NEWS & VIEWS

SPECTROSCOPY

Handedness in quick time

Patrick H. Vaccaro

The handedness of chiral molecules can be probed spectroscopically, but acquiring data can take hours, which is a problem for time-resolved studies. The latest method records such data in a flash.

Most daily encounters with chirality and chiral recognition go unnoticed, including such mundane events as putting on shoes, shaking hands with someone and admiring the helical patterns of seashells. But the concept that certain objects and interactions have an intrinsic 'handedness' permeates the entire fabric of science, and has a crucial role in diverse physical, chemical and biological processes. Of special importance for the molecular sciences are the different chemical and biochemical reactivities displayed by enantiomeric (mirror-image) compounds. Indeed, many drugs are single enantiomers; their mirror-image versions are often ineffective, or even harmful.

The development of tools to discriminate between enantiomers and to work out how chiral molecules interact is therefore an ongoing challenge. In particular, the unique signatures that can be obtained using optical probes that are based on chiral — circularly polarized — light (Fig. 1a) represent a long-standing, yet constantly evolving avenue of research[1]. On page 310 of this issue, Rhee *et al.*[2] demonstrate a significant advance that promises to open new vistas in the realm of chiroptical spectroscopy, including the tantalizing possibility of interrogating chirospecific phenomena with femtosecond temporal resolution (1 femtosecond is 10^{-15} seconds).

When electromagnetic radiation passes through a randomly oriented (isotropic) ensemble of non-chiral molecules — such as those in a gas, liquid or glass — its state of polarization, defined by the direction of oscillation of the electric-field vector, remains unaltered. This implies that the sample's frequency-dependent refractive index, n, and absorption index, κ, are independent of optical polarization. These parameters govern the speed and intensity of a wave propagating through the medium.

In contrast, light traversing a chiral medium experiences an intrinsic helical anisotropy, commonly referred to as optical activity, which causes right-circularly (R) and left-circularly (L) polarized light to undergo distinct retardation ($n_L \neq n_R$) and attenuation ($\kappa_L \neq \kappa_R$) processes[1]. The differential retardation ($\Delta n = n_L - n_R$) leads to the phenomenon of circular birefringence (CB, Fig. 1b), the observable effect of which is the rotation of linearly polarized light from its original angle; the differential attenuation ($\Delta\kappa = \kappa_L - \kappa_R$) causes circular dichroism (CD, Fig. 1c), in which R- and L-polarized light are absorbed unequally.

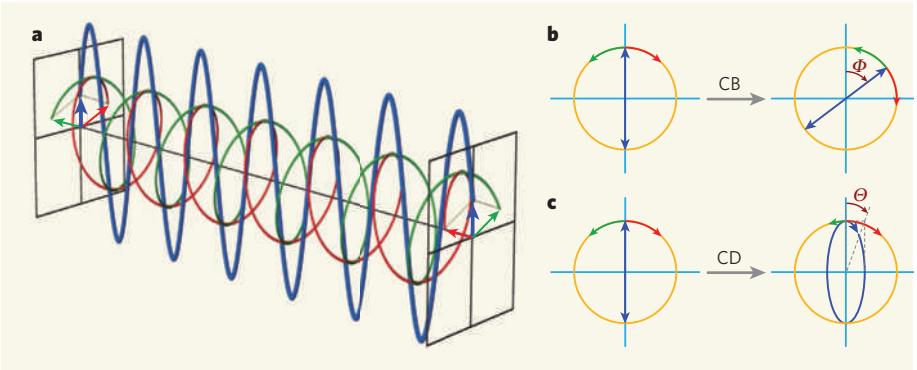

Figure 1 | Optical polarization and optical activity. a, The oscillating electric-field vector of linearly polarized electromagnetic radiation (blue) can be broken down into equal contributions of right-circular (red) and left-circular (green) polarized waves. **b**, Chiral media are optically active — they impose an intrinsic anisotropy on linearly polarized light. Here, the electric-field vector oscillates in a plane defined by the blue arrow. The phenomenon known as circular birefringence (CB) causes one component of circular polarization to propagate faster than the other, so that the plane of the electric-field vector rotates by an angle Φ. **c**, In circular dichroism (CD), one component of circular polarization is absorbed more strongly than the other, leading to net polarization ellipticity at an angle Θ. Rhee *et al.*[2] report a technique for acquiring information about the optical activity of chiral compounds on a femtosecond timescale, in principle opening the way to ultrafast time-resolved studies of chirospecific chemical and biochemical processes.

The two enantiomeric forms of a chiral compound display optical activities (Δn and $\Delta\kappa$) of equal magnitude, yet opposite sign, reflecting the chirospecific nature of their interactions with oscillating electric and magnetic fields. The patterns obtained by recording CB or CD as a function of wavelength thus afford a spectral 'fingerprint' for a given enantiomer and its environment. The majority of such measurements rely on electronic excitations caused by radiation in the visible and ultraviolet regions of the spectrum. But analogous methods involving infrared light — which excites molecular vibrations — have also emerged as powerful probes of molecular structure and function[1,3]. Rhee *et al.* build on the techniques of vibrational circular dichroism (VCD)[4,5], in which the unique chiroptical behaviour displayed by each vibrational feature of an infrared absorption spectrum conveys information about the configuration and conformation of the molecule under investigation.

Unfortunately, the chiroptical response of an isotropic solution is exceedingly small, and so any viable probe of CD must be able to distinguish a minuscule chiral absorption signal from a substantially larger background of achiral absorption. For VCD (where the ratio of signal to background absorption is typically 10^{-5}–10^{-6}), exquisitely sensitive detection schemes are needed to isolate the slight differences in sample absorption revealed by alternating bursts of R- and L-polarized infrared light. Even when the most efficient data-collection methods are used (such as Fourier-transform infrared spectroscopy[4]), hours of continuous averaging are often required to produce statistically meaningful VCD spectra, thus limiting the usefulness of the technique for time-resolved studies.

But Rhee *et al.*[2] have developed a strategy that permits both vibrational CB (known as vibrational optical rotatory dispersion, VORD) and VCD to be recorded without the need to

differentially discriminate minute signals. In their technique, a short infrared pulse is used to coherently excite vibrations in a dissolved chiral compound, thereby creating a macroscopic assembly of oscillating dipoles. This induced 'polarization' radiates the vibrational properties of the target molecules in the form of a signal known as a free-induction decay. This signal has chirospecific information encoded in the amplitude and phase of its electric field — in the same way that the characteristic sound of a ringing bell reflects its size and shape.

So how is the weak chiroptical signal discriminated from the strong achiral background? The authors rely on the fact that linearly polarized light always develops another polarized component (perpendicular to the original direction of polarization) as it crosses a chiral medium (Fig. 1b, c). Using polarization analysers of exceptional quality aligned to detect only signals orthogonal to the impinging (linearly polarized) infrared beam, the authors were able to isolate the desired optical-activity response in their experiments.

To amplify and interpret the responses, Rhee *et al.* exploited the phenomenon of interference, in which the superposition of oscillating waves gives rise to distinctive patterns of new waves. They thus combined the free-induction decay from the chiral sample with an infrared reference pulse of much greater intensity. The coherently beating portion of the resultant pattern — the product of the weak signal field and the strong reference field — can be isolated and analysed using Fourier-transform manipulations[6], yielding information about the sample.

Ultimately, the authors were able to ascertain a property of chiral molecules known as the complex susceptibility. This quantity consists of a part that is related to Δn and another part that is related to $\Delta\kappa$. In this way, VORD and VCD spectra can be acquired for each incident pulse of infrared radiation, with the timescale of each measurement essentially being given by the pulse duration (less than 100 femtoseconds).

Rhee *et al.* validated their approach by examining the enantiomers of a simple chiral molecule (limonene) dissolved in solution. They showed that the resulting spectra match those obtained by conventional VCD spectrometers and agree with theoretical VCD predictions. In principle, the techniques and concepts introduced in this work[2] should be applicable to a variety of chiroptical studies on systems in equilibrium, including those based on electronic (rather than vibrational) phenomena, provided that polarization analysers of sufficient quality are available for the targeted spectral region.

A time-resolved analysis of optical activity has yet to be performed, but the present results establish the basic principles required for studies in which changes in chirality induced by an abrupt pulse of 'pump' light can be monitored at subsequent points in time using a synchronously delayed infrared 'probe'. The resulting arrays of VORD and VCD spectra should allow an unprecedented glimpse of the ultrafast structural and conformational changes that accompany chirospecific chemical and biochemical transformations, such as those that occur during asymmetric catalysis or protein folding. Once the practical details are sorted out, the successful execution of time-resolved measurements will revolutionize our ability to interrogate chiral molecules and their interactions, in much the same way that the advent of high-speed photography overcame the limitations of visual perception. ■

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CANCER

The nuances of therapy

Lee M. Ellis and David A. Reardon

Oncologists use drugs that limit a tumour's blood supply to prevent its growth. Although the initial effects of these drugs are beneficial to patients, new data suggest that their long-term effects warrant further study.

Angiogenesis — the formation of new blood vessels — is a hallmark of cancer, and allows tumour growth. Anti-angiogenic therapy offers great promise and is often used to treat cancer, either alone or in combination with chemotherapy. But, like all anticancer therapies, agents that inhibit tumour angiogenesis are prone to either intrinsic or acquired resistance. Pàez-Ribes *et al.*[1] and Ebos *et al.*[2] show in two preclinical (*in vitro* and animal) studies[1,2] published in *Cancer Cell* that, depending on treatment conditions, anti-angiogenic therapy could theoretically increase the likelihood of tumour invasiveness and spread.

One protein with a central role in promoting angiogenesis is vascular endothelial growth factor (VEGF), and anti-VEGF agents are therefore commonly used to treat cancer. Nonetheless, as highlighted by several studies, host and tumour responses to loss of VEGF-mediated signalling can be complex[3].

In mice, deletion of a single copy of the *VEGF* gene causes embryonic death[4], suggesting that it is essential for survival. Loss of this gene specifically in endothelial cells, which line blood vessels, increases the probability of angiogenesis-related disease conditions such as thrombosis, haemorrhage and fibrosis[5]. What's more, VEGF inhibition affects not only tumour vasculature but also healthy host tissues. When VEGF activity is impaired in tumour-free mice, for example, compensatory pathways are activated that in theory could augment tumour invasion and metastasis in patients with cancer. Ebos *et al.* have previously shown[6] that anti-VEGF therapy increases levels of

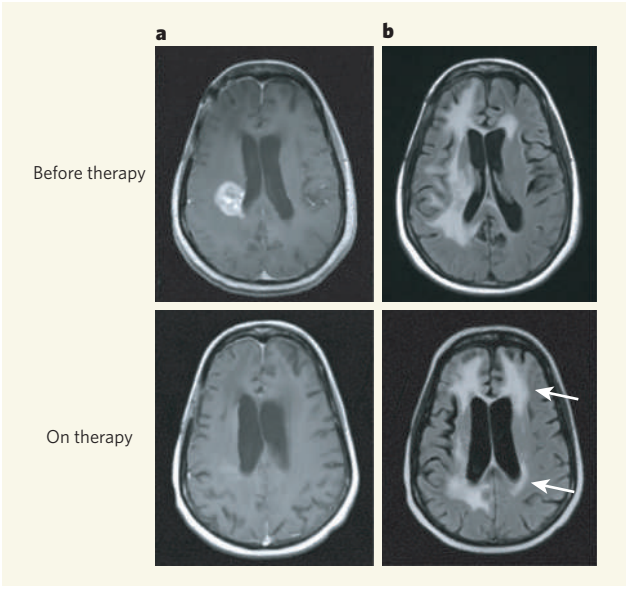

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Figure 1 | The MRI evidence. a, In agreement with the latest preclinical data[1,2], MRI scans from a patient with recurrent glioblastoma show that, after treatment with the VEGF-neutralizing antibody bevacizumab and the chemotherapeutic agent irinotecan, the macroscopic 'enhancing' tumour disappears, consistent with a complete response. **b,** However, microscopic tumour infiltration to other brain regions (arrows) is detectable in the patient after this therapy, using a different type of MRI that highlights brain inflammation and swelling[11].