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**Spotlight on EMF Research**

# Spotlight on recent progress in research on the Radical Pair Mechanism

## Category [theory/molecular mechanism]

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Competence Centre Electromagnetic Fields (KEMF)

## 1 A short introduction to the Radical Pair Mechanism by the BfS

In the following summary of the papers [1-6] we give an update on recent research activities on the Radical Pair Mechanism (RPM). Magnetic fields (MFs) penetrate biological tissue and therefore might influence any biophysical process within living systems. However, effects of weak MF's caused by magnetic force-field action are conventionally considered as negligibly small compared to the ubiquitous noise in a biological environment. Effects are only conceivable as subtle statistical or quantum mechanical effects. The basic idea behind the RPM is that in a variety of chemical reactions, radicals serve as transient reaction intermediates and can be influenced by external MFs. The changed reactions could possibly influence biological processes and thus have an impact on human health and the environment.

For a short time-interval, the electrons in a pair of radicals can form quantum mechanically correlated spin[1] states: Singlet in case of total spin zero and triplet in case of total spin one. Interaction of the spins with nuclei in the surrounding molecules leads to an interconversion between singlet and triplet states. A well-defined fraction of radicals then proceeds the reaction as a singlet product and the rest as a (different) triplet product. External MF's alter the coherent interconversion and result in a different singlet-triplet ratio, i.e. a different reaction outcome. Biological processes sensitive to the outcome of such radical reactions can therefore be influenced by external MFs. This is the Radical Pair Mechanism [7-10]. The RPM involves two contrarotating effects, depending on the strength of the external MF. In case of MF's in the range of mT, the Zeeman splitting[2] reduces the singlet-triplet interconversion rate with increasing MF, which is termed "high field effect". For weak MFs in the µT range, the increasing probability to fluctuate (compared to zero MF) leads to an increasing interconversion rate between the states with increasing MF, which is termed "low field effect" (LFE) [11]. The LFE is an intriguing candidate to explain avian magnetoreception. Its significance for other biological processes, in particular in humans, is lively debated [12] and currently investigated by many research groups including the BfS.

[1] *Spin*: A specific form of quantum mechanical angular momentum, carried by elementary particles. For electrons, spin can be either parallel or anti-parallel to a specific direction (e.g. the direction of the external MF). For two electrons, the spins either add up to a total spin of 1 or a total spin of 0.

[2] *Zeeman effect*: Splitting of energy levels in the electron shell of an atom by an external MF, which interacts with the spins of the electrons. Due to the different values of the spin, energy levels of electrons split into at least two separate values. This was first observed by P. Zeeman in spectral lines of sodium.

# 2 Results and conclusions from the authors perspective

For the magnetic compass in migratory species, the ability to detect weak MFs is indispensable. A crucial parameter for magneto-sensitivity of a radical pair (RP) is its life-time. The genuine quantum property of two electrons being in a correlated state is rapidly destroyed by any interaction with the environment (decoherence). To investigate the effect of protein motion in the environment of an RP on its quantum properties, in particular the sensitivity of the magnetic compass, [1] compared RP dynamics in *Erithacus rubecula* (European robin, migratory) Cryptochrome (ErCry4a), and *Columba livia* (pigeon, non-migratory) Cryptochrome (ClCry4a) with *Arabidopsis thaliana* (plant) Cryptochrome (AtCry1). These three species of Cryptochrome have differing magneto-sensitivities. In all of them, the RP consists of the blue-light activated flavin (FAD) cofactor and an oxidized tryptophan (Trp) and is denoted [$FAD^{\bullet-}$ $Trp^{\bullet+}$]. There is however the difference that in ErCry4a (and ClCry4a), the electron donor is either the third or the fourth tryptophan residue whereas in AtCry1 it is the third Trp. The analysis of the RP environment was done by first simulating the Cryptochrome by all-atom molecular dynamics[3] (for 400ns, after equilibration), followed by a density functional theory[4] (DFT) calculation of specific parts of the protein containing the $Trp^{\bullet+}$ and $FAD^{\bullet-}$. With the extracted hyperfine structure data, the singlet and triplet yield of the RP coherent interconversion was calculated by numerically solving the corresponding stochastic Liouville equation[5]. A significant change in the reaction yields is caused by variations in the hyperfine structure[6] and Zeeman couplings. These are influenced by angular changes in specific molecular axes (dihedral angles) and relative orientations of aromatic rings of the $FAD^{\bullet-}$ and $Trp^{\bullet+}$ (librational angles). Such changes are induced by random thermal motion of the protein components. The authors extracted probability distributions of these parameters from the molecular dynamics trajectories and determined the influence on the hyperfine and Zeeman couplings by DFT calculations utilizing the extracted data. Finally, the result on directional magneto-sensitivity was investigated by determining the singlet yield directional anisotropy (with respect to the external MF direction). The authors find larger motions in $Trp^{\bullet+}$ than $FAD^{\bullet-}$. The $Trp^{\bullet+}$ librational motion is larger in plant AtCry1 than in animal ErCry4a and ClCry4a and differs from the third to fourth Trp residue (reflecting the different environments). The authors identify the hyperfine interactions most affected by changes in the dihedral and librational parameters (the Hβ protons in both $FAD^{\bullet-}$ and $Trp^{\bullet+}$). However, the authors also observe that anisotropic MF effects and hence the sensitivity of the magnetic compass, strongly decrease as soon as more hyperfine coupled nuclei are included in the calculations.

Anisotropic hyperfine interactions are the basis of directional MF sensing. The significance of the interaction of a RP with nuclear magnetic moments (hyperfine interaction) of the surrounding molecular environment is investigated in [2]. The authors use the fact that changing the properties of the nuclei involved in hyperfine interactions leads to varying MF directional sensitivity and in turn gives insight into the hyperfine structure of the protein environment in the vicinity of an RP. Nuclear properties are changed by substituting a nucleus with an isotope. In particular the authors changed the isotopes $^{1}H \rightarrow {}^{2}H$, $^{12}C \rightarrow {}^{13}C$ and $^{14}N \rightarrow {}^{15}N$ in FAD-Trp and FAD-Z RP's, where Z denotes a component without hyperfine coupling. Hyperfine structure changes were calculated using DFT and then followed by spin dynamics simulation (similar to [1]) to determine changes in directional MF sensitivity. The authors find that Nitrogen substitution does not have a significant effect, in line with the similarity of magnetic moments of the two Nitrogen isotopes. Deuteration

[3] *Molecular dynamics*: A simulation method, in which Newton's equations of motion (together with a stochastic background) are solved numerically for all atoms in a molecular system (e.g. a protein in water). Interactions of the molecule, such as bonds and electrostatic forces determine the potential energy in the equations.

[4] *Density functional theory*: Computational method to determine the quantum mechanical state of a large quantum mechanical system of several components, e.g. several electrons.

[5] *Liouville equation*: Differential equation describing the quantum mechanical time evolution of one or more particles. It can be seen as the quantum analogue of classical mechanical equations of motion and is closely related to the von Neumann equation for a closed quantum system.

[6] *Hyperfine structure*: The quantum-mechanical interaction of electron spins in an electron shell with the nuclear magnetic moments in an atom leads to a substructure of electron energy levels, which is termed hyperfine structure.

increases the directional MF sensitivity, whereas carbon substitution decreases it. In general, the observed effects are very small. The changes lie below 0.1%. It remains an open question if and how such small effects are amplified by magnetic fields *in vivo* in the bird's retina.

The influence of the molecular environment of an RP due to hyperfine interactions is further investigated in [3] for a [$FAD^{\bullet-}$ $Trp^{\bullet+}$] RP. One way to quantify the latter is the so-called half-field parameter $B_{1/2}$ which expresses the MF strength at which the change in reaction products lies in the middle between the value without external MF and its saturation at very high external MFs. To determine the reaction product yields, the authors performed several numerical simulations, ranging from perturbative methods (Schulten-Wolynes method [13]) over quantum mechanical Monte-Carlo calculations to explicitly calculating the quantum dynamics. Depending on the method, different aspects of the molecular environment of the RP have been taken into account: Varying number of nuclear spins, various degrees of accuracy in hyperfine couplings and inclusion of other interactions, such as dipolar couplings[7]. The detailed simulations reveal a discrepancy to earlier results [14]. It is concluded that the latter are only applicable in the case of short-lived, rapidly tumbling RPs, in contrast to the slowly tumbling Cryptochrome macromolecule. For this, the authors find $B_{1/2} = 2.46\,mT$. It is further observed that inclusion of anisotropic hyperfine couplings abolishes the LFE - and thus the sensitivity of the magnetic compass for weak MFs - in the simulations, which is partly in contradiction to experimental findings (e.g. the LFE in AtCry1). Further simulations and experiments are proposed to clarify this issue.

A further, yet more speculative way how the molecular environment might influence the dynamics of an RP and thus the sensitivity of the magnetic compass of migrating birds, is investigated in [4]. If the vicinity of the RP has a chiral electronic structure (as it is the case in the helical electrostatic potential in protein subdomains), the interaction of the spin of the electrons with their linear momentum (due to the movement) inhibits the propagation of one of the spin polarizations. Consequently, after electron transfer, the RP is not in a pure singlet or triplet state, but rather in a superposition of both. This is the chiral-induced spin selectivity (CISS) effect (we refer to [15] for a treatment in context of the RPM). The authors of [4] investigate the influence of CISS on MF sensitivity, the domain of extremal sensitivity ("functional window") and on electron-electron interactions. They use a simple toy model as well as a more realistic 4-nuclei, 2-electron RP model together with typical CISS initial states as presented in [15]. It is found that with certain combinations of reaction rates, an enhancement of MF sensitivity is possible. However, in this case the symmetry of reaction yields with respect to inversion of the MF direction is lost. The functional window changes its domain for the realistic model. Finally, the attenuating effect of electron-electron interactions on magnetic field effects is mitigated for certain reaction rate combinations. The nuclei in the vicinity of an RP strongly determine the strength of MF effects, in particular the possibility of directional sensitivity. The authors of [5] investigate a sharp minimum in the recombination yield of a [$FAD^{\bullet-}$ $Trp^{\bullet+}$] RP (the "quantum needle effect") by analyzing analytically and numerically the dependency of the reaction yields on the hyperfine structure. For an idealized model, comprising of the RP and the nitrogen nuclei N5 in $FAD^{\bullet-}$ and N1 in $Trp^{\bullet+}$, the authors determine the energy spectrum and the corresponding eigenstates of the system dependent on the hyperfine structure and the external MF. They observe that in case of anisotropic hyperfine structure, a directionally varying external MF leads to a flip in certain spin states (at a specific angle with respect to the external MF) and henceforth to a sharp variation in the reaction yields. This contributes to the understanding of the mechanism of directional sensitivity in the RPM at the quantum level.

On the experimental side, the authors of [6] investigate the sensitivity of the magnetic compass of the pied flycatcher *Ficedula hypoleuca* with respect to perturbations by an oscillating MF. The birds were exposed to

[7] *Dipolar coupling*: Similar to the interaction energy of magnetic dipoles, the spins of two electrons (e.g. in an RP) exhibit a specific interaction energy, characterized by their spin quantum numbers and a dipolar coupling parameter, reflecting the distance and orientation of the spins.

an oscillating MF of frequency 1.41 MHz (the Larmor frequency[8] of an electron spin in a natural MF of 50.4 µT) with amplitudes of 17nT and 190nT. The exposure was generated by single-loop coils of 0.75m diameter, together with a Rigol DG4162 high frequency generator. The animals' paths were recorded in plastic Emlen funnels. In control experiments, where birds were only subjected to the local geomagnetic field, paths were recorded in aluminum Emlen funnels. Whereas in case of 17nT, the bird's orientation was not disrupted, in case of 190nT, orientation was lost. The results confirm the disruption of the magnetic compass of migratory bird species by weak oscillating MF. However, the effect appears at MF strengths of about two orders of magnitude higher than in the most studied species (*Sylvia borin*). For the garden warbler *Sylvia borin*, compass disruption already occurs at MF strengths of the order of 1nT. Explanations of these very different sensitivities range from biophysical differences in the corresponding Cryptochromes and their environments to ecological adaptations of the species. The authors emphasize that the results highlight the complexity of the avian magnetic compass, biophysically as well as biologically.

# 3 Comments by the BfS

The methods applied by the authors of [1] include classical molecular dynamics simulations as well as DFT calculations of specific excised parts of the Cryptochrome proteins. Spin dynamics simulations are performed to evaluate singlet and triplet quantum yields which in turn determine directional MF sensitivity. The work reveals new insight into the influence of both, size and motion of the Cryptochrome environment on the (directional) MF sensitivity of [$FAD^{\bullet-}$ $Trp^{\bullet+}$] radical pairs. The results are crucial to assess the significance of MF effects in realistic biological environments. The authors of [2] use computational methods similar to [1], which are the current state of the art. The applied isotope substitutions unravel the significance of the protein environment on directional MF sensing in realistic biological systems, in particular for avian magnetoreception. The computational methods used in [3] cover a broad range of available tools to simulate large spin systems. Their finding, that inclusion of anisotropic hyperfine interactions might lower or even abolish the LFE in specific Cryptochrome RPs has consequences to the relevance of RPM effects in detecting weak MFs in the order of µT. A further refinement of the simulations as well as experimental investigations of possible LFEs in ErCry4a and its mutants are required to clarify inconsistencies between simulations and experiments. The CISS effect investigated in [4] is a more speculative variant of local environmental interaction with an RP. In particular, the anisotropy of MF effects with respect to changing the sign of the external MF seems to be in contradiction with current animal experiments. Nevertheless, it is an intriguing possibility of how the helical protein subdomains could in principle enhance the MF sensitivity in the RPM. On a technical level, computational details such as the selected numerical algorithms are not mentioned in [4], so that the validity of the numerical results cannot be assessed. The authors of [5] derive a very precise explanation of directional sensitivity effects in the RPM by using an idealized RP model. The latter is suitable to clearly exhibit the quantum mechanisms for directional sensitivity at the expense of being less realistic. In particular, fast spin relaxation as well as inter-radical effects such as dipolar couplings and exchange interactions will significantly reduce directional sensitivity. Furthermore, it is known that considering a larger number of nuclei will in general impede MF sensitivity. Nevertheless, the authors clearly give a mechanistic explanation of directional effects in the RPM.

In conclusion, the presented papers highlight the enormous effort that is currently undertaken to better understand the effects of relatively weak magnetic fields on biological systems at a fundamental level. The role of the RPM for the magnetic compass in migratory songbirds, which was the main topic of the articles discussed here, still remains unanswered. Even if such processes at the fundamental level turn out to be

[8] *Larmor frequency*: Similar to a classical gyroscope, the magnetic moment of the electron precesses in an external MF. The precession frequency is called Larmor frequency and is given by $\frac{eg}{2m}B$, where $e$ is the elementary charge, $g$ is the electron g-factor, $m$ is the electron mass and $B$ denotes the MF.

consistently proved, it still remains unclear if they would result in biological effects that ultimately would lead to adverse human health effects.

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