

Optical Spectroscopy

1. Absorption spectroscopy (UV/vis)
2. Circular dichroism (optical activity)
 - CD / ORD
3. Fluorescence spectroscopy and energy transfer

Electromagnetic Spectrum

	Electronic		Molecular Vibrations			Molecular Rotations		NMR, ESR
	UV	VIS	near IR	mid IR	far IR	Microwave		Radio
$\lambda(\text{m})$			10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
$\lambda(\text{cm})$			10^4	10^3	10^2	0.1	1	10
$\tilde{\nu}(\text{cm}^{-1})$			10,000	1,000	100	10	1	0.1
$\nu(\text{Hz})$			3×10^{14}	3×10^{13}	3×10^{12}	3×10^{11}	3×10^{10}	3×10^9
ΔE (kcal/mol)			~30	3	0.3	0.03	0.003	0.0003
					↑	kT at room temperature		

Absorption Spectroscopy

$$\epsilon = h\nu \quad \text{energy of a photon}$$

$$E = N \epsilon \quad (\text{Einstein: energy of a mole of photons})$$

$$E = \frac{Nhc}{\lambda} \quad \text{since } \nu = \frac{c}{\lambda}$$

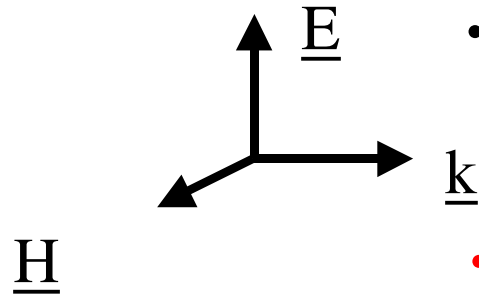
$$E = \frac{28.6}{\lambda} \text{ kcal/mol} \quad (\lambda \text{ in microns})$$

Energy of a photon of ultraviolet (UV) or visible light has enough energy to break covalent bonds and result in photochemistry

- UV light (~ 250 nm), $E \cong 114$ kcal/mol
- visible light (~ 500 nm), $E \cong 57$ kcal/mol

Bond	E (kcal/mol)
C-H	100
C-C	50-80
C=C	120-140

2 Electromagnetic waves



- 1. **Direction of propagation**

- $\underline{E}, \underline{H}, \underline{k}$ mutually perpendicular
- $\underbrace{\hspace{1.5cm}}$
oscillating

- 2. **Frequency and phase**

- $E = E_0 \cos(2\pi\nu t + \phi)$

- $H = H_0 \cos(2\pi\nu t + \phi)$

- $\phi \equiv$ phase

- $\nu =$ frequency

- $2\pi\nu = \omega$, circular frequency

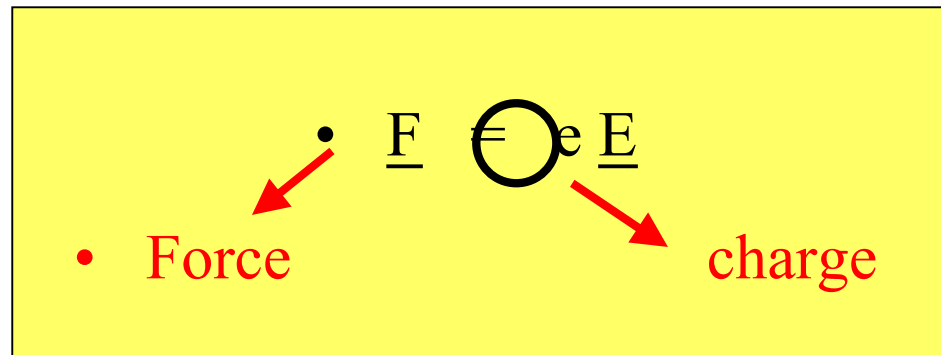
- 3. **Energy density** (energy per

volume)

- is proportional to the square of the electric field.

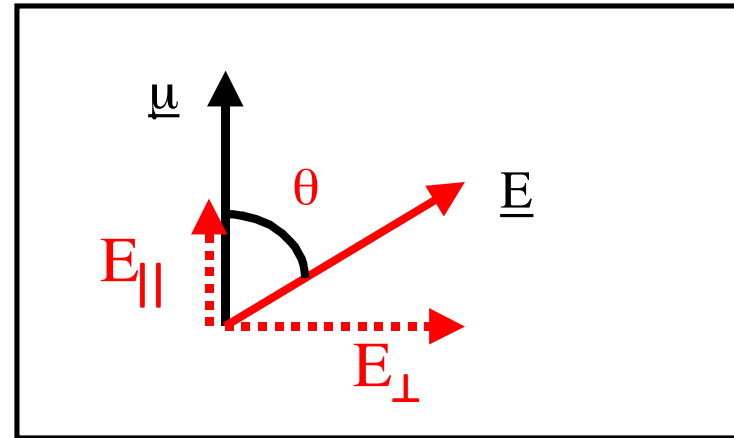
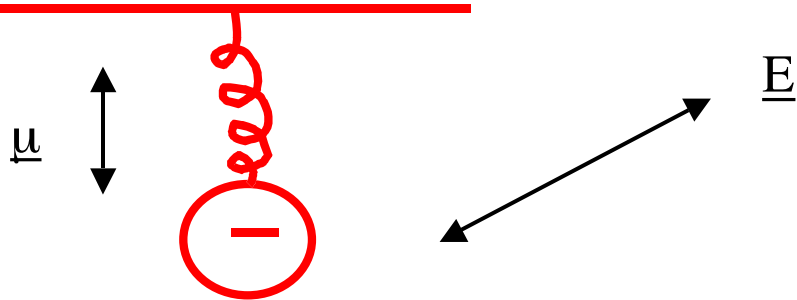
- $\rho(\nu) \propto |\underline{E}|^2$

- b Main interaction between electromagnetic radiation and electrons is *via* the Electric Field Vector \underline{E}



- **Classical view is an oscillating electric field vector interacting with an electron on a spring.**

Electron on a spring



Classical view:

- harmonic oscillator
 - electron on a spring
 - preferred direction of oscillation
- } equivalent to quantum mechanical
transition dipole moment $\underline{\mu}$

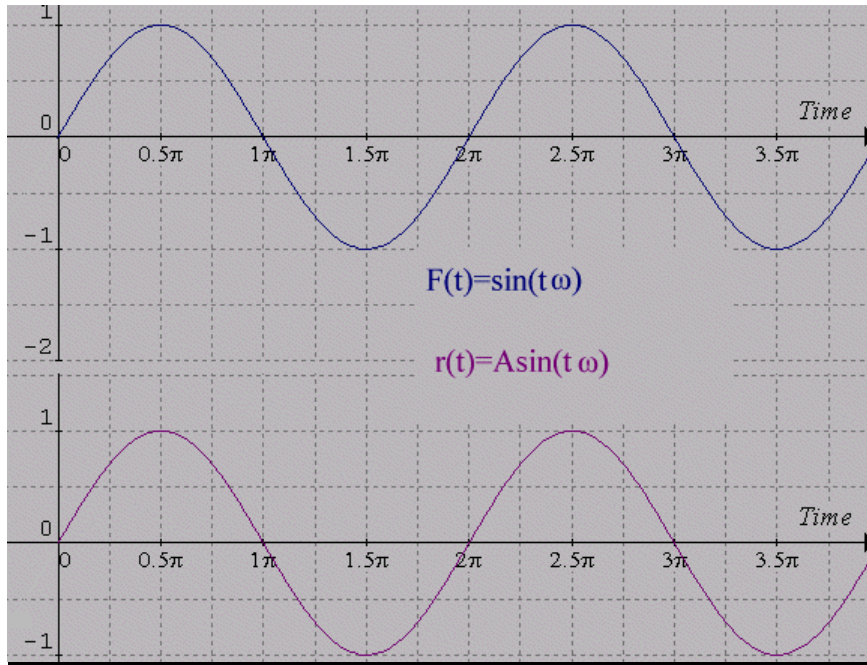
Effective interaction is with the portion of \underline{E} parallel to $\underline{\mu}$

$$E_{||} = |\underline{E}| \cos \theta$$

Absorption rate is proportional to **Energy Density**

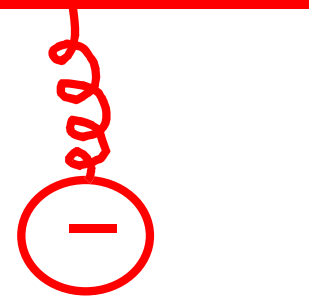
* $\text{Absorption rate} \propto E^2 \cos^2 \theta$ *

Force and Response: A classical view

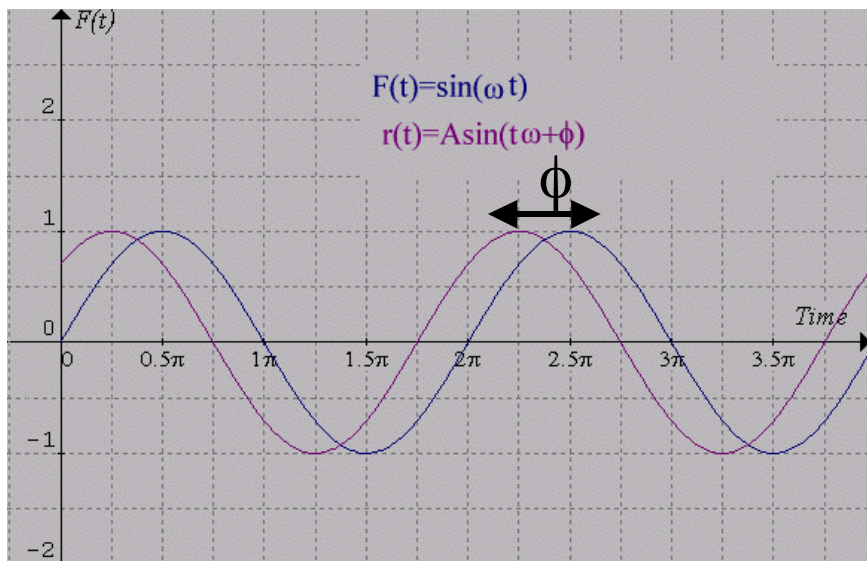


$$F(t) = \sin(\omega t)$$

In a system with no resistance, the response is in phase with the force



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



In a system where there is resistance, the response is out of phase. This corresponds classically to energy

Dissipation --absorption

$$F(t) = A \sin(\omega t + \phi)$$

$$r(t) = \underbrace{\alpha \sin(\omega t)}_{\text{in phase}} + \underbrace{\beta \cos(\omega t)}_{\text{out of phase}}$$

Response of electron (harmonic oscillator) to an oscillating electric field

$$r(t) = \alpha \sin(\omega t) + \beta \cos(\omega t) = A \sin(\omega t + \varphi)$$

in-phase polarizability

α

related to index of refraction, n

Dispersion Spectrum

n vs λ

or

α vs λ

out-of-phase polarizability

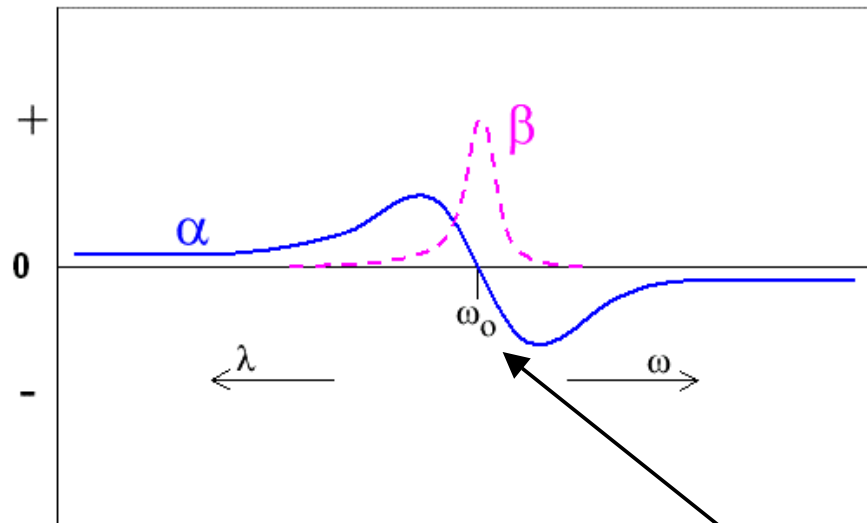
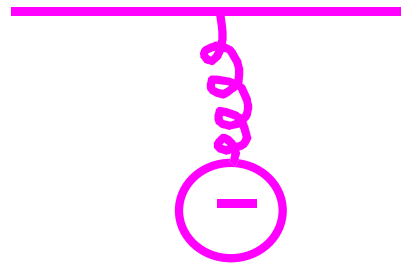
β

related to energy dissipation
or absorption

ϵ , extinction coefficient

Absorption Spectrum

ϵ vs λ



Natural frequency of spring

Main Points:

energy dissipation

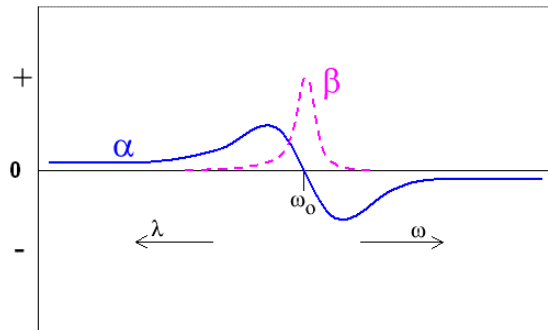
speed of light through sample

1 **Absorption** (ϵ) and **Dispersion** (n) result from the same fundamental interaction

- They are mathematically related
- If you know $n(\omega)$ at all frequencies you can calculate the absorption spectrum

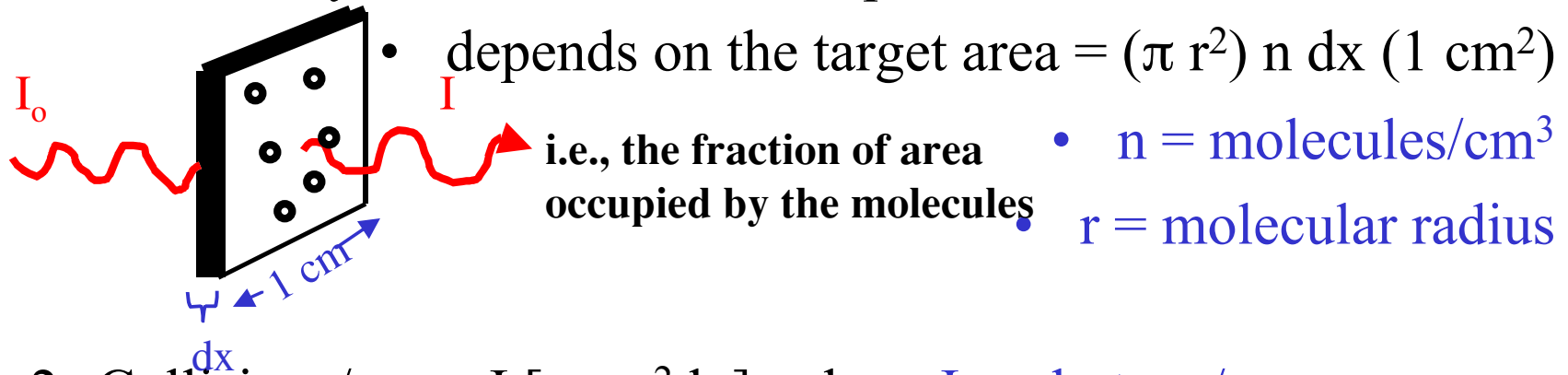
2 **Absorption** is restricted to region near resonance frequency, ω_0

- But dispersion goes far beyond that
- example: sugars absorb at $\lambda < 250$ nm, but the influence on the index of refraction is easily measured in the visible (~ 500 nm)



Quantifying Absorption Probability: Beer's Law and the extinction coefficient

1 Probability of collision between photon and molecule



2 Collisions/sec = $I [n \pi r^2 dx]$ where $I = \text{photons/sec}$

3 Photons absorbed/sec ($-dI$)

• $-dI = I [n \pi r^2 dx] P$ where $P = \text{absorption probability}$

• $-dI = I n [P\pi r^2] dx$

• $-dI = I n \sigma (dx)$

• Where we define σ as the cross section for absorption

4 Integrate from $x (0, l)$ and (I_0, I)

- $\frac{dI}{I} = -n \sigma dx$

•

- $\log \left[\frac{I}{I_0} \right] = \frac{-n \sigma l}{2.303}$

•

5 Change units to mol/liter (c)

where $N = \text{Avogadro's number}$

- $n = \frac{cN}{1000}$

•

- $\log \left[\frac{I}{I_0} \right] = \frac{-c N \sigma(\lambda) l}{(2.303) (1000)}$

•

- Define the extinction coefficient: $\epsilon(\lambda) = \frac{N \sigma(\lambda)}{2.303}$

•

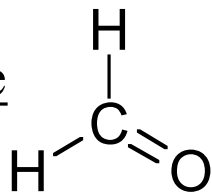
- $A = \log_{10} \left[\frac{I_0}{I} \right] = \epsilon c l$

∇ ϵ is proportional to σ , the cross-section of absorbance

- Upper limit is around $\epsilon \approx 10^5 \text{ M}^{-1}\text{cm}^{-1}$

π , n , and π^* orbitals

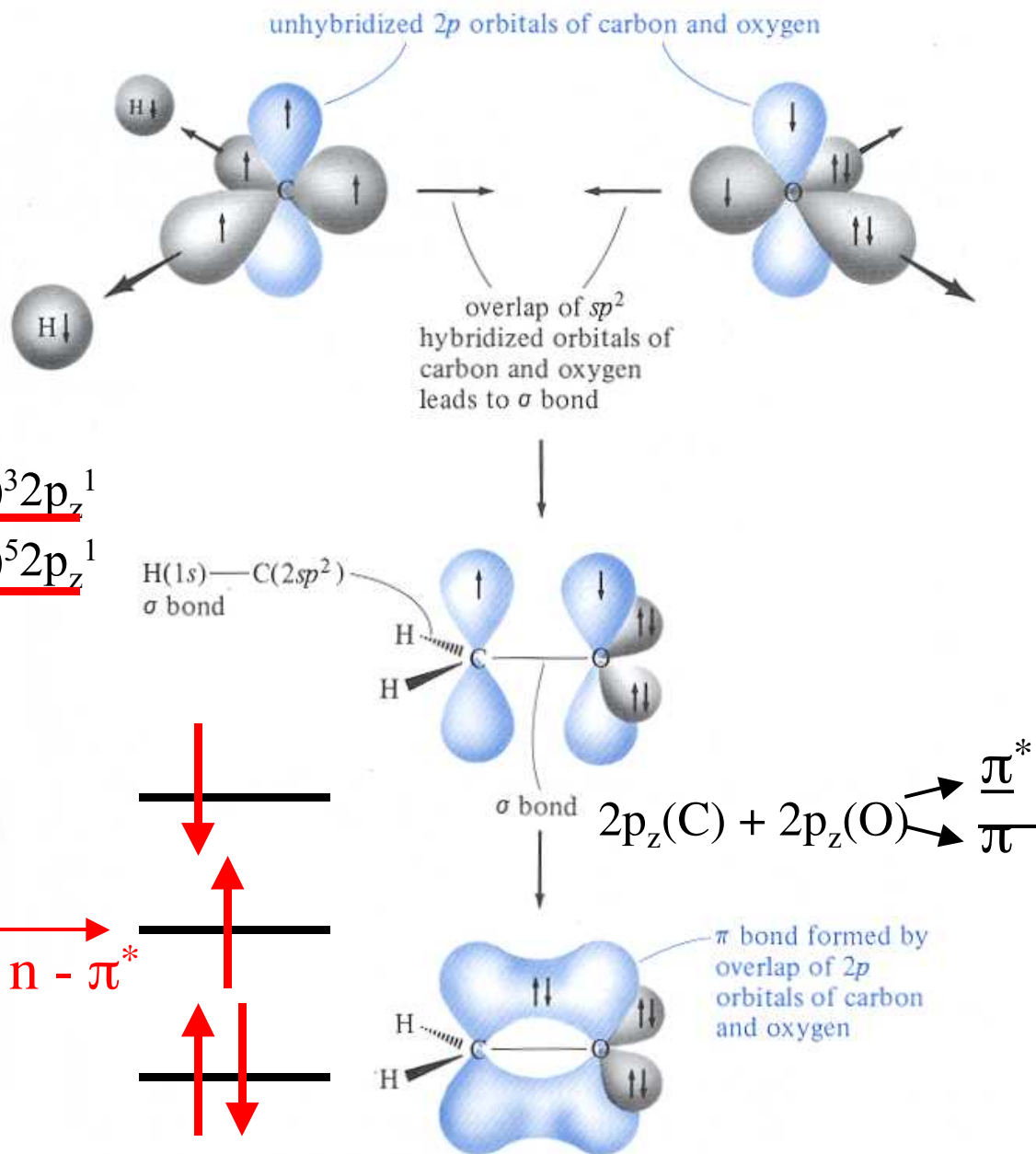
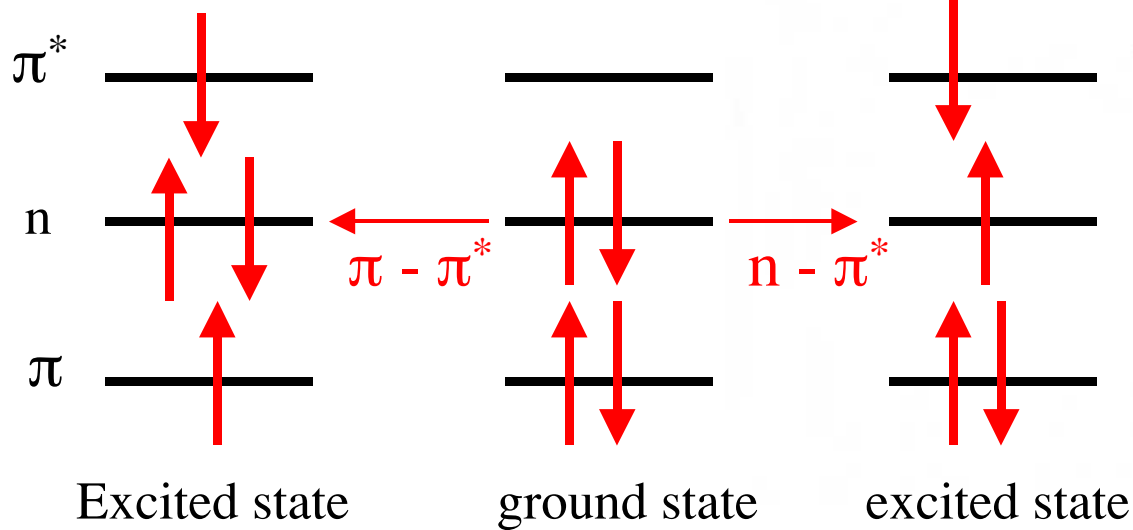
Formaldehyde



1e- H: 1s

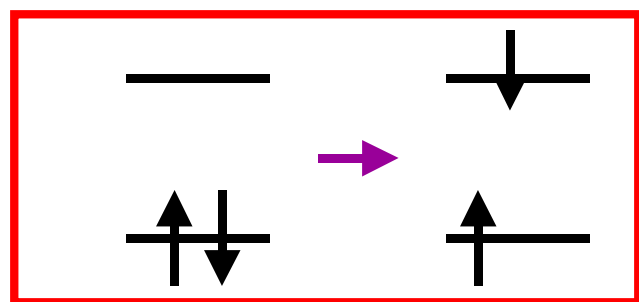
6e- C: $1s^2 2s^2 2p_x 2p_y \rightarrow 1s^2 (2sp^2)^3 2p_z^1$

8e- O: $1s^2 2s^2 2p_y^2 2p_x^2 \rightarrow 1s^2 (2sp^2)^5 2p_z^1$

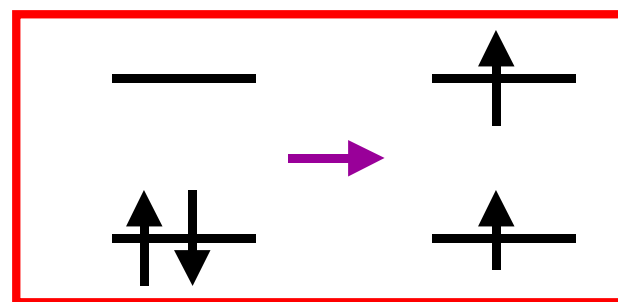


What determines the probability of absorption (size of ϵ)

1. Shape of the ground state and excited state molecular orbitals
 - overlap
 - symmetry
 - ($n - \pi^*$) transitions are overlap forbidden (low ϵ)
 - ($\pi - \pi^*$) are “allowed” (high ϵ)
2. Multiplicity: changes in electron spin are “not allowed”
requires interaction with environment to allow spin flip



VS

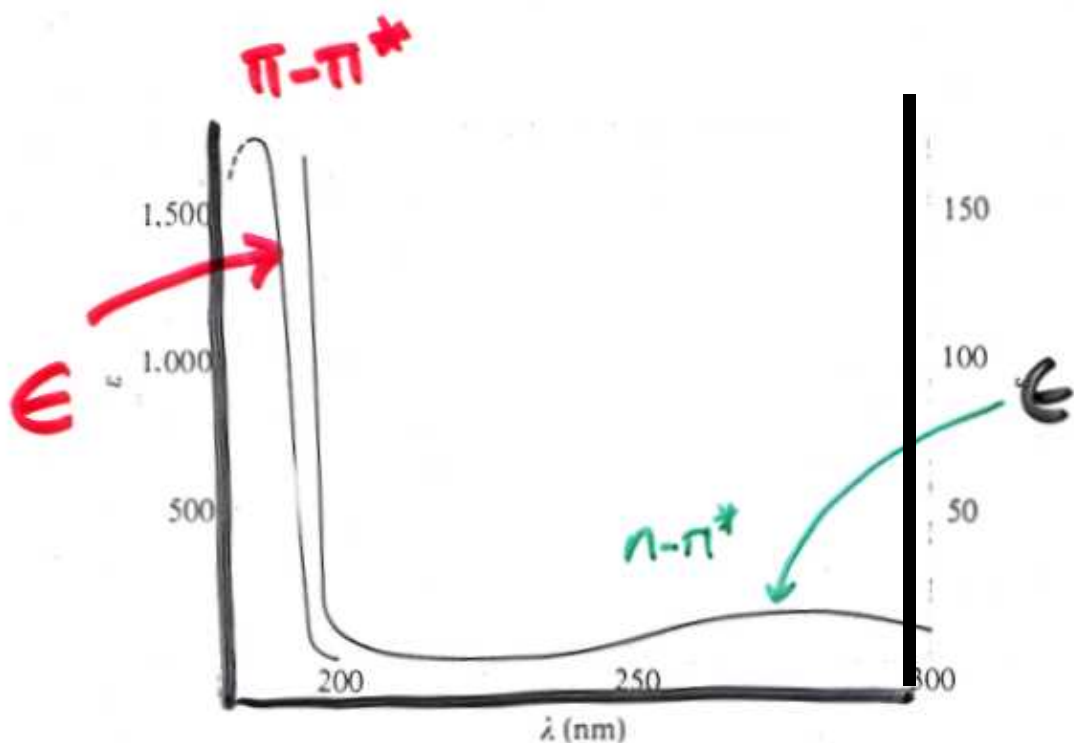
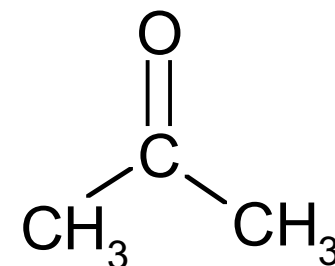


Lowest triplet
excited state

Ground state Lowest singlet excited state

$\sim 10^6$ less likely transition

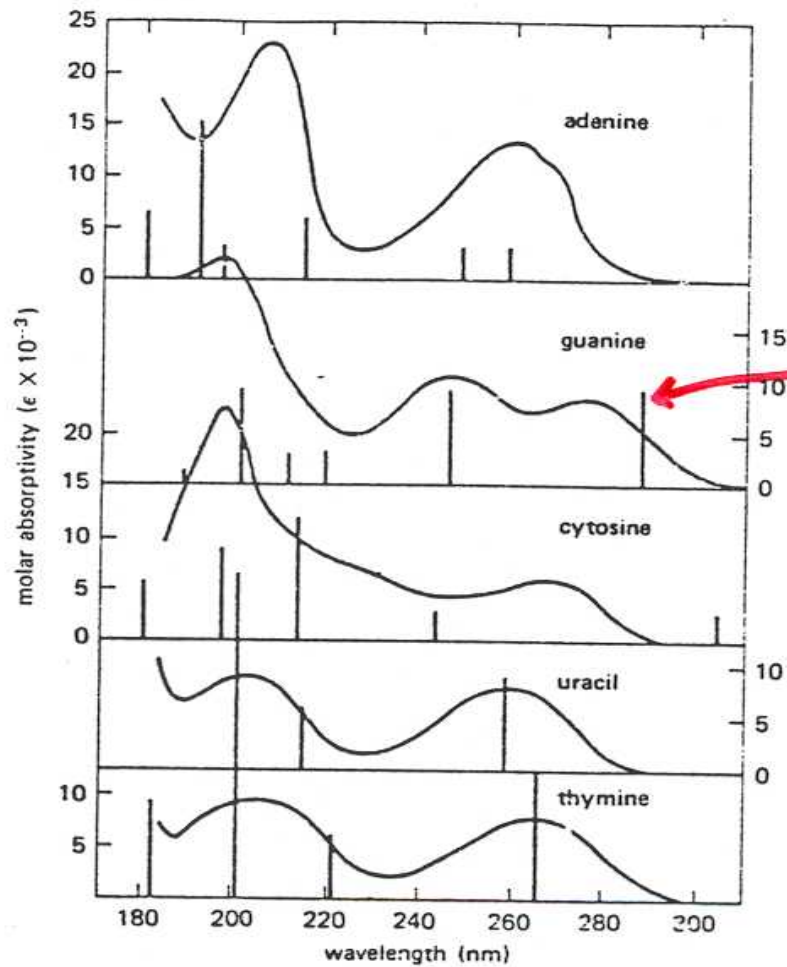
example: spectrum of acetone



($M^{-1} \text{ cm}^{-1}$)

$\pi-\pi^*$ higher energy
higher extinction

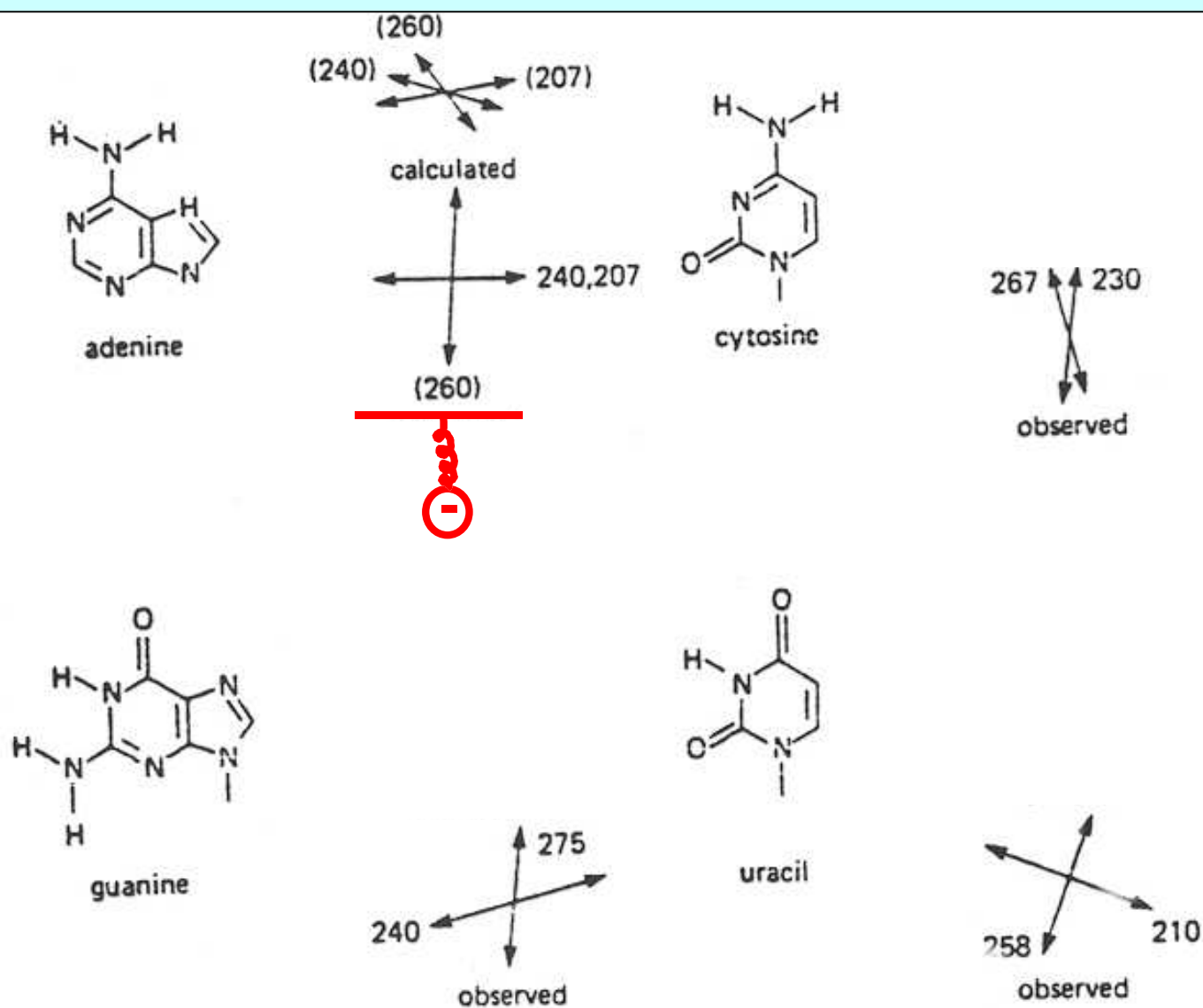
$n-\pi^*$ lower energy
very low extinction



**UV spectra of purines
and pyrimidines
 $\pi-\pi^*$ transitions**

Lines are calculated
transitions

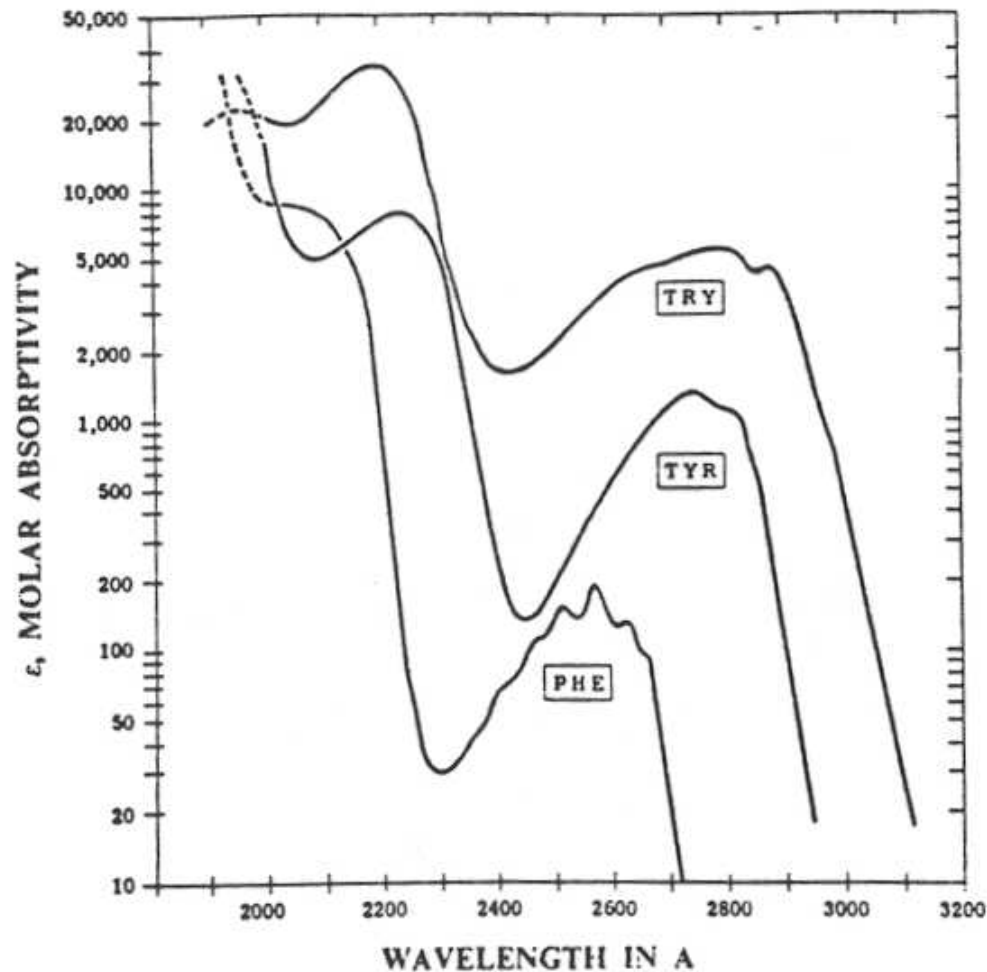
π - π^* transition dipoles are within the plane of the aromatic ring in purines and pyrimidines



Absorption of Aromatic Amino Acids in Proteins

Note that tryptophan is responsible for A_{280}

ABSORPTION SPECTRA OF THE AROMATIC AMINO ACIDS AT pH 6 .

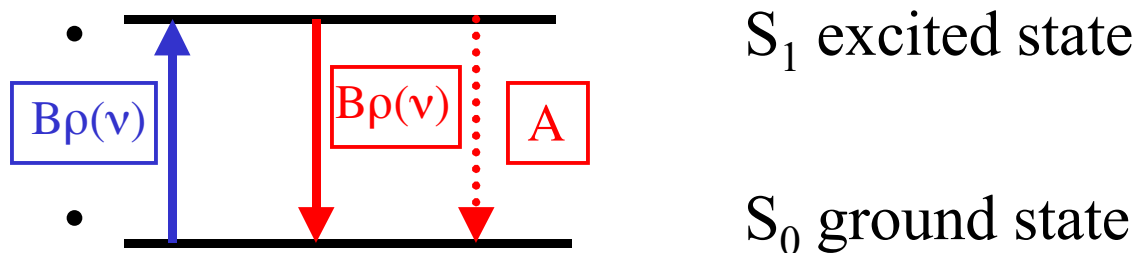


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4 Einstein coefficients and the concept of saturation

- Phenomenological equations
- Treat absorption as a second-order kinetic process

- 2 energy-level system:



- rate up($S_0 \rightarrow S_1$) = $B \rho(\nu) [S_0]$

- rate constant depends on the energy density and the concentration of ground state molecules

- rate down($S_1 \rightarrow S_0$) = $\underline{B \rho(\nu)[S_1]} + \underline{A[S_1]}$

induced emission rate spontaneous emission

rate

- in Steady State: rate up = rate down

Steady State: $k_{0-1} [S_0] = k_{1-0} [S_1]$

for a typical spectrophotometer

$$k_{0-1} \sim 10 \text{ sec}^{-1}$$

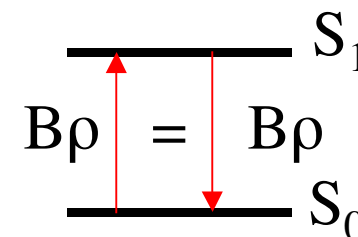
k_{1-0} is dominated by the A term and is typically $\sim 10^8 \text{ sec}^{-1}$
fast

conclude: $[S_1] \lll [S_0]$

We don't come close to "saturating" the system. Hence, we need not worry about effects due to varying light intensity in doing typical absorbance measurements.

- this is not the case with magnetic resonance (NMR, ESR) where spontaneous relaxation can be slow.

i.e. $[S_1] = [S_0]$, at high $\rho(\nu)$



In NMR and EPR, there will be no net absorption if the power is too high since energy is both absorbed and emitted at equal rates. This does not happen in UV/vis spectroscopy