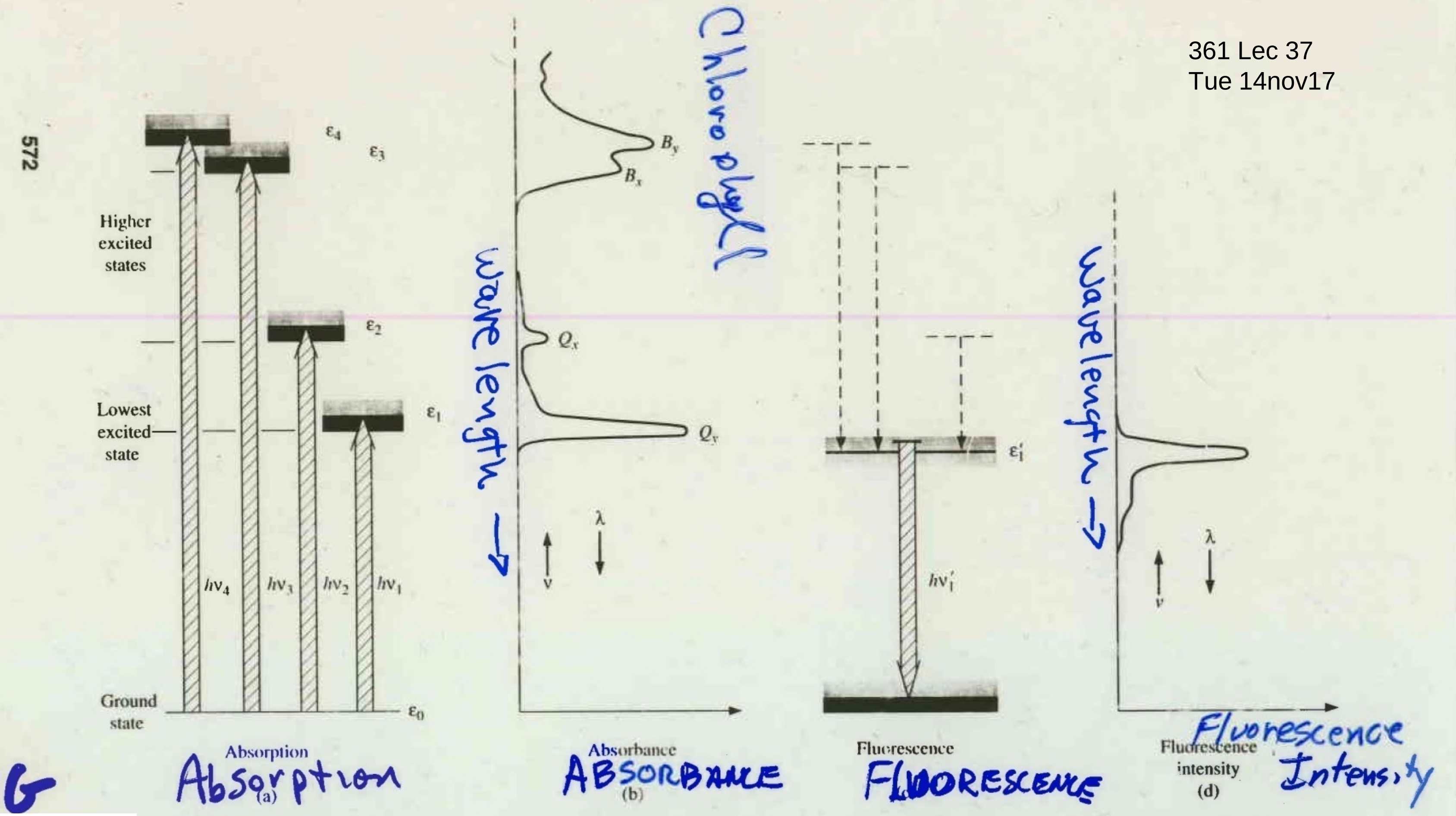
361 Lec 37
Tue 14nov17



361 Lec 38
Thur. 13nov14

Hypochromic Effect: due to Stacking of bases.

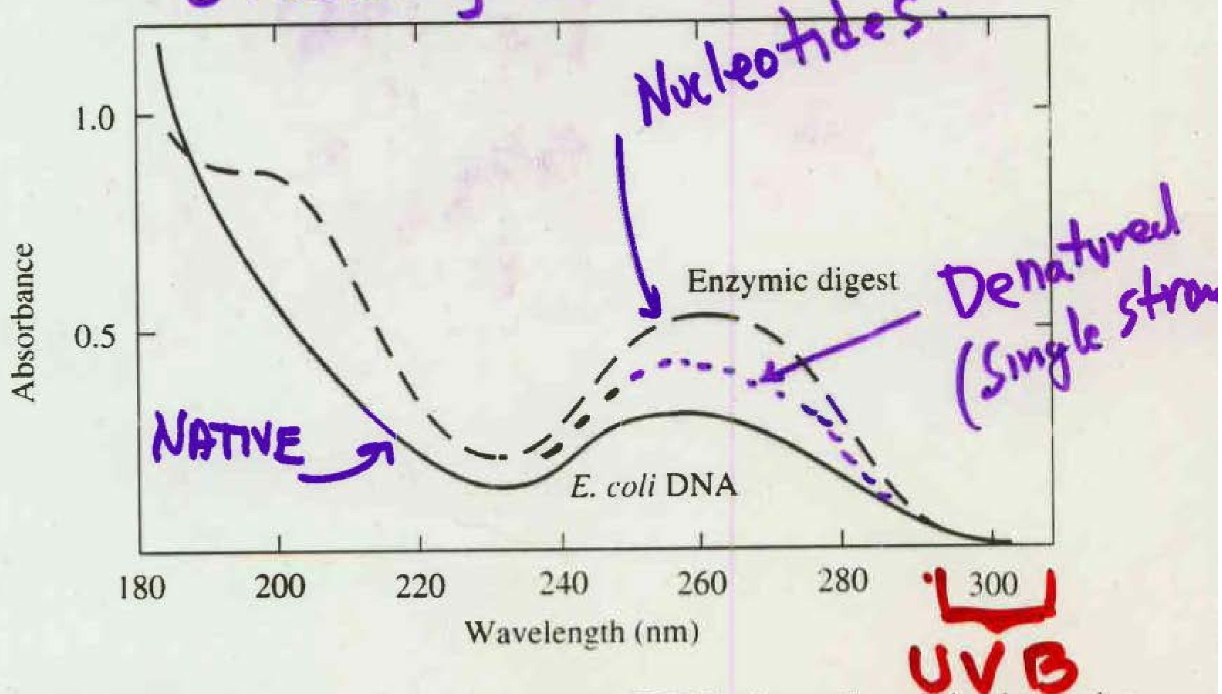
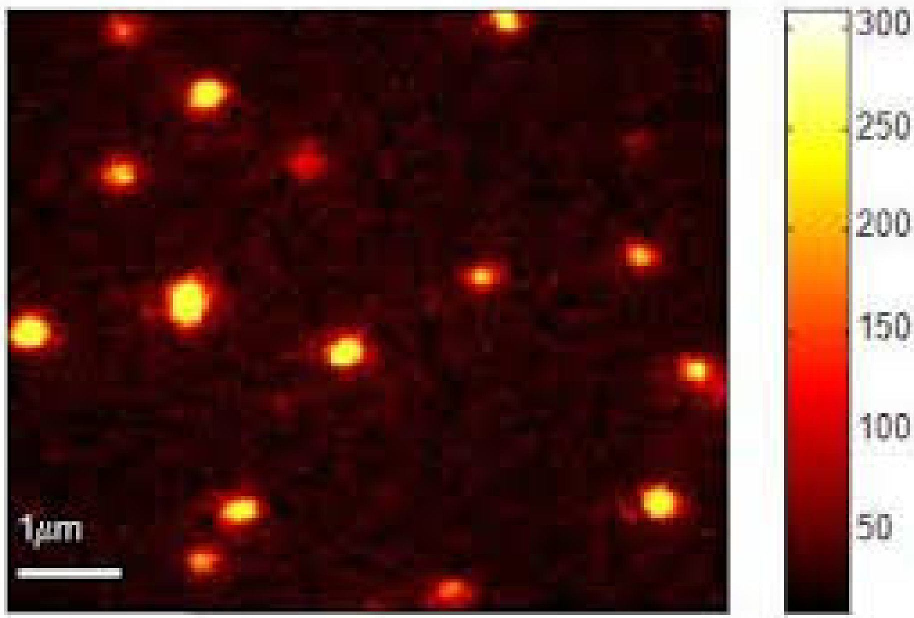


Fig. 10.9 Ultraviolet absorption spectrum of DNA from *E. coli* in the native form at 25°C (solid curve) and as an enzymic digest of nucleotides (dashed curve). [From D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers 1*, 193 (1963).] Reprinted with permission of John Wiley and Sons, Inc.

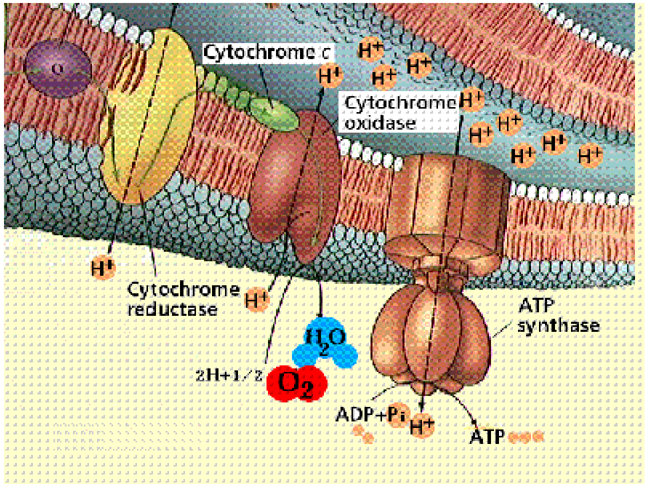
are sufficiently general that the ratio of absorbances A_{260}/A_{280} has been used to deterr quantitatively the ratio of nucleic acid to protein in a mixture of the two. This requires c ful calibration, however, because proteins differ significantly in their content of aron amino acids.

Single Molecule Spectroscopy

The Nobel Prize in Chemistry 2014 was awarded jointly to Eric Betzig, Stefan W. Hell and William E. Moerner *"for the development of super-resolved fluorescence microscopy"*.



http://www.k2.phys.waseda.ac.jp/Movies.html



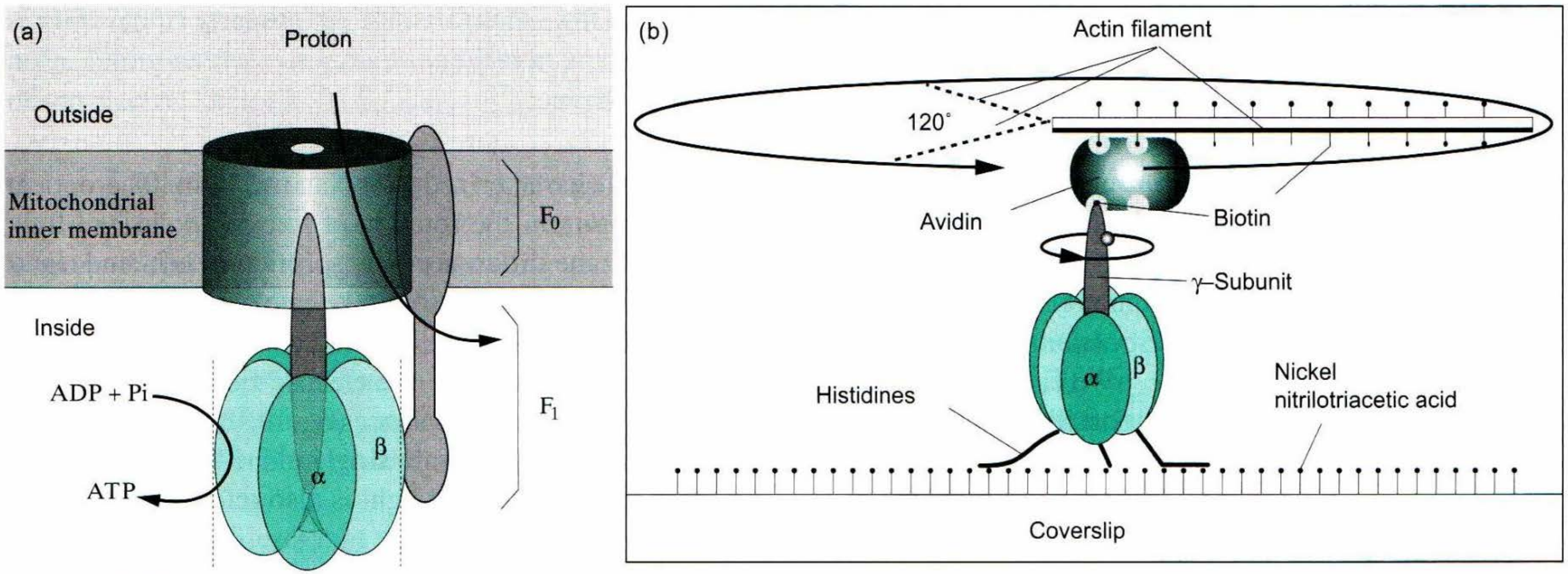


FIGURE 13.27 Single-molecule studies of F_1-ATPase. (a) The F_0F_1-ATP synthesase is shown schematically. The F_0 subunit is embedded in the membrane and is a proton pump. The F_1-ATPase is a rotary motor that synthesizes ATP from ADP and inorganic phosphate. (b) In the experiment, the F_1 subunit was attached to a glass coverslip through nickel–histidine linkages, and a fluorescent actin filament was attached to the gamma subunit. The rotation of a single actin filament could be observed by fluorescence miscroscopy.

http://www.k2.phys.waseda.ac.jp/Movies.html

http://www.k2.phys.waseda.ac.jp/Movies.html

Moves clockwise if ATP synthesized
Moves counter-clockwise if ATP being used

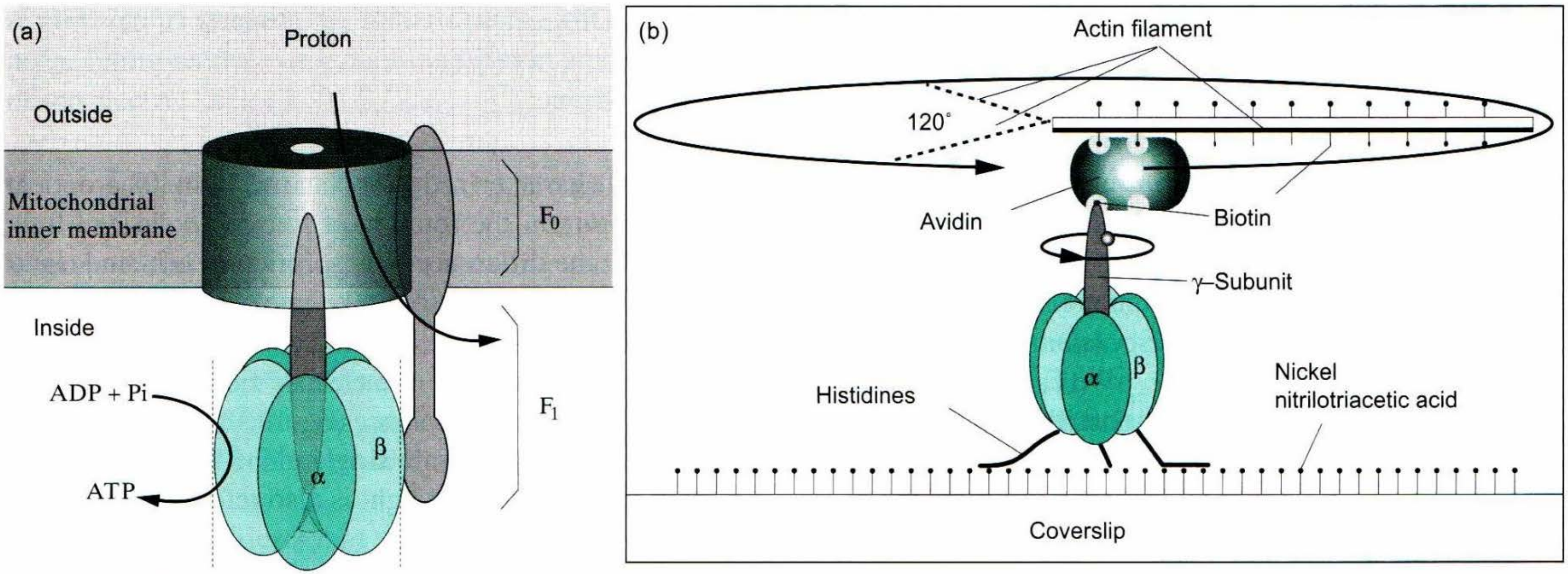
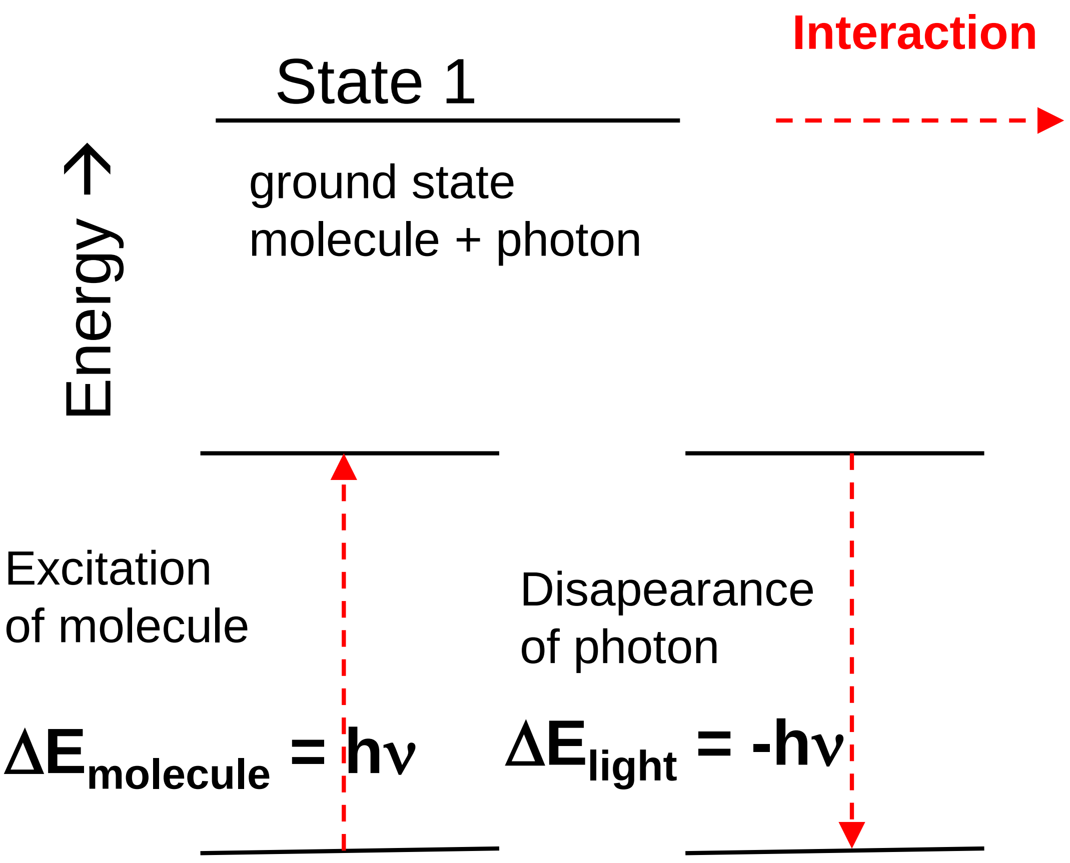


FIGURE 13.27 Single-molecule studies of F_1-ATPase. (a) The F_0F_1-ATP synthesase is shown schematically. The F_0 subunit is embedded in the membrane and is a proton pump. The F_1-ATPase is a rotary motor that synthesizes ATP from ADP and inorganic phosphate. (b) In the experiment, the F_1 subunit was attached to a glass coverslip through nickel–histidine linkages, and a fluorescent actin filament was attached to the gamma subunit. The rotation of a single actin filament could be observed by fluorescence miscroscopy. (Reprinted from Cell, 93 (1), F1-ATPase: A Rotary Motor Made of a Single Molecule, pp. 21, Copyright © 1998, with permission from Elsevier.)

Resonance: What we call “happening” is the world moving between states in RESONANCE. i.e., two states of the UNIVERSE obeying the **FIRST LAW**

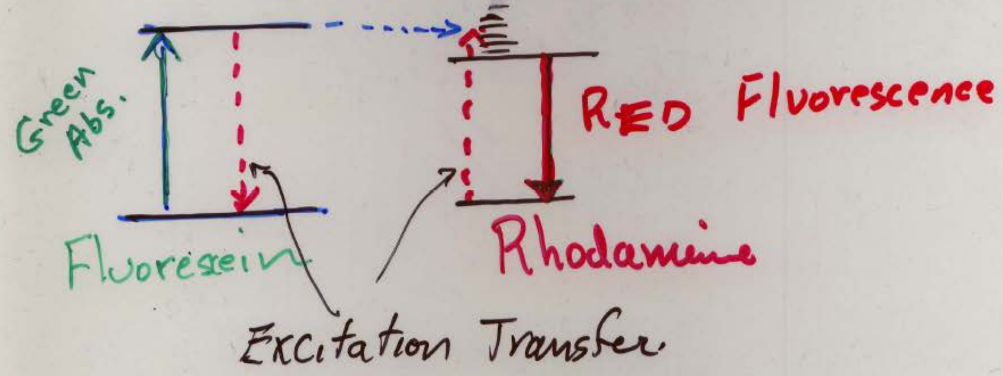


$\Delta E_{universe}$ = 0 at every instant!!!

FRET☹ (not really ☺)

Only ***half*** the story

FRET ("Fluorescence" Resonant Energy Transfer or Förster)



Not emission + absorption.

It is through space Coulomb Law interaction of electrons

$$\text{Efficiency} \propto \frac{1}{(\text{distance apart})^6}$$

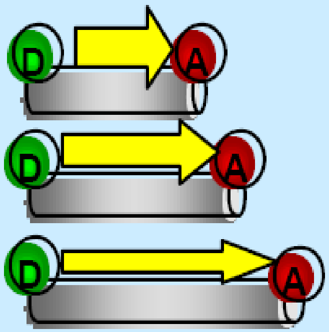
"Molecular ruler"

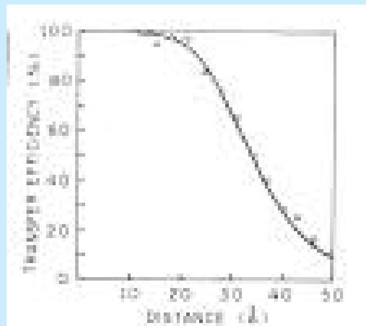
<u>London dispersion force</u> (the very same force that holds liquid nitrogen together.

FRET: APPLICATIONS

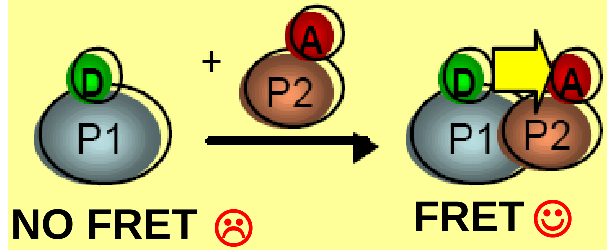
Measure distances within macromolecules; generate structures of large complexes

FRET as a "molecular ruler": Stryer and Haugland, 1967

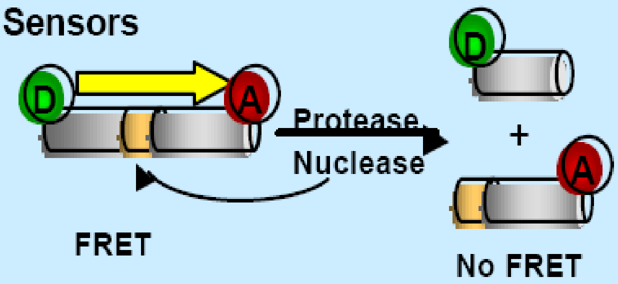




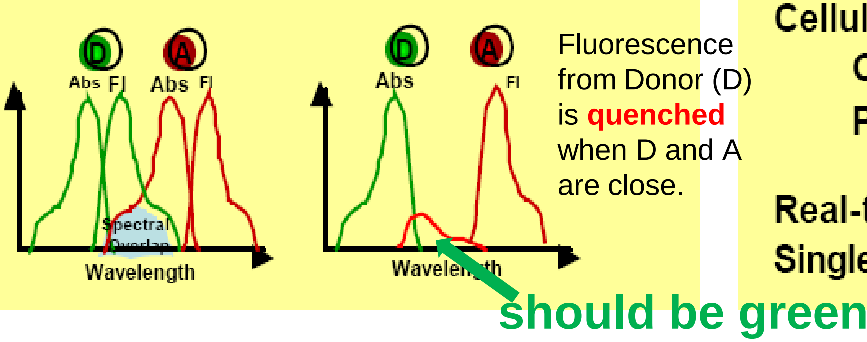
Monitor interactions & their kinetics



Sensors



Generate new probes with large Stokes shift ("energy transfer probes") by placing D-A at $R \ll R_0$



Fluorescence from Donor (D) is **quenched** when D and A are close.

Conformational transitions

Cellular work

- **Chameleons (Ca^{2+} sensors)**
- **Protein-protein interaction (GFPs)**

Real-time PCR

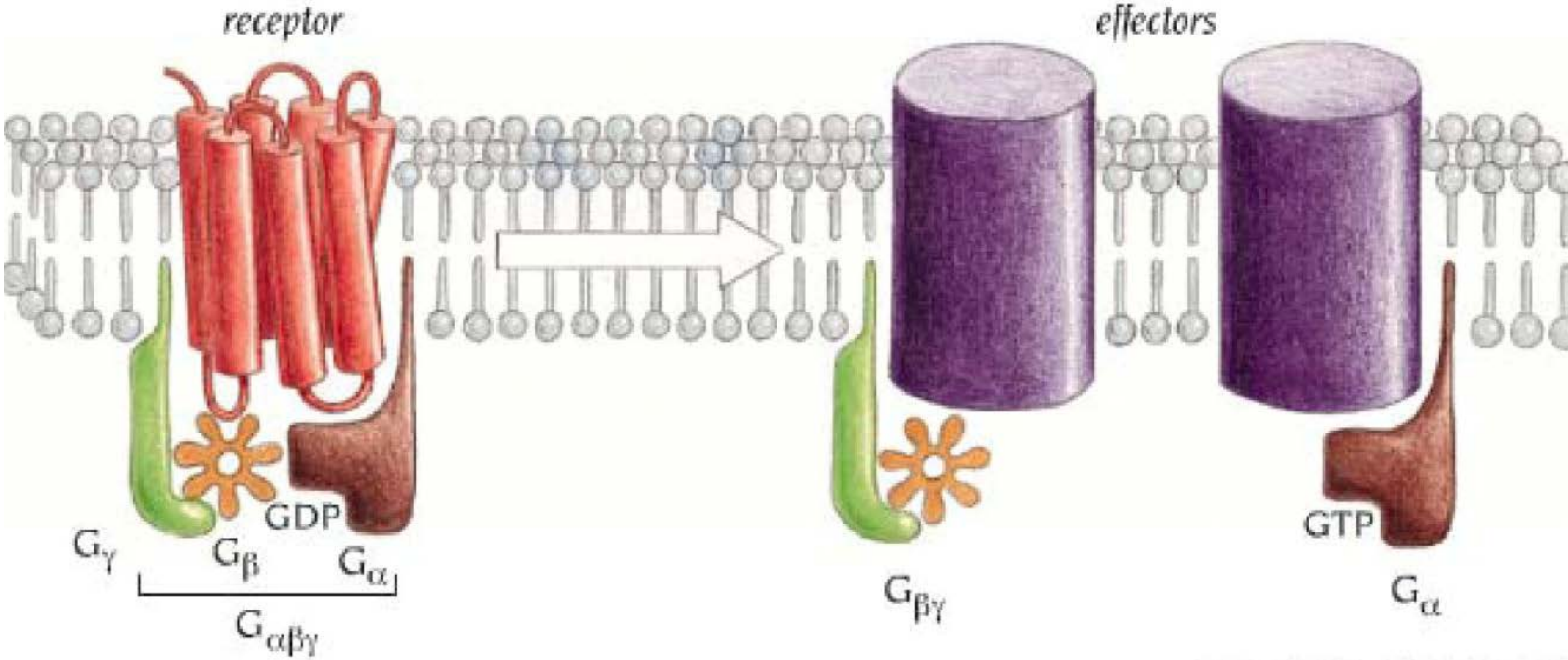
Single -molecule DNA sequencing

Shimon Weiss http://laxmi.crump.ucla.edu:8248/weiss/class033.pdf

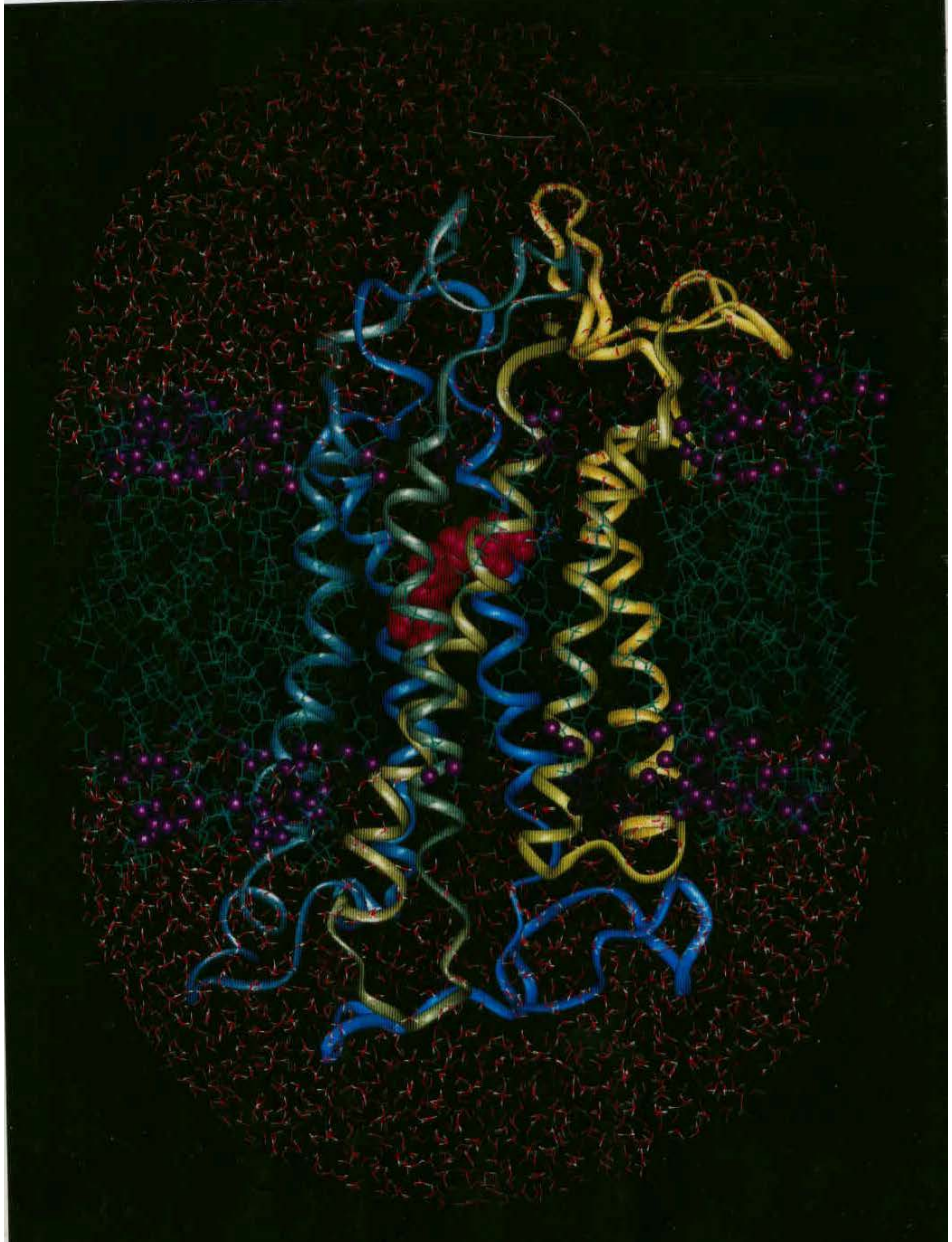
- G-Protein-Coupled receptors have emerged over the last 20 years as a MAJOR biological signaling motif--constituting perhaps 10% of the human genome, and the target of 60% of commercial drug research .

- Examples:

Receptor	**Mediates**	**Drug/Hormone (ligand)**
Adrenergic	hypertension	beta blockers/adrenaline
Cannabinoid	lots of things	marijuana
………… dozens more		
Rhodopsin	vision	retinal Schiff base + light


receptor
effectors
GDP
GTP
G_γ
G_β
G_α
$G_{\alpha\beta\gamma}$
$G_{\beta\gamma}$
G_α

Rhodopsin, harboring the visual pigment retinal (pink) is a member of the large class of signal transducing proteins called G-protein coupled receptors.



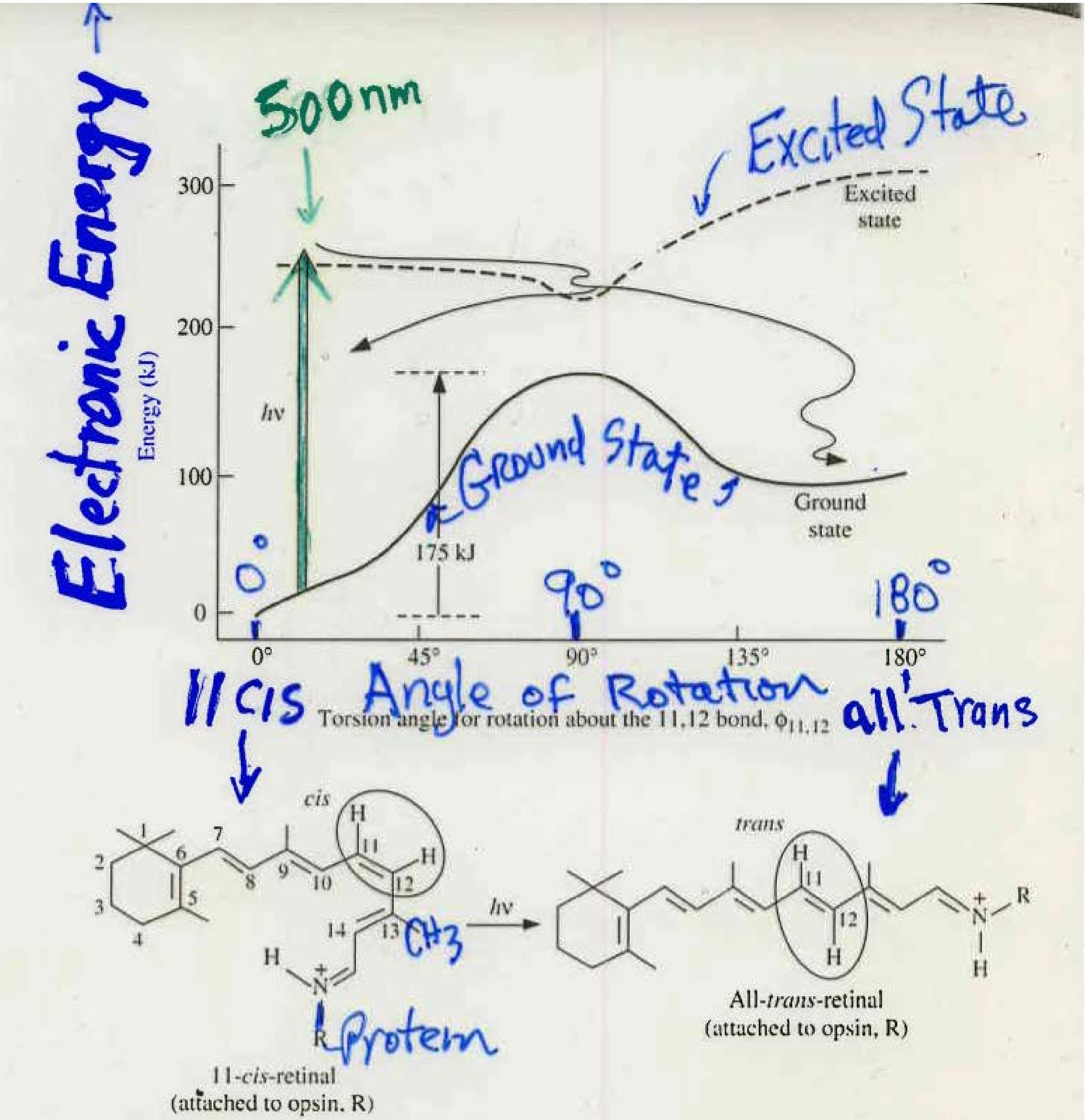
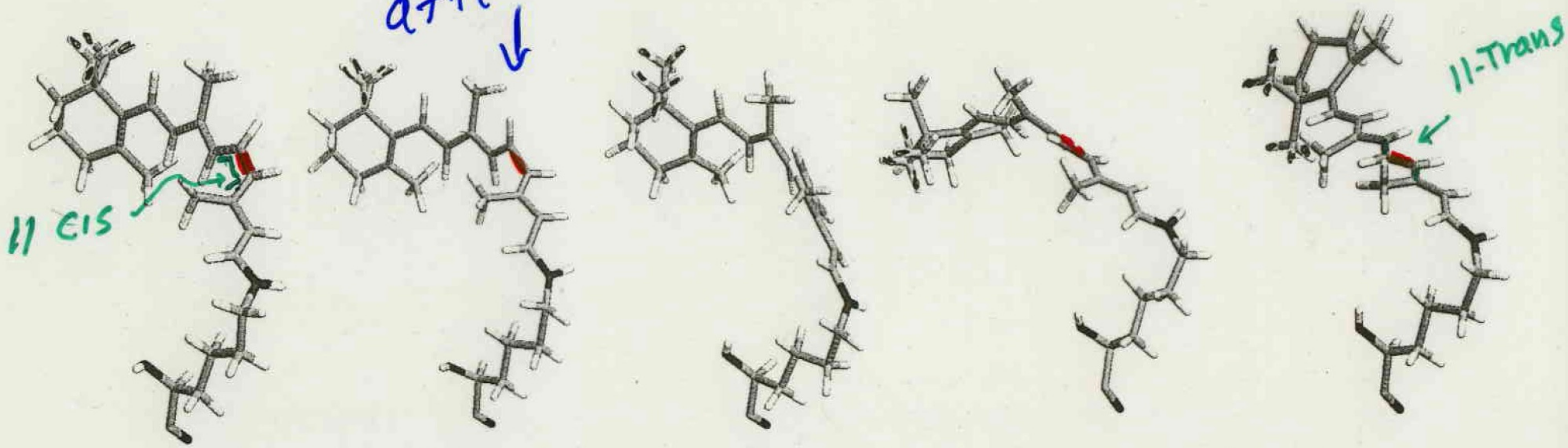


Fig. 9.1 Light-driven isomerization of 11-*cis*-retinal attached to opsin (R) by a Schiff's base linkage. Theoretical calculations of the energy of both the ground state and an excited electronic state suggest a possible path whereby absorption of a photon will lead to conversion from the *cis* to the *trans* configuration about 60% of the time. The angle of rotation about the 11,12-double bond is plotted as the horizontal axis.

$1\ fs = 1\ \text{femto second} = 1 \times 10^{-15}\ s$
$1\ ps = 1\ \text{pico}\ " = 1 \times 10^{-12}\ s$
$1\ ns = 1\ \text{nano}\ "\ \ 1 \times 10^{-9}\ s$

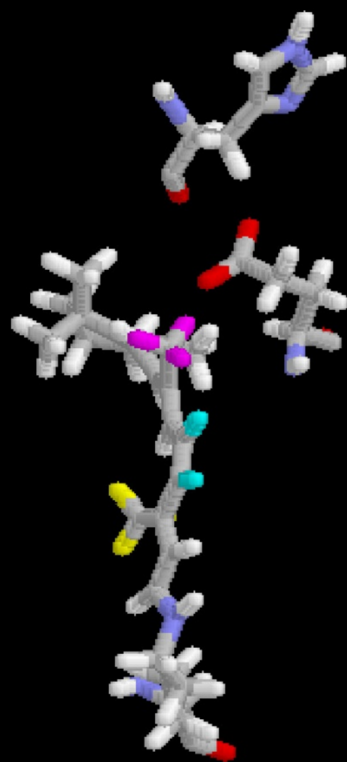
30 fs
after excitation



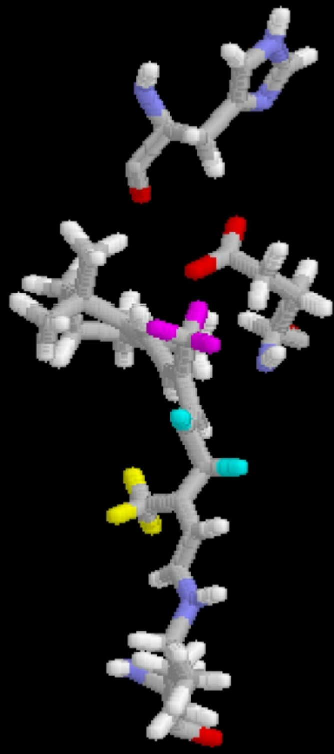
0 30 fs 530 fs 30 ps 1 ns

Snapshots of simulated isomerization of retinal Schiff base of rhodopsin (1hzx)

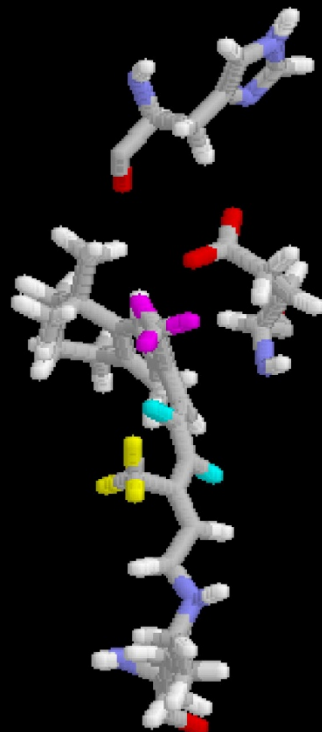
Retinal: 11-cis



excited state



all-trans



Optical Rotary Dispersion (ORD) and Circular Dichrorism (CD) of Nulcleic Acids

Very useful when biopolymers absorb light strongly and have ***chirality***

A **chiral molecule** is a type of molecule that has a non-superimposable mirror image.

A chiral molecule will rotate the plane of LINEARLY polarized light.

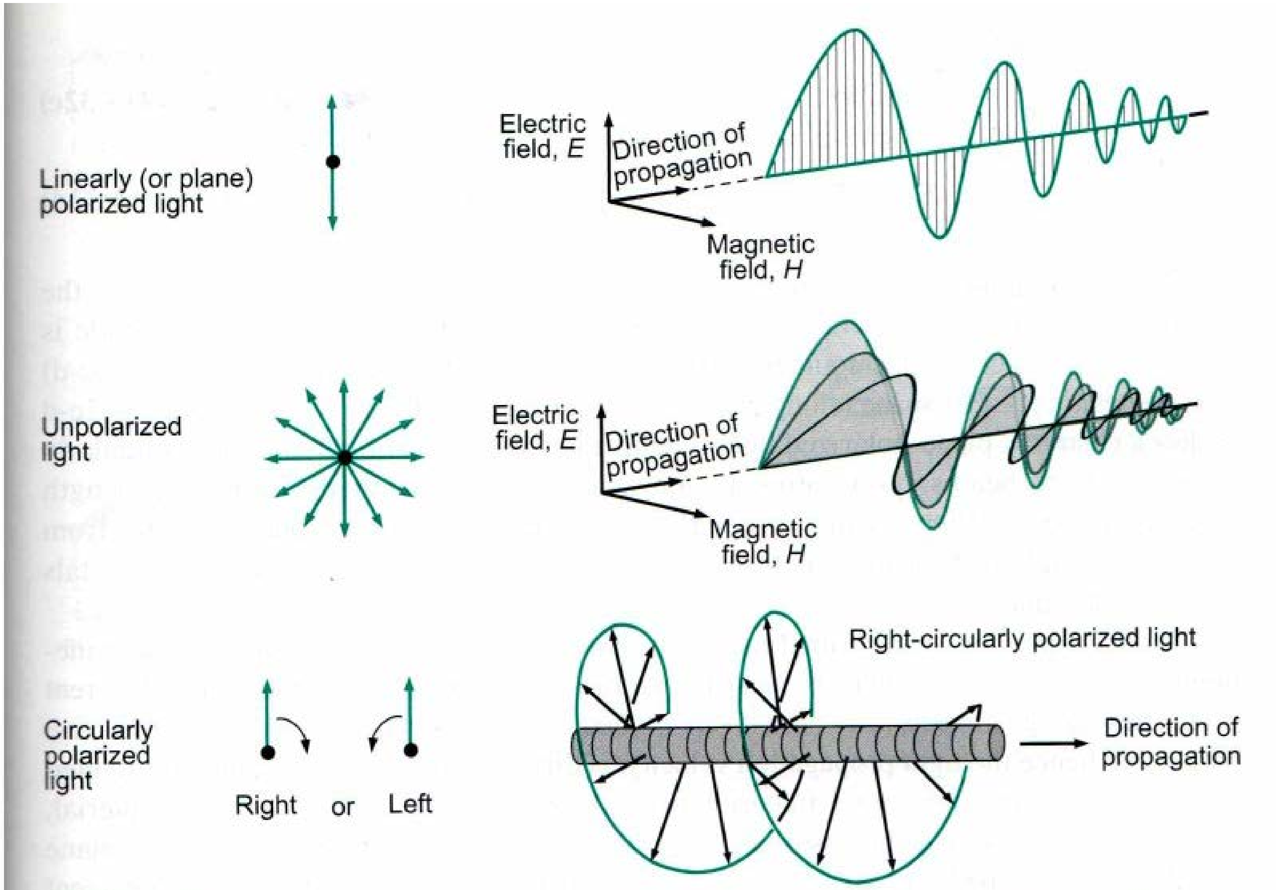


FIGURE 13.28 Different types of polarized light. In the middle column, the arrows represent the electric vector of the light as seen by an observer moving with the light (or equivalently viewing the light down the direction of propagation). The light is moving into the page.

Every photon of ***linearly polarized*** light shakes electrons back and forth in same direction.

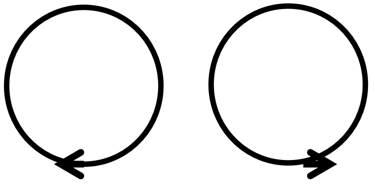
Photons of ***unpolarized*** light have ***random*** polarization directions.

The polarization of ***circularly polarized*** light photons depends on time and position in a sinusoidal manner.

What is CIRCULARLY polarized light?

With LINEARLY polarized light the electric field shake electrons back and forth in straight line.

CIRCULARLY polarized light the field continually direction rotates in a **circle** **Electrons move around in a small circle**



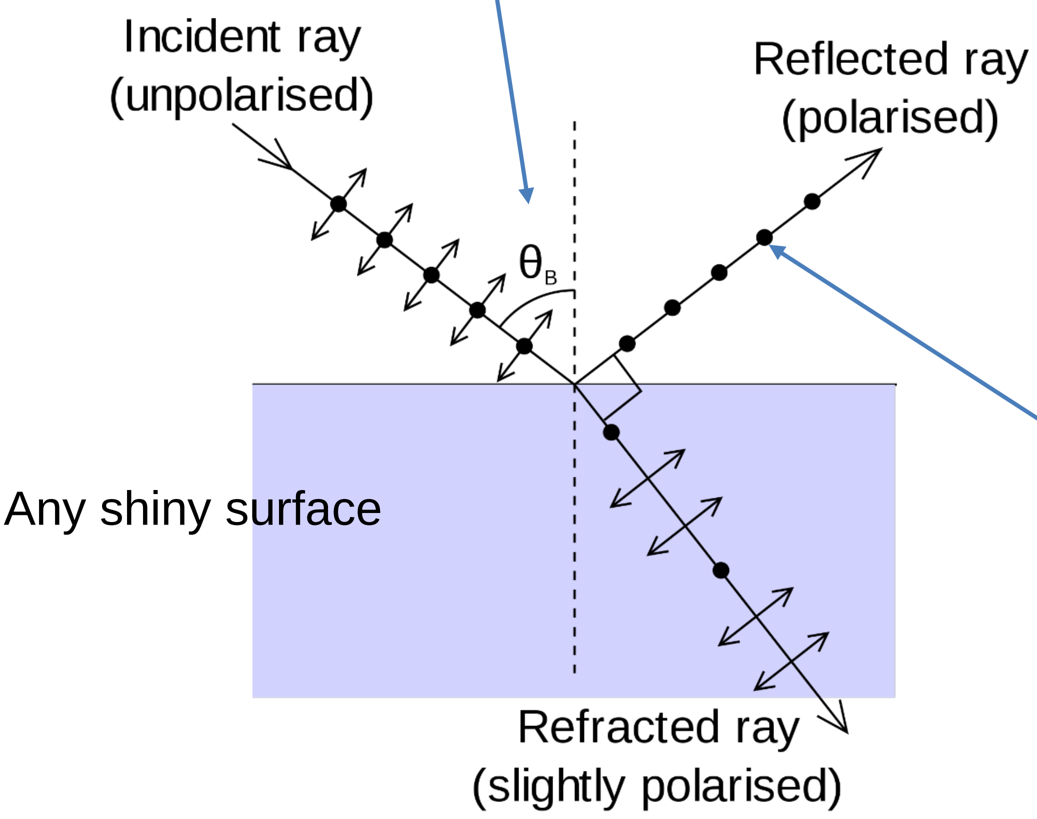
Circular Dichroism

CD signal = $\varepsilon_{\text{Left}} - \varepsilon_{\text{Right}}$

i.e., the ***difference*** of UV absorbance using **left** and **right** *CIRCULARLY* polarized light.

Suppose you are in a store to buy polaroid sunglasses: How to check whether sun glasses are polaroid or not

Brewster's Angle = ~55º



Rotate the glasses while view a relection off the floor at about 50 degree angle.

The reflected light is almost ALL HORIZONTALLY POLARIZED,
which does not pass through the sunglasses in the normal orientation

[Brewster's angle - Wikipedia]

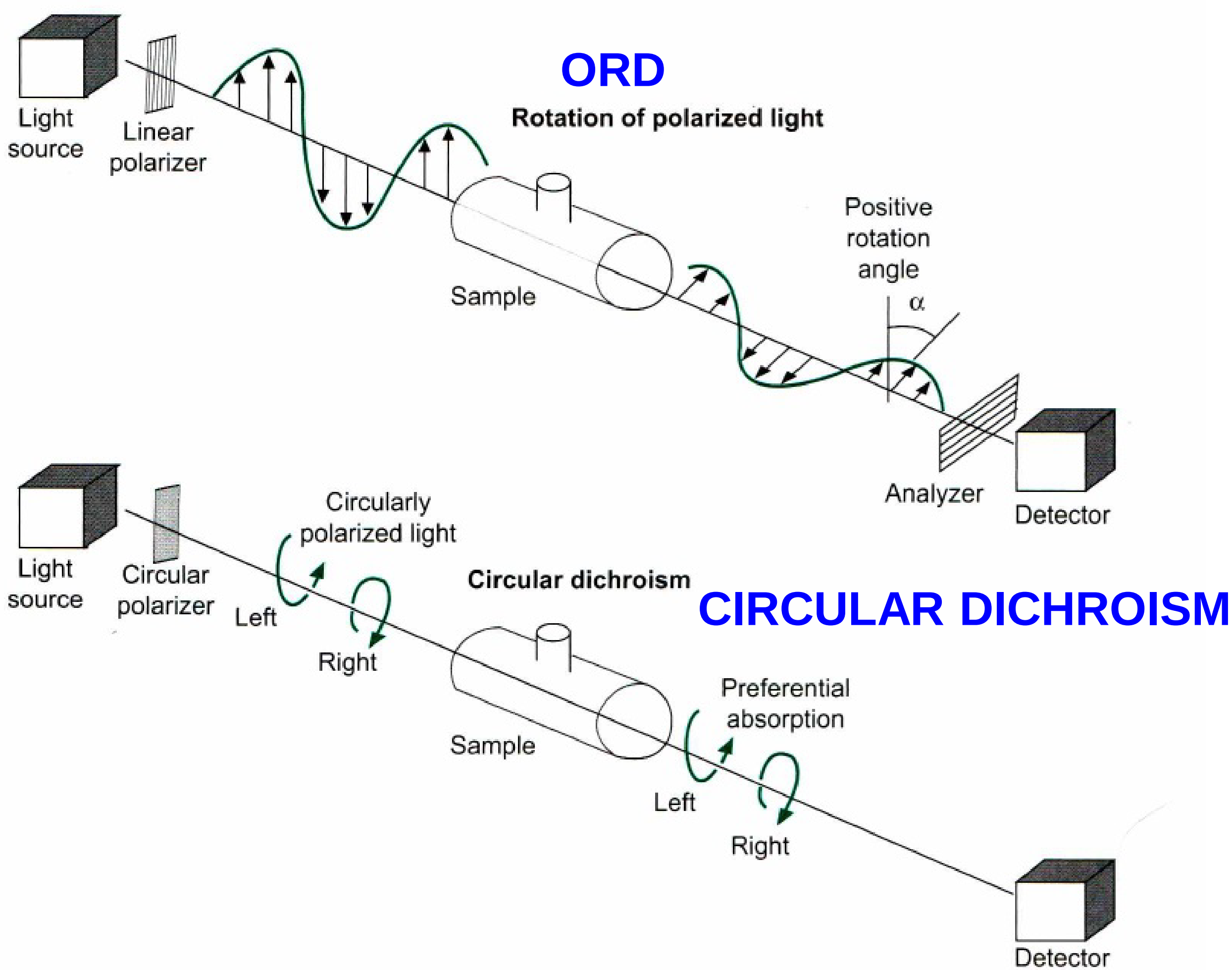


FIGURE 13.29 (top) Measurement of the rotation of linearly polarized light. If the sample in the top cell has significant absorbance, the transmitted light will be elliptically polarized (figure 13.30). (bottom) Measurement of circular dichroism, the preferential absorption of circularly polarized light. The detector measures the difference in absorbance of the right- and left-circularly polarized light.

RNA and DNA easily distinguished with CD

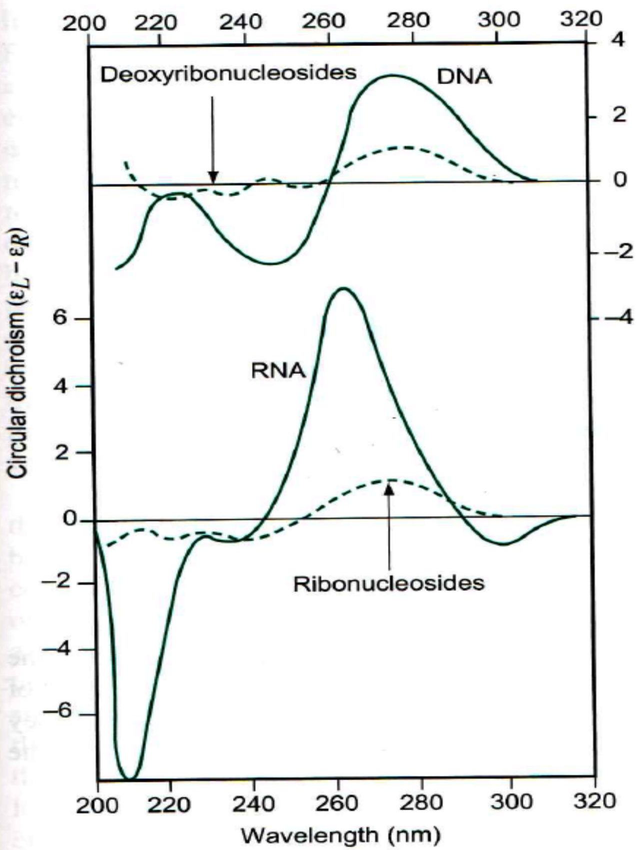


FIGURE 13.31 Circular dichroism of double-stranded DNA and RNA compared with their component mono-nucleosides.
(*M. lysodeikticus* DNA data from F. Allen et al., 1972, *Biopolymers* 11:853. Rice dwarf virus RNA data from T. Samejima et al., 1968, *J. Mol. Biol.* 34:39. Nucleoside spectra calculated from the base composition (72% G + C for the DNA; 44% G + C for the RNA) and CD data of C. R. Cantor et al., 1970, *Biopolymers* 9:1059, 1079.) (From V. A. Bloomfield, D. M. Crothers, and I. Tinoco, Jr., 1974, *Physical Chemistry of Nucleic* Acids (New York: Harper & Row), 134.)

3 forms of DNA easily distinguished with CD

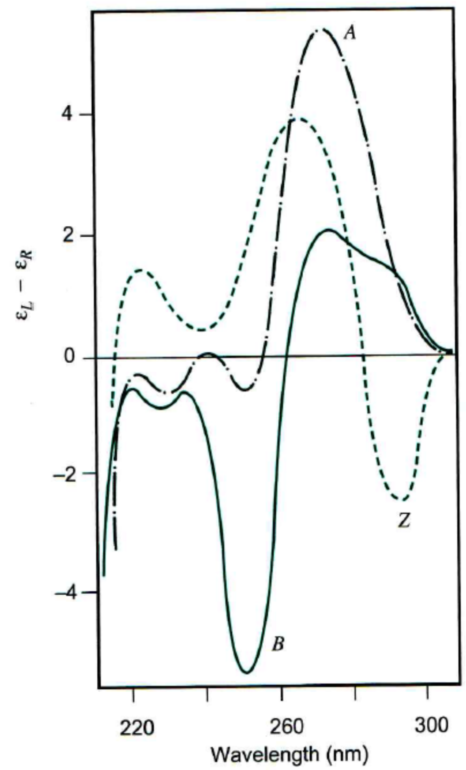


FIGURE 13.32 Circular dichroism of the synthetic polynucleotide poly(dG-dC). (poly(dG-dC) in different conformations. The poly-nucleotide is a double-stranded helix; each strand has a sequence of alternating deoxyguanylic acid (dG) and deoxycytidilic acid (dC). Different conformations are obtained by changing the solvent. The B form is obtained in 0 to 40% ethanol or 10^{-3} M to 2 M NaCl; it is a right-handed helix with about 10 base pairs per turn of the double helix. The *Z* form is obtained in 56% ethanol or 3.9 M NaCl; it is a left-handed helix. The A form is obtained in 80% ethanol; it is a right-handed helix with about 11 base pairs per turn.
(Reprinted by permission from Macmillan Publishers Ltd: *Nature*, Polymorphism of a synthetic DNA in solution, by F. M. Pohl, vol. 260, p. 365, copyright © 1976. (http://www.nature.com/nature/journal/v260/n5549/pdf/260365a0.pdf)