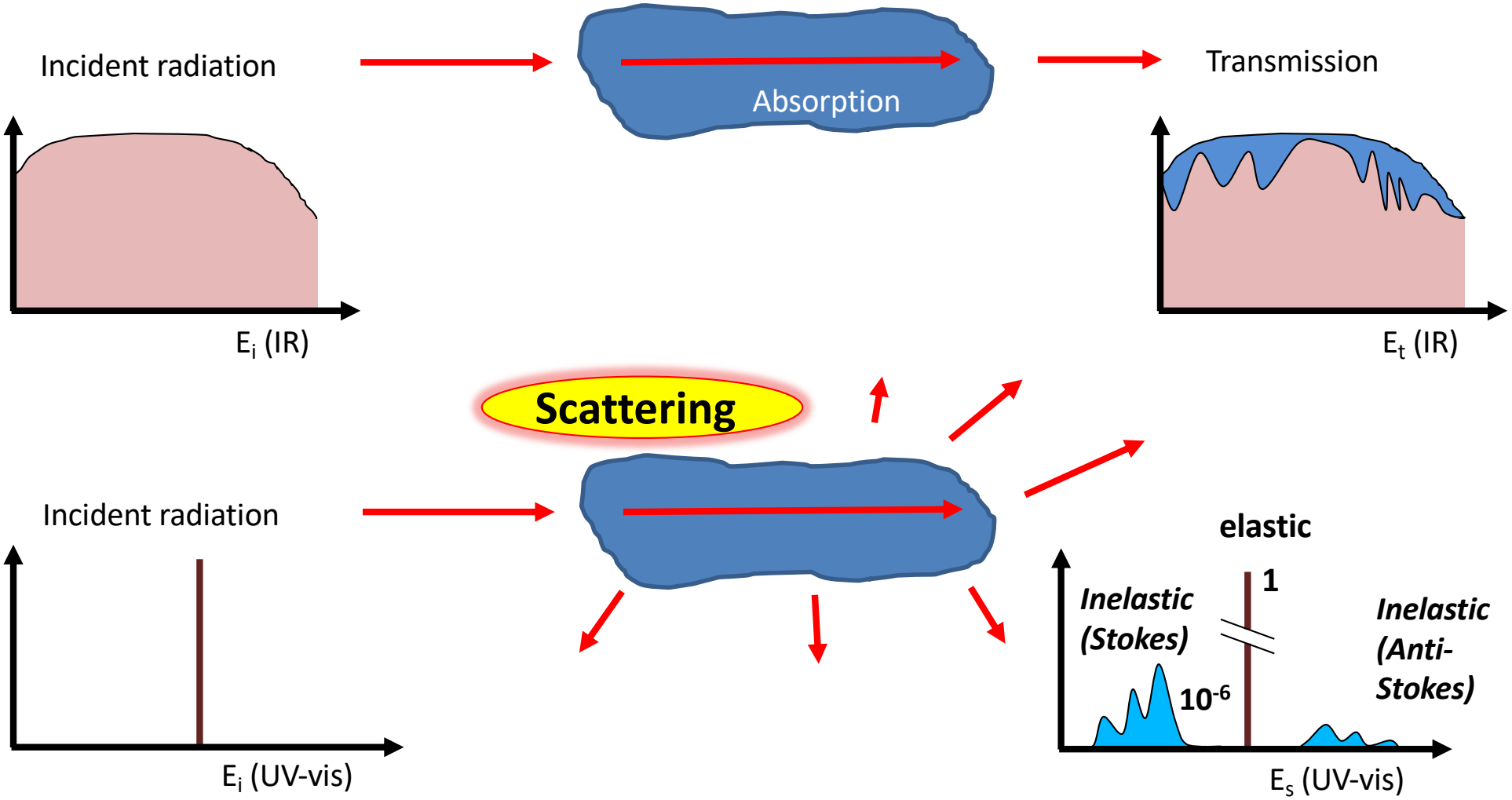
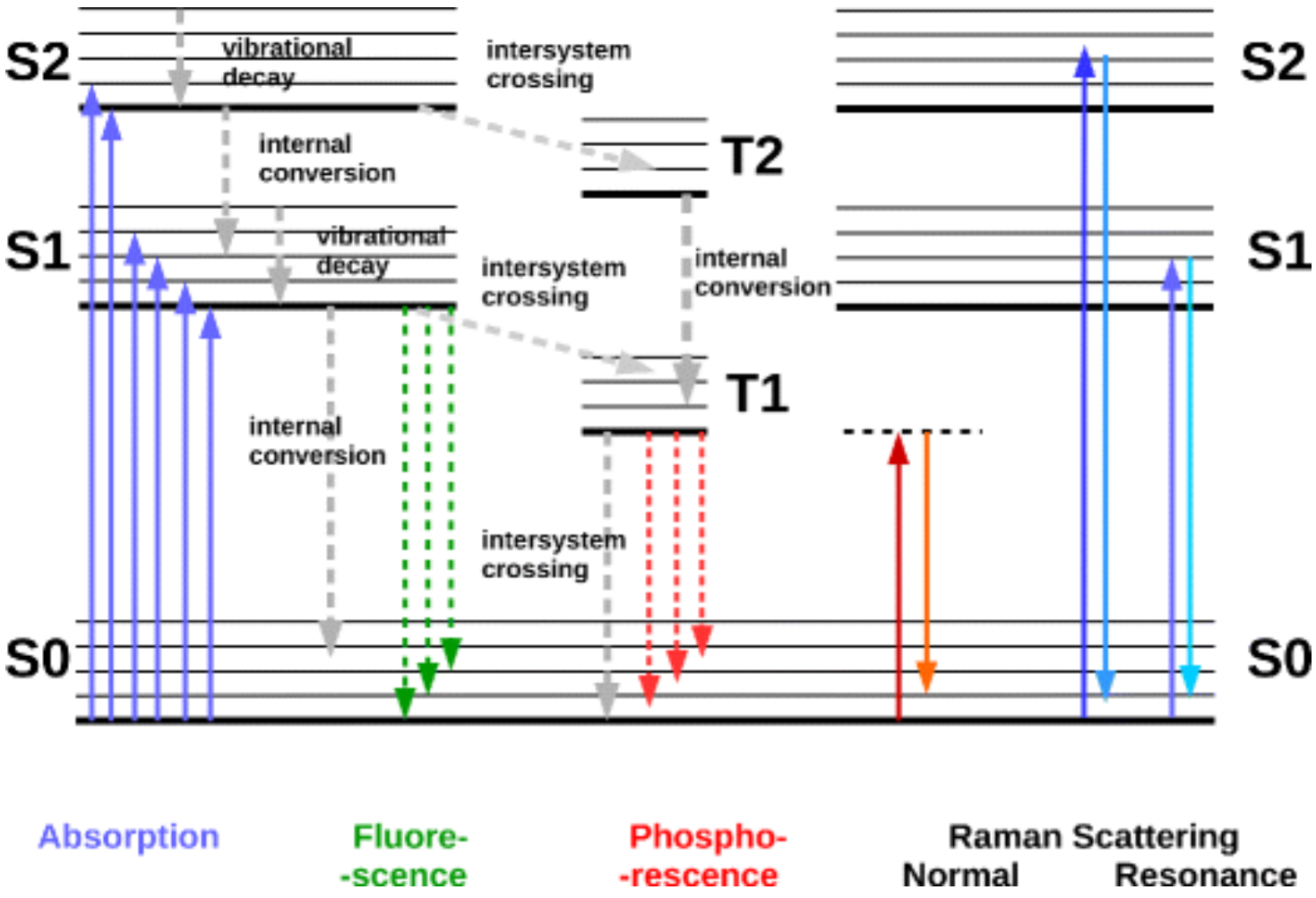
UV-vis **absorption** spectroscopy

Interaction of radiation with matter




S2
vibrational decay
intersystem crossing
internal conversion
T2
S1
vibrational decay
intersystem crossing
internal conversion
T1
internal conversion
intersystem crossing
S0
S2
S1
S0
Absorption
Fluore--scence
Phospho--rescence
Raman Scattering
Normal
Resonance

Absorpion of Light: basics

λ(m)	ν (Hz s^{-1})	E (kJ $mole^{-1}$)	Method
~3	$\sim 10^{8}$	$\sim 10^{-4}$	Radio-frequency NMR
~0.1–0.01	$\sim 10^{9}$–10^{10}	$\sim 10^{-1}$–10^{-2}	Microwave rotational and EPR spectroscopy
$\sim 10^{-4}$–10^{-5}	$\sim 10^{12}$–10^{13}	~1–10	IR vibrational spectroscopy
$\sim 8\times10^{-7}$–4×10^{-7}	$\sim 5\times10^{14}$–10^{15}	~200	Visible electronic spectroscopy
$\sim 10^{-7}$	$\sim 4\times10^{15}$	$\sim 10^{3}$	UV electronic spectroscopy
$\sim 10^{-10}$	$\sim 10^{18}$	$\sim 10^{6}$	X-ray absorption electronic spectroscopy
$\sim 10^{-13}$	$\sim 10^{21}$	$\sim 10^{9}$	γ-ray Mössbauer spectroscopy

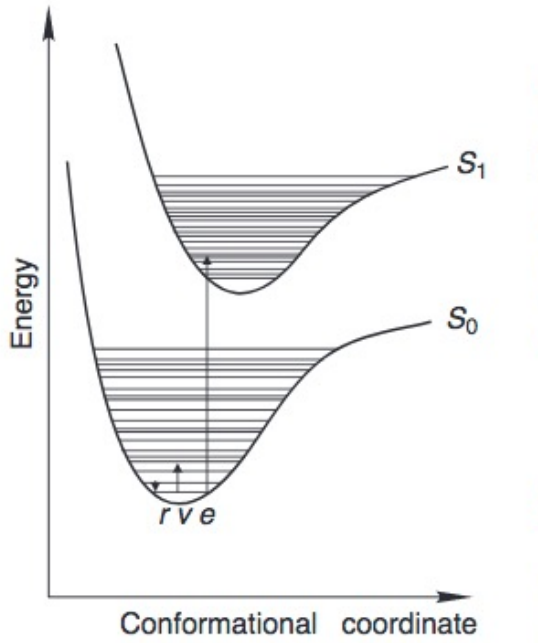
- Near-UV, wavelength 300 nm energies ≈ 4 eV (400 kJ $mole^{-1}$)
- Visible radiation, wavelengths 400 ÷ 800 nm and energies ≈2 eV (200 kJ $mole^{-1}$)

correspond to **transitions between electronic orbital states**, and can be used to probe the **different types of bonds** in molecules.

- IR, wavelengths 10 µm ÷ 100 µm and energies 100 ÷ 10 meV (10 kJ $mole^{-1}$ ÷ 1 kJ $mole^{-1}$)

includes thermal energy at ambient temperature (about 25 meV) **and can be used to study vibrational states and molecular dynamics**

UV-vis spectroscopy



The **UV--visible spectral range** (**transition energies 100-1000 kJ mole^{-1}**) matches the difference between the ground (lowest-energy) and first excited electronic states (S_0 and S_1).

These energies are much greater than thermal energy at ambient temperature (2.5 kJ mole^{-1} at 300 K). Each electronic state is itself split into finer energy levels arising from rotational and vibrational energies of the molecule itself.

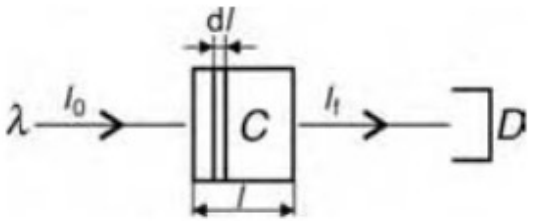
For **small molecules**, the **energy differences between vibrational states are ≈ 40 kJ mole^{-1}**, also larger than ambient thermal energy.

It results from statistical quantum mechanics that all the molecules can be considered as occupying the lowest vibrational energy levels in the electronic ground state

Energy states corresponding to **molecular rotations** are **separated by less than 5 kJ mole^{-1}**, so that many rotational energy levels could be occupied at ambient T.

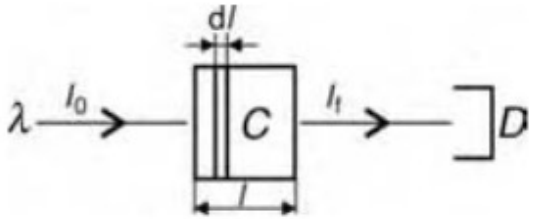
Absorpion of Light

In an absorption spectroscopy experiment, electromagnetic radiation of a given wavelength is observed after it passes through a sample to see how much of it has been absorbed.



The extinction coefficient and absorbance

The intensity of light absorbed, −dI, by the molecules in the thin sample slab of thickness, dl, and unit area is proportional to the number of moles in the slab, Cdl , and to the incident intensity, I:



$$-\mathrm{d}I = C\varepsilon'(\lambda)I\,\mathrm{d}l$$

Where ε', the proportionality constant, depends on the wavelength and on the particular molecular type. It is called the **molar extinction coefficient** and has usual units of (mole litre^{-1})$^{-1}$cm^{-1},or M^{-1} cm^{-1}. Integrating over the sample path length l:

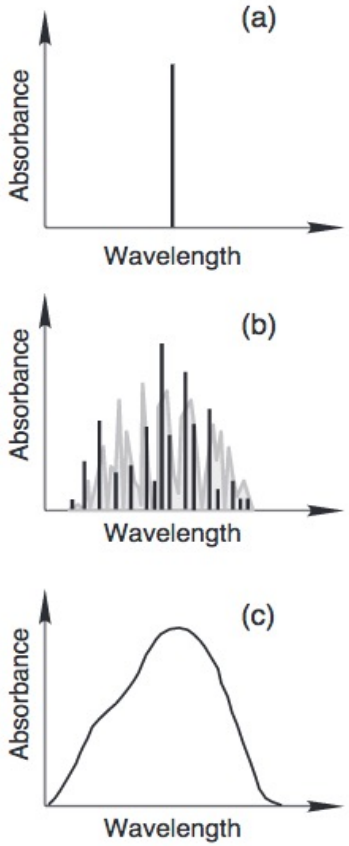
$$\log(I_0/I_\mathrm{f}) = C\varepsilon(\lambda)l \equiv A(\lambda)$$

Beer-Lambert law

Where I_f is the final intensity transmitted by the sample.

A (λ) is called the absorbance or optical density (OD)
Measurable OD: 0.1 ÷ 3 (3 means 1/1000 of the intensity is transmitted)

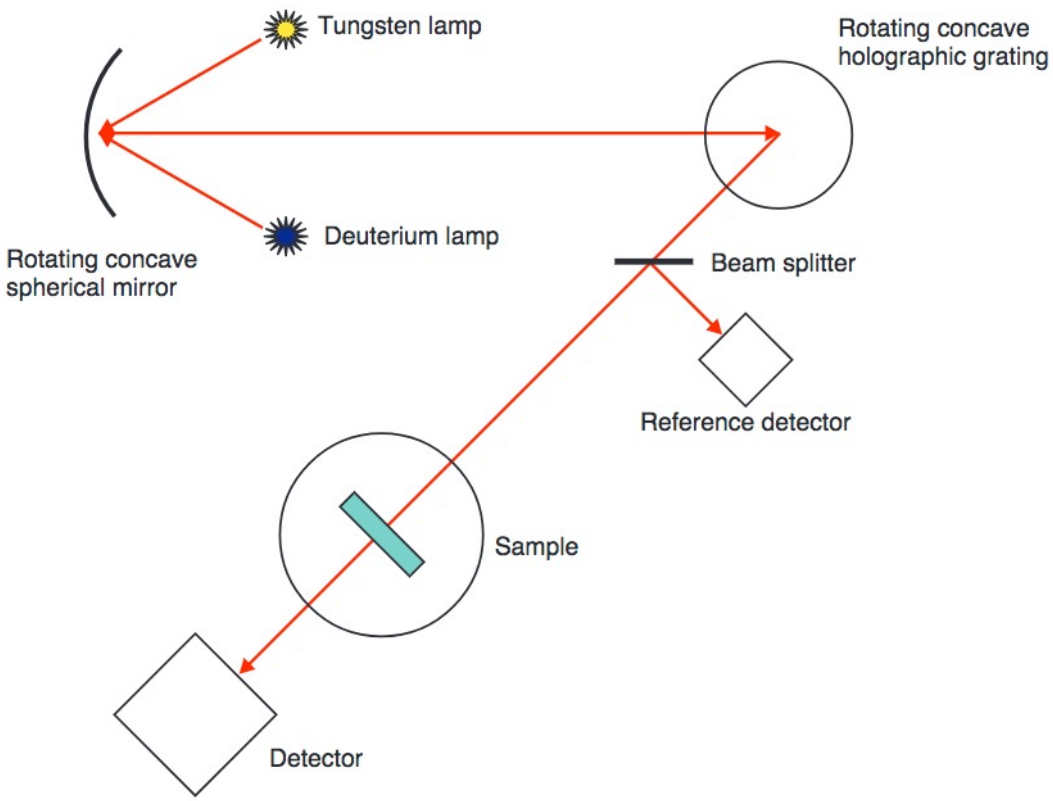
UV-vis spectroscopy



Absorption spectrum of a molecule with one type of electronic transition:
(a) theoretical, at very low temperature;
(b) theoretical at ambient temperature;
(c) Observed (broadening due to an inhomogeneous local molecular environment or coupling with solvent if the molecule is in solution).

In principle, absorption shape and broadening contains info on the vibrational/rotational states and can be used to follow protein molecular dynamics.

Spectrophotometer



A beam splitter diverts part of the beam to a reference detector for continuous calibration.
In a single-beam instrument sample solution and solvent absorbance are measured sequentially; the solvent A (λ) curve is stored on the instrument's computer and automatically subtracted from the sample solution curve.

In double-beam spectrophotometer the ratio of light intensity on two different optical paths (e.g. through a sample solution cell and through a cell containing solvent alone) is measured simultaneously.

UV absorption spectra of proteins

In proteins, **only a few amino acid residues strongly absorb UV light**, mainly in the **200–300 nm** range. These residues contain **aromatic or conjugated groups**, which are responsible for UV absorption.

Tryptophan (Trp, W)

- **Strongest UV absorber** ($\varepsilon \approx 5500\ M^{-1}cm^{-1}$ at 280 nm)
- Peak: **≈280 nm**
- Dominates protein UV spectra when present

Tyrosine (Tyr, Y)

- Moderate absorption
- Peak: **≈274–276 nm**

Phenylalanine (Phe, F)

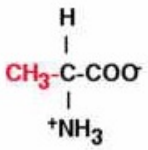
- Weakest aromatic absorber
- Peak: **≈257–260 nm**

The 20 amino acids:

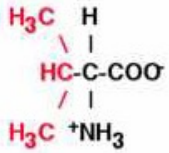
hydrophobic

hydrophilic

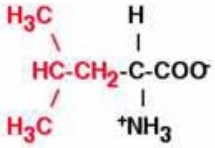
aromatic



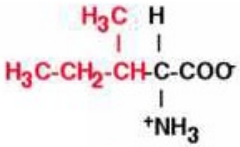
alanine (Ala)



valine (Val)

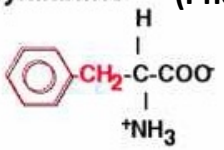


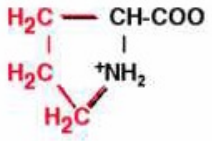
leucine (Leu)



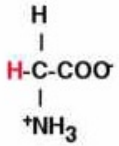
isoleucine (Ile)

phenylalanine (Phe)

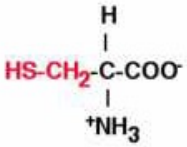




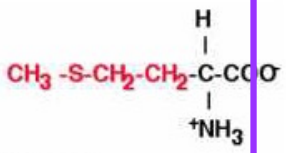
proline (Pro)



glycine (Gly)

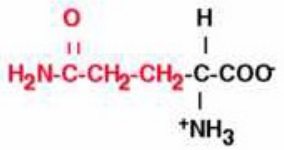


cysteine (Cys)

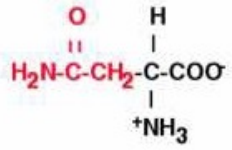


methionine (Met)

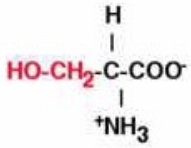
tryptophan (Trp)



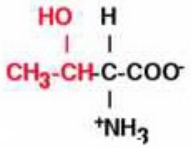
glutamine (Gln)



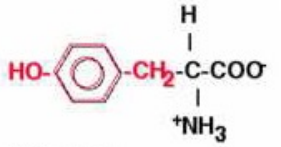
asparagine (Asn)



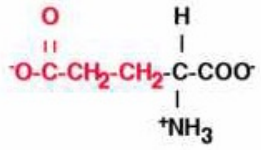
serine (Ser)



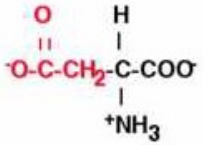
threonine (Thr)



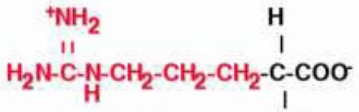
tyrosine (Tyr)



(Glu) glutamic acid

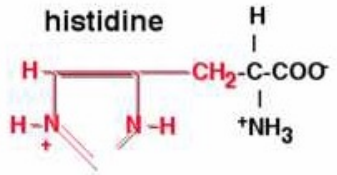


(Asp) aspartic acid

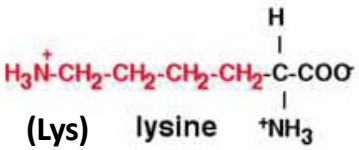


arginine (Arg)

histidine

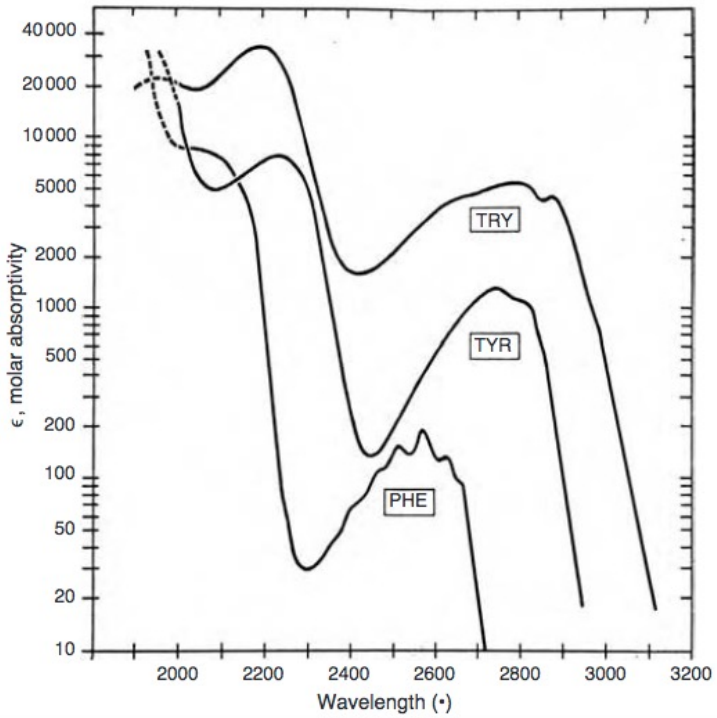


(His)



(Lys) lysine

UV absorption spectra of proteins



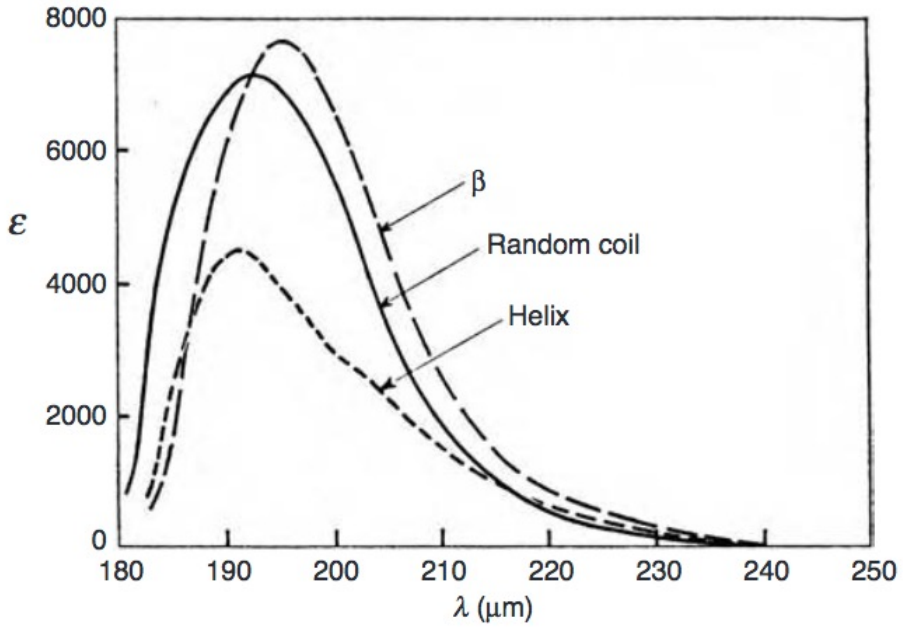
Fine structure can be seen in the curve maxima (it is especially clear in the spectrum for Phe) resulting from electronic transitions to different vibrational states. These features become sharper at low temperatures, at which broadening due to coupling with rotational modes is reduced.

π-π^*

Histidine, tryptophan, tyrosine and phenylalanine side-chains (aromatics) present extinction coefficients greater than 5000 in the peptide absorption region (190--210 nm). Above 230 nm, the strongest individual amino acid contribution to absorption in proteins is from tryptophan, followed by tyrosine, then phenylalanine and cysteine.

UV absorption spectra of proteins

In proteins, **only a few amino acid residues strongly absorb UV light**, mainly in the **200–300 nm** range. These residues contain **aromatic or conjugated groups**, which are responsible for UV absorption.



Colour in a protein is always due to a prosthetic group that absorbs in the visible region of the spectrum.

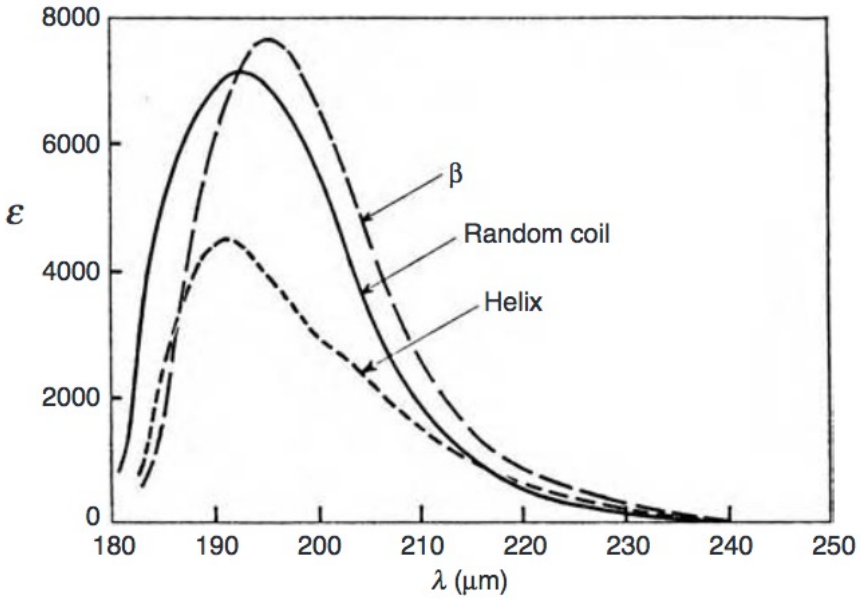
Even proteins lacking aromatics still absorb UV strongly in the **far-UV range** due to **π → π*** transitions in the peptide bond.

- Peak: **≈190–200 nm**
- Used for **secondary structure analysis** by CD or UV absorbance

To summarize:

- Electronic bands in the **peptide group** (at **170--220 nm**)
- **aromatic amino acid side-chains** close to **280 nm**
- **prosthetic groups, cofactors, enzyme substrates or inhibitors** (in the **full UV--visible range** depending on the group)

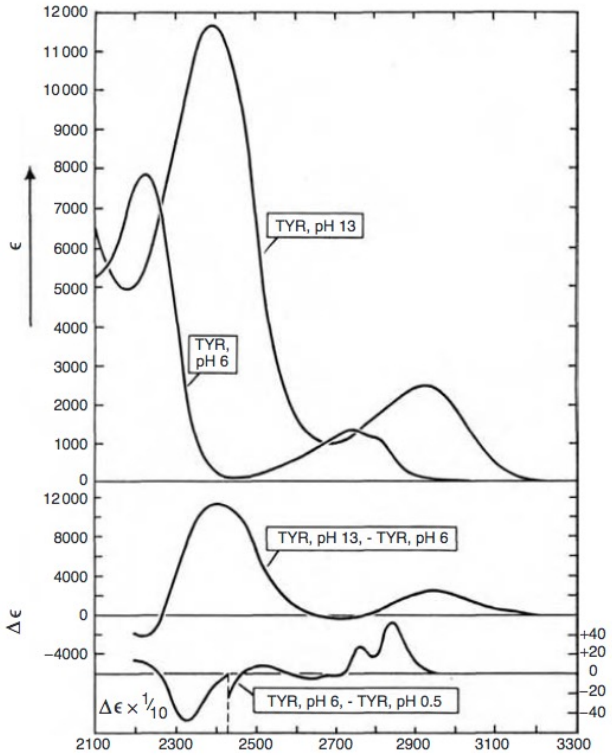
UV absorption spectra of proteins



attempts have been made to estimate the α-helix content in proteins from measured molar extinction values at 190 nm by interpolating between the random coil and helix values. Such measurements are not entirely satisfactory, however, because of the low reliability of the assumptions involved with respect to the effects of other than helix and random coil conformations, as well as with respect to corrections for amino acid side-chain contributions

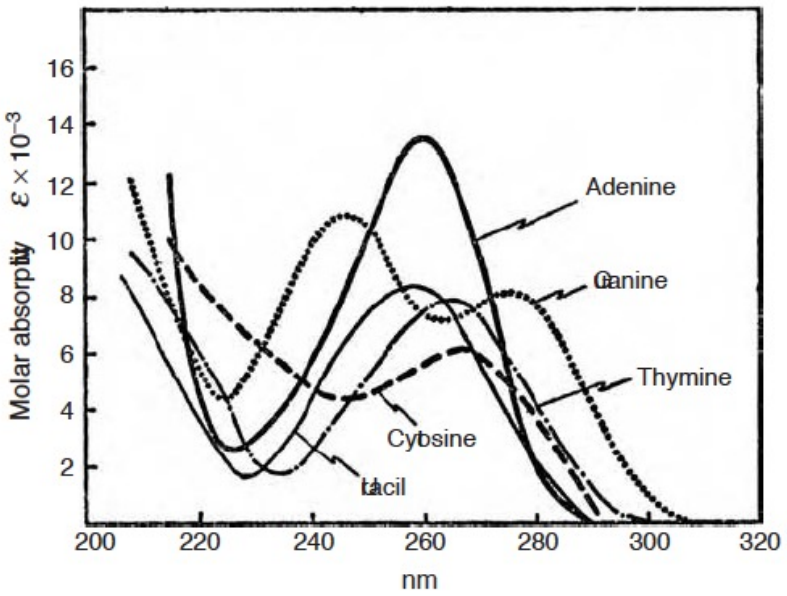
- π-π*, n-π*, σ-σ*, and n-σ* transition involved, π-π* beaing the strongest ones

UV absorption spectra of proteins



The UV absorption curve of Tyr depends on its various ionisation states and
is especially sensitive to pH. Between pH 6 and pH 13, the peak maximum in the
near-UV, at 274.6 nm, shifts to higher wavelengths by about 20 nm and increases in intensity by almost a factor of 2.
This sensitivity has been used in the accurate titration of Tyr residues in proteins as well as in the separate determination of Tyr and Trp contributions to an observed absorption spectrum

UV absorption: DNA



Base	pH	λ_{max}(nm)
Adenine	1	262.5
Adenine	7	260.5
Adenine	12	269
Cytosine	1	276
Cytosine	7	267
Cytosine	14	282
Guanine	1	248, 276
Guanine	7	246, 276
Guanine	11	274
Thymine	4	264.5
Thymine	7	264.5
Thymine	12	291
Uracyl	4	259.5
Uracil	7	259.5
Uracil	12	284

[a]Data taken from Sober (1997).

The electronic transitions of the **sugar and phosphate moieties of a nucleotide are well beyond 200 nm** in the UV and the **aromatic bases dominate nucleic acid absorption at more easily observable wavelengths**. Chemical derivatives of purine and pyrimidine bases exhibit strong absorption in the **260--290 nm** spectral range, through the electronic states of their conjugated double-bond structures. The purine and pyrimidine bases in aqueous solution have distinct absorption spectra, which vary characteristically with pH.

The maxima all occur close to 260 nm, and the minima close to 230 nm.

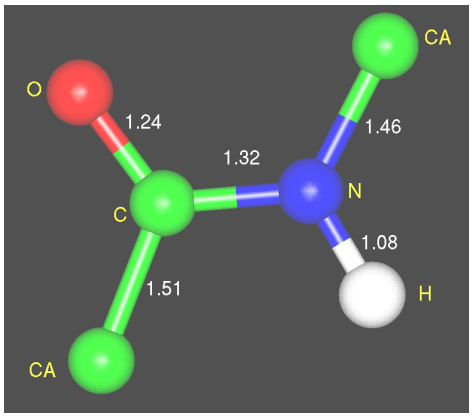
A 260 nm absorption in a protein solution is a clear sign of DNA contamination.

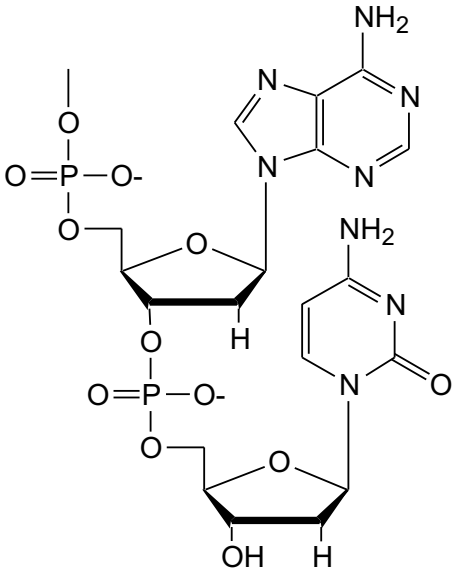
IR absorption spectroscopy

Absorpion of Light: Infrared (IR)

IR absorption excites the vibrational states in molecules. IR spectroscopy is one of the classical tools for the study of the structures and interactions of small molecules.

At first, it appeared to be too ambitious to apply this technique to biological macromolecules, because of their enormous number of vibrational modes. It was thought that the resulting overlap in absorption bands would not allow the extraction of detailed structural information from the IR spectra. **Biological macromolecules, however, exhibit an intrinsic order of repeating units**: the peptide bond in the protein backbone, the phosphate ester bond between the 3-hydroxyl group on the sugar residue of one nucleotide and the 5-phosphate group of the next nucleotide in nucleic acids and the two-dimensional array of a limited class of molecules in lipid membranes.





Absorpion of Light: Infrared (IR)

IR spectra of biological macromolecules are then simpler than at first expected and detailed band analysis provides useful information on macromolecular structure and interactions.

In the case of **proteins**, **linear IR spectroscopy** provides insights into **secondary structure.**
For **nucleic acids**, information can be obtained on the **overall structure and interactions with small molecules** such as intercalating drugs or metal ions.

The **IR difference spectra** formed between different states of an enzyme, for example, only contain bands of those groups that undergo changes during the transition from one state to the other.

In parallel experiments, **time-resolved IR spectroscopy** provides information on the **reaction kinetics**.

IR absorption spectroscopy

The IR region of the electromagnetic spectrum encompasses the wavelength range from 0.78 to 1000 μm.

In IR spectroscopy, the frequency of a band is usually expressed in terms of wave numbers in units of cm^{-1} (inverse of the wavelength).

Table E1.3. *IR spectral regions*

Region	Wavelength range, μm	Wave number range, cm^{-1}	Frequency range, Hz
Near	0.78–2.5	12 800–4000	$3.8\times10^{14} - 1.2\times10^{12}$
Middle	2.5–50	4000–200	$1.2\times10^{14} - 6.0\times10^{12}$
Far	50–1000	200–10	$6.0\times10^{12} - 3.0\times10^{11}$
Most used	2.5–15	4000–670	$1.2\times10^{14} - 2.0\times10^{13}$

IR absorption spectroscopy

Measurements in the ***near- IR*** region are made with a spectrophotometer similar in design and components to the instruments described for UV--visible spectrometry.

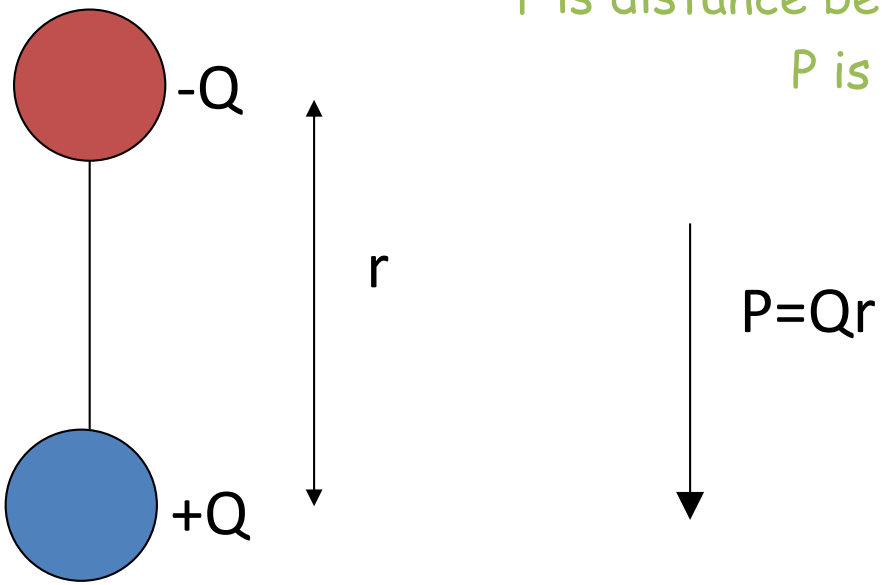
Until the early 1980s, instruments for the ***mid-IR*** region were largely of the dispersive type, based on diffraction gratings. Since that time, however, there has been a dramatic change in mid-IR instrumentation. Current instruments are of a Fourier transform type and the method is called **Fourier transform IR (FTIR) spectroscopy.**

In the past, the ***far-IR*** region of the spectrum has had limited use because of significant experimental difficulties. The few available sources of this type of radiation are notoriously weak and are further attenuated by the need for order-sorting filters that must be used to prevent radiation of higher grating orders from reaching the detector. FTIR spectrometers, with their much higher throughput, have largely overcome this problem and made the far-IR spectral region much more accessible to chemists and biologists

IR absorption spectroscopy

In a **classical mechanics** description, the interaction of an electromagnetic wave with matter is treated in terms of induced dipoles.
Depending on its frequency and on how strongly the charges are maintained in place (the polarisability), it causes positive and negative charges to oscillate with opposite phase in the electric field plane ➔**induction of dipoles with an oscillating dipole moment** of certain defined energies and in certain defined directions with respect to the beam, because of the properties of the atoms and bonding patterns involved.

Q is charge
r is distance between the charges
P is dipole



IR and water: Attenuated total reflection (ATR)

IR transmission measurements of biological molecules are often impeded by the**intense absorption of water near 1650 and 3300 cm^{-1} bands**. For this reason most transmission measurements must be made on **very thin samples (10 µm)** in **D_2O, or under partially dehydrated conditions**.

Attenuated total reflection (ATR) avoids many problems associated with transmission measurements by limiting the effective sample thickness to a thin layer near the surface of internal reflection element (IRE). At each point of internal reflection there is an **evanescent electromagnetic wave that penetrates through surface** into the medium of lower refractive index.

In the case of a 3 mm thick by 50mm long IRE crystal, it is routine to obtain 10--20 reflections. The actual penetration depth is described by Eq:

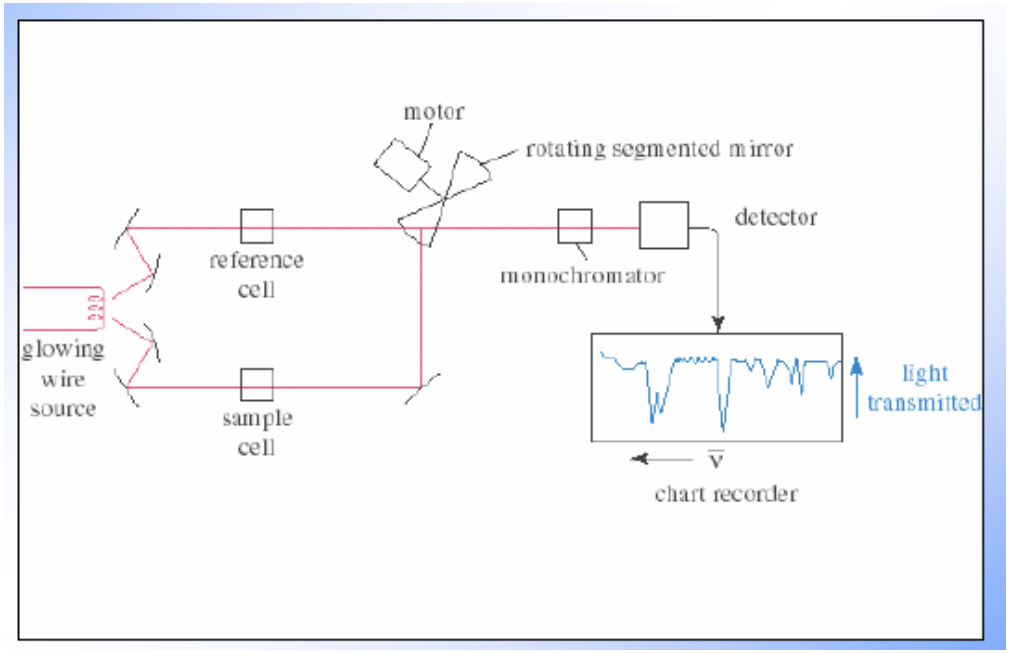
$$d_p = \frac{\lambda_0 / n_1}{4\pi \sqrt{\sin^2 \theta - (n_2/n_1)}}$$

For the case of crystalline germanium surrounded by an aqueous medium, this equation gives a penetration depth of∼5 µm at 1000 cm−1.

Thus ATR measurements solve the problem of thickness of the sample and create appropriate conditions for the transmission measurement of biological samples (i.e. biomembranes).

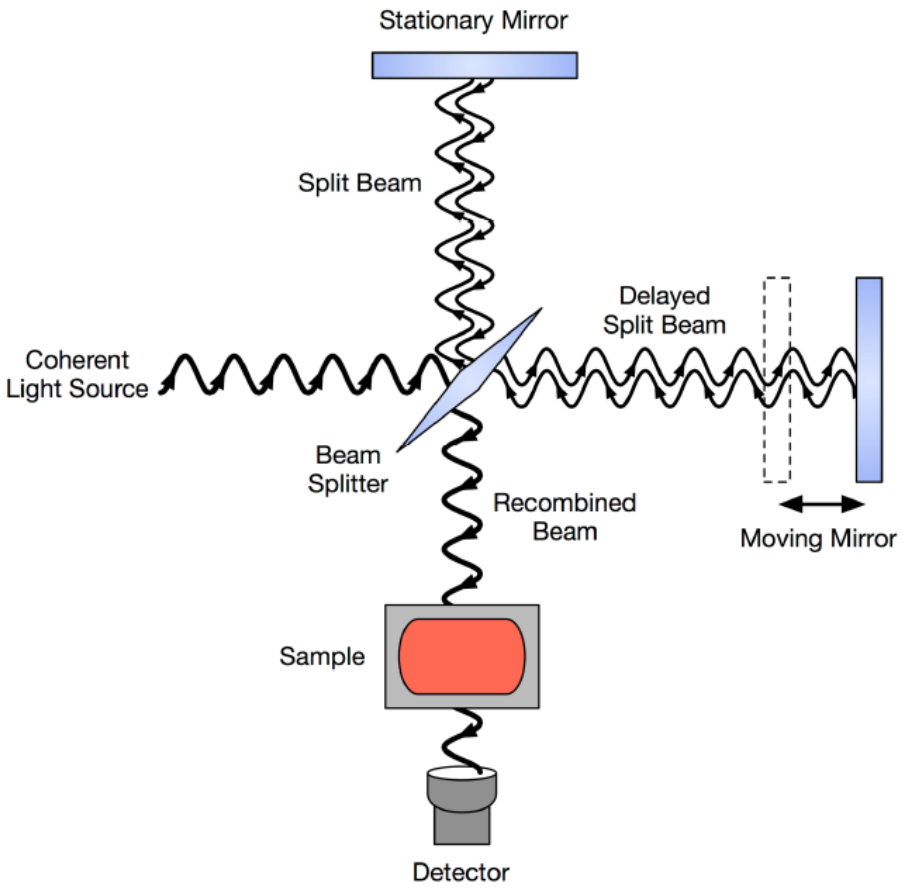
Fourier transform infrared (FTIR) spectrometers

All IR spectrometers include a broad-band source (usually an incandescent ceramic material), optics for collimating the IR light and directing it through a sample, an IR detector, and some means for analysing the wavelength dependence of transmitted light. In conventional dispersive spectrometers, this last function is accomplished by spatially spreading the light with a diffraction grating, and selecting individual spectral elements with a narrow slit. Only a narrow wavelength range reaches the sample and detector at any time. The collection of a spectrum covering a broad wavelength range requires sequential scanning over different spectral elements.



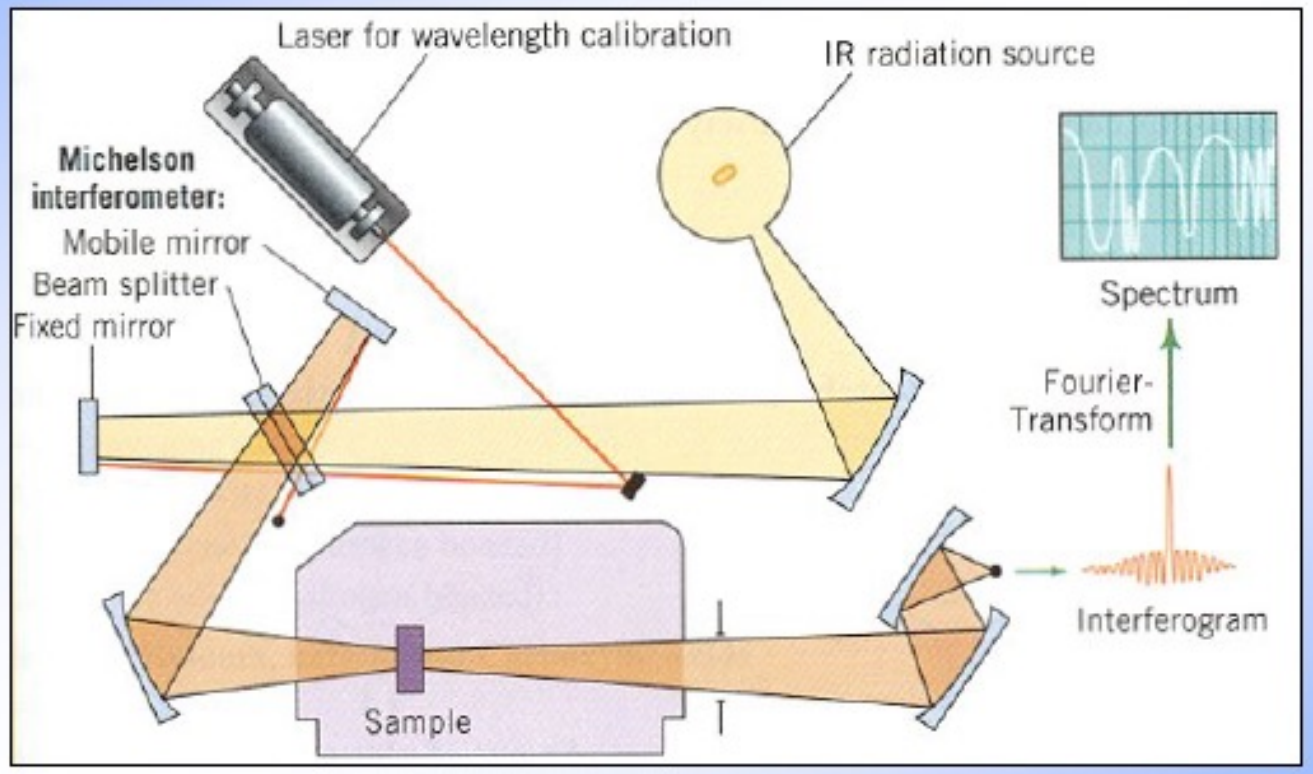
Fourier transform infrared (FTIR) spectrometers

An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time.



In FTIR spectrometers the broad-band IR beam passes through an interferometer before sending it through the sample and then to a single very sensitive detector .
In this case, the intensity of each wavelength component varies as a cosine function of the optical path length difference, L, from a beam splitter to fixed and moving mirrors; the frequency of each cosine function is the reciprocal of the associated IR wavelength (i.e. the wave number **ν**). The single-beam spectrum (the detected IR intensity as a function of **ν**) is obtained from a Fourier transform of the interferogram, which is the detected intensity as a function of L. Most modern IR spectrometers are Fourier transform instruments.

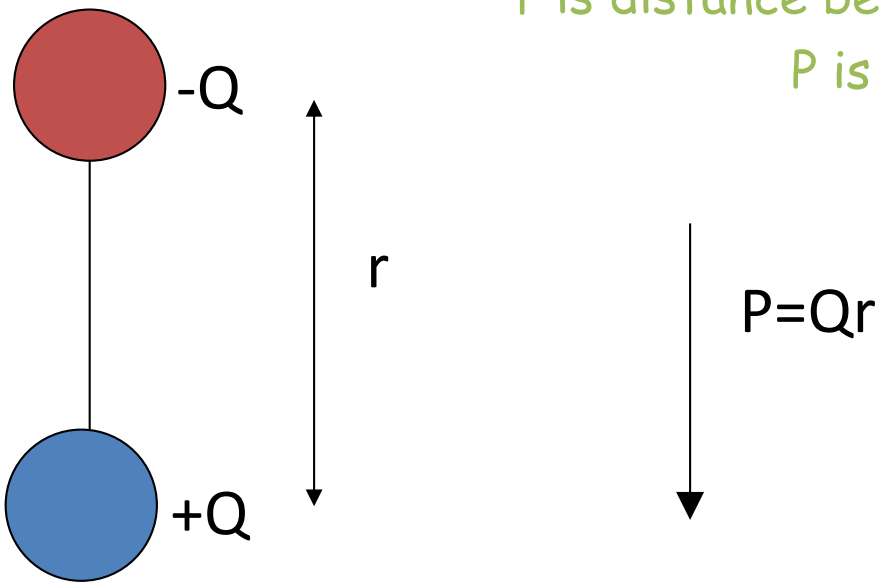
FTIR


Laser for wavelength calibration
IR radiation source
Michelson interferometer:
Mobile mirror
Beam splitter
Fixed mirror
Spectrum
Fourier-Transform
Interferogram
Sample

IR absorption spectroscopy

In a **classical mechanics** description, the interaction of an electromagnetic wave with matter is treated in terms of induced dipoles.
Depending on its frequency and on how strongly the charges are maintained in place (the polarisability), it causes positive and negative charges to oscillate with opposite phase in the electric field plane ➔**induction of dipoles with an oscillating dipole moment** of certain defined energies and in certain defined directions with respect to the beam, because of the properties of the atoms and bonding patterns involved.

Q is charge
r is distance between the charges
P is dipole

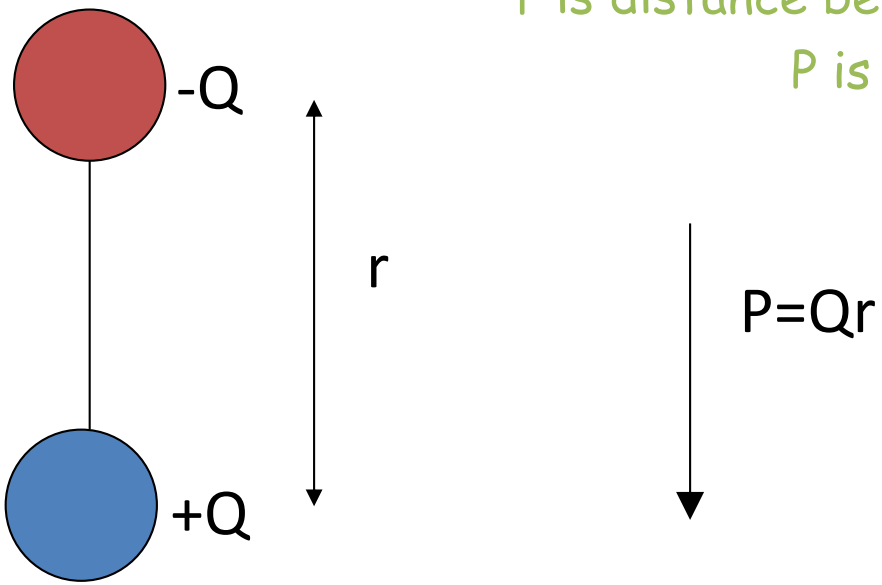


IR absorption spectroscopy

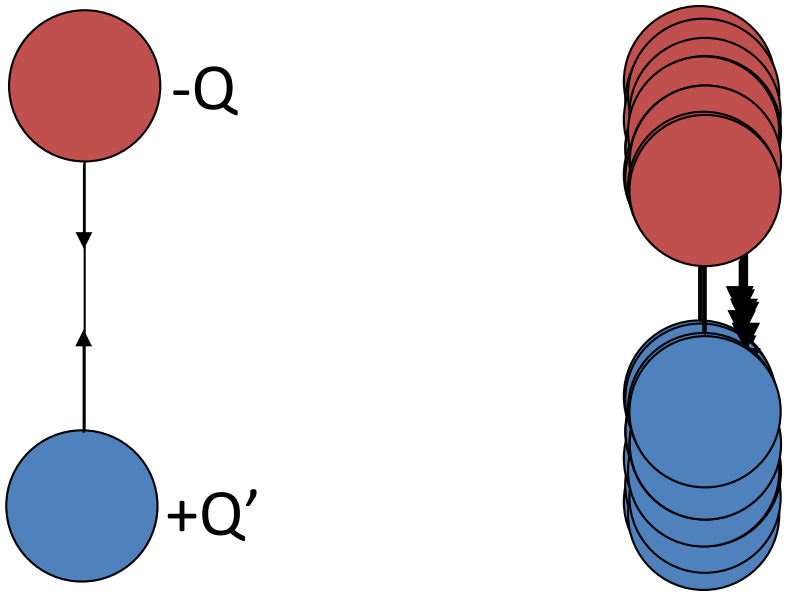
Which of the following molecules are dipoles:

O_2, CO, N_2, NO?

Q is charge
r is distance between the charges
P is dipole



What is a Dynamic Dipole



Separated charge distribution that is moving

A **permanent dipole moment** is associated with heteronuclear diatomic molecule (or its chemical bond), because the electronegative values of the bonded atoms are different so that there is a separation between the centres of negative and positive charge.

If the bond vibrates with the normal mode frequency, ν_m, then the dipole moment oscillates at the same frequency. According to classical electromagnetic theory, an oscillating dipole absorbs electromagnetic radiation of the same frequency as that of its oscillation.

A **normal mode of** vibration that gives rise to an oscillating dipole in the IR spectral range is said to be ***IR-active.***

Conversely, a **normal mode of vibration that does not give rise to an oscillating dipole moment** (e.g. the stretching of a homonuclear diatomic molecule) cannot lead to IR absorption and is said to be ***IR- inactive.***

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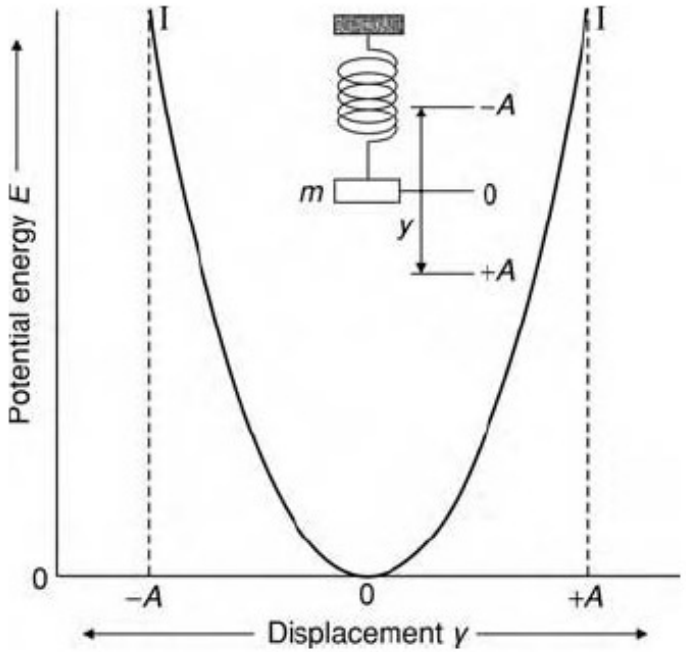


Potential energy

Harmonic oscillator (1 mass):

$$F = -ky = m\frac{d^2y}{dt^2}$$

$$y = A\cos\omega_m t$$

$$\omega_m = \sqrt{k/m}$$

$$dE = -F\,dy \qquad E = \tfrac{1}{2}ky^2$$

Total energy, exchanged between Ekin e Ep

$$E_{total} = \tfrac{1}{2}kA^2$$

$$m_{1,2} = \frac{m_1 m_2}{m_1 + m_2} \qquad \omega_m = \sqrt{\frac{k}{m_{1,2}}} = \sqrt{\frac{k(m_1 + m_2)}{m_1 m_2}}$$

Fig. A3.11 Two masses connected by a spring of force constant, *k*.

The energy for each vibration is dependent upon the mass and spring constant, IR absorption spectroscopy is chemically specific.

$$E = \left(n + \frac{1}{2}\right)\frac{h}{2\pi}\sqrt{\frac{k}{m_{1,2}}} \qquad m_{1,2} = \frac{m_1 m_2}{m_1 + m_2}$$

n= vibrational quantum number (integer)

Transitions between vibrational energy levels can be brought about by the absorption of radiation, only if the energy of the radiation exactly matches the difference in energy between levels and certain selection rules are obeyed

$$\Delta E = h\nu_m = \frac{h}{2\pi}\sqrt{\frac{k}{m_{1,2}}}$$

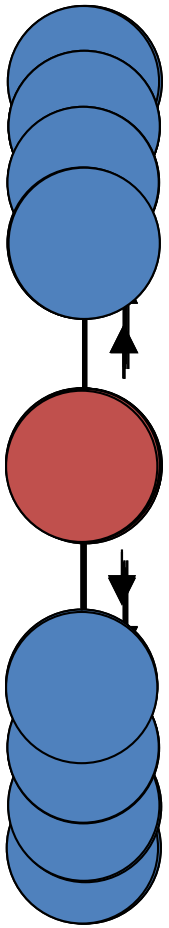
where ν_m can be seen as a fundamental frequency associated with the set of vibrational modes. In IR spectroscopy, the frequency of a band is usually expressed in terms of wave numbers

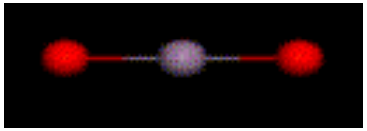
$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{k}{m_{1,2}}} = 5.3 \times 10^{-12}\sqrt{\frac{k}{m_{1,2}}}$$

where $\bar{\nu}$ is the wave number of an absorption peak in cm^{-1}

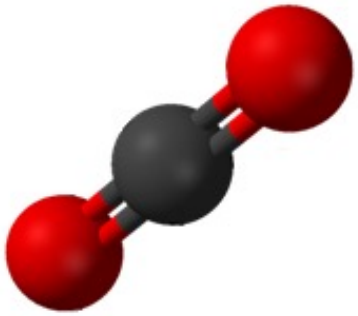
Selection rule: $\Delta n = \pm 1$
(indeed anarmonicity (e.g.: coulomb repulsion) induces the presence of overtones)

CO_2 Vibrations: Stretching Modes



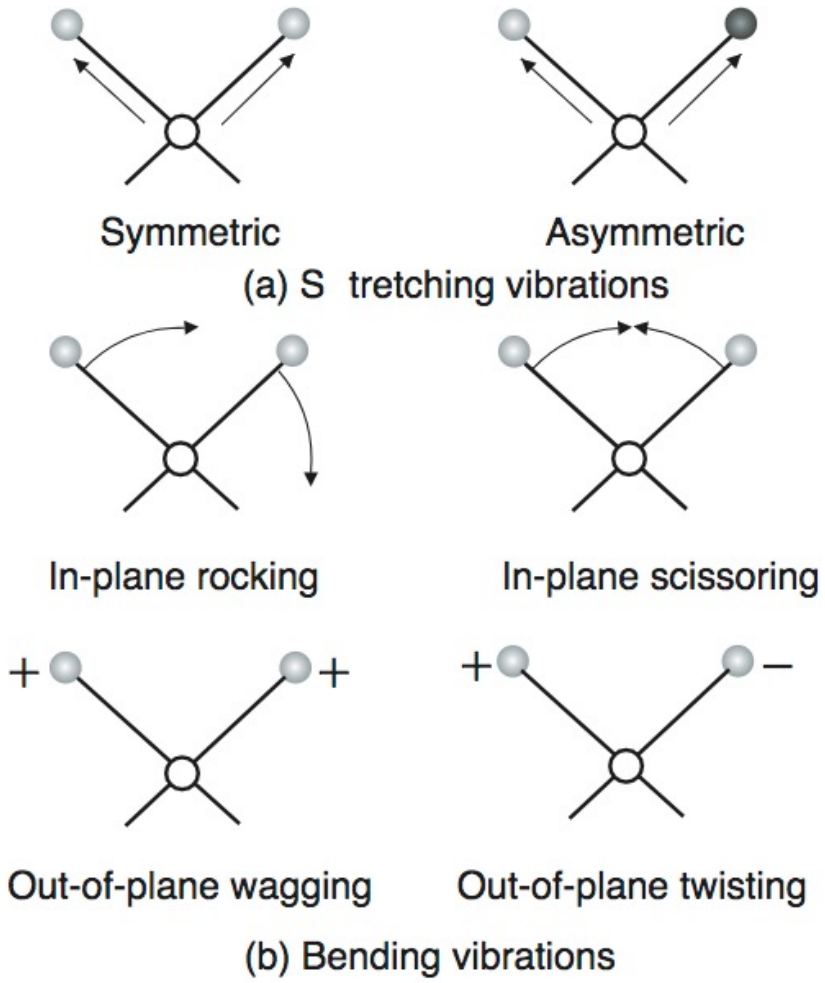


CO_2 IR spectrum



four normal modes of vibration (3N-5 for linear mol; 3N-6 for any mol.).
Symmetry considerations dictate that two of these are bond stretching modes, and two are valence angle bending modes.
One stretching mode is symmetrical (simultaneous extension or the simultaneous contraction of each oxygen--carbon double bond). This mode is **IR-inactive**, no molecular dipole moment change accompanies the in-phase displacements of the two oxygen atoms with respect to the central carbon.
The other stretching mode is antisymmetrical; the carbon-oxygen bonds vibrate out of phase: linearly displacing the central carbon atom between two fixed oxygen atoms. This mode **is IR-active**, associated with a large dipole moment fluctuation

3 nuclei vibration modes



continuous change in interatomic distance along the axis of the bond

change in the angle between two bonds

Such an analysis becomes difficult if not impossible for molecules made up of more atoms. Not only do they contain a large number of vibrating centres, but also interactions can occur amongst them and must be taken into account.

Molecule with N atoms: 3N-6 normal modes

All these modes are highly localised—meaning that, to a good approximation, each involves only a small group of atoms moving as if they were isolated from the vibrations of other molecular groups.

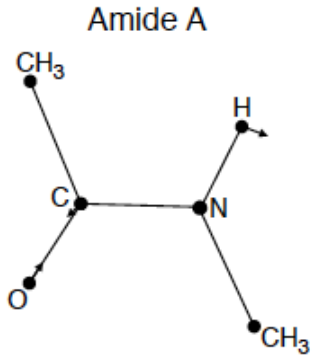
Consider the case of the **amide I mode of an α-helical polypeptide chain** of K residues. To a first approximation, each peptide—COOH group in the α -helix will have the same vibrational frequency, resulting in the appearance of a single amide I band, rather than k distinct bands.

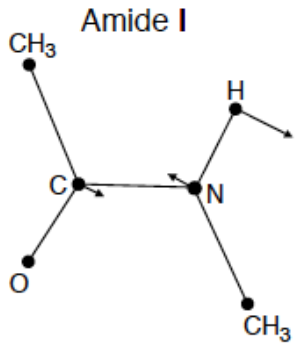
In general, if the N atoms of the protein are distributed among K sets of identical groups each of n atoms, and if each such group exhibits the same or nearly the same conformation and environment in the macromolecule, then the collection of **3nk- 6 hypothetical spectral bands is reduced to 3n−6**.

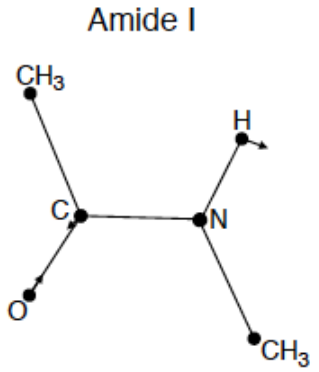
The vibrational frequencies of polyatomic molecules are determined, in principle, by the masses and geometrical arrangement of their constituent atoms and the interatomic forces resulting from the distortion of the equilibrium configuration. The frequencies and atomic displacements can be calculated by using empirical, classical mechanics, potential energy functions (rigorously up to 10 atoms only…).

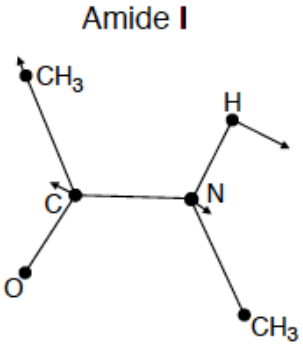
AMIDES

In-plane modes	
Amide A	~3300 cm^{-1}
Amide B	~3100 cm^{-1}
Amide I	1597–1672 cm^{-1}
Amide II	1480-1575 cm^{-1}
Amide III	1229–1301 cm^{-1}
Amide IV	625–767 cm^{-1}
Out-of-plane modes	
Amide V	640–800 cm^{-1}
Amide VI	537–606 cm^{-1}
Amide VII	~200 cm^{-1}









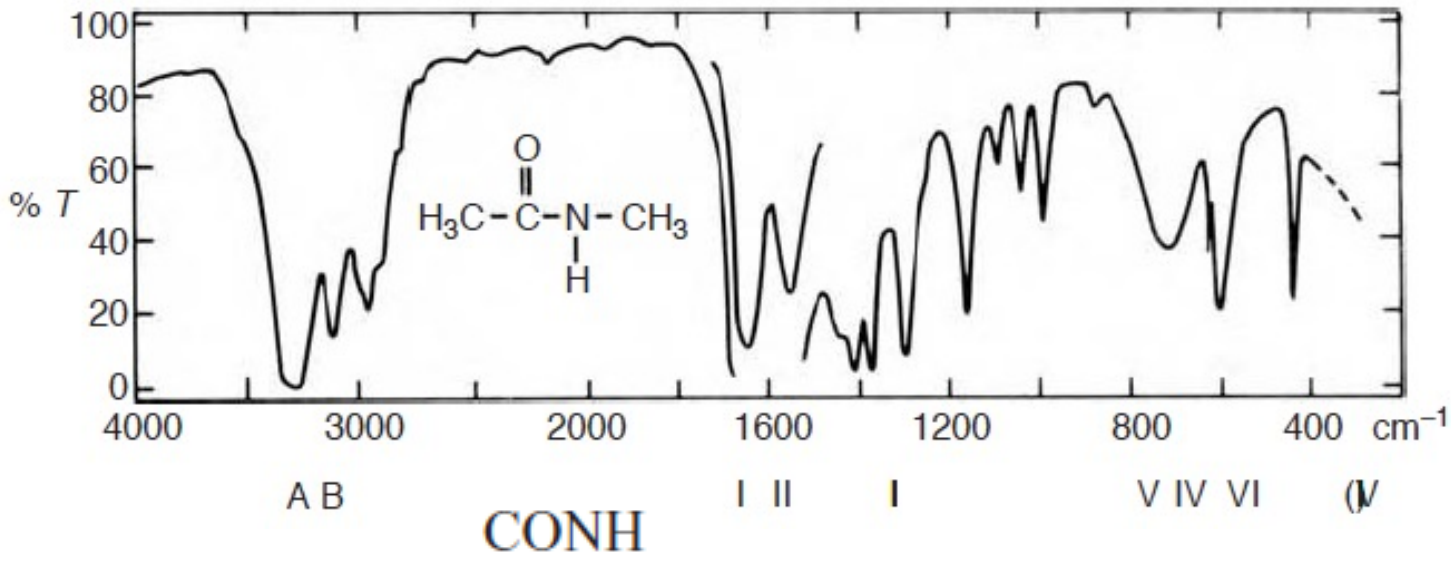
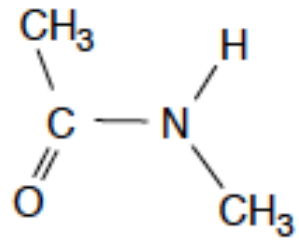
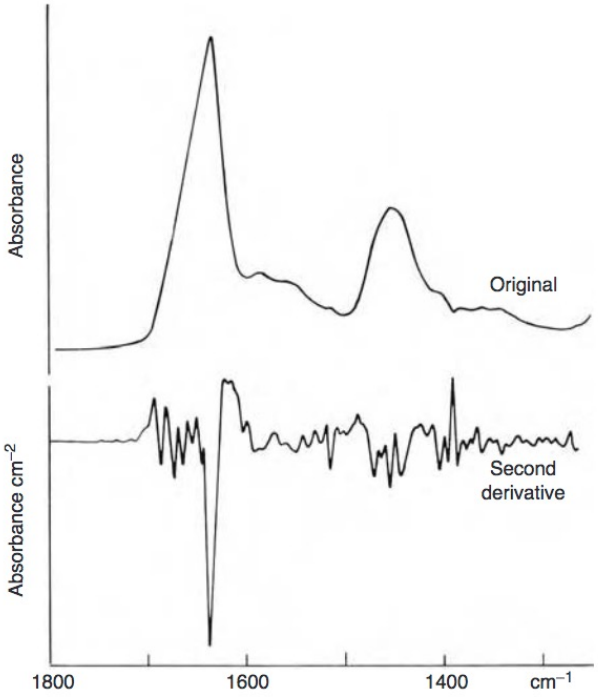


Fig. E1.23 Characteristic amide bands as exhibited by a capillary film of N-methylacetamide. The amide VII band is not shown.



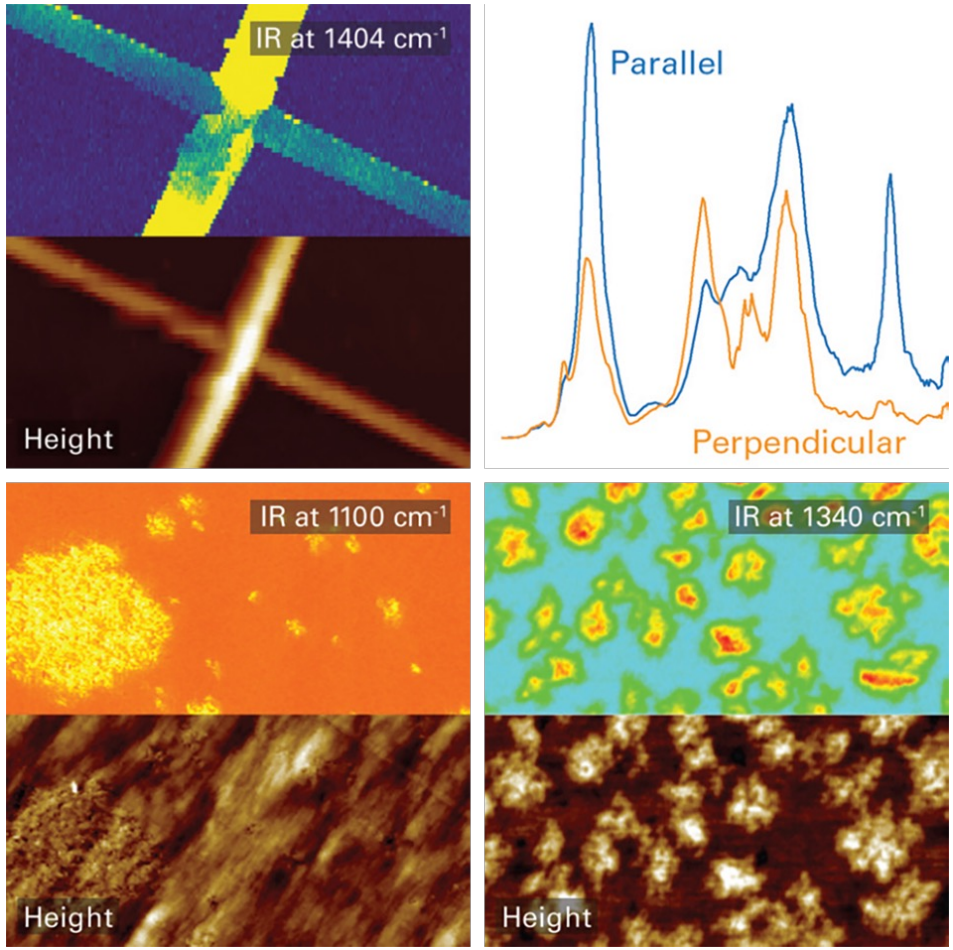
Second derivative spectra

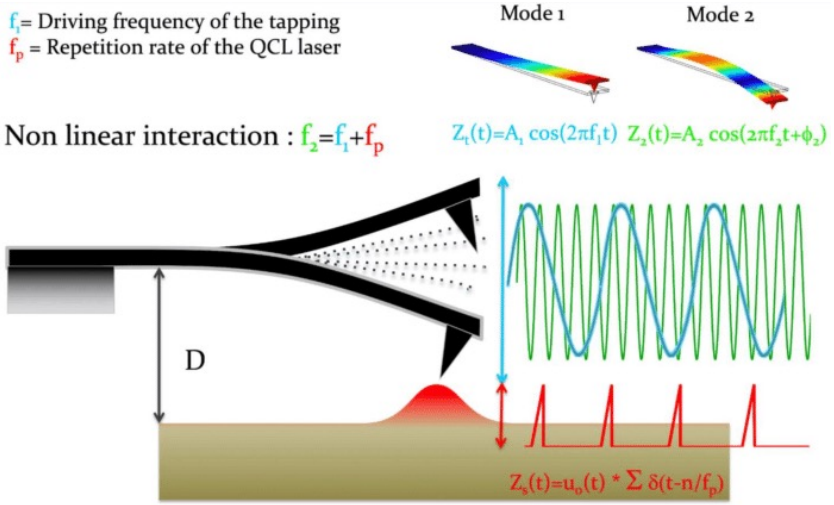


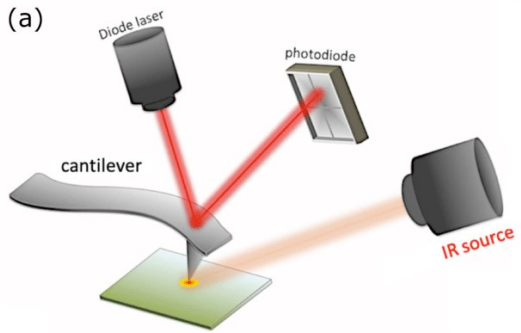
The peak height of the second derivative is proportional to the original peak height (with opposite sign) and inversely proportional to the square of the original half-width.

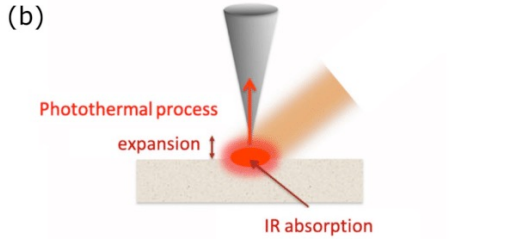
The main advantage of using second derivative spectra lies in the ease with which the peak frequencies of unresolved components can be identified.

Frontier: AFM-IR



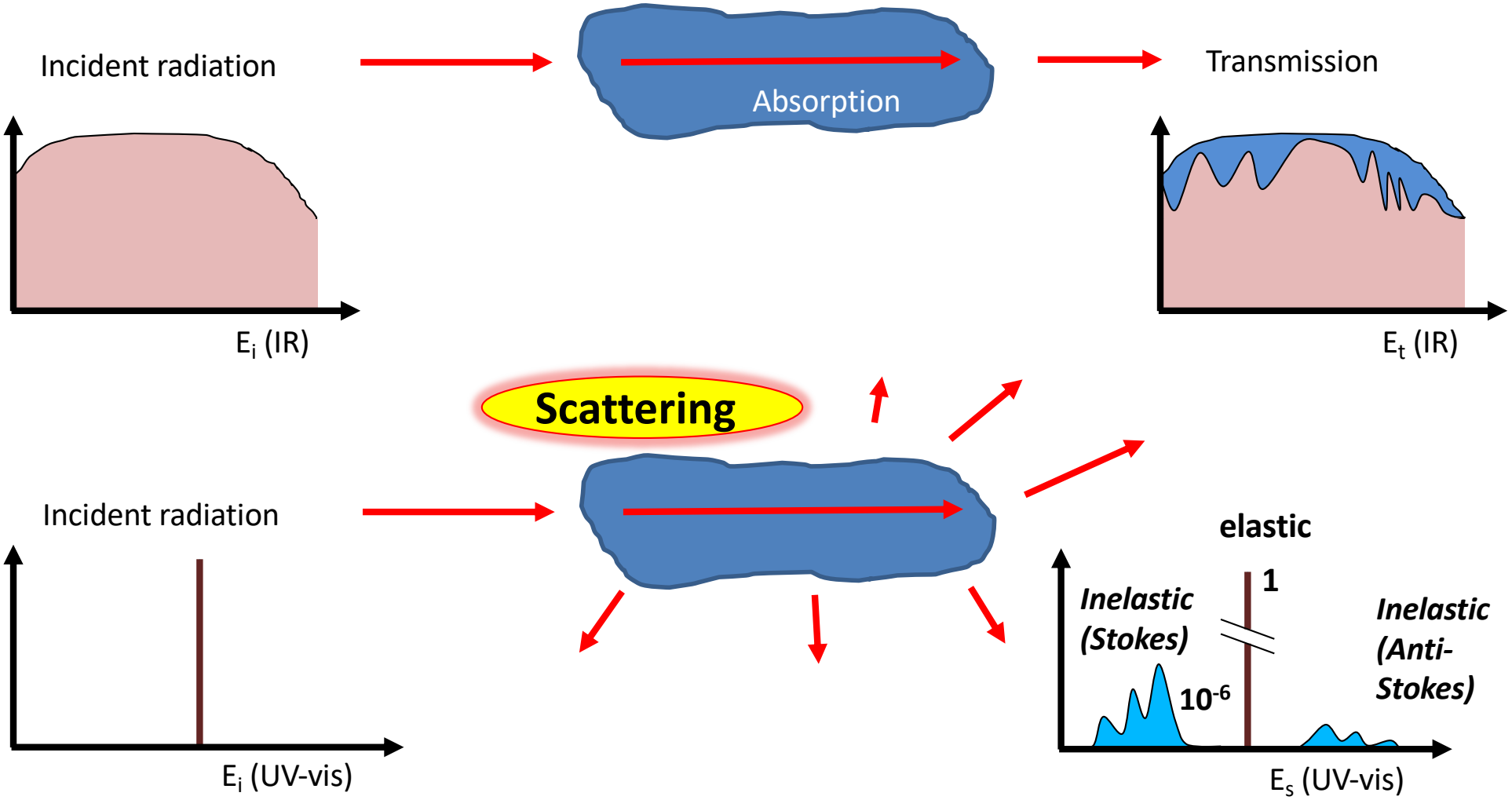




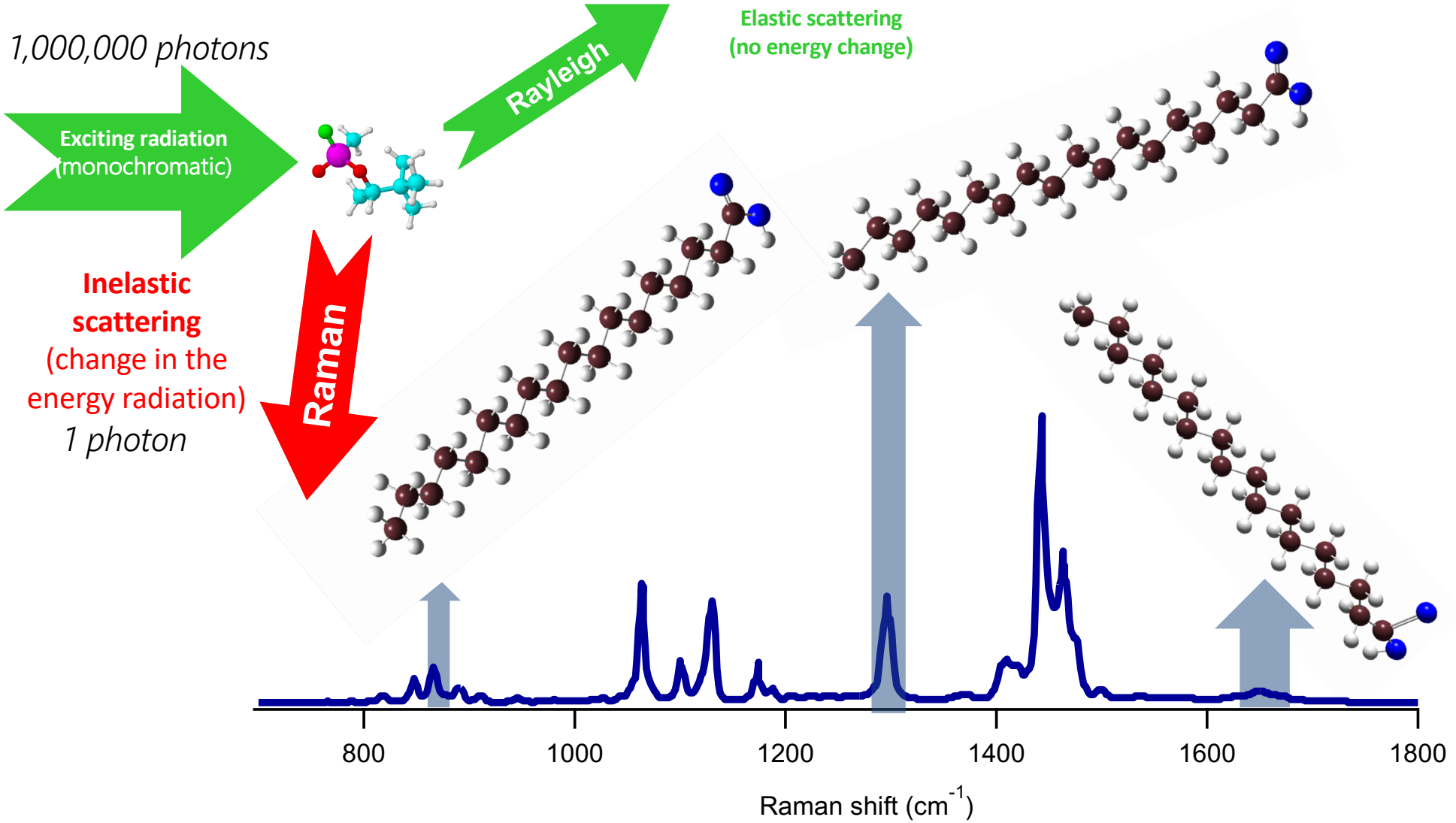


Raman spectroscopy

Interaction of radiation with matter

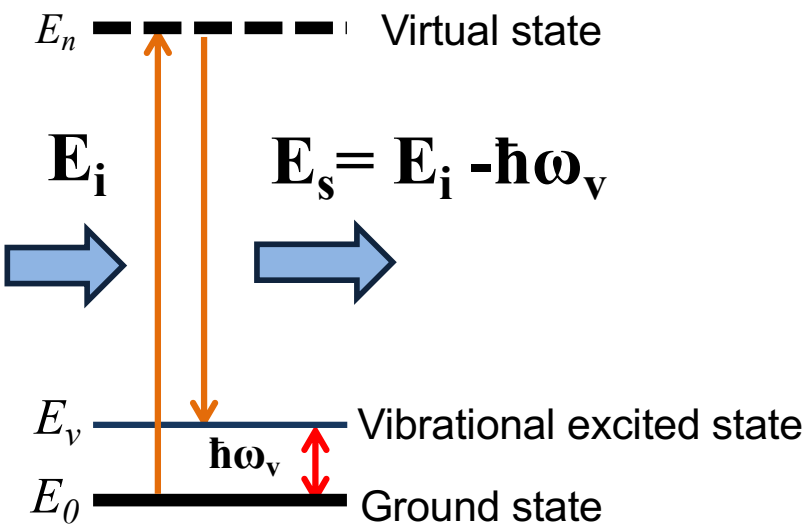


Raman scattering

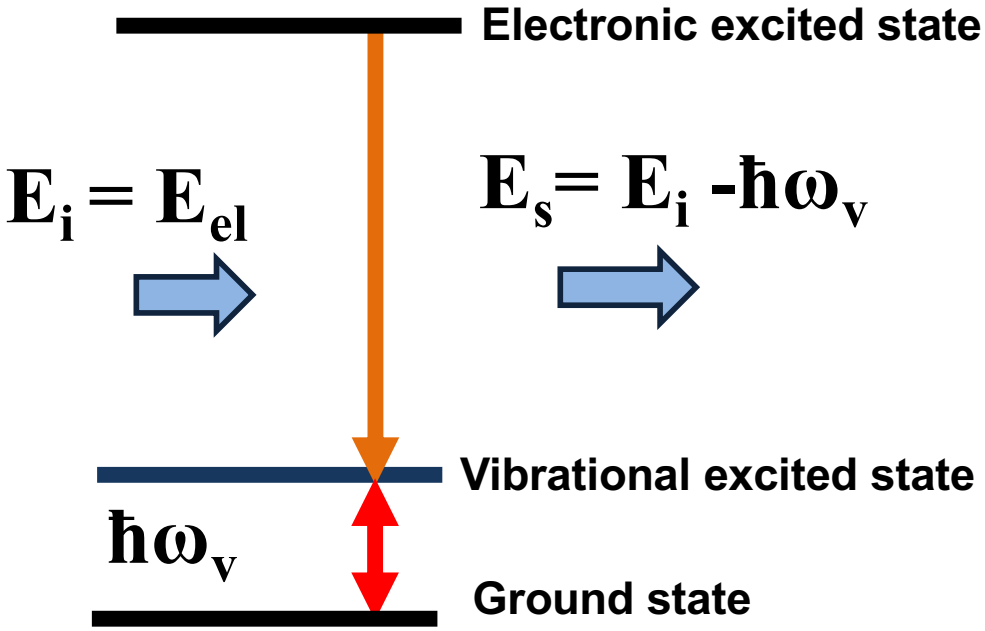


Resonant Raman scattering

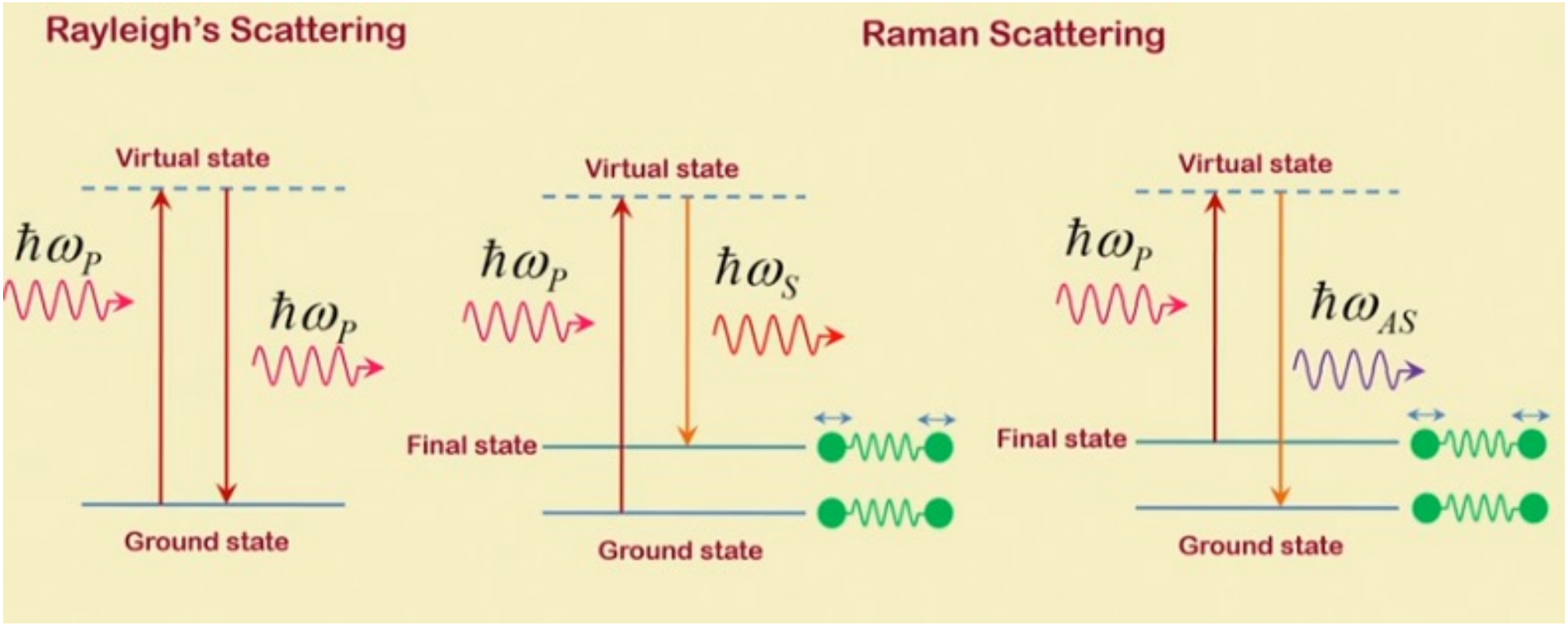
Spontaneous Raman



Resonant Raman



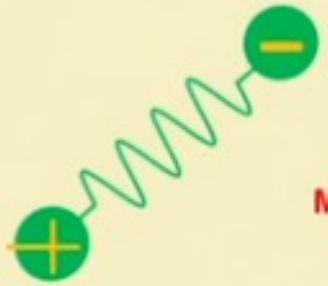
Raman cross section increased


Rayleigh's Scattering
Virtual state
$\hbar\omega_P$
$\hbar\omega_P$
Ground state
Raman Scattering
Virtual state
$\hbar\omega_P$
$\hbar\omega_S$
Final state
Ground state
Virtual state
$\hbar\omega_P$
$\hbar\omega_{AS}$
Final state
Ground state

Classical Theory of Raman Scattering

$$E_i = E_0 \cos(\omega_p t)$$

Monochromatic Field



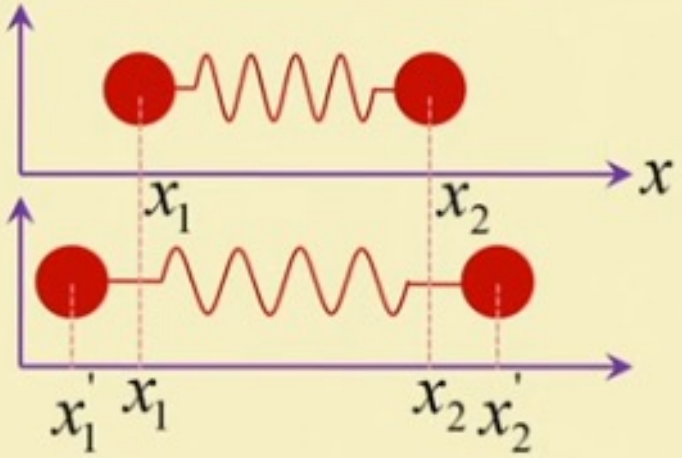
$$p = \epsilon_0 \alpha E_i$$

Microscopic Polarization

Microscopic Susceptibility

$$\alpha(Q) = \alpha_0 + \left.\frac{d\alpha}{dQ}\right|_0 Q + ...$$

Generalized Coordinate



Change in length

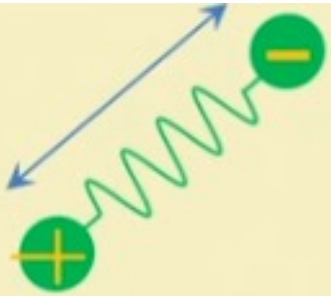
$$\left(x_2' - x_1'\right) - \left(x_2 - x_1\right)$$
$$= \left(x_2' - x_2\right) - \left(x_1' - x_1\right)$$
$$= q_2 - q_1$$

p = polarizability

Q = vibrational coordinate

$$E_i = E_0 \cos(\omega_p t)$$

Monochromatic Field



$$Q = Q_0 \cos(\Omega t)$$

$$p = \epsilon_0 \alpha E_i$$

$$p \approx \epsilon_0 \left[\alpha_0 + \left. \frac{d\alpha}{dQ} \right|_0 Q \right] E_i$$

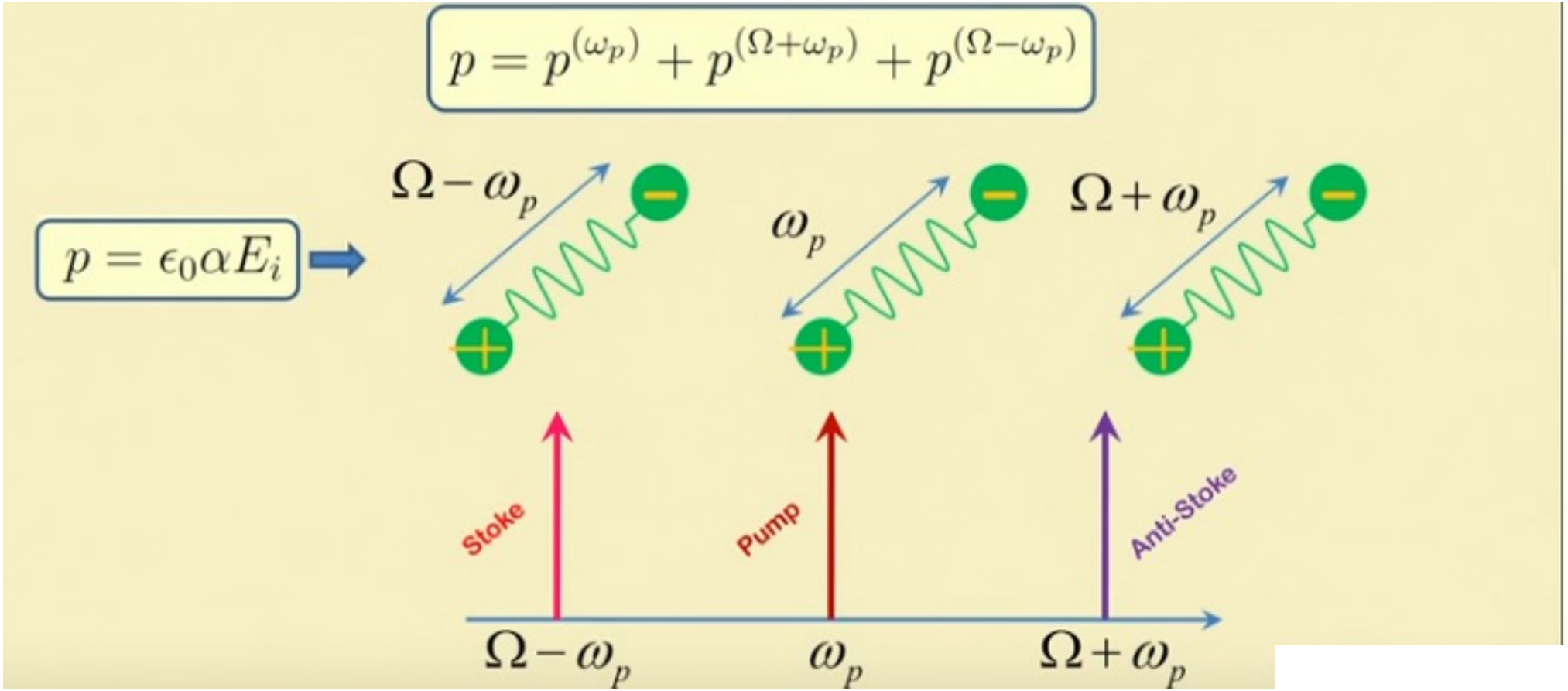
$$p \approx \epsilon_0 \left[\alpha_0 + \left. \frac{d\alpha}{dQ} \right|_0 Q \right] E_0 \cos(\omega_p t)$$

$$p = \epsilon_0 \alpha_0 E_0 \cos(\omega_p t) + \left. \frac{d\alpha}{dQ} \right|_0 Q E_0 \cos(\omega_p t)$$

$$p = \epsilon_0 \alpha_0 E_0 \cos(\omega_p t) + \left.\frac{d\alpha}{dQ}\right|_0 Q_0 \cos(\Omega t) E_0 \cos(\omega_p t)$$

$$p = \epsilon_0 \alpha_0 E_0 \cos(\omega_p t) + \left.\frac{E_0 Q_0}{2}\frac{d\alpha}{dQ}\right|_0 [\cos(\Omega + \omega_p)t + \cos(\Omega - \omega_p)t]$$

$$p = p^{(\omega_p)} + p^{(\Omega+\omega_p)} + p^{(\Omega-\omega_p)}$$


$p = p^{(\omega_p)} + p^{(\Omega+\omega_p)} + p^{(\Omega-\omega_p)}$
$p = \epsilon_0 \alpha E_i$
$\Omega - \omega_p$
ω_p
$\Omega + \omega_p$
Stoke
Pump
Anti-Stoke
$\Omega - \omega_p$
ω_p
$\Omega + \omega_p$

6.4 Raman Scattering selection rules

Scattering is not an oscillating dipole phenomenon! (no TDM)

The presence of an electric field *E* induces a polarization in an atom/ molecule given by $\mu_{ind} = \alpha E$ (polarizability)

If the field is oscillating (e.g., photon) $\mu_{ind} = \alpha E_0 cos\left(2\pi\nu t\right)$

In atoms the polarizability is isotropic, and the atom acts like an antenna and re-radiates at the incident frequency – Rayleigh Scattering only

In molecules the polarizability may be anisotropic, and depends on the rotational and vibrational coordinates. This can also give rise to Raman Scattering.

Gross Selection Rule:

To be Raman active a molecule must have anisotropic polarizability

[Less restrictive than the need for a dipole moment, symmetric molecules can be Raman active]

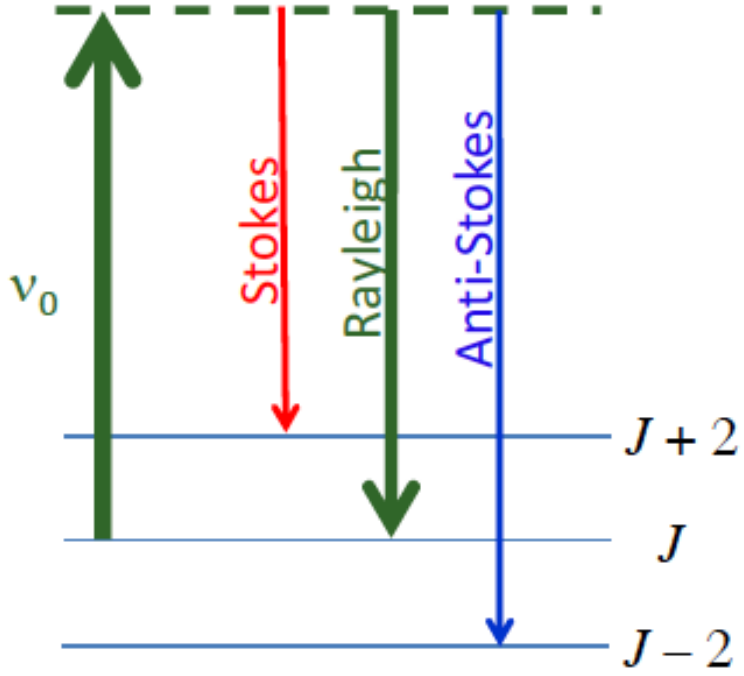
6.5 Rotational Raman

6.5.1 Linear Molecules: The polarizability tensor is anisotropic ($\alpha_{\perp} \neq \alpha_{||}$)

As a molecule rotates the polarizability presented to the *E* field changes:

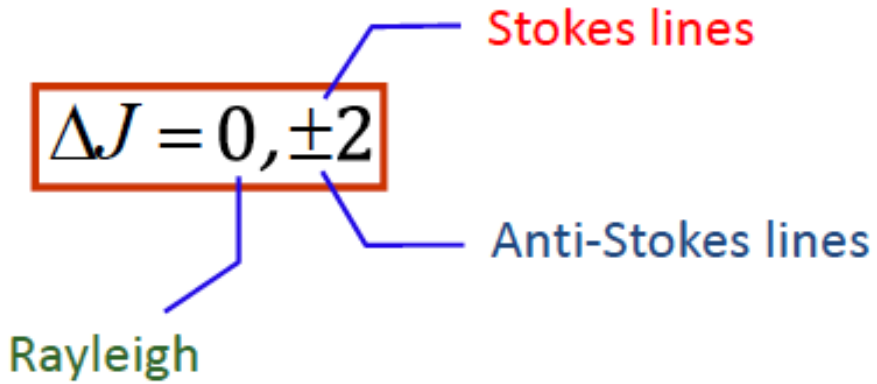
→ the induced dipole is modulated by rotation

→ results in rotational transitions



Specific Selection Rule:

Effective two-photon process and ∴



Even non-polar molecules (O_2, N_2, CO_2) exhibit rotational Raman Spectra

6.6 Vibrational Raman

Gross Selection Rule: The polarizability must change during the vibration $\left(\frac{d\alpha}{dq}\right)_{q=0} \neq 0$

In practice this means the normal mode must transform with the same symmetry as the quadratic forms (x^2, xy, *etc.*)

6.6.1 Diatomics:

Even homonuclear diatomics satisfy the gross selection rule and exhibit Raman spectra

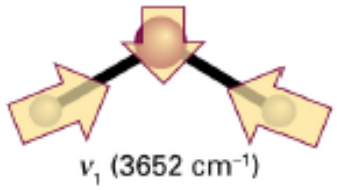
Specific Selection Rule: $\Delta v = \pm 1$ (+ Stokes, – Anti-Stokes)

n.b. Anti-Stokes rarely observed because v > 0 weakly populated

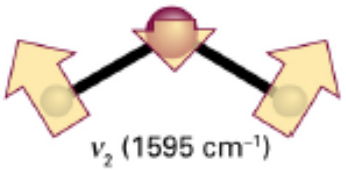
6.6.2 Polyatomics:

Need to check each normal mode against the gross selection rule:

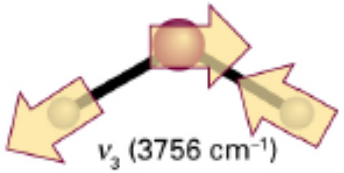
H_2O



Raman Active



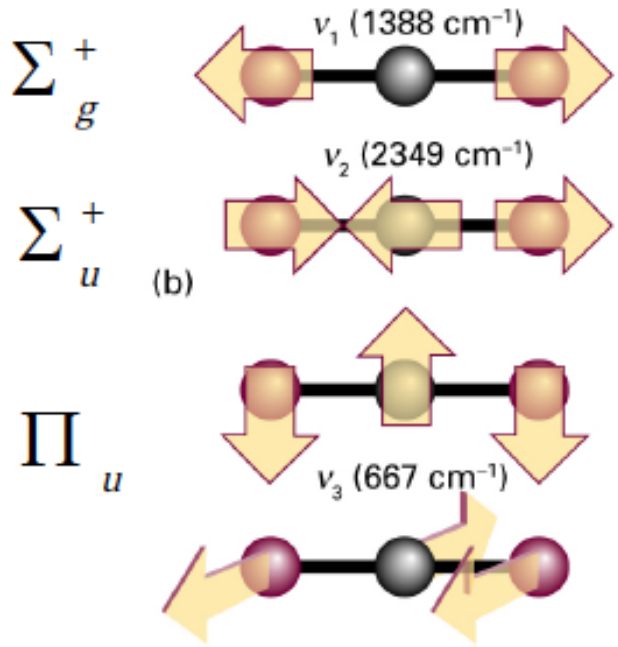
Raman Active



Raman Active

CO_2: $D_{\infty h}$

$D_{\infty h}$	E	$2C_\infty^\phi$	…	$\infty\sigma_v$	i	$2S_\infty^\phi$	…	∞C		
Σ_g^+	1	1	…	1	1	1	…	1		x^2+y^2, z^2
Σ_g^-	1	1	…	−1	1	1	…	−1	R_z	
Π_g	2	$2\cos\phi$	…	0	2	$-2\cos\phi$	…	0	(R_x, R_y)	(xz, yz)
Δ_g	2	$2\cos 2\phi$	…	0	2	$2\cos 2\phi$	…	0		$(x^2-y^2, 2xy)$
…	…	…	…	…	…	…	…	…		
Σ_u^+	1	1	…	1	−1	−1	…	−1	z	
Σ_u^-	1	1	…	−1	−1	−1	…	1		
Π_u	2	$2\cos\phi$	…	0	−2	$2\cos\phi$	…	0	(x,y)	
Δ_u	2	$2\cos 2\phi$	…	0	−2	$-2\cos 2\phi$	…	0		
…	…	…	…	…	…	…	…	…		



IR Inactive — Raman Active

IR Active — Raman Inactive

IR Active — Raman Inactive

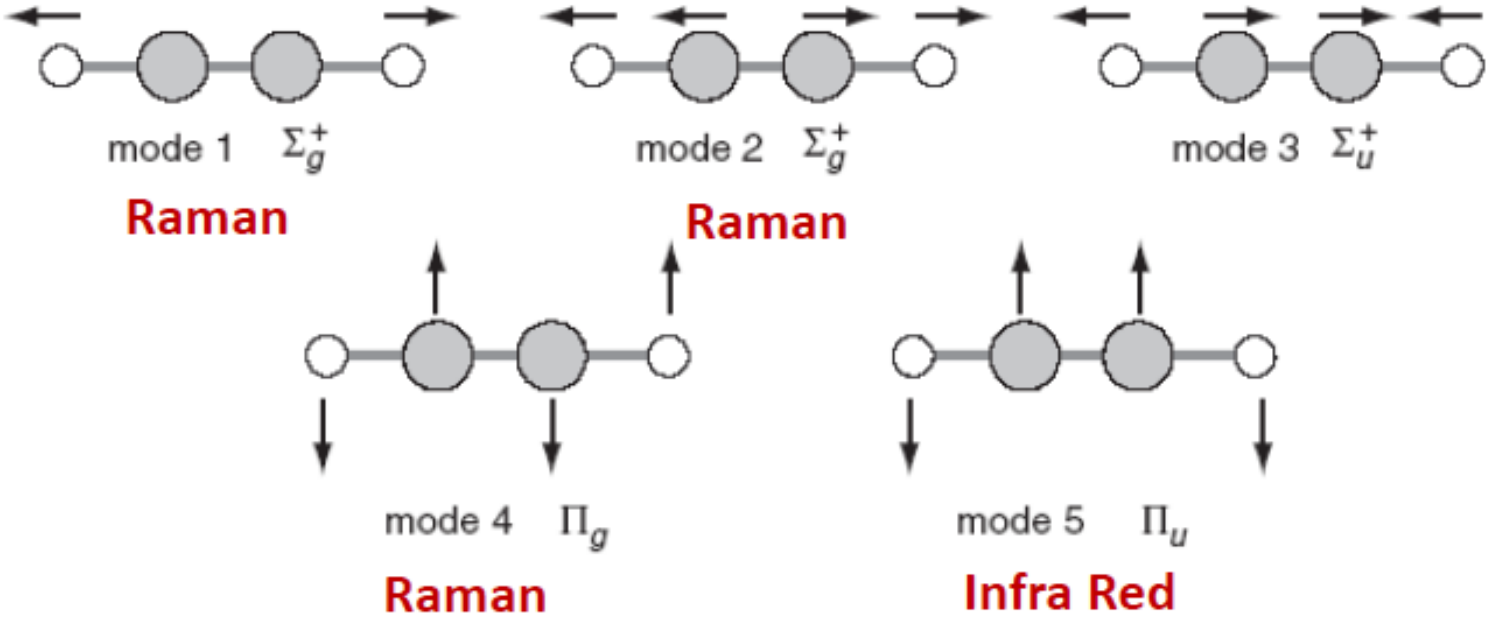
6.7 The Rule of Mutual Exclusion

In the case of CO_2 it is not coincidence that those modes which are Raman active are IR inactive and *vice versa*. This is an example of the rule of mutual exclusion which states:

> In a centrosymmetric molecule (*i.e.*, one with a centre of inversion symmetry) a vibrational mode may be either IR active or Raman active but not both.

acetylene

$D_{\infty h}$



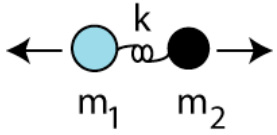
Raman Spectroscopy: Classical Treatment

- Number of peaks related to degrees of freedom

  DoF = 3N - 6 (bent) or 3N - 5 (linear) for N atoms

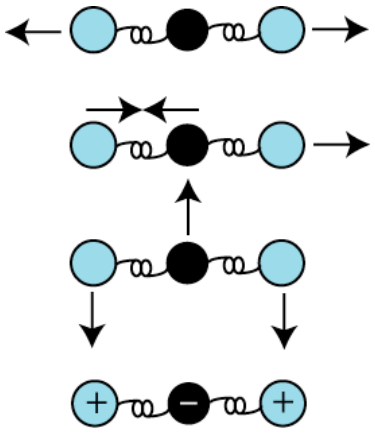
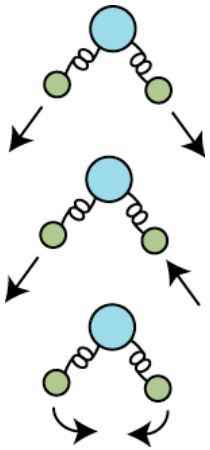
- Energy related to harmonic oscillator

$$\sigma \text{ or } \Delta\sigma = \frac{c}{2\pi}\sqrt{\frac{k(m_1+m_2)}{m_1 m_2}}$$

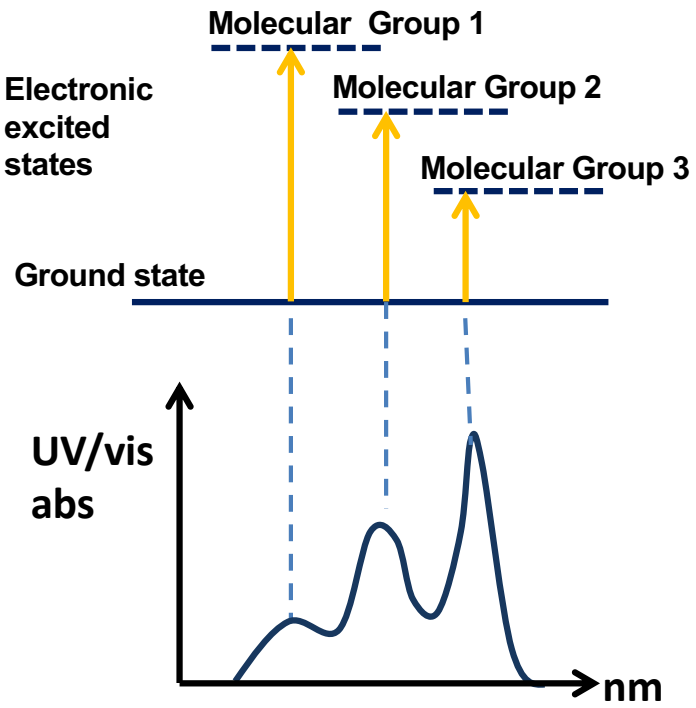


- Selection rules related to symmetry

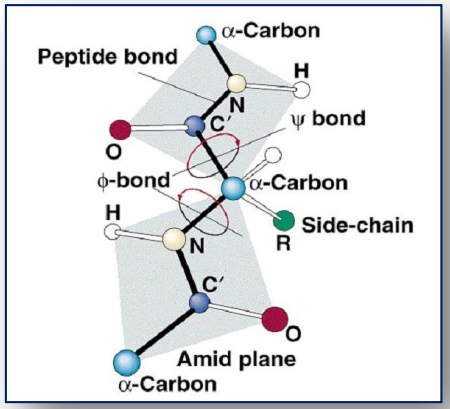
  Rule of thumb: symmetric=Raman active, asymmetric=IR active

CO_2	H_2O
 Raman: 1335 cm^{-1} IR: 2349 cm^{-1} IR: 667 cm^{-1}	 Raman + IR: 3657 cm^{-1} Raman + IR: 3756 cm^{-1} Raman + IR: 1594 cm^{-1}

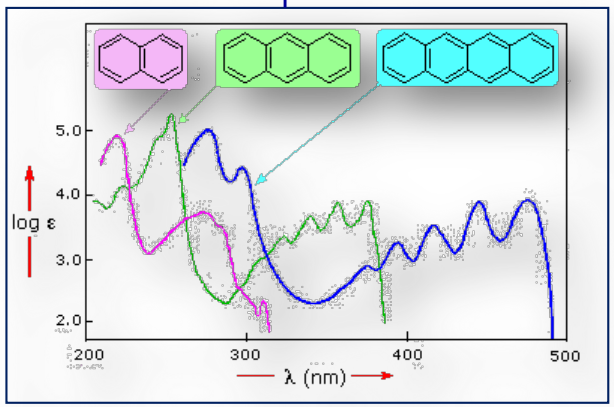
UV Resonant Raman scattering



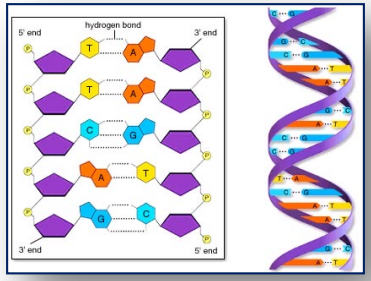
200-220 nm
Peptide bonds



200-300 nm
Aromatic compounds



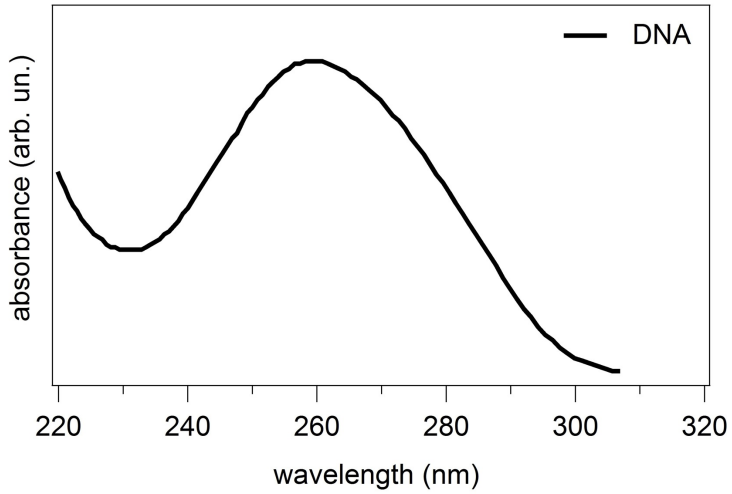
225-300 nm
DNA nitrogenous bases

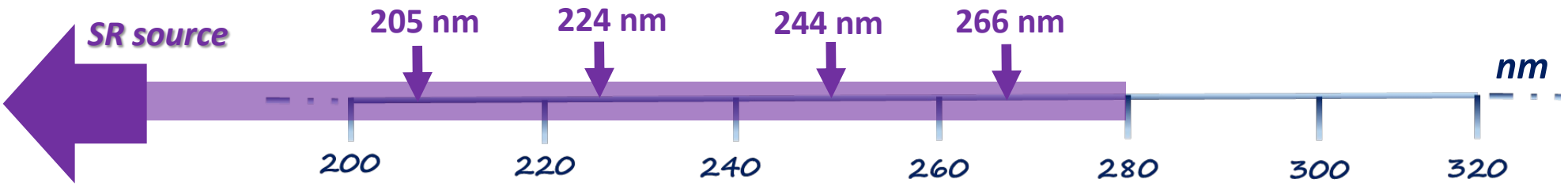


S. A. Oladepo et al. *Chem. Rev.* 2012.

Exciting wavelength selectivity

- *Laser: fixed wavelength sources*
- *SR: **tunable** CW wavelength source → better selectivity*





Exciting wavelength selectivity

- *Laser: fixed wavelength sources*
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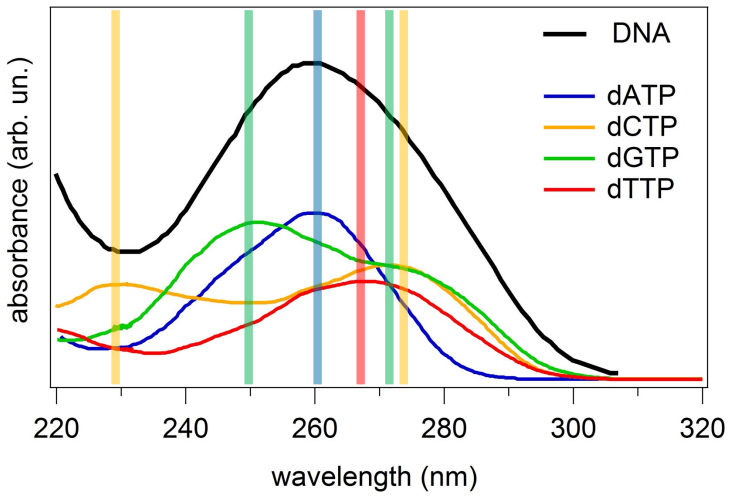

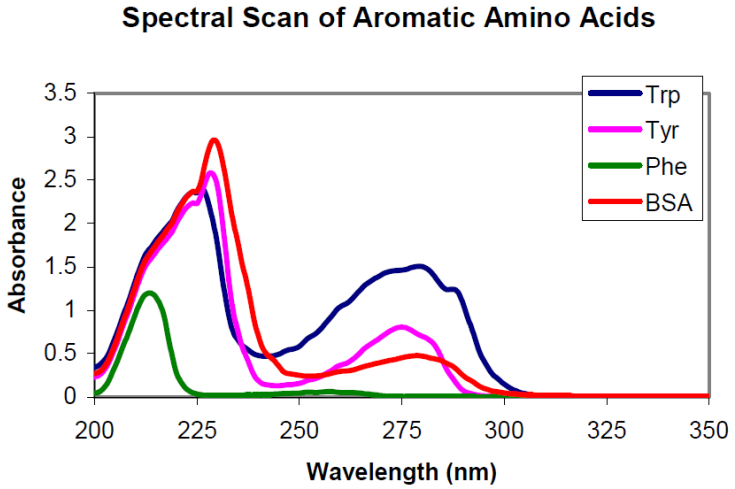

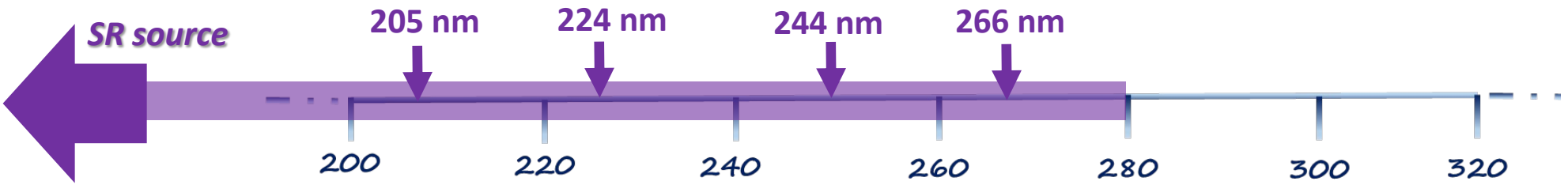

Further advantages on the use of UVRR

- **Absence** of *fluorescence background* on the spectra
- Measurements in **water** and/or **buffer solutions** at low solute concentrations
- **Higher Raman cross section** with respect to the Raman scattering performed exploting visible near/IR laser sources