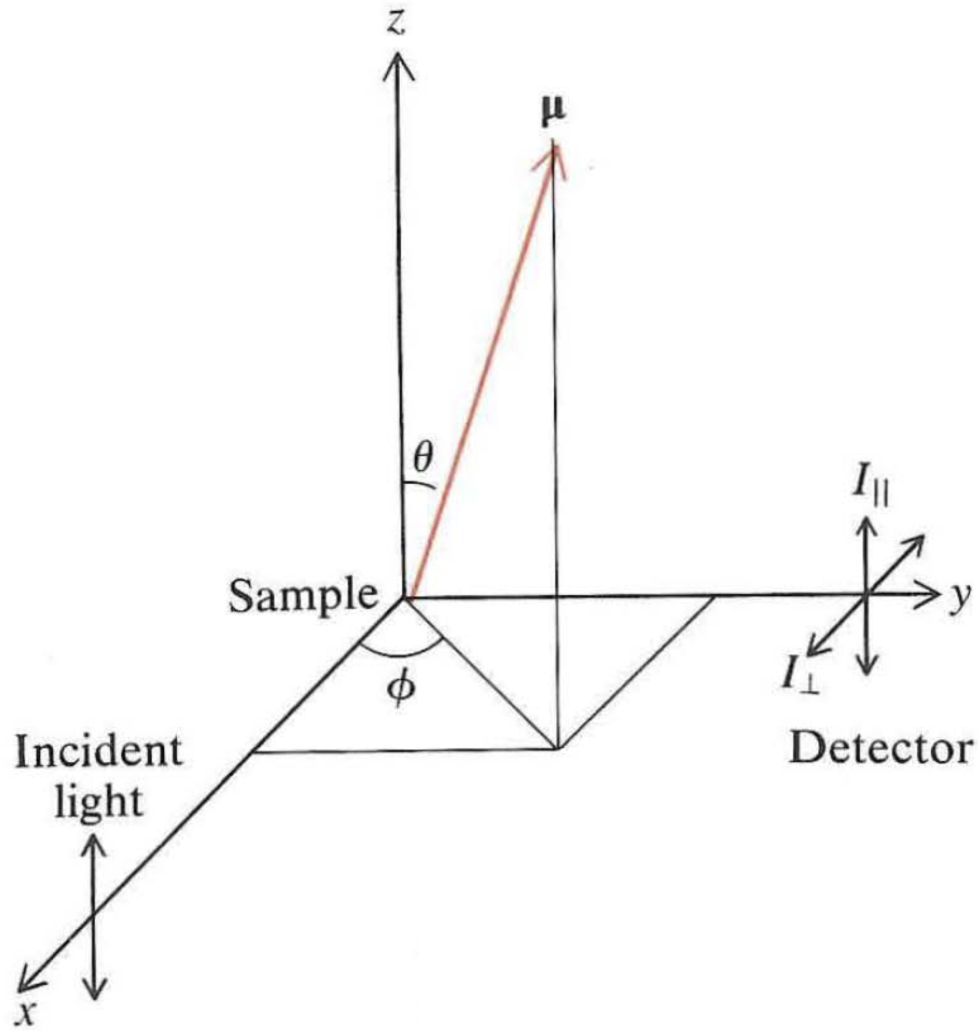
Fluorescence Polarization



From *Biophysical Chemistry, Part II*
Cantor & Schimmel, Chapt. 8, p. 456

Excited Chromophore Distribution

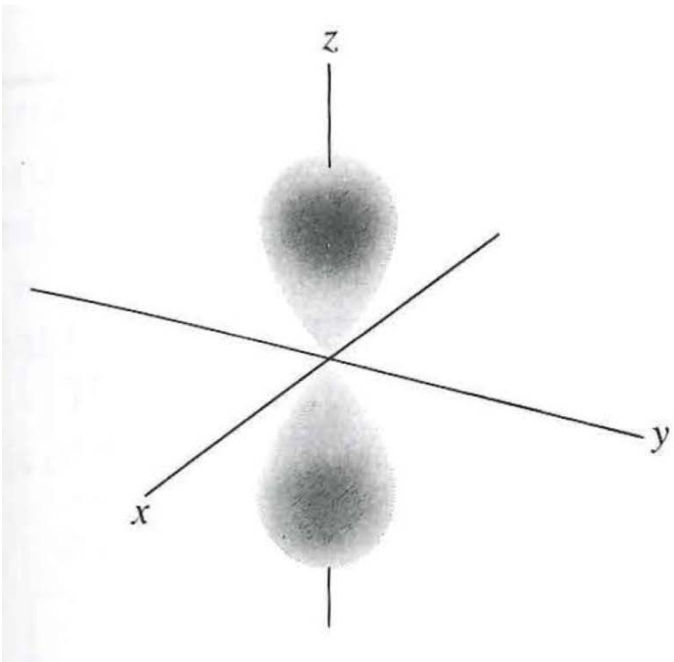


Figure 8-22

Distribution of excited chromophores produced by exciting a sample with z-polarized light propagating along the x axis. The density of the shading is proportional to the probability of finding an excited molecule with its transition dipole at that particular orientation.

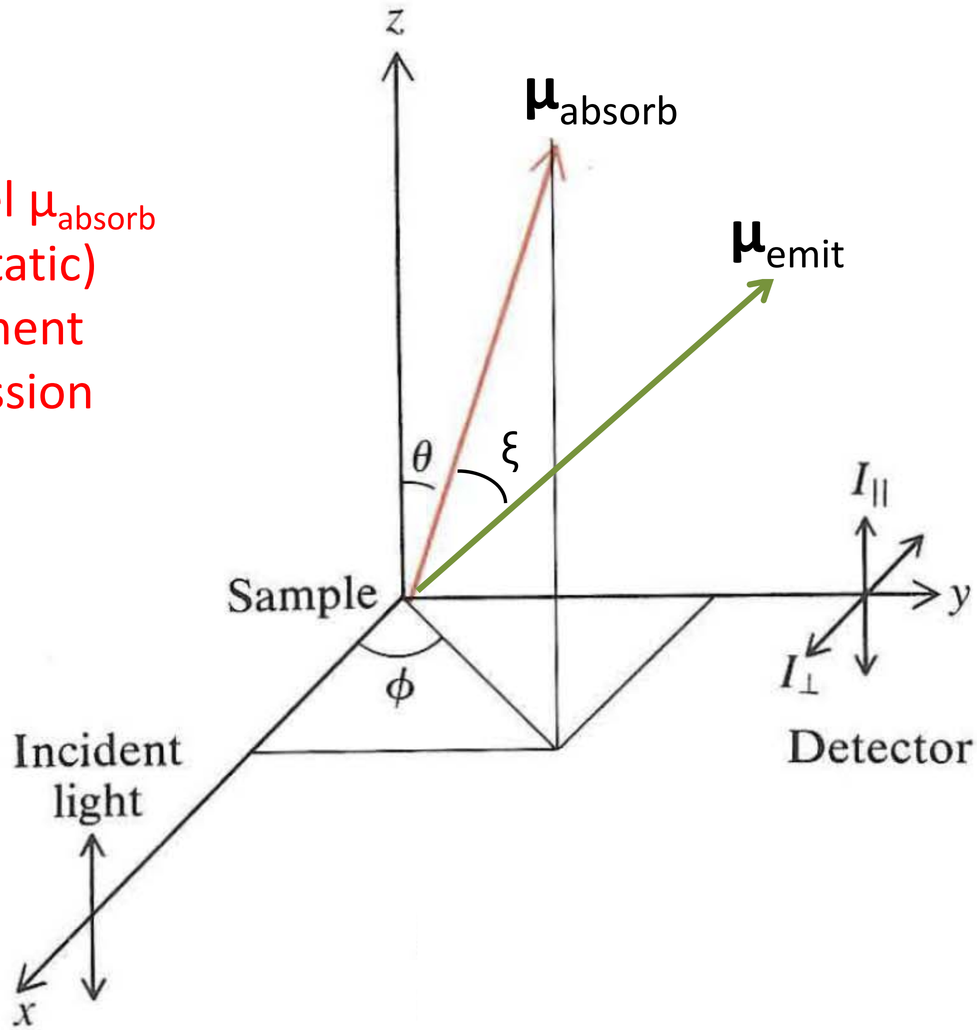
$$W(\theta,\varphi) = \left(\frac{3}{4\pi}\right)\cos^2\theta\sin\theta$$

From *Biophysical Chemistry, Part II*
Cantor & Schimmel, Chapt. 8, p. 457

Dynamics from Fluorescence

$r_0 \neq 2/5$ ➔

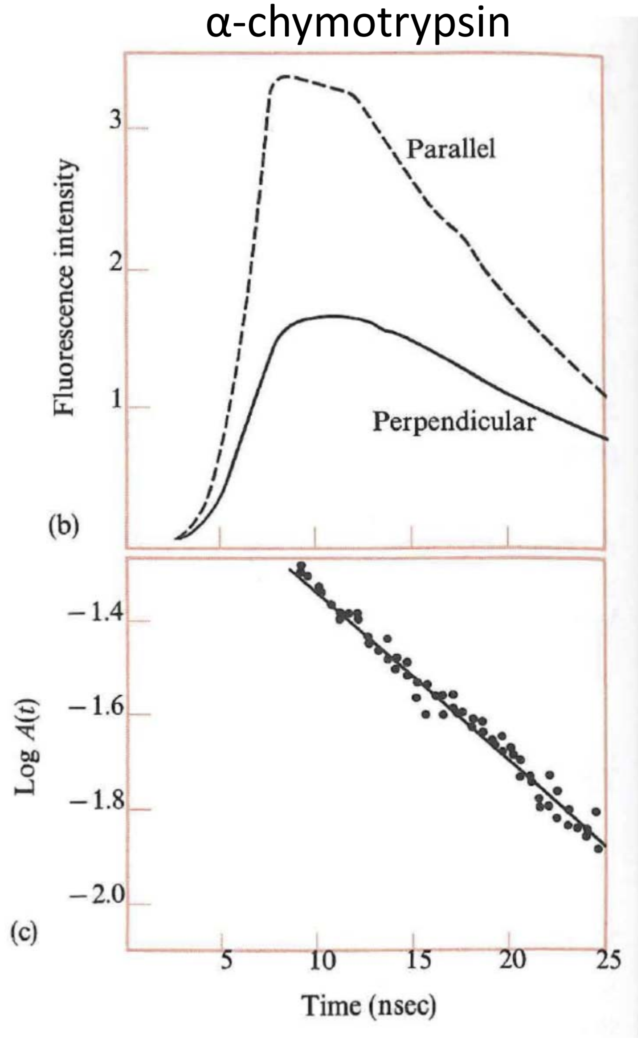
- Non-parallel μ_{absorb} and μ_{emit} (static)
- Rearrangement before emission (dynamic)



$$r_0 = \frac{3\cos^2\xi - 1}{5}$$

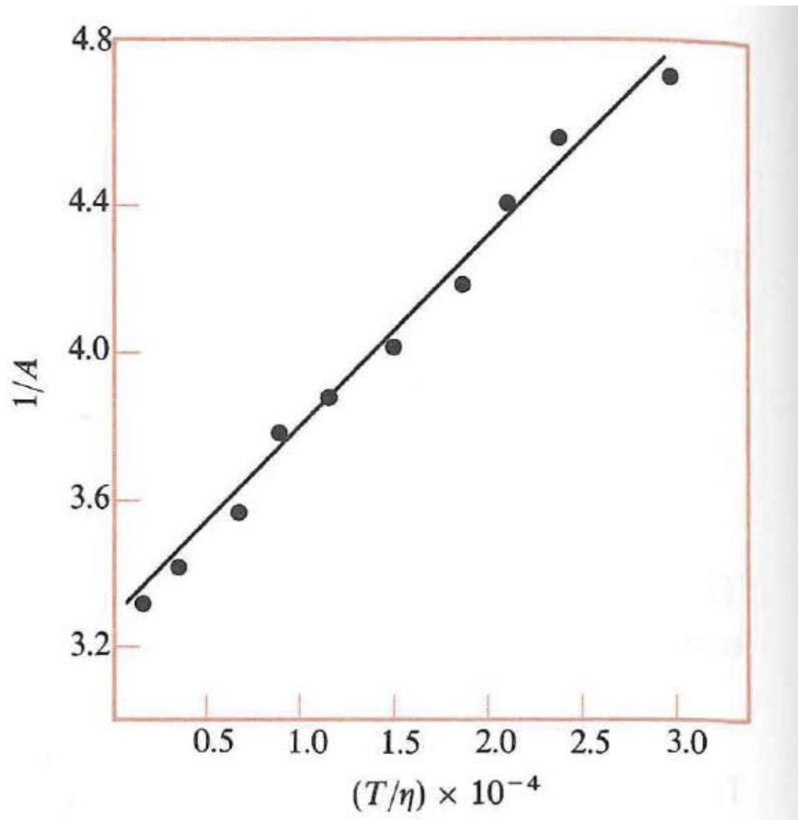
Time-Dependent Fluorescence Anisotropy

α-chymotrypsin

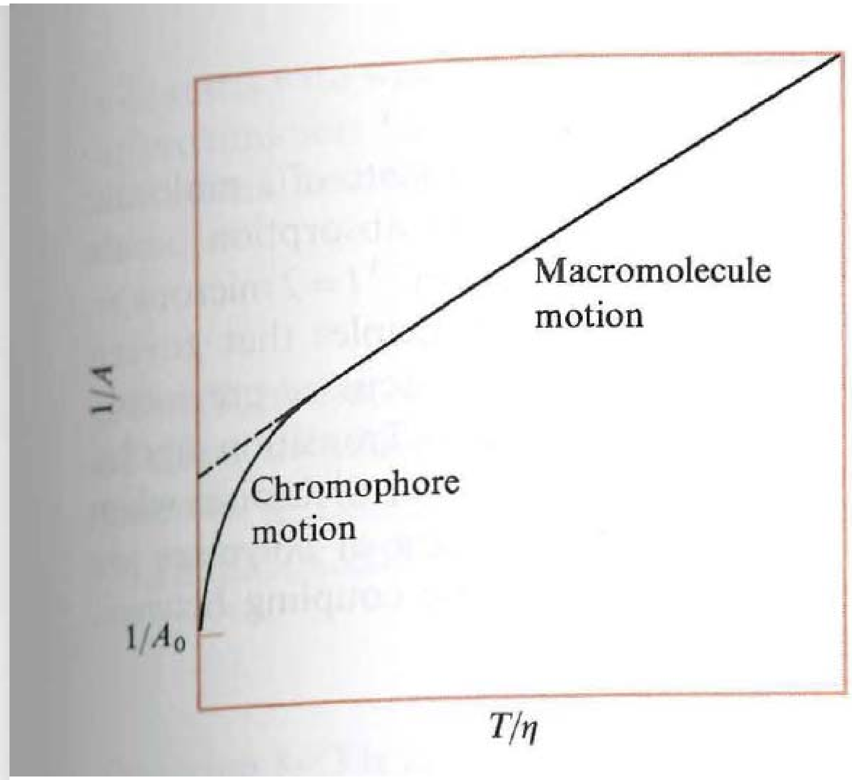


From *Biophysical Chemistry, Part II*
Cantor & Schimmel, Chapt. 8, p. 462

Perrin Plots



“Rigid” Chromophore
(fluorophore reorientation > τ_{obs})



“Rigid” Chromophore
(fluorophore reorientation ~ τ_{obs})

From *Biophysical Chemistry, Part II*
Cantor & Schimmel, Chapt. 8, p. 464-5

Summary

- Preferential absorption of polarized light will give rise to polarized fluorescence
- Molecular motion will result in fluorescence depolarization
  - Over time, fluorescence aniosotropy r → 0
- Decay rate (τ_c) is related to rotational diffusion coefficient
- Steady-state depolarization can be used to estimate τ_c (and hydrodynamic volume) if τ_{obs} for a fluorophore is known (Perrin plot)