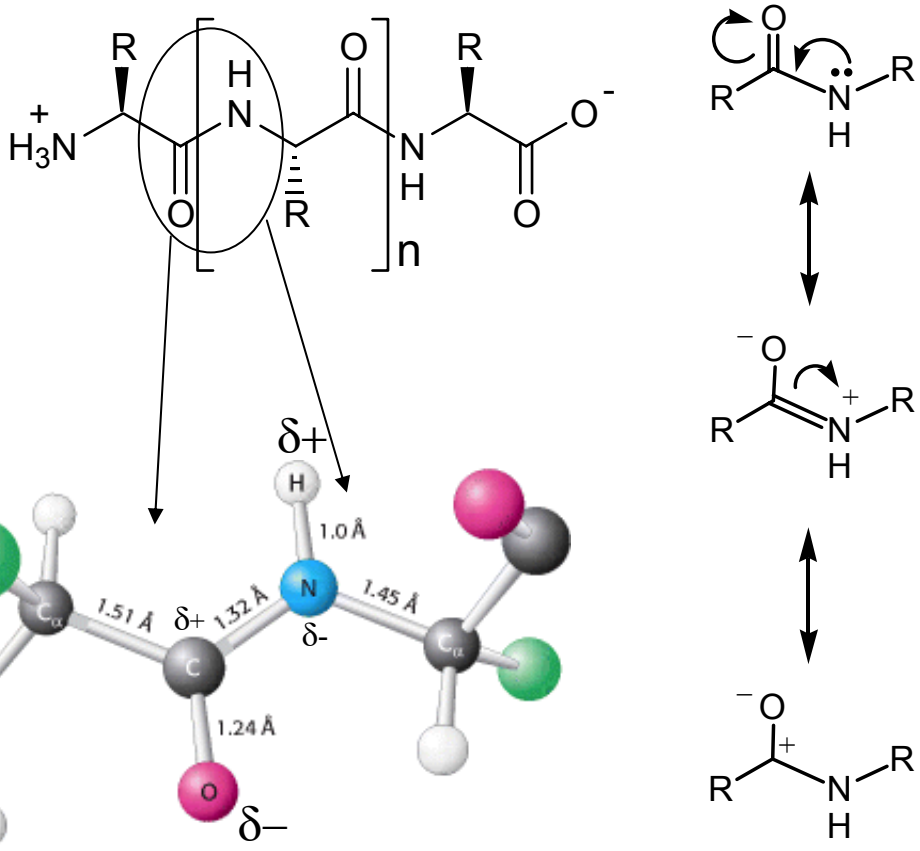
Peptide Bonds: Structure

Peptide primary structure

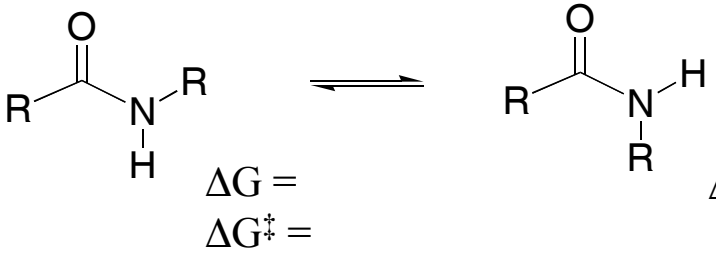
The amino acid sequence, from N- to C-terminus, determines the primary structure of a peptide or protein. The amino acids are linked through amide or peptide bonds.



The C-N distance in a peptide bond is typically 1.32 Å, which is intermediate between the values expected for a C-N single bond (1.49 Å) and a C=N double bond (1.27 Å).

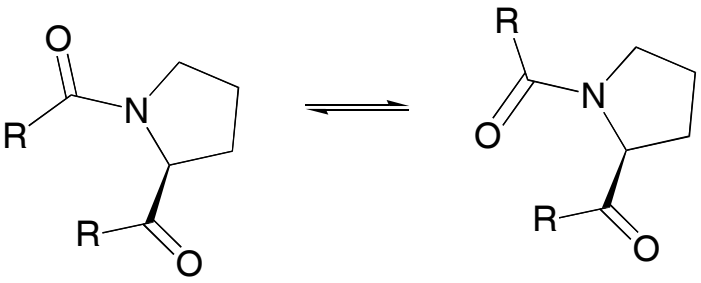
Cis-trans isomerism

Having partial double bond character, the peptide bond is planar. For steric reasons, the *trans* configuration is normally favored in peptides and proteins. Only rarely (ca. 0.03%) are *cis*-peptide bonds seen in natively folded protein crystal structures. Proline is an exception, where the proportion in the *cis* form is larger (ca. 5.2% of Xaa-Pro in protein crystal structures; or 10-30% of the *cis* form in linear unfolded peptides in water). The energy differences have been estimated in small model peptides:



ΔG =
ΔG‡ =

ΔG = -RT lnQ
Q =



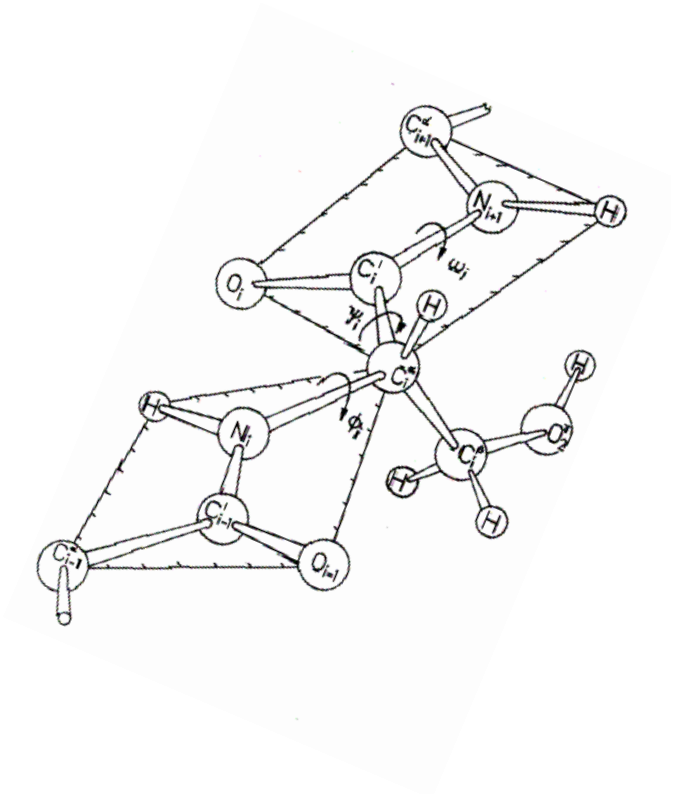
ΔG =

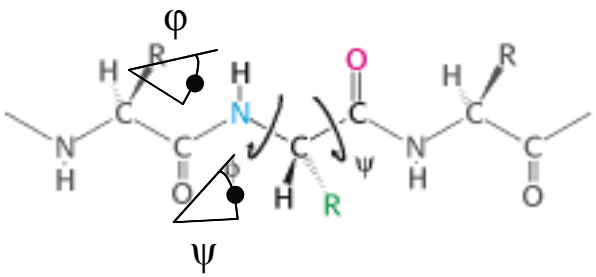
ΔG‡ =

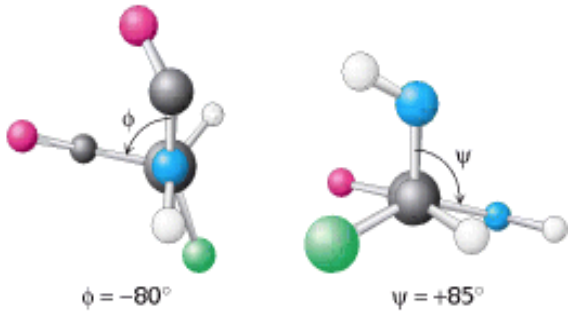
Peptide Conformation

Backbone Conformation

In order to describe the backbone conformation of a peptide or protein it is necessary to define the torsion angles ϕ, ψ und ω for each residue. The angle of rotation about the bond between the nitrogen and the α-carbon atoms is called phi (φ). The angle of rotation about the bond between the α-carbon and the carbonyl carbon atoms is called psi (ψ). A clockwise rotation about either bond as viewed from the front to the back group corresponds to a positive value. The torsion angle is zero when the neighboring backbone atoms are in *cis*-conformation. When the backbone atoms are *trans* (anti-periplanar) the torsion angle is 180º.





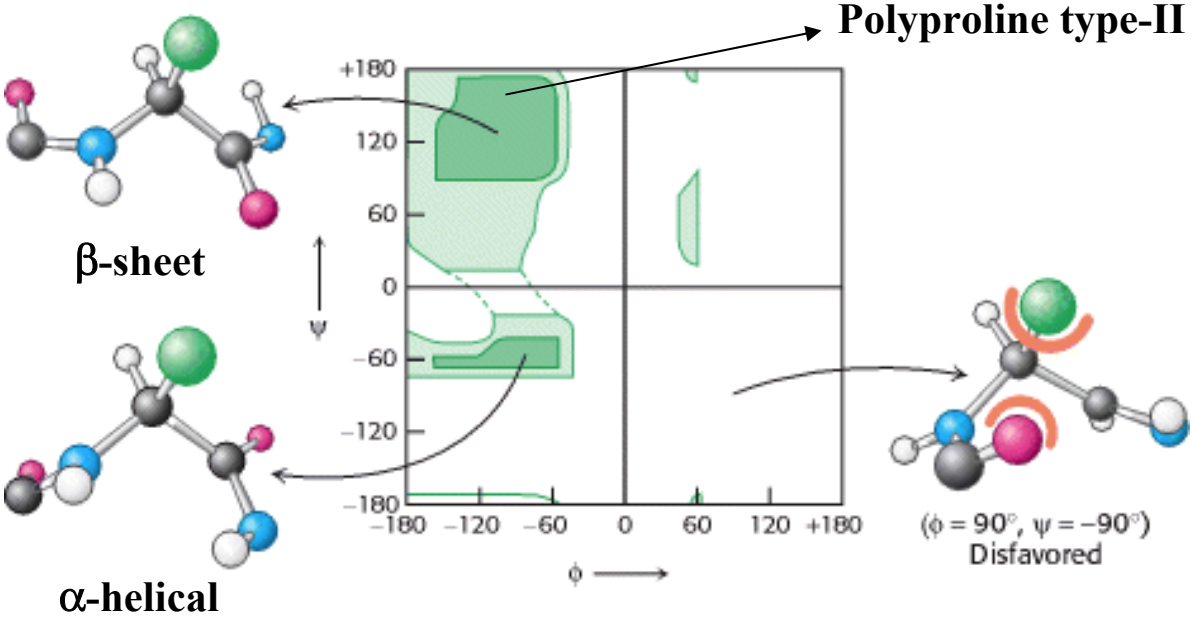


Peptide Conformation

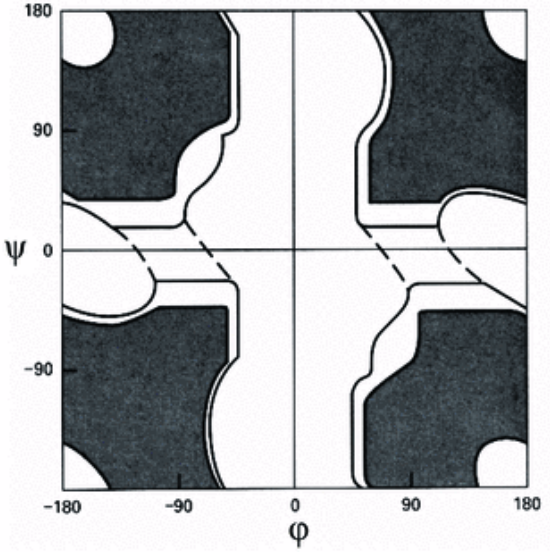
Backbone Conformation

In 1951, Linus Pauling and Robert Corey proposed two periodic structures for polypeptides called the alpha helix (α-helix) and the beta pleated sheet (β-sheet). They hypothesized that preferred backbone torsion angles for φ and ψ in polypeptides would give rise to these secondary structures (next slides).

In 1968, G. N. Ramachandran cataloged all combinations of φ and ψ forbidden because of steric collisions between atoms. He showed that the allowed values can be visualized on a two-dimensional plot, which became known as a Ramachandran plot. The classic example showing the allowed regions (green) for all amino acids except Gly and Pro is shown below. Over three-quarters of the possible (φ, ψ) combinations are excluded simply by local steric clashes. In the allowed regions, the dihedral angles characteristic of the three common secondary structures are found:



What if Gly?

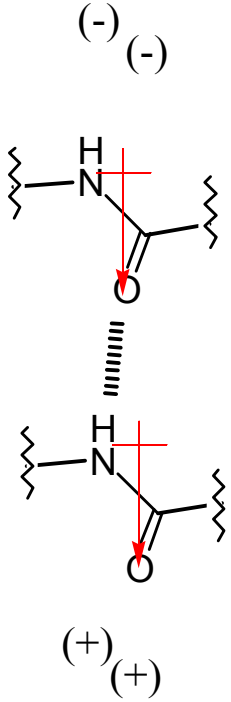


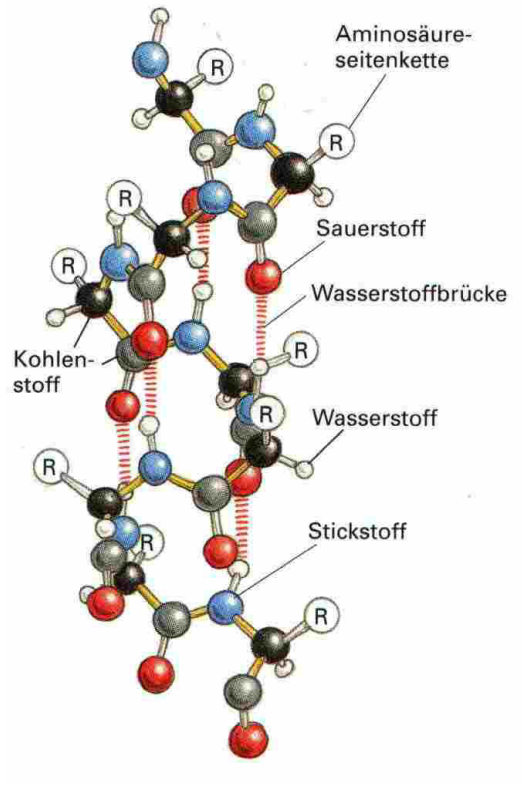
Secondary Structure

α-helix:
H-Bonds are formed between the carbonyl CO-atom of residue n and the N-H of residue n+4. The overall helix, therefore, has a dipole moment aligned along the helix axis, which is positive at the N-terminus and negative at the C-terminus.

This "macro-dipole" can be stabilized, by capping the N- and C-terminus, and/or by placing acidic residues at the N-terminus and basic residues at the C-termius.

The tendency of a peptide chain to adopt helical secondary structure depends on its amino acid sequence and length:





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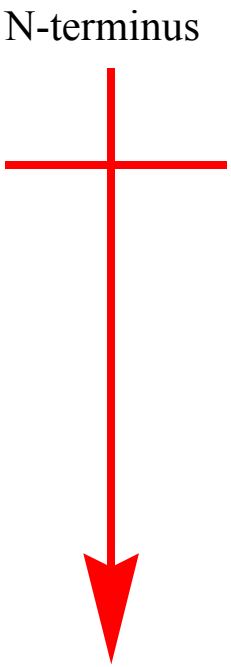
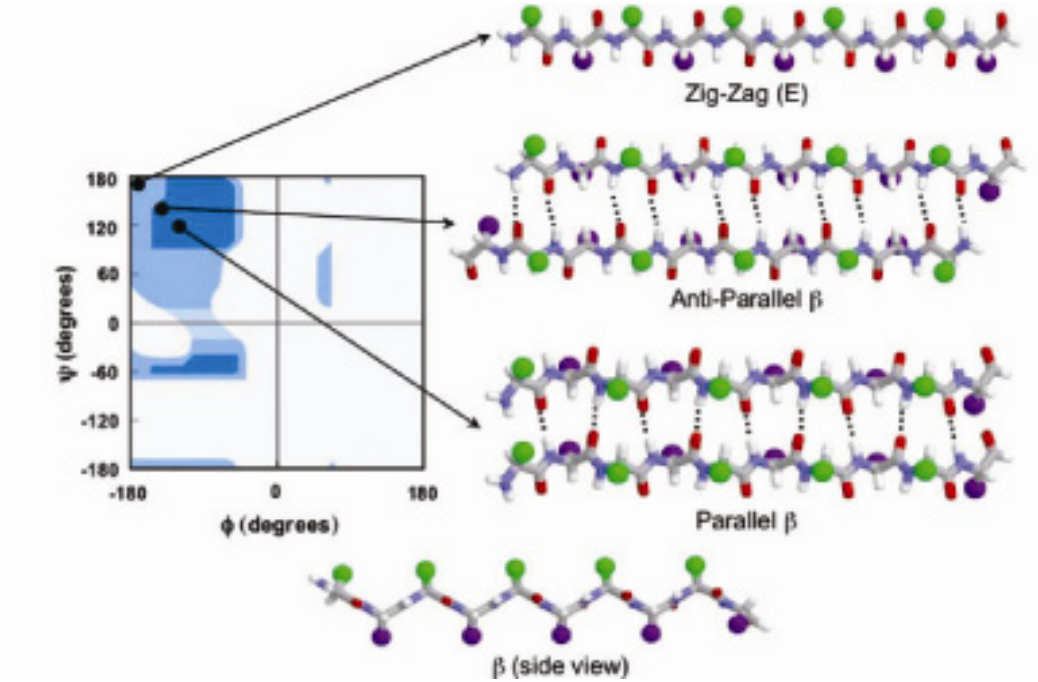


No. Amino Acids:	<5	5	6	7	8	20	40
No. of H-bonds:	0	1	2	3	4	16	36
% of possible:	0	20	33	43	50	80	90

Peptides: Secondary and Tertiary Structure

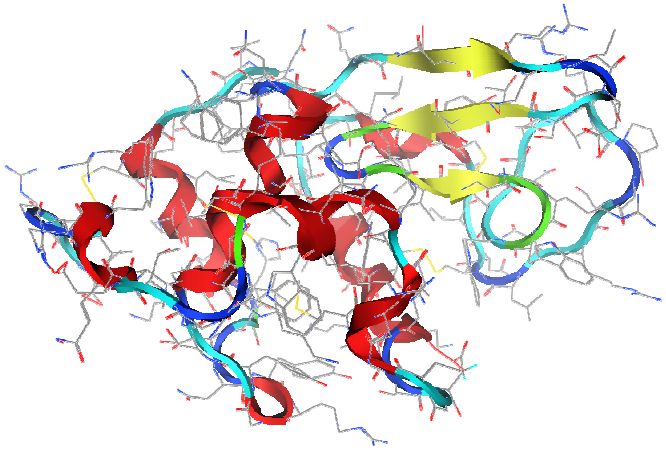
β-sheets:

Pleated ß-sheets were also predicted by Pauling and Corey in 1951. The first example was seen in the structure of hen egg-white lysozyme (*Nature*, **1965**, *206*, 759). In a **ß-sheet two or more polypeptide chains run alongside each other** and are linked in a regular manner by hydrogen bonds between the main chain C=O and N-H groups. Therefore all hydrogen bonds in a ß-sheet are between different segments of polypeptide. This contrasts with the α-helix where all hydrogen bonds involve the same element of secondary structure. The side chains of neighboring residues in a ß-strand point in opposite directions.



Tertiary Structure:

The complex interactions between the secondary structures that drive protein folding are typically mediated by **hydrophobic interactions**. Interestingly, the **isolated secondary structures** from most proteins are not usually stable outside the context of the protein.

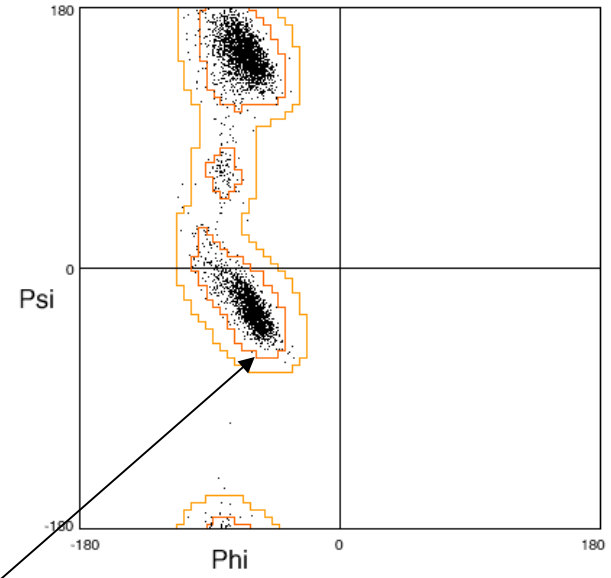


Biomimetic chemistry:

One important direction in chemical biology is the development of small molecules or constrained peptides used to mimic small regions of folded proteins.

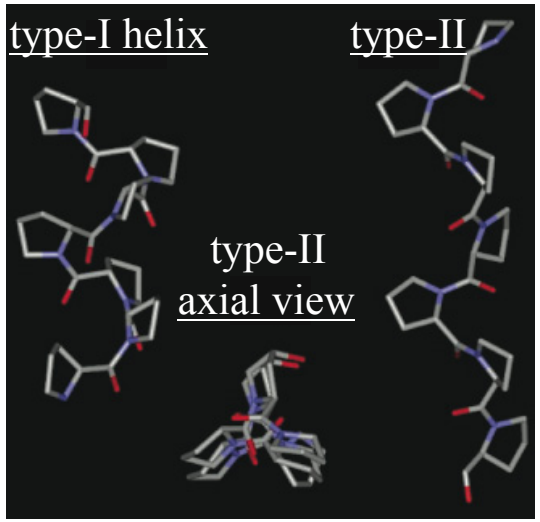
Conformational Constraints of Peptides and Proteins

Proline:
This cyclic residue can only adopt a very limited number of stable conformations. It is known as an "**α-helix breaker**" as none of these conformations are compatible with the α-helix. Polymers of proline in water adopt a left-handed polyproline type-II helical conformation, a secondary structure found in ~50% of proteins and possibly the predominant conformation of denatured proteins.

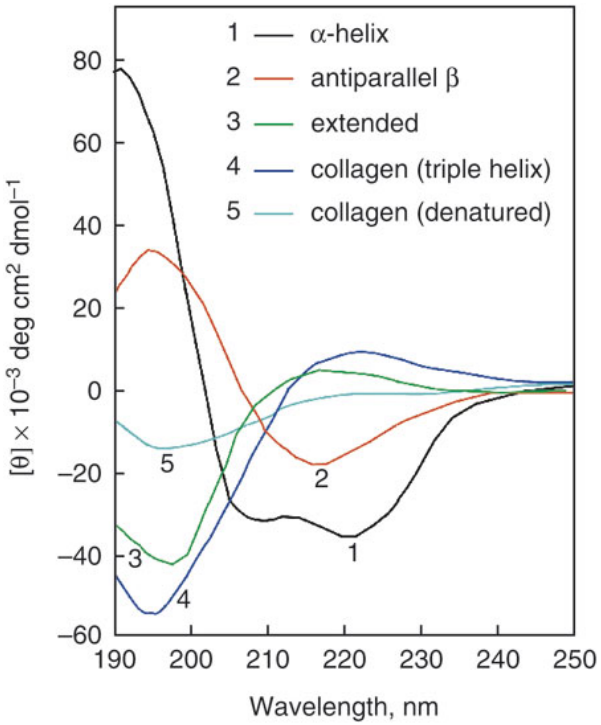


α-helix

Polyproline helicies:
Type-I helix is observed in organic solvents. Polyproline in water adopts a type-II helix (also collagen). The type-II helix is a common secondary structure observed in many other proteins too. Unfolded proteins often exhibit CD data consistent with type-II structure.



Circular dichroism (CD) spectra:

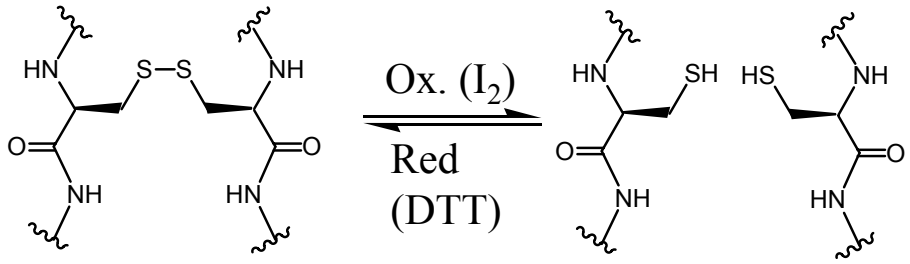


Nature Protocols 1, 2527 - 2535 (2007)

CD spectra of poly-L-lysine in: (1) α-helical (black, pH 10), (2) antiparallel β-sheet (red, pH 11), and (3) extended conformation (green, pH 7). Placental collagen in its (4) native triple-helical (blue) and (5) denatured (cyan) forms. Note that the extended conformation of poly-L-lysine was original described as a "random coil", but its spectrum is similar to the conformation of poly-L-proline II, which forms an extended left-handed helix.

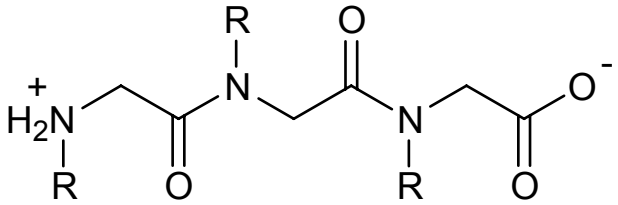
Conformational Constraints of Peptides and Proteins

Disulfide bond formation (natural):
In secreted proteins disulfide bonds between Cys residues help to maintain the protein's tertiary structure.

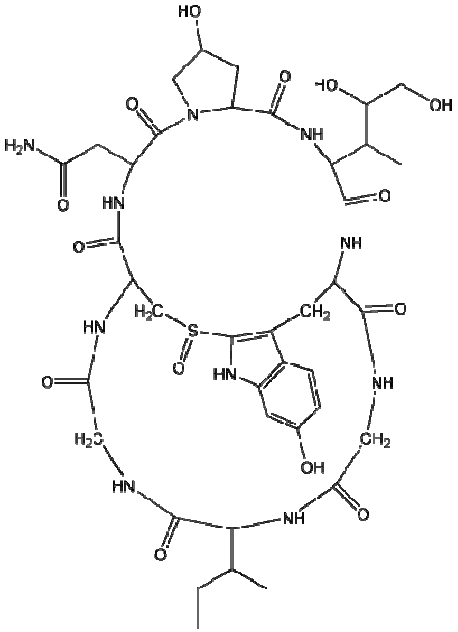


Cysteine Cystine

Peptoids (synthetic):
Poly-N-substituted glycines, are a class of peptidomimetics whose side chains are appended to the nitrogen atom of the peptide backbone. Peptoids with alpha-chiral bulky side chains are known to adopt a Polyproline-type I-like conformation.



Cyclization (natural):
Many natural peptides, and man made mimetics are backbone and/or side-chain cyclized. This natural product **α-amanitin**, is a potent inhibitor of RNA polymerase II and has an LD50 of 0.1 mg/kg in mammals.



β-Peptides (synthetic):
β-peptides consist of β amino acids. Many types of helix structures consisting of β-peptides have been reported. These conformation types are distinguished by the number of atoms in the hydrogen-bonded ring that is formed in solution; 10-helix, 12-helix, 14-helix, have been reported. Generally speaking, β-peptides form a more stable helix than α-peptides