

## 審査の結果の要旨

論文提出者氏名 ルイス マーティン アンデル

The work described in this thesis aims to address an unsolved mystery in the behavioural biology of migratory birds and many other animals; the mechanism by which they are able to sense the very weak geomagnetic field and in the case of migratory birds, exploit compass information from it for navigation. The currently favoured mechanism for such magnetoreception is the radical pair mechanism (RPM) hypothesis. This mechanism proposes that the magnetosensitivity is delivered through a magnetically sensitive photochemical reaction which proceeds through a special transient reaction intermediate known as a spin-correlated radical pair. In avian magnetoreception, the radical pair is believed to be generated by blue light photoexcitation of a protein called cryptochrome, located in the bird's retina, with information being transferred by the optic nerve along with that associated with vision. Chemical studies have verified that purified cryptochromes extracted from plants, frogs and fruit flies show magnetic field dependent photochemistry *in vitro*. This work aims at developing a microscope and related imaging techniques capable of studying cryptochrome and related model photochemical reactions at submicron spatial resolution, in an attempt to observe the photochemical processes taking place directly in living cells and related biomimetic systems. This is understood to be a highly novel and ambitious project. The thesis consists of six chapters of which chapter one is an introduction to animal magnetoreception, spin chemistry and flavin photochemistry, and chapter six presents the conclusions of the work. The central four chapters focus on experimental development, measurements and analysis and are described below.

The second chapter introduces the new microscope and covers technical aspects of its operation. The candidate began working with the microscope as construction was just completed. The instrumental work was substantial, and the chapter highlights the development of two new imaging techniques, TOAD (Transient Optical Absorption Detection) and MIM (Magnetic Intensity Modulation) developed by the candidate and the application of these techniques to a model magnetosensitive reaction system based on the photochemistry of flavin adenine dinucleotide (FAD), the active cofactor in cryptochrome. The chapter highlights the spatial resolution and very high sensitivity of the instrument and also describes additional instrumental developments including a spiral stage scanning method for working with non-cyclic photochemical processes and a 3-beam modification for transient species imaging that simultaneously exploits two different observation wavelengths. Two publications have arisen from the work in this chapter, both in important international journals. In particular, the first paper is published in *Angewandte Chemie*, which is one of the highest profile general chemistry journals in the world.

The third chapter describes the use of the instrument to study, in detail, the photochemistry of FAD over a wide range of pH values from 2 to 8. Previous work has not been sufficiently sensitive to study the magnetic field behaviour above pH 3.6 as a large proportion of the FAD molecules are rendered photochemically inactive under such

conditions. Here, kinetic and magnetic field effect data are provided, and a detailed analysis allows the construction of a reaction scheme for the photochemistry at higher (and importantly including physiological) pH for the first time. This is particularly relevant as unbound FAD is known to be present in many cells, including those in humans, and the effect of a magnetic field is shown to increase the lifetime of transient radical species generated on photoexcitation. The work in this chapter has been published in the *Journal of Physical Chemistry Letters* which has, perhaps, the highest impact factor among physical chemistry journals.

The fourth chapter looks at the application of the microscope to flavin photochemistry in biomimetic vesicle environments, for both small unilamellar vesicles and giant unilamellar vesicles. For the former, photoinduced electron transfer reaction across lipid monolayers and bilayers were studied using flavin based photochemistry and its magnetic field dependence. For the latter, the newly developed TOAD imaging technique was used to directly image the formation of flavin based radical pairs in individual vesicles, a world first. The work on small unilamellar vesicles has already been submitted to the journal, *Molecular Physics* for publication and is currently undergoing minor revision based on the reviewers' comments. The work on giant vesicles has not yet been published. Measurements continue, and the work will be published in the near future.

The fifth chapter describes work in which the microscope was used to study real biological systems. The first of these is the photochemistry and magnetic field effects thereon in purified cryptochrome extracted from *Drosophila melanogaster*. The observed behaviour is in contrast to a recent publication on this system and a new three spin mechanism based on photoexcitation of the semiquinone anion form of the FAD is proposed to explain experimental results. In the second part of this chapter, preliminary studies on the observation of FAD photochemistry in living cells is presented along with the work the candidate has done in establishing cell culturing facilities in the laboratory, for future studies.

All of the examiners were impressed by the quality and number of important scientific achievements made in this work. The candidate delivered a clear presentation explaining, in detail, the background and motivation of the work and summarizing the various scientific problems addressed in each chapter and the new conclusions that could be drawn as a result of the measurements made. The candidate was questioned by all examiners on both theoretical and experimental aspects of the work and provided answers to their satisfaction. In particular, the external examiner, who is a world leader in this field, was very impressed with the scientific progress made in this work and congratulated the candidate on his achievements. The thesis itself was assessed to be of suitable quality in terms of the content, writing and presentation.

Therefore, the committee members agreed unanimously that the awarding of a doctoral degree (Environmental Sciences) is appropriate.