

Ultraviolet And Fluorescence Spectroscopy

from chapter _____ in the recommended text

A. Introduction

B. Fundamental Physics

Quanta of UV radiation are *more* energetic than those corresponding to vibrational states less energetic than quanta corresponding to the *X-ray* range of the electromagnetic

UV region corresponds to promotion of an electron from a *ground* state energy level to a(n) *excited*

maximum absorption of a UV peak ($\lambda_{\text{max abs}}$ in nm) is *inversely* proportional to the *energies* of the quanta involved, and the degree of absorbance is *directly* related to their *number*.

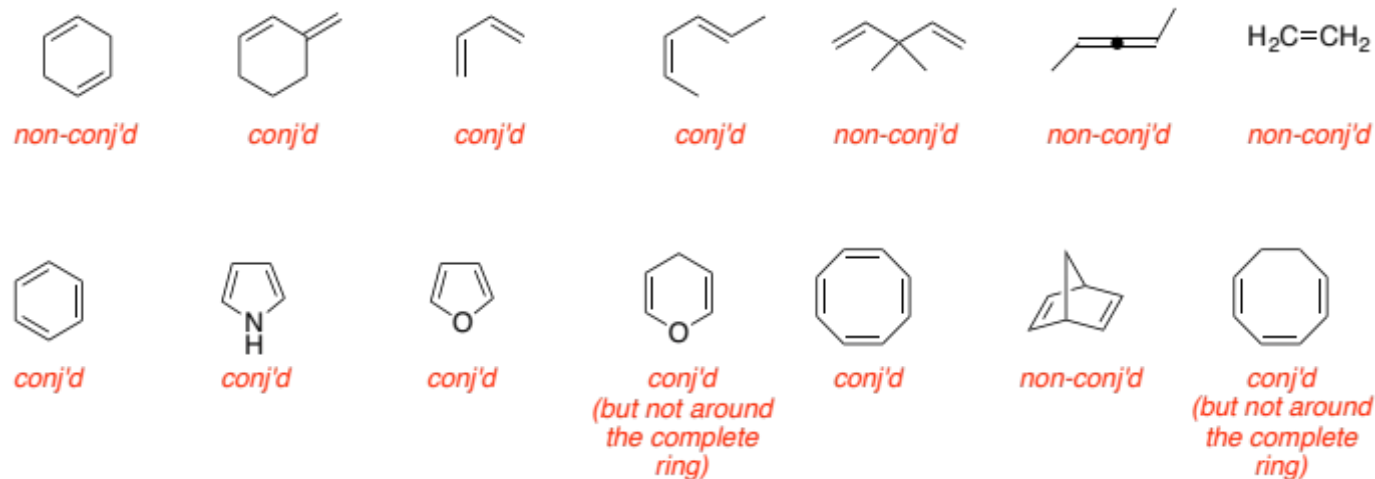
UV absorbances tend to be *broad* peaks because promotion of an electron can place it within different *IR-vibrational* states in the excited energy level.

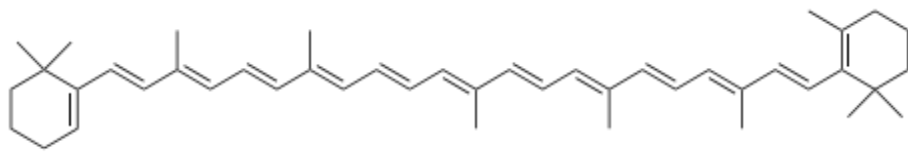
Groups that absorb quanta in the UV are called *chromophores* and these include aromatic systems,

the more extended a conjugated system is, the larger its UV *cross-section* and the *more* photons it

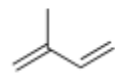
C. Molecular Orbital Diagrams Of Alkenes, Dienes, and Polyenes

ones in which C=C double bonds are linked *just one* C-C bond.

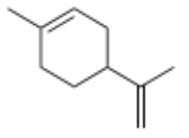




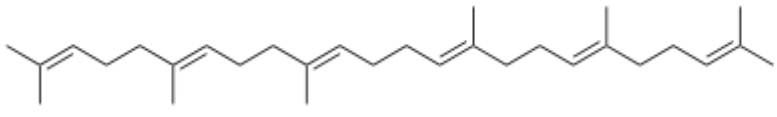
β-carotene conj'd



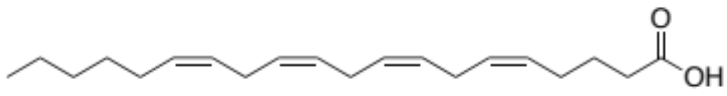
isoprene conj'd



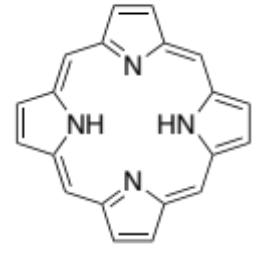
limonene non-conj'd



squalene non-conj'd

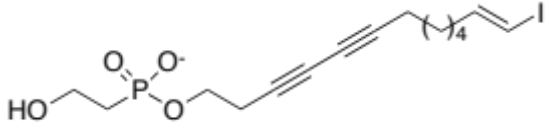


arachidonic acid non-conj'd

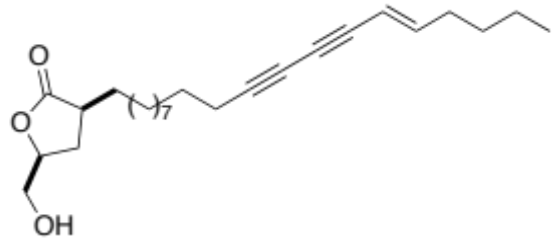


porphyrin conj'd

Conjugation *is* possible between unsaturated double and triple bonds,



phosphatidylserine non-conj'd



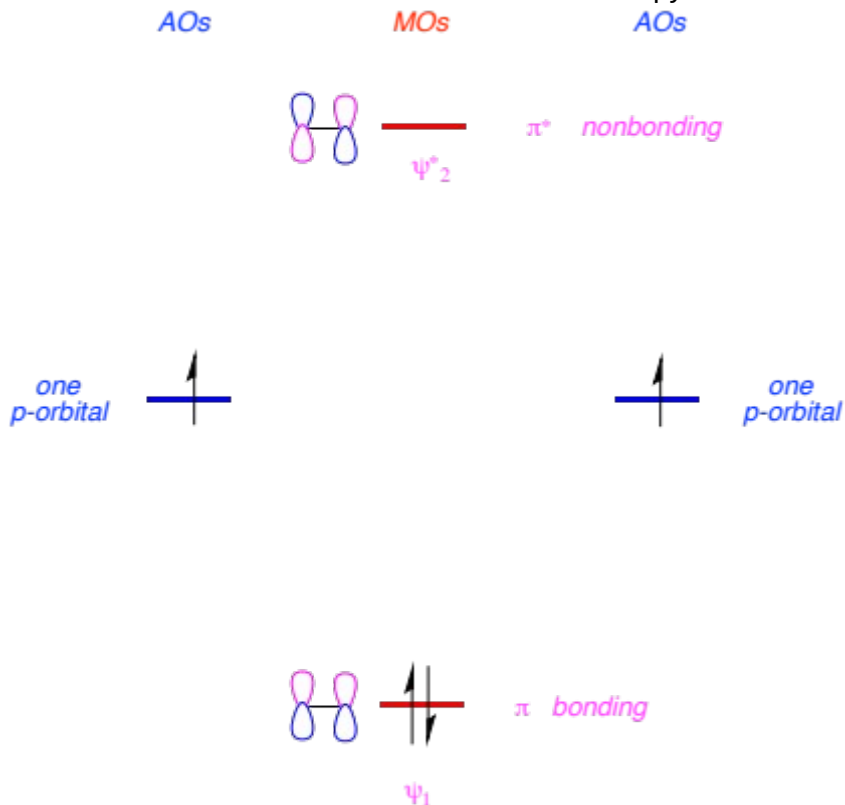
debrisoquine conj'd

Molecular orbital theory is *an alternative to* valence bonding to describe chemical bonding.

When n atomic orbitals mix they *always* form n molecular orbitals.

two π -molecular orbitals (MOs) are formed from the 2 parallel, unhybridized p-orbitals

the other has more, and these are called *bonding* - and *antibonding* π^* -orbitals, respectively.
Maximal overall stabilization occurs when $2e$ occupy the π -orbital.



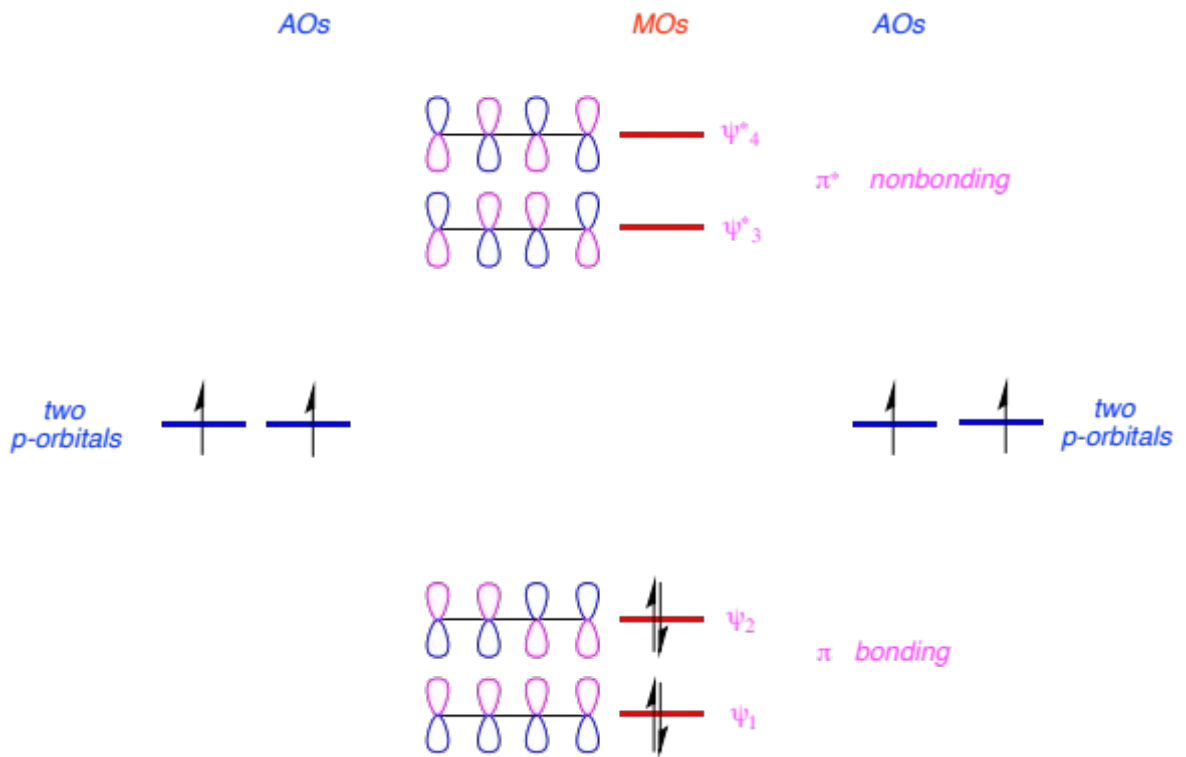
π - to a π^* -orbital that corresponds to quanta in the *ultraviolet excited* state
loss of *IR*

Systems with more double bonds in conjugation have *larger* cross-sections, therefore they absorb *more* photons from a white light source

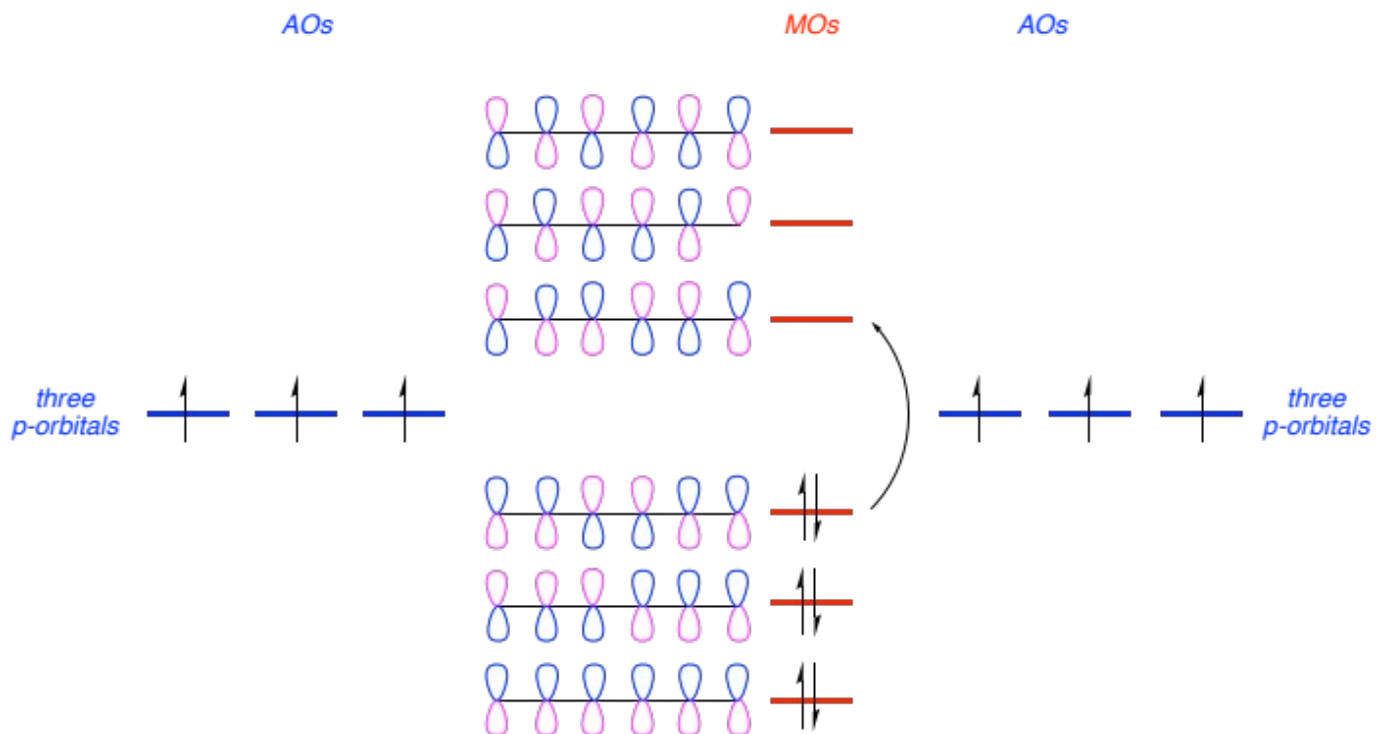
This corresponds to the *absorbance* of the *chromophore* and that is expressed in terms of *extinction coefficients*.

A more conjugated polyene has a *larger* extinction coefficient than a less conjugated one.

1,3-butadiene:



1,3,5-hexatriene:



D. UV Spectroscopy

More conjugation *decreases* gaps between the highest occupied molecular orbital
This is because the HOMO has a *lower* energy, and the LUMO is *higher*, relative to ethene.

corresponds to absorbance of *lower* energy quanta of *increased* wavelengths.

infra-red (IR) absorptions correspond to transitions between *vibrational* energy states, while *electronic* transitions correspond to quanta

Thus *IR* transitions correspond to stretching bonds
electrons are promoted from one molecular orbital to one higher in energy by quanta in the *UV* range

Energies involved for *UV* transitions are greater than ones for *IR*.

Transitions **A**, **B** and **C** correspond to *IR* transitions, while **D**, **E**, and **F** are between *UV* states.

transitions **D**, **E**, and **F** are likely to be *greater* than for transitions like **G**.

they involve transitions between *multiple* vibrational level(s)

10 °C enables a molecule to overcome a *smaller*

E. Quantifying Absorbance: Extinction Coefficients

The peak(s) with lowest absorbance is (are) at **a**.

Maximal absorbance ($\lambda_{\text{max abs}}$) for state **b** is red-shifted relative to **c** and blue shifted relative to **a**.

The $\lambda_{\text{max abs}}$ for state **b** is around **795** nm, and that for **c** occurs at about **810** nm.

The most intensely colored state is **b**, while that which absorbs photons of highest energy is **a**.

The equation below is *Beer-Lambert's* Law, where the absorbance of the material is **A**, the molar extinction coefficient is ϵ , the molar concentration is **c**, and the pathlength is **l**.

$$\epsilon = A / cl$$

Units of molar extinction coefficient are therefore $M^{-1}cm^{-1}$.

Molar extinction coefficients tend to be *directly* correlated with the “cross section”

1,3-butadiene with has a *lower* molar extinction coefficient, a *smaller* cross section, and a *lower* $\lambda_{\text{max abs}}$.

slope of this line is *directly* correlated with the molar extinction coefficient of the compound.

Assays that use UV to measure amounts of colored compounds are a form of *colorimetry*.

F. Fluorescence Spectroscopy

following diagram correspond to *UV* and transitions between *electronic* energy levels.

it may relax via transitions like *d – g* that correspond to *vibrational* emissions.

After half-lives that are typically in the *nano*-second, the molecule will relax via emission of *fluorescence*

Fluorescence will only occur for relatively *rigid* molecules for which competing rotational

relatively brilliant hence fluorescence spectroscopy can be a *sensitive* way of detecting “fluors”.

Fluorescence can be used to detect fluor-labeled organelles in cells with *higher* sensitivity than UV spectroscopy, and much *higher* sensitivity than IR.

Many DNA sequencing techniques rely on *fluorescence* spectroscopy

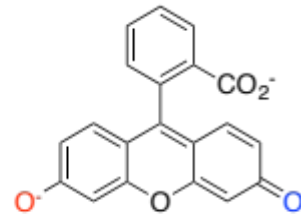
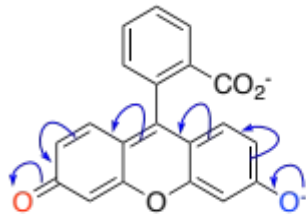
cell sorting (FACS) detects numbers of cells labeled with different *fluors*.

tend to be *not very* sensitive because a colored analyte is generated *stoichiometrically*.

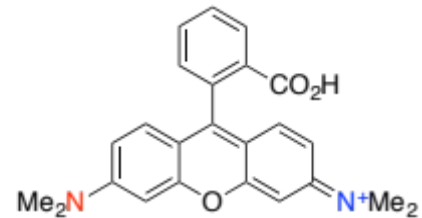
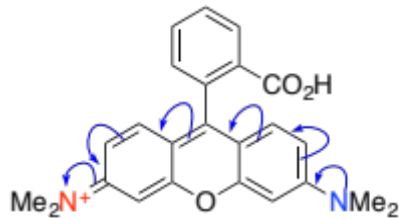
Fluorescent compounds tend to be relatively *rigid* structures that shift charges



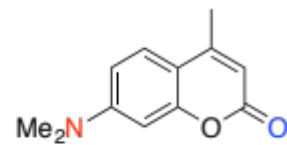
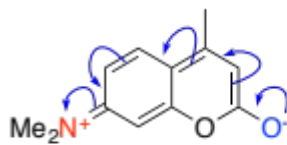
a BODIPY



a fluorescein



a rhodamine



a coumarin

the fluorescence of the molecule will tend to be *insensitive* to solvent polarity;
solvatochromaticity

Fluorescence of the *coumarin* is likely to be most sensitive to the dipole moment of the solvent it is in because the oscillation of charge in this molecule is unsymmetrical.

most pH sensitive fluorescence around 7 are the *fluorescein / coumarin*, whereas the *BODIPY* is least sensitive to pH.

This is because *fluorescein / coumarins* contain phenolate-O⁻ groups that can be protonated as the pH

highest energy light *V*, and which of them emit at the longest wavelength *R*.

