Theory of photoselection by intense light pulses
Influence of reorientational dynamics and chemical kinetics on absorbance measurements

Anjum Ansari and Attila Szabo
Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases,
National Institutes of Health, Bethesda, Maryland 20892 USA

ABSTRACT The theory of absorbance measurements on a system (e.g., chromophore(s) in a protein) that undergoes a sequence of reactions initiated by a linearly polarized light pulse is developed for excitation pulses of arbitrary intensity. This formalism is based on a set of master equations describing the time evolution of the orientational distribution function of the various species resulting from excitation, reorientational dynamics, and chemical kinetics. For intense but short excitation pulses, the changes in absorbance (for arbitrary polarization directions of the excitation and probe pulses) and the absorption anisotropy are expressed in terms of reorientational correlation functions. The influence of the internal motions of the chromophore as well as the overall motions of the molecules is considered. When the duration of the excitation pulse is long compared to the time-scale of internal motions but comparable to the overall correlation time of the molecule that is reorienting isotropically, the problem of calculating the changes in absorbance is reduced to the solution of a set of first-order coupled differential equations. Emphasis is placed on obtaining explicit results for quantities that are measured in photolysis and fluorescence experiments so as to facilitate the analysis of experimental data.

1. INTRODUCTION

With advances in laser technology, photolysis experiments have emerged as the most powerful method for investigating the kinetics and dynamics of ligand binding and conformational changes in heme proteins. In these experiments photodissociation of the hemebound ligand is produced by excitation with a linearly polarized laser pulse. Subsequent events are usually monitored by optical absorption measurements of the heme chromophore. Photoselection and rotational diffusion influence the measured absorbances. Photoselection produces an anisotropic distribution of species in the solution because molecules having a larger projection of their transition moment onto the electric vector of the excitation pulse are preferentially photodissociated. The resulting optical anisotropy can be detected by measuring the absorbance with light linearly polarized both parallel and perpendicular to the electric vector of the excitation pulse. As in the case of fluorescence depolarization experiments, there is dynamical information contained in these measurements. The optical anisotropy, however, also interferes with accurate determination of ligand rebinding and conformational relaxation kinetics if data from only a single polarization is employed.

To understand these effects on time-resolved polarized absorption measurements we have developed a comprehensive theory of photolysis experiments. Our formulation is based on a set of master equations describing the rate of change of the orientational distribution functions of the various species resulting from excitation, reorientational dynamics, and kinetics (1). Special emphasis is placed on the influence of intense excitation pulses which, with some notable exceptions (2–4), has received relatively little attention. When the excitation pulses are sufficiently intense, the system no longer responds linearly and one cannot simply convolute the response with the pulse profile. In this paper, we obtain expressions for the change of absorption and the absorption anisotropy as a function of time and laser intensity for both δ-function and finite excitation pulses. We consider the effects of both overall motions of the molecule and internal motions of the chromophore and the non-ideal (i.e., deviations from linear or circular) absorption properties of the chromophore. In the following paper we apply these results to experimental measurements on myoglobin (5).

This paper is organized as follows. Section 2 discusses the general formalism in the absence of chemical kinetics. The observables are expressed in terms of orientational distribution functions that are a function of orientation dependent extinction coefficients and that satisfy master equations. In section 2.1 we show that for weak excitation pulses, the absorbance changes can be obtained by convoluting the intensity profile with a response that depends on certain reorientational correlation functions. In section 2.2 we consider intense excitation pulses that are sufficiently short so that no reorientational dynamics occurs. In section 3 explicit expressions for the orientation dependent extinction coefficients are invoked. We consider absorbers that are ellipsoids of revolution (linear and circular absorbers are special cases). General ellipsoidal absorbers are treated in the Appendix. In sections 3.1 and 3.2 we obtain explicit expressions for the change in absorbance and absorbance anisotropy for weak and intense but short excitation pulses. In section 3.3 we consider the influence of internal motions. Section 3.4 shows how to handle intense excitation pulses that are so long that significant overall rotational diffusion occurs during the pulse. In section 4 the formalism is extended to include chemical kinetics that occurs on a time-scale longer than the dura-

0006-3495/93/03/838/14 $2.00

tion of the excitation pulse. In section 5 the theory is further generalized to the case in which both rotational diffusion and chemical kinetics occur during excitation. Finally, in section 6 we make some concluding remarks.

2. GENERAL FORMALISM WITHOUT KINETICS

We consider an isotropic distribution of molecules that are initially in state A and that undergo the reaction

$$\mathrm{A} \rightarrow \mathrm{B}, \tag{2.1}$$

which is initiated when a photon is absorbed by A. The orientation of the chromophore in the laboratory frame is specified by the Euler angles Ω. Let $p_{\mathrm{eq}}^{\mathrm{A}}(\Omega)$ be the normalized equilibrium orientational distribution function of the chromophore in state A. Suppose that at $t = 0$ the system is excited by a linearly polarized laser pulse. For an optically thin sample, the time-dependent orientational distribution function of the chromophore in state A, denoted by $p_{\mathrm{A}}(\Omega, t)$, satisfies

$$\frac{\partial p_{\mathrm{A}}(\Omega, t)}{\partial t} = \mathcal{L}_{\mathrm{A}} p_{\mathrm{A}}(\Omega, t) - \epsilon_{\mathrm{A}}(e, \Omega) I(t) p_{\mathrm{A}}(\Omega, t), \tag{2.2}$$

where $\mathcal{L}_{\mathrm{A}}$ is an operator that describes the reorientational dynamics of the chromophore in A. $\epsilon_{\mathrm{A}}(e, \Omega)$ is the extinction coefficient of the chromophore in state A with orientation Ω, at the wavelength (λ_e) and polarization direction $(\hat{\mathbf{e}})$ of the excitation beam. $I(t)$ is proportional to the intensity of the excitation beam and is given by

$$I(t) = \frac{\ln(10) n_e(t) \phi}{N_{\mathrm{A}}}, \tag{2.3}$$

where $n_e(t)$ is the number of incident photons per unit area per unit time, N_{A} is Avogadro's number, and ϕ is the probability that A, after absorption of a photon, will undergo the reaction Eq. 2.1. Eq. 2.2 must be solved subject to the initial condition $p_{\mathrm{A}}(\Omega, 0) = p_{\mathrm{eq}}^{\mathrm{A}}(\Omega)$.

The orientational distribution function of the chromophore in state B satisfies

$$\frac{\partial p_{\mathrm{B}}(\Omega, t)}{\partial t} = \mathcal{L}_{\mathrm{B}} p_{\mathrm{B}}(\Omega, t) + \epsilon_{\mathrm{A}}(e, \Omega) I(t) p_{\mathrm{A}}(\Omega, t), \tag{2.4}$$

where $\mathcal{L}_{\mathrm{B}}$ describes the reorientational dynamics in state B. Eq. 2.4 must be solved subject to the initial condition $p_{\mathrm{B}}(\Omega, 0) = 0$. In the special case that the reorientational dynamics in the two states are identical ($\mathcal{L}_{\mathrm{A}} = \mathcal{L}_{\mathrm{B}} = \mathcal{L}$, $p_{\mathrm{eq}}^{\mathrm{A}}(\Omega) = p_{\mathrm{eq}}^{\mathrm{B}}(\Omega) = p_{\mathrm{eq}}(\Omega)$), the time-dependent orientational distribution function in the two states is related by

$$p_{\mathrm{A}}(\Omega, t) + p_{\mathrm{B}}(\Omega, t) = p_{\mathrm{eq}}(\Omega). \tag{2.5}$$

The relative populations of A and B can be monitored by observing the change in absorbance after excitation, at a probe wavelength (λ_p) where the two states differ in their absorbance. In general, the chromophore that is excited may not be the same as the chromophore that is probed. Furthermore, the orientation of the chromophore that is probed in states A and B may also differ. We define $\epsilon_{\mathrm{I=A,B}}(p, \Omega)$ as the extinction coefficient of the chromophore that is probed in state I with orientation Ω, at the probe wavelength (λ_p) and polarization direction $(\hat{\mathbf{p}})$ of the probe beam. ϵ_{I}^0 is the isotropic extinction coefficient in state I:

$$\epsilon_{\mathrm{I}}^0(\lambda_p) = \int \epsilon_{\mathrm{I}}(p, \Omega) p_{\mathrm{eq}}^{\mathrm{I}}(\Omega)\, \mathrm{d}\Omega \quad \mathrm{I} = \mathrm{A}, \mathrm{B}. \tag{2.6}$$

The change in absorbance of linearly polarized light, normalized by the maximum change that would be observed if all the molecules in state A were excited to state B, is

$$f(t) = \frac{1}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)} \left[\int \epsilon_{\mathrm{A}}(p, \Omega) p_{\mathrm{A}}(\Omega, t)\, \mathrm{d}\Omega + \int \epsilon_{\mathrm{B}}(p, \Omega) p_{\mathrm{B}}(\Omega, t)\, \mathrm{d}\Omega - \epsilon_{\mathrm{A}}^0(\lambda_p) \right] \tag{2.7}$$

In the special case that the reorientational dynamics in the two states is the same, Eq. 2.7 simplifies to

$$f(t) = \frac{\int [\epsilon_{\mathrm{B}}(p, \Omega) - \epsilon_A(p, \Omega)](p_{\mathrm{eq}}(\Omega) - p_{\mathrm{A}}(\Omega, t))\, \mathrm{d}\Omega}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)}, \tag{2.8}$$

where we have used Eqs. 2.5 and 2.6.

To proceed, one must solve Eqs. 2.2 and 2.4 for $p_{\mathrm{A}}(\Omega, t)$ and $p_{\mathrm{B}}(\Omega, t)$. In two limiting cases it is possible to obtain formal solutions for these quantities and express $f(t)$ in terms of reorientational correlation functions. We now consider these in turn.

2.1. Weak excitation pulses

Consider an excitation pulse with an arbitrary profile but which is sufficiently weak so that only a negligible fraction of the molecules in state A are excited. In this case Eq. 2.2 can be approximated as

$$\frac{\partial p_{\mathrm{A}}(\Omega, t)}{\partial t} \approx \mathcal{L}_{\mathrm{A}} p_{\mathrm{A}}(\Omega, t) - \epsilon_{\mathrm{A}}(e, \Omega) I(t) p_{\mathrm{eq}}^{\mathrm{A}}(\Omega). \tag{2.9}$$

The solution of this equation can be expressed in terms of the Green's function $G_{\mathrm{A}}(\Omega t \,|\, \Omega_0 0)$ of the operator $\mathcal{L}_{\mathrm{A}}$, as (6)

$$p_{\mathrm{A}}(\Omega, t) = p_{\mathrm{eq}}^{\mathrm{A}}(\Omega) - \int_0^t \mathrm{d}\tau I(t - \tau) \times \int \mathrm{d}\Omega_0 G_{\mathrm{A}}(\Omega \tau \,|\, \Omega_0 0) \epsilon_{\mathrm{A}}(e, \Omega_0) p_{\mathrm{eq}}^{\mathrm{A}}(\Omega_0). \tag{2.10}$$

The Green's function $G_{\mathrm{A}}(\Omega t \,|\, \Omega_0 0)$ is simply the probability density that the orientation of the chromophore in state A is Ω at time t given that it was Ω_0 at time zero. Similarly, the solution for the corresponding equation for $p_{\mathrm{B}}(\Omega, t)$ is

$$p_{\mathrm{B}}(\Omega, t) = \int_0^t \mathrm{d}\tau I(t-\tau) \times \int \mathrm{d}\Omega_0 G_{\mathrm{B}}(\Omega\tau \mid \Omega_0 0)\epsilon_{\mathrm{A}}(e, \Omega_0) p_{\mathrm{eq}}^{\mathrm{A}}(\Omega_0), \quad (2.11)$$

where $G_{\mathrm{B}}(\Omega t \mid \Omega_0 0)$ is the Green's function of the operator $\mathcal{L}_{\mathrm{B}}$. Using Eqs. 2.10 and 2.11 in Eq. 2.7 we find

$$f(t) = \int_0^t \mathrm{d}\tau I(t-\tau) \frac{1}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)} \times (\langle\langle \epsilon_{\mathrm{B}}(p, \Omega(\tau))\epsilon_{\mathrm{A}}(e, \Omega(0))\rangle\rangle - \langle \epsilon_{\mathrm{A}}(p, \Omega(\tau))\epsilon_{\mathrm{A}}(e, \Omega(0))\rangle) \quad (2.12)$$

where $\langle x(\Omega(t))y(\Omega(0))\rangle$ denotes an equilibrium correlation function

$$\langle x(\Omega(t))y(\Omega(0))\rangle = \iint \mathrm{d}\Omega\, \mathrm{d}\Omega_0 x(\Omega) G_{\mathrm{A}}(\Omega t \mid \Omega_0 0) y(\Omega_0) p_{\mathrm{eq}}^{\mathrm{A}}(\Omega_0), \quad (2.13)$$

whereas $\langle\langle x(\Omega(t))y(\Omega(0))\rangle\rangle$ denotes the nonequilibrium correlation function

$$\langle\langle x(\Omega(t))y(\Omega(0))\rangle\rangle = \iint \mathrm{d}\Omega\, \mathrm{d}\Omega_0 x(\Omega) G_{\mathrm{B}}(\Omega t \mid \Omega_0 0) y(\Omega_0) p_{\mathrm{eq}}^{\mathrm{A}}(\Omega_0). \quad (2.14)$$

The reason that Eq. 2.14 is a nonequilibrium correlation function is that while the dynamics occurs in state B, the equilibrium distribution is that of state A. In the special case that the reorientational dynamics in the two states are the same, $G_{\mathrm{B}}(\Omega t \mid \Omega_0 0) = G_{\mathrm{A}}(\Omega t \mid \Omega_0 0)$ and $\langle\langle x(\Omega(t))y(\Omega(0))\rangle\rangle$ becomes identical to $\langle x(\Omega(t))y(\Omega(0))\rangle$, and Eq. 2.12 simplifies to

$$f(t) = \int_0^t \mathrm{d}\tau I(t-\tau) \times \frac{\langle [\epsilon_{\mathrm{B}}(p, \Omega(\tau)) - \epsilon_{\mathrm{A}}(p, \Omega(\tau))]\epsilon_{\mathrm{A}}(e, \Omega(0))\rangle}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)}. \quad (2.15)$$

2.2. Short intense excitation pulses

If no reorientational motion of the chromophore occurs during the excitation pulse, the first term on the right hand side of Eq. 2.2 can be ignored during excitation, and the solution of the resulting equation is

$$p_{\mathrm{A}}(\Omega, t) = p_{\mathrm{eq}}^{\mathrm{A}}(\Omega) \exp\left[-\epsilon_{\mathrm{A}}(e, \Omega) \int_0^t I(\tau)\, \mathrm{d}\tau\right]. \quad (2.16)$$

It is convenient to shift the time axis so that the time immediately after the pulse is denoted by 0^+. The orientational distribution of A at $t = 0^+$ becomes

$$p_{\mathrm{A}}(\Omega, 0^+) = p_{\mathrm{eq}}^{\mathrm{A}}(\Omega) \exp(-\epsilon_{\mathrm{A}}(e, \Omega) I_0), \quad (2.17)$$

where I_0 denotes the time integral of $I(t)$ over the duration of the pulse. After the pulse $I(t) = 0$ in Eq. 2.2 and $p_{\mathrm{A}}(\Omega, t)$ satisfies

$$\frac{\partial p_{\mathrm{A}}(\Omega, t)}{\partial t} = \mathcal{L}_{\mathrm{A}} p_{\mathrm{A}}(\Omega, t). \quad (2.18)$$

The solution of Eq. 2.18 subject to the initial condition (2.17) is

$$p_{\mathrm{A}}(\Omega, t) = \int G_{\mathrm{A}}(\Omega t \mid \Omega_0 0^+) p_{\mathrm{A}}(\Omega_0, 0^+)\, \mathrm{d}\Omega_0 = \int G_{\mathrm{A}}(\Omega t \mid \Omega_0 0^+) \times \exp(-\epsilon_{\mathrm{A}}(e, \Omega_0) I_0) p_{\mathrm{eq}}^{\mathrm{A}}(\Omega_0)\, \mathrm{d}\Omega_0. \quad (2.19)$$

The orientational distribution of B at $t = 0^+$ prior to any reorientational dynamics is

$$p_{\mathrm{B}}(\Omega, 0^+) = p_{\mathrm{eq}}^{\mathrm{A}}(\Omega) - p_{\mathrm{A}}(\Omega, 0^+) = p_{\mathrm{eq}}^{\mathrm{A}}(\Omega)[1 - \exp(-\epsilon_{\mathrm{A}}(e, \Omega) I_0)], \quad (2.20)$$

and, after the pulse, $p_{\mathrm{B}}(\Omega, t)$ is given by

$$p_{\mathrm{B}}(\Omega, t) = \int G_{\mathrm{B}}(\Omega t \mid \Omega_0 0^+) \times [1 - \exp(-\epsilon_{\mathrm{A}}(e, \Omega_0) I_0)] p_{\mathrm{eq}}^{\mathrm{A}}(\Omega_0). \quad (2.21)$$

Substituting Eqs. 2.19 and 2.21 into Eq. 2.7 and using the definitions in Eqs. 2.13 and 2.14 we have

$$f(t) = \frac{1}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)} \times (\langle\langle \epsilon_{\mathrm{B}}(p, \Omega(t))[1 - \exp(-\epsilon_{\mathrm{A}}(e, \Omega(0^+)) I_0)]\rangle\rangle - \langle \epsilon_{\mathrm{A}}(p, \Omega(t))[1 - \exp(-\epsilon_{\mathrm{A}}(e, \Omega(0^+)) I_0)]\rangle) \quad (2.22)$$

In the case where the reorientational dynamics in the two states is identical, Eq. 2.22 simplifies to

$$f(t) = \frac{1}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)} \times \langle (\epsilon_{\mathrm{B}}(p, \Omega(t)) - \epsilon_{\mathrm{A}}(p, \Omega(t))) \times [1 - \exp(-\epsilon_{\mathrm{A}}(e, \Omega(0^+)) I_0)]\rangle. \quad (2.23)$$

Eqs. 2.12 and 2.22 are the principal results of this section. Using these formal expressions as the starting point, we shall now obtain more explicit results by exploiting the isotropic (rotationally invariant) nature of our system.

3. LINEAR, CIRCULAR, AND ELLIPSOIDAL ABSORBERS

To proceed we need explicit expressions for the orientation dependent extinction coefficients, $\epsilon_{\mathrm{A}}(e, \Omega)$ and $\epsilon_{\mathrm{I=A,B}}(p, \Omega)$. We first consider the extinction coefficients in state A. Let $\hat{\mathbf{n}}_i$ ($i = 1, 2, 3$) be three orthogonal axes that are fixed on the chromophore in state A and α_i be the ratio of the extinction coefficient in the direction $\hat{\mathbf{n}}_i$ to the isotropic extinction coefficient ϵ_{A}^0. Then $\epsilon_{\mathrm{A}}(e, \Omega)$ may be written as

$$\epsilon_A(e, \Omega) = \epsilon_A^0(\lambda_e) \sum_{i=1}^{3} \alpha_i(\lambda_e)(\hat{\mathbf{n}}_i \cdot \hat{\mathbf{e}})^2. \tag{3.1}$$

Since $\epsilon_A^0(\lambda_e)$ is by definition $\int \epsilon_A(e, \Omega)\, d\Omega/4\pi$, we require that

$$\sum_{i=1}^{3} \alpha_i(\lambda_e) = 3. \tag{3.2}$$

The general case of an ellipsoidal absorber, defined in Eq. 3.1, is treated in the Appendix. Here we shall consider, for the sake of simplicity, absorbers for which $\alpha_2 = \alpha_3 = \alpha_\perp$ and $\alpha_1 = \alpha_\parallel$ (ellipsoids of revolution), so that

$$\begin{aligned} \epsilon_A(e, \Omega) &= \epsilon_A^0(\lambda_e)[\alpha_\parallel(\lambda_e)(\hat{\mathbf{n}}_1 \cdot \hat{\mathbf{e}})^2 \\ &\quad + \alpha_\perp(\lambda_e)((\hat{\mathbf{n}}_2 \cdot \hat{\mathbf{e}})^2 + (\hat{\mathbf{n}}_3 \cdot \hat{\mathbf{e}})^2)] \\ &= \epsilon_A^0(\lambda_e)[\alpha_\parallel(\lambda_e)(\hat{\mathbf{n}}_1 \cdot \hat{\mathbf{e}})^2 \\ &\quad + \alpha_\perp(\lambda_e)(1 - (\hat{\mathbf{n}}_1 \cdot \hat{\mathbf{e}})^2)], \end{aligned} \tag{3.3}$$

where we have used the fact that $\sum_i (\hat{\mathbf{n}}_i \cdot \hat{\mathbf{e}})^2 = 1$. The unit vector $\hat{\mathbf{n}}_1$ specifies the direction of the unique symmetry axis. For a linear absorber, $\alpha_\perp = 0$, so $\hat{\mathbf{n}}_1$ points along the transition dipole. For a circular absorber such as the heme, $\alpha_\parallel = 0$ and $\hat{\mathbf{n}}_1$ is normal to the heme plane. It will prove convenient to express $(\hat{\mathbf{n}}_1 \cdot \hat{\mathbf{e}})^2$ in Eq. 3.3 in terms of the second Legendre polynomial $P_2(x) = {}^1\!/_2(3x^2 - 1)$. Denoting the unique symmetry axis ($\hat{\mathbf{n}}_1$) of the chromophore that is excited in state A at the excitation wavelength λ_e by $\hat{\mathbf{n}}_{Ae}$, Eq. (3.3) becomes

$$\epsilon_A(e, \Omega) = \epsilon_A^0(\lambda_e)[1 - \sigma_A(\lambda_e) P_2(\hat{\mathbf{n}}_{Ae} \cdot \hat{\mathbf{e}})], \tag{3.4}$$

with

$$\sigma_A(\lambda_e) = 2\frac{\alpha_\perp(\lambda_e) - \alpha_\parallel(\lambda_e)}{\alpha_\parallel(\lambda_e) + 2\alpha_\perp(\lambda_e)} = 1 - \alpha_\parallel(\lambda_e), \tag{3.5}$$

where we have used Eq. 3.2. For a linear absorber $\sigma = -2$ whereas for a circular absorber $\sigma = 1$. Similarly one can write

$$\epsilon_A(p, \Omega) = \epsilon_A^0(\lambda_p)[1 - \sigma_A(\lambda_p) P_2(\hat{\mathbf{n}}_{Ap} \cdot \hat{\mathbf{p}})], \tag{3.6}$$

where $\hat{\mathbf{n}}_{Ap}$ points along the unique symmetry axis of the chromophore in state A at the probe wavelength λ_p. Finally,

$$\epsilon_B(p, \Omega) = \epsilon_B^0(\lambda_p)[1 - \sigma_B(\lambda_p) P_2(\hat{\mathbf{n}}_{Bp} \cdot \hat{\mathbf{p}})], \tag{3.7}$$

and

$$\sigma_B(\lambda_p) = 1 - \beta_\parallel(\lambda_p), \tag{3.8}$$

where $\beta_\parallel(\lambda_p)$ is the ratio of the extinction coefficient in the direction $\hat{\mathbf{n}}_{Bp}$ to the isotropic extinction coefficient $\epsilon_B^0(\lambda_p)$ in state B.

We are now in a position to simplify the correlation functions that appear in Eq. 2.12 (low intensity pulses) and in Eq. 2.22 (arbitrary intensity but short pulses). Owing to the isotropic nature of the system we can use the following identities:

$$\langle P_l(\hat{\mathbf{n}}(t) \cdot \hat{\mathbf{a}}) \rangle = \delta_{l0}, \tag{3.9}$$

and

$$\begin{aligned} &\langle P_2(\hat{\mathbf{n}}(t) \cdot \hat{\mathbf{a}}) P_l(\hat{\mathbf{m}}(0) \cdot \hat{\mathbf{b}}) \rangle \\ &\qquad = \frac{\delta_{l2}}{5} P_2(\hat{\mathbf{a}} \cdot \hat{\mathbf{b}}) \langle P_2(\hat{\mathbf{n}}(t) \cdot \hat{\mathbf{m}}(0)) \rangle, \end{aligned} \tag{3.10}$$

to express our results in terms of correlation functions that depend on the reorientational dynamics of the chromophore. Eq. 3.10 is a straightforward generalization of an identity derived by Berne and Pecora (7). Identical relations hold for nonequilibrium correlation functions (i.e., for $\langle\langle(\cdots)\rangle\rangle$).

3.1. Weak pulses

To simplify Eq. 2.12, let us consider $\langle \epsilon_A(p, \Omega(t)) \times \epsilon_A(e, \Omega(0)) \rangle$. Using Eqs. 3.4 and 3.6, we have

$$\begin{aligned} &\langle \epsilon_A(p, \Omega(t)) \epsilon_A(e, \Omega(0)) \rangle \\ &\quad = \langle \epsilon_A^0(\lambda_p)[1 - \sigma_A(\lambda_p) P_2(\hat{\mathbf{n}}_{Ap}(t) \cdot \hat{\mathbf{p}})] \\ &\qquad \times \epsilon_A^0(\lambda_e)[1 - \sigma_A(\lambda_e) P_2(\hat{\mathbf{n}}_{Ae}(0) \cdot \hat{\mathbf{e}})] \rangle \\ &\quad = \epsilon_A^0(\lambda_p) \epsilon_A^0(\lambda_e)[1 + ({}^1\!/_5)\sigma_A(\lambda_p)\sigma_A(\lambda_e) \\ &\qquad \times P_2(\hat{\mathbf{e}} \cdot \hat{\mathbf{p}}) \langle P_2(\hat{\mathbf{n}}_{Ap}(t) \cdot \hat{\mathbf{n}}_{Ae}(0)) \rangle], \end{aligned} \tag{3.11}$$

where we have used the identities in Eqs. 3.9 and 3.10. Similarly,

$$\begin{aligned} &\langle\langle \epsilon_B(p, \Omega(t)) \epsilon_A(e, \Omega(0)) \rangle\rangle \\ &\quad = \epsilon_B^0(\lambda_p) \epsilon_A^0(\lambda_e)[1 + ({}^1\!/_5)\sigma_B(\lambda_p)\sigma_A(\lambda_e) P_2(\hat{\mathbf{e}} \cdot \hat{\mathbf{p}}) \\ &\qquad \times \langle\langle P_2(\hat{\mathbf{n}}_{Bp}(t) \cdot \hat{\mathbf{n}}_{Ae}(0)) \rangle\rangle]. \end{aligned} \tag{3.12}$$

Using Eqs. 3.11 and 3.12 in Eq. 2.12, we finally have

$$\begin{aligned} f(t) &= \epsilon_A^0(\lambda_e) \int_0^t d\tau I(t - \tau) \\ &\quad \times \left[1 + \frac{\sigma_A(\lambda_e) P_2(\hat{\mathbf{e}} \cdot \hat{\mathbf{p}})}{5} \left(\frac{\epsilon_B^0(\lambda_p)\sigma_B(\lambda_p) \langle\langle P_2(\hat{\mathbf{n}}_{Bp}(\tau) \cdot \hat{\mathbf{n}}_{Ae}(0)) \rangle\rangle - \epsilon_A^0(\lambda_p)\sigma_A(\lambda_p) \langle P_2(\hat{\mathbf{n}}_{Ap}(\tau) \cdot \hat{\mathbf{n}}_{Ae}(0)) \rangle}{\epsilon_B^0(\lambda_p) - \epsilon_A^0(\lambda_p)} \right)\right]. \end{aligned} \tag{3.13}$$

The absorption anisotropy is defined as

$$r(t)=\frac{f_{\|}(t)-f_{\perp}(t)}{f_{\|}(t)+2f_{\perp}(t)}$$

$$=\frac{1}{10}\frac{\sigma_{\mathrm{A}}(\lambda_e)}{\epsilon_{\mathrm{B}}^0(\lambda_p)-\epsilon_{\mathrm{A}}^0(\lambda_p)}\frac{\int_0^t \mathrm{d}\tau I(t-\tau)[\epsilon_{\mathrm{B}}^0(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p)\langle\langle P_2(\hat{\mathbf{n}}_{\mathrm{B}p}(\tau)\cdot\hat{\mathbf{n}}_{\mathrm{A}e}(0))\rangle\rangle-\epsilon_{\mathrm{A}}^0(\lambda_p)\sigma_{\mathrm{A}}(\lambda_p)\langle P_2(\hat{\mathbf{n}}_{\mathrm{A}p}(\tau)\cdot\hat{\mathbf{n}}_{\mathrm{A}e}(0))\rangle]}{\int_0^t \mathrm{d}\tau I(t-\tau)}, \tag{3.14}$$

where $f_{\|}$ and $f_{\perp}$ are defined from Eq. 3.13 as the normalized change in absorbance when $\hat{\mathbf{e}}\cdot\hat{\mathbf{p}}=1$ and $\hat{\mathbf{e}}\cdot\hat{\mathbf{p}}=0$, respectively. Eqs. 3.13 and 3.14 are the principal results of this subsection and involve two reorientational correlation functions. It can be seen from Eq. 3.13 that when the excitation and probe polarizations are at the magic angle, defined by $P_2(\hat{\mathbf{e}}\cdot\hat{\mathbf{p}})=0$, $f(t)$ is independent of the reorientational dynamics and is, from Eq. 2.11, equal to $\int p_{\mathrm{B}}(\Omega,t)\,\mathrm{d}\Omega$; at the magic angle the change in absorbance monitors the fraction of molecules that are excited in state B. It should be emphasized that the result at the magic angle is correct only for optically thin samples. As Lewis and Kliger (8) have pointed out, dichroic samples behave as polarizers owing to the differential absorption in the directions parallel and perpendicular to the polarization direction of the excitation beam ($\hat{\mathbf{e}}$). An incident probe beam polarized in some arbitrary direction ($\hat{\mathbf{p}}$) will in fact rotate toward the dichroic sample's least absorbing axis and hence the molecules at the back end of the sample will not be probed at the same angle as the molecules in the front. In addition, the linearly polarized probe beam will become elliptically polarized owing to the birefringent properties of the dichroic sample.

3.2. Short intense pulses

We now evaluate the correlation functions in Eq. 2.22. For uniaxially symmetric absorbers the orientation dependent extinction coefficients are defined in Eqs. 3.4, 3.6, and 3.7, and the derivation presented in the previous subsection can be readily generalized by expanding $\exp(-\epsilon_{\mathrm{A}}(e,\Omega)I_0)$ in a series of Legendre polynomials, i.e.,

$$\begin{aligned}&\exp(-\epsilon_{\mathrm{A}}(e,\Omega)I_0)\\&\quad=\exp(-\epsilon_{\mathrm{A}}^0(\lambda_e)I_0[1-\sigma_{\mathrm{A}}(\lambda_e)P_2(\hat{\mathbf{n}}_{\mathrm{A}e}(0^+)\cdot\hat{\mathbf{e}})])\\&\quad=\sum_{l=0}^{\infty}a_l(\lambda_e)P_l(\hat{\mathbf{n}}_{\mathrm{A}e}(0^+)\cdot\hat{\mathbf{e}}),\end{aligned} \tag{3.15}$$

where $P_l(x)$ is the lth Legendre polynomial. The coefficients $a_l(\lambda_e)$ can be evaluated using the orthogonality properties of these polynomials. Thus,

$$a_l(\lambda_e)=\frac{2l+1}{2}\int_0^{\pi}\exp(-\epsilon_{\mathrm{A}}^0(\lambda_e)I_0[1-\sigma_{\mathrm{A}}(\lambda_e)P_2(\cos\theta)])\times P_l(\cos\theta)\sin\theta\,\mathrm{d}\theta. \tag{3.16}$$

For future reference, note that

$$\begin{aligned}a_0(\lambda_e)&=\int_0^1\exp(-\epsilon_{\mathrm{A}}^0(\lambda_e)I_0[1-\sigma_A(\lambda_e)P_2(x)])\,\mathrm{d}x\\&=1-\epsilon_{\mathrm{A}}^0(\lambda_e)I_0\\&\quad+\frac{1}{2}\epsilon_{\mathrm{A}}^0(\lambda_e)^2I_0^2\left(1+\frac{\sigma_{\mathrm{A}}(\lambda_e)^2}{5}\right)\cdots I_0\rightarrow 0,\end{aligned} \tag{3.17}$$

and

$$\begin{aligned}a_2(\lambda_e)&=5\int_0^1\exp(-\epsilon_{\mathrm{A}}^0(\lambda_e)I_0[1-\sigma_{\mathrm{A}}(\lambda_e)P_2(x)])P_2(x)\,\mathrm{d}x\\&=\epsilon_{\mathrm{A}}^0(\lambda_e)I_0\sigma_{\mathrm{A}}(\lambda_e)\\&\quad-\epsilon_{\mathrm{A}}^0(\lambda_e)^2I_0^2\sigma_{\mathrm{A}}(\lambda_e)\left(1-\frac{\sigma_{\mathrm{A}}(\lambda_e)}{7}\right)\cdots I_0\rightarrow 0,\end{aligned} \tag{3.18}$$

where we have set $x=\cos\theta$. Using Eq. 3.15 in Eq. 2.20 and averaging over all orientations Ω, we note that $1-a_0(\lambda_e)$ is the fraction of molecules that undergo the reaction A → B; $a_2(\lambda_e)$ is shown below to be proportional to the linear dichroism induced by excitation of an isotropic system with linearly polarized light.

To calculate $f(t)$ we use Eqs. 3.6 and 3.7, and the expansion 3.15 in Eq. 2.22 and then use the identities 3.9 and 3.10 to simplify the correlation functions. Thus,

$$\begin{aligned}f(t)&=1-a_0(\lambda_e)+\frac{a_2(\lambda_e)}{5}P_2(\hat{\mathbf{e}}\cdot\hat{\mathbf{p}})\frac{1}{\epsilon_{\mathrm{B}}^0(\lambda_p)-\epsilon_{\mathrm{A}}^0(\lambda_p)}\\&\quad\times(\epsilon_{\mathrm{B}}^0(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p)\langle\langle P_2(\hat{\mathbf{n}}_{\mathrm{B}p}(t)\cdot\hat{\mathbf{n}}_{\mathrm{A}e}(0^+))\rangle\rangle\\&\quad-\epsilon_{\mathrm{A}}^0(\lambda_p)\sigma_A(\lambda_p)\langle P_2(\hat{\mathbf{n}}_{\mathrm{A}p}(t)\cdot\hat{\mathbf{n}}_{\mathrm{A}e}(0^+))\rangle).\end{aligned} \tag{3.19}$$

The absorption anisotropy (Eq. 3.14) is

$$\begin{aligned}r(t)&=\frac{1}{10}\frac{a_2(\lambda_e)}{1-a_0(\lambda_e)}\frac{1}{\epsilon_{\mathrm{B}}^0(\lambda_p)-\epsilon_{\mathrm{A}}^0(\lambda_p)}\\&\quad\times(\epsilon_{\mathrm{B}}^0(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p)\langle\langle P_2(\hat{\mathbf{n}}_{\mathrm{B}p}(t)\cdot\hat{\mathbf{n}}_{\mathrm{A}e}(0^+))\rangle\rangle\\&\quad-\epsilon_{\mathrm{A}}^0(\lambda_p)\sigma_{\mathrm{A}}(\lambda_p)\langle P_2(\hat{\mathbf{n}}_{\mathrm{A}p}(t)\cdot\hat{\mathbf{n}}_{\mathrm{A}e}(0^+))\rangle).\end{aligned} \tag{3.20}$$

In the limit that $I_0\rightarrow 0$ it follows from Eqs. 3.17 and 3.18 that

$$\frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} = \sigma_A(\lambda_e) \times \left[1 - \epsilon_A^0(\lambda_e) I_0 \left(\frac{1}{2} - \frac{\sigma_A(\lambda_e)}{7} - \frac{\sigma_A(\lambda_e)^2}{10} \right) + \cdots \right] \quad (3.21)$$

For an arbitrary ellipsoid of revolution, $\sigma_A(\lambda_e)$ can take on values between -2 and 1, and the term in the square brackets in Eq. 3.21 is always less than unity. Thus, for a short excitation pulse, high light intensity does not influence the time dependence of the absorption anisotropy but merely reduces the value of the limiting anisotropy observed at $t = 0^+$. Note that the absorption anisotropy defined in Eq. 3.20 depends not only on the shape of the absorber in states A and B (characterized by σ_A and σ_B) but also by the relative magnitudes of the isotropic extinction coefficients at the probe wavelength in the two states. Eqs. 3.19 and 3.20 are the main results of this subsection. We will now relate the general formalism developed in this paper to specific problems in the literature and derive some special relations starting from Eqs. 3.19 and 3.20.

Our primary motivation for developing the photoselection theory was to analyze optical photolysis experiments on heme proteins. In these experiments, state A is a heme-ligand complex. The excitation pulse photodissociates the ligand and then the absorbance of the hemes is probed. If the reorientational dynamics of the liganded (A) and unliganded (B) hemes are the same ($\mathcal{L}_A = \mathcal{L}_B$) and the normal to the heme plane points in the same direction at the excitation and probe wavelengths in the liganded state ($\hat{\mathbf{n}}_{Ae} = \hat{\mathbf{n}}_{Ap} = \hat{\mathbf{n}}$) but in a different direction in the unliganded state, and if the heme is rigidly attached to an isotropically diffusing molecule, then

$$\langle P_2(\hat{\mathbf{n}}_{Bp}(t) \cdot \hat{\mathbf{n}}_{Ae}(0^+)) \rangle = P_2(\cos\gamma) \exp(-6D_r t), \quad (3.22)$$

where D_r is the rotational diffusion coefficient of the molecule and γ is the angle between the normals to the heme planes in the liganded and unliganded states. Eq. 3.20 simplifies to

$$r(t) = \frac{1}{10} \frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} \times \left[\frac{\epsilon_B^0(\lambda_p)\sigma_B(\lambda_p)P_2(\cos\gamma) - \epsilon_A^0(\lambda_p)\sigma_A(\lambda_p)}{\epsilon_B^0(\lambda_p) - \epsilon_A^0(\lambda_p)} \right] \times \exp(-6D_r t) \quad (3.23)$$

If $\gamma = 0$, then Eq. 3.23 may be written more generally, for any reorientational dynamics of the heme, as

$$r(t) = \frac{1}{10} \frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} \left[\frac{\epsilon_B^0(\lambda_p)\sigma_B(\lambda_p) - \epsilon_A^0(\lambda_p)\sigma_A(\lambda_p)}{\epsilon_B^0(\lambda_p) - \epsilon_A^0(\lambda_p)} \right] \times \langle P_2(\hat{\mathbf{n}}(t) \cdot \hat{\mathbf{n}}(0)) \rangle. \quad (3.24)$$

If both hemes have the same axial ratios ($\sigma_A(\lambda_p) = \sigma_B(\lambda_p) = \sigma(\lambda_p)$) then Eq. 3.24 further simplifies to

$$r(t) = \frac{1}{10} \frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} \sigma(\lambda_p) \langle P_2(\hat{\mathbf{n}}(t) \cdot \hat{\mathbf{n}}(0^+)) \rangle \quad (3.25)$$

and becomes independent of the extinction coefficients in the two states.

Next, consider the optical-infrared photoselection experiments that have been used to determine the geometry of the Fe-CO bond in the carboxyheme complexes (9–11). In this experiment state A is a carboxyheme that is photodissociated by an excitation pulse. The system is then probed with an infrared beam that is absorbed by the CO. The probe wavelengths are chosen so as to monitor the bound CO and, since the absorption spectra of the bound and the photodissociated CO do not overlap, $\epsilon_B(\lambda_p) = 0$. $\hat{\mathbf{n}}_{Ae} = \hat{\mathbf{n}}$ is a unit vector along the unique symmetry axis of the heme (perpendicular to the heme plane). Assuming that the CO is a linear absorber, $\sigma_A(\lambda_p) = -2$ and $\hat{\mathbf{n}}_{Ap} = \hat{\mathbf{n}}_{CO}$ where $\hat{\mathbf{n}}_{CO}$ is a unit vector along the absorption dipole of the CO. Under these conditions Eq. 3.20 reduces to

$$r(t) = -\frac{1}{5} \frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} \langle P_2(\hat{\mathbf{n}}_{CO}(t) \cdot \hat{\mathbf{n}}(0^+)) \rangle, \quad (3.26)$$

where $a_0(\lambda_e)$ and $a_2(\lambda_e)$ are given by Eqs. 3.17 and 3.18, respectively. A similar result has been obtained by Hansen et al. (4) where they assumed that the heme was a circular absorber at their excitation wavelength ($\sigma_A(\lambda_e) = 1$ in Eqs. 3.17 and 3.18).

In the study of the photoselection effects on flash experiments on bacteriorhodopsin (3), the chromophore is a linear absorber in states A and B ($\sigma_A = \sigma_B = -2$) and is assumed to undergo no significant reorientational motion. The absorption anisotropy from Eq. 3.20 reduces to

$$r(t) = -\frac{1}{5} \frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} P_2(\hat{\mathbf{n}}_{Bp} \cdot \hat{\mathbf{n}}_{Ae}), \quad (3.27)$$

where $a_0(\lambda_e)$ and $a_2(\lambda_e)$ are given by Eqs. 3.17 and 3.18 with $\sigma_A(\lambda_e) = -2$. If the transition dipoles of A and B are colinear, then this result is equivalent to that obtained by Nagle et al. (3) (see their Eqs. 10–12).

Finally, Magde (2) has obtained expressions for the dichroic ratio ($f_{\parallel}/f_{\perp}$) after excitation with intense light pulses and in the absence of any reorientational dynamics. For absorption measurements in which $\epsilon_A^0(\lambda_p) = 0$, the dichroic ratio is given by

$$\frac{f_{\parallel}}{f_{\perp}} = \frac{1 - a_0(\lambda_e) + \frac{a_2(\lambda_e)}{5} \sigma_B(\lambda_p) P_2(\hat{\mathbf{n}}_{Bp} \cdot \hat{\mathbf{n}}_{Ae})}{1 - a_0(\lambda_e) - \frac{a_2(\lambda_e)}{10} \sigma_B(\lambda_p) P_2(\hat{\mathbf{n}}_{Bp} \cdot \hat{\mathbf{n}}_{Ae})}. \quad (3.28)$$

If A and B are linear absorbers ($\sigma_A = \sigma_B = -2$) we recover Eqs. 48 and 50 of Magde when the transition dipoles of A and B are collinear ($\hat{\mathbf{n}}_{Bp} \cdot \hat{\mathbf{n}}_{Ae} = 1$) and perpendicular ($\hat{\mathbf{n}}_{Bp} \cdot \hat{\mathbf{n}}_{Ae} = 0$), respectively. If A is a planar absorber ($\sigma_A = 1$) we recover his Eqs. 77 and 81 for the two orientations of the linear absorber B. (Note that there are some typographical errors in his Eqs. 50 and 81).

Although the primary focus of this paper is the theoretical description of photoselection effects on absorption measurements, the fluorescence emission anisotropy, observed using short intense excitation pulses, may be easily obtained by a suitable reinterpretation of the parameters in Eq. 3.20. Let A be the ground state of the fluorophore and B the excited state. Identify λ_p with the wavelength at which B fluoresces (λ_f) and $\hat{\mathbf{n}}_{Bp} = \hat{\mathbf{n}}_{Bf}$ as the symmetry axis of the ellipsoid-of-revolution of the emitter ($\sigma_B(\lambda_f) = -2$ for a linear emitter, $\sigma_B(\lambda_f) = 1$ for a circular one). Since A does not fluoresce, one can formally set $\epsilon_A^0(\lambda_p) = 0$ and Eq. 3.20 reduces to

$$\begin{aligned} r(t) &= \frac{1}{10}\frac{a_2(\lambda_e)}{1-a_0(\lambda_e)}\sigma_B(\lambda_f)\langle\langle P_2(\hat{\mathbf{n}}_{Bf}(t)\cdot\hat{\mathbf{n}}_{Ae}(0^+))\rangle\rangle \\ &= \frac{\sigma_A(\lambda_e)\sigma_B(\lambda_f)}{10}\left[1-\epsilon_A^0(\lambda_e)I_0\left(\frac{1}{2}-\frac{\sigma_A(\lambda_e)}{7}-\frac{\sigma_A(\lambda_e)^2}{10}\right)\right. \\ &\quad \left. + \cdots\right]\langle\langle P_2(\hat{\mathbf{n}}_{Bf}(t)\cdot\hat{\mathbf{n}}_{Ae}(0^+))\rangle\rangle, \qquad (3.29) \end{aligned}$$

where we have used Eq. 3.21 in the limit $I_0 \to 0$. Note that the reorientational correlation function is in general a nonequilibrium one, since the equilibrium orientational distribution of the ground and excited states can differ (i.e., $\mathcal{L}_A \neq \mathcal{L}_B$) because of differences in the charge distributions. If $p_{eq}^A = p_{eq}^B$, as is usually assumed, the correlation function becomes an equilibrium one involving the reorientational dynamics of the excited state.

3.3. Reorientation correlation functions and the influence of fast internal motions

For a δ-function excitation pulse ($I(t) = I_0\delta(t)$) the formalism developed in the previous subsection is valid for any model of the reorientational dynamics of the chromophore. The motion of the chromophore influences $f(t)$ and $r(t)$ only through the reorientational correlation functions, which do not depend on the intensity or polarization direction of the excitation or probe beams. For a chromophore rigidly attached to a molecule that is undergoing isotropic rotational diffusion, the correlation functions in Eqs. 3.19 and 3.20 are of the form in Eq. 3.22. A comprehensive treatment of how internal motions alter this result can be found in the paper by Szabo (1). Here we shall consider only a simple model of the fast internal dynamics of the chromophore in an isotropically reorienting system. Suppose that the normal to the heme plane ($\hat{\mathbf{n}}$) fluctuates ("wobbles") in an axially symmetric way about a protein fixed axis ($\hat{\mathbf{m}}$). Let $p_{eq}^f(\theta)$ be the normalized ($\int_0^\pi p_{eq}^f(\theta)\sin\theta\, d\theta = 1$) equilibrium probability density that the angle between $\hat{\mathbf{n}}$ and $\hat{\mathbf{m}}$ is θ. The correlation function $\langle P_2(\hat{\mathbf{n}}(t)\cdot\hat{\mathbf{n}}(0))\rangle$ starts out at unity at $t = 0$ and then begins to decay due to fast internal motions. If the internal motions are fast and independent of the overall diffusive motion, then, for times long compared to the time-scale of the internal motions (τ_f), the correlation function becomes (12)

$$\langle P_2(\hat{\mathbf{n}}(t)\cdot\hat{\mathbf{n}}(0))\rangle = S^2\exp(-6D_r t) \quad t \gg \tau_f, \qquad (3.30)$$

where S is an order parameter ($0 \leq S \leq 1$) defined as

$$S = \int_0^\pi P_2(\cos\theta)p_{eq}^f \sin\theta\, d\theta. \qquad (3.31)$$

$S = 1$ if there is no motion while $S = 0$ in the limit that the motion is isotropic. If the internal motions are modelled as wobbling in a cone of semiangle θ_0

$$\begin{aligned} p_{eq}^f(\theta) &= [2\pi(1-\cos\theta_0)]^{-1} \qquad && 0 \leq \theta \leq \theta_0 \\ &= 0 && \theta \geq \theta_0 \quad . \qquad (3.32) \\ S &= {}^1\!/_2 \cos\theta_0(1+\cos\theta_0) \end{aligned}$$

From Eq. 3.30 we note that if the time-scales of the measurements are such that the experiment cannot resolve the fast internal motions, the apparent or extrapolated value of the correlation function at $t = 0$ is reduced by a factor S^2, and from Eq. 3.24, this results in a reduction of the extrapolated value of the limiting anisotropy. The apparent initial anisotropy, extrapolated from measurements on a time-scale much slower than τ_f is, therefore, reduced by a factor S^2.

The presence of fast motions can also affect the apparent angle between the CO bond and the heme plane, obtained from the optical infrared measurements discussed above. For a δ-function excitation pulse, it follows from Eq. 3.26 that the initial anisotropy is

$$\begin{aligned} r(0) &= -\frac{1}{5}\frac{a_2(\lambda_e)}{1-a_0(\lambda_e)}\langle P_2(\hat{\mathbf{n}}_{CO}\cdot\hat{\mathbf{n}})\rangle \\ &= -\frac{1}{5}\left(1-\frac{9}{35}\epsilon_A^0(\lambda_e)I_0+\cdots\right)\langle P_2(\hat{\mathbf{n}}_{CO}\cdot\hat{\mathbf{n}})\rangle, \qquad (3.33) \end{aligned}$$

where we have used the expansion in Eq. 3.21 for a circular absorber ($\sigma_A(\lambda_e) = 1$). The apparent angle (α_{app}) between the CO and the heme normal is obtained by equating the initial anisotropy to $-{}^1\!/_5 P_2(\cos\alpha_{app})$, so that

$$\begin{aligned} P_2(\cos\alpha_{app}) &= \frac{a_2(\lambda_e)}{1-a_0(\lambda_e)}\langle P_2(\hat{\mathbf{n}}_{CO}\cdot\hat{\mathbf{n}})\rangle \\ &= \left(1-\frac{9}{35}\epsilon_A^0(\lambda_e)I_0+\cdots\right)\langle P_2(\hat{\mathbf{n}}_{CO}\cdot\hat{\mathbf{n}})\rangle. \qquad (3.34) \end{aligned}$$

As discussed by Hansen et al. (4), the effect of finite excitation intensities is to lower the initial anisotropy and therefore increase α_{app}. Moreover, even if on the average the geometry is linear, α_{app} can deviate from zero. Suppose $\hat{\mathbf{n}}_{CO}$ wobbles relative to the heme normal in a cone of semiangle γ then $\langle P_2(\hat{\mathbf{n}}_{CO}\cdot\hat{\mathbf{n}})\rangle =$

½ cos γ(1 + cos γ) and α_{app} in Eq. 3.34 is non-zero due to the motion of the CO. If, in addition, the heme wobbles in a protein or in a solvent cage on a time-scale faster than the resolution of the experiment, then the apparent angle is further increased. If the motions of the heme normal and the CO bond relative to the heme normal are independent, then

$$\begin{aligned} P_2(\cos \alpha_{\text{app}}) &= S^2 \frac{a_2(\lambda_e)}{1 - a_0(\lambda_e)} \langle P_2(\hat{\mathbf{n}}_{\text{CO}} \cdot \hat{\mathbf{n}}) \rangle \\ &= S^2 \left(1 - \frac{9}{35} \epsilon^0_{\text{A}}(\lambda_e) I_0 + \cdots \right) \langle P_2(\hat{\mathbf{n}}_{\text{CO}} \cdot \hat{\mathbf{n}}) \rangle, \end{aligned} \quad (3.35)$$

where S is the order parameter describing the heme wobble. Thus, intense excitation pulses, the motion of the CO relative to the heme normal, and the wobble of the heme, all tend to increase the apparent angle between the CO and the heme normal.

The above discussion is valid when the excitation pulse is sufficiently short so that even the fast internal motions do not occur within the pulse. Let us now consider the situation when the internal motions are so fast that during the excitation pulse, the system reaches equilibrium with respect to the fast motions. The overall diffusive motion of the protein is still assumed to be slow on the time-scale of excitation. In this case the system behaves as if it had an effective extinction coefficient $\epsilon^{\text{eff}}_{\text{A}}(e, \Omega)$ which can be obtained by averaging the instantaneous extinction coefficient (Eq. 3.4) over the equilibrium distribution for fast motions,

$$\epsilon^{\text{eff}}_{\text{A}}(e, \Omega) = \langle \epsilon^0_{\text{A}}(\lambda_e)[1 - \sigma_{\text{A}}(\lambda_e) P_2(\hat{\mathbf{n}}_{\text{A}e} \cdot \hat{\mathbf{e}})] \rangle_f. \quad (3.36)$$

Assuming that $\hat{\mathbf{n}}_{\text{A}e}$ wobbles in an axially symmetric way about an axis $\hat{\mathbf{m}}_{\text{A}e}$, and using the addition theorem (13) we have

$$\begin{aligned} \langle P_2(\hat{\mathbf{n}}_{\text{A}e} \cdot \hat{\mathbf{e}}) \rangle_f &= \langle P_2(\hat{\mathbf{n}}_{\text{A}e} \cdot \hat{\mathbf{m}}_{\text{A}e}) \rangle_f P_2(\hat{\mathbf{m}}_{\text{A}e} \cdot \hat{\mathbf{e}}) \\ &= S P_2(\hat{\mathbf{m}}_{\text{A}e} \cdot \hat{\mathbf{e}}), \end{aligned} \quad (3.37)$$

where S is the order parameter defined in Eq. 3.31. From Eqs. 3.36 and 3.37, it follows that

$$\begin{aligned} \epsilon^{\text{eff}}_{\text{A}}(e, \Omega) &= \epsilon^0_{\text{A}}(\lambda_e)[1 - S\sigma_{\text{A}}(\lambda_e) P_2(\hat{\mathbf{m}}_{\text{A}e} \cdot \hat{\mathbf{e}})] \\ &= \epsilon^0_{\text{A}}(\lambda_e)[1 - \sigma^{\text{eff}}_{\text{A}}(\lambda_e) P_2(\hat{\mathbf{m}}_{\text{A}e} \cdot \hat{\mathbf{e}})], \end{aligned} \quad (3.38)$$

and, from Eq. 3.5,

$$\sigma^{\text{eff}}_{\text{A}}(\lambda_e) = S\sigma_{\text{A}}(\lambda_e) = S(1 - \alpha_{\|}(\lambda_e)) = 1 - \alpha^{\text{eff}}_{\|}(\lambda_e), \quad (3.39)$$

where $\alpha^{\text{eff}}_{\|}(\lambda_e) = 1 - S + \alpha_{\|}(\lambda_e)$. Similarly, for any given orientation of the molecule in the laboratory frame, the probe pulse samples the equilibrium orientational distribution of the chromophore within the cone, and the effective extinction coefficient in the direction of polarization of the probe beam becomes

$$\begin{aligned} \epsilon^{\text{eff}}_I(p, \Omega) &= \epsilon^0_I(\lambda_p)[1 - S\sigma_I(\lambda_p) P_2(\hat{\mathbf{m}}_{Ip} \cdot \hat{\mathbf{p}})] \\ &= \epsilon^0_I(\lambda_p)[1 - \sigma^{\text{eff}}_I(\lambda_p) P_2(\hat{\mathbf{m}}_{Ip} \cdot \hat{\mathbf{p}})], \\ I &= \text{A, B} \end{aligned} \quad (3.40)$$

where

$$\begin{aligned} \sigma^{\text{eff}}_{\text{A}}(\lambda_p) &= S(1 - \alpha_{\|}(\lambda_p)) \\ \sigma^{\text{eff}}_{\text{B}}(\lambda_p) &= S(1 - \beta_{\|}(\lambda_p)). \end{aligned} \quad (3.41)$$

Since Eqs. 3.38 and 3.40 have the same form as Eqs. 3.4, 3.6, and 3.7 we can immediately adapt the formalism of Section 3.3 by replacing σ by σ^{eff} and $\hat{\mathbf{n}}$ by $\hat{\mathbf{m}}$. For a circular absorber ($\alpha_\perp = 1, \alpha_{\|} = 0$) $\alpha^{\text{eff}}_{\|} = 1 - S$. Thus, fast motions within the excitation pulse cause a circular absorber to behave as if it had an out-of-plane component. Clearly it is not possible to distinguish between a circular absorber undergoing fast axially symmetric librational motion during excitation and a rigid absorber with an out-of-plane component if the time resolution of the experiment is long compared to the time-scale of the fast motions.

3.4. Rotational diffusion within the excitation pulse

We now consider intense excitation pulses that are so long that significant overall rotational diffusion occurs within the pulse width. The general formalism to treat such a problem has been developed in Section 2: we need to solve Eqs. 2.2 and 2.4 subject to the appropriate boundary conditions and then calculate the change in absorbance using Eq. 2.7. We will consider only the simple case in which (1) the dynamics of A and B are the same ($\mathcal{L}_{\text{A}} = \mathcal{L}_{\text{B}} = \mathcal{L}$), (2) A and B undergo isotropic rotational diffusion ($\mathcal{L} = D_r \nabla^2_\Omega$, where D_r is the rotational diffusion coefficient of the molecule), and (3) both A and B are ellipsoids-of-revolution absorbers with the same symmetry axis ($\hat{\mathbf{n}}$) at both the excitation and probe wavelengths. Under these conditions, and using Eq. 3.4 for $\epsilon_{\text{A}}(e, \Omega)$, Eq. 2.2 becomes

$$\begin{aligned} \frac{\partial p_{\text{A}}(\Omega, t)}{\partial t} &= D_r \nabla^2_\Omega p_{\text{A}}(\Omega, t) - \epsilon^0_{\text{A}}(\lambda_e) \\ &\quad \times [1 - \sigma_{\text{A}}(\lambda_e) P_2(\hat{\mathbf{n}} \cdot \hat{\mathbf{e}})] I(t) p_{\text{A}}(\Omega, t), \end{aligned} \quad (3.42)$$

which must be solved subject to the initial condition $p_{\text{A}}(\Omega, 0) = p_{\text{eq}}(\Omega)$. We expand $p_{\text{A}}(\Omega, t)$ in a series of Legendre polynomials

$$p_{\text{A}}(\Omega, t) = p_{\text{eq}}(\Omega) \sum_{l=0}^{\infty} a_l(\lambda_e, t) P_l(\hat{\mathbf{n}} \cdot \hat{\mathbf{e}}), \quad (3.43)$$

and determine the expansion coefficients a_l by requiring Eq. 3.43 to satisfy Eq. 3.42 (see below). We obtain the normalized change in absorbance ($f(t)$) from Eq. 2.8 using Eqs. 3.6 and 3.7 for $\epsilon_{\text{A}}(p, \Omega)$ and $\epsilon_{\text{B}}(p, \Omega)$ and with $\hat{\mathbf{n}}_{\text{A}p} = \hat{\mathbf{n}}_{\text{B}p} = \hat{\mathbf{n}}$. Using the following identities

$$\int p_{\mathrm{eq}}(\Omega)P_l(\hat{\mathbf{n}}\cdot\hat{\mathbf{e}})\,\mathrm{d}\Omega=\delta_{l0} \tag{3.44a}$$

$$\int p_{\mathrm{eq}}(\Omega)P_2(\hat{\mathbf{n}}\cdot\hat{\mathbf{p}})P_l(\hat{\mathbf{n}}\cdot\hat{\mathbf{e}})\,\mathrm{d}\Omega=(^1/_5)\delta_{l2}P_2(\hat{\mathbf{e}}\cdot\hat{\mathbf{p}}) \tag{3.44b}$$

Eq. 2.8 may be rewritten as

$$f(t)=1-\mathrm{a}_0(\lambda_e,t)+\frac{a_2(\lambda_e,t)}{5}\times P_2(\hat{\mathbf{e}}\cdot\hat{\mathbf{p}})\left[\frac{\epsilon^0_{\mathrm{B}}(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p)-\epsilon^0_{\mathrm{A}}(\lambda_p)\sigma_{\mathrm{A}}(\lambda_p)}{\epsilon^0_{\mathrm{B}}(\lambda_p)-\epsilon^0_{\mathrm{A}}(\lambda_p)}\right]. \tag{3.45}$$

The corresponding absorption anisotropy is

$$r(t)=\frac{1}{10}\frac{a_2(\lambda_e,t)}{1-a_0(\lambda_e,t)}\times\left[\frac{\epsilon^0_{\mathrm{B}}(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p)-\epsilon^0_{\mathrm{A}}(\lambda_p)\sigma_{\mathrm{A}}(\lambda_p)}{\epsilon^0_{\mathrm{B}}(\lambda_p)-\epsilon^0_{\mathrm{A}}(\lambda_p)}\right]. \tag{3.46}$$

Eq. 3.46 is formally analogous to the result obtained for short excitation pulses (Eq. 3.24) with the time dependence now absorbed in the expansion coefficients $a_0(\lambda_e, t)$ and $a_2(\lambda_e, t)$.

In order to obtain these coefficients we substitute Eq. 3.43 into Eq. 3.42. Setting $\hat{\mathbf{n}}\cdot\hat{\mathbf{e}} = \cos\theta$ and using the orthogonality properties of Legendre polynomials, we find

$$\frac{\mathrm{d}a_l(\lambda_e,t)}{\mathrm{d}t}=-\sum_{l'}(D_{ll'}+E_{ll'}(t))a_{l'}(\lambda_e,t), \tag{3.47}$$

where

$$D_{ll'}=l(l+1)D_r\delta_{ll'} \tag{3.48}$$

and

$$E_{ll'}(t)=\epsilon^0_{\mathrm{A}}(\lambda_e)I(t)\delta_{ll'}-\frac{2l+1}{2}\epsilon^0_{\mathrm{A}}(\lambda_e)I(t)\sigma_{\mathrm{A}}(\lambda_e)\times\int_0^\pi P_l(\cos\theta)P_2(\cos\theta)P_{l'}(\cos\theta)\sin\theta\,\mathrm{d}\theta. \tag{3.49}$$

The integral of the product of the three Legendre polynomials in Eq. 3.49 can be expressed in terms of Wigner 3-*j* coefficients (13) as

$$\int_{-1}^{1}P_l(x)P_2(x)P_{l'}(x)\,\mathrm{d}x=2\begin{pmatrix} l & 2 & l' \\ 0 & 0 & 0 \end{pmatrix}^2. \tag{3.50}$$

This coefficient is non-zero when $l' = l$, $l \pm 2$ and has values

$$\begin{pmatrix} l & 2 & l \\ 0 & 0 & 0 \end{pmatrix}^2=\frac{l(l+1)}{(2l+3)(2l+1)(2l-1)} \tag{3.51a}$$

$$\begin{pmatrix} l & 2 & l-2 \\ 0 & 0 & 0 \end{pmatrix}^2=\frac{3}{2}\frac{l(l-1)}{(2l+1)(2l-1)(2l-3)} \tag{3.51b}$$

$$\begin{pmatrix} l & 2 & l+2 \\ 0 & 0 & 0 \end{pmatrix}^2=\frac{3}{2}\frac{(l+1)(l+2)}{(2l+5)(2l+3)(2l+1)}. \tag{3.51c}$$

Thus we have reduced the solution of the partial differential equation (3.42) to the solution of a set of coupled ordinary equations for the expansion coefficients. From Eqs. 3.47 to 3.51 we note that there is no mixing between even and odd coefficients a_l; since $f(t)$ and $r(t)$ depend only on $a_0(\lambda_e, t)$ and $a_2(\lambda_e, t)$ we need to solve Eq. 3.47 only for the even coefficients. For an isotropic system that is initially in state A, the initial condition, from Eq. 3.43, is $a_l(\lambda_e, 0) = \delta_{l0}$.

For a square excitation pulse of width t_p ($I(t) = I_0$ for $t \le t_p$ and $=0$ for $t \ge t_p$), the solution of Eq. 3.47 for $t \le t_p$ can be written as

$$\mathbf{a}(\lambda_e,t)=e^{-(\mathbf{D}+\mathbf{E})t}\mathbf{a}(\lambda_e,0)=\mathbf{U}^{-1}e^{-\boldsymbol{\lambda}t}\mathbf{U}\mathbf{a}(\lambda_e,0), \tag{3.52}$$

where $\boldsymbol{\lambda}$ is a diagonal matrix containing the eigenvalues of $\mathbf{D} + \mathbf{E}$ and $\mathbf{U}$ is a matrix whose columns are the corresponding eigenvectors. In practice one must, of course, truncate the matrix $\mathbf{D} + \mathbf{E}$. We found that truncation at $l = 8$ gives values for a_l that are accurate to six significant figures. Finally, for an excitation pulse of arbitrary shape, Eq. 3.47 can be solved iteratively

$$\mathbf{a}(\lambda_e,t+\delta t)=(\mathbf{I}-(\mathbf{D}+\mathbf{E}(t))\delta t)\mathbf{a}(\lambda_e,t). \tag{3.53}$$

For $t > t_p$ the matrix elements of $\mathbf{E}$ are zero and the solution of Eq. 3.47 simplifies to

$$a_l(\lambda_e,t)=e^{-l(l+1)D_r(t-t_p)}a_l(\lambda_e,t_p), \tag{3.54}$$

and the absorption anisotropy, from Eq. 3.46, becomes

$$r(t>t_p)=\frac{1}{10}\frac{a_2(\lambda_e,t_p)}{1-a_0(\lambda_e,t_p)}\times\left[\frac{\epsilon^0_{\mathrm{B}}(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p)-\epsilon^0_{\mathrm{A}}(\lambda_p)\sigma_{\mathrm{A}}(\lambda_p)}{\epsilon^0_{\mathrm{B}}(\lambda_p)-\epsilon^0_{\mathrm{A}}(\lambda_p)}\right]e^{-6D_r(t-t_p)} \tag{3.55}$$

The above formalism can be readily extended to allow for fast axially symmetric internal motions that occur during excitation. If the correlation time for internal motions is much shorter than the duration of the pulse, the above expressions can be applied by simply scaling all the σ's by the order parameter of the internal motions (i.e., by using $\sigma_I^{\mathrm{eff}}(\lambda) = S\sigma_I(\lambda)$, $I = A, B$ in Eqs. 3.45, 3.46 and 3.49). If $\sigma_{\mathrm{A}}^{\mathrm{eff}} = \sigma_{\mathrm{B}}^{\mathrm{eff}} = S$, Eq. 3.55 is identical to Eq. 7 of Jones et al. (14) in their analysis of the absorption anisotropy measurements on hemoglobin.

4. REORIENTATIONAL DYNAMICS AND KINETICS AFTER EXCITATION

So far we have only considered the reaction (Eq. 2.1) where a molecule in state A absorbs a photon and is excited into a long-lived state B. We now consider a more complicated reaction scheme:

$$A \underset{k_1}{\overset{h\nu}{\rightleftharpoons}} B \underset{k_3}{\overset{k_2}{\rightleftharpoons}} C. \tag{4.1}$$

We will assume that the molecule is spectroscopically identical in states B and C, i.e., $\epsilon_B(p, \Omega) = \epsilon_C(p, \Omega)$. The normalized change in absorbance is given by

$$f(t) = \frac{1}{\epsilon_B^0(\lambda_p) - \epsilon_A^0(\lambda_p)}\left[\int \epsilon_A(p, \Omega)p_A(\Omega, t)\,d\Omega + \int \epsilon_B(p, \Omega)[p_B(\Omega, t) + p_C(\Omega, t)]\,d\Omega - \epsilon_A^0(\lambda_p)\right], \tag{4.2}$$

where $p_C(\Omega, t)$ is the time-dependent orientational distribution function in state C. $p_I(\Omega, t)$ $(I = A, B, C)$ satisfy the following set of equations

$$\begin{aligned}
\frac{\partial p_A(\Omega, t)}{\partial t} &= \mathcal{L}_A p_A(\Omega, t) - \epsilon_A(e, \Omega)I(t)p_A(\Omega, t) + k_1 p_B(\Omega, t)\\
\frac{\partial p_B(\Omega, t)}{\partial t} &= \mathcal{L}_B p_B(\Omega, t) + \epsilon_A(e, \Omega)I(t)p_A(\Omega, t)\\
&\quad - (k_1 + k_2)p_B(\Omega, t) + k_3 p_C(\Omega, t)\\
\frac{\partial p_C(\Omega, t)}{\partial t} &= \mathcal{L}_C p_C(\Omega, t) + k_2 p_B(\Omega, t) - k_3 p_C(\Omega, t),
\end{aligned} \tag{4.3}$$

where $\mathcal{L}_C$ describes the reorientational dynamics of the chromophore in state C. The coupled set of equations (4.3) must be solved subject to the initial conditions $p_A(\Omega, 0) = p_{eq}^A(\Omega)$, $p_B(\Omega, 0) = p_C(\Omega, 0) = 0$. In this section we will consider the case where no reorientational dynamics or kinetics occurs during the pulse. The orientational distribution of the chromophore in the three states immediately after the pulse ($t = 0^+$) is

$$\begin{aligned}
p_A(\Omega, 0^+) &= p_{eq}^A(\Omega)\exp(-\epsilon_A(e, \Omega)I_0)\\
p_B(\Omega, 0^+) &= p_{eq}^A(\Omega)[1 - \exp(-\epsilon_A(e, \Omega)I_0)]\\
p_C(\Omega, 0^+) &= 0,
\end{aligned} \tag{4.4}$$

where I_0 is the time integral of $I(t)$. After the pulse Eq. 4.3 simplifies to

$$\begin{aligned}
\frac{\partial p_A(\Omega, t)}{\partial t} &= \mathcal{L}_A p_A(\Omega, t) + k_1 p_B(\Omega, t)\\
\frac{\partial p_B(\Omega, t)}{\partial t} &= \mathcal{L}_B p_B(\Omega, t) - (k_1 + k_2)p_B(\Omega, t) + k_3 p_C(\Omega, t)\\
\frac{\partial p_C(\Omega, t)}{\partial t} &= \mathcal{L}_C p_C(\Omega, t) + k_2 p_B(\Omega, t) - k_3 p_C(\Omega, t).
\end{aligned} \tag{4.5}$$

Since the kinetics of going from one state to another are independent of the reorientational dynamics of the molecules within each state, the solution to the coupled equations (4.5) may be written in terms of the Green's function of $\mathcal{L}_I$ as

$$p_I(\Omega, t) = \int d\Omega_0 G_I(\Omega t\,|\,\Omega_0 0^+) \sum_{J=A,B,C} P(It\,|\,J0^+)p_J(\Omega_0, 0^+), \qquad I = A, B, C \tag{4.6}$$

where $P(It\,|\,J0^+)$ is the probability that a molecule at time t is in state I, given that at $t = 0^+$ it is in state J and may be evaluated by setting the terms containing $\mathcal{L}_I$ in Eq. 4.5 to zero and solving the rate equations. We will assume negligible thermal rate coefficient for the reaction step $A \rightarrow B$, i.e., $P(At\,|\,A0^+) = 1$ and $P(Bt\,|\,A0^+) = P(Ct\,|\,A0^+) = 0$. Using Eqs. 4.4 and 4.6 we have

$$\begin{aligned}
p_A(\Omega, t) &= \int d\Omega_0 G_A(\Omega t\,|\,\Omega_0 0^+)\\
&\quad \times [p_A(\Omega_0, 0^+) + p_B(\Omega_0, 0^+)P(At\,|\,B0^+)]\\
&= \int d\Omega_0 G_A(\Omega t\,|\,\Omega_0 0^+)p_{eq}^A(\Omega_0)\\
&\quad \times [e^{-\epsilon_A(e,\Omega_0)I_0} + (1 - e^{-\epsilon_A(e,\Omega_0)I_0})P(At\,|\,B0^+)]\\
&= p_{eq}^A(\Omega) - [1 - P(At\,|\,B0^+)]\\
&\quad \times \left[\int d\Omega_0 G_A(\Omega t\,|\,\Omega_0 0^+)p_{eq}^A(\Omega_0)(1 - e^{-\epsilon_A(e,\Omega_0)I_0})\right].
\end{aligned} \tag{4.7}$$

We now assume that the reorientational dynamics of B and C are the same ($\mathcal{L}_B = \mathcal{L}_C$) and write

$$\begin{aligned}
&p_B(\Omega, t) + p_C(\Omega, t)\\
&\quad = \int d\Omega_0 G_B(\Omega t\,|\,\Omega_0 0^+)[p_B(\Omega_0, 0^+)\\
&\qquad \times P(Bt\,|\,B0^+) + p_B(\Omega_0, 0^+)P(Ct\,|\,B0^+)]\\
&\quad = [1 - P(At\,|\,B0^+)]\\
&\qquad \times \int d\Omega_0 G_B(\Omega t\,|\,\Omega_0 0^+)p_{eq}^A(\Omega_0)(1 - e^{-\epsilon_A(e,\Omega)I_0}),
\end{aligned} \tag{4.8}$$

where we have used

$$\sum_{I=A,B,C} P(It\,|\,J0^+) = 1 \tag{4.9}$$

Using Eqs. 4.7 and 4.8 in Eq. 4.2 we have

$$\begin{aligned}
f(t) &= [1 - P(At\,|\,B0^+)]\frac{1}{\epsilon_B^0(\lambda_p) - \epsilon_A^0(\lambda_p)}\\
&\quad \times (\langle\langle \epsilon_B(p, \Omega(t))[1 - \exp(-\epsilon_A(e, \Omega(0^+))I_0)]\rangle\rangle\\
&\quad - \langle \epsilon_A(p, \Omega(t))[1 - \exp(-\epsilon_A(e, \Omega(0^+))I_0)]\rangle)
\end{aligned} \tag{4.10}$$

where we have used the definitions in Eqs. 2.13 and 2.14 for the correlation functions. The form of Eq. 4.10 is identical to that of Eq. 2.22 multiplied by a factor that depends upon the reaction mechanism and is in fact the fraction of molecules that were excited and have not decayed back to A. For absorbers that are ellipsoids of revolution, the formalism in section 3.2 follows through for the evaluation of the correlation functions and $f(t)$ is given by Eq. 3.19 multipled by $(1 - P(At\,|\,B0^+))$. Since the kinetics influence $f(t)$ via a multiplicative factor, it follows that the absorption anisotropy is independent of the kinetics and is still given by Eq. 3.20.

When $k_3 = 0$, the kinetic equations in Eq. 4.5 may be solved analytically to yield

$$P(At \mid B0^+) = \frac{k_1}{k_1 + k_2}\left(1 - e^{-(k_1+k_2)t}\right). \tag{4.11}$$

For a circular absorber rigidly fixed in an isotropically diffusing macromolecule, when the excitation polarization is parallel to the probe polarization ($\hat{\mathbf{e}} = \hat{\mathbf{p}}$), using Eqs. 4.10 and 3.19 and setting $\sigma = 1$ and the reorientational correlation function equal to $\exp(-6D_r t)$, we find

$$f(t) = \left[\frac{k_2}{k_1 + k_2} + \frac{k_1}{k_1 + k_2} e^{-(k_1+k_2)t}\right] \times \left[1 - a_0(\lambda_e) + \frac{a_2(\lambda_e)}{5} e^{-6D_r t}\right], \tag{4.12}$$

where $a_0(\lambda_e)$ and $a_2(\lambda_e)$ are given by Eqs. 3.17 and 3.18. This result is identical to Eq. 10 of Hofrichter et al. (15) who showed experimentally that the change in absorbance is influenced both by the rotational diffusion of the molecule (which changes the projection of the absorption dipole onto the probe beam), and the kinetics (which changes the fraction of molecules in the various states). The anisotropy decay, however, in the limit where the pulse-width is short compared to the rotational diffusion time of the molecule and the decay time of the excited state B, is insensitive to the kinetics and depends only on the rotational diffusion of the molecule.

5. ROTATIONAL DIFFUSION AND KINETICS DURING EXCITATION

In this section we will consider the general case of a finite excitation pulse-width, allowing for both rotational diffusion and kinetics during excitation. We will consider the reaction scheme of Eq. 4.1 and assume, as before, that B and C are spectroscopically identical. We will further assume that the reorientational dynamics in the three states are identical ($\mathcal{L}_I = \mathcal{L}$; $p_{\mathrm{eq}}^I(\Omega) = p_{\mathrm{eq}}(\Omega)$; $I = A, B, C$). Thus,

$$p_{\mathrm{A}}(\Omega, t) + p_{\mathrm{B}}(\Omega, t) + p_{\mathrm{C}}(\Omega, t) = p_{\mathrm{eq}}(\Omega) \tag{5.1}$$

and Eq. 4.2 simplifies to

$$f(t) = 1 - \frac{\int [\epsilon_{\mathrm{B}}(p, \Omega) - \epsilon_{\mathrm{A}}(p, \Omega)] p_{\mathrm{A}}(\Omega, t)\, \mathrm{d}\Omega}{\epsilon_{\mathrm{B}}^0(\lambda_p) - \epsilon_{\mathrm{A}}^0(\lambda_p)}. \tag{5.2}$$

The time-dependent orientational distribution functions ($p_I(\Omega, t)$) satisfy Eq. 4.3 and the orientation-dependent extinction coefficients for ellipsoid-of-revolution absorbers are given by Eqs. 3.4, 3.6, and 3.8.

We will now follow the formalism of section 3.3 and expand $p_I(\Omega, t)$ in a series of Legendre polynomials with time-dependent coefficients,

$$\begin{aligned} p_{\mathrm{A}}(\Omega, t) &= p_{\mathrm{eq}}(\Omega) \sum_l a_l(\lambda_e, t) P_l(\hat{\mathbf{n}} \cdot \hat{\mathbf{e}}) \\ p_{\mathrm{B}}(\Omega, t) &= p_{\mathrm{eq}}(\Omega) \sum_l b_l(\lambda_e, t) P_l(\hat{\mathbf{n}} \cdot \hat{\mathbf{e}}) \\ p_{\mathrm{C}}(\Omega, t) &= p_{\mathrm{eq}}(\Omega) \sum_l c_l(\lambda_e, t) P_l(\hat{\mathbf{n}} \cdot \hat{\mathbf{e}}). \end{aligned} \tag{5.3}$$

We have assumed that A, B, and C have the same symmetry axis ($\hat{\mathbf{n}}$). Using Eqs. 3.4, 3.6 and 3.8 and 5.3 in Eq. 5.2 and using the identities in Eq. 3.44 we recover Eqs. 3.45 and 3.46 for the normalized change in absorbance and the absorption anisotropy; the expansion coefficients $a_0(\lambda_e, t)$ and $a_2(\lambda_e, t)$ now depend on the excitation intensity and the reorientational dynamics of the chromophore as well as the rate coefficients in the kinetic scheme.

We now proceed to solve for these expansion coefficients. Substituting Eqs. 3.4 and 5.3 in Eq. 4.3, setting $\mathcal{L} = D_r \nabla_\Omega^2$, and using the orthogonality properties of Legendre polynomials we have

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} \mathbf{a}(\lambda_e, t) \\ \mathbf{b}(\lambda_e, t) \\ \mathbf{c}(\lambda_e, t) \end{pmatrix} = \mathbf{M}(t) \begin{pmatrix} \mathbf{a}(\lambda_e, t) \\ \mathbf{b}(\lambda_e, t) \\ \mathbf{c}(\lambda_e, t) \end{pmatrix} = \begin{pmatrix} -[\mathbf{D} + \mathbf{E}(t)] & k_1\mathbf{I} & 0 \\ \mathbf{E}(t) & -[\mathbf{D} + (k_1 + k_2)\mathbf{I}] & k_3\mathbf{I} \\ 0 & k_2\mathbf{I} & -[\mathbf{D} + k_3\mathbf{I}] \end{pmatrix} \times \begin{pmatrix} \mathbf{a}(\lambda_e, t) \\ \mathbf{b}(\lambda_e, t) \\ \mathbf{c}(\lambda_e, t) \end{pmatrix}, \tag{5.4}$$

where **a**, **b**, **c** are vectors whose elements are the time-dependent coefficients a_l, b_l, and c_l, respectively; **I** is the unit matrix and **D** and $\mathbf{E}(t)$ are matrices that describe the rotational diffusion and excitation, respectively, with elements given by Eqs. 3.48 and 3.49. The set of coupled differential equations defined in Eq. 5.4 need to be solved subject to the initial conditions $a_l(0) = \delta_{l0}$, $b_l(0) = c_l(0) = 0$. Since $f(t)$ and $r(t)$ depend only on coefficients with $l = 0$ and $l = 2$ and, as discussed in section 3.3, there is no mixing between the even and odd coefficients, we need to solve only for the even coefficients a_l, b_l, c_l ($l = 0, 2, 4, \cdots$).

For a square excitation pulse of width t_p, the time dependent matrix in Eq. 5.4 becomes $\mathbf{M}(t) = \mathbf{M}_0$ for $t \le t_p$ and the solution to Eq. 5.4 may be written as

$$\begin{pmatrix} \mathbf{a}(\lambda_e, t) \\ \mathbf{b}(\lambda_e, t) \\ \mathbf{c}(\lambda_e, t) \end{pmatrix} = \mathbf{e}^{\mathbf{M}_0 t} \begin{pmatrix} \mathbf{a}(\lambda_e, 0) \\ \mathbf{b}(\lambda_e, 0) \\ \mathbf{c}(\lambda_e, 0) \end{pmatrix} = \mathbf{U}^{-1} e^{\lambda t} \mathbf{U} \begin{pmatrix} \mathbf{a}(\lambda_e, 0) \\ \mathbf{b}(\lambda_e, 0) \\ \mathbf{c}(\lambda_e, 0) \end{pmatrix} \tag{5.5}$$

and for an excitation pulse of arbitrary shape Eq. 5.4 may be solved iteratively:

$$\begin{pmatrix} \mathbf{a}(\lambda_e, t + \delta t) \\ \mathbf{b}(\lambda_e, t + \delta t) \\ \mathbf{c}(\lambda_e, t + \delta t) \end{pmatrix} = [\mathbf{I} + \mathbf{M}(t)\delta t] \begin{pmatrix} \mathbf{a}(\lambda_e, t) \\ \mathbf{b}(\lambda_e, t) \\ \mathbf{c}(\lambda_e, t) \end{pmatrix}. \tag{5.6}$$

After the pulse ($I(t) = 0$) the coupled equations (5.4) simplify to

$$\frac{da_0(\lambda_e, t)}{dt} = k_1 a_0(\lambda_e, t)$$

$$\frac{da_2(\lambda_e, t)}{dt} = -6D_r a_2(\lambda_e, t) + k_1 b_2(\lambda_e, t)$$

$$\frac{db_0(\lambda_e, t)}{dt} = -(k_1 + k_2) b_0(\lambda_e, t) + k_3 c_0(\lambda_e, t)$$

$$\frac{db_2(\lambda_e, t)}{dt} = -(6D_r + k_1 + k_2) b_2(\lambda_e, t) + k_3 c_2(\lambda_e, t)$$

$$\frac{dc_0(\lambda_e, t)}{dt} = k_2 b_0(\lambda_e, t) - k_3 c_0(\lambda_e, t)$$

$$\frac{dc_2(\lambda_e, t)}{dt} = k_2 b_2(\lambda_e, t) - (6D_r + k_3) c_2(\lambda_e, t) \quad (5.7)$$

If $k_3 \rightarrow 0$, then Eq. 5.7 may be solved analytically to yield

$$a_0(\lambda_e, t) = a_0(\lambda_e, t_p) + \frac{k_1}{k_1 + k_2} b_0(\lambda_e, t_p)(1 - e^{-(k_1+k_2)(t-t_p)})$$

$$a_2(\lambda_e, t) = \left[a_2(\lambda_e, t_p) + \frac{k_1}{k_1 + k_2} b_2(\lambda_e, t_p)(1 - e^{-(k_1+k_2)(t-t_p)})\right] \times \exp(-6D_r(t - t_p)) \quad (5.8)$$

where $a_l(\lambda_e, t_p)$ and $b_l(\lambda_e, t_p)$ are the values of the coefficients at the end of the pulse. The anisotropy decay after the pulse becomes

$$r(t > t_p) = \frac{1}{10}\left[\frac{a_2(\lambda_e, t_p) + \frac{k_1}{k_1 + k_2} b_2(\lambda_e, t_p)(1 - e^{-(k_1+k_2)(t-t_p)})}{1 - a_0(\lambda_e, t_p) - \frac{k_1}{k_1 + k_2} b_0(\lambda_e, t_p)(1 - e^{-(k_1+k_2)(t-t_p)})}\right] \times \left[\frac{\epsilon^0_{\mathrm{B}}(\lambda_p)\sigma_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)\sigma_{\mathrm{A}}(\lambda_p)}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)}\right] \exp(-6D_r(t - t_p)). \quad (5.9)$$

An interesting consequence of the above result is that there is a change in the absorption anisotropy after excitation with a finite pulse that is due to both kinetics and rotational diffusion of the macromolecule. This is because at the end of the pulse states B and C are populated with different orientational distributions; since B and C are spectroscopically identical but decay at different rates, there is a change in the orientational distribution of the photoproduct resulting in a change in the anisotropy. If $k_1 = 0$ (no change in the total population of B + C after the pulse) or $k_2 = 0$ (C is never populated), the anisotropy decay simplifies to the result obtained in Eq. 3.55 and decays only when the macromolecule reorients.

6. CONCLUDING REMARKS

We have developed a comprehensive and unified theory of the absorbance of dynamic systems in which an intense excitation pulse produces a photoselected initial population. We have considered a variety of situations and complications (e.g., reorientational motion that is slower, faster, or on the same time-scale as the duration of the excitation pulse, the influence of internal motions and chemical kinetics, deviations of the absorption properties of the chromophore from linear or circular. Many of the results derived here have already proved useful in the analysis of experimental data on heme proteins (5, 14, 15).

In this paper we have attempted to reach a compromise between simplicity and generality. For example, the short intense excitation pulse results for the absorbance change (Eq. 3.19) and the absorption anisotropy (Eq. 3.20), while valid for arbitrary reorientational dynamics, are based on the assumption that the chromophores are ellipsoid-of-revolution absorbers. The treatment of general ellipsoidal absorbers, presented in the Appendix, is restricted to isotropic overall diffusion. The theoretical framework, developed for linearly polarized excitation and probe pulses, can be extended to include circularly polarized or unpolarized light pulses by appropriately modifying the definitions of the orientation dependent extinction coefficients in section 3. Our discussion of chemical kinetics was based on a simple illustrative reaction scheme with two spectrally distinct species, but the approach can be readily generalized to treat more complicated situations. Thus, in addition to presenting a variety of explicit results that can be used to interpret experimental data, this paper serves as a useful guide to treating complex photoselection problems.

APPENDIX

Here we derive an expression for the change in absorbance and anisotropy decay when an isotropically diffusing absorber of arbitrary shape is excited with a short pulse of linearly polarized light. Eq. 2.23 describes the change in absorbance when the molecule is excited from state A to state B, and the reorientational dynamics in the two states is identical. The extinction coefficients in the direction of polarization of the excitation and probe beams are:

$$\begin{aligned}\epsilon_{\mathrm{A}}(e,\Omega) &= \epsilon_{\mathrm{A}}^{0}(\lambda_e)\sum_i \alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\\ \epsilon_{\mathrm{A}}(p,\Omega) &= \epsilon_{\mathrm{A}}^{0}(\lambda_p)\sum_i \alpha_i(\lambda_p)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{p}})^2\\ \epsilon_{\mathrm{B}}(p,\Omega) &= \epsilon_{\mathrm{B}}^{0}(\lambda_p)\sum_i \beta_i(\lambda_p)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{p}})^2,\end{aligned}\tag{A1}$$

where $\hat{\mathbf{n}}_i$ (i = 1, 2, 3) are orthogonal axes fixed on the absorber and $\sum\alpha_i = \sum\beta_i = 3$. Combining Eqs. 2.23 and A1 we get

$$f(t) = \frac{1}{\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)}\left\langle\sum_i(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))(\hat{\mathbf{n}}_i(t)\cdot\hat{\mathbf{p}})^2\left[1-\exp\left(-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i(0^+)\cdot\hat{\mathbf{e}})^2\right)\right]\right\rangle. \tag{A2}$$

For an isotropic system, the change in absorbance is proportional to the fraction of molecules excited. An additional term that decays with the rotational correlation time of the molecule is introduced as a result of photoselection. Analogous to the solution for a uniaxially symmetric absorber (Eq. 3.19) we may rewrite Eq. A2 as a sum of two terms:

$$f(t) = 1 - y + zP_2(\hat{e}\cdot\hat{p})\exp(-6D_rt) \tag{A3}$$

with the absorption anisotropy given by

$$r(t) = \frac{z}{2(1-y)}\exp(-6D_rt). \tag{A4}$$

$(1 - y)$ is the normalized change in absorbance observed at the magic angle and is thus the fraction of molecules that are excited. z is proportional to the linear dichroism introduced in the sample; the linear dichroism decays exponentially for an isotropically diffusing absorber. y and z are functions of the excitation intensity and depend upon the properties of the absorber at the excitation and probe wavelengths. We now proceed to solve for y and z. Since y is the fraction of molecules in state A that are not excited, we have

$$\begin{aligned}y &= \left\langle\exp\left(\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\right)\right\rangle\\ &= \frac{1}{4\pi}\int_0^{2\pi}\mathrm{d}\phi\int_0^{\pi}\sin\theta\,\mathrm{d}\theta\exp(-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0[\alpha_1(\lambda_e)\cos^2\theta+\alpha_2(\lambda_e)\sin^2\theta\cos^2\phi+\alpha_3(\lambda_e)\sin^2\theta\sin^2\phi]).\end{aligned}\tag{A5}$$

We evaluate z by setting $\hat{\mathbf{e}}\cdot\hat{\mathbf{p}} = 1$ and $t = 0^+$ in Eqs. A2 and A3 and equating the results to yield

$$\begin{aligned}f(0^+) &= 1 - y + z\\ &= \frac{1}{\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)}\left\langle\sum_i(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))(\hat{\mathbf{n}}_i(0^+)\cdot\hat{\mathbf{e}})^2\left[1-\exp\left(-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i(0^+)\cdot\hat{\mathbf{e}})^2\right)\right]\right\rangle\\ &= 1-\frac{1}{\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)}\left\langle\sum_i(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))\left(\frac{2P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})+1}{3}\right)\exp\left(-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\right)\right\rangle\\ &= 1-y-\frac{2}{3(\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p))}\left\langle\sum_i(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})\exp\left(-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\right)\right\rangle,\end{aligned}\tag{A6}$$

where we have used Eq. A5. Therefore,

$$z = \frac{-2}{3(\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p))}\left\langle\sum_i(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})\exp\left(-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\right)\right\rangle. \tag{A7}$$

Eqs. A5 and A7 are the principal results of this appendix.

In the limit of low intensities ($I_0 \rightarrow 0$) it can be shown that

$$y \approx \left\langle 1-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\right\rangle = \left\langle 1-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)\left(\frac{2P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})+1}{3}\right)\right\rangle = 1-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0 \tag{A8}$$

and

$$\begin{aligned}z &\approx \frac{-2}{3(\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p))}\left\langle\sum_i(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})\left[1-\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0\sum_i\alpha_i(\lambda_e)(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})^2\right]\right\rangle\\ &= \frac{2}{3}\frac{\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0}{\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)}\sum_{ij}(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))\alpha_j(\lambda_e)\left\langle P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})\left(\frac{2P_2(\hat{\mathbf{n}}_j\cdot\hat{\mathbf{e}})+1}{3}\right)\right\rangle\\ &= \frac{4}{9}\frac{\epsilon_{\mathrm{A}}^{0}(\lambda_e)I_0}{\epsilon_{\mathrm{B}}^{0}(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)}\sum_{ij}(\epsilon_{\mathrm{B}}^{0}(\lambda_p)\beta_i(\lambda_p)-\epsilon_{\mathrm{A}}^{0}(\lambda_p)\alpha_i(\lambda_p))\alpha_j(\lambda_e)\langle P_2(\hat{\mathbf{n}}_i\cdot\hat{\mathbf{e}})P_2(\hat{\mathbf{n}}_j\cdot\hat{\mathbf{e}})\rangle,\end{aligned}\tag{A9}$$

where we have used the fact that for an isotropic system $\langle P_2\rangle = 0$. Using $\langle P_2(\hat{m}_i\cdot\hat{e})P_2(\hat{m}_j\cdot\hat{e})\rangle = P_2(\hat{e}\cdot\hat{e})P_2(\hat{\mathbf{m}}_i\cdot\hat{\mathbf{m}}_j)/5 = P_2(\hat{\mathbf{m}}_i\cdot\hat{\mathbf{m}}_j)/5$ yields

$$z \approx \frac{4}{9} \frac{\epsilon^0_{\mathrm{A}}(\lambda_e) I_0}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)} \sum_{ij} (\epsilon^0_{\mathrm{B}}(\lambda_p)\beta_i(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)\alpha_i(\lambda_p))\alpha_j(\lambda_e) \frac{P_2(\hat{\mathbf{n}}_i \cdot \hat{\mathbf{n}}_j)}{5}$$

$$= \frac{4}{45} \frac{\epsilon^0_{\mathrm{A}}(\lambda_e) I_0}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)} \sum_{ij} (\epsilon^0_{\mathrm{B}}(\lambda_p)\beta_i(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)\alpha_i(\lambda_p))\alpha_j(\lambda_e) \left[\frac{3}{2}\delta_{ij} - \frac{1}{2}\right], \quad \text{(A10)}$$

and, since $\sum \alpha_i = \sum \beta_i = 3$, we get

$$z \approx \frac{2}{15} \frac{\epsilon^0_{\mathrm{A}}(\lambda_e) I_0}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)} \left[\sum_i (\epsilon^0_{\mathrm{B}}(\lambda_p)\beta_i(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)\alpha_i(\lambda_p))\alpha_i(\lambda_e) - 3(\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p))\right]. \quad \text{(A11)}$$

In the limit of low excitation intensities, the absorption anisotropy becomes

$$r(t, I_0 \rightarrow 0) = \frac{1}{2}\left[\frac{2}{15} \sum_i \frac{(\epsilon^0_{\mathrm{B}}(\lambda_p)\beta_i(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)\alpha_i(\lambda_p))\alpha_i(\lambda_e)}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)} - \frac{2}{5}\right] \exp(-6D_r t)$$

$$= \frac{2}{5} P_2\left(\frac{1}{3}\sqrt{\frac{\sum_i (\epsilon^0_{\mathrm{B}}(\lambda_p)\beta_i(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)\alpha_i(\lambda_p))\alpha_i(\lambda_e)}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)}}\right) \exp(-6D_r t). \quad \text{(A12)}$$

Therefore, for a linear absorber ($\alpha_1 = \beta_1 = 3; \alpha_2 = \alpha_3 = \beta_2 = \beta_3 = 0$) we get $r(0^+) = 0.4$ and for a circular absorber ($\alpha_1 = \alpha_2 = \beta_1 = \beta_2 = 3/2; \alpha_3 = \beta_3 = 0$) $r(0^+) = 0.1$. Eq. A12 shows that, in general, the absorption anisotropy depends upon the isotropic extinction coefficients $\epsilon^0_{\mathrm{A}}(\lambda_p)$ and $\epsilon^0_{\mathrm{B}}(\lambda_p)$. If $\alpha_i(\lambda_p) = \beta_i(\lambda_p)$, i.e., the shape and orientation of the absorber is unchanged in going from state A to state B, then Eq. A12 simplifies to

$$r(t, I_0 \rightarrow 0) = \frac{2}{5} P_2\left(\frac{1}{3}\sqrt{\sum_i \alpha_i(\lambda_p)\alpha_i(\lambda_e)}\right) \exp(-6D_r t), \quad \text{(A13)}$$

and the dependence on the extinction coefficients disappears. If the molecule is a circular absorber in state A, i.e., $\alpha_1 = \alpha_2 = 3/2$ and $\alpha_3 = 0$, then Eq. A12 reduces to

$$r(t, I_0 \rightarrow 0) = \frac{2}{5} P_2\left(\frac{1}{3}\sqrt{\frac{3}{2}\frac{\epsilon^0_{\mathrm{B}}(\lambda_p)(\beta_1(\lambda_p) + \beta_2(\lambda_p)) - 3\epsilon^0_{\mathrm{A}}(\lambda_p)}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)}}\right) \exp(-6D_r t)$$

$$= \frac{1}{10}\left(1 - \frac{\beta_3(\lambda_p)\epsilon^0_{\mathrm{B}}(\lambda_p)}{\epsilon^0_{\mathrm{B}}(\lambda_p) - \epsilon^0_{\mathrm{A}}(\lambda_p)}\right) \exp(-6D_r t). \quad \text{(A14)}$$

Therefore if the molecule is a circular absorber in state A and has no out-of-plane component in state B (i.e., $\beta_3 = 0$) then Eq. A14 shows that the limiting anisotropy $r(0^+) = 0.1$ independent of the relative values of β_1 and β_2.

We thank William Eaton for suggesting this problem.

Received for publication 8 April and in final form 20 October 1992.

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