Supplementary Information

Victoire Déjean, Marcin Konowalczyk, Jamie Gravell, Matthew J. Golesworthy, Catlin Gunn, Nils Pompe, Olivia Foster Vander Elst, Mark Oxborrow, Dirk G. A. L. Aarts, Stuart R. Mackenzie and Christiane R. Timmel

S1 Imaging parameters

System	Figure in the main text	Field of view/ μm^2	Laser power/ μW	Pixel dwell time/ μ s
FMN/HEWL solution	4	50.6×50.6	42	1.61
AtCry1 solution	5	50.6×50.6	15	0.95
FMN/HEWL crystal	6	202.03×202.03	37	12.79
	7	101.8×101.8	0.9	1.27
	8	50.6×50.6	37	1.61
Tetracene crystal	9	50.6×50.6	70	10.24

S2 HEWL crystals X-ray parameters

	HEWL crystals	FMN/HEWL crystals
a-axis length / Å	76.776	78.776
b-axis length / Å	76.776	78.776
c-axis length / Å	36.99	37.09
Unit cell volume / \AA^3	21805	23 0 20

S3 HEWL electrostatic potential



Fig. S1: Electrostatic potential of HEWL calculated with an adaptive Poisson-Boltzmann solver at pH 4.2 and drawn with UCSF Chimera.^{1,2} The tryptophan residues involved in the radical pair mechanism (in green) are located in a positively charged region (mainly due to lysine and arginine residues), fostering the interaction between the flavin (via the negatively charged phosphate group) and HEWL.

S4 Fitting equations

The MFE step data in Fig. 4b and 5 were fitted with a square wave. The square wave can be defined as the sign function of the sinusoid as follows:

$$f_1(x \mid \alpha, \beta, T) = \frac{\alpha - \beta}{2} (1 + \operatorname{sgn}(\sin(2\pi x/T))) + \beta$$

This square wave oscillates between low α and high β level with a period *T*. The equation above applies to a function centred at the origin with zero phase. For fitting, f_1 was shifted to the centre of the field step and *T* was fixed at the field switching period. Only the values of α and β were fitted.

The time-varying MFE and anisotropy steps in Fig. 8 and 9a were fitted with a piecewise single exponential step, akin to that used in the work by Kattnig *et al.*³ The exponential step function can be defined as follows:

$$E(x \mid \alpha, \beta, \tau, T) = \begin{cases} (\alpha - \beta) \exp\left(-\frac{x}{\tau}\right) + \beta & x \ge 0\\ (\beta - \alpha) \exp\left(-\frac{x + T/2}{\tau}\right) - \beta & x < 0 \land x \ge -T/2\\ -\alpha & x < -T/2 \end{cases}$$

For x < -T/2, this function takes a constant value of $-\alpha$. Starting at x = -T/2, it levels off to a value of $-\beta$ with a single exponential time constant τ . Then, at x = 0, it jumps to a value of $+\alpha$ and levels off to a value of $+\beta$ with the same single exponential time constant τ . A sawtooth function with period p can be defined in terms of a floor function, or a modulus as follows:

$$W(x | T) = x - T \lfloor x/T - 1/2 \rfloor - T = mod(x - T/2, T) - T/2$$

Defined in this way, $W(x | T) \equiv x$ in the range (-T/2, T/2). A periodic piecewise exponential step function is then simply:

$$f_2(x \mid \alpha, \beta, \tau, T) = E(W(x) \mid \alpha, \beta, \tau, T)$$

Similarly to the square wave above, f_2 was shifted to the centre of the field step and T was fixed at the field switching period. Moreover, since f_2 oscillates between $\pm \max(|\alpha|, |\beta|)$, an additional fit parameter was added to allow for vertical shift of the step and was allowed to vary.

The decay of the overall fluorescence intensity (Fig. S6, S8 and S15) was found to be well modelled by a bi-exponential decay. The bi-exponential function can be defined as follows:

$$f_3(x \mid a_1, \tau_1, a_2, \tau_2) = a_1 \exp\left(-\frac{x}{\tau_1}\right) + a_2 \exp\left(-\frac{x}{\tau_2}\right)$$

If all the parameters are positive, this function decays from $a_1 + a_2$ to 0 with two time constants τ_1 and τ_2 . We attribute the fast component to the immediate response of the sample to change in its average light exposure (as the sample is kept in the dark before the beginning of the experiment). The slow step is attributed to the diffusion-controlled establishment of a steady state with the diffusion of fresh fluorophores into the frame and the focal plane. Since the fluorescence intensity in the frame establishes a steady-state with the recovery and diffusion of new fluorophores, and because of the dark noise of the detector, the fluorescence intensity levels off at a non-zero value. To account for this, an additional parameter was therefore included and allowed to vary during the fit.

The fitting was performed with least squares optimisation using fminsearch() - a Nelder-Mead optimiser⁴ implemented in MATLAB.

S5 Additional details of data analysis

The slices though the spatially-resolved data in Fig. 6 were obtained by using a linear 2D interpolation. The axis onto which the slice was interpolated was constructed to have approximately the same sample spacing as the axis of the image.

The slices through the MFE images in Fig. 6 were taken through an image smoothed with a Gaussian blur with radius of 0.8 pixels.

To obtain Fig. 4b, 5, 8 and 9a in the main text, a baseline subtraction was performed by determining the centre points between each pair of u-d and d-u segments. To do so, both segments were fitted to smoothing polynomials (usually quadratics). These polynomials were then evaluated at the inner edges of the two segments, and finally the midpoint of the two values was used as an estimate of the centre point. Note that the centre points were used for the baseline subtraction instead of the field-off segments because, in some cases (for instance in Fig. 8 in the main text), the MFE evolves within the field-off segments which, therefore, do not constitute a stable reference. Thus, using the centre points as the baseline makes the dynamics of the MFE within one step visible. The error bars correspond to the standard deviation of the averaged field steps.



Fig. S2: Top: Fitting of the magnetic field induced change in fluorescence intensity ΔI . A triplet of up-down-up steps is shown, but an analogous procedure is applied to the down-up-down steps. **Bottom:** Fitting of the centreline of the steps. In this case, three points were removed from the edges of each step to make the illustration clearer.

S6 Tetracene simulations

The simulations used to model the fluorescence output follow that of Johnson and Merrifield⁵ with the only difference being that the exciton pair is assumed to be formed by singlet fission, rather than by two independently formed triplet excitons. This change does not affect the outcome of the simulations, but differs only by a scaling factor. The reaction scheme is modelled according to the reaction scheme shown in Fig. 3 in the main text. The rate constant for the formation of the triplet exciton pair (${}^{1}P^{*} + P \rightarrow {}^{1} ({}^{3}P^{3}P)$), k_{0} , is kinetically zeroth order as a result of the continuous photoexcitation of the samples during the experimental work. The first order rate of dissociation (${}^{1/3/5}({}^{3}P^{3}P) \rightarrow {}^{3}P + {}^{3}P$), k_{d} , and spin-selective triplet-triplet annihilation (${}^{1}({}^{3}P^{3}P) \rightarrow {}^{1}P^{*} + P$), k_{s} , are both magnetically sensitive.

Considering the equation of motion of the density operator of the triplet exciton pair (TT), yields:

$$\frac{\mathrm{d}\hat{\boldsymbol{\rho}}}{\mathrm{d}t} = -\left[\hat{\mathbf{H}}, \hat{\boldsymbol{\rho}}\right] - k_{\mathrm{d}}\hat{\boldsymbol{\rho}} - \frac{1}{2}k_{\mathrm{s}}\left(\hat{\mathbf{P}}^{\mathrm{S}}\hat{\boldsymbol{\rho}} + \hat{\boldsymbol{\rho}}\hat{\mathbf{P}}^{\mathrm{S}}\right) + k_{0}\hat{\mathbf{P}}^{\mathrm{S}}$$

where $\hat{\mathbf{H}}$ is the spin Hamiltonian and $\hat{\mathbf{P}}^{S}$ is the singlet projection operator. The terms on the right hand side of the equation describe the evolution of the density operator under the Hamiltonian, the loss of pair states due to dissociation, the loss of pair states to triplet-triplet annihilation, and the formation of the triplet pairs by singlet fission, respectively. The third term, relating to triplet-triplet annihilation, is modelled using the Haberkorn theory of spin-selective recombination.⁶ Rewriting the equation in Liouville space yields:

$$\frac{\mathrm{d}|\rho\rangle}{\mathrm{d}t} = -i\hat{\mathbf{H}}|\rho\rangle - k_{\mathrm{d}}|\rho\rangle - \hat{\mathbf{K}}|\rho\rangle + k_{0}|\mathbf{P}^{\mathrm{S}}\rangle$$
$$= -\hat{\mathbf{L}}|\rho\rangle + k_{0}|\mathbf{P}^{\mathrm{S}}\rangle$$

where $|\rho\rangle$ is an 81-element row-flattened version of the 9 × 9 Hilbert space density matrix (ρ), $\hat{\mathbf{H}}$ is the spin Hamiltonian superoperator, and $\hat{\mathbf{K}} = \frac{1}{2}k_s\hat{\mathbf{Q}}$, where $\hat{\mathbf{Q}}$ is the singlet projection superoperator.

Under steady-state conditions, such as continuous photoexcitation, $\frac{d|\rho}{dt} = 0$ and we obtain: $\hat{\mathbf{L}}|\rho\rangle = k_0|\mathbf{P}^{\rm S}\rangle$ from which $|\rho\rangle$ can be solved: $|\rho\rangle = k_0\hat{\mathbf{L}}^{-1}|\mathbf{P}^{\rm S}\rangle$. The fluorescence intensity can then be obtained by scaling the fraction of the pairs in the singlet state by $k_{\rm s}$, i.e.:

$$I_{\rm f} = k_{\rm s} \operatorname{Tr} \left[\boldsymbol{\rho} \mathbf{P}^{\rm S} \right]$$
$$= k_{\rm s} \langle \mathbf{P}^{\rm S} | \boldsymbol{\rho} \rangle$$
$$= k_{\rm o} k_{\rm s} \langle \mathbf{P}^{\rm S} | \hat{\mathbf{L}}^{-1} | \mathbf{P}^{\rm S} \rangle$$

An additional factor worth considering is that of the basis in which the simulations are run. With crystals, it is easiest to work in the crystal axis basis, however it is uncommon that the crystal axes lie along the zero-field splitting (ZFS) axes. It is, therefore, important that the basis of the Zeeman and dipolar axes are adjusted in the simulation.

ZFS and orientational values were taken from Yarmus *et al.*⁷ Kinetic values were estimated from zero- and high-field fluorescence measurements.^{8,9} The qualitative change in fluorescence intensity between the *a* and *b* axes is insensitive to these kinetic parameters, both their order of magnitude $(10^8 < k_s/s^{-1} < 10^{11})$ and ratio (up to $k_d = k_s$), the latter being of greater importance.¹⁰ The same qualitative change is also true of the ZFS parameters after adjustment by the process described by Tedder.^{11,12} The parameters used are listed below:

Parameter	$k_{\rm s} \; / \; {\rm s}^{-1}$	$k_{\rm d} \; / \; {\rm s}^{-1}$	D^* / mT	<i>E</i> * / mT
Value	7.0×10^{9}	3.5×10^{9}	-6.63	26.53

S7 Angular field measurements

The magnetic field magnitude was measured as a function of its angle. The field was found to be slightly eccentric, with the maximum error in its magnitude found to be ca. 2%. The absolute magnitude difference between 0° and 90° was 0.21 mT. The simulations of the largest possible effect on the anisotropy are shown in Fig. S4. The change in fluorescence intensity ΔI due to anisotropy of tetracence is predicted to be 16% (~24 counts). The *largest* estimate of the same effect coming from the 2% field magnitude mismatch between 0° and 90° is ca. 0.2% (~0.3 counts). Note how that compares to the observed ΔI in Fig. S16.



Fig. S3: Magnetic field magnitude measurement as a function of the angle. The measurement was completed using a 3D hall probe, set in the centre of the coils - where the sample would normally be located. The angle of the field was swept though the 360° arc over 15 s, and the magnitude of the field at each angle was recorded. The datapoints shown are $60 \times 6^\circ$ angular bins with associated (1σ) error bars. The corresponding orientation of the *a*- and *b*-axes of the crystal are indicated.



Fig. S4: Anisotropy simulation with error bounds corresponding to $\pm 5\%$ field magnitude error. The 0° and 90° points, corresponding to the *b*- and *a*-axes, can be seen to be only very weakly affected.

S8 Complete datasets and additional data

This section shows details of the datasets from the main text. Some additional data are also presented.



S8.1 FMN/HEWL solution

Fig. S5: MFE measurement of an FMN/HEWL solution **Top:** Average pixel intensity I. The centre points as well as the resulting centreline are shown. The centreline is an interpolant though the centre points. **Bottom:** Magnification of the 200–600 s region.



Fig. S6: Bi-exponential fit of I from Fig. S5 - FMN / HEWL solution. $a_{\text{fast}} = 67.0 \pm 0.8 \text{ counts}, \tau_{\text{fast}} = 23.1 \pm 0.7 \text{ s}, a_{\text{slow}} = 40.6 \pm 0.6 \text{ counts}, \tau_{\text{slow}} = 244.6 \pm 6.0 \text{ s}, \text{ offset} = 49.6 \text{ counts}, \text{RMS residuals} = 0.4 \text{ counts}$

S8.2 AtCry



Fig. S7: Average pixel intensity I for the measurement of the %MFE in *At*Cry1. The initial drop is fitted to a bi-exponential decay ($a_{\text{fast}} = 5.4 \pm 0.2 \text{ counts}$, $\tau_{\text{fast}} = 2.4 \pm 0.2 \text{ s}$, $a_{\text{slow}} = 8.3 \pm 0.1 \text{ counts}$, $\tau_{\text{slow}} = 59.1 \pm 1.3 \text{ s}$, offset = 44 counts, RMS residuals = 0.2 counts) as described in section S4. The sample volume was extremely small (single drop, ~50 µL), so the drop after ca. 300 s is attributed to photodegradation and the unavailability of fresh fluorophores diffusing from outside the field of view. Only the four MFE steps between ca. 25 and 247 s have been used to obtain the average step shown in Fig. 5.



Fig. S8: Top: Magnification of the 0–300 s region the data presented in Fig. S7. The MFE steps can be clearly seen. **Bottom:** Magnification of the 350–600 s region of the data presented in Fig. S7. No MFE steps can be seen. The decay fits well to a single exponential ($a = 10.9 \pm 0.2$ counts, $\tau = 57.1 \pm 0.1$ s, offset = 16 counts, RMS residuals = 0.1 counts).



Fig. S9: Data from Fig. S8, Top. Change in fluorescence intensity ΔI induced by the applied magnetic field after subtraction of the centreline. These steps were averaged together to yield an average MFE step shown in Fig. 5.

S8.3 Spatially-resolved MFEs

Fig. 6b,d in the main text were obtained using the following time bins for averaging:

1. 044.2 : 103.1 s 2. 103.1 : 164.5 s 3. 164.5 : 224.4 s 4. 224.4 : 284.3 s 5. 284.3 : 344.2 s 6. 344.2 : 404.1 s 7. 404.1 : 464.0 s

The first 36 frames were discarded as the gradient of the intensity at early times makes the calculation of the MFE inaccurate.

Images obtained with different time bins are presented below.



Fig. S10: Average fluorescence intensity (**Top**) and %|MFE| (**Bottom**) images for the data presented in Fig. 6. Each image is an average over approx. 17 s from time $t \approx 36$ s to $t \approx 450$ s. The colour scaling of the intensity images is scaled to the magnitude of the data, but the %|MFE| images are all displayed on the same scale. A movie showing the time evolution of the fluorescence profile across the crystal is included, large_crystal_video.mp4.



Fig. S11: Selected fluorescence intensity images (**Top**) and slices though them (**Bottom**) at early times for the data presented in Fig. 6. The slices shown are though the main diagonal (top left corner to bottom right corner) of the images shown, similarly to the data shown in the main text. The images shown are single, unaveraged frames. The frame number, as well as the corresponding time bins are indicated on each frame. The colour scaling of the intensity images is scaled to the magnitude of the data.

S8.4 Spatially-resolved MFEs - additional dataset



Fig. S12: Average fluorescence intensity (**Top**) and ΔI (**Bottom**) images for an FMN/HEWL crystal. Each image is an average over approx. 17 s from time $t \approx 36 \text{ s}$ to $t \approx 450 \text{ s}$. The colour scaling of the intensity images is scaled to the magnitude of the data, but the ΔI images are all displayed on the same scale. The brightest point in the top images corresponds to ca. 1.6 counts while the darkest to ca. 0 count. The %|MFE| is not displayed for this data since the intensity I of the fluorescence in the solution is very low and hence the division of ΔI by I is unreliable. A movie showing the time evolution of the fluorescence profile across the crystal is included, edge_crystal_video.mp4.

S8.5 FRAP

Frame #1	Frame # 2	000 : 017 s.	017 : 035 s.	035 : 052 s
052 : 069 s.	069 : 087 s.	087 : 105 s	105 : 122 s.	122 : 139 s
139 : 157 s.	157 : 174 s.	174 : 191 s	191 : 209 s	209 : 226 s
226 : 243 s	243 : 261 s	261 : 278 s	278 : 296 s	296 : 313 s
313 : 331 s	331 : 348 s.	348 : 365 s.	365 : 383 s	383 : 393 s



Fig. S13: Top: Selected images of the FRAP experiment. The first and second frames are shown explicitly. **Bottom:** Time evolution of consecutive slices though the bleached square. The slice is a *vertical bin* though the middle 80 pixels of each image. The slices are therefore *horizontal* slices with respect to how the images above are displayed. The brightest/dimmest points in both figures correspond to ca. 30/130 counts respectively.

S8.6 FMN/HEWL crystal



Fig. S14: Average pixel intensity I for the MFE measurement of an FMN/HEWL crystal. The centre points as well as the resulting centreline are shown. The centreline is an interpolant though the centre points smoothed using a 1^{st} order Savitsky-Golay filter over three points.^{13,14}



Fig. S15: Bi-exponential fit of I from Fig. S14 - HEWL co-crystallised with FMN. $a_{\text{fast}} = 124.7 \pm 2.0 \text{ counts}, \quad \tau_{\text{fast}} = 2.9 \pm 0.1 \text{ s}, \quad a_{\text{slow}} = 20.8 \pm 2.0 \text{ counts}, \quad \tau_{\text{slow}} = 23.0 \pm 3.0 \text{ s},$ offset = 21.9 counts, RMS residuals = 0.7 counts

S8.7 Tetracene

The fluorescence intensity for the measurement of the tetracene crystal is shown in Fig. S16. The excitation wavelength was 458 nm. An applied magnetic field was maintained at 16 mT and its orientation was changed from parallel to perpendicular with respect to the *b*-axis of the crystal every ~8 s. The centreline subtracted steps can be seen to slowly decrease in magnitude level off to a constant value at ~400 s. Furthermore, the evolution of I within the individual steps appears to change over the course of the experiment. This can be seen in Fig. S18. All of these effects were found to be broadly reproducible. The ΔI steps were *always* observed. The origin of these effects, as well as the behaviour of the overall fluorescence intensity are not yet fully understood, but we speculate they are related to anisotropic exciton diffusion though the crystal.



Fig. S16: Top: Average pixel intensity I for the tetracene crystal MFE anisotropy measurement. The centre points as well as the resulting centreline are shown. The centreline is an interpolant though the centre points smoothed by using a 1st order Savitsky-Golay filter over five points.^{13,14} **Bottom:** Magnification of the first 100 s of the above data.



Fig. S17: Data from Fig. S16. The centreline subtracted from the intensity steps showing the change in fluorescence intensity ΔI induced by the *orientation* of the applied magnetic field.

006 : 084 s	085 : 163 s	163 : 241 s	242 : 320 s	321 : 399 s
399 : 477 s	478 : 556 s	557 : 635 s	635 : 713 s	698 : 776 s

Fig. S18: Exponential step fits to consecutive time regions of the ΔI data from Fig. S17. The error shadows shown are the centre points ± 1 standard deviation of the averaged steps.

References

- [1] Baker, N. A.; Sept, D.; Joseph, S.; Holst, M. J.; McCammon, J. A. Proc. Natl. Acad. Sci. U.S.A. 2001, 98, 10037–10041.
- [2] Pettersen, E.; Goddard, T.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. J. Comput. Chem. 2004, 25, 1605–1612.
- [3] Kattnig, D. R.; Evans, E. W.; Déjean, V.; Dodson, C. A.; Wallace, M. I.; Mackenzie, S. R.; Timmel, C. R.; J., H. Nat. Chem. 2016, 8, 384–391.
- [4] Nelder, J. A.; Mead, R. Comput. J. 1965, 7, 308-313.
- [5] Johnson, R. C.; Merrifield, R. E. Phys. Rev. B 1970, 1, 896–902.
- [6] Haberkorn, R.; Dietz, W. Solid State Commun. 1980, 35, 505–508.
- [7] Yarmus, L.; Rosenthal, J.; Chopp, M. Chem. Phys. Lett. 1972, 16, 477-481.
- [8] Kalinowski, J.; Godlewski, J. Chem. Phys. Lett. 1975, 36, 345–348.
- [9] Geacintov, N.; Pope, M.; Vogel, F. Phys. Rev. Lett. 1969, 22, 593-596.
- [10] Smith, M. B.; Michl, J. Annu. Rev. Phys. Chem. 2013, 64, 361–386.
- [11] Tedder, S. H. Chem. Phys. 1976, 14, 455–459.
- [12] Tedder, S. H. Chem. Phys. 1977, 24, 287.
- [13] Savitzky, A.; Golay, M. J. E. Anal. Chem. 1964, 36, 1627–1639.
- [14] MathWorks Inc., Savitzky-Golay filtering. https://uk.mathworks.com/help/signal/ref/sgolayfilt.html.