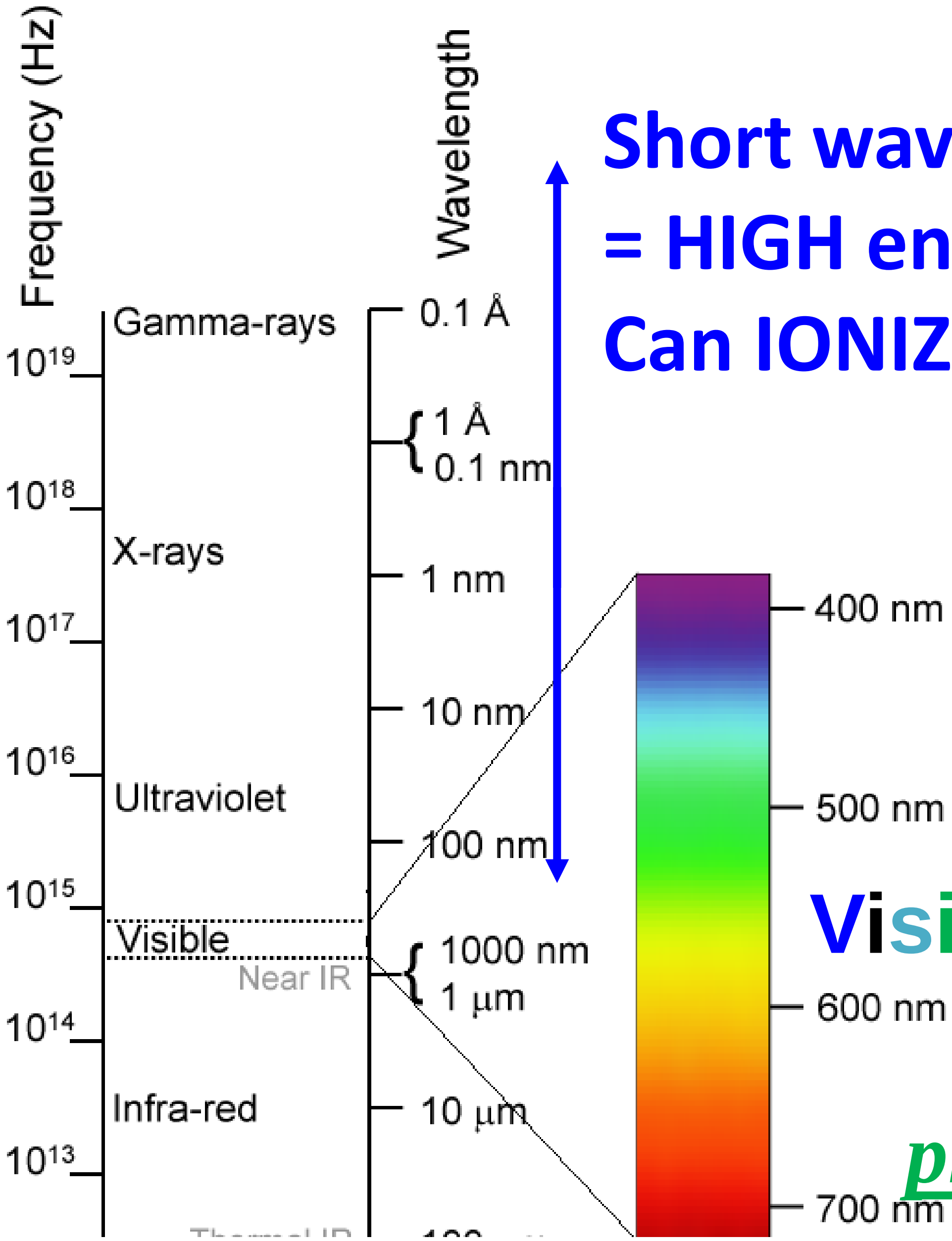


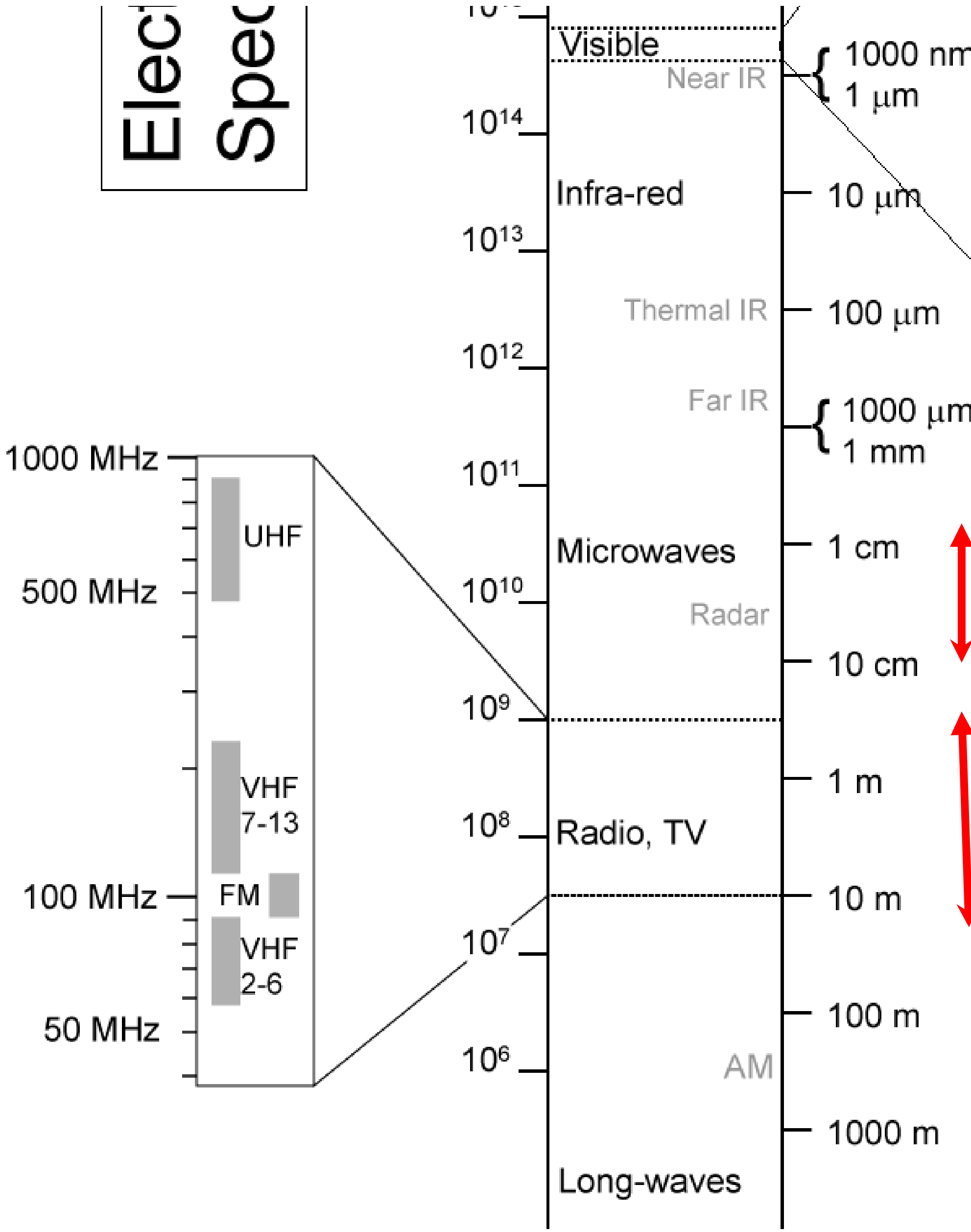

# Short wavelengths = HIGH energy photons Can IONIZE < 250 nm

**UV C <300 nm**
**UV B 310-290 nm**
**UV A 310-400 nm**

**Photochemistry out to 700 nm**

**Vision**

***photosynthesis.***




**Makes molecule VIBRATE**

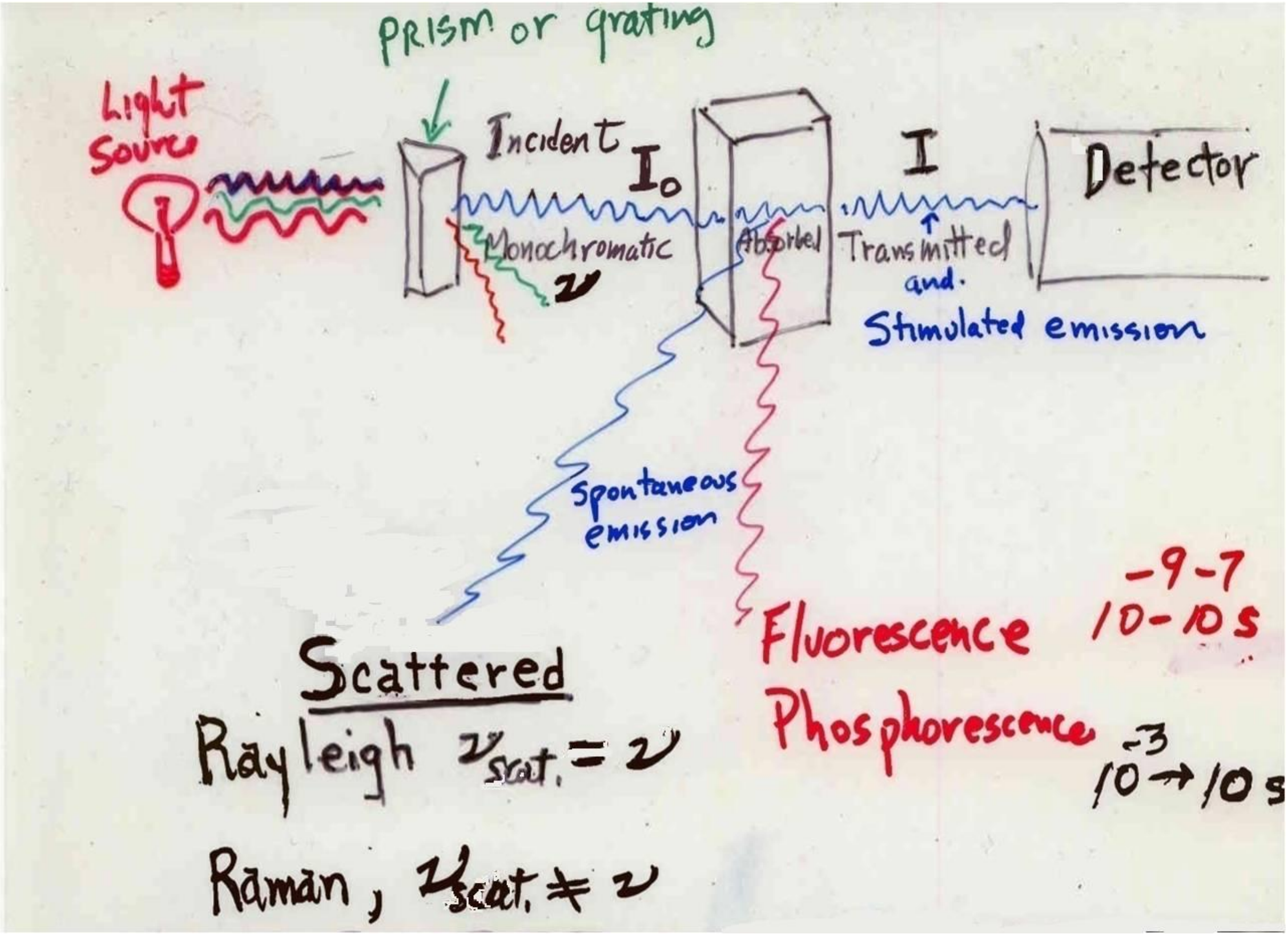
**Flip electron spin**

**Make molecules ROTATE**

**CELL phones**

**Flip NUCLEAR spin**

# <u>Long</u> wavelengths = Very LOW energy photons


PRISM or grating
Light Source
Incident
$I_0$
$I$
Detector
Monochromatic
$\nu$
Absorbed
Transmitted
and
Stimulated emission
Spontaneous emission
Fluorescence $10^{-9}-10^{-7}$ s
Phosphorescence $10^{-3} \rightarrow 10$ s
Scattered
Rayleigh $\nu_{scat.} = \nu$
Raman, $\nu_{scat.} \neq \nu$

# Beer-Lambert Law (A = εcx )

photon + M ---> M* (electronically excited molecule)

$$\frac{d[\text{photon}]}{dt} = -k[M][\text{photon}] = -k\,[M]\,I$$

$$[\text{photon}]\text{ is proportional to light intensity } = I$$

$$\frac{dI}{dt} = -k[M]I\text{, where } k[M] \text{ is a } \textit{pseudo first-order rate constant}$$

$$\text{for the disappearance of photons (time, } t = x/\text{speed of light)}$$

$$f = \frac{I}{I_0} = e^{-k[M]t} = e^{-k[M]x/c} = 10^{\frac{-k[M]x}{2.303c}} = 10^{-\varepsilon[M]x} = 10^{-A}$$

In this context **f = fraction of photons remaining after travelling distance x**

i.e., **f= Transmittance = T = $10^{-\varepsilon cx}$ = $10^{-A}$**

**These two are <u>EXACTLY</u> the <u>SAME</u> thing. Learn and use <u>BOTH</u>**

**$10^{-A}$** is just telling you that **A = εcx = –log T**

# Beer-Lambert Law ($A = \varepsilon c x$)

suppose **A = 2** What fraction of light is transmitted?

$\mathbf{10^{-2} = 0.01 = 1\ \%}$

Now, double the concentration.
What fraction of light is transmitted?

$\mathbf{10^{-4} = 0.0001 = 0.01\ \%}$

Now, double the path length using this concentration.
What fraction of light is transmitted?

$\mathbf{10^{-8} = 10^{-6}\ \%}$

Now, change the wavelength until $\varepsilon$ is doubled.
What fraction of light is transmitted at this wavelength?

$\mathbf{10^{-16} = 10^{-14}\ \%}$

# UV absorption of Amino Acids



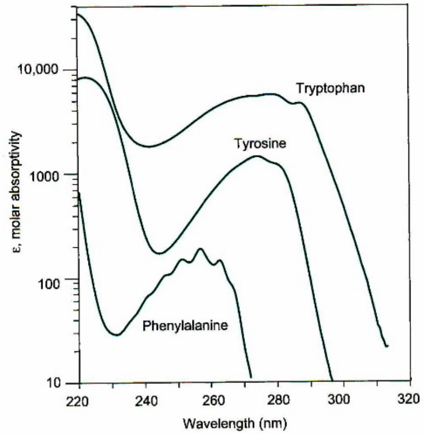



**FIGURE 13.15** Absorption spectra of the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) at pH 6.

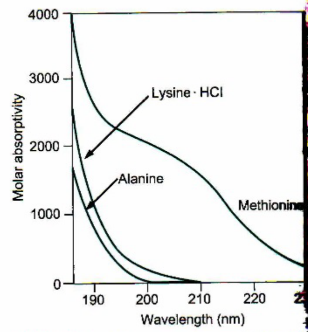
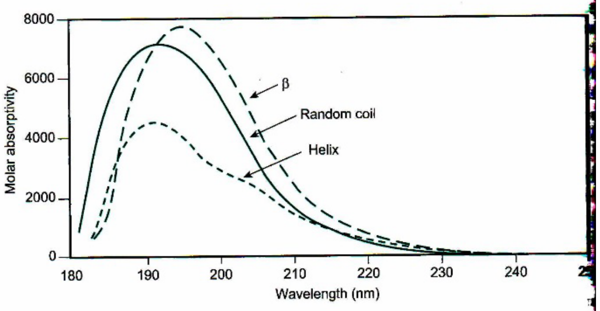


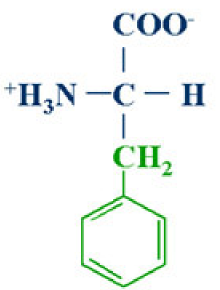

**FIGURE 13.16** UV-absorption spectra of three α-amino acids in aqueous solution at pH 5.

## Polypeptide Spectra

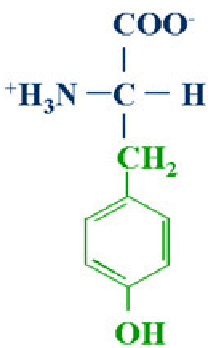
The contribution of the amide linkages to the absorption spectra can be see by comparing the spectrum of lysine hydrochloride in figure 13.16 with that of poly-L-lysine hydrochloride in the random-coil form (figure 13.17). The broad absorp tion centered at 192 nm ($\varepsilon_{192} = 7100\ M^{-1}\ cm^{-1}$) is characteristic of the amide linka in poly-L-lysine and increases the absorbance in this region by eightfold over that the free amino acid. All proteins have contributions to the absorption spectra in the region around 190 nm (180 to 200 nm) from the polypeptide backbone; however, the are accompanied by absorption contributions from certain of the side chains, especial the aromatic ones.

**FIGURE 13.17** UV-absorption spectra of poly-L-lysine hydrochloride in aqueous solution; random coil, pH 6.0, 25°C; helix, pH 10.8, 25°C; β form, pH 10.8, 52°C.
(K. Rosenheck and P. Doty, The Far Ultraviolet Absorption Spectra of Polypeptide and Protein Solutions and Their Dependence on Conformation PNAS 1961 47 (11) 1775–1785. Reprinted by permission of the Estate of Paul M. Doty.)

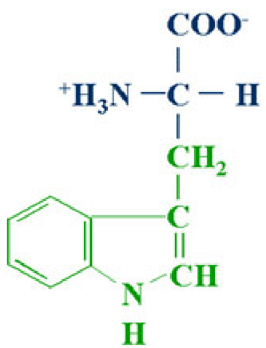



Phenylalanine




Tyrosine




Tryptophan

# UV absorption of Amino Acids

504 Chapter 13 | Optical Spectroscopy

**Note the Log scale**

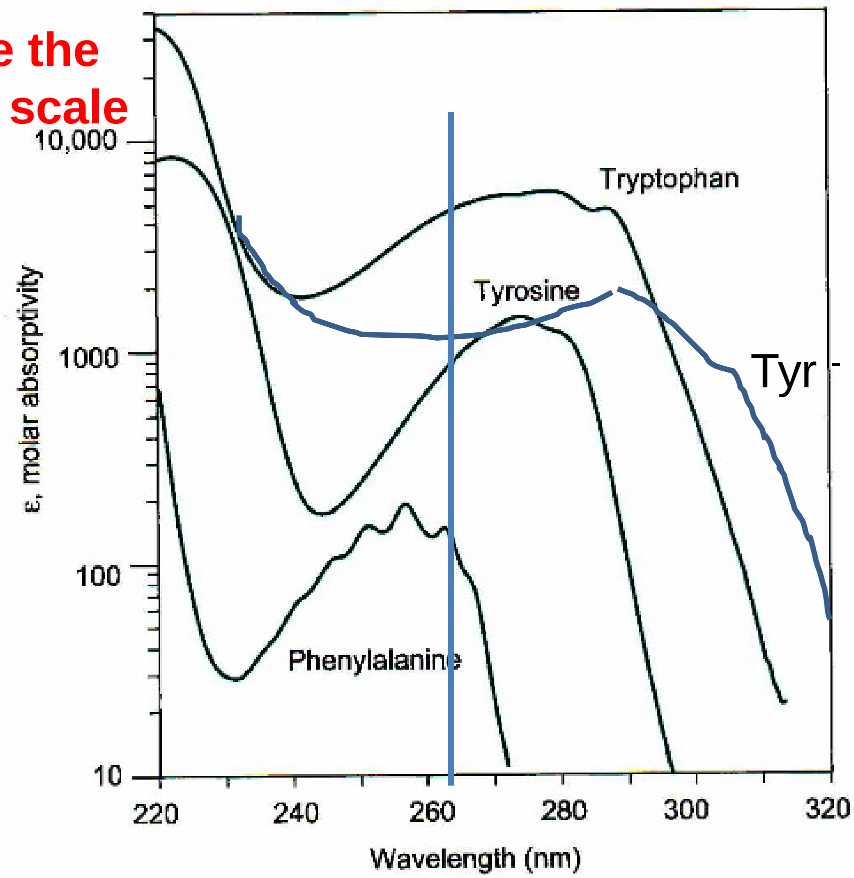
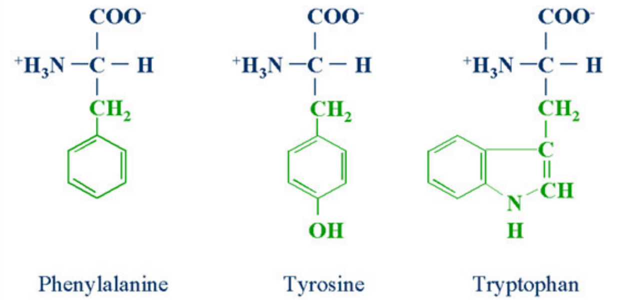



FIGURE 13.15 Absorption spectra of the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) at pH 6.



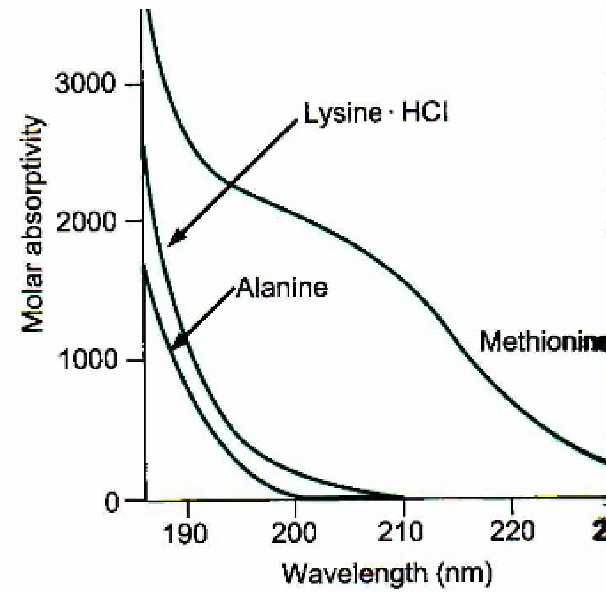




FIGURE 13.16 UV-absorption spectra of three α-amino acids in aqueous solution at pH 5.

What is absorbance of a 0.01 M solution of **phenylalanine** if path is 1 cm

**A = εcx** **A = 100 x .01 x 1= 1** **Fraction transmitted = ?** **0.1**

What is absorbance of a 0.01 M solution of **tyrosine** is path if 1 cm

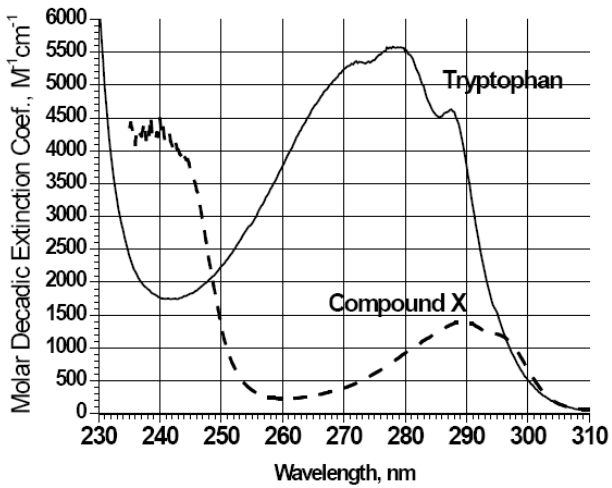
**A = 1000 x .01 x 1= 10** **Fraction transmitted = ?** **$10^{-10}$**

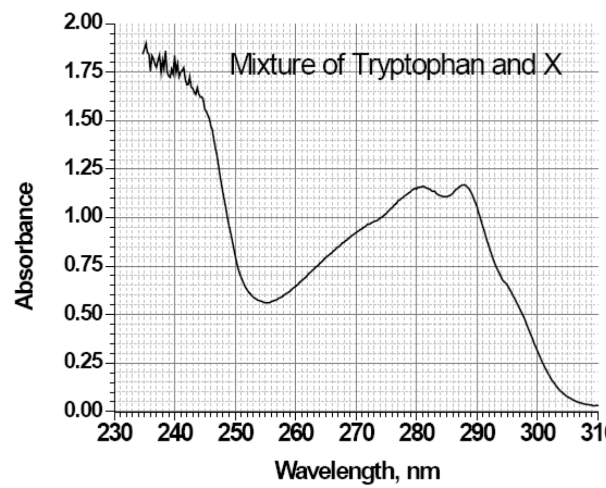
**HOMEWORK #7**

**CHMY 361** **HANDOUT #10**
**Nov. 18, 2013**

**Due Fri., Nov. 22 in class**

**1.** Given the information on the left hand plot, find the molar concentrations of tryptophan and compound X in the solution that gives the absorbance spectrum on the right. The path length is ***0.10 cm***.(Note To have reasonable accuracy, use wavelengths with A = 0.2 or greater and extinction coef. = 500 or greater to solve the problem. (realize that the problem cannot be solved if you pick two wavelengths for which the ratio of extinction coefficients is the same.) That will give you two identical equations, but you need two independent equations to solve for 2 unknowns. Therefore try to pick two wavelengths such that the ratio of extinction coefficients differs by a factor of 2 or greater.




**2**. Assuming the absorption band at 240 nm for compound X is not due to the lowest excited electronic state, make a drawing of the fluorescence spectrum you expect if you excite the molecule with 240 nm light.

## Using the Beer-Lambert Law to determine concentrations of a mixture of ***two*** absorbing species

Absorbance is additive:

Consider 2 absorbers M & N

$A_1 = \epsilon_1^M [M] + \epsilon_1^N [N]$ at wavelength 1

$A_2 = \epsilon_2^M [M] + \epsilon_2^N [N]$ at " 2 $\quad l = 1$

What if $\varepsilon^M{}_1 = \varepsilon^N{}_1$??? (*isosbestic point*)

**Then $A_1 = \varepsilon[M] + \varepsilon[N] = \varepsilon\,([M] + [N])$**

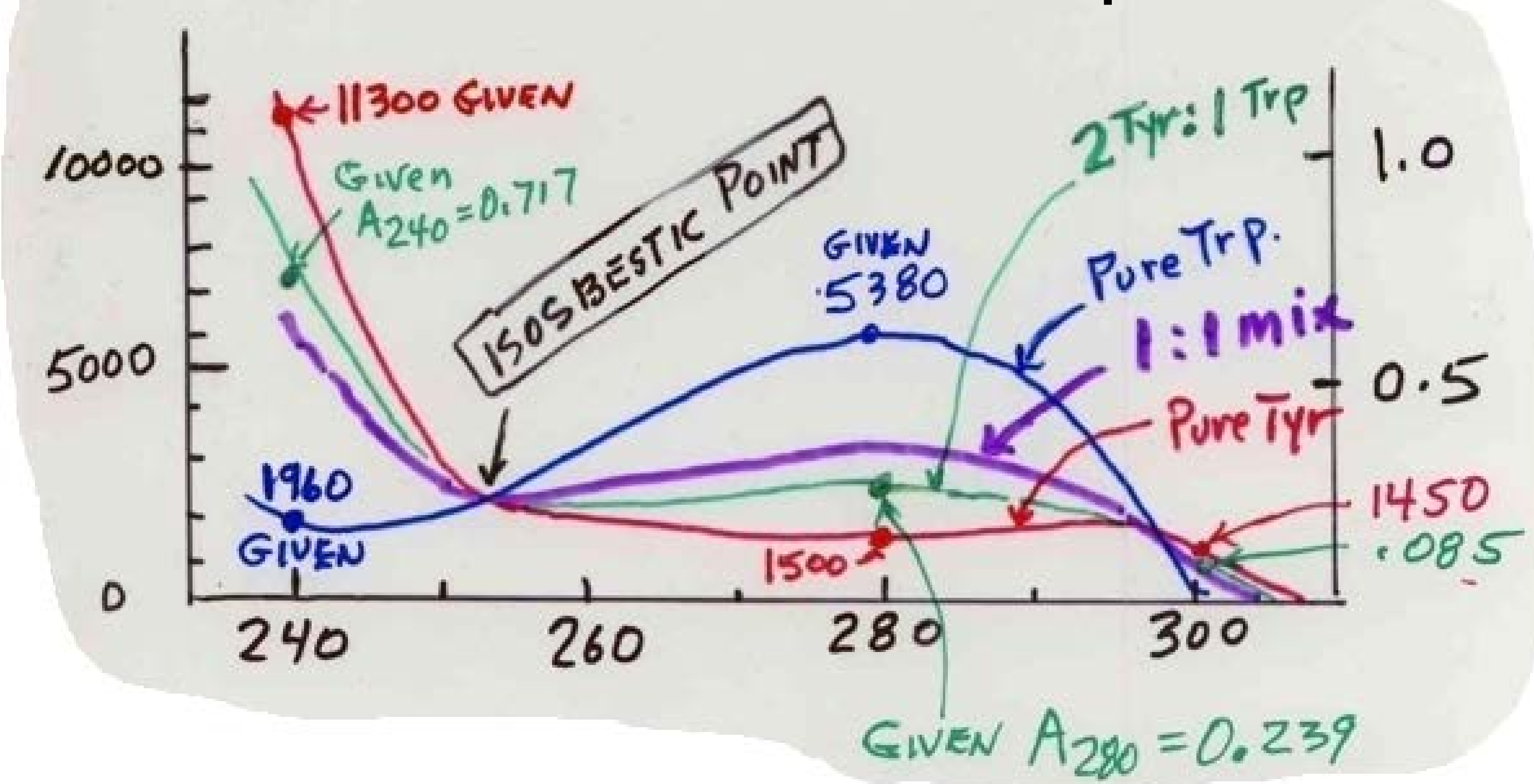
**In other words at isosbestic point you get the <u>TOTAL</u> concentration**

Green is the unknown mixure of Trp and Tyr

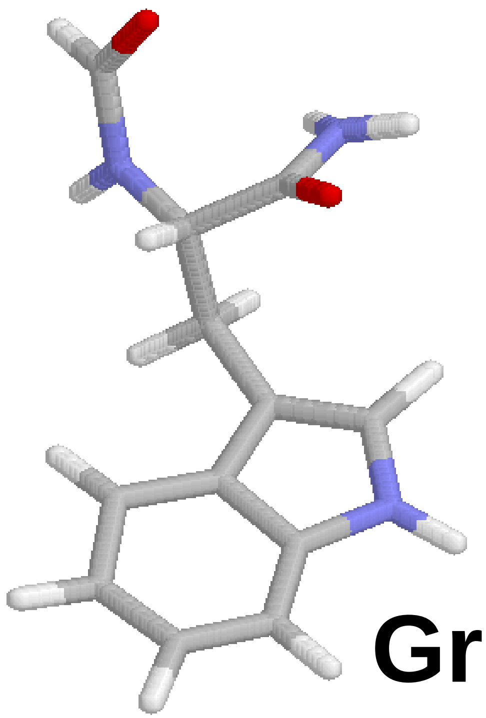
Red is pure Tyr $^-$ Blue is pure Trp

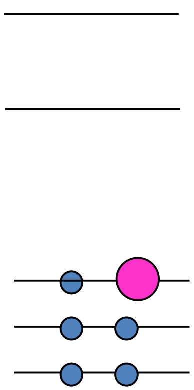
Purple is 50-50 Trp + Tyr $^-$

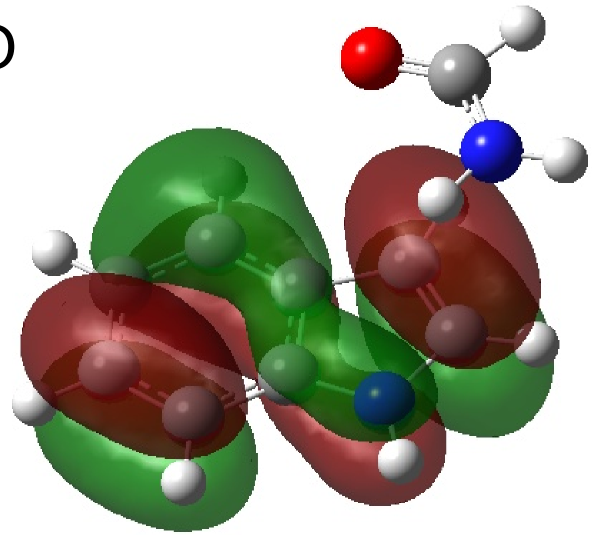
**Example 13.1**



# What happens during absorption of light by Tryptophan?

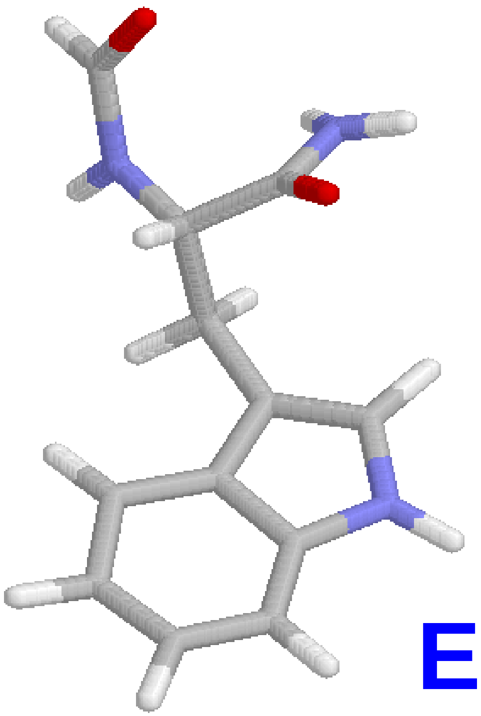


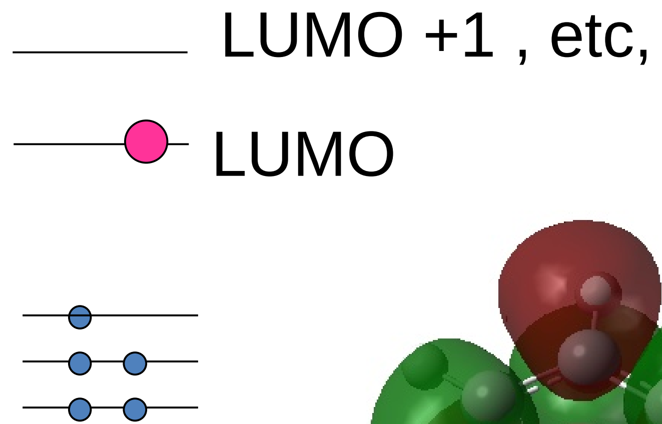


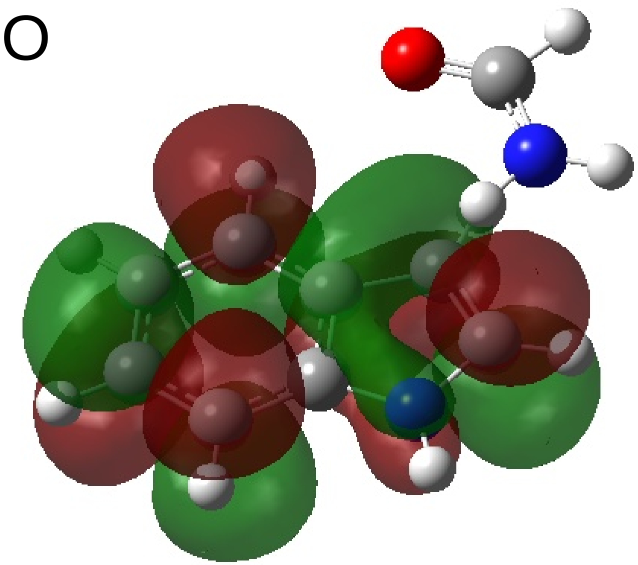



**Ground State**

**Highest Occupied Molecular Orbital (a linear combination of atomic p orbitals)**

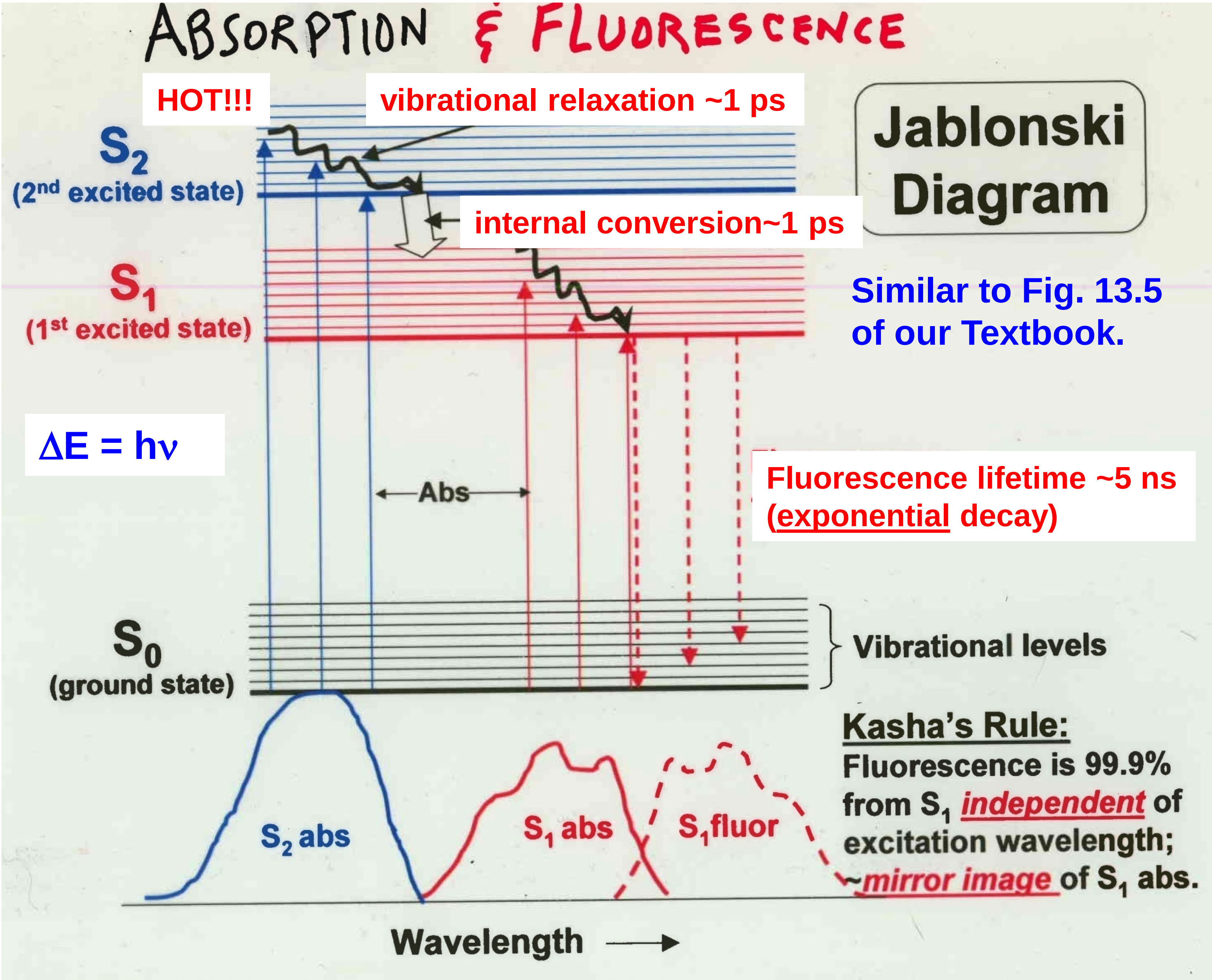




**Excited State (fluorescing state)**

**Lowest Unoccupied Molecular Orbital (electron excited)**


ABSORPTION & FLUORESCENCE
HOT!!!
vibrational relaxation ~1 ps
Jablonski Diagram
$S_2$
($2^{nd}$ excited state)
internal conversion~1 ps
$S_1$
($1^{st}$ excited state)
Similar to Fig. 13.5 of our Textbook.
$\Delta E = h\nu$
Abs
Fluorescence lifetime ~5 ns
(exponential decay)
$S_0$
(ground state)
Vibrational levels
Kasha's Rule:
Fluorescence is 99.9% from $S_1$ independent of excitation wavelength; ~mirror image of $S_1$ abs.
$S_2$ abs
$S_1$ abs
$S_1$fluor
Wavelength