Non-covalent Interactions in Biological Sciences

EVROPSKÁ UNIE
Evropské strukturální a investiční fondy
Operační program Výzkum, vývoj a vzdělávání

MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY

What are non-covalent interactions?

What are the major non-covalent interactions in biological systems?

What are the major non-covalent interactions in biological systems?

electrostatic (Coulombic) interactions – interactions between charged groups, partial charges or dipoles

hydrogen bonds – X-H...Y interaction, where X and Y are eletronegative atoms, they are not special from the physical point of view because they can be viewed as a combination of electrostatic and London dispersion forces

alternative hydrogen bonds and other interactions (pi-pi, CH/pi, cation/pi, halogen bonds...)

van der Waals interactions – other than H-bonds and electrostatic Interactions, much weaker interactions between charged or uncharged atoms originating from London dispersion forces

hydrophobic interactions – are not real (physical) interactions measurable in vacuum at 0 K, but rather an interplay between solvation and desolvation in water environment, entropy driven

Where are non-covalent interactions important in biological systems?

Where are non-covalent interactions important in biological systems?

protein structure formation, protein-protein, protein-nucleic acid, protein-ligand interactions, cytoskeleton, membranes,
formation of DNA double-helix, RNA structure formation, antibody-antigen, receptor-hormone,
drug-target interactions, enzyme-substrate interactions

How could non-covalent interactions be exploited in lab, industry etc.?

How could non-covalent interactions be exploited in lab, industry etc.?

application of antibodies (identification of proteins, bacteria etc., immunochemical assays, flow cytometry, immunohistochemistry, fluorescence microscopy, affinity purification, therapeutic antibodies etc.)

drug design, drug targeting, enzyme inhibition, receptor activation/inhibition

diagnostics

affinity-based isolation of biomolecules, chromatography

...

How could be non-covalent interactions studied?

How could be non-covalent interactions studied?

Calorimetry, affinity measurements,
optical measurements, affinity chromatography, enzyme assays, structural biology

Determination of binding thermodynamics (ΔH, ΔG, ΔS),
saturation thermodynamics measurements (by measuring any optical, NMR, electrochemical or other variables differing for bound and unbound states)
direct measurements of concentrations of equilibrium mixture components, affinity chromatography and other biophysical methods,
measurement of *Ki* for enzyme inhibitors,
determination of 3D structures of molecular complexes.

How do non-covalent interactions stabilize native structures of proteins and nucleic acids?

How they can be denaturated?

How do non-covalent interactions stabilize protein structure?

secondary structure – hydrogen bonds, α-helices and β-sheets are formed by hydrogen bonds between the main chain atoms

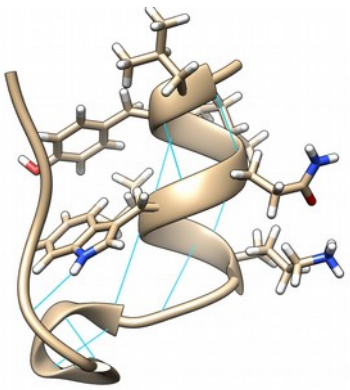
tertiary structure – all kinds of interactions, hydrophobic effect as the major driving force

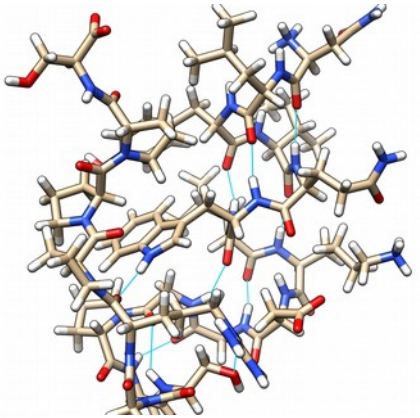
quaternary structure – all kinds of interactions

Denaturation:
- heat
- urea, guanidinium chloride
- surfactants, non-polar solvents

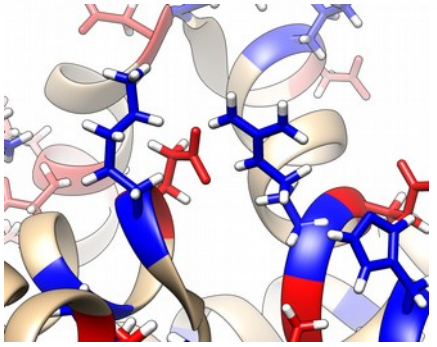
How do non-covalent interactions stabilize protein structure?

Hydrogen bonds

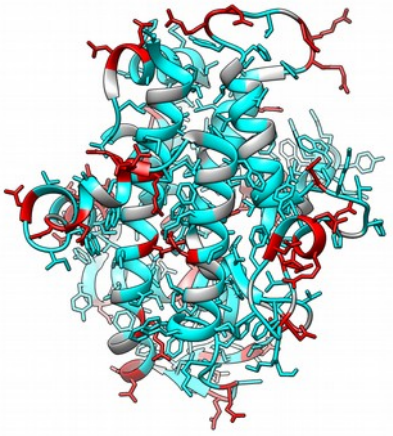




Electrostatic interactions



Hydrophobic



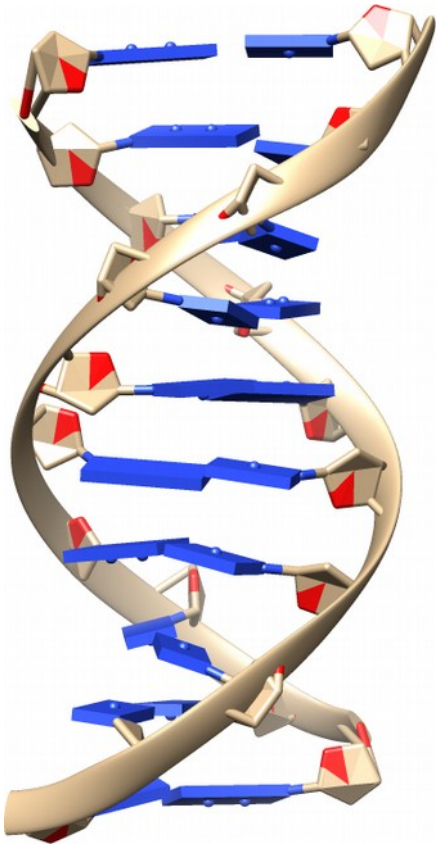
How do non-covalent interactions stabilize nucleic acids?

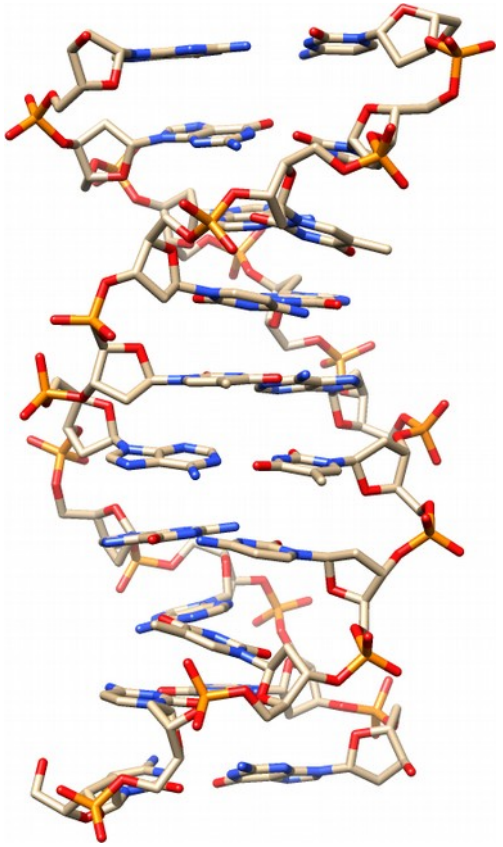
base pairing – hydrogen bonds between A-T (two) and G-C (three), that is the reason why GC-rich nucleic acids are more stable

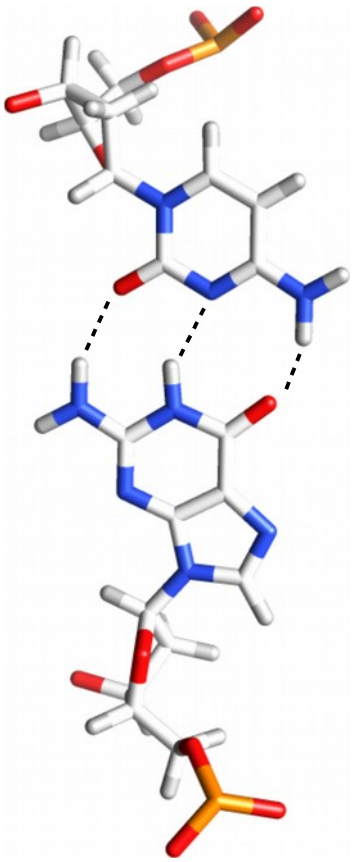
pi-pi interactions – between DNA stacks

Denaturation:
- heat

How do non-covalent interactions stabilize nucleic acids?







How do non-covalent interactions stabilize membranes?

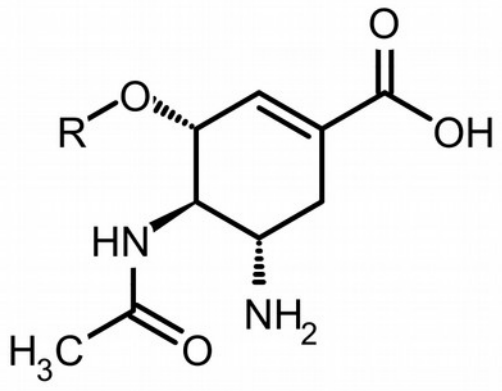
hydrophobic effect

https://www.youtube.com/watch?v=Im-dAvbI330

Membranes are stabilized by hydrophobic effect of hydrophobic fatty acid chains.

How do non-covalent interactions stabilize drug-target interactions?

Tamiflu (inhibitor of influenza neuraminidase)



R:	K*i* (nM)
–H	6 300
–CH	3 700
$-CH_2CH_3$	2 000
$-CH_2CH_2CH_3$	180
$-CH_2CH_2CF_3$	225
$-CH_2OCH_3$	2 000
$-CH_2CH{=}CH_2$	2 200

R:	K*i* (nM)
$-CH_2CH_2CH_2CH_3$	300
$-CH_2CH(CH_3)_2$	200
$-CH(CH_3)CH_2CH_3$	10
$-CH(CH_2CH_2CH_3)_2$	16
–cyklopentyl	22
–cyklohexyl	60
–fenyl	530
$-CH(CH_2CH_3)_2$	1

The process of drug discovery can be seen as optimization of non-covalent bonds to achieve highest possible affinity.

Affinity chromatography

Antibody-antigen – antibody can be immobilized on a resin in a column, magnetic beads or other material. This material can be used to isolate corresponding proteins.

Protein-ligand – the same can be done for ligands to isolate proteins or other way around

enzyme-substrate (or substrate analogue)

Famous interaction pairs

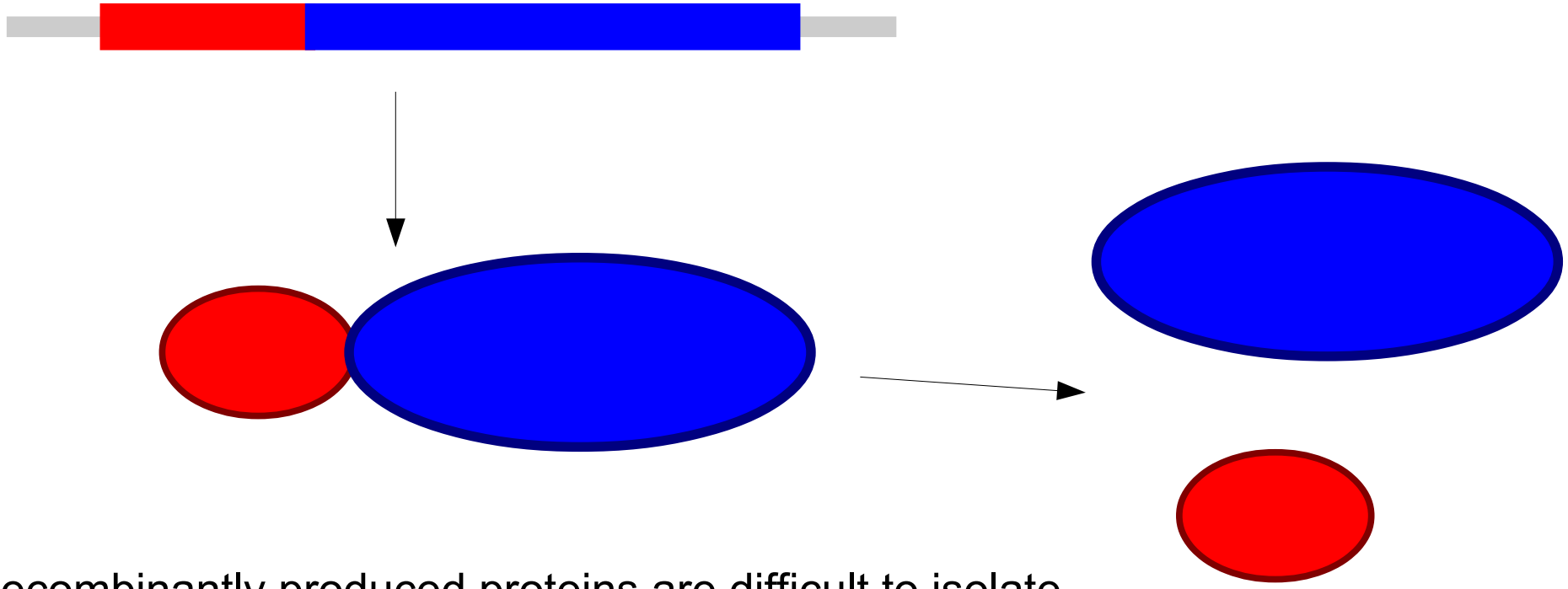
streptavidin-biotin, avidin-biotin – extremely high affinity, often used as affinity tags in molecular biology

protein A – IgG antibody – protein A is a protein with affinity to IgG produced by *S. aureus*

affinity tags in recombinant protein production – next page

Affinity tags in recombinant protein production

poly-His – Ni^{2+}
maltose-binding protein – amylose
glutathione-S-transferase – glutathione



Recombinantly produced proteins are difficult to isolate because producing organisms produce many other protein. Therefore, it is useful to modify their genes to produce a chimeric protein composed of the protein and a tag on N- or C- terminus. The resulting chimeric protein can be isolated and the tag can be removed by a protease, if necessary.

How can affinity be quantified?

equilibrium (dissociation/association) constants

$$P + L \rightleftharpoons PL$$

$$K_{dissoc.} = \frac{[P][L]}{[PL]} \qquad K_{assoc.} = \frac{[PL]}{[P][L]}$$

Gibbs/Helmholz free energy, Affinity

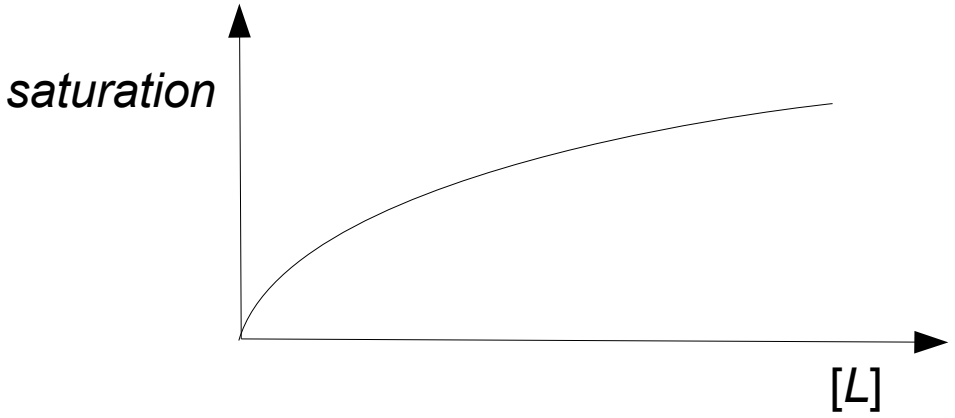
$$\Delta G^0 = -RT \ln K$$
$$\Delta G = \Delta H - T\Delta S$$

How does formation of a complex depend on concentration(s)?

Saturation:

binding of a ligand to a protein

$$saturation = \frac{[L]}{[L] + K_S}$$



How does formation of a complex depend on concentration(s)?

Saturation:

enzyme kinetics

$$v = \frac{v_{max}[S]}{[S] + K_M}$$

Binding of ligand to protein or substrate to enzyme follows saturation thermodynamics. At low concentrations of ligand/substrate there is a quasi-linear relationships between concentration and saturation. Absolute saturation would be achieved at infinite concentration.