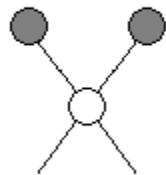


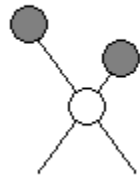
# IR spektroskopie

Excitace vibračních a rotačních přechodů

## Stretching vibrations

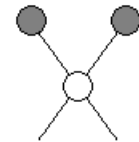


Symmetric

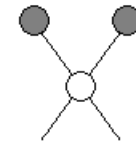


Asymmetric

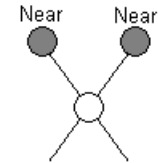
## Bending vibrations



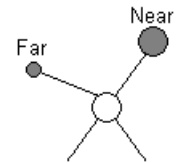
In-plane rocking



In-plane scissoring



Out-of-plane wagging



Out-of-plane twisting

Valenční vibrace

$\nu$

Deformační vibrace

$\delta$

# IR spektroskopie

$N$  atomů =  $3N$  stupňů volnosti

Vibrační modus: molekulární pohyb, ve kterém atomy vibrují ve fázi se stejnou frekvencí

Lineární molekuly:  $3N - 5$  modů

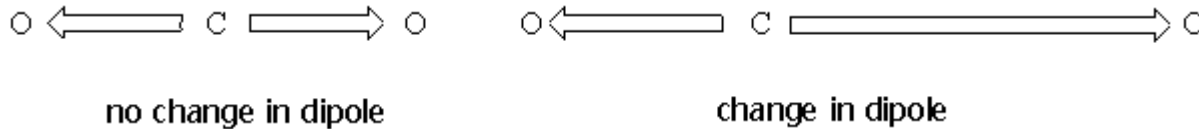
Nelineární molekuly  $3N - 6$  modů

# IR spektroskopie

Podmínka viditelnosti absorpce v IR spektru – vibrace musí být provázena změnou dipólového momentu

(např. asymetrické vibrace)

(Elmg. záření je oscilující dipolový moment)



# IR spektroskopie

$$E = h\nu$$

$$\text{Vlnočet} = \text{cm}^{-1}$$

Blízká	0.78 - 2.5 $\mu\text{m}$	12800 – 4000 $\text{cm}^{-1}$
<b>Střední</b>	<b>2.5 – 50 <math>\mu\text{m}</math></b>	<b>4000 – 200 <math>\text{cm}^{-1}</math></b>
Vzdálená	50 -1000 $\mu\text{m}$	200 – 10 $\text{cm}^{-1}$

X – H	3600 – 2700
X=Y	2700 – 1900
X=O	1900 – 1500
X-Y	1500 - 500

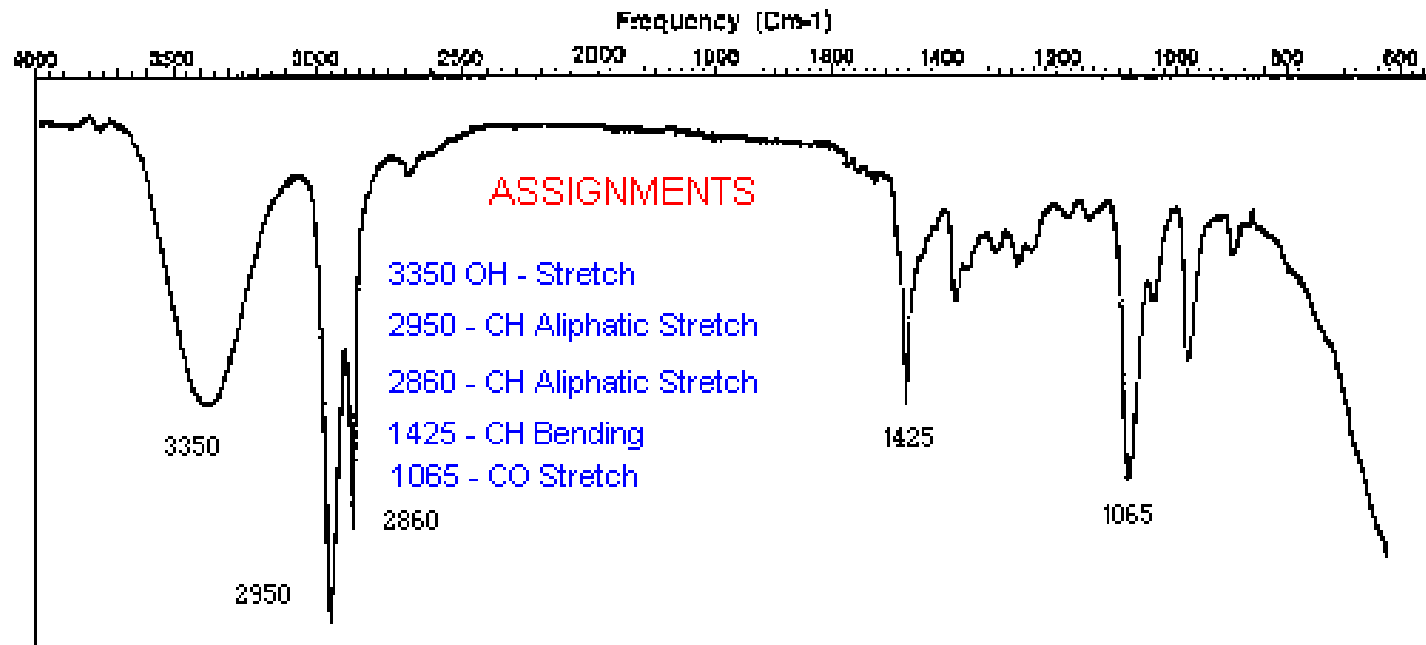


$$\nu_{\text{bar}} = 1/2\pi (k_{\text{AB}}/\mu_{\text{AB}})^{1/2} \text{ kde } \mu_{\text{AB}} = M_{\text{A}}M_{\text{B}}/(M_{\text{A}} + M_{\text{B}})$$

Kde  $k$  je silová konstanta,  $M_{\text{A}}$ ,  $M_{\text{B}}$  hmotnosti atomů,  $\nu$  vlnočet

# IR spektroskopie

Typické IR spektrum



## Příklady vibrací

cm<sup>-1</sup>

v OH (alkohol)	3300-3700
v NH <sub>2</sub> (amin)	3200-3500
v SH (thioly)	2500-2600
δ HOH (voda)	1550-1650

Polohy abs. pásu podle typu vazeb

### biopolymery

v SH (cystein)	2600-2700
v NH <sub>2</sub> (amidy)	3200-3400
v C=O (Asp, Glu)	1710-1720
v COO-	1410-1570
v C-N	1625
v NH <sub>3</sub> <sup>+</sup>	1670
δ jádro (Tyr)	1515
v NH (Trp)	3400
v O-PO (fosfát)	900
δ jádro (nukl. báze)	1520-1570
v CO, δNH (pep. vazba)	1620-1670
δCN, δNH	1500-1550

X-H	3600 – 2700
X=Y	2700 – 1900
X≡Y	1900 – 1500
X-Y	1500 - 500

# IR spektroskopie

## IR spektra proteinů

Amid I -  $1655\text{ cm}^{-1}$   $\nu$  N-CO

Amid II –  $1550\text{ cm}^{-1}$   $\delta$  NH CN

## Závislost spektra peptidové vazby na typu struktury

Amid I ( $\text{D}_2\text{O}$ )

$\alpha$ -helix                    1651 -1657

$\beta$ - struktura                1620 -1640

1670 -1680

RC                            1640 -1648

# IR spektroskopie

## FT-IR spektroskopie

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

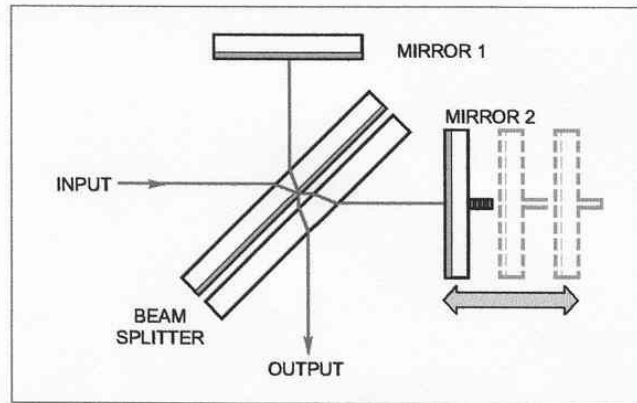


Fig. 1 A Schematic of a generic Michelson interferometer.

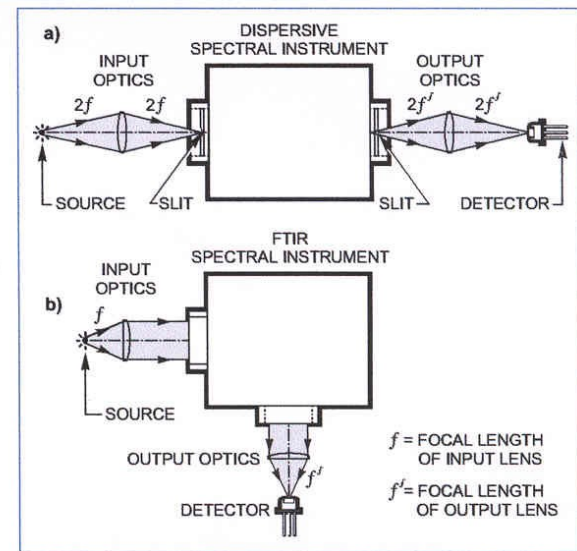


Fig. 5 Diagram of a dispersive spectral instrument and an FT-IR spectral instrument.



# IR spektroskopie

## FT-IR spektra proteinů

CaF<sub>2</sub>, D<sub>2</sub>O, 60 mg/ml, 10 μl

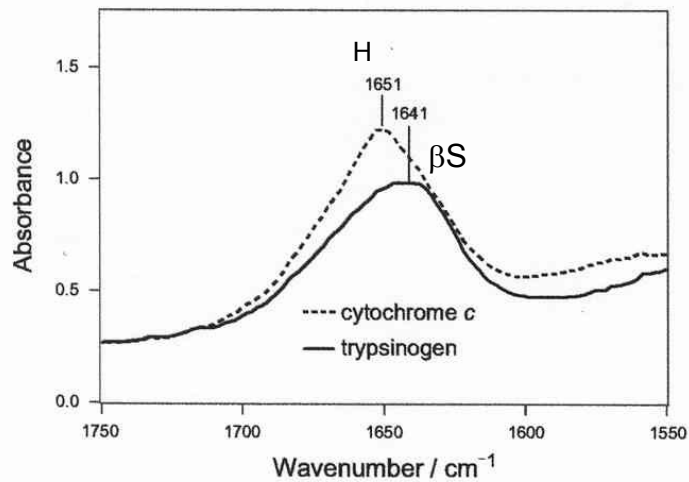


Figure 1. Absorption spectra of ~60 mg/mL solutions of cytochrome *c* and trypsinogen dissolved in D<sub>2</sub>O. Ten microliters of each protein sample was placed between calcium fluoride windows separated by a 15-μm spacer. The data were recorded at 4-cm<sup>-1</sup> resolution, and each spectrum is the result of 50 scans.

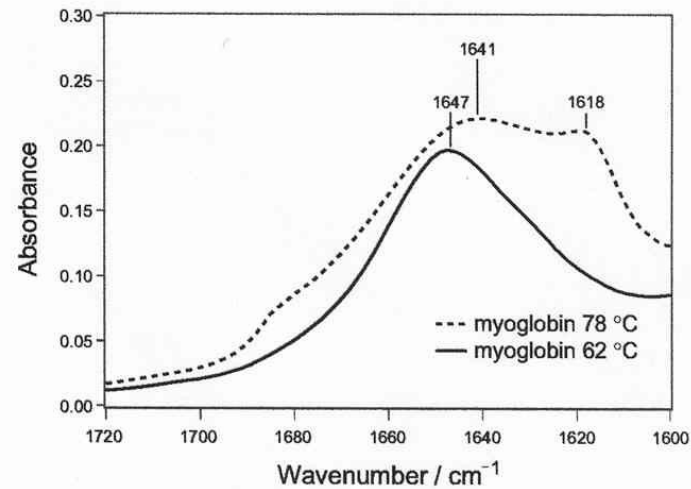
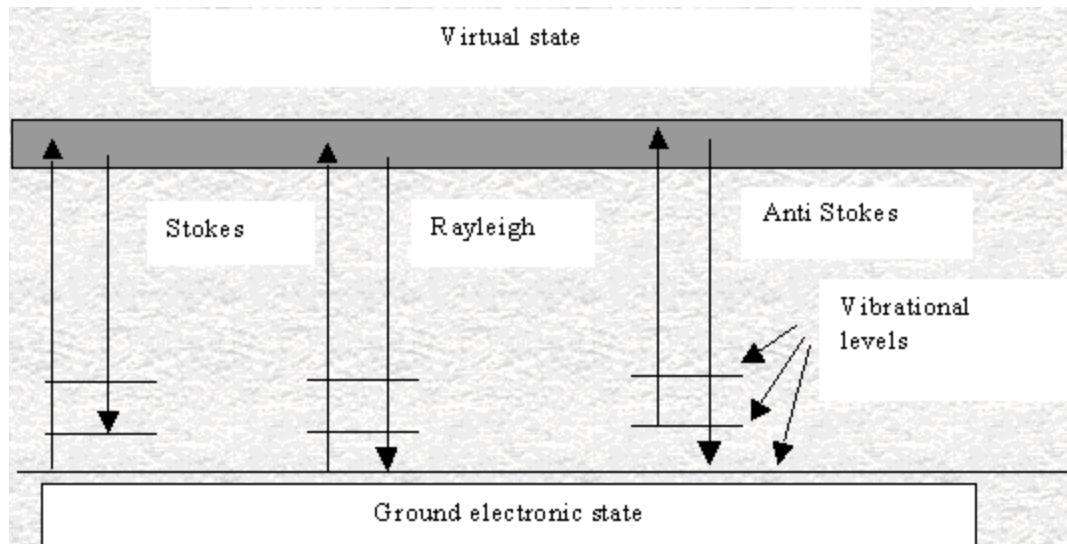


Figure 2. Absorption spectra of ~60 mg/mL myoglobin solutions in D<sub>2</sub>O preincubated at 62 and 78 °C for 15 min. Spectral conditions are identical to those described in Figure 1.

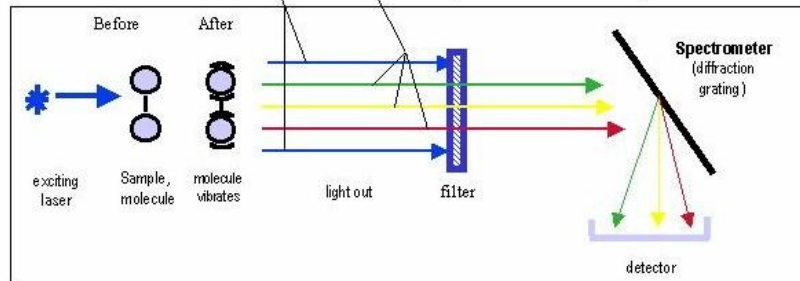
# Ramanova spektroskopie



# Ramanova spektroskopie

## The Principle of Raman Spectroscopy

- The sample is excited with laser light
- elastically scattered light (Rayleigh)
- inelastically scattered light (Raman)
- Filter eliminates intense Rayleigh scattering (typically  $10^8$  times stronger than the Raman scattering)
- Grating disperses the light onto a detector to generate a spectrum

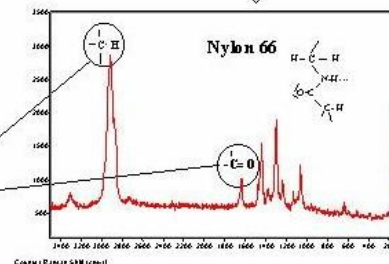


Light loses energy to the molecule vibration

Raman shift:

$$\nu_{\text{laser}} - \nu_{\text{scattered}} = \Delta\nu_{\text{Raman}}$$

The Spectrum gives informations about molecule bondings



**Raman spectroscopy** combined with a **conventional optical microscope** opens the possibility of Raman microstructural investigations on the  $\mu\text{m}$  scale.

# Ramanova spektroskopie

$$\nu_R = \nu_0 - \nu_{\text{vibr}}$$

$$\begin{aligned}\Delta\nu &= \nu_0 - \nu_R \\ &= \nu_0 - (\nu_0 - \nu_{\text{vibr}})\end{aligned}$$

$$\Delta\nu = \nu_{\text{vibr}}$$

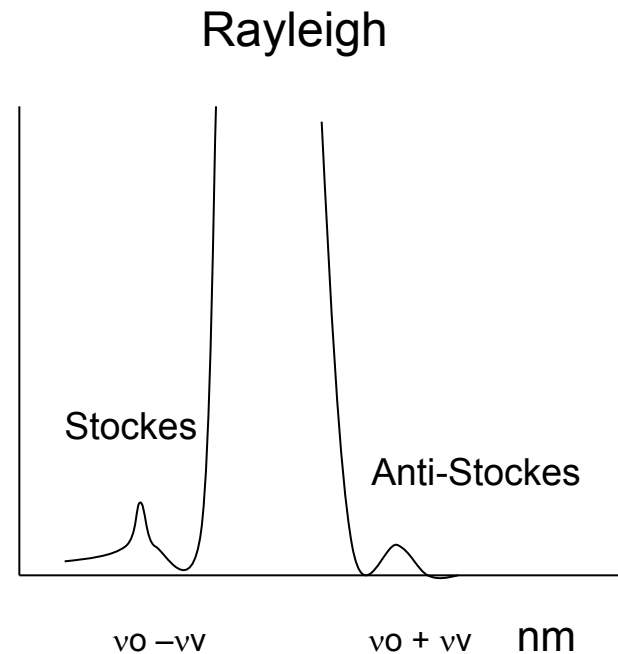
$$\lambda_0 = 555 \text{ nm}$$

$$\nu_0 = 18\,000 \text{ cm}^{-1}$$

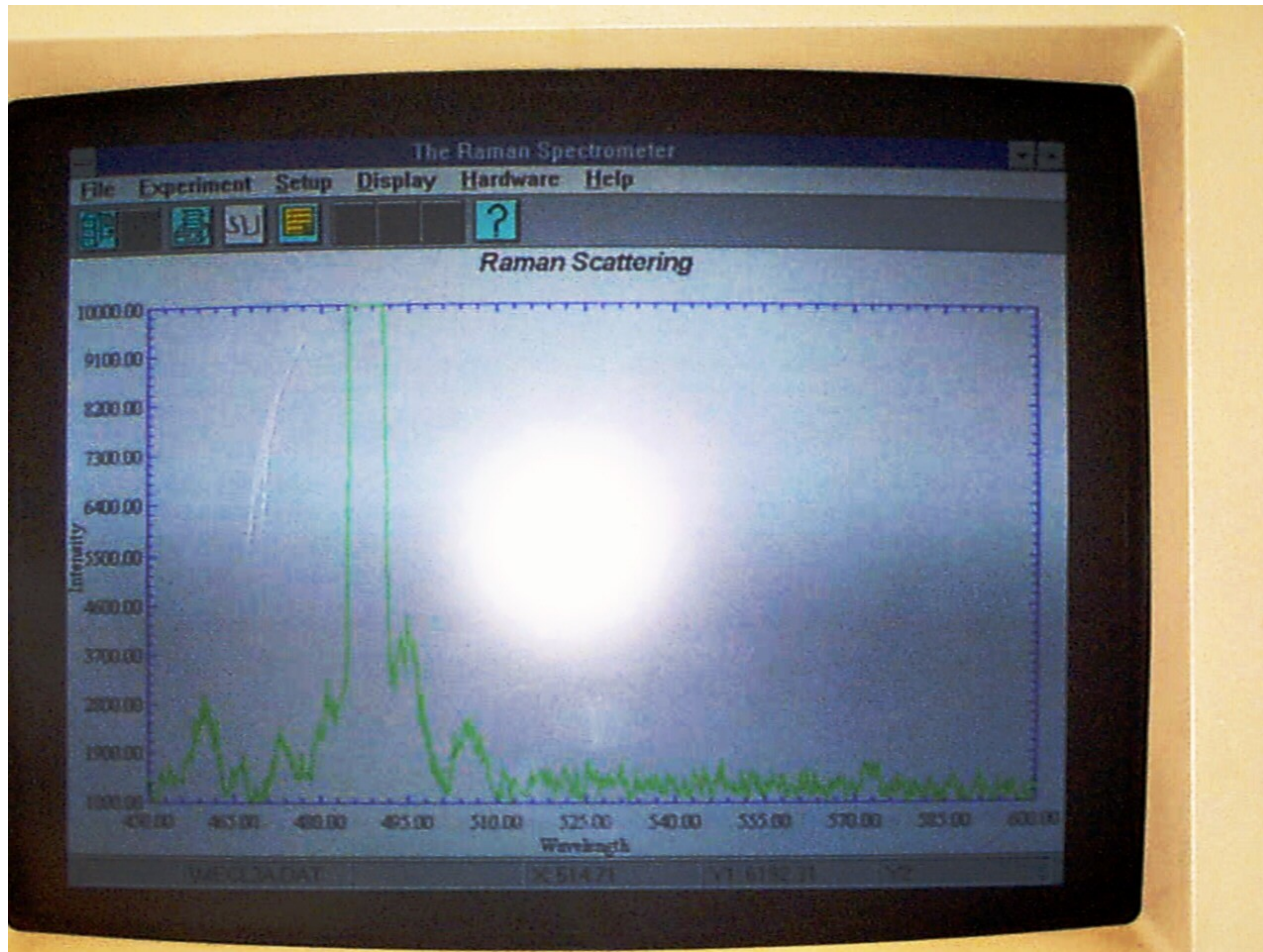
$$\text{Pokud } \nu_{\text{vibr}} = 1600 \text{ cm}^{-1}$$

$$\nu_R = 18\,000 - 1\,600 = 16\,400 \text{ cm}^{-1}$$

$$\lambda_R = 610 \text{ nm}$$



# Ramanova spektroskopie

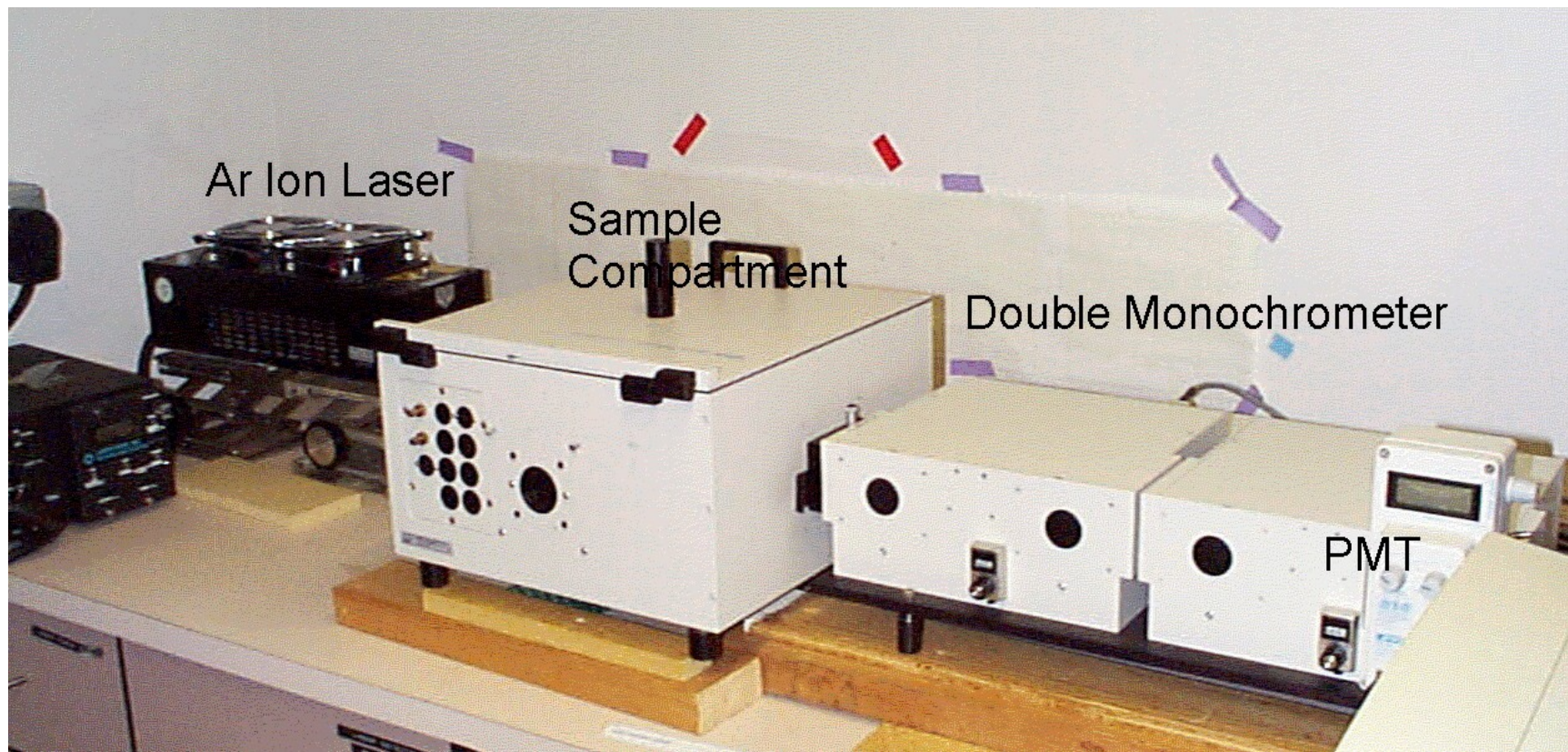


Zdroje: lasery

He-Ne – 633 nm

Ar        488 nm

# Ramanova spektroskopie

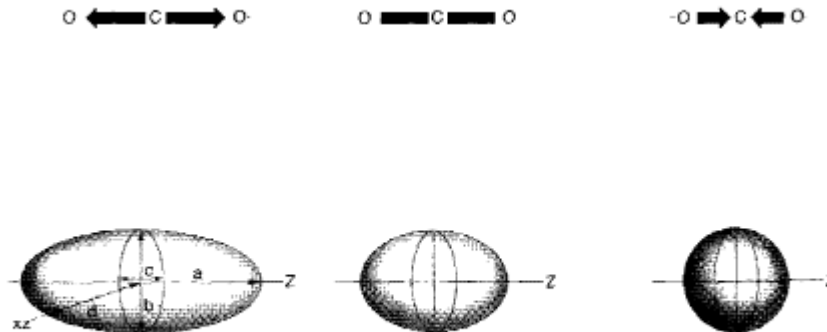


# Ramanova spektroskopie

Polarizovatelnost molekuly a vibrační mody

Viditelnost vibrace v Ramanově spektru vyžaduje změnu **polarizovatelnosti**

Symetrické vibrace CO<sub>2</sub>



# Ramanova spektroskopie

Indukce dipólu P v elektrickém poli E<sub>i</sub>

$$P = \alpha \cdot E_i \quad \alpha - \text{polarizovatelnost}$$

$$E_i = E_0 \cos(2\pi\nu_i t) \quad \nu_i \text{ vlnčet záření}$$
$$\alpha = \alpha_0 + \alpha_v \cos(2\pi\nu_v t) \quad \nu_{\text{vib}} \text{ vibr. frekvence molekuly}$$

$$p = E_0 \alpha_0 \cos(2\pi\nu_i t) + E_0 \alpha_v \cos(2\pi\nu_i t) \cdot \cos(2\pi\nu_v t)$$
$$+ E_0 \alpha_v [\cos 2\pi t(\nu_i + \nu_v) + \cos 2\pi t(\nu_i - \nu_v)]$$

Poměr intenzit Stokesových a anti-Stokesových linií

$$I_A/I_S = \frac{(\nu_i - \nu_v)^4}{(\nu_i + \nu_v)^4} \cdot e^{(-h\nu_v/kT)}$$

Anti-Stokesovy linie jsou mnohem slabší



# Ramanova spektroskopie

	Infrared	Raman
<b>Fyzikální efekt</b>	Absorpce Změna diplového momentu (silné, iontové vazby jako O-H, N-H)	Rozptyl Změna polarizovatelnosti (silné: kovalentní vazby jako C=C, C-S, <u>S-S</u> )
<b>Příprava vzorku</b>	Optimální tloušťka (transmission mode) kontakt vzorku nutný	Bez kontaktu, destrukce jednoduchý příprava voda nebo sklo neruší měření
<b>Komplikace</b>	Silná absorpce skla, vody, CO <sub>2</sub>	Fluorescece
<b>Materiály</b>	Zejména organické látky	Neomezeno
<b>Frekvenční rozsah</b>	4000 - 700 cm <sup>-1</sup>	4000 - 50 cm <sup>-1</sup> (Stokes and Antistokes)

# Ramanova spektroskopie

## **Rezonanční Ramanova spektroskopie**

Elektronové a vibrační přechody – excitace světlem  $E \geq \Delta E = E_{\text{ex}} - E_z$

$\lambda$  světla je kratší než  $\lambda$  odpovídajícího el. přechodu

( $\lambda$  chromoforu 550 nm,  $\lambda$  světla 500 - 400 nm, laditelný laser)

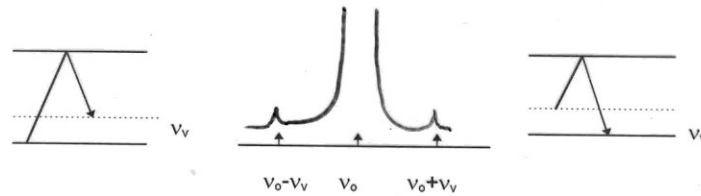
Rezonanční Ramanův efekt – zesílení určitých linií

## **Běžná Ramanova spektroskopie**

Excitace světlem  $E \leq \Delta E = E_{\text{ex}} - E_z$

Např.

$\lambda$  chromoforu 220 nm,  $\lambda$  světla 488 nm,



skupina	$\nu$ (cm <sup>-1</sup> )	skupina	$\nu$ (cm <sup>-1</sup> )
C-C	800-860	C-N	880-930
C=C	1600-1650	C=N	1650
C=C	2100-2250	C=N	2150
C-O	820 - 880	C-H <sub>alif</sub>	2930
C=O	1710-1750	C-H <sub>ar</sub>	3050

### Ramanova spektra bílkovin

#### peptidová vazba

$\nu$  C-N,  $\delta$  N-H  
 1235 ( $\beta$  skládaný list)  
 1275 ( $\alpha$  šroubovice)  
 1235 -1245 široký pás RC

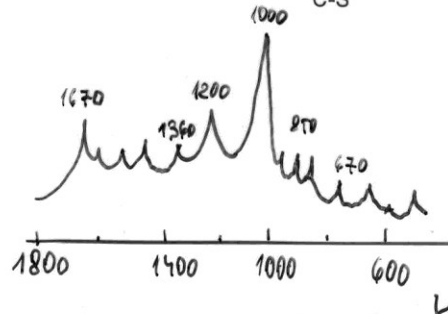
$\nu$  C=O  
 1635 ( $\alpha$  šroubovice)  
 1670 ( $\beta$  skládaný list)  
 1665 široký pás RC

#### aromatické AK

Phe (kruh) dýchání 1000  
 $\delta$  C-H 1203

Tyr  $\delta$  C-H 1210  
 830,850  
 $I_{850}/I_{830}$  0,5 skrytý  
 1,4 v roztoku

Try (kruh) 1361  
 -S-S- 510, 525, 540  
 C-S 630, 670, 700



# Ramanova spektroskopie

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L. Rivas et al. / Journal of Molecular Structure 565–566 (2001) 193–196

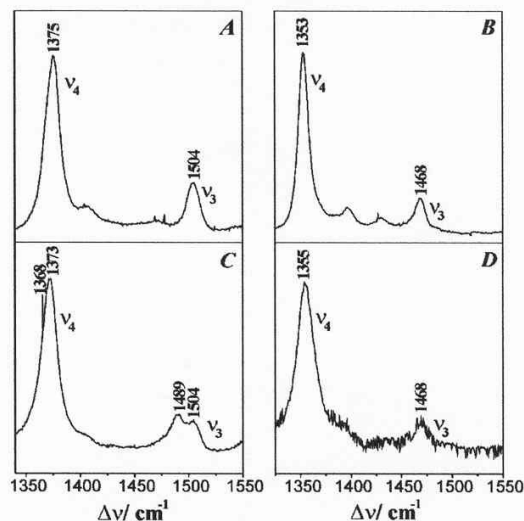
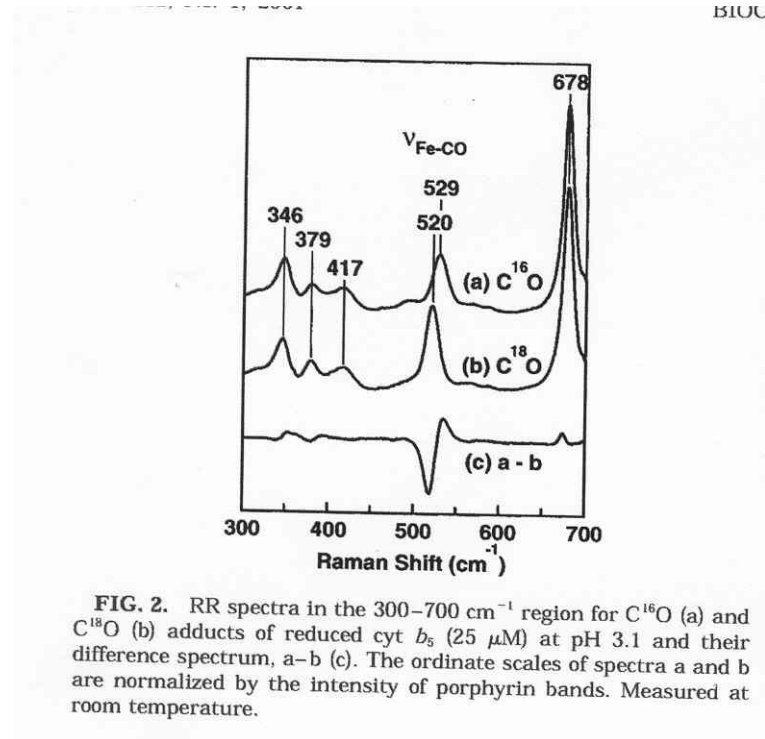


Fig. 1. RR and SERR spectra of Cyt-c<sup>ox</sup> in the marker band region excited at 413 nm. The SERR measurements were carried out after adsorption of the protein at 0.0 V. (A) RR spectra of Cyt-c<sup>ox</sup> in solution; (B) RR spectra of Cyt-c<sup>red</sup> in solution; (C) SERR spectra of Cyt-c<sup>ox</sup> measured at 0.0 V; and (D) SERR spectra of Cyt-c<sup>red</sup> measured at -0.7 V.

Resonanční Ramanova spektra cyt c: 1340 – 1550 cm<sup>-1</sup>: oxidační a vazebný stav hemového Fe. 1375 a 1504 cm<sup>-1</sup> – oxidovaný stav, bis-His hemu (6cLS konfigurace). Redukovaný stav: 1353 – 1468 cm<sup>-1</sup> (5cHS konfigurace)

# Ramanova spektroskopie



Vazba izotopů CO na cytochrom b5.

# Ramanova spektroskopie

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JONES, PATEL, AI

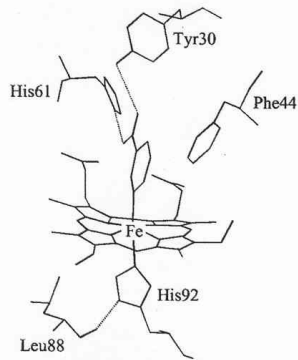


FIG. 1. The active site of the nicotinate derivative of ferric soybean leghemoglobin *a* (22). Hydrogen bonds are indicated with dashed lines.

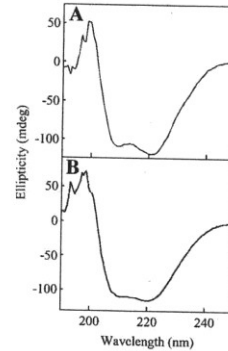


FIG. 3. Circular dichroism spectra of (A) rLb and (B) Leu88Asp in the far-UV region. Conditions: sodium phosphate, pH 7.0,  $\mu = 0.10$  M, 25.0°C.

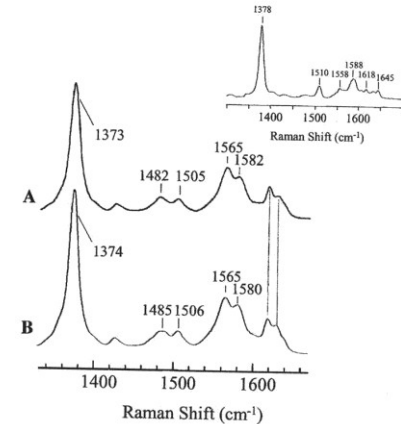


FIG. 4. Room temperature resonance Raman spectra of (A) ferric rLb and (B) Leu88Asp recorded using 413-nm excitation. Inset: Low-temperature (90 K) resonance Raman spectra of ferric Leu88Asp recorded using 413-nm excitation. Conditions: sodium phosphate, pH 7.0,  $\mu = 0.10$  M.

Analýza mutantů Leu88Asp

Prakticky totožná CD spektra

RR: 1482, 1565 – 6 koordinační Fe, s vysokým spinem

1505, 1582 – 6 koordinační, nízký spin

divoký kmen a mutant prakticky totožné

# Ramanova spektroskopie

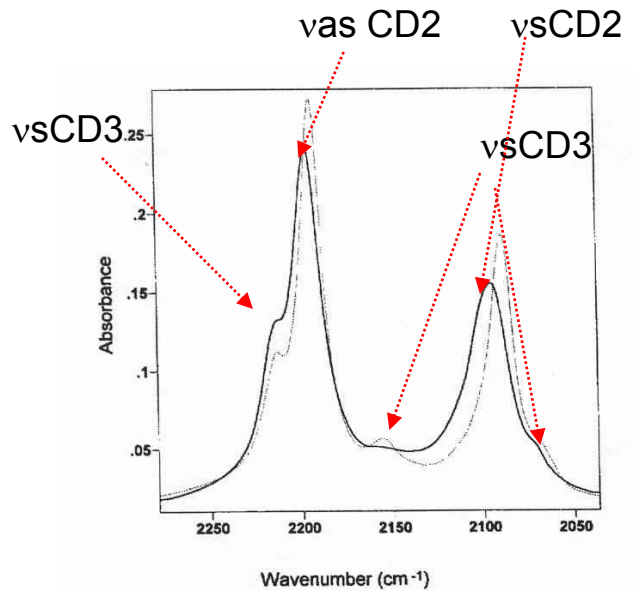


Fig. 1. Infrared spectra of DPPC-d<sub>62</sub> at 22°C (···) and 55°C (—) in aqueous dispersion.

Spektrum deuterovaného DPPC