Fig. 10.4 Absorption and fluorescence of bacteriochlorophyll. (a) Energy-level diagram showing spectral transitions (vertical arrows). The energy levels are broadened (shading) by vibrational sublevels that are not usually resolved in solution spectra. (b) Absorption spectrum corresponding to energy levels of part (a). This spectrum is turned 90° from the usual orientation to show the relation to the energy levels. (c) Radiationless relaxation (dashed arrows) and fluorescence (shaded arrow). (d) Fluorescence emission spectrum corresponding to part (c). Note the red shift of the fluorescence compared with the corresponding $Q_{v}$ absorption illustrated in parts (a) and (b). (From K. Sauer, in Bioenergetics of Photosynthesis, Govindjee, ed., Academic Press, New York, 1975, pp. 115–181.)
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 determine quantitatively the ratio of nucleic acid to protein in a mixture of the two. This requires careful calibration, however, because proteins differ significantly in their content of aromatic 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".
FIGURE 13.27 Single-molecule studies of F₁-ATPase. (a) The F₀F₁-ATP synthase is shown schematically. The F₀ subunit is embedded in the membrane and is a proton pump. The F₁-ATPase is a rotary motor that synthesizes ATP from ADP and inorganic phosphate. (b) In the experiment, the F₁ 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 microscopy. (Reprinted from Cell, 93 (1), F₁-ATPase: A Rotary Motor Made of a Single Molecule, pp. 21, Copyright © 1998, with permission from Elsevier.)

http://www.k2.phys.waseda.ac.jp/Movies.html
FIGURE 13.27 Single-molecule studies of F$_1$-ATPase. (a) The F$_0$ F$_1$-ATP synthase 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 microscopy. (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

State 1

- ground state molecule + photon

State 2

- excited state molecule and NO photon

Excitation of molecule

\[ \Delta E_{\text{molecule}} = h\nu \]

Disappearance of photon

\[ \Delta E_{\text{light}} = -h\nu \]

\[ \Delta E_{\text{universe}} = 0 \text{ at every instant!!!} \]

FRET (not really 😊)

Only *half* the story
London dispersion force (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

NO FRET ☹️

FRET 😊

Generate new probes with large Stokes shift ("energy transfer probes") by placing D-A at R<<R₀

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

Sensors

Protease Nuclease

FRET

No FRET

Conformational transitions

Cellular work

Chameleons (Ca²⁺ sensors)

Protein-protein interaction (GFPs)

Real-time PCR

Single-molecule DNA sequencing

Should be green

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:**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Mediates</th>
<th>Drug/Hormone (ligand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenergic</td>
<td>hypertension</td>
<td>beta blockers/adrenaline</td>
</tr>
<tr>
<td>Cannabinoid</td>
<td>lots of things</td>
<td>marijuana</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhodopsin</td>
<td><strong>vision</strong></td>
<td>retinal Schiff base + light</td>
</tr>
</tbody>
</table>
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.
Snapshots of simulated isomerization of retinal Schiff base of rhodopsin (1hzx)
Retinal: 11-cis
excited state
Optical Rotary Dispersion (ORD) and Circular Dichroism (CD) of Nucleic 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.

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 sunglasses are polaroid or not

Brewster’s Angle = ~55°

Rotate the glasses while viewing a reflection 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
**ORD**

Rotation of polarized light

**CIRCULAR DICHROISM**

**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

3 forms of DNA easily distinguished with CD

**FIGURE 13.31** Circular dichroism of double-stranded DNA and RNA compared with their component mononucleosides.


(From V. A. Bloomfield, D. M. Crothers, and I. Tinoco, Jr., 1974, Physical Chemistry of Nucleic Acids (New York: Harper & Row), 134.)

**FIGURE 13.32** Circular dichroism of the synthetic polynucleotide poly(dG-dC), (poly(dG-dC) in different conformations. The polynucleotide 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.