Chemical compass model of avian magnetoreception

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Approximately 50 species, including birds, mammals, reptiles, amphibians, fish, crustaceans and insects, are known to use the Earth's magnetic field for orientation and navigation[1]. Birds in particular have been intensively studied, but the biophysical mechanisms that underlie the avian magnetic compass are still poorly understood. One proposal, based on magnetically sensitive free radical reactions[2,3], is gaining support[4–11] despite the fact that no chemical reaction in vitro has been shown to respond to magnetic fields as weak as the Earth's ($\sim$50 μT) or to be sensitive to the direction of such a field. Here we use spectroscopic observation of a carotenoid–porphyrin–fullerene model system to demonstrate that the lifetime of a photochemically formed radical pair is changed by application of $\leq$50 μT magnetic fields, and to measure the anisotropic chemical response that is essential for its operation as a chemical compass sensor. These experiments establish the feasibility of chemical magnetoreception and give insight into the structural and dynamic design features required for optimal detection of the direction of the Earth's magnetic field.

Two principal mechanisms for animal magnetoreception have been put forward (reviewed in ref. 1), based on specialized deposits of magnetic iron minerals and magnetically sensitive photochemical reactions, respectively. The latter, the radical pair mechanism, is well established as the source of a variety of magnetic effects on free radical reactions *in vitro*[12,13]. It has been suggested that the avian compass mechanism relies on magneto-sensitive radical pairs formed by photoinduced intramolecular electron transfer reactions in an array of aligned photoreceptors located in the retina[2,3]. A promising candidate radical pair comprises the reduced flavin cofactor and an oxidized tryptophan residue in a cryptochrome flavoprotein[3,8,10]. Such a photochemical process could, in principle, account for two fundamental behavioural characteristics of the avian compass: its dependence on the wavelength of the ambient light[14] and the fact that birds respond to the inclination, rather than the polarity, of the geomagnetic field[15]. This proposal has been corroborated, in part, by the detection of cryptochromes in the retinae of migratory birds[5,6], and the finding that these proteins are expressed when the birds perform magnetic orientation[6], and by the observation of light-dependent, cryptochrome-mediated magnetic field effects on plant growth[11]. Theoretical work has also confirmed the principle and clarified some of the details[7–10]. Further compelling evidence for the involvement of radical pairs has come from the observation that weak radiofrequency magnetic fields, which can have profound effects on radical pair reactions *in vitro*[16], can disrupt the ability of birds to orient in the Earth's magnetic field[4,17].

Despite numerous studies[12], it has never been demonstrated that a static magnetic field as weak as that of the Earth can produce detectable changes in chemical reaction rates or product yields. Nor has a radical pair reaction been shown to respond to the direction of such a field, an essential requirement for a compass sensor. For this, it is essential that at least one of the radicals is immobilized so that its anisotropic magnetic interactions are preserved[2].

Here we demonstrate, as a proof-of-principle, that a photochemical reaction can act as a magnetic compass. The molecule selected for this purpose is a triad composed of linked carotenoid (C), porphyrin (P) and fullerene (F) groups (Fig. 1)[18]. Green-light irradiation efficiently produces the spin-correlated electronic singlet state of the radical pair (or biradical) $^S[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$ by sequential intramolecular electron transfers (Fig. 1). $^S[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$ undergoes reverse electron transfer, either directly to the ground state, with rate constant k_S, or to the excited triplet state ^{T}C–P–F, with rate constant k_T, having first converted to the triplet radical pair, $^T[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$. This last process is controlled by the magnetic interactions of the two unpaired electrons and is the magnetic-field-sensitive step. As has been observed for related triads[19,20], an applied field alters the observed lifetime of $[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$ by modifying the singlet–triplet character of its spin states, so changing the relative contributions of k_S and k_T to the overall kinetics.

We began by characterizing the effects of applied magnetic fields on the disappearance kinetics of the radical pair in isotropic solution. The transient absorption signal of $C^{\bullet+}$ in $[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$ at 133 K (Fig. 2a, top), which has a lifetime of $\sim$190 ns in zero field, was markedly increased to $\sim$380 ns in an 8-mT field. The amplitude of the magnetic field effect decreased as the temperature increases and the difference signals were biphasic below $\sim$200 K (Fig. 2a, bottom). Both of these properties are characteristic of a singlet-born radical pair with $k_T < k_S$, undergoing spin-lattice relaxation at a rate comparable to its recombination[21].

The magnetic field dependence of the $[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$ transient absorption at 119 K (Fig. 2b) shows the biphasic magnetic field response expected for a long-lived radical pair[22]. The change in sign below $\sim$1 mT is the 'low field effect': normally observed for the product yields of radical reactions in solution[12,13], it was manifested here as a change in the radical pair kinetics. The effect of the applied field on the radical pair absorption was opposite at 100 and 400 ns, as expected from the biphasic time dependence in Fig. 2a (bottom).

Finally, experiments performed in magnetic fields comparable to that of the Earth (Fig. 2c) revealed changes in radical pair absorption of up to $\sim$1.5%. The biphasic time dependence observed at higher fields (Fig. 2a) was inverted here because of the low field effect (Fig. 2b). Thus, for $t > 400$ ns, $[C^{\bullet+}\text{–}P\text{–}F^{\bullet-}]$ recombined more rapidly in a $\sim$50-μT field than it did in zero field, which was in turn faster than when the field exceeded 1 mT. This seems to be the first observation of a chemical effect of a magnetic field as weak as $\sim$50 μT. The electron Zeeman interaction in such a magnetic field is more than a million times smaller than the thermal energy, $k_B T$, implying a negligible effect on the position of a chemical equilibrium or the kinetics of an activated reaction. However, such considerations are irrelevant for the interconversion of singlet and triplet states of radical pairs, a process that is activationless and far from equilibrium.

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To function as a chemical compass, a radical pair magnetoreceptor must respond anisotropically to an external magnetic field. We demonstrated this property for $[C^{\bullet+}–P–F^{\bullet-}]$ using an aligned sample, obtained by freezing C–P–F in the nematic phase of a liquid crystal in the presence of a strong (800 mT) magnetic field. Transient absorption signals, $A(\theta)$, were recorded in a 3.1-mT magnetic field, the orientation of which (defined by the angle θ; Fig. 3a) was varied with respect to the alignment axis. The measured $[C^{\bullet+}–P–F^{\bullet-}]$ absorption (Fig. 3b) shows a clear dependence on θ which, similar to the avian magnetic compass, is invariant to inversion of the field direction; that is, $A(\theta) = A(\theta + \pi)$.

To obtain additional insight, the anisotropy was also measured by a transient absorption photoselection method (Fig. 3a) using unpolarized laser pump pulses, polarized probe light and a frozen solution of randomly oriented molecules. Calculations (see Supplementary Information) show that the transition dipole moment of the $C^{\bullet+}$ absorption at 980 nm is almost parallel to the long axis of C–P–F, so that molecules aligned with the polarization axis are preferentially detected. The θ-dependence of the $[C^{\bullet+}–P–F^{\bullet-}]$ absorption, $A'(\theta)$, where θ is now the angle between the polarization axis and the magnetic-field vector, is essentially identical to that observed in the aligned sample (Fig. 3b, c).

A consideration of the orientational averaging involved in these two experiments (see Supplementary Information) suggests that in both cases, the observed signal should be a weighted sum of even powers of $\sin\theta$, but that in the photoselection measurement all terms beyond $\sin^2\theta$ vanish; that is:

$$A(\theta) \propto \sum_{n=0}^{\infty} a_{2n} \sin^{2n}\theta; \quad A'(\theta) \propto a'_0 + a'_2 \sin^2\theta$$

Within experimental error, both $A(\theta)$ and $A'(\theta)$ have a $\sin^2\theta$ dependence, consistent with $a_{2n} \approx 0$ for all $n > 1$ and a simple orientation dependence of the underlying magnetic field effect (see Supplementary Information).

Our demonstration that a radical pair can act as a chemical compass yields valuable information on the design of a sensitive *in vivo* magnetoreceptor. Although not ideal as a compass, $[C^{\bullet+}–P–F^{\bullet-}]$ 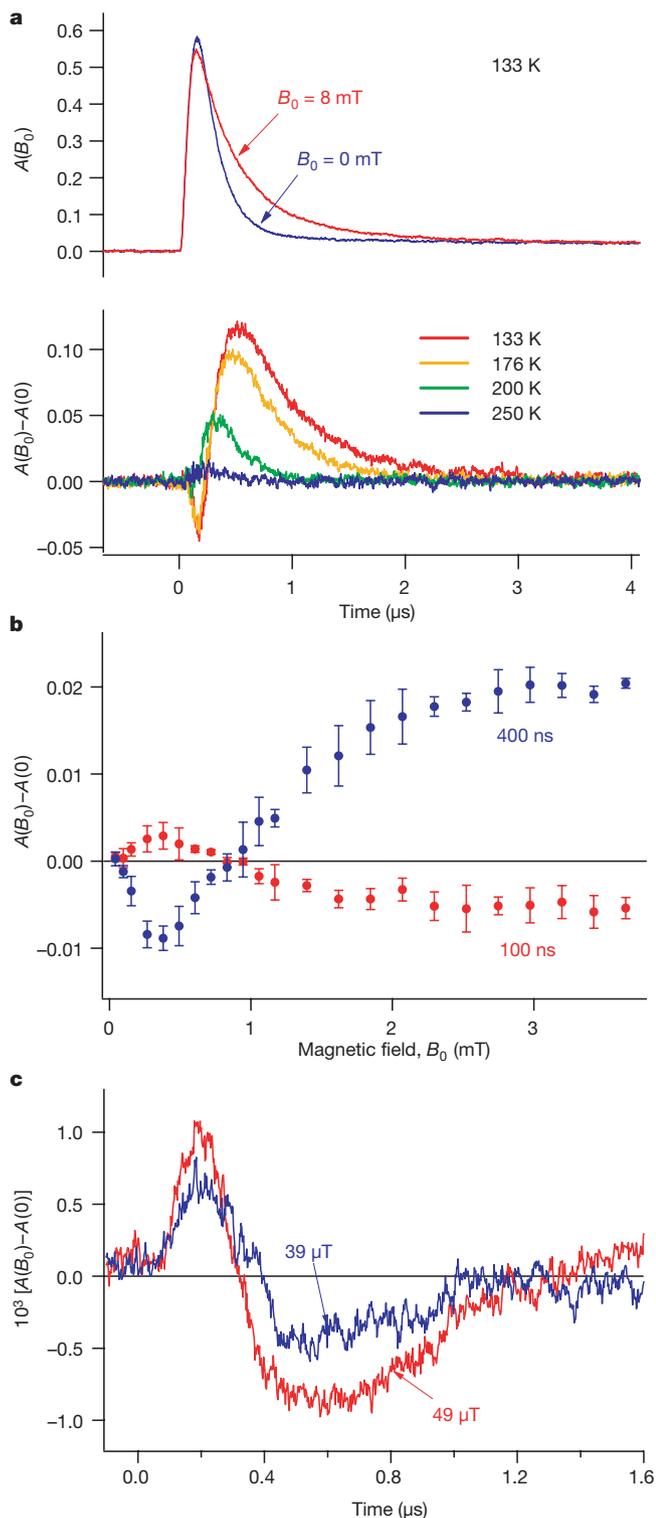does have several favourable properties—it is rapidly and efficiently formed by absorption of light and is long enough lived to allow a $\sim$50 µT magnetic field to have a detectable effect on its spin dynamics. Furthermore, the radical centres are sufficiently separated (which minimizes interference from radical–radical exchange and dipolar interactions[22]), whereas their fixed relative orientations and restricted molecular motion in frozen solution result in minimal static and dynamic averaging of the crucial anisotropic magnetic interactions. A further attractive feature of $[C^{\bullet+}–P–F^{\bullet-}]$ is its highly unsymmetrical distribution of magnetic nuclei (Fig. 1), a feature associated with optimum isotropic and anisotropic magnetic field effects[23]. However, both its recombination and spin relaxation, jointly responsible for the biphasic time dependence (Fig. 2a, c), are fast enough (even at 113 K) to attenuate the $\sim$50-µT signal (Fig. 2c). Responses closer to the theoretical maximum of 20–40% (ref. 22), expected for spin correlation lifetimes $\geq$1 µs would have allowed the anisotropy measurements (Fig. 3) to be performed at much lower fields than $\sim$3 mT. An additional factor responsible for a reduction in the magnetosensitivity of $[C^{\bullet+}–P–F^{\bullet-}]$ is the extent of delocalization of the unpaired electron in $C^{\bullet+}$ and the associated cancellation of the anisotropic effects of the many, differently aligned, magnetic hyperfine tensors[8].

In principle, greater sensitivity to weak magnetic fields would be possible for radical pairs formed in a specialized photoreceptor such as cryptochrome[3,10]. By analogy with photosynthetic charge separation[24], efficient sequential electron transfer along a chain of tryptophan residues to the flavin cofactor in cryptochrome could produce a well-separated ($\geq$2 nm) radical pair with weak inter-radical interactions[25] within 10 ns (allowing negligible loss of spin correlation), with slow back electron transfer ($\geq$1 µs)[10]. A suitably aligned and immobilized array of such photoreceptors could, at physiological temperatures, have the dynamical properties required for slow spin relaxation ($\geq$1 µs), a condition that is only approached by $[C^{\bullet+}–P–F^{\bullet-}]$ at the lowest temperatures studied here. For example, flavin–tyrosine radical pairs that retain their spin correlation for up to 20 µs have been detected in photolyase (a flavoprotein closely related to cryptochrome) at 278 K (ref 26). In addition, the radicals formed in a photoactive protein may have just a few dominant hyperfine interactions with tensor properties that lead to reinforcement, rather than
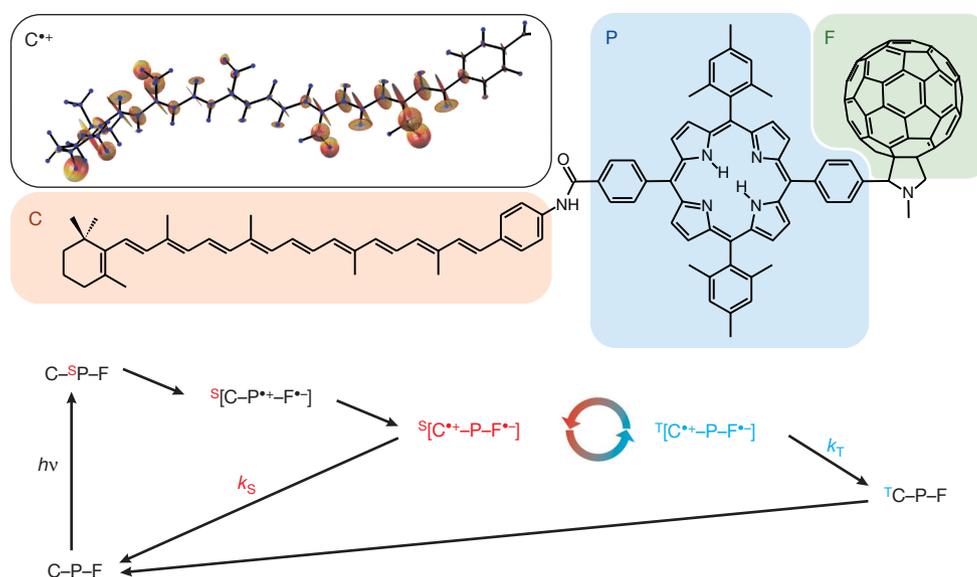
Figure 1 | C–P–F triad. Structure (top) and reaction scheme (bottom) of the C–P–F triad used to demonstrate the principle of a chemical compass. The interconversion of the singlet (S) and triplet (T) states of the radical pair $[C^{\bullet+}–P–F^{\bullet-}]$ is driven by magnetic hyperfine interactions and is modulated by the Zeeman interaction with an external magnetic field. $[C^{\bullet+}–P–F^{\bullet-}]$ can recombine spin-select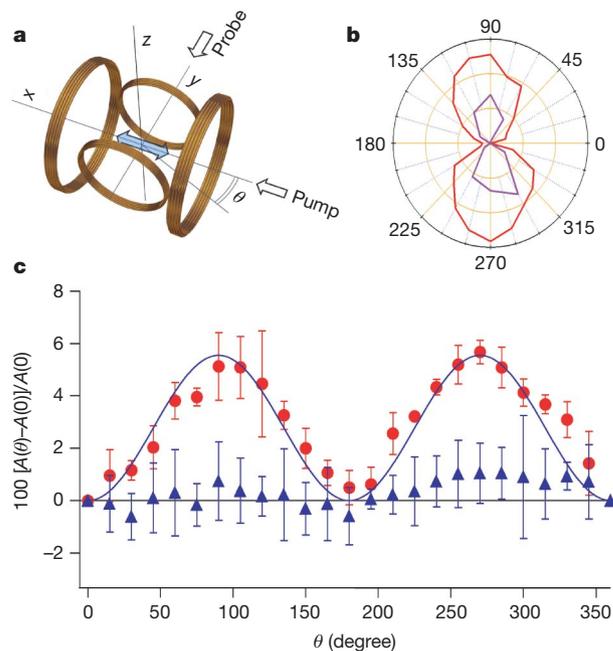ively, with rate constants k_S and k_T. As the hyperfine interactions are anisotropic, and $k_S \neq k_T$, the lifetime of the radical pair reaction depends on its orientation with respect to the external magnetic field. The inset at top left is a representation of the anisotropic hyperfine interactions in $C^{\bullet+}$; 22 of the 46 protons in this radical have isotropic hyperfine couplings larger than 100 µT. $F^{\bullet-}$, by contrast, is almost devoid of hyperfine couplings. $h\nu$: light excitation.

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cancellation, of the anisotropic effects, as seems to be the case for two of the nitrogen atoms in the radical form of the flavin adenine dinucleotide cofactor, FADH[•](ref. 8). The favourable asymmetric distribution of hyperfine interactions in $[C^{•+}–P–F^{•-}]$ might also be found in a cryptochrome. For example, reoxidation of the photochemically reduced flavin cofactor in flavoproteins is mediated by molecular oxygen[27]. Both O_2 and its reduced form, superoxide $O_2^{•-}$, are paramagnetic, have no hyperfine interactions, and if paired with FADH[•] might allow the reoxidation kinetics to be magnetic field dependent, although it is not clear whether $O_2^{•-}$, in particular, would meet the requirement of slow spin relaxation.

These possibilities could be tested *in vitro* in a variety of ways. Spectroscopic measurements of the kind described here could be performed on isolated cryptochromes subject to applied magnetic fields. Radiofrequency magnetic fields could also be used as a diagnostic test for the formation of transient radical pairs and might allow their identification[28]. The involvement of O_2 could be established by spin-trapping of $O_2^{•-}$ or by a ^{17}O magnetic isotope effect[29].

Figure 2 | Isotropic magnetic field effects on C–P–F. Transient absorption data recorded for frozen solutions of C–P–F showing the magnetic field-dependence of the recombination of the radical pair state, $[C^{•+}–P–F^{•-}]$. Apart from the upper part of **a**, all data are field-on minus field-off differences: $A(B_0) − A(0)$. **a**, Top: absorption with and without an 8-mT applied field at 133 K. Bottom: difference signals as a function of temperature. All signals rise with the instrumental time constant (~50 ns). **b**, Changes in the transient absorption of $[C^{•+}–P–F^{•-}]$ at 119 K averaged over a 50-ns period centred at 100 ns and 400 ns after its formation. **c**, Changes in the transient absorption of $[C^{•+}–P–F^{•-}]$ at 113 K caused by 39-μT and 49-μT applied magnetic fields. Error bars, ±1 s.d.

Figure 3 | Operation of C–P–F as a chemical compass. a, Schematic of the experimental arrangement used to measure the anisotropy of the magnetic field effect on the disappearance kinetics of $[C^{•+}–P–F^{•-}]$. The direction of the applied magnetic field generated by two orthogonal pairs of Helmholtz coils is varied in 15° steps in the *x*–*y* (horizontal) plane. The pump laser pulses and continuous probe light propagate along the *x* and *y* axes, respectively. The central blue arrow on the *x* axis represents the direction of the alignment axis in the experiments on C–P–F dissolved in a frozen nematic liquid crystal, and the probe light polarization axis in the photoselection measurements. θ is the angle between the magnetic field vector and the *x* axis. **b**, Polar plot of the anisotropy of the magnetic field effect, $[A(\theta) − A(0)]/A(0)$ (as a function of θ, in degrees), on the transient absorption of $[C^{•+}–P–F^{•-}]$ detected using an aligned sample (purple, 3.1 mT, 193 K) and by photoselection (red, 3.4 mT, 88 K). The maximum magnetic field effects in the two cases were ~1.5% and ~5%, respectively. The data for the aligned sample have been doubled for clarity. The anisotropy is smaller in the liquid crystal measurement than in the photoselection experiment mainly because of the faster spin relaxation at the higher temperature of the former. **c**, Data from the photoselection measurements. The red dots show the dependence of the $[C^{•+}–P–F^{•-}]$ absorption on the direction of the magnetic field, θ. The solid line is the best fit to a $\sin^2 \theta$ form. Also shown (blue) are the signals detected when the polarization axis of the probe light was *z* (that is, vertical). No θ-dependence is expected or seen. Error bars, ±1 s.d.

METHODS SUMMARY

The C–P–F triad was synthesized according to previously described procedures[18]. Isotropic magnetic field effects were measured using $100\,\mu M$ frozen solutions of C–P–F in 2-methyl-tetrahydrofuran. C–P–F was excited at 532 nm with 7-ns, 5-mJ, 10-Hz repetition rate pulses from a frequency-doubled Nd:YAG laser and the radical pair was detected by means of the $C^{\bullet+}$ absorption band at 950 nm using light from a xenon arc lamp. Anisotropic magnetic field effects were measured on an aligned sample and on a frozen isotropic sample using photoselection. For the aligned sample, the conditions were C–P–F ($100\,\mu M$) aligned in E7 liquid crystal (Merck), with the alignment axis parallel to the x axis (Fig. 3a). The magnetic field strength was 3.1 mT. The $C^{\bullet+}$ absorption was monitored at 900 nm with unpolarized light (xenon arc lamp) and averaged over the period 400 ± 200 ns. Measurements were performed at 193 K. For the frozen isotropic sample, the Nd:YAG pump pulses (x direction) were depolarized. The probe light (y direction) was supplied by a diode laser ($\lambda = 980$ nm, 100 mW), and was polarized in the x or z direction (see Fig. 3a). The magnetic field strength was 3.4 mT. Measurements were performed at 88 K. For the liquid crystal and photoselection experiments, the magnetic field was generated by means of two sets of orthogonal Helmholtz coils aligned with the x and y directions (Fig. 3a). They had the same number of turns but different diameters (125 mm and 85 mm, respectively) and carried different currents.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Author Contributions K.M., K.B.H. and F.C. performed the experiments. K.M., K.B.H and C.R.T analysed the data. P.A.L. and D.G. synthesized the triad molecule. C.T.R. and P.J.H. analysed the orientational averaging. I.K. performed *ab initio* calculations. F.C., C.R.T. and P.J.H. designed the study. C.R.T. co-ordinated the study. P.J.H. wrote the paper. All authors discussed the results and commented on the manuscript.

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METHODS

Isotropic magnetic field effects. Transient absorption signals of $C^{\bullet+}$ in 2-methyl-tetrahydrofuran (MTHF) were detected with an Oriel 77250 Series 1/8 m monochromator, a grating optimized for maximum reflectivity at 1,000 nm and a Hamamatsu photomultiplier R5108. The magnetic field was produced by a pair of Helmholtz coils (radius 125 mm). Alternate measurements with and without the applied magnetic field were performed in synchrony with the laser. The temperature of the sample, held in a cryostat, was controlled with flowing nitrogen gas. The cryostat and field coils were enclosed in a mu-metal box to shield the sample from the Earth's magnetic field. The field inside the box was $0 \pm 10\,\mu T$ (measured with high-sensitivity one- and three-dimensional Hall transducers).

Anisotropic magnetic field effects. C–P–F ($100\,\mu M$) was dissolved in E7 liquid crystal (Merck) and aligned in a 800-mT magnetic field by cycling the temperature three times between 20 °C and 60 °C (between the nematic and isotropic phases, respectively). The aligned sample was allowed to cool in the magnetic field and was then transferred into the cryostat and cooled further to 193 K. The solution was sufficiently viscous that the cooling and subsequent transfer could be done without loss of alignment.

For the photoselection experiments, a Halbo Optics WDQ15M depolarizer was used to depolarize the Nd:YAG pump pulses. A Thorlabs L980P100 diode laser supplied the probe light, which was polarized using a Glan Thompson polarizer.

Transient absorption data were measured for each of 24 equally spaced values of θ (Fig. 3a) in random order and sorted according to θ in the computer memory. This process was repeated 300 times for the photoselection experiments or 1,500 times for the liquid crystal sample with a different sampling order each time, and the resulting signals averaged for each θ. In all other respects, the experiments were performed essentially as described for the MTHF solutions.

To confirm the anisotropic behaviour of the magnetic field effect, the experiment was repeated with a 2.6-mT field in the x–z plane, and the probe light still directed along the y axis. When the polarization axis of the probe light was rotated by $\pm 45°$ in the x–z plane, the absorption was phase-shifted by $\pm 45°$ (Supplementary Fig. 1).

Magnetic fields. Calibration measurements on the two sets of orthogonal Helmholtz coils used for the liquid crystal and photoselection experiments revealed that a 46% larger current was required in the x coils to achieve the same magnetic field strength as the y coils. The currents through the two coils were:

$$I_x(\theta_n) = 1.46\ I_0 \cos \theta_n$$

$$I_y(\theta_n) = I_0 \sin \theta_n$$

where $\theta_n = n\delta$, $\delta = 15°$ and $n = 0, 1, \ldots 23$.

The magnetic fields produced by the two sets of coils were measured with a 3D Hall probe and are shown as the dashed lines in Supplementary Fig. 2. The black dashed line is $\sqrt{I_x^2(\theta_n) + I_y^2(\theta_n)}$. The slight variation in this quantity with θ_n was reduced by modifying the currents using a table of correction factors g_n:

$$I_x = 1.46\ I_0 \cos \theta_n / g_n$$

$$I_y = I_0 \sin \theta_n / g_n$$

The values of g_n were in the range 0.996–1.027. The resulting current (Supplementary Fig. 2, solid black line) showed a reduced variation with θ. The averaged magnetic field for the photoselection experiments was 3.4 mT, with a standard deviation of 0.02 mT.