Beer-Lambert Law \((A = \varepsilon cx)\)

suppose \(A = 2\)  
What fraction of light is transmitted?

\[ f = \text{Transmittance} = T = 10^{-\varepsilon cx} = 10^{-A} = 10^{-2} = 0.01 = 1\% \]

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

\[ 10^{-4} = 0.0001 = 0.01\% \]

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

\[ 10^{-8} = 10^{-6}\% \]

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

\[ 10^{-16} = 10^{-14}\% \]
UV absorption of Amino Acids

Polypeptide Spectra

The contribution of the amide linkages to the absorption spectra can be seen 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 absorption centered at 192 nm (ε₁₉₂ ≈ 7100 M⁻¹ cm⁻¹) is characteristic of the amide linkages in poly-l-lysine and increases the absorbance in this region by eightfold over that of 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, these are accompanied by absorption contributions from certain of the side chains, especially the aromatic ones.
What is absorbance of a 0.01 M solution of phenylalanine if path is 1 cm

\[ A = \varepsilon_{\text{cx}} \]

\[ A = 100 \times 0.01 \times 1 = 1 \]

Fraction transmitted = ? 0.1

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

\[ A = 1000 \times 0.01 \times 1 = 10 \]

Fraction transmitted = ? \(10^{-10}\)
Using the Beer-Lambert Law to determine concentrations of a mixture of \textit{two} absorbing species

Absorbance is additive:
Consider 2 absorbers \(M \& N\)

\[ A_1 = \varepsilon^M [M] + \varepsilon^N [N] \text{ at wavelength } 1 \]

What if \(\varepsilon^M_1 = \varepsilon^N_1??\)

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

In other words at isosbestic point you get the \textbf{TOTAL} concentration
Green is the unknown mixture 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)
Lowest Unoccupied Molecular Orbital (electron excited)

Excited State (fluorescing state)

LUMO +1, etc.

LUMO
Fluorescence lifetime ~5 ns (exponential decay)

Similar to Fig. 13.5 of our Textbook.

HOT!!!

vibrational relaxation ~1 ps

internal conversion ~1 ps

$\Delta E = h\nu$

Kasha’s Rule:
Fluorescence is 99.9% from $S_1$ independent of excitation wavelength; ~mirror image of $S_1$ abs.
What is fluorescence lifetime?

\[
d(\text{Intensity})/dt = -k \cdot \text{Intensity}
\]

Fluor. intensity at time \(t\) = (Fluor. Intensity at time 0) \(\times e^{-kt}\)

\[
= (\text{Fluor. Intensity at time 0} \times e^{-t/\tau})
\]

\(\tau = \text{“lifetime = 1/k} \)

\(\tau = \text{inverse of 1}\text{st order rate constant} \)

**Fluorescence “Quenching”**

A reaction with another molecule that competes with the rate of fluorescence
Highest Occupied Molecular Orbital (a linear combination of atomic p orbitals)

Ground State
Lowest Unoccupied Molecular Orbital (electron excited)

Excited State (fluorescing state)

Excited State (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)
Iodide ion collides; (has higher HOMO) will quench fluorescence
Electron transfer from I⁻ to indole makes a radical pair that cannot fluoresce. (would violate Pauli exclusion)

Radical Pair (can’t fluoresce)

Electron transferred from iodide to vacancy in HOMO of ring i.e., QUENCHING
Phosphorescence

$S_1$
(1st excited state)

$S_0$
(ground state)

Intersystem crossing, ns

Fluorescence:
~ 5 ns

Lowest triplet state
(unpaired spins)

Phosphorescence
Slow, forbidden ($10^{-6}$ to 10 seconds)

Requires rigid, oxygen free environment

Wavelength ->
Fig. 10.12 Absorption and fluorescence of bacteriochlorophyll. (a) Energy-level diagram showing spectral transitions (vertical arrows). The energy levels are broadened (shading) by vibrational sublevels that are not usually resolved in solution spectra. (b) Absorption spectrum corresponding to energy levels of part (a). This spectrum is turned 90° from the usual orientation to show the relation to the energy levels. (c) Radiationless relaxation (dashed arrows) and fluorescence (shaded arrow). (d) Fluorescence emission spectrum corresponding to part (c). Note the red shift of the fluorescence compared with the corresponding $Q_v$ absorption illustrated in parts (a) and (b). (From K. Sauer, in Bioenergetics of Photosynthesis, Govindjee, ed., Academic Press, New York, 1975, pp. 115–181.)