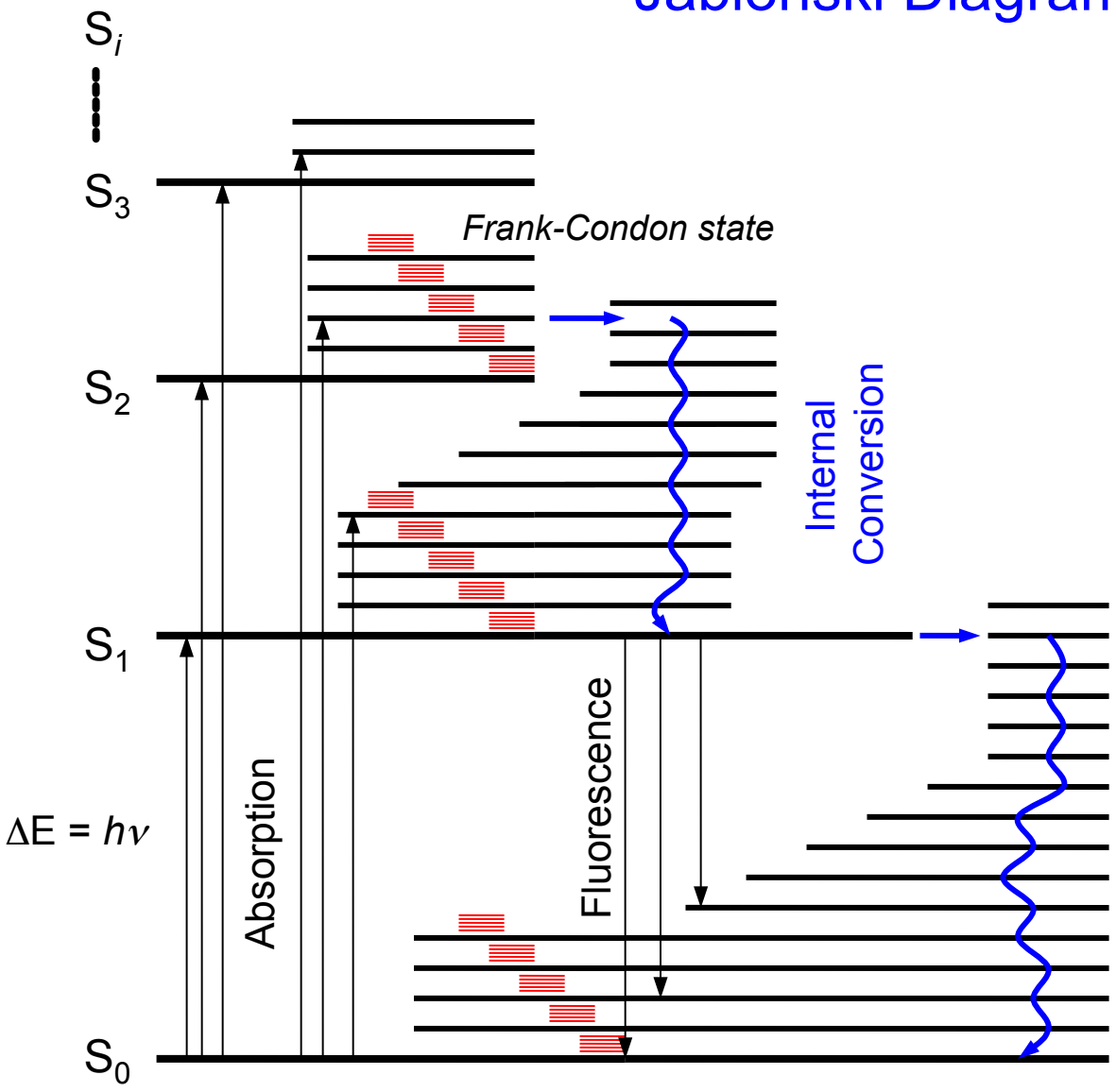
Spectroscopy

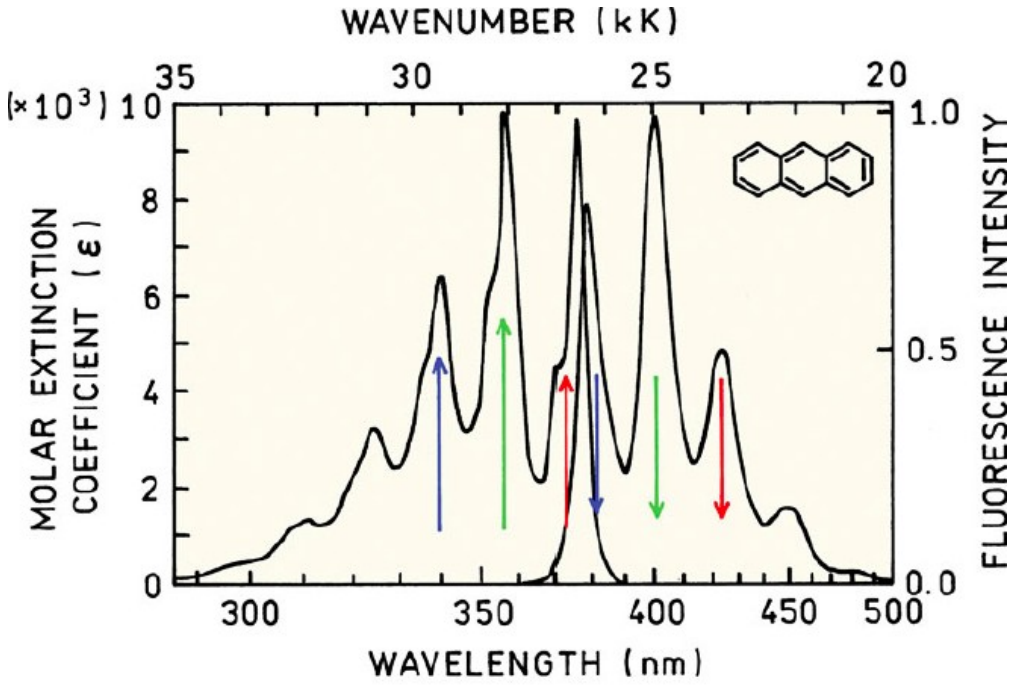
Jabłonski Diagram

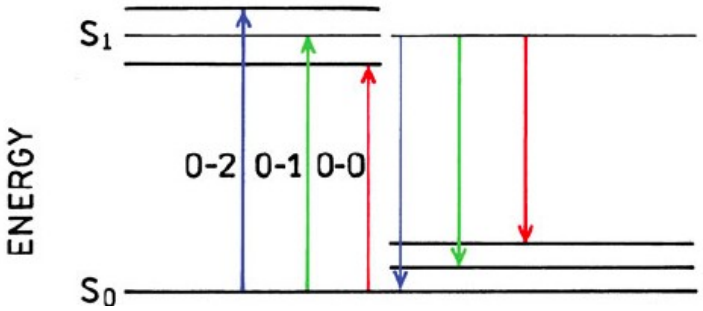


Time Scales

Abs.: ~ 10^{-15} s
IC.: < 10^{-12} s
Fluor: ~ 10^{-9} s

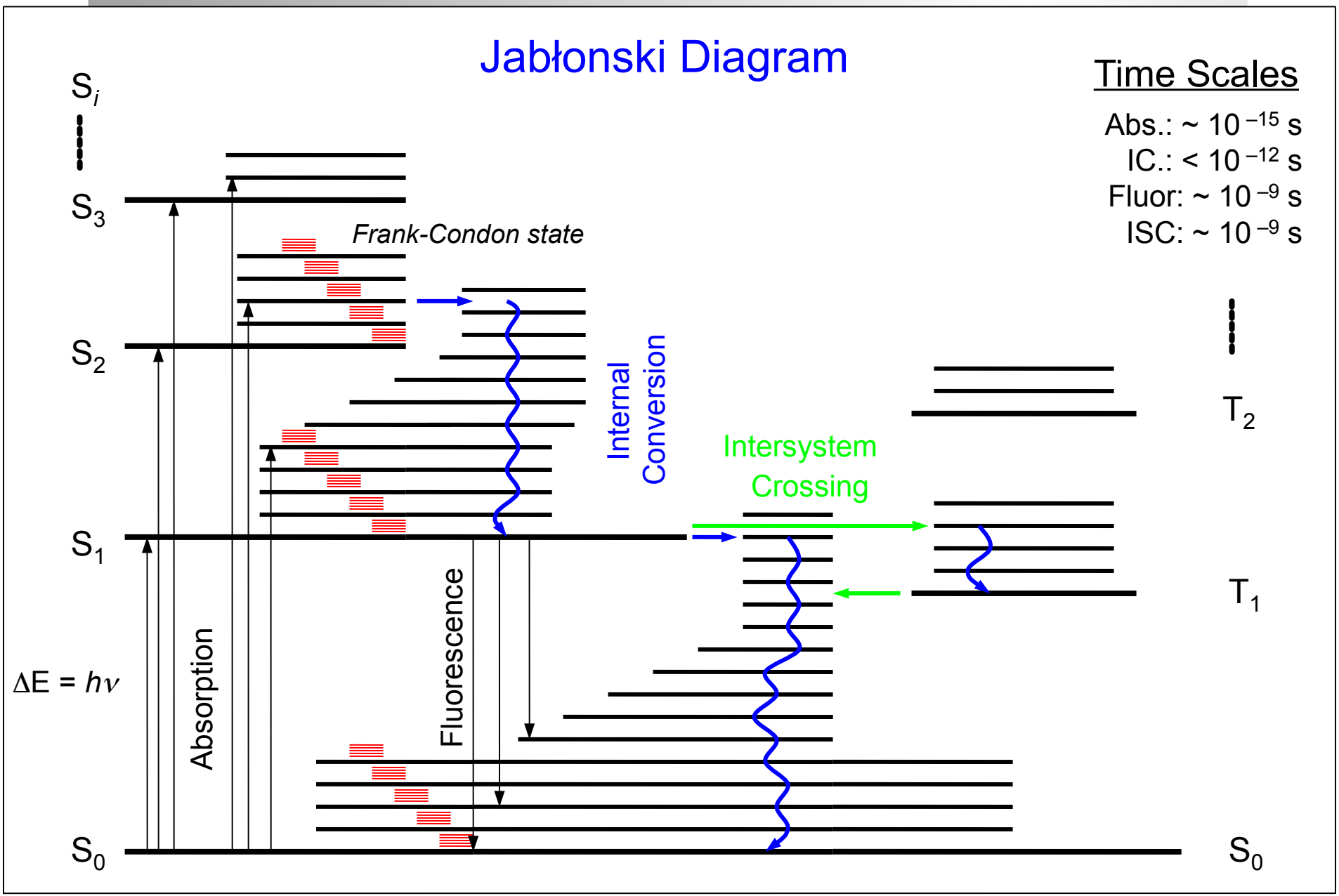
Spectroscopy



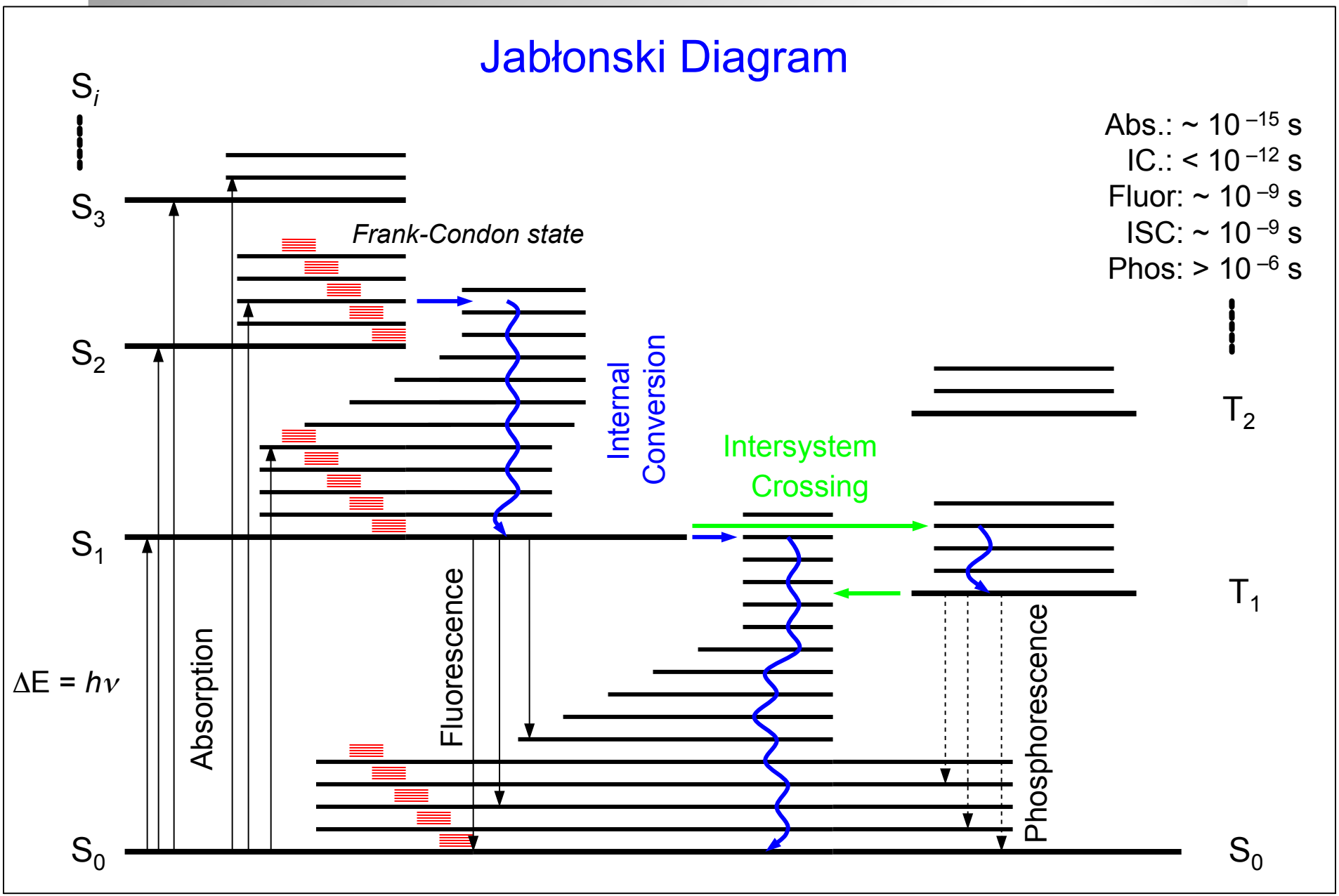


Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy

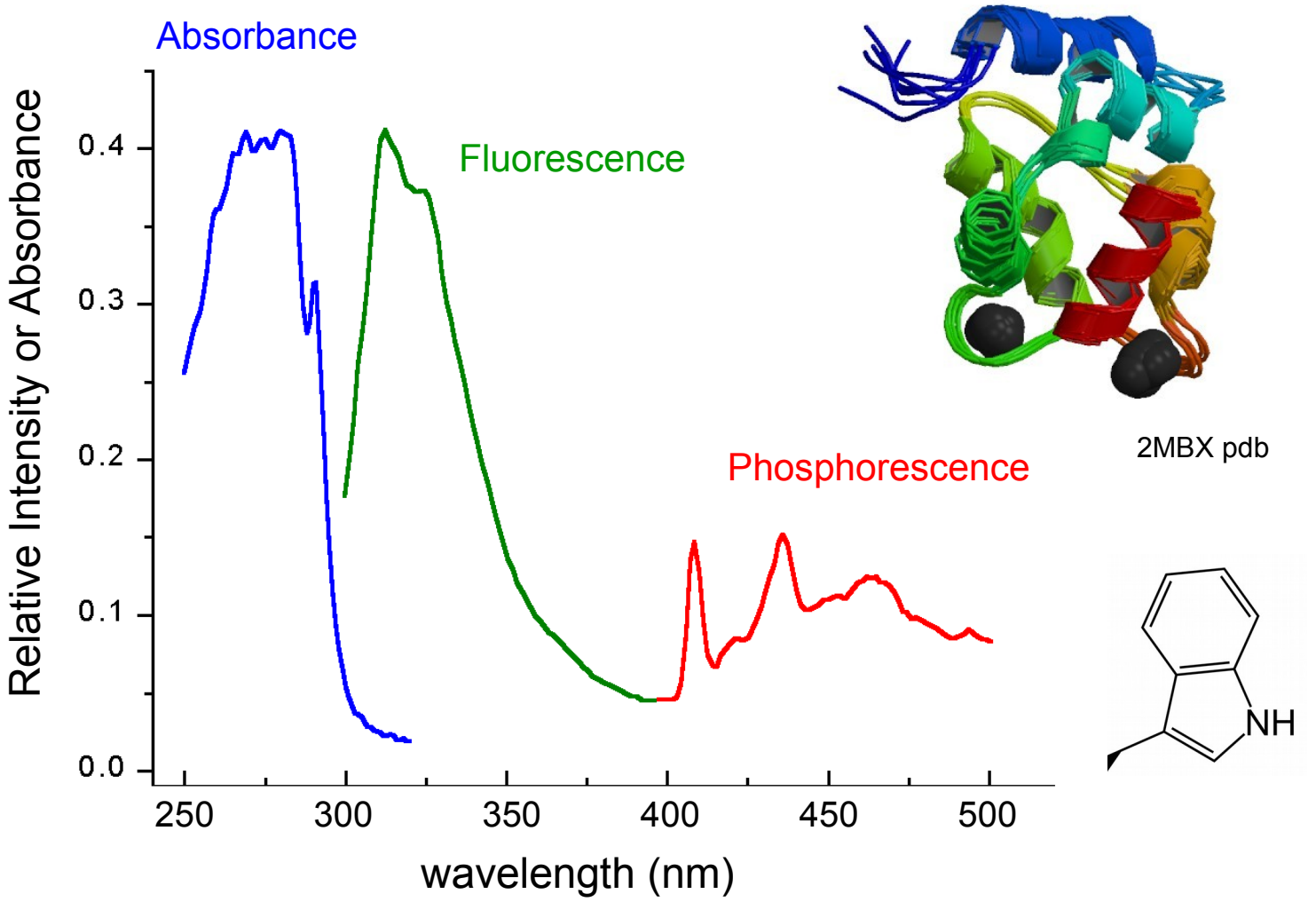


Spectroscopy



Spectroscopy

Single Trp Residue in Cod Parvalbumin; 77 K



2MBX pdb

Spectroscopy

Loss of Energy from Excited State back to Ground State

- Internal Conversion (IC; mainly through vibrational relaxation)
- Quenching: collisions with solvent, solutes, or groups of chromophore
- Intersystem Crossing (ISC)
  phosphorescence from long-lived triplet state
- Förster Resonance Energy Transfer (FRET)
- Emission of a photon
  fluorescence from lower energy than from initial Frank-Condon state; Stoke's shift
- Excited-State Reactions

Excited-State Reactions

- Bond Breaking (UV, x-ray)
- Bleaching
  - reactions with O_2, etc.
  - photorecovery experiments
- Labeling Reactions
- Generation of New Emitters
  - proton transfer ($A^* \leftrightarrow B^{-*} + H^+$)
  - excimer formation (excited-state dimer: $A^* + A \leftrightarrow A\,A^*$)
- Solvent (dipolar) Relaxation

  $S_1 \rightarrow S_1' \rightarrow \rightarrow S_1'' \rightarrow \rightarrow \rightarrow S_1''' \rightarrow \rightarrow \rightarrow \rightarrow \ldots..$

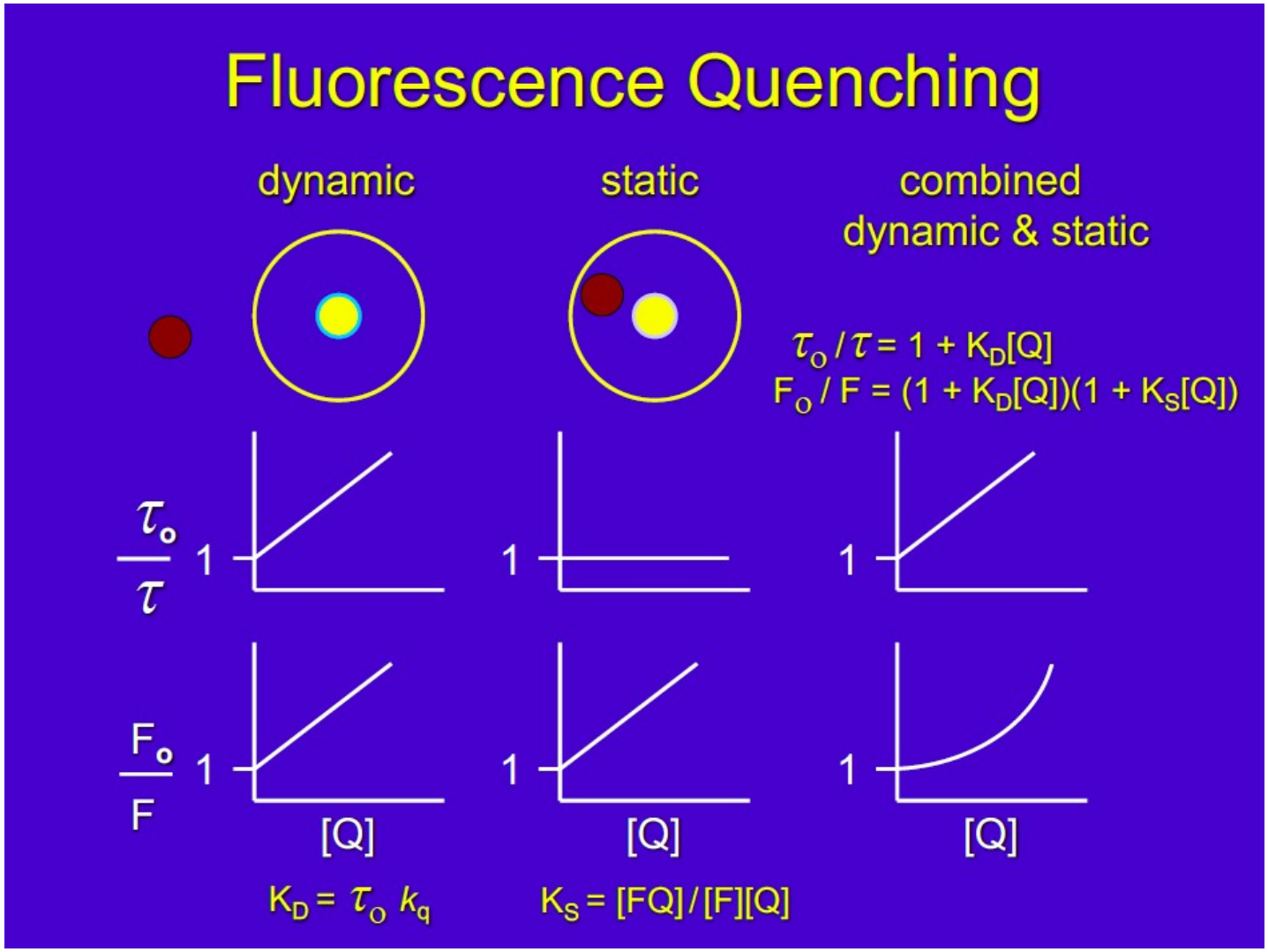
Lifetime and quantum yield

$\tau = 1 / (K_f + \Sigma K_{nr})$ excited-state lifetime

$\varphi = K_f / (K_f + \Sigma K_{nr})$ excited-state quantum yield

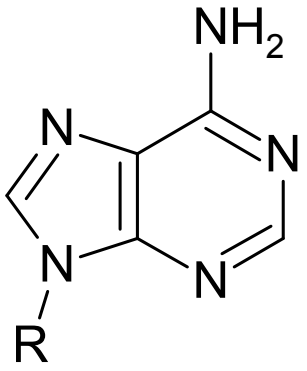
$F_\lambda = \varphi A_\lambda = \varphi \varepsilon_\lambda c l$ fluorescence intensity

ΣK_{nr} = IC + ISC + e^- transfer + ….. other dynamic processes

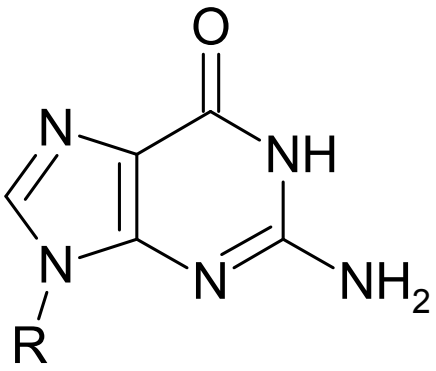

Fluorescence Quenching
dynamic
static
combined
dynamic & static
$\tau_o / \tau = 1 + K_D[Q]$
$F_o / F = (1 + K_D[Q])(1 + K_S[Q])$
$\frac{\tau_o}{\tau}$
$\frac{F_o}{F}$
1
[Q]
$K_D = \tau_o k_q$
$K_S = [FQ]/[F][Q]$

Spectroscopy

Natural Bases

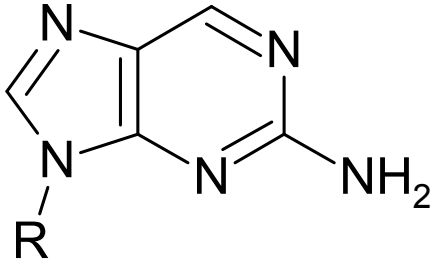


Adenine (6-aminopurine)

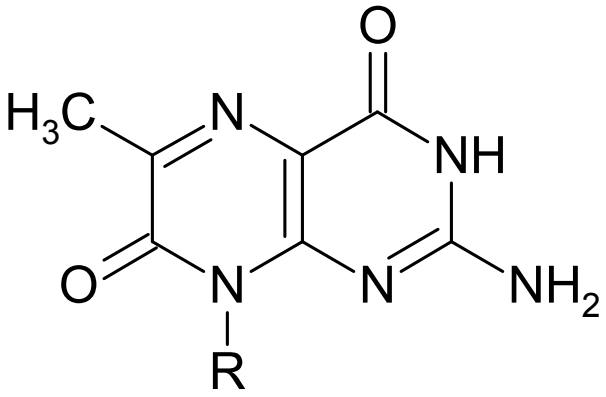


Guanine

Fluorescent Analogs

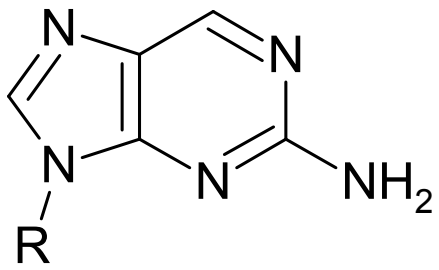


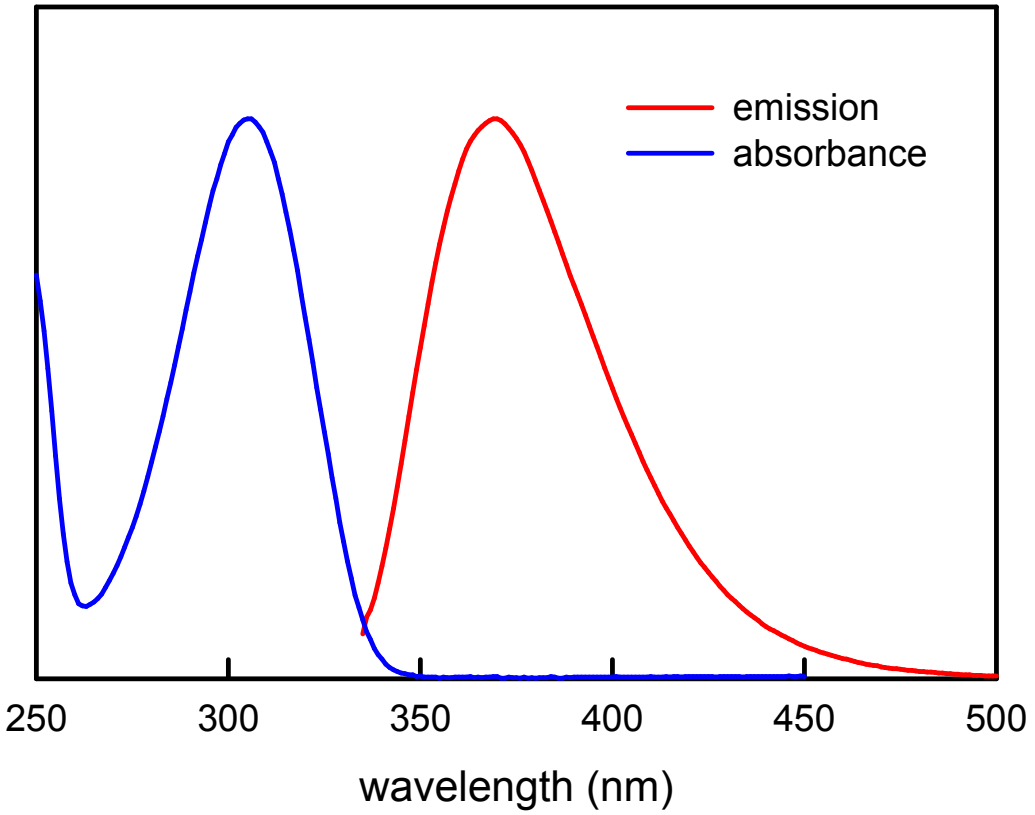
2-Aminopurine (2AP)



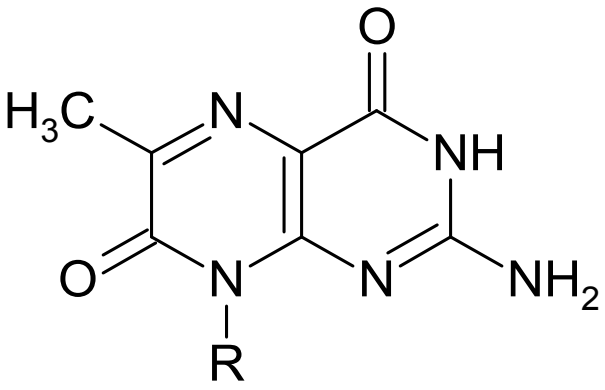
6-Methylisoxanthopterin (6MI)

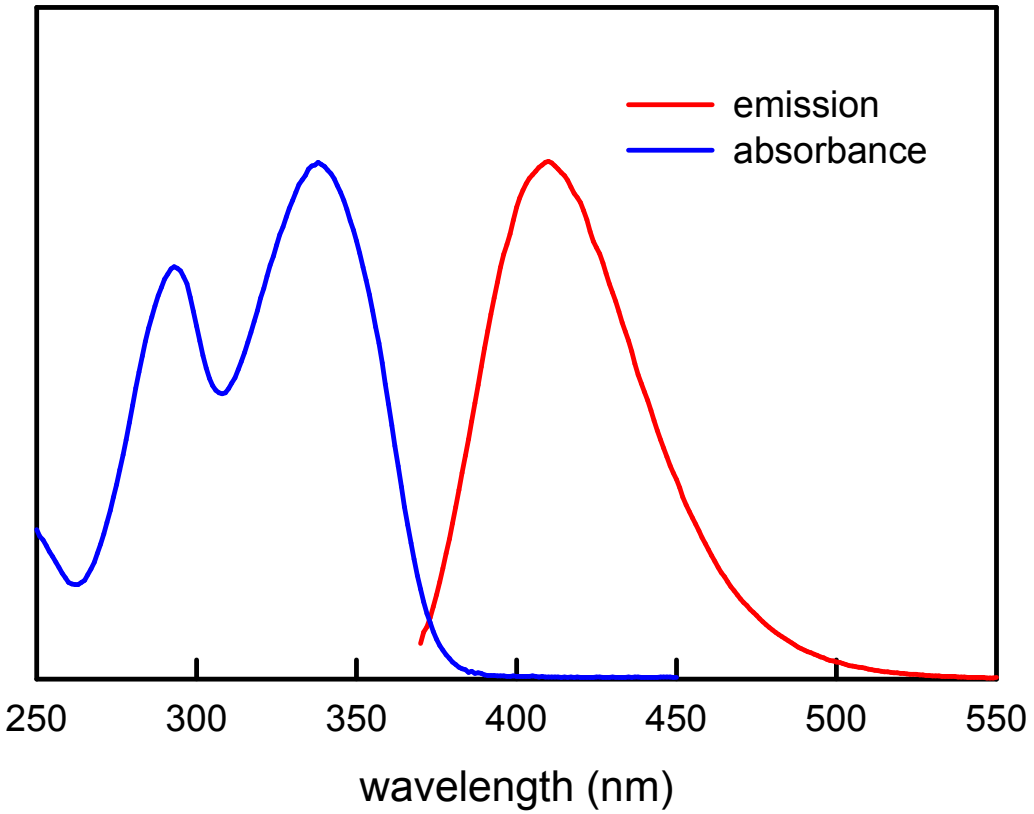
2-Aminopurine (2AP)





6-Methylisoxanthopterin (6MI)





Spectroscopy

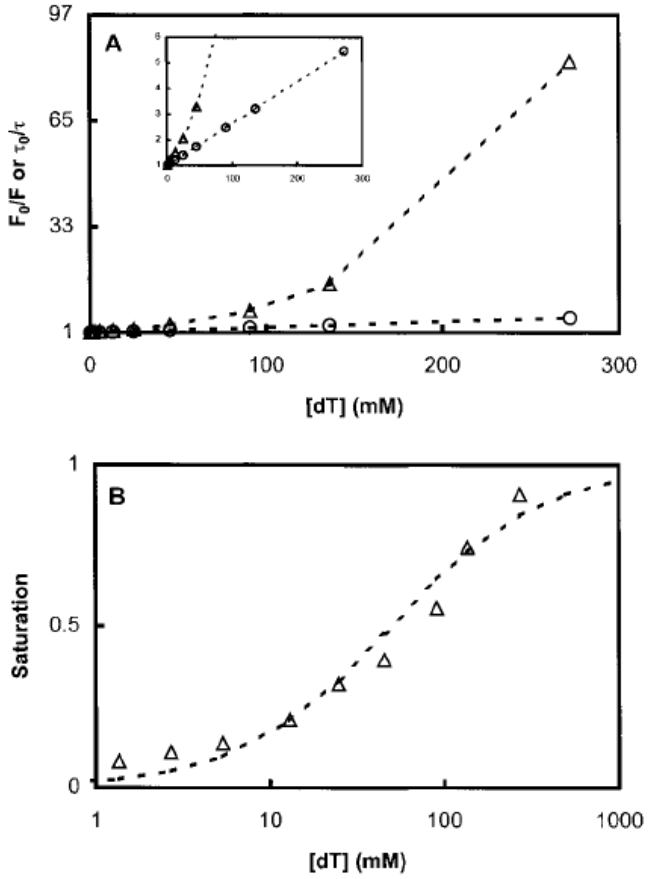
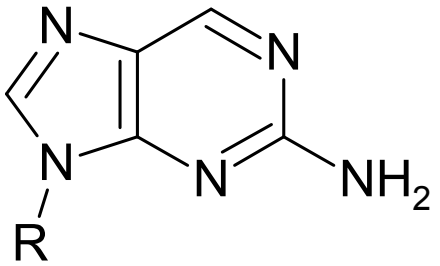
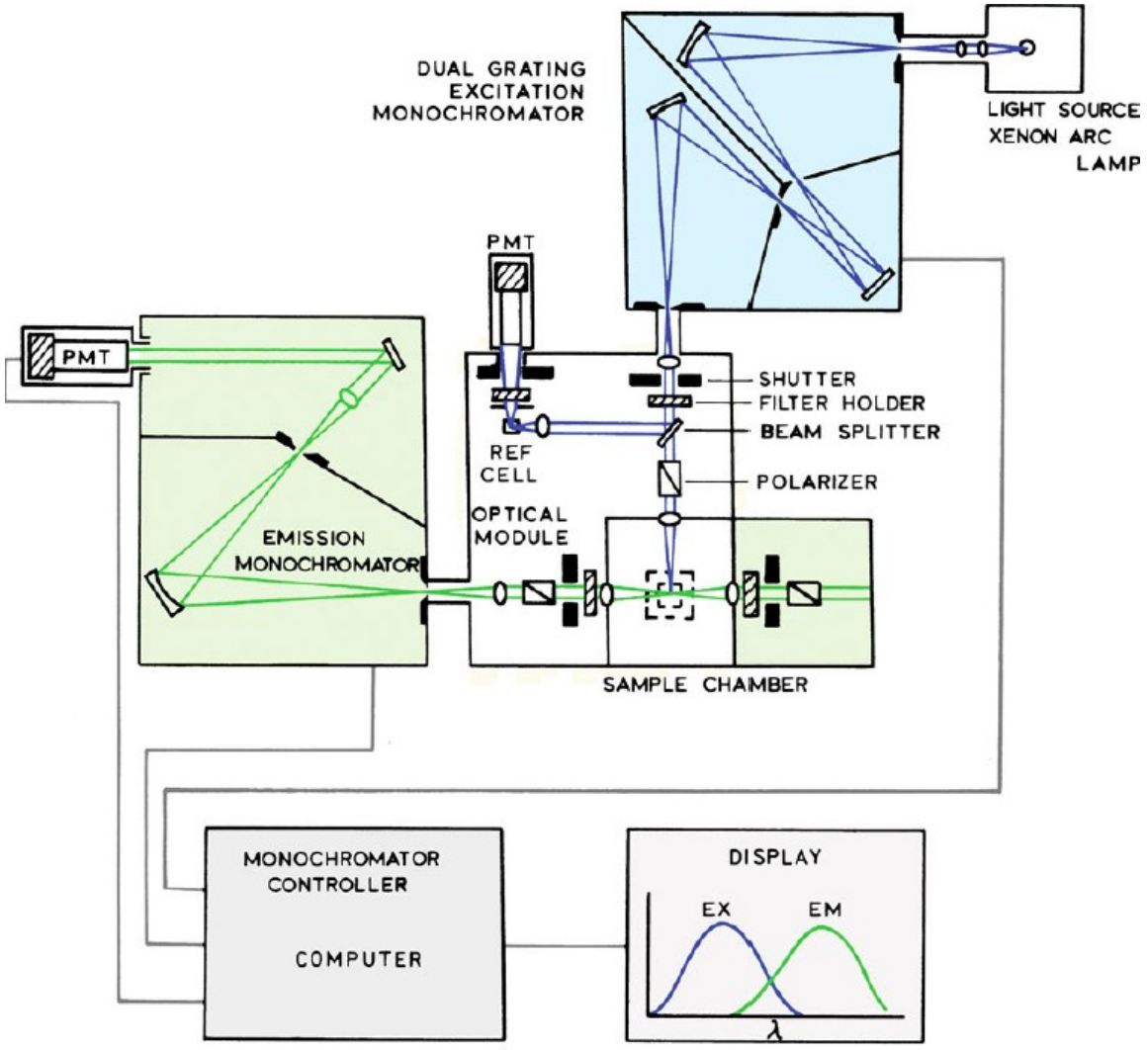


FIGURE 1: Quenching of d2AP by dT. (A) Stern−Volmer plot of F_0/F (triangles) and τ_0/τ (circles) vs [dT]. The inset shows an expansion of the ordinate axis; (B) Fractional saturation of dT: d2AP binding interaction. The points represent experimental values; the line represents the best fit of eq 8 to these values.



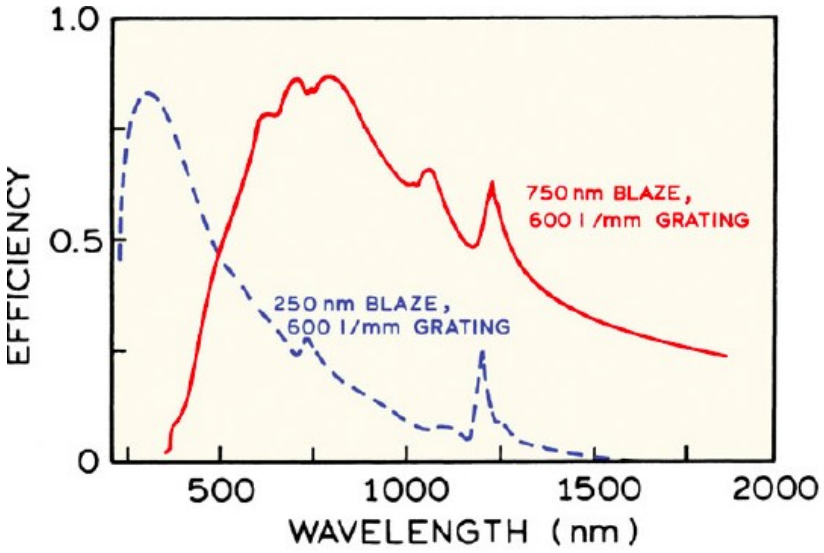
Rachofsky et al (2001)
Biochemistry 40, 946

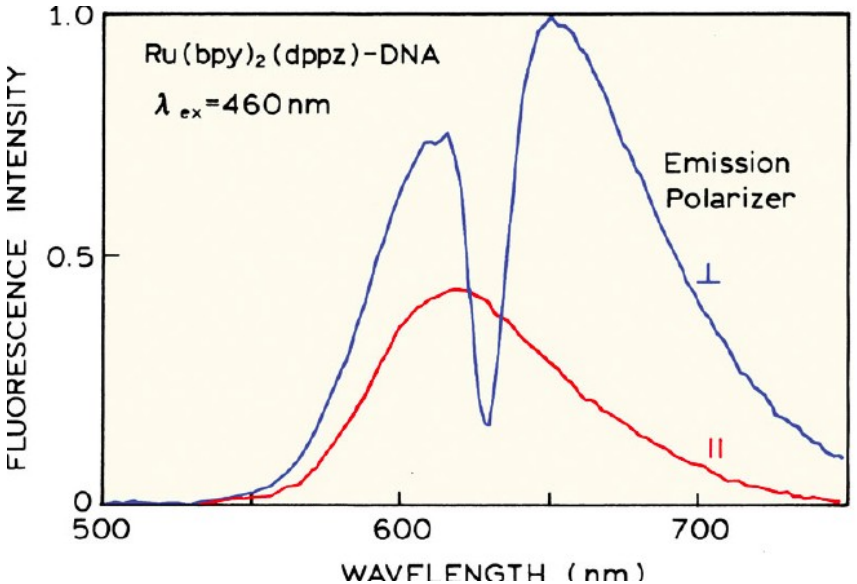
Spectroscopy



Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

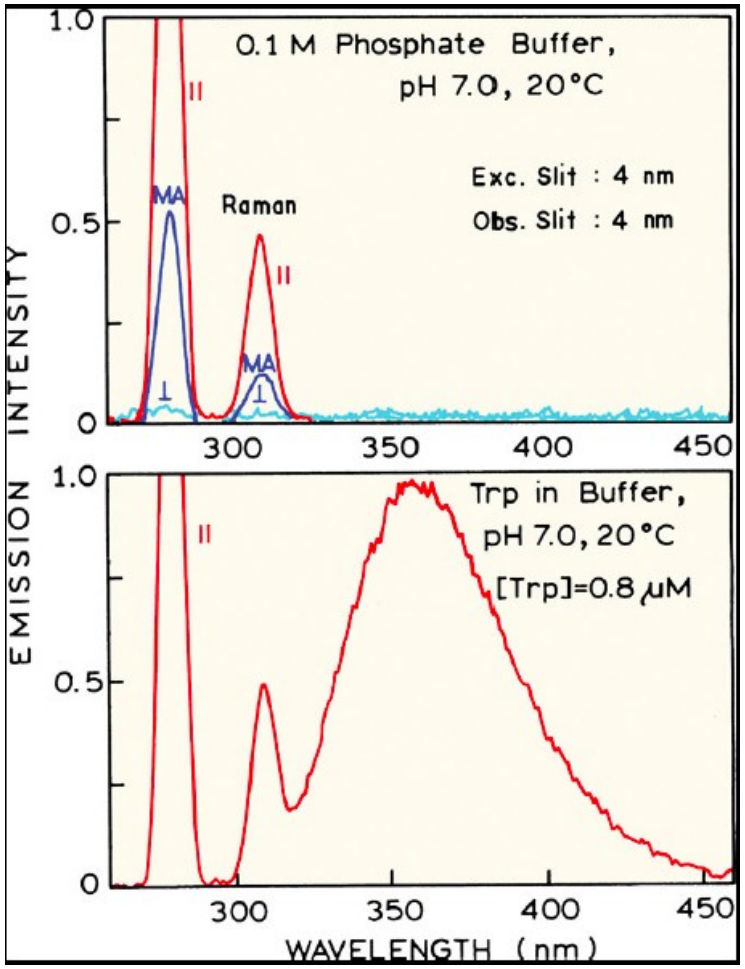
Spectroscopy

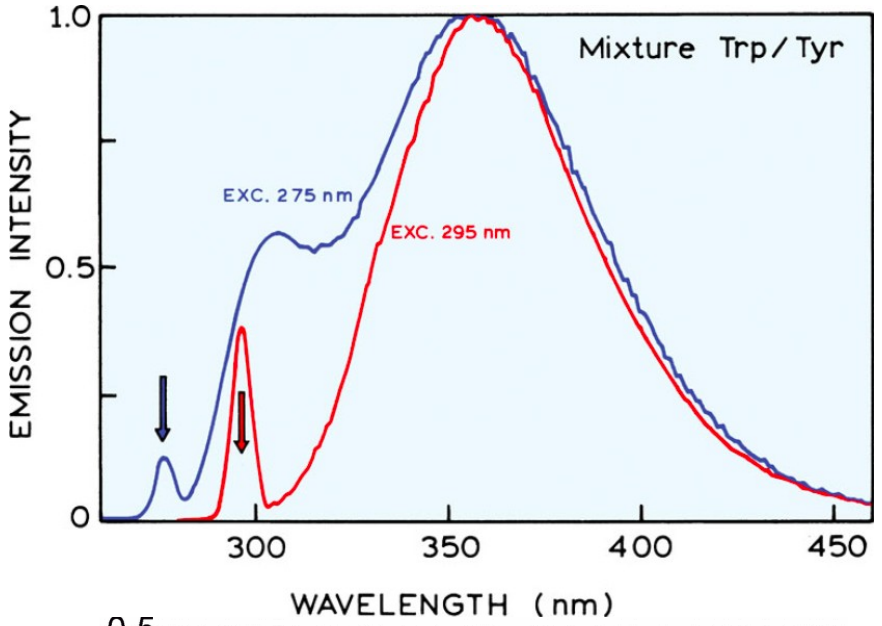


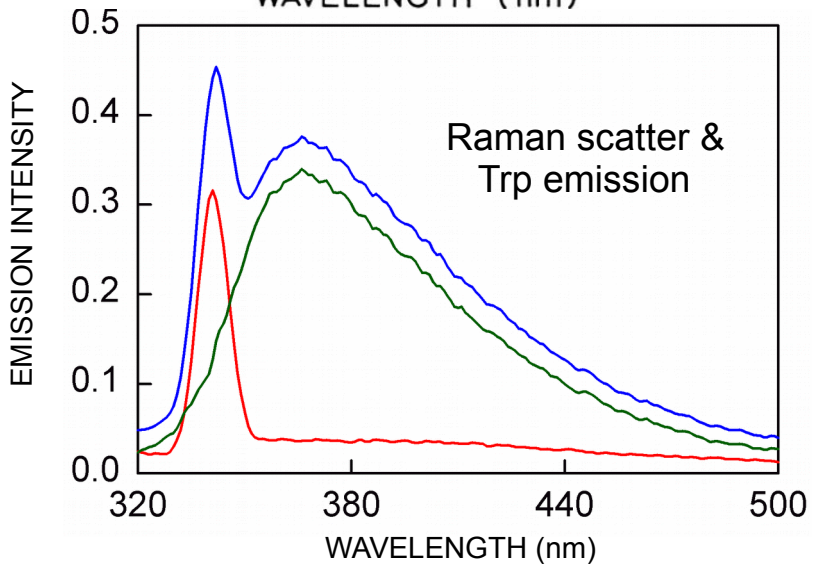


Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy

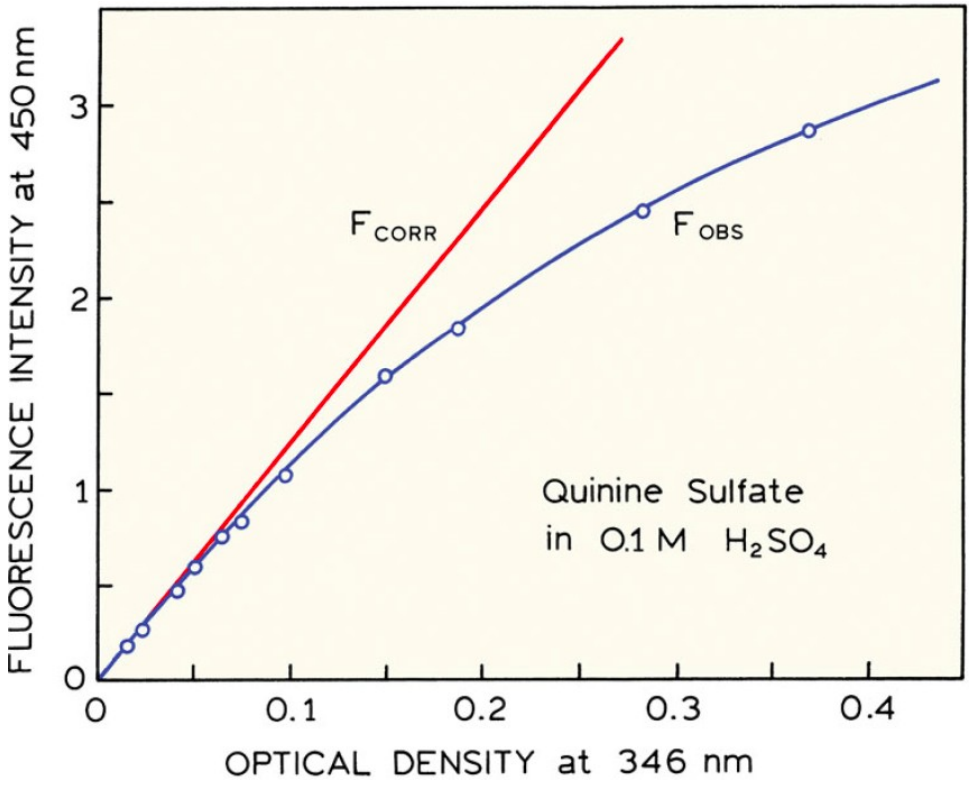


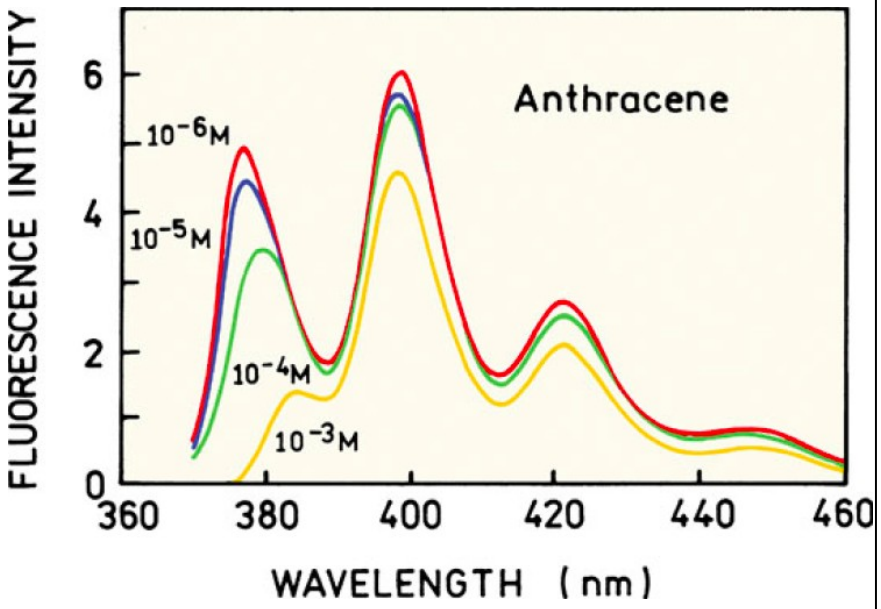




Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy





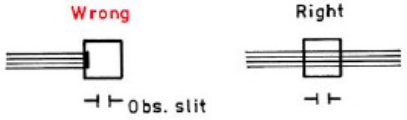
Primary and secondary inner filter errors:

$$F_{corr} = F_{obs} \log ((OD_{ex} + OD_{em}) /2)$$

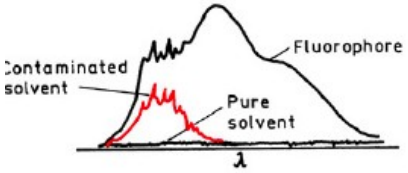
Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy

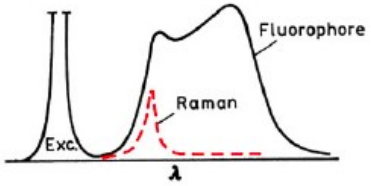
Fluorophore concentration too high



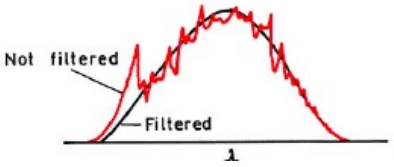
Contaminated solvent and/or cuvette

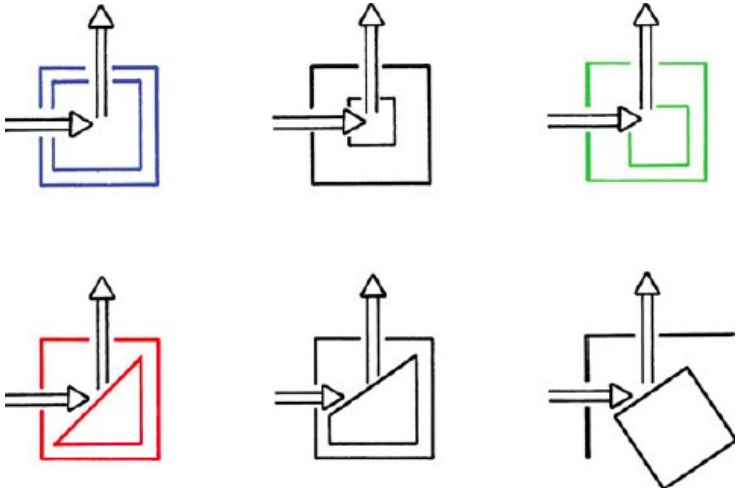


Scattered light



Particles in solution





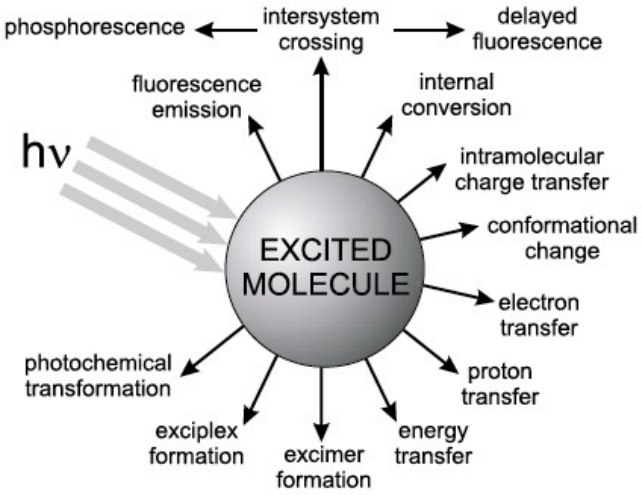
Geometry considerations:

most common is center focus (upper left and upper middle)

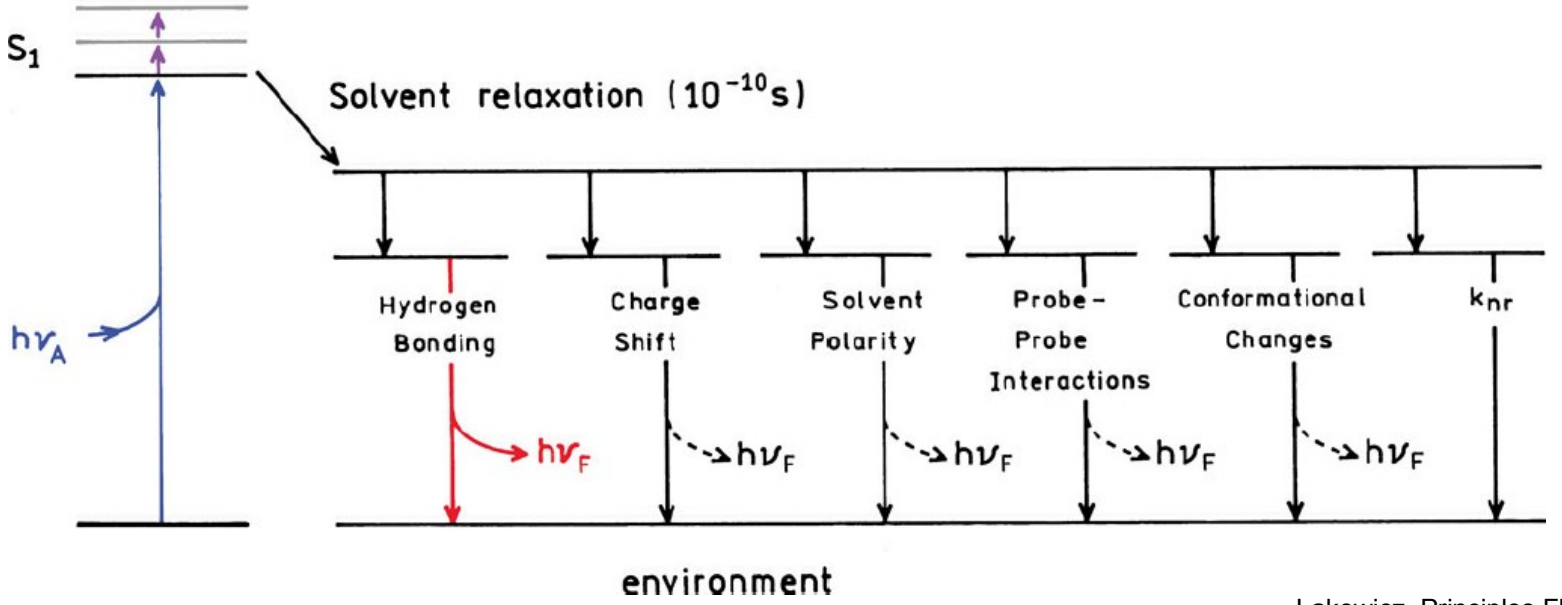
front-face illumination, used for optically thick samples, should be either at 30° or 60°, not 45°. Excitation reflection angle makes this obvious.

Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Spectroscopy

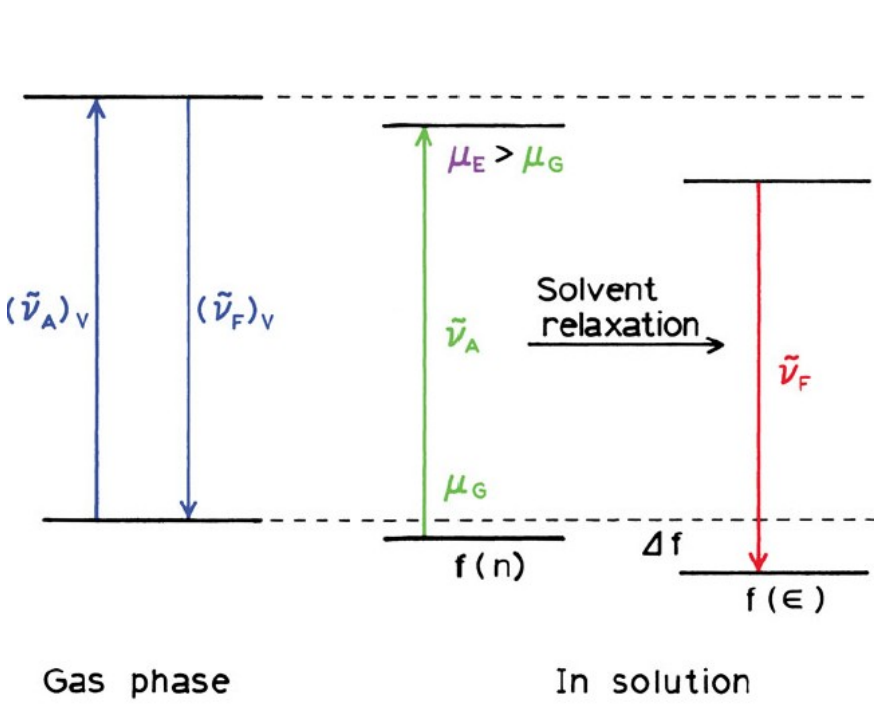


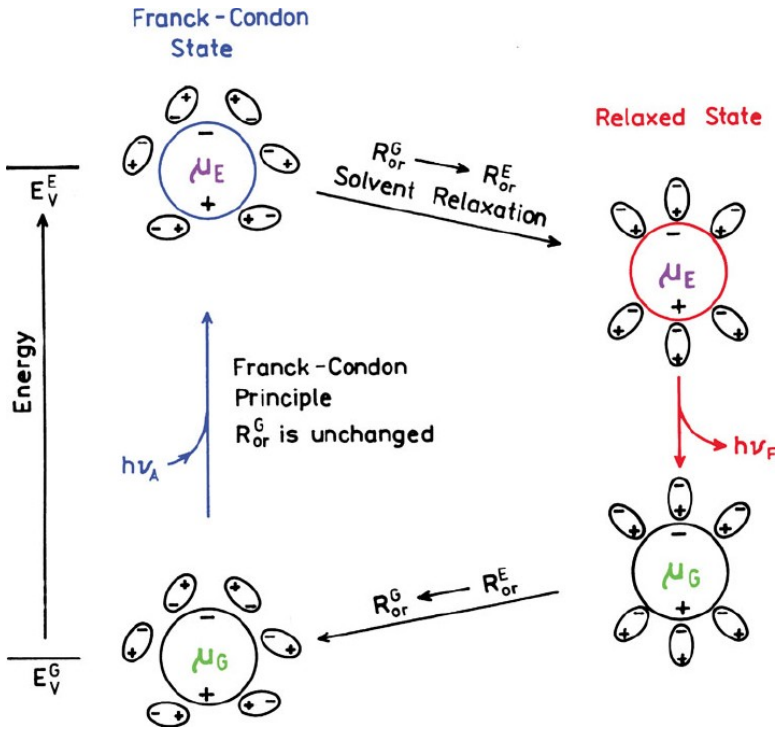
Valeur and Berbaran-Santos, Molecular Fluorescence, 2nd Ed., 2012



Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

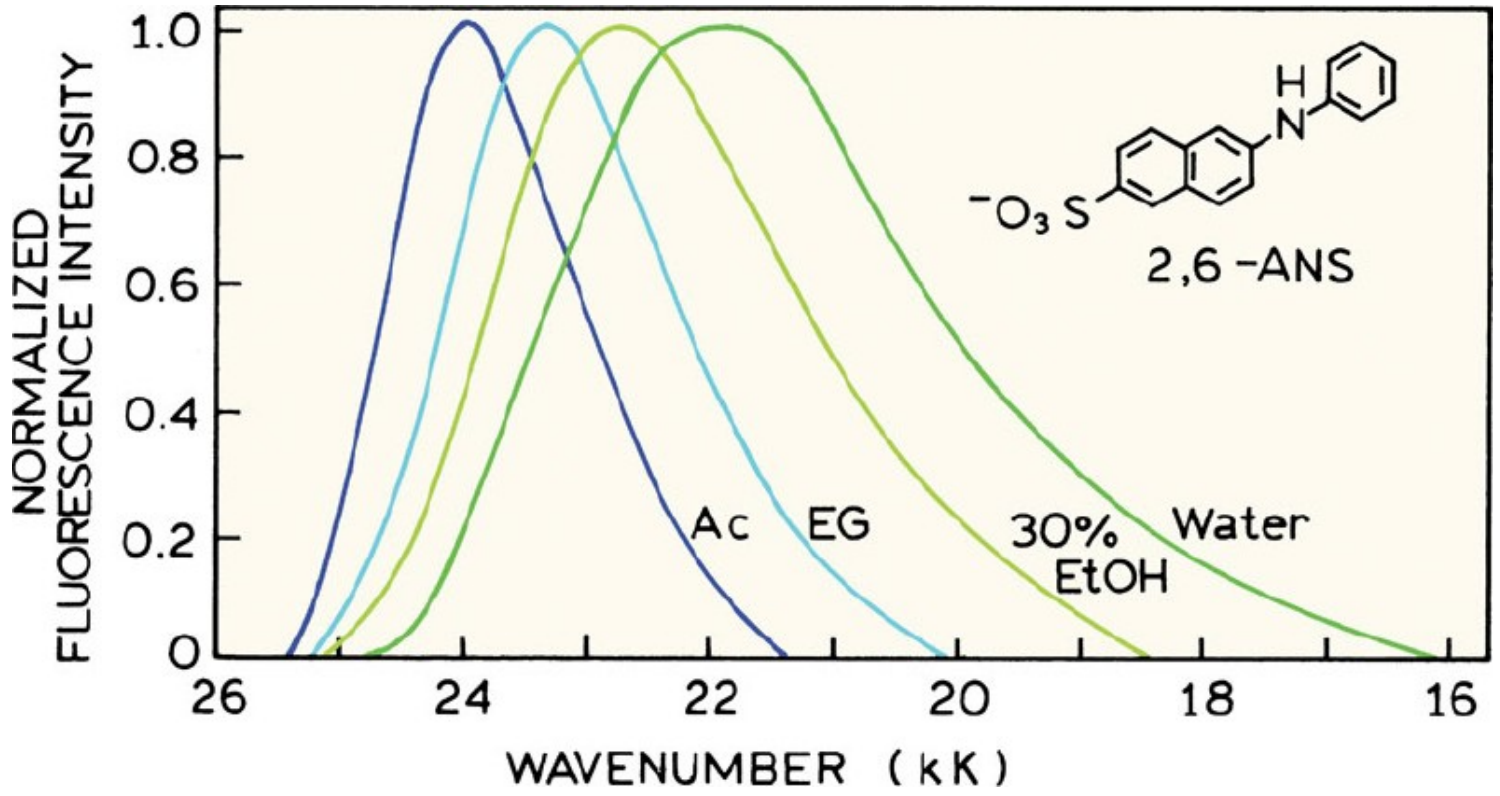
Excited-State Reactions: Dipolar Relaxation





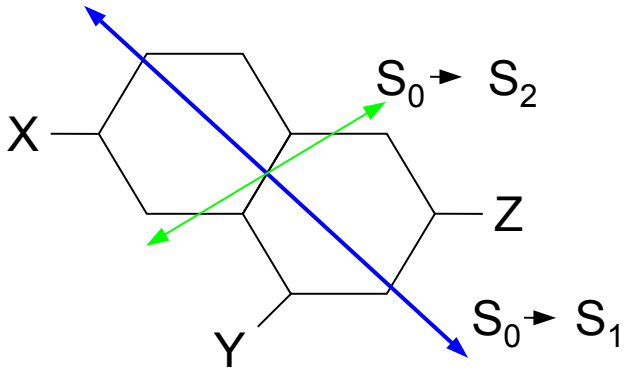
Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Excited-State Reactions: Dipolar Relaxation



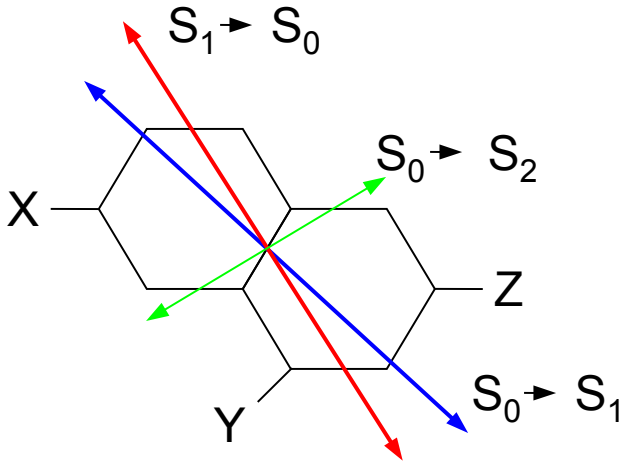
Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Absorption and Emission Transition Dipole Moments



Absorption transition moments: unique directions with respect to the molecular axes

Absorption and Emission Transition Dipole Moments



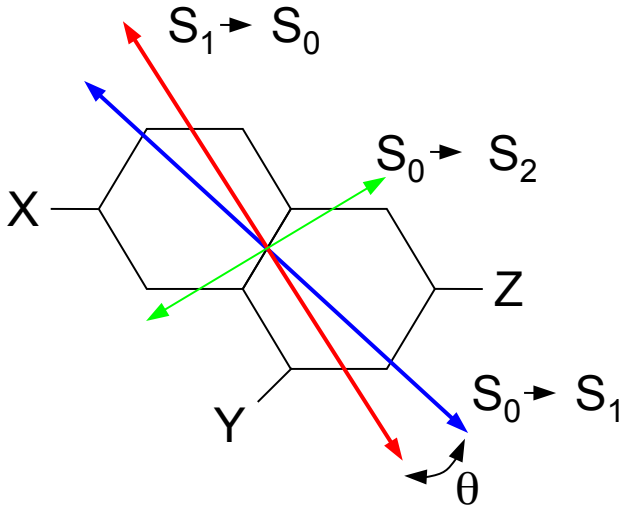
Emission transition moment: a unique direction with respect to the molecular axes

emission spectrum (shape) is independent of excitation wavelength

Kasha's rule: emission is from the lowest energy excited state

Vavilov's rule: quantum yield is independent of excitation wavelength

Absorption and Emission Transition Dipole Moments



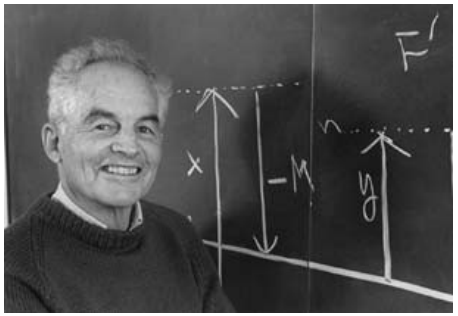
Anisotropy (Jabłonski, 1960):

$$r = (I_V - I_H)/(I_V + 2\,I_H) = (I_V - I_H)/I_{total})$$

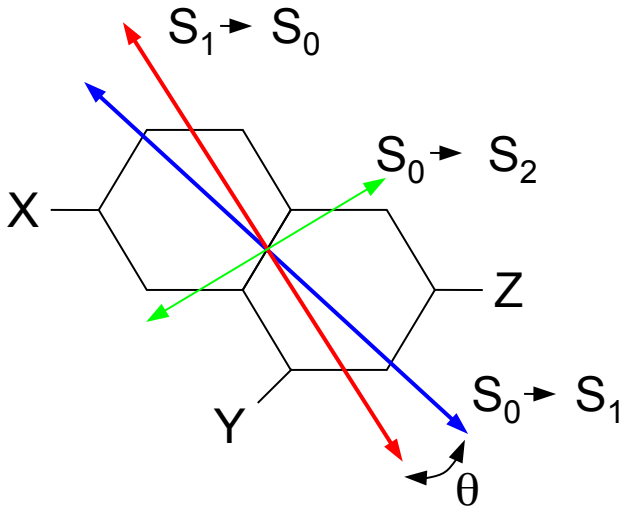
depends on the angle, θ, between absorption and emission transition moments

Absorption and Emission Transition Dipole Moments

Principle of Photoselection (Albrecht, 1961)



Andreas Albrecht, 1927-2002



Anisotropy (Jabłonski, 1960):

$r = (I_V - I_H)/I_{total}$)

depends on the angle, θ, between absorption and emission transition moments

with random molecular orientation ⟶ $r_0 = (3\cos^2\theta - 1)/5$

if θ = 0° (parallel), then r_0 = 0.4

or if θ = 90° (perpendicular), then r_0 = -0.2

Principle Polarization Spectrum

Determination of r_0 as a function of excitation wavelength

at a constant λ_{em}

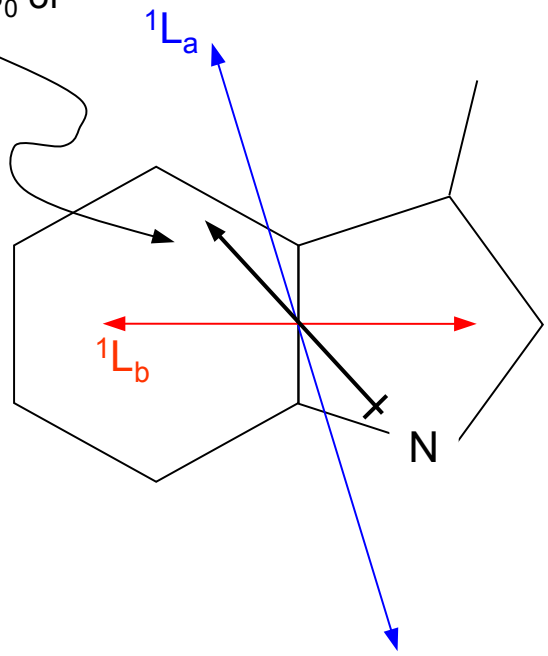
prevent depolarizing motions

scan λ_{ex} for all 4 sets of polarizer angles

calculate and plot r_0 vs λ_{ex}

see Figs. 10.6, 10.7, 10.29 in Lakowicz (3rd ed.)

permanent dipole moment in S_0 of ~ 2 debye



Information obtained

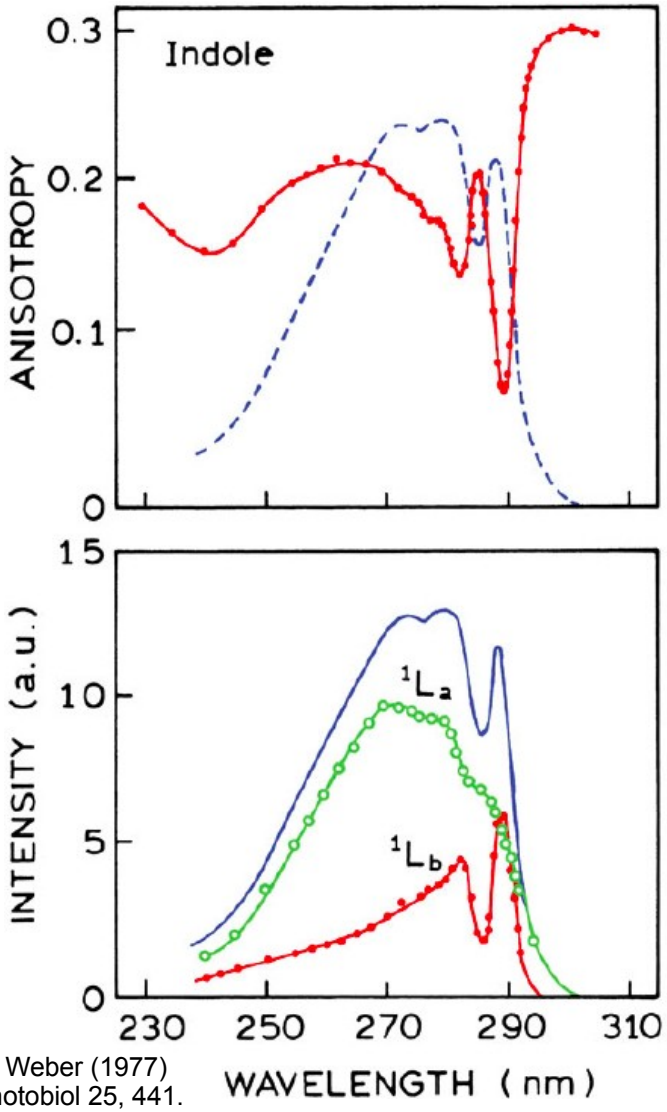
r_0 for different electronic transitions

thus calculate θ between abs. and em. dipole moments

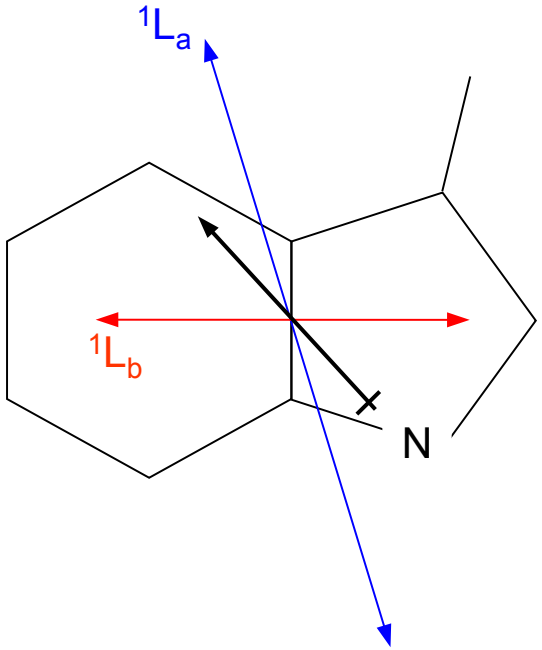
as in indole, find 'hidden' transitions →

for indole spectrum, see Fig. 10.8 in Lakowicz (3rd ed.)

Principle Polarization Spectrum

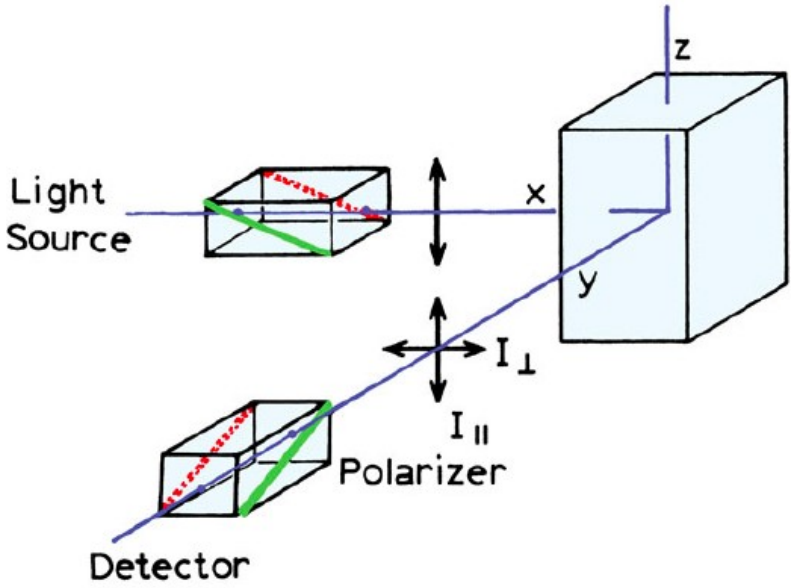


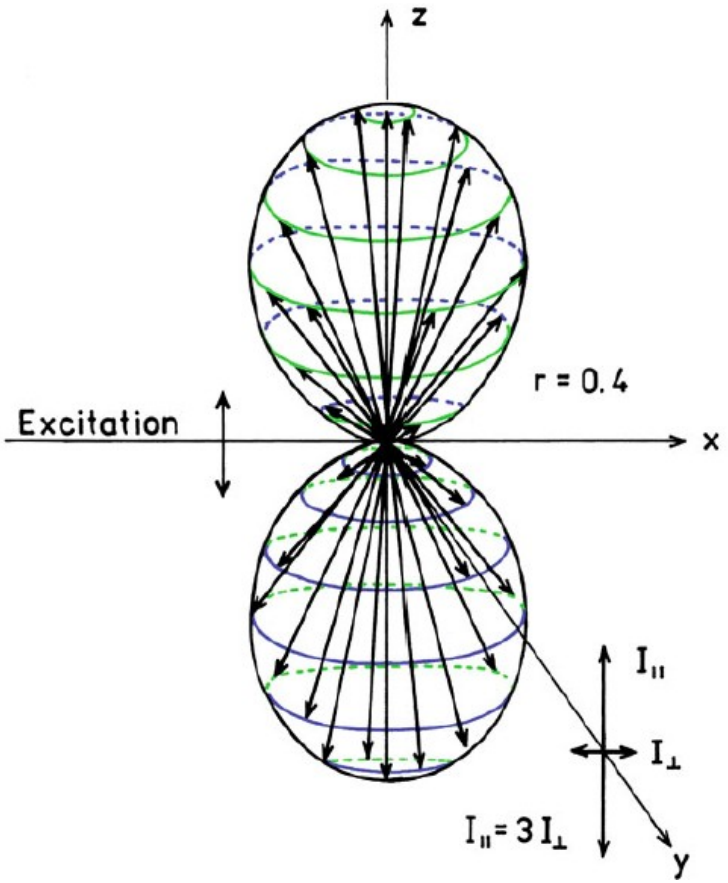
From Valeur & Weber (1977)
Photochem Photobiol 25, 441.



Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

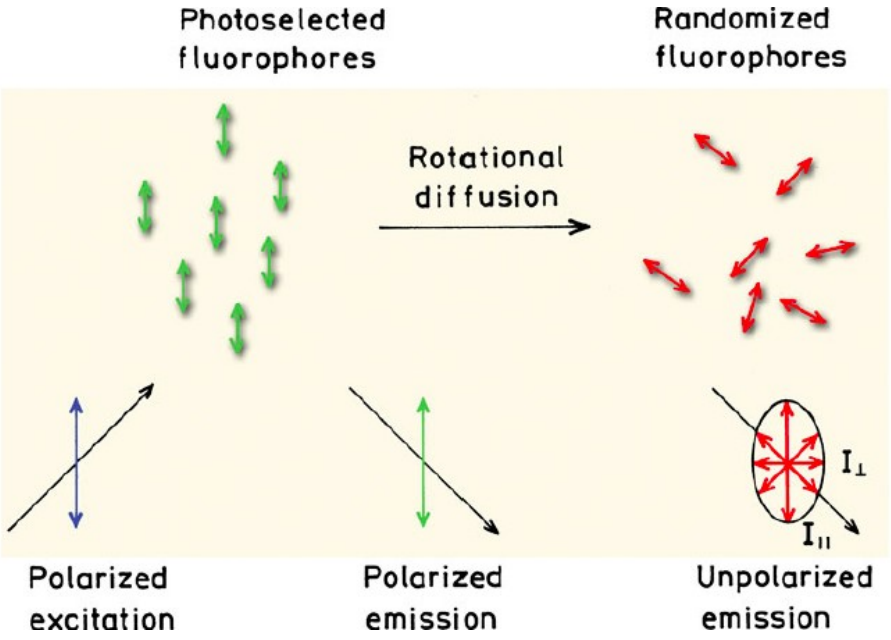
Anisotropy and Photoselection

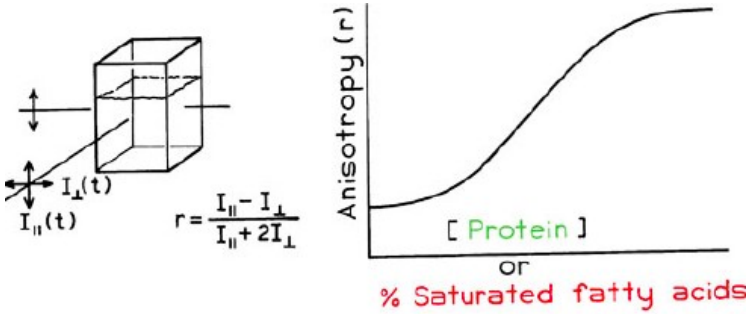


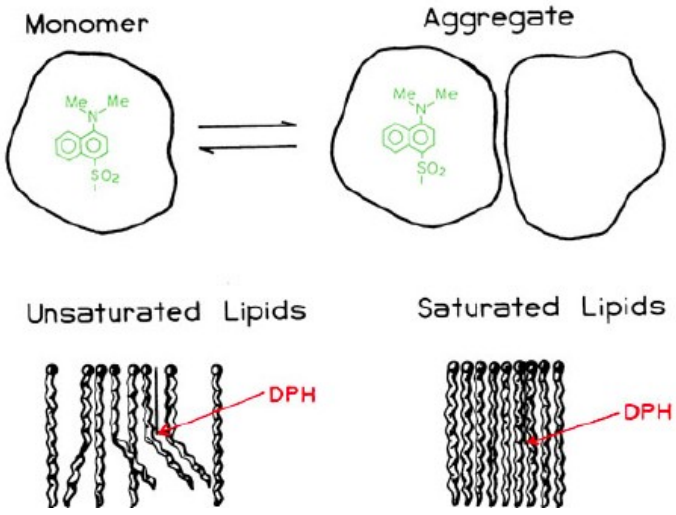


Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Anisotropy and Photoselection







Lakowicz, Principles Fluorescence Spectroscopy 3rd Ed., 2006

Anisotropy and Photoselection

β (deg)	r_0	p_0
0	0.4	0.5
45	0.1	0.143
54.7	0	0
90	–0.2	–0.333

Perrin equation for spherical rotor: $r = r_0/(1 + \tau/\theta)$