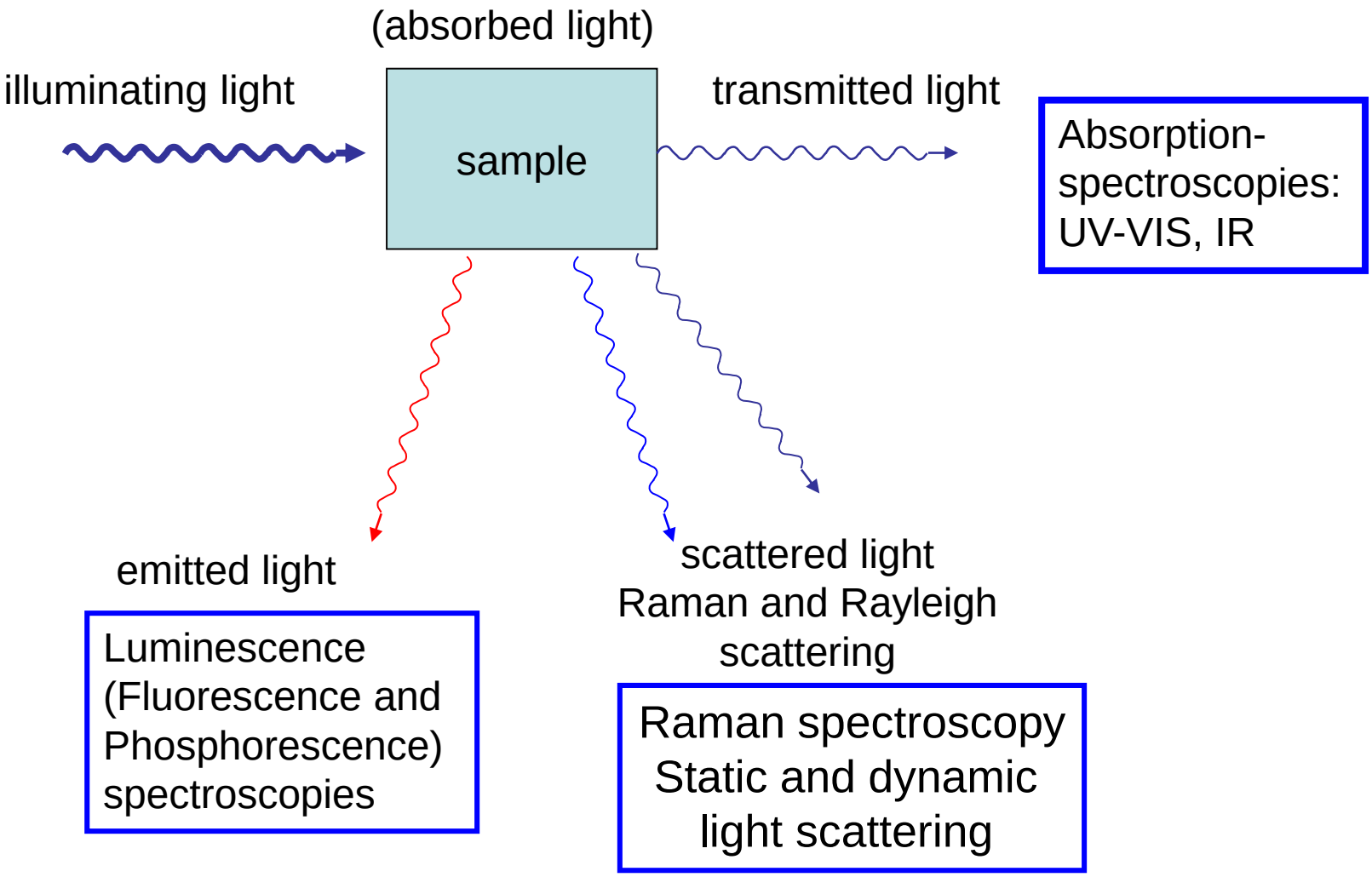
Methods for investigation of macromolecular structures: IR, CD

What happens if a sample is illuminated by light?



Spectroscopy
(Absorption and emission spectroscopy)

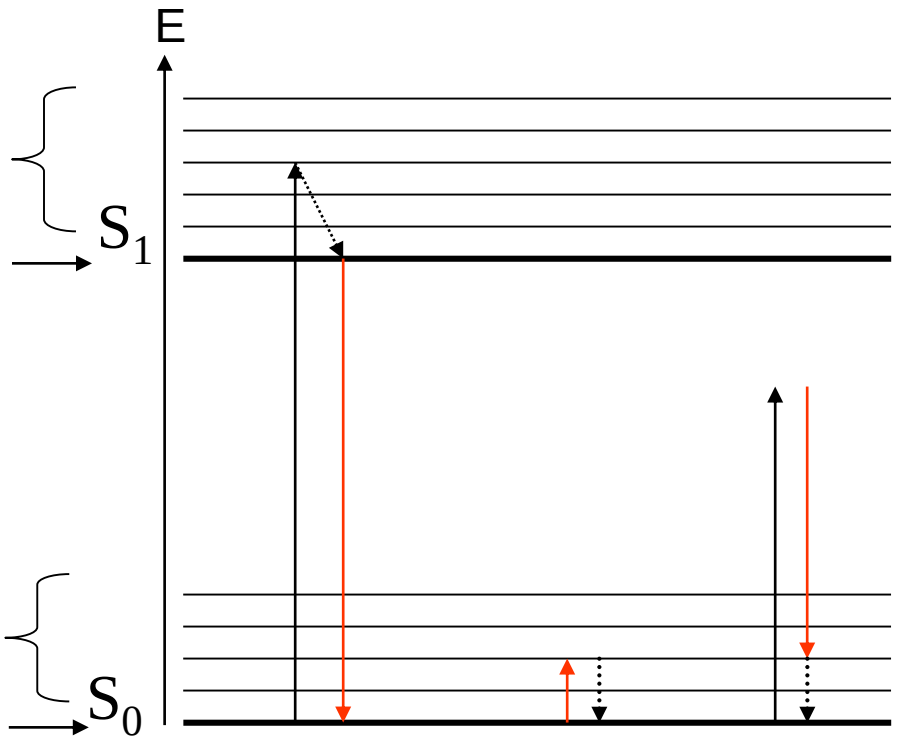
- Analysis of the wavelength dependence of the transmitted or emitted light.
- Information:
  - identification of atoms and molecules,
  - detection of changes in the molecular structure (conformation)
  - determination of the concentration

Why is light absorbed or emitted?

Jablonski diagram

Excited electron and
excited vibrational state*

Excited electron state

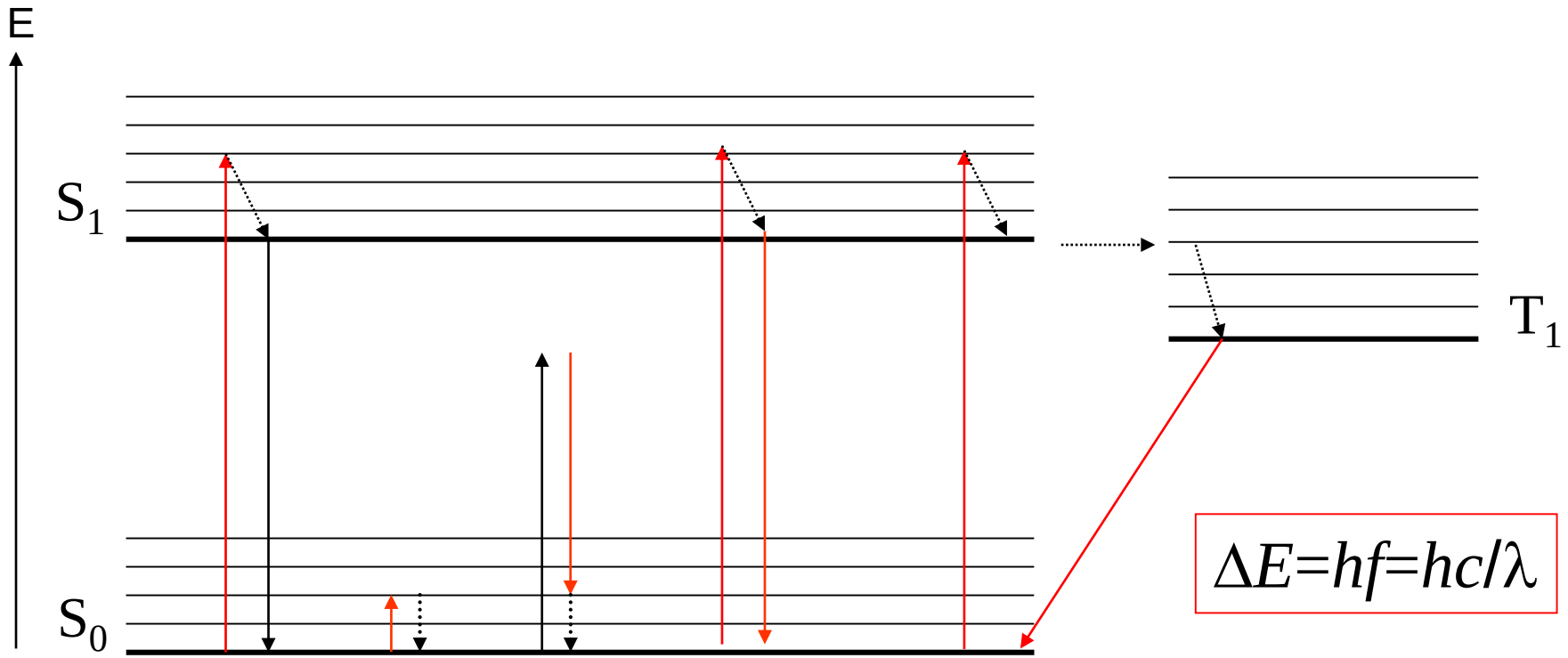


Vibrational excited state.*

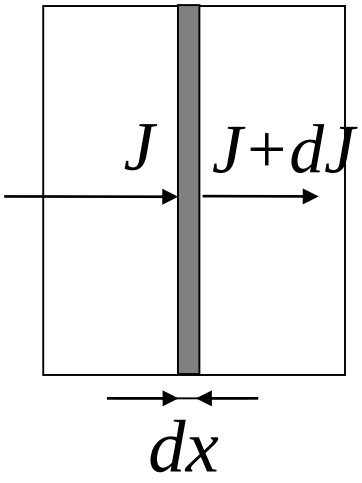
Ground state

*only for molecules! (not for atoms)

Absorption and emission of light



Absorption spectroscopy
The law of absorption



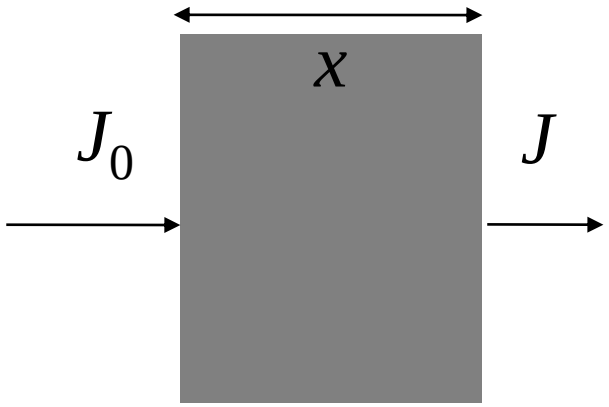
$$\left.\begin{aligned} dJ &\propto J \\ dJ &\propto dx \end{aligned}\right\} \quad dJ = -\mu J dx$$

$$\frac{dJ}{J} = -\mu dx$$

$$\int \frac{dJ}{J} = \int -\mu dx$$

$$\ln J = -\mu x + \text{const}$$

$$J = J_0 e^{-\mu x}$$



Absorption spectroscopy
Lambert-Beer law

Law of absorption: $J=J_0 \cdot e^{-\mu x}$ where μ(material ,c, λ)

Lambert-Beer law:

$$A = \lg \frac{J_0}{J} = \varepsilon(\lambda) c x$$

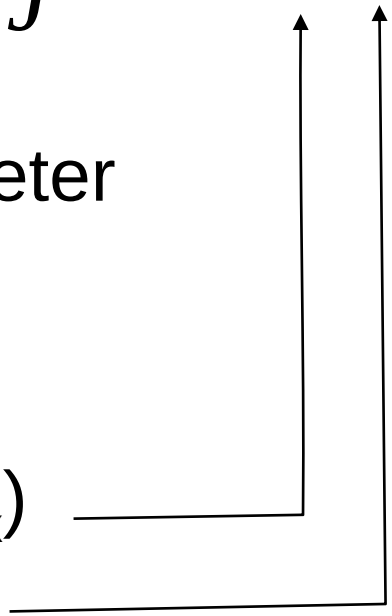
spectrum: $A(\lambda)$

measurement: spectrophotometer

reference solution (J_0)

information: identification (λ_{max})

concentration (A)



UV-VIS absorption spectroscopy

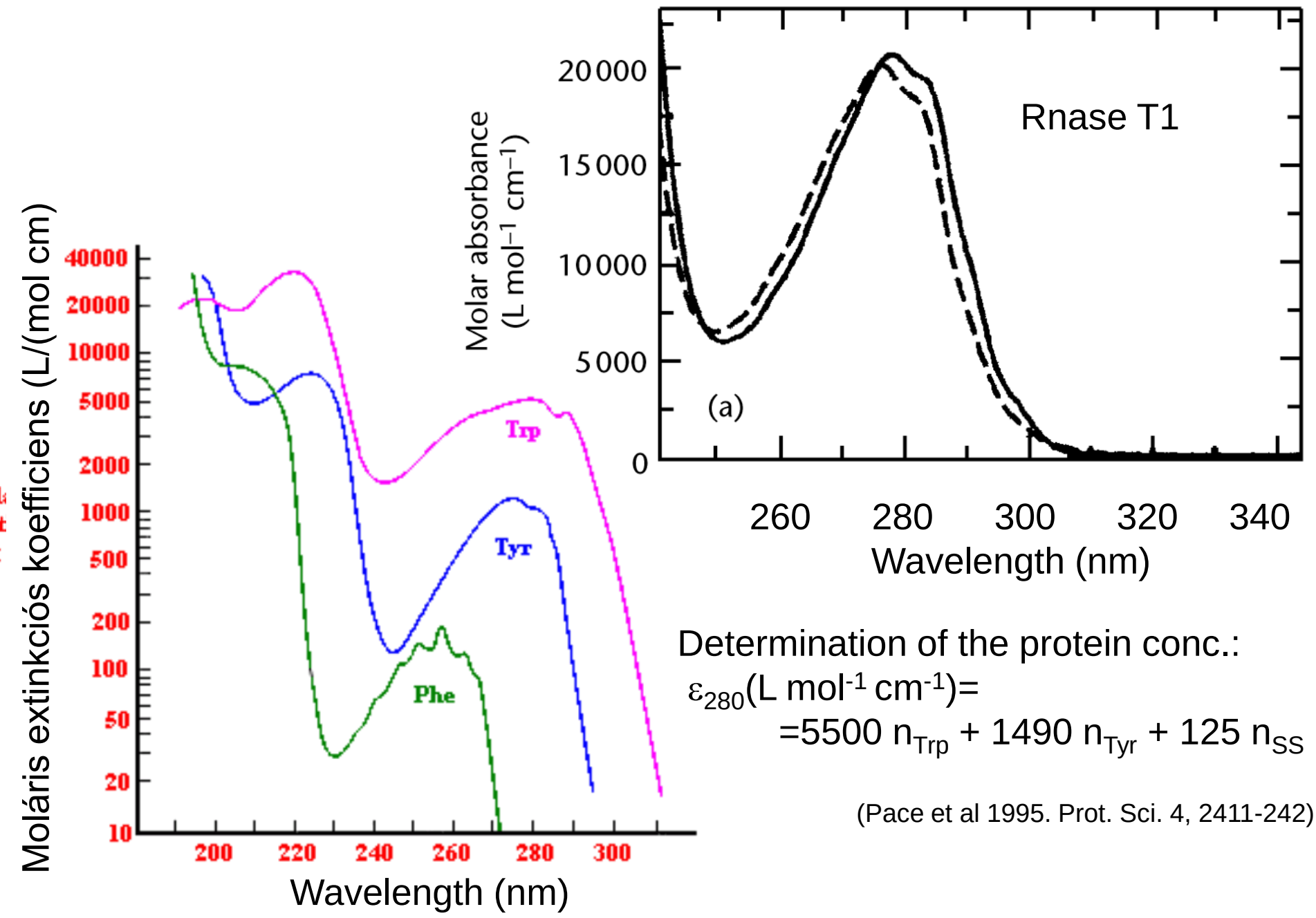
Proteins:

Absorbing site	λ_{max}(nm)	ε(L/cm mol)
Trp	280	5600
Tyr	274	1400
Phe	257	200
Disulfide bridge	250-270	300
Peptide bond	190-230	

Determination of the protein concentration:

$$\varepsilon_{280}(\text{L mol}^{-1}\text{cm}^{-1}) = 5500\, n_{Trp} + 1490\, n_{Tyr} + 125\, n_{SS}$$

(Pace et al 1995. Prot. Sci. 4, 2411-242)



Determination of the protein conc.:

ε_{280}(L mol^{-1} cm^{-1})=

$=5500\ n_{Trp} + 1490\ n_{Tyr} + 125\ n_{SS}$

(Pace et al 1995. Prot. Sci. 4, 2411-242)

Infrared spectroscopy

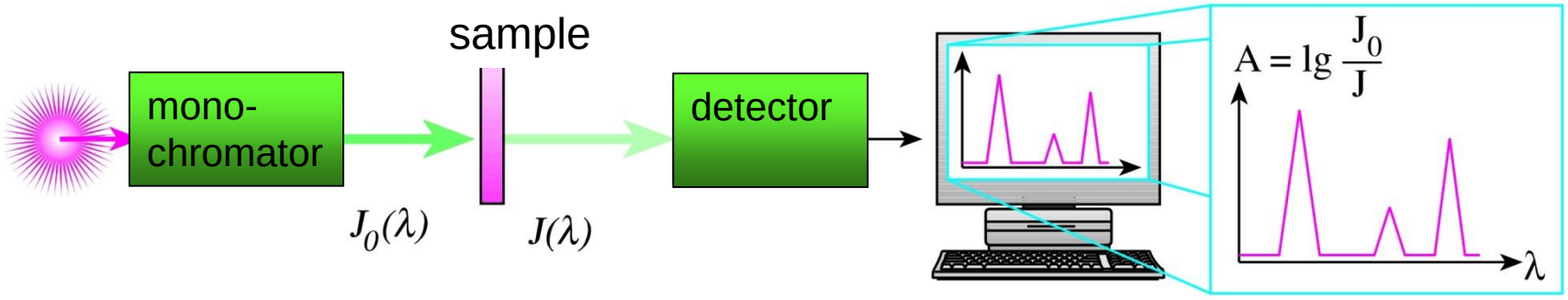
- Infrared light: λ=800 nm - 1 mm

  MIR (mid-infrared) : 2,5-50 μm
- absorption spectroscopy
- the absorbed infrared radiation excite molecular vibrations
- very specific for the structure of the molecule
- special method for detection:

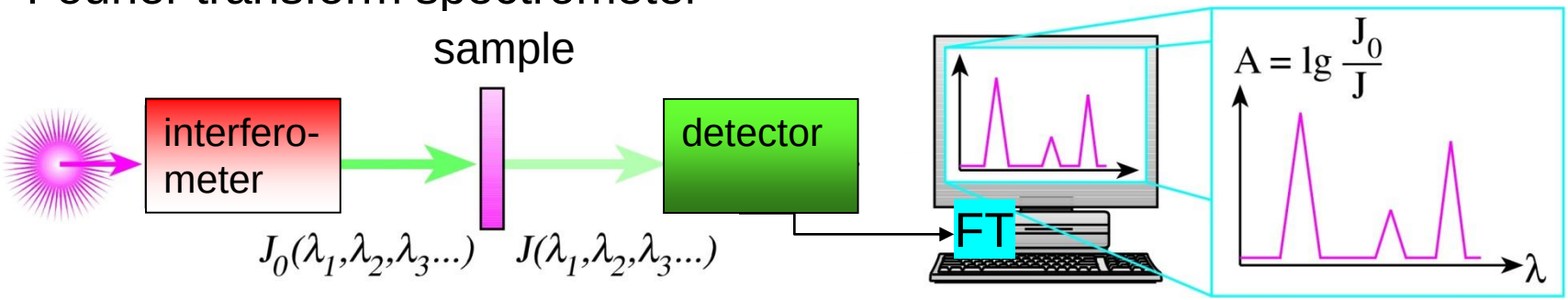
  FT spectrometer

The technique of the measurement : Fourier transform spectrometer (FTIR)

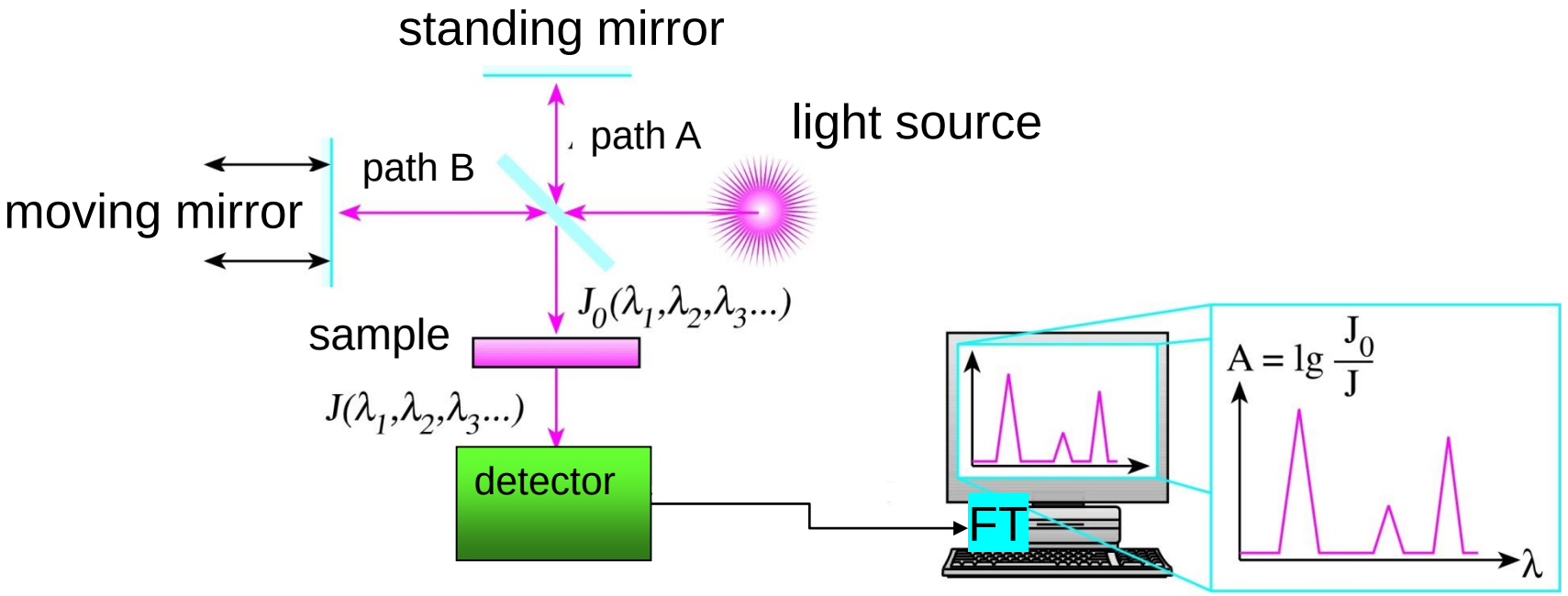
conventional (dispersion) spectrometer



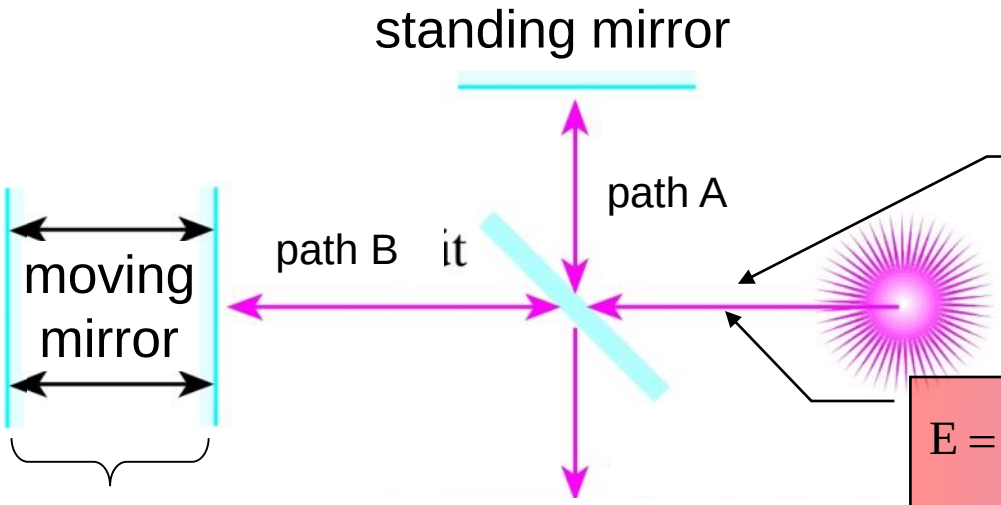
Fourier transform spectrometer



The Fourier transform spectrometer



Principle of the FTIR technique



$E = A \sin(\omega t)$

$$E = \sum_{i=1}^{n} A_i \sin(\omega_i t) \qquad E = \int_0^{\infty} \frac{dA}{d\omega} \sin(\omega t) d\omega$$

J = const (1-cos($2\pi x/\lambda$))
= J_{DC}+const cos($2\pi x/\lambda$)

$$J_{AC} = J_0 \sum_{i=1}^{n} \cos(2\pi x/\lambda_i)$$

$$J_{AC} = \int_0^{\infty} \frac{dJ_0(\lambda)}{d\lambda} \cos(2\pi x/\lambda) d\lambda$$

Fourier transformation

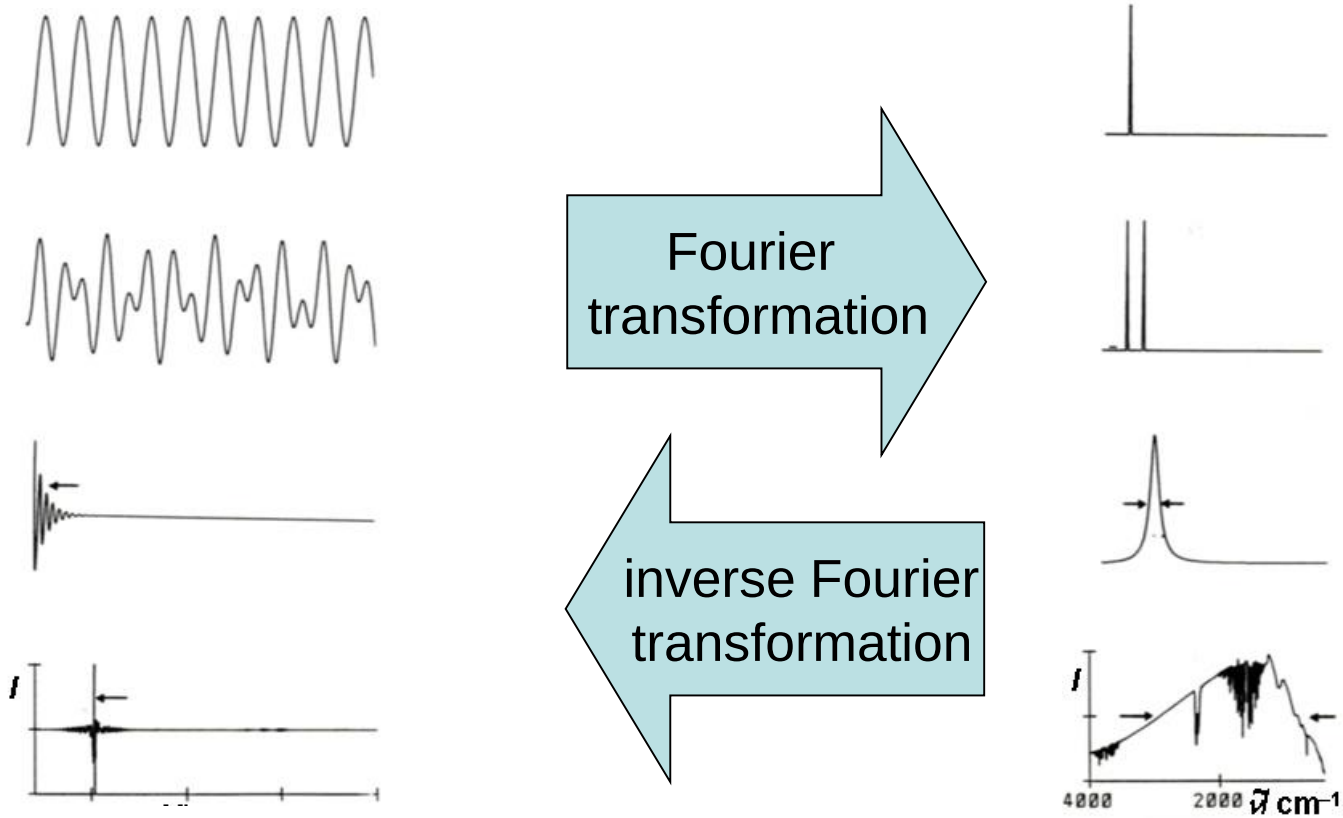
$g(\nu)$ is the Fourier transform of the function $f(t)$:

$$F(f(t)) = \int_{-\infty}^{\infty} f(t) e^{-2\pi i \nu t} dt = g(\nu)$$

Inverse Fourier transformation:

$$F^{-1}(g(\nu)) = \int_{-\infty}^{\infty} g(x) e^{2\pi i \nu t} d\nu = f(t)$$

Fourier transformation



How to obtain the spectrum in an FT spectrometer?

The intensity of the radiation passed through the interferometer:

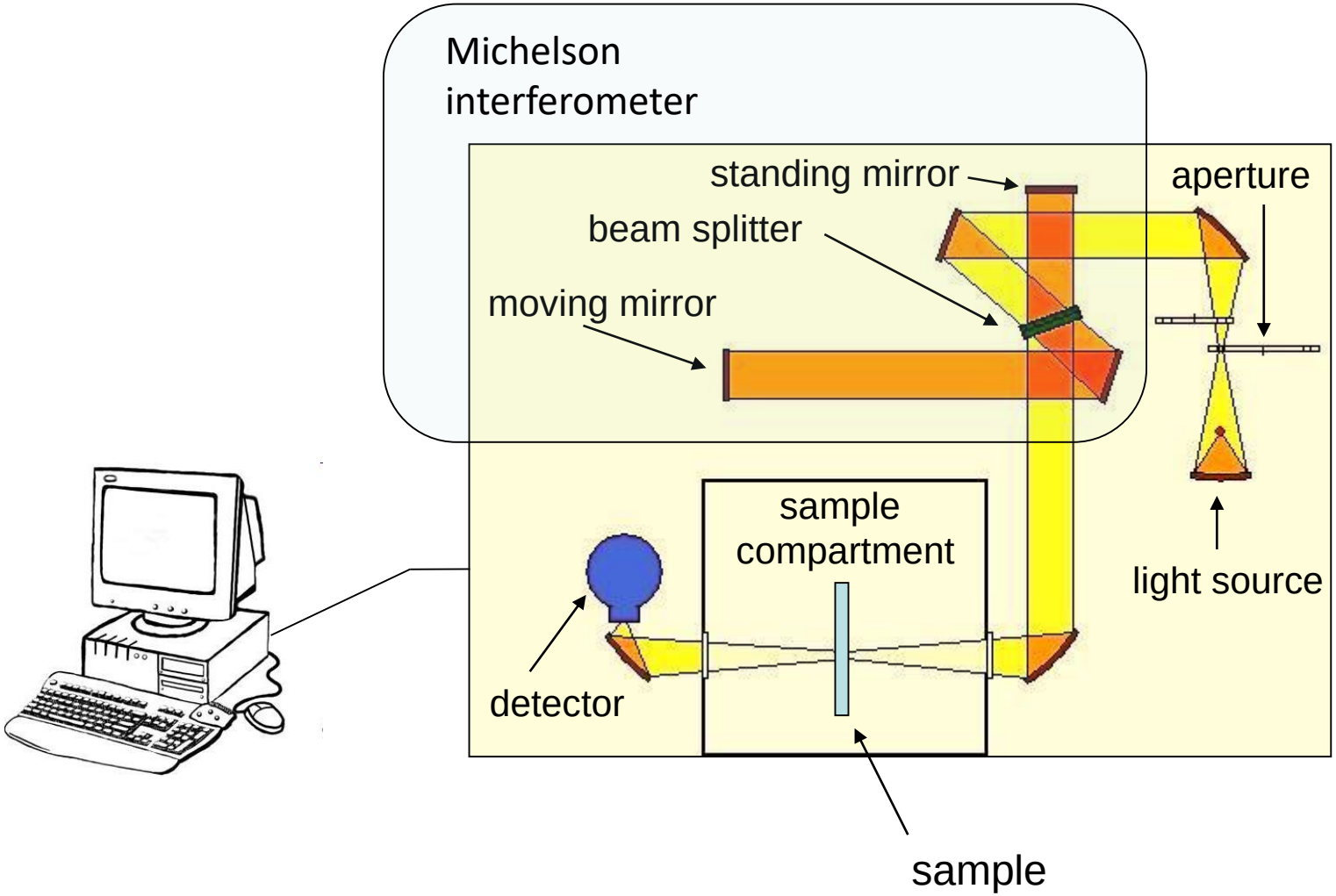
$$J_{AC} = \int_0^{\infty} \frac{dJ_0(\lambda)}{d\lambda} \cos(2\pi x/\lambda) d\lambda$$

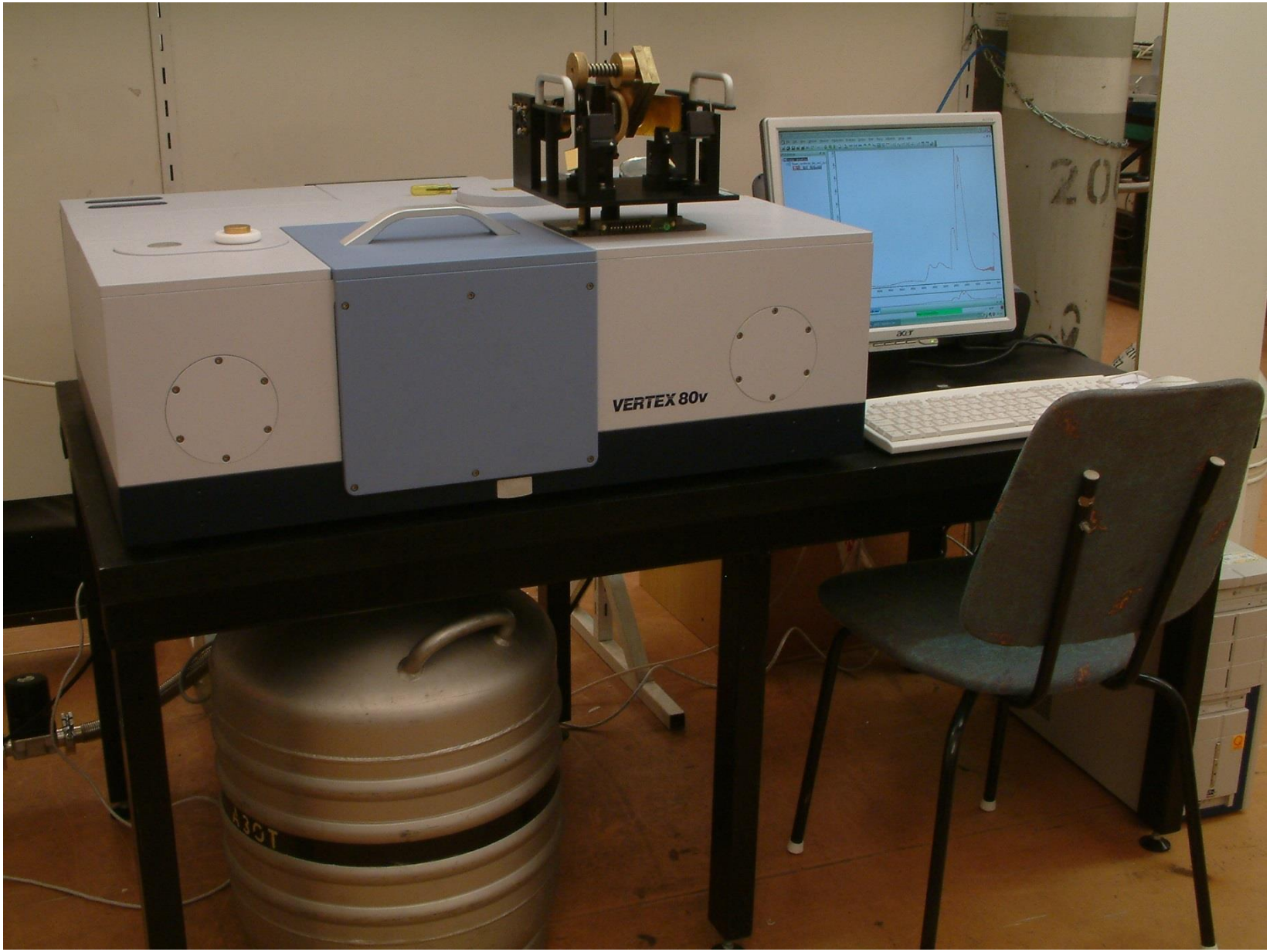
is the Cosine transform of $\frac{dJ_0(\lambda)}{d\lambda}$

After the sample it reduces to $\frac{dJ(\lambda)}{d\lambda}$

The transmission spectrum is: $T(\lambda) = \frac{J(\lambda)}{J_0(\lambda)} = \frac{\frac{dJ(\lambda)}{d\lambda}}{\frac{dJ_0(\lambda)}{d\lambda}}$

The absorption spectrum is: $A(\lambda) = lg\ (1/T(\lambda))$


Michelson
interferometer
standing mirror
aperture
beam splitter
moving mirror
sample
compartment
light source
detector
sample

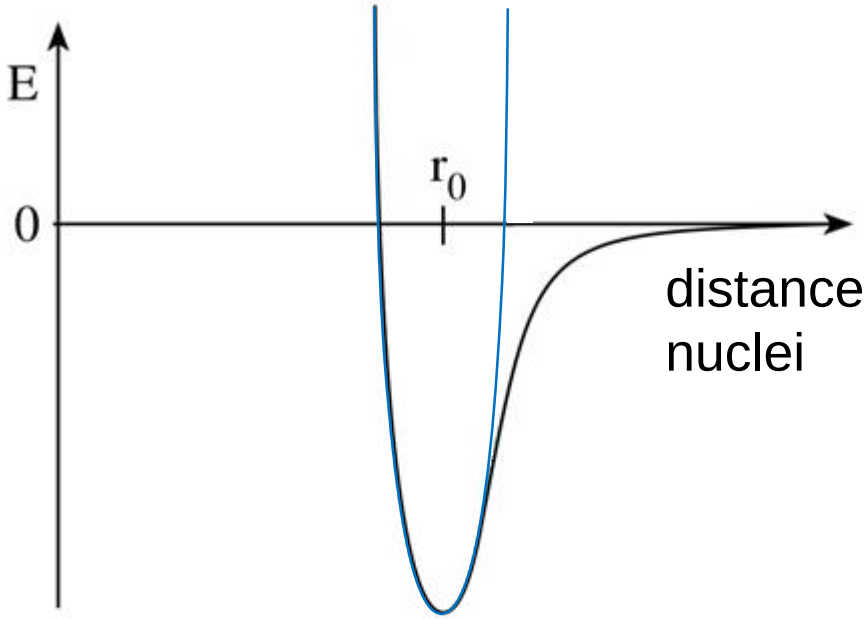

VERTEX 80v

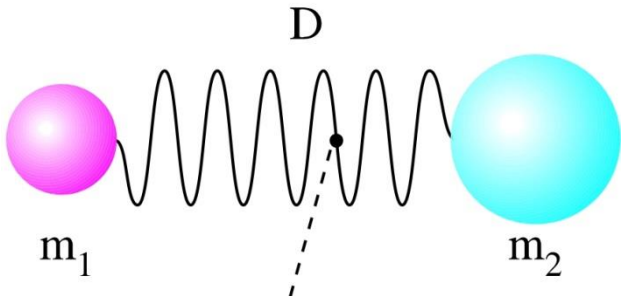
Molecular vibrations

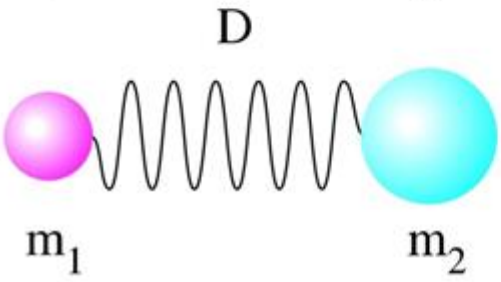
The electrons are light ($m_e << m_{nucleus}$), they can follow the movements of the nuclei easily, therefore the movements of the nuclei are independent of the movements of the electrons.

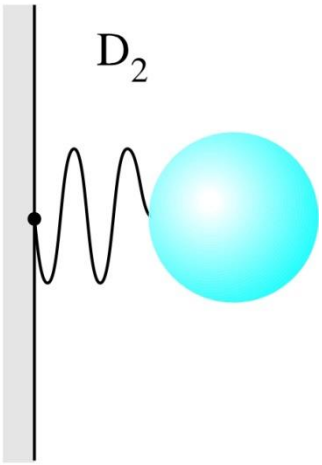
Classical physical description: the chemical bond is represented by a spring.

Molecular vibrations:

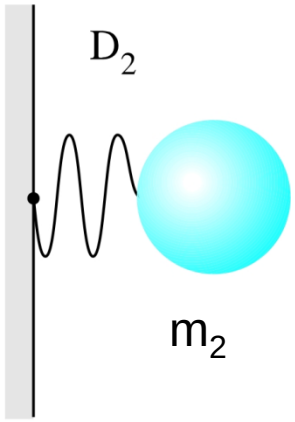






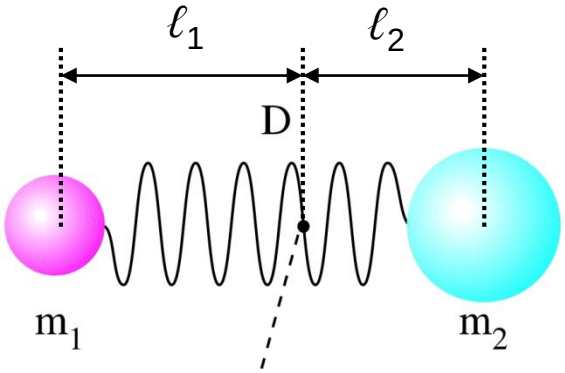


known from elementary mechanics:



$$f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$$

$$\frac{m_2}{m_1} = \frac{\ell_1}{\ell_2}$$

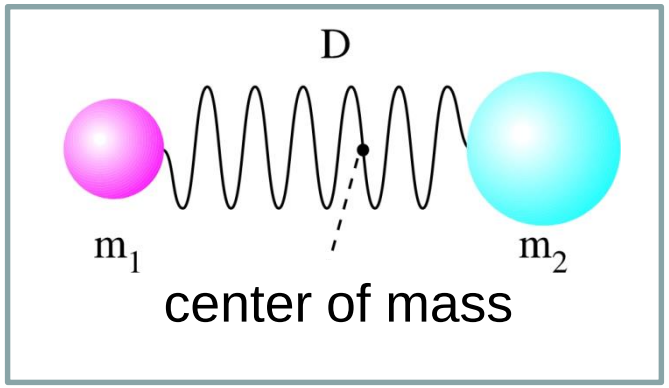


$F = D\Delta\ell$

$$\frac{D_2}{D} = \frac{F/D}{F/D_2} = \frac{\Delta\ell}{\Delta\ell_2} = \frac{\ell}{\ell_2} =$$

$$= \frac{\ell_1 + \ell_2}{\ell_2} = \frac{\ell_1}{\ell_2} + 1 = \frac{m_2}{m_1} + 1 = \frac{m_1 + m_2}{m_1}$$

substituting $\frac{m_1 + m_2}{m_1} = \frac{D_2}{D}$ into $f = \frac{1}{2\pi}\sqrt{\frac{D_2}{m_2}}$



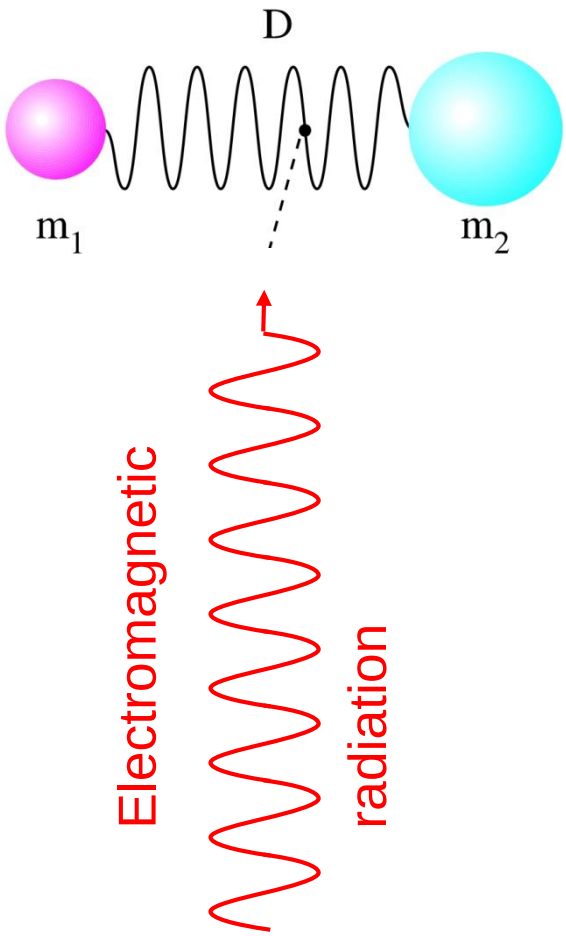
The frequency of the vibration:

$$f = \frac{1}{2\pi}\sqrt{\frac{D(m_1 + m_2)}{m_1 m_2}}$$

$m_{red} = \frac{m_1 m_2}{m_1 + m_2}$ is called as reduced mass

Frequency with the reduced mass: $f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$

How can we induce molecular vibrations?



$$f_{E.M.rad} = f_{vibration} = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$$

Resonance

⇩

absorption of the energy of the radiation

The wavelength of this radiation is:

$$\lambda = \frac{c}{f} = 2\pi c\sqrt{\frac{m_{red}}{D}}$$

In the IR spectroscopy the wavenumber (ν) is used, which is the reciprocal of λ:

$$\nu = \frac{1}{\lambda} = \frac{1}{2\pi c}\sqrt{\frac{D}{m_{red}}}$$

ν: number of waves in a unit length [cm^{-1}]

Example: CO

The measured wavenumber: ν= 2143 cm^{-1}

⇨ λ=4,67μm ⇨ f =6,43 10^{13} Hz

m_C=2·10^{-26} kg, m_O=2,7·10^{-26} kg

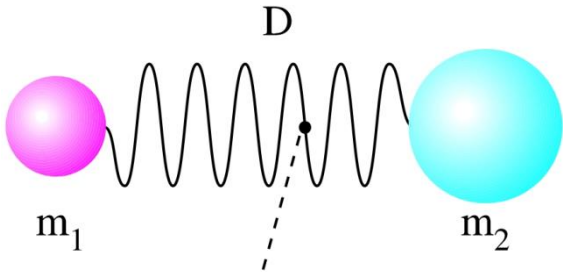
} ⇨ D=1875 N/m

if ν is known, *D* can be calculated

if *D* is known, ν can be calculated

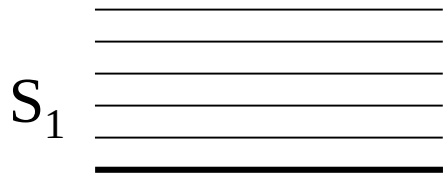
Classical vs. quantum physics

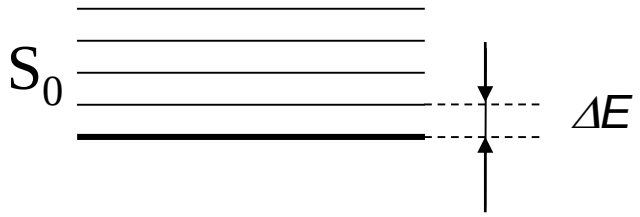
Classical physical picture



$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$$

Quantum mechanical picture





resonance with the light with frequency f

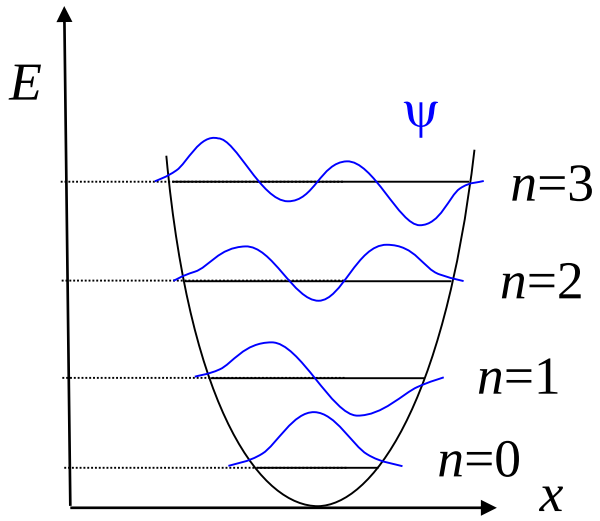
$$\Delta E = hf$$

=

Quantummechanical description

Quantum harmonic oscillator

Particle in a harmonic potential



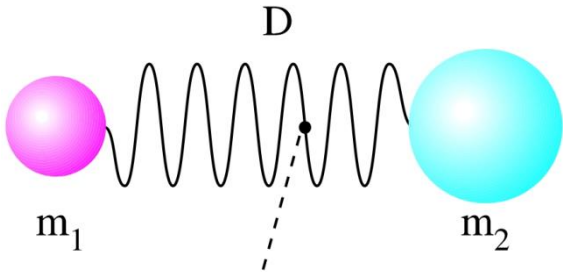
Energy eigenvalues

$$E_n = hf\,(n + \tfrac{1}{2})$$

$$n = 0,\ 1,\ 2\ldots.$$

Classical vs. quantum physics

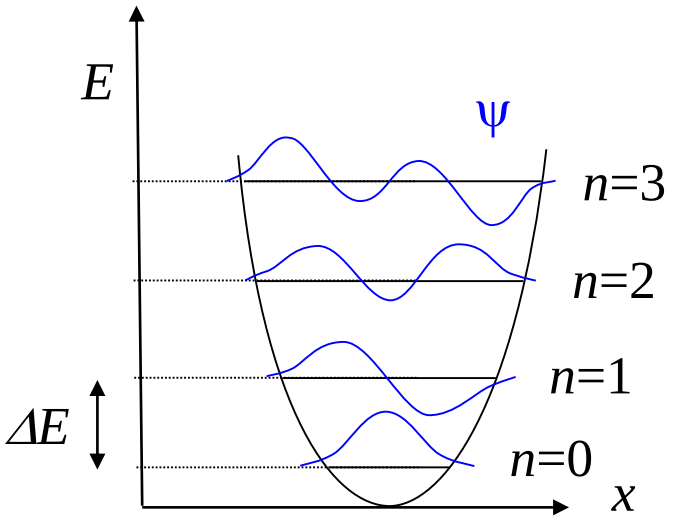
Classical physical picture



$$f = \frac{1}{2\pi}\sqrt{\frac{D}{m_{red}}}$$

resonance with the light with frequency f

Quantum mechanical picture



$$\Delta E = hf$$

=

Dependence of the vibrational frequency on the mass of the atoms and on the strength of the chemical bond.

mass: ⇩

Infravörös rezgési frekvenciák (cm^{-1})				
B-H 2400	C-H 3000	N-H 3400	O-H 3600	F-H 4000
Al-H 1750	Si-H 2150	P-H 2350	S-H 2570	Cl-H 2890
	Ge-H 2070	As-H 2150	Se-H 2300	Br-H 2650

Water (O-H): 3600 => D_2O: 2600 cm^{-1}

Bond strength:

C-N: 1100 cm^{-1},

C=N: 1660 cm^{-1},

C≡N: 2220 cm^{-1}.

Vibrations of the large molecules

Molecule consisting of N atoms:

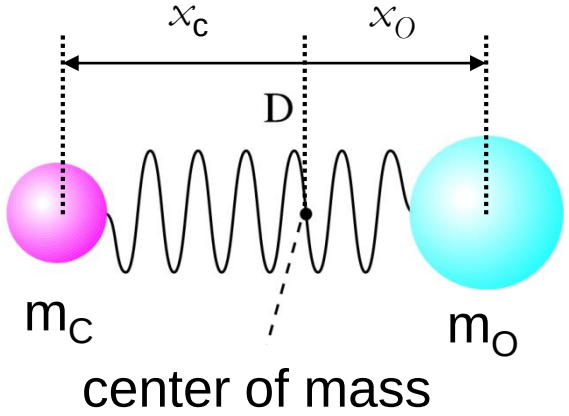
- 3N degree of freedom,

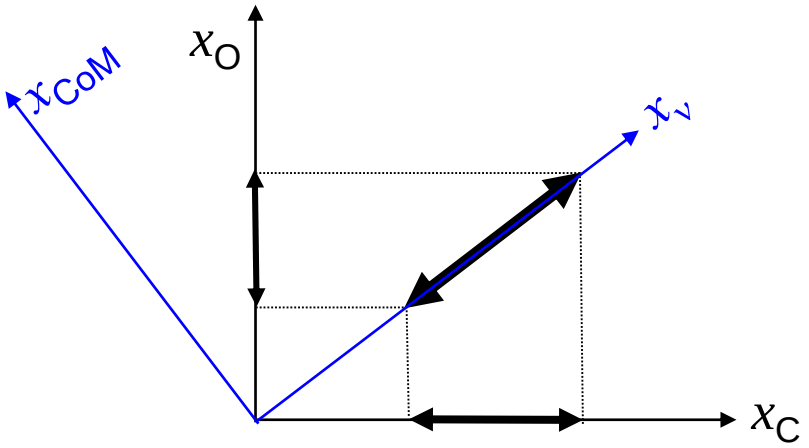
  3-3 are the rotations and translations of the whole molecule

- 3N-6 vibrational degree of freedom (3N-5 for the linear molecules)
- 3N-6 independent normal vibrations

Normal coordinates

Shown in case of a simple molecule of two atoms:





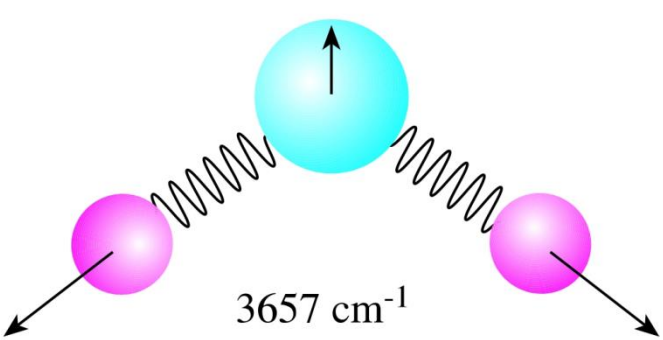
In general case one has to rotate a 3N dimensional coordinate system. Linear transformation (matrix operation)

Normal vibrations

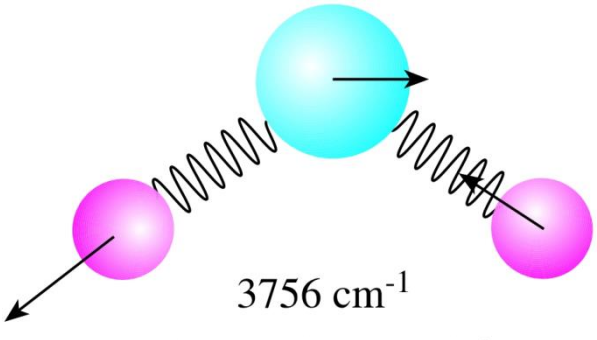
All the atoms vibrate

- with the same frequency but
- with different amplitude and
- in different direction.

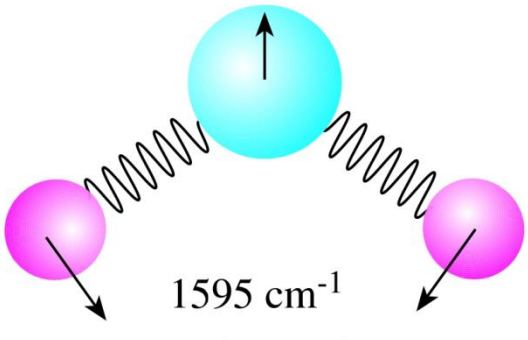
Example: water



symmetric stretching

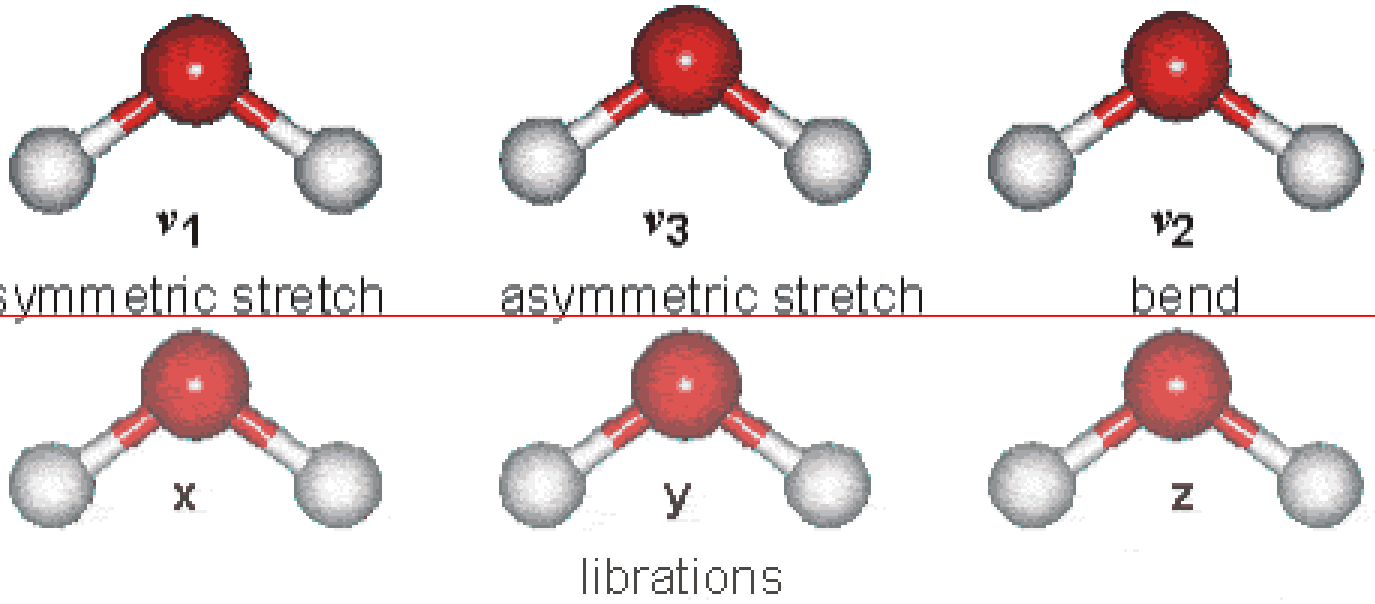


asymmetric stretching



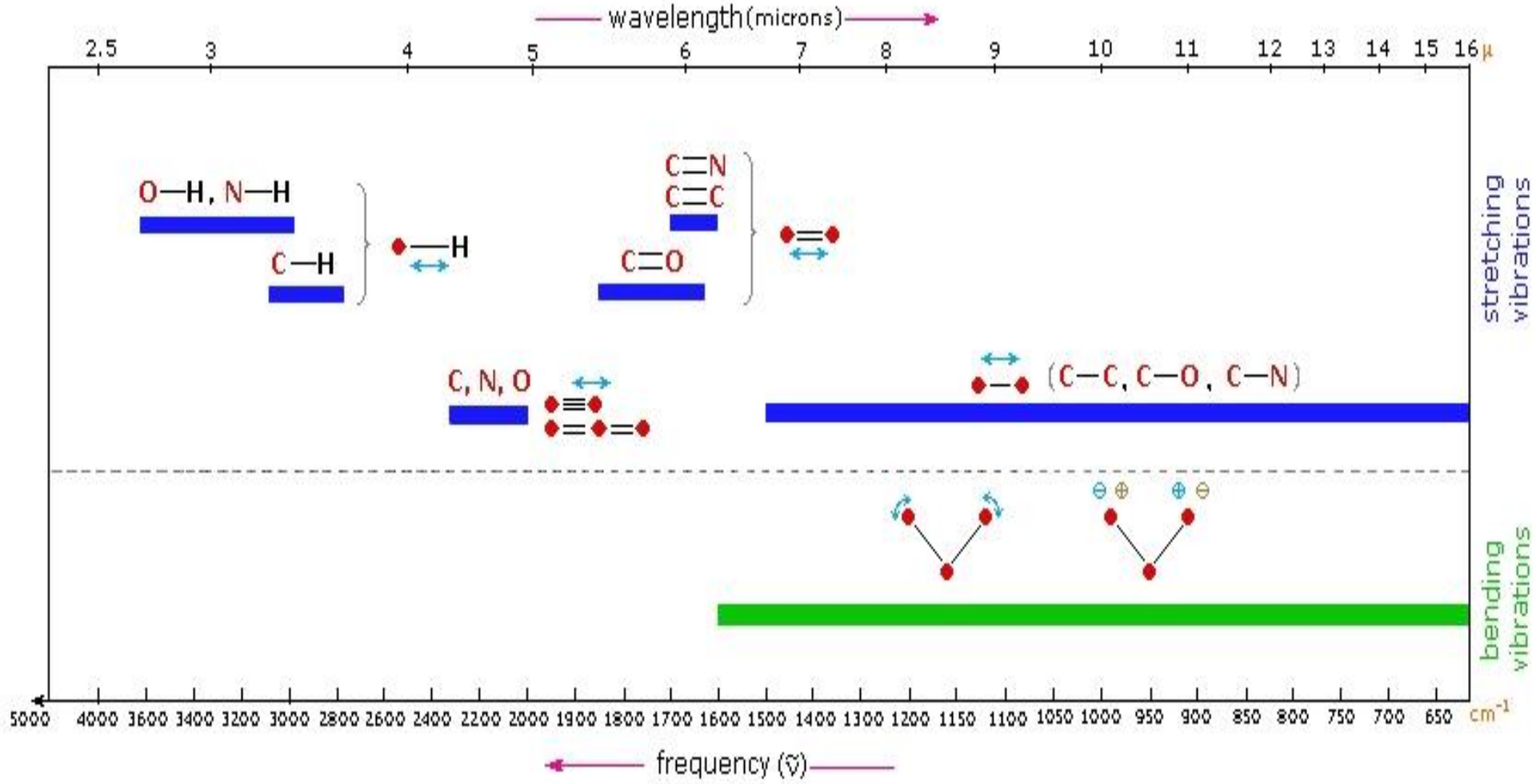
bending

Normal vibrations of water

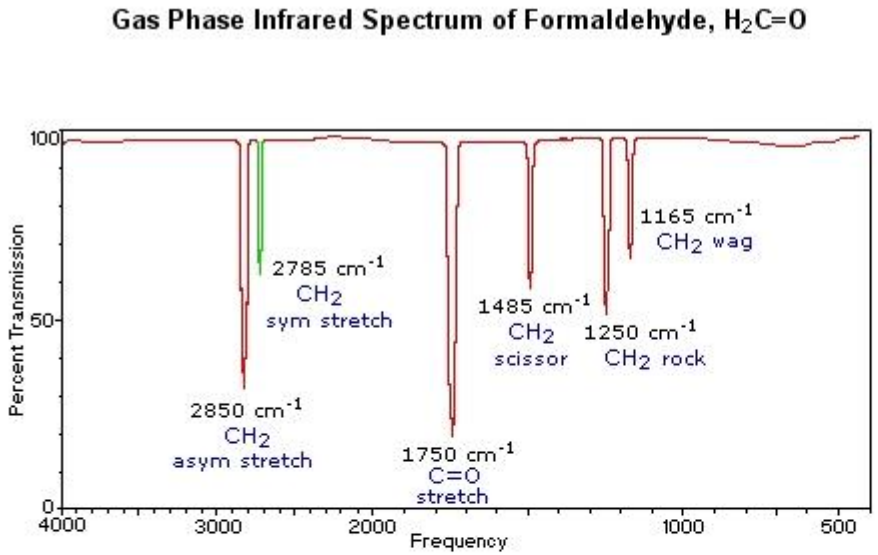


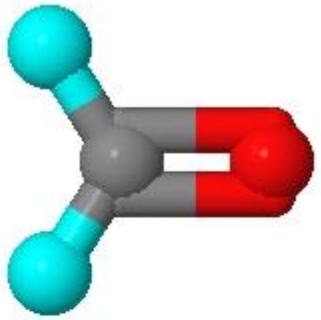
These are no vibrations! These are rotations!

Typical vibrational frequencies (wavenumbers)



Example: Formaldehyde



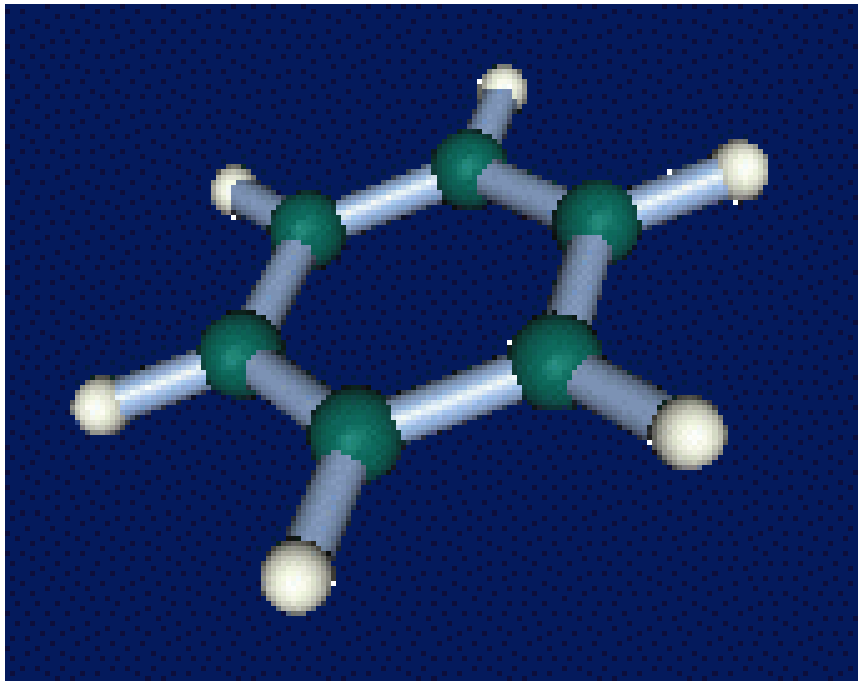


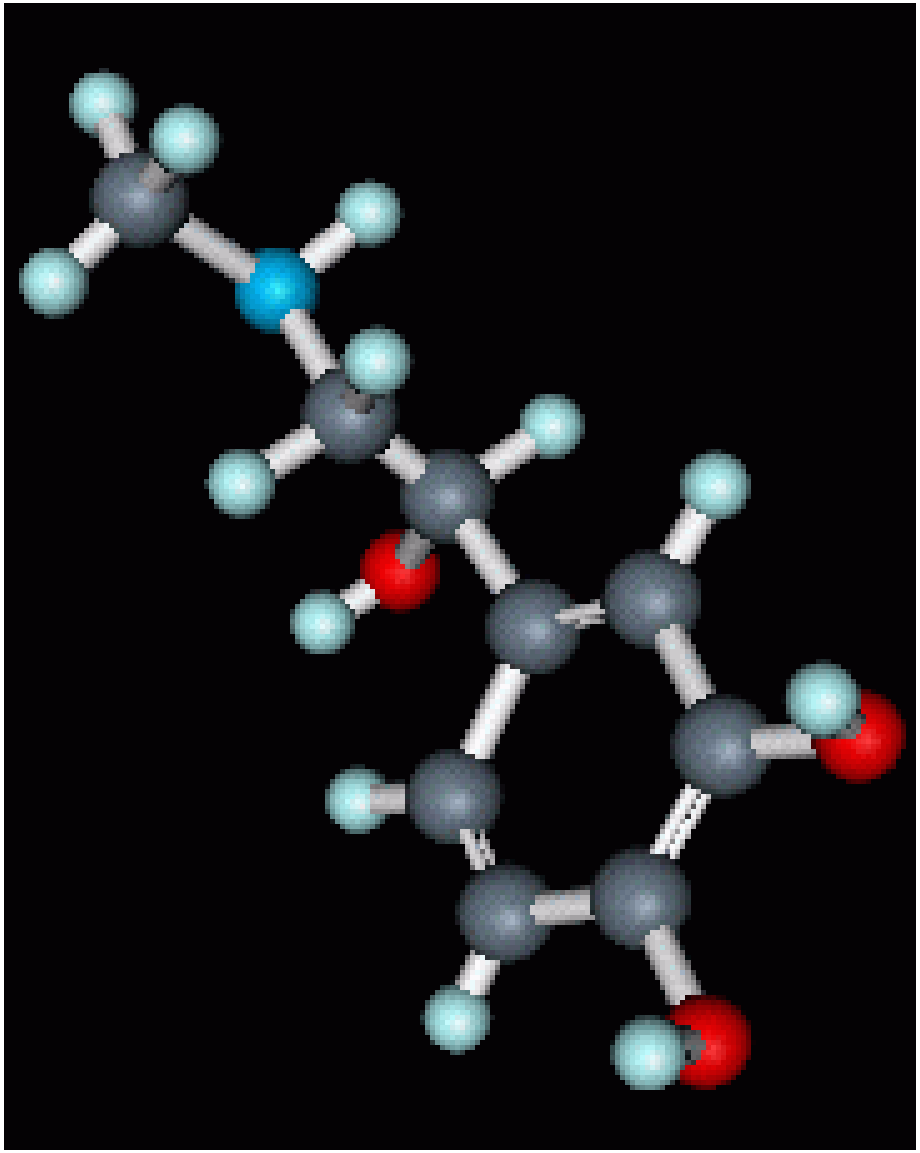
- O View CH_2 Asymmetric Stretch
- O View CH_2 Symmetric Stretch
- O View C=O Stretch
- O View CH_2 Scissoring
- O View CH_2 Rocking
- O View CH_2 Wagging

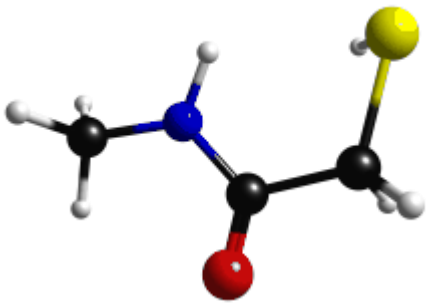
- O Ball&Stick Model
- O Spacefill Model
- O Stick Model
- O Motion Off

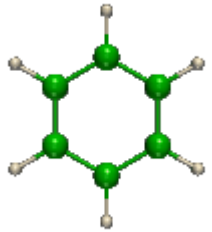
http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

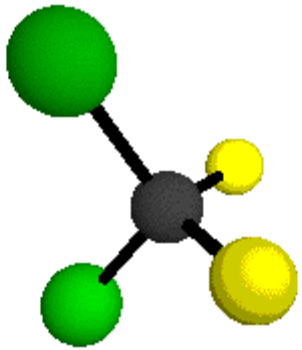
Benzol









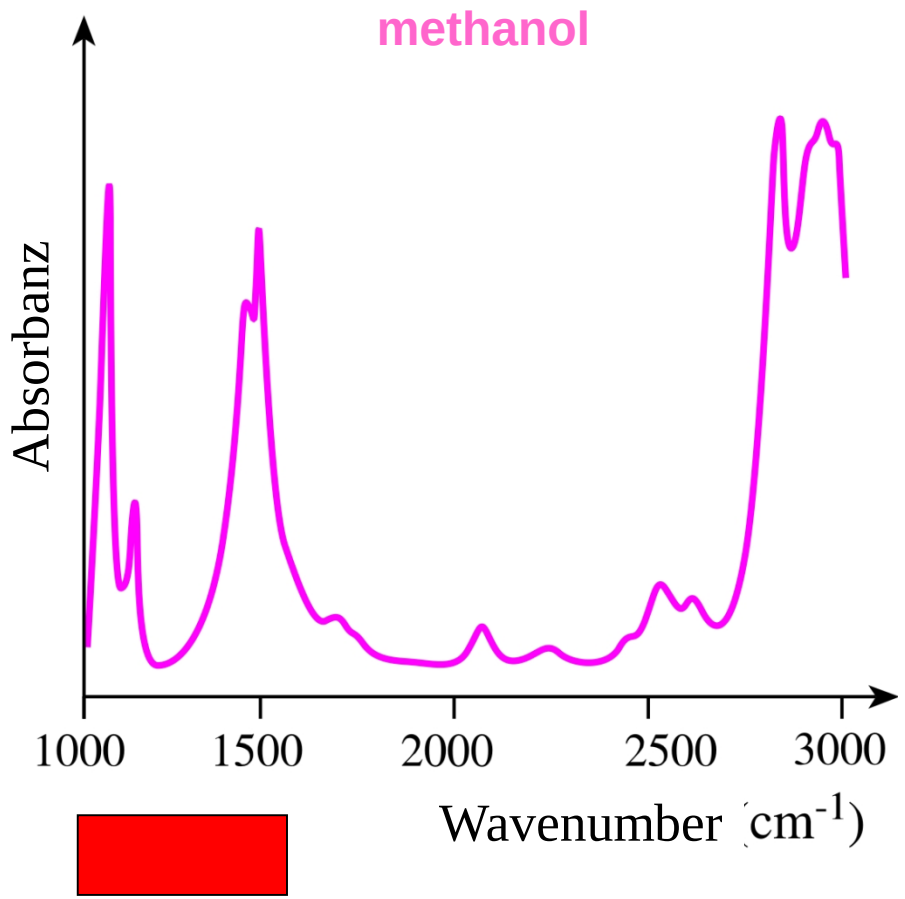


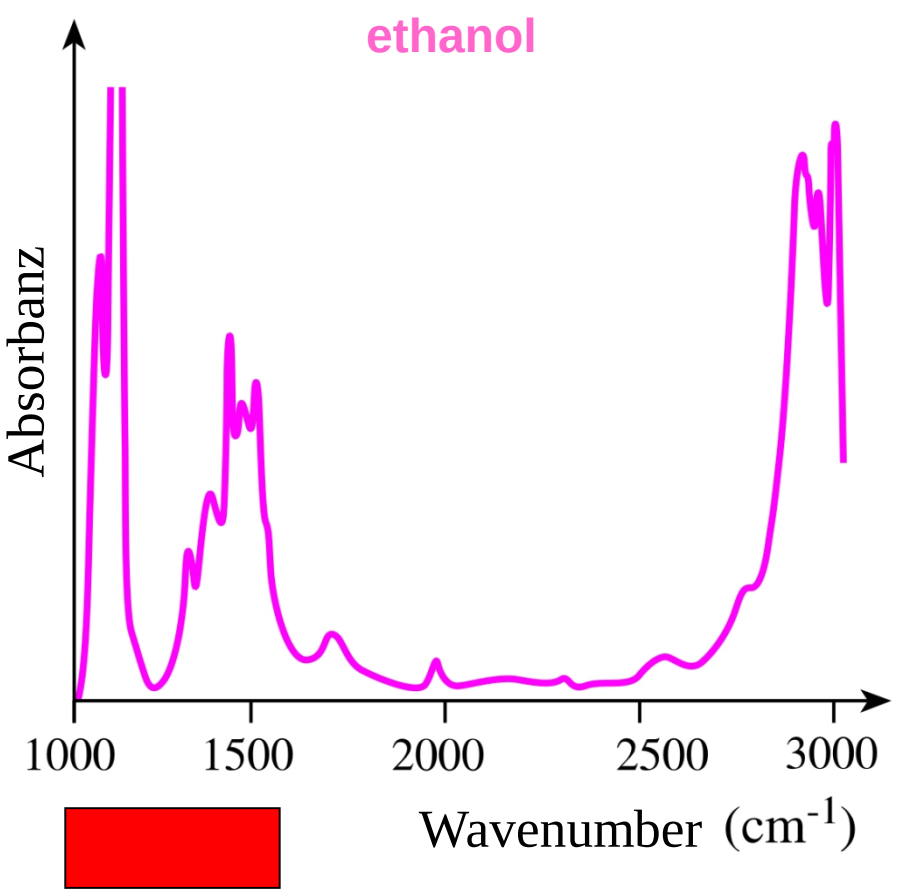
Analytical applications

- synthesis: identification of the intermediate and the end product
- determination and justification of the molecular structure
- detection of the metabolites
- quality control (purity)

- Remark.: Lambert-Beer law is valid, determination of concentration is possible

Identification of the Molecules

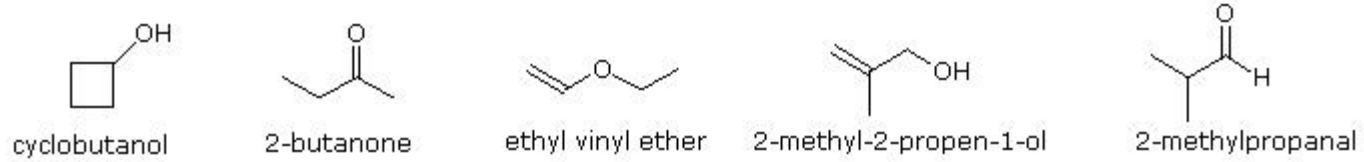




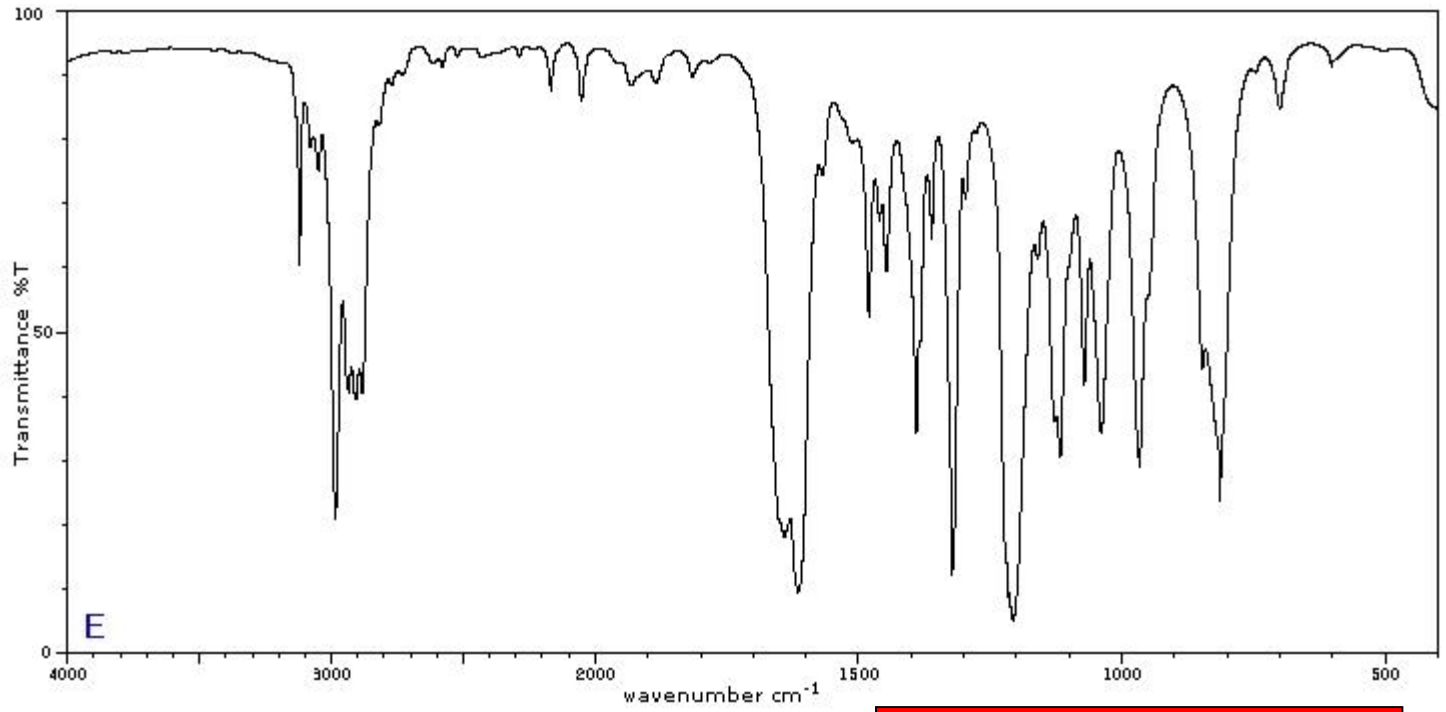
Fingerprint Region

Example: Identification of molecules

C_4H_8O



cyclobutanol | 2-butanone | ethyl vinyl ether | 2-methyl-2-propen-1-ol | 2-methylpropanal



Fingerprint Region

http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm

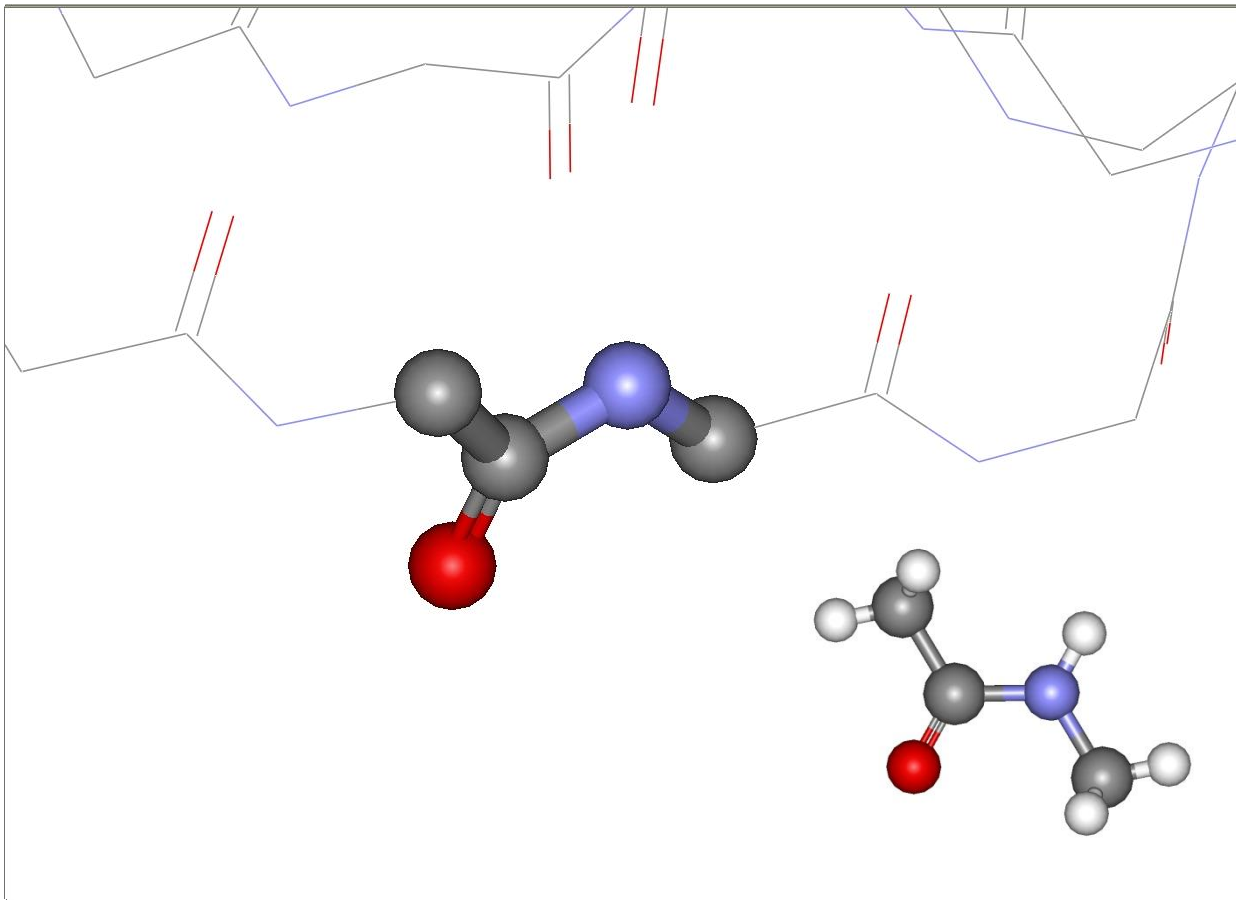
Vibrations of the macromolecules

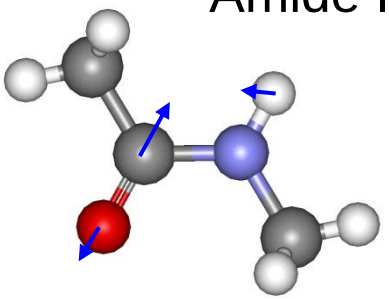
Complex global vibrations

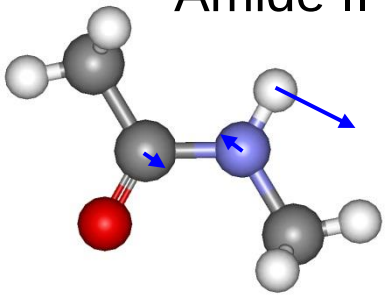
Localized vibrations, e.g.:

- amid vibrations of proteins
  (obtained from N-methylacetamide)
- CH2 vibrations of the lipids

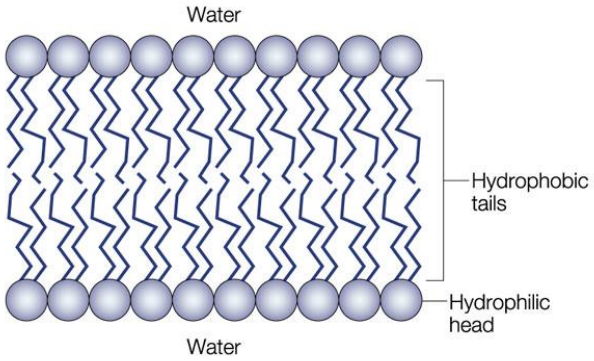
Proteins: model of the polypeptide backbone

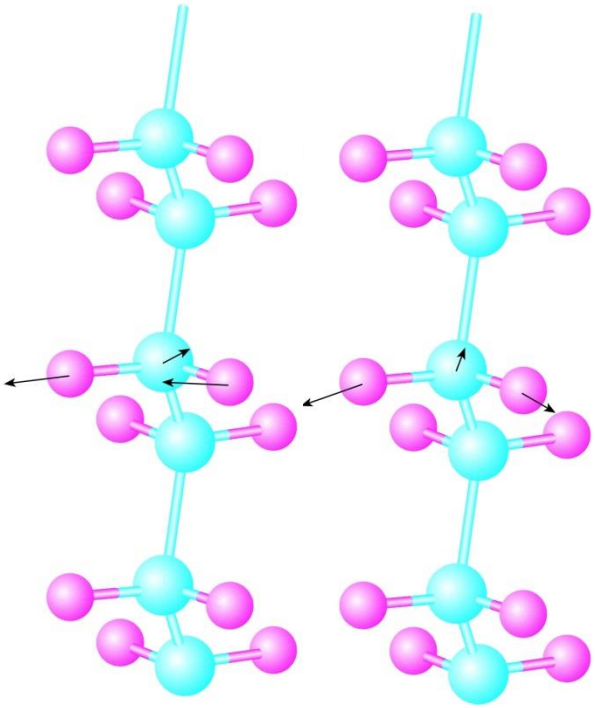


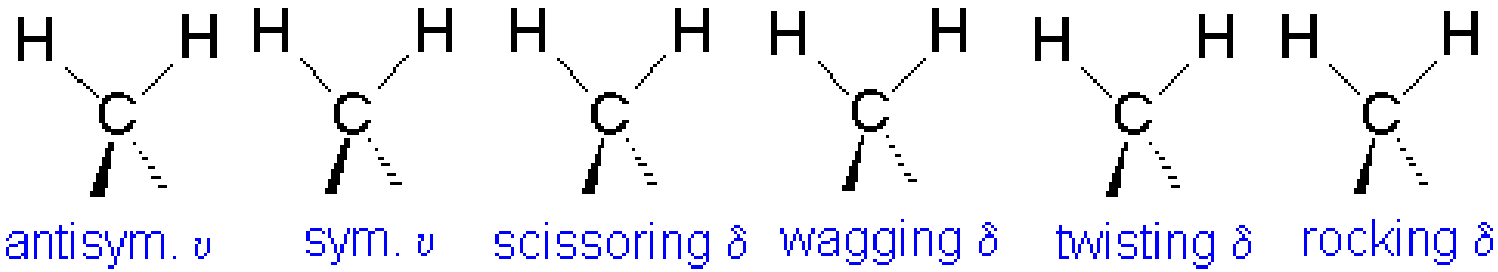




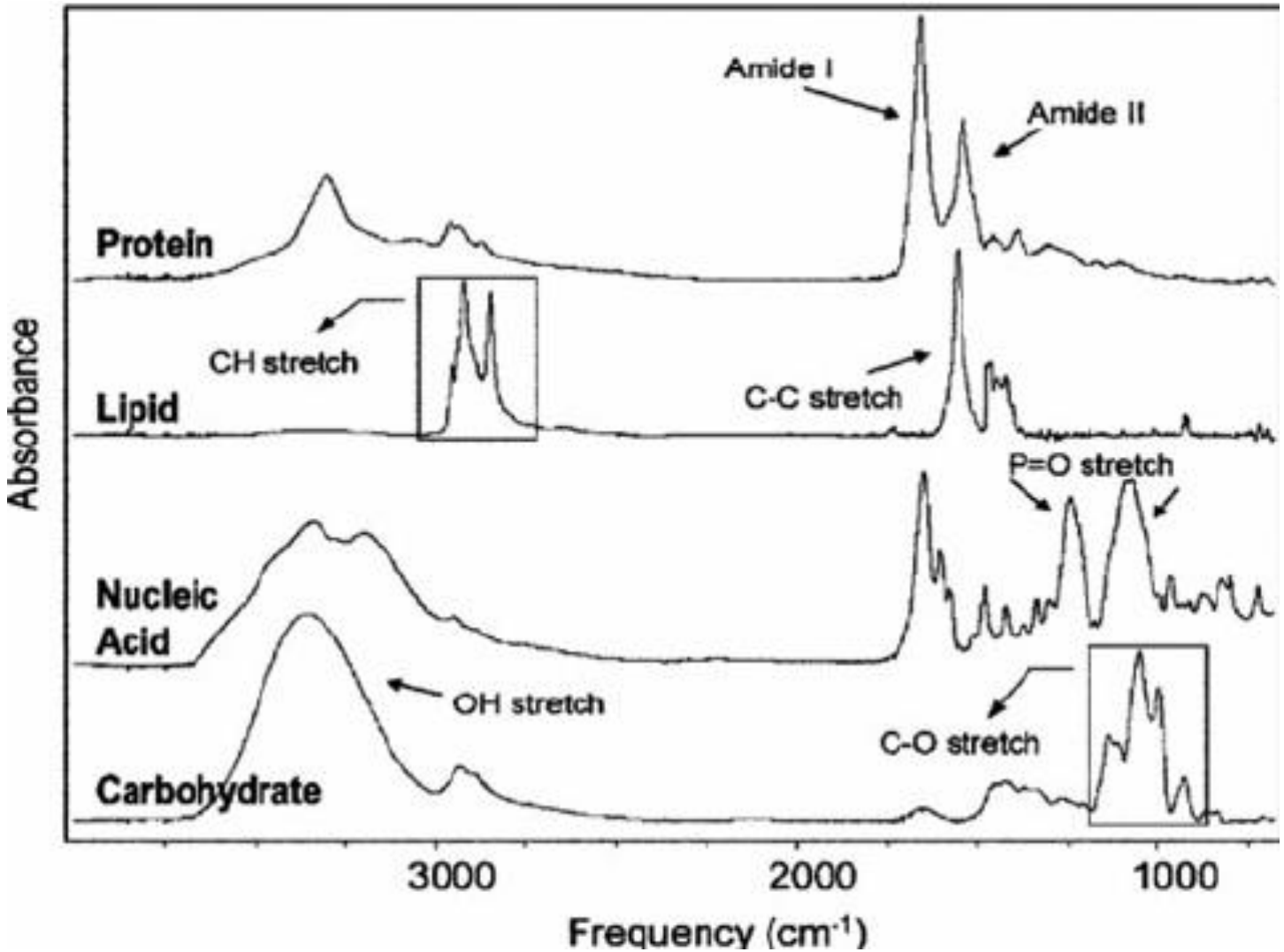
Localized vibrations: CH_2 vibrations of the lipids



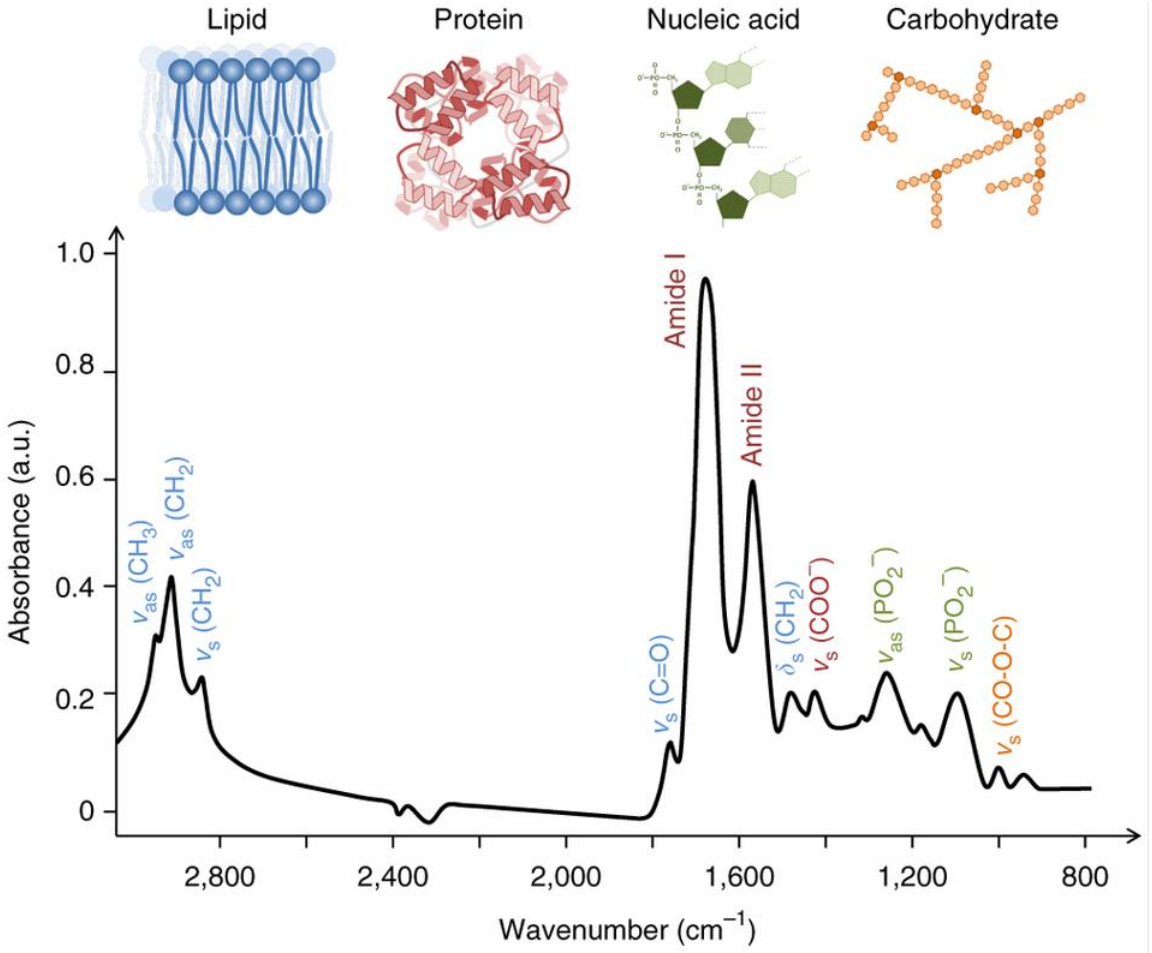




Typical infrared spectra of biological macromolecules



Infrared spectrum of the cell

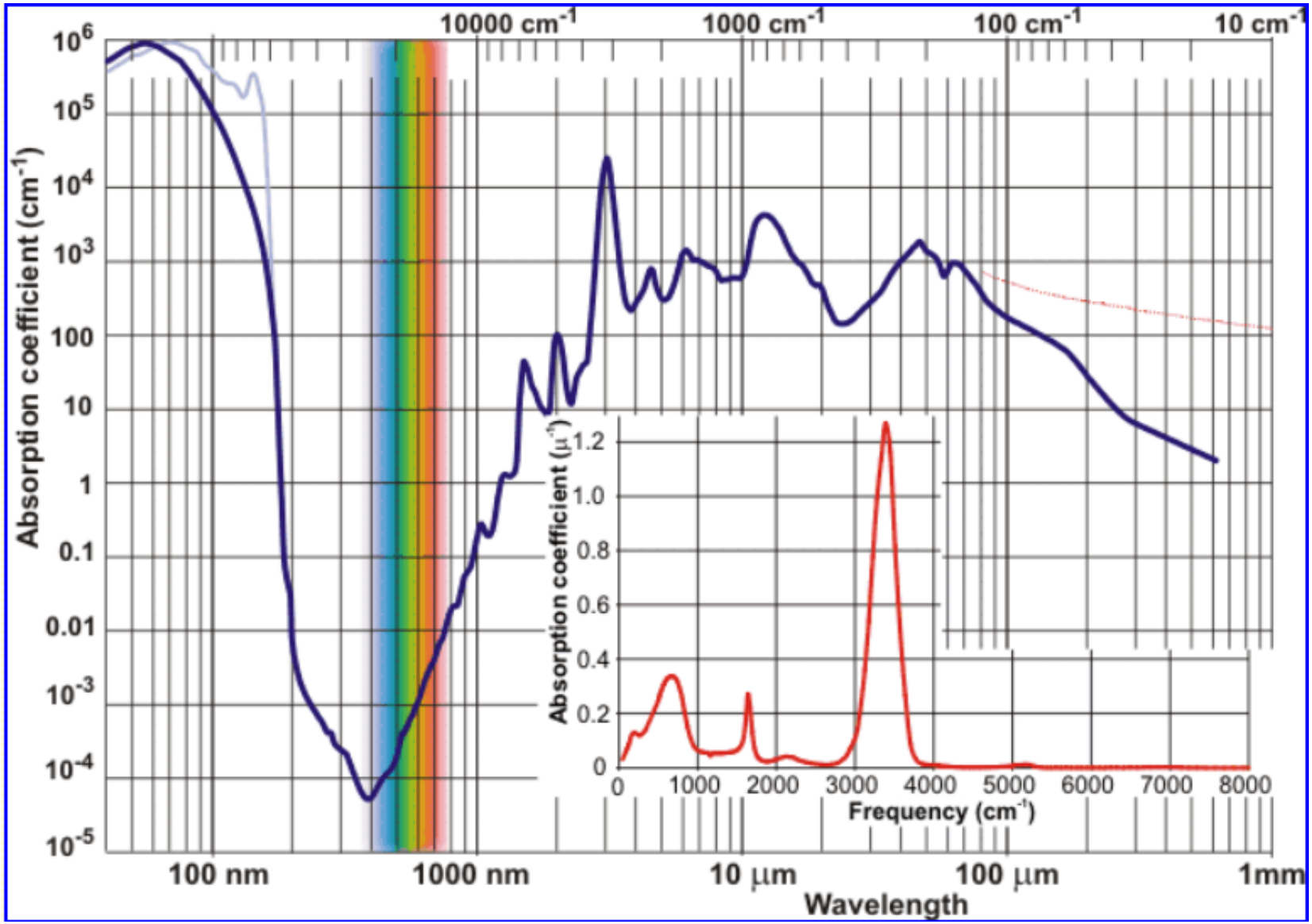


IR spectroscopy of proteins

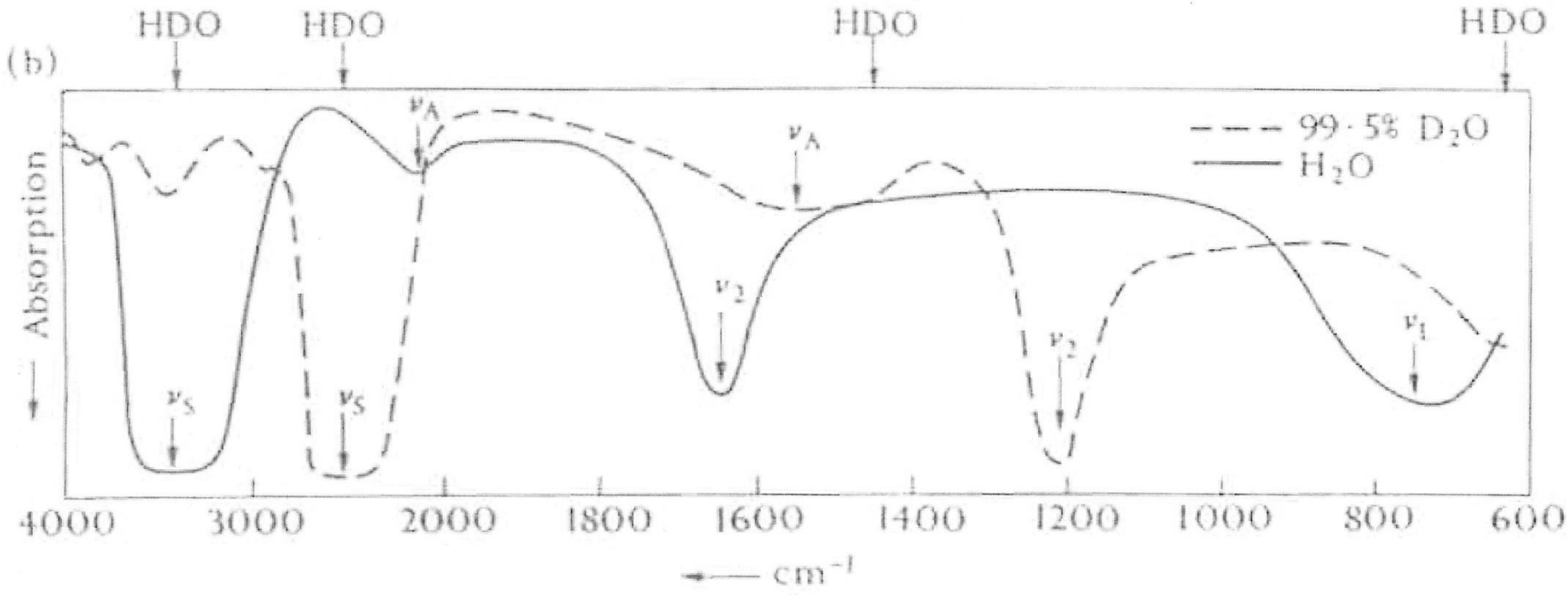
- Backbone: amide vibrations
  - conformation (secondary structure)
  - H/D exchange, (tertiary structure)
- Side chains
  - interaction with other molecules
  - Ca^{2+} binding

Technical point: heavy water solutions (D_2O)

Absorption spectrum of the water

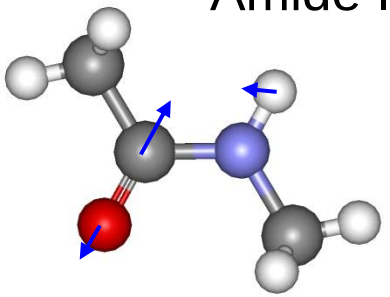


Absorption spectra of water and heavy water

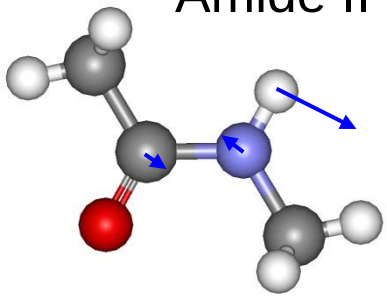


Amide vibrations of proteins

Amide I



Amide II



amide I

C=O vibration.

Sensitive to the secondary structure because of the hydrogen bond.

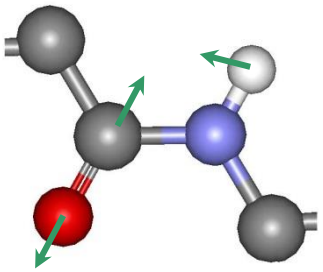
amide II

N-H in plane bending vibration

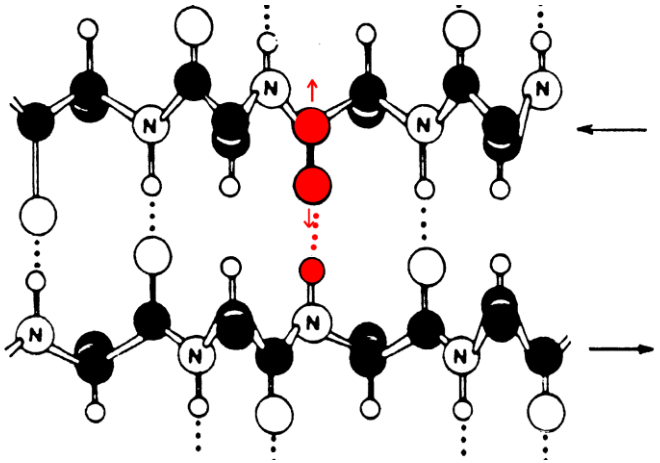
Sensitive to the H-D exchange i.e. to the compactness of the molecule.

(tertiary structure)

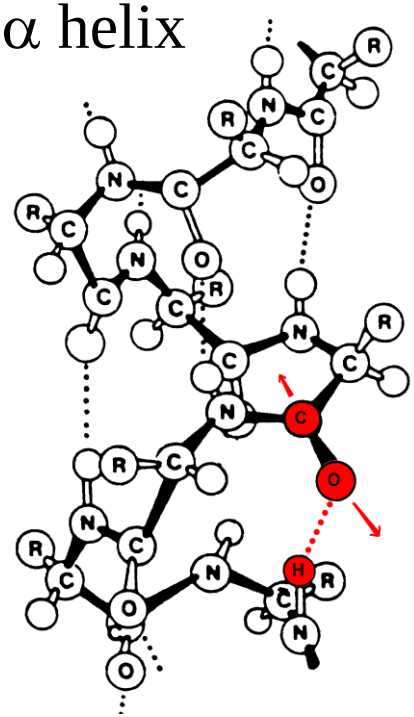
The amide I vibration and the secondary structure



β sheer



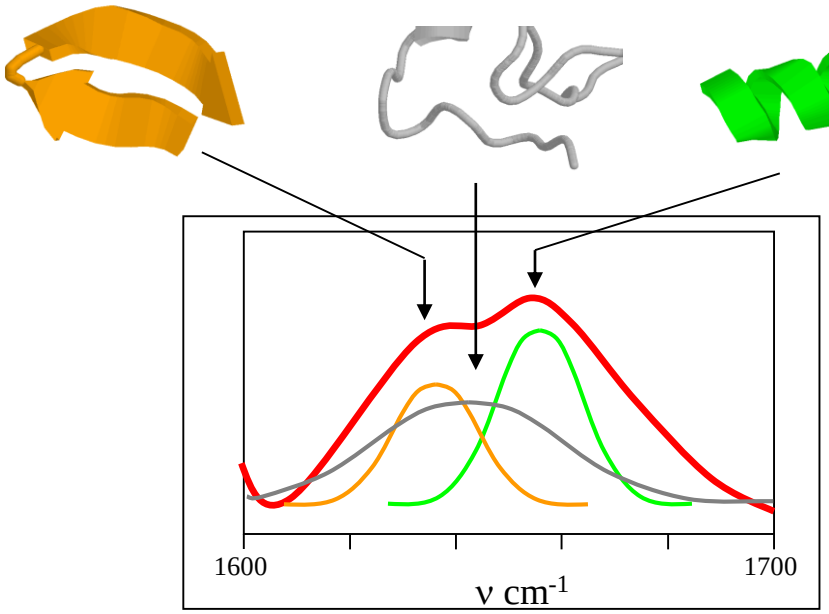
α helix



Position of the amide I band in different secondary structures

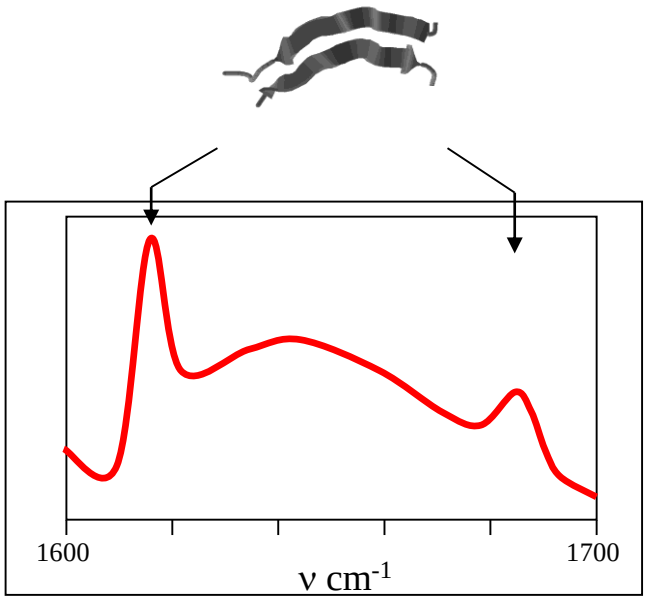
Intramolecular structure

β-sheet unordered α-helix

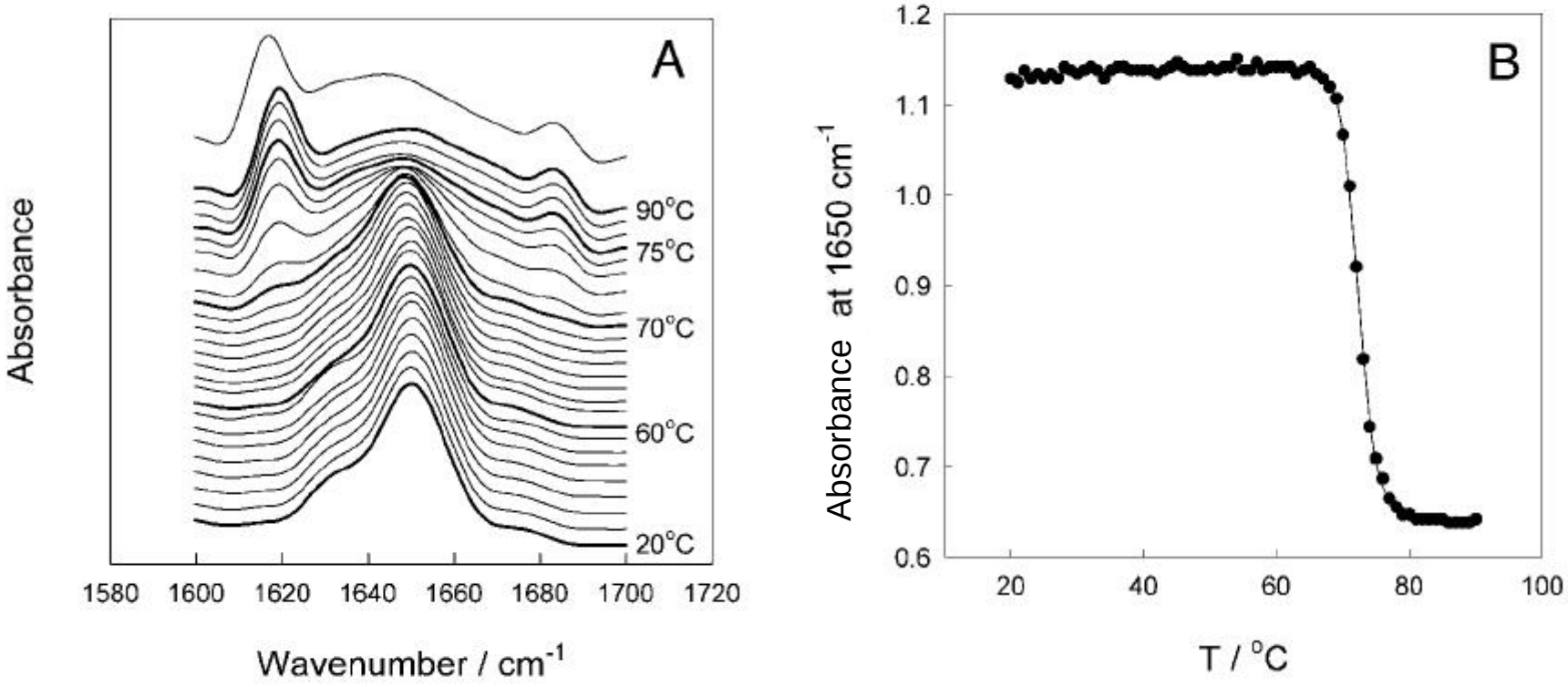


Intermolecular interaction

Intermolecular antiparallel β-sheet

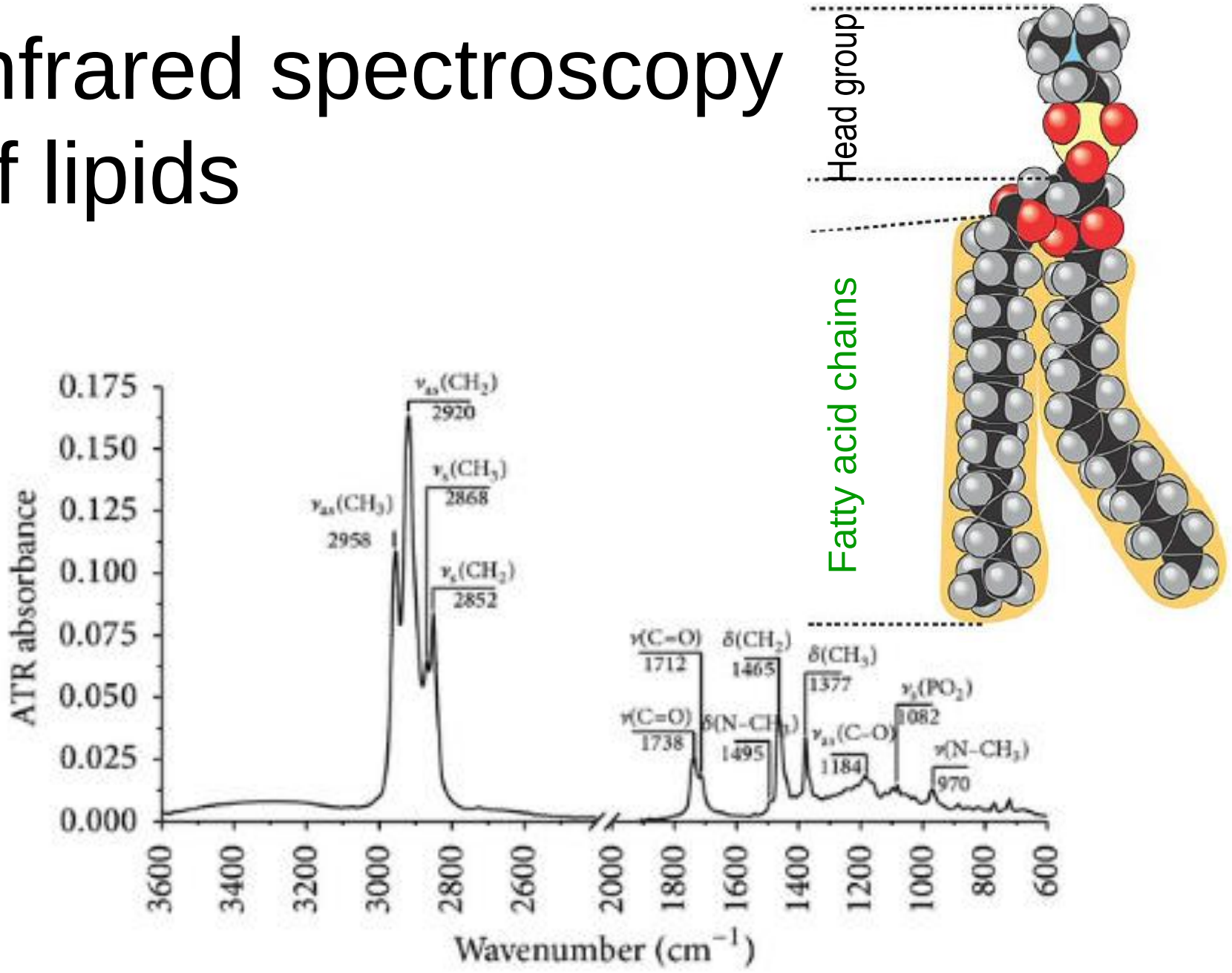


Applications: Denaturation

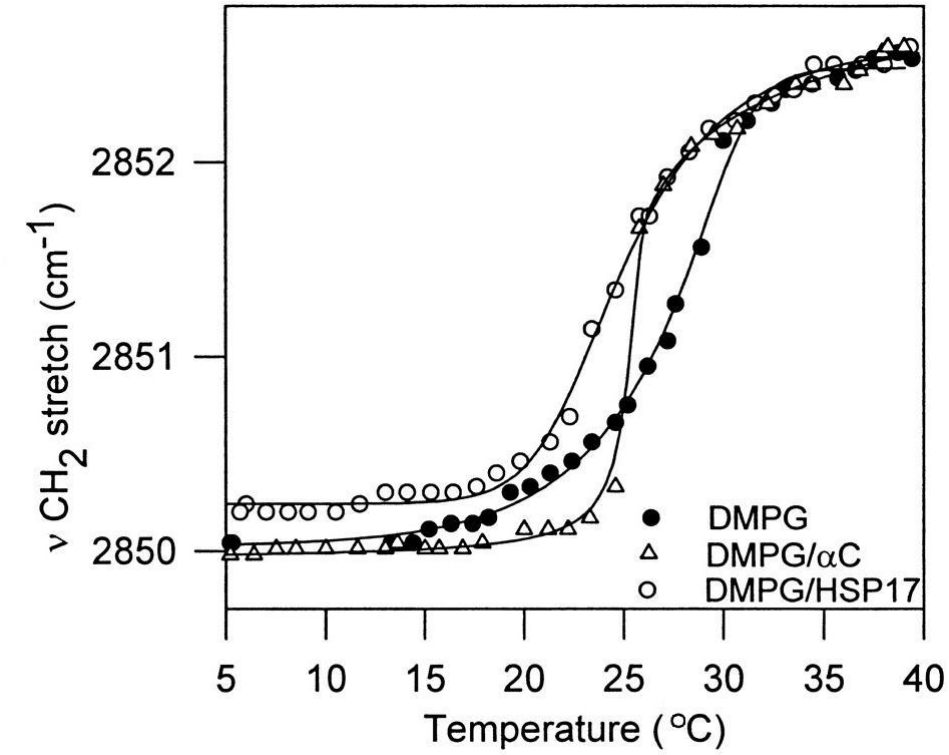


Meersman et al. Biophys J.

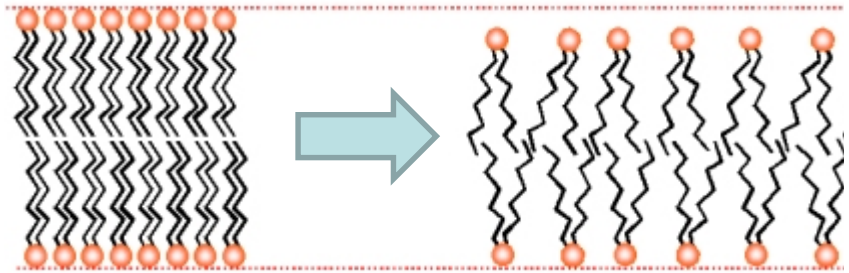
Infrared spectroscopy of lipids



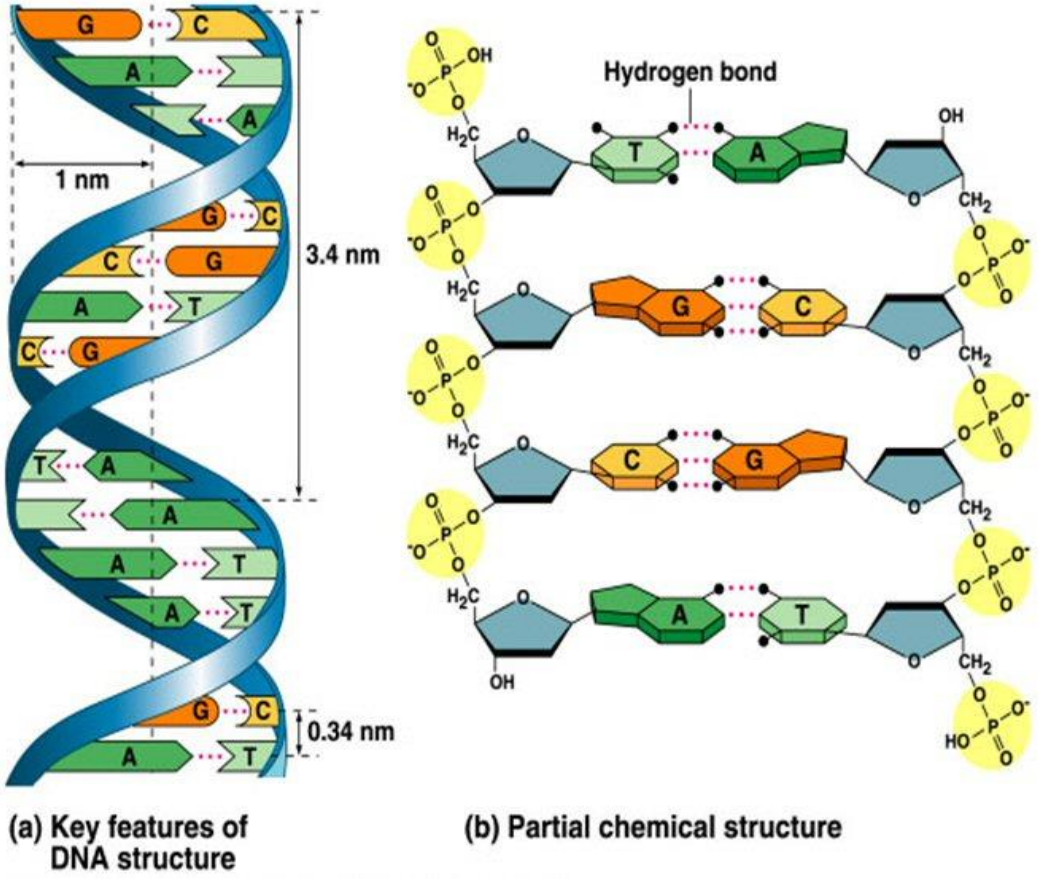
Applications: lipid phase transitions



Tsvetkova és mtsai PNAS 2002 | vol. 99 | no. 21 | 13504-13509



Nucleic acids



Bases (H bond)

Phosphate group. (PO^-_2)

Sugar

DNA

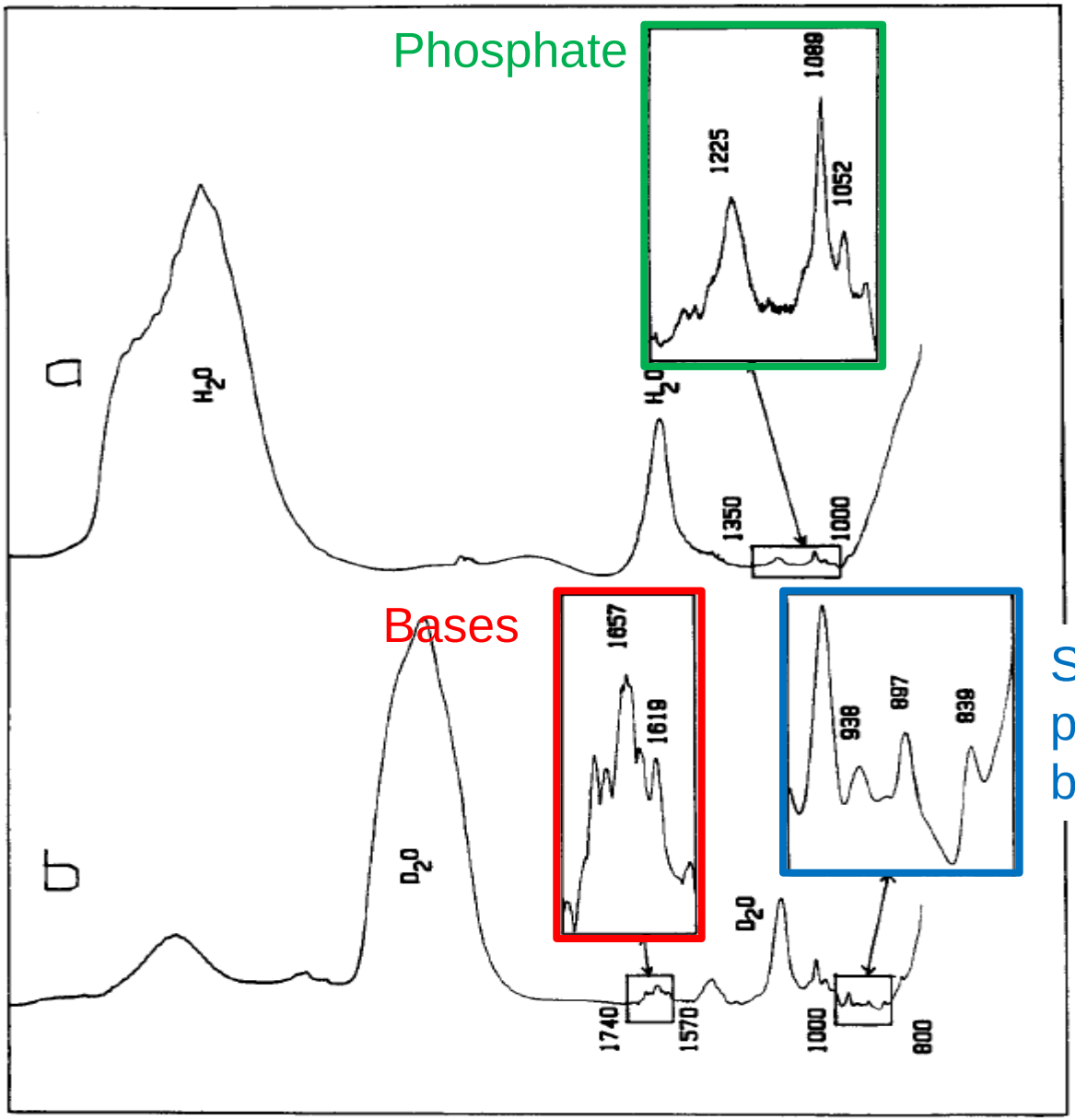


FIG. 1. FT-IR spectra of DNA in solution. (a) H_2O solution; (b) D_2O solution. Enlarged parts of the spectra present the absorptions involving mainly the vibrations of the phosphate groups (top), the double bonds of the bases in their plane (bottom left), and the sugar–phosphate backbone (bottom right).

DNA, RNA A and B structures

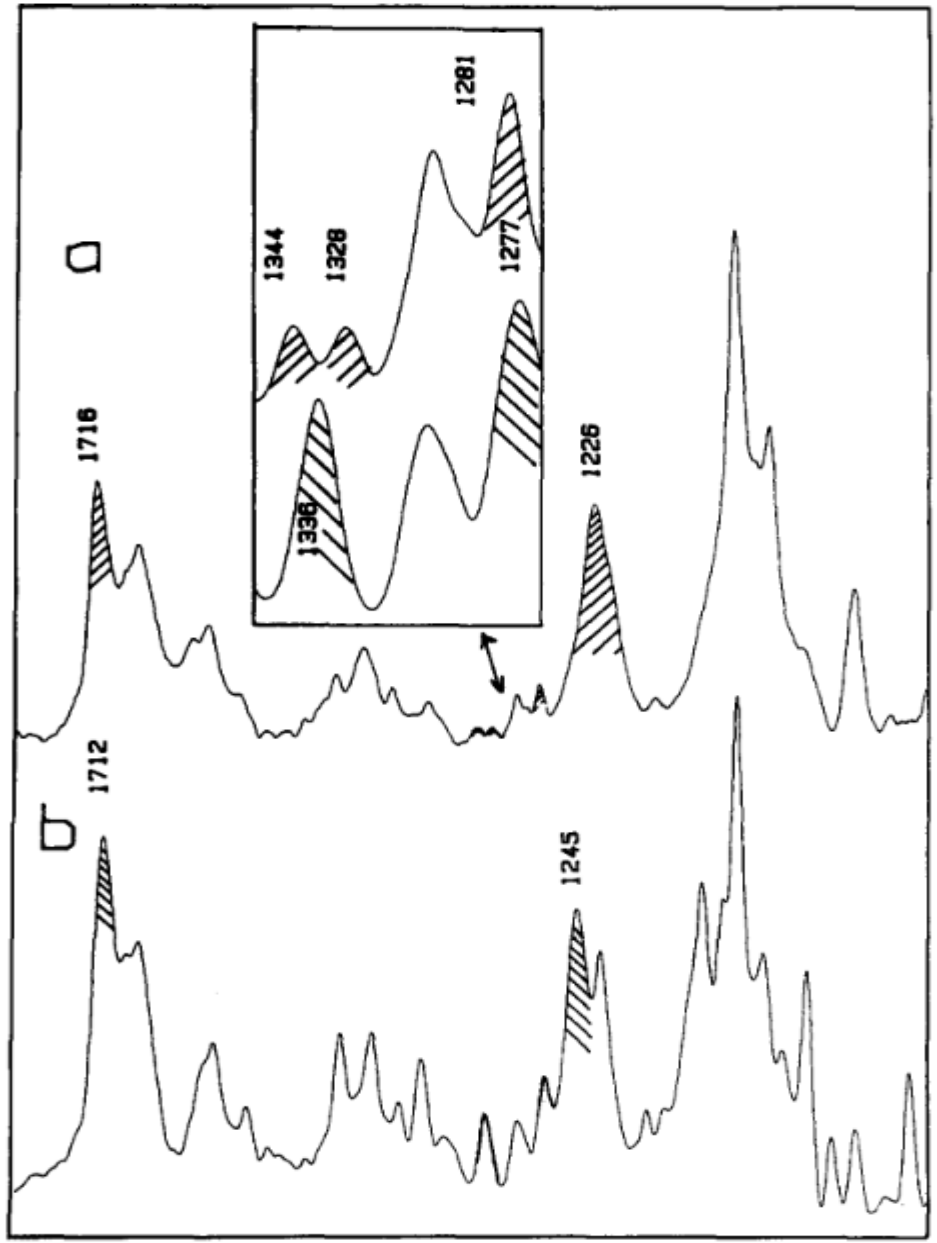
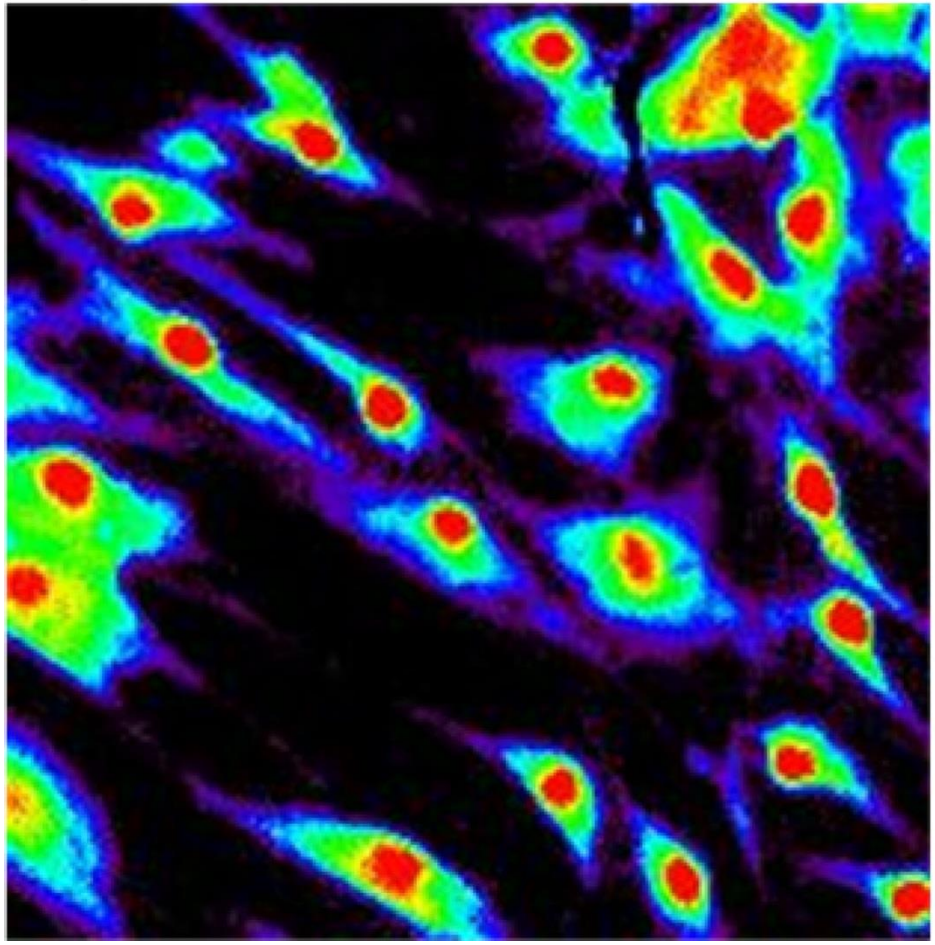
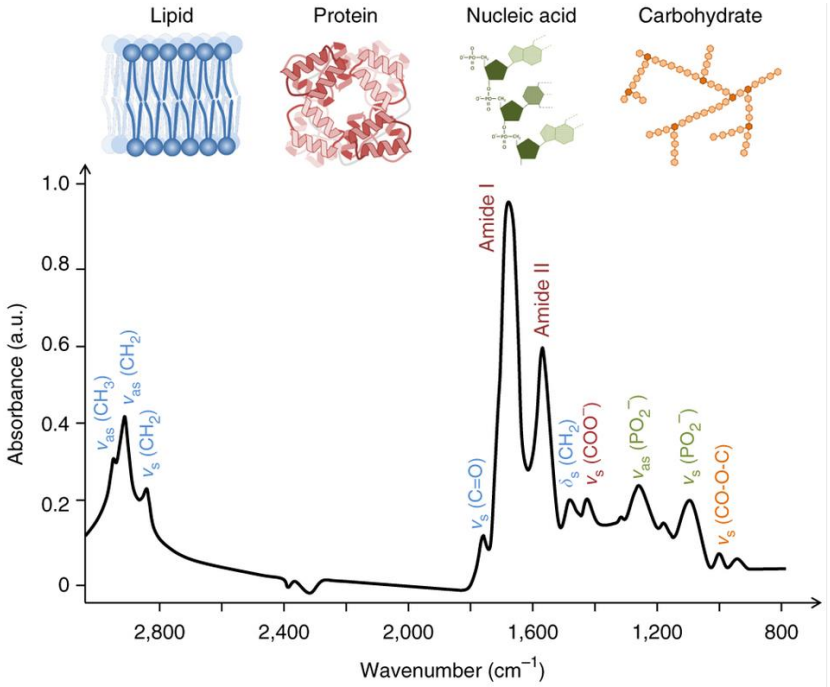


FIG. 3. FT-IR spectra of H_2O solutions of $d(A\text{-}U)_n$ (a) and $r(A\text{-}U)_s$ (b). The enlarged area between 1350 and 1270 cm^{-1} shows absorptions characteristic of A (\\\\\\) and B (//////) geometries.

Special IR methods: IR Microscope

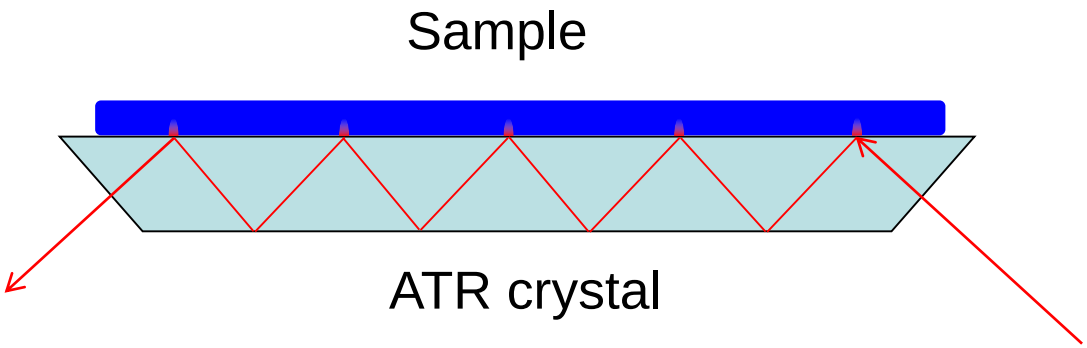


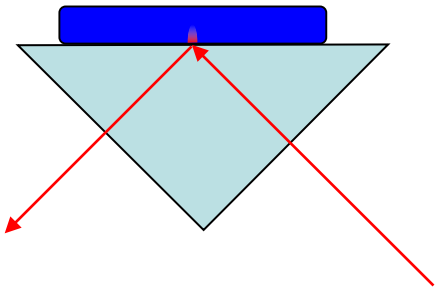


dermal fibroblasts imagined at 1224 cm^{-1}

Portable FTIR specrometer

ATR technique
(Attenuated Total Reflection)



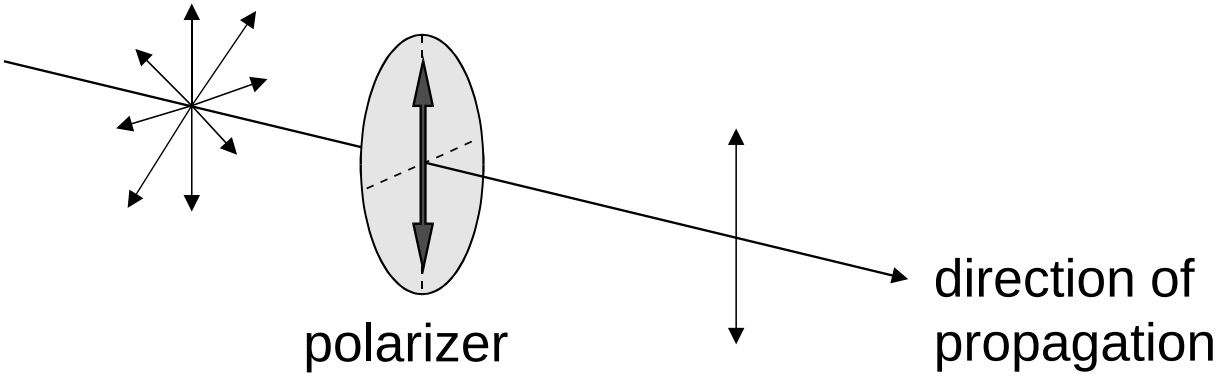


CD

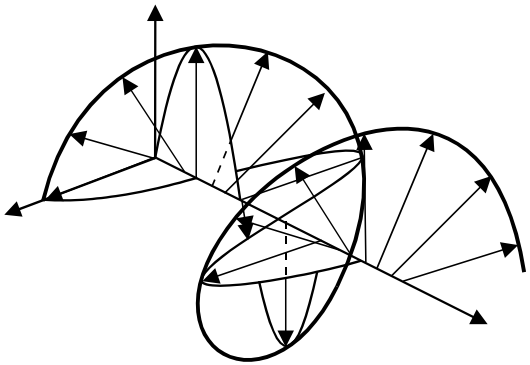
Circular dichroism spectroscopy

Polarized light

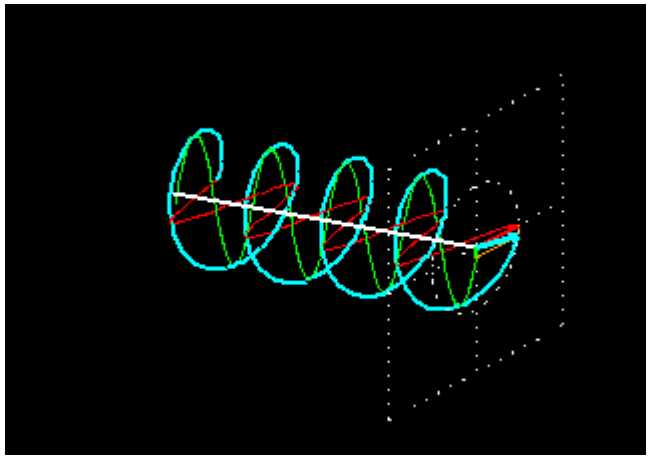
Linear polarized:



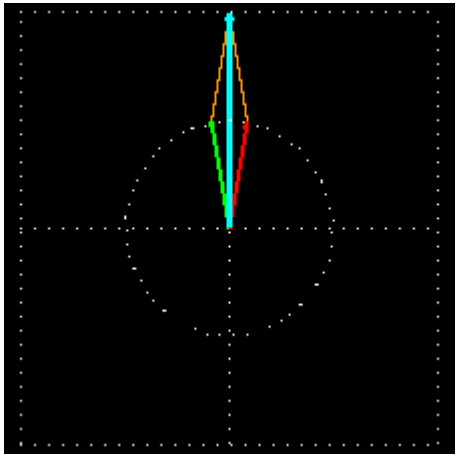
Circular polarized



Circular
polarized light:



lin. pol light=
right +
left circ. pol.

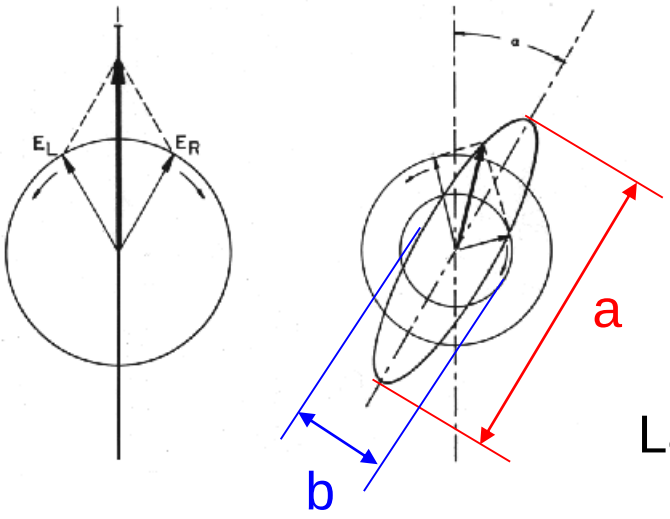


Animations can be found here: http://www.enzim.hu/~szia/cddemo/demo0.htm

Interaction of chiral molecules with left and right polarized light is different.
The absorbance difference is:

$$\Delta A = A_L - A_R = \Delta\varepsilon \; c \; x$$

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R$$



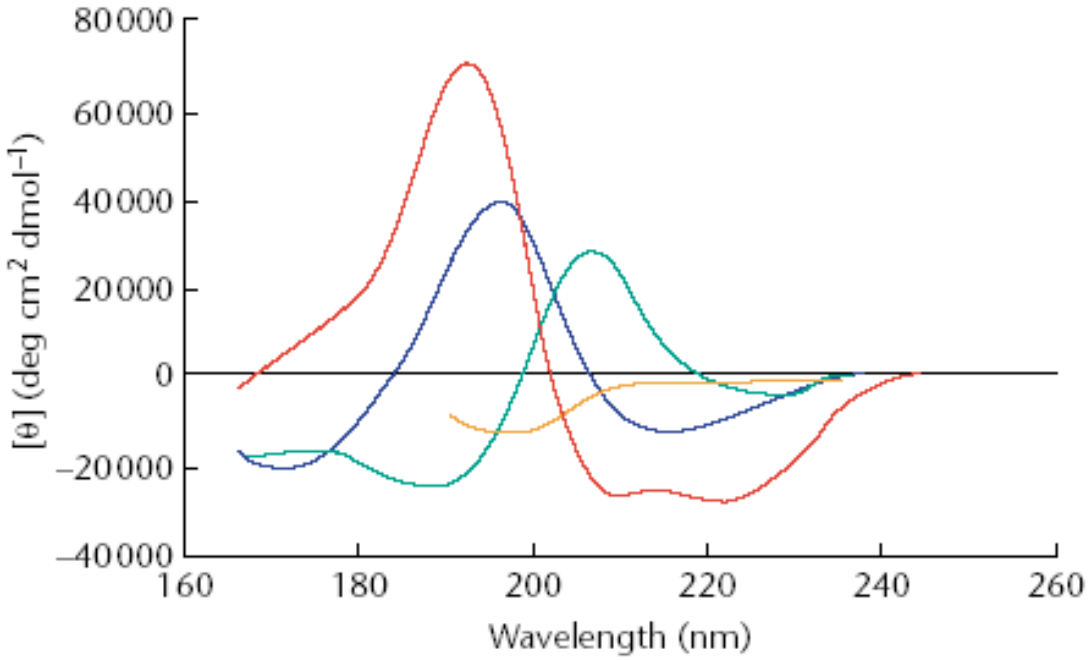
Ellipticity: θ tg θ = b/a

$$\theta = \frac{2.303}{4} \cdot (A_L - A_R) \cdot \frac{180}{\pi} \quad \text{[deg]}$$

Lambert-Beer like law: $\theta = c \cdot l \cdot \theta_m$

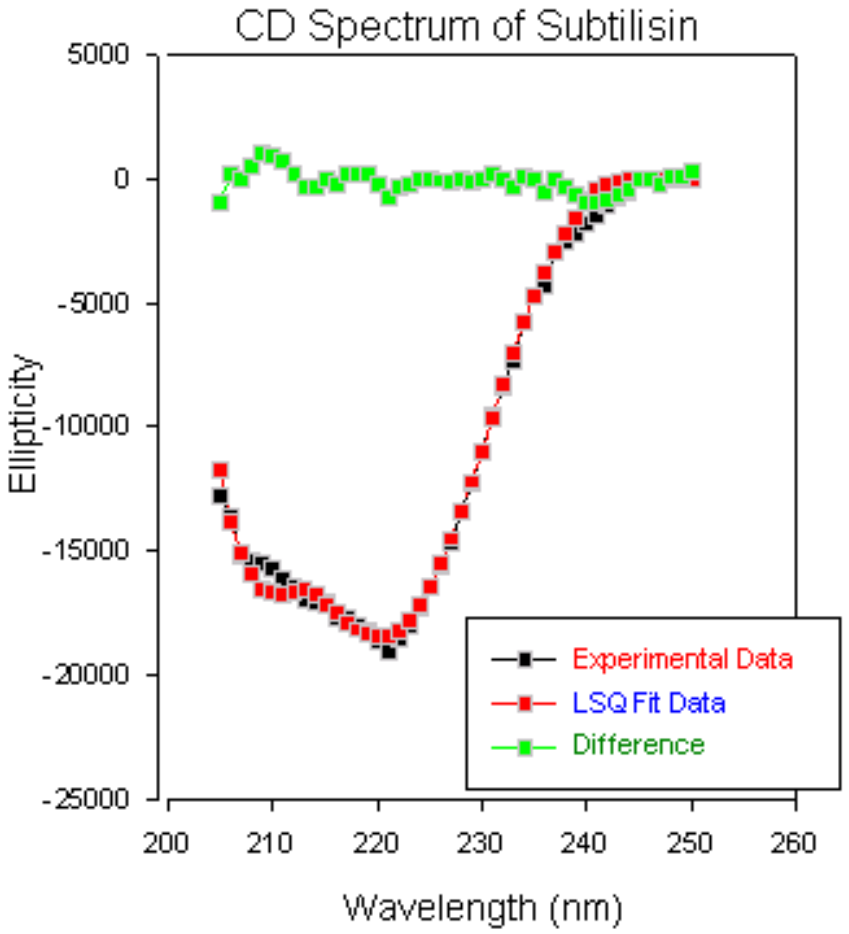
(θ_m: molar ellipticity)

CD and protein structure



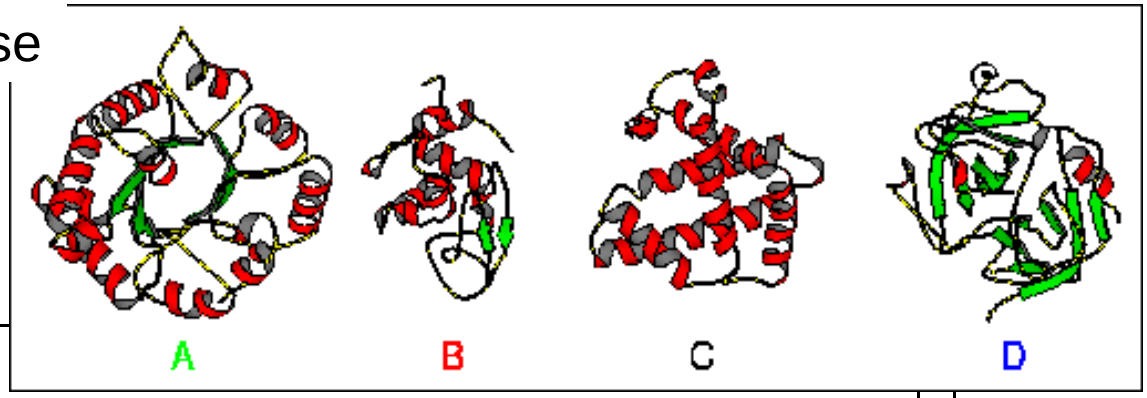
The far-UV CD spectra associated with various types of secondary structure elements in proteins. Red: α-helix; blue: antiparallel β-sheet; green: type I β-turn; orange: irregular structure.
(Data taken from the Encyclopedia of Life Sciences)

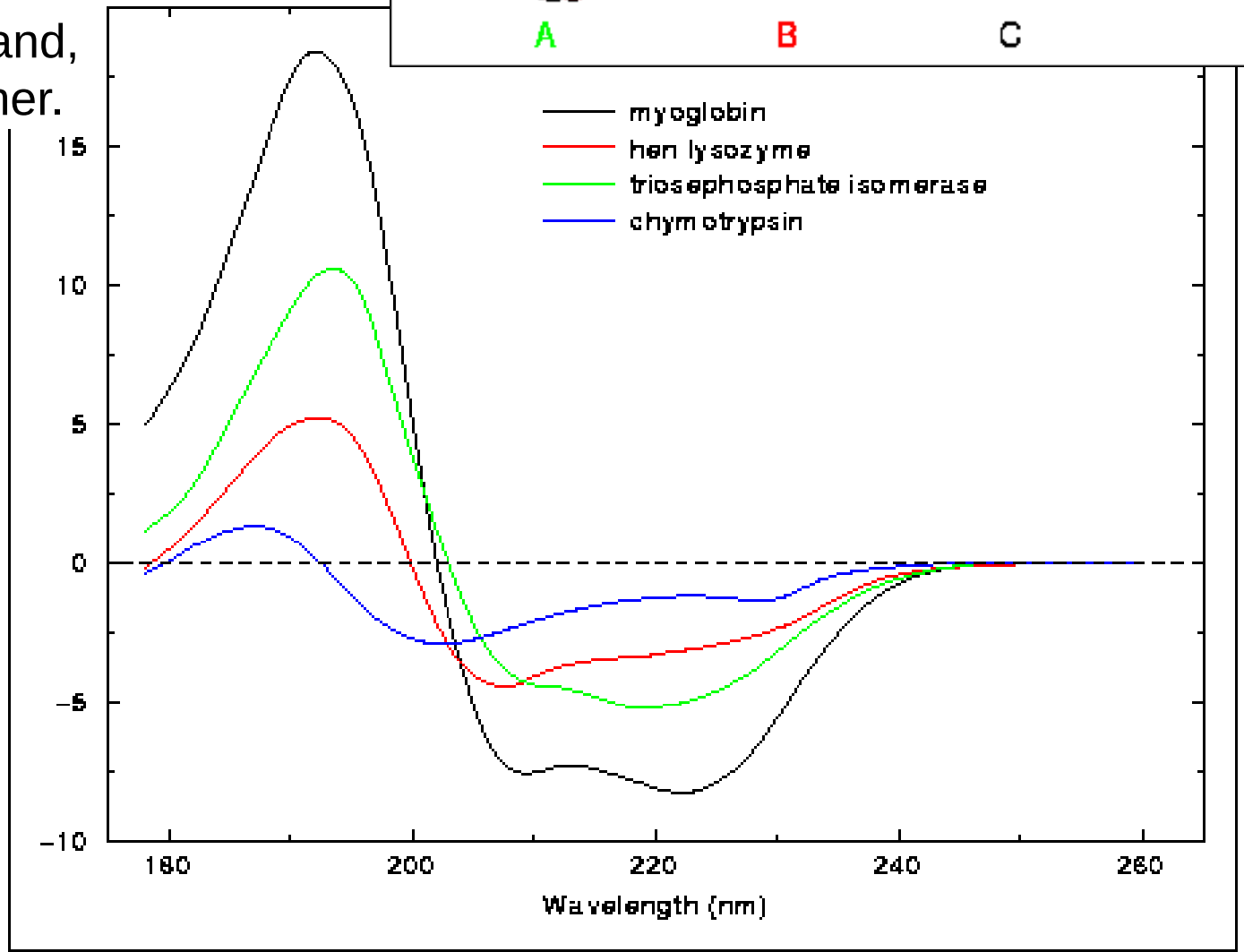
The Structure and CD spectrum of Subtilisin



helix	sheet	coil
57.92	26.22	15.85

A) triosephosphate isomerase
B) hen egg lysozyme
C) myoglobin
D) chymotrypsin
red: helix.
green: strand,
yellow: other.





End

The CD spektrometer