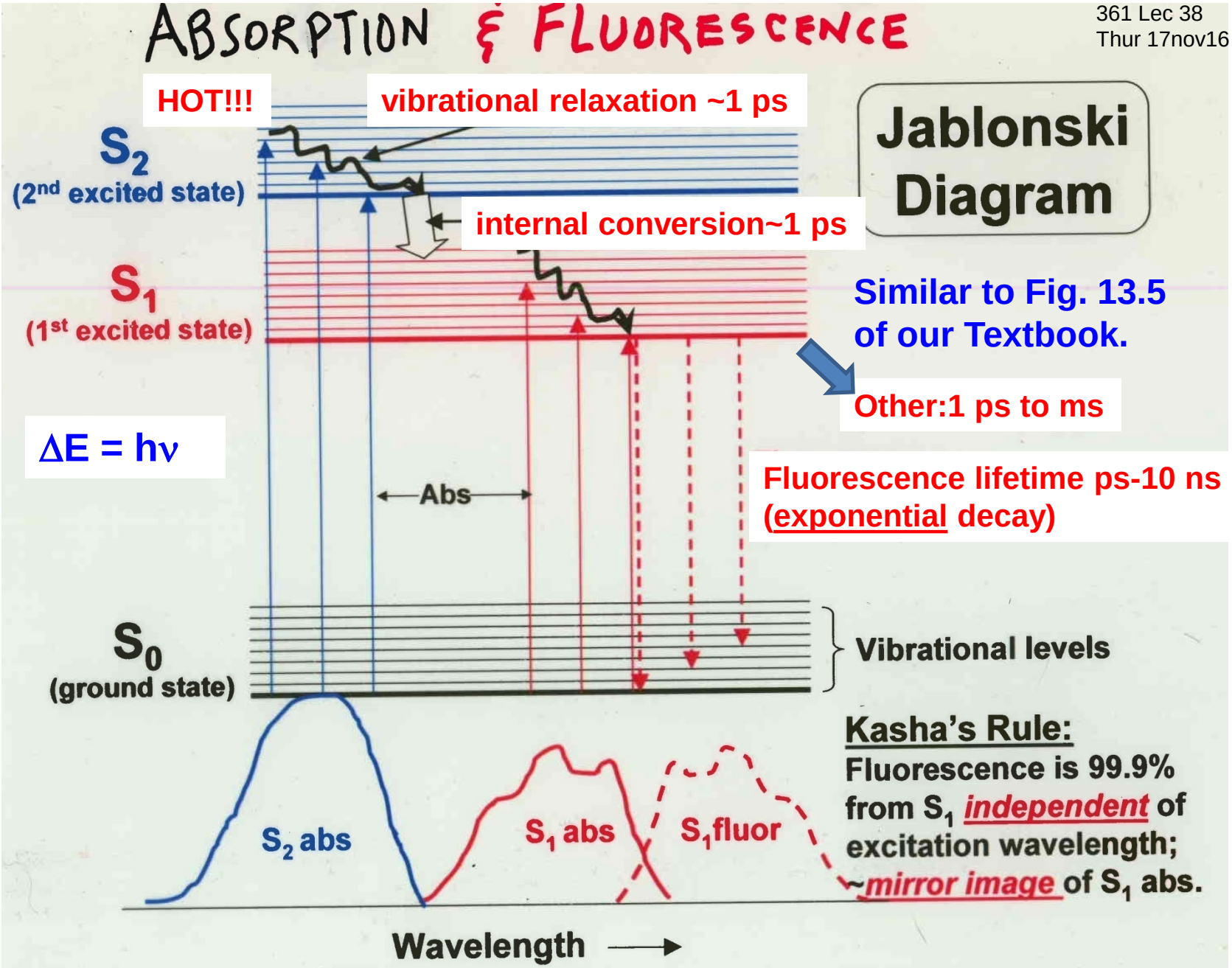
ABSORPTION & FLUORESCENCE



What is fluorescence lifetime?

d(Intensity)/dt = -k (Intensity)

Fluor. intensity at time t = (Fluor. Intensity at time 0) x e^{-kt}

= (Fluor. Intensity at time 0 x) $e^{-t/\tau}$

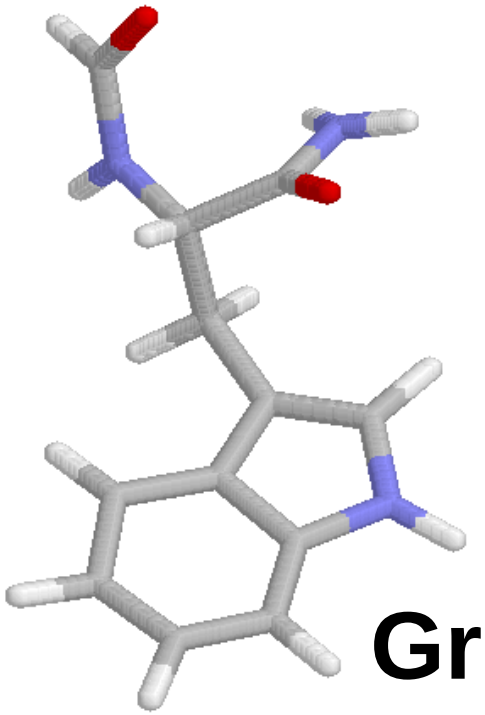
τ = "lifetime = 1/k

τ = inverse of 1st order rate constant

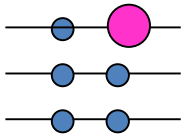
Also internal conversion to ground state, or triplet, or photochemistry will shorten the lifetime (e.g., DNA bases at room temperature, 1ps)

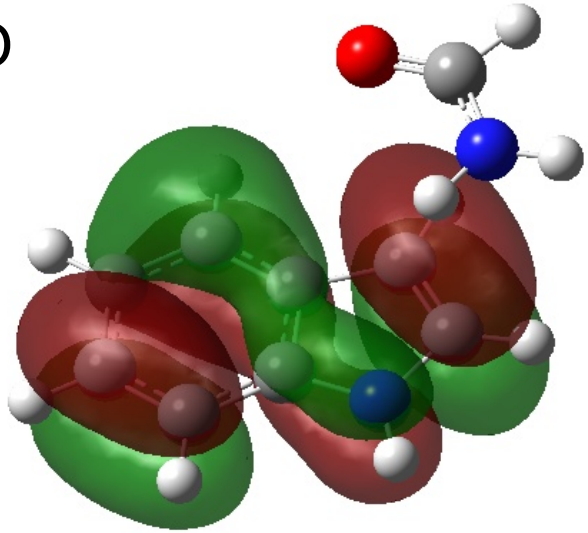
and Fluorescence "Quenching"

A reaction with another molecule that competes with the rate of fluorescence



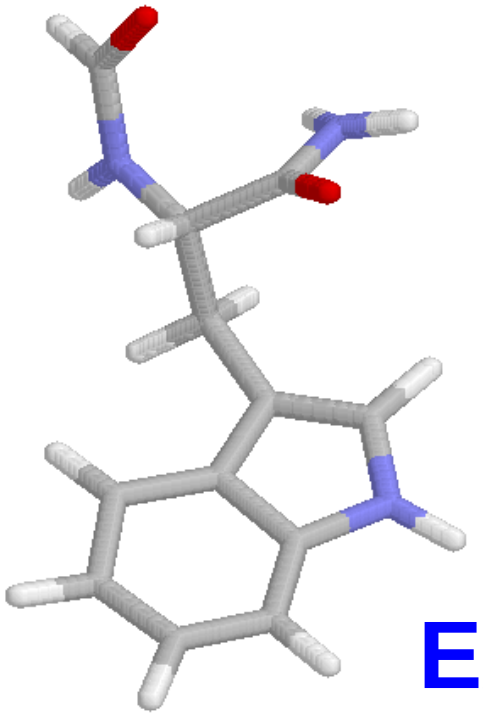
LUMO

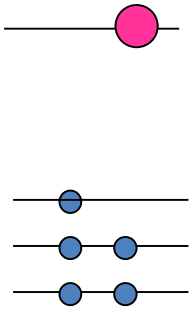


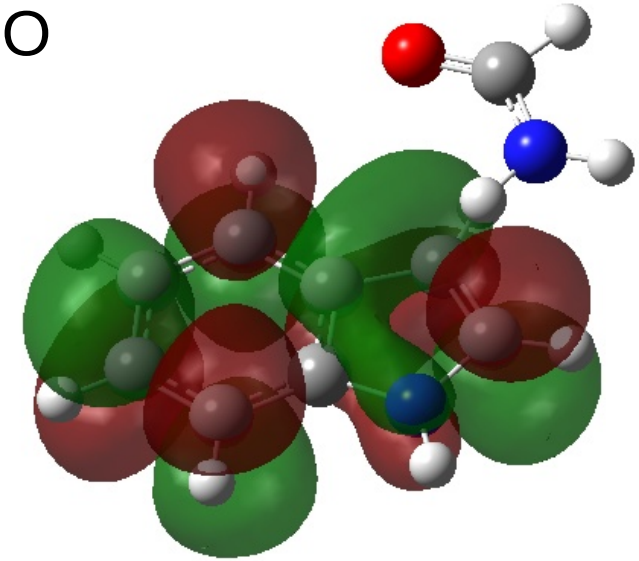


Ground State

Highest Occupied Molecular Orbital (a linear combination of atomic p orbitals)

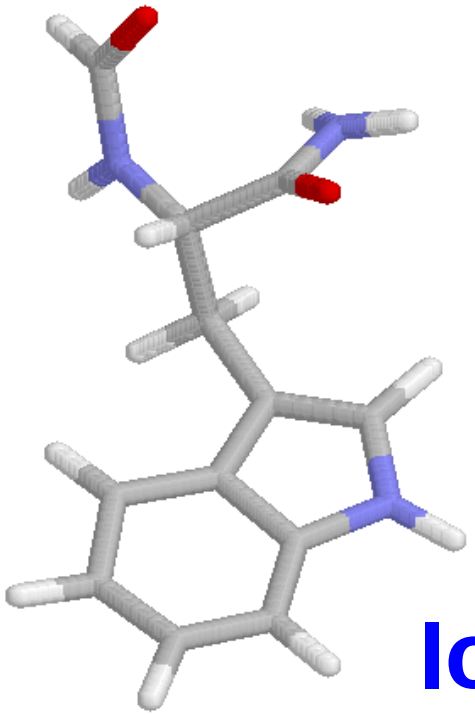


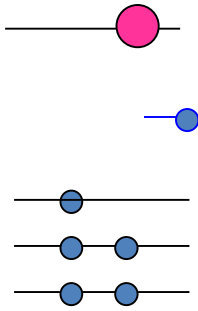


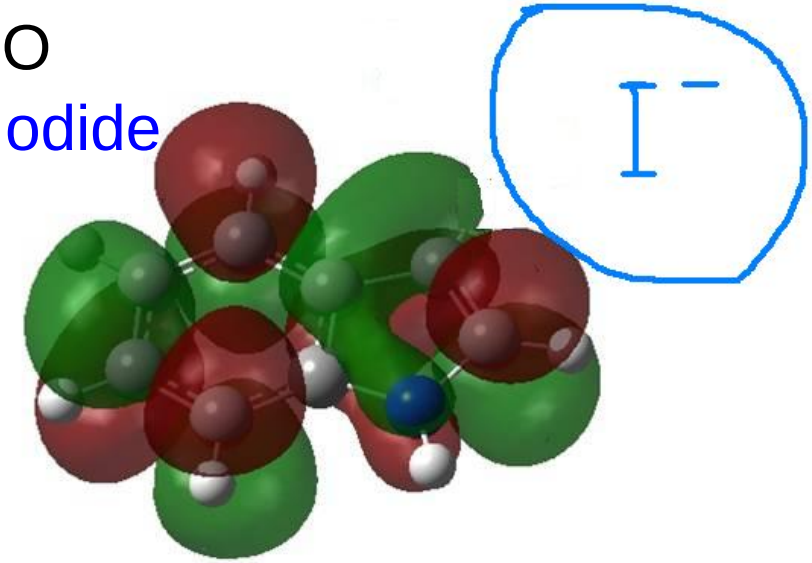


Excited State (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)

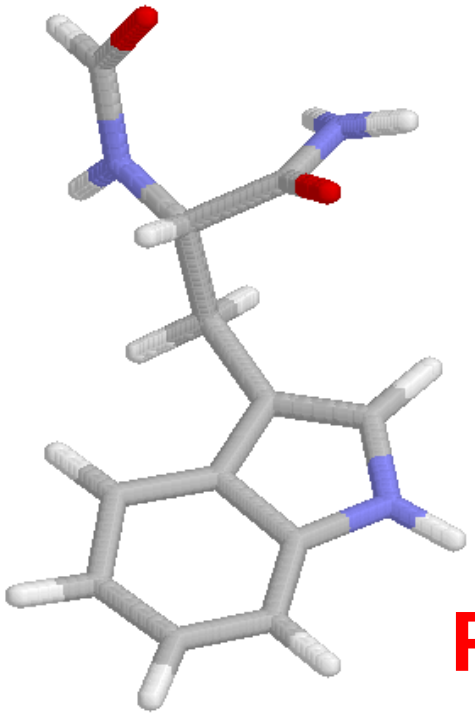


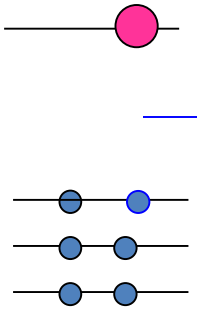


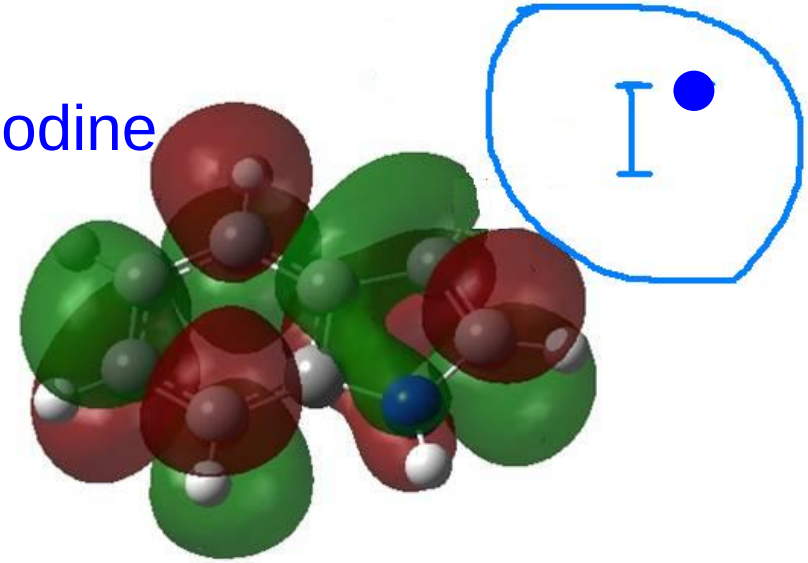


Iodide ion collides;
(has higher HOMO)
will quench fluorescence

Electron transfer from I^- to indole makes a radical pair that cannot fluoresce. (would violate Pauli exclusion)





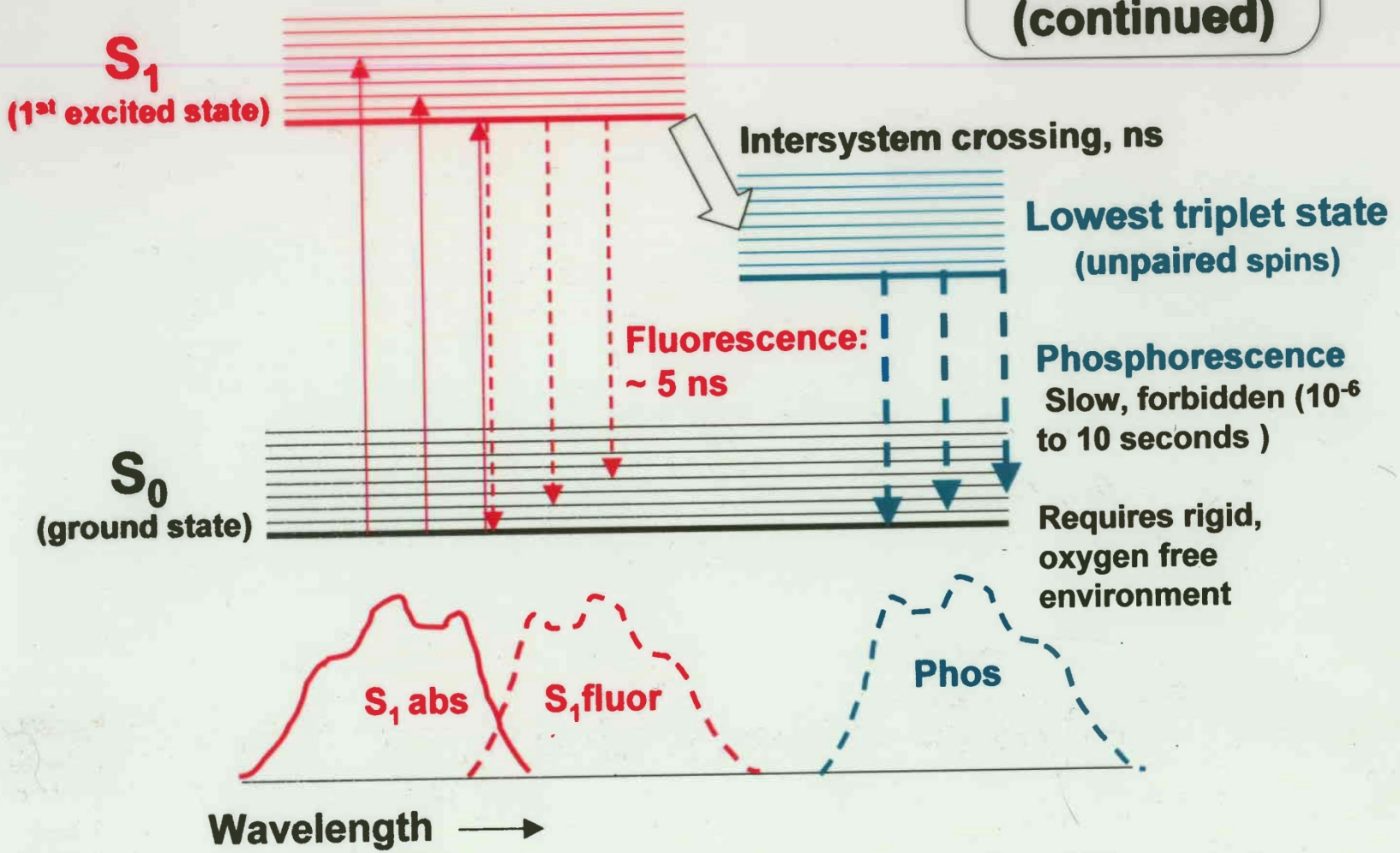


Radical Pair (can't fluoresce)

Electron transferred from iodide to vacancy in HOMO of ring
i.e., <u>QUENCHING</u>

Phosphorescence

Jablonski Diagram (continued)



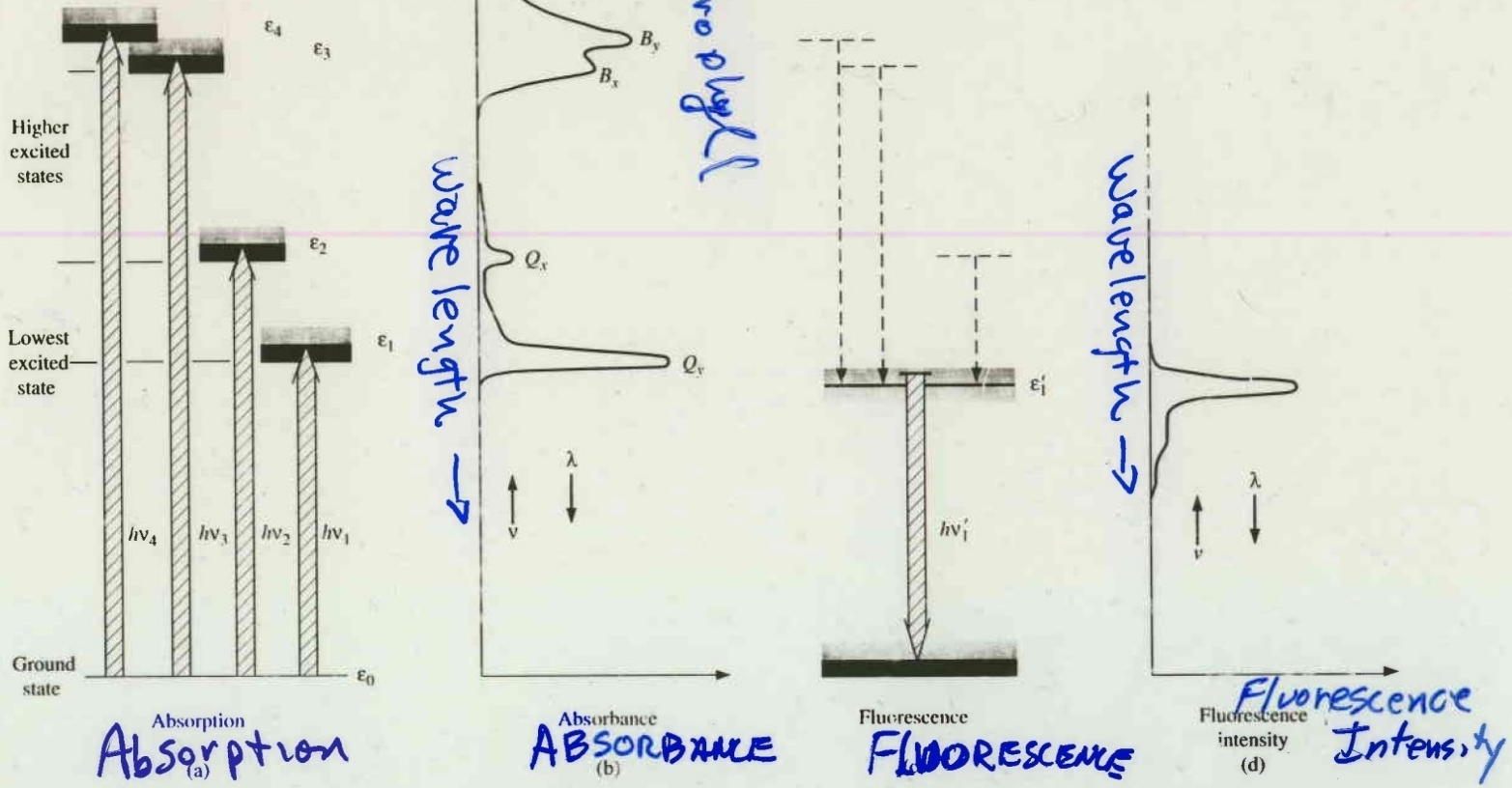
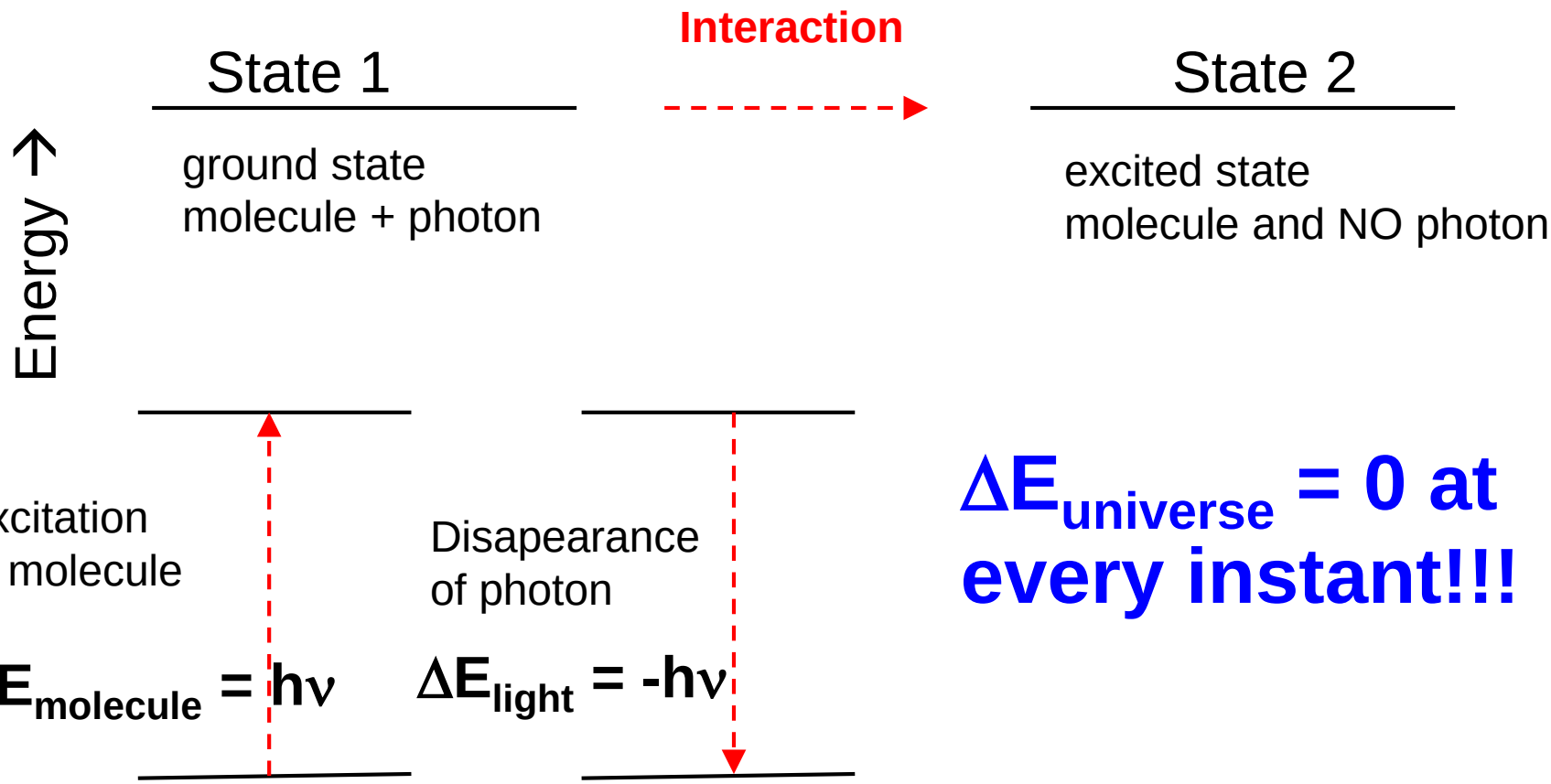


FIG 13.22

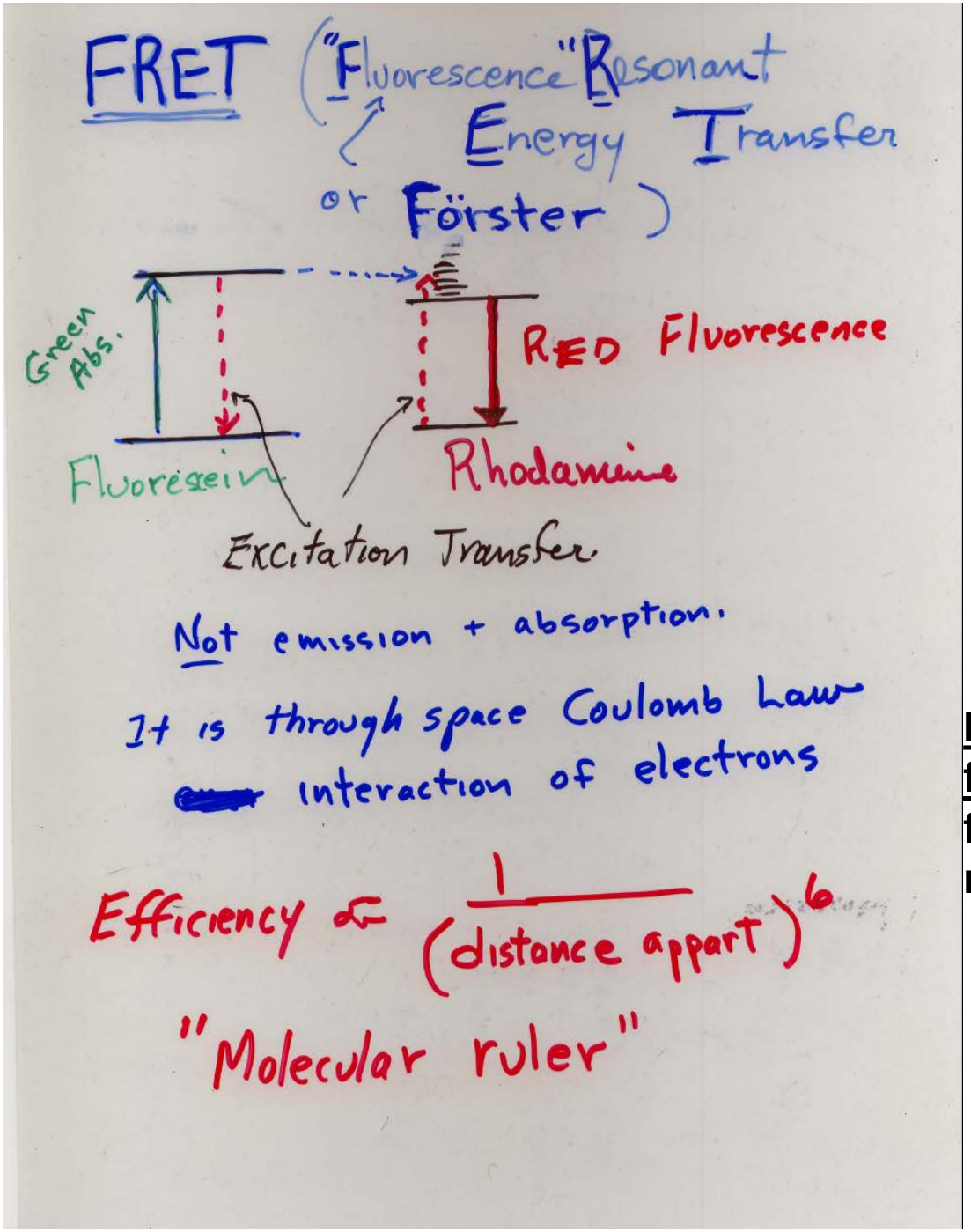
Fig. 10.16 Absorption and fluorescence of bacteriochlorophyll. (a) Energy-level diagram showing spectral transitions (vertical arrows). The energy levels are broadened (shading) by vibrational sublevels that are not usually resolved in solution spectra. (b) Absorption spectrum corresponding to energy 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_y absorption illustrated in parts (a) and (b). (From K. Sauer, in *Bioenergetics of Photosynthesis*, Govindjee, ed., Academic Press, New York, 1975, pp. 115–181.)

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!!!

Only ***half*** the story

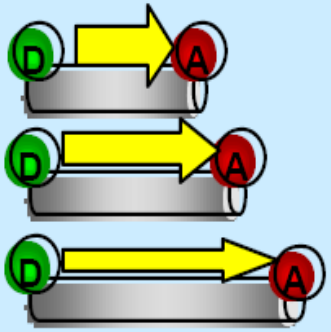


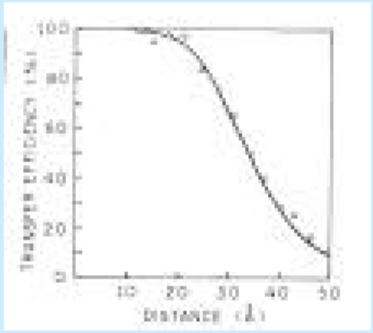
London dispersion force (the very same force that holds liquid nitrogen together.

FRET: APPLICATIONS

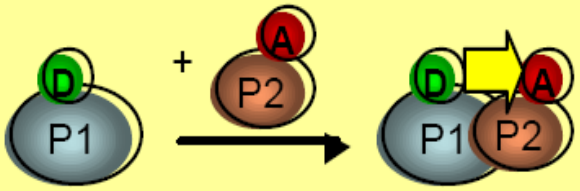
Measure distances within macromolecules; generate structures of large complexes

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



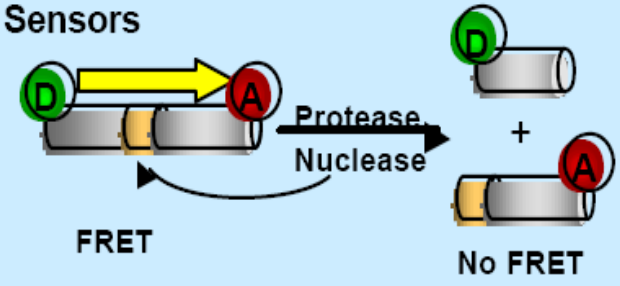


Monitor interactions & their kinetics



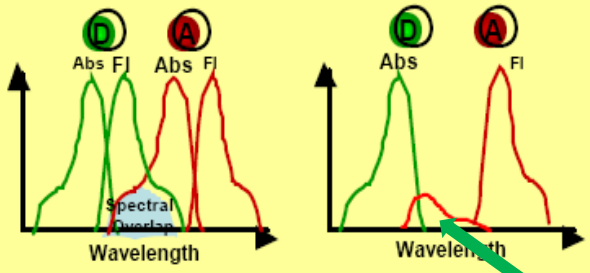
NO FRET ☹ FRET ☺

Sensors



FRET No FRET

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



should be green

- 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

Hypochromic Effect due to Stacking of bases.

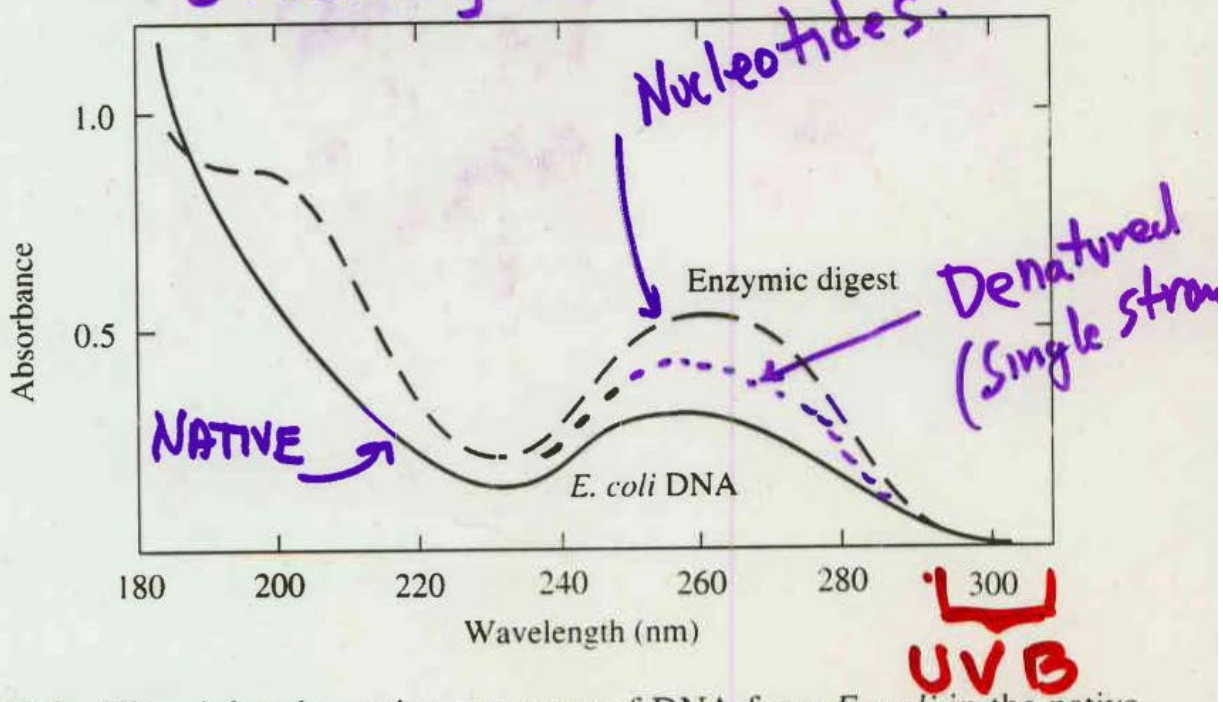
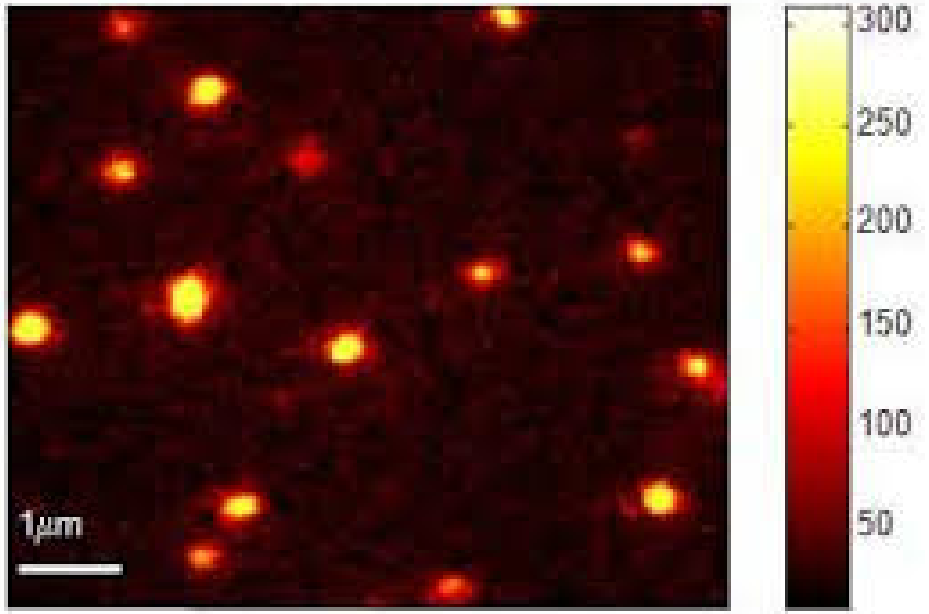


Fig. 10.9 Ultraviolet absorption spectrum of DNA from *E. coli* in the native form at 25°C (solid curve) and as an enzymic digest of nucleotides (dashed curve). [From D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers 1*, 193 (1963).] Reprinted with permission of John Wiley and Sons, Inc.

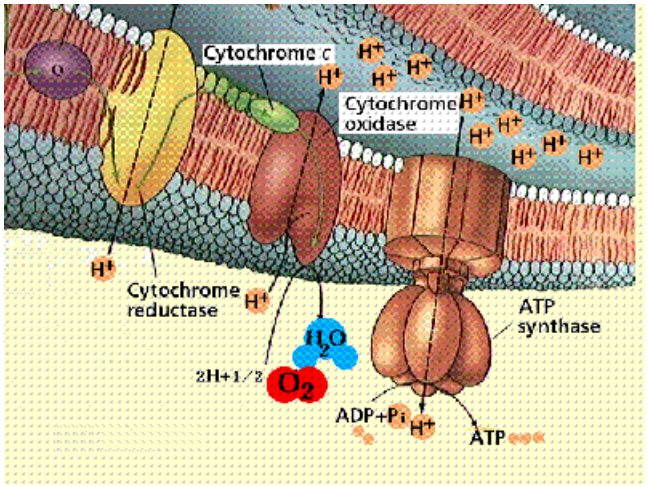
are sufficiently general that the ratio of absorbances A_{260}/A_{280} has been used to deter quantitatively the ratio of nucleic acid to protein in a mixture of the two. This requires ful calibration, however, because proteins differ significantly in their content of aron amino acids.

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



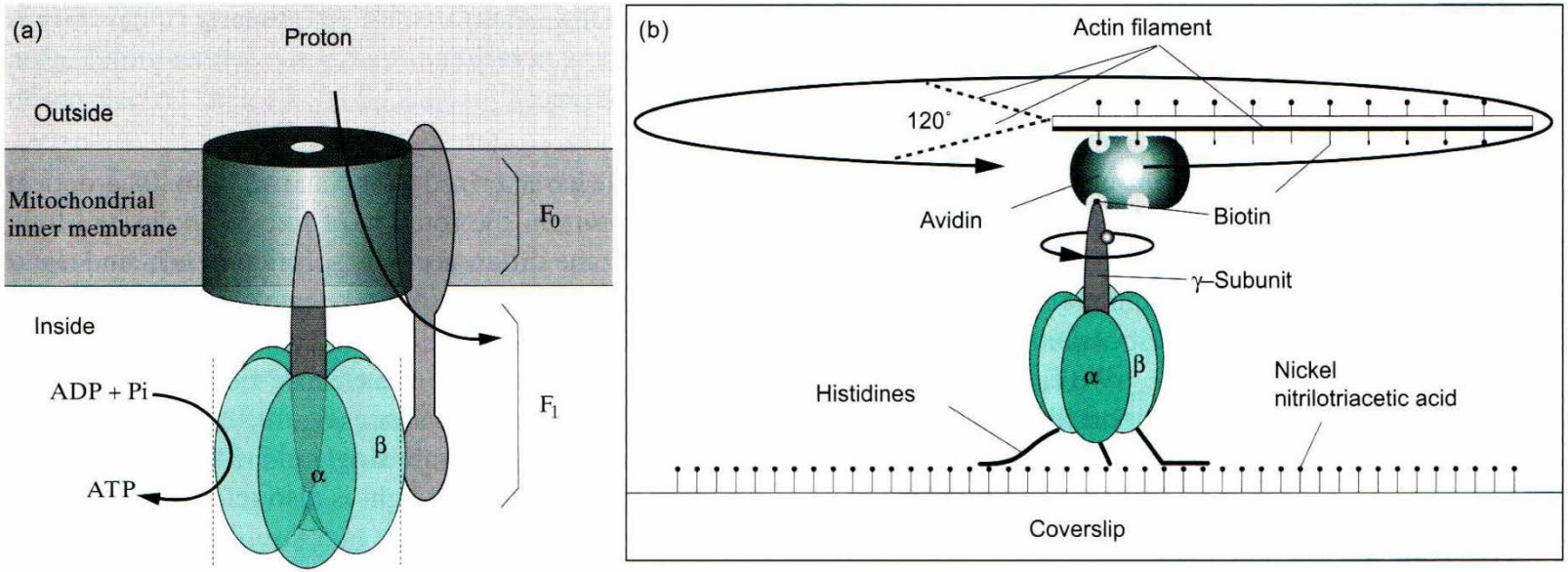


FIGURE 13.27 Single-molecule studies of F_1-ATPase. (a) The F_0F_1-ATP synthesase is shown schematically. The F_0 subunit is embedded in the membrane and is a proton pump. The F_1-ATPase is a rotary motor that synthesizes ATP from ADP and inorganic phosphate. (b) In the experiment, the F_1 subunit was 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.
(Reprinted from Cell, 93 (1), F1-ATPase: A Rotary Motor Made of a Single Molecule, pp. 21, Copyright © 1998, with permission from Elsevier.)