evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: How to analyze nucleic acid?

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Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

How to analyze nucleic acid?

Sequence determination

- Blotting
- PCR
- Electrophoresis
- Microarray
- Sequencing

Structure determination

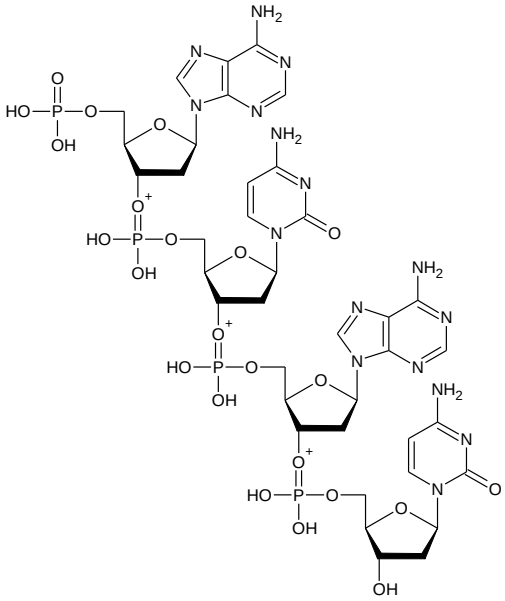
- NMR
- X-ray spectroscopy
- Circular dichroism

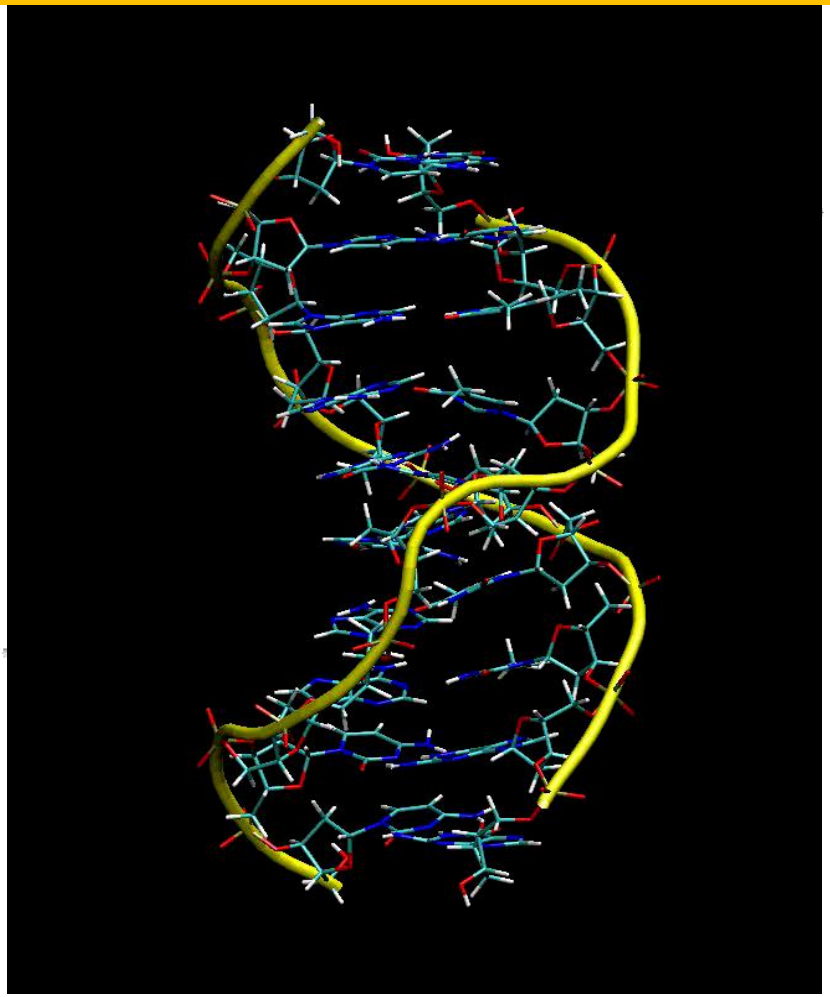
Quantification

- UV/VIS spectroscopy
- Electrochemical methods

Structure of nucleic acid

Simple or complicated?





Structural motifs of nucleic acid

single strands

duplex

A-Form helix

B-Form helix

Z-Form helix

parallel stranded

triplexes

quadruplexes

junctions

aptamers

pseudoknots

hairpins

stem-loops

bulges

Mismatches

DNA-RNA hybrid

and complexes with:

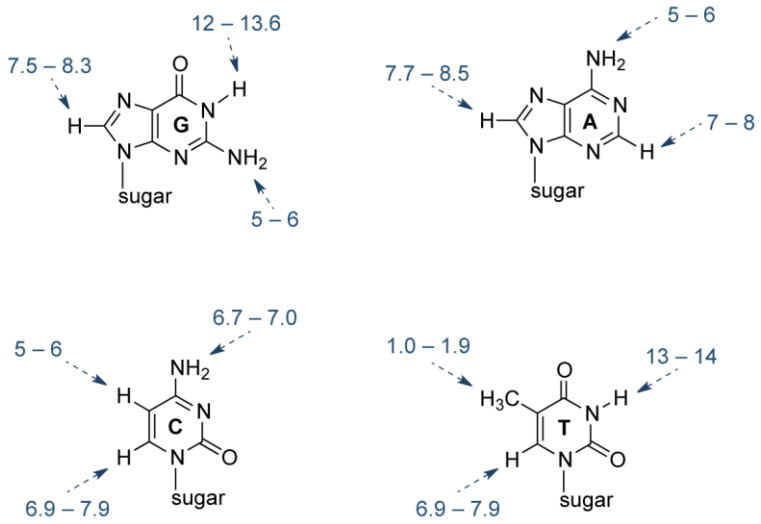
proteins and peptides

ligands (drugs)

- covalent
- intercalated
- groove binding

Nuclear magnetic resonance (NMR)

NMR is a highly developed and powerful spectroscopic technique that is valuable in the investigation of the structural, thermodynamic and kinetic properties of nucleic acids. The technique can be used to study DNA duplexes, triplexes, quadruplexes, hairpin loops, RNA duplexes and other secondary and tertiary RNA structures. Primarily, ^{1}H NMR is used in aqueous buffers with water suppression.
NMR should always be used in conjunction with other physical techniques such as circular dichroism (indicative of conformation, A, B, Z), Ultraviolet melting of duplexes, triplexes and quadruplexes (thermodynamic information), X-ray diffraction (high resolution structure analysis), and fluorescence resonance energy transfer (distance measurements).

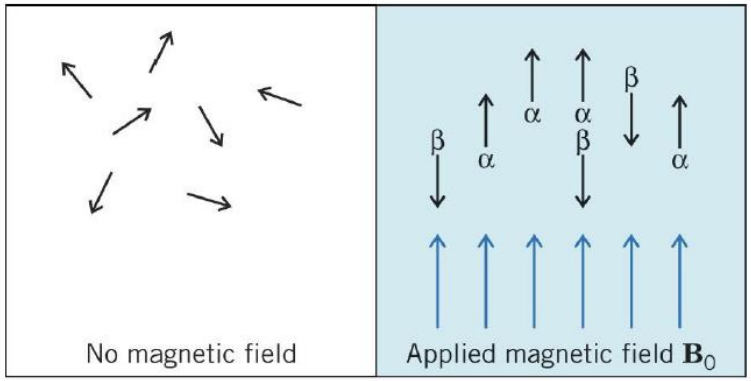


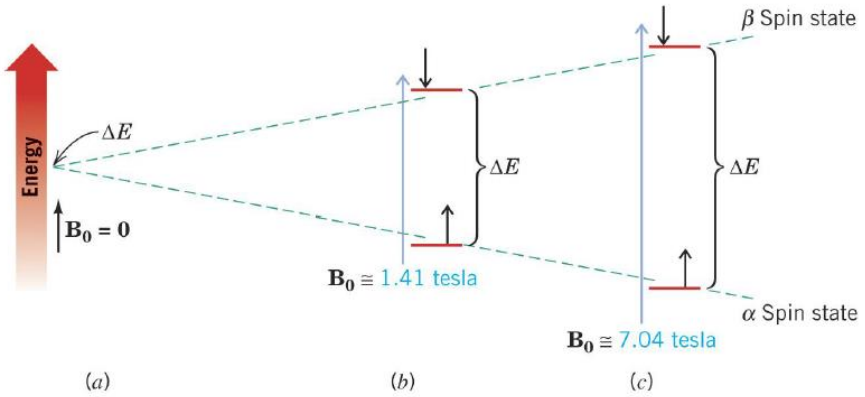
Typical NMR chemical shifts of base and sugar protons

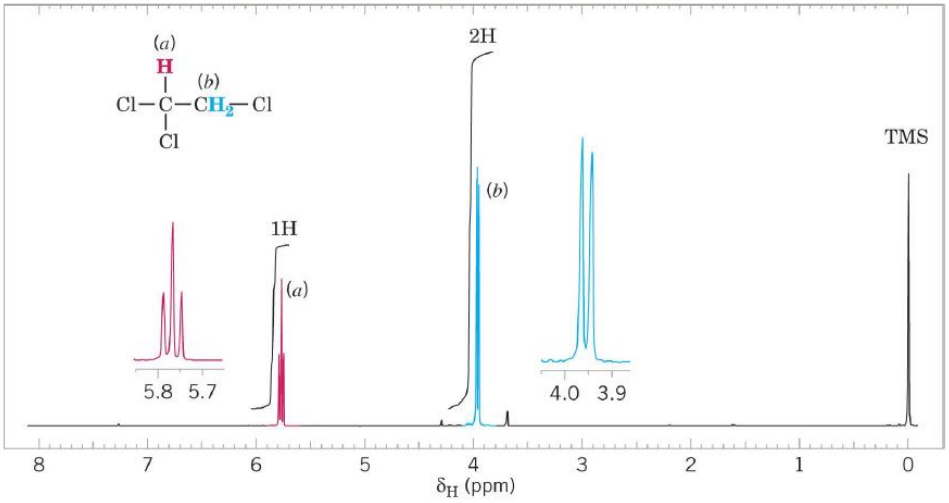
Nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; A key feature of NMR is that the resonance frequency of a particular substance is directly proportional to the strength of the applied magnetic field.

NMR

Orientation of magnetic moments of nuclei





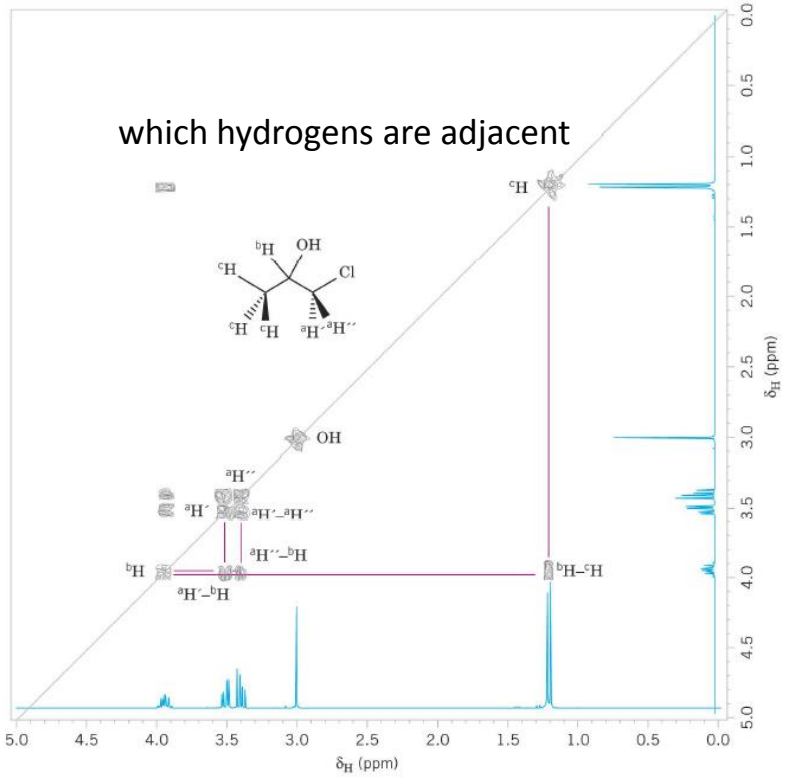


$$\upsilon = \frac{\gamma B_o}{2\pi}$$

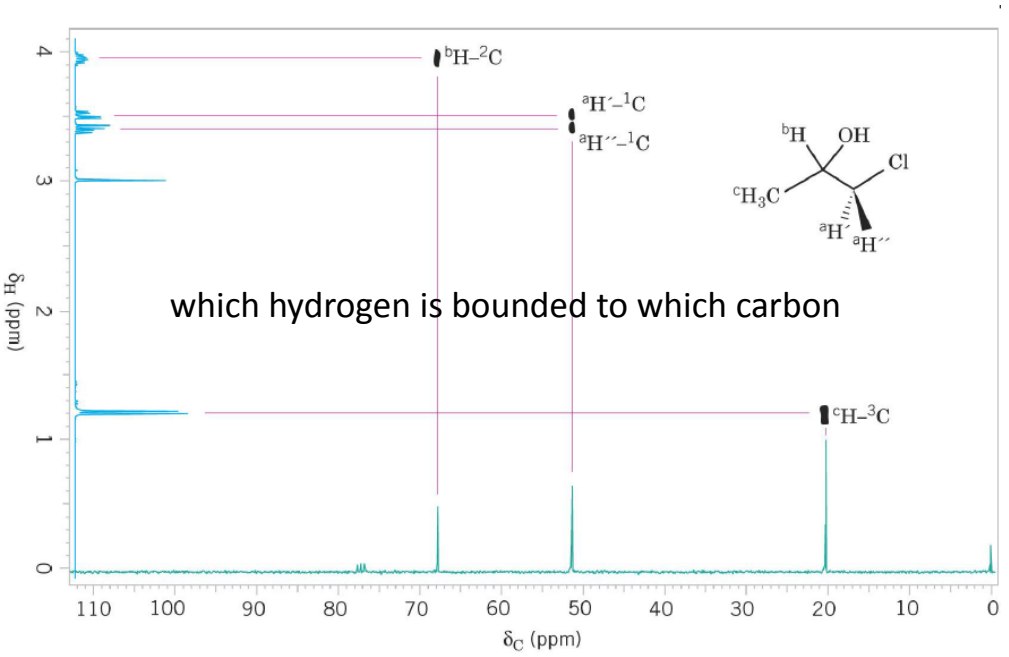
- **počet signálů** počet chemicky neekvivalentních jader
- **poloha signálů** chemický posun (δ [ppm])
- **štěpení signálů** nepřímá spin-spinová interakce (***J*** [Hz])
- **intenzita signálů** počet chemicky ekvivalentních jader v signálu

2D NMR

2D homonuclear correlation spectroscopy

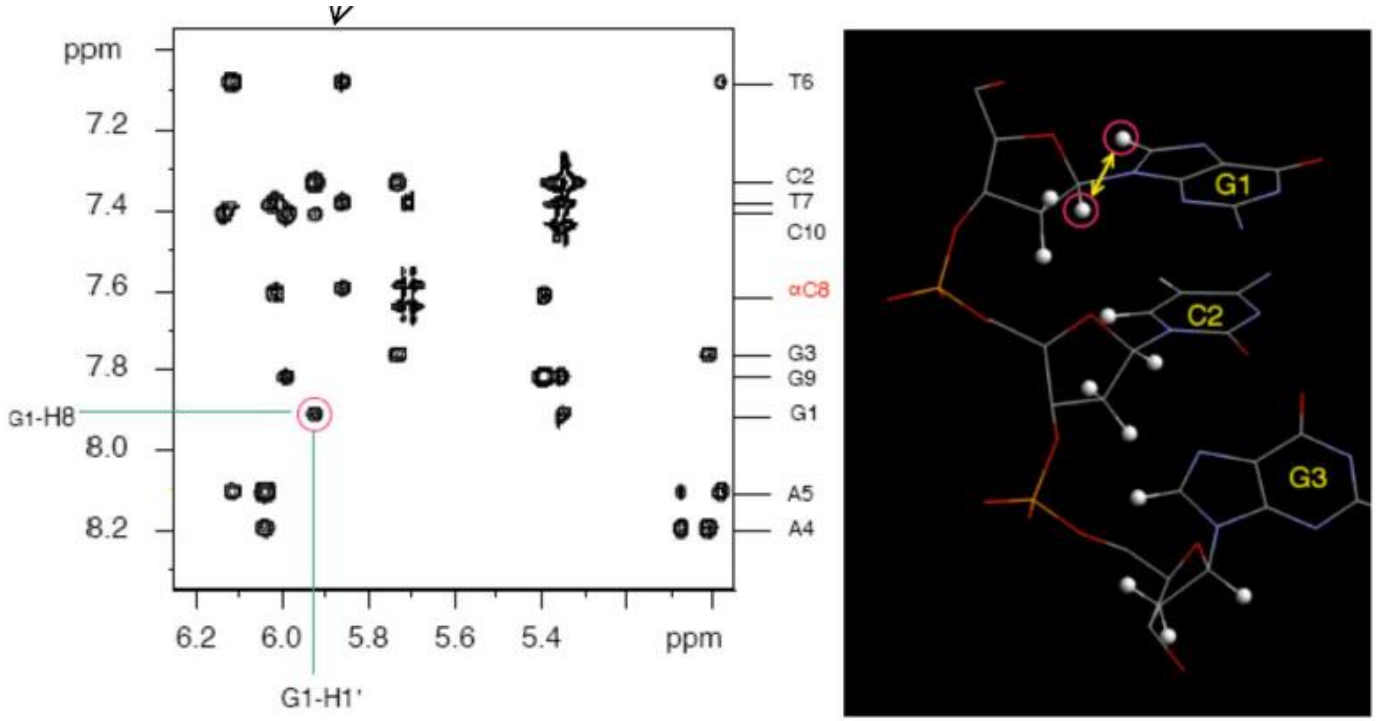


2D heteronuclear correlation spectroscopy



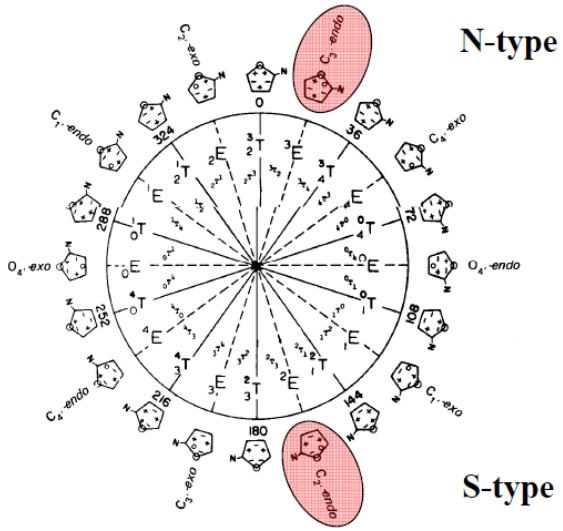
Structure of Nucleic Acid

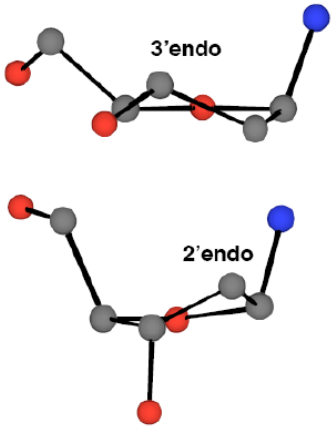
2D homonuclear correlation spectroscopy

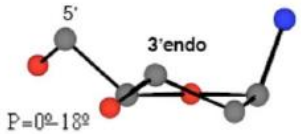


The Sugar Pucker

Very weak $J_{H1'-H2'}$ and strong $J_{H3'-H4'}$ cross-peaks correspond to *pure N-type conformation* (preferred conformation in RNA). Strong $J_{H1'-H2'}$ and weak $J_{H3', H4'}$ cross-peaks correspond to *pure S-type conformation*. $J_{H2'-H3,}$ is similar in both states. Intermediate intensities indicate an equilibrium between N and S states.

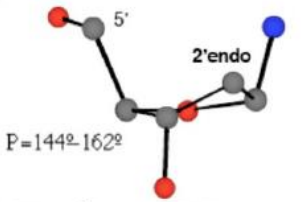






Ribose: $^3J_{H1'-H2'} \approx 1$ Hz

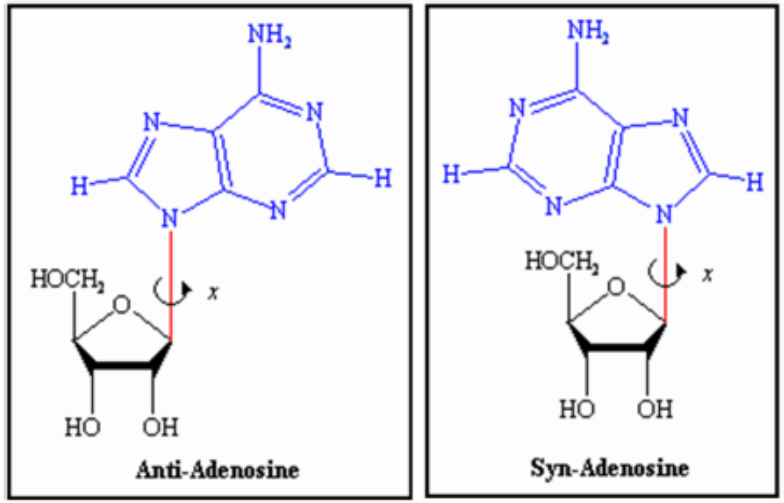
Deoxyribose: $^3J_{H1'-H2'} \approx 1.8$ Hz



Ribose: $^3J_{H1'-H2'} \approx 7.9$ Hz

Deoxyribose: $^3J_{H1'-H2'} \approx 10$ Hz

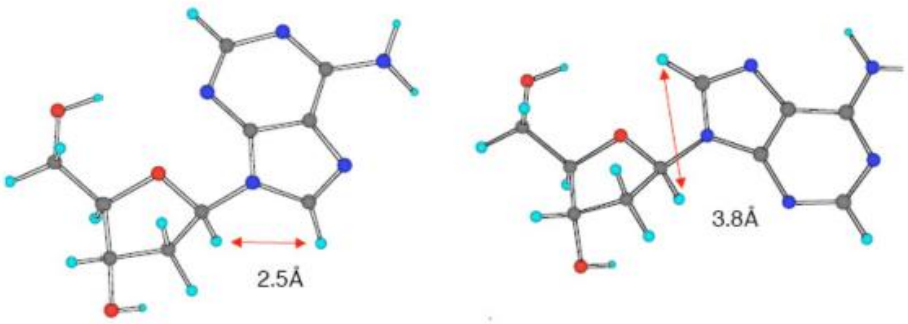
The Glycosidic Torsion Angle Measuring Using Distances



The base can exist in 2 distinct orientations about the N-glycosidic bond. These conformations are identified as, ***syn*** and ***anti*.**

The *anti* conformation predominates.

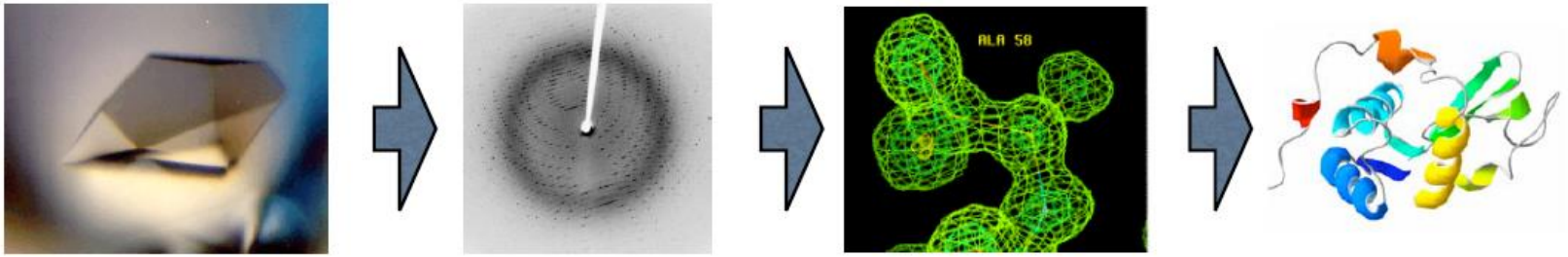
Distance information (H8 - H1') determines the glycosidic torsion angle



Furthermore, although H8 resonances from nucleotides in the *anti* glycosidic conformation are in close contact to several sugar proton resonances, in the *syn* conformation no close contact to sugar protons is expected (except to the anomeric resonances).

X-ray crystallography

Advances in solid-phase oligonucleotide synthesis and purification have allowed a large number of oligonucleotides to be crystallized and their molecular structures solved. This has provided important information on both the B and A forms of DNA, confirming predictions made from earlier fibre-diffraction studies, and providing high-resolution information on the sequence dependence of nucleic acid structure.



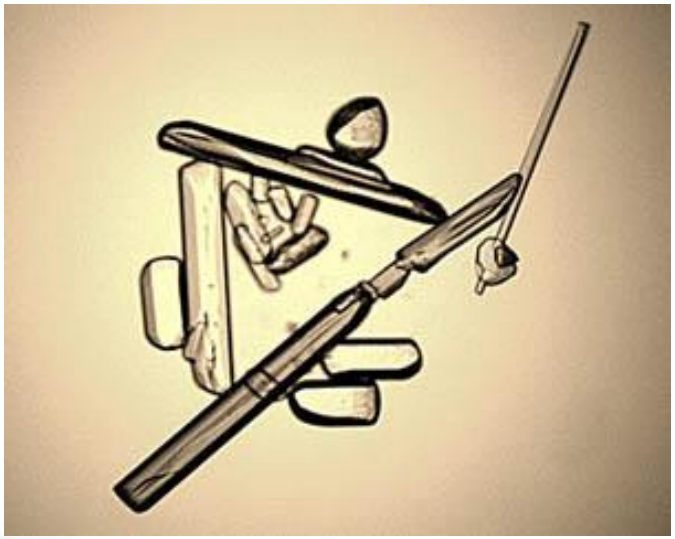
When a crystal is placed in the path of an X-ray source it will generate a diffraction pattern, from which, with the help of some complicated mathematics, a three-dimensional picture of the unit cell and the molecules present can be generated.

X-ray crystallography

X-ray crystallography requires highly ordered crystals, and obtaining suitable crystals of nucleic acids is normally the slowest part of the process.

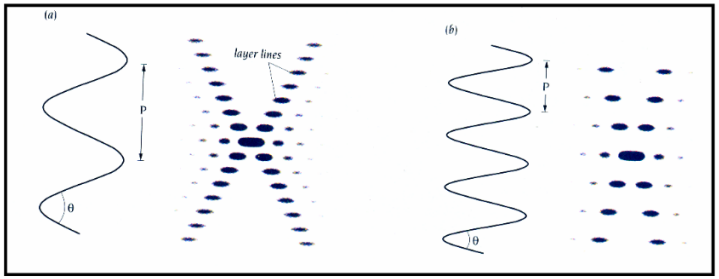
A highly concentrated solution of an oligonucleotide is required to provide a thermodynamic driving force for crystal formation. The solubility limit is exceeded by gradually removing water from the oligonucleotide solution using an external dehydrating agent, or diffusing a precipitant such as isopropanol into the solution. High-resolution structures of A, B and Z-DNA have been obtained by X-ray crystallography and the technique has been used to study DNA-drug complexes in atomic detail.

In most cases the resolution of the structure does not extend beyond 2 Å (0.2 nm), but this is sufficient to provide a reasonably clear picture of the heterocyclic bases and sugar-phosphate backbone.



Fiber diffraction

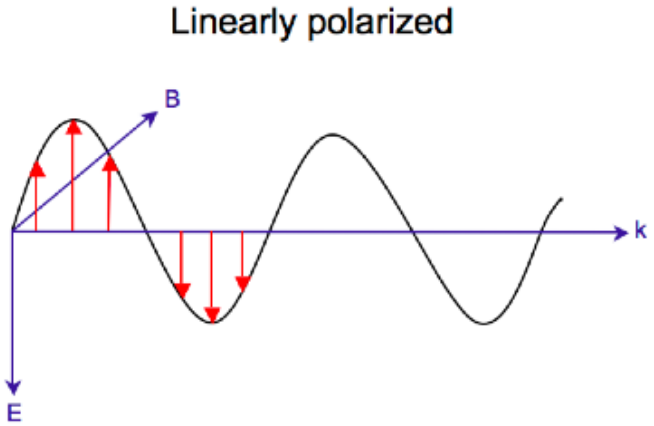
Sample in solution - one axis of rotation fixed

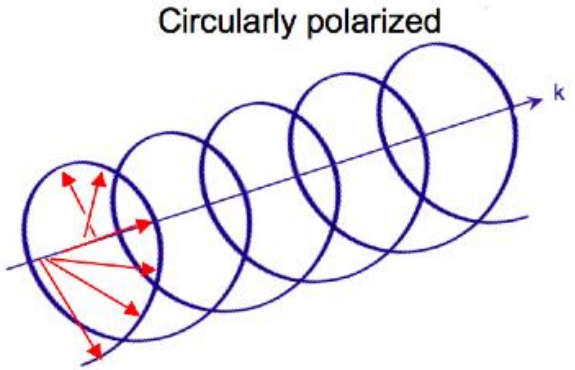


Molecules with helical symmetry

Circular dichroism

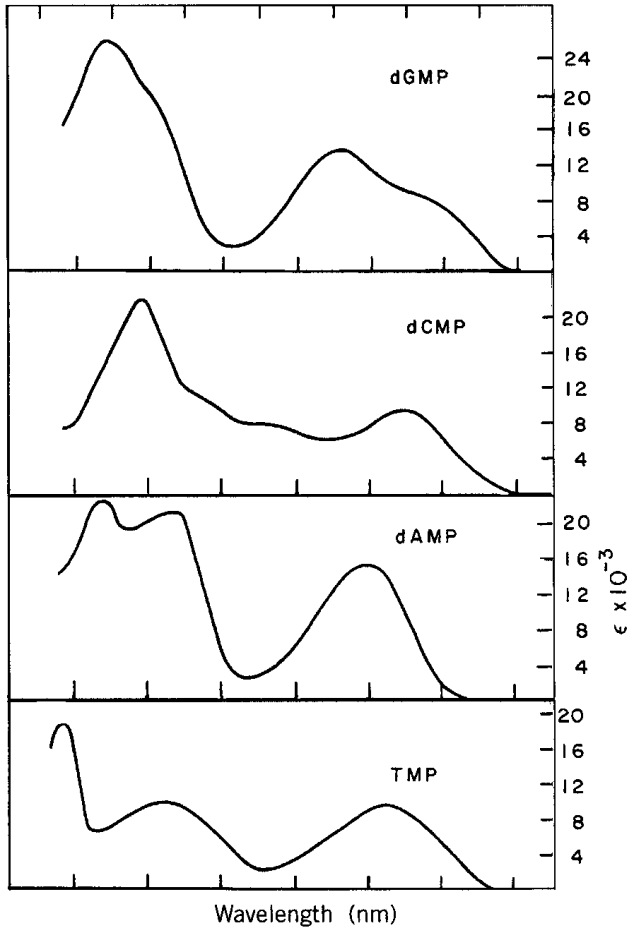
- differential absorption of left and right circularly polarized light
- it is exhibited in the absorption bands of optically active chiral molecules
- when circularly polarized light passes through an absorbing optically active medium, the speeds between right and left polarizations differ ($c_L \neq c_R$) as well as their wavelength ($\lambda_L \neq \lambda_R$) and the extent to which they are absorbed ($\varepsilon_L \neq \varepsilon_R$). *Circular dichroism* is the difference $\Delta\varepsilon \equiv \varepsilon_L - \varepsilon_R$.



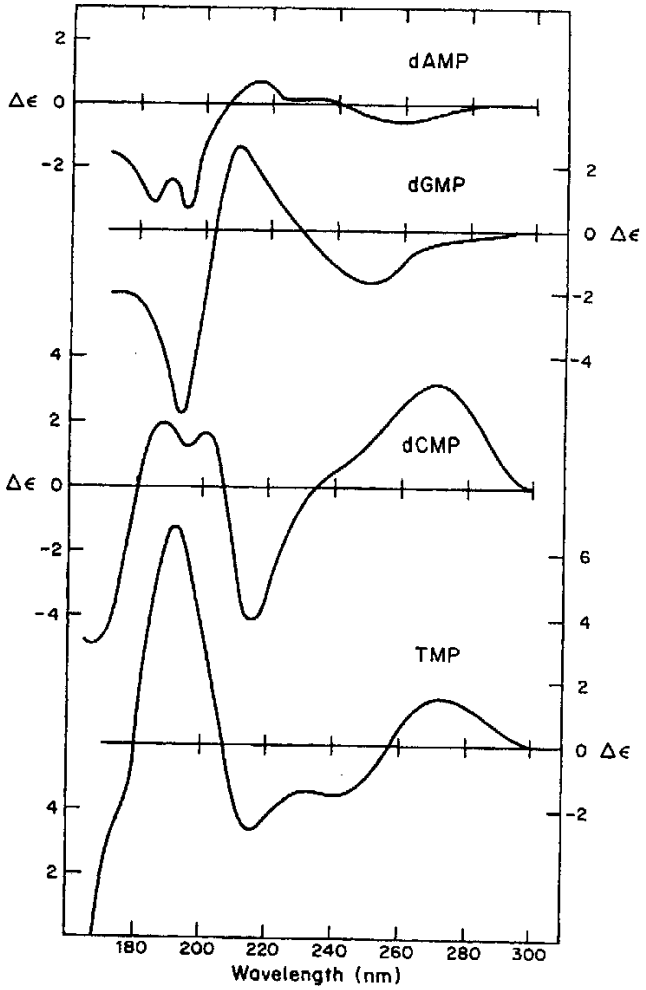


Chromophores of NA

Absorption Spectra

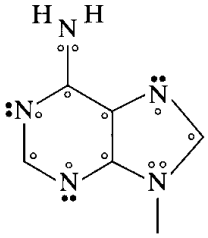


CD Spectra

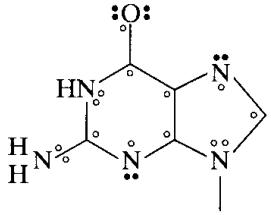


• σ
o π

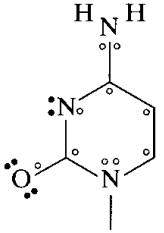
Adenine



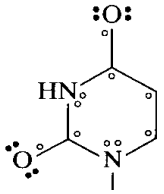
Guanine



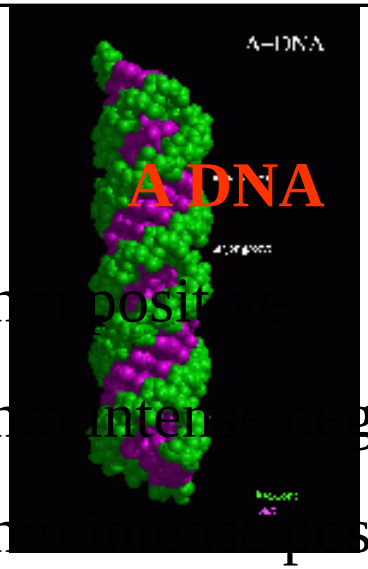
Cytosine



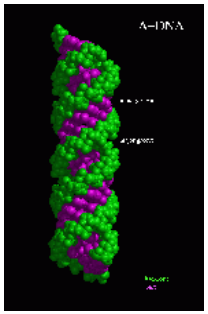
Uracil
(Thymine)



Structure of DNA



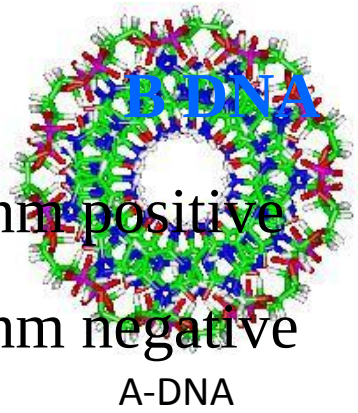
A DNA



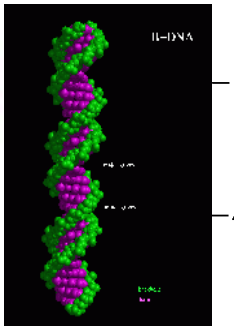
260 nm positive

210 nm intense negative

190 nm intense positive



B DNA

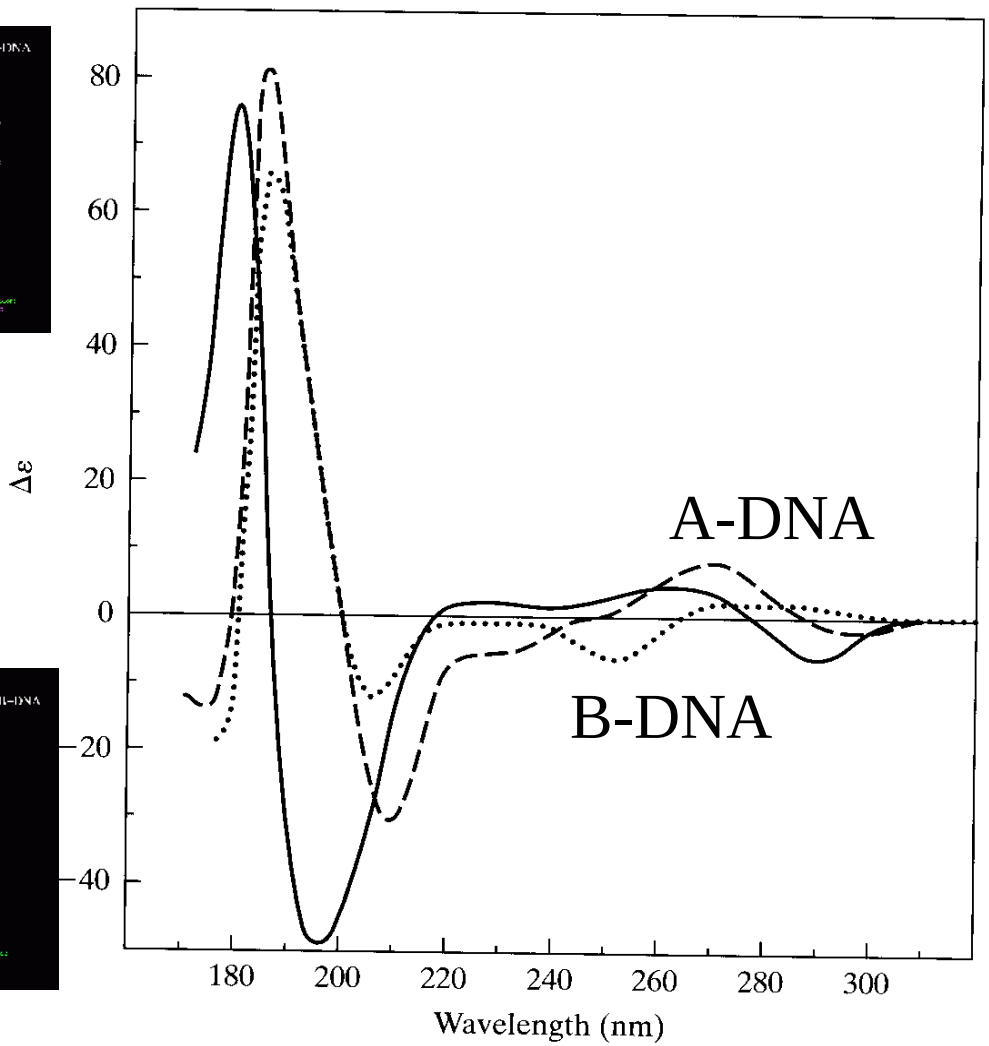


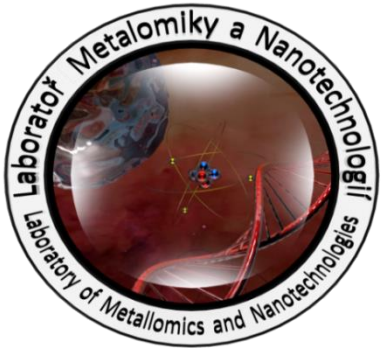
275 nm positive

240 nm negative

A-DNA

258 nm crossover





Mendel University in Brno

Thank you for your attention!