), polar
- interactions between sites…



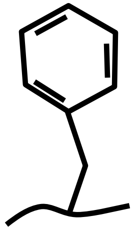
Side chain properties

properties

- big / small
- neutral / polar / charged
- special (…)

example

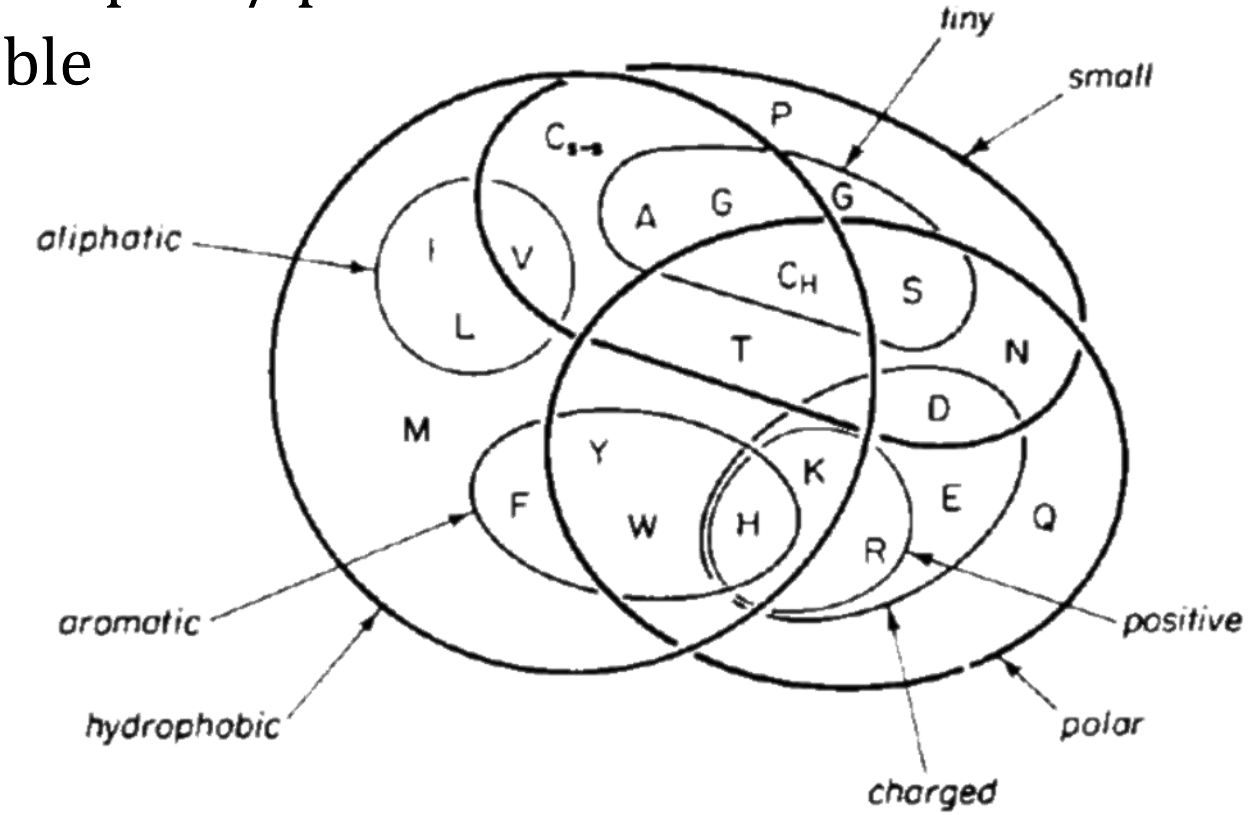
- phenylalanine side chain looks like benzene (benzin)
  - very insoluble
  - benzene would rather interact with benzene than water
  - what if you have phe-phe-phe… poly-phe ?
    - does not happen in nature (can be made)
    - would be insoluble
    - not like a real peptide
  - phe is a constituent of real proteins – has a role



Properties are not clear cut

You can be big / small, hydrophic / polar

- combinations are possible



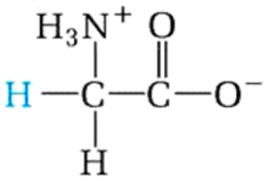
Do not memorise this figure

Taylor, W.R. (1986) J. Theor. Biol., 215-218

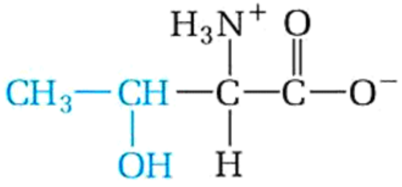
Sidechain interactions

- ionic (if the sidechains have charge)
- hydrophobic (insoluble sidechains)
- H-bonds (some donors and acceptors)
- repulsive

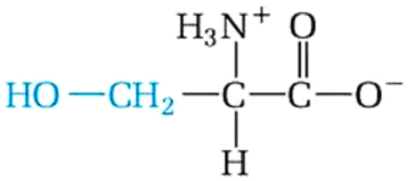
Summary of amino acids (first dozen)



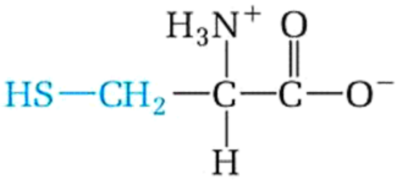
Glycin (Gly)



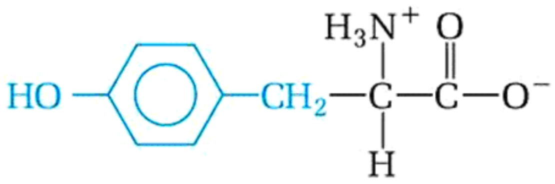
Threonin (Thr)



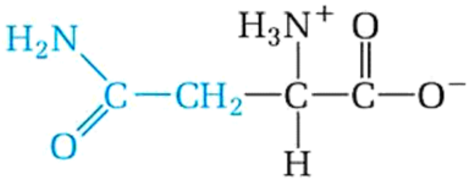
Serin (Ser)



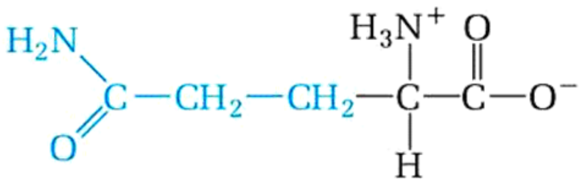
Cystein (Cys)



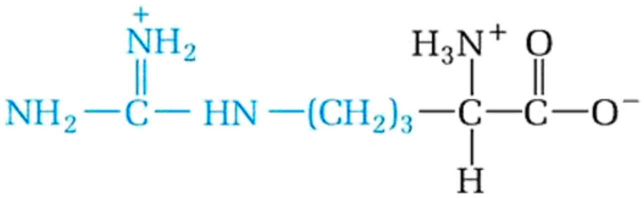
Tyrosin (Tyr)



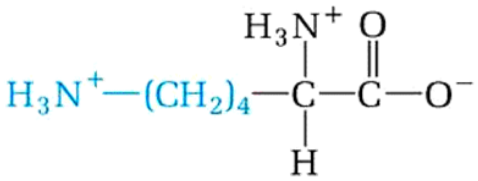
Asparagin (Asn)



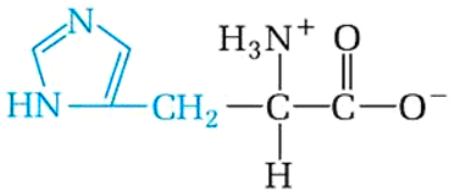
Glutamin (Gln)



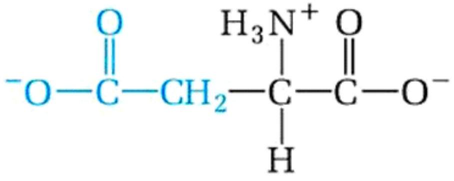
Arginin (Arg)



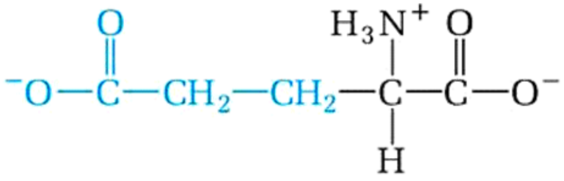
Lysin (Lys)



Histidin (His)

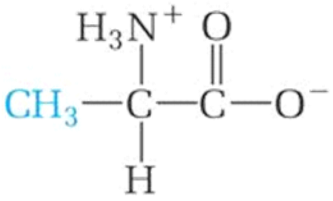


Asparaginsäure (Asp)

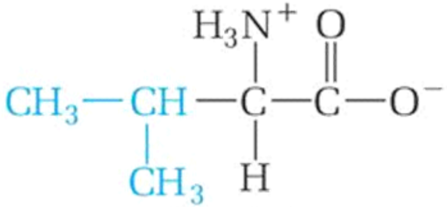


Glutaminsäure (Glu)

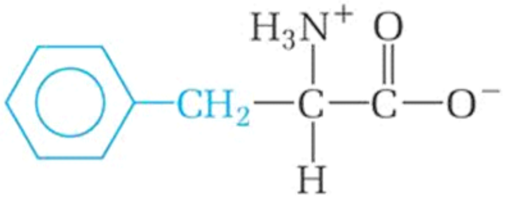
summary of amino acids (part 2)



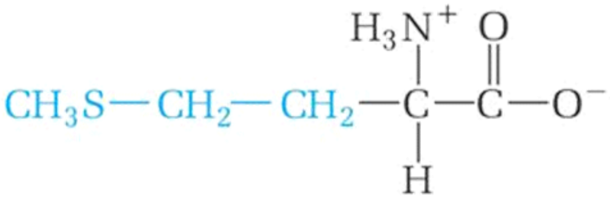
Alanin (Ala)



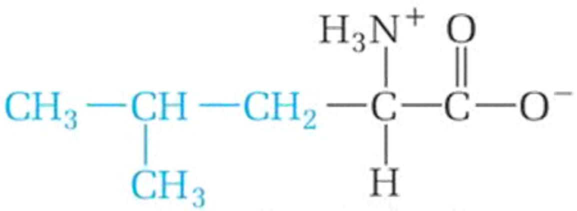
Valin (Val)



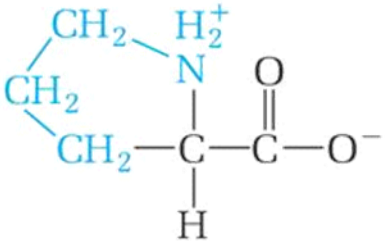
Phenylalanin (Phe)



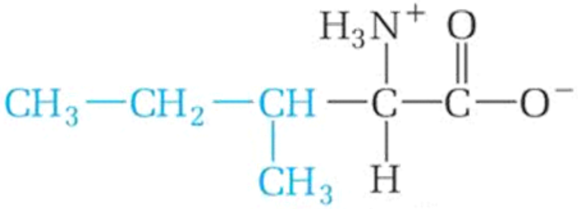
Methionin (Met)



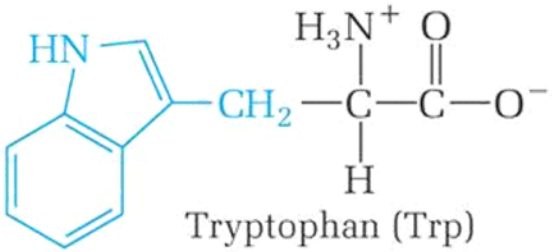
Leucin (Leu)



Prolin (Pro)



Isoleucin (Ile)

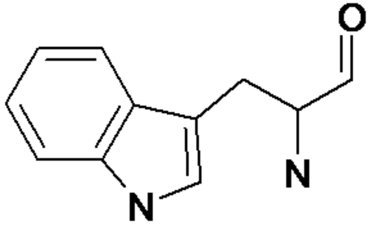


Tryptophan (Trp)

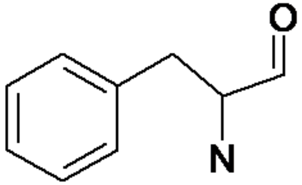
Amino Acids by property

aromatic

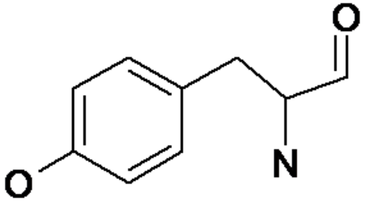
tryptophan



phenylalanine

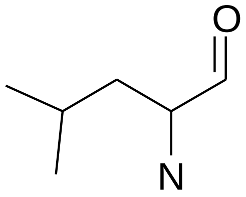


tyrosine

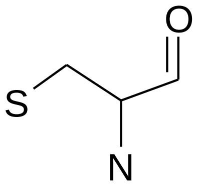


rather hydrophobic

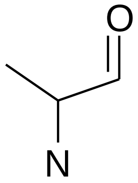
leucine



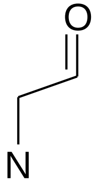
cysteine



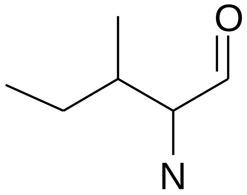
alanine



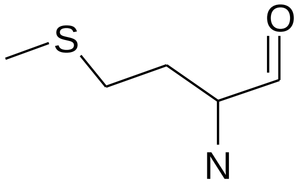
glycine



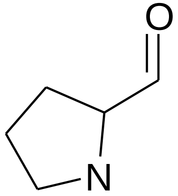
isoleucine



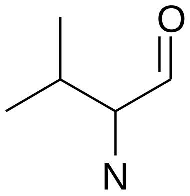
methionine



proline

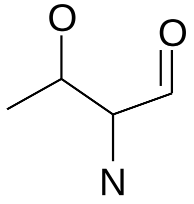


valine

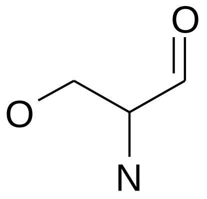


Polar

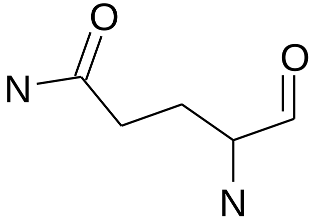
threonine



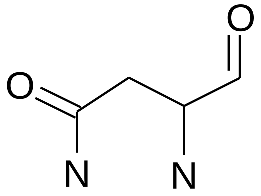
serine



glutamine

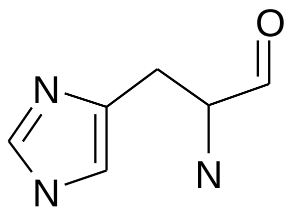


asparagine

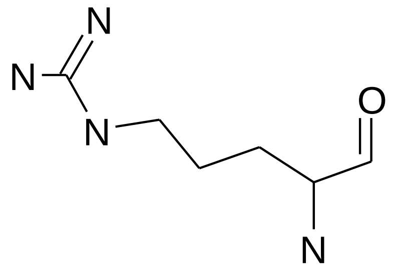


charged

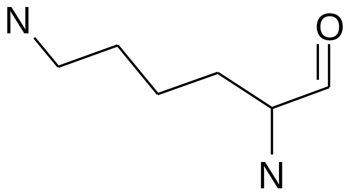
histidine



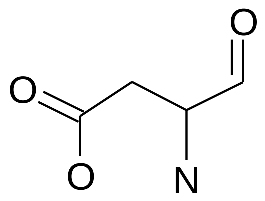
arginine



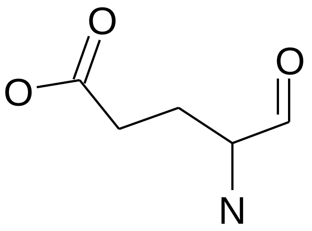
lysine



aspartate



glutamate



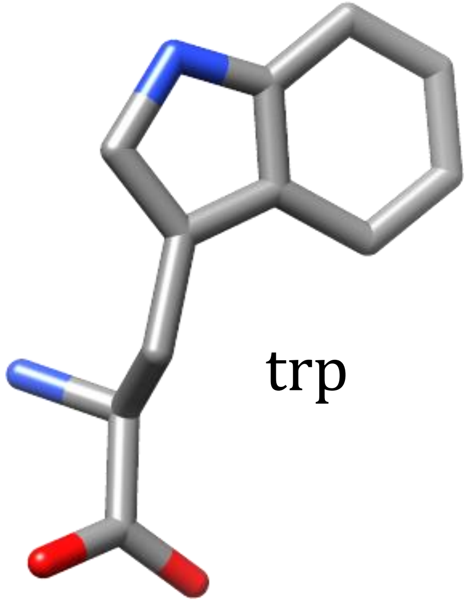
- Muss ich alle Strukturen für die Klausur wissen ?

Hydrophobicity – how serious ?

Very serious, but simplified

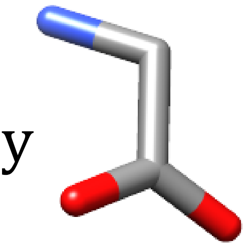
- the lists above are
  - pH dependent
  - difficult to measure experimentally (some aspects)
- Is there a single definition for hydrophobicity ?

Other properties - size

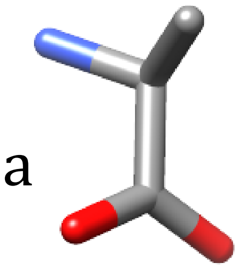


trp

big … small



gly

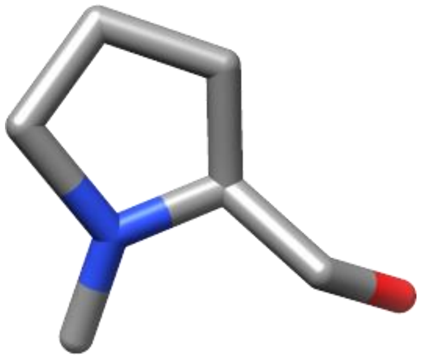


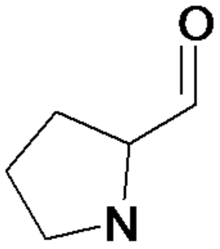
ala

Other properties – chemistry / geometry

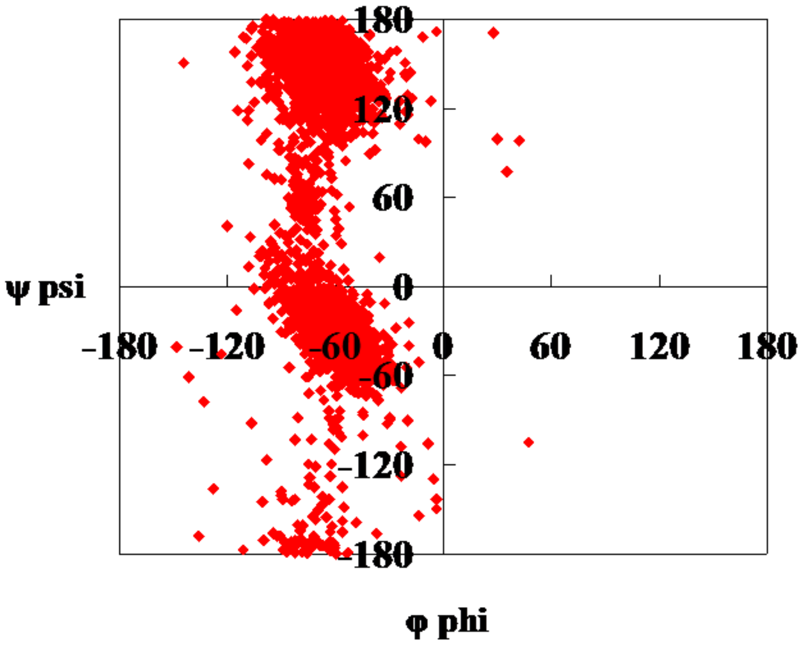
Proline

- only one rotatable angle !
- peptide bond sometimes *cis*

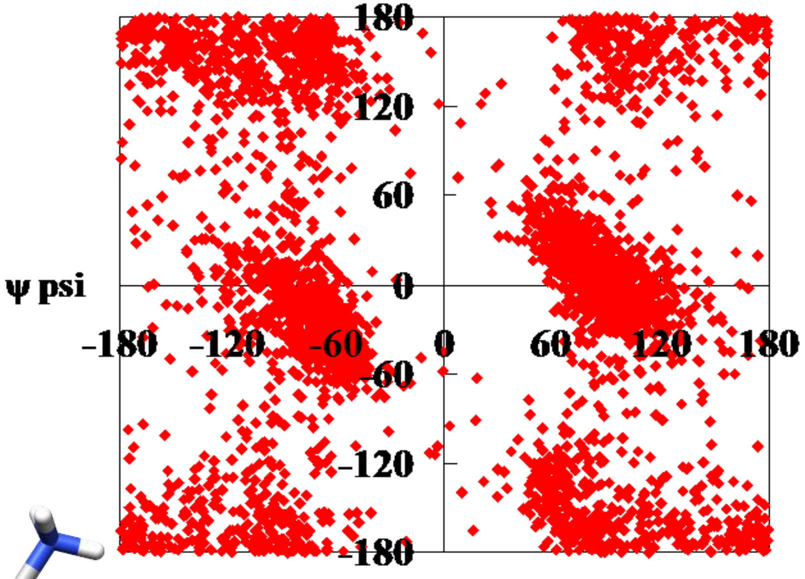




- pro ramachandran plot



gly and cys

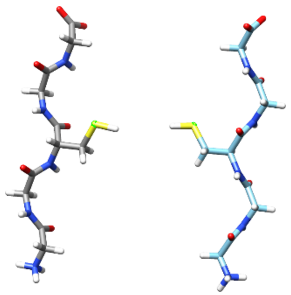


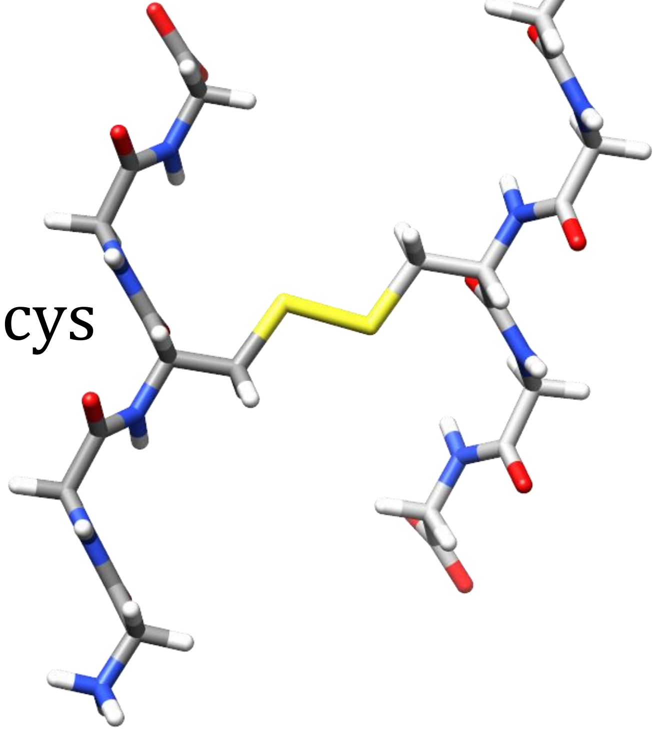
glycine

- no side chain
- can visit forbidden parts of phi-psi map

cysteine

- forms covalent links with other cys





Summary so far

- proteins are heteropolymers
- backbone forms α-helices and β-strands (and more)
  - not sequence specific
- side-chains determine the
  - pattern of secondary structure
  - overall protein shape
- special amino acids
  - cys (forms disulfide bridges)
  - gly (can visit "forbidden" regions of ramachandran plot)
  - pro (no H-bond donor)
- how many sequences can one have ? 20^n

Nomenclature

Some rules are unavoidable

Alanine	Ala	A
Cysteine	Cys	C
Aspartic acid	Asp	D
Glutamic acid	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asn	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y

Always write from N to C terminal (convention)

Definitions, primary, secondary …

More definitions

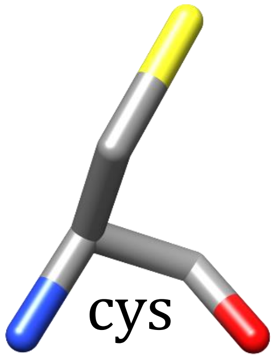
- primary structure
  - sequence of amino acids
    - ACDF (ala cys asp phe…)
- secondary structure
  - α-helix, β-sheet (+ few more)
    - structure defined by local backbone
- tertiary structure
  - how these units fold together
  - coordinates of a protein

distributions of residue types

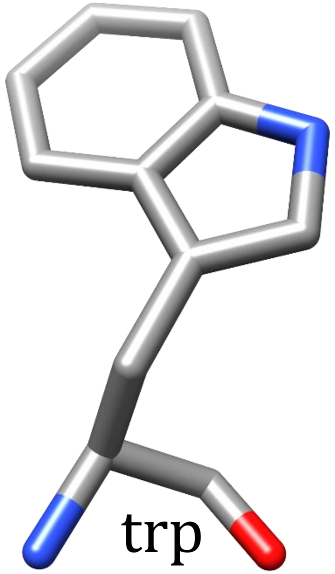
Surprise coming

- 20 amino acid types – are they all equally common ?
- Are you made of $^1/_{20}$= 5 % of ala, leu, cys, … ?

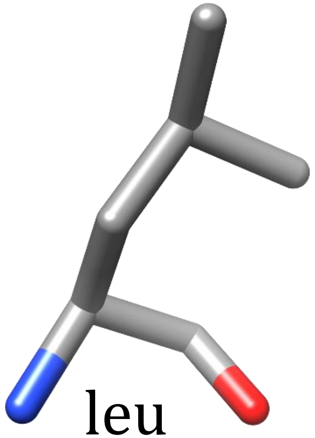
rare



cys

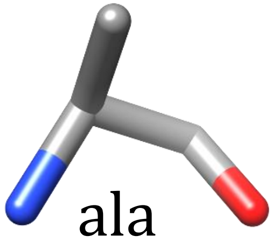


trp



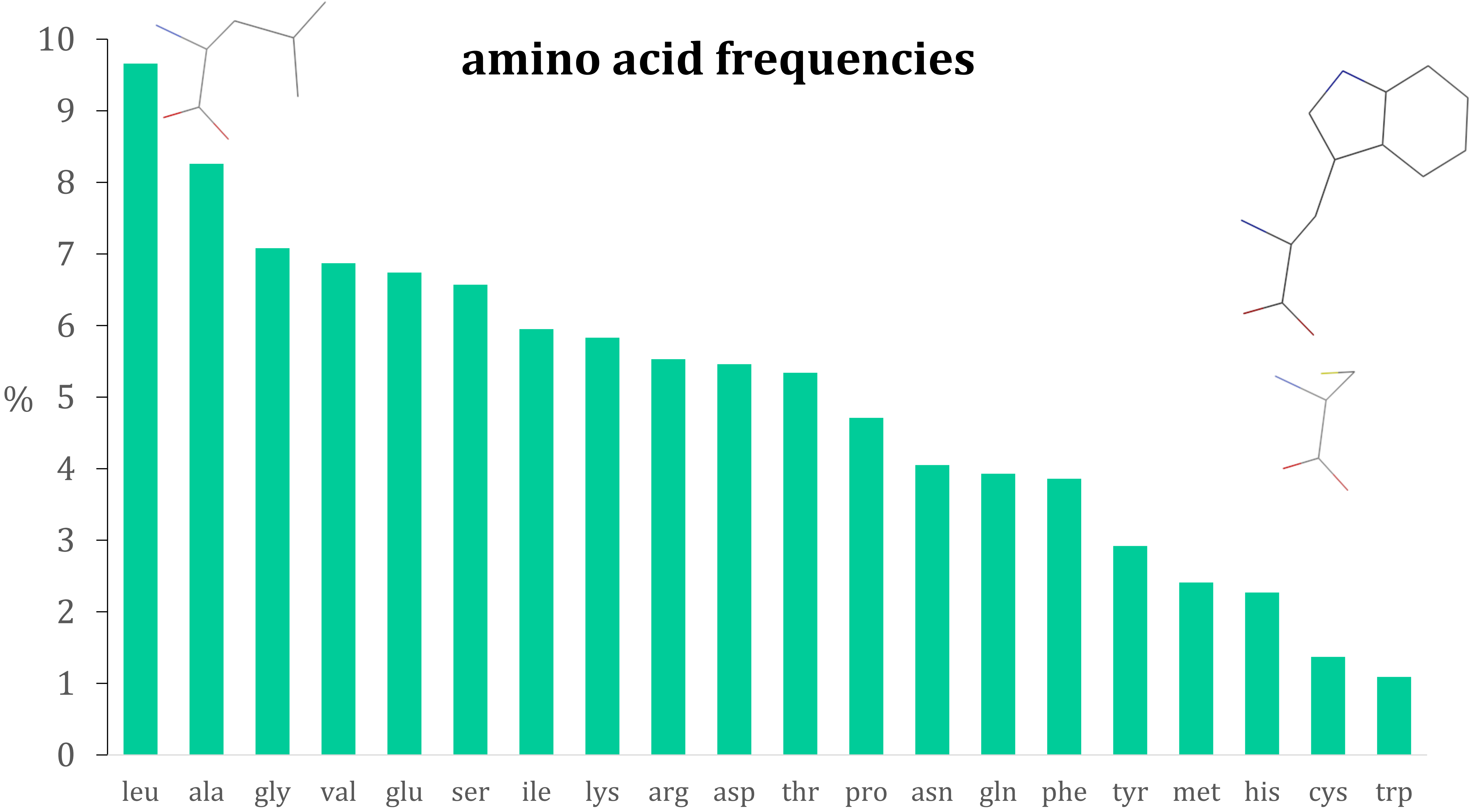
leu

common



ala

amino acid frequencies



swissprot (2014)

What would Darwin say ?

Why ?

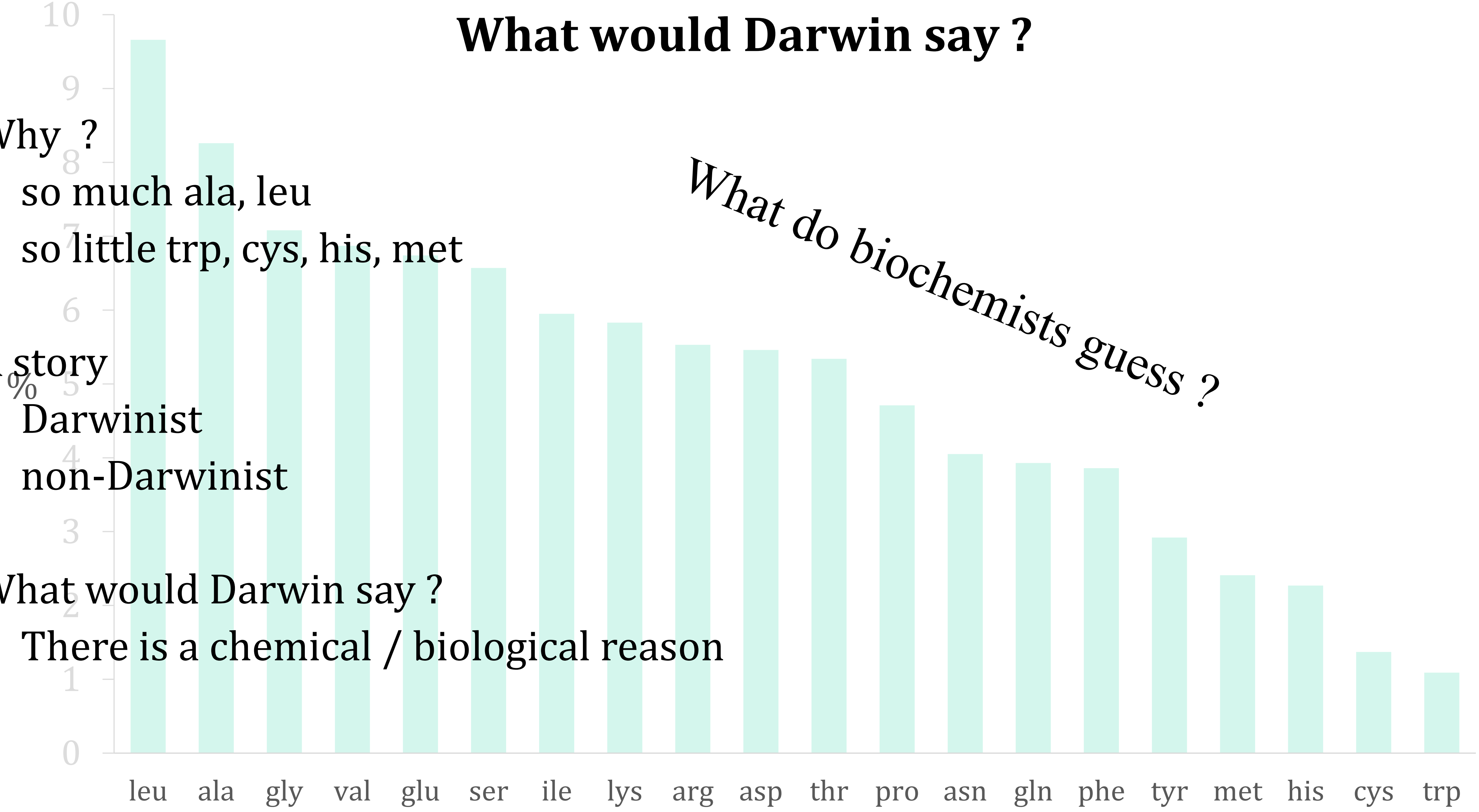
- so much ala, leu
- so little trp, cys, his, met

A story

- Darwinist
- non-Darwinist

What would Darwin say ?

- There is a chemical / biological reason



What do biochemists guess ?

Think Darwinist

Empirical fact

- trp, cys, met are rare in proteins

Consequence

- too much trp is bad for you / expensive / dangerous

Possibilities

- metabolic cost issues
  - does it cost energy / nutrients to make trp ? cys with its sulfur ?
- protein structure – lots of chemical differences between amino acid types
  - if you put lots of trp / cys / met in a protein
    - does it not fold ?   Does it become unstable ?
- if free trp toxic ?

Common amino acids

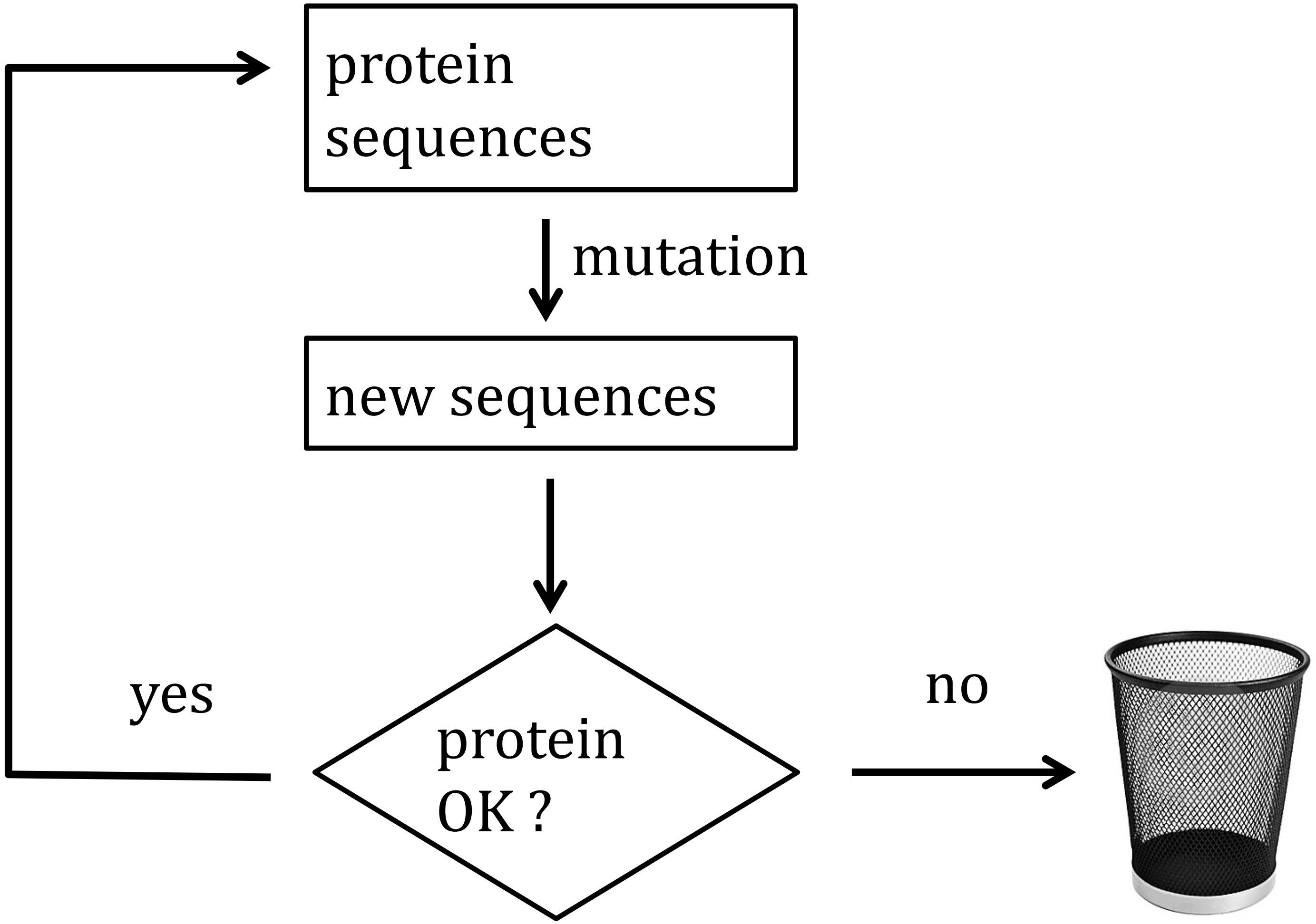
Leu and ala

- cheap to synthesise ?
- do you get them as by-products from other biochemistry ?
- what is their advantage in protein structure ?
    - stability ? rigidity ? flexibility ?

Forget Darwin – think neutral evolution

- what do we mean by Darwinism ?

Very Darwinist



protein sequences

mutation

new sequences

yes

protein OK ?

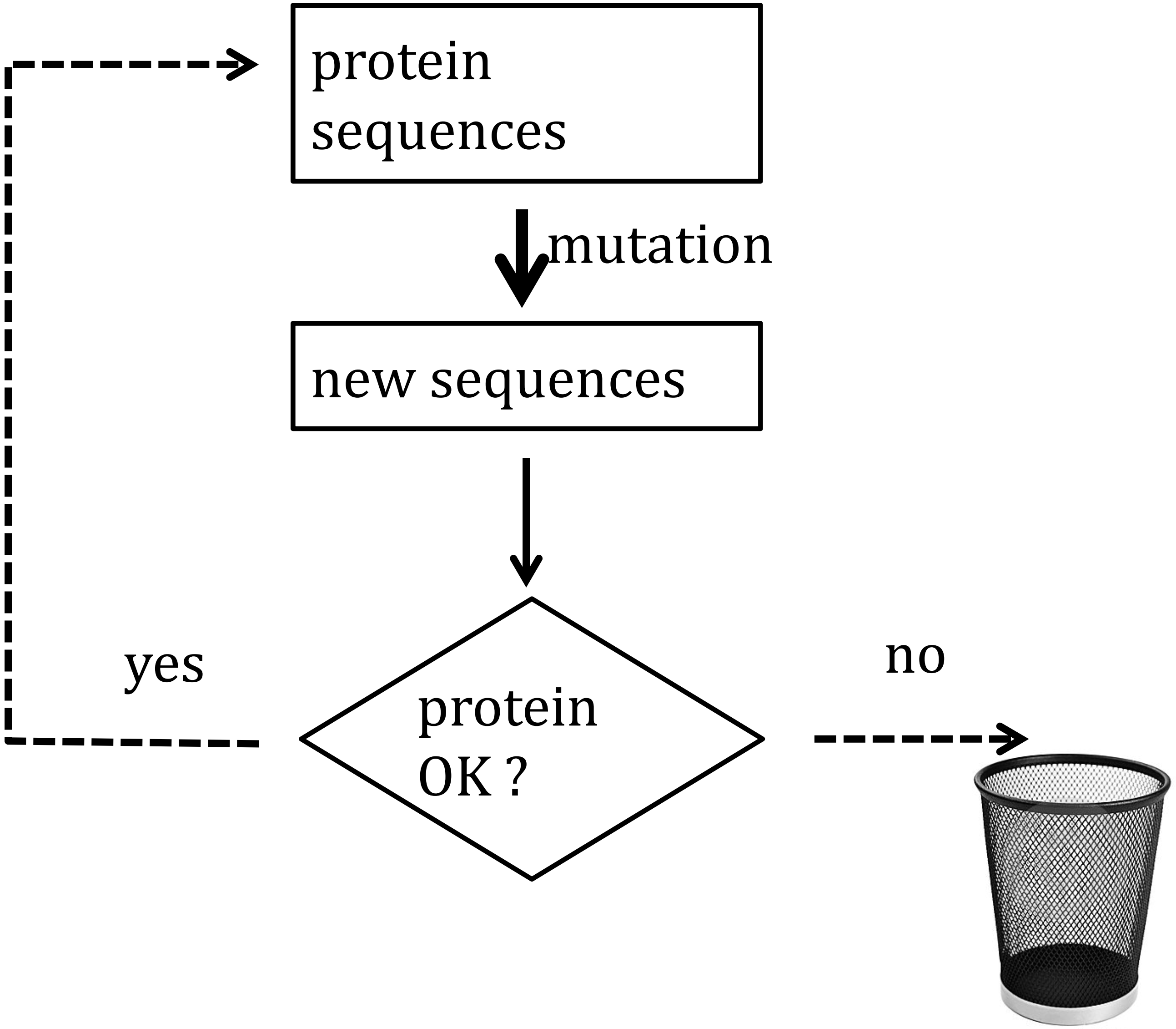
no

Think neutralist

- OK/not OK step (selection) less important
- What determines the sequences you see ?
  - "mutation" step

- mutation step looks very simple
  - not really

- consider the meaning and biases



Codon bias

- look at the most rare amino acids…
- number of codons not quite everything

ser	UCU, UCA, UCC, UCG, AGU, AGC
leu	CUU, CUA, CUC, CUG, UUA, UUG
…	
his	CAU, CAC
met	AUG
trp	UGG

- some bases are more common than others

$$p(\text{his}) = 0.22 \cdot 0.3 \cdot 0.22 + 0.22 \cdot 0.30 \cdot 0.22 \approx 0.03$$

U	22 %
A	30 %
C	22 %
G	26 %

- does this predict the probability of all amino acids ?
- if yes, there is no selection for amino acids
  - Darwinism at the amino acid selection level

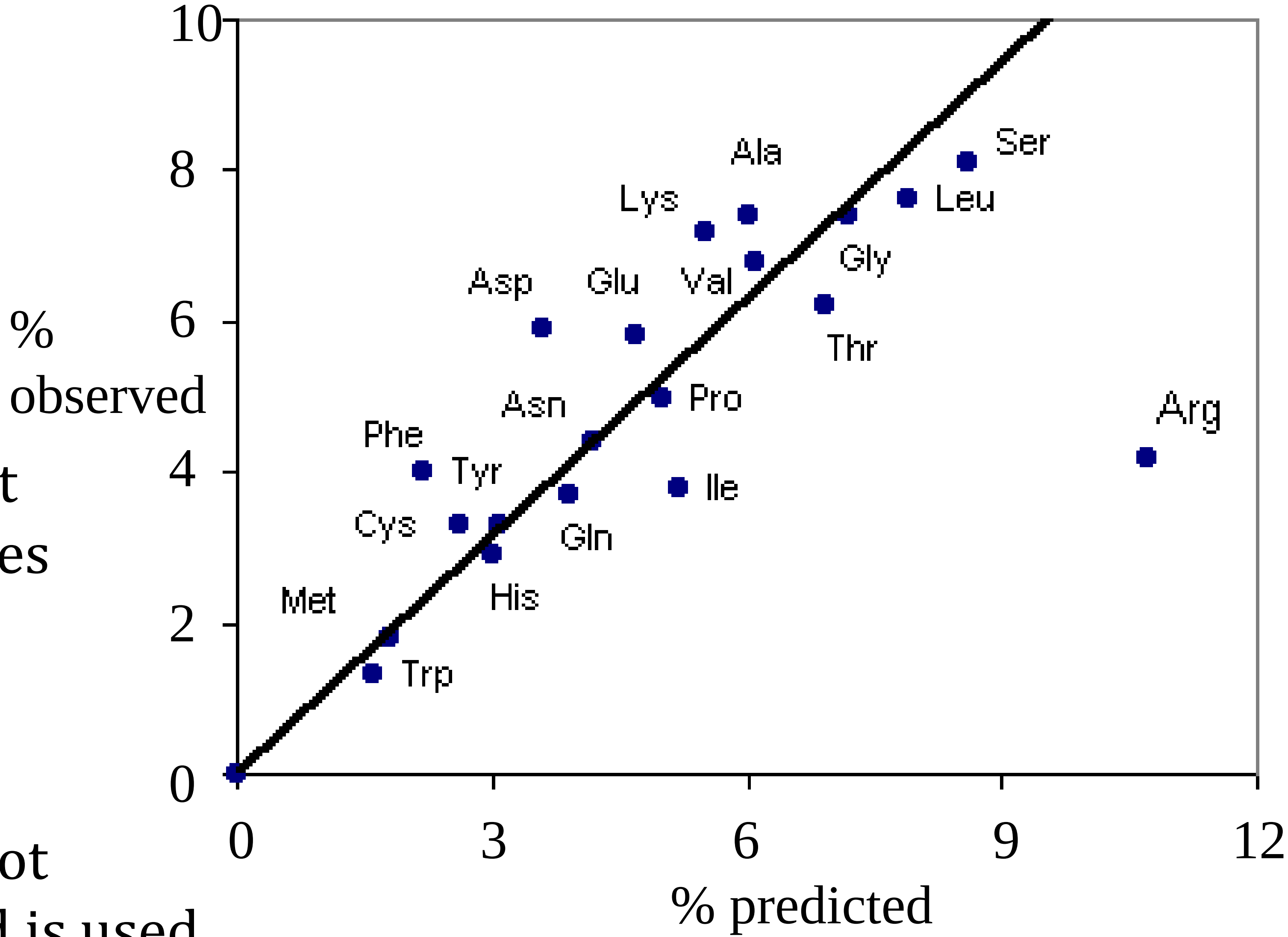
How relevant is Darwinism ?

One outlier (arg)

Little evidence of Darwinist selection in amino acid types

Logical consequence

- there are many sites in proteins where it does not matter which amino acid is used



King, J.L., Jukes, T.H. Science 164, 788 (1969)

Forget Darwinism and selection of amino acids ?

No

- arg example
- lots of mutation data
    - for an enzyme
        - most mutations are a bit bad, some do not matter

- Do not be a pure Darwinist
- do not interpret everything you see in terms of fitness

Representation

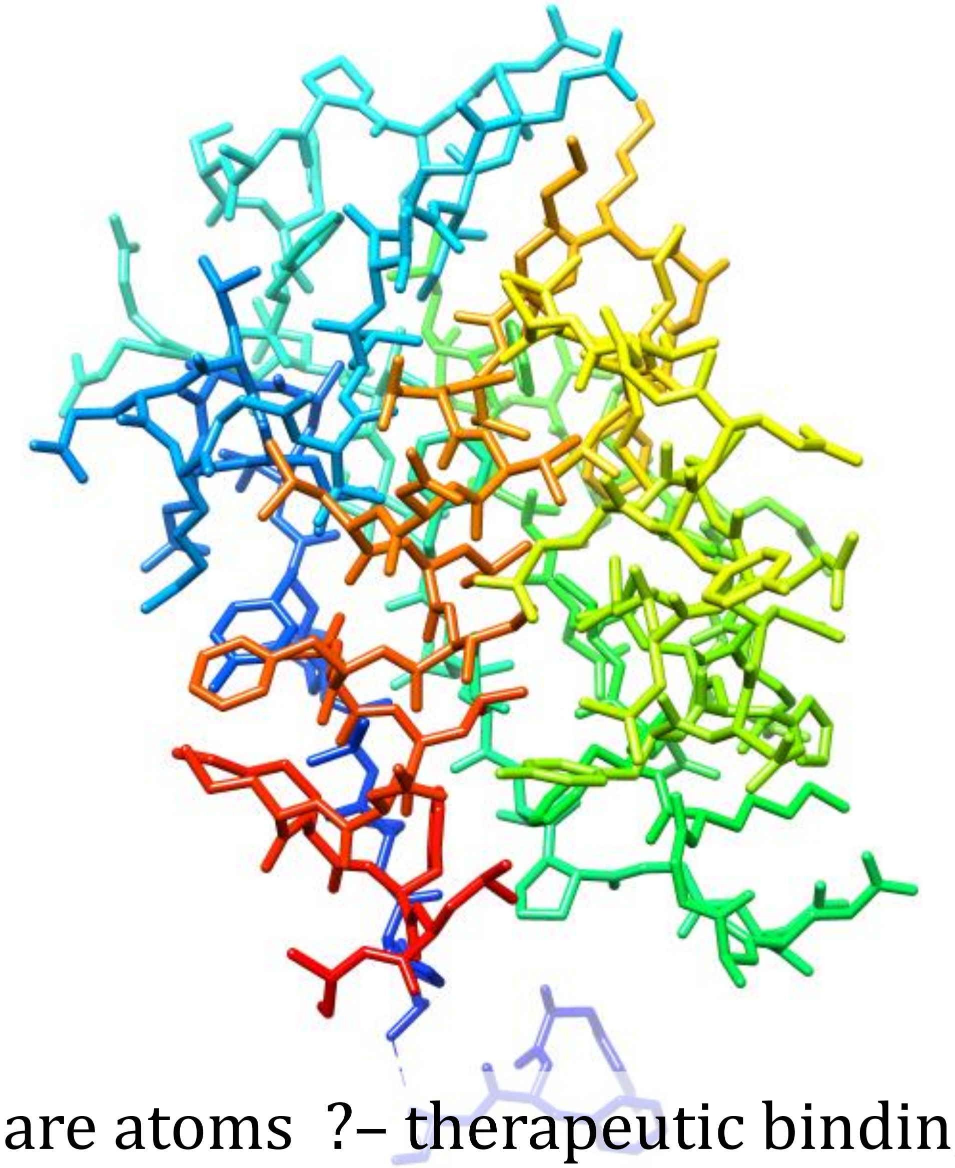
Ultimately, our representation of a structure…

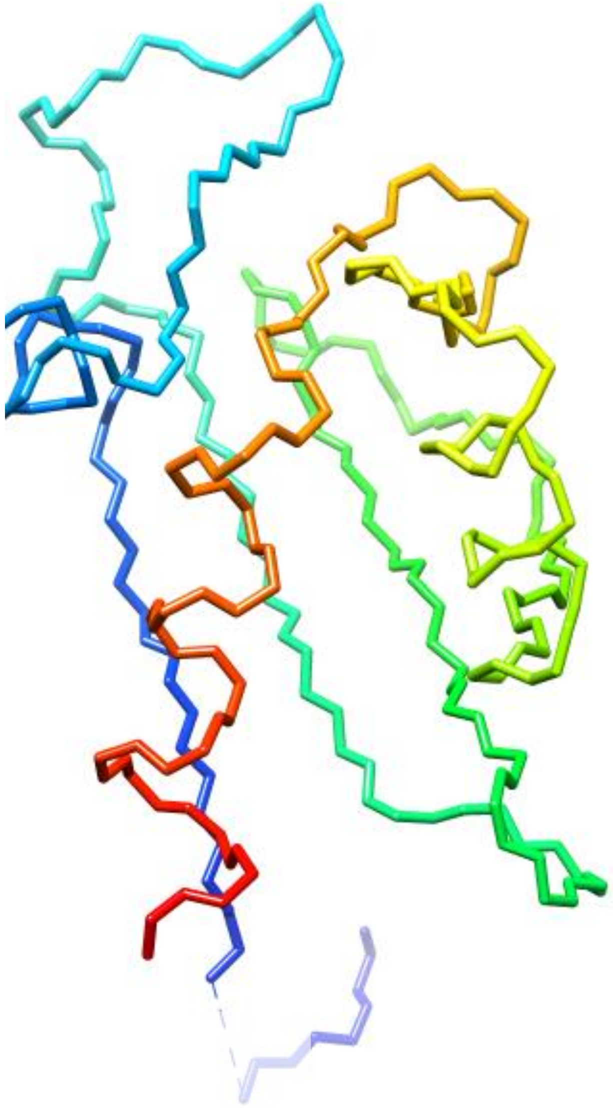
```
ATOM      1  N   ARG     1      31.758  13.358 -13.673  1.00 18.79      1BPI 137
ATOM      2  CA  ARG     1      31.718  13.292 -12.188  1.00 14.26      1BPI 138
ATOM      3  C   ARG     1      33.154  13.224 -11.664  1.00 18.25      1BPI 139
ATOM      4  O   ARG     1      33.996  12.441 -12.225  1.00 20.10      1BPI 140
ATOM      5  CB  ARG     1      30.886  12.103 -11.724  1.00 16.74      1BPI 141
ATOM      6  CG  ARG     1      29.594  11.968 -12.534  1.00 15.96      1BPI 142
ATOM      7  CD  ARG     1      28.700  13.182 -12.299  1.00 15.45      1BPI 143
ATOM      8  NE  ARG     1      27.267  12.895 -12.546  1.00 12.82      1BPI 144
ATOM      9  CZ  ARG     1      26.661  13.087 -13.727  1.00 17.38      1BPI 145
ATOM     10  NH1 ARG     1      27.370  13.558 -14.735  1.00 18.38      1BPI 146
ATOM     11  NH2 ARG     1      25.367  12.797 -13.838  1.00 25.73      1BPI 147
ATOM     12  N   PRO     2      33.800  13.936 -10.586  1.00 17.07      1BPI 148
ATOM     13  CA  PRO     2      34.976  13.367  -9.840  1.00 14.99      1BPI 149
ATOM     14  C   PRO     2      34.960  11.922  -9.660  1.00 13.11      1BPI 150
ATOM     15  O   PRO     2      33.962  11.306  -9.391  1.00 10.57      1BPI 151
ATOM     16  CB  PRO     2      34.922  14.145  -8.523  1.00 15.81      1BPI 152
ATOM     17  CG  PRO     2      34.058  15.391  -8.737  1.00 18.91      1BPI 153
ATOM     18  CD  PRO     2      33.371  15.273 -10.096  1.00 19.41      1BPI 154
ATOM     19  N   ASP     3      36.192  11.817  -9.707  1.00  8.73      1BPI 155
```

x, y, z coordinates

Drawing the structure ?

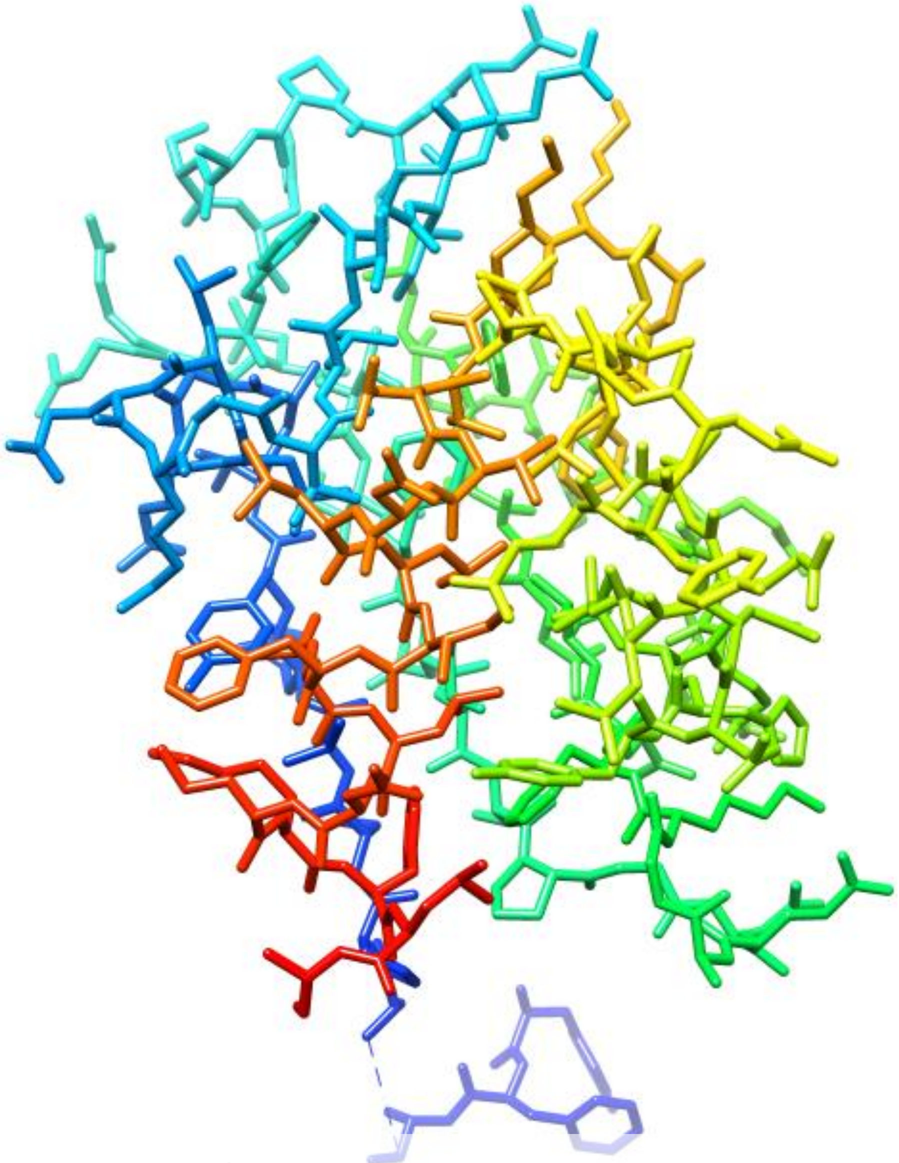
Representations

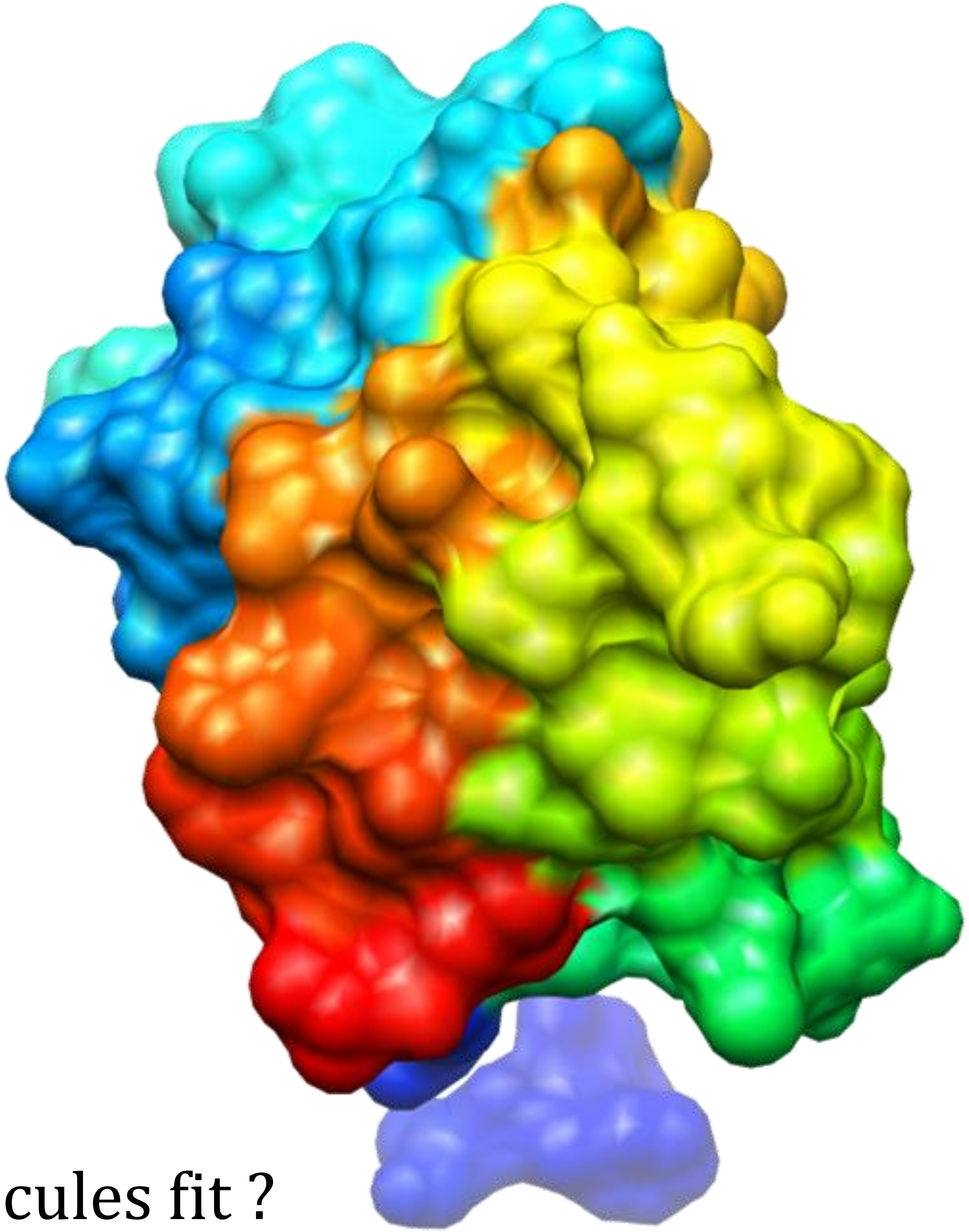




- where are atoms ?– therapeutic binding
- which residues could be involved in interactions ?

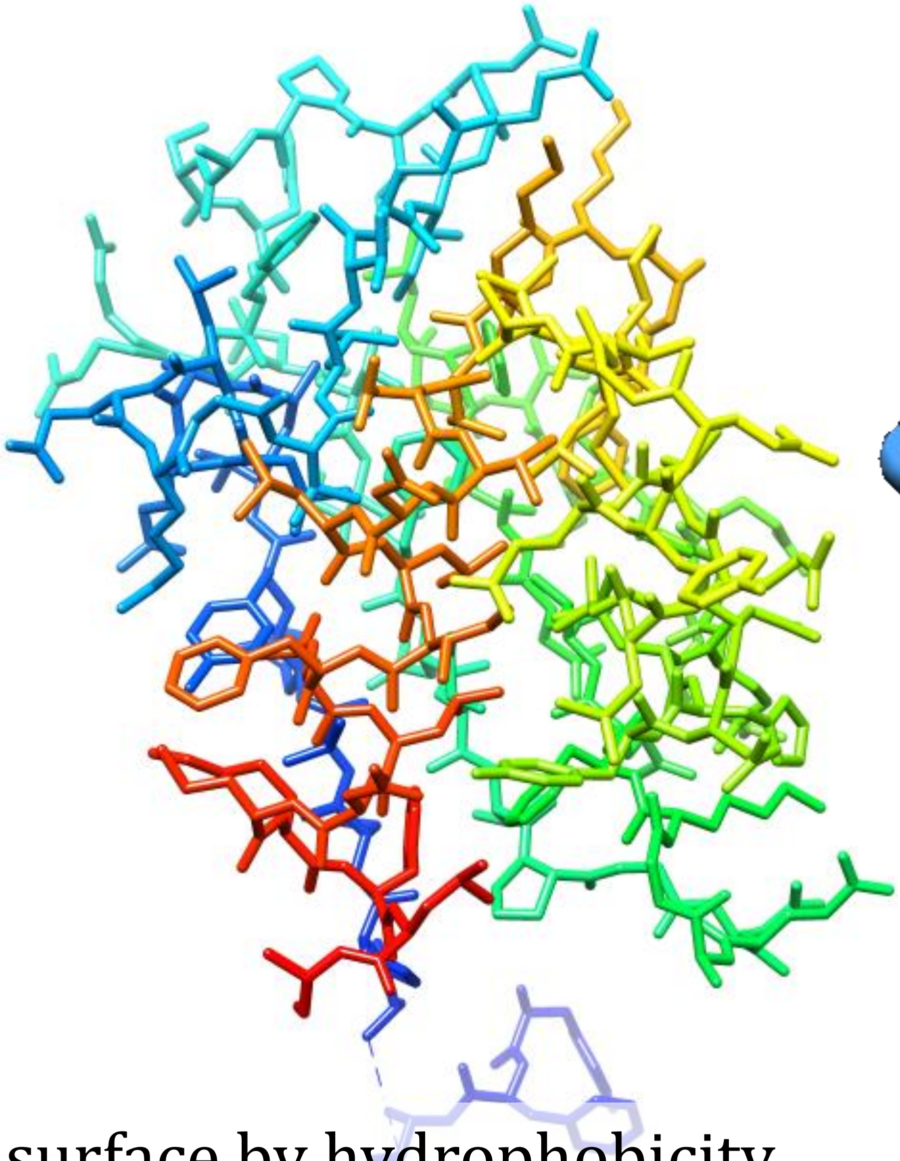
Representations

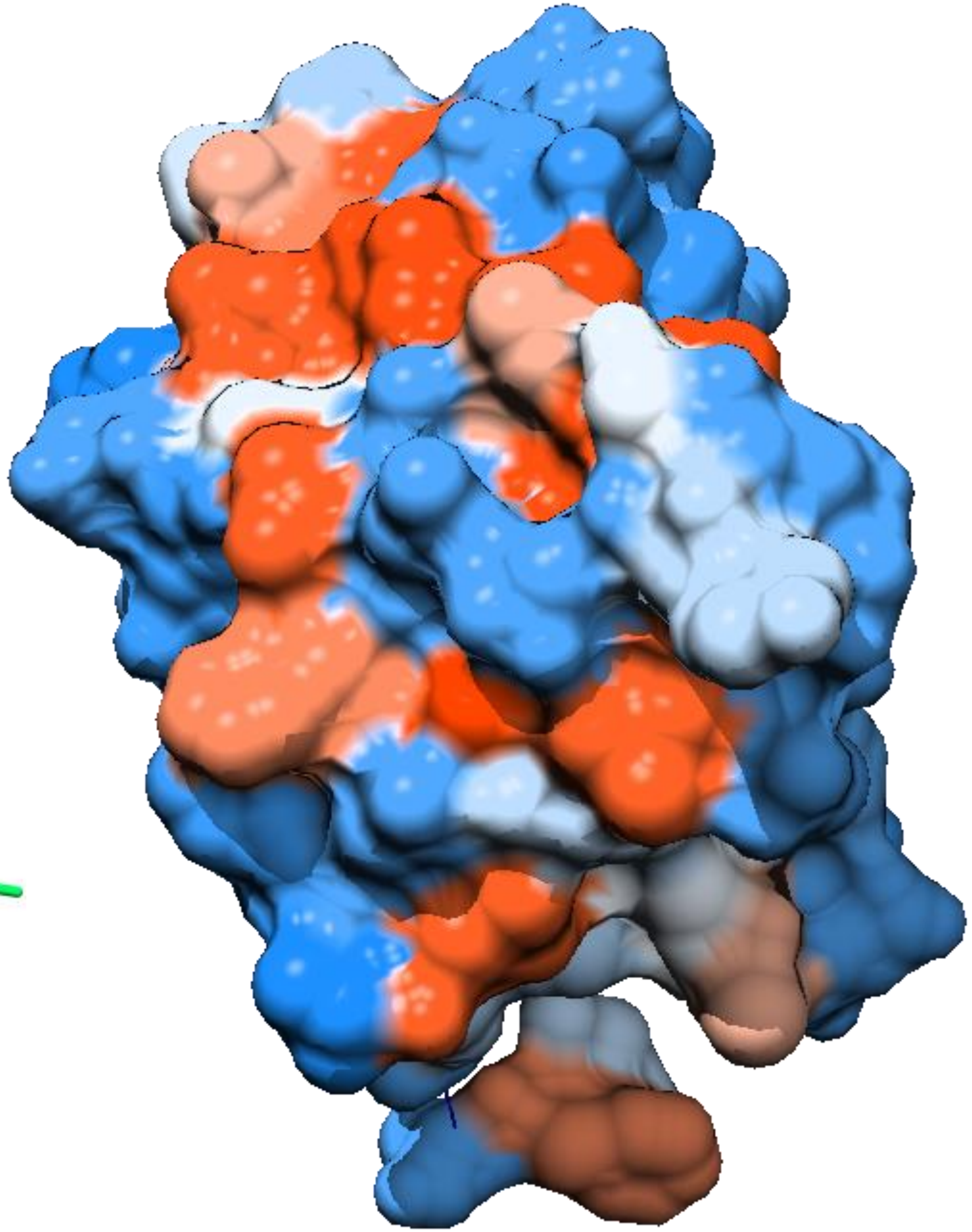




What is the surface ? where could molecules fit ?

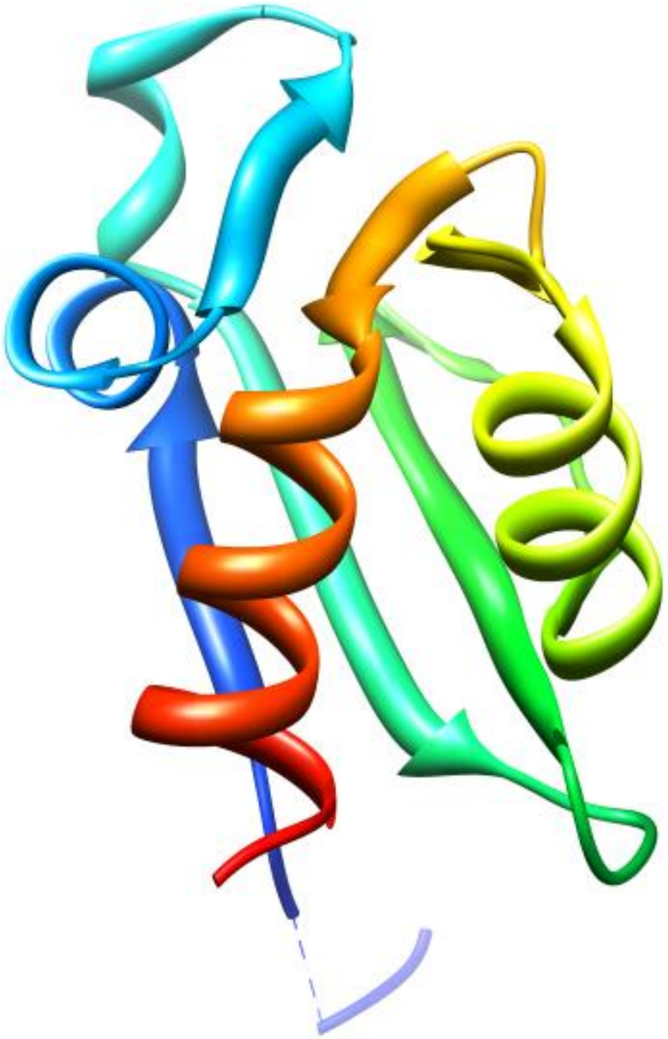
Representations

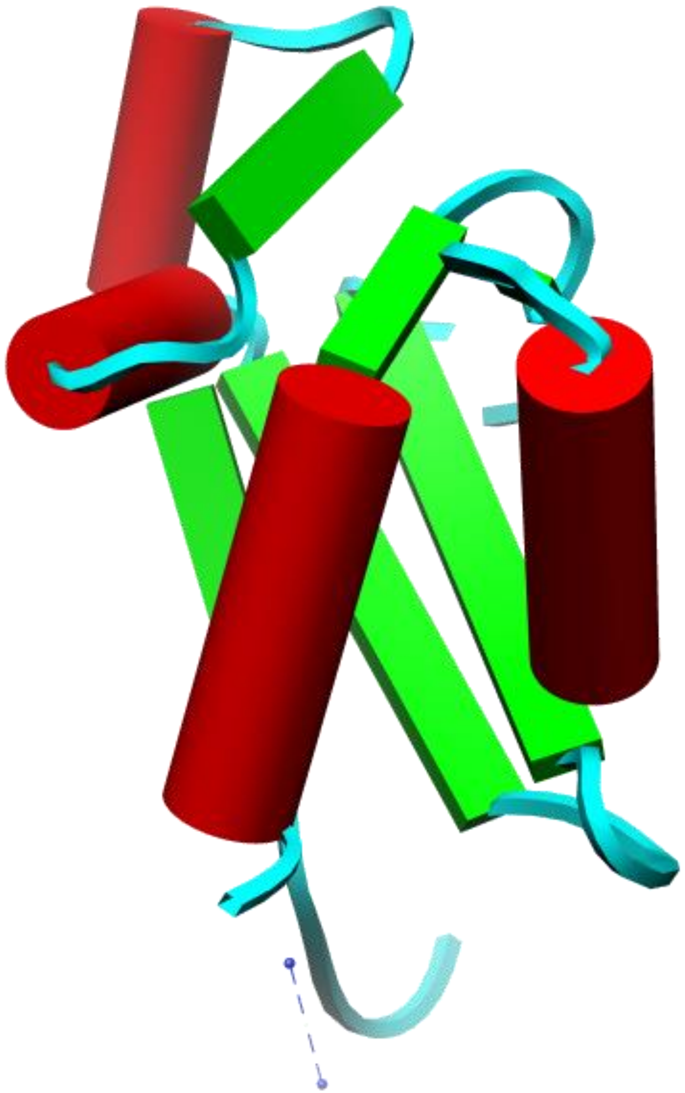




Colour surface by hydrophobicity

Representations





Highlight / emphasise regular structures

Why does structure matter ?

- what residues can I change and preserve function ?
- what is the reaction mechanism of an enzyme ?
- what small molecules would bind and block the enzyme ?
- is this protein the same shape as some other of known function ?

Where do structures come from ?

- X-ray crystallography
- NMR
- + a bit of small angle X-ray scattering, electron diffraction, neutron diffraction…

resolution, precision, accuracy

Coordinates `27.370  13.558 -14.735`

  - what do they mean ?

Random errors

- non-systematic / noise / uncertainty
- should be scattered around correct point

X-ray crystallography has model for data

- uncertainty (probability)
- resolution (experimental)
  - < 1 Å (unusually good)
  - > 5 Å (bad, but examples..
    3LJ5 Full Length Bacteriophage P22 Portal Protein
    3M0C X-ray Crystal Structure of PCSK9 in Complex with the LDL receptor

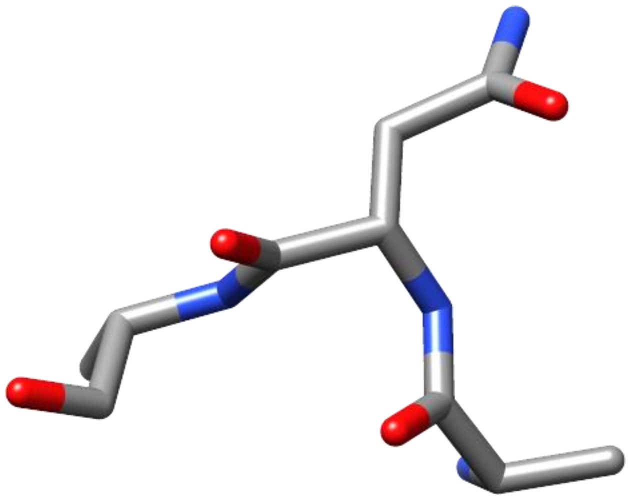
X-ray crystallography

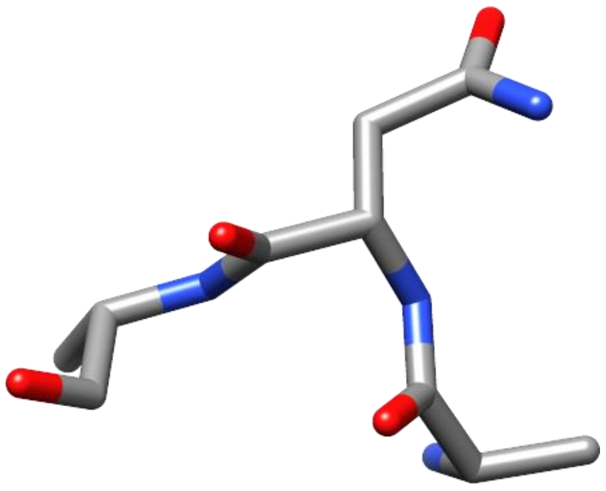
Non-systematic errors

- small problems: (O and N look the same)
- few huge problems
- newer structures are better

Proteins are not static

- overall motion
- local motion





NMR structures

Different philosophy to X-ray

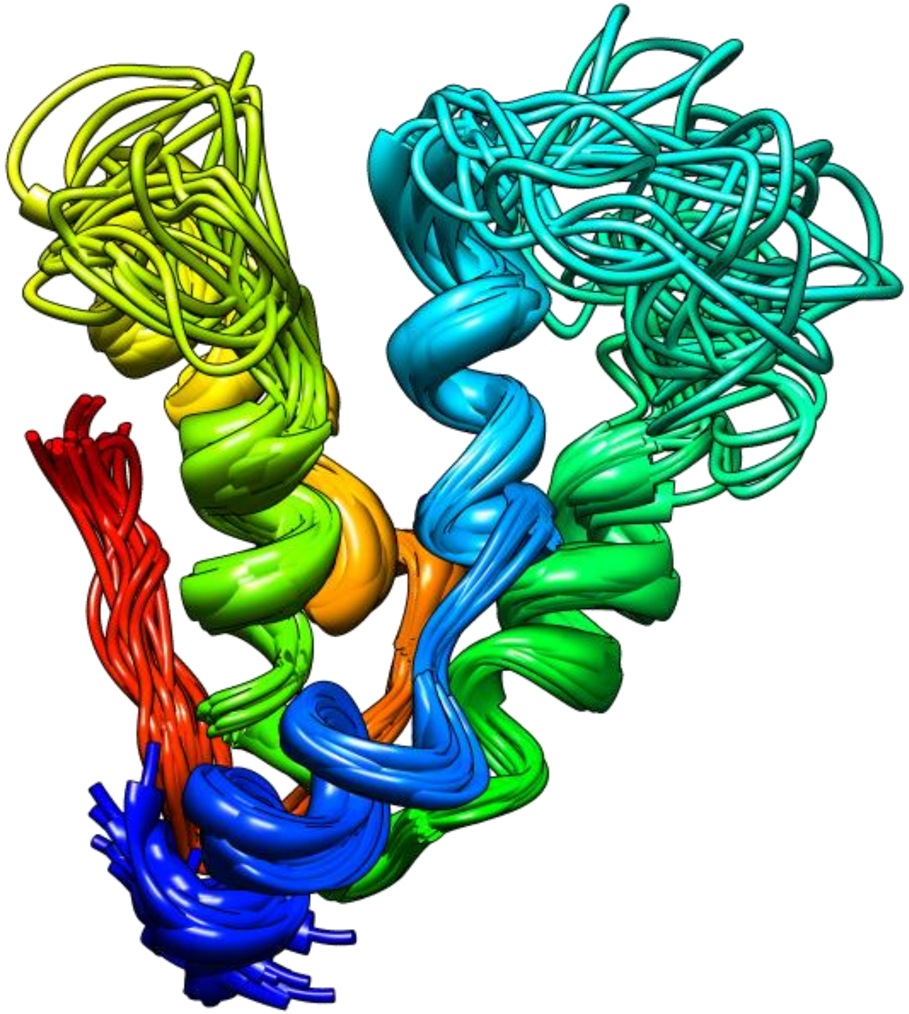
- lots of little internal distances
- do not quite define structure

Generate 50 or 10^2 solutions

- look at scatter of solutions

As with X-ray

- some parts are well defined
- some not



structure 1sm7

Summarise and stop

- roles of proteins
- heteropolymers – 20 types of amino acid / residue
- geometry – avoiding atomic clashes, forming H bonds
  - leads to regular secondary structure
- chemistry of amino acids very different to another
- unique structure for a sequence reflects these differences
- representations of structures
- structures in PDB are experimental – have errors

some questions

- $(\mathrm{Asp})_{100}$
  - is it soluble ? Is it acidic / basic ?
  - would it form a compact regular structure ?
- How big is sequence space ? How much has been tried by evolution ?
- if you have a protein of poly-trp, would it form a specific structure ? How would it behave in solution ?
- for length *n*, do all / many / few of the n^{20} sequences form specific structures ?
- how would a Darwinist explain the uneven distribution of amino acid usage ?
- why would you want to represent a protein by its surface ?
- why might you draw it as a series of helices and strands ?
- what is the biggest chain in the protein data bank ? Examples
  - fatty acid synthase > 2×10^3 residues/chain
  - dynein heavy chain motor domain > 4×10^3 residues/chain