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# Fluorescence-detected magnetic field effects on radical pair reactions from femtolitre volumes

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## ELECTRONIC SUPPLEMENTARY INFORMATION

### MATERIALS AND METHODS

#### Buffers

All measurements were made in citric acid-phosphate buffer (pH 2.3, 12 mM Na<sub>2</sub>HPO<sub>4</sub> and 94 mM citric acid).

#### Microscopy

Solutions for measurement were placed in tunnel slides made from two perpendicular coverslips separated by a single layer of double sided tape (total sample volume ~30 μL). The sample was excited through a 60× oil-immersion 1.45 N.A. TIRF objective on a Nikon TE-2000 inverted microscope using a 473 nm laser reflected by a dichroic filter (Semrock Brightline FF497-Di03), giving a <100 fL probe volume. Fluorescence transmitted through the dichroic filter was passed through an emission filter (Semrock Brightline 550/88) before fluorescence detection using an Andor iXon+ electron-multiplying CCD camera (emCCD, gain: 300) (Fig. 1a). Illumination intensity at the sample was 0.5 kW cm<sup>-2</sup> neglecting losses inside the microscope body and optics. Both camera firing and magnetic field switching were controlled by the output of a Quantum Composers 9520 pulse generator to ensure complete synchrony. The magnetic field was switched (on or off) every ten camera frames (102.4 ms) for the duration of recording (usually 1000 frames).

#### Calibration and determination of the rise time of the solenoid

Magnetic fields were generated using a solenoid wound around a 3 mm diameter ferrite core and fixed into a non-metallic mount using epoxy resin. A custom-built power supply drove the solenoid at an input frequency of up to 2 kHz and provided a maximum field strength at the tip of the

solenoid of over 27 mT. Calibration of the solenoid was performed using a gaussmeter based upon a Hall effect probe with 3  $\mu$ s rise time (Honeywell SS94A). The rise time of the magnetic field generated by the solenoid was found to be less than 70  $\mu$ s at the maximum achievable field strength.

The solenoid assembly was attached via a cantilever to a micrometer-resolution 3-axis translation stage mounted securely on the body of the inverted microscope. This arrangement allowed the solenoid position relative to the microscope objective to be reproducibly controlled, while an independent 2-axis translation stage was used to adjust the horizontal position of the sample. To centre the solenoid over the objective, coarse calibration was performed using bright-field microscopy images of the solenoid, followed by a magnetic field mapping experiment in which the MFE measured in a TIRF experiment was plotted against micrometer position to determine the centre field in the horizontal plane. All further MFE experiments were performed with the solenoid centred relative to the objective lens.

### Fluorescence data processing

Camera frames (one every 10.24 ms) were separated according to the presence or absence of magnetic field, and the mean pixel intensity across each frame determined to give a single data point per frame. In order to ensure that data collected at the moment of field switching did not contain contributions from both field conditions, the data from such frames were discarded. To determine the magnetic field effect, eight values of mean pixel intensity within each sequence of ten points (representing measurements in the presence / absence of magnetic field, but excluding frames which may contain contributions from both conditions) were fit with a straight line. The line was extrapolated to the moment of field-switching and the step height between field on and off measurements determined by subtraction.

The value of  $F(0) - F(B_0)$  varied over the first few data points collected, although it was constant at longer times (Fig. S4 a,b). This apparent time-dependence was also observed for FAD/Trp in the absence of magnetic fields (Fig. S4 c,d) and for FMN (which shows no magnetic field effect) in the presence of magnetic fields (Fig. S4 e,f). It is an artefact introduced by the initial steepness of the measured decay curves for the flavins, hence it was not observed for the photostable dye Alexa Fluor® 488 (Fig. S4 g,h). In order to enable as much of the data as possible to contribute to the final value, traces of  $mfe_F$  versus time were fit by an exponential decay plus a straight line and the y-intercept of the extrapolated straight line taken as the measured  $mfe_F$  value.

### Transient absorption

Transient absorption spectra were observed using custom-built equipment. A flow system was used to transfer the sample into a quartz optical cell (optical path length 4 mm) where photochemical reactions were initiated by a laser pulse. The third harmonic ( $\lambda = 355$  nm) of a Nd:YAG Laser (Spectra Physics GCR-3) was used as the excitation light source. The energy of the laser was adjusted to 15 mJ per pulse. The laser pulse repetition rate was 5 Hz. A 500 W Xe lamp (Ushio UXL-500SX) was used as a probe light source. The transient absorption signal was detected by a photomultiplier tube (Hamamatsu R928) preceded by a monochromator (Jasco CT-25) and was recorded by a digital oscilloscope (LeCroy LT-344).

## SUPPLEMENTARY FIGURES

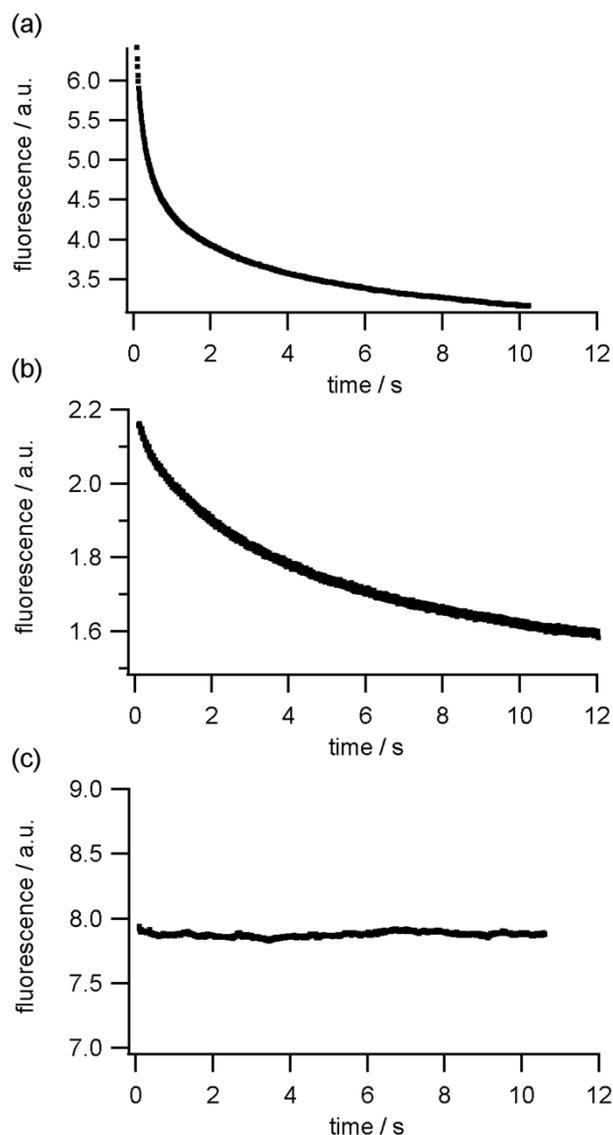


Fig. S1: Fluorescence decay curves for control fluorophores. (a) 1  $\mu$ M FAD + 300  $\mu$ M tryptophan in the absence of magnetic fields; (b) 1  $\mu$ M fluorescein and (c) 1  $\mu$ M Alexa Fluor<sup>®</sup> 488 in the presence of a square wave modulated magnetic field (same conditions as in Fig. 1b). The fast decay rate for fluorescein is 2-3 times slower than that for FAD and FMN. Alexa Fluor<sup>®</sup> 488 is over 40 times as bright as FAD and FMN, and so fluorescence in (c) was measured at  $\sim$ 15 times lower laser power and 0.5-0.7 times the gain on the emCCD camera compared with (a), (b) and Fig. 1b-c.

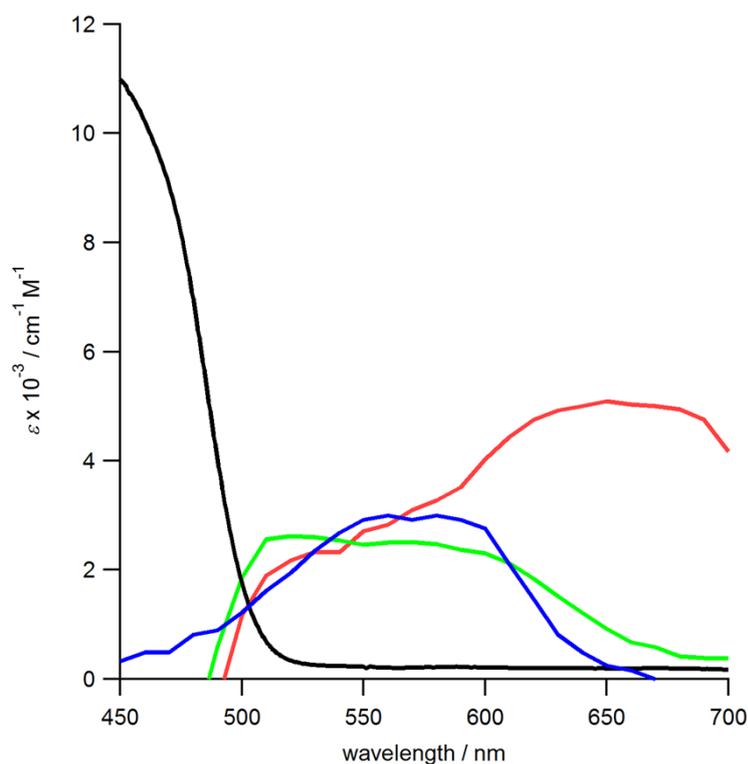


Fig. S2: Absorption spectra of flavin and tryptophan species. Absorption of FAD (black) measured at pH 2.6, absorption of flavin excited triplet state (red) and protonated flavin radical (green) taken from Ref 1, absorption of protonated Trp<sup>\*\*</sup> radical (blue) taken from Ref 2. Tryptophan itself absorbs below 300 nm.

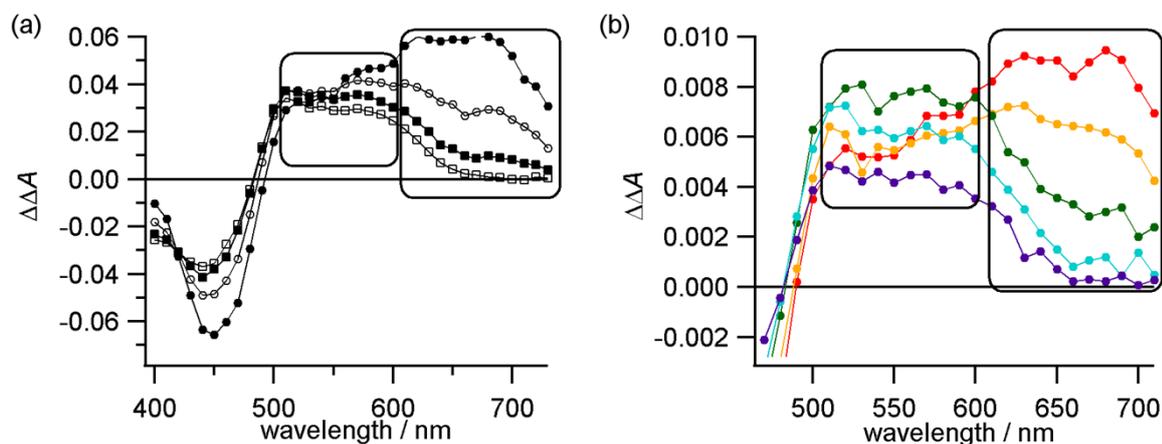


Fig. S3: Magnetic sensitivity of FAD absorption in the visible spectrum as measured by transient absorption. (a) Magnetic sensitivity over time for 0.2 mM FAD + 0.5 mM Trp. Filled circles – 0.2  $\mu$ s; open circles – 0.5  $\mu$ s; filled squares – 1.0  $\mu$ s; open squares – 5.0  $\mu$ s. (b) Magnetic sensitivity at different concentrations of Trp. Data averaged 0.5-2.0  $\mu$ s. Red – 0 mM Trp; yellow – 0.1 mM Trp; green – 0.5 mM Trp; cyan – 1.0 mM Trp; purple – 2.0 mM Trp. Boxes indicate regions of the spectrum averaged in the  $\Delta\Delta A$  measurements reported in the main text.

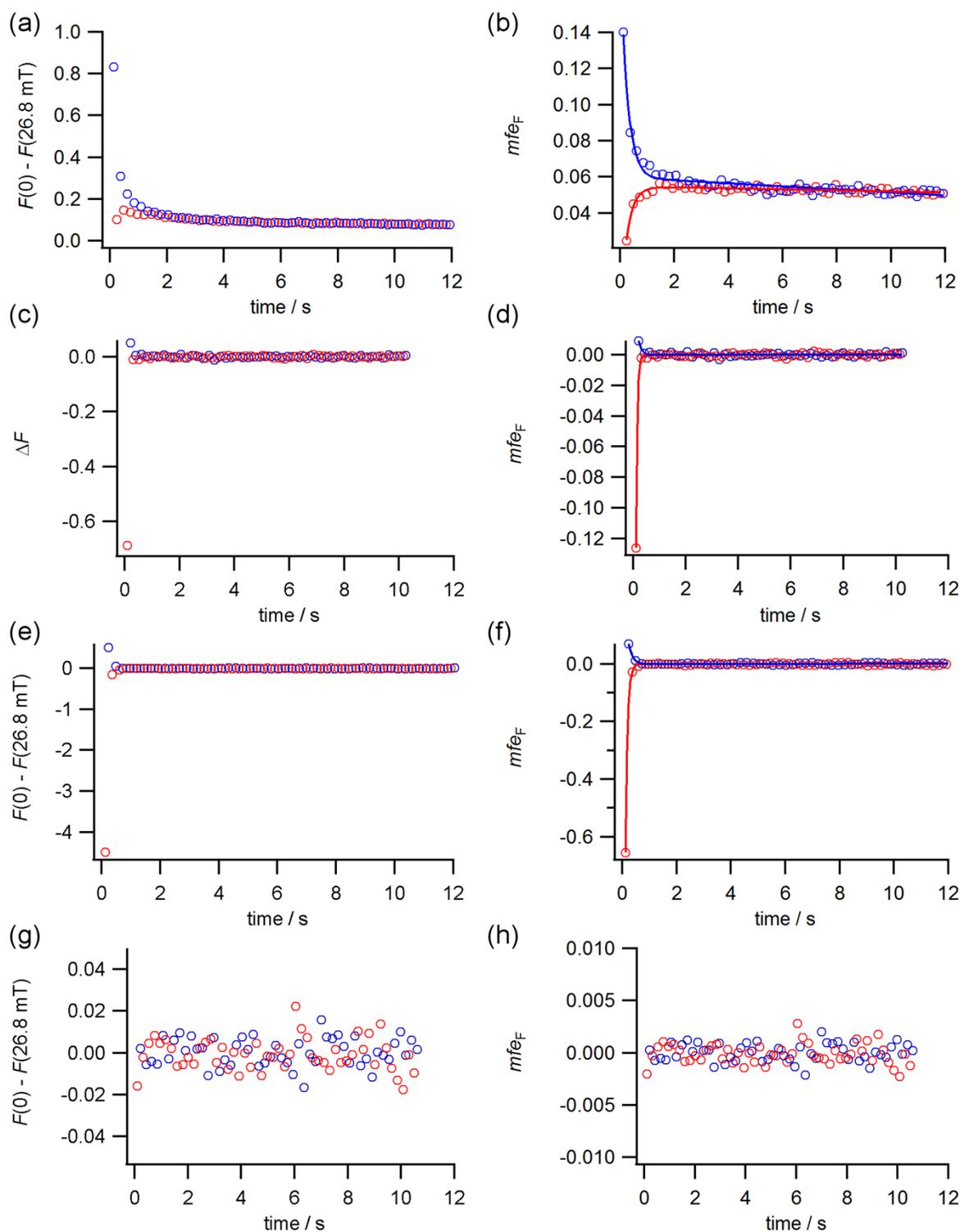


Fig. S4: Apparent time-dependence in  $mfe_F$  calculations. (a, b)  $1 \mu\text{M}$  FAD +  $300 \mu\text{M}$  Trp in the presence of a pulsed  $26.8 \text{ mT}$  magnetic field. (c, d)  $1 \mu\text{M}$  FAD +  $300 \mu\text{M}$  Trp in the absence of an applied magnetic field. Quantities calculated as ‘mock’ measurements using exactly the same methodology as panels (a), (b) and (e)–(h). The difference in  $y$ -axis label for panel (c) reflects the absence of magnetic fields in this ‘mock’ measurement. (e, f)  $1 \mu\text{M}$  FMN in the presence of a pulsed  $26.8 \text{ mT}$  magnetic field. (g, h)  $1 \mu\text{M}$  Alexa Fluor<sup>®</sup> 488 in the presence of a pulsed  $26.8 \text{ mT}$  magnetic field. Note the scale used on the Alexa measurements. Solid lines in panels (b), (d) and (f) are fits to an exponential decay plus a straight line. The extrapolation of this straight line back to the  $y$ -axis was taken to be the time-independent value of  $mfe_F$ . Blue: measurements originating in a step down (from field-off to field-on), red: measurements originating in a step up (from field-on to field-off).

## REFERENCES

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