Magnetoreception Through Cryptochrome May Involve Superoxide (Supplementary Material)

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Choice of hyperfine coupling constants for the [FADH$^\bullet$ + $O_2^{\bullet -}$] radical pair

For a qualitative understanding of the feasibility of a magnetic field effect in the suggested reaction we consider only one representative spin-1/2 nucleus on the FADH$^\bullet$ radical with anisotropic hyperfine coupling tensor A, as also assumed in (1), given by

$$A = \begin{pmatrix} 10\text{ G} & 0 & 0 \\ 0 & 10\text{ G} & 0 \\ 0 & 0 & 0 \end{pmatrix}. \tag{S1}$$

The values of the hyperfine coupling tensor are chosen to be close to the values, which were calculated (2, 3) and measured (3–5) for the nuclei of the FADH$^\bullet$ radical at different conditions. The main purpose of the suggested generic model is to demonstrate to what extent the interplay among hyperfine coupling strength, magnetic field strength, and rate constants k_b, k_et affects the FADH$^\bullet$+$O_2^{\bullet -}$→FADH$^-$ +O_2 reaction in cryptochrome. The goal is solely to illustrate the feasibility of a magnetic compass in birds based on the reaction in Fig. 2 rather than to present a quantitative model for the magnetoreception capability.

Substituting Eqs. (10)-(12) and Eq. (S1) into Eqs. (8)-(9) one obtains

$$\hat{H}_\text{FADH} = \frac{g\mu_B}{2}\left[B_x(\hat{\sigma}_x \otimes E_2) + B_z(\hat{\sigma}_z \otimes E_2)\right] + \frac{a\mu_B}{4}\left[\hat{\sigma}_x \otimes \hat{\sigma}_x + \hat{\sigma}_y \otimes \hat{\sigma}_y\right] \tag{S2}$$

$$\hat{H}_\text{O2-} = \frac{g\mu_B}{2}\left[B_x\hat{\sigma}_x + B_z\hat{\sigma}_z\right], \tag{S3}$$

where $a = 10$ G is the hyperfine coupling, which follows from Eq. (S1); the first factor of the tensor product in Eq. (S2) acts on the electron spin, the second factor acts on the nuclear spin, and the operators in Eq. (S3) act on the electron spin.

Calculation of FADH$^\bullet$+O$_2^{\bullet-}$→ FADH$^-$+O$_2$ reaction energies

In determining the energy difference between ^{1}[FADH$^\bullet$ + O$_2^{\bullet-}$] and ^{1}[FADH$^-$ + O$_2$] states we proceed as follows. The structure and energy of the stable FADH$^\bullet$, FADH$^-$, O$_2^{\bullet-}$ and O$_2$ molecules was determined using density functional theory used in physics and chemistry to investigate the electronic structure of many-body systems. With this theory, the properties of a many-electron system can be determined. We use the so-called B3LYP density functional as it has been proven to be successful for the calculation of properties of various organic molecules (6–10).

To determine the energy of a molecule within the framework of the density functional theory one has to solve the Kohn-Sham equations (11, 12) and determine the wavefunction of the system. This is often achieved via expanding the wavefunction in an a priori set of orthonormal functions, known as the basis functions. For the computations we used the program Gaussian 03 (13), and employed the widely used cc-pVTZ set of basis functions developed by Dunning and coworkers (14).

From the calculated energies of the individual molecules involved in the FADH$^\bullet$ +O$_2^{\bullet-}$ → FADH$^-$+O$_2$ reaction we calculate its enthalpy difference ΔE.

Exchange and Dipolar Interactions in the Radical pair Hamiltonian

The part of the Hamiltonian accounting for electron-electron exchange and dipolar interactions is

$$\hat{H}_{\text{int}} = \hat{H}_{\text{J}}(R) + \hat{H}_{\text{D}}(R, \vec{n}), \tag{S4}$$

where $\hat{H}_{\text{J}}$ and $\hat{H}_{\text{D}}$ are the exchange and the dipolar interactions respectively, defined as (15, 16)

$$\begin{aligned} \hat{H}_{\text{J}}(R) &= -\mu_B J_0 \exp\left[-\beta R\right] \left(\frac{1}{2} + 2\hat{\vec{S}}_1 \cdot \hat{\vec{S}}_2\right) && \text{(S5)} \\ \hat{H}_{\text{D}}(R, \vec{n}) &= \frac{g^2 \mu_B^2}{R^3} \left[\hat{\vec{S}}_1 \cdot \hat{\vec{S}}_2 - 3(\hat{\vec{S}}_1 \cdot \vec{n})(\hat{\vec{S}}_2 \cdot \vec{n})\right]. && \text{(S6)} \end{aligned}$$

Here R is the edge-to-edge distance between the radicals, $\vec{n}$ is the unit vector in the direction of $\vec{R}$ which specifies the orientation of the radical pair, J_0 is the exchange coupling constant, and β is a range parameter. According to Eqs. (S5)-(S6) the strength and the distance dependence of the dipolar and the exchange coupling parameters can be expressed as an effective magnetic field, i.e., in units of Gauss (15, 16), namely as

$$\begin{aligned} J(R) &= J_0 \exp\left[-\beta R\right] && \text{(S7)} \\ D(R) &= -\frac{3}{2}\frac{g^2 \mu_B}{R^3}. && \text{(S8)} \end{aligned}$$

The eliminated factor $-\mu_B\,(\frac{1}{2} + 2\hat{\vec{S}}_1 \cdot \hat{\vec{S}}_2)$ in Eq. (S5) assume values $+\mu_B$ and $-\mu_B$ in case of electron spin singlet and triplet states, respectively. The eliminated factor $-\frac{2}{3}\mu_B\,[\hat{\vec{S}}_1 \cdot \hat{\vec{S}}_2 - 3\,(\hat{\vec{S}}_1 \cdot \hat{\vec{n}}_1)\,(\hat{\vec{S}}_2 \cdot \hat{\vec{n}}_2)]$ likewise assume values 0 and $-\mu_B(\,m^2 - \frac{2}{3})$. An exchange interaction comparable to or larger than the Zeeman interaction lifts the zero-field degeneracies of the singlet and triplet states and, thereby, suppresses the effect of the external magnetic field (17). A dipolar interaction produces a similar result (15). Thus, if $|D(R)|$ or $|J(R)|$ in Eqs. (S7)-(S8) are too large compared to Zeeman and hyperfine interaction, singlet↔triplet interconversion that would otherwise be driven by the weak Zeeman interaction will be blocked. According to Eqs. (S7)-(S8) for $|D(R)|$ or $|J(R)|$ to be $\lesssim 0.5$ G, the radicals should be $R \gtrsim 35$ Å apart. Such large separations R are inconsistent with some other conditions that the [FADH$^\bullet$ + $O_2^{\bullet -}$] radical pair must satisfy to act as a magnetoreceptor (e.g. the size of the molecular pocket in cryptochrome is less than 20-25 Å; the electron transfer rate from $O_2^{\bullet -}$ to FADH$^\bullet$ is expected to be too low at large radical separations, as discussed below).

A possible way out of this dilemma has been suggested recently by Efimova and Hore (16), who showed that at certain conditions exchange and dipolar interactions can become of approximately the same size, but possessing different signs, i.e., then cancel each other. The cancelation condition can be derived if one considers the matrix elements of the exchange and dipolar Hamiltonians in the basis of singlet state S_0 and triplet states T_m ($m = 0, \pm 1$) (16, 18)

$$\begin{aligned} E_S &= \langle S|\hat{H}_{int}|S\rangle = \langle S|\hat{H}_J + \hat{H}_D|S\rangle = J && \text{(S9)} \\ E_T &= \langle T_m|\hat{H}_{int}|T_m\rangle = \langle T_m|\hat{H}_J + \hat{H}_D|T_m\rangle = -J + D\left(m^2 - \frac{2}{3}\right), && \text{(S10)} \end{aligned}$$

The exchange and dipolar interactions cancel each other for $E_S = E_T$, resulting in the condition (16)

$$D = \frac{2J}{m^2 - \frac{2}{3}}, \quad m = 0, \pm 1. \tag{S11}$$

According to Eq. (S11) the partial cancelation of the effects of the exchange and dipolar interactions occurs at

$$D = 6J, \quad \text{or} \quad D = -3J. \tag{S12}$$

Since D is negative the first case can arise when $J_0 < 0$ and the later case when $J_0 > 0$.

In (16) it was demonstrated that the cancelation conditions Eqs. (S12) can be realized in the photoactivation cycle of cryptochrome if the separation distance between the radical partners is ∼18-22 Å. These distances are consistent with the separation between the FADH$^\bullet$ and Trp324$^+$ radicals involved in the photoactivation reaction of cryptochrome (16, 19). However the distance at which the cancelation conditions, Eqs. (S12), are fulfilled strongly depend on the exchange coupling constant J_0 and the range parameter β (see Eqs. (S7)-(S8)). For the estimates performed in (16) the values $\beta = 1.4$ Å^{-1} and $J_0 = 1.6 \times 10^{12}$ G were used, which were taken from the primary radical pair in the reaction center of the purple photosynthetic bacterium (16, 20–22). The values for J_0 and β are different for the acylketyl biradicals, namely $J_0 = 7 \times 10^9$ G and $\beta = 2.14$ Å^{-1} (15, 23).

The exchange interaction parameters for the [FADH$^\bullet$+O$_2^-$] radical pair are not known and, in principle, should be different from exchange interaction parameters in the photosynthetic bacterium or in the acylketyl biradicals. To study the feasibility of magnetic field effects in the [FADH$^\bullet$+O$_2^-$] radical pair the cancelation conditions Eqs. (S12) are considered for a wide range of parameters β and J_0. According to the literature (15, 16, 20–23) the exchange coupling constant is expected to lie in the range $\sim 10^9 - 10^{13}$ G with $\beta \sim 1 - 2$ Å^{-1}.

Using Eqs. (S12) one can estimate the radical pair separation distance at which $J - D$ cancelation arises. Figure S2 shows the radical pair separation as a function of J_0 and β. In the present paper the value of J_0 is assumed positive and, therefore, the distances shown in Fig. S2 are obtained as the solution of $D = -3J$. Assuming a distance between FADH$^\bullet$ and O$_2^{\bullet -}$ of 10-12 Å, which conforms with the 20-25 Å depth of cryptochrome's pocket shown in Fig. S1, Fig. S2 shows that exchange and dipolar interactions cancel each other rather well for J_0 and β in the range $J_0 \in (10^9...10^{12})$ G and $\beta \in (1.8...2.2)$ Å^{-1}, i.e., parameters also employed in earlier studies (15, 16, 20–23).

For the stated choice of exchange and dipolar interaction strength, the sum of the interactive terms can be neglected and a magnetic field effect in the [FADH$^\bullet$+O$_2^-$] radical pair reaction becomes possible.

Another key factor determining the feasibility of a magnetic field effect on the back-reaction of cryptochrome is the value of the electron transfer rate k_{et} which depends sensitively on the edge-to-edge distance R arising also in Eqs. (S7)-(S8). It was shown (24, 25) that electron transfer in proteins obeys the empirical relationship

$$\log_{10} k_{\mathrm{et}} = 15 - 0.6R - 3.1\frac{(\Delta E + \lambda)^2}{\lambda}. \tag{S13}$$

Here λ is the (reorganization) energy required to repolarize the protein matrix upon electron transfer and ΔE is the driving force for the electron transfer (16, 19). The optimum electron transfer rate occurs when $\Delta G = -\lambda$ (16, 25), in which case holds

$$R = [25 - 0.7238 \log(k_{\mathrm{et}})] \text{ Å}. \tag{S14}$$

Using Eq. (S14) one can estimate the characteristic distance at which the electron transfer between the radicals occurs at a rate k_{et}. Then k_{et}, R, $\Delta E \sim \lambda$ are in units of s^{-1}, Å, and eV.

Duration of the FADH$^\bullet$+O$_2^{\bullet -}$→FADH$^-$+O$_2$ reaction

Since the O$_2^{\bullet -}$ radical can escape from both spin states of the radical pair, its escape yield can be written

$$\Phi_{\mathrm{b}}^{(n)} = k_{\mathrm{b}} \int_0^\infty \mathrm{Tr}\left[(Q^S + Q^T)\rho^{(n)}(t)\right] dt = k_{\mathrm{b}} \int_0^\infty \mathrm{Tr}\left[\rho^{(n)}(t)\right] dt. \tag{S15}$$

The superscript (n) denotes that the $[\mathrm{FADH}^\bullet + \mathrm{O}_2^{\bullet -}]$ radical pair was created for the n-th time. The initial condition for the density matrix in this case is

$$\rho^{(n)}(0) = \Phi_{\mathrm{b}}^{(n-1)} \frac{\hat{Q}^S + \hat{Q}^T}{\mathrm{Tr}\left[\hat{Q}^S + \hat{Q}^T\right]} = \Phi_{\mathrm{b}}^{(n-1)} \rho^{(1)}(0). \tag{S16}$$

Substituting Eq. (S16) into Eq. (5) and the resulting equation into Eq. (S15) one obtains

$$\Phi_{\mathrm{b}}^{(n)} = k_{\mathrm{b}} \Phi_{\mathrm{b}}^{(n-1)} \int_0^\infty \mathrm{Tr}\left[\rho^{(1)}(t)\right] dt = \Phi_{\mathrm{b}}^{(n-1)} \Phi_{\mathrm{b}}^{(1)}. \tag{S17}$$

With $\Phi_{\mathrm{b}}^{(1)} \equiv 1-\alpha$, where α is the probability that an $\mathrm{FADH}^\bullet + \mathrm{O}_2^{\bullet -}$ encounter actually leads to reduction of $\mathrm{FADH}^\bullet$ and to an end of cryptochrome signalling, follows

$$\Phi_{\mathrm{b}}^{(n)} = (1-\alpha)^n. \tag{S18}$$

If a radical pair is created for the n-th time the corresponding electron transfer yield from the $\mathrm{O}_2^{\bullet -}$ radical to the $\mathrm{FADH}^\bullet$ radical is $\Phi_{\mathrm{et}}^{(n)} = \Phi_{\mathrm{b}}^{(n-1)} - \Phi_{\mathrm{b}}^{(n)}$. Substituting Eq. (S18) one obtains

$$\Phi_{\mathrm{et}}^{(n)} = \alpha(1-\alpha)^{n-1}. \tag{S19}$$

Accordingly, the electron transfer yield is described by a geometrical series with initial value α and common ratio $(1-\alpha)$. Since $0 < 1-\alpha < 1$ holds it follows from Eq. (S19), $\sum_{n=1}^\infty \Phi_{\mathrm{et}}^{(n)} = 1$, i.e., at the end of the $\mathrm{FADH}^\bullet + \mathrm{O}_2^{\bullet -} \rightarrow \mathrm{FADH}^- + \mathrm{O}_2$ reaction the probability to find the FAD cofactor in its reduced FADH^- form equals unity, as expected.

It follows from Eq. (S19) that the electron transfer yield decreases with n, i.e., with time. Let $\tau_{\mathrm{ox}} = 1/k_{\mathrm{ox}}$ be the characteristic time scale at which the $[\mathrm{FADH}^\bullet + \mathrm{O}_2^{\bullet -}]$ radical pair is formed. Due to the low concentration of $\mathrm{O}_2^{\bullet -}$ (26, 27) this time is fairly long, likely in the millisecond range (see below). The expected value of the reaction duration time $\langle\tau\rangle$ can be calculated according to probability theory (28) using

$$\langle\tau\rangle = \sum_{n=1}^\infty n\tau_{\mathrm{ox}} p(n). \tag{S20}$$

Here $n\tau_{\mathrm{ox}}$ describes the time needed for n attempts of forming an $[\mathrm{FADH}^\bullet + \mathrm{O}_2^{\bullet -}]$ radical pair, neglecting the radical pair lifetime in comparison with τ_{ox}. $p(n) \equiv \Phi_{\mathrm{et}}^{(n)}$ is the probability that the formation of FADH^- occurs in the n-th encounter of $\mathrm{FADH}^\bullet$ and an $\mathrm{O}_2^{\bullet -}$. Substituting Eq. (S19) into Eq. (S20) one obtains

$$\langle\tau\rangle = \tau_{\mathrm{ox}} \sum_{n=1}^\infty n\Phi_{\mathrm{et}}^{(n)} = \tau_{\mathrm{ox}}\alpha \sum_{n=1}^\infty n(1-\alpha)^{n-1}. \tag{S21}$$

The summation in Eq. (S21) gives

$$\sum_{n=1}^\infty n(1-\alpha)^{n-1} = -\frac{\partial}{\partial\alpha}\sum_{n=0}^\infty (1-\alpha)^n = -\frac{\partial}{\partial\alpha}\left(\frac{1}{\alpha}\right) = \frac{1}{\alpha^2}. \tag{S22}$$

Substituting Eq. (S22) into Eq. (S21) one obtains

$$\langle \tau \rangle = \frac{\tau_{\mathrm{ox}}}{\alpha} \tag{S23}$$

where α is magnetic field dependent.

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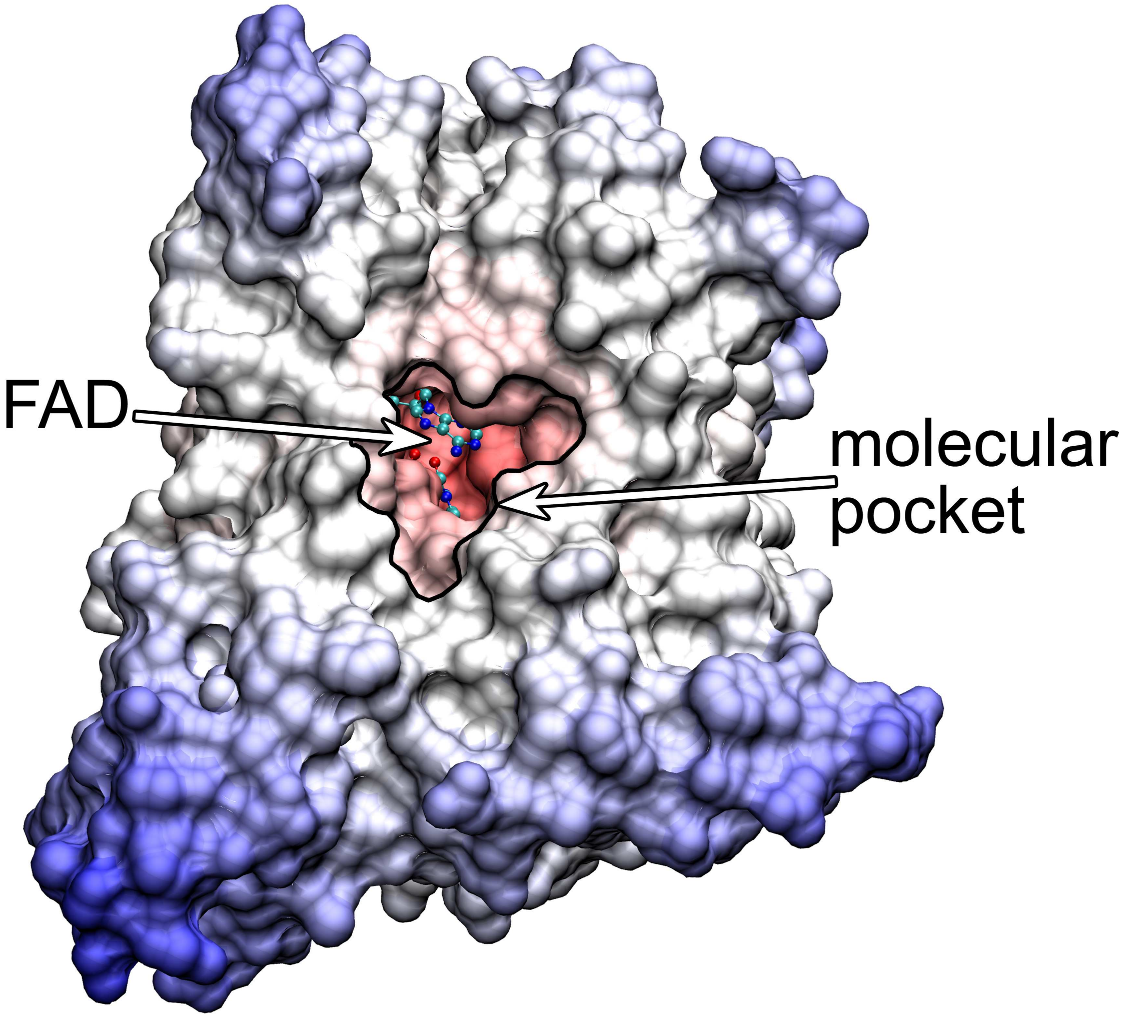

Figure S1: Structure of *Arabidopsis thaliana* cryptochrome-1. Shown is the solute accessible surface of cryptochrome; the molecular pocket directed towards the FAD cofactor is clearly visible. The structure is taken from (29). The coloring of different atoms in the protein shows the distance of the corresponding atom from the center of cryptochrome, i.e., the most central atoms are marked red, while the peripheral residues are colored blue.

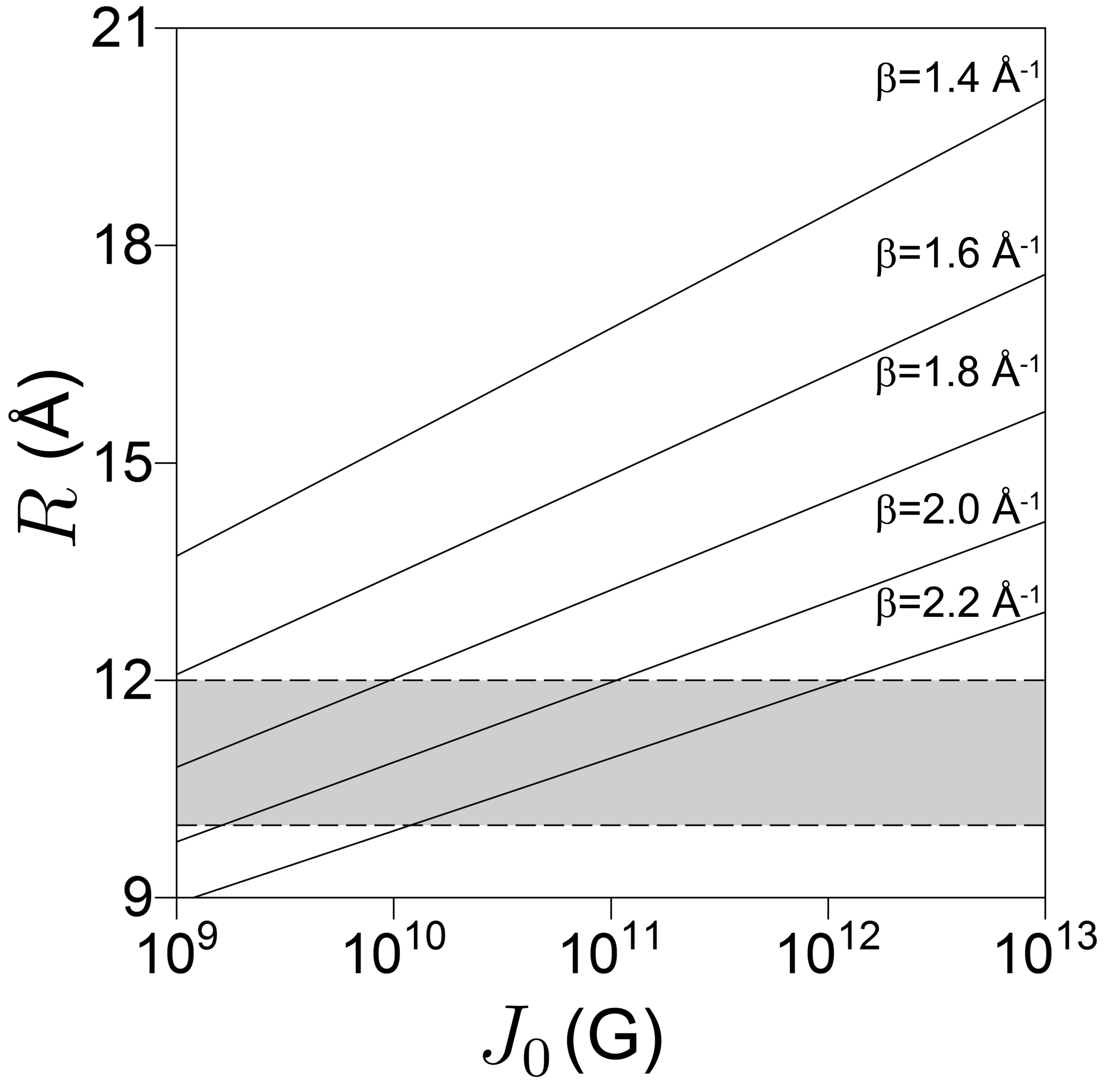

Figure S2: Dependence of the radical pair separation distance R on the exchange interaction parameters J_0 and β according to Eq. (S7). The shaded area marks the distances expected in cryptochrome.