Protein modeling

Dr. Amanda Souza Câmara

Introduction to Bioinformatics
Spring school for early stage plant scientists
IPK Gatersleben · May 13-16, 2025

PLANT 2030 ACADEMY

Schedule

- 13:30 – 15:00 Part 1 – Theory on protein structure and the challenge of solving it
- 15:00 – 15:30 Coffee break
- 15:30 – 17:00 Part 2 – Hands-on modelling, visualizing and analyzing with AlphaFold and PyMOL

Everything, including this presentation, is on GitHub:
https://github.com/InsilicoGenebankProteomics/ProteinModeling-Plant2020SpringSchool

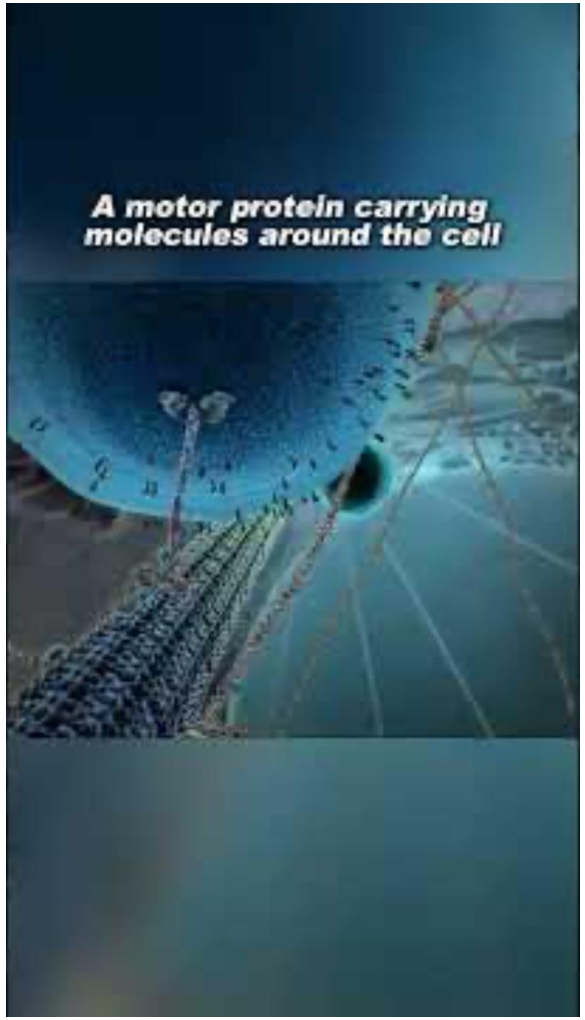
Bioinformatics from 1D ....

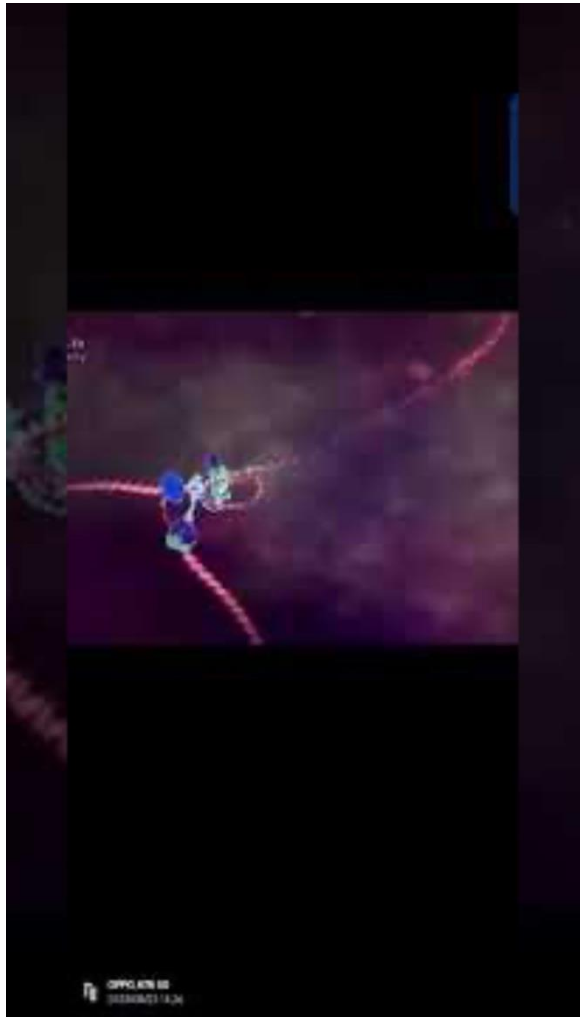
... to 3D!

Goals for part 1

- Diversity of proteins shapes and functions
- Relationship between structure and function, and between sequence and structure
- Main characteristics of protein folding and structure
- The Protein Data Bank and the coordinates file
- AlphaFold, understand and interpret

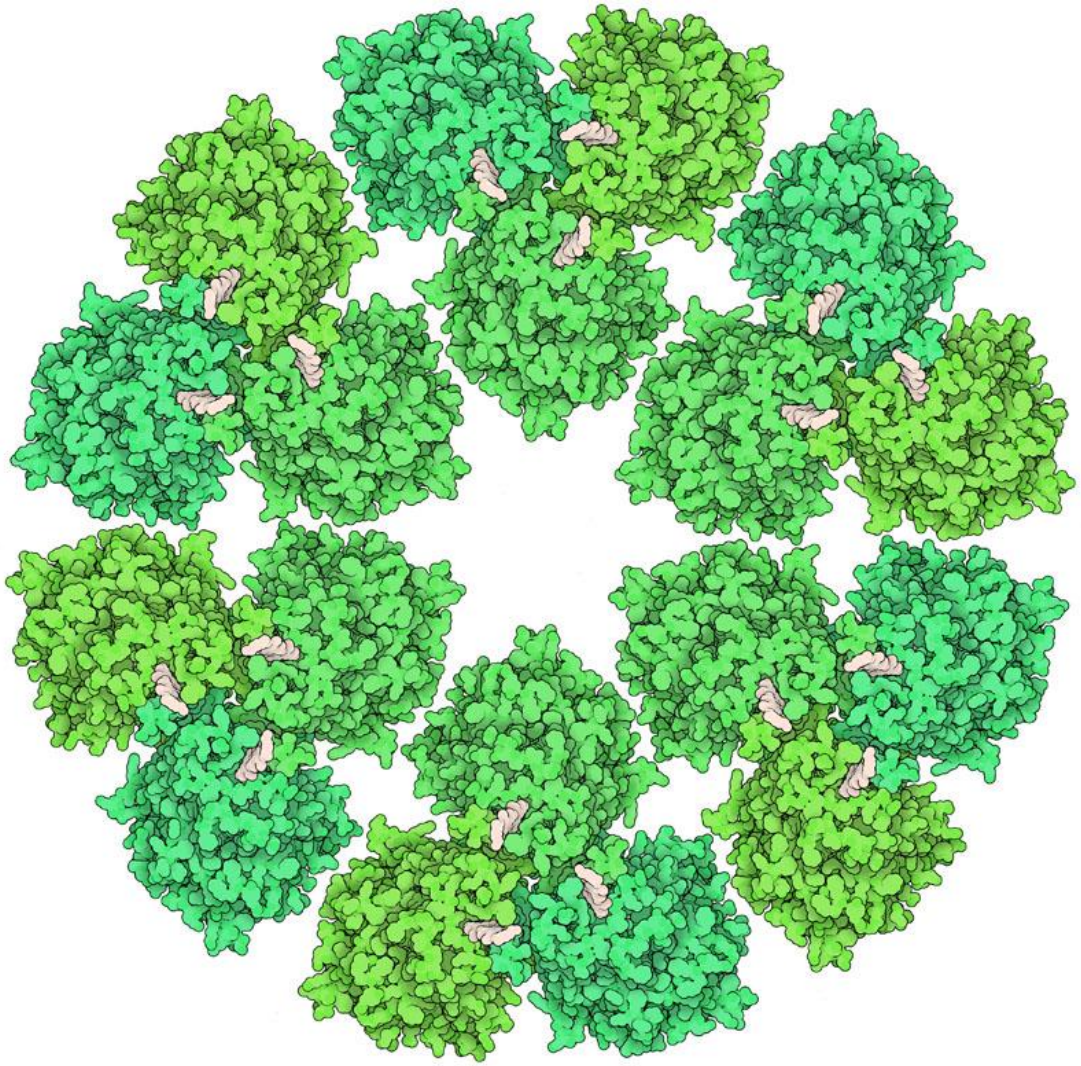
Proteins variety of shapes and functions

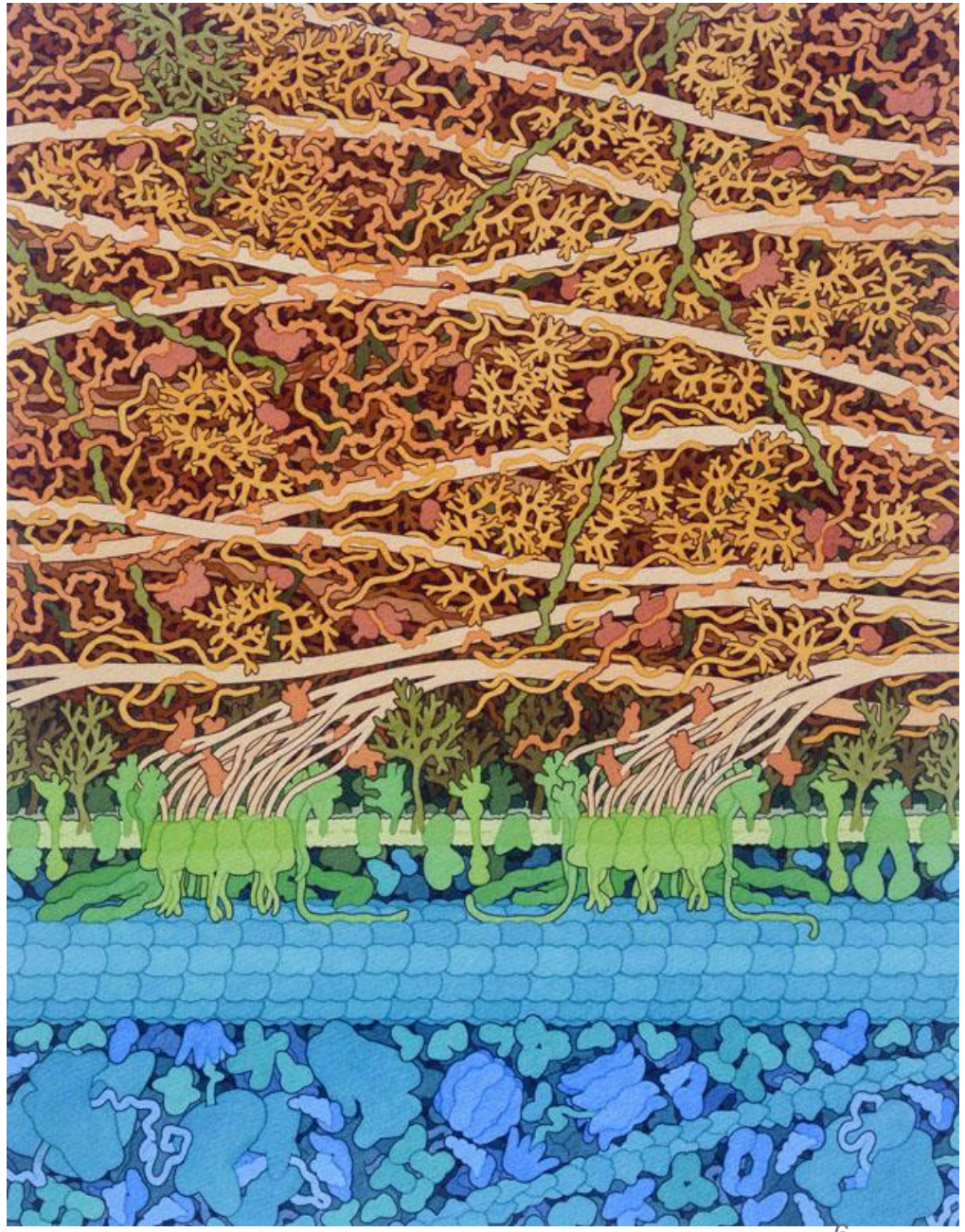




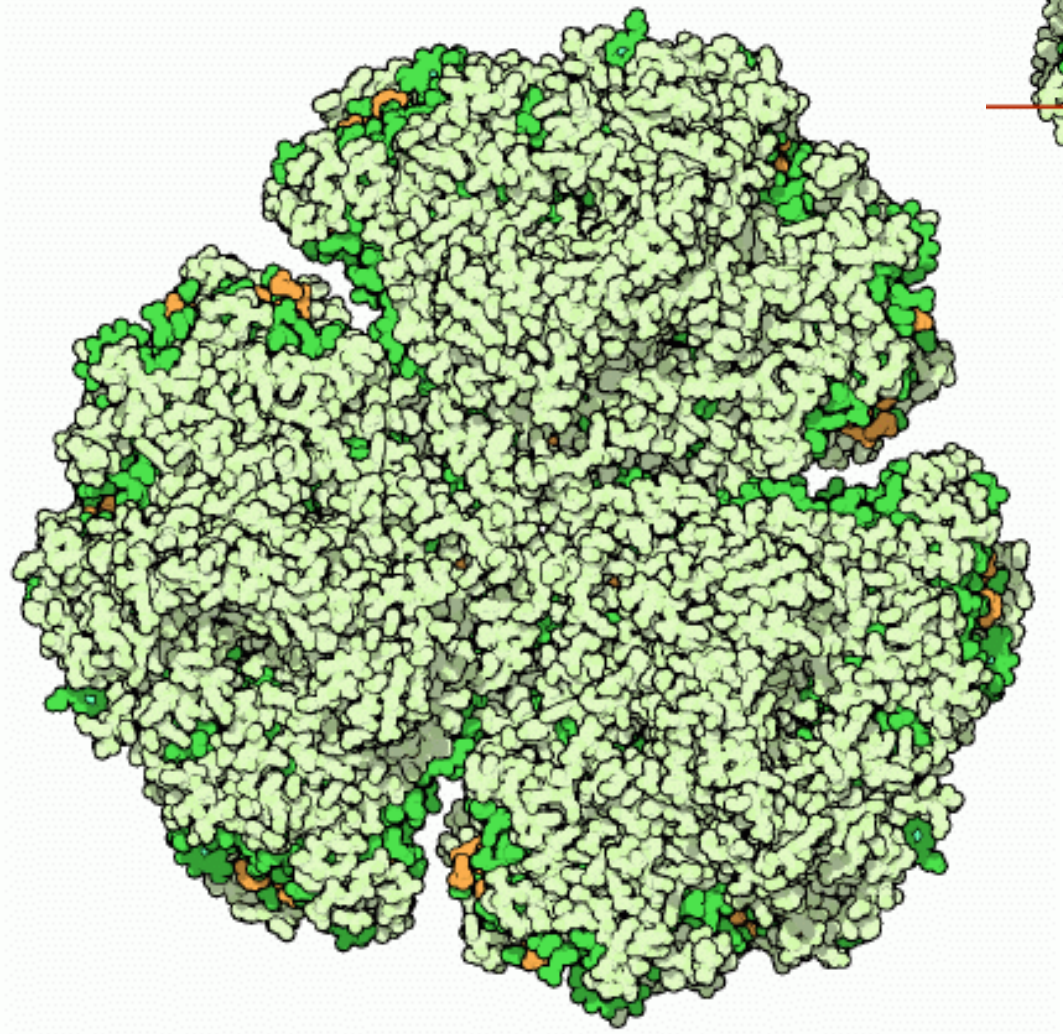
ATP synthase or
Ions pump

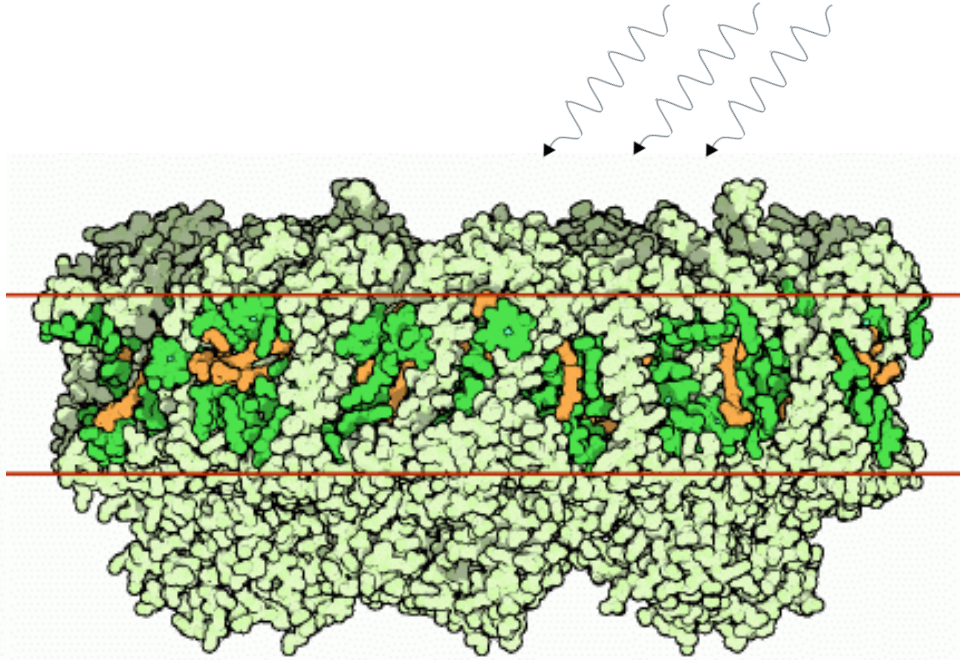
Cellulose synthase

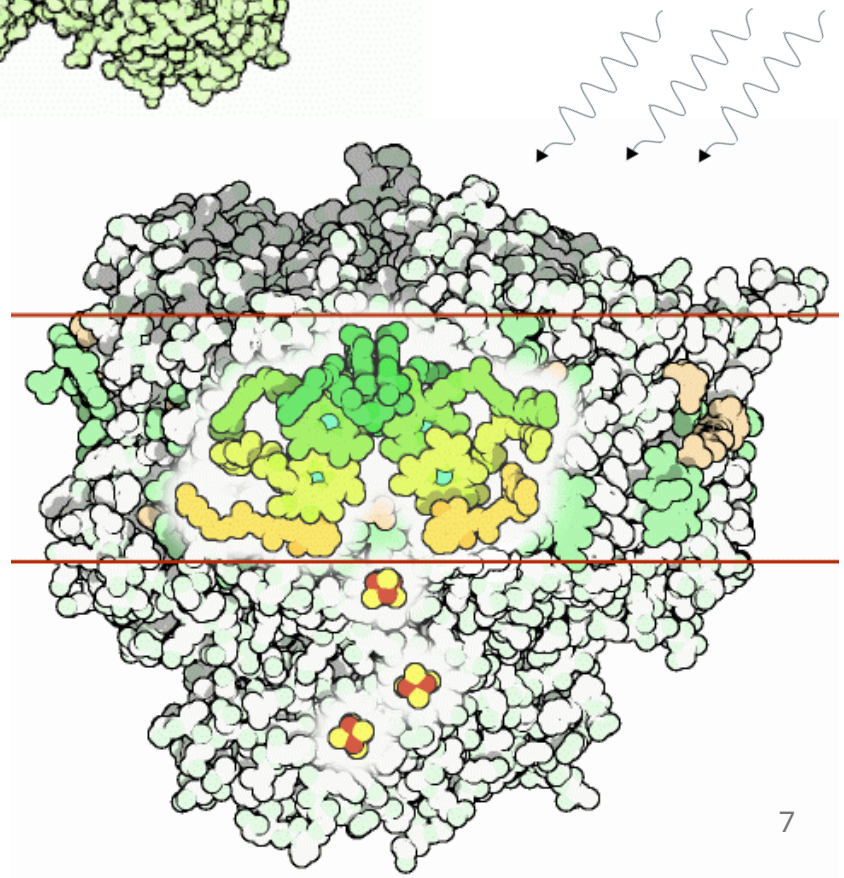




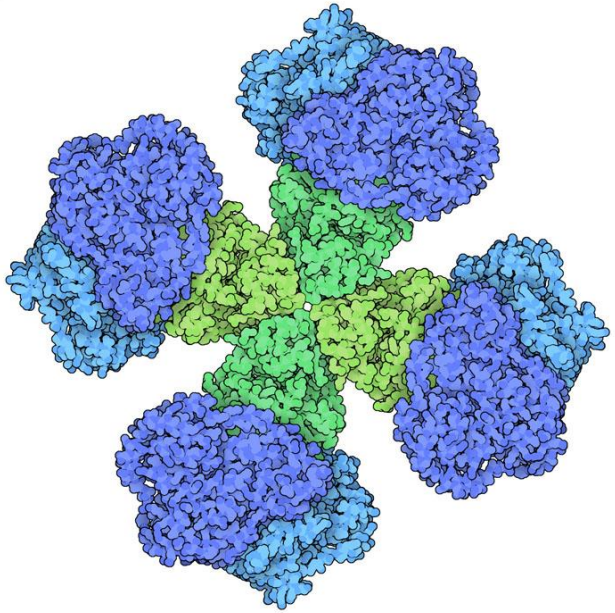
Photosystem I



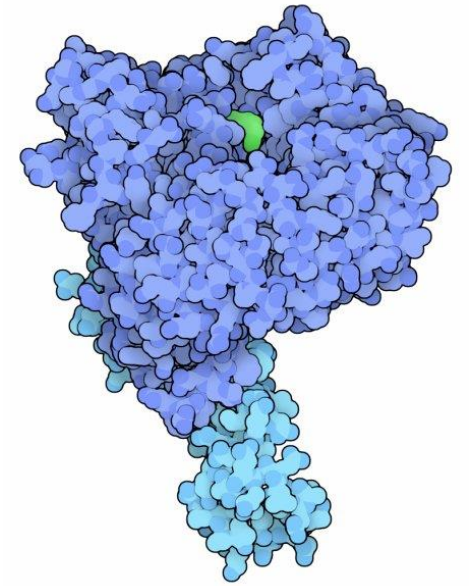




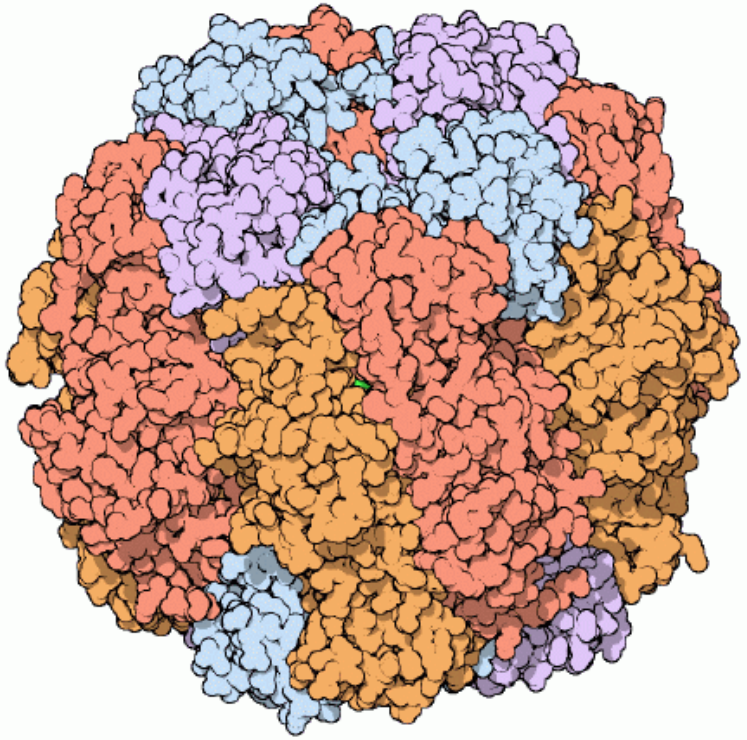
Other plant specific proteins



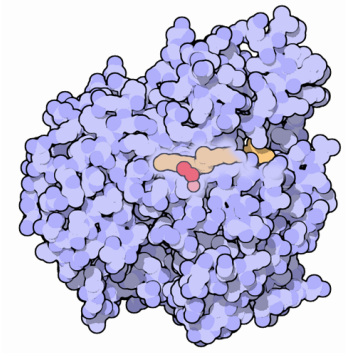
Acetohydroxyacid Synthase
Synthesis of three essential amino acids



TIR ubiquitin ligase with auxin (green)

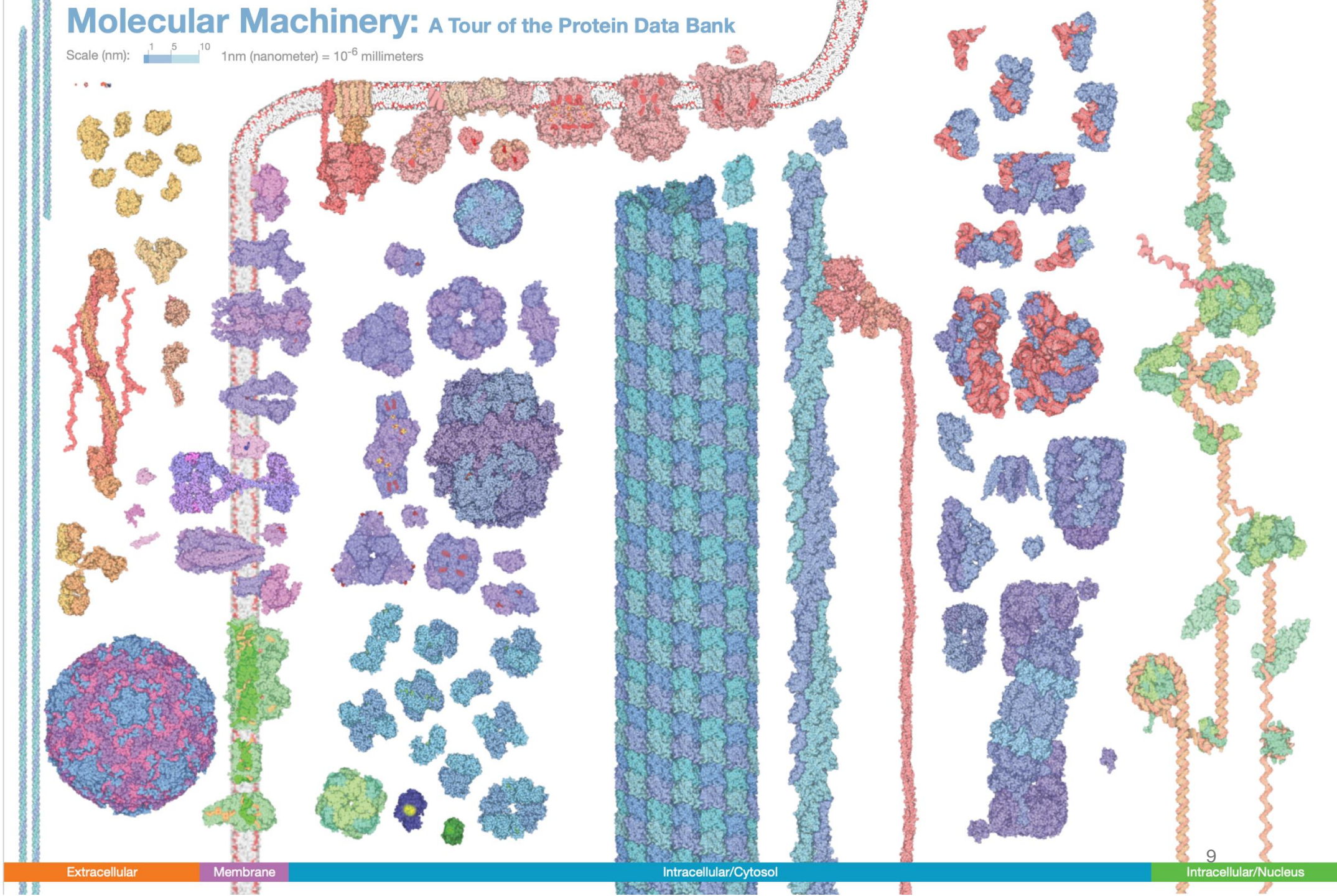


Rubisco fixes atmospheric carbon dioxide into bioavailable sugar molecules

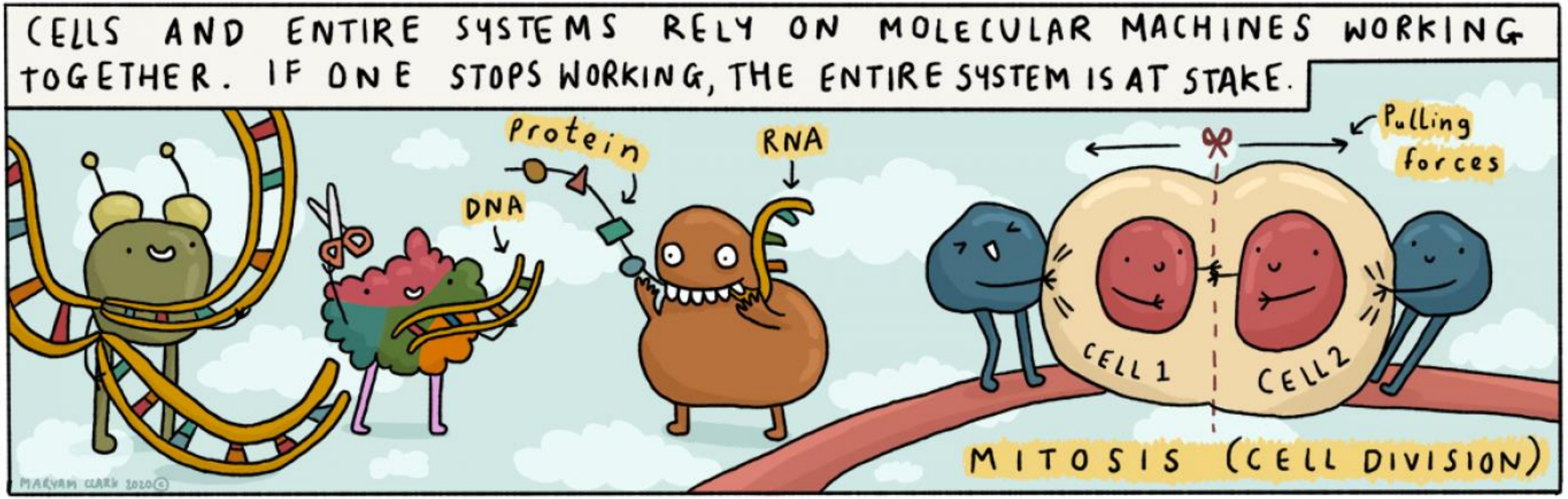


Carotenoid oxygenase, with a carotenoid molecule.

Proteins diversity



Lifeless molecular machines that perform vital processes



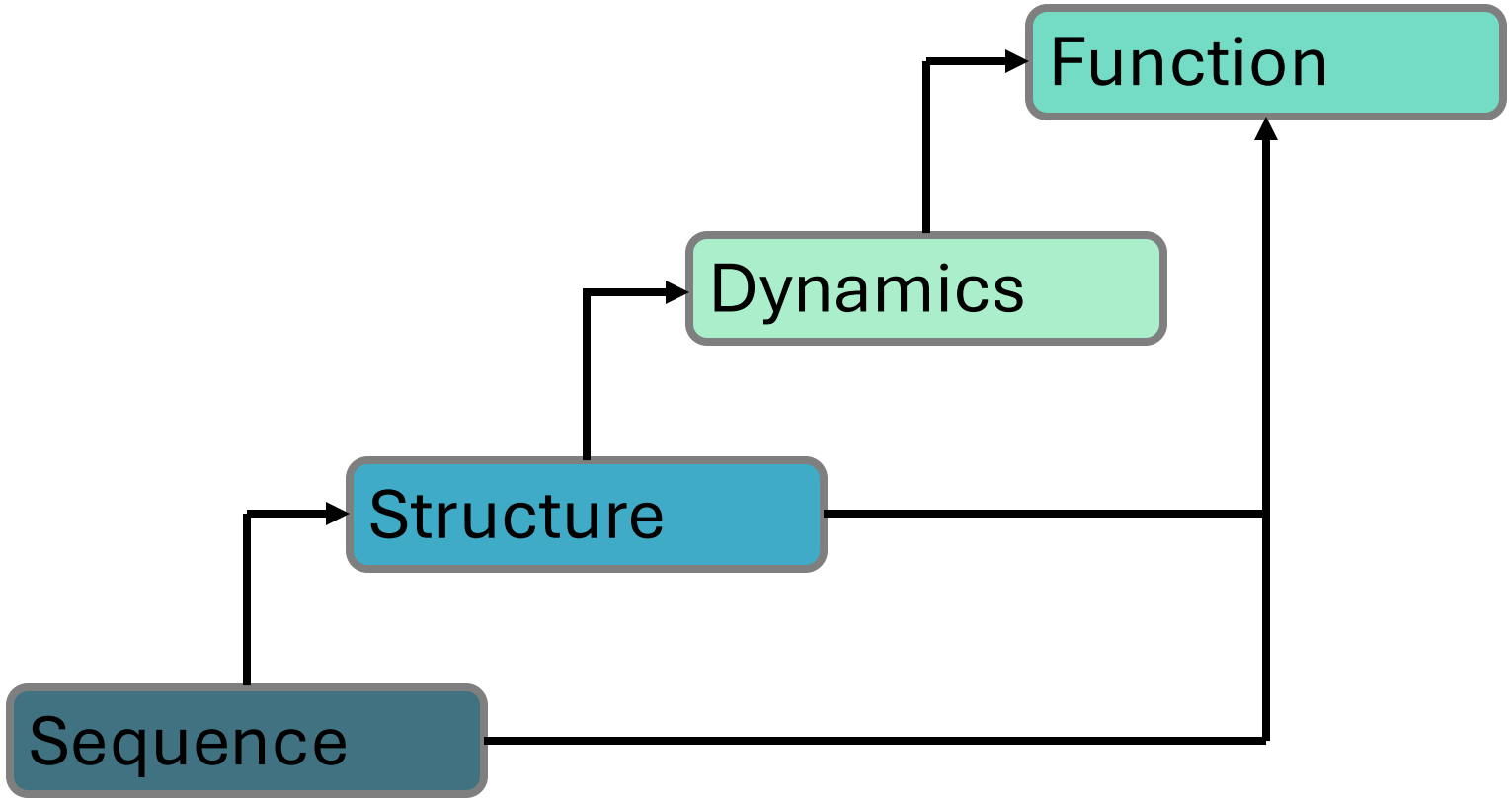
https://www.ucl.ac.uk/

They move around and move themselves according to their physical and chemical properties.

“Everything that living things do can be understood in terms of the jigglings and wigglings of atoms.”

Richard Feynman

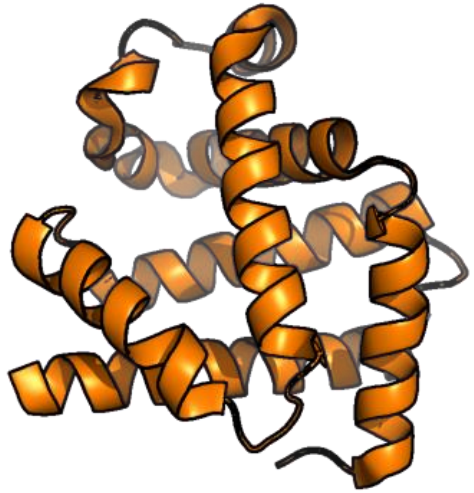
Pillars of function



But nothings is straight forward.

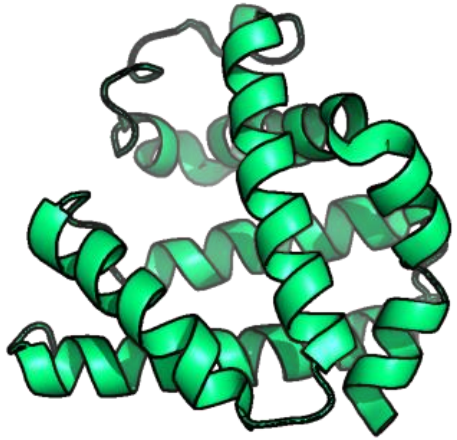
Low sequence similarity but high structural homology

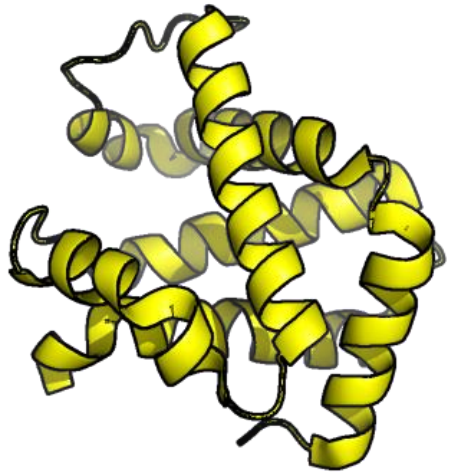
Human myoglobin



African elephant myoglobin
80% sequence identity

Pigeon myoglobin
25% sequence identity

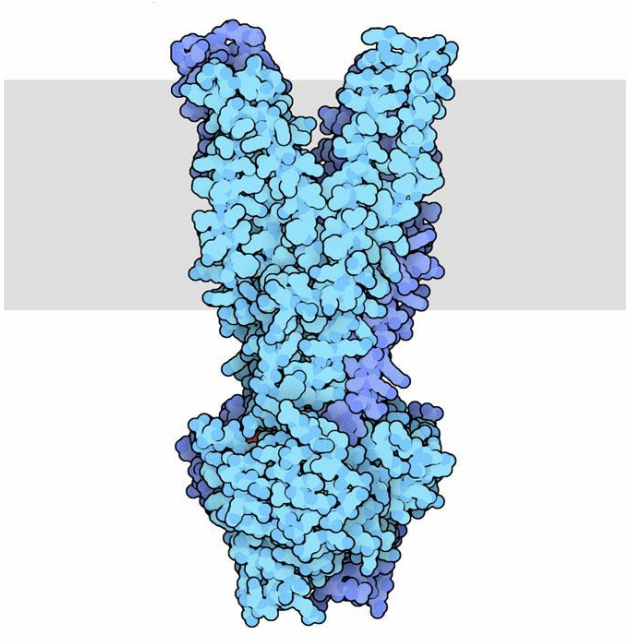


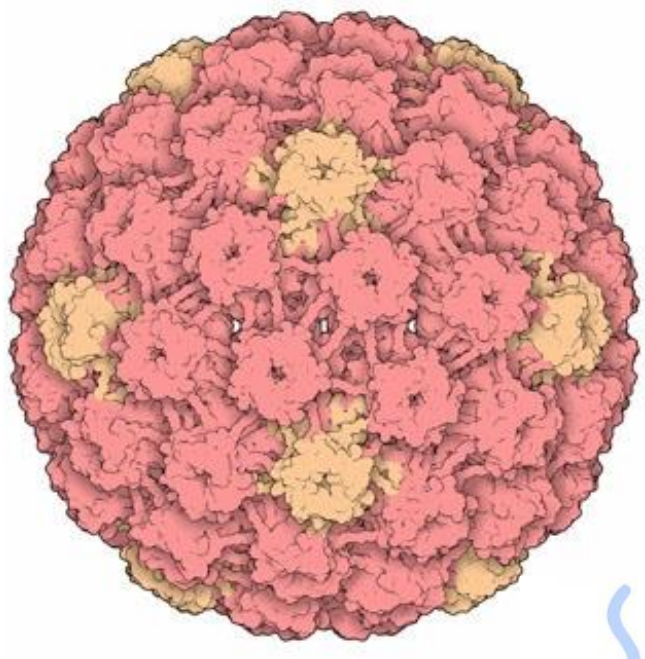


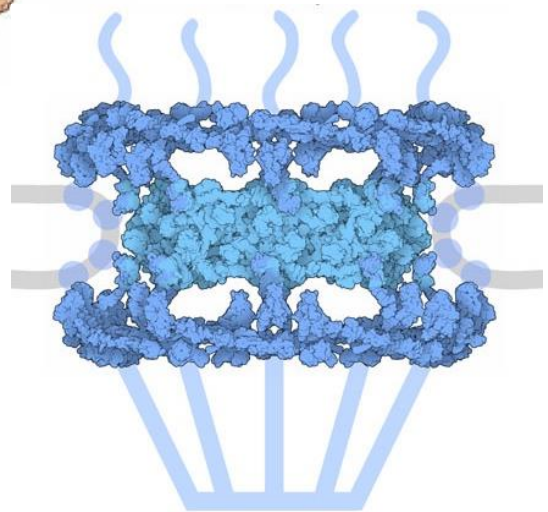
Black-fin tuna myoglobin
45% sequence identity

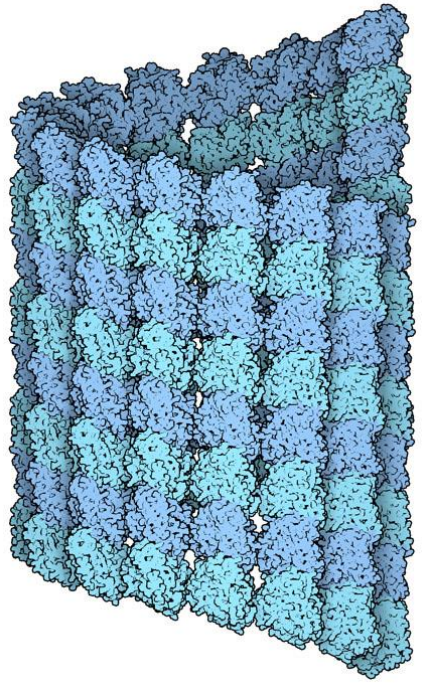
Rubiera, https://www.blopig.com/blog/

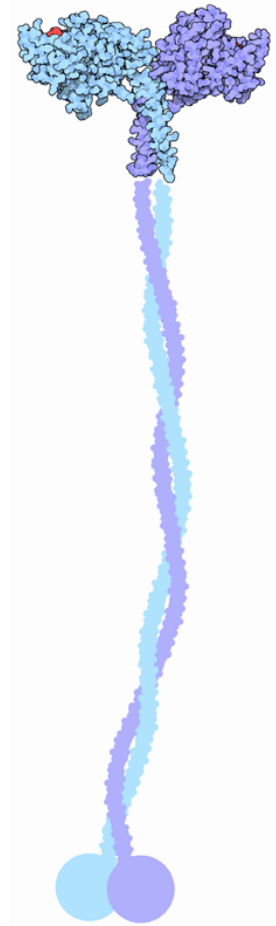
Some structures are of obvious function, or not…





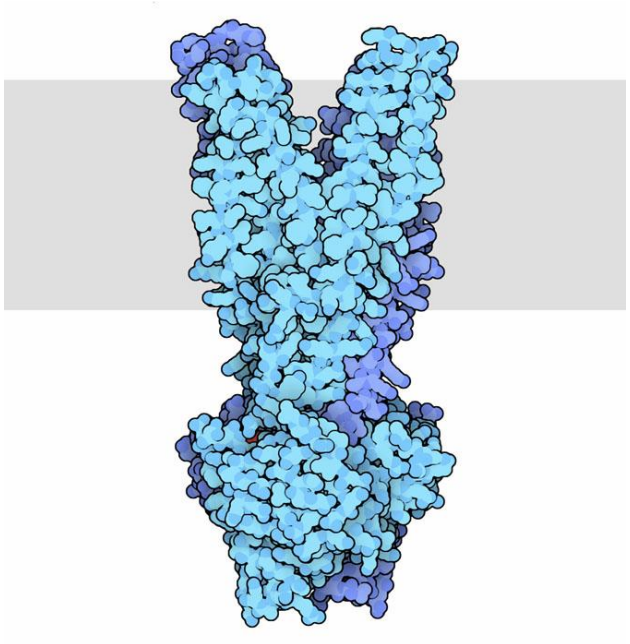


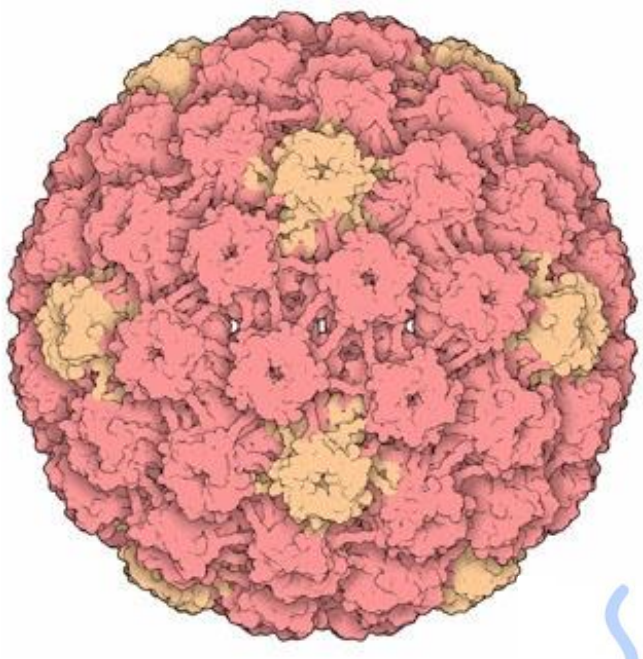




Some structures are of obvious function, or not…

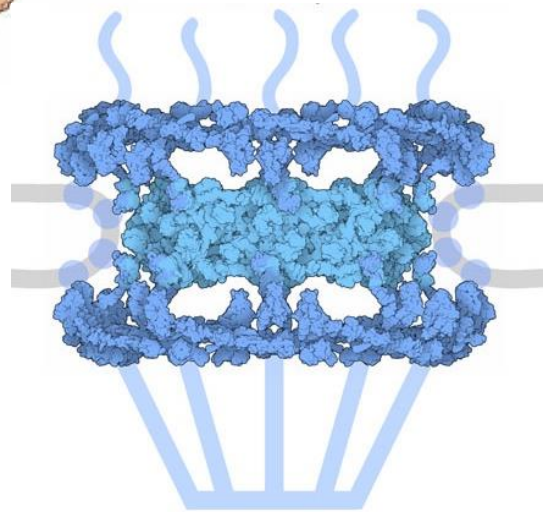
Transporter

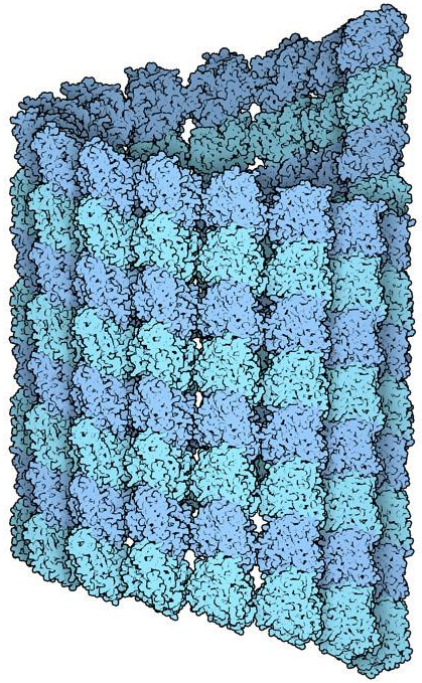




Virus capsid

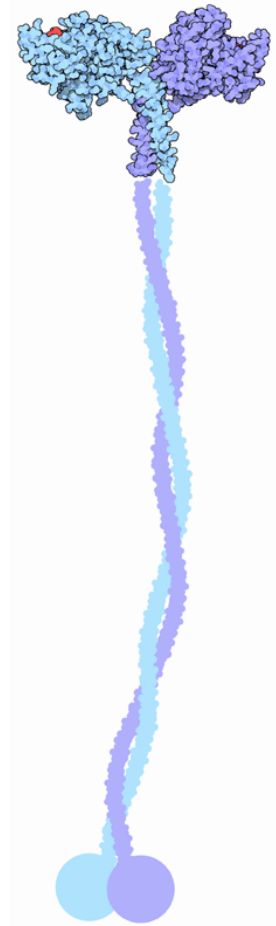
Pore



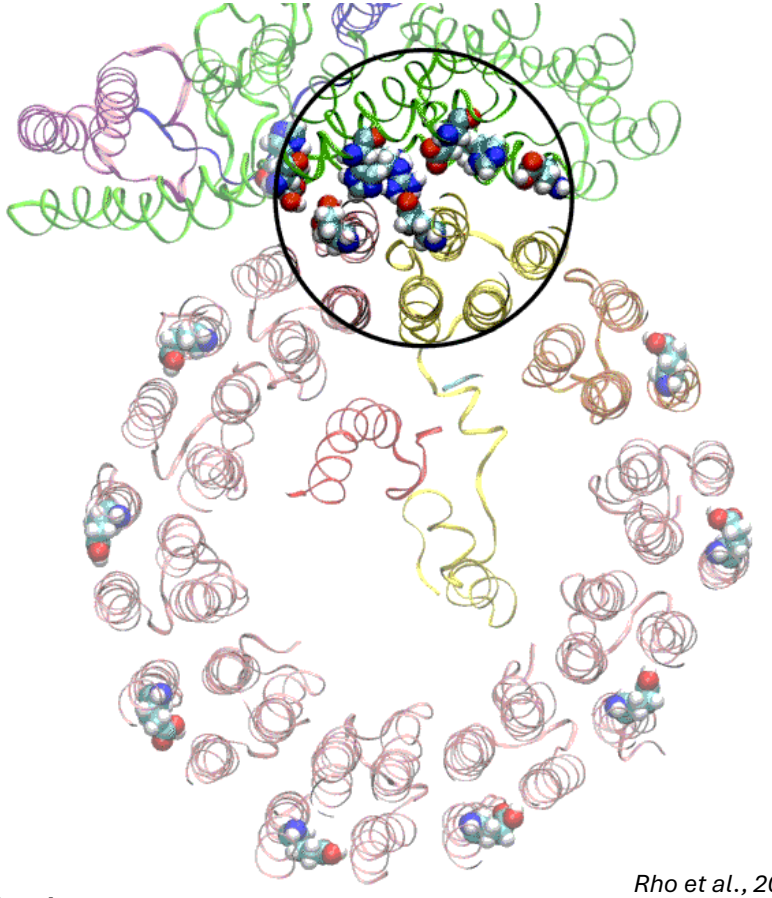


Microtubule

Cargo transporter

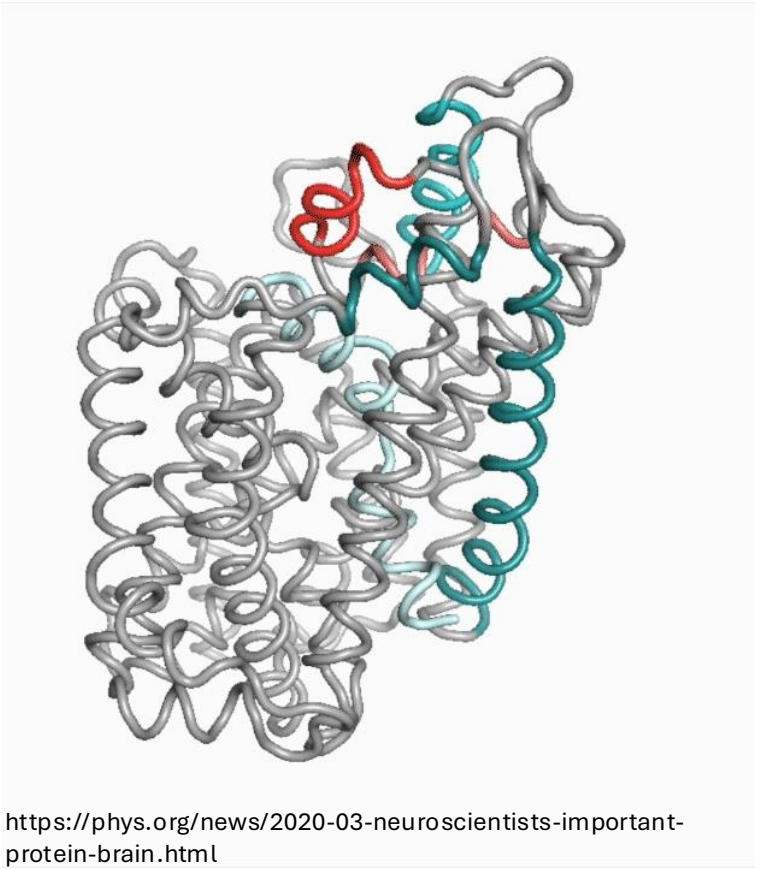


Dynamics can be calculated to see them in action



Rho et al., 2020

Proton pump

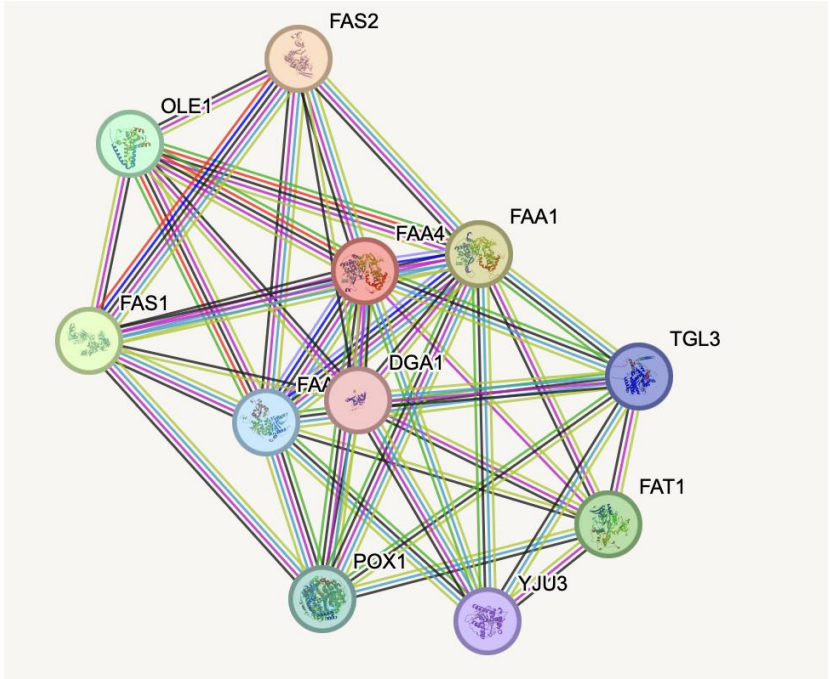


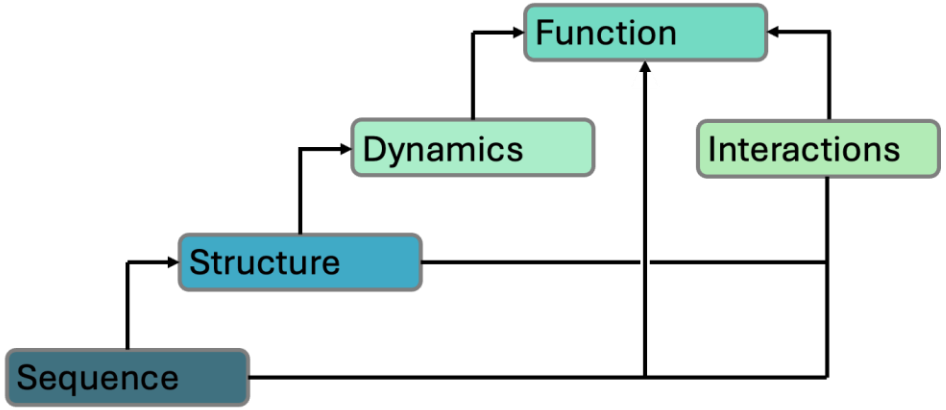
https://phys.org/news/2020-03-neuroscientists-important-protein-brain.html

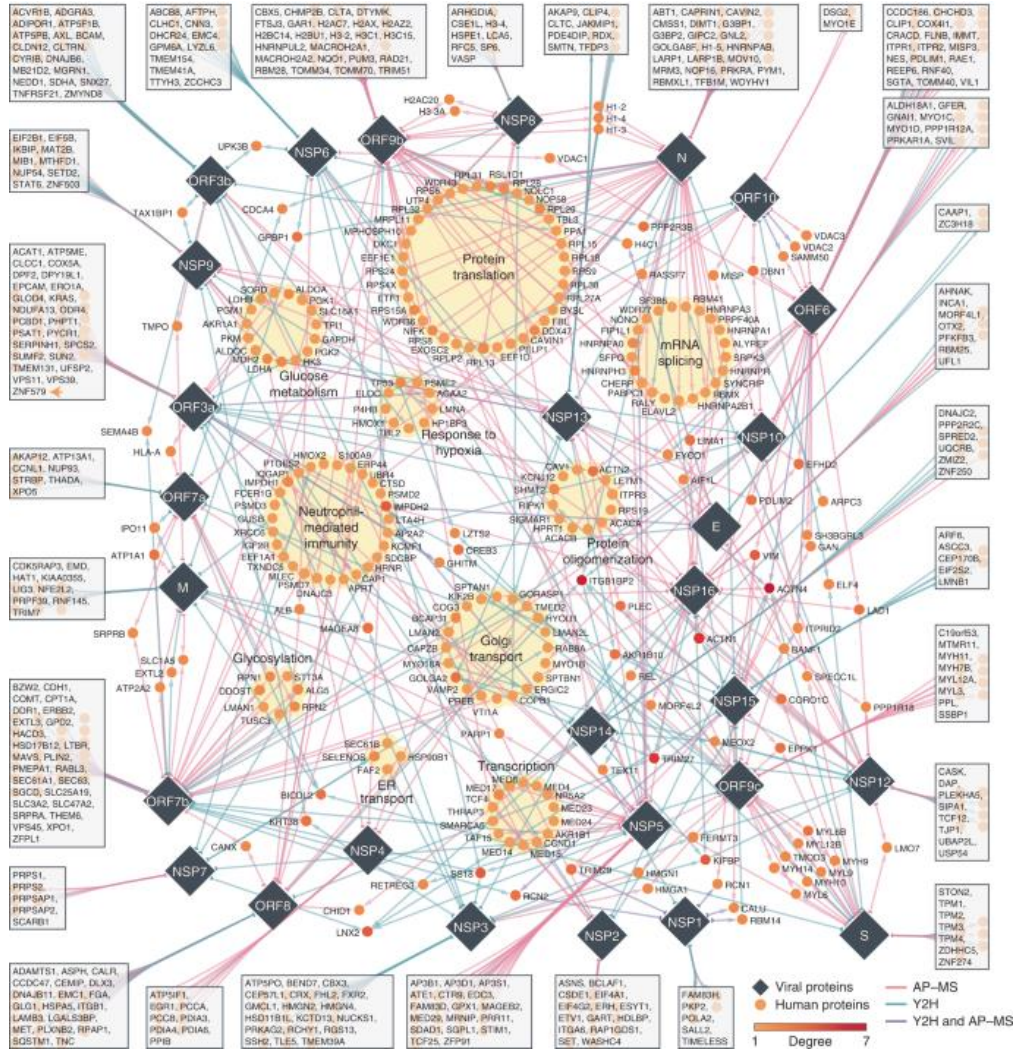
Neurotransmiter transporter

But some previous knowledge is required, and the computational costs are big.

Interactome







Zhou et al., 2023

SARS-CoV-2 proteins

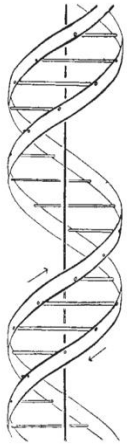
Human proteins

Solve the structure, solve the function

The relationship between structure and function is interdisciplinary.
It helps us build functional objects and understand biological objects.

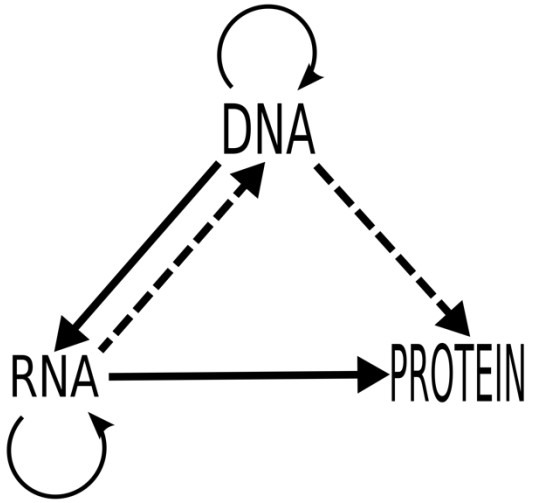
Solving the structure of biomacromolecules

DNA structure

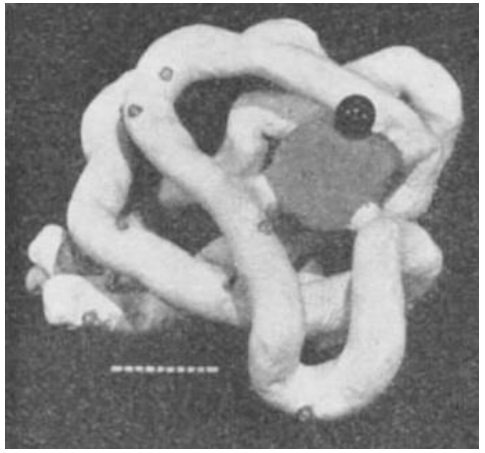


Watson and Crick, 1953

Molecular biology central dogma



Myoglobin structure



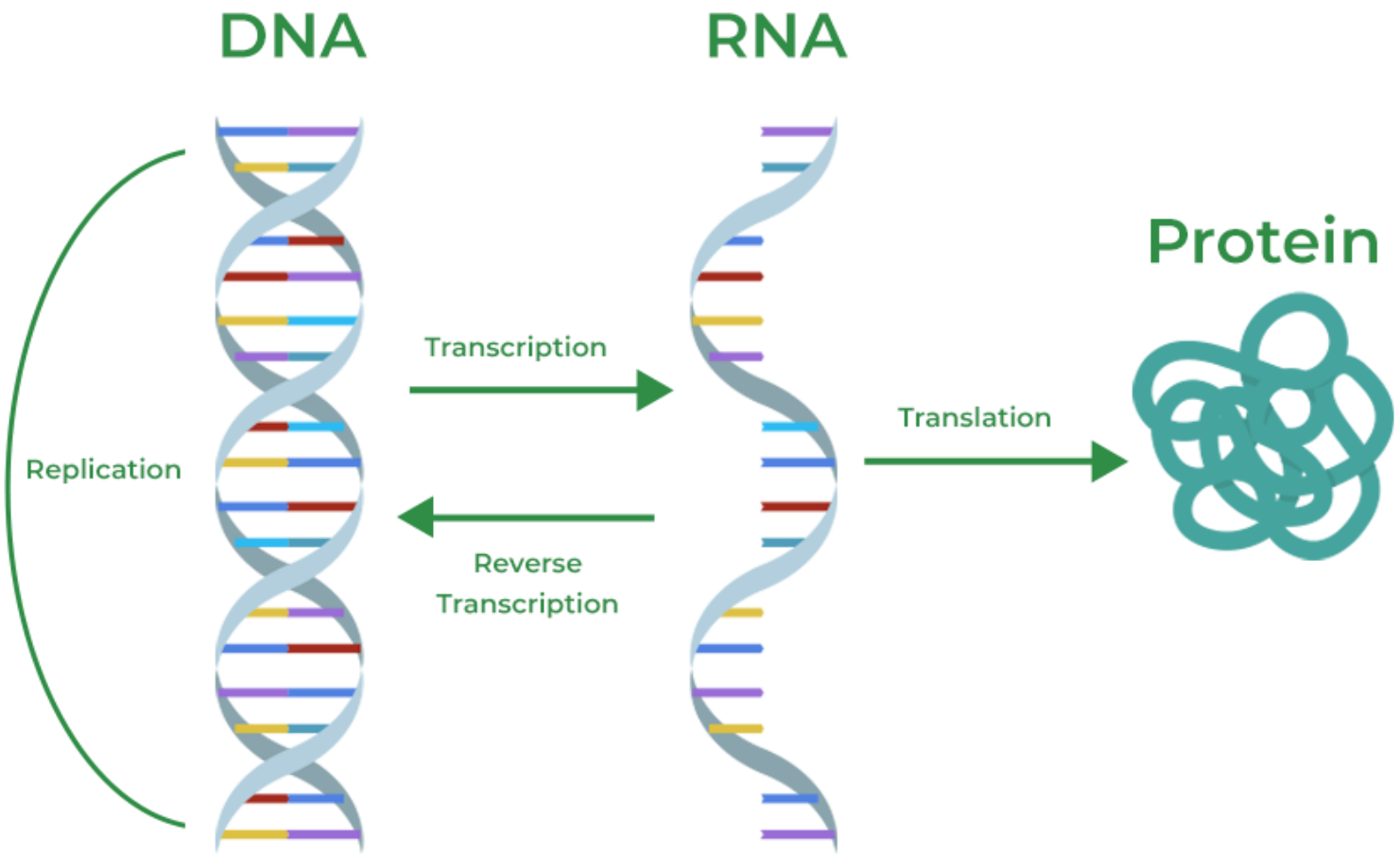
Kendrew et al., 1958

1953

1957

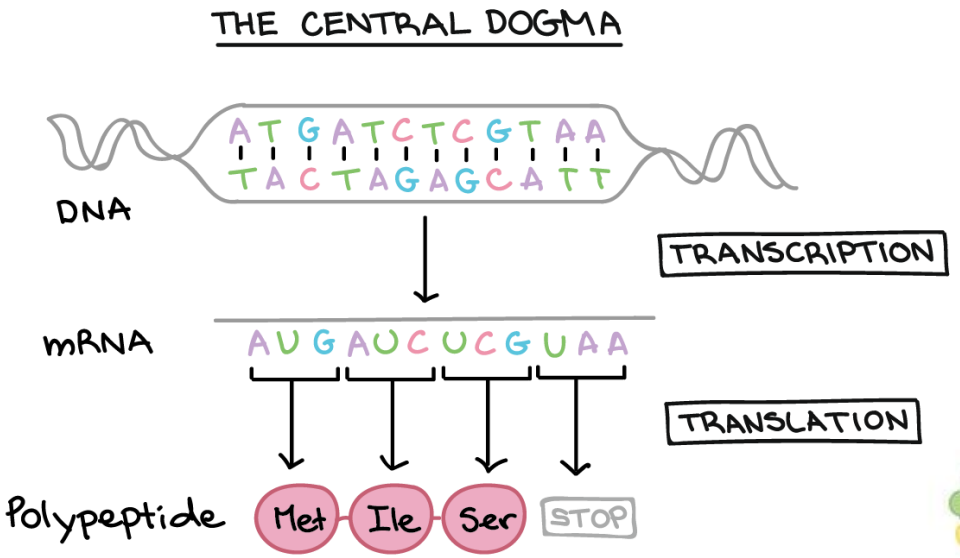
1958

Molecular biology central dogma

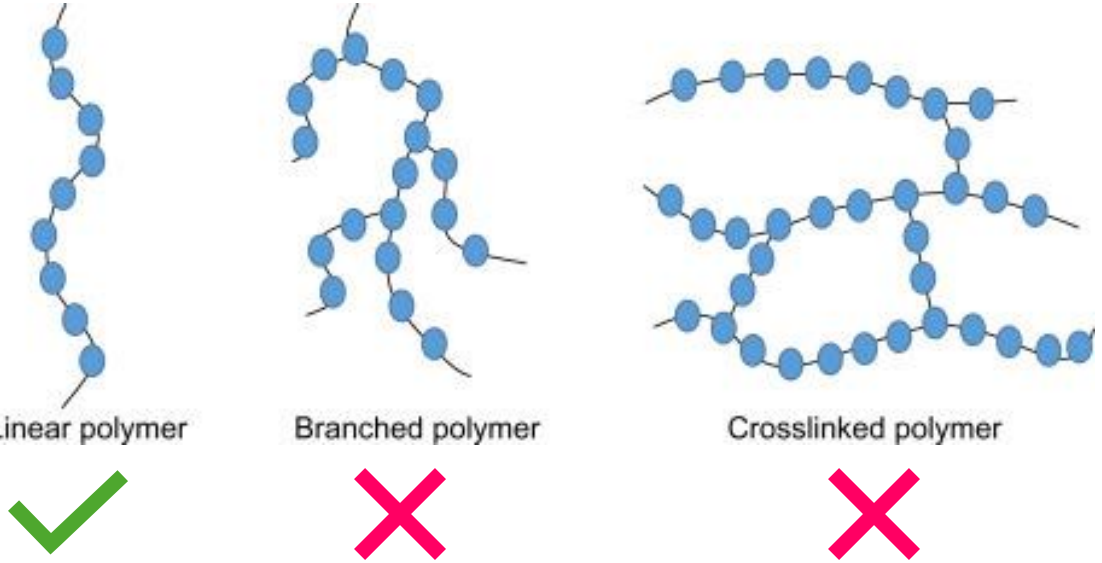


https://www.geeksforgeeks.org/central-dogma-steps-guide/

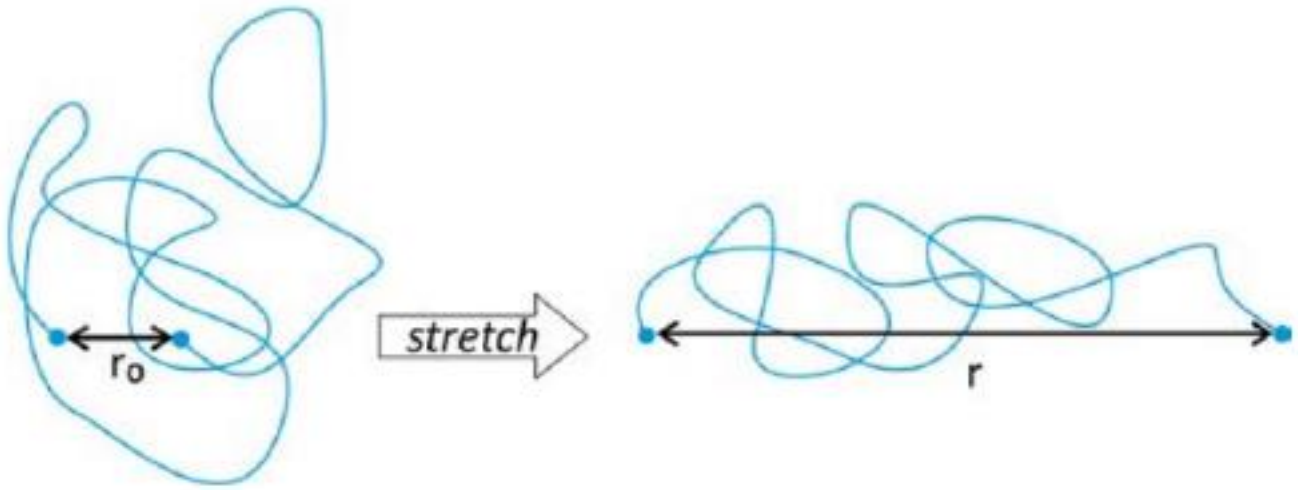
Proteins are linear heteropolymers



https://www.khanacademy.org



Conformations of a linear polymer



The completely stretched conformation is just one among countless possibilities.
But these conformations have **restrictions**!

Amino acids are the building blocks

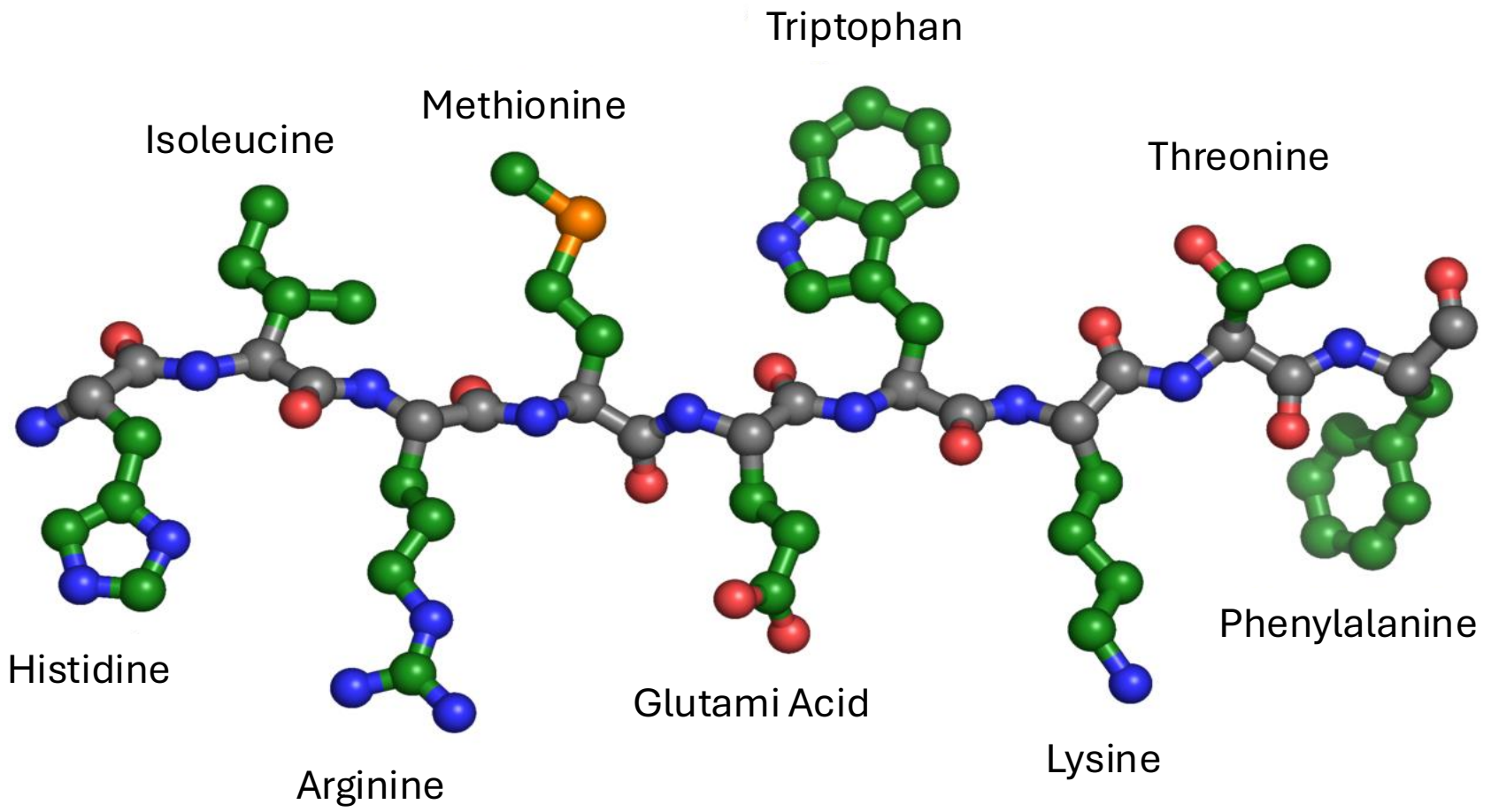
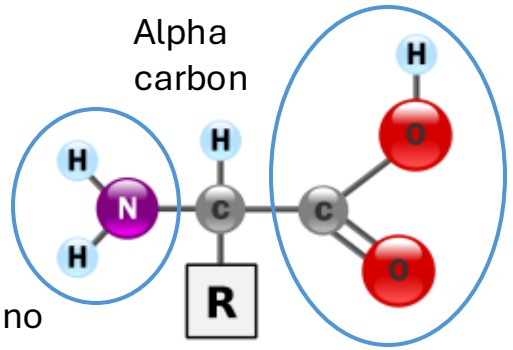
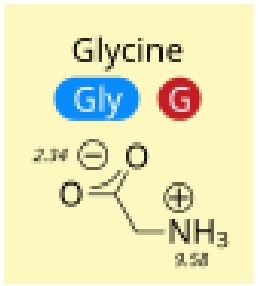


Figure by Richard Garratt

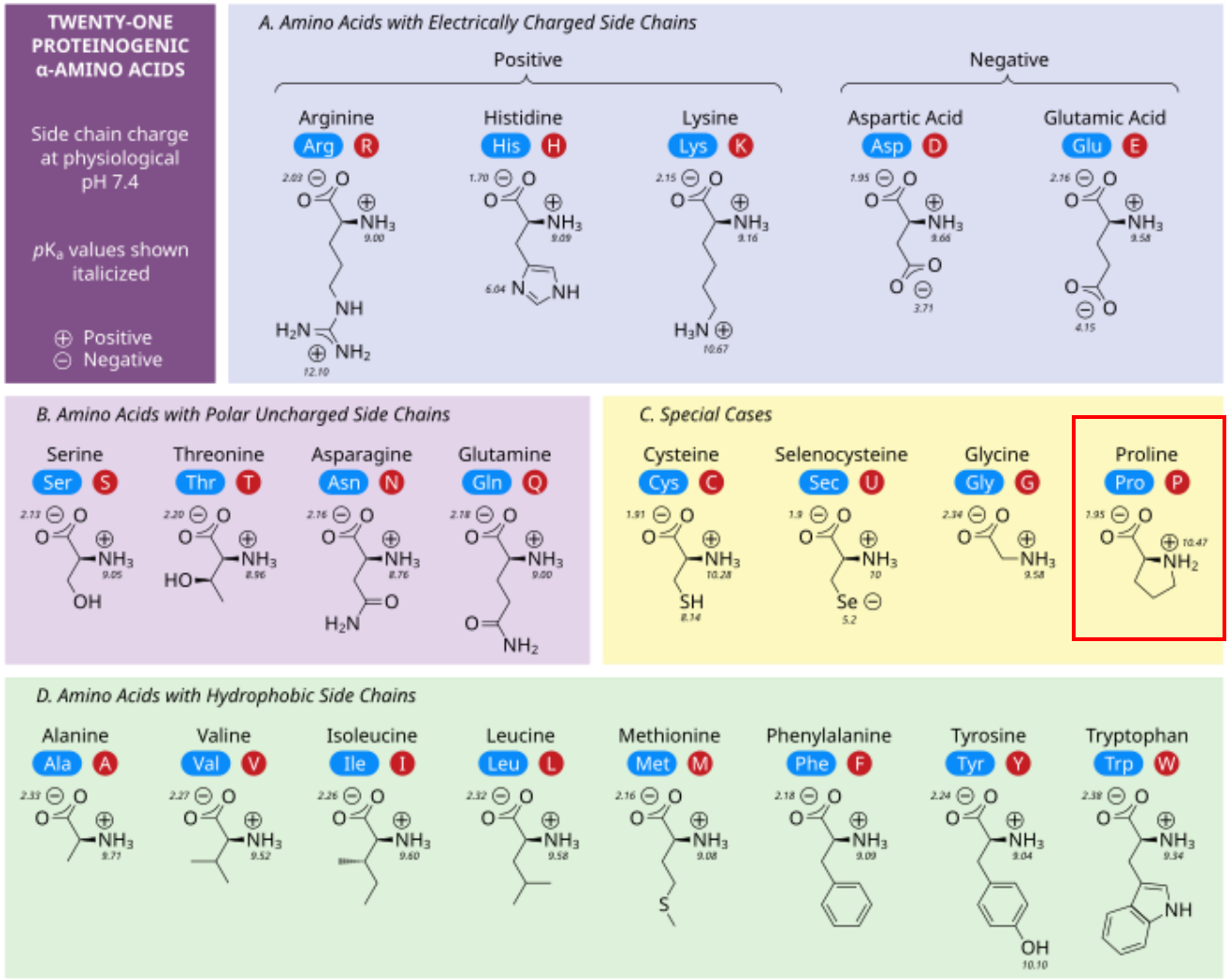
The twenty amino acids

Basic structure





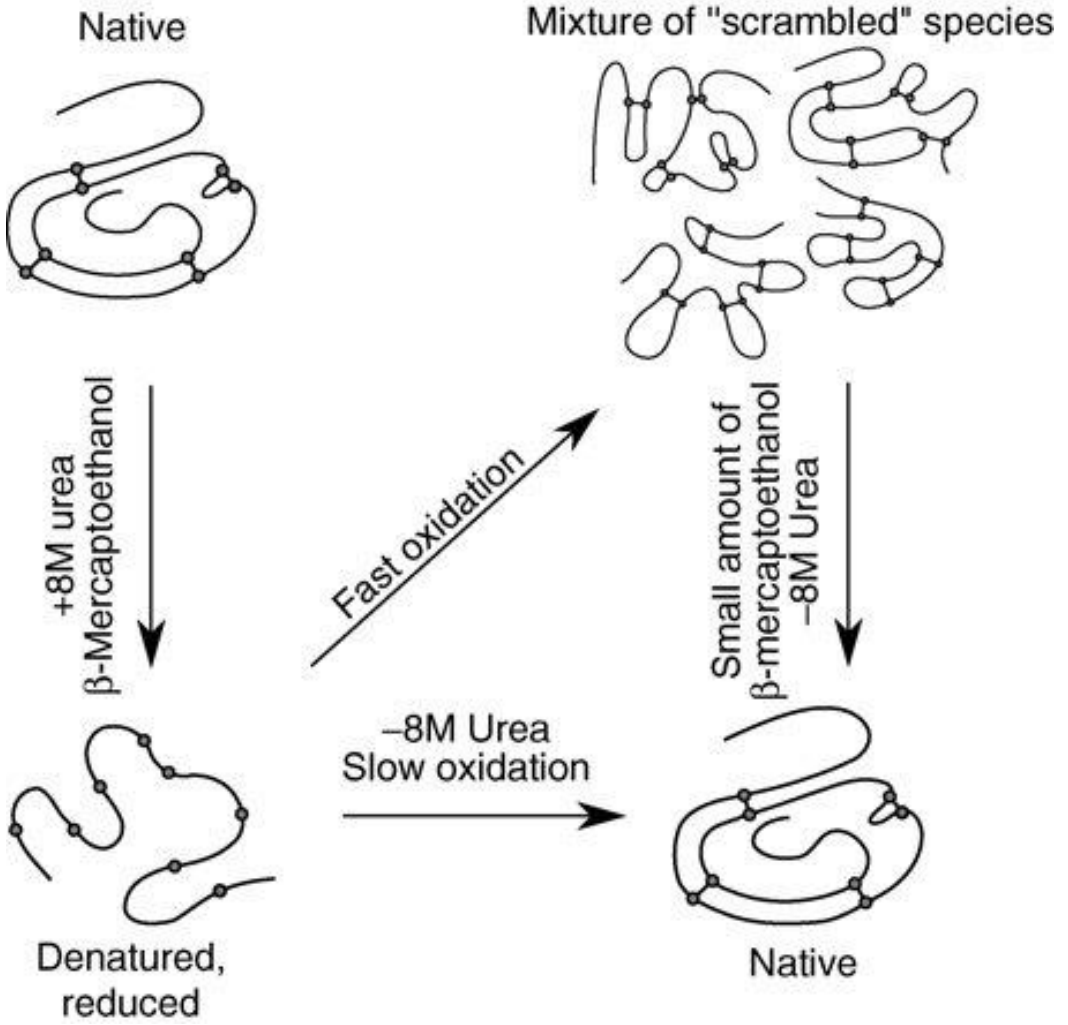
\+ different side chains



Anfinsen's experiment (1961)

Urea
disrupts non-covalent interactions like hydrogen bonds

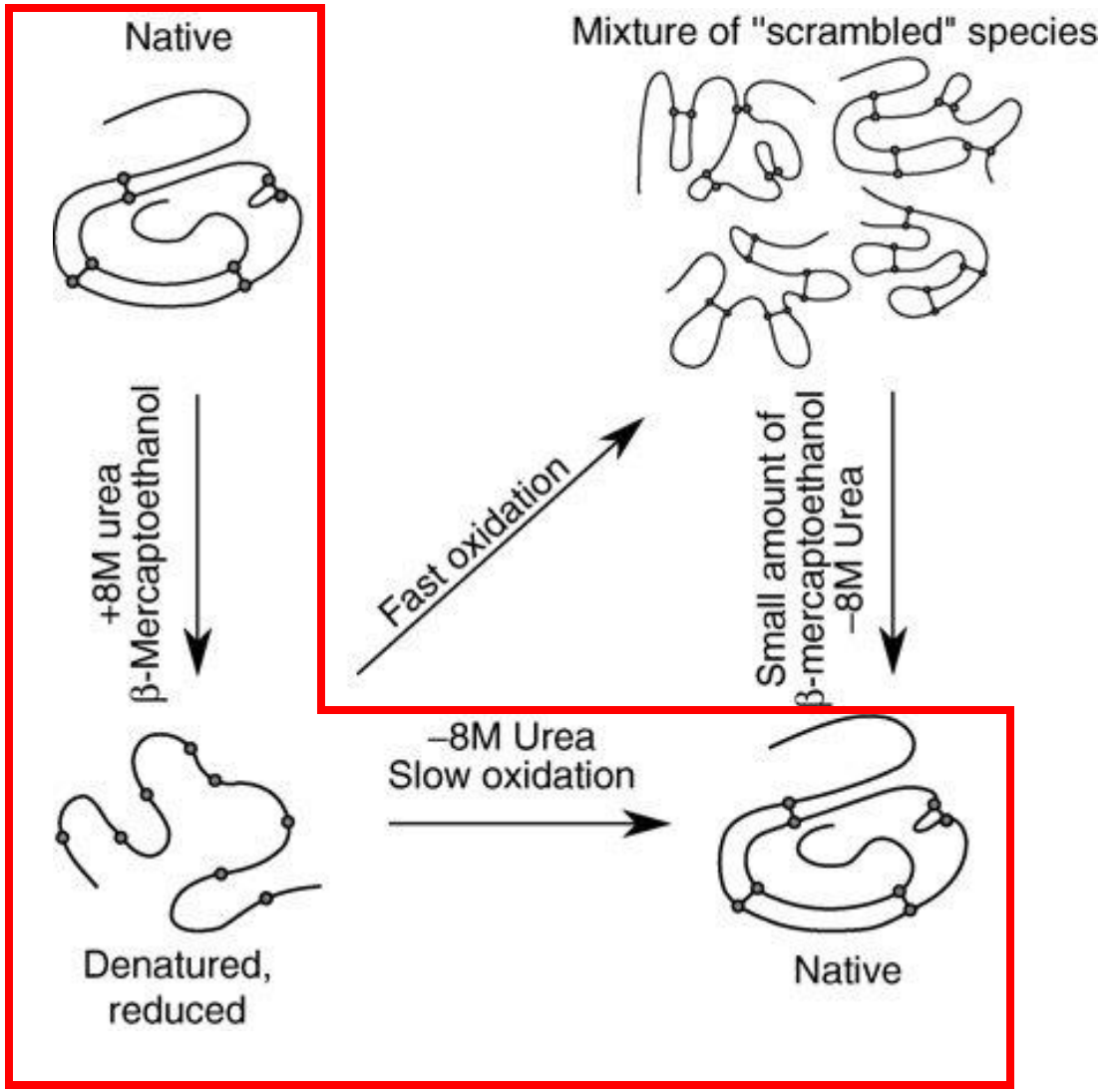
β-mercaptoethanol
reduces disulfide bonds



Christian B. Anfinsen

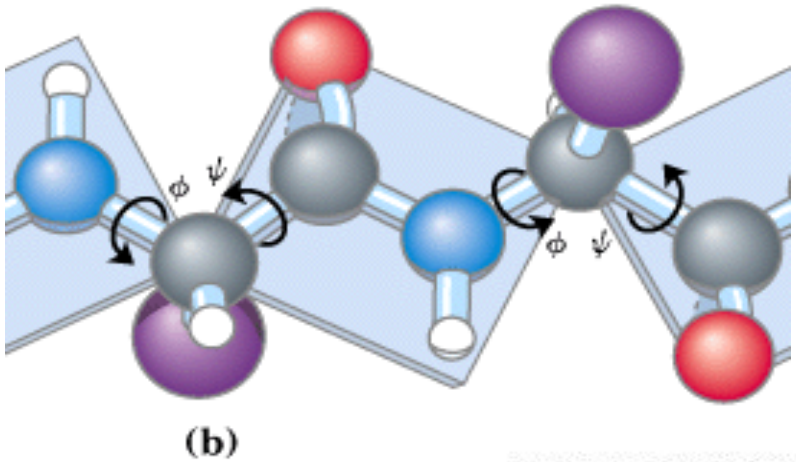
Anfinsen's hypothesis

The information for correct protein folding is entirely contained in its amino acid sequence.



Levinthal's paradox (1969)

- 3 stable conformations per angle (alpha helix, beta sheet and loop)
- For a protein with 101 amino acids, $3^{100} = 5 \times 10^{47}$ conformations

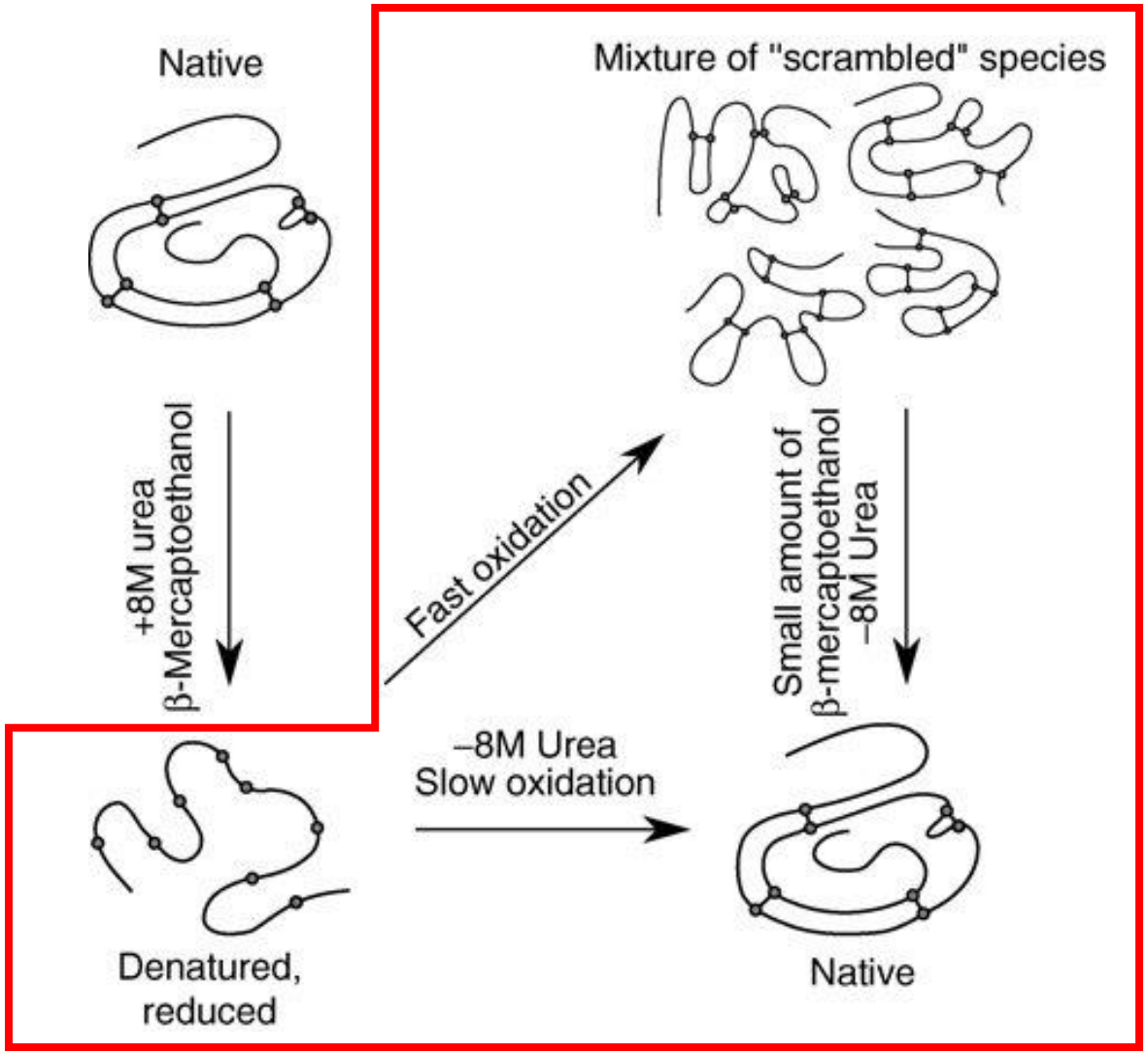


- Bond rotation rate of 10^{13} conformations per second
- It will take **10^{27} years to try them all!**

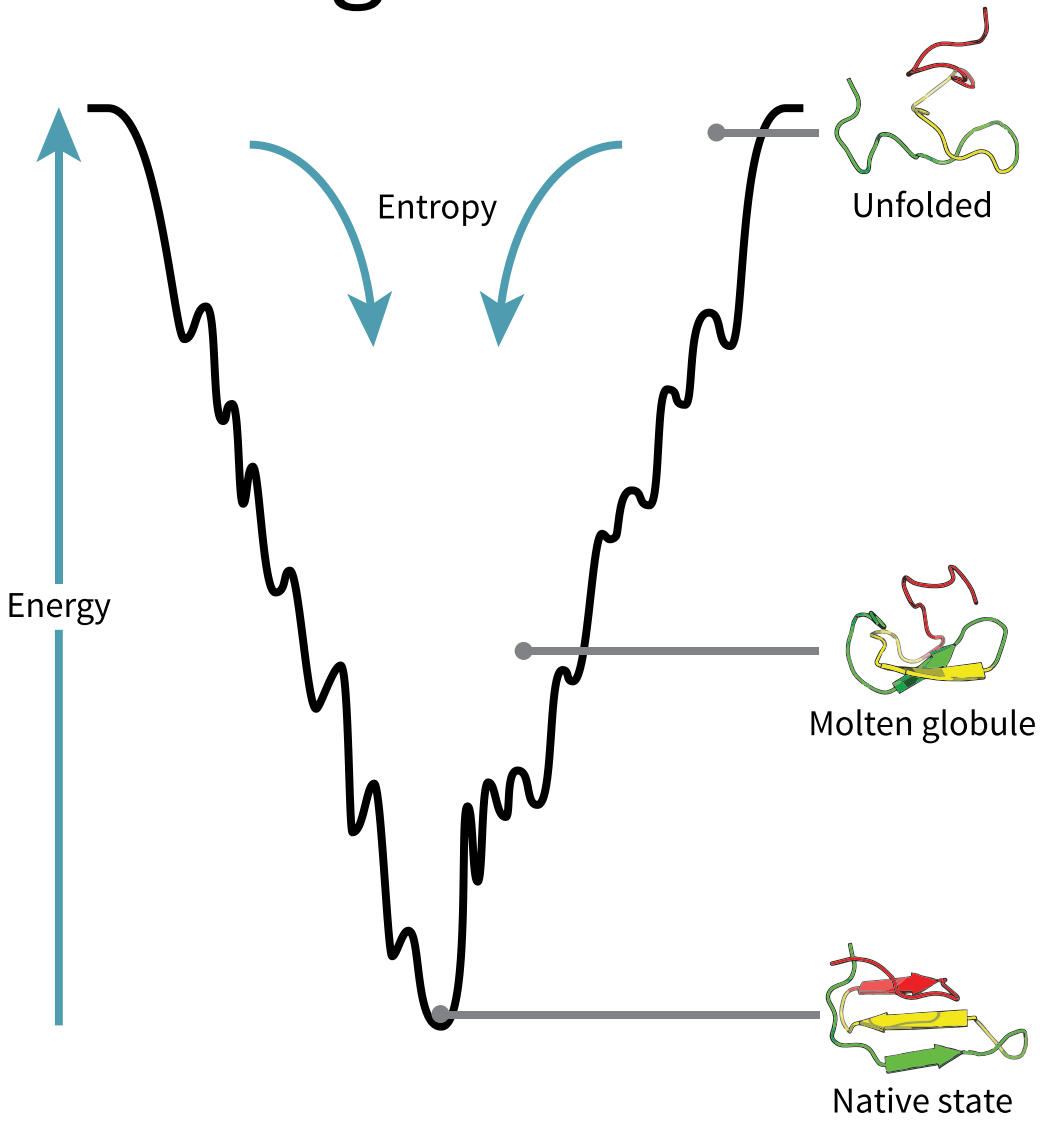
Order of hydrogen bonds and disulfide bonds

The order and timing of bond formation (non-covalent before covalent) are crucial.

Hydrogen bonds help *find* the native structure, while **disulfide bonds help *stabilize* it**.



Folding funnel



Extended conformations have low probability and high energy

Globular conformations have a hydriphobic core

Lacking water, polar groups of the main chain make hydrogen bonds with each other, forming Secondary Structures (helices and sheets)

Secondary Structures arrange into motives and domains, which arrange into Tertiary Structures

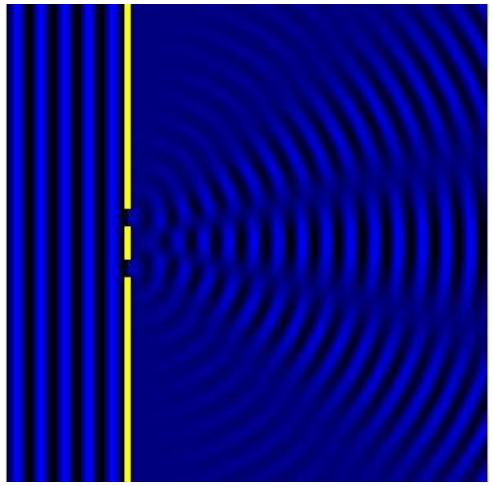
Other protein chains arrange to form Quaternary Structures

The quest to solve protein structure

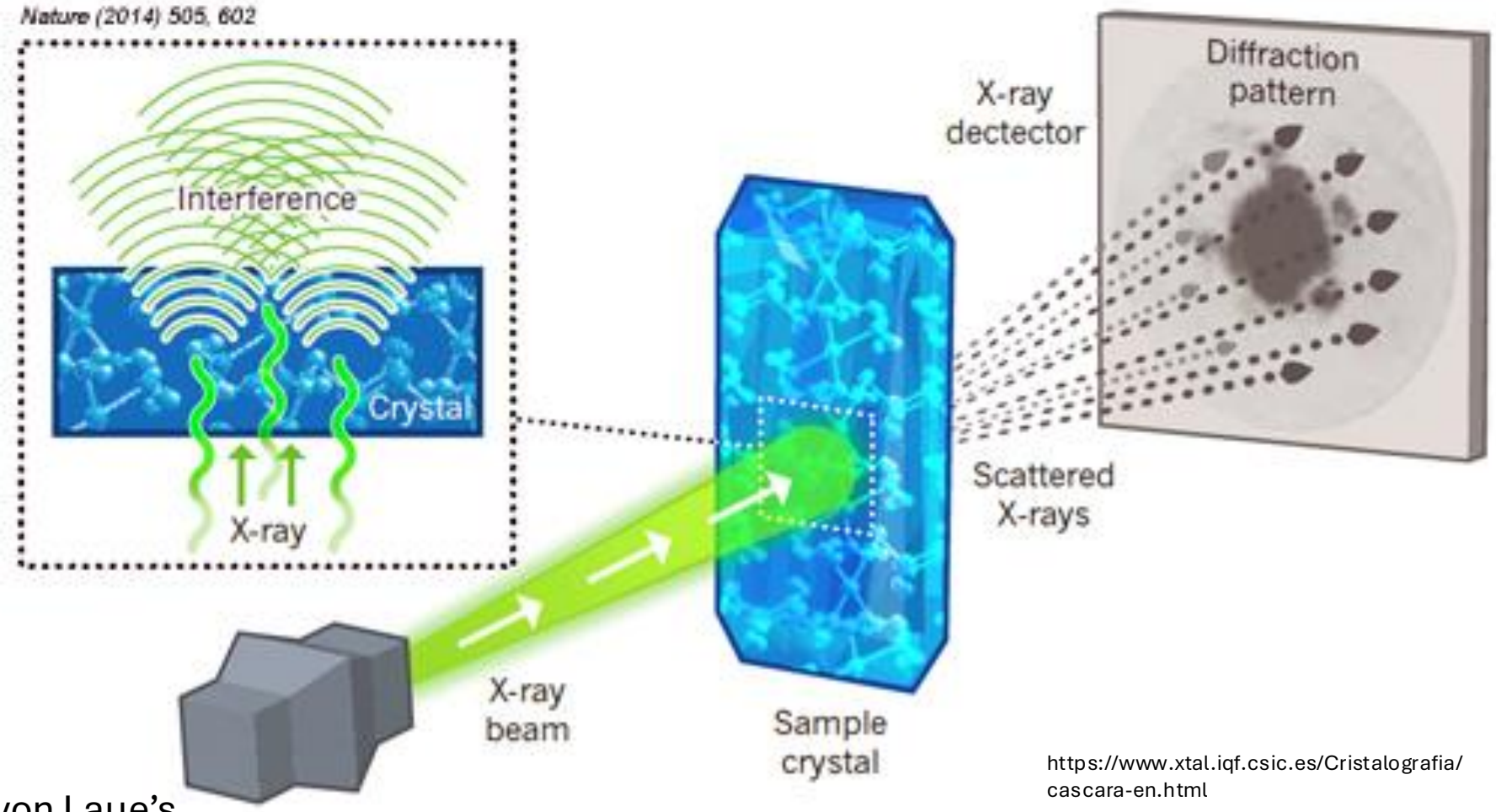
- Experiments – Crystallography, Nuclear Magnetic Resonance and Cryo-Electron Microscopy

- Modeling – Simulate folding and by homology with known structures

Crystallography



Max von Laue's
x-ray machine, 1912



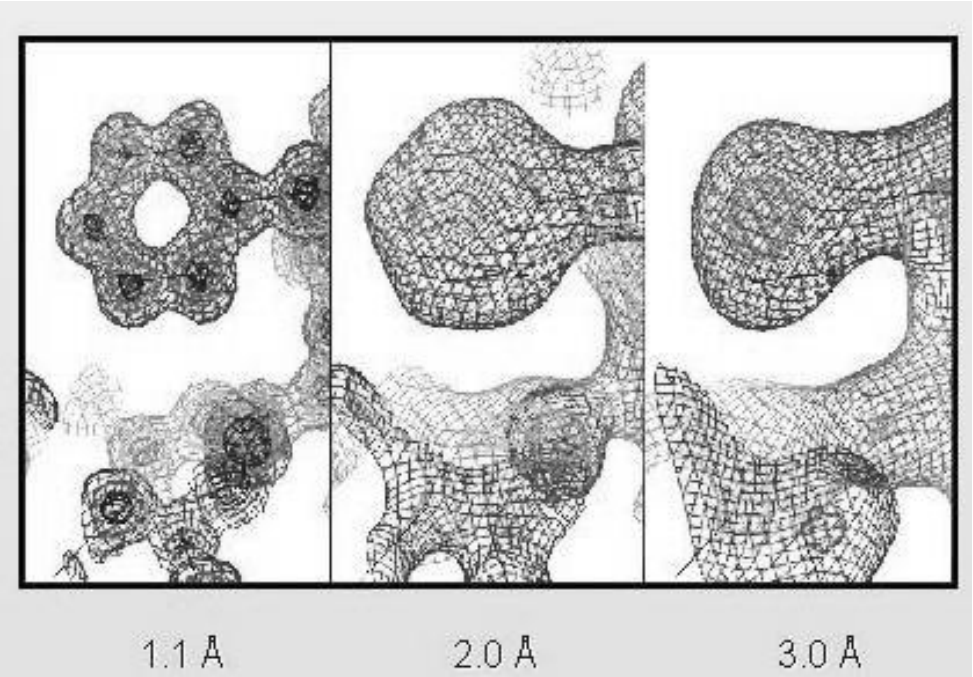
https://www.xtal.iqf.csic.es/Cristalografia/cascara-en.html

The phase problem

Diffraction data gives the **intensities** of reflected X-rays, which are proportional to the **amplitudes** of the structure factors. However, **phases** are lost in the experiment, and you need both **amplitudes and phases** to reconstruct the electron density map (via Fourier transform).

Molecular replacement solves the phase problem by:

1. Using a previously solved **homologous structure** as a **model**.
2. Placing and orienting the model within the new crystal's unit cell.
3. Calculating initial phase estimates from this positioned model.
4. Refining the model against the observed data.



http://www.bmsc.washington.edu/people/verlinde/experiment.html

A model that fits the experimental data.

Identification of common structural features

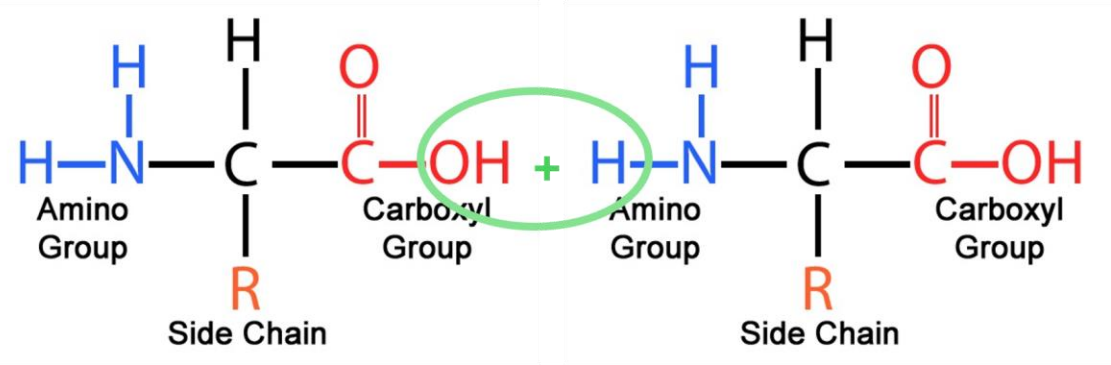
- Not all conformations are allowed because some cause clashes between atoms

- Foldings are hierarchicaly organized into secondary, tertiary and quaternary structures

Prohibited conformations

Calculated prohibited conformations coming from the restrictions of the peptide bond.

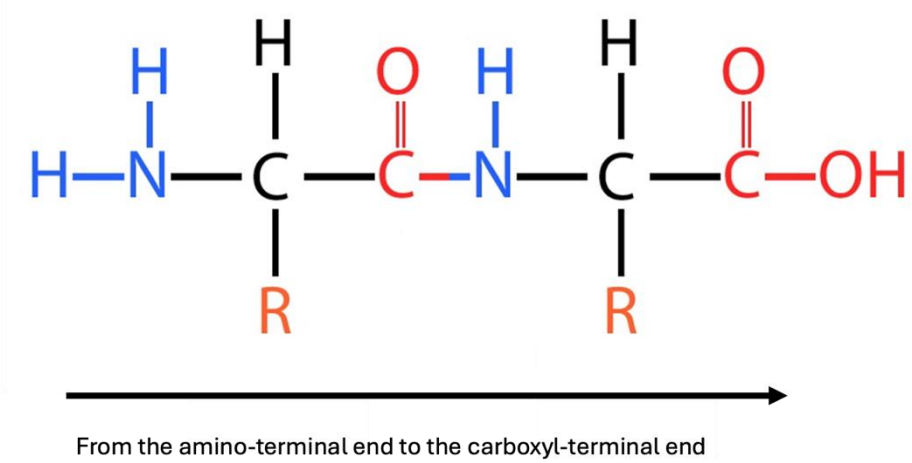
G.N. Ramachandran



Prohibited conformations

Calculated prohibited conformations coming from the restrictions of the peptide bond.

G.N. Ramachandran

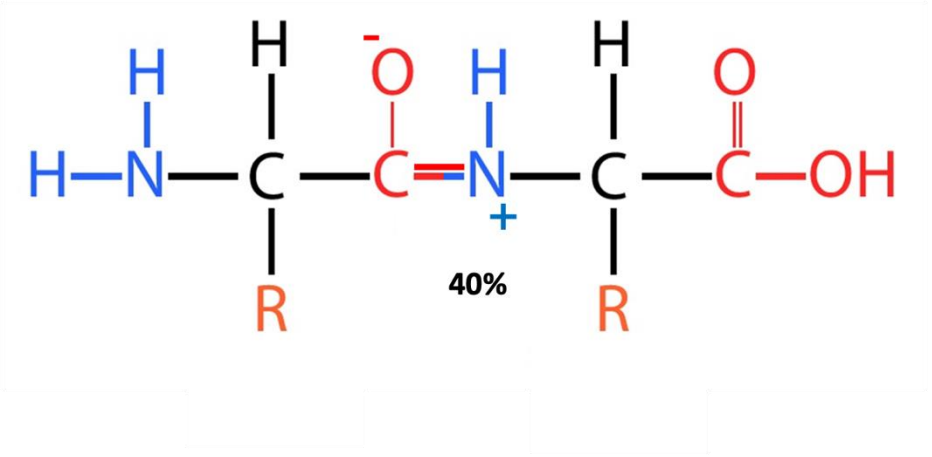


2 amino acid residues

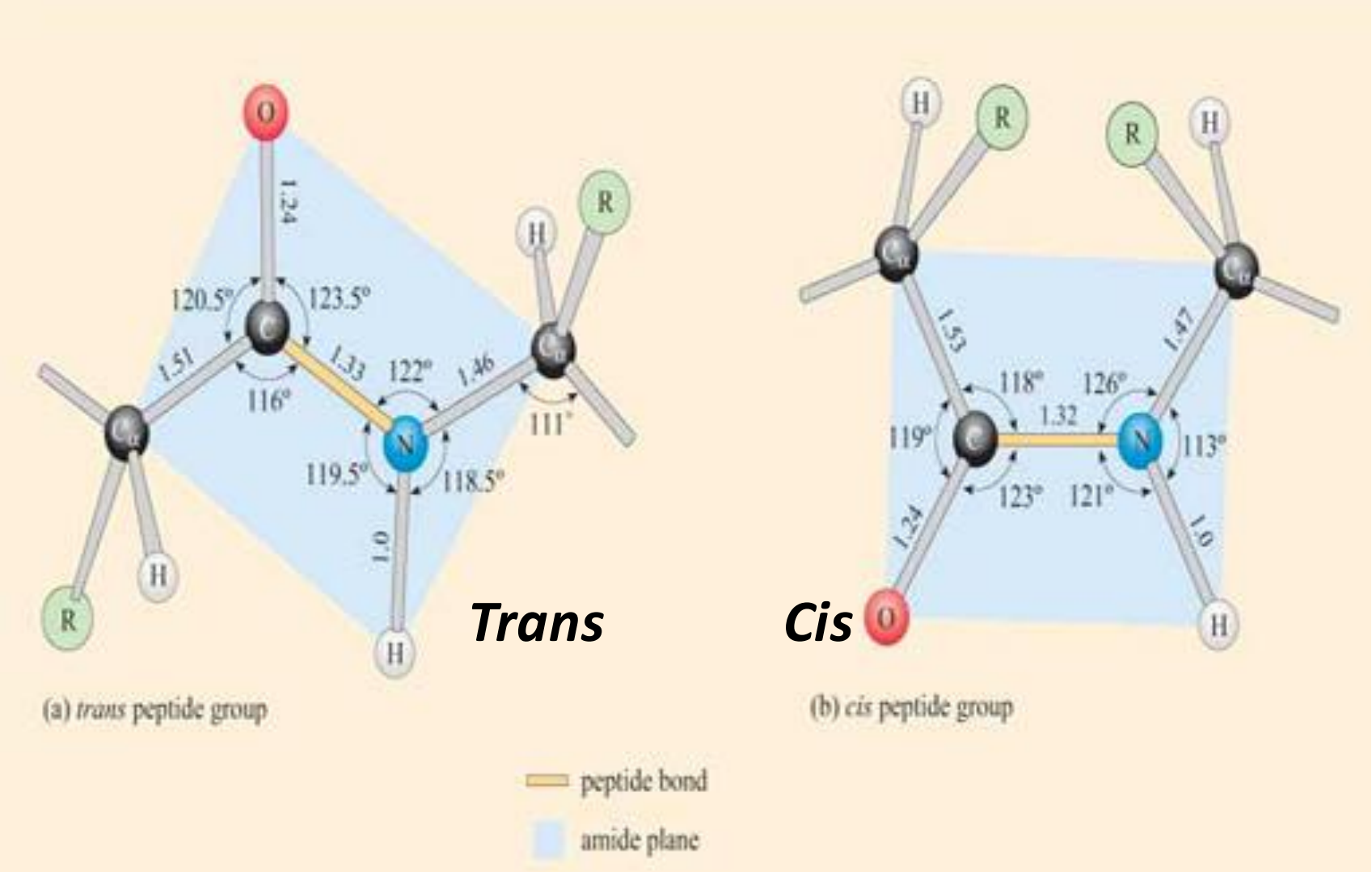
Prohibited conformations

Calculated prohibited conformations coming from the restrictions of the peptide bond.

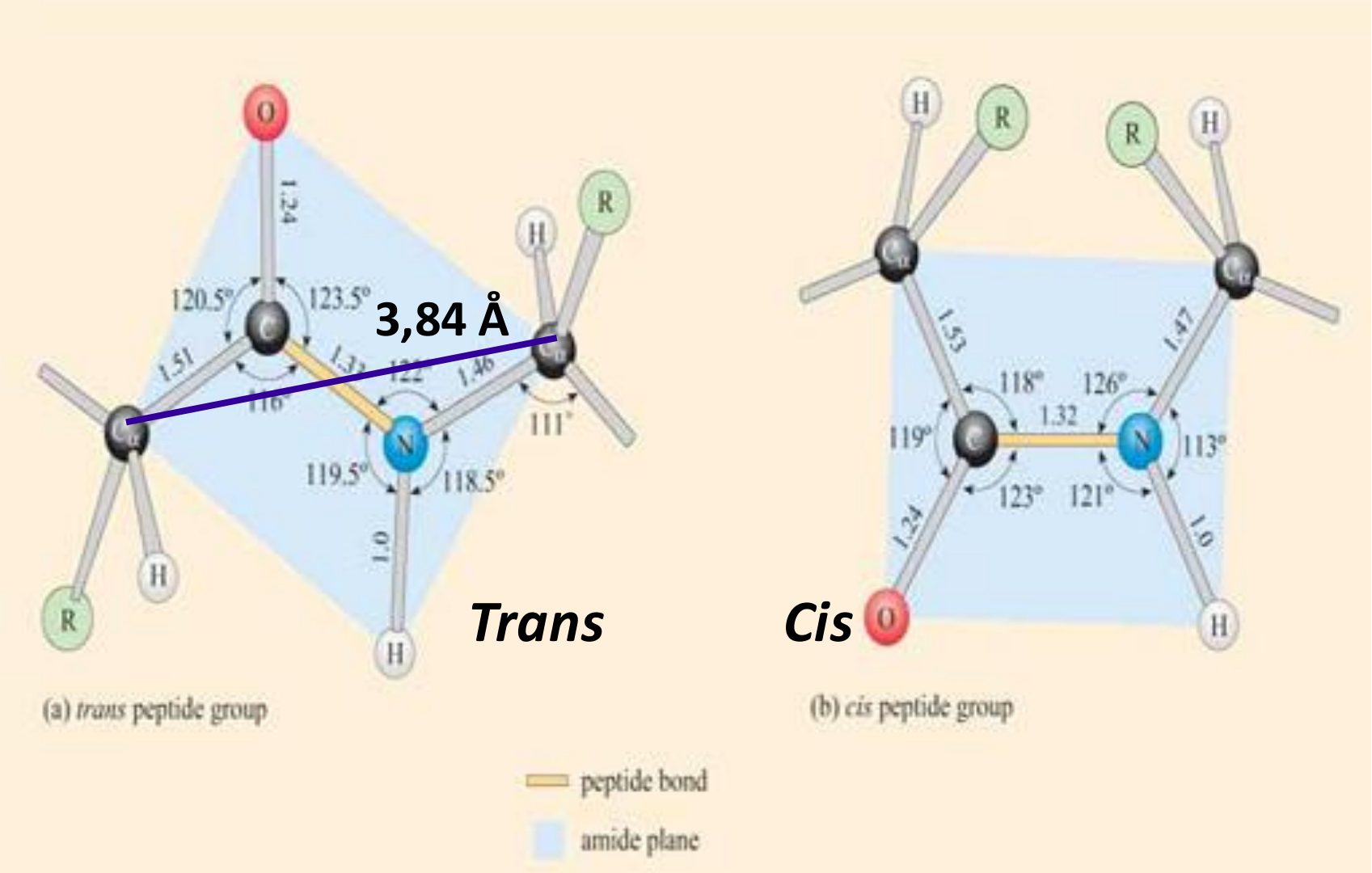
G.N. Ramachandran



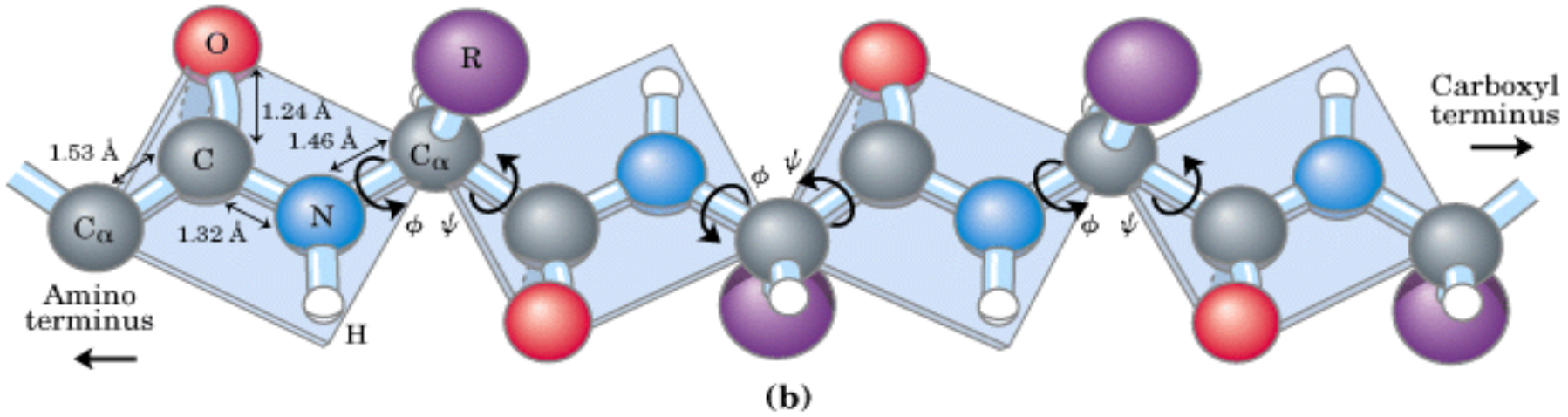
The peptide bond, cis or trans conformations



The peptide bond, cis or trans conformations



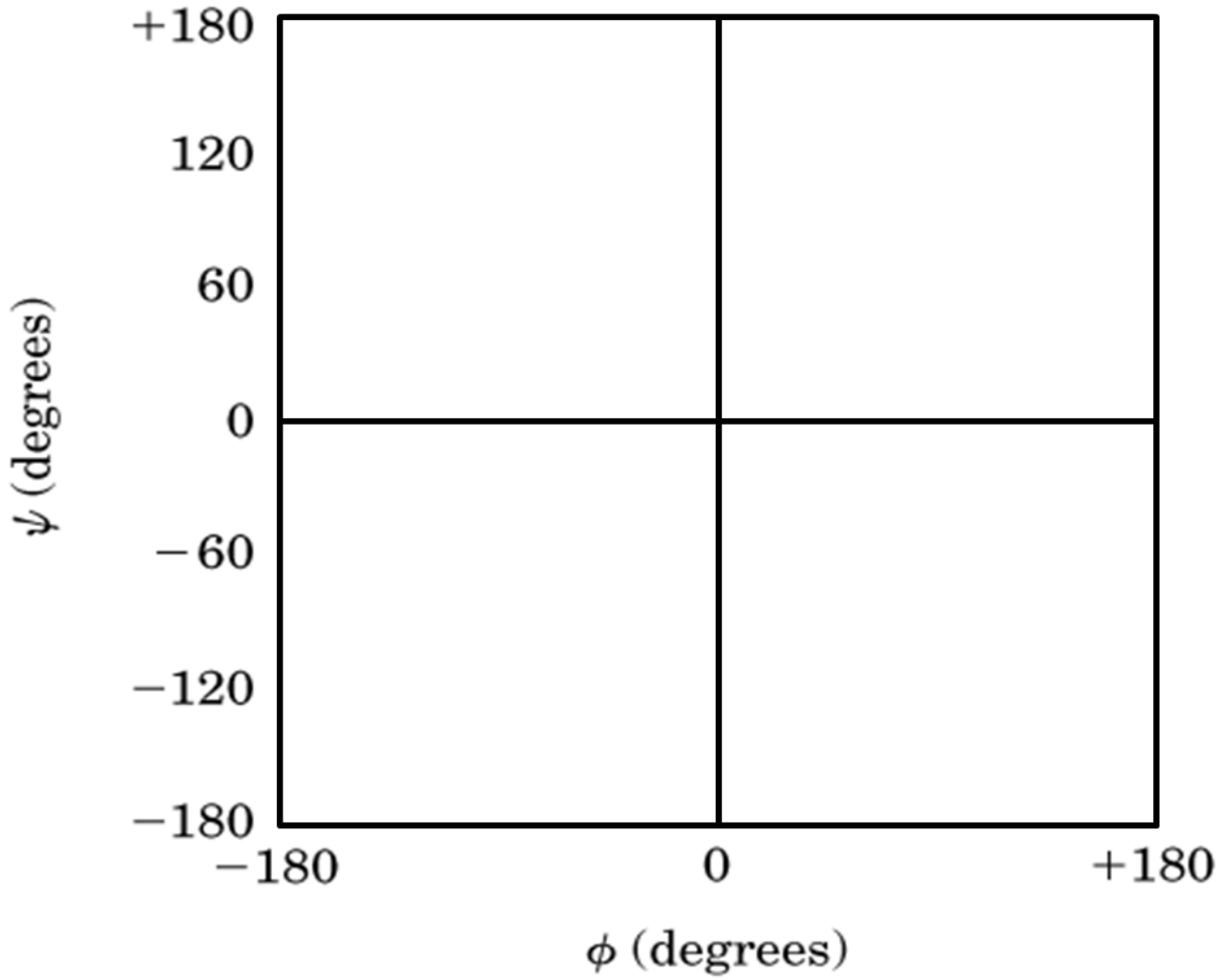
Between peptide planes are two torsion angles

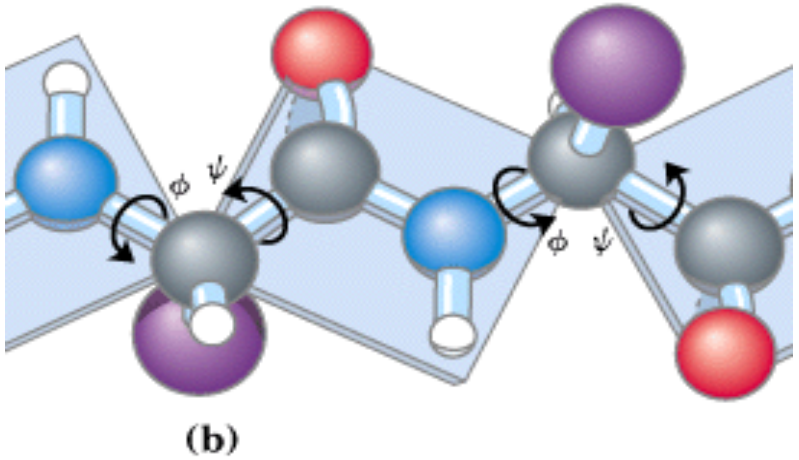


Only the ϕ and ψ angles are free to rotate

And the positions of the main-chain atoms can be entirely defined by them.

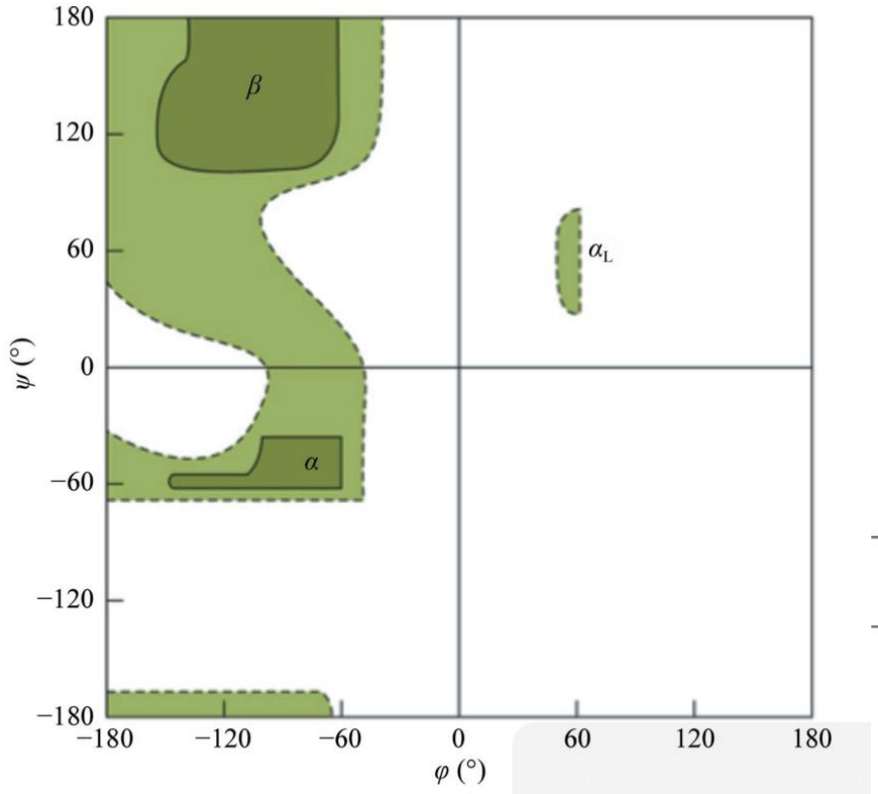
The Ramachandran plot



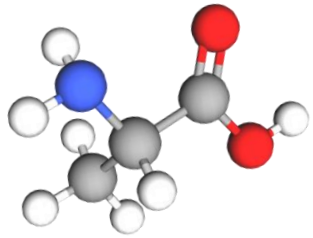


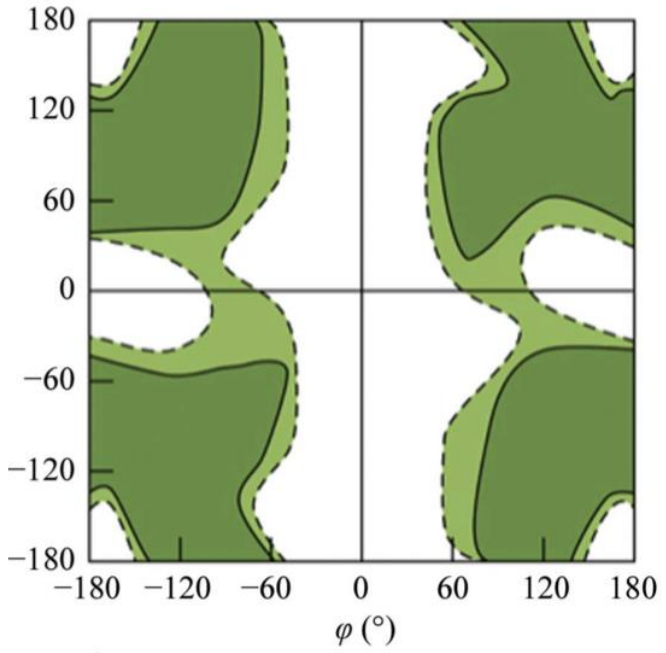
(b)

Allowed angles depending on side chain

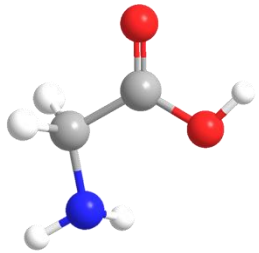


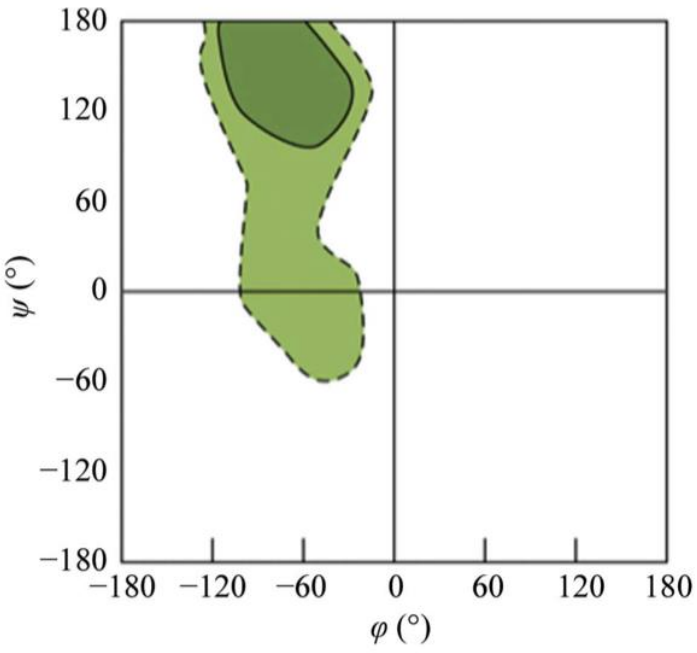
Most amino acids



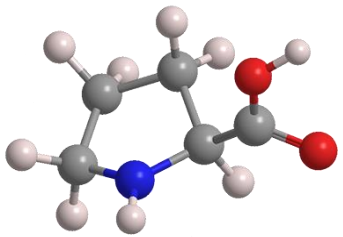


Glycine

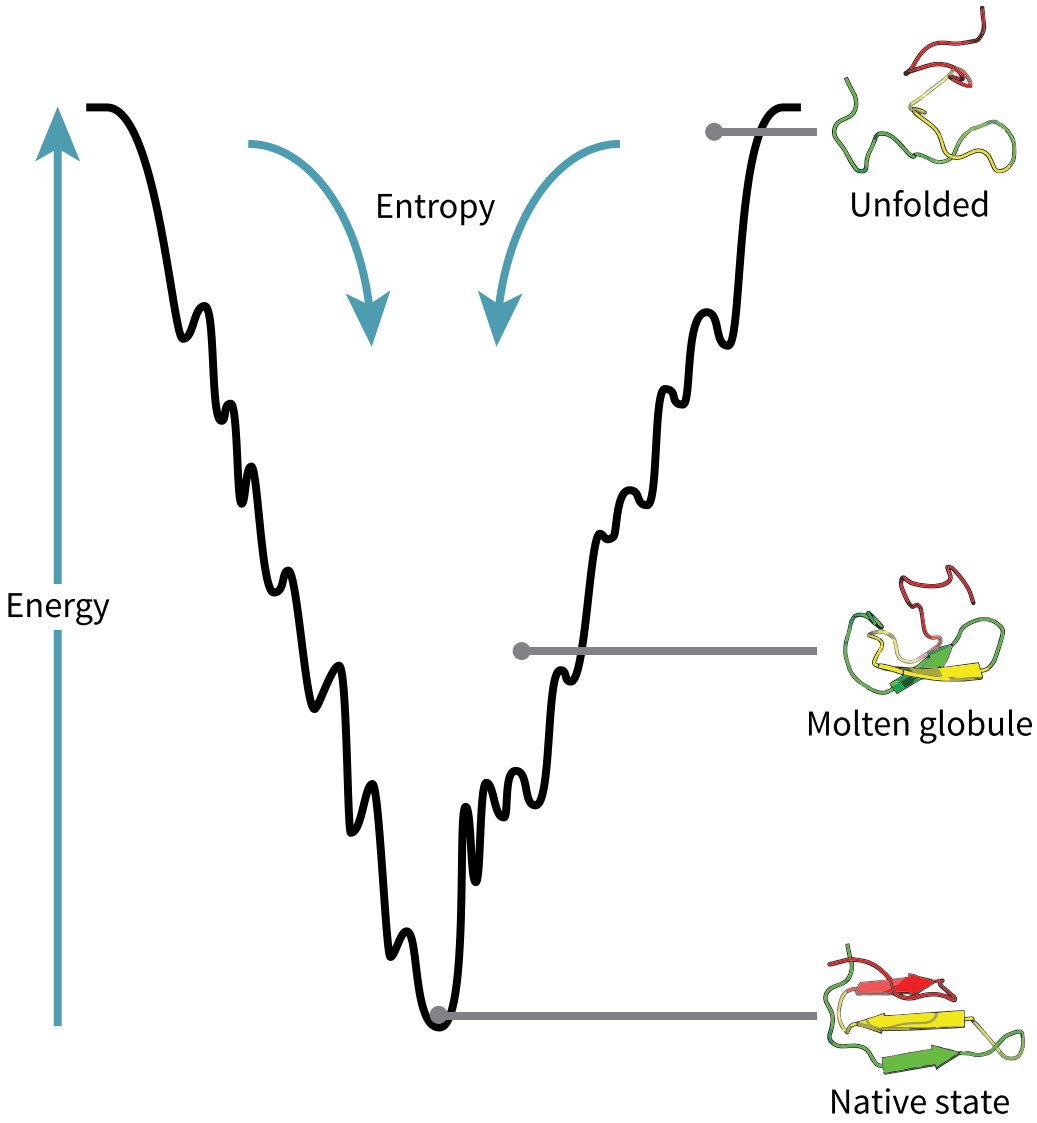




Proline



Caruso and Djinovic-Caruso, 2013



Extended conformations have low probability and high energy

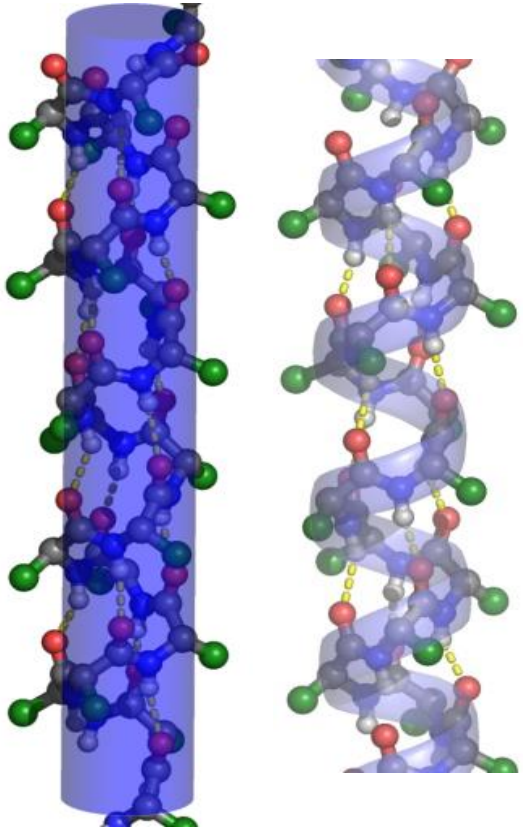
Globular conformations have a hydriphobic core

Lacking water, polar groups of the main chain make hydrogen bonds with each other, forming Secondary Structures (helices and sheets)

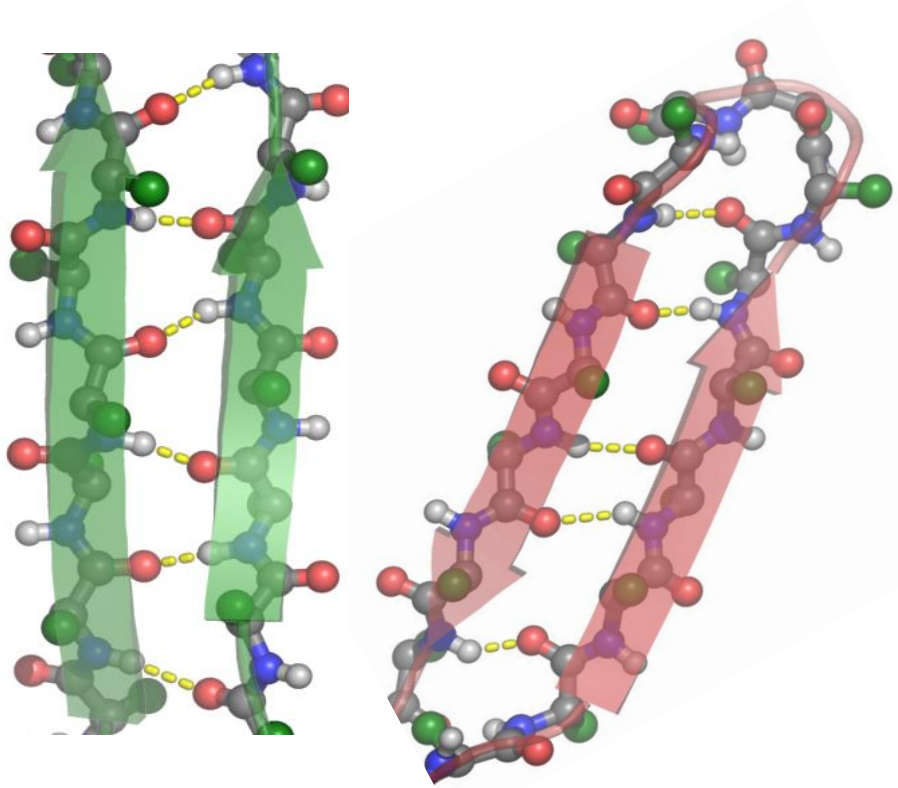
Secondary Structures arrange into motives and domains, which arrange into Tertiary Structures

Other protein chains arrange to form Quaternary Structures

Secondary structure



α-helices



β-sheets

Ramachandran plot

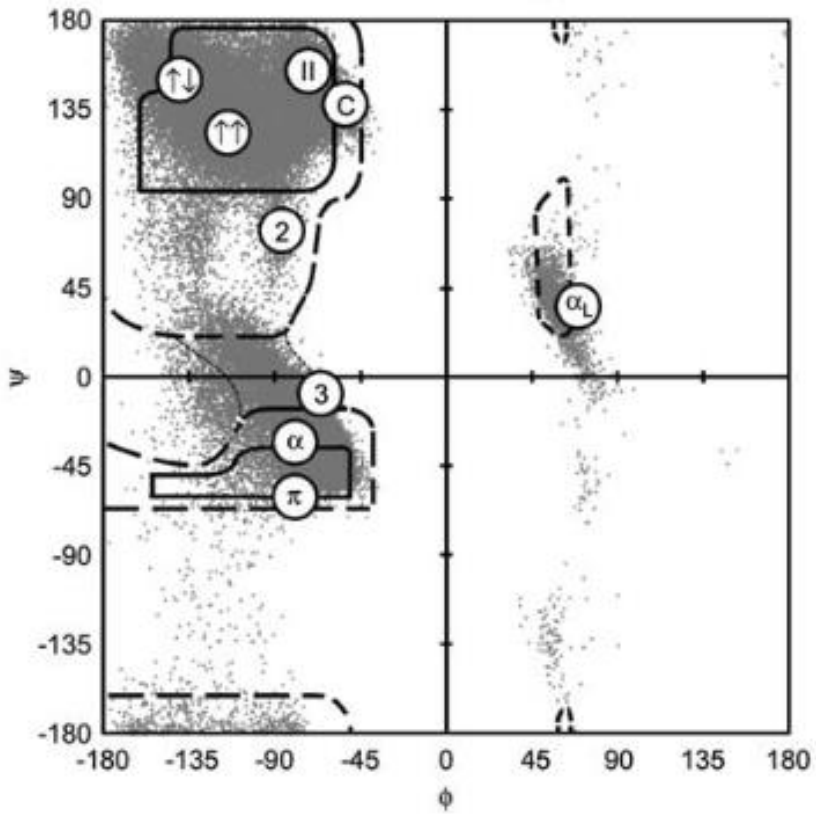


Figure by Richard Garratt

α-helices

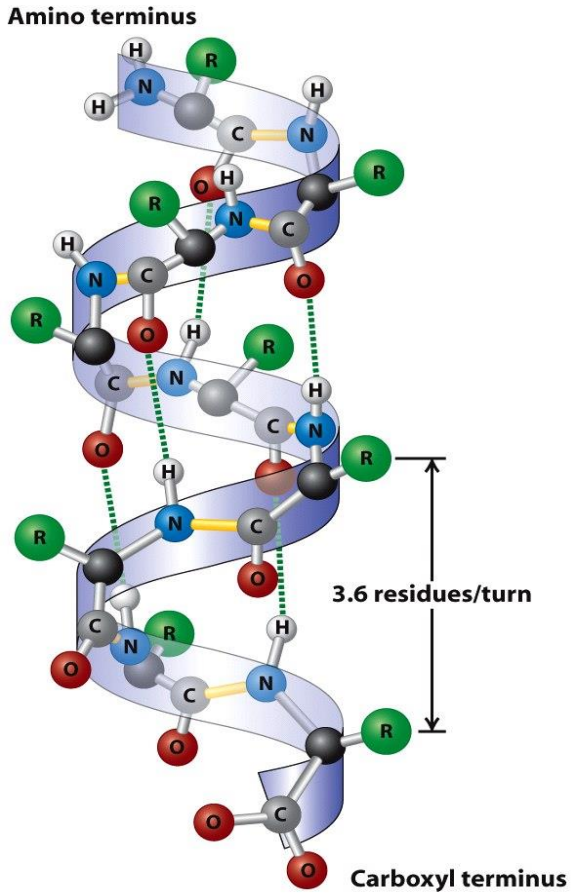
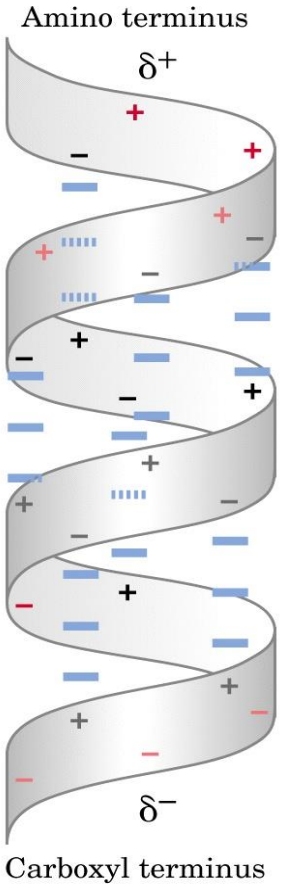
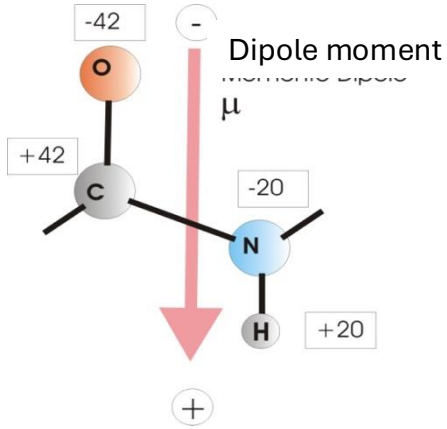


Figure 3-4
Molecular Cell Biology, Sixth Edition



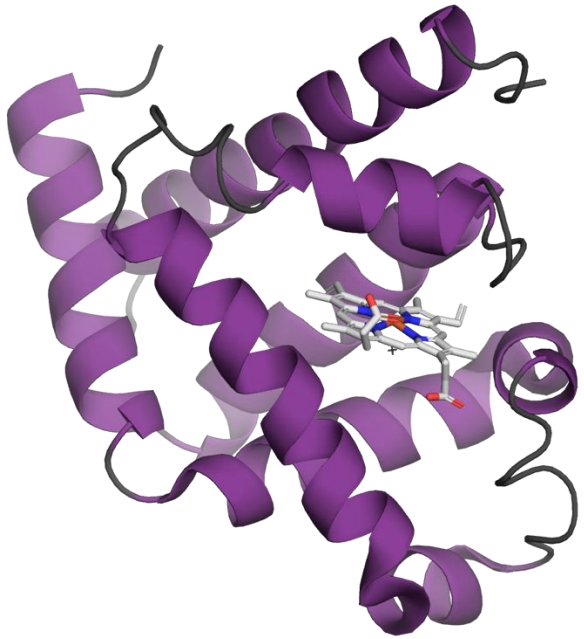


Biological Function

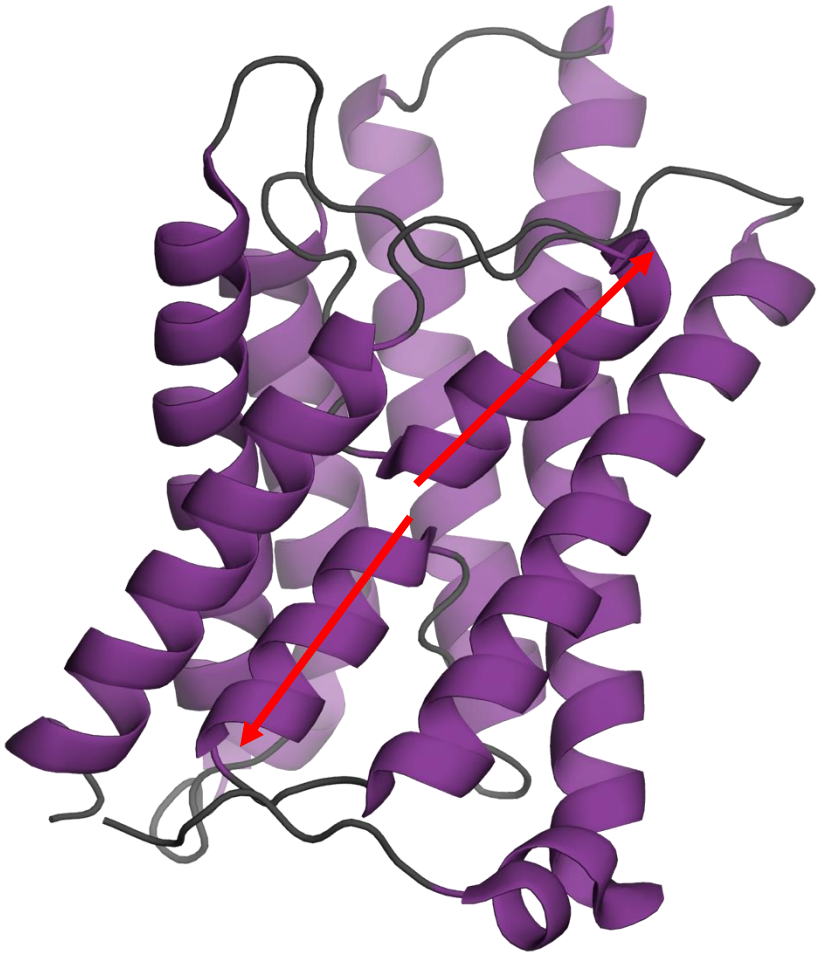
- Help in binding charged cofactors
- Long range attraction (K^+ and Cl^- channels)
- Change the nucleophilic properties of neighbouring residues for catalysis

Slide by Richard Garratt

All alpha protein example



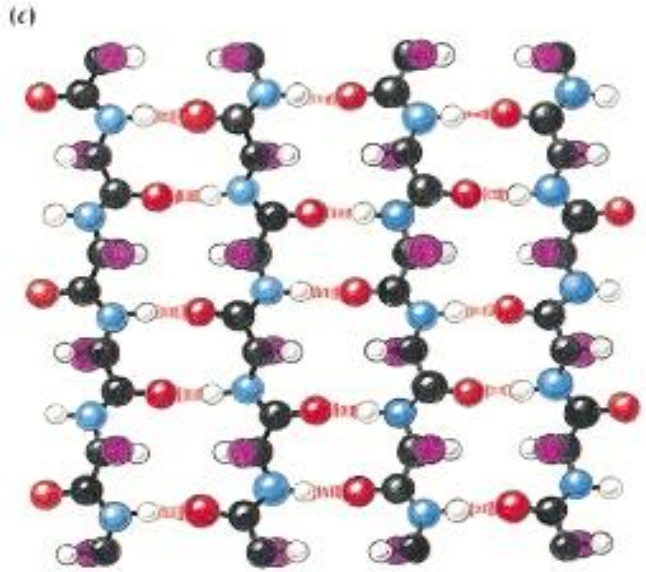
Myoglobin, 1976

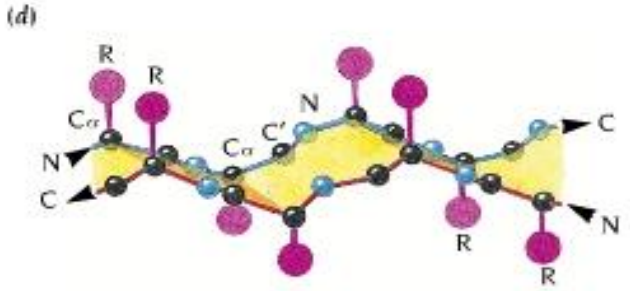


Aquaporin

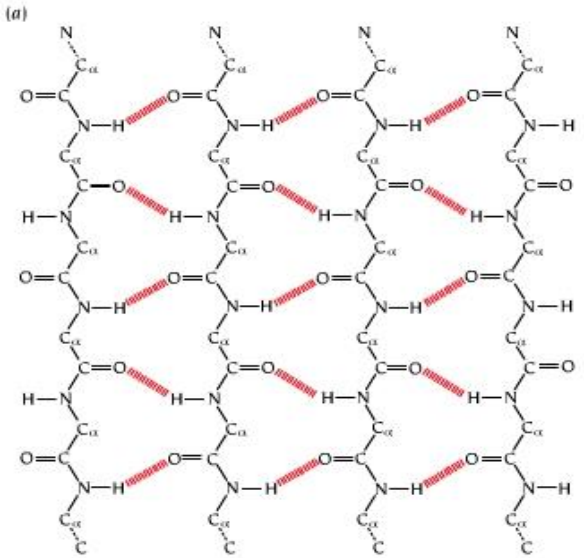
β-sheets

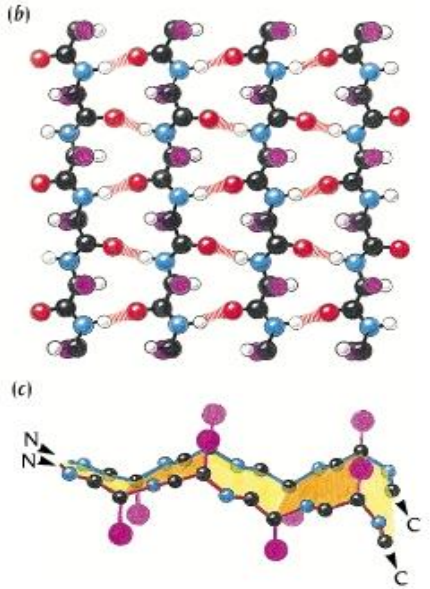
Anti-parallel β-sheet



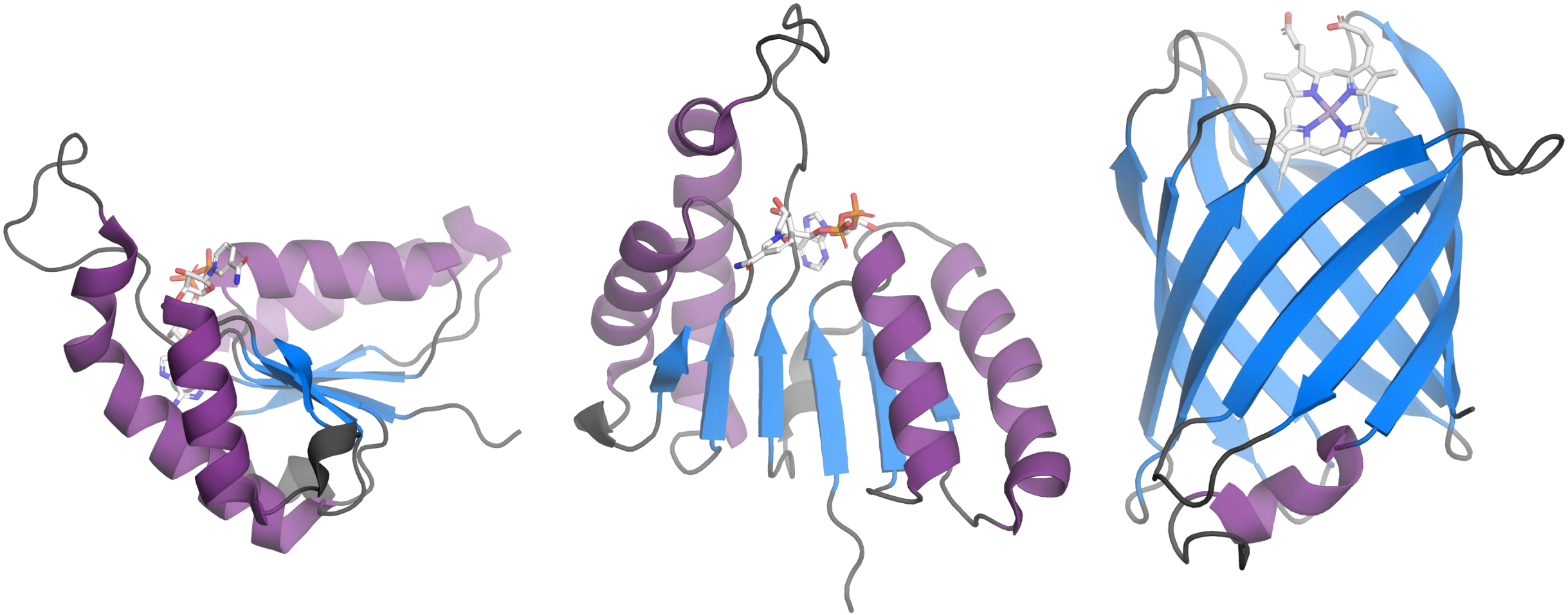


Parallel β-sheet



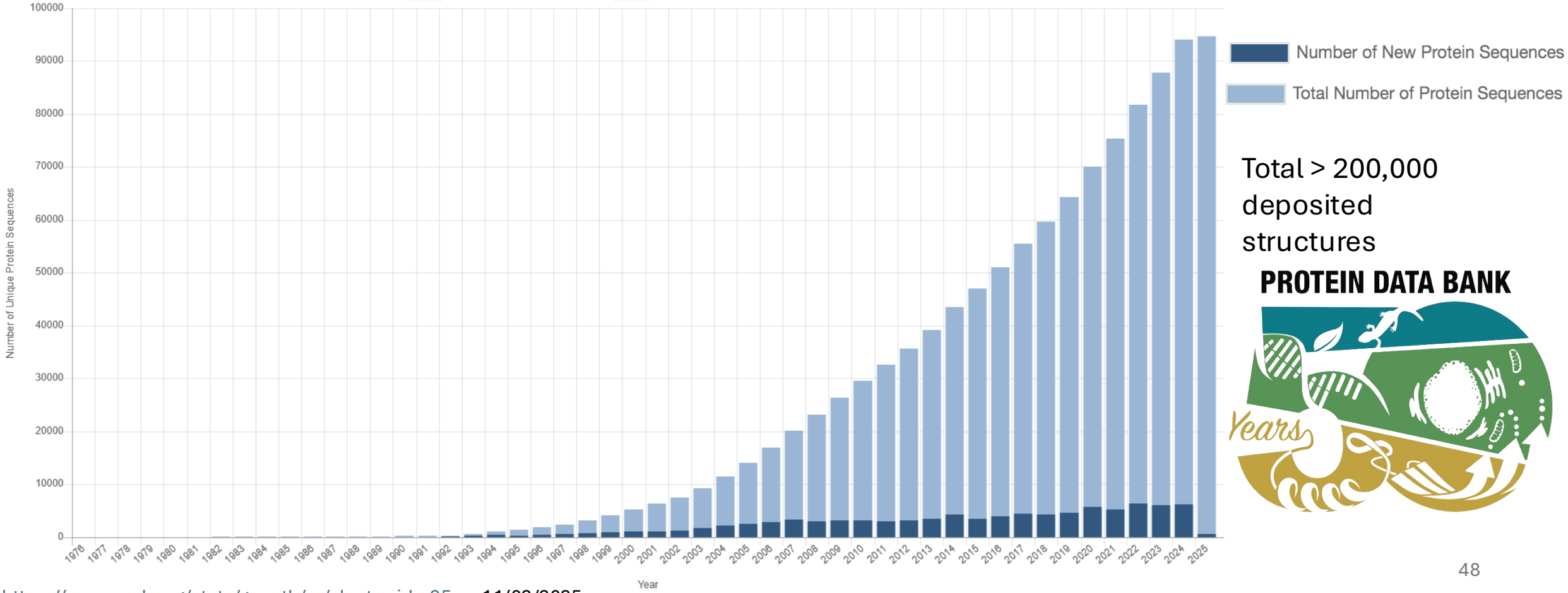


Example of beta sheet with twisted beta strands



Protein structure as a digital resource

- 1971 was stablished the Protein Data Bank with only 7 structures



Total > 200,000 deposited structures

PROTEIN DATA BANK

Years

https://www.rcsb.org/stats/growth/nr/cluster-ids-95 on 11/02/2025

Protein structure as a digital resource

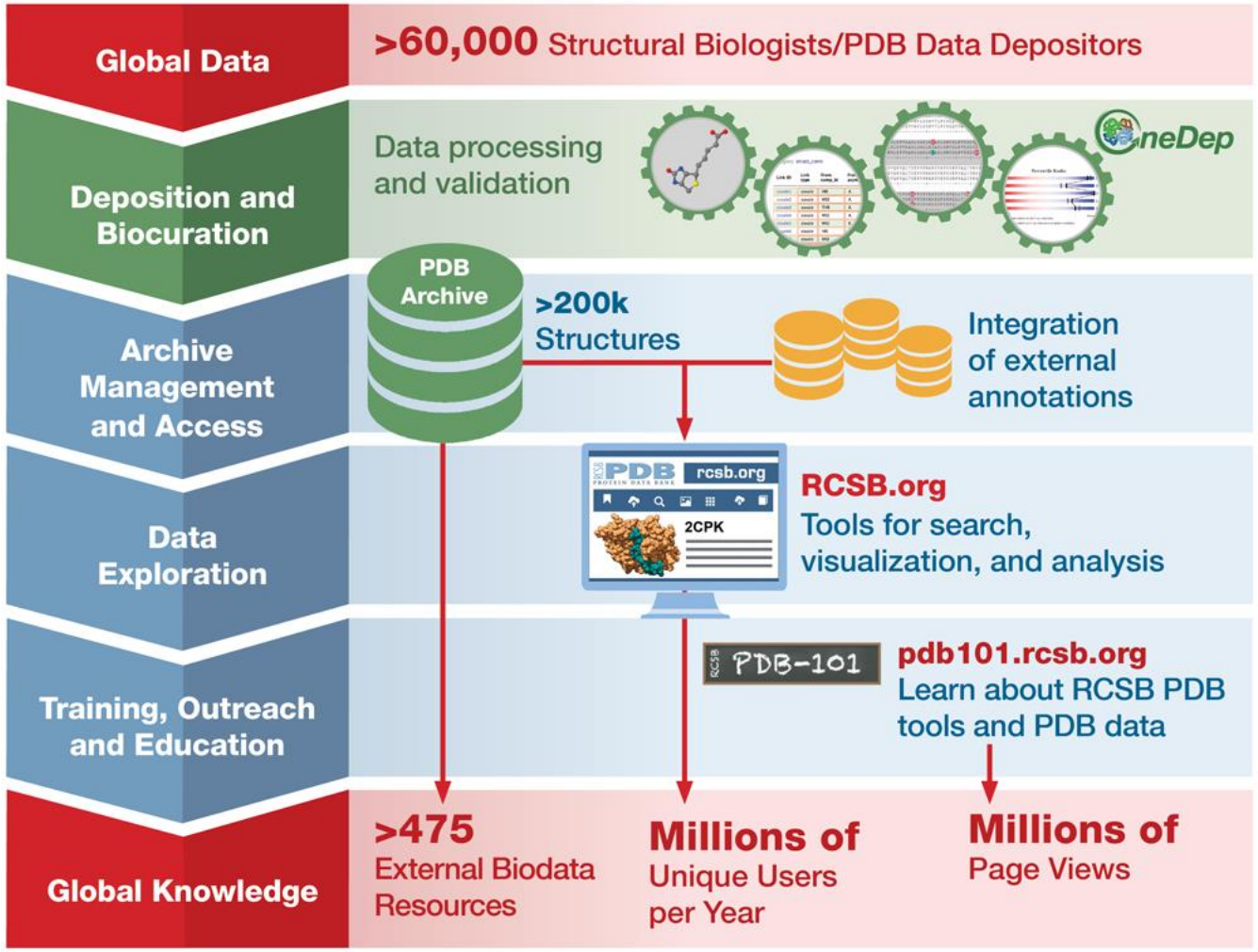
- 2003 was announced the worldwide Protein Data Bank - wwPDB

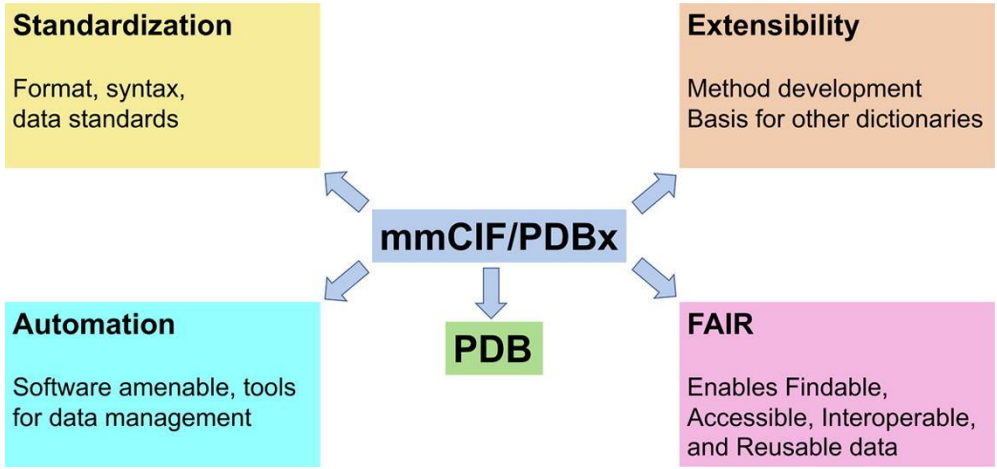
https://www.rcsb.org/pages/about-us/index

The PDB website

https://www.rcsb.org/

Global outreach of the Protein Data Bank





https://www.rcsb.org/pages/about-us/index

Westbrook et al., 2022

The coordinates files

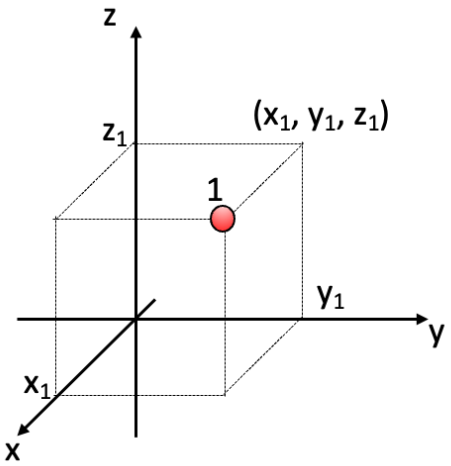
.pdb

```
ATOM      1  N   PRO A  27      18.254  54.186 -22.797  1.00 53.35           N
ATOM      2  CA  PRO A  27      17.690  52.844 -22.682  1.00 53.37           C
ATOM      3  C   PRO A  27      18.564  51.908 -21.783  1.00 53.24           C
ATOM      4  O   PRO A  27      19.115  50.895 -22.257  1.00 50.82           O
ATOM      5  CB  PRO A  27      17.659  52.369 -24.147  1.00 51.91           C
ATOM      6  CG  PRO A  27      18.673  53.271 -24.894  1.00 52.61           C
ATOM      7  CD  PRO A  27      19.231  54.261 -23.898  1.00 53.07           C
ATOM      8  N   TYR A  28      18.669  52.246 -20.493  1.00 51.56           N
ATOM      9  CA  TYR A  28      19.634  51.581 -19.613  1.00 49.67           C
ATOM     10  C   TYR A  28      19.310  50.171 -19.219  1.00 49.67           C
ATOM     11  O   TYR A  28      20.210  49.351 -19.134  1.00 49.06           O
ATOM     12  CB  TYR A  28      19.819  52.335 -18.325  1.00 48.01           C
ATOM     13  CG  TYR A  28      20.340  53.693 -18.509  1.00 48.13           C
ATOM     14  CD1 TYR A  28      21.705  53.916 -18.629  1.00 46.64           C
ATOM     15  CD2 TYR A  28      19.460  54.772 -18.573  1.00 48.85           C
ATOM     16  CE1 TYR A  28      22.204  55.188 -18.792  1.00 48.05           C
ATOM     17  CE2 TYR A  28      19.939  56.058 -18.741  1.00 50.76           C
ATOM     18  CZ  TYR A  28      21.319  56.261 -18.850  1.00 50.78           C
ATOM     19  OH  TYR A  28      21.781  57.551 -19.020  1.00 52.62           O
```

.cif

```
ATOM   1    N N   . PRO A 1 27 ? 18.254 54.186 -22.797 1.00 53.35 ? 27   PRO A N   1
ATOM   2    C CA  . PRO A 1 27 ? 17.690 52.844 -22.682 1.00 53.37 ? 27   PRO A CA  1
ATOM   3    C C   . PRO A 1 27 ? 18.564 51.908 -21.783 1.00 53.24 ? 27   PRO A C   1
ATOM   4    O O   . PRO A 1 27 ? 19.115 50.895 -22.257 1.00 50.82 ? 27   PRO A O   1
ATOM   5    C CB  . PRO A 1 27 ? 17.659 52.369 -24.147 1.00 51.91 ? 27   PRO A CB  1
ATOM   6    C CG  . PRO A 1 27 ? 18.673 53.271 -24.894 1.00 52.61 ? 27   PRO A CG  1
ATOM   7    C CD  . PRO A 1 27 ? 19.231 54.261 -23.898 1.00 53.07 ? 27   PRO A CD  1
ATOM   8    N N   . TYR A 1 28 ? 18.669 52.246 -20.493 1.00 51.56 ? 28   TYR A N   1
ATOM   9    C CA  . TYR A 1 28 ? 19.634 51.581 -19.613 1.00 49.67 ? 28   TYR A CA  1
ATOM   10   C C   . TYR A 1 28 ? 19.310 50.171 -19.219 1.00 49.67 ? 28   TYR A C   1
ATOM   11   O O   . TYR A 1 28 ? 20.210 49.351 -19.134 1.00 49.06 ? 28   TYR A O   1
ATOM   12   C CB  . TYR A 1 28 ? 19.819 52.335 -18.325 1.00 48.01 ? 28   TYR A CB  1
ATOM   13   C CG  . TYR A 1 28 ? 20.340 53.693 -18.509 1.00 48.13 ? 28   TYR A CG  1
ATOM   14   C CD1 . TYR A 1 28 ? 21.705 53.916 -18.629 1.00 46.64 ? 28   TYR A CD1 1
ATOM   15   C CD2 . TYR A 1 28 ? 19.460 54.772 -18.573 1.00 48.85 ? 28   TYR A CD2 1
ATOM   16   C CE1 . TYR A 1 28 ? 22.204 55.188 -18.792 1.00 48.05 ? 28   TYR A CE1 1
ATOM   17   C CE2 . TYR A 1 28 ? 19.939 56.058 -18.741 1.00 50.76 ? 28   TYR A CE2 1
ATOM   18   C CZ  . TYR A 1 28 ? 21.319 56.261 -18.850 1.00 50.78 ? 28   TYR A CZ  1
ATOM   19   O OH  . TYR A 1 28 ? 21.781 57.551 -19.020 1.00 52.62 ? 28   TYR A OH  1
```

Atom indices and type



ATOM 1 C CA 1 A x_1 y_1 z_1

The coordinates files

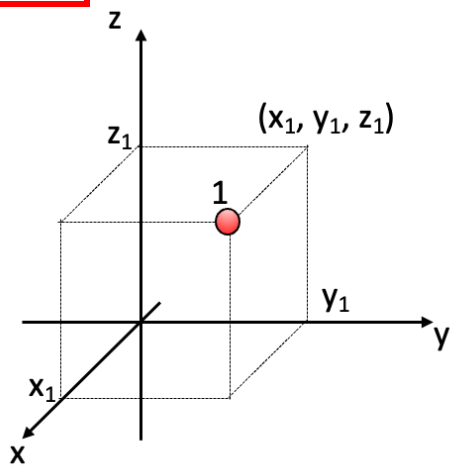
.pdb

x, y and z coordinates

```
ATOM      1  N   PRO A  27      18.254  54.186 -22.797  1.00 53.35           N
ATOM      2  CA  PRO A  27      17.690  52.844 -22.682  1.00 53.37           C
ATOM      3  C   PRO A  27      18.564  51.908 -21.783  1.00 53.24           C
ATOM      4  O   PRO A  27      19.115  50.895 -22.257  1.00 50.82           O
ATOM      5  CB  PRO A  27      17.659  52.369 -24.147  1.00 51.91           C
ATOM      6  CG  PRO A  27      18.673  53.271 -24.894  1.00 52.61           C
ATOM      7  CD  PRO A  27      19.231  54.261 -23.898  1.00 53.07           C
ATOM      8  N   TYR A  28      18.669  52.246 -20.493  1.00 51.56           N
ATOM      9  CA  TYR A  28      19.634  51.581 -19.613  1.00 49.67           C
ATOM     10  C   TYR A  28      19.310  50.171 -19.219  1.00 49.67           C
ATOM     11  O   TYR A  28      20.210  49.351 -19.134  1.00 49.06           O
ATOM     12  CB  TYR A  28      19.819  52.335 -18.325  1.00 48.01           C
ATOM     13  CG  TYR A  28      20.340  53.693 -18.509  1.00 48.13           C
ATOM     14  CD1 TYR A  28      21.705  53.916 -18.629  1.00 46.64           C
ATOM     15  CD2 TYR A  28      19.460  54.772 -18.573  1.00 48.85           C
ATOM     16  CE1 TYR A  28      22.204  55.188 -18.792  1.00 48.05           C
ATOM     17  CE2 TYR A  28      19.939  56.058 -18.741  1.00 50.76           C
ATOM     18  CZ  TYR A  28      21.319  56.261 -18.850  1.00 50.78           C
ATOM     19  OH  TYR A  28      21.781  57.551 -19.020  1.00 52.62           O
```

.cif

```
ATOM   1    N N   . PRO A 1 27 ? 18.254 54.186 -22.797 1.00 53.35 ? 27   PRO A N   1
ATOM   2    C CA  . PRO A 1 27 ? 17.690 52.844 -22.682 1.00 53.37 ? 27   PRO A CA  1
ATOM   3    C C   . PRO A 1 27 ? 18.564 51.908 -21.783 1.00 53.24 ? 27   PRO A C   1
ATOM   4    O O   . PRO A 1 27 ? 19.115 50.895 -22.257 1.00 50.82 ? 27   PRO A O   1
ATOM   5    C CB  . PRO A 1 27 ? 17.659 52.369 -24.147 1.00 51.91 ? 27   PRO A CB  1
ATOM   6    C CG  . PRO A 1 27 ? 18.673 53.271 -24.894 1.00 52.61 ? 27   PRO A CG  1
ATOM   7    C CD  . PRO A 1 27 ? 19.231 54.261 -23.898 1.00 53.07 ? 27   PRO A CD  1
ATOM   8    N N   . TYR A 1 28 ? 18.669 52.246 -20.493 1.00 51.56 ? 28   TYR A N   1
ATOM   9    C CA  . TYR A 1 28 ? 19.634 51.581 -19.613 1.00 49.67 ? 28   TYR A CA  1
ATOM   10   C C   . TYR A 1 28 ? 19.310 50.171 -19.219 1.00 49.67 ? 28   TYR A C   1
ATOM   11   O O   . TYR A 1 28 ? 20.210 49.351 -19.134 1.00 49.06 ? 28   TYR A O   1
ATOM   12   C CB  . TYR A 1 28 ? 19.819 52.335 -18.325 1.00 48.01 ? 28   TYR A CB  1
ATOM   13   C CG  . TYR A 1 28 ? 20.340 53.693 -18.509 1.00 48.13 ? 28   TYR A CG  1
ATOM   14   C CD1 . TYR A 1 28 ? 21.705 53.916 -18.629 1.00 46.64 ? 28   TYR A CD1 1
ATOM   15   C CD2 . TYR A 1 28 ? 19.460 54.772 -18.573 1.00 48.85 ? 28   TYR A CD2 1
ATOM   16   C CE1 . TYR A 1 28 ? 22.204 55.188 -18.792 1.00 48.05 ? 28   TYR A CE1 1
ATOM   17   C CE2 . TYR A 1 28 ? 19.939 56.058 -18.741 1.00 50.76 ? 28   TYR A CE2 1
ATOM   18   C CZ  . TYR A 1 28 ? 21.319 56.261 -18.850 1.00 50.78 ? 28   TYR A CZ  1
ATOM   19   O OH  . TYR A 1 28 ? 21.781 57.551 -19.020 1.00 52.62 ? 28   TYR A OH  1
```



ATOM 1 C CA 1 A x_1 y_1 z_1

The coordinates files

Because proteins are flexible!!!

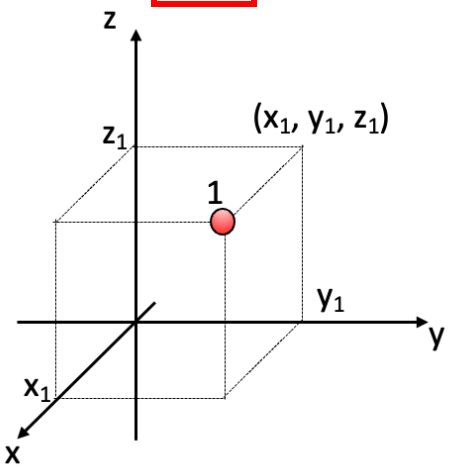
Atomic displacement

.pdb

```
ATOM      1  N   PRO A  27      18.254  54.186 -22.797  1.00 53.35           N
ATOM      2  CA  PRO A  27      17.690  52.844 -22.682  1.00 53.37           C
ATOM      3  C   PRO A  27      18.564  51.908 -21.783  1.00 53.24           C
ATOM      4  O   PRO A  27      19.115  50.895 -22.257  1.00 50.82           O
ATOM      5  CB  PRO A  27      17.659  52.369 -24.147  1.00 51.91           C
ATOM      6  CG  PRO A  27      18.673  53.271 -24.894  1.00 52.61           C
ATOM      7  CD  PRO A  27      19.231  54.261 -23.898  1.00 53.07           C
ATOM      8  N   TYR A  28      18.669  52.246 -20.493  1.00 51.56           N
ATOM      9  CA  TYR A  28      19.634  51.581 -19.613  1.00 49.67           C
ATOM     10  C   TYR A  28      19.310  50.171 -19.219  1.00 49.67           C
ATOM     11  O   TYR A  28      20.210  49.351 -19.134  1.00 49.06           O
ATOM     12  CB  TYR A  28      19.819  52.335 -18.325  1.00 48.01           C
ATOM     13  CG  TYR A  28      20.340  53.693 -18.509  1.00 48.13           C
ATOM     14  CD1 TYR A  28      21.705  53.916 -18.629  1.00 46.64           C
ATOM     15  CD2 TYR A  28      19.460  54.772 -18.573  1.00 48.85           C
ATOM     16  CE1 TYR A  28      22.204  55.188 -18.792  1.00 48.05           C
ATOM     17  CE2 TYR A  28      19.939  56.058 -18.741  1.00 50.76           C
ATOM     18  CZ  TYR A  28      21.319  56.261 -18.850  1.00 50.78           C
ATOM     19  OH  TYR A  28      21.781  57.551 -19.020  1.00 52.62           O
```

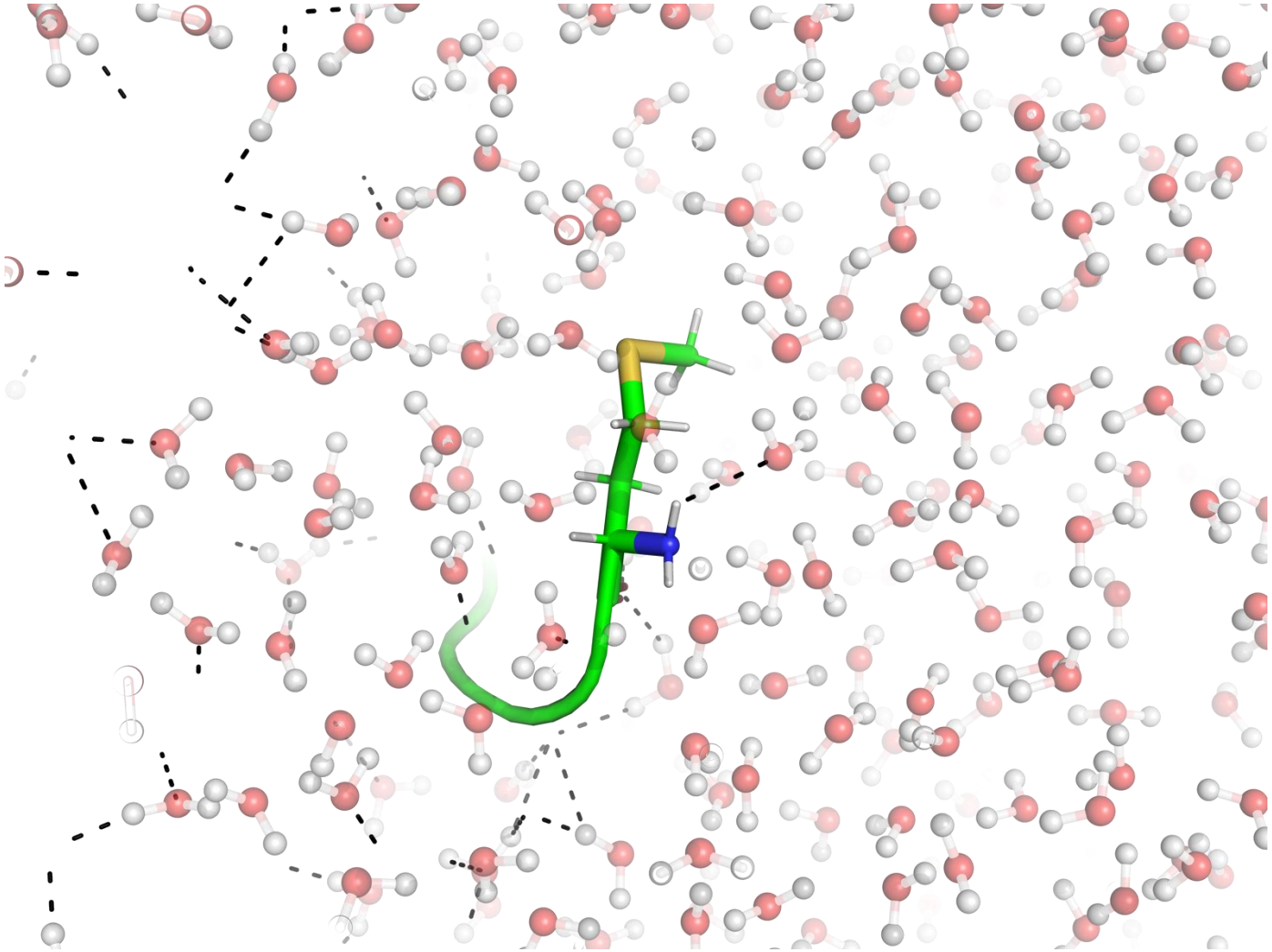
.cif

```
ATOM   1    N N   . PRO A 1 27  ? 18.254 54.186 -22.797 1.00 53.35 ? 27  PRO A N   1
ATOM   2    C CA  . PRO A 1 27  ? 17.690 52.844 -22.682 1.00 53.37 ? 27  PRO A CA  1
ATOM   3    C C   . PRO A 1 27  ? 18.564 51.908 -21.783 1.00 53.24 ? 27  PRO A C   1
ATOM   4    O O   . PRO A 1 27  ? 19.115 50.895 -22.257 1.00 50.82 ? 27  PRO A O   1
ATOM   5    C CB  . PRO A 1 27  ? 17.659 52.369 -24.147 1.00 51.91 ? 27  PRO A CB  1
ATOM   6    C CG  . PRO A 1 27  ? 18.673 53.271 -24.894 1.00 52.61 ? 27  PRO A CG  1
ATOM   7    C CD  . PRO A 1 27  ? 19.231 54.261 -23.898 1.00 53.07 ? 27  PRO A CD  1
ATOM   8    N N   . TYR A 1 28  ? 18.669 52.246 -20.493 1.00 51.56 ? 28  TYR A N   1
ATOM   9    C CA  . TYR A 1 28  ? 19.634 51.581 -19.613 1.00 49.67 ? 28  TYR A CA  1
ATOM   10   C C   . TYR A 1 28  ? 19.310 50.171 -19.219 1.00 49.67 ? 28  TYR A C   1
ATOM   11   O O   . TYR A 1 28  ? 20.210 49.351 -19.134 1.00 49.06 ? 28  TYR A O   1
ATOM   12   C CB  . TYR A 1 28  ? 19.819 52.335 -18.325 1.00 48.01 ? 28  TYR A CB  1
ATOM   13   C CG  . TYR A 1 28  ? 20.340 53.693 -18.509 1.00 48.13 ? 28  TYR A CG  1
ATOM   14   C CD1 . TYR A 1 28  ? 21.705 53.916 -18.629 1.00 46.64 ? 28  TYR A CD1 1
ATOM   15   C CD2 . TYR A 1 28  ? 19.460 54.772 -18.573 1.00 48.85 ? 28  TYR A CD2 1
ATOM   16   C CE1 . TYR A 1 28  ? 22.204 55.188 -18.792 1.00 48.05 ? 28  TYR A CE1 1
ATOM   17   C CE2 . TYR A 1 28  ? 19.939 56.058 -18.741 1.00 50.76 ? 28  TYR A CE2 1
ATOM   18   C CZ  . TYR A 1 28  ? 21.319 56.261 -18.850 1.00 50.78 ? 28  TYR A CZ  1
ATOM   19   O OH  . TYR A 1 28  ? 21.781 57.551 -19.020 1.00 52.62 ? 28  TYR A OH  1
```

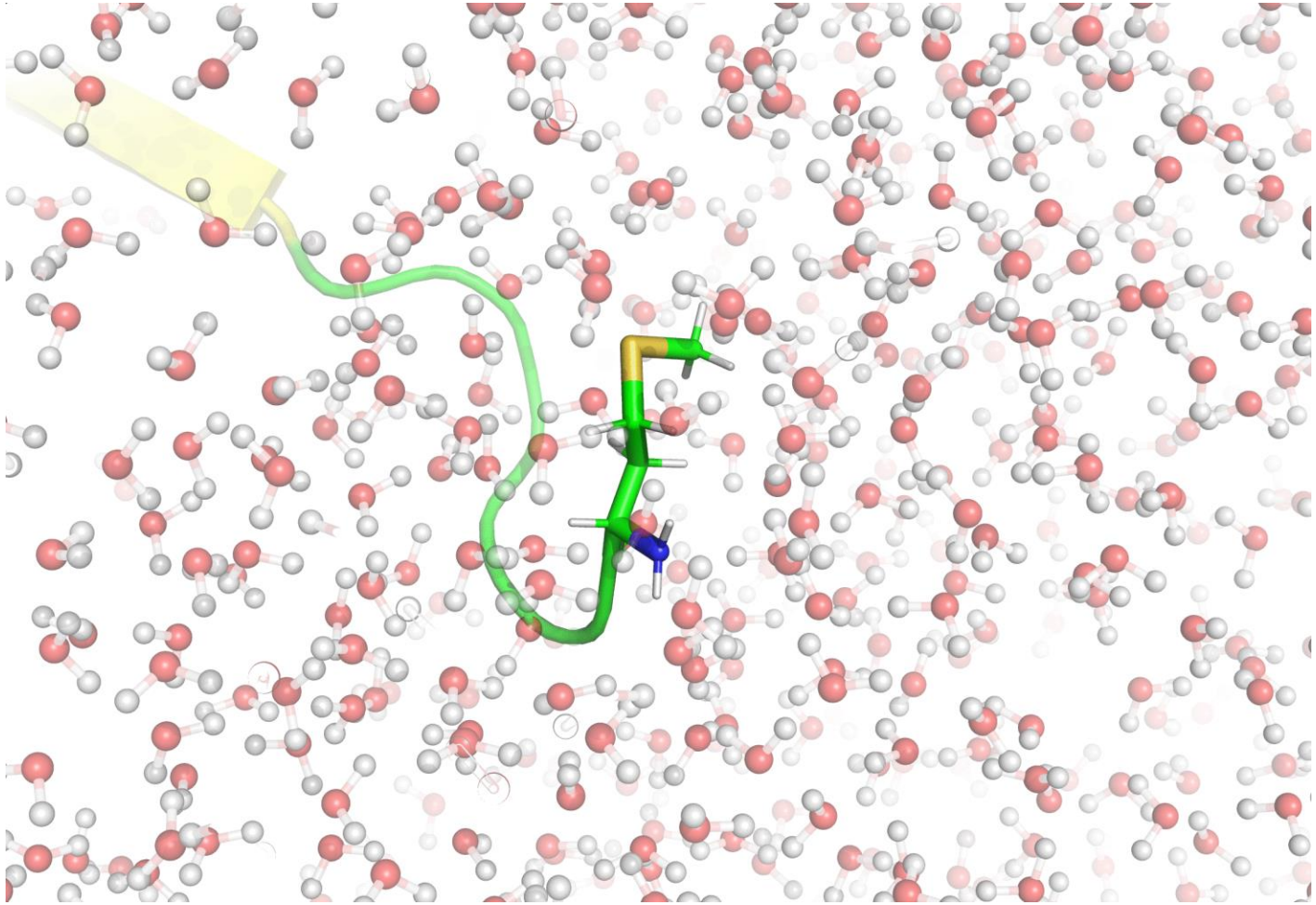


ATOM 1 C CA 1 A x_1 y_1 z_1

Atomic coordinates in 3D

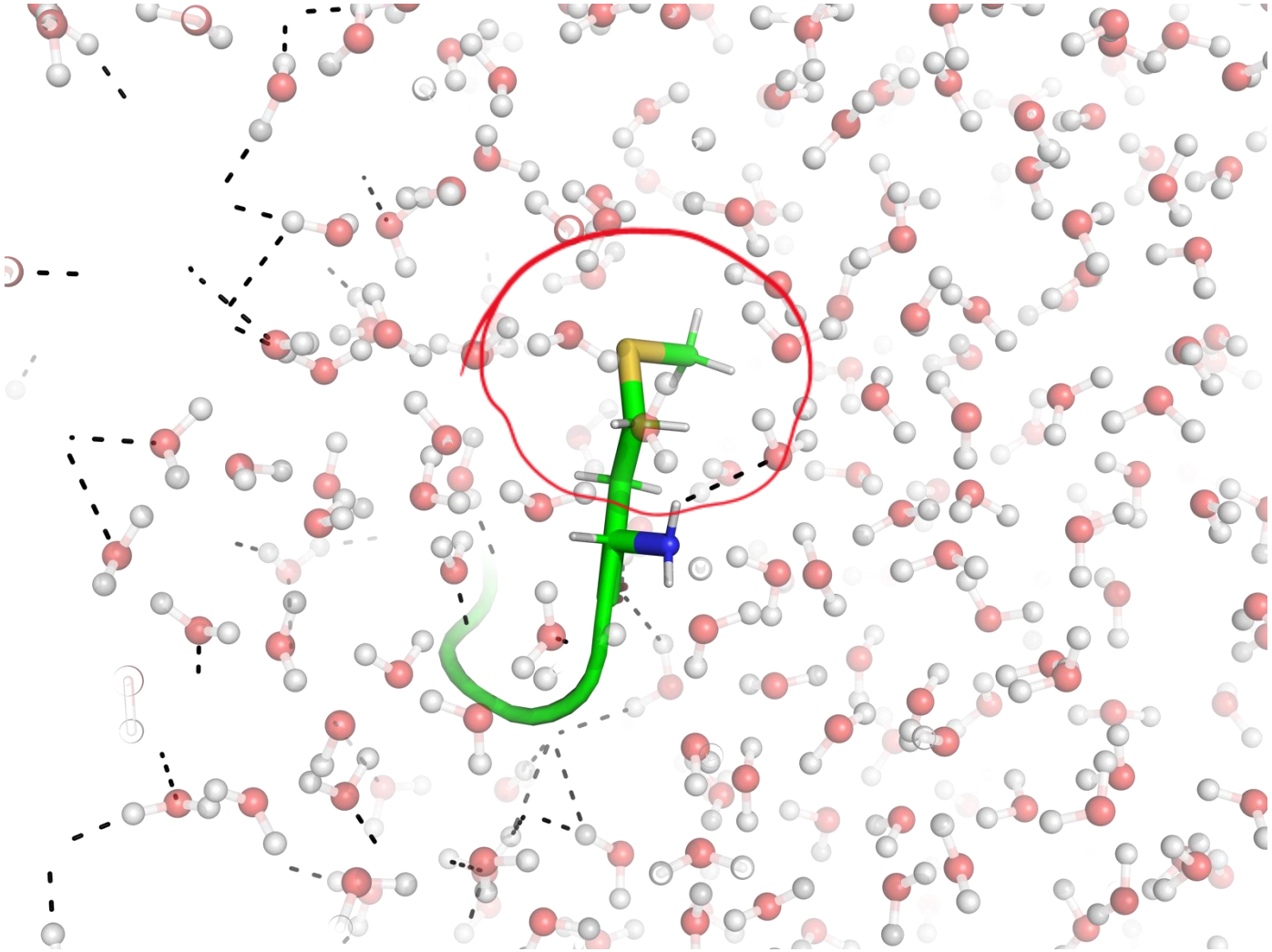


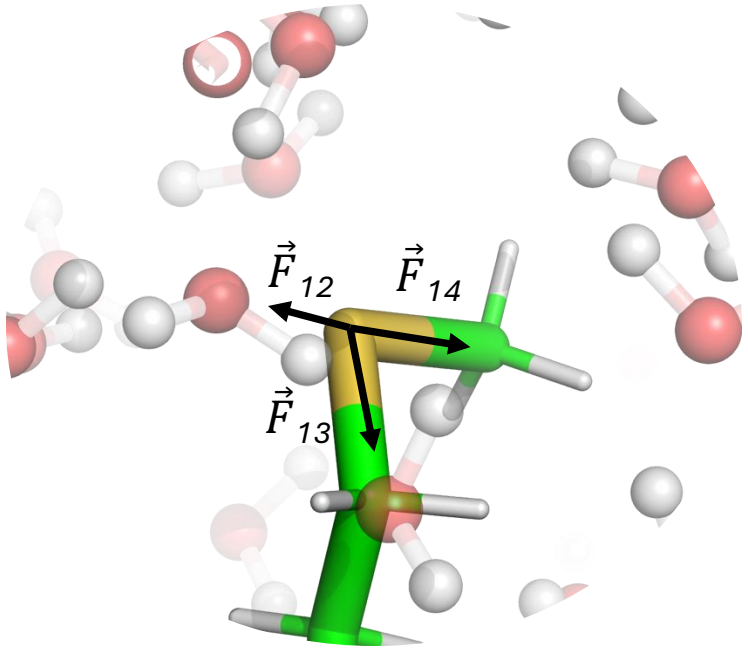
Wigglings and jigglings of atoms



... but 10^{10} times faster!

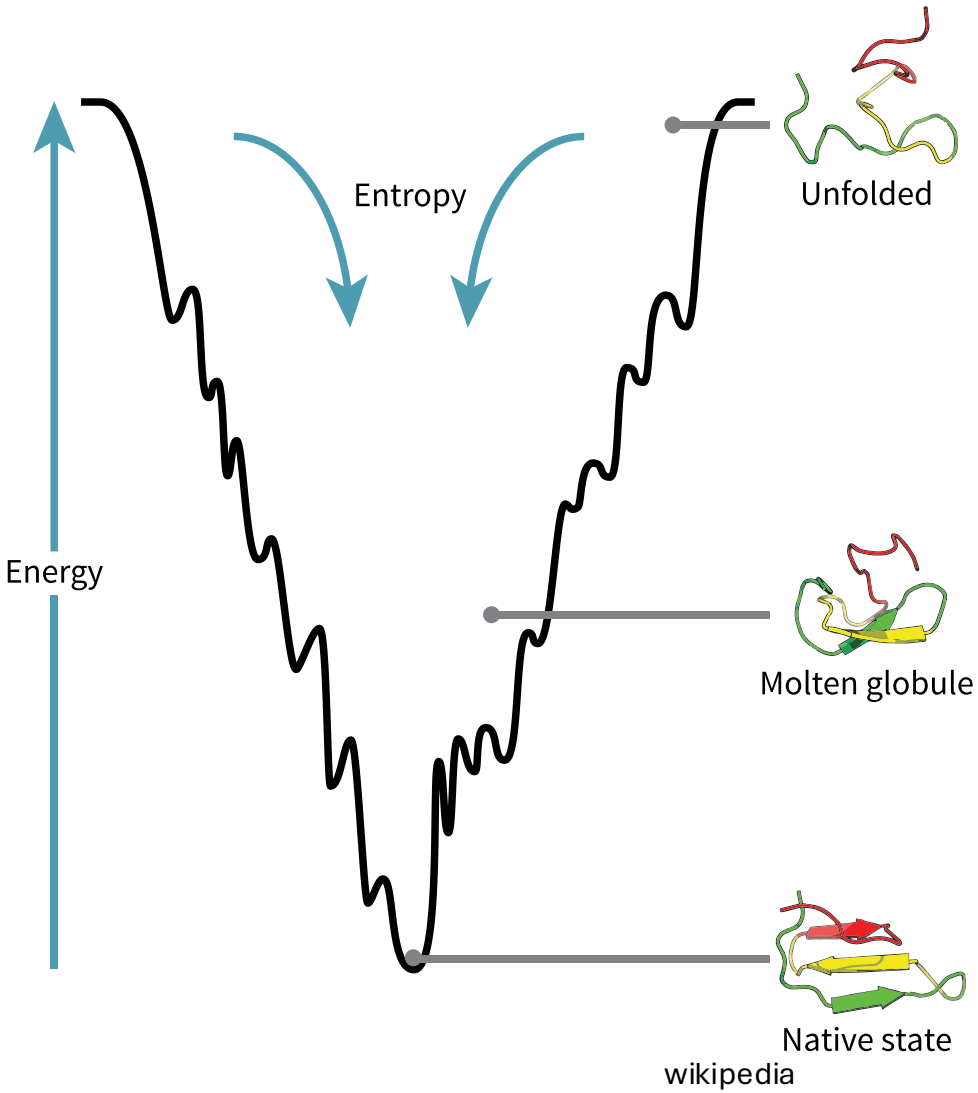
Atomic forces





$$\overline{E_K} = \frac{1}{2} m \bar{v}^2 = \frac{3}{2} k_B T$$

- The higher the temperature the more agitated are the molecules
- Interactions forming secondary structures are lost with higher temperature



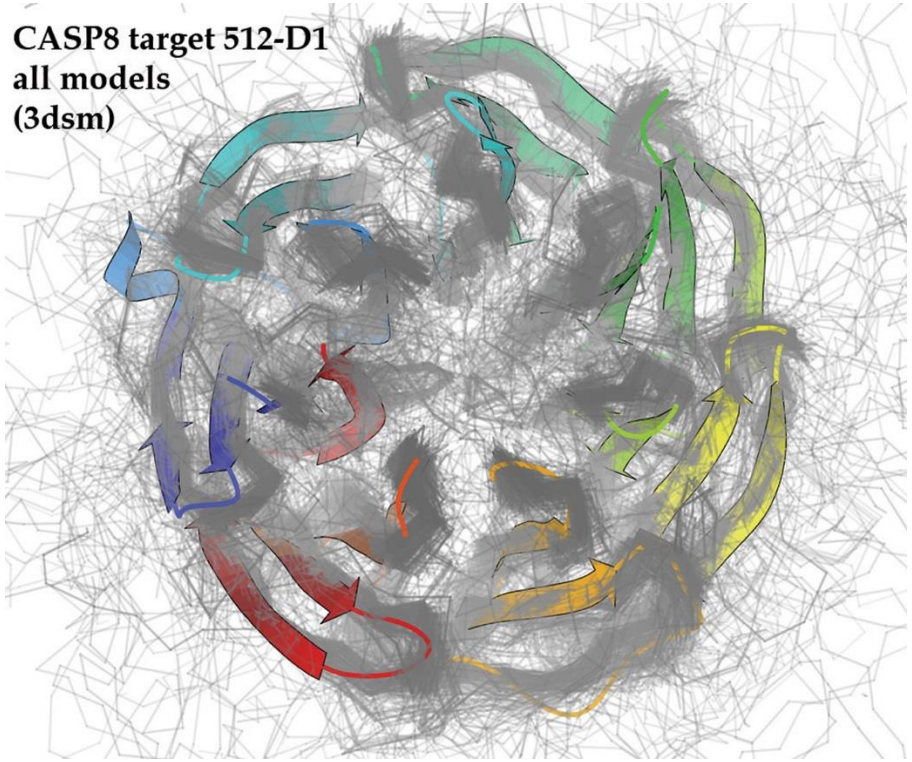
Modeling strategies

- Homology modelling – comparison to homologous proteins

- *De novo* or *ab initio* – prediction from primary structure

CASP

- **Critical Assessment of Structure Prediction**



- Every 2 years since 1994
- An experiment to objectively evaluate the predictions of a community
- Help advance prediction methods
- „world championship“
- Target structures were very recently solved and not yet published

Alphafold will change everything

nature

Explore content | About the journal | Publish with us | Subscribe

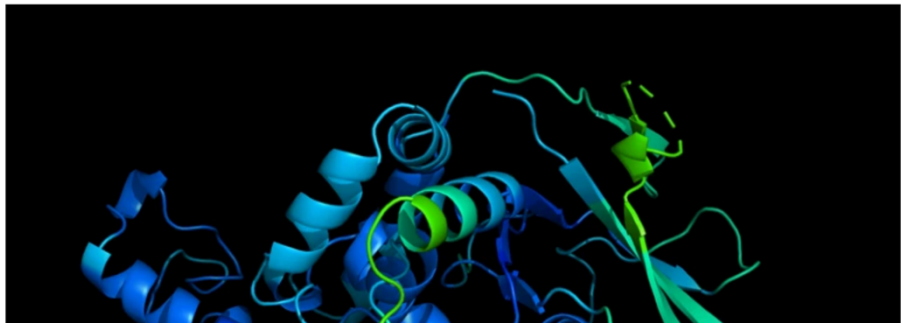
nature > news > article

NEWS | 30 November 2020

'It will change everything': DeepMind's AI makes gigantic leap in solving protein structures

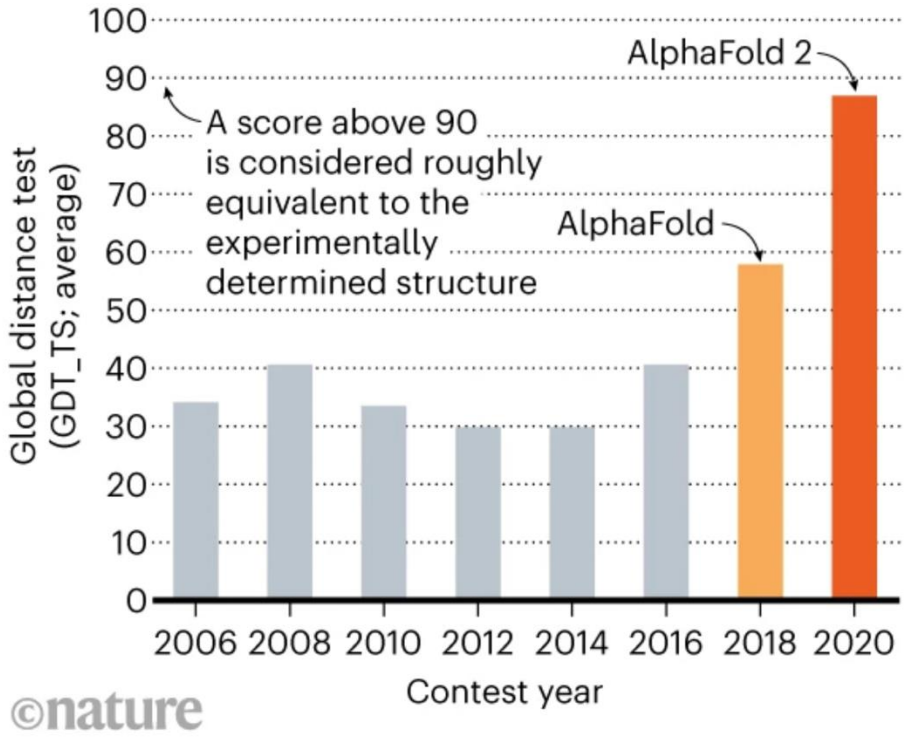
Google's deep-learning program for determining the 3D shapes of proteins stands to transform biology, say scientists.

Ewen Callaway

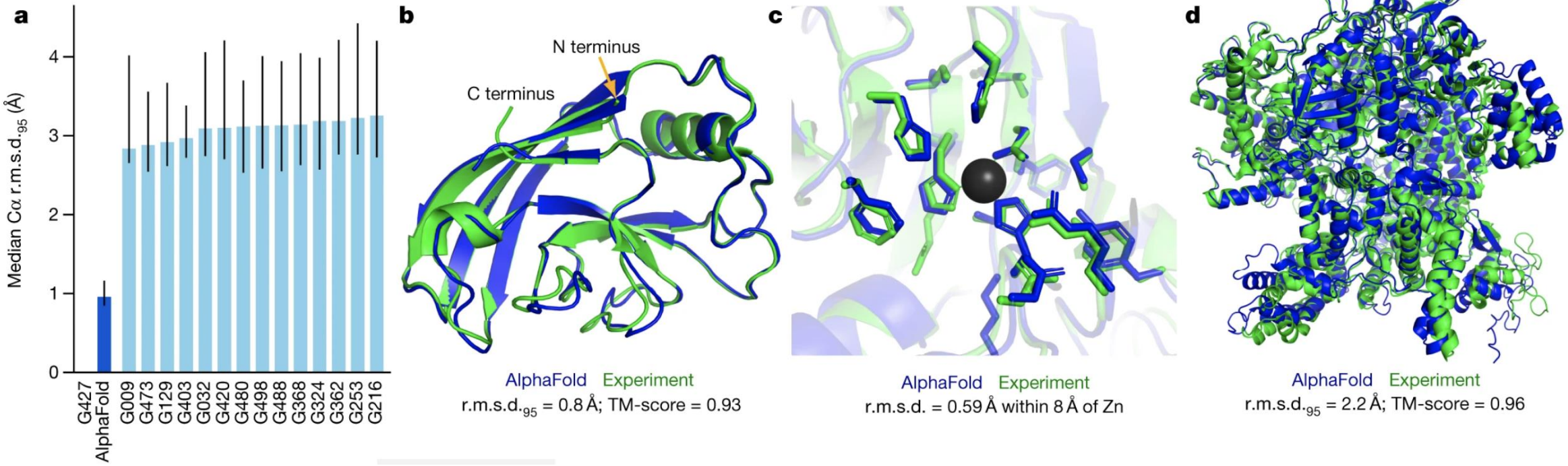


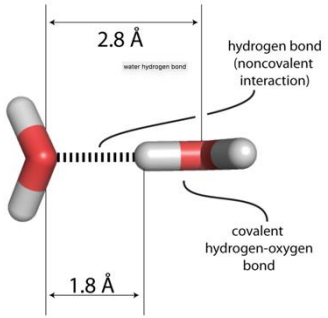
STRUCTURE SOLVER

DeepMind's AlphaFold 2 algorithm significantly outperformed other teams at the CASP14 protein-folding contest — and its previous version's performance at the last CASP.



AlphaFold accuracy

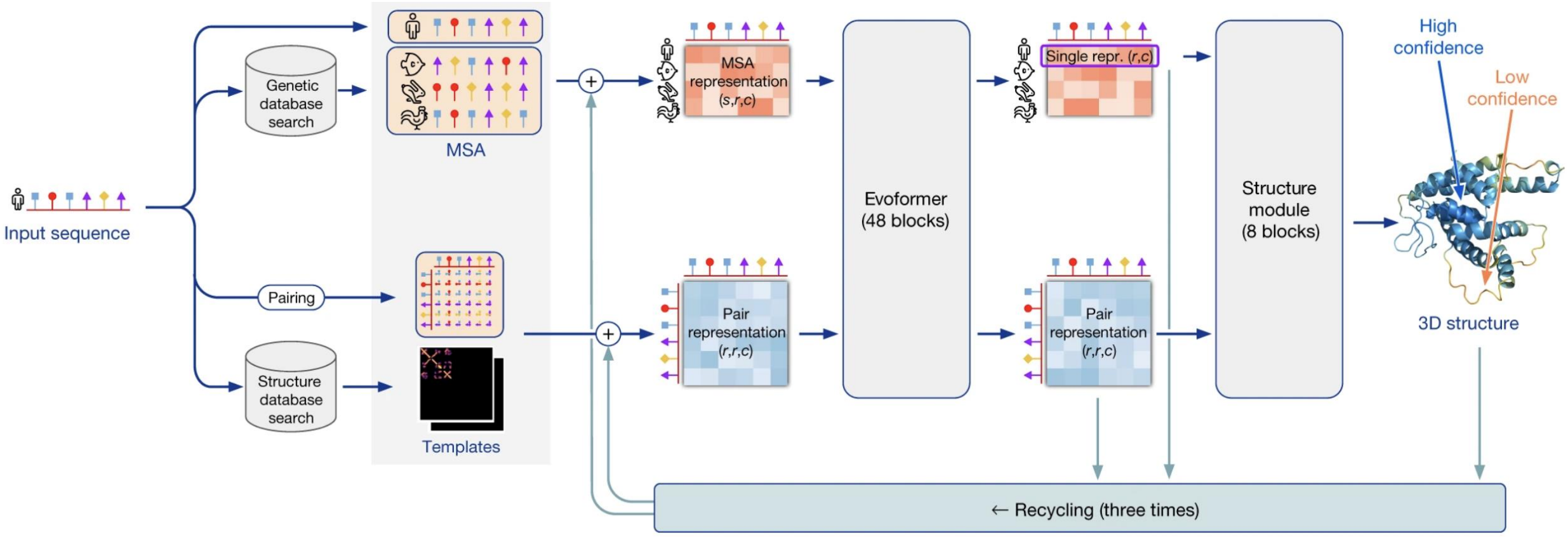




- Compared by RMSD and TM-score

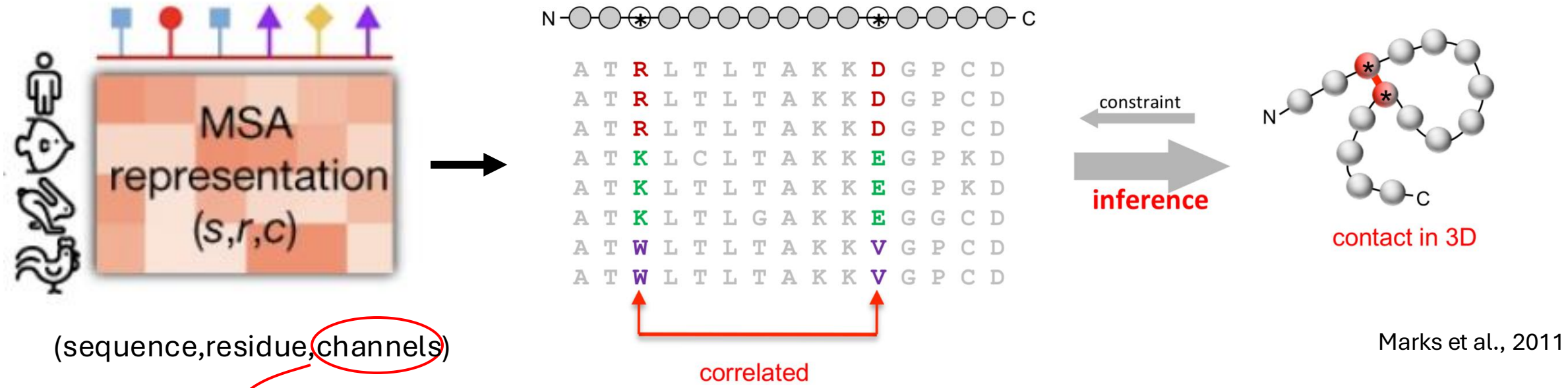
Jumper et al., 2021

AlphaFold pipeline



Jumper et al., 2021

Multiple Sequence Alignment representation

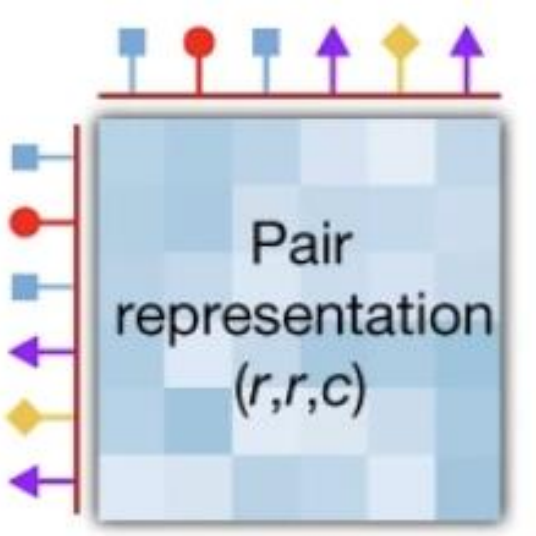


(sequence,residue,channels)

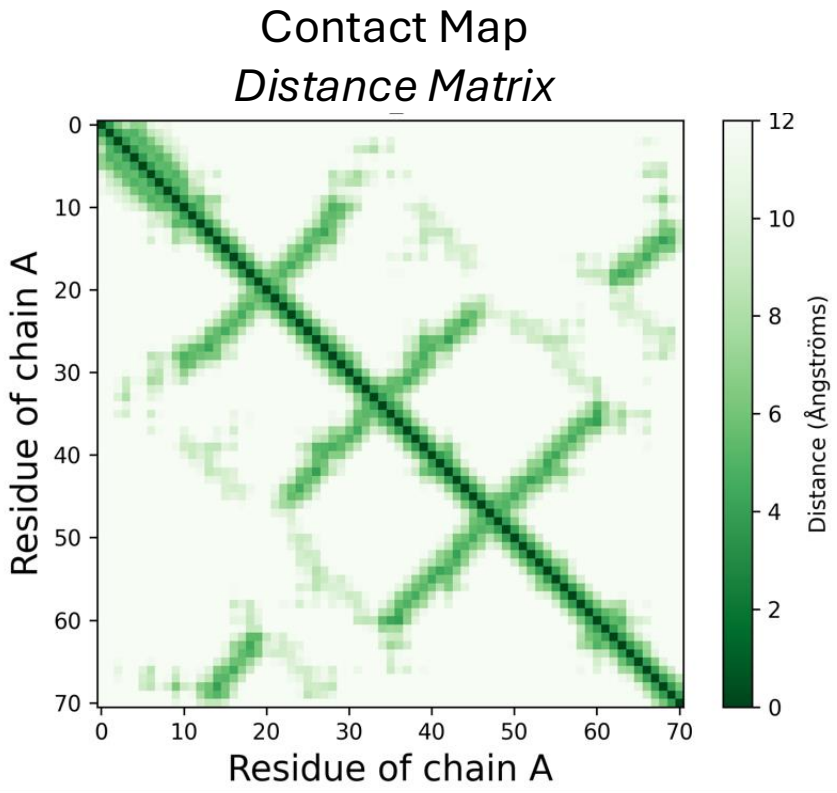
Many layers with learned features, like more geometric or evolutionary relationships and attention features

Marks et al., 2011

Pair representation



(residue,residue,channels)



Like a contact map, but with more than 100x more information

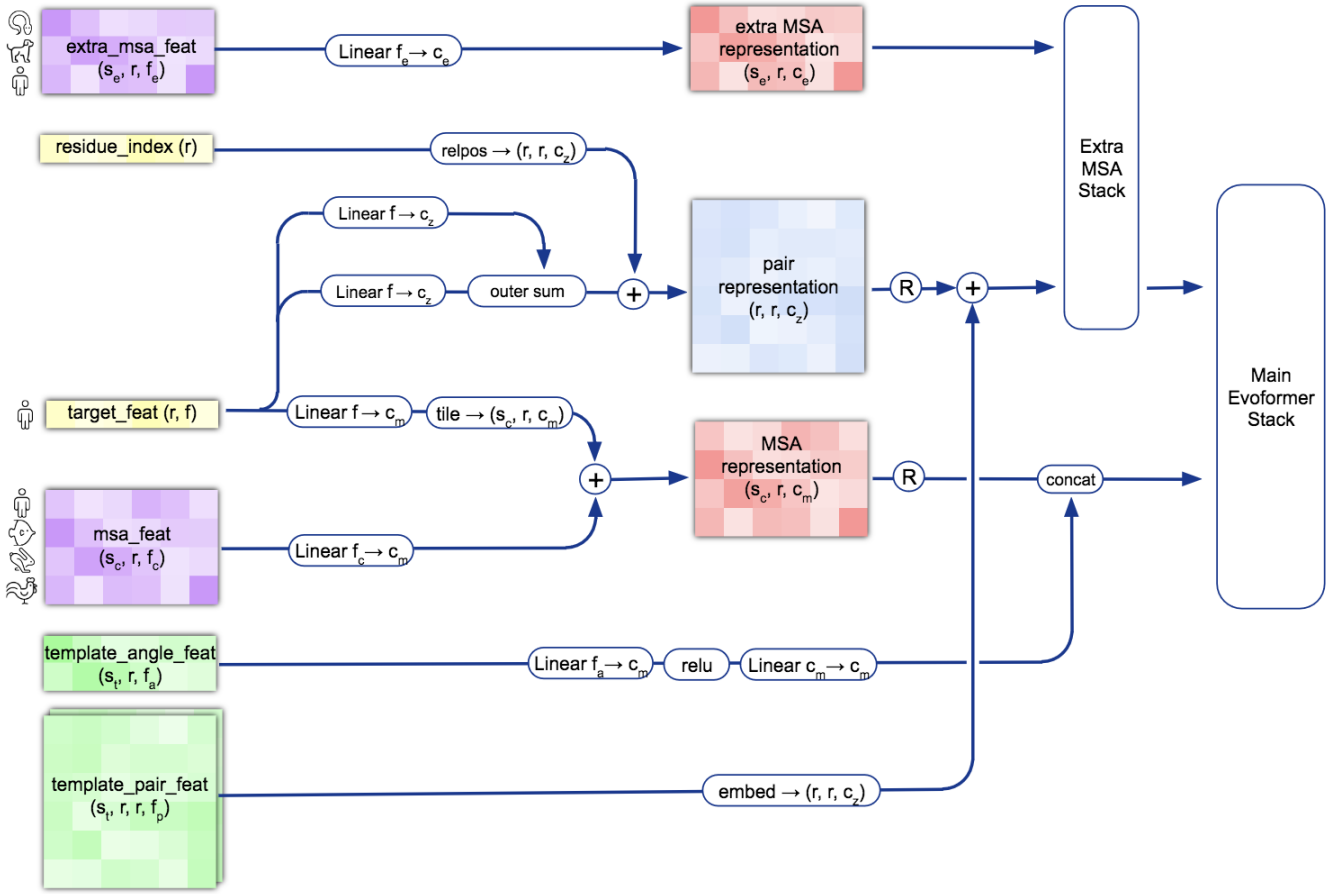
Many layers with learned features, like more geometric or evolutionary relationships and attention features

Keys to success of AlphaFold2?

- A great database
    - **Big Fantastic Database -** 65,983,866 families , 2,204,359,010 protein sequences from reference databases, metagenomes and metatranscriptomes
    - **PDB**
    - **PDB70**
    - **Uniref90**
    - **Uniclust30**
    - **Uniprot**
    - **MGnify**

Keys to success of AlphaFold2?

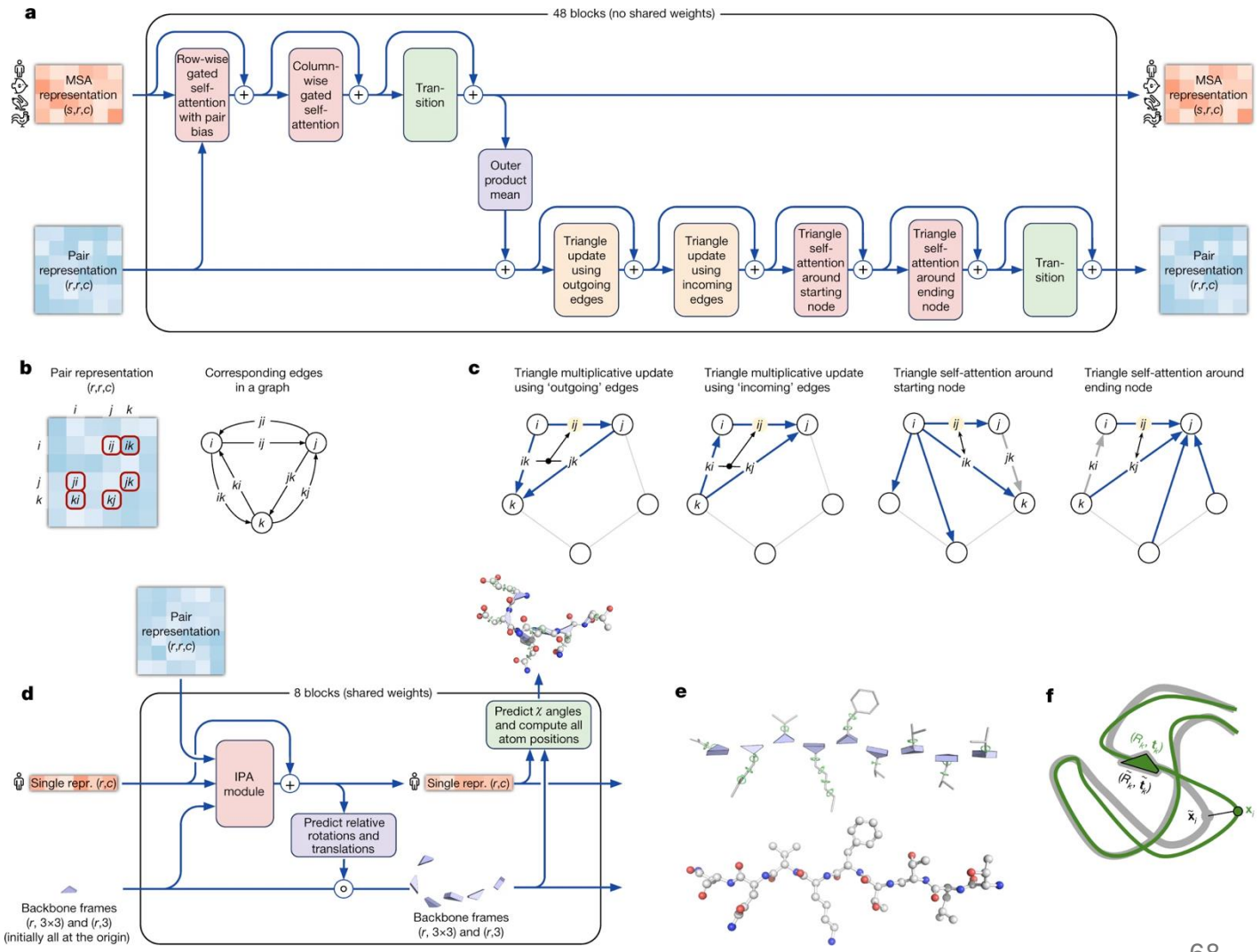
- Abbility to handle complex (a great team of experts and a great infrastructure)



Jumper et al., 2021

Keys to success of AlphaFold2?

- A neural network that alternates between structural and geometrical data and evolutionary data (Evoformer)
- And other clever architectural tricks and assumptions like a „gas of residues“



Jumper et al., 2021

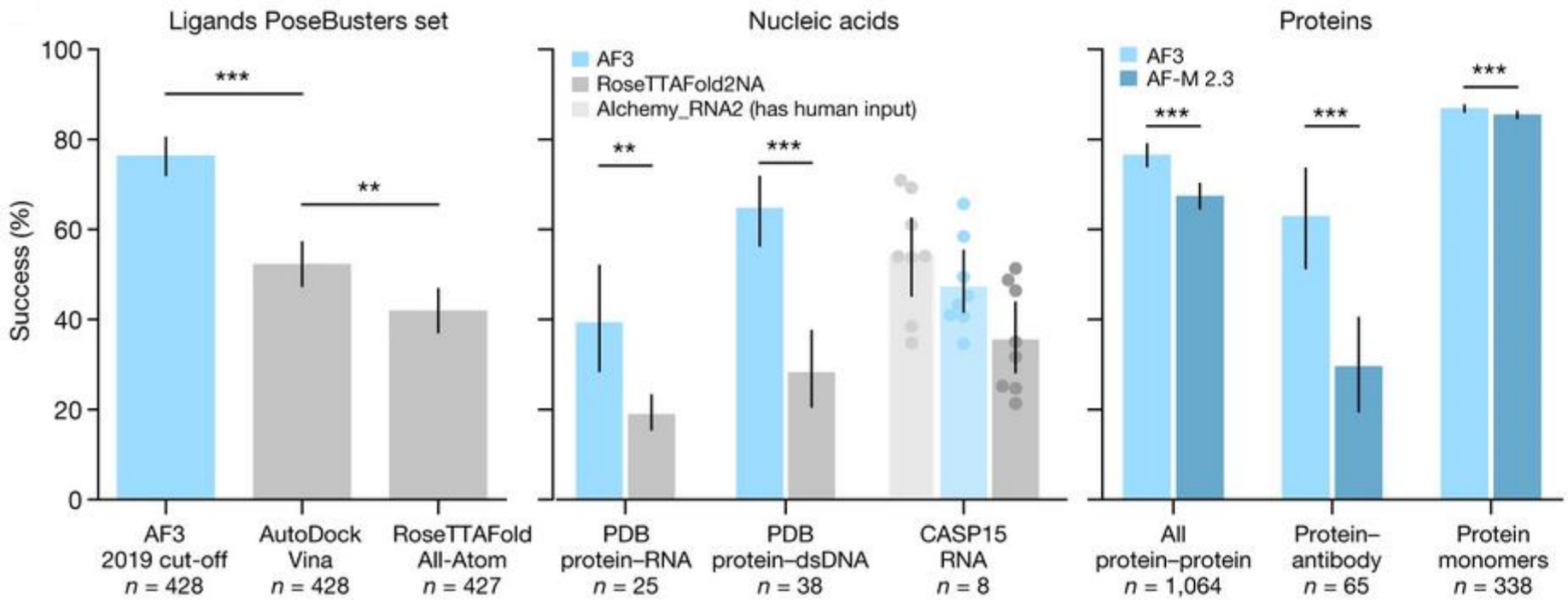
More on how Alphafold works

- **Highly Accurate Protein Structure Prediction with AlphaFold | SimonKohl**
  - **https://www.youtube.com/watch?v=tTN0MM2CQLU**
- Oxford Protein Informatics Group - **AlphaFold 2 is here: what's behind the structure prediction miracle**
  - **https://www.blopig.com/blog/2021/07/alphafold-2-is-here-whats-behind-the-structure-prediction-miracle/**

Novelties of AlphaFold3

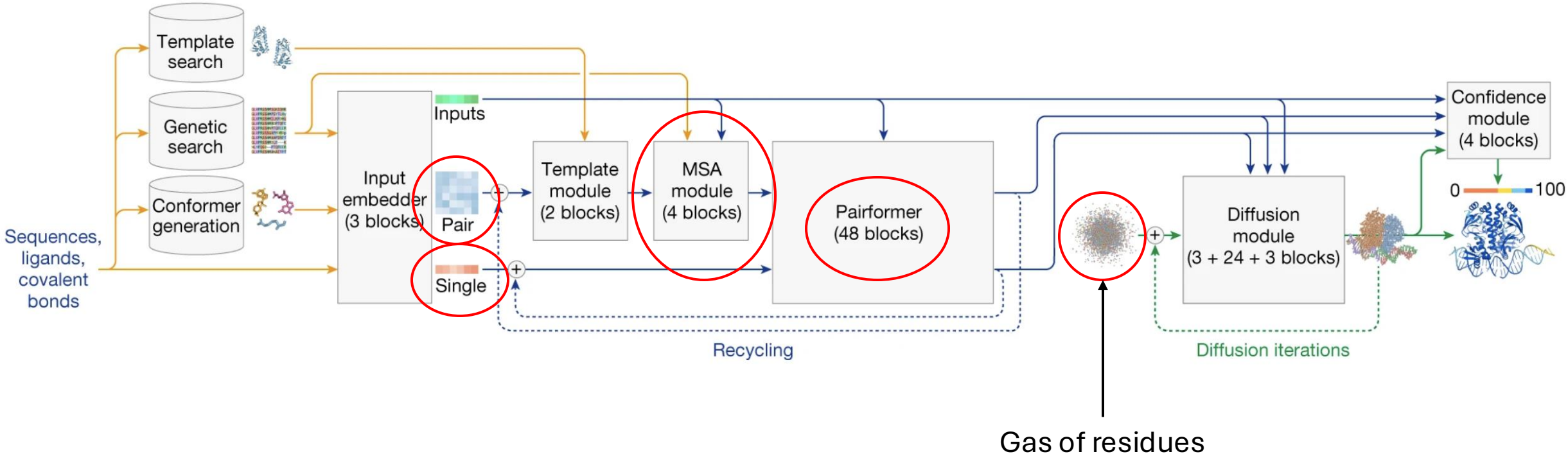
Prediction of proteins with DNA and some small ligands.

Improvements of AlphaFold3



Abramson et al., 2024

Novelties of AlphaFold3



Abramson et al., 2024

Interpretation of predicted structures

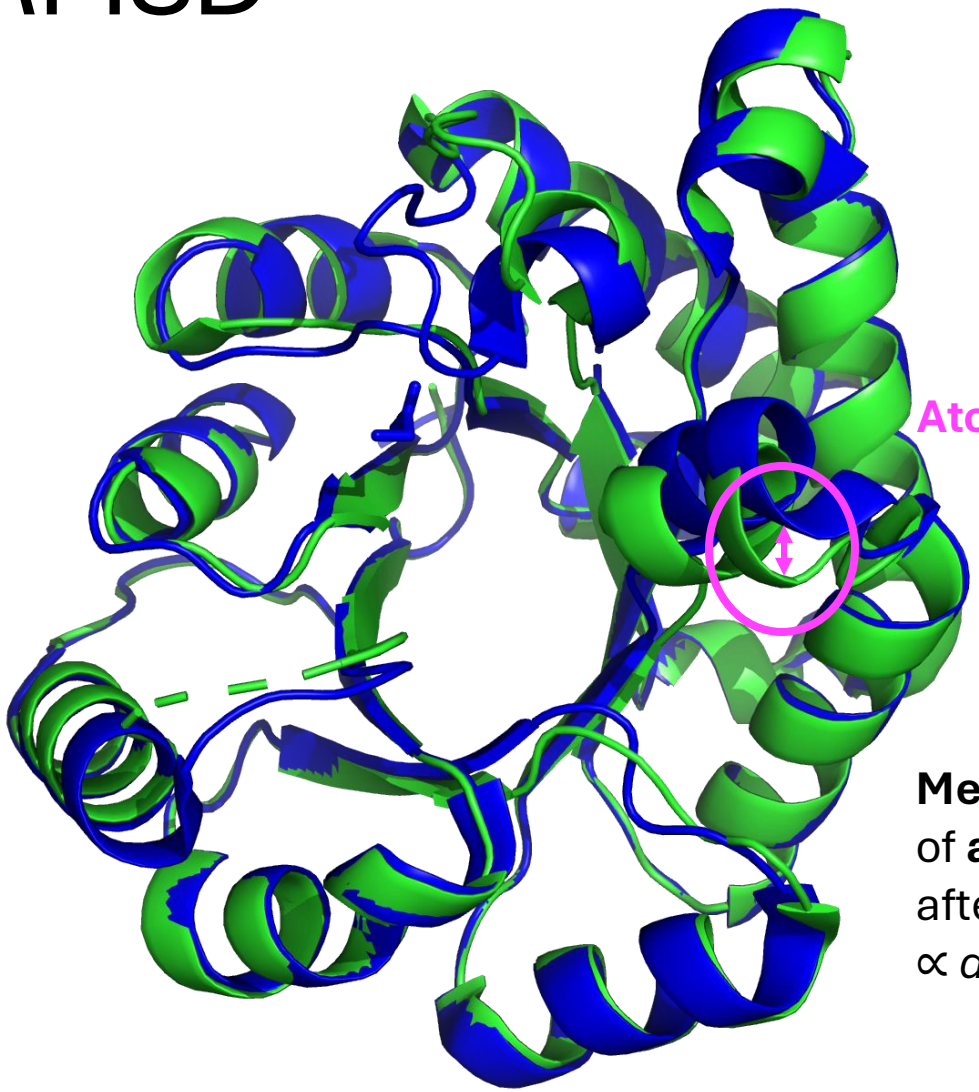
Usual metrics

- RMSD – Root Mean Square Deviation
- TM-score – Template Modelling score
- LDDT – Local Distance Difference Test
- GDT – Global Distance Test (used by CASP)

AlphaFold metrics

- pLDDT – predicted LDDT
- PAE – Predicted Aligned Errors

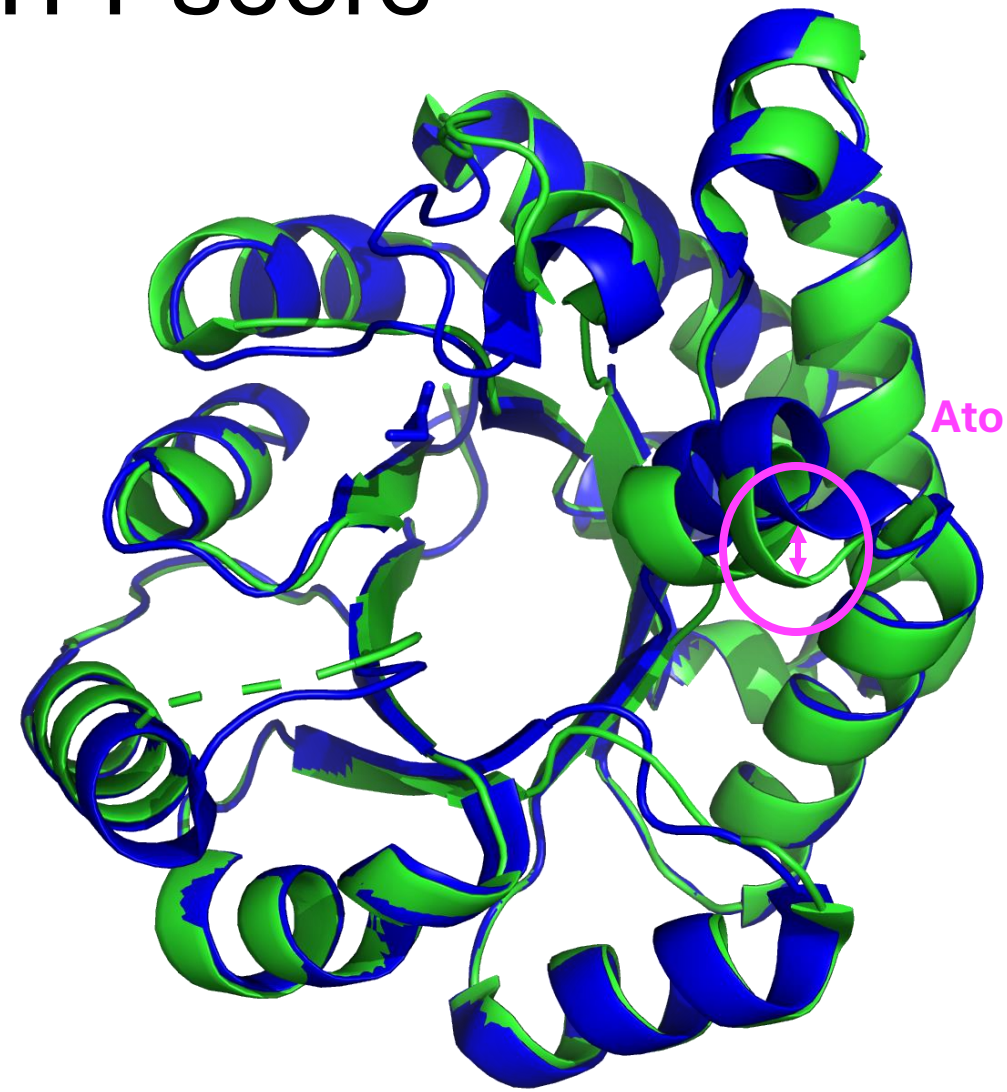
RMSD



Atomic deviation d_i

Mean deviation
of **all** aligned atoms
after **superposition**
$\propto d_i$

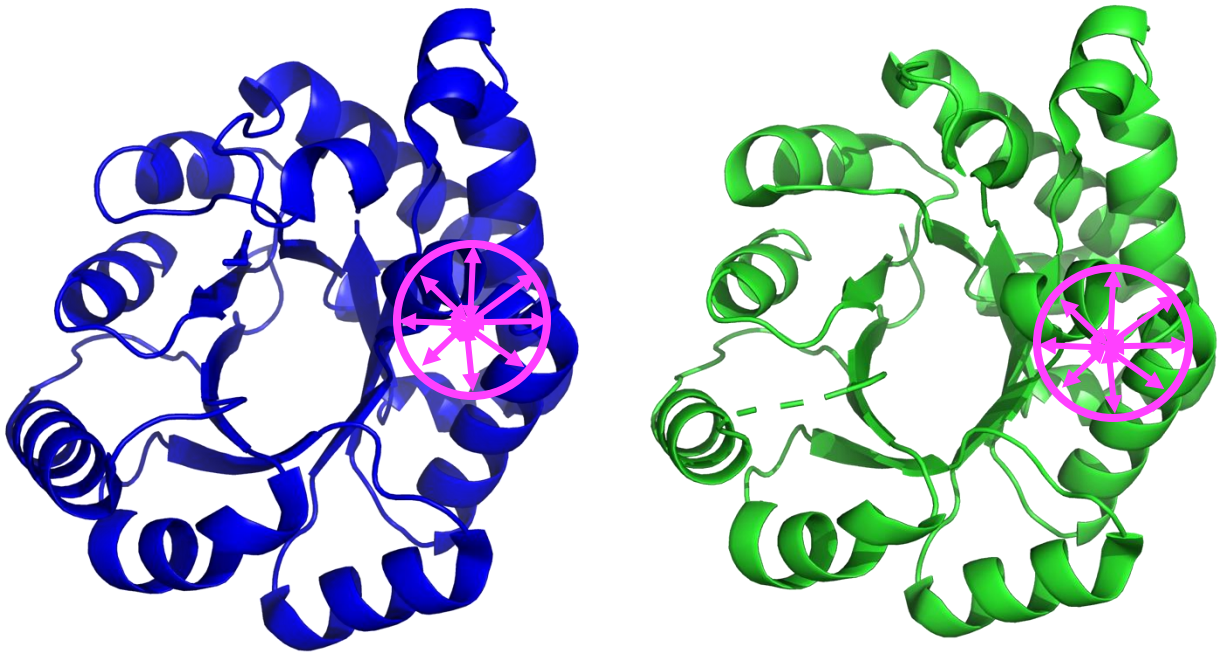
TM-score



A **score** between (0,1] considering **all** aligned atoms after **superposition**
$\propto 1/d_i$

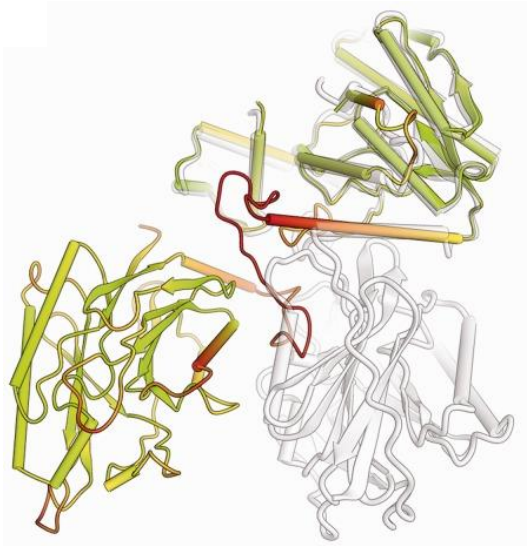
Intended to be more accurate because smaller deviations weigh more than larger deviations.

LDDT



List of d_i *within a threshold*

Superposition free,
percentage of conserved
local inter-atomic distances



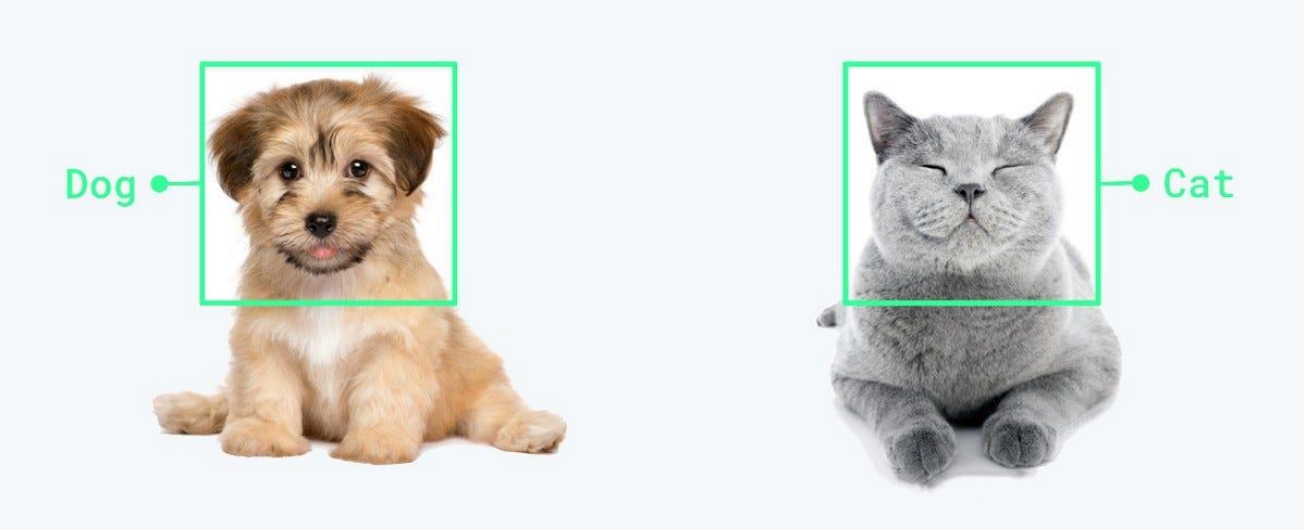
Mariani et al., 2013

Good for comparison of structures with separate domains

Correct in relation to others

Ground truth

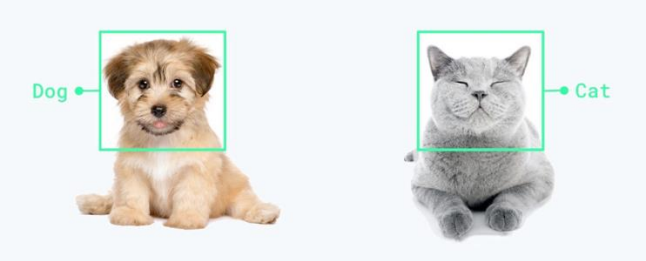
Datasets and labels

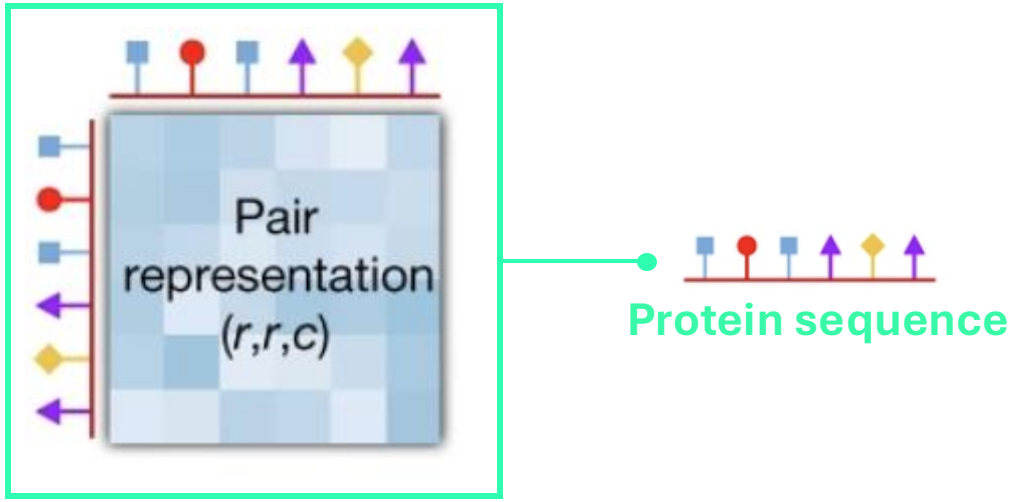


Correct in relation to others

Ground truth

Datasets and labels

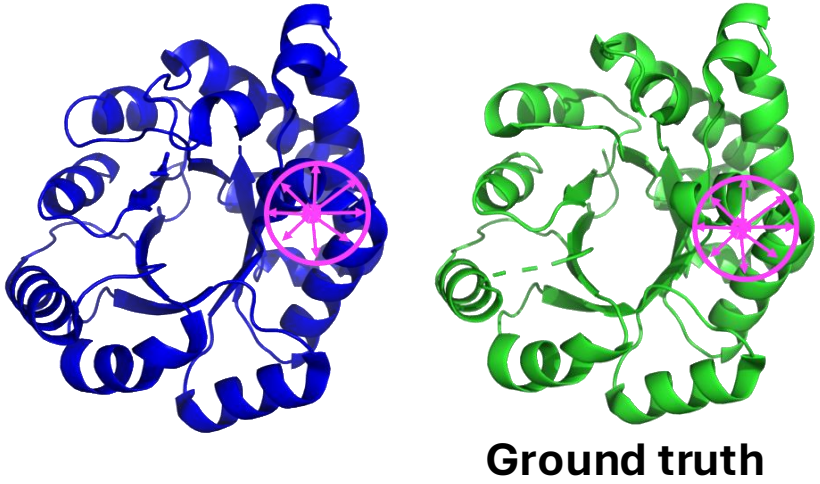




PDB

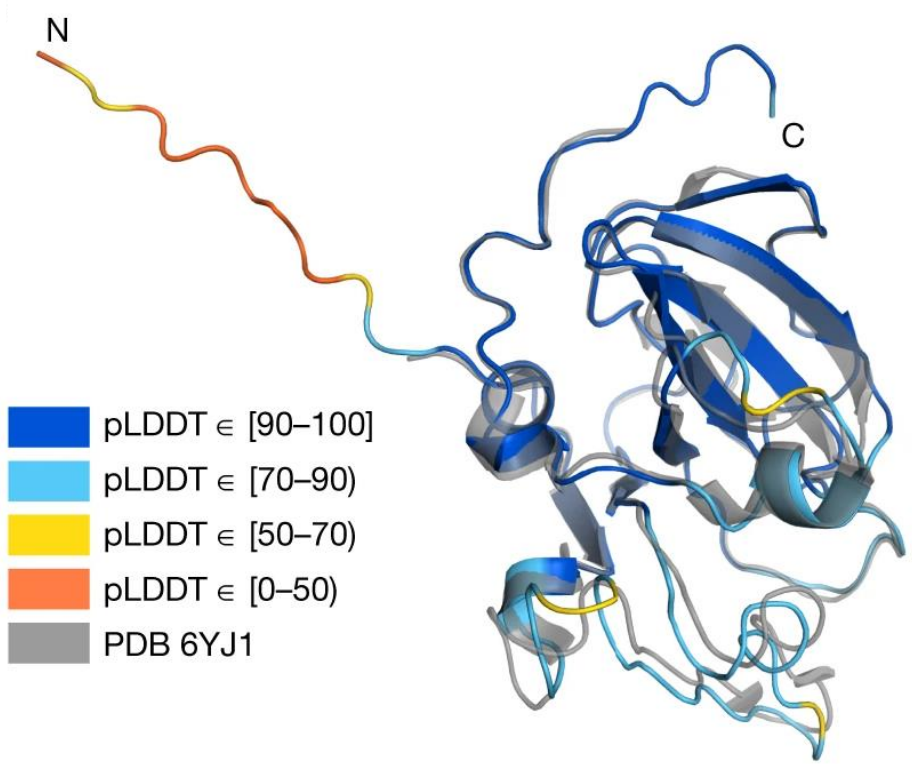
Contact map-like
(with more information)

pLDDT



Ground truth

Per residue score

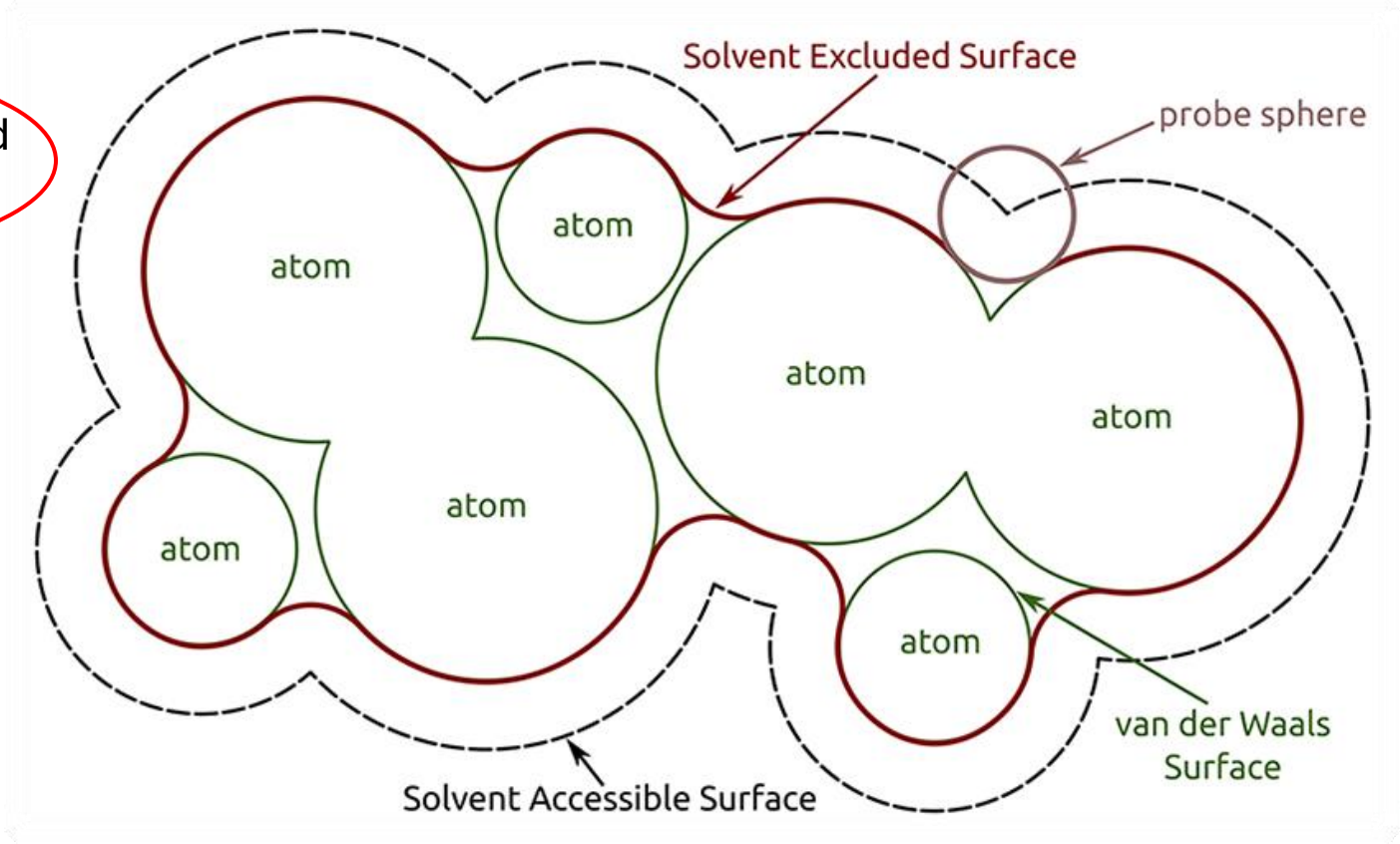


pLDDT > 90 – high confidence
pLDDT > 70 – confident / generally correct backbone
pLDDT < 70 – low confidence
pLDDT < 50 - not confident / disordered

RASA with AlphaFold3

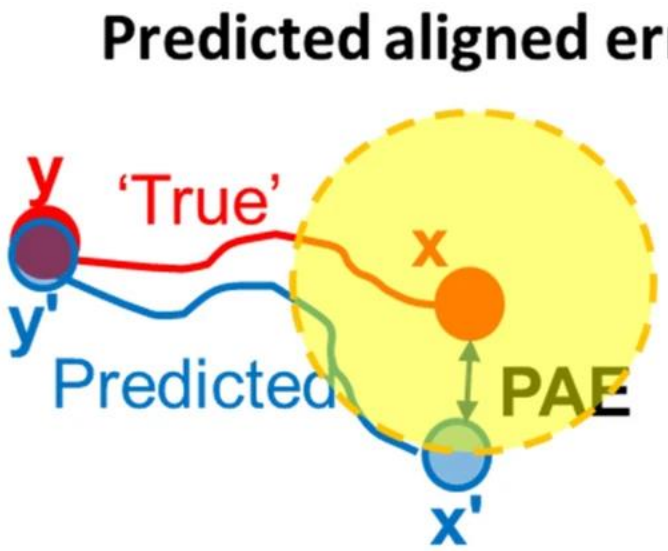
- Relative solvent Accessible Surface Area

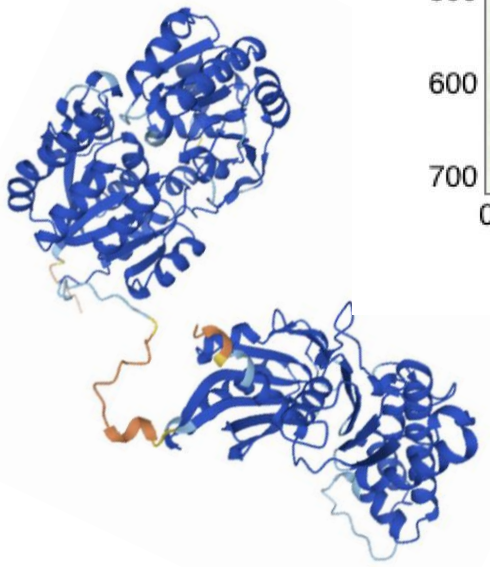
Compared to extended conformation

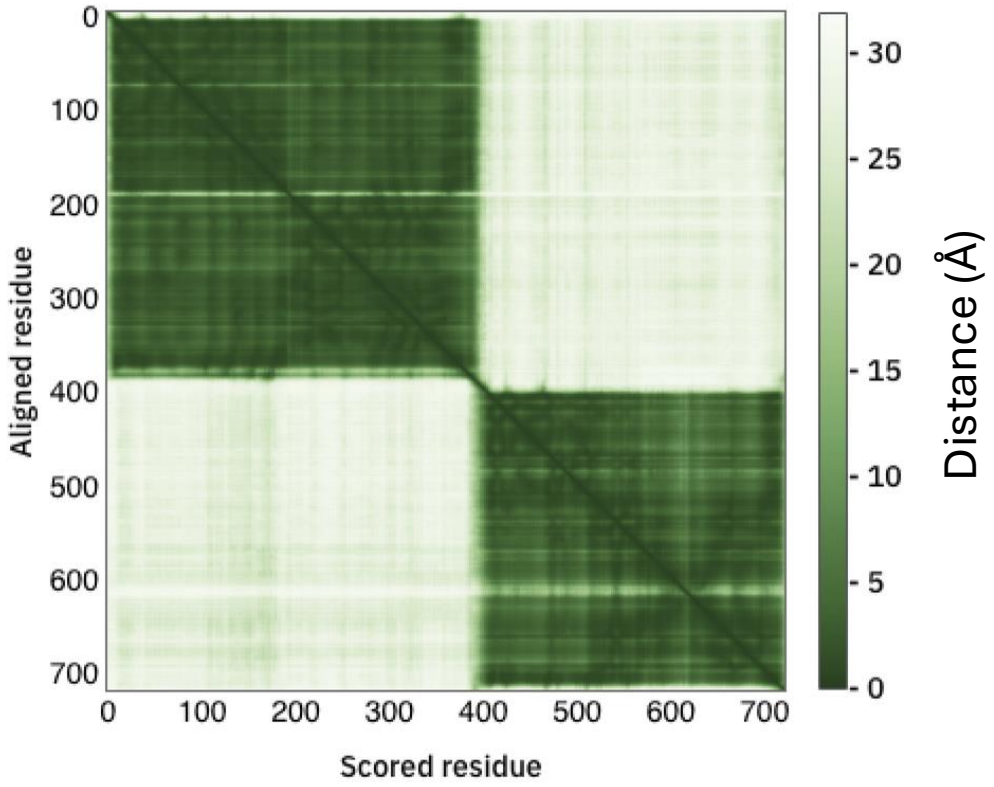


https://www.cgl.ucsf.edu/chimera/docs/UsersGuide/representation.html

PAE


Predicted aligned error (PAE)
y
'True'
x
y'
Predicted
PAE
x'



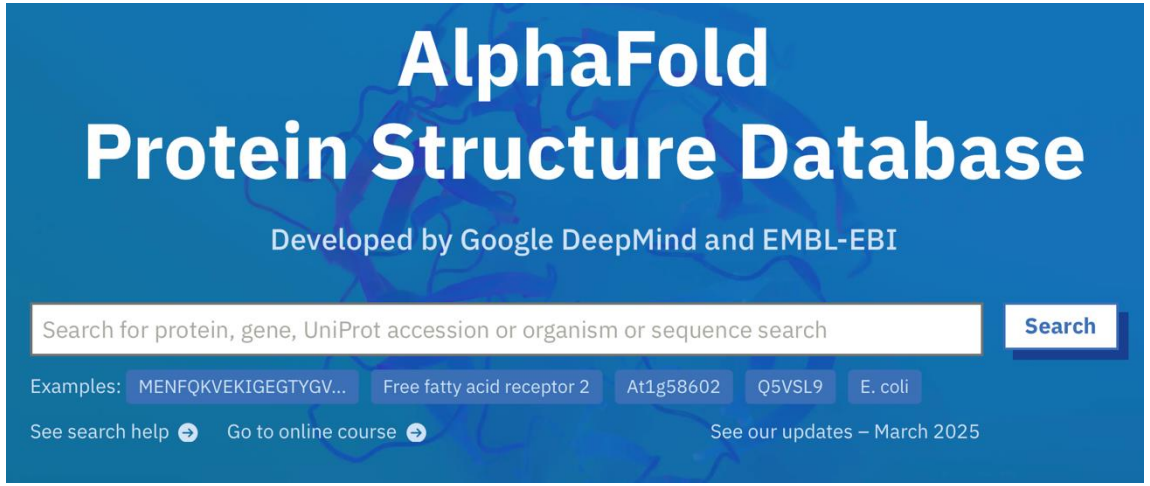

Aligned residue
Scored residue
0
100
200
300
400
500
600
700
30
25
20
15
10
5
0
Distance (Å)

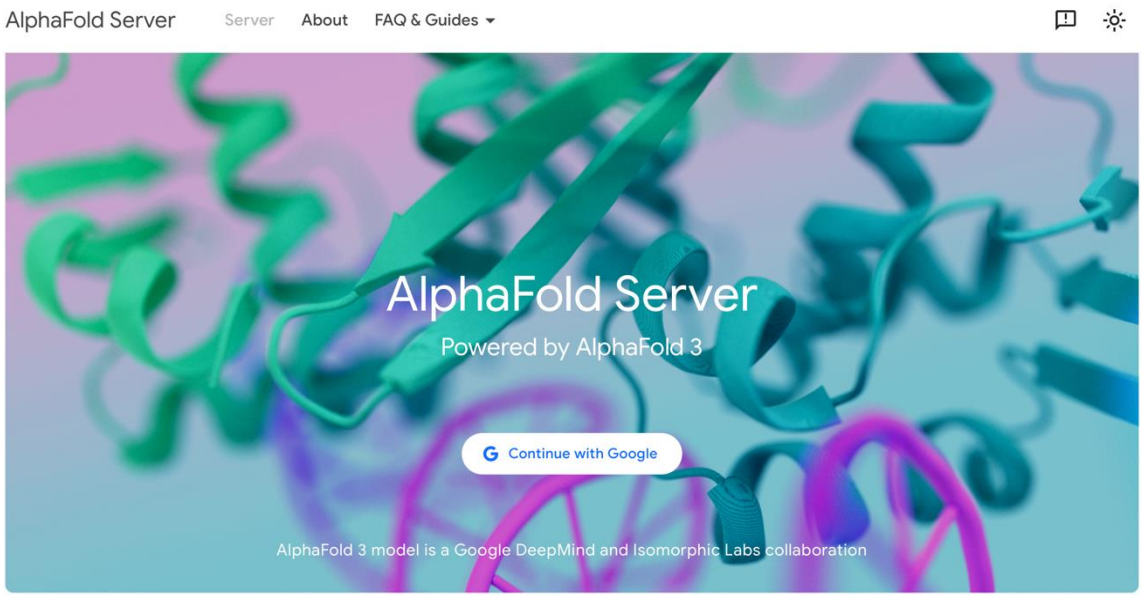
What are the difficulties of AlphaFold?

- Proteins that are experimentally hard to solve are also hard to predict because they lack background data
- The effects of single point mutations are underestimated
- Multimeric predictions will always give a result, even when there is no real interaction

Did AlphaFold solve how proteins fold?

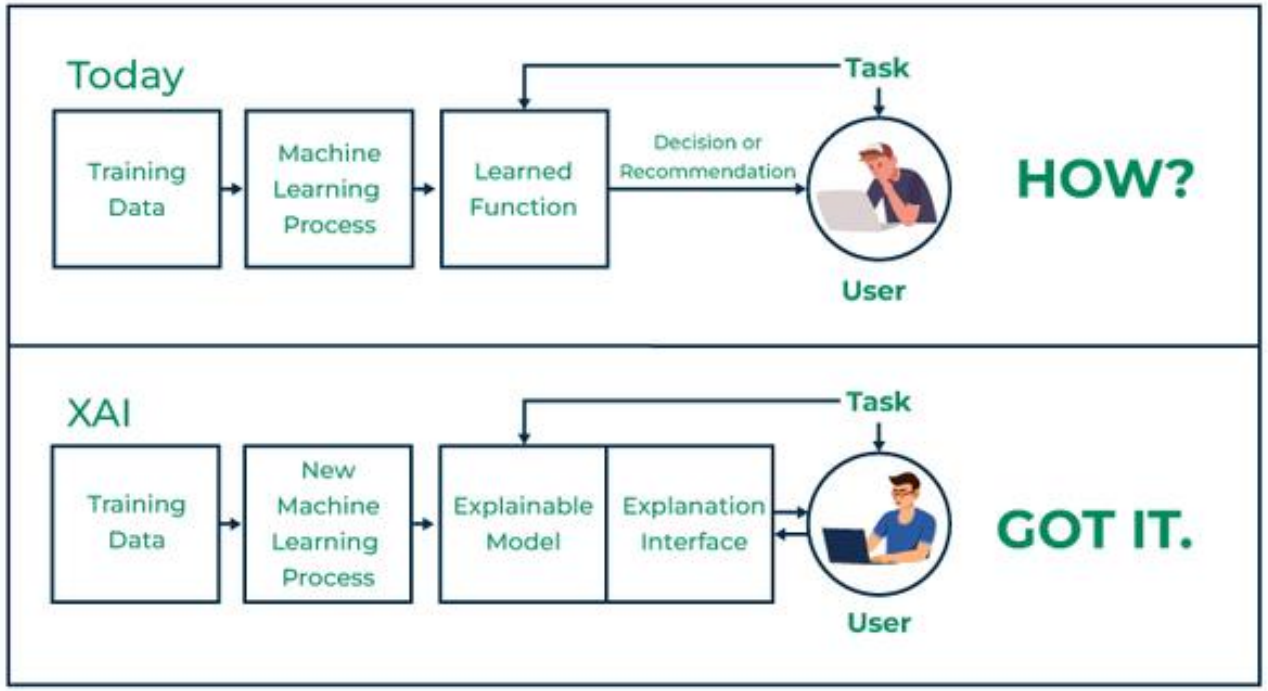
No, but it helps a lot!





Millions of protein structures
Several entire proteomes

Explanable AI



If an AI predicts that a loan should be denied, XAI might reveal:

- 60% of the decision was due to low income,
- 30% due to high debt,
- 10% due to recent credit inquiries.

Transparency, interpretability, justification and trust

Coffee break

"All models are wrong, but some are useful".

George Box

Alphafold outputs many models

	initial training	first fine-tuning		second fine-tuning				
Model	1	1.1	1.2	**1.1.1**	**1.1.2**	**1.2.1**	**1.2.2**	**1.2.3**
Parameters initialized from	Random	Model 1	···	Model 1.1	···	Model 1.2	···	···
Number of templates N_{templ}	4	4	0	4	···	0	···	···
Sequence crop size N_{res}	256	···	···	384	···	···	···	···
Number of sequences N_{seq}	128	512	···	···	···	···	···	···
Number of extra sequences $N_{\text{extra\_seq}}$	1024	···	···	5120	1024	5120	···	1024
Initial learning rate	10^{-3}	$5 \cdot 10^{-4}$	···	···	···	···	···	···
Learning rate linear warm-up samples	128000	0	···	···	···	···	···	···
Structural violation loss weight	0.0	1.0	···	···	···	···	···	···
"Experimentally resolved" loss weight	0.0	0.01	···	···	···	···	···	···
Training samples ($\cdot 10^6$)	9.2	1.1	1.7	0.3	0.6	1.4	1.1	2.4
Training time	6d 6h	1d 10h	2d 3h	20h	1d 13h	4d 1h	3d	5d 12h

5 models

AlphaFold Multimer runs each of the 5 models with 5 different random seeds for MSA sampling, resulting in 25 different models.

equations

- Rmsd

$$\mathrm{RMSD}(\mathbf{v}, \mathbf{w}) = \sqrt{\frac{1}{n}\sum_{i=1}^{n} \|v_i - w_i\|^2}$$
$$= \sqrt{\frac{1}{n}\sum_{i=1}^{n} ((v_{ix} - w_{ix})^2 + (v_{iy} - w_{iy})^2 + (v_{iz} - w_{iz})^2)}$$

- Tm score

$$\text{TM-score} = \max\left[\frac{1}{L_{\text{target}}}\sum_{i}^{L_{\text{common}}}\frac{1}{1+\left(\frac{d_i}{d_0(L_{\text{target}})}\right)^2}\right]$$

- https://en.wikipedia.org/wiki/Template_modeling_score
- iptm https://www.biorxiv.org/content/10.1101/2021.10.04.463034v1.full.pdf

Pillars of function