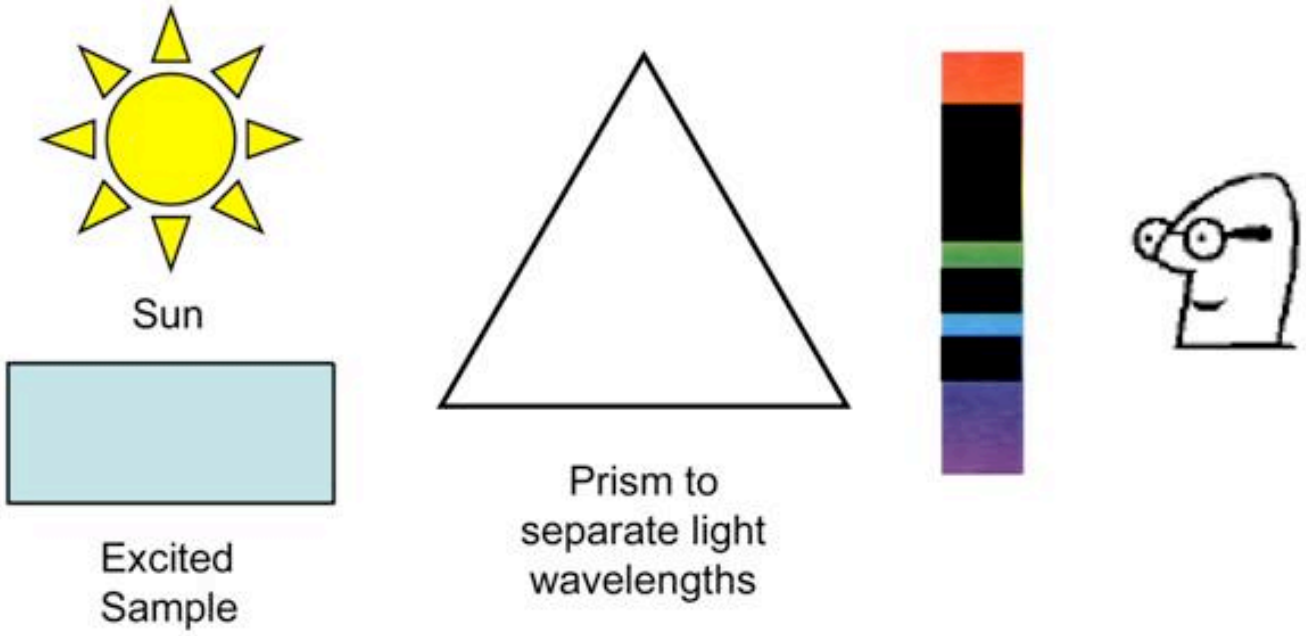
Introduction to the Spectroscopy of Dye Molecules

Emission Spectroscopy

Late 19th century: many atomic spectra were collected.

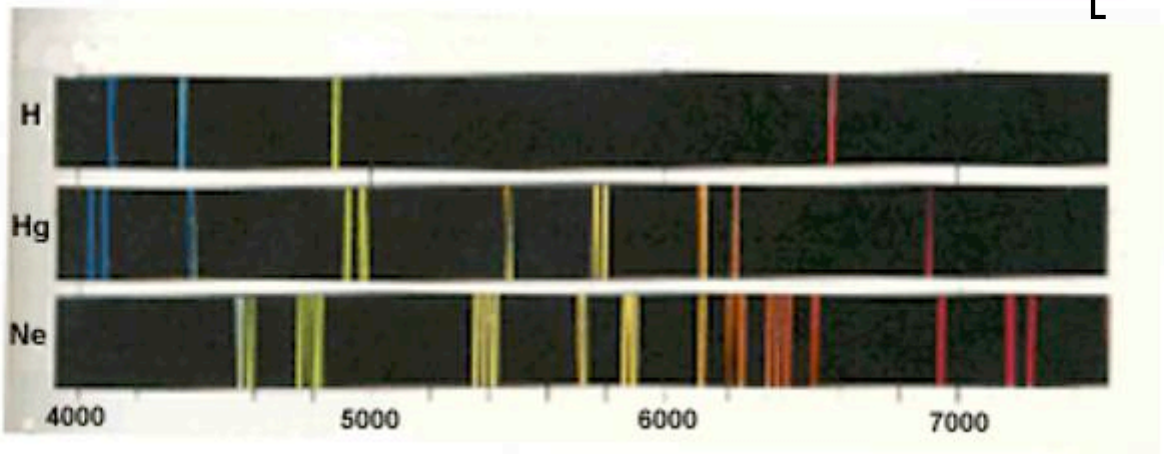
How?



Emission Spectroscopy

1885: J.J. Balmer: Hydrogen

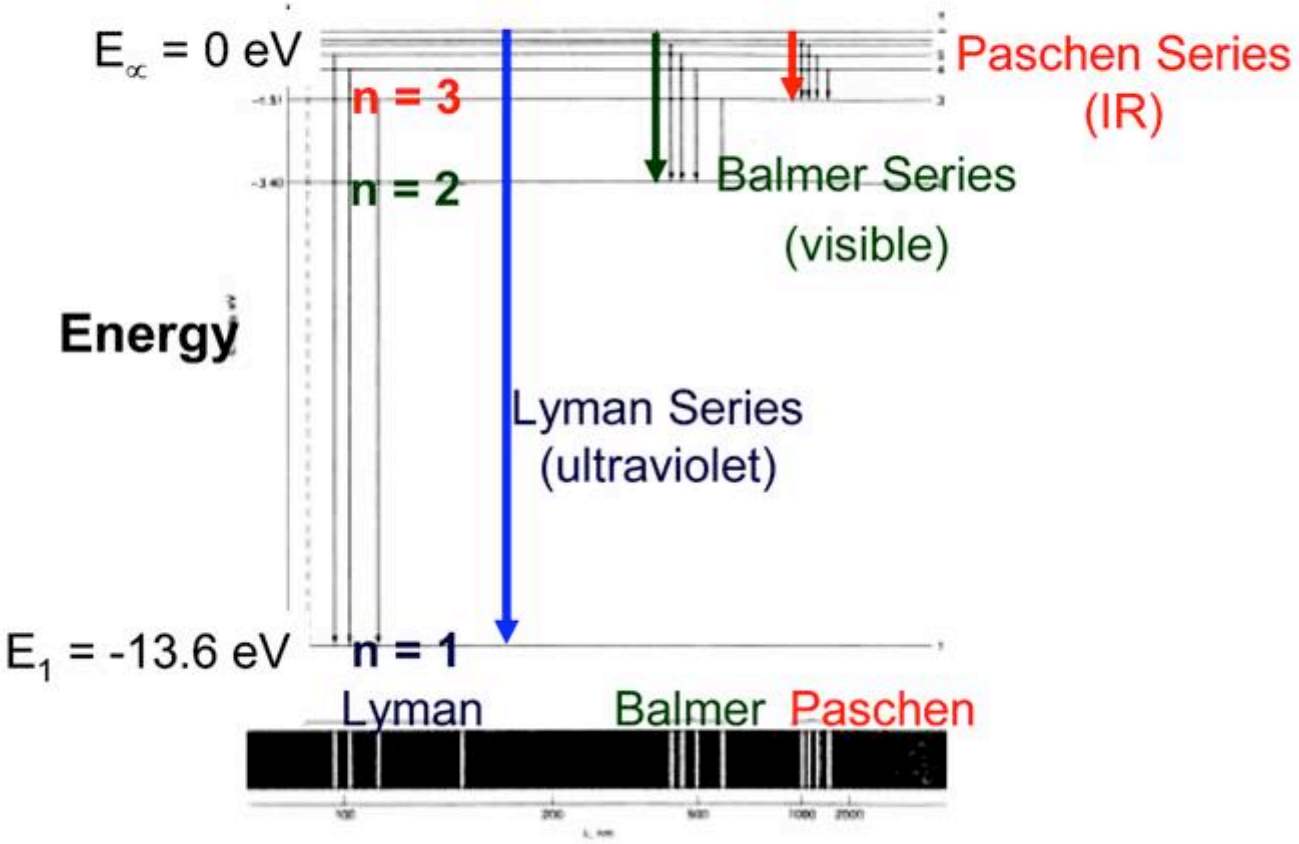
$$\lambda = 364.56\ nm \left[\frac{n^2}{n^2 - 2^2} \right]$$



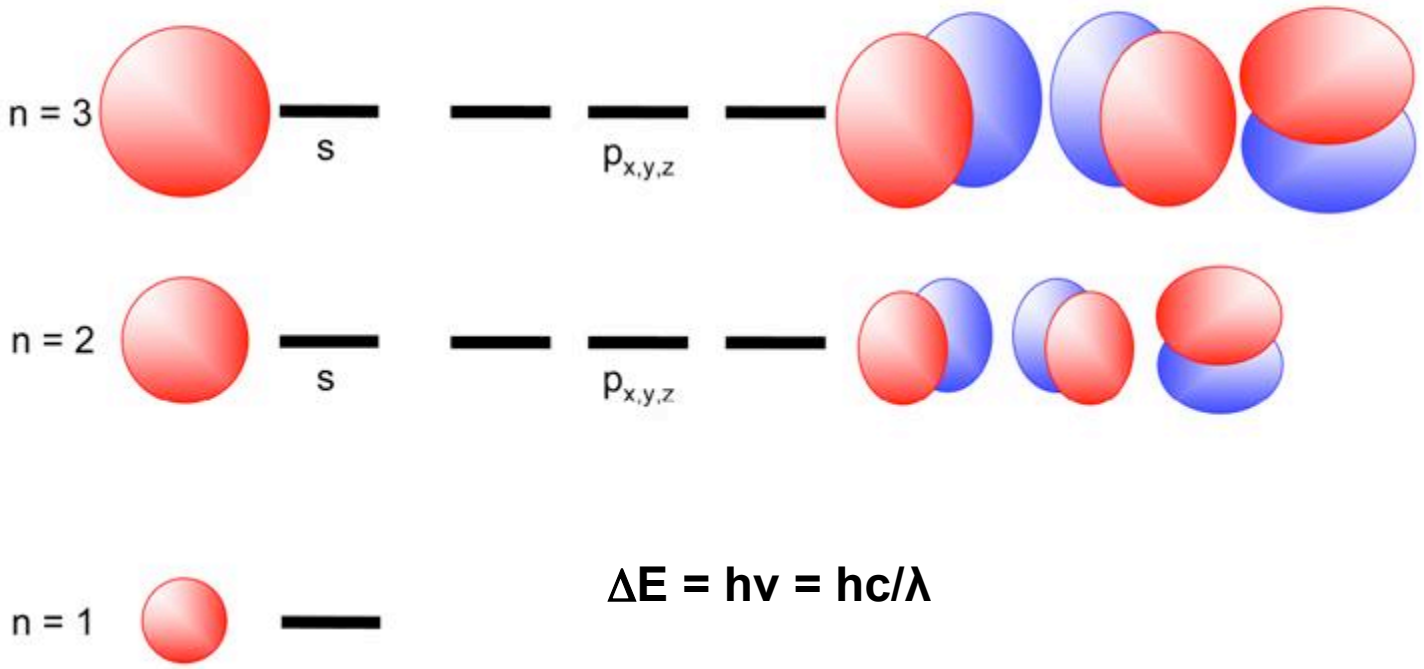
Angstroms (10 Å =1 nm)

n	3	4	5	6	7	8	9	10
λ	656	486	434	410	397	389	383	380
	Red	Green	Blue	Indigo	Violet	Not visible		

Emission Spectroscopy
Hydrogen Energy Levels



Hydrogen Energy Levels
What is happening?



$$\Delta E = h\nu = hc/\lambda$$

Emission: Atom is excited by an external source; light is given out as it goes to its ground state.

Absorption: Atom absorbs light; atom becomes excited.

$E_{photon} = h\nu = E_{upper\ state} - E_{lower\ state}$

True for atoms, true for molecules

To interpret the color of an object we must know the array of possible energy levels for its molecules.

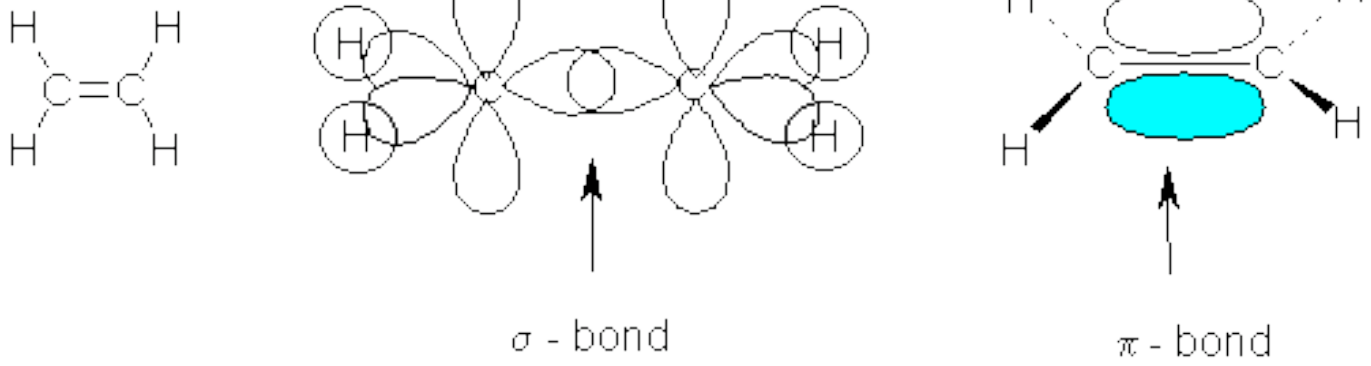
Visible absorptive coloration arises when visible photons are absorbed and excite molecules from their ground or lowest-energy electronic state to a higher-energy **electronic** state.

Transitions between electronic states are responsible for the majority of the colors we see in the natural world.

Q: Why are most substances colorless or white?

A: Most molecular substances are colorless because the spacing between the highest occupied electronic energy level and the lowest unoccupied level typically is larger than the energy of any photon in the visible range.

σ and π Bonds in Ethylene and in Conjugated Systems

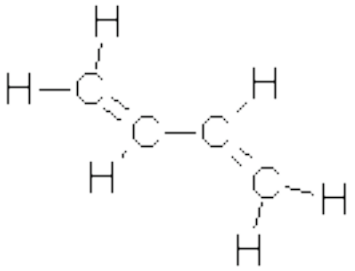


C – sp^3 hybrid tetrahedral bonding
diamond

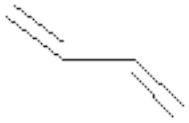
C – sp^2 hybrid equilateral triangle
graphite

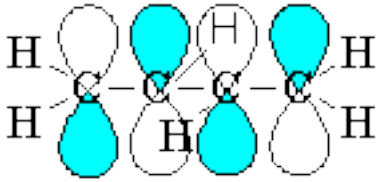
Polyenes

Organic molecules that contain alternating single and double bonds are said to be conjugated. The simplest example is butadiene (C_4H_6), whose structure is shown below.



also represented as





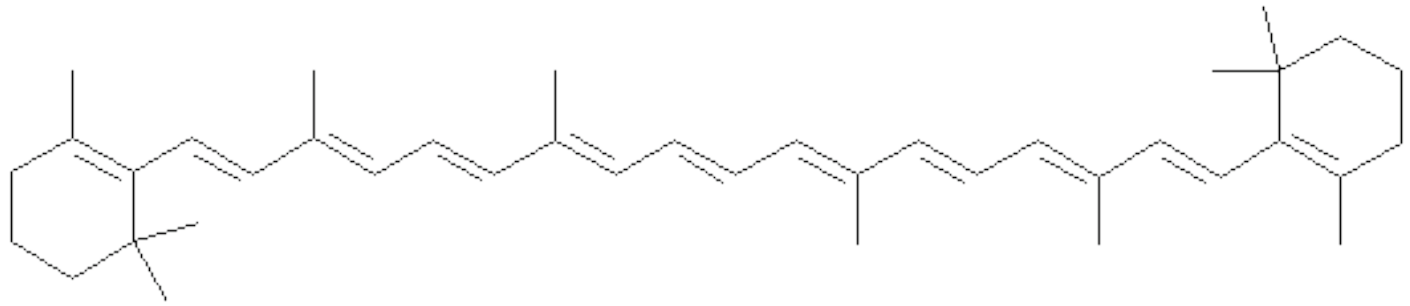
If we examine the p-bonding arrangement in butadiene we can imagine each carbon atom with a p-orbital overlapping in a side-on fashion, with each p-orbital containing 1 electron.

BIG CONCEPT: **delocalized electrons**

One example is β-carotene, the pigment that makes carrots orange and makes butter yellow.

In such molecules the electrons in the π-bonds can be considered to be delocalized over all the atoms of the conjugated chain, and to a crude approximation the electrons can be thought of as moving freely along the length of the carbon skeleton.

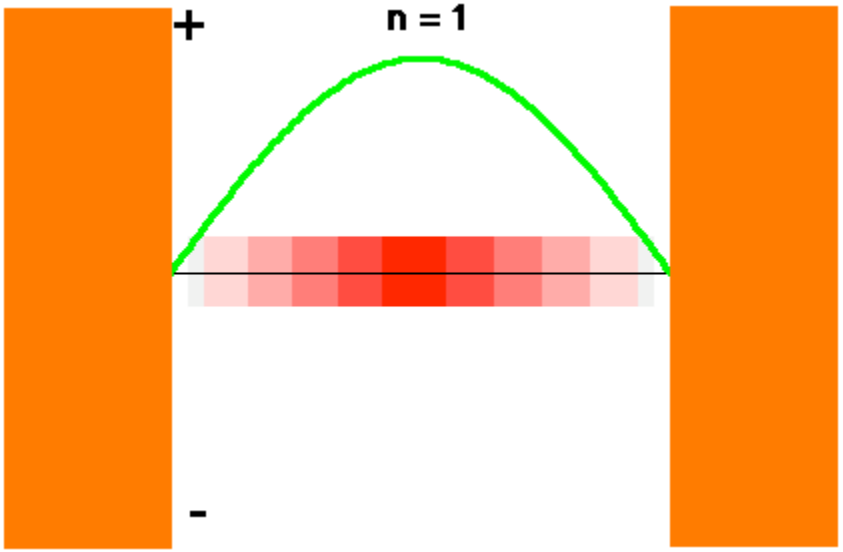
The color of β -carotene arises from an absorption in the visible spectrum with λ_{max} at 450 nm.

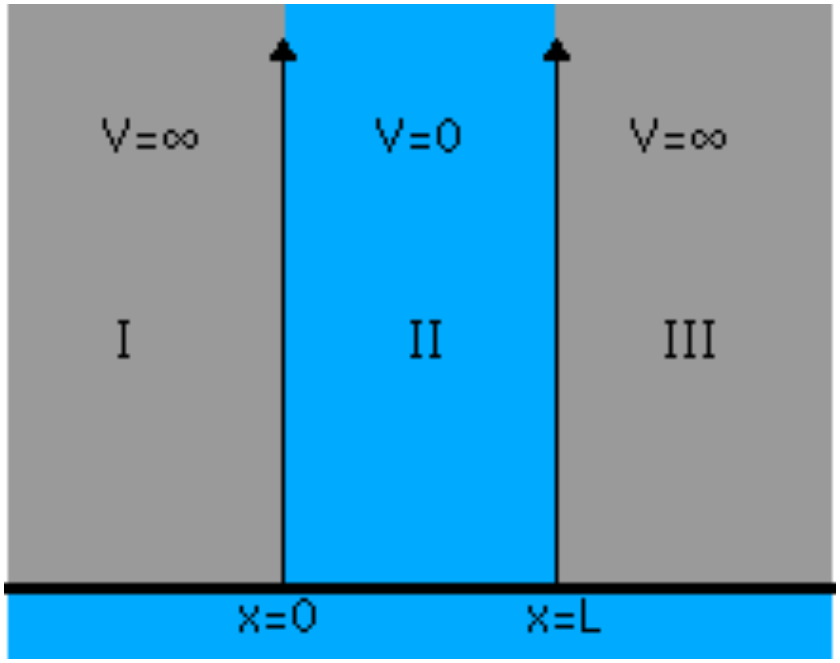


β - carotene

Particle In A Box

Quantum Treatment



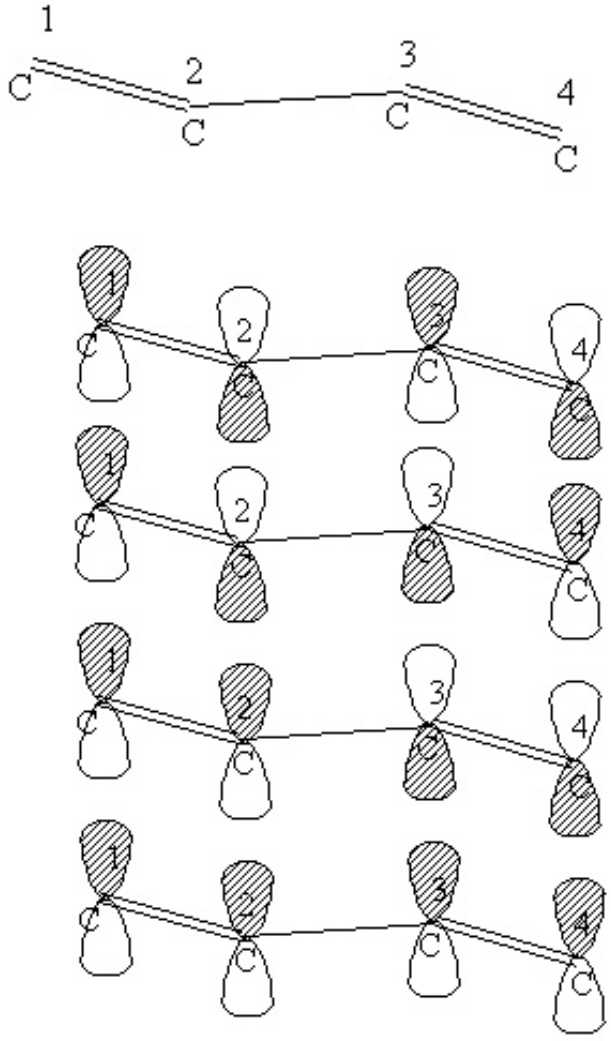


$$\psi_n = \sqrt{\frac{2}{L}} \sin\left(\frac{n\pi x}{L}\right)$$

$$E_n = \frac{n^2 h^2}{8mL^2}$$

n= 1,2,3,…

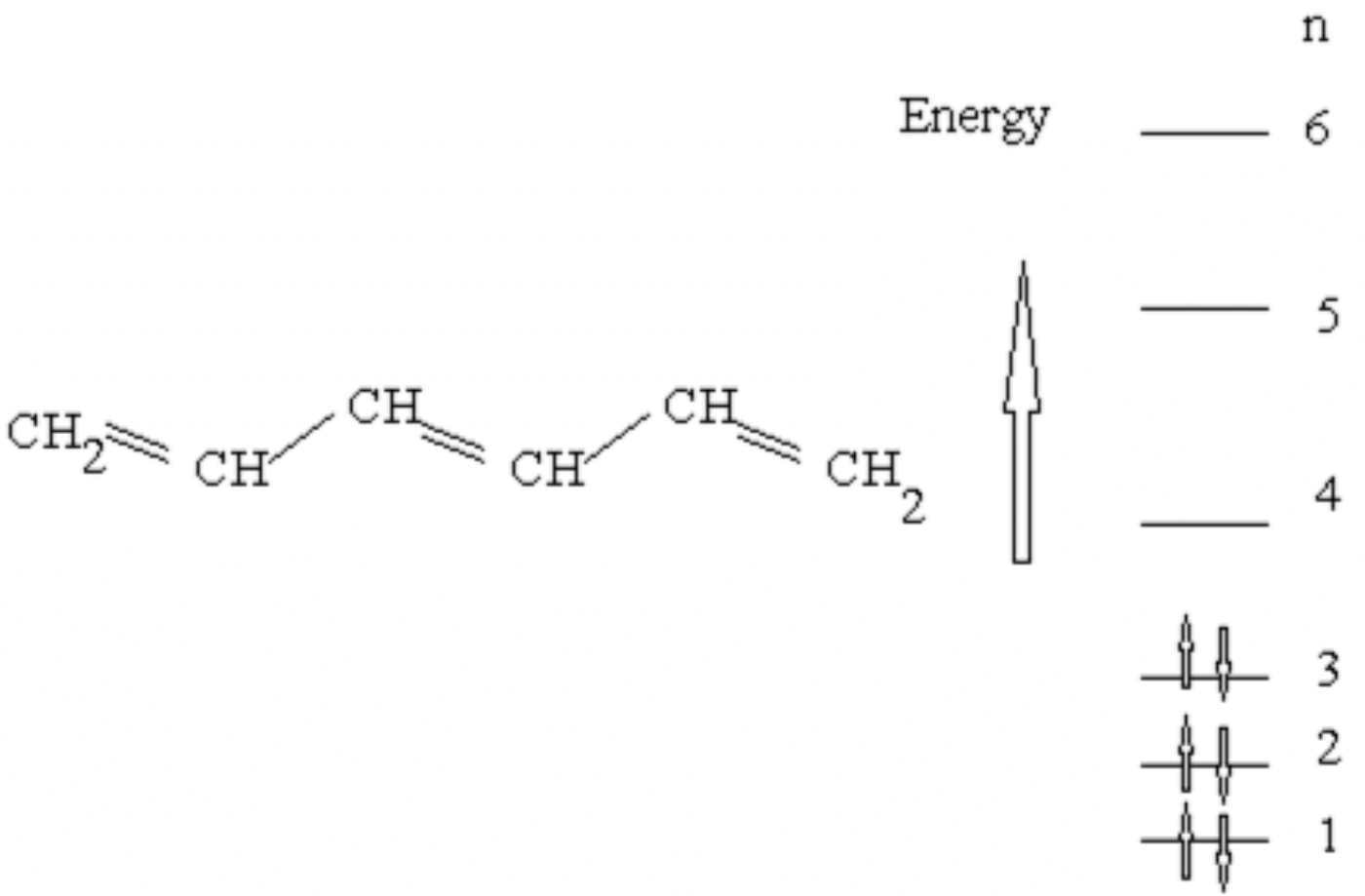
Reconsideration of Butadiene



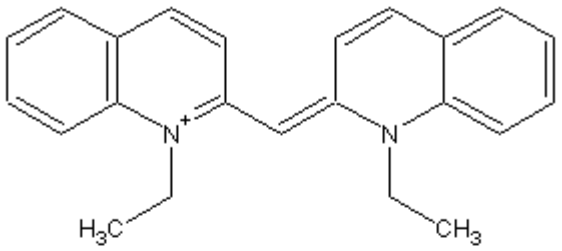
Each π orbital can hold two electrons, one spin up, the other spin down. In 1,3 butadiene, there are 4 π electrons, so the first two π MOs are filled.

$$\Delta E = E_{LUO} - E_{HFO} = \frac{h^2}{8mL^2}\left(n_{LUO}^2 - n_{HFO}^2\right)$$

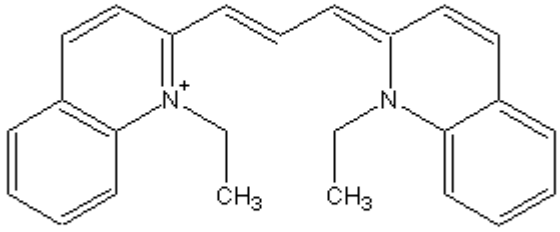
What 1,3,5 hexatriene looks like …



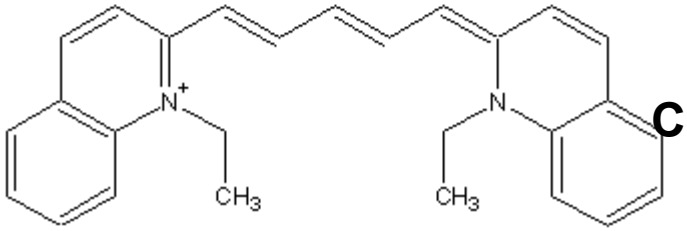
So How Well Does This Crude Theory Work?



A



B



C

Assume L = (2k+2)b, where:

k = number of double bonds along the chain

b = 139 pm (C-C length in benzene)

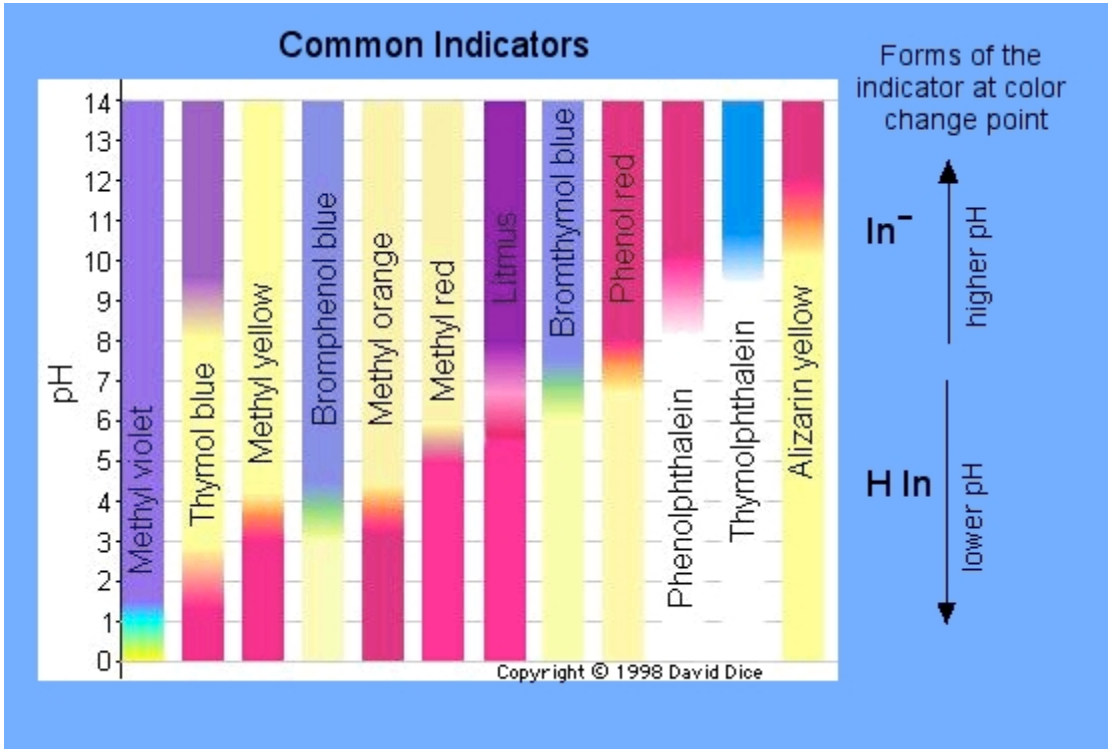
The Result:

	L (pm)	Theory	Experiment
A	556	328 nm	523 nm
B	834	453 nm	605 nm
C	1112	580 nm	706 nm

The simple one-dimensional particle-in-the-box model does not match the experimental results exactly, but it does show the same trend of decreasing energy (longer wavelength) as the "box" gets larger.

Acid Base Indicators

- Indicators are weak acids or weak bases in which the undissociated form has a different color than the dissociated (ionized) form.

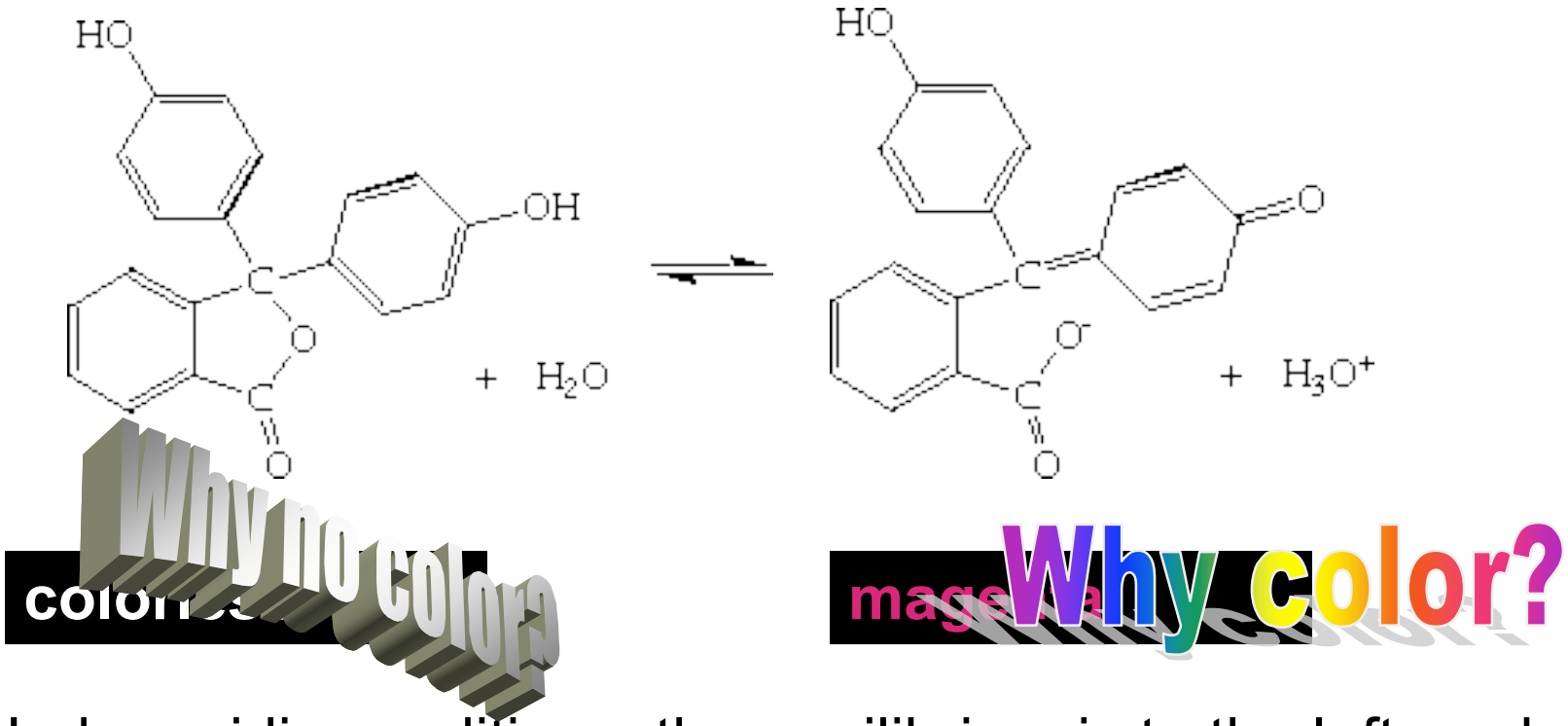


$$H\,In \leftrightarrow H^{+} + In^{-}$$

$$K = \frac{[H^{+}][In^{-}]}{[H\ In]}$$

$$[H^{+}] = K\frac{[H\ In]}{[In^{-}]}$$

Phenothalein



Under acidic conditions, the equilibrium is to the left, and the concentration of the anions is too low for the magenta color to be observed.

Under alkaline conditions, the equilibrium is to the right, and the concentration of the anion becomes sufficient for the magenta color to be observed.

What Can Light Tell us About a Molecule and its Environment?

After a molecule is excited

(1) it is more reactive than before it absorbed light

(2) it takes time for the molecule to emit light and return to the starting molecule (ground state).

During this time, various things can happen:

(1) **solvent rearrangement**

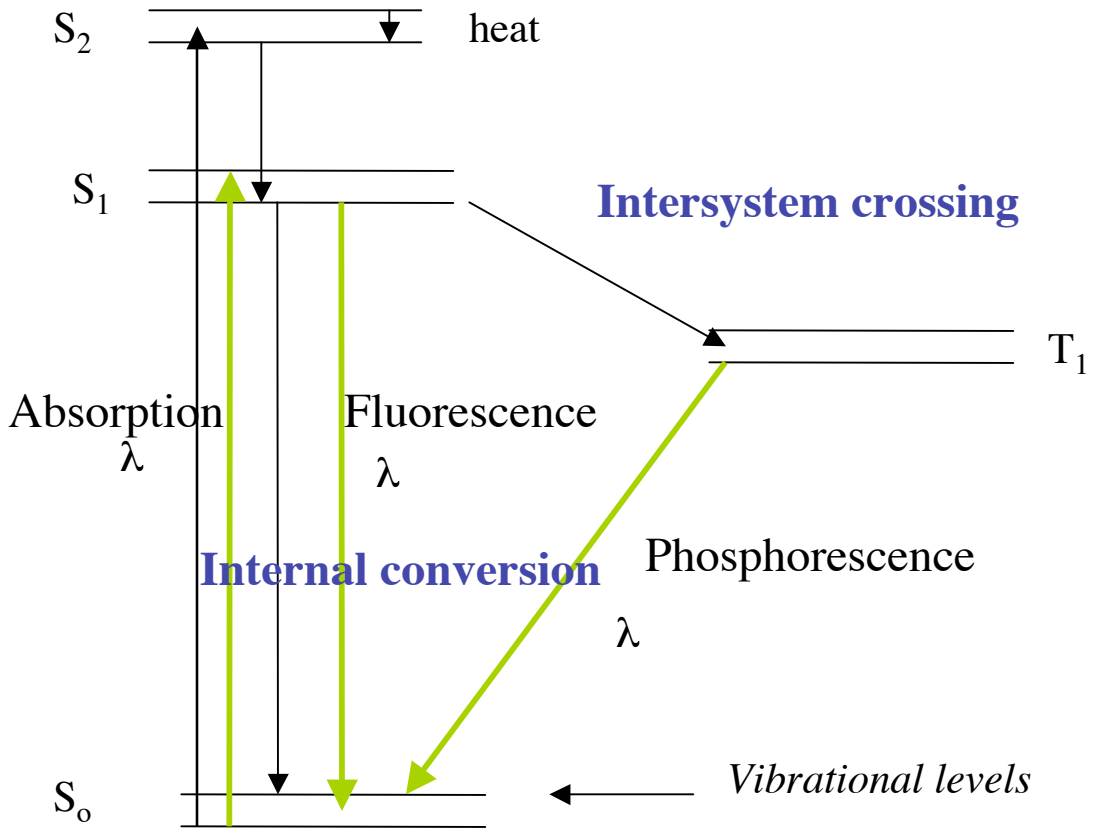
(2) **rotation** of the molecule

(3) reaction (**quenching**)

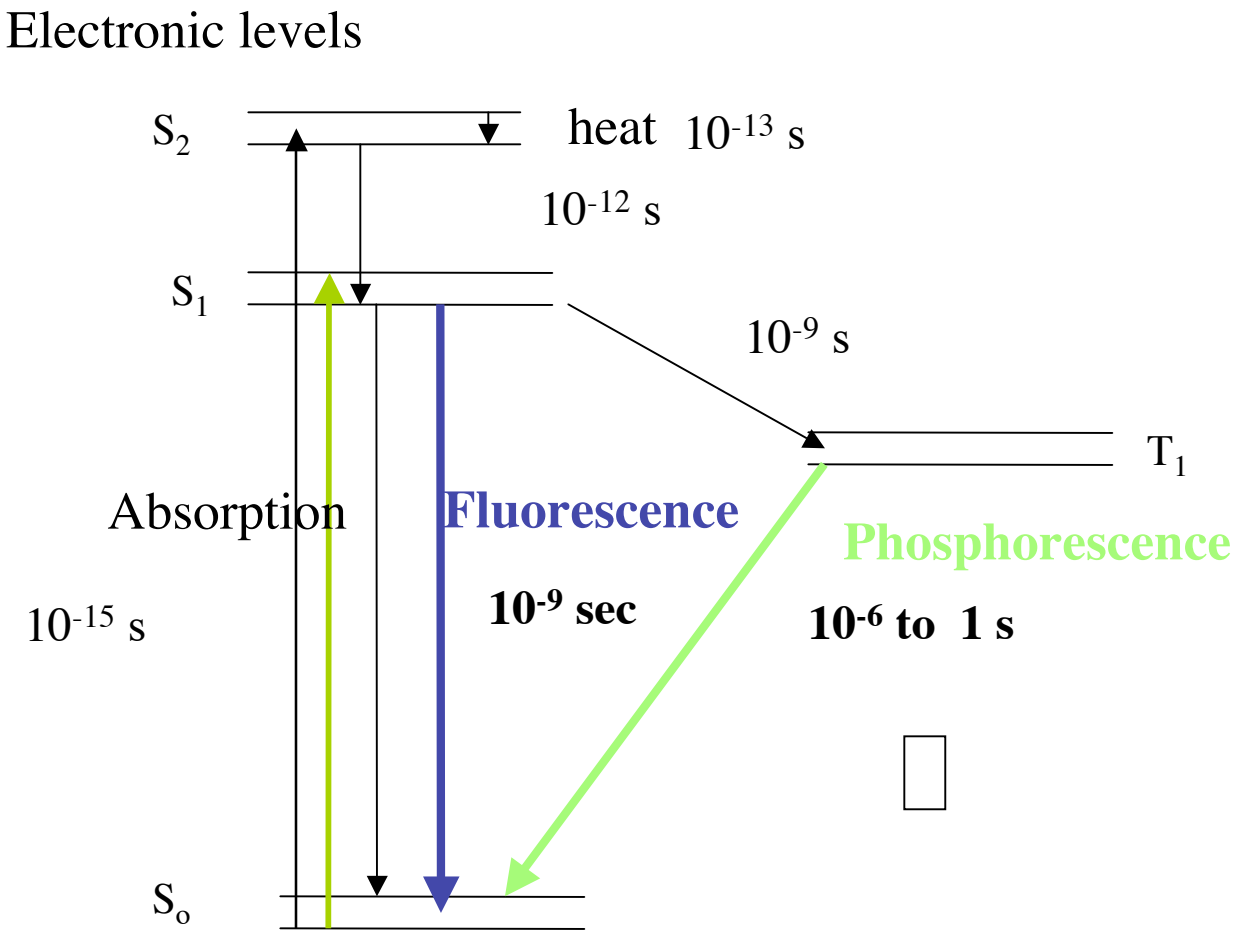
(4) loss of energy to a neighboring molecule (**energy transfer**) in a process that is **distance** related

Energy Diagram

Electronic levels



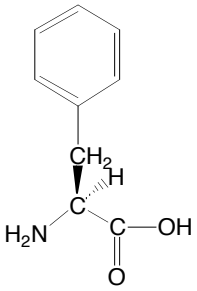
Energy Diagram
Rates of Relaxation



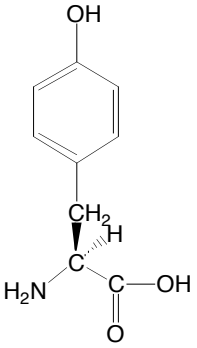
Naturally Fluorescent Chromophores

Amino acids

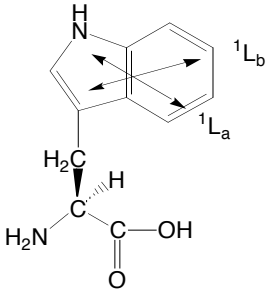
phenylalanine



tyrosine

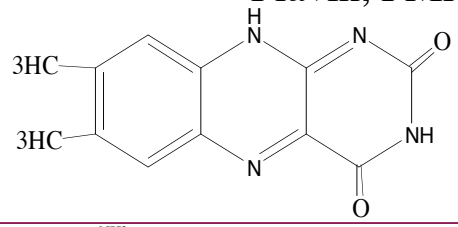


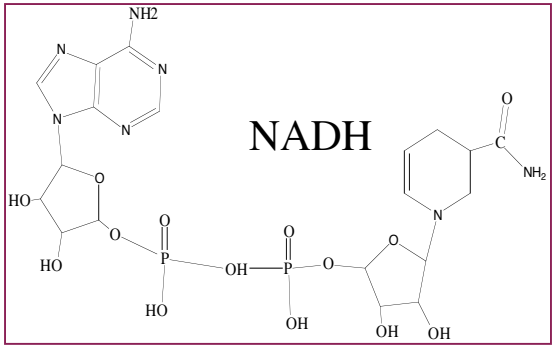
tryptophan



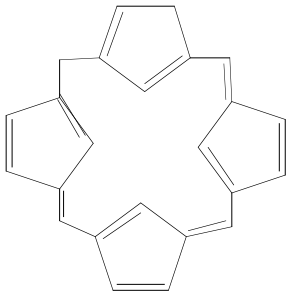
Cofactors

Flavin, FMN

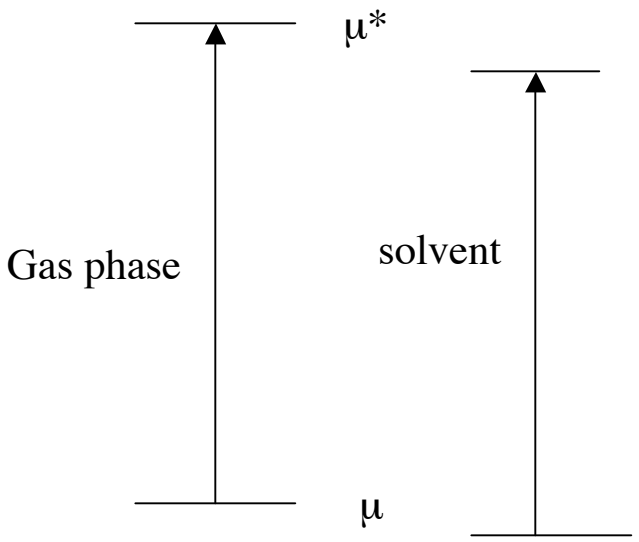




porphyrin



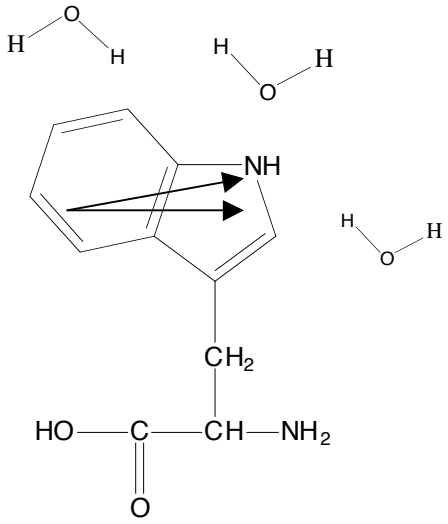
Local Electric Fields Cause Spectral Shifts



Solvent can affect the ground state and excited state molecules causing spectral shifts

Example: H bonding to tryptophan. Changes its absorption by about 10 nm Changes its emission by about 60 nm

Solvent Relaxes Around Excited State Trp



Ground state dipole moment: 2.1D

Excited state dipole moment: 5.4 D

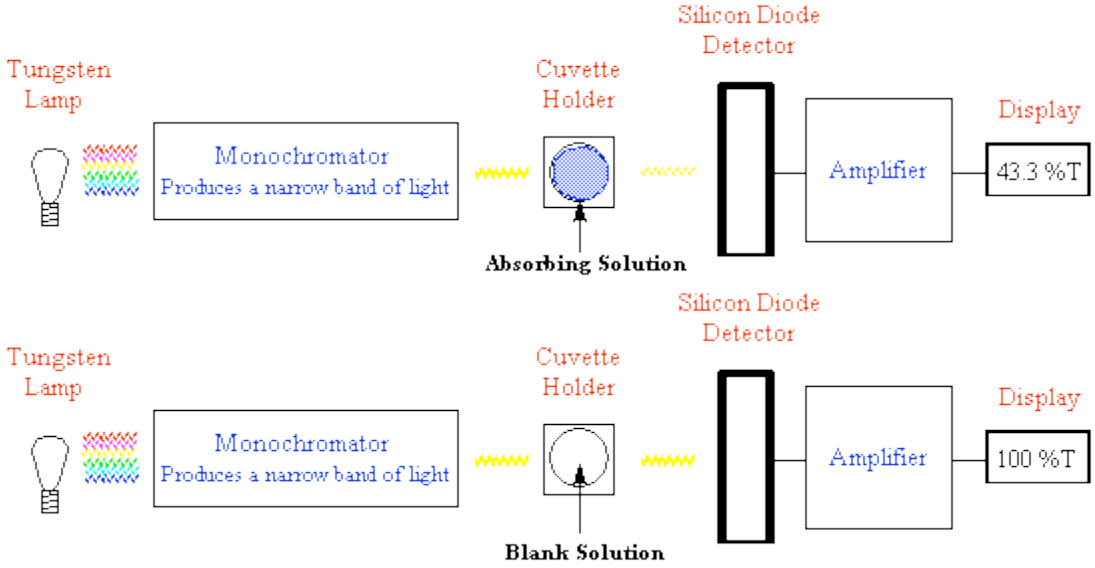
More polar in the excited state

Fluorescence spectral shifts are sensitive indicators of conformational changes

Spectrophotometer Instrumentation

Let me begin with my favorite movie illustrating how such a device works … …

Single-Beam Spectrophotometer



Shimadzu BioSpec Mini

Hardware Specifications

Spectral Bandwidth	5 nm
Wavelength Range	190 – 1100 nm
Wavelength Accuracy	±1 nm
Recording Range	-399 to 399% transmittance
Photometric Accuracy	±0.005 abs at 1.0 abs;
	±0.003 abs at 0.5 abs

Spectronic Genesys 20

Spectronic GENESYS 20 Spectrophotometer
Part Number 4001 & 4004 Series

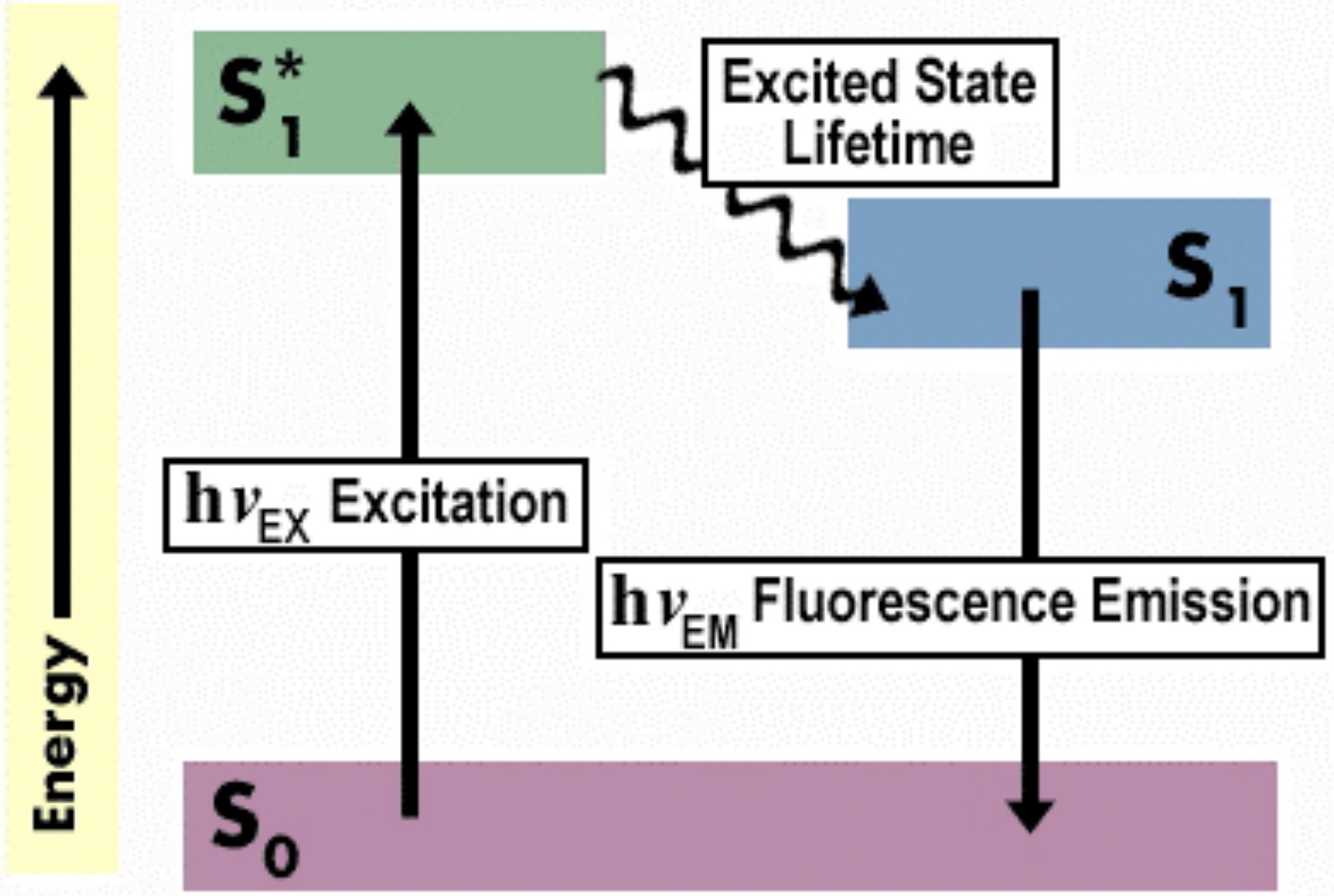
Spectral Slitwidth	8 nm
Optical System	Grating-based, 1200 lines/mm
Wavelength	
Range	325 to 1100 nm
Accuracy	± 2.0 nm
Display	20-character, 2-line LCD
Photometric	
Range	0 to 125 % Transmittance – 0.1 to 2.5 Absorbance 0 to 1999 Concentration ± 0.1 to ± 9990 Factor
Accuracy*	0.003 A from 0.0 to 0.3 A 1.0% from 0.301 A to 2.5 A
Noise (at 500 nm)	≤ 0.001 A at 0 A ≤ 0.004 A at 2 A
Drift	0.003 A/hour

Spectrofluorimeter

L geometry for optical layout

Jablonski Diagram

Illustrating the Fluorescence Processes



Stage 1: Excitation

A photon of energy hv_{EX} is supplied by an external source such as an incandescent lamp or a laser and absorbed by the fluorophore, creating an excited electronic singlet state (S_1'). This process distinguishes fluorescence from chemiluminescence, in which the excited state is populated by a chemical reaction.

Stage 2 : Excited-State Lifetime

The excited state exists for a finite time (typically 1–10 x 10^{-9} seconds). During this time, the fluorophore undergoes conformational changes and is also subject to a multitude of possible interactions with its molecular environment.

These processes have two important consequences.

First, the energy of S_1' is partially dissipated, yielding a relaxed singlet excited state (S_1) from which fluorescence emission originates.

Second, not all the molecules initially excited by absorption (Stage 1) return to the ground state (S_0) by fluorescence emission. Other processes such as collisional quenching, fluorescence energy transfer, and intersystem crossing (see below) may also depopulate S_1.

The fluorescence quantum yield, which is the ratio of the number of fluorescence photons emitted (Stage 3) to the number of photons absorbed (Stage 1), is a measure of the relative extent to which these processes occur.

The entire fluorescence process is cyclical. Unless the fluorophore is irreversibly destroyed in the excited state (an important phenomenon known as photobleaching), the same fluorophore can be repeatedly excited and detected. This concept is key to single-molecule spectroscopy.

For polyatomic molecules in solution, the discrete electronic transitions represented by hv_{EX} and hv_{EM} in the Jablonski diagram are replaced by rather broad energy spectra called the fluorescence excitation spectrum and the fluorescence emission spectrum, respectively.

The bandwidths of these spectra are parameters of particular importance for applications in which two or more different fluorophores are simultaneously detected. With few exceptions, the fluorescence excitation spectrum of a single fluorophore species in dilute solution is identical to its absorption spectrum.

Under the same conditions, the fluorescence emission spectrum is independent of the excitation wavelength, owing to the partial dissipation of excitation energy during the excited-state lifetime.

Stage 3 : Fluorescence Emission

A photon of energy hv_{EM} is emitted, returning the fluorophore to its ground state S_0. Owing to energy dissipation during the excited-state lifetime, the energy of this photon is lower, and therefore of longer wavelength, than the excitation photon hv_{EX}.

The difference in energy or wavelength represented by

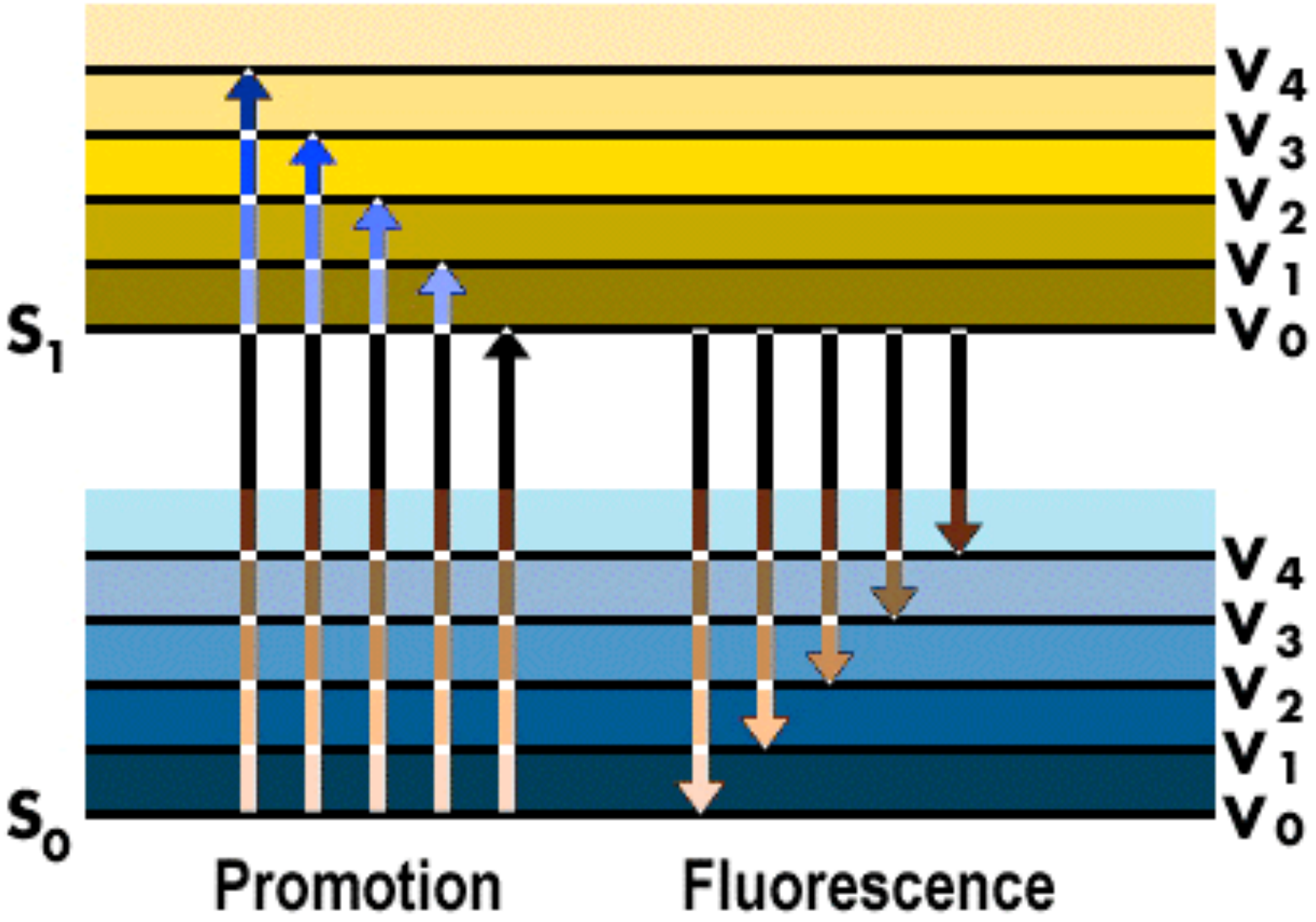
$$(hv_{EX}–hv_{EM})$$

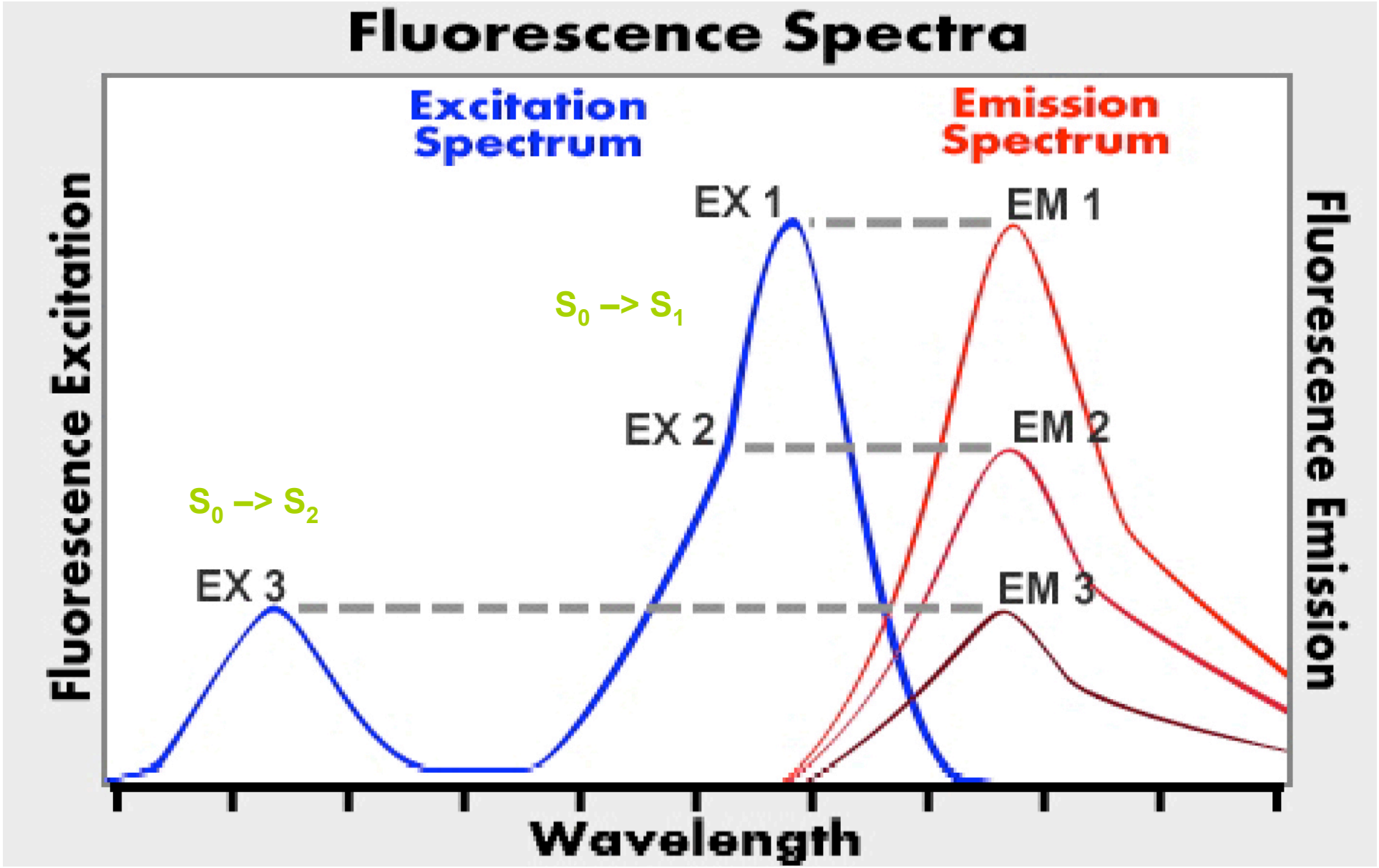
is called the **Stokes shift**.

The Stokes shift is fundamental to the sensitivity of fluorescence techniques because it allows emission photons to be detected against a low background, isolated from excitation photons.

In contrast, absorption spectrophotometry requires measurement of transmitted light relative to high incident light levels at the same wavelength.

Promotion and Fluorescence between S_1 and S_0 states.




Fluorescence Spectra
Excitation Spectrum
Emission Spectrum
EX 1
EM 1
S_0 –> S_1
EX 2
EM 2
S_0 –> S_2
EX 3
EM 3
Fluorescence Excitation
Fluorescence Emission
Wavelength

The Fluorescence Quantum Yield

The fluorescence quantum yield (Φ_F) is the ratio of photons absorbed to photons emitted through fluorescence.

In other words the quantum yield gives the probability of the excited state being deactivated by fluorescence rather than by another, nonradiative mechanism.

$0 \leq \Phi_F \leq 1$