Fundamentals in Biophotonics

Franck Condon rule- Chromophores

Aleksandra Radenovic
aleksandra.radenovic@epfl.ch

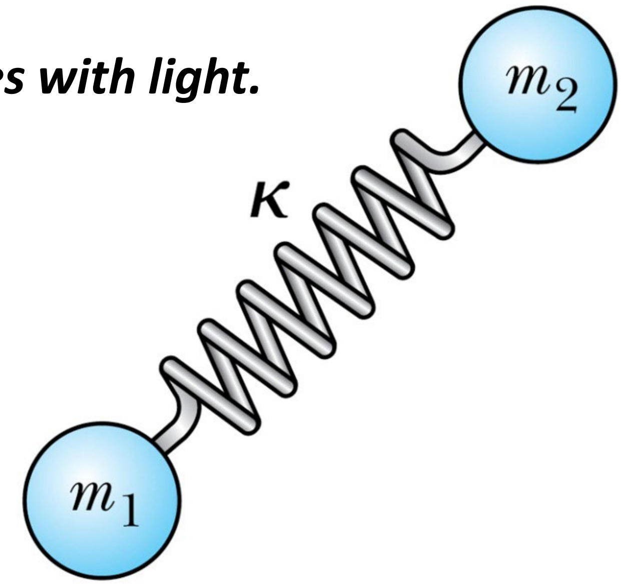
EPFL – Ecole Polytechnique Federale de Lausanne
Bioengineering Institute IBI

EPFL

03. 29. 2021.

Vibrational States

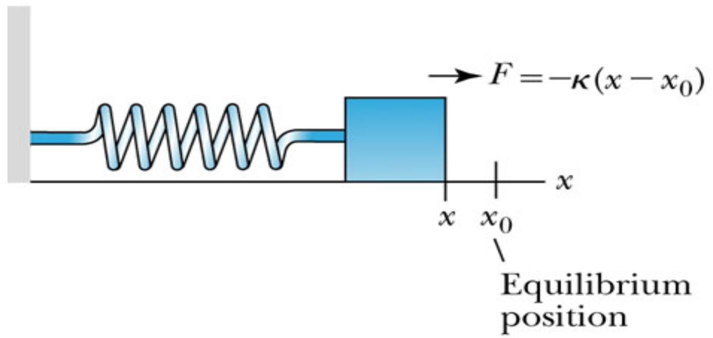
- A vibrational energy mode can also be excited.
- Thermal excitation of a vibrational mode can occur.
- ***It is also possible to stimulate vibrations in molecules with light.***

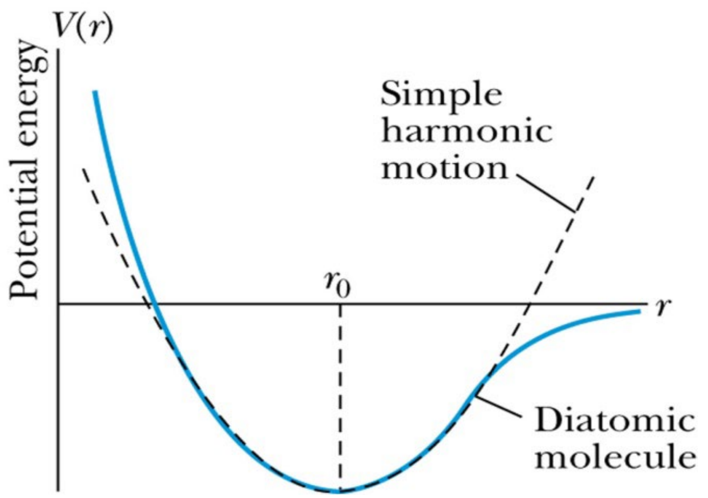


Assume that the two atoms are point masses connected by a massless spring with simple harmonic motion.

Vibrational motion: a simple harmonic oscillator

The simple harmonic oscillator accurately describes a diatomic molecule, as well as more complex molecules.





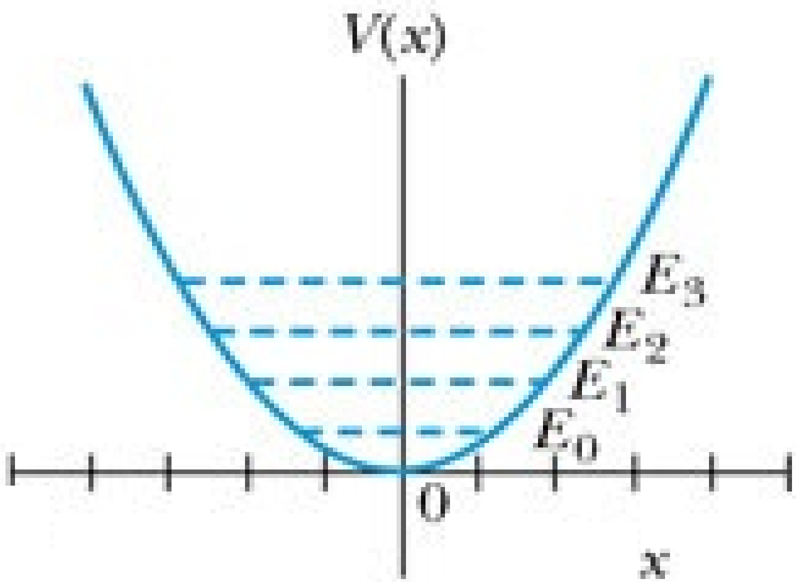
Vibrational States

- The energy levels are those of a quantum-mechanical oscillator

$$E_{vibr} = (n + \tfrac{1}{2})\,\hbar\omega$$

$$\omega = \sqrt{\kappa / M_r}$$

$$m_{\rm red} = \mu = \frac{1}{\frac{1}{m_1} + \frac{1}{m_2}} = \frac{m_1 m_2}{m_1 + m_2},$$



n is called the **vibrational quantum number**. Don't confuse it for n, **the principal quantum number of the electronic state.**

Vibrational-transition selection rule:

$$\Delta n = \pm 1$$

The only spectral line is ω!

However, deviations from a perfect parabolic potential allow other transitions, called **overtones**, but they're much weaker.

Vibrational States

- The energy levels are those of a quantum-mechanical oscillator

$$E_{vibr} = (n + \tfrac{1}{2})\,\hbar\omega$$

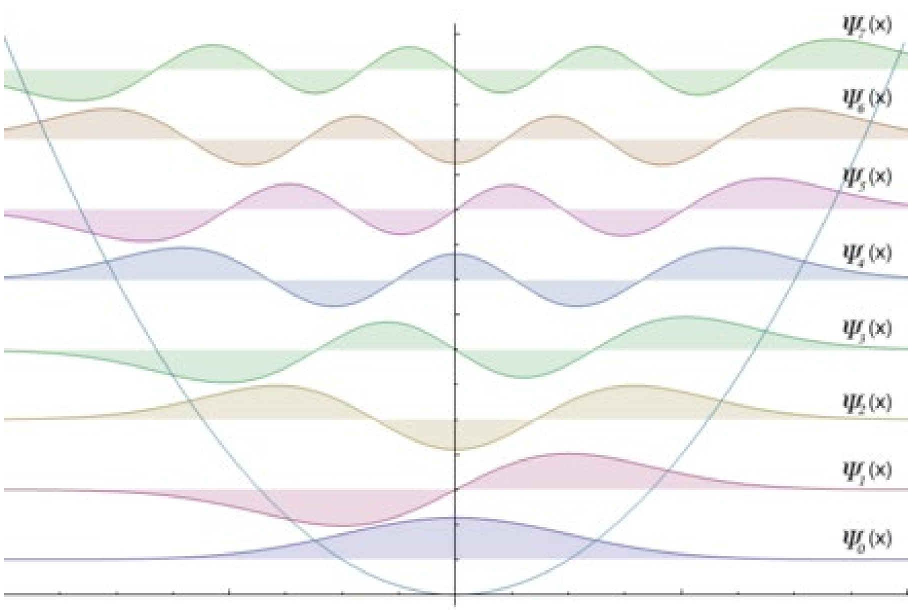
$$\omega = \sqrt{\kappa / M_r}$$

$$m_{\text{red}} = \mu = \frac{1}{\frac{1}{m_1} + \frac{1}{m_2}} = \frac{m_1 m_2}{m_1 + m_2},$$

n is called the **vibrational quantum number**. Don't confuse it for $\boldsymbol{n}$, **the principal quantum number of the electronic state.**

Vibrational-transition selection rule:

$$\Delta n = \pm 1$$

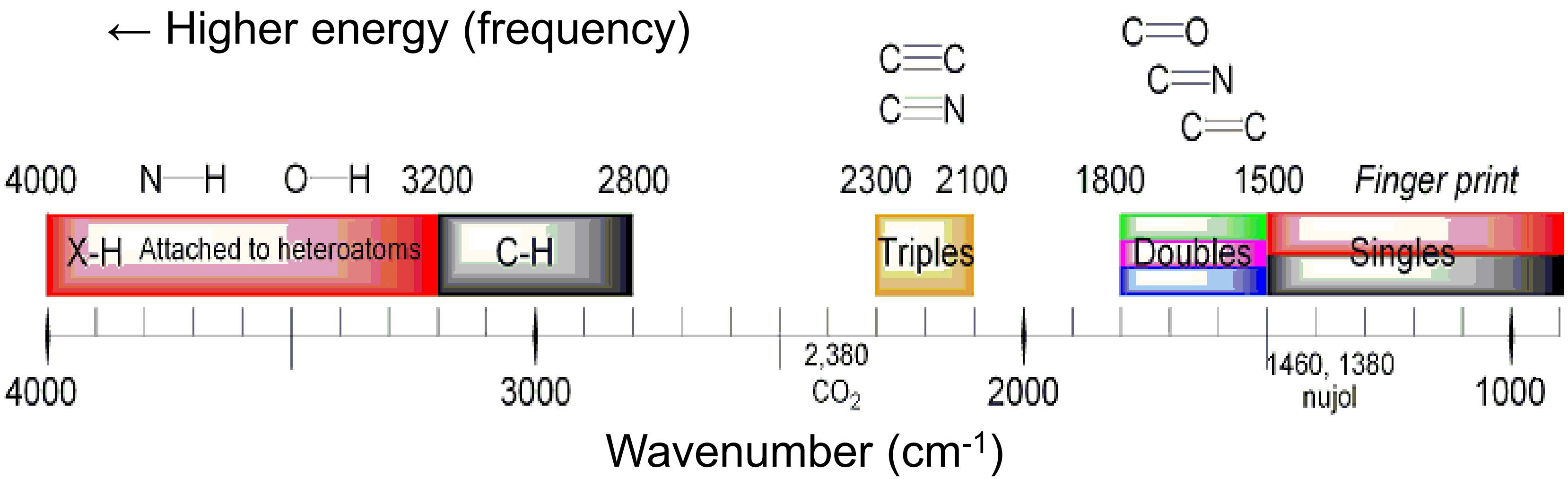


The only spectral line is ω!

However, deviations from a perfect parabolic potential allow other transitions, called **overtones**, but they're much weaker.

Vibrational frequencies for various bonds

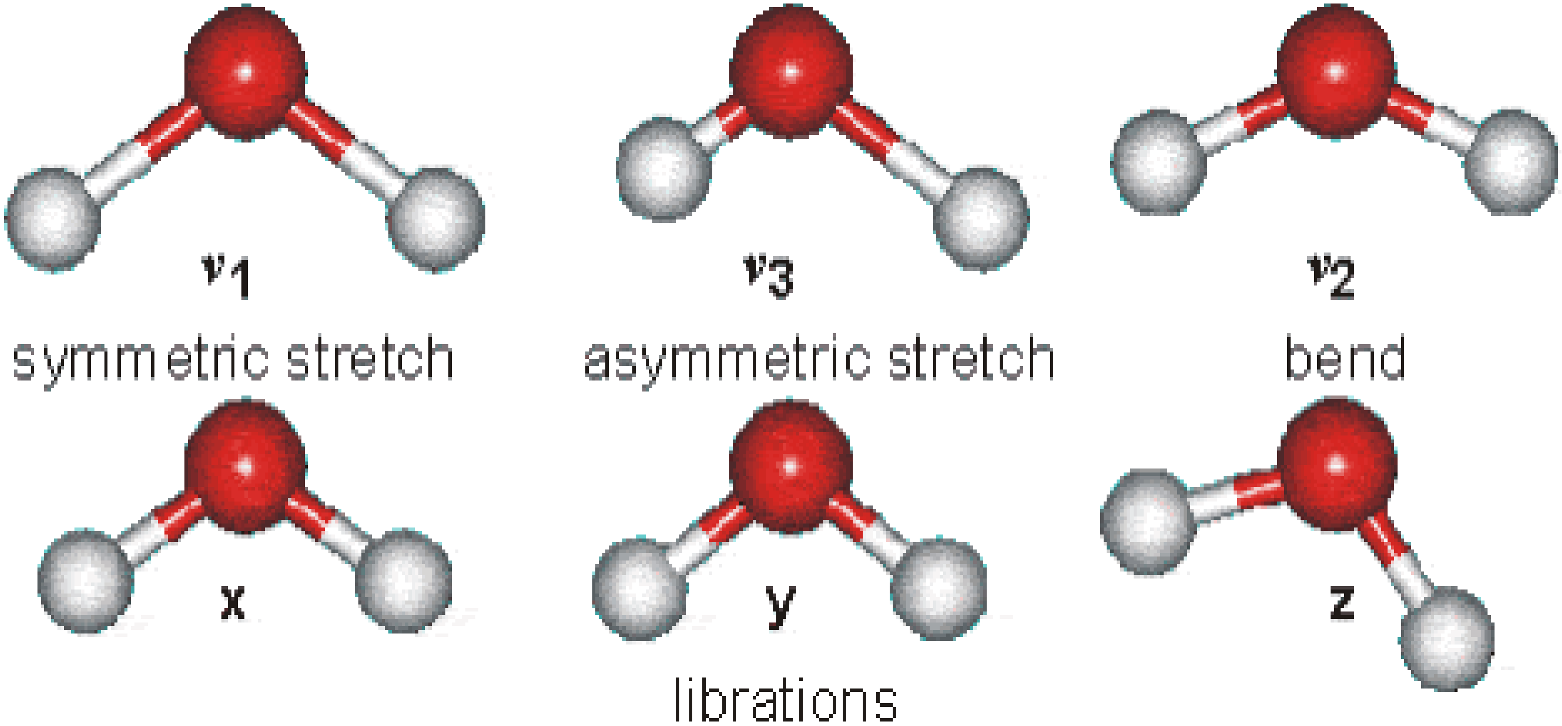
- Different bonds have different vibrational frequencies (which are also affected by other nearby atoms).



Notice that bonds containing Hydrogen vibrate faster because H is lighter.

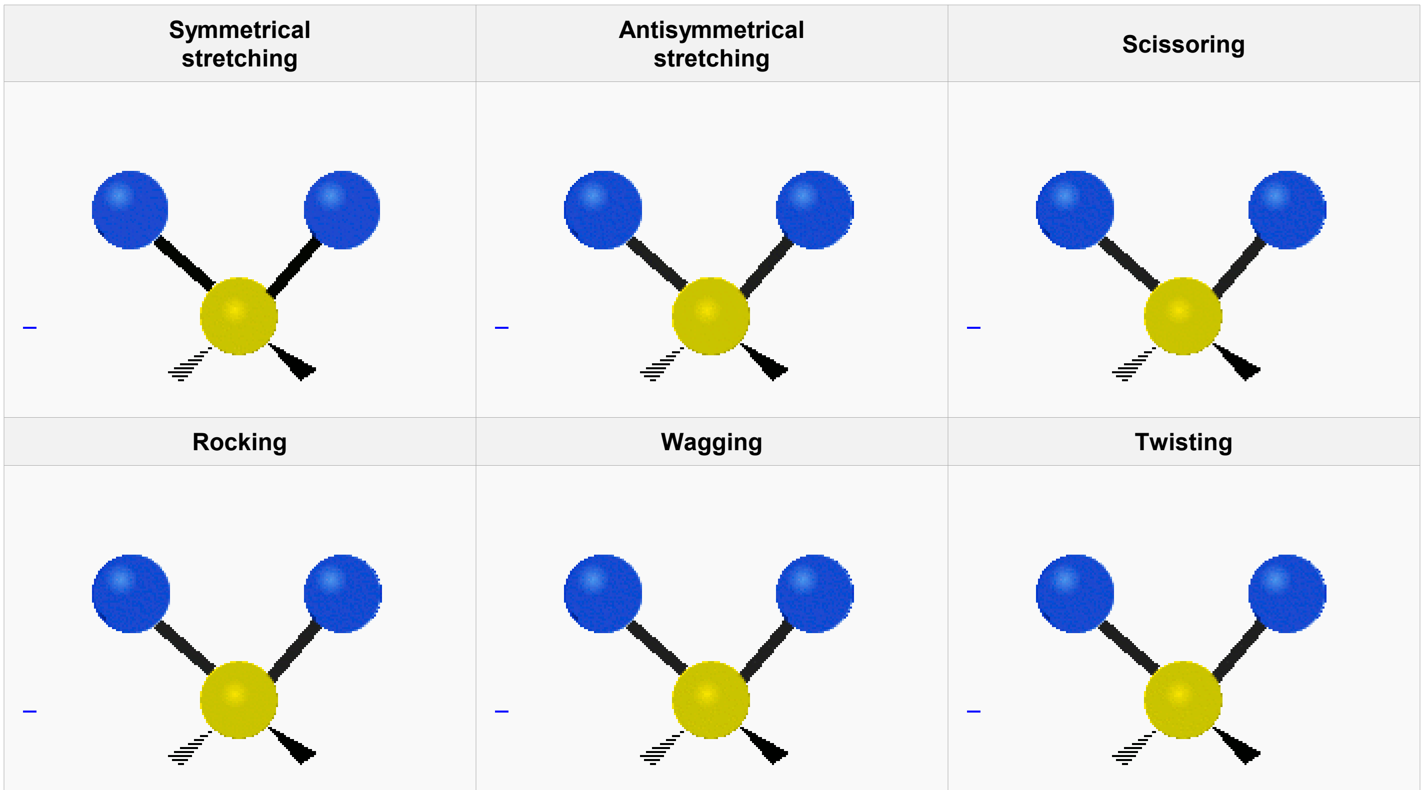
Unlike the energy levels of the hydrogen atom and the rotating diatomic molecule, the vibrational energy levels of the diatomic molecule **are equally spaced**. In practice, however, the potential-energy curves for most molecules become anharmonic as the energy increases, resulting in a diminution of energy-level separations

Water vibrations-vibrating triatomic molecules



Carbon-Hydrogen-vibration-vibrating triatomic molecules

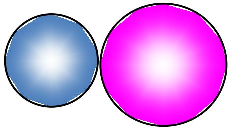
- CH2-Carbon-Hydrogen bonds are important in the chemistry of life-laser medium



As with diatomic molecules, each vibrational level is split into many closely spaced rotational levels

Rotational states

- Consider diatomic molecules



•A diatomic molecule may be thought of as two atoms held together with a massless, rigid rod (*rigid rotator model*).

•In a purely rotational system, the kinetic energy is expressed in terms of the angular momentum L and rotational inertia I.

$$E_{rot} = \frac{L^2}{2I}$$

•L is quantized

$$L = \sqrt{\ell(\ell+1)}\,\hbar$$

•The energy levels are

$$E_{rot} = \frac{\hbar^2 \ell(\ell+1)}{2I}$$

•E_{rot} varies only as a function of the quantum number ℓ.

Rotational states

- The energy separations of rotational energy levels typically lie in the range 10^{-4} 10^{-2} eV, corresponding to photons in the **microwave and far-infrared regions of the spectrum.**
- The energy spacing between successive rotational energy levels **increases with increasing quantum rotational number,** in contrast to the spacing between successive electronic energy levels of the hydrogen atom, **which decrease with increasing quantum number**
- Diatomic molecules with identical nuclei (such as N_2) have no permanent electric dipole moment; they therefore do not exhibit pure rotational spectra.

Vibration and Rotation Combined

- It's possible to excite rotational and vibrational modes simultaneously.
- Total energy of a simple vibration-rotation system:

$$E = E_{rot} + E_{vibr} = \frac{\hbar^2 \ell(\ell+1)}{2I} + \left(n + \frac{1}{2}\right)\hbar\omega$$

- Vibrational energies are spaced at regular intervals.

- Transitions from ℓ +1 to ℓ :
- Photons will have energies at regular intervals (plus the vibrational energy difference):

$$E_{ph} = \frac{\hbar^2}{2I}\left[(\ell+1)(\ell+2) - \ell(\ell+1)\right]$$

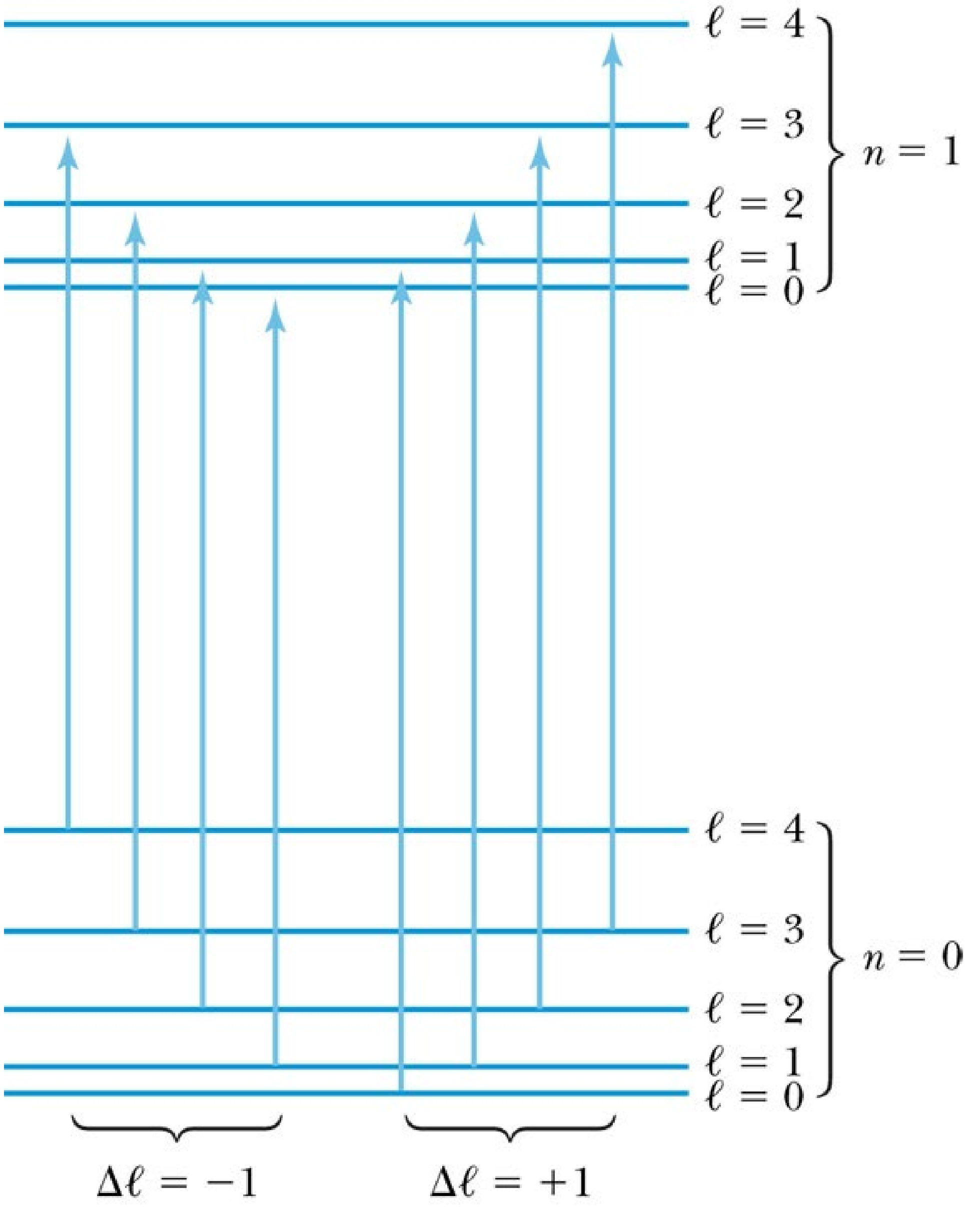
$$= \frac{\hbar^2}{2I}\left[\ell^2 + 3\ell + 2 - \ell^2 - \ell\right] = \frac{\hbar^2}{I}(\ell+1)$$

Vibration and Rotation Combined

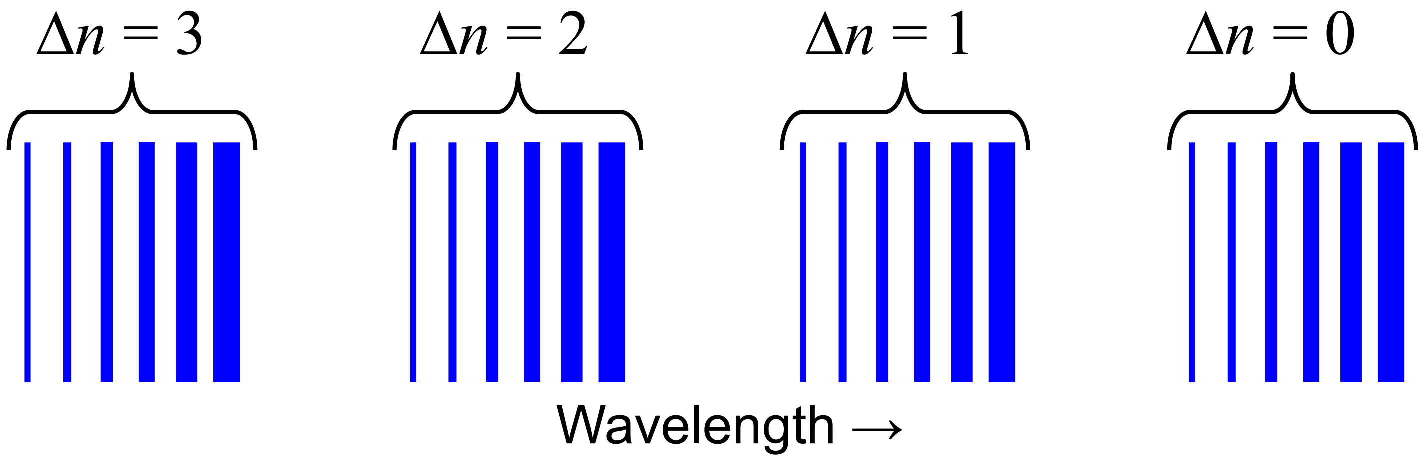
- ΔE increases linearly with ℓ.
- Many transitions are forbidden by the selection rules that requires $\Delta \ell = \pm 1$ and $\Delta n = \pm 1$

- The emission (and absorption) spectrum spacing varies with ℓ.
- The higher the starting energy level, the greater the photon energy.

- Vibrational energies are greater than rotational energies. For a diatomic molecule, this energy difference results in band structure.
- The line strengths depend on populations of the states and the vibrational selection rules, however.

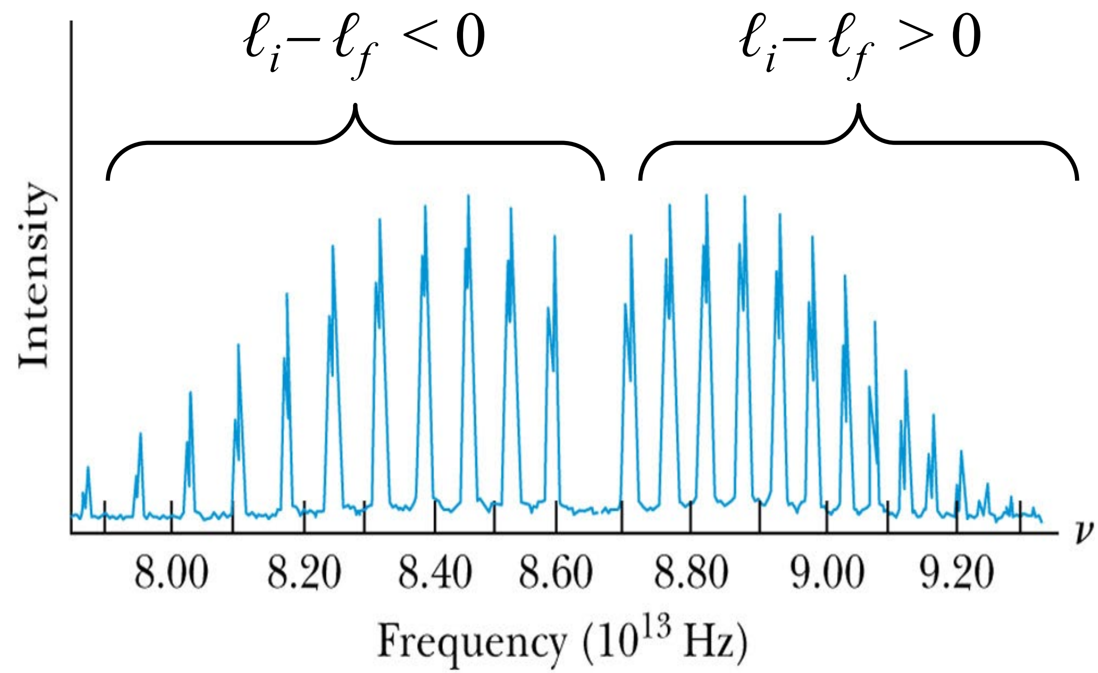


Vibration and Rotation Combined



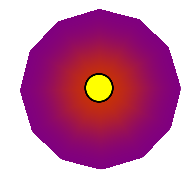
- In the absorption spectrum of HCl, the spacing between the peaks can be used to compute the rotational inertia I.
- The missing peak in the center
- corresponds to the forbidden
- $\Delta\ell = 0$ transition.

$$n_i - n_f = 1$$

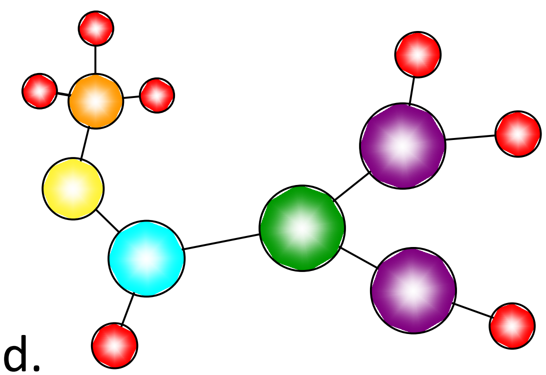


Motion frequencies in atoms and molecules

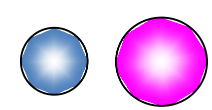
- Electrons vibrate in their motion around nuclei
- High frequency: ~10^{14} - 10^{17} cycles per second.



- Nuclei in molecules vibrate with respect to each other
- Intermediate frequency: ~10^{11} – 10^{13} cycles per second.



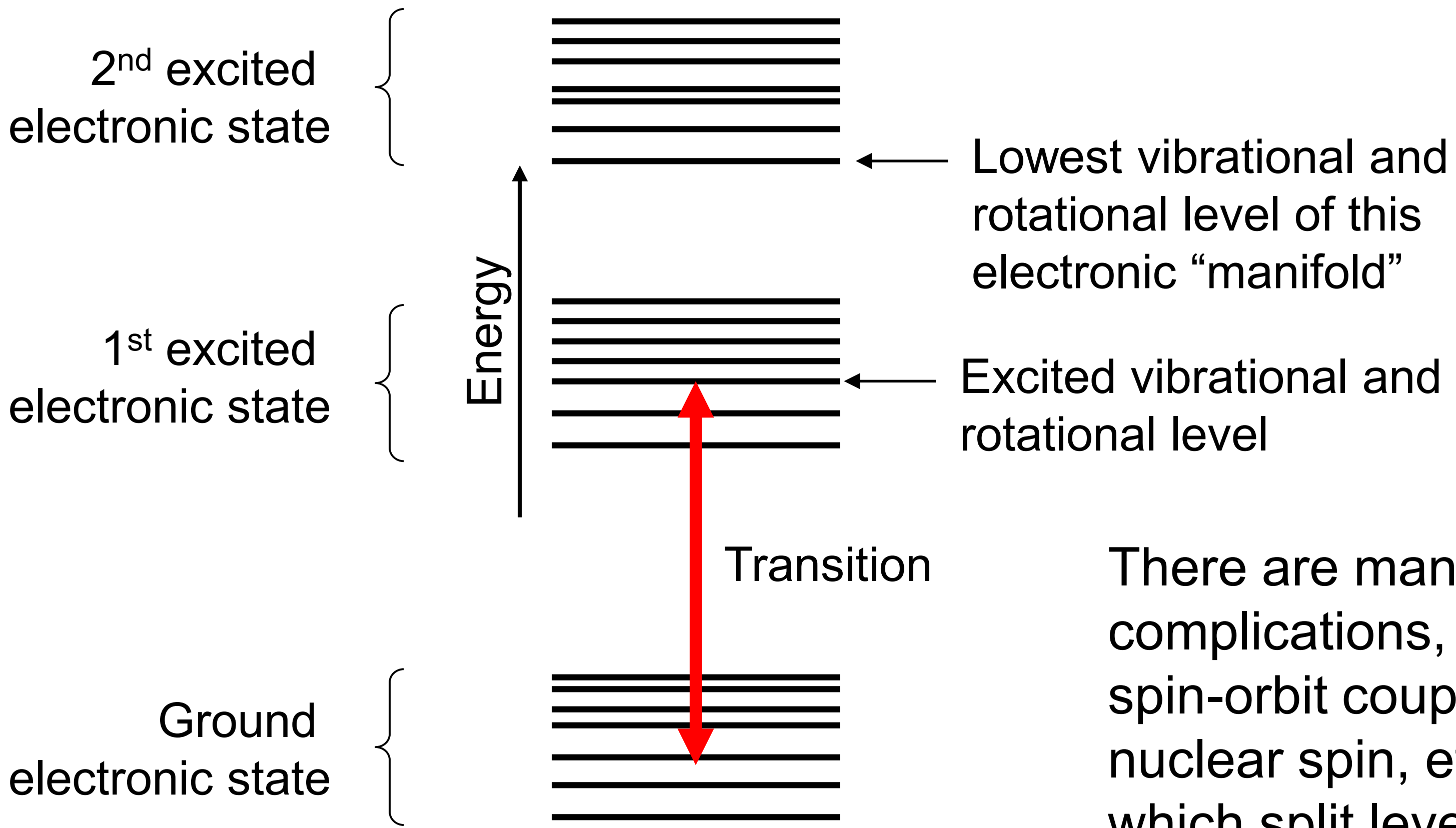
- Nuclei in molecules rotate
- Low frequency: ~10^{9} - 10^{10} cycles per second.



Including electronic energy levels

- A typical large molecule's energy levels

$$E = E_{electonic} + E_{vibrational} + E_{rotational}$$

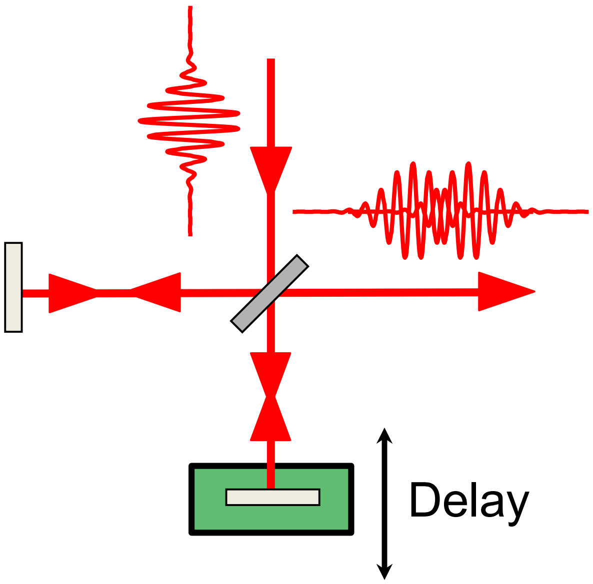


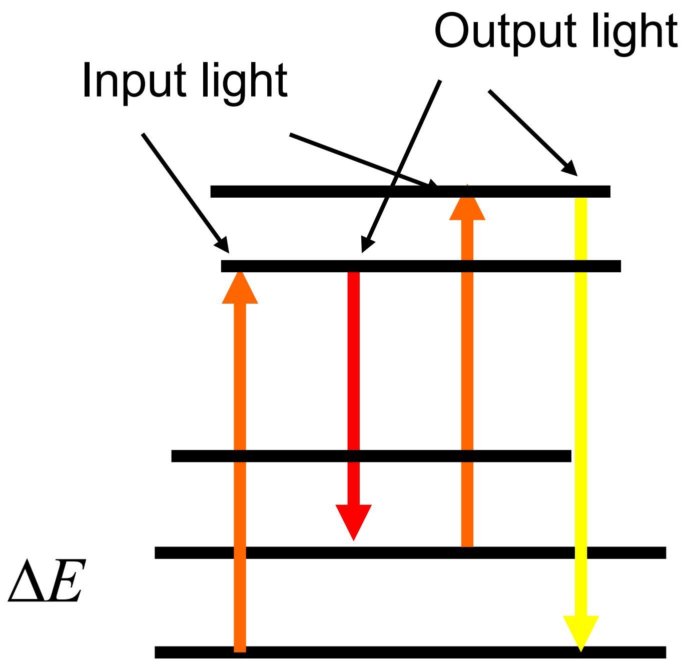
There are many other complications, such as spin-orbit coupling, nuclear spin, etc., which split levels.

As a result, molecules generally have **very complex spectra.**

Studying Vibrations and Rotations

- **Fourier transform infrared (FTIR) spectroscopy**
- The Fourier transform of the output intensity vs. delay of a Michelson Interferometer is the spectrum





Absorption/Transmittance;

- Net absorption **depends on the difference between the populations of the energy levels**
- **The more populated the ground state, the more intense the net absorption is**
- Two factors that influence absorption are the **energy level spacing and the temperature**
- UV Visible absorption spectroscopy involves transitions between electronic energy levels

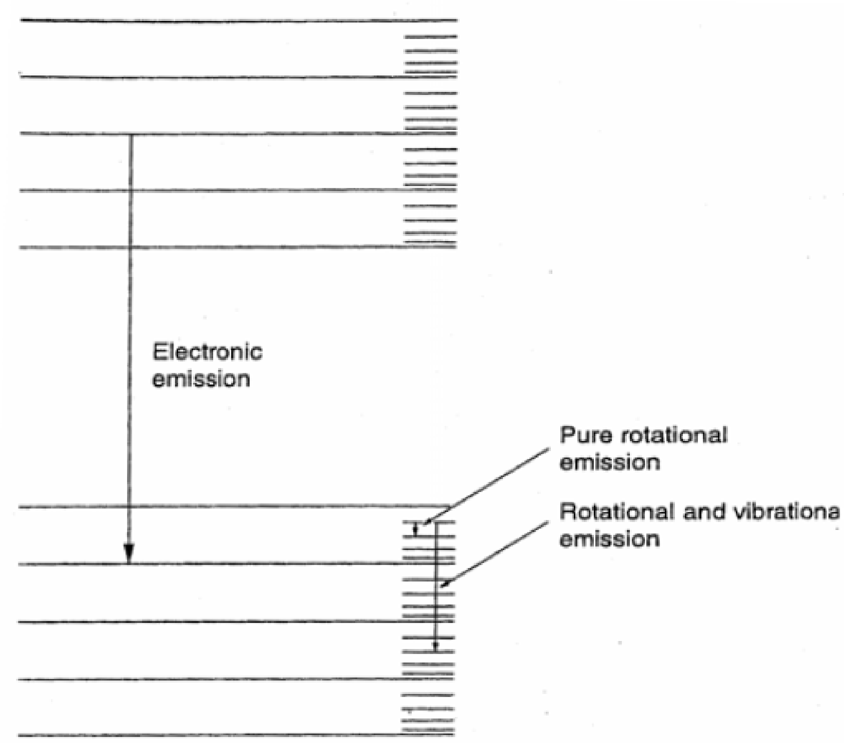
Electron transition rules

Energy is absorbed by transitions induced between different electronic energy states of a molecule

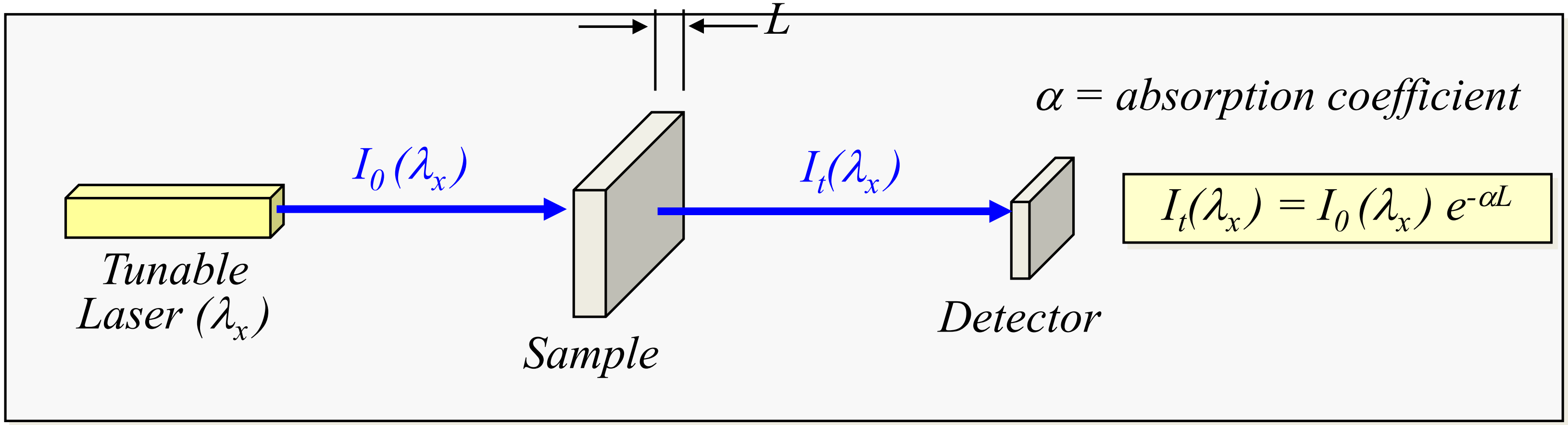
Transition occurs only if there is an induced dipole moment

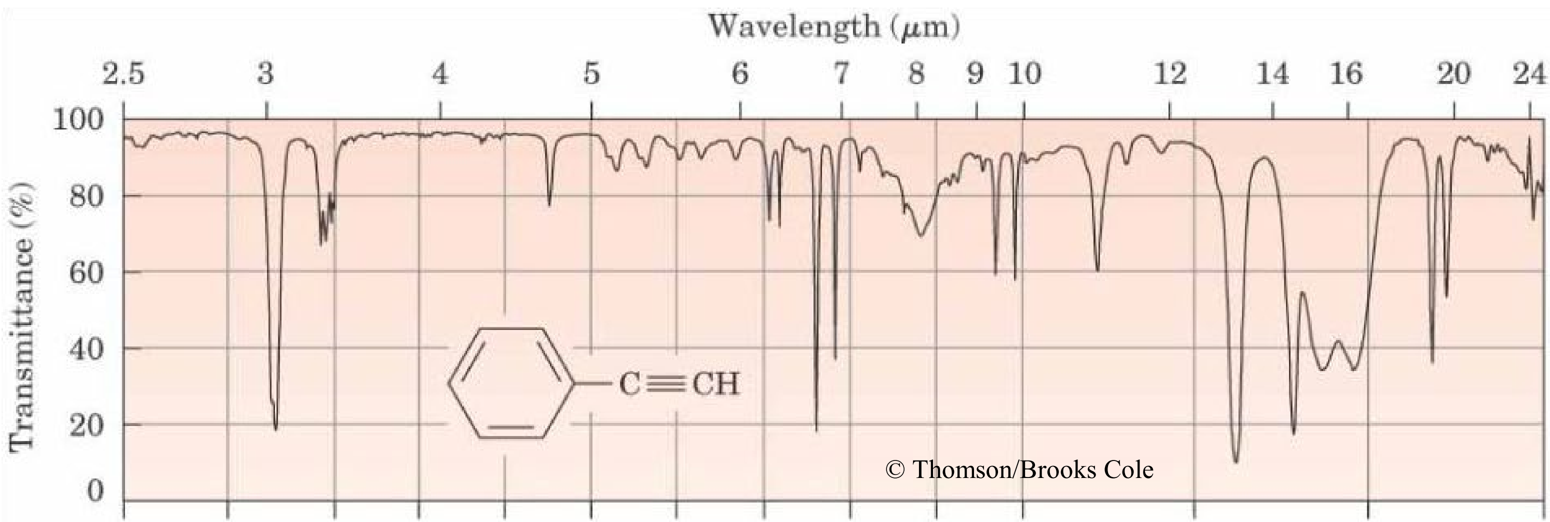
Resonance condition; the frequency of radiation must be equal to the frequency of the dipole

$$\Delta E = h\nu$$



Absorption/Transmittance;

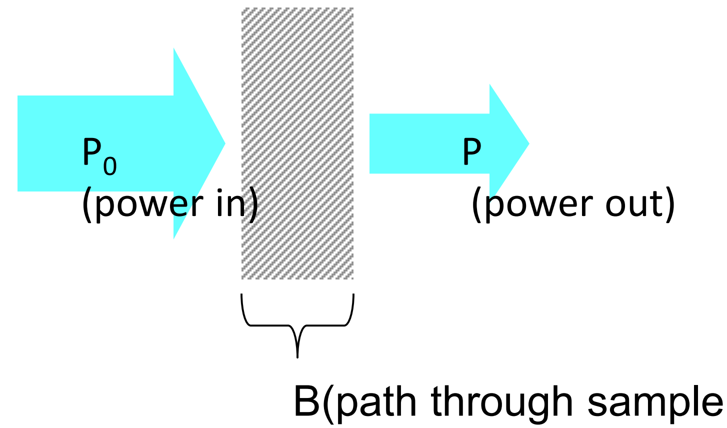


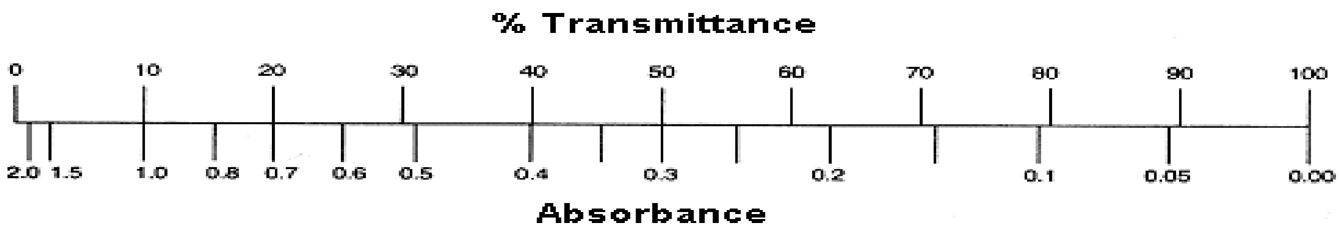


The Quantitative Picture

- Transmittance:
- $T = P/P_0$
- Absorbance:

$A = -\log_{10} T = \log_{10} P_0/P$





- The Beer-Lambert Law (a.k.a. Beer's Law):

$A = \varepsilon bc$ or αL where $\alpha = \varepsilon c$

Where the absorbance A has no units, since $A = \log_{10} P_0 / P$

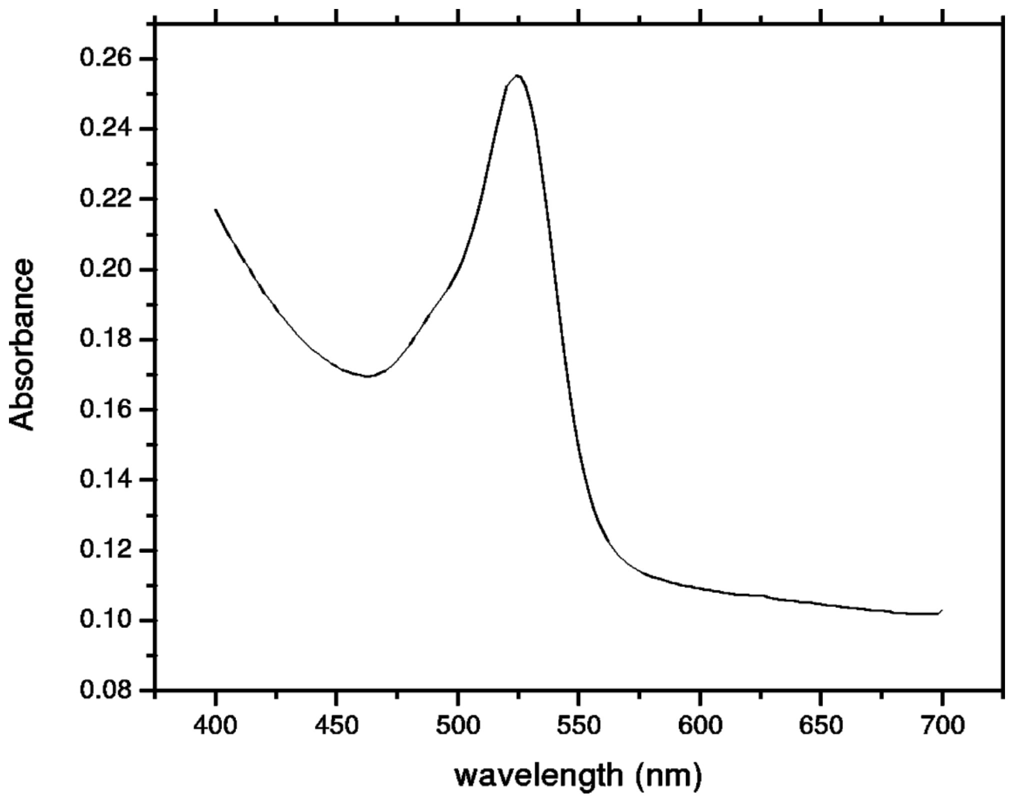
ε is the molar absorbtivity with units of $L\ mol^{-1}\ cm^{-1}$

b is the path length of the sample in cm

c is the concentration of the compound in solution, expressed in $mol\ L^{-1}$ (or M, molarity)

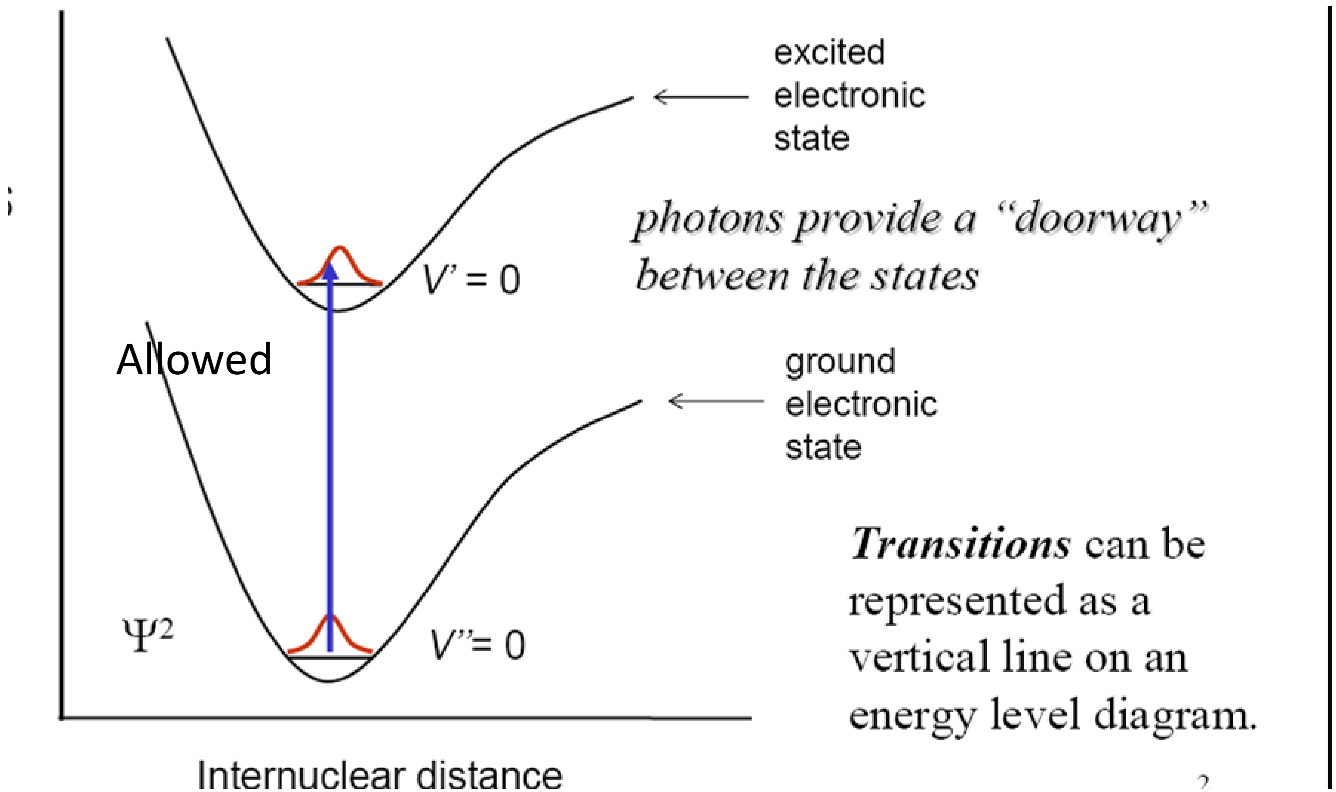
Beer-Lambert Law

- Linear absorbance with increased concentration--directly proportional
- Makes UV useful for quantitative analysis and in **High-performance liquid chromatography** detectors
- Above a certain concentration the linearity curves down, loses direct proportionality--**Due to molecular associations at higher concentrations**. Must demonstrate linearity in validating response in an analytical procedure

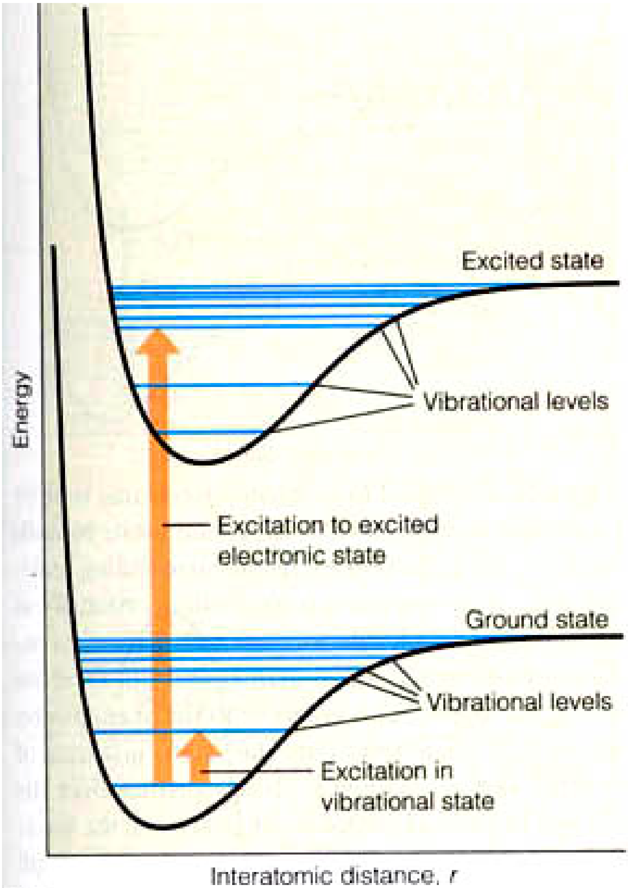


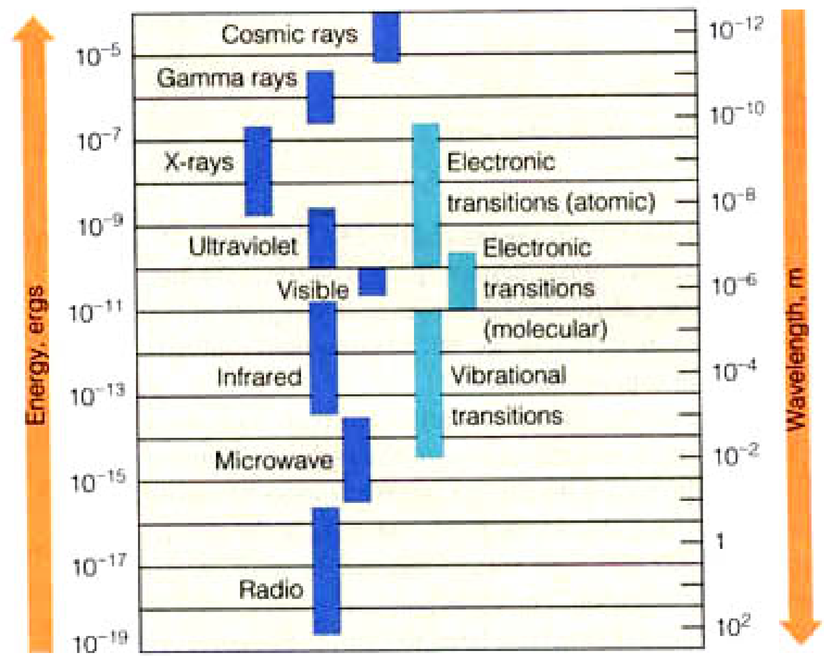
Franck Condon principle

- Electronic transitions can terminate in different vibrational and rotational states, resulting in broad electronic absorption/emission bands. There are no simple selection rules here, but the *Franck-Condon principle* states **that the vibrational coordinate should not change during a transition, (see below).** Since the electrons have highest probability of being at the extreme positions of their excursions, this controls which transitions are most possible, which strongly affects the shape of the absorption band.



Franck Condon principle

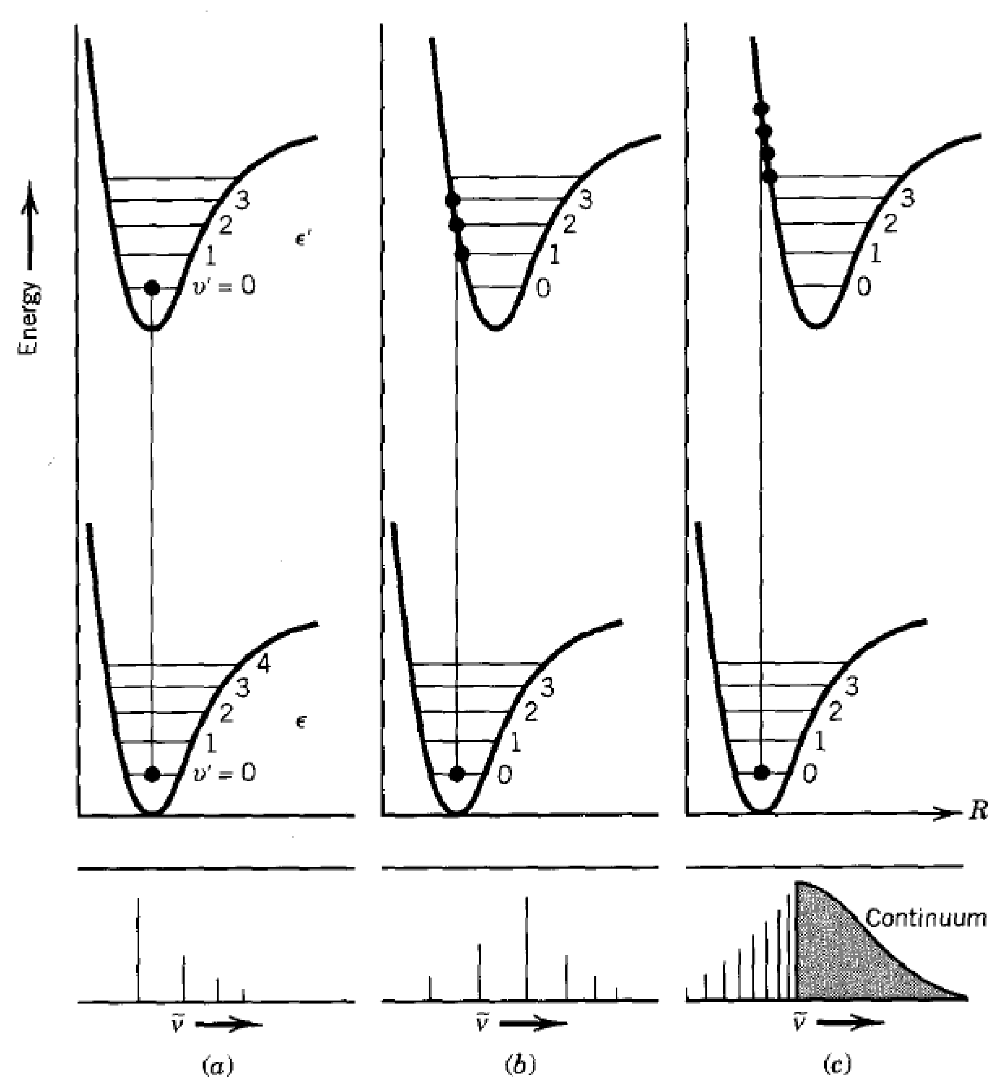




- The nuclear motion (10^{-13} s) is negligible during the time required for an electronic excitation (10^{-16} s).
- Since the nuclei do not move during the excitation, **the internuclear distances remain constant and "the most probable component of an electronic transition involves only the vertical transitions".**

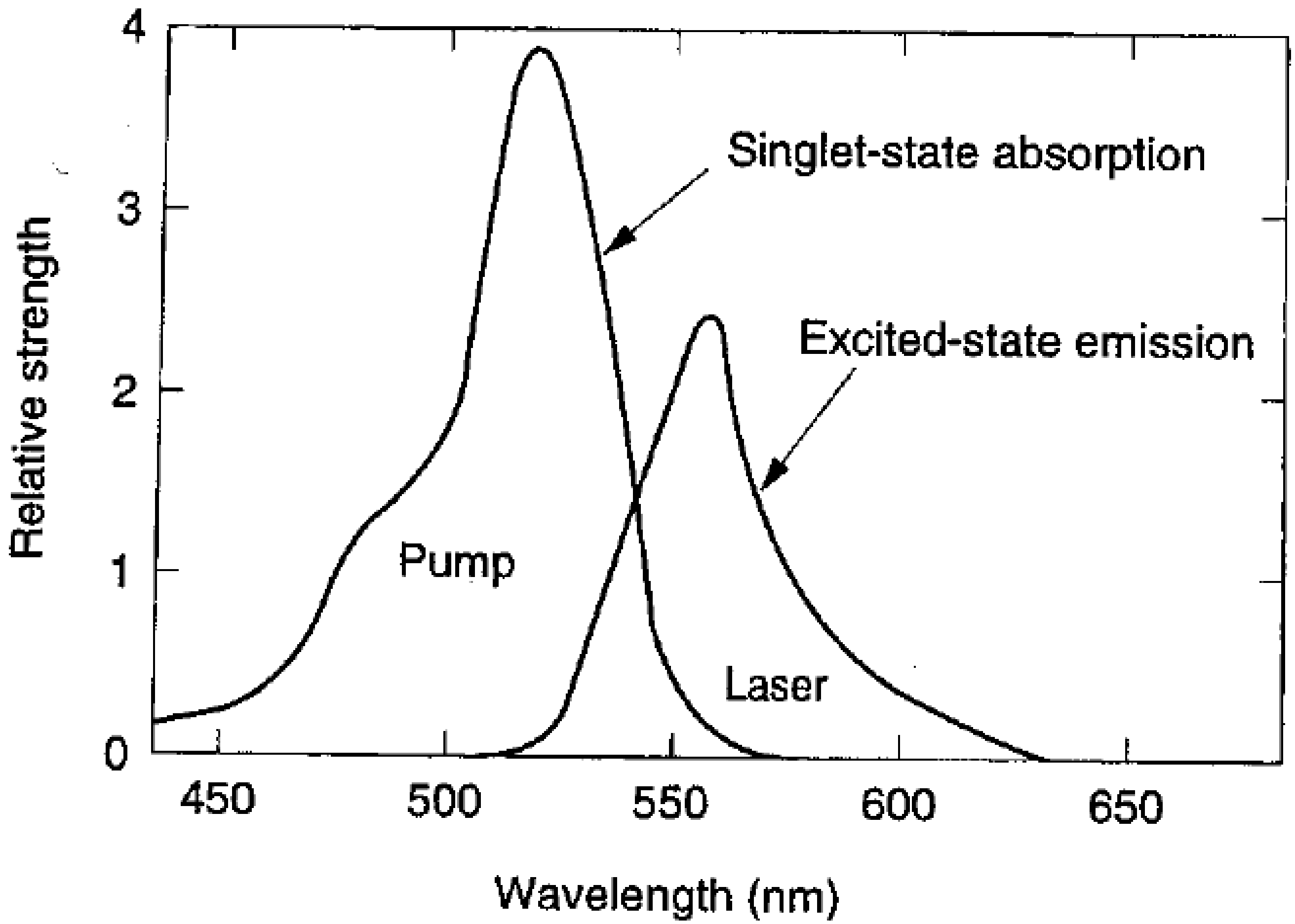
Franck Condon principle

- The time **for an electronic transition is: $t=1/\nu = \lambda/c \sim 10^{-15}$ s (at 420 nm)**
- Franck Condon principle: electronic transitions occur so rapidly that during the transition the nuclei are static
- Thus, all electronic transitions are vertical (internuclear distance doesn't change)
- As the optical transition becomes less ***vertical the absorption spectra shift due to the change in the Franck-Condon patterns.***
- With enough of these transitions the absorption spectrum looks **more like a smooth curve rather than a line**

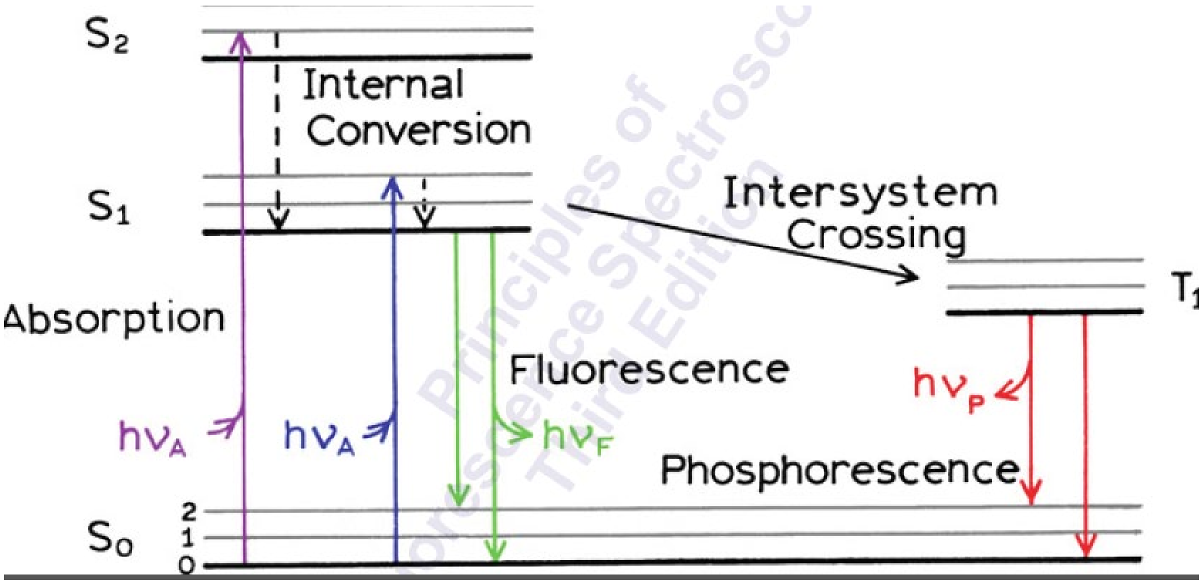


Franck Condon principle

- Due to the Franck-Condon ***principle and the tendency for molecules to relax to the***
- ***bottom of the vibration bands, the emission spectrum is shifted to longer wavelengths than the absorption spectrum, and the emission band usually looks like a mirror image of the absorption band***



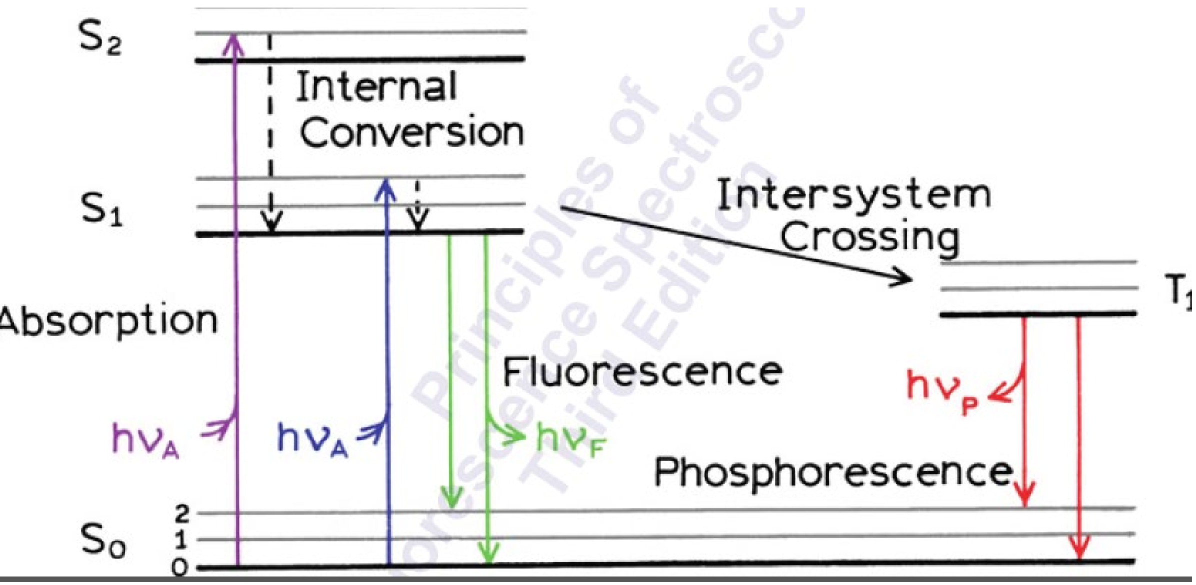
Fluorescence

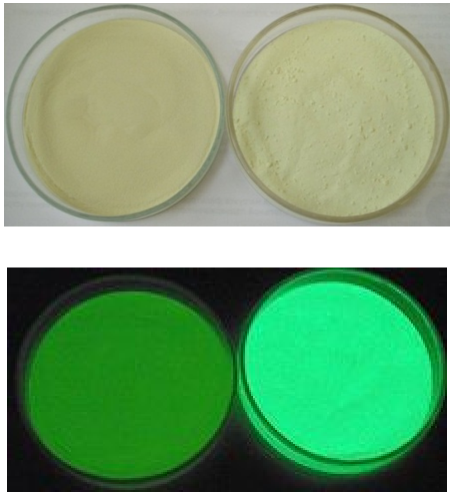


- **Fluorescence** is the emission of light by a substance that has absorbed light or other electromagnetic radiation. In most cases, emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation.

Alexander Jablonski (1898-1980)

Phosphoresence





ZnS powder

- **Phosphorescence** is a specific type of photoluminescence related to fluorescence. Unlike fluorescence, a phosphorescent material does not immediately re-emit the radiation it absorbs. The slower time scales of the re-emission are associated with "forbidden" energy state transitions in quantum mechanics. As these transitions occur very slowly in certain materials, absorbed radiation may be re-emitted at a lower intensity for up to several hours after the original excitation.

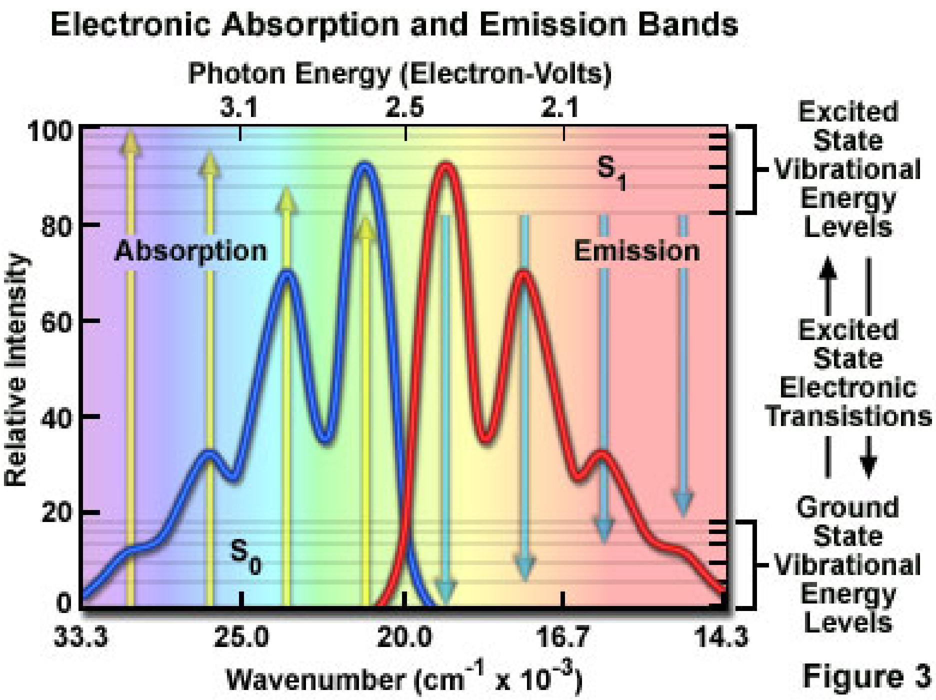
Fluorescence and Phosphorescence

Fluorescence – return from excited singlet state to ground state; does not require change in spin orientation (more common of relaxation)

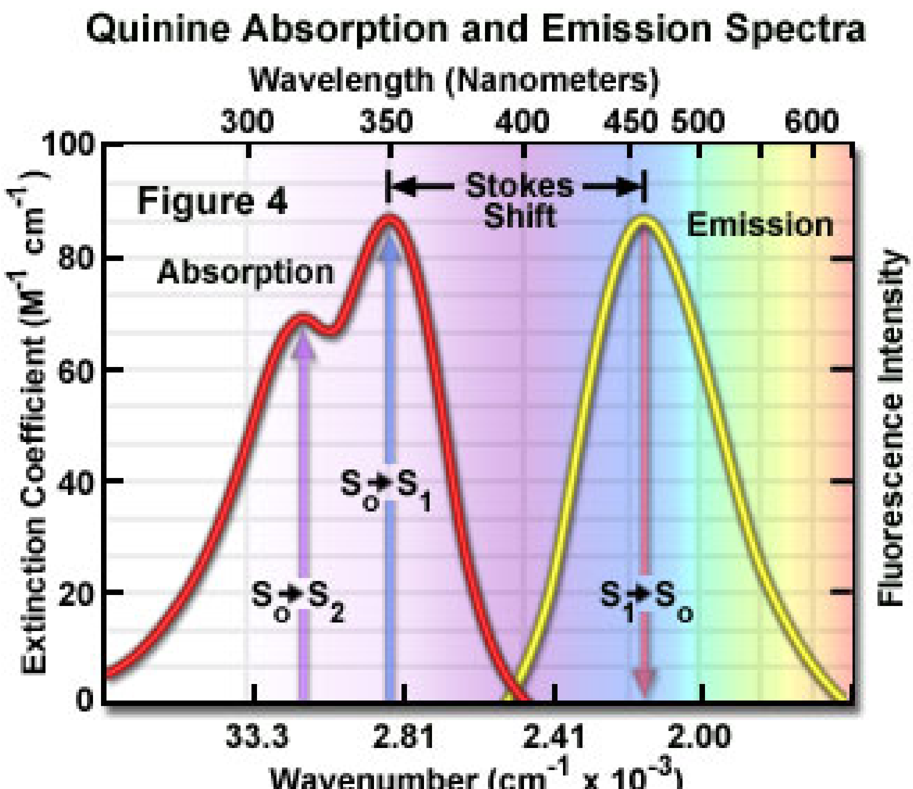
Phosphorescence – return from a triplet excited state to a ground state; **electron requires change in spin orientation**

Emissive rates of fluorescence are several orders of magnitude faster than that of phosphorescence

- The fluorescence light is red-shifted (longer wavelength than the excitation light) relative to the absorbed light ("Stokes shift”).
- Internal conversion -can affect Stokes shift
- Solvent effects and excited state reactions can also affect the magnitude of the Stoke’s shift



- Mirror-image rule typically applies when only $S_0 \rightarrow S_1$ excitation takes place



Deviations from the mirror-image rule are observed when $S_0 \rightarrow S_2$ or transitions to even higher excited states also take place

Quantum yield and lifetime

- Quantum yield of fluorescence, Φ_f, is defined as

$$\Phi_f = \frac{\text{number of photons emitted}}{\text{number of photons absorbed}}$$

- In practice, is measured by comparative measurements with reference compound for which has been determined with high degree of accuracy.

- Ideally, reference compound should have

- the same absorbance as the compound of interest at given excitation wavelength
  - similar excitation-emission characteristics to compound of interest (otherwise, instrument wavelength response should be taken into account)
  - Same solvent, because intensity of emitted light is dependent on refractive index (otherwise, apply correction

$$\frac{\Phi_f^u}{\Phi_f^s} = \frac{I_f^u}{I_f^s} \times \frac{n^2(u)}{n^2(s)}$$

  - Yields similar fluorescence intensity to ensure measurements are taken within the range of linear instrument response

Fluorescence Intensities

The fluorescence intensity (F) at a particular excitation (λ_x) and emission wavelength (λ_m) will depend on the absorption and the quantum yield:

$$\mathbf{F}(\lambda_{\mathbf{x}}, \lambda_{\mathbf{m}}) = \mathbf{I}_{\mathbf{A}}(\lambda_{\mathbf{x}})\phi(\lambda_{\mathbf{m}})$$

where,

I_A – light absorbed to promote electronic transition

ϕ – quantum yield

From the Beer-Lambert law, the absorbed intensity for a dilute solution (very small absorbance)

$$\mathbf{I}_{\mathbf{A}}(\lambda_{\mathbf{x}}) = \mathbf{2.303 I}_{\mathbf{o}}\varepsilon(\lambda_{\mathbf{x}})\mathbf{CL}$$

$$\textbf{for } \varepsilon(\lambda_{\mathbf{x}})\mathbf{CL} << \mathbf{1}$$

where,

I_o – Initial intensity

ε – molar extinction coefficient

C – concentration

L – path length

Fluorescence intensity expression

The fluorescence intensity (F) at a particular excitation (λ_x) and emission wavelength (λ_m) for a dilute solution containing a fluorophore is:

$$\mathbf{F}(\lambda_{\mathbf{x}}, \lambda_{\mathbf{m}}) = \mathbf{I}_{\mathbf{o}} 2.303 \varepsilon(\lambda_{\mathbf{x}}) \mathbf{CL} \phi(\lambda_{\mathbf{m}})$$

where,

I_o – incident light intensity

C – concentration

L – path length

ϕ – quantum yield

ε – molar extinction coefficient

Florescence remarks

- Because of the differing rotational, vibrational and even electronic states a molecule can have**, peaks are BROAD.**

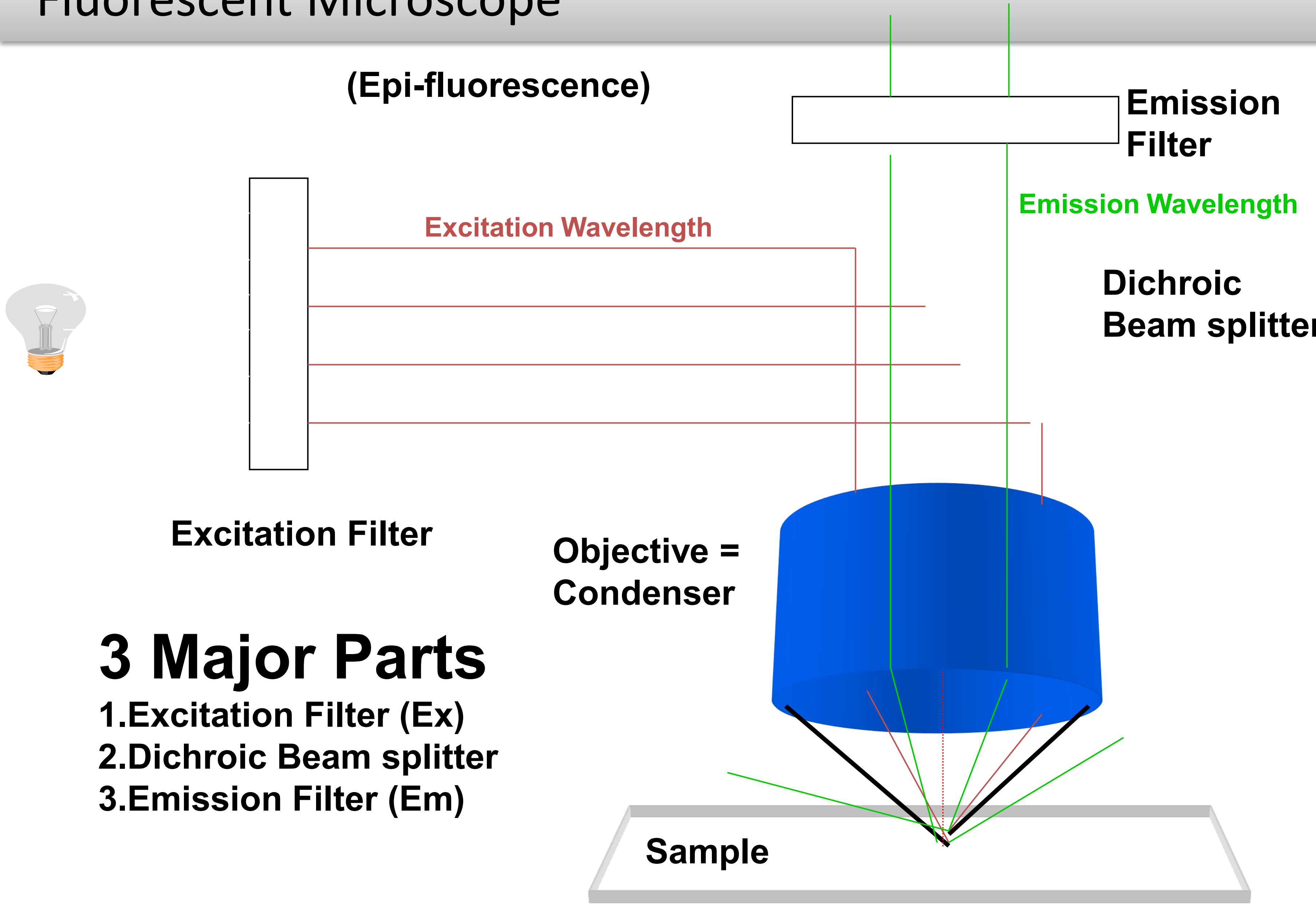
- ***Each spectrum is characteristic of a given molecule.***

- Before returning to its normal state, the electron remain at the excited level for approximately 10^{-15} seconds.

- Because the excited state **is HIGHLY REACTIVE it can "bleach" (react with other molecules).**

Fluorescent Microscope

(Epi-fluorescence)



3 Major Parts

1. Excitation Filter (Ex)
2. Dichroic Beam splitter
3. Emission Filter (Em)

Chromophore

- A **chromophore** is the part of a molecule responsible for its color. The color arises when a molecule absorbs certain wavelengths of visible light and transmits or reflects others. The chromophore is a region in the molecule where the energy difference between two different molecular orbitals falls within the range of the visible spectrum. Visible light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state.

- In biology, molecules that serve to capture or detect light energy, the chromophore is the moiety that causes a conformational change of the molecule when hit by light.

- **Fluorophore** (or fluorochrome, similarly to a chromophore) is a fluorescent chemical compounds that can re-emit light upon light excitation. Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds.

Wikipedia

Biological Fluorophores /Chromophores

- **Endogenous Fluorophores**
  - structural proteins
  - enzymes and co-enzymes
  - vitamins
  - lipids
  - Porphyrins
- **Exogenous Fluorophores**
  - Rhodamines, fluoresceins
  - Coumarins, carbocyanine dyes
  - aromatic hydrocarbons and derivatives: pyrenes, perylenes, anthracenes
  - Molecular markers – GFP, etc.

Endogenous fluorophores	Excitation maxima (nm)	Emission maxima (nm)
Amino acids		
Tryptophan	280	350
Tyrosine	275	300
Phenylalanine	260	280
Structural proteins		
Collagen	325	400, 405
Elastin	290, 325	340, 400
Enzymes and coenzymes		
FAD, flavins	450	535
NADH	290, 351	440, 460
NADPH	336	464
Vitamins		
Vitamin A	327	510
Vitamin K	335	480
Vitamin D	390	480
Vitamin B_6 compounds		
Pyridoxine	332, 340	400
Pyridoxamine	335	400
Pyridoxal	330	385
Pyridoxic acid	315	425
Pyridoxal 5′-phosphate	330	400
Vitamin B_{12}	275	305
Lipids		
Phospholipids	436	540, 560
Lipofuscin	340–395	540, 430–460
Ceroid	340–395	430–460, 540
Porphyrins	400–450	630, 690

FAD, flavin adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; AND(P)H, reduced nicotinamide adenine dinucleotide phosphate.

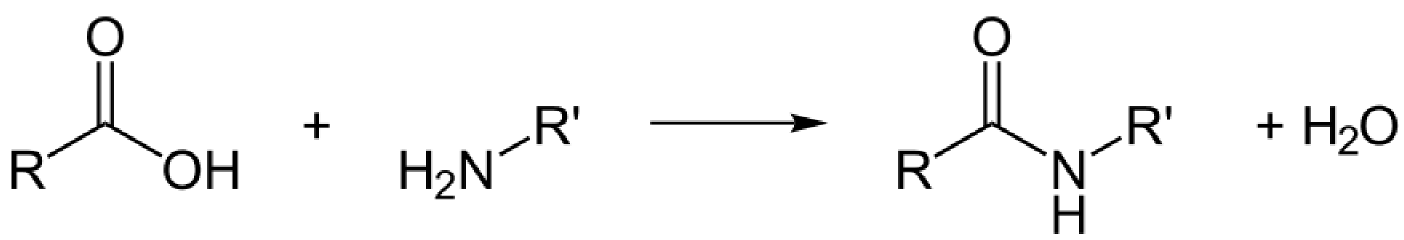
Biological chromophores

1. The peptide bonds and amino acids in proteins
   - The p electrons of the peptide group are delocalized over the carbon, nitrogen, and oxygen atoms. The n-π* transition is typically observed at 210-220 nm, while the main π-π* transition occurs at ~190 nm.
   - Aromatic side chains contribute to absorption at λ> 230 nm

2. Purine and pyrimidine bases in nucleic acids and their derivatives

3. Highly conjugated double bond systems

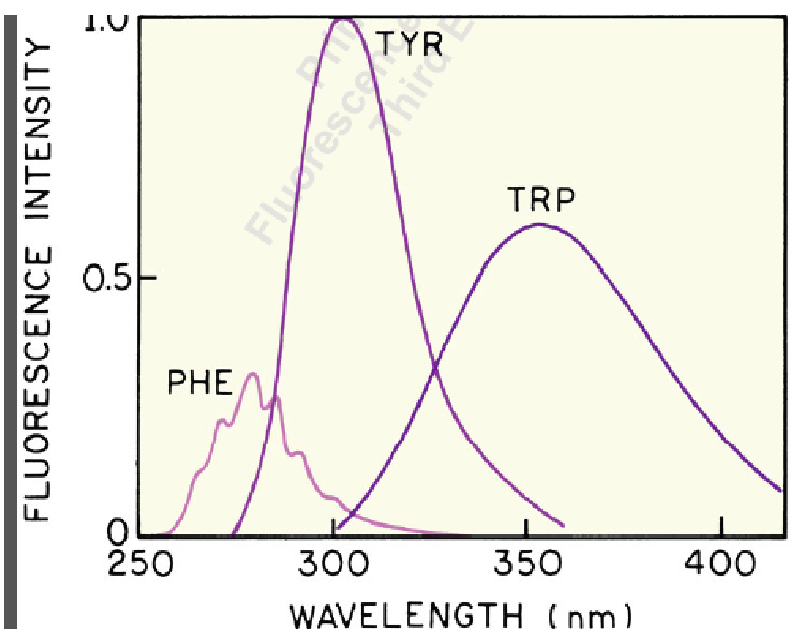
A **peptide bond** (**amide bond**) is a covalent chemical bond formed between two molecules when the carboxyl group of one molecule reacts with the amino group of the other molecule,

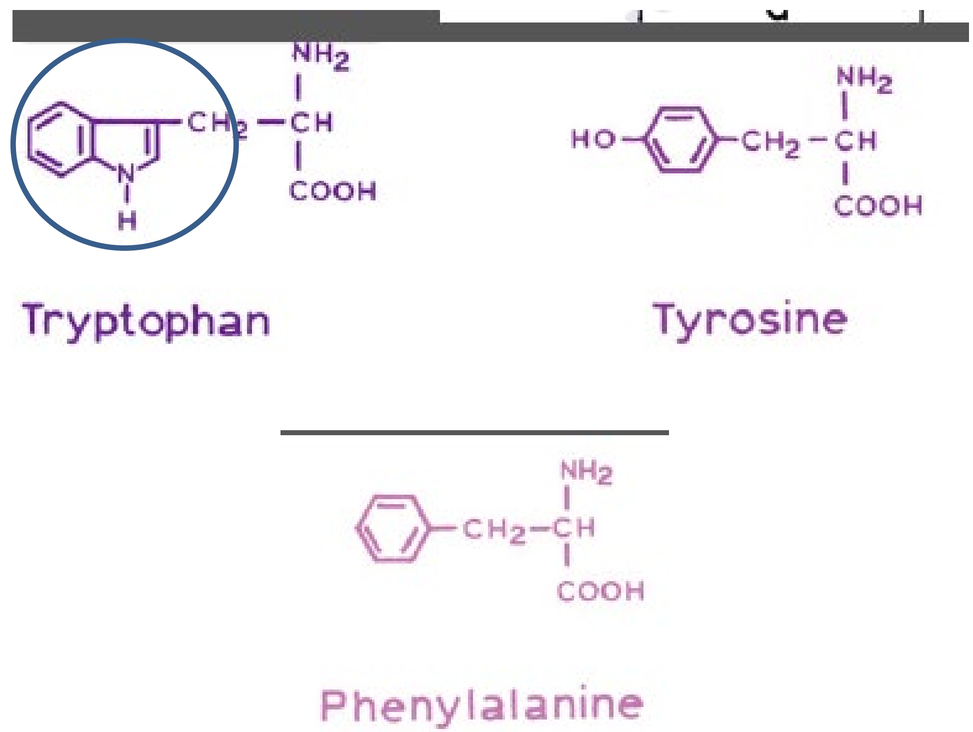


Principles of Fluorescence Spectroscopy 2nd Edition (1999) Lakowicz, J.R. Editor, Kluwer Academic/Plenum Publishers, New York, New York

Biological chromophores/fluorophores

Species	λ max (nm)	λ max (nm)	Bandwidth	Quantum yield	Lieftime (ns)
Phenylalanine	260	282	-	0.02	6.8
Tyrosine	275	304	34	0.2	3.6
Tryptophan	295	353	60	0.13	3.1





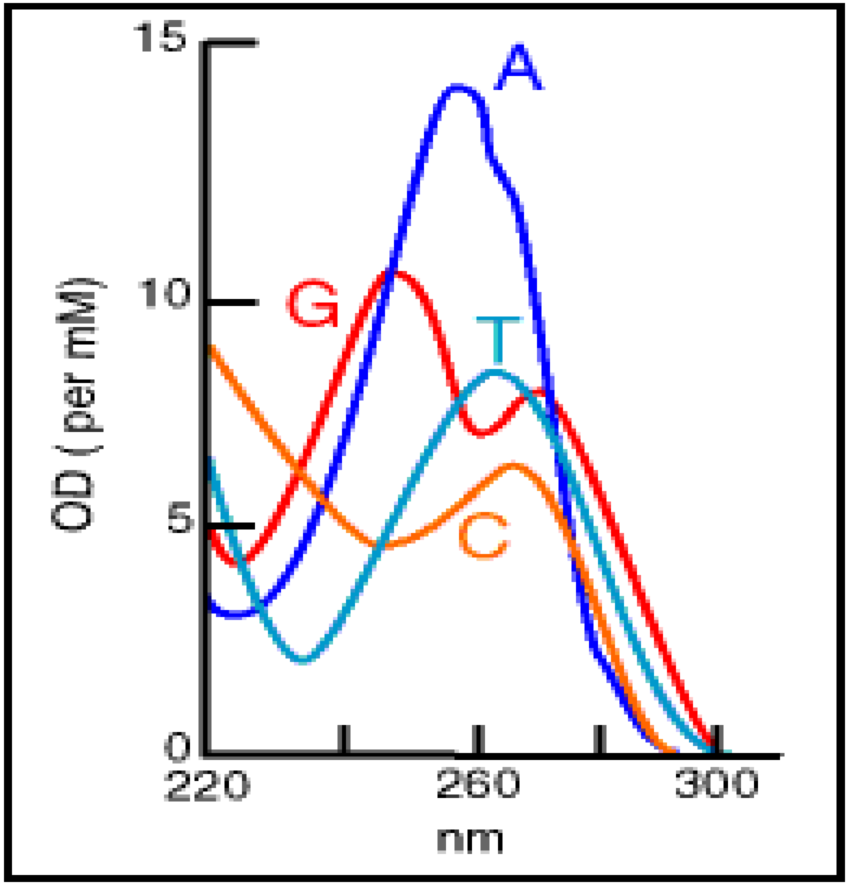
Principles of Fluorescence Spectroscopy 2nd Edition (1999) Lakowicz, J.R. Editor, Kluwer Academic/Plenum Publishers, New York, New York

Nucleic Acids

- The rings of the bases (A, C, G, T, U) are made up of alternating single and double bonds.
- Such ring structures absorb in the U.V.
- Each of the four nucleotide bases has a slightly different absorption spectrum, and the spectrum of DNA is the average of them.

- Molar Extinction Coefficients of Bases

Base	ε (Epsilon) (molar extinction coefficient) at OD260
Adenine	15,200
Cytosine	7,050
Guanine	12,010
Thymine	8,400



Nucleic Acids

- When a DNA helix is denatured to become single strands the absorbance is increased about 30 percent.
- This increase, (the hyperchromic shift) indicates that the double-stranded molecule is quenching fluorescence.
- So, you always need to know if your DNA is double or single stranded when measuring it using the spectrophotometer

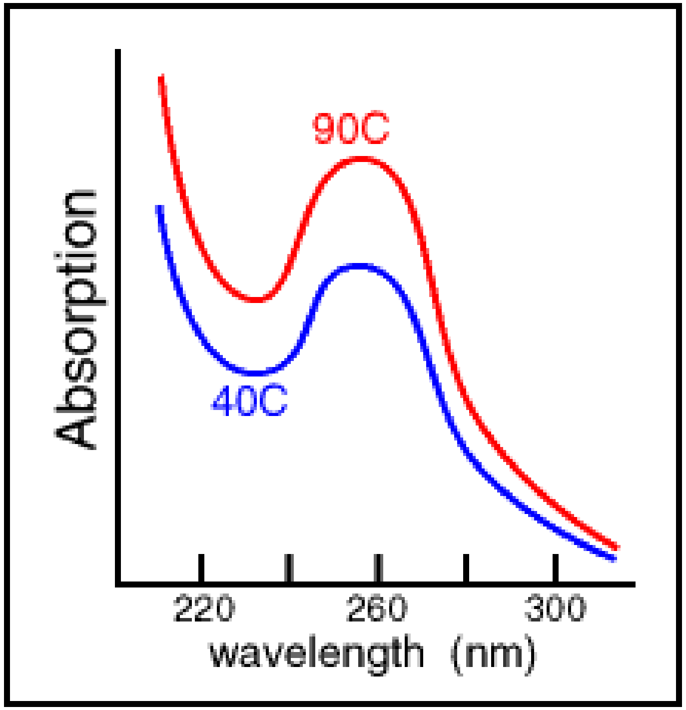
The values of **Molar Extinction Coefficient**

E used here are as follows:

ssDNA, 0.027 $(ug/ml)^{-1}cm^{-1}$

dsDNA, 0.020 $(ug/ml)^{-1}cm^{-1}$

ssRNA, 0.025 $(ug/ml)^{-1}cm^{-1}$



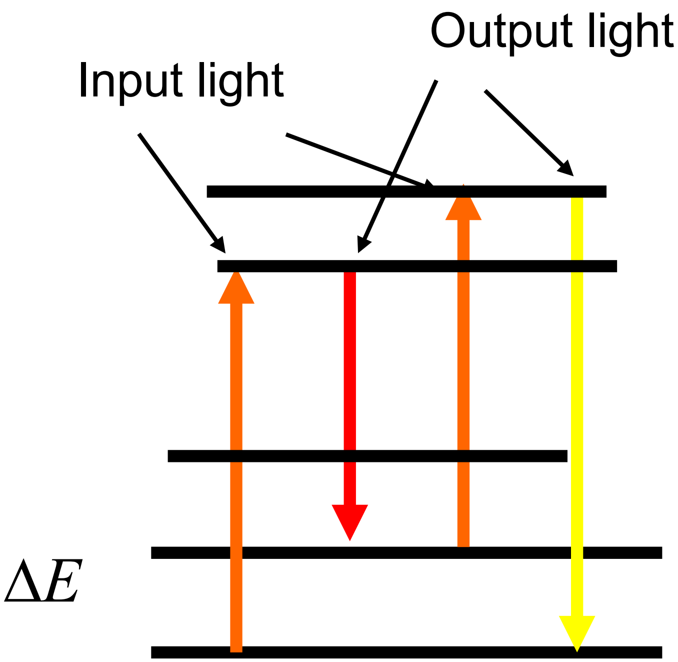
Nucleic Acids-UV Quantitation of DNA

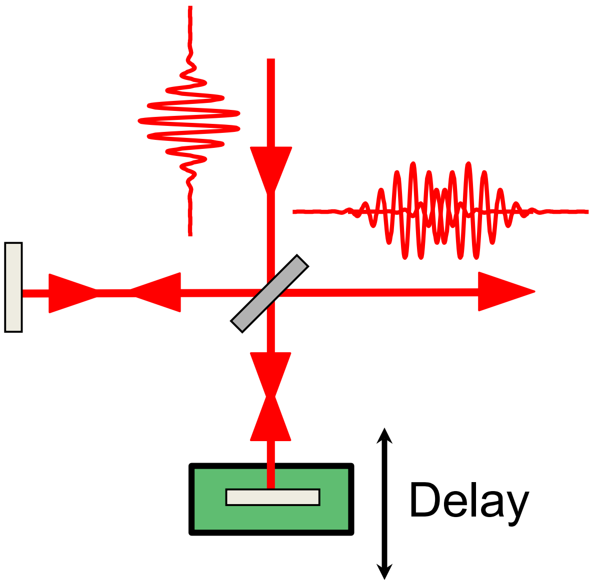
- Using these calculations, an A_{260} of 1.0 indicates:
- 50 ug/ml double-stranded DNA
- ~ 37 ug/ml single-stranded DNA
- ~ 40 ug/ml single- stranded RNA

- The detection limit of absorption spectroscopy will depend on the sensitivity of the spectrophotometer and any UV-absorbing contaminants that might be present.
- The lower limit is generally ~0.5 to 1 ug nucleic acid.

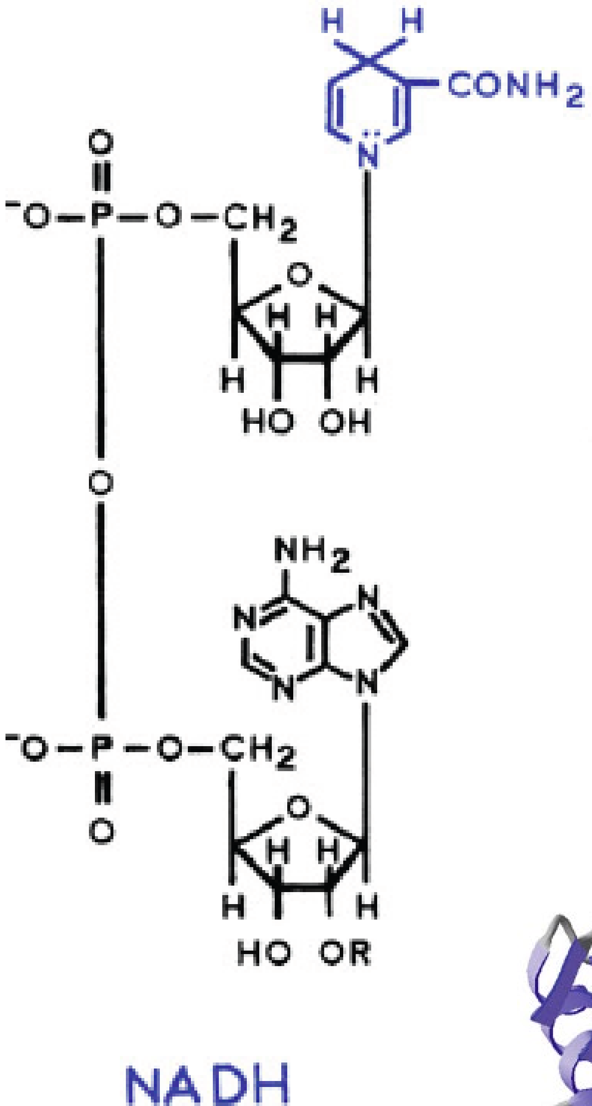
Studying Vibrations and Rotations

- **Fourier transform infrared (FTIR) spectroscopy**
- The Fourier transform of the output intensity vs. delay of a Michelson Interferometer is the spectrum



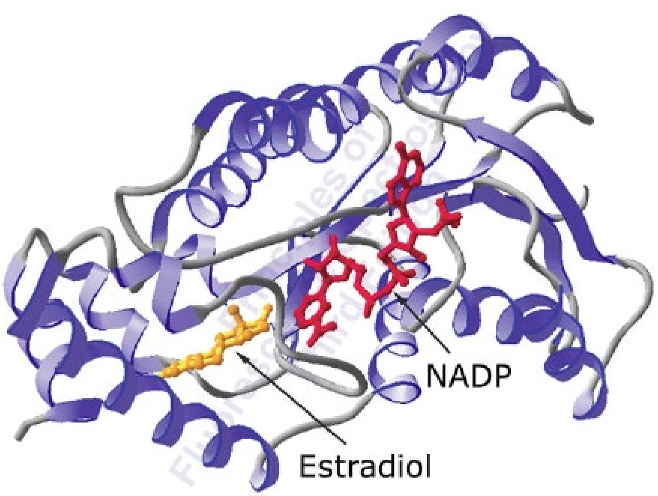


NADH



Nicotinamide adenine dinucleotide, abbreviated **NAD⁺**, is a coenzyme found in all living cells. The compound is a dinucleotide, since it consists of two nucleotides joined through their phosphate groups. One nucleotide contains an adenine base and the other nicotinamide.

NADH fluorescence can increase or decrease upon protein binding-mostly increases for **17β-hydroxysteroid dehydrogenases** (17β-HSD enzyme)



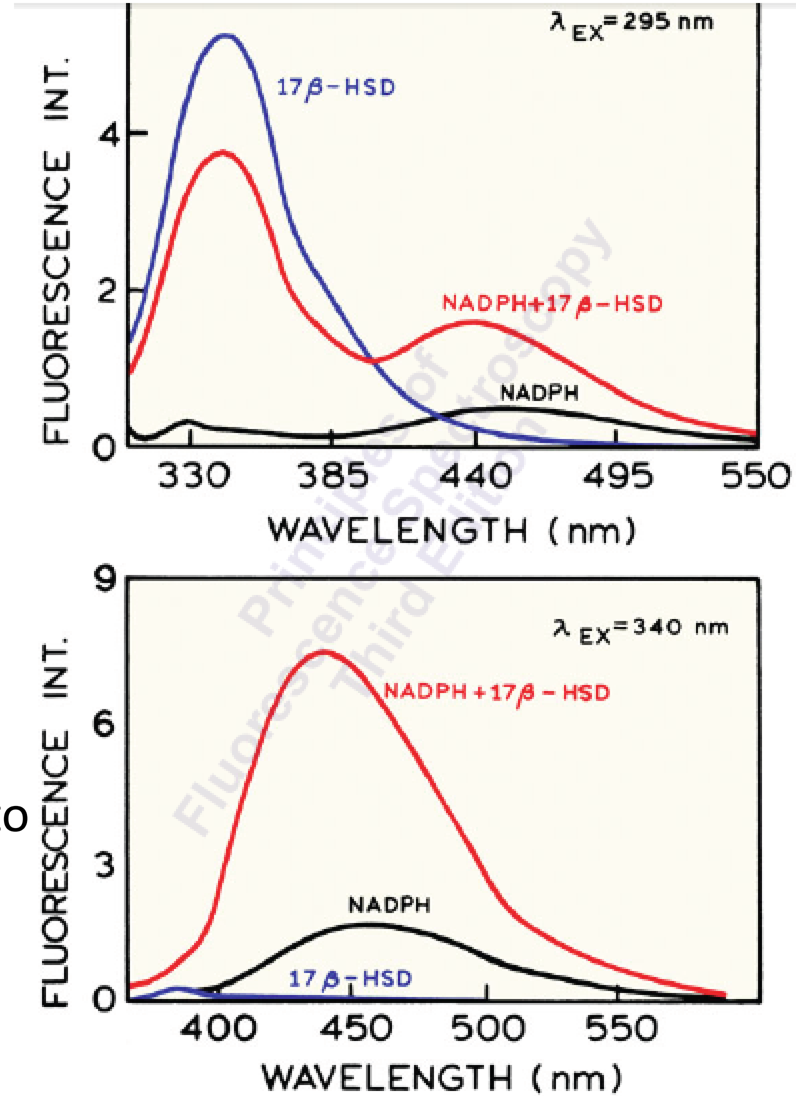
Quenching of nicotinamide by adenine is prevented **and intensity gets higher**!

NADH

- NADH in solution has an emission peak at 460 nm and a fluorescence lifetime of 0.4 nanoseconds, while the oxidized form of the coenzyme does not fluoresce.[7]

- The properties of the fluorescence signal changes when NADH binds to proteins, so these changes can be used to measure dissociation constants, which are useful in the study of enzyme kinetics.[7][8]

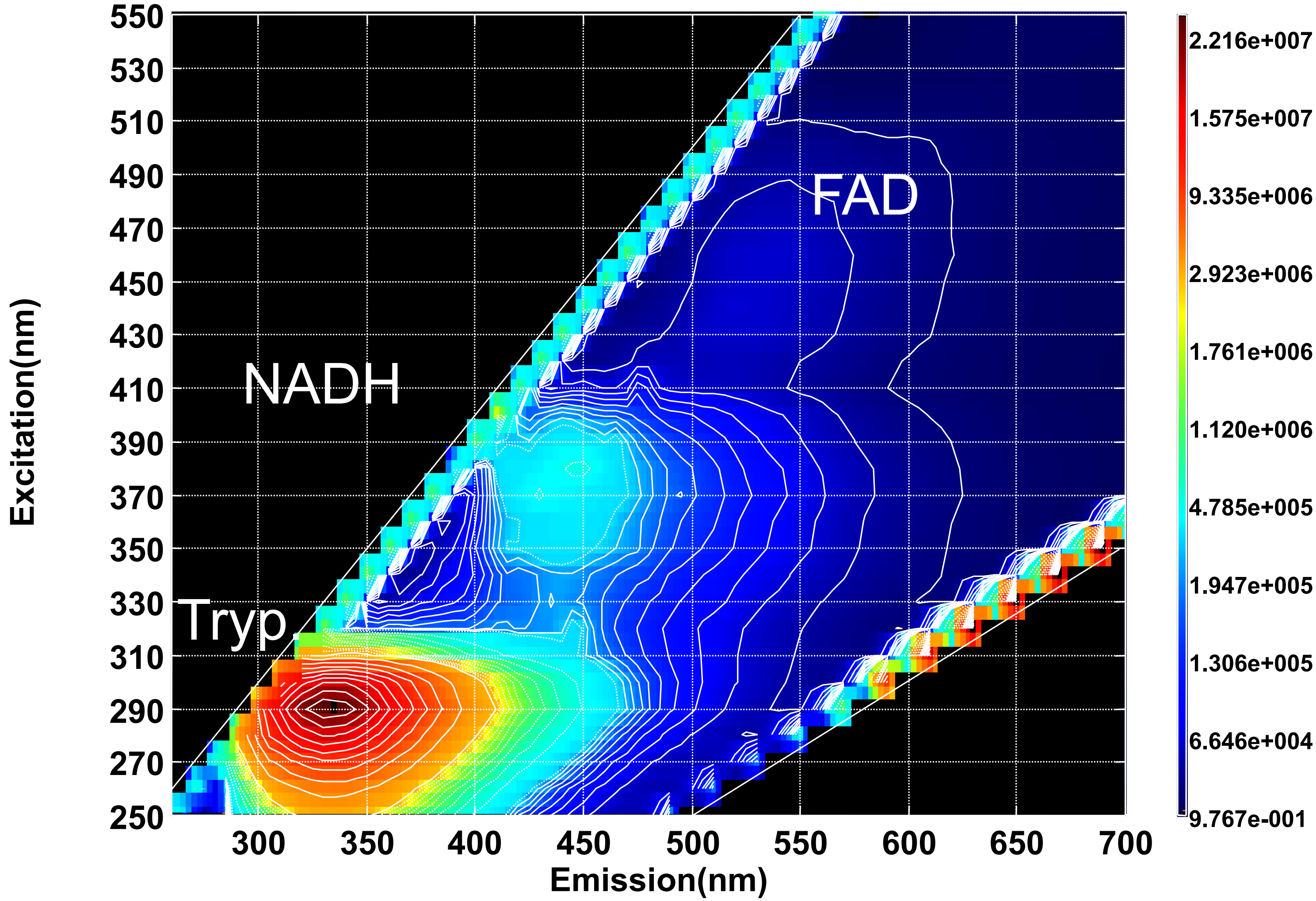
- These changes in fluorescence are also used to measure changes in the redox state of living cells, through fluorescence microscopy.[



Much of the cell auto-fluoresence is due to NADH and flavins

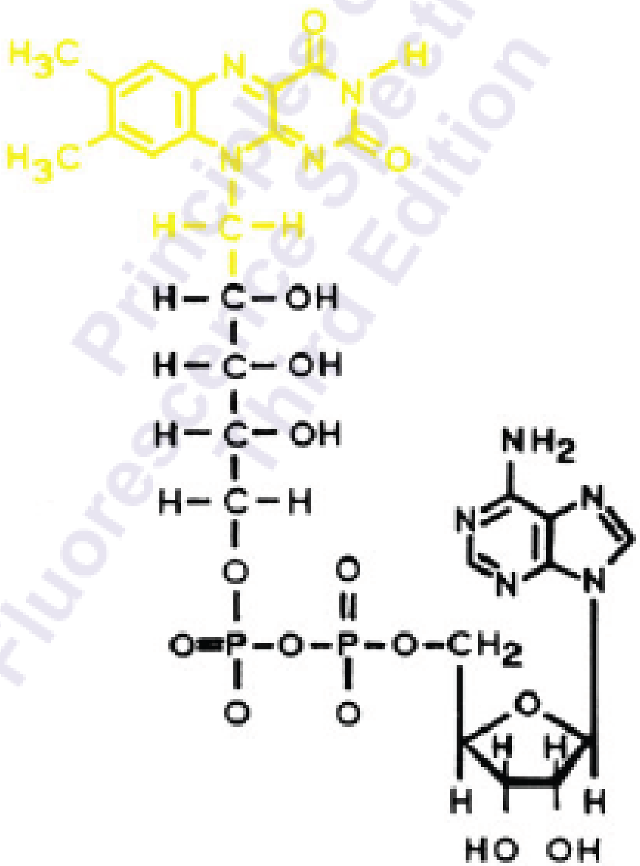
Epithelial Cell Suspension

- **Fluorescence intensity excitation-emission matrix**



FAD

- **Flavin adenine dinucleotide** (**FAD**) is a redox cofactor involved in several important reactions in metabolism. FAD can exist in two different redox states, which it converts between by accepting or donating electrons.

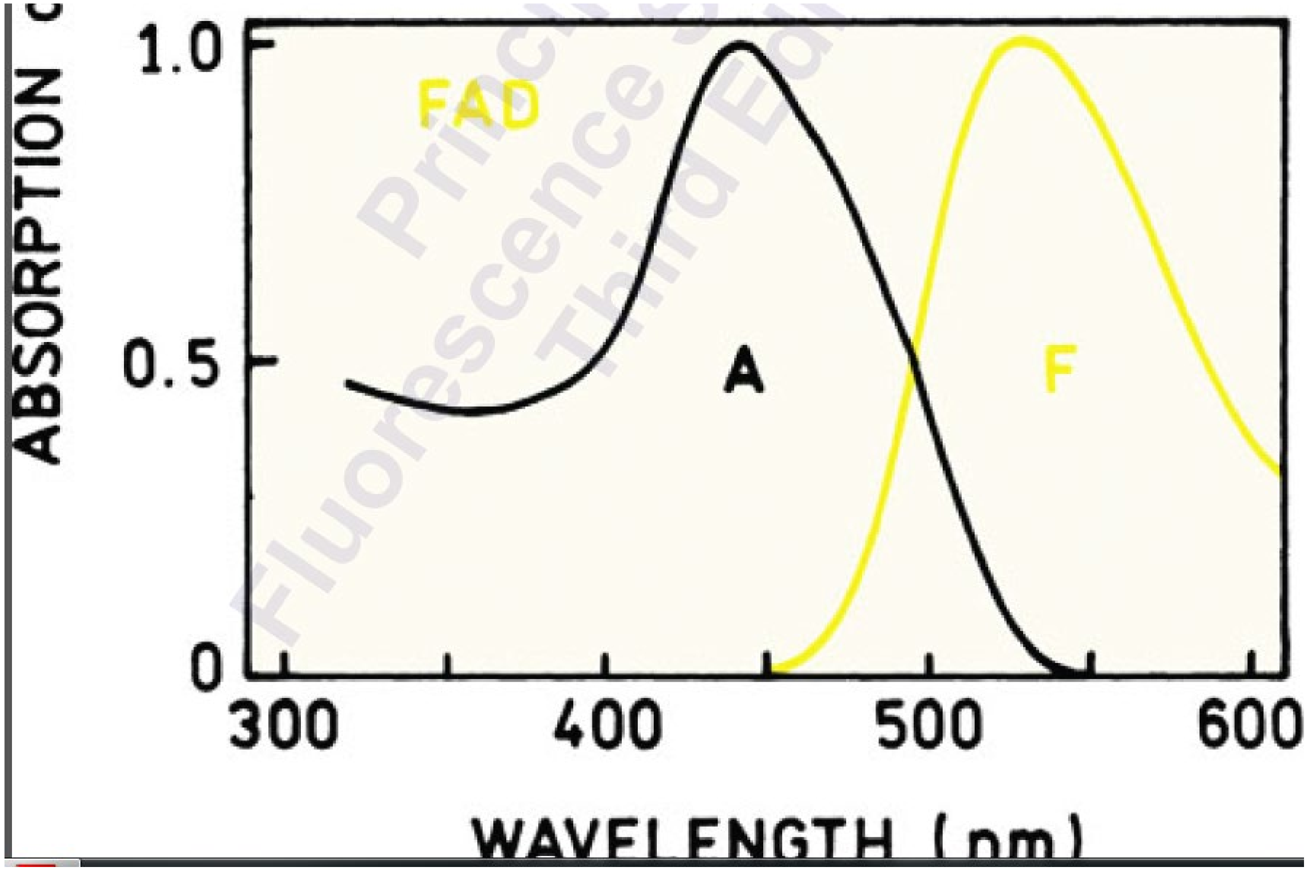


FAD

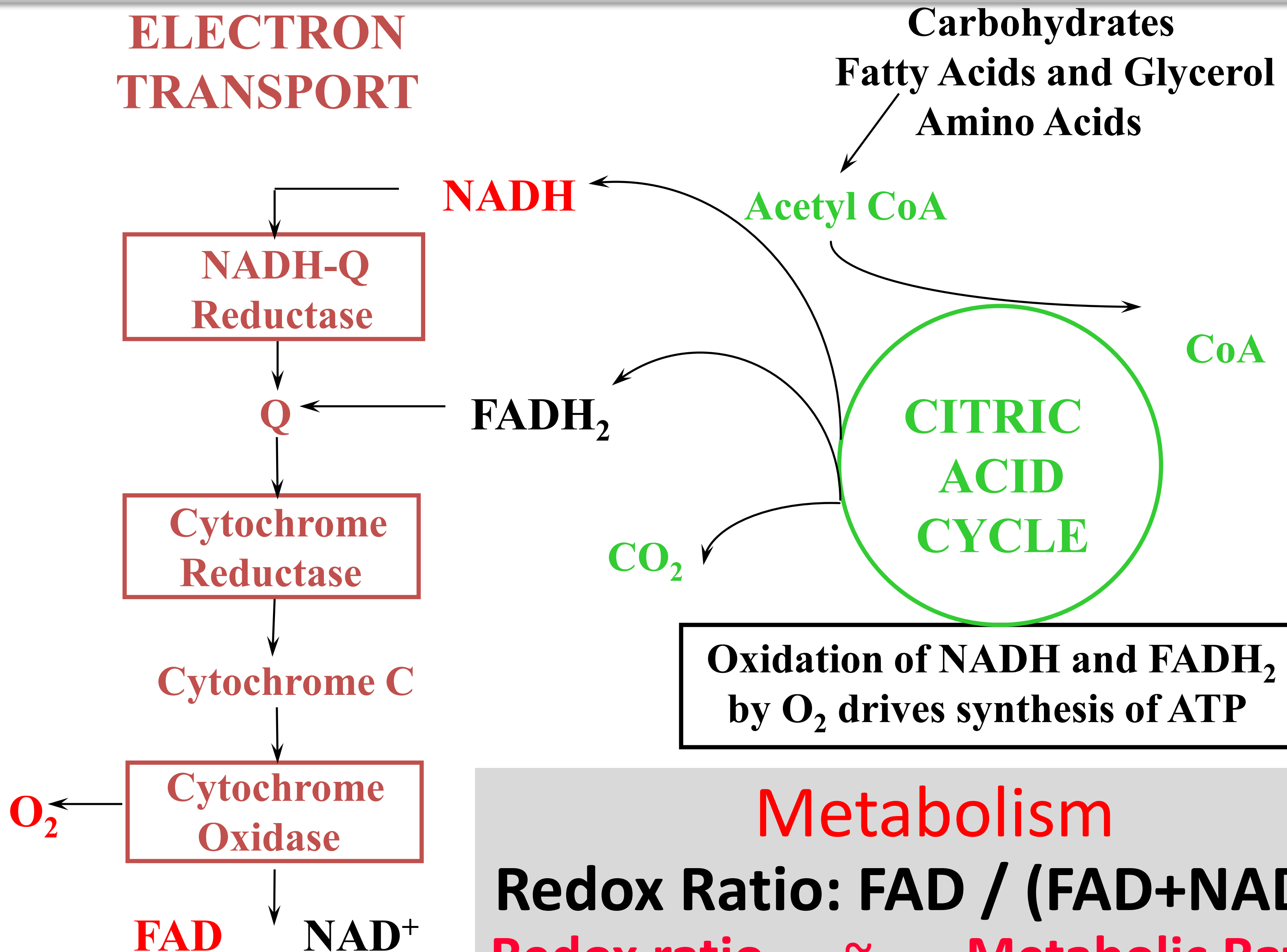
The molecule consists of a riboflavin moiety (vitamin B_2) bound to the phosphate group of an ADP molecule. The flavin group is bound to ribitol, a sugar alcohol, by a carbon-nitrogen bond, not a glycosidic bond. Thus, riboflavin is not technically a nucleotide; the name *flavin adenine dinucleotide* is a misnomer

FAD as NADH binds to proteins and it can be used to estimate the concentration of the protein . In contrast to NADH where if bound to protein we observe an increase in emission **FAD when bound to the protein exhibits decrease.**

FAD absorption and emission spectra



FAD and NADH metabolic indicators



Metabolism

Redox Ratio: FAD / (FAD+NADH)

Redox ratio ~ Metabolic Rate

Biological intrinsic fluorophores

- the extracellular matrix can also contribute to autofluorescence because of the intrinsic properties of collagen and elastin.

- **Collagen**

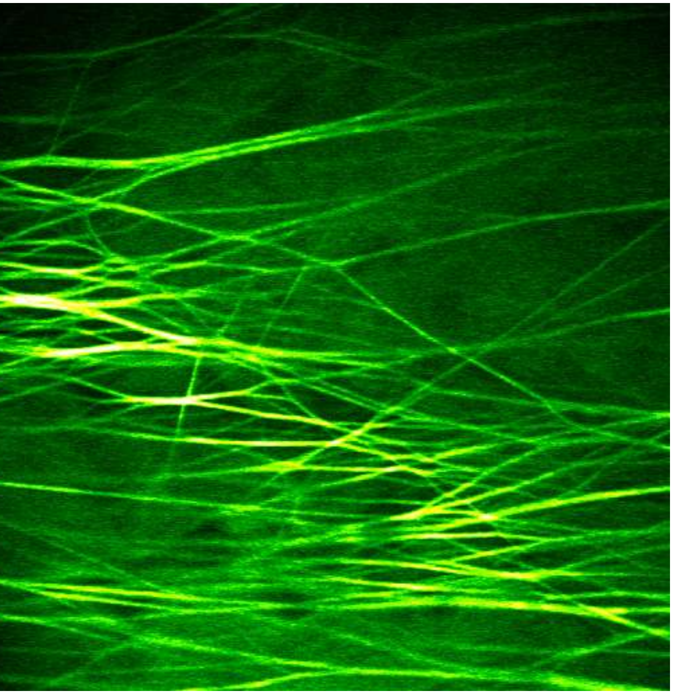
- It is the major extracellular matrix component, which is present to some extent in nearly all organs and serves to hold cells together in discrete units

- Collagen fluorescence in load-bearing tissues is associated with cross-links, hydroxylysyl pyridoline (HP) and lysyl pyridinoline (LP).

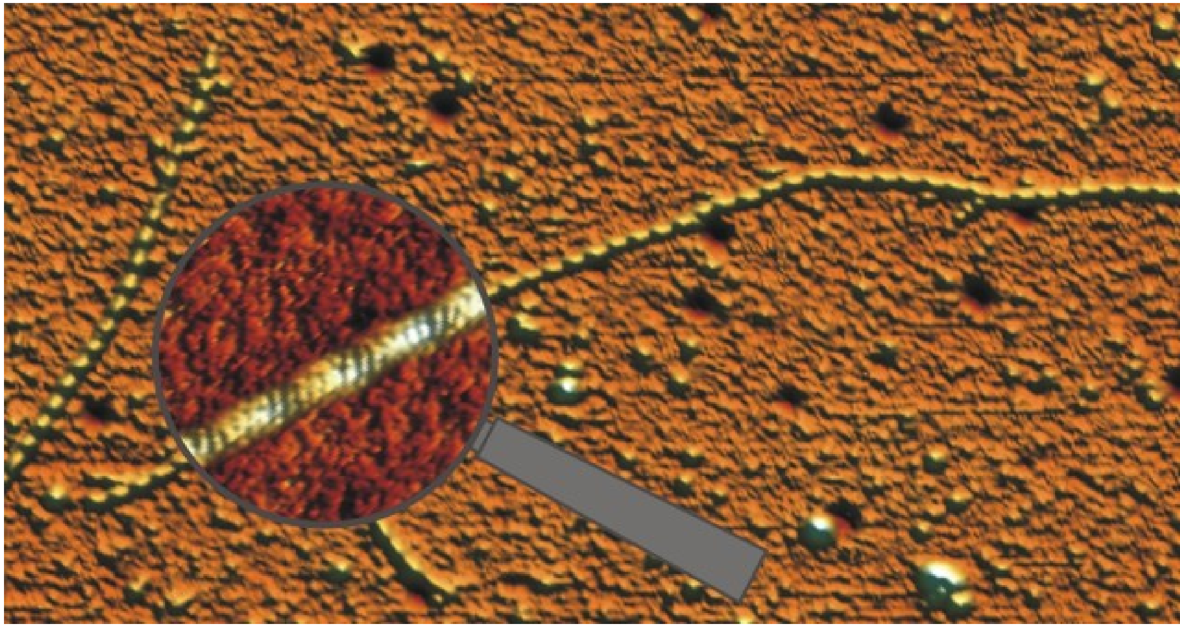
- Collagen crosslinks are altered with age and with invasion of cancer into the extracellular matrix

Collagen and elastin

- 2-photon auto-fluorescence image (right) of a bovine mesenteric collecting lymphatic vessel

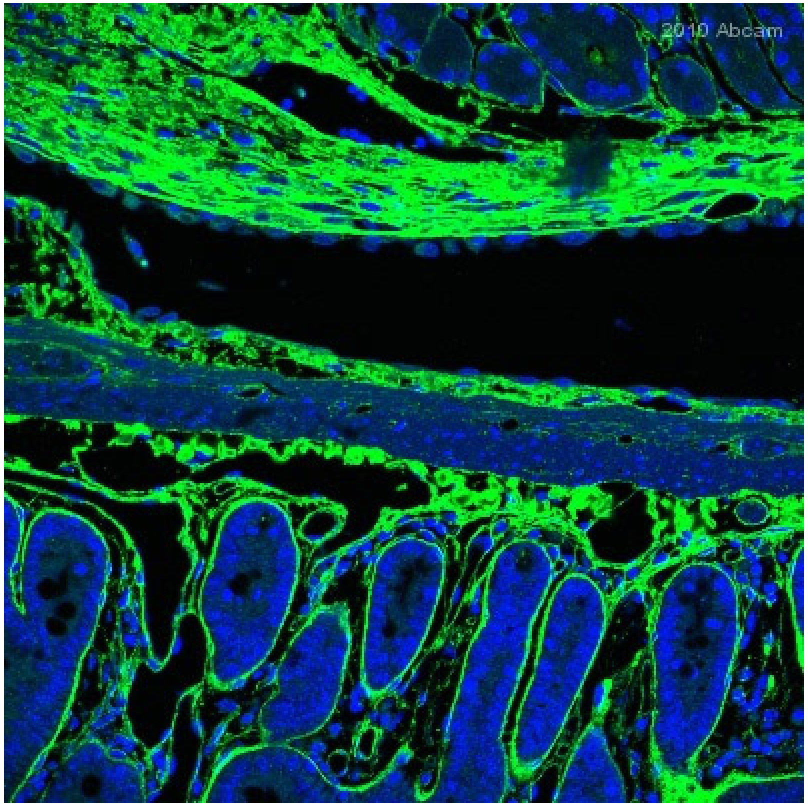


The stretched elastin fibers are clearly visible as bright straight lines, mostly in the direction of the vessel (left/right). There is a dimmer green background which is the spill over of the collagen fluorescence



Extrinsic Fluorophores

- Frequently molecules of interest are non-fluorescent or intrinsic florescence is not adequate



Collagen I antibody (ab21286) =secondary

Antibody labeled with FITC

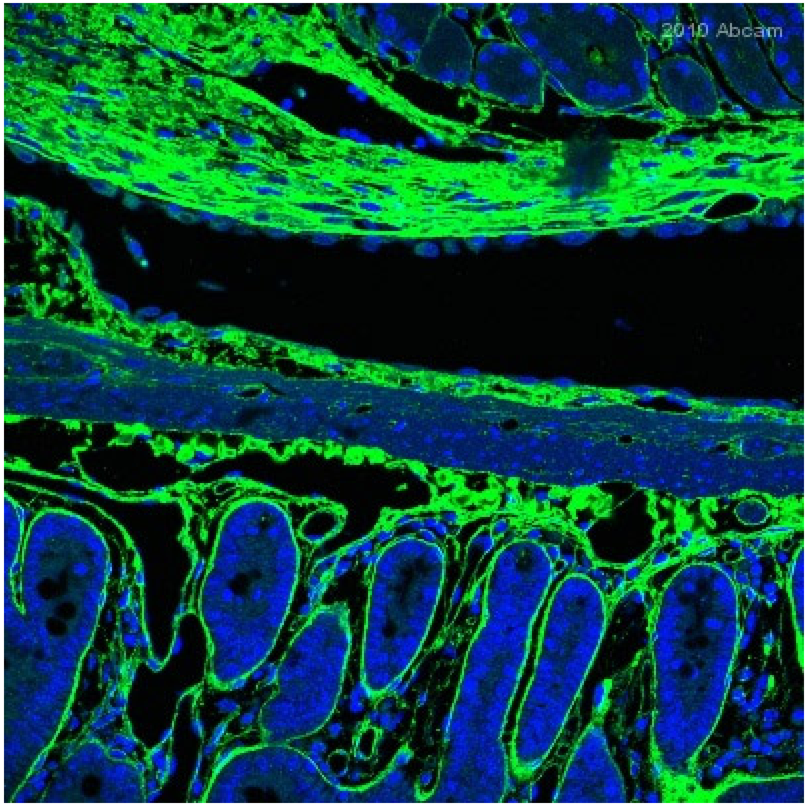
Number of extrinsic Fluorophores increased dramatically over the past decade

Nice list of the current **available** fluorophores can be found on the Molecular probes handbook

http://www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook.html

Extrinsic Fluorophores

- Frequently molecules of interest are non-fluorescent or intrinsic florescence is not adequate



Collagen I antibody (ab21286) =secondary

Antibody labeled with FITC

Number of extrinsic Fluorophores increased dramatically over the past decade

Nice list of the current **available** fluorophores can be found on the Molecular probes handbook

http://www.invitrogen.com/site/us/en/home/References/Molecular-Probes-The-Handbook.html

Lipids

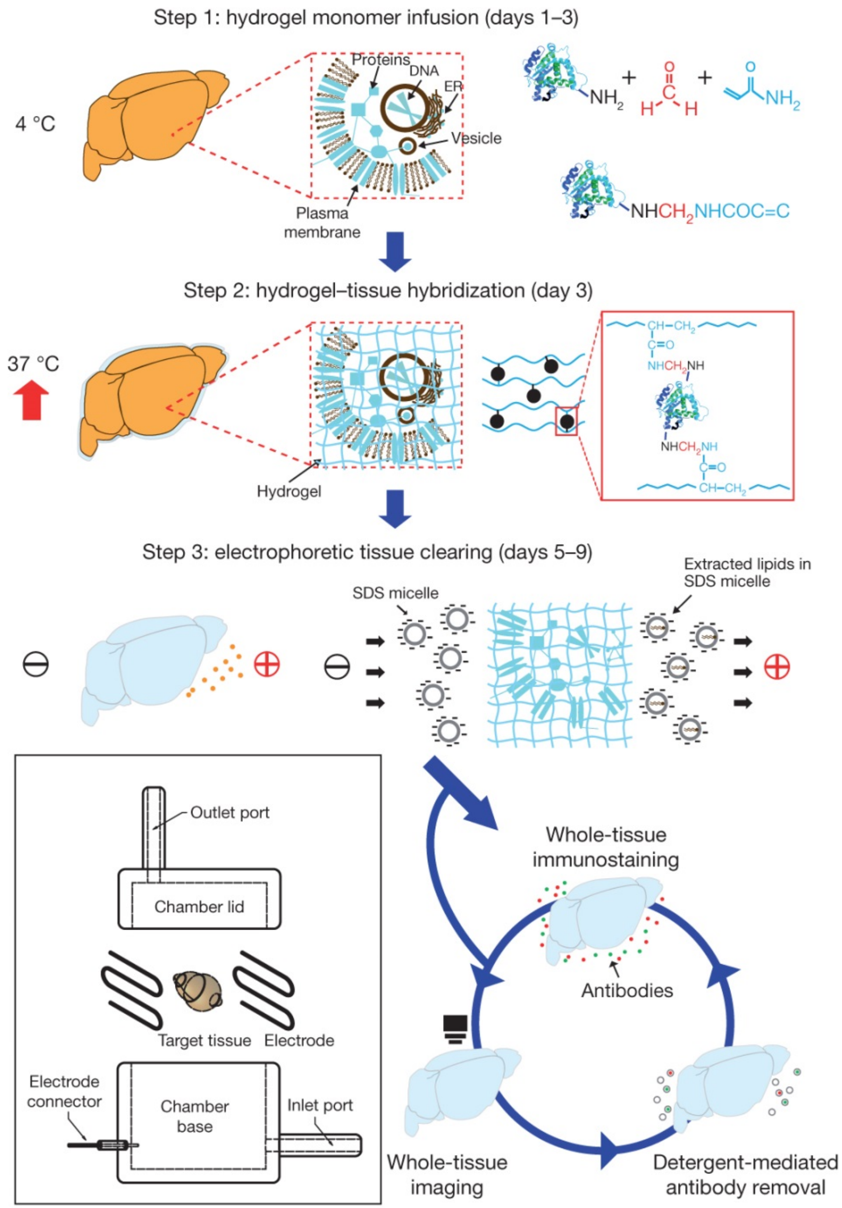
- Lipids are non-polar (hydrophobic) compounds, soluble in organic solvents.
- Most membrane lipids are amphipathic, having a non-polar end and a polar end.
- Fatty acids consist of a hydrocarbon chain with a carboxylic acid at one end.
- A 16-C fatty acid: **$CH_3(CH_2)_{14}$-COO^-**
- **Non-polar** **polar**

- A 16-C fatty acid with one cis double bond between C atoms 9-10 may be represented as 16:1 cis D9.

Lipids		
Phospholipids	436	540, 560
Lipofuscin	340–395	540, 430–460
Ceroid	340–395	430–460, 540
Porphyrins	400–450	630, 690

FAD, flavin adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; AND(P)H, reduced nicotinamide adenine dinucleotide phosphate.

CLARITY method



Tissue is crosslinked with formaldehyde (red) in the presence of hydrogel monomers (blue), covalently linking tissue elements to monomers that are then polymerized into a hydrogel mesh (followed by a day-4 wash step; Methods). Electric fields applied across the sample in ionic detergent actively transport micelles into, and lipids out of, the tissue, leaving fine-structure and crosslinked biomolecules in place. The ETC chamber is depicted in the boxed region

K Chung *et al. Nature* **000**, 1-6 (2013) doi:10.1038/nature12107

SHOW MOVIES