Absorption Spectroscopy as a probe of the chromophore environment

topics

1 **Spectroscopic Broadening**

- vibronic structure
- solvent induced heterogeneity

2 **Solvent - chromophore interactions**

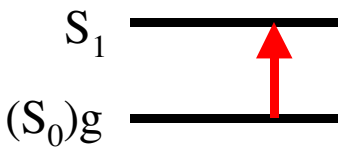
- red shift / blue shift
- polarity vs polarizability

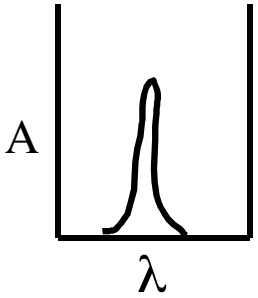
3 **Chromophore - chromophore interactions**

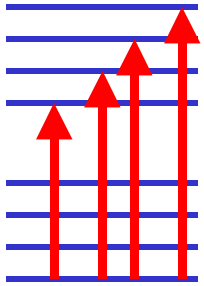
- hypochromism
- excitons

Causes of Spectroscopic Broadening

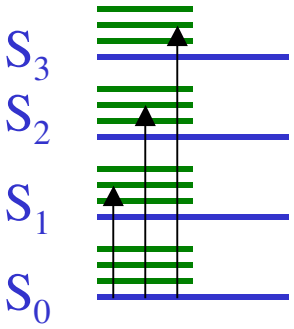
1 Vibrational Structure

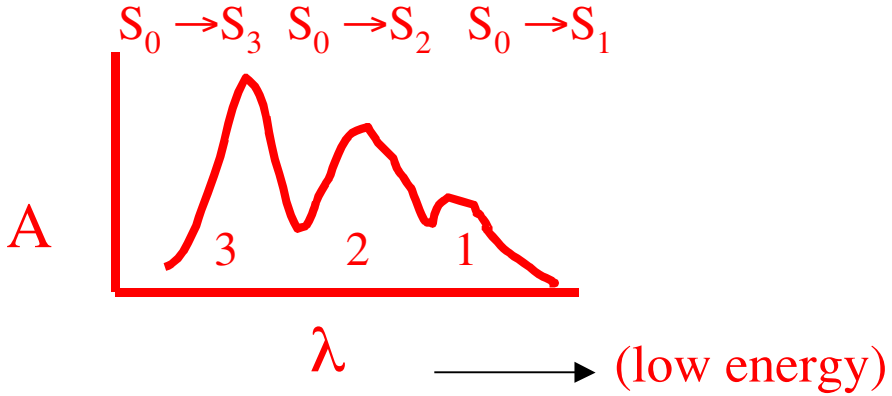






2 Overlapping Electronic Bands





3 Solvent-Induced Heterogeneity

Franck-Condon Principle

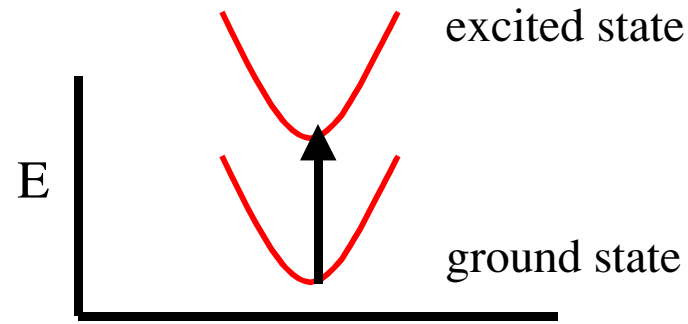
Nuclei can be considered as not moving during an electronic transition.

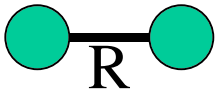
electronic transition ~ 10^{-15} sec

nuclear motions ~ 10^{-11} sec

Results in a Vertical Transition:

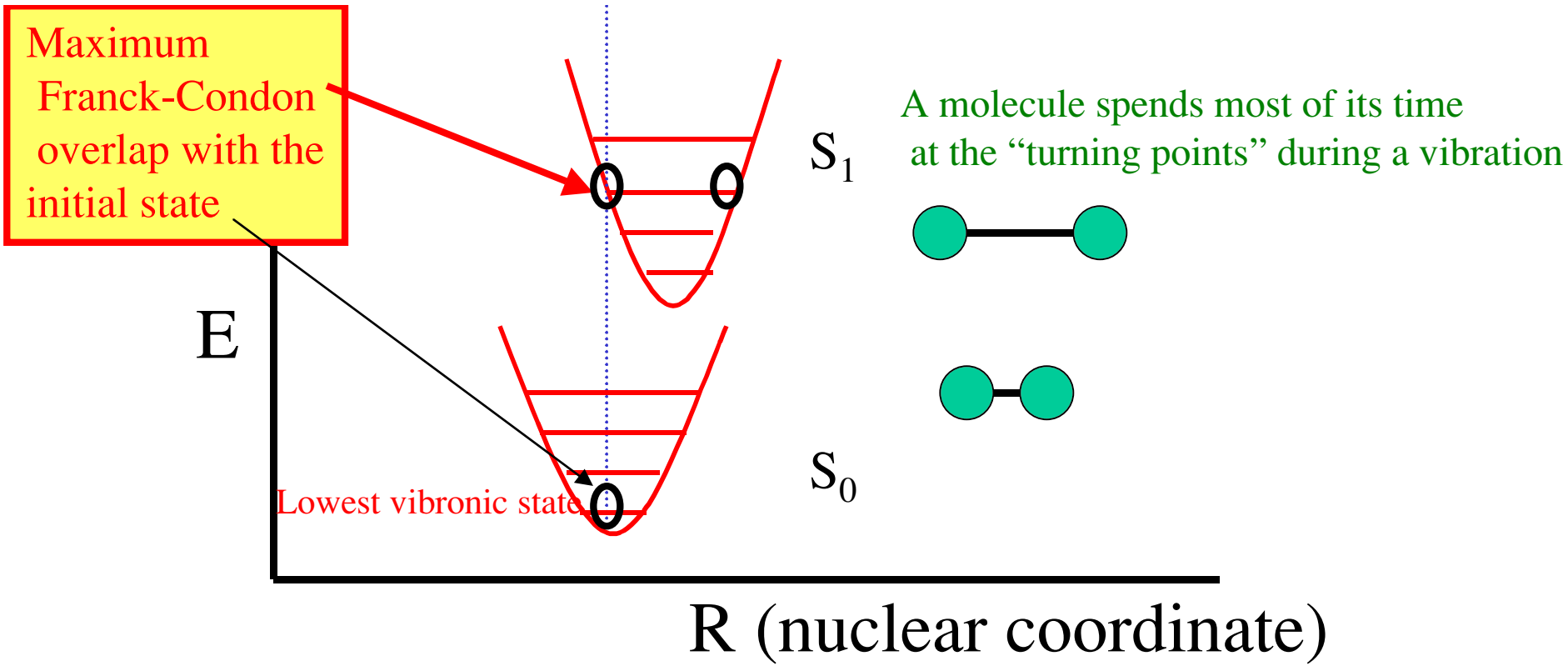
no change in nuclear coordinates





Probability of promoting a molecule from one vibronic state to another depends on the probability of the two states sharing the same nuclear coordinates.

This overlap factor is called the **Franck-Condon Factor**



Probability of promoting a molecule from one vibronic state to another depends on the probability of the two states sharing the same nuclear coordinates

The Franck-Condon factors are a measure of this probability and determine the shape of both

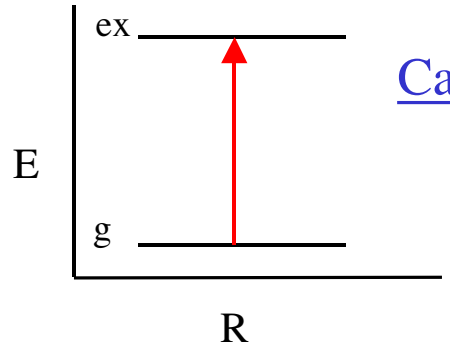
Absorbance

and

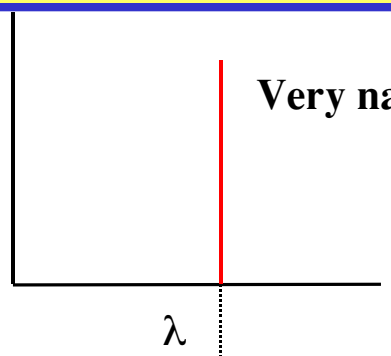
Fluorescence

Spectra

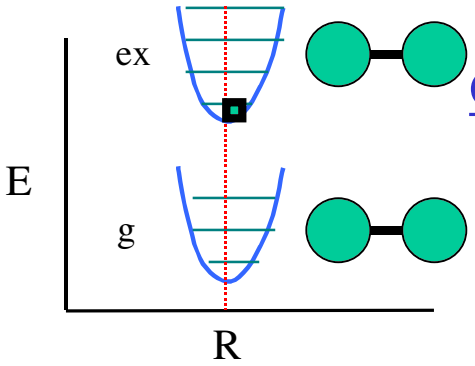
Vibrational Structure and the Franck-Condon principle: vertical transitions



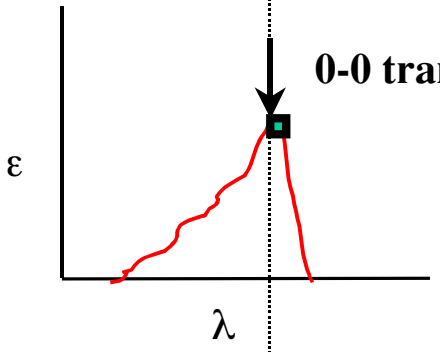
Case I: No vibronic structure



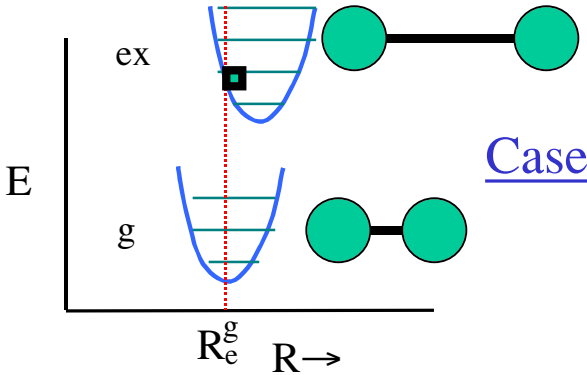
Very narrow spectrum



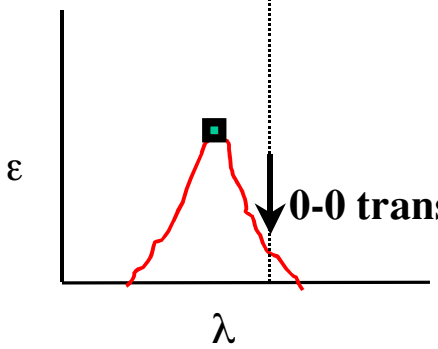
Case II $R_e^{\,g} \approx R_e^{ex}$:



0-0 transition



Case III $R_e^{\,g} \neq R_e^{ex}$:



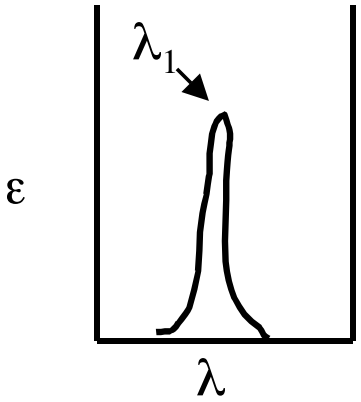
0-0 transition

Solvent Broadening

Hypothetical case:

(A) Vapor spectrum - sharp, centered at λ_1 each molecule in the vapor is in the same exact environment

(B) Solution spectrum - broad, often λ_{max} shifted



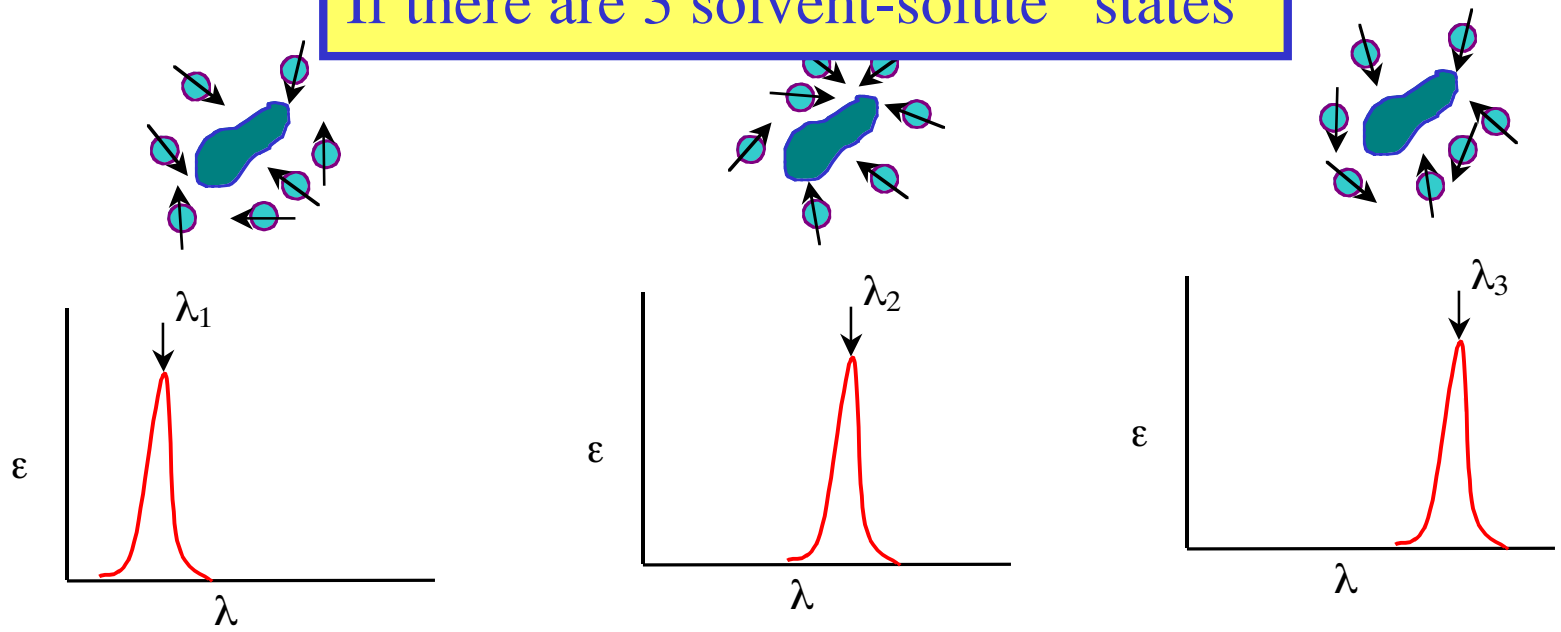
environmental heterogeneity

At any instant in time, each molecule has a different arrangement of solvent molecules surrounding it

recorded spectrum is a **SUM**, not an average

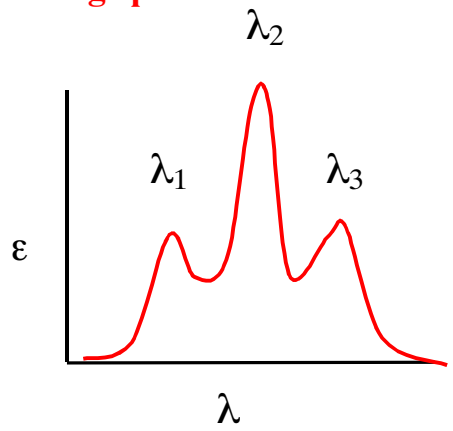
- Like an instantaneous snapshot
- Very different from situation in NMR where tumbling molecules can each sense the same average environment

If there are 3 solvent-solute "states"



If at any time the population is: **25% λ_1 (state 1)**
50% λ_2 (state 2)
25% λ_3 (state 3)

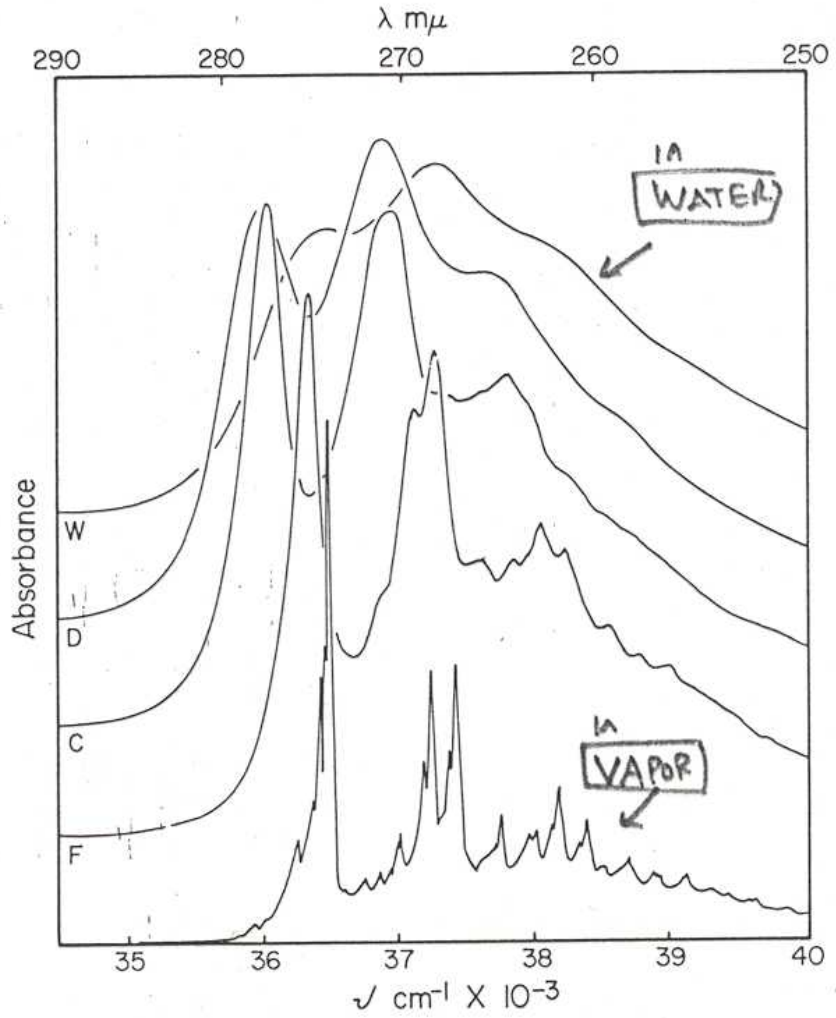
The resulting spectrum:

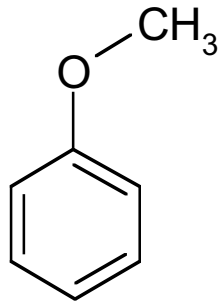


Broad, shifted spectrum due to solvent interactions

The inside of a protein can be less heterogeneous than a real solvent ⇒ sharper spectra from buried aromatic amino acid side chains such as tyrosine

Solvent Effects on the Absorption Spectrum of Anisole





solvent

water

dioxane

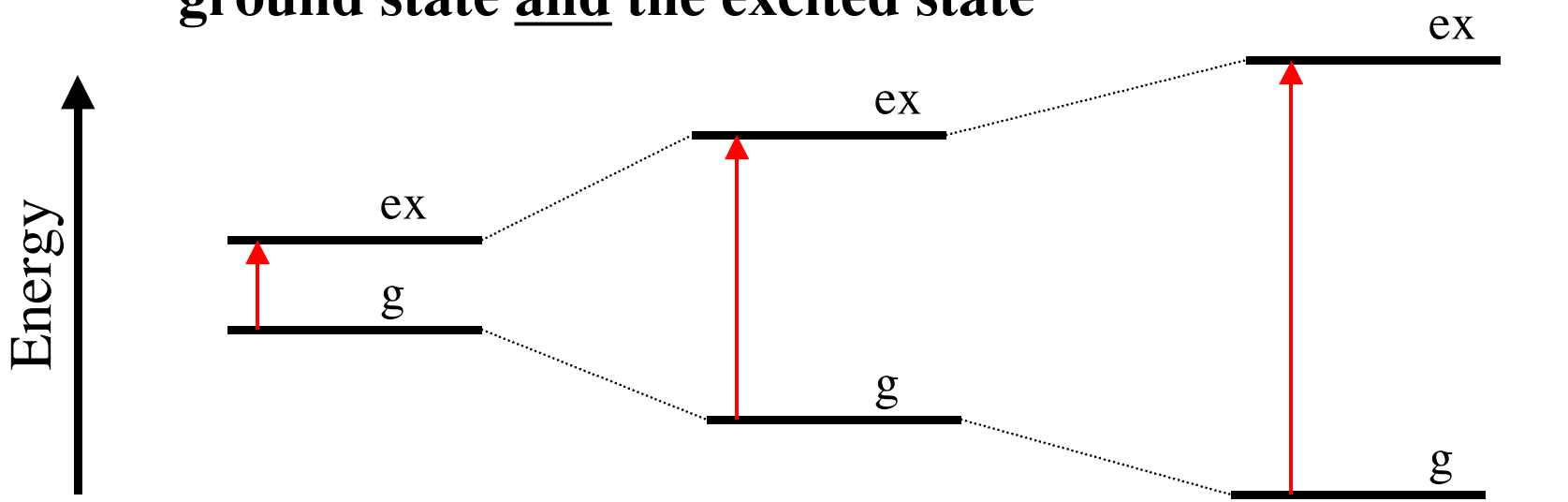
cyclohexane

perfluoro-octane

vapor

Solvent effects on Absorption

1 **The solvent can influence the energy levels of both the ground state and the excited state**



1 Solvent 2 Solvent 1 Solvent 3

1 red shift blue shift

1 (lower ΔE) (higher ΔE)

Polarizability vs Polarity

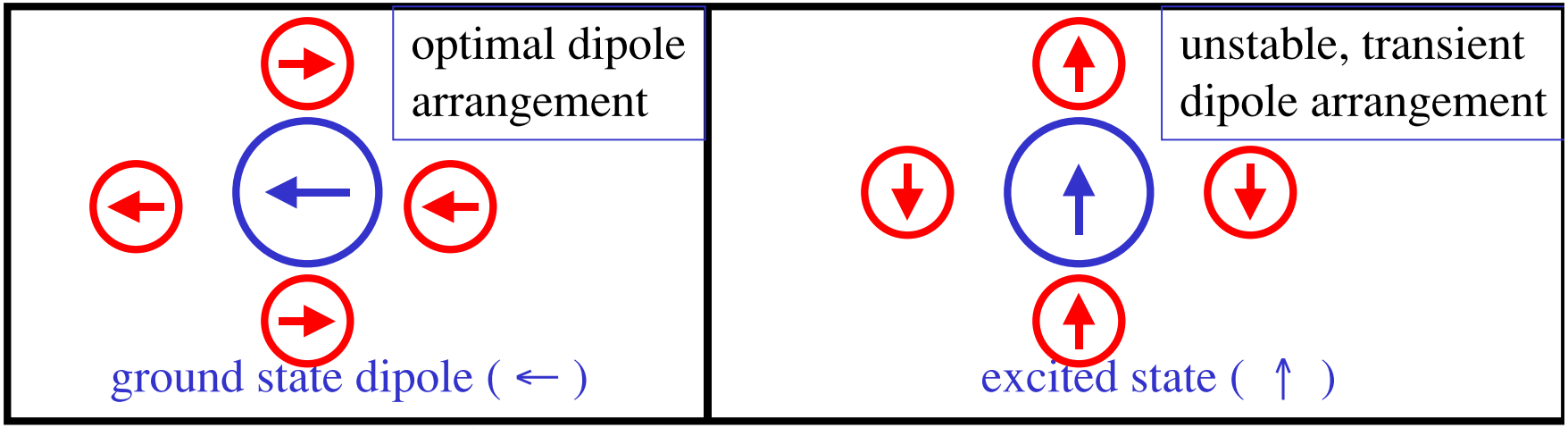
1 1 Solvent **polarizability**:

- high frequency response of electrons in solvent to the induced dipole in the chromophore

  (induced dipole)

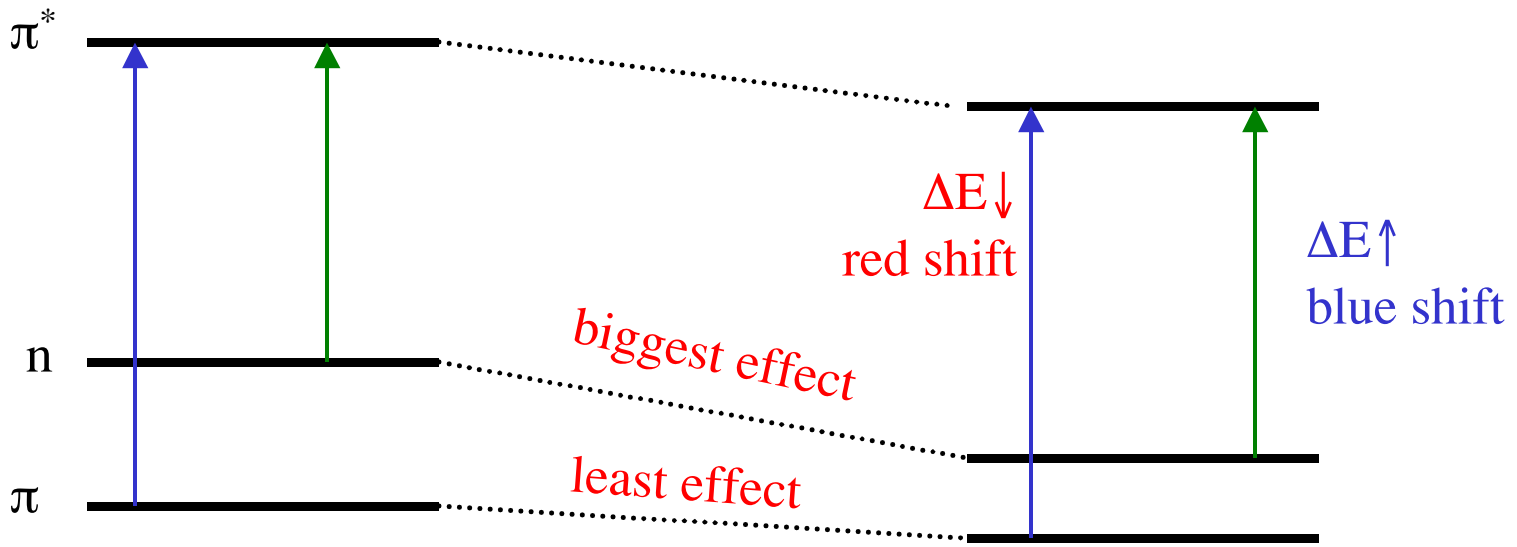
- measured by the **index of refraction**
- can respond to changes in chromophore electronic distribution instantaneously

  (no nuclear motions are required)



Result: the MORE polar state (g, ex) of the chromophore is stabilized to greater extent

Influence on the energy levels of π, n, π* orbitals by solvent polarizability



Low polarizability solvent or high polarizability solvent

A π - π* transitions: red shift in more polarizable solvent

- π* interacts with solvent dipoles more strongly than π

B n - π* transitions: blue shift in more polarizable solvent

- n interacts with solvent dipoles more strongly than π*

Tyrosine and Tryptophan respond to solvent polarizability

$\pi - \pi^*$ transitions

Solvent shifts in some non-polar chromophores.

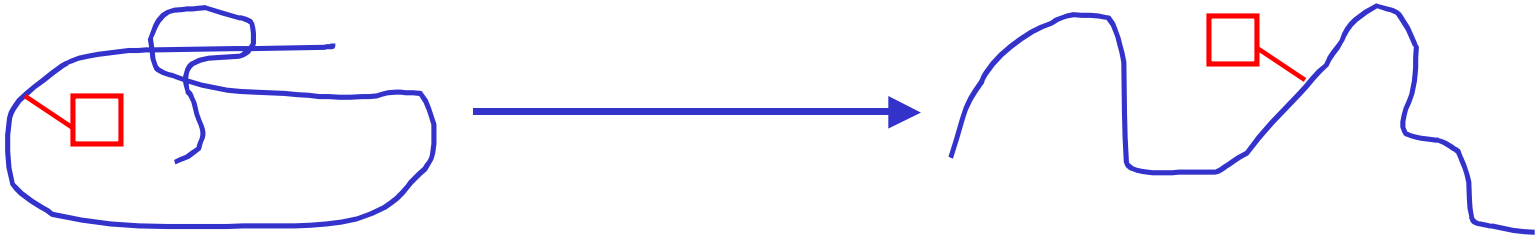
polarizability

red shift

Solvent	n_D^{25}	Wavelength shift, $\Delta\lambda$ (nm) relative to iso-octane		
		Benzene	Phenol	indol
perfluoropentane	1.239	-1.4	-2.3	-
water	1.333	-0.8	-1.4	-0.9
ethanol	1.362	-0.2	-	-
iso-octane	1.392	0	0	0
chloroform	1.446	+0.8	+0.5	+2.0
carbontetrachloride	1.463	+1.2	+1.5	+3.4

red shift

Protein denaturation



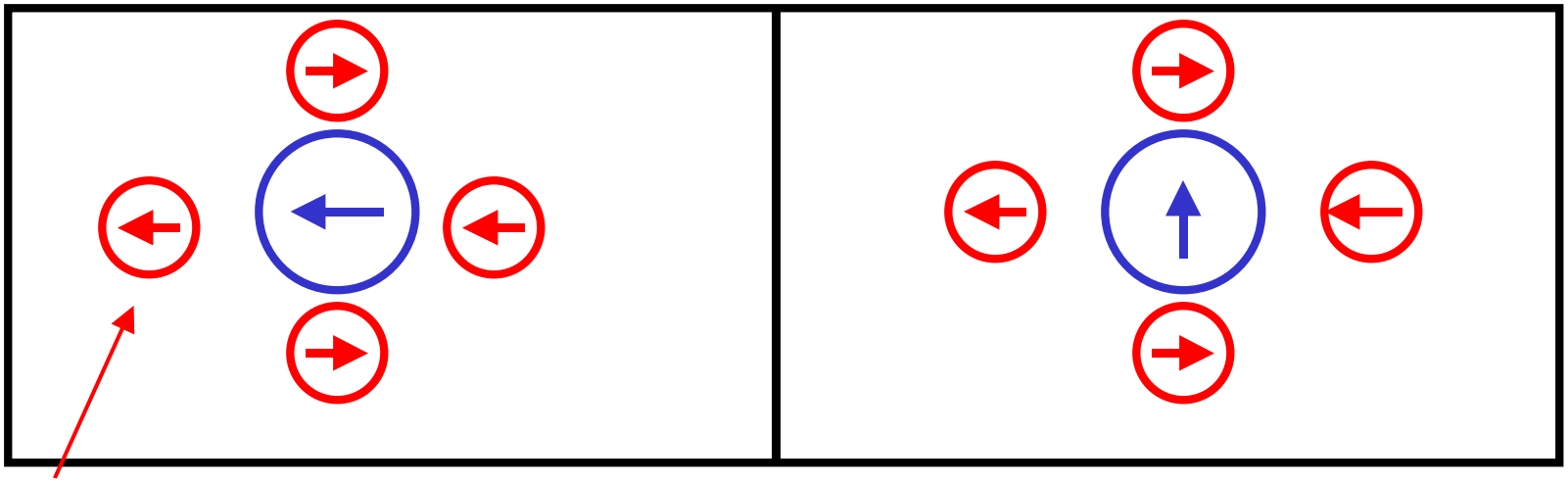
High polarizability
(inside protein)

low polarizability
(water)

Blue shift of tryptophan absorbance

2 Solvent **polarity**

- Due to the permanent dipole of the solvent interacting with the dipole of the chromophore in the ground and excited states.
- low frequency response of the solvent molecules to the changing of an electric field
- measured by the dielectric constant of the solvent (ε)
- more difficult to predict the influence of the solvent permanent dipoles since the solvent molecules cannot re-orient sufficiently rapidly to altered electronic distribution of the chromophore excited state



Permanent dipoles:
nuclear re-orientation ~10^{-11} sec

g ⟶ ex
10^{-15} sec

Example: Effect of solvent polarity on the absorption of mesityl oxide, a polar chromophore

Mesityl oxide:

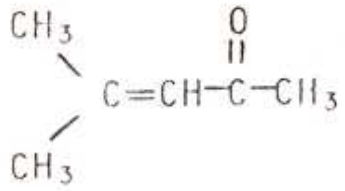


Table 1: Solvent shifts of a polar chromophore.

band ↓	ε $M^{-1}cm^{-1}$ ↓	solvent	λmax (nm) hexane	ether	ethanol	methanol	water
		dielectric constant→	2	4.3	25.8	31	81
π-π*	12,600		229.5	230	237	238	244.5
n-π*	40		327	326	315	312	305

Red shift

Blue shift

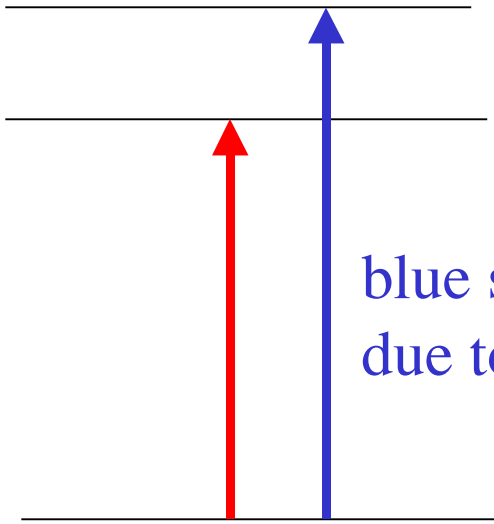
Solvent shift in this case correlates with permanent dipole of solvent

The dielectric constant measures polarity, due to permanent molecular dipoles

Note: The influence on λ_{max} is the opposite as predicted by polarizability in this case

An example tuning the absorption spectrum of a chromophore bound to a protein by different electrostatic and steric interactions with the ground and excited states

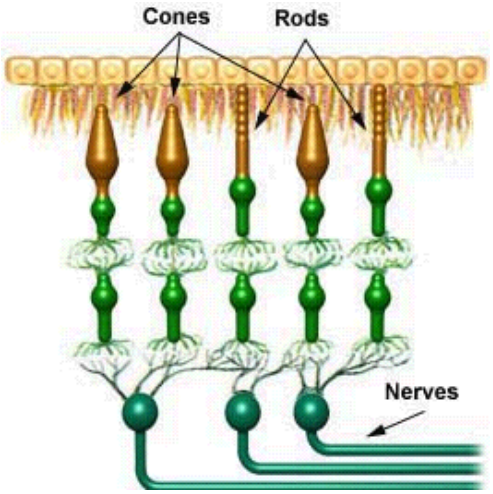
Visual Pigments: rhodopsin family



excited state

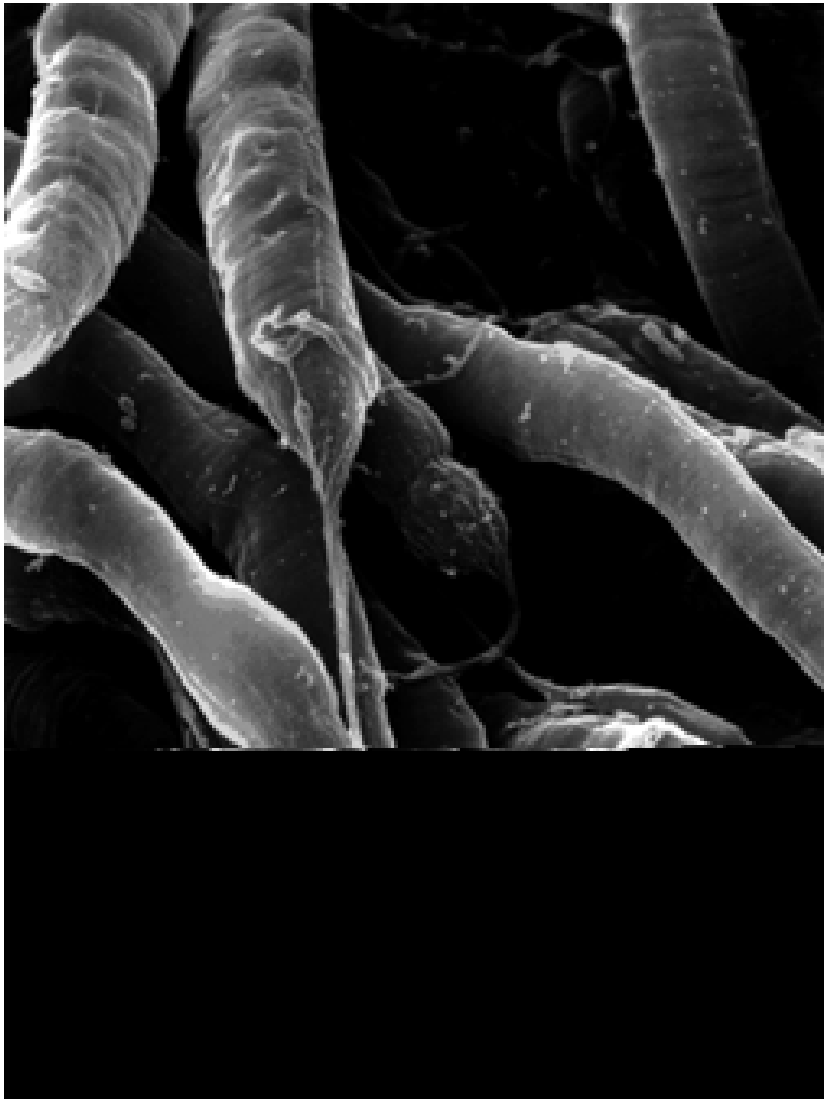
blue shift if excited state has higher energy
due to interaction with an amino acid side chain

ground state

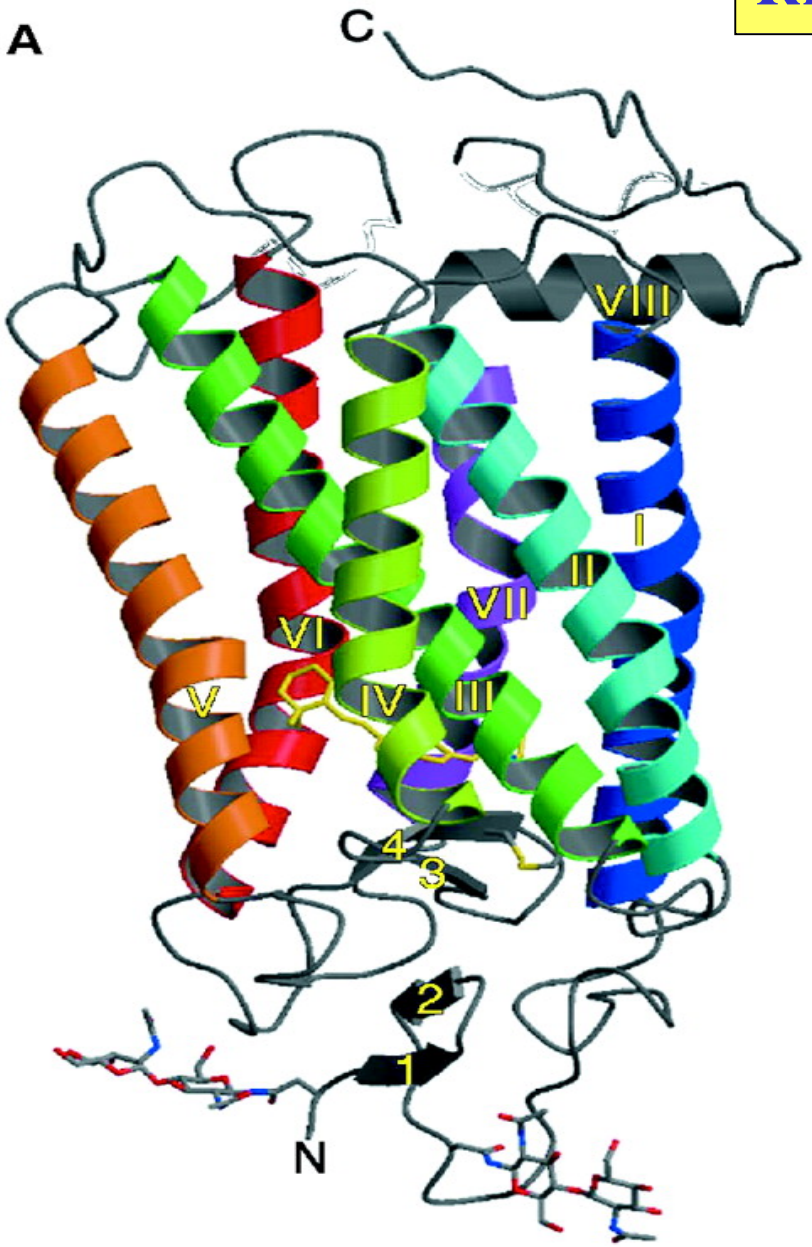


rods: black/white vision

cones: color vision



Rhodopsin



visual pigment in the eye rod cells responsible for dim light vision

λ_{max} = 500 nm

color vision is due to similar pigment proteins in cone cells:

λ_{max} = 414 nm (blue)

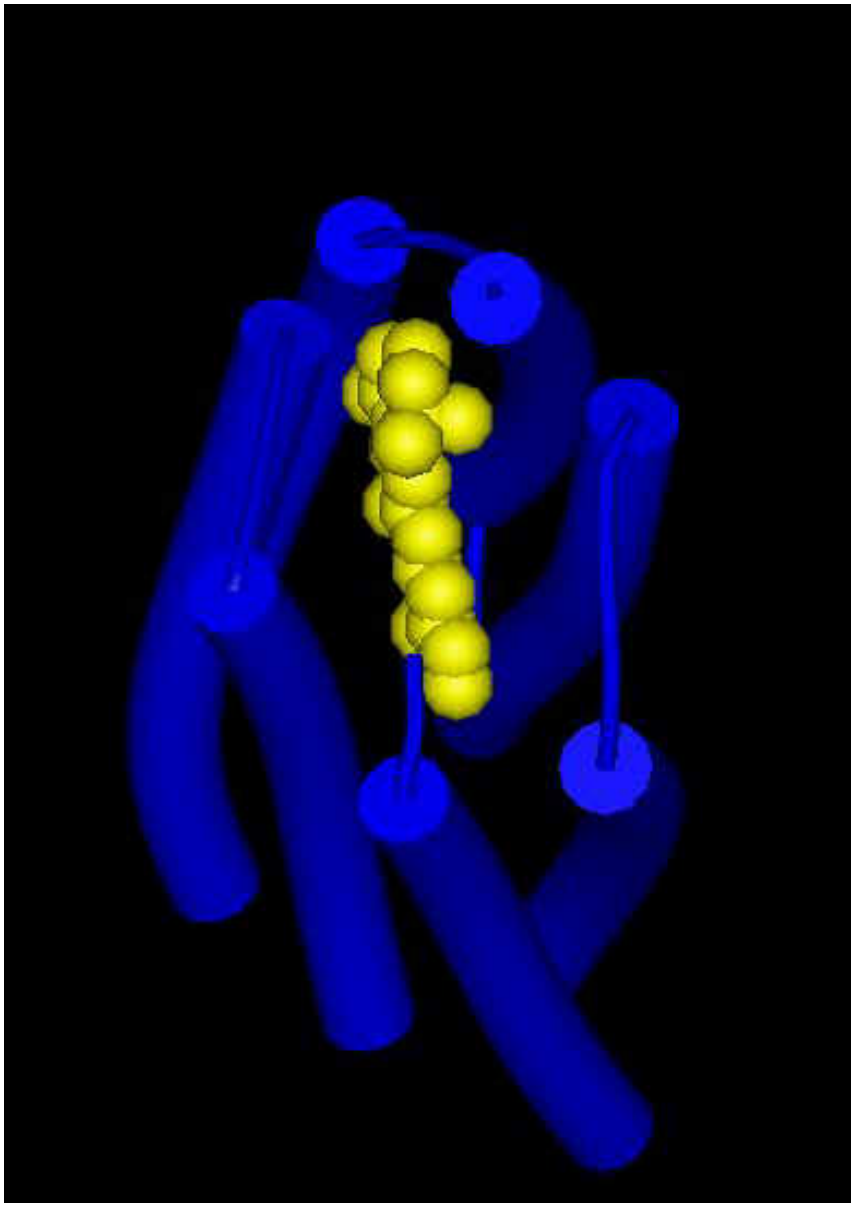
λ_{max} = 533 nm (green)

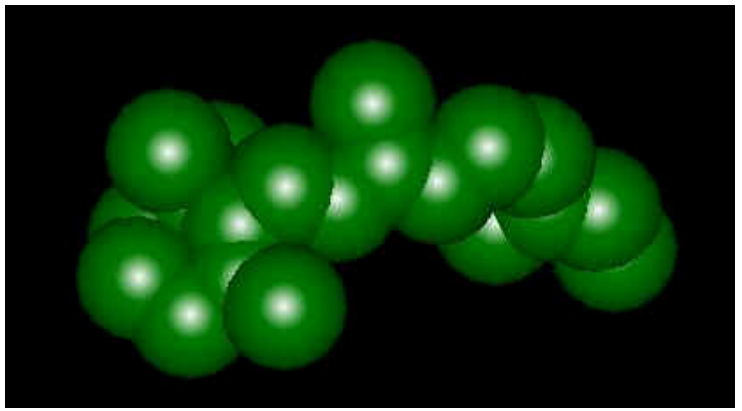
λ_{max} = 560 nm (red)

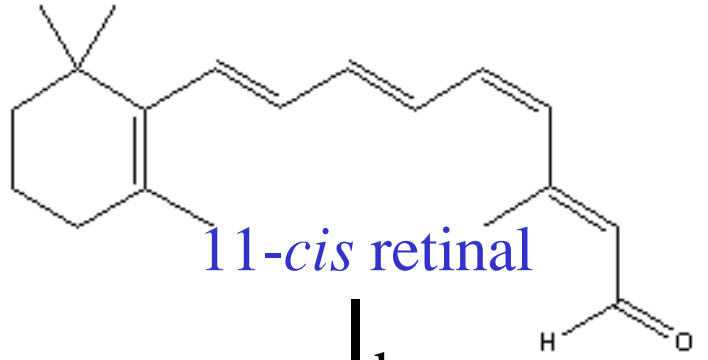
Same chromophore: 11-*cis* retinal

"spectral tuning" by interaction with amino acid residues nearby

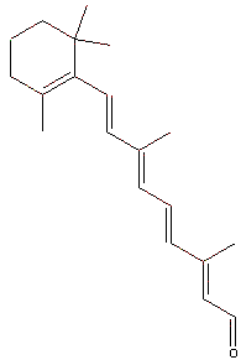
Biochem (2001) 40, 7219-7227





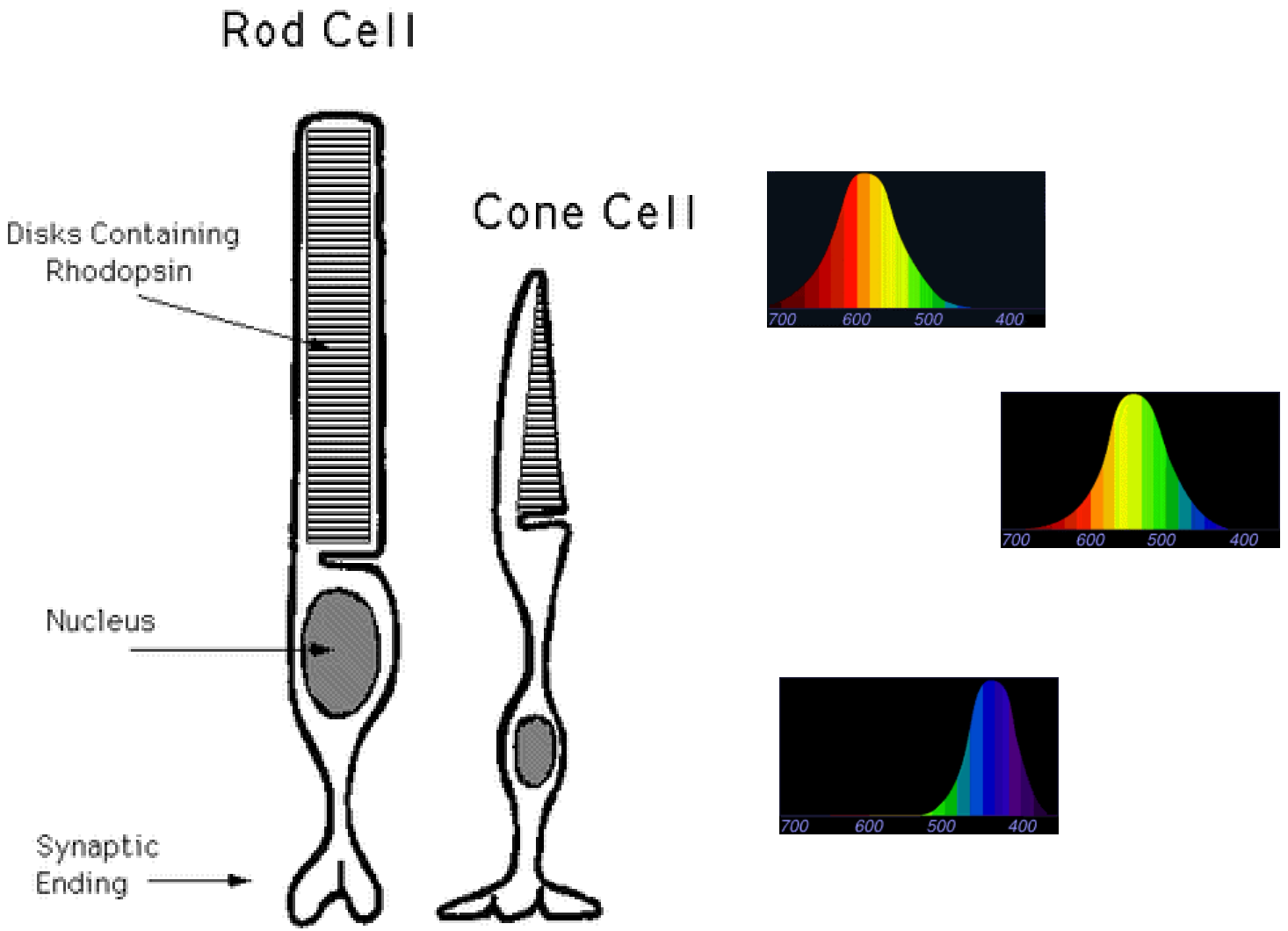


hν



all *trans* retinal

www.isat.jmu.edu/users/klevicca/isat351/vision.ppt



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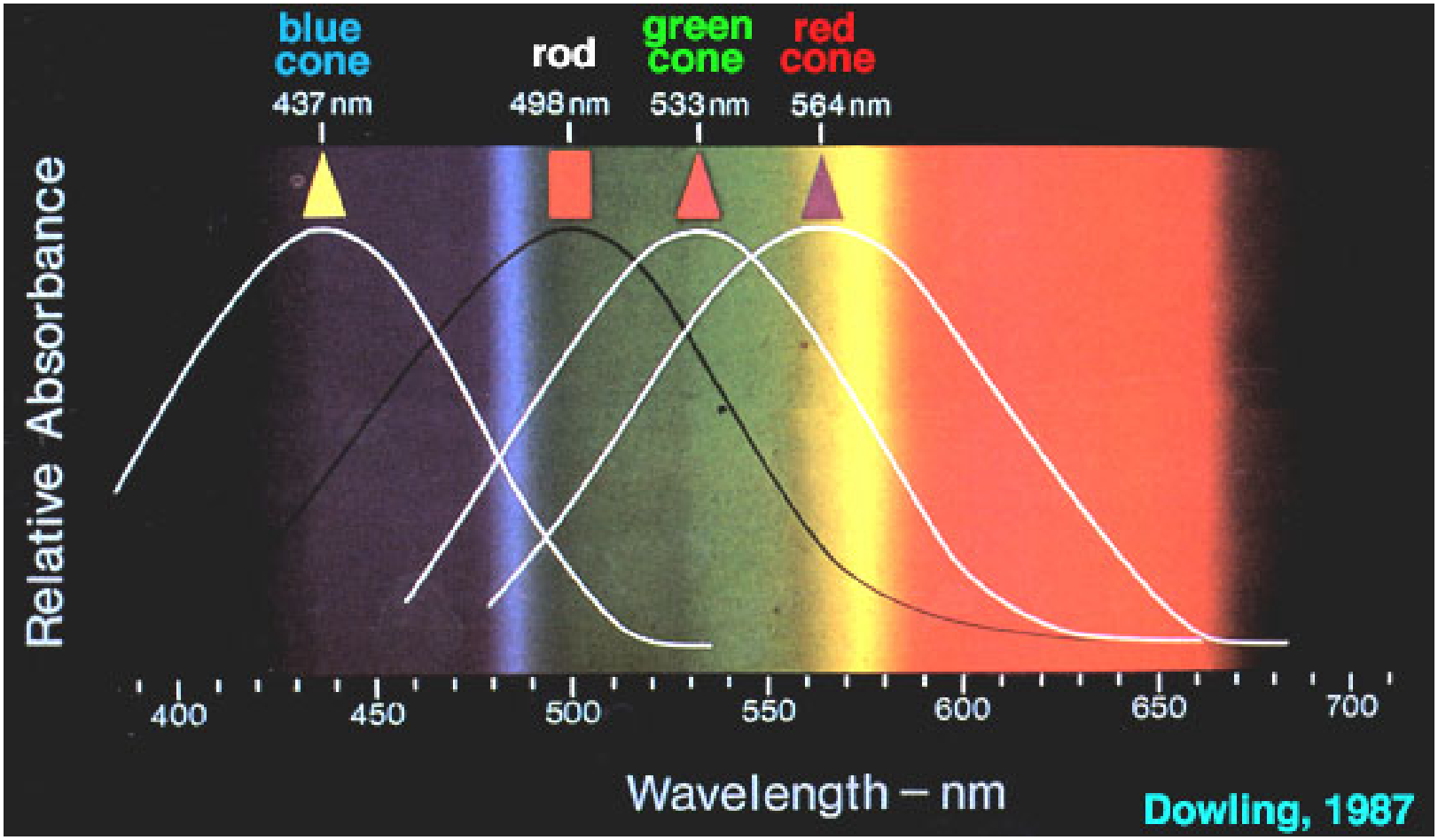
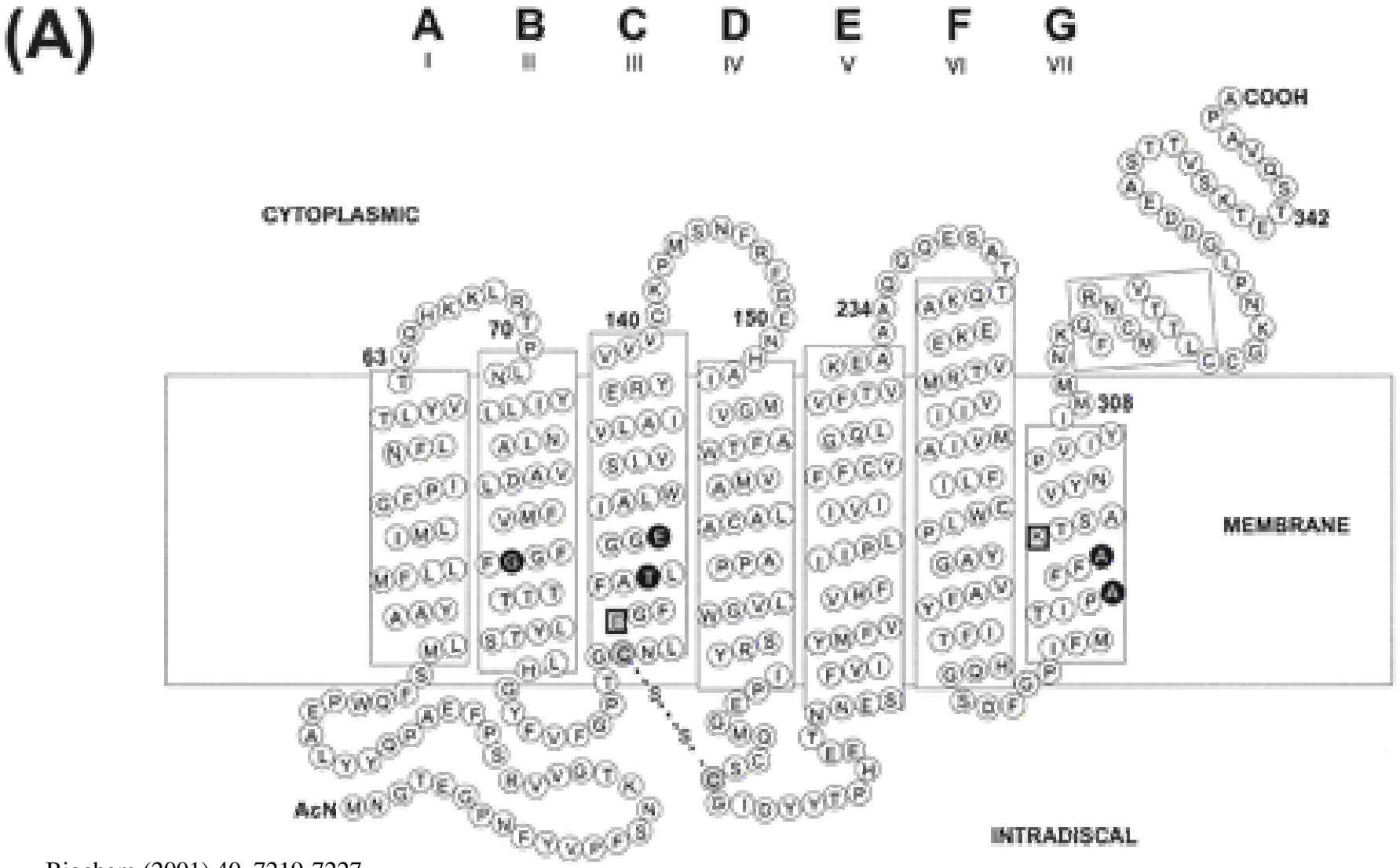


http://insight.med.utah.edu/Webvision/imageswv/spectra.jpeg

Residues altered to shift the spectrum of rhodopsin



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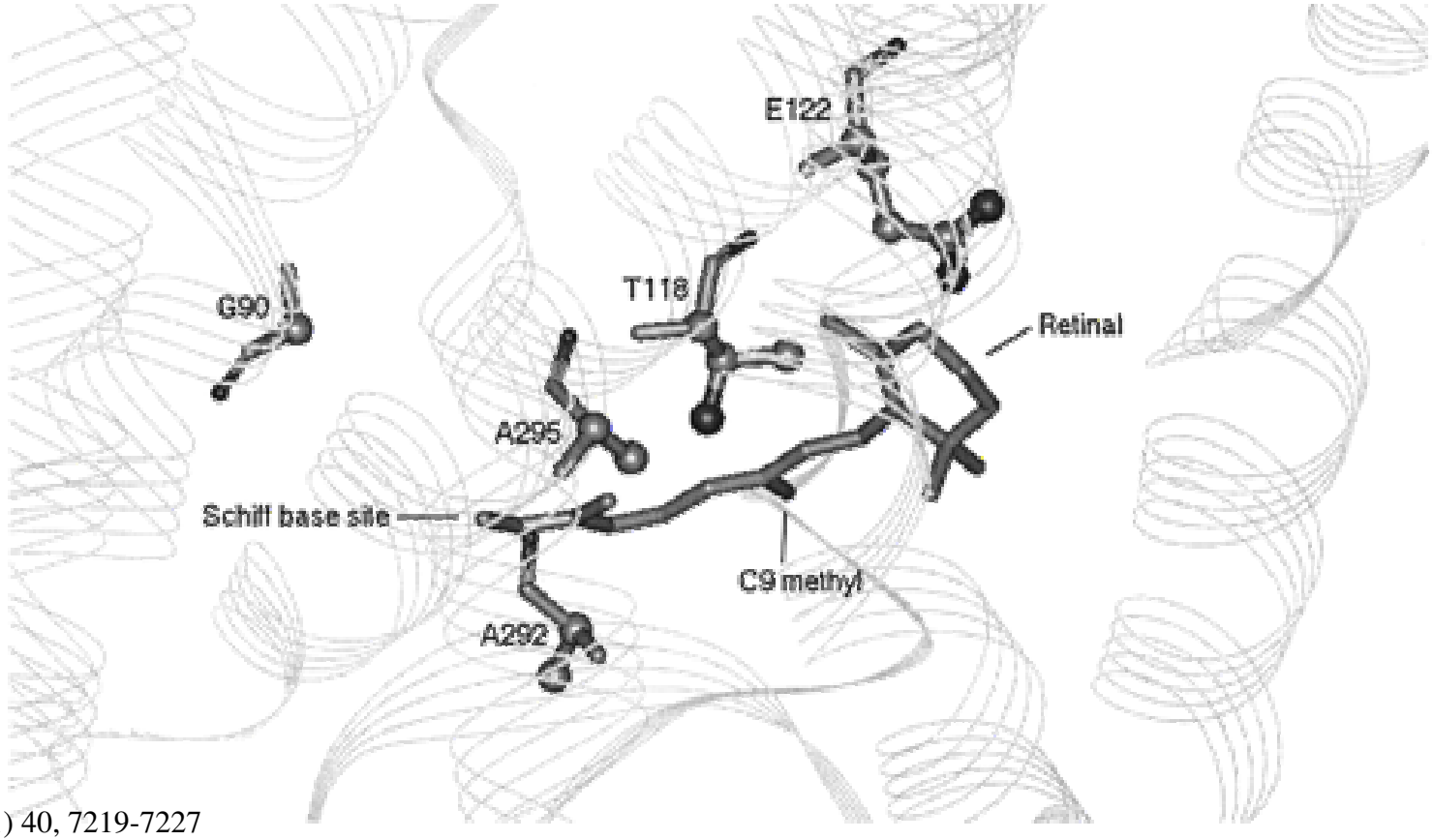
Residues in Rhodopsin near the retinal chromophore



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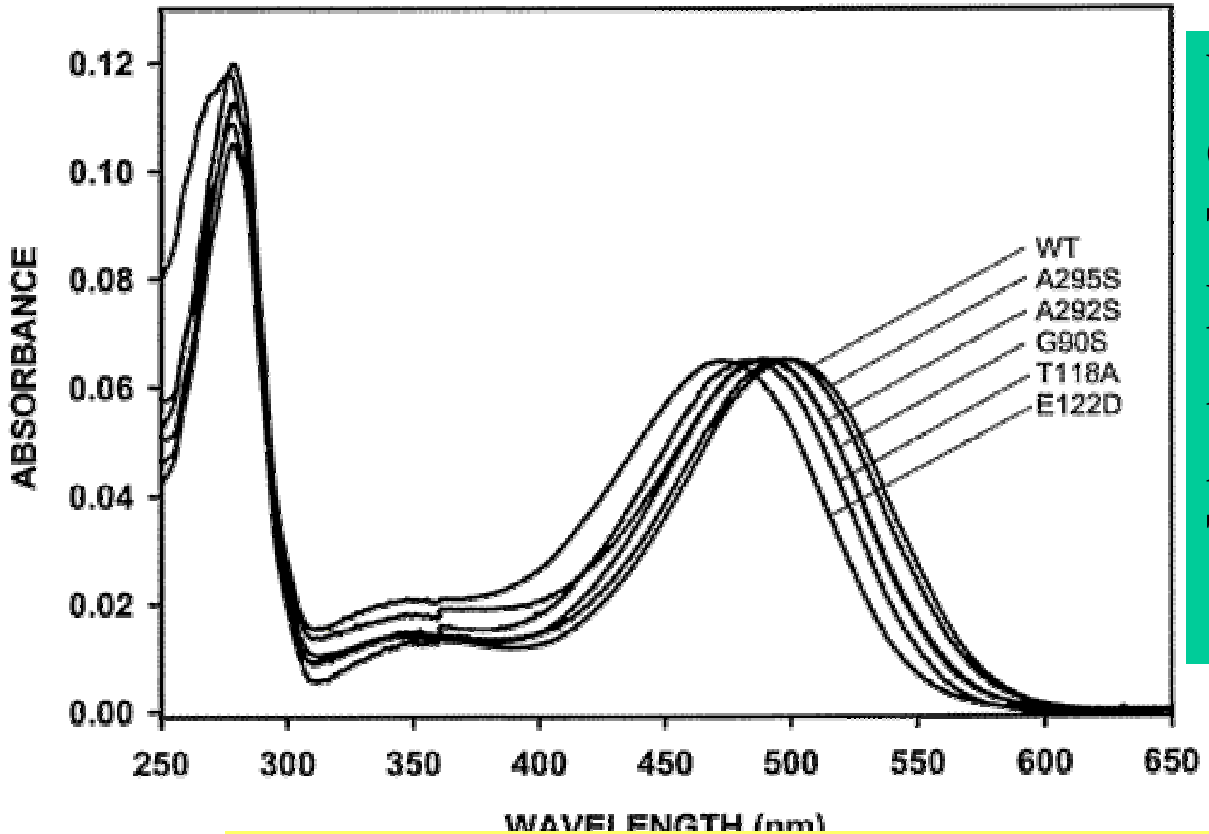
Blue shift of the rhodopsin spectrum due to altering the residues in the protein binding site



WT: 500 nm
G90S: 487 nm
T118A: 484 nm
E122D: 477 nm
A292S: 489 nm
A295S: 498 nm
T/E/A triple mutant 453 nm

different electrostatic and steric interactions with the ground state and excited state of retinal alter their relative energy difference

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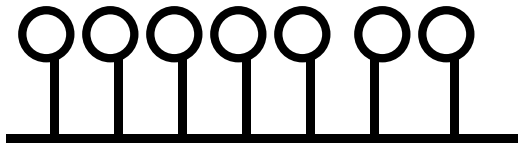
Chromophore - Chromophore Interactions

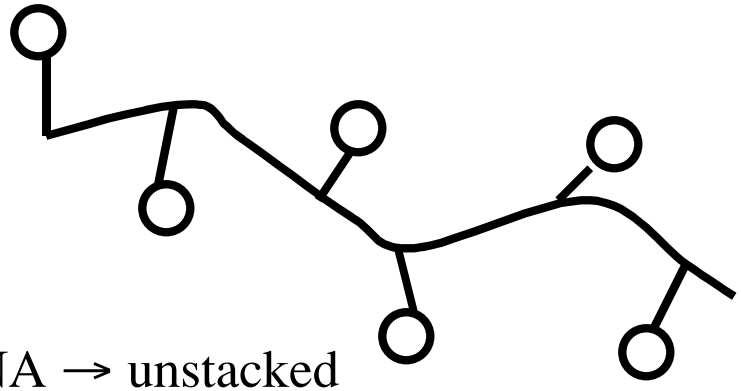
1 Important for determining optical properties of nucleic acids and proteins

2 **Primarily short range interactions**

- **stacked bases in DNA, RNA**
- **Amide groups in α-helix**

3 Useful to monitor ordered $\rightleftharpoons$ disordered transitions





- protein α-helix → coil
- stack single strand DNA or RNA → unstacked
- Double-strand DNA or RNA → single-strand

Two Classes of Interactions

1 Interactions between electronic bands of similar energy

- excitons
- energy transfer

2 interactions between electronic bands of different energies

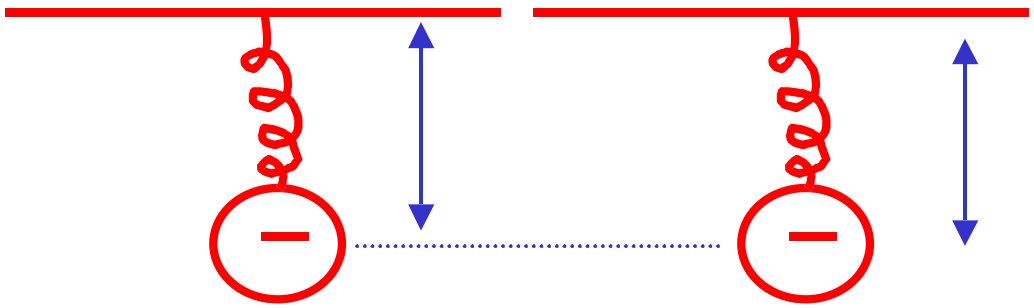
- hyperchromism
  (borrowed intensity)

3 **in all cases: the total oscillator strength is constant**

3 (i.e., the area of the spectrum is constant)

3 If ε goes down in one place then ε must decrease elsewhere

classical view
coupled harmonic oscillators



Quantum Mechanical View

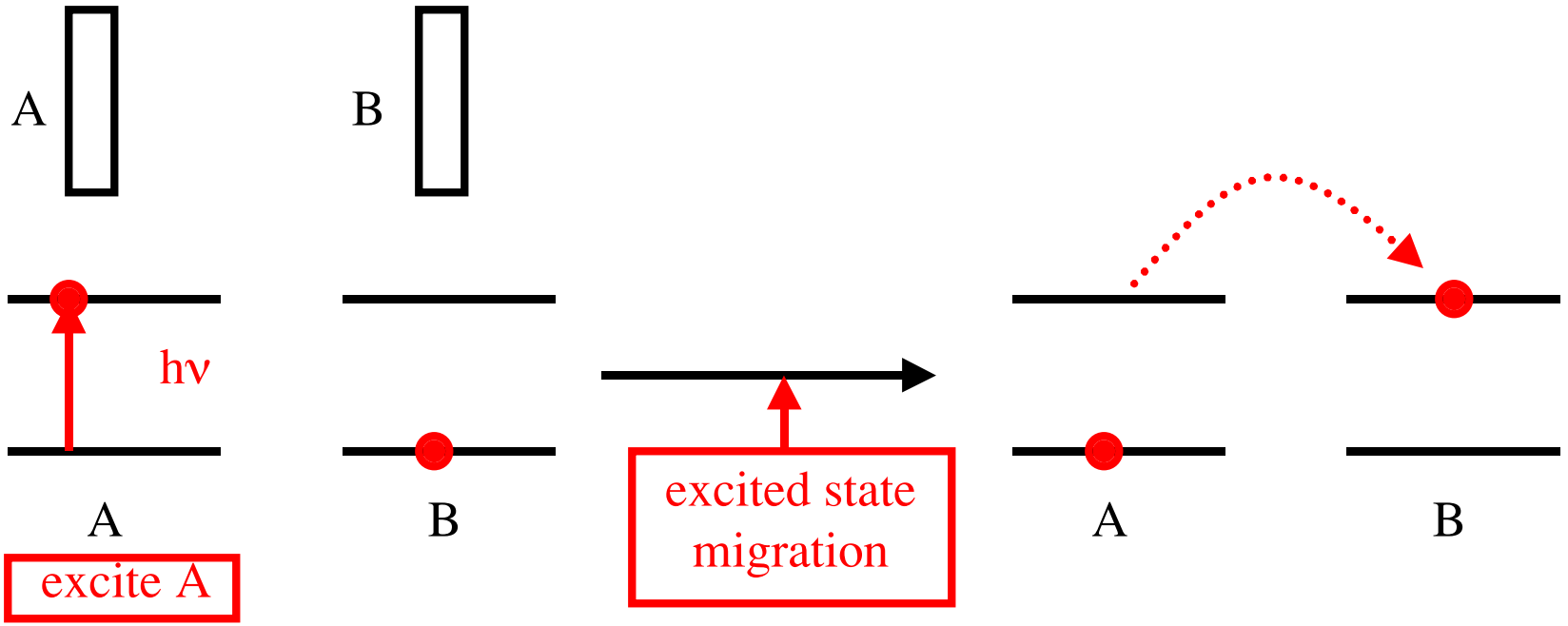
Mixed wave functions resulting in altered transition dipoles

Both views incorporate the idea of "in-phase" and "out-of-phase" modes of coupled oscillations

Note: interactions are highly dependent on molecular geometry

- angles and distances.

Excitons and Energy Transfer



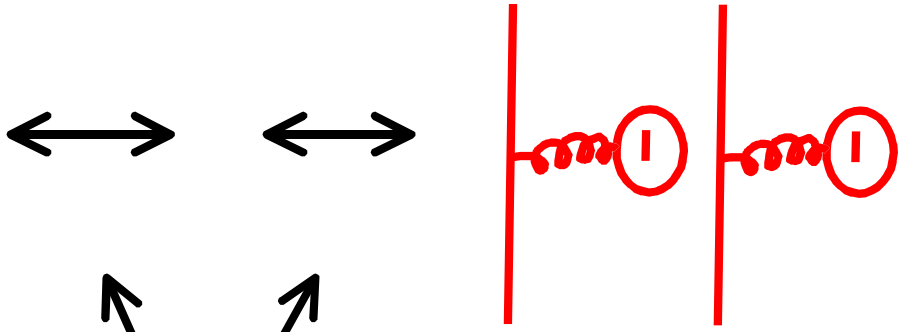
1 If excitation hops very fast, it cannot be localized in a single molecule
   - excited state covers both A and B (or more)
   - called exciton band
   - can view excitation as diffusing from one molecule to next.

2 Slow hopping (10^8 sec^{-1}) is measurable
   - This leads to **energy transfer**, which provides a way to experimentally measure the distance between **A** and **B**

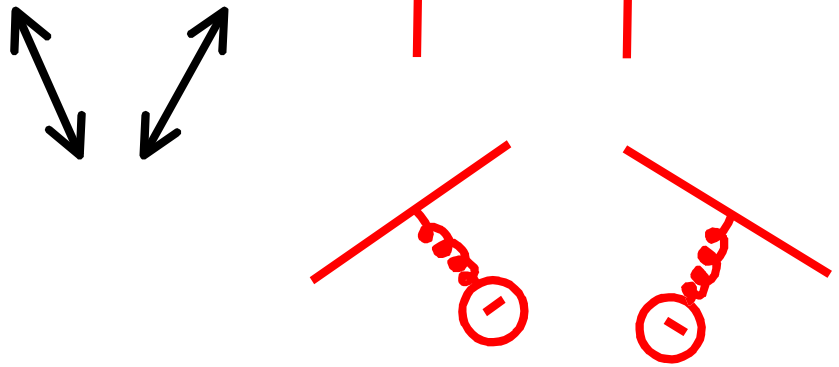
Three general situations to consider geometrically

I Card stack geometry

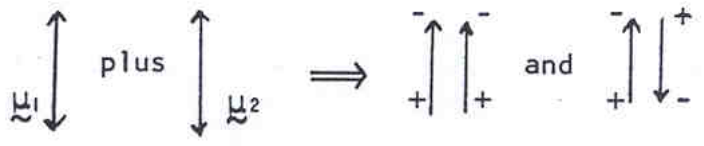
II Head to tail geometry



III Herringbone geometry

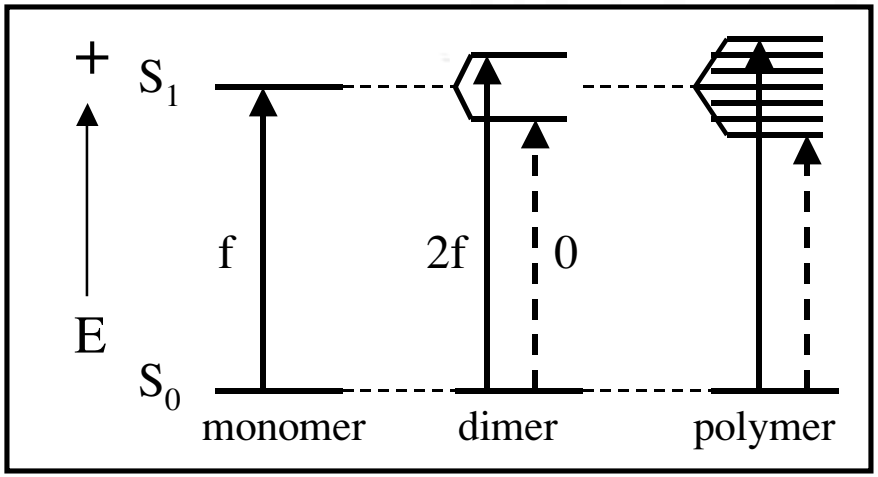


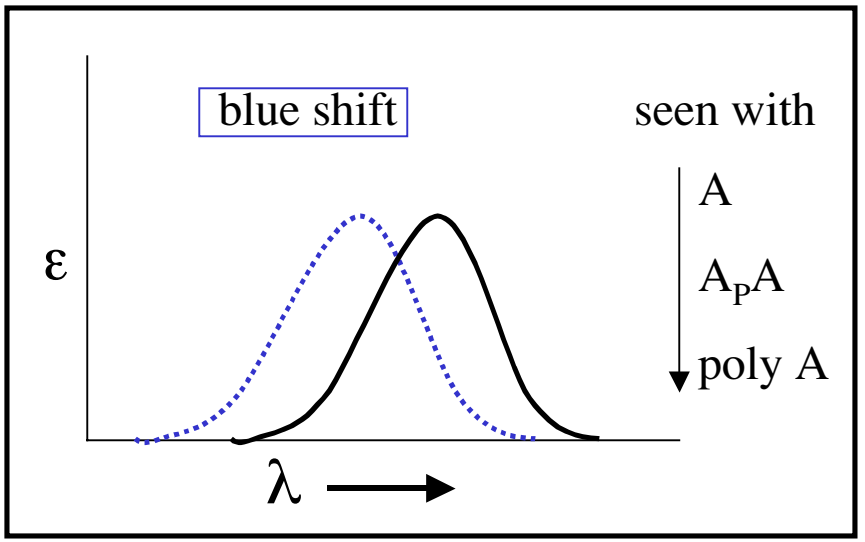
Case I: Card stack geometry



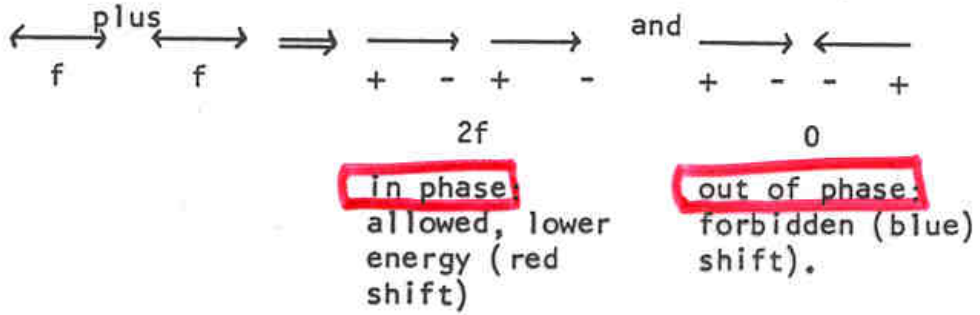
in phase: charge repulsion results in raising the energy of the transition; transition dipoles add to each other thus increasing the oscillator strength.

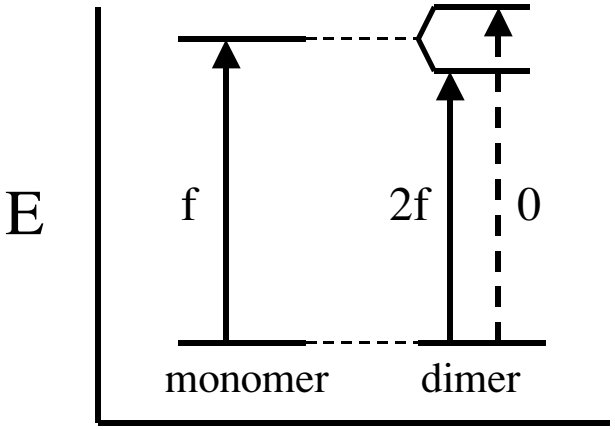
out of phase: lower energy transition due to opposite charge orientation; but transition dipoles cancel each other, so this transition is forbidden and is never seen.

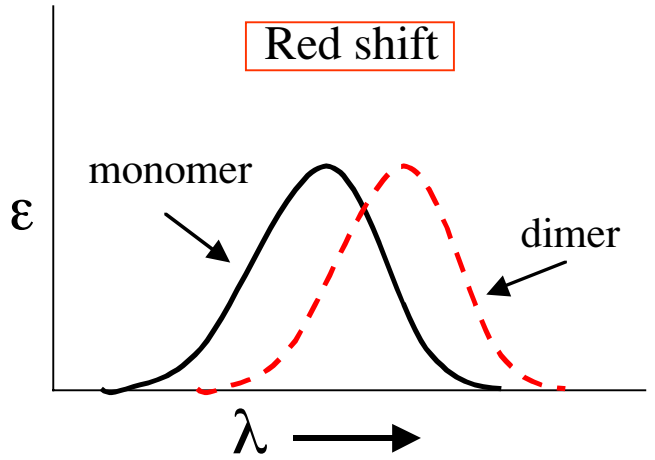




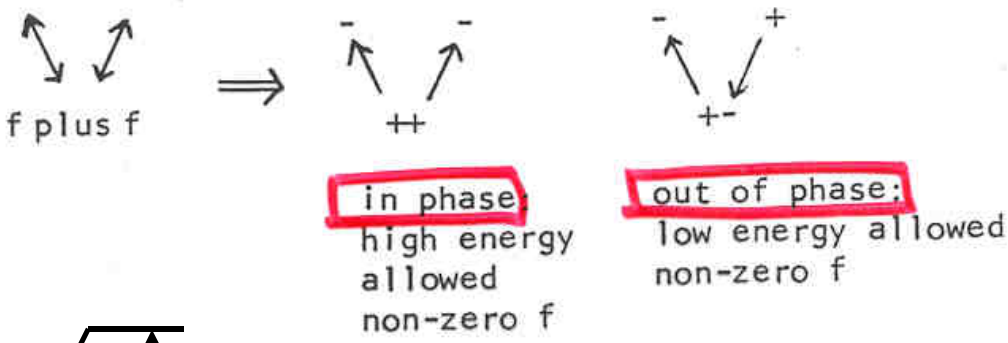
Case 2: Head-to-tail geometry

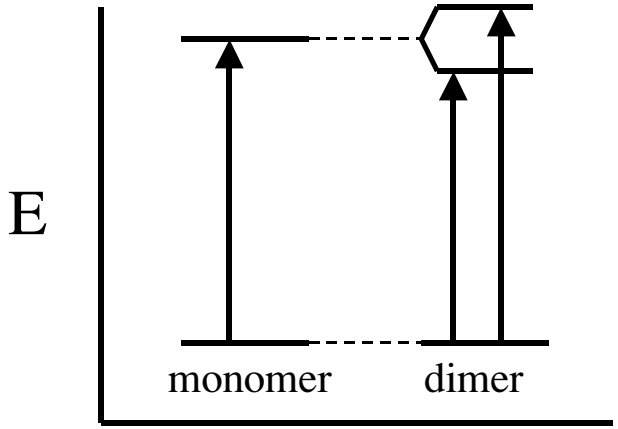


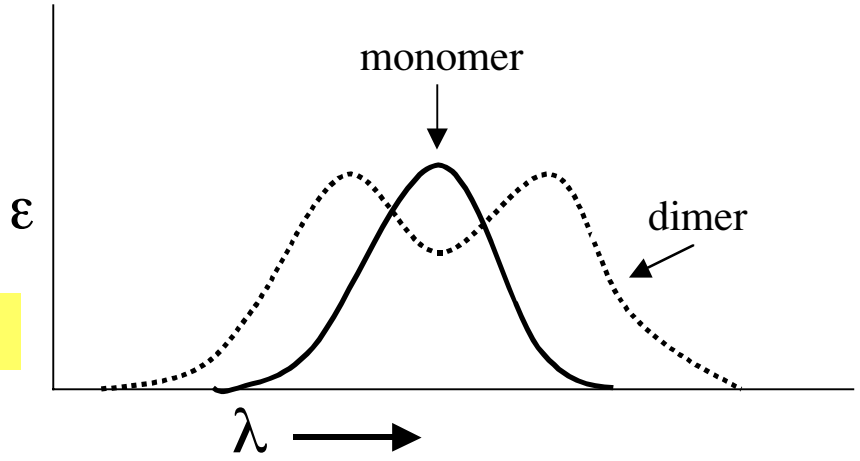




Case 3: Herringbone geometry







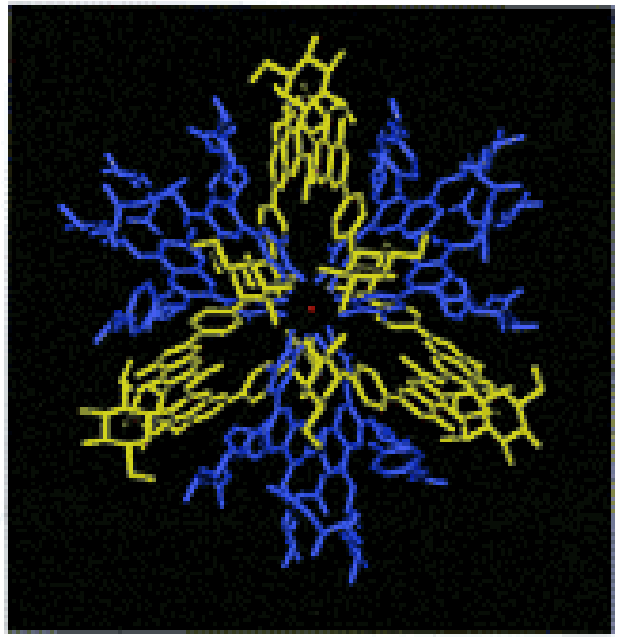
This is called **Davydov** splitting

An example of shifts in the absorption spectrum due to molecular complex formation

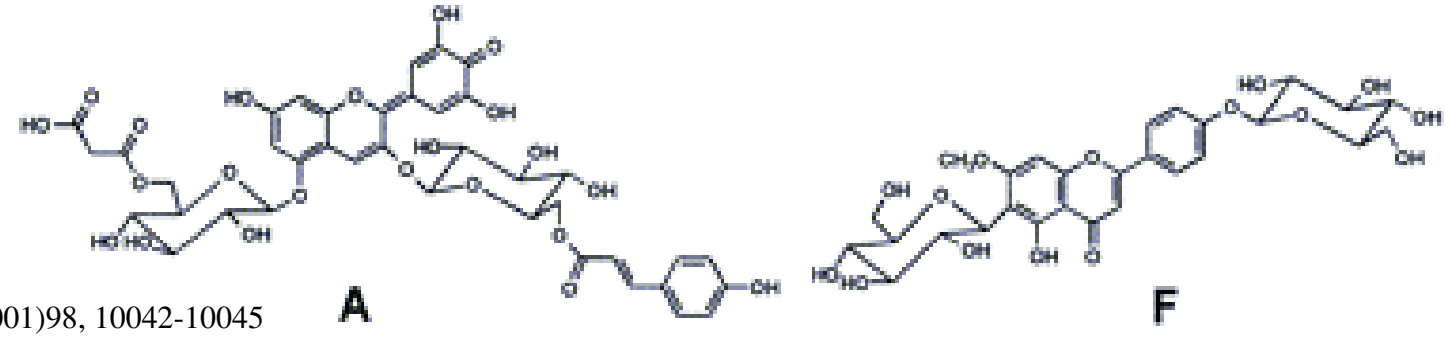
anthocyanin complexes responsible for the colors of flowers and fruits cover the entire visible spectrum

Exiton couplings are responsible for many of the colors of flowers and fruits
Due to non-covalent hydrogen bonded complexes of anthocyanins

a)

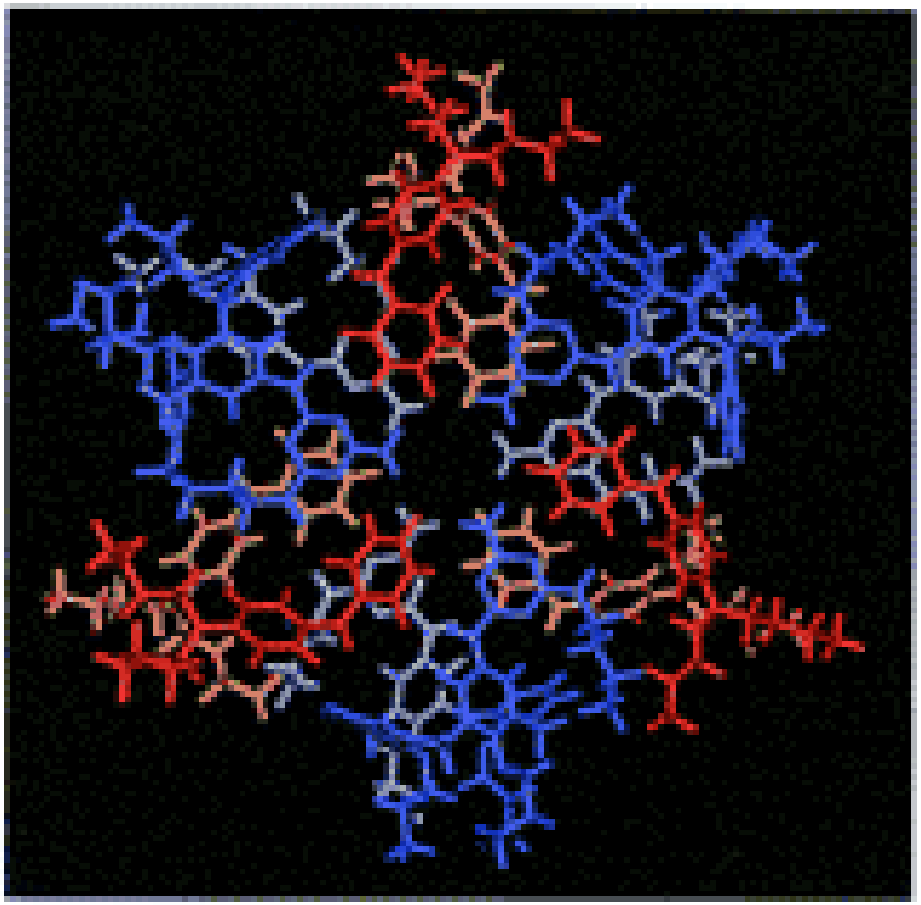


Commelinin is composed of six anthocyanin (A/blue) and 6 flavocommelins (yellow, F)



PNAS (2001)98, 10042-10045

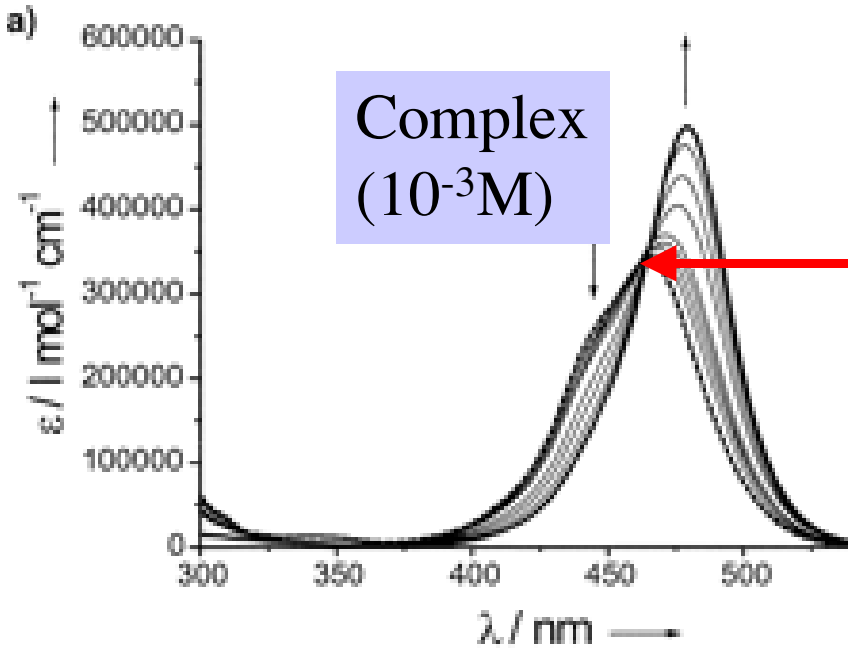
Model of the synthetic complex between a dimelamine and barbituate



PNAS (2001)98, 10042-10045

Dilution causes the complex to dissociate and results in color change: wavelength change of the absorption spectrum

No Complex (10^{-6}M)



Complex (10^{-3}M)

NOTE: ISOSBESTIC POINT

all the spectra pass through a common point

complex behaves like card stack geometry: blue shift upon complex formation

PNAS (2001)98, 10042-10045

An isobestic point implies that there are only two species in equilibrium being observed

Main point: if there were three or more species,the chances they all have the same absorbance at any given wavelength is very small.

$$[A] \rightleftharpoons [B]$$
$$C_{tot} = [A] + [B]$$

Absorbance = $[A]\varepsilon_A l + [B]\varepsilon_B l$

Absorbance = $(frac_A C_{tot})\ \varepsilon_A l + (frac_B C_{tot})\ \varepsilon_B l$

when $\varepsilon_A = \varepsilon_B$ (at the isobestic point)

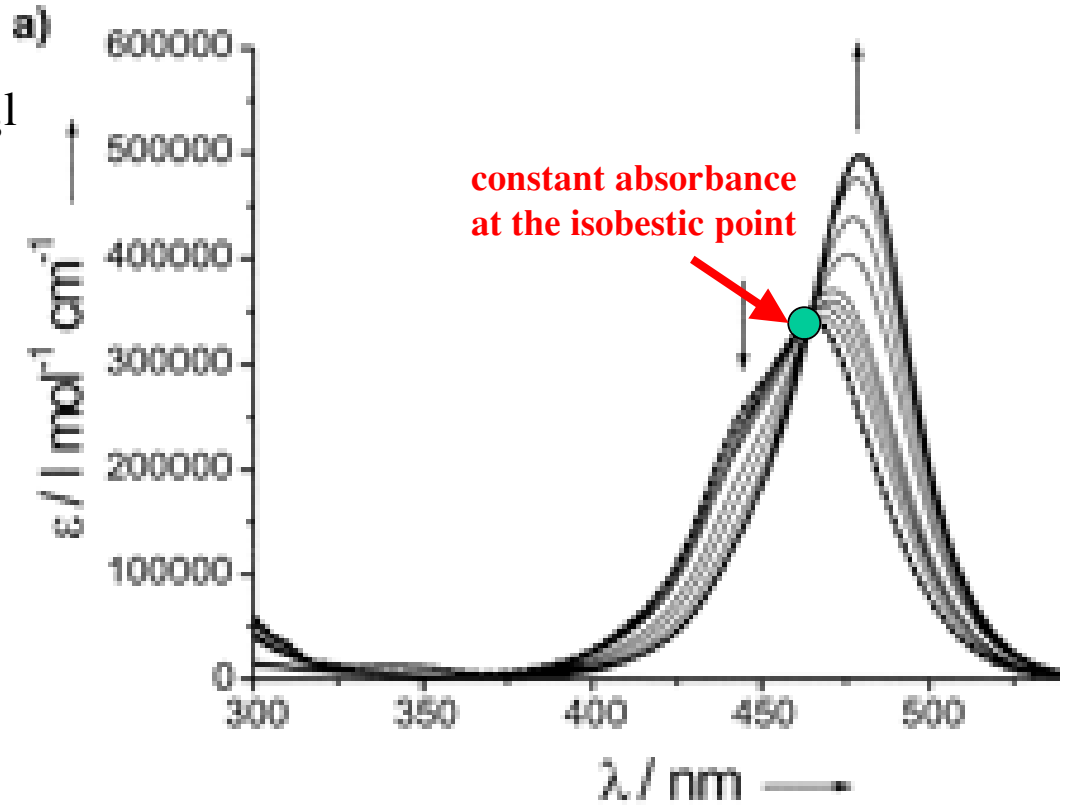
then

Absorbance = $\varepsilon_{iso} C_{tot}(frac_A + frac_B) l$

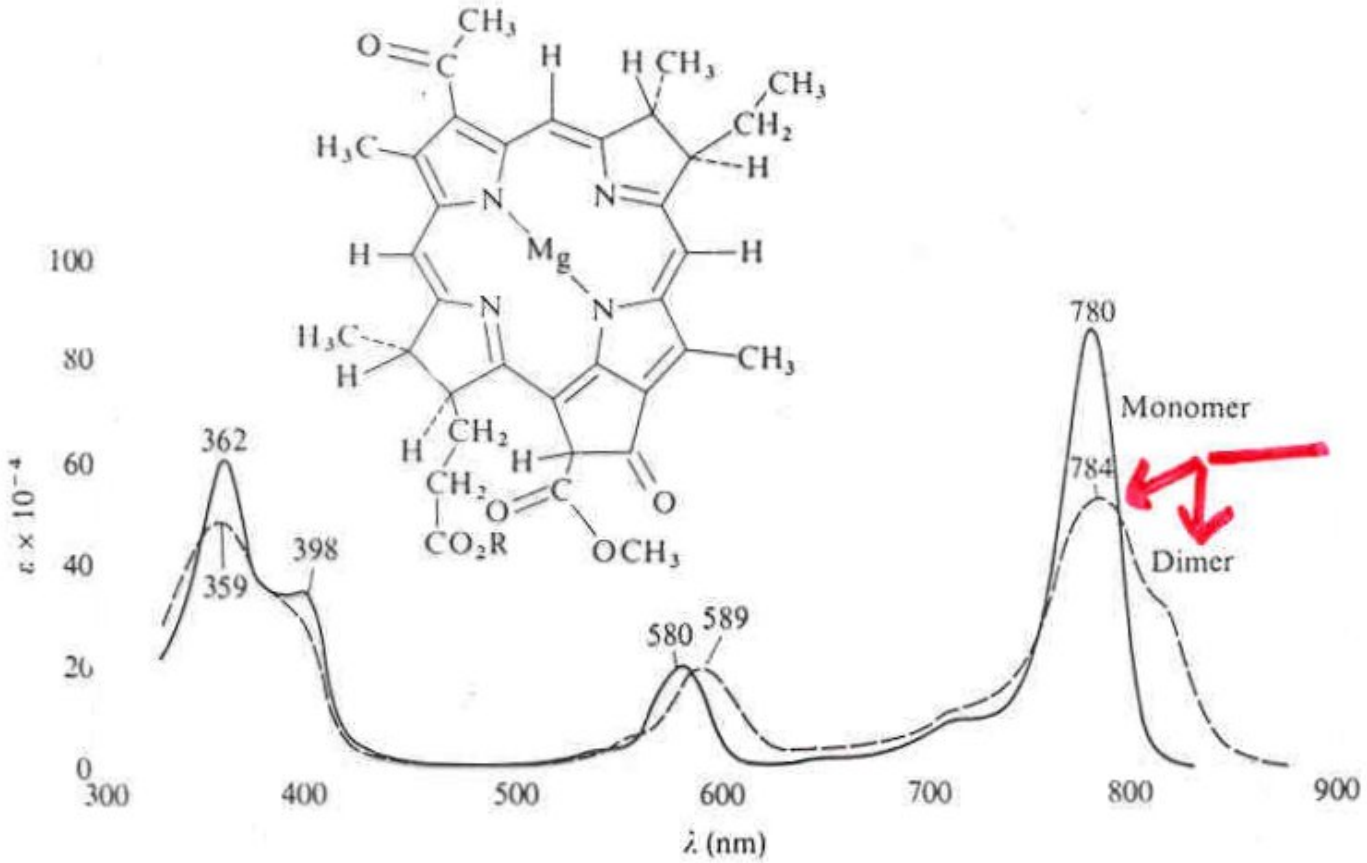
but $(frac_A + frac_B) = 1$

so Absorbance = $\varepsilon_{iso} C_{tot} l$

does not change as the ratio of A and B change



Bacteriochlorophyll Dimer - Davydov splitting observed in long wavelength band



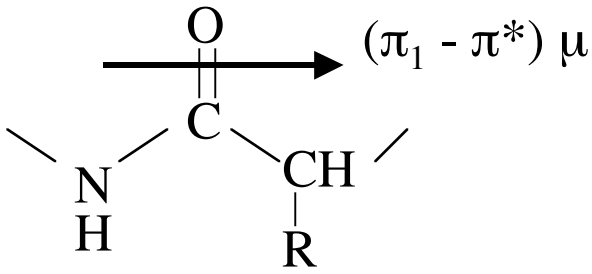
Monomer and dimer spectra for solutions of bacteriophyll. A pronounced splitting of the longest-wavelength band in the dimer is visible.

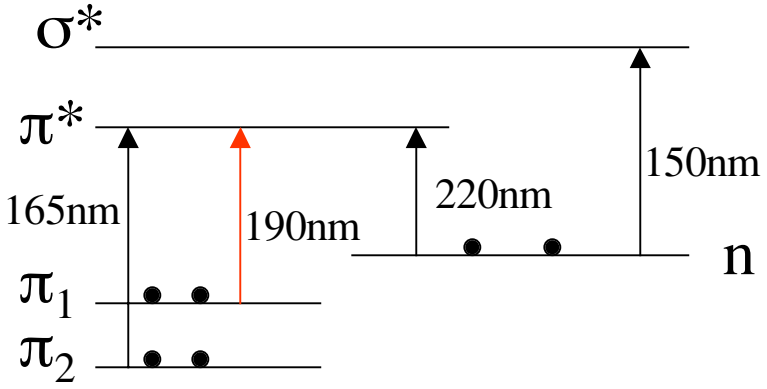
JACS 88:2681 (1996)

A prominent example of Davydov Splitting:

Far UV spectrum of protein α-helix

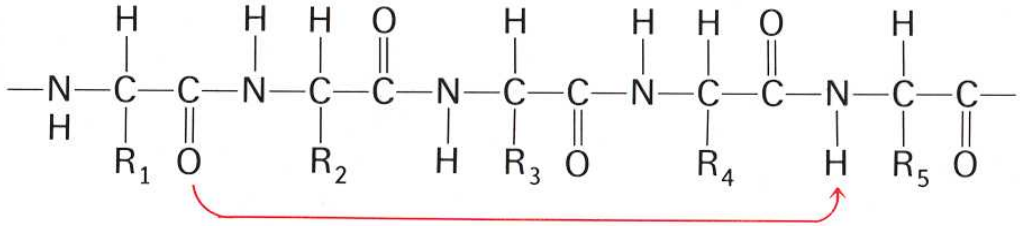
π - π* bands of amide groups interact with each other in the helix

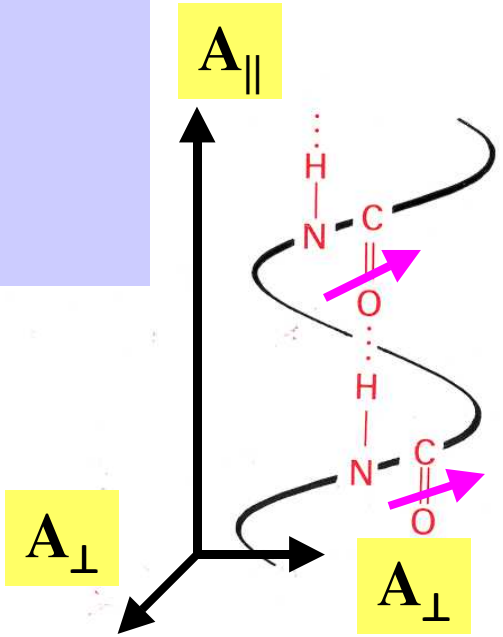




3-dimensional version of herringbone geometry in α-helix

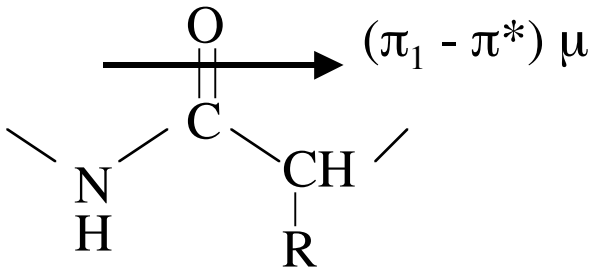
Interaction of the carbonyls along the helix results in splitting into 3 absorption bands: one parallel to the helix axis and two bands perpendicular to the helix axis

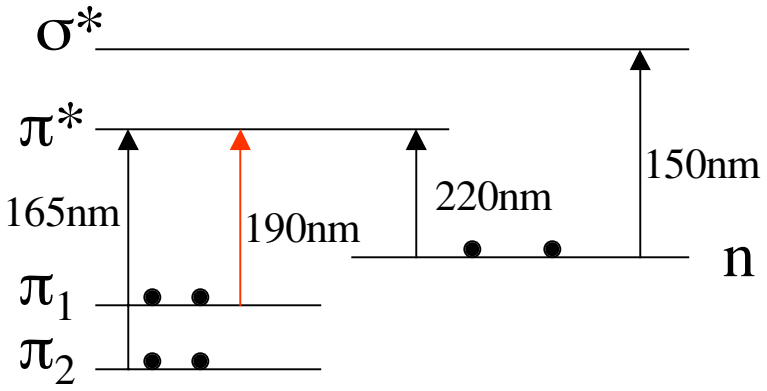


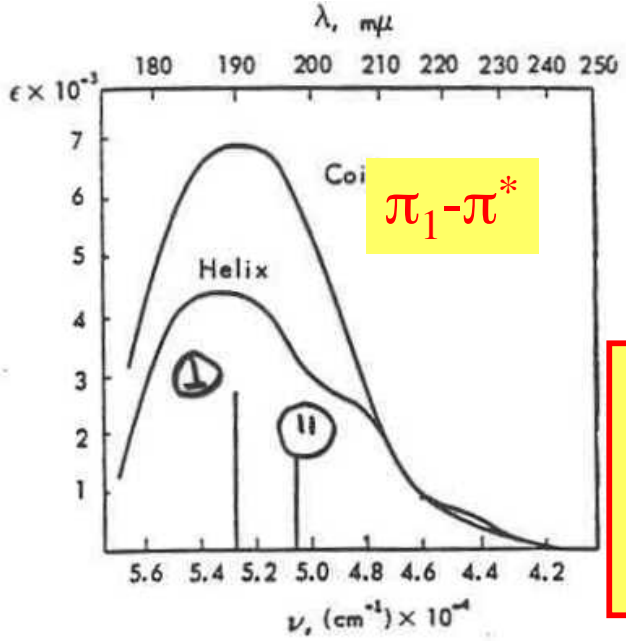


A prominent example of Davydov Splitting:

Far UV spectrum of protein α-helix







π_1-π^*

Results in Davydov splitting

One absorption band (at low energy):

μ parallel to helix

Two degenerate absorption bands (higher energy):

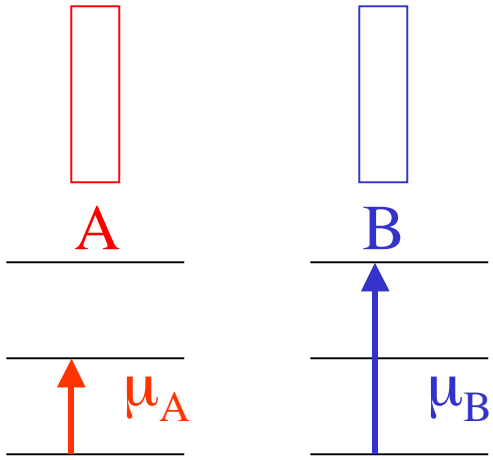
μ ⊥ to helix axis

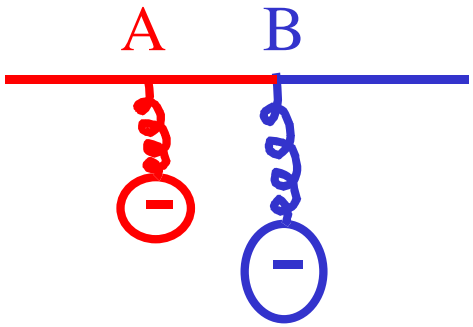
Note that there are two effects upon forming α-helix:

Hypochromisim and **exciton splitting** observed upon helix formation of polyglutamic acid. Lines indicate calculated band positions

Hyperchromism

Due to interactions of neighboring molecules where a transition in molecule A interacts with different transitions (higher or lower energy) of molecule B



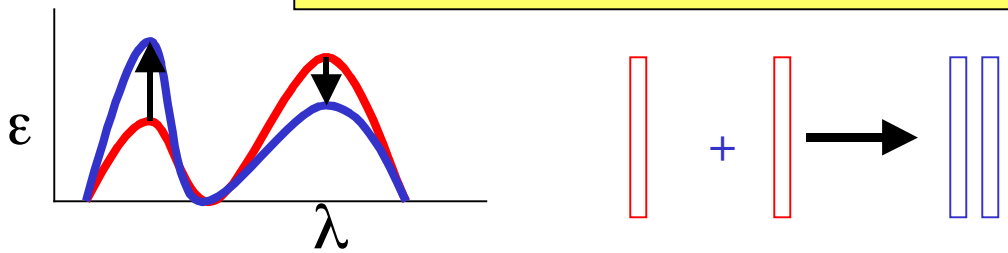


Mixing of wavefunctions of higher excited states results in **intensity borrowing**

-Oscillator strengths of different transitions can increase or decrease

(hyperchromism) (hypochromism)

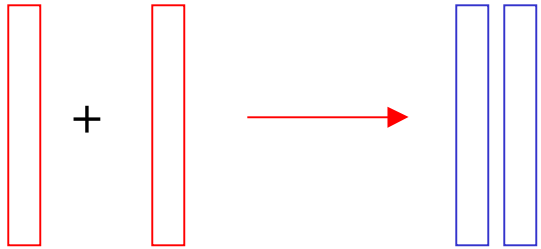
Kuhn-Thomas Sum Rule:



Area of total Absorption spectrum is constant.

Molecular interactions can increase or decrease particular bands - but the net area under the spectrum is not changed by the molecular interactions.

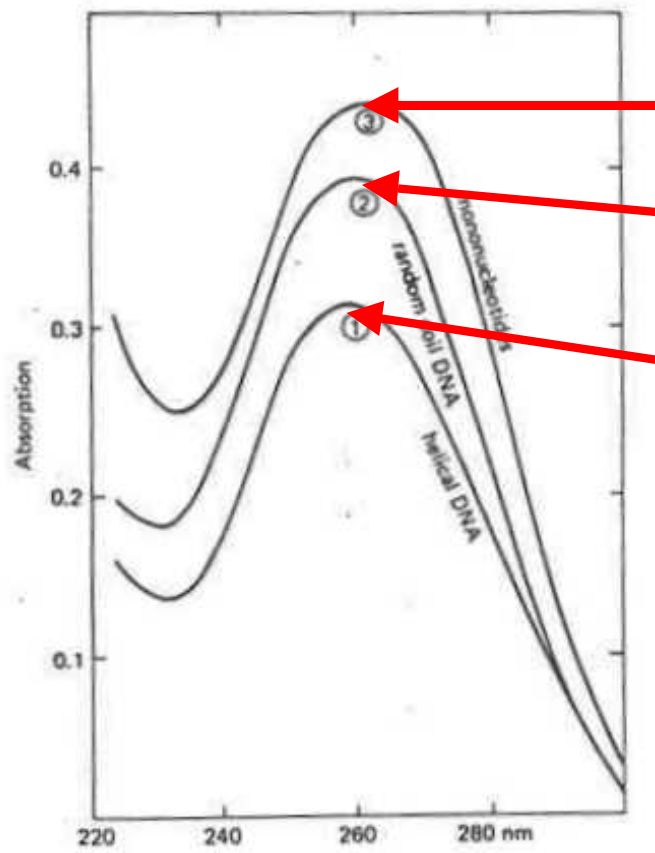
e.g. **Hypochromism** in nucleic acids:
lower absorption in one region of the spectrum means there must be an increased absorption elsewhere.



Stacking results in decrease in the intensity of the UV band, but an increase in a far UV band.

An Example of Hypochromisim

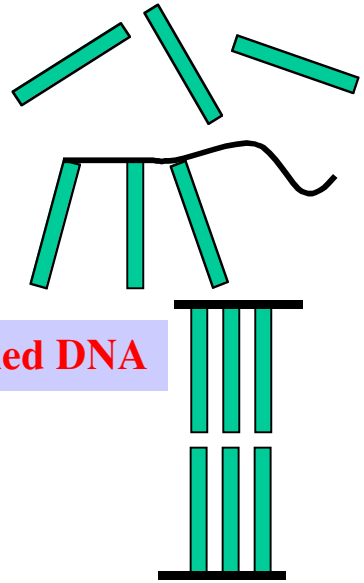
Lower absorbance at 260 nm due to stacking in DNA and RNA



Mononucleotides

Single-strand unstacked DNA

Double-strand stacked DNA



Monitoring "melting" of double-strand DNA (from *E. coli*) by the absorbance change at 260 nm an example of hypochromism: lower ε_{260} for double-strand vs single-strand DNA