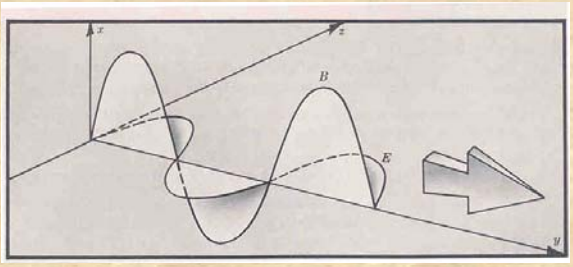
Polarization

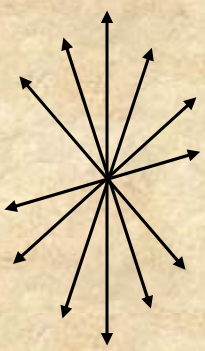
As stated earlier, light can be considered as oscillations of an electromagnetic field – characterized by electric and magnetic components - perpendicular to the direction of light propagation.



In these lectures we shall be concerned only with the electric component.

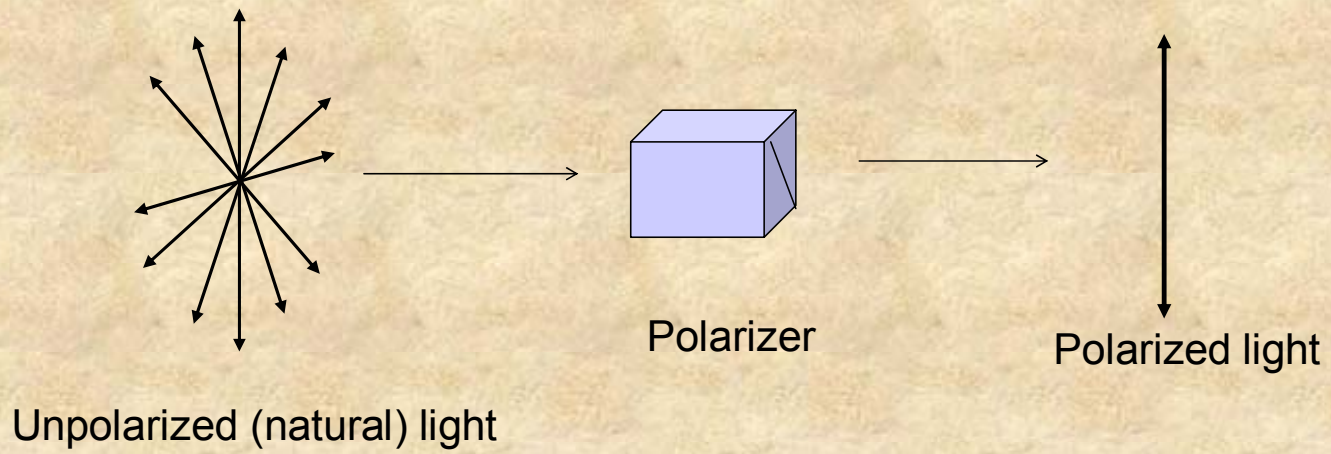
In natural light the electric field vector can assume any direction of oscillation perpendicular or normal to the light propagation direction.

Unpolarized (natural) light



Light Propagation Direction

Polarizers are optically active devices that can isolate one direction of the electric vector.

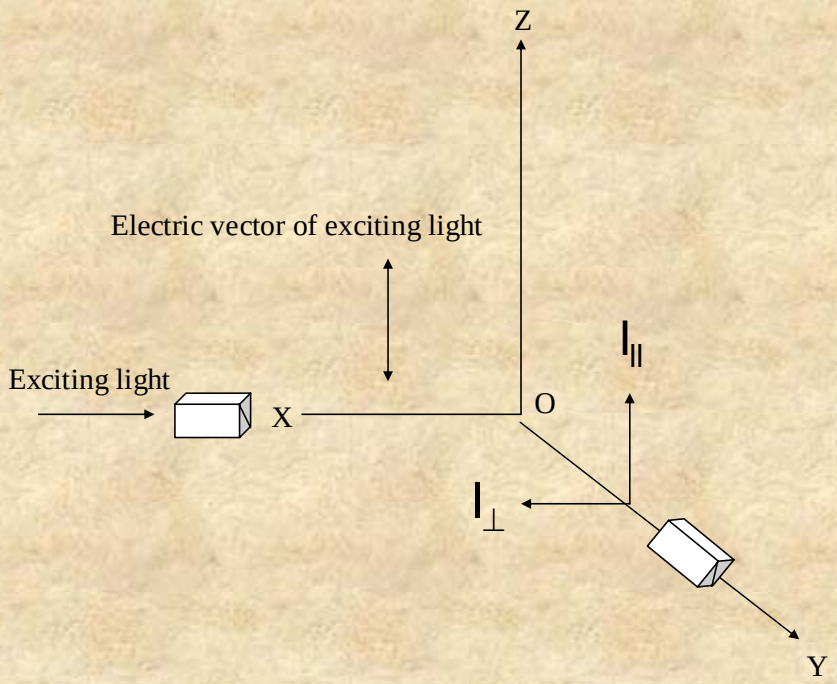


The most common polarizers used today are (1) dichroic devices, which operate by effectively absorbing one plane of polarization (e.g., Polaroid type-H sheets based on stretched polyvinyl alcohol impregnated with iodine) and (2) double refracting calcite ($CaCO_3$) crystal polarizers - which differentially disperse the two planes of polarization (examples of this class of polarizers are Nicol polarizers, Wollaston prisms and Glan-type polarizers such as the Glan-Foucault, Glan-Thompson and Glan-Taylor polarizers)

Polarizers have, in fact, been in use for a very long time - the Vikings used a "sunstone" (now thought to have been composed of the mineral cordierite, a natural polarizing material) to observe the location of the sun on foggy or overcast days. Since scattered sunlight is highly polarized compared to light coming along the direction to the sun, the distribution of the sky's brightness could be observed through the sunstone and hence the sun's position could be localized and, if the time of day were known, the compass directions.

In 1920, F. Weigert discovered that the fluorescence from solutions of dyes was polarized. Specifically, he looked at solutions of fluorescein, eosin, rhodamine and other dyes and noted the effect of temperature and viscosity on the observed polarization. Wiegert discovered that polarization increased with the size of the dye molecule and the viscosity of the solvent, yet decreased as the temperature increased. He recognized that all of these considerations meant that fluorescence polarization increased as the mobility of the emitting species decreased.

Consider an XYZ coordinate framework with a fluorescent solution placed at the origin, as shown below, where XZ is in the plane of the page.



In this system, the exciting light is traveling along the X direction. If a polarizer is inserted in the beam, one can isolate a unique direction of the electric vector and obtain light polarized parallel to the Z axis which corresponds to the vertical laboratory axis.

This exciting light will be absorbed by the fluorophore at the origin and give rise to fluorescence which is typically observed at 90° to the excitation direction, i.e., from along the Y axis.

We initially consider that this fluorescence can have any direction of polarization. The actual direction of the electric vector of the emission can be determined by viewing the emission through a polarizer which can be oriented alternatively in the parallel or perpendicular direction relative to the Z axis or laboratory vertical direction. Polarization is then defined as a function of the observed parallel ($I_{||}$) and perpendicular intensities ($I_{\perp}$) :

$$P = \frac{I_{||} - I_{\perp}}{I_{||} + I_{\perp}}$$

If the emission is completely polarized in the parallel direction, i.e., the electric vector of the exciting light is totally maintained, then:

$$P = \frac{1-0}{1+0} = 1$$

If the emitted light is totally polarized in the perpendicular direction then:

$$P = \frac{0-1}{0+1} = -1$$

The limits of polarization are thus +1 to -1

Another term frequently used in the context of polarized emission is anisotropy (usually designated as either A or r) which is defined as:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

By analogy to polarization, the limits of anisotropy are +1 to -0.5.

A comment about the difference between polarization and anisotropy:

Given the definition of polarization and anisotropy, one can show that:

$$r = \frac{2}{3}\left(\frac{1}{P} - \frac{1}{3}\right)^{-1} \quad \text{or} \quad r = \frac{2P}{3 - P}$$

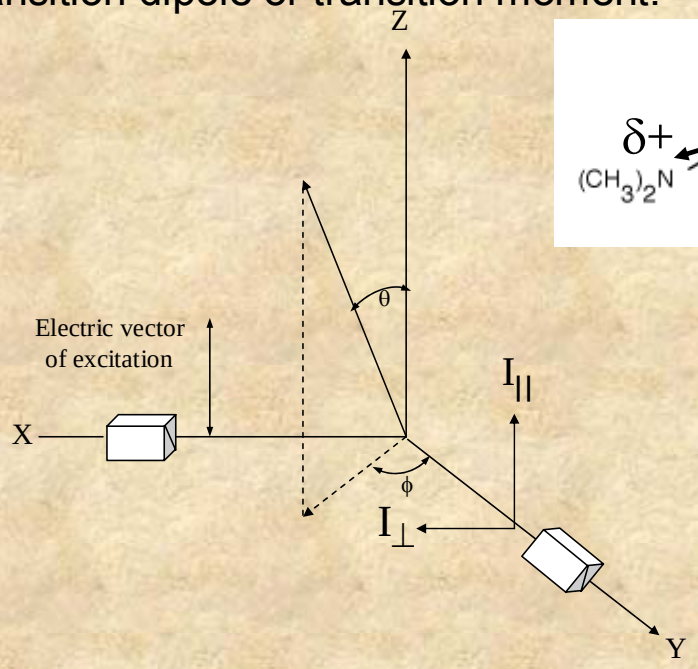
For example:

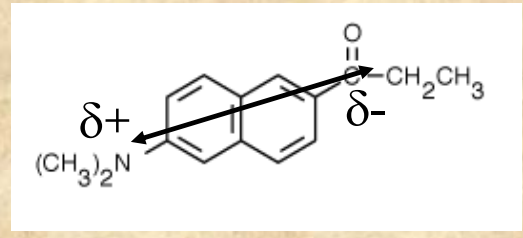
P	r
0.50	0.40
0.30	0.22
0.10	0.069

Clearly, the information content in the polarization function and the anisotropy function is identical and the use of one term or the other is dictated by practical considerations as will be discussed later.

In solution these limits (e.g., +/-1) are not realized. Consider, as shown below, a fluorophore at the origin of our coordinate system.

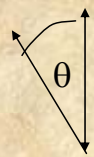
Upon absorption of an exciting photon a dipole moment is created in the fluorophore (usually of different magnitude and direction from the ground state dipole). The orientation of this dipole moment relative to the nuclear framework, and its magnitude, will be determined by the nature of the substituents on the molecule. This excited state dipole moment is also known as the transition dipole or transition moment.



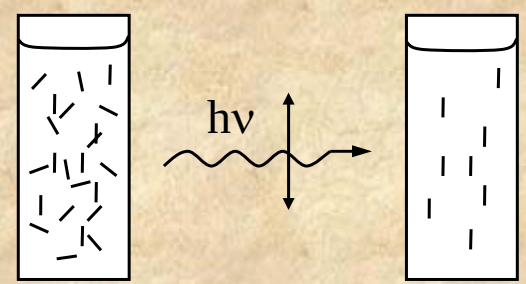


In fact, if light of a particular electric vector orientation (plane polarized light) impinges on a sample, only those molecules which are properly oriented relative to this electric vector can absorb the light.

Specifically, the probability of the absorption is proportional to the cosine squared of the angle θ between the exciting light and the transition dipole ($\cos^2 \theta$).



Hence, when we excite an ensemble of randomly oriented fluorophores with plane-polarized light we are performing a *photoselection* process, creating a population of excited molecules which nominally have their excited dipoles lines up with the polarization direction of the excitation. This process is illustrated below:



Potential dipoles **Excited state dipoles**

FLUORESCENCE POLARIZATION

Fluorescence polarization is used to obtain information on size, shape and flexibility of macromolecules.

Consider a frozen isotropic sample. In the figure (x, y, z) indicates the laboratory coordinate system.

A molecule has a transition dipole μ oriented at angle θ with respect to the z-axis. An incident light beam is polarized in the xz plane and travels in the x direction as shown.

The probability to excite the chromophore is

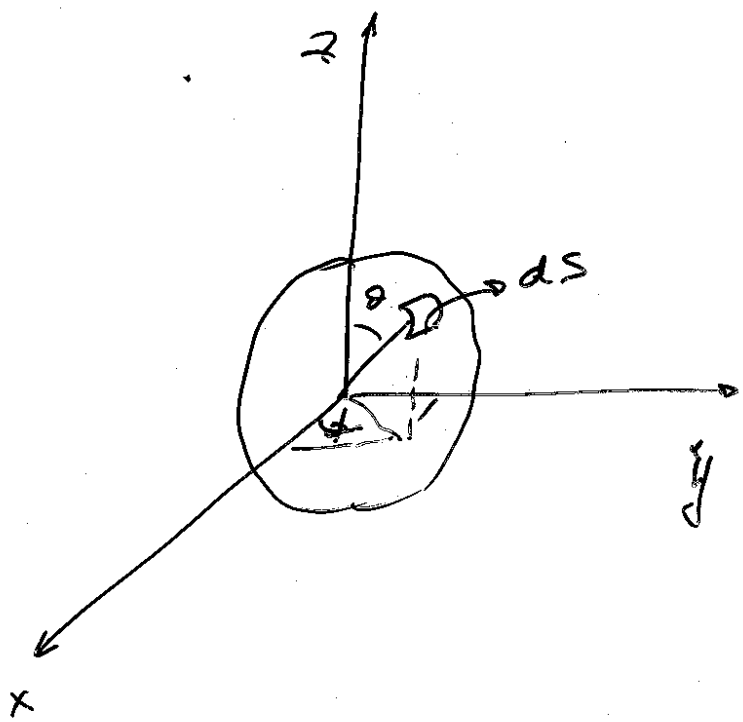
$$P \propto (\mu \bullet E)^2 \propto \cos^2 \vartheta$$

Only molecules with an orientation component of μ on the z axis will be excited **(Photoselection)**

Consider all possible orientations for a molecule. In our picture we can imagine they are distributed uniformly over a sphere of radius ρ. The probability of finding

a molecule with orientation between θ and $\theta+\delta\theta$ is proportional to the element of sphere

$$dS = \sin\theta \, d\theta \, d\phi$$



The probability for absorption is

$$P(\theta,\phi)d\theta d\phi = \cos^2\theta \sin\theta d\theta d\phi \,.P_0$$

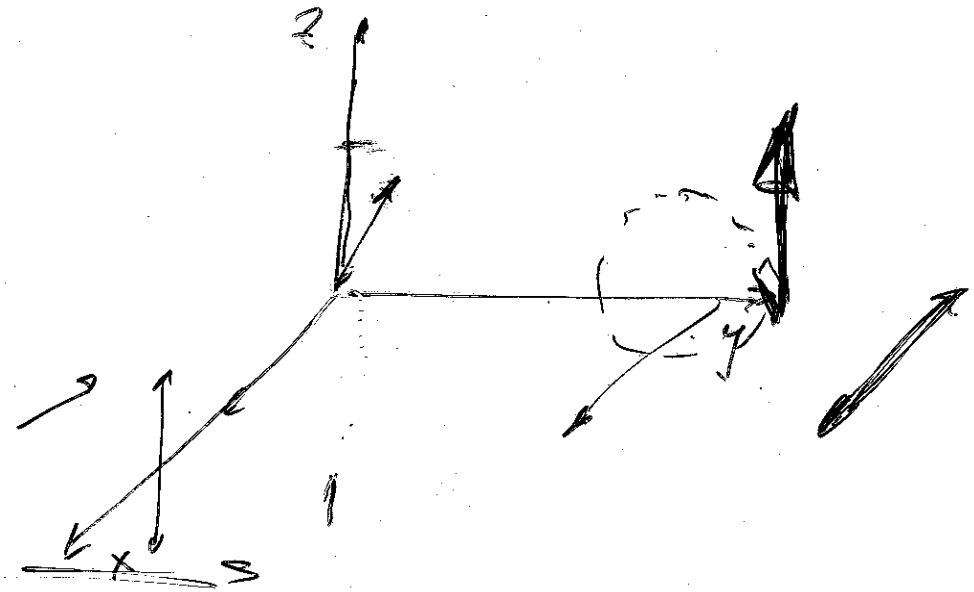
We can better express this quantity in term of fraction of excited molecules

$$W(\vartheta,\varphi)d\vartheta d\varphi = \frac{P(\vartheta,\varphi)d\vartheta d\varphi}{\int_0^{\pi} d\vartheta \int_0^{2\pi} d\varphi \cos^2\vartheta \sin\vartheta}$$

$$W(\vartheta,\varphi)d\vartheta d\varphi = \frac{3}{4\pi}\cos^2\vartheta \sin\vartheta d\vartheta d\varphi$$

This distribution of excited molecules is independent of ϕ

It is a distribution cylindrical symmetrical around z axis.



Since the distribution of excited molecules is non isotropic, the emission will be non isotropic.

Generally the absorption and emission dipole do not have the same orientation. However, suppose they have the same orientation: the absorbing and the emitting dipole are parallel.

The probability of emission along the x, y and z axis is, respectively

$$P_{E,x} \propto |\mu \cdot i|^2$$

$$P_{E,y} \propto |\mu \cdot j|^2$$

$$P_{E,z} \propto |\mu \cdot k|^2$$

The measured quantities are the intensity of emission along the y direction polarized in the z and x directions ($I_{||}$ and $I_{\perp}$ in the figure).

It is easy to show that

$$|\mu \cdot k|^2 \propto \cos^2 \vartheta$$

$$|\mu \cdot i|^2 \propto \sin^2 \vartheta \cos^2 \varphi$$

Then $I_{||}$ is proportional to the fraction of molecules with orientation θ and ϕ times $\cos^2\theta$ integrated over all possible θ and ϕ.

$$I_{\parallel} \propto \int_0^{2\pi} d\varphi \int_0^{\pi} d\vartheta \cos^2 \vartheta W(\vartheta,\varphi) = \frac{3}{5}$$

$$I_{\perp} \propto \int_0^{2\pi} d\varphi \int_0^{\pi} d\vartheta \sin^2 \vartheta \cos^2 \varphi W(\vartheta,\varphi) = \frac{1}{5}$$

The maximum value for the ratio $I_{||}/I_{\perp}$ is 3

We define the following two quantities

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} = \frac{\frac{I_{\parallel}}{I_{\perp}} - 1}{\frac{I_{\parallel}}{I_{\perp}} + 1}$$

POLARIZATION

$$A = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} = \frac{\frac{I_{\parallel}}{I_{\perp}} - 1}{\frac{I_{\parallel}}{I_{\perp}} + 2}$$

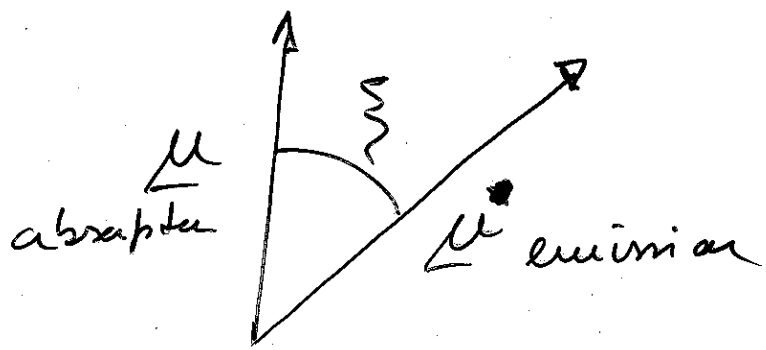
ANISOTROPY

For our model: rigid system with parallel emitting and absorbing dipoles

$$P = \frac{1}{2}$$

$$A = \frac{2}{5}$$

In the case of a rigid system with different orientation for the absorbing and emitting dipole



The polarization and anisotropy are given by

$$P_0 = \frac{3\cos^2\xi - 1}{\cos^2\xi + 3}$$

$$A_0 = \frac{3\cos^2\xi - 1}{5}$$

$$A = \frac{2P}{3 - P}$$

These quantities are called limiting polarization and limiting anisotropy**.**

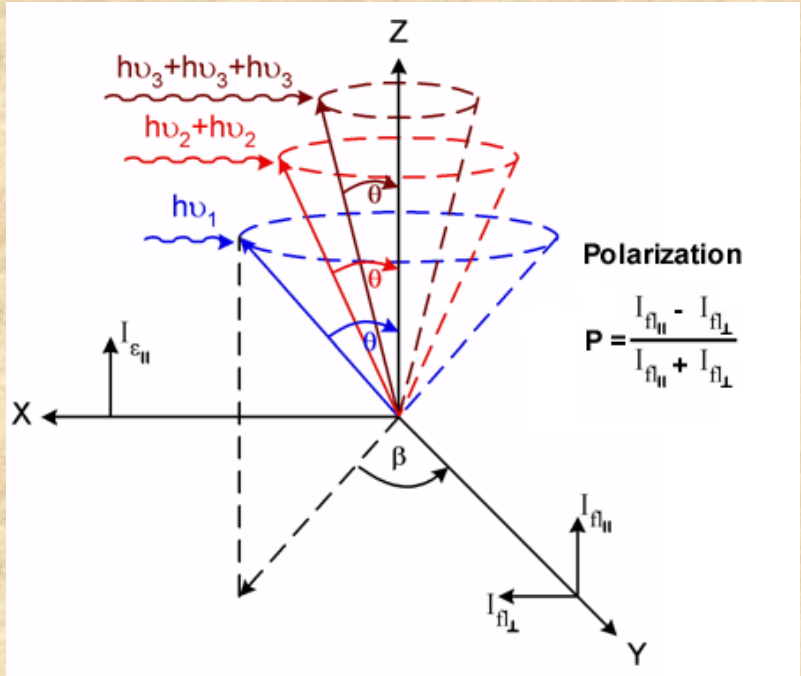
The polarization (and the anisotropy) can be negative. The polarization is zero when

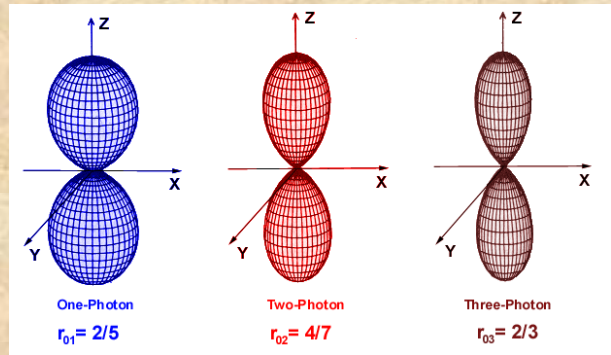
$$0 = 3\cos^2\xi - 1$$

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

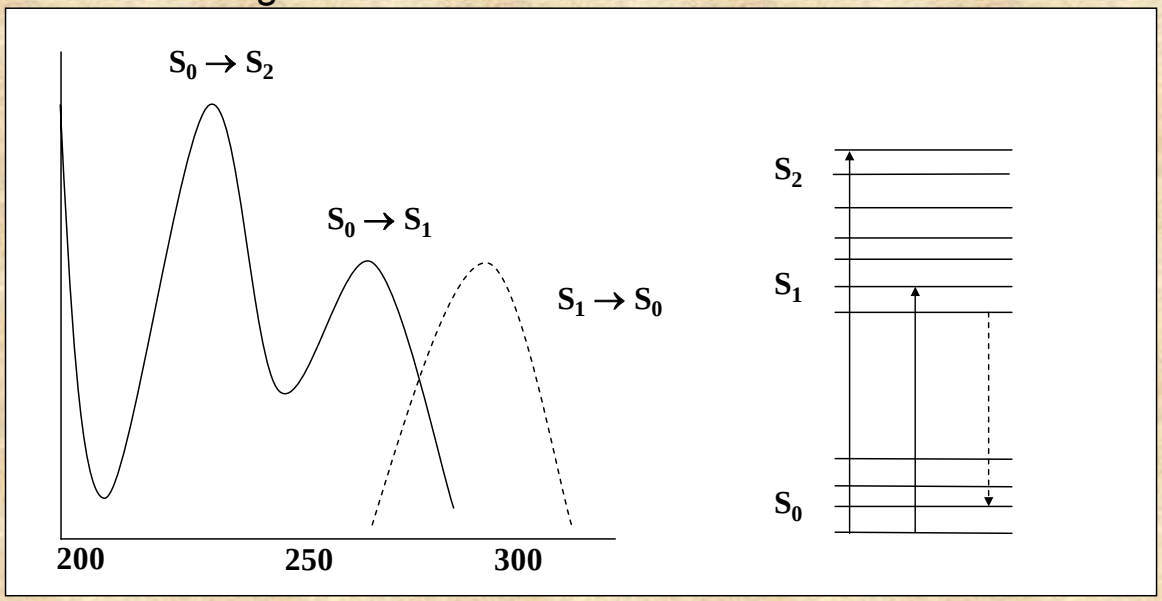
$$\xi = 54.7^\circ$$

Note: in the case of multi-photon excitation the limits differ



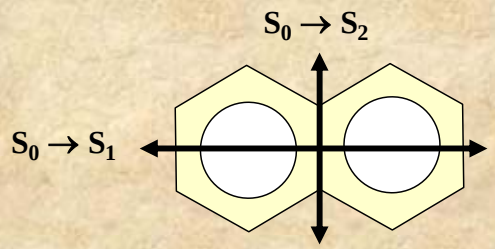


Consider the general case shown below:



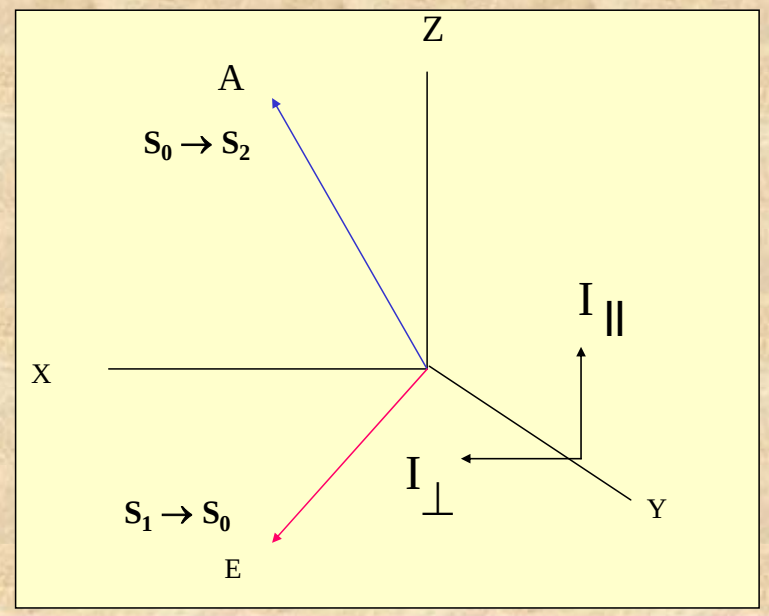
Here are depicted two principle absorption bands for a compound along with and the emission band. The energy level diagram corresponding to this system is also depicted.

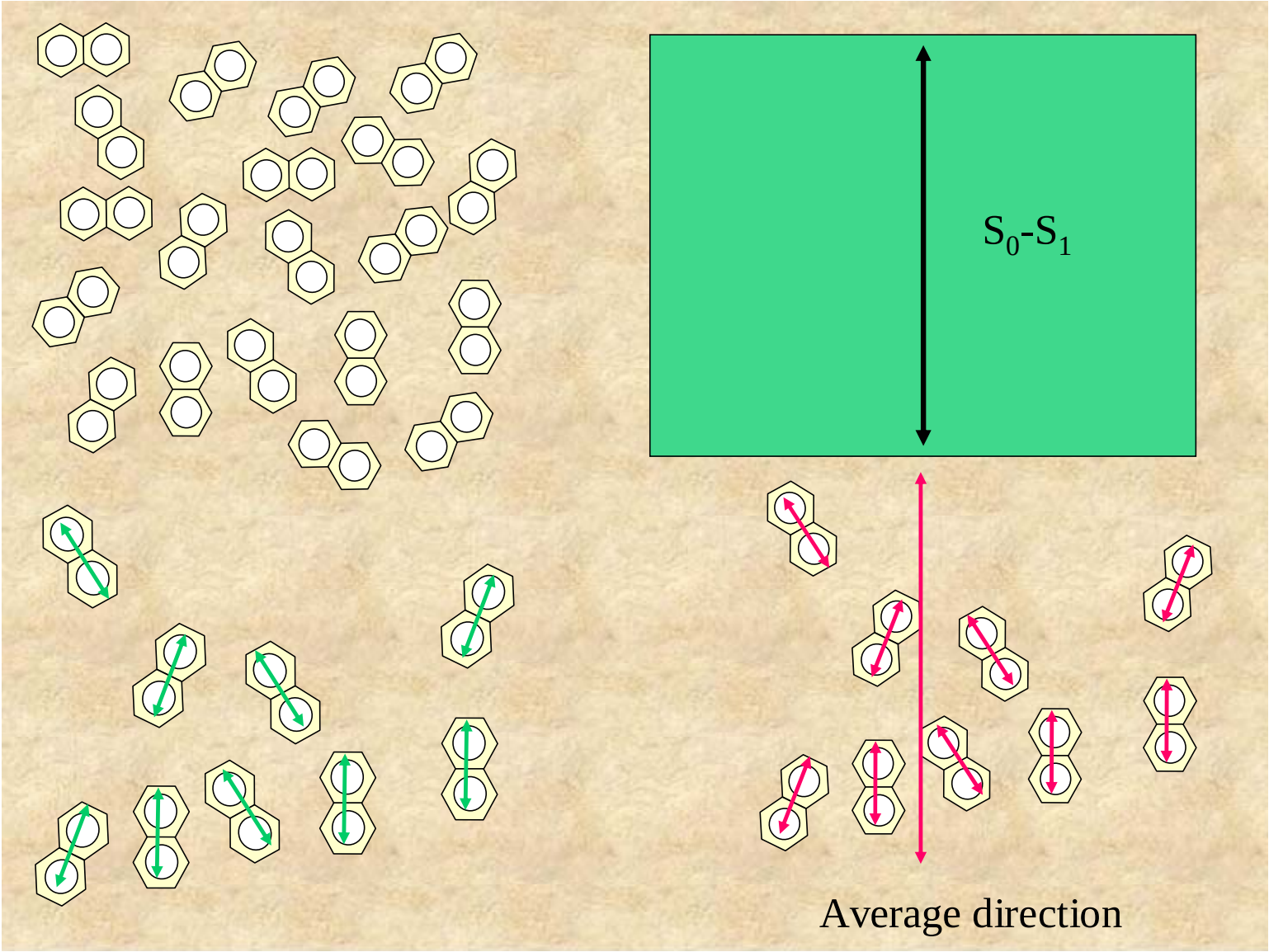
The directions of the absorption dipoles – relative to the nuclear framework – may differ greatly for the two transitions as illustrated on the right.

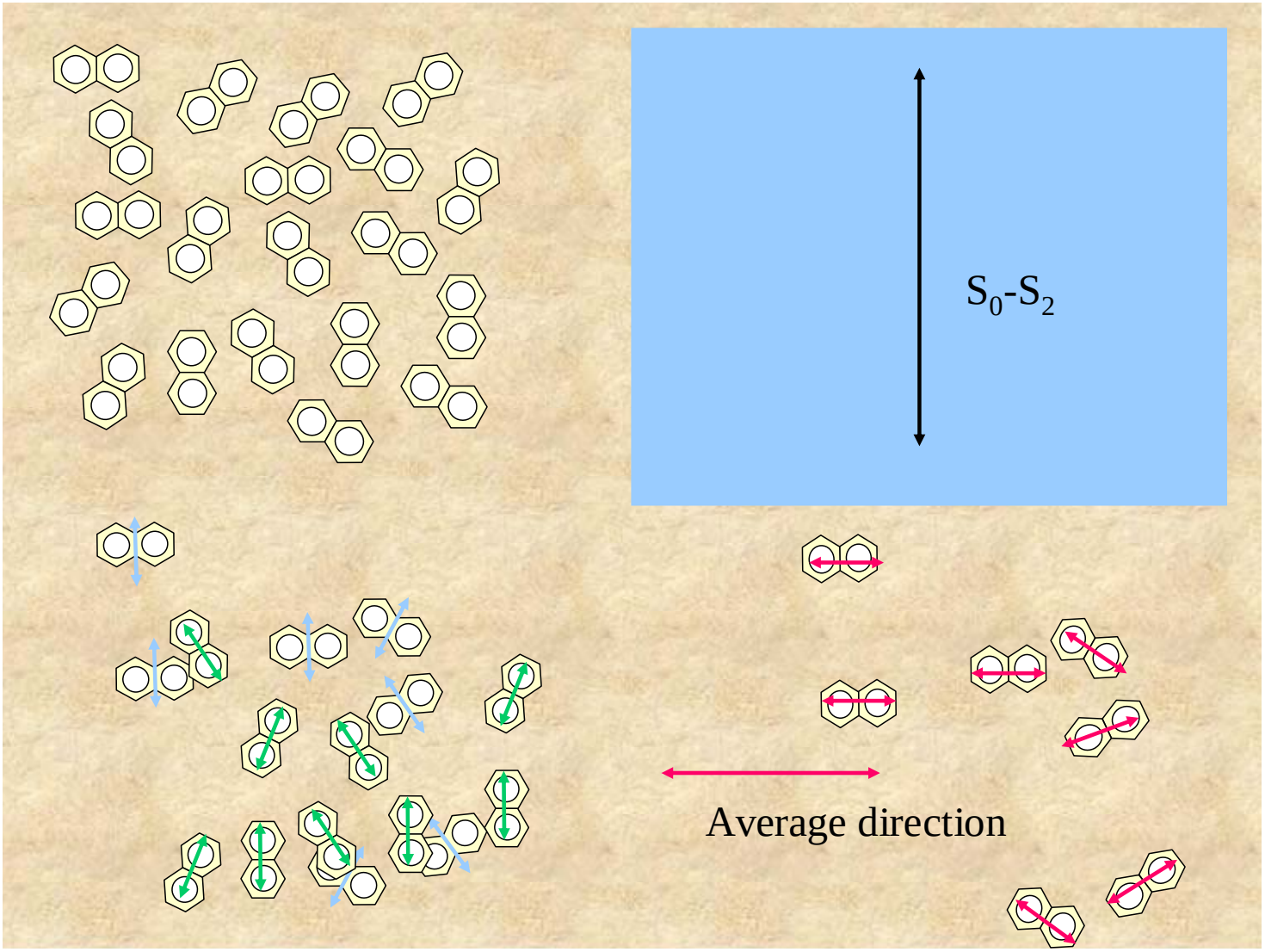


So we see that the two excited dipoles corresponding to the $S_0 \rightarrow S_1$ and the $S_0 \rightarrow S_2$ transitions may be oriented at an arbitrary angle - in the extreme case this angle could be 90°. After the excitation process, however, regardless of whether the absorption process corresponded to the $S_0 \rightarrow S_1$ or the $S_0 \rightarrow S_2$ transition, rapid thermalization leaves the excited fluorophore in the S_1 level. The orientation of the excited dipoles will thus now possess a different average orientation than the absorption dipoles originally photoselected by the exciting light.

This situation is depicted below:




S_0-S_1
Average direction


S_0-S_2
Average direction

Consider the excitation polarization spectrum for phenol (in glycerol at - 70 C).

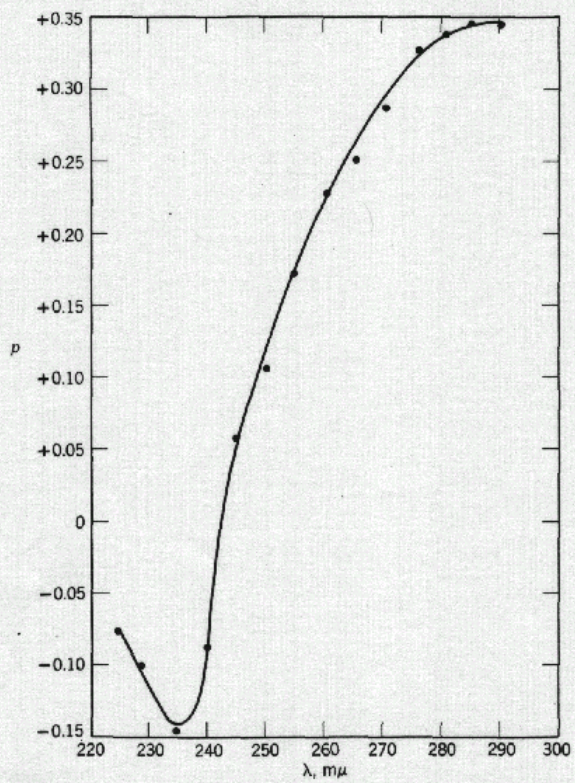


Fig. 8-5. Fluorescence polarization spectrum of phenol at −70°C in propylene glycol. Ordinate = polarization, p; abscissa, exciting wavelength in mμ. Redrawn from Weber (18).

In cases where there are multiple overlapping absorption bands at various angles, the excitation polarization spectrum can be somewhat complex as shown below for indole.

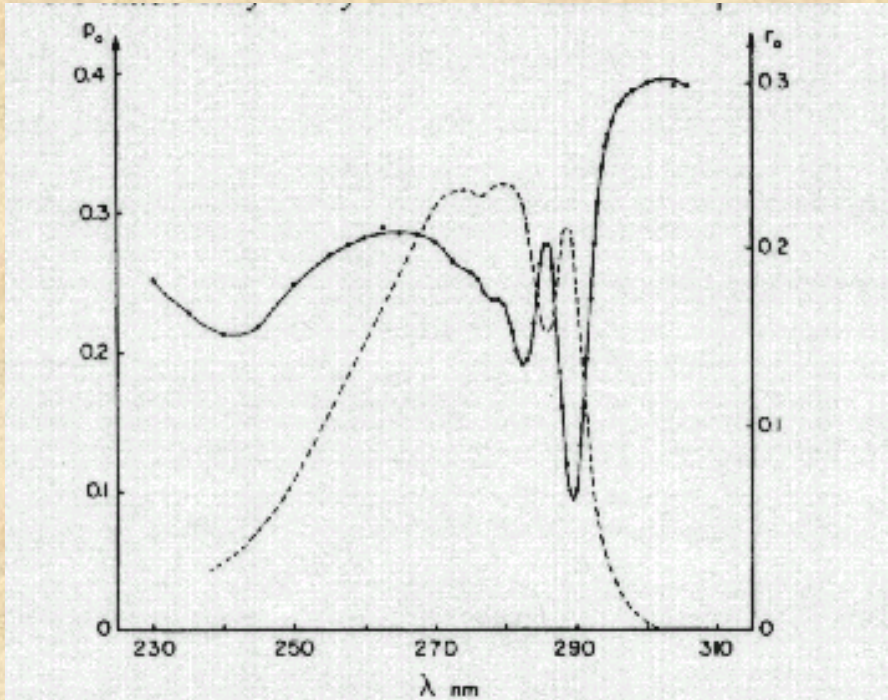
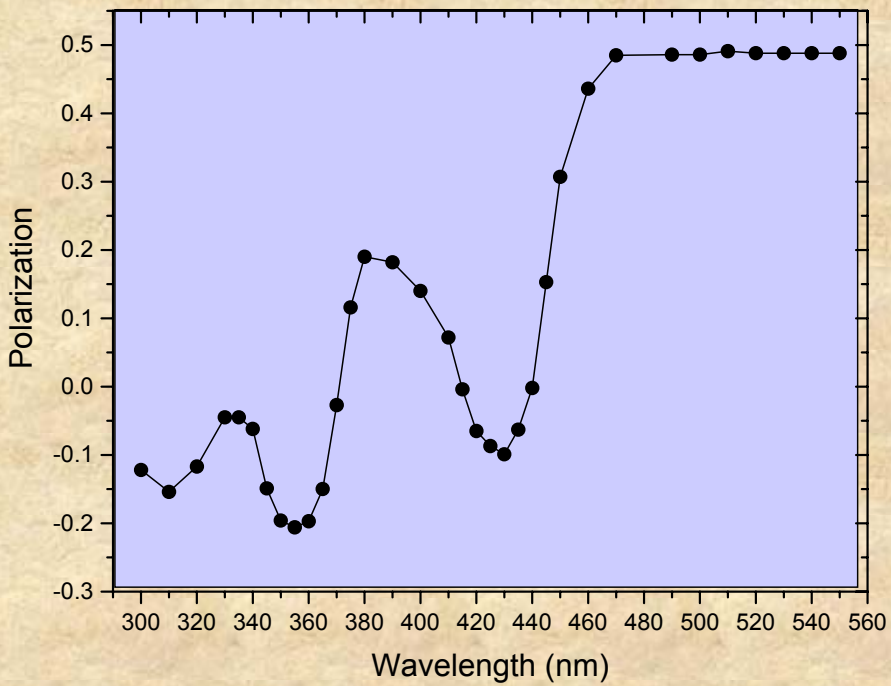
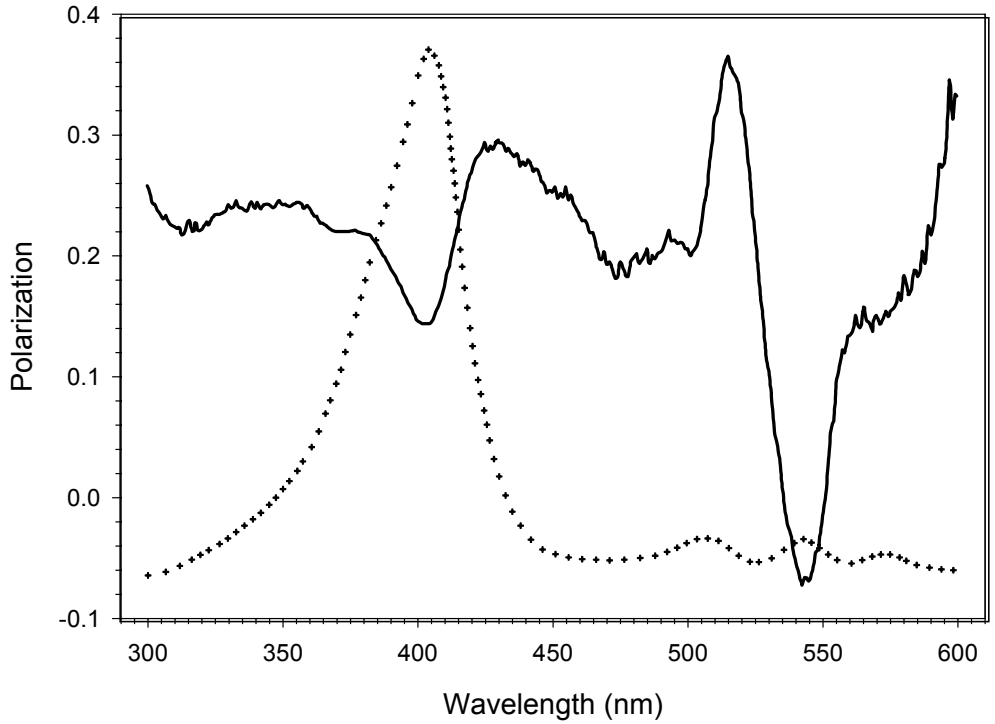


Figure 2. Corrected excitation spectrum (broken line) and excitation polarization spectrum of indole in propylene glycol at −58 . The fluorescence is observed through a Corning 7-39 filter.

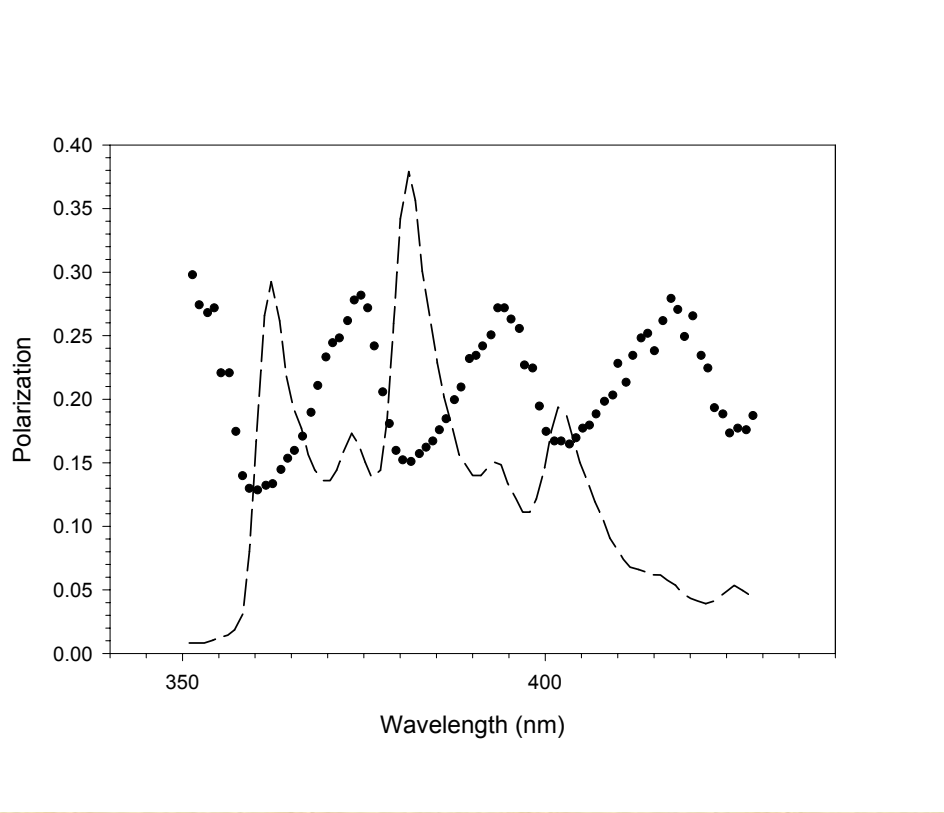
Excitation polarization spectra of rhodamine B embedded in a Lucite matrix at room temperature. Emission was viewed through a cut-on filter passing wavelengths longer than 560nm; slits were ~4nm.



Another example is protoporphyrin IX in glycerol at –20C



In fact, the limiting polarization can also vary across the emission band, as shown here for chrysene in glycerol at –60C



Fluorescence polarization: the effect of molecular motion.

Until now we have considered the fluorescence emitted by a fixed dipole, in practice, the emission takes place 10^{-8} to 10^{-9} s after excitation.

During this time the molecule can change the orientation.

In this case the polarization of the emitted radiation will be different with respect to the one we have calculated.

Consider a sample excited at time t=0 by a pulse of light. The sample will decay in a very short time to the bottom of the first excited electronic level and then will start to decay to the ground level with an exponential characteristic time τ.

If simultaneously the molecule is **rotating,** the first photon emitted will have a polarization P_o and the last will be depolarized. The **rate of change of the polarization** (or anisotropy) is dependent on the rotational rate.

The transition dipole moment can be represented by a point on the surface of a sphere. The rotational process can be described by a **random walk on the surface of the sphere.**

The equation describing the motion is

$$\frac{dW(\vartheta,\varphi,t)}{dt} = D_{rot}\nabla^2 W(\vartheta,\varphi,t)$$

W is the probability for the molecule to have orientation θ and ϕ at time t.

The **rotational** diffusion coefficient is given by the Einstein formula

$$D_{rot} = \frac{kT}{6V_h\eta}$$

where η is the viscosity and V_h the hydrated volume

To calculate the **emission intensity** we must account that the population decays as

exp $(-t/\tau_F)$

so that $W(\theta, \underline{f}, t)$ exp $(-t/\tau_F)$ is the probability to find a molecule with an angle θ and ϕ at time t.

We have calculated the probability distribution of excited molecules at time t=0.

$$W(\vartheta, \varphi, t) = \frac{3}{4\pi} \cos^2 \vartheta \sin \vartheta$$

The probability of emission along z or x directions is $\cos^2\theta$ and $\sin^2\theta\cos^2\phi$ respectively.

Combining all these relationships and solving the diffusion equation we obtain

$$I_{|}(t) = [\frac{1}{3} + \frac{4}{15} e^{-6 D_{rot} t} \frac{3\cos^2 \xi - 1}{2}] e^{-t/\tau_F}$$

$$I_{\perp}(t) = [\frac{1}{3} - \frac{2}{15} e^{-6 D_{rot} t} \frac{3\cos^2 \xi - 1}{2}] e^{-t/\tau_F}$$

The decay of the emission of a sample excited with polarized light and analyzed in the parallel and perpendicular directions is a **double exponential.**

The decay of the emission anisotropy is given by

$$A(t) = \frac{I_{\parallel} - I_{\perp}}{2I_{\parallel} + I_{\perp}} = \frac{2e^{-6D_{rot}t}}{5}\frac{3\cos^2\xi - 1}{2}$$

and is **independent** upon the lifetime τ_F,

Notice that at time t=0 $\Rightarrow$ A=A_o and at time t=$\infty \Rightarrow$ $A = 0$;

Estimation of the value of $\mathbf{D_{rot}}$**:**

$$D_{rot} = \frac{kT}{6V_h\eta}$$

In water η = 0.01 p.

$$D_{rot} = \frac{2 \times 300}{6 \times 12000 \times 0.01} \frac{cal}{M \frac{ml}{M} \frac{gm}{cm \times \sec \times 10^2}} = 3.4 \times 10^9 \, s^{-1}$$

For lysozyme V_h=12000 ml/mole

This value is in the same range as the fluorescence decay time.

Notice that the volume of the protein is the **hydrated volume.**

STEADY-STATE values in the presence of rotational motion.

Generally what is measured is the average value of the parallel and the perpendicular polarization component.

$$\overline{I_{\parallel}} = \frac{1}{\tau_F}\int_0^{\infty} I_{\parallel} dt$$

$$\overline{I_{\perp}} = \frac{1}{\tau_F}\int_0^{\infty} I_{\perp} dt$$

We define the average value of the anisotropy

$$\overline{A} = \frac{\overline{I_{\parallel} - I_{\perp}}}{I_{\parallel} + I_{\perp}} = \frac{3\cos^2\xi - 1}{5(1 + \frac{\tau_F}{\tau_C})}$$

$$\tau_C = \frac{1}{6D_{rot}} = \frac{V_h \eta}{kT}$$

If T is very small or η is very high such that τ_c is very long, then

$$\overline{A} \Longrightarrow \overline{A_0} = A_0$$

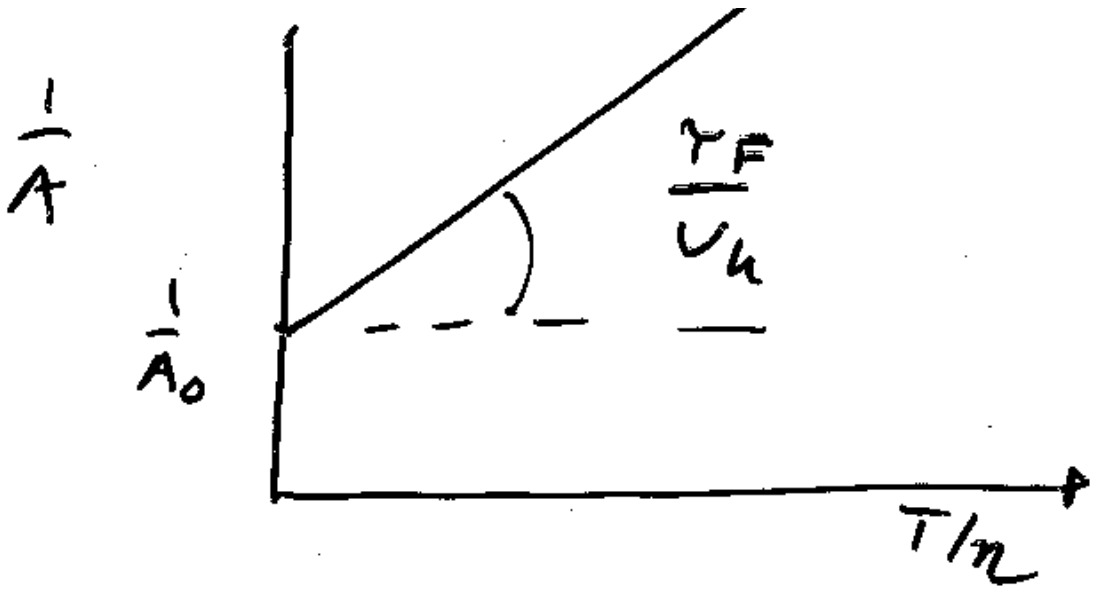
this is the limiting case.

The Perrin equation

Introducing the expression of the limiting anisotropy into equation 1 we obtain the Perrin equation

$$\frac{1}{A} = \frac{1}{A_0}(1 + \frac{\tau_F}{\tau_C}) = \frac{1}{A_0}(1 + \frac{\tau_F kT}{V_h \eta})$$

If the values of the 1/anisotropy are plotted as a function of T/η, according to the Perrin equation a **straight line** should result.



If the value of τ_F is known, the **volume of the protein** can be measured.

Comments on the Perrin equation

1. The Perrin equation is valid for a spherical molecule. In general, proteins are non spherical. The rotational diffusion rate can be different for different molecular

axis of the protein. A more complex equation can be obtained (Weber-Perrin equation). As a general result the equation will contain as many as five exponentials. Generally the accuracy of the data does not allow more than two exponentials to be fitted. What is found is that the apparent rotational rate will be larger for the same molecular weight protein if the shape is non-spherical.

2. We have considered rotations of the entire molecule and we have assumed that the emitting chromophore is rigid in the macromolecular frame. In many cases a residual mobility is present. This effect lowers the value of the anisotropy.

The Perrin plot is not a straight line if there is residue rotational mobility.

F. Perrin related the observed polarization to the excited state lifetime and the rotational diffusion of a fluorophore: *Perrin, F. 1926. Polarisation de la Lumiere de Fluorescence. Vie Moyene des Molecules Fluorescentes. J. Physique. 7:390-401.*

Specifically:

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_o} - \frac{1}{3}\right)\left(1 + \frac{RT}{\eta V}\tau\right)$$

where V is the molar volume of the rotating unit, R is the universal gas constant, T the absolute temperature, η the viscosity and τ the excited state lifetime.

We can rewrite this equation as:

$$\frac{1}{P} - \frac{1}{3} = \left(\frac{1}{P_o} - \frac{1}{3}\right)\left(1 + \frac{3\tau}{\rho}\right)$$

Where ρ is the Debye rotational relaxation time which is the time for a given orientation to rotate through an angle given by the arccos e^{-1} (68.42^o).

For a spherical molecule:

$$\rho_o = \frac{3\eta V}{RT}$$

For a spherical protein,
it follows that:

$$\rho_o = \frac{3\eta M(\upsilon + h)}{RT}$$

Where M is the molecular weight, ν is the partial specific volume and h the degree of hydration.

* *Rotational relaxation time* versus *rotational correlation time*.

We should note that it is not uncommon to see the term "rotational correlation time", often denoted as τ_c, used in place of the Debye rotational relaxation time. The information content of these terms is similar since $\rho = 3\tau_c$ but we have observed that some people become rather fervently attached to the use of one term or the other.

In the original development of the theories of rotational motion of fluorophores Perrin and others used the rotational relaxation time, as originally defined by Debye in his studies on dielectric phenomena. Only later (in the 1950's) during the development of nuclear magnetic resonance was the term rotational correlation time used by Bloch. It thus seems reasonable for fluorescence practitioners to use ρ but certainly adoption of either term should not lead to confusion. In terms of anisotropy and rotational correlation times, then, the Perrin equation would be:

$$\frac{r_o}{r} = \left(1 + \frac{\tau}{\tau_c}\right)$$

If the molecule is not spherical then the relevant term is the harmonic mean of the rotational relaxation times (ρ_h) about the principle rotational axes

$$\rho_h^{-1} = \left(\frac{\rho_1^{-1} + \rho_2^{-1} + \rho_3^{-1}}{3} \right)$$

A plot of 1/P - 1/3 versus T/η predicts a straight line, the intercept and slope of which permit determination of P_o and the molar volume (if the lifetime is known). Shown below is such a plot (termed a Perrin-Weber plot) for protoporphyrin IX associated with apohorseradish peroxidase - the viscosity of the solvent is varied by addition of sucrose.

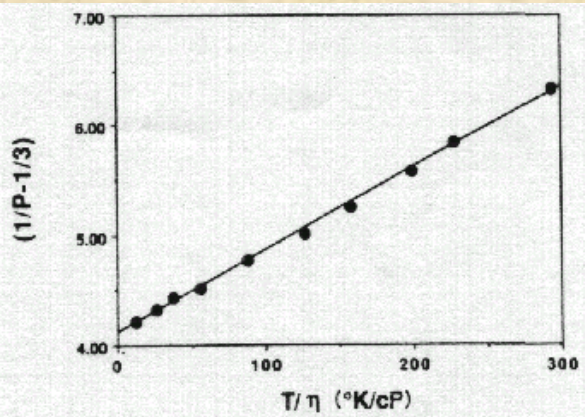


Fig. 3. Perrin plot for HRP(desFe) at 22° C; viscosity was varied by sucrose addition. Excitation wavelength was 514 nm; emission was observed through a Corion LL 600 cuton filter which passed $\lambda > 600$ nm.

The polarization observed in buffer alone was 0.151 while the limiting polarization obtained from the intercept on the Y-axis was 0.225, which is the same value one obtains for upon excitation of protoporphyrin IX in glycerol at low temperatures. From the Perrin equation:

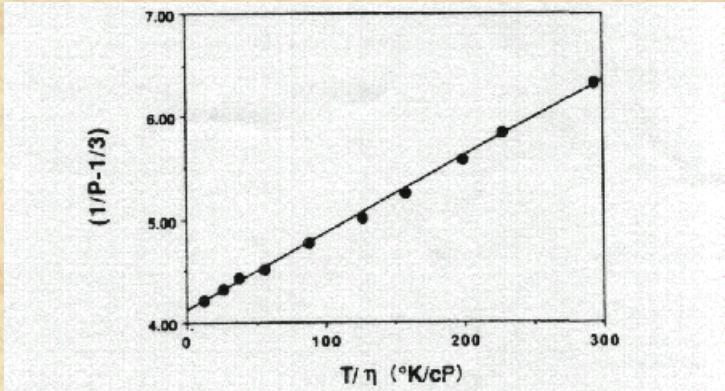


Fig. 3. Perrin plot for HRP(desFe) at 22° C; viscosity was varied by sucrose addition. Excitation wavelength was 514 nm; emission was observed through a Corion LL 600 cuton filter which passed λ > 600 nm.

$$\frac{1}{P}-\frac{1}{3}=\left(\frac{1}{P_o}-\frac{1}{3}\right)\left(1+\frac{3\tau}{\rho}\right)$$

and knowing the lifetime of 16.9 ns, one can calculate a rotational relaxation time of 96 ns for the protein-porphyrin complex:

$$\frac{1}{0.151}-\frac{1}{3}=\left(\frac{1}{0.225}-\frac{1}{3}\right)\left(1+\frac{3\text{x}16.9\text{ns}}{\rho}\right)$$

$$\rho = 96 \text{ ns}$$

$$\rho_o = \frac{3\eta M(\upsilon + h)}{RT}$$

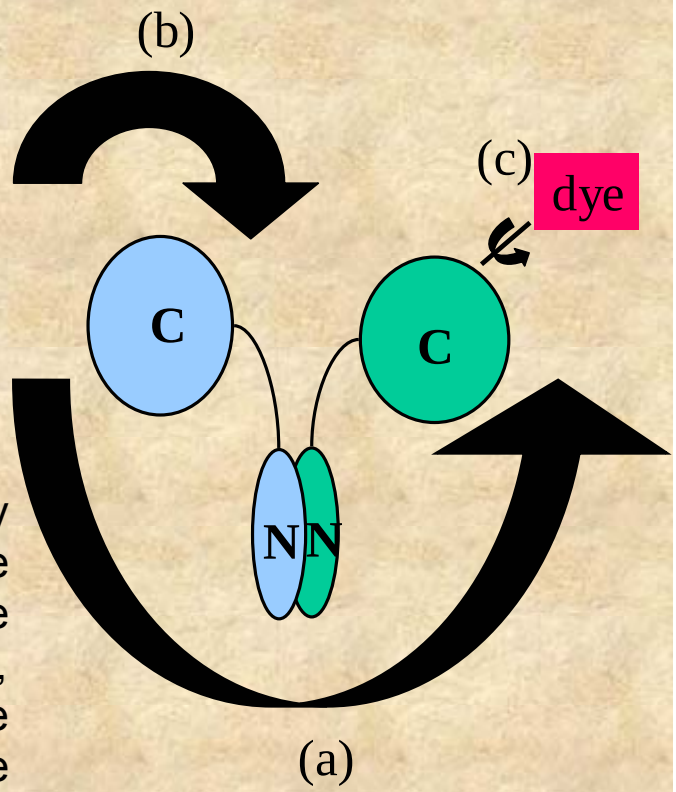
For a spherical protein of 44,000 daltons and assuming a partial specific volume of 0.74 and 0.3 ml/mg for the hydration, one can then calculate:

ρ_o = (3)(0.01)(44000)(0.74+0.3)/(8.31x10^7)(293) = ~56 ns

Thus it appears as if this protein is non-spherical

In the case of fluorescence probes associated non-covalently with proteins, (for example porphryins, FAD, NADH or ANS to give but a few systems), the probe is held to the protein matrix by several points of attachment and hence its “local” mobility, that is, its ability to rotate independent of the overall “global” motion of the protein, is very restricted.

In the case of a probe attached covalently to a protein, via a linkage through an amine or sulfhydryl groups for example, or in the case of tryptophan or tyrosine sidechains, considerable “local” motion of the fluorophore can occur. In addition, the protein may consist of flexible domains which can rotate independent of the overall “global” protein rotation. This type of mobility hierarchy is illustrated on the right for the case of a probe covalently attached to a dimeric protein



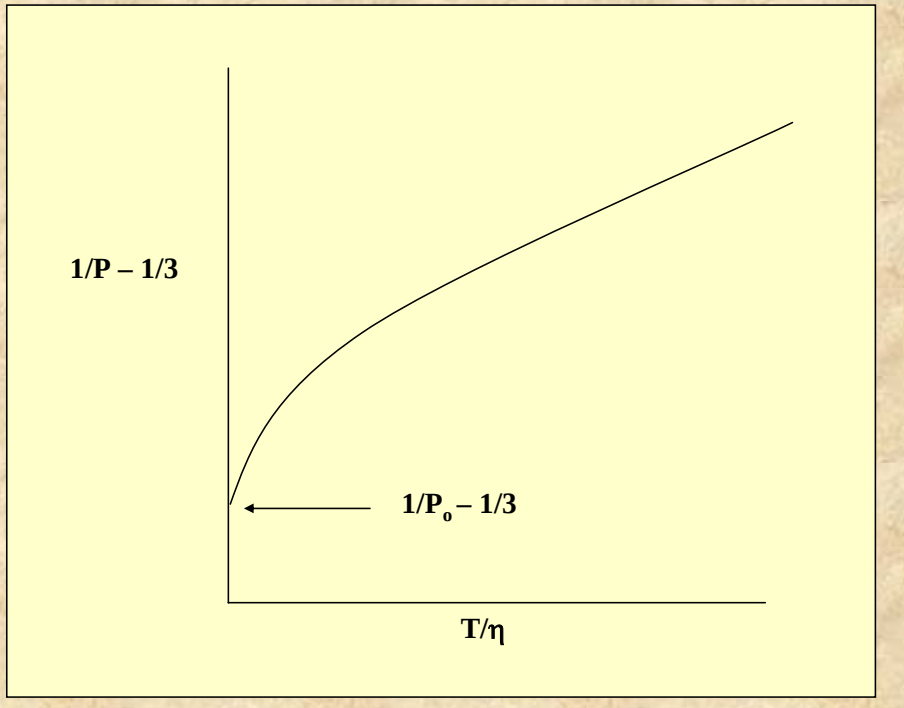
Rotational Modalities

(a) overall L7/L12 rotation

(b) movement of one C-domain relative to other domains

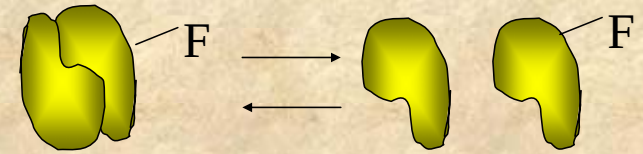
(c) movement of dye molecule around its point of attachment

In such a system one would see a downward curvature in the Perrin-Weber plots as illustrated below:

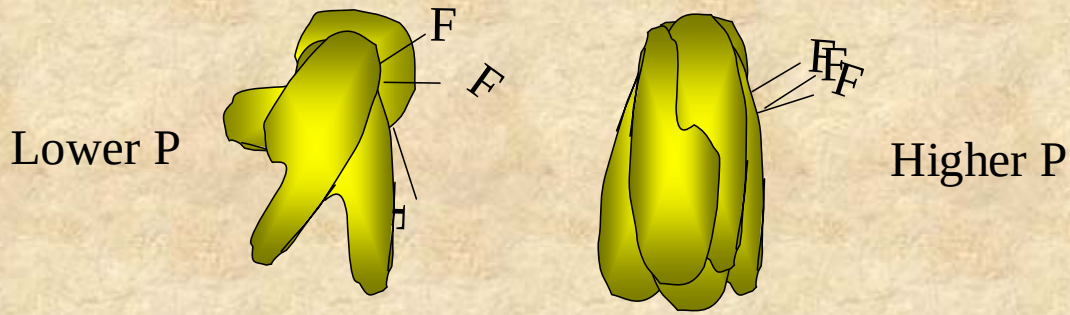


A detailed analysis of the rotational modalities in such a system requires time-resolved measurements, which will be discussed later.

Polarization methods are ideally suited to study the aggregation state of a protein. Consider, for example the case of a protein dimer - monomer equilibrium.



Following either intrinsic protein fluorescence (if possible) or by labeling the protein with a suitable probe one would expect the polarization of the system to decrease upon dissociation of the dimer into monomers since the smaller monomers will rotate more rapidly than the dimers (during the excited state lifetime).



Hence for a given probe lifetime the polarization (or anisotropy) of the monomer will be less than that of the dimer

In the concentration range near the dimer/monomer equilibrium constant, one expects to observe a polarization intermediate between that associated with either dimer or monomer. One can relate the observed polarization to the fraction of dimer or monomer using the additivity of polarizations first described by Weber (1952) namely:

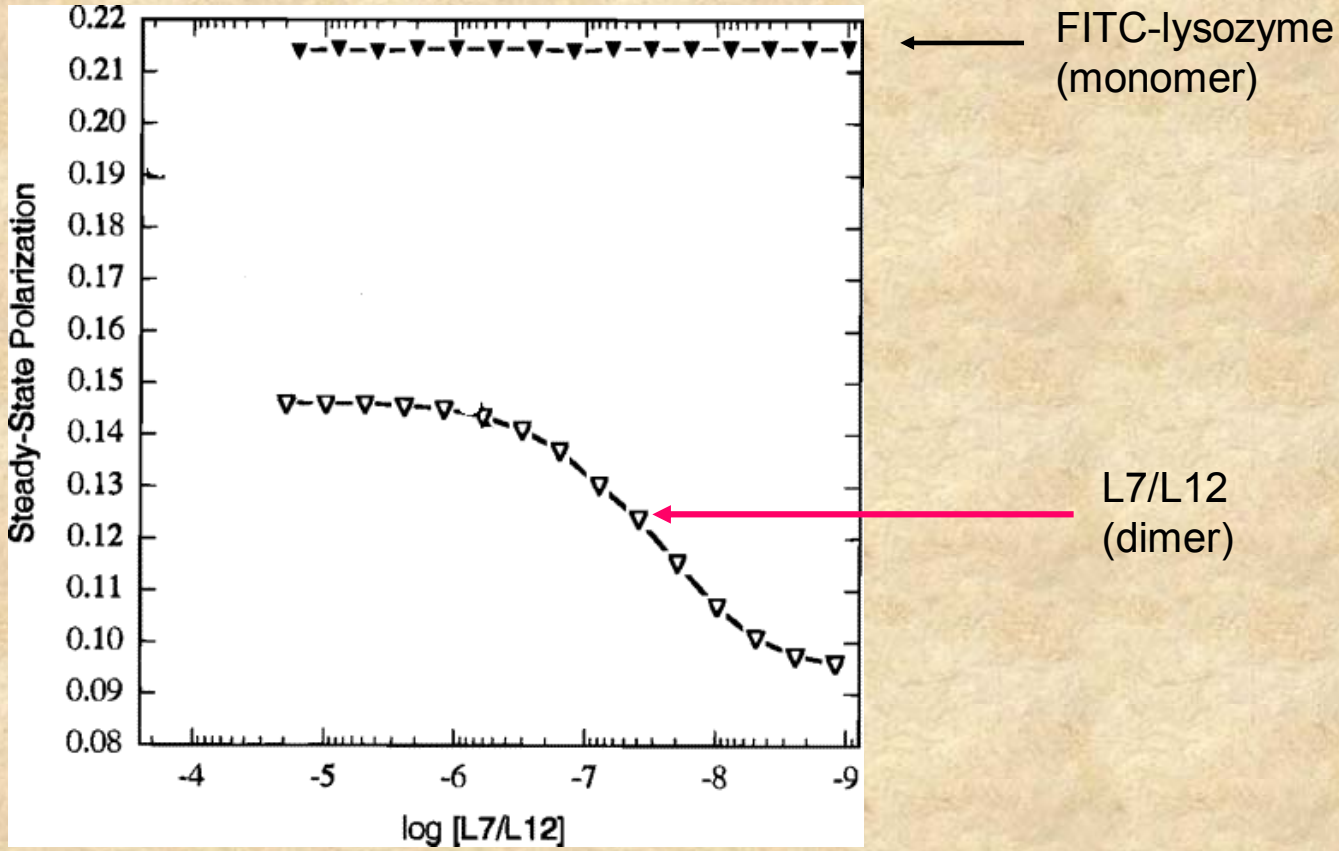
$$\left(\frac{1}{<P>}-\frac{1}{3}\right)^{-1}=\sum f_i\left(\frac{1}{P_i}-\frac{1}{3}\right)^{-1}$$

where <P> is the observed polarization, f_i is the fractional intensity contributed by the ith component and P_i is the polarization of the ith component. One must then relate the fractional intensity contributions to molar quantities which means that one must take into account any change in the quantum yield of the fluorophore associated with either species.

The anisotropy function is directly additive (owing to the fact that the denominator represents the total emitted intensity) and hence:

$$<r>=\sum f_i r_i$$

So to determine the dissociation constant, one can dilute the protein and observe the polarization (or anisotropy) as a function of protein concentration as shown below.



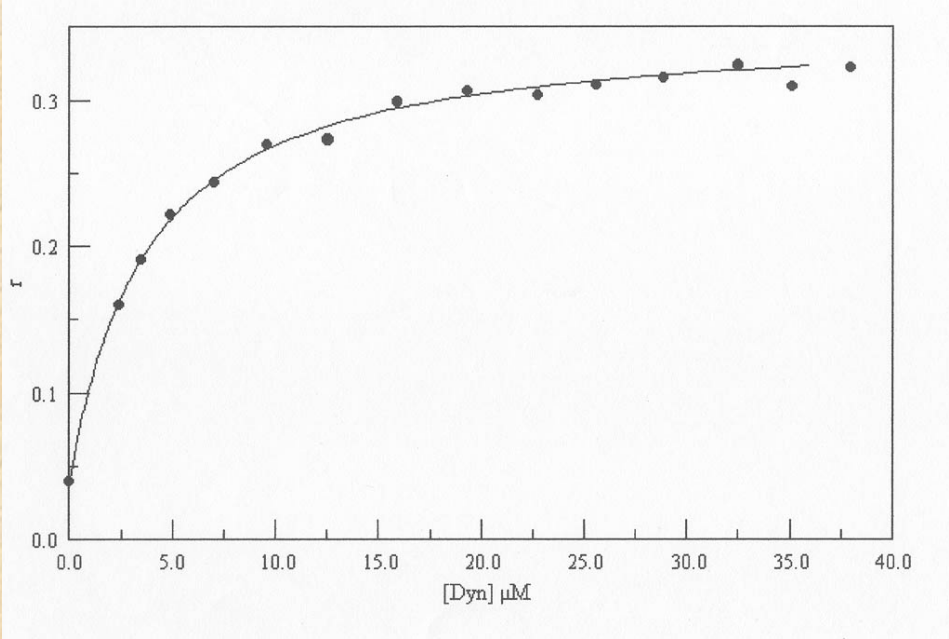
The polarization/anisotropy approach is also very useful to study protein-ligand interactions in general.

The first application of fluorescence polarization to monitor the binding of small molecules to proteins was carried out by D. Laurence in 1952 using Gregorio Weber's instrumentation in Cambridge. Specifically, Laurence studied the binding of numerous dyes, including fluorescein, eosin, acridine and others, to bovine serum albumin, and used the polarization data to estimate the binding constants.

Although many probes (such as fluorescein) do not significantly alter their quantum yield upon interaction with proteins, one should not take this fact for granted and would be well advised to check. If the quantum yield does in fact change, one can readily correct the fitting equation to take the yield change into account. In terms of anisotropy the correct expression relating observed anisotropy (r) to fraction of bound ligand (x), bound anisotropy (r_b), free anisotropy (r_f), and the quantum yield enhancement factor (g) is:

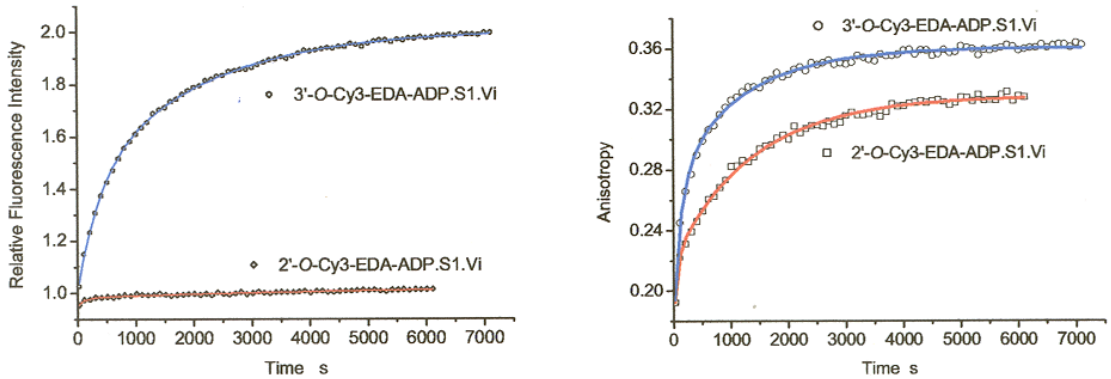
$$x = \frac{r - r_f}{r_b - r_f + (g-1)(r_b - r)}$$

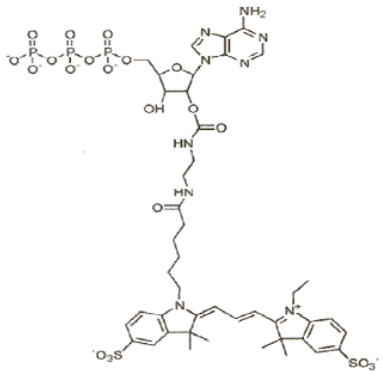
A typical plot of polarization versus ligand/protein ratio is shown below:



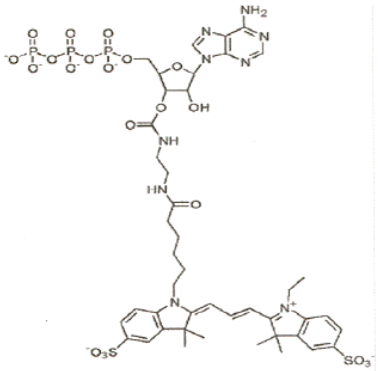
In this experiment, 1 micromolar mant-GTPγS (a fluorescent, non-hydrolyzable GTP analog) was present and the concentration of the GTP-binding protein, dynamin, was varied by starting at high concentrations followed by dilution. The binding curve was fit to the anisotropy equation (in this case the yield of the fluorophore increased about 2 fold upon binding). A K_d of 8.3 micromolar was found

Another example of the utility of polarization/anisotropy data is shown here for the case of cyanine analogs of ADP binding to myosin subfragment. The 3'-isomer shows increased intensity upon binding while the 2'-isomer does not. But anisotropy data indicate binding of both isomers (from Oiwa et al 2003 Biophys. J. 84:634)





2'



3'

FPIA – Fluorescence Polarization ImmunoAssay

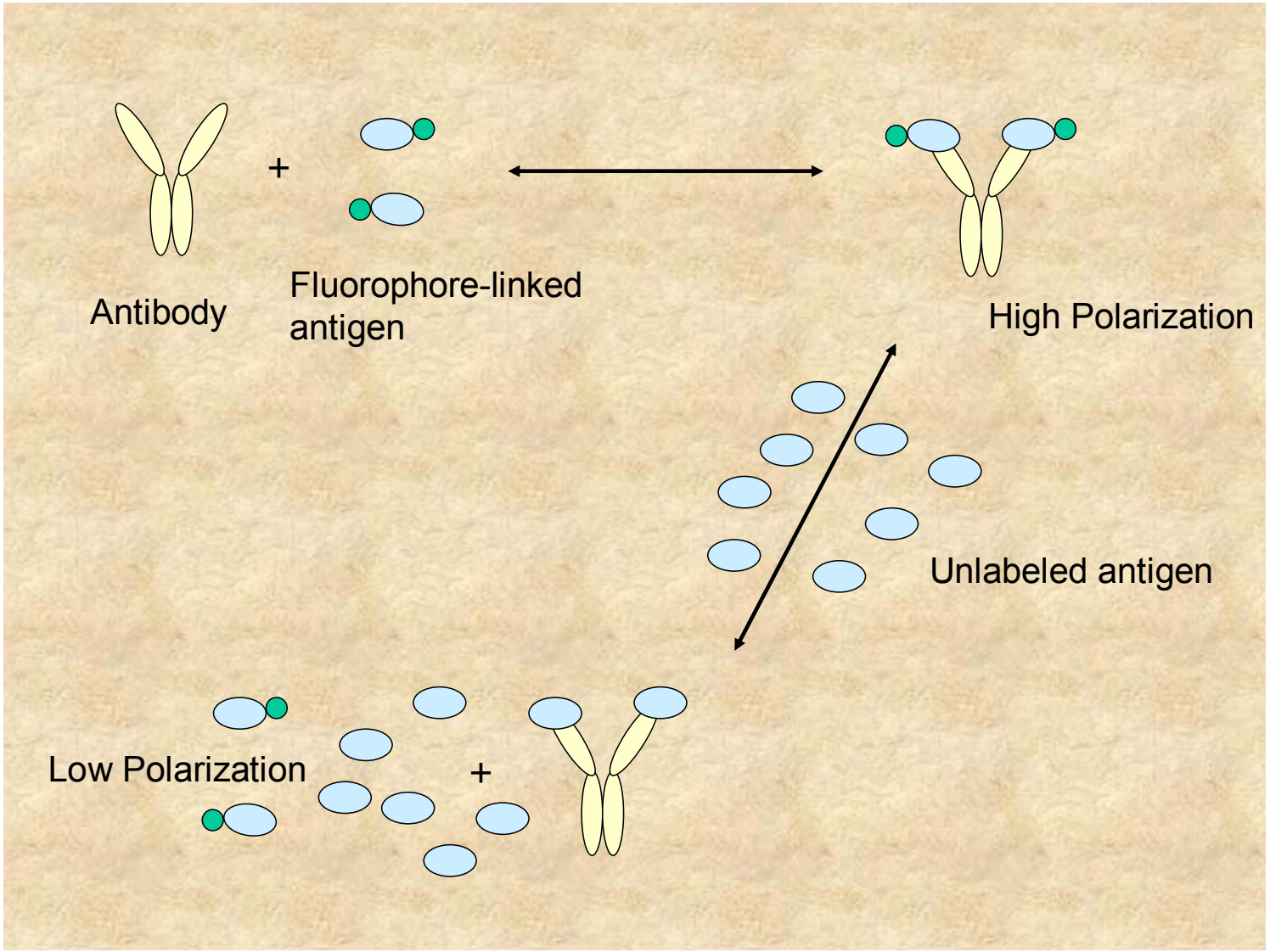
Among the first commercial instruments designed to use a fluorescence polarization immunoassay for clinical diagnostic purposes was the Abbott TDx – introduced in 1981.

The basic principle of a polarization immunoassay is to:

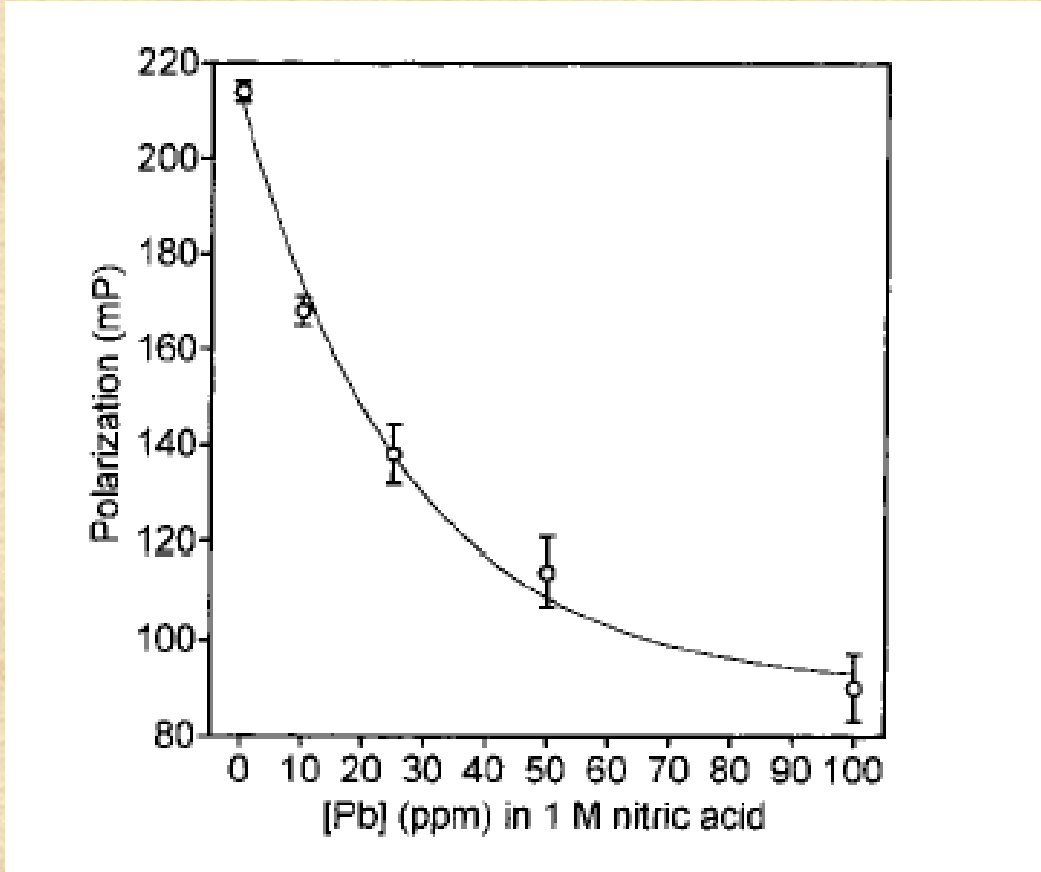
(1) Add a fluorescent analog of a target molecule – e.g., a drug – to a solution containing antibody to the target molecule

(2) Measure the fluorescence polarization, which corresponds to the fluorophore bound to the antibody

(3) Add the appropriate biological fluid, e.g., blood, urine, etc., and measure the decrease in polarization as the target molecules in the sample fluid bind to the antibodies, displacing the fluoroescent analogs.


Antibody
+
Fluorophore-linked antigen
High Polarization
Unlabeled antigen
Low Polarization
+

Example of FPIA data using an antibody against a lead-chelating agent



Johnson et al., (2002) Environ. Sci. Tech. 36:1042-1047.