Coherent vibrational climbing in carboxyhemoglobin

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We demonstrate vibrational climbing in the CO stretch of carboxyhemoglobin pumped by midinfrared chirped ultrashort pulses. By use of spectrally resolved pump-probe measurements, we directly observed the induced absorption lines caused by excited vibrational populations up to $v = 6$. In some cases, we also observed stimulated emission, providing direct evidence of vibrational population inversion. This study provides important spectroscopic parameters on the CO stretch in the strong-field regime, such as transition frequencies and dephasing times up to the $v = 6$ to $v = 7$ vibrational transition. We measured equally spaced vibrational transitions, in agreement with the energy levels of a Morse potential up to $v = 6$. It is interesting that the integral of the differential absorption spectra was observed to deviate far from zero, in contrast to what one would expect from a simple one-dimensional Morse model assuming a linear dependence of dipole moment with bond length.

The recent availability of ultrashort and intense midinfrared pulses in a compact setup (1) provides a convenient means for controlling the nuclear motion of molecules (2). With infrared pulses, only vibrational transitions are addressed while the molecule remains in its electronic ground state during the entire interaction. This makes such an approach particularly suited to complex systems such as proteins. Another fascinating possibility opened up by infrared vibrational control is the prospect of exploring the potential energy surface far from the harmonic region up to the transition state of intraprotein reactions.

Coherent vibrational climbing consists of exciting a molecular vibration with an ultrashort midinfrared pulse, the broadband spectrum of which encompasses several vibrational transitions of the molecule. Indeed, these transition frequencies become smaller and smaller while climbing the ladder because of the molecular anharmonicity. Therefore, the climbing efficiency can be increased dramatically when using a negatively chirped pulse so that its high-frequency components, resonant with the lower transitions of the ladder, precede its low-frequency components, resonant with the upper transitions of the ladder. Coherent vibrational climbing can be viewed also as a rapid adiabatic passage leading to efficient excitation of the upper vibrational states, with an efficiency that in theory can be close to 100% because of the coherent nature of the interaction. Vibrational climbing has been demonstrated in small molecules, such as $\text{W}(\text{CO})_6$ (3–5), NO (6), CO adsorbed on a Ru (001) surface (7), $\text{Cr}(\text{CO})_6$ (8), $\text{Mo}(\text{CO})_6$ (5), $\text{Fe}(\text{CO})_5$ (5), and CH_2N_2 (9). In this article, we report on the demonstration of coherent vibrational climbing in a biological system, carboxyhemoglobin (HbCO). This system was chosen rather than carboxymyoglobin, in which different CO-bound configurations result in an inhomogeneous broadening of the absorption line (10).

Experimental Methods

The 800-nm pulses produced by a titanium/sapphire regenerative amplifier (Hurricane, Spectra-Physics) running at a repetition rate of 1 kHz were used to pump an optical parametric amplifier seeded with a continuum produced in a sapphire crystal. The generated signal and idler beams were then mixed in a 1-mm-thick AgGaS_2 crystal to produce tunable midinfrared pulses. These pulses, tuned at a center frequency at $\approx 1,900 \text{ cm}^{-1}$,

had a spectral full width at half-maximum equal to 170 cm^{-1} and a duration of 100 fs. They were stretched by being transmitted through materials of known dispersion, either positive (Ge) or negative (CaF_2) (11). The chirp was characterized by the second-order derivative of the spectral phase in the frequency domain taken at the pulse center frequency ω_0 , i.e., $\varphi'' = (d^2\varphi/d\omega^2)(\omega_0)$. We used values of $\varphi'' = 6,000 \text{ fs}^2$, $-6,000 \text{ fs}^2$, and $-32,000 \text{ fs}^2$, corresponding to pulse durations of 300 fs in the first two cases and 1.4 ps in the third case. The main part of the midinfrared beam was used as the pump beam, whereas a smaller part, reflected off a CaF_2 wedge, was used as the probe beam. To be able to make a quantitative comparison between measurements obtained for different values of the pump chirp, we chose to place the dispersive material before the pump-probe splitting. Indeed, placing the dispersive material after the pump-probe splitting would require a realignment of the pump-probe superposition when the slightly prismatic dispersive material was replaced, rendering a comparison questionable. The drawback of our experimental procedure was that it resulted in chirping the probe pulses, giving a picosecond time resolution instead of the 100 fs expected for transform-limited probe pulses. However, because the vibrational relaxation times were much longer than 1.4 ps, our time-domain measurements did not suffer from this probe duration.

The time delay between the pump and probe pulses was controlled by using a delay line scanned with a $0.1\text{-}\mu\text{m}$ stepping motor. The two beams were focused on a $40\text{-}\mu\text{m}$ -diameter spot by using ZnSe lenses ($f = 50 \text{ mm}$). We used human hemoglobin at a heme concentration of $\approx 20 \text{ mM}$ in a 50 mM Tris-HCl buffer in $^2\text{H}_2\text{O}$ ($\text{pD} = 7.6$). The samples were degassed, reduced with sodium dithionite, exposed to 1 atm ($1 \text{ atm} = 101.3 \text{ kPa}$) of CO for several minutes, and then loaded between 2-mm-thick CaF_2 windows by using a spacer of thickness $L = 50 \mu\text{m}$. The sample was rotated slowly to avoid long-term heating. In all reported measurements, the energy of the pump pulse focused on the sample was equal to $2.2 \mu\text{J}$, whereas the probe-pulse energy was 50 times smaller. The probe pulses transmitted through the sample were spectrally resolved by using a Fourier transform spectrometer based on a Michelson interferometer with an optical path that was continuously scanned and accurately monitored by using a helium-neon laser. The interferometer delay range was $\pm 5 \text{ ps}$, corresponding to a frequency resolution of 3 cm^{-1} . The signal provided by a HgCdTe detector (JD15D22, Judson, Montgomeryville, PA) was digitized for each laser shot and divided by a reference signal provided by an identical HgCdTe detector monitoring part of the probe beam before transmission through the sample, thus reducing the effect of the 1% shot-to-shot fluctuations of the midinfrared pulses. Furthermore, the pump beam was modulated at half of the laser repetition rate by using a synchronized chopper, thus providing efficient detection of the differential pump-probe signal. By averaging the acquisition points in different memory slots depending on the pump state (on or off) and the interferometer delay, we obtained two interferograms corresponding to the

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Fig. 1. Experimental results for different chirp values. (Upper) Midinfrared absorption spectrum of HbCO around the CO vibration frequency. (Lower) Differential spectra $-\Delta\alpha(\omega)L$ obtained by using stretched midinfrared pulses centered at $1,890\text{ cm}^{-1}$ with a chirp value $\varphi'' = 6,000\text{ fs}^2$ (curve a), $-6,000\text{ fs}^2$ (curve b), and $-32,000\text{ fs}^2$ (curve c). The pump-pulse energy is $2.2\text{ }\mu\text{J}$, and the pump-probe time delay is equal to 16 ps . Curves b and c are shifted up for clarity. The noise observed for frequencies of $<1,775\text{ cm}^{-1}$ results from the vanishing probe spectral intensity in this spectral region.

linear autocorrelation of the probe electric field in the presence and in the absence of the pump beam. After Fourier transforming these two interferograms, the differential spectra were obtained by computing $\Delta\ln T(\omega) = \ln(\text{transmission}_{\text{pump on}}/\text{transmission}_{\text{pump off}}) = -\Delta\alpha(\omega)L$, which is therefore of opposite sign with respect to the absorption change $\Delta\alpha(\omega)$. As a consequence, a positive signal means a reduced absorption.

Results and Discussion

Fig. 1 shows the differential absorption spectra obtained for a pump-probe delay of $\tau = 16\text{ ps}$. The positive peak at frequency ω_{10} , associated with the transition $v = 0 \rightarrow v = 1$, results from the decrease in absorption caused by the depletion of the ground state. The negative peaks correspond to induced absorption associated with the transitions $v \rightarrow v + 1$, with $v \geq 1$. The fact that all of these peaks are negative indicates that, in this case, induced absorption overcomes stimulated emission, which means that the vibrational population decays monotonically when v increases. As expected, vibrational climbing is observed to be much more efficient when using a midinfrared pulse of large negative chirp (c) rather than positive chirp (a): in case c, all levels up to $v = 6$ are populated, whereas only levels $v = 1$ and 2 are significantly populated in case a. Two important spectroscopic results can be deduced directly from the data shown in Fig. 1. The first observation is that the transitions are perfectly equidistant, in agreement with the energy levels expected for a Morse potential (12):

$$E_v = \hbar\omega_0 \left[\left(v + \frac{1}{2} \right) - \chi_e \left(v + \frac{1}{2} \right)^2 \right]. \quad [1]$$

According to the data shown in Fig. 1, the difference between two consecutive transitions, $\omega_{v+2,v+1} - \omega_{v+1,v} = -2\chi_e\omega_0$, is equal to -25 cm^{-1} , in good agreement with the value previously measured in the perturbative regime on the transition $v = 1 \rightarrow v = 2$ using either pump-probe spectroscopy (13) or vibrational echo beats (14). This anharmonicity corresponds to $\chi_e \approx 0.63\%$.

Fig. 2. Differential spectrum recorded for a pump pulse centered at $1,920\text{ cm}^{-1}$ and for a time delay $\tau = 7\text{ ps}$ (curve a). The other experimental conditions are the same as those described for Fig. 1, curve c, i.e., $\varphi'' = -32,000\text{ fs}^2$. Note the stimulated-emission peak observed at frequency ω_{54} . As a reference on the noise level, we also plot the differential spectrum obtained for a time delay of 140 ps (curve b), where only a small positive peak remains at frequency ω_{10} . Curve b is shifted down for clarity.

The second result apparent in Fig. 1 is that the induced absorption line widths are roughly identical for all transitions, taking values between 6 and 7 cm^{-1} , which corresponds to a dephasing time of $\approx 1.6\text{ ps}$. This value is long enough to ensure that the interaction with the midinfrared pulse is coherent. Indeed, even for the longest pulse (1.4 ps), each individual transition takes place in a time significantly shorter than the pulse duration and consequently shorter than the transition dephasing time. Therefore, the measured dephasing times are compatible, with a coherent process and hence with a high climbing efficiency. One expected manifestation of a coherent excitation is the occurrence of population inversion, which indeed is shown in Fig. 2 for $\varphi'' = -32,000\text{ fs}^2$, and a pump-probe delay of $\tau = 7\text{ ps}$ such that the effect of vibrational relaxation is less significant than in the case of Fig. 1. In contrast with the previous case, a stimulated-emission peak is clearly observed at frequency ω_{54} , demonstrating that population inversion has been achieved: the population in level $v = 5$ is larger than the population in level $v = 4$. Furthermore, the small negative peak observed at frequency ω_{87} may be attributed to a population in level $v = 7$.

Fig. 3 shows time-domain data for which we plotted the amplitudes of the induced absorption or transmission lines as a function of the pump-probe delay. For several transitions, the induced absorption is shown to first increase and then decay. For a given vibrational state, this behavior results from the increase in population associated with the relaxation of upper states followed by the decay of the population of the considered state itself. However, note that the plotted values do not directly represent the populations but the population differences. Furthermore, for each transition the signal is proportional to the transition dipole squared, which is not known accurately (see below), so that the population of each individual vibrational state cannot be deduced from the experimental data. It is possible, however, to determine the population relaxation times for the lower states by use of the experimental data corresponding to time domains in which the population of upper states can be neglected. As shown in Fig. 4, when only level $v = 1$ is populated ($\tau > 75\text{ ps}$), an exponential decay was observed for the differential signals at ω_{10} and ω_{21} , yielding a population relaxation time $T_{1,0} = 25(3)\text{ ps}$ for level $v = 1$. Similarly, the induced absorption at ω_{32} for $\tau \geq 40\text{ ps}$ when only levels $v = 2$ and 1 are populated yields the $v = 2$ relaxation time, $T_{2,1} = 15(3)\text{ ps}$. Using the same approach, we found $T_{3,2} = 12(4)\text{ ps}$. The noise for the absorption lines at smaller frequencies prevented us from determining the population relaxation times of upper states. The value that we report for $T_{1,0}$ is in good agreement with previous

Fig. 3. Dependence on pump-probe delay. (Lower) Variation of the transition line amplitudes as a function of pump-probe delay in the same experimental conditions as described for Fig. 2. The dotted lines show the zeros of the vertically offset curves. (Upper) Sum of all contributions $\Sigma [-\Delta\alpha(\omega_{v+1,v})L]$. Solid lines are included as a guide for the eye.

measurements performed in the perturbative regime on similar systems (15–17), and it was identical to the value reported by Lim *et al.* (18) in HbCO. The general trend of shorter relaxation times for upper levels was similar to that reported by Arrivo *et al.* (3) in W(CO)₆ using a similar technique. It also roughly followed the law expected for a harmonic oscillator linearly coupled to a thermal bath (19) with decay rates proportional to the vibrational quantum number.

Integral of the Differential Absorption Spectra. In the case of a Morse potential, the matrix elements of the position operator x are known exactly (20, 21):

$$\begin{aligned} \langle v+1|x|v\rangle &= \langle 1|x|0\rangle \sqrt{v+1} \frac{1-2\chi_e}{1-2\chi_e(v+1)} \\ &\cdot \sqrt{\frac{[1-(2v+1)\chi_e][1-(2v+3)\chi_e]}{[1-(v+1)\chi_e](1-3\chi_e)}}, \end{aligned} \quad [2]$$

which simply approximates to $\langle 1|x|0\rangle \sqrt{v+1} (1+\chi_e v/2)$ when χ_e is small. Considering the small value of the anharmonicity in our case (0.63%), we can even assume that the x matrix elements simply scale as $\sqrt{v+1}$ as in a harmonic oscillator. Let us first neglect electrical anharmonicity so that the matrix elements of the dipole operator μ are simply proportional to those of x and hence also scale as $\sqrt{v+1}$. The absorption corresponding to the transition $v \rightarrow v+1$ is proportional to the square of the transition dipole times the population difference between the two levels, namely,

$$\alpha(\omega_{v+1,v}) \propto |\langle v+1|\mu|v\rangle|^2 (\rho_{v,v} - \rho_{v+1,v+1}), \quad [3]$$

where ρ is the density matrix and $\rho_{v,v}$ is the vibrational population in level v . Summing over all vibrational transitions, and assuming the transition dipoles scale with $\sqrt{v+1}$, we obtain

Fig. 4. Time dependence of the transition line amplitudes for the lower transitions (symbols) with exponential fits (solid lines) of time constants equal to 25 ps (ω_{10} and ω_{21}), 15 ps (ω_{32}), and 12 ps (ω_{43}). The dotted lines show the zeros of the vertically offset curves. The vertical scale of the last curve has been expanded by a factor of 2. The experimental conditions are identical to those described for Figs. 2 and 3.

$$\begin{aligned} \sum_v \alpha(\omega_{v+1,v}) &\propto \sum_v |\langle v+1|\mu|v\rangle|^2 (\rho_{v,v} - \rho_{v+1,v+1}) \\ &= |\mu_{10}|^2 \sum_v (\sqrt{v+1})^2 (\rho_{v,v} - \rho_{v+1,v+1}) \\ &= |\mu_{10}|^2 \sum_v \rho_{v,v} = |\mu_{10}|^2. \end{aligned}$$

[4]

This result implies that the sum over all absorption lines is independent of the population distribution such that the frequency-integrated differential absorption spectra should vanish. It is obvious from Fig. 2 that this is not verified in the experiment. Even in the case of Fig. 1, the integrated differential spectra yield, respectively, 0.08 (a), 0.25 (b), and 0.55 (c) in units of the integrated induced bleaching at frequency ω_{10} . Similarly, Fig. 3 shows that the sum over all differential absorption lines takes significant values at early time delays.

At least four hypotheses can be raised to address this issue. First, a significant part of the population could be promoted to levels $v \geq 9$. The corresponding induced absorption lines lie outside the probe spectrum and hence do not contribute to the integrated spectra. However, apart from the fact that the pump spectrum is also vanishing for direct resonant excitation of such levels, it is quite unlikely that the decay of this large population from upper levels would not result in a more significant subsequent increase of the population of levels $v = 5$ and 6 (see Fig. 3). Therefore, we discarded the possibility that a significant fraction of the vibrational population lies in levels $v \geq 9$. The second hypothesis is that CO dissociated from the heme (for example, because of a coupling between the CO and the FeC bonds), resulting in a breaking of the latter. Because dissociated CO absorbs at a different frequency (22) outside the probe spectrum, this effect would indeed appear as a net decrease in infrared absorption. To check this hypothesis, we measured the sample transmission by using a visible probe pulse with a center wavelength of 530 nm. The dissociation yield was measured to be $<2\%$, a value that is far too small to explain the observed decrease in infrared absorption mentioned above. Therefore, we also discarded the possibility that a significant fraction of CO molecules dissociate from the heme in this excitation regime. The third hypothesis is that the reduction in infrared absorption results from a coupling between the CO vibration and other vibrational modes that are populated through the decay of upper CO vibrational levels. Although the effect of such a coupling should decay with the same rate as the population of these other vibrational

modes, i.e., with a time constant probably shorter than the ≈ 30 ps observed for the recovery of the infrared absorption (see Fig. 3 *Upper*), we do not rule out this hypothesis. The fourth hypothesis is that the transition dipoles do not scale with $\sqrt{\nu + 1}$. From the small value of the integral (0.08) in the case of Fig. 1, curve a, we note that the deviation from the $\sqrt{\nu + 1}$ law is rather small for the first three transitions. In contrast, our data obtained in the case of a stronger excitation regime indicate that for upper vibrational levels, the transition dipoles differ quite dramatically from the $\sqrt{\nu + 1}$ law. This effect could result from either the electrical anharmonicity of the CO dipole moment μ (nonlinear variation of μ with respect to the bond length x caused by the rearrangement of the charge distribution) or an increasing effect of mode coupling on the transition dipoles when ν increases because of a breakdown of the simple one-dimensional Morse-oscillator model. Elaborate theoretical calculations outside the scope of this work (taking into account the multidimensional potential-energy and dipole-moment surfaces as well as the vibrational dynamics) would be needed to account for our experimental observations.

Vibrational Populations. As discussed above, the poor knowledge of the transition dipoles makes it difficult to determine accurately the relative populations of the different vibrational states. However, a few important results can still be obtained from the experimental data. First, Fig. 2 shows a rather strong bleaching of the ω_{10} absorption line, equal to $\approx 50\%$ of the corresponding unperturbed absorption. If we were to assume that all of the population excited from the ground state were in level $\nu = 1$, the measured 50% bleaching would correspond to a 25% depletion of level $\nu = 0$ and a 25% population of level $\nu = 1$. However, this is clearly not the case because Fig. 2 does not exhibit the corresponding induced absorption that would then be expected at frequency ω_{21} . In contrast, Fig. 2 corresponds to a situation in which the excited population is spread over all vibrational states from $\nu = 1$ up to 7. This means that the induced bleaching observed at frequency ω_{10} results almost entirely from the decrease in absorption caused by the depletion of level $\nu = 0$ and not to the stimulated emission from $\nu = 1$ to 0. As a consequence, the corresponding depletion of the ground state must be close to 50%. Therefore it can be concluded that almost half of the molecules have been excited by the midinfrared pump pulse. Furthermore, it should be emphasized that the present experiments have been achieved in a liquid sample so that the measured spectra actually result from an averaging over the different orientations of the excited molecules. Additionally, the pump and probe have identical spot size so that there is also an averaging because of the pump-intensity profile within the focal spot. Therefore, an average excitation of 50% of the molecules means a much greater efficiency for the molecules at the center of the focal spot and aligned parallel to the pump polarization. More generally, the averaged differential absorption can be written as follows:

$$\Delta\alpha(\omega) = \int_0^\pi \sin\theta \, d\theta \int_0^\infty r dr \Delta\alpha(I_p \cos^2\theta e^{-2\frac{r^2}{w^2}}, \omega) \cos^2\theta e^{-2\frac{r^2}{w^2}}, \quad [5]$$

where $\Delta\alpha(I, \omega)$ is the differential absorption expected for a molecule aligned parallel to a homogeneous pump field of peak intensity I , I_p is the pump peak intensity, and w is the beam waist diameter. The expression shown above takes into account an effective pump intensity ηI_p , where $\eta = \cos^2\theta \exp[-2(r^2/w^2)]$ for a molecule at a distance r from the beam center and oriented

with an angle θ from the pump-field polarization. Eq. 5 can be written more simply as follows:

$$\Delta\alpha(\omega) = \int_0^1 \Delta\alpha(\eta I_p, \omega) g(\eta) d\eta, \quad [6]$$

where $g(\eta)$ is the effective intensity distribution resulting from the orientational and spatial inhomogeneities. It reads

$$g(\eta) = \int_0^\pi \sin\theta \, d\theta \int_0^\infty r dr \delta\left(\eta - \cos^2\theta \exp\left(-2\frac{r^2}{w^2}\right)\right) \cdot \cos^2\theta e^{-2\frac{r^2}{w^2}} = 3(1 - \sqrt{\eta}). \quad [7]$$

Let us consider the population in level $\nu = 6$ in the case of Fig. 1, curve c. Because the transition dipoles are not known, we use the harmonic oscillator values in $\sqrt{\nu + 1}$ and deduce from the measured differential signal that the corresponding average population is 0.5%. Note that this is clearly a lower estimate of the population, because the actual transition dipoles are known to be smaller, which implies a larger population to explain the observed differential signal. If we assume that the $\nu = 6$ population obeys perturbation theory, we expect a signal proportional to the sixth power of the pump intensity. We therefore must integrate $\eta^6 g(\eta)$ from 0 to 1 to get the orientational and spatial average, which yields an average value 35 times smaller than in the case of a homogeneous excitation of the same intensity. In fact, our experiment is in the strong-field regime so that the above perturbative law breaks down and a numerical calculation must be performed to determine the exact dependence of $\Delta\alpha(I, \omega)$ on the pump intensity. We performed such a calculation by numerically integrating the Bloch equation using the Runge-Kutta technique and then calculated the average value weighted by using the function $g(\eta)$. We found that the measured signal is consistent with a population equal to 5% for the molecules at the center of the pump spot and aligned parallel to the pump polarization, which means that the effect of averaging decreases the efficiency by a factor of 10 instead of 35 in the perturbative approach, in which saturation effects were neglected. Still, the corresponding population is far greater than what would be expected for an incoherent excitation ($1/2^6 \approx 0.8\%$) and, therefore, is additional evidence of the coherent nature of the vibrational climbing.

Finally, an issue that should be addressed is the quantum interference between sequential and direct multiphoton paths that have been reported in other systems (23–25). This effect results in oscillations in the signal as a function of the pump chirp. However, our numerical calculations indicate that the combined effect of a large number of possible paths in a vibrational ladder and the averaging over a broad range of intensities weighted with function $g(\eta)$ results in a strong reduction of the oscillation contrast. Consequently, the general trend that the climbing efficiency increases when $|\varphi'|$ increases remains essentially correct, in agreement with Fig. 1.

Conclusions

We have demonstrated vibrational climbing in a biological system, HbCO, by the use of stretched midinfrared optical pulses. We have shown that this process results in vibrational population inversion and that vibrational states as high as $\nu = 6$ could be significantly populated. The measured induced absorption lines were observed to be equidistant, in agreement with a Morse potential for the CO stretch potential energy surface. The dephasing times of all vibrational transitions were

measured to be 1.6 ps, thus compatible with a coherent excitation regime. The vibrational relaxation measured for the lower states was found to agree qualitatively with the law expected for a harmonic oscillator linearly coupled to a thermal bath, namely decay rates increasing with the vibrational quantum number. In contrast, the nonvanishing integral of the differential absorption spectra could not be explained within a simple model, and we expect that our experimental results will trigger future theoretical work, which should help in gaining a better understanding of the vibrational motion of this system far from the harmonic region. Finally, because our experiments were conducted in the liquid phase, a great improvement of the climbing efficiency can be expected in protein crystals in which the CO molecules would be perfectly

aligned with respect to the infrared field polarization. Together with optimal control strategies and the use of broader infrared spectra, this might lead to a new approach for exciting proteins to the transition state by use of infrared fields, which have the advantage that the molecule remains in its electronic ground state during the entire excitation process.

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1. Kaindl, R. A., Wurm, M., Reimann, K., Hamm, P., Weiner, A. M. & Woerner, M. (2000) *J. Opt. Soc. Am. B* **17**, 2086–2094.
2. Chelkowski, S., Bandrauk, A. D. & Corkum, P. B. (1990) *Phys. Rev. Lett.* **65**, 2355–2358.
3. Arrivo, S. M., Dougherty, T. P., Grubbs, W. T. & Heilweil, E. J. (1995) *Chem. Phys. Lett.* **235**, 247–254.
4. Kleiman, V. D., Arrivo, S. M., Melinger, J. S. & Heilweil, E. J. (1998) *Chem. Phys.* **233**, 207–216.
5. Windhorn, L., Witte, T., Yeston, J. S., Proch, D., Motzkus, M., Kompa, K.-L. & Fus, W. (2002) *Chem. Phys. Lett.* **357**, 85–90.
6. Maas, D. J., Duncan, D. I., Vrijen, R. B., van der Zande, W. J. & Noordam, L. D. (1998) *Chem. Phys. Lett.* **290**, 75–80.
7. Bonn, M., Hess, C. & Wolf, M. (2000) *Phys. Rev. Lett.* **85**, 4341–4344.
8. Witte, T., Hornung, T., Windhorn, L., Proch, D., DeVivie-Riedle, R., Motzkus, M. & Kompa, K. L. (2003) *J. Chem. Phys.* **118**, 2021–2024.
9. Windhorn, L., Yeston, J. S., Witte, T., Füsse, W., Motzkus, M., Proch, D., Kompa, K.-L. & Moore, C. B. (2003) *J. Chem. Phys.* **119**, 641–645.
10. Ansari, A., Berendzen, J., Braunstein, D., Cowen, B. R., Frauenfelder, H., Hong, M. K., Iben, I. E. T., Johnson, J. B., Ormos, P., Sauke, T., *et al.* (1987) *Biophys. Chem.* **26**, 337–355.
11. Demirdöven, N., Khalil, M., Golonzka, O. & Tokmakoff, A. (2002) *Opt. Lett.* **27**, 433–435.
12. Morse, P. M. (1929) *Phys. Rev.* **34**, 57–64.
13. Owrutsky, J. C., Li, M., Locke, B. & Hochstrasser, R. M. (1995) *J. Phys. Chem.* **99**, 4842–4846.
14. Rector, K. D., Kwok, A. S., Ferrante, C., Tokmakoff, A., Rella, C. W. & Fayer, M. D. (1997) *J. Chem. Phys.* **106**, 10027–10036.
15. Hill, J. R., Tokmakoff, A., Peterson, K. A., Sauter, B., Zimdars, D., Blott, D. D. & Fayer, M. D. (1994) *J. Phys. Chem.* **98**, 11213–11219.
16. Blott, D. D., Fayer, M. D., Hill, J. R., Rella, C. W., Suslick, K. S. & Ziegler, C. J. (1996) *J. Am. Chem. Soc.* **118**, 7853–7854.
17. Fayer, M. D. (1996) *J. Phys. Chem.* **100**, 12100–12107.
18. Lim, M., Hamm, P. & Hochstrasser, R. M. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 15315–15320.
19. Fourkas, J. T., Kawashima, H. & Nelson, K. A. (1995) *J. Chem. Phys.* **103**, 4393–4407.
20. Rosenthal, J. E. (1935) *Proc. Natl. Acad. Sci. USA* **21**, 281–285.
21. Heaps, H. S. & Herzberg, G. (1952) *Z. Phys.* **133**, 48–64.
22. Lim, M., Jackson, T. A. & Anfinrud, P. A. (1995) *J. Chem. Phys.* **102**, 4355–4366.
23. Balling, P., Maas, D. J. & Noordam, L. D. (1994) *Phys. Rev. A At. Mol. Opt. Phys.* **50**, 4276–4285.
24. Maas, D. J., Rella, C. W., Antoine, P., Toma, E. S. & Noordam, L. D. (1999) *Phys. Rev. A At. Mol. Opt. Phys.* **59**, 1374–1381.
25. Chatel, B., Degert, J., Stock, S. & Girard, B. (2003) *Phys. Rev. A At. Mol. Opt. Phys.* **68**, 041402.