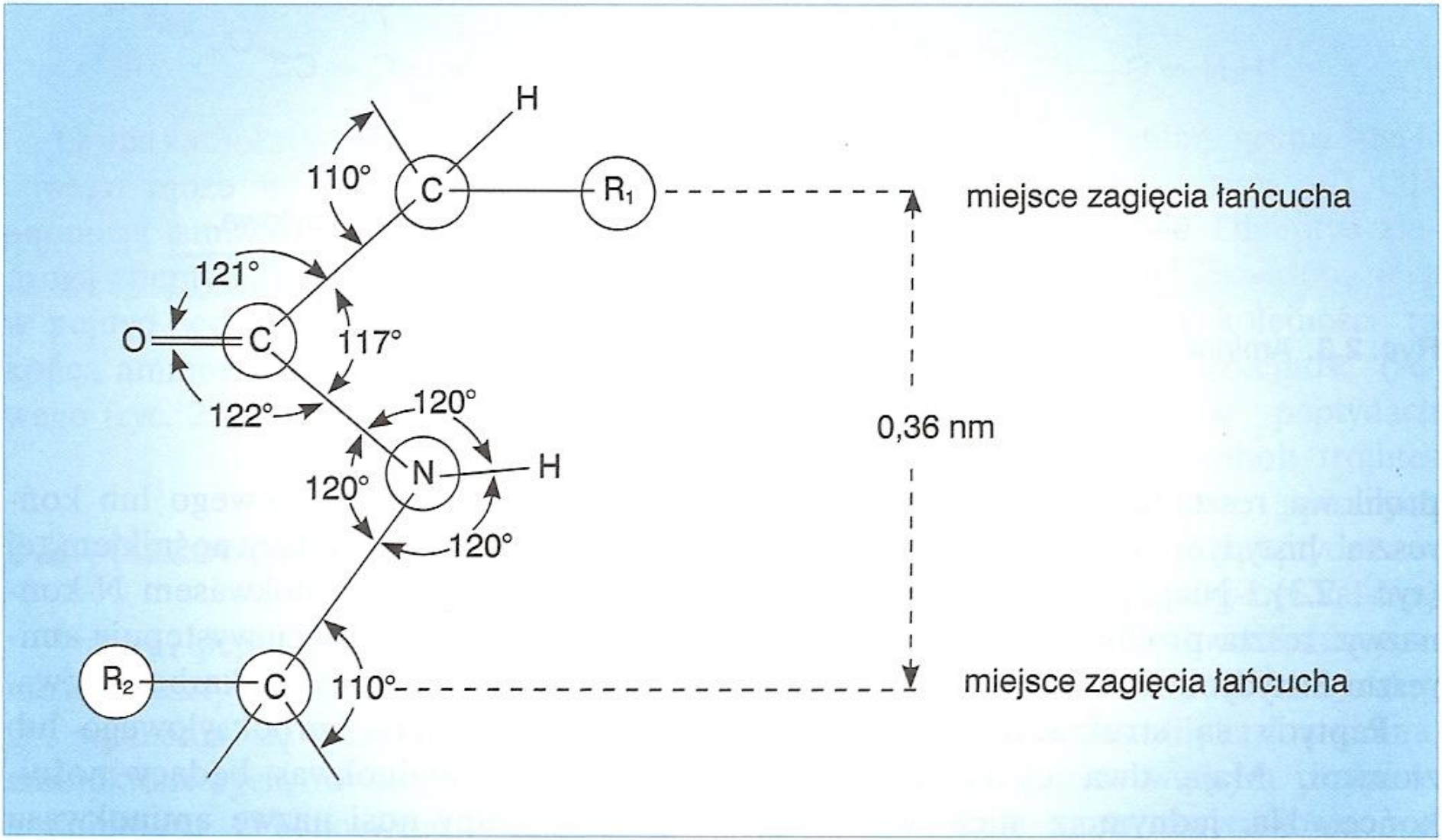
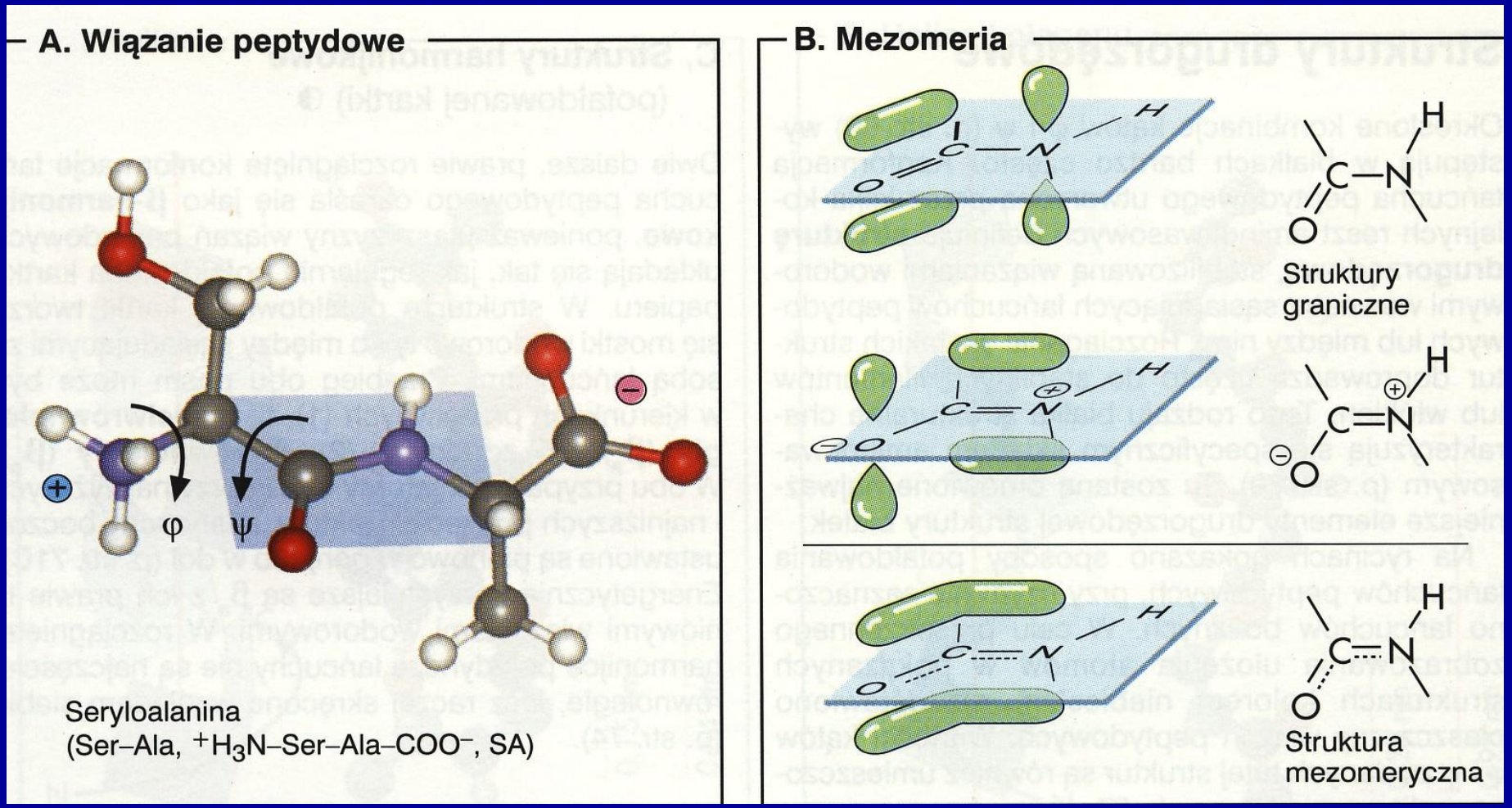
Quantitative methods for protein determinations

Peptide bond

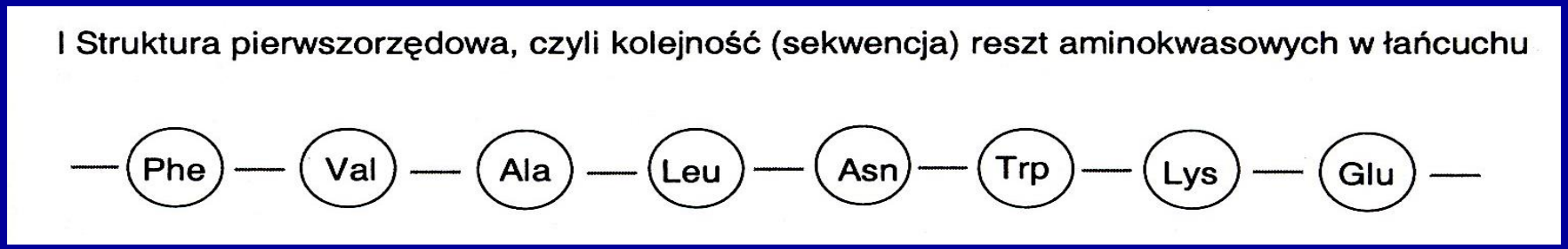


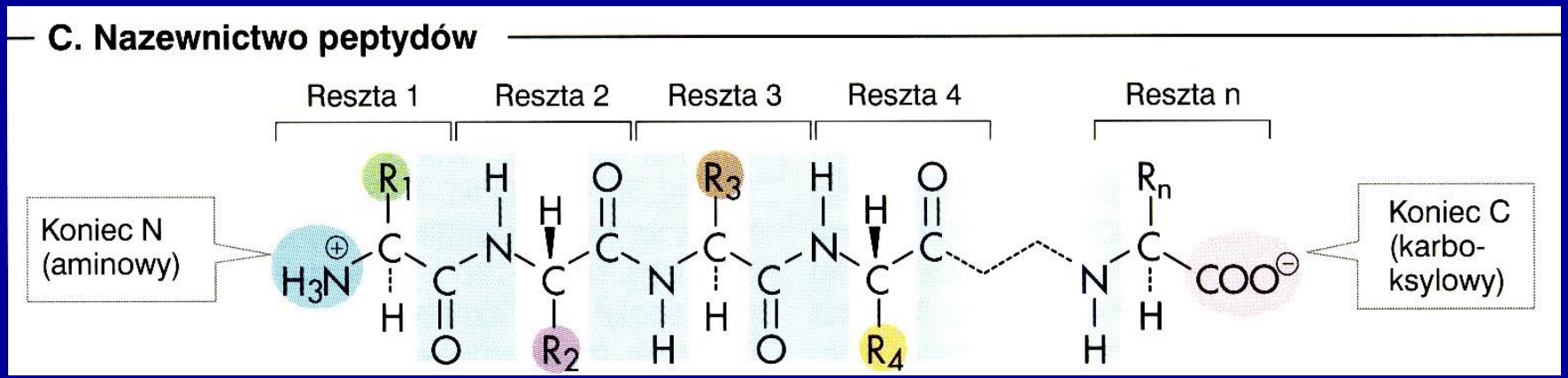
Properties of peptide bond: polar, planar, semi-nonsatureted, trans-configurated



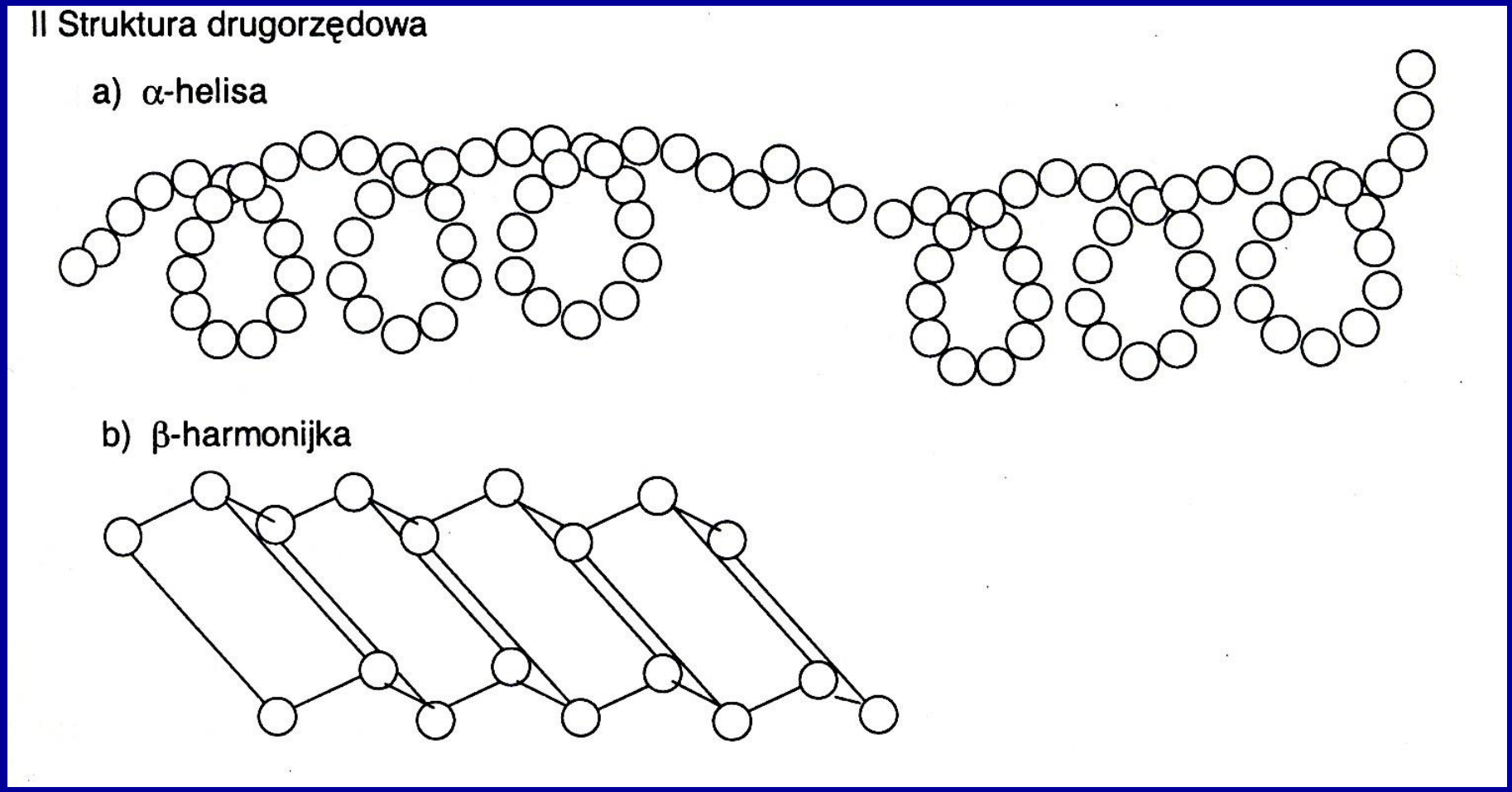
The division of aminoacids in accordance to Karlson is based on the participation of particular aminoacids in tertiary structure of proteins

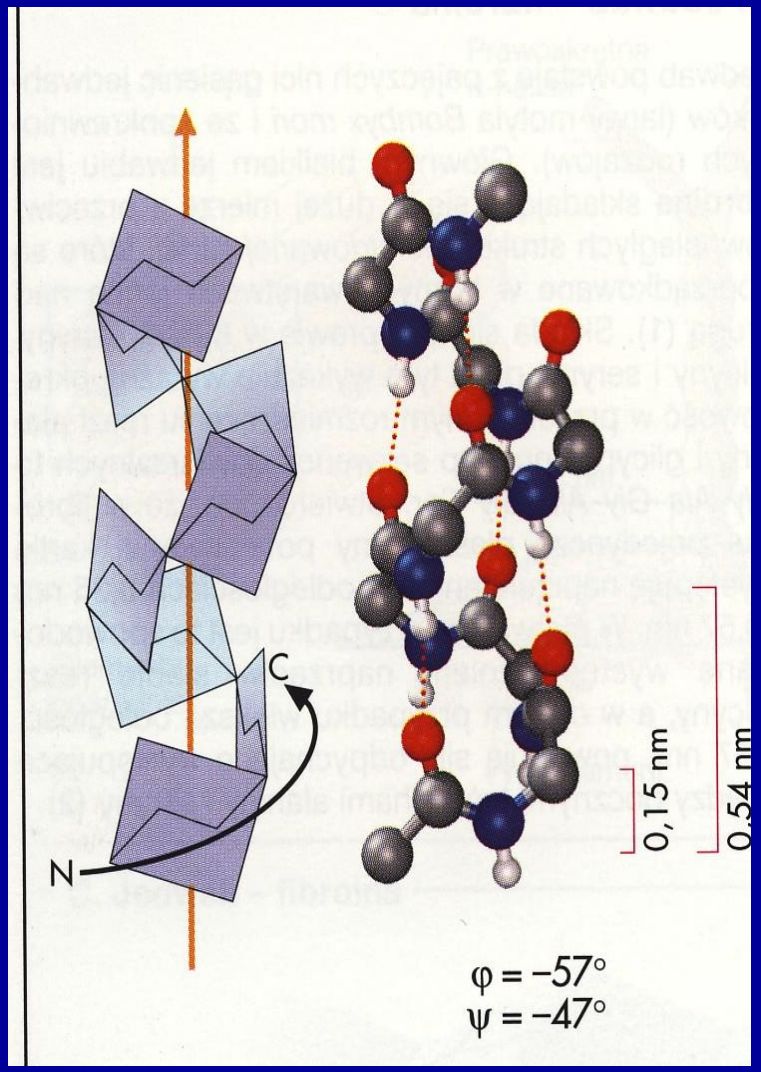
- We use the following terms to describe protein structure: primary, secondary, tertiary, quaternary structure
- **Primary** structure is defined as the sequence of aminoacids in chain which are bound by peptide bond

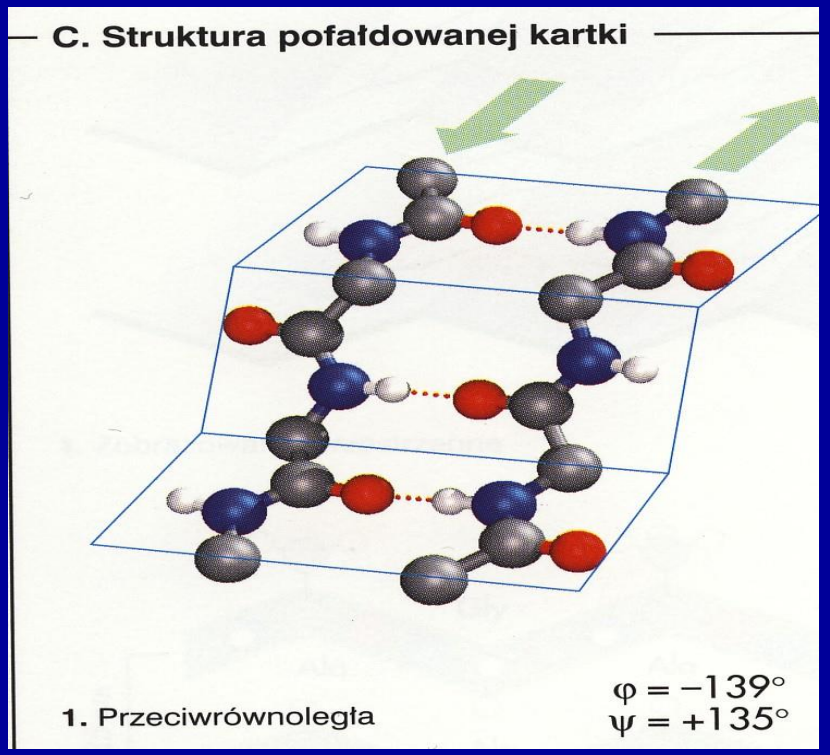


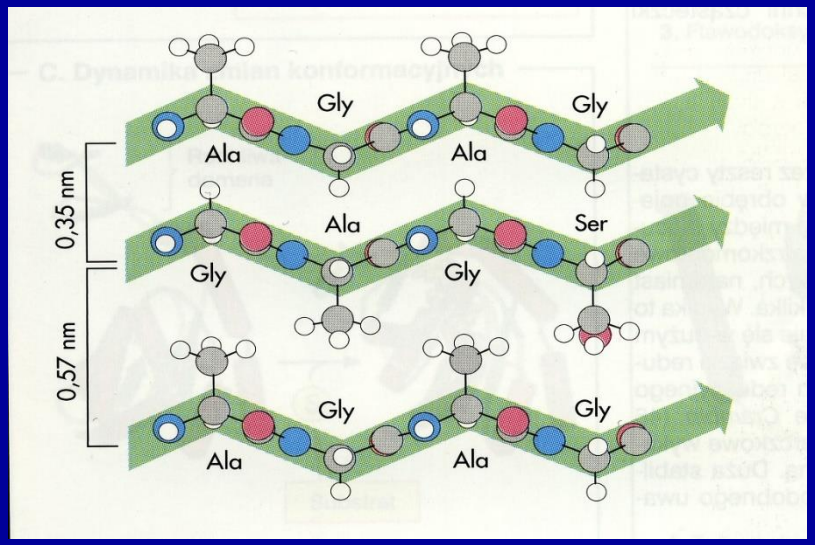


In **secondary structure** the chain is twisted in two ways (alpha helix and beta pleated sheet). The structure is stabilized by hydrogen bonds which bind peptide bonds – between hydrogen atom in =NH of first peptide bond and oxygen of =C=O, belonging to 4th peptide bond in the chain

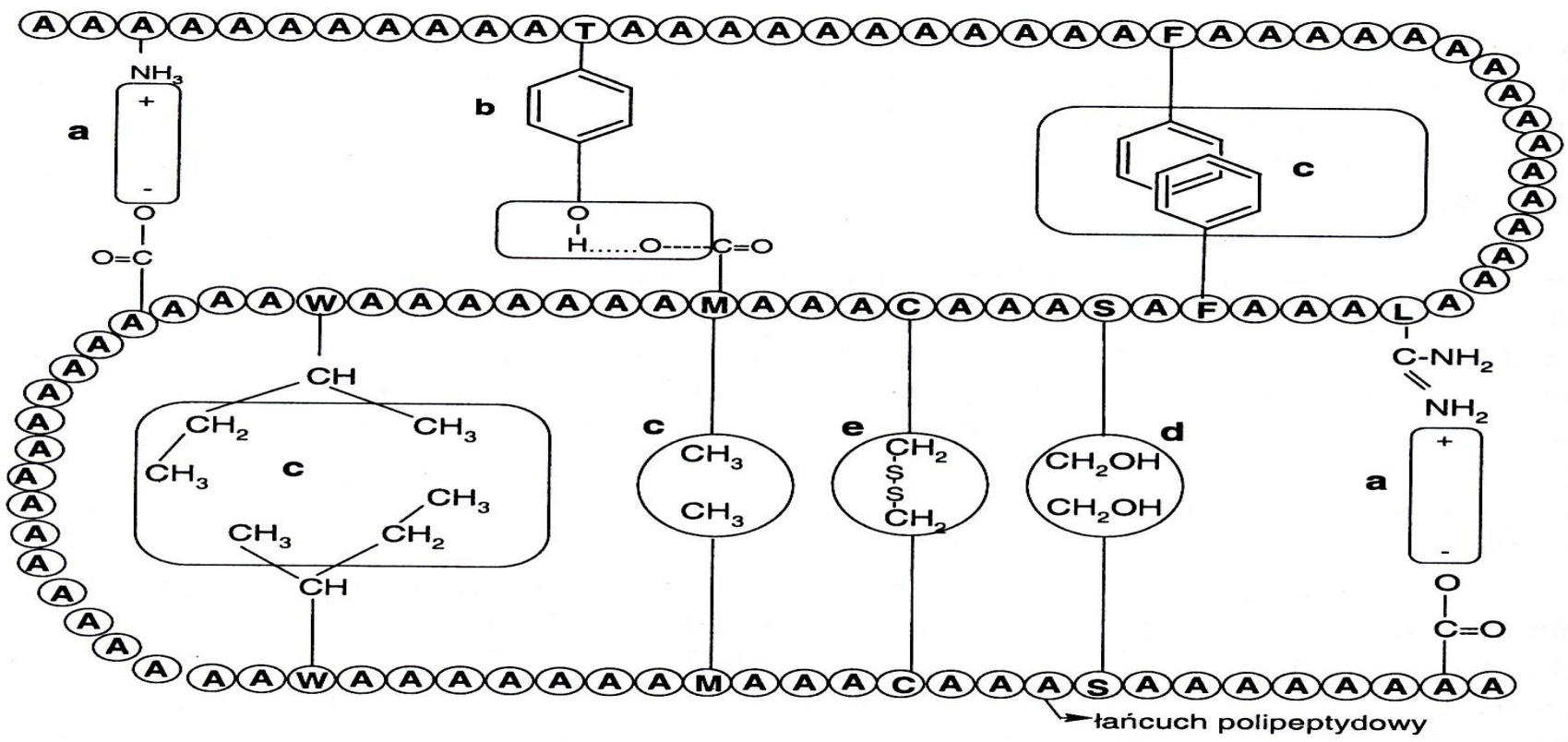



C
N
0,15 nm
0,54 nm
φ = −57°
ψ = −47°

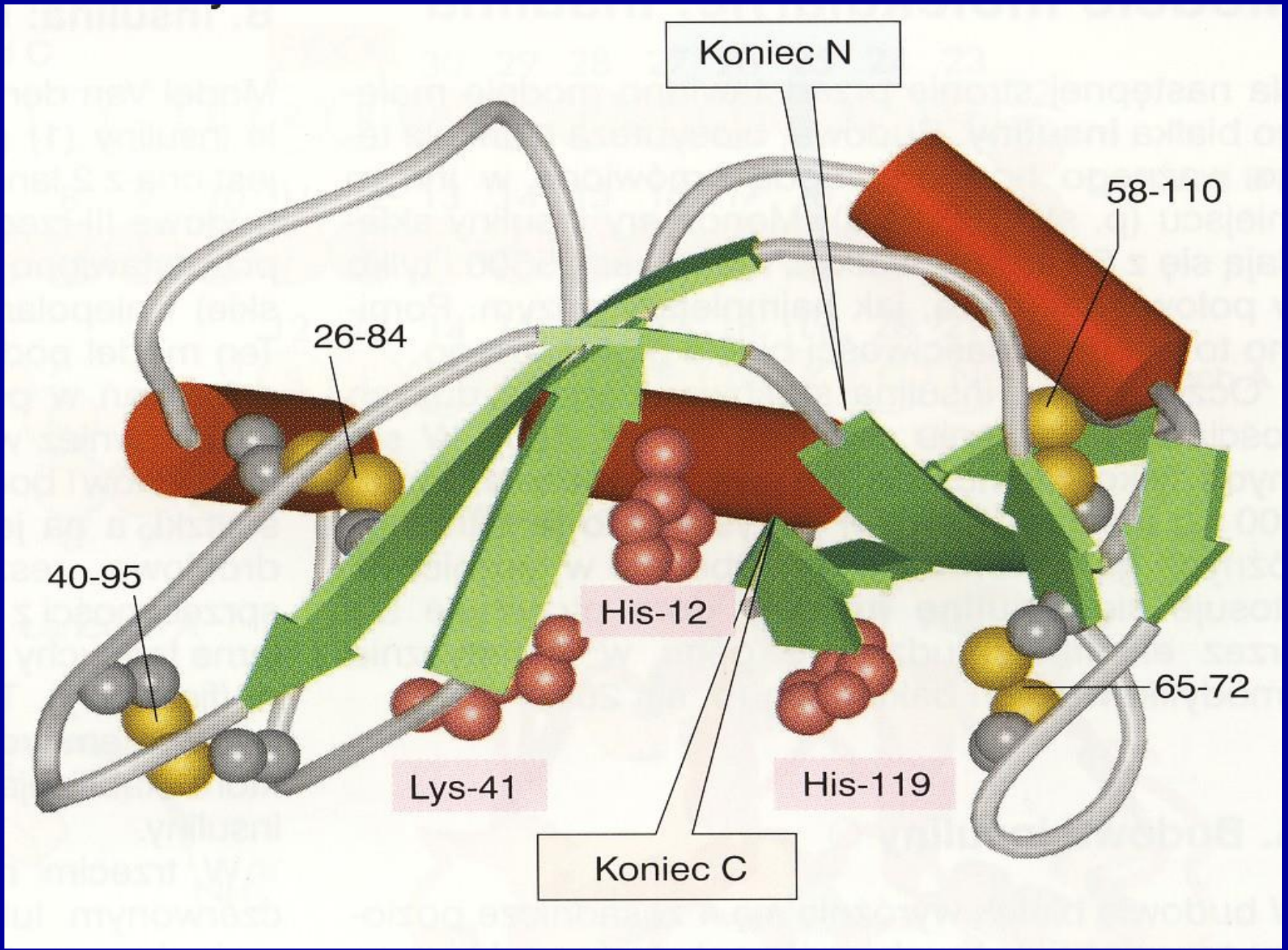

C. Struktura pofałdowanej kartki
1. Przeciwrównoległa
φ = −139°
ψ = +135°


Gly
Gly
Ala
Ala
0,35 nm
Ala
Ser
Gly
Gly
0,57 nm
Gly
Gly
Ala
Ala

Primary and secondary structures along with the interactions give rise to **tertiary structure**. It depends on the presence of particular aminoacids and their interactions - hydrogen bonds, hydrophobic bonds, disulfide linkage, dipole forces



Rys. 9. Typy wiązań uczestniczące w formowaniu cząsteczki białka globularnego: a – wiązanie jonowe, b – wiązanie wodorowe, c – oddziaływania hydrofobowe, d – oddziaływania dipolowe, e – wiązanie disiarczkowe (wg Kulki i Rejowskiego 1994)

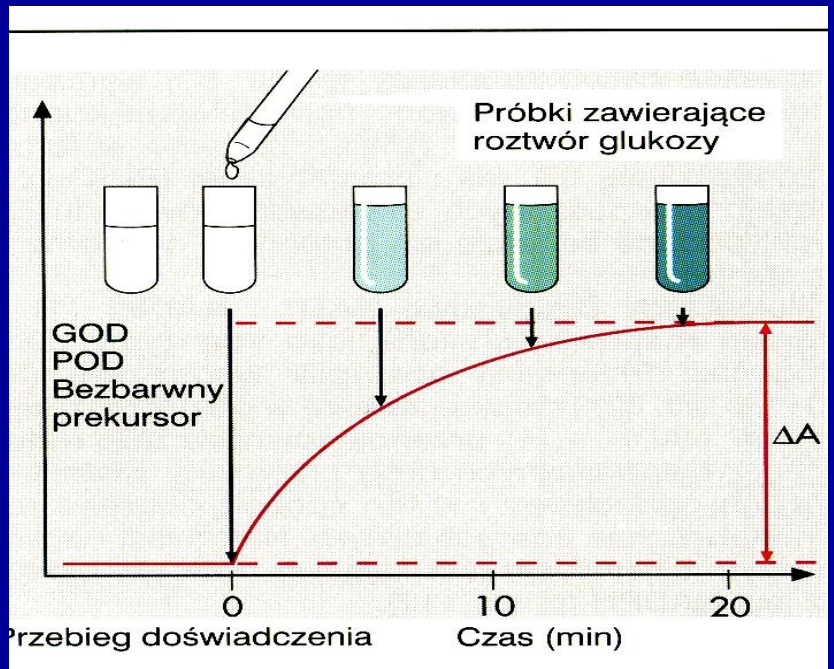

Koniec N
58-110
26-84
40-95
His-12
65-72
Lys-41
His-119
Koniec C

Quantitative determination of protein – biuret method

- The need to determine the concentration of protein in biological sample appears very often in veterinary practice.
- Biuret method is the most commonly used
- The name is related to biuret – the product of condensation of two molecules of urea which binds to cooper ions II giving characteristic blue colour. Cu^{2+} ion binds to two peptide bonds in peptide or protein. The intensity of colour depends directly on the concentration of protein in the solution

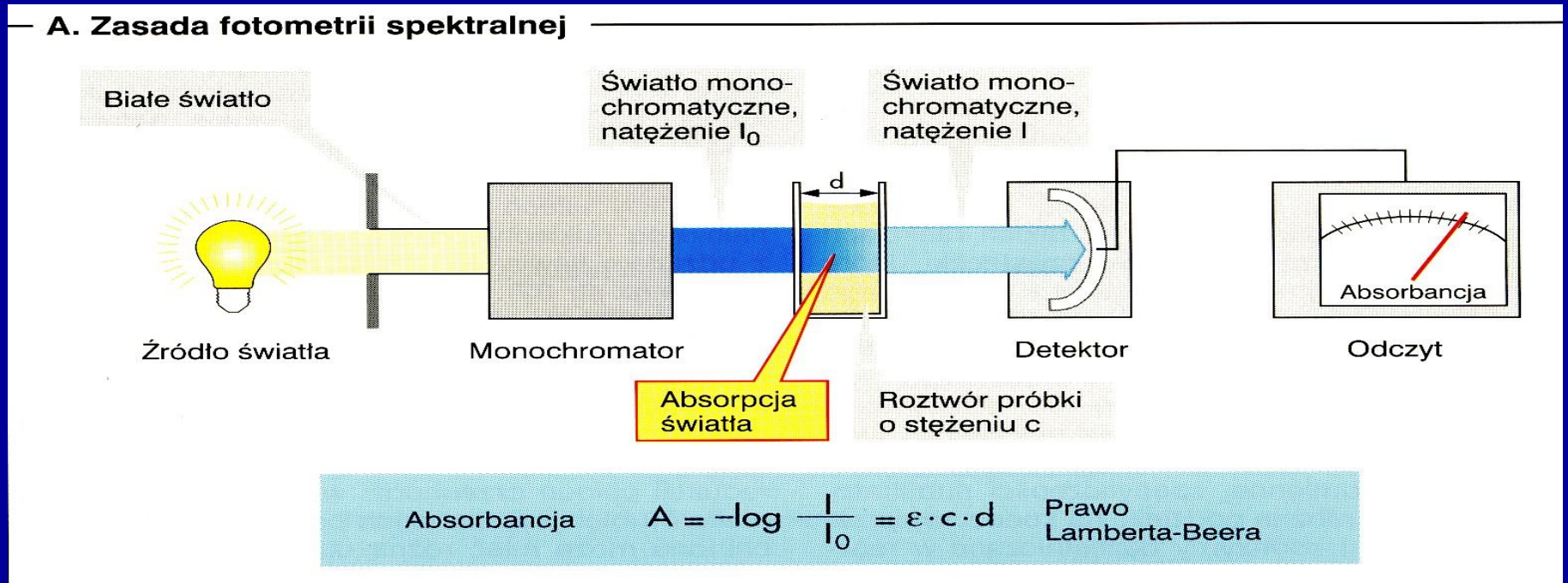
Colorimetric methods are used in the definition of the intensity of colour of solution

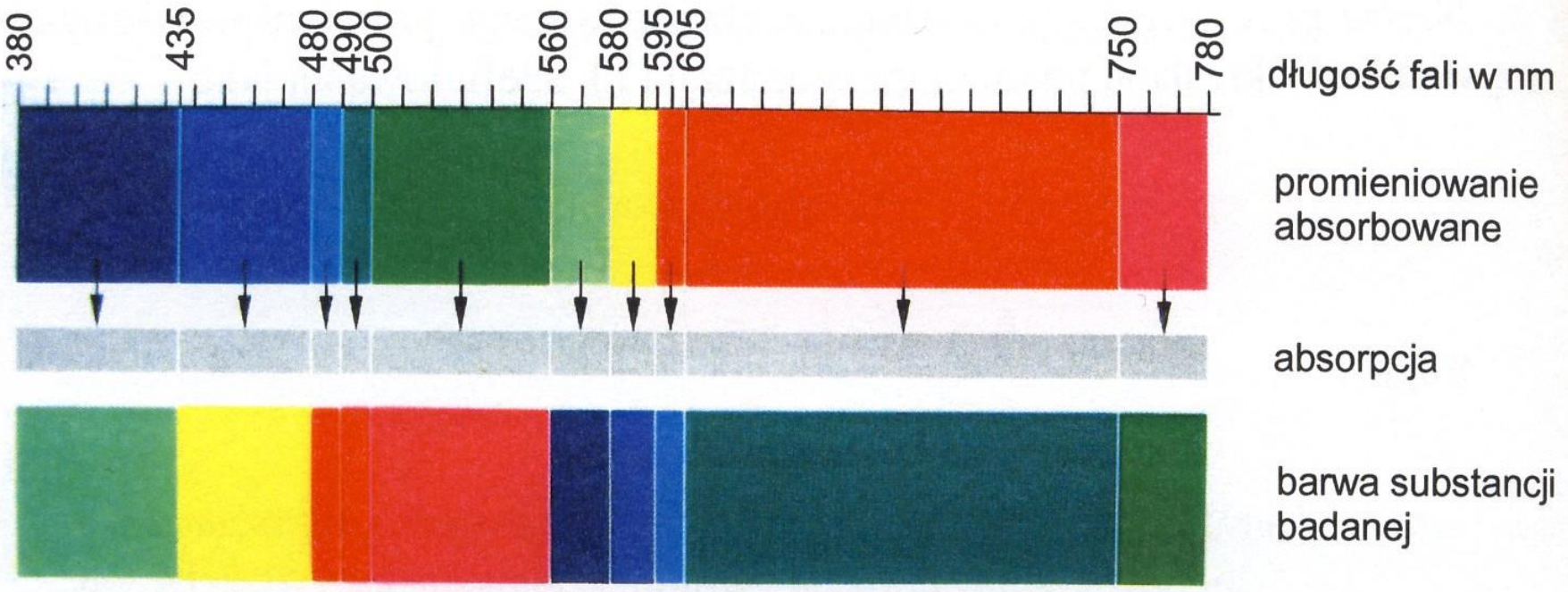
- Previously the scale of colours of known concentrations was the standard. Scientist compared the standard with examined sample subjectively and estimated approximate concentration



Absorptive Spectrophotometry

- Is based on the measurement of the level of absorption of electromagnetic radiation (light) by examined solution so called **absorbancy**
- Absorbancy is directly proportional to the intensity of colour (the concentration of solution) and the length of the path that radiation passes in solution





Rys. 25. Barwy zasadnicze substancji badanych i promieniowanie dopełniające (maksymalnie absorbowane)

Standard curve

- Colorimetric methods are indirect methods. They use standard curve for the definition of the concentration of examined parameter. It can be prepared in advance and used many times when necessary.

Method of Lowry

- One of the most sensitive quantitative method for protein determination.
- Allows for the determination of protein in concentration of 5 -10 micrograms in 1 cm^3 ($\mu g/cm^3$)
- Method is based on color reaction between peptide bond and aromatic aminoacids with Folin-Ciocalteu reagent. The course of reaction requires 2 stages:
- The first is based on joining cooper ions to peptide bond in peptide chains what leads to biuret reaction

- In the second stage the reduction of phosphomolybdic and phosphovolfram acids to appropriate oxides occurs. It is connected with cooper ions bound to protein as well as tyrosine and tryptophan which are in examined protein.
- Absorbancy of colour is measured at 750 nm
- Always standard curve have to be prepared because straight line dependency does not occur.
- Some compounds may give colour metabolites with Folina-Ciocalteu reagent that is why interfere in the determination of protein
- Among others are: phenols, purines, pyrimidines, uric acid. Ether, methanol, urea, 0,5% glycine decrease the intensity of colour.

Method of Bradford

- The method allows for the determination of protein concentration in micrograms per 1 cm^3 ($\mu g/cm^3$)
- The method is based on coloured reaction with Coomassie Brilliant Blue
- Absorbance is measured at wavelength of 595 nm

Absorbance in ultraviolet

- Aromatic aminoacids can absorb the light in ultraviolet. Due to their presence in protein chain we can measure the concentration of this protein at 280 nm wavelength.
- The method allows for the determination of protein concentration in micrograms per 1 cm^3 ($\mu g/cm^3$)

Task 1. Quantitative determination of protein by use of biuret method – preparation of standard curve

Preparation of standard curve.

Prepare different dilutions of standard solution of protein in accordance to table:

No of tube	Casein (1 mg/cm^3)	H_2O dest	Concentration	Absorbance
0	–	1 cm^3	0,000	
1	1,0 cm^3	–	1%	
2	0,8 cm^3	0,2 cm^3	0,8%	
3	0,6 cm^3	0,4 cm^3	0,6%	
4	0,4 cm^3	0,6 cm^3	0,4%	
5	0,2 cm^3	0,8 cm^3	0,2%	

Add 4 cm^3 of cooper reagent to each tube and incubate at room temperature for 25-30 min. Measure absorbance at wavelength of 545 nm against blank. Prepare the plot of dependencies between the concentration of casein and absorbance.