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Towards spatially resolved magnetic field effect measurements in biological systems

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[Introduction] An important question addressed by spin chemists has been that of whether magnetic fields can exert measurable influences in biology. Indeed the observation of such effects has proved quite controversial due to the difficulty of reproducing observed effects across different laboratories. The most promising target for the observation of robust and significant magnetic field effects in biology has come from the attempt to understand the ability of many species to navigate in the earths magnetic field. Much progress has been made since 2000, when Ritz *et al.* reinvigorated Shulten's original 1978 proposal of the radical pair as a magnetic compass[1]. This work identified the eye as the likely location of the sensor, and a group of newly discovered proteins, called cryptochromes, as the likely source of the radical pair. From one direction, spin chemists have tried to study both model reactions and simplified systems involving cryptochromes and related photolyases *in vitro*[2]. From the other direction, biologists have increasingly improved our understanding of the nature of the magneto-sensing ability and the physical location and characteristics of the sensing apparatus. These two communities have yet to meet in the middle and this research aims at trying to build a bridge between them

The presented research studies the effects of external magnetic fields on biologically relevant, spin correlated radical pairs. A novel application of a continuous-wave laser detection system has yielded improved resolution of the magnetic field effects in a test system based on the interaction between flavin mono-nucleotide (FMN) and hen egg white lysozyme (HEWL). The detection system is being combined with a purpose built microscope that will directly observe the species involved in spin-selective reactions within the sub-cellular structures in which they are found in nature. Although we focus our efforts on the study of flavoproteins in general and cryptochromes in particular, we aim at developing general experimental methods to observe magnetic field effects on reactions within microstructures in a spatially-resolved manner.

The origin of the magnetic effects observed within spin correlated radical pairs lies in the coupling of their electron spins to the nuclear spins and also to external magnetic fields[3]. The radical pairs examined in this work are born from the electron transfer between a lysozyme and a flavin based molecule in an excited state. The radical pair formed remains spin correlated. Application of an external magnetic field alters the spin state of the radical pair and can ultimately affect the products formed upon re-encounter/reaction between the two radicals. A schematic representation of the pathways involved in the FMN-HEWL system are shown in Figure 1.

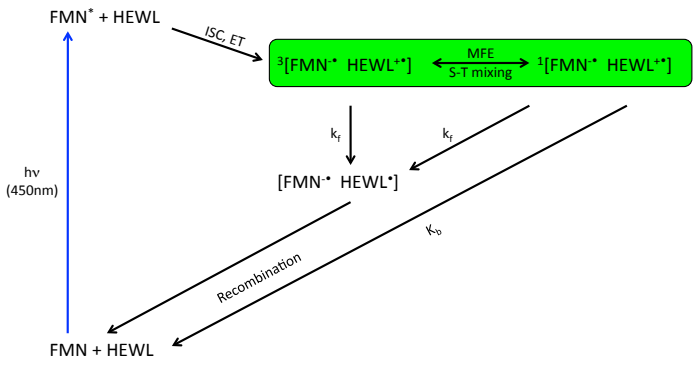

Figure 1: The proposed photochemical reaction scheme of the FMN-HEWL system.

[Experiment] In the experiments discussed FMN was excited from the ground state by the output of a Coherent CUBE laser at 449 nm. The output of this laser can either be continuous-wave or modulated at up to 150 MHz by a TTL signal. The output of this laser is beam combined with that of a Coherent Sapphire LP 532. The 532 nm laser light is used to monitor changes in the populations of the radical pair states via absorption spectroscopy. The absorption signal is detected by a Nirvana auto-balanced photoreceiver (Model 2007) which can reduce noise from laser intensity fluctuations by over 50 dB. The output from this detector is then passed into a Stanford Research Systems lock-in amplifier (Model SR830). Modulation of the absorption signal is produced either through modulation of the 'pump' laser (449 nm) output or by application of an AC magnetic field (provided by a custom built Helmholtz coil pair). The effects of an applied magnetic field can then be observed.

Initial experiments have been performed utilising aqueous solutions in a standard cuvette. These experiments have enabled the characteristics of the entire system to be studied before the added complexity that arises from the implementation of microscopy. These experiments have also yielded interesting results not previously observed in the FMN-HEWL system. A custom built transmission mode, confocal microscope has also been constructed (see Figure 2) and absorption imaging tests have been performed. The beam combined output of the two lasers can be diverted from the cuvette experiment and launched into a single-mode fiber. This provides a beam with very clean spatial mode, which then passes through a high NA, super apochromat objective lens. The transmitted light is then collected through an identical lens and directed to the Nirvana photoreceiver. A Thorlabs CMOS camera is also utilised for direct imaging (and navigation) of the sample. The sample is mounted to a Physik Instrumente P-545.3R7 3-axis peizo stage. This stage provides 200 μm translation in all three axes with a maximum step resolution of 1 nm.

[Results and Discussion] By using both methods of signal modulation (via 'pump' light modulation or through an AC component in the applied magnetic field) we have been able to determine the absolute change in the signal due to an external magnetic field and sensitively detect the changes due to the field. The combined data from these complimentary methods provide the data required to perform detailed simulations to determine many of the reaction kinetics of the scheme outlined in Figure 1.

The two methods of detecting the magnetic field effects in this system also has important implications for imaging within the confocal microscope. Areas of interest can initially be determined by utilising the larger signals obtained using pump beam modulation. These areas can then be scanned at higher resolution and using the more sensitive technique. To date only the initial scanning method has been implemented. The latest results will be discuss in detail during the presentation.

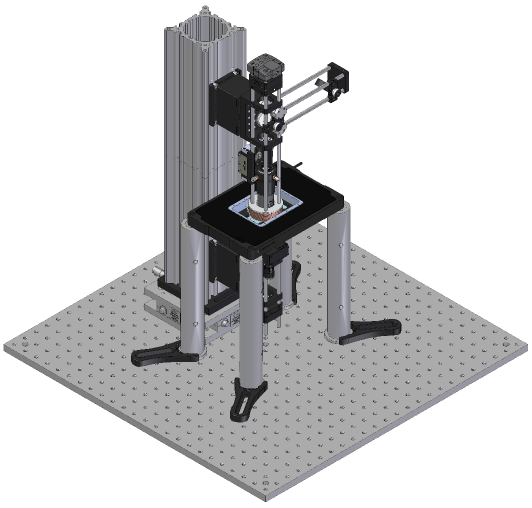

Figure 2: A 3D design drawing of the confocal microscope apparatus.

[1] Ritz *et al.*, *Biophys. J.* **2000**, 78, 707-718.; Schulten *et al.*, *Z. Phys. Chem.* **1978**, NF111:1-5.

[2] See recently, Maeda *et al.*, *PNAS* **2012**, 109, 4774-4779.

[3] See Steiner and Ulrich, *Chem. Rev.* **1989**, 89, 51-147 and references therein.