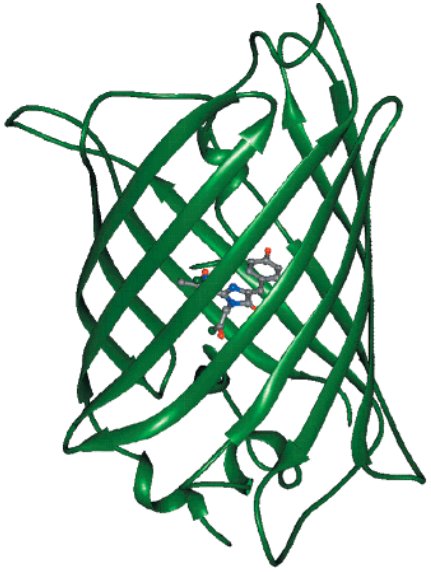
Ch 1b Lecture 8
January 24th, 2013

Next few lectures – *Light/matter interactions*, or, An Introduction to Spectroscopy.

Today: Electronic spectroscopy.

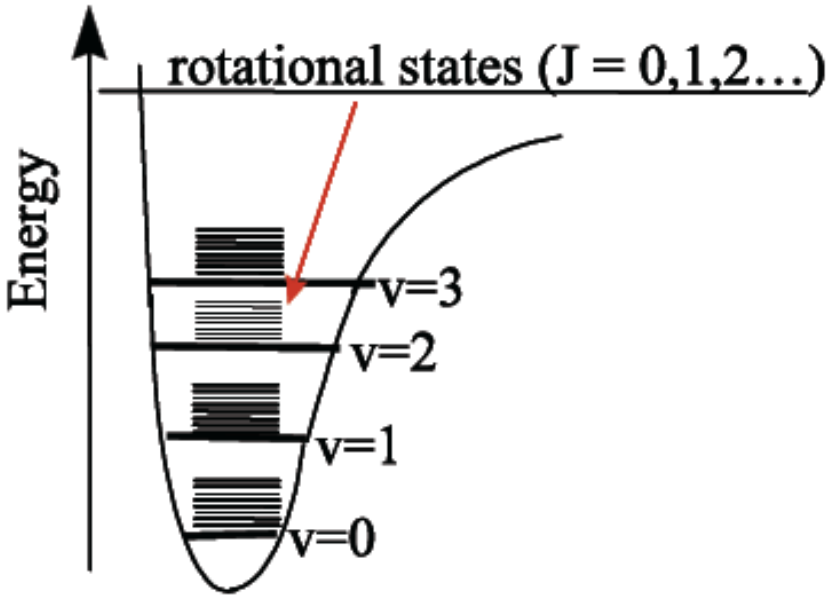


Reading: Review OGC sections 4.5-4.7 as needed, OGC sections 20.1-20.3; RC Sections 9-4 to 9-7, 9-9

Quick review of last lecture:

- The Born-Oppenheimer approximation suggests we can decouple electronic and nuclear degrees of freedom.
- The rotational degrees of freedom can be accessed when the molecule has a permanent dipole moment, and are sensitive to the structure of the molecule.
- Vibrational spectroscopy probes the stiffness of bonds, and can be used to assess the presence/absence of certain functional groups. Need $d\mu/dR \neq 0$.

Pictorially:



The ground electronic state potential energy well of a diatomic molecule, with rotational and vibrational states shown. Each vibrational state has its own manifold of rotational states.

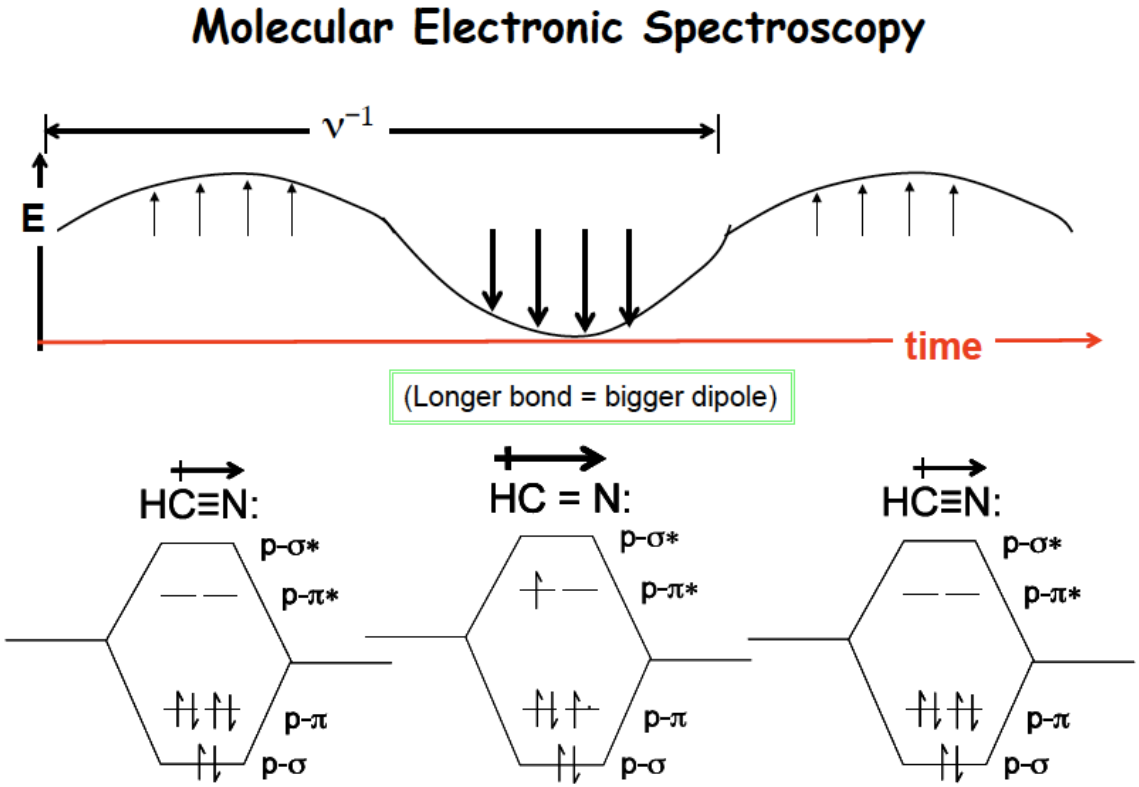
$m_p = 1836 m_e$ Born-Oppenheimer (Section 6.1.2, OGC):

The ground electronic state is described by a potential energy well

Rotational states & vibrational states can be put into context within this energy surface.

What about different molecular orbital configurations?

Electronic Spectroscopy:



As with rotation & vibration, the dipole moment needs to change.

And, for most simple, closed shell molecules with sigma bonds, the orbital energies as such that the photons involve lie well into the ultraviolet (think about the colors of various solvents, etc.).

Electronic Spectroscopy: Diatomics

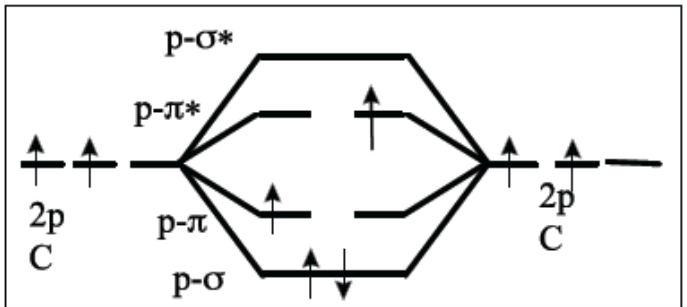


Fig. Sp.13. A highly excited triplet state of C_2.

Higher energy electronic excited (triplet) state

'triplet' implies 2 unpaired electrons

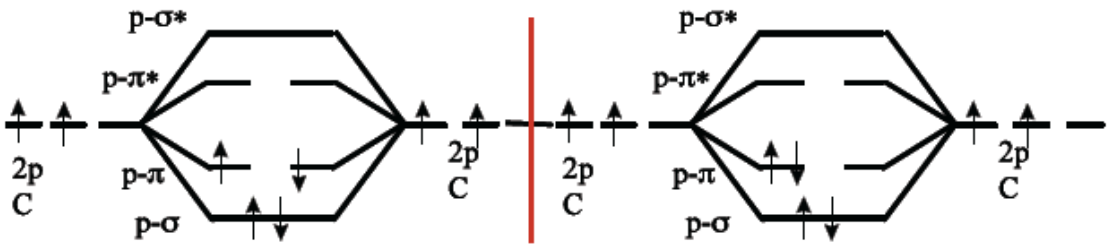


Fig. Sp.12. Two low-energy singlet states of C_2.

Two low-lying excited electronic (singlet) states

'singlet' implies 0 unpaired electrons

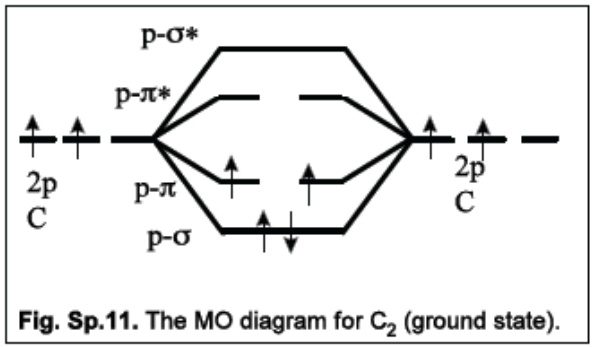
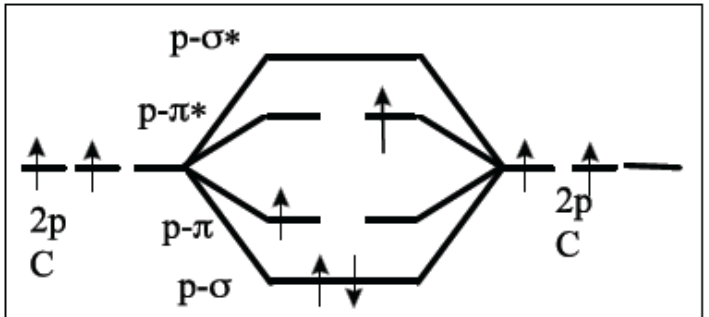


Fig. Sp.11. The MO diagram for C_2 (ground state).

Ground state (triplet)

Electronic Spectroscopy: Diatomics



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Higher energy electronic excited (triplet) state

Bond enthalpies

C=C = **615** kJ mol^{-1}

C-C = **348** kJ mol^{-1}

ΔE = the energy of a C=C π bond

615-**348** = **267**

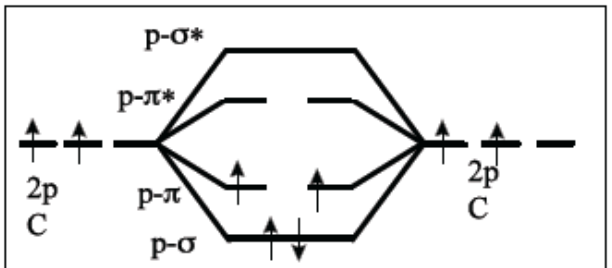


Fig. Sp.11. The MO diagram for C_2 (ground state).

Ground state (triplet)

Electronic Spectroscopy: Diatomics

Upon excitation, what do we expect to see?

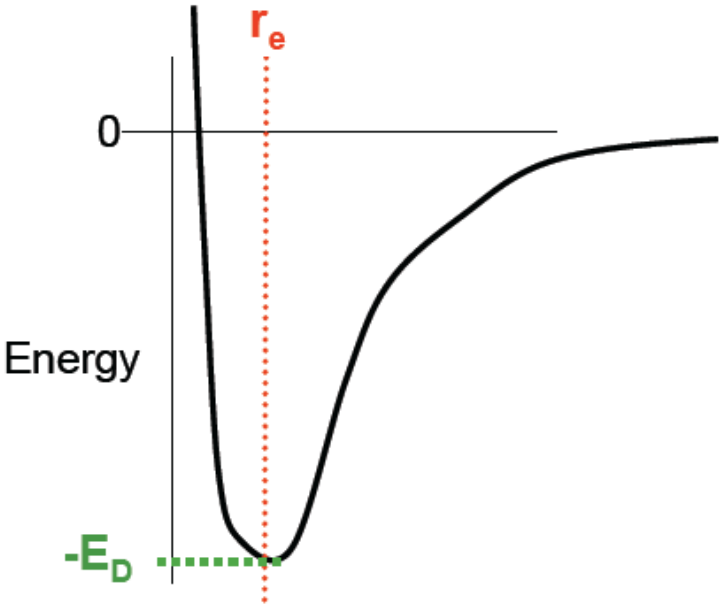
C=C becomes C-C

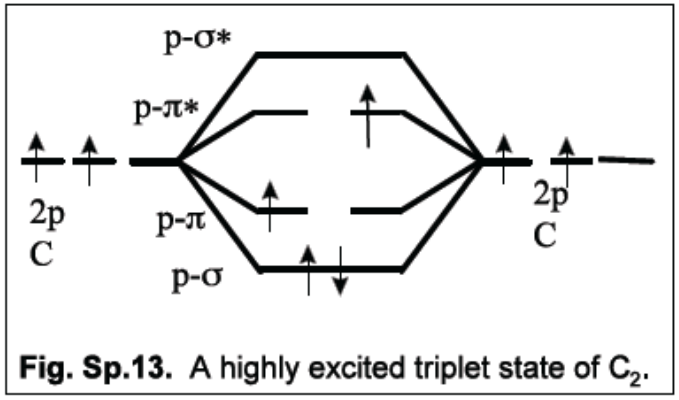
r_e increases significantly

(vibrational well shifts)

Bond enthalpy decreases by 267 kJ mol^{-1}

vibrational well **depth** is shallower





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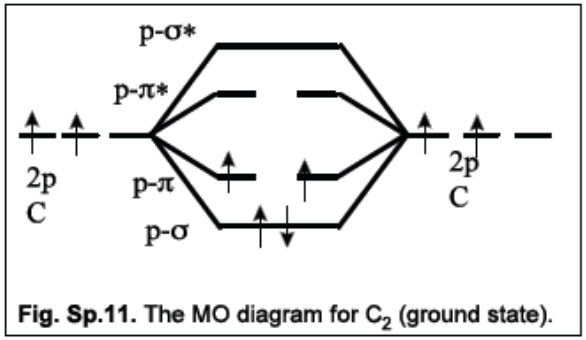

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Electronic Spectroscopy: Diatomics

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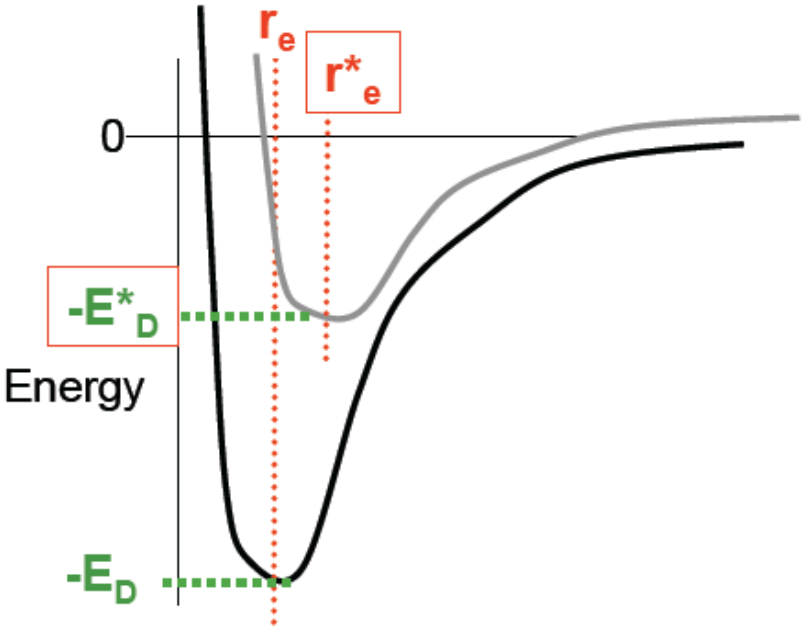

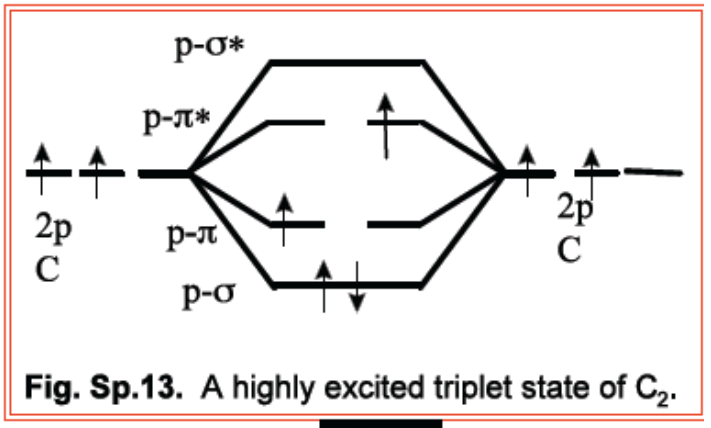

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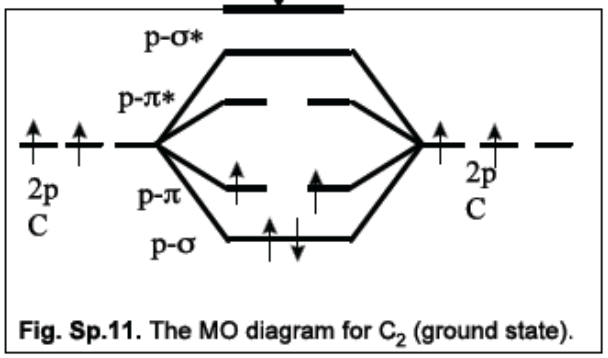

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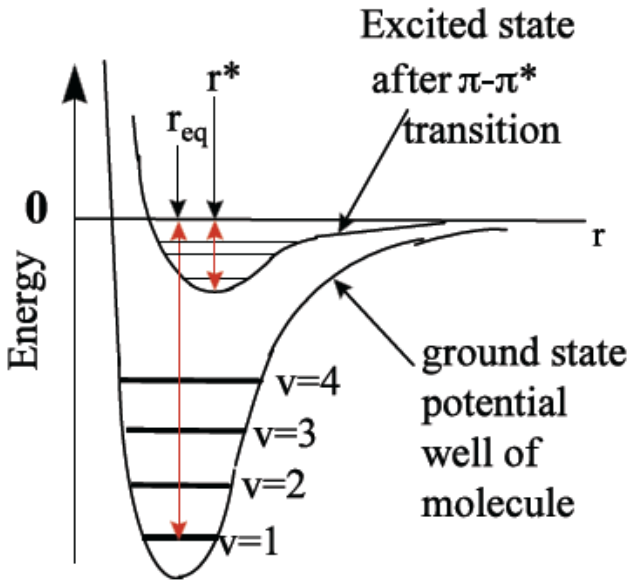
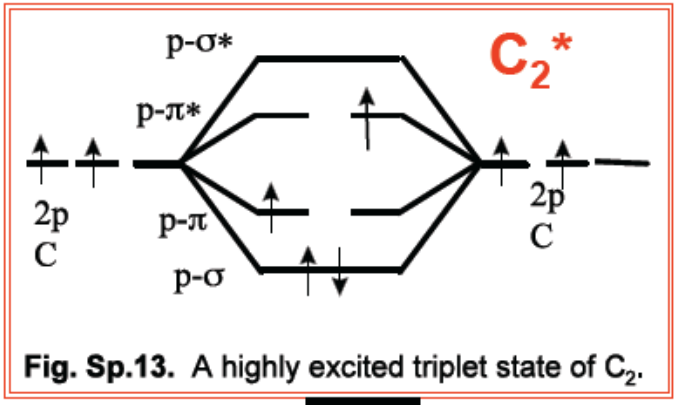
Electronic Spectroscopy: Diatomics





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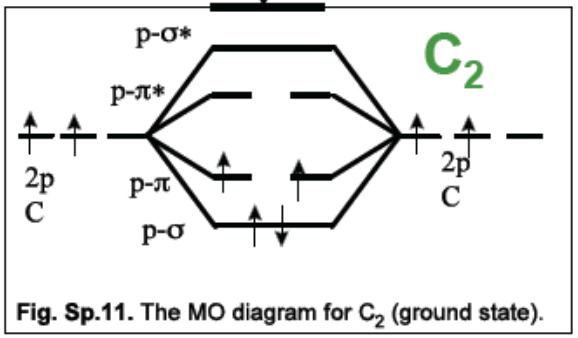


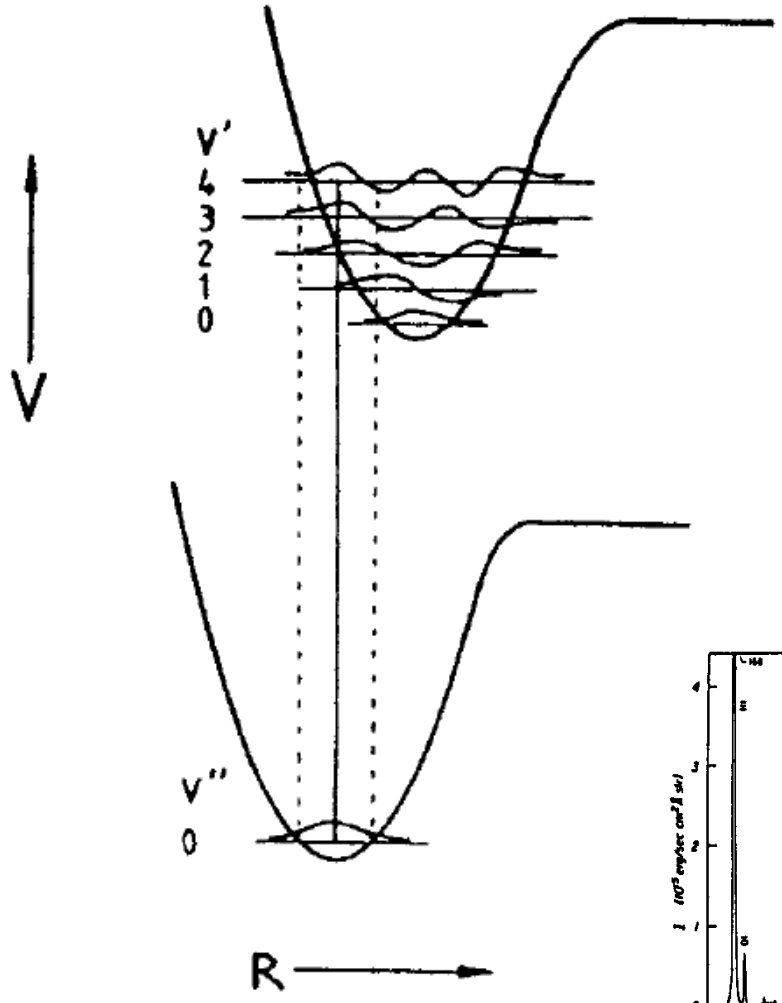
Fig. Sp.11. The MO diagram for C_2 (ground state).

C_2 → C_2*

- **Bond energy decreases**
- **Bond length increases**
- **Rotational constant (B) decreases**
- **vibrational frequency ν decreases**
- **vibrational force constant decreases**

$$B = \hbar^2 / 2I \qquad I = \mu R^2$$

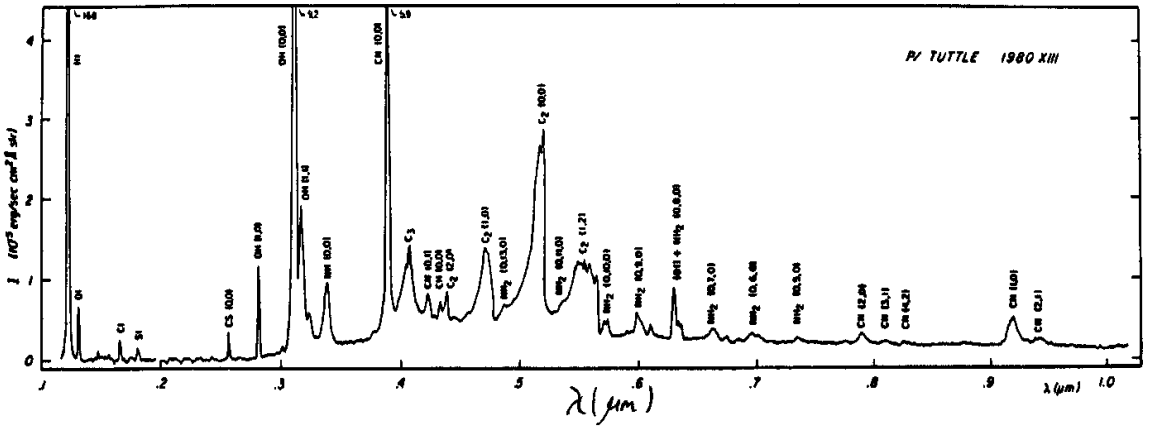
Electronic Spectroscopy: Diatomics



Can access many excited vibrational or electronic states, even from only v=0.

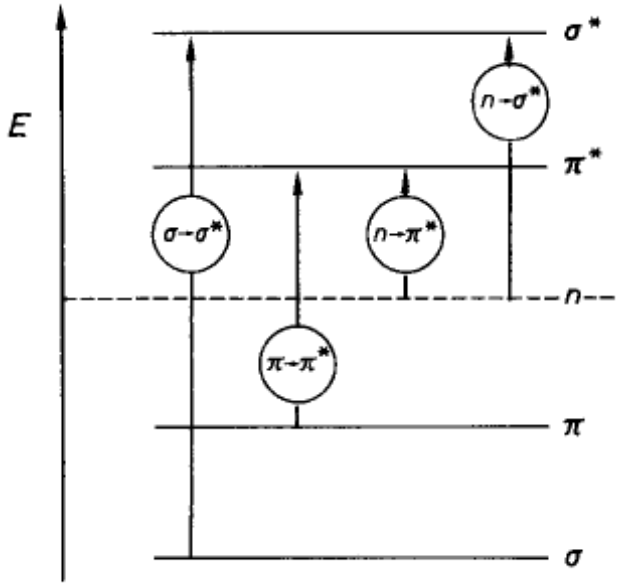
Optical spectra of comets dominated by radicals such as C_2 (first seen in 1881), CN, NH_2 (fragments of closed shell species in the nucleus).

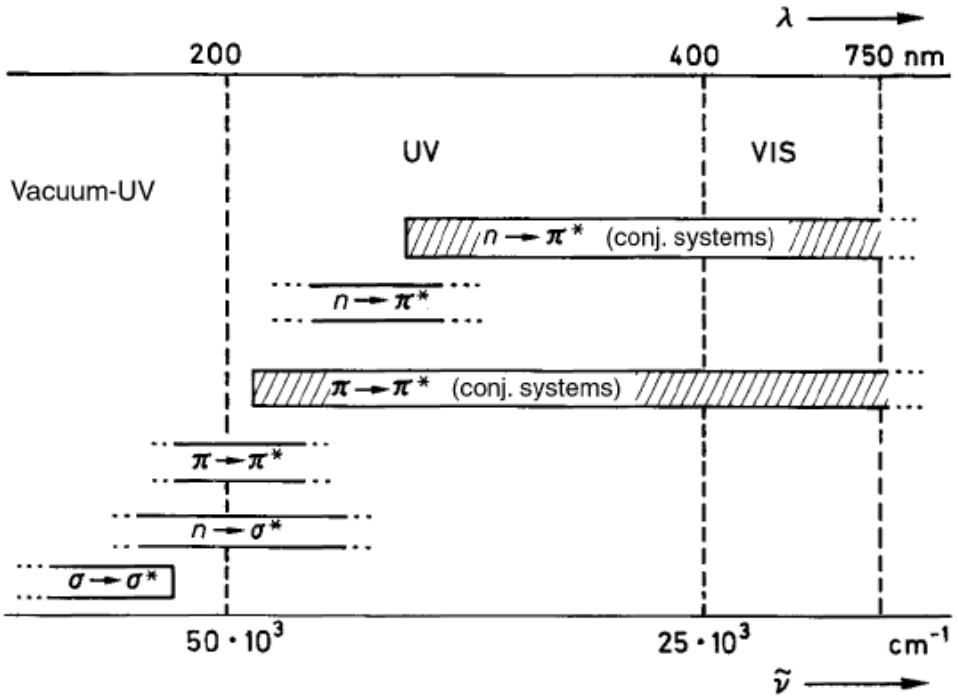
UV/Vis Spectrum of Comet P/ Tutle (1980 XIII)



A'Hearn & Festou, in Physics & Chemistry of Comets, p. 92
W.F. Huebner, ed., Springer-Verlag 1990.

Electronic Spectroscopy: Organics





Absorption of sufficiently energetic photons provides access to the HOMO-LUMO gap, the transitions are most often labeled by the nature of the MO's involved.

Typically, such absorption occurs in the UV region for small, closed shell molecules, but can extend into the visible with conjugation.

Electronic Spectroscopy: Organics

Chromophore	Transition	Example	λ_{max} (nm)	ε_{max}
C–H	$\sigma \rightarrow \sigma^*$	CH_4	122	intense
C–C	$\sigma \rightarrow \sigma^*$	H_3C-CH_3	130	intense
–Ö–	$n \rightarrow \sigma^*$	H_2O	167	1500
	$n \rightarrow \sigma^*$	H_3C-OH	183	200
	$n \rightarrow \sigma^*$	$C_2H_5-O-C_2H_5$	189	2000
–S̈–	$n \rightarrow \sigma^*$	H_3C-SH	235	180
	$n \rightarrow \sigma^*$	$H_3C-S-CH_3$	228	620
	$n \rightarrow \sigma^*$	$C_2H_5-S-S-C_2H_5$	250	380
–N̈–	$n \rightarrow \sigma^*$	NH_3	194	5700
	$n \rightarrow \sigma^*$	$C_2H_5-NH_2$	210	800
	$n \rightarrow \sigma^*$	$C_2H_5-NH-C_2H_5$	193	3000
	$n \rightarrow \sigma^*$	$(C_2H_5)_3N$	213	6000
–Hal	$n \rightarrow \sigma^*$	H_3C-Cl	173	200
	$n \rightarrow \sigma^*$	H_3C-Br	204	260
	$n \rightarrow \sigma^*$	H_3C-I	258	380
	$n \rightarrow \sigma^*$	CHI_3	349	2170
>C=C<	$\pi \rightarrow \pi^*$	$H_2C=CH_2$	165	16000
		$C_2H_5-CH=CH-C_2H_5$	185	7940
–C≡C–	$\pi \rightarrow \pi^*$	$HC\equiv CH$	173	6000
	$\pi \rightarrow \pi^*$	$H-C\equiv C-C_2H_5$	172	250
>C=Ö	$n \rightarrow \pi^*$	$H_3C-CH=O$	293	12
	$\pi \rightarrow \pi^*$	$H_3C-\overset{O}{\overset{\|\|}{C}}-CH_3$	187	950
	$n \rightarrow \pi^*$	$H_3C-\overset{O}{\overset{\|\|}{C}}-CH_3$	273	14
>C=S̈	$n \rightarrow \pi^*$	$H_3C-\overset{S}{\overset{\|\|}{C}}-CH_3$	460	weak
>C=N–	$\pi \rightarrow \pi^*$	$H_3C-CH=N-OH$	190	8000
	$n \rightarrow \pi^*$	$H_3C-CH=N-OH$	279	15
–N̈=N̈–	$n \rightarrow \pi^*$	$H_3C-N=N-CH_3$	347	15
–N̈=Ö	$n \rightarrow \pi^*$	$(H_3C)_3C-NO$	300	100
		$(H_3C)_3C-NO$	665	20
$-NO_2$		H_3C-NO_2	210	10000
			278	10

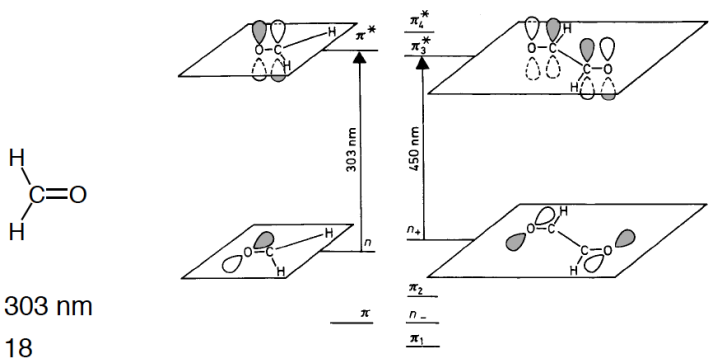
Chromophores are characterized by absoprtion wavelength & strength:

$$A = \log \frac{I_0}{I} = \varepsilon \cdot c \cdot l$$

ε = molar extinction coefficient

c = concentration, *l* = pathlength

Isolated chromophores will give a UV-Vis spectrum that is ~the additive contribution of the individual groups.



λ_{max} = 303 nm

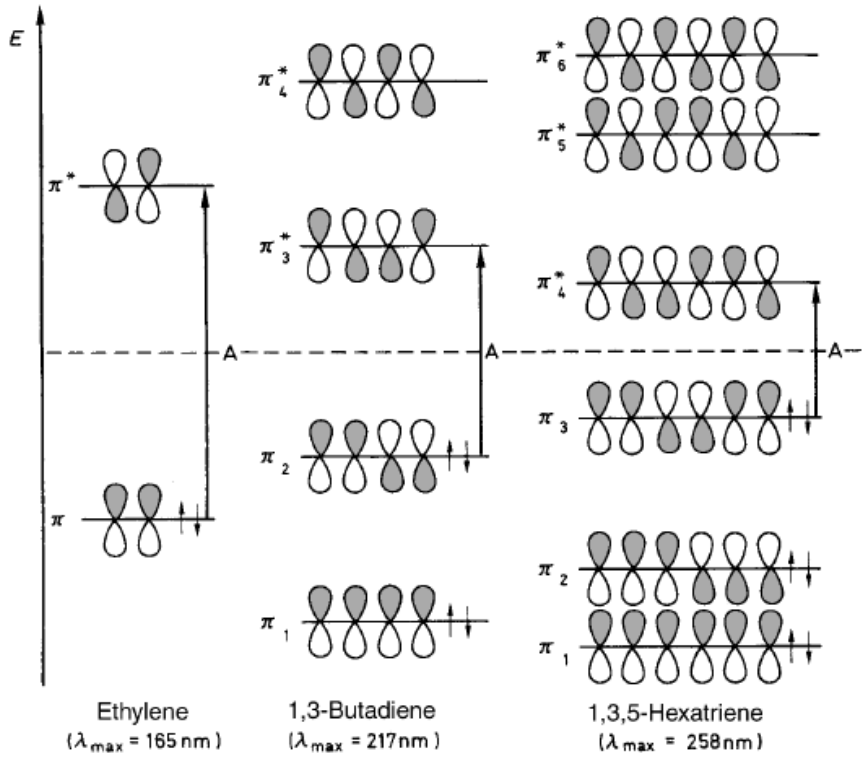
ε = 18

λ_{max} = 450 nm

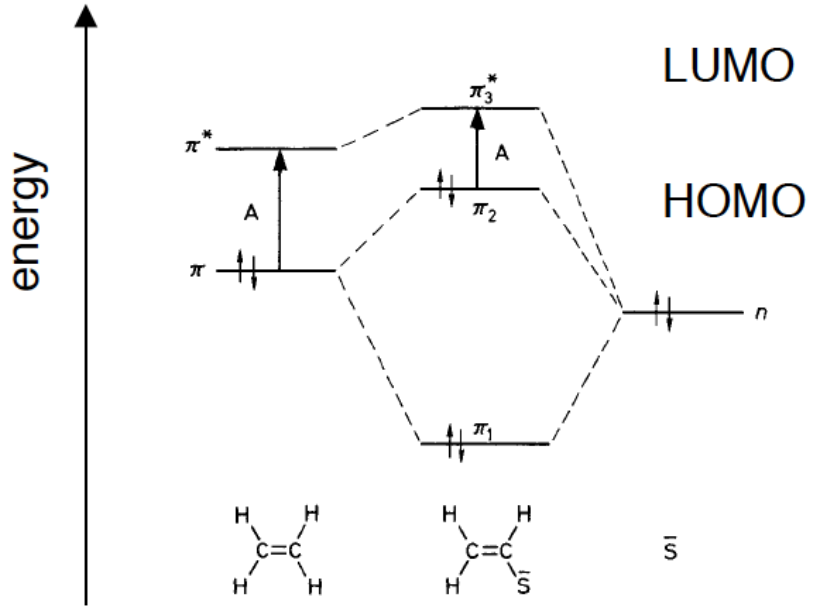
ε = 5

This is not the case for extended chromophores, with increasing conjugation the absorption energy and intensity decreases steadily.

Electronic Spectroscopy: Olefins

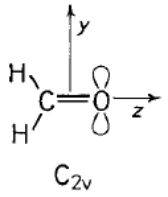


The π ⟶ π* transition moves steady to the red with increasing conjugation, and the molar extinction coefficient increases.

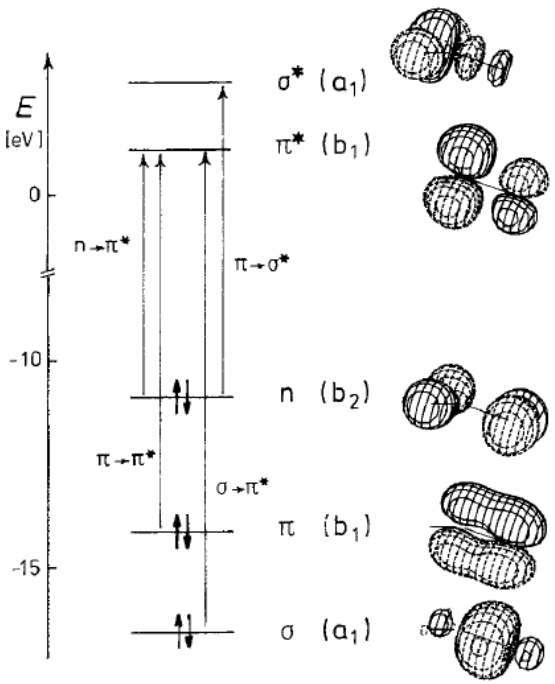


Groups with non-bonding electrons (so called auxochromic groups, -OH, -OR, -SH, -SR, $-NH_2$, $-NR_2$) result in bathochromic shifts, because the HOMO-LUMO energy difference is decreased:

Electronic Spectroscopy: Carbonyls

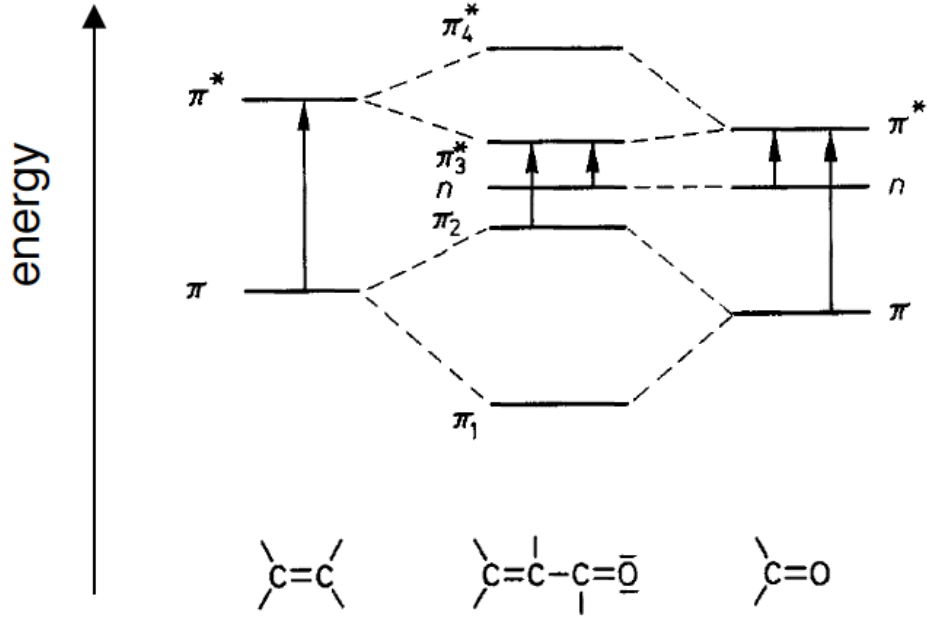


C_{2v}

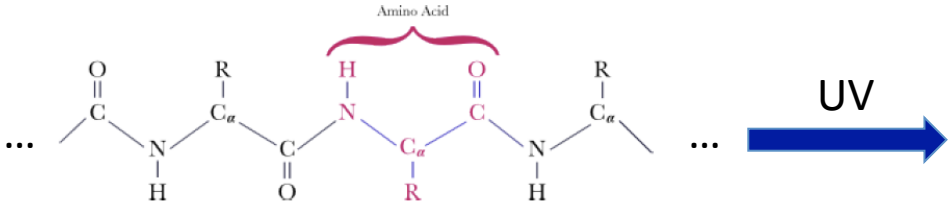


Excitation can occur to the anti-bonding π* or σ* orbitals, but the lowest energy *n* ⟶ π* transition is forbidden (with ε = 15-30). For saturated ketones or aldehydes the strong π ⟶ π* and *n* ⟶ σ* bands occur in the vacuum-UV. Auxochromic substituents increase the transition energies (blue-shift) by increasing the π* energy and decreasing the non- bonding orbital energy.

Conjugation largely leaves the *n* orbital alone, but splits the π orbitals such that the π/π* energy difference is greatly reduced (absorption in the visible):

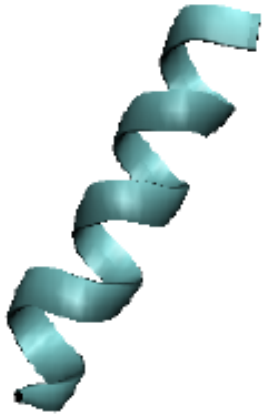


UV Spectroscopy, Polymers/Proteins:

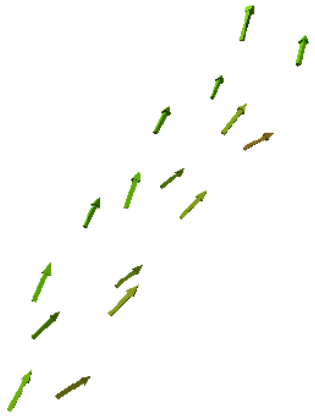


Many copies of functional groups, can lead to complex, broadened spectral features. For proteins, the amino acid order is called the primary structure (more later).

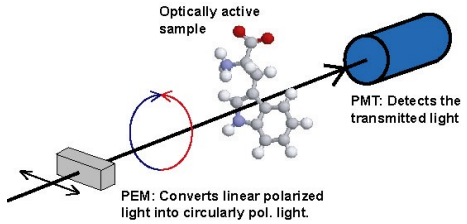
UV Circular Dichroism (CD), where the protein interacts with polarized light, is sensitive to secondary structure due to the *spatial ordering* of the transition dipole moments:

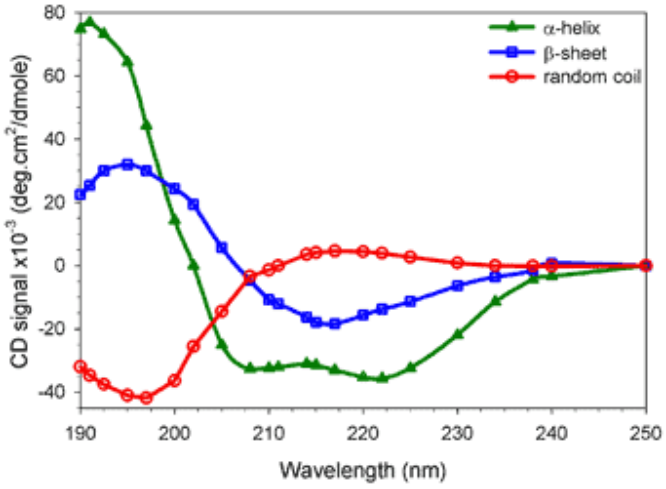


α Helix

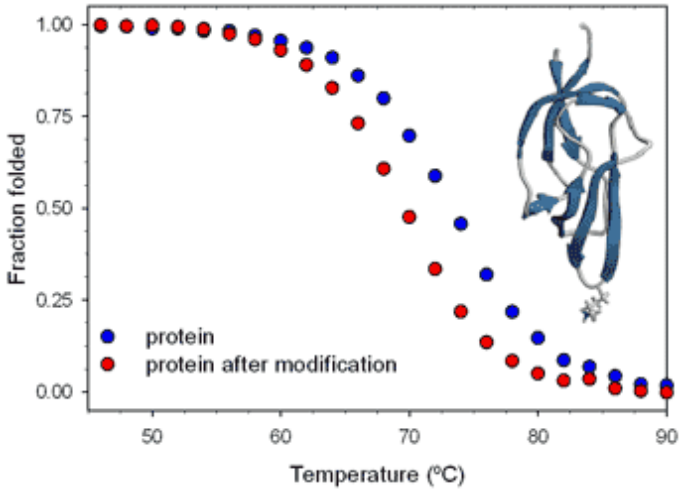


Amide Dipoles

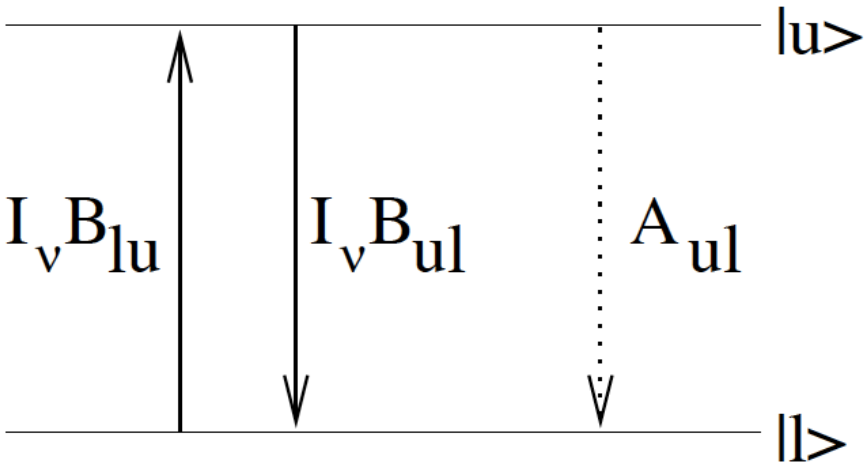




CD signals correlated with secondary structure, can sense folding.



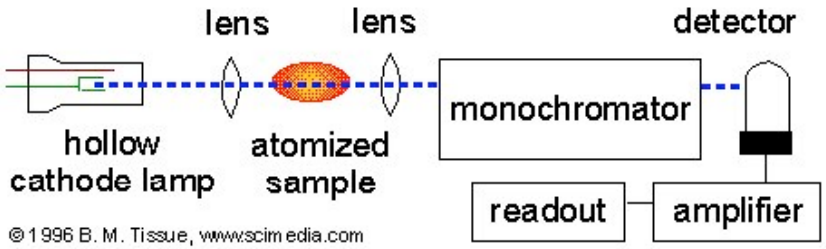
Electronic Spectroscopy: Abs. versus Emission

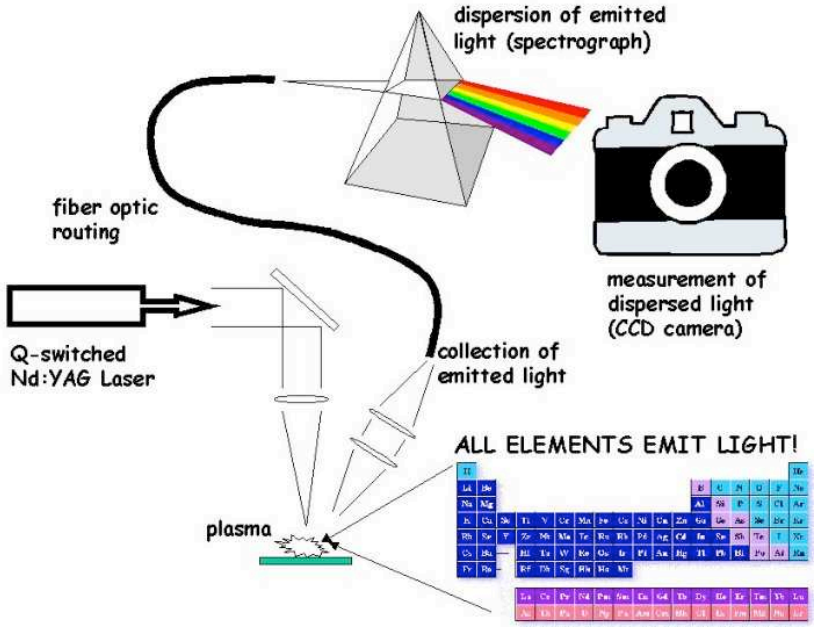


Stimulated Absorption/Emission

Spontaneous Emission

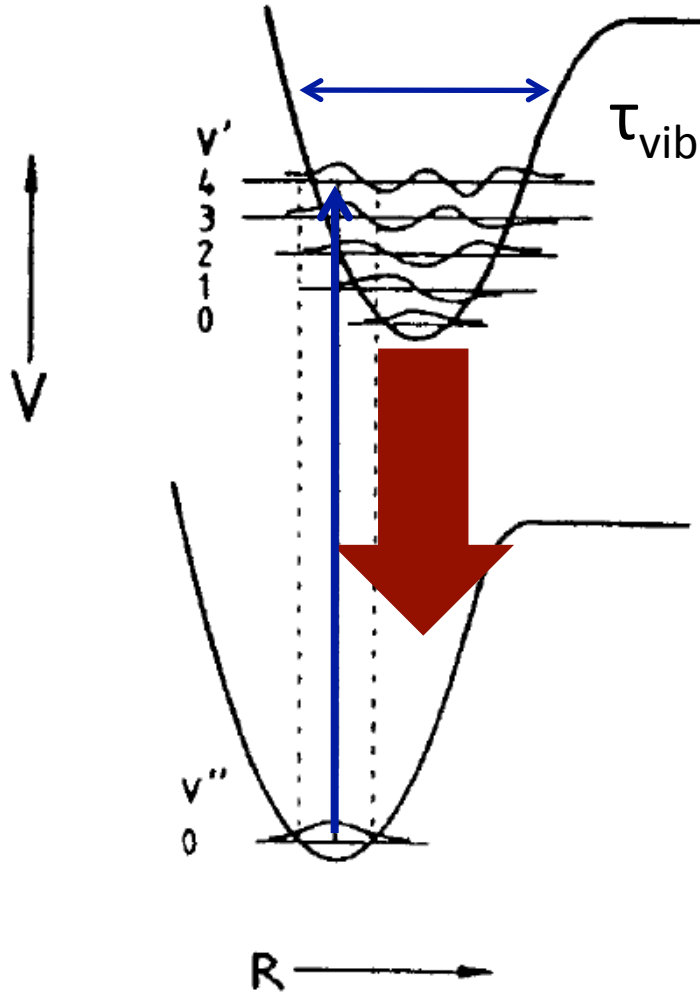
- Here, $\Delta E >> k_B T$, so no thermal excitation available, need light or unusual conditions.
- Spontaneous emission rate, or fluorescence, steeply frequency dependent, can be very fast (up to 10^8 s^{-1}) in visible/UV.





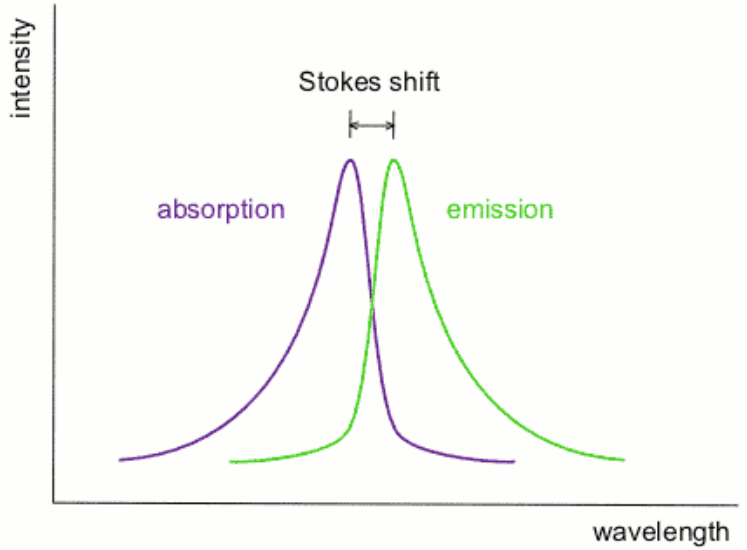
Atomic absorption schematic at left, emission at right (Laser Induced Breakdown Spec, or LIBS).

Electronic Spectroscopy: Abs. versus Emission



$\tau_{vib} << \tau_{emission}$

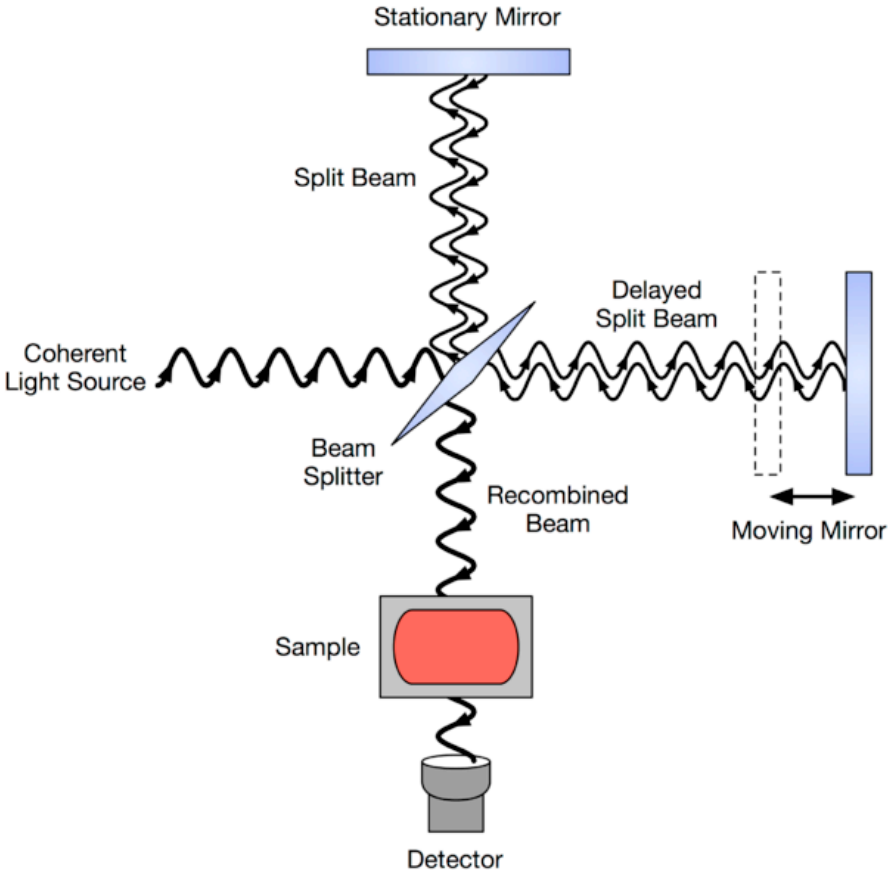
If the geometry change is large in the excited state, the emission can occur at quite different wavelengths. The $\Delta\lambda$ from abs. to emission is called the *Stokes Shift*.

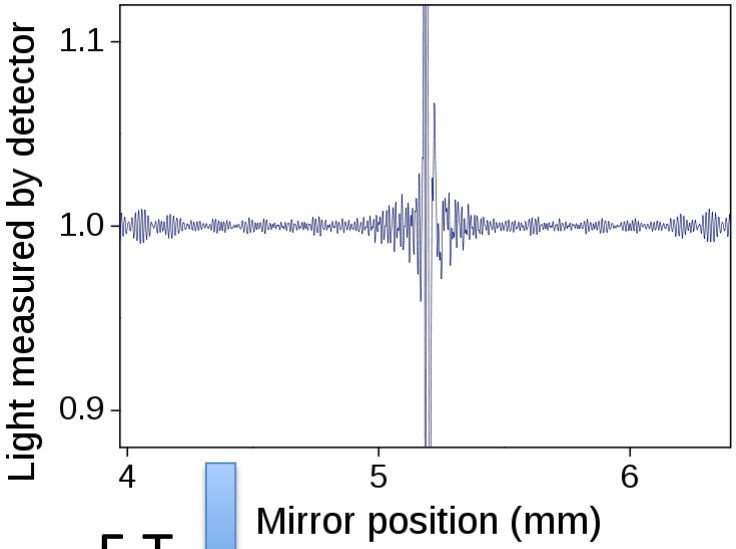


Can access many excited vibrational or electronic states, even from only v=0.

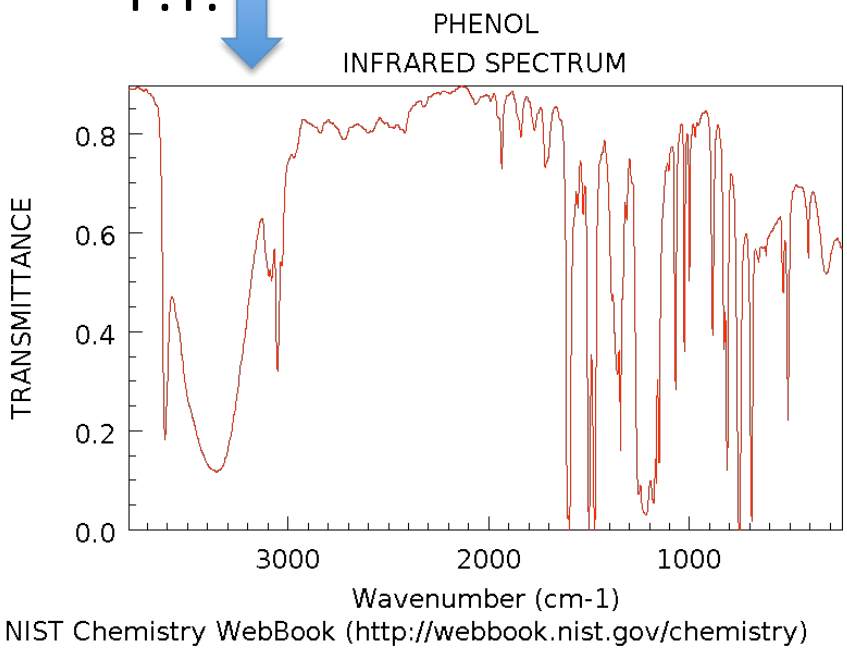
Critical to many techniques (DNA sequencers, for example).

Vibrational Spectroscopy, Slow Emission:





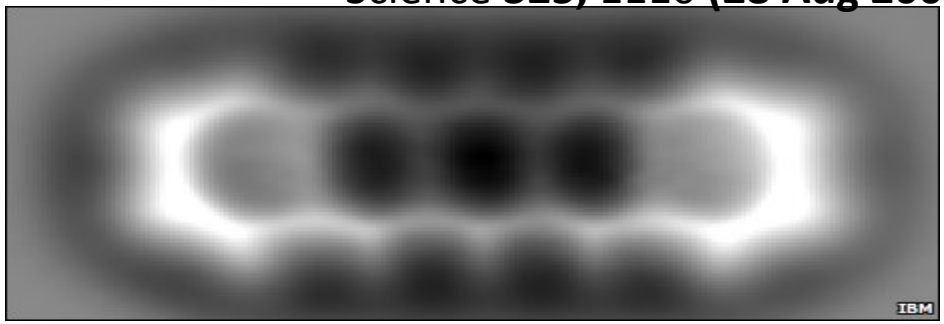
F.T.



Thus, absorption techniques are used, typically Fourier Transform methods, or *FT-IR* (more in NMR lecture).

Electronic Spectroscopy: Demo showcased emission…

Science **325,** 1110 **(28 Aug 2009)**

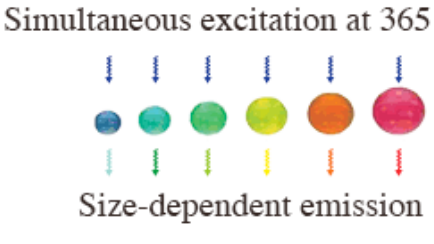


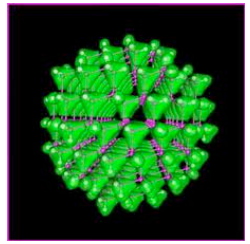
http://news.bbc.co.uk/2/hi/8225491.stm

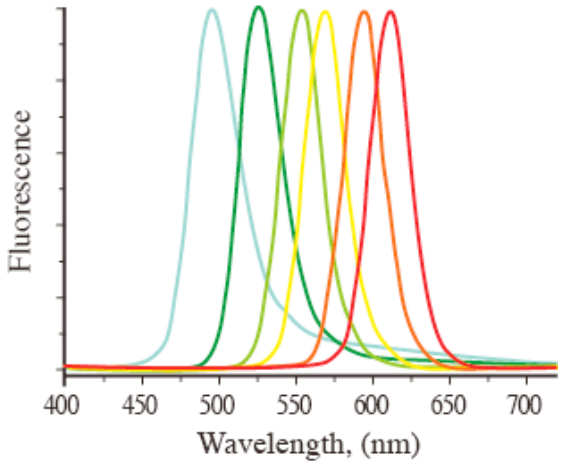
Go to larger molecules, esp. those with that are aromatic to delocalize the electrons (think about the PiB solutions).

Why do we care?

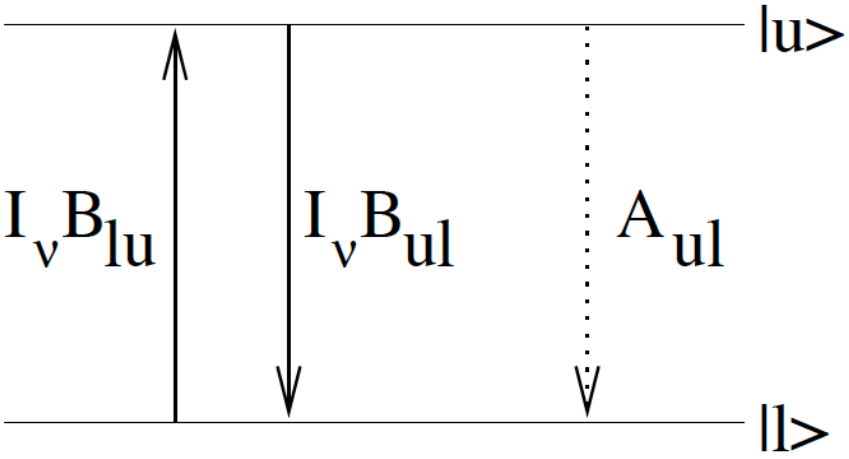
Create "artificial atoms": CdSe Quantum Dots





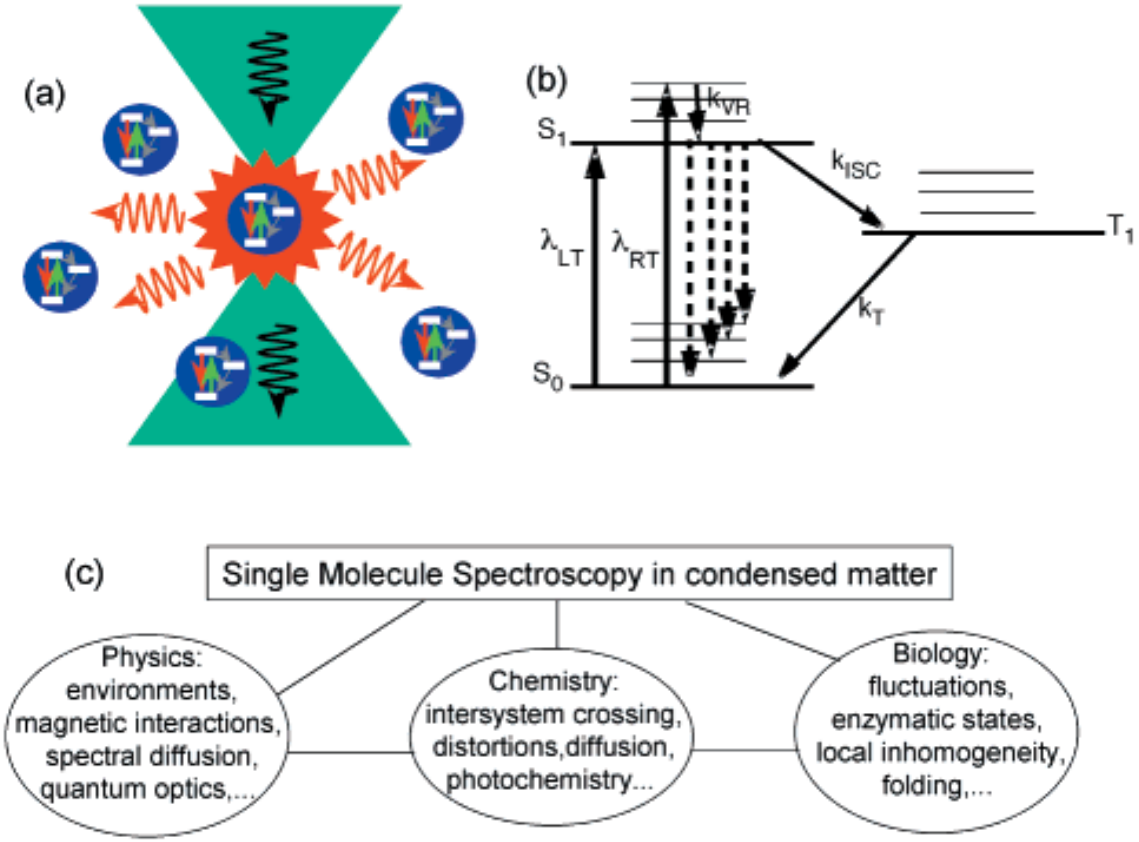


One answer is: Single molecule spectroscopy!

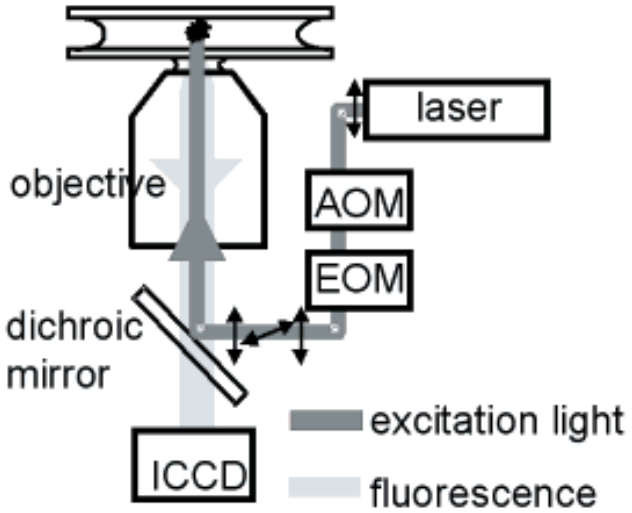


For atoms/truly two level systems, photon emission rate can approach A s^{-1}, emitted isotropically. Even with very high numerical aperture optics, you can only collect ~10% of the emitted photons.

For complex molecular systems, a variety of processes must be considered and optimized… but for the "right" systems, the fluorescence from single atoms or molecules can be detected!

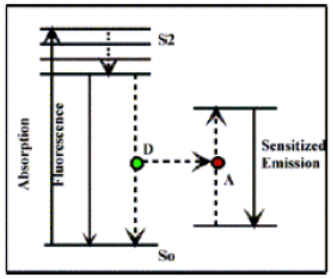


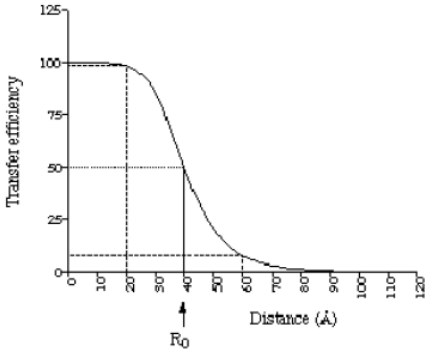
OK, so we can get decent signals. Why is this hard?

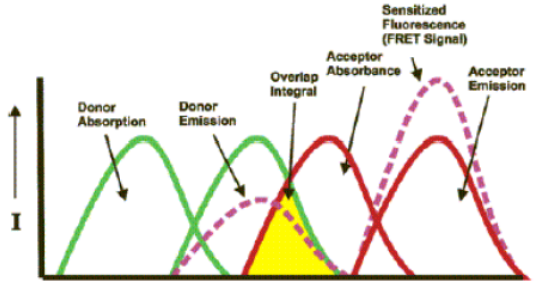


Suppose you can get 10^6 photons/s. What is the intrinsic SNR? Shot noise says 1000:1 in one second. But… must collect only the emitted (and not scattered) photons!

Forster energy transfer is one way to get a huge Stokes shift that is distance dependent, and so all the cool kids are working on single molecule FRET experiments…

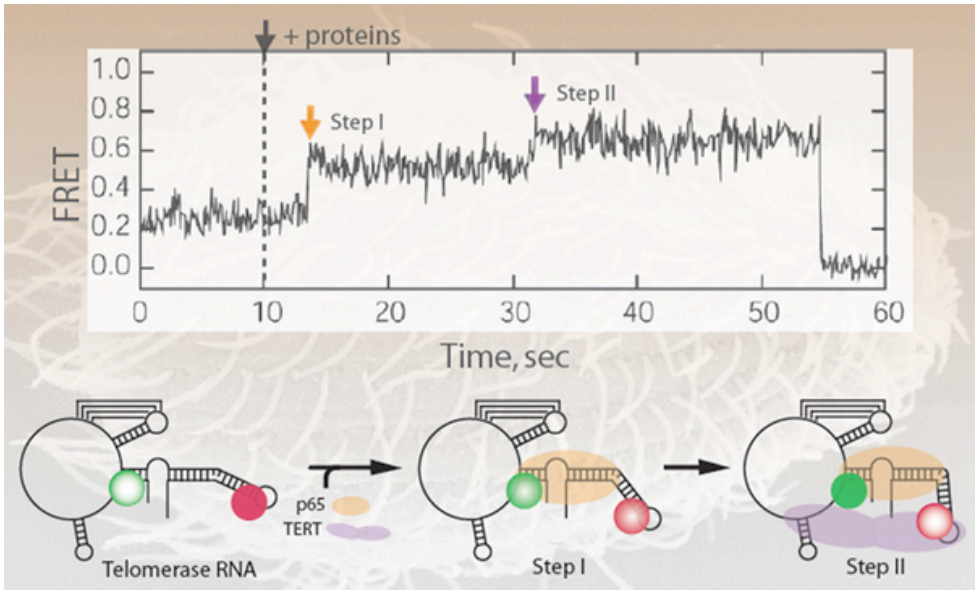






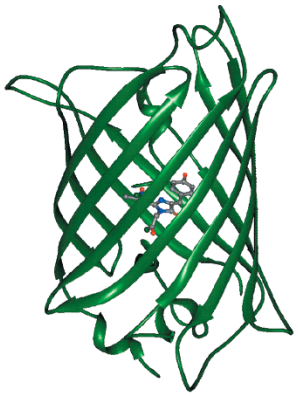
The Forster radius defines the spacing between fluorphores: nanometer scale optical propagation.

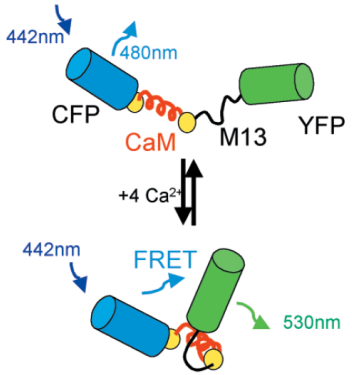
Single molecule FRET examples:



Hierarchical assembly pathway of the telomerase ribonucleoprotein (RNP) revealed by single-molecule FRET. Superimposed on the faint background of a Tetrahymena image are the single-molecule FRET time trace showing the stepwise assembly process of Tetrahymena telomerase (upper panel) and the hierarchical assembly model (lower panel). The p65 protein binds to the telomerase RNA first and causes an intermediate conformation of the RNA where the two TERT binding sites are brought closer together (reflected by the first step of FRET increase). Then the TERT binds and snatches the RNA into its final functional form (reflected by the second step of FRET increase). The green and red spheres indicate the fluorescent donor and acceptor dye molecules. Figure adapted from Nature 446, 458-461 (2007), Zhuang group (Harvard).

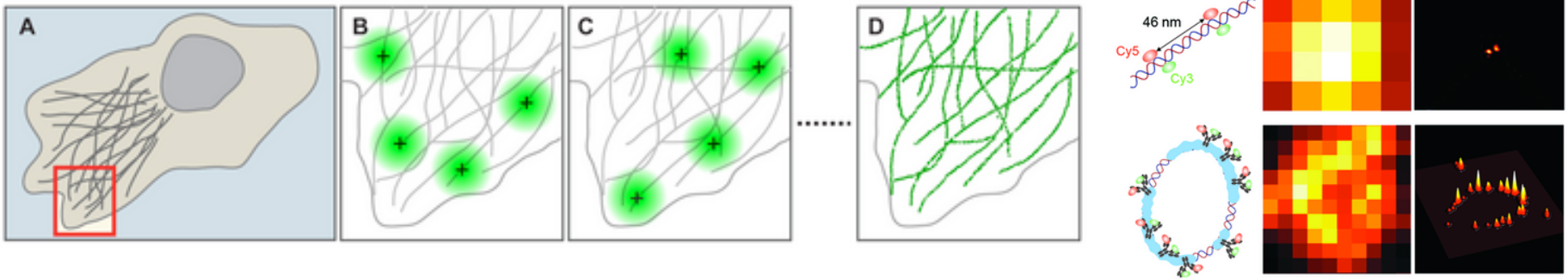
Fluorescent proteins now enable the full power of biochemistry to be applied (GFP, CFP, YFP):





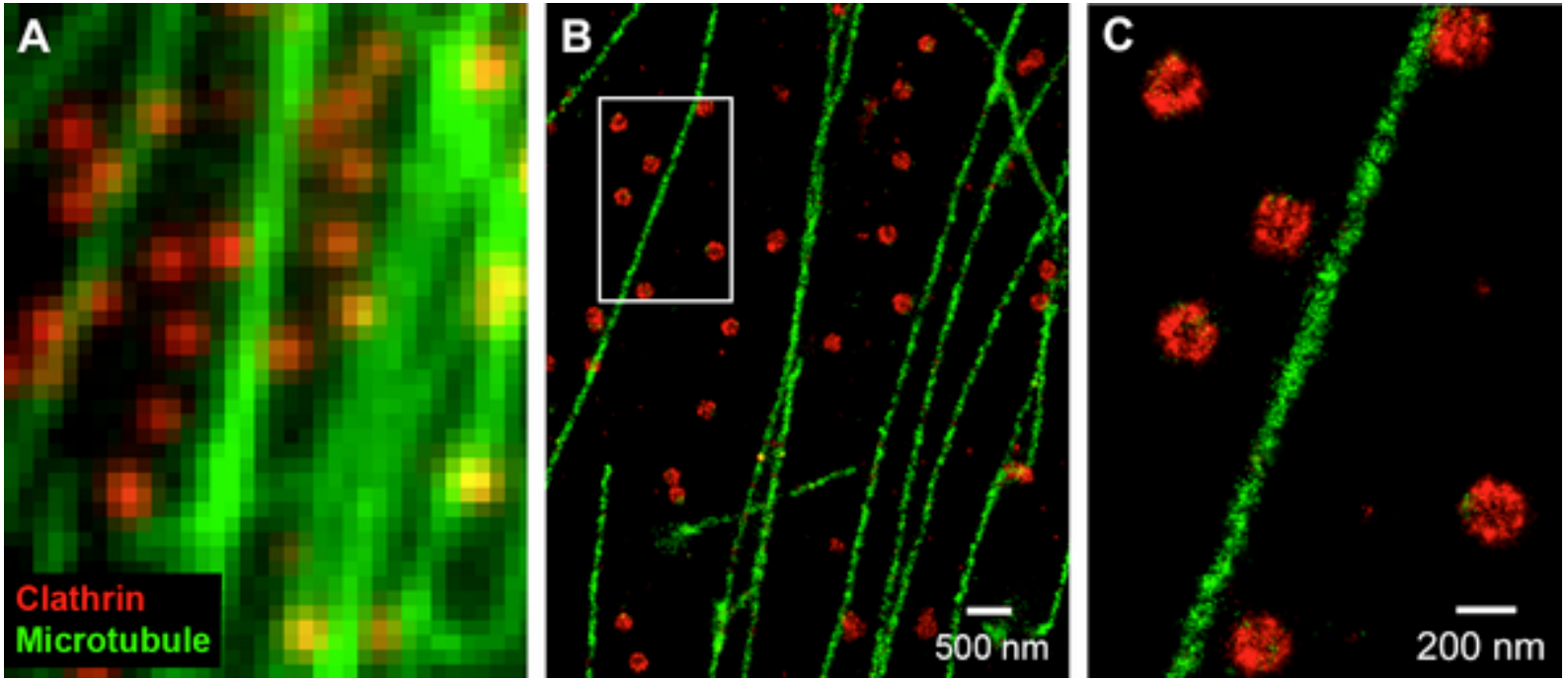
Single molecule microscopy (there are many flavors!):

Stochastic Optical Reconstruction Microscopy (STORM)



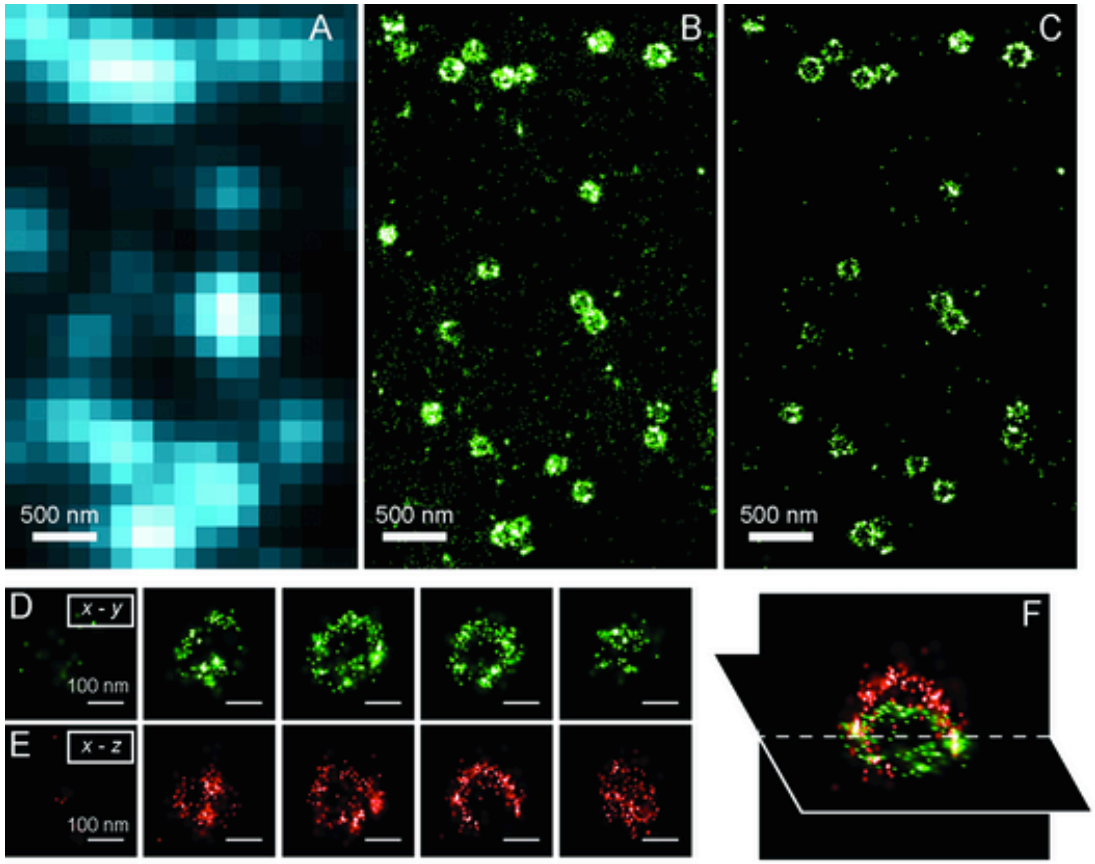
STORM imaging. Panel A shows a cell in which the structure of interest is labeled with photoswitchable fluorescent molecules. Panels B-D show a close-up view of the boxed region in A. In the first activation cycle (B), a subset of fluorophores are activated at a sufficiently low density such that the image of each fluorophore appears as isolated spots (green circles). This allows the exact position of the fluorophores to be determined by finding the centroid of each spot (black crosses). The activated fluorophores are then switched off. In a subsequent cycle (C), a different set of fluorophores are activated and localized. After a sufficient number of fluorophores have been localized, an image is constructed by plotting the positions of the fluorophores (green dots in D). The resolution of the STORM image is not limited by diffraction, but instead by the accuracy of determining the fluorophore positions. Figure at right, ***Nat. Meth.* 8**, 793-795 (2006), Zhuang group.

Single molecule microscopy (there are many flavors!):



Comparison of conventional (A) and STORM (B,C) images of microtubules and clathrin-coated pits (CCPs) in a cell. Microtubules (green) and clathrin (red) were stained with antibodies labeled with the above mentioned activator-reporter pairs. Panel C shows a magnified view of the boxed region in B. Images adapted from Science 317, 1749-1753 (2007).

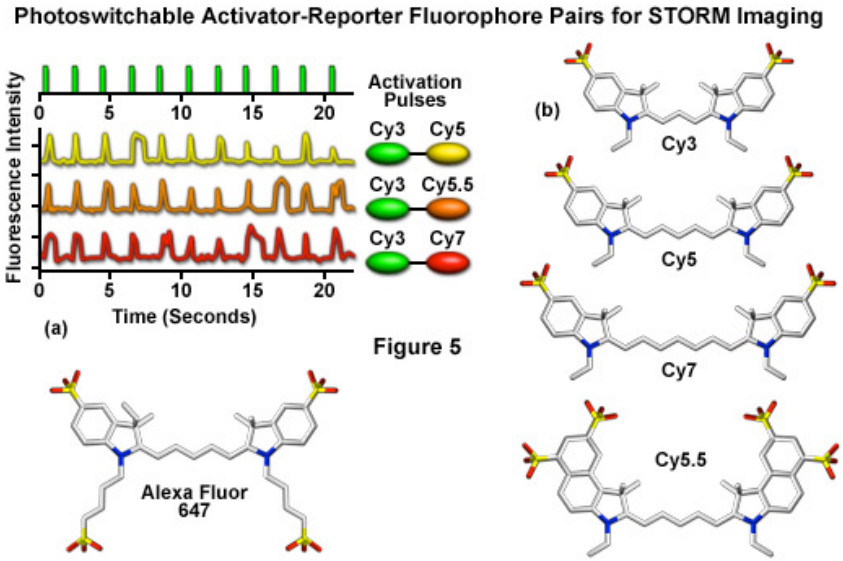
Single molecule microscopy (there are many flavors!):

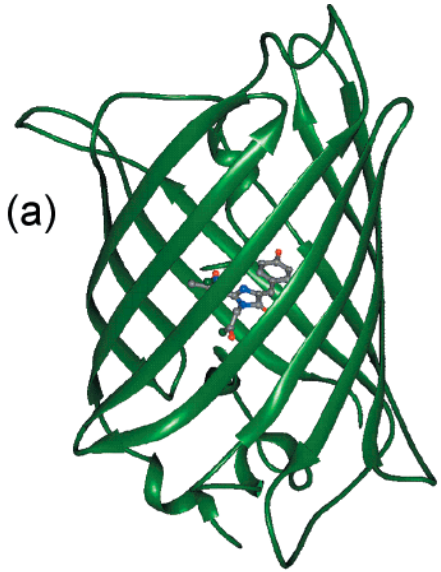


Resolution is not just enhanced in (x,y), but also with depth. Prof. Cai's and Shan's groups in CCE work on single molecule techniques & science.

Comparison of conventional and 3D STORM images of clathrin-coated pits in a cell. (A) Conventional immunofluorescence image of clathrin. (B) The STORM image of the same area with all localizations at different z positions stacked. (C) An x-y cross-section of the same area. (D,E) Serial x-y cross-sections (D) and x-z cross-sections (E) of a coated pit. (F) An x-y and an x-z cross section presented in 3D perspective. Images adapted from Science 319, 810-813 (2008), Zhuang group (Harvard, http://zhuang.harvard.edu).

Ultimately, this is a (bio)chemical problem:

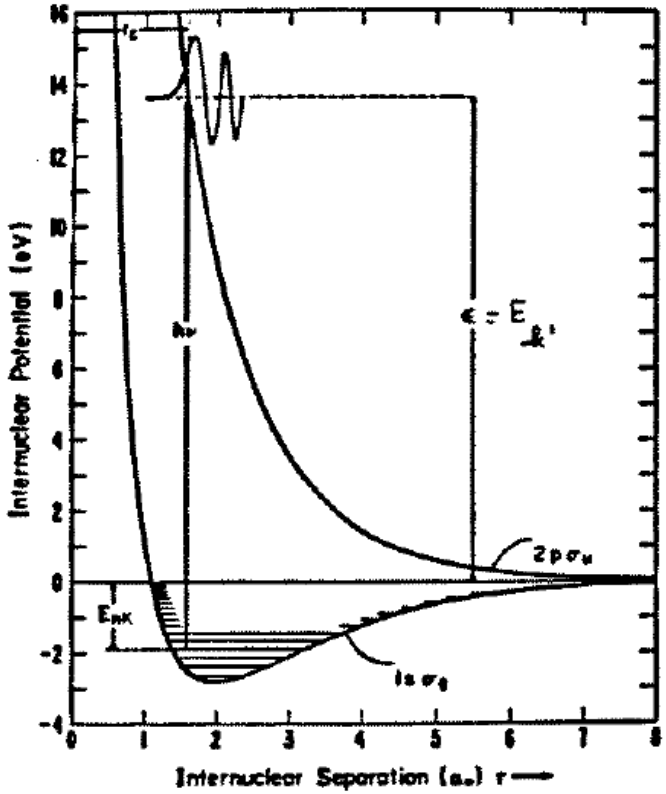




Need to design probes that are:

- Highly photo-switchable (on/off) but stable.
- Have high emission efficiency/A-values
- Are non-toxic.
- Can pass through cell membranes.
- Can bind to specific targets in the cell.

Electronic Spectroscopy - Dissociative Transitions



Let's go back to H_2^+ for a moment. Remember, the excited state in anti-bonding, so there is no bound state!

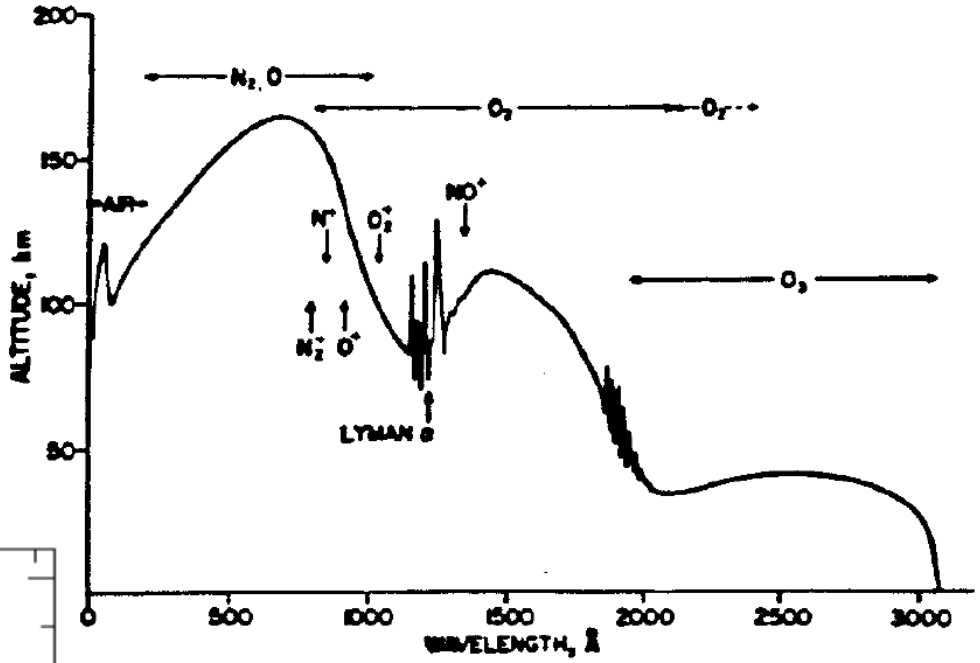
Thus, upon absorbing a photon of appropriate energy, the ion falls apart, or *photodissociates*.

What do you notice about the energy required to access the excited state?

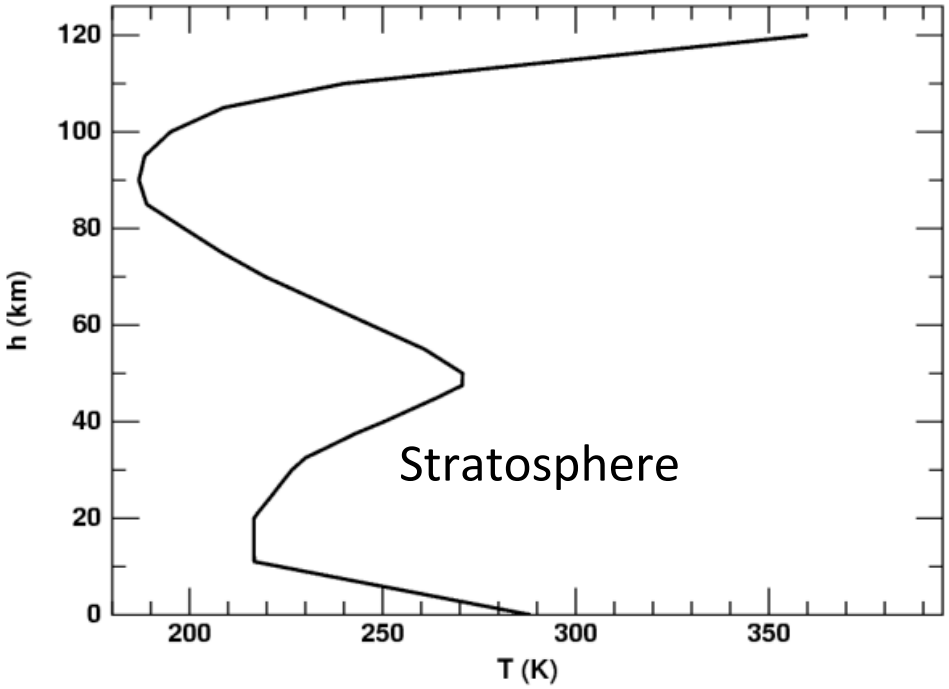
Remember, 1 eV has an equivalent $\langle k_B T \rangle$ that corresponds to T~11, 600 K.

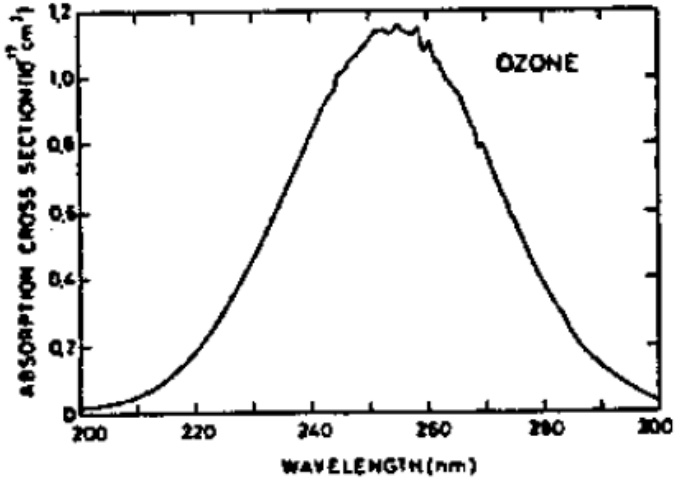
Electronic Spectroscopy - Dissociative Transitions

Thus, gas in which photodissociation is important is *heated*!



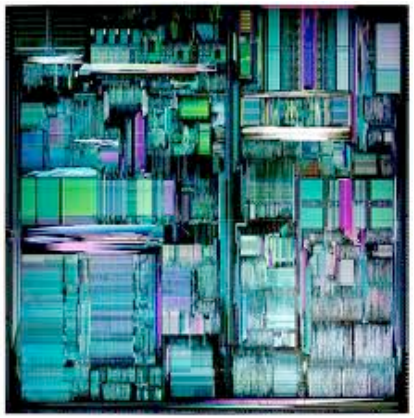
Standard Atmosphere

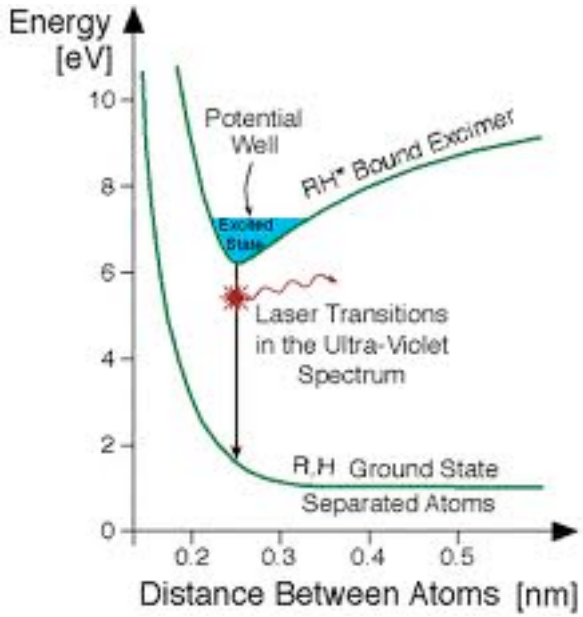




Electronic Spectroscopy - Dissociative Transitions

Excimer Lasers ⟷ Photolithography





ArF (193 nm), KrF (248 nm), XeCl (351 nm)

Excited states bound, ground states repulsive, formed in discharges.

Feature scale determined by wavelength, how far can you go before damage induced by dissociation becomes pervasive?