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Review article

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Navigation of migratory songbirds: a quantum magnetic compass sensor

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Abstract: The remarkable ability of migratory birds to navigate accurately using the geomagnetic field for journeys of thousands of kilometres is currently thought to arise from radical pair reactions inside a protein called cryptochrome. In this article, we explain the quantum mechanical basis of the radical pair mechanism and why it is currently the dominant theory of compass magnetoreception. We also provide a brief account of two important computational simulation techniques that are used to study the mechanism in cryptochrome: spin dynamics and molecular dynamics. At the end, we provide an overview of current research on quantum mechanical processes in avian cryptochromes and the computational models for describing them.

Keywords: cryptochrome; magnetoreception; molecular dynamics; radical pair mechanism; spin dynamics.

Zusammenfassung: Zugvögel können mit Hilfe des Erdmagnetfeldes über tausende Kilometer hinweg akkurat navigieren. Es wird heutzutage angenommen, dass quantenmechanische Vorgänge im Cryptochromprotein diese Fähigkeit ermöglichen. Bei diesen Prozessen handelt es sich um Radikalpaar-Reaktionen. In diesem Artikel werden wir den Radikalpaarmechanismus erklären und erläutern, warum er momentan die vorherrschende Theorie ist. Des Weiteren werden wir kurz zwei wichtige, rechnerische Simulationstechniken vorstellen, die benutzt werden um Cryptochrome zu studieren: Spindynamik und Molekulardynamik. Abschließend geben wir einen Überblick über aktuelle Forschungsfragen.

Schlüsselwörter: Magnetsinn; Cryptochrom; Radikalpaarmechanismus; Molekulardynamik; Spindynamik.

Introduction

To secure better environmental conditions, migratory birds fly thousands of kilometres every year. Their ability to navigate over vast distances with seemingly little effort has fascinated scientists and non-scientists alike. It is clear that birds use a multitude of cues for navigation over different geographic scales including the sun, stars, olfaction and landmarks (Mouritsen, 2018; Spiecker et al., 2021). In this article, we concentrate on the magnetic compass sense.

Given the low intensity of the Earth's magnetic field ($\sim 5 \times 10^{-5}$ T)—about 100 times weaker than the field experienced near a small fridge magnet—it is remarkable that living organisms have a magnetic sense. Although avian magnetoreception has been researched for the last 50 years, it is still unclear how this feat is achieved at the molecular level. The broad range of length scales (Figure 1) involved means that a highly multidisciplinary approach, as realised in SFB 1372, is essential for an understanding of the mechanism.

A functioning magnetic compass sense requires a receptor molecule to translate the direction of the geomagnetic field into a chemical signal which can be amplified and sent to the brain for processing. We focus here on the dominant hypothesis, known as the radical pair mechanism (RPM), in which the signal is derived from magnetically sensitive reactions of short-lived pairs of radicals (reactive molecules containing odd numbers of electrons) inside a protein called cryptochrome (Cry). A full description of this mechanism requires a quantum mechanical treatment (Fay et al., 2020). An additional hypothesis, based on biogenic magnetite (Fe_3O_4), is beyond the scope of this article (Winklhofer, 2010).

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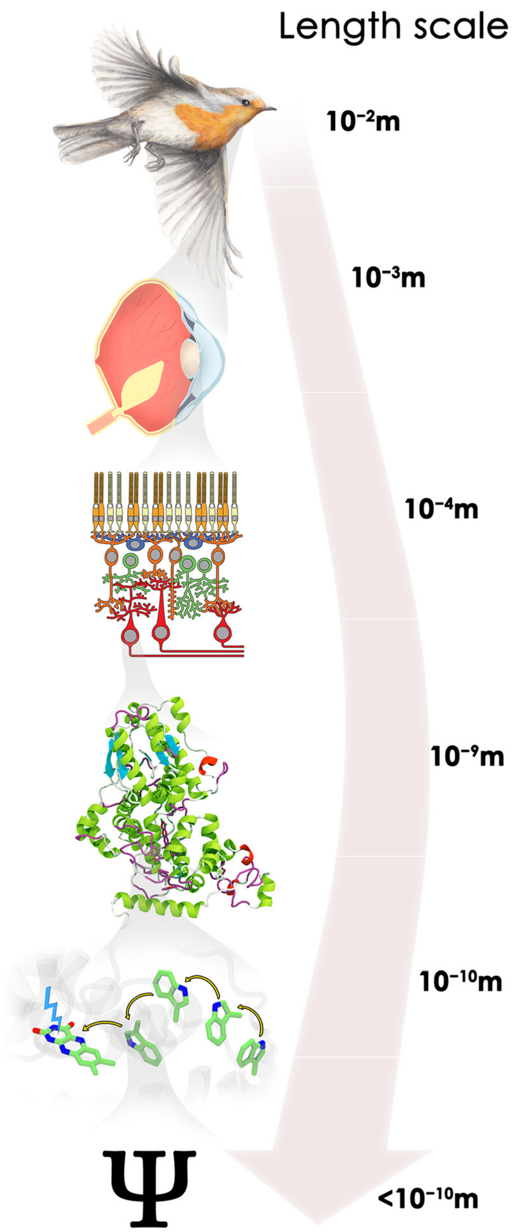

Figure 1: The range of distances involved demands a multidisciplinary approach to understand exactly how birds sense the direction of the Earth's magnetic field. Experiments to determine how birds (~10^{-2} m) respond to a magnetic field requires specialists in animal behaviour. The anatomy of the bird's eye (~10^{-3} m), and the cells in the retina (~10^{-4} m) where the magnetoreceptors are located, are studied by cell biologists. To sense the magnetic field, the cells must contain molecules that respond to magnetic stimuli having first been activated by light. The protein thought to fulfil this role is cryptochrome 4 (~10^{-9} m) whose properties are explored using biochemical and biophysical techniques. Inside the cryptochrome, light-induced magnetically sensitive radical pairs are formed by a series of electron-transfer reactions from tryptophan amino acid residues to a flavin chromophore (~10^{-10} m). The quantum nature of this 'radical pair mechanism' (<10^{-10} m) takes us to the realm of quantum physics (Greek letter Psi, Ψ) in order to understand the primary events of compass magnetoreception.

To date, members of the cryptochrome class of proteins are the only candidate radical pair magnetoreceptors. Their ability to form radical pairs when activated by blue light is consistent with behavioural studies of migratory birds which clearly show that the magnetic compass sense is light dependent (Wiltschko et al., 2010). Of the various types of avian cryptochrome, the requirement for photo-excitation makes cryptochrome 4 (Cry4) the most likely to have a magnetic sensing function (Xu et al., 2021). Cry4 binds a flavin adenine dinucleotide (FAD) cofactor which enables it to absorb visible light and is present in the retina where the magnetoreceptors are known to be located (Günther et al., 2018). SFB 1372 has recently discovered that at least two forms of Cry4 exist in migratory birds (Einwich et al., 2020). Cryptochromes are found in many organisms, including plants where they were first reported (Ahmad and Cashmore, 1993; Yu et al., 2010). While plant, insect and avian cryptochromes *in vitro* are sensitive to magnetic fields (Maeda et al., 2012; Sheppard et al., 2017; Xu et al., 2021), this may only be of biological significance for long-distance migrants. One aspect of SFB 1372's work is therefore to compare cryptochromes from different species.

In the following pages, we outline the quantum mechanical basis of the radical pair mechanism (Hore and Mouritsen, 2016), summarise what we hope to learn from computational and biophysical studies of Cry4 in SFB 1372, and how it will complement behavioural studies.

Radical pair mechanism

The key to understanding the radical pair mechanism is the fundamentally quantum property known as 'spin'. Electrons and atomic nuclei such as hydrogen and nitrogen have spin and therefore behave like microscopic magnets. We represent electron spins here by means of arrows (↑ and ↓). Molecules containing an even number of electrons usually have them arranged in pairs (↑↓) such that their magnetic moments cancel. Radicals have an odd number of electrons, meaning that at least one electron cannot have a partner with opposite spin. In a radical pair, the two odd electrons, one in each radical, can either have their spins antiparallel (↑↓) or parallel (↑↑). These states are known as singlet and triplet, respectively.

Figure 2(A) shows a simplified radical pair reaction scheme in which A and D represent electron acceptor and electron donor molecules. When one of the reactants absorbs light an electron is transferred from D to A and a radical pair, $[A^{\bullet-}\ D^{\bullet+}]$, is formed where the superscripts denote the odd electrons (dots) and the charges on the radicals. $[A^{\bullet-}\ D^{\bullet+}]$ is formed exclusively in the singlet (↑↓)

state and subsequently interconverts spontaneously, rapidly and reversibly with the triplet state (↑↑). The magnetic interactions of the odd electrons with the spins of atomic nuclei and with the geomagnetic field drive this interconversion at frequencies of around a million times per second (Hore and Mouritsen, 2016). While this is going on, the radical pair can either (a) recombine to form the initial reactants (A and D), or (b) react to form a product (P). Since pathway (a) involves pairing up the electrons again, it can only occur from the singlet state. Pathway (b) may occur for both singlet and triplet states. If the radical pair spends less time in the singlet state, less recombination will occur and more product will be formed, and vice versa. Since the external magnetic field is one of the driving forces of the interconversion, its direction can influence the yield of the chemical product via its effect on the relative amounts of singlet and triplet radical pairs. It is the oscillatory interchange of singlet and triplet that is fundamentally quantum in nature.

Spin relaxation (of which more later) is the enemy of the radical pair compass. After it is born as a pure singlet, the pair starts to relax towards its equilibrium state in which the two electron spins are completely uncorrelated, at which point the geomagnetic field can no longer influence the spin dynamics. For a sensitive compass, it is therefore crucial to maintain a non-equilibrium state for as long as possible, which in practice means for at least a microsecond (Kattnig et al., 2016a,b). Normally, weak magnetic interactions like the ones discussed here have negligible influence on the outcome of chemical reactions because they pale in comparison to the random fluctuations in energy experienced by all molecules at physiological temperatures. Radical pair reactions, by contrast, can respond to minute magnetic interactions, more than a million times smaller than thermal energies (~$k_B T$, where k_B = Boltzmann's constant and T = temperature), because the electron spins are far from equilibrium. Having been created as a reaction intermediate using the large amount of energy (>>$k_B T$) carried by photons of blue light (Figure 2(A)), it only takes a tiny additional amount of magnetic energy to tip the singlet-triplet balance and so cause more pairs to proceed to the product and fewer to return to the reactants, or vice versa. Thereby, a radical pair–based magnetoreception mechanism could make animals sensitive to environmental stimuli more than a million times lower in energy than previously thought possible.

The RPM is a genuine mechanism solidly backed by theory and experiment, with hundreds of laboratory studies reporting effects of external magnetic fields on radical pairs (Rodgers and Hore, 2009; Steiner and Ulrich, 1989; Woodward, 2002). Several behavioural observations support the RPM hypothesis. One is the finding that birds require light to use their compass sense (Wiltschko et al., 2010). Another is that birds have an inclination compass: instead of distinguishing north from south, they detect the difference in the directions of the magnetic pole and magnetic equator (Wiltschko and Wiltschko, 1995, 1972). This accords precisely with the RPM in which the yield of the reaction product is unaffected by an exact inversion of the magnetic field vector. Finally, migratory birds are unable to use their magnetic compass in the presence of weak radiofrequency fields (Engels et al., 2014; Ritz et al., 2004; Schwarze et al., 2016; for more details, see Spiecker et al., 2021 in this issue). This, too, could be consistent with radical pairs which are known to be affected by electromagnetic fields in resonance with their singlet-triplet interconversion frequencies (Hiscock et al., 2017).

There is clear evidence that suggests that magnetoreception starts in the bird's retinas (for more details, see

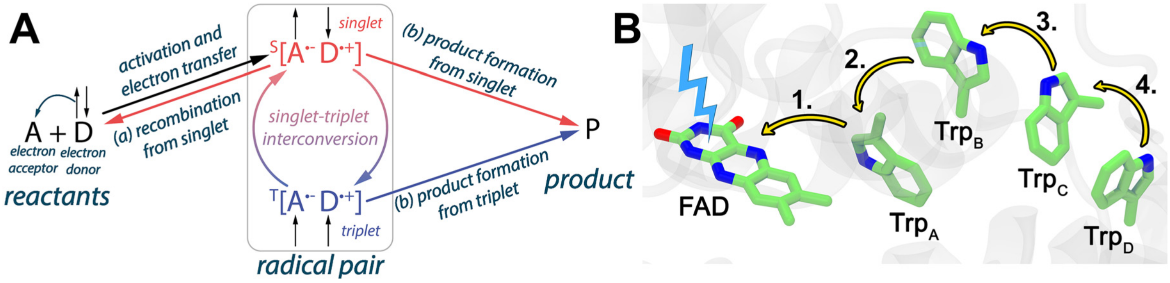

Figure 2: (A) A simplified scheme of the radical pair mechanism. The radical pair $[A^{\bullet-}\ D^{\bullet+}]$ is formed from reactants A and D by light-absorption (black arrow) and interconverts between singlet and triplet states (curved red/blue arrows). The singlet state can recombine to regenerate the reactants or react to form the product P (red arrows), while the triplet state can only form P (blue arrow). (B) Electron transfer in Cry4 to FAD along a chain of four tryptophan (Trp) residues. This process occurs after the protein has absorbed a photon of blue light and results in the formation of a radical pair comprising the FAD and the terminal tryptophan.

Haase et al., 2021 in this issue): the signal is carried to the brain along the optical nerve to Cluster N, a part of the brain region that processes visual information (Mouritsen et al., 2005). Cluster N has not been found in non-migratory songbirds, and chemical inactivation abolishes the ability of night-migratory birds to orient in a magnetic field (Zapka et al., 2009).

Cryptochrome

With strong experimental evidence pointing towards a radical pair–based magnetoreceptor in the birds' visual system, we turn our attention to cryptochromes, the only class of vertebrate proteins that can form long-lived radical pairs on exposure to light. More than 20 years ago, Ritz et al. (2000) suggested that the magnetically sensitive radical pair could be formed inside a cryptochrome by sequential electron transfers along a chain of three tryptophan (TrpH) residues to the FAD cofactor, triggered by blue-light activation of the FAD. Type-4 cryptochromes have four tryptophans (Einwich et al., 2020; Günther et al., 2018; Müller et al., 2015) all of which are involved in photoreduction of FAD (Hochstoeger et al., 2020; Xu et al., 2021).

Figure 2(B) shows the FAD excited by blue light (depicted by a lightning bolt) and the four TrpH residues in Cry4, where the yellow arrows indicate the sequential electron transfers. The wavelength of light required to excite FAD is around 450 nm, which is within the range of wavelengths (373–565 nm) the birds require to be able to use their magnetic compass in behavioural studies (Wiltschko et al., 2010). Depending on whether the fourth electron transfer occurs, the net result is the formation of either $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_CH}^{\bullet+}]$ or $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_DH}^{\bullet+}]$. Evidence of the sensitivity of these radical pairs to external magnetic fields comes from spectroscopic measurements of the photochemical yields of radicals in purified cryptochromes subject to millitesla magnetic fields (Maeda et al., 2012; Sheppard et al., 2017). Xu et al. (2021) have recently shown that both radical pairs contribute to the magnetic sensitivity of European robin Cry4 and that this protein, from a migratory bird, shows larger magnetic field effects than Cry4s from non-migratory chicken and pigeon.

It has not yet proved possible to detect effects of Earth-strength magnetic fields (~50 μT) on cryptochrome photochemistry. A demonstration of the sensitivity of cryptochromes to the direction of an external magnetic field would provide strong evidence to support the RPM hypothesis. Such a measurement would require the proteins to be aligned and immobilised (Lau et al., 2010), a challenge that has yet to be met experimentally.

The reactions of $[\mathrm{FAD}^{\bullet-}\ \mathrm{TrpH}^{\bullet+}]$ in cryptochromes are analogous to (a) and (b) in Figure 2(A) (Hore and Mouritsen, 2016): (a) is the spin-selective recombination of the singlet state of $[\mathrm{FAD}^{\bullet-}\ \mathrm{TrpH}^{\bullet+}]$, and (b) is the non-spin-selective deprotonation of $\mathrm{TrpH}^{\bullet+}$ to form a neutral $\mathrm{Trp}^{\bullet}$ radical. Ideally, these two processes should occur on similar timescales and result in a radical pair lifetime of a few microseconds (Rodgers and Hore, 2009). If the lifetime is too short, there will be insufficient time for the direction of the geomagnetic field to be encoded in the product yield, greatly impacting the sensitivity of the compass. Behavioural experiments in which birds are subjected to radiofrequency electromagnetic fields suggest that the lifetime of radical pairs in the bird's retinas is around 2–10 μs (Engels et al., 2014; Kobylkov et al., 2019).

The deprotonation of $\mathrm{TrpH}^{\bullet+}$ to give $\mathrm{Trp}^{\bullet}$ results in a stabilised, longer-lived form of the protein (corresponding to the product in Figure 2(A)), which can be monitored spectroscopically (Maeda et al., 2011; Neil et al., 2014). *In vivo*, this stabilised form of the protein is presumed to lead to the signalling state. As explored in Bartölke et al., 2021 in this issue, the signalling process is thought to be initiated by conformational changes in the C-terminal region of the cryptochrome molecule. Information on the C-terminal tail is therefore vital not just for an understanding of signal transduction but also because its interaction with binding partners may help optimise the structure of the cryptochrome for magnetic sensing. Unfortunately, no crystallographic data are available for this crucial part of the protein. As we now discuss, computer simulations can shed light on this and other issues.

Computational studies of cryptochrome

Modern computational techniques are powerful and versatile and form an integral part of the multidisciplinary approach of SFB 1372. Biophysicists aim to provide increasingly accurate estimates of various properties of proteins, allowing experimental observations to be rationalised and theoretical predictions to be tested in experiments. Using a 'computational microscope', it is possible to address questions about the dynamics of the protein and the radical pairs within it. We start with molecular dynamics (MD) simulations.

Molecular dynamics simulations

The MD method involves solving Newton's equations of motion to obtain the instantaneous velocity and direction of movement of every single atom in the protein. This information is used to calculate where all the atoms would move to after a short time-step, typically $\sim 10^{-15}$ s. Using these new positions, Newton's equations are solved again and the atoms moved accordingly. The whole process is repeated $\sim 10^{8}$ times to produce a 'trajectory' that is essentially a stop-motion movie of the structure of the protein at atomic resolution. Solvent molecules and ions can be included so that the protein is in an environment resembling that *in vivo*.

An example of the use of MD in the context of magnetoreception is our attempt to model the C-terminal tail of cryptochrome. As mentioned before, there is no crystal structure of any full-length avian Cry4 incorporating the C-terminal tail because the intrinsic disorder of this part of the protein has so far precluded crystallisation. To learn more about how the conformation and position of the C-terminal tail might change when the protein is photoreduced, we are trying to determine its structure computationally. One approach uses the software Pep McConst to randomly generate a multitude of structures (Schuhmann et al., 2021) on which we can perform MD simulations to investigate their properties.

One of the many properties that MD simulations provide is interatomic distances. The separation of the FAD radical and its partner, $\mathrm{TrpH}^{\bullet+}$, can for example be used to estimate the fluctuations in the strength of the magnetic coupling of the two radicals. As these interactions affect singlet-triplet interconversion and spin relaxation of the radical pair, realistic estimates are important for an accurate description of the spin dynamics. Figure 3(A)(ii) and (iii) show an example for European robin Cry4. As well as studying intraprotein distances, one can use statistical tools to filter out the structural differences produced by changes in the redox state of the protein, for example when radical pairs are formed (Kattnig et al., 2018), to calculate the root-mean-square deviation (RMSD) in the positions of the atoms as a measure of the stability of the simulated structure (Figure 3(A)(i)), and to determine the energies of the different radical pair states. Using such methods, Xu et al. (2021) found evidence that the third radical pair, $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_CH}^{\bullet+}]$, in robin Cry4 interconverts rapidly with the fourth, $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_DH}^{\bullet+}]$. It was argued that such an equilibrium could allow migratory birds to evolve a magnetoreceptor in which the sensing and signalling functions were independently optimised.

Software for MD simulations is widely available. NAMD (Phillips et al., 2020, 2005), for example, allows the calculations to be automated and performed using parallel computing architectures. VMD (Humphrey et al., 1996) aids in the visualisation of trajectories and provides tools for analysis. The online platform VIKING (Korol et al., 2020), which is continuously developed and updated within SFB 1372, allows users to set up simulations with no need for in-depth computational knowledge.

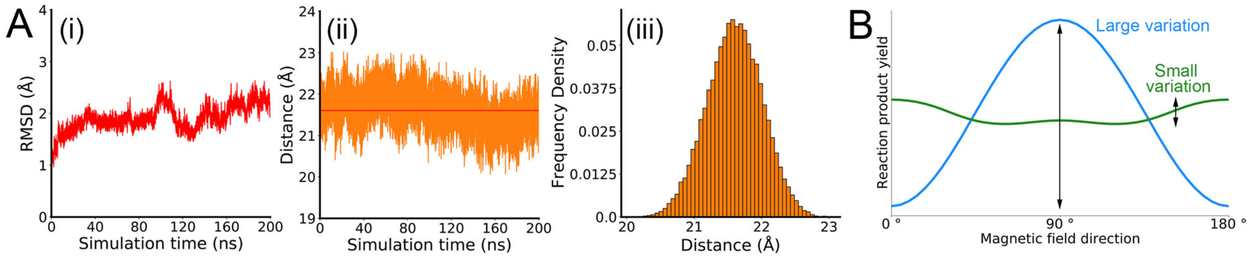

Figure 3: (A) Three graphs obtained from an MD simulation of European robin Cry4. (i) The root-mean-square deviation (RMSD) of the atomic positions over the course of the simulation. The stability of this structure is shown by the levelling out of the RMSD. (ii) and (iii) The centre-to-centre distance between FAD and $\mathrm{Trp_DH}$: (ii) gives the variation as a function of time while (iii) shows the distribution of distances. (B) Calculated variation of the yield of the reaction of two radical pairs as a function of the direction of a 50 μT magnetic field. The green and blue lines are $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_CH}^{\bullet+}]$ and $[\mathrm{FAD}^{\bullet-}\ \mathrm{Z}^{\bullet}]$, respectively, where the $\mathrm{Z}^{\bullet}$ radical lacks the internal magnetic interactions present in $\mathrm{Trp_CH}^{\bullet+}$. The much smaller signal for $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_CH}^{\bullet+}]$ has been magnified tenfold to make the variation easier to see. Simulation parameters were taken from Lee et al. (2014), and the relative orientations of FAD and $\mathrm{Trp_CH}$ came from the crystal structure of pigeon Cry4 (Zoltowski et al., 2019). Further examples of such spin dynamics calculations can be found in (Hiscock et al., 2016).

Modelling protein dynamics using MD simulations is a valuable source of information on spin relaxation. Caused principally by the vibrations of chemical bonds within the protein, fast equilibration of the electron spins can drastically reduce the sensitivity of a radical pair to the geomagnetic field. How this process could be slow enough to make a protein-based radical pair viable as a geomagnetic sensor is one of the important research questions to be addressed in SFB 1372.

Spin dynamics simulations

The RPM has a strong theoretical foundation making it possible to simulate the responses of even quite complex radical pairs to the geomagnetic field (Hiscock et al., 2016; Lewis et al., 2014; Manolopoulos and Hore, 2013). Requiring quantum mechanical modelling of the singlet-triplet interconversion including chemical reactions and spin relaxation, these calculations have three motivations: (1) to interpret data from spectroscopic studies of purified cryptochromes (e.g. Maeda et al., 2012) and from behavioural experiments (e.g. Kobylkov et al., 2019); (2) to suggest and guide future experiments (e.g. Hiscock et al., 2017; Worster and Hore, 2018); and (3) to explore aspects of the radical pair hypothesis that are currently too challenging to study experimentally (e.g. Ren et al., 2021; Wong et al., 2021). Input parameters for these simulations typically come from crystallography and MD simulations (protein structure and dynamics) and from molecular orbital calculations (magnetic interactions). Available software includes MolSpin (Nielsen and Solov'yov, 2019) and Spinach (Hogben et al., 2011).

As outlined above, the influence of spin relaxation on radical pair magnetoreceptors can be explored by combining molecular and spin dynamics simulations. The seminal work in this area is Kattnig et al. (2016a,b) who used information derived from all-atom MD trajectories to model the magnetic sensitivity of $[\mathrm{FAD}^{\bullet-}\ \mathrm{TrpH}^{\bullet+}]$ radical pairs in a plant cryptochrome. Although, the relaxation caused by molecular vibrations could be compatible with geomagnetic sensing, it was clearly too fast to account for the disruptive effects of weak radiofrequency fields that have been observed in behavioural studies (Engels et al., 2014; Hiscock et al., 2017). The conclusion was that a cryptochrome sensor *in vivo* would need different dynamics from the isolated protein, e.g. as a result of binding to signalling partners and/or the structures required for its alignment and immobilisation. In SFB 1372, this work will be extended to European robin Cry4 with a focus on identifying any mechanisms that might shield the radicals from the dynamical modes of their environment. The long-term plan is to model the surroundings of the cryptochrome and develop a general picture of spin relaxation including all relevant magnetic and electronic interactions.

It is clear that the magnetic field effects that have been observed in spectroscopic experiments on purified cryptochromes derive from $[\mathrm{FAD}^{\bullet-}\ \mathrm{TrpH}^{\bullet+}]$ radical pairs (Maeda et al., 2012; Sheppard et al., 2017; Xu et al., 2021). *In vivo*, however, there could be contributions from the $\mathrm{FAD}^{\bullet-}$ radical paired with a radical simpler than $\mathrm{TrpH}^{\bullet+}$ (Ritz et al., 2009). Possibilities such as this can be explored by simulating the magnetic responses of a range of radicals. In this way, Lee et al. (2014) found that the magnetic properties of the $\mathrm{FAD}^{\bullet-}$ radical are near-optimal for compass sensitivity. However, much of that sensitivity is lost if $\mathrm{FAD}^{\bullet-}$ is partnered with $\mathrm{TrpH}^{\bullet+}$ but could be regained if $\mathrm{TrpH}^{\bullet+}$ were replaced by a hypothetical radical, denoted $\mathrm{Z}^{\bullet}$, that is devoid of the internal magnetic interactions in $\mathrm{TrpH}^{\bullet+}$. A simplified version of the calculation by Lee et al. (2014) is shown in Figure 3(B): even in this simple case, the reaction product yields of $[\mathrm{FAD}^{\bullet-}\ \mathrm{Z}^{\bullet}]$ are much more sensitive to the magnetic field direction than those of $[\mathrm{FAD}^{\bullet-}\ \mathrm{Trp_CH}^{\bullet+}]$. However, as most biological radicals have multiple internal interactions, there are limited options for the identity of $\mathrm{Z}^{\bullet}$. One candidate, superoxide $(\mathrm{O}_2^{\bullet-})$, has the serious drawback that it probably undergoes extremely rapid spin relaxation (Hogben et al., 2009; Player and Hore, 2019). Other species that contain fewer magnetic nuclei than tryptophan have also been proposed, such as the radical form of ascorbic acid (Lee et al., 2014; Nielsen et al., 2017). As already mentioned, many of the predictions of these spin dynamics simulations can be tested experimentally by *in vitro* spectroscopic measurements on recombinantly expressed and purified proteins. In a typical experiment, changes in the yields and rates of formation and decay of potential signalling states are measured as a function of time after absorption of a short laser light pulse. Intermediate states of the proteins, including the radical pair forms, can be identified from their characteristic UV-visible absorption spectra. Observations with and without applied magnetic fields give key clues to the factors that could make cryptochromes sensitive magnetic sensors *in vivo*. Such measurements allowed Xu et al. (2021) to claim that "Cry4 from the night-migratory European robin seems fit for purpose a magnetic sensor". The next step will be to see whether it is possible to manipulate Cry4 in the eyes of a migratory bird.

In conjunction with behavioural experiments (Engels et al., 2014; Kobylkov et al., 2019; Schwarze et al., 2016), extensions of these calculations can be used to explore the identities and properties of the radicals involved *in vivo*. This work exploits the effect of weak radiofrequency magnetic fields on birds' ability to use their magnetic compass. Spin dynamics simulations can predict the radiofrequency conditions in which these effects should occur for different radical pairs (Hiscock et al., 2017). Radiofrequency fields with cleverly chosen intensities and frequency ranges can then be used in behavioural studies to discriminate between different radical pair models (for details, see Spiecker et al., 2021 in this issue). The close collaboration within SFB 1372 will allow an efficient iterative process, where the first experimental findings will shape the next set of simulations, which in turn will allow informed choices for subsequent experimental design.

In summary, among the open questions we will attempt to solve within the SFB using computer simulations combined with multidisciplinary collaboration are: is the radical pair *in vivo* really [$\mathrm{FAD}^{\bullet-}$ $\mathrm{TrpH}^{\bullet+}$] and how might evolution have optimised the spin relaxation of the radical pair *in vivo*?

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