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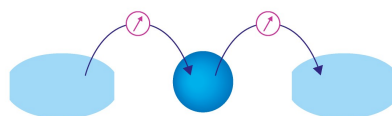
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ABSTRACT

The avian compass and many other of nature's magnetoreceptive traits are widely ascribed to the protein cryptochrome. There, magnetosensitivity is thought to emerge as the spin dynamics of radicals in the applied magnetic field enters in competition with their recombination. The first and dominant model makes use of a radical pair. However, recent studies have suggested that magnetosensitivity could be markedly enhanced for a radical triad, the primary radical pair of which undergoes a spin-selective recombination reaction with a third radical. Here, we test the practicality of this supposition for the reoxidation reaction of the reduced FAD cofactor in cryptochrome, which has been implicated with light-independent magnetoreception but appears irreconcilable with the classical radical pair mechanism (RPM). Based on the available realistic cryptochrome structures, we predict the magnetosensitivity of radical triad systems comprising the flavin semiquinone, the superoxide, and a tyrosine or ascorbyl scavenger radical. We consider many hyperfine-coupled nuclear spins, the relative orientation and placement of the radicals, their coupling by the electron–electron dipolar interaction, and spin relaxation in the superoxide radical in the limit of instantaneous decoherence, which have not been comprehensively considered before. We demonstrate that these systems can provide superior magnetosensitivity under realistic conditions, with implications for dark-state cryptochrome magnetoreception and other biological magneto- and isotope-sensitive radical recombination reactions.

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INTRODUCTION

Magnetoreception presents itself as a mysterious sensor modality. Despite having been documented for all major taxonomic groups of vertebrates, the sensory mechanism has remained opaque.¹ The two main mechanisms discussed attribute the phenomenon either to ferrimagnetic or superparamagnetic particles² or magnetic field dependent spin dynamics in radical pairs (RP), presumably in the flavo-protein cryptochrome.³ The latter hypothesis has nurtured interest beyond sensory biology because it serves as an example for a putative quantum-processes underpinning biology in a non-trivial manner, i.e., a subject of the emerging field of quantum biology.^{4,5} The idea of magnetoreception underpinned by quantum mechanics is mostly developed and researched in the context of the avian magnetoreception, where it is widely attributed to a light-dependent

inclination compass that is susceptible to interference from weak radio frequency magnetic fields.³

The quantum hypothesis of magnetoreception, as conceptually suggested in 1978 by Schulten and co-workers⁶ and sophisticated to its current form by Ritz *et al.*,⁷ builds on the magnetosensitivity of a radical pair recombination reaction in the protein cryptochrome.³ Such magnetosensitive radicals are, for example, generated in cryptochromes in the photo-reduction of the non-covalently bound flavin cofactor.^{8–10} In this system, a succession of intra-protein electron transfer processes generates an electron spin-correlated pair of radicals, comprising the flavin anion radical and tryptophan radical cation.¹¹ The pair's electronic singlet and triplet states coherently interconvert due to magnetic interactions in the radicals and with the geomagnetic field, thus rendering the reaction outcome—either charge recombination or structural

rearrangement—sensitive to the applied magnetic fields. This mechanism has been clearly demonstrated for various cryptochromes *in vitro*, albeit in magnetic fields typically exceeding the geomagnetic fields by orders of magnitude.^{8–10}

Besides the photo-reduction, the reoxidation has been implicated with magnetosensitivity. A transient flavin semiquinone/superoxide radical pair has been suggested^{12,13} and amply discussed.^{14,15} Such a radical pair is, e.g., expected to result from the oxidation of the fully reduced flavin cofactor in cryptochrome by molecular oxygen.¹⁶ Although a demonstration of a clear-cut magnetic field effect (MFE) of this radical pair has so far not been delivered *in vitro*, the hypothesis has gained new impetus through a series of *in vivo* experiments demonstrating magnetoreception in (intermittent) darkness, i.e., under conditions for which the photo-reduction has been ruled out.^{17–19} From the conceptual point of view, a superoxide/semiquinone radical pair offers potential advantages but also raises fundamental questions. As such a radical pair is of the so-called reference-probe coupling topology, i.e., lacks significant hyperfine interactions in one of the radicals, much larger magnetic field effects are predicted by models employing the radical pair mechanism (RPM) than is the case for flavin/tryptophan pairs.^{20,21} On the other hand, superoxide is known to be subject to fast spin-rotational relaxation, which would abolish the magnetic field sensitivity of the reaction if the anion was freely tumbling in solution.^{14,22}

A previous work has suggested that the issue posed by fast spin relaxation could be overcome in an extended model relying on a radical triad instead of a radical pair.^{10,15} Specifically, it has been

shown that a scavenging reaction of a radical pair by a third radical can markedly boost the magneto-sensitivity of the recombination reaction (observed for the primary pair) both for flavin/tryptophan and flavin/superoxide reactions and even if the primary pair does not recombine (on the relevant time scale).¹⁰ The enhancement process is robust to fast spin relaxation in one member of the primary pair, which, at least conceptually, allows for the inclusion of superoxide and other reactive oxygen species (ROS) beleaguered by fast spin relaxation.¹⁵ In addition, a recent study demonstrated that the effect remains effective in the presence of the unavoidable but oft-neglected electron–electron dipolar (EED) interactions.²³ While these previous studies are promising, the models used had been simplified in many respects, thus elucidating the principal idea rather than their practicality for magnetoreception.

While the scavenged three-radical processes offers many virtues, its obvious downside is the increased complexity resulting from the need to recruit a scavenging radical to interact with the primary radical pair. Reference 15 suggests that this could be realized through a reaction scheme, where the scavenger radical is generated in the photo-reduction and conserved in the form of a persistent radical until demanded in the three-radical reoxidation. In the avian cryptochromes, such reoxidation processes could be induced by molecular oxygen from the fully reduced cryptochrome (see Fig. 1).²⁴ In cryptochromes that do not undergo complete photo-reduction, such as the cryptochrome from *D. melanogaster* (*DmCry*),²⁵ the radical triad could result in an encounter processes with a pre-formed superoxide. Many persistent radicals are known in nature; in the context of cryptochromes, long-lived

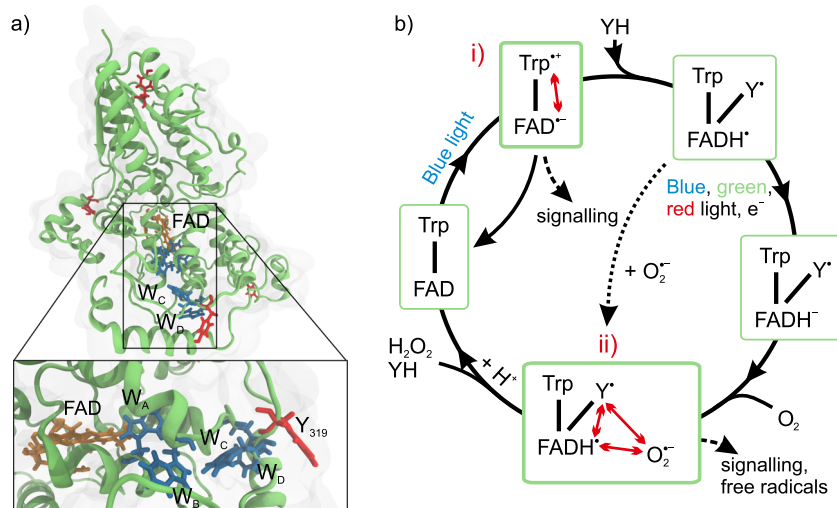
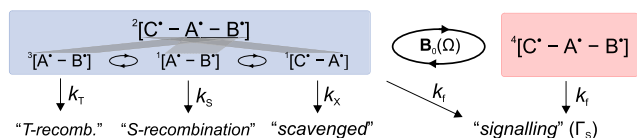


FIG. 1. (a) Schematic representation of pigeon cryptochrome 4, *C/Cry4*. The inset highlights the FAD (orange), the tryptophan tetrad (blue), and the surface-exposed tyrosine residue, Y319, extending the latter. Selected tyrosine residues are shown in red: Y319, Y32, Y441, and Y134. These sites can give rise to large magnetosensitivity in the FADH[•]/O₂^{•-}/Y^{•-} radical triad system with fast relaxing O₂^{•-}, provided that the scavenging reaction is sufficiently rapid (see “Discussion”). (b) Photo-cycle of the cryptochrome undergoing a two-step photoreduction and reoxidation. The photo-reduction is assumed to lead to the formation of the persistent radical Y^{•-}, which acts as a scavenger to FADH[•] in the subsequent re-oxidation with molecular oxygen. The redox-cycle involves two potentially magnetosensitive radical pairs, FAD^{•-}/Trp^{•+} in the first photo-reduction (labeled i) and the FADH[•]/O₂^{•-} radical pair, resulting from the oxidation of the fully reduced FADH[•] by oxygen. A mobile O₂^{•-} will not be magnetosensitive in the geomagnetic field due to swift spin relaxation. Engaging a scavenging reaction in FADH[•]/Y^{•-} resurrects the magnetosensitivity. Y^{•-} could be a long-lived tyrosine radical or a radical derived from a radical scavenger, such as ascorbic acid. Cryptochromes that cannot be fully photo-reduced (e.g., *DmCry*) could form flavin/O₂^{•-}/Y^{•-} radical triad systems by correlating with a pre-formed O₂^{•-} in a random encounter (dashed arrow).

tyrosine radicals are well-documented.^{26,27} With this motivation in mind, we here are set to elucidate the directional magnetic field effects (MFEs) that the model from Ref. 15 predicts for a tyrosine radical-scavenged flavin semiquinone superoxide radical pair if the electron–electron dipolar coupling and the scavenger radical identity (and thus its orientation) are taken into account. Specifically, we ask the question if one of the tyrosine residues, as present in prototypical cryptochromes, could function as a radical scavenger to boost the magnetosensitivity of the reoxidation of cryptochrome. We base our analysis on the recently resolved crystal structure of the pigeon cryptochrome 4 (*ClCry4*), close in sequence to the European Robin Cryptochrome 4 (*ErCry4*) and, for comparison, *DmCry*.^{24,28} The latter has been used widely as a model cryptochrome system to study magnetoreception.^{29,30} This includes the original studies proposing the three-radical model.^{15,31} *ClCry4* is the only bird cryptochrome for which the crystal structure has been resolved so far.²⁴ It shares broad sequence homology with cryptochromes from migratory birds, for which a compass sense has been extensively studied (85.6% sequence identity with *ErCry4*). Using a variety of template structures, our study thus reveals the range of magnetic sensitivities realizable in realistic, i.e., existing, structures. While it is understood that the sensor *in vivo* will most likely deviate from these established structures,⁸ they provide a well-defined frame of reference and target for systematic exploration. We present data for reasonably complex radical systems as a function of the scavenging rate constant and discuss the effects of spin relaxation in the superoxide and the electron–electron dipolar coupling of radicals. A comprehensive evaluation of the effect for the various tyrosine sites allows predicting the most apt residue. The majority of the analysis has been carried out in the limit of infinitely fast spin relaxation within the superoxide anion, thereby highlighting the design principles that allow realizing large magnetosensitivity in reactions involving swiftly relaxing species.

THEORY

We consider systems of three radicals, henceforth denoted A', B', and C'. Radicals A' and B' are thought to form the primary radical pair (RP), while C' assumes the role of the scavenger (Scheme 1). The latter reacts with A' in a spin-selective reaction forming a diamagnetic product distinct from the signaling state. For the majority of this paper, A', B', and C' are thought to be realized by FADH[•], O₂^{•-}, and Y' in cryptochrome (cf. Fig. 1). In the “Discussion,” we will also consider the ascorbyl radical in place of C'. The spin dynamics and magnetic field effects of this system are described in terms of the population weighted spin density operator in the combined Hilbert



SCHEME 1. Reaction scheme underpinning the theoretical model. The sub-states shown for the doublet manifold (underlined in blue) only show the reacting pair of radicals and are not mutually exclusive, i.e., orthogonal.

space of the three radicals, $\hat{\rho}(t, \Omega)$. Its equation of motion is of the form

$$\frac{d}{dt}\hat{\rho}(t, \Omega) = -i [\hat{H}(\Omega), \hat{\rho}(t, \Omega)] + \hat{K}\hat{\rho}(t, \Omega) + \hat{R}\hat{\rho}(t, \Omega) \quad (1)$$

and comprises a conservative part describing the coherent evolution of the spin system under the spin Hamiltonian $\hat{H}(\Omega)$ and a non-conservative part that accounts (phenomenologically) for the two important consequences of the open nature of the quantum system, namely, chemical reactions and environment induced relaxation, in the form of the linear superoperators \hat{K} and \hat{R} , respectively. Here, $[\hat{A}, \hat{B}] = \hat{A}\hat{B} - \hat{B}\hat{A}$ denotes the commutator, and the spin Hamiltonian $\hat{H}(\Omega)$ comprises the Zeeman interaction with the geomagnetic field, hyperfine interactions with surrounding nuclear spins, the electron–electron dipolar (EED) coupling, and the exchange interactions. Specifically,

$$\hat{H}(\Omega) = \sum_{K \in \{A, B, C\}} \hat{H}_K(\Omega) + \sum_{K \neq L \in \{A, B, C\}} \hat{H}_{K, L}, \quad (2)$$

with $\hat{H}_K(\Omega)$ and $\hat{H}_{K, L}$ given in angular frequency units by

$$\hat{H}_K(\Omega) = \omega_0 \cdot \hat{\mathbf{S}}_K + \sum_j^{N_K} \hat{\mathbf{S}}_K \cdot \mathbf{A}_{Kj} \cdot \hat{\mathbf{I}}_{Kj} \quad (3)$$

and

$$\hat{H}_{K, L} = -J_{KL} \left(\frac{\hat{1}}{2} + 2\hat{\mathbf{S}}_K \cdot \hat{\mathbf{S}}_L \right) + d_{KL} (\hat{\mathbf{S}}_K \cdot \hat{\mathbf{S}}_L - 3(\hat{\mathbf{S}}_K \cdot \mathbf{n}_{KL})(\hat{\mathbf{S}}_L \cdot \mathbf{n}_{KL})). \quad (4)$$

$\hat{\mathbf{S}}_K$ and $\hat{\mathbf{I}}_{Kj}$ denote the vector operators of electron spin $K \in \{A, B, C\}$ and of nuclear spin j in radical K , respectively. For spin- $\frac{1}{2}$ particles in their respective sub-Hilbert spaces, the spin operators as used here are related to the Pauli operators by $\hat{S}_{Kj,a} = \frac{1}{2}\hat{\sigma}_a$, where $a \in \{x, y, z\}$. The sum in Eq. (3) runs over all N_K magnetic nuclei with hyperfine tensor \mathbf{A}_{Kj} in radical K . $\omega_0 = g\mu_B \hbar^{-1} \mathbf{B}_0(\Omega)$, with $\mathbf{B}_0(\Omega)$ denoting the applied magnetic field (of assumed 50 μT intensity; Larmor precession frequency: $g\mu_B \frac{\|\mathbf{B}_0(\Omega)\|}{\hbar} \approx 1.4$ MHz) in the coordinate frame of the protein, as specified by the combined polar and azimuthal angles Ω . Note that the geomagnetic field is weak enough that differences in g -factors of the three electron spins can be neglected (in particular, $g \approx 2$). $2J_{KL}$ is the energy difference of the singlet and triplet states of pair (K, L) due to the exchange coupling. The electron–electron dipolar coupling [second term in Eq. (4)] has been considered in the point-dipole approximation. The dipolar coupling constant is given by $d_{KL} = \mu_0 g^2 \mu_B^2 / (4\pi r_{KL}^3 \hbar)$, with r_{KL} denoting the distance of the centers of spin density of radicals K and L . \mathbf{n}_{KL} is a unit vector parallel to the line joining the centers of the two radicals.

The superoperator \hat{K} accounts for chemical reactions of the radical triad. Specifically, following the Haberkorn approach,³² \hat{K} can be expressed as

$$\begin{aligned} \hat{K}\hat{\rho}(t, \Omega) = & -\frac{1}{2}k_X \left[\hat{P}_{AC}^{(S)}, \hat{\rho}(t, \Omega) \right]_+ - \frac{1}{2}k_S \left[\hat{P}_{AB}^{(S)}, \hat{\rho}(t, \Omega) \right]_+ \\ & - \frac{1}{2}k_T \left[\hat{P}_{AB}^{(T)}, \hat{\rho}(t, \Omega) \right]_+ - k_f \hat{\rho}(t, \Omega), \end{aligned} \quad (5)$$

where k_X and k_f are the rate constants of radical scavenging and for spin-independent generation of the signaling state, respectively. k_S and k_T denote the rate constants of singlet and triplet pair recombination in the primary RP. Here, $[\cdot]_+$ denotes the anti-commutator and $\hat{P}_{KL}^{(S)}$ and $\hat{P}_{KL}^{(T)} = \hat{1} - \hat{P}_{KL}^{(S)}$ are the singlet and triplet projection operators in the subspace of the electron spins of radicals K and L , respectively. The former can be expressed as $\hat{P}_{KL}^{(S)} = \frac{1}{4}\hat{1} - \hat{S}_K \cdot \hat{S}_L$. Equation (5) is of the truncated Lindblad form (the inclusion of “shelving” states for the reaction products would reveal its complete Lindblad form; see e.g., Refs. 33 and 34) and accommodates minimal reaction-related singlet–triplet-dephasing of the reacting pair.^{4,35} We will refer to k_f^{-1} as the “lifetime” of the radical system.

We treat spin relaxation in terms of the generic Lindbladian in the diagonal form

$$\hat{K}\hat{\rho}(t, \Omega) = \sum_n \gamma_n \frac{1}{2} (2\hat{A}_n \hat{\rho}(t, \Omega) \hat{A}_n^\dagger - \hat{\rho}(t, \Omega) \hat{A}_n^\dagger \hat{A}_n - \hat{A}_n^\dagger \hat{A}_n \hat{\rho}(t, \Omega)), \quad (6)$$

where the \hat{A}_n are jump operators and the γ_n are the associated noise rates. In the sections titled Results, we shall present simulations for random-field relaxation, for which $\hat{A}_n \in \{\hat{S}_{K,x}, \hat{S}_{K,y}, \hat{S}_{K,z}\}$ and the three associated coupling coefficients are assumed identical (corresponding to uncorrelated, i.e., incoherently modulated field noise in the three space dimensions). For the K th radical, these assumptions lead to

$$\hat{K}_K \hat{\rho}(t, \Omega) = \gamma_K \left(\sum_{i \in \{x,y,z\}} S_{K,i} \hat{\rho}(t, \Omega) S_{K,i} - \frac{3}{4} \hat{\rho}(t, \Omega) \right), \quad (7)$$

which is equal to the phenomenological relaxation superoperator popular with magnetic resonance applications with equal spin-lattice (T_1) and spin–spin relaxation times (T_2), i.e., $\gamma_K = T_{1,K}^{-1} = T_{2,K}^{-1}$. Equations (6) and (7) generally apply to Markovian noise, which is deemed applicable to describe the wider system–bath coupling effects (as, e.g., the discussion in Ref. 33). Alternative descriptions based on Bloch–Redfield theory^{36,37} and spin-boson models³⁸ have been employed (which likewise are Markovian or have been applied in this limit).

Equation (1) is here solved subject to the initial condition

$$\hat{\rho}(0, \Omega) = \hat{P}_{AB}^T / \text{Tr}[\hat{P}_{AB}^T] \quad (8)$$

which corresponds to an unpolarized triplet state of the AB-radical pair, in line with its assumed generation from the fully reduced FADH[−] and triplet oxygen, in the presence of an initially uncorrelated radical C. Encounters of initially uncorrelated radicals (F-pairs/radical cluster) give rise to qualitatively comparable MFEs.³⁹

The quantum yield of the signaling state, once all radicals have reacted, is given by

$$Y_S(\Omega) = k_f \int_0^\infty \text{Tr}[\hat{\rho}] dt = k_f \text{Tr}[\hat{\rho}(\Omega)], \quad (9)$$

with $\hat{\rho}(\Omega) = \int_0^\infty \hat{\rho}(t, \Omega) dt$ denoting the accumulated density operator. The directional dependence of the yield dictates the performance of the reaction as a compass sensor. We use as fidelity measures the absolute (Δ_S) and the relative (Γ_S) anisotropy of the yield of the signaling state, which are defined as follows:

$$\Delta_S = \max_\Omega [Y_S(\Omega)] - \min_\Omega [Y_S(\Omega)] \quad \text{and} \quad \Gamma_S = \frac{\Delta_S}{\langle Y_S \rangle},$$

where

$$\langle Y_S \rangle = \frac{1}{4\pi} \int Y_S(\Omega) d\Omega. \quad (10)$$

Assume that the spin relaxation is fast and local to B' (i.e., acting on radical B', the superoxide radical anion, only). In the limit that the associated $\gamma_n \rightarrow \infty$, B-coherences are eradicated and B-population differences vanish. In this scenario, the spin dynamics of the system can be described in terms of the reduced density operator,

$$\hat{\sigma}(t, \Omega) = \text{Tr}_B[\hat{\rho}(t, \Omega)]$$

or

$$\langle a, b | \hat{\sigma}(t, \Omega) | c, d \rangle = \langle a, \uparrow, b | \hat{\rho}(t, \Omega) | c, \uparrow, d \rangle + \langle a, \downarrow, b | \hat{\rho}(t, \Omega) | c, \downarrow, d \rangle, \quad (11)$$

for which the spin-states of the B-radical have been traced out (a and c and b and d stand for the spin states of radical A and C, respectively; hyperfine interactions have been assumed to be absent for B'). $\hat{\sigma}(t, \Omega)$ obeys an effective equation of motion of the form

$$\frac{d}{dt} \hat{\sigma}(t, \Omega) = -i [\hat{H}'(\Omega), \hat{\sigma}(t, \Omega)] + \hat{K}' \hat{\sigma}(t, \Omega) + \hat{R}' \hat{\sigma}(t, \Omega), \quad (12)$$

where $\hat{H}'(\Omega)$ is the Hamiltonian pertinent to A and C only, i.e., $\hat{H}'(\Omega) = \hat{H}_A(\Omega) + \hat{H}_C(\Omega) + \hat{H}_{A,C}$, \hat{K}' is the relaxation superoperator in the AC-subspace, and the recombination superoperator obeys

$$\hat{K}' \hat{\sigma}(t, \Omega) = -\frac{1}{2} k_X [\hat{P}_{AC}^S, \hat{\sigma}(t, \Omega)]_+ - \left(k_f + \frac{k_S}{4} + \frac{3k_T}{4} \right) \hat{\sigma}(t, \Omega). \quad (13)$$

Equations (12) and (13) suggest that the spin dynamics of the three-radical system in the limit of fast spin relaxation in B' resemble that of a radical pair composed of A' and C' with the effective singlet recombination rate $k'_S = k_X$ and modified decay rate $k'_f = k_f + \frac{k_S}{4} + \frac{3k_T}{4}$. Equation (12) is to be solved subject to the random initial condition, $\hat{\sigma}(0, \Omega) \propto \hat{1}$. This applies both to $\hat{\rho}(0)$ given by Eq. (8) and F-pair/triad initial conditions. The majority of simulations described here will be conducted in the limit of infinitely fast spin relaxation in B', for simplicity, reduction of the number of parameters, and in the expectation that this limit is, in fact, closely realized for a freely tumbling superoxide. Hyperfine parameters are reported for the molecular frames of the FADH[−] and Y radicals in the [supplementary material](#) (Tables S1–S3). The identity of the scavenger radical within the protein, i.e., sequence number, impacts on the spin dynamics via the relative orientation of radicals and, for models including the electron–electron dipolar coupling, the inter-radical distance.

RESULTS

For context, we first consider a radical pair model of the reoxidation reaction. We adopt the prototype model of a triplet-born radical pair comprising the flavin semiquinone, FADH^\cdot , and superoxide anion radical, $\text{O}_2^{\cdot-}$ (hyperfine parameters are reported in Table S3 in the supplementary material). Assuming that the superoxide is mobile allows us to neglect its electron–electron dipolar interactions, which have a suppressive effect on the magnetic field effects from radical pair reactions in general and in flavin-containing radical pair models of the cryptochrome charge recombination in particular.^{23,40,41} We consider a “short-lived” ($k_f^{-1} = 1 \mu\text{s}$) and “long-lived” ($k_f^{-1} = 10 \mu\text{s}$) radical pair, with lifetimes chosen to elicit marked sensitivity of the geomagnetic field but still obeying lifetime bounds derived from the inference of avian magnetoreception by radio frequency electromagnetic fields⁴² and the expected spin relaxation times of cryptochrome-bound flavin radicals (i.e., for longer lifetimes, spin relaxation in the flavin will strongly attenuate the effect).³⁷ The radical pair recombination has been accounted for subject to the frequent assumptions $k_S = k_f$ and $k_T = 0$. Modeling spin relaxation in the superoxide anion radical by Eq. (7), we predict the dependence of the relative anisotropy [Eq. (10)] of the quantum yield of signaling state formation on the relaxation rate constant γ_B , as shown in Fig. 2(a). In the absence of spin relaxation ($\gamma_B \rightarrow 0$), the model yields marked anisotropy of the yield of the signaling state of the order of $\sim 5\%$ for both radical pair lifetimes considered. As the relaxation rate increases, the effects are, however, quickly attenuated, whereby the long-lived pair is more susceptible to the noise process. For the superoxide anion radical, the predominant source of the spin relaxation in weak magnetic fields is the spin-rotational interaction, the dephasing effect of which is directly proportional to the rotational correlation time.⁴³ Estimating the latter based on the Stokes–Einstein relation and using parameters from Ref. 43, the relaxation rate of superoxide freely tumbling in an aqueous solution is estimated as $\gamma_B = 1.8 \times 10^9 \text{ s}^{-1}$, i.e., γ exceeds the range of γ -values considered in Fig. 2. Allowing for the fact that the dynamic viscosity of hydrophobic cellular compartments surpasses that of water (80 mPa s vs 1 mPa s for water⁴⁴), the intracellular spin-rotational relaxation rate is, in fact, expected to be smaller: $\gamma_B = 2.2 \times 10^7 \text{ s}^{-1}$ (i.e., the system loses coherences on a timescale of $\gamma_B^{-1} = 45 \text{ ns}$). However, the MFEs are strongly attenuated (for the here more favorable $k_f^{-1} = 1 \mu\text{s}$, from 5.2% to $<0.04\%$; worse for $k_f^{-1} = 10 \mu\text{s}$), thereby making obvious that the model of a mobile superoxide is irreconcilable with the demand of providing a sensitive compass in the geomagnetic field.

We next consider the extension of the radical pair model by a scavenging reaction with a third radical as shown schematically in Fig. 1 and Scheme 1. We assume that the signaling state is induced by the semiquinoid form of FAD.⁴⁵ The scavenging reaction correspond to the oxidation of FADH^\cdot by the tyrosine radical, thereby re-forming the fully oxidized resting state of FAD, which is also formed via the oxidation of the semiquinone by $\text{O}_2^{\cdot-}$. Overall, the reaction products are thus the activated protein or its resting state. For definiteness, we arbitrarily identify the scavenger radical with the neutral tyrosine radical corresponding to Y319 as present in the crystal structure of *DmCry*. This residue is separated by 10.2 Å from the flavin (OH–N5 distance) and thus, in principle, well positioned to serve as scavenger of the flavin semiquinone based on the mere

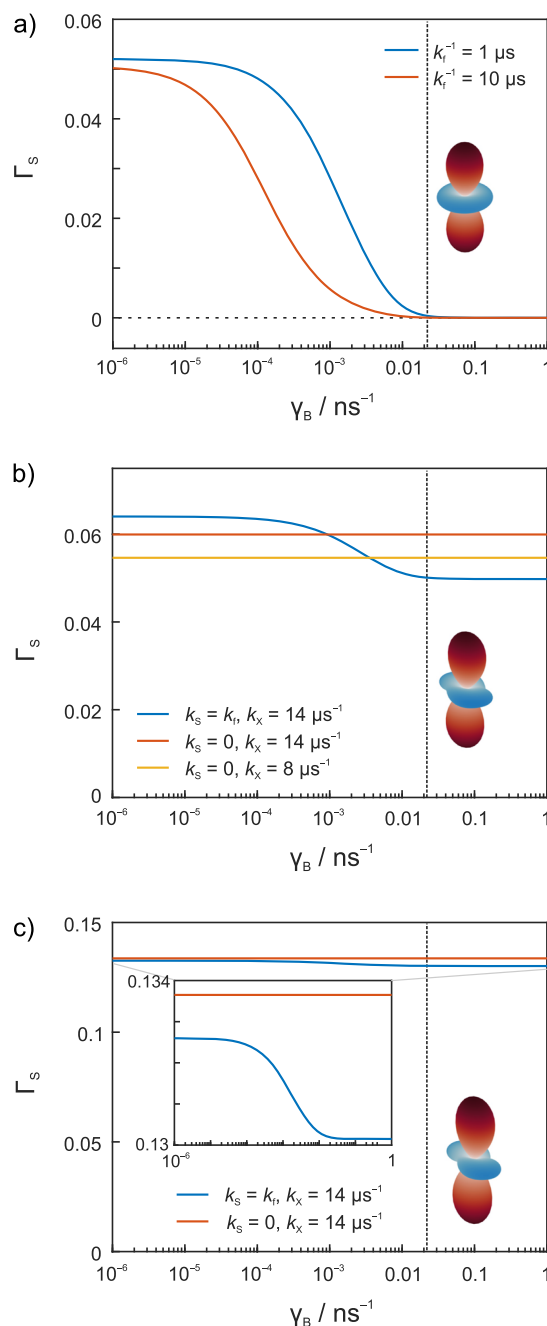


FIG. 2. Directional magnetic field effects of radical pair and triad systems of topology $\text{FADH}^\cdot/\text{O}_2^{\cdot-}$ or $\text{FADH}^\cdot/\text{O}_2^{\cdot-}/\text{Y}^\cdot$ subject to random-field spin relaxation in $\text{O}_2^{\cdot-}$. (a) $\text{FADH}^\cdot/\text{O}_2^{\cdot-}$ for $k_f^{-1} = 1$ and $10 \mu\text{s}$, $k_S = k_f$ and $k_T = 0$; (b) and (c) $\text{FADH}^\cdot/\text{O}_2^{\cdot-}/\text{Y}^\cdot$ for $k_f^{-1} = 1$ and $10 \mu\text{s}$, respectively, different k_S and k_X as indicated in the legends, and $k_T = 0$. The Y[•] has been modeled after Y319 in *DmCry*. For the three-radical systems, the hyperfine interactions of N5, N10, and H5 in FADH^\cdot and of the two ortho-protons in Y[•] have been considered; (a) additionally included H β 1 and H6 in FADH^\cdot . The insets on the right illustrate the anisotropy of the yield of the signaling state evaluated for $\gamma_B = 0.022 \text{ ns}^{-1}$, the relaxation rate expected for a viscous cellular environment, and the conditions represented by the blue lines. These diagrams are not drawn to scale.

criterion of distance; beyond that, we do not want to imply mechanistic relevance of this system. For a moderately fast scavenging rate of $k_X \sim 10 \mu\text{s}^{-1}$, Figs. 2(b) and 2(c) again show the dependence of the relative reaction yield on the rate of spin relaxation, γ_B , in the superoxide radical. It is apparent that the three-radical model predicts MFEs that exceed those of the RPM-based model, in particular, for the longer lifetime of $k_f^{-1} = 10 \mu\text{s}$ [Fig. 2(c)] and that, importantly, the effects are robust to spin relaxation in the superoxide radical anion. Specifically, we find that the anisotropy is unaffected by B'-spin relaxation if $k_S = k_T = 0$. For non-zero recombination in the primary pair, e.g., $k_S = k_f$, the spin relaxation has an attenuating effect, but effects remain large even for $\gamma_B \rightarrow \infty$. The diminishing effect of primary pair recombination in this limit is consistent with the observation [as expressed in Eqs. (12) and (13)] that, for $\gamma_B \rightarrow \infty$, the radical-triad dynamics correspond to that of the AC-pair but with decreased lifetime, i.e., increased effective k_f' . As the effective lifetime is decreased for $k_S > 0$ [see Eq. (13)], the sensitivity to the magnetic field is reduced, which is particularly pronounced for systems with lifetimes close to the “detection limit” for the geomagnetic field (i.e., for the simulations with $k_f^{-1} = 1 \mu\text{s}$; the time of at least one Larmor precession, i.e., ~ 700 ns, is required to elicit a marked response to the magnetic field). Figure 2 also shows the anisotropy of the MFE for $\gamma_B = 0.022 \text{ ns}^{-1}$, the relaxation rate expected for a viscous cellular environment, as an inset. A comparable directionality of the radical-pair and radical-triad systems is observed (while the size of the effects is markedly different).

For the calculations on triad systems with explicit spin relaxation taken into account, we have been able to include the five largest hyperfine interactions. Subsequent simulations will assume the extreme relaxation limit, $\gamma_B \rightarrow \infty$, for simplicity and efficiency while also being in line with the spin relaxation rates as estimated above. In this limit, we will be able to consider the eight largest hyperfine-coupled nuclei, yielding a more realistic description of the spin dynamics in these triad systems. As might be expected in view of the combined simulation data published for various radical pair systems,^{20,29,30} this increase in complexity is associated with a (modest) decrease in Γ_S .

Figure 3(a) shows the dependence of the relative anisotropy of the flavin semiquinone/superoxide/Y319 triad system on the rate constant of scavenging for $k_f^{-1} = 1$ and $10 \mu\text{s}$. Spin relaxation in the superoxide anion has been accounted for in the $\gamma_B \rightarrow \infty$ limit; EED interactions have initially been neglected (see below). The data reveal substantial MFEs for scavenging rate constants $k_X \sim 10 k_f$. For $k_f^{-1} = 1$ and $10 \mu\text{s}$, the effect peaks at $k_X = 13 \mu\text{s}^{-1}$ and $k_X = 6 \mu\text{s}^{-1}$, respectively. With increasing lifetime, the maximally attainable Γ_S increases and the associated scavenging rate constant k_X decreases. A similar trend is obtained for the absolute orientational spread of the reaction yield, Δ_S , except for that the $k_f^{-1} = 10 \mu\text{s}$ data appear to peak at lower k_X ($k_X = 0.9 \mu\text{s}^{-1}$ for $k_f = 0.1 \mu\text{s}^{-1}$; see the supplementary material). Figures 3(b) and S1 in the supplementary material provide comparable data for Y319 from ClCry4 and Y388 from ErCry4. The latter is the tyrosine residue closest to FAD according to a homology model taken from Ref. 46 (FAD-N5-OH-distance: 13.8 Å). Y319 in ClCry4 is a surface-exposed tyrosine extending the tryptophan tetrad. Radical formation at this site has been implicated with the photo-reduction.²⁴ Despite different relative orientations of these tyrosine residues compared to Y319 in DmCry, the predicted

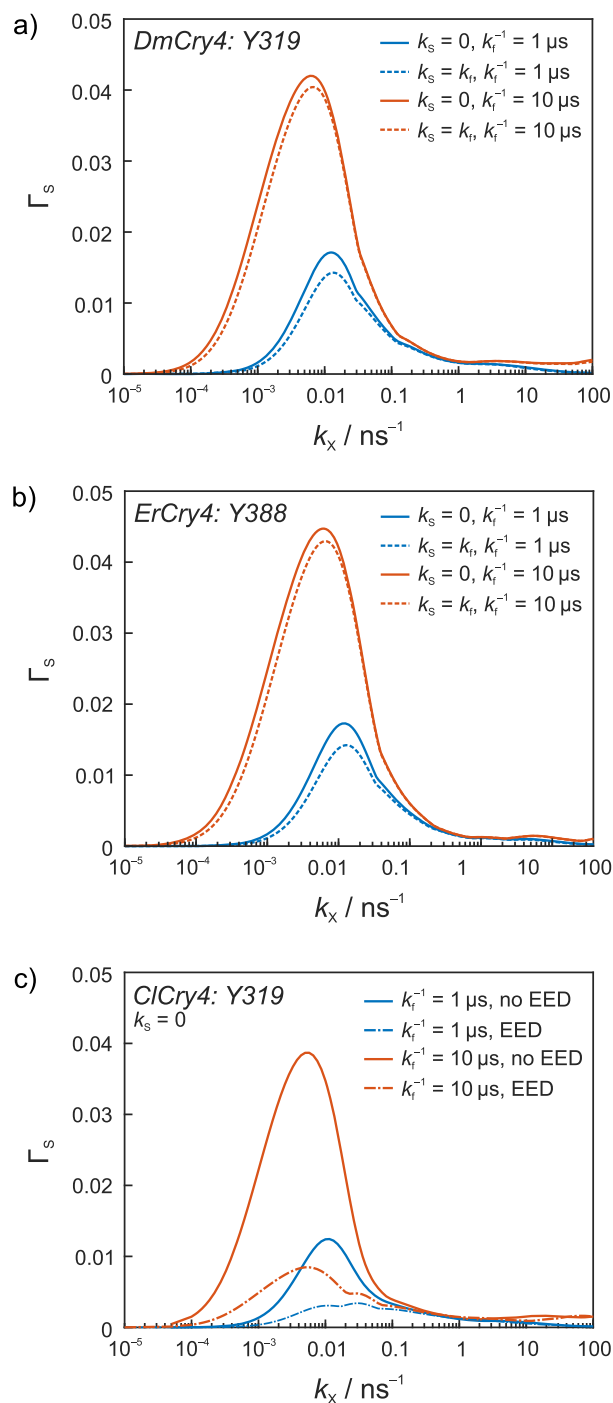


FIG. 3. Directional magnetic field effects evaluated for models of $\text{FADH}^+/\text{O}_2^{\cdot-}/\text{Y}$ in the limit of infinitely fast spin relaxation in $\text{O}_2^{\cdot-}$ presented as a function of the scavenging rate constants k_X . The scavenger radical Y has been identified with (a) Y319 in DmCry, (b) Y388 in ErCry4, and (c) Y319 in ClCry4, respectively. k_S and k_f are specified in the legends; $k_T = 0$. All simulations included the hyperfine interactions of N5, N10, H5, H6, and H β 1 in FADH^+ and Ho1, Ho2, and H β 2 in Y. For (a) and (b), EED interactions have been neglected; (c) provides simulations with (dashed-dotted lines) and without (solid lines) EED interactions.

anisotropies of the three systems are comparable, as is shown in Fig. 3. In Figs. 3(a) and 3(b), we summarize simulations for $k_S = k_f$ in addition to $k_S = 0$. It is apparent that in the presence of significant scavenging, the effect of primary pair recombination, i.e., k_S , is minor, which is in agreement with the findings in the original publication for flavin/tryptophan radical pairs scavenged by radicals devoid of hyperfine interactions.³¹ Figures S2 and S3 in the supplementary material further explore the dependence of the MFE on the reaction yield and its anisotropy on the intensity of the applied magnetic field. The simulations predict large relative anisotropies, up to 30%, at fields below 10 mT (for larger fields, the anisotropy is reduced) and huge MFEs of the reaction yield of several 100%. It is interesting to note that these MFEs monotonically increase with the applied field without going through an anti-phased low-field feature, as typical for the RPM under the conditions applying for magnetoreception.⁹

The electron–electron dipolar interaction of radicals is inevitably significant in magnetosensitive radical recombination reactions, both in radical pair and radical triad scenarios, where radicals are restrained at a distance allowing for mutual recombination, typically by distant electron transfer.^{23,40} Nonetheless, EED interactions have traditionally been neglected for simplicity, reduction of computational effort, and possibly on conceptual grounds, as the RPM places an emphasis on the hyperfine-driven spin-state conversion. Recently, unexpected rich MFEs have been predicted for three-radical systems due to the EED coupling alone,^{47,48} while the suppressive effect of EED interactions on the low-field sensitivity has raised doubts about the RPM hypothesis of magnetoreception.²³ Both observations motivate the exploration of the profound effects of EED coupling in the scavenged FADH[•]/O₂^{•−} systems.

In the limit of fast O₂^{•−} relaxation, the EED-interactions involving O₂^{•−} are effectively averaged. Thus, the spin dynamics are independent of the location of O₂^{•−} relative to the flavin and tyrosine radicals that governs its EED coupling [see Eq. (4)]. EED interactions will still impact on the MFEs via the FADH[•]/Y coupling. Possibly not unexpected in view of the suppressive effect found in Ref. 23, we find that here too the directional MFEs are strongly suppressed by the EED coupling. This is demonstrated for Y319 in Fig. 3(c). For $k_f^{-1} = 1 \mu\text{s}$, Γ_S of this system is reduced from 1.3×10^{-2} to 3.4×10^{-3} , whereby a larger k_X is necessary to elicit this maximal effect. For Y319 in *DmCRY*, the suppression is even stronger, which is in line with the larger EED coupling present at the shorter inter-radical distance. For this system with $k_f^{-1} = 1 \mu\text{s}$, Γ_S is reduced from 1.7×10^{-2} to a mere 2.3×10^{-3} . Moreover, the peak of sensitivity of this system is shifted to markedly larger scavenging rate constants k_X (8.2 ns^{-1} as opposed to 0.013 ns^{-1} without EED). Figure S4 illustrates the dependence of the MFE on k_X for various tyrosine sites.

While the suppressive effect of the EED coupling is discouraging, the effect is expected to strongly vary with distance and orientation of the radicals involved. This led us to carry out a systematic exploration of the suitability of the various tyrosine sites in the cryptochrome protein to sustain large three-radical MFEs. These data are the main results of this contribution. We opted to use *CiCry4* and *DmCry* as template structures (for which the crystal structures have been resolved, at least in part^{24,49}) and again considered lifetimes of $k_f^{-1} = 1$ and $10 \mu\text{s}$. The primary radical recombination rate constant was set to $k_S = 0 \mu\text{s}^{-1}$, as the predictions

are broadly insensitive to this choice. The exchange coupling of the scavenger and the flavin semiquinone was neglected in order to reduce the number of parameters to the manageable set of k_X and tyrosine-location in the protein (experimental values of the exchange interaction are available for the terminal tryptophans of the triads/tetrads of selected cryptochromes and photolyases, for which they appear to be negligibly small⁵⁰). The eight largest hyperfine interactions, five in FADH[•] and three in Y, have been taken into account. Figure 4 summarizes the dependence of the directional MFEs for the 18 tyrosine residues in *CiCry4*. The maximal Γ_S 's range from 1.1×10^{-3} to 0.021 and 2.6×10^{-3} to 0.065 for $k_f^{-1} = 1$ and $10 \mu\text{s}$, respectively. Here, we find that for many tyrosine residues in *CiCry4*, the MFEs remain large in the presence of EED coupling. This applies, in particular, for (in the order of decreasing

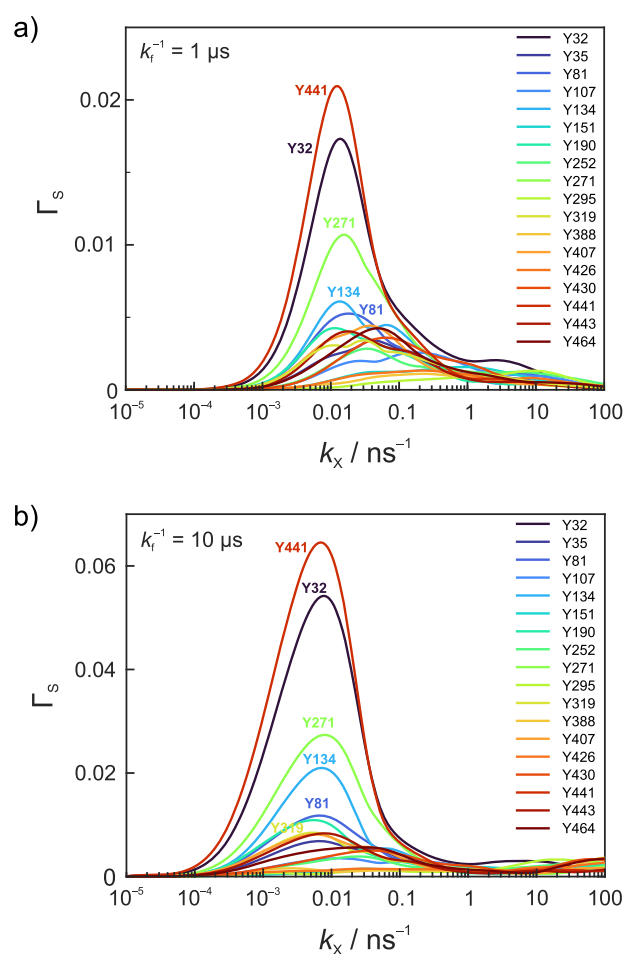


FIG. 4. Directional magnetic field effects evaluated for various FADH[•]/O₂^{•−}/Y radical triads based on the crystal structure of *CiCry4* for $k_f^{-1} = 1 \mu\text{s}$ (a) and $10 \mu\text{s}$ (b). The simulations have assumed infinitely fast spin relaxation in O₂^{•−}, $k_S = k_f$ and included the hyperfine interactions of N5, N10, H5, H6, and Hβ1 in FADH[•] and Ho1, Ho2, and Hβ2 in Y. The effect of the EED coupling was fully taken into account. The systems differ in the assumed position of the Y, which is indicated in the legend in terms of the residue number in the crystal structure of *CiCry4* (PDB ID: 6PU0).

maximal Γ_S) Y441, Y32, Y271, Y134, Y81, and Y190, for which large effects are observed for both $k_f^{-1} = 1$ and $10 \mu\text{s}$. Note further that for these systems, the maximal MFEs occur at the “usual” scavenging rate constants of $k_X \sim 10 \mu\text{s}^{-1}$ and that the longer lifetime, i.e., $k_f^{-1} = 10 \mu\text{s}$, gives rise to larger sensitivity, as expected. Figure S4 provides a similarly exhaustive exploration for the tyrosine residues in *DmCry* for $k_f^{-1} = 1 \mu\text{s}$, both in the presence and absence of EED coupling. Maximal Γ_S 's are of the order of 2% and 1% in the absence and presence of EED coupling, respectively. If the EED interaction is ignored, the largest effects are predicted for Y328, Y317, and Y319; with EED coupling included, the MFEs of the two surface-exposed residues Y90 and Y211 exceed those of other sites by more than a factor of three at their respective optimal k_X .

DISCUSSION

Magneto-sensitivity of the reoxidation

The potential magneto-sensitivity of the cryptochrome reoxidation reaction has been widely discussed following up on works of Ritz *et al.*, proposing the involvement of molecular oxygen, and later Maeda *et al.*, proposing superoxide.^{12,51} The flavin semiquinone/superoxide radical pair is central to this hypothesis.^{13,14,16} In terms of its hyperfine interaction structure alone, this model promises superior magneto-sensitivity, vastly exceeding that of flavin/tryptophan radical pairs.^{20,21} However, in free solution, superoxide is subject to fast spin relaxation as a consequence of its orbitally degenerate ground state and thus non-zero angular momentum, which gives rise to huge g-tensor anisotropy and eventually swift spin relaxation via the spin-rotational mechanism.⁴³ It is for this reason that the model has widely been viewed as improbable,¹⁴ leading to the widespread practice of postulating a radical, usually denoted Z',²⁰ which shares superoxide favorable features in terms of being devoid of hyperfine interaction but is freed of its stigma of fast spin relaxation. The recent observations of dark-state magneto-sensitivity, however, have brought the superoxide hypothesis back into the spotlight of cryptochrome's magneto-sensitivity.^{17–19} Further indirect support has been found in the observation of magnetoreception being affected by green/red light in some experiments and its feasibility under low light conditions.⁵² Note, however, that as for now, no consensus as to the identity of the radical pair has been reached.

A frequent argument suggests that $\text{FADH}^{\cdot}/\text{O}_2^{\cdot-}$ could be feasible if the superoxide's rotational tumbling was hindered and/or its molecular symmetry reduced, i.e., its angular momentum quenched.^{14,22} This implies that the radical would have to strongly interact with its environment, i.e., be essentially immobilized. This notion, however, is equally unsuccessful as immobilization at a feasible reaction distance⁵³ gave rise to larger, non-averaged EED coupling, which in model calculations effectively suppresses the magneto-sensitivity of the radical pair recombination in weak magnetic fields.²³

Magneto-sensitivity enabled by three-radical interactions

We here suggest that this impasse can be overcome by a model involving three radicals, whereby the primary flavin semiquinone

superoxide radical pair is scavenged in a spin-selective recombination reaction with a third radical. This model was conceptually introduced in Ref. 15, where the authors demonstrated vastly enhanced directional sensitivity and resilience to spin relaxation in the non-scavenged radical of the primary pair, at least for simple model systems and neglecting the EED interaction. Here, we have subjected this idea to test under more realistic conditions, whereby we focus on the hypothetical scavenging reaction from a long-lived tyrosine radical inside of the protein for concreteness. We have realized a systematic survey for 18 tyrosine residues in the vicinity of the FAD cofactor in *CiCry4*, whereby a realistic but necessarily simplified model of the hyperfine structure, the relative orientation of the radicals, and their unavoidable EED coupling have been considered. In the limit of fast spin relaxation in the superoxide, the spin dynamics have been found to essentially correspond to that of the radical pair of FADH^{\cdot} and Y', subject to an F-pair initial condition, spin-selective recombination due to the scavenging reaction, and an altered lifetime. While thus showing similarity to strongly asymmetric radical pair recombination reactions,⁵⁴ the model permits the radical pair generation via dark-state reactions, such as the direct oxidation of the fully reduced FAD with molecular oxygen under production of superoxide. The fast relaxation of the superoxide could in this model offer an advantage, as it averages the large EED coupling of the $\text{FADH}^{\cdot}/\text{O}_2^{\cdot-}$ pair at the short reaction distance for hydroperoxide formation (a study has identified a likely binding site of $\text{O}_2^{\cdot-}$ in immediate vicinity of H5 of FADH^{\cdot} ⁵⁵), which otherwise exerted a strongly suppressive effect on the magneto-sensitivity. While this suppression can, in principle, be overcome in slowly relaxing three-radical schemes, as has been shown in Refs. 23 and 41, it requires comparable EED coupling of the involved radicals. This, however, implies that all three radicals had to be close in space, which could turn out impractical/improbable in practice. In this sense, the fast relaxation in $\text{O}_2^{\cdot-}$ is recognized to simplify the model prerequisites.

We have here found that the three-radical scheme $\text{FADH}^{\cdot}/\text{O}_2^{\cdot-}/\text{Y}'$ with quickly relaxing $\text{O}_2^{\cdot-}$ predicts comparably larger anisotropy of the MFEs even if the suppressive effect of the EED coupling is accounted for. Specifically, the model delivers yield anisotropies exceeding those of the realistic model of $\text{FAD}^{\cdot-}/\text{W}^+$ even when the suppressive EED is neglected in the latter.^{29,30} Several auspicious Y-residues have been identified in *CiCry4* that sustained large MFEs. Inspecting the location of the radicals relative to the FAD, one realizes, however, that particularly large MFEs arise from distant Ys typically located at the protein's surface (see Fig. 1), i.e., the data appear to reflect an intrinsic tendency to reduce the EED coupling by increasing the inter-radical distance. Indeed, we find a strong correlation of the maximally attainable relative anisotropy Γ_S (for the optimal k_X) and the inter-radical distance. For $k_f^{-1} = 1 \mu\text{s}$, Pearson's correlation coefficient of the FAD-N5-Y-OH -distance and Γ_S amounts to $R = 0.82$ for the combined *DmCry* and *CiCry4* data; for $k_f^{-1} = 10 \mu\text{s}$ in *CiCry4*, we find $R = 0.79$ (the correlation of Γ_S and dipolar interaction strength, which scales with r^{-3} , is weaker; $R = -0.49$). Further note that the simple correlation of the MFE and distance appears to be a consequence of essentially reducing the system dynamics to that of the pair, i.e., this tendency had not been identified for slowly relaxing three-radical systems in Ref. 41.

Scavenging rates vs inter-radical interactions

The effect requires an efficient scavenging reaction with the rate constant $k_X \sim 10 \mu\text{s}^{-1}$ or more for small inter-radical distances. Thus, a balance needs to be struck between reducing the EED coupling by increasing the inter-radical distance and the need to sustain this reaction rate. Among the Ys identified for *CiCry4* to potentially give rise to sizable effects (if a suitable k_X could be realized), Y319 has attracted particular attention ($\Gamma_S = 8.5 \times 10^{-3}$ for $k_f^{-1} = 10 \mu\text{s}$).¹⁵ Y319 extends the highly conserved electron transfer pathway provided by the tryptophan tetrad (W_A , W_B , W_C , and W_D in Fig. 1; the Y319- W_D distance is only 3.9 Å).¹¹ Is it conceivable that the very same pathway that facilitates the charge separation in the photo-reduction could enable the scavenging as previously hypothesized?¹⁵ The smallest tyrosine-to-isoalloxazine distance (R) amounts to 17.1 Å (between O in Y319 and C7M in FAD). If we assume that the scavenging reaction proceeds by diabatic, long-distance electron transfer, we can estimate the scavenging rates using the Moser–Dutton ruler,⁵⁵

$$\log_{10} k_X \cong 13 - \frac{\beta}{2.3} (R - 3.6\text{\AA}) - 3.1 \frac{(\Delta G_{et} + \lambda)^2}{\lambda}. \quad (14)$$

In the most favorable activation-less limit ($\Delta G_{et} = -\lambda$, $\Delta G^\ddagger = 0$) and using typical values for β (which represents the exponential decay of the coupling-matrix element-squared with distance) ranging from 0.9 and 1.4 Å⁻¹,^{56,57} one obtains values of k_X between 6×10^5 and $5 \times 10^8 \text{ s}^{-1}$. Thus, provided that the coupling is efficient, the required k_X is at least not infeasible. However, it should be noted that the electron transfer rates would have to be modulated throughout the photo-cycle. In particular, the charge recombination ought to be slow in the fully reduced state (to avoid premature reoxidation of the flavin) and fast once the re-oxidation was initiated by reaction with molecular oxygen. That such modulation is, in principle, possible is hinted at in several studies. A recent molecular dynamics investigation of the photo-activation of *CiCry4* identified significant changes of the FAD- W_D distance, and thus electron transfer rates, set in motion by the charge redistribution associated with the photoreduction.⁴⁵ Metabolite binding and (de-)protonation reactions can strongly modulate electron transfer rates in cryptochromes.⁵⁸ The requirement could also be fulfilled if the FADH⁻/Y⁻ recombination is in the Marcus inverted region, while the less exergonic FADH⁻/Y⁻ is close to activationless. While experimental insights do not yet allow a serious assessment of the feasibility of a three-radical processes involving Y319, it is reassuring that Y319 appears to enhance the efficiency of photo-reduction in *CiCry4* *in vitro*, thereby hinting at the possibility of formation of a long-lived tyrosine radical at this location.²⁴ It is also worth mentioning that the mechanism as suggested here correctly predicts the enhancement of the signaling state yield in modest magnetic fields (0.5 mT) as observed for the dark-state MFEs in plants (while the radical-pair mechanism will predict a decrease for the triplet-born FADH⁻/O₂⁻ radical pair due to the low-field effect).¹⁹ Finally, note that while the MFE predicted for Y319 in *CiCry4* is not among the largest observed here, further enhancement appear realizable by optimizing the relative position of the radicals in space, even when keeping the inter-radical distance constant. That this is possible in principle is, e.g., demonstrated by Y134 in *CiCry4*, for which large MFEs

($\Gamma_S = 0.021$) are predicted despite its smaller distance from FAD compared to Y319.

In view of the systematic study of *CiCry4* presented above, it is furthermore remarkable that the optimal k_X tends to increase with decreasing distance, at least for FAD-Y distances smaller than ~25 Å; for larger distances, the distance dependence is weak. Thus, at least in part the deleterious effect of large electron–electron dipolar couplings at small reaction distances appears to be compensated by increased scavenging reaction rate constants. This is not unknown for radical pair reactions, to which the spin dynamics of the radical triad system are here effectively reduced due to the assumed fast spin relaxation in superoxide. Specifically, Kominis and co-worker previously showed that the effect of the exchange coupling can be ameliorated by fast triplet recombination via the quantum Zeno effect, i.e., a decreased decay rate of quantum states subject to frequent measurement, reaction events, or other decoherence generating processes.^{59,60} It appears that here a similar principle is at play. However, the compensation results from the fast singlet recombination that the scavenging reaction equates to in the limit of fast O₂⁻ relaxation. Indeed, following Ref. 59, a simple model based on the coherent interconversion of degenerate singlet and triplet states with frequency ω subject to fast singlet recombination with rate k_X shows that one of the eigenstates of the Liouvillian decays with rate $4\omega^2/k_X$. This quantum Zeno scaling, i.e., the larger the interrogation rate k_X , the slower the decay, can enhance the sensitivity to weak magnetic fields. Note that while a fast triplet recombination is not expected in the avian compass system,³⁶ here the asymmetric relativity is introduced indirectly via the scavenging processes, for which a variety of processes are conceivable.

Chemical requirements of the tyrosyl radical hypothesis

Here, we have focused on the requirements that would enable a FADH⁻/O₂⁻/Y⁻-magnetosensor from the point of view of spin dynamics. In addition, such a system is obviously constrained by its chemistry.¹⁵ First and foremost, the Y-radical has to be formed. Ideally this radical ought to be persistent to enable “on-demand” magnetoreception, for which the “charging” (i.e., the accumulation of scavenger radicals) of the compass and its readout (i.e., light-independent reoxidation) are temporally separated to also work in the night.

As for Y⁻-formation, the process could be facilitated by photo-activation and accompany the photoreduction as suggested above for Y319 in *CiCry4*.¹⁵ It could also be realized by chemical, i.e., dark, one-electron oxidation of tyrosine by mechanisms that include oxidation by hydrogen peroxide and peroxidases, hydroxyl radical- and metal-catalyzed oxidations, and electron or hydrogen transfer to other radicals.⁶¹ In fact, dark-state tyrosyl radicals are part of the enzymatic catalytic cycle of, for example, ribonucleotide reductase, prostaglandin synthase, and lactoperoxidase and are also formed on myoglobin and hemoglobin during reaction with hydrogen peroxide (see Ref. 61 and references therein).

As for the lifetime constraint, long-lived tyrosine radicals have been observed as a product of the photo-reduction in the animal-like cryptochrome in the green alga *C. reinhardtii* (lifetime: 2.6 s²⁶) and a cryptochrome from *A. thaliana* (100 μs ²⁷). Arguably, these lifetimes are too short to allow ample radical accumulation under

light for later readout in the dark, but it could still rationalize the flickering-light experiments. On the other hand, more persistent tyrosyl radicals are well known in biochemistry. The classical example is provided by the radical of the redox-active tyrosine residue Y_D in the photosystem II, which exhibits lifetimes up to hours, depending on preparation.⁶² Although for now no such long-lived tyrosyl radical has been observed in avian cryptochromes (or other magnetosensitive animal cryptochromes), its occurrence is not infeasible and, in our opinion, should be considered a target of experimental efforts.

In general, chemical factors cannot currently be assessed beyond speculation. The true magnetoreceptor is not identical to free cryptochrome, as recently discussed.⁸ Protein–protein interactions, immobilization, and association with the appropriate signaling partners are deemed essential to realize sensitivity to magnetic fields of around $50 \mu\text{T}$ *in vivo*, all of which will impact on the feasibility of $\text{FADH}^{\cdot-}/\text{O}_2^{\cdot-}/Y^{\cdot}$.

It has become clear from the discussion that several requirements (k_X , scavenger location, chemistry) have to be met for the three-radical processes to function as a sensitive compass as originally envisaged. We think that it is not unconceivable that such a complex but more sensitive mechanism has resulted as the apex of an evolutionary optimization process. One could imagine that rudimentary magnetosensitivity was present in the photo-reduction but succeeded by a three-radical process realized on the basis of the pre-existing, basic building blocks. This could have offered greater sensitivity (limited by the lacking symmetry of hyperfine interactions in W^+ and by EED interactions; see Refs. 20, 30, and 63) and reception on demand/despite low light levels, for which the accuracy of the photo-reduction-based compass is likely insufficient.⁵²

Ascorbyl radical: A better model?

We here focus on a mechanism *within* cryptochrome and have hence assumed the scavenger in the form of tyrosine radicals, putatively generated in the preceding photo-reduction. This was a deliberate decision for this study made for concreteness but does not present a necessity. In fact, alternative choices could be more favorable. Assume, for example, that the oxidation equivalent made available in the photo-reduction, via the tryptophan tetrad, ends up in a stable radical derived from a radical scavenger (in the original sense of the term), such as, for example, ascorbic acid, AscH_2 . Following the reaction cycle as in Fig. 1, but with the ascorbyl radical ($\text{Asc}^{\cdot-}$) instead of the tyrosine residue, could give rise to a $\text{FADH}^{\cdot-}/\text{O}_2^{\cdot-}/\text{Asc}^{\cdot-}$ radical triad. Figure 5 shows the directional MFEs calculated for this system as a function of the rate of the $\text{FADH}^{\cdot-}/\text{Asc}^{\cdot-}$ -recombination reaction. Huge I_S 's of the order of 60% appear feasible for this system. This large effect could, however, come at the cost of less tight control (compared to $\text{FADH}^{\cdot-}/\text{O}_2^{\cdot-}/Y^{\cdot}$), as it involved a freely diffusing $\text{Asc}^{\cdot-}$. It is interesting to note that the $\text{FAD}^{\cdot-}/\text{Asc}^{\cdot-}$ -radical pair has been previously suggested in the context of the photo-reduction as a more potent radical pair²⁰ but rejected as the formation of the ascorbyl radical from the primary $\text{FAD}^{\cdot-}/W^+$ could not compete with its recombination.⁶⁴ This is not a limitation here as the $\text{Asc}^{\cdot-}$ formation precedes the radical triad processes and could thus be realized on a slow timescale either via the photo-reduction or independent pathways.

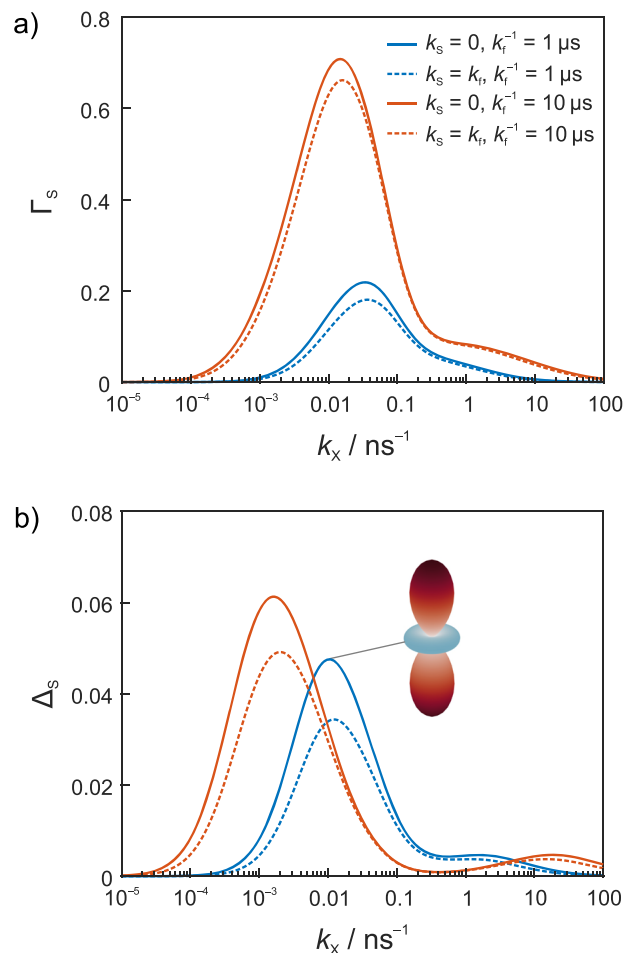


FIG. 5. Directional magnetic field effects evaluated for the $\text{FADH}^{\cdot-}/\text{O}_2^{\cdot-}/\text{Asc}^{\cdot-}$ radical triad, where $\text{Asc}^{\cdot-}$ denotes a freely diffusing ascorbyl radical. The relative anisotropy (a) and absolute spread of the reaction yield of the signaling state are shown in (a) and (b), respectively. k_f and k_S are indicated in the legend of (a), which applies throughout. The simulations have included the hyperfine interactions of N5, N10, H5, H6, and H β 1 in $\text{FADH}^{\cdot-}$ and the isotropic interaction of H4 in $\text{Asc}^{\cdot-}$. The model does not depend on the identity of the cryptochrome.

Superoxide MFEs beyond magnetoreception

Eventually, we want to point out that superoxide containing radicals have been suggested to underpin a broad range of magnetic field and isotope-sensitive reactions beyond the established paradigm of cryptochrome magnetoreception. Usselman *et al.* used oscillating magnetic fields at the Zeeman resonance to impact on reactive oxygen species (ROS) partitioning and thus cellular bioenergetics, which the authors attributed to the $\text{FADH}^{\cdot-}/\text{O}_2^{\cdot-}$ -radical pair.^{65,66} Sherrard *et al.* demonstrated that exposure to weak magnetic fields can induce ROS production in human cells and that this process requires the presence of the cryptochrome receptor.⁶⁷ Smith and co-workers suggested that isotope effects on xenon-induced anesthesia⁶⁸ and the lithium treatment of bipolar disorder⁶⁹ could both be linked to transient, superoxide containing radical

pairs. Zhang *et al.* demonstrated that hypomagnetic fields attenuate hippocampal neurogenesis and cognition in mice and that this effect is correlated with a reduction of reactive oxygen species.⁷⁰ While in these contexts magnetosensitivity appears to emerge as a serendipitous side effect, the suggested processes share much similarity with the FADH/O₂^{•-}-model of cryptochrome magnetoreception. Here too, the question of fast spin relaxation in O₂^{•-} appears pertinent. While it is unlikely that a complex three-radical processes of the marking FADH/O₂^{•-}/Y[•] could be relevant here too, because the complexity of the mechanism likely mandated evolutionary optimization, a FADH/O₂^{•-}/Asc^{•-} process appears possible. Highly reactive radicals are quickly sequestered in biological systems, usually under the formation of persistent, less reactive species, such as Asc^{•-}, which could engage in the suggested three-radical process. In this context, it is interesting to note that many observations of dark-state MFEs appear to implicate a relation with oxidative stress⁷¹ and thus the overall radical concentration, in line with the expectations of the three-radical hypothesis.

Suggested experiments

The reoxidation of cryptochrome and its putative magnetosensitivity remain a mystery. Here, we have suggested a way by which these processes could elicit magnetosensitivity despite its involvement of quickly relaxing reactive oxygen species, such as superoxide. It is clear that many questions about feasibility and concrete implementation, including the identity of the scavenger radical, remain speculative. We hope nonetheless that this theoretical analysis is able to instigate dedicated experiments. A simple experiment could be built around the cyclic photo-reduction and dark re-oxidation of a cryptochrome *in vitro*. Magnetic fields could be applied selectively during the dark reoxidation, while the fluorescence of the flavin cofactor could be used to readout the oxidation yield during the photo-reduction cycle. Probing the magnetosensitivity of the processes while varying the frequency of photo-reduction and reoxidation cycles, optionally in the absence and presence of, e.g., ascorbic acid, would allow valuable, needed insights to solve the cryptochrome puzzle. In addition, the identification of putatively long-lived tyrosyl radicals in avian cryptochromes and, in particular, their assemblies *in vivo* is suggested.

CONCLUSIONS

In summary, we have presented comprehensive simulations of radical triad systems comprising the flavin semiquinone, superoxide, and a tyrosine radical in cryptochrome. Assuming that the tyrosine radical can undergo a spin-selective recombination reaction with the flavin radical, this model delivers sizable directional magnetic field effects in the geomagnetic field, even if the superoxide is subject to fast spin relaxation and electron–electron dipolar interactions are fully accounted for. Specifically, we have shown that in the limit of infinitely fast spin relaxation in the superoxide, the spin dynamics observables correspond to those of the flavin semiquinone tyrosine system with random initial spin configuration, spin-selective recombination, and an altered effective lifetime. For the model to elicit marked magnetosensitivity in the geomagnetic field, the scavenging reaction ought to proceed swiftly, i.e., with rate $k_X \sim 10^7 \text{ s}^{-1}$ or larger.

This requirement and a systematic exploration of the effect for the tyrosine residues in pigeon cryptochrome 4 show that Y319 is a possible candidate to facilitate such enhanced three-radical MFEs. Being located at the end of the tryptophan tetrad, Y319 appears to form long-lived tyrosine radicals that could act as scavengers of FADH[•], generated in the reoxidation of FADH[•], via a long-range electron transfer processes mediated by the tryptophan tetrad.

The aim of this work was to test the hypothesis of a scavenging-enhanced MFE in a more realistic context than previously explored. In particular, the complexity arising from many hyperfine-coupled nuclear spins, the relative orientations of radicals, and the electron–electron dipolar coupling have been considered. The requirement to sustain a fast scavenging reaction while simultaneously reducing the EED coupling has been identified as the limiting factors in the employed FADH[•]/O₂^{•-}/Y[•] model. In the limit of fast relaxation in the O₂^{•-}, the location of the superoxide relative to the other radicals is inconsequential, i.e., radicals at close contact can be accommodated, but the processes is more susceptible to the electron–electron dipolar coupling in the FADH[•]/Y[•]. Here, slowly relaxing, i.e., immobilized O₂^{•-} could provide the additional degrees of freedom needed to compensate the mutual dipolar coupling and further boost the magnetosensitivity.⁴¹ Fast relaxation in O₂^{•-} reduces the spin dynamics to that of an effective radical pair system that has lesser degrees of freedom to compensate for the delirious effect of the mutual EED coupling.

The FADH[•]/O₂^{•-}/Y[•] system here has been elected for study for concreteness, but we also acknowledge that systems involving a freely diffusing scavenger radical besides the quickly relaxing superoxide might fare better in sustaining large MFEs, as they avoid the suppressive effect of the electron–electron dipolar interaction by design. With this in mind, FADH[•]/O₂^{•-}/Asc^{•-}, where Asc^{•-} is a freely diffusing stable radical resulting from a radical scavenger, such as ascorbic acid, has been realized as an exquisitely magnetosensitive system. The simplicity and general abundance of radical scavenger-derived radicals suggests that this potent spin chemical scheme could be relevant for many recent observations or suggestions of magnetic field and isotope effects involving reactive oxygen species. Overall, we find that the three-radical model involving a scavenging reaction is feasible in principle. The issue of the electron–electron dipolar coupling suppressing the effect can be sidestepped for freely diffusing systems and mitigated in systems of three immobilized radicals by suitable placement of the radicals.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for a detailed listing of simulation parameters, the dependence of the magnetic field effect of selected radical triad systems on the field intensity, and results on the FADH[•]/O₂^{•-}/Y[•] radical triads in *DmCry*.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

DATA AVAILABILITY

The data that support the findings of this study are available within the article and its [supplementary material](#). Raw simulation data providing the recombination/escape yield as a function of the magnetic field direction are provided by the corresponding author upon reasonable request.

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