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LETTERS

Femtosecond characterization of vibrational optical activity of chiral molecules

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Optical activity[1–3] is the result of chiral molecules interacting differently with left versus right circularly polarized light. Because of this intrinsic link to molecular structure, the determination of optical activity through circular dichroism (CD) spectroscopy has long served as a routine method for obtaining structural information about chemical and biological systems in condensed phases[4–6]. A recent development is time-resolved CD spectroscopy, which can in principle map the structural changes associated with biomolecular function[7] and thus lead to mechanistic insights into fundamental biological processes. But implementing time-resolved CD measurements is experimentally challenging because CD is a notoriously weak effect (a factor of 10^{-4}–10^{-6} smaller than absorption). In fact, this problem has so far prevented time-resolved vibrational CD experiments. Here we show that vibrational CD spectroscopy with femtosecond time resolution can be realized when using heterodyned spectral interferometry to detect[8–10] the phase and amplitude of the infrared optical activity free-induction-decay field in time (much like in a pulsed NMR experiment). We show that we can detect extremely weak signals in the presence of large achiral background contributions, by simultaneously measuring with a femtosecond laser pulse the vibrational CD and optical rotatory dispersion spectra of dissolved chiral limonene molecules. We have so far only targeted molecules in equilibrium, but it would be straightforward to extend the method for the observation of ultrafast structural changes such as those occurring during protein folding or asymmetric chemical reactions. That is, we should now be in a position to produce 'molecular motion pictures'[11] of fundamental molecular processes from a chiral perspective.

CD spectroscopy is considered an incisive tool for determining the secondary structure of proteins in solution[4–6,12,13]. But biomolecules participating in chemical or biological reactions often undergo ultrafast structural changes that modulate their chiro-optical properties. The desire to map the chirality changes of such reactive systems over time, as a means to gain insight into the underlying structural changes, thus naturally led to the development of transient electronic CD measurement techniques[7,14–16]. One approach to improve the relatively poor time resolution of electronic CD spectrometry and overcome the weak-signal problems uses an ellipsometric detection scheme[7,14], but its time resolution limit is set by the (limited) speed of the measurement electronics, such as amplifying gates or transient digitizers. Another approach combines an ultrashort laser with an electro-optic modulator or a Babinet-Soleil compensator to measure electronic CD or optical rotatory dispersion (ORD)[15,16] signals with subpicosecond time resolution. These approaches should in principle enable one to monitor time-resolved CD spectra by first optically triggering the chemical or biochemical reaction, and then measuring the differential absorption ΔA of time-delayed light pulses that are left- and right-circularly polarized (LCP and RCP) or left- and right-elliptically polarized (LEP and REP) (that is, $\Delta A = A_{\mathrm{LCP}} - A_{\mathrm{RCP}}$ or $\Delta A = A_{\mathrm{LEP}} - A_{\mathrm{REP}}$). But the mode-locked ultrafast lasers used in such pump-probe measurements usually lack the intensity stabilities of approximately 0.001% that are needed to discriminate minuscule CD signals from the large achiral background signal.

Vibrational circular dichroism (VCD) is the vibrational analogue of the more popular and extensively used electronic CD. It is useful for differentiating between discrete structural conformations of biomolecules[3,12,13,17] and for determining absolute configurations of chiral molecules and drugs. Time-resolved VCD spectrometry could thus play a key role in unravelling the mechanisms of important biological or chemical processes, by monitoring the structural evolution of biomolecules or chiral molecules during reaction. But time-resolved VCD experiments are considered even more challenging than electronic CD experiments, mainly because the chiral susceptibilities for nuclear vibrations are much smaller than those for electronic degrees of freedom; consequently, neither an experimental set-up nor actual measurements involving ultrafast time-resolved VCD have been reported to date. Our approach to realizing femtosecond vibrational optical activity (CD and ORD) measurements involves detecting the phase and amplitude of the coherently emitted infrared optical activity free-induction-decay (FID) field (Fig. 1) using a novel cross-polarization detection scheme (Fig. 1a).

Figure 1a sketches the key principles of our experiment. Molecules with opposite chiral properties absorb LCP and RCP light differently. Therefore, an excitation pulse of linearly polarized light—an equal superposition of LCP and RCP—is transformed into LEP or REP light on interacting with chiral molecules. The interaction with the chiral molecules will at the same time give rise to circular birefringence, so that RCP light is transmitted with a different velocity from LCP light: the molecules rotate the major axis of the polarization ellipse arising from the CD phenomenon by an amount given by their circular birefringence Δn, defined as the differential index of refraction of LCP and RCP beams ($\Delta n = n_{\mathrm{LCP}} - n_{\mathrm{RCP}}$). Particularly relevant here is that in the transmitted elliptically polarized light, information on the optical activity and hence chiral properties of the molecules is almost exclusively contained in the minor-axis field component (horizontal electric field component in Fig. 1a, $\tilde{E}_{\perp}$); in contrast, the major-axis field component (vertical electric field component, $\tilde{E}_{\parallel}$) is mostly influenced by all-electric-dipole-allowed achiral features of the molecule. As a result, a polarizer oriented perpendicular to the polarization direction of the excitation beam will select only the chiral (CD and ORD) components of the transmitted elliptically polarized light. It is the phase of the minor-axis field ($\tilde{E}_{\perp}$) that provides information on the handedness of the chiral molecule[18]. We found that the

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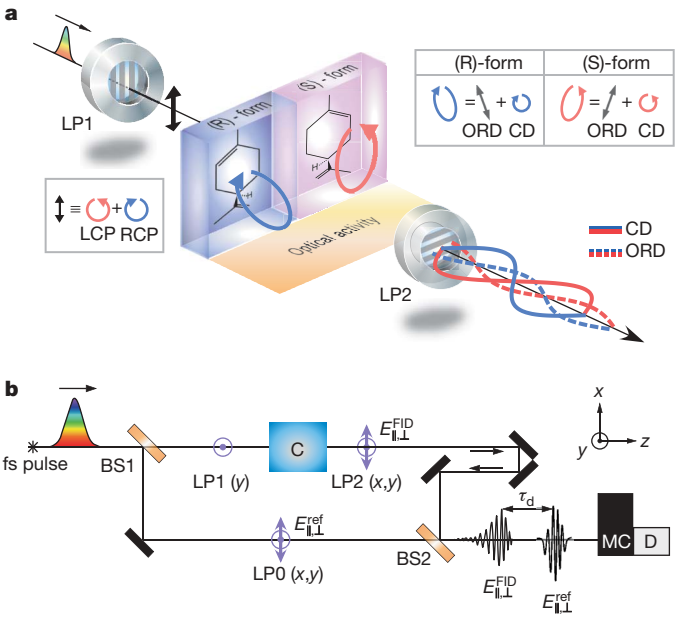

Figure 1 | Heterodyned detection of optical activity free induction decay. **a**, Sketch of the basic principles of the cross-polarization detection in our optical activity FID measurements. We first linearly polarize a coherent femtosecond light pulse with linear polarizer LP1. The pulse is then incident on the chiral sample, and the left- and right-circularly polarized components (LCP, RCP) of the linearly polarized field are differentially absorbed. Depending on the chirality of the sample, this differential absorption (circular dichroism, CD) transforms the pulse into one with an eccentric left- or right-elliptical polarization (LEP, REP). Furthermore, the circular birefringence of the chiral molecules causes an optical rotation of the major (vertical) axis of the field polarization ellipse created through CD. After passage through the sample, the light pulse passes through linear polarizer LP2. LP2 is oriented perpendicular to LP1, so most of the transmitted field parallel to the incident beam is rejected while the perpendicular field components are retained. These perpendicular components are out of phase with each other after interaction of the light with two different enantiomers, the (*R*)-form (blue) and (*S*)-form (red); the individual CD (solid lines) and ORD (dashed lines) components are approximately in quadrature if the optical rotation angle is small. **b**, Sketch of the set-up for Fourier transform spectral interferometry experiments. The input pulse is split in two by a beam splitter (BS1). One beam is linearly *y*-polarized by LP1 and used to create electronic or vibrational coherences in the chiral sample (C), while the other is used to amplify and characterize the emitted FID field. For measurements of both parallel and perpendicular components of the FID fields ($E^{\mathrm{FID}}_{\parallel,\perp}$) emitted from C, the transmission axes of the linear polarizers LP0 and LP2 are aligned along the *y*- and *x*-axis, respectively. The reference pulse ($E^{\mathrm{ref}}_{\parallel,\perp}$)and the signal field are combined by another beam splitter (BS2), with the former preceding the latter by a finite time, τ_{d}. The heterodyned signal field is dispersed by a monochromator (MC) and detected by detector D.

relationship between $\tilde{E}_{\perp}(\omega)$ and $\tilde{E}_{\parallel}(\omega)$, where ω is frequency, is given by theory as (see Supplementary Information for details)

$$\tilde{E}_{\perp}(\omega) \propto \Delta\chi(\omega)\tilde{E}_{\parallel}(\omega) \qquad (1)$$

where $\tilde{E}_{\parallel}(\omega)$ is the complex transmitted electric field spectrum that results from the interference between the input field and the induced electric-dipole-allowed optical FID field. The complex function $\Delta\chi(\omega)$ is the optical activity susceptibility; its imaginary part directly corresponds to the CD spectrum $\Delta\alpha(\omega)$, and its real part to the frequency-dependent ORD spectrum. Although the minor-axis component $\tilde{E}_{\perp}(\omega)$ is extremely weak, the optical heterodyning allows us to amplify the weak signal field to give readily measurable levels of phase and amplitude information. Once the minor- and major-axis field components, $\tilde{E}_{\perp}(\omega)$ and $\tilde{E}_{\parallel}(\omega)$, are measured, from equation (1) the complex susceptibility $\Delta\chi(\omega)$ can be directly obtained.

Our femtosecond measurement technique of vibrational optical activity utilizes heterodyned Fourier transform spectral interferometry[8–10,19–22] as sketched in Fig. 1b (see Supplementary Information for more details regarding the experimental set-up). Briefly, we use an infrared pulse (1 kHz repetition rate, 2 μJ, centre wavelength of ~3.4 μm, and pulse width of ~60 fs) generated by difference frequency mixing of a signal and an idler pulse from an optical parametric amplifier. To measure $\tilde{E}_{\perp}(\omega)$, the linear polarizers LP0 and LP2 are directed along the *x* axis while the linear polarizer LP1 is oriented along the *y* axis. In this cross-polarization configuration, only the $\tilde{E}_{\perp}(\omega)$ component of the elliptically polarized light pulse that is transmitted through the sample is allowed to pass through LP2 for heterodyne detection. (A high-resolution motorized rotational stage is used to finely control the relative orientation of LP1 with incremental resolution of 0.0005°.) This FID signal and the reference pulse are then combined and spectrally dispersed with a monochromator, and the cross-polarization spectral interferogram $S_{\perp}(\omega)$ is detected. To measure the parallel-polarization spectral interferogram $S_{\parallel}(\omega)$ that originates from $\tilde{E}_{\parallel}(\omega)$, we rotate LP1 by a mere 0.5°; this alters the beam pathway and experimental set-up only minimally, and the phase change induced by the rotated linear polarizer is thus negligibly small. We find that the performances of the polarizers (LP1 and LP2) are critical for the success of the experiment because $\tilde{E}_{\perp}(\omega)$ is typically several orders of magnitude smaller than $\tilde{E}_{\parallel}(\omega)$ when LP1 ∥ LP2. We therefore chose calcite plate polarizers[23] for the main polarizer pair that removes the large achiral signal contribution $\tilde{E}_{\parallel}(\omega)$: unlike usual prism-type polarizers (with extinction ratios of $\sim 10^{-6}$), calcite plate polarizers utilize dichroic absorption that results in exceptionally small extinction ratios (10^{-8}–10^{-9}) within limited infrared spectral regions (3.35–3.5 μm, 3.9–4.1 μm, and so on).

To test both the cross-polarization detection scheme as a means for realizing femtosecond vibrational optical activity spectrometry (Fig. 1a) and our experimental set-up (Fig. 1b), we performed proof-of-principle measurements on the (*R*)- and (*S*)-enantiomers of limonene and their racemic mixture. Even though the working ranges of our calcite dichroic polarizers are limited, most of the C–H stretching mode frequencies of limonene fall within one of their spectral windows. Figure 2a shows the dispersed spectral interferograms (that is, $S_{\perp}(\omega)$ and $S_{\parallel}(\omega)$) measured in the 2,840 to 3,000 cm^{-1} wavenumber range; all three spectral interferograms in Fig. 2a exhibit systematic differences in terms of oscillating amplitudes and phases. We note here that the heterodyne signal measured for the racemic mixture is finite (though relatively small) rather than zero; this signal is due to an optical imperfection of the linear polarizers, and originates from interference between the reference pulse and a weak horizontal light pulse component leaked from LP1. Although not ideal, this achiral measured signal component contributes only to the VCD spectrum as a background offset (Supplementary Fig. 3) and can thus be removed.

The CD and ORD spectra are obtained by transforming the measured spectral interferograms $S_{\perp}(\omega)$ and $S_{\parallel}(\omega)$ into the electric field spectra $\tilde{E}_{\perp}(\omega)$ and $\tilde{E}_{\parallel}(\omega)$ and then taking the ratio $\tilde{E}_{\perp}(\omega)/\tilde{E}_{\parallel}(\omega)$; the imaginary and real parts of this ratio then yield the CD and ORD spectra, respectively. The spectral characteristics (amplitude and phase) of the reference pulse and an additional phase term produced by the time delay τ_{d} between the signal field and the reference pulse (Fig. 1b) contribute equally to $S_{\perp}(\omega)$ and $S_{\parallel}(\omega)$, so their contributions automatically cancel out when taking the ratio of $\tilde{E}_{\perp}(\omega)$ to $\tilde{E}_{\parallel}(\omega)$. This means that as long as the two paths in the interferometer have a constant delay time τ_{d} and are sufficiently stable (in terms of phase fluctuations) during measurement, CD and ORD spectra can be retrieved from the measured interferograms without requiring a detailed characterization of the reference pulse or precise determination of the value of the delay time τ_{d}. This feature is crucial for significantly boosting the sensitivity of our method.

Figure 2b depicts VCD spectra $\Delta\alpha(\omega)$ extracted from the spectral interferograms measured with samples of (*R*)- and (*S*)-limonene and their racemic mixture. The VCD peak positions of (*R*)- and (*S*)-limonene are identical and their signs opposite to each other, and

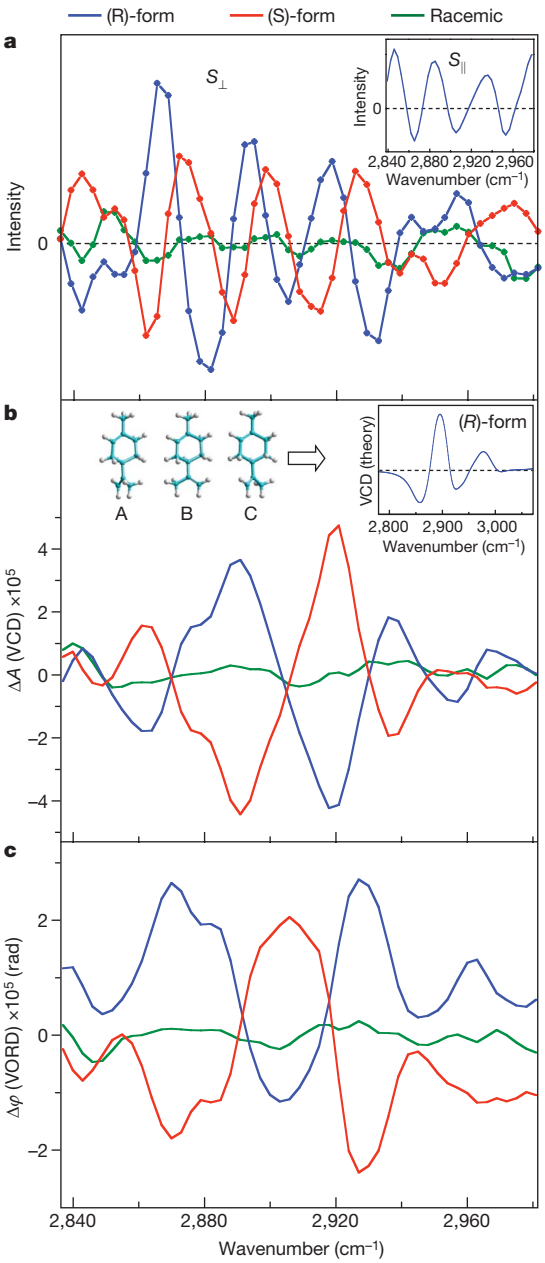

Figure 2 | Vibrational optical activity signals of chiral limonenes. a, Cross-polarization spectral interferograms $S_\perp(\omega)$ of (*R*)-limonene (blue line) and (*S*)-limonene (red line) and their 1:1 racemic solution (green line) dissolved in CCl_4. (Analyte concentration, 1.2–1.5 M; path length, ~50 μm; maximum absorbance, 1.1 for (*R*)-limonene and racemic solution, and 1.4 for (*S*)-limonene.) Parallel-polarization spectral interferograms $S_\parallel(\omega)$ are required to retrieve VCD and VORD spectra, and the inset therefore gives that of (*R*)-limonene. Coloured dots give the measured data points for comparison, with the dashed line indicating zero. Note that the three limonene samples have distinctive spectral phases and amplitudes, which are different from one another. **b**, VCD spectra retrieved from the measured spectral interferograms in **a**, with linear baseline correction to enable a clear comparison. Absolute VCD values (ΔA) were directly calculated according to $(4/2.303)\mathrm{Im}[\tilde{E}_\perp(\omega)/\tilde{E}_\parallel(\omega)]$ (Supplementary Information). *Ab initio* calculations using MP2/6-311++G** identified the three major (*R*)-limonene conformers A, B and C (top-left inset) and gave the simulated C–H stretch VCD spectrum shown in the top-right inset. **c**, VORD spectra extracted from the real part of $\Delta\chi(\omega)$. The absolute VORD values ($\Delta\varphi$) were also calculated by $\mathrm{Re}[\tilde{E}_\perp(\omega)/\tilde{E}_\parallel(\omega)]$ (Supplementary Information), and the maximum optical rotation angle (at 2,927 cm^{-1}) found to be about 1.6×10^{-3} degrees.

the spectra are quantitatively consistent with spectra measured using a continuous wave Fourier transform infrared (FT-IR) VCD spectrometer[24] (Supplementary Fig. 5). This indicates that our technique is robust and reliable. The racemic spectrum does not show any notable structure, as expected. A series of concentration-dependent VCD measurements confirmed that the attenuation of the signal field by self-absorption does not affect the spectrum as long as the experimental optical density is close to or less than 1. We note that the data collection time needed to obtain the VCD spectra in Fig. 2b is only tens of minutes, whereas measurements with the commercial FT-IR VCD spectrometer required multiple hours of signal averaging. The enhanced sensitivity of our method is due to the fact that the measurements are non-differential, free from achiral background signal, and based on heterodyned detection.

Finally, Fig. 2c shows the vibrational ORD (VORD) spectra of the (*R*)- and (*S*)-limonene and their racemic mixture obtained directly from the real part of $\Delta\chi(\omega)$. The spectra illustrate successful location of a maximum in the angle of optical rotation of an infrared beam by (*R*)-limonene in solution that is as small as about 10^{-5} rad. We note that resonant VORD spectra of a liquid crystal[25] and off-resonant VORD spectra of polypeptides[26] have been measured before; but the direct measurement of the resonant VORD spectrum of a small chiral molecule in solution presumably posed significant experimental challenges due to the minute extent of optical rotation and the large attenuation of the signal in the resonant frequency region. To enable a comparison between theory and experiment, we carried out high-level *ab initio* calculations of an isolated (*R*)-limonene molecule and quantum mechanical/molecular mechanical simulations of limonene/CCl_4 solutions. The calculations identified three major conformers (Fig. 2b top left inset) and yielded a simulated VCD spectrum (Fig. 2b top right inset) in good agreement with experiment; this illustrates that the current quantum chemistry calculation method, when properly combined with molecular dynamics simulation techniques, is able to provide a quantitative description of the absolute configuration of a given chiral molecule in solution[27–29].

Although we have not yet carried out time-resolved spectroscopy, the present work clearly establishes that femtosecond optical pulses can be used to simultaneously measure VCD and VORD spectra with a time resolution that is only limited by the optical activity FID time. Time-resolved CD (ORD) experiments using the current technique can therefore be implemented by adding a pump pulse to trigger chemical or physical changes (such as protein unfolding) and then using a femtosecond optical pulse to probe the subsequent relaxation process after varying waiting times, T_w. This would result in a series of VCD and VORD spectra as a function of T_w, and provide information on the conformational change of the target molecule. In most experiments so far, it was found that the pump-induced changes result in signals that are too weak to be discriminated from large static signals when trying to differentiate measured CD spectra at different times; thus, a pump modulation technique that allows one to measure only the pump-induced changes would be required to increase sensitivity and enable measurements. In contrast to previous time-resolved CD experiments relying on differential measurements, the present method does not require any polarization modulation of the probe pulse and should thus enable the development of a highly sensitive time-resolved CD version in a straightforward manner.

Our phase-and-amplitude measurement technique overcomes both the small-signal and non-zero-background problems of conventional electronic and vibrational CD techniques relying on differential absorption measurement, owing to its capability of coherent amplification of a weak signal field. Although the present work demonstrated infrared CD and ORD measurements, the key concepts and the technique are sufficiently general that they can be readily applied to the measurement of other related chiro-optical properties. We therefore anticipate that the present method, which is a CD analogue of pulsed NMR, will trigger novel developments to

further improve time-resolved optical activity spectroscopy for use in studies of biomolecular dynamics in aqueous solution at physiological conditions.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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