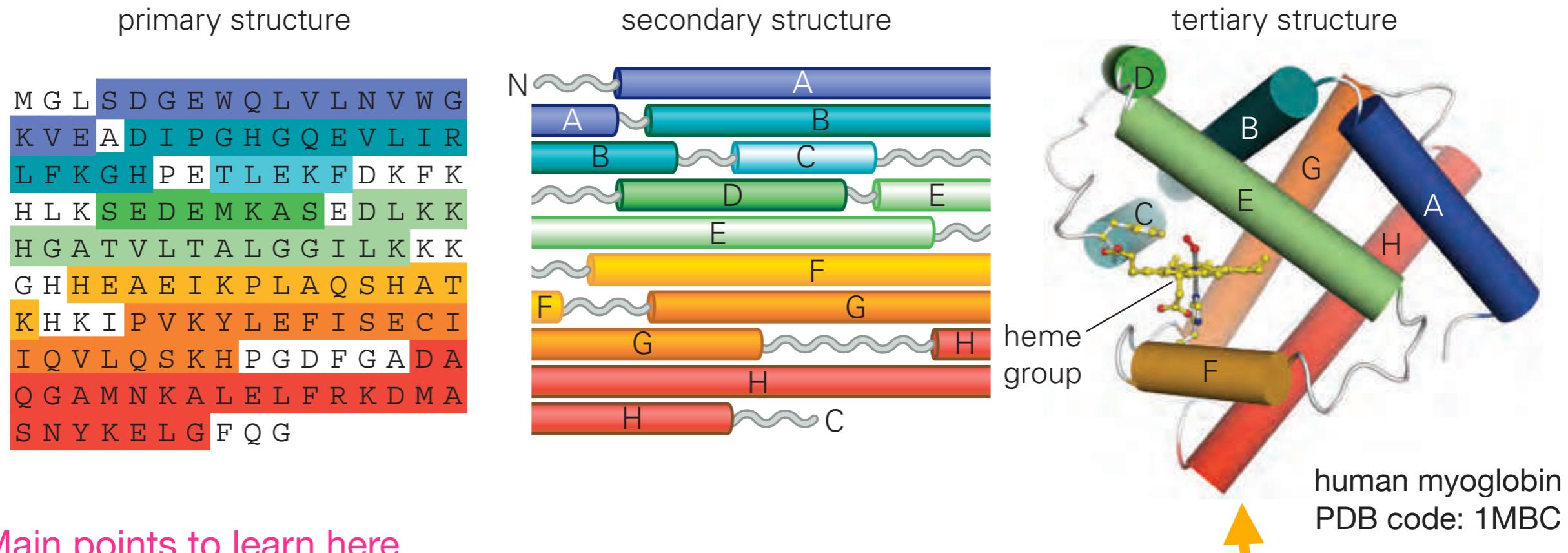


Today's class:

Protein structure

*This lecture follows the chapter 4 in the book
'The Molecules of Life' by Kuriyan, Konforti & Wemmer, 1st Ed, 2013*

Protein structures display a hierarchical organization

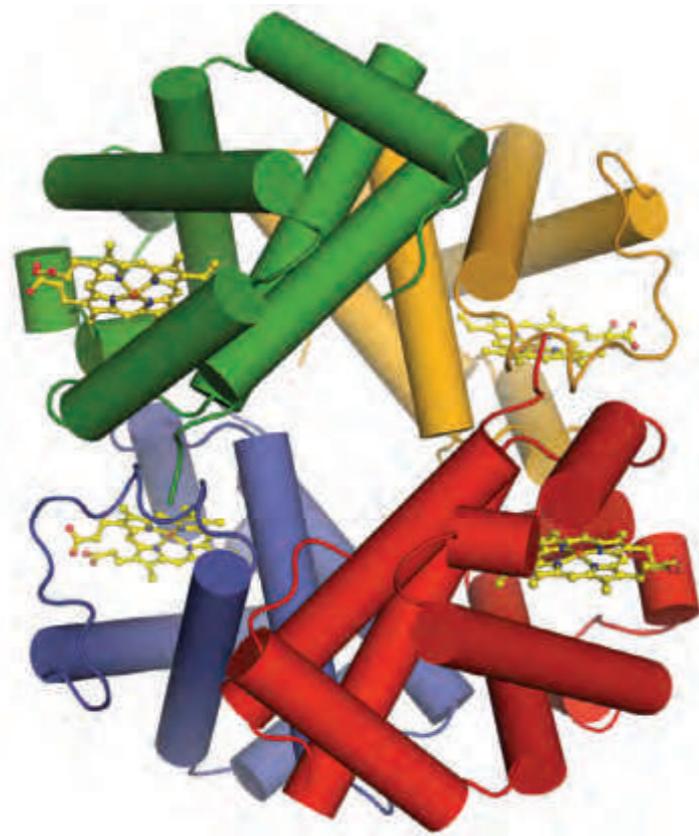


Main points to learn here

- A general rule is that the residues forming interfaces between secondary structural elements are hydrophobic
- Packing of secondary structural elements results in a structural domain.
- Specific organization of secondary structural elements in a domain is called the protein fold e.g. globin fold
- A protein can have several structural domains

The visually recognizable arrangement of α helices and β strands in the three-dimensional structure of a protein is referred to as the protein fold. Different proteins can have the same fold, as is the case for myoglobin and the subunits of hemoglobin

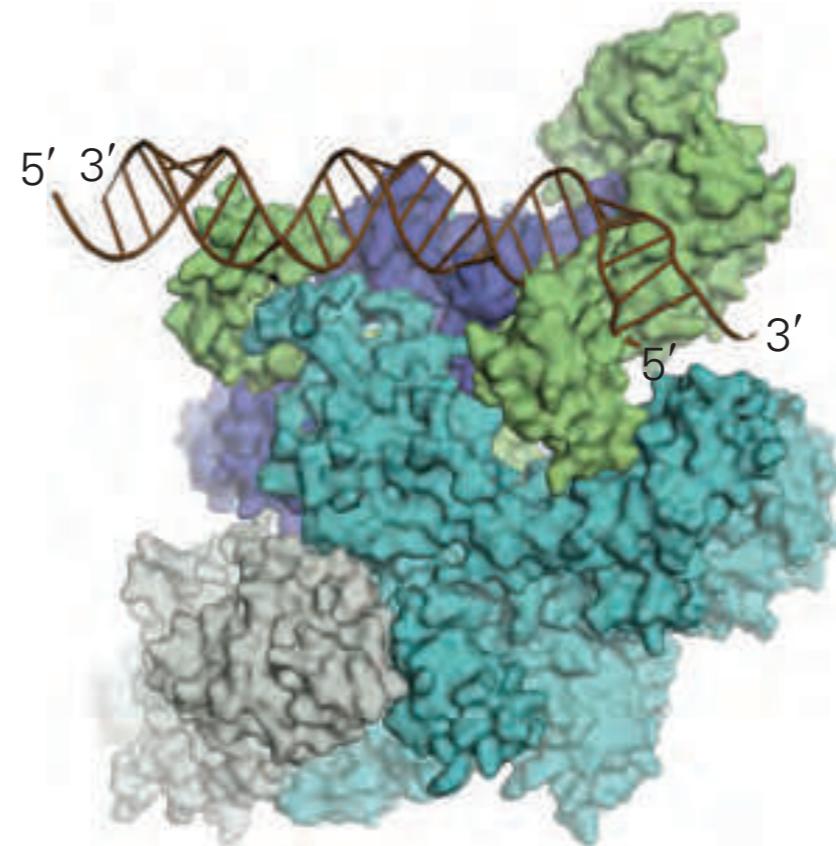
Protein structural domains organize into quaternary structure



Human hemoglobin

PDB code: 1A00

Made of two copies of each
of two different subunits



Bacterial RNA polymerase

PDB code: 1L9Z

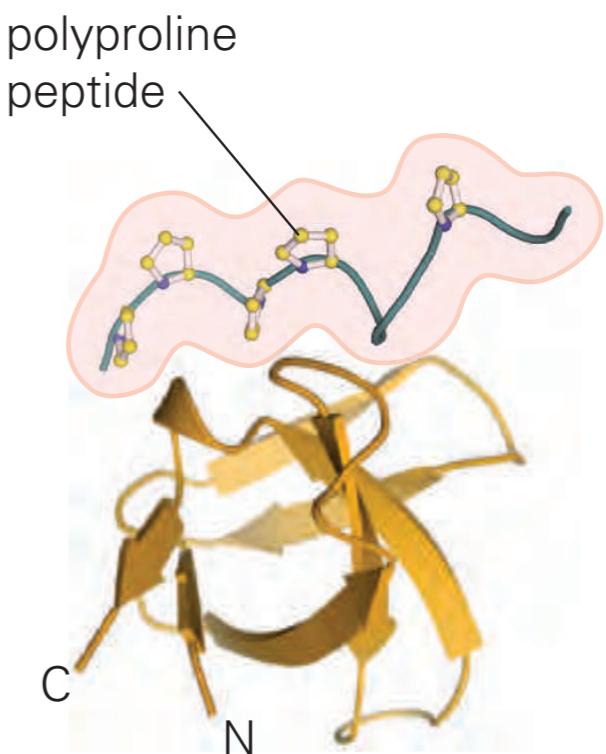
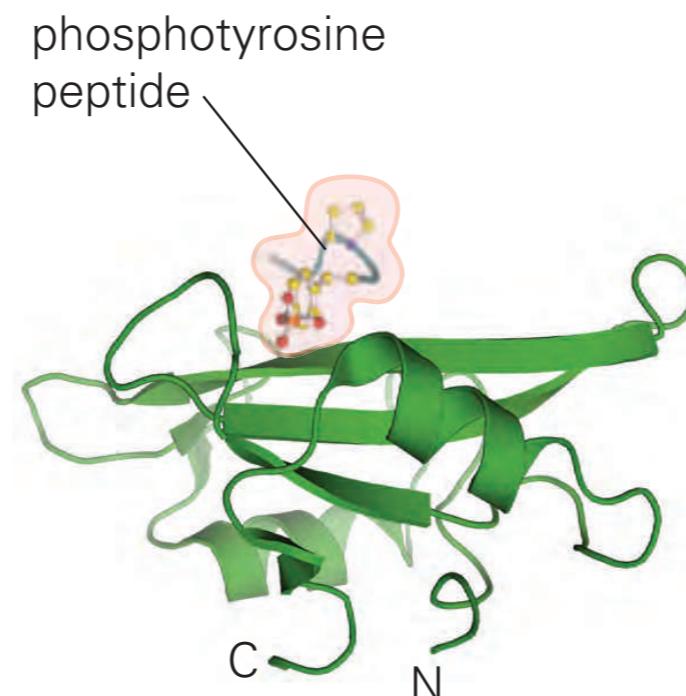
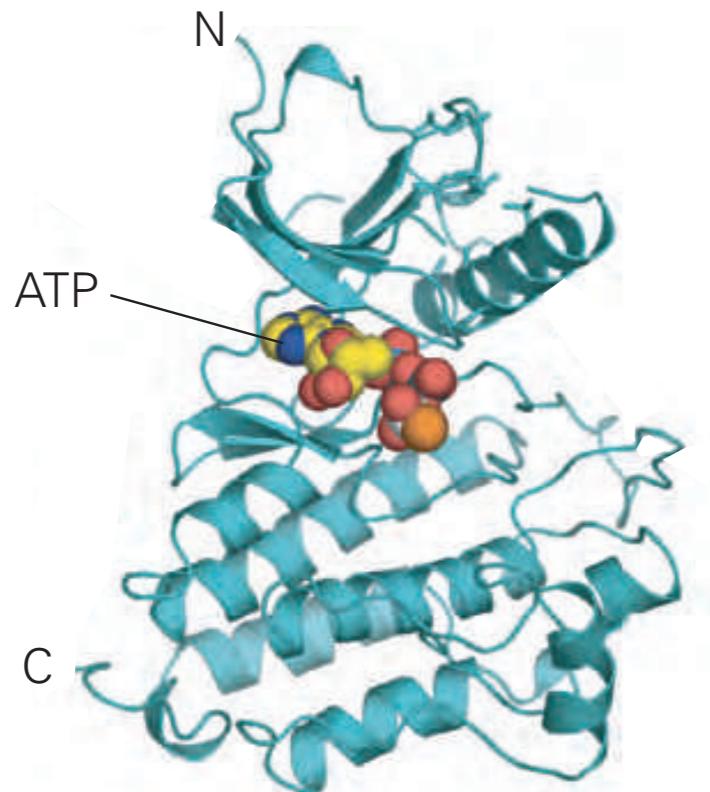
Made of several different
subunits

Structural domains are the building blocks for complex protein structures

Gene duplication and genetic recombination have resulted in the combinatorial creation of multifunctional proteins by mixing and matching structural domains.

Example: Src Homology domains SH1, SH2 and SH3

Src is a protein that causes sarcoma



SH1
(tyrosine kinase)

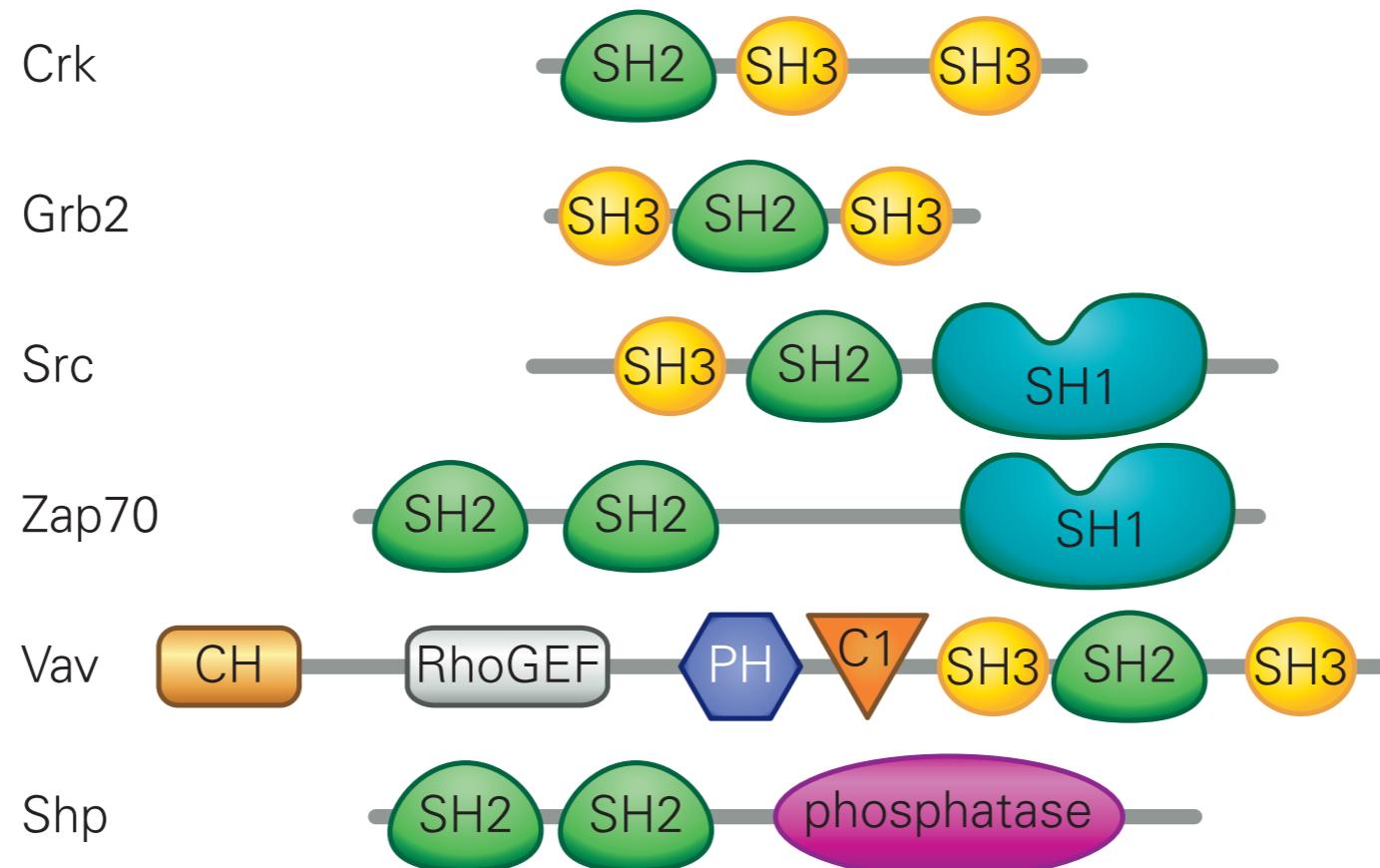
Catalyzes tyrosine phosphorylation

Transfer of terminal phosphate group from ATP to the target

Binds to phosphorylated tyrosines

Binds to peptides having proline residues at specific positions

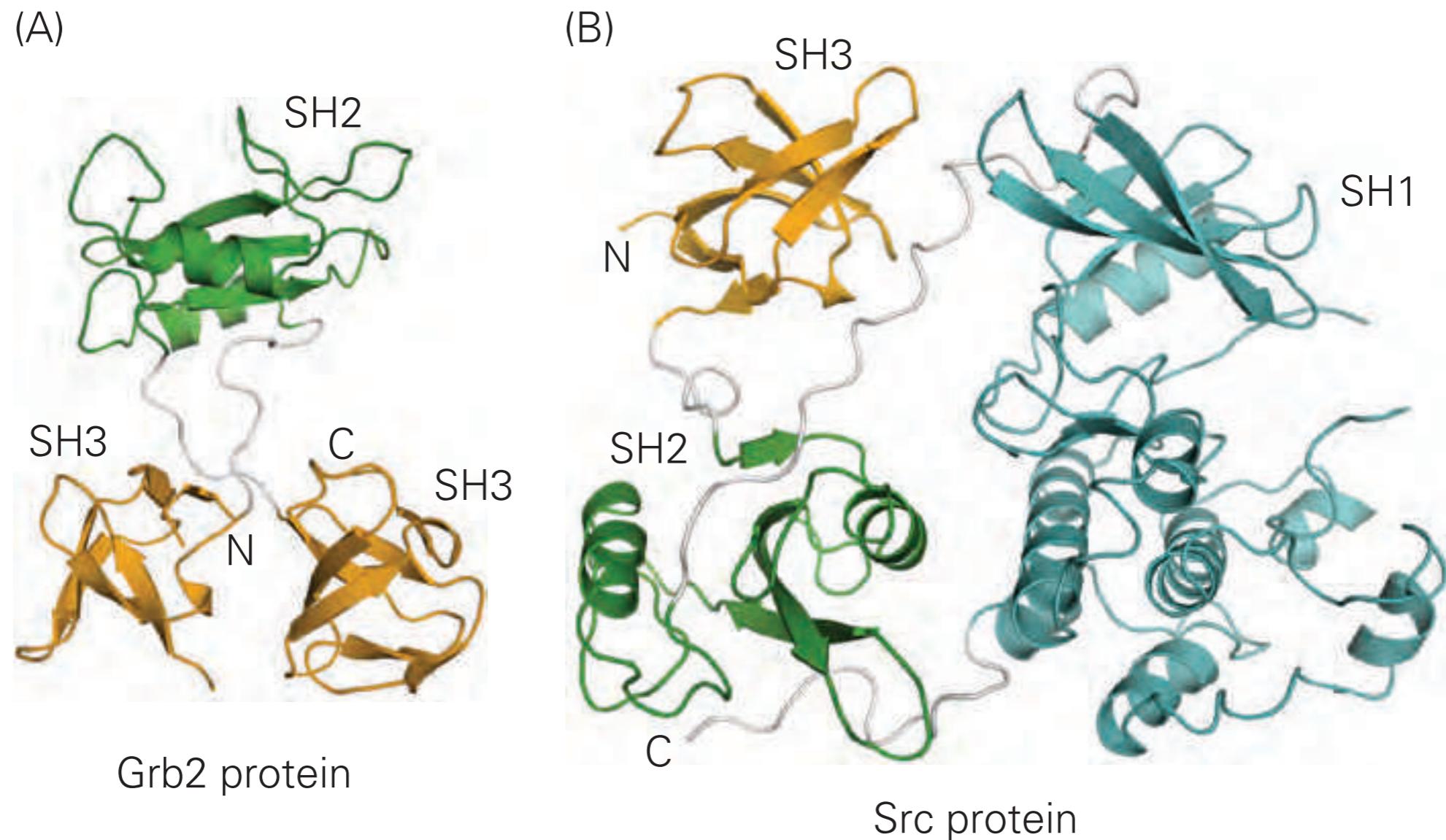
Diversity of proteins containing SH domains



Knowing the other domains apart from SH

- CH = actin binding domain
- PH and C1 = specific membrane lipid binding domains
- RhoGEF = causes dissociation of GTP or GDP from Rho family proteins
- Phosphatase = enzyme domain that catalyzes removal of phosphate from phosphotyrosine residues

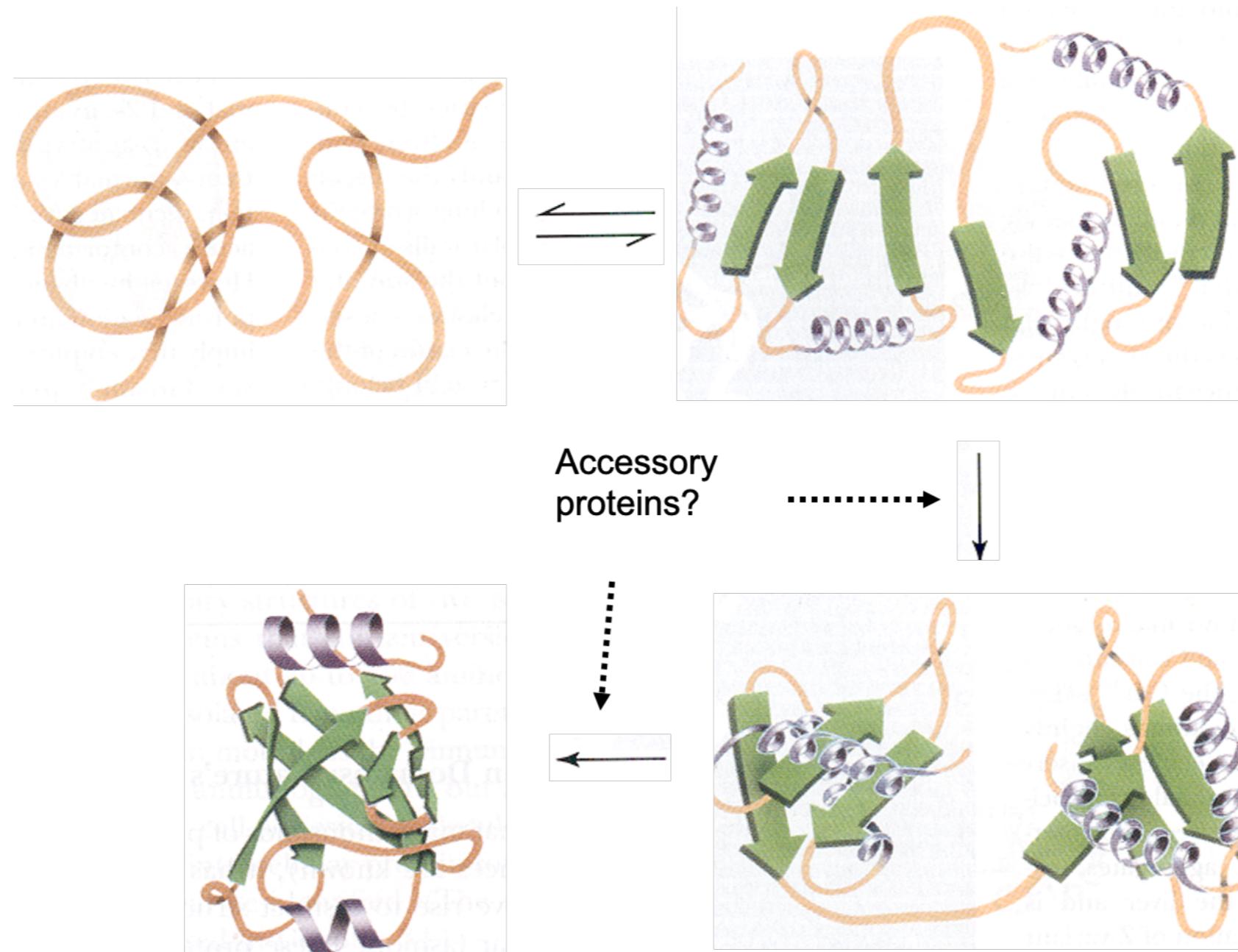
3D organization of structural domains can differ from protein to protein



Salient points

- Amino acid sequence of a given domain may vary from protein to protein
- Interactions of these domains with different other domains lead to slightly different structures
- Although the protein fold remain conserved, the 3D arrangement of the domains can be very different in different proteins

Protein folding: the expected process

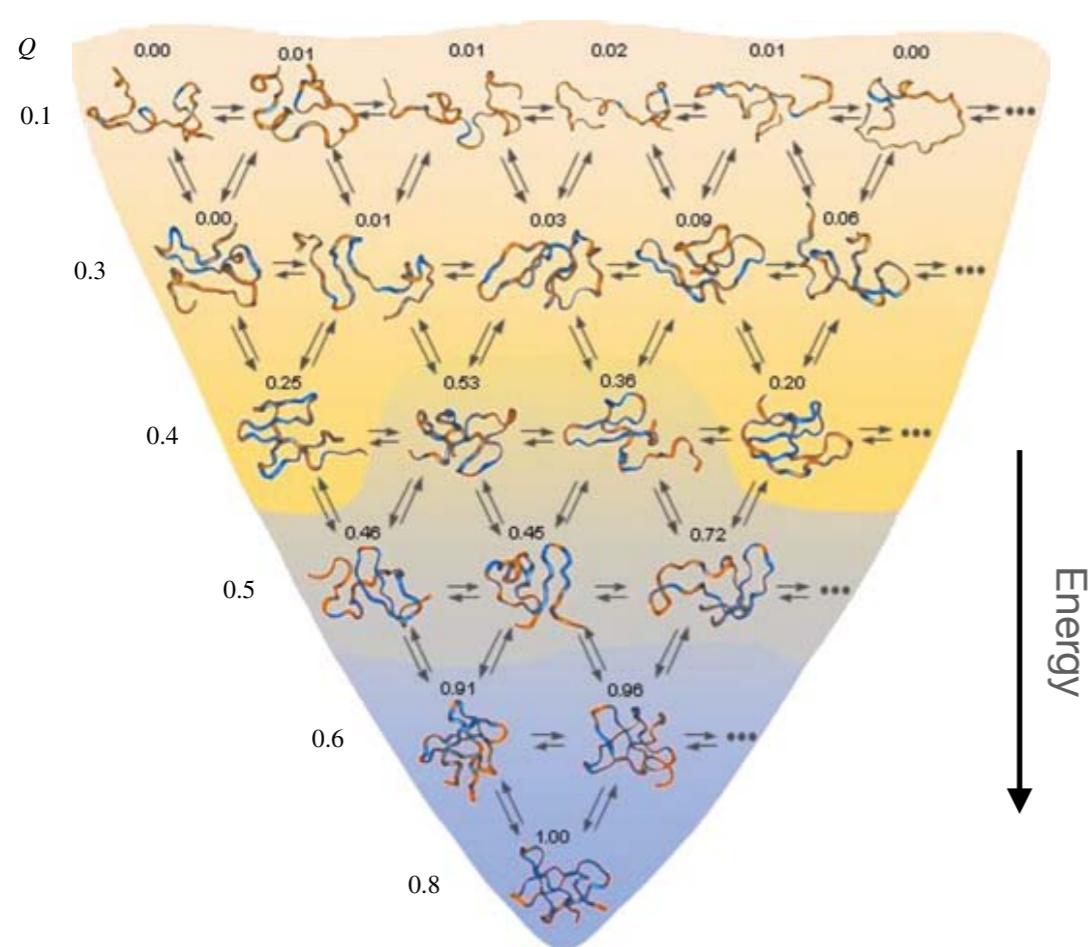


Folding through random search would not be possible in any physically meaningful time

But proteins do fold in milli-microseconds timescale

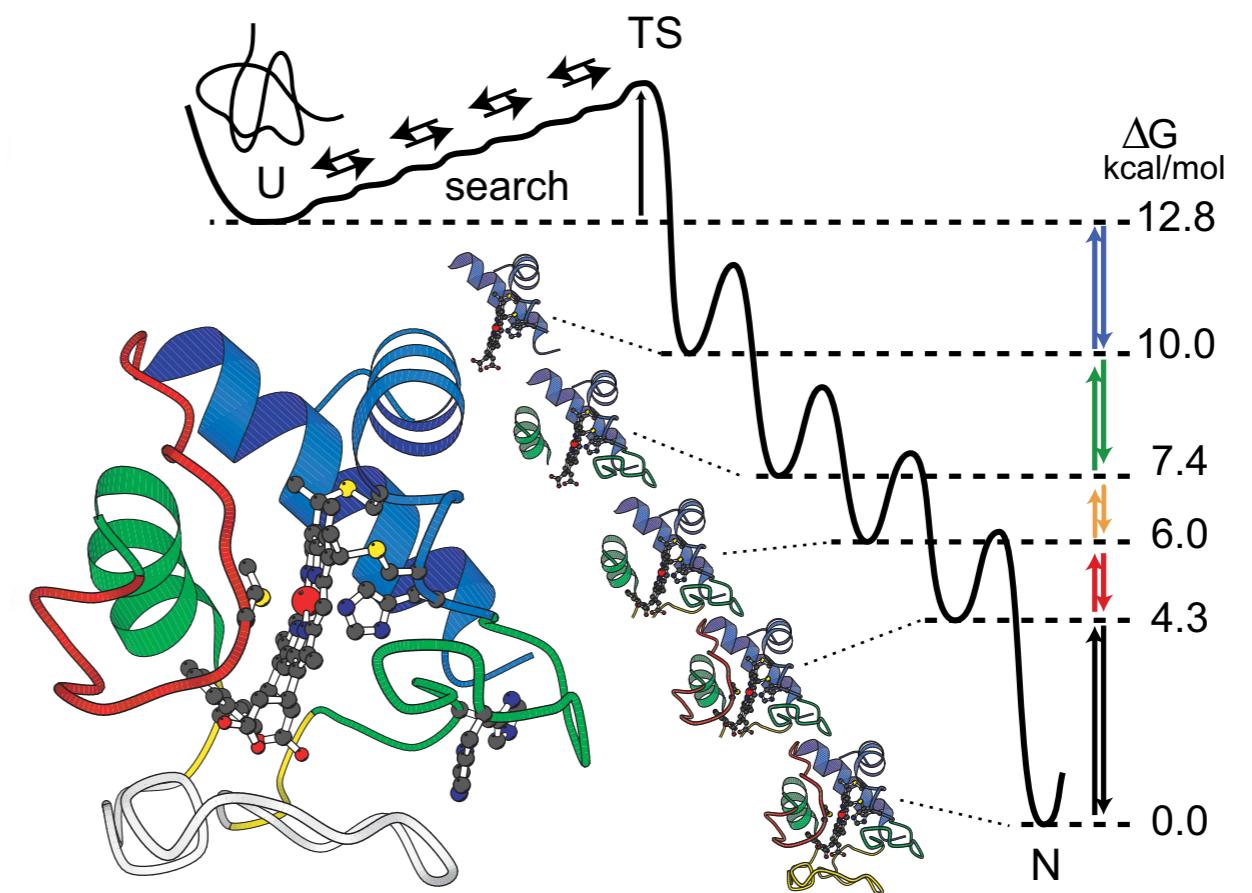
Levinthal's paradox 1969

Protein folding: the debate continues..



Energy Landscape Theory of protein folding

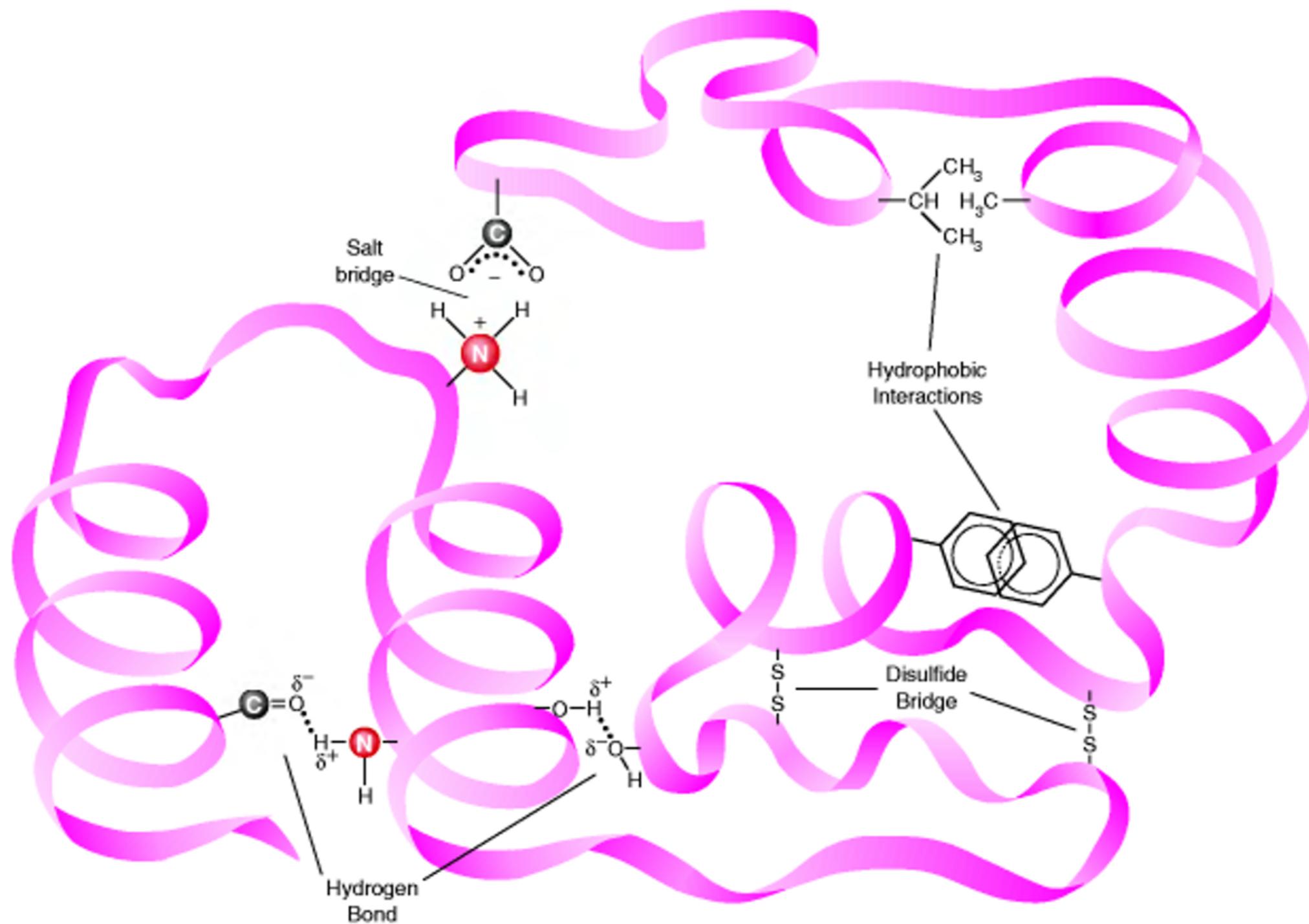
Onuchic, Wolynes and co-workers - 1990's



Protein folding via "Foldon" units

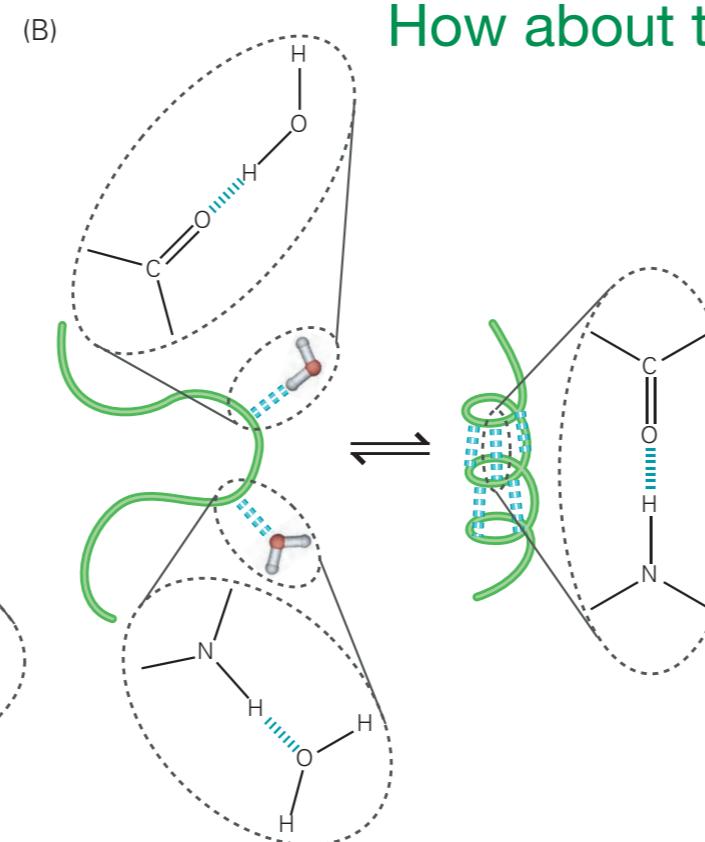
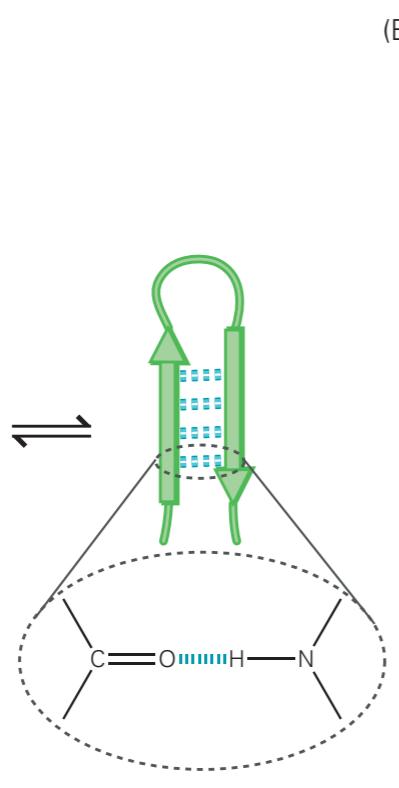
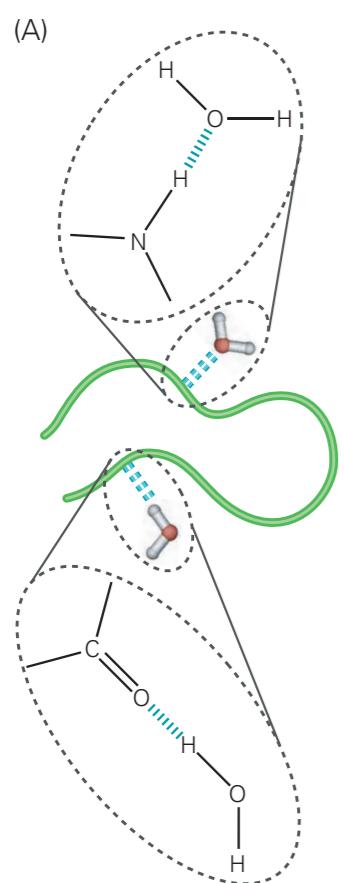
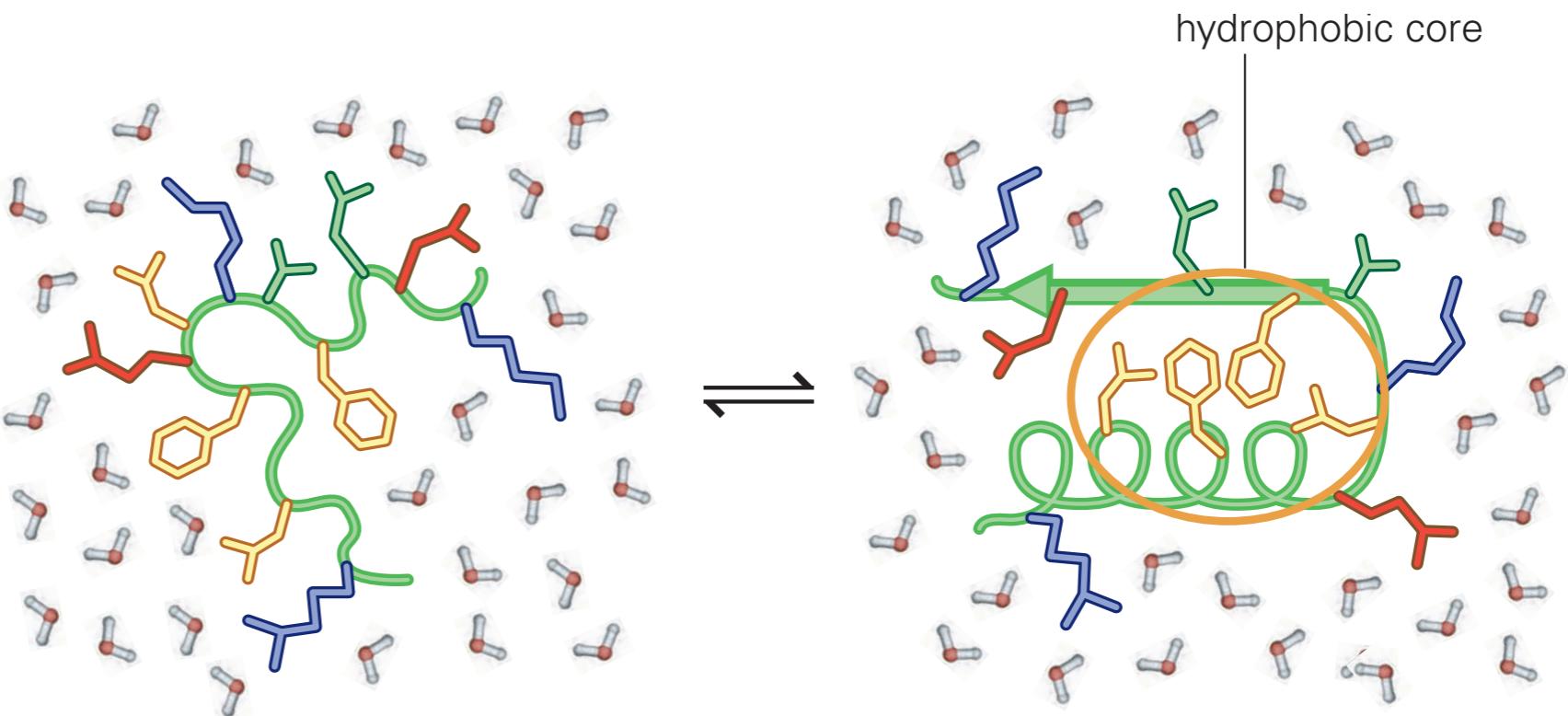
Englander and coworkers, 2003

Molecular forces involved in stabilization of protein folding



Protein folding is dominated by hydrophobic interactions

Packing of secondary structural elements leads to a hydrophobic core

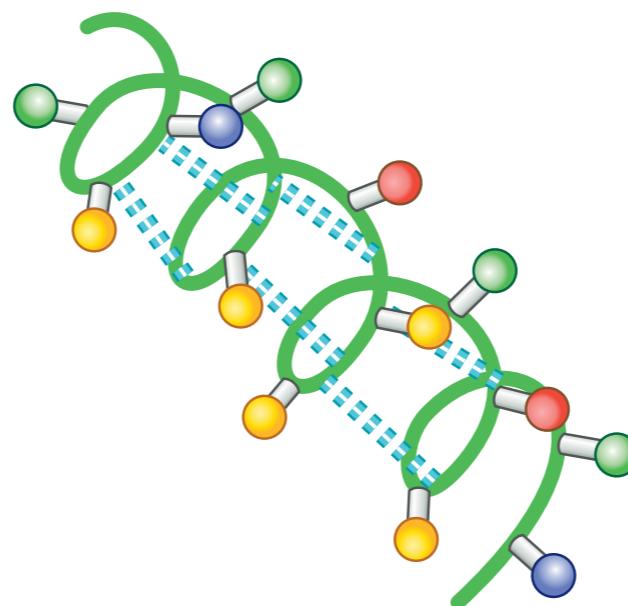
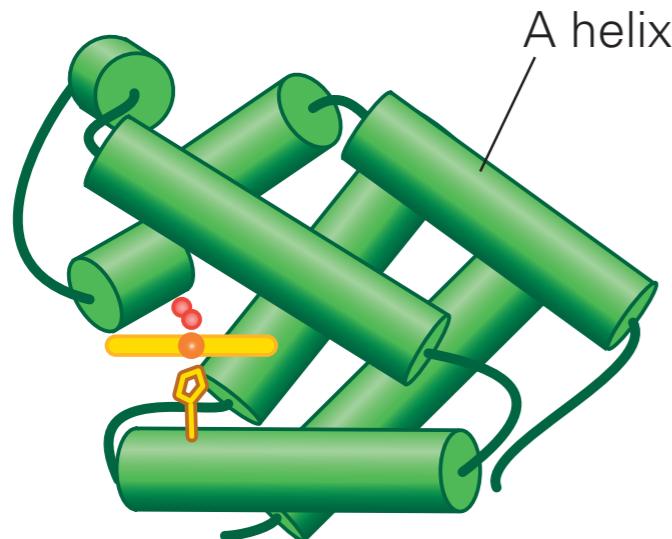


How about the stabilization from H-bonding?

As proteins fold, contribution from H-bonds remain almost unchanged as H-bonds with water gets replaced by H-bonds formed within secondary structural elements

Importance of the hydrophobic effect: isolated protein fragments don't fold

(A)

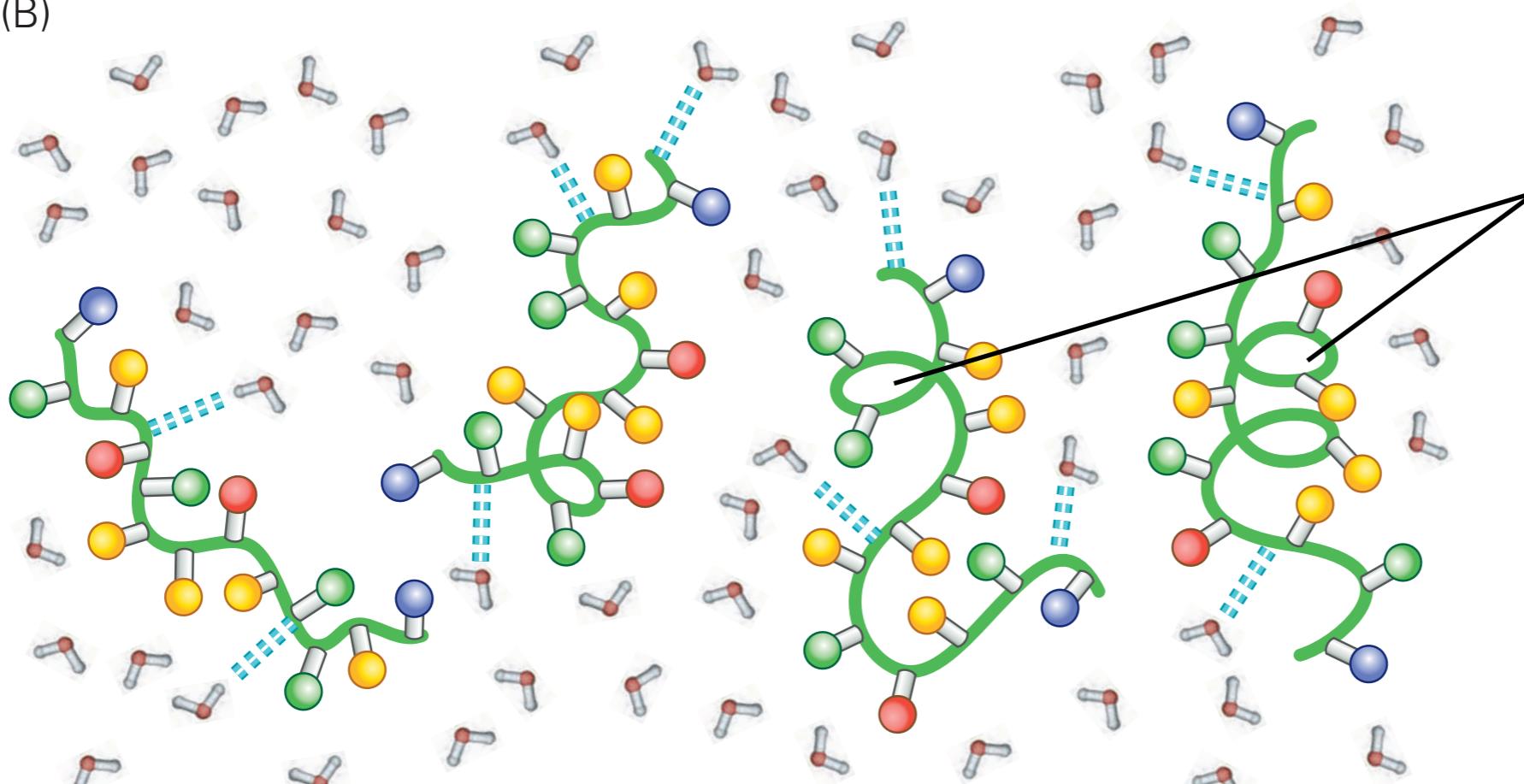


Hydrophobic
Polar
Charged

A legend on the right side of the image. 'Hydrophobic' is represented by a yellow square. 'Polar' is represented by a green square. 'Charged' is represented by a red square next to a blue square.

structure of A helix in protein

(B)

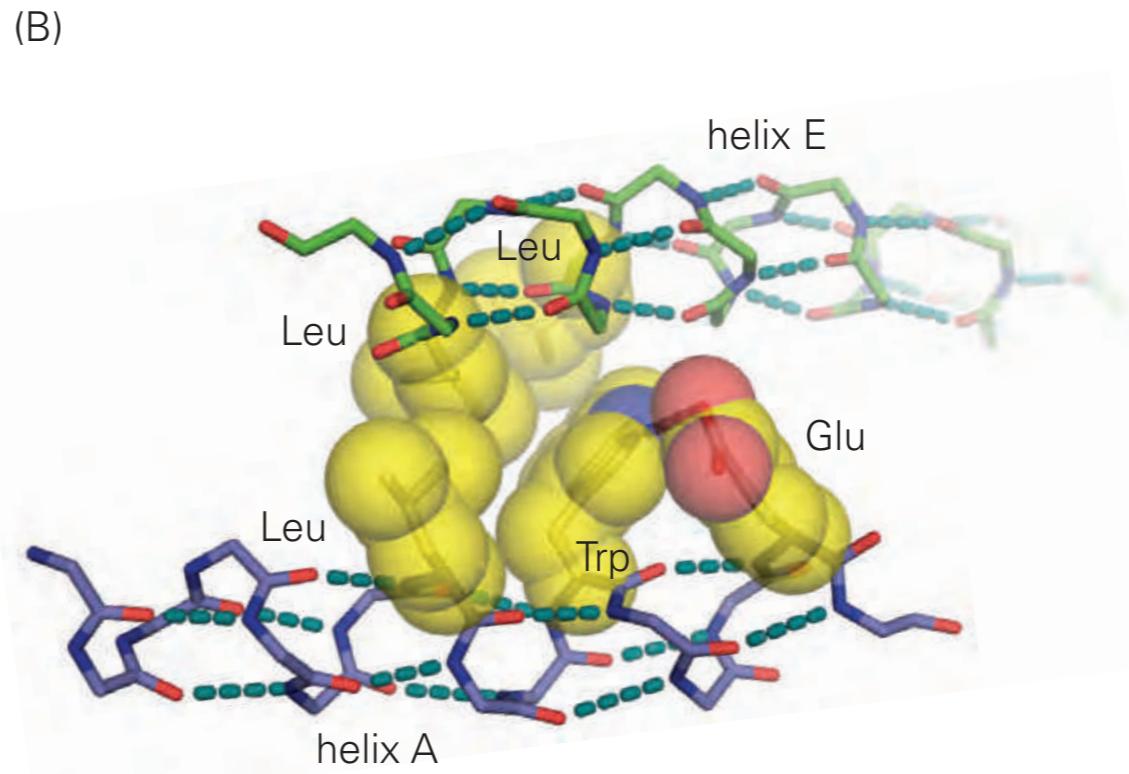
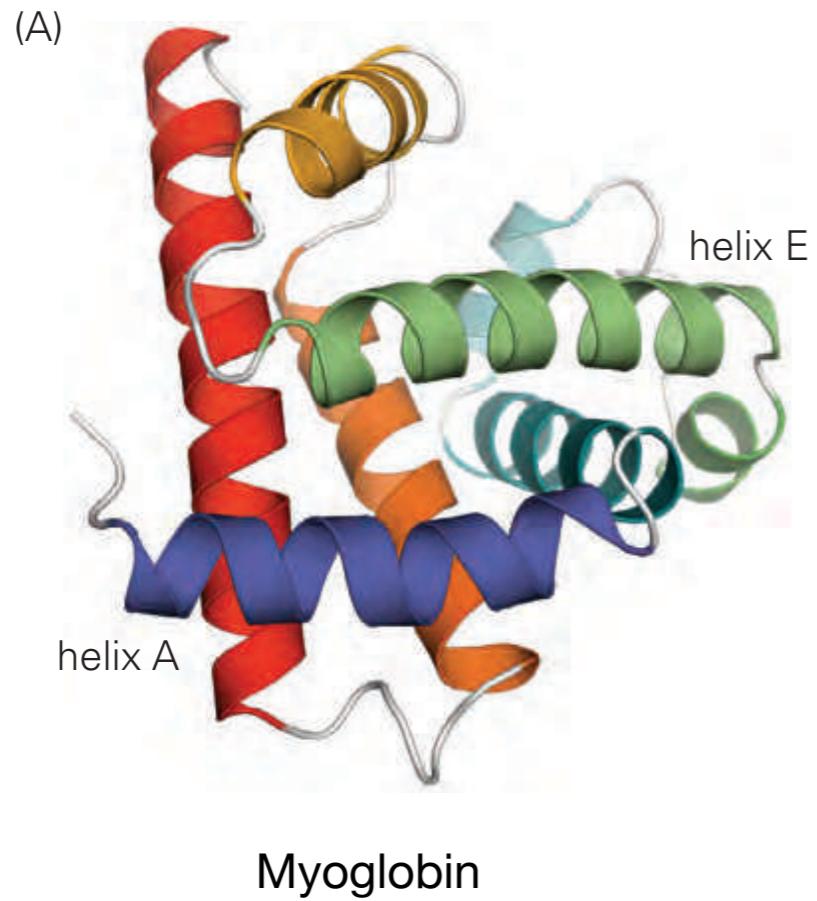


Transient helical turns

Overall it can't fold to create a hydrophobic core as the packaging partners to the different groups are missing

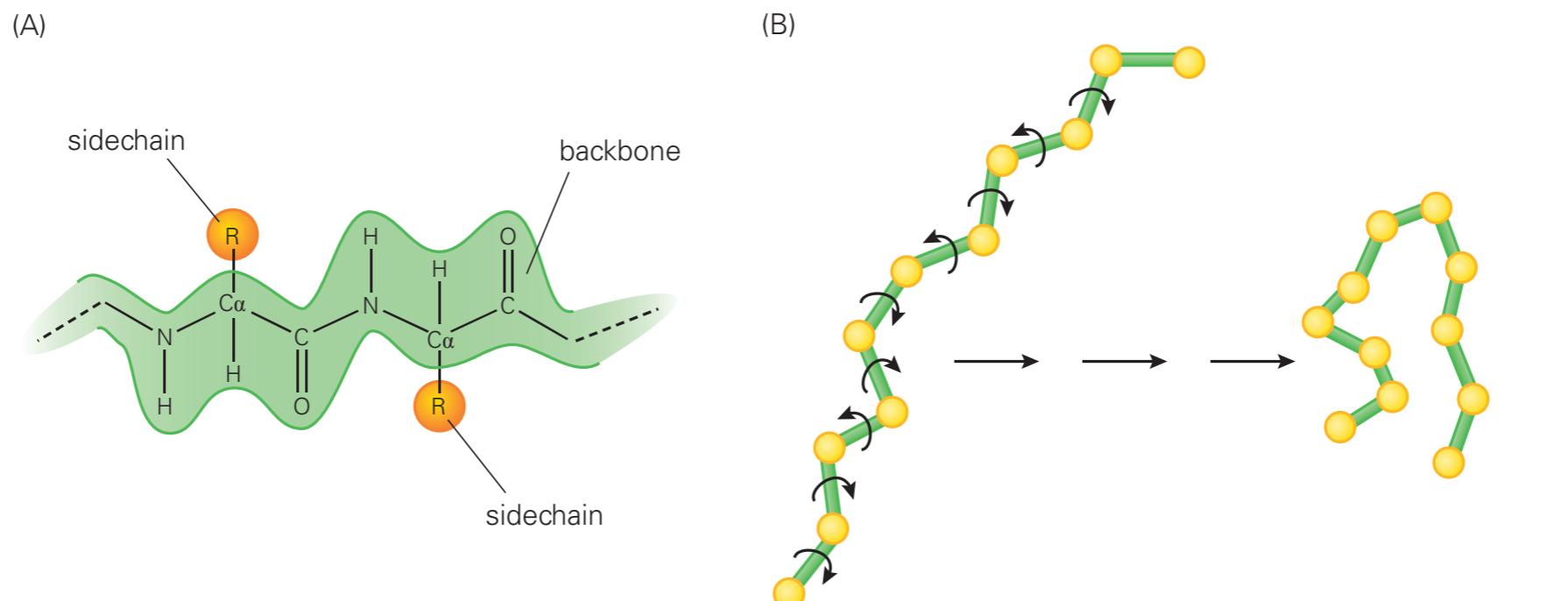
structures of isolated A-helix peptide

H-bonding drives formation of secondary structure elements



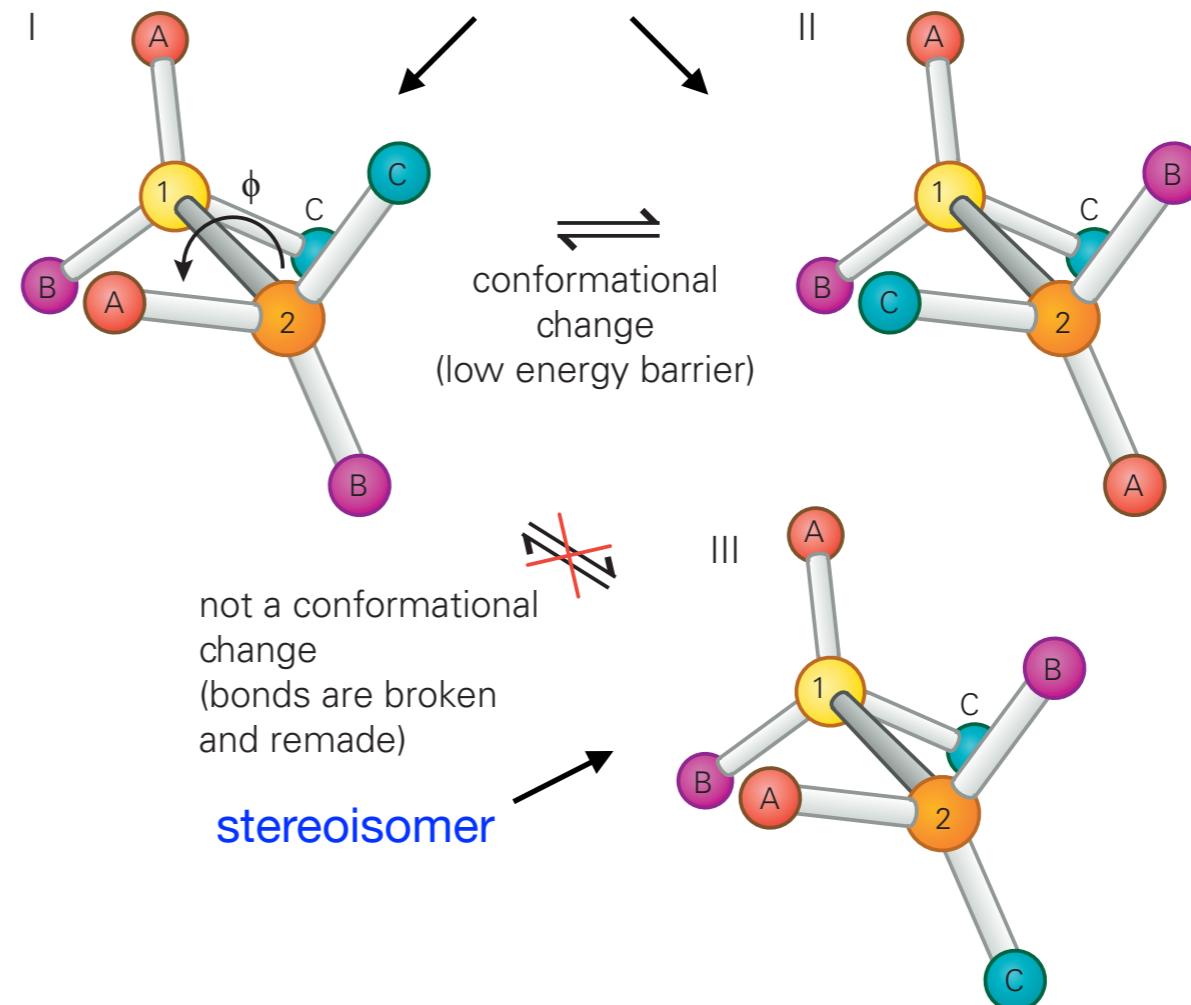
Formation of the α helices here satisfies the H-bonding requirements in the folded state. The H-bonds with water are lost and they are replaced by those along the protein backbone

Folding involves conformational changes of the peptide backbone

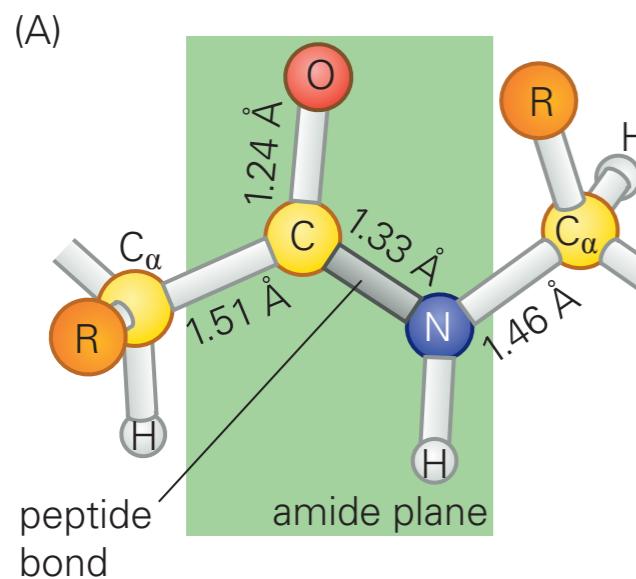


Conformational change

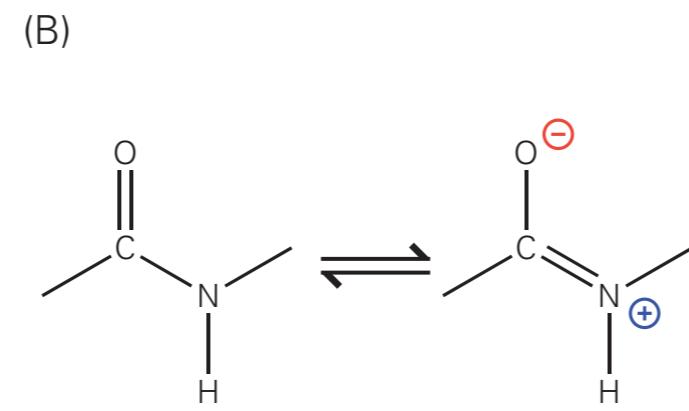
A change in structure that arises solely from rotations about covalent bonds is called a conformational change. Conformational rearrangements do not involve breaking and forming covalent bonds, and can often occur readily at room temperature.



Backbone torsion angles determine the conformations



the bond between the C atom in the first residue and the N atom in the second residue

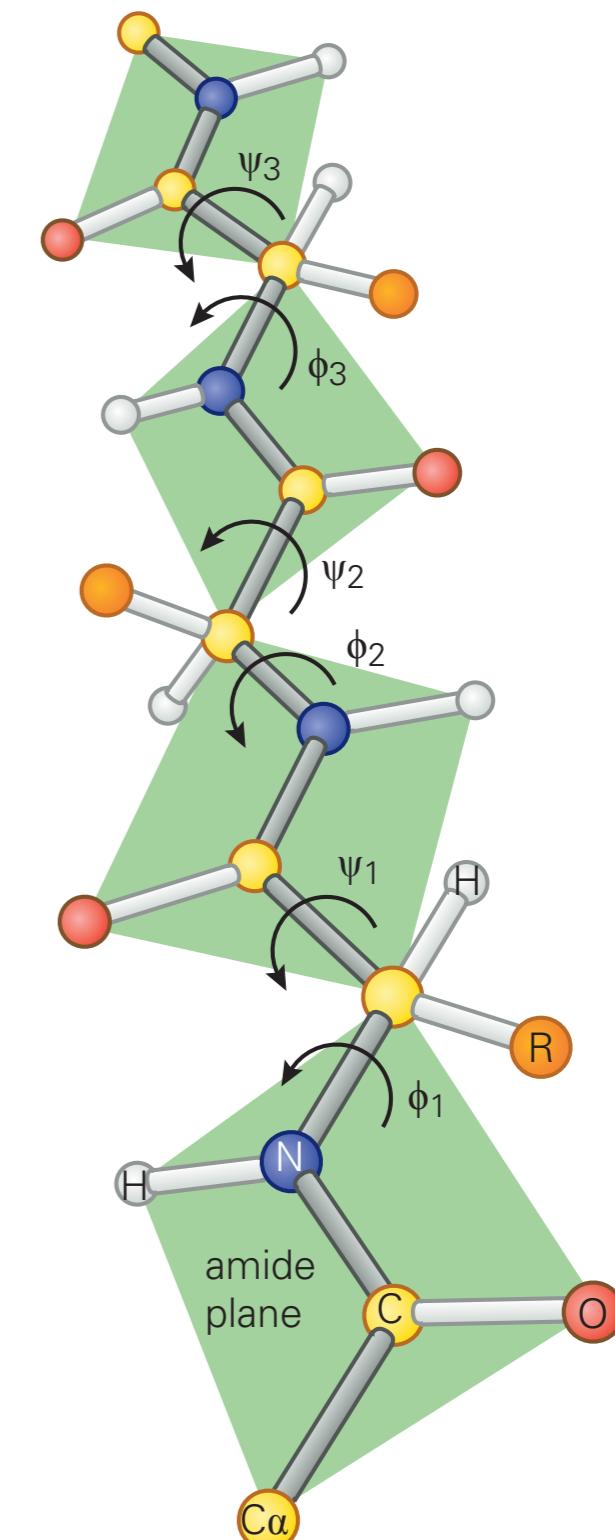
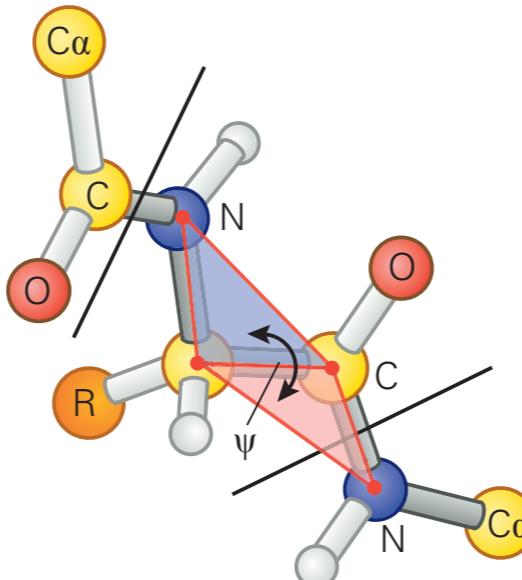
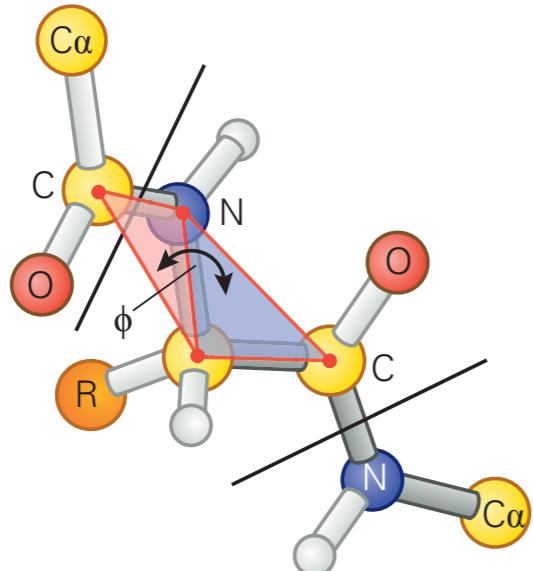


The peptide bond is planar due to partial double bond character

So rotation is allowed only at the point where these planes meet

ϕ = Rotation about $N - C_\alpha$

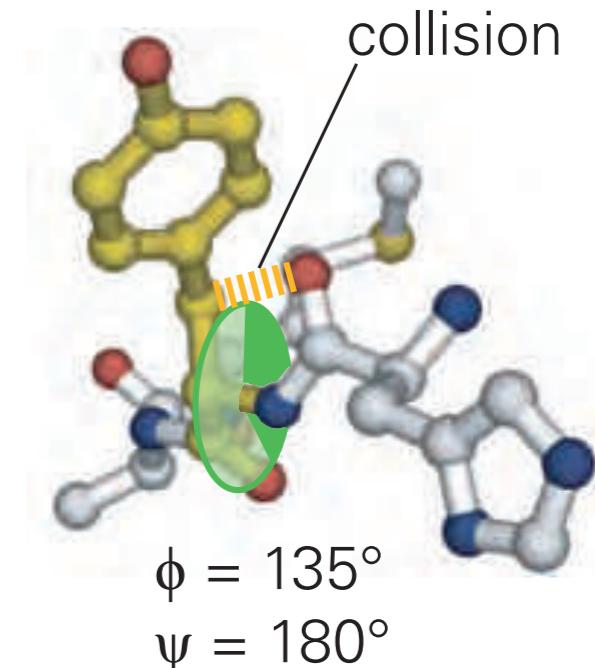
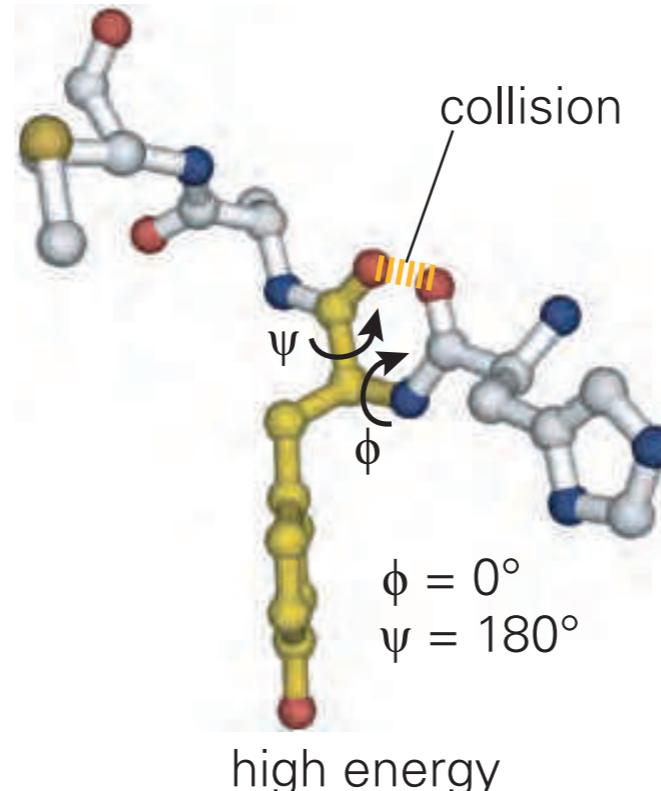
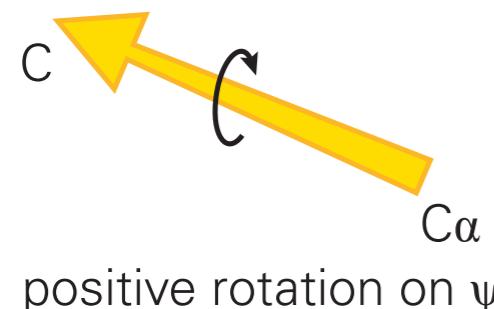
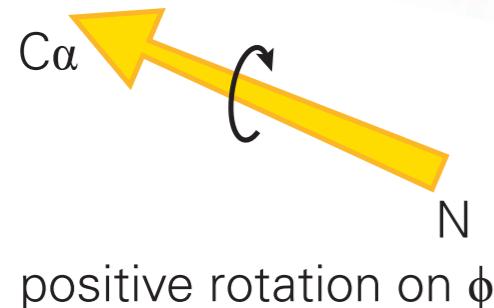
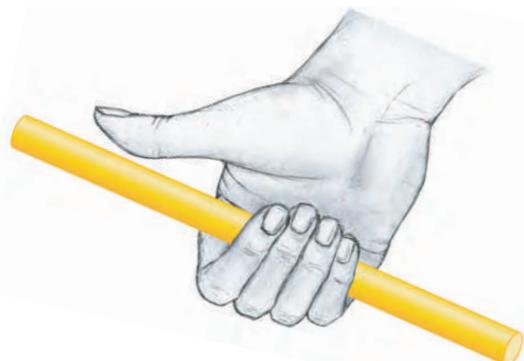
ψ = Rotation about $C_\alpha - C$



Sets of ϕ and ψ completely specify the 3D structure of peptide backbone

Not all values of ϕ and ψ are accessible to the peptide backbone

Convention of measuring torsion angles



Forbidden combinations of ϕ and ψ for a Tyr-His peptide

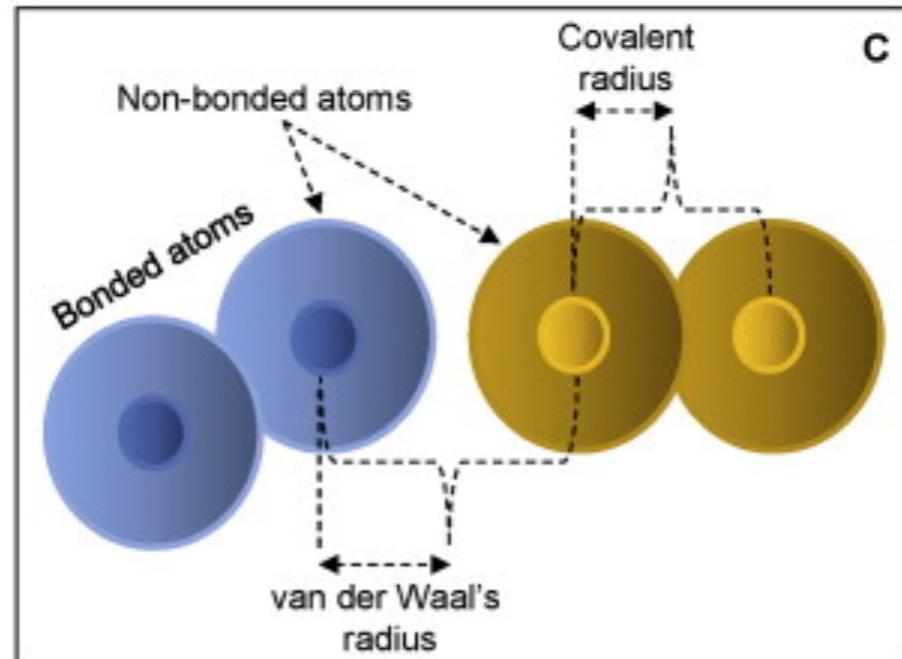
van der Waals repulsion is the factor responsible for these restricted conformations

How to quantify them?

The Ramachandran diagram provides a measure of disallowed conformations



GN Ramachandran

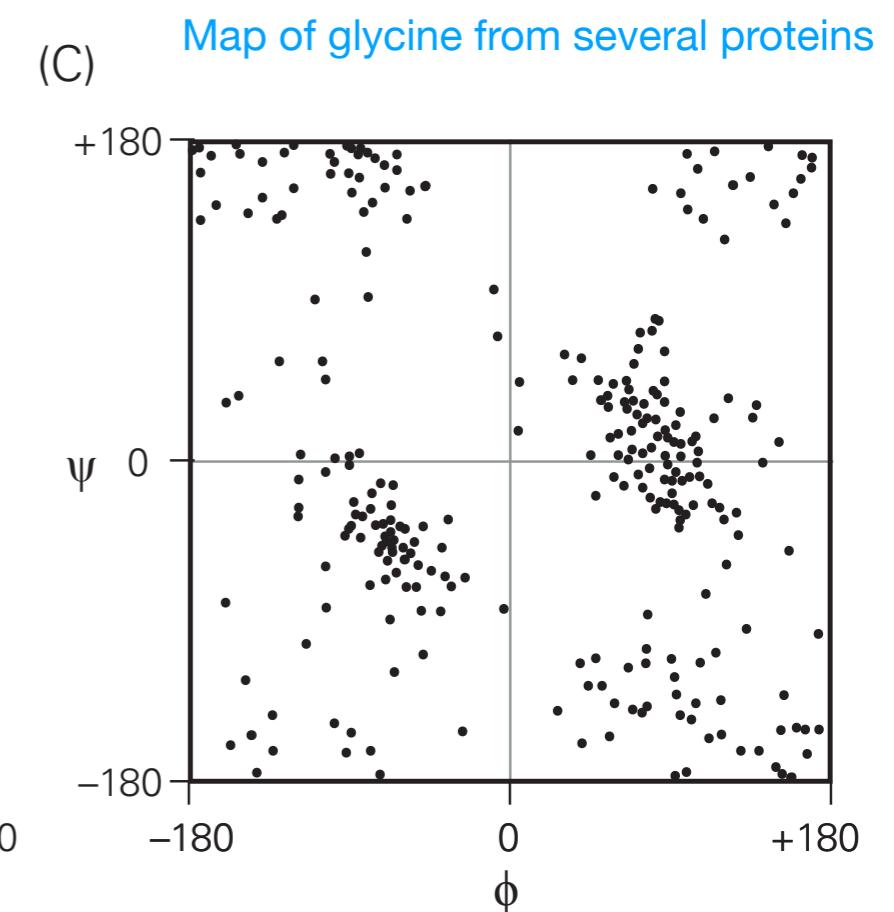
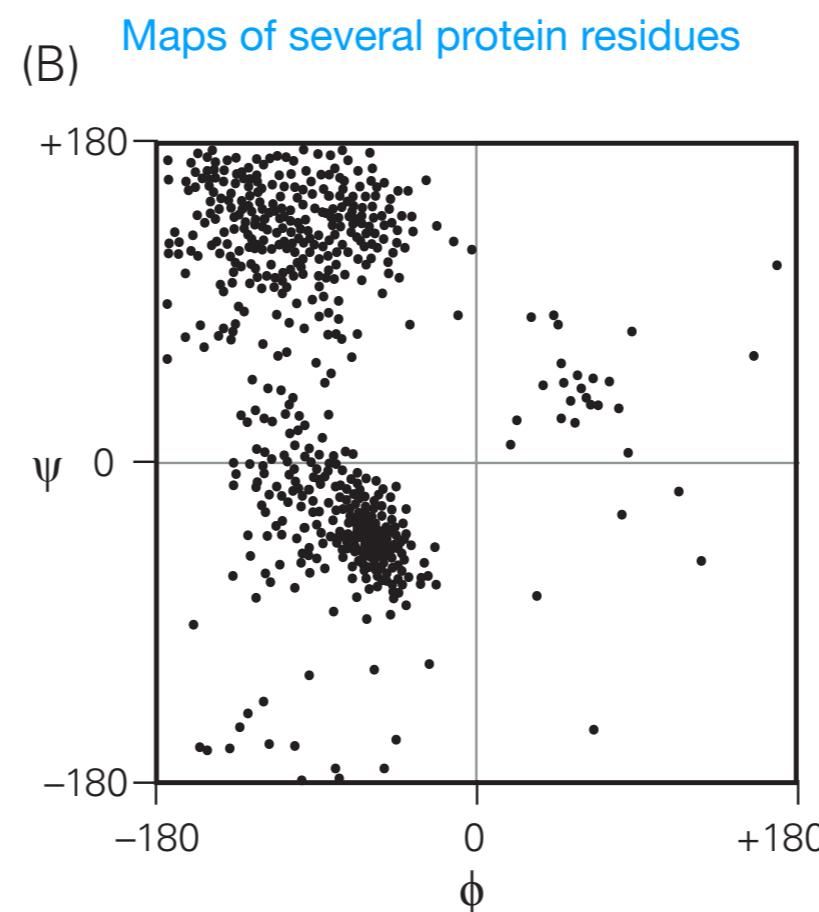
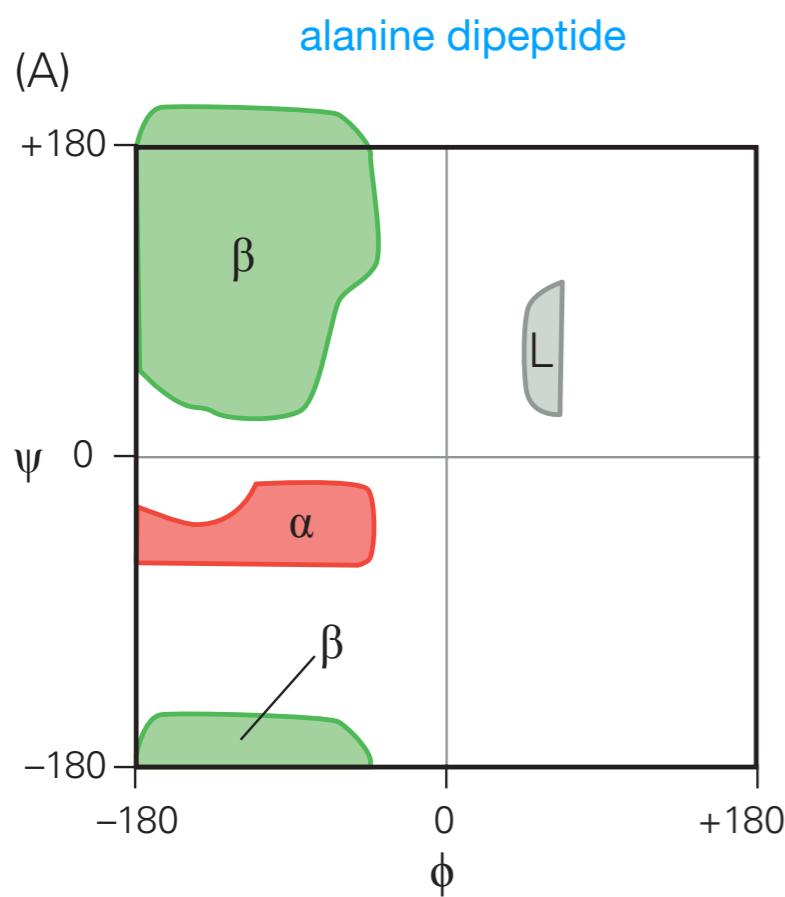


Ramachandran and colleagues considered atoms as hard spheres

Any conformation is disallowed if non-bonded atoms collide

Calculations were done for alanine dipeptide

If a conformation is disallowed for alanine dipeptide, it is disallowed for any residue except glycine



Robustness of the Ramachandran diagram

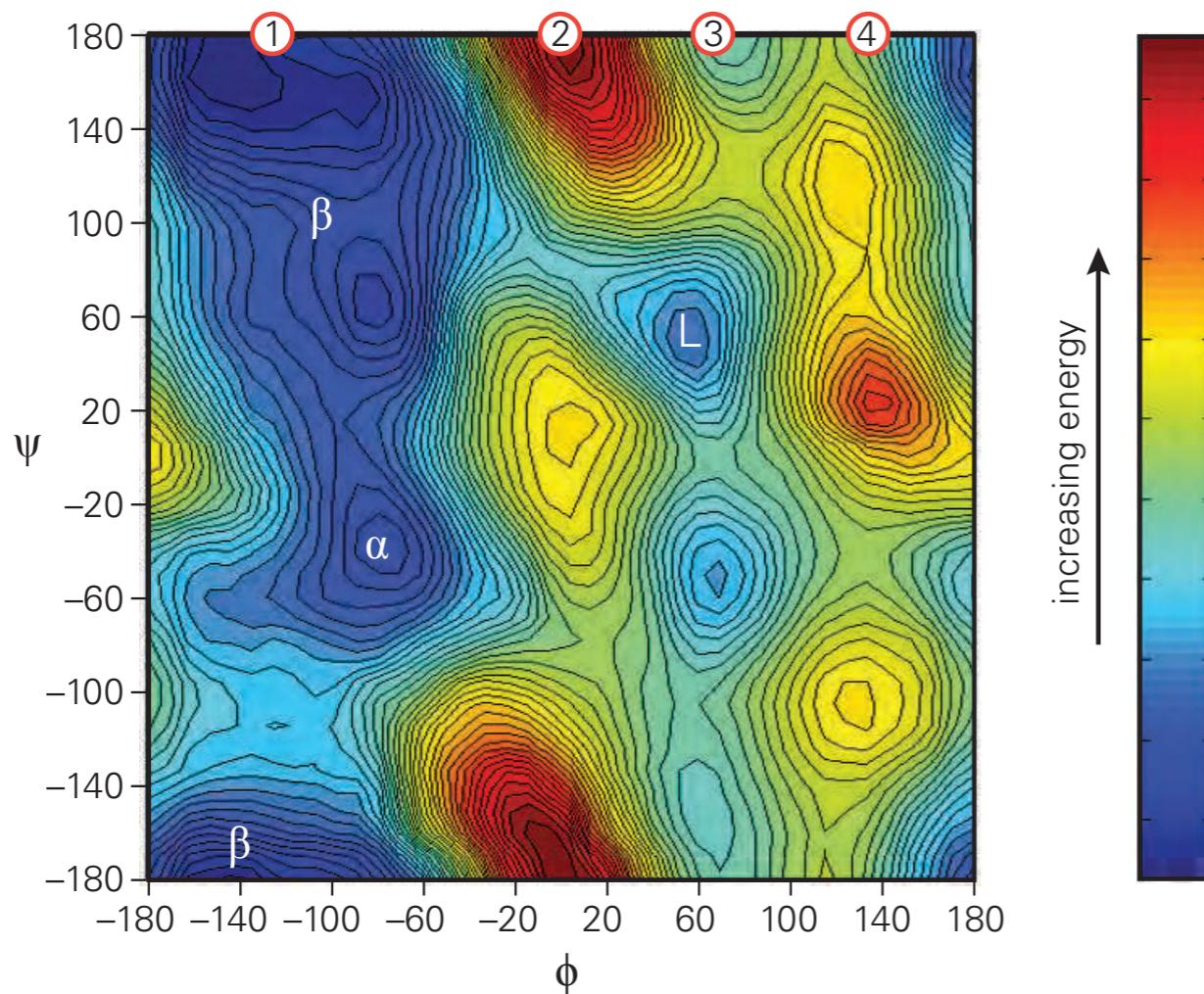
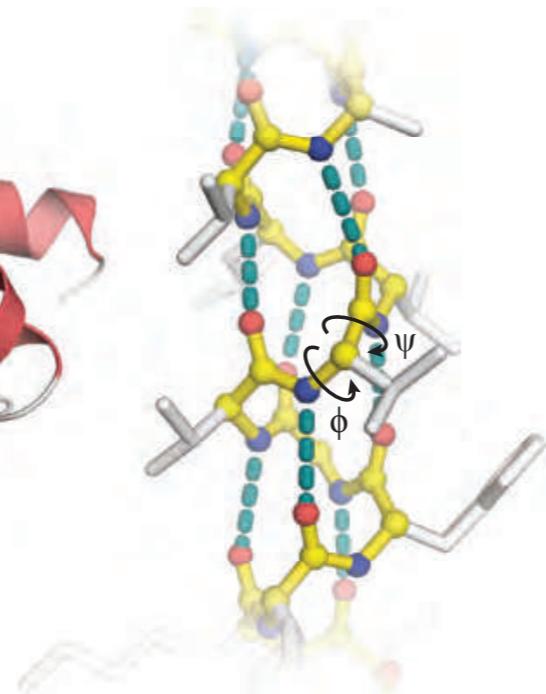


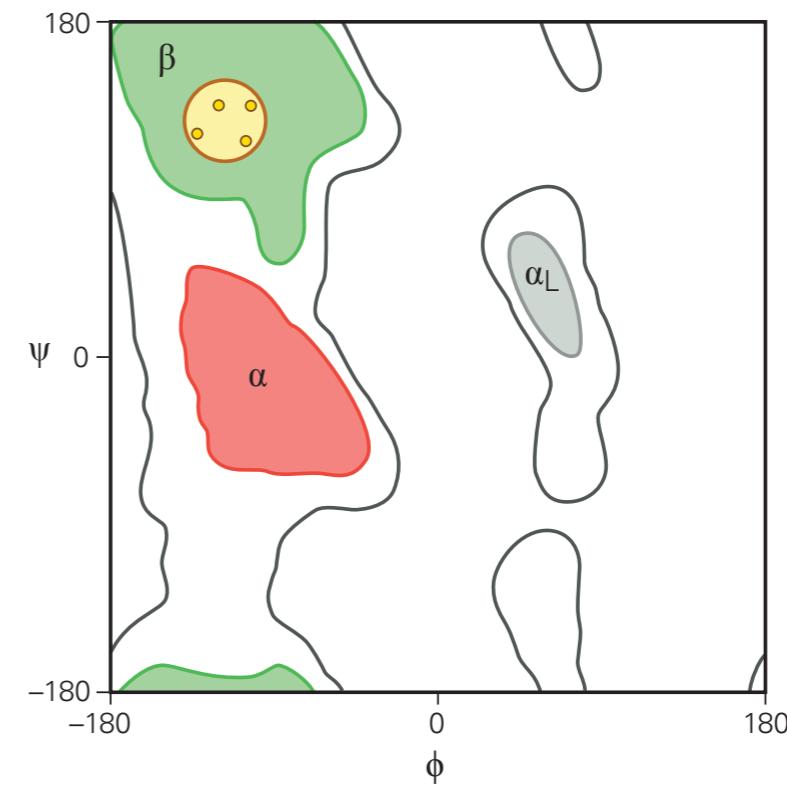
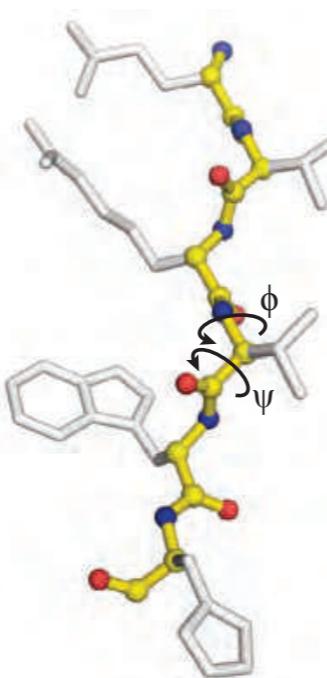
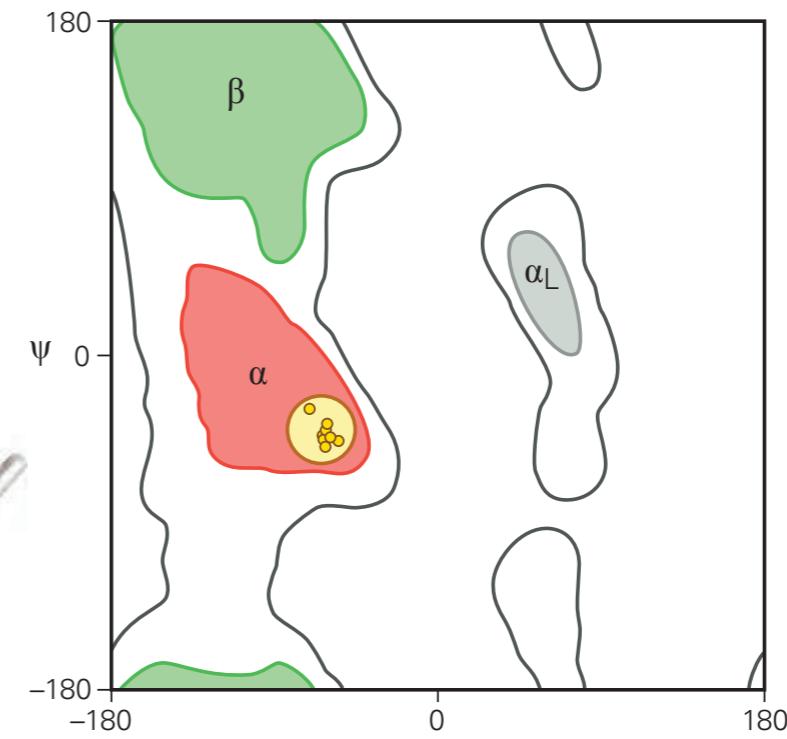
Figure 4.21 A sophisticated version of the Ramachandran diagram.

Shown here is a Ramachandran diagram for the alanine dipeptide that uses a sophisticated evaluation of the energy of each conformation. Hydrogen bonding is accounted for, as is the interaction of the peptide with water molecules. Low- and high-energy regions are in *blue* and *red*, respectively. Notice that the lowest energy regions of this diagram are the same as those in Figure 4.20A. The conformations of a peptide with ϕ, ψ values corresponding to the points labeled 1–4 are shown in Figure 4.22. (Adapted from D.S. Chekmarev, T. Ishida, and R.M. Levy, *J. Phys. Chem. B* 108: 19487–19495, 2004. With permission from the American Chemical Society.)

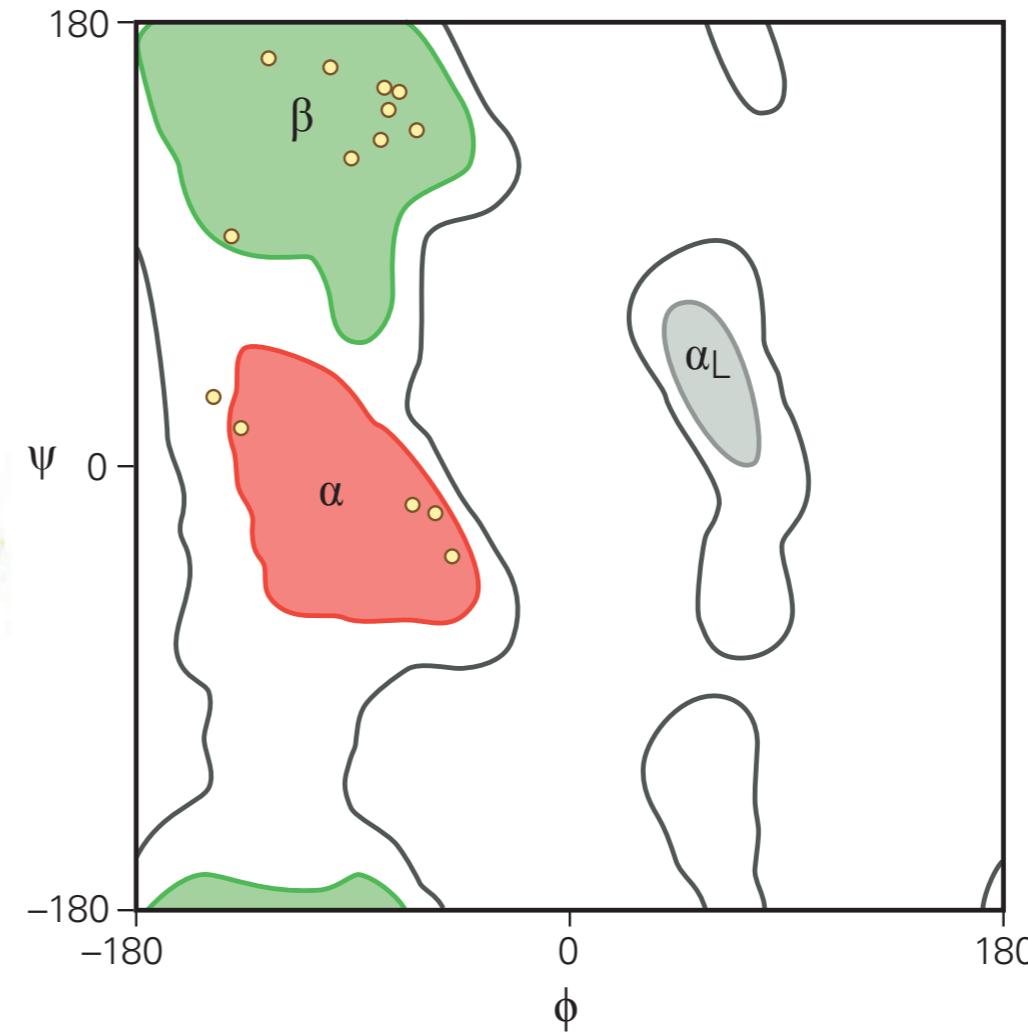
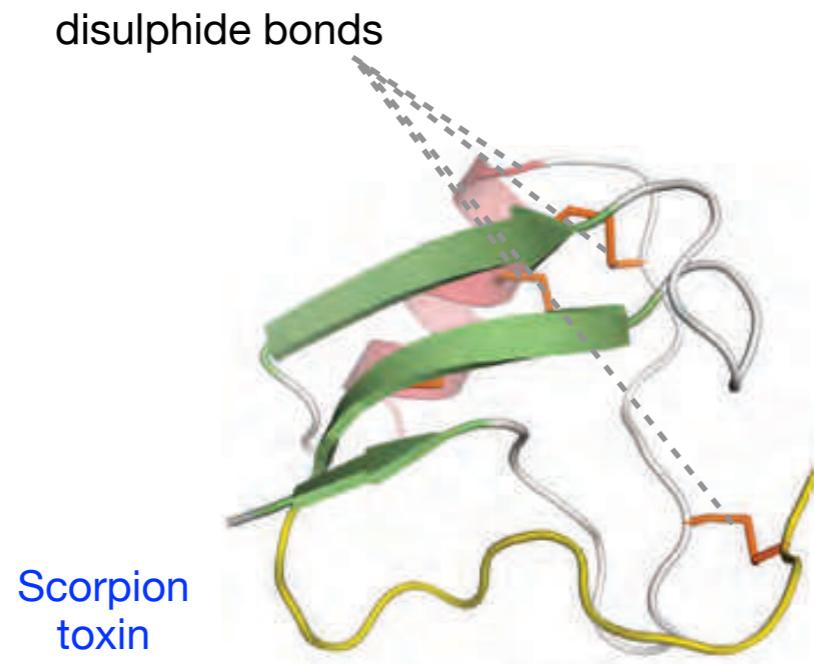
α helices and β strands form when consecutive residues adopt similar ϕ and ψ



Myoglobin



Loop segments have residues with very different values of ϕ and ψ



Usually protein structures are stabilized by helices and strands and the hydrophobic core

How is such a big loop stabilized?

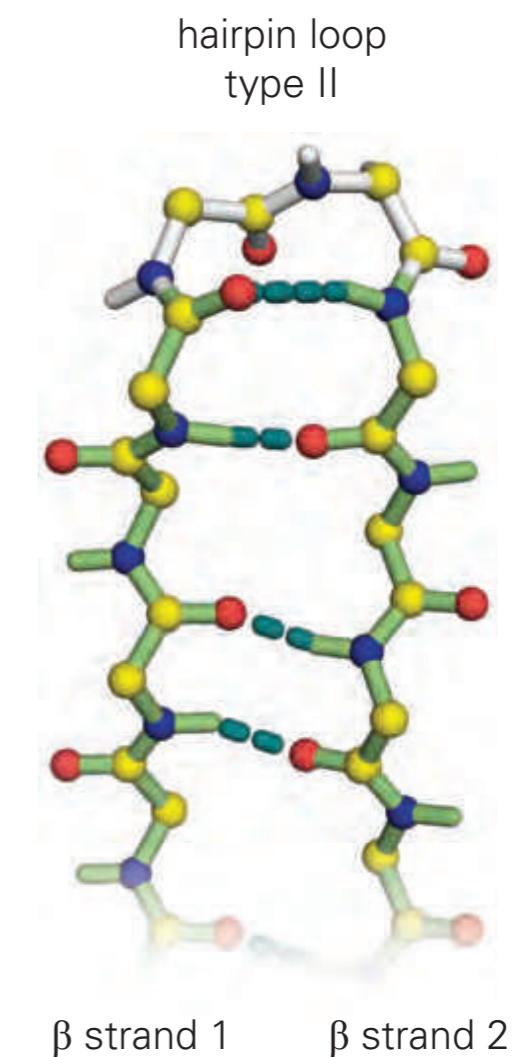
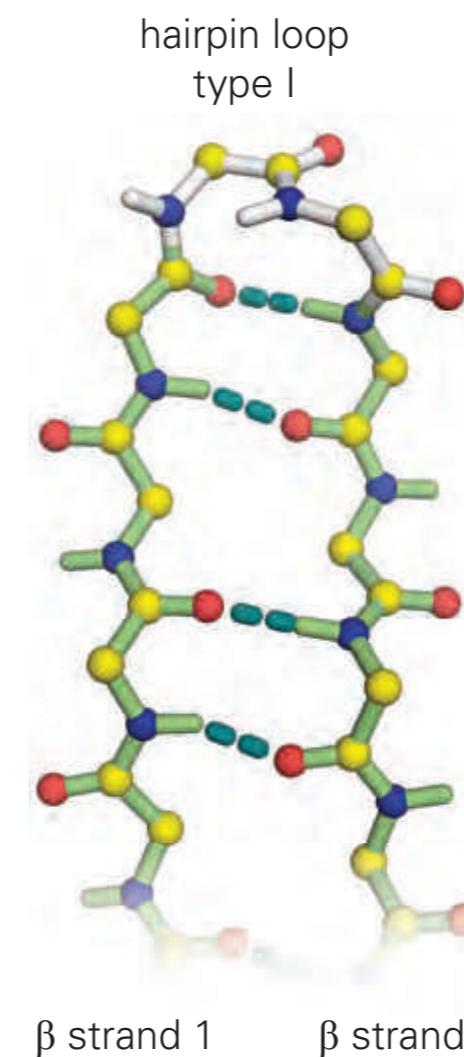
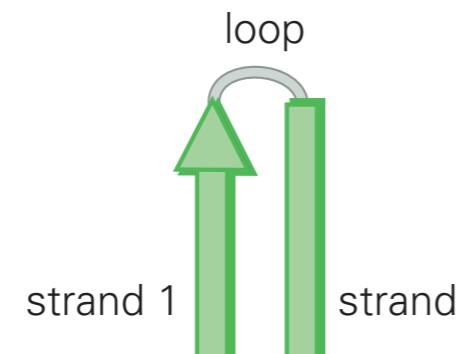
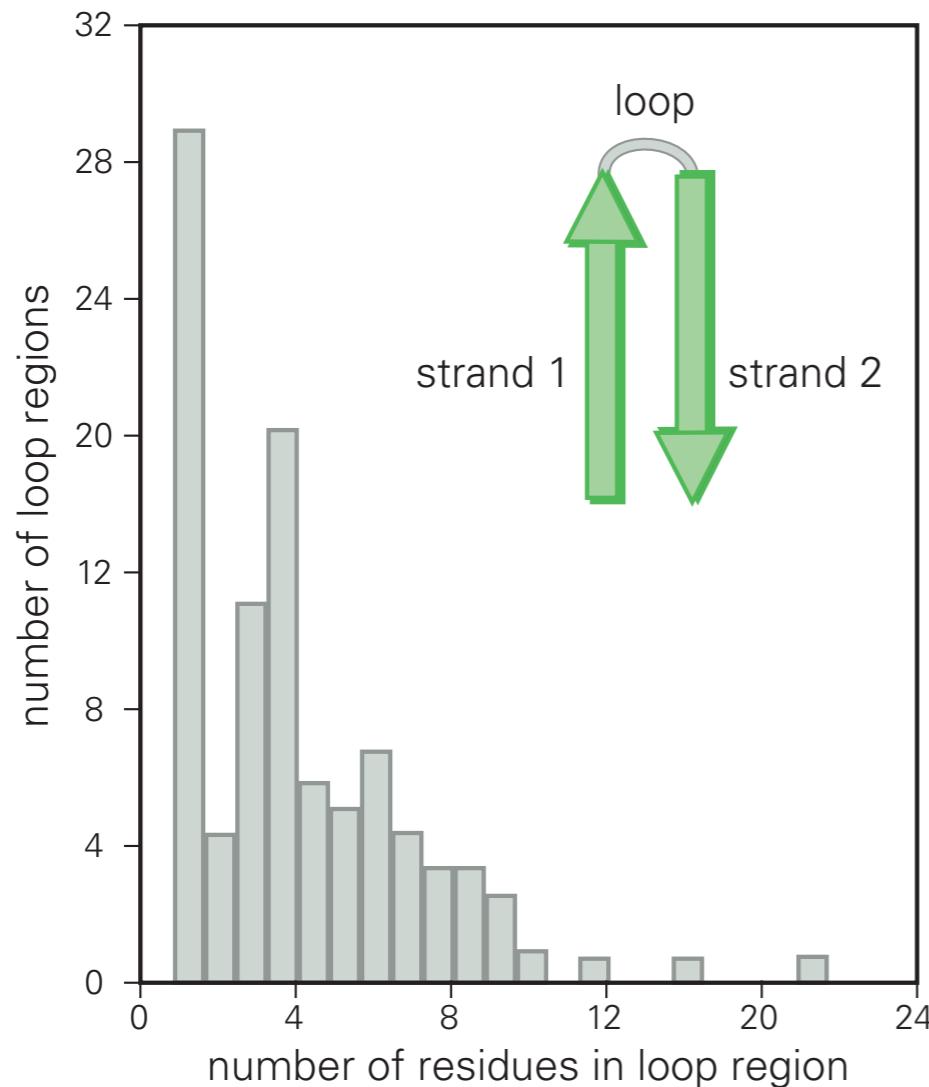
This loop is stabilized by disulphide bonds which are unstable inside the cell due to presence of reducing agents

Such disulphide bonds are common in secreted proteins

The back-bone C=O and NH groups of the loop regions are exposed to water, and no need to engage in H-bonds within the protein. This feature relaxes the necessity to form a repeating structure that satisfies backbone H-bonding requirements, as in α helices and β sheets.

As a consequence, the values of ϕ and ψ in consecutive residues in a loop segment can be quite different.

Another type of loop segment commonly observed: β -hairpin loops



β -hairpin loops are usually quite small

β -hairpin loops are usually of two kinds that allow the anti-parallel architecture of the polypeptide chain