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Nonlinear Optical Properties of Bacteriorhodopsin: Assignment of the Third-order Polarizability Based on Two-Photon Absorption Spectroscopy.

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ABSTRACT

The third-order π -electron polarizability, γ_π , of bacteriorhodopsin in the 0.0 - 1.2 eV optical region is assigned based on an analysis of the experimental two-photon properties of the low-lying singlet state manifold. The following selected values of γ_π (units of 10^{-36} esu) are observed: $\gamma(0;0,0,0) = 2482 \pm 327$; $\gamma(-3\omega; \omega, \omega, \omega) = 2976 \pm 385$ ($\omega = 0.25$ eV), 5867 ± 704 ($\omega = 0.5$ eV), 14863 ± 1614 ($\omega = 0.66$ eV), 15817 ± 2314 ($\omega = 1.0$ eV), 10755 ± 1733 ($\omega = 1.17$ eV). The third-order polarizability of this protein, which contains an all-trans retinyl protonated Schiff base chromophore with six double bonds, is comparable to that observed for much longer chain polyenes [for example, dodecapreno β -carotene, a polyene with 19 double bonds, exhibits a third-order π -electron polarizability at 0.66 eV of $17000 \pm 6000 \times 10^{-36}$ esu {J.P. Hermann and J. Ducuing, *J. Appl. Phys.* **45**, 5100-5102 (1974)}]. We attribute the enhanced third-order nonlinearity associated with the protein bound chromophore of bacteriorhodopsin to two mutually enhancing origins. First, the chromophore is protonated, and the resultant charge reorganization enhances the polarizability in a fashion that is similar to that known to occur for polaronic and bipolaronic chromophores. We estimate protonation generates a five-fold enhancement in γ_π . Second, the protein bound chromophore exhibits a large change in dipole moment upon excitation into the lowest-lying, strongly-allowed ${}^1B_u^+$ -like state [$\Delta\mu = 13.5$ D, R.R. Birge and C.F. Zhang, *J. Chem. Phys.* **92**, 7178-7195 (1990)]. The latter property is responsible for a Type III enhancement of the third-order polarizability, and yields at least a 20-fold increase in γ_π .

1. INTRODUCTION

Recent studies have demonstrated the significant potential of organic molecules in applications requiring large third-order polarizabilities.¹⁻⁹ These studies suggest that long-chain polyenes provide some of the best potential due to the extended π -electron system coupled with a low-lying strongly allowed excited state.⁴⁻⁷ One problem associated with long-chain polyenes is that the effective conjugation length reaches a limiting value that reduces the effectiveness of adding additional double bonds. Theoretical calculations suggest that the formation of the polaron (polyene charge, $q, ++1$) or bipolaron ($q, ++2$) mediates this problem and enhances both the third-order polarizability as well as the effective conjugation length of long-chain polyenes.⁶ Other studies have suggested that long-chain polyenes with reduced symmetry have enhanced third-order properties due to additional enhancement of the third-order polarizability associated with a low-lying allowed excited state with a large change in dipole moment relative to the ground state.⁵ The above two contributions are not mutually exclusive, and this observation prompted our interest in investigating the third-order polarizability of bacteriorhodopsin, a protein which contains a protonated all-trans retinyl Schiff base polyene. The positively charged chromophore in bacteriorhodopsin exhibits some of the key electronic properties which are observed in bipolarons, and has

a lowest-lying strongly-allowed ${}^1B_u^{*+}$ state which exhibits a large change in dipole moment relative to the ground state ($\Delta\mu = 13.5$ D).¹⁰ Thus, based on previous experimental and theoretical studies, we anticipated that bacteriorhodopsin might exhibit highly enhanced third-order susceptibility. The present investigation provides a quantitative evaluation of γ_π to test this prediction.

Bacteriorhodopsin (MW \approx 26,000) is the light transducing protein in the purple membrane of *Halobacterium halobium*.¹¹⁻¹⁴ The purple membrane, which contains the protein bacteriorhodopsin in a 3:1 protein:lipid matrix, is grown by the bacterium when the concentration of oxygen becomes too low to sustain the generation of ATP via oxidative phosphorylation. The absorption of light by the light-adapted protein initiates a photocycle which pumps protons from the inside (cytoplasmic) to the outside (extracellular) of the membrane. The resulting pH gradient ($\Delta\text{pH} \sim 0.2$) generates a proton-motive force which is used to synthesize ATP from inorganic phosphate and ADP. *Halobacterium halobium* is thus capable of either respiratory or photochemical ATP synthesis.

2. METHODS

Our goal is to assign the third-order polarizability associated with a static electric field, $\gamma(0;0,0,0)$ and the third-order polarizability associated with third-harmonic generation, $\gamma(-3\omega;\omega,\omega,\omega)$. These two polarizabilities, which are invariant to molecular orientation, are defined in terms of the molecular components by the following two expressions:

$$\gamma(-3\omega;\omega,\omega,\omega) = \frac{1}{5} \left[\gamma_{xxxx} + \gamma_{yyyy} + \gamma_{zzzz} + \gamma_{xxyy} + \gamma_{yyxx} + \gamma_{xxzz} + \gamma_{zzxx} + \gamma_{yyzz} + \gamma_{zzyy} \right] \quad (1)$$

$$\gamma(0;0,0,0) = \frac{1}{5} \left[\gamma_{xxxx} + \gamma_{yyyy} + \gamma_{zzzz} + 2\gamma_{xxyy} + 2\gamma_{xxzz} + 2\gamma_{yyzz} \right] \quad (2)$$

where the simplification introduced in Eq. 2 is associated with Kleinman symmetry (e.g. $\gamma_{xxyy} = \gamma_{yyxx}$), which can be applied only for static fields (see discussion in Ref. 7). Both of the above third-order polarizabilities can be calculated in terms of a perturbation expansion summing over all of the excited electronic states:⁷

$$\begin{aligned} \gamma_{abcd}(-3\omega;\omega,\omega,\omega) &= \left[\frac{e^4(2\pi)^3}{h^3} \right] K(-3\omega;\omega,\omega,\omega) \\ &\times \left\{ \sum_{\mathcal{P}} \left[\sum_{i \neq 0} \sum_{j \neq 0} \sum_{k \neq 0} \frac{\langle k|\Delta\mathbf{r}_a|0\rangle \langle k|\Delta\mathbf{r}_b|j\rangle \langle j|\Delta\mathbf{r}_c|i\rangle \langle i|\Delta\mathbf{r}_d|0\rangle}{(\omega_{k0}-3\omega)(\omega_{j0}-2\omega)(\omega_{i0}-\omega)} \right] \right. \\ &\left. - \sum_{\mathcal{P}} \left[\sum_{j \neq 0} \sum_{k \neq 0} \frac{\langle j|\Delta\mathbf{r}_a|0\rangle \langle j|\Delta\mathbf{r}_b|0\rangle \langle k|\Delta\mathbf{r}_c|0\rangle \langle k|\Delta\mathbf{r}_d|0\rangle}{(\omega_{j0}-3\omega)(\omega_{k0}-\omega)(\omega_{k0}+\omega)} \right] \right\} \quad (3) \end{aligned}$$

where $K(-3\omega;\omega,\omega,\omega)$ is a multiplier that adjusts the magnitude of the right-hand-side so that $\gamma_{abcd}(-3\omega;\omega,\omega,\omega)$ [which requires $K=1/8$] is equal to $\gamma_{abcd}(0;0,0,0)$ [which requires $K=1$], $\sum_{\mathcal{P}}$ indicates summation of terms obtained by permuting i, j and k , and $\Delta\mathbf{r}$ represents the transition length delta-operator ($\Delta\mathbf{r} = \mathbf{r}$ if $i \neq j$; $\langle i|\Delta\mathbf{r}|i\rangle = \langle i|\mathbf{r}|i\rangle - \langle 0|\mathbf{r}|0\rangle$). Explicit expansion of the above equation clearly showing the permutations is given in the appendix of Ref. 7.

Before assigning the third-order properties of bacteriorhodopsin, we will demonstrate briefly the relationship between the two-photon absorptivity and the third-order polarizability.

2.1 Perturbation treatment of the two-photon absorption process.

The two-photon absorptivity of the sth excited state is a function not only of the properties of the molecule but also the laser polarization and energies used to generate a simultaneous two-photon absorption in the molecule:¹⁵

$$\delta_{\nu}^{\text{so}} = \frac{8\pi^4 e^4}{(ch)^2} \tilde{\nu}_{\lambda} \tilde{\nu}_{\mu} g(\tilde{\nu}_{\lambda} + \tilde{\nu}_{\mu}) |S_{\text{so}}(\lambda, \mu)|^2 \quad (4)$$

where $\tilde{\nu}_{\lambda}$ and $\tilde{\nu}_{\mu}$ are the frequencies of the two laser beams, $g(\tilde{\nu}_{\lambda} + \tilde{\nu}_{\mu})$ is the normalized lineshape function (see below) and $S_{\text{so}}(\lambda, \mu)$ is the two-photon tensor:

$$S_{\text{so}}(\alpha, \beta) = \sum_{j=0}^N \left(\frac{(\alpha \cdot \langle j | \mathbf{r} | 0 \rangle) (\langle s | \mathbf{r} | j \rangle \cdot \beta)}{\tilde{\nu}_j - \tilde{\nu}_{\alpha} + i\Gamma_j} + \frac{(\beta \cdot \langle j | \mathbf{r} | 0 \rangle) (\langle s | \mathbf{r} | j \rangle \cdot \alpha)}{\tilde{\nu}_j - \tilde{\nu}_{\beta} + i\Gamma_j} \right) \quad (5)$$

where α and β are the unit vectors defining the polarization of the two photons and $\tilde{\nu}_j$ and Γ_j are the transition frequency and the homogeneous linewidth of state j , respectively. As we examine in more detail below, the summation is over all electronic states of the molecule including the ground and final states.¹⁶ Eq. 4 includes a factor of 1/2 that is required in order to make the theoretical and the experimental definitions identical.¹⁷ The normalized lineshape function will be approximated for the present analysis of bacteriorhodopsin by using a log-normal distribution.^{18,19,20}

$$g(\tilde{\nu}_{\mu} + \tilde{\nu}_{\lambda}) = g_{\text{max}} \exp - \left\{ \frac{\ln 2}{(\ln \rho)^2} \left[\ln \left(\frac{(\tilde{\nu}_{\mu} + \tilde{\nu}_{\lambda} - \tilde{\nu}_{\text{so}})(\rho^2 - 1)}{\Delta \tilde{\nu} \rho} + 1 \right) \right]^2 \right\} \quad (6a)$$

$$\tilde{\nu}_{\mu} + \tilde{\nu}_{\lambda} > \tilde{\nu}_{\text{so}} - [\Delta \tilde{\nu} \rho / (\rho^2 - 1)], \quad (6a)$$

$$g(\tilde{\nu}_{\mu} + \tilde{\nu}_{\lambda}) = 0, \quad \tilde{\nu}_{\mu} + \tilde{\nu}_{\lambda} \leq \tilde{\nu}_{\text{so}} - [\Delta \tilde{\nu} \rho / (\rho^2 - 1)] \quad (6b)$$

where,

$$g_{\text{max}} = \left(\frac{4 \ln 2}{\pi c^2 \Delta \tilde{\nu}^2} \right)^{1/2} \left\{ \frac{2 \rho (\ln \rho)}{(\rho^2 - 1)} \exp \left(\frac{(\ln \rho)^2}{4 \ln 2} \right) \right\}^{-1} \quad (7)$$

$\tilde{\nu}_{\text{so}}$ is the wavenumber at maximum two-photon absorptivity, $\Delta \tilde{\nu}$ is the full-width at half-maximum (FWHM) in wavenumbers and ρ is the skewness. The units of g (sec) derive from the fact that this function is normalized to unity in frequency space.²⁰ The skewness is a dimensionless parameter which is an indirect measure of the distribution of vibronic activity into higher vibrational modes due to Franck-Condon activity, vibronic coupling and/or vibronic resonance effects. A log-normal fit to the two-photon double resonance spectrum of bacteriorhodopsin (see below) yields a value of g_{max} for all bands of 8.828×10^{-15} sec ($\rho = 1.6$, $\Delta \tilde{\nu} = 3420 \text{ cm}^{-1}$).

The following three-state approximation for the two-photon absorptivity can be used to predict the absorptivity of low-lying (non-resonantly enhanced) allowed or forbidden states.¹⁰

$$\delta_{\max}^{s<-0} = \frac{4\pi^4 e^4 \tilde{\nu}_\lambda^2}{15c^2 h^2} g_{\max} \left\{ \frac{(a+b) [\langle i|\mathbf{r}|o\rangle \cdot \langle s|\mathbf{r}|i\rangle]^2}{(\tilde{\nu}_{io} - \tilde{\nu}_\lambda)^2} + \frac{b |\langle i|\mathbf{r}|o\rangle|^2 |\langle s|\mathbf{r}|i\rangle|^2}{(\tilde{\nu}_{io} - \tilde{\nu}_\lambda)^2} \right\} \quad (8a)$$

$$+ \frac{8\pi^4 e^3 \tilde{\nu}_\lambda^2}{15c^2 h^2} g_{\max} \left\{ \frac{(a+2b) |\langle i|\mathbf{r}|o\rangle| |\langle s|\mathbf{r}|i\rangle| |\langle s|\mathbf{r}|o\rangle| |\Delta\mu_{so}| \cos(\phi_{ave})^2}{(\tilde{\nu}_{io} - \tilde{\nu}_\lambda)^2} \right\} \quad (8b)$$

$$+ \frac{4\pi^4 e^2}{15c^2 h^2} g_{\max} \{ (a+b) (\Delta\mu_{so} \cdot \langle s|\mathbf{r}|o\rangle)^2 + b \Delta\mu_{so}^2 |\langle s|\mathbf{r}|o\rangle|^2 \}, \quad (8c)$$

where

$$\Delta\mu_{so} = \mu_s - \mu_o. \quad (9)$$

and a and b are photon polarization and propagation variables (see Refs. 20 & 21) and ϕ_{ave} is the RMS average of the following ten transition/dipole vector angles with the specified weightings: $\langle \langle i|\mathbf{r}|o\rangle \langle s|\mathbf{r}|i\rangle \rangle$, $\langle \langle s|\mathbf{r}|o\rangle \Delta\mu_{so} \rangle$, $2\langle \langle i|\mathbf{r}|o\rangle \Delta\mu_{so} \rangle$, $2\langle \langle s|\mathbf{r}|o\rangle \langle s|\mathbf{r}|i\rangle \rangle$, $2\langle \langle s|\mathbf{r}|o\rangle \langle i|\mathbf{r}|o\rangle \rangle$, $2\langle \langle s|\mathbf{r}|i\rangle \Delta\mu_{so} \rangle$. The cross term in Eq. 8b has been simplified and is rigorous only when $\phi_{ave} = 0$. We can informally describe Eq. 8 as dividing the two-photon absorption process into three contributions, an electronic term (8a), a dipolar (or charge transfer) term (8c) and a cross term (8b). The above equation has been shown to work successfully in predicting the absorptivity of the low-lying ${}^1A_g^*$ and ${}^1A_g^{*-}$ -like states in polyenes (where Eq. 8a dominates) and the low-lying ${}^1B_u^{*+}$ states in polar polyenes (where Eq. 8c dominates).^{15,22-28}

2.2 Relationship between two-photon process and the third-order polarizability.

There are some obvious similarities between the two-photon and third-order polarizability perturbation expansion. For example, we can write the two-photon tensor in a form which emphasizes the similarities:

$$\delta_s \propto g_s(\omega_\lambda) \sum_{j=0}^N \left\{ \frac{\omega_\lambda \omega_\lambda \langle j|\mathbf{r}|o\rangle \langle j|\mathbf{r}|o\rangle \langle s|\mathbf{r}|j\rangle \langle s|\mathbf{r}|j\rangle}{(\omega_j - \omega_\lambda) (\omega_j - \omega_\lambda)} \right\}$$

This result shows that both the two-photon and third-order polarizability tensors have numerators with products of four transition lengths and denominators with products of the energy differences between excited states and the radiation field. Less obvious is the fact that for molecules with inversion symmetry, or near inversion symmetry, couplings between the low-lying strongly allowed excited state and forbidden state(s) dominate the tensors. For example, Pierce has shown that in linear polyenes, a simple three state approximation yields a good estimate for $\gamma_\pi(0;0,0,0)$:

$$\gamma_\pi(0;0,0,0) \approx \left[\frac{6 e^4 (2\pi)^3}{5} \right] \frac{|\langle B|\mathbf{r}|0\rangle|^2}{E_B^2} \left[\frac{|\langle B|\mathbf{r}|nA\rangle|^2}{E_{nA}} - \frac{|\langle B|\mathbf{r}|0\rangle|^2}{E_B} \right] \quad (10a)$$

where B represents the low-lying 1B_u one-photon allowed state and nA represents a higher excited 1A_g excited state which couples strongly via a transition length to the B state. If this equation is compared with the three state approximation for the two-photon absorptivity (Eq. 8), we observe that similar transition lengths contribute to both $\gamma_\pi(0;0,0,0)$ and δ_s , provided s is properly chosen. If we drop terms 8b and 8c (which are zero for linear polyenes), assume linearly polarized light ($a = b = 8$) of the correct frequency to access the final state, and assume the transition lengths are similarly polarized Eq. 8 reduces to:

$$\delta_{\max}^{nA \leftarrow 0} \cong \frac{24\pi^4 e^4 E_{nA}^2}{15c^2 h^2} g_{\max} \left\{ \frac{|\langle B | \mathbf{r} | o \rangle|^2 |\langle B | \mathbf{r} | nA \rangle|^2}{(E_B - \frac{1}{2} E_{nA})^2} \right\} \quad (10b)$$

It can be seen from the above example that the same transition lengths and energies that are important in defining the third-order polarizability are also important in defining the two-photon absorptivity of the nA state. The cross-terms that remain, and the differences in the energy denominators, preclude the derivation of a simple relationship. A more global analysis yields the following set of equations [Birge *et al.* (to be published)]:

$$\gamma_\pi\{\delta\} = \xi_g + \xi_u + \xi_{gu} + \xi_{ug} \quad (11)$$

$$\xi_g = \frac{5 c^2 h^2 \delta_{g \leftarrow 0}}{8 \pi^4 E_g^3 g_{\max}} \mathcal{E}_{ii}[E_g, E_\lambda] \quad (12)$$

$$\xi_u = \frac{5 c^2 h^2 \delta_{u \leftarrow 0}}{8 \pi^4 E_u^3 g_{\max}} \mathcal{E}_{ii}[E_u, E_\lambda] \quad (13)$$

$$\xi_{gu} = \frac{3 e^4 h^2 f_g |\langle g | \mathbf{r} | u \rangle|^2}{2 m_e \pi^2 E_g^3 E_u} \mathcal{E}_{ij}[E_g, E_u, E_\lambda] \quad (14)$$

$$\xi_{ug} = \frac{3 e^4 h^2 f_u |\langle g | \mathbf{r} | u \rangle|^2}{2 m_e \pi^2 E_u^3 E_g} \mathcal{E}_{ij}[E_u, E_g, E_\lambda] \quad (15)$$

where $\delta_{g \leftarrow 0}$ is the two-photon absorptivity of the low-lying gerade (or gerade-like) (g) excited state, E_g is the Franck-Condon excitation energy of the g excited state, g_{\max} is defined in Eq. 7, $\delta_{u \leftarrow 0}$ is the two-photon absorptivity of the low-lying ungerade (or ungerade-like) (u) excited state, E_u is the Franck-Condon excitation energy of the u excited state, f_g is the oscillator strength of the g excited state, $|\langle u | \mathbf{r} | g \rangle| = |\langle g | \mathbf{r} | u \rangle|$ is the absolute values of the transition length vector coupling the u and g states, f_u is the oscillator strength of the u excited state, and the dimensionless energy terms are given by:

$$\mathcal{E}_{ii}[E_i, E_\lambda] = \frac{E_i^6 + E_i^4 E_\lambda^2}{E_i^6 - 14 E_i^4 E_\lambda^2 + 49 E_i^2 E_\lambda^4 - 36 E_\lambda^6} \quad (16)$$

$$\mathcal{E}_{ij}[E_i, E_j, E_\lambda] = \frac{E_i^2 E_j (3 E_j E_\lambda - E_j^2 E_j - 4 E_i E_\lambda^2)}{(E_\lambda - E_i) (E_\lambda + E_i) (2E_\lambda - E_j) (2E_\lambda + E_j) (3E_\lambda - E_i) (3E_\lambda + E_i)} \quad (17)$$

The above equations assume that the g state is the lowest excited state with gerade (or gerade-like) symmetry and that the u state is the lowest excited state with ungerade (or ungerade-like) symmetry. Note

that for a molecule with inversion symmetry, $\delta_{u \leftarrow 0} = 0$ and $f_g = 0$, and therefore ξ_{gu} and ξ_u vanish and only Eqs. 12 and 14 contribute to $\gamma_\pi\{\delta\}$. It is important to note that the above equations were derived assuming that the lowest gerade excited state, and not the higher "nA" gerade state referenced in Eq. 10, is the "g state". It is essential to use the lowest gerade excited state to avoid resonance effects that are unpredictable. This formalistic requirement does not preclude higher gerade states from making significant contributions to γ . Indeed, Pierce has demonstrated that coupling between a high energy ${}^1A_g^*$ excited state and the strongly allowed, low-lying ${}^1B_u^{*+}$ state is the dominant contribution to γ in linear polyenes. Nevertheless, as we demonstrate below, the above two-photon equations accurately predict the full sum-over-states value despite the fact that contributions from higher A_g states are not explicitly included.

In order to test the validity of the above relationships, we will compare the third-order π -electron polarizability $\gamma_\pi(0;0,0,0)$ calculated by using the sum-over-states treatment embodied in Eq. 3 and the value calculated by using Eqs. 11 - 17. The INDO-SDCI molecular orbital investigation by Pierce provides most of the necessary theoretical data for an analysis of three polyenes (butadiene, hexatriene and octatetraene) in both trans and cis forms as well as benzene.⁷ We need only calculate values for ξ_g and ξ_{ug} because ξ_u and ξ_{gu} vanish due to symmetry. The only parameter that is not provided is the transition length $\langle g|r|u \rangle$ between the ${}^1A_g^*$ and ${}^1B_u^{*+}$ states. Our calculations based on the use of the identical INDO formalism indicate that $\langle {}^1A_g^*|r|{}^1B_u^{*+} \rangle$ falls between 0.4 and 0.7 Å for the molecules studied here. Our calculations assume $\langle g|r|u \rangle = 0.5\text{Å}$. (The modest contribution of the ξ_{gu} term relegates this assignment to one of minor consequence for the longer chain polyenes.) The results are shown in Fig. 1.

As can be seen from an analysis of Fig. 1, our two-photon based relationships provide an excellent approximation to the full sum-over-states treatment. The above results should not be used to support replacing the sum-over-states calculation embodied in Eq. 3 in favor of calculating the two-photon absorptivity (Eq. 5) and then using Eqs. 11-17 to calculate γ_p . Equation 3 will invariably provide a more accurate result. Nevertheless, there are many instances when the experimental measurement of the two-photon absorptivity is more convenient than the measurement of the third-order polarizability. Our evaluation of the second order polarizability of bacteriorhodopsin is one such example.

3. RESULTS AND DISCUSSION

The two-photon double-resonance spectrum of light adapted bacteriorhodopsin in D_2O at room temperature is shown in Fig. 2.¹⁰ This spectrum is unique relative to other two-photon spectra measured for the visual chromophores and pigments^{19,25-30} in that it exhibits two low-lying band maxima. The lowest energy band maximum at 560nm ($\delta = 290$ GM) corresponds within experimental error with the one-photon absorption maximum at 568nm, and is assigned to the ${}^1B_u^{*+} \leftarrow S_0$ transition. The higher energy two-photon band at ~488nm ($\delta = 120$ GM) is assigned to the ${}^1A_g^{*-} \leftarrow S_0$ transition. A full discussion of the two-photon properties of bacteriorhodopsin can be found in Ref. 10.

We are interested in calculating both $\gamma(0;0,0,0)$ and $\gamma(-3\omega;\omega,\omega,\omega)$ for bacteriorhodopsin. Although Eqs. 11 - 17 can be used to calculate both terms, it is important to include an energy damping factor in the denominators of the energy terms (Eqs. 16 and 17) to prevent resonances from generating unrealistic enhancements. The approximate approach that we adopt is to replace all occurrences of the term $(nE_\lambda - E_j)$, where $n=1,2$ or 3, with the term $[\Gamma^2 + (nE_\lambda - E_j)^2]^{1/2}$. This change is obvious for Eq. 17, but expansion of the denominator of Eq. 16 into factors is required to introduce damping, which yields the following result:

$$\mathcal{E}_{ii}[E_i, E_\lambda, \Gamma] = \frac{E_i^6 + E_i^4 E_\lambda^2}{[(E_i - 3E_\lambda)^2 + \Gamma^2]^2 [(E_i - 2E_\lambda)^2 + \Gamma^2]^2 [(E_i - E_\lambda)^2 + \Gamma^2]^2 (E_i + E_\lambda) (E_i + 2E_\lambda) (E_i + 3E_\lambda)} \quad (18)$$

where Γ is the damping factor. To a first approximation, Γ is equal to the homogeneous linewidth, but larger values should normally be used to reflect the vibronic distribution of the excited state absorption band. We will explore this issue below.

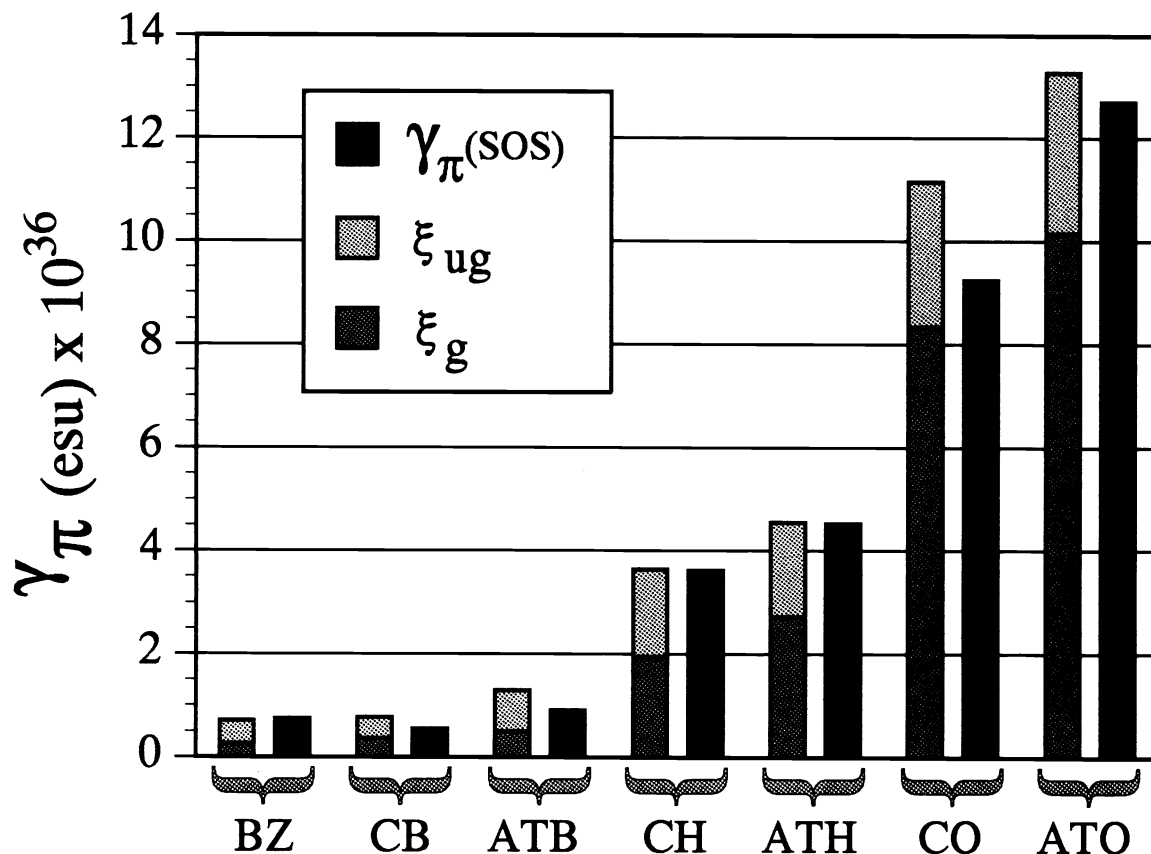


Figure 1. Comparison of third-order π -electron polarizabilities at $\omega=0$ [$\gamma_{\pi}(0;0,0,0)$] calculated by using two-photon based equations (Eqs. 11-15) versus those calculated by using the full sum-over-states (Eq. 3). The molecules from left to right are as follows: BZ=benzene, CB=s-cis butadiene, ATB=all-trans butadiene, CH=3-cis hexatriene, ATH=all-trans hexatriene, CO=3,5-dicis octatetraene, ATO=all-trans octatetraene. The vertical axis is in units of 10^{-36} esu. The height of the vertical bar to the left of each molecule code is equal to $\gamma_{\pi}(\delta)$ [Eq. 10] and is divided into the ξ_g (bottom section; based on Eq. 12) and ξ_{ug} (top section; based on Eq. 15). The height of the dark vertical bar to the right of each molecule code is equal to $\gamma_{\pi}(\text{SOS})$ taken from the full sum-over-states results reported by Pierce.⁷ The transition length $\langle g|r|u \rangle$ is assumed to be 0.5\AA . Remaining parameters required for the $\gamma_{\pi}(\delta)$ calculations (i.e. g_{max} , E_g , δ_g , E_u , f_u) were taken from Ref. 7.

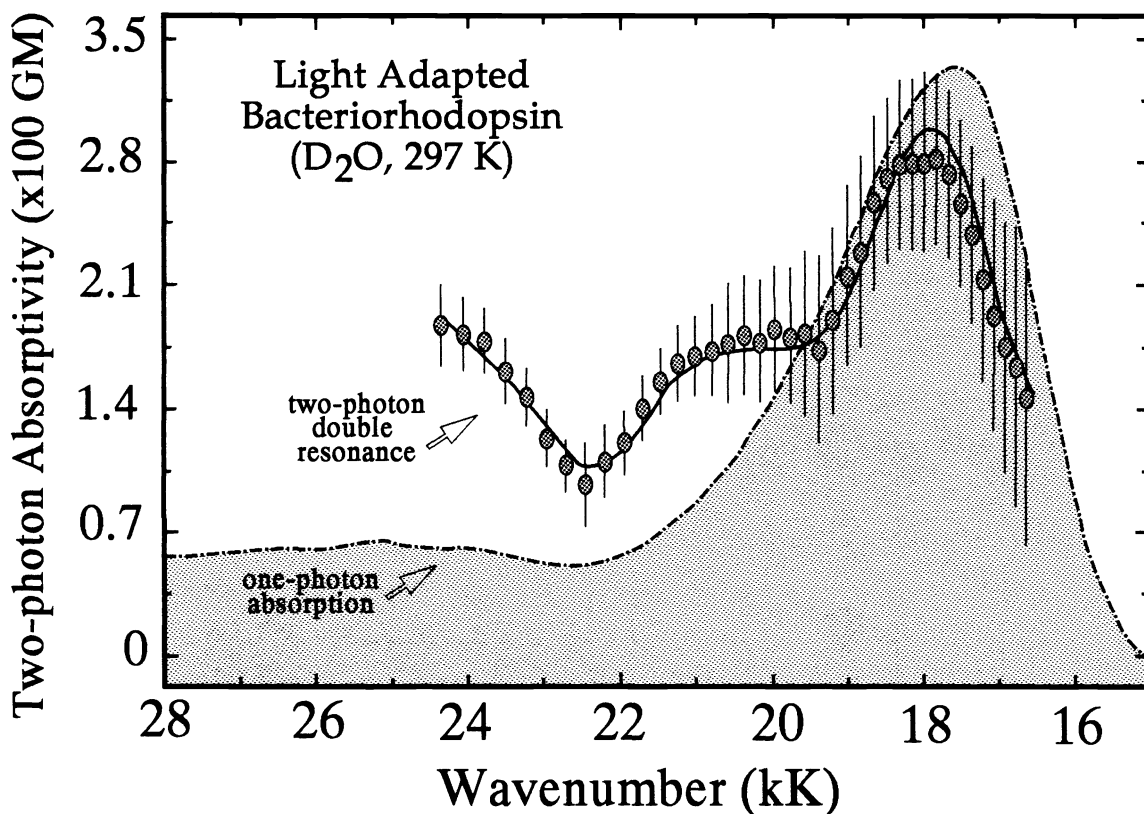


Figure 2. Comparison of the two-photon double resonance spectrum with the one-photon absorption spectrum of light-adapted bacteriorhodopsin in D_2O at ambient temperature. The height of the vertical error bar on each of the two-photon data points is equal to the standard deviation of the two-photon signal determined via least-squares regression of the double-resonance signal as a function of laser pulse energy. The solid line through the two-photon data was calculated by applying a triangular slit function with a full-width at half-maximum of 1000 cm^{-1} . Data from Ref. 10.

The chromophore in bacteriorhodopsin is protonated and ξ_u and ξ_{gu} no longer vanish by symmetry as was the case for the molecules investigated in section 2. Thus, we must assign values for all the terms that appear in Eqs. 12 - 15. We assign the g state to the ${}^1A_g^{*-}$ state and the u state to the ${}^1B_u^{*+}$ state, both of which generate two-photon resonances (see Fig. 2). The following assignments are based on the experimental data from Ref. 29: $\delta_{g\leftarrow 0} = 120\text{ GM}$, $\delta_{u\leftarrow 0} = 290\text{ GM}$, (where $1\text{ GM} = 10^{-50}\text{ cm}^4\text{ sec/molecule-photon}$), $f_g = 0.3$, $f_u = 0.8$, $E_g = 2.62\text{ eV}$, $E_u = 2.17\text{ eV}$, and $g_{\text{max}} = 8.83 \times 10^{-15}\text{ s}$. A value for $|\langle g|\hat{r}|u\rangle|$ of 0.6 \AA is assumed based on arguments presented in Ref. 29. The results for a selected set of photon energies based on $\Gamma = 0.25\text{ eV}$ are given in Table I.

Examination of Table I indicates that $\gamma_\pi\{\delta\}$ is dominated by ξ_u with important contributions from ξ_g . The cross-terms ξ_{gu} and ξ_{ug} combined contribute less than 5% to the total calculated susceptibility. Thus, our assignment of $\langle g|\hat{r}|u\rangle$ is not critical to evaluation of $\gamma_\pi\{\delta\}$, which is fortunate because this transition length is the only parameter which could not be assigned experimentally from the one- and two-photon spectra shown in Fig. 2.

In order to more accurately assess the affect of the damping factor, the dispersion of $\gamma_\pi\{\delta\}$ as a function of photon energy is presented in Fig. 3 for two values of Γ (0.05 and 0.25 eV).

Table I. Third order polarizability of light-adapted bacteriorhodopsin as a function of the energy of the incident polarizing radiation.^(a)

$E_\lambda(\text{eV})^{(b)}$	$\xi_g^{(c)}$	$\xi_u^{(d)}$	$\xi_{gu}^{(e)}$	$\xi_{ug}^{(f)}$	$\gamma_\pi\{\delta\}^{(g)}$
0.00	461 \pm 173	1940 \pm 167	16.6 \pm 2.7	64.4 \pm 10.4	2482 \pm 327
0.25	527 \pm 198	2354 \pm 203	19.5 \pm 3.2	76.2 \pm 12.4	2976 \pm 385
0.5	840 \pm 315	4849 \pm 418	33.5 \pm 5.4	144.5 \pm 23.4	5867 \pm 704
0.66	1511 \pm 567	12937 \pm 1115	66.3 \pm 10.8	349.1 \pm 56.6	14863 \pm 1614
1.00	3677 \pm 1379	11691 \pm 1008	246.2 \pm 39.9	203.4 \pm 33.0	15817 \pm 2314
1.17	3082 \pm 1156	7335 \pm 632	118.1 \pm 19.1	220.7 \pm 35.8	10755 \pm 1733

- (a) Calculations assume $\Gamma = 0.25$ eV and are based on the following experimental data from Ref. 29: $\delta_{g \leftarrow 0} = 120$ GM, $\delta_{u \leftarrow 0} = 290$ GM, (where $1\text{GM} = 10^{-50}$ cm⁴ sec/ molecule-photon), $|\langle g|r|u \rangle| = 0.6$ Å, $f_g = 0.3$, $f_u = 0.8$, $E_g = 2.62$ eV, $E_u = 2.17$ eV, $g_{\text{max}} = 8.83 \times 10^{-15}$ s, where the g state is the "¹A_g^{*}" state and the u state is the "¹B_u^{*}" state. The error ranges given are based on the error ranges assigned to the above parameters in Ref. 29.
- (b) Energy of the incident radiation field in electron volts.
- (c) Component of the third-order polarizability based on Eq. 12 (units of 10^{-36} esu).
- (d) Component of the third-order polarizability based on Eq. 13 (units of 10^{-36} esu).
- (e) Component of the third-order polarizability based on Eq. 14 (units of 10^{-36} esu).
- (f) Component of the third-order polarizability based on Eq. 15 (units of 10^{-36} esu).
- (g) Total third-order polarizability based on Eq. 11 (units of 10^{-36} esu).

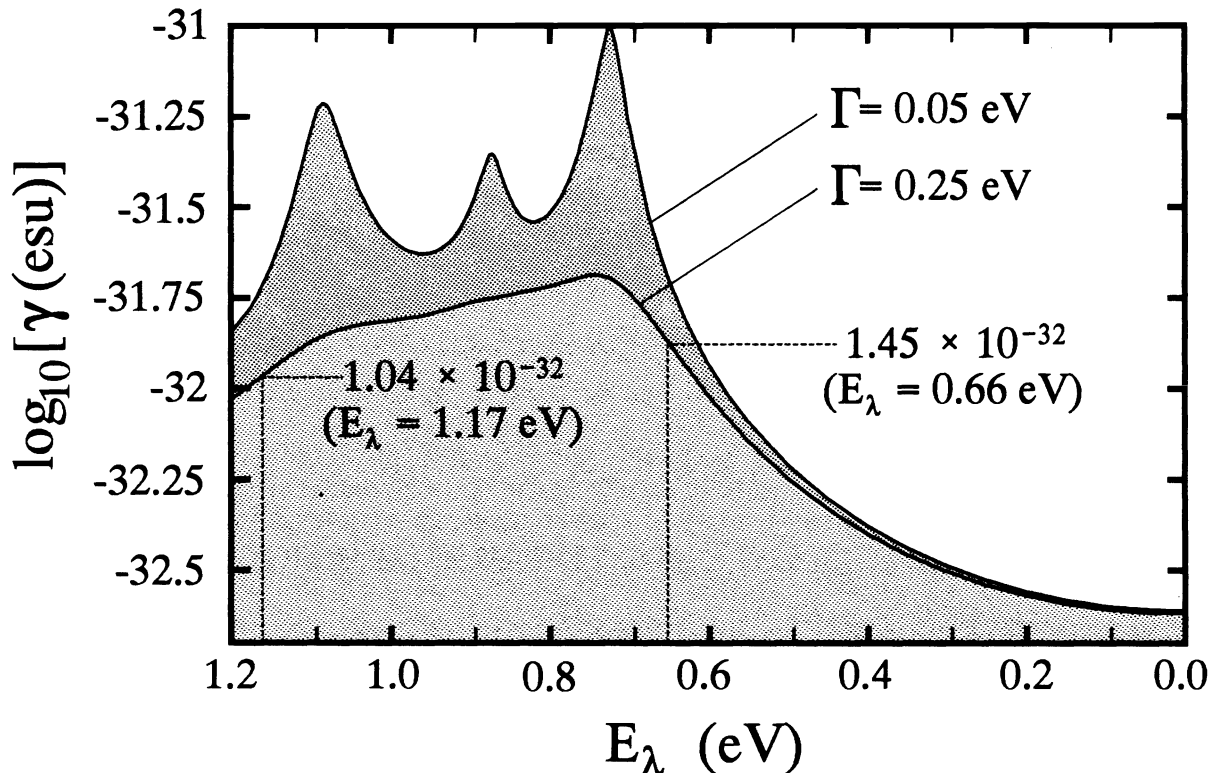


Figure 3. Dispersion in the third-order π -electron polarizability of bacteriorhodopsin as a function of two values of the damping function [$\Gamma = 0.25$ eV, foreground (lower) curve; $\Gamma = 0.05$ eV, background (upper) curve]. The vertical axis displays $\log_{10}[\gamma_\pi(\delta)]$ based on Eq. 10 (see text).

As can be seen from the dispersion curve calculated for $\Gamma=0.05$ eV in Fig. 3 there are three resonances that occur in γ_π in the 0 - 1.2 eV region. The most intense resonance occurs at $E_\lambda = 0.723$ eV due to the $E_i - 3 E_\lambda$ term in \mathcal{E}_{ij} (Eq. 18), where E_i is the transition energy of the ${}^1B_u^{*+}$ state absorption maximum ($E_u = 2.17$ eV). The second resonance at $E_\lambda = 0.873$ eV is associated with the same $E_i - 3 E_\lambda$ term but where E_i is the transition energy of the ${}^1A_g^{*-}$ state absorption maximum ($E_g = 2.62$ eV). The third resonance at $E_\lambda = 1.085$ eV is associated with the $E_i - 2 E_\lambda$ where E_i is the transition energy of the ${}^1B_u^{*+}$ state absorption maximum ($E_u = 2.17$ eV). These same resonances also occur in the \mathcal{E}_{ij} function (Eq. 17), but have a smaller impact on the dispersion curve due to the modest contributions from the cross-terms. The use of a non-zero Γ value is essential to prevent these resonances from generating infinite (and obviously unrealistic) calculated values for γ_π . We consider the values generated for $\Gamma=0.25$ eV (Table I and Fig. 3) more realistic than those generated for $\Gamma=0.05$ eV.

In evaluating the third-order properties of bacteriorhodopsin, it is instructive to compare the γ_π values for bacteriorhodopsin with those measured by Hermann and Ducuing for a series of polyenes by using third-harmonic generation.⁴ The comparisons are shown in Table II and suggest that bacteriorhodopsin has a surprisingly large third-order polarizability given the fact that the chromophore has only six double bonds. In fact, it has a γ_π value that is comparable to that measured for dodecapreno β -carotene, a polyene with eleven double bonds (see Table II).

Table II. Third order polarizabilities of selected molecules.

molecule	# dbl bonds	conditions ^(a)	$\gamma_\pi[0.50 \text{ eV}]^{(b)}$	$\gamma_\pi[0.66 \text{ eV}]^{(b)}$	Source ^(c)
allo-ocime	3	pure		7 \pm 2	H&D
all-trans retinol	5	molten		46 \pm 12	H&D
all-trans retinol	5	EPA(77K)	42 \pm 7	55 \pm 9	TPA
all-trans retinal	6	molten		90 \pm 20	H&D
all-trans retinal	6	DMSO		130 \pm 40	H&D
all-trans retinal	6	EPA(77K)	180 \pm 21	274 \pm 64	TPA
bacteriorhodopsin	6	D ₂ O	5867 \pm 704	14863 \pm 1614	TPA
trans β -carotene	11	benzene	1100 \pm 500	1400 \pm 700	H&D
cis-trans bixine	11	DMSO		300 \pm 80	H&D
dodecapreno β -carotene	19	benzene	\sim 4000	17000 \pm 6000	H&D

(a) Conditions list the solvent (assumed ambient temperature unless specified otherwise) or the state of the pure material.

(b) Third-order π -electron polarizability (units of 10^{-36} esu) at E_λ given in brackets.

(c) H&D = Hermann and Ducuing measurements based on third harmonic generation⁴; TPA = two-photon measurements based on Eqs. 11 - 15 (two-photon data for all-trans retinol from Ref. 29, two-photon data for all-trans retinal from Ref. 19, two-photon data for bacteriorhodopsin from Ref. 10).

Garito and coworkers have examined theoretically the electronic contributions to the third-order polarizability of polyenes and note that the principal contributions to γ_π can be divided into the three types shown in Fig. 4.⁵ Type I is associated with the allowedness of the low-lying ungerade (or ungerade-like) excited singlet state. If we label this state B, the Type I contribution is proportional to $|\langle B | \mathbf{r} | o \rangle|^4$. Type II is associated with the sequence $S_0 \rightarrow B \rightarrow nA \rightarrow B \rightarrow S_0$, and is assigned by Pierce⁷ to be the dominant contributor to γ_π in linear polyenes (see discussion above). The Type II contribution is proportional to $|\langle B | \mathbf{r} | o \rangle|^2 |\langle B | \mathbf{r} | nA \rangle|^2$. Type III is only relevant to polar molecules and is associated with the product $\langle o | \mathbf{r} | B \rangle \langle B | \Delta \mathbf{r} | B \rangle \langle B | \Delta \mathbf{r} | B \rangle \langle B | \mathbf{r} | o \rangle$, where $\langle B | \Delta \mathbf{r} | B \rangle = \Delta \mu_B / e$ ($\Delta \mu_B$ = the change in dipole moment upon excitation into the B state). Garito and coworkers have noted that this term typically contributes

more than one-order of magnitude to γ_π in polar long-chain polyenes when $\Delta\mu_B \geq 10$ D. Unfortunately, our two-photon equations do not equate directly with the three types illustrated in Fig. 4, but approximate relationships are defined in the caption to Fig. 4. The protein-bound chromophore in bacteriorhodopsin exhibits a large change in dipole moment upon excitation into the lowest-lying, strongly-allowed ${}^1B_u^{*+}$ -like state ($\Delta\mu = 13.5$ D).¹⁰ Based on the results shown in Table I, we conclude that Type III enhancement of the third-order polarizability yields at least a 20-fold increase in γ_π .

More subtle is the enhancement associated with the protonation of the chromophore in bacteriorhodopsin. De Melo and Silbey have demonstrated theoretically that polarons and bipolarons have enhanced third-order polarizabilities.⁶ While protonated and "polaronic" chromophores are not electronically identical, the effect of protonation is similar with respect to bond alternation effects and excited state manifold perturbation. Based on simulations, we conclude that protonation yields approximately a five-fold enhancement in the third-order polarizability.

In summary, we attribute the enhanced third-order nonlinearity associated with the protein bound chromophore of bacteriorhodopsin to two mutually enhancing origins. First, the chromophore is protonated, and the resultant charge reorganization enhances the polarizability in a fashion that is similar to that known to occur for polaronic and bipolaronic chromophores. We estimate protonation generates a five-fold enhancement in γ_π . Second, the protein bound chromophore exhibits a large change in dipole moment upon excitation into the lowest-lying, strongly-allowed ${}^1B_u^{*+}$ -like state ($\Delta\mu = 13.5$ D). The latter property is responsible for a Type III enhancement of the third-order polarizability and yields at least a 20-fold increase in γ_π .

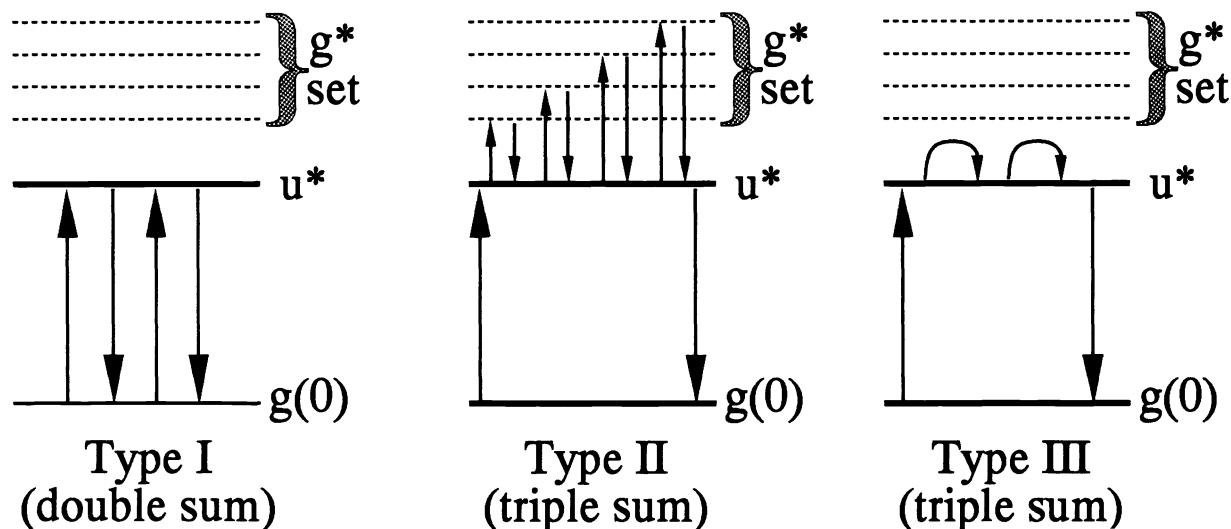


Figure 4. Schematic diagrams illustrating the major contributions to the π -electron third-order polarizability for non-polar chromophores (Type I & Type II only) as well as polar chromophores (Types I, II & III) following the definitions proposed in Ref. 5. The ground state is labelled " $g(0)$ ", and the low-lying strongly-allowed excited state of ungerade or ungerade-like symmetry is labelled " u^* ". The manifold of excited gerade or gerade-like excited states are labelled " g^* set". The " u^* " state is not necessarily the lowest-lying excited singlet state, but is shown in that position for clarity. The Type I and Type II contributions are embodied in the ξ_g and ξ_{ug} terms, but there is no direct correspondence. The Type III contribution is embodied in the ξ_u term and will usually dominate all other contributions to the third-order polarizability in polar chromophores with extended conjugated systems.

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