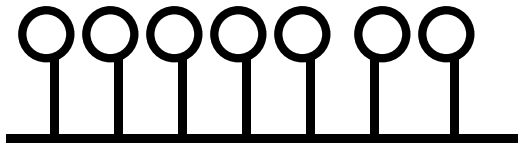
Chromophore - Chromophore Interactions

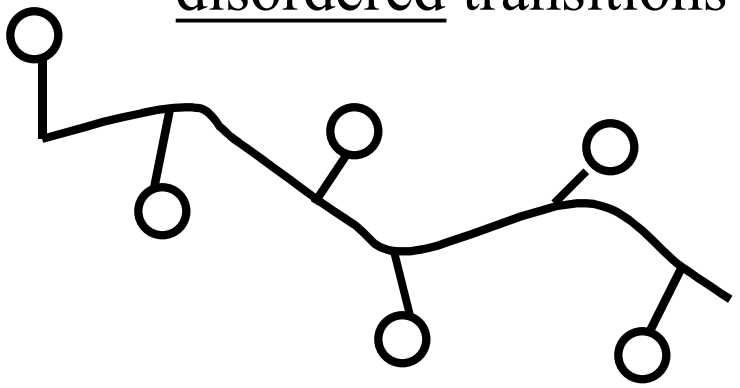
1 Important for determining optical properties of nucleic acids and proteins

2 **Primarily short range interactions**

- **stacked bases in DNA, RNA**
- **Amide groups in α-helix**
- **Chlorophylls in light harvesting photosynthetic systems**

3 Useful to monitor ordered ⇌ 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

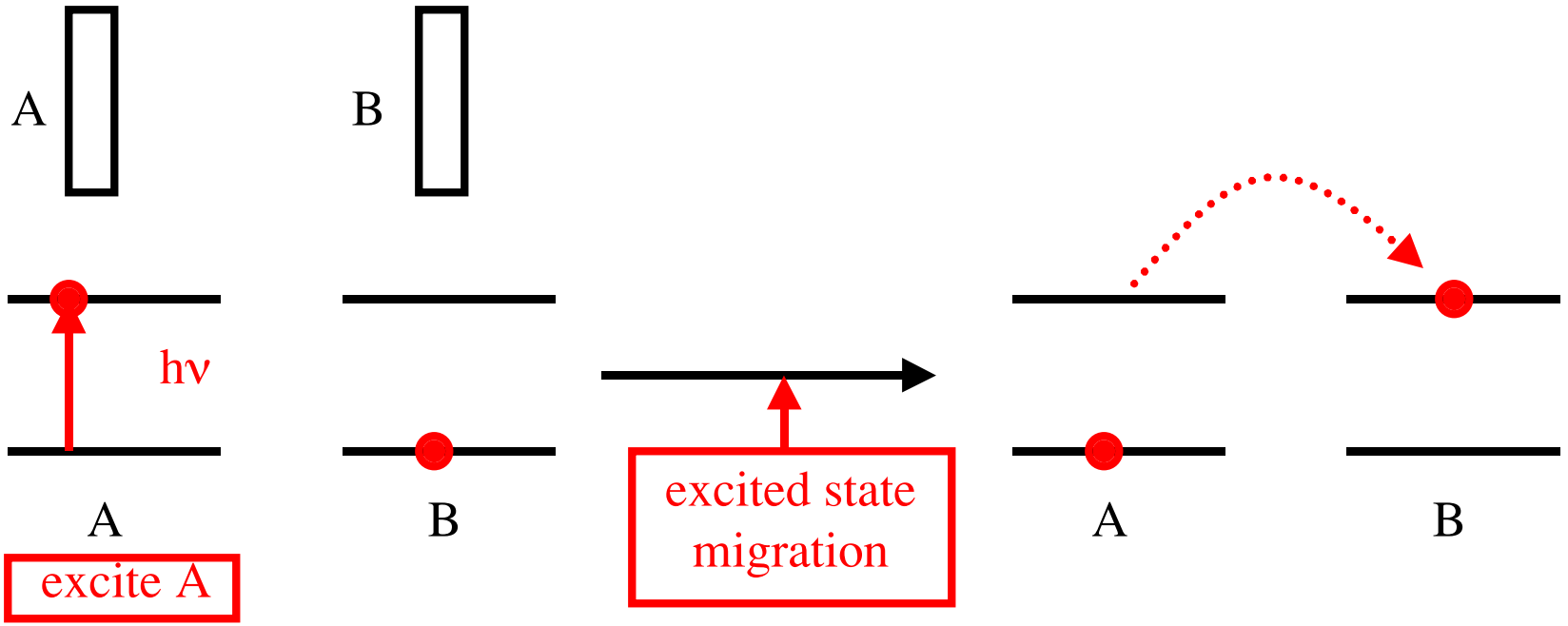
- hyperchromism
  (borrowed intensity)

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

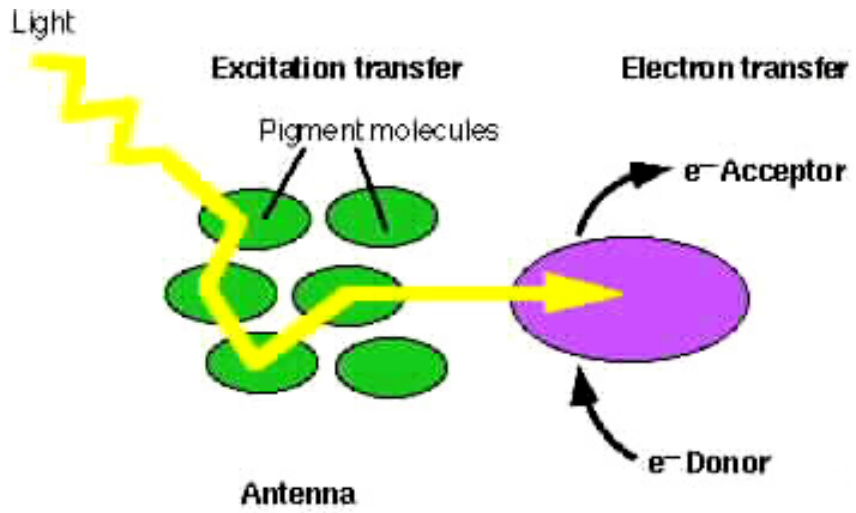
Excitons and Energy Transfer



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.

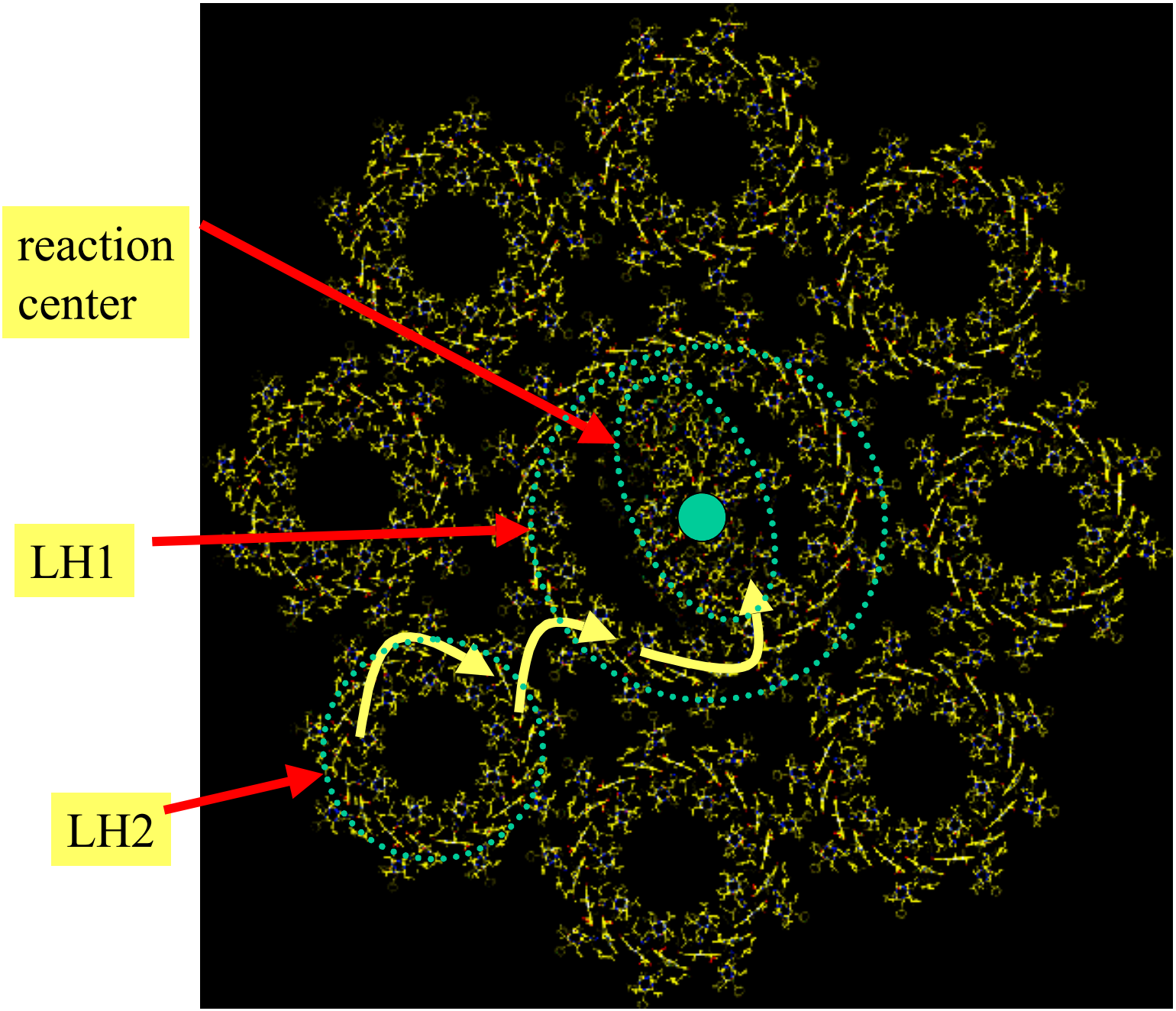
2 Slow hopping (10^8 sec^{-1}) is measurable
   - This leads to **energy transfer**, which provides a way to experimentally measure the distance between **A** and **B**

Excitons and Energy Migration in Photosynthesis

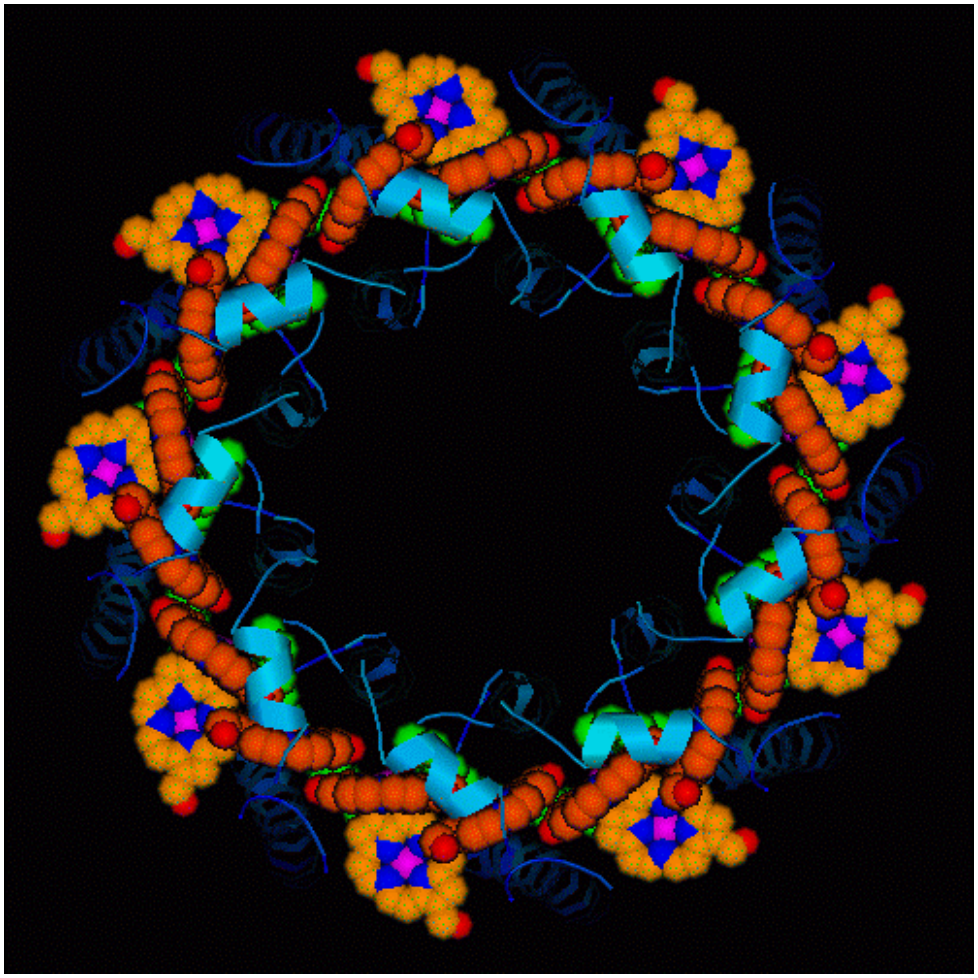


In purple photosynthetic bacteria (R. sphaeroides) there are
two light harvesting complexes:
LH2→LH1 →Reaction Center

http://photoscience.la.asu.edu/photosyn/education/antenna.html


reaction center
LH1
LH2

LH2 Complex:

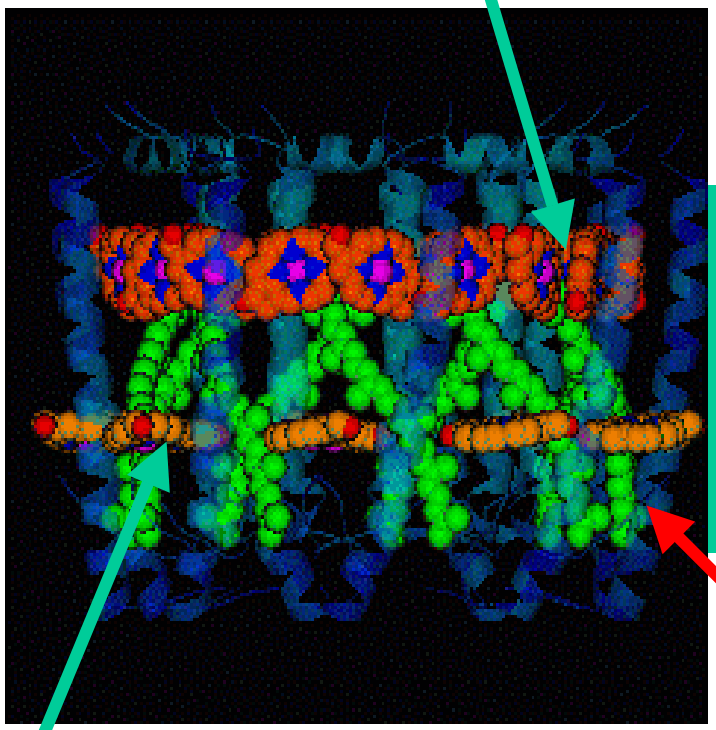


9 copies of protomers consisting of
1 α subunit
1 β subunit
3 bacteriochlorophyll
1 rodopin glucoside
1β octyl glucoside

The Structure of LH2

ring of 9 dimeric overlapping chlorophyl a's at the top of the molecule

side view



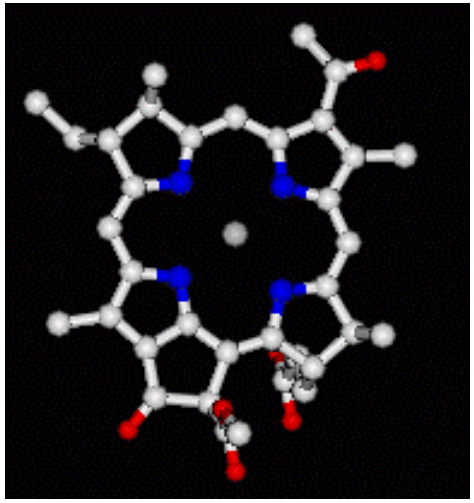
membrane

carotenoids

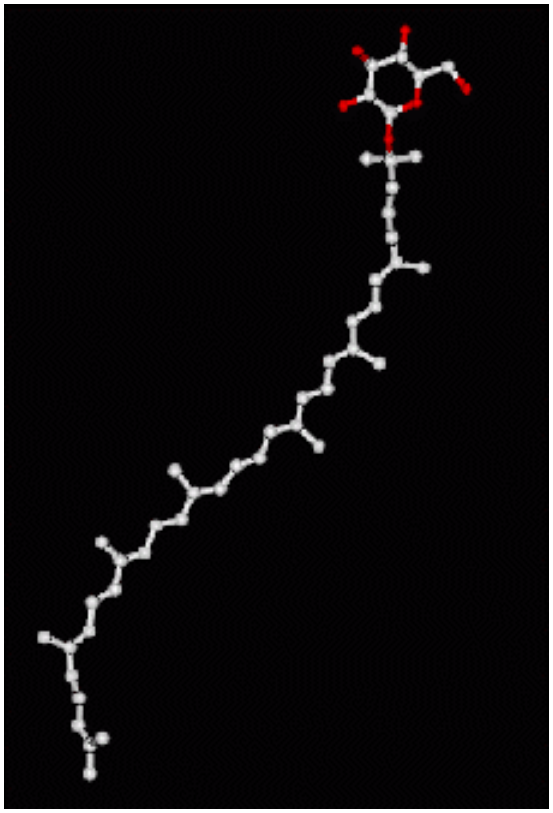
ring of 9 chlorophyl a's in middle of membrane

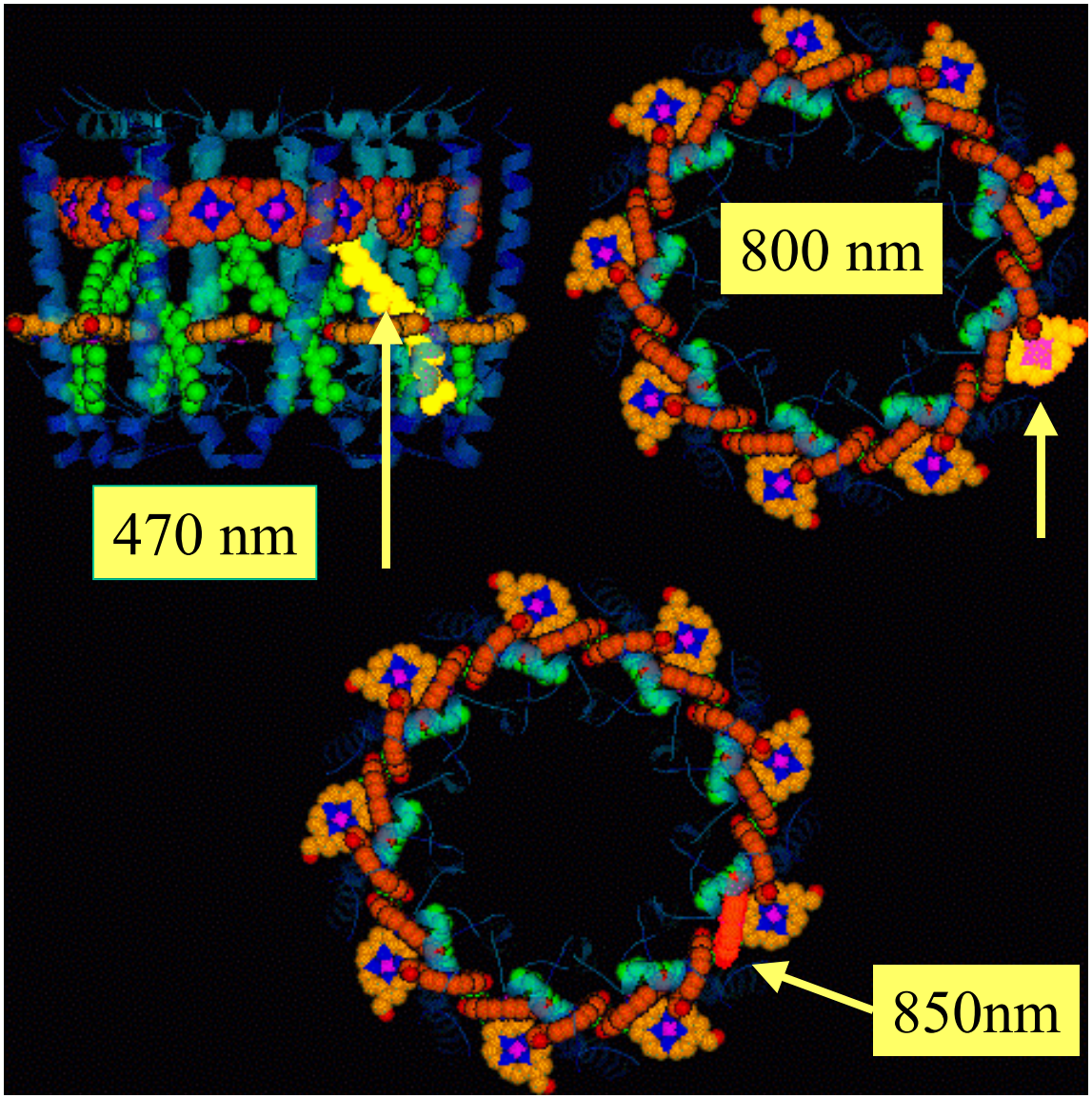
http://www.chem.gla.ac.uk/protein/LH2/lh2struc.html

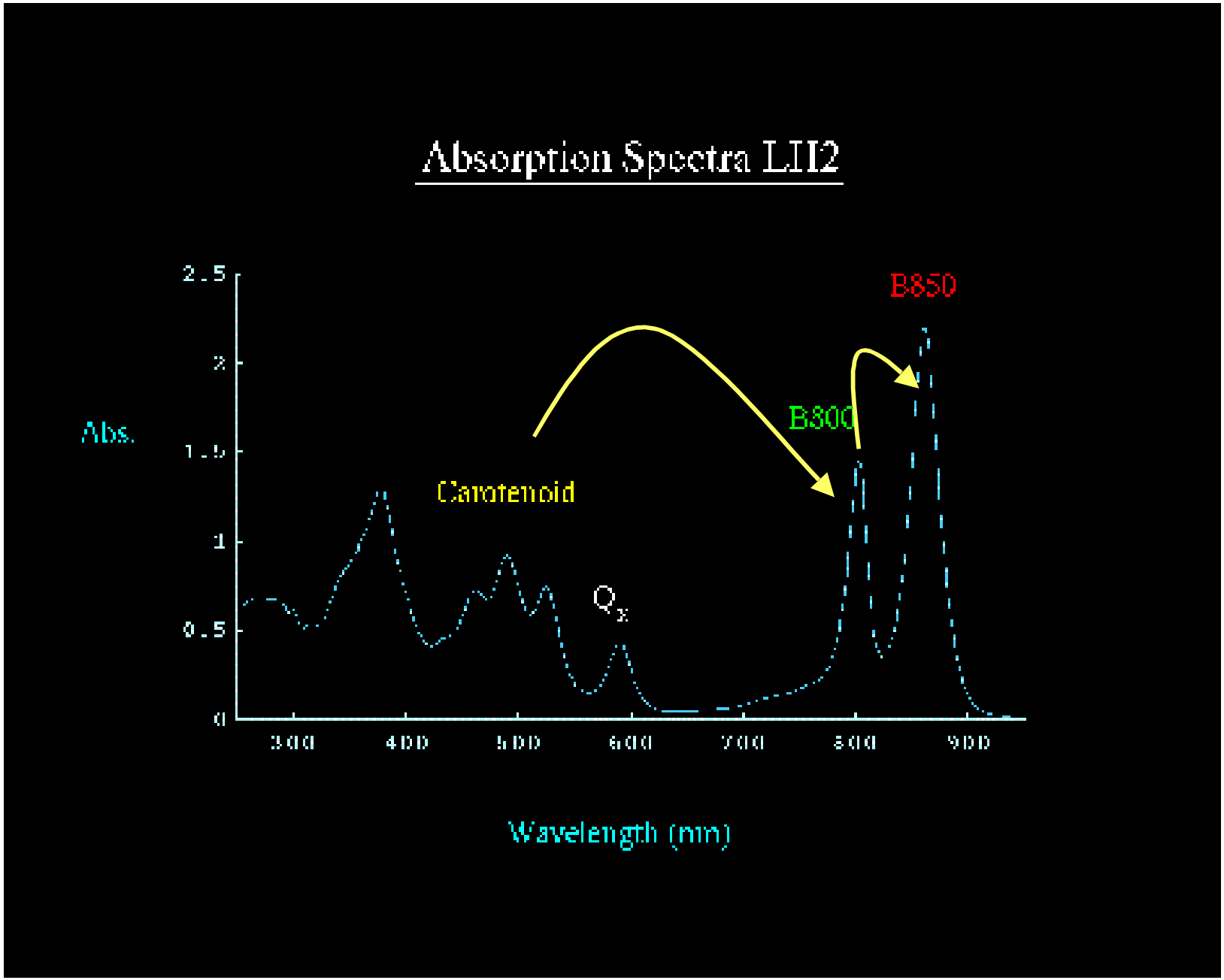
bacteriochlorophyll a



rhodopin glucoside

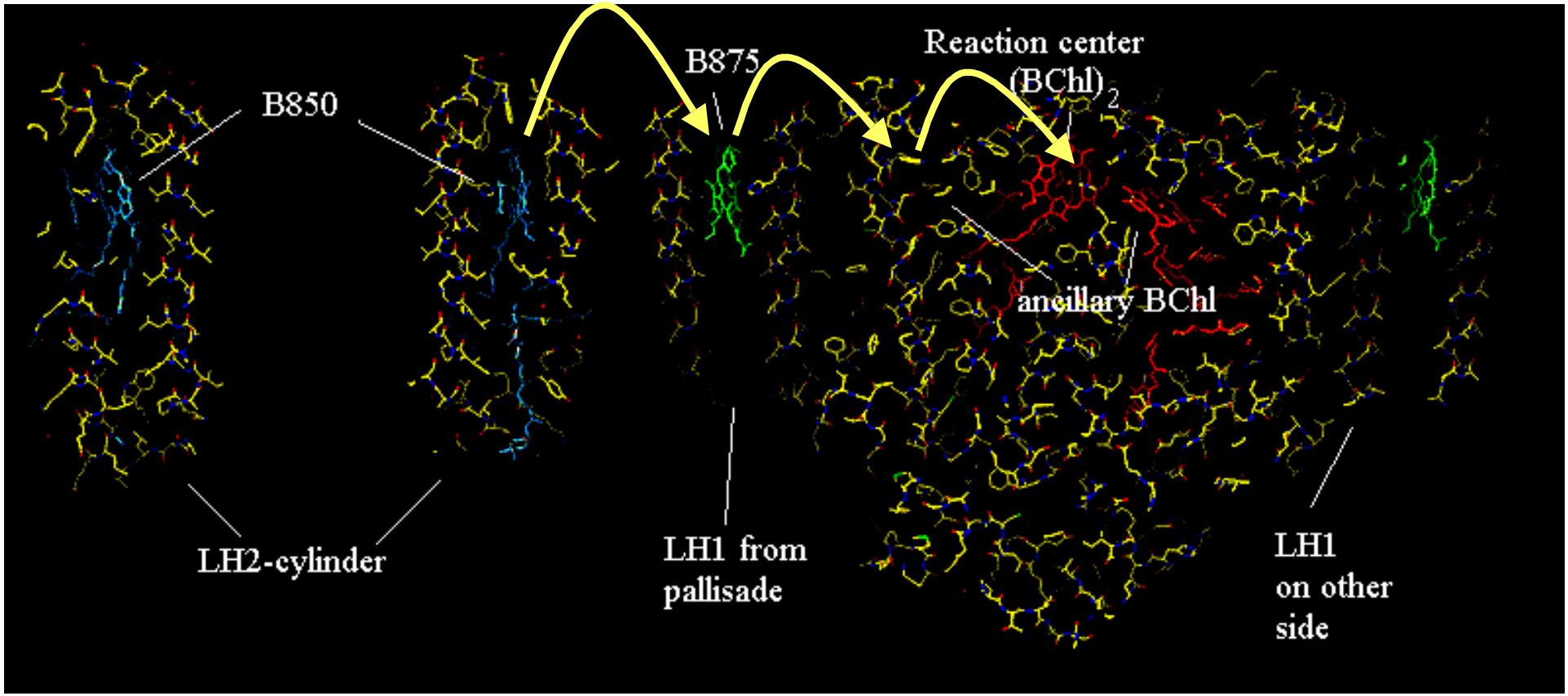



800 nm
470 nm
850nm

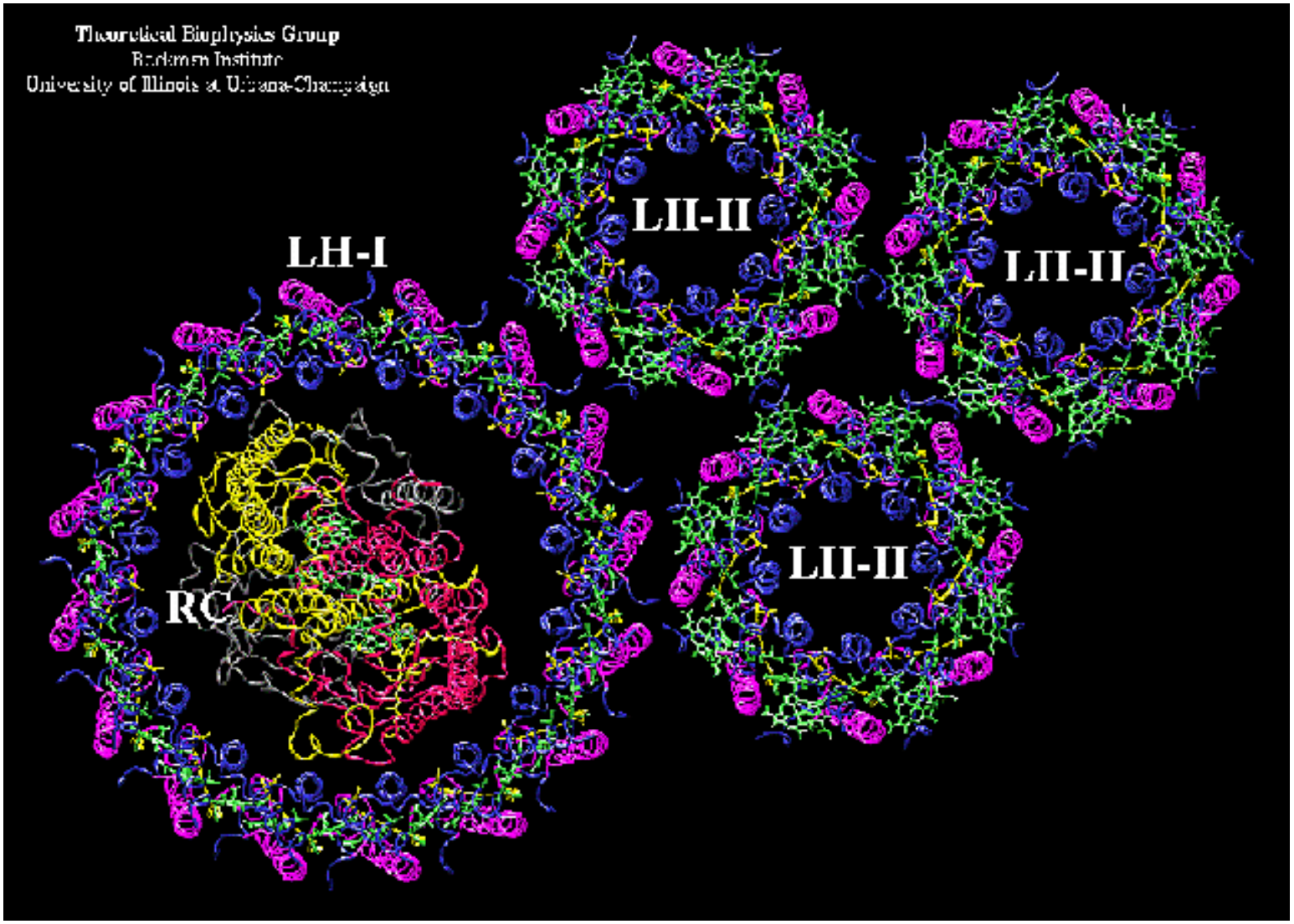


http://www.chem.gla.ac.uk/protein/LH2/etrans.html

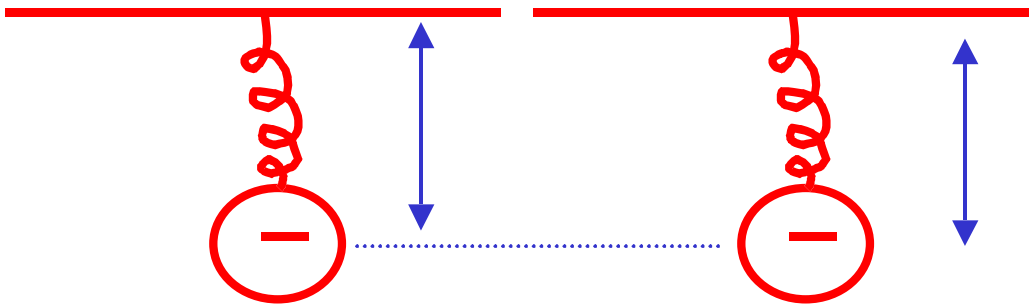
Section through the LH2-LH1-Reaction Center
Exciton transfers in picoseconds after light absorption



http://www.life.uiuc.edu/crofts/bioph354/lect20.html


Theoretical Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign
LH-I
LII-II
LII-II
LII-II
RC

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

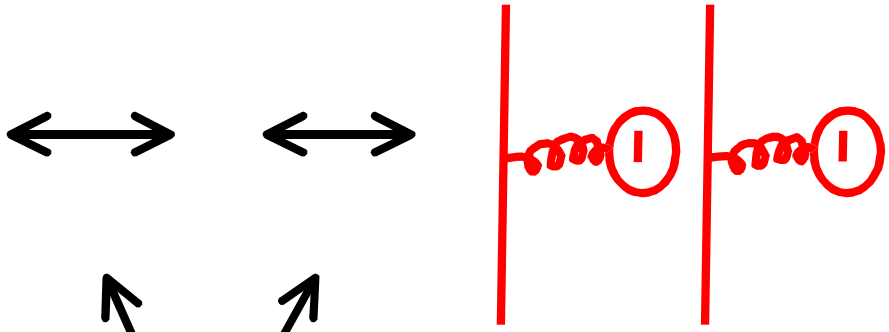
Note: interactions are highly dependent on molecular geometry

- angles and distances.

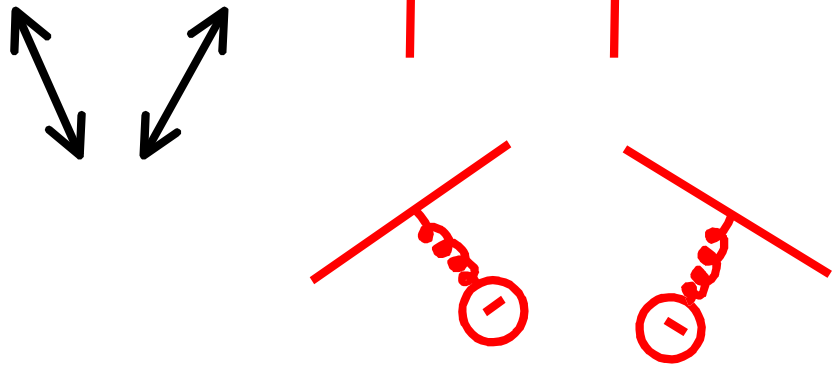
Three general situations to consider geometrically

I Card stack geometry

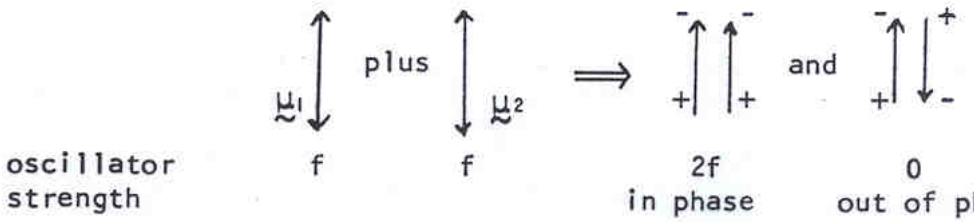
II Head to tail geometry

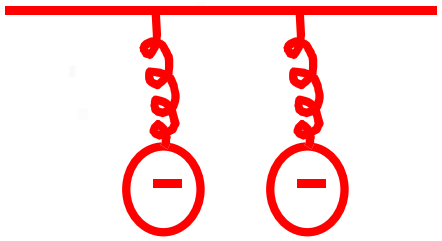


III Herringbone geometry



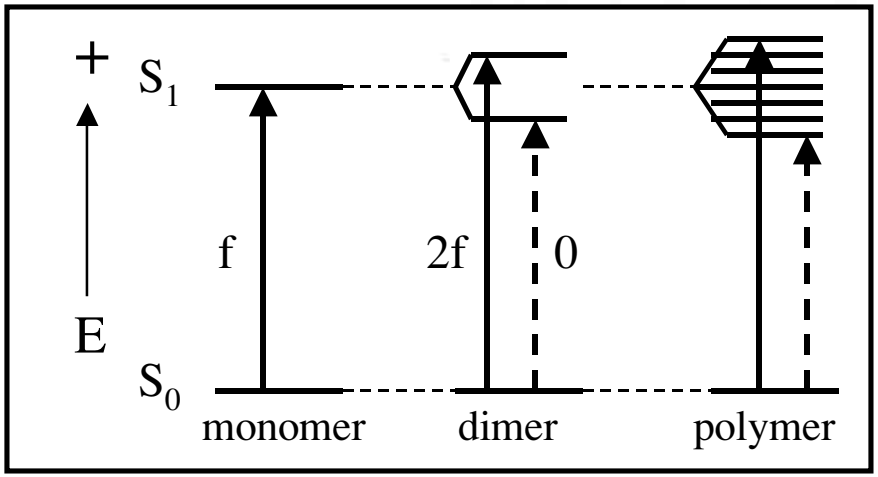
Case I: Card stack geometry

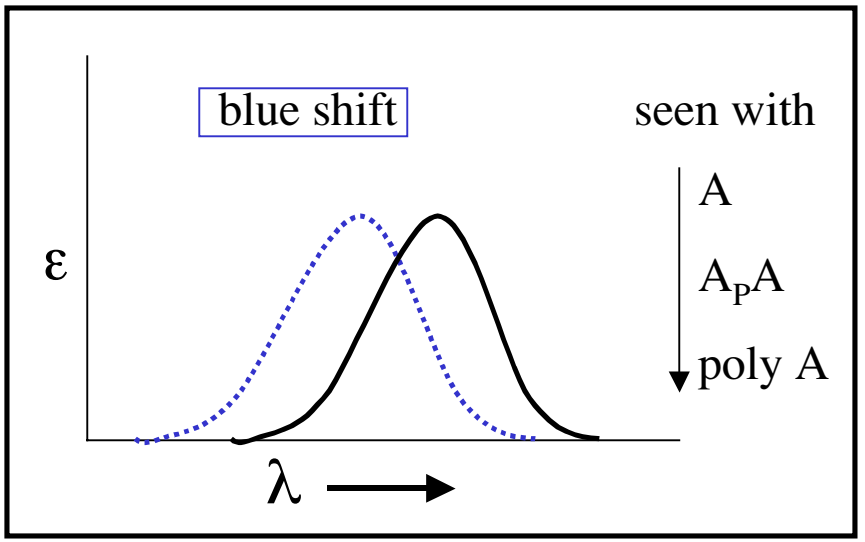




in phase: charge repulsion results in raising the energy of the transition; transition dipoles add to each other thus increasing the oscillator strength.

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.

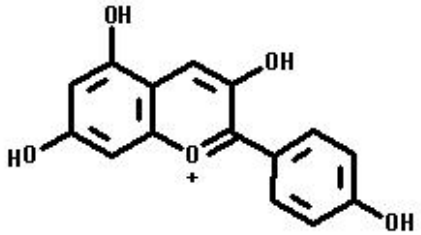




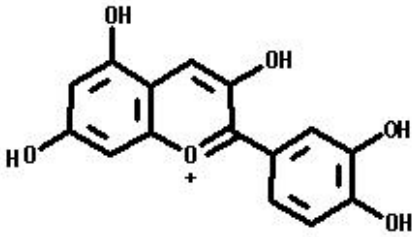
An example of shifts in the absorption spectrum due to molecular complex formation

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

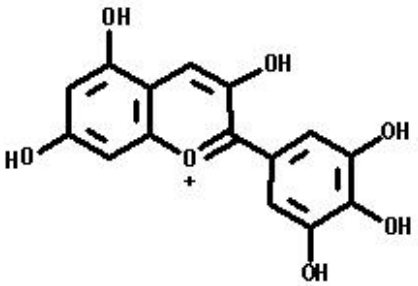
ANTHOCYANIDINS



Pelargonidin



Cyanidin

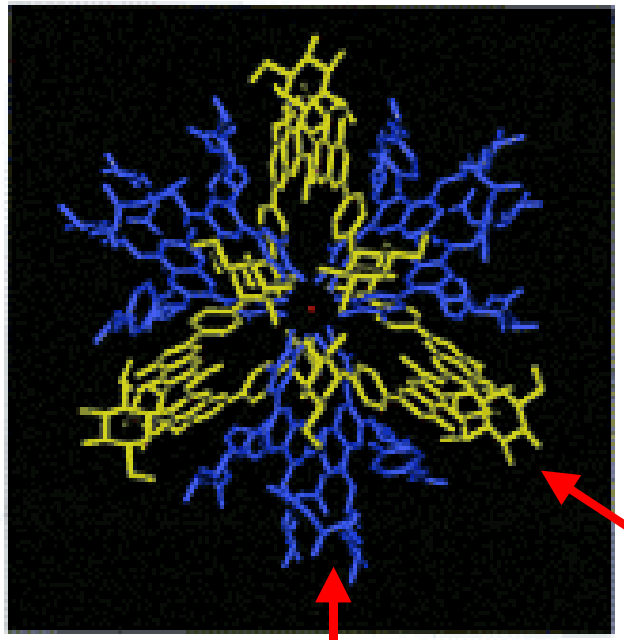


Delphinidin

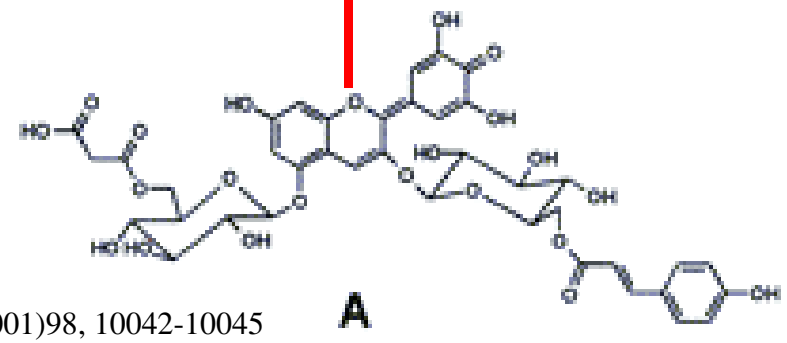
three anthocyanins

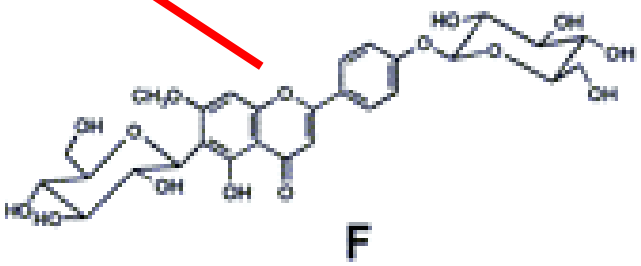
Exciton couplings are responsible for many of the colors of flowers and fruits
Due to non-covalent hydrogen bonded complexes of anthocyanins

a)



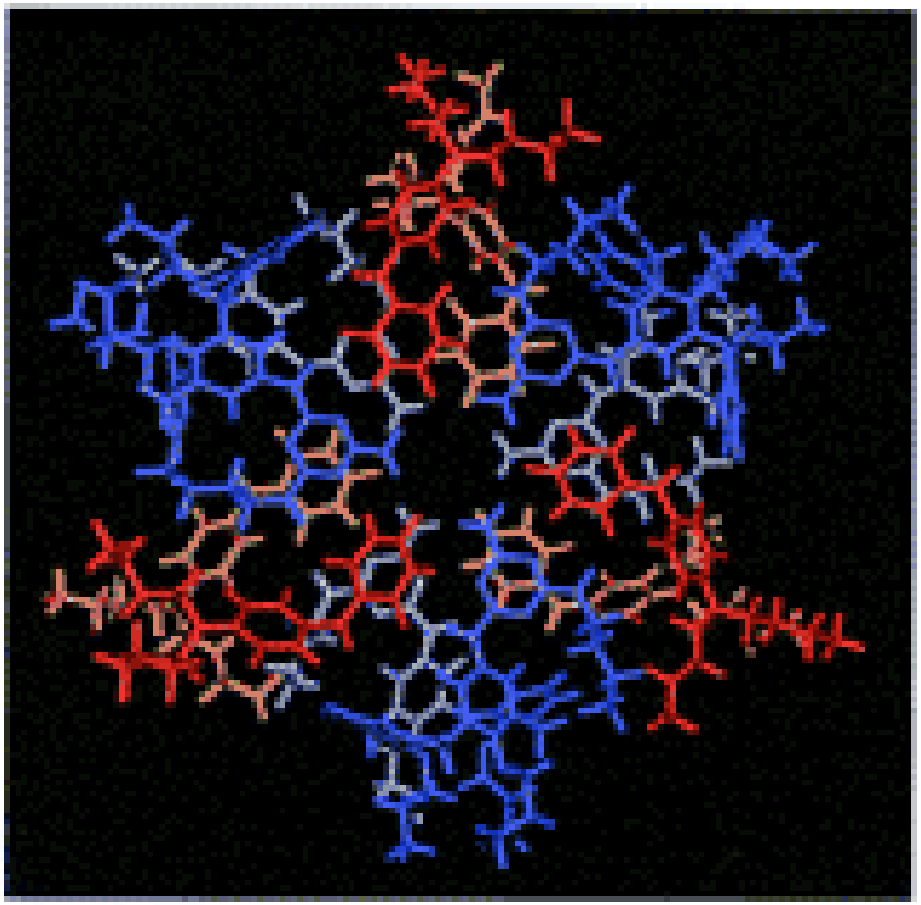
Commelinin is composed of six anthocyanin (A/blue) and 6 flavocommelins (yellow, F)





PNAS (2001)98, 10042-10045

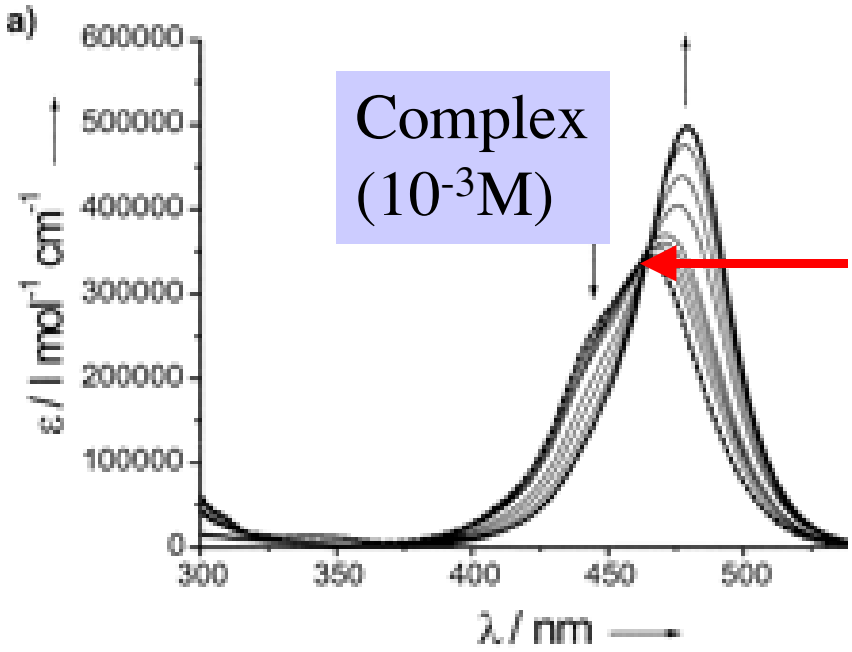
Model of the synthetic complex between a dimelamine and barbituate



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)

all the spectra pass through a common point

This is called an ISOSBESTIC POINT

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

PNAS (2001)98, 10042-10045

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

Key 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 isosbestic point)

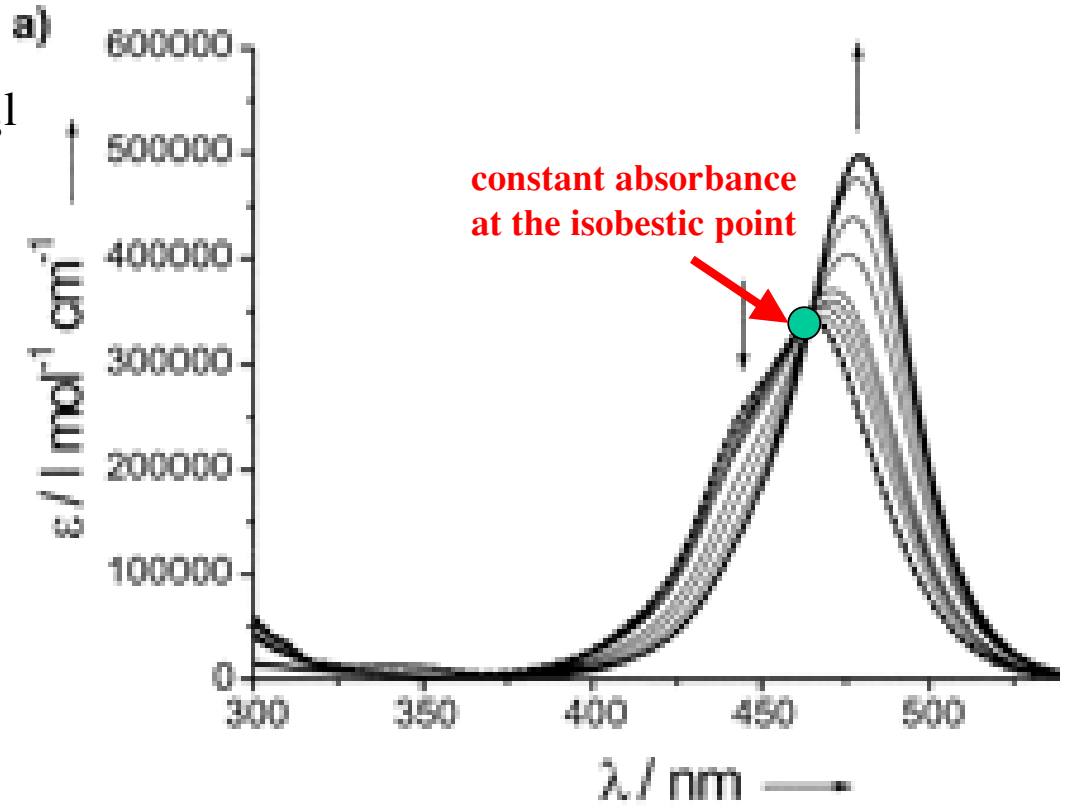
then

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

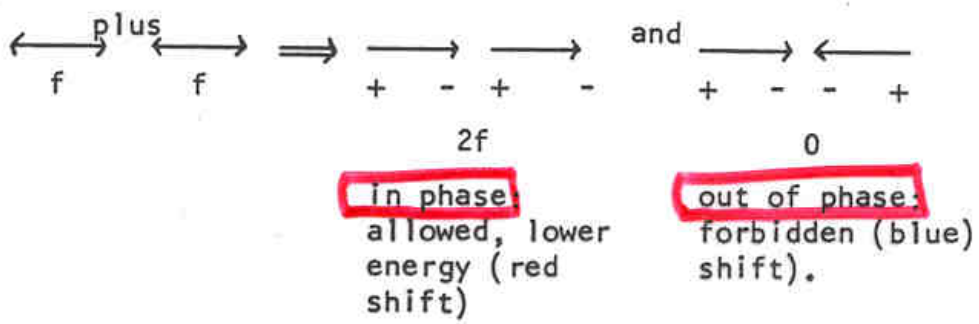
but $(frac_A + frac_B) = 1$

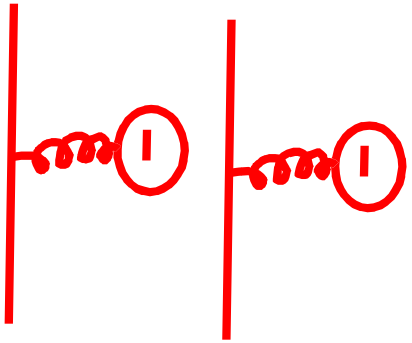
so Absorbance = $\varepsilon_{iso} C_{tot} l$

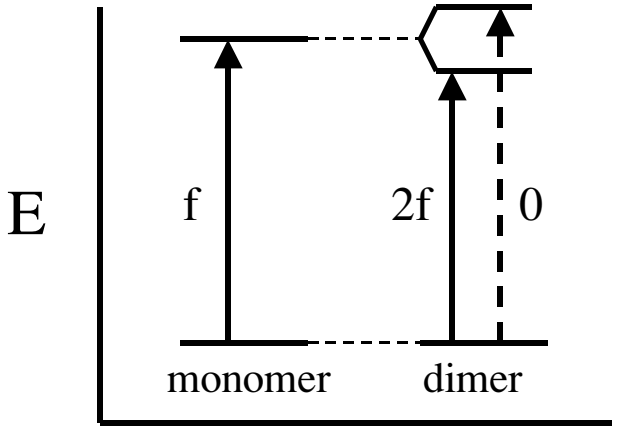
does not change as the ratio of A and B change

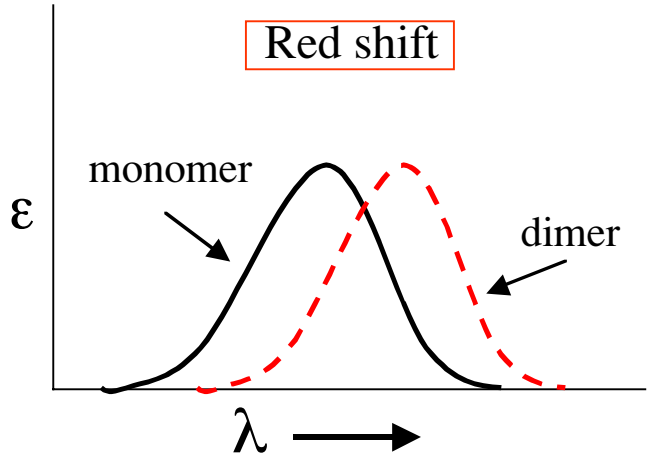


Case 2: Head-to-tail geometry

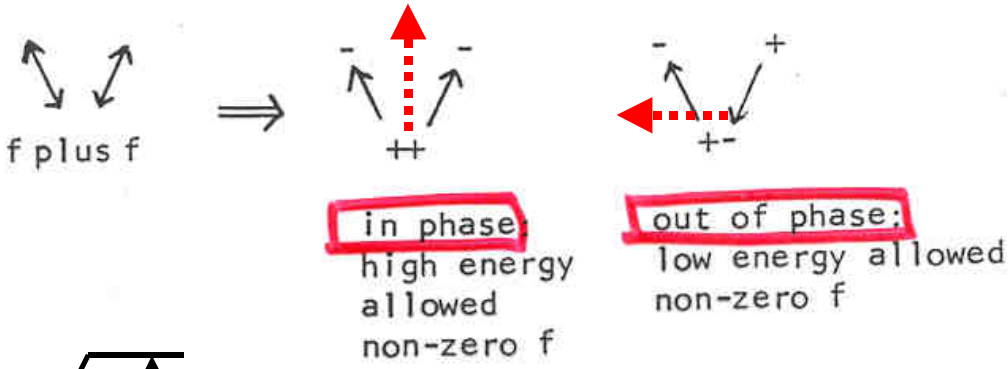


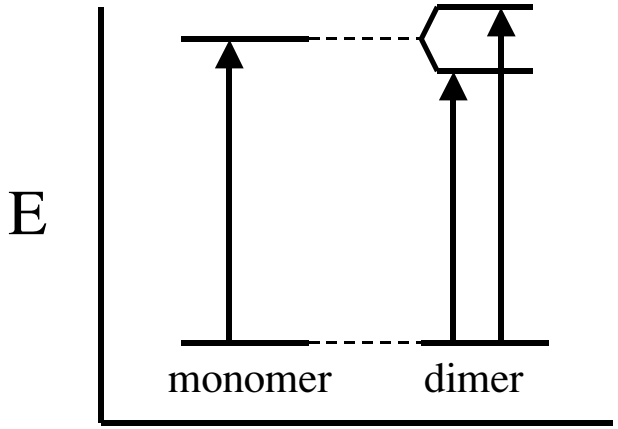


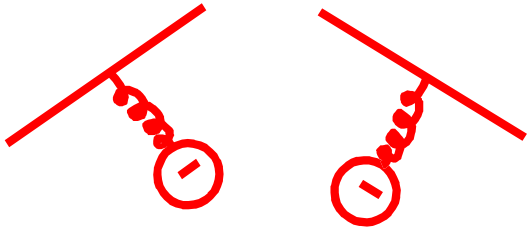


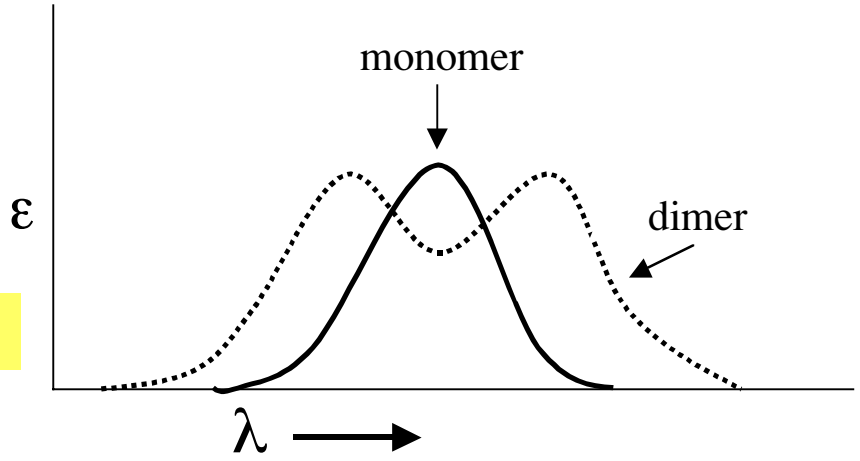


Case 3: Herringbone geometry



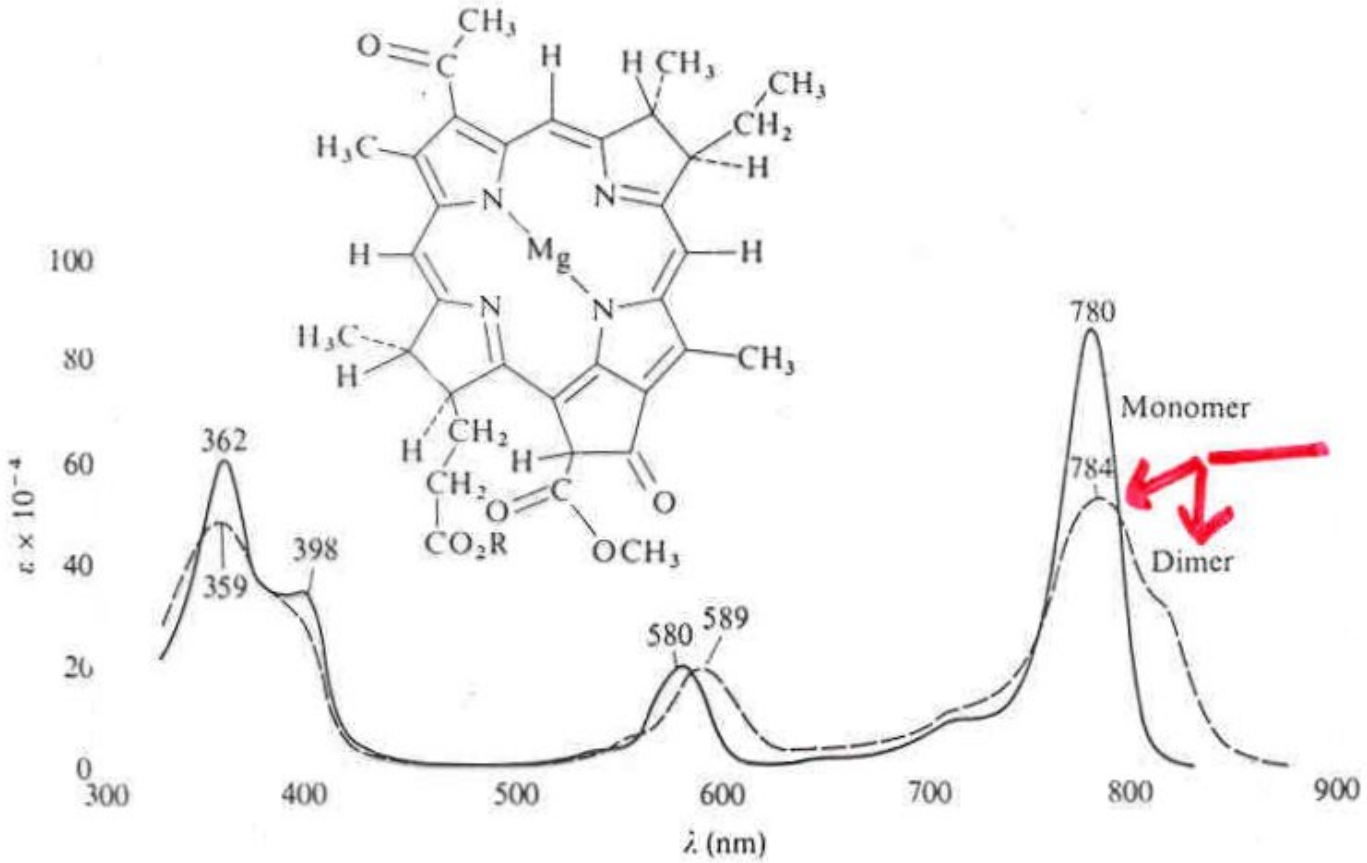






This is called **Davydov** splitting

Bacteriochlorophyll Dimer - Davydov splitting observed in long wavelength band



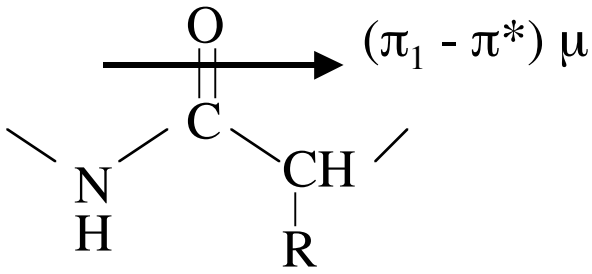
Monomer and dimer spectra for solutions of bacteriophyll. A pronounced splitting of the longest-wavelength band in the dimer is visible.

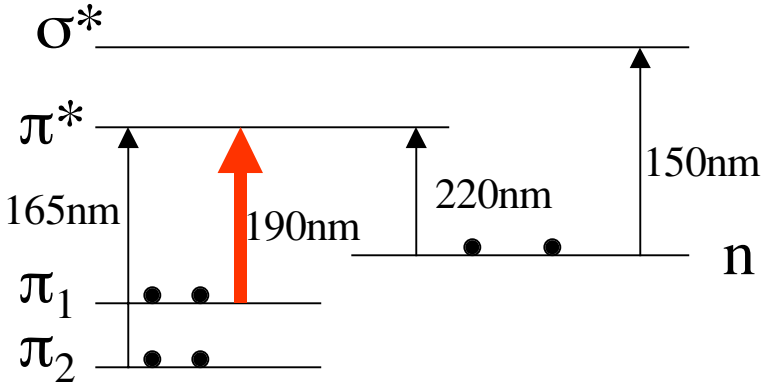
JACS 88:2681 (1996)

A prominent example of Davydov Splitting:

Far UV spectrum of protein α-helix

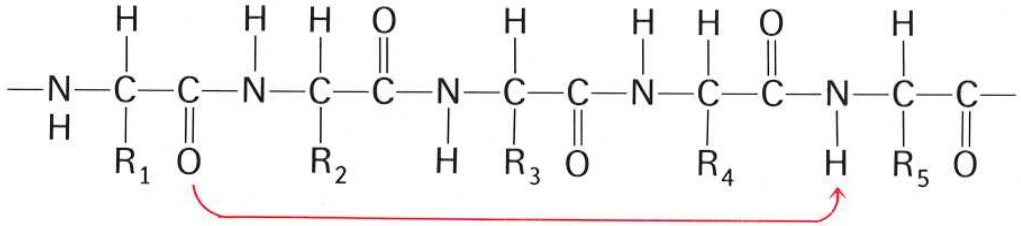
π - π* bands of amide groups interact with each other in the helix

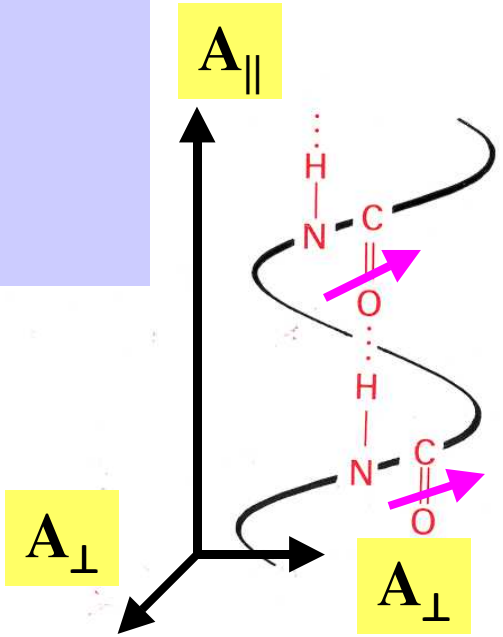




3-dimensional version of herringbone geometry in α-helix

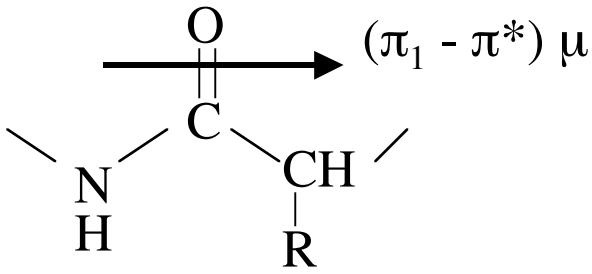
Interaction of the carbonyls along the helix results in splitting into 3 absorption bands: one parallel to the helix axis and two bands perpendicular to the helix axis

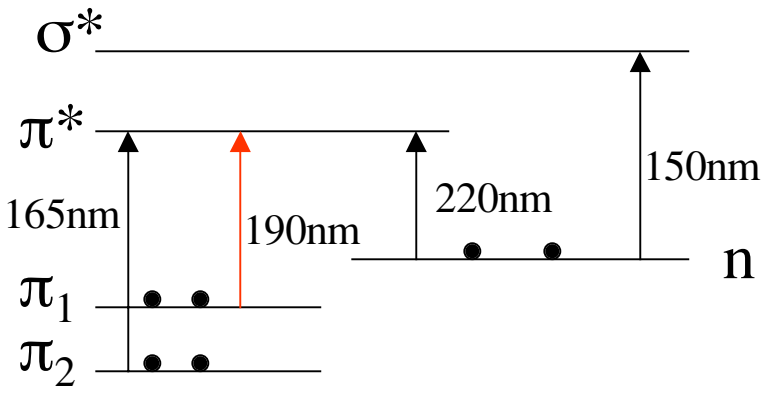


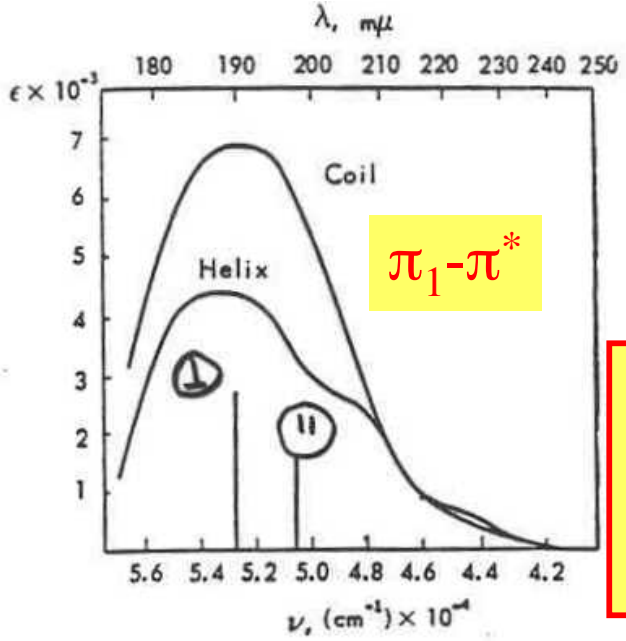


A prominent example of Davydov Splitting:

Far UV spectrum of protein α-helix







Results in Davydov splitting

One absorption band (at low energy):

μ parallel to helix

Two degenerate absorption bands (higher energy):

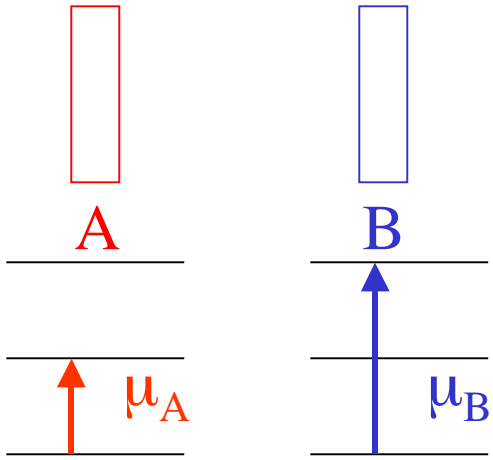
μ ⊥ to helix axis

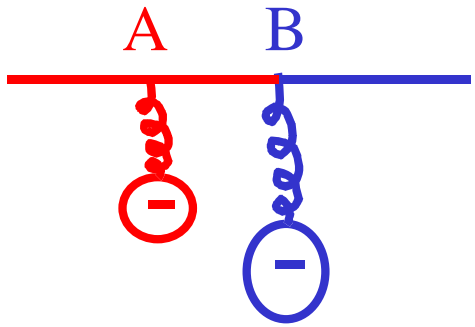
Note that there are two effects upon forming α-helix:

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



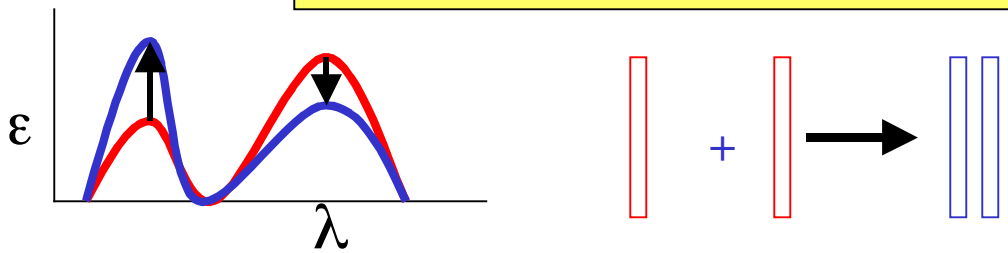


Mixing of wavefunctions of higher excited states results in **intensity borrowing**

-Oscillator strengths of different transitions can increase or decrease

hyperchromism **hypochromism**

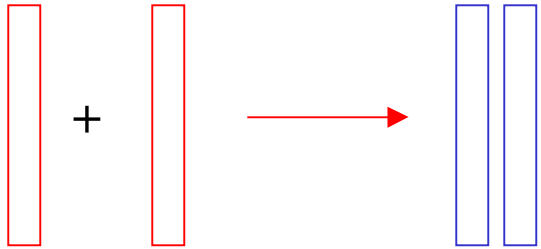
Kuhn-Thomas Sum Rule:



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.

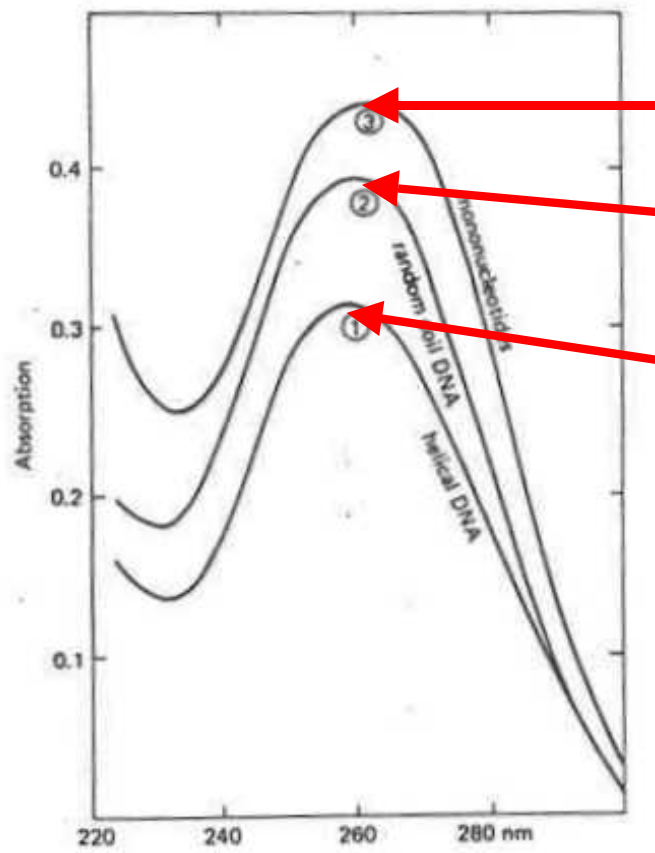
e.g. **Hypochromism** in nucleic acids:
lower absorption in one region of the spectrum means there must be an increased absorption elsewhere.



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

An Example of Hypochromisim

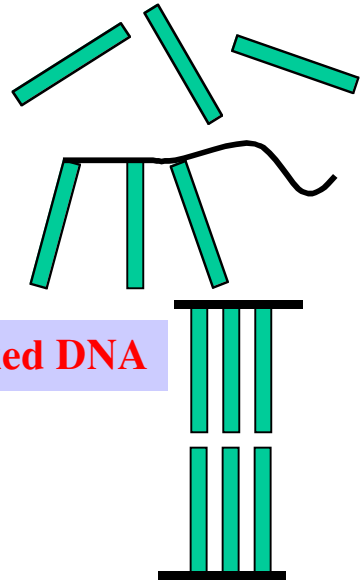
Lower absorbance at 260 nm due to stacking in DNA and RNA



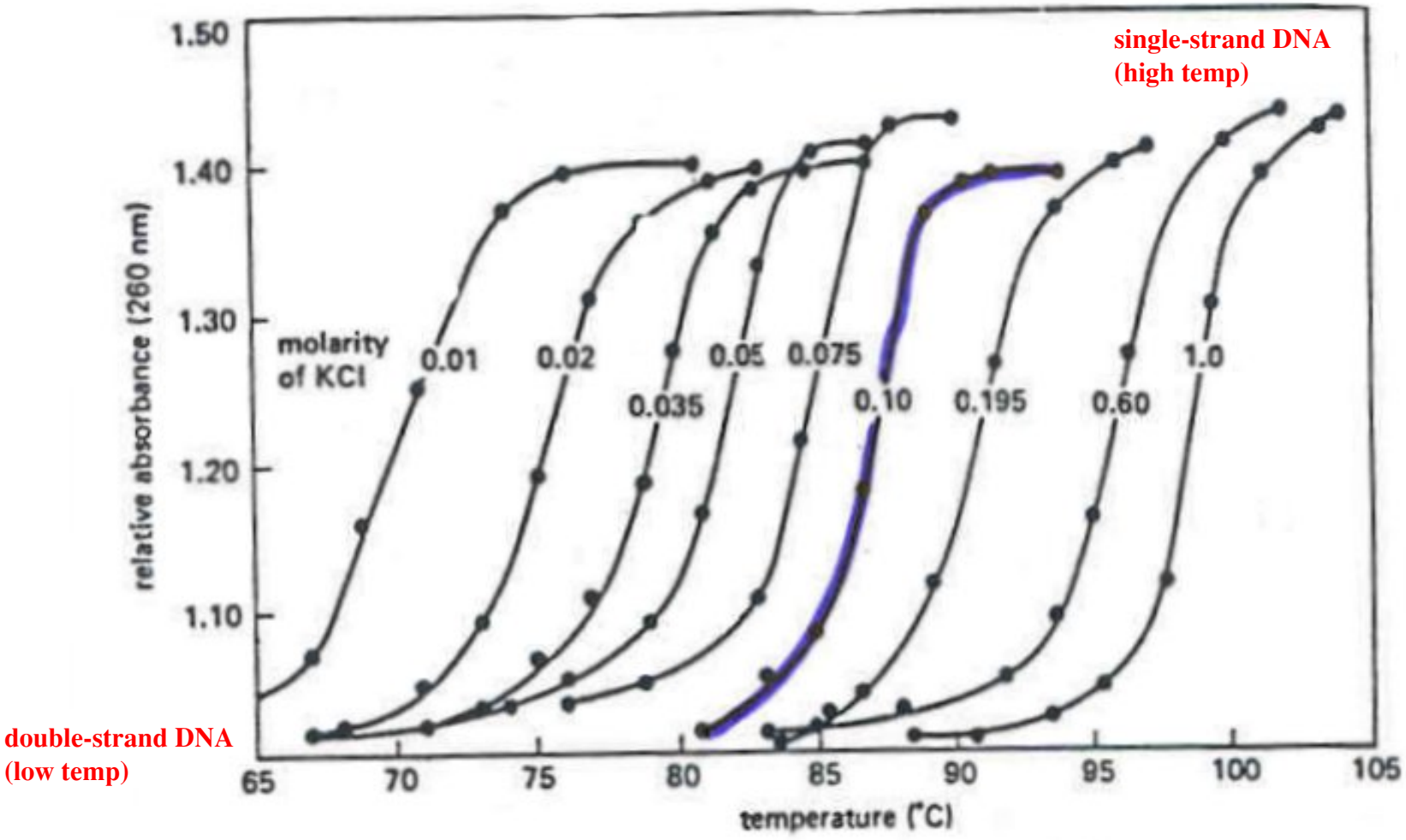
Mononucleotides

Single-strand unstacked DNA

Double-strand stacked DNA



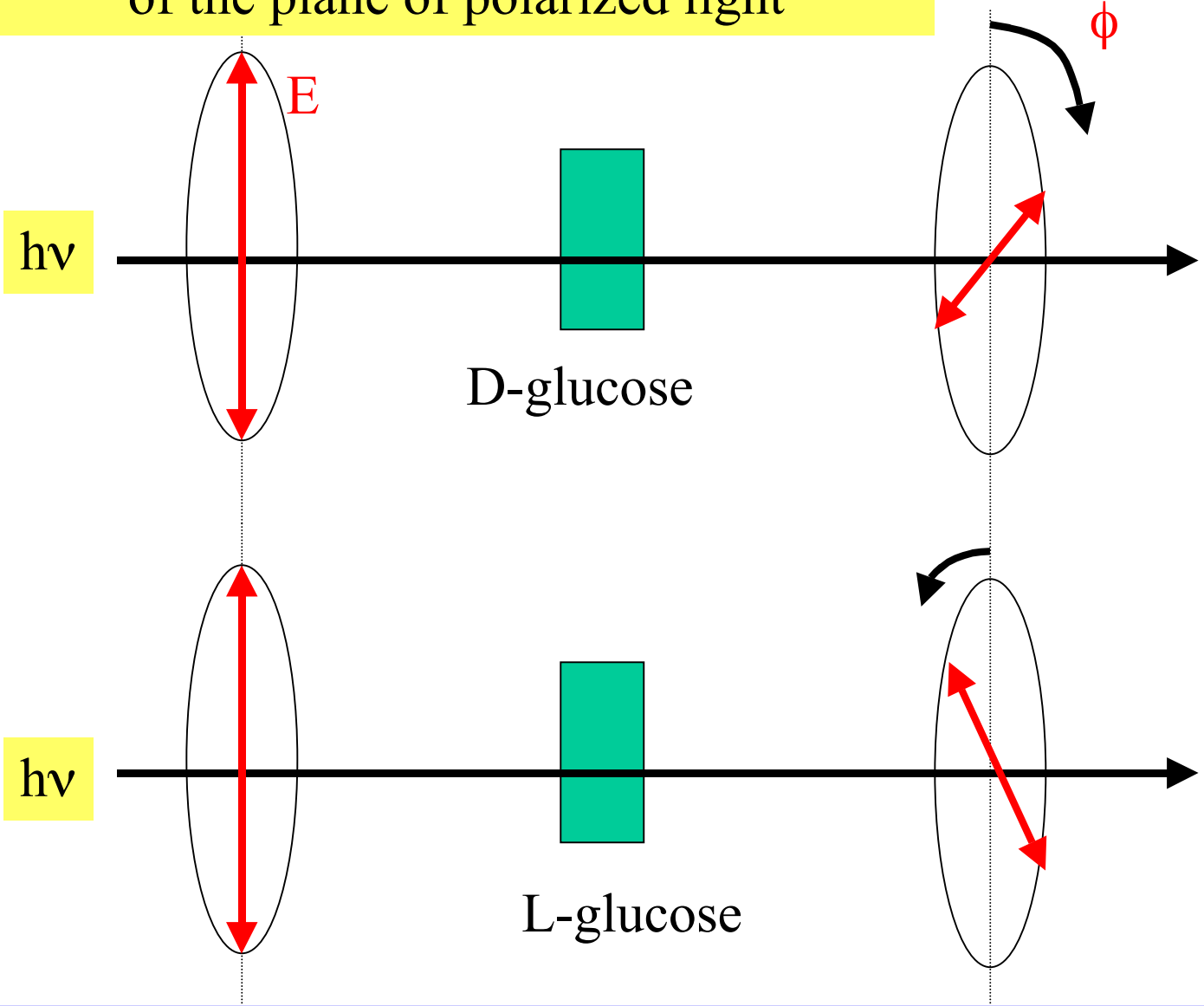
Monitoring "melting" of double-strand DNA (from *E. coli*) by the absorbance change at 260 nm an example of hypochromism: lower ε_{260} for double-strand vs single-strand DNA



Circular Dichroism (CD) and Optical Rotatory Dispersion (ORD)

optical activity of chiral molecules

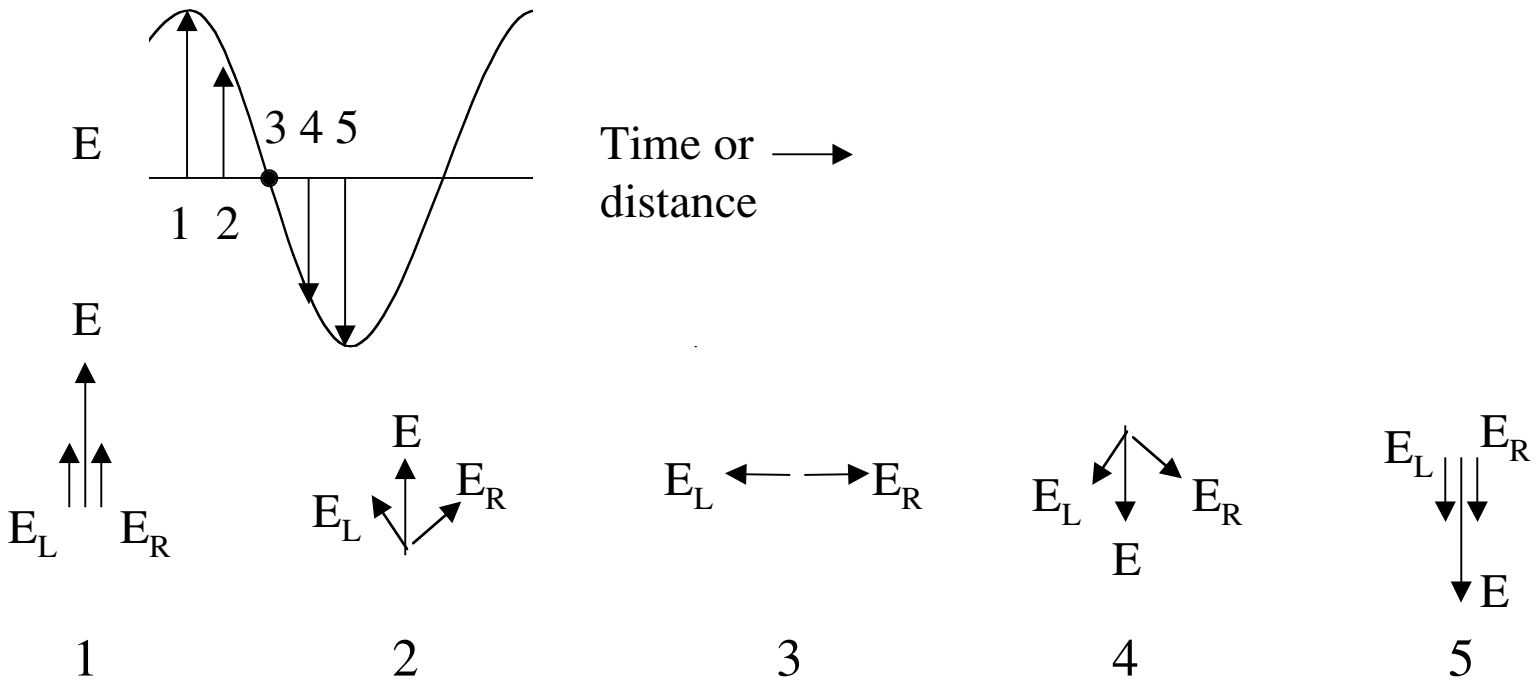
An optically active sample causes rotation of the plane of polarized light



DUE TO DIFFERENT INTERACTIONS OF THE MOLECULES WITH RIGHT HANDED AND LEFT HANDED POLARIZED LIGHT

Plane polarized light and circularly polarized light

= the sum of left and right circularly polarized light



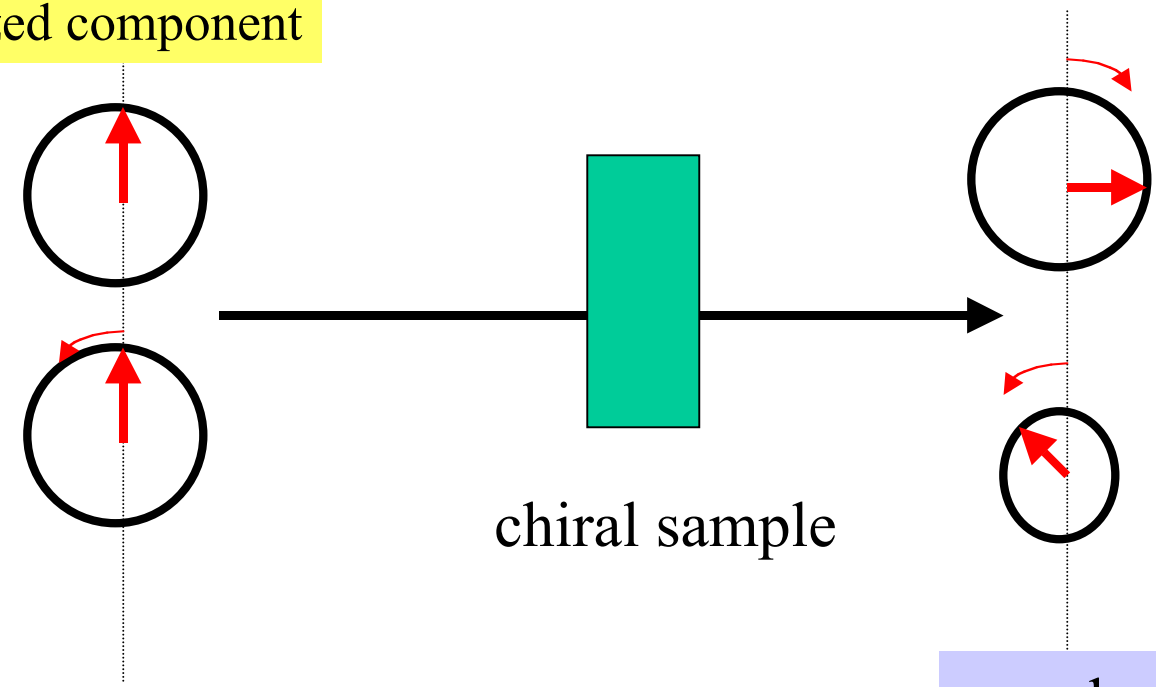
-left and right handed circular polarized light can be physically separated (Pockel cell)

-molecules that are asymmetric (chiral) interact differently with right and left handed circularly polarized light).

This is the cause of optical activity

Different index of refraction and different extinction coefficients for right and left components

right circularly polarized component



chiral sample

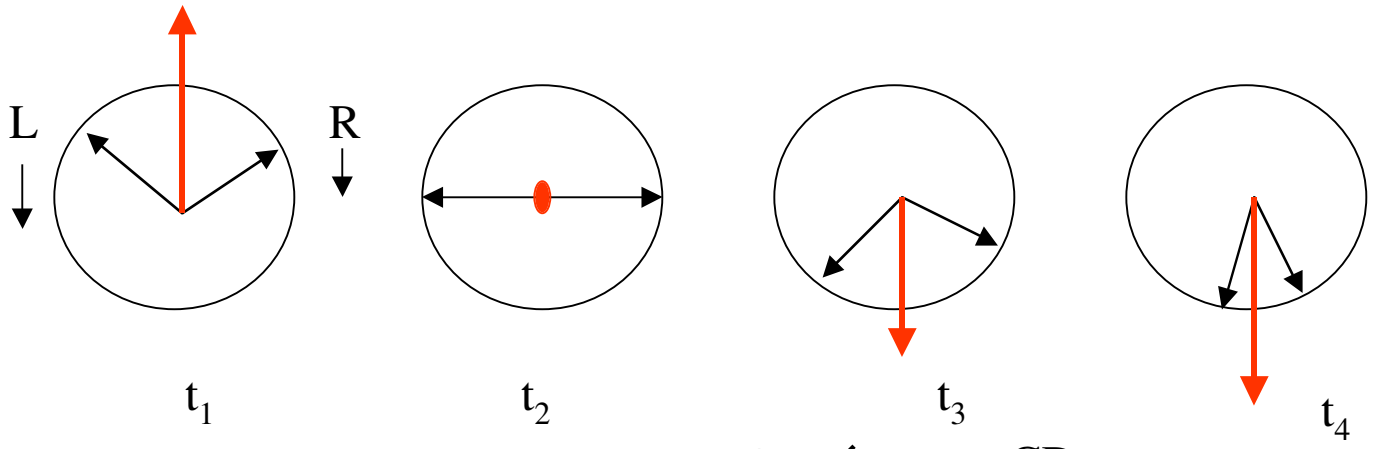
left circularly polarized component

speed of light of the two components differ

absorption of the two components differ

CD/ORD

Plane polarized light $\Longrightarrow$ left + right circularly polarized light



$\varepsilon = \varepsilon_R + \varepsilon_L$

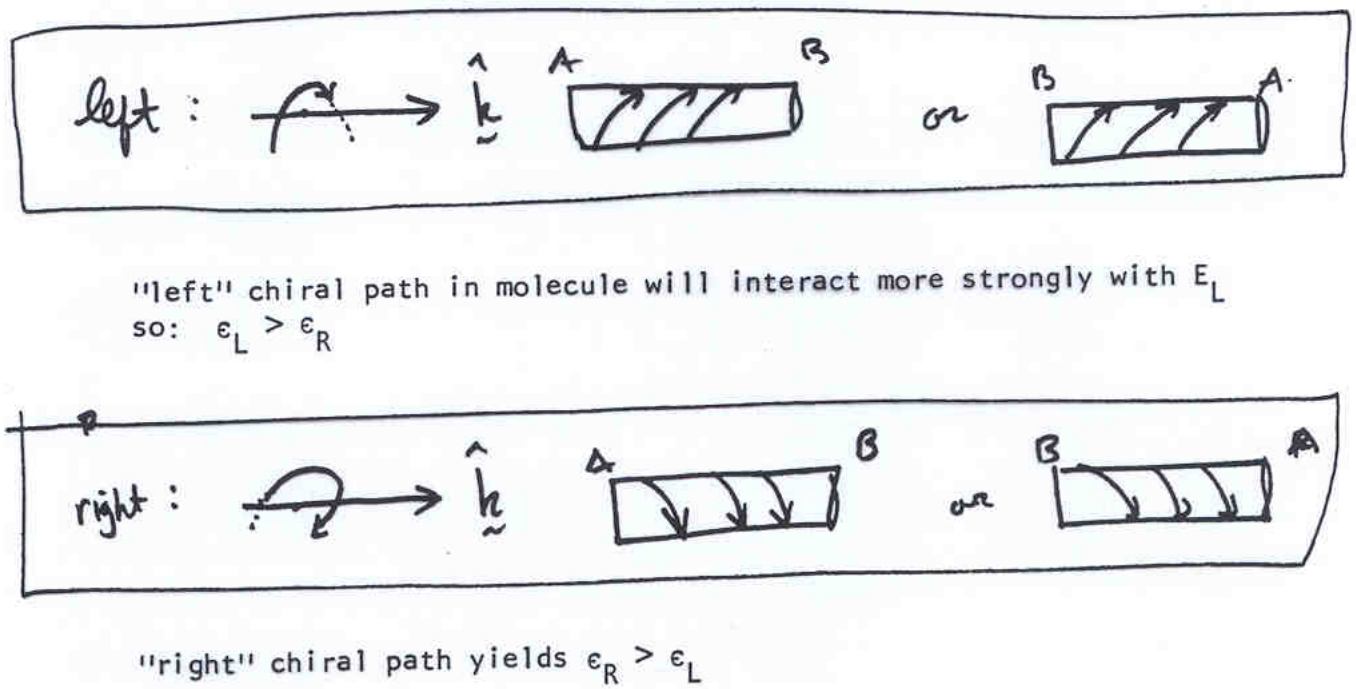
$\varepsilon_R \neq \varepsilon_L$ CD

$n_R \neq n_L$ ORD

	Absorption	Dispersion
1	ε	n
2	$\varepsilon_{\parallel} - \varepsilon_{\perp}$ linear dichroism	$n_{\parallel} - n_{\perp}$ birefringence
3	$\varepsilon_L - \varepsilon_R$ (CD)	$n_L - n_R$ (ORD)

ε and n related mathematically by Kronig-Kramers relation

Classically, optical activity results from an electron oscillating along a helical or chiral path



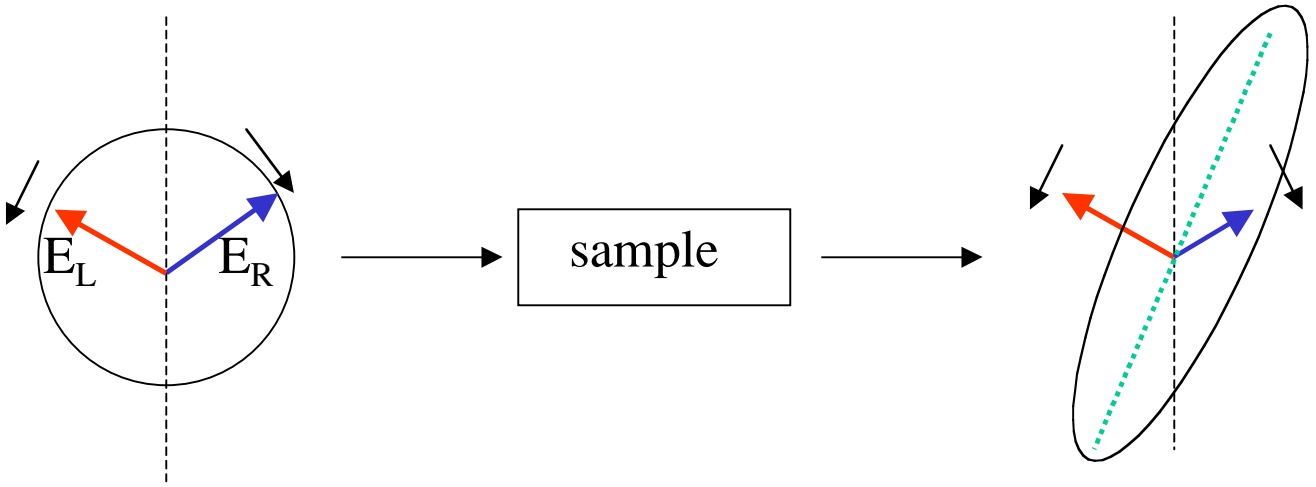
Note: random orientation of the molecule with respect to the light does not negate the asymmetry

chirality is an intrinsic property of the molecule

Mirror image of "left chiral" molecule is "right chiral"

-stereo-isomers or enantiomers-

CD/ORD



Plane polarized incident light

After passing through a chiral sample

(1) $n_L \neq n_R$ so one component of light is retarded (slower velocity) passing through sample. This results in ORD

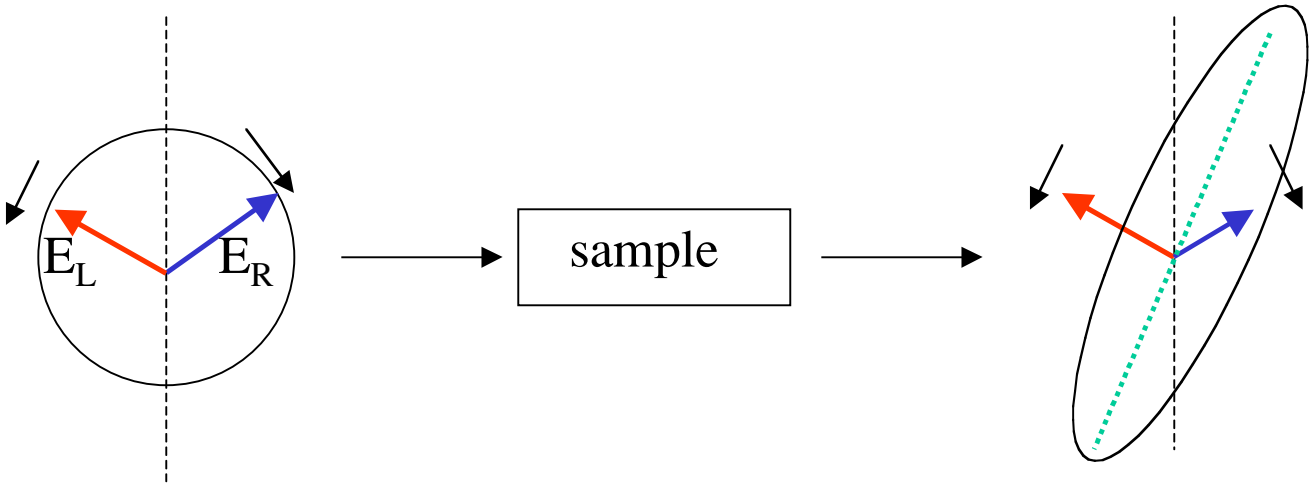
(2) $\varepsilon_L \neq \varepsilon_R$, so amplitude of one component will be less than other component. This results in CD

(3) sum of $\underline{E_R}$ and $\underline{E_L}$ after passing through the sample is an **ellipse**

elliptically polarized light

Note: elliptically polarized light has a major and minor axis

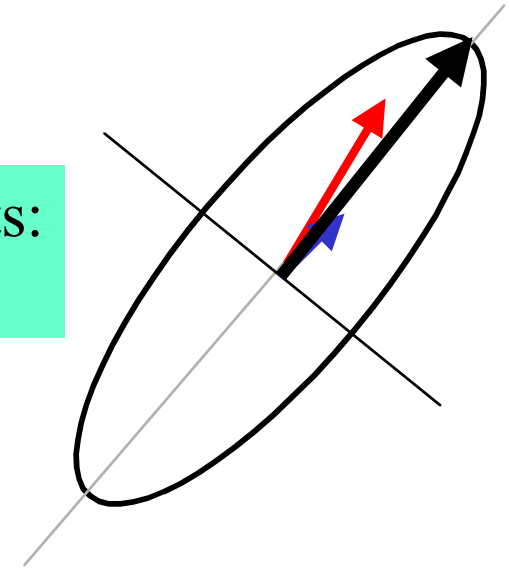
CD/ORD: Elliptically polarized light

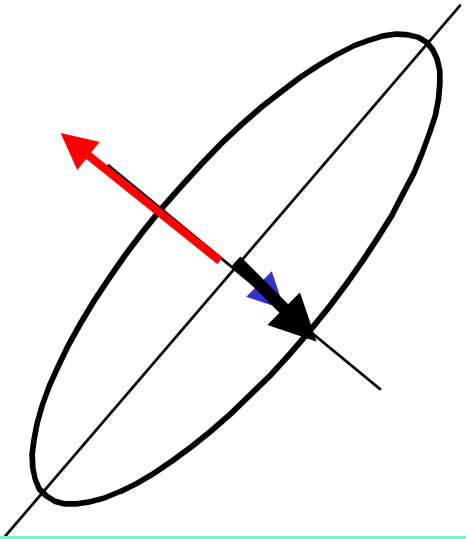


Plane polarized incident light

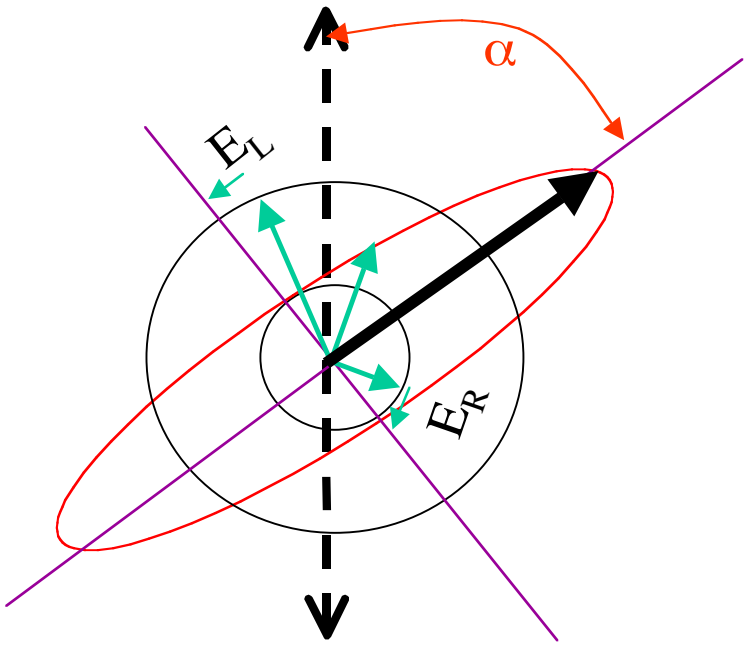
After passing through a chiral sample

Sum of components: major axis





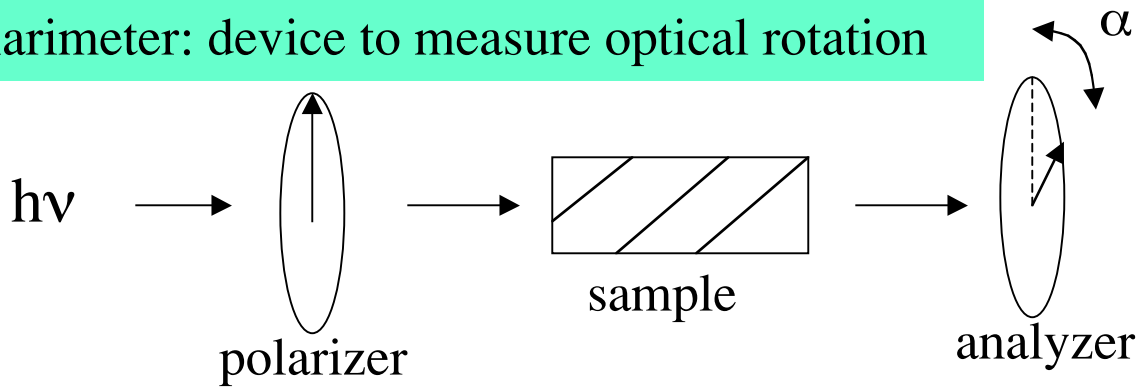
Difference of components minor axis



α = optical rotation

the angle between the direction of the electric field vector of the plane polarized light entering the sample and the major axis of the elliptically polarized light exiting the sample

Polarimeter: device to measure optical rotation



Rotate to maximize transmittance

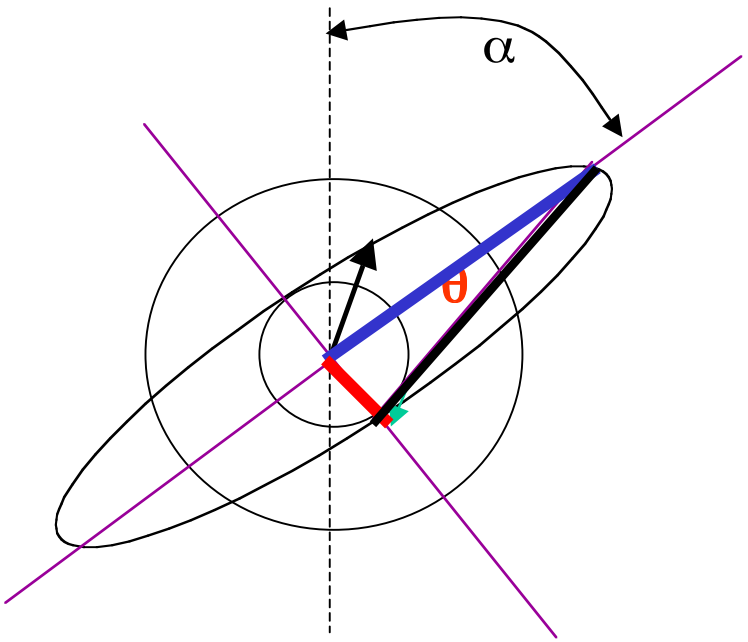
(The major axis is much larger than the minor axis so the resultant appears plane polarized by this measurement)

Measure $[\alpha]_D$

Sodium "D" line in visible

α due to bands in ultraviolet (for sugars)

Circular dichroism



CD: θ

Angle of the triangle formed by the major and minor axes of the elliptically polarized light

plane polarized light ⟶ optically active sample ⟶ elliptically polarized light

$$\theta \approx \tan\theta = \frac{\text{minor axis}}{\text{major axis}} = \frac{I_R - I_L}{I_R + I_L} = \frac{\text{difference}}{\text{sum}}$$

I = light intensity (right or left components)

Molar ellipticity $[\theta] = 3300\ \Delta\varepsilon$

Optical activity: Every electronic transition has a rotational strength: R_{0i}

The value of R is zero when the molecule has either a

(1) a plane of symmetry

(2) a center of symmetry

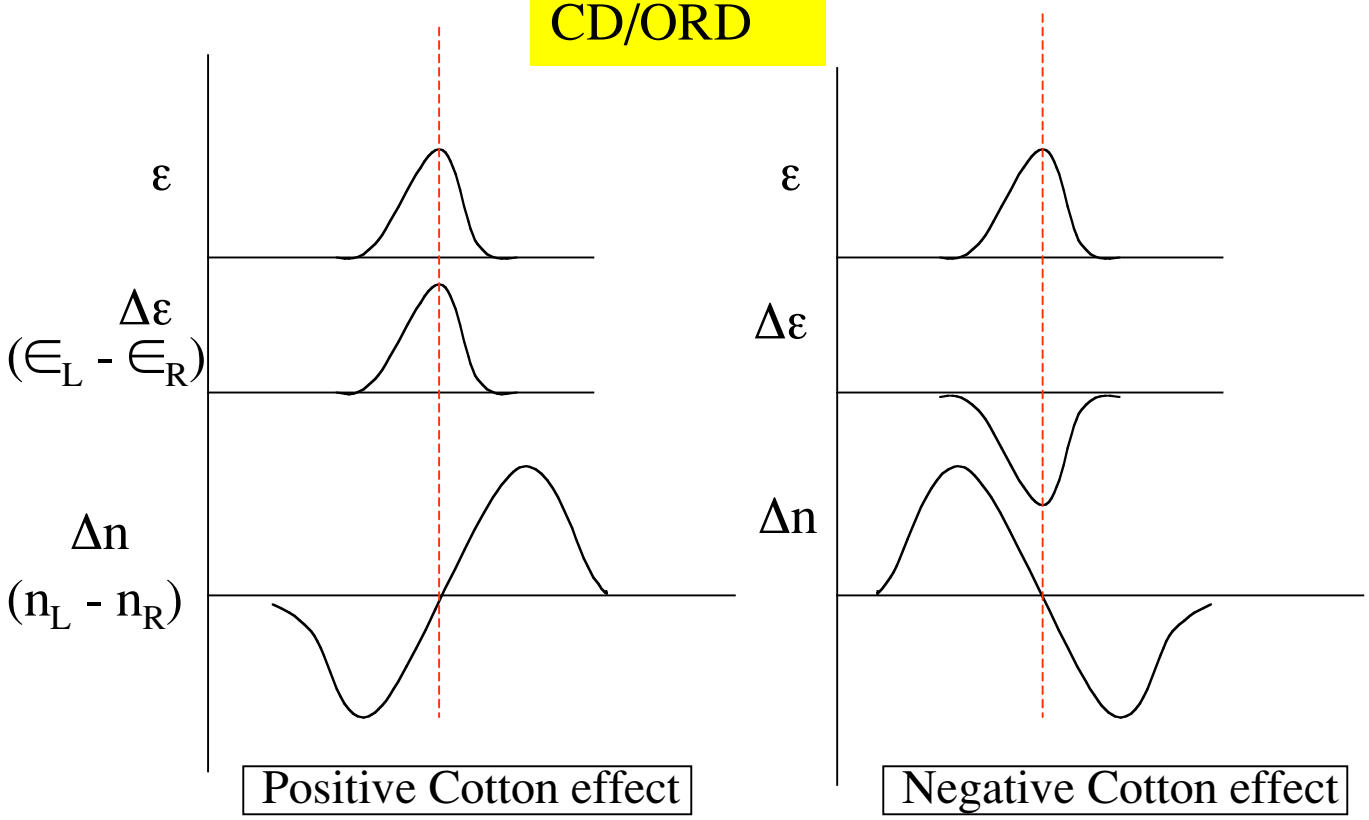
→ If there is no chirality the molecule is not optically active

Optical activity is measured in two modes

Absorption (Δε) : CD

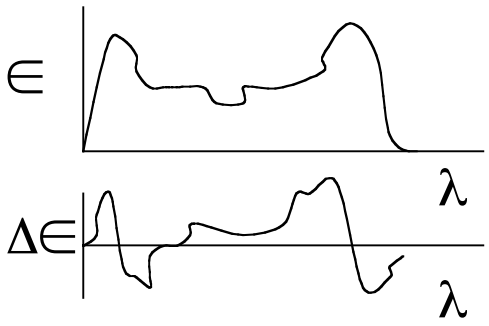
Dispersion (Δn) : ORD

CD/ORD



Area under Absorption curve: related to f_{oi}

Area under CD curve : related to R_{oi}



$$\sum_{\text{all i}} f_{oi} = N \quad \text{(\# electrons)}$$

$$\sum_{\text{all i}} R_{oi} = 0$$

Which chromophores are optically active?

(1) Any molecule which does not have a plane or center of symmetry

(2) A chromophore is an asymmetric environment can have an induced optical activity

flavin or heme bound to a protein

tyrosine or tryptophan in a protein

dyes bound to DNA (intercalaters)

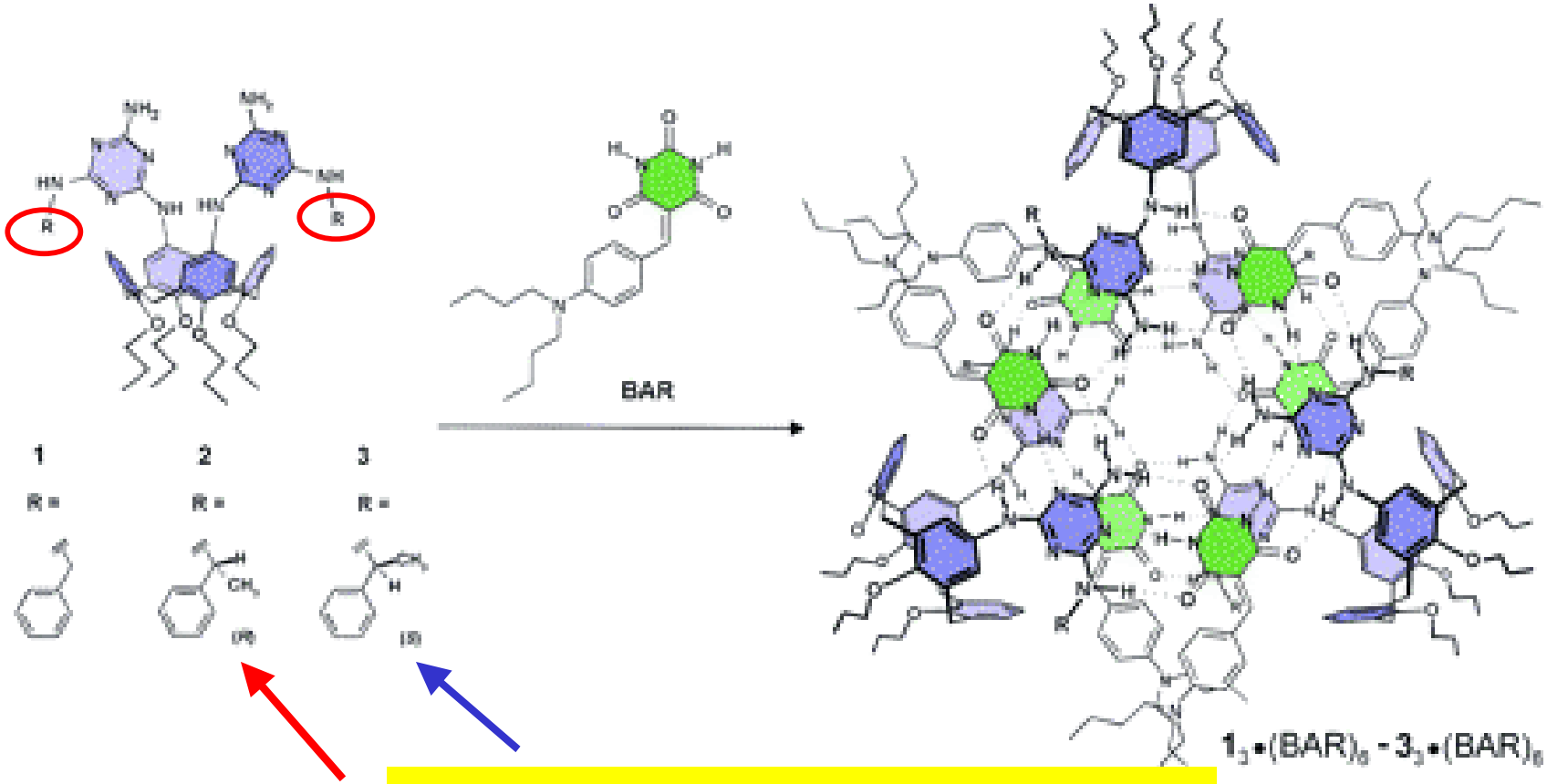
(3) Chromophores which are strongly interacting can exhibit chirality

→ CD is an important measure of secondary structure

protein α, β structure

DNA, RNA stacking

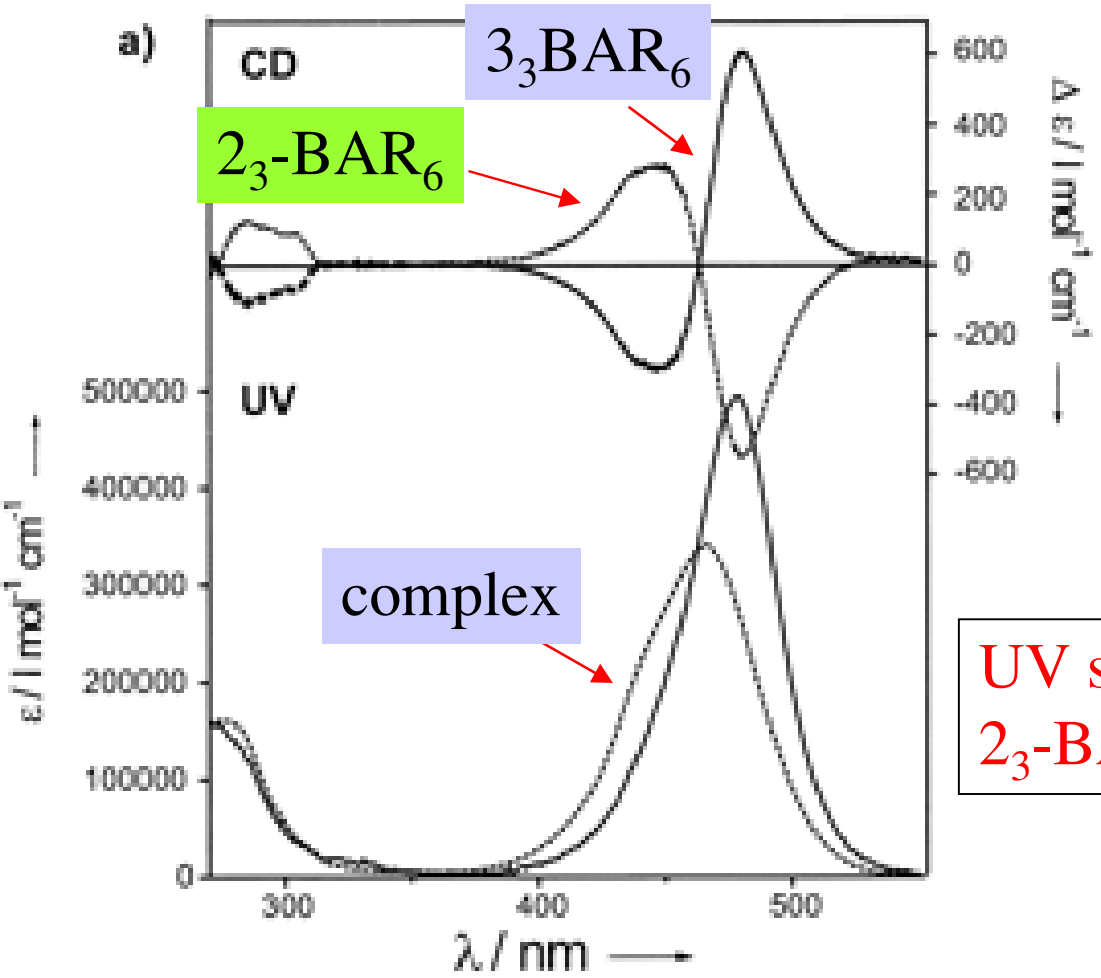
A complex between a dimelamine and barbituate exhibits exciton coupling that shifts the absorption spectrum and has induced CD



"R" group determines chirality of the complex

PNAS (2001)98, 10042-10045

Different complexes of melamines and barbituates exhibit enhanced CD and different chiralities



CD spectra of two different complexes show reversed helicity

UV spectrum of 2_3-BAR_6 and BAR alone

PNAS (2001)98, 10042-10045

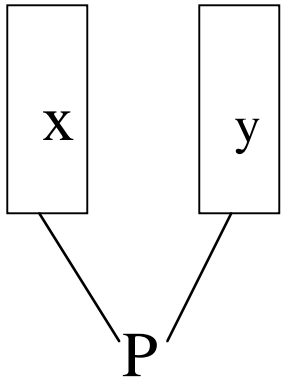
CD of Nucleic Acids

(1) largely due to nearest neighbor stacking interaction

(2) Very sensitive to secondary structure

-helix geometry (or turns, loops, etc)

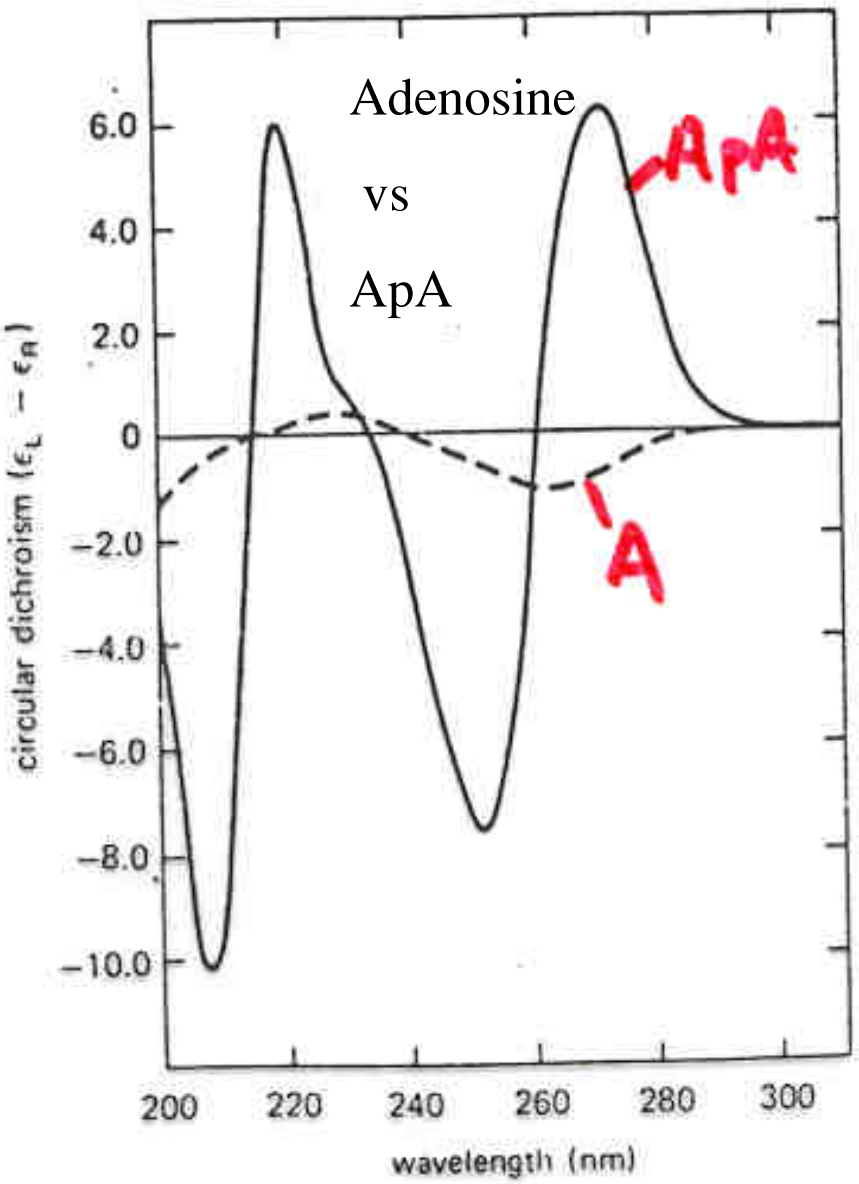
-sequence dependent



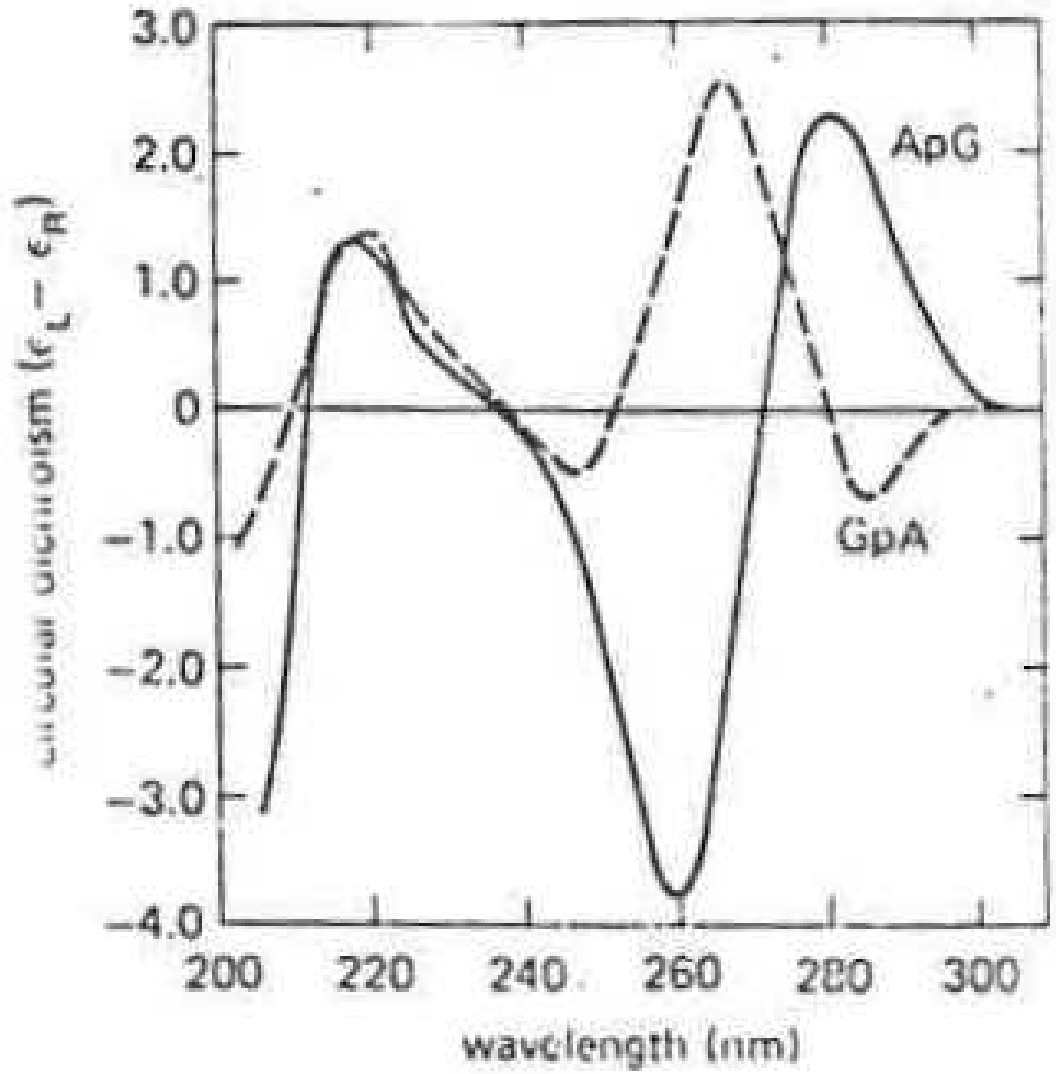
-sequence dependence is observed for short oligomers

-sequence dependence is averaged out for natural DNA, RNA

Stacking is important for CD of nucleic acids



Sequence Dependence of CD of Nucleic Acids



CD of proteins

(1) **Primarily used to measure secondary structure**

-Absorption bands in the far UV (190 nm-250 nm) due to amide groups

-Sensitive to geometry of regular structure
α, β(↑↑), β(↑↓), β turn, "random"

each has a characteristic CD spectrum

The best way to determine protein secondary structure if not known from X-ray analysis

Also observe **changes** in secondary structure (e.g. induced by ligand binding)

(2) **CD is used to monitor aromatic amino acids, hemes, flavins, and other groups**

-environmental asymmetry, interactions (heme-heme) etc...

Secondary structure determination of proteins by CD

Strategy is to fit the CD spectrum of an unknown protein to a weighted sum of spectra of pure α, β, and "random" polypeptide

at any λ : $[\theta] = f_\alpha[\theta]_\alpha + f_\beta[\theta]_\beta + f_R[\theta]_R$

f_α = fraction of protein in α helix, etc

$f_\alpha + f_\beta + f_R = 1$

One needs the spectra for pure α, β, and random polypeptide structures as a basis to analyze the secondary structural content of an unknown protein

Two ways to get $[\theta]_\alpha(\lambda)$ etc

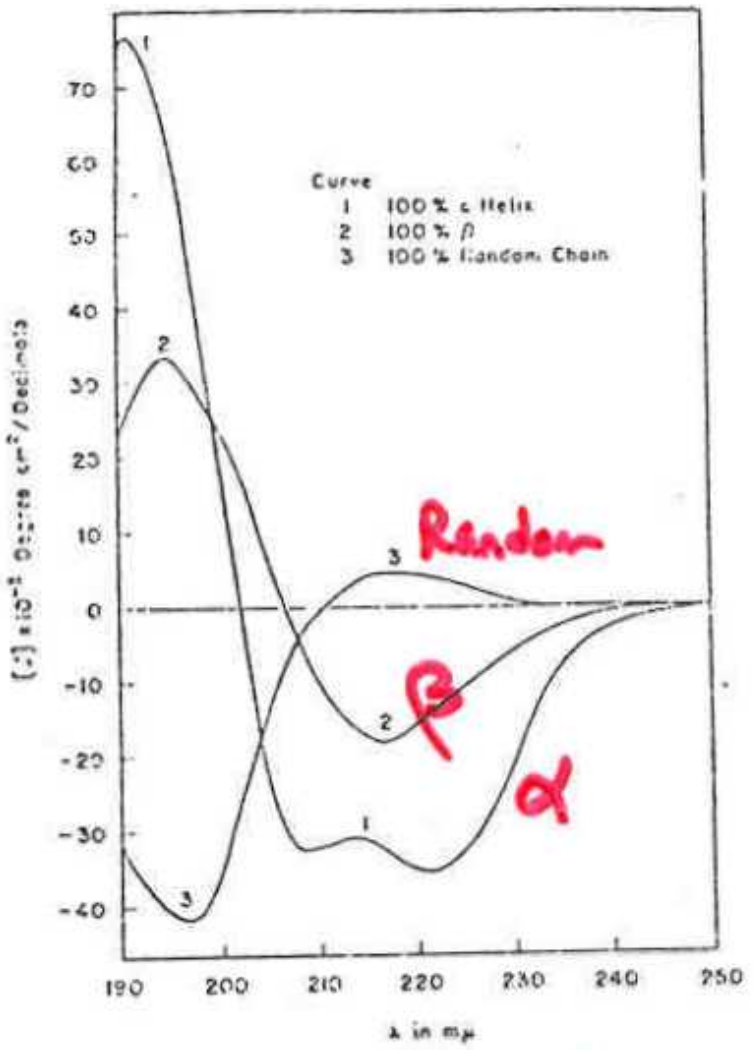
I. Measure poly amino acids known to be in α, β, R forms

II. Deconvolute CD spectra of proteins whose values of f_α, f_β, f_R are known

The CD spectra of poly-L-lysine

3 solution conditions used to get the CD of

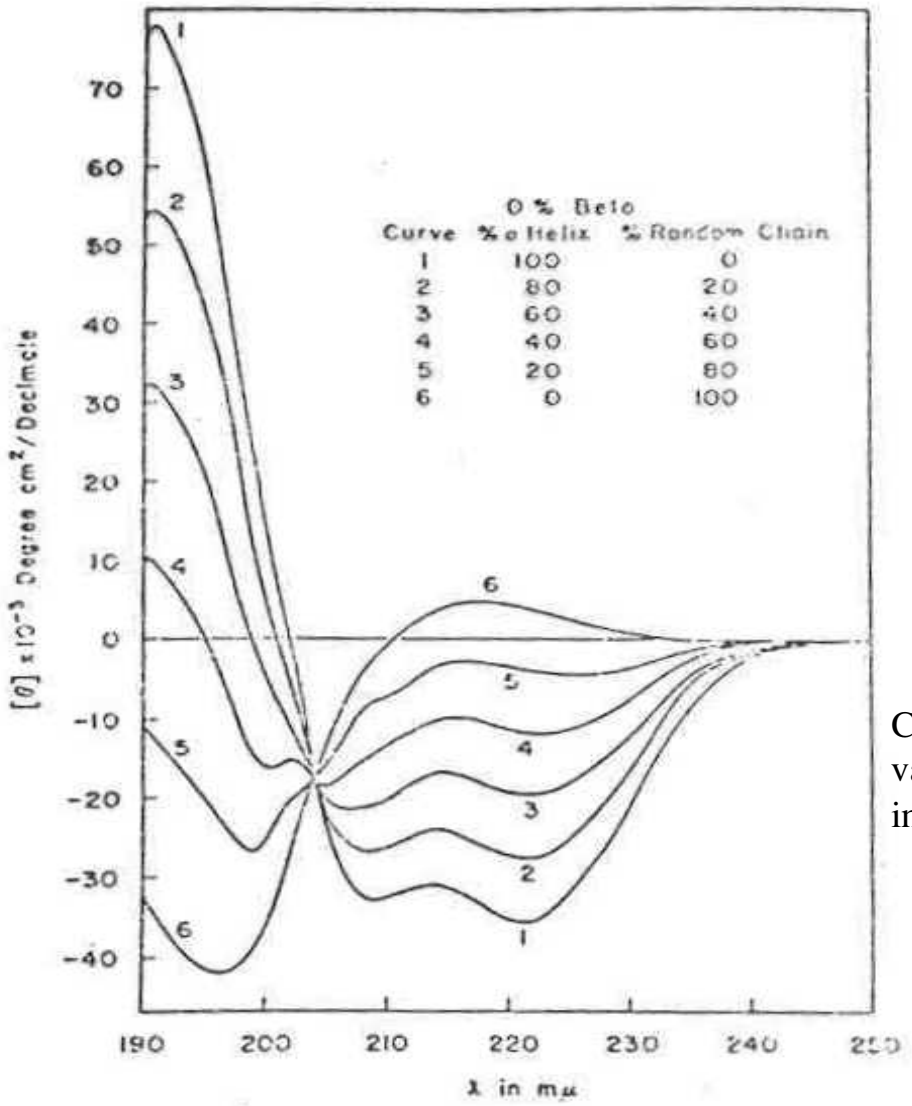
100% α, β, or Random polypeptide



Combining basis set spectra to get the best fit

Computer adds $[\theta]_\alpha$, $[\theta]_\beta$, etc... in optimum way to fit the unknown spectrum

$\Longrightarrow f_\alpha, f_\beta, f_R$



Example

vary % α

% random coil

Calculated CD of poly-L-lysine containing 0% β and varying percentages of α helix and random coil, as indicated.