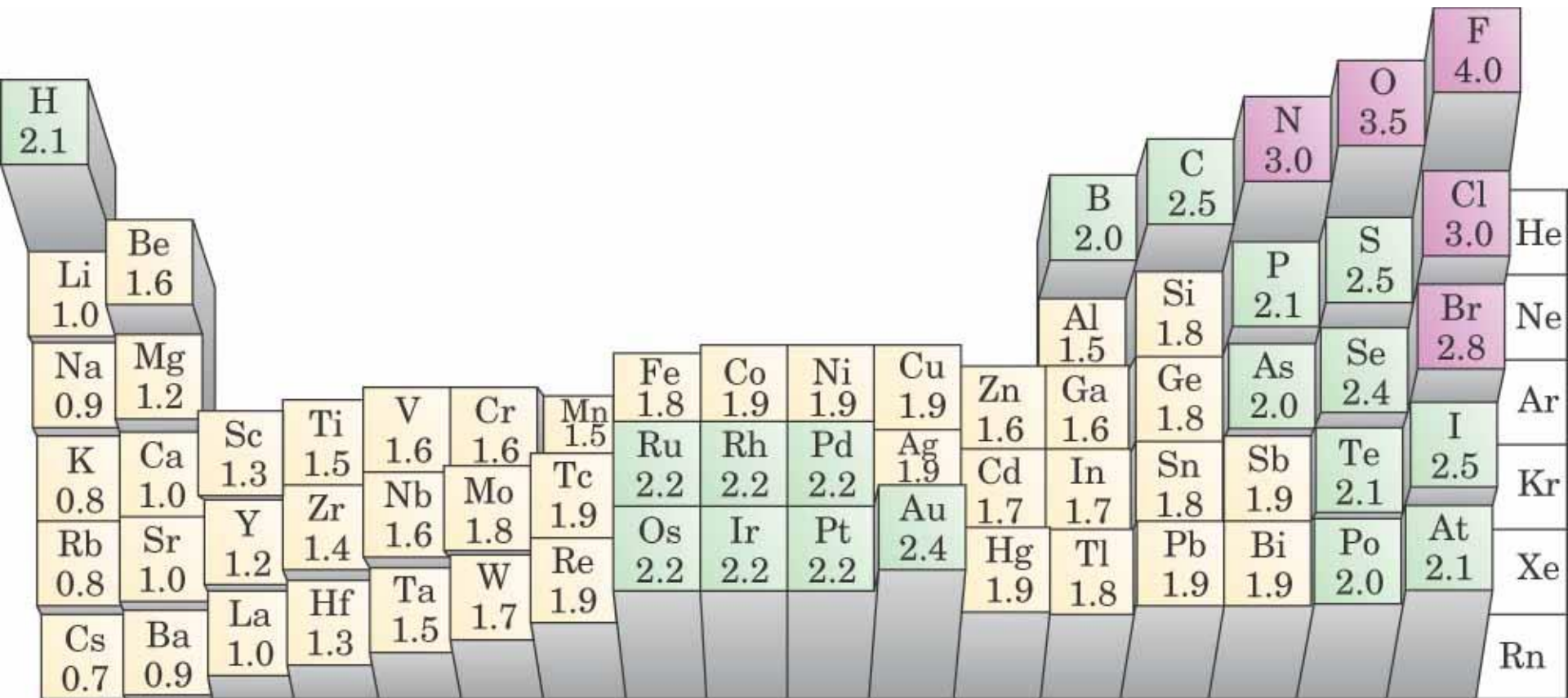


Biologické analýzy I.

Prednáška 1

Elektronegativita

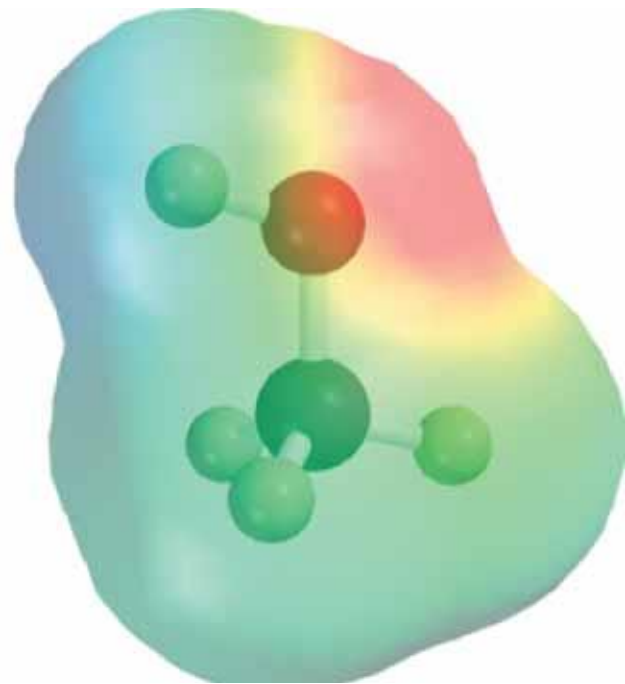
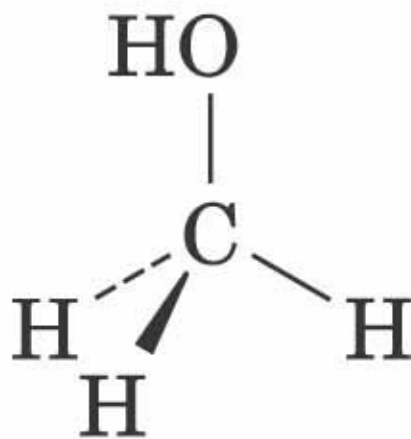


Clasifikácia väzieb

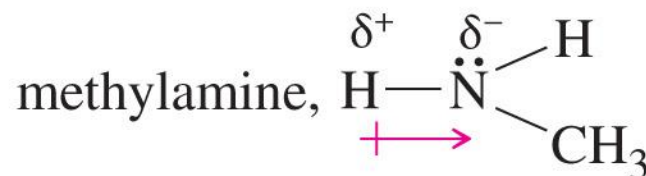
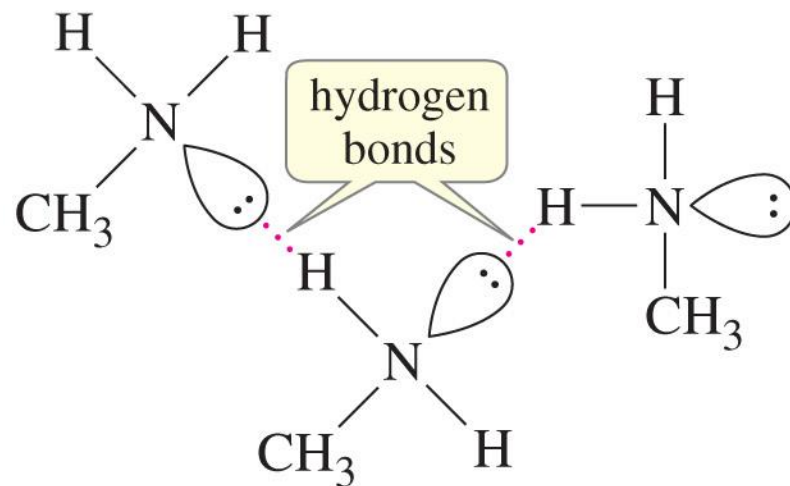
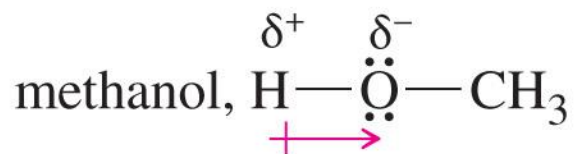
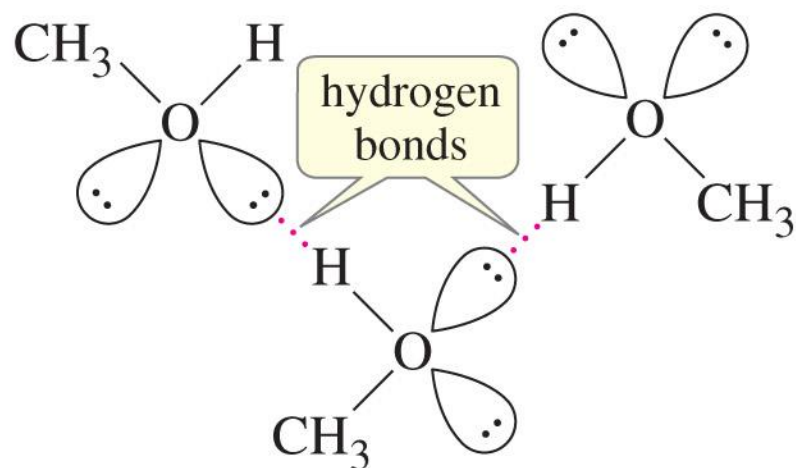
Väzba	Rozdiel v elektronegativite
Nepolárna Kovalentná	$0 \rightarrow 0.4$
Polárna Kovalentná	$0.5 \rightarrow 1.9$
Iónová	$2.0 \rightarrow 4.0$

Metanol

Methyl alcohol

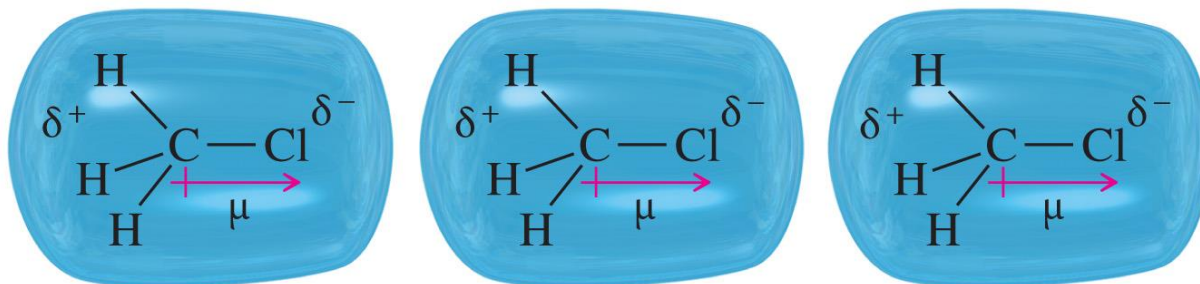


Vodíková väzba

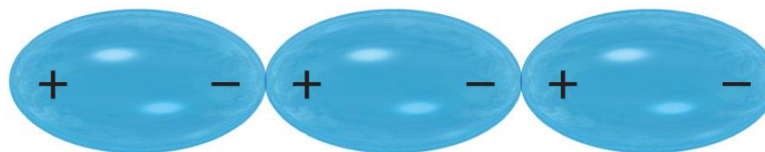


Dipole–Dipole Interaction

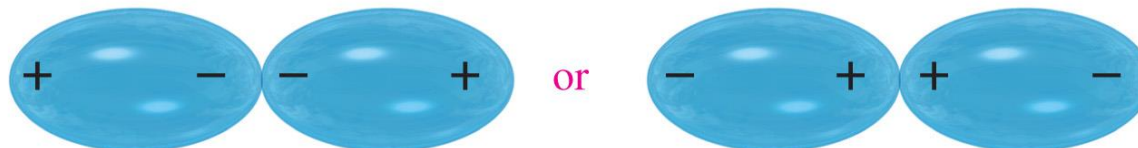
attraction (common)



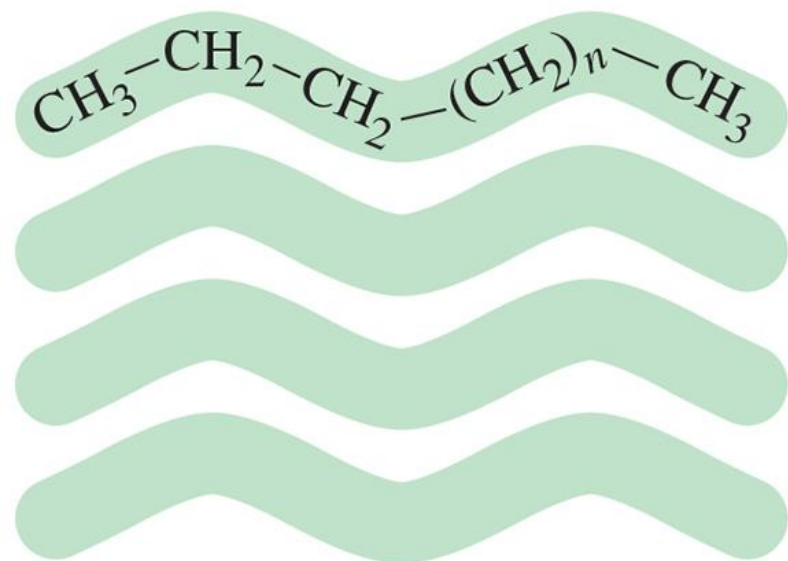
symbolized by



repulsion (uncommon)

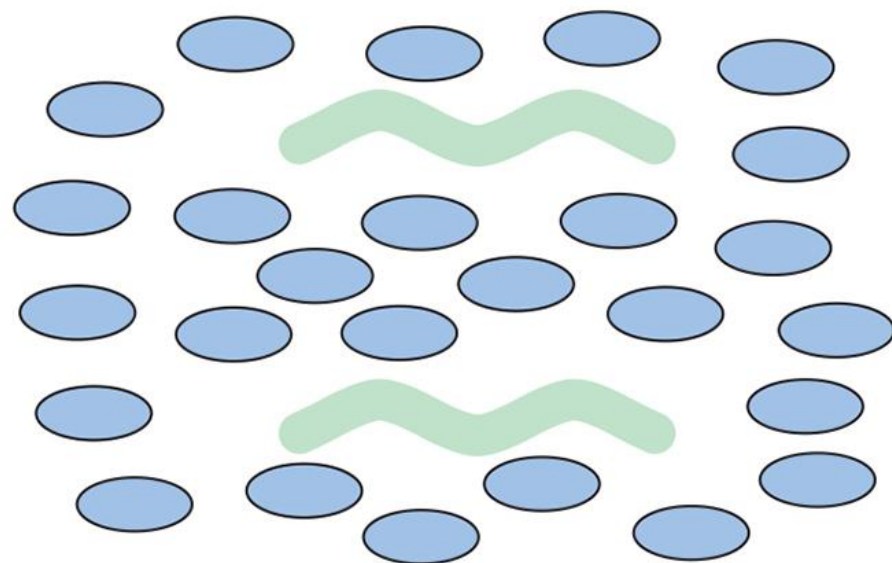


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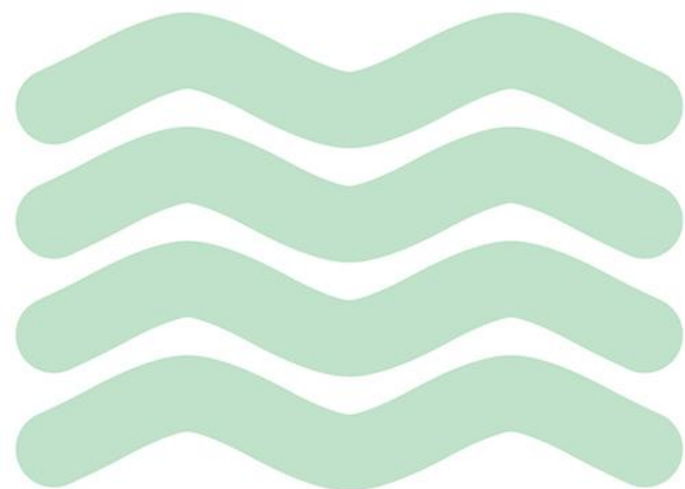


nonpolar solid
(weak intermolecular forces)

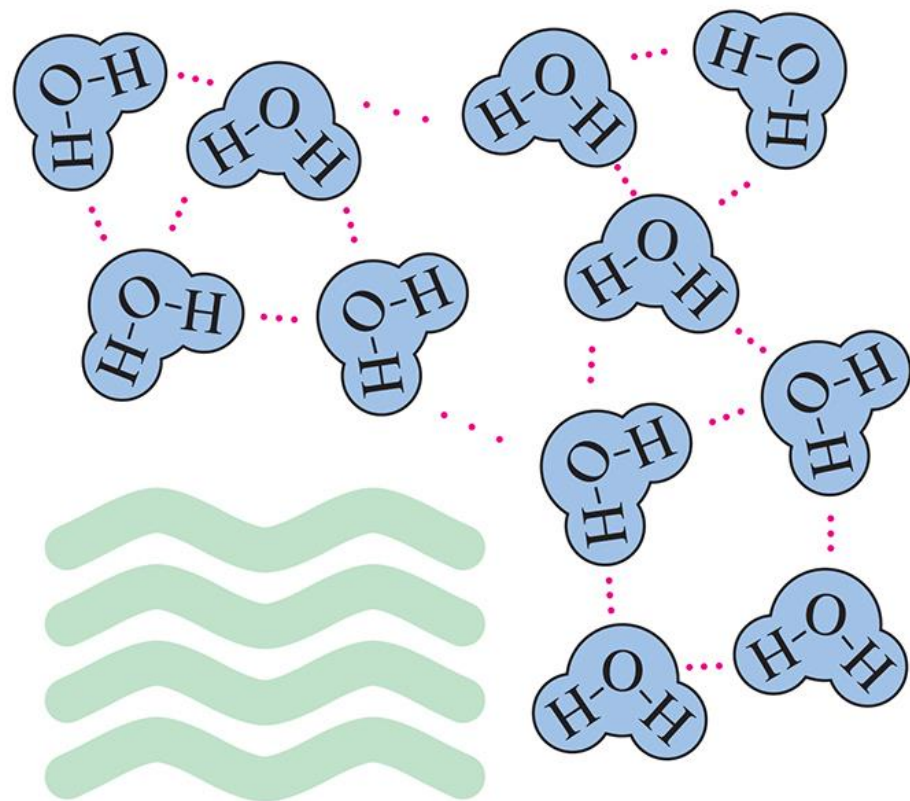
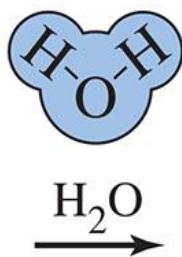
nonpolar
solvent
→



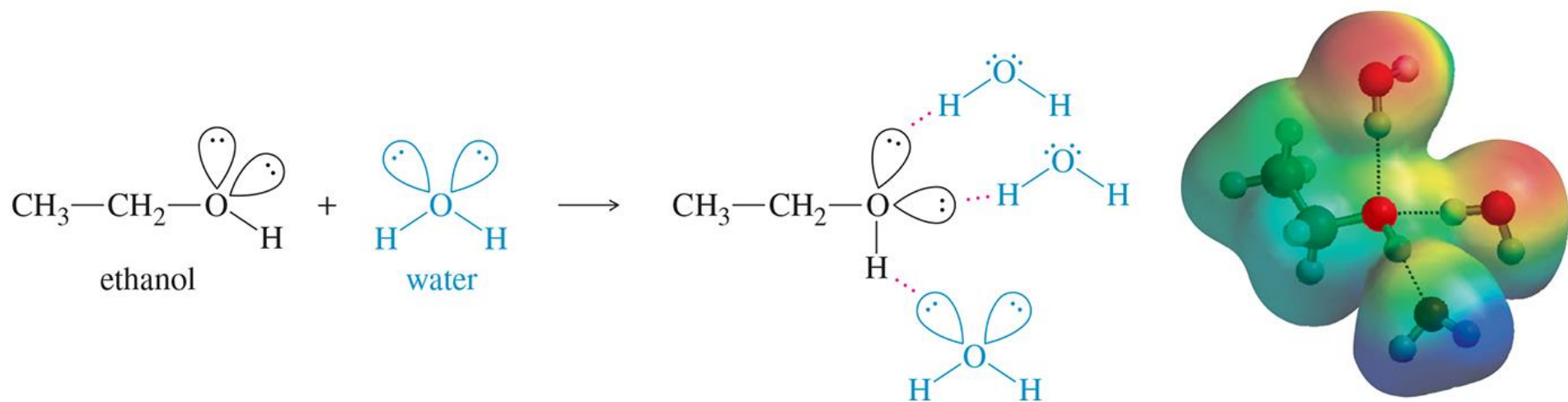
dissolves



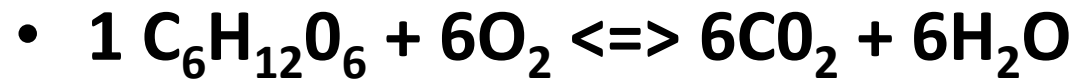
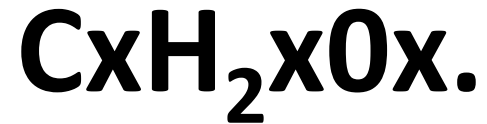
nonpolar solid



does not dissolve



Uhlohydráty





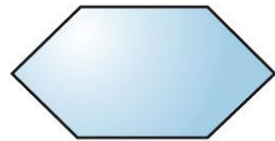
Jednoduché

VS

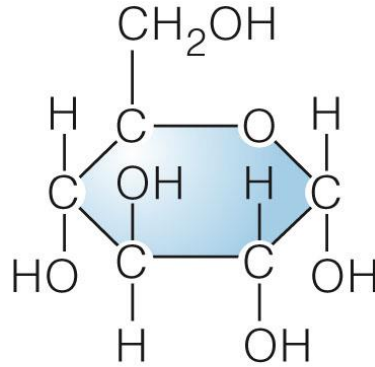
Komplexné

Jednoduché

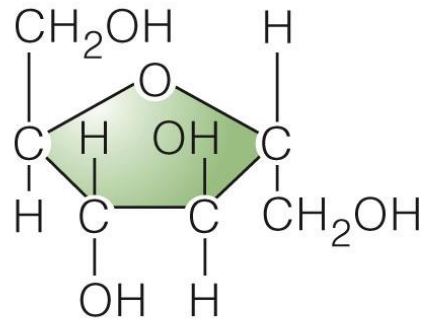
Monosaccharides



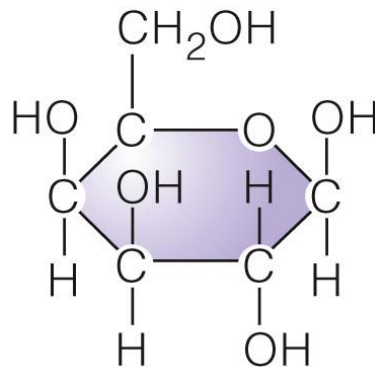
Glucose



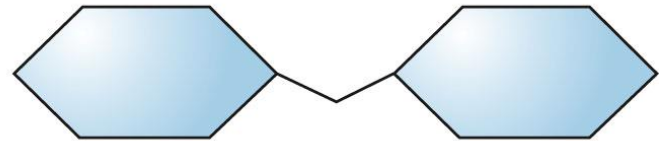
Fructose



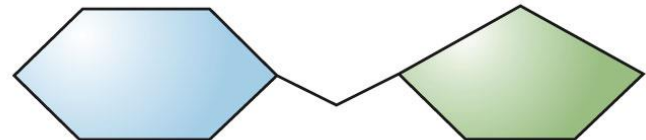
Galactose



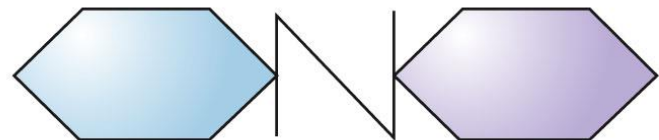
Disaccharides



Maltose

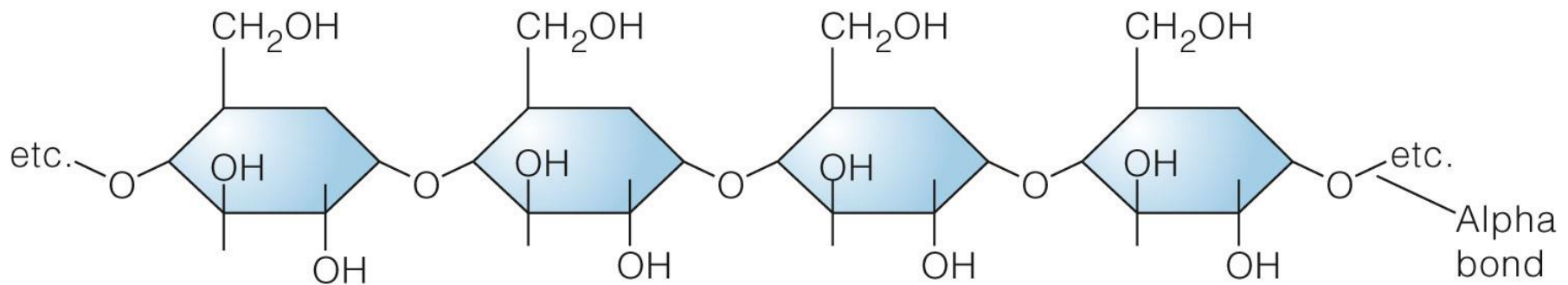


Sucrose

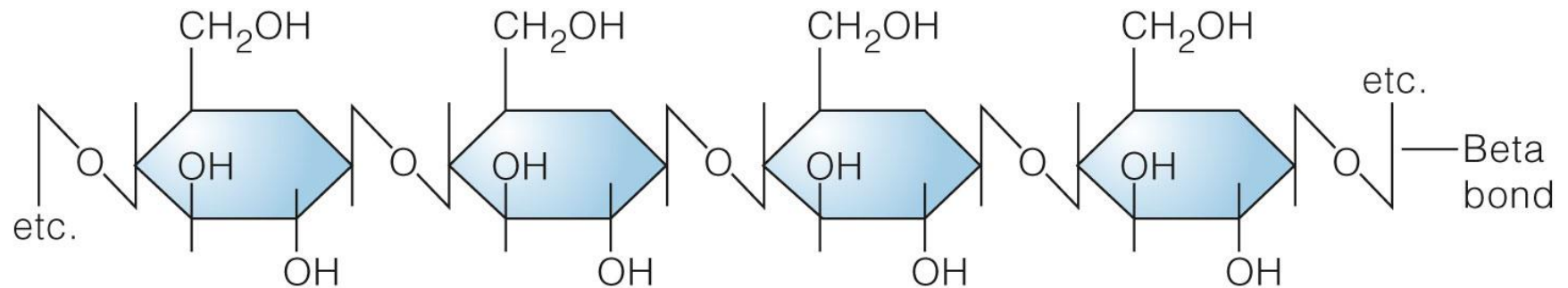


Lactose

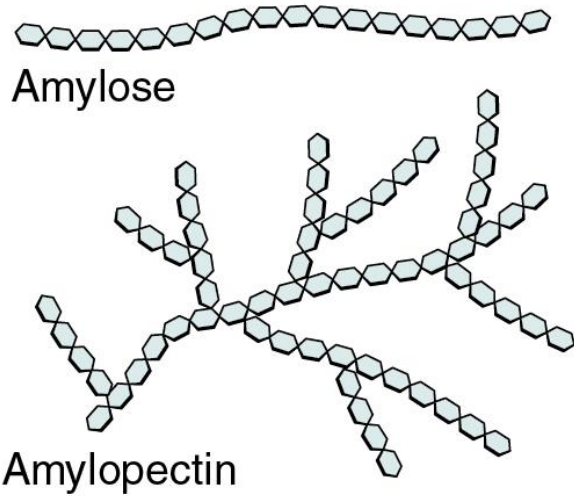
Komplexné



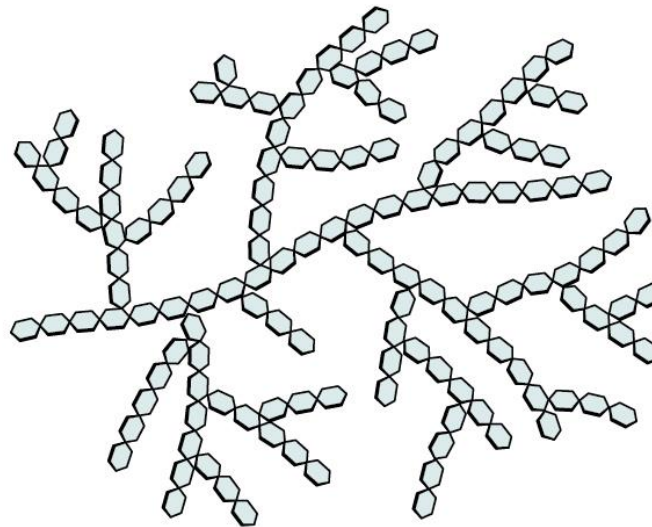
Alpha-amylose: a starch



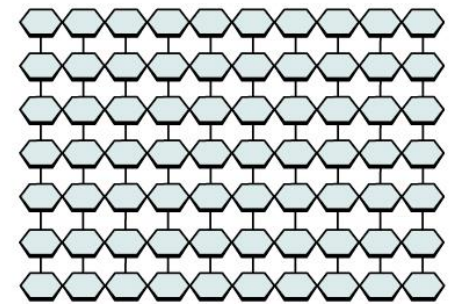
Beta-cellulose: a fiber



Starch



Glycogen



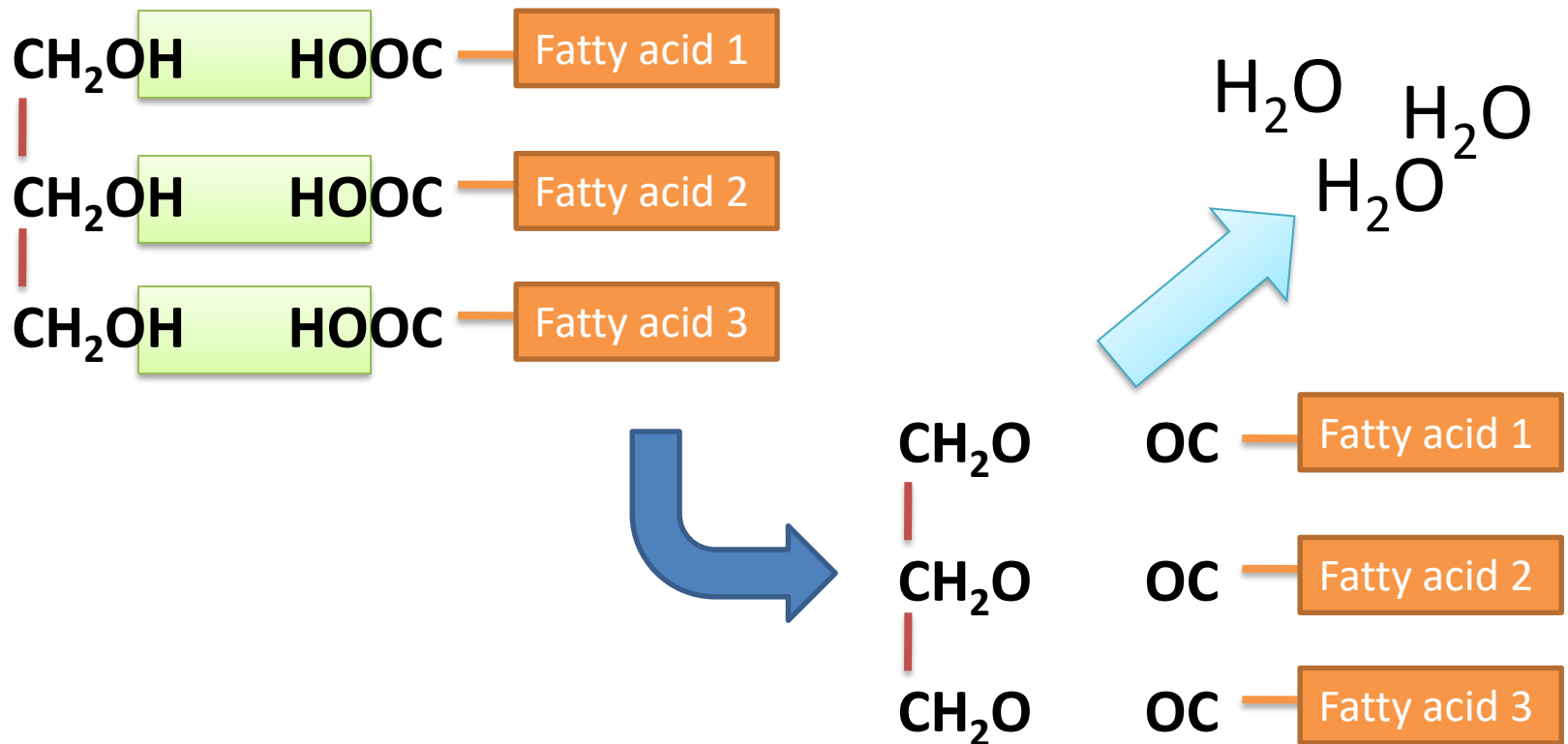
Cellulose (fiber)

LIPIDY

- Ako väčšina biochemických zlúčenín obsahujú **uhlík**, **vodík** a **kyslík**.
- Podiel kyslíka je **menší** ako u uhlohydrátov.
- Sú tvorené molekulou **GLYCEROLU** a molekulou **mastnej kyseliny**.



Triglyceridy

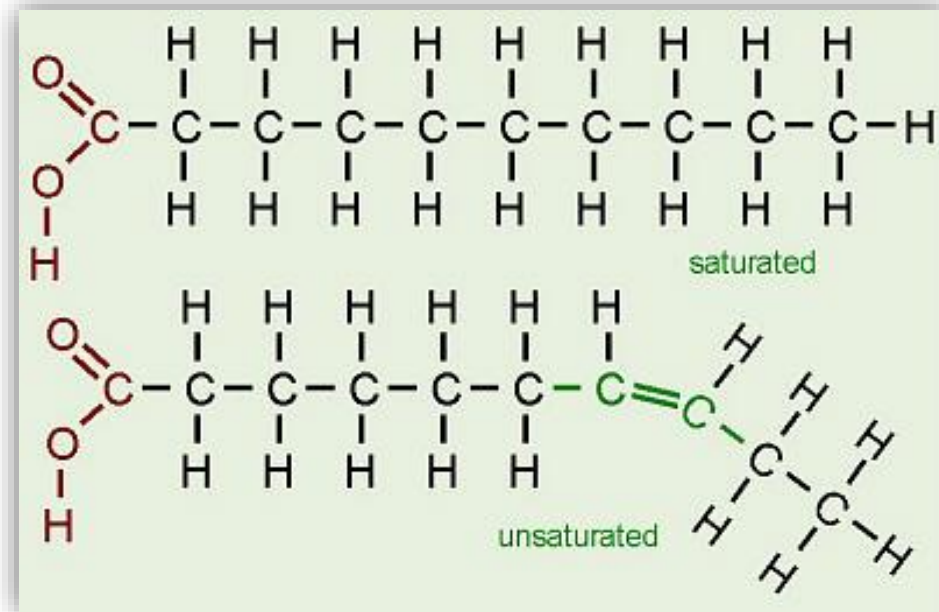


Mastné kyseliny

- Schématicky

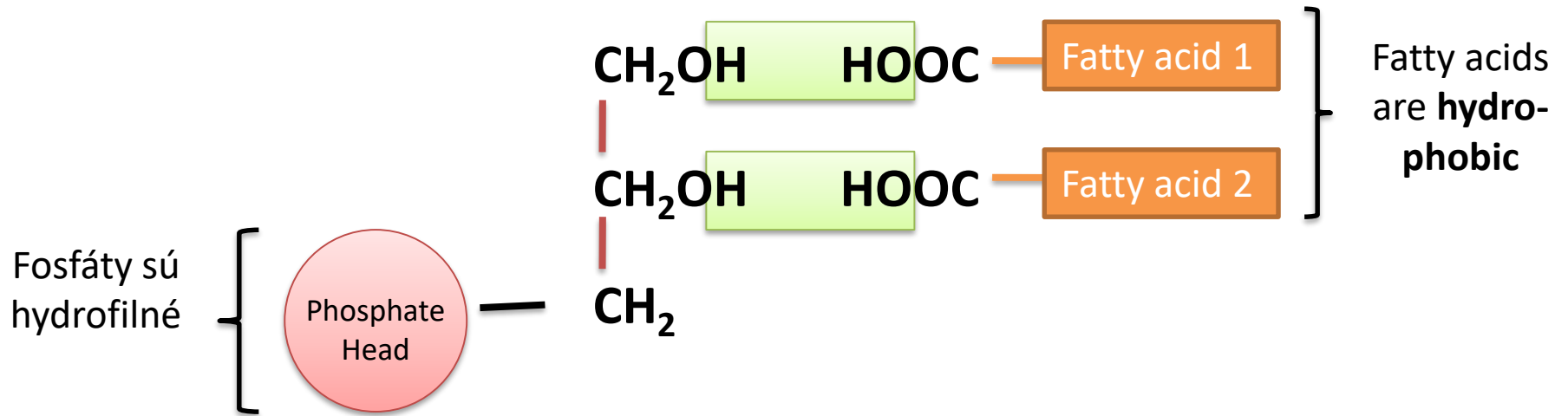


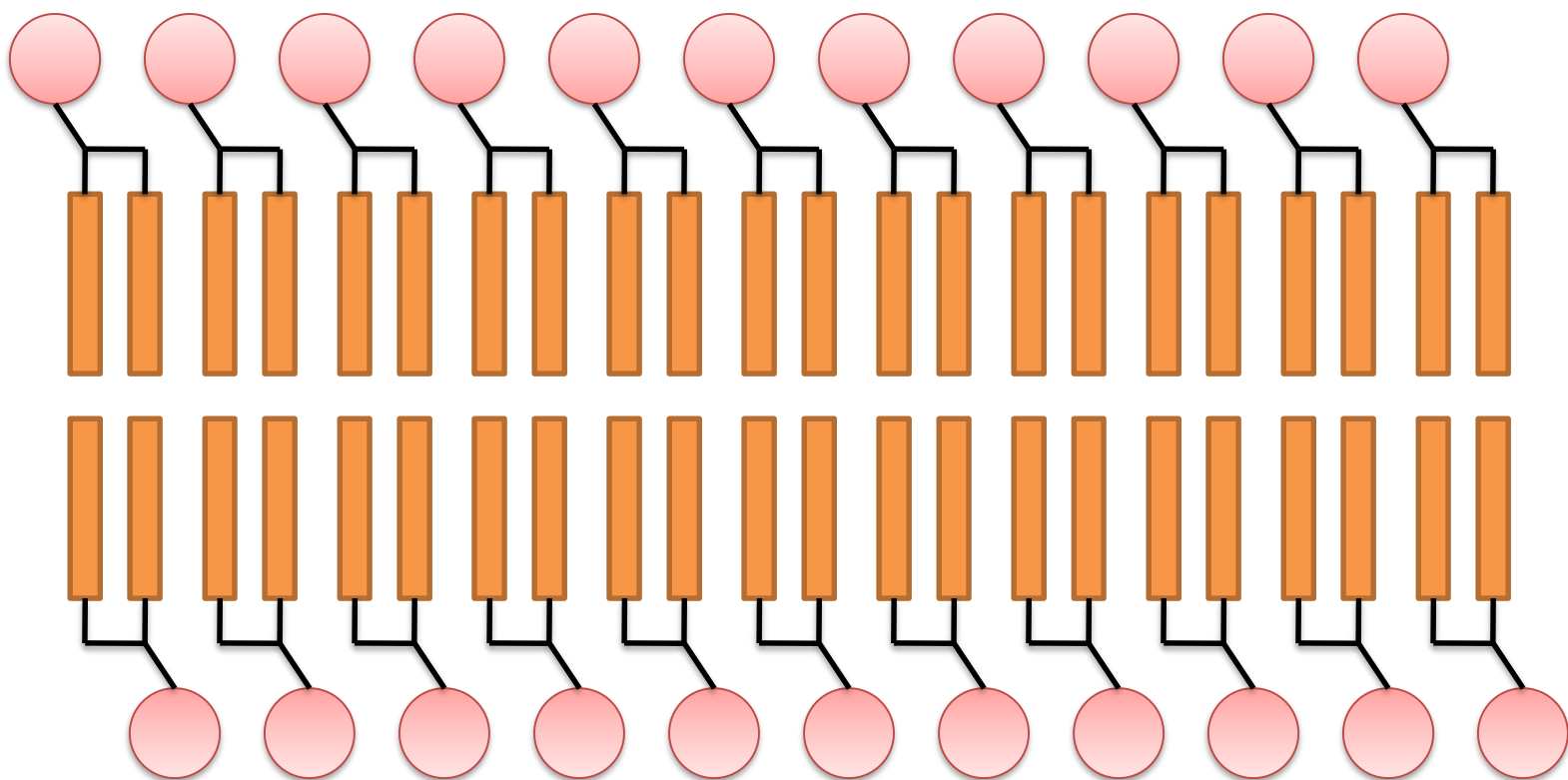
- Naozaj:

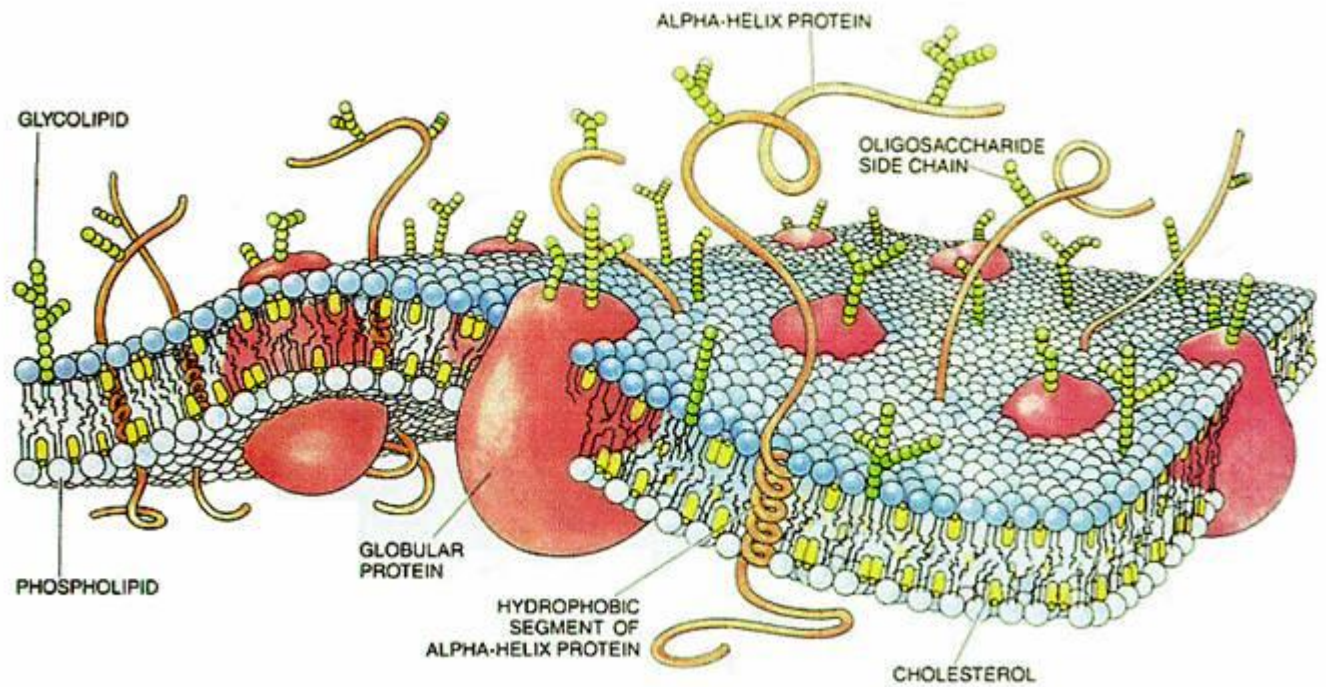
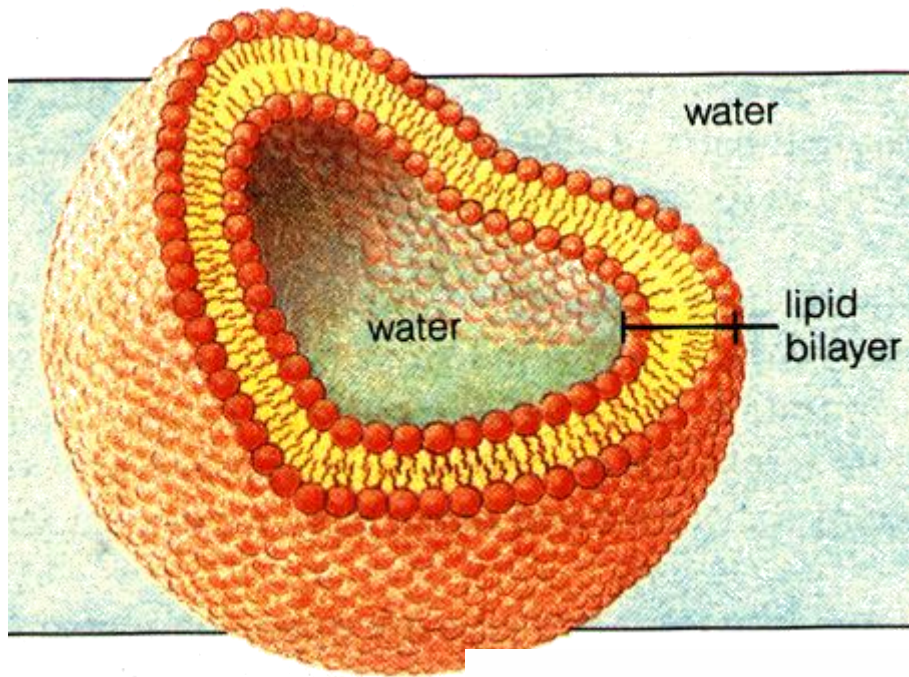


- Okolo **70** rôznych mastných kyselín.
- Všetky majú karboxylovú skupinu (-COOH), čo ich robí kyselinami.

Fosfolipidy

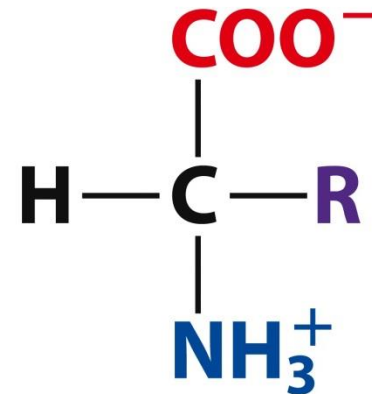




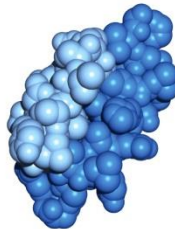


Štruktúra aminokyselín

- Alpha carbon
- Vedľajší reťazec
- Karboxilová skupina
- Amino skupina

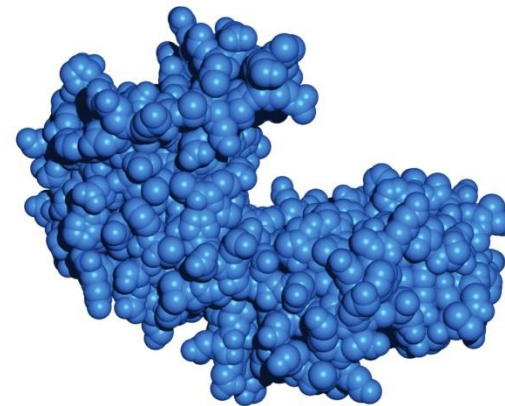


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Insulin (pig)
Released from the pancreas to signal the availability of the metabolic fuel glucose (more in Section 19-2)

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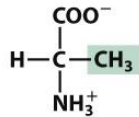


Phosphoglycerate kinase (yeast)
Catalyzes one of the central reactions in metabolism (more in Section 13-1)

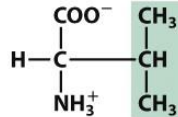
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Náboj

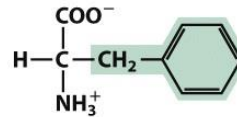
Hydrophobic amino acids



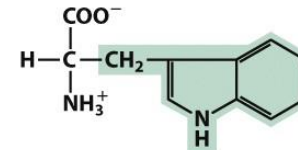
Alanine (Ala, A)



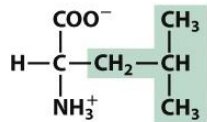
Valine (Val, V)



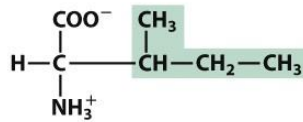
Phenylalanine (Phe, F)



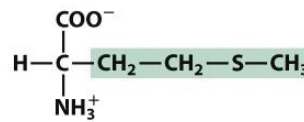
Tryptophan (Trp, W)



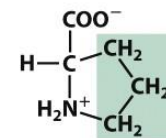
Leucine (Leu, L)



Isoleucine (Ile, I)

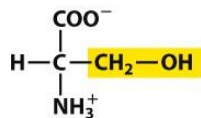


Methionine (Met, M)

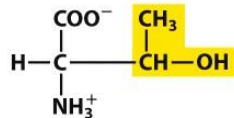


Proline (Pro, P)

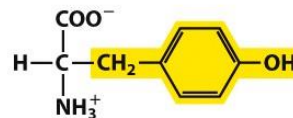
Polar amino acids



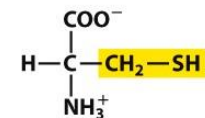
Serine (Ser, S)



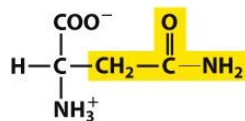
Threonine (Thr, T)



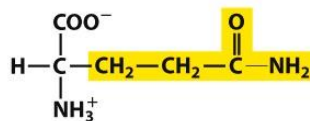
Tyrosine (Tyr, Y)



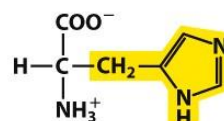
Cysteine (Cys, C)



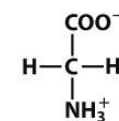
Asparagine (Asn, N)



Glutamine (Gln, Q)

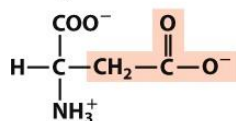


Histidine (His, H)

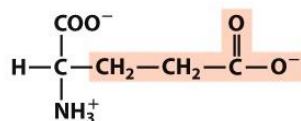


Glycine (Gly, G)

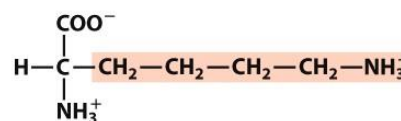
Charged amino acids



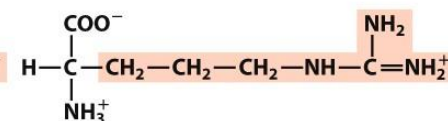
Aspartate (Asp, D)



Glutamate (Glu, E)

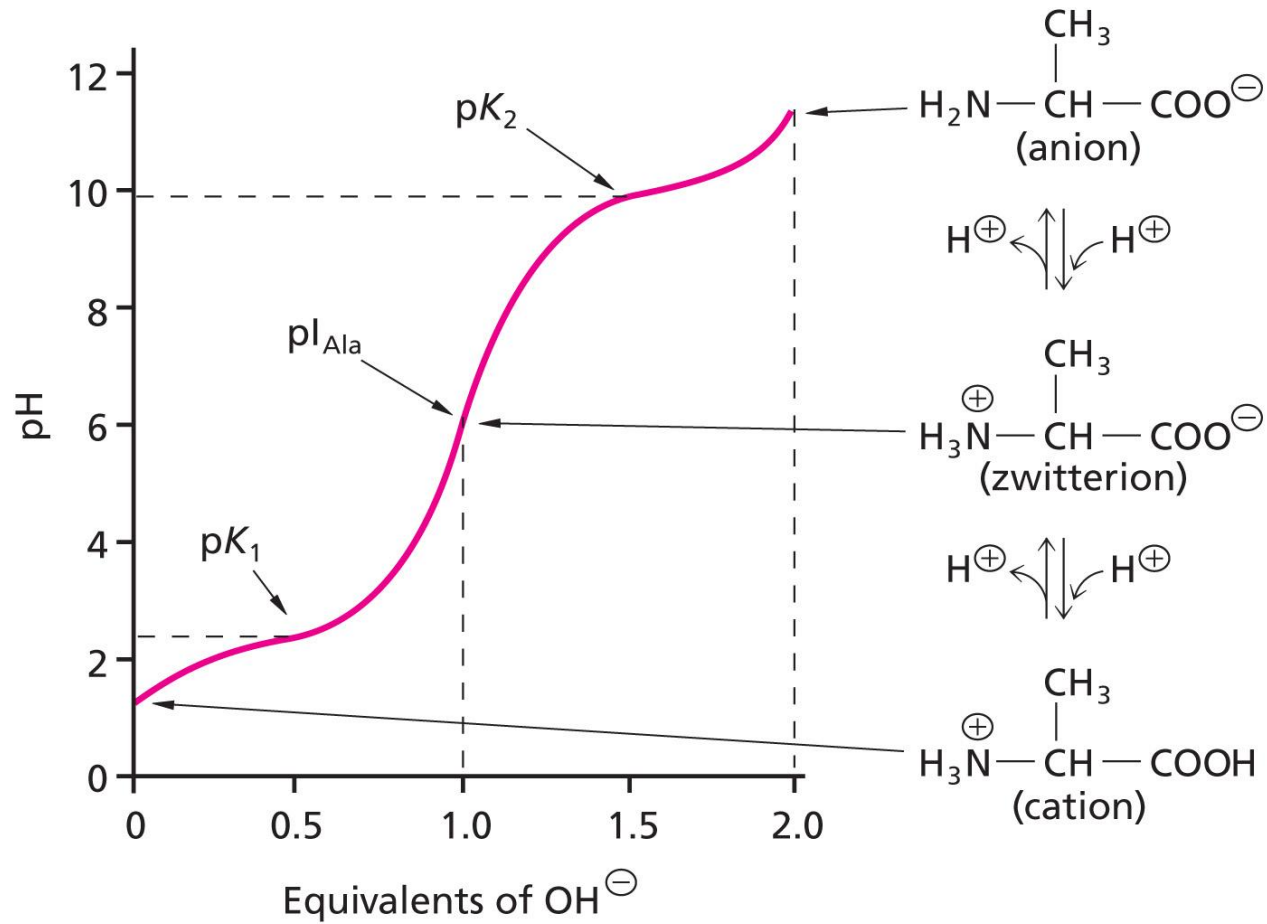


Lysine (Lys, K)

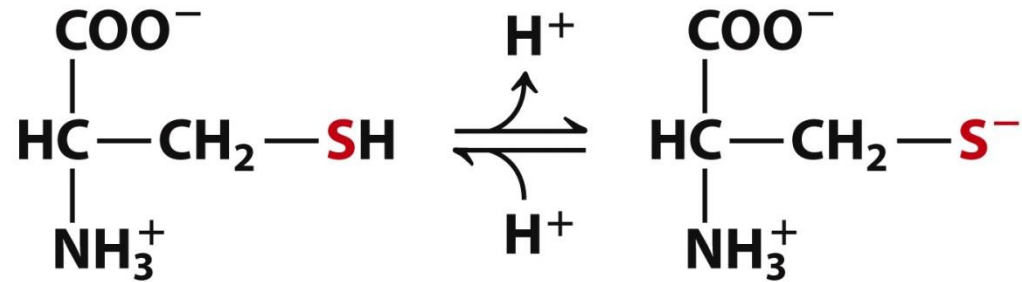


Arginine (Arg, R)

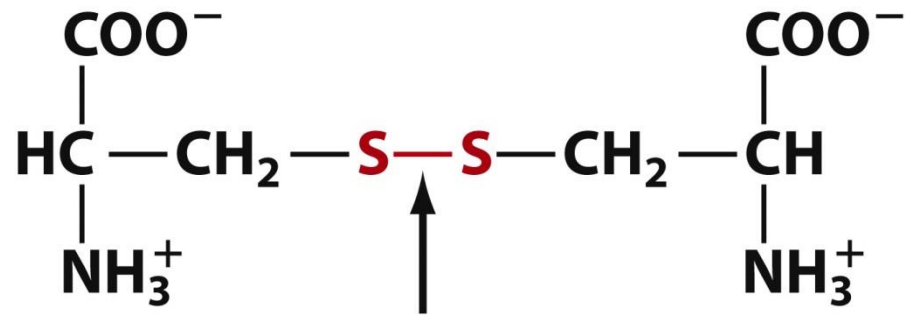
Titrační krivka



Disulfidická väzba



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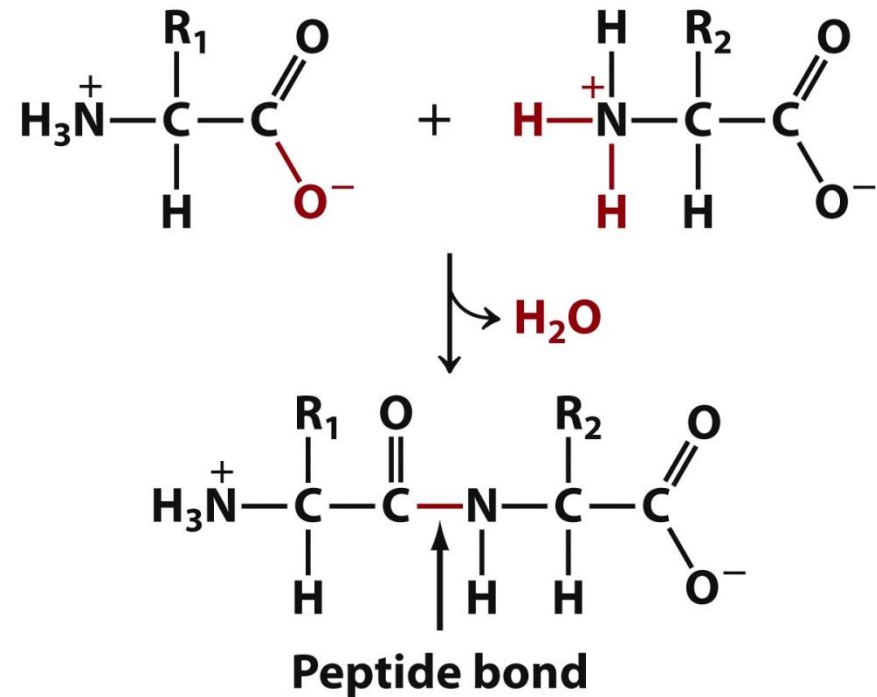


Disulfide bond

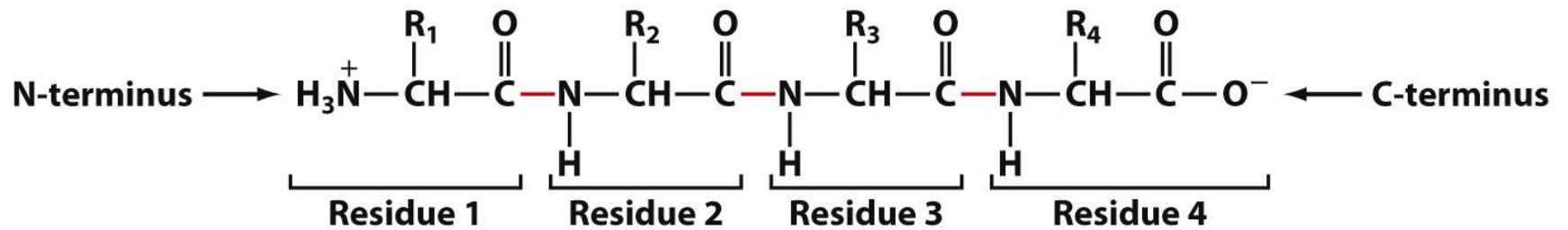
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Peptidická väzba

- Primárna štruktúra
- N- a C-terminus
- Kondenzácia a hydrolýza



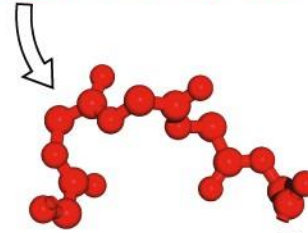
Polypeptid



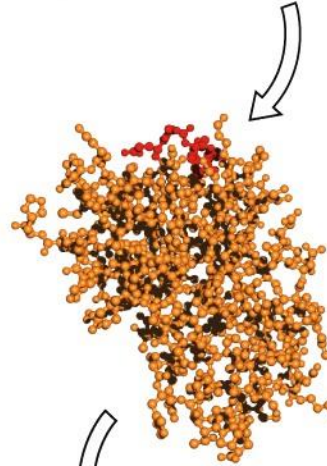
Primary structure
The sequence of
amino acid residues

-Glu-Ser-Phe-Gly-Asp-

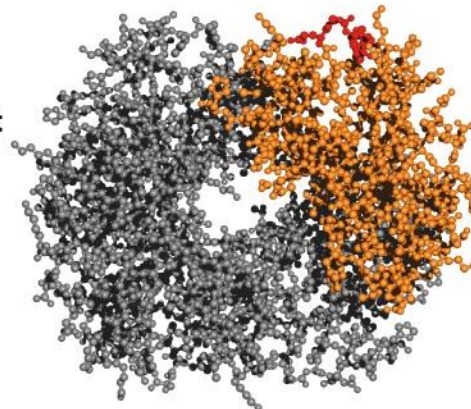
Secondary structure
The localized
conformation of
the polypeptide backbone



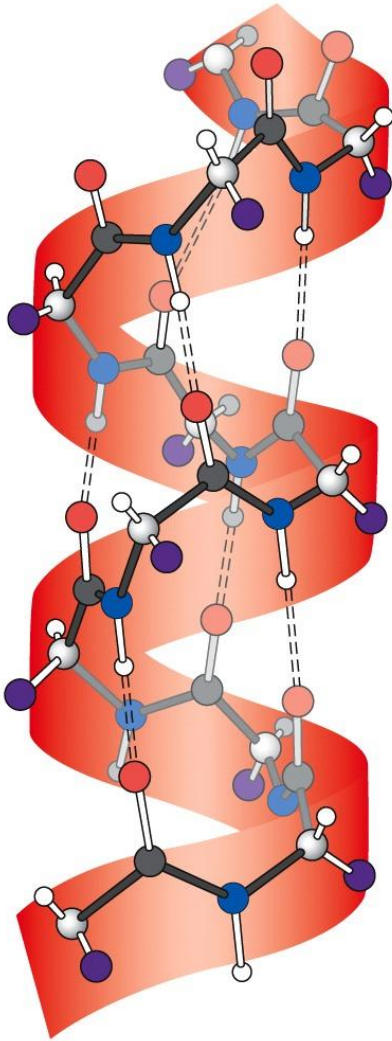
Tertiary structure
The three-dimensional
structure of an entire
polypeptide, including
all its side chains



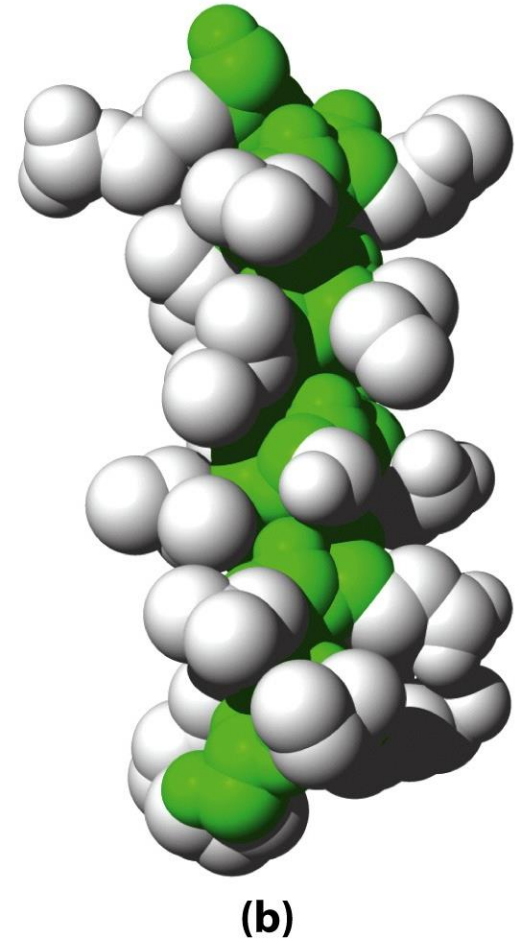
Quaternary structure
The spatial arrangement
of polypeptide chains
in a protein with
multiple subunits



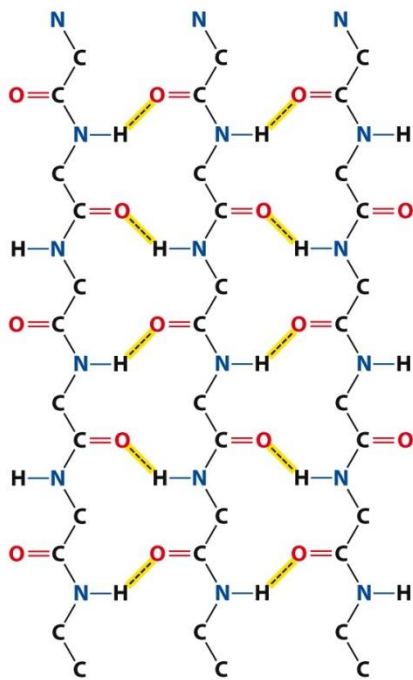
Alpha Helix



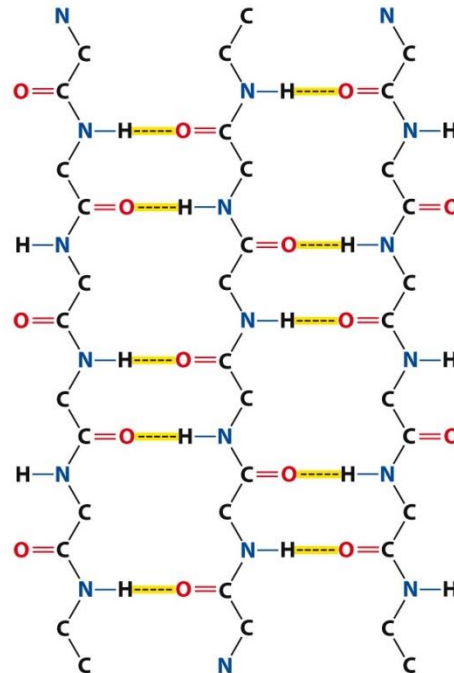
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Beta Sheets



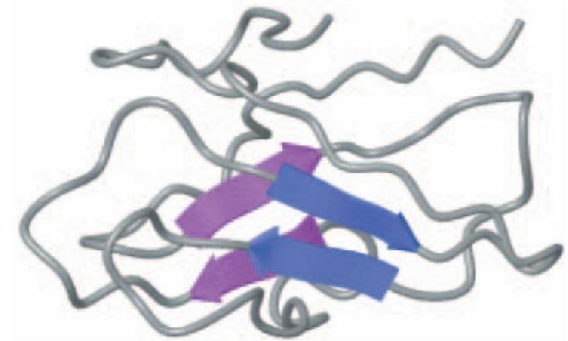
Parallel



Antiparallel

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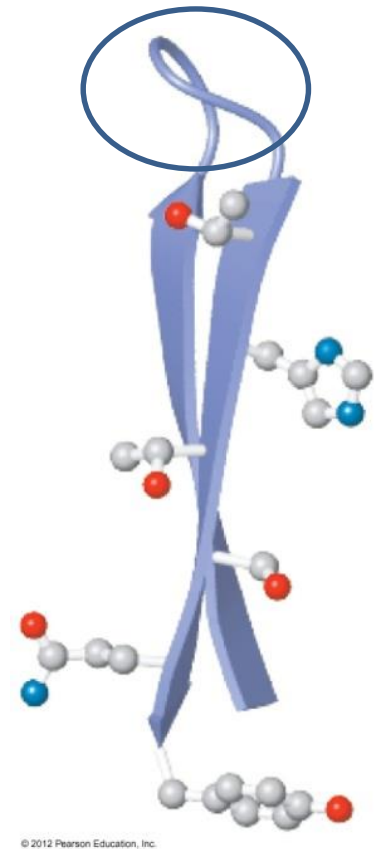
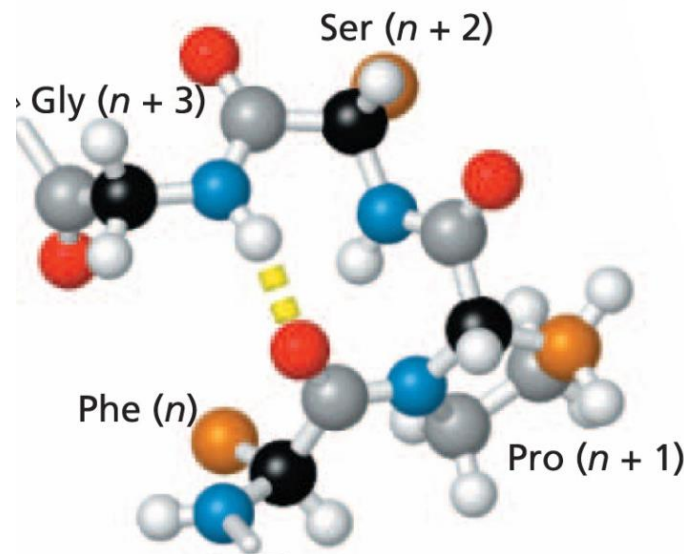
(a)



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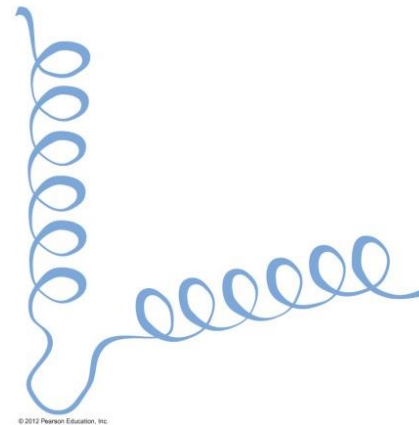
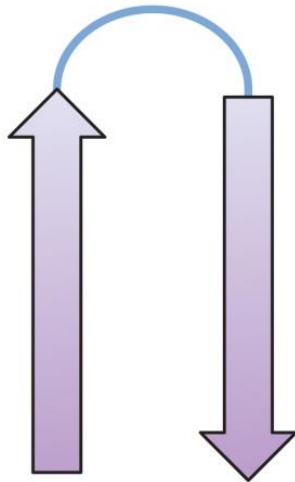
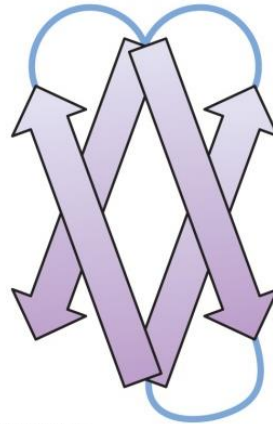
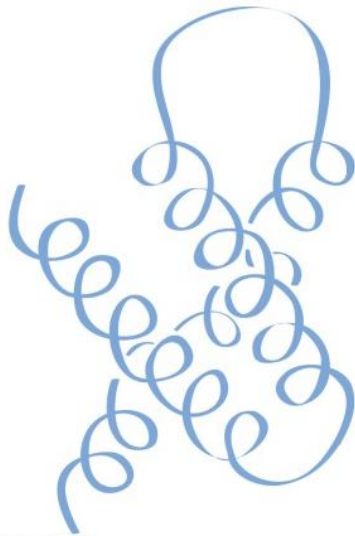
Nepravidelné sekundárne štruktúry

- Slučky a obrátky
- Zmena smeru
- Vnútorne vodíkové väzby
- Gly, Pro



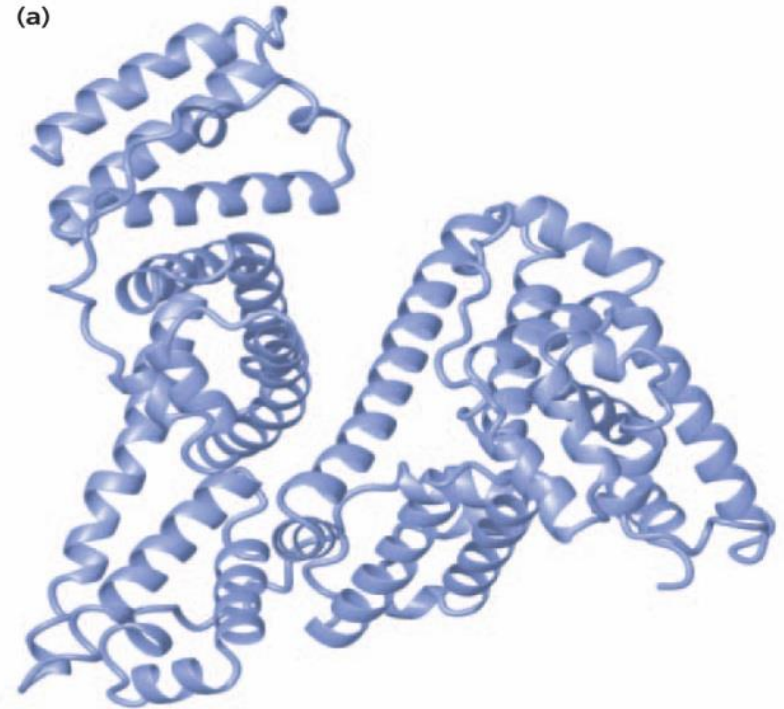
Motív

(Kombinácia sekundárnych štruktúr)



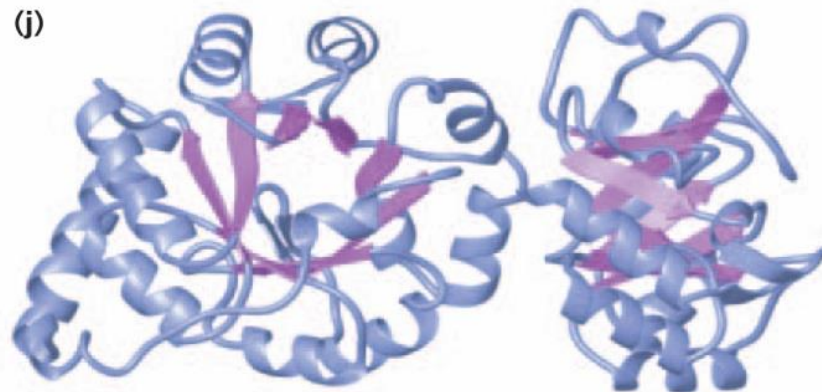
Domény

- Jedinečné oblasti molekuly
- Môžu mať rôzne aktivity ako napríklad “ATP binding domain” alebo “catalytic domain”
- Podobná aktivita medzi viacerými proteínmi



Human serum albumin

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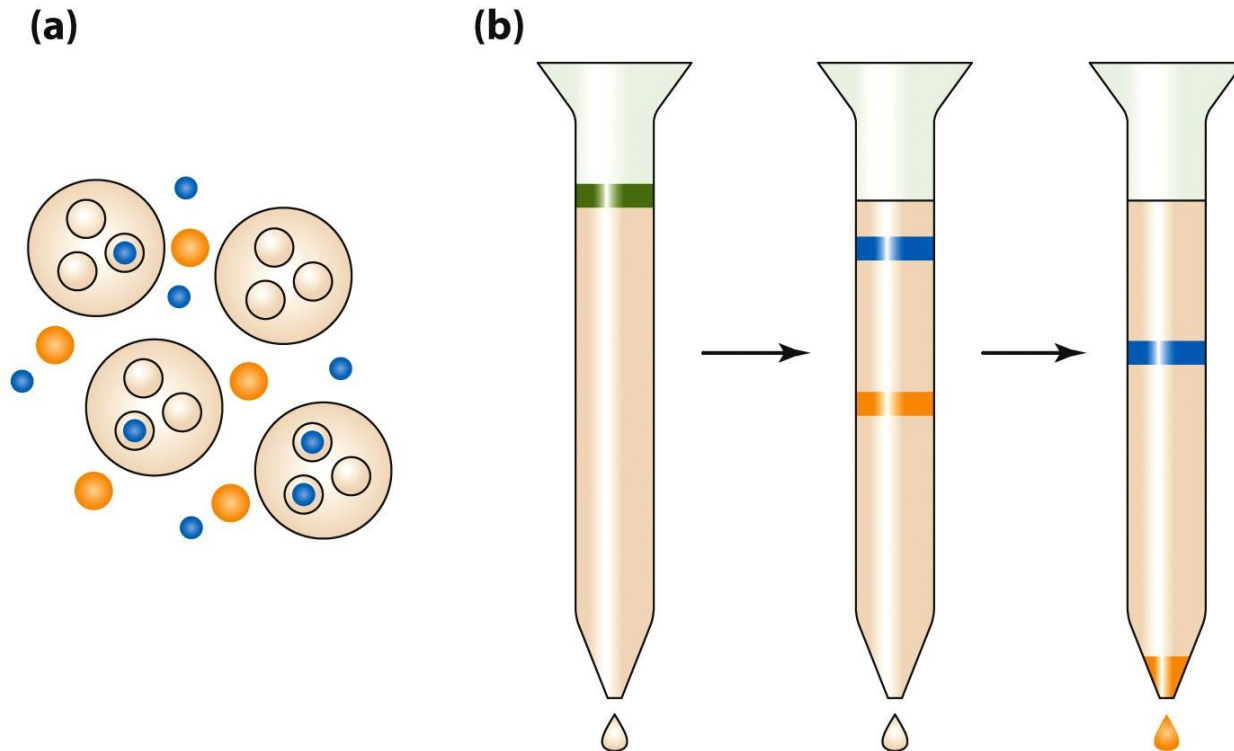
E. coli tryptophan biosynthesis enzyme

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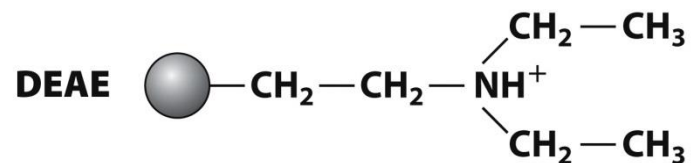
Analýza proteínov

- Chromatografia
 - Affinity
 - HPLC
 - Gélová filtrácia
 - Ionová výmena
- Electrophoresis
 - Isoelektrická fokusácia
 - SDS-PAGE

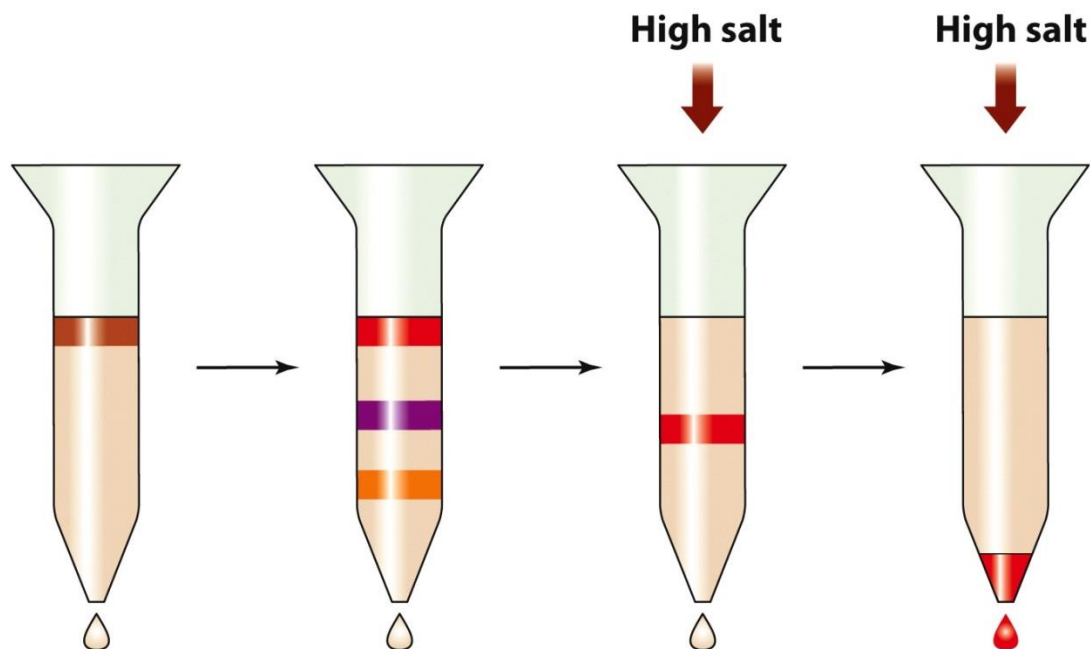
Gélová filtrácia



Ionová výměna



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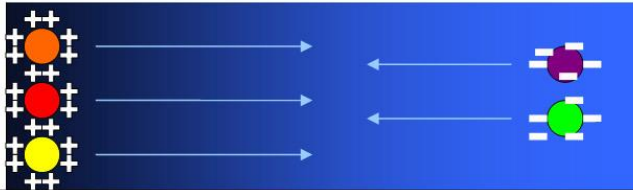
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Stable pH gradient

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

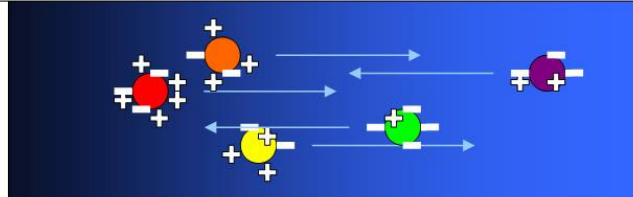


+



At low pH, most proteins have a positive charge while at high pH, most proteins have a negative charge.

+



When an electric field is present, the cathode and anode ends pull the proteins to their isoelectric point where each individual protein possesses a neutral charge.

+



The proteins stopped migrating because they've reached their isoelectric point at a unique pH level.

● = Isoelectric point at pH 7.5

● = Isoelectric point at pH 6.8

● = Isoelectric point at pH 8.5

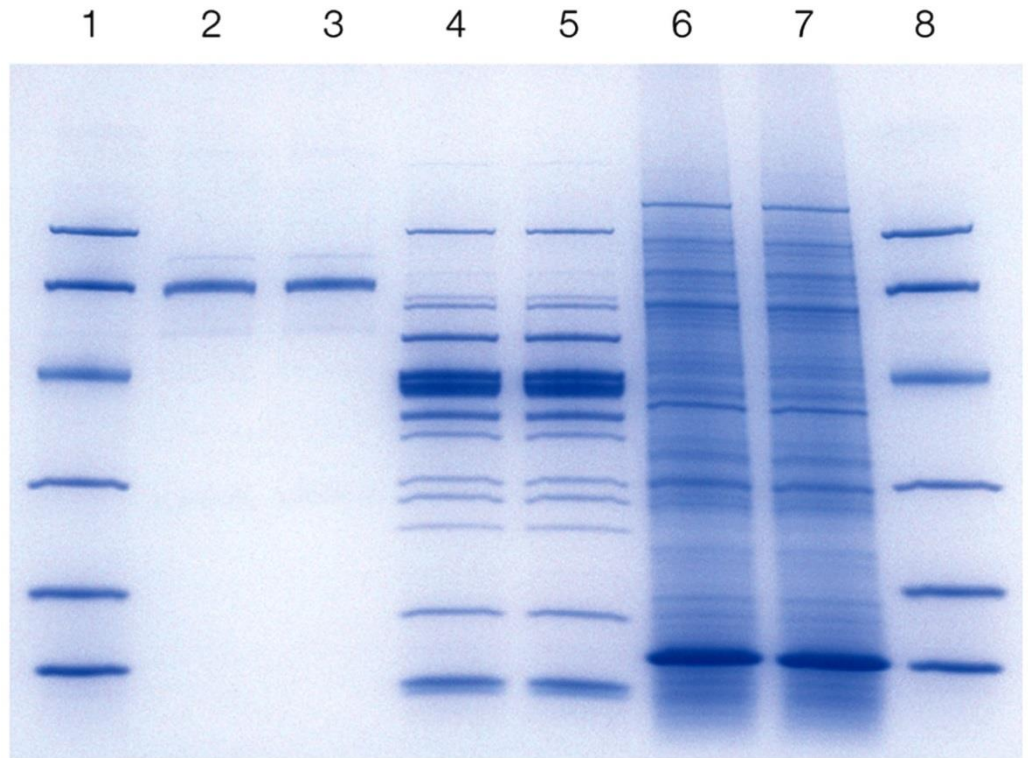
● = Isoelectric point at pH 10.1

● = Isoelectric point at pH 5.6

Izoelektrická fokusácia

SDS PAGE

- Sodium dodecyl sulfate
- Electroforéza na polyakrilamidovom géle



[Courtesy of Bio-Rad Laboratories, ©2012.]