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Mechanism of the Chiral SHG Activity of Bacteriorhodopsin Films

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Second order nonlinear optical measurements have been shown to be remarkably sensitive to chirality.[1−4] In recent years, three primary models have emerged for interpreting chiral effects in second harmonic generation (SHG) measurements of oriented assemblies.[1] Magnetic dipole interactions and/or interference between electric and magnetic dipoles have been implicated as contributing to the chiral response in a manner qualitatively similar to linear chiroptical activity.[2] Additionally, intrinsic chirality within the local chromophore has also been shown to arise within the electric-dipole approximation, often through at least one pair of coupled oscillators.[3] More recently, macromolecular orientational effects have been suggested as possibly driving many second-order chiral optical responses of uniaxial systems without requiring intrinsic chirality within the chromophore or coupling.[4] In this communication, theoretical and experimental studies were performed on a system with a well-established structure and orientation to quantitatively evaluate the relative contributions from the three most commonly invoked mechanisms for describing the chiral response.

Bacteriorhodopsin (bR), a seven helical transmembrane protein found in the purple membrane (PM) of *Halobacterium salinarum*, provides an excellent model for this objective. The nonlinear optical response is dominated by the all-trans retinal chromophore, which is bound to the lysine 216 residue via a protonated Schiff base (PSB). The PSB retinal chromophore exhibits a large two-photon absorption cross-section and second-order nonlinear polarizabilities when residing inside the binding pocket of bR.[5,6] Persoons and co-workers and El-Sayed and co-workers observed relatively large nonlinear chiral effects in Langmuir−Blodgett (LB) films of bR.[7,8] Furthermore, both the structure of the chromophore and its orientation with respect to the molecular frame (i.e., protein cage) are known to a reasonably high degree of accuracy from previous crystallography measurements.[9,10]

For an individual chromophore near resonance with the second harmonic frequency with state n, the hyperpolarizability tensor $\boldsymbol{\beta}^{(2)}$ has recently been shown to reduce to the direct product of the transition moment and the two-photon absorption (TPA) tensor, $\beta_{ijk} = -S_\omega \cdot \mu^i_{0n}(\alpha^{jk}_{n0})_{\mathrm{TPA}}$, where S_ω is a line shape function.[11,12] A diagrammatic representation of the $\boldsymbol{\beta}^{(2)}$ tensor for retinal is shown in Figure 1 (see Supporting Information for computational details). From this representation, the relative magnitudes and signs of the different elements within the $\boldsymbol{\beta}^{(2)}$ tensor of the retinal chromophore can be determined by simple projection of the principal elements of the TPA tensor $\boldsymbol{\alpha}^{(1)}$ and the transition moment $\boldsymbol{\mu}$ onto any arbitrary coordinate system.[13]

Inspection of Figure 1 suggests that the dominant element within the resonant $\boldsymbol{\beta}^{(2)}$ tensor for the $S_1 \leftarrow S_0$ transition should be β_{zzz} in the coordinate system shown, given by the projections of μ and α_c (i.e., α_{zz}) onto the molecular z-axis. Furthermore, β_{zzz} should be positive in sign (expressed as $\boldsymbol{\mu} \otimes \boldsymbol{\alpha}$), since the projection of μ on the molecular z-axis is negative and α_c, which lies nearly coparallel to the z-axis, is also negative in sign. Similarly, the next largest $\boldsymbol{\beta}^{(2)}$ tensor element is β_{xzz}, which is positive in sign.

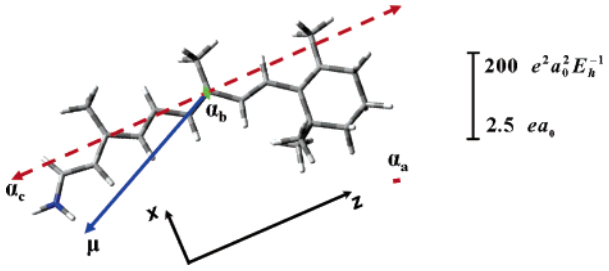

Figure 1. Structure and diagrammatic representation of the corresponding $\boldsymbol{\beta}^{(2)}$ tensor for the PSB retinal chromophore. Atom notation is as follows: H, white; C, gray; and N, blue. Double sided arrows represent the principal elements of the TPA tensor (i.e., α_c, α_b, α_a) (red indicates negative sign and green positive sign) and the blue single-sided arrow represents the electric transition moment $\boldsymbol{\mu}$. α_a is the out-of-plane TPA contribution.

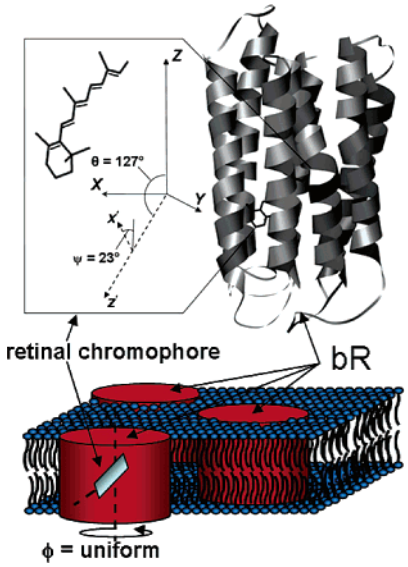

Figure 2. The coordinate systems of the PSB retinal chromophore with respect to the protein cage. The Euler angles θ and ψ describes the tilt and twist angle of the PSB retinal chromophore within the protein cage.

Since the crystal structure of bR is known,[9,10] the orientational averages connecting the retinal frame to the protein frame are also known with a high degree of accuracy. Furthermore, the orientation of the integral membrane protein with respect to the laboratory frame can be reasonably expected to be fairly narrow in highly ordered films, such that the chiral and achiral $\boldsymbol{\chi}^{(2)}$ tensor elements can be predicted with no adjustable parameters from the calculated $\boldsymbol{\beta}^{(2)}$ tensor (See Figure 2).

Table 1 contains a comparison of the predicted SHG optical rotary dispersion (ORD) angles using the crystal structure for PSB retinal (PDB ID 1c3w) and the experimentally measured values obtained in reflection, transmission, and total internal reflection. The SHG-ORD measurements for reflection at multiple angles of incidence were performed previously by El-Sayed and co-workers.[8]

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Table 1. Experimental and Predicted SHG-ORD Angles for bR LB Films

	angle of incidence	measured	predicted[c]
reflection[a]	13°	−4.5°	−6.7°
	25°	−6.8°	−8.4°
	28°	−8.7°	−9.3°
TIR[b]	73°	−4.3°	−11°
transmission[b]	45°	3.7°	3.1°

[a] Reflection measurements reproduced from ref 8. [b] Present work. [c] Protein Databank ID 1c3w. Predicted values were based solely on electric dipole interactions and for a uniaxially oriented film.

Complementary measurements performed in transmission and total internal reflection geometries were acquired in the present study using similar experimental protocols (see Supporting Information). The predicted SHG-ORD angles were obtained using the calculated $\boldsymbol{\beta}^{(2)}$ tensor elements and assuming asymmetry in twist angle ψ .

The predicted and measured SHG-ORD angles are in remarkably good agreement, both qualitatively and quantitatively. Specifically, the calculations correctly recover both the signs and magnitudes of the SHG-ORD angles measured in all three experimental configurations. Furthermore, the trend of increasing SHG-ORD angle with increasing angle of incidence in reflection is also recovered.[8]

The accuracy of the predictions allows quantitative assessment of the importance of the three proposed contributions to the macroscopic chiral response (e.g., magnetic dipole, intrinsic chirality, macromolecular orientational chirality). The magnetic dipole contributions were calculated to be ~100-fold smaller in magnitude than the electric dipole terms. These results appear to conflict with previous reports by Persoons and co-workers of significant magnetic dipole contributions.[7] Interestingly, the calculations also do not support a mechanism proposed previously by El-Sayed and co-workers, in which SHG-ORD was interpreted to indicate the presence of twisting of the conjugated polyene chain.[8] In that work, Volkov et al., implicitly assumed a uniform distribution in ψ about the long *z*-axis of the PSB retinal chromophore.[8] Within this limit, the macroscopic electric-dipole allowed chiral response reduces to $\chi_{XYZ} \propto \langle\cos^2\theta\rangle(\beta_{xyz} - \beta_{yxz})$. However, the diagrammatic representations (Figure 1) and quantum chemical calculations suggest that the minor distortions away from planarity have a negligible effect on the intrinsic chirality of the PSB retinal chromophore. Furthermore, intrinsic chirality within the PSB retinal chromophore (i.e., the residual change in the resonant $\boldsymbol{\beta}^{(2)}$ tensor following mirror-plane reflection[13]) was calculated to be only ~0.0005%. Consistent with these trends, the predicted SHG-ORD angles, assuming a uniform distribution in ψ , yielded angles of much less than a degree in magnitude.

Surface chirality can potentially arise from the orientational averages connecting the retinal frame and the laboratory frame. Although the predictions in Table 1 were evaluated including all nonzero elements within the molecular $\boldsymbol{\beta}^{(2)}$ tensor, identical results, to within the precision of the measurements, were obtained by considering just the dominant elements of the molecular tensor generated from inspection of the diagrammatic representation in Figure 1. The orientational averages for the macroscopic chiral $\boldsymbol{\chi}^{(2)}$ tensor elements considering just these dominant in-plane $\boldsymbol{\beta}^{(2)}$ tensor elements are given by the following equation.

$$\chi_{XYZ} \simeq \frac{1}{2} N_s \begin{bmatrix} \langle \sin^2\theta \sin\psi \cos\psi \rangle (\beta_{xzx} - \beta_{zxx}) \\ \langle \sin\theta \cos\theta \sin\psi \rangle (\beta_{xzz} + \beta_{zzx}) \end{bmatrix} \quad (1)$$

Equation 1 clearly indicates that chirality within the chromophore is not a necessary requirement for generating a macroscopic NLO chiral response provided there is asymmetry in the distribution in the twist angle ψ.[4] The chiral response described by eq 1 is loosely analogous to the chirality in a propeller composed of achiral "blades" twisted relative to each other by an angle ψ. Furthermore, no exciton coupling between the nominally achiral PSB retinal chromophores is required in the mechanism described by eq 1. The effect is purely orientational. This chiral mechanism has no simple analogue in linear spectroscopy, in which the molecular absorptivity is described by a vector (within the electric dipole approximation) and is invariant to twist about that vector. From these collective results, it appears that electric dipole allowed orientational chirality plays the dominant role in describing the measured SHG-ORD chiral activity of oriented bR films. It is reasonable to suggest that similar orientational effects may be important in describing the chiral responses of other oriented thin film assemblies composed of nominally planar chromophores.

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Supporting Information Available: Computational methods and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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