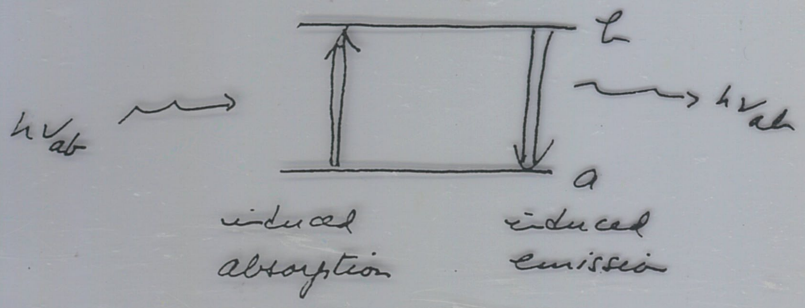
Biophysical Chemistry
for Life Scientists
NTU Fall 2000
Lecture 14
January 5, 2001

Reading Assignment
Eisenberg & Crothers, Chapter 13

Absorption or Emission Spectroscopy



$$B_{ab} = B_{ba} = \left(\frac{2}{3}\right)\left(\pi/\hbar^2\right)\left|\int \phi_b^* \vec{\mu} \phi_a \, d\vec{r}\right|^2$$

where $\vec{\mu} = -|e|\vec{r}$ for an electron

or $\vec{\mu} = \sum_i q_i x_i = \sum_i z_i |e| x_i$ for a molecule.

or $\int \rho(x)\, x \, d\vec{r}$

above result from time-dependent quantum mechanics.

Integral $\int \phi_b^* \vec{\mu} \phi_a \, d\vec{r}$ called transition dipole

$= \mu_{ab}$ for short

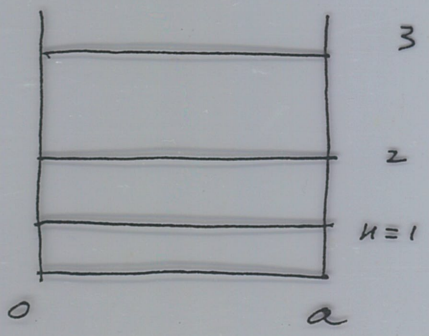
or μ_{ba}

Important concept or quantity, because unless $\mu_{ba} \neq 0$, there is no transition induced by $\vec{E}^*$ of light, or no

electric dipole transition.

Selection rules for simple situations

a) Particle in a box (1D)



$$\phi_n(x) = \frac{\sqrt{2}}{\sqrt{a}} \sin \frac{n\pi x}{a}$$

$$W_n = \frac{n^2 h^2}{8ma^2} \qquad (n = \text{integer})$$

$$\mu_{n\to m} = \mu_{nm} = \int_0^a \phi_m^*(x)\, \mu\, \phi_n(x)\, dx$$

$$= -|e| \left(\sqrt{\frac{2}{a}}\right)^2 \int_0^a \sin \frac{m\pi x}{a} \cdot x \cdot \sin \frac{n\pi x}{a}\, dx$$

Integral vanishes unless $m = n \pm 1,\ n \pm 3,\ n \pm 5, \ldots$

Follows from symmetry of problem.

x is odd if reflected about $x = a/2$
i.e., changes sign — middle of box

$\phi_n(x)$ is odd function for n odd (antisymmetric fn)
$\phi_n(x)$ is even function for n even (symmetric fn)

$\phi_m^*(x)\,\phi_n(x)$ is odd function for $m = n \pm 1,\ n \pm 3,\ n \pm 5 \ldots$

even function otherwise

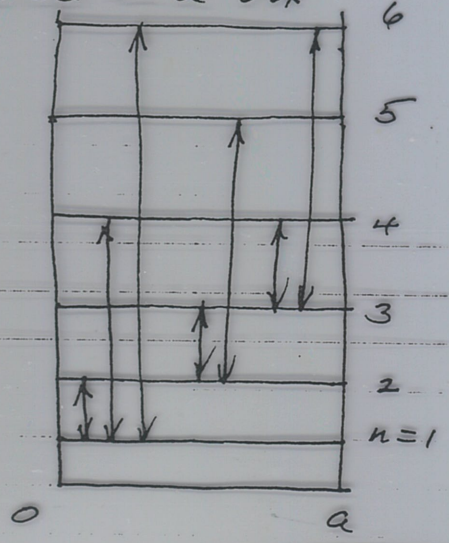
For integral not to vanish, integrand $\phi_m^*(x) \cdot x \cdot \phi_n(x)$

must be an even function upon reflection about $x = \frac{a}{2}$.

or $\phi_m^*(x)\,\phi_n(x)$ must be an odd function to compensate for x being an odd function

or $m = n \pm 1,\ n \pm 3,\ n \pm 3$

Selection rule for electric dipole transitions for a particle in a box



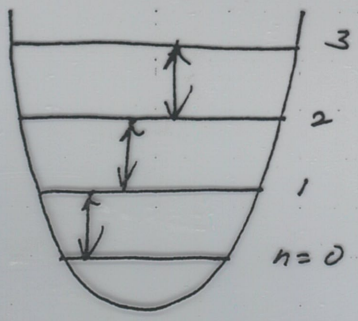
$\Delta n = \pm 1$ strongest

$\Delta n = \pm 3$ weaker, i.e., M_{nm} smaller

$\Delta n = \pm 5$ weaker yet

b) e^- in a harmonic potential

or harmonic oscillator



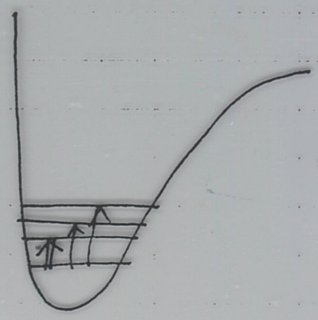
$W_n = h\nu_0 (n + \frac{1}{2})$

Selection rules for electric dipole transitions:

$\Delta n = \pm 1$ only

Note $\Delta n \pm 3, \pm 5$ transitions allowed by symmetry, but the integrals all turn out to vanish.

c) Anharmonic oscillator with cubic anharmonicity



no longer symmetry restriction

Selection rules

$\Delta n = \pm 1$ strongest

$\Delta n = \pm 2$ allowed, but weak

$\Delta n = \pm 3$

$0 \rightarrow 1$ transition usually called "fundamental"

$0 \rightarrow 2$ transition "1st overtone"

$1 \rightarrow 2$ transition "1st hot band"

d) Anharmonic oscillator with quartic anharmonicity

Problem again has symmetry

Selection rules for electric dipole transition

$\Delta n = \pm 1, \pm 3, \pm 5$ etc as for particle in the box

c) Hydrogen-like atom

Quantum state are identified by quantum # n, l, m_l

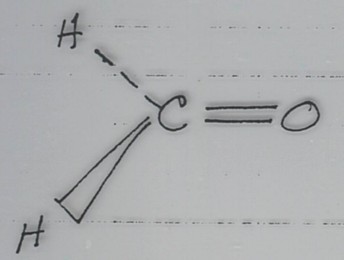
Selection rules for electric dipole transitions

$\Delta n = \pm 1, \pm 2, \ldots$ no restriction

$\Delta l = \pm 1$ only $s \leftrightarrow p$, $p \leftrightarrow d$, $d \leftrightarrow f$ transitions only

$\Delta m_l = \pm 1, 0$

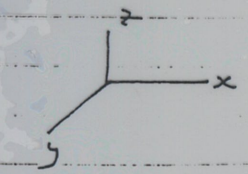
A more complex situation : Formaldehyde



Bonding description

Carbon : sp^2 hybridized

or $1s^2, (2sp^2)^3 2p_z$



2 C-H bonds are formed by carbon hybrid sp^2 orbital overlapping with hydrogen 1s

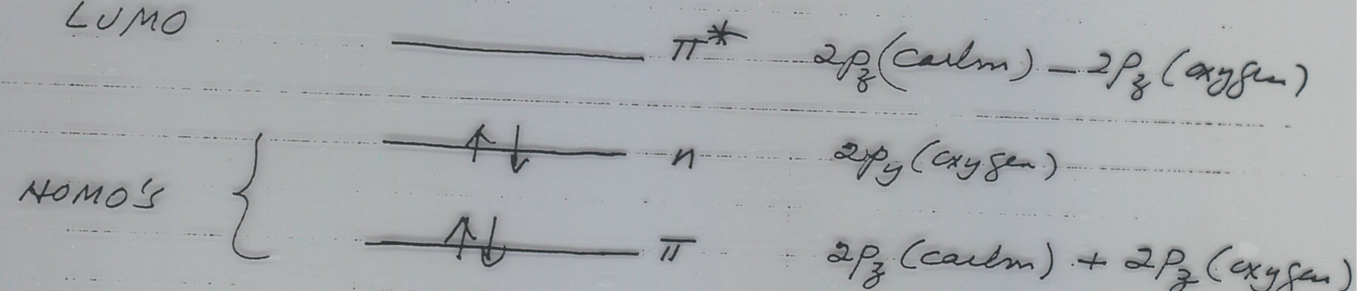
Third sp^2 orbital of Carbon overlaps with O $2p_x$ orbital to form C–O sigma bond

C $2p_z$ & O $2p_z$ orbitals form π-bond (π-MO with 2e⁻'s)

and remaining two oxygen electrons are in $2p_y$ orbital of the oxygen.

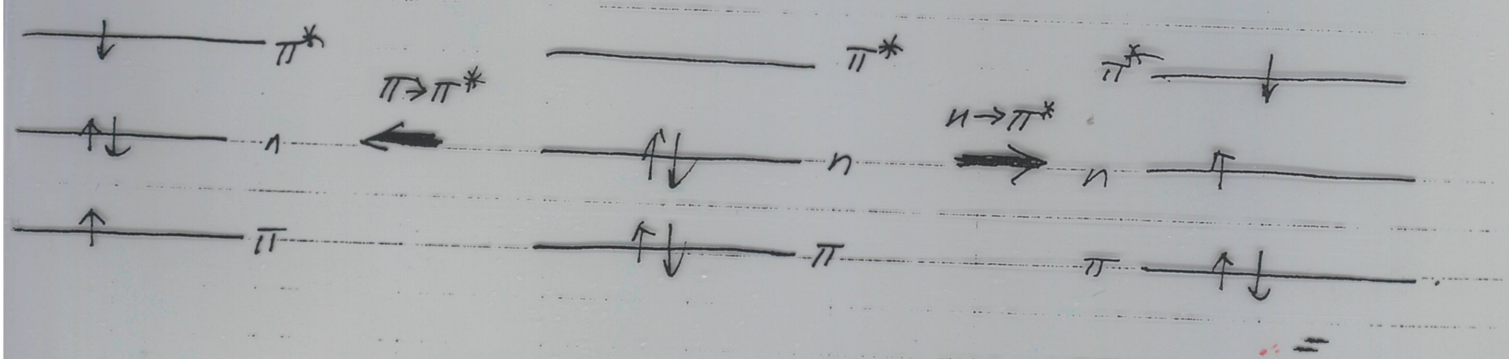
HOMO & LUMO's

highest occupied MO's
lowest unoccupied MO's



Ground State

Transitions (lowest energy ones)



$\pi - \pi^*$ transition

$$\int \phi_{\pi^*}^* \vec{\mu} \phi_\pi \, d\vec{\tau}$$

$$= \vec{i} \int \phi_{\pi^*}^* \mu_x \phi_\pi \, d\vec{\tau} + \vec{j} \int \phi_{\pi^*}^* \mu_y \phi_\pi \, d\vec{\tau} + \vec{k} \int \phi_{\pi^*}^* \mu_z \phi_\pi \, d\vec{\tau}$$

only $\int \phi_{\pi^*}^* \mu_x \phi_\pi \, d\vec{\tau}$ nonzero

others vanish by symmetry

Result transition dipole along $C=O$ bond

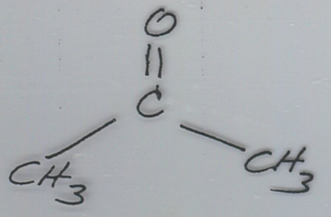
or intense absorption can only occur when $\vec{E}^*$ of light wave is parallel to $C=O$ bond; i.e., transition is polarized along $C=O$ bond.

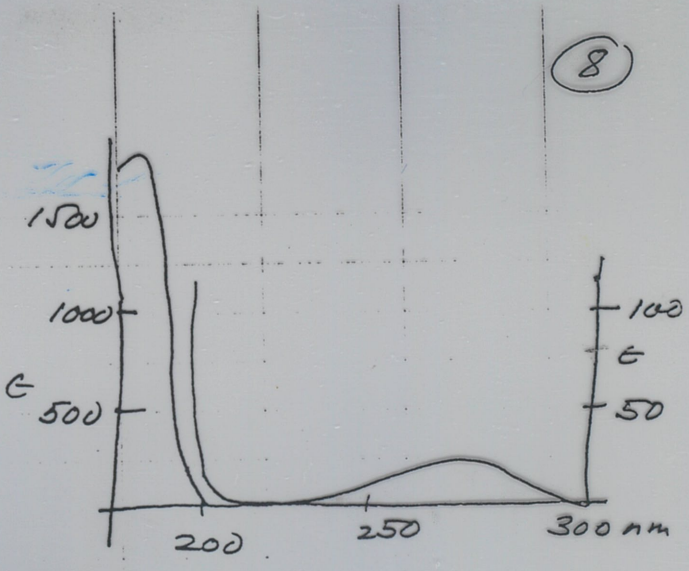
$n \rightarrow \pi^*$ transition

$$\int \phi_\pi^* \vec{\mu} \phi_n \, d\vec{\tau} = 0 \quad \text{for } \mu_x, \mu_y \ll \mu_z$$

$n \rightarrow \pi^*$ transition is symmetry forbidden; in practice can be observable, but extremely weak. Typically $n \rightarrow \pi^*$ absorption has an intensity ~ 1% of $\pi - \pi^*$ transition

Absorption spectrum of acetone





$\pi - \pi^*$
at 180 nm

$n \rightarrow \pi^*$
at 280 nm

Biological chromophores

→ Protein chromophores

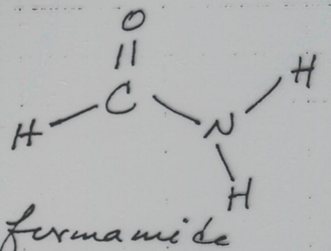
peptide bond
amino acid side chain (typ, tyr, phe)
prosthetic groups (hemes, flavins, blue copper)

→ Nucleic acid chromophores

bases

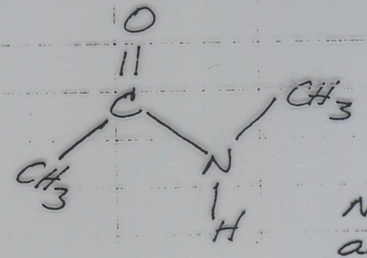
→ Peptide bond

typical models



formamide

or



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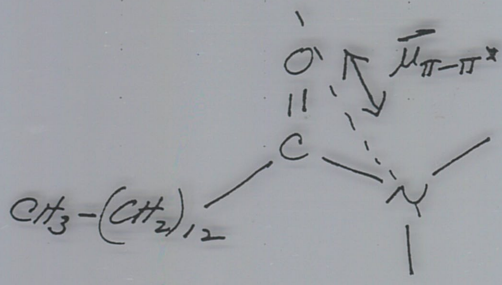
N-methyl acetamide

→ peptide bond

$n \rightarrow \pi^*$ absorption 210 – 220 nm $\varepsilon_{max} \sim 100$ weak

$\pi \rightarrow \pi^*$ 190 nm $\varepsilon_{max} \sim 7000$



myristamide

$\vec{\mu}_{\pi-\pi^*}$ not along C=O, but along a line between O & N in plane of peptide bond

determined by polarized absorption of single crystals

→ amino acid side chains

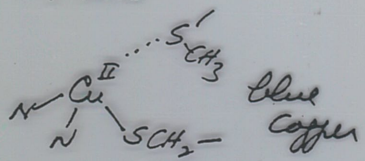
Trp (tryptophan) most intense 240 – 290 nm absorption complex (3 transitions of indole ring

but not present in large amounts in proteins

Tyr (tyrosine) 274 nm $\pi-\pi^*$ ($\varepsilon_{max} \sim 1400$)
analogous to 271 nm absorption in phenol

Phe (phenylalanine) 250 nm weak $\pi-\pi^*$
sym. forbidden
analogous to 256 nm absorption in benzene

→ **Prosthetic groups**

Protein	Prosthetic group	Longest wavelength absorption band λ_{max} nm	$\varepsilon_{max} \times 10^{-4}$	Second longest λ absorption λ_{max}	$\varepsilon_{max} \times 10^{-4}$
Amino acid oxidase rat kidney	FMN	455	1.27	358	1.07
Azurin, P. fluorescens also Plastocyanin, spinach stellacyanin	 blue copper	781	0.32	625 $Cu^{II} \leftarrow S$ charge transfer	0.35
Ceruloplasmin (human)	8 copper type 1, 2, 3	794	2.2	610	1.13
Cytochrome c (reduced) heme	Fe^{II}–heme	550	2.77		
Ferredoxin	$2Fe^{III}$-$2S^-$ cluster	421	0.98	330	1.33
Flavodoxin (C. pasteurianum)	FMN	443	0.91	372	0.79
pyruvate dehydrogenase (E. coli)	FAD	460	1.27	438	1.46
Rhodopsin (bovine)	retinal–lys	498	4.2	350	1.1
Rubredoxin (M. aerogenes)	$(Fe^{III}, 4\,Cys)$ tetrahedral	570	0.35	490	0.76
Xanthine oxidase	Fe, Mo	550	2.2		
Threonine deaminase (E. coli)	4 pyridoxal phosphate	415	2.6		

Others

retinal in bacteriorhodopsin

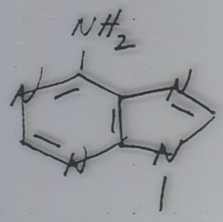
chlorophylls in reaction centers of cyanobacteria
PS I & II

hemes and coppers in cytochrome oxidase	heme	λ_{max}	420 nm 600 nm
	Cu		830 nm

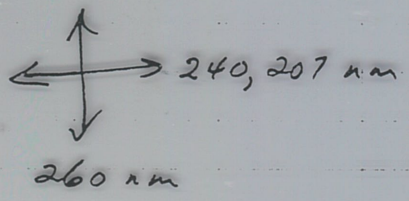
→ Nucleic Acid Bases

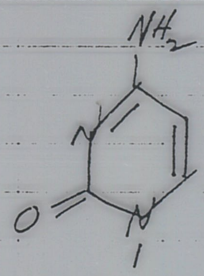
In DNA, RNA, absorption dominated by nucleic acid bases.

A
G
T, U
C



A





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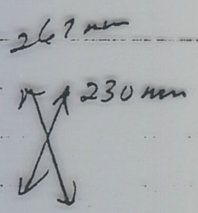

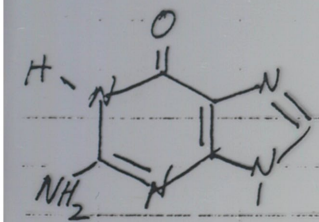

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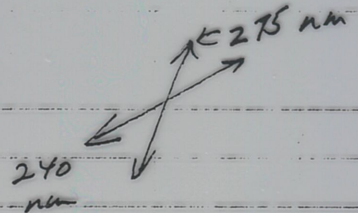

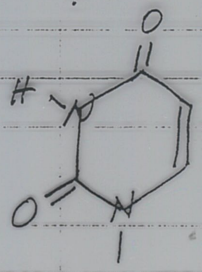

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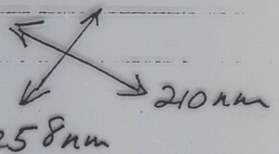


$\pi - \pi^*$ transition moments lie in plane of base

Vibrational Spectroscopy / Rotational Spectroscopy

Absorption arises from interaction of dipole moment of molecule with $\vec{E}^*$ of light wave.

Dipole moment of molecule $= \vec{\mu}(\underset{\sim}{R})$

$$= \vec{\mu}(\underset{\sim}{R}_0) + \sum_i \left(\frac{\partial \vec{\mu}}{\partial R_i}\right)_{R_0^i} (R_i - R_0^i) + \cdots$$

- $\vec{\mu}(\underset{\sim}{R}_0)$: permanent dipole moment → responsible for pure rotational spectroscopy
- $\left(\frac{\partial \vec{\mu}}{\partial R_i}\right)_{R_0^i}$: dipole derivative → responsible for
  - (a) vibrational-rotational spectroscopy of gases
  - (b) vibrational spectroscopy of liquids

→ **For diatomic molecule:**

a) Rotational spectroscopy

- No absorption unless $\mu(\underset{\sim}{R}_0)$ = permanent dipole moment $\neq 0$
- Selection rules $\Delta J = \pm 1$

H_2, N_2, F_2	no rotational absorption spectrum
HCl, HF	rotational absorption spectrum

b) Vibrational spectroscopy

No vibrational excitation unless $\left(\frac{\partial \mu}{\partial R}\right)_{R_0} \neq 0$

e.g. H_2 dipole derivative

Selection rule: $\Delta n = \pm 1, \ (\pm 2, \ldots)$

strong weak

→ Linear triatomic molecule

CO_2 no $\mu(R_0)$ or permanent dipole moment

vibrations

4 normal modes (normal coordinate Q_i)

symmetric stretch $\leftarrow O=C=O \rightarrow$ $\left(\frac{\partial \mu}{\partial Q_i}\right)_0 = 0$

asymmetric stretch $\overrightarrow{O}=\overleftarrow{C}=O \rightarrow$ $\left(\frac{\partial \mu}{\partial Q_i}\right)_0 \neq 0$

bending

$O=\overset{\uparrow}{C}=O$ (O ↓, O ↓) $\left(\frac{\partial \mu}{\partial Q_i}\right)_0 \neq 0$

$\overset{\oplus}{O}=\overset{\ominus}{C}=\overset{\oplus}{O}$ $\left(\frac{\partial \mu}{\partial Q_i}\right)_0 \neq 0$

Accordingly

symmetric stretch — infrared inactive

asymmetric stretch, bending — infrared active

→ Peptide Group

Characteristics of principal infrared absorption bands

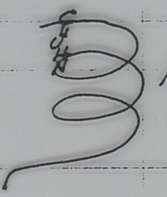
Vibration $\left(\frac{\partial \mu}{\partial Q}\right)$		Hydrogen-bonded forms: α-helix frequency	dichroism	β-sheet* frequency	dichroism*	Non-hydrogen bonded forms frequency
N–H stretch	←N–H→ ↔	3290–3300 cm^{-1}	∥	3280–3300 cm^{-1}	⊥	~3400 cm^{-1}
Amide I C=O stretch	←C=O→ ↔	1650–1660 cm^{-1}	∥	1630 cm^{-1}	⊥	1680–1700 cm^{-1}
Amide II	←C–N→ ↙	1540–1550 cm^{-1}	⊥	1520–1525 cm^{-1}	∥	<1520 cm^{-1}

polarized near C–N bond or ⊥ N–H bond

* α-helix

N–H ···· O=C hydrogen bonds ∥ helix axis

→ α-helix axis



↑ α-helix axis

β-sheet

N–H ···· O=C

↑ long-axis of extended peptide chain