CHEMICAL SYNTHESIS OF BIOPOLYMERS

2. Proteomics

A number of very important physiological and biochemical functions of life are influenced by peptides:

- **Peptides** are involved as **neurotransmitter** and **hormones** in **receptor-mediated signal transduction**.
- **More than 100 peptides** with functions in the **central and peripheral nervous system**, in **immunological processes**, in the **cardiovascular system**, and in the **intestine** are known.
- Peptides are involved in **metabolism**, **pain**, **reproduction**, and **immune response**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

- The **isolation of peptides** from natural sources often is problematic. In many cases, the concentration of a specific peptide range from **10^{-15} to 10^{-12} mol per mg fresh weight of tissue.**
- Studies on **structure-activity relationships** involve a large number of synthetic peptide analogues with sequence variation and the introduction of **nonproteinogenic building blocks** as well as **selectively labelled amino acids**.
- Synthetic peptides serve as **antigens to raise antibodies**.
- Synthetic peptides serve as **enzyme substrate to map the active site** requirements of an enzyme under investigation.
- Synthetic peptides act as **enzyme inhibitors to influence signalling pathways**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

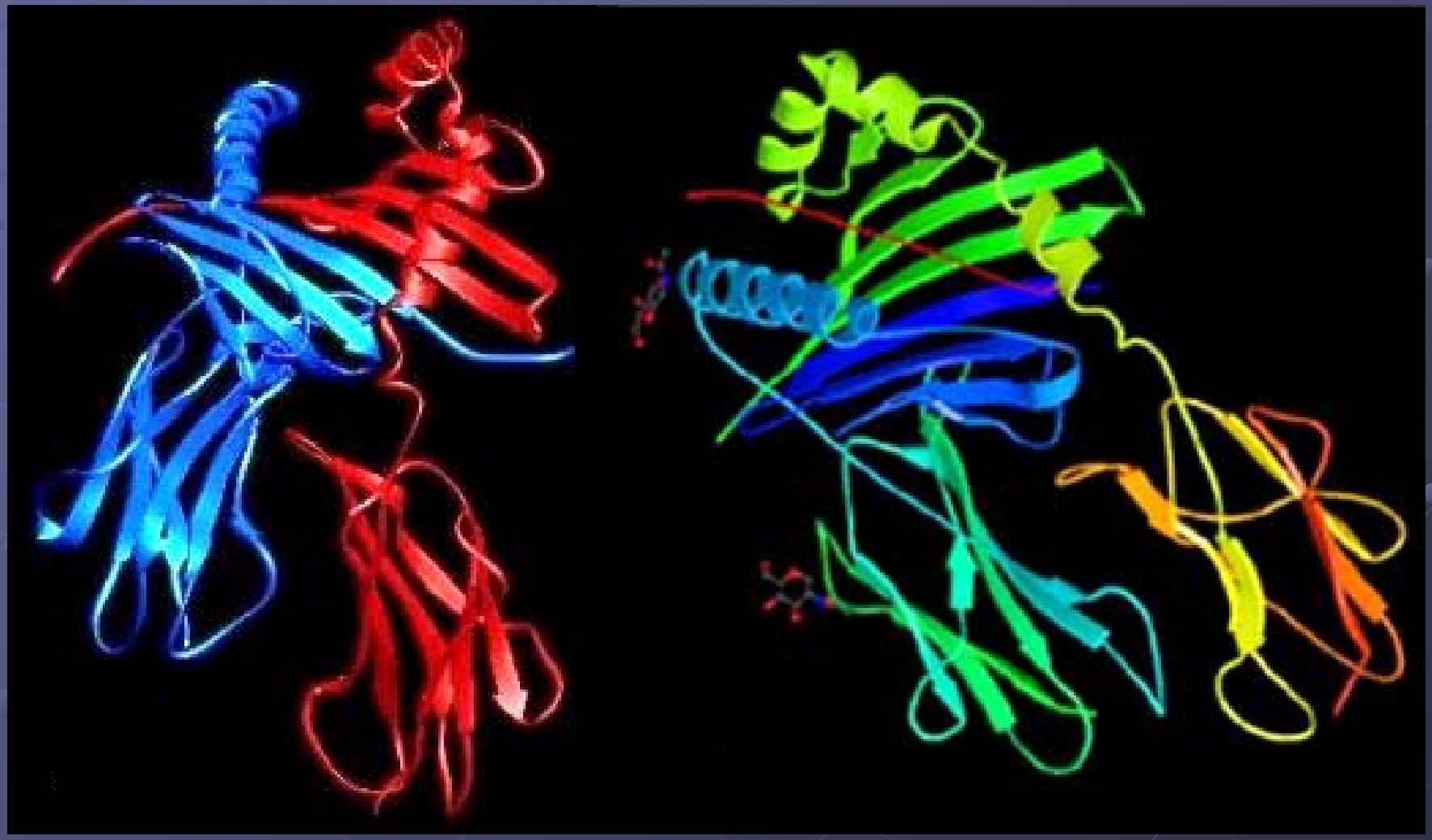
The **chemical peptide synthesis** is the classical method which has been mainly developed during the past four decades, although the foundations were laid in the early 20th century by **Theodor Curtius** and **Emil Fischer**.

Theodor Curtius

Hermann Emil Fischer
Nobel Prize in Chemistry 1902

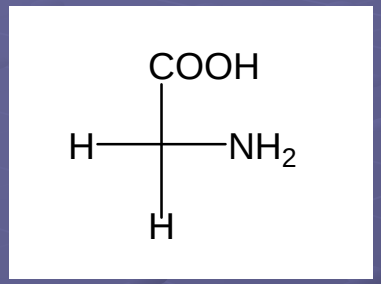
CHEMICAL SYNTHESIS OF BIOPOLYMERS

2.1 Structure and Chemistry

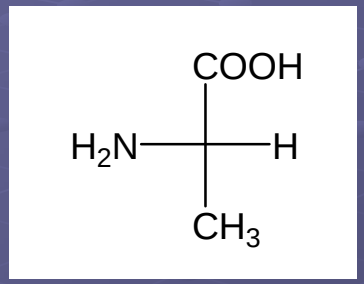


CHEMICAL SYNTHESIS OF BIOPOLYMERS

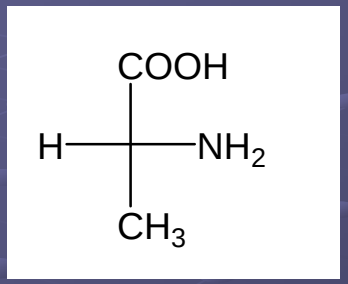
α-Amino Acids



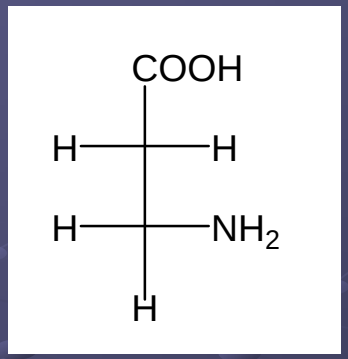
Glycin (**Gly**)



L-Alanin (**Ala**)

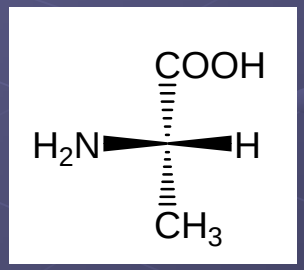


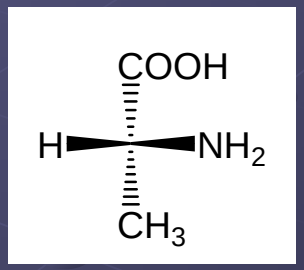
D-Alanin

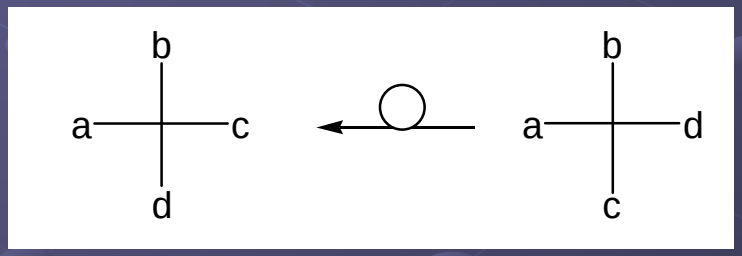


β-Alanin

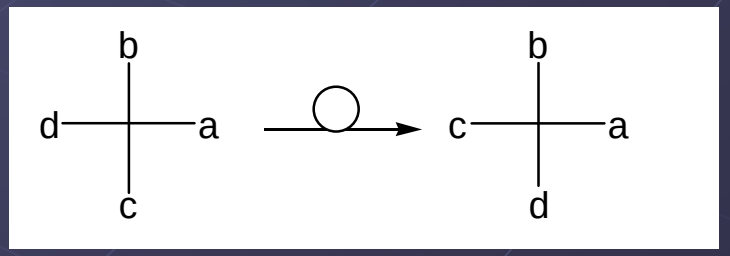
D,L-Nomenklatur – *R,S*-Nomenklatur







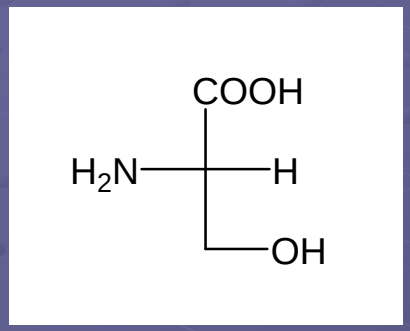
R ***S***



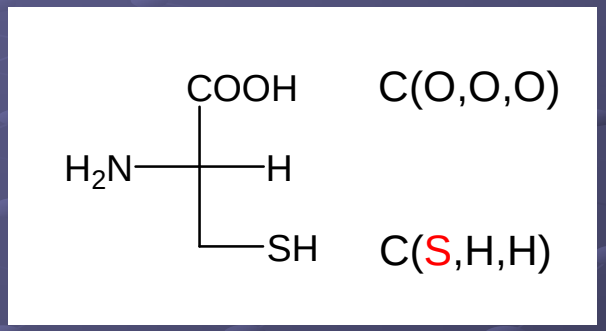
R ***S***

CHEMICAL SYNTHESIS OF BIOPOLYMERS

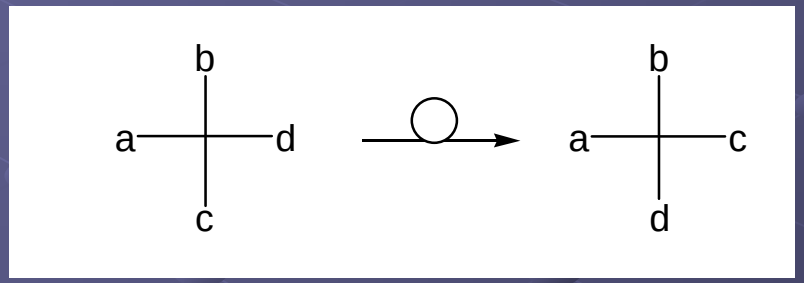
α-Amino Acids



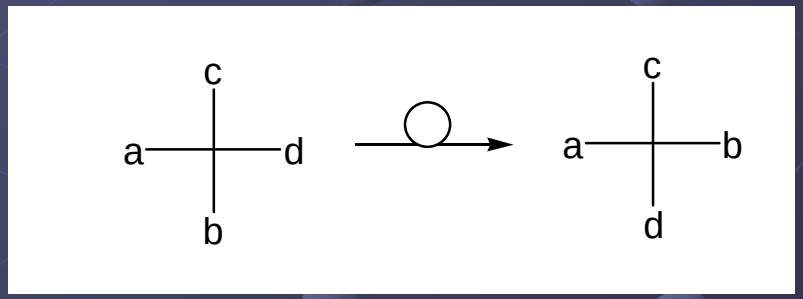
L-Serin (**Ser**)



L-Cystein (**Cys**)



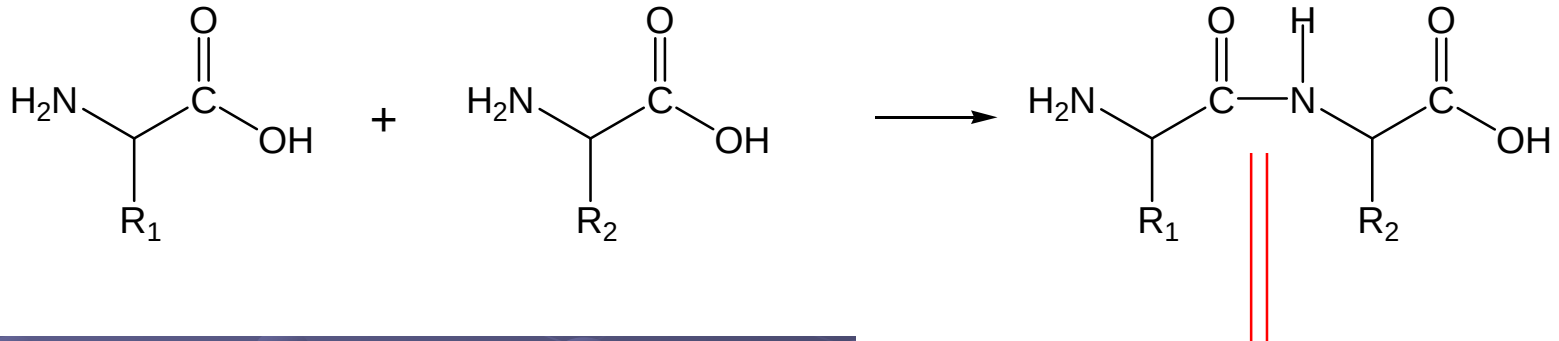
S ***R***

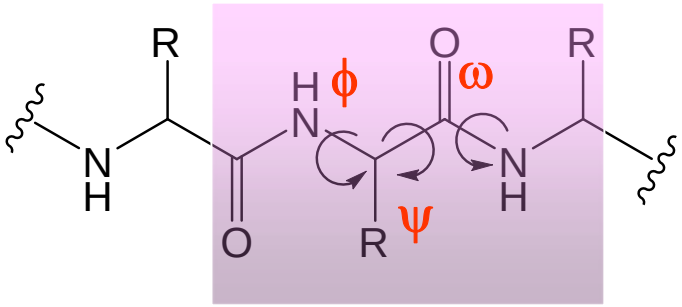


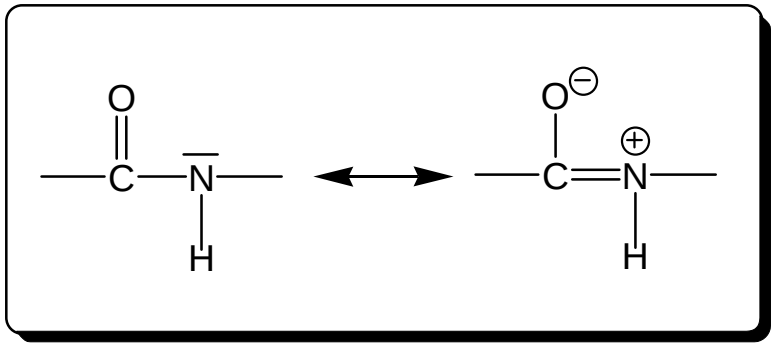
R ***S***

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Formal formation of peptide bonds







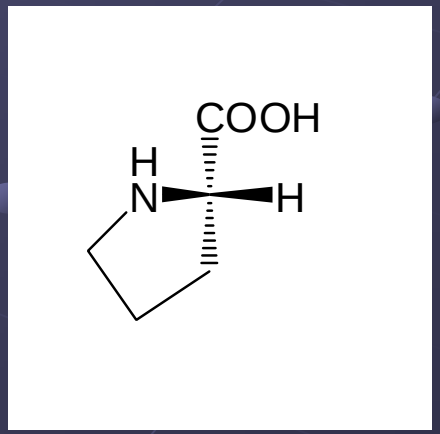
CHEMICAL SYNTHESIS OF BIOPOLYMERS

The **free rotation around the C-N-amide bond is drastically restricted** because of the partial double bond character with a rotational barrier of ~ **105 kJ mol^{-1}**.

Consequently, two rotamers of the peptide bond exist: the ***trans*-configurated** and the ***cis*-configurated peptide bond**.

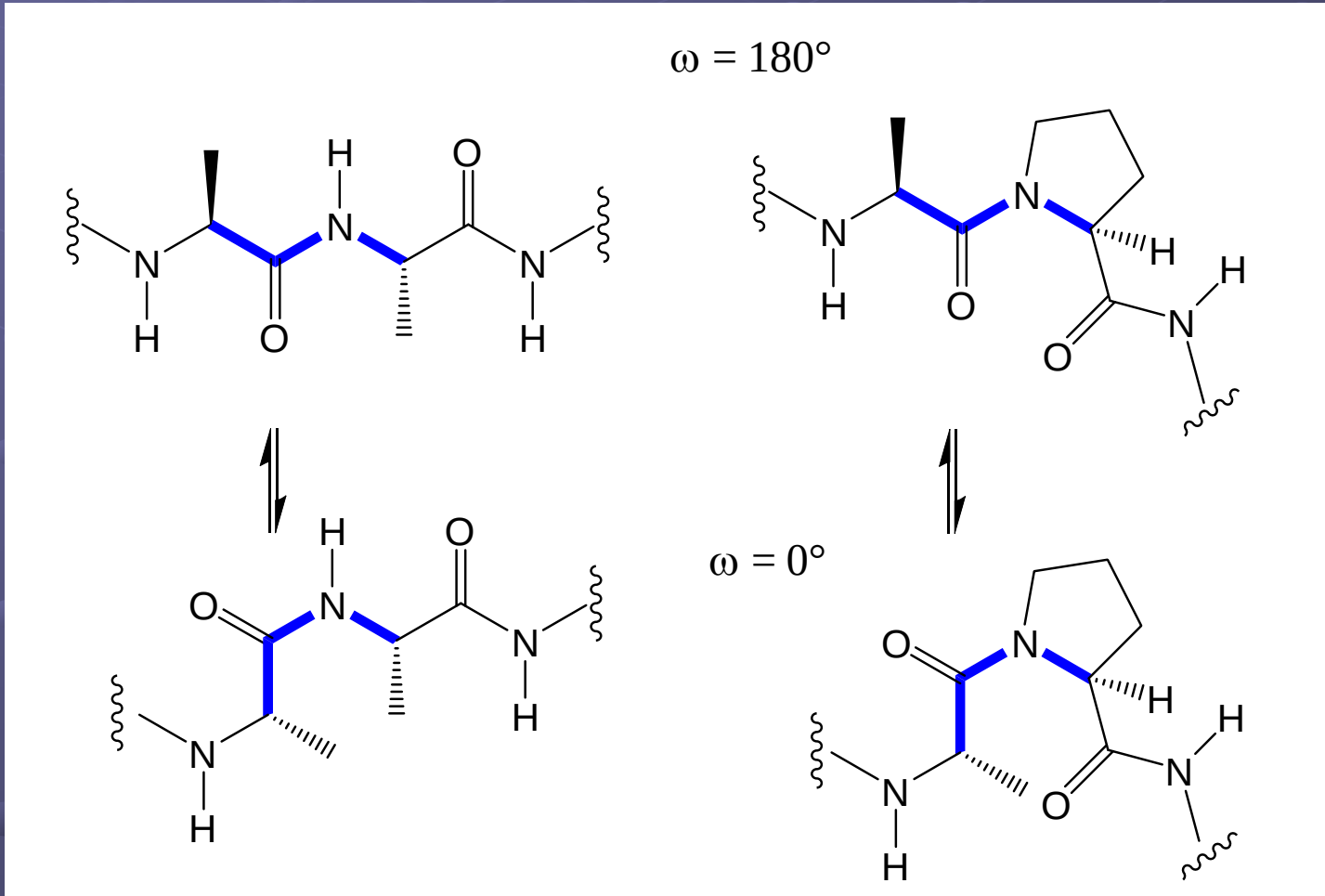
The ***trans*-configurated peptide** bond is energetically **favoured** by **8 kJ mol^{-1}** and is found in most peptides that **do not contain proline**.

The percentage of ***cis*-configurated proline** bonds (**6.5%**) is approximately two orders of magnitude higher **compared to *cis* peptide bonds between all other amino acids (0.05% *cis*)**.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

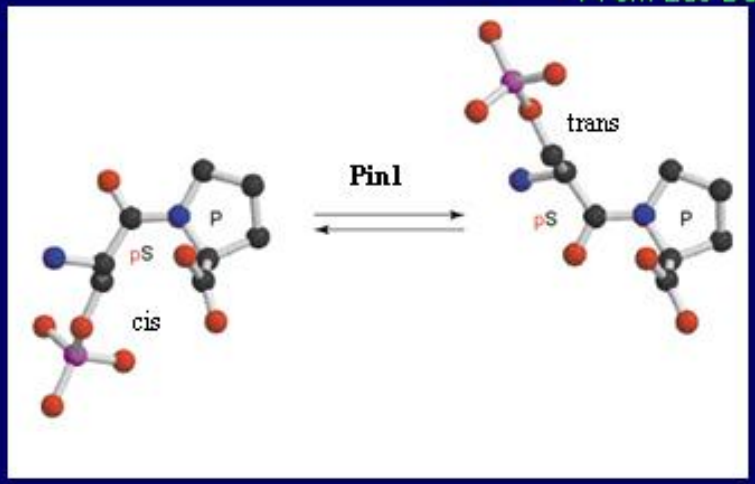
This ***cis/trans* isomerization** of peptide bonds involving the imino group of proline usually takes place in many proteins, and has a half-life between 10 s and 17 min.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Peptidyl prolin-cis/trans isomerases accelerate significantly this conformational transition.

From Luc Buée



Pin1 is a peptidyl prolyl cis/trans isomerase

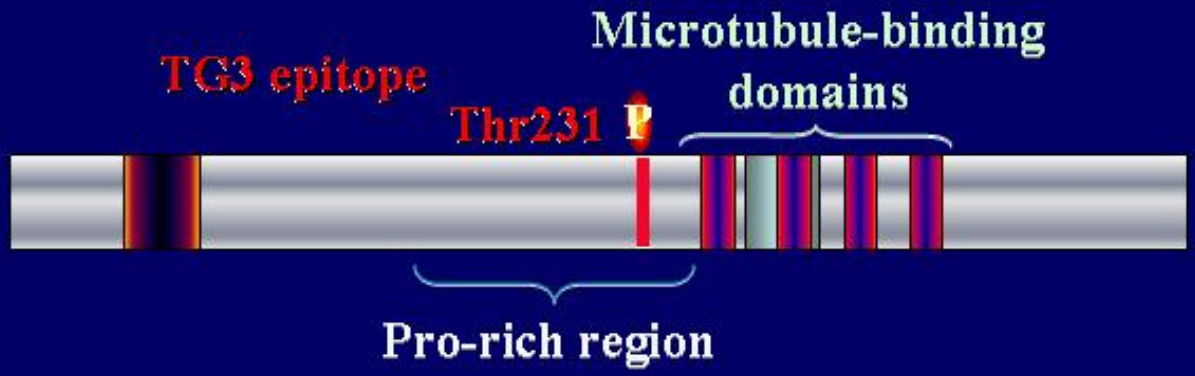
Pin1 is made of two domains:

* WW domain
* catalytic domain (rotamase)

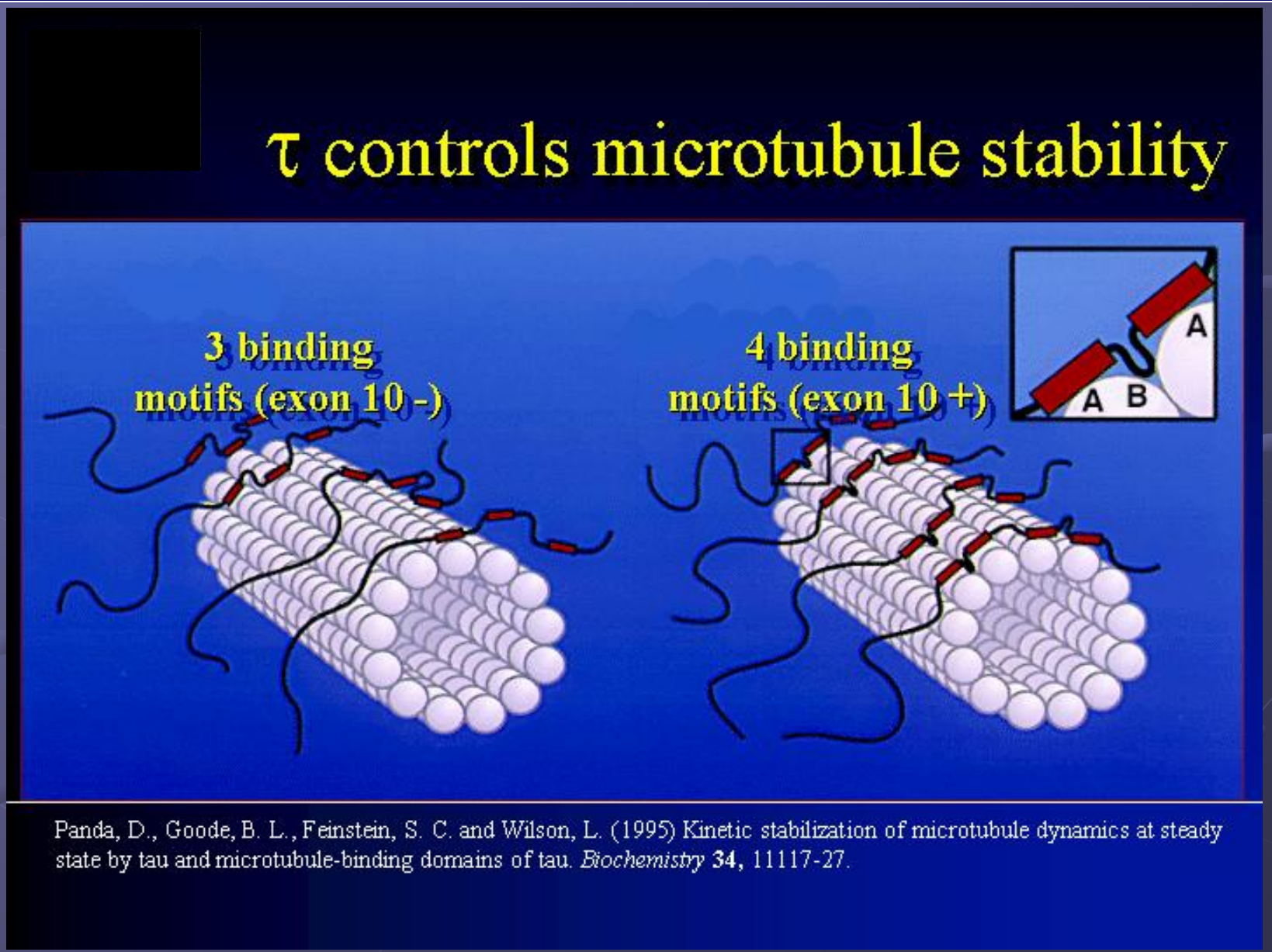
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Pin1 and Tau

- Interaction between Pin1 and Tau
  - Single interaction site Pin1-Tau: pThr231.



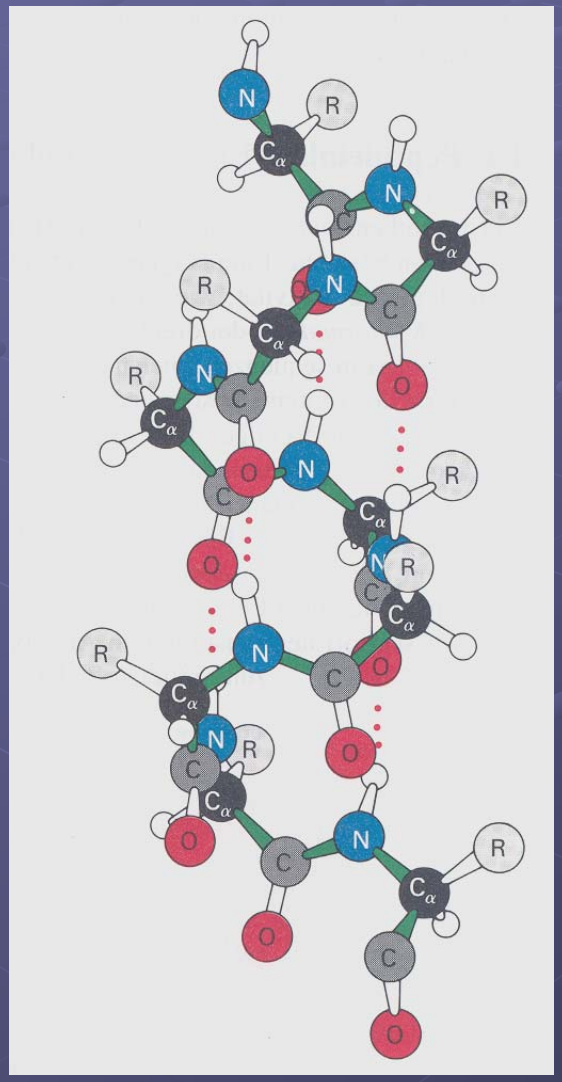
CHEMICAL SYNTHESIS OF BIOPOLYMERS



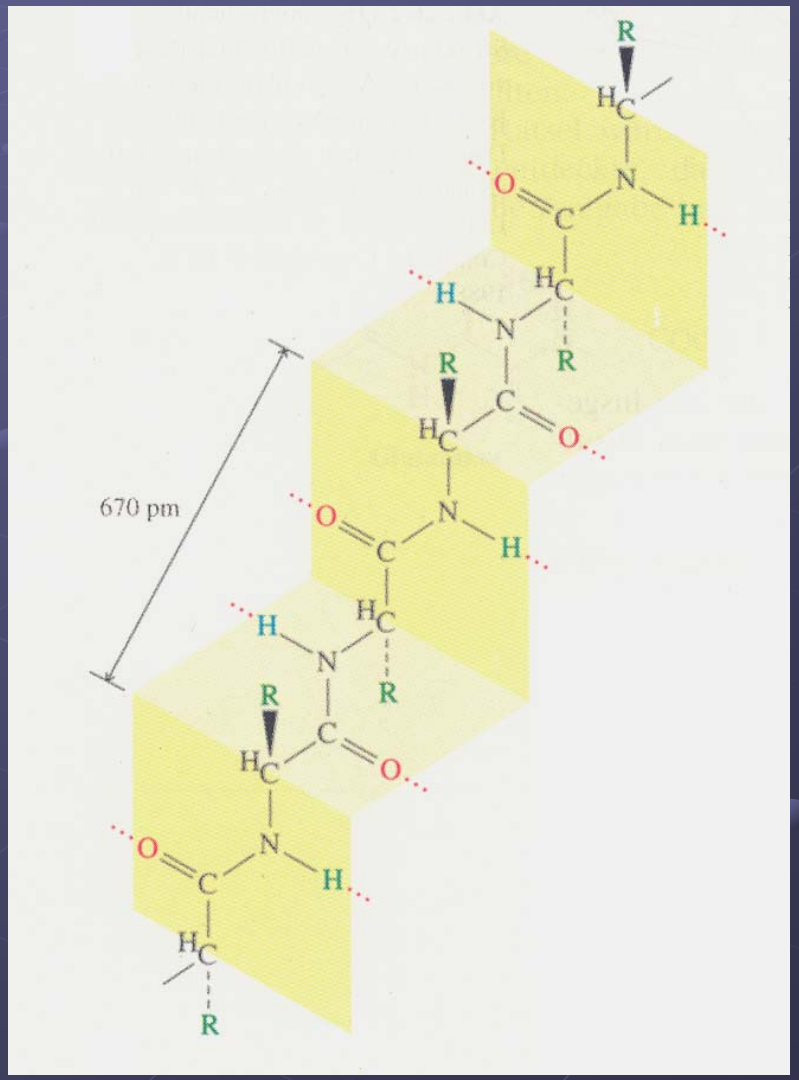
Panda, D., Goode, B. L., Feinstein, S. C. and Wilson, L. (1995) Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. *Biochemistry* **34**, 11117-27.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

α-helix structure



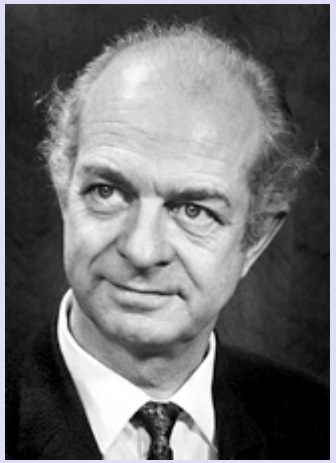
β-pleated sheet structure



CHEMICAL SYNTHESIS OF BIOPOLYMERS

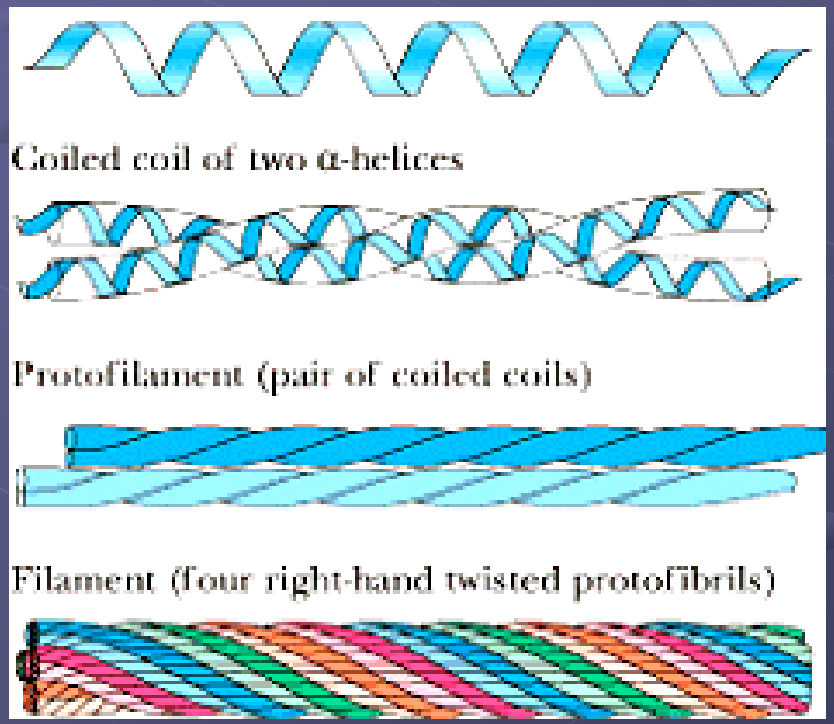
α-Keratins are main constituent in the hair (including wool), horns, nails, claws and hooves of mammals.

The harder **β-keratins** found in nails, in the scales and claws of reptiles, their shells (turtle), and in the feathers, beaks, claws of birds and quills of porcupines.

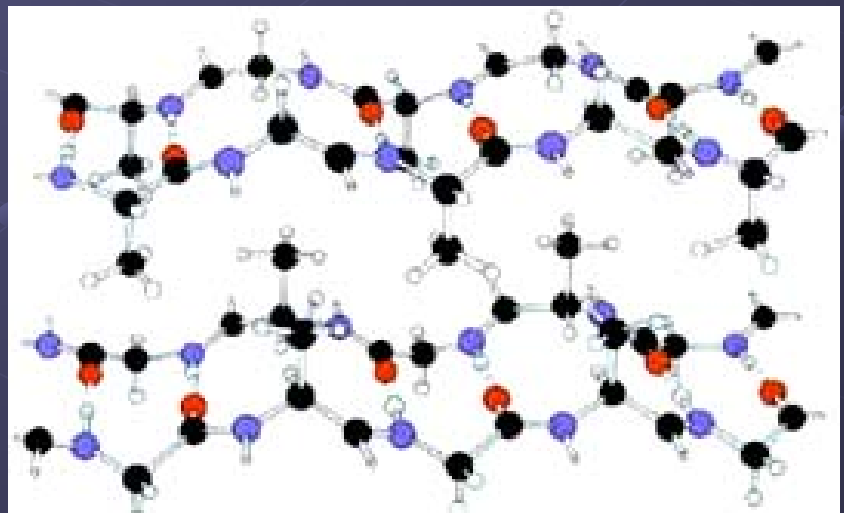


Prof. Linus Pauling
Nobel Prize
1954

α-helix structure



β-pleated sheet structure

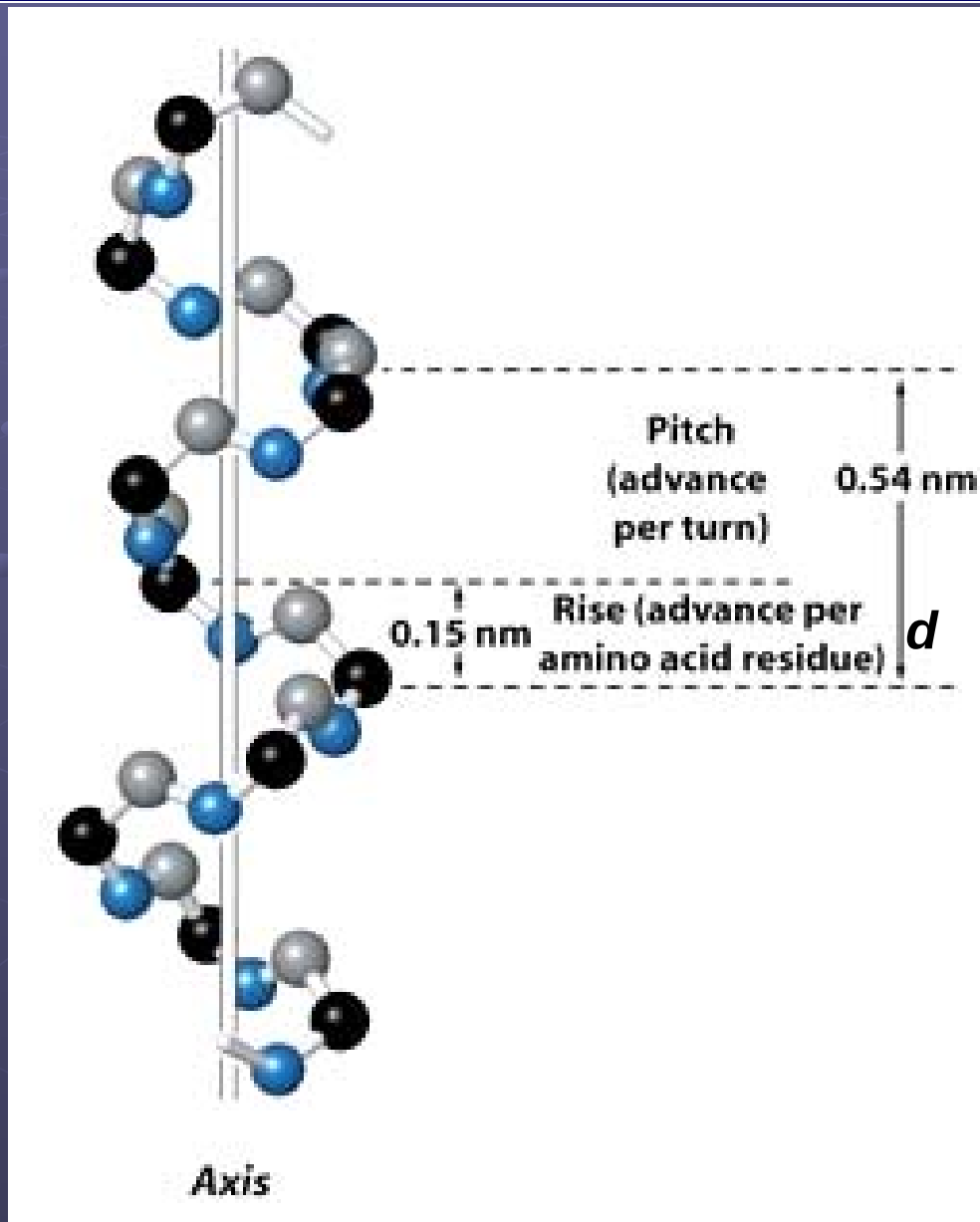


CHEMICAL SYNTHESIS OF BIOPOLYMERS

Helix

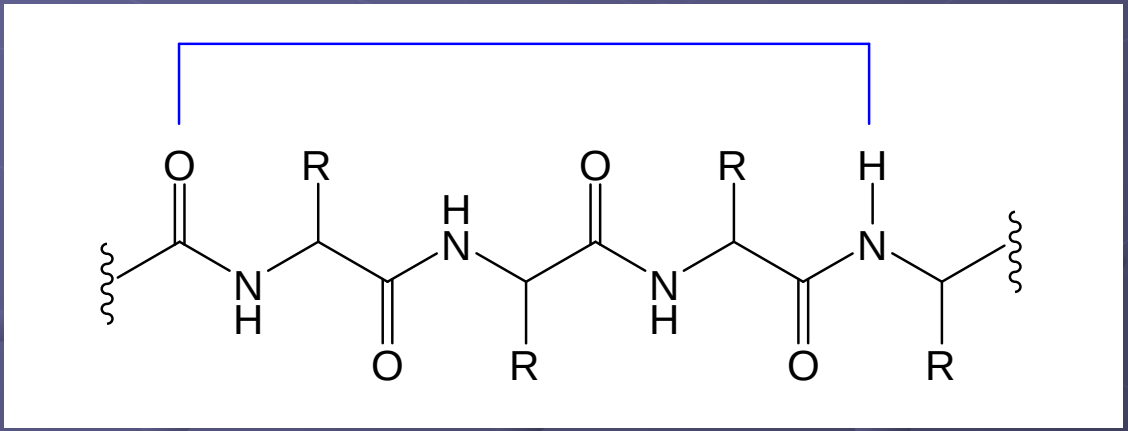
A helix is characterized by a well-defined **number of amino acid residues per turn** (**n, repeating units**)
and the **helix pitch**
(**p**, **repeated distance**).

$$d = \frac{p}{n}$$



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Further characterization is the **number of skeleton atoms** incorporated into the **ring formed by Intramolecuar hydrogen bond**.



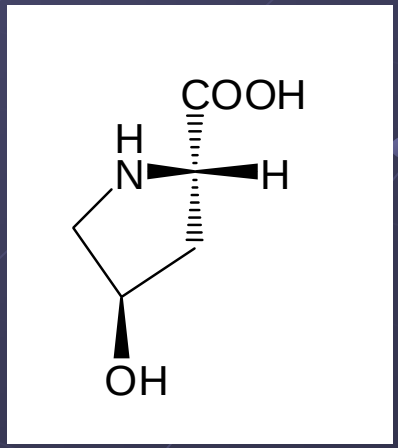
3.6_{13} P-helix (α-helix)

Helices are **chiral objects**, the direction of the helical turn being given by the letters **P** (plus) for **clockwise** and **M** (minus) for **anti-clockwise** helices.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The **nature of the amino acid side chain** is of crucial importance for **helix stability**. The general criteria are:

- **the steric requirements of the amino acid side-chain**
- **the electrostatic interaction of the amino acid side-chain**
- **the presence of proline and hydroxyproline**
- **the electrostatic dipole moment of the helix**



The amino acids **proline** and **hydroxyproline** are not able to act as hydrogen bond donors, and display high **helix-breaking properties**.

α-Helices can only formed by peptide chains of **homochiral building Blocks**. They contain then exclusively D- or exclusively L-amino acids.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

β-Sheet

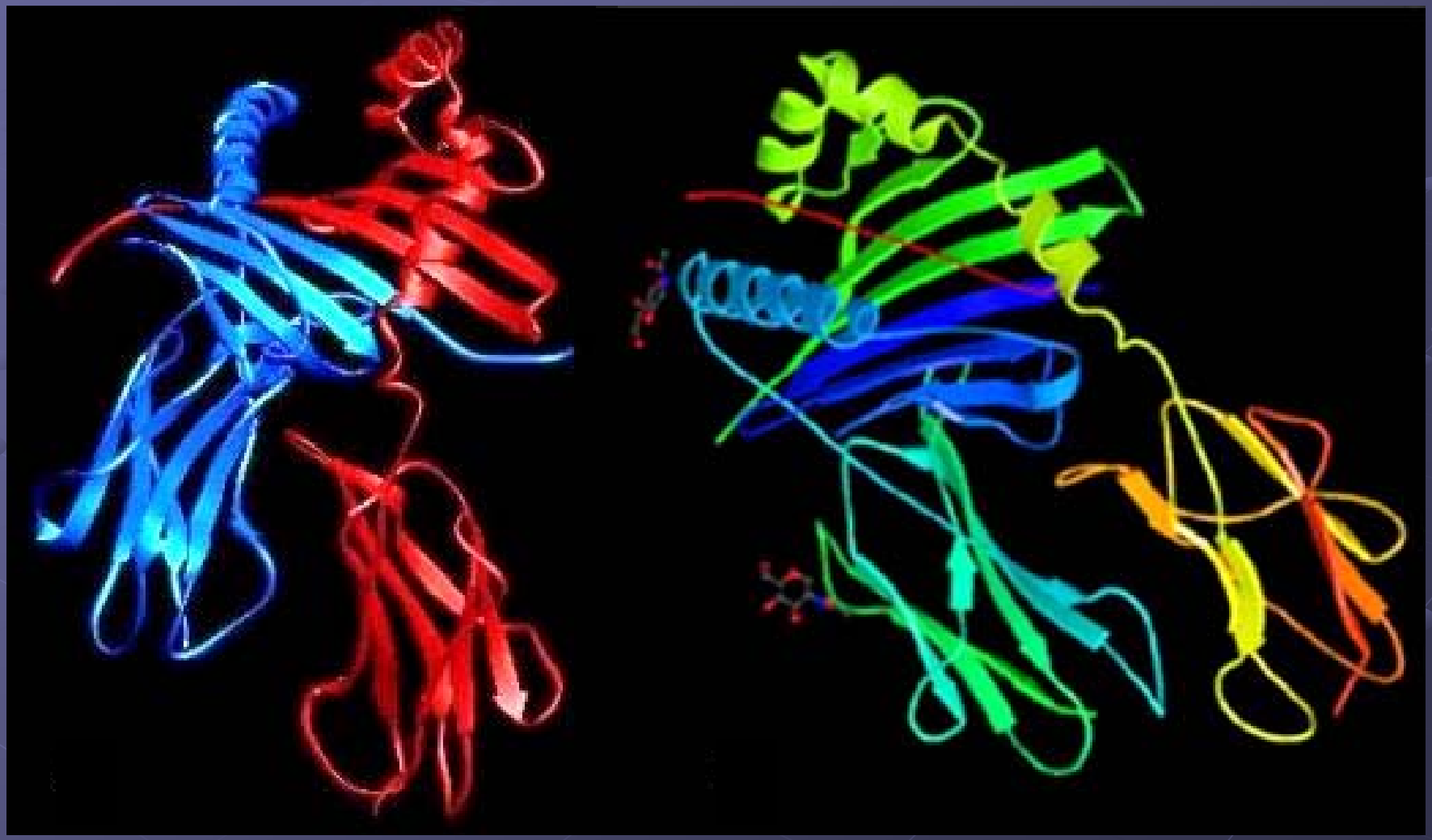
Two major variants of β-sheet structures may be distinguished. The **parallel β-sheet**, were the chains are aligned in a parallel manner, and
the **antiparallel β-sheet**, where two neighbouring peptide chains are aligned in a antiparallel manner.

β-Sheet structure is much more complex than a simple diagram might Imply: **β-sheets** may occur in a ***twisted***, ***curled*** or ***backfolded*** form.

β-Sheets often occur in the **hydrophobic core** of a protein.

The **β-pleated sheet** has been postulated as a structure into which any amino acid could substitute.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Turns

Loops of a polypeptide chain are characterized by an inversion of the chain direction (**reverse turn**).

A **polypeptide** chain **cannot fold into a compact globular structure without involving tight turns** that usually occur on exposed surface of proteins.

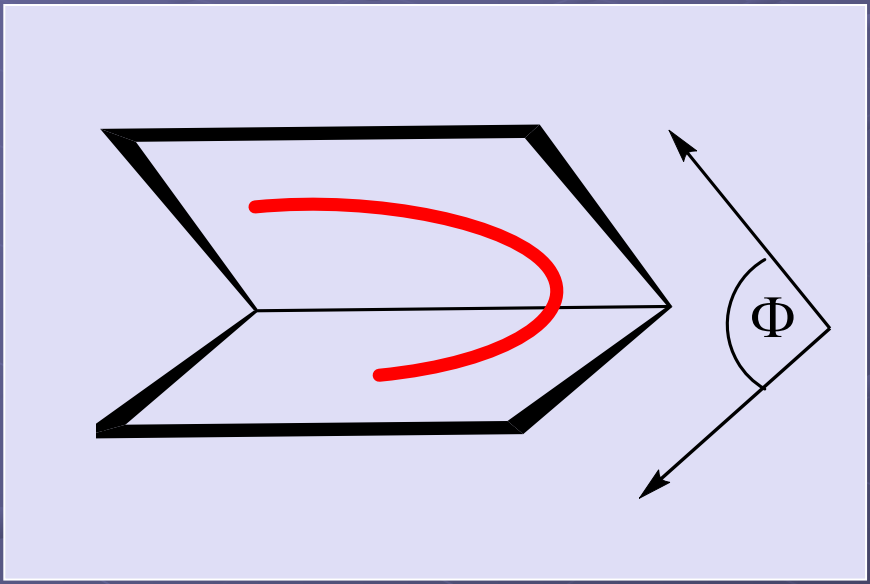
Turns are classified according to the number of amino acid residues involved:
3 amino acids – **γ-turn**; 4 amino acids – **β-turn**; 5 amino acids – **α-turn**;
6 amino acids – **π-turn**.

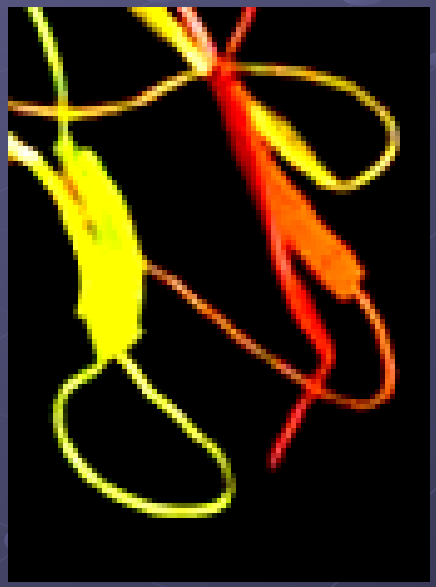
Turns are additionally classified by **dihedral angles**.

Some types of **β-turns** are **stabilized** intrinsically by certain amino acids such as **proline**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Turns are additionally classified by **dihedral angles**.



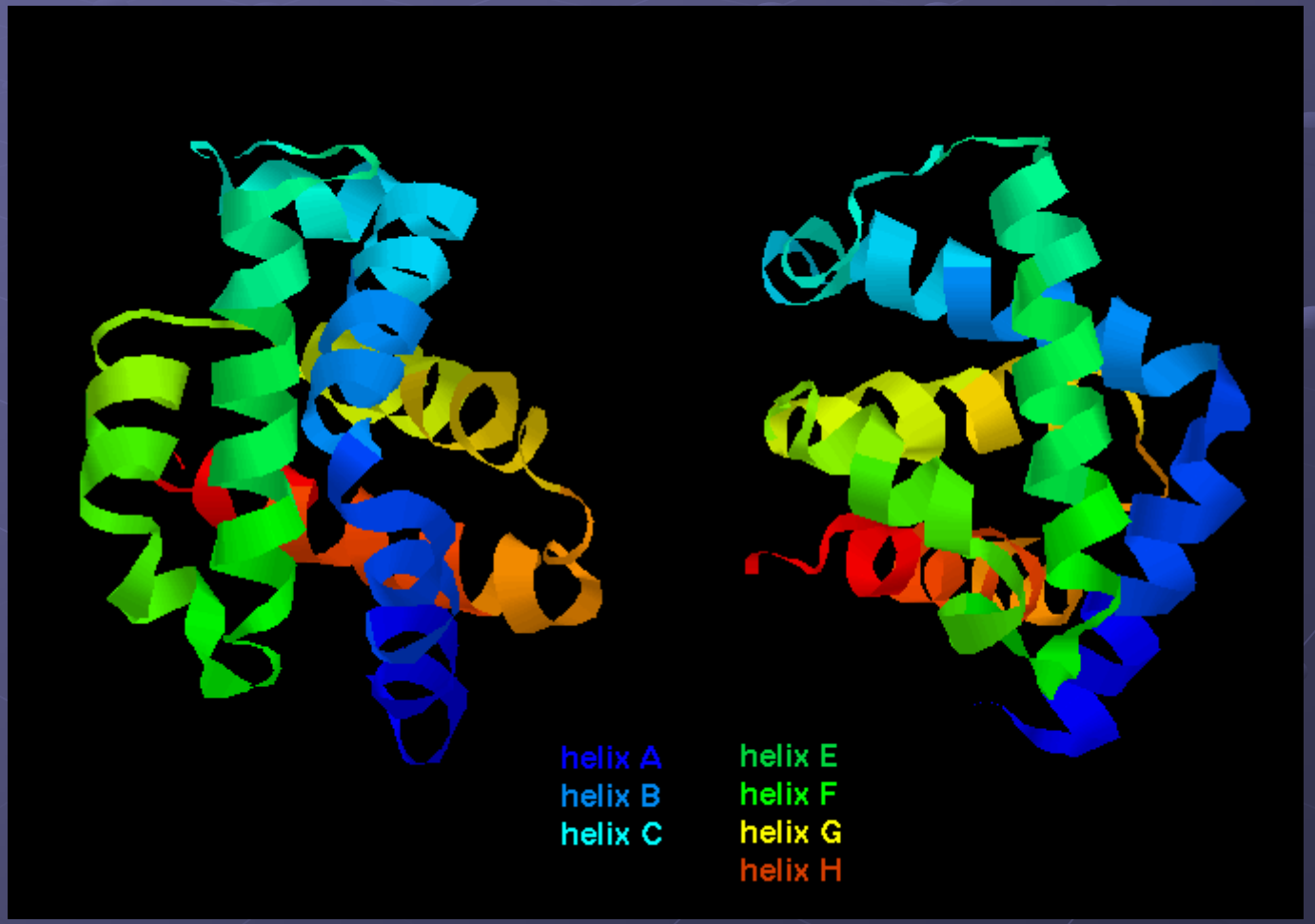


Some types of β-**turns** are **stabilized** intrinsically by certain amino acids such as **proline**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Hemoglobin is a hetero-tetramer, consisting of two alpha subunits and two beta subunits. (a "dimer of dimers"[a(2):b(2)])

globular

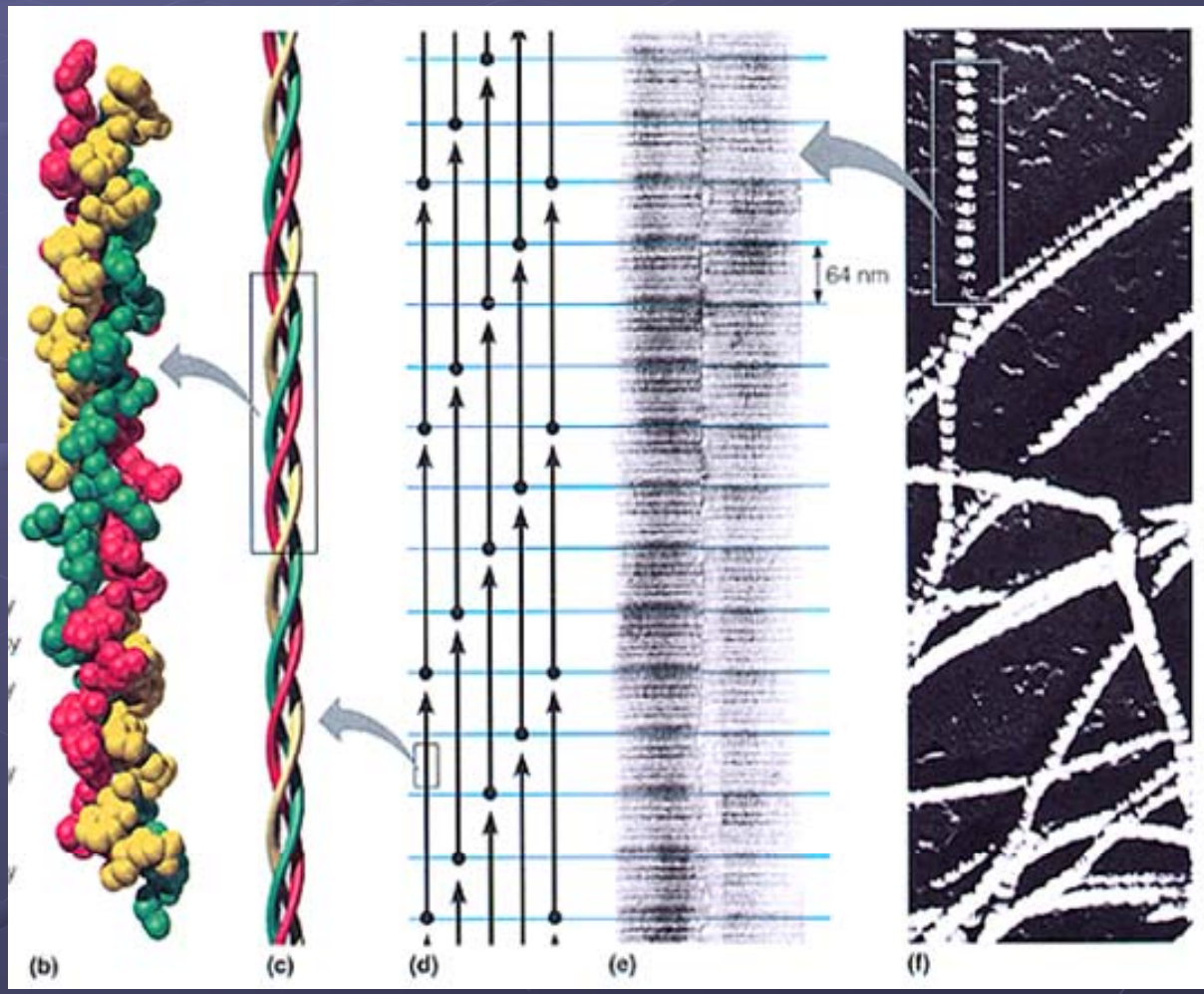


CHEMICAL SYNTHESIS OF BIOPOLYMERS

Collagen is a group of naturally occurring proteins found in animals, especially in the flesh and connective tissues of mammals.

Three left-handed helices are twisted together into a right-handed triple helix or "super helix",

fibrillar



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Primary structure comprises the number and sequence of amino acids connected consecutively by peptide bonds within the peptide chain.

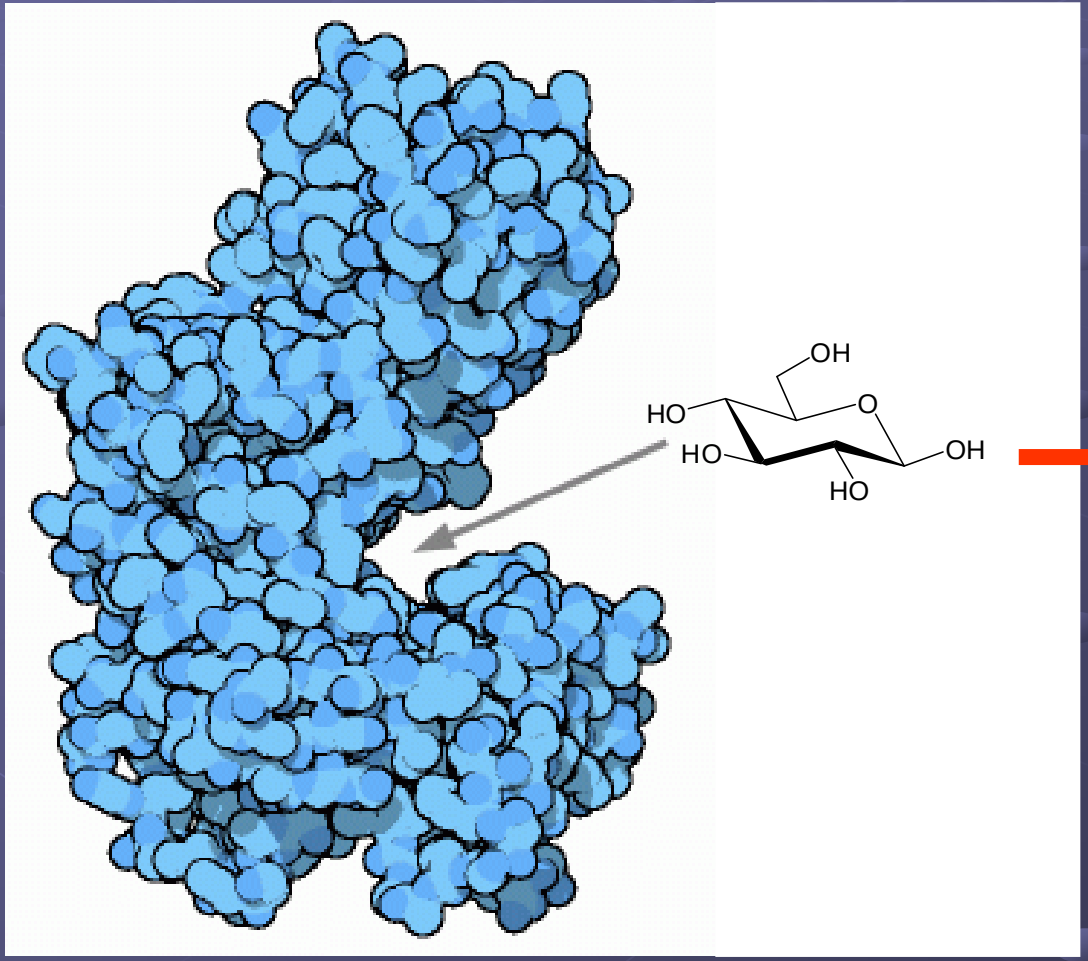
Secondary structure describes the local three-dimensional arrangement off the peptide backbone (***β-pleated sheet***, ***α-helix, turns***).

Tertiary structure describes the tree-dimensional structure or overall shape of a single peptide chain resulting from the intramolecular interactions between secondary structure elements (***globular***, ***fibrillar***).

The term **quaternary structure** refers to the spatial arrangement of two or more poylpeptide chains associated by noncovalent interactions, or in special cases linked by disulfide bonds, forming definite oligomer complexes.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The term **domain** is applied to describe globular clusters within a protein molecule with more than **~200 amino acids**.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Peptides are classified with Greek prefixes as **di-, tri-, tetra-….decapeptides**, etc., according to the number of amino acid residues incorporated.

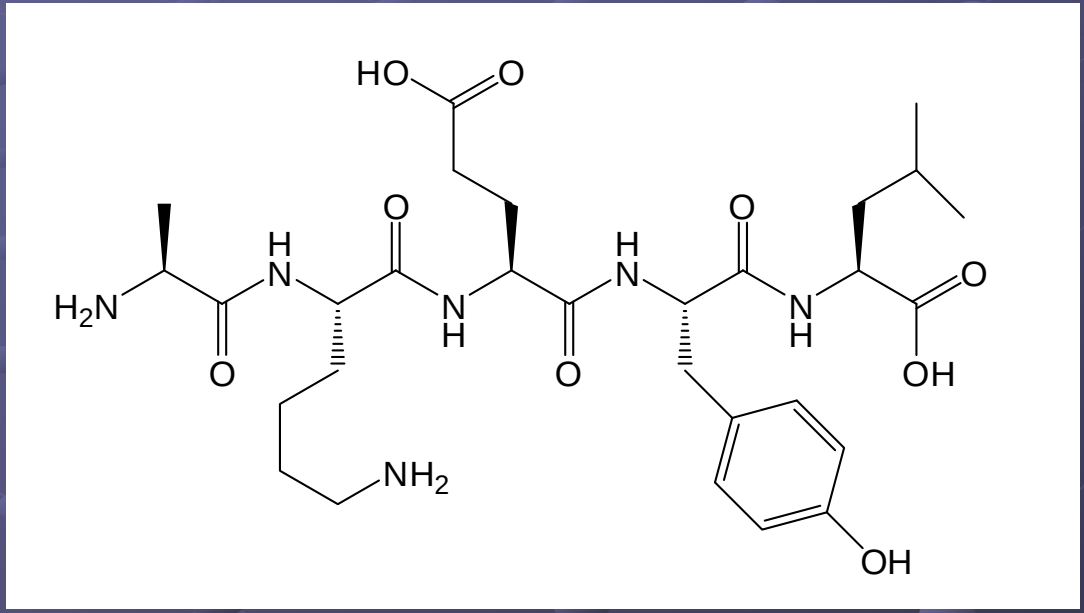
Longer peptides such as **dodecapeptide** may be called **12-peptide**.

Peptides containing **fewer than 15 amino acid** residues were classified as **oligopeptides**, whereas peptides with **15 - 50 amino acids** residues were called **polypeptides**.

For peptides with **more than 50 amino acid** residues the expression **protein** is used.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

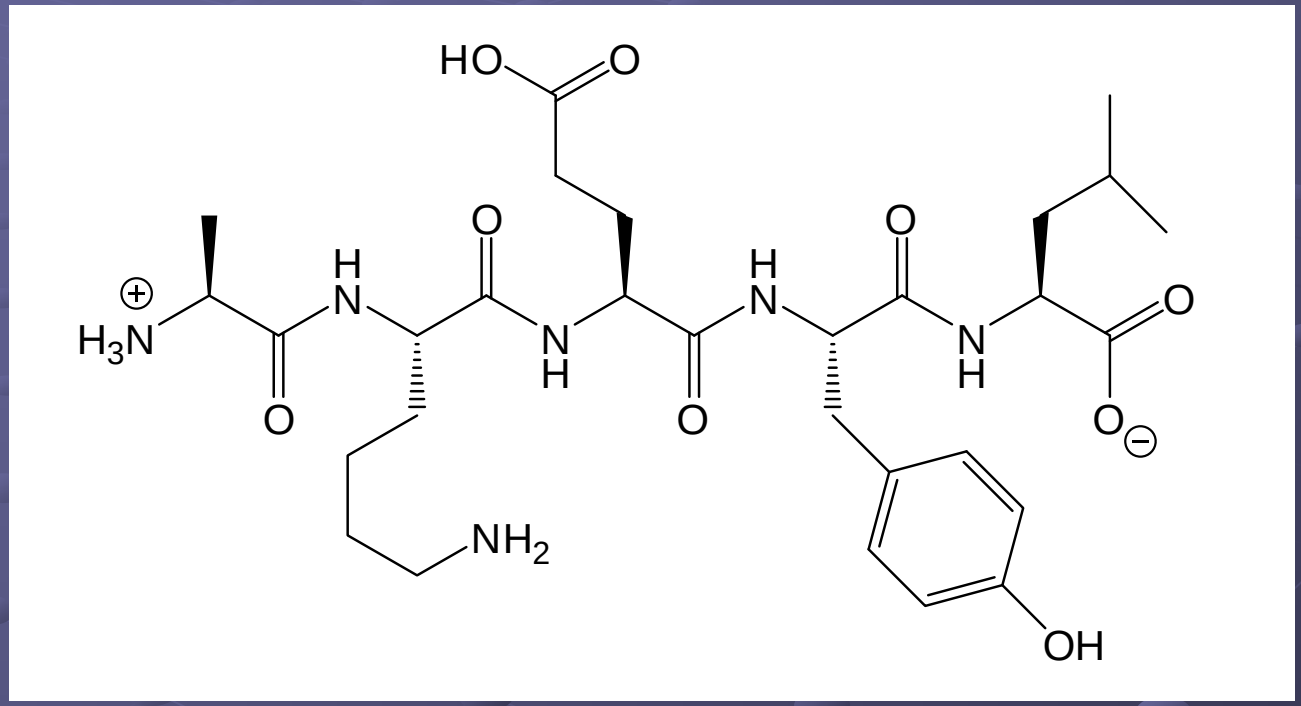
The nomenclature formally considers **peptides** as ***N*-acyl amino acids**. Only the amino acid **residue at the carboxy terminus** of the peptide chain keeps the original name **without suffix**, **all others** are used with the original name and the suffix **–yl**.



H—Ala—Lys—Glu—Tyr—Leu—OH

alanyl-lysyl-glutamyl-tyrosyl-leucine

CHEMICAL SYNTHESIS OF BIOPOLYMERS

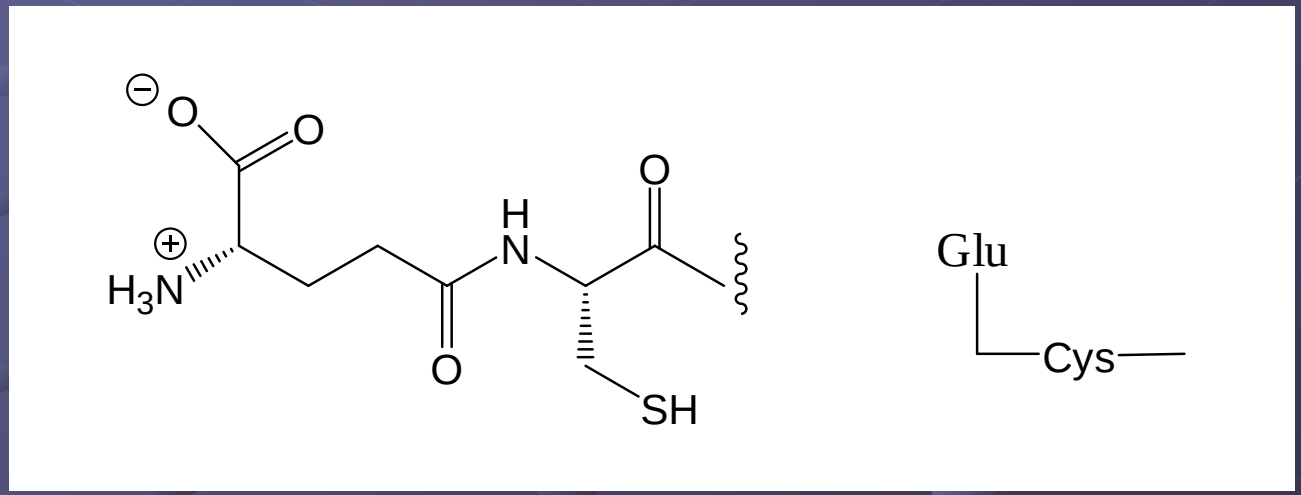


$^{\oplus}H_2$—Ala—Lys—Glu—Tyr—Leu—$O^{\ominus}$

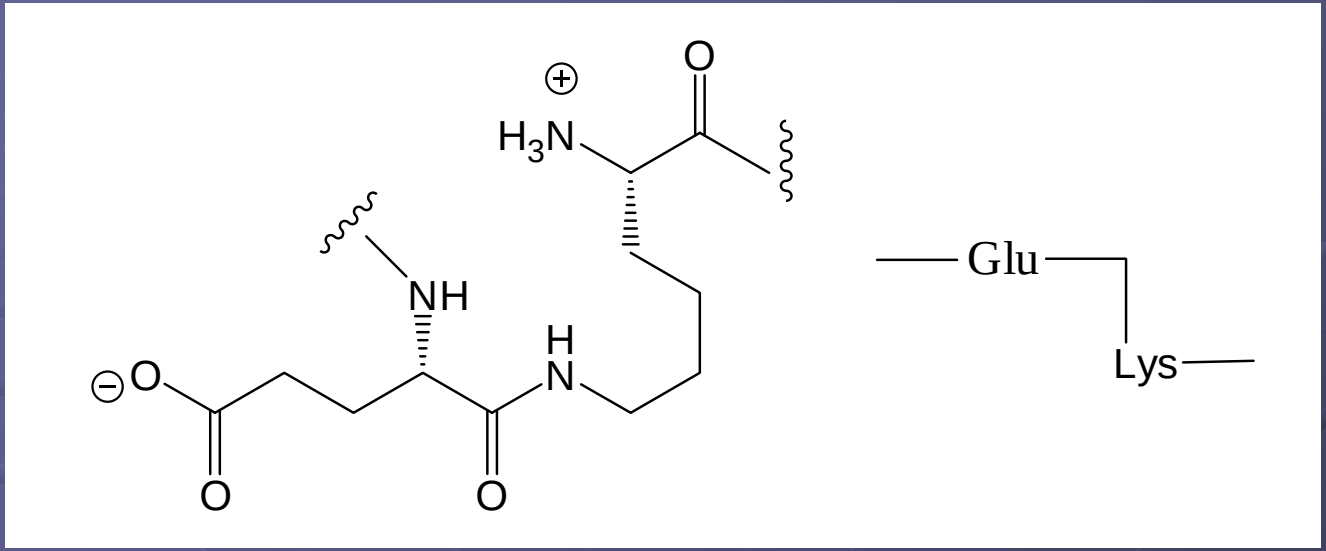
CHEMICAL SYNTHESIS OF BIOPOLYMERS

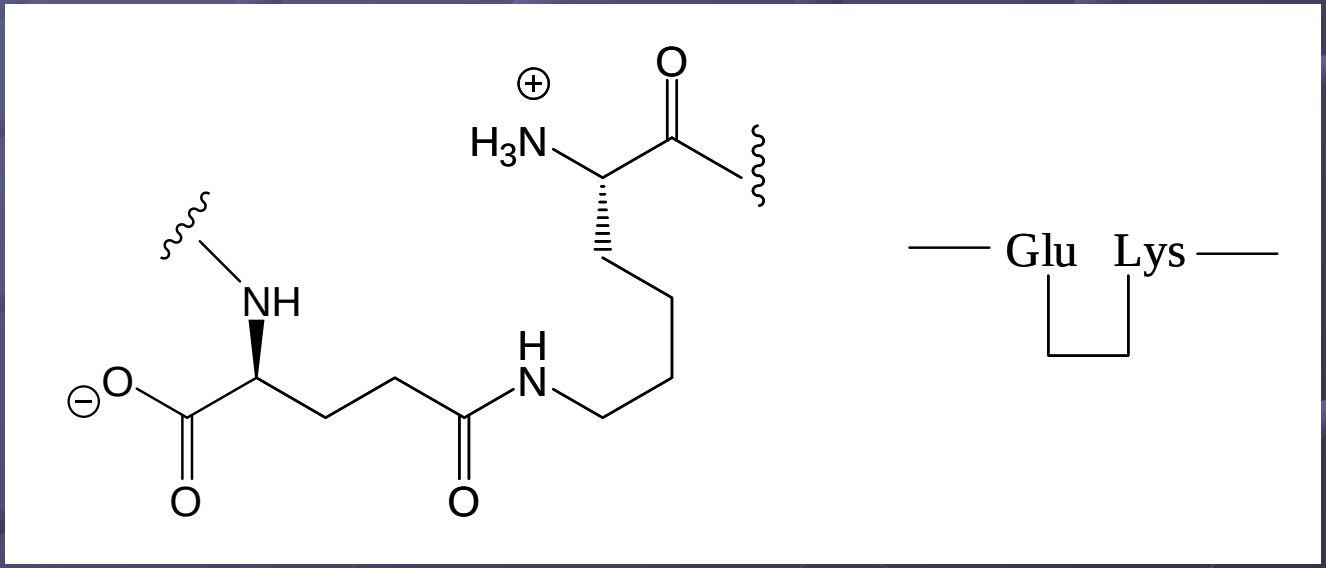
The **three-letter code** usually precludes that trifunctional amino acids with additional amino or carboxy functions located in the side chains (Lys, Glu, Asp) are connected by **α-peptide bonds**.

But biochemical important peptides comprises besides α-peptide bonds also **δ-glutamyl-cyctyl-**, ***N*ε-α-glutamyl-lysyl-**, and ***N*ε-δ-glutamyl-lysyl bonds**.



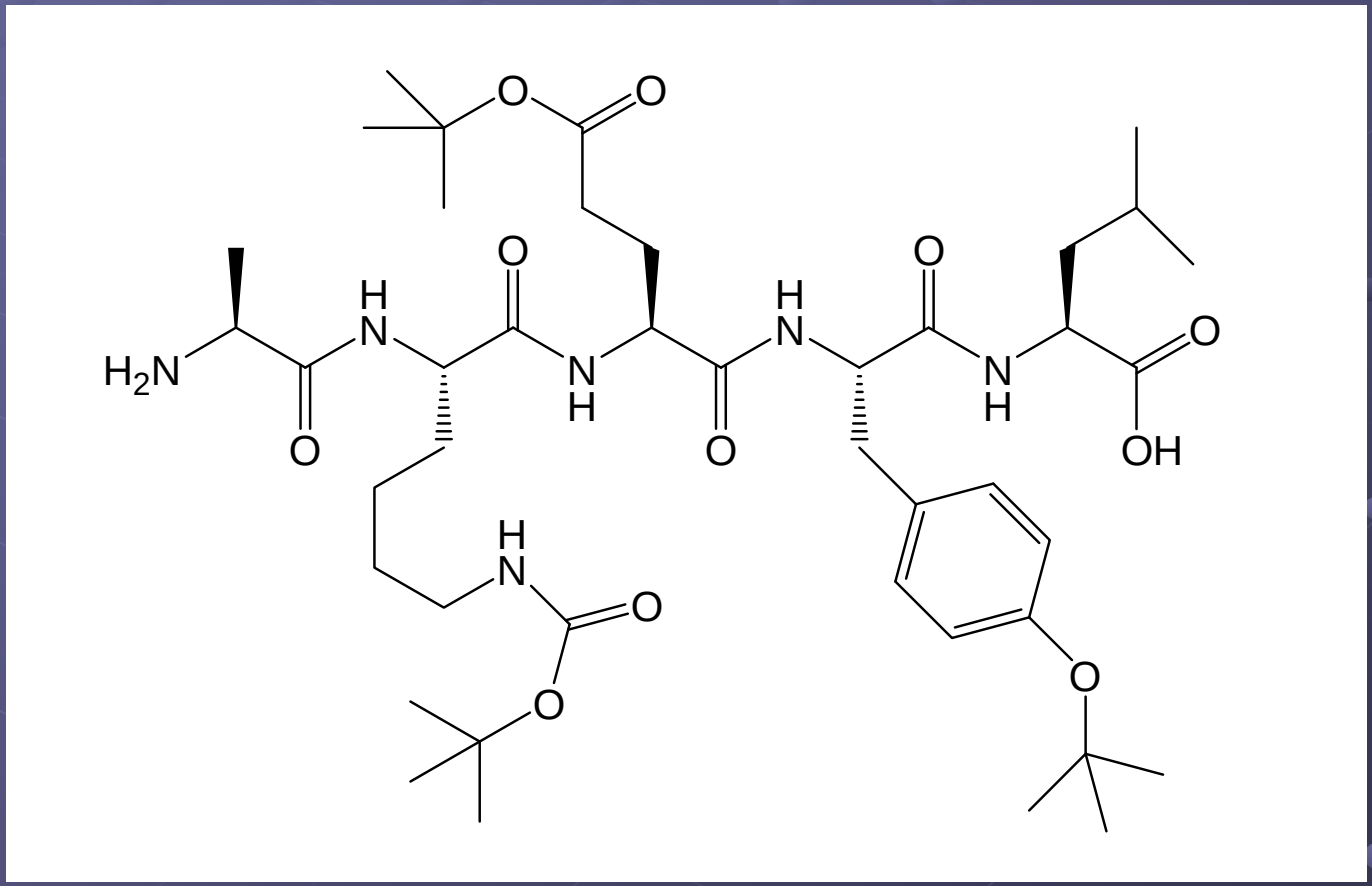
CHEMICAL SYNTHESIS OF BIOPOLYMERS





CHEMICAL SYNTHESIS OF BIOPOLYMERS

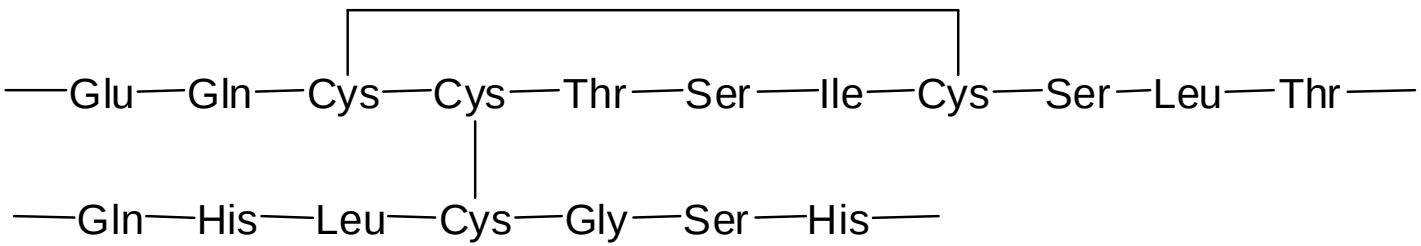
The **side-chain substituents** (**protecting groups**) are displayed by an **abbreviation in brackets** immediately after the three-letter abbreviation.



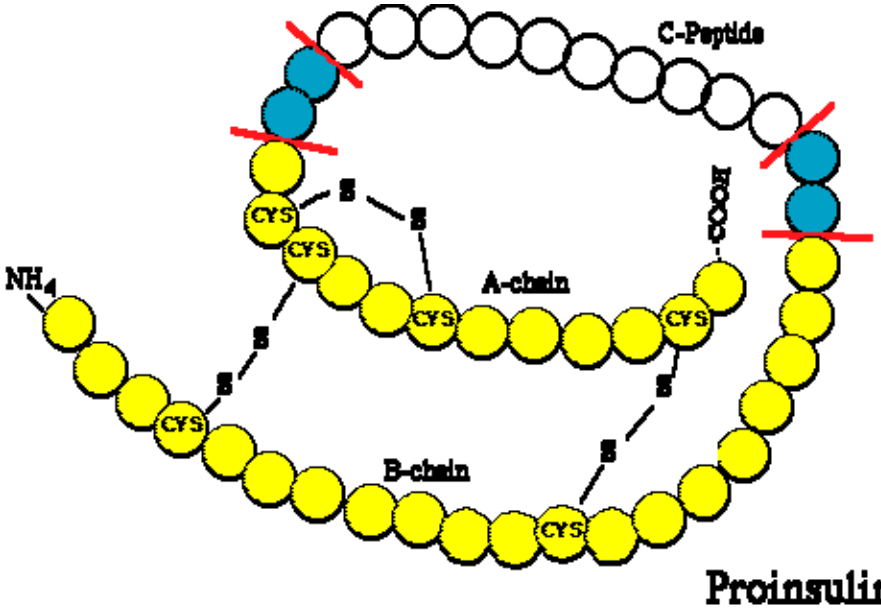
H—Ala—Lys(Boc)— Glu(OtBu)— Tyr(tBu)—OH

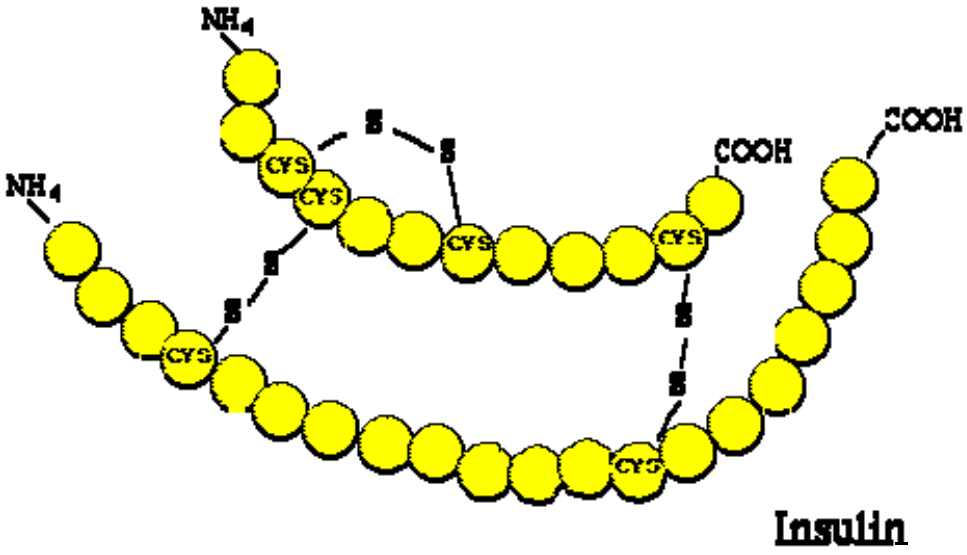
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Covalent bonds between side-chain functional groups are also possible For the amino acid **cysteine**. **Intramolecular (intrachain)** and **intermolecular (interchain) disulfide bonds** are observed.

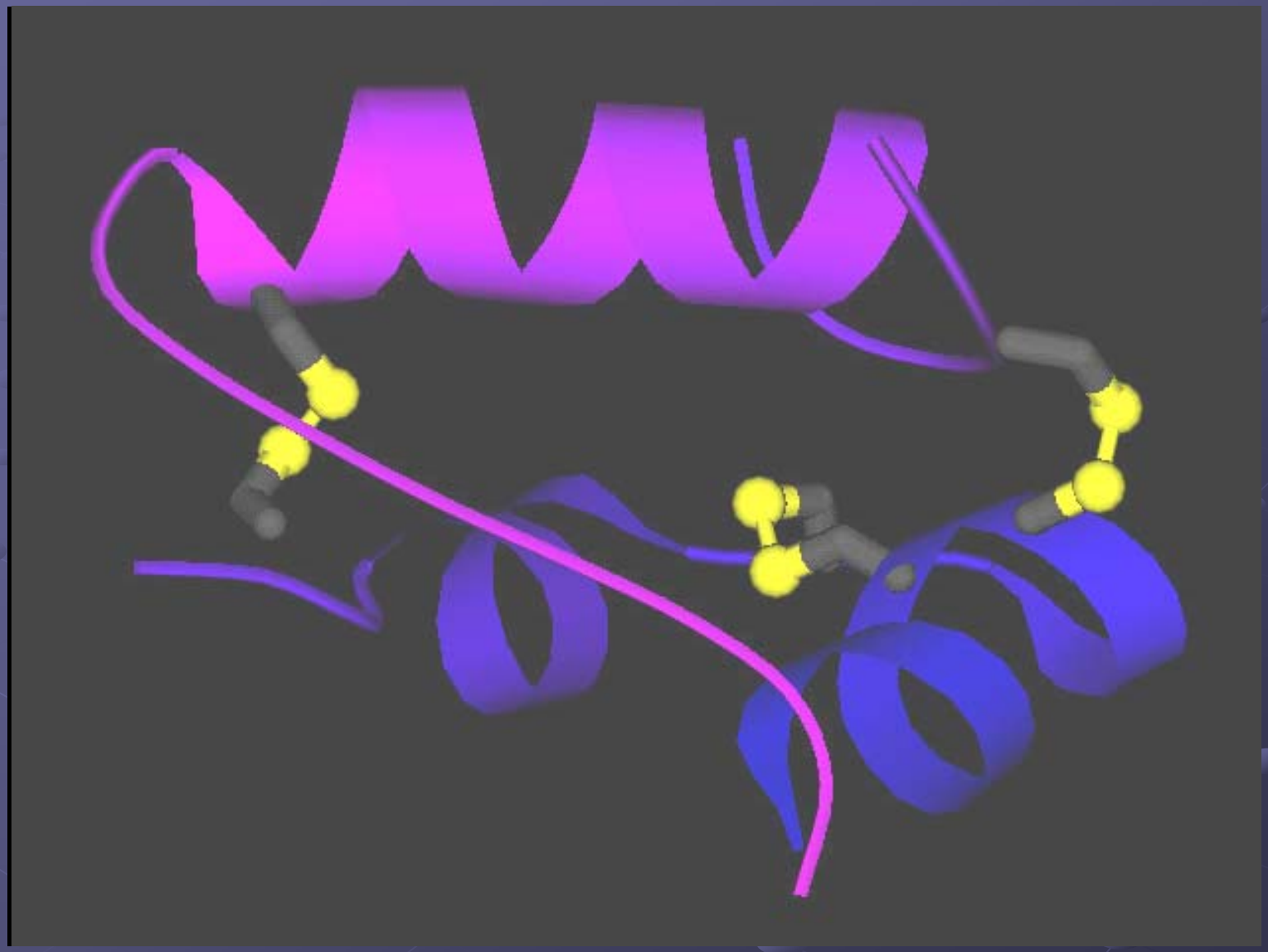


CHEMICAL SYNTHESIS OF BIOPOLYMERS





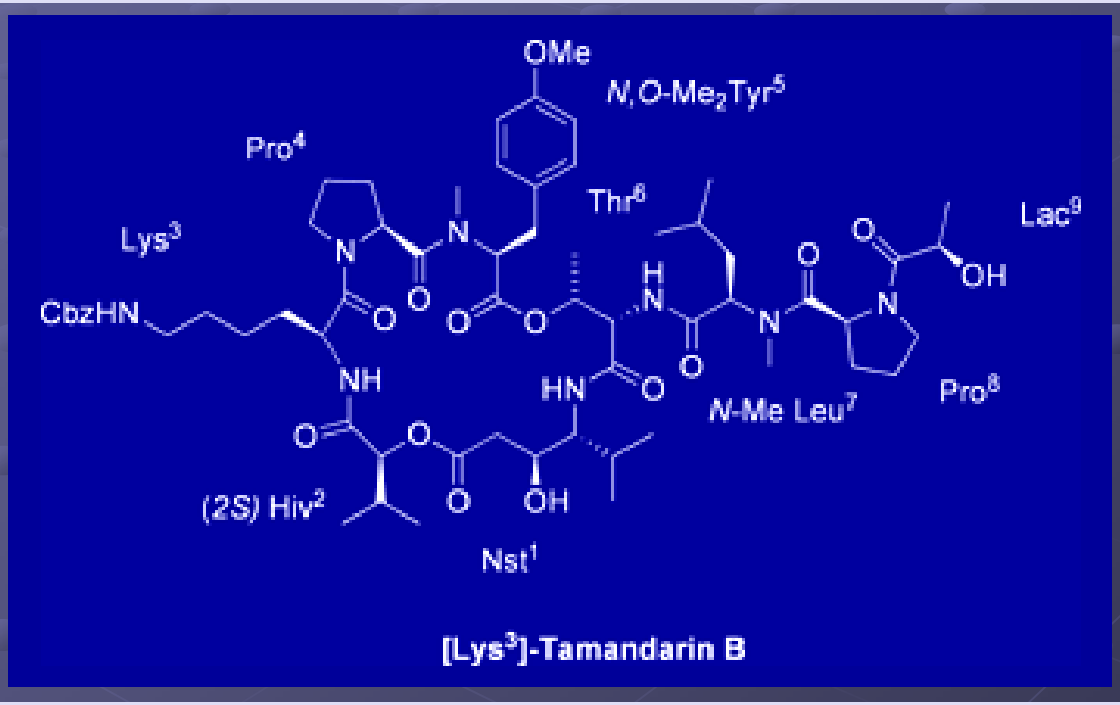
CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Homomeric peptides are composed solely of **proteinogenic amino acids**.

Heteromeric peptides contain also **nonproteinogenic building blocks**
Such as hydroxycarboxyl acids, D-amino acids, and *N*-alkyl-amino acids.

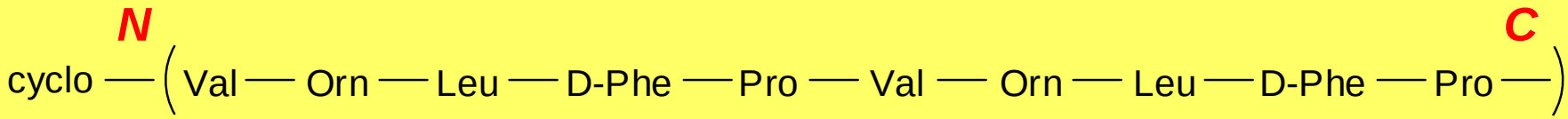


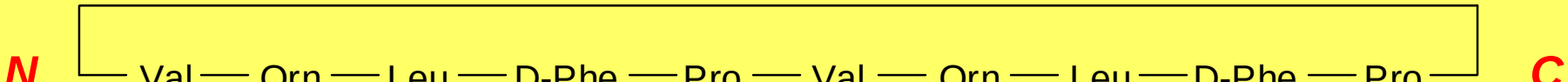
Homodetic peptides contain exclusively peptide bonds (N^{α} amide bonds).

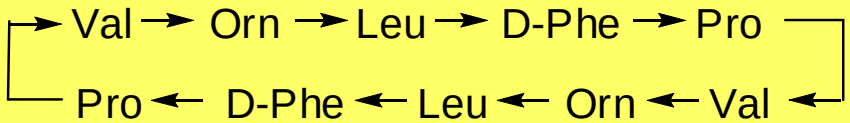
Heterodetic peptides may also contain ester, disulfide, thioester bonds.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The sequence of a **cyclic homodetic homomeric peptide** can be written in three different ways as shown next slide.

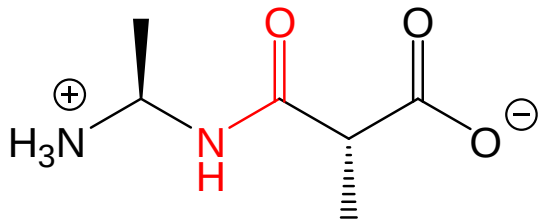




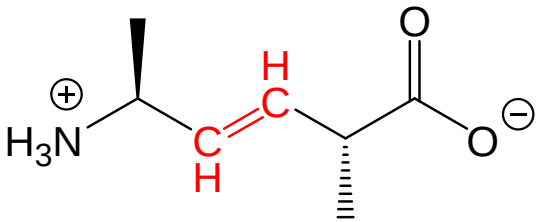


CHEMICAL SYNTHESIS OF BIOPOLYMERS

Analogues of peptides, in which the **–CO-NH-group** that joins residues is **replaced** by another grouping, may be named by placing Greek psi (ψ), followed the replacing group in parentheses, between the residue symbols where the change occurs:



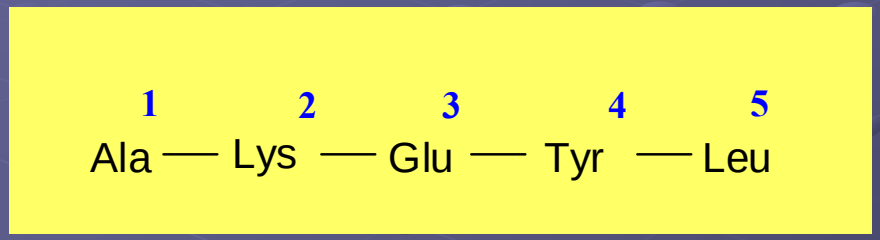
Ala —[ψ](NH—CO)— Ala



Ala —[ψ](HC═CH , *trans*)— Ala

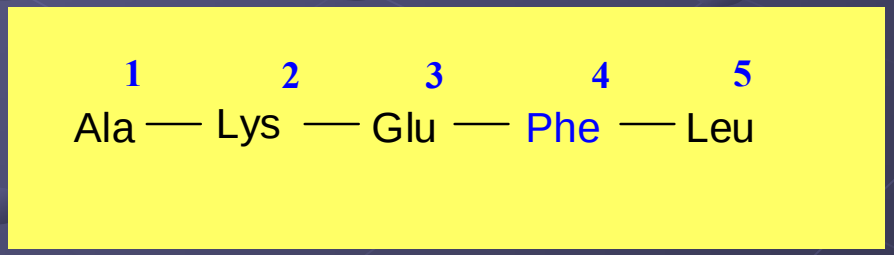
CHEMICAL SYNTHESIS OF BIOPOLYMERS

As **analogues of biologically active peptides** are often synthesized in order to study the relationship between structure and activity, a brief introduction into the rules of the nomenclature are given here for the example of a hypothetical peptide with the name ***aturobin***.



aturobin

The **exchange of amino acid resiudes** in a peptide is symbolized by the trivial name of the corresponding peptide preceded by the full name or the three-letter code of the amino acid replacement and its position given in square brackets.

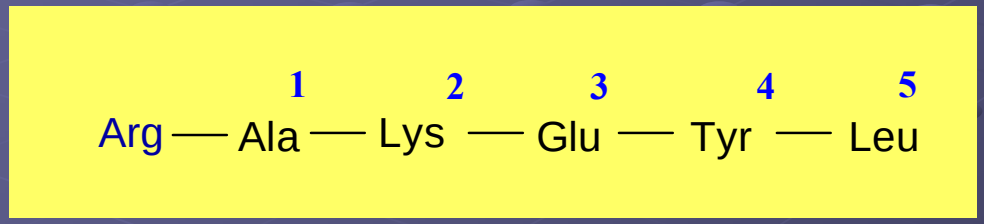


[4-phenylalanine]aturobin

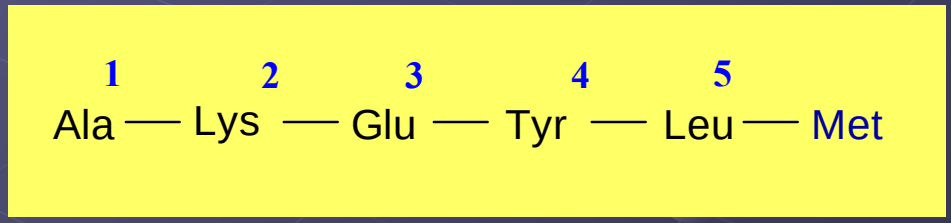
[Phe^4]aturobin

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The **extension of a peptide** may occur N-terminally as well as C-terminally. The modified name is generated according to the previously discussed rules.

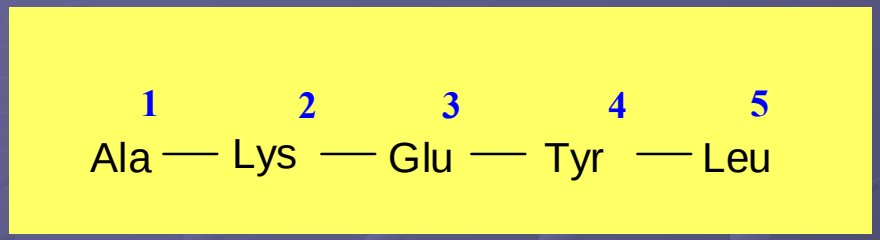


arginyl-aturobin



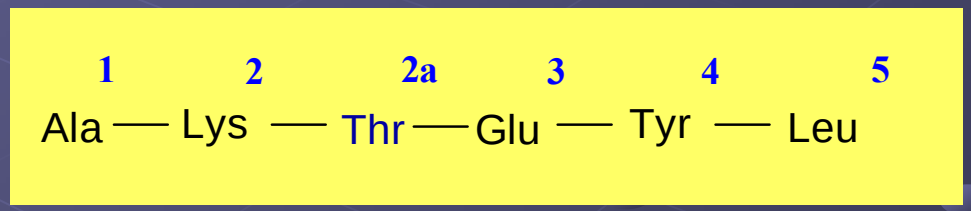
aturobyl-methionin

CHEMICAL SYNTHESIS OF BIOPOLYMERS



aturobin

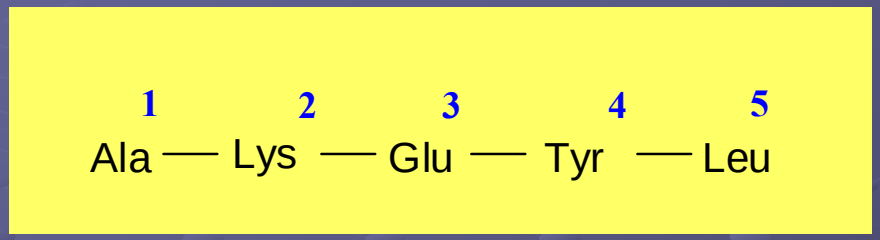
An **insertion of an additional amino acid residue** is indicated by the prefix ***endo*** in combination with the number of the sequence position.



endo-2a-threonine-aturobin

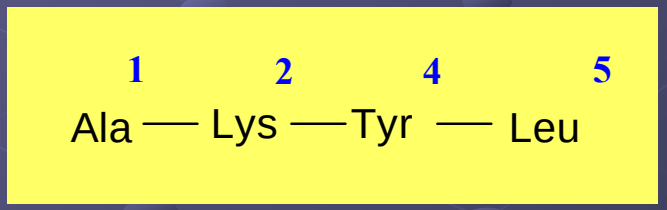
endo-Thr^{2a}-aturobin

CHEMICAL SYNTHESIS OF BIOPOLYMERS



aturobin

The **omission of an amino acid residue** is symbolized by the prefix ***de*** and the position.

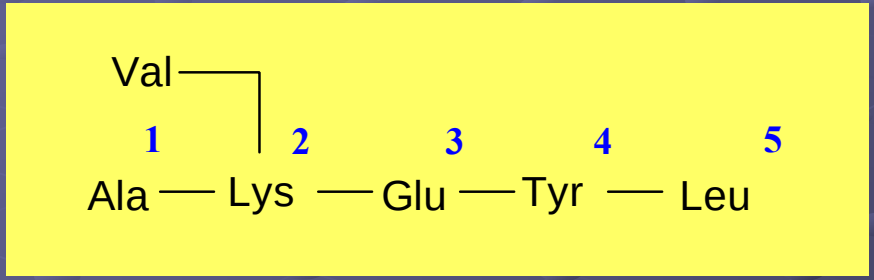


de-3-glutamic acid-aturobin

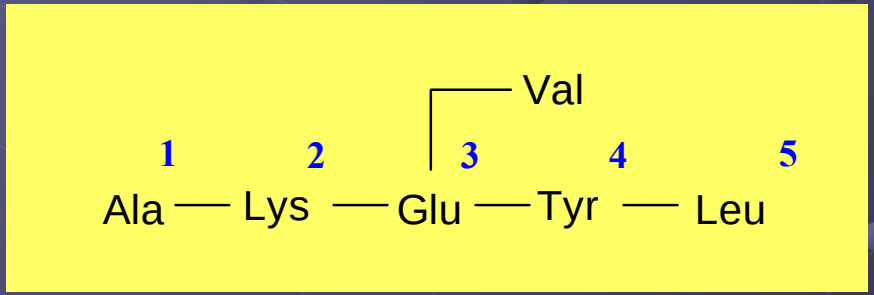
de-Glu^{3}-aturobin

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Substitution on side-chain amino groups or side chain carboxy groups are symbolized considering the general rules.



Nε²-Valyl-aturobin or Nε²-Val-aturobin

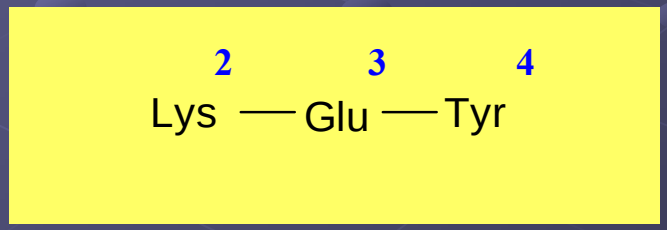


N-(aturobin-Cδ³-yl)-valine or aturobin-Cδ³-yl-Val

CHEMICAL SYNTHESIS OF BIOPOLYMERS

aturobin

The nomenclature for **partial sequences** that are derived from peptides with a trivial name uses the trivial name, followed by the numbers of sequence positions of the first and last amino group within the partial sequence in brackets.



aturobin-(2-4)-peptide