# Quantification of Sudden Light-Induced Polarization in Bacteriorhodopsin by Optical Rectification

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Upon population of its excited state, the retinal chromophore in the membrane protein bacteriorhodopsin (bR) undergoes a sudden (less than ∼10 fs) change in dipole moment, $\Delta\mu$, that can be visualized in a direct way by optical rectification of a broadband visible femtosecond light pulse to the infrared but has not been quantified in this way. Here we show that a transparent thick $AgGaS_2$ crystal delivers infrared radiation with the same spectral profile as bR and is a suitable reference for quantifying conversion efficiency. Using this reference, we estimate the projection of $\Delta\mu$ on the membrane normal at 11 D, corresponding to the displacement of a full charge over approximately half the length of the retinal chromophore. This result may help to evaluate models describing the interplay between the initial polarization change and the subsequent isomerization of the retinal.

## Introduction

The order and physiological relevance of the primary photoinduced events in retinal proteins is subject to intense debate. A change in dipole moment, $\Delta\mu$, in the excited state of the retinal chromophore gives rise to strong second-order nonlinear properties, which have been correlated with functional properties of the proteins in early work.[1,2] In the "model" retinal protein bacteriorhodopsin (bR), we have recently succeeded in directly visualizing the ultrafast (∼10 fs or less) charge separation process by interferometrically monitoring the associated low-frequency emission in dry macroscopically ordered films (optical rectification).[3] The retinal all-trans → 13-cis isomerization subsequently takes place in few hundreds of femtoseconds[4−6] and appears associated with a relaxation of $\Delta\mu$.[7−9] The isomerization kinetics and yield were found to be unaltered in dried films with respect to purple membrane suspensions.[10]

The magnitude of $\Delta\mu$ associated with excited-state formation has been previously evaluated using Stark spectroscopy,[11] off-resonance second-harmonic generation,[12] two-photon absorption spectroscopy,[13] and off-resonance quadratic Kerr effect measurements.[14] These techniques are sensitive to the magnitude of the dipole moment change associated with the vertical Franck−Condon transition between the ground and excited state but do not time resolve the dipole moment change. In our resonance coherent emission experiments, the ensemble of charge displacements upon photon absorption following direct absorption of the absorption band is monitored and time-resolved. Figure 1 illustrates the principle of the experiment for the case of bacteriorhodopsin.

In our previous work,[3] however, we were unable to accurately determine the magnitude of $\Delta\mu$, because the relevant nonlinear properties of the used, efficient but highly absorbing, reference material GaAs are not straightforwardly accessible. Here we report the direct quantitative determination of $\Delta\mu$ associated with the excited-state population in bR, using a transparent $AgGaS_2$ crystal as a reference. The results are compared with the previous experiments, and the origin of differences is discussed.

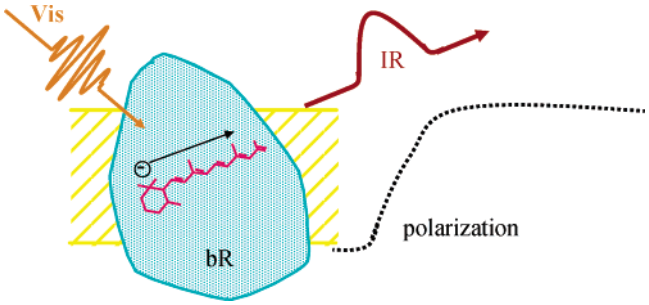


**Figure 1.** Schematic view of principle of coherent emission generation in bacteriorhodopsin. A short visible light pulse is incident on an oriented multilayer of bacteriorhodopsin-containing purple membranes. Upon absorption by retinal, an intramolecular charge displacement yields a fast-rising transmembrane polarization, which gives rise to the emission of a half-cycle infrared pulse. This pulse is subsequently detected (not depicted) and time-resolved by letting it interfere with a reference pulse on an infrared detector.

## Materials and Methods

Bacteriorhodopsin-containing purple membranes were obtained from strain R1 of *Halobacterium salinarum* using standard methods,[15] electrophoretically deposited[16] on germanium plates, and subsequently dried under ∼50% relative humidity.[3]

The experimental apparatus has been described in detail previously.[3,17] Briefly, the output of a noncollinear parametric amplifier (NOPA) centered close to the maximum absorption of bR at 560 nm, and precompensated for minimal pulse duration in the reference beam, was split in two parts. One part

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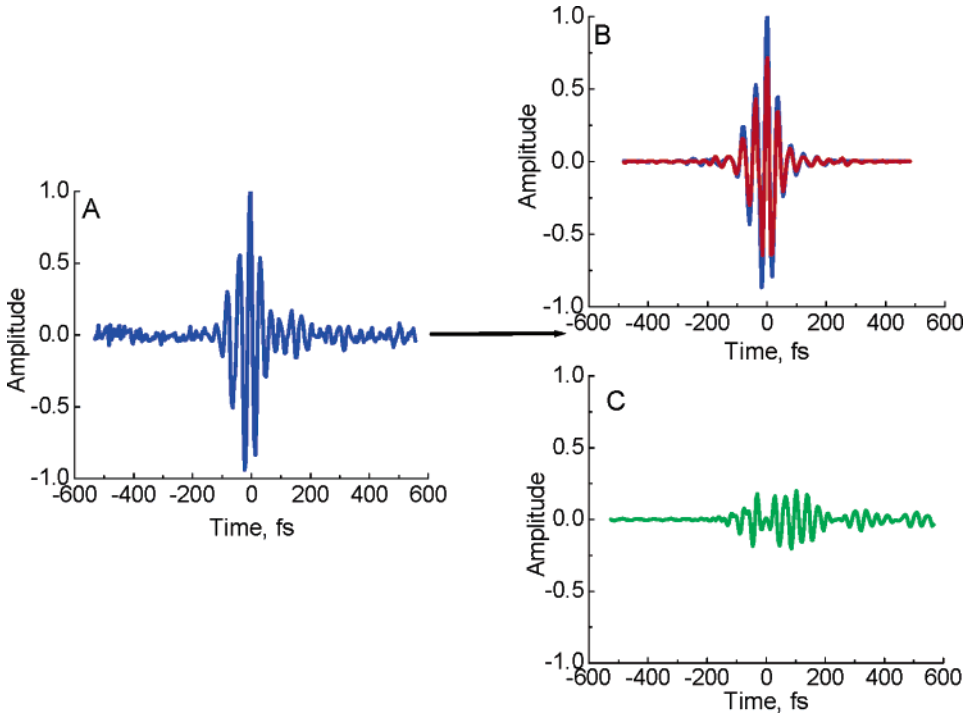


**Figure 2.** Decomposition of the interferogram between $AgGaS_2$ and bR infrared emissions filtered by an $AgGaS_2$ crystal (A) in electronic (B, blue line) and vibrational (C) responses. Red line: AgGaS2/AgGaS2 interferogram, filtered by the bR sample.

(13 fs duration, ~80 nJ, further attenuated in the case of bR) was focused either on the bR sample rotated at ~6 Hz or on a silver thiogallate crystal ($AgGaS_2$, type II, 1 mm thick). Both emit in the infrared through optical rectification.[18] Another fraction of the NOPA output (11 fs duration) is used to create the reference infrared pulse in a second identical $AgGaS_2$ crystal. Both infrared (IR) beams are focused on a HgCdTe detector, and an interferogram is constructed by varying the time delay between the two visible excitation pulses. To symmetrize total absorption of the IR beams, a bR sample is placed in front of the detector when recording the $AgGaS_2/AgGaS_2$ interferogram, and an $AgGaS_2$ crystal, respectively, when recording the $bR/AgGaS_2$ interferogram.

## Results and Discussion

The $bR/AgGaS_2$ interferogram is shown in Figure 2A. Due to the lower conversion efficiency of $AgGaS_2$ with respect to that of GaAs, the signal-to-noise ratio is somewhat poorer than in the previously reported bR/GaAs interferograms.[3] Nevertheless, apart from the instantaneous broadband infrared emission, characteristic of electronic optical rectification, long-lasting modulations at positive time can be distinguished that reflect the infrared-active vibrational response of the retinal/protein system.[3,17] The electronic and vibrational responses were numerically separated as follows (Figure 2, parts B and C). The electronic response was described by a simple two-level model[3] based on Liouville space pathways,[19] i.e., a pulse-limited polarization rise causing an emission well approximated by a "half-cycle". The vibrational motions were described by exponentially damped sinusoidal functions. Taking into account the instrument response (pulse shapes of the visible pulses, detector spectral response), the interferogram was fitted by sums of these responses. With the use of the "half-cycle" ($AgGaS_2$-like, see below) emission profile for the electronic response, no overdamped vibrational modes (with damping times <30 fs) were required to fit the interferogram. We stress that this is not the case when the GaAs emission profile is taken as a reference,

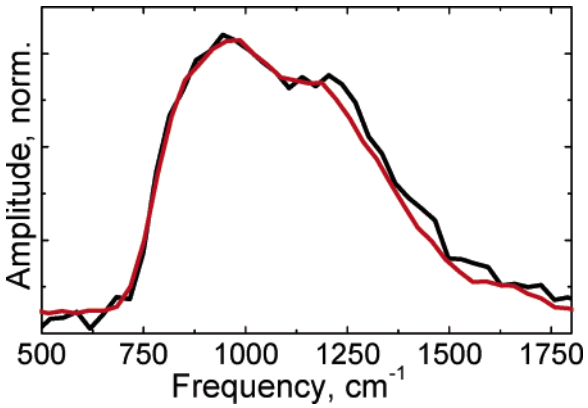


**Figure 3.** Normalized FT spectra of the $bR/AgGaS_2$ electronic interferogram filtered by the second $AgGaS_2$ crystal (black line) and of the $AgGaS_2/AgGaS_2$ interferogram filtered by the bR sample (red line).

presumably because overdamped vibrational modes must be introduced to compensate the noninstantaneous response of this absorbing material.[3] The detailed analysis of the vibrational response will be described elsewhere; in the remainder of the paper we will refer only to the "electronic interferogram" (Figure 2B).

Figure 3 highlights that the Fourier transform spectrum of this interferogram is very similar to that of $AgGaS_2/AgGaS_2$. Our modeling of the propagation of the visible and IR pulses shows that, somewhat surprisingly, for thick, transparent nonlinear materials used under non-phase-matching conditions, such as the $AgGaS_2$ crystals used in this study, the infrared emitted signal is directly proportional to the polarization created (itself proportional to the square of the electric field of the visible exciting beam), i.e., a "half-cycle" following the visible pulse envelope (see the Supporting Information). Therefore, the finding of Figure 3 implies that (a) the bR electronic interferogram is indeed due to "half-cycle" emission and (b) we can directly calibrate the bR emission on the $AgGaS_2$ emission,

although the shape similarity of the two signals is a result of rather different mechanisms. By straightforward comparison of the amplitudes of the interferograms we find a value of 1.4 for the ratio $E_{bR}/E_{AgGaS_2}$ of the amplitudes of the emitted electric fields.

$E_{AgGaS_2}$ can be evaluated (see the Supporting Information) at

$$E_{AgGaS_2} = \frac{(1 + n_\nu)L_{eff}\chi^{(2)}|\underline{E}(t)|^2}{2cT(n_\nu + n_{IR})(1 + n_{IR})} \quad (1)$$

Here, $n_{IR}$ (2.3) and $n_v$ (2.6) are the infrared and visible refraction indexes of $AgGaS_2$, respectively. $L_{eff}$ is the characteristic length of infrared generation, which is essentially determined by the dispersion between the infrared and the visible pulses and evaluated, using the model outlined in the Supporting Information, for visible and IR pulses centered at 560 nm and 10 $\mu$m, respectively, at $L_{eff} = 12$ $\mu$m. $\chi^{(2)}$ is the $AgGaS_2$ nonlinear susceptibility, which equals 13 pm/V,[20] $c$ is the speed of light in air, and $T$ is the pulse length. $\underline{E}$ is the complex visible incident field.

$E_{bR}$ can be expressed according to the second-order nonlinear polarization created in the sample[21]

$$E_{bR} = \frac{\mu_o c}{2n_{IR,bR}} \frac{\partial \underline{P}^{(2)}(z,t)}{\partial t} l_o \quad (2)$$

Here, $\mu_o$ is the magnetic permeability in vacuum, $n_{IR,bR}$ is the bacteriorhodopsin refractive index, and $l_o$ is the effective infrared generation length. Since the lifetime of the excited state of bR ($\sim$500 fs) is much longer than the pulse length, the polarization created in the sample in the case of a two-level system is well approximated by[22]

$$P^{(2)}(t) = \eta N \epsilon_0 \Delta\mu \int_{-\infty}^{t} |\underline{E}(t')|^2 \, dt' \quad (3)$$

where $N$ is the density of dipoles, with a probability $\eta$ of being excited by the incident pulse. In this expression, we suppose the regime incoherent, which is an approximation in our case as the bR absorption spectrum and the visible beam spectrum widths are roughly the same. Hence, taking into account the Fresnel's coefficient transmission contribution in the bacteriorhodopsin layer for the visible beam, we can write

$$E_{bR} = \frac{2\cos\theta_i}{\cos\theta_i + n_{v,bR}\cos\theta_s} \frac{\epsilon_0 \mu_o c}{2n_{IR,bR}} \eta N l_o \Delta\mu |\underline{E}(t')|^2 \quad (4)$$

in which $\theta_i$ is the tilt angle of the bR film (45°) and $\theta_s$ is the transmission angle. The term $\eta N l_o$ is the surface density of excited dipoles. As the bR film is highly absorbing at 560 nm, this quantity equals the surface density of visible photons on the sample.

With the use of eqs 1 and 4, the ratio of $E_{bR}/E_{AgGaS_2}$ directly gives the projection of $\Delta\mu$ on the membrane symmetry axis. We found this value to be 11 D. If one assumes that the dipole moment is along the transition moment of all-trans retinal, at 71° with respect to the membrane normal,[23] we find $|\Delta\mu| \approx 34$ D. This value corresponds to displacement of a full charge of $\sim$7 Å, roughly half the length of the retinal chromophore.

Thus, we have been able to directly determine the change in dipole moment of retinal in a protein upon resonant excitation on the time scale of $\sim$10 fs. Values of $\sim$10−16 D were deduced from early Stark spectroscopic measurements of retinal in solution,[2] and in particular a value of $|\Delta\mu| = 16$ D was reported for all-trans retinal. Recently, quantum-mechanical models have been used to estimate the dipole moment changes along the photoisomerization pathways, and in particular, for isolated 11-*cis*-retinal a sudden polarization of 14 D was calculated.[24] In the bR protein, $|\Delta\mu|$ has been estimated from frequency-doubling experiments;[12] a value of 21 D was deduced using nanosecond nonresonant excitation of purple membrane−PVA films.[12] With the same technique, lower values ($\sim$14 D) were found for Langmuir−Blodgett retinal films,[25] suggesting substantial influence of the retinal environment.[12] However, values of $\sim$13 D for $|\Delta\mu|$ in the bR protein were reported from Stark spectroscopy,[11] two-photon absorption spectroscopy,[13] and off-resonance quadratic Kerr effect measurements.[14]

The value obtained by time-resolving the dipole moment change in the present work is of the same order of magnitude as these previous studies but significantly higher. Different factors can be invoked to explain this difference. These include possible surface effects and local field effects on the emitted field ignored in our work, but these effects are not expected to explain a factor of 2−3 difference. We recall, however, that in our measurements the ensemble of charge displacements within the $\sim$10 fs pulse length contribute to the signal. Thus, near-instantaneous charge movements triggered by retinal photon absorption, but not associated with the Frank−Condon excited state, could lead to effective dielectric enhancement of $|\Delta\mu|$. Such movements need not be along the retinal transition moment axis (as in the case of the $\Delta\mu$ contributions detected by the other techniques). Indeed, in view of the 71° angle of the transmission moment with the membrane normal, any charge displacement in the transmembrane direction would relatively strongly contribute to the signal we detect, $\sim$3-fold more than a charge displacement along the retinal transition moment. We note that these changes should be near instantaneous and much faster than the $\sim$50−70 fs inertial response phase of the protein[7,8] that would be too slow to contribute to the infrared emission signal.

Our result thus indicates that the protein environment may help to enhance the transmembrane component of the dipole moment associated with the sudden polarization. Further work is required to explore this hypothesis, including investigations of the influence of charges in the close environment of the retinal cofactor on the amplitude of the induced polarization.

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**Supporting Information Available:** Simulation of temporal shape of IR emission from a thick transparent dispersive nonlinear material under non-phase-matching conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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