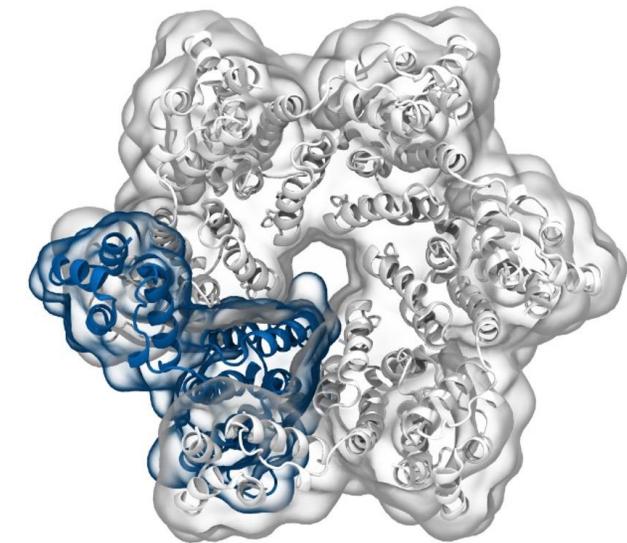
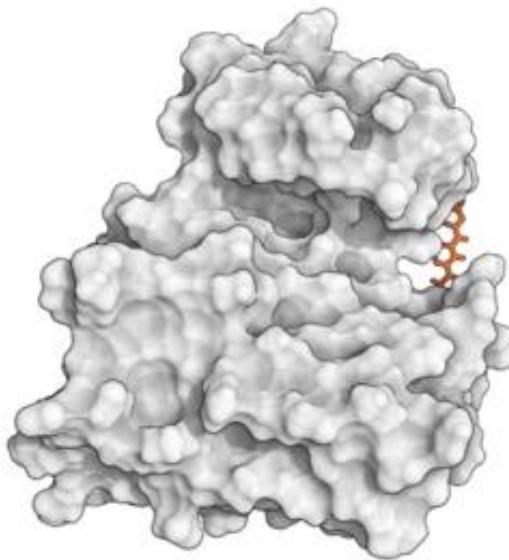


Simulation of Biomolecules

Introduction



Dr Matteo Degiacomi

Durham University

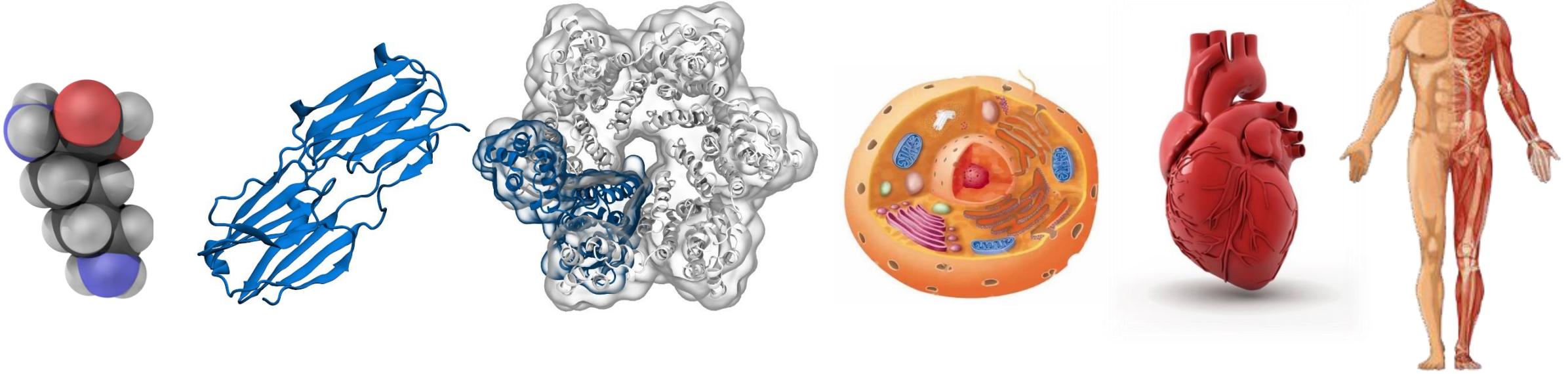
matteo.t.degiacomi@durham.ac.uk

Dr Antonia Mey

University of Edinburgh

antonia.mey@ed.ac.uk

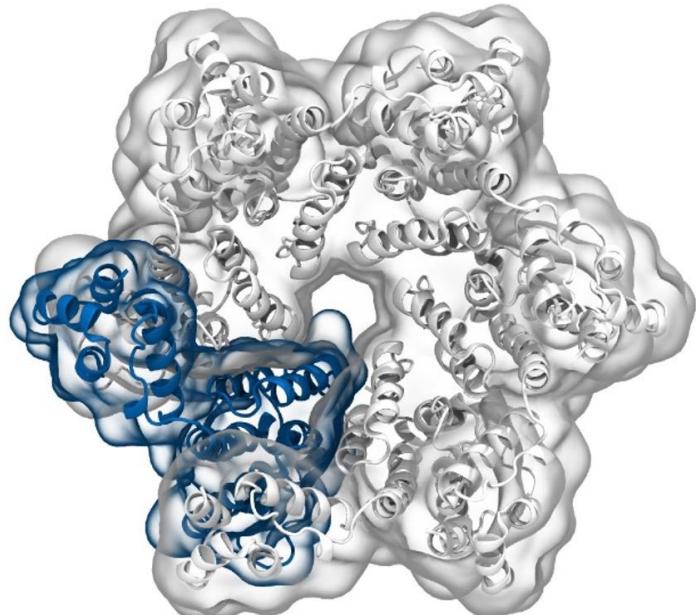
Life emerges from molecular assembly



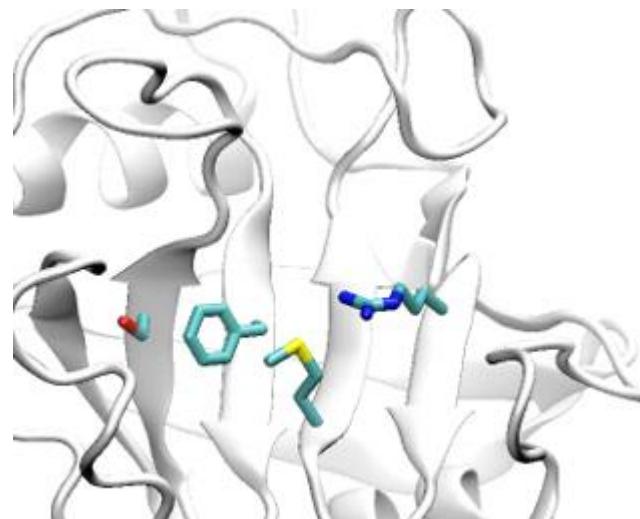
COMPLEXITY

Structure and dynamics determine protein (mal)function

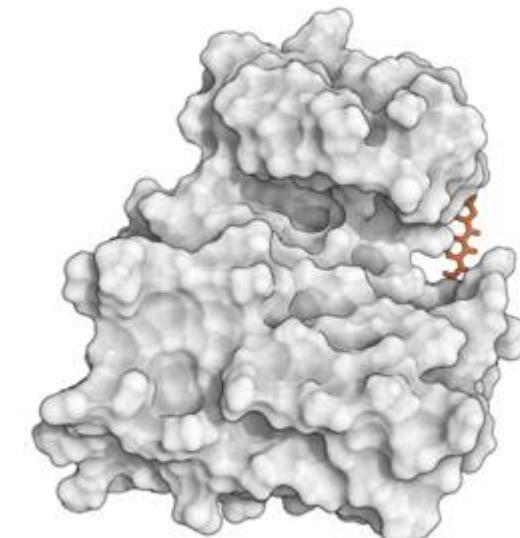
HIV Capsomer



Cyclophilin



Tyrosine kinase —dasatanib

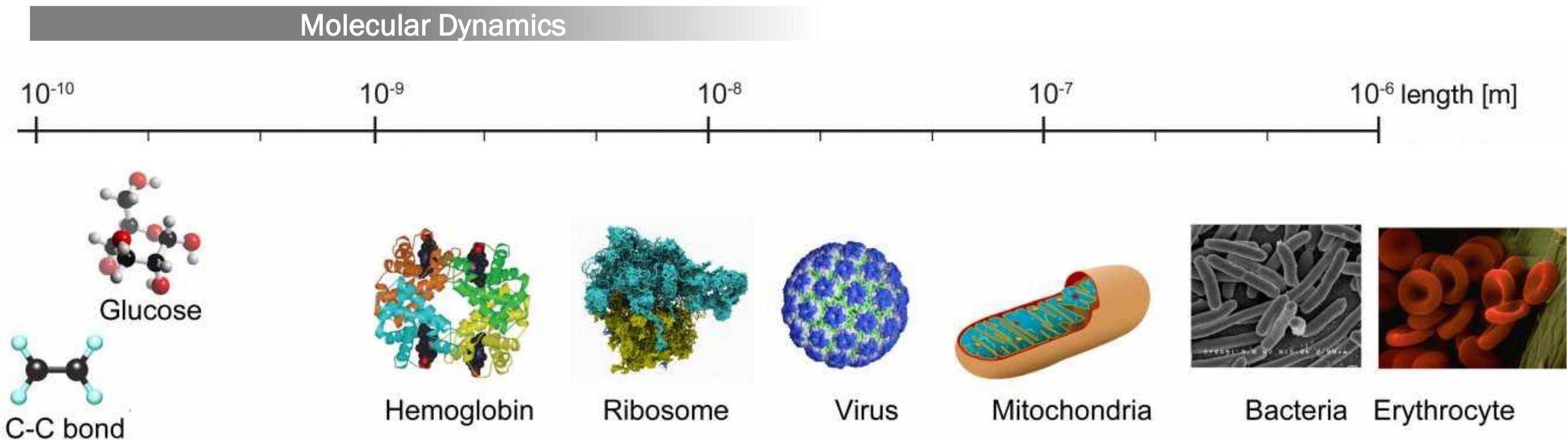


M.T. Degiacomi, *Structure*, 2019

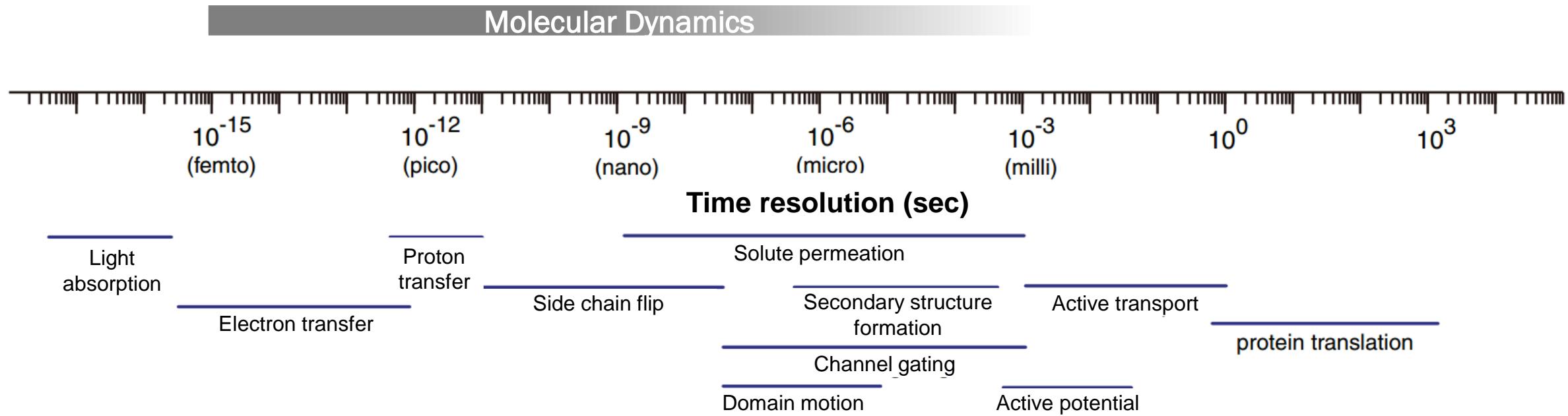
Wapeesittipan, Mey, et al., *Comms. Chem.*, 2019

Y Shan et al. *JACS*, 2011

Sizes in biochemistry



Timescales in biochemistry

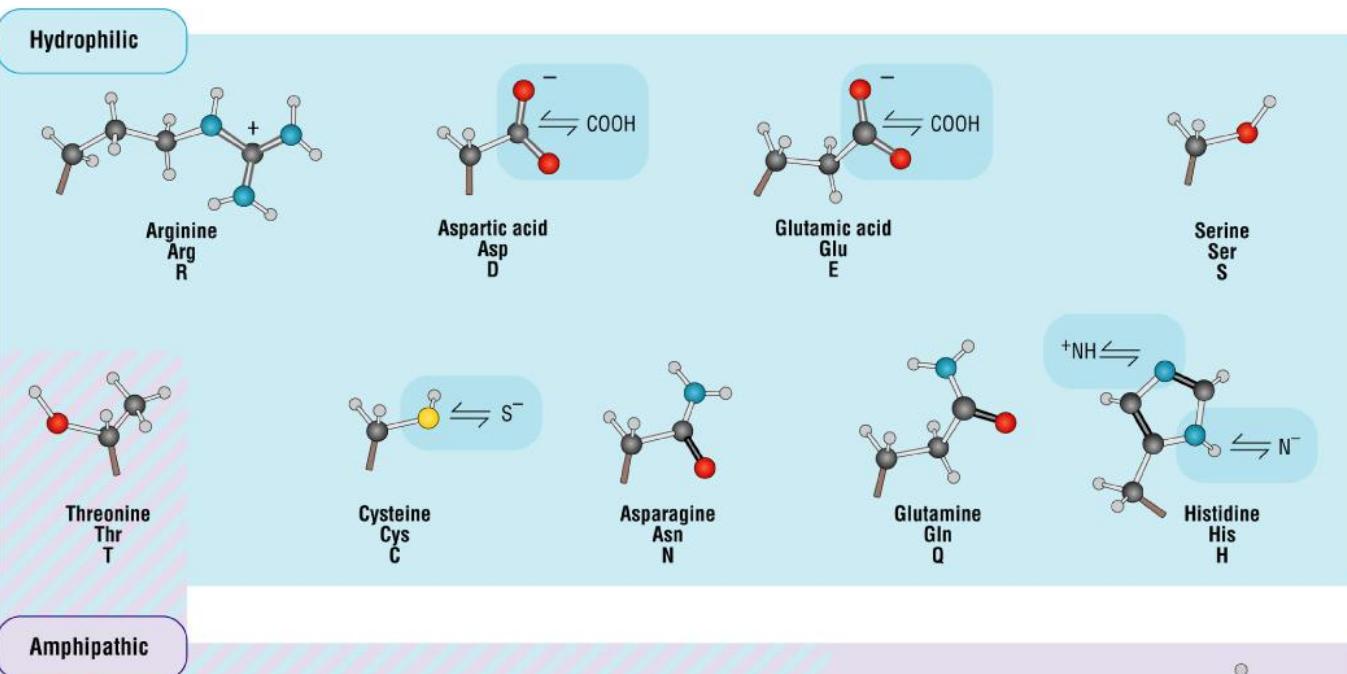
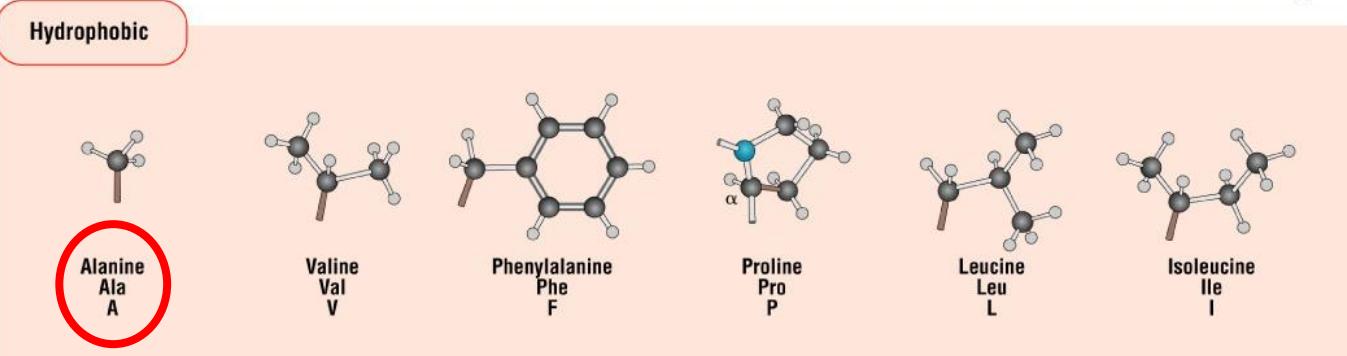
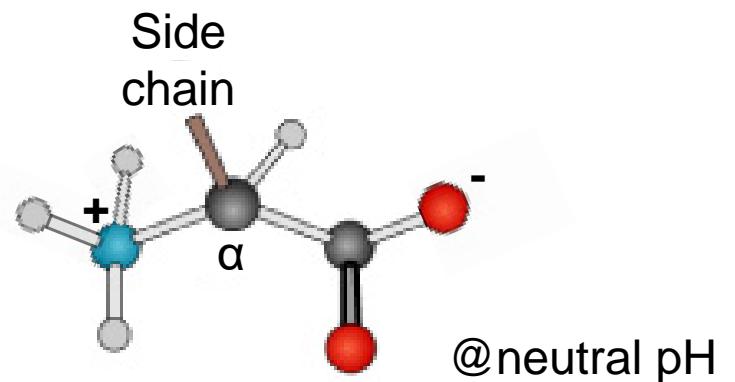


Part 1: What is a protein?

Proteins are amino acids polymers

Amino acids are composed of:

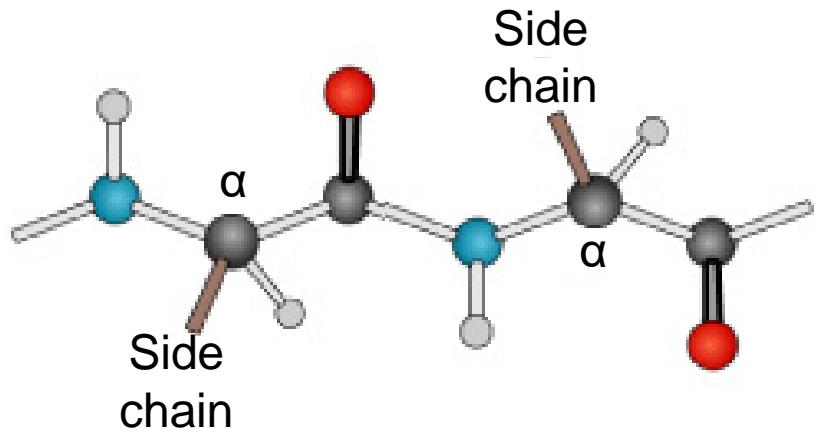
- **Backbone** (conserved)
- **Side chain** (variable)



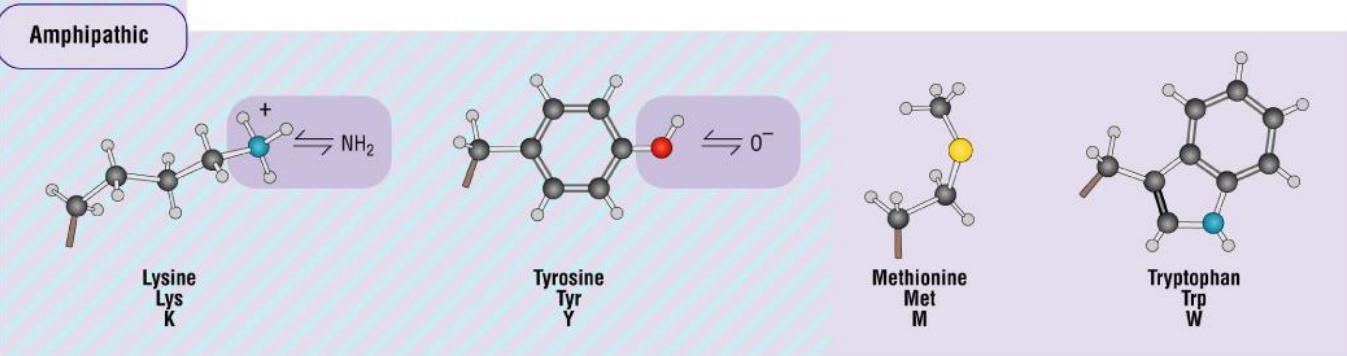
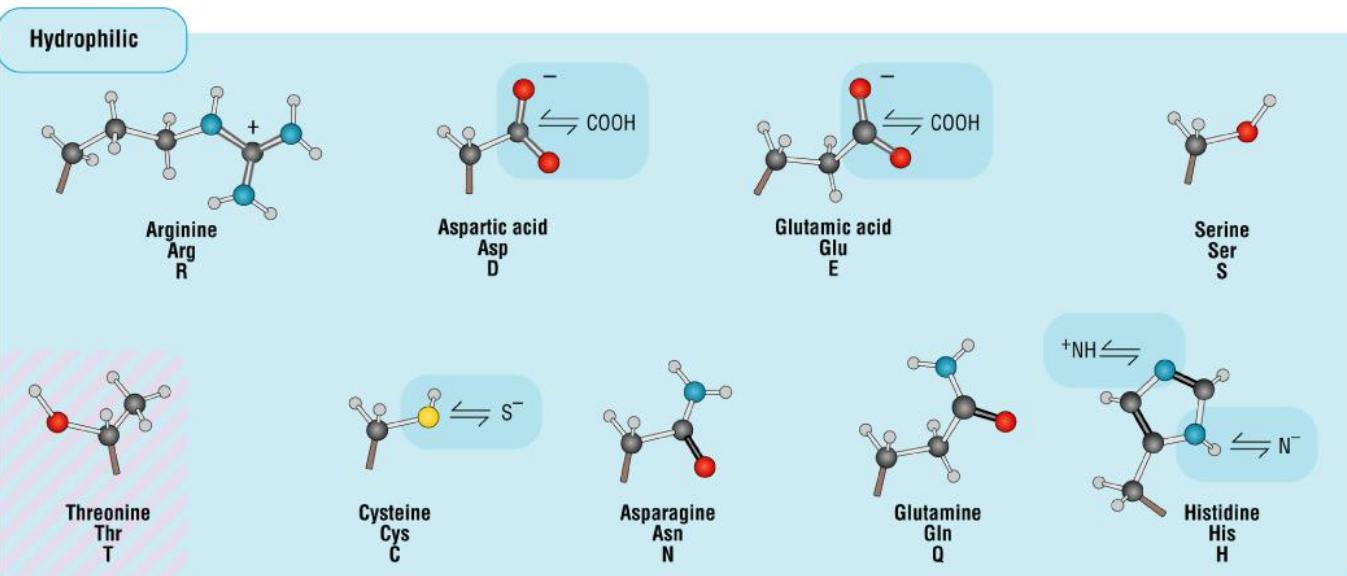
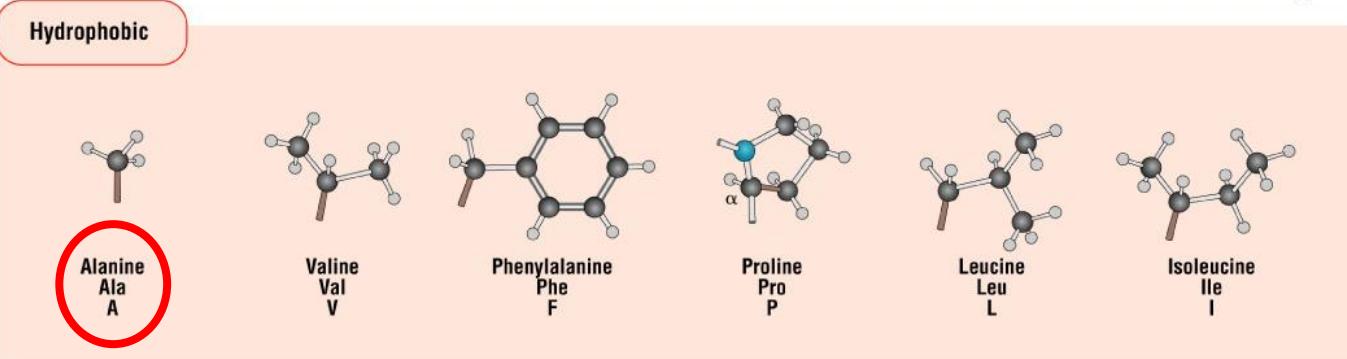
Proteins are amino acids polymers

Amino acids are composed of:

- **Backbone** (conserved)
- **Side chain** (variable)



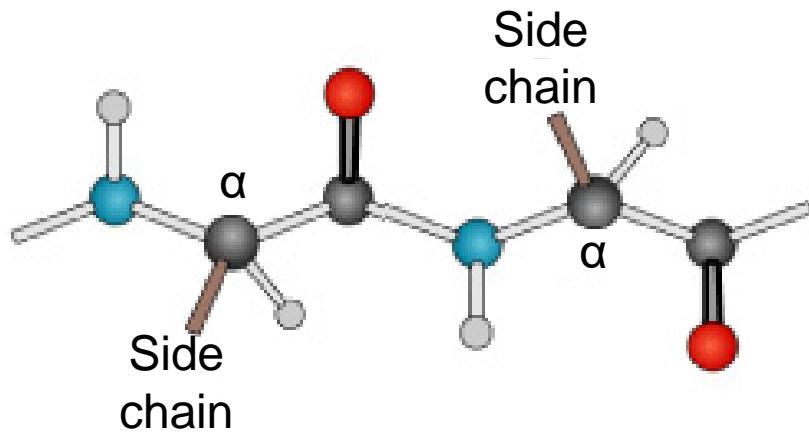
Amino acids polymerize forming a *peptidic bond* (condensation)



Proteins are amino acids polymers

Amino acids are composed of:

- **Backbone** (conserved)
- **Side chain** (variable)



Amino acids polymerize forming a *peptidic bond* (condensation)

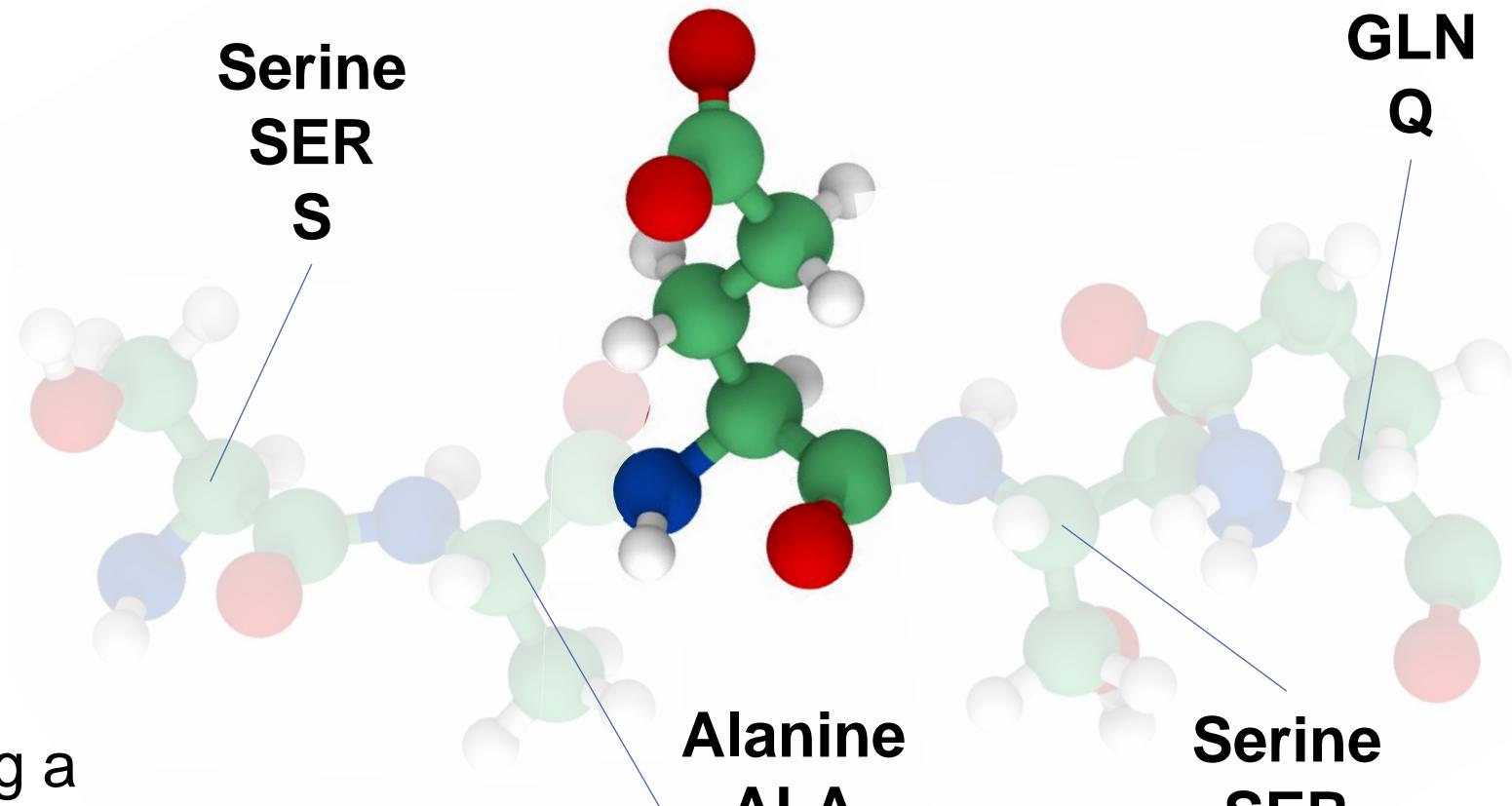
Glutamic acid
GLU
E

Glutamine
GLN
Q

Serine
SER
S

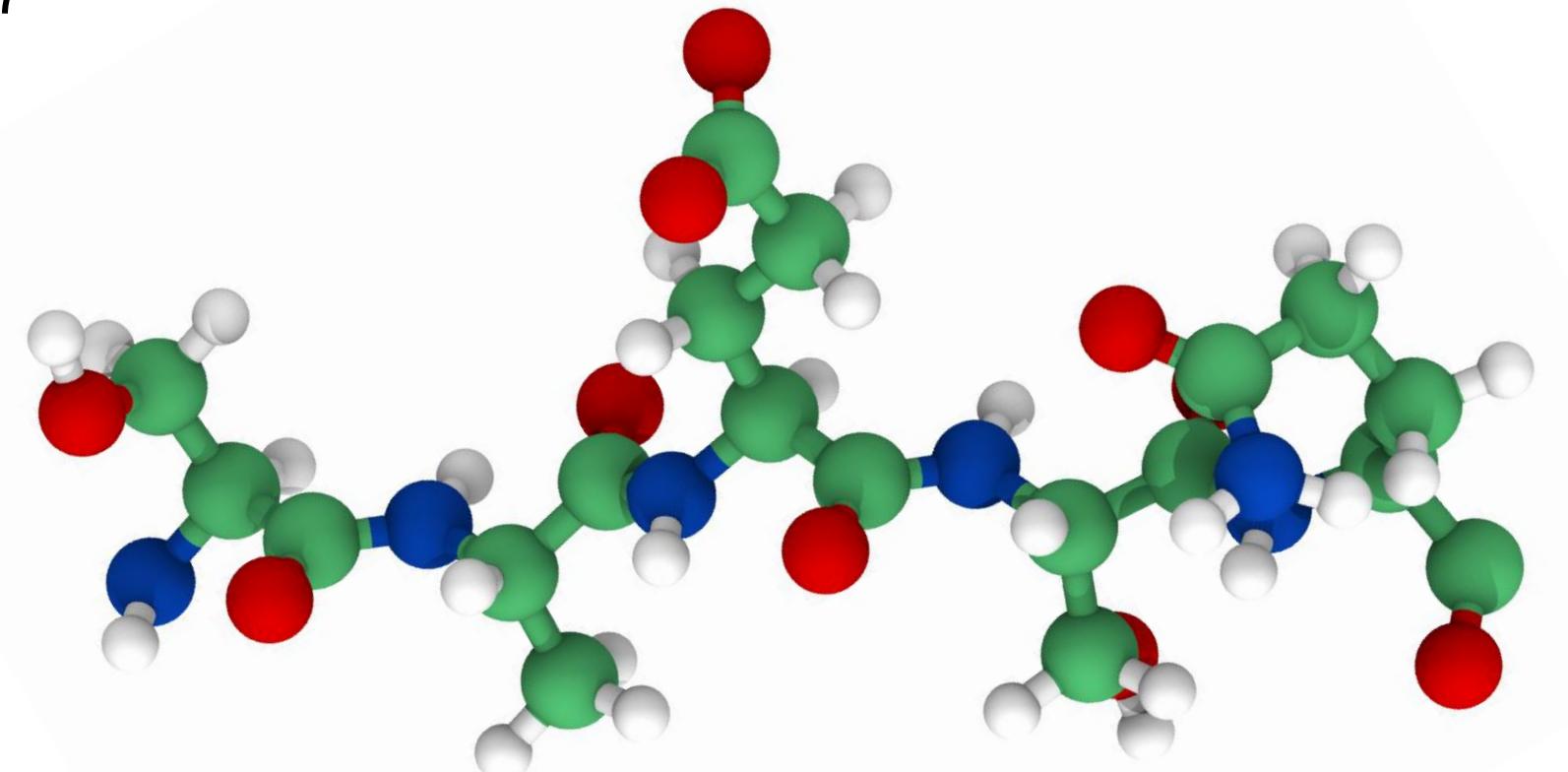
Alanine
ALA
A

Serine
SER
S



Protein Primary Structure: Sequence

SAESQ

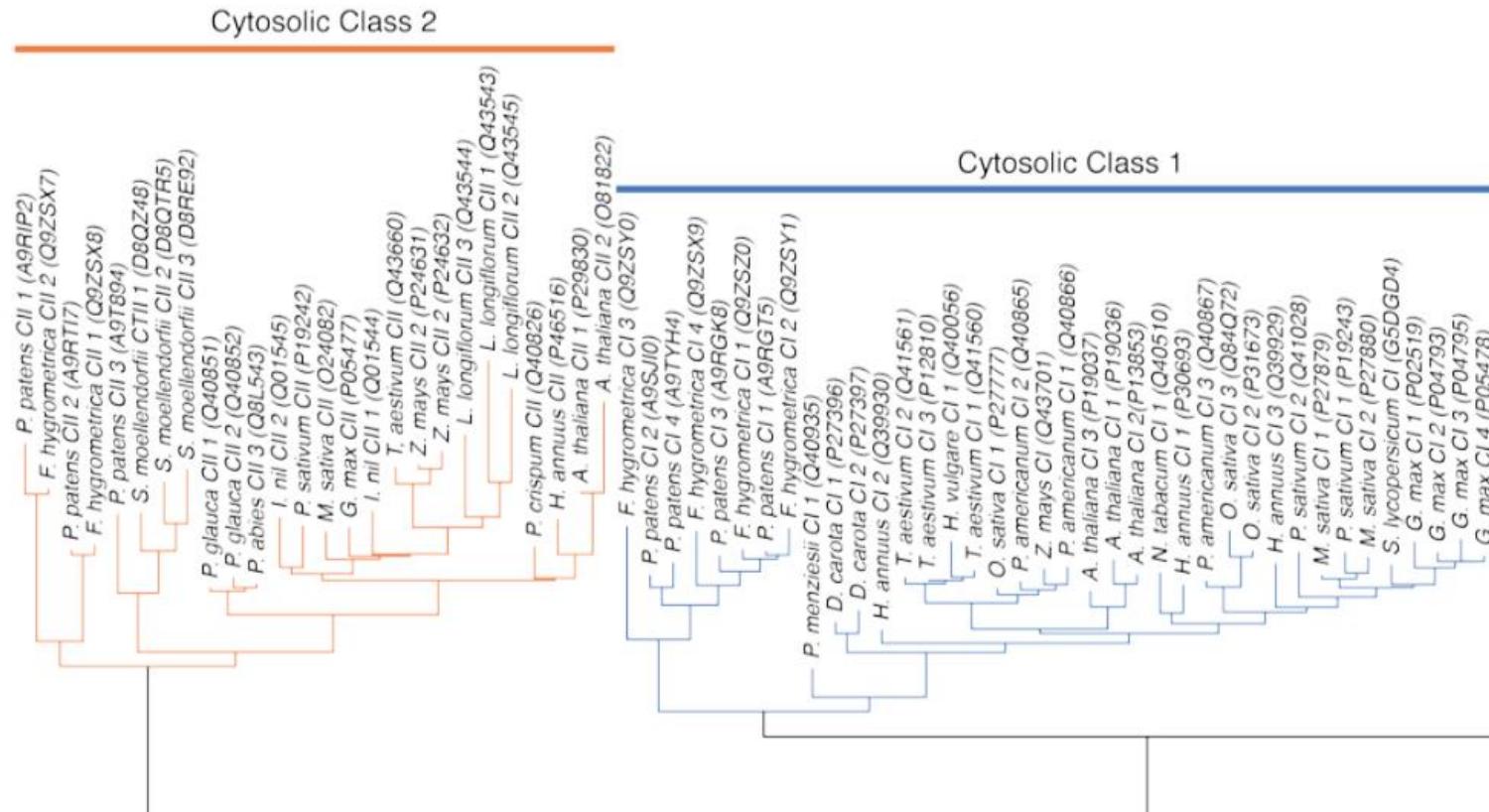


Proteins with similar sequence, likely:

- have similar functions in an organism
- are evolutionarily related

Protein Primary Structure: Sequence

Angiosperm 1 MSLIPSFFSGRRSNVFD-PF--SL-DVWDPLKD-FPFSNSSPSASPRENPAFV-STRVDWKETPEAHVFKA
DLPGLKKEEVKVEVE
Gymnosperm 1 MSIIPSFFGRRSSSAFD-PF--SL-DVWDPFRAFTDLGGPGSQFVNEASAVA-NTQIDWKETPEAHIFKA
DLPGLKKEEVKIELE
Bryophyte 1 MAL--SLFGSRGNGVFD-PF--EFGSVWDPFSA---PESGLSRKLAGDAHAGA-NTRIDWRETPEAHIFKA
DLPGLRKEEVKIQVV
Angiosperm 2 -----MDLDSPLFNTLHHIMDLTDDTTEKNLNAPTRTYVRDAKAMA-ATPADVKEHPNSYVFMVDMPGVKSGDIKVQVE
Gymnosperm 2 -----MAMD-PSLITVQHLLGVPDD-LEKLLNAPTHSYMRDTKAMA-STPVDVKEYPNSYVFIIDMPGLKSNDIKVQVE
Bryophyte 2 -----MEFVVFDTD-PFLTSLHQHVHEPESDLERKIKRKRRSQHDEPRHVTIATPVDVKEKKDAYLFIA
DVPGLQKTDIEVQIE

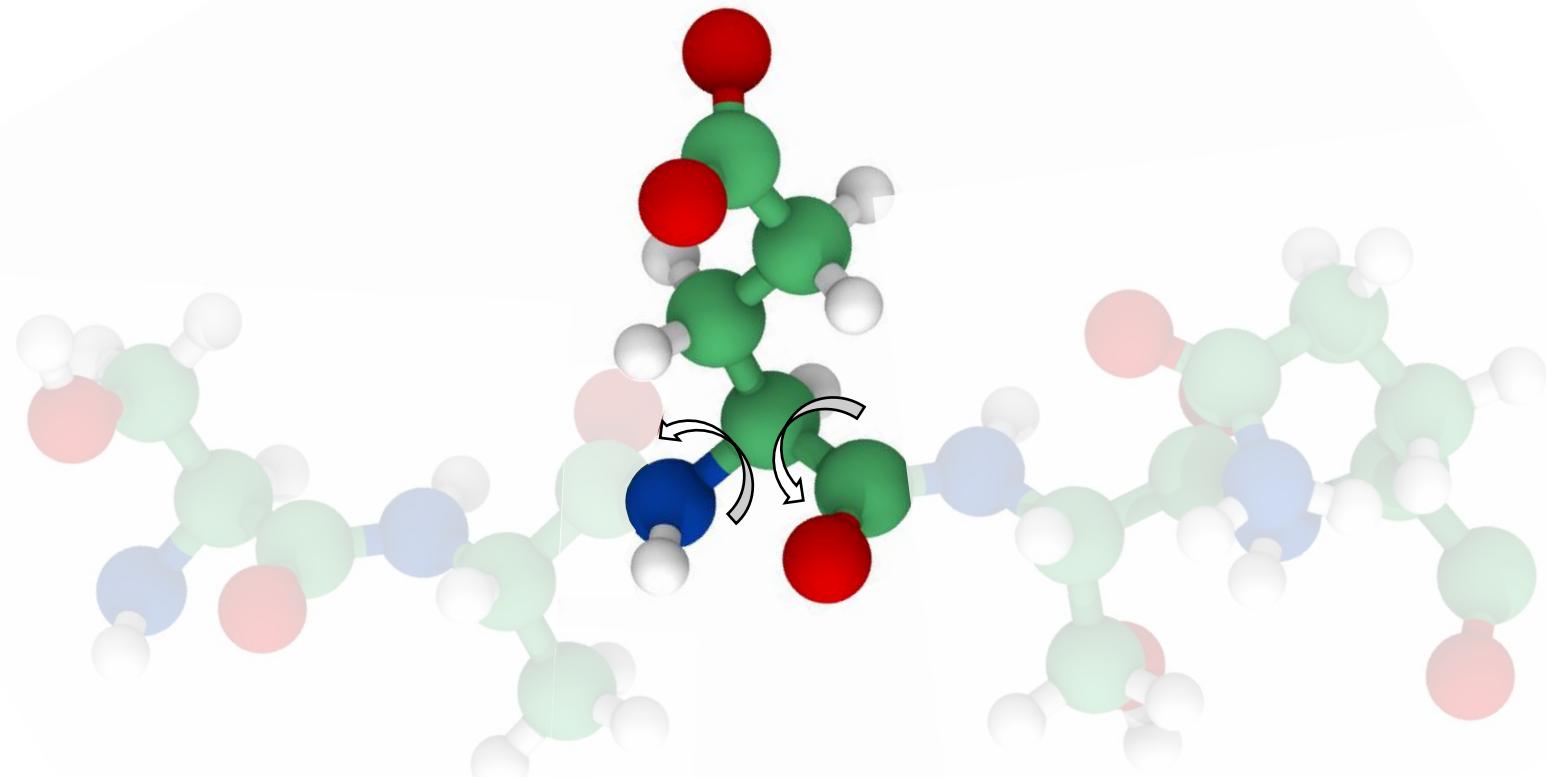


Proteins with similar sequence, likely:

- have similar functions in an organism
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Protein Secondary Structure

The amino acid chain path is determined by
backbone torsional angles

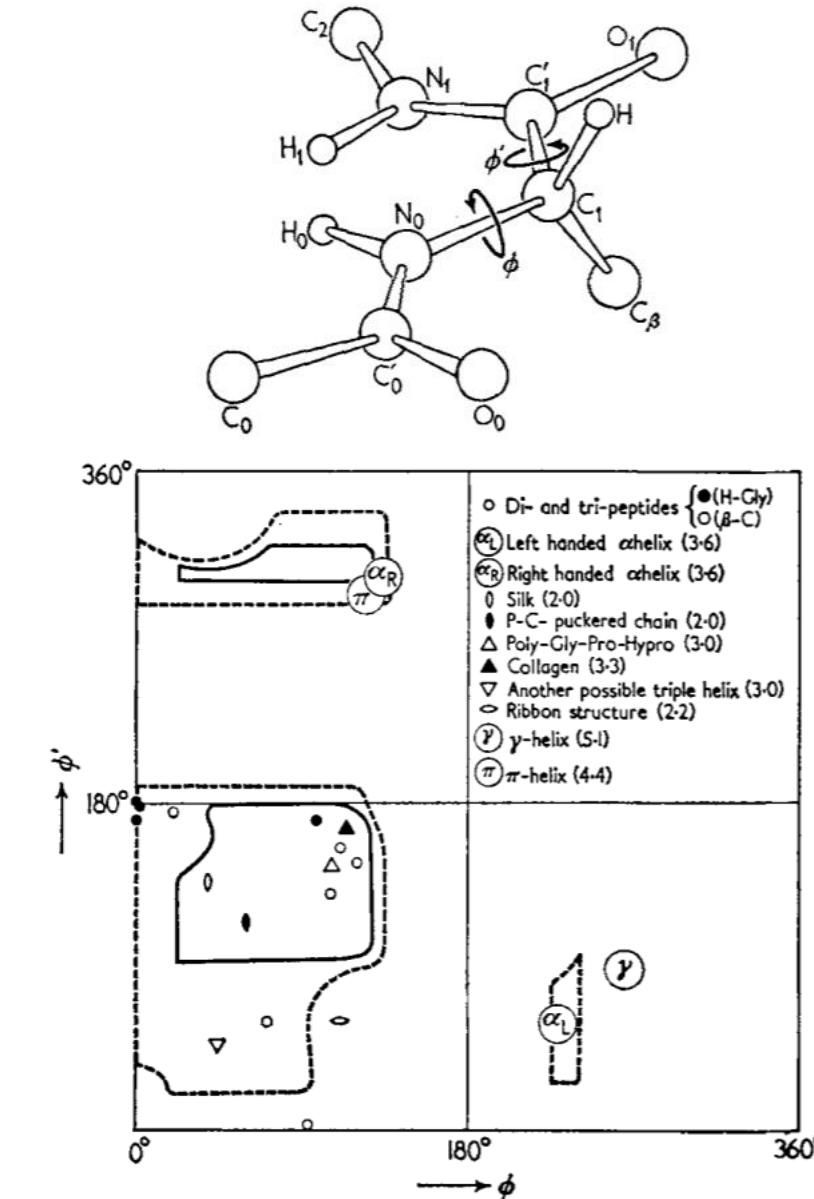


Protein Secondary Structure

The amino acid chain path is determined by backbone torsional angles

Ramachandran plot: scatter plot of amino acids backbone torsional angles

G.N. Ramachandran, C. Ramakrishnan, V. Sasisekharan. *Stereochemistry of polypeptide chain configurations. Journal of Molecular Biology*, 1963



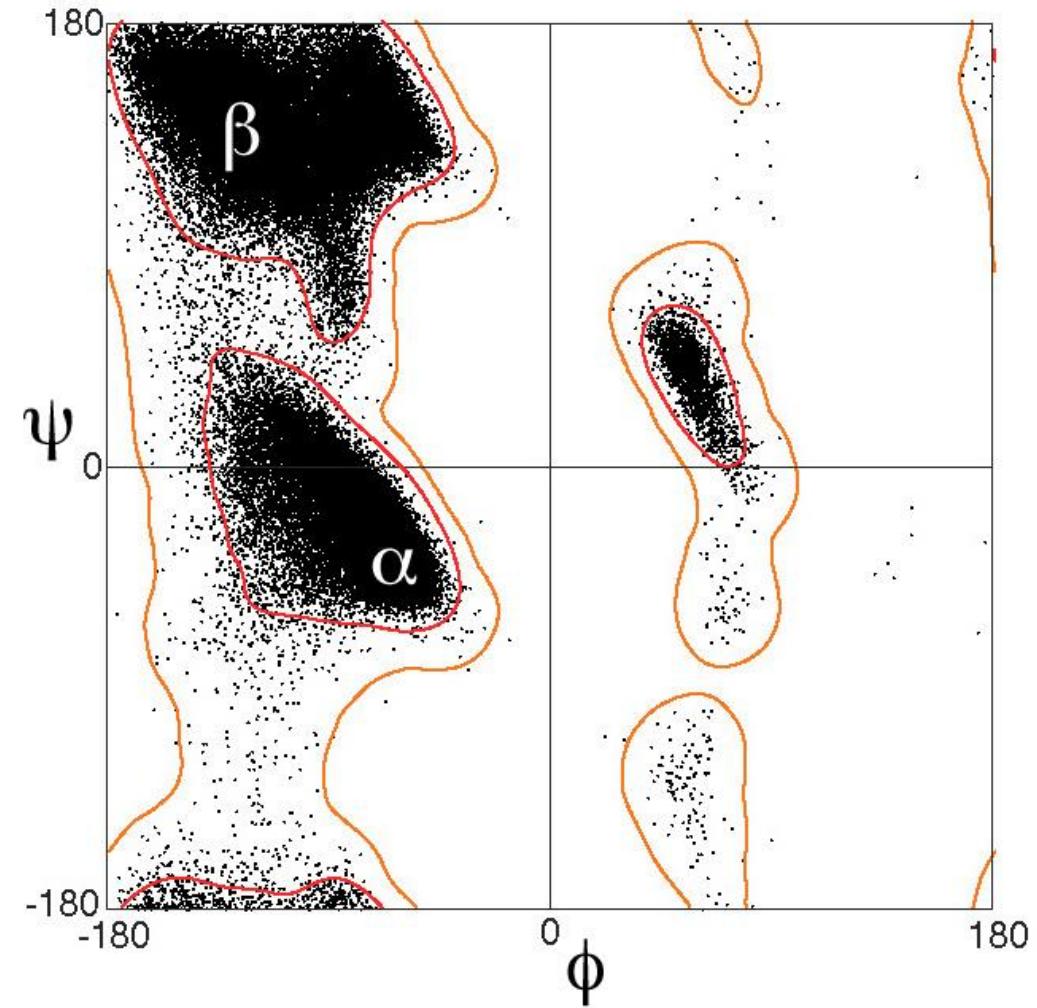
Protein Secondary Structure

The amino acid chain path is determined by backbone torsional angles

Ramachandran plot: scatter plot of amino acids backbone torsional angles

Dictionary of Secondary Structure in Proteins (DSSP) classification defines 7 secondary structure elements (regions in the plot):

- H = α -helix
- G = 3_{10} helix
- I = π -helix
- B = residue in isolated β -bridge
- E = extended strand
- T = hydrogen bonded turn
- S = bend



[Extra] Protein Secondary Structure

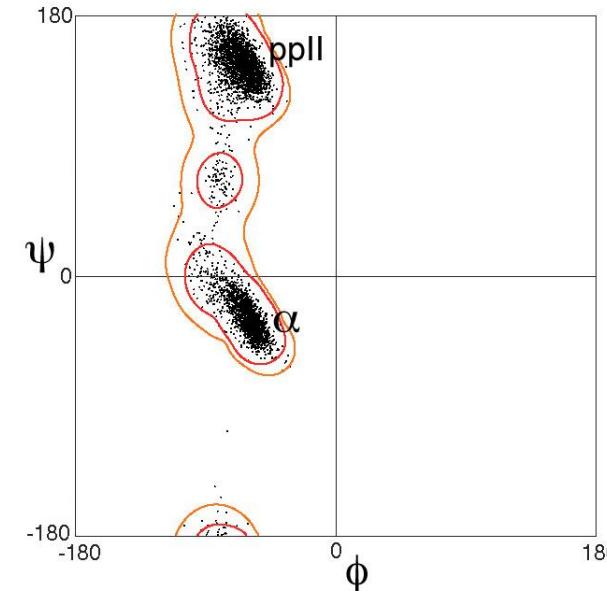
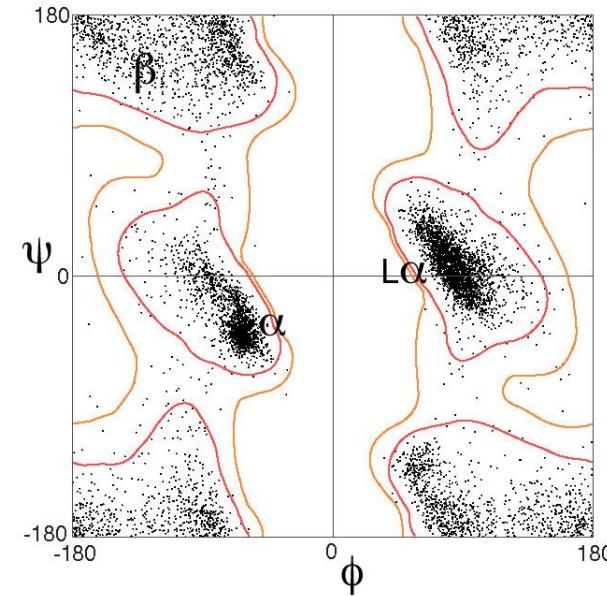
The amino acid chain path is determined by backbone torsional angles

Ramachandran plot: scatter plot of amino acids backbone torsional angles

Dictionary of Secondary Structure in Proteins (DSSP) classification defines 7 secondary structure elements (regions in the plot):

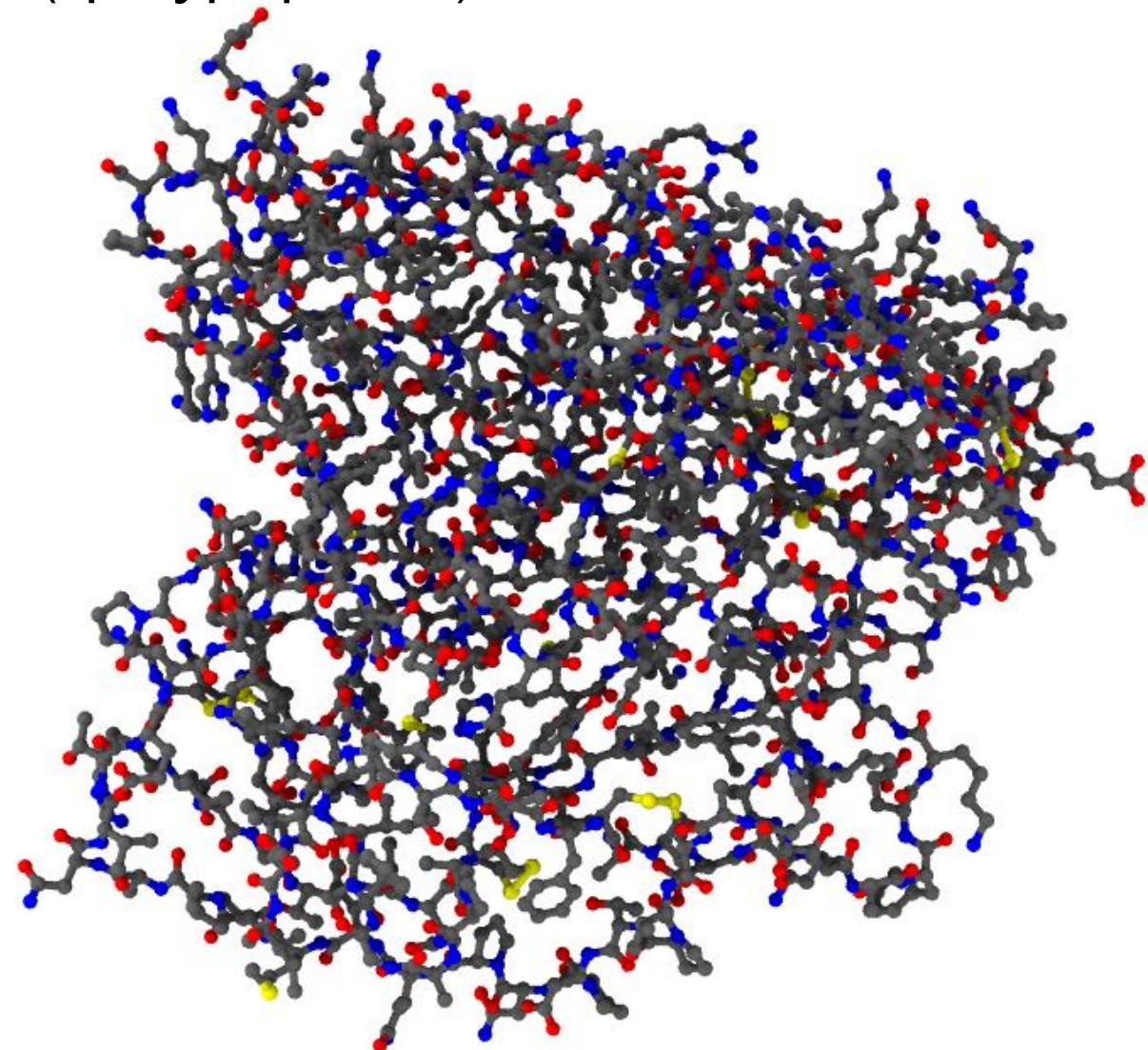
- H = α -helix
- G = 3_{10} helix
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- B = residue in isolated β -bridge
- E = extended strand
- T = hydrogen bonded turn
- S = bend

W. Kabsch and C. Sander, *Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features*, *Biopolymers*, 1983



Protein tertiary structure: folding

Protein («polypeptide»): 10 to >1000 amino acids



Protein tertiary structure: folding

Protein («polypeptide»): 10 to >1000 amino acids



Cartoon
representation:
helices

Protein tertiary structure: folding

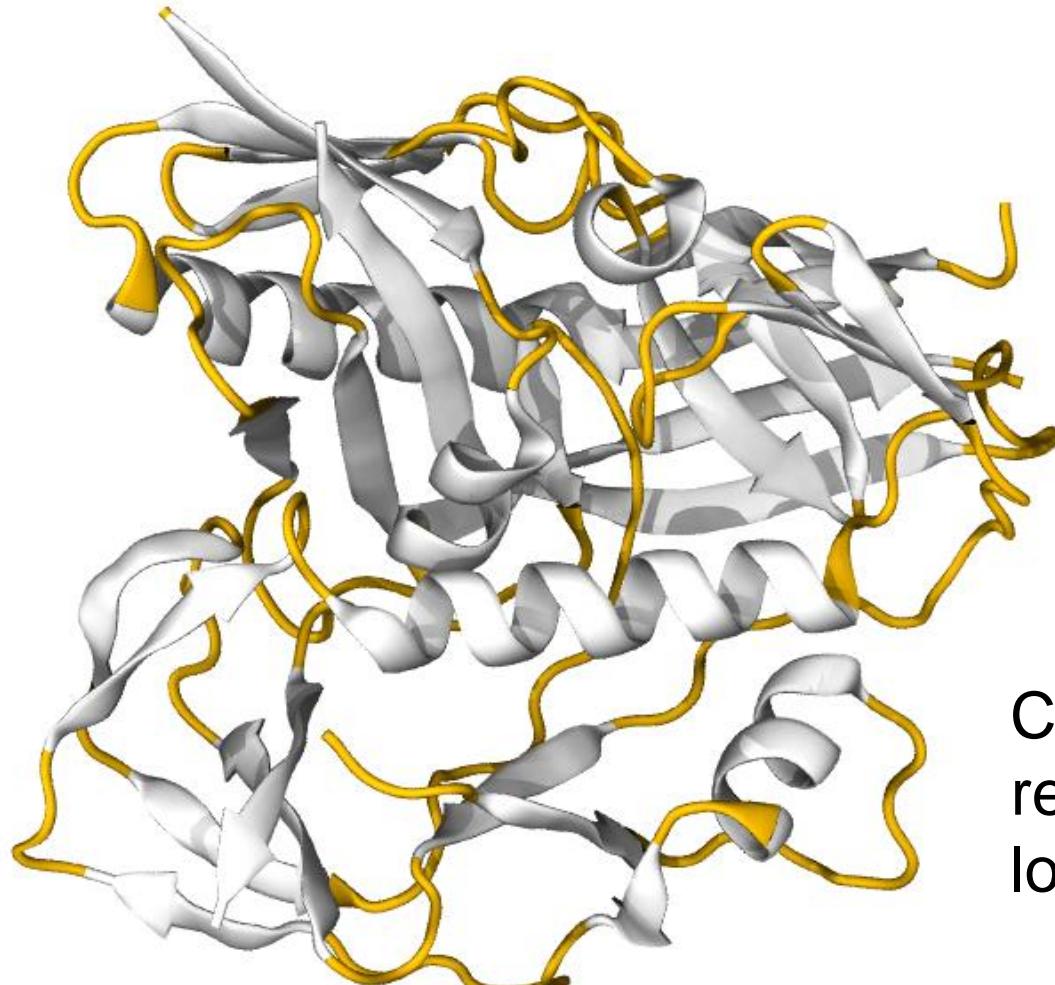
Protein («polypeptide»): 10 to >1000 amino acids



Cartoon
representation:
sheets

Protein tertiary structure: folding

Protein («polypeptide»): 10 to >1000 amino acids



Cartoon
representation:
loops

Protein tertiary structure: folding

Protein («polypeptide»): 10 to >1000 amino acids



Anfinsen's dogma

The three-dimensional structure of a protein in its native environment is solely determined by its amino acid sequence.



Christian Anfinsen. *Principles that govern the folding of protein chains*, *Science*, 1973

Hydrogen bonds

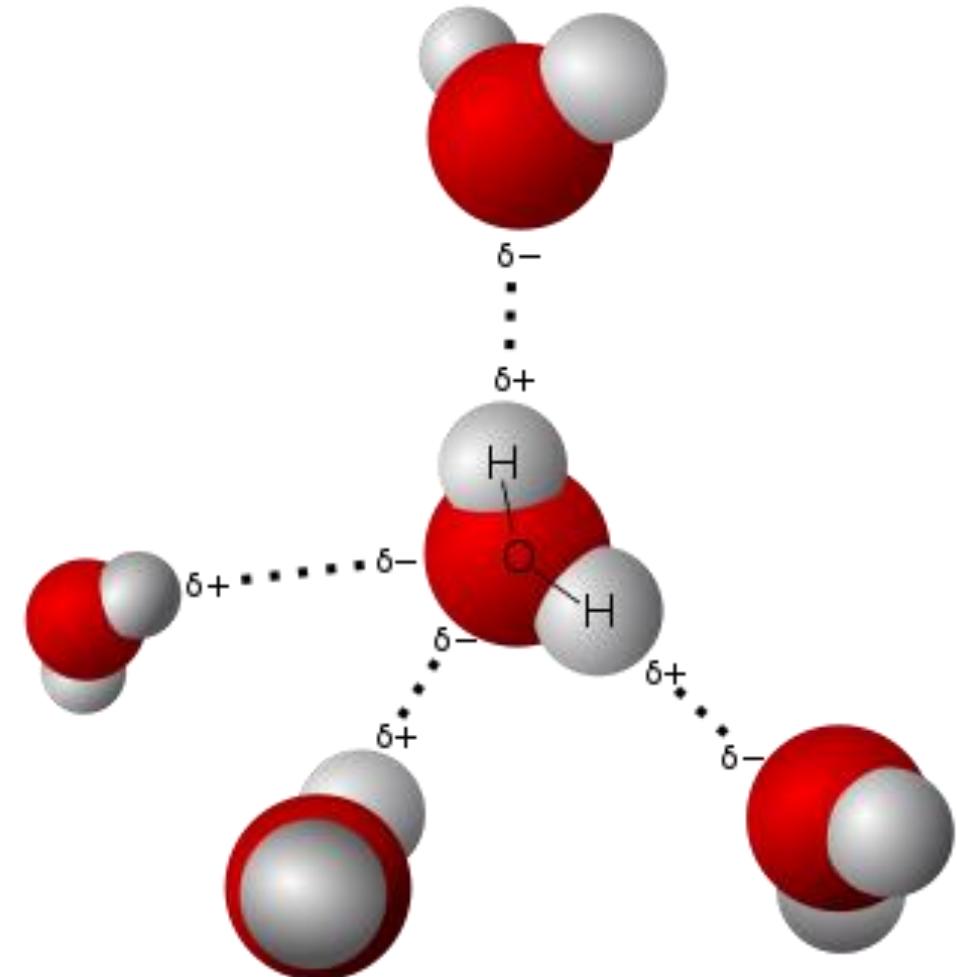
Electrostatic interaction. Structure:

- donor-acceptor distance typically 1.6-2 Å
- donor-acceptor-hydrogen angle must be small

Hydrogen bond energy in biomolecular systems typically 5-25 kJ/mol, e.g.:

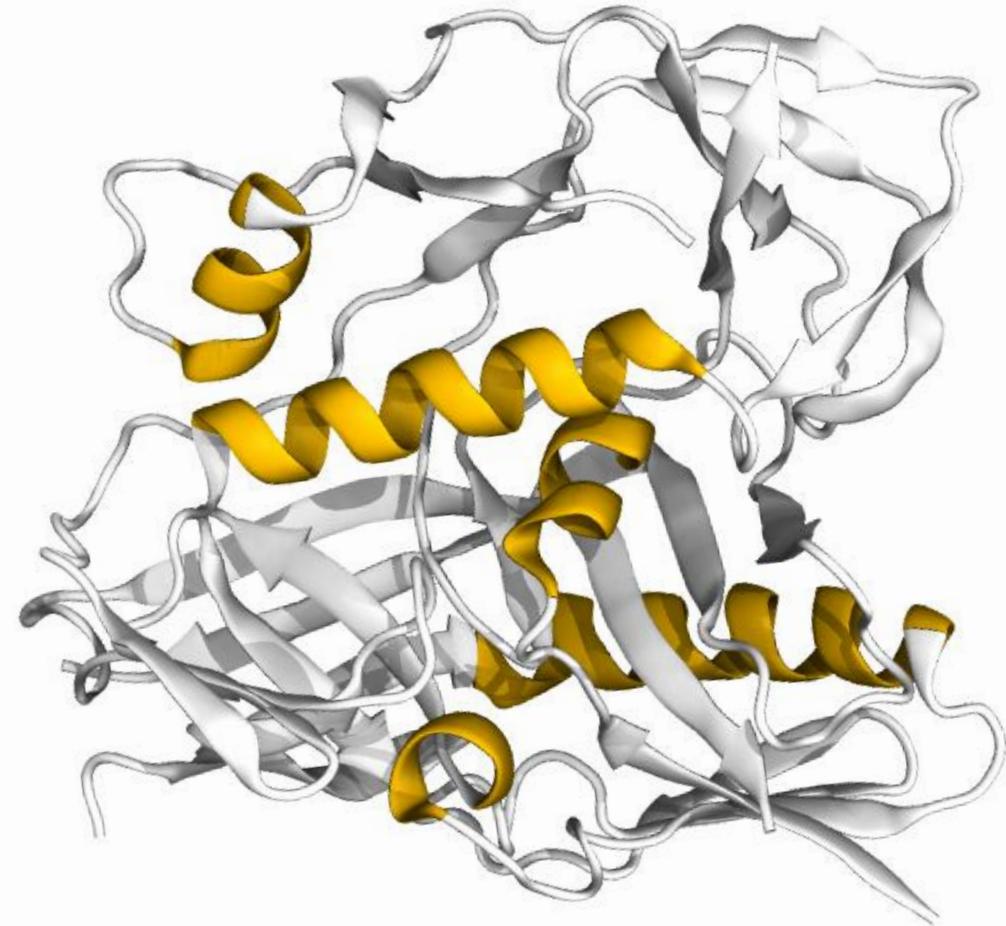
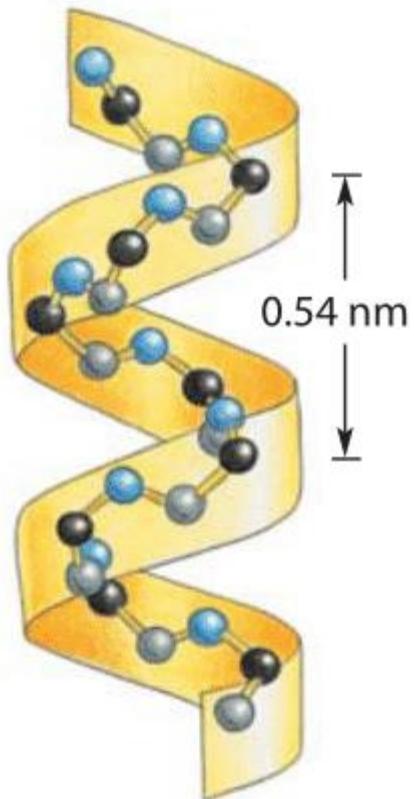
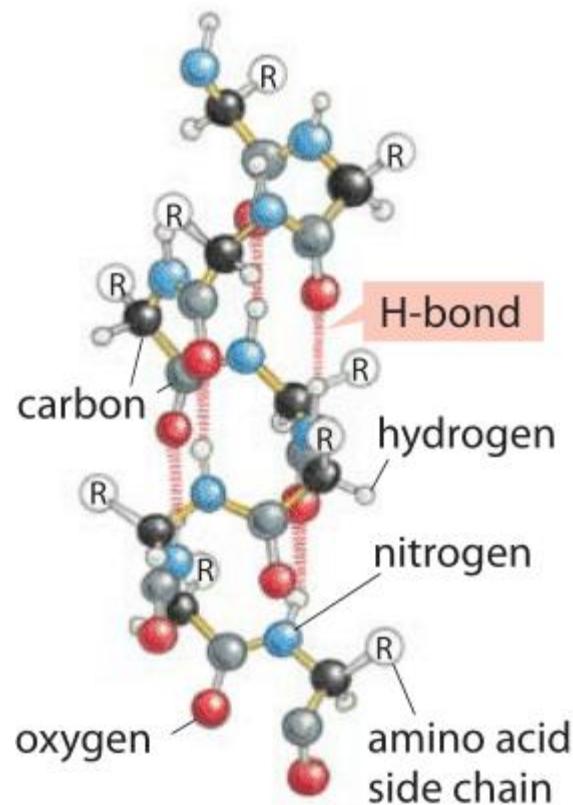
- O-H \cdots :O , 21 kJ/mol (5.0 kcal/mol)
- N-H \cdots :O , 8 kJ/mol (1.9 kcal/mol)

amino acids' backbone and polar side chains can be donor/acceptor



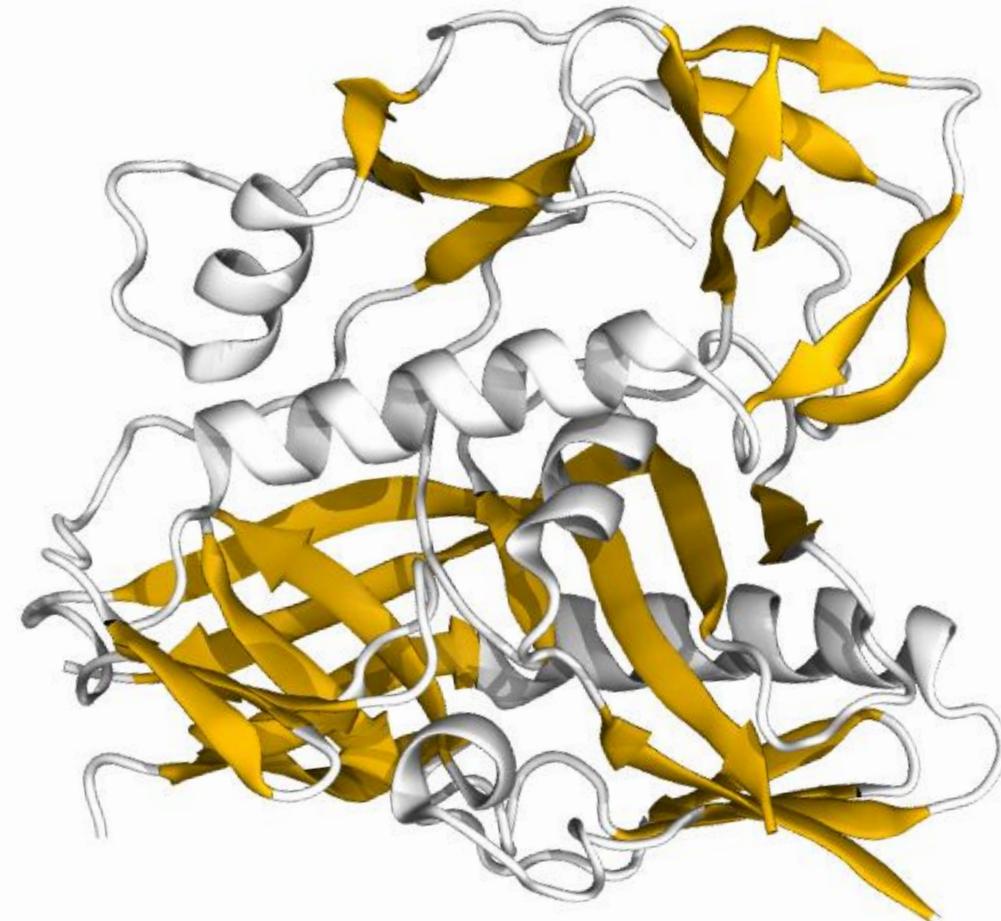
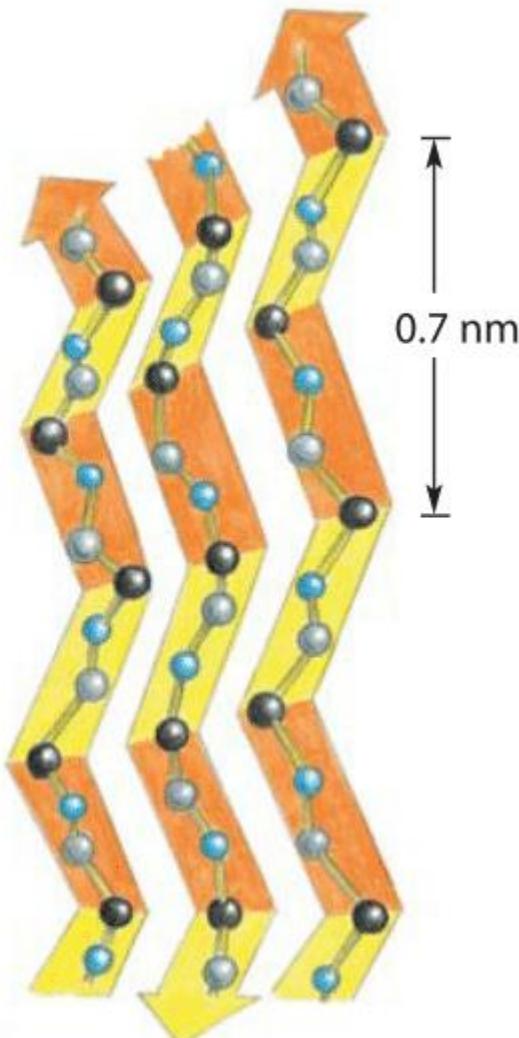
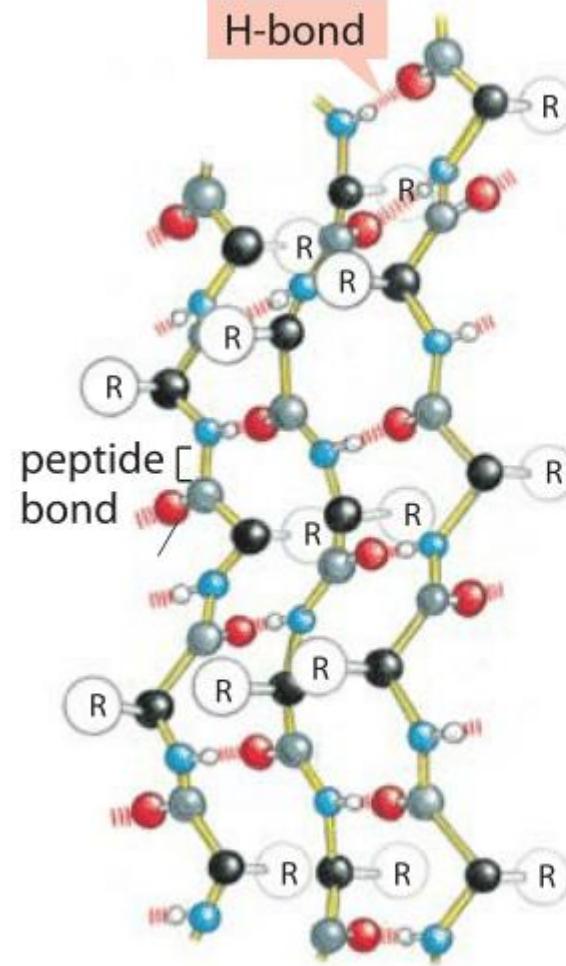
Hydrogen bonding on protein backbone

alpha helix

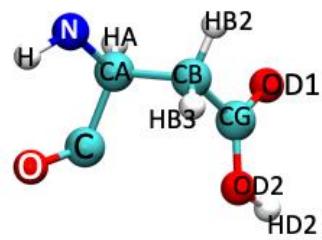


Hydrogen bonding on protein backbone

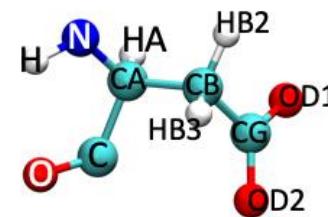
beta sheet



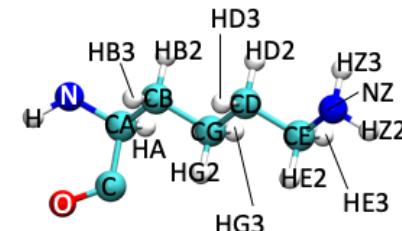
Hydrogen bonding: effect of *local* pH



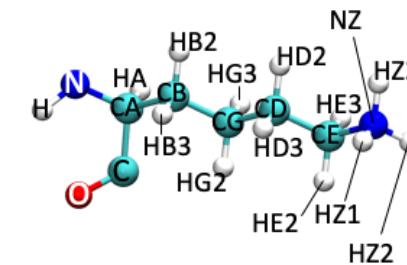
ASH, Aspartic acid*, D



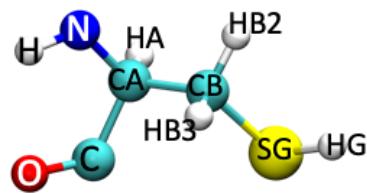
ASP, Aspartate, D



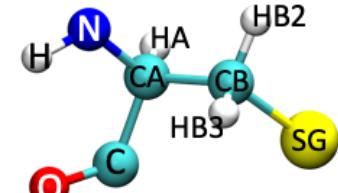
LYN, Lysine*, K



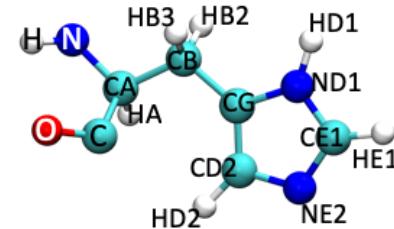
LYS, Lysine, K



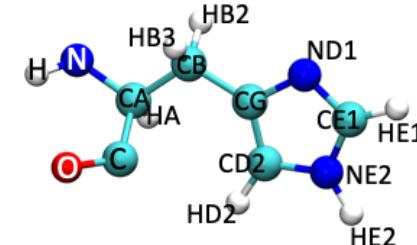
CYS, Cysteine, C



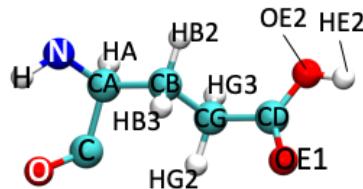
CYX, Cysteine**, C



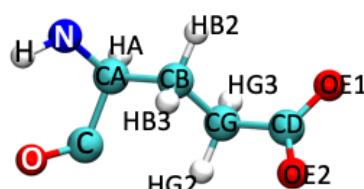
Histidine*, H



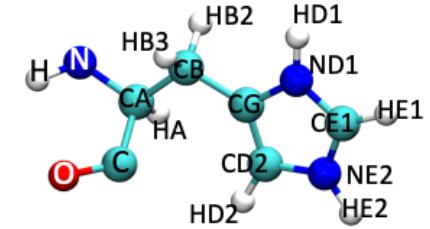
HIE, Histidine, H



GLH, Glutamic Acid*, E



GLU, Glutamate, E

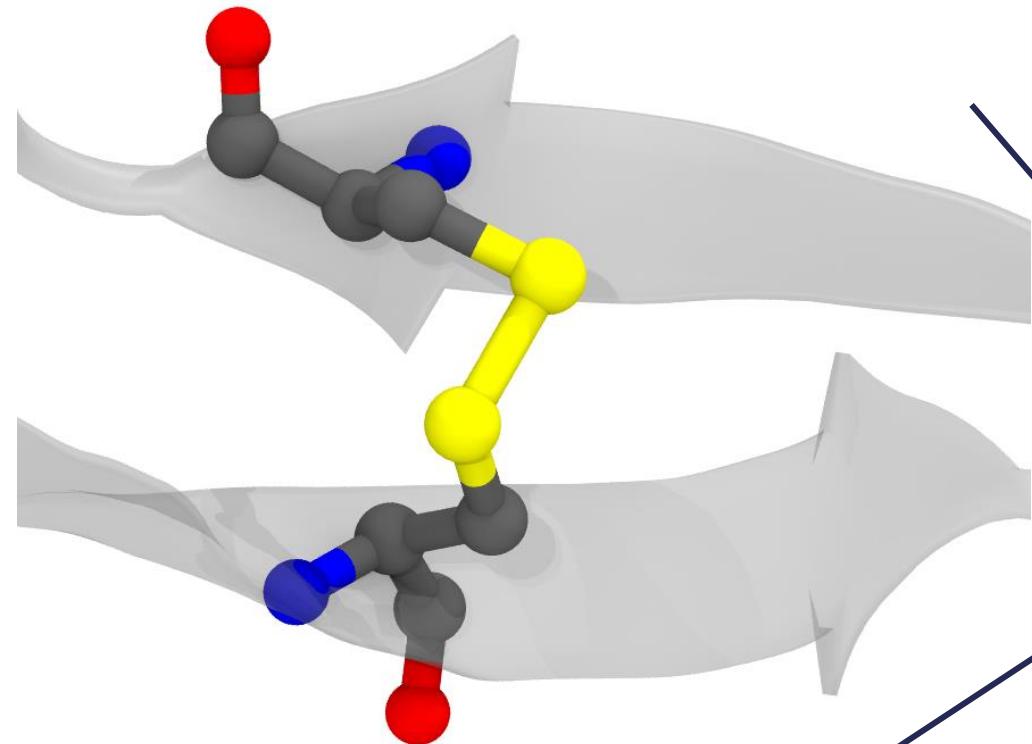


HIP, Histidine*, H

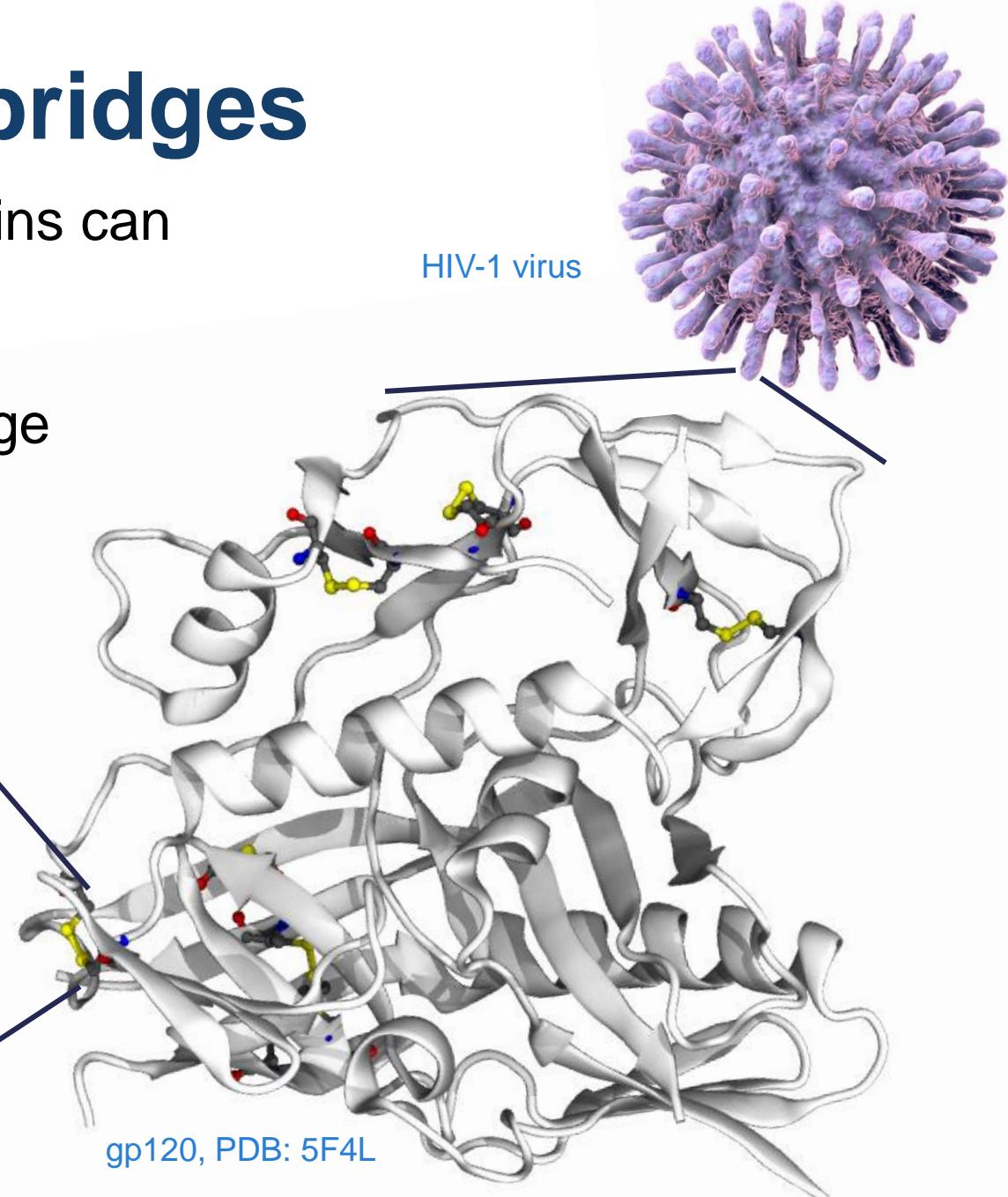
Disulfide bridges

Under *oxidising* conditions, cysteine side chains can form a *covalent* bond.

- Protein more resistant to denaturation
- Changes in conditions → structural change



HIV-1 virus



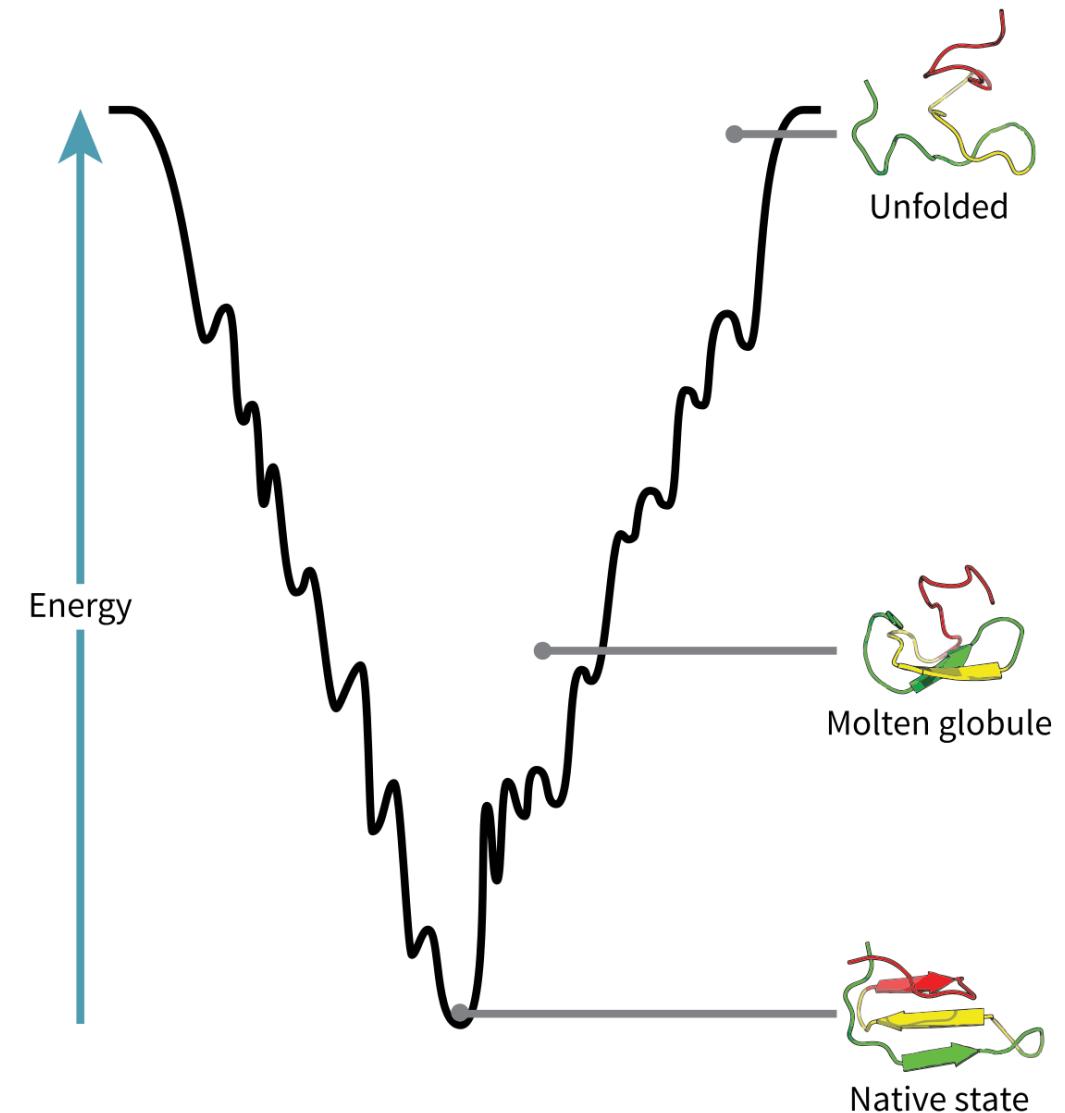
Proteins fold in low energy structures

$$\Delta G = \Delta H - T\Delta S$$

Proteins fold spontaneously, i.e. $\Delta G < 0$

$\Delta S_{\text{protein}}$ is < 0

$\Delta S = \Delta S_{\text{protein}} + \Delta S_{\text{water}} > 0$



Proteins fold in low energy structures

$$\Delta G = \Delta H - T\Delta S$$

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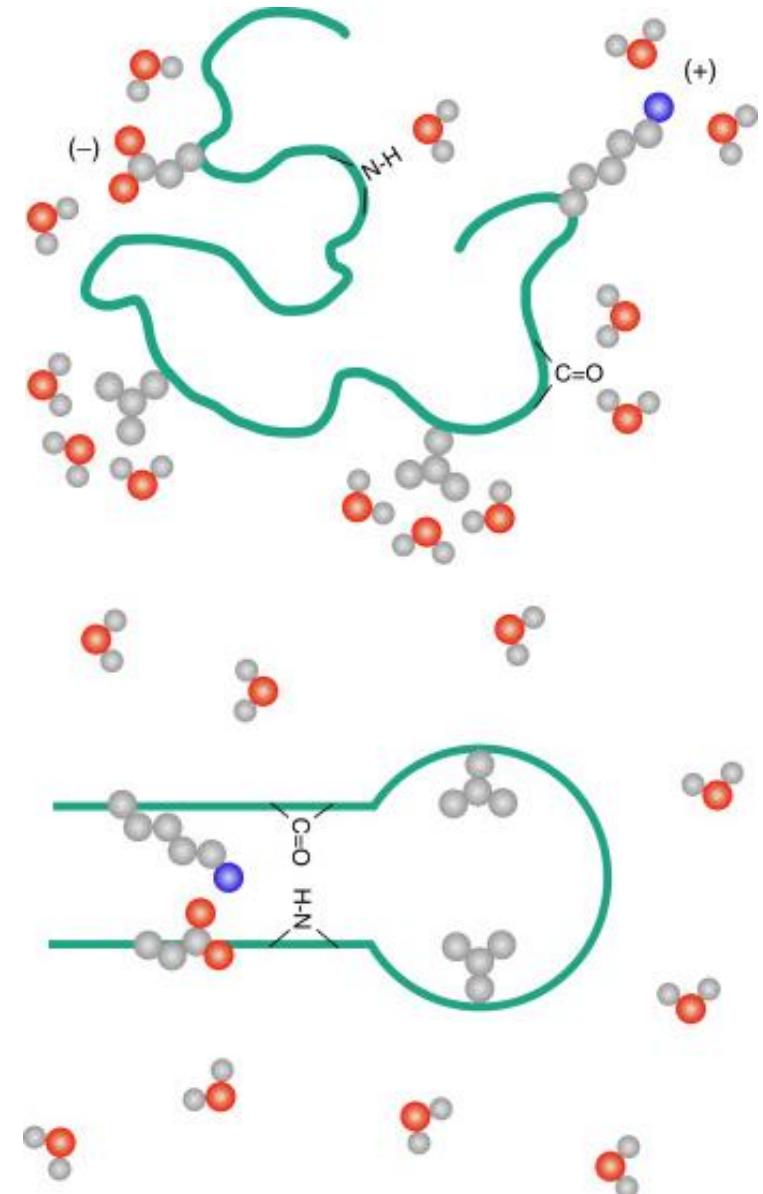
$$\Delta S = \Delta S_{\text{protein}} + \Delta S_{\text{water}} > 0$$

$H = U + PV$; at physiological conditions

$\Delta H \approx \Delta U$

ΔH is small: a folded protein forms bonds with itself, an unfolded one forms bonds with water.

Hydrophobic collapse drives folding!



[Extra] Levinthal's paradox

How can proteins find their unique fold in sub-second timescales?

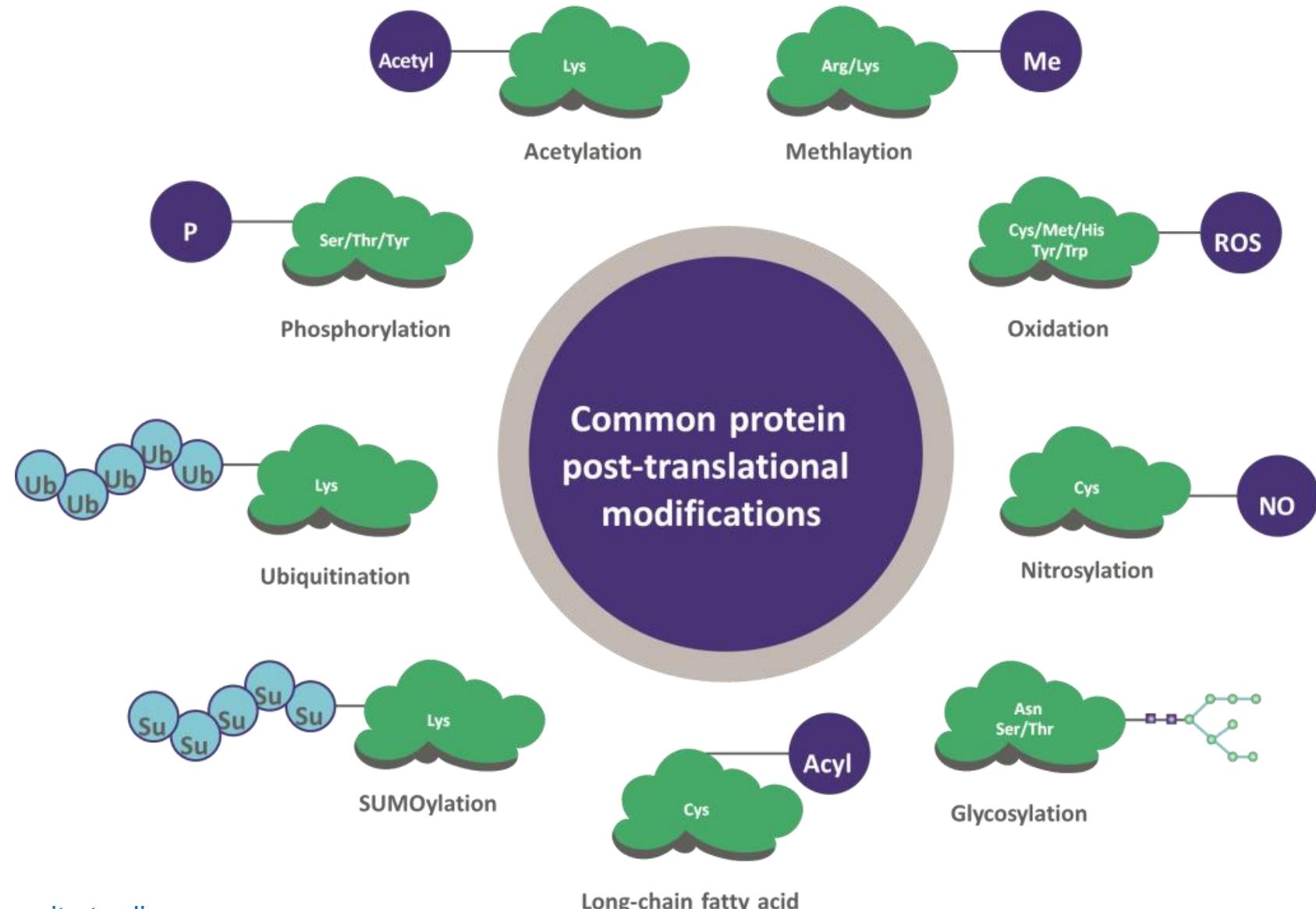
- 100 amino acids-long protein
- $2 \times 100 - 2 = 198$ backbone torsional angles
- each torsional angle has on average 3 stable positions
- **3^{198} different configurations**
- side chain rotation in the fs timescale (10^{-12} s)
- random search worst case scenario: $10^{-12} \times 3^{198} \approx 10^{-12} \times 10^{99} = 10^{87}$ s
- age of the universe ≈ 13.7 billion years = **4.32×10^{17} s**

Protein folding cannot be a random process

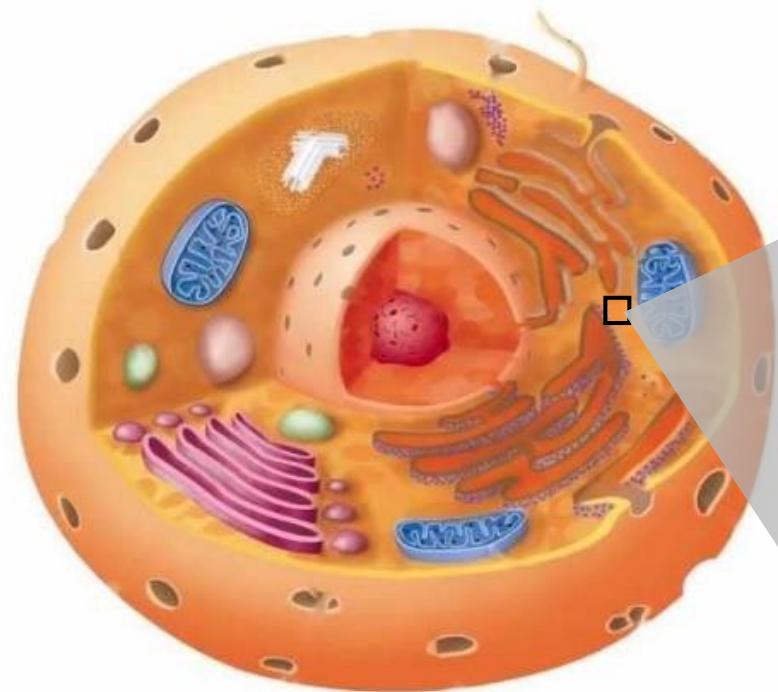
Post-translational modifications (PTMs)

Once expressed, proteins can be modified by the covalent binding of other molecules.

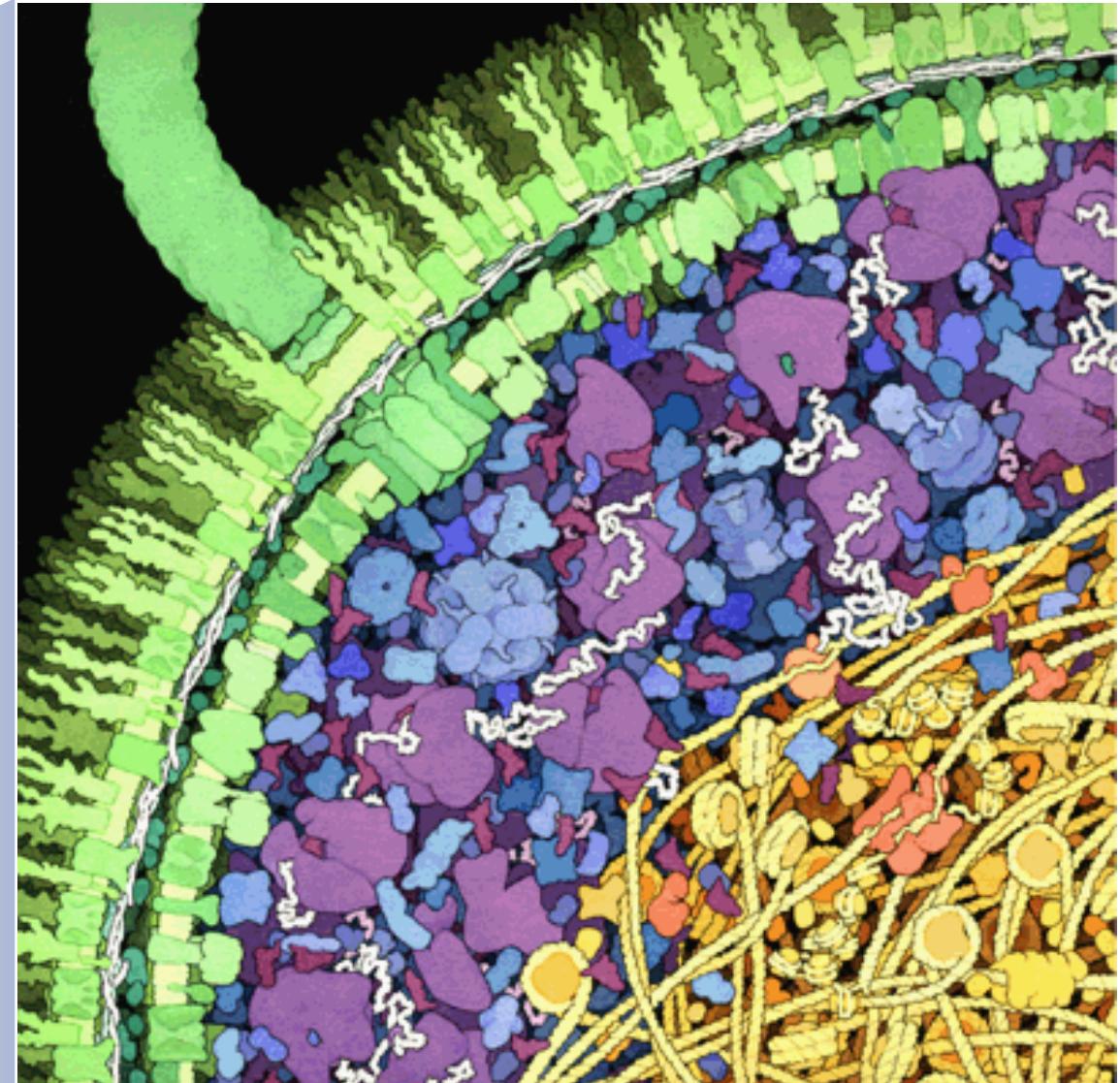
The addition/removal of most PTMs is controlled by the organism, to modulate protein function.



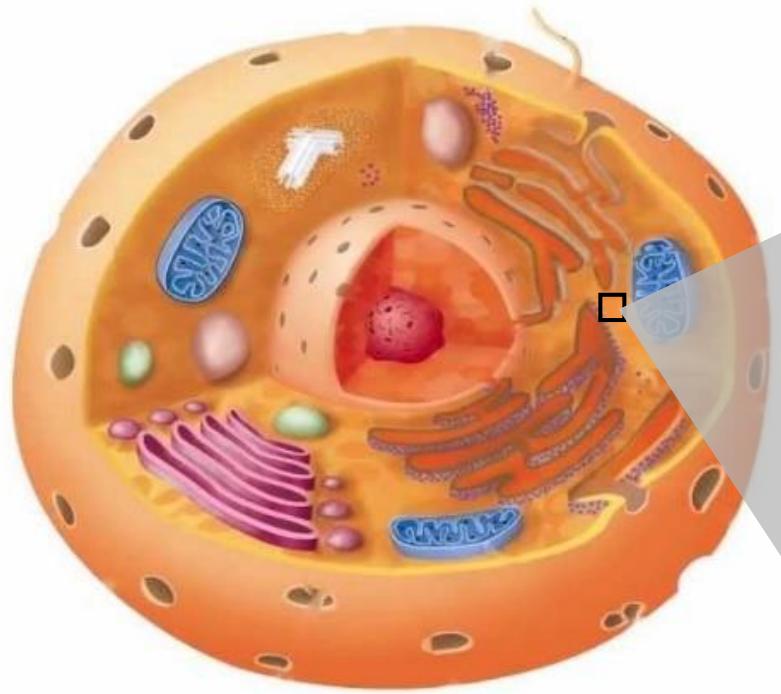
The intracellular space



Crowded environment!



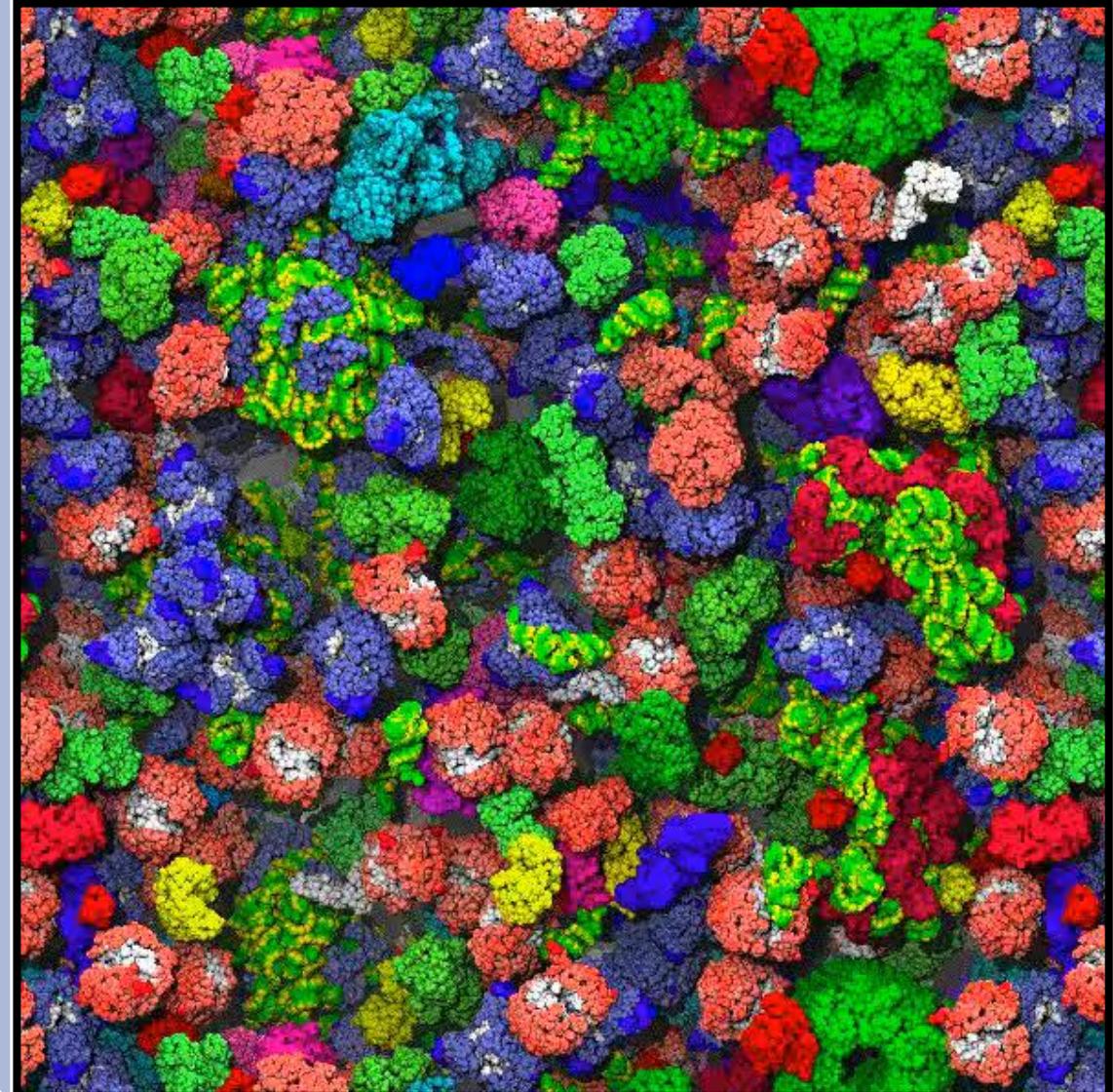
The intracellular space



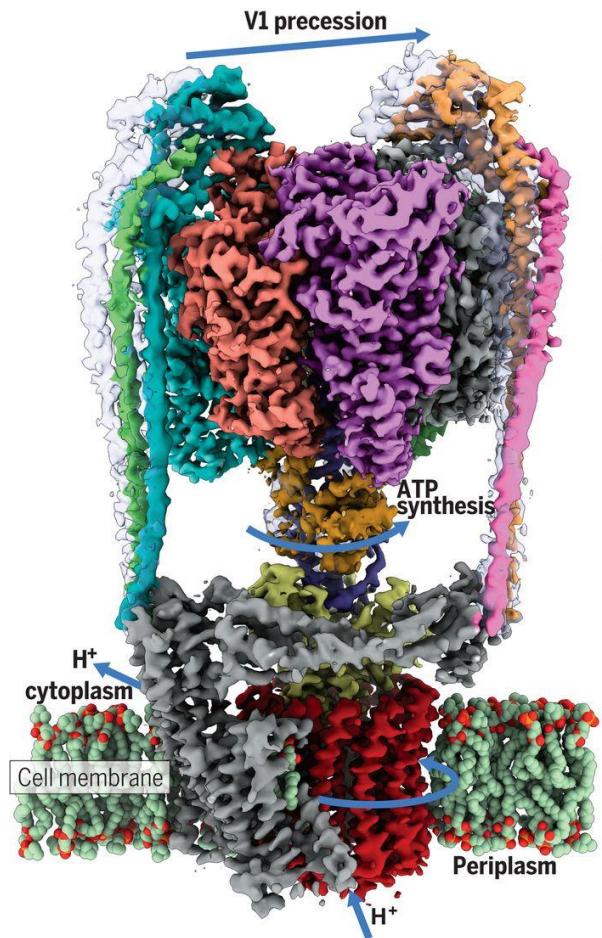
Crowded environment!

Brownian motion: proteins bump into other molecules all the time!

Most contacts are short-lived (ns- μ s)

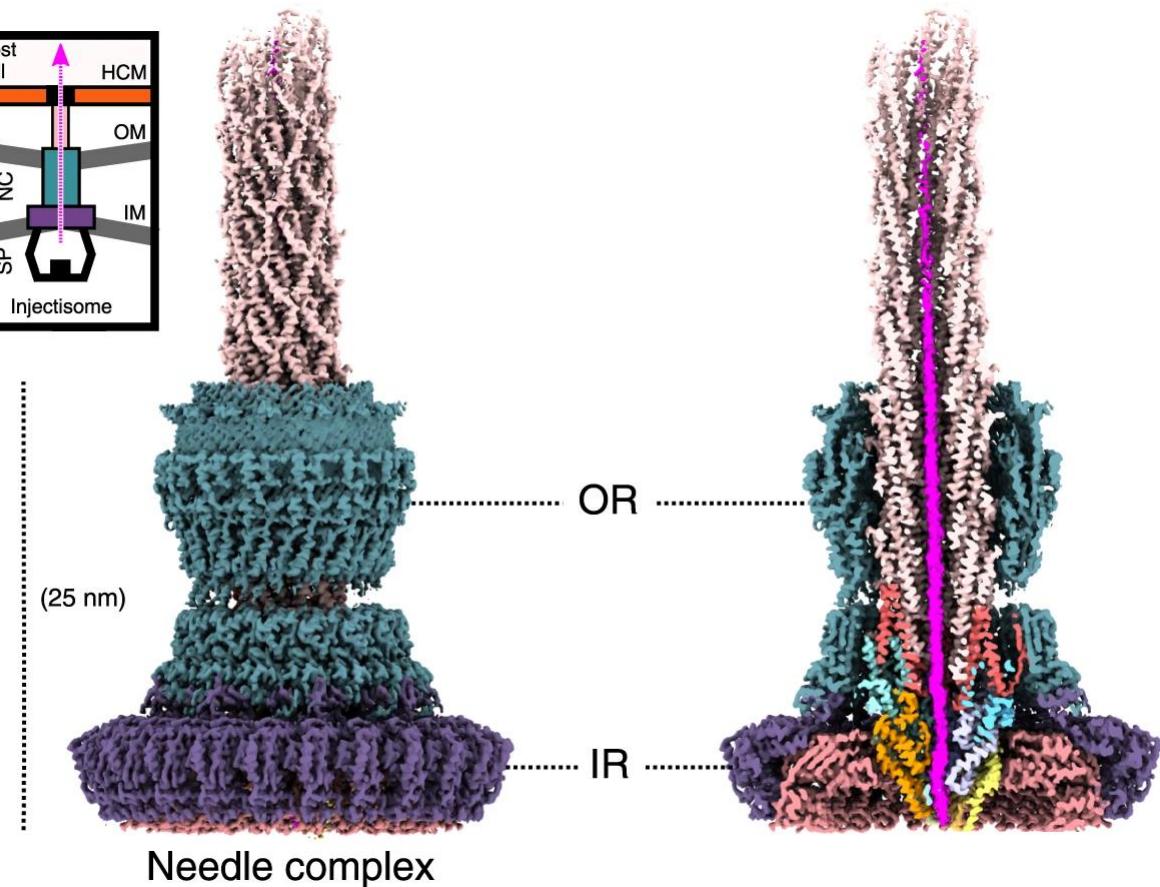
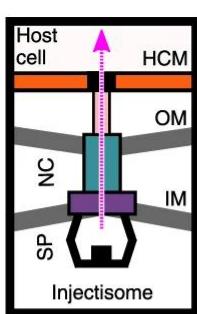


Protein quaternary structure: assembly



ATP syntase

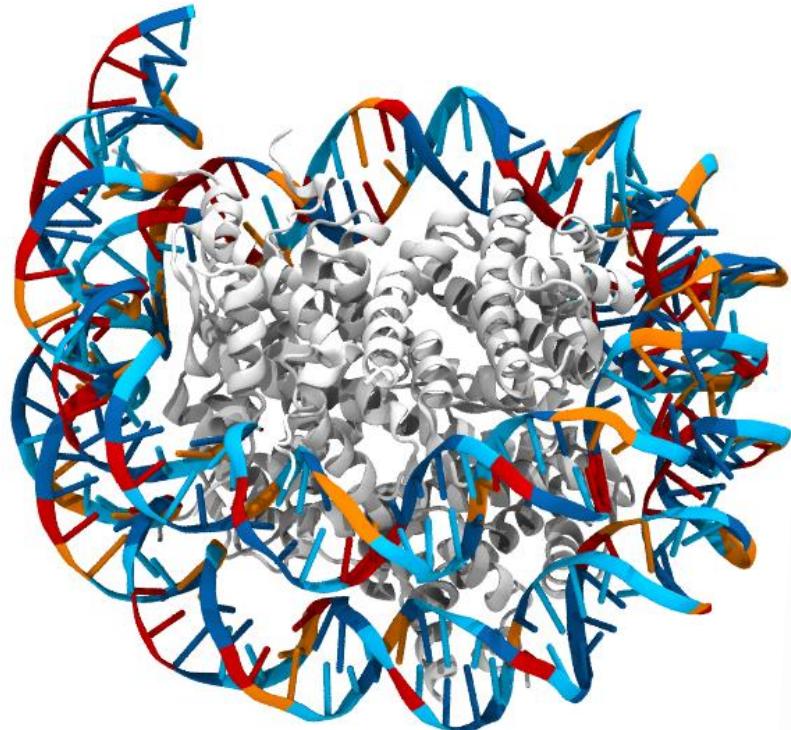
L. Zhou and L. A. Sazanov, *Structure and conformational plasticity of the intact Thermus thermophilus V/A-type ATPase*, *Science*, 2019



Injectisome

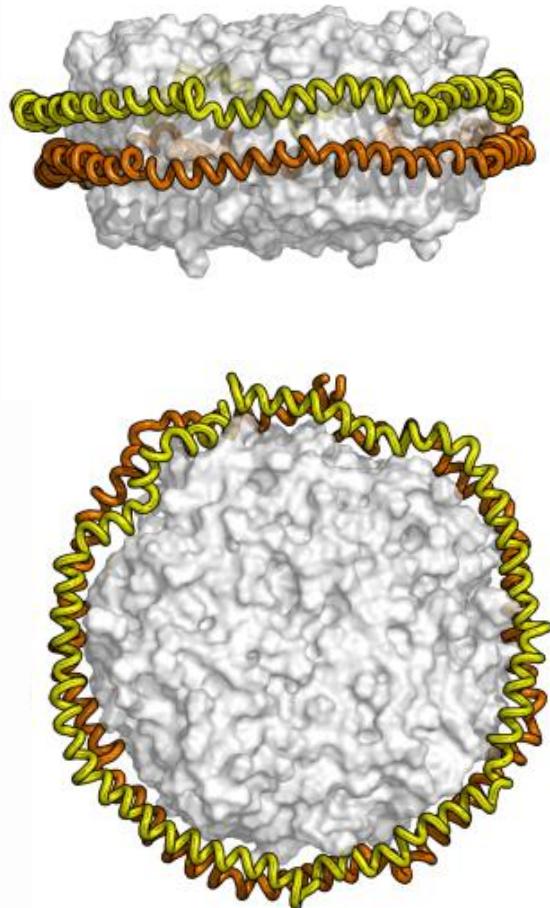
S. Miletic et al., *Substrate-engaged type III secretion system structures reveal gating mechanism for unfolded protein translocation*, *Nature Comms*, 2021

The structure determines the function



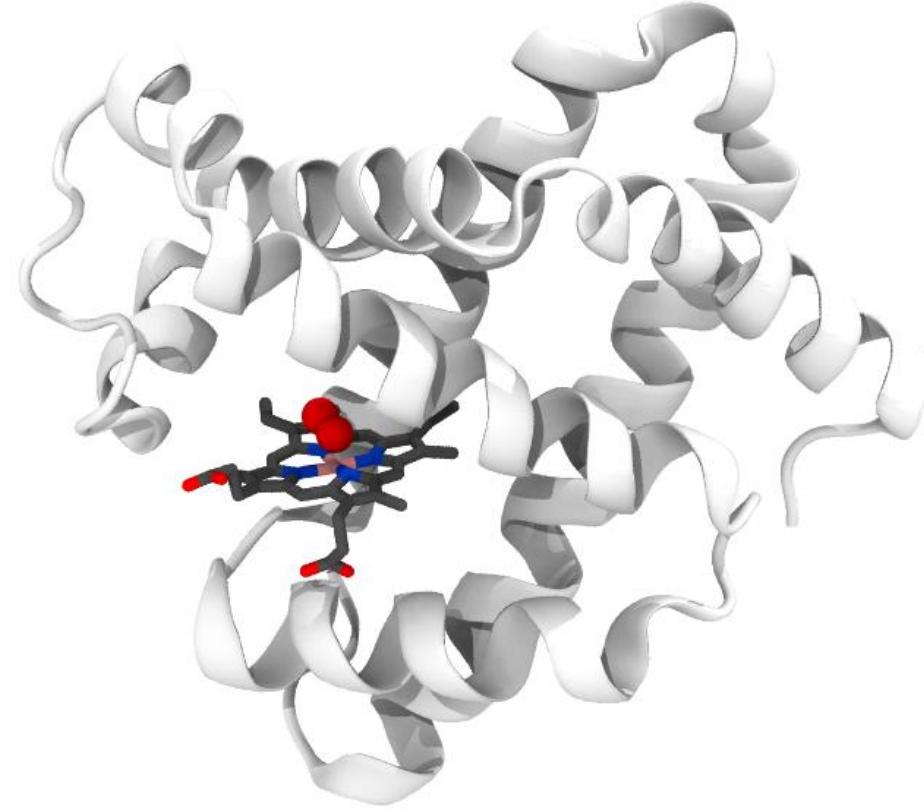
Nucleosome (PDB: 5CPI)

K. Luger et al., *Crystal Structure of the nucleosome core particle at 2.8 Å resolution*, *Nature*, 1997



Lipoprotein (PDB: 1AV1)

D.W. Bohrani et al., *Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation*, *PNAS*, 1997



Myoglobin (PDB: 1MBO)

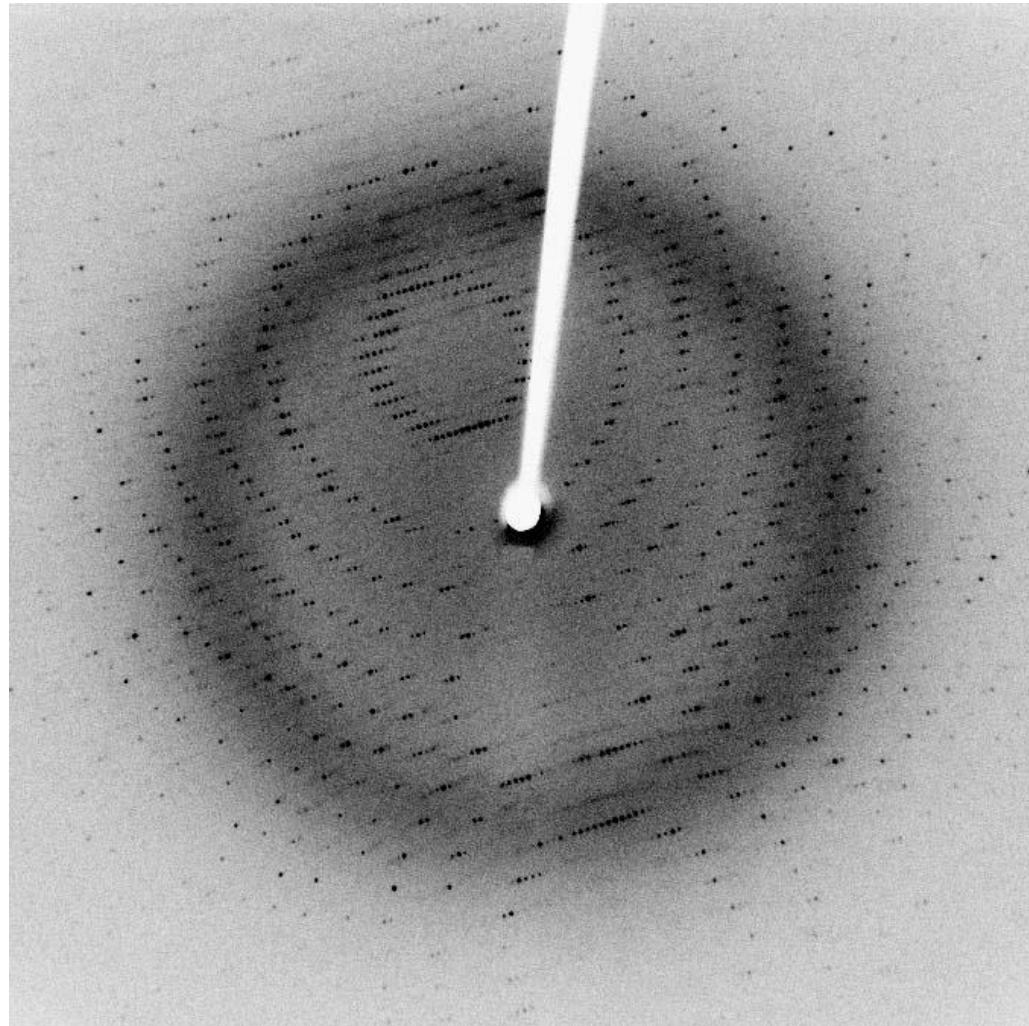
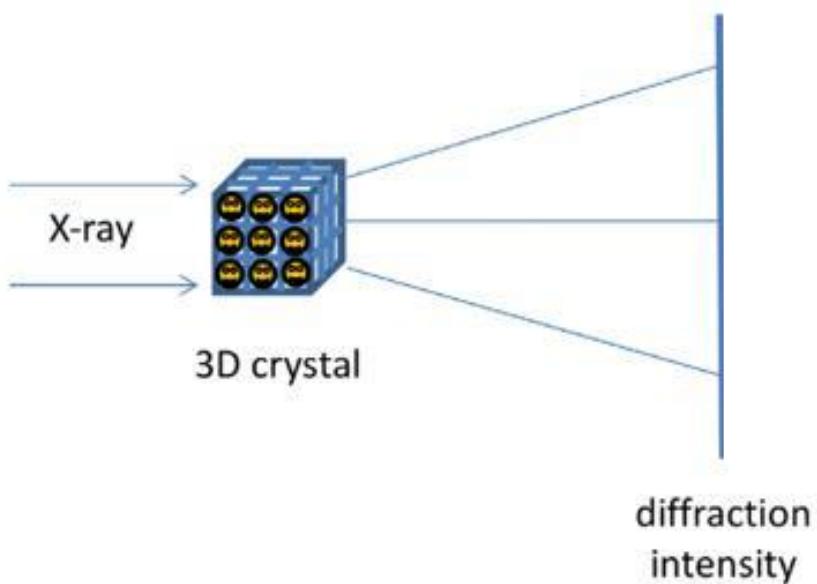
J.C. Kendrew et al., *A three-dimensional Model of the Myoglobin Molecule obtained by X-Ray Analysis*, *Nature*, 1958

Proteins, illness, and drug design

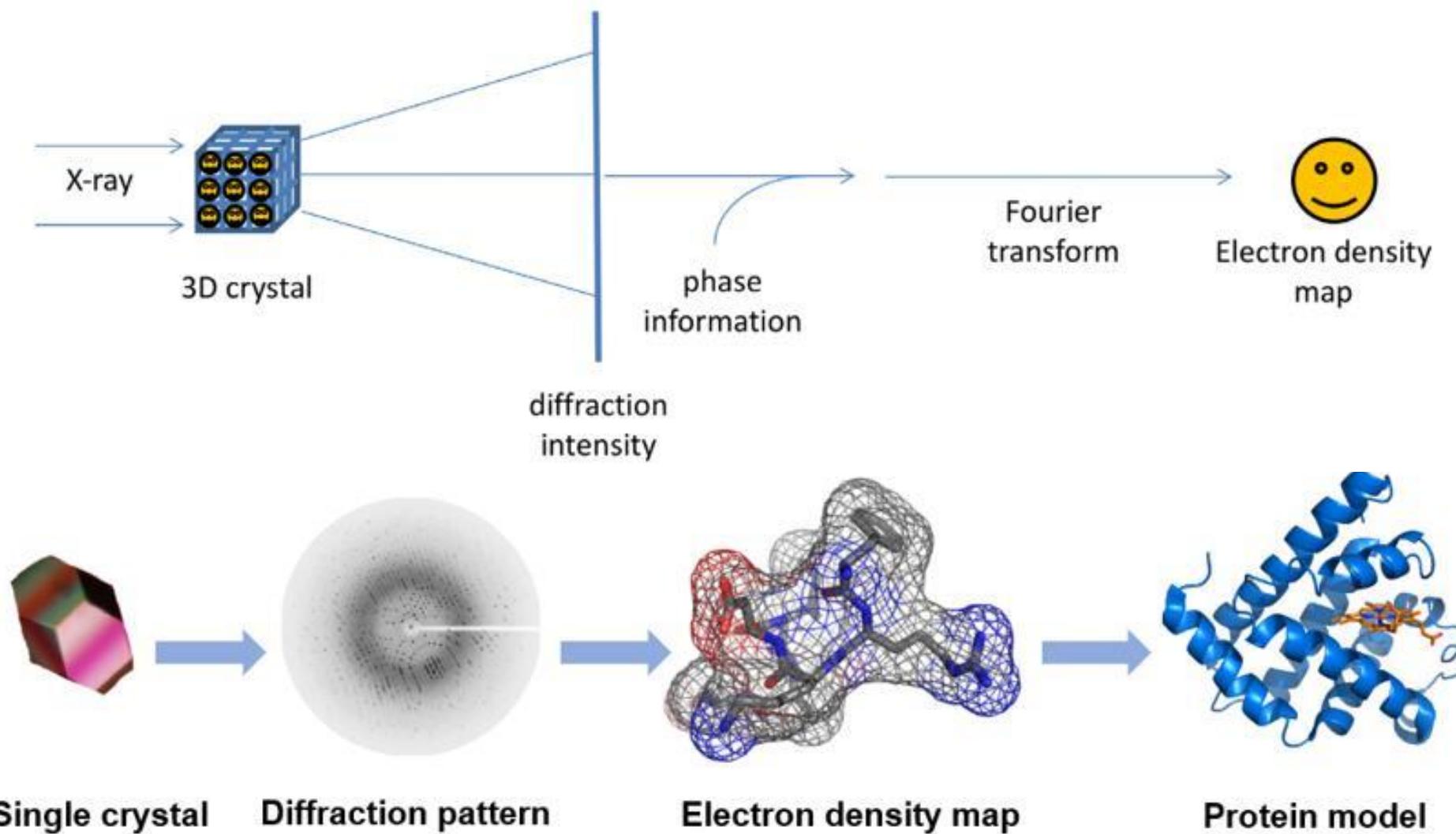
- **Proteins in diseases** (e.g., COVID-19, salmonella, flu, ...)
 - pathogen's own metabolism/structure
 - pathogen's weapon
- **Proteins in disorders** (e.g., Cancer, Alzheimer's, ...)
 - own protein misfolds
 - own protein folds, but has different dynamics
- **Drug**
 - Small molecule designed to specifically bind to a protein, so as to affect its function

Part 2: structure determination

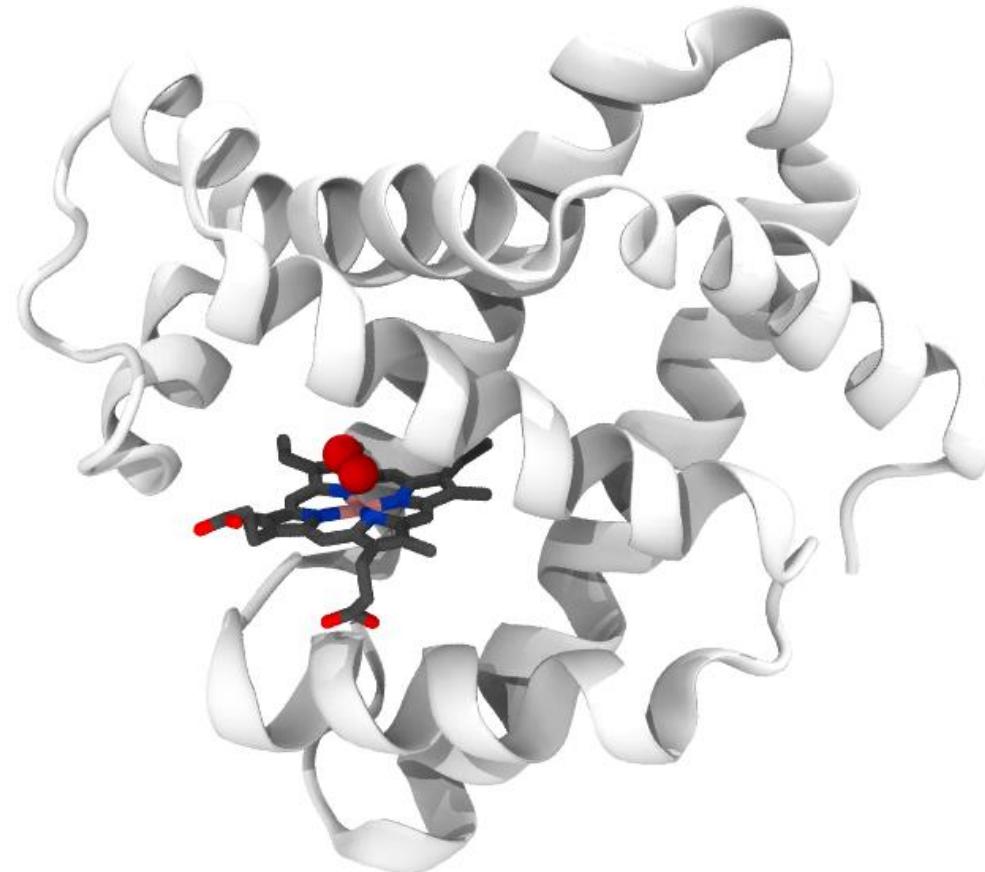
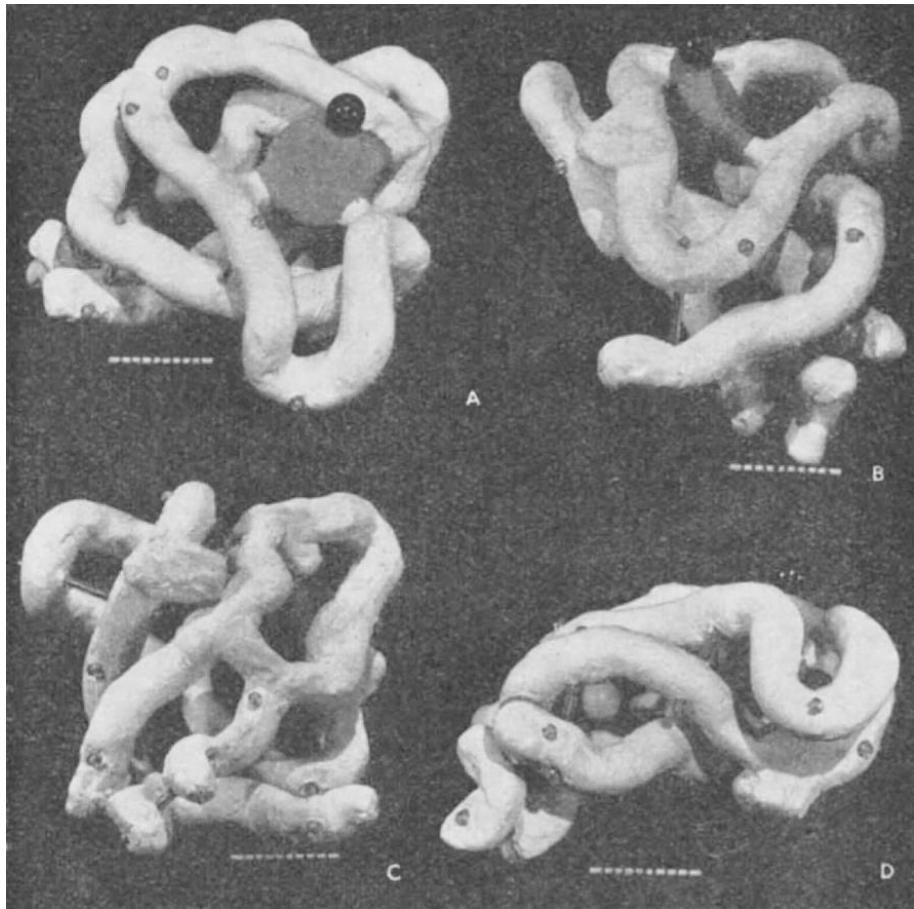
Structure determination: X-ray crystallography



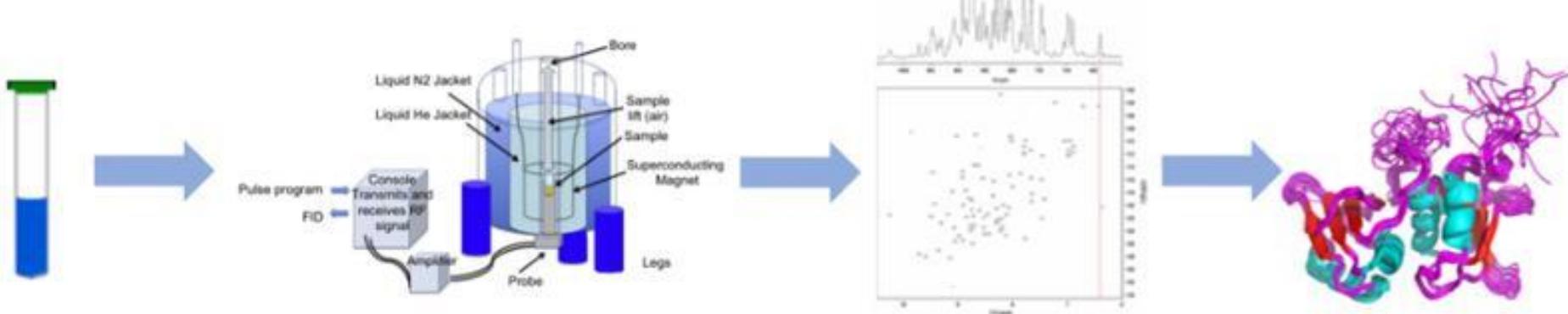
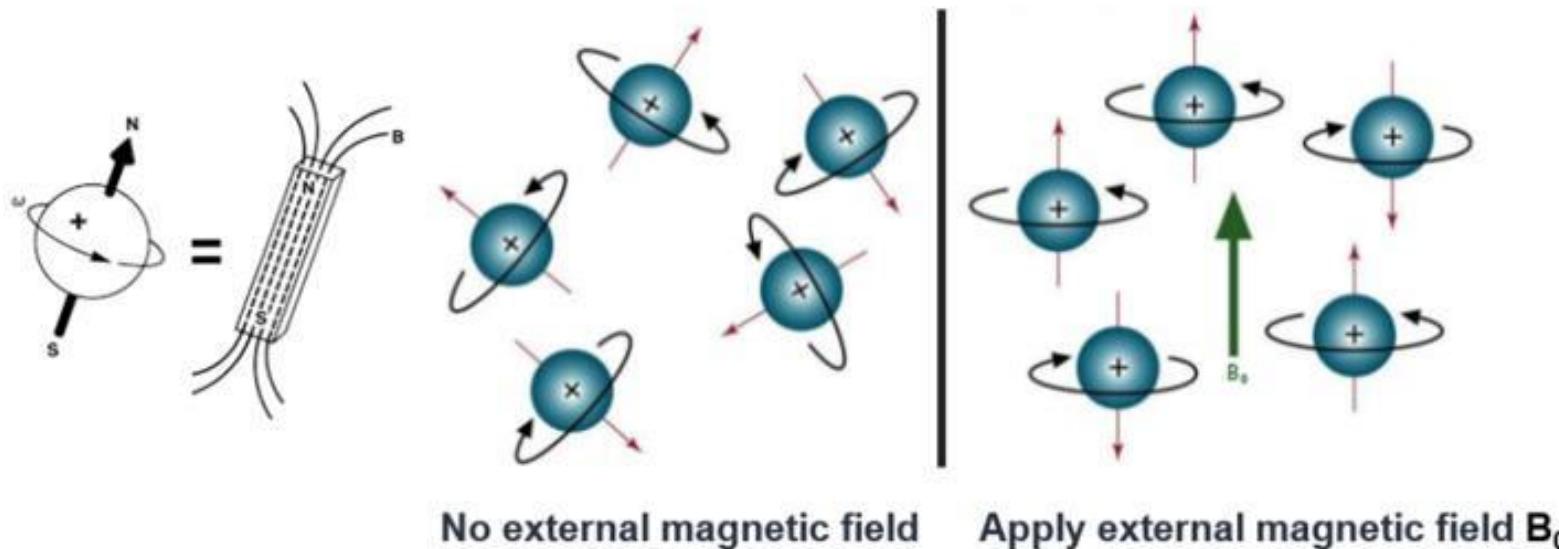
Structure determination: X-ray crystallography



Structure determination: X-ray crystallography



Structure determination: Nuclear Magnetic Resonance (NMR)



Sample preparation

Data acquisition

Spectral processing

Structural analysis

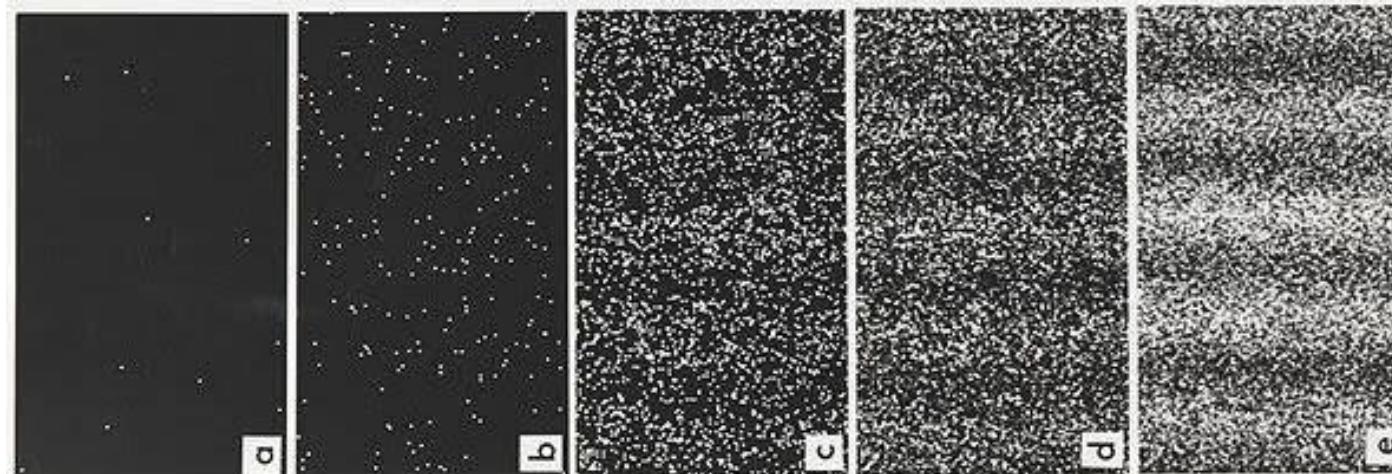
[Extra] Structure determination: Electron Microscopy (EM)

- Particles as «waves that transfers energy and momentum»

$$\lambda = \frac{h}{p}$$

λ : wavelength
 p : momentum
 h : Planck constant

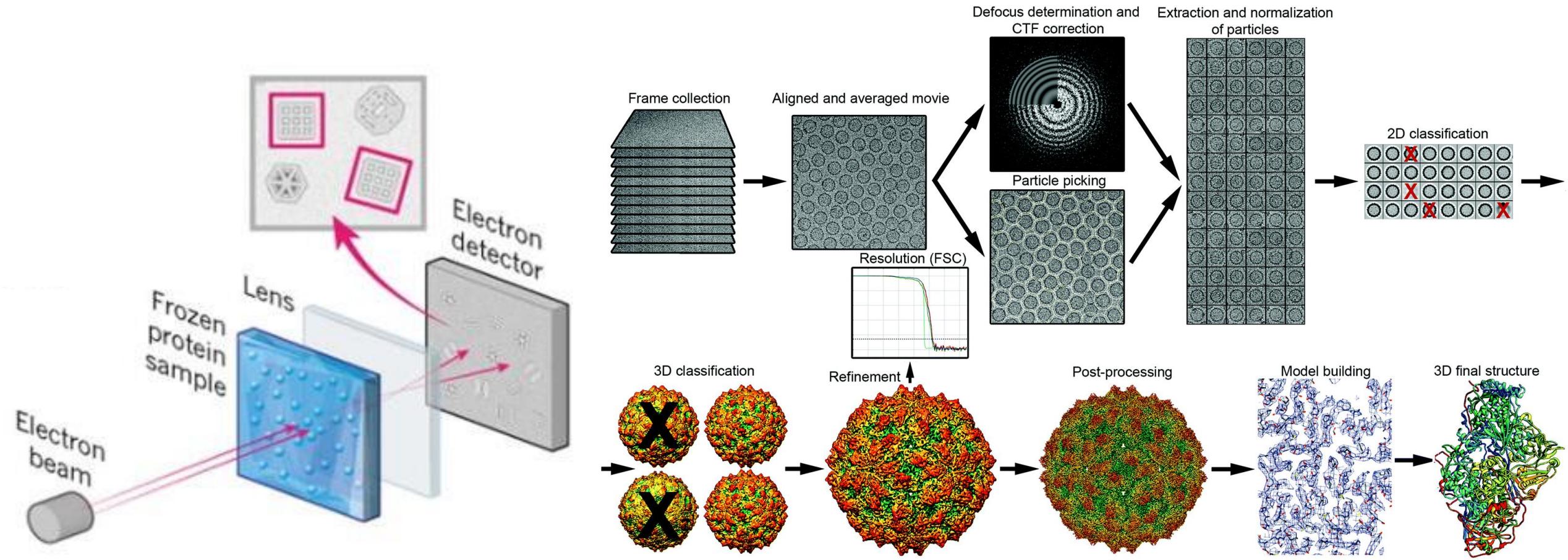
- Davisson–Germer experiment: electrons diffract too!



Louis De Broglie
1892-1987

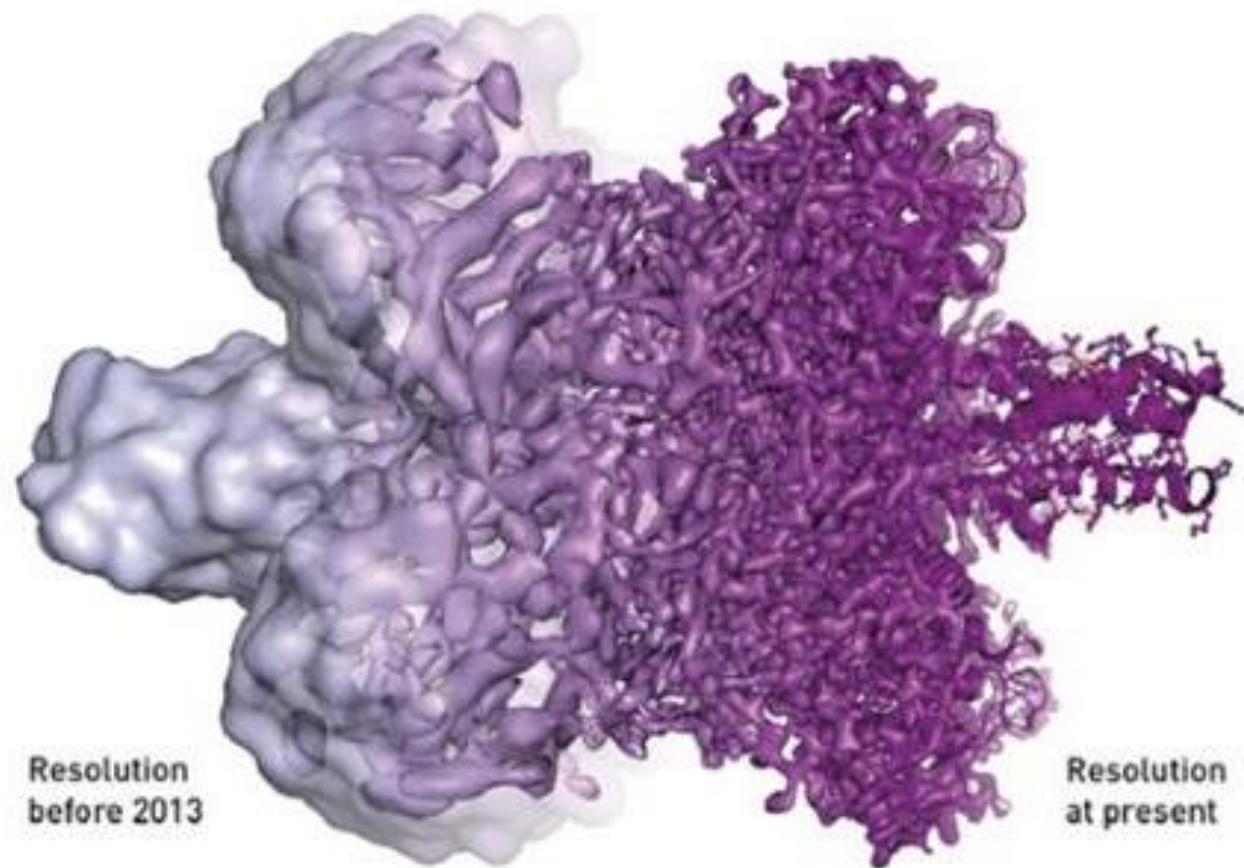
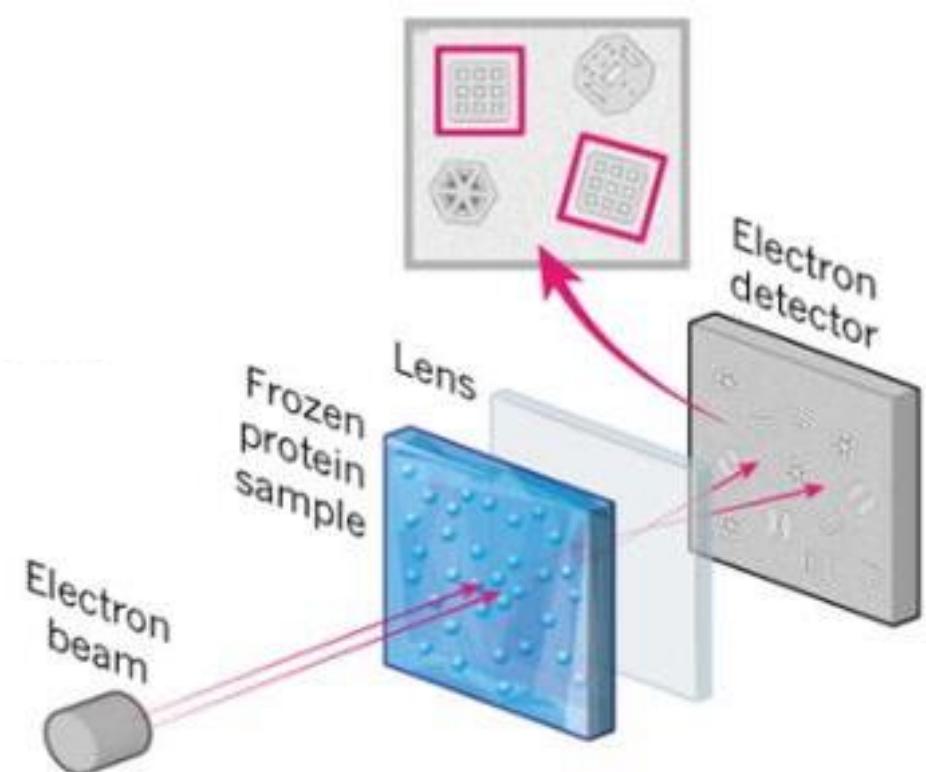
A. Tonomura et al., *Demonstration of single-electron buildup of an interference pattern*, *American Journal of Physics*, 1989

Structure determination: Electron Microscopy (EM)



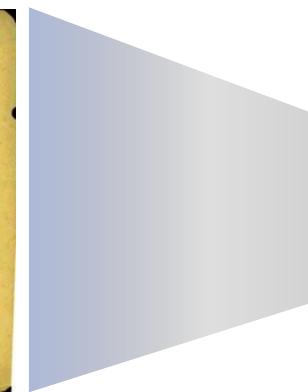
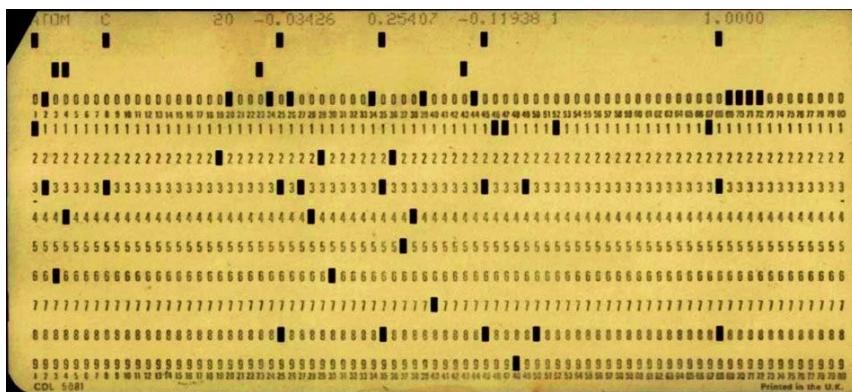
Structure determination: Electron Microscopy (EM)

Resolution Revolution



The Protein Data Bank (PDB)

- Molecular structures are deposited in the Protein Data Bank (PDB)
 - 1971: foundation of PDB at Brookhaven National Laboratory



The Protein Data Bank (PDB)

- Molecular structures are deposited in the Protein Data Bank (PDB)
 - 1971: foundation of PDB at Brookhaven National Laboratory
 - 2003: wwPDB founded
 - now with four deposition centres



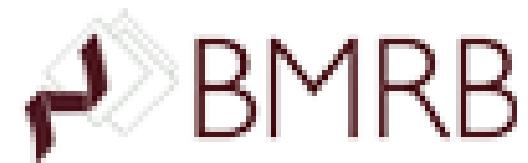
www.rcsb.org



www.ebi.ac.uk/pdbe



www.pdbj.org



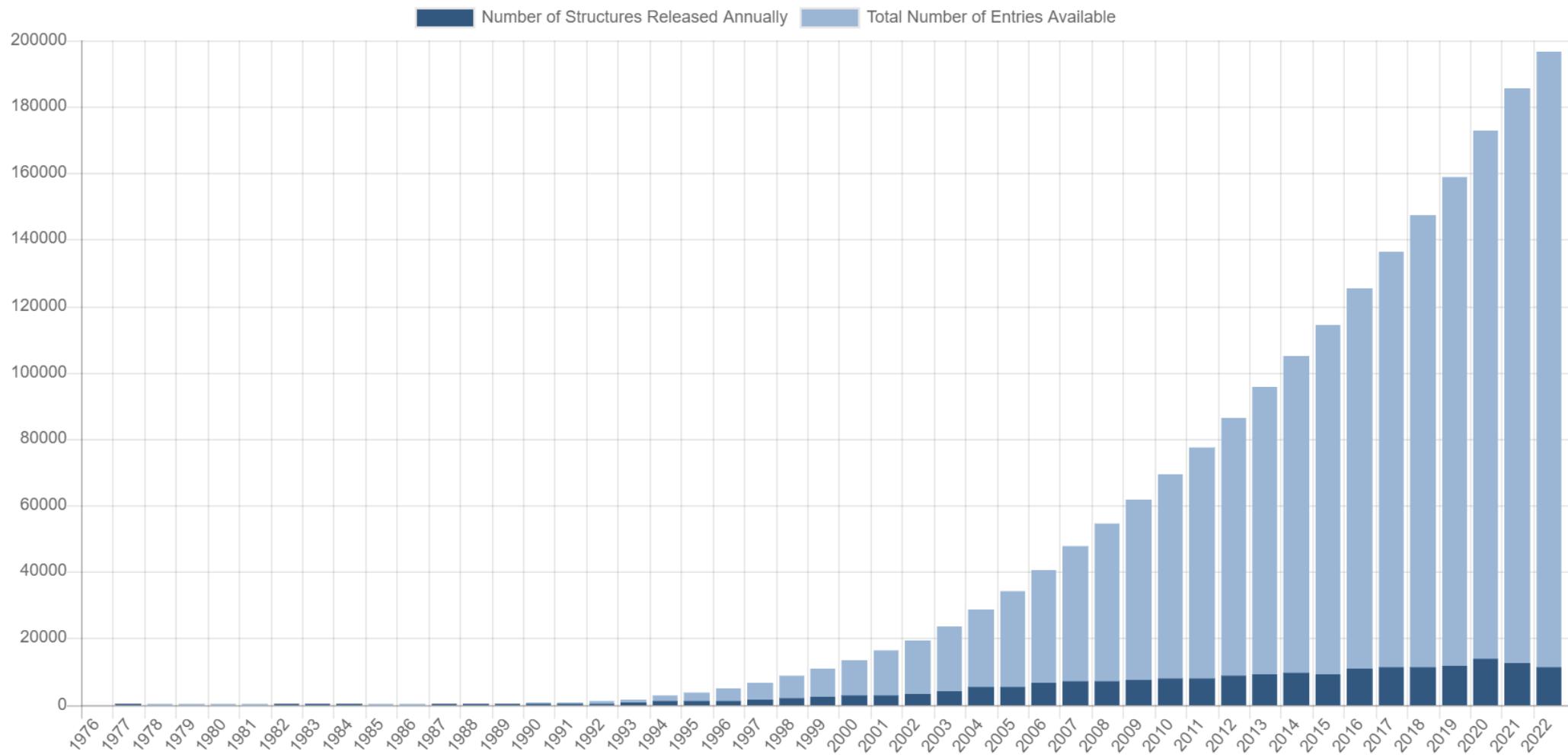
www.bmrb.io

- Each molecule assigned a unique **4-characters code** (e.g., 1MBO, 1AV1, ...)

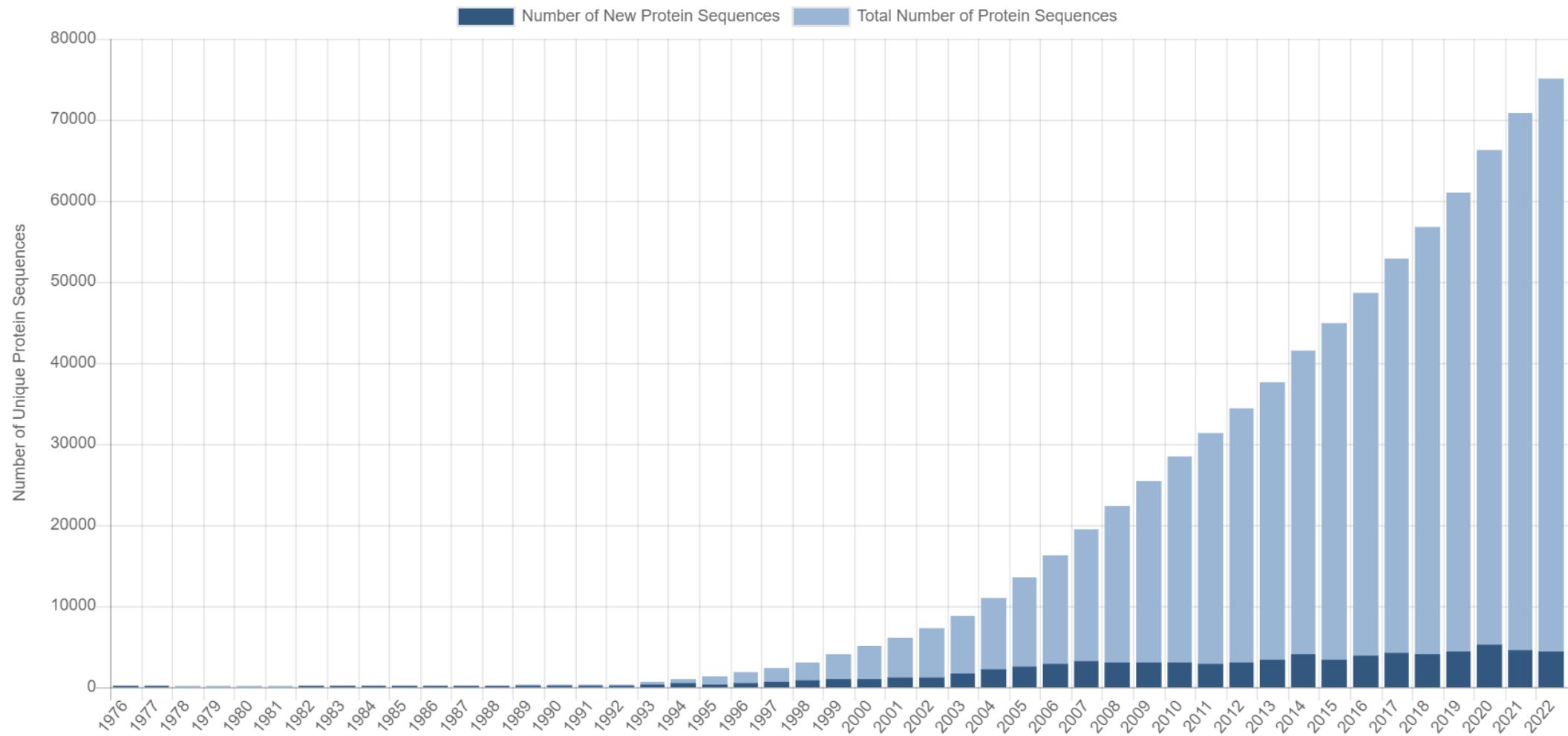
H.Berman, K.Henrick and H. Nakamura, *Announcing the worldwide Protein Data Bank*, *Nature Structural & Molecular Biology*, 2003

wwPDB consortium , *Protein Data Bank: the single global archive for 3D macromolecular structure data*, *Nucleic Acids Research*, 2019

The Protein Data Bank (PDB)



The Protein Data Bank (PDB)



known protein **structures**: ~80'000 (*PDB, 90% identity*)

known protein **sequences**: ~190'000'000 (*UNIPROT*)

Protein fold prediction

Protein Sequence

SQETRKKCTEMKKFKNCEVRCDESNCVRCSDTKYTL



prediction

Structure



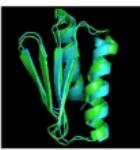
CASP, since 1994 biennial competition on protein fold prediction: predictioncenter.org

Protein fold prediction

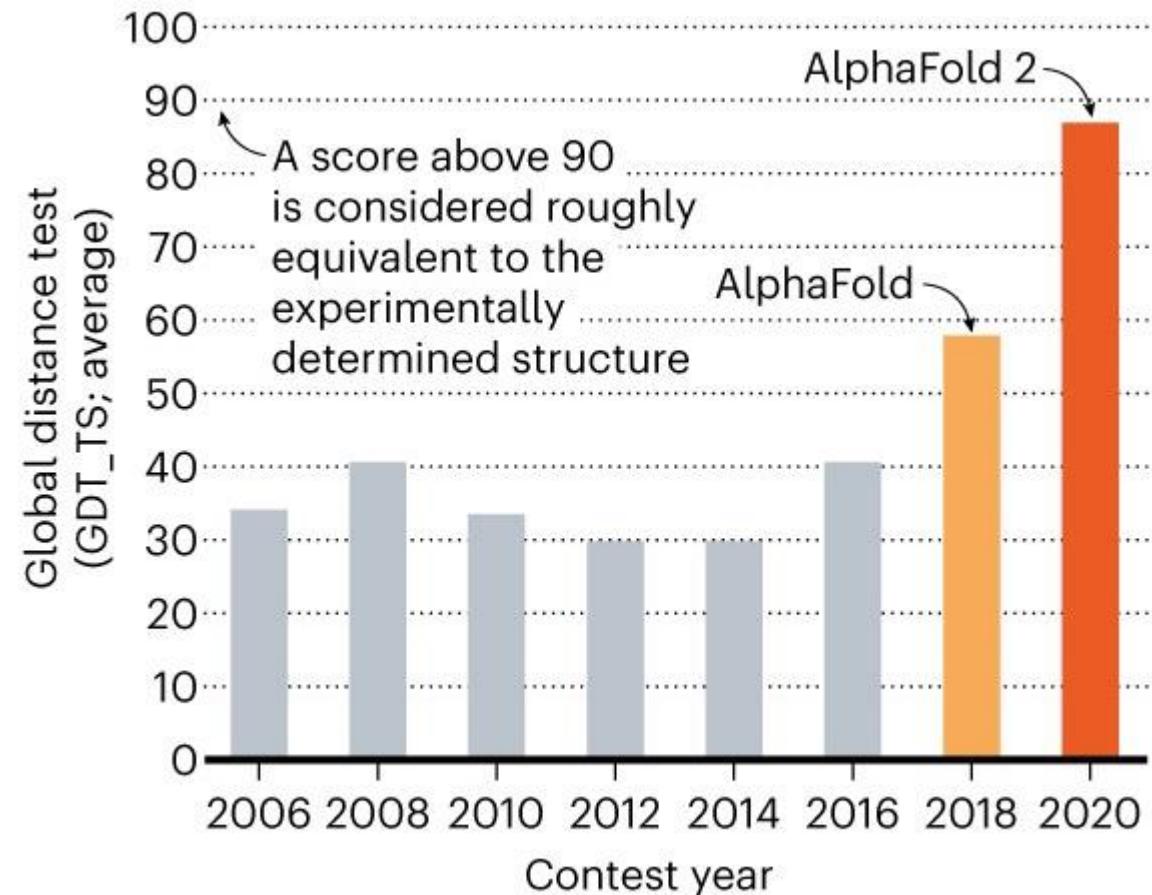
Protein Sequence

SQETRKKCTEMKKFKNCEVRCDESNHCVRCSDTKYTLC

prediction



Structure

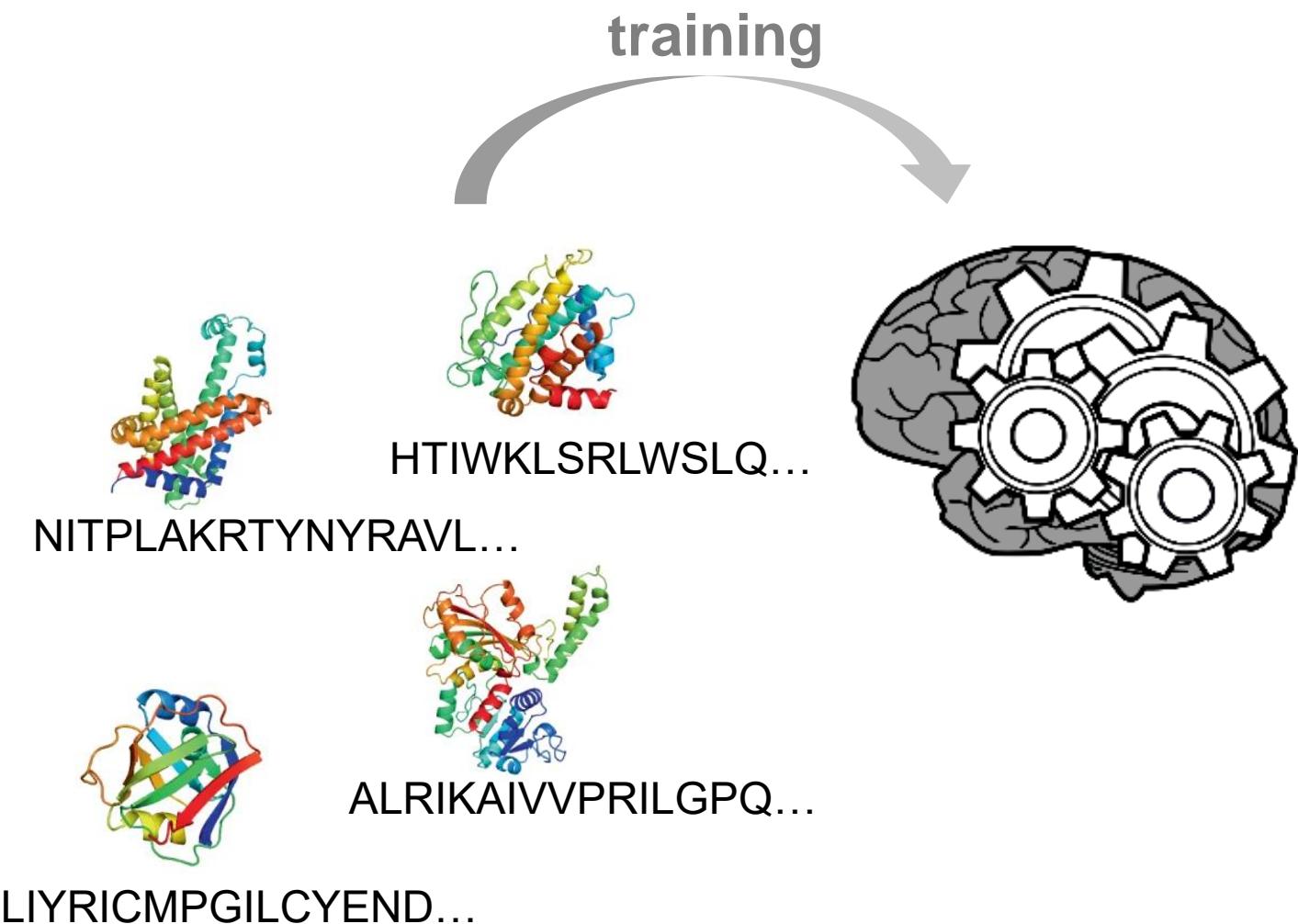


A.W. Senior et al., *Improved protein structure prediction using potentials from deep learning*, *Nature*, 2020

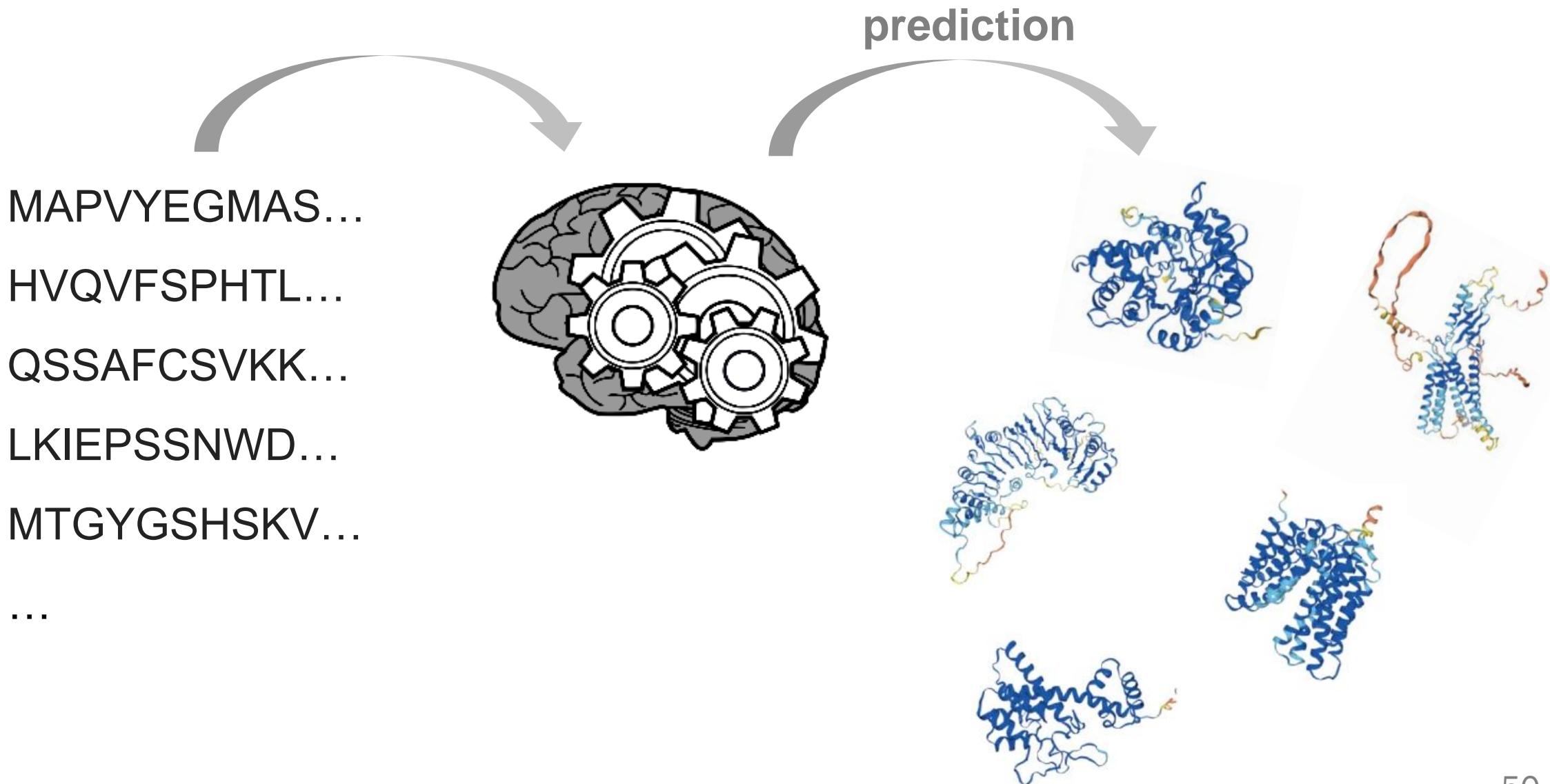
J. Jumper et al., *Highly accurate structure prediction with AlphaFold*, *Nature*, 2021

E. Callaway, 'It will change everything': DeepMind's AI makes gigantic leap in solving protein structures, *Nature*, 2021

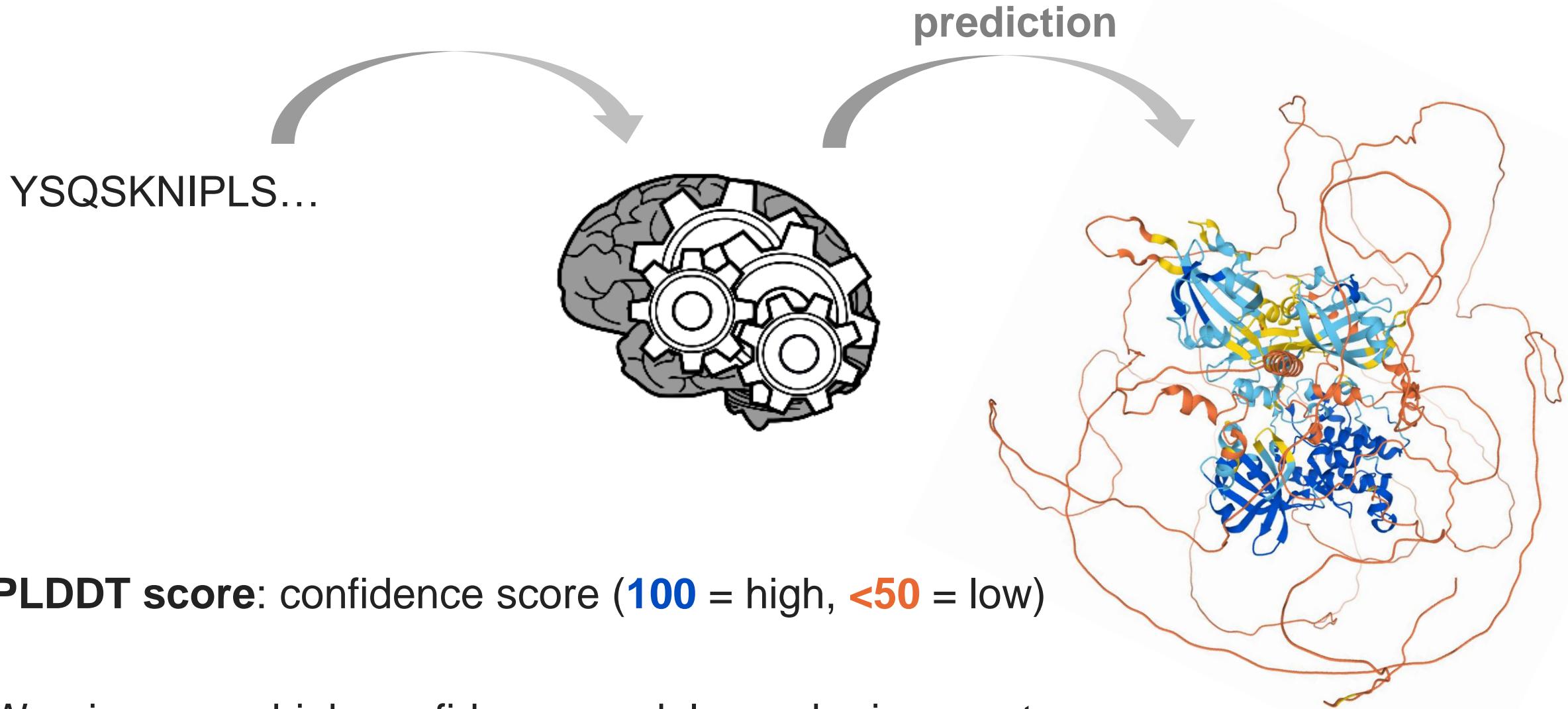
Protein fold prediction: AlphaFold



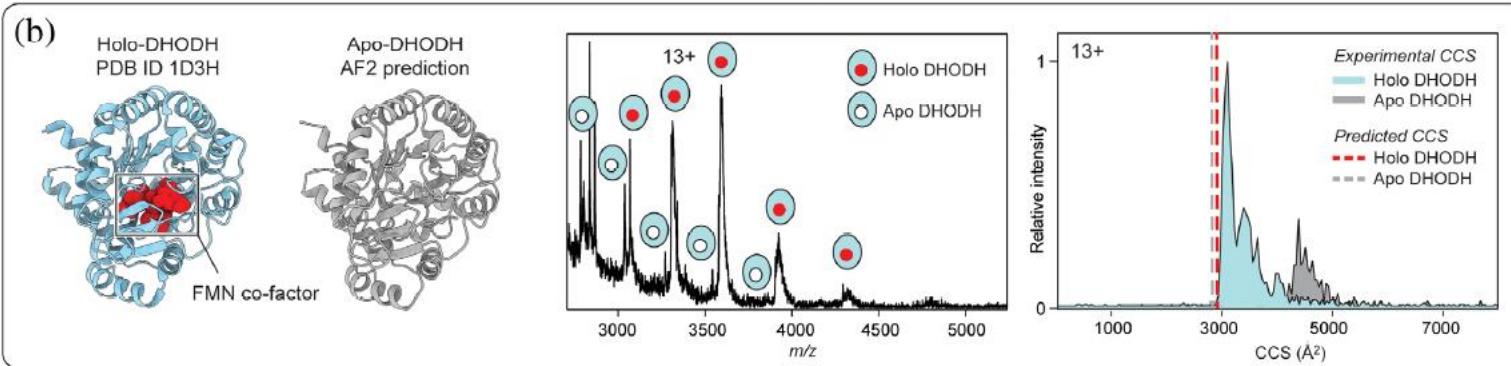
Protein fold prediction: AlphaFold



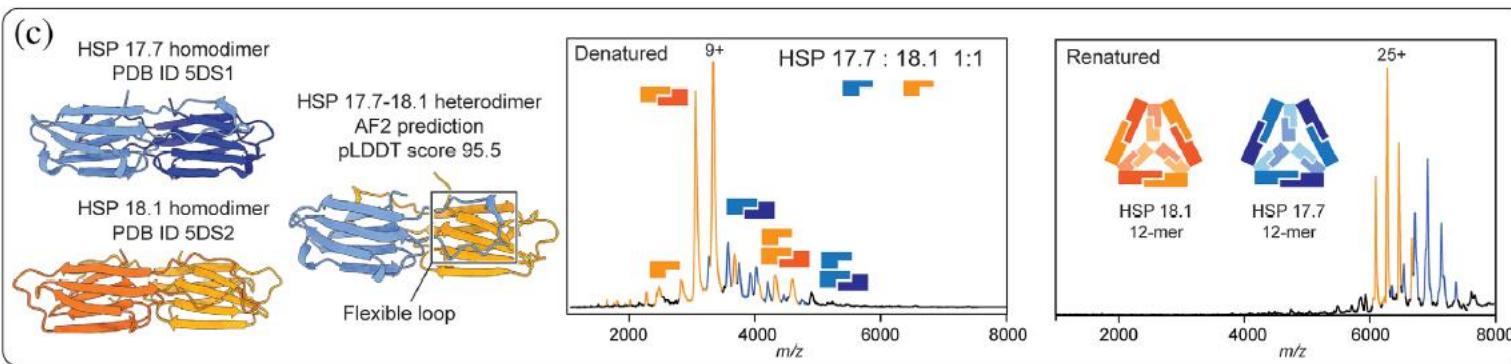
Protein fold prediction: AlphaFold



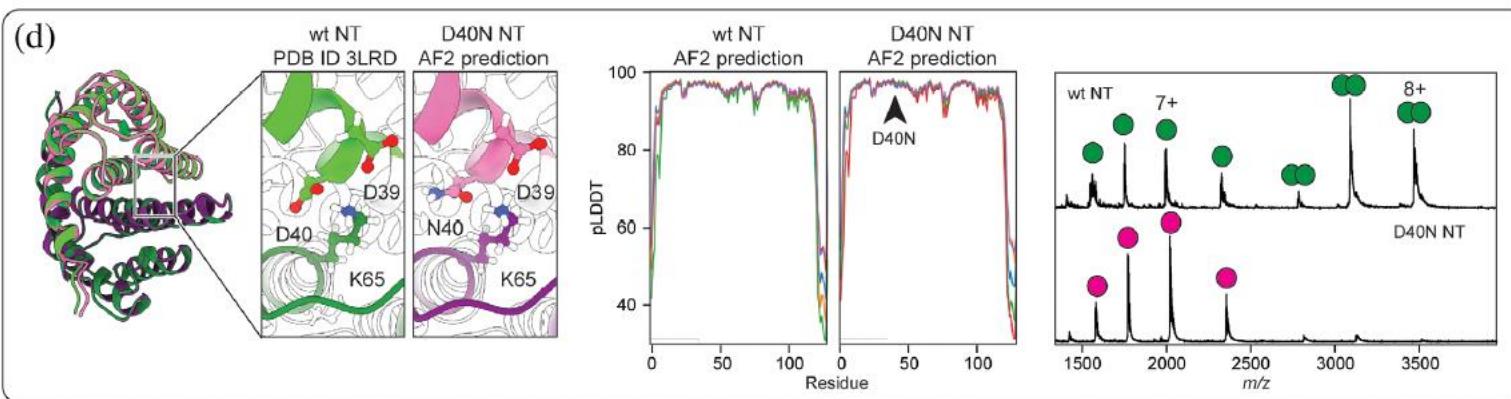
Protein fold prediction: warning!



Apo protein predicted folded like holo state, but it should be unfolded



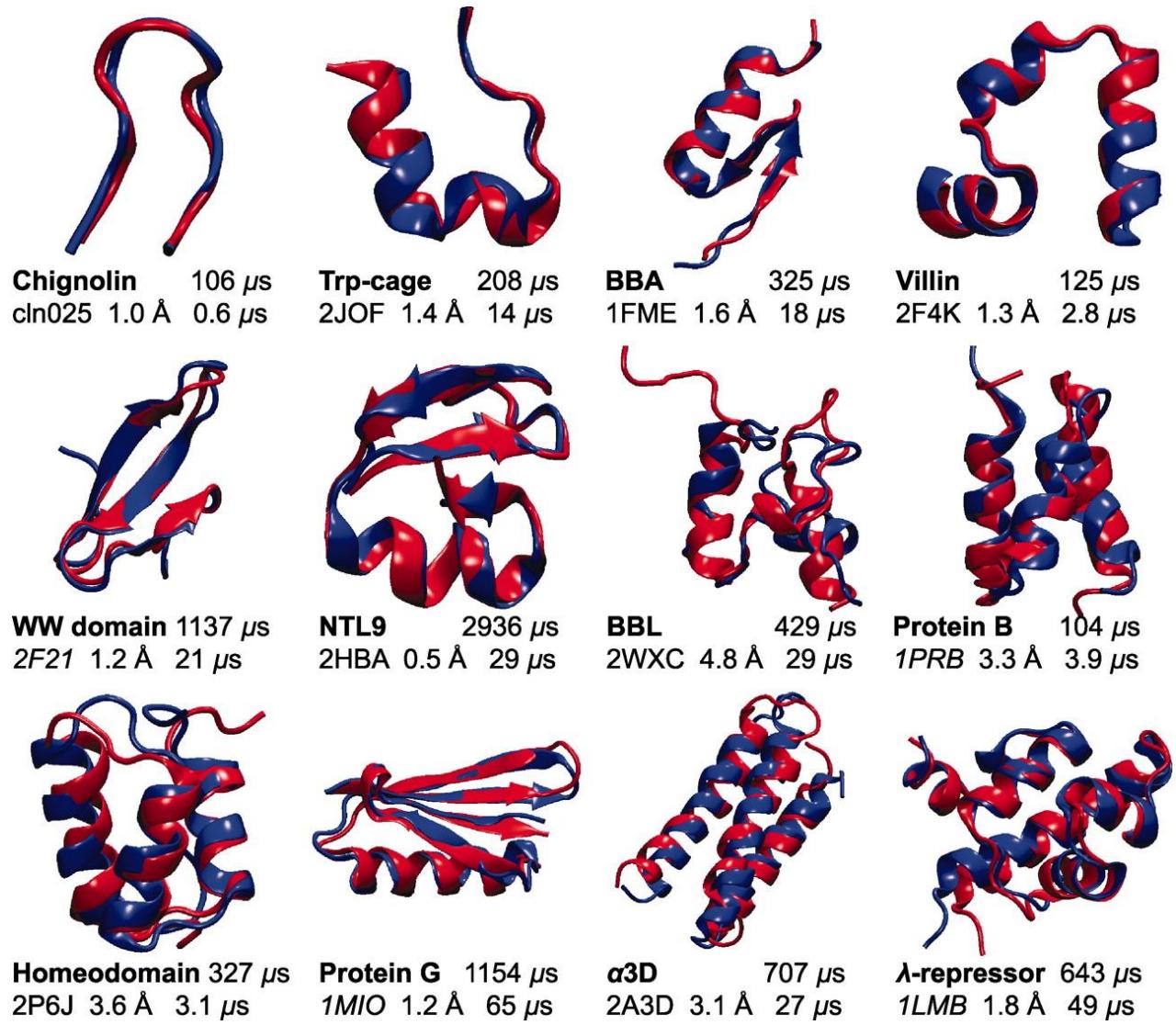
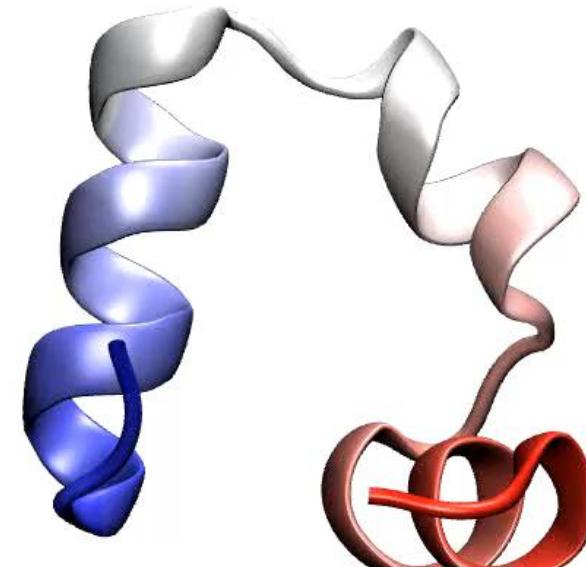
High-confidence hetero multimer predicted, but proteins do not co-assemble



High-confidence homodimer of mutated protein predicted, but mutation abolishes complex formation

Watching proteins fold: simulation

- Following experimentally the *folding pathway* of a protein is difficult
- Folding of small fast-folding (<100 μ s) proteins can be studied via simulation

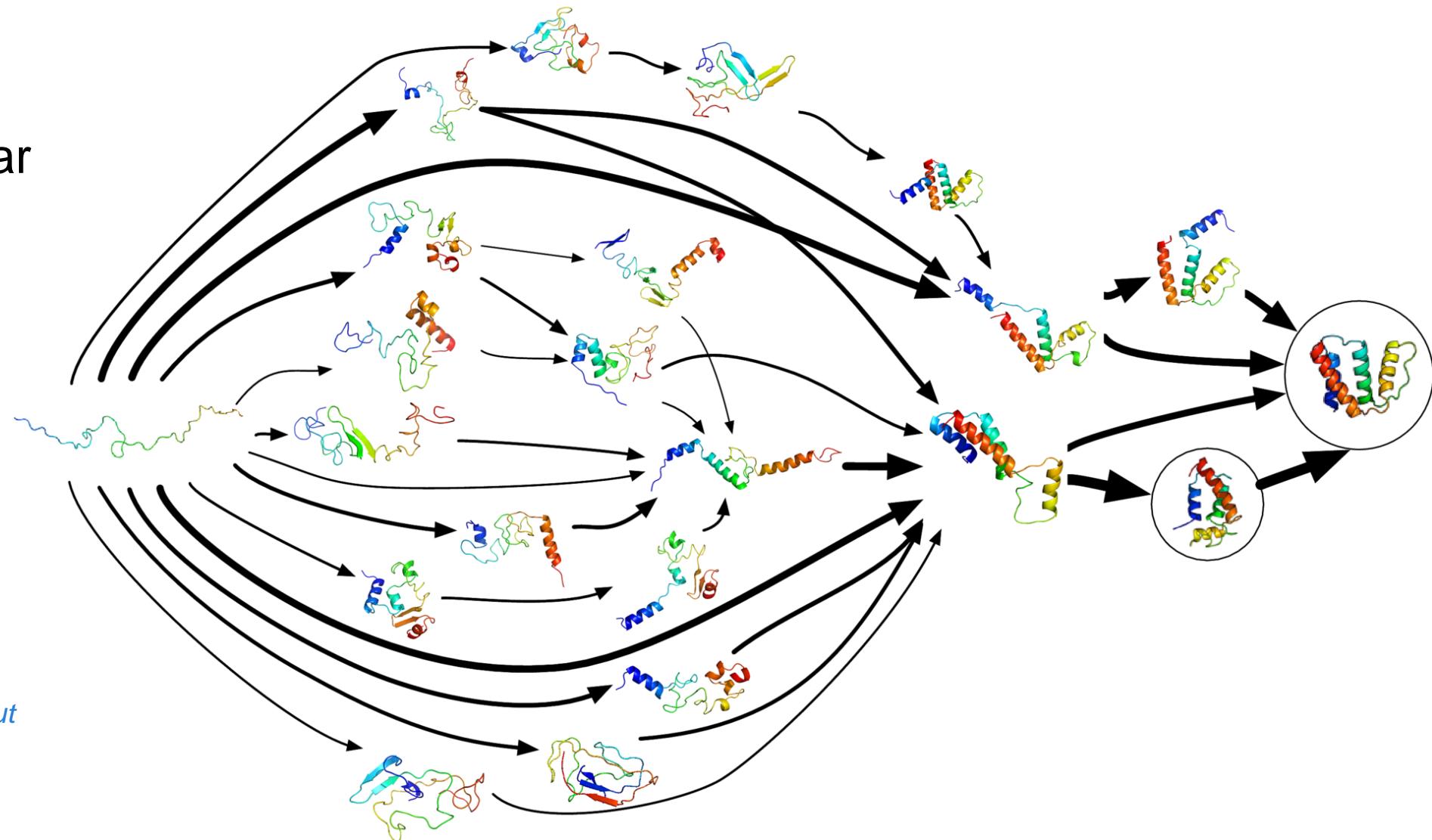


Folding@Home

Combine distributed computing, molecular simulation and Markov State Modelling (MSM) to predict protein folding pathways.

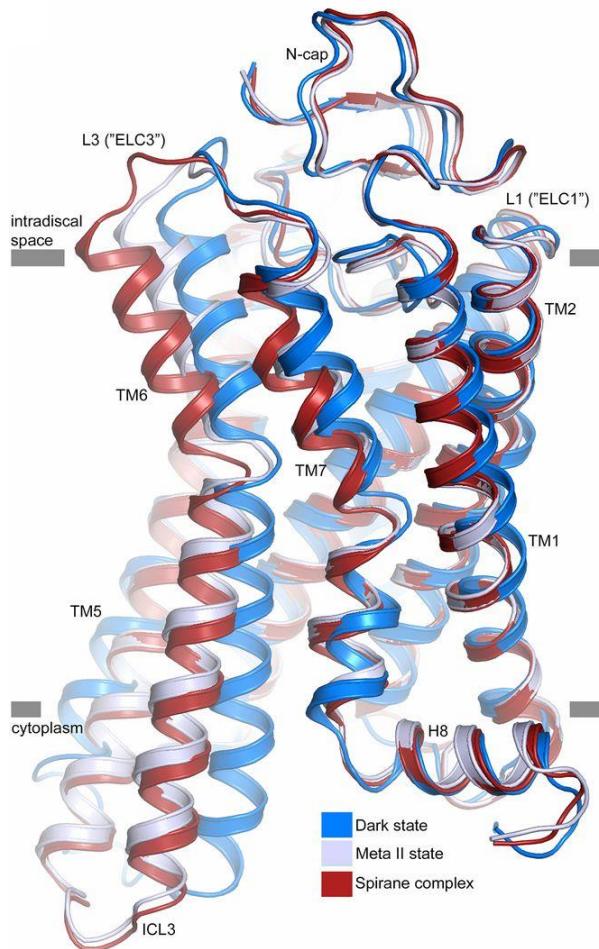
www.foldingathome.org

V. S. Pande, K. Beauchamp, G. R. Bowman, *Everything you wanted to know about Markov State Models but were afraid to ask. Methods*, 2010

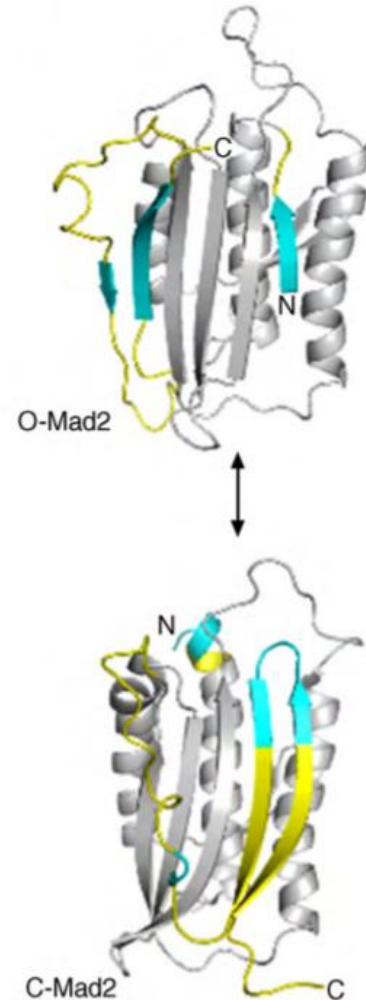


Protein dynamics

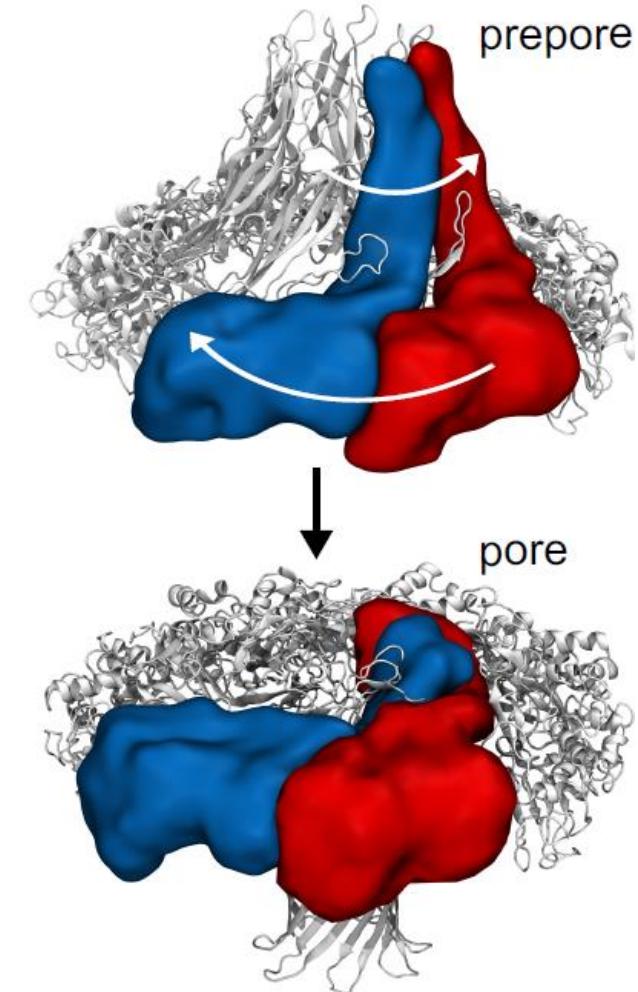
sequence + interaction with environment = conformational space



D. Mattle et al., *Ligand channel in pharmacologically stabilized rhodopsin*, *PNAS*, 2018



P.N. Bryan and J. Orban, *Proteins that switch folds*, *Curr. Op. Struct. Biol.*, 2010

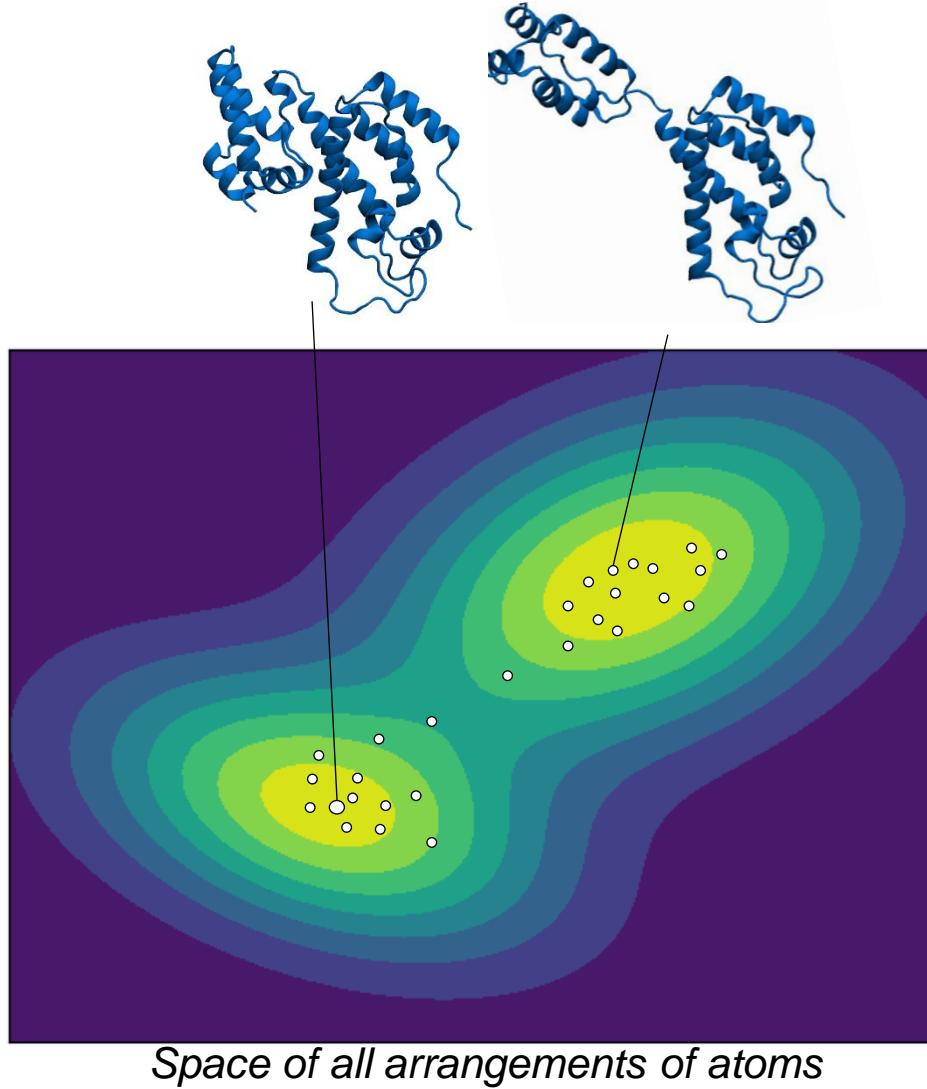


M.T. Degiacomi et al., *Molecular assembly of the aerolysin pore reveals a swirling membrane-insertion mechanism*, *Nat. Chem. Biol.*, 2013

Sampling the conformational space

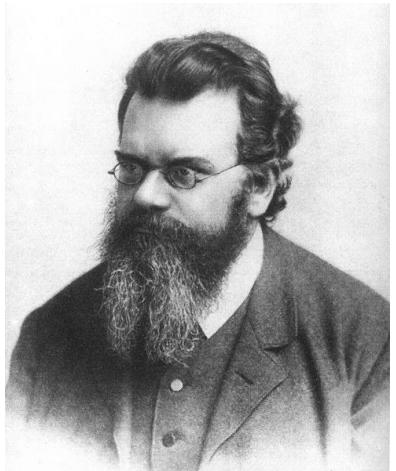
Low-energy conformations are explored more often than high-energy ones

The higher the energy barrier between two states, the longer the time needed to observe a transition



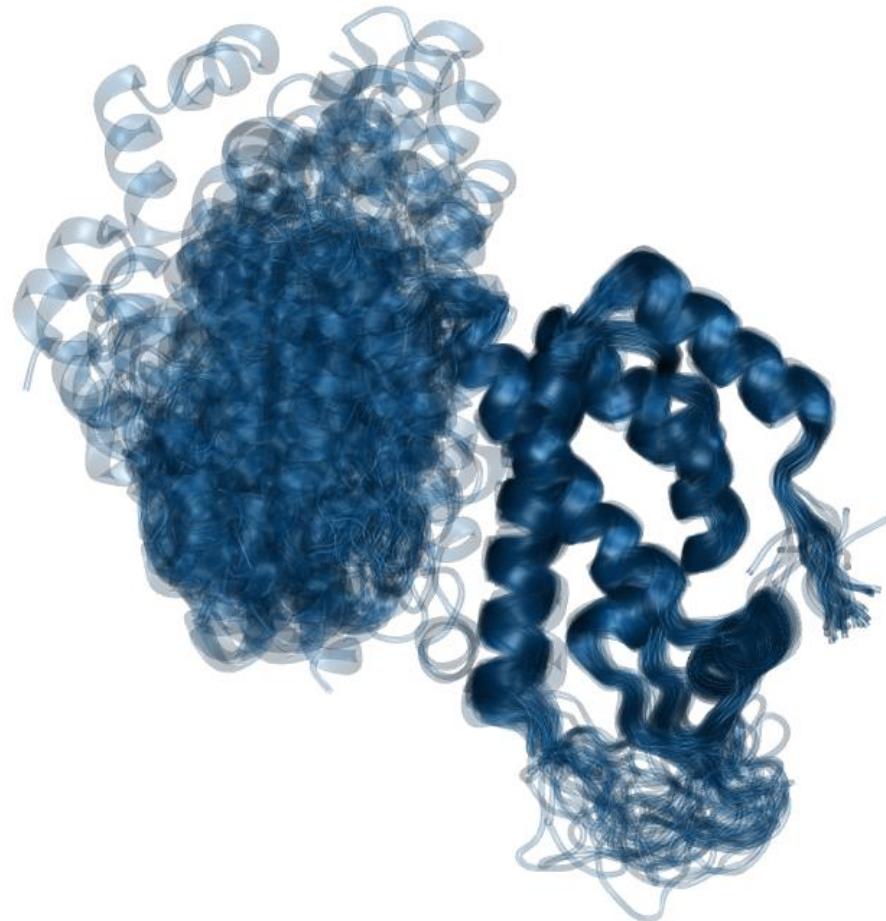
[Extra] sampling the conformational space

The Boltzmann distribution



$$p_i = \frac{1}{Q} e^{-\varepsilon_i/kT}$$

$$Q = \sum_{i=1}^M e^{-\varepsilon_i/k_B T}$$

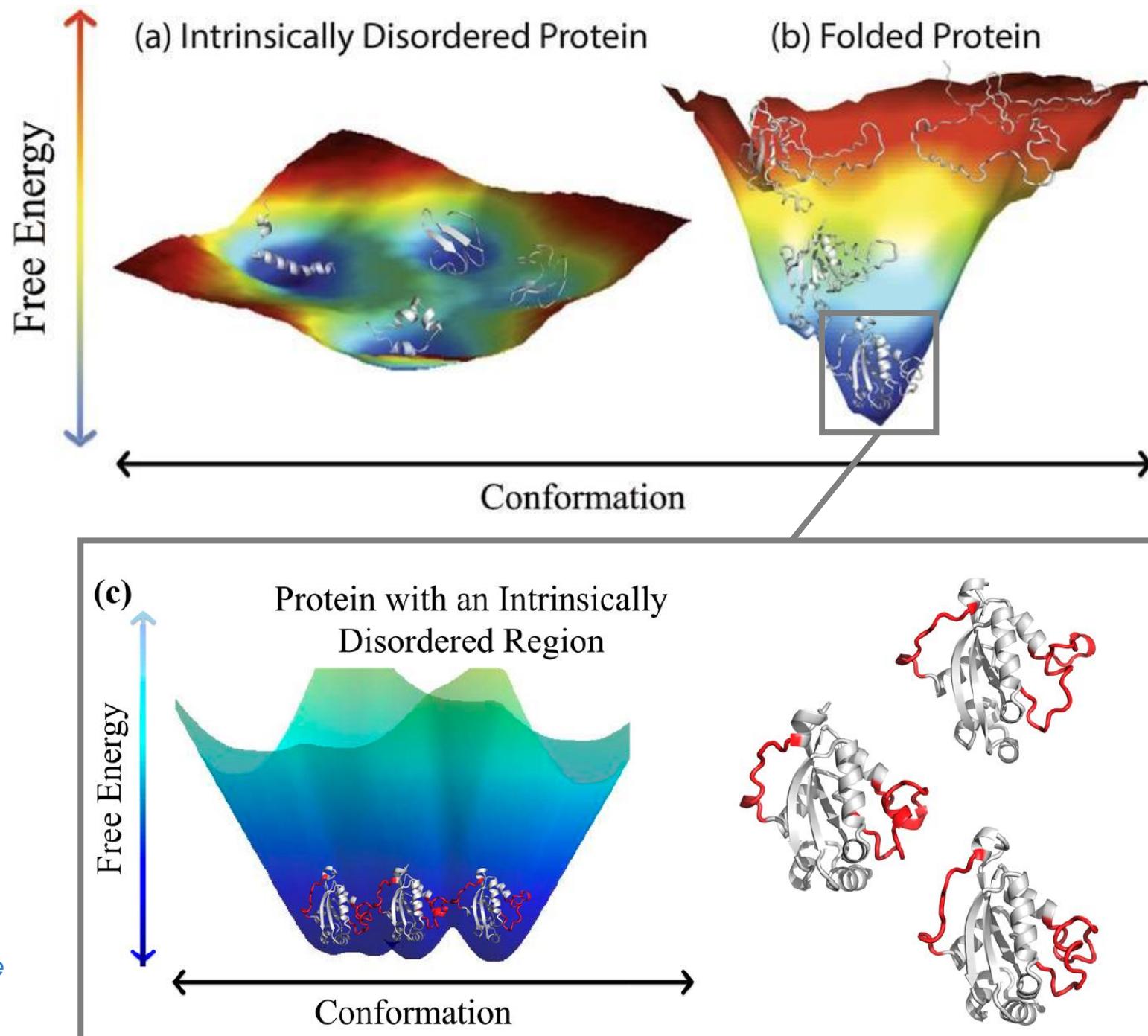


Not all proteins fold

Intrinsically Disordered Proteins (IDP):

- have a shallow energy landscape
- do not occupy a well-defined ensemble of states

Proteins may contain Intrinsically Disordered Regions (IDR)



**Next: how to prepare a protein
structure so that it is ready for
molecular modelling?**