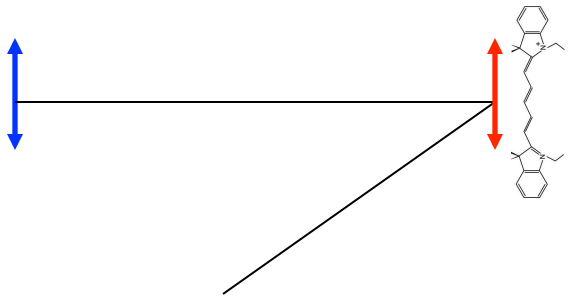
A - Polarization Anisotropy

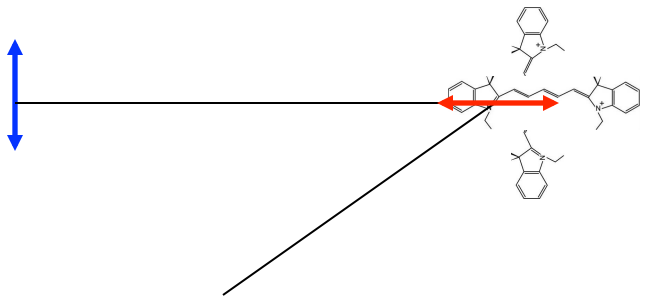
1. Molecules aligned parallel to the electric field absorb light, molecules aligned perpendicular do not absorb light efficiently. In solution, where molecules tumble rapidly, light absorption is the spatial average of the transition dipole moment.

$$\left|\left\langle \Psi_b \left| \hat{\mu} \right| \Psi_a \right\rangle \cdot \hat{E}_0 \right|^2 = \frac{1}{3}\left(\mu_x^2 + \mu_y^2 + \mu_z^2\right)E_0^2$$

2. Without motion, the polarization of the emitted light is parallel to the excitation source. *(assuming that the excitation and emission transition dipole moments are parallel)*



3. With rapid motion, the emission polarization can either be parallel or perpendicular to the polarization of the excitation source



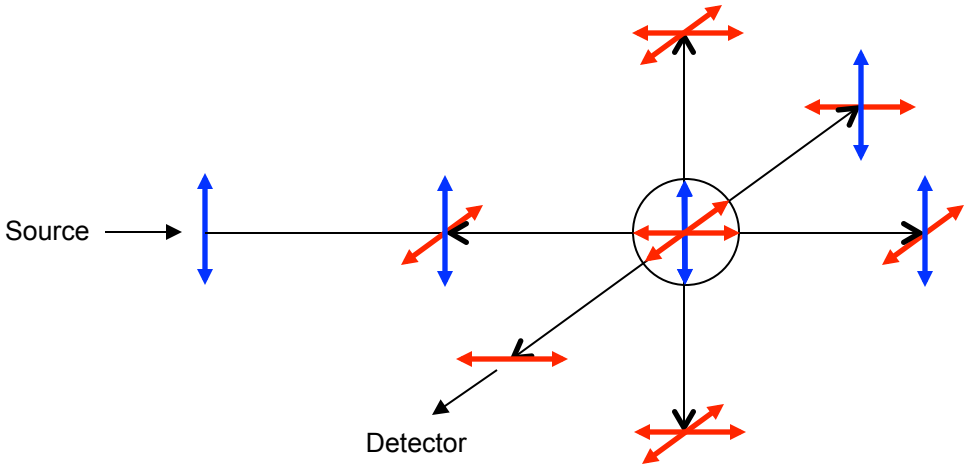
Anisotropy - Definition

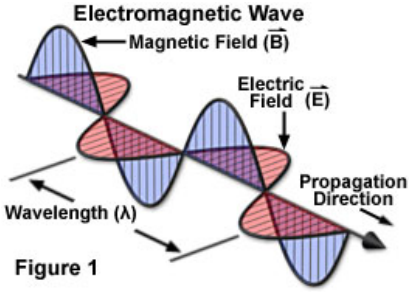
1. The anisotropy indicates the extent to which emission polarization is retained

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

2. $I_{\parallel} - I_{\perp}$: the difference in the detected intensities of || & ⊥ polarized light

3. $I_{\parallel} + 2I_{\perp}$: the total isotropic fluorescence intensity





(E and B are ⊥ to direction of propagation)

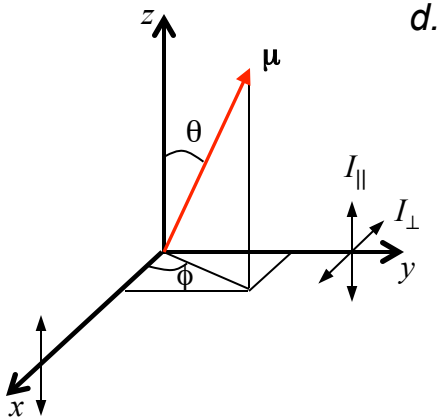
1. What are the Maximum and Minimum Values of *A*?

a. $A_{MIN} = 0.0$ $A_{MAX} = 1.0$

b. $A_{MIN} = 0.2$ $A_{MAX} = 0.5$

c. $A_{MIN} = 0.0$ $A_{MAX} = 0.4$

d. $A_{MIN} = 0.0$ $A_{MAX} = 0.5$

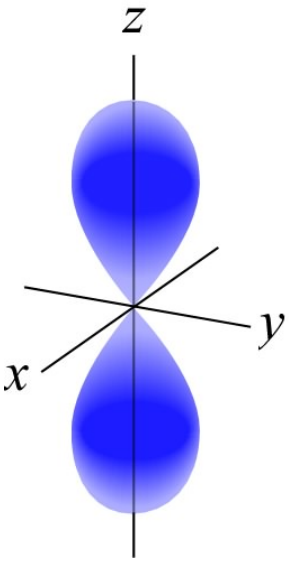


1a. Relative number of excited molecule oriented between θ to $\theta + d\theta$ and ϕ to $\phi + d\phi$:

$$P(\theta,\phi)\, d\theta d\phi \;\propto\; \cos^2\theta \sin\theta\, d\theta d\phi$$

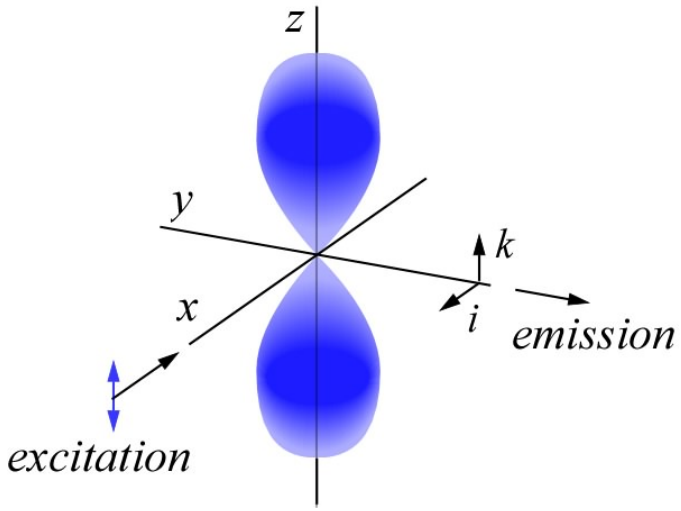
1b. The fraction of all the excited molecules that are between θ to $\theta + d\theta$ and ϕ to $\phi + d\phi$:

$$W(\theta,\phi)\, d\theta d\phi = \frac{P(\theta,\phi)\, d\theta d\phi}{\int_0^{2\pi}\int_0^{\pi} \cos^2\theta \sin\theta\, d\theta d\phi} = \frac{3}{4\pi}\cos^2\theta \sin\theta\, d\theta d\phi$$



Photoselected Population

1c. $I_{\|} \propto |\boldsymbol{\mu}\, \boldsymbol{k}|^2$ and $I_{\perp} \propto |\boldsymbol{\mu}\, \boldsymbol{i}|^2$, which are proportional to $\cos^2\theta$ and $(\sin\theta\cos\phi)^2$, respectively. Integrating over all values of θ and ϕ



Result:

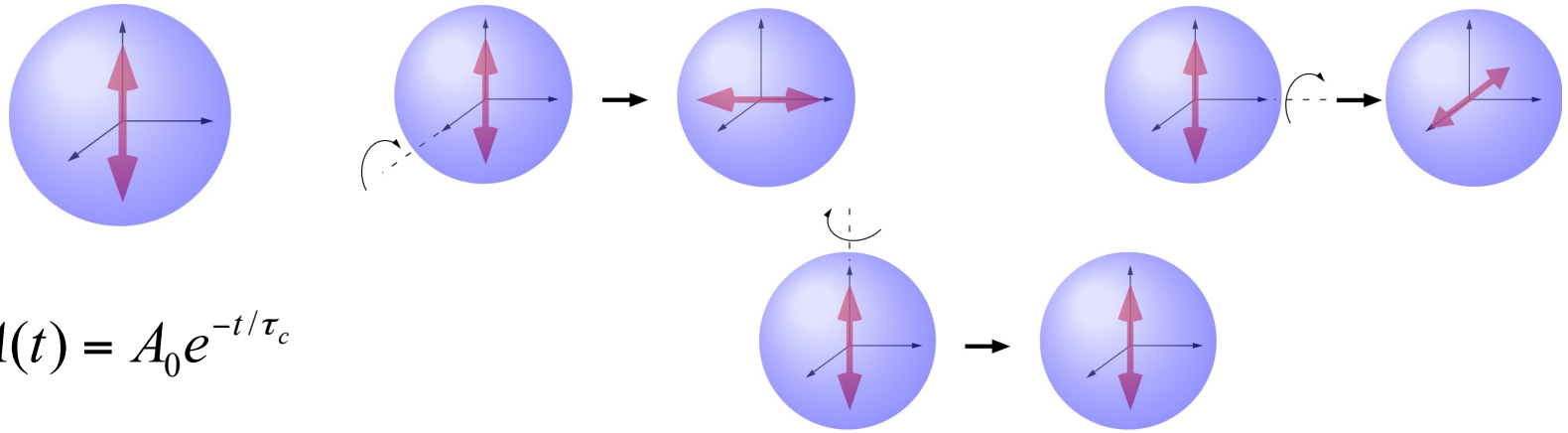
$$A_{\mathrm{MAX}} = \frac{I_{\|} - I_{\perp}}{I_{\|} + 2I_{\perp}} = \frac{3/5 - 1/5}{3/5 + 2/5} = \frac{2}{5} = 0.4$$

$$I_{\|} \propto \int_0^{2\pi}\int_0^{\pi} \cos^2(\theta) W(\theta,\phi) d\theta d\phi = \frac{3}{4\pi}\int_0^{2\pi}\int_0^{\pi} \cos^4(\theta)\sin(\theta)\, d\theta d\phi = \frac{3}{5}$$

$$I_{\perp} \propto \int_0^{2\pi}\int_0^{\pi} \sin^2(\theta)\cos^2(\phi) W(\theta,\phi)\, d\theta d\phi = \frac{3}{4\pi}\int_0^{2\pi}\int_0^{\pi} \cos^2(\phi)\cos^2(\theta)\sin^3(\theta)\, d\theta d\phi = \frac{1}{5}$$

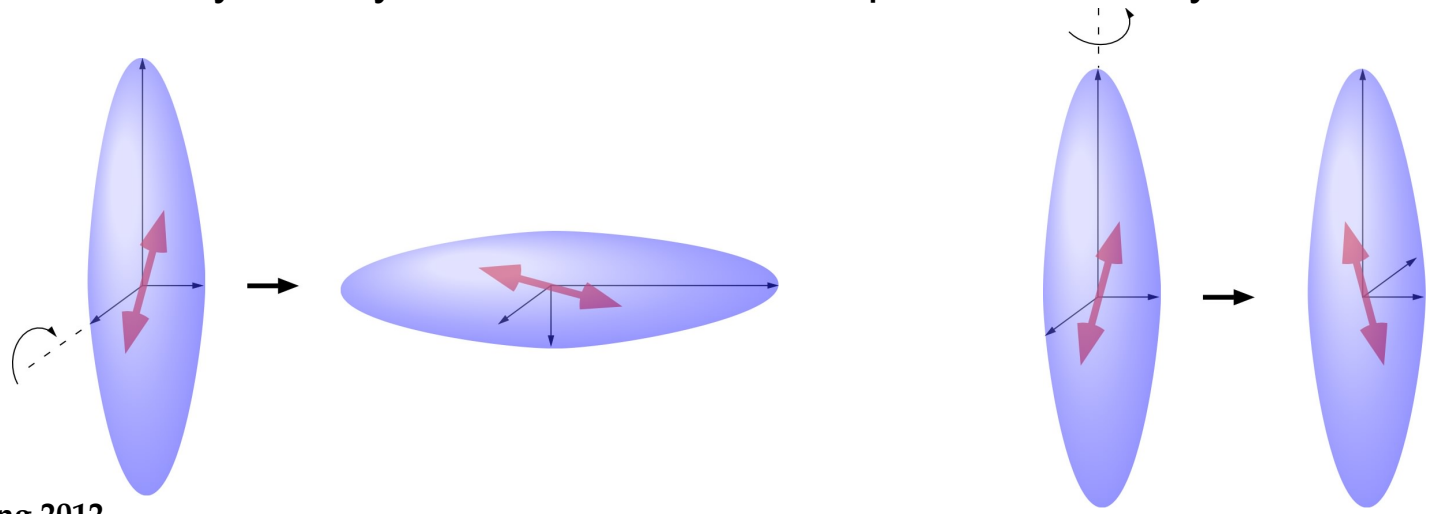
Anisotropy Decay is Sensitive to Macromolecule Shape and Motional Restrictions

1. Spherical Molecule in Solution → Single Exponential Decay



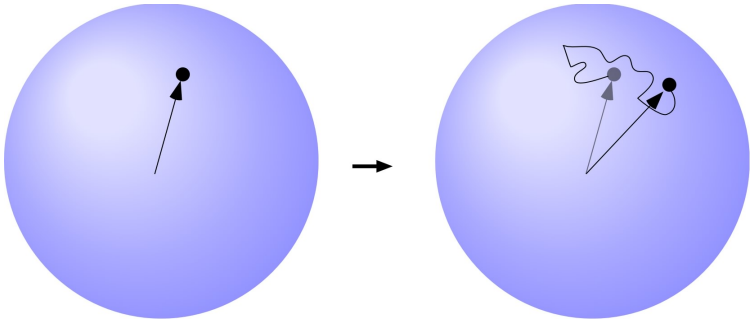
$$A(t) = A_0 e^{-t/\tau_c}$$

2. Lower symmetry Molecules → Multi-exponential Decays



Decay by Diffusion

$$\frac{dW(\theta,\phi,t)}{dt} = D_{Rot}\nabla^2 W(\theta,\phi,t)$$



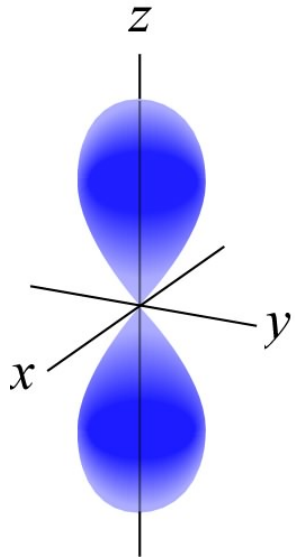
by analogy to the one dimensional translational diffusion and random walk equations:

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}$$

$$\langle x^2 \rangle = 2Dt$$

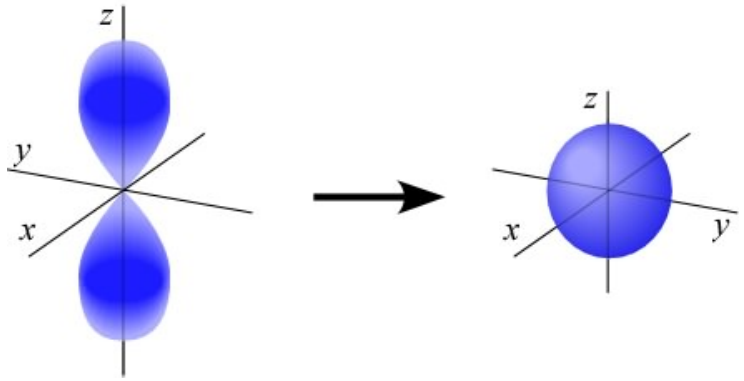
Initial Condition:

$$W(\theta,\phi,0) = \frac{3}{4\pi}\cos^2\theta\sin\theta$$



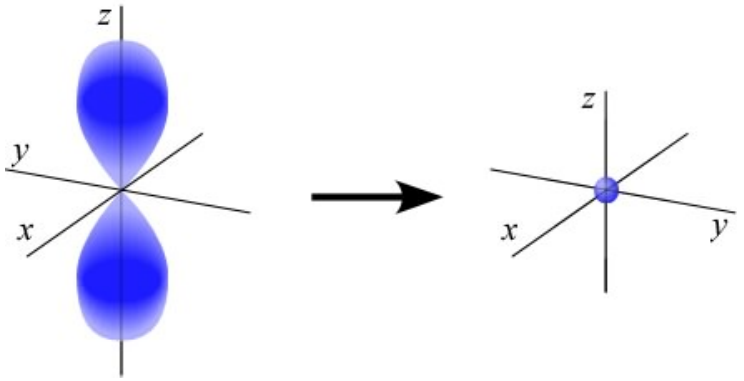
Photoselected Population

Photo-selected Population will Decay by Diffusion to an Isotropic Distribution



…. But the Fluorescence also Decays, as exp(-t/τ_F).

$\tau_F = [k_F + k_{ic} + k_{is} + k_q(Q)]^{-1}$



$$W(\theta,\phi,t)e^{-t/\tau_F}$$

Two time-dependent terms in the anisotropy:

1. W(θ,ϕ,t) describes changes in angular distribution of the photoselected population by rotational diffusion
2. $e^{-t/\tau}$ describes fluorescence decay

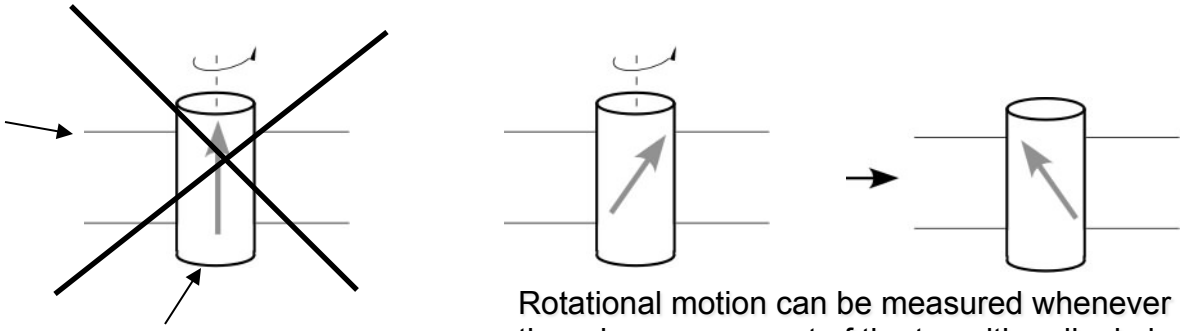
$$I_{\parallel} = \left[\left(\frac{1}{3}\right) + \left(\frac{4}{15}\right)e^{-6D_{Rot}t}\right]e^{-t/\tau_F}$$

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

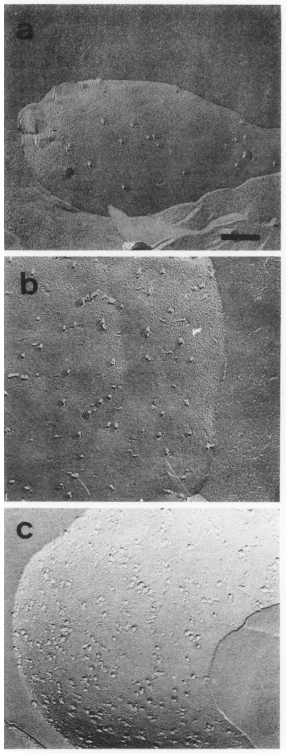
$$A(t) = \left(\frac{2}{5}\right)e^{-6D_{Rot}t}$$

where $D_{\mathrm{Rot}} = kT/f_{\mathrm{Rot}} = kT/6V_{\mathrm{h}}\eta$

Rotational Motion of Membrane Proteins



Rotational motion can be measured whenever there is a component of the transition dipole in the plane of the membrane



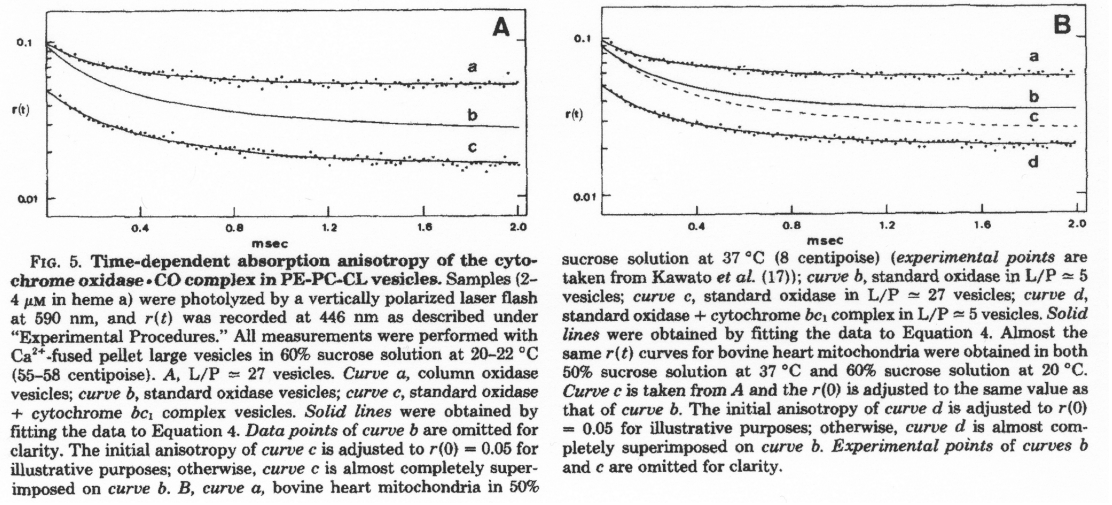


FIG. 5. **Time-dependent absorption anisotropy of the cytochrome oxidase·CO complex in PE-PC-CL vesicles.** Samples (2–4 μM in heme a) were photolyzed by a vertically polarized laser flash at 590 nm, and $r(t)$ was recorded at 446 nm as described under "Experimental Procedures." All measurements were performed with Ca^{2+}-fused pellet large vesicles in 60% sucrose solution at 20–22 °C (55–58 centipoise). *A*, L/P ≃ 27 vesicles. *Curve a*, column oxidase vesicles; *curve b*, standard oxidase vesicles; *curve c*, standard oxidase + cytochrome bc_1 complex vesicles. *Solid lines* were obtained by fitting the data to Equation 4. *Data points* of *curve b* are omitted for clarity. The initial anisotropy of *curve c* is adjusted to $r(0) = 0.05$ for illustrative purposes; otherwise, *curve c* is almost completely superimposed on *curve b*. *B*, *curve a*, bovine heart mitochondria in 50% sucrose solution at 37 °C (8 centipoise) (*experimental points* are taken from Kawato *et al.* (17)); *curve b*, standard oxidase in L/P ≃ 5 vesicles; *curve c*, standard oxidase in L/P ≃ 27 vesicles; *curve d*, standard oxidase + cytochrome bc_1 complex in L/P ≃ 5 vesicles. *Solid lines* were obtained by fitting the data to Equation 4. Almost the same $r(t)$ curves for bovine heart mitochondria were obtained in both 50% sucrose solution at 37 °C and 60% sucrose solution at 20 °C. *Curve c* is taken from *A* and the $r(0)$ is adjusted to the same value as that of *curve b*. The initial anisotropy of *curve d* is adjusted to $r(0) = 0.05$ for illustrative purposes; otherwise, *curve d* is almost completely superimposed on *curve b*. *Experimental points* of *curves b* and *c* are omitted for clarity.

Kawato *et al.* 1981. Rotation of cytochrome oxidase in phospholipid vesicles. Investigations of interactions between cytochrome oxidases and between cytochrome oxidase and cytochrome bc1 complex. *J. Biol. Chem.* **256:**7518-27.