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Ultrafast Spectroscopy of DNA Binding Systems

By Caitriona M. Creely

Thesis Submitted for the degree of
Doctor of Philosophy
in the
University of Dublin

Department of Physics
Trinity College Dublin

April 2004
DECLARATION

I declare that the work in this thesis has not been previously submitted as an exercise for a degree to this or any other university.

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Caitriona Mary Creely

8th of March 2004
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Summary

Ultrafast transient spectroscopy was used to investigate the properties of various systems which bind to DNA. Visible and IR pump and probe was employed to elucidate excited state process of the DNA binding complexes. Ultrafast inter and intra molecular electron transfer reactions were monitored, via changes in the kinetics of transient species formed. Starting with a simple intercalating dye system where only one chromophore is present, we subsequently studied multi-ligand DNA binding complexes, where the geometries of the interacting species were less well defined.

Thionine, an intercalating dye was bound to DNA and found to undergo a femtosecond electron transfer, which quenched the thionine fluorescence.

Ru(TAP)$_2$dpdz complexes were studied in aqueous and acetonitrile solutions. There was similarly evidence for an electron transfer, although proceeding on a picosecond timescale for this complex. Changes in the part of the molecule bound to DNA resulted in small changes in the excited state dynamics. The substitution of D$_2$O for H$_2$O slowed the kinetics appreciably, which suggested that the electron transfer may be proton-coupled.

Ru(phen)$_2$dpqa acts as a light switch in the presence of DNA, exhibiting strong fluorescence. The kinetics for the visible and IR region were different in organic and aqueous media, as expected. The excited state deactivates in water, with similar kinetics measured by visible IR transient absorption.

Re(CO)$_3$py dpdz complexes investigated showed very different excited state behaviour for when the binding dpdz ligand was substituted with fluorine atoms. The complex is deactivated in aqueous solution, showing biexponential kinetics. It undergoes an ET reaction, in the presence of guanine. Results show how adjusting substituents on the intercalating ligand can sensitise molecular probes to different solvent environments.

The investigation of dpdz studied the sensitivity of the ligand to substitution and solvent polarity.
1. MOTIVATION

Ultrafast reactions, which occur on the picosecond or femtosecond timescale, are a hot topic amongst today’s chemists, especially since the recent 1999 Nobel prize for Chemistry was given to Ahmed Zewail for his work in the field. However the study of ultrafast reactions is also extremely interesting and challenging for physicists because of the information on energy transfer gained at a sub-picosecond level and the experimental techniques and sophisticated lasers needed to break the picosecond barrier. In recent years lasers, employing sophisticated non-linear optics, have been developed to operate in the femtosecond regime and the diagnostic equipment has also been modified to keep up with the rate of information acquisition required.

Most ultrafast processes take place with minimal losses in terms of energy and hence information. This is interesting in terms of designing and developing materials for photonics and photochemical reactions. Fields of research that can benefit from ultrafast processes range from telecommunications development to computer processor design and drug synthesis.

Electron transfer is the key reaction in many biological processes such as photosynthesis, respiration and drug metabolism. Femtosecond electron transport processes in DNA have received renewed attention both experimentally and theoretically. Many studies are being pursued in order to fully understand the nature of ET in DNA and its importance in DNA damage and repair. Many of the current studies involve visible wavelength excitation, where the wavelength used will not be absorbed directly by the DNA, but instead by a molecule bound to it (called a photosensitiser).

These molecules act as molecular probes for DNA and are generating great interest in research at the moment. The simplest type of molecular probe is one which activates in the presence of DNA, displaying some easily observable optical property. Many of these probes contain fluorescent species and upon binding, the change in photophysical properties indicates the presence of DNA. More sophisticated probes are activated by specific sequences of DNA, allowing for example the detection of certain diseases.
From an experimental perspective, optical excitation of a bound dye is one method by which electrons or holes can be rapidly injected into DNA strands, allowing the elucidation of inter and intrastrand electron transfer.

Different theories have been proposed in terms of the mechanisms of intrastrand electron transport. This area has been termed the molecular wire debate, as one of the theories is the molecular wire model where the DNA strand is treated as a metallic wire and the base pair stacking has no effect on the ET process. This has lately been shown to be an incorrect generalization as some experiments have demonstrated that certain parts of the DNA strand can act as potential barriers to charge transport, so the idea of a homogeneous medium through which charge can travel is oversimplified. Another aspect to the debate is how fast the electron transfer occurs, does it occur on a time-scale that is faster than predicted for other biological structures such as proteins?

In addition to elucidating electron transfer pathways, intercalated (bound) dyes can be used as probes of the dynamics of DNA. It has been suggested that the flexibility of DNA strands is the key to its functionality, in an analogous way to the tertiary structure of proteins defining its function. To this end Fiebig et al have recently reported on the photophysics of ethidium complexes with mononucleotides and polynucleotides in water. In this study it was necessary to consider the interplay between orientational motion and electron transfer, since both occurred on a picosecond timescale.

The selective interaction of molecular probes with DNA has great potential from the point of view of medical applications. Since the interaction is selective, ie the molecule only binds to the faulty DNA, then only that specific strand should suffer oxidative damage, leaving other DNA intact. This potential has been realized in the development of photodynamic therapy, used mainly to treat skin cancers, obstructive lung cancers and more recently to halt the advance of macular degeneration, which can lead to blindness. This treatment has advantages over surgical procedures since it is less invasive and hence involves less trauma of the patient and minimizes the pathways for infection to enter the body. It involves intravenous administration of an anti tumor agent, such as a porphyrin to a patient. The drug is selectively uptaken by cancerous tissue, and the tumor is later irradiated with a certain frequency of light. The treatment works by photocatalysis of the photodynamic drug (normally a porphyrin) to create toxic oxygen species. This involves an
electron transfer process. Tissue damage then occurs in the tumor, usually resulting from damaged vasculature. It is important to discern the mechanisms behind these effects. Light activated ultrafast investigations of drug DNA complexes can provide useful information about possible reaction intermediates. Knowing more about the exact reaction mechanisms can aid in the design process of future drugs. Using this information together with toxicity studies from biology should aid synthetic chemists to provide more efficient anti-cancer drugs. Apart from the aforementioned medical benefits, electron transfer processes during photosynthesis are also of interest with regard to the development of more efficient solar cells. Not withstanding these applied areas of study, electron transfer in DNA is still a rich area for fundamental research.

Starting with simpler systems that bind with DNA and are well characterised on the nanosecond timescale, this study involves becoming familiar with ultrafast processes and their interpretation from transient absorption spectra. Then more complex systems can be investigated at the ultrafast level.


(e) B. Giese, S. Wesely, M. Spormann, U. Lindemann, E. Meggers, M. E. Michel-Beyerle

3 Hop to it, B. Giese Chemistry in Britain 44-46 (2000)


2. INTRODUCTION

2.1 Femtosecond science

2.1.1 Overview
A key part of research today is optimisation. Constant improvement of materials, techniques and diagnostic equipment allows scientists to develop tailored drugs, better materials and faster computers. However this optimisation has the greatest implications when it crosses a barrier. The femtosecond time regime is one such leap in knowledge where we can probe events occurring on $10^{-15}$ seconds. At this timescale nuclear motions can be observed and the making or breaking of chemical bonds monitored in real time. Today coherent control of these ultrafast processes is possible.

2.1.2 Ultrafast Spectroscopy
Photochemical reactions occur widely in nature, and the ubiquity in the natural world of ultrafast photoreactions is a testament to their evolutionary success. The 200fs cis-trans isomerisation of rhodopsin in the eye allowing sight is one of many such processes. If we want to study processes as rapid as these we require specialised tools.
Optical spectroscopy uses the interaction of light with matter as a means to extract information about the system under observation. This can occur in the steady-state or as a time-resolved experiment, where the time evolution of a system is of importance. In excited state spectroscopy a pump pulse creates a number of excited states in a system, which can be observed at some time later. The changes in quantum states of the system are inferred by interpreting the spectroscopic data and, depending on the time resolution of the experiment, different information can be elucidated.
Advances in laser technology have made it possible to probe systems on an ultrafast timescale in the condensed phase. It is extremely important to study biological systems in the liquid phase as their natural conformations occur in aqueous solutions. Ideally we require a form of spectroscopy that is non-destructive, can be performed in aqueous solution and can be used
together with others spectroscopic techniques to build up a clear picture about the ultrafast processes taking place in biological systems.

Ultrafast spectroscopy allows us to study processes that are of particular interest to physicists and photochemists. How DNA interacts with drugs is of great importance to medical science and can also yield more information about DNA itself. Conformational information about DNA or proteins can be extracted in real-time, (studying the dynamics of protein folding for example elucidates protein function). Biological systems can be examined given a whole range of initial parameters: solvent, temperature, pH value, salt concentration etc., Sometimes the initial conditions are chosen to be similar to those found in vivo, where experiments will be performed in aqueous solution and at neutral pH values. Other times it is useful to choose the initial conditions in order to extract the maximum information from the system, for example using a different solvent other than water.

Figure 2.1 shows a time-line for different processes ranging from the age of the universe to electronic motions in the region of femtoseconds, and corresponding rate constants. Rate constants are widely used by chemists to indicate the likelihood of a reaction, with a large rate constant indicating a favorable reaction.
2.1.3 Flash Photolysis

This procedure was pioneered by Norrish and Porter in 1949 which led to their being awarded a Nobel Prize in Chemistry (1967). The methodology involved one flash-lamp (pump) which produced excited species in a sample and another (lower-energy) flash-lamp to interrogate (probe) the system at a later time. These were termed flash photolysis experiments. The two flashes were electronically delayed with respect to one another which provided the
experimental time-resolution. This is an experiment which is still used today in the nanosecond regime by many chemists.

2.1.4 Pump and Probe

The invention of the laser in 1960 and subsequent development of pulsed laser systems meant that laser pulses became available as a way of pumping and probing the sample. The method of using two ultrafast laser pulses as pump and probe overcomes some problems inherent in previous methods that restricted the experimental time resolution. For example experiments where the detection is dependent on a fast photomultiplier tube were restricted by electronic limitations. The impedance of electronic devices can cause the rise time (dependent on the RC time constant) of the circuitry to exceed 1ns, which was the limiting factor as regards ultrafast time resolution.

Pump and probe measurements, where a sample is photoexcited with a ultrashort pulse of light and changes in its excited states are probed using a second ultrashort pulse, overcame this difficulty. The main components are a pump beam, a probe beam and a mechanical delay line that changes the distance of travel of one or both of the beams. This spatial delay allows us to control the optical path length and hence the time-delay between the initial excitation and the arrival of the probe pulse. Given the high repetition rates of ultrafast lasers today we can look at the average of a huge number of ultrafast excitation/deactivation processes at each time delay over a short period of time. The time resolution for the experiment is dependent on the longest pulse duration, pump or probe, an ultrafast detector is unnecessary as we are looking at an average signal at each delay. This method is widely used for performing ultrafast methods with the longest time delay being approximately 10 ns (3 m optical path difference). A time delay much greater than this would require either a lot more bench space than is normally used in labs, or a delicate optical alignment using numerous mirrors and zig-zagging the probe beam back and forth to generate a long time delay. Short times delays will depend on the increments available from the mechanical delay line.
2.2 Absorption Spectroscopy

2.2.1 Introduction
Absorption spectroscopy studies what happens when light of a certain frequency is partially absorbed by a sample. The Beer-Lambert Law describes the variation of intensity of light at a certain wavelength passing through a homogeneous sample.

\[ I_f = I_0 e^{-\alpha c l} \]  

\( I_f \) = final intensity  
\( I_0 \) = initial intensity  
\( \alpha \) = absorption coefficient  
\( c \) = concentration of the absorbing sample  
\( l \) = length of sample the light passes through.

The absorption is,

\[ A = \log \frac{I_0}{I_f} = -\log T \]  

Where \( T \) is the transmittance. The absorption can also be expressed in terms of \( A = \varepsilon c l \), where \( \varepsilon \) is the extinction coefficient of a material at a certain wavelength. This relationship is useful for calculating concentrations using a spectrum obtained from a sample in a known absorption cell length.

2.2.2 Absorption Spectra
When molecules absorb light in the visible or UV part of the spectrum, electrons are excited from lower to upper electronic energy levels (the nature of excited states will be discussed in the following chapter). The absorption peak is very broad as each electronic level has vibrational and rotational levels associated with it. Absorption spectra of this kind are normally displayed with wavelength or electron volts on the x-axis.

Infra red radiation does not contain enough energy to excite the electrons, but it will cause changes in the frequency and amplitude of vibrations about the covalent bonds of certain atoms or groups of atoms. These vibrations are quantized, and absorb IR photons from different parts of the IR spectrum. The location of an IR band is specified by its wavenumber.
(in units of cm$^{-1}$) which is the inverse of wavelength and hence is directly proportional to energy, since

$$\nu = \frac{1}{\lambda} \quad (2.3)$$

where $\lambda$ is the wavelength of radiation in cm. Briefly the frequency of a given vibration is related to both the masses of the bonded atoms (lighter atoms vibrating at a higher frequency) and the relative stiffness of the bond itself (triple bonds are stiffer and vibrate at higher frequencies than double bonds and so forth). In order to absorb IR radiation the dipole moment of the molecule must change, a symmetric vibration about a bond will not cause an absorption.

### 2.3 Biochemistry of DNA

#### 2.3.1 Overview

DNA is a complex macromolecular system that is being studied by scientists of myriad disciplines. The structure has two main components: the phosphate-sugar backbone and the basepairs (nucleotides) (Figure 2.2). There are four bases in DNA: the purines, guanine and adenine and the pyrimidines, cytosine and thymine. These nucleotides form the Watson-Crick basepairs where guanine binds to cytosine and adenine binds to thymine via hydrogen bonds, (see Figure 2.3). It is the sequence of successive base-pairs that provides the blueprint for DNA and for producing all the components of our bodies. The 5 carbon sugar rings (pentoses) that connect the phosphates to each other have different conformations with respect to the bases. These are called sugar puckers and affect the angles of the basepairs with respect to the phosphate backbone. DNA itself is a polyanion, the negative charge residing on the phosphate atom. The pentose rings have 2 different carbons that the phosphate can bind to 5'(5 endo) and 3' (3 endo). This gives a direction when reading the base-pair sequence of a DNA strand starting at 5'.
Figure 2.2 A single strand of phosphate-sugar (pentose) backbone showing each of the nucleotides
Picture: Solomons, pg 1196

Figure 2.3 Nucleobases of the Watson-Crick base pairs
Picture: Solomons, pg 1199
2.3.2 DNA Conformations

The conformation of DNA most commonly seen in nature is B DNA but A, Z and C types are also well characterised. These conformations occur under different humidities. There exist other more complex structures such as coiled and cruciform DNA, which depend more on the base-pair sequence. Only B-DNA will be discussed here, both for simplicity and because it is the type found in cells (the A helix is also important biologically as the form of double-stranded RNA). It is sufficient to know that the physical characteristics such as pitch, base pairs per turn and sugar puckers differ from one form to another.

B-DNA has base pairs lying almost perpendicular to the phosphate-sugar backbone. A complete turn of this right-handed double helix comprises 10 base pairs with a distance of 3.4 Å between each one. Viewed from above the diameter of the helix is 20 Å and the centre is hollow. DNA grooves are the indentations in the surface of a double helix that arise from the progression of bases and backbone around the helix axis (Figure 2.4). Their geometry and nature depends on the type of helix formed. It should be noted that the values given above are average values and the local environment can vary greatly depending on interactions with other molecules.

The major groove in B DNA is wide and deep and the minor groove is narrow and deep and tends to contain water, known as the spine of hydration. The sugars are on the same side of the base pairs. These base pairs can move around, allowing in some cases molecules to slide in between them. The base pairs can also slide, tilt and roll w.r.t. each other and the backbone. The base pair binding consists of hydrogen bonding, of which a G-C pair has 3 bonds and an A-T has 2, making successive G-C base pairs more stable and causing lesser propeller twists (analogous to torsion around C=C double bond c.f. C-C).
When the double-stranded structure of DNA separates it is termed denaturing. This can occur in a number of ways, i.e. by heating, changing the pH level or the salt concentration of the DNA solution. Denaturing by heating occurs above 60 degrees Celsius for neutral pH solutions of DNA, and different types of DNA will have a characteristic melting temperature. DNA must also be kept in a medium having a minimum ionic strength to shield the outer phosphate groups from each other. For these reasons DNA used in experiments is normally kept in buffered solution and experiments are performed at room temperature, to ensure the stability of the structure.
2.3.3 Mononucleotides/Polynucleotides

In some studies it is useful to differentiate between the properties of individual base pairs in a reaction. Mononucleotides describe a single nucleobase, sugar and phosphate(s). Depending on the base these are GMP (guanosine monophosphate), AMP (adenosine monophosphate), CMP (cytidine monophosphate) and TMP (thymidine monophosphate). Below is shown dAMP, the deoxygenated form of AMP, with varying numbers of phosphate groups.

![Deoxyadenosine-5'-monophosphate (dAMP)](image)

![Deoxyadenosine-5'-diphosphate (dADP)](image)

![Deoxyadenosine-5'-triphosphate (dATP)](image)

Figure 2.5 Picture showing adenine mononucleotides, from University of Arizona Biology Learning Centre webpage.

Polynucleotides usually refer to an ordered strand(s) of base pairs so that the base-pair sequence is fully known. This can be useful for studies where different modes of binding are necessary, or when the different redox potentials of the bases are employed. There is also an entire industry based around creating synthetic oligonucleotides that bind to a duplex DNA or RNA and thus inhibit transcription of a faulty gene. Figure 2.5 is a representation of the [poly(dG-dC)]$_2$ polynucleotide.

\[
\begin{align*}
    \text{G} & \quad - \quad \text{C} \\
    \text{G} & \quad - \quad \text{C} \\
    \text{C} & \quad - \quad \text{G} \\
    \text{G} & \quad - \quad \text{C}
\end{align*}
\]

Figure 2.6 Simplified representation of [Poly (dG-dC)]$_2$, the sugar phosphate backbone is represented by — and hydrogen bonding between Watson-Crick base-pairs is represented by |.
2.3.4 DNA Binding Modes

The two types of binding modes are groove binding and intercalation. The shape and charge of the molecule binding to the DNA is vitally important in deciding the mode of binding. The sequence of base pairs also affects binding, since the electron affinities of the base pairs are different.

2.3.4.1 Groove Binding

Minor groove binding molecules must be able to expel the water that is normally in these grooves and bond to one of the base pairs. Normally showing a preference for A-T regions the minor groove binding molecules do not significantly perturb the DNA structure. They are stabilised by Van der Waals interactions (attractive, short range forces arising from dipoles) as well as being hydrogen bonded to nitrogen on adenine or oxygen on thymine. Isohelicity is a concept common in such minor groove binding molecules, where the curvature of the molecule complements the convex nature of the groove floor and the helicity of the DNA. Major groove binders also have a preference for A-T sites and here steric hinderance can be less of a problem. The molecules fitting in here do not need to have as much torsional freedom as the minor groove binders.

2.3.4.2 Intercalation

Intercalation occurs when the binding molecule is a chromophore: an electron deficient, planar, aromatic structure about the same size as a base pair. Here the molecule inserts between adjacent base pairs causing slight unwinding of the helix at the site. The interaction is mainly mediated by Van der Waals forces but can also involve hydrogen bonding to adjacent base pairs. Intercalators can also cause other distortions of the backbone. This effect can be used to cleave the helix through excitation of the intercalator. Molecules can intercalate into more than one site, bisintercalators occupy 2 binding sites in between base pairs. Intercalators prefer to bind to G-C sites because of their electrostatic potentials. Figure 2.6 below shows intercalative binding to a CG site and a GC site. These sites are not identical because of the aforementioned directionality of the DNA strand.
2.4 Spectral Properties of DNA and Polynucleotides

2.4.1 Visible region
The 4 nucleobases that make up the DNA have absorption spectra with slightly different $\lambda_{\text{max}}$. The DNA polynucleotides absorb in the region of 260 nm, less than the sum of the individual absorptions of the nucleobases. This property, known as hypochromicity, is thought to arise from the interactions of the stacked base-pairs with each other.

2.4.2 IR region – [poly(dG-dC)]$_2$

Figure 2.8 FTIR spectrum of [poly(dG-dC)]$_2$ in buffered D$_2$O, indicated are bands arising mainly from Guanine(G) or Cytosine(C) vibrations. Picture: J. Dyer PhD thesis.
Figure 2.7 shows the FTIR ground state spectrum of the polynucleotide [poly(dG-dC)]. The signals arise from characteristic absorption due to the C=O stretching, NH bending and skeletal stretching. The vibrations due to absorptions of the sugars and phosphodiester backbone occur below 1500 cm⁻¹. The positions of these bands and the vibrations associated with them are described below. Figure 2.8 shows the GC Watson-Crick basepair and the numbering of the carbons in each structure.

1685 cm⁻¹ Guanine in-plane double-bond stretching vibration, involving mainly the C6 carbonyl group
1649 cm⁻¹ Cytosine in-plane double bond stretching vibration, involving mainly the C2 carbonyl group
1619 cm⁻¹ Cytosine in-plane ring vibration
1579 cm⁻¹ Guanine in-plane vibration of largely C=N stretching character
1561 cm⁻¹ guanine in-plane ring vibration

Figure 2.9 Watson-Crick pairing of Cytosine(C) and Guanine(G), carbons are numbered for both nucleobases. dR: deoxyribose. Picture: J. Dyer PhD thesis.
2.4.3 IR region - \([\text{poly(dA-dT)}]_2\):

Figure 2.10 FTIR spectrum of \([\text{poly(dA-dT)}]_2\) in buffered D$_2$O, indicated are bands arising mainly from Thymine(T) or Adenine(A) vibrations. Picture: J. Dyer PhD thesis.

Figure 2.9 shows the corresponding ground state FTIR for \([\text{poly(dA-dT)}]_2\), note the magnitude of the signal is much less due to the smaller extinction coefficient of the AT polymer. The corresponding absorption bands for this polymer are given below:

1691 cm$^{-1}$ Thymine in-plane double bond stretching vibration, involving mainly the C2 carbonyl group

1663 cm$^{-1}$ Thymine in-plane double bond stretching vibration, involving mainly the C4 carbonyl group

1644 cm$^{-1}$ Thymine in-plane ring vibration

1617 cm$^{-1}$ Adenine in-plane ring vibration of largely C=N stretching character

1575 cm$^{-1}$ Adenine in-plane ring vibration of largely C=C stretching character

Figure 2.11 Watson-Crick pairing of Thymine (T) and Adenine(A), carbons are numbered for both nucleobases. dR: deoxyribose. Picture: J. Dyer PhD thesis.

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2.5 Transition Metal Complexes

Transition metals react with a variety of groups of molecules called ligands to form transition metal complexes. In doing so the ligands donate electrons to vacant orbitals of the metal. These bonds are typically covalent but have some polar character. Where a ligand shares a $\pi$ bond with the central metal electrons are donated from the $\pi$ bond to the metal. Electrons are also donated from the d orbital (of the metal) back to the empty $\pi^*$ orbital of the carbon compound. This is called back bonding. The complexes can assume various geometries depending on the metal and number of surrounding ligands. This in turn can greatly affect how the complex interacts with other molecules such as DNA.

When the metal binds directly to a carbon atom it is called an organometallic complex. Organometallic compounds are as a rule toxic. This toxicity varies from compound to compound and can exist even though the metal itself is non-toxic. This toxicity was harnessed in some early treatments of sleeping sickness at the beginning of this century where it was hoped that the disease-causing organisms would experience greater toxicity than the surrounding healthy tissue. Organometallics have been used in chemotherapeutics as highly selective binding agents that can be photo activated, as mentioned in the previous chapter. Metallo-organic complexes contain ligands where, although the ligands are organic, there is no carbon-metal bond. In some of these cases there are metal to nitrogen bonds. The extent of back bonding as a method of stabilization is small for most of these complexes.

2.6 Electron transfer in DNA

The Motivation stated how important electron transfer was from both a materials science perspective and also because of its ubiquity in biological processes. In the molecular wire debate different methods have been employed to study intramolecular electron/hole transport mechanisms. This ranges from DNA binding moieties acting as external redox active materials along the strand to synthetic DNA bases embedded in the helix which have specific redox properties to induce oxidation/reduction.
This latter method has been employed to alter the properties of a DNA strand in a very ordered manner, after irradiation produces a photoinduced reaction. The initial electron transfer can lead to enhanced yield of strand breaks and to adduct\(^*\) formation.\(^5\) Afterwards the amount of strand breakages due to oxidative damage in a particular reaction can easily be ascertained by quantitative methods such as gel electrophoresis.\(^6\) Although \textit{in vivo} treatment of disease will not involve ordered, synthetic strands with altered bases at particular places, studying these systems can reveal important information about inter and intramolecular processes.

The study of compounds intercalated into DNA are appropriate both from the standpoint of theoretical approaches and from an applied point of view. Electron transfer reactions, being fast chemical processes are well suited to study by ultrashort laser pulse excitation of various sensitising dyes such as phenothiazonium, anthraquinones and also metallic complexes. Ethidium is one compound that is both widely used in experimental studies (in its bromide form for gel electrophoresis) and in theoretical studies of ET mechanisms, due to its binding with DNA.\(^7\) An attractive benefit of these intercalating complexes is that the mutual orientation of electron donor and acceptor is well defined. It is these intercalating complexes that will be investigated in this work.


Also Gerber’s group in Würzburg

\(^3\) See: S. Neidle, M. Waring (Eds.), \textit{Molecular Aspects of Anticancer Drug-DNA Interactions 1} (1993)


\(^*\) An adduct is the product of a Diels-Alder reaction, widely used in synthetic chemistry

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3. THEORY

3.1 Overview

This chapter details the basic ideas behind laser operation, explains ultrashort pulse formation and propagation and the properties of ultrashort pulses. An explanation of time-resolved experiments is given and the subject of organic photochemistry is introduced. The optical properties of DNA and polynucleotides are also discussed. An understanding of the theory in this chapter was needed in order to operate an ultrafast laser system, set up a time-resolved experiment and interpret the results accordingly. For a complete treatment of the theory in this section see Svelto or Siegman, theory used in this chapter has been taken from or adapted from these texts and also from the Coherent Laser manuals supplied with the ultrafast laser system in Trinity College Dublin.

3.2 Ultrafast Laser Physics

A very brief section will follow on laser operation. In the following subsection terminology will be introduced and examples given of NLO effects and how they are utilised to form stable ultrashort pulses for use in ultrafast laser spectroscopy. The exact details of the laser systems used will be given in Chapter 4: Experimental Methods.

3.2.1 Lasers

A laser is an optical oscillator which creates a highly directional beam at a certain wavelength. Each laser system requires a pump (method of excitation), a gain medium and an optical cavity. The manner of operation is as follows. The gain medium inside the optical cavity contains some number of atoms in their ground states. The pump excites the medium by some means, until population inversion has been achieved. Light is then amplified as a result of stimulated emission from these upper states and laser light leaves the cavity by the output coupler, as a highly directional coherent beam. In order to explain the workings of a laser we introduce the ideas of absorption, spontaneous emission and stimulated emission.
3.2.2 Spontaneous Emission
Consider an atom which has two energy levels, E₁ and E₂ such that E₁ < E₂. The population of the levels in atoms per unit volume are given correspondingly by N₁ and N₂. At time t = 0 there are some N₂ atoms in level E₂. As E₂ > E₁, the atom will tend to decay to level E₁. When the atom does this by emitting energy in the form of an EM wave the process is known as spontaneous emission, and the energy emitted has frequency ν, where:

\[ \nu = \frac{E_1 - E_2}{h} \quad (3.1) \]

where h is Planck’s constant. The rate of spontaneous emission is proportional to the amount of atoms present in level E₂ as:

\[ \left( \frac{dN_2}{dt} \right)_p = -AN_2 \quad (3.2) \]

A is called the spontaneous emission probability or the Einstein coefficient, and is related to the spontaneous emission lifetime \( \tau_{sp} \) by:

\[ \tau_{sp} = \frac{1}{A} \quad (3.3) \]

The numerical value of A depends on the particular transitions involved.

3.2.3 Stimulated emission
When an atom is initially in level E₂ an EM wave with the same frequency \( \nu \) (from Eq. 3.1) can stimulate it to return to a state E₁. This process is called stimulated emission. This process differs to that of spontaneous emission in that the photon emitted is in phase with the incident photon, which is crucial for the operation of lasers.

The rate for this process is given by:

\[ \left( \frac{dN_2}{dt} \right)_{st} = -W_{21}N_2 \quad (3.4) \]

where \( W_{21} \) is called the stimulated emission transition probability, this depends not only on the specific transition but also on the intensity of the incident EM wave. For a plane EM wave we can write \( W_{21} \) as:

\[ W_{21} = \sigma_{21}F \quad (3.5) \]
Where $\sigma_{21}$ is called the stimulated emission cross-section and $F$ is the photon flux of the incident wave.

### 3.2.4 Absorption

Absorption can now be described by using similar terms. An EM wave is incident on an atom where there is some number of atoms in level $E_1$. If the EM wave has frequency $\nu$ (Eq. 3.1), then there is a probability that the atom will be raised to level $E_2$. The rate of absorption for this process is given by:

$$\frac{dN_1}{dt} = -W_{12}N_1 \quad (3.6)$$

Where:

$$W_{12} = \sigma_{12}F \quad (3.7)$$

So this process like stimulated emission is dependent on the intensity of the EM wave. Einstein showed that $\sigma_{12} = \sigma_{21} = \sigma$ which is called the transition cross section.

### 3.2.5 Population Inversion

If an EM wave travels a distance $dx$ through a medium the photon flux will change according to

$$dF = \sigma F (N_2 - N_1) \, dx \quad (3.8)$$

If $N_1$ is greater than $N_2$ absorption will occur and the change in flux will be negative. For stimulated emission to occur $N_2$ must be greater than $N_1$. At equilibrium $N_1 > N_2$ according to Boltzmann statistics relating to the absolute temperature $T$ of the medium:

$$\frac{N_2}{N_1} = e^{-\frac{E_2 - E_1}{kT}} \quad (3.9)$$

Where $k$ is Boltzmann’s constant. The process achieving a non-equilibrium condition such that $N_2 > N_1$ is called population inversion. A material which has achieved population inversion is called an “active material”.

3-3
3.2.6 Laser Components

Each laser requires a pump, an active material (gain medium) and an optical cavity to amplify light.

A pump is the means used to raise the atoms of the active material to the higher level $E_2$ to achieve population inversion. When population inversion has been reached, the first few photons emitted spontaneously can go on to produce stimulated emission as described above. To achieve laser gain population inversion must be reached, after which stimulated emission will be dominant over absorption.

Different laser systems use different methods of pumping; a commonly used pump is a "flash-lamp" where the energy of the photons emitted by the lamp corresponds to differences in energy levels in the atoms of the active material. Electrical discharge is used in ion lasers whereby the discharge causes the gaseous atoms to lose some of their electrons (in noble gases anywhere from 1-3 electrons), forming a plasma.

![Figure 3.1 Light propagating in a laser cavity, mirror 2 is the output coupler. Picture: Svelto pg 5](image)

The light energy released through stimulated emission is stored in the optical cavity (Figure 3.1). At one end there is a mirror of almost 100% reflectivity and at the other end is a mirror where the reflectivity is slightly less, called the output coupler.

In the early stages of the laser process spontaneous and stimulated photons are emitted in every direction; however any photon emitted on the axis of the two mirrors will be reflected back through the gain medium, where it is available to stimulate the emission of another atom. This axial beam will bounce back and forth between the two reflectors, creating an optical feedback loop. This gives rise to the term oscillator, as the beam bounces back and forth.

Laser action occurs where the gain from stimulated emission is sufficient to overcome the loss in the cavity due to the leakage from the output coupler, the light which leaves the output coupler is called laser light.

The threshold for laser gain or simply lasing occurs at a critical value of the population inversion, the critical inversion:
Here $R_1$ and $R_2$ are the reflectivities of the two mirrors in the cavity and $l$ is the length of the active material.

The light leaving the cavity will have a highly directional beam as the gain depends strictly on the geometry of the beam, and the beam collimation can be augmented further by the design of the mirrors at either end of the cavity.

### 3.2.7 Multi-level systems

We can see that a simple two level system will not allow us to achieve population inversion, as when $N_2 = N_1$ the processes of absorption and stimulated emission will cancel each other out. For stimulated emission to be the dominant process a multi-level system is necessary. Schematically the levels of a three level and four level system are given in Figure 3.2.

![Figure 3.2 Three-level (a) and four-level (b) laser system. Picture: Svelto pg 7.](image)

In the three-level system population inversion is reached when $N_2 > N_1$. In the four-level system population inversion requires that $N_2 > N_1$, however in the latter case $N_1$ is only weakly populated by thermal excitation at room temperature, so almost all atoms arriving at level $E_2$ are available for stimulated emission. So in the 4 level system population inversion is easier to achieve.

(When there is more than one set of energy levels that can support stimulated emission and hence amplification the laser is said to operate in multi-line or multi-wavelength mode.)
3.2.8 Gain-bandwidth

A single line laser produces light at one point in the spectrum and appears monochromatic. However, this is not actually the case and the laser line contains a random mixture of closely spaced wavelength components. In the case of an ion laser a gain vs frequency plot shows that there are a number of modes where the gain exceed the loss from the output coupler. This gain profile derives its shape from the intrinsic properties of the ions and from the pumping mechanism. Measuring the width of this gain profile gives us the gain bandwidth. For most ion lasers this is 6 GHz, which only constitutes a tiny fraction of the visible spectrum ~ 375 x 10^{12} Hz (400-800 nm) and can be considered monochromatic.

3.2.9 Cavity Modes

Electromagnetic energy stored in the laser cavity is distributed among what is known as its transverse and longitudinal modes. These can be explained as follows: a wave of light propagating within the cavity takes on a standing wave configuration. There must be a node at either end of the cavity and for this to happen there must be an integer number of half-wavelengths in the cavity. This is related to the distance between the two mirrors (length of the cavity), L, by

$$m = \frac{L}{\lambda/2} \quad (3.11)$$

Where m is the number of modes that can resonate in the cavity. And the frequency of each mode is given by

$$\nu = \frac{mc}{2L} \quad (3.12)$$

these are called longitudinal modes, and occur in the axis of the laser propagation, the distance between each successive mode given by

$$\Delta \nu = \frac{c}{2L} \quad (3.13)$$

It can be seen that the length of the laser cavity used will determine both the number of modes and the resultant output energy. Although there are an infinite number of modes available the laser will not support laser action in all of them because of mode competition. For a thorough discussion of laser mode competition see Siegman Chapter 25, Section 4. In simple terms this
phenomenon arises because the homogeneous linewidth within the gain profile is much greater than the spacing between the modes. All atoms capable of emitting within that bandwidth contribute to one single mode. The strongest mode takes all the gain and neighboring modes are eliminated.

Transverse modes occur in the planes perpendicular to the axis of the beam. These mode patterns may be observed by examining the intensity of the cross-section of the beam at any distance along the beam path. In the simplest of these patterns the maximum light intensity is found at the center of the beam and this drops off smoothly as a function of increasing distance from the centre. This is called TEM $_{00}$, standing for transverse electromagnetic mode and the output in this case will be a Gaussian beam. This is the most frequently used output of a laser, as it will pass through optical systems in close agreement with Gaussian formulae. See Siegman pages 44-49 for discussion of higher order modes.

3.2.10 Mode-locking

Mode-locking is discussed in detail in Chapter 27 of Siegman, see also references within this chapter. Mode-locking allows the formation of ultrashort laser pulses and refers to the fact that the modes of the cavity are made to oscillate with comparable amplitudes and their phases "locked" together. Any of the longitudinal modes described above can propagate in the laser cavity. When several of the modes are lasing simultaneously with random phases they can add to each other such that at certain times the light from all the modes will add to give an intense burst of light and at other times the modes will add to zero. If the phase between each successive lasing mode can be adjusted non-randomly then the peak powers available in the laser become much higher and a stable pulse is formed. The higher the number of modes available the higher the peak power and the narrower in time the pulse will be. If the cavity length is fluctuated at the right frequency a pulse with high enough instantaneous power for mode-locking can be created in the cavity. This high peak power pulse can act as a gate for opening whatever system is being used to propagate the mode-locked pulse versus other random pulses. In the case where the laser is mode-locked we have a large gain bandwidth. One method for passive mode-locking is described in the Section 4.1.3.
Within the cavity of the mode-locked laser, a single short pulse of light bounces back and forth between the mirrors. At each bounce from the output coupler a small portion of the pulse escapes to form the output of the laser. The time between pulses is equal to the time it takes for light to make one round trip of the laser, \(2L/c\). The inverse of this time gives the amount of pulses per second, which is the repetition rate of the laser. Once a pulse is formed within the cavity most of the atoms that were in their excited state, prepared to emit light, have done so by the passage of the pulse through the medium. There will be a period of time afterwards where there are not enough atoms in the excited state to form and amplify another pulse. This means that only a single pulse can be created at a time, so the mode-locked output consists of this one pulse periodically being emitted from the output coupler. This is called saturable absorption.

### 3.2.11 Q-switching

Q-switching is a technique used to generate a short laser pulse with high peak power. It uses some form of intracavity shutter to stop lasing action. While the cavity is shut, population inversion can reach a far higher value than the threshold needed for lasing. The shutter is then opened suddenly and the stored energy is emitted as an intense short pulse. (\(Q\) refers to the quality factor, related to the photon lifetime in the cavity, and is proportional to the energy stored/energy lost over one oscillation in the cavity). When Q-switching occurs the \(Q\) factor goes from a very high to a very low value in a short time. Q-switching can be active or passive. An active Q-switch uses some type of shutter to hold off lasing in the cavity, and the rep rate of the output pulse is limited by the number of times the shutter can open and shut in a second. A passive Q-switch uses some intrinsic property of a material in the cavity to act as a shutter, which can be modulated very rapidly if required.

### 3.2.12 Time-bandwidth product

For a bandwidth limited pulse the frequency bandwidth is of the same order as the reciprocal of the width of the pulse, or:

\[
\Delta \nu \propto \frac{1}{\Delta t} \quad (3.14)
\]
so the bandwidth of an ultrashort pulse must be correspondingly large. The time-bandwidth of any pulsed signal is constrained by the uncertainty principle where \( \Delta \nu_{\text{rms}} \Delta t_{\text{rms}} \geq 0.5 \), where \( \Delta \nu_{\text{rms}} \) and \( \Delta t_{\text{rms}} \) are the root-mean-square widths of the signal in frequency and time, respectively (Siegman pg 334). For a Gaussian pulse the intensity of the pulse envelope, \( I(t) \) is given by:

\[
I(t) = e^{-\left[\frac{4(\ln 2)\nu^2}{\tau_p/2}\right]} (3.15)
\]

Where \( \tau_p \) is the FWHM of the intensity envelope function in seconds. Subsequently \( \Delta \nu \Delta \tau_p \) for a Gaussian pulse is 0.441.

3.2.13 Linewidth

The term linewidth must be used with care when describing the output of a single frequency laser. Mechanical variations of the laser resonator cause the frequency of the selected cavity mode to vary with time. The effective linewidth for any physical interaction between the laser light and other materials depends on the range of frequencies that occur during the time period of the measurement. This concept is extremely important for certain application that depend on long-term phase coherence of the light. This property of the laser is described as the coherence length, \( l \) defined by:

\[
l = \frac{c}{\Delta \nu} (3.16)
\]

Where \( \Delta \nu \) is the FWHM (Full Width Half Maximum) of the laser frequency range over the course of the measurement. The coherence length is the maximum path length difference over which two beams split from the same laser will form an interference with good contrast ratios. An important source of frequency variation in lasers is slow drift deriving from thermal changes in the resonator cavity. As the spacing between the intra-cavity mirrors changes the frequency position of the cavity modes will also change.
3.3 Non-linear optics (NLO)

3.3.1 Overview
In optics the linear regime is used to describe the operating region where the amount of light put into a system is linearly proportional to the amount coming out. The field of NLO deals with the interaction of intense (usually laser) light with matter. The first demonstration of a NLO response in a material was in 1961 by Franken et al., a year after Theodore Maiman devised the first working laser. Franken noticed that the intensity of the light generated at the second harmonic frequency increased as the square of the intensity of the incident light. This process is known as second harmonic generation (SHG) and its use is employed in the laser system in Trinity for production of UV light. Today, forty years on, the field is still expanding with many different applications possible. The operation of ultrafast laser systems is only possible by fully utilizing these NLO effects. A description of the linear regime is given below, followed by the non-linear (NL) regime. The NLO effects necessary for the working of the ultrafast systems are detailed below.

3.3.2 Linear regime
When light interacts with matter it causes changes in the electric dipoles present. These are composed of the charged parts of atoms and molecules. The refractive index $n$ of a material depends on these interactions in a certain material. It is defined as:

$$n = \frac{c}{v} \quad (3.17)$$

$c$ is the speed of light in a vacuum and $v$ is the speed of light in the medium.

The refractive index is a macroscopic quantity and can depend on the temperature of the medium. It also depends on the frequency of the incident light - this dependence is called chromatic dispersion and was first demonstrated when Isaac Newton used a prism to split up sunlight into its different frequencies. It arises from the difference in refractive index of the medium at different frequencies, (frequencies close to resonance for a particular medium are absorbed, for glass this occurs in the UV).
3.3.3 Nonlinear refractive index

See Chapter 4 of Boyd for an in depth discussion of the non-linear refractive index. Briefly many optical materials have a refractive index that is dependent on the intensity of the incident light. The refractive index can be thus written

\[ n = n_0 + n_2 \langle E^2 \rangle \] (3.18)

Where \( n_0 \) is the normal weak-field index of refraction and \( n_2 \) is the non-linear index of refraction, which increases with increasing optical intensity. The brackets indicate the time averaged quantity of the square of the electric field. This can also be written as

\[ n = n_0 + n_2 I \] (3.19)

So the non-linear refractive index is now a function of the optical intensity.

3.4 Some results from NLO

3.4.1 Self-focusing

One of the results from NLO is that under intense light the refractive index of a medium can change, becoming non-uniform over the diameter of the incident light. The higher the refractive index, the lower the velocity of light. If the velocity of light is different for different parts of the beam the propagating beam will be bent or reshaped according to refraction. A lens works in the same way, by having the thickness of glass in the centre different from the edges, or by doping the glass differently at the edges etc. This can focus or de-focus the beam depending on what is required.

Self-focusing (SF) occurs when the intensity of the incident beam is greater in the centre and the material can act as a graded index lens, i.e. the refractive index increases towards the centre of the beam. This is also called a Kerr lens and its use is described below for Mira passive modelocking.

3.4.2 Group-velocity dispersion

An ultrashort pulse of light contains a distribution of wavelengths to either side of a central wavelength. Chromatic dispersion shows that refractive index depends on wavelength. A
normal dispersion curve plots $n$ vs $\lambda$. At a given wavelength $n(\lambda)$ determines the phase velocity. The change of refractive index with wavelength, $dn(\lambda)/d(\lambda)$, determines the velocity of the pulse with a central wavelength $\lambda$. The second derivative, the group velocity dispersion (GVD) determines which frequency components of the pulse travel faster in a given medium. This depends on the initial wave packet spectrum and the dispersive material used (Figure 3.3).

![Graphs showing refractive index vs wavelength and GVD vs wavelength](image)

Figure 3.3 (Left) refractive index vs wavelength for some common materials: (a) Fused silica, (b) Schott BK7, (c) Schott SF10 and (d) sapphire. (Right) the GVD vs wavelength for 1mm of material derived from the fits. Picture: Reid pg 4, adapted.

An imbalance in the GVD of a pulse will be manifested as "chirp", so named because it imitates the way a bird chirps, with the frequency packet changing over time. A pulse is said to be positively chirped if the instantaneous frequency increases from the leading edge to the trailing edge of the pulse, and is the type of chirp that occurs after passing through some "normal" material, i.e. from red to blue. Anomalous dispersion material will impose a negative chirp or GVD on the pulse. Without compensation for this effect, each successive trip through the various elements of the laser system would mean broadening and pulse substructure would occur.

Thus elements have to be included in the laser cavity that account for chirp and compensate accordingly, if a stable ultrashort pulse is required. GVD compensation is discussed in Section 3.5.2.
3.4.3 Self-phase Modulation
Self phase modulation (SPM) is the temporal analogue of SF. Intense light pulses propagating through a NLO medium can create a graded index lens. However the creation of the lens is intensity dependent, so the leading and trailing edge of the pulses will cause the least change while the centre of the pulse will cause the greatest. The different frequency components of the ultrashort pulse will be phase shifted differently depending on where they occur in the pulse. These frequency components are inherently positively chirped. The leading edge of the pulse is converted to IR wavelengths and the trailing edge is converted to UV. The result should give more UV because of the 10 fs time response of the non-linearity meaning the trailing edge will have time to see more SF and SPM.

![Figure 3.4 Typical white light continuum spectrum. Picture: OPA manual.](image)

3.4.4 White light generation
Spectral broadening of intense short pulses was observed early on in the development of lasers. This effect can be used to create an ultrashort white-light continuum pulse, given the
correct initial conditions. Figure 3.4 shows a spectrum of a typical white light continuum formed after an 800 nm ultrashort pulse is focused into a sapphire crystal.

The dominant mechanism in the formation of the continuum is Self-phase modulation, with possible additional mechanisms such as four-wave mixing and self-focusing. The beam collapses in on itself to form a single filament, where the intensity is high enough to permit extensive SPM giving rise to the spectral broadening to either side of the input wavelength.

3.4.5 Sum-frequency Generation

The idea of sum frequency generation is that a wave at frequency $\omega_1$ can be produced as a sum of two other waves with frequencies $\omega_1$ and $\omega_2$ interacting in a non-linear crystal. Second Harmonic generation is a limiting case of this where $\omega_1 = \omega_2 = \omega$, $\omega_3 = 2\omega$. The wave $\omega_1$ will beat with that of $\omega_2$ generating a polarization component at

$$\omega_1 + \omega_2 = \omega_3 \quad (3.20)$$

So we can write

$$h \omega_1 + h \omega_2 = h \omega_3 \quad (3.21)$$

This infers that two photons with frequencies $\omega_1$ and $\omega_2$ disappear, and another photon of frequency $\omega_3$ appears. Expecting photon momentum to be conserved, we write

$$h k_1 + h k_2 = h k_3 \quad (3.22)$$

Where $k$ denotes vectors. This expresses the phase-matching condition for sum frequency generation. The most common way of satisfying the phase matching criteria is by using a birefringent crystal and angle tuning, where the crystal is precisely oriented w.r.t. the incident light. See Boyd Chapter 2, Section 7 for a discussion of phase matching criteria.

3.4.6 Difference Frequency Generation and Optical Parametric Amplification

In the case of difference frequency generation a wave at frequency $\omega_3$ generates two waves, called the idler and signal waves, at frequencies $\omega_1$ and $\omega_2$, so that the total photon energy and momentum is conserved.

$$h k_3 = h k_1 + h k_2 \quad (3.23)$$

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The physical process involves a strong wave at frequency $\omega_3$, (the pump) and a weak wave with frequency $\omega_1$ present in the non-linear crystal. The wave at $\omega_3$ will beat with the wave at $\omega_1$. This will give rise to give a polarization component at frequency $\omega_2$. This generated wave at $\omega_2$ (at phase-matching conditions, where $n_3V_3 = n_2V_2 + n_1V_1$) will beat with the wave at $\omega_3$ to give a polarization component at $\omega_1$. This polarization causes the wave at $\omega_1$ to grow also. So power is transferred from the pump wave at $\omega_3$ to other wavelengths, $\omega_1$ and $\omega_2$. The wave at $\omega_1$ can be supplied by an external source (another laser). However parametric noise generated in the non-linear anisotropic crystal can act as the source of $\omega_1$. If mirrors that are highly reflecting at the frequencies of $\omega_1$ and/or $\omega_2$, then these frequencies can be amplified, leading to the term optical parametric amplification. For theory of this subject see Boyd Section 2.5.

3.5 Final Ultrashort pulse formation

3.5.1 Pulse Broadening
As the GVD is changed and the pulse formed becomes shorter in duration, it also becomes more intense, assuming the energy loss is minimal for passing through each medium and so the pulse energy is the same. Thus the effect of SPM will be more severe, which will in turn lead to more pulse broadening. Thus the formation of a stable ultrashort pulse in a particular cavity is a dynamic process, which will result in a stable pulse formation of a specific width and pulse amplitude, which will remain unchanged through one trip of the cavity. This stable pulse is self-modulating in that an increase in intensity of the pulse will lead to more broadening which will in turn reduce the intensity of the pulse.

3.5.2 Pulse compression
One such technique for GVD compensation is to use a prism pair. This technique uses the fact that the blue and red components of the cavity beam will have different pathlengths in the dispersive medium which can compensate for chirp. The prisms can be translated in and out of the beam to change the amount of GVD compensation. A single pass through a prism pair is shown in Figure 3.5. The process of GVD compensation is a dynamic one as changes in GVD
which shorten the pulse in turn will cause increased SPM, which creates more spectral broadening.

Figure 3.5 GVD change by a prism pair.

3.5.3 Chirped Pulse Amplification (CPA)
Ultrashort pulse trains of the order of nanojoules per pulse often need to be amplified for use in applications such as spectroscopy. However their amplification could cause problems because of the pulse energies involved. A 180 fs pulse focused down to just 1 mm² spot size with a pulse power of 1mJ has a peak fluence of $5 \times 10^{11}$ W cm², this is around the damage threshold for most optical materials. To avoid damage the pulse is first stretched in time, and then amplified before finally being recompressed. The pulse broadening can be induced by multiple passes through a dispersive element which induces chirp in the pulse. Amplification occurs in the same cavity as broadening, where for each pass through the dispersive element the pulse also passes through a gain medium. After a certain number of passes through the optical elements the pulse is normally Q-switched out of the cavity by some means, when it can be recompressed by compensating for the induced chirp.
3.6 Thermodynamics of Chemical Reactions

For a simple bimolecular charge transfer reaction where species A reacts with species B, we can express the reaction as:

\[ [A] + [B] \rightarrow [A^+] + [B^-] \] (3.24)

where \([X]\) denotes the concentration of a species \(X\). We can now define a rate equation for the reaction at equilibrium, in terms of an equilibrium constant

\[ K_{eq} = \frac{[A^+] [B^-]}{[A] [B]} \] (3.25)

We can use this equilibrium constant to predict how likely a reaction is to proceed using:

\[ \Delta G^\circ = -2.303 RT \log K_{eq} \] (3.26)

where \(\Delta G^\circ\) is the standard free energy change (Gibbs free energy change) for the reaction, \(R\) is the gas constant (1.987cal K\(^{-1}\) mol\(^{-1}\)), \(T\) is the absolute temperature in Kelvin. A negative value for \(\Delta G^\circ\) is associated with the formation of products in a reaction at equilibrium, and for which \(K_{eq} > 1\).

Apart from changes in the concentrations of reactants and products it is necessary to describe some reactions in thermodynamic quantities. A thermodynamic system is one which can interact with or exchange energy with its surroundings. A chemical example of this is a
molecule being dissociated in water where the molecule is the system and water is the surroundings. Another way of describing $\Delta G^\circ$ for a reaction is in terms of its two thermodynamic components, $\Delta H^\circ$ and $\Delta S^\circ$, where $\Delta H^\circ$ is the enthalpy change in a reaction and $\Delta S^\circ$ is the associated change in entropy.

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \hspace{1cm} (3.27)$$

$\Delta H^\circ$ is associated with changes in bonding during a reaction, a positive value of $\Delta H^\circ$ means an endothermic reaction is occurring which requires energy to proceed, whereas a negative value for $\Delta H^\circ$ (exothermic reaction) will make $\Delta G^\circ$ more negative and thus more likely.

$\Delta S^\circ$ is associated with a change in entropy, where a positive change in $\Delta S^\circ$ denotes goes from a more ordered to a less ordered total system. In any thermodynamic process when all the systems taking part in a process are counted, the entropy stays constant or increases. This is another way of stating the second law of thermodynamics i.e. that the entropy of the universe is increasing to a maximum. As the entropy term in Equation 3.27 has a minus sign in front of it we can see that an increase in the disorder of a system makes the reaction more likely to go to completion. In the simple bimolecular system where the number of products equals the number of reactants the change in entropy is normally small and, unless $T$ is very large, the enthalpy will normally be the deciding factor of the $\Delta G^\circ$ equation. Where $\Delta G^\circ$ has a negative value the reaction is said to be exergonic, when positive it is endergonic. However this relates to the final outcome of the reaction.

When treating intermediate states another term, $\Delta G$ (different from $\Delta G^\circ$ above), the free energy of activation for the intermediate state is important. The difference in free energy between the products and reactants is termed the free energy of activation, given by

$$\Delta G = \Delta H - T\Delta S \hspace{1cm} (3.28)$$

Where $\Delta H$, the enthalpy of activation is involved in bond breaking and bringing the reactants close together. $\Delta S$, the entropy of activation is a function of the amount of orientations possible in the transitions state. $\Delta S$ will be more negative (and $\Delta G$ more positive) if the transition state must be more ordered than the reactants. $\Delta G$ is related to the rate at which the reaction proceeds given by

$$k = k_0e^{-\Delta G/RT} \hspace{1cm} (3.29)$$
Where $k$ is the rate constant for the reaction, $k_0$ is the rate constant at which all transition states proceed to products, $T$ is the temperature in Kelvin and $R$ is the gas constant. The theory in this section can be found in more detail in most undergraduate chemistry texts such as Atkins’ Physical Chemistry.

3.7 Organic Photochemistry

3.7.1 Introduction
Organic chemistry is simply the chemistry of compounds of carbon. Classical chemistry involves reactions of molecules in their electronic ground states. Photochemistry deals with reactions that occur from electronically excited molecules. In this way reactions that were thermodynamically unfavorable in their ground states can now occur.

3.7.2 Excited states
Electronic excitations that produce photochemical reactions usually result from absorption in the UV or visible part of the EM spectrum. When a molecule absorbs the electromagnetic radiation, its energy increases by an amount equal to the energy of the absorbed photon ($E$).

\[ E = h\nu = \frac{hc}{\lambda} \quad (3.30) \]

$h =$ Planck constant
\( \nu \) = frequency of radiation
\( \lambda \) = wavelength of radiation
\( c \) = velocity of light

The Born-Oppenheimer approximation allows us to treat the energy absorbed $E_{TOT}$ as the sum of a number of molecular modes viz. the electronic, vibrational, rotational and translational energy.

\[ E_{TOT} = E_{ELEC} + E_{VIB} + E_{ROT} + E_{TRANS} \quad (3.31) \]

As translational energy is not quantised it is not normally included in theoretical treatments of the energy levels. Because the nuclei are so much more massive than the electrons, during an electronic transition the nuclei of interacting molecules are taken to be stationary.

Figure 3.7 shows a potential energy diagram (Morse curve) where the potential energy varies as the interatomic distance is varied in a diatomic system for a given electronic state. The
vibrational levels are represented as horizontal lines within the energy curve. The solution to the vibrational energy levels derive from the simple harmonic oscillator, (around $r_{EQ}$ the curve traces a parabola). For this reason the lowest vibrational level $v_0$ does not correspond to the minimum of the curve, having the $\hbar v/2$ zero-point energy of the harmonic oscillator. The separation of the rotational levels are another hundred times smaller than the vibrational levels, for this reason they are not normally shown, suffice to know that each vibrational level has an associated set of rotational levels. In 3.7 $r_{EQ}$ is the equilibrium distance, the interatomic distance that the molecule maintains in the absence of any other perturbations. This figure also shows the continuum of states available after the molecule is dissociated, i.e. when $r$ reaches infinity. When the molecule is provided with enough energy to dissociate using light, i.e. the continuum of states is reached along the vertical axis, the process is known as photolysis.

![Figure 3.7 Variation of potential energy with distance in diatomic molecule. Picture: Kopecky pg 18.](image)

The initial excitation caused by photon absorption is $1/\text{freq.}$ and takes about 1 fs for 400 nm light. This forms the Franck-Condon state. According to the Franck-Condon principle the most intense vibronic excitation is from the ground state to the vibrational state lying directly above it, this occurs when a photon initiates an electron jump where the relative positions of the nuclei remain the same before and after the jump. These transitions are often visually represented on potential energy surface diagrams, where the Schrödinger equation has been solved for the electron at varying distances from the nuclei and having different electronic energies. It approximates to an electron moving in a potential field of the nuclei. Figure 3.8
shows a potential energy surface (PES) for a diatomic molecule and possible relaxation pathways after electronic excitation.

Figure 3.8 Ground and excited state potential energy surfaces and some radiative transitions. Picture: Kopecky pg 5

Excited singlet states ($S_1$, $S_2$, $S_3$, etc) are formed after absorption of the photon. The spins of the $\pi$ electrons are paired in singlet states. The photochemical reactions occur mainly from the lowest excited singlet states, due to the very fast rate of internal conversion from the higher to the lower excited singlet states. The radiative transition $S_1$-$S_0$ is called fluorescence.

The lowest triplet state ($T_1$) is normally formed from non-radiative inter-system crossing from $S_1$. The spins of the $\pi$ electrons are unpaired in the triplet states. The radiative transition ($T_1$ – $S_0$) is called phosphorescence. Once excited, the excitation energy of a molecule may be dissipated by radiative transitions such as those mentioned above, radiationless transitions, bimolecular deactivation and dissociation.

Additional vibrational levels in the region where the vertical line touches the excited state potential are excited (Fig. 3.8). The conventional model of what happens next is normally intramolecular vibrational relaxation (IVR), followed by internal conversion (IC) to the lowest singlet excited state. After this there can be radiative and non-radiative processes or intersystem crossing (ISC) to a triplet state and from there relaxation can occur.
Ultrafast reactions are different in that they are not in thermally equilibrated states. In ultrafast reactions, however, more than one relaxation can seem to happen at the same time. Sometimes no discernible intermediates are present, or vibrationally excited (hot) states can be formed with relaxation occurring afterwards.

A non-adiabatic process in thermodynamic terms is when heat flows in or out of the system (to the surroundings) during the process. A non-adiabatic reaction in electronic terms is one in which an electronic transition involves more than one potential energy surface. These non-adiabatic reactions may be thought of in terms of two main species

Radiative transitions: fluorescence, phosphorescence
Non-radiative transitions: IC, ISC and most-photoreactions

3.7.3 Molecular Orbital (MO) Theory
Molecular Orbital (MO) Theory assumes that each electron moves in the field of the nuclei but also of the other electrons present. MOs were proposed to describe the wave functions of electrons, taking into account the Pauli Exclusion Principle which states that no two electrons can have identical spin and orbital functions. MOs for the valence electrons are shown in Figure 3.9, classified further according to nodal planes where the wavefunctions vanish. \( \pi \) MOs exist only in planar segments of molecules. HOMO (Highest Occupied), LUMO (Lowest Unoccupied) and SOMO (Singly Occupied) are used to describe electrons in these orbitals.
The conventions most commonly used to describe electronically excited states in organic photochemistry are the enumerative and Kasha (MO) systems.

An electronic configuration with antiparallel spins is a Singlet state. Triplet states contain parallel spins, i.e., they involve a spin flip.

**Enumerative**

\[ S_0, S_1, ..., S_n \quad S_0 \rightarrow S_1, S_2 \rightarrow S_0, S_1 \rightarrow S_0 \]

\[ T_1, T_2, ..., T_n \quad S_1 \rightarrow T_1, T_2 \rightarrow T_1 \]

**Kasha**

\[ ^1(n, \pi^*), \quad ^3(n, \pi^*) \quad n \rightarrow \pi^*, \pi \rightarrow \pi^* \]

\[ ^1(n, \sigma^*), \quad ^1(\sigma, \pi^*) \quad n \rightarrow \sigma^* \]

### 3.7.4 Solvent interactions

Energies of electronic transitions in solution are specifically affected by solvent, see Kopecky Section 5.7. By interaction with lone electron pairs, polar and protic solvents from either van der Waals complexes or, more often, hydrogen bonds of various stabilities. These complex formations, along with the effect of dipole-dipole or dipole-induced dipole interactions, cause a stabilization of a polar molecule in both ground and excited states and can change the
ordering of the excited states. As a consequence, the energy of the $n \rightarrow \pi^*$ transition is usually raised (giving rise to a spectral blue-shift) and the energy of the $\pi \rightarrow \pi^*$ transition is lowered which leads to a red-shift in the spectrum of the excited state. The solvent effect is sometimes used to assign the type of electronic transition.

3.7.5 Lifetimes and Deactivation
There are decay times $\tau$ associated with these excited states, the time taken for deactivation of the molecule. The time it takes for the population of the excited state to decay to $1/e$ of its initial value is

$$\tau = \frac{1}{k_d} \quad (3.32)$$

$k_d$ = rate constant for decay

$k_d$ may comprise of only one type of decay process such as fluorescence, but more likely will be a sum of constants for different processes. Radiationless processes lower the quantum yield $Q$ which is the fraction of photons absorbed that lead to a radiative process. The decrease in the quantum yield of fluorescence ($Q_f$) is called fluorescence quenching. This can be caused by a variety of mechanisms. Most are caused when the energy transfers to a non-fluorescent species such as in collisional quenching, or when a non-emissive complex is formed due to a high concentration of the second molecule. High concentrations of the molecule itself may lead to non-emissive excited dimers (excimers) forming. In these latter cases $Q_f$ should exhibit a concentration-dependent dependence, described by the Stern-Volmer equation:

$$\frac{Q_f^0}{Q_f} = \frac{k_l + k_d + k_q(Q)}{k_l + k_d} \quad (3.33)$$

$Q_f^0$ = quantum yield in absence of quenchers

$k_l =$ luminescent rate constant

$k_d =$ deactivation rate constant (heat etc)

$k_q =$ quenching constant

Another way for fluorescence quenching which will be discussed in the following sections is by electron transfer.
3.8 Energy transfer

This is the process where an excited donor ($D^*$) can transfer its excitation to an acceptor (A) molecule

$$D^* + A \rightarrow D + A^* \quad (3.34)$$

Two types of transfer will be discussed here: Förster and Dexter Transfer

3.8.1 Förster/Resonance Excitation Transfer

Förster transfer occurs when the dipoles of $D^*$ and A interact and energy transfer occurs by Coloumbic interaction. The Förster radius $R_O$ is the key parameter controlling the rate of energy transfer. At this radius the rate of energy transfer equals the sum of the radiative and non-radiative deactivation of D. It can be calculated from the overlap of the emission spectrum of D and the absorption spectrum of A using

$$R_O = \frac{9000 \ln 10 f^2}{128 \pi^5 N_l N_s^4} Q_{r} J \quad (3.35)$$

$f^2$ = an orientation factor (0.6 for random orientation)

$N_l$ = Avagadro’s number

$n_s$ = the refractive index of D

$J$ = the spectral overlap function of the emission of D and absorption spectrum of A.

The spectral overlap function is given by

$$J = \int f(\lambda) e_A(\lambda) A^d d\lambda \quad (3.36)$$

Where

$$f(\lambda) = \frac{F_D(\lambda)d\lambda}{\int F_D(\lambda)d\lambda} \quad (3.37)$$

And $F_D$ is the fluorescence emission intensity of the donor at wavelength $\lambda$ in cm. This type of excitation transfer can occur over a distance of 50-100Å. No electron transfer occurs in this first case. Only singlet-singlet transitions are involved.
3.8.2 Dexter/ Electron transfer

Electron transfer or Dexter transfer can also be described using a donor acceptor model, where D donates an electron to A. Excitation transfer occurs as a result of an electron exchange mechanism. It requires an overlap of the wavefunctions of the energy donor and the energy acceptor and is the dominant mechanism in triplet-triplet energy transfer. For this mechanism the spin conservation rules are obeyed. With Dexter transfer both singlet-singlet or triplet-triplet transitions are involved.

This process is dependent on the Redox potentials of the two molecules and their intermolecular distance, ignoring for the moment the effects of the medium. Scheme 3.1 illustrates the difference between the Dexter and Förster transfer, showing the transfer of an electron and subsequent back electron transfer in the electron exchange case. This latter electron exchange case will be examined in this thesis.

Scheme 3.1 Förster (Coulombic Interaction) and Dexter (Electron Exchange) schematic. Picture from weblecture(hackman.mit.edu).
ET can occur in the ground or excited state of one or both molecules. In photoinduced ET, the extra excitation energy can make $\Delta G^\circ$ favourable for the reaction. A substance with a positive reduction potential likes to be reduced - it is a good oxidising agent. An electron transfer process will be more likely to occur if the A is closely bound to D and if A has a more positive reduction potential than D.

The likelihood of an electron transfer occurring is measured by the positive magnitude of its standard redox potential in volts. This must be positive for an electron transfer to spontaneously occur. $E_{\text{react}}$ is the redox potential for an electron transfer reaction.

$$E_{\text{react}} = E_{\text{red}} + E_{\text{ox}} \quad (3.38)$$

Where $E_{\text{red}}$ is the standard reduction potential for the reaction and $E_{\text{ox}}$ is the standard oxidational potential in Volts. A favorable value for a species to be reduced is $E_{\text{red}}$ being large and positive, a favourable value for the oxidation of a species is $E_{\text{ox}}$ being small and negative.

In relation to the free energy for the reaction, $\Delta G$

$$\Delta G = -nF E_{\text{react}} \quad (3.39)$$

Where n is the number of moles in the reaction, F is the Faraday constant. Here we can see that a negative value for $E_{\text{react}}$ will yield an endergonic reaction pathway, whereas a large positive value for $E_{\text{react}}$ predicts a strongly exergonic reaction, when the concentration is kept constant.

$$\Delta G_f = nF(E_{\text{Nu}^+/\text{Nu}}^0 - E_{D/D^+}^0) - \Delta E_{0,0}$$

$$\Delta G_r = -nF(E_{\text{Nu}^+/\text{Nu}}^0 - E_{D/D^+}^0) \quad (3.40)$$

Equation 3.40 is used for calculating Gibbs energy for the forward ($\Delta G_f$) and reverse ($\Delta G_r$) photoinduced electron transfer reactions. $E^0(\text{Nu}^+/\text{Nu})$ is the standard reduction potential of the donor and $E^0(D/D^+)$ the standard reduction potential of the dye. $\Delta E_{0,0}$ is the electronic excitation energy of the excited partner that drives the forward reaction, this is absent for the back electron transfer. Scheme 3.2 illustrates the point that excited states are both more easily oxidize and reduced.
3.8.3 Marcus Theory

The Marcus equation relates the rate constants for non-adiabatic ET to the Gibbs free energy ($\Delta G^o$).\(^\text{11}\)

\[
K_{ET} = \frac{4\pi |V(r)|^2}{h} \frac{1}{\sqrt{4\pi\lambda_kT}} \exp\left(-\frac{(\Delta G^o + \lambda)^2}{4\lambda_kT}\right) \quad (3.41)
\]

$\lambda$ = reorganisation energy  
$|V|$ = electronic coupling matrix element

A rate constant would normally be expected to increase as $\Delta G^o$ becomes more negative, however a key result of Marcus theory is that $k_{ET}$ increases to a maximum as a function of $\Delta G^o$, after which it decreases, exhibiting a parabolic dependence on $\Delta G^o$. Reactions occurring near the maximum of this curve are in the ultrafast region.

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Scheme 3.2 Excited states are both more easily oxidized and reduced. Picture from weblecture(hackman.mit.edu).

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The region where $K_{ET}$ decreases with increasing $\Delta G^\circ$ is called the "inverted region". Mechanisms of ET have been proposed to act over short (10-15 Å) distances by superexchange (exponential distance dependence) or over longer distances by electron hopping or sometimes as a combination both. For example Giese et al\textsuperscript{12} have demonstrated ET through DNA can be mediated by electron hopping from G to GGG base sequences and by superexchange (tunnelling) through successive AT base pairs. This was shown to mediate ET at \textasciitilde70\% efficiency over a distance of 70 Å. After ET occurs the ET products can separate or back electron transfer can occur depending on the rate constants for the reactions.

Photoinduced processes are extremely useful for generating ET as excitation in the UV/visible gives energies ranging from 4.95 eV (250 nm light) to 1.65 eV (750 nm light). Given that excited state energies are upwards of around 1.5 eV we can see that excitation using UV/visible could easily promote the occurrence of ET.

If we consider that the D and A are not in isolation but are normally surrounded by solvent or indeed connected via some bridging element, i.e. an optically inert matrix such as PMMA or a sequence of base pairs in the DNA polynucleotide, how does this effect the ET? It has been proposed that there are some inelastic tunneling processes that occur with this bridge and it makes sense that energy can be lost to solvent or matrix when averaging over many such processes, which is what generally is being investigated in spectroscopy (apart from single

Figure 3.10 Parabolic dependence of $k_{ET}$ on $\Delta G^\circ$ values. Picture: website of University of Illinois, Biophysics 354, Lecture 19.
molecule optical studies). There are also ways in which the solvent used can contribute to the ET process.

One process which has been studied in this regard is proton-coupled electron transfer. This effect is detected using different isotopic mixtures of H₂O and D₂O in aqueous reactions, and noting that the rate of ET is faster in the presence of H₂O. The ratio of k_{ET} in H₂O/D₂O can vary widely depending on the reaction.

3.9 Time-resolved Measurements

3.9.1 Visible Transient Absorption

To record a transient absorption spectrum we first pump the sample with the appropriate wavelength light and measure its absorption spectrum at a later time to probe the evolution of the excited states formed. When these wavelengths are in the visible or UV region we are probing electronic states coupled to vibrational states of the material (vibronic levels).

3.9.2 IR Transient Absorption

Early ultrafast transient absorption IR measurements were typically performed using identical frequency picosecond IR pulses for both pumping and probing a vibrational mode of condensed phase molecules. In this case an intense, narrow bandwidth tunable pump pulse excites the population from \( V = 0 \) to the \( V = 1 \) state of a particular vibrational level, in a manner analogous to formation of an excited singlet state. A much weaker probe pulse then interrogates the recovery of the ground state vibrational absorption.

As technology progressed “two-colour” pump and probe experiments became available, usually as a result of two OPA’s being used to separately tune both pump and probe pulse. This meant that other parts of the spectrum could be monitored while the bleach was recovering and new species could be detected. However, using narrowband pulses to take a frequency-scanned spectrum with single element detectors at each time delay requires huge amounts of data acquisition time. Also problems with laser stability or depending on the exciting wavelength sample degradation could arise. The experimental section suggests how these problems are overcome.
3.9.3 Interpretation of Transient Absorption spectra

Ground state species have a characteristic absorption spectrum. The excitation source for a time-resolved experiment is chosen to be absorbed within this region, as close as possible to $\lambda_{\text{max}}$, ignoring other experimental considerations for the moment. In the case of a transient absorption spectrum there are three different regions that we can examine to elucidate information about the system under study.

The bleach region.

After absorption of an exciting pulse, a “bleach” can be created in the material. This occurs when the electrons are promoted to upper vibronic levels leaving the ground state levels of many molecules unpopulated. If this happens in enough molecules, there will be no ground state absorbing species left by the time the interrogating pulse comes, shortly afterwards. The time taken for the ground state levels to repopulate is related to the spontaneous emission lifetime for the molecule. The material becomes transparent to the interrogating pulse at certain wavelengths, hence the term “bleaching”. In most molecules the time take for the bleach to recover is the time taken for spontaneous $S_1 \rightarrow S_0$ transition. (There are exceptions to this rule, some molecules spontaneously emit from $S_2 \rightarrow S_0$ for example). The $\Delta$ abs. spectrum will be negative in this region as the $\text{abs}_{\text{pumped}} < \text{abs}_{\text{unpumped}}$. This will appear like an inverted absorption spectrum in the lower wavelength region, when using a broadband probe and monitoring multiple wavelengths at once.

The absorption region.

This is where the features resulting from excited state absorption and subsequent processes appear. Changes in absorption related to charge transfer/energy transfer are monitored in this region. The TA in the UV-vis region will produce quite broad and featureless bands, as the electronic bands are smeared by superposition with vibrational (and rotational) bands. Monitoring transient signals in the infra-red part of the spectrum can yield a much clearer picture of reactions as the vibrations themselves can be monitored. In certain “finger print” regions vibrational levels associated with molecules appear as very clear peaks in the spectrum. The magnitude of these bands will of course be much smaller than the vibronic bands seen in the visible spectrum. There are limitations associated with the IR regions such as the strong absorption of water in some regions of biological interest and also the need for ligands with strong IR absorptions in order to give a large enough signal for transient
measurements. However transient IR and UV-vis can be used as complementary techniques in some cases, and by comparing values for kinetics found in each technique one can postulate a reaction pathway.

The gain region.

Excited states are normally formed vibrationally excited. The difference in energy between the vibrationally excited (hot) and the vibrationally relaxed or cooled state can translate to be over 100 nm. The gain region results from stimulated emission from the cooled vibronic state, caused by the incident light. It will appear as a photobleach type region in the spectrum around the wavelength corresponding to the cooled state. The signal is negative as more light leaves the system than is absorbed.

If we are looking for evidence of electron transfer we will normally look in the absorption region of the spectrum. In a general case the absorption spectrum should be known for the acceptor (A) and the reduced, excited form of the acceptor (A*). Then over the time-scale of the experiment if electron transfer occurs from donor to acceptor we expect to see a spectral contribution from A decrease, as it is reduced and the spectral contribution from A* to appear as the electron transfer proceeds. In the systems studied in this thesis the DNA is the electron donor. How can we know that Förster transfer is not occurring and thus will make our system more difficult to elucidate? Förster transfer requires that the fluorescence of the Donor overlaps with the absorption of the Acceptor. We will excite the system at a wavelength where only the acceptor will be excited; i.e. the DNA will not fluoresce. Thus we can exclude Förster transfer from our calculations if we choose appropriate experimental conditions.


8 T. Forster Discussions Of the Faraday Society 27 7-17 (1959)


4. EXPERIMENTAL METHODS

4.1 Ultrafast laser system

4.1.1 Overview
Three different ultrafast systems were used, all slightly different in specifications. However all operated with the same basic components, i.e. an oscillator to form short modelocked pulses, a Regenerative Amplifier for pulse amplification and an Optical Parametric Amplifier for tunable probe wavelength operation (if required). The components of the system in Trinity College Dublin (TCD) are described in detail below, to explain the workings of an ultrafast system. The system in TCD comprises an Ar⁺ ion pumped, mode-locked Titanium Sapphire laser system yielding sub-200 fs pulses at 800 nm, with outputs of UV and white light or a choice of tunable visible outputs at 2-3 μJ per pulse from an optical parametric amplifier. The autocorrelation of the output light from the regenerative amplifier before the OPA is about 200 fs at 250 kHz rep rate.

4.1.2 Pump Laser (Ar⁺ ion)
The main pump laser was a Sabre R series Innova Ar⁺ ion laser from Coherent with maximum output of 24 W operating in CW mode. The normal output is 22 W. The gain medium was Argon gas, ionized to produce population inversion. The plasma tube operated in multi-line mode with outputs UV, 488 nm and 514 nm. The normal operation of the laser uses a tube current of 50 A, to create the electrical discharge for operation. This tube was cooled by means of surrounding heat-exchanging coils on a closed loop, which in turn are cooled a second heat exchange system fed by cold water from the mains. In this way the temperature was maintained at a constant 30 °C. The pump laser was very sensitive to changes in ambient/water temperature, and also to the build up of ozone around the Brewster window produced by the UV radiation. The sensitivity of this type of pump laser to external factors such as temperature change is one of the reasons why diode lasers are more popular as the stability of the pump laser is paramount to effective system operation.
4.1.3 Oscillator (Mira)

The Mira Model 900-B laser is a mode-locked ultrafast laser that uses Titanium doped Sapphire as its gain medium and is tunable from 740 to 1070 nm (the gain bandwidth of Ti:Sapph). The Mira cavity is shown in Figure 4.1.

![Cavity Diagram](image)

Figure 4.1 The Mira cavity, M: Mirror, BP: Brewster Prism, L: Lens, BRF: Birefringent Filter. M8 and M9 provide an auxiliary cavity to aid alignment. Picture: Mira users' manual pg 2-4.

The system used Kerr-lens passive mode-locking to produce pulses 180 fs in length at the peak of the gain bandwidth of Ti-Sapph (800 nm). The pump used was the Ar⁺ ion laser output split to give 8 W for the oscillator. The passive Kerr lens system used for stable mode-locking operation works as follows: The butterfly starting mechanism oscillated in the beam, rapidly changing the cavity length at high frequency. This introduced very high power fluctuations in the beam and the laser began pulsed operation. The high intensity of the pulses induced NLO effects as the beam passed through the Ti:Sapph, causing self-focusing of the beam. A variable diameter slit in front of the output coupler was closed down to introduce loss for the larger CW component of the laser beam and to allow passage for the smaller diameter mode-locked beam (Figure 4.2). In this way the CW component was lost from the cavity and stable passive mode-locking was achieved with pulse durations of approx 180 fs at 800 nm (as measured by a Timewarp autocorrelator, Edinburgh Instruments). The mode-locking process was monitored with an oscilloscope connected to the Mira photodiode output, where any CW component was seen as low-level noise superimposed on the mode-locked pulses.
An SF-10 prism pair was used in the cavity to compensate for GVD effects arising from dispersive intracavity elements. By translating one of the prisms in or out of the beam and adjusting the pump mirrors to compensate for increased SPM a stable ultrashort pulse was achieved. The wavelength output of the Mira was tunable by rotating an intracavity birefringent filter (BRF), to select an output wavelength. The prisms were subsequently adjusted to compensate for any changes in pulse width. These two steps were iterated to produce a stable pulse at the required wavelength. Although the Mira output was tunable from 740-1070 nm, for optimum RegA operation the peak of the gain bandwidth (800 nm) was used. The average CW power at 800 nm from the Mira was ~ 1.8 W.

### 4.1.4 RegA

The RegA is a regenerative amplifier system that increases the pulse energy from the modelocked source. The regenerative amplifier used was a RegA Model 9000 from Coherent, pumped with 14 W CW from the Ar⁺ laser. The power from the Ar⁺ laser was used to pump a second Ti:Sapph crystal as the gain medium in the RegA for amplification. The Mira pulses were steered into the RegA by way of external mirrors and amplified at a 250 kHz rep rate as follows:
The incoming Mira beam passed through a cube polariser and Faraday Isolator (FI) and a single pulse was injected into the REGA by a SiO$_2$ acousto-optic Cavity Dumper (CD), operating at 380 MHz RF excitation. Q-switching took place in an intracavity TeO$_2$ crystal, also acousto-optically modulated to allow for shutter action. The 20 to 30 round trips needed for amplification in the Ti:Sapphire crystal stretched the pulse to around 30 ps, using chirped pulse amplification. The cavity dumper extracted the Q-switched pulse, which contained a few μJ of energy per pulse, and this went back through the FI, and entered the compressor optics. The amplified pulses were compressed by a 4-pass grating assembly, using a holographic gold-coated diffraction grating (2000 lines/mm). The output from the RegA was 800 nm, 200 fs pulses with pulse energies of < 2μJ at 200 kHz rep. rate.
4.1.5 Electronics (Timing)

The timing for the Mira and RegA systems was controlled externally by a RegA 900 Controller Box. This is a CPU controlled electronics unit that provides the necessary RF pulses to drive the acousto-optic modulators in the RegA. The timings related back to the length of the cavity of the Mira and RegA and hence the rep rate 76 MHz for the Mira and varying from 9 kHz to 250 kHz for the RegA.

Figure 4.4 Timing scheme for the RegA. Picture: RegA users’ manual pg 4-13.
Line 1 shows the 76 MHz signal from the Mira photodiode is used to generate the 38 MHz master clock for the RegA RF pulse timings.

Line 2 shows that rep rate synch out pulse that marks the start of each timing cycle. In the RegA the 38 MHz clock is divided to give the rep rate ranging from 9 kHz to 250 kHz.

Line 3 shows the Q-switch action, where the RegA is prevented from lasing until the pulse has been suitably amplified. When the Q-switch is on the beam is diffracted out of the cavity. The Q-switch is supplied with continuous 80 MHz RF excitation, until a set delay has been reached when it is switched off. It stays off for the duration of the Q-switch width (variable in increments of 26 ns).

Line 4 shows the timings of the cavity dumper RF pulses. The injection of the Mira pulse into the RegA cavity is timed to be just after the Q-switch turns off to allow amplification of the pulse. Injection and ejection electronics allow the modulation of the injected and ejected pulses delay and phase.

Line 5 shows the measurement of the amplifying pulse inside the RegA cavity after each round trip. The intracavity intensity is seen to rise and saturate before the ejection pulse extraction removes the amplified pulse. For efficient extraction, which allows for maximum amplification with minimum pulse stretching, the pulse should be extracted just as the injected pulse reaches its maximum value. The actual amount of round trips required for amplification is 20-30.

Line 6 shows the output intensity of the RegA pulse measured with a fast detector and oscilloscope. Measuring the pulse length requires an autocorrelation method.

4.1.6 OPA
This system was used to convert the ultrashort pulses from the REGA to different wavelengths. Figure 4.5 shows the optical layout of a commercial system. The output of the RegA entered the OPA 9400 (Coherent) and was split in the ratio 75/25 by a beam-splitter. 75% was focused (200 mm focal length) into a 1 mm BBO type 1 crystal to produce 400 nm (SHG). The other 25% produced a white light continuum in the range 460 to 1200 nm by focusing the 800 nm beam with a 35 mm lens into a 3 mm Sapphire crystal. The white light output was focused with a 20 mm achromat and exited the output aperture for white light/UV operation.
Alternatively the UV and white light beams were focused into the OPA crystal (BBO) for wavelength conversion. In this configuration the beams made 2 passes through the OPA crystal with an adjustable optical delay on each leg for path length matching to create the tunable visible/IR output which then exited the OPA via the signal and idler remnant beam output mirrors. The angle of the SHG and OPA crystals was tuned externally for phase matching.

4.2 Transient Absorption Set-up

4.2.1 Overview

Three separate pump and probe set-ups are described in the following sections, the initial set-up in Trinity College Dublin, the Ultrafast Facility at the Chemistry dept. of Leeds University and both the visible and IR pump and probe at the Rutherford Appleton Laboratory (Lasers for Science: Chemistry) facility.
4.2.2 Trinity College Dublin (TCD)

In the laboratory in TCD the mode of operation used was 400 nm pump and a white light probe. The system operation is explained in Section 4.1. The pump and probe set-up is in Figure 4.6. The pump beam is the 400 nm SHG output of the OPA. The white light continuum exited the OPA parallel to the pump beam. Al or Ag front-coated mirrors were used throughout the experiment instead of back-coated mirrors to minimise pulse stretching due to passing through glass. The pump beam was sent on a delay line comprising a 35 mm diameter Al coated retroreflector fixed atop a 50 mm side driven macro-mech delay line (Coherent) with a 37-1112 encoder driver from LOT Oriel. The delay line allowed 110 mm path length for the delay line, providing approx. 360 ps time delay in total. The pump and probe beams were spatially overlapped in the sample cuvette, using rhodamine 6G solution to visualise the beams clearly during initial alignment.

The pump and probe beams were overlapped at the sample, keeping the angle between the beams as small as possible, i.e. $\leq 5^\circ$. Lateral beam “walk-off” was then checked for, again using rhodamine 6G, and by moving the delay line to opposite ends of its thread to look for any relative movement.
As the concentration of excited states decreases exponentially on traversing the sample as optical path-length in the sample increases, a small pathlength cuvette (1 mm) was used to maximise the excited states that the probe encounters.

After the pump beam left the sample it entered a photodiode where it was used to monitor the laser stability by taking 100 consecutive measurements and verifying that the standard deviation of $V_{\text{max}}$ (max. voltage) did not exceed 10%.

The spectrum analyser used was an Oriel MS257 containing 2 zero-order diffraction gratings (blaze $\lambda = 500$ nm), with 1800 and 300 lines/mm. An Andor CCD (EEVCCD 40-11) was used with the spectrum analyser, having $1024 \times 128$ pixels each sized 26 $\mu$m$^2$, capable of being cooled to 243 K. A standard mercury lamp was used for calibration of pixel to wavelength on the CCD array.

The software used (LABVIEW 5.0) is a graphical interface that can be programmed to drive and externally control both data acquisition and motion control, via a GPIB controller. LABVIEW was used to drive the mechanical delay line that gives the time resolution required for the experiment. Details of the LABVIEW program used to control the experiment are given in Appendix A, Grace Jordan of TCD provided the programming.

In order to time the measurements of pump on and pump off a shutter was needed in the pump beam before the sample. After passing through the sample the probe beam entered the spectrum analyser. The CCD took a measurement and LABVIEW used the shutter synch out signal to indicate the state of the input (pump on or pump off). A one bladed chopper operating at 1 Hz was tried for this purpose using the synch out signal, but the triggering was unstable and led to beating between “pump on” and “pump off” signals. Both LABVIEW and the CCD have a certain amount of inherent “dead-time” with regard to measuring before they follow the instructions sent via GPIB. This limits how fast we could switch from measuring one signal to another, so using a shutter that has an open or closed configuration would be preferable for this kind of timing, since it needs no fast electronics. LABVIEW saved the pump on/off data in a 2D array as a function of time delay in spreadsheet format. The data files were then subtracted as mentioned before for a transient absorption signal. The data was further corrected for intensity as a function of wavelength to finally yield the $\Delta \text{Abs.}$ signal.
In general the change in transmission of the signal $\Delta T$ is given by:

$$\Delta T = \frac{I_{\text{probe}}}{I_{\text{ref \_ pump on}}} - \frac{I_{\text{probe}}}{I_{\text{ref \_ pump off}}} \quad (3.1)$$

This leads to a rolling average signal, accumulated over $N$ measurements as:

$$\Delta T = \frac{\left[ I_{\text{probe}} / I_{\text{ref \_ pump on}} - \left( I_{\text{probe}} / I_{\text{ref \_ pump off}} \right) \right]_N + \Delta T_{N-1} (N - 1)}{N} \quad (3.2)$$

The average change in absorbance, $\Delta A_N$ is then given by:

$$\Delta A_N = -\log \left( 1 + \frac{I_R}{I_P} \Delta T_N \right) \quad (3.3)$$

Where $I_R$ and $I_P$ are the final averages of the pump off spectra on the reference and probe side respectively.

### 4.2.3 Leeds University

This laser set-up was designed and aligned by Dr. G. D. Reid of the University of Leeds. The laser set-up is an entirely home-built system (Figure 4.7).

![Diagram](image_url)  

*Figure 4.7 The pump and probe set-up in University of Leeds.*
It comprises a Kerr-lens mode-locked Ti:Sapphire oscillator which produces 13 fs pulses at 800 nm, pumped by a multi-line 6.5 W CW Ar$^+$ ion Coherent Innova 310 laser. These pulses pass through a regenerative amplifier pumped by an intra-cavity doubled, 2.9 kHz Nd:YAG, with an acousto-optic modulator (QS27-4SN, Gooch and Housego Ltd.). The laser produces two 8.5 W beams at 532 nm driven by a flash-lamp current of 14 A pumping a 100 mm rod.

The RegA is a three mirror cavity containing a fast Pockels cell (Medox Electrooptics) to inject and dump the pulses and a 20 mm Ti:Sapph rod to absorb ca. 90% of the pump light. The RegA pulses (300 µJ) are compressed to between 30-40 fs with an ultimate energy of 150 µJ per pulse.

A combination of high refractive index prisms (SF10) and unequal groove density diffraction grating in the stretcher (1200 L mm$^{-1}$) allows the correction of higher phase terms up to and including 4$^{th}$ order.

The Leeds set-up allows generation of separately tunable pump and probe wavelengths, by means of two independent non-collinear OPA’s. A seed pulse consisting of a single filament white-light continuum is generated in 1 mm path length of sapphire. This is amplified by the SHG generated (in 1 mm LBO) output of the amplifier (10 µJ) in a non-collinear arrangement using a 2 mm BBO crystal. The pump beam generates a cone of superfluorescence from the BBO crystal, the angle of which is adjusted so that the superfluorescence shows no spatial dispersion. Directing the seed beam along the cone axis allows a large spectral bandwidth to be phase-matched simultaneously. The centre wavelength and bandwidth of the pulses can be changed by tuning the relative delay between pump and seed pulses and controlling the chirp on the seed pulse, using a pair of BK7 prisms. The result of which is ultrashort visible pulses continuously tunable from 480-750 nm with a pulse duration of between 10-30 fs depending on wavelength (Figure 4.8).
Figure 4.8 Schematic of a NOPA producing ultrashort pulses. Inset is the arrangement of the seed beam (signal) relative to the pump and the generated superfluorescence. Picture: Reid pg 12

The pump beam was chopped synchronously (New Focus 3501) at half of the rep rate of the amplifier. The sample was probed using pulses from the second parametric amplifier, with 25 fs resolution, either at 600 nm to observe recovery of the ground state or at 670 nm to record the change in the gain signal over time. The central wavelength and bandwidth of the pump and probe beams were measured using a spectrograph and CCD camera. The relative polarisation of the pump and probe beams was set to 54.7° ('magic angle') in order to remove any contributions from orientational effects.

The probe beam was split into two parts before the sample and the intensities were measured on a pair of large area photodiodes (New Focus 2031) interfaced to a PC, to record the difference in absorbance ($\Delta$ abs.) between the excited and ground state species as a function of the pump-probe delay. A $\Delta$ abs. of $\leq 1 \times 10^{-4}$ could be detected by averaging for 1 s at each time delay. Typically 20-60 scans were averaged to remove long time drifts in pump energy. The estimated error on the measured lifetimes was $\pm 5\%$. 
4.2.4 Rutherford Appleton Laboratory (RAL)

The set-up for both of the following experiments were designed and aligned by Dr. P. Matousek and Dr. M. Towrie of the Central Laser Facility, Oxfordshire.

4.2.4.1 Visible Pump and Probe

The set-up in the Rutherford lab in Oxfordshire, England is shown in Fig 4.9. An incoming pulse train from a Ti:Sapph amplified system comes in at 150 fs, 3 mJ at 800 nm, 1 kHz repetition rate, and is split into two. A white light continuum is generated by a water cell. Light at 400 nm is generated by an SHG crystal. The UV serves as a pump and the white light is used to probe across a range of visible wavelengths. The final resolution is 400 fs. The data collected from the diode pixel arrays is stored as a function of time delay. This is later converted to intensity vs wavelength and manipulated to arrive at a plot of delta absorption over all wavelengths for the subsequent delays.

The equipment for femtosecond transient absorption is described in detail elsewhere. Briefly this system comprises a Spectra Physics/Positive Light Ti-Sapphire regenerative amplifier system producing 200 fs pulses at 800 nm and 0.6 kHz repetition rate. The pulse train was beam-split in the ratio 1:3, with 25% converted to either the 2nd (400 nm) or 3rd (267 nm) harmonic to pump the sample and the other 75% generating a white light continuum probe from a 10 mm pathlength fused silica water flow cell. This white light beam was split to give the probe and a reference beam which bypassed the sample. The pump beam (pulse energy typically 6 μJ) traveled along a delay line and later passed through the 5 mm pathlength Suprasil cuvette containing the sample, intersecting the probe beam at a small angle. The probe beam was much less energetic, typically of the order of tens of nanojoules. The pump beam diameter was ~250 μm, with the probe beam the same or slightly smaller.
The beams were overlapped spatially as close to the front of the cell as possible. A bandpass filter was inserted after the cell in the probe and reference beams to exclude residual pump light. The spatial overlap of pump and probe beams was optimised daily to ensure a good signal to noise ratio. The signals from the sample and reference beams were detected on two diode array detectors.
This set-up provided either a 267 nm or 400 nm pump and probed the region 430-650 nm. We initially had a temporal range from -50 ps → 1.5 ns. Further adjustments made to the delay-line yielded an extended range of up to 5 ns. A calibrating sample with a large \( \Delta \text{abs.} \) signal was used to ensure the beam overlap was not adversely affected by this extra optical path-length. Samples were continuously stirred during each experiment with a magnetic bar stirrer, or a flow cell was used where there was enough solution available. The experiments could be made with as little as 300 µL of solution in the 5 mm path-length cuvette, or 25 mL in the 1mm path-length flow cell (also quartz Suprasil). Steady-state UV/Vis absorption were taken before and after each run over the range 200-800 nm and showed sample decomposition over the course of the experiments to be negligible.

4.2.4.2 IR pump and probe (PIRATE)

The Picosecond Infra-Red Absorption and Transient Excitation (PIRATE) facility is also located at the Rutherford Appleton Laboratory in the UK. It uses ultrafast laser spectroscopy to probe the mid-IR region (1000-3000 cm\(^{-1}\)) on a femtosecond or picosecond timescale. The pump laser is a Ti:Sapph RegA operating a 1 kHz, 800 nm with an energy of 2-3 mJ per pulse. In picosecond mode the pulse is frequency doubled and split to pump two OPAs. Spectral filtering then delivers 1ps pulses with \(-35 \text{ cm}^{-1}\) bandwidth (or optionally 2-3 ps with 10-15 cm\(^{-1}\) with pulse energies of \(-10 \mu J\) across the tuning range of 470-700 nm (signal). Combined with upconversion and including the idler tunability, the overall tuning range is 205-2800 nm. The PIRATE mid-IR outputs are generated by frequency down-conversion of the signal and idler outputs of an 800 nm pumped OPA in silver gallium arsenide. For high-brightness narrow linewidth \((-25 \text{ cm}^{-1}\) mid-IR light the picosecond mode of the pump laser is used. Given that mid-IR spectroscopy probes the vibrations of molecules, the signals are normally very much less than that of the electronic transitions probed by experiments carried out in the visible. With notable exceptions such as the metal carbonyls, it is necessary to detect \( \Delta \text{abs.} \) of \(10^4 - 10^5\). This places heavy constraints on the pulse to pulse stability in such experiments and the sensitivity of the detectors. To reduce the overall effect of fluctuations in power etc, the researchers at RAL utilized two 64 channel mid-IR arrays. These mercury cadmium telluride (MCT) photoconductive detectors (MCT-13-64el, Infrared Associates Inc.) and preamplifiers (MCT-64000, Infrared
Systems Development Corp.) detected the reference and probe signals. A custom-made analogue readout system was designed and built, capturing the data in pump-on/pump-off pairs, and creating a rolling average. Other software discrimination was used to account for fluctuations in laser power and bubbles in the sample flow on a shot-by-shot basis. The simultaneous outputs of both MCTs were fed into a modified HX2 analogue multiplexer test board (PC20001/5) designed by the RAL Instrument Development Group. Each integrated circuit on the HX2 integrates charge with all readouts multiplexed into a single output with a sequence of 64 analogue voltages. A second HX2 board slaved to the first is used to acquire the signals from the second MCT detector. The outputs from both boards are read out in simultaneous bursts of 64 analogue signals, with each burst separated by 1 ms as defined by the laser rep rate. The HX2 board provides trigger and clock pulses to synchronise with the Analogue to Digital Converter (ADC). The ADC card (Datel 416J, 8 channels) is housed in a standard 500 MHz PC. The card has the capacity to acquire at up to 250 kHz on each channel. The data is then streamed to the PC RAM where data analysis such as signal discrimination, normalisation and averaging between detector channels is carried out on a shot-by-shot basis. The experiments are carried out in the pump/probe configuration where the pump pulses is switched on/off using an optical galvanometer synchronized to half the rep rate of the laser. A third channel of the ADC is used to monitor the pump on/pump off status of the laser. The pump pulse was the SHG of 800 nm light from the RegA.

Spectrally dispersed time-resolved IR spectroscopy uses the femtosecond mode to generate the broadband (~150 cm⁻¹) probe beam. The mid-infrared probe beam generated from the OPA is split into a reference and probe beam using a 50% germanium beamsplitter. The probe beam is focused to about 150μm diameter into the sample cell using a 300 mm focal length gold coated spherical mirror. The transmitted light from the sample is then imaged onto the spectrometer input slit that is set to 0.5 mm width. A similar optical relay is used for the reference arm. Two CVI DKSP240 ¼ m spectrometers disperse the IR beams. They have the option of 150 lines/mm (4000 nm blaze) and 300 lines/mm (2400 nm blaze) gold gratings with 25.6 and 12.8 nm/mm dispersion respectively. An adaptation of the exit port and mirror increased the spectral window of the spectrometer from 25 mm to 35 mm, matching the dimensions of the MCT diode array.

4-16
Pump and probe beam were overlapped in a standard IR flow cell (Harrick Scientific Corp., with a pathlength of 56 μm), in a closed flow system.

Fig 4.10 PIRATE set-up in RAL. The position of the sample is indicated by the green square in the diagram.
Picture from ref (1).

4.3 Zero-point Measurement

Before one can proceed with a pump and probe experiment, it is necessary to find the zero-point for the set-up in question. This occurs where the pump and the probe pulse arrive at the same time at the same point in the sample. One can first make a crude estimate by measuring the path-lengths of the pump and probe beams with a ruler/tape measure. This is normally an easier task with a home-built system as the components are more spread out, as opposed to the compact commercial systems where there is less room to manoeuvre. Once a good spatial estimate has been found, the delay line can be set-up and scanned back and forth over the path-length range to find the zero-point. Various methods are given below.

4.3.1 Two photon absorption.
In this method a solution of rhodamine 6G is used in place of the sample. When the zero-point is reached and the two pulses coincide the combined two photon absorption (TPA) will be observed as a lessening of intensity of light passing through the sample. By monitoring the output at 400 nm in this case with a photodiode one can look for a drop in intensity.
4.3.2 Second Harmonic Generation.
SHG could be attempted using the peak of the SHG intensity generated by a crystal to find the overlap point. For a pump wavelength of 400 nm this may not be as useful as one needs a UV detector for the SHG.

4.3.3 Fast Photodiode
With a fast enough photodiode in place of the sample, two pulses, the more energetic one being the pump, can be seen and made to overlap by adjusting the delay-line. This is quite a good system, however the picosecond photodiodes can be quite expensive. This method will give a general idea normally within 100 ps or so of where the zero-point is. A more exact method can subsequently be used.

4.3.4 Pinhole
When there is little overall noise in the system and the paths lengths of the two beams are already very nearly matched, the two beams can be overlapped through a pin-hole and at the zero-point the noise should increase due to the interference of the two pulses, monitored on a photodiode. The system in TCD is not stable enough for this method to be applied.

4.3.5 Kerr Gating
Kerr gating occurs where the pump pulse (overlapping with the probe) is linearly polarised and passes through a solution of carbon disulphide. The pulse polarises the solution in the same direction for a short space of time (circa 300 ps). The probe pulse is detected after the sample with a photodiode. A crossed polariser on the probe pulse means that only when the pump pulse arrives 300 ps or less before the probe can any light be seen beyond the second crossed polariser. Once within a few picoseconds of the zero-point a pump-probe experiment of a known system can be performed to find it exactly. This requires 800 nm light to be used as the pump. The OPA system would have to have the SHG crystal removed to use this method.
4.3.6 Techniques Used

In TCD we used Coumarin 152 in ethanol as a sample. This absorbs strongly at 400 nm, which was the pump wavelength. We were able to detect a bleach signal when moving from negative to positive time delays. Some of the traces of before and after the zero-point can be seen in the Appendix B. As a shutter was not available at the time to provide pump on and pump off the measurement was taken at each delay with the pump first blocked and then unblocked.

The pinhole method has been used successfully in Leeds, where the system is stable enough to support it. The Kerr gating method, using CS$_2$ as a sample, has been successfully used in RAL. In each case, when the zero-point has been approximately found, a transient absorption scan can be performed on a known substance with a ps decay time to find the exact zero-point.

4.4 Experimental considerations for pump and probe

4.4.1 Non-linearity of signal

Normally in each experiment the exciting pulse is selected so that it is preferentially absorbed by one material in the system and not by the other, making it easier to elucidate the possible excitation/relaxation pathways. Where possible, multiple excitation wavelengths are used to try to separate out the earliest processes. At the high instantaneous pump powers present in femtosecond experiments other non-linear effects can occur. One example of this is two-photon absorption in solutions containing water when the exciting in the UV. This can lead to the formation of solvated electrons which in turn drown out other features in the TA spectrum (see Chapter 11). Thus it was necessary to verify the linearity of the signal with pump power. In RAL this was performed by modulating the pump power using suitable neutral density filters. This also excludes the possibility that we are inducing amplified spontaneous emission by using too strong a pump pulse.

4.4.2 Residual photoproducts

Due to the nature of some of the materials used i.e. the organometallics, it would not be surprising if some short-lived photoproducts were temporarily formed, and interacted with the
next probe pulse. The Δ abs. signals we are dealing with are quite small (~ 10⁻⁴) and any photoproducts could lead to a change in the base-line of the TA spectrum, which in turn would adversely affect kinetics. The low rep-rate of the system means there is a pulse arriving at a given point every 1.67 ms, which would give any ultrafast process time to conclude. This enabled artefacts from saturation and photoexcitation of incompletely relaxed reaction centres to be avoided. In the materials examined most processes were finished on a nanosecond timescale, having previously been examined by other time-resolved studies such as nanosecond flash photolysis.

4.4.3 Sample concentration

The concentrations of the samples used had to be dilute enough to allow sufficient light through the sample but concentrated enough to provide a good excited state absorption signal. IR signals will be much smaller than their visible counterparts which necessitates using higher concentrations. In this case much smaller pathlengths were needed (56 μm c.f. 1mm), to allow enough light through the sample. Also the solvent itself is absorbing which also requires the pathlength to be small.

CaF₂ was used instead of glass to have minimal absorption in the IR. Acetonitrile and water were the solvents used. The concentration was also chosen to avoid dimers or aggregates in the sample. Choosing a more suitable pump wavelength when possible, where more excited states will be created, is one way of avoiding this.

4.4.4 Orientation of pump and probe beams

It is of utmost importance in a pump and probe experiment that the area being probed contains as many excited states as possible when the pump is “on”. If not enough excited states are monitored the signal/noise ratio will be low. The number of excited reaction centres decreases exponentially with distance as the pump passes through the sample according to Beers law. Thus pump and probe beam need to be overlapped as close to the front of the cell as possible, to probe as much excited state absorption as possible. Careful alignment ensures that the pump and probe beam overlap each other well, with no transverse beam “walk-off” as the delay is changed. The pump and probe polarisations were set at an angle of 54.7° to each other.
to remove orientational effects (G.R. Fleming, Chemical Ultrafast Applications of Ultrafast Spectroscopy 1986).

4.4.5 Thermal Effects
Ideally thermal effects in solution are avoided by each successive excitation encountering fresh solution. A sample cell which is stationary over the course of the experiment may be prone to thermal effects and photodegradation from the pump beam. Some form of agitation or stirring can be used to refresh the solution during the sample, e.g. a magnetic stirrer bar. It should be noted that the part of the solution that is probed is necessarily close to the cell wall and friction will make the rate of refreshing the sample less at this point.

Using a small cell and a stirrer bar close to the overlapped incoming beams allows the part of solution under excitation to be refreshed over the course of a whole experiment. It is rather unlikely that the solution is refreshed between successive excitations when using a rep rate of 1 kHz, i.e. every ms.

A flow-cell would be the best way to avoid degradation and thermal effects. The draw-back to using a flow-cell for every experiment is the larger quantity of sample required, e.g. enough to make a 25 mL solution.

4.4.6 Biological Samples and Sample quality
Samples containing DNA are especially prone to degradation, and or denaturing, so some form of stirring or agitation was used for each biological sample solution, and where possible, fresh samples were used for each successive experiment, (the use of a flow-cell was not possible due to the small quantity and expense of each DNA sample). Buffered solutions were stored under the correct refrigeration conditions to prevent bacterial growth. DNA was checked for other biological contaminants (such as proteins) by verifying the ratio of absorbance at 260/280 nm was between 1.75-2.10. 4,5
Absorption spectra were taken before and after a run checking for degradation occurring over the course of the experiment.

Bubbles present in solution can lead to experimental artefacts, samples were checked by eye for bubbles before each run. In some cases the samples were heated gently to release excess gases present in solution.

4.5 Data processing and analysis

4.5.1 Leeds University
The PC performed the processing on the raw data, converting the CCD readings to Δ abs. arb. vs time., and saving the data in ASCII format. The kinetic data were calculated by fitting exponential decays to the time-resolved data. Origin 6.0 was used to fit the kinetic data.

4.5.2 Rutherford Appleton Laboratory

4.5.2.1 Visible Pump and Probe
At RAL the raw data consisted of the readouts from two CCD arrays over a range of 400-750 nm as a function of time delay, saved as one set of ASCII data. This data was saved as a value of the ref/probe signal at each of the 512 pixels for every time delay.

Each experimental run comprised a number of consecutive cycles, with the order of time delays in each cycle randomly varied, and averaged over 20 s at each delay position. The PC averaged the data over all the cycles (normally 5 for each run), and saved it as a single ASCII file. This raw data had to be corrected for absorption, as the reference beam did not pass through the sample. The correction data was taken with each sample in place for 30 seconds with no pump on, and saved as a separate calibration file. Finally the pixel to wavelength calibration was taken once a day. This consisted of two consecutive measurements using two different bandpass filters, one blue and one red, in the probe beam without the sample in place. The average CCD readings accumulated over 20 s were saved as ASCII files and the peak of intensity profile was used to calibrate pixel to wavelength in each case.

The raw experimental data was imported into an excel spreadsheet and the separate files for absorption correction and wavelength calibration were incorporated step-by-step.
Origin 6.0 was used to plot the $\Delta$ abs data as a function of time delay and fitted the data to exponentials decays. For the visible TA spectra wavelength ranges consisted of 10 pixels, ~ 5 nm. The fits normally consisted of single exponential decays where,

$$\Delta\text{abs.} = A_1 e^{-t/\tau} + y_\infty \quad (3.2)$$

$y_\infty$ is the offset value at time infinity. A value of zero for this constant suggested the transient absorption processes had completely finished. Otherwise, for biexponential fitting an equation of the form was used

$$\Delta\text{abs.} = -A_1 e^{-t/\tau_1} + B_1 e^{-t/\tau_2} + y_\infty \quad (3.3)$$

See Appendix C.

4.5.2.2 IR Pump and Probe

The data was processed to produce transient absorption spectra by a program written by Xue-Zhong Sun of the Nottingham group. The excel files containing TA data were imported into Origin 6.0.

It was sometimes necessary to join together two data ranges (or windows) to give a single TA spectrum over an extended wavenumber region. In these cases the first few and last few data points were discarded when the wavenumber ranges were pieced together as these data are sometimes unreliable. Single pixel kinetic traces were fitted to exponential decays to provide kinetic data for IR time-resolved data.

4.6 Errors

Experiments were normally repeated at least once, when not repeated it was due to time constraints. For each experimental run operating at a laser rep. rate of 1 kHz and averaging over 20 seconds at each time delay, 20,000 pump and probe measurements occur. This multiplied in turn by the number of excited species we have $N = n N_A$, (where $n$ is the number of moles contained in the excited area which approximates as a cylinder and $N_A$ is Avagadro’s constant). Therefore, many processes are being monitored in the space of a minute, although
this is necessary as TA signals are normally quite small, of the order of $10^4$ for vibronic transitions.

To get the optimum data with a good signal to noise ratio, without requiring a very long acquisition time some points had to be addressed:

The spatial overlap of pump and probe beam was checked daily to allow for thermal drift over time (from thermal expansion of optics holders for example) to provide the strongest signal.

(This was also verified for the longer time delays: a drop in TA signal from the sample used for calibration at the longer delays would have indicated that the two beams were moving with respect to each other).

Negative time delays were measured, to subtract from the raw data, as these should provide a base-line for a time-resolved experiment.

Where values of $\tau$ for specific processes are given as an average value of a set of separate experiments, the individual values are tabulated. Values for $\tau$ may differ over different wavelength ranges. Experimental results from simple single step processes show kinetics for the absorption and depletion region to be within 10% error of each other. Therefore we assume that different values for $\tau$ at different regions in the spectra are “real”, i.e. not arising because of instrumental errors.

N.B. some of the earlier measurements were performed with no data points between 200 and 1000 ps. This could produce an extra error not accounted for in the error calculated by Origin 6.0 fitting program as it is an error associated with the quality of data available. In some cases it will shorten the kinetics, as the baseline is slightly raised to what it would be if there were more points present, due to the fitting program. This should not matter much if we only compare results fitted over the same data ranges. Different data ranges were used to extract kinetic data for the very fast and the slower processes. Some experimental runs were performed with most of the data points taken during the early time delays. These were more useful for elucidating the earlier process.

We estimate the error for the TA experiments to be the error for the fitted exponential decays in Origin 6.0 or $\pm 10\%$, whichever is larger.
It must be pointed out at this point that although the quality of the PIRATE data appears to be good, there was only one experiment performed for each sample (due to time constraints) and so one cannot get an average value for $\tau$. Also single point kinetics were used which will induce greater errors.

Due to the dispersion of optical components the broadband white light probe beam can sometimes contain a positive chirp, where the "red" part of the probe arrives at the sample before the "blue". This can result in a spurious signal for the earliest time delays, and when detected that trace is deleted and is not included for kinetics. A theoretical treatment of chirped probe pulses has been performed by Sorokin et al.  

4 O. Warburg Biochem. Z. 310 384 (1942)
5 J.A. Glasel BioTechniques 18 (1) 62-63 (1994)
5. MATERIALS AND SAMPLE PREPARATION

Deionised water was used throughout each experiment.

![Figure 5.1 The structure of thionine](image)

5.1 Thionine

Solutions of thionine (50\(\mu\)M, Aldrich) in 5mM phosphate buffer containing either GMP (100mM), \([\text{poly(dG-dC)}]_2\) (0.4mM), or calf thymus DNA (1.2mM) were studied. Purification was by G. Reids group as follows: the thionine was purchased from Aldrich, purified on neutral alumina, recrystallised three times from ethanol, and dried under vacuum. The dye purity was confirmed by NMR and visible absorption and emission spectroscopies. The polynucleotides were purchased from Sigma and used as supplied. The thionine systems were made up in a phosphate buffer at pH 6.9, to keep the DNA helix stable. The aim was to simulate as close to physiological conditions as possible for these types of experiment. At the concentrations used it is expected that the thionine would be mostly bound 96-99\%.2

![Figure 5.2 The structure of Ru(TAP)\_2 dppz](image)

5.2 Ru(TAP)\_2 dppz complexes

Ru(TAP)\_2 dppz (1) and [Ru(TAP)\_2 F\_2 dppz] (2) (TAP = 1,4,5,8 tetraazaphenanthrene; dppz = dipyrido-[3,2-a: 2',3'-c]-phenazine,) were synthesized and purified as previously described, by Benjamin Elias of the Brussels group.3 4 The PF\_6\^- salt of the ruthenium
complex was used in organic solvents and the \( \text{Cl}^- \) salt for aqueous solutions in deionised water. The compounds were characterized by NMR and electrospray mass spectrometry. Solutions of \( 8.5 \times 10^{-5} \text{ M} \) with/without \( 1.7 \times 10^{-3} \text{ M} \) polynucleotide were prepared either in 10 mM phosphate (pH 6.9) buffer, buffered \( \text{D}_2\text{O} \) (spectroscopic grade, Aldrich) or spectroscopic grade acetonitrile (Aldrich). We had access to ultrapure water used for photolithography in Rutherford and ran this as a comparative solvent run, this showed no difference to the deionised water used throughout the rest of the experiments.

The concentrations used for Transient absorption for both complexes were \( 1.68 \times 10^{-4} \text{ M} \) (1), \( 3.3 \times 10^{-3} \text{ M} \) (polynucleotide) in 2001 and \( 8.5 \times 10^{-5} \text{ M} \) (1) \( 1.7 \times 10^{-3} \text{ M} \) (polynucleotide) in 2002. Polynucleotides were purchased from Pharmacia-Amersham (GC 27-7910, AT 27-7870), or Sigma (GC P9389, AT P5782), and used as received. Typically 300 \( \mu \text{L} \) of solutions with a nucleotide/Ru binding ratio of 20:1 were prepared to ensure that most of the complex was bound and to avoid aggregation.

![Figure 5.3 The structure of Ru(phen)\(_2\)dpqa](image)

**5.3 Ru(phen)\(_2\)dpqa complex**

\([\text{Ru(phen)}_2\text{dpqa}]^{2+} \) (phen = 1,10-phenanthroline, dpqa = 2-dipyridoquinoxaline pentylamide), was synthesized by K. O’Donoghue formerly of the Kelly group in Trinity College Dublin. First Medpq was synthesized from 1,10-phenanthroline-5,6-dione and 1,2-diaminopropane according to a similar procedure for dpq, involving the oxidation and subsequent esterification of Medpq. The amide was then produced by reaction of the ester intermediate with amylamine. The ruthenium bis-phenanthroline complexes were then synthesised according to literature procedures. The complex was purified by HPLC and the proposed structures agreed with elemental analysis, NMR, ESMS and UV-vis. Spectroscopy.
Solutions for transient absorption of $1.2 \times 10^{-4}$ M were prepared in H$_2$O and D$_2$O, and $1.4 \times 10^{-4}$ M in spectroscopic grade acetonitrile. Solutions of $2.8 \times 10^{-3}$ M in D$_2$O and acetonitrile were used for PIRATE measurements.

![Figure 5.4 The structure of Re (CO)$_3$(py) dppz, $X_1$ =H, $X_2$ = H.](image)

5.4 Re (CO)$_3$(py)dppz complexes

fac-[Re(CO)$_3$py(R$_2$ dppz)] complexes (R = F, H, Me) were prepared by J. Dyer of the Nottingham group, and also by S. Hudson formerly of the Kelly group in TCD, by adapting the method by Schanze et. al. Solutions for transient absorption in 2001 were made up in acetonitrile solution (R = F, H, Me) ($1.7 \times 10^{-4}$ M, $1.5 \times 10^{-4}$ M and $1.7 \times 10^{-4}$ M resp. Solutions for transient absorption in 2002 were made up in 10 mM phosphate buffered H$_2$O and D$_2$O solutions, (R = F, H) ($8.8 \times 10^{-4}$ M and $8.8 \times 10^{-4}$ M resp.). Solutions for PIRATE measurements were (R = F, H) ($1 \times 10^{-3}$ M and $1.2 \times 10^{-3}$ M resp.), for experiments with polynucleotides the concentration of complex (R= F) was $0.3 \times 10^{-3}$ M, however the concentration of complex (R= H) was $1.2 \times 10^{-3}$ M, with [poly(dG-dC)]$_2$ and $1.1 \times 10^{-3}$ M with [poly(dA-dT)]$_2$. In all cases the ratio of complex to polynucleotide was 1:20. Polynucleotides were purchased as in 5.2 above.
5.5 Dppz ligands

The \( R_2 \) dppz ligand (\( R = F, H, Me \)) were synthesized following literature procedures by A. M. Selmi and D. A. McGovern of the Kelly group at TCD, rigorously purified by chromatography on neutral aluminium oxide, recrystallised and characterized by IR, NMR and ESMS.  

Solutions for transient absorption were prepared using spectroscopic grade acetonitrile. In order to study the effect of water on the photophysical properties of dppz derivatives, 50/50 (w/v) acetonitrile solutions were used. The concentrations in acetonitrile (\( R = F, H, Me \)) were \( 1.86 \times 10^{-5} \) M, \( 2.02 \times 10^{-5} \) M and \( 1.89 \times 10^{-5} \) M resp., and the concentrations in the acetonitrile/water mix were \( 1.24 \times 10^{-5} \) M, \( 2.19 \times 10^{-5} \) M and \( 2.24 \times 10^{-5} \) M resp. (2002 values).

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4. [Ru(TAP)\(_2\)dppz\(^{2+}\) : A DNA intercalating complex which luminesces strongly in water and undergoes photo-induced proton-coupled electron transfer with guanosine-5'-monophosphate I. Ortmans, B. Elias, J. M. Kelly, C. Moucheron, A. Kirsch-De Mesmaker (to be submitted)


9 MSc Photophysical Studies of a Family of Re(I) Complexes based on dipyridophenazine ligands: Effects of Solvents and Substituents S. Hudson, MSc. (*University of Dublin October 2002*)
6. RESULTS: Thionine

6.1 Introduction

Ultrafast electron transfer in DNA has attracted much attention both theoretically and experimentally. Various applications are envisaged using the principles of electron transport in DNA including molecular electronics. Photoinduced damage of DNA involving site-specific cleavage of the polynucleotide strand and adduct formation have possible uses in the field of photodynamic therapy.

In nature DNA is normally protected from such photodegradation by its extremely short-lived states. Photodynamic degradation of DNA may however be induced by ultrafast redox reactions. Using redox-active dyes bound to DNA is one way of injecting charge into the DNA strand. Here, the phenothiazine family of dyes, of which thionine is part of, has attracted considerable attention because the excited states of the dyes are strongly quenched when they bind near guanine bases. This quenching is believed to be due to electron transfer from the guanine to the dye excited state. Quenching by adenine has been found to be less favourable, due to the redox properties of the nucleobase. Indeed it is this increased oxidation potential of guanine that makes successive GC sites susceptible to mutations in the base-pair sequence - which can be induced by singlet oxygen, or by other endogenous or exogeneous photosensitisers, such as UVC or ionizing radiation. The studies in this area of DNA damage and repair are ongoing.

Studies of methylene blue, another phenothiazine dye, had indicated that its excited singlet state was efficiently quenched by species containing guanine, taking 4ps. Thionine had previously been studied with a resolution of 150fs and a similar sub-picosecond process was also detected in the presence of guanine. It was hoped that better femtosecond resolution could elucidate this ultrafast process.

Figure 6.1 The structure of thionine, left and methylene blue, right.
The structure of thionine is shown in Figure 6.1. This molecule is very similar structurally to that of methylene blue (MB), which is known to intercalate into DNA, and undergo reduction by guanine containing species after excitation. The suggested model for intercalative binding to \([\text{poly(dG-dC)}]_2\) is one in which the MB is intercalated fully and symmetrically between the base pairs. It is expected then that in the presence of polynucleotides the thionine chromophore will similarly intercalate into \([\text{poly(dG-dC)}]_2\), and that the electron deficient ring structure should make it an ideal candidate for ultrafast electron transfer from the guanine nucleobase.

The resolution for the experiments was 50fs. All measurements were carried out in the ultrafast facility at the department of Chemistry in the University of Leeds under the supervision of Prof. J. M. Kelly. Details of the experimental set-up are in Chapter 4.

![Figure 6.2](image_url)

**Figure 6.2** The absorption spectrum of the 50 µM thionine complex in water (The absorption spectrum of thionine bound to \([\text{poly(dG-dC)}]_2\) is red shifted by \textit{circa} 17 nm but the shape remains almost unchanged.)

### 6.2 UV/vis Measurements

The absorption spectrum of free thionine in water is shown in Figure 6.2. At the excitation wavelength for the experiments we should be exciting near the peak of the thionine absorption and should expect a good signal. The DNA/polynucleotides will not absorb at this longer wavelength. The addition of DNA had the effect of blue-shifting the thionine IL absorption maximum, with \(\lambda_{\text{max}}\) going from 600 nm for free thionine in aqueous buffered solution to 617 nm for thionine bound to \([\text{poly(dG-dC)}]_2\) in the ratio 1:20.
6.3 Transient Absorption (TA)

All transient absorption measurements were carried out in collaboration with Dr. G. D. Reid, D. J. Whittaker, M. A. Day and M. Johnston.

6.3.1 Thionine and GMP (guanosine mono-phosphate)

The TA decays of thionine/GMP systems are shown below. The ground state recovery occurs in 1.21 ± 0.04 ps (Fig 6.3) however the decay at 670 nm occurs much more quickly, taking approx. 880 fs (Fig 6.4). This indicates that the singlet decays to a transient before returning to the ground state.

![Figure 6.3 TA results for 50 μM thionine with 100 mM GMP pump and probe 600 nm. Red line is the fitted kinetics at 600 nm.]

![Figure 6.4 TA results for 50 μM thionine with 100 mM GMP pump 600 nm and probe 670 nm. Red line is the fitted kinetics for 670 nm.]

Figure 6.3 TA results for 50 μM thionine with 100 mM GMP pump and probe 600 nm. Red line is the fitted kinetics at 600 nm.

Figure 6.4 TA results for 50 μM thionine with 100 mM GMP pump 600 nm and probe 670 nm. Red line is the fitted kinetics for 670 nm.
Two solutions of thionine/GMP were made up with 100 mM and 2 mM of GMP to vary the binding ratio. Figure 6.5 shows the gain region decay of the 2 mM GMP solution, fitted with a bi-exponential decay. The second time constant was set to be 320 ps, the decay time of free thionine in water. A decay of $(860 \pm 90)$ fs was calculated for the deactivation of the singlet state. This indicates that the small amount of bound thionine is decaying rapidly but the main part of the signal is due to the free thionine decay.

![Graph](image)

Figure 6.5 TA results for 50 $\mu$M thionine with 2 mM GMP pump 600 nm and probe 670 nm. Red line is the fitted kinetics at 670 nm.

### 6.3.2 Thionine and [poly (dG-dC)]$_2$

In this system the probe wavelengths were again 600 nm and 670 nm. There was a more pronounced difference between the photobleach recovery and singlet decay times. The concentration of polynucleotide was calculated so that most of the thionine would be bound (96%). Monitoring the kinetics at 600 nm allowed us to calculate the recovery of the bleach to take $760 \pm 20$ fs (Figure 6.6) and the singlet decay time is $260 \pm 10$ fs (Figure 6.7). Therefore the excited state is quenched more efficiently than in the mononucleotide case.
Figure 6.6 TA results for 50 μM thionine with 0.4 mM [poly(dG-dC)]₂ pump and probe 600 nm. Red line is the fitted kinetics at 600 nm.

Figure 6.7 TA results for 50 μM thionine with 0.4 mM [poly(dG-dC)]₂ pump 600 nm and probe 670 nm. Red line is the fitted kinetics at 670 nm.
Figure 6.8 TA results for 50 μM thionine with 1.4 mM ct-DNA pump 600 nm and probe 600 nm. Red line is the fitted kinetics at 600 nm.

6.3.3 Thionine and ct-DNA

TA spectra were taken with thionine and double stranded calf thymus DNA. The graph is shown in Figure 6.8 with a multi-exponential fit using 760 fs for recovery of the ground state of thionine bound between two GC pairs, 2.5 ps when bound between one AT and 1 GC pair and 2500 ps when bound between 2 AT pairs (see 6.4 Discussion). Here we note that the quality of the DNA data is very much poorer, yielding a signal which is approximately 4.5 times smaller than that for thionine bound to [poly(dG-dC)]$_2$. Time constraints prevented us from repeating the experiments to try to improve the quality of data.

6.4 Discussion

Buffered solutions of thionine were studied after addition of GMP, [poly(dG-dC)]$_2$ or calf thymus DNA. The transient species formed by excitation with 600 nm light, were monitored either by following the reformation of ground state thionine at 600 nm, i.e. looking at the bleach, or by monitoring the loss of its singlet state by observing the stimulated emission at 670 nm. At this latter wavelength neither the ground state nor the excited state absorb significantly.

The results indicated that the thionine excited state, when bound to the polynucleotide, undergoes a reduction from guanine and is strongly quenched with a single exponential lifetime of 260 fs. The lifetime of free thionine in the absence of the polynucleotide is 320 ps, more than a factor of 1200 longer. Monitoring the transient bleaching at 600 nm
allows one to follow reformation of the ground state as the reaction products recombine. The signal recovers with a single exponential lifetime of 760 fs and this decay will be a lower limit for the return electron transfer rate, $k_r$. The forward rate is actually the average of rates to two GC binding sites, this derives from the fact that a polynucleotide strand has directionality given by either the 5' endo or 3' endo strand.

Similarly, Figure 6.4 shows that with the mononucleotide, GMP, the excited state lifetime is 880 fs and the bleach recovery is 1.2 ps, indicating a similar, but slower, quenching of the excited state of thionine. At the concentrations used approximately 98% of the thionine was bound and 1:2 complexes were expected to predominate for GMP.

It should be noted here that the distance from chromophore to base is fairly well characterised in a polynucleotide strand where the chromophore is fully intercalated. This is not necessarily the case when a mononucleotide is used. The interaction length may not be as clearly defined because of the extra rotational freedom available in the latter case, and indeed the interaction radius will often be larger in the case of the mononucleotide binding, which may in turn affect the rate of electron transfer. The slower measured rate for the mononucleotide could also reflect a slightly larger separation between donor and acceptor in the GMP complex than when intercalated in the polynucleotide.

The results of the TA of thionine bound to DNA were not as clear. As mentioned in the results section the quality of the data was not as good as had been previously recorded for the mono and polynucleotides, due to time constraints and pump laser instability. However the binding site for thionine in ct-DNA is as not well defined as it is in the former two cases.

Firstly the binding may not all be intercalative, although the studies done for MB indicate that intercalation predominantly occurs, this is true for GC polymers and not necessarily AT sites. AT sites are known to favour groove binding and so not all of the thionine may be intercalated, which would results in a range of different nucleotide to dye distances. For simplicity let us say that the binding in ct-DNA is entirely intercalative, and electron transfer only occurs to an adjacent basepair.

Secondly, the GC content of calf-thymus DNA is 42%, so the probability that thionine will be bound between two GC pairs is $0.42 \times 0.42 = 0.1764$. The probability that thionine is bound between two AT pairs is 0.3364, whereas the probability that the dye is intercalated between 1AT and 1GC pair is 0.4872. If the data for the ct-DNA was of better quality we
could fit the data as a combination of exponential recoveries for thionine bound to GC pairs only (760 fs), to AT only (> 2500 ps) and to thionine in a site between 1 GC and 1 AT pair (2.5 ps), weighted in favour of the latter time constant.

The previous solution ignores any quenching contribution from distant basepairs. It is known that electron transfer can occur via bases at a distance from the binding site, and this contribution could also affect the kinetics measured for electron transfer in DNA \(^8,^9\). The thionine will show different rates for electron transfer if intercalated directly adjacent to a GC pair or some distance away. For all these reasons it is hard to elucidate the DNA transient data.

To estimate the driving force for Reaction 1 we need the oxidation potential of guanine and the reduction potential of the thionine excited singlet state. The value for \(E^* (\text{Th}^+/\text{Th})\) has been given as 2.003 V. \(^2\) However the value for the oxidation potential of guanine is still contentious. A value of ca. 1.33 V \(^{10}\) had previously been determined for guanosine. The value from the literature for the reduction potential of adenine is given as 1.75 V. \(^2\)

\[
\text{Th}^+, G \xrightarrow{k_f} \left[ \text{Th}^+, G^{**} \right] \xrightarrow{k_r} \text{Th}^+, G
\]

Reaction 1: Reduction of excited thionine by guanine forming radical and radical cation respectively before the back electron transfer.

More recent pulse radiolysis studies give figures for the oxidation potential for guanosine as 1.58 V (GMP), 2.03 V for adenosine (AMP) and 1.53 V for [poly(dG-dC)]\(_2\). \(^{11}\) These pulse radiolysis values have been found to give a good fit for the electron transfer reactions of thionine and various polynucleotides, the value for [poly(dA-dT)]\(_2\) is taken to be the same as that for (AMP) \(^{12}\) We shall take these as the oxidational potential values for the nucleobases throughout this thesis.

This gives the standard redox potential for guanine and adenine as -0.473 V, and +0.027 V respectively. So the reduction of thionine by guanine will be strongly exergonic whilst the reaction with adenine will be mildly endergonic. This is reflected in the ultrafast timescale of the ET process. Using the same potentials we would predict slower rates for the forward and return steps in the GMP complex also, which is what is observed.
Work done on thionine (and methylene blue) since then using different polynucleotides has allowed estimation of $\lambda$, the reorganisation energy and $|V|$, the electronic coupling matrix element at 298 K, using the measured forward, $k_f$ and return, $k_r$ rates from the [poly(dG-dC)]$_2$ measurements. These gave estimates of $\lambda \sim 0.87$ eV (~7000 cm$^{-1}$) and $|V| \sim 0.015$ eV (~120 cm$^{-1}$). The maximum classical rate, i.e. when the driving force is equal to the reorganisation energy, corresponds to a rate on the order of $1/260$ fs ($3.8 \times 10^{12}$ s$^{-1}$), placing our measured forward rate near the maximum on the Marcus curve. A different rate for quenching was found when thionine was bound to [poly(dG-dT). Poly(dC-dA)], as in this case only one guanine is adjacent to each intercalated thionine. This seems to suggest that intrastrand electron transfer does not seem to play a part on the ultrafast timescale of this ET process.

These results also show that both the forward and reverse reactions are extremely rapid on the timescale of molecular reorientation. The rotation time of thionine in water at 298 K is 63 ps and it is impossible that the dye molecules rotate through any significant angle on the timescale of electron transfer.

### 6.5 Conclusions

This study of thionine bound to DNA shows ultrafast fluorescence quenching, arising due to electron transfer from guanine to the intercalated base. The process is favoured in [poly(dG-dC)]$_2$ by both its exogenicity and the small electron donor to acceptor distance. The rate for quenching is $1/260$ fs, which is near the maximum of the Marcus curve. This is one of the simplest systems available to study intermolecular ET from DNA, as the mode of binding with DNA is well-characterised, and there is only one chromophore available to accept an electron. The ultrafast reaction involved necessitates that femtosecond spectroscopy is used to fully elucidate the electron transfer process.

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7. RESULTS: $[\text{Ru(TAP)}_2\text{dppz}]^{2+}$ complexes

7.1 Introduction

Metal polypyridyl complexes are finding extensive uses both as photophysical probes and as photochemical reagents.\(^1\) The great structural variability of these complexes allows the redox properties to be tuned over a broad range. This in turn can have a great effect on the optical properties of a material and can lead to emission enhancement or in some cases fluorescence quenching. Changing the metal or the ancilliary ligand in heteroleptic (different ligands) complexes allows for further control of the photophysical properties. Together with their excited state photoreactivity these make ideal complexes for the study of photoinduced electron transfer.

Polypyridines form stable compounds with most transition metals. They are bidentate ligands which bond via the nitrogens (7.1) and are good $\sigma$ donors. They also have low-lying $\pi^*$ orbitals which can accept electrons upon reduction.

![Polypyridyl compounds](image)

Figure 7.1 Two polypyridyl compounds, bpy(2, 2’bipyridine) and phen(1,10-phenanthroline), commonly used as ligands for metallo-organic complexes

Ruthenium polypyridyl complexes, in particular, have been studied as excellent sensitisers for probing various properties of DNA, as their photophysical and electrochemical properties can be readily varied in a controlled fashion. Ideally a complex was required that would bind in a well-defined manner to DNA, whose photophysical properties could be tuned and whose redox properties could induce obvious changes when bound to DNA facilitating electron transfer study.

For applications with DNA, dipyridophenazine (dppz) complexes have attracted special attention as the dppz ligand can intercalate between the base pairs of the polynucleotide,
giving a well-defined donor-acceptor geometry for electron transfer. In particular 
[\text{Ru(phen)}_2\text{(dppz)}]^2+ (phen = 1,10-phenanthroline) has been studied in detail, as the complex, 
which is essentially non-luminescent in water, becomes emissive when bound to DNA. This 
is known as the “light-switch” effect. The reasons for this behaviour have been elucidated by 
picosecond spectroscopy. Light switches will be discussed in further detail in the following 
chapters.

Another group of ruthenium complexes, which show interesting behaviour with DNA, are 
complexes such as [\text{Ru(TAP)}_2\text{(L)}]^2+, (TAP = 1,4,5,8-tetraazaphenanthrene; L = 2, 
2’-bipyridine, phen, TAP), because the electron accepting ability of the TAP ligand makes the 
excited states capable of photooxidising guanine in either nucleotides (GMP or dGMP) or in 
the double stranded polynucleotides. While direct evidence for electron transfer has been 
obtained with guanosine monophosphate, the process in DNA is too fast to measure using 
nanosecond methods and must occur on a picosecond time scale. A further disadvantage of 
complexes such as [\text{Ru(TAP)}_2\text{(phen)}]^2+ is that their mode of binding is uncertain. It is likely 
that they are groove-bound with some partial intercalation between the base-pairs. On the 
other hand complexes of dipyrido[3,2-a: 2’,3’-c] phenazine such as [\text{Ru(phen)}_2\text{(dppz)}]^2+ are 
known to intercalate fully by the dppz ligand and such species are therefore more appropriate 
for detailed kinetic studies. However the excited state of [\text{Ru(phen)}_2\text{(dppz)}]^2+ is insufficiently 
oxidising to produce the guanine radical cation and we have therefore studied 
[\text{Ru(TAP)}_2\text{(dppz)}]^2+, which has similar redox chemistry to that of [\text{Ru(TAP)}_2\text{(phen)}]^2+, but 
unlike that species it binds to DNA in a well-defined intercalating fashion. Preliminary 
picosecond studies have indicated that the kinetic behaviour of transient species formed from 
[\text{Ru(TAP)}_2\text{(dppz)}]^2+ bound to DNA is markedly different to that of the complex free in 
aqueous solution.

These measurements were performed to further elucidate the processes occurring in \text{Ru(TAP)}_2 
dppz (1) in various solvent environments and in the presence of DNA and polynucleotides. 
Two sets of measurements were carried out for this complex, in July 2001 and December 
2002. The results from the first year indicated an electron transfer process was occurring from 
guanine to the ruthenium complex. The following year due to adjustments to the
experimental set-up it became possible to search for the predicted back electron transfer on the correct time-scale.

In the second year a second complex was available for study, the difluoro substituted \([\text{Ru(TAP)\textsubscript{2} F\textsubscript{2}dppz}]^{2+}\) (2). It was thought that any observed electron transfer process might be influenced by an electron withdrawing substituent on the dppz ligand. Concurrent with this work in the second year a picosecond infra-red study was performed on 1 with polynucleotides to further corroborate kinetics. Work was also carried out in deuterated water, both to discover whether any solvent isotope effect was present and also to compare visible TA work directly with the PIRATE data. All measurements were carried out in the Central Laser Facility in the Rutherford Appleton Laboratory, under the supervision of Prof. J. M. Kelly. Details of the experimental set-up are in Chapter 4: Experimental Methods.

![Figure 7.2 The structure of [Ru(TAP)\textsubscript{2} dppz]\textsuperscript{2+}](image)

![Figure 7.3 The structure of di-fluoro substituted dppz. Coloured letters are schematic representation of the area of the dppz ligand associated with phenanthroline (ABC) or phenazine (BDE) character.](image)
7.2 UV/vis Measurements

The structure of 1 is shown in Figure 7.2, note the extended dppz chromophore which facilitates intercalation between the DNA base-pairs. In 2 the dppz ligand is substituted with fluorine atoms as depicted in Figure 7.3. This figure also shows a schematic representation of the dppz ligand having both phenanthroline and phenazine character, yielding photophysically different MLCT states, MLCT Ru-(phen) and Ru-(phz).

The absorption spectrum of the complex in aqueous buffer is shown in Figure 7.4. The complex shows a strong absorption in the region 400-500 nm, as expected for MLCT transitions. Interestingly the strong bands corresponding to the dppz (π-π*) intraligand transitions found for each of [Ru(phen)2(dppz)]2+, fac-[(dppz)Re(CO)3(py)]1 and the free ligand in the region 350 - 400 nm are not readily visible with this complex.

![Absorption spectrum of \( [\text{Ru(TAP)}_2 \text{dppz}]^{2+} \) and \( [\text{Ru(TAP)}_2 \text{dppz}]^{1+} \).](image)

Figure 7.4 Absorption spectrum of \( 8 \times 10^{-5} \text{ M } [\text{Ru(TAP)}_2 \text{dppz}]^{2+} \) in aqueous 10 mM phosphate buffer. Arrow indicates pump wavelength.

The absorption spectrum of the difluoro substituted dppz ligand shows a red shift of the \(^1\text{IL}\) band, indicating a lowering of energy of this band, possibly because of the lesser degree of electron density in the extended chromophore resulting from the electron withdrawing fluorines. The shifts for the different complexes are given in Table 7.1.
Complex & $\lambda_{\text{max}}$ (nm) & $\lambda'_{\text{max}}$ (nm) \\
\hline
[ Ru(TAP)$_2$ dppz$^{2+}$ ] & 273 & 414 \\
[ Ru(TAP)$_2$ F$_2$ dppz$^{2+}$ ] & 277 & 408 \\
[ Ru(TAP)$_2$ dppz$^{2+}$ and [ poly(dG-dC) ]$_2$ (1:20) ] & 258 & 418 \\
\hline

Table 7.1 Wavelength maxima for 1 (8 x 10$^{-5}$ M) and 2 (8.5 x 10$^{-5}$ M) in aqueous 10 mM phosphate buffer.

At the excitation wavelength of 400 nm it is probable that the molecule will be excited partly to a dppz-localised ($\pi-\pi^*$) excited state and partly to its MLCT Ru(TAP) states. At the other excitation wavelength available, 267 nm, we expect direct excitation of IL TAP or dppz bands. There is a sizeable shift of the absorption maxima for the IL transitions on binding of 1 to [poly(dG-dC)]$_2$. The lower wavelength band red-shifts by 15 nm. However the band excited around 400 shows only a small blue-shift of 4 nm on binding to the polynucleotide.

### 7.3 Transient absorption (TA) – 2001

#### 7.3.1. Aqueous Solution

The transient spectra of [Ru(TAP)$_2$(dppz)$^{2+}$] in aqueous buffer were recorded at intervals between 0.5 and 1500 ps after excitation with 400 nm light. It was observed that there were substantial spectroscopic changes occurring only over the first 100 ps, after which time a long-lived species is formed. Figure 7.5 shows the spectra at 4 ps, 100 ps and 1000 ps. The nature of this fast process (lifetime 43 ± 4 ps) is uncertain but it could involve conversion from an initially formed IL ($\pi-\pi^*$) state to the lowest-lying MLCT excited state. The latter is a long-lived (1090 ns) emissive state and has been shown by transient resonance Raman spectroscopy (TR$^3$) to correspond to an excited state where the electron is located on one of the TAP ligands. $^5,8$
Figure 7.5 Transient absorption (TA) spectra of [Ru(TAP)$_2$(dppz)]$^{2+}$ ($8 \times 10^{-5}$ M) in 10 mM phosphate buffer, observed at 4, 100 and 1000 ps following laser excitation $\lambda_{exc} = 400$ nm.

This figure also shows a substantial TA signal over the experimental range, 430-650 nm. (In some experiments an experimental artefact appeared in the bleach region, prompting us to rely on kinetics from the absorption region. This artefact will be discussed further in the 2002 results section.)

7.3.2 Aqueous Solution and Polynucleotides

The samples that were examined were 1 alone, with [poly(dA-dT)$_2$] (P/Ru ratio 20:1), with ct-DNA 6:1, with ct-DNA 20:1 and finally with [poly(dG-dC)$_2$] 20:1, all in aqueous buffer. The concentration of 1 = $1.68 \times 10^{-4}$ M in 10 mM phosphate buffer. The transient spectra of 1 in aqueous buffer is shown in Figure 7.6. A decay is evident around 515nm and likewise when 1 is bound to [poly(dA-dT)$_2$] (not shown). Figure 7.6 shows a close up view of the region that we were examining for evidence of electron transfer, as the reduced complex [Ru(TAP)$_2$dppz]$^-$ absorbs in the region around 500 nm. The bleach region is not shown as it contains the artefact.
Kinetics were calculated for these data. The kinetics of 1 with [poly(dA-dT)]$_2$ also showed a rapid partial decay forming a long-lived state. This long-lived state was formed in 36 ± 8 ps, which is similar to that of the complex alone in solution. In the presence of DNA an increase in absorbance is seen at 515 nm. Analysed qualitatively it was seen that an increase in the GC content of the sample led to an increase in the absorbance seen at around 515 nm.

Considering the small decrease in absorption observed in the same region when 1 is bound to [poly(dA-dT)]$_2$, we can be fairly certain that the guanine is responsible for the process we are observing. The signal increased by at least two-fold when [poly(dG-dC)]$_2$ was substituted for DNA (Figure 7.7).
Figure 7.7 TA spectra of [Ru(TAP)_2(dppz)]^+ (1.68x 10^{-4} M) in 10 mM phosphate buffer with [poly(dG-dC)]_2 1:20 following laser excitation λ_{exc} = 400 nm showing an increasing signal around 515 nm.

Figure 7.8 shows the increase in signal seen as increasing amounts of GC were present. In the presence of [poly(dG-dC)]_2 the strong increase in absorption in this wavelength region is observed proceeding with a lifetime of 508 ± 53 ps. (Figure 7.9) The kinetics were performed over the range 508-513 nm as this seemed to be the region of greatest change in absorption given the experimental data. This is consistent with the formation of [Ru(TAP)_2(dppz)]^+ through electron transfer from the guanine to the excited ruthenium complex.

Figure 7.8 Showing the increase in signal from negative to positive on each subsequent addition of GC, as measured at 515 nm,
Figure 7.9 TA spectra observed at 4, 100 and 1000 ps following laser excitation $\lambda_{exc} = 400$ nm of $[\text{Ru(TAP)}_2(\text{dppz})]^2^+ (8 \times 10^{-5} \text{M})$ in 10 mM phosphate buffer in the presence of $[\text{poly(dG-dC)}]_2 (1.6 \times 10^{-3} \text{M}$ nucleotide). Inset: Kinetics at 508-513 nm

Note the magnitude of the TA signal in this case, $\Delta \text{abs.} = +0.002$. While we were convinced that what we were seeing was most probably the reduction of the ruthenium complex with electron transfer occurring on a picosecond timescale we were unable to detect any back electron transfer occurring on the time-scale of 0-1500 ps. Nanosecond flash photolysis studies from 20 ns onwards found no reduced species present.  

7.4 Artefact

There was an artefact that appeared in the bleach region in certain measurements in 2001 and again in 2002, Figure 7.10 shows an example of the artefact. One of the issues that had to be resolved was to discover if this was indeed an experimental artefact and, if so, how to eradicate it. This artefact had also appeared in the work of a colleague performed on the same ruthenium complex the previous year.

The artefact appeared in the bleach region when using $[\text{Ru(TAP)}_2(\text{dppz})]^2^+$ in both water and acetonitrile, so it did not seem to be dependent on solvent polarity or the extent of hydrogen bonding in the sample. It occurred when using either 400 nm or 267 nm excitation wavelength and absorbed in the same part of the spectrum for each consecutive time-delay in a measurement.

7-9
Some preliminary runs were performed to discover what concentration of $[\text{Ru(TAP)}_2\text{(dppz)}]^{2+}$ would give an optimum TA signal and it was realised that the appearance of the artefact coincided with increasing concentration of the absorbing molecule. This prompted us to find an optimum concentration which would minimise the artefact whilst maintaining a good signal to noise ratio. This dependence on concentration was non-linear. When 1 was diluted by a half the gain signal decreased by a third, however the magnitude of the bleach signal increased 40-fold. Figure 7.11 shows a diminished artefact due to using a smaller concentration of complex.
The appearance of the artefact seemed somewhat like interference fringes. The mutual alignment of the pump and probe beams did not seem to have any effect on it, so it should not be a result of interference between the pump and probe beam (which were almost collinear). Also the fact that it appeared when the sample was excited at 267 nm would exclude interference as a factor.

It was thought that perhaps scattering could have been a factor. The scattered flux density is proportional to the fourth power of the frequency, so for 400 nm light, the frequency is 750 THz, and it will be scattered 1.25 times more than 500 nm (600 THz) light. Scattering does not cut off above a certain threshold, whereas in each experimental run the artefact is not seen much above 500 nm.

A possible factor is a thermal lensing (thermal blooming) effect, formed by the exciting pulse.10 This is a non-linear effect where a pump pulse encounters the sample and through a localised heating effect produces a change in the local refractive index. Thermal lensing normally occurs when the rep. rate of the laser is too high and heating of the sample occurs, however the increased concentration of the sample could make it harder for heat to dissipate from the complex to the solvent. Thermal lensing is sometimes used as a sensitive method for measuring concentrations. The threshold for the onset of the thermal lensing effect with
increasing rep. rate is lowered when the exciting pulse is close to an absorption band of the sample.

Another fact that supports the presence of a thermal effect is that the artefact also appears in the negative time delays (on a much smaller scale), indicating that the additional heat has not had time to dissipate completely between excitations. The artefact did not occur when the flow-cell was used (in other experiments where there were larger quantities of complex available for solutions), but only when the small 5 mm² cell was used which had a stirrer bar agitating the sample. In this small cell the stirring may not have been enough to supply new complex for every subsequent excitation, especially as the pump and probe beams were overlapped close to the cell wall (in order to probe the most excited states).

Due to time restrictions the artefact itself was not investigated fully. Further investigation would be required to verify that the artefact seen was due to interference and, if so, how it arises. Some of the 2001 data shown was of better quality than others, the more concentrated solutions of 1 contributed to a larger TA signal, but unfortunately also quite a sizeable artefact. In 2002 not only was an optimum concentration found for the ruthenium complexes where the artefact had little effect but also the signals were checked more rigorously for any mutual drift. This meant that the size of the TA signals in the gain region were much greater, increasing the signal to noise ratio from 23 to 111, measured for 1 in aqueous solution in the bleach region.

7.5 Transient absorption (TA) – 2002

7.5.1 Aqueous Solution
The TA spectra for [Ru(TAP)₂(dppz)]⁺⁺(I) in buffered H₂O is shown in Figure 7.12. The inset kinetics show evidence for a short-lived species. Thus monitoring at 515 nm reveals a partial decrease in the transient absorption giving a first order decay rate constant of 2.5 ± 0.3 x 10¹⁰ s⁻¹ (41 ± 4 ps) (Δ abs. arb = 0.003) and similar rate constants are obtained at other wavelengths, whether in the transient bleaching or absorption regions. The same decay rate constant is found in buffered D₂O solution.
Figure 7.12 \([\text{Ru(TAP)}_2\text{dppz}]\text{Cl}_2\ (8.5 \times 10^{-3} \text{ M})\) in 10 mM aqueous phosphate buffer, following laser excitation \(\lambda_{\text{exc}} = 400\ \text{nm}\). For clarity only traces at 10, 20, 70 and 1000 ps shown. Inset: kinetics at 515 nm

Figure 7.13 \([\text{Ru(TAP)}_2\text{F}_2\text{dppz}]\text{Cl}_2\ (8.5 \times 10^{-3} \text{ M})\) in aqueous solution, following laser excitation \(\lambda_{\text{exc}} = 400\ \text{nm}\). Inset: kinetics at 515 nm

In \(\text{H}_2\text{O}\) buffered solution \([\text{Ru(TAP)}_2\text{F}_2\text{dppz}]^{2+}\) (2) shows similar features in its TA spectra. A partial decay is seen over all wavelengths, Figure 7.13. At 515 nm the kinetics were \(\tau = 6 \pm 2\ \text{ps}\). A similar increase of the bleaching is seen in the region below 500 nm, as seen with 1 in aqueous solution.
7.5.2 Acetonitrile Solution

Analogous results are also found when the transient absorption spectra of 1 are determined in acetonitrile (Figure 7.14), as these also show that the excited state is formed is long-lived and that there is also evidence of a very rapid process, (with a lifetime of $9 \pm 2$ ps) although the signal is weaker than in water, ($\Delta$ abs. arb = .0012), even though the concentration of 1 is the same.

![Figure 7.14](image)

It was mentioned in section 7.2 that excitation of 1 at 400 nm will induce transitions to both dppz IL and MLCT, and it is probable that the transient signals determined both in water and acetonitrile are a consequence of inter-state processes. To test this hypothesis we also carried out excitation of 1 in acetonitrile solution at 267 nm, where the initial species produced is expected to be predominantly IL (dppz) or IL (TAP). The results obtained were similar ($10 \pm 4$ ps from Figure 7.15) to those found on excitation at 400 nm, indicating that, as with other metal polypyridyl complexes, deactivation of the singlet excited states by internal conversion takes place very rapidly. \cite{11, 12}
To see if 1 in H₂O would yield similar results we examined the kinetics of 1 in aqueous solution after excitation at the shorter wavelength.

We see that a similar long-lived species is formed with single exponential kinetics of 18 ± 6 ps, (Figure 7.16).
7.5.3 Aqueous Solution and Polynucleotides – [poly(dG-dC)]₂

The dominant feature in Figure 7.17 is strong absorption in the region 500-550 nm, which grows in with a lifetime of $\tau = 507 \pm 50$ ps ($2 \times 10^5$ s$^{-1}$) at 515 nm, this is an average value for 4 separate measurements of the same sample concentration in H$_2$O. This is consistent with our previous measurements for the formation of the reduced complex [Ru(TAP)$_2$(dppz)]$^{2+}$, as a result of electron transfer from guanine in H$_2$O buffer, (2001 results $508 \pm 53$ ps). Additionally a very rapid (4 ± 2 ps) process occurs before the onset of the grow-in. This ultrafast process could be an interstate process similar to that suggested for the complex in solution, the value being closer to that observed for the organic solvent, possibly indicating that the process is sensitive to the solvent polarity. The bleach region is recovering quite substantially unlike the case of 1 in aqueous solution alone.

It may be noted that in the experimental results displayed in Figure 7.17 the reduced product of 1 appears to be stable at 1500 ps. However in earlier nanosecond flash photolysis experiments we were unable to find evidence for electron transfer products when 1 was bound to [poly(dG-dC)]$_2$. This implies that the transient must have a lifetime at least less than 50 ns. Transient absorption studies were subsequently performed using the longer delay line,
allowing measurements to be made out to 5 ns. These data (Figure 7.18) provide evidence for partial decay of the 510 nm absorbing species. Assuming that the species decays to zero absorbance a decay rate constant of $1.1 \pm 0.2 \times 10^8$ s$^{-1}$ (9 ns) can be derived for this process.

![Figure 7.18](image)

Figure 7.18 $[\text{Ru(TAP)2dppz}]^2\text{Cl}_2$ (8.5 x 10$^{-5}$ M) in 10 mM aqueous phosphate buffer in the presence of [poly(dG-dC)]$_2$ (1.7 x 10$^{-5}$ M nucleotide) following laser excitation $\lambda_{exc} = 400$ nm. Long times shown. Black arrow indicates grow-in from forward ET, red arrow indicates decay from back ET.

To determine whether the photoreduction process was affected by the deuteration of the solvent (and hence isotopic substitution of exchangeable protons in the DNA bases), the transient absorption of 1 intercalated in [poly(dG-dC)]$_2$ in buffered D$_2$O solution was performed. Figure 7.19 shows the data compared to that in H$_2$O. It was found that both the forward and back reactions were significantly slower, taking 723 ± 90 ps (average value for 2 measurements) and 16.9 ns (with rate constants of $1.4 \pm 0.3 \times 10^9$ s$^{-1}$ and $0.6 \pm 0.2 \times 10^8$ s$^{-1}$ respectively) than in H$_2$O, yielding isotope effects of approximately 1.4 for the forward ET and 1.9 for the back ET, when data was fitted to single-exponential fits. The kinetic fits in Figure 7.19 are biexponential functions which appear to fit the experimental data well, the results are tabulated in Table 7.2.
Figure 7.19  TA data and kinetics at 515 nm for [Ru(TAP)2dpzp]Cl₂ (8.5 x 10⁻³ M) in the presence of [poly(dG-dC)]₂ (1.7 x 10⁻³ M nucleotide) in 10 mM aqueous phosphate buffer prepared with H₂O and D₂O, following laser excitation λ₀ = 400 nm

The results from different experimental runs fitted to the biexponential function indicate a slightly faster process occurring for the back electron transfer process in both solvents than the single exponential fits show.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent</th>
<th>Grow-in (ps)</th>
<th>Grow-in (s⁻¹)</th>
<th>Decay (ps)</th>
<th>Decay (s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 with [poly(dG-dC)]₂</td>
<td>H₂O</td>
<td>526 ± 35</td>
<td>1.9 x 10⁻⁹</td>
<td>8872 ± 620</td>
<td>0.1 x 10⁻⁹</td>
</tr>
<tr>
<td>1 with [poly(dG-dC)]₂</td>
<td>H₂O</td>
<td>528 ± 35</td>
<td>1.9 x 10⁻⁹</td>
<td>8810 ± 597</td>
<td>0.1 x 10⁻⁹</td>
</tr>
<tr>
<td>1 with [poly(dG-dC)]₂</td>
<td>D₂O</td>
<td>667 ± 51</td>
<td>1.5 x 10⁻⁹</td>
<td>14043 ± 1770</td>
<td>7.1 x 10⁻⁷</td>
</tr>
</tbody>
</table>

Table 7.2 Kinetic data fitted with biexponential fits for [Ru(TAP)2dpzp]Cl₂ (8.5 x 10⁻³ M) in the presence of [poly(dG-dC)]₂ (1.7 x 10⁻³ M nucleotide) in 10 mM aqueous phosphate buffer prepared with H₂O and D₂O.
Figure 7.20 [Ru(TAP)$_2$F$_2$dpdz]Cl$_2$ (8.5 x 10$^{-5}$ M) in the presence of [poly(dG-dC)]$_2$ in 10mM phosphate H$_2$O buffer, following laser excitation $\lambda_{exc} = 400$ nm.

Figure 7.20 shows the TA spectra for 2 in aqueous solution. The data in the absorption region indicates that the same process is occurring as for the parent complex. Figure 7.21 overleaf shows the data at 515nm for the grow-in and decay of the parent complex and the substituted complex. It is clear that the forward ET kinetics seem to be slower for 2 in water than 1 in water. The time taken for the forward electron transfer is 554 ± 86 ps which gives a rate constant of $1.8 \times 10^9$ s$^{-1}$ and the back electron transfer takes 20.2 ± 6 ns ($5 \times 10^7$ s$^{-1}$), fitted as a biexponential function. Figure 7.21 shows the kinetic fits to normalised TA data for 1 and 2 in H$_2$O buffer and with 1 in buffered D$_2$O for additional comparison. The fluoro substitution causes the initial electron transfer process to slow, compared with the parent complex in H$_2$O buffer. The back electron transfer process of 2 in water is also slower compared with 1 in D$_2$O.
Figure 7.21 Kinetic biexponential fits to normalised TA data of 1 \((8.5 \times 10^{-5} \text{ M})\) and 2 \((8.5 \times 10^{-5} \text{ M})\) in the presence of \([\text{poly(dG-dC)}]_2\) in 10 mM phosphate \(\text{H}_2\text{O}\) or \(\text{D}_2\text{O}\) buffer, (no data points shown). Kinetics shown at 515 nm.

Unfortunately there was not enough time to make measurements on the deuterated solution, to measure if an isotope effect was also present for the difluoro derivative.

7.5.4 Aqueous Solution and Polynucleotides – \([\text{poly(dA-dT)}]_2\)

Experiments conducted with 1 bound to \([\text{poly(dA-dT)}]_2\) (1:20) revealed a behaviour quite different from that with the GC - polynucleotide (Figure 7.22). There is only a weak increase in absorption observed in the 510 nm region, after the initial fast process is complete. On the timescale of this experiment the bleach is seen to undergo a partial recovery, as for 1 bound to \([\text{poly(dG-dC)}]_2\).
Figure 7.22 TA spectra of [Ru(TAP)2dppz]Cl2 (8.5 x 10^{-5} M) in 10 mM aqueous phosphate buffer in the presence of [poly(dA-dT)]2 (1.7 x 10^{-3} M nucleotide) following laser excitation $\lambda_{\text{exc}} = 400$ nm. Inset: data at 515nm

The kinetics for the recovery in the bleach at 460 nm give $\tau = 4420 \pm 553$ ps. This is approximately twice as slow as the recovery in the presence of the GC polymer. There were no studies performed at long delay times with this polynucleotide.

Similarly, with [poly(dA-dT)]2 shows a small recovery in the bleach, with $\tau = 9622 \pm 1853$ for the process to go back to zero (Figure 7.23).

Figure 7.23 TA spectra of [Ru(TAP)2 F2 dppz]Cl2 (8.5 x 10^{-5} M) in 10 mM aqueous phosphate buffer in the presence of [poly(dA-dT)]2 (1.7 x 10^{-3} M nucleotide) following laser excitation $\lambda_{\text{exc}} = 400$ nm.

Table 7.3 overleaf shows the results for all the visible transient absorption results fitted to single exponential kinetics.
<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>SOLVENT</th>
<th>Time-scale</th>
<th>τ at 460 nm</th>
<th>τ at 515 nm</th>
</tr>
</thead>
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<td>(1) in CH$_3$CN</td>
<td>CH$_3$CN</td>
<td>Short</td>
<td>10 ± 4</td>
<td></td>
</tr>
<tr>
<td>(1) in H$_2$O buffer</td>
<td>H$_2$O</td>
<td>Short</td>
<td>18 ± 6</td>
<td></td>
</tr>
<tr>
<td>(1) in CH$_3$CN</td>
<td>CH$_3$CN</td>
<td>Short</td>
<td>9 ± 2</td>
<td></td>
</tr>
<tr>
<td>(1) in H$_2$O buffer</td>
<td>H$_2$O</td>
<td>Short</td>
<td>39 ± 3</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>(1) in D$_2$O</td>
<td>D$_2$O</td>
<td>Short</td>
<td>43 ± 5</td>
<td>37 ± 4</td>
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<tr>
<td>(1) with dG-dC</td>
<td>H$_2$O</td>
<td>Short</td>
<td>1376 ± 373</td>
<td>2346 ± 108</td>
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<tr>
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<td></td>
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<td>473 ± 30</td>
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<td>(50-1500 ps)</td>
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<td>(1) with dG-dC</td>
<td>H$_2$O</td>
<td>Short</td>
<td>546 ± 74</td>
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<td>(50-1500 ps)</td>
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<td>(1) with dG-dC</td>
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<td>Short</td>
<td>702 ± 92</td>
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<td>(75-1500 ps)</td>
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<td>(1) with dG-dC</td>
<td>D$_2$O</td>
<td>Short</td>
<td>744 ± 99</td>
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<td>(45-1500 ps)</td>
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<td>(1) with dA-dT</td>
<td>H$_2$O</td>
<td>Short</td>
<td>271 ± 55</td>
<td>Grow</td>
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<td>4420 ± 553</td>
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<td>(y$_w$=0)</td>
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<tr>
<td>(1) with dG-dC</td>
<td>H$_2$O</td>
<td>Long</td>
<td>1676 ± 200</td>
<td>2864 ± 212</td>
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<td>355±30,</td>
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<td>9.2±0.3</td>
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<tr>
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<td>ns</td>
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<tr>
<td>(1) with dG-dC</td>
<td>H$_2$O</td>
<td>Long</td>
<td>1685 ± 116</td>
<td>2932 ± 200</td>
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<td>434±38, 8.3</td>
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<td>± 0.7</td>
</tr>
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<td>ns</td>
</tr>
<tr>
<td>(1) with dG-dC</td>
<td>D$_2$O</td>
<td>Long</td>
<td>1831 ± 130</td>
<td>3830 ± 294</td>
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<td>17.0±0.9</td>
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<tr>
<td>(2)</td>
<td>H$_2$O</td>
<td>Short</td>
<td>Decay</td>
<td>6</td>
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<td>(2) with dA-dT</td>
<td>H$_2$O</td>
<td>Short</td>
<td>260 ± 133</td>
<td>508 ± 148</td>
</tr>
<tr>
<td>(2) with dG-dC</td>
<td>H$_2$O</td>
<td>Short</td>
<td>Grow</td>
<td>545 ± 87</td>
</tr>
<tr>
<td>(2) with dG-dC</td>
<td>H$_2$O</td>
<td>Long</td>
<td>1611 ± 295</td>
<td>3130 ± 288</td>
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<td>20.3±2</td>
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Table 7.3 Kinetics for TA results for [Ru(TAP)$_2$dpdp$_2$$]^{3+}$ (1) and [Ru(TAP)$_2$F$_2$dpdp$_2$$]^{3+}$ (2002 ), following laser excitation $\lambda_{exc} = 400$ nm, or ($\lambda_{exc} = 267$ nm). Single exponential fits. Non-italicised numbers denote decay kinetics, bold italics denote a "grow-in" Kinetics calculated over 5 nm, centre wavelength indicated. Numbers in brackets indicate the range over which kinetics were calculated. Values for $\tau$ are in picoseconds unless otherwise indicated. All aqueous solutions phosphate buffered to 10 mM.
7.6 IR Transient absorption (PIRATE)

7.6.1 Aqueous Solution and [poly(dG-dC)]$_2$

These measurements were carried out by D. C. Grills, J. Dyer (Nottingham University) and Prof. J. M. Kelly. The PIRATE measurements were carried out using 400 nm excitation and probing the bands associated with the polynucleotides. 1 in aqueous solution alone will not show any absorption in the fingerprint region for DNA c. 1620-1680 cm$^{-1}$, it is only the DNA bound complex that will show absorption in this region. By pumping at 400 nm we are only directly exciting (1), as the absorption of the DNA bases is below 300 nm.

Samples of 1 in D$_2$O buffer were made up with both [poly(dA-dT)]$_2$ and [poly(dG-dC)]$_2$, to investigate if the results from transient visible absorption could be corroborated by IR measurements. In the DNA fingerprint region (1600-1690 cm$^{-1}$) 1 does not show any bands. However it is expected that the bound complex will show bands associated with the electron transfer process proceeding from guanine and possibly of the protonation of cytosine. The signals in the IR region are due to the carbonyl bonds on each of the nucleobases.

Figure 7.24 shows the DNA fingerprint region and it can clearly be seen that some processes are proceeding on a very short time-scale, with $\tau <10$ ps. The kinetics of the guanine band at 1680 cm$^{-1}$ were studied first. Figure 7.25 shows the longer time delay kinetics fitted at 1680 cm$^{-1}$ (single-point kinetics). A single-exponential decay fitted to these data yields $\tau = 678 \pm 290$ ps. This slower process is preceded by a fast process which
takes 10 ± 1 ps. The decay process falls within the error margin of the grow-in associated with the formation of the reduced form of 1 in D$_2$O, proceeding with $\tau = 744 \pm 99$ ps. So at first glance it appears that the PIRATE data agrees with the visible data for 1.

Figure 7.25 PIRATE results for [Ru(TAP)$_2$dpdz]Cl$_2$ (1.7 x 10$^{-3}$ M) in the presence of [poly(dG-dC)$_2$] (1:20) in 10 mM aqueous phosphate buffer D$_2$O, kinetics of guanine band at 1680 cm$^{-1}$

The cytosine band at 1649 cm$^{-1}$ was fitted to single point kinetics and the connected data points are shown in Figure 7.26. Again two processes are taking place, the fast process takes 15 ± 4 ps to grow in, and subsequently shows a decay over 392 ± 94 ps, fitted from 75-2000 ps. This suggests that the process associated with cytosine proceeds at a faster rate than for guanine. The kinetics representing the forward electron transfer were fitted over the same time ranges as those for the visible TA results.

Figure 7.26 PIRATE results for [Ru(TAP)$_2$dpdz]Cl$_2$ (1.7 x 10$^{-3}$ M) in the presence of [poly(dG-dC)$_2$] (1:20) in 10 mM aqueous phosphate buffer D$_2$O, kinetics of cytosine band at 1649 cm$^{-1}$

7-24
Figure 7.27 PIRATE results for [Ru(TAP)2dppz]Cl2 (1.7 x 10^{-3} M) in the presence of [poly(dG-dC)]2 (1:20) in 10 mM aqueous phosphate buffer D2O, time delays shown less 10 ps at which time vibrational cooling should be finished.

Figure 7.27 shows the PIRATE results for 1 and [poly(dG-dC)]2, less the TA signal at 10 ps. It is thought that by this time all vibrational cooling processes should have finished. This data has been fitted by Marina Kuimova in Nottingham University, who fit a series of Lorentzian curves to the data. This fitting shows a band that appears after 300 ps, which they associate with the electron transfer process.
7.6.2 Aqueous Solution and [poly(dA-dT)]$_2$

Figure 7.28 PIRATE of [Ru(TAP)$_2$dppz]Cl$_2$ (1.7 x 10$^{-3}$ M) in the presence of [poly(dA-dT)]$_2$ (1:20) in 10 mM aqueous phosphate buffer in the (1.7 x 10$^{-3}$ M nucleotide) following laser excitation $\lambda_{exc} = 400$ nm.

Figure 7.28 shows the PIRATE results for 1 in the presence of [poly(dA-dT)]$_2$. Note the magnitude of the signal is much less as this polynucleotide has a smaller extinction coefficient than its GC analogue. The kinetics for the thymine main band are shown in Figure 7.29. Similarly a fast process is seen followed by a slow process. This fast process takes 15 ± 8 ps.

Figure 7.29 Kinetics of [Ru(TAP)$_2$dppz]Cl$_2$ (8.5 x 10$^{-5}$ M) in 10 mM aqueous phosphate buffer in the presence of [poly(dA-dT)]$_2$ (1.7 x 10$^{-3}$ M nucleotide) following laser excitation $\lambda_{exc} = 400$ nm.
7.7 Discussion

7.7.1 Visible TA

All TA spectra showed a strong bleaching between 450 and 500 nm and a region of weaker positive absorption above 500 nm. It is clear that for the ruthenium complexes in buffered solution an excited state species is produced, which has a lifetime greater than 10 ns, consistent with the previous emission lifetime studies which showed that the Ru-TAP MLCT state has a lifetime of 1090 ns in aqueous solution.\(^5\) (The assignment of this excited state has been confirmed by transient resonance Raman spectroscopy.\(^8\)) This behaviour contrasts with that of \([\text{Ru(phen)}_2(\text{dppz})]^{2+}\) (2), where two excited states are formed which have lifetimes of 3-7 ps and 250 ps in aqueous solution.\(^{13}\) Our results indicate that an electron transfer process occurs in the presence of guanine to the excited Ruthenium complexes. The appearance of a signal in the absorption region of the TA spectra (circa 515 nm) was associated with the formation of the reduced \([\text{Ru(TAP)}_2\text{dppz}]^{2+}\) complex. Initial results (7.3.2) suggested that the ET process only occurred in the presence of guanine.

DNA was not used for the measurements in 2002 as it was felt that the system was too complex. A well-defined system was preferred where every complex of 1 should be bound adjacent to a guanine. The different ratios of binding sites available to chromophores in DNA, and how this produces multieponential kinetics for ET were discussed in the previous chapter (section 6.4).

Studies performed in 2002 showed a small increase in the absorption signal around 515 nm for the AT polynucleotide, albeit much less than for the GC polymer. However the kinetics in the bleach region of the spectrum showed a partial recovery of the bleach, as for 1 in the presence of guanine. This was in contrast to 1 and 2 in buffer alone, where the long-lived emissive state formed shows a stable TA signal after ~ 40 ps.

This small effect for the AT polymer was not observed at all during the initial experiments in 2001 as the experimental artefact had wildly distorted results in the bleach region. Subsequently no experiment was performed at long times with the ruthenium complexes and \([\text{poly(dA-dT)}]_2\); as at the time the possible significance of the bleach region went unnoticed. An experiment performed on 1 at long delays with the AT polynucleotide could have allowed us to calculate kinetics for the forward and back ET reaction with adenine.
The driving force for ET involving complex 1 can be estimated from knowing the reduction potential for the excited state \( \text{E}^0(\text{[Ru(TAP)}_2\text{(dppz)]})^{2+}/[\text{Ru(TAP)}_2\text{(dppz]}^+ \) (1.42 V) (vs NHE) and the oxidation potential of the polynucleotide bases. The values of 1.53 V for guanine in \([\text{poly(dG-dC)}])_2\), and 2.03 V for adenine in \([\text{poly(dA-dT)}])_2\). This gives a standard redox potential of +0.11 V for the reaction involving guanine and +0.61 V for the corresponding process involving adenine. These indicate that the forward ET process is weakly endergonic in the presence of guanine and much more so in the presence of adenine, proving the reaction involving adenine to be much less thermodynamically favoured. The fact that there is only a weak thermodynamic driving force for the reactions leads to much slower kinetics than for thionine, examined in the previous chapter.

The di-fluoro substituted analogue of 1 was studied in the same solvent environments and at similar concentrations to determine how this would affect the kinetics for the system. Figure 7.3 shows the dppz ligand with fluorine substitution, which will be intercalated into the polynucleotides. The fluoro-substituted ligand was chosen so that the electron-withdrawing effect of the fluoro might in turn affect the kinetics of the reaction. The small size of the fluorine should not cause the polynucleotide to unravel much more than in the normal intercalative manner for dppz.

If the electron transfer took place via the dppz ligand a more bulky substituent might increase the dppz-guanine distance enough for the kinetics to be affected. This of course would give an indication of the exact pathway that the electron transfer is taking, i.e. whether the electron travels through-bond or through space. If the electron transfer occurs through space (or through solvent) then changing the dppz-guanine distance may not have much of an effect on the ET kinetics. It was also noted that in the ground-state absorption we are not seeing a large contribution from the MLCT dppz, and the nature of the excited state of 1 in aqueous solution has been determined as TAP based.

We did however see a change in the kinetics for the difluoro substituted ligand. The rate of ET was slowed in 2 compared with the parent complex. Although the change was not great for the forward ET process, \( (k_2 = 1.8 \times 10^9 \text{ s}^{-1}, \text{ compared with } k_1 = 1.9 \times 10^9 \text{ s}^{-1}) \), the rate did change drastically for the back electron transfer process \( (k_2 = 5 \times 10^7 \text{ s}^{-1}, k_1 = 0.1 \times 10^9 \text{ s}^{-1}) \).
The rates of both the forward and back electron transfer reactions are shown to be significantly slower in deuterated water, demonstrating a solvent isotope effect. From this effect and supported by the weak thermodynamic driving force behind electron transfer, it is most probable that we are observing a proton-coupled electron transfer (PCET). \(^{15,16,17}\)

(Scheme 7.1)

\[
[Ru(TAP)\_2(dppz)]^{2+} + G
\]

\[
\begin{align*}
\text{k} &= 2 \times 10^9 \text{ s}^{-1} \text{ in } H_2O \\
\text{k} &= 1.4 \times 10^8 \text{ s}^{-1} \text{ in } D_2O \\
\end{align*}
\]

In the previous chapter electron transfer was monitored in a system where only one chromophore was present. This system is more complex however because of the presence of multiple chromophores in each ET reaction. However the fact that the emissive state of \(1\) in water is one in which charge resides on the TAP ligand, should enable us to postulate a suitable pathway for the ET reaction. \(^8\)

(Scheme 7.2: Schematic representation of PCET between 1 and guanine, the reduced guanine nucleobase is coloured red. Picture courtesy of M. Feeney.)
Due to the fact that the lowest excited state of 1 is TAP based, we expect the initial excitation to rapidly form an MLCT Ru-TAP state (in < 10 ps, according to the ultrafast early process), after which electron transfer occurs from the guanine to the ruthenium centre. This process is coupled to a deprotonation of the guanine where the proton ends up on the cytosine nucleobase. The distance between electron donor and the metal acceptor is greater than that of an intercalated acceptor, as was the case in the previous chapter. Although the rate for electron transfer is ultrafast, it is much slower than for thionine intercalated into the GC polynucleotide.

Scheme 7.2 shows the relative positions of 1 and the guanine nucleobase for the electron transfer process. Scheme 7.3 shows the process of deprotonation of the guanine radical cation after electron transfer. In PCET the deprotonation of the nucleobase is the rate determining step, where the electron transfer precedes the deprotonation of the radical cation. In D$_2$O the hydrogens indicated will have been substituted by deuterium atoms. It is expected that the perturbation of this system should give a signal in the IR associated with the guanine and cytosine bands, particularly at 1649 cm$^{-1}$, where the carbonyl group is directly involved with the proton transfer.

![Scheme 7.3: Proton transfer from guanine to cytosine as part of a proton coupled electron transfer process.](image)

**Scheme 7.3: Proton transfer from guanine to cytosine as part of a proton coupled electron transfer process.**

*Picture: J. Dyer PhD thesis.*

### 7.7.2 PIRATE

For the first time we report the direct monitoring of the vibrational spectra of the nucleobases on a picosecond timescale, providing evidence for oxidation and other ultrafast processes.
When interpreting the PIRATE data one must remember that we are not probing the excited complex directly, but the vibrations of the nucleotides bound to the complex. The PIRATE results for I show kinetics for guanine that agree with the kinetics from the visible TA, where a fast decay process $10 \pm 1$ ps is followed by a slower grow-in process $\tau = 678 \pm 290$ ps, monitored at 1680 cm$^{-1}$. According to Scheme 7.3, the carbonyl group located on the guanine will not be directly involved in the proton transfer.

The signal at 1649 cm$^{-1}$ is associated with cytosine, specifically the C2 carbonyl group which would be involved directly in the proton transfer (Figure 7.30). The kinetics of this band give $\tau = 392 \pm 94$ ps, which is hard to explain given the fact that the proton is thought to transfer after the electron, so the process should take longer than the ET.

For I with [poly(dA-dT)]$_2$ it may be harder to see a signal resulting from electron transfer because the extinction coefficient is lower for this polynucleotide. Also because the electron would be expected to transfer from adenine and there are no C=O groups on adenine itself, (thymine has two), any signal from the initial electron transfer might be harder to monitor for an AT process (Figure 7.31). One could speculate that if there were a proton coupled electron transfer that the signal associated with the C4 carbonyl group which would be accepting the proton would be the most perturbed vibrational signal, absorbing at 1663 cm$^{-1}$. The process seen associated with thymine vibrations at this band is again a fast ($15 \pm 8$ ps) process followed by a much slower process. However the kinetics seen do not follow a decay and subsequent grow-in as do the kinetics for the GC polymer.
7.8 Conclusions

We present evidence of a proton coupled electron transfer reaction occurring predominantly from the guanine nucleobase to the excited state ruthenium complexes. Redox potentials for 1 support the result that ET is more likely from guanine than the adenine nucleobase, although there is evidence for the process occurring on a much smaller scale in the AT nucleotide. This suggests that with the AT polynucleotide photoreduction is a minor process, and is consistent with our suggestion from the previous year that the excited state of 1 can efficiently oxidize guanine but not the other nucleobases of DNA.

The fluorescence of 1 and 2 is quenched by the presence of DNA. The substitution of the intercalating ligand can alter the rate of ET, however the driving force of the reaction appears to be from the TAP ligand as the lowest lying excited state. From the evidence presented we suggest that the electron transfer process is responsible for this quenching.

Monitoring the nucleobases with PIRATE can give additional evidence for the ET process in the GC nucleotide, however the results give conflicting evidence for a proton-coupled process.


8. RESULTS: \([\text{Ru(phen)}_2\text{dpqa}]^{2+}\) complex

8.1 Introduction

There is great interest in photophysical sensors for nucleic acids and particularly for DNA-recognising molecules, which, while essentially non-luminescent in water, become luminescent when DNA is present in solution. This effect is termed “light-switching”, and it contrasts with the fluorescence quenching effect that the previous ruthenium complex studied (\([\text{Ru(TAP)}_2\text{dppz}]^{2+}\)) showed in the presence of guanine (Chapter 7). A number of transition metal complexes were investigated for this effect. One type of molecule, which has attracted great interest for this purpose is \([\text{Ru L}_2\text{(dppz)}]^{2+}\) (\(L = 2,2’\)-bipyridyl or 9,10-phenanthroline). The reason for its activity has been shown to be due to the fact that its lowest excited state is long-lived in organic solvents (and also when intercalated between the base-pairs of DNA) but to be very short-lived in water, due to a complex photochemistry which has been revealed by ultrafast visible transient absorption and Raman spectroscopic techniques.

A new complex was synthesized which has the properties of a light-switch and can be studied using both transient visible absorption and picosecond IR transient spectroscopic (PIRATE) measurements. This is the first case of a ruthenium complex where the light-switch effect has been activated by a simple change in the ligand substituent.

![Figure 8.1](image)

Figure 8.1 the structure of \([\text{Ru(phen)}_2\text{dpqa}]^{2+}\), picture courtesy of K. O’Donoghue
The complex \([\text{Ru(phen)}_2\text{dpq}]^{2+} \) (4) (\text{phen} = 1,10-phenanthroline, dpq = dipyridoquinoxaline) is closely related to \([\text{Ru(phen)}_2\text{dppz}]^{2+} \) (5) (dppz = dipyrido[3,2-a: 2',3'-c] phenazine) but shows quite distinct photophysical properties. Thus 4 is strongly luminescent in both aqueous and organic solution (lifetime is 335 ns in water and 760 ns in acetonitrile). In aqueous solution the luminescence of 4 is enhanced by the presence of double stranded DNA, behaviour similar to that of many other ruthenium polypyridyl complexes. This is not ideal light switch behaviour however. Ideally there would be no emission at all (or very little) in aqueous solution and then strong emission in the presence of DNA as in 5.

By contrast the amide derivative of 4, \([\text{Ru(phen)}_2\text{dpqa}]^{2+} \) (dpqa = 2- dipyridoquinoxalinepenylamide), illustrated in Figure 8.1 shows dramatically different behaviour as it is essentially non-luminescent in water but emits strongly in acetonitrile (lifetime 1104 ns). This behaviour is analogous to that of 5. It also acts as a very effective light switch for nucleic acids as the quantum yield of fluorescence in the presence of double-stranded DNA is greater than that of the standard system 4. Another feature of this complex is the carbonyl bond on the amide, which can facilitate IR studies. To further understand the different natures of the excited state of \([\text{Ru(phen)}_2\text{dpqa}]^{2+} \) in aqueous and organic media we have examined the transient spectroscopic processes in both the visible and IR regions.

Measurements were performed of the complex \([\text{Ru(phen)}_2\text{dpqa}]^{2+} \) (3) in acetonitrile, H\(_2\)O and D\(_2\)O (non-buffered), using visible TA and PIRATE. The use of D\(_2\)O together with water allowed direct comparison between visible TA and PIRATE, and the additional possibility of detecting any isotope effect. All measurements were carried out in the Central Laser Facility in the Rutherford Appleton Laboratory under the supervision of Prof. J. M. Kelly. Details of the experimental set-up are in Chapter 4: Experimental Methods.
8.2 UV/vis Measurements

The absorption spectra of 3 in the three different solvents used are shown in Figure 8.2, with the excitation wavelength for the transient absorption and IR measurements indicated, \( \epsilon_{444} = 19700 \text{ dm}^3 \text{ M}^{-1} \text{ cm}^{-1} \) in CH\(_3\)CN, \( \epsilon_{443} = 15800 \text{ dm}^3 \text{ M}^{-1} \text{ cm}^{-1} \) in H\(_2\)O). This also looks very similar to the parent compound 4, indicating that the two complexes act in a similar way in their ground states. The bands from 350-500 nm are associated mainly with MLCT Ru (phen) and MLCT Ru (dpqa) transitions. The higher energy bands are predominately associated with IL (dpqa) transitions, although as with most polypyridyl complexes there is most likely some overlap between absorption bands. By exciting the complex at 400 nm we expect to produce mostly MLCT transitions.

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<th>Solvent</th>
<th>( \lambda_{\text{max}} ) (nm) IL band</th>
<th>( \lambda_{\text{max}} ) (nm) MCLT band</th>
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<td>CH(_3)CN</td>
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<td>H(_2)O</td>
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</tr>
<tr>
<td>D(_2)O</td>
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Table 8.1 Absorption maxima of [Ru(phen)\(_2\)dpqa]\(^{2+}\) in CH\(_3\)CN (1.4 \times 10^{-4} \text{ M}), H\(_2\)O(1.2 \times 10^{-4} \text{ M}) and D\(_2\)O(1.2 \times 10^{-4} \text{ M}).

Table 8.1 shows the absorption maxima for 3 in the three solvents used. There is a red shift in the absorption of the lowest excited state going from D\(_2\)O to CH\(_3\)CN.
8.3 Transient absorption (TA)

8.3.1 Acetonitrile Solution
Measurements were first performed of 3 in acetonitrile solution, probing over the range 430-650 nm. The spectra in Figure 8.3 show an artefact in the bleach (450-490 nm) region, this experimental artefact was discussed in the previous chapter and should not have to adverse an effect on the kinetics of the absorption region. There is a small decay in the $\Delta$ abs. signal at 570 nm, $\tau = 3 \pm 3$ ps, which forms a long-lived emissive state shown from previous studies to decay with $\tau = 1104$ ns (c.f. 760 ns for 2).

![Figure 8.3 Transient Absorption of [Ru(phen)$_2$dpqa]$^{2+}$ in acetonitrile $1.4 \times 10^{-4}$ M, following laser excitation $\lambda_{\text{exc}} = 400$ nm.](image)

8.3.2 H$_2$O Solution
Figure 8.4 shows the transient absorption spectra of 3 taken in water. The spectra show that the excited state decays ($\lambda > 511$ nm) and the ground state recovery ($\lambda < 511$ nm) both occur following first order kinetics with a lifetime of 663 ± 70 ps (c.f. 335 ns for 4), which is more than three orders of magnitude shorter than for the excited state in acetonitrile.

8-4
It may also be noted in Figure 8.5 that there is a precursor to this excited state, which forms with lifetimes of 6 ± 2 ps in H$_2$O, 8 ± 2 ps in D$_2$O and of 3 ± 3 ps in CH$_3$CN. While interstate processes such as intersystem crossing, vibrational relaxation or solvation, are possible candidates, it is probable that these would be significantly faster and we suggest that the most likely origin of the observed kinetics is an interligand electron transfer (ILET) from a Ru-phen (MLCT) (initially formed by 400 nm excitation of 3) to a lower-lying Ru (dpqa) MLCT state.
Figure 8.5 Transient Absorption kinetics at 570 nm for [Ru(phen)$_2$dpqa]$^{2+}$ in H$_2$O $1.2 \times 10^{-4}$M, following laser excitation $\lambda_{\text{exc}} = 400$ nm. Red line shows kinetics fit, red points are not included in kinetics.

Figure 8.6 Transient Absorption of [Ru(phen)$_2$dpqa]$^{2+}$ in D$_2$O $1.2 \times 10^{-4}$M, following laser excitation $\lambda_{\text{exc}} = 400$ nm.

8.3.3 D$_2$O Solution

The experiment carried out in deuterated water is shown in Figure 8.6, which gave a lifetime of $1526 \pm 150$ ps, indicating that the decay process is subject to a solvent isotope effect of ca. $2.3 \pm 0.4$ ps. This non-radiative pathway, which is much faster than the corresponding process for its dpq analogue 4, must involve specific interactions of water with the dpqa ligand. It is likely that the charge transfer state of 3 is an interligand electron...
transfer (ILET) involving the movement of an electron from the metal to the dpqa ligand and that the rapid deactivation is associated with appreciable electron density residing on the carbonyl function.

\[
[Ru(phen)_2dpqa]^{2+} \quad \text{Wavelength (nm)} \quad \tau \text{ for } y \to 0 \quad \tau \text{ for } y \neq 0 \quad \text{Short lifetime (ps)}
\]

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<td><strong>Average D(_2)O</strong></td>
<td></td>
<td><strong>1526</strong></td>
<td><strong>1230</strong></td>
<td><strong>7.75</strong></td>
</tr>
</tbody>
</table>

Table 8.2 Visible TA: Results for single exponential kinetic decays fitted to data for \([Ru(phen)_2dpqa]^{2+}\) in water and D\(_2\)O

The results for the TA kinetics of the visible region are shown in Table 8.2, showing the single exponential fits of the data from measurements in water and D\(_2\)O. Acetonitrile is not included because apart from the ultrafast 3 ps kinetics it forms a long-lived state and shows no other kinetics on a picosecond timescale. The kinetics of the data were plotted using either \(y = 0\), or \(y \neq 0\), the former value was chosen to represent complete deactivation of the excited state.

### 8.4 IR Transient absorption (PIRATE)

These measurements were carried out by D. C. Grills (Nottingham University) and Prof. J. M. Kelly. The absorption of the samples for the PIRATE experiments was estimated using \(\varepsilon_{260} \sim 60,000 \text{ dm}^3 \text{ M}^{-1} \text{ cm}^{-1}\).

#### 8.4.1 Deutero-Acetonitrile Solution

The ground state FTIR of 3 in deutero-acetonitrile shows a characteristic absorption band at 1680 cm\(^{-1}\) (CD\(_3\)CN), which is assigned to the CO stretch of the dpqa ligand.
Excitation of the complexes at 400 nm causes depletion of this band at 1680 cm$^{-1}$. In CD$_3$CN solution the result is the formation of a long-lived species with strong absorption bands at 1670 cm$^{-1}$ and 1509 cm$^{-1}$ (Figure 8.7). The 1670 cm$^{-1}$ band was assigned to the amide C=O stretching vibration, the small change in its position compared to that of the ground state being consistent with an excited state in which there are only small changes in the electron density in the pyrazine moiety of the dpqa ligand. The kinetics for each of the bands are fast, proceeding with $\tau \approx 10$ ps.

Figure 8.8 shows a plot of kinetic data for 1509 cm$^{-1}$ showing the formation of a long-lived state in ~10 ps. This data is fitted over the range 0 - 75 ps. This feature at 1509 cm$^{-1}$ could be assigned to the formation of a MLCT charge transfer state resulting from an electron transfer from Ru$\rightarrow$dpqa.
8.4.2 D$_2$O Solution

The ground state FTIR of 3 in D$_2$O (not shown) exhibits a C=O stretching band at 1650 cm$^{-1}$, which is bleached after excitation at 400 nm. The excited state behaviour of 3 in D$_2$O is strikingly different to that in CD$_3$CN, as while excitation initially leads to a rapid rise in the absorption in the 1620 cm$^{-1}$ region, it is then followed by a decrease in less than 10 ps and the parallel formation of bands at 1570 cm$^{-1}$ and 1600 cm$^{-1}$ (Figure 8.9).

The kinetics of the data were plotted using either $y_\infty = 0$, or $y_\infty \neq 0$, the former case for when we believe a photophysical process goes to completion. The results for different bands are shown in Table 8.2 at the end of this section for both fits of the data.

The same $\Delta$ abs. scale was used as for deuterio-acetonitrile, the magnitude of the signal in water is much less.
The band observed at 1650 cm$^{-1}$ is associated with the bleaching of the ground state C=O band. Figure 8.10 shows the data points for this band. As in CD$_2$CN this band has not shifted appreciably with respect to the ground state. The kinetics for these data give 9 ± 1 ps for the growing in of the bleach and 1701 ± 155 ps for the recovery of the bleach. This is within error of the value of 1526 ± 150 ps assigned to the deactivation of the excited complex in D$_2$O.
The data for the band at 1620 cm$^{-1}$ was fitted with two single exponential functions over the range for the longer decay process (75-2500 ps) giving a decay of $1370 \pm 301$ ps, and $4 \pm 1$ ps for the fast process.

Figure 8.11 shows the data points for 1600 cm$^{-1}$. A fast grow-in is observed proceeding with $\tau = 10 \pm 1$ ps. This is followed by a decay with $\tau = 1590 \pm 160$ ps. Again this agrees well with kinetics plotted for visible TA data.

![Figure 8.11 PIRATE of [Ru(phen)$_2$dpqa]$^{2+}$ in D$_2$O 2.8 x 10$^{-3}$M, following laser excitation $\lambda_{\text{exc}}$ = 400 nm. Data at 1600 cm$^{-1}$, red line shows kinetics fit, red points are not included in kinetics.](image)

The band at 1509 cm$^{-1}$, tentatively assigned to charge transfer, is present. However at the same concentration of 3 the magnitude of the signal is far less than in CD$_3$CN, and the band is decreasing over time, not increasing as in CD$_3$CN. The early kinetics of this band were not straightforward however and single exponential kinetics were unable to be extracted for a formation of the band.

<table>
<thead>
<tr>
<th>Wavenumber</th>
<th>$\tau$ for $y \to 0$</th>
<th>$\tau$ for $y \neq 0$</th>
<th>Short lifetime</th>
</tr>
</thead>
<tbody>
<tr>
<td>1650 (bleach)</td>
<td>$1701 \pm 155$</td>
<td>$1288 \pm 480$</td>
<td>$9 \pm 1$</td>
</tr>
<tr>
<td>1620</td>
<td>$1072 \pm 75$</td>
<td>$1370 \pm 301$</td>
<td>$4 \pm 1$</td>
</tr>
<tr>
<td>1600</td>
<td>$1590 \pm 135$</td>
<td>$2163 \pm 1077$</td>
<td>$10 \pm 1$</td>
</tr>
<tr>
<td>1570</td>
<td>$1320 \pm 156$</td>
<td>$1684 \pm 884$</td>
<td>$6 \pm 1$</td>
</tr>
<tr>
<td>1509</td>
<td>$1880 \pm 288$</td>
<td>$1910 \pm 1694$</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 8.3 PIRATE: Results for single exponential kinetic decays fitted to data for [Ru(phen)$_2$dpqa]$^{2+}$ in D$_2$O
8.5 Discussion

It is clear from these results that although \([\text{Ru(phen)}_2\text{dpqa}]^{2+}\) has very similar character in the ground state of both aqueous solution and acetonitrile, the excited states in the two solvents differ greatly.

The visible TA suggests that after excitation to a \(^1\text{MLCT}\) state deactivation of the complex occurs in aqueous solution on a picosecond timescale, but not in acetonitrile solution. It is known that 3 in acetonitrile emits with \(\tau = 1104\) ns. The visible-TA data shows that in all three solvents a fast process occurs < 10 ps. This could be assigned to a Ru (phen) \(^1\text{MLCT}\) to Ru (dpqa) \(^3\text{MLCT}\). The rate for this process goes as CH\(_3\)CN > H\(_2\)O > D\(_2\)O which could be explained from the size of the red shift in absorption maximum in the MLCT region of the Uv/vis spectra. The larger red-shift of 3 in CH3CN giving rise to a faster rate for ILET. The triplet state formed from this transition is long-lived in acetonitrile and shorter lived in H\(_2\)O and D\(_2\)O. A solvent isotope effect was observed with the deactivation process occurring \(2.3 \pm 0.4\) times faster in H\(_2\)O.

The PIRATE results performed in both D\(_2\)O and CD\(_3\)CN also show large differences in their spectra. Over the wavenumber range examined we can monitor the changes associated with the carbonyl group on the dpqa ligand. The ground state FTIR shows a band associated with C=O stretching in D\(_2\)O at 1650 cm\(^{-1}\) and in CD\(_3\)CN at 1680 cm\(^{-1}\). In both solvents these bands bleach however in D\(_2\)O the band recovers in 1701 ± 155 ps, whereas CD\(_3\)CN shows a long-lived bleach. Initially a band forms in D\(_2\)O at 1633 cm\(^{-1}\), which decays with a lifetime of ca. 7 ps producing several bands at lower frequency as excited state solvates. These also decay away within 1000 ps.

It has been mentioned that the charge transfer state of 3 in water involves the movement of an electron from the metal to the dpqa ligand and that the rapid deactivation is associated with appreciable electron density residing on the carbonyl group. This charge transfer band has been tentatively assigned to 1509 cm\(^{-1}\). This band is present in both D\(_2\)O and CD\(_3\)CN, in the case of CD\(_3\)CN the band grows-in and then shows no further change over the course of the experiment, which would be consistent with the charger building up on the dpqa ligand and then residing there. In D\(_2\)O the band is present however it does not show clear kinetics over the first short times (0-50 ps). Also the band is much smaller and
it decays away over the course of the experiment. This is consistent with competing processes of electron transfer to the dpqa moiety and subsequent deactivation over the first 100 ps or so after which the ET process is complete and deactivation of the dpqa moiety is the dominant process exhibiting a signal which decays away. Perhaps the fact that in water hydrogen bonding has solvated the complex (via the carbonyl bond on the dpqa ligand), allows for direct coupling from the $^3$ MLCT Ru-(dpqa) excited complex to the water solvent as opposed to encountering an additional energy barrier associated with solvating the complex. Although the lowest excited state band (which we are exciting into at 400nm) is shifted slightly in the ground state when going from acetonitrile to aqueous environment we suggest that the dramatic change in excited state dynamics observed can not simply be explained due to increasing or decreasing the relative energies of the lowest excited states. This could be further investigated by the use of other non-polar solvents where the emission (if any) can be investigated as a function of $\lambda_{\text{max}}$ MLCT.

8.6 Conclusions

Previous studies had shown that the complex [Ru(phen)$_2$dpqa]$^{2+}$ (3), synthesised by substitution of the parent Ruthenium complex [Ru(phen)$_2$dpq]$^{2+}$ (4) resulted in very different excited state properties. It was show that 3 showed no emission in aqueous solution but in acetonitrile or in the presence of DNA it was emissive, exhibiting classic “light-switch” characteristics. Our studies suggest that the difference in excited state behaviour between aqueous and organic solutions of 3 is due to an ILET process resulting in substantial reduction in the C=O bond order. There is an increase in the negative charge on the oxygen atom of the amide group of the dpqa ligand, which is stabilised by strong hydrogen bonding in aqueous solution. This allows for rapid deactivation of the excited state of 3 in aqueous solution.

Picosecond kinetics from visible TA experiments propose a rate constant $> 1 \times 10^{11}$ s$^{-1}$ for the fast process, suggested as being the ILET process to the dpqa ligand. The deactivation process proceeded with a rate constant of $1.5 \times 10^{9}$ s$^{-1}$ for H$_2$O and $0.7 \times 10^{9}$ s$^{-1}$ for D$_2$O showing an appreciable isotope effect.

The carbonyl group on the dpqa ligand of 3 allowed study by PIRATE. The results supported our visible region results for different excited state character in aqueous and organic solvents. Similar kinetics were extracted for D$_2$O as for the visible TA from the
bleaching band. Similarly a fast process was observed in both solvents, proceeding with similar rate constant $k > 1 \times 10^{11}$ s$^{-1}$, assigned to $k_{\text{LET}}$.

As it is known from previous work that 3 acts as a light switch in the presence of DNA, it would be interesting to perform PIRATE on the complex bound to the polynucleotide. The measurements performed over the same wavenumber range could probe the proposed charger transfer band and the DNA fingerprint region within the range of 1750 – 1500 cm$^{-1}$ simultaneously to further elucidate the light switch effect. Future studies of this complex should also include an investigation of the exact binding mode of 3 to DNA. Other studies have inferred that the alkyl tail intercalates between the DNA basepairs (see references within ref[6]), as this may have the effect of protecting the carbonyl group from solvent interactions in water. Ideally we would have DFT calculations as supplemental results, to infer where the electron density resides for each excited state.

7 Synthesis and Photophysical Characterisation of the [Ru(phen)$_2$Rdpq]$^{2+}$ Family in Different Media, K. A. O’Donoghue, J. M. Kelly, P. E. Kruger, J. C. Penedo (*to be submitted*)
9. RESULTS: [Re(CO)$_3$ (py) dppz]$^+$ complexes

9.1 Introduction

The suitability of various ruthenium complexes as molecular probes has been explored in the previous chapters in terms of changes in photophysical properties in the presence of DNA. Rhenium carbonyls with polypyridyl ligands are another group of complexes whose tuneable photophysical properties make them good candidates as molecular probes.$^{1,2}$ The character and behaviour of the lowest excited state are strongly dependent on both the axial and the polypyridyl ligand. Complexes such as fac-[Re(CO)$_3$py dppz]$^+$ will possess a strong mid-IR signal due to the presence of the carbonyl groups, which make these systems suitable for monitoring with PIRATE, as well as steady-state techniques. They will also bind to DNA in a well-defined fashion via intercalation of the dppz ligand. Another reason for the potential of these complexes as molecular probes is the sensitivity of the lowest excited state to the solvent polarity. These complexes differ from the previous dppz complex studied ([Ru(TAP)$_2$dppz]$^{2+}$) as it is known that the lowest excited state is associated with the dppz ligand in both aqueous solution and acetonitrile.

The elucidation of the processes resulting from alterations in the polypyridyl ligand or solvent based effects were considered. The complexes fac-[Re(CO)$_3$py dppz]$^+$ (6), fac-[Re(CO)$_3$py F$_2$ dppz]$^+$ (7) and fac-[Re(CO)$_3$py Me$_2$dppz]$^+$ (8), (Figure 9.1) were studied in aqueous and organic solvent using visible Transient Absorption and PIRATE. Transient IR should be an effective means of determining the nature of the excited state (it is known that for similar dppz complexes the triplet dppz ($\pi$- $\pi^*$) and MLCT states lie close in energy$^2$) and of identifying the species formed in redox reactions. As a preliminary to picosecond infrared transient absorption (PIRATE) measurements, we carried out UV/vis TA studies. The derivatives have been selected as we anticipate that both the oxidising power and nature of the lowest excited state can be controlled by the electron-withdrawing or electron-donating properties of the substituent on the dppz ligand.

All measurements were carried out in the Central Laser Facility in the Rutherford Appleton Laboratory, under the supervision of Prof. J. M. Kelly. Details of the experimental set-up are in Chapter 4: Experimental Methods.
9.2 UV/vis Measurements

9.2.1 Acetonitrile Solution
The UV/Visible spectra (Figure 9.2) of 6, 7 and 8 have analogous features, each exhibiting a strong absorption around 290 nm and a weaker absorption in the region 350-400 nm. The lower wavelength region shows the most change upon substitution of the dppz ligand, with the methyl substitution causing a significant red-shift of 12 nm and the difluoro substitution causing a blue shift of 4nm. In the region of 350-400 nm it can be seen that the ratio of the lowest absorption to the next lowest changes quite substantially upon substitution of the dppz ligand.
Figure 9.2 UV/visible absorption Spectra of fac-\((R_2dppz)Re(CO)_3(py)^+\), (R = F, H, Me) (1.7 x 10^{-4} M; 1.5 x 10^{-4} M and 1.7 x 10^{-4} M resp.) in acetonitrile solution. Picture: S. Hudson MSc thesis.

Table 9.1 \(\lambda_{\text{max}}\) for complex 6, 7 and 8 (1.7 x 10^{-4} M; 1.5 x 10^{-4} M and 1.7 x 10^{-4} M resp.) in acetonitrile solution

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\lambda_{\text{max}})</th>
<th>(\lambda_{\text{max}})</th>
<th>(\lambda_{\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re(CO)_3 py Me_2 dppz</td>
<td>289</td>
<td>371</td>
<td>391</td>
</tr>
<tr>
<td>Re(CO)_3 py dppz</td>
<td>277</td>
<td>361</td>
<td>380</td>
</tr>
<tr>
<td>Re(CO)_3 py F_2 dppz</td>
<td>273</td>
<td>361</td>
<td>379</td>
</tr>
</tbody>
</table>

Table 9.1 \(\lambda_{\text{max}}\) for the three complexes in acetonitrile. The deconvolution of these spectra show the presence of a band at 370 nm.\(^4\) This band at ca. 370 nm is thought to be MLCT in nature, based upon the phen MO of dppz. This phen based assignment of the 370 nm feature is further supported by Figure 9.3, which shows the contribution due to a Re-phen transition (dashed line) which is absent in the spectrum of the dppz ligand in acetonitrile alone. It seems that the electron-donating methyl group serves to both lower the energy of the two lowest transitions and increase the energy gap between the two lowest energy levels.
9.2.2 Aqueous Solution

The absorption spectra were subsequently performed in buffered H₂O. Figure 9.4 show the absorption spectra of 6 and 7. Again we see that the ratio between the lowest (circa 380 nm) and the next lowest (circa 365 nm) energy transitions is bigger for the substituted dppz complex. No new features are seen in the spectra with this solvent.

Figure 9.4 Normalised Absorption spectra of 6(8 × 10⁻⁴ M) and 7 (8.8 × 10⁻⁴ M) in H₂O buffer
The energies of all the transitions are lowered in aqueous solution. The lowest energy transitions are red-shifted by 3 nm, the MLCT transition of 6 is lowered slightly more than that of 7, compared with the complexes in acetonitrile.

In both solvents we see that the dppz IL and Re-dppz(phen)MLCT transitions lie close in energy to one another. Exciting at 400 nm initially populates MLCT and IL excited states, and excitation at 267 nm should initially populate excited IL states.

9.3 Transient Absorption (TA) - 2001

9.3.1 Acetonitrile Solution

Figure 9.5 shows the TA spectra for 6 in acetonitrile after excitation at 400 nm. At 4 ps a broad absorption with a maximum at 470 nm and a shoulder at ca. 540 nm is evident. An increase in intensity (ca. 5 %) at the maximum is then observed over the first 100 ps, with
only very small changes occurring elsewhere. A long-lived state “grows in” in 21 ± 7 ps, and at 1000 ps the final spectrum is essentially identical to that reported by Schanze and coworkers for \( \text{fac-[(dppz)Re(CO)_3(4-Mepy)]}^+ \). This long-lived state has been assigned to the \(^3\text{IL} \pi-\pi^* \text{(dppz)} \) excited state and is expected to be of phz character. The nature of the process occurring in the first 100 ps is still uncertain. Possibilities include (i) the conversion of an initially formed MLCT state to the \( \pi-\pi^* \text{(dppz)} \) or (ii) the conversion from singlet to triplet state.

Figure 9.6 Transient absorption spectra observed at various delays \( \text{fac-[(Me}_2\text{dppz)Re(CO)_3(py)]}^+ \) in acetonitrile solution, following laser excitation \( \lambda_{\text{exc}} = 400 \text{ nm} \).

Figure 9.7 Transient absorption spectra observed at various delays \( \text{fac-[(F}_2\text{dppz)Re(CO)_3(py)]}^+ \) in acetonitrile solution, following laser excitation \( \lambda_{\text{exc}} = 400 \text{ nm} \).
Changing the electron density in the dppz ring might be expected to affect the relative energies of the MLCT and IL π-π* (dppz) excited states.

Figure 9.6 shows a similar process takes place in 8 with the long-lived state forming in 27 ± 8 ps. The spectrum of the fac-\([(\text{Me}_2\text{dppz})\text{Re(CO)}_3(\text{py})]^+\) looks similar to that of the parent complex (the features being somewhat broader). However although the difluoro complex also shows this long-lived state forming (15 ± 10 ps), a new feature is seen at longer wavelengths, (Figure 9.7). This might be due to the electron-withdrawing fluorine substituent causing a stabilisation of the MLCT state of the complex.

9.3.2 Aqueous Solution

We then studied the effect of solvent polarity and DNA binding on the TA spectra of fac-\([(\text{dppz})\text{Re(CO)}_3(\text{py})]^+\). It was observed that the change of the solvent from acetonitrile to water caused a significant red shift of the band maximum of approximately 10 nm. The TA spectrum of 6 in water contrasts with the acetonitrile results in that it shows a small decrease in intensity over 1000 ps, seen in Figure 9.8.

Figure 9.8 Transient absorption spectra of \([(\text{dppz})\text{Re(CO)}_3(\text{py})]^+ (1.7 \times 10^{-4} \text{M})\) in 10 mM phosphate buffer, observed at 4 ps and 1000 ps following laser excitation (λ_{exc} = 400 nm)
9.3.3 Aqueous Solution and [poly(dG-dC)]<sub>2</sub>

By contrast when 6 is bound to [poly(dG-dC)]<sub>2</sub> (1:20) there is a substantial decrease in absorption over the first 100 ps, seen in Figure 9.9. We speculated that this change is due to electron transfer from the guanine but if this is the case then the spectra of both the excited state and the reduced complex must be similar. It was hoped that this matter could be resolved by PIRATE measurements, and the difference between the parent complex and that of the difluoro complex could be more fully elucidated. It was also hoped to improve the quality of the TA data in future analyses.

![Figure 9.9 Transient absorption spectra of [(dppz)Re(CO)<sub>3</sub>(py)]<sup>+</sup> (1.7 x 10<sup>-4</sup>M) in 10 mM phosphate buffer in the presence of [poly(dG-dC)]<sub>2</sub> (3.4 x 10<sup>-3</sup>M nucleotide), observed at 4 ps and 1000 ps following laser excitation λ<sub>exc</sub> = 400 nm.](image)

9.4 Transient Absorption (TA) - 2002

9.4.1 Aqueous Solution

Transient absorption was performed on 6 in aqueous solution again, using a more concentrated solution to improve the quality of the data. The TA were performed in both H<sub>2</sub>O and D<sub>2</sub>O to see if any isotopic effect was present. The changes in the visible transient absorption spectra of the parent complex 7 in buffered D<sub>2</sub>O (Figure 9.10) showed a small decrease in absorption over the first 100 ps and a steady absorption thereafter, confirming the results of the previous year.
Figure 9.10 Re(CO)$_3$(py) dppz in D$_2$O buffer, $\lambda_{exc} = 400$ nm, $8 \times 10^{-4}$ M, following laser excitation $\lambda_{exc} = 400$ nm.

By contrast the excited state of 7 in water decays rapidly (Figure 9.11), following biexponential kinetics ($\tau_1 = 108 \pm 20$ ps; $\tau_2 = 1190 \pm 200$ ps), (assuming that the absorbance goes back to the baseline). The decay kinetics show a very modest solvent effect, decaying with lifetimes of $\tau_1 = 113 \pm 20$ ps; $\tau_2 = 1410 \pm 280$ ps in D$_2$O.

The quality of the TA data is better than the first year, allowing us to fit reasonable kinetics to the data.

Figure 9.11 Re(CO)$_3$(py)F$_2$dppz in D$_2$O buffer, $\lambda_{exc} = 400$nm, $8.8 \times 10^{-4}$ M, following laser excitation $\lambda_{exc} = 400$ nm.
<table>
<thead>
<tr>
<th>Re (CO)$_2$F$_2$dp pz</th>
<th>Wavelength (nm)</th>
<th>$Y_{\infty \to 0}$</th>
<th>$Y_{\infty \neq 0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>470</td>
<td>109 ± 24, 1012 ± 57</td>
<td>87 ± 32, 824 ± 171</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>114 ± 21, 1191 ± 68</td>
<td>72 ± 18, 761 ± 95</td>
</tr>
<tr>
<td></td>
<td>535</td>
<td>100 ± 15, 1306 ± 68</td>
<td>62 ± 9, 758 ± 63</td>
</tr>
<tr>
<td></td>
<td>570</td>
<td>107 ±17, 1243 ± 68</td>
<td>69 ±14, 770 ± 94</td>
</tr>
<tr>
<td>Average H$_2$O</td>
<td></td>
<td>107.5, 1188</td>
<td>72.5, 778</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>470</td>
<td>99 ± 27, 1203 ± 74</td>
<td>67 ± 28, 862 ± 179</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>120 ± 26, 1398 ± 107</td>
<td>86 ± 33, 888 ± 250</td>
</tr>
<tr>
<td></td>
<td>535</td>
<td>136 ± 25, 1546 ± 123</td>
<td>87 ± 29, 824 ±190</td>
</tr>
<tr>
<td></td>
<td>570</td>
<td>97 ±17, 1488 ± 90</td>
<td>61 ±13, 812 ± 115</td>
</tr>
<tr>
<td>Average D$_2$O</td>
<td></td>
<td>113, 1409</td>
<td>75, 846.5</td>
</tr>
</tbody>
</table>

Table 9.3 Values of kinetics for 7 in both H$_2$O and D$_2$O, averaged over a range of wavelengths

9.4.2 Aqueous Solution and [poly(dG-dC)]$_2$

Both 6 and 7 were studied upon addition of [poly(dG-dC)]$_2$( P:Re = 20:1). The parent complex 6 shows the formation of a long-lived state on the time-scale of the experiment. The initial decay is quite rapid taking 16 ± 2 ps in D$_2$O (Figure 9.12).

![Figure 9.12](image-url)
Although a similar rapid decay occurs with 7 in the presence of [poly(dG-dC)]$_2$ (Figure 9.13), the kinetics for the system are quite different.

![Graph](image)

Figure 9.13 Re(CO)$_3$(py)F$_2$dpdz in D$_2$O buffer, $\lambda_{\text{exc}} = 400$ nm, $8.8 \times 10^{-4}$ M, with [poly(dG-dC)]$_2$ (1:20) following laser excitation $\lambda_{\text{exc}} = 400$ nm.

In the region around 470 nm there is a decay taking 23 ± 4 ps. The kinetics around 580 nm can be modeled as a decay followed by a biexponential growth (Figure 9.14), setting a time of 20 ps for the fast decay and 22 ± 8 ps and 6779 ± 1452 ps for the biexponential growths.

![Graph](image)

Figure 9.14 Re(CO)$_3$(py)F$_2$dpdz in D$_2$O buffer, $\lambda_{\text{exc}} = 400$ nm, $8.8 \times 10^{-4}$ M, with [poly(dG-dC)]$_2$ (1:20) following laser excitation $\lambda_{\text{exc}} = 400$ nm, red line is kinetics fit at 580 nm.
9.5 IR Transient absorption (PIRATE)

9.5.1 Deutero-Acetonitrile Solution

The measurements in 9.5.1 and 9.5.2 were carried out by D. C. Grills, J. Dyer (Nottingham University) and Prof. J. M. Kelly. Figure 9.15 compares the transient spectra of [Re(CO)₃(dppz)py]Cl (6) and [Re(CO)₃(F₂dppz)py]Cl (7) in deutero-acetonitrile.

![Figure 9.15 ps-TRIR spectra for Re(CO)₃(py)dppz (left) and Re(CO)₃(py)F₂dppz(right) in CD₃CN (1.2 ×10⁻³ M and 1. ×10⁻³ M resp.), following laser excitation λ_{exc} = 400 nm.](image)

The spectra of both compounds show bands shifted to lower frequency. This is entirely consistent with the formation of the π-π* excited state. Both 6 and 7 show a fast partial excited state decay and a recovery of bleach with τ = 25 ± 5 ps for 6 and 18 ± 2 ps for the difluoro complex in CD₃CN.
9.5.2 Aqueous Solution

By contrast the spectra of compounds 6 and 7 in D\textsubscript{2}O are markedly different. The dppz complex 6 shows two CO bands at 2031 and 1916 cm\textsuperscript{-1}, which may be assigned to the π-π\textsuperscript{*} state. These bands show a partial decay in 14 ± 2 ps. The vibrational cooling proceeds with a lifetime of ca. 20 ps. With the F\textsubscript{2}dppz complex 7 the dominant species at longer times has CO bands at 2108, 2029, 2005 cm\textsuperscript{-1}, which decay biexponential kinetics of τ = 14 ± 2 ps and 339 ± 100 ps. The shift of the bands to high frequency indicates that there is a substantial build-up of positive charge at the metal centre, consistent with an MLCT (pyrazine) excited state where the electron is located primarily on the pyrazine part of the molecule. Detailed studies show that at very early times a π-π\textsuperscript{*} species, which decays with a lifetime of ca. 10 ps, is present which converts to the MLCT (pyrazine) species.

![Figure 9.16 TRIR spectra for Re(CO)\textsubscript{3}(py)dppz(left) and Re(CO)\textsubscript{3}(py)F\textsubscript{2}dppz(right) in D\textsubscript{2}O buffer, (1.2 \times 10\textsuperscript{-3} M and 1 \times 10\textsuperscript{-3} M resp.), following laser excitation λ\textsubscript{exc} = 400 nm.](image-url)
9.5.3 Aqueous Solution and \([\text{poly(dG-dC)}]_2\)

Figure 9.17 Re (CO\(_3\))(py) dppz (1.2\(\times\)10\(^{-3}\) M) with [poly(dG-dC)]\(_2\) (1:20) in D\(_2\)O buffer, following laser excitation \(\lambda_{\text{exc}} = 400\) nm.

Figure 9.17 shows the PIRATE data for 6 in the presence of [poly(dG-dC)]\(_2\) (1:20). The signal at 2022 cm\(^{-1}\) is shown below in Figure 9.18. It has single exponential kinetics that gives \(\tau = 12 \pm 2\) ps for the process occurring. The bleach signals at 2038 cm\(^{-1}\) and 1935 cm\(^{-1}\) both show a partial recovery over 15 \(\pm\) 3 ps, in both cases these are preceded by an ultrafast grow-in of the bleach which shows \(\tau < 2\) ps in each case (see Figure 9.19).

Figure 9.18 Re (CO\(_3\))(py) dppz (1.2\(\times\)10\(^{-3}\) M) with [poly(dG-dC)]\(_2\) (1:20) in D\(_2\)O buffer, following laser excitation \(\lambda_{\text{exc}} = 400\) nm, red line is kinetics fitted at 2022 cm\(^{-1}\).
Figure 9.19 Re (CO)$_3$(py) dppz (1.2×10$^{-3}$ M) with [poly(dG-dC)]$_2$ (1:20) in D$_2$O buffer, following laser excitation $\lambda_{\text{exc}} = 400$ nm, kinetics fit not shown.

Figure 9.20 Re (CO)$_3$(py) F$_2$ dppz (0.3 $\times 10^{-3}$ M) with [poly(dG-dC)]$_2$ (1:20) in D$_2$O buffer, following laser excitation $\lambda_{\text{exc}} = 400$ nm.

Figure 9.20 shows the PIRATE data for 7 in the presence of GC polynucleotides. The two sets of spectra for 6 and 7 with the addition of the GC nucleotide show differences in their kinetics. Unfortunately the signal for 7 in D$_2$O is quite small because of the lower concentration used (the limiting factor is the amount of polynucleotide available as 20 times the concentration is needed, and they are quite expensive). The data shown in Figure 9.21 is the bleach recovery signal which shows multiexponential kinetics, indicating that a more complicated process is occurring, than for 6 with [poly(dG-dC)]$_2$, (compared with Figure 9.19). The magnitude of the signal for 7 is much less.
9.6 Discussion

Previous TA studies on \([\text{Re(CO)}_3(\text{dppz})\text{py}]^+\) (6) and its difluoro-analogue \([\text{Re(CO)}_3(\text{F}_2\text{dppz})\text{py}]^+\) (7) show formation of a long-lived state within 100ps. This agrees with showing that the related complex in acetonitrile forms a triplet excited state in 30 ps, assigned to an IL dppz emissive state. The TA spectra of 7 did, however show a new feature at longer wavelengths.

The results from Transient Absorption shows that the excited states of the two complexes behave quite differently in aqueous solution. Complex 6 forms a long-lived state in water, showing no change after the first 100 ps. In contrast the excited state of 7 deactivates following biexponential kinetics of 108 and 1188 ps. It is known that the triplet MLCT produced in aqueous solution is based on the phen MO orbital. The fast process that occurs in both complexes in water can be assigned to the formation of the Re-(phz) \(^3\)MLCT state via the Re-(phen) \(^3\)MLCT which is stable in acetonitrile solution. The fast process taking 108 ± 20 ps in water and 113 ± 20 ps in D\(_2\)O does not exhibit any appreciable solvent isotope effect. This Re-(phz) \(^3\)MLCT state deactivates in aqueous solution following kinetics of \(\tau = 1190 ± 200\) ps in water and 1410 ± 280 ps in D\(_2\)O, (showing a modest solvent isotope effect). Spectroelectrochemical work has suggested that the first reduction involves the phenazine part of the dppz ligand. This is the part of the ligand that should be
most sensitive to the difluoro substitutions and also to the solvent polarity, where the lone pairs on the phenazine nitrogens can experience stabilisation from a polar solvent such as water.

As the lowest excited state of the complex is dppz based, binding to polynucleotides can enhance luminescence by protection from deactivation by solvent interaction. Complex 6 is known to act as a light switch in the presence of DNA, enhancing the luminescence of the polynucleotide considerably. When both complexes bind to the GC nucleotide a long-lived state is formed. In the presence of [poly(dG-dC)]₂ 6 shows fast decay kinetics of 16 ± 2 ps. The transient spectra has the same form as for 1 in water alone, indicating that the same MLCT (phz) state is formed, although more rapidly than in water alone.

However the transient spectra of 7 shows a decay over all wavelengths but a subsequent increase in absorption in the 580 nm region. A transient signal in this region (centred on 600 nm) is associated with the reduced rhenium complex. We can infer from this that electron transfer is occurring from guanine to the excited state of [Re(CO)₃ py F₂dppz]⁺. Furthermore 7 is a likely candidate for photoreduction, indicated by emission studies and spectroelectrochemical work by the Nottingham group, where the process for oxidation of guanine is favourable for 7 but not for the parent complex in aqueous solution. ΔG for 6 was found to be 0.02 eV whereas ΔG for 7 the difluoro analogue was found to be −0.06 eV, indicating the likelihood of photoreduction for the difluoro compound, (with oxidation of the adenine bases much less likely).

The PIRATE studies are expected to monitor the vibrations of CO coordinated to a metal ion as a probe for changes in the electron density in the excited states of metal carbonyl derivatives. Although the carbonyl ligands provide a good signal for transient measurement the lowest excited state can not be probed directly, unlike the previously studied dpqa complex.

The PIRATE studies show bleaching for both complexes in deuterio-acetonitrile solution, with a partial recovery of the bleach in < 20 ps. The difference between the excited state behaviour of 7 and the parent complex in D₂O is evident from the kinetics. While 6
exhibits single exponential kinetics for a partial recovery of the bleach, the kinetics for 7 are biexponential, although the times taken to recover $14 \pm 2$ ps and $339 \pm 100$ ps differ from the values measured in the visible part of the spectrum.

Additional PIRATE measurements on 6 and 7 in the DNA base region when the complexes are bound to $[\text{poly(dG-dC)}]_2$ show that for 6: a fast partial recovery occurs with $\tau = 15 \pm 4$ ps, and then a long-lived state is formed. This can be assigned to the emissive IL state of dppz. This long-lived state formation supports what was seen in the carbonyl region. When the excited state of 7 reacts with $[\text{poly(dG-dC)}]_2$ a transient band at 1695 cm$^{-1}$ shows a partial recovery $\tau = 23$ ps, but then a subsequent rebleaching, $\tau = 79$ ps. This coincides with a rebleaching of a band at 2038 cm$^{-1}$ for 7 in the carbonyl region.

9.7 Conclusions

Both rhenium complexes show enhancement of emission on binding to DNA, 6 showing a large enhancement of emission. However 6 in aqueous solution alone also shows emission, so this complex does not exhibit a clear-cut ‘off’ state in aqueous solution and an ‘on’ state only when bound to DNA.

The difluoro complex however shows hardly any emission in water and then emission enhancement in the presence of DNA. The drawback with this complex as a non-invasive probe for DNA is the chemical reaction with the DNA strand in the form of photooxidation of guanine.

It was hoped that as these complexes only possess one polypyridyl ligand that it should make the assignment of the excited state, and vibrational spectra easier, as there is no ambiguity with respect to the acceptor ligand involved in the MLCT transition. Both complexes have ligands which facilitate TRIR studies as well as visible transient absorption. The PIRATE results agree qualitatively with the TA results, however there were some problems with the quality of the PIRATE data due to low concentration of 7 used. The preliminary results suggest that picosecond TA and TRIR can be used as complementary techniques for ultrafast studies. 

9-18


3 S. Hudson, MSc. Thesis University of Dublin 2000


10. RESULTS: DPPZ

10.1 Introduction

Despite the enormous interest in the photophysical properties of its metal complexes, such as [Ru(phen)$_2$dpdz]$^{2+}$, there have been few studies on the photophysics of the free ligand (dipyrido [3,2-a:2',3'-c] phenazine) itself. Thus while there have been brief reports of weak emission from the ligand in ethanol solution, the origin of this is uncertain as its excitation spectrum differs markedly from the absorption spectrum of the compound. A possibility is that this emission is due to the presence of dimers. Nanosecond flash photolysis studies carried out with dpdz in methanol have revealed that the $\pi$-$\pi^*$ triplet state is formed in good yield. Interestingly this species decays to give a long-lived transient, whose nature is unknown but may be an isomer. Weak emission has also been reported from dipyrido[3,4-b:2',3'-d]phenazine, an isomer of dpdz and in this case it has been proposed that the emission originates from an upper excited state. As part of our study of the ruthenium and rhenium complexes of dpdz in solution and when bound to DNA visible transient absorption was performed on study of dpdz derivatives in acetonitrile and aqueous acetonitrile solution. These compounds have been selected as we anticipate that both the oxidising power and nature of the lowest excited state can be altered by the addition of electron-withdrawing (F) or electron-donating (Me) properties of the substituent on the dpdz ligand.

[Diagram of substituted dpdz with $X_1 = F, H, CH_3$]

Figure 10.1 Structure of the substituted dpdz, $X_1 = F, H, CH_3$
All measurements were carried out in the Central Laser Facility in the Rutherford Appleton Laboratory, under the supervision of Prof. J. M. Kelly. Details of the experimental set-up are in Chapter 4.

10.2 UV/vis Measurements

![Absorption spectra](image)

Figure 10.2 Absorption spectra of dppz (2.02x10^5 M), Me_2dppz (1.89x10^5 M) and F_2dppz (1.86x10^5 M) in acetonitrile

10.2.1 Acetonitrile Solution
The highest wavelength, lowest energy absorption band has been assigned to the IL (phz) 3\pi-\pi* band, with the next lowest bands associated with the IL (phen) character triplet states. The absorptions around 260 nm are of IL (phz) 1\pi-\pi* character. These absorption maxima shift for both Me_2dppz and F_2dppz. In the triplet band region, the Me_2dppz substitution shows the greatest shift, see Table 10.1.
10.2.2 Acetonitrile/Water Solution
The ground state absorption spectra show that change of the solvent from acetonitrile to the water/acetonitrile mixture causes only a small red shift of the absorption maxima in each case, slightly more for F<sub>2</sub>dppz at the singlet absorption maximum.

Table 10.1 Absorption maxima for dppz complexes in CH<sub>3</sub>CN and CH<sub>3</sub>CN/H<sub>2</sub>O

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10.3 Transient Absorption (TA) – 2001
We investigated the excited state dynamics produced by exciting the dppz free ligand (9) in acetonitrile. The excitation wavelength used was 400 nm.

Figure 10.3 shows the TA spectra of 9 in acetonitrile, in which two interconverting species are present. The data was smoothed using adjacent averaging of 5 points. Kinetics were fitted to unsmoothed data. The initially formed species shows a broad maximum at 470 nm and a shoulder at ca. 530 nm. This species converts (lifetime 63 ± 12 ps) to another, which possesses a narrower band at 460 nm and a better resolved peak at 550 nm. It is probable that
these species are the singlet and triplet excited states of the heteroaromatic compound. While these spectra are both reasonably similar to those of that observed for fac-
[(dppz)Re(CO)3(py)]+, it appears unlikely that the spectroscopic changes noted within the first 30 ps of excitation of the rhenium complex are due to singlet to triplet conversion.7

![Graph](10.4 Transient absorption spectra of 9 in acetonitrile following laser excitation λ_{exc} = 400 nm (Adjacent average smoothing of data points))

10.4 Transient absorption (TA) - 2002

10.4.1 Acetonitrile Solution
From Figure 10.2 it can be seen that dppz absorbs only weakly at 400 nm so the signals were necessarily quite weak even when working with solutions close to the solubility limit. In our later work the dppz samples were rigorously purified, so as to eliminate any possible effect of impurities and the compounds were excited at 267 nm close to their maximum absorption. This allowed us to work at much lower concentrations than previously, obviating any possible problems arising from aggregation.
Figure 10.5 TA spectra of dppz (2.02×10⁻⁵ M), in acetonitrile, λ<sub>exc</sub> = 267 nm

Figure 10.5 shows TA spectra for dppz in acetonitrile over a range of time delays. Two transient species are clearly visible, the initial one giving a strong broad absorption peaking at 467 nm with a shoulder at 530 nm. This converts to another species with a well-resolved shoulder at ca. 580 nm. Monitoring the time course at 500 nm reveals that the first species decays exponentially with a lifetime of 42 ± 5 ps. (Figure 10.6 below shows a sample kinetic data).

Figure 10.6 Decay kinetics monitored at 500 nm for dppz in acetonitrile, λ<sub>exc</sub> = 267 nm
Figure 10.7 TA spectra recorded 4 ps and 1000 ps after 267 nm excitation of dppz (2.02×10⁻⁵ M), Me₂dppz (1.89×10⁻⁵ M) and F₂dppz (1.86×10⁻⁵ M) in acetonitrile.

The transient absorption spectra of Me₂dppz and F₂dppz were also obtained. Figure 10.7 compares the spectra for the three dppz derivatives, recorded at 4 ps and 1000 ps. While these are rather similar that of F₂dppz at 1000 ps does not show as prominent a shoulder as exhibited by the other two compounds. The kinetics were determined at 500 nm, yielding an average value for the decay of the initial transient of 42 ± 5 ps (for dppz), 50 ± 5 ps (for Me₂dppz) and 34 ± 6 ps (for F₂dppz).
10.4.2 Acetonitrile/Water Solution

The initial and final TA data for each ligand in acetonitrile and water are presented in Figure 10.8. The presence of water markedly affects the excited state dynamics. Thus the lifetimes for dppz and Me₂dppz are slightly increased to 50 ± 5 ps (for dppz) and to 65 ± 6 ps (for Me₂dppz), whereas that for F₂dppz seems to be shortened appreciably from 34 ± 6 ps in acetonitrile alone, to 14 ± 7 ps.

![Figure 10.8 TA spectra recorded 4 ps and 1000 ps after 267 nm excitation of dppz (2.19 × 10⁻⁵ M), Me₂dppz (2.24 × 10⁻⁵ M) and F₂dppz (1.24 × 10⁻⁵ M) in acetonitrile/water.](image)

The kinetics data is less trustworthy for the difluoro complex. Figure 10.9 shows the fitted data, where a single exponential fit incurs quite a large error.
10.5 Discussion

For each compound the TA data reveals two inter-converting species. Excitation of the dppz ligand at 267 nm produces an upper excited state. With most organic compounds it is expected that the decay to the lowest excited singlet state (S₁) will be very rapid. Although it has been reported\(^5\) that the fluorescence of an isomer of dppz occurs from the S₂ level, we nevertheless believe that for our isomer of dppz the probable assignment of the first transient is the S₁ state. This is supported by the fact that the data obtained in the current investigation for dppz is closely similar to that reported in our earlier study\(^8\) using a 400 nm pump, an excitation wavelength which is expected to yield lower excited singlet states. For this reason we believe that internal conversion, at least from the high energy singlet states, is very rapid.

The second transient which formed from the first with a lifetime of 42 ± 5 ps for dppz has a spectrum which is similar to the species reported by Schanze et al.\(^5\), which had a lifetime of 3 μs in methanol. This species, assigned to the triplet state of dppz, exhibited a peak at 470 nm and a broad, weaker absorption over the rest of the visible spectrum. Therefore it is probable that the longer-lived transient in our experiment is the triplet state of dppz, and the interconversion that we observe is inter-system crossing, most probably S₁ to T₁.
Two species with spectra similar to those recorded in acetonitrile are also observed in aqueous acetonitrile solution. Interestingly the decay of the \( S_1 \) state is shorter for \( \text{F}_2\text{dppz} \), while the lifetime of this state is somewhat lengthened for the other compounds. This may be due to changes in the solvation, possibly involving hydrogen bonding of the phenazine nitrogen atoms, as has been proposed for the excited states of the \([\text{Ru(phen)}_2\text{dppz}^2+]\).  

### 10.6 Conclusions

UV/vis transient absorption measurements taken over a timescale of 1000 ps were used to probe the transient species produced from initially formed higher excited states of three dppz compounds. The similar process of one species converting to another seems to indicate the formation of a triplet species by intersystem crossing from the rapidly formed \( S_1 \) state. The kinetics for the three compounds differ slightly in acetonitrile and more so in the case of \( \text{F}_2\text{dppz} \) when water is present. The results indicate that changes in the electron distribution on the phenazine part of dppz, can alter the photophysical properties of the ligand.

11. RESULTS: Solvated Electron

11.1 Introduction

The photolysis of certain molecules in solution can lead to their electrons being trapped in the solvent, the classic example of which is ammonia. In the case of water the electron is solvated by surrounding polar water molecules. The electron stays in this ‘cage’ until recombining with the water molecule some time later. ¹

![Solvated electron “cage”](image)

Figure 11.1 Solvated electron “cage”. The hydrogens of the water are blue, these have a $\delta^-$ charge. The oxygen atoms are coloured red.

This solvated electron has a broad absorbance, centred on 650 nm, for a review of solvated electron dynamics see ² and references therein. The process can occur in water if sufficient pump energy is available for absorption by the water.

![TA spectra](image)

Figure 11.2 TA spectra of buffered water excited at 267 nm, full input power.
11.2 Transient Absorption (TA)

TA spectra were performed on a number of different complexes in water, excited at 267 nm. In some of the more dilute complexes a broad absorption was seen in the absorption region. This was further investigated to see if buffered water was responsible for the signal. Figure 11.2 shows the TA spectra produced after excitation at 267 nm. Although the signal is noisy a feature could be seen around 650 nm when using full excitation power (15 MW). We could not investigate further into the IR as our system was limited to probing the region between 430 - 650 nm. To check that this feature was not incurred by the use of buffer a run was performed on ultrapure water alone, and the same feature was seen, Figure 11.3.

Figure 11.3  TA Spectra of ultrapure H₂O, full input intensity.

Figure 11.4 Transient absorption of H₂O, Input intensity 1/3
To check whether there was any intensity dependence the input intensity was taken down by the use of neutral density filters to a third of its initial value. The resulting drop in signal was non-linear.

At 267 nm two-photon absorption at the correct intensity could produce absorption in water. This is a $\chi_3$ process and is dependent on the square of the input intensity. A log log plot of signal vs input intensity should give a slope of 2 for such a process.

![Figure 11.5 Log-Log plot of integrated signal at 10 ps vs input intensity](image)

The graph in Figure 11.5 plotting the integral of the TA signal at 10 ps in both cases has a slope of ~1.7. This is close enough to 2 to suggest a $\chi_3$ process occurring.

A small amount of HCl (1.85% w/v) was added to the sample as it was expected that the $H^+$ ions should extract the solvated electrons from solution. Figure 11.6 shows the decrease of the signal over the time-scale of the experiment, suggesting that this is what occurred.
11.3 Conclusions

The broad absorption around 650 nm seen in aqueous solutions when excited at 267 nm is thought to arise from the presence of solvated electrons. These are produced by the water molecules which undergo a 2-photon absorption process at the input intensity used for the TA experiments. The log-log plot shows a slope of ~1.7, close enough to 2 to be characteristic of a $\chi_3$ process. The addition of acid is seen to cause the solvated electron contribution to disappear as the protons in solution capture the electrons.

When exciting aqueous solutions at 267 nm, it was necessary to have the complex dilute enough not to cause a large artifact (see section 7.4), yet concentrated enough to not incur a large solvated electron contribution in the TA spectra.

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This thesis investigated the ultrafast photophysical and photochemical processes of a variety of DNA binding species. Starting with a simple intercalating dye system where only one chromophore is present, we subsequently studied multi-ligand DNA binding complexes, where the geometries of the interacting species were less well defined.

Thionine interacts with DNA on a femtosecond time scale. It was shown that the reduced dye underwent reduction by the guanine nucleobase in \([\text{poly(dG-dC)}]_2\) with a rate of \(1/260\) fs. The rate was slower in GMP (1/880 fs) where the interaction distance is expected to be greater. The reaction with AT base pairs is less well defined. Some results suggest enhanced on binding solely to AT pairs as the excited state is protected from deactivation form intercalation. Thus the data for DNA were not simple biexponential kinetics. The probability of binding next to a GC pair was also discussed.

Ru(TAP)\(_2\)dppz complexes were studied in aqueous and acetonitrile solutions. There is evidence for an electron transfer from the guanine to the Ru* complex, proceeding with a rate of \(2 \times 10^9\) s\(^{-1}\) in water. Unlike thionine, it is not expected that the electron transfers to the intercalated ligand but transfers to the Ru metal centre. The kinetics support a fast formation of the Ru-TAP MLCT state, followed by a reduction of the metal centre. A back electron transfer follows with kinetics of \(1.1 \times 10^8\) s\(^{-1}\). A solvent isotope is present, with the forward electron transfer rate constant as \(1.4 \times 10^9\) s\(^{-1}\) in D\(_2\)O, and the back electron transfer proceeding with a rate of \(0.6 \times 10^8\) s\(^{-1}\). It was proposed that the redox reaction we see is a proton-coupled effect. The electron transfer causes quenching of Ru(TAP)\(_2\)dppz complexes, in aqueous solutions when bound to DNA.

PIRATE results in D\(_2\)O gave a rate for forward electron transfer (monitoring the guanine base vibrations) as 1/678 ps. Although the kinetics for the cytosine show a faster rate (1/392 ps) this would conflict with PCET, where the electron transfer precedes the proton transfer. However, the visible TA results and the electrochemistry suggest that reduced Ru* is formed from oxidation of guanine.

The study of the Ru(phen)\(_2\)dpqa complex (known to exhibit a light switch effect in the presence of DNA), showed the kinetics for the visible and IR region to be very different in organic and aqueous media. The excited state complex in D\(_2\)O deactivates with a rate of
1/1526 ps, with similar kinetics measured by PIRATE. These measurements may have agreed better because the Ru-dpqa complex could be monitored directly using the carbonyl groups on the dpqa ligand.

The rhenium complexes were investigated using visible TA and PIRATE. The CO ligands produced a strong IR signal, allowing direct monitoring of the metal complex in the IR as well as in the DNA base region, when bound to polynucleotides. The results show very different excited state behaviour for Re(CO)$_3$py dppz (1) and Re(CO)$_3$py F$_2$ dppz (2) in water. 1 showing a long lived state where 2 is deactivated, showing biexponential kinetics. When bound to the GC polynucleotide 2 undergoes an ET reaction, where the formation of the reduced complex is observed growing in around 580 nm. The PIRATE results agree qualitatively with the visible picosecond results. Results show how adjusting substituents on the intercalating ligand can sensitise molecular probes to different solvent environments.

The investigation of dppz showed the sensitivity of the ligand to the solvent was not pronounced. However the kinetics were affected slightly on changing from organic to an organic/aqueous mixture.

The solvated electron investigation, while not new work, gave an example of erroneous results that can be incurred when working at high peak pulse intensities.
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<td>intramolecular vibrational relaxation</td>
</tr>
<tr>
<td>L</td>
<td>ligand</td>
</tr>
<tr>
<td>LUMO</td>
<td>lowest unoccupied molecular orbital</td>
</tr>
<tr>
<td>MB</td>
<td>methylene blue</td>
</tr>
<tr>
<td>MCT</td>
<td>mercury cadmium telluride</td>
</tr>
<tr>
<td>Me</td>
<td>methyl</td>
</tr>
<tr>
<td>ML</td>
<td>metal to ligand</td>
</tr>
<tr>
<td>MLCT</td>
<td>metal to ligand charge transfer</td>
</tr>
<tr>
<td>MO</td>
<td>molecular orbital</td>
</tr>
<tr>
<td>NHE</td>
<td>normal hydrogen electrode</td>
</tr>
<tr>
<td>NL</td>
<td>non-linear</td>
</tr>
<tr>
<td>NLO</td>
<td>non-linear optics/ non-linear optical</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OPA</td>
<td>Optical parametric amplification</td>
</tr>
<tr>
<td>OPO</td>
<td>optical parametric oscillator</td>
</tr>
<tr>
<td>P</td>
<td>polymer</td>
</tr>
<tr>
<td>PCET</td>
<td>proton-coupled electron transfer</td>
</tr>
<tr>
<td>PES</td>
<td>potential energy surface</td>
</tr>
<tr>
<td>Phen</td>
<td>phenanthroline</td>
</tr>
<tr>
<td>Phz</td>
<td>phenazine</td>
</tr>
<tr>
<td>PIRATE</td>
<td>picosecond infra red absorption and transient excitation</td>
</tr>
<tr>
<td>PMMA</td>
<td>poly methyl methacrylate</td>
</tr>
<tr>
<td>ps</td>
<td>picosecond(s)</td>
</tr>
<tr>
<td>py</td>
<td>pyridine</td>
</tr>
<tr>
<td>RAL</td>
<td>Rutherford Appleton Laboratory</td>
</tr>
<tr>
<td>Re</td>
<td>Rhenium</td>
</tr>
<tr>
<td>RegA</td>
<td>regenerative amplifier</td>
</tr>
<tr>
<td>Rep</td>
<td>rate repetition rate</td>
</tr>
<tr>
<td>RF</td>
<td>radio frequency</td>
</tr>
<tr>
<td>Ru</td>
<td>Ruthenium</td>
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<tr>
<td>SF</td>
<td>self focusing</td>
</tr>
<tr>
<td>SF10</td>
<td>dense flint glass</td>
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<tr>
<td>SHG</td>
<td>second harmonic generation</td>
</tr>
<tr>
<td>SOMO</td>
<td>singly occupied molecular orbital</td>
</tr>
<tr>
<td>SPM</td>
<td>self phase modulation</td>
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<tr>
<td>T</td>
<td>thymine</td>
</tr>
<tr>
<td>TA</td>
<td>transient absorption</td>
</tr>
<tr>
<td>TAP</td>
<td>tetra-aza phenanthrene</td>
</tr>
<tr>
<td>TCD</td>
<td>Trinity College Dublin</td>
</tr>
<tr>
<td>TEM</td>
<td>transverse electromagnetic mode</td>
</tr>
<tr>
<td>Th</td>
<td>thionine</td>
</tr>
<tr>
<td>Ti:Sapph</td>
<td>titanium sapphire</td>
</tr>
</tbody>
</table>
TPA  two photon absorption
TR$^3$  Transient Resonance Raman
TRIR  Transient Infra-red
UV  ultraviolet (below 400 nm)
UVC  ultraviolet (below 300 nm)
Vis  visible
YAG  Yttrium Aluminium Garnet
A.1 Labview program

Sequence of events:

User enters parameters: Number of observations
- Delay steps (distance to be moved on each step)
- Dwelling time (time spent at each delay before moving)
- Exposure time (for accumulation on the CCD)
- Acquisition mode single scan/accumulate
- Trigger ext/int (ext trigger – shutter)

Press (GO)
GO!

LB Initialises delay-line (opens communication line, sets step size)

LB Initialises CCD (opens communication, cools it down)

USER PROMPT: Begin Scan?

YES

CCD takes spectrum with shutter open (pump on)
CCD takes spectrum - shutter closed (pump off)
LB Reads delay position
Moves delay line (calculated step size)

Until

Set number of observations reached

USER PROMPT: Choose file to read

USER PROMPT: Choose file to write (save as excel or ascii)

LB saves file

LB Calibrates file

Pixel to Wavelength calibration

LB Closes communication

END
B.1 Zero-point Measurement

Coumarin 152 in ethanol was used as a sample to test where the zero-point was. The pump wavelength used was 400 nm, and the probe was a white light continuum. The transmission was measured for pump on and pump off signals, using the set-up described in Chapter 4, by blocking and unblocking the pump beam. The reference signal was taken without the sample in place. As the delay line was scanned along the length of its travel the Δ abs. signal from the sample changed from negative to positive, indicating that we were passing through the zero-point where the pump and probe pulses coincide.

The actual origin of the signal could be that we are going from absorption in the sample at negative time delays, to stimulated emission in the sample at positive time delays. Two traces are shown, B.2 and B.3.
B.2 A trace of a positive time-delay for the Coumarin sample

B.3 A trace of a negative time delay for the Coumarin sample.
C.1 Biexponential Kinetics

An equation was needed to calculate the rates for forward and back electron transfer in the system where the electron transfer is photoinduced. What is measured is the change in absorption over time in the transient absorption spectra, which is related to the concentration of the absorbing species, \( c \).

\[
\Delta \text{Abs.} \propto c \quad (1.0)
\]

The reaction that occurs is

\[
A^* + B \rightarrow A^- + B^+ \rightarrow A + B \quad (1.1)
\]

In the part of the spectrum where we take our data from for electron transfer it is expected that neither the ground state of A or B absorb appreciably.

To measure the rate of electron transfer we need to know the rate for formation of either \( A^- \) or \( B^+ \), in the case of our sample the species \( A^- \) absorbs in the region that we are measuring in.

\( C_2(t) \) is the concentration of the reduced state as a function of time. This depends on the formation of state B from \( A^* \) and its subsequent decay. This is an inflow - outflow equation.

\[
\frac{dC_2}{dt} = AC_1(t) - BC_2(t) \quad (1.2)
\]

\[
\frac{dC_1}{dt} = AC_1 \quad (1.3)
\]

\[
\frac{1}{C_1} dC_1 = Adt \quad (1.4)
\]

\[
\Rightarrow \int_{C_0}^{C_1} \frac{1}{C_1} dC_1 = A \int_{t_0}^{t_1} dt \quad (1.5)
\]

\[
\Rightarrow \ln C_1 - \ln C_0 = A(t_1 - t_0) \quad (1.6)
\]

\[
\Rightarrow \ln \frac{C_1}{C_0} = At_1 \quad (1.7)
\]

Assuming \( t_0 = 0 \). The solution of this gives:

\[
C_1 = C_0 e^{At_1} \quad (1.8)
\]