Electrochemical and Luminescence Studies of
Ruthenium and Iridium Metal Chelates

by

Emily M. Kerr

B.Sc. (Hons)

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Deakin University

February, 2017
I am the author of the thesis entitled

Electrochemical and Luminescence Studies of Ruthenium and Iridium Metal Chelates

submitted for the degree of Doctor of Philosophy

This thesis may be made available for consultation, loan and limited copying in accordance with the Copyright Act 1968.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: EMILY KERR

Signed: Signature Redacted by Library

Date: 22/05/2017
I certify the following about the thesis entitled (10 word maximum)

Electrochemical and Luminescence Studies of Ruthenium and Iridium Metal Chelates

submitted for the degree of Doctor of Philosophy

a. I am the creator of all or part of the whole work(s) (including content and layout) and that where reference is made to the work of others, due acknowledgment is given.

b. The work(s) are not in any way a violation or infringement of any copyright, trademark, patent, or other rights whatsoever of any person.

c. That if the work(s) have been commissioned, sponsored or supported by any organisation, I have fulfilled all of the obligations required by such contract or agreement.

d. That any material in the thesis which has been accepted for a degree or diploma by any university or institution is identified in the text.

e. All research integrity requirements have been complied with.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: Emily Maria Kerr

Signed: [Signature Redacted by Library]

Date: 19/2/2017
Don’t let the muggles get you down

J. K. Rowling
Table of Contents

Abstract ..................................................................................................................................................i

Acknowledgements .................................................................................................................................. iii

List of Publications .................................................................................................................................. iv

Abbreviations ........................................................................................................................................ vi

Chapter One: Introduction ...................................................................................................................... 1

1.1 Electrogenerated Chemiluminescence ............................................................................................... 1

1.1.1 Annihilation ECL .............................................................................................................................. 2

1.1.2 Co-Reactant ECL ............................................................................................................................... 2

1.1.3 Electrochemical Methods for ECL ..................................................................................................... 3

1.1.4 Thermodynamic and Kinetic Properties of ECL Systems ................................................................. 6

1.1.5 Electronic Transitions of Metal Chelates ....................................................................................... 7

1.2 Novel Metal Chelates for ECL and Chemiluminescence Detection .................................................... 10

1.3 Bioanalytical Applications of ECL .................................................................................................... 14

1.4 Multiplexed ECL Detection .............................................................................................................. 20

1.4.1 Array Based Multiplexed ECL ........................................................................................................ 20

1.4.2 Potential Resolved Multiplexed ECL Detection ............................................................................. 22

1.4.3 Multi-Colour, Multiplexed ECL Detection .................................................................................... 23

1.5 Linking Statement ............................................................................................................................. 34

Chapter Two: Annihilation Electrogenerated Chemiluminescence of Mixed Metal Chelates in Solution: Modulating Emission Colour by Manipulating the Energetics ................................................................................................. 35
Chapter Two: New Perspectives on the Annihilation Electrogenerated Chemiluminescence of Mixed Metal Complexes in Solution

3.1 Abstract..................................................................................................................................64
3.2 Introduction............................................................................................................................65
3.3 Experimental..........................................................................................................................69
  3.3.1 Chemicals.........................................................................................................................69
  3.3.2 Experimental Procedure.................................................................................................69
3.4 Results and Discussion .........................................................................................................71
3.4.1 The [Ru(bpy)$_3$]$^{2+}$ – [Ir(ppy)$_3$] Mixed Annihilation ECL System .......... 71
3.4.2 Energy Transfer in Mixed Annihilation ECL Systems .................. 75
3.4.3 An Additional Route for Enhancement in Mixed Annihilation ECL Systems ........................................................................................................... 79
3.4.4 A Comparison of Mixed Metal Complex Annihilation ECL Systems . 81
3.5 Conclusions .................................................................................. 87
3.6 Acknowledgements ....................................................................... 87

Chapter Four: Blue Electrogenerated Chemiluminescence from Water-Soluble Iridium Complexes Containing Sulfonated Phenylpyridine or Tetraethylene Glycol Derivatised Triazolylpyridine Ligands ....................... 88

4.1 Abstract ..................................................................................... 91
4.2 Introduction ................................................................................ 92
4.3 Experimental Section .................................................................. 95
4.3.1 Absorption and Photoluminescence Emission Spectra .............. 95
4.3.2 Electrochemistry and ECL ....................................................... 95
4.3.3 Synthesis and Characterisation ............................................... 97
4.3.4 Computational Methods .......................................................... 97
4.4 Results and Discussion ............................................................... 99
4.4.1 Design and Preparation of Iridium(III) Complexes .................. 99
4.4.2 Electronic Spectroscopy ........................................................... 102
4.4.3 Electrochemistry ...................................................................... 104
4.4.4 Theoretical Calculations ......................................................... 104
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4</td>
<td>Results and Discussion</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>6.4.1 Electrochemical Properties</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>6.4.2 SEM Characterisation</td>
<td>147</td>
</tr>
<tr>
<td></td>
<td>6.4.3 Electrogernated Chemiluminescence</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>6.4.4 Effect of the Addition of Surfactants on ECL Intensity</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>6.4.5 Stability of ECL Over Multiple Potential Cycles</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>6.4.6 Considerations and Future Work</td>
<td>158</td>
</tr>
<tr>
<td>6.5</td>
<td>Conclusions</td>
<td>159</td>
</tr>
<tr>
<td>6.6</td>
<td>Acknowledgements</td>
<td>159</td>
</tr>
</tbody>
</table>

**Conclusions and Future Work** | 160

**References** | 162

**Appendices** | 174

Appendix I | 174
Appendix II | 200
Appendix III | 214
Appendix IV | 218
Abstract

Electrogenerated chemiluminescence (ECL) is luminescent emission at the surface of an electrode resulting from energetic redox reactions of electrogenerated species in solution. ECL is routinely employed in a variety of clinical diagnostic assays in commercial analysers, to provide sensitive and selective detection of a range of biological compounds of interest. All commercially available ECL analysers use one luminophore, tris(2,2’-bipyridineruthenium(II) ([Ru(bpy)_3]^{2+}) together with the co-reactant tripropylamine (TPrA). This system, however, suffers from a significant limitation; the quantum yield of [Ru(bpy)_3]^{2+} is low (3.9% in water) compared to the quantum yields frequently reported from cyclometalated iridium complexes. For example, the quantum yield of tris(2-phenylpyridinato)iridium(III) ([Ir(ppy)_3]) is reported to be 99% in acetonitrile. Cyclometalated iridium complexes also allow colour-tuning of the emission maxima of the complex across the visible region and even into the UV; this presents exciting opportunities for ‘mixed-ECL’ from solutions containing multiple luminophores.

In this work, previously reported iridium and ruthenium metal chelates have been used to investigate multi-colour ECL of mixed solutions of metal chelates in anhydrous solvents. It is possible to predict the ECL colour intensity in these experiments, simply by calculating the exergonicity of the ground and excited state pathways. In numerous systems, distinct emissions from multiple luminophores were observed and it was possible to tune the ECL emission colour by controlling the applied electrochemical potential. A thorough comparison of three mixed metal chelate systems was then conducted to consolidate several previously reported conflicting observations. Multi-coloured annihilation ECL was observed from all three systems examined, but only when the applied potential was sufficient to generate the
excited state and the concentration of the iridium complex was sufficiently high to
minimise quenching by the ruthenium complex. These results provide an interesting
insight into previously unexplored physical and analytical characteristics of ECL and
are particularly important for the development of light emitting devices.

A range of water-soluble iridium complexes was evaluated, with emissions
spanning from orange to blue, for their ECL and chemiluminescence properties. The
ECL intensity of the most efficient ECL emitter $[\text{Ir(df-ppy)}_{2}(\text{pt-TEG})]^{+}$ (df-ppy = 2-(2,4-difluorophenyl)pyridine anion and pt-TEG = 1-(2-(2-(2-
hydroxyethoxy)ethoxy)ethoxy)ethyl)-4-(2-pyridyl)-1,2,3-triazole) was 102% that of
$[\text{Ru(bpy)}_{3}]^{2+}$ in aqueous solution. This result is promising for the development of
multi-colour analytical ECL detection strategies and for the enhancement of pre-
existing bioanalytical ECL assays. Moreover, $[\text{Ir(df-ppy)}_{2}(\text{pt-TEG})]^{+}$ showed a
marked difference in chemiluminescence selectivity to $[\text{Ru(bpy)}_{3}]^{2+}$ and an intense
chemiluminescence signal was observed from the reaction of $[\text{Ir(df-ppy)}_{2}(\text{pt-TEG})]^{+}$
with furosemide.

Lastly, a range of commercially available screen printed electrodes (SPEs) was
evaluated for their suitability for analytical ECL applications. Using cyclic
voltammetry, unmodified carbon-based SPEs produced the highest relative ECL
intensities (45-100% depending on electrode variety). SPEs composed of
combinations of carbon and carbon nanomaterials exhibited comparatively poor ECL
intensities ranging from 21-48%. Gold (9%) and platinum-based (16%) electrodes
showed the lowest relative ECL intensities when examined using cyclic voltammetry;
however, significant cathodic ECL was observed for both electrode varieties.
Acknowledgements

I would like to start by thanking my primary supervisor, Professor Paul Francis; thanks for always making time for me and for never failing to refresh my enthusiasm for my work when my patience was wearing thin. Thanks to my associate supervisor, Dr Egan Doeven, for putting up with me in the lab for three years, for answering my many, many questions with so much patience and for being a constant source of guidance and support. To Dr Gregory Barbante, thanks so much for your unwavering support and for always keeping me on my toes. To Professor George Whitesides, thank you so much for the opportunity to work in your lab for the final year of my PhD, it has been a wonderful experience, thank you for our stimulating discussions and for always encouraging me to ‘think bigger’.

To my co-workers and friends Deakin, Harvard and La Trobe, thanks for making my time in the lab enjoyable. I would like to thank Associate Professor Conor Hogan, Dr Zoe Smith, Dr Richard Alexander, Dr David Bower, Dr Yi Heng Nai and Kara Spilstead, for their specific contributions to various projects, as outlined in each section.

I couldn’t have achieved what I have without the unwavering support of my parents, Mum and Dad, thanks for being amazing.

Chris and Freya, the two of you have made my life spectacular, thanks for going along with all my crazy plans and for always making me smile.
List of Publications

The following publications are included in this thesis


Other publications


Abbreviations

\( \lambda \) – wavelength

A – working electrode area

AFP – alpha-fetoprotein

Alq\(_3\) – aluminium tris-(8-hydroxyquinoline)

biq – 2,2′-biquinoline

BPS – bathophenanthroline-disulfonate

bpy – 2,2′-bipyridine

bt – phenylbenzothiazole

\( c \) – the speed of light \((3.00 \times 10^8 \text{ m s}^{-1})\)

CA – carcinoma antigen

CA153 – carcinoma antigen 153

CA159 – carcinoma antigen 159

CEA – carcinoembryonic antigen

CCD – charge coupled device

CIE – Commission Internationale de l’Eclairage

CL – chemiluminescence

CNF – carbon nanofiber

CNF – carbon nanotube

CT – charge transfer
D – diffusion coefficient

DBAE – 2-(dibutylamino)ethanol

df-MeppyH – 2-(2,4-difluorophenyl)-5-methylpyridine

df-phtl – 1-benzyl-4-(2,4-difluoro-phenyl)-1H-1,2,3-triazole

df-ppy – 2-(2,4-difluorophenyl)pyridine

df-ppz – 1-(2,4-difluorophenyl)pyrazole

DFT – density functional theory

dim-bpy – 4,4’-dimethylbipyridine

DIPEA – N, N-diisopropylethylamine

DIPEA-OH – 2-(diisopropylamino)ethanol

dma-bpy-dma – 4,4’-(dimethylamino-2,2’-bipyridine)

dm-bpy-dc – dimethyl 2,2’-bipyridine-4,4’-dicarboxylate

DNA – deoxyribonucleic acid

DPA – 9,10-diphenylanthracene

DPVBi – 4,4’-bis(2,2’-diphenylvinyl)-1,1’-biphenyl

DS – DropSens

dtb-bpy – 4,4’-di(tert-butyl)-2,2’-bipyridine

$E_1$ – first applied potential

$E_2$ – second applied potential

$E_{AA^-}$ – peak potential for reduction,
$E_{D,D^+}$ – peak potential for oxidation

$E_{es}$ – emission energy

ECL – electrogenerated chemiluminescence

ELISA – enzyme linked immunosorbent assay

EMCCD – electron multiplying charged coupled device

eV – electron volt

F – faraday constant

$\text{Fc}^{0/+}$ – ferrocene/ferrocenium redox couple

Fab – fragment, antigen-binding region

$\Delta G$ – Gibbs free energy

$\Delta G_{es}$ – Gibbs free energy associated with the formation of excited state products

$\Delta G_{gs}$ – Gibbs free energy associated with the formation of ground state products

GC – glassy carbon

GNP – gold nanoparticle

GNP-HBV – gold nanoparticle, hepatitis B virus bioconjugate

GNP-HCV – gold nanoparticle, hepatitis C virus bioconjugate

GPH – graphene

HIV – human immunodeficiency virus

HOMO – highest occupied molecular orbital

$h$ – Planck’s constant ($4.13 \times 10^{-15}$ eV s$^{-1}$)
hv – photon
IgG – Immunoglobulin G
IL-6 – interleukin-6
$i_p$ – peak current
ITO – indium tin oxide
L – ligand
LC – ligand centred transition
LL – ancillary ligand
LMCT – ligand to metal charge transfer
LOD – limit of detection
LUMO – lowest unoccupied molecular orbital
MC – metal centred transition
M – metallic centre
MLCT – metal to ligand charge transfer
MLLCT – metal to ligand to ligand charge transfer
MO – molecular orbital
mRNA – messenger ribonucleic acid
$n$ – number of electrons transferred in a given redox reaction
NA – nucleic acid
NHS – N-hydroxysuccinimide
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPB</td>
<td>N,N′-di(naphthalene-1-yl)-N,N'-diphenyl-benzidine</td>
</tr>
<tr>
<td>OLEDs</td>
<td>organic light emitting devices</td>
</tr>
<tr>
<td>OMC</td>
<td>ordered mesoporous carbon</td>
</tr>
<tr>
<td>PAMAM</td>
<td>poly(amido amine)</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffer solution</td>
</tr>
<tr>
<td>PCM</td>
<td>polarizable continuum model</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>(PF$_6$)$^-$</td>
<td>hexafluorophosphate</td>
</tr>
<tr>
<td>pic</td>
<td>2-carboxypyridyl</td>
</tr>
<tr>
<td>PICH$_2$</td>
<td>2-(4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline</td>
</tr>
<tr>
<td>piq</td>
<td>phenylisoquinoline</td>
</tr>
<tr>
<td>pmi</td>
<td>1-phenyl-3-methylimidazolin-2-ylidene</td>
</tr>
<tr>
<td>PMMA</td>
<td>poly(methyl methacrylate)</td>
</tr>
<tr>
<td>PMT</td>
<td>photomultiplier tube</td>
</tr>
<tr>
<td>ppy</td>
<td>2-phenylpyridine</td>
</tr>
<tr>
<td>PPV</td>
<td>polyphenylenevinylene</td>
</tr>
<tr>
<td>ppz</td>
<td>phenylpyrazole</td>
</tr>
<tr>
<td>PSA</td>
<td>prostate specific antigen</td>
</tr>
<tr>
<td>ptb</td>
<td>2-[1-(phenylmethyl)-1H-1,2,3-triazol-4-yl]pyridine</td>
</tr>
<tr>
<td>ptp</td>
<td>2-(3-phenyl-1H-1,2,4-triazol-5-yl)pyridinato</td>
</tr>
</tbody>
</table>
Q – charge at the electrode surface

QD – quantum dot

QD-HBV – quantum dot, hepatitis B virus conjugate

QD-HCV – quantum dot, hepatitis C virus conjugate

RGB-ECL – red blue green electrogenerated chemiluminescence

ROS – reactive oxygen species

RNA – ribonucleic acid

RUB – rubrene

Ru-COOH – bis(2,2’-bipyridine)-4-methyl-4’-carboxypropyl-2,2’-bipyridine)ruthenium(II)-bis(hexafluorophosphate)

SEM – scanning electron microscopy

SCRF – self-consistent reaction field

SECLDs – solid state electrogenerated chemiluminescence devices

ssDNA – single stranded deoxyribonucleic acid

SPE – screen printed electrode

SWCNT – single walled carbon nanotube

TBA – tetrabutylammonium

TBAPF$_6$ – tetrabutylammonium hexafluorophosphate

TEG – tetraethylene glycol

TOPO – trioctyl phosphine oxide
TPrA – tripropylamine

TSH – thyrotropin

TX – polyethylene glycol tert-octylphenyl ether

T20 – polyethylene glycol sorbitan monolaurate

$\nu$ – scan rate
Chapter One: Introduction

1.1 Electrogenerated Chemiluminescence

Electrogenerated chemiluminescence or electrochemiluminescence (ECL) is luminescent emission resulting from electron transfer reactions between electrochemically generated species at the surface of an electrode.\(^1\) The first comprehensive analysis of ECL was detailed by Hercules and Bard in the 1960’s.\(^2\)-\(^4\) However, the phenomenon appeared in the literature as early as the 1920’s.\(^5\) The field of ECL has evolved from this initial discovery to incorporate a variety of generation mechanisms, analytical applications and luminophores.\(^6\)-\(^9\) The vast majority of ECL studies have focussed on tris(2,2’bipyridine)ruthenium(II) ([Ru(bpy)\(_3\)]\(^{2+}\); \(\lambda_{\text{max}} = 620\) nm, Figure 1.1). [Ru(bpy)\(_3\)]\(^{2+}\) ECL was the first account of inorganic ECL, observed by Tokel and Bard\(^10\) in 1972; after investigations into the luminescent properties of the complex was undertaken by Paris and Brandt\(^11\) Currently, [Ru(bpy)\(_3\)]\(^{2+}\) is the only metal chelate used in commercially available ECL diagnostic systems; due to its excellent electrochemical, energetic and spectroscopic properties in both aqueous and organic media.\(^12\) ECL from [Ru(bpy)\(_3\)]\(^{2+}\), can be produced by one of two general pathways, termed ‘annihilation’ and ‘co-reactant’.\(^13\)

![Figure 1.1: Structure of [Ru(bpy)\(_3\)]\(^{2+}\).](image-url)
1.1.1 Annihilation ECL

Annihilation ECL involves the electrochemical formation of oxidised and reduced species at the surface of the electrode because of alternating the applied potential between the oxidation and reduction potentials of the complex. These oxidised and reduced species then annihilate, with one undergoing excited state formation and radiative decay. This reaction pathway is further detailed in reactions 1-4.

(1) \( A \rightarrow A^{++} + e^- \)
(2) \( A + e^- \rightarrow A^{--} \)
(3) \( A^{--} + A^{++} \rightarrow A + A^* \)
(4) \( A^* \rightarrow h\nu + A \)

Where \( h\nu \) represents a photon. The primary advantage of annihilation ECL, when compared to co-reactant ECL, is the simplicity of the experimental setup; only the luminophore, solvent and supporting electrolyte are required for ECL to occur. However, solvent selection is limited to organic solvents, as the accessible potential window of water is insufficient to facilitate both oxidation and reduction of most luminophores. Therefore, most commercial ECL applications are based on co-reactant, as opposed to annihilation, ECL systems.\(^{14}\)

1.1.2 Co-Reactant ECL

Co-reactant ECL differs from annihilation ECL in that it is possible to generate ECL from a single applied electrochemical potential as a result of the addition of a reagent (co-reactant) to the experimental system.\(^{15}\) This potential causes the co-reactant present in the solution to form a product capable of reacting with the oxidised or reduced form of the emitting complex to form excited states and light emission.\(^{13,16}\) An example of a co-reactant ECL system is described by reactions 5-13, which detail
the reaction of Ru(bpy)$_3^{2+}$ with co-reactant tripropylamine (TPrA) as described by Bard, *et al*.$^{17}$

\[
(5) \quad [\text{Ru(bpy)}_3^{2+} - e^- \rightarrow [\text{Ru(bpy)}_3]^{3+}
\]

\[
(6) \quad \text{TPrA} - e^- \rightarrow \text{TPrA}^{++}
\]

\[
(7) \quad [\text{Ru(bpy)}_3]^{3+} + \text{TPrA} \rightarrow [\text{Ru(bpy)}_3]^{2+} + \text{TPrA}^{++}
\]

\[
(8) \quad \text{TPrA}^{++} \rightarrow \text{TPrA}^{+} + \text{H}^+
\]

\[
(9) \quad [\text{Ru(bpy)}_3]^{3+} + \text{TPrA}^+ \rightarrow [\text{Ru(bpy)}_3]^{2+*} + \text{other products}
\]

\[
(10) \quad [\text{Ru(bpy)}_3]^{2+} + \text{TPrA}^+ \rightarrow [\text{Ru(bpy)}_3]^{+} + \text{other products}
\]

\[
(11) \quad [\text{Ru(bpy)}_3]^+ + [\text{Ru(bpy)}_3]^{3+} \rightarrow [\text{Ru(bpy)}_3]^{2+*} + [\text{Ru(bpy)}_3]^{2+}
\]

\[
(12) \quad [\text{Ru(bpy)}_3]^+ + \text{TPrA}^{++} \rightarrow [\text{Ru(bpy)}_3]^{2+*} + \text{TPrA}
\]

Following excited state formation, (10) $[\text{Ru(bpy)}_3]^{2+*}$ returns to the ground state \textit{via} reaction 13.

\[
(13) \quad [\text{Ru(bpy)}_3]^{2+*} \rightarrow [\text{Ru(bpy)}_3]^{2+} + hv
\]

Co-reactant ECL with TPrA first documented by Leland and Powell.$^{18}$ It is the most extensively studied co-reactant as it has been successfully exploited in numerous commercially available analytical ECL applications.$^{7, 14, 16, 18, 19}$ Alternative co-reactants, such as 2-(dibutylamino)ethanol (DBAE), have also been employed in several analytical ECL applications.$^{6, 14, 20-23}$

1.1.3 Electrochemical Methods for ECL

The electrochemical method used and hence the applied electrochemical potential, varies depending on both the luminophore and the analytical application. Cyclic voltammetry is an electrochemical technique where the applied potential is scanned linearly from an initial potential to a set end point (forward sweep), then reversed to a
set end point (reverse sweep). Cyclic voltammetry is used in ECL to ascertain qualitative, quantitative, kinetic and mechanistic information about the redox processes which facilitate ECL. The peak current ($i_p$) resulting from oxidation and reduction of analytes in a cyclic voltammogram relates to crucial information about the experimental system including the diffusion coefficient ($D$, cm²/s), analyte concentration (moles/cm³) electrode area ($A$, cm²) and scan rate ($v$, V/s) and these values can be calculated using the equation (I),

$$i_p = (2.69 \times 10^5) A D C v^{1/2}$$

A representative cyclic voltammogram of [Ru(bpy)₃]²⁺ is shown in Figure 1.2. From Figure 1.2, it is possible to observe the reversible oxidation of the complex in the anodic scan range; in the reverse sweep, the three accessible ligand reductions of the complex are evident. Generally, if redox processes are stable and the forward and reverse scans display identical peak magnitudes, the system is said to be electrochemically reversible.

![Figure 1.2: Cyclic voltammogram of [Ru(bpy)₃]²⁺ vs Fe⁰/⁺ (ferrocene/ferrocenium redox couple) Conditions: [Ru(bpy)₃]²⁺ 1 mM, 0.1 M tetrabutylammonium](image-url)
hexafluorophosphate (TBAPF$_6$) electrolyte dissolved in acetonitrile, scan rate 0.1 V s$^{-1}$.

In a chronoamperometry experiment, the potential of the working electrode is stepped (or ‘pulsed’) between desired potentials (Figure 1.3), usually a potential sufficient to induce oxidation or reduction of the reactant or a ‘rest’ potential (0 V), and the resulting current from Faradaic processes occurring at the electrode is monitored as a function of time.$^{27}$ As the duration of the applied potential (t) increases, a growing diffusion layer of product is formed at the electrode surface causing an exponential decay in the current (i) in accordance with the Cottrell equation (II),$^{25}$

$$i = \frac{FACD^2}{(\pi t)^{\frac{1}{2}}}$$

Chronoamperometry is frequently employed to generate the emission profiles of luminophores in both annihilation and co-reactant ECL.$^{27}$ In ECL, chronoamperometry experiments are generally synchronised with the acquisition of a light detecting instrument, such as a charged coupled device (CCD) spectrometer, digital camera, photodiode or photomultiplier tube (PMT), to facilitate the quantitative determination of ECL intensity.$^{7,14,16,28}$ By integrating the current measurements and comparing this value with the integrated response from a light detector, information about the ECL efficiency (the probability that an electron transfer event will result in light emission, $\Phi_{\text{ECL}}$) of the experimental system can also be obtained. The analysis of both the electrochemical and luminescent properties of different luminophores provides significant insight into their emissive, mechanistic and kinetic properties.
1.1.4 Thermodynamic and Kinetic Properties of ECL Systems

The applied electrochemical potential determines the amount of free energy available for ECL to occur in any given system and dictates which electron transfer reactions are thermodynamically feasible. Electron transfer reactions, contrastingly to reactions involving transfer of atoms, occur almost instantaneously. Whilst the surrounding solvent molecules reorientate themselves to balance the excess energy generated from the ‘electron jump’, a high energy intermediate is produced, in accordance with the Franck-Condon principle. This high energy intermediate, can then react to form an excited state product capable of undergoing radiative or non-radiative decay to the ground state. Marcus first used chemiluminescent electron transfer reactions to examine the theories of outer sphere redox processes in 1965. Marcus found that as electron transfer reactions became increasingly exothermic, the formation of ground state products from the reactive intermediates became less favourable; the excess energy associated with the return to the ground state reaction pathway could not be dissipated into either the surrounding solvent or the vibrational modes of the molecule. In such systems, the production of an excited state product
from the highly reactive intermediates is kinetically favoured, even though it is comparatively thermodynamically disfavoured, allowing ECL to occur.\textsuperscript{33} It is possible to directly calculate the Gibbs free energy ($\Delta G$) associated with both the formation of ground ($\Delta G_{gs}$) or excited state ($\Delta G_{es}$) products from the oxidised and reduced intermediate species using both the formal potentials (obtained from a cyclic voltammogram of the complex) and the emission energy ($E_{es}$) of the complex in accordance with equations III and IV respectively.\textsuperscript{14}

\begin{align*}
\text{III.} & \quad \Delta G_{gs} \approx E_{A,A^-}^o - E_{D,D^+}^o \\
\text{IV.} & \quad \Delta G_{es} \approx (E_{A,A^-}^o - E_{D,D^+}^o) + E_{es}
\end{align*}

Where; $E_{A,A^-}^o =$ peak potential for reduction, $E_{D,D^+}^o =$ peak potential for oxidation, and

\begin{align*}
\text{V.} & \quad E_{es} = \frac{hc}{\lambda}
\end{align*}

Where; $h =$ Planck’s constant ($4.13 \times 10^{-15}$ eV s\textsuperscript{-1}), $c =$ the speed of light ($3.00 \times 10^8$ m s\textsuperscript{-1}) and $\lambda =$ emission wavelength (m). In ECL, there is constant competition between the formation of ground and excited state products from the oxidised and reduced intermediate species; ultimately, the kinetic and thermodynamic properties of the experimental system will determine if a luminophore undergoes radiative or non-radiative decay to the ground state.

\textbf{1.1.5 Electronic Transitions of Metal Chelates}

ECL can occur through a variety of electron transfer processes. Typical transition metal complexes, such as [Ru(bpy)]\textsuperscript{$3^{2+}$}, exhibit several possible electronic transition processes, as shown in the Figure 1.4, and provide an appropriate model for developing an understanding of the electron transfer processes that produce ECL.\textsuperscript{7,13}
The molecular orbitals (MOs) of transition metal chelates are located on either the metallic centre (M) or ligands (L) of the complex. Hence, the corresponding π and σ-bonding molecular orbitals are labelled as either M or L depending on which MO is the major contributor to the bond. For example, when \([\text{Ru(bpy)}_3]^{2+}\) is in a stable oxidation state, the ligand orbitals (σ_L and π_L) are completely filled and the metal orbitals (π_M) are partially filled.

**Figure 1.4:** Schematic energy-level diagram representing the molecular orbitals and energy transitions of typical octahedral transition metal complexes. Image from reference.\(^3^4\)

Three types of electronic transitions can occur to produce ECL:\(^3^4\)

1. Metal centred transitions (MC), between different MOs of the metal centre;
2. Ligand centred transitions (LC), between different MOs of the ligands;
3. Charge transfer transitions (CT), from a metal based orbital to a ligand centred orbital (MLCT) or *vice versa* (LMCT).
Transitions which result in ECL from metal chelates are principally MLCT transitions where electrons undergo strong spin-orbit coupling and intersystem crossing (due to the relatively high lying $\sigma$ orbitals of the heavy metal centre and comparatively low lying $\pi^*$ orbitals of the ligands) to form an excited triplet state then relax back to the ground state via the emission of light (Figure 1.5).\textsuperscript{34}

![Diagram showing intersystem crossing in metal chelate annihilation ECL. Image adapted from reference.\textsuperscript{14}](image)

**Figure 1.5:** Diagram showing intersystem crossing in metal chelate annihilation ECL. Image adapted from reference.\textsuperscript{14}

It is important to note that the order in which the energies of the excited states fall varies between different complexes.\textsuperscript{34} The ligand $\pi^*$ orbital is also often referred to as the lowest unoccupied molecular orbital (LUMO). The highest occupied molecular orbital (HOMO) is commonly metal centred ($\pi_M$ orbital). The energy difference between the HOMO and LUMO orbitals of a complex is termed the HOMO-LUMO gap and is proportional to the emission energy of the complex.\textsuperscript{14} Complexes with larger HOMO-LUMO gaps have higher energy wavelength emissions.\textsuperscript{14} As the oxidation potential of a complex is determined by the amount of energy required to
remove an electron from the HOMO of a complex and the reduction potential is
determined by the amount of energy required to populate the LUMO of a complex
with an electron, the HOMO and LUMO of a complex are also directly related to the
oxidation and reduction potentials of a complex.

The ability to systematically alter the structure, and hence, molecular orbital
energies, of metal chelates allows the fine tuning of both their emission energies and
electronic properties. This has led to the development of metal chelate complexes with
emissions spanning almost the entire visible spectrum, and many of these newly
synthesised complexes are ideal candidates for incorporation into both analytical ECL
applications and solid state displays.\textsuperscript{28, 35, 36}

1.2 Novel Metal Chelates for ECL and Chemiluminescence Detection

Despite the comprehensively studied and extensively applied \([\text{Ru(bpy)}_3]^2+\) and
TPrA ECL system, it suffers from limitations, namely a low luminescence quantum
yield (3.9\% in aerated H\(_2\)O, this work, section 4.4.2) and inefficient population \textit{via}
electron transfer of the \([\text{Ru(bpy)}_3]^2+\) state. To combat these shortcomings, numerous
research groups have focussed on developing alternative iridium chelates. Since the
first demonstrations of ECL from iridium chelates by Demas, \textit{et al.}\textsuperscript{37} and Watts, \textit{et al.}\textsuperscript{38}, numerous luminophores have been synthesised with many exhibiting both high
luminescence quantum yields and efficient ECL with co-reactant TPrA.\textsuperscript{37-56} A recent,
comprehensive review by Kapturkiewicz\textsuperscript{43}, has provided an extensive overview of
iridium chelates and their applications in various ECL detection systems.

Iridium chelates are particularly promising as alternatives to ruthenium chelates
because the incorporation of cyclometalating ligands into the octahedral structure of
the complex allows electronic interactions between the d orbitals of the metallic centre
and \(\sigma\) orbitals of the ligands, permitting HOMO modulation \textit{via} systematic modification of the phenyl ring structure and, together with strong spin-orbit coupling due to the heavy metal centre, significantly enhancing luminescence efficiencies.\textsuperscript{36, 39, 55} Furthermore, substitution of one cyclometalating ligand with an ancillary ligand, permits modulation of both the HOMO and LUMO of the complex.\textsuperscript{45, 53, 57-61} The ability to tune the HOMO and LUMO of a complex, and, therefore, both the redox properties and emission wavelength, has enormous potential for the development of multiplexed ECL systems where different analytes are selected on the basis of colour or potential, or both, negating the need for spatial or flow based separation methods.

An efficient, blue emitter is highly desirable for ECL applications to facilitate spectral distinction from orange \([\text{Ru(bpy)}_3]^{2+}\). Various methods have been employed in attempts to increase the HOMO-LUMO gap, by either stabilisation of the HOMO or destabilisation of the LUMO of the archetypal cyclometalated iridium complex, to obtain a blue emission.\textsuperscript{39, 41, 42, 49, 62, 63} Three approaches proposed have been found to be the most successful in obtaining efficient, blue emission from iridium metal chelates:

1. Addition of electron withdrawing groups, such as fluorine, to the phenyl ligand fragment, stabilising the HOMO, with minimal effect on the LUMO.

2. Use of ancillary ligands (LL) that result in the stabilisation of HOMO levels, such as \([\text{Ir(ppy)}_2(\text{LL})]\) or \([\text{Ir(df-ppy)}_2(\text{LL})]\) (where ppy is 2-phenylpyridine and df-ppy is 2-(2,4-difluorophenyl)pyridine).

3. Substitution of pyridine ring fragments with comparatively higher LUMO level fragments, such as phenylpyrazolyl (ppz).
A recent study by Barbante, *et al.*[^36] assayed a variety of emitters developed using these strategies and determined that combinations of strategies 1 and 2 were found to be the most effective at obtaining efficient, blue ECL with co-reactant TPrA. One heteroleptic complex, $\text{[Ir(df-ppy)2(ptb)]}^+$ (where ptb is 2-[1-(phenylmethyl)-1H-1,2,3-triazol-4-yl]pyridine), exhibited blue ECL ($\lambda_{\text{max}} = 455$ nm) 16 fold higher than $\text{[Ir(ppy)3]}$ (Figure 1.5). Many obstacles, including poor water solubility and unfavourable energetics with TPrA co-reactant, have hampered the development of analytical assays using iridium chelates.[^12] Nevertheless, in recent years, a handful of analytical chemiluminescence (CL) and ECL assays have been developed which employ iridium chelates as the emissive species.[^9] [^64-66]

![Figure 1.5: [Ir(df-ppy)2(ptb)]^+ structure and ECL spectrum (concentration 0.1 mM, in acetonitrile, 0.1 M TBAPF_6).][^36]

Due to the direct relation between metal chelate CL and ECL reaction mechanisms, metal chelates developed for the purposes of ECL applications are often subsequently employed as reagents in CL assays and *vice versa.*[^7] [^43] Analytical CL systems employ an oxidant (such as cerium (IV) sulfate) to induce the chemical generation of excited state products. The general reaction mechanism for $\text{[Ru(bpy)3]}^{2+}$ CL is detailed in reactions 14-16;

\begin{align*}
(14) \quad & \text{[Ru(bpy)3]}^{2+} + \text{Oxidant} \rightarrow \text{[Ru(bpy)3]}^{3+} \\
(15) \quad & \text{[Ru(bpy)3]}^{3+} + \text{Analyte} \rightarrow \text{[Ru(bpy)3]}^{2+*}\
\end{align*}
\[
(16) \quad \text{[Ru(bpy)}_3])^{2+*} \rightarrow \text{[Ru(bpy)}_3]^{2+} + h\nu
\]

CL applications of iridium chelates include the detection of cysteine, tryptophan
and sulphite.\textsuperscript{67}-\textsuperscript{69} Shin, \textit{et al.}\textsuperscript{66} successfully detected a variety of analytes including
tryptophan, histidine and epinephrine using a neutral iridium chelate in a flow based
ECL experimental manifold. Previous investigations into the CL of green emissive
water soluble iridium complexes by Kiran, \textit{et al.}\textsuperscript{70} along with a follow up study by
Truong, \textit{et al.}\textsuperscript{71} demonstrated efficient CL of water soluble iridium chelates exhibiting
sulfonate functional groups in acidic aqueous media.\textsuperscript{52} Yu, \textit{et al.}\textsuperscript{72} later went on to
demonstrate their ECL properties. Another strategy to enhance water solubility,
developed by Li, \textit{et al.}\textsuperscript{48}, is the addition of sugar groups to an ancillary ligand, iridium
chelates produced using this method have been incorporated into assays for the
detection of thrombin and antibiotics.\textsuperscript{48, 73}

Alternative approaches to ECL assays do not rely on the water solubility of iridium
(III) chelates, in stead, immobilising the reagents on nanomaterials, such as multi-
walled carbon nanotubes, gold microparticles, cell surfaces or magnetic microbeads.
Zanarini, \textit{et al.}\textsuperscript{77} obtained ECL from an insoluble neutral iridium complex with
co-reactant DBAE, \textit{via} its incorporation into silica nanoparticles with an outer shell
exhibiting highly polar polyethylene glycol (PEG) substituents (Figure 1.6). \textsuperscript{48, 77-79}
The variety of avenues to both develop and immobilise iridium chelates capable of
producing ECL in aqueous environments provides great promise for the development
of new, ultra-sensitive and multiplexed detection systems for bioanalytical ECL
applications.
1.3 Bioanalytical Applications of ECL

Recent advances in the field of ECL have led to the development of several commercially available ECL diagnostic systems for biologically important compounds. Almost all the commercially available ECL kits are based on immunoassay or nucleic acid (NA) bioconjugation analysis techniques.

Immunoassays are diagnostic techniques which exploit the high degree of selectivity exhibited in endogenous antibody-antigen systems. When the human body overcomes an infection caused by a specific virus or bacteria, it produces a range of B-cells and T-helper cells which exhibit highly specific antigens on their surface, capable of binding to an epitope expressed on the surface of the corresponding pathogen. The antibody contains three primary structural units, the antigen binding site, known as the fragment antigen-binding region (Fab), two ‘heavy’ chains and two ‘light’ chains (Figure 1.7).

Although there is a high degree of similarity between the heavy and light chains of different antigens, (5 mammalian heavy chain varieties and 2 mammalian light chain varieties) the antibody binding site, is varied and specific, rendering it an ideal candidate for selective assays for the corresponding antigen.
The most common form of immunoassay used in immunodiagnostics is the enzyme linked immunosorbent assay (ELISA). Since Yalow and Berson first used an ELISA for the detection of insulin in 1959, the field of immunodiagnostics has expanded to facilitate a multitude of biological applications from the diagnosis of cancer, endocrine malfunctions, metabolic diseases, infertility, pregnancy, diabetes and haematological disturbances to the detection of various viral, parasitic and bacterial infections. In fact, ELISA based immunoassays were the first widely employed test for the diagnosis of infection with Malaria in the 1970’s and human immunodeficiency virus (HIV) in the 1980’s. The ELISA may be direct, where the primary antibody is attached to an enzyme which reacts directly with a substrate, or indirect (sandwich ELISA), where a secondary antibody is added which contains the reactive enzyme (Figure 1.8). The primary disadvantages of ELISA based immunoassays are the limitations of the various enzymes used to produce the measured response, usually a chemiluminescent colour change or electrical current. These enzymes can be recycled and reused to produce many colour changes, although this is advantageous in terms of amplifying the response, it makes quantitative evaluation difficult and careful timing of mixing of reagents and detection steps is critical for obtaining accurate quantitative results.
Figure 1.8: a) Direct ELISA (1) virus adhered to surface, (2) addition of enzyme containing antibody, (3) addition of substrate resulting in measurable (chemiluminescent or electrochemical) response upon reaction with enzyme. b) Indirect Sandwich ELISA (1) capture antibody immobilised on surface, (2) addition of virus containing sample (3) addition of detection antibody, (4) addition of secondary enzyme-linked antibody, (5) substrate added resulting in measurable response upon reaction with enzyme.

The readily controllable ECL of [Ru(bpy)$_3$]$_{2+}$ presents a convenient solution to the drawbacks of traditional ELISA immunoassays.$^{110}$ Enhancement of both the temporal and spatial control of the immunoassay is achieved in ECL because the experiment can be initiated by the accurate application of an electrochemical potential, ECL is also spatially restricted to the surface of the working electrode.$^{14, 111}$ The first ECL immunoassay was conducted by Ikariyama, et al.$^{112}$ in 1985, using an antigen labelled with pyrene to detect albumin.$^{112}$ Although this initial experiment yielded acceptable results (LOD of 1 µM) pyrene exhibits poor luminescence efficiency when compared to other luminophores, such as [Ru(bpy)$_3$]$_{2+}$. Future ECL immunoassays, such as those developed by Blackburn, et al.$^{113}$ used a derivatised [Ru(bpy)$_3$]$_{2+}$ analogue exhibiting an N-hydroxysuccinimide (NHS) ester on one of the three bipyridine ligands (Figure 1.9) to facilitate antigen binding.$^{113}$ Previous studies had found that the binding of the
NHS-derivatised $[\text{Ru(bpy)}_3]^{2+}$ chelates to bulky deoxyribonucleic acid (DNA) molecules had no effect on their luminescent properties. Blackburn, et al.\textsuperscript{113} was able to successfully detect digoxin, thyrotropin (TSH), carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) using various ECL immunoassay procedures.\textsuperscript{113}

![Image of $[\text{Ru(bpy)}_3]^{2+}$](image)

**Figure 1.9:** NHS-derivatised $[\text{Ru(bpy)}_3]^{2+}$.

Blackburn, et al.\textsuperscript{113} also used previously established procedures for DNA amplification (polymerase chain reaction, PCR) of HIV-1 gene along with a DNA probe with an emissive $[\text{Ru(bpy)}_3]^{2+}$ tag to develop a proof-of-concept DNA probe assay.\textsuperscript{115} In an ECL NA probe assay (detailed in Figure 1.10), the single stranded DNA (ssDNA) or ribonucleic Acid (RNA) of interest is first immobilised on the electrode surface, a complimentary ssDNA, which is labelled with a $[\text{Ru(bpy)}_3]^{2+}$ tag, is then added. The complimentary ssDNA hybridises with the immobilised analyte ssDNA, and after washing to remove unbound complimentary strands, an electrochemical potential is applied, allowing $[\text{Ru(bpy)}_3]^{2+}$ to luminesce; the resulting response can be measured with either a PMT or CCD. DNA probe assays using $[\text{Ru(bpy)}_3]^{2+}$ ECL have since been applied to a multitude of analytical applications, including improved detection of various viral infections and messenger RNA (mRNA) of numerous cancer markers.\textsuperscript{116-124}
Figure 1.10: ECL DNA probe assay. Image from reference.125

All automated commercial ECL instruments, such as the Roche ELECSYS system, employ TPrA as a co-reactant with a derivatised [Ru(bpy)_3]^{2+} label that is immobilised on either a DNA probe, antibody or antibody (depending on the desired analysis), bound to a paramagnetic microparticle.125, 126 The paramagnetic microparticle allows precise manipulation of the spatial position of the bioconjugated complex, which permits both the washing of unbound labels without the loss of sample and the precise positioning of the analyte and label directly above the electrode surface. Once the bioconjugate is positioned directly above the electrode, an appropriate electrochemical potential is applied, forming the [Ru(bpy)_3]^{2+*} state, resulting in light emission which is proportional to the amount of analyte, this light emission is measured using a PMT (Figure 1.11).126 Alternative systems, such as those developed by meso scale discoveries, do not require washing of uncoordinated components prior to analysis as the background emission is low, due to the low concentration of unbound labels compared to bound labels at the electrode surface.80
Figure 1.11: Diagram illustrating sandwich immunoassay using paramagnetic nanoparticle isolated antibody/antigen complexes with [Ru(bpy)$_3$]$_{2+}$ label and co-reactant TPrA ECL at the electrode surface. Image from reference.$^{127,128}$

The ability to ‘load’ ECL labels onto platforms such as magnetic microbeads, polystyrene microspheres, silica nanoparticles or carbon nanotubes has facilitated significant enhancement of the ECL signal and, therefore, sensitivity of various ECL assays.$^{118,129-131}$ Other ECL based immunoassays, such as those proposed by Venkatanarayanan, et al.$^{129}$ use inkjet printed single walled carbon nanotube (SWCNT) forests on indium tin oxide (ITO) electrodes as a platform for ECL.$^{129}$ SWCNTs, when compared to conventional graphite electrodes, can accommodate up to 17 fold more antibody conjugates, enhancing the signal and, therefore, the sensitivity of the assay.$^{132}$ Expanding on previous work conducted by the same group employing SWCNT forests for amperometric immunosensors, Venkatanarayanan, et al.$^{129}$ applied the same platform for ECL detection in an array based immunoassay.$^{129,133}$ By functionalising the carboxylated end groups of the SWCNTs with
immunoglobulin G (IgG) antibodies and functionalising silica nanospheres with both [Ru(bpy)$_2$PICH$_2$]$^{2+}$ (where PICH$_2$ is 2-(4-carboxyphenyl)imidazo[4,5-f][1,10]phenanthroline) and poly(amido amine) (PAMAM), a dendrimer capable of binding IgG, Venkatanarayanan, et al.$^{129}$ were able to detect IgG at concentrations as low as 1.1 pM with sodium oxalate as a co-reactant, as shown in Figure 1.12.$^{129}$

![Figure 1.12: Detection of IgG analyte using SWCNT forest array immunoassay. Image from reference.$^{129}$](image)

### 1.4 Multiplexed ECL Detection

Although ECL has been successfully used for several different assay formats with an extensive range of analytes and platforms, one major limitation still exists; no commercially available ECL diagnostic kits can detect more than one ECL label. However, various research groups have developed multiplexed assays employing electrochemically, spectrally, or spatially resolved emitters.$^{134-139}$

#### 1.4.1 Array Based Multiplexed ECL

The simplest array based ECL assays, such as those developed by meso-scale discovery, consist of a micro-well plate with disposable printed carbon ink electrodes.$^{80}$ Each well within the plate contains multiple binding domains for different analytes of interest.$^{14, 80}$ Comparatively complex multiplexed array based
assays, such as those developed by Sardesai, et al.\textsuperscript{140} use a similar SWCNT based platform as described in Figure 1.13.\textsuperscript{135} However, spatially separated well plates containing SWCNTs functionalised with different antibodies facilitate very sensitive multiplexed detection of two analytes, prostate specific antigen (PSA) and interleukin-6 (IL-6), at concentrations as low as 1 pg mL\textsuperscript{-1} and 0.25 pg mL\textsuperscript{-1} respectively.\textsuperscript{140}

**Figure 1.13:** Multiplexed ECL immunoassay developed by Sardesai, et al.\textsuperscript{140} using separated well plates with SWCNTs functionalised with different capture antibodies to facilitate multiplexed detection of PSA and IL-6.

Alternative array based devices, such as those developed by Deiss, et al.\textsuperscript{141} use a variety of capture antibody modified microspheres encoded with corresponding concentrations of a fluorescent dye for each antibody and a [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} tag to facilitate ECL.\textsuperscript{141} These microspheres were isolated in an etched gold coated fibre optic bundle, which also acts as the working electrode for the experiment. Detection using an electron multiplying charged coupled device (EM-CCD), after a 3-step preparation process, detailed in Figure 1.14, facilitated the resolution of ECL occurring at individual microspheres, allowing the synchronous detection of three different analytes.\textsuperscript{141}
Figure 1.14: 3 step ECL microarray immunoassay using fluorescent dye encoded capture antibody functionalised microspheres. a) microspheres added to etched gold coated fibre optic and allowed to self-assemble on surface. b) analyte added. c) ECL tag labelled antibody added.\textsuperscript{141}

Although all the previous multiplexed systems have made significant advances towards the detection of multiple analytes, they still require the physical separation of either the capture antibody or the emissive species.

1.4.2 Potential Resolved Multiplexed ECL Detection

Although spatially resolved ECL detection systems predominate multiplexed ECL applications, some research groups have exploited potential resolution strategies for ECL detection of analytes of importance.\textsuperscript{138,142} Notably, Wang, \textit{et al.}\textsuperscript{139}, developed a multiplexed ECL device employing both spatial- and potential-resolved ECL strategies for the multiplexed detection of four cancer biomarkers in human serum samples; AFP, carcinoma antigen 153 (CA153), carcinoma antigen 199 (CA199) and CEA. Capture antibodies for two biomarkers were immobilised on each of the two separate working electrode zones, in each capture zone, \([\text{Ru(bpy)}_3]^{2+}\) was used as a label for one biomarker and carbon nanodots were used as a label for the second biomarker. First, using a battery, a potential of 1.2 V (\textit{vs} Ag/AgCl) was applied at each of the two working area zones in turn, producing ECL from the \([\text{Ru(bpy)}_3]^{2+}\) labelled bioconjugates with TPrA as a co-reactant. Second, the electrode connections to the
battery were reversed, and a potential of -1.2 V (vs Ag/AgCl) was applied at each of the two working area zones in turn, producing ECL from the carbon nanodot labelled bioconjugates with persulfate as a co-reactant.

Although the aforementioned study presents interesting possibilities for multiplexed ECL detection systems, more extensive investigation will be required before they can be applied to commercially available systems or developed into readily available technologies. Recent innovations have led to the development of numerous luminophores exhibiting ECL emissions spanning the entire visible spectrum.¹³ Such luminophores provide exceptional promise in the development of multi-colour ECL, and will allow the development of multi-coloured multiplexed bioanalytical ECL detection systems and facilitate further understanding of the electron transfer processes which underpin ECL.

1.4.3 Multi-Colour, Multiplexed ECL Detection

The notion of using multiple metal chelates to produce multi-colour ECL systems was first postulated in proof-of-concept experiments conducted by Bruce and Richter¹⁴ and Muegge and Richter¹⁴⁵ where [Ru(bpy)₃]²⁺ was combined with green emissive [Ir(ppy)₃] (where ppy is 2-phenylpyridine, λₘₐₓ = 530 nm) and a blue-green emissive iridium complex; [Ir(df-ppy)₂(pic)] (where df-ppy is 2-(2,4-difluorophenyl)pyridine and pic is 2-carboxypyridyl, λₘₐₓ = 498 nm) respectively. It was possible to observe concomitant emissions from the two complexes at equal concentration with co-reactant TPrA as shown in Figure 1.15. Muegge and Richter¹⁴⁵ also attempted to detect three different metal chelate complexes using a combination of [Ru(bpy)₃]²⁺, [Ir(df-ppy)₂(pic)] and [Ir(ppy)₃]. However, insufficient spectral resolution between [Ir(df-ppy)₂(pic)] and [Ir(ppy)₃] (λₘₐₓ = 468 nm and λₘₐₓ = 517 nm respectively) resulted in the appearance of a single peak. Since this initial postulation,
several research groups have focussed on the development of both luminophores and detection systems which facilitate efficient and spectrally resolved metal chelate ECL.\textsuperscript{28, 36, 46, 47, 137, 146-149}

![Figure 1.15](image)

**Figure 1.15:** a) (A) Synchronous ECL from $[\text{Ir(ppy)}_3]$ and $[\text{Ru(bpy)}_3]^{2+}$ (both 10 $\mu$M, acetonitrile, 0.05 M TPrA, 0.1 M TBAPF$_6$), (B) ECL from $[\text{Ir(ppy)}_3]$, (10 $\mu$M, acetonitrile, 0.05 M TPrA, 0.1 M TBAPF$_6$) (C) ECL from $[\text{Ir(ppy)}_3]$, 10 $\mu$M (acetonitrile/water 50:50 v/v, 0.1 M KH$_2$PO$_4$). b) $[\text{Ir(df-ppy)}_3]_{(pic)}$ (A, 100 $\mu$M) and $[\text{Ru(bpy)}_3]^{2+}$ (B, 100 $\mu$M) 0.05 M TPrA, 0.1 M TBAPF$_6$ in acetonitrile. Image from references.\textsuperscript{144, 145}

Other approaches to multi-colour ECL have focussed on solid-state thin film metallopolymers doped with luminophores such as $[\text{Ru(bpy)}_3]^{2+}$. Expanding on previous work using single centre thin film metallopolymers, dual-colour ECL was reported by Dennany, *et al.*\textsuperscript{150} from a thin film metallopolymer, deposited on ITO, containing both $[\text{Ru(bpy)}_3]^{2+}$ and $[\text{Os(bpy)}_3]^{2+}$ centres.\textsuperscript{151} Using guanine and 8-
oxoguanine as co-reactants ECL was successfully elicited from both the [Ru(bpy)₃]²⁺ chelate at 600 nm and [Os(bpy)₃]²⁺ chelate at 760 nm. Solid state ECL has led to the development of a variety of multi-colour ECL systems for incorporation into organic light emitting devices (OLEDs). A notable experiment by Welter, et al.¹⁵² combined two [Ru(bpy)]²⁺ complexes with four phenylene spacer units and mixed the complex with a polyphenylenevinylene derivative (PPV) to form a homogenous layer on ITO. When a positive bias of 4 V was applied, efficient, red emission from the [Ru(bpy)₃]²⁺ centres was observed; contrastingly, when a negative bias of -4 V was applied, green emission was produced, resulting from charge recombination of the PPV polymer.

The recent development of solid state electrogenerated chemiluminescence devices (SECLDs) has combatted the various weaknesses of OLEDs including poor colour contrast and low reproducibility along with dull, unstable and inefficient emission.³⁵,¹⁵³-¹⁵⁹ A study by Wang, et al.¹⁵⁷ produced two separate devices exhibiting voltage controllable green to red colouring in device I and blue to red colouring in device II. Device I contained ITO, [Ru(bpy)₃][(PF₆)₂] (30 nM), N,N’-di(naphthalene-1-yl)-N,N’-diphenyl-benzidine (NPB, 50 nM), aluminium tris-(8-hydroxyquinoline) (Alq₃, 50 nM), Mg:Ag (10:1150 nM), Ag (10 nM) layers and device II contained [Ru(bpy)₃][(PF₆)₂] (30 nM), NPB (50 nM), 4,4’-bis(2,2’-diphenylvinyl-1,1’-biphenyl (DPVBi, 25 nm), Alq₃ (15 nM), Mg:Ag (10:1150 nM), Ag (10 nM) layers.¹⁵⁷ Emission was found to change in both devices with the applied bias. In device I, at a forward bias of 10 V, efficient emission at 530 nm was observed from the Alq₃ layer, as the applied bias was increased, emission from the [Ru(bpy)₃][(PF₆)₂] layer emerged and eventually dominated the corresponding spectra at 19-20 V, achieving efficient, voltage controlled green and red emission.¹⁵⁷ At a low driving bias, electrons do not have high enough energy to transport across the NPB/Alq₃ interface, and hence
accumulate at the interface. At a higher driving bias electrons fill ‘traps’ in the organic layer in accordance with the Poole-Frenkel principle, allowing increased mobility of electrons and, eventually, a high enough potential for electrons to inject into the NPB layer and transfer into the π* orbitals of the [Ru(bpy)₃][(PF₆)₂] layer, where efficient [Ru(bpy)₃]²⁺ state formation and radiative decay can occur. Device II exhibited similar characteristics, with an efficient colour change from blue to red light occurring with increased forward bias as a result of increasing electron mobility. This process is outlined in Figure 1.16. This was the first demonstration of efficient blue, green and red multi-coloured ECL.

![Figure 1.16: Device I (a) and Device II (b) colour changes with changes in applied forward bias. Image from reference.](image)

Following the production of efficient, multi-coloured displays, various researchers sought to obtain ‘white ECL’ for incorporation into SECLDs. For example, Zhen, et al. developed a solid-state device where the emission from a thin film containing [Ru(bpy)₂(dim-bpy)]²⁺ (where dim-bpy is 4,4'-dimethylbipyridine), Alq₃, NPB and Ag layers on ITO (Figure 1.17), changed in colour depending on the time of the applied bias. At a reverse bias of –10 V, emission from the [Ru(bpy)₂(dim-bpy)]²⁺ was observed at 625 nm. However, upon application of a forward bias (9 V) a colour change over time from green, to yellow-white occurred (Figure 1.17). Initially, dominant emission at 519 nm from the Alq₃ charge recombination zone was observed.
This is due to efficient electron transfer from the $\pi^*$ orbitals of the $[\text{Ru(bpy)}_2(\text{dim-bpy})]^2+$ ligands to the Alq$_3$ layer. At higher applied potentials, due to the accumulation of anions at the interface of the two layers, the injection of holes from the Alq$_3$ to the $[\text{Ru(bpy)}_2(\text{dim-bpy})]^2+$ layer becomes more favourable and efficient emission is observed from both the $[\text{Ru(bpy)}_2(\text{dim-bpy})]^2+$ and Alq$_3$ recombination zones, resulting in a change from green, to yellow-white emission.

**Figure 1.17:** (1) composition of layers of the hybrid ECL device and the molecular structure contained in the corresponding layers. (2) ECL spectra taken at 20 s, 40 s, 100 s and 150 s (1, 2, 3 and 4 respectively) after application of the forward bias. Image from reference.\(^{158}\)

This initial attempt at obtaining ‘white ECL’, was later improved upon by Su, et al.\(^{160}\), who combined $[\text{Ir(df-ppz)}_2(\text{dtb-bpy})]^+$ (where df-ppz is 1-(2,4-difluorophenyl)pyrazole and dtb-bpy is 4,4′-di(tert-butyl)-2,2′-bipyridine) a blue – green emitter ($\lambda_{\text{max}} = 487$ nm in thin film) and $[\text{Ir(ppz)}_2(\text{biq})]^+$ (where ppz is 1-phenylpyrazole and biq is 2,2′-biquinoline) a red emitter ($\lambda_{\text{max}} = 622$ nm in thin film) in a thin film to produce ECL with near pure white Comission Internationale de l’Eclairage (CIE) coordinates of (x, y) = (0.34, 0.37); pure white CIE coordinates are (x, y) = (0.33, 0.33). Nobeshima, et al.\(^{161}\) also combined a blue – green (9,10-diphenylanthracene, DPA) and red (rubrene, RUB) emissive species to produce white ECL, but used solutions of DPA (20 mm) and RUB (5 mm) sandwiched between two
ITO electrodes as opposed to a solid-state device. When a bias of 1000Hz was applied using an alternating current device, only emission from RUB was observed. However, when a bias of 300 Hz was applied, white ECL was observed resulting from synchronous emission from both RUB and DPA.

The ability to distinguish electrochemically and spectroscopically between different luminophores in solution and thin film has led to a variety of studies on multi-nuclear complexes and their multi-colour ECL properties in solution. A report by Schmittel, et al.\(^9\) demonstrated the ability of a tri-nuclear Ir(III)-Ru(II)-Ir(III) complex to emit at three different wavelengths (605 nm, 645 nm and 684 nm) depending on which electronic transitions are the major contributor to the emission. The complex was found to have three distinct oxidation potentials, 0.90 V, 0.93 V, from oxidation of the two iridium HOMOs and 1.01 V from oxidation of the ruthenium HOMO. Therefore, three distinct ECL waves of varying intensity and distribution were observed; ECL at 605 nm, 645 nm and 684 nm resulted from reductions of Ir-Ru-Ir\(^{3+}\), Ir-Ru-Ir\(^{2+}\) and Ir-Ru-Ir\(^+\) respectively.

The ECL of multi-centre luminophores has now been extensively studied and applied to various detection platforms including the detection of quinolone containing antibiotics.\(^9, 162-167\) Recently, Sun, et al.\(^162\) have developed a ratiometric ECL detection system using a heterodinuclear complex containing both orange \([\text{Ru(bpy)}_3]^{2+}\) and deep red \([\text{Os(bpy)}_3]^{2+}\) labels. In this study, the ratios of emissions from the two distinct metallic centres \((\Delta \lambda_{\text{max}} = 113 \text{ nm})\) were compared to yield an accurate measurement of co-reactant concentration as a proof-of-concept demonstration as shown in Figure 1.18.
**Figure 1.18:** a) ECL of heterodinuclear Os-Ru dual emission label (50 µM) with increased TPrA concentration, 0.1 M TBAPF₆ in acetonitrile. b) Calibration curve of ECL ratios of emission at 731 nm and 618 nm, with added TPrA in concentration range of 1 mM to 12 mM in acetonitrile.

Another promising area for multi-coloured analytical ECL is the use of quantum dots (QDs), as alternatives to metal chelate complexes as labels. QDs have been successfully applied to a variety of analytical applications including ECL immunoassays and DNA probe assays.¹⁶⁸⁻¹⁷¹ QDs are nanocrystals of semi-conducting materials, most commonly a CdSe core coated with a ZnS outer layer, capable of emitting at a range of different wavelengths dependant on the size of the QD.¹⁶⁹ In 2009 Zhou, *et al.*¹⁷² described the potential controlled red and blue emission from CdSe nanocrystals capped with trioctyl phosphine oxide (TOPO). Red ECL ($\lambda_{\text{max}} = 653$ nm) from the surface state structure was observed at potentials between $–1.60$ and $–1.80$ V and combinations of red blue ECL ($\lambda_{\text{max}} = 486$ nm) from the CdSe core and surface structures was observed at potentials between $–2.00$ and $–2.20$ V. Guo, *et al.*¹³⁷ successfully immobilised two different QDs on a graphene-chitosan composite with distinct green and orange ($\lambda_{\text{max}}$ at 525 nm and 625 nm respectively) emission wavelengths, to detect two separate tumour markers, AFP and CEA.

Recently, Liu, *et al.*¹³⁶ developed a similar protocol for the detection of Hepatitis B and C viral NAs, employing a graphene conducting bridge to anchor two QD varieties with yellow and orange emissions ($\lambda_{\text{max}}$ at 551 nm and 621 nm respectively),
bioconjugated to hepatitis B (QD-HBV) and C (QD-HCV) DNA capture probes respectively. A separate DNA probe for both hepatitis B and C, bioconjugated with gold nanoparticles (GNP-HBV and GNP-HCV respectively), conjugates to unreacted capture DNA at the electrode surface, and quenches the ECL from the QDs. Therefore, with a lower sample concentration of hepatitis B or C virus, less QD capture DNA sites would be occupied, and many GNP-HBV and GNP-HCV probes will be present at the electrode surface, quenching the ECL from QD-HBV and QD-HCV. The analysis had good LODs for both hepatitis B and C NAs, of 0.082 pmol L\(^{-1}\) and 0.34 pmol L\(^{-1}\) respectively and was successfully applied to human serum samples. Although QD ECL offers great promise, the ECL intensity from QD labels is typically lower than that observed from more commonly employed \([\text{Ru(bpy)}_3]^{2+}\) labels and significant improvement in the efficiencies of QDs is critical for the development highly sensitive QD ECL detection systems.\(^\text{110}\)

Alternative approaches to multi-colour ECL have focussed on eliciting different coloured emissions from a single luminophore. Initial experiments conducted by Swanick, \textit{et al.}\(^\text{173}\) showed the ability of an iridium complex exhibiting two tertiary amine substituents on the bipyridine ligand ([Ir(df-phtl)\(_2\)(dma-bpy-dma)]\(^+\); where df-phtl is 1-benzyl-4-(2,4-difluoro-phenyl)-1H-1,2,3-triazole; and dma-bpy-dma is 4,4’-(dimethylamino-2,2’-bipyridine)) to ‘auto-enhance’ the ECL of the complex. Swanick, \textit{et al.}\(^\text{173}\) found that [Ir(df-phtl)\(_2\)(dma-bpyd-ma)]\(^+\) exhibited four distinct oxidation potentials at 1.42, 1.75, 1.94 and 2.18 V corresponding to oxidation of the df-phtl ligand, oxidation of the two dimethylamino substituents and an oxidation localised on the triazole ligand. Pulsing past the fourth oxidation potential was found to increase the intensity of the ECL emission by 16-fold and shift the emission from green (\(\lambda_{\text{max}} = 543\) nm) to yellow (\(\lambda_{\text{max}} = 588\) nm). The ECL spectrum at 588 nm was deconvoluted
and found to consist of three peak wavelengths of 543, 608 and 651 nm corresponding to emissions from three different excited states; $[\text{Ir(df-phtl)}_2(\text{dma-bpy-dma})]^+$, $[\text{Ir(df-phtl)}_2(\text{dma-bpy-dma}^+)]^+$ and $[\text{Ir(df-phtl)}_2(\text{dma}^{++-}\text{bpy-dma}^{+})]^+$ respectively. In a subsequent study by Ladouceur, et al. the same principle was applied to two new iridium metal chelates exhibiting the same dma-bpy-dma ancillary ligand, but different cyclometalating ligands; 2-(2,4-difluorophenyl)-5-methylpyridine (df-MeppyH) and df-ppy. With emission shifting from green ($\lambda_{\text{max}} = 535$ nm and $\lambda_{\text{max}} = 528$ nm) to orange ($\lambda_{\text{max}} = 610$ nm and $\lambda_{\text{max}} = 605$ nm) with both $[\text{Ir(df-ppy)}_2(\text{dma-bpy-dma})]^+$ and $[\text{Ir(df-MeppyH)}_2(\text{dma-bpy-dma})]^+$. The ability of luminophores to both ‘auto-enhance’ and change their emission wavelength have interesting potential applications in both SECLDs and bioanalytical ECL diagnostic systems. Although an interesting prospect for enhancing LODs in diagnostic applications, the spectroscopic changes of these emissive species mean that the detection of multiple luminophores in concert, would be more challenging when compared to other combinations of multiple, distinct emitters.

A study by Doeven, et al. successfully detected three, distinct metal chelates, a red-shifted Ruthenium emitter $([\text{Ru(bpy)}_2(\text{dm-bpy-dc})]^2+$, where dm-bpy-dc = dimethyl 2,2′-bipyridine-4,4′-dicarboxylate; $\lambda_{\text{max}} = 685$ nm), green $[\text{Ir(ppy)}_3]$ and a blue shifted iridium emitter (either $[\text{Ir(df-ppy)}_3], \lambda_{\text{max}} = 495$ nm or $[\text{Ir(df-ppy)}_2(\text{ptp})], \lambda_{\text{max}} = 463$ and 492 nm) using both a CCD and a digital camera in 2014 (Figure 1.19). This work expanded significantly on previous work conducted by the same research group which solved the problem of spectral overlap between the two iridium emitters experienced by Muegge and Richter. Doeven, et al. demonstrated that emission from $[\text{Ir(ppy)}_3]$, when reacted using excess TPrA, is quenched at high overpotentials.
‘switch-off’ phenomenon is a result of interactions between [Ir(ppy)₃] and the TPrA co-reactant. The long half-life of the TPrA⁺⁺ of ~0.2 ms results in high concentrations of the oxidised form of the TPrA co-reactant at high over-potentials. In the presence of excess TPrA⁺⁺, the excited state [Ir(ppy)₃]* will preferentially reduce the TPrA⁺⁺ to return to the ground state as opposed to undergoing radiative decay. This interaction results in no light emission from the [Ir(ppy)₃] complex at high overpotentials, as described in reactions 17-22.

17 [Ir(ppy)₃] → [Ir(ppy)₃]⁺ + e⁻
18 TPrA → TPrA⁺⁺ + e⁻
19 TPrA⁺⁺ → TPrA⁺ + H⁺
20 [Ir(ppy)₃]⁺ + TPrA⁺ → [Ir(ppy)₃]* + P₁
21 [Ir(ppy)₃]* → [Ir(ppy)₃] + hv
22 [Ir(ppy)₃]* + TPrA⁺⁺ → [Ir(ppy)₃]⁺ + P₂

This excited state quenching was also observed (but to a lesser extent) with co-reactants 2-(diisopropylamino)ethanol (DIPEA-OH) and N,N-diisopropylethylamine (DIPEA) in a study conducted by Barbante, et al. Because of this quenching, emission from [Ir(ppy)₃] with TPrA was only observed at low applied electrochemical potentials (0.0 to 0.4 V vs Fe⁰/⁺) subsequently, the red (0.72 V vs Fe⁰/⁺) and blue (0.97 V vs Fe⁰/⁺) emissive species were oxidised and emitted concomitantly. Distinction between the red and blue luminophores was facilitated through their spectral distribution, resulting in red, green and blue ECL from a single experimental solution.
Many previously developed ECL detection systems use multiple ECL labels with spectroscopically distinct emissions; however, most rely solely on their different electrochemical properties to resolve the ECL of different emitters, with detection limited to a PMT.\textsuperscript{139, 174-176} The ability to detect ECL from different luminophores using a digital camera as opposed to PMT or CCD as a photodetector was successfully illustrated by Doeven, \textit{et al.}\textsuperscript{28}. Doeven, \textit{et al.}\textsuperscript{28} employed ImageJ software to isolate the individual red, blue and green colour channels of photographs of ECL at the electrode surface, allowing sensitive detection of the red, green and blue luminophores at concentrations as low as 0.07, 0.4 and 0.2 µM respectively. The promising concept of using cameras as photodetectors, combined with recent advances in mobile phone and paper-based microfluidic technology has enormous potential for the development of low cost, portable and sensitive multiplexed ECL detection systems.\textsuperscript{83, 139, 174, 177, 178}
1.5 Linking Statement

The papers presented in this thesis focus on the central theme of ECL. A well-rounded set of experiments have been carried out from fundamental investigations into the electron transfer and light emission processes occurring in mixed metal chelate annihilation ECL experiments, to aqueous systems with novel electrode materials and novel iridium complexes. Chemiluminescence studies of these novel iridium metal chelates have also been carried out, Chemiluminescence is a technique which marries well with ECL, as it simply employs a chemical means of oxidation of the metal complex as opposed to electrochemical.
Chapter Two: Annihilation Electrogene\(r\)ated Chemiluminescence of Mixed Metal Chelates in Solution: Modulating Emission Colour by Manipulating the Energetics

Emily Kerr \(^{a}\), Egan H. Doeven \(^{a}\), Gregory J. Barbante \(*\), Conor F. Hogan \(*\), David J. Bower \(^{a}\), Paul S. Donnelly \(^{a}\), Timothy U. Connell \(^{c}\) and Paul S. Francis \(*\).

\(^{a}\)Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment, Deakin University, Geelong, Victoria 3220, Australia. E-mail: paul.francis@deakin.edu.au; g.barbante@deakin.edu.au

\(^{b}\)Department of Chemistry, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria 3086, Australia. E-mail: C.Hogan@latrobe.edu.au

\(^{c}\)School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne 3010, Australia

Published in Chemical Science, Royal Society of Chemistry, 2015, volume 6, issue 1, pages 472-479. Reproduced with permission from the Royal Society of Chemistry.
Authorship Statement

1. Details of publication and executive author

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/institute/division if based at Deakin Organization and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul Francis</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td><a href="mailto:paul.francis@deakin.edu.au">paul.francis@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

2. Inclusion of publication in a thesis

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>If Yes, please complete Section 3. If No. go straight to Section 4.</td>
<td></td>
</tr>
</tbody>
</table>

3. HDR thesis author’s declaration

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above: (If the same, write &quot;as above&quot;)</th>
<th>School/institute/division if based at Deakin</th>
<th>Thesis Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emily Kerr</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td>Electroluminescence Studies of Ruthenium and Iridium Metal-Chelates</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

Designed and planned experiments with assistance from co-authors. Collected all published or data, developed and optimised procedures for analysis; cyclic voltamograms and ICL spectra, photographs collected with assistance from Egan Dooven. I completed the first manuscript draft and assisted with subsequent revisions, including conceptualisation of methods for presenting data.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.  

Signature and date

4. Description of all author contributions

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan H. Dooven</td>
<td>Assisted with taking photographs of ICL. Assisted with planning experiments. Assisted with revision of manuscript.</td>
</tr>
</tbody>
</table>
5. Author Declarations
I agree to be named as one of the authors of this work and confirm:

i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,

ii. that there are no other authors according to these criteria,

iii. that the description in Section 4 of my contribution(s) to this publication is accurate,

iv. that the data on which these findings are based are stored as set out in Section 7 below.

If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further
v. consent to the incorporation of the publication into the candidate's HDR thesis submitted to Deakin University and, if the higher degree is awarded, the subsequent publication of the thesis by the university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan H. Deaven</td>
<td></td>
<td>15/12/16</td>
</tr>
<tr>
<td>Gregory J. Barbante</td>
<td></td>
<td>15/12/16</td>
</tr>
<tr>
<td>Conor F. Hogan</td>
<td></td>
<td>16/12/16</td>
</tr>
<tr>
<td>David J. Bowker</td>
<td></td>
<td>16/12/16</td>
</tr>
<tr>
<td>Paul S. Donnelly</td>
<td></td>
<td>16/12/16</td>
</tr>
<tr>
<td>Timothy U. Connell</td>
<td></td>
<td>15/12/16</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td></td>
<td>16/12/2016</td>
</tr>
</tbody>
</table>

6. Other contributor declarations
I agree to be named as a non-author contributor to this work.

<table>
<thead>
<tr>
<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
</table>

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author.

7. Data storage
The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB</td>
<td>WP K4 3.127</td>
<td>19/02/2017</td>
<td>Paul Francis</td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based. If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
2.1 Abstract

We demonstrate the mixed annihilation electrogenerated chemiluminescence of tris(2,2′-bipyridine)ruthenium(II) with various cyclometalated iridium(III) chelates. Compared to mixed ECL systems comprising organic luminophores, the absence of T-route pathways enables effective predictions of the observed ECL based on simple estimations of the exergonicity of the reactions leading to excited state production. Moreover, the multiple, closely spaced reductions and oxidations of the metal chelates provide the ability to finely tune the energetics and therefore the observed emission colour. Distinct emissions from multiple luminophores in the same solution are observed in numerous systems. The relative intensity of these emissions and the overall emission colour are dependent on the particular oxidized and reduced species selected by the applied electrochemical potentials. Finally, these studies offer insights into the importance of electronic factors in the question of whether the reduced or oxidized partner becomes excited in annihilation ECL.
2.2 Introduction

Electrogenerated chemiluminescence (ECL) is the luminescence arising from electron transfer reactions in which at least one reactant has been electrochemically generated. This phenomenon has not only provided an excellent means for highly sensitive chemical detection,¹, ⁷, ¹³, ¹⁴, ¹⁷⁹-¹⁸² but also enabled extensive exploration of exergonic electron-transfer reactions in solution.¹⁴⁶, ¹⁸³ In fact, ECL investigations provided the first experimental verifications of the ‘inverted region’ of Marcus electron transfer theory,²⁹, ³³, ¹⁸⁴, ¹⁸⁵ where the electron transfer rates of highly exergonic reactions decrease with increasing free energy. Thus, under certain circumstances, the formation of (luminescent) excited states occur at much faster rates than the thermodynamically favoured ground state products.¹⁸⁶, ¹⁸⁷

Central to ECL is the ‘annihilation’ pathway, in which oxidized and reduced species are formed, usually sequentially, at two different electrode potentials,¹, ⁷, ¹³, ¹⁴, ¹⁷⁹-¹⁸² with the subsequent comproportionation of these species generating an emissive excited state. For example, the singlet excited state of an organic luminophore may be generated via recombination of its electrogenerated radical anion and cation as in reactions 1–4 (known as the S-route). The oxidized and reduced species can also be generated from different parent compounds, which is referred to as a ‘mixed’ ECL system.

\[
\begin{align*}
(1) \quad & \text{R} + e^{-} \rightarrow \text{R}^- \\
(2) \quad & \text{R}^- e^{-} \rightarrow \text{R}^+ \\
(3) \quad & \text{R}^- + \text{R}^+ \rightarrow \text{R} + \text{R}^* \\
(4) \quad & \text{R}^* \rightarrow \text{R} + h\nu
\end{align*}
\]
For organic ECL systems, the above excitation process is frequently in competition with generation of the lower-lying triplet excited-state (5). In solution at room temperature, organic triplets are generally non-emissive, but triplet-triplet annihilation can generate the emissive excited state (known as the T-route) (6), even in reactions that lack sufficient energy for direct singlet population (3).  

\[(5) \quad R^- + R^+ \rightarrow R + ^3R^*\]

\[(6) \quad ^3R^* + ^3R^* \rightarrow R + ^1R^*\]

Accordingly, annihilation ECL is somewhat simplified for luminophores exhibiting efficient phosphorescence at room temperature. This includes various transition metal complexes such as tris(2,2′-bipyridine)ruthenium(II) ([Ru(bpy)₃]²⁺), in which the emission of light from the lowest lying triplet state is facilitated by spin-orbit coupling induced by the heavy-metal ion.

Mixed annihilation systems combining more than one organic compound, or a transition metal chelate with a non-emissive organic compound, have been widely used to study bimolecular electron-transfer reactions and the competitive generation of excited states. Demonstrations of ECL from cyclometalated iridium(III) chelates in recent years, with emission maxima spanning the entire visible region, has created new opportunities for multi-colour ECL. Several reports of mixed metal chelate co-reactant ECL systems incorporating ruthenium(II) and iridium(III) complexes have emerged, where excitation is achieved solely by applied oxidative potentials. This includes cases in which the emissions were resolved by selective excitation at different potentials. Similarly, the co-reactant ECL of [Ru(bpy)₃]²⁺ and peroxydisulfate has recently been used in
conjunction with luminol ECL detection in a potential-resolved immunoassay of two different antigens at a cell surface.175

Ruthenium(II) and iridium(III) chelates have also been extensively utilized in light-emitting devices,182, 193-199 and several research groups have combined an electrochemiluminescent [Ru(bpy)₃]²⁺ derivative with an electroluminescent material for bias- or potential-controlled switching between emission colours.152,157,158 The use of multiple transition metal chelates in this context offers several major advantages, as demonstrated by Su, et al.160 who combined a blue-green and red emitting iridium(III) complex in a solid-state electrochemical cell to generate white electroluminescence, and Moon, et al.200 who recently created emissive plastic displays based on the mixed annihilation ECL of [Ru(bpy)₃]²⁺ and [Ir(dfppy)₂(bpy)]⁺ in block-copolymer-based ion-gels. This approach enabled Moon, et al.200 to set the emission colour from orange-red to green, based on the mole ratio of the incorporated complexes.200

Despite these impressive advances towards multiplexed ECL detection systems and colour-tuneable light-emitting technologies, annihilation ECL from mixed transition metal-chelate systems in simple solution is yet to be explored. This is surprising, as the fundamental understanding gained from such studies may underpin new developments in these areas. One basic mechanistic question that has remained unanswered in relation to annihilation ECL is whether the reduced or oxidized partner becomes excited following the comproportionation reaction. In the case of ruthenium complexes, formation of an excited state from the reduced parent requires a metal-to-metal electron transfer, whereas formation of an excited state from the oxidized species involves a ligand-to-ligand electron transfer. This suggests that the latter route ought to predominate due to more a favourable electronic factor. By exploring mixed
ECL systems where the components have differing localizations of electron density associated with their frontier orbitals, we hope to gain insight into the importance of electronic factors in annihilation ECL.

Utilizing an electrochemical cell coupled with a CCD spectrometer for instantaneous collection of emission spectra, we have examined the multi-colour emissions from a series of mixed annihilation ECL systems containing [Ru(bpy)_3]^{2+} and various cyclometalated iridium(III) chelates exhibiting green or blue luminescence, to understand and control the relative emission intensities of these novel ECL systems.
2.3 Experimental Section

2.3.1 Chemicals

Acetonitrile (Ajax Finechem, Australia) was distilled over calcium hydride under grade 5 argon. Solutions were degassed with argon prior to analysis. The chemical structure and luminescence chromaticity of each ruthenium and iridium complex used in this study is shown in Figure 2.1. Tetrabutylammonium hexafluorophosphate (TBAPF$_6$, 99.5%, electrochemical grade) was purchased from Sigma-Aldrich (Australia). The hexafluorophosphate salt of tris(2,2′-bipyridine-κN1,κN1′)ruthenium(2+) ([Ru(bpy)$_3$][(PF$_6$)$_2$]) was prepared from Ru(bpy)$_3$Cl$_2$·6H$_2$O (Strem Chemicals, USA). $fac$-Tris[2-(2-pyridinyl-κN)phenyl-κC]iridium (tris(2-phenylpyridinato-C$^2$,N)iridium(III); [Ir(ppy)$_3$], 99%) and tris[4,6-difluoro-2-(2-pyridyl)phenyl-C$^2$,N]iridium(III) ([Ir(df-ppy)$_3$]) were purchased from Sigma-Aldrich (Australia). Tris[2-(1H-pyrazol-1-yl-κN$^3$)phenyl-κC]iridium (tris(phenylpyrazole)iridium(III)); [Ir(ppz)$_3$], >99%) and $fac$-tris(1-phenyl-3-methylimidazolin-2-ylidene-C,C(2′))iridium(III) ([Ir(pmi)$_3$], >99%) were purchased from LumTech (Taiwan). Bis[3,5-difluoro-2-(2-pyridinyl-κN)phenyl-κC][2-[1-(phenylmethyl)-1H-1,2,3-triazol-4-yl-κN3]pyridine-κN]iridium(1+) hexafluorophosphate(1−) ([Ir(df-ppy)$_2$(ptb)][PF$_6$]), was synthesized and characterized as previously described. 36
Figure 2.1: (a) Ruthenium and iridium complexes used in this study. (b) CIE chromaticity characterization of the photoluminescence of individual complexes (black squares) and the ECL from mixtures of complexes (white circles). The ECL CIE coordinates were calculated using the mean RGB values for the circular area of the electrode in the photographs shown in subsequent figures. The colour space representation was generated with efg's Computer Lab software.
2.3.2 Experimental Procedure

An Autolab PGSTA12 potentiostat was used to perform chronoamperometry and cyclic voltammetry experiments (Metrohm Autolab B.V., Netherlands). A custom-built, light-tight faraday cage encased the electrochemical cell, which consisted of a cylindrical glass cell with a quartz window base and Teflon cover with spill tray. A conventional three-electrode assembly was used throughout, consisting of a glassy carbon (3 mm diameter) working electrode shrouded in Teflon (CH Instruments, Austin, USA), Ag/AgNO₃ (0.02 M) reference electrode and platinum wire counter electrode. The glassy carbon working electrode was polished using 0.30 and 0.05 μm alumina on a felt pad with water, rinsed in freshly distilled acetonitrile and dried with nitrogen. The electrode was positioned ∼2 mm from the bottom of the cell.

For cyclic voltammetry measurements, the complexes were prepared at equal concentrations in acetonitrile (0.1 M TBAPF₆ supporting electrolyte) and the potentials obtained were referenced to the formal potential of the ferrocene/ferrocenium couple (1 mM); measured in situ in each case. Prior to analysis, solutions were degassed with grade 5 argon. ECL spectra were obtained using a model QE65pro CCD spectrometer (Ocean Optics). The spectrometer was interfaced with the electrochemical cell through an optic fibre (1 m, 1 mm core diameter) and collimating lens using a custom-built electrochemical cell holder. A HR 4000 Break-Out box was programmed to initiate acquisition at the initiation of the experiment using NOVA software. For annihilation ECL experiments, appropriate concentrations of the complexes (in freshly distilled acetonitrile with 0.1 M TBAPF₆ supporting electrolyte) were selected to generate similar emission intensities. Solutions were degassed for 15 min prior to analysis using grade 5 argon. ECL spectra were recorded using a 14 s integration time with Spectra Suite software. NOVA software was
employed to configure the potentiostat to apply a 12 s 2-step chronoamperometry pulse to the appropriate applied potentials. Oxidative and reductive potentials for chronoamperometry were determined by cyclic voltammograms prior to each set of ECL experiments.
2.4 Results and Discussion

2.4.1 Preliminary Experiments – [Ru(bpy)$_3$]$^{2+}$ with [Ir(ppy)$_3$]

Cyclic voltammetric scans of a mixture of [Ru(bpy)$_3$]$^{2+}$ and [Ir(ppy)$_3$] in acetonitrile (containing 0.1 M TBAPF$_6$) show a combination of the characteristic electron-transfer processes of the two metal chelates (Figure 2.2b).$^{10,36,46}$ This system offers numerous possible reactants for annihilation ECL, which will depend on the applied oxidation and reduction potentials of the alternating electrochemical process. For example, when pulsing 0.1 V beyond the first reduction and oxidation potentials of [Ru(bpy)$_3$]$^{2+}$ (as in Expt 1; Figure 2.2c), we form not only [Ru(bpy)$_3$]$^+$ and [Ru(bpy)$_3$]$^{3+}$, but also [Ir(ppy)$_3$]$^+$, for which a series of subsequent reactions to form ground and excited state products can be considered. The free energy ($\Delta G$) of each reaction can be estimated from the separation of the formal potentials of the reactants, and for excited states, from the emission energy (eqn I and II).$^{183,201,202}$

I. $\Delta G_{gs} \approx E_{A/A^-}^{\circ} - E_{D+/D^-}^{\circ}$

II. $\Delta G_{es} \approx (E_{A/A^-}^{\circ} - E_{D+/D^-}^{\circ}) + E_{es}$

Where $\Delta G_{gs}$ and $\Delta G_{es}$ are the free energies of the reactions leading to the ground and excited states respectively; $E_{A/A^-}^{\circ}$ and $E_{D+/D^-}^{\circ}$ are the formal potentials of the acceptor and donor species in the annihilation reaction and $E_{es}$ is the energy of the excited state in eV from the emission maximum. These estimations omit the Coulomb repulsion energy required to bring the reactants into the active complex and the vibrational levels of the radiative transition, but as these contributions are small and often opposing, they can (at least to a first approximation) be reasonably neglected.$^{202,203}$ In subsequent experiments, we show this approach to be an effective predictor of the observed emissions.
Figure 2.2: (a) Cyclic voltammogram of 0.25 mM [Ru(bpy)_3]^{2+} and 0.25 mM [Ir(ppy)]_3, in acetonitrile containing 0.1 M TBAPF_6. (b) Relevant reduction and oxidation potentials of the two metal chelates. (c) Illustration of potentials used in annihilation ECL experiments. (d–f) Spectra and photographs of the ECL at the working electrode of selected annihilation ECL experiments using 0.003 mM [Ru(bpy)]_3^{2+} and 0.25 mM [Ir(ppy)]_3, in acetonitrile containing 0.1 M TBAPF_6.

Ground-state products:

\[
\begin{align*}
\text{(7)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ru(bpy)}_3]^{3+} \rightarrow 2 [\text{Ru(bpy)}_3]^{2+} \\
\text{(8)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}]^- \rightarrow [\text{Ru(bpy)}_3]^{2+} + [\text{Ir(ppy)}],
\end{align*}
\]

\[\Delta G_{gs} = -2.64 \text{ and } -2.08 \text{ eV, respectively.}\]

One excited-state product:

\[
\begin{align*}
\text{(9)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ru(bpy)}_3]^{3+} \rightarrow [\text{Ru(bpy)}_3]^{2+*} + [\text{Ru(bpy)}_3]^{2+} \\
\text{(10)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ru(bpy)}_3]^{3+} \rightarrow [\text{Ru(bpy)}_3]^{2+} + [\text{Ru(bpy)}_3]^{2+*} \\
\text{(11)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}]^- \rightarrow [\text{Ru(bpy)}_3]^{2+*} + [\text{Ir(ppy)}], \\
\text{(12)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}]^- \rightarrow [\text{Ru(bpy)}_3]^{2+} + [\text{Ir(ppy)}]^*
\end{align*}
\]

\[\Delta G_{es} = -0.63, -0.63, -0.07, \text{ and } +0.27 \text{ eV, respectively.}\]

Two excited-state products:

\[
\begin{align*}
\text{(13)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ru(bpy)}_3]^{3+} \rightarrow 2 [\text{Ru(bpy)}_3]^{2+*} \\
\text{(14)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}]^- \rightarrow [\text{Ru(bpy)}_3]^{2+*} + [\text{Ir(ppy)}]^*
\end{align*}
\]

\[\Delta G_{es} = +1.37, \text{ and } +2.28 \text{ eV, respectively.}\]

Reactions 7 and 8, which form only ground state products, are so exergonic (\[\Delta G \ll 0]\) that they fall into the Marcus inverted region, and thus are kinetically unfavourable compared to the generation of excited states.\(^{188}\) On the other hand,
reactions 13 and 14, which would form two excited state products, are not thermodynamically feasible ($\Delta G \gg 0$).

The formation of $[\text{Ru(bpy)}_3]^{2+*}$ from the annihilation of $[\text{Ru(bpy)}_3]^+$ and $[\text{Ru(bpy)}_3]^{3+}$ is well-known.\textsuperscript{1, 10, 14} There has been ongoing interest in the subtle question of which of the two parent species forms the excited state,\textsuperscript{188} as reactions 9 and 10 are thermodynamically equivalent, and co-reactant ‘oxidative-reduction’\textsuperscript{204} and ‘reductive-oxidation’\textsuperscript{205} ECL show that either reactant is capable of forming the excited state.\textsuperscript{1, 14} The question of HOMO $\rightarrow$ HOMO $\textit{versus}$ LUMO $\rightarrow$ LUMO electron transfer is not easily resolved by experiment, but in the case of the annihilation mechanism, simple orbital overlap arguments suggest that the formation of $[\text{Ru(bpy)}_3]^{2+*}$ from the oxidized parent (involving ligand-to-ligand electron transfer) will be kinetically favoured over formation from the reduced parent (which requires metal-to-metal electron transfer).\textsuperscript{188} The investigation of mixed inorganic ECL systems involving iridium complexes offer an interesting means to gain insight into this question because iridium complexes of the type investigated here often have the electron density of their HOMO delocalized over their ligands as well as on the metal. For example, the HOMO of $[\text{Ir(ppy)}_3]$ has been estimated to be less than 50% metal based, on the basis of DFT calculations.\textsuperscript{52} Therefore, the HOMO $\rightarrow$ HOMO electron transfer route ought to be relatively less disfavoured when an iridium complex is the oxidant compared to the case where a ruthenium complex with a purely metal-based HOMO is used.

On the basis of eqn II, the reaction of $[\text{Ru(bpy)}_3]^+$ with $[\text{Ir(ppy)}_3]^+$ is sufficiently exergonic to attain $[\text{Ru(bpy)}_3]^{2+*}$ ($\Delta G_{cs} < 0$), but not $[\text{Ir(ppy)}_3]^*$ ($\Delta G_{cs} > 0$). Indeed, only the characteristic orange-red emission of $[\text{Ru(bpy)}_3]^{2+*}$ was observed in Expt 1 (Figure 2.2d). We can isolate reaction 11 by applying suitable voltages to generate
only \([\text{Ru(bpy)}_3]^+\) and \([\text{Ir(ppy)}_3]^+\) (Expt 2). Although this isolated mixed system is less exergonic than conventional \([\text{Ru(bpy)}_3]^{2+}\) annihilation ECL, the orange-red emission of \([\text{Ru(bpy)}_3]^{2+}\) is still observed as predicted (Figure 2.2e). The observation of intense orange-red ECL from Expt 2–4 of comparable intensity to Expt 1, shows that the HOMO → HOMO electron transfer route to the excited state is not significantly inhibited in this system.

The reaction of \([\text{Ru(bpy)}_3]^+\) with \([\text{Ir(ppy)}_3]^+\) does not generate \([\text{Ir(ppy)}_3]^*\), but the ruthenium(II) chelate exhibits two additional closely spaced ligand reductions that could be exploited to increase the exergonicity of the \([\text{Ir(ppy)}_3]^+\) reduction. Pulsing between the potentials required for the oxidation of \([\text{Ir(ppy)}_3]^+)\) and the second reduction of \([\text{Ru(bpy)}_3]^{2+}\) (Expt 3) did not change the spectral distribution, but pulsing between potentials suitable for the oxidation of \([\text{Ir(ppy)}_3]^+)\) and the third reduction of \([\text{Ru(bpy)}_3]^{2+}\) (Expt 4) provided sufficient energy to populate the \([\text{Ir(ppy)}_3]^*\) state (\(\Delta G_{\text{es}} < 0\); reaction 10). Experimentally, we observed the combined orange-red ECL of \([\text{Ru(bpy)}_3]^{2+}\) and green ECL of \([\text{Ir(ppy)}_3]^*\) as a yellow emission (Figure 2.2e, Expt 4), which can be confidently ascribed to reactions 11 and 16.

\[
\begin{align*}
\text{(15)} & \quad \text{Ru(bpy)}_3 + [\text{Ir(ppy)}_3]^+ \rightarrow [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}_3]^* \\
\text{(16)} & \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}_3]^+ \rightarrow [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}_3]^* \\
\end{align*}
\]

\(\Delta G_{\text{es}} = +0.09\) eV, and \(-0.15\) eV, respectively.

In the previously explored annihilation-ECL systems containing mixtures of organic molecules,\(^2\) the luminescence was often found to emanate from the lowest lying singlet excited state of one of the emitters, after either direct population or efficient energy transfer. One explanation for the simultaneous ECL from two distinct emitters in Expt 4 could be the large difference in the concentration of the two metal chelates, and thus an insufficient concentration of \([\text{Ru(bpy)}_3]^{2+}\) for significant energy
transfer from the higher energy \([\text{Ir}(ppy)_3]^*\) emitter. However, there is very little overlap in their respective absorption and emission bands (due in part to the large Stokes shift of their phosphorescent emissions). Furthermore, mixed electrochemiluminophore co-reactant ECL experiments have shown that the emission from \([\text{Ir}(ppy)_3]^*\) can occur in the presence of \([\text{Ru}(bpy)_3]^{2+}\) without significant energy transfer.\(^{28,148}\)

In Expt 1–4, the limiting reactants for annihilation ECL in terms of concentration are the reduced ruthenium complexes. However, when \([\text{Ir}(ppy)_3]\) was also reduced (e.g. Expt 5), relatively high concentrations of both \([\text{Ir}(ppy)_3]^+\) and \([\text{Ir}(ppy)_3]^−\) were formed, and the characteristic green emission of \([\text{Ir}(ppy)_3]^*\) was dominant (reaction 17 and Figure 2.2f).

\[
\text{17} \quad [\text{Ir}(ppy)_3]^− + [\text{Ir}(ppy)_3]^+ \rightarrow [\text{Ir}(ppy)_3]^* + [\text{Ir}(ppy)_3] \\
\Delta G_{\text{es}} = −0.65 \text{ eV}.
\]

The mean RGB data from the photographs of the mixed annihilation ECL near the electrode surface were used to calculate the CIE chromaticity. For experiments in which the emission was found to occur from only one metal complex (e.g., \([\text{Ru}(bpy)_3]^{2+}\) in Expt 2, or \([\text{Ir}(ppy)_3]^*\) in Expt 5), the CIE coordinates were in reasonable agreement with those obtained from photoluminescence experiments using a spectrometer with integrating sphere and corrected CCD detector (Figure 2.1b), despite difference in the responses of the digital camera and CCD detector over the visible region. For experiments that led to more than one emitting species (e.g., \([\text{Ru}(bpy)_3]^{2+}\) and \([\text{Ir}(ppy)_3]^*\) in Expt 4), intermediate CIE coordinates were obtained (Figure 2.1b).
2.4.2 Mixing Emissive and Non-Emissive Metal Chelate Species.

\([\text{Ru(bpy)}_3]^{2+}\) with \([\text{Ir(ppz)}_3]\)

The above findings suggest that annihilation ECL should also be possible using a mixture of \([\text{Ru(bpy)}_3]^{2+}\) and a non-emissive iridium complex with similar oxidation potential to \([\text{Ir(ppy)}_3]\), without needing to generate the oxidized \([\text{Ru(bpy)}_3]^{3+}\) species. For this experiment, we selected \([\text{Ir(ppz)}_3]\), which has a photoluminescence quantum yield below 0.01 at room temperature, due to efficient population of a non-emissive metal centred (\(^{\text{MC}}\)) excited state.\(^{206}\) The reduction potential of \([\text{Ir(ppz)}_3]\) is outside the potential window of the acetonitrile solvent, but its oxidation potential (0.38 V vs. Fe\(^{0+/+}\)) is marginally higher than that of \([\text{Ir(ppy)}_3]\) (0.33 V vs. Fe\(^{0+/+}\)), ensuring \(\Delta G < 0\) for the generation of \([\text{Ru(bpy)}_3]^{2+*}\) (reaction 18). The application of alternating potentials sufficient to create these precursors (Expt 6, Figure 2.3) resulted in the characteristic emission from \([\text{Ru(bpy)}_3]^{2+*}\) (Figure 2.3d).

(18) \([\text{Ru(bpy)}_3]^{+} + [\text{Ir(ppz)}_3]^{+} \rightarrow [\text{Ru(bpy)}_3]^{2+*} + [\text{Ir(ppz)}_3]\)

\(\Delta G_{\text{es}} = -0.12\) eV.

Population of the \([\text{Ru(bpy)}_3]^{2+*}\) excited state via an energy transfer pathway can effectively be ruled out due to the low luminescent quantum yield of \([\text{Ir(ppz)}_3]\). Therefore, the intense orange-red emission observed in this system is due to efficient HOMO → HOMO electron transfer in reaction 12.
Figure 2.3: (a) Cyclic voltammogram of 0.5 mM [Ru(bpy)₃]²⁺ and 0.5 mM [Ir(ppz)₃] in acetonitrile containing 0.1 M TBAPF₆. (b) Relevant reduction and oxidation potentials of the two metal chelates. (c) Illustration of potentials used in annihilation ECL experiments. (d) Spectrum and photograph of the ECL at the working electrode of annihilation ECL Expt 6, using 0.01 mM [Ru(bpy)₃]²⁺ and 0.5 mM [Ir(ppz)₃] in acetonitrile containing 0.1 M TBAPF₆.

2.4.3 An Energy Insufficient Metal Chelate System – [Ru(bpy)₃]²⁺ with [Ir(pmi)₃]

Although the reactions of [Ru(bpy)₃]⁺ with [Ir(ppy)₃]⁺ (Expt 2) or [Ir(ppz)₃]⁺ (Expt 6) are less exergonic than that of [Ru(bpy)₃]⁺ with [Ru(bpy)₃]³⁺, they can each directly populate the [Ru(bpy)₃]²⁺* excited state. The oxidation potential of [Ir(pmi)₃] (0.22 V vs. Fe⁰/⁺), however, is lower than that of [Ir(ppy)₃], and although both species are photoluminescent, the generation of [Ru(bpy)₃]²⁺* or [Ir(pmi)₃]* from the reaction of
[Ru(bpy)₃]⁺ with [Ir(pmi)₃]⁺ (Expt 7; data not shown) is not feasible (reactions 19 and 20).

19) [Ru(bpy)₃]⁺ + [Ir(pmi)₃]⁻ → [Ru(bpy)₃]²⁺* + [Ir(pmi)₃]

20) [Ru(bpy)₃]⁺ + [Ir(pmi)₃]⁻ → [Ru(bpy)₃]²⁺ + [Ir(pmi)₃]⁺

ΔG° = +0.04, and +1.26 eV, respectively.

As predicted, no significant ECL was observed from this experiment. Unlike many ‘energy-insufficient’ systems comprising organic molecules, the initial population of lower energy excited states, followed by up-conversion to generate the emissive excited state, is not possible in these mixed metal-chelate systems where the emission occurs from short-lived triplets.ΔG° = +0.04, and +1.26 eV, respectively.

2.4.4 Manipulating Emission in Mixed Metal Chelate ECL Systems

(i) Dominant emission determined by reduced species – [Ru(bpy)₃]²⁺ with [Ir(df-ppy)₃].

The oxidation potential of [Ir(df-ppy)₃] is 0.21 V less positive than [Ru(bpy)₃]²⁺, and the reduction potential of [Ir(df-ppy)₃] is 0.36 V more negative than the third reduction of [Ru(bpy)₃]²⁺. Under these experimental conditions, pulsing 0.1 V beyond the first reduction of [Ru(bpy)₃]²⁺ in conjunction with one or both oxidized metal chelates (e.g. Expt 8 or 10) produced a strong orange-red emission from [Ru(bpy)₃]²⁺* and a weak blue emission from [Ir(df-ppy)₃]⁺. In contrast, pulsing beyond the reduction of [Ir(df-ppy)₃] in conjunction with one or both oxidized metal chelates (e.g. Expt 9 or 11) generated a large emission from both [Ir(df-ppy)₃]⁺ and [Ru(bpy)₃]²⁺*. For example, the ECL spectra for Expt 8 and 9 are shown in Figure 2.4d. Once again the unexpectedly high efficiency of the HOMO → HOMO electron
transfer route to the excited state is evident in the intense orange-red ECL emission observed in Expt 8 and 9.

Figure 2.4: (a) Cyclic voltammogram of 0.25 mM [Ru(bpy)$_3$]$^{2+}$ and 0.25 mM [Ir(df-ppy)$_3$] in acetonitrile containing 0.1 M TBAPF$_6$. (b) Relevant reduction and oxidation potentials of the two metal chelates. (c) Illustration of potentials used in annihilation ECL experiments. (d) Spectra and photographs of the ECL at the working electrode of selected annihilation ECL experiments using 0.01 mM [Ru(bpy)$_3$]$^{2+}$ and 0.25 mM [Ir(df-ppy)$_3$] in acetonitrile containing 0.1 M TBAPF$_6$.

In both Expt 8 and 9, [Ru(bpy)$_3$]$^{2+}$* is formed by the oxidation of [Ru(bpy)$_3$]$^+$ (reaction 15), but in Expt 9 this immediate precursor is not initially the dominant form of the ruthenium chelate at the electrode. In Expt 8, small amounts of [Ir(df-ppy)$_3$]* are formed from the reduction of [Ir(df-ppy)$_3$]$^+$ (reaction 22, $\Delta G_m \approx 0$),
but in Expt 9 this emitter may be formed from reactions 23 and 24. At these metal chelate concentrations, [Ru(bpy)]$_3^{2+}$* dominates in Expt 8, and the emission from [Ir(df-ppy)$_3$]$^*$ is greater in Expt 9 (Figure 2.4d). Similar reasoning can be presented for Expt 10 and 11. The dominant emission colour in this system is therefore largely determined by the applied reduction potential of the electrochemical process, if the applied oxidation potential is at least sufficient to achieve the first metal-chelate oxidation.

$$\text{(21)} \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(df-ppy)}_3]^+ \rightarrow [\text{Ru(bpy)}_3]^{2+} + [\text{Ir(df-ppy)}_3]$$

$$\text{(22)} \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(df-ppy)}_3]^+ \rightarrow [\text{Ru(bpy)}_3]^{2+} + [\text{Ir(df-ppy)}_3]^*$$

$$\text{(23)} \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(df-ppy)}_3]^+ \rightarrow [\text{Ru(bpy)}_3]^{2+} + [\text{Ir(df-ppy)}_3]^*$$

$$\text{(24)} \quad [\text{Ir(df-ppy)}_3]^+ + [\text{Ir(df-ppy)}_3]^+ \rightarrow [\text{Ir(df-ppy)}_3]^+ + [\text{Ir(df-ppy)}_3]^*$$

$\Delta G_{\alpha} = -0.63, +0.07, -0.35, \text{ and } -0.71 \text{ eV, respectively.}$

(ii) Dominant emitter determined by oxidized species – [Ru(bpy)$_3$]$^{2+}$ with [Ir(df-ppy)$_2$(ptb)]$^+$.

The first reduction and oxidation potentials of [Ru(bpy)$_3$]$^{2+}$ lie inside those of [Ir(df-ppy)$_2$(ptb)]$^+$ (Figure 2.5). This enables the selective generation of [Ru(bpy)$_3$]$^{2+}$* without any electrochemical interaction between [Ir(df-ppy)$_2$(ptb)]$^+$ and electrode (Expt 12). The ECL intensity in this case, however, was weak, because of the low concentration of [Ru(bpy)$_3$]$^{2+}$ (the precursor to both the oxidized and reduced reactants in Expt 12) in this group of experiments. Nevertheless, extending the applied voltages to include the oxidation (Expt 13) or reduction (Expt 14) of [Ir(df-ppy)$_2$(ptb)]$^+$ resulted in ECL from both [Ru(bpy)$_3$]$^{2+}$* and [Ir(df-ppy)$_2$(ptb)]$^*$, but at considerably different ratios (Figure 2.5d). All mixed annihilation reactions in these experiments are sufficiently exergonic to form either emitter (reactions 25-28).
ΔG_{es} = −0.19, −0.91, −0.30, and −1.02 eV, respectively.

Notably in Expt 14, it is the iridium excited state that is populated by electron transfer from the HOMO of the ruthenium complex to the HOMO of the iridium complex, though with seemingly lower efficiency than the previously cases where the iridium HOMO is the donor.

Further extending the applied potential range to include both the reduction and oxidation of [Ir(df-ppy)_2(ptb)]^+ (Expt 15) resulted in a similar ECL spectral distribution to that of Expt 13. Therefore, in contrast to the above system, the dominant emission in this case is largely determined by the applied oxidation potential, so long as the other applied potential was at least beyond the first reduction. In Expt 13 and Expt 9, the combined emissions spanned the entire visible region, resulting in near-white luminescence (Figures 2.1b, 2.4d, and 2.5d).
Figure 2.5: (a) Cyclic voltammogram of 0.5 mM [Ru(bpy)$_3$]$_2^{2+}$ and 0.5 mM [Ir(df-ppy)$_2$(ptb)]$^{+}$ in acetonitrile containing 0.1 M TBAPF$_6$. (b) Relevant reduction and oxidation potentials of the two metal chelates. (c) Illustration of potentials used in annihilation ECL experiments. (d) Spectra and photographs of the ECL at the working electrode of selected annihilation ECL experiments using 0.004 mM [Ru(bpy)$_3$]$^{2+}$ and 0.4 mM [Ir(df-ppy)$_2$(ptb)]$^{+}$ in acetonitrile containing 0.1 M TBAPF$_6$. 

59
2.5 Conclusions

The mixed annihilation ECL of metal chelates provides an alternative approach to multi-colour ECL, in which the relative intensity of the emissions from multiple luminophores (and hence overall emission colour) can be controlled initially by selection of the electrochemical and spectroscopic properties of the complexes, and then the applied electrochemical potentials. The absence of the up-conversion processes often encountered in ‘energy insufficient’ organic mixed systems simplifies predictions of excited state generation. Furthermore, the numerous closely spaced reductions and oxidations of the mixed systems enable fine tuning of the reaction energy and hence control of the resulting ECL emission colour. Apart from the relevance of these studies to research into voltage controllable light emitting devices, the observation of efficient HOMO → HOMO electron transfer pathways in these mixed systems offers interesting insights into the somewhat intractable question of whether the reduced or oxidized partner becomes the excited state in classic annihilation ECL experiments. In the case of ruthenium complexes where the HOMO is almost exclusively metal-based, it is generally believed that electron transfer between LUMOs is strongly preferred due to more favourable orbital overlap compared with the alternative HOMO → HOMO transfer where the reduced partner becomes excited. Our results tend to support this analysis, because the delocalized nature of the HOMO in the case of the iridium complexes studied here renders this electronic factor less unfavourable, resulting in higher than expected ECL intensities in cases where \([\text{Ru(bpy)}_3]^+\) forms an excited state by loss of an electron from its HOMO.

2.6 Acknowledgements

This research was funded by the Australian Research Council (FT100100646).
Chapter Three: New Perspectives on the Annihilation

Electrogenerated Chemiluminescence of Mixed Metal Complexes in Solution

Emily Kerr\textsuperscript{a}, Egan H. Doeven\textsuperscript{b*}, Gregory J. Barbante\textsuperscript{a}, Conor F. Hogan\textsuperscript{c}, David J. Hayne\textsuperscript{d}, Paul S. Donnelly\textsuperscript{d} and Paul S. Francis\textsuperscript{*a}

\textsuperscript{a}Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment, Deakin University, Geelong, Victoria 3220, Australia. E-mail: paul.francis@deakin.edu.au

\textsuperscript{b}Centre for Regional and Rural Futures, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment, Deakin University, Geelong, Victoria 3220, Australia. E-mail: egan.doeven@deakin.edu.au

\textsuperscript{c}Department of Chemistry and Physics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria 3086, Australia

\textsuperscript{d}School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne 3010, Australia

Published in Chemical Science, Royal Society of Chemistry, 2016, volume 7, issue 1, pages 5271-5279. Reproduced with permission from the Royal Society of Chemistry.
### Authorship Statement

#### 1. Details of publication and executive author

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Perspectives on the Annihilation Electrogenated Chemiluminescence of Mixed Metal Complexes in Solution</td>
<td><em>Chemos. Sci.</em>, 2016, 7, 5271-5276</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organization and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul Francis</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td><a href="mailto:paul.francis@deakin.edu.au">paul.francis@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

#### 2. Inclusion of publication in a thesis

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>If Yes, please complete Section 3 if No, go straight to Section 4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes/</td>
<td></td>
</tr>
</tbody>
</table>

#### 3. HDR thesis author's declaration

<table>
<thead>
<tr>
<th>Name of HDR thesis author different from above: (If the same, write &quot;as above&quot;)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emily Kerr</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td>Electrochemical and Luminescence Studies of Ruthenium and Iridium Metal-Chelates</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author's contribution to the publication. For example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

**Signature and date**: 14/12/16

#### 4. Description of all author contributions

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (e.g., conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan H. Goosen</td>
<td>Assisted with taking photographs at ECL. Assisted with planning experiments. Assisted with revision of manuscript.</td>
</tr>
<tr>
<td>Gregory J. Barthane</td>
<td>Involved in conceptualization of project. Assisted with planning experiments. Assisted with revision of manuscript.</td>
</tr>
<tr>
<td>Conor F. Hogan</td>
<td>Involved in conceptualization of project. Involved with revision of manuscript and contributed to intellectual content of manuscript.</td>
</tr>
<tr>
<td>David J. Kayne</td>
<td>Completed all chemical synthesis and prepared relevant sections of manuscript.</td>
</tr>
<tr>
<td>Paul B. Donnelly</td>
<td>Assisted with revision of manuscript and overview aspects of chemical synthesis.</td>
</tr>
<tr>
<td>Paul B. Francis</td>
<td>Involved in conceptualization of project. After receiving initial draft, was involved in the revision process, contributed to intellectual content of manuscript and finalised submission to journal.</td>
</tr>
</tbody>
</table>
5. Author Declarations
I agree to be named as one of the authors of this work, and confirm:

i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,

ii. that there are no other authors according to these criteria,

iii. that the description in Section 6 of my contribution(s) to this publication is accurate,

iv. that the data on which these findings are based are stored as set out in Section 7 below.

If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further

v. consent to the incorporation of the publication into the candidate’s HDR thesis submitted to Deakin University and, if the higher degree is awarded, the subsequent publication of the thesis by the university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan H. Dooven</td>
<td></td>
<td>15/12/16</td>
</tr>
<tr>
<td>Gregory J. Bartanie</td>
<td></td>
<td>15/12/16</td>
</tr>
<tr>
<td>Conor F. Hogan</td>
<td></td>
<td>16/12/16</td>
</tr>
<tr>
<td>David J. Kayne</td>
<td></td>
<td>20/12/2016</td>
</tr>
<tr>
<td>Paul S. Donnelly</td>
<td></td>
<td>16/12/16</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td></td>
<td>16/12/2016</td>
</tr>
</tbody>
</table>

6. Other contributor declarations
I agree to be named as a non-author contributor to this work.

<table>
<thead>
<tr>
<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
</table>

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author.

7. Data storage
The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB</td>
<td>WP Ka 3.127</td>
<td>19/02/2017</td>
<td>Paul Francis</td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based.

If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
3.1 Abstract

Preliminary explorations of the annihilation electrogenerated chemiluminescence (ECL) of mixed metal complexes have revealed opportunities to enhance emission intensities and control the relative intensities from multiple luminophores through the applied potentials. However, the mechanisms of these systems are only poorly understood. Herein, we present a comprehensive characterisation of the annihilation ECL of mixtures of tris(2,2′-bipyridine)ruthenium(II) hexafluorophosphate ([Ru(bpy)$_3$][(PF$_6$)$_2$]) and fac-tris(2-phenylpyridine)iridium(III) ([Ir(ppy)$_3$]). This includes a detailed investigation of the change in emission intensity from each luminophore as a function of both the applied electrochemical potentials and the relative concentrations of the two complexes, and a direct comparison with two mixed (Ru/Ir) ECL systems for which emission from only the ruthenium-complex was previously reported. Concomitant emission from both luminophores was observed in all three systems, but only when: (1) the applied potentials were sufficient to generate the intermediates required to form the electronically excited state of both complexes; and (2) the concentration of the iridium complex (relative to the ruthenium complex) was sufficient to overcome quenching processes. Both enhancement and quenching of the ECL of the ruthenium complex was observed, depending on the experimental conditions. The observations were rationalised through several complementary mechanisms, including resonance energy transfer and various energetically favourable electron-transfer pathways.
3.2 Introduction

Electrogenerated chemiluminescence (also known as electrochemiluminescence or ECL) is the emission of light resulting from reactions between electrochemically generated species.\textsuperscript{13, 14, 182} ECL is a consequence of the so-called ‘inverted region’ of Marcus electron transfer theory.\textsuperscript{29, 33, 184} It transpires because the energy available from the homogeneous electron-transfer processes is too large to be dissipated on the timescale of the vibrational modes of the emitter's molecular framework.

ECL is often categorised into two general pathways: annihilation and co-reactant.\textsuperscript{13, 14, 182} Annihilation ECL involves the direct electrochemical formation of both oxidised and reduced species, normally as a result two-directional potential stepping. These oxidised and reduced species may then react to form electronically excited products capable of emitting light, as shown in reactions 1-7, where \( A \) and \( D \) may be the same or in the case of ‘mixed’ ECL systems, different molecules.

\begin{align*}
(1) \quad A + e^- & \rightarrow A^- \\
(2) \quad D - e^- & \rightarrow D^+ \\
(3) \quad A^- + D^+ & \rightarrow A + D^* \\
(4) \quad A^- + D^+ & \rightarrow A^* + D \\
(5) \quad A^- + D^+ & \rightarrow A + D \\
(6) \quad D^* & \rightarrow D + \hbar \nu \\
(7) \quad A^* & \rightarrow A + \hbar \nu
\end{align*}

For annihilation ECL systems, in most cases the Gibbs free energy associated with the formation of either ground (\( \Delta G_{gs} \)) (reaction 5) or excited (\( \Delta G_{es} \)) state (reactions 3 or 4) products can be reasonably estimated from the respective electrochemical potentials and the emission energy, as shown in eqn I and II (with further details in the Appendix 1).\textsuperscript{183, 184, 209}
\[ \Delta G_{\text{gs}} \approx E^{\circ}_{D+/D} - E^{\circ}_{A/A^-} \]

II. \[ \Delta G_{\text{es}}(D^*) \approx (E^{\circ}_{D+/D} - E^{\circ}_{A/A^-}) + E_{\text{es}}(D^*) \]

III. \[ \Delta G_{\text{es}}(A^*) \approx (E^{\circ}_{D+/D} - E^{\circ}_{A/A^-}) + E_{\text{es}}(A^*) \]

where \( E_{\text{es}}(D^*) \) and \( E_{\text{es}}(A^*) \) are the excited-state energies of complexes A and D, obtained from their photoluminescence emission spectra. For simplicity, minor contributions from factors such as the Coulomb repulsion of bringing the reactants together and the Franck–Condon energy of the emissive product have been omitted from these equations.\(^{202, 203, 209}\)

The question of whether the oxidised or reduced partner becomes the electronically excited species responsible for the emission (i.e. whether the system proceeds through reactions 3 and 6 or reactions 4 and 7) is important from both a fundamental and a practical standpoint.\(^{200, 209, 210}\) In the classic tris(2,2′-bipyridine)ruthenium(II) ([Ru(bpy)_3]^{2+}) annihilation ECL system,\(^{10}\) for example, reactants A and D are both [Ru(bpy)_3]^{2+}, and therefore reactions 3 and 4 are thermodynamically equivalent. Simple orbital overlap arguments, however, suggest that ligand-to-ligand electron transfer to form [Ru(bpy)_3]^{3+*} from the oxidised parent will be kinetically favoured over metal-to-metal electron transfer to form the excited state from the reduced parent.\(^{188}\) Nevertheless, co-reactant ‘oxidative-reduction’ and ‘reductive-oxidation’ ECL pathways\(^{20, 204, 205}\) show that either the oxidised or reduced intermediate can become the emitting species.

Mixed annihilation ECL systems can be more complicated, as the oxidation and/or reduction of both reactants, and two possible emitting species, need to be considered.\(^{142}\) Nevertheless, the emission spectra of many mixed ECL systems comprising organic reactants have shown that these reactions almost always generate
a single emitting species,\textsuperscript{183} which can be attributed to the rates of the competing electron transfer processes and the relative energies of the possible excited states. Mixed systems that combine a transition metal complex with an organic compound have been used to generate exceptionally high annihilation ECL efficiencies from the metal complex.\textsuperscript{46, 147}

In our recent preliminary report of the mixed annihilation ECL of transition metal complexes (combining [Ru(bpy)]\textsuperscript{2+} with a variety of iridium(III) complexes),\textsuperscript{209} we observed simultaneous emissions from multiple emitters, and showed that the ratio of these emissions (and hence the overall colour of the luminescence) could be tuned through the applied electrode potentials, exploiting the multiple, closely spaced reductions and oxidations of the reactants. In a subsequent study, Swanick, \textit{et al.}\textsuperscript{210} examined the ECL of a ruthenium(II)–iridium(III) complex ‘soft salt’,\textsuperscript{211} comprising a [Ru(dtb-bpy)]\textsuperscript{2+} cation (where dtb-bpy = 4,4′-di-t-butyl-2,2′-bipyridine) and two [Ir(ppy)\textsubscript{2}(CN)\textsubscript{2}]\textsuperscript{−} anions (where ppy = 2-phenylpyridine), in solution. In an unanticipated result, Swanick, \textit{et al.}\textsuperscript{210} unlike the case with the photoluminescence of the soft salt under closely related conditions,\textsuperscript{212} Swanick, \textit{et al.}\textsuperscript{210} observed ECL solely from the ruthenium(II) complex (\textit{i.e.}, no emission from the iridium(III) complex). This contrasts with our reports of multiple ECL emissions from mixtures of ruthenium(II) and iridium(III) complexes involving annihilation\textsuperscript{209} or co-reactant\textsuperscript{20, 28, 144, 145, 148, 149, 213, 214} ECL pathways. Swanick, \textit{et al.}\textsuperscript{210} attributed the absence of ECL from the iridium(III) luminophore (and enhancement of the [Ru(dtb-bpy)]\textsuperscript{2+} emission) to the rapid consumption of electrochemically reduced iridium species through electron transfer to the ruthenium complex, which precluded the formation of the iridium(III) emitter. Similarly, seeking to fabricate a colour-tuneable ECL-based light-emitting device, Moon, \textit{et al.}\textsuperscript{200} incorporated [Ru(bpy)]\textsuperscript{2+} and [Ir(df-
ppy)₂(bpy)⁺ (where df-ppy = 2-(2,4-difluorophenyl)pyridine) into an ion-gel cast onto an ITO-coated flexible substrate. ECL was observed from only the ruthenium(II) complex, but the inclusion of the iridium(III) species was found to enhance the emission intensity up to 2-fold. In this case, the absence of ECL from the iridium(III) complex was attributed to electron transfer quenching of the excited state.

Herein, we reconcile these seemingly disparate findings through an examination of concentration effects and energy transfer in mixed annihilation ECL, whilst introducing both a novel three-dimensional representation of the phenomenon (annihilation ECL intensity versus emission wavelength and the applied reduction potential) and a simple graphical depiction of the energetics of annihilation and co-reactant ECL systems to explore electron-transfer quenching pathways.
3.3 Experimental

3.3.1 Chemicals

Acetonitrile (Ajax Finechem, Australia) was distilled over calcium hydride under nitrogen and solutions were degassed with grade 5 argon prior to analysis. Tetrabutylammonium hexafluorophosphate (TBAPF$_6$, 99.5%, electrochemical grade) and fac-tris[2-(2-pyridinyl-κN)phenyl-κC]iridium (tris(2-phenylpyridinato-C$_2$N)iridium(III); [Ir(ppy)$_3$, 99%) were purchased from Sigma-Aldrich (Australia). Tris(2,2’-bipyridine)ruthenium(II) dichloride hexahydrate ([Ru(bpy)$_3$]Cl$_2$$\cdot$6H$_2$O) was purchased from Strem Chemicals (USA) and converted to the hexafluorophosphate salt ([Ru(bpy)$_3$]([PF$_6$])$_2$). Tris[2-(1H-pyrazol-1-yl-κN$^\text{N}$)phenyl-κC]iridium (tris(phenylpyrazole)iridium(III); [Ir(ppz)$_3$, >99%) was purchased from LumTech (Taiwan). Details of the synthesis and characterisation of [Ir(df-ppy)$_2$(bpy)][PF$_6$], [Ru(dtb-bpy)$_3$][PF$_6$]$_2$, [TBA][Ir(ppy)$_2$(CN)$_2$], and the [Ru(dtb-ppy)$_3$][Ir(ppy)$_2$(CN)$_2$]$_2$ soft salt are included in Appendix I.

3.3.2 Experimental Procedure

An Autolab PGSTAT 101 or PGSTAT 128N potentiostat was used to perform chronoamperometry and cyclic voltammetry experiments (Metrohm Autolab B.V., Netherlands). The instrumental configuration was equivalent to that described previously.$^{40}$ For cyclic voltammetry measurements, the complexes were prepared at 0.25 mM in degassed, freshly distilled acetonitrile (0.1 M TBAPF$_6$ supporting electrolyte) and referenced to the formal potential of the ferrocene/ferrocenium couple (1 mM), measured in situ in each case. ECL spectra were obtained using a model QE65pro CCD spectrometer (Ocean Optics) interfaced with the working electrode through a collimating lens and custom built cell holder (Figure A1.1); the potentiostat applied a two-step chronoamperometry pulse at 0.5 Hz (i.e. alternating 1 s oxidative...
potential with 1 s reductive potential) for 12 s, unless otherwise stated. Intensities were calculated from the average integrated peak area of three replicates. For convenience, the arbitrary intensity units from spectrometer were divided by $10^3$. To generate the 3D profiles (intensity versus emission wavelength and applied reduction potential) of annihilation ECL, appropriate concentrations of the complexes were prepared in freshly distilled acetonitrile with 0.1 M TBAPF$_6$ supporting electrolyte, and solutions were degassed with grade 5 argon prior to analysis. NOVA software was configured to apply a two-step 0.5 Hz pulse from the oxidative potential to corresponding reduction potentials, for 12 s, with a 30 s wait time between each pulse sequence, to allow for degassing (15 s) between the collection of each spectrum.
3.4 Results and Discussion

3.4.1 The [Ru(bpy)$_3$]$_{2}^{2+}$ – [Ir(ppy)$_3$] Mixed Annihilation ECL System

Cyclic voltammetric scans of an equimolar mixture of [Ru(bpy)$_3$]$_{2}^{2+}$ and [Ir(ppy)$_3$] in acetonitrile containing 0.1 M TBAPF$_6$ (Figure 3.1b) exhibit a combination of the characteristic electron-transfer processes of the two metal complexes (Figure 3.1a and c).

![Cyclic voltammograms](image)

**Figure 3.1**: Cyclic voltammograms of: (a) [Ir(ppy)$_3$]; (b) a mixture of [Ir(ppy)$_3$] and [Ru(bpy)$_3$]$_{2}^{2+}$; and (c) [Ru(bpy)$_3$]$_{2}^{2+}$, showing $E^0$ values. All complexes at 0.25 mM with 0.1 M TBAPF$_6$ supporting electrolyte in acetonitrile. Scan rate: 0.1 V s$^{-1}$.

In our previous report of annihilation ECL from mixtures of [Ru(bpy)$_3$]$_{2}^{2+}$ with various iridium(III) complexes (including [Ir(ppy)$_3$]),$^{209}$ we observed, under certain circumstances, simultaneous emissions from both luminophores. Moreover, the
relative intensity of the emissions could be manipulated through the applied voltages, which generated different redox forms of the complexes, thus modifying the energetics of the light-producing reactions. For example, when alternately pulsing slightly beyond the first reduction potential of \([\text{Ru(bpy)}_3]^{2+}\) and the oxidation potential of \([\text{Ir(ppy)}_3]^-\), we form \([\text{Ru(bpy)}_3]^+\) and \([\text{Ir(ppy)}_3]^+\) (but at these potentials, neither \([\text{Ir(ppy)}_3]^-\) nor \([\text{Ru(bpy)}_3]^{3+}\) is formed). Estimations of the \(\Delta G\) of the subsequent reaction between \([\text{Ru(bpy)}_3]^-\) and \([\text{Ir(ppy)}_3]^+\) (eqn II and III) indicated that the generation of \([\text{Ru(bpy)}_3]^{2+*}\) and \([\text{Ir(ppy)}_3]^+\) (reaction 8) was energetically favourable, but there was insufficient energy to produce \([\text{Ir(ppy)}_3]^*\) and \([\text{Ru(bpy)}_3]^{2+}\). Under these conditions, the characteristic orange-red emission of the \([\text{Ru(bpy)}_3]^{2+}\) complex was observed. However, by pulsing to further negative potentials, more reductive intermediates were formed, which upon reaction with \([\text{Ir(ppy)}_3]^+\), enabled the \([\text{Ir(ppy)}_3]^*\) species to be attained \((\Delta G < 0)\). Pulsing beyond the third reduction potential of \([\text{Ru(bpy)}_3]^{2+}\) and the oxidation potential of \([\text{Ir(ppy)}_3]^-\) gave an overall yellow emission from a combination of emissions from reactions 8 and 9. Whereas pulsing beyond the reduction and oxidation potentials of \([\text{Ir(ppy)}_3]^-\) gave the characteristic green emission of the \([\text{Ir(ppy)}_3]^-\) complex predominantly via reaction 10.

\[
\begin{align*}
(8) \quad [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}_3]^+ & \rightarrow [\text{Ru(bpy)}_3]^{2+*} + [\text{Ir(ppy)}_3]^+ \\
(9) \quad [\text{Ru(bpy)}_3]^- + [\text{Ir(ppy)}_3]^+ & \rightarrow [\text{Ru(bpy)}_3]^+ + [\text{Ir(ppy)}_3]^* \\
(10) \quad [\text{Ir(ppy)}_3]^- + [\text{Ir(ppy)}_3]^+ & \rightarrow [\text{Ir(ppy)}_3]^* + [\text{Ir(ppy)}_3]^+ 
\end{align*}
\]

This simple comparison of the ECL generated at a few sets of applied potentials shows that free energy considerations (eqn II) provide a basis for understanding the potential dependence of the ECL in observed in such mixed annihilation systems, but it is an incomplete characterisation due to the possibility of other ground state and excited state interactions between the species that are present. With this in mind, we
adapted our 3D ECL approach that was previously used to examine the parameters of mixed co-reactant ECL systems. This involved an automated pulsing cycle from 100 mV beyond a single oxidative potential to a series of evenly spaced reductive potentials at 50 mV intervals over the range of interest, whilst monitoring the ECL spectra with a CCD spectrometer (Figure 3.2).

Figure 3.2: (a) A 3D representation of the ECL of the [Ru(bpy)₃]²⁺–[Ir(ppy)₃] mixed annihilation system showing ECL intensity versus emission wavelength and applied reductive potential, with an alternating oxidative potential of 0.98 V (to generate both [Ir(ppy)₃]⁺ and [Ru(bpy)₃]³⁺), using 0.01 mM [Ru(bpy)₃]²⁺ and 0.24 mM [Ir(ppy)₃] in acetonitrile with 0.1 M TBAPF₆. A similar graph was obtained using an oxidative potential of 0.43 V, which generated [Ir(ppy)₃]⁺, but not [Ru(bpy)₃]³⁺ (Figure A1.2). (b) The corresponding portion of a cyclic voltammogram of 0.25 mM [Ru(bpy)₃]²⁺ and 0.25 mM [Ir(ppy)₃] (0.1 M TBAPF₆, acetonitrile), showing: (1) [Ru(bpy)₃]⁴⁺; (2) [Ru(bpy)₃]¹⁺; (3) [Ru(bpy)₃]⁻⁰; (4) a combination of [Ir(ppy)₃]¹⁻⁰ and [Ru(bpy)₃]²⁻⁻. All potentials shown versus the Fe⁶⁺⁻ redox couple.

The relevant portion of the cyclic voltammogram was superimposed on the graph so that the electrochemical generation of various reduced species could be easily correlated with the emission processes. In agreement with our previous results, homogeneous electron transfer to [Ir(ppy)₃]⁺ generates the [Ir(ppy)₃]⁺ emitter, but only with reducing agents at least as strong as [Ru(bpy)₃]⁺, and the relative
emission intensities from [Ru(bpy)$_3$]$^{2+}$* and [Ir(ppy)$_3$]$_2*$ thereafter were highly dependent on the applied potential.

In our previous study,$^{209}$ we sought concentrations of the two complexes that would generate similar ECL intensities, to demonstrate the control of the emission ratio (and overall emission colour) through the applied potentials. In ECL system shown in Figure 3.2a, the ratio of iridium to ruthenium complex is 24 : 1, which is much greater than those used in the studies by Moon, et al.$^{200}$ (up to 4 : 1) and Swanick, et al.$^{210}$ (2 : 1), in which ECL from only one luminophore was observed.

Applying the above comprehensive approach to explore the [Ru(bpy)$_3$]$^{2+}$–[Ir(ppy)$_3$] mixed annihilation ECL system at a range of metal complex concentrations (e.g., Figure A1.3) confirmed that the contrasting observations of these previous studies$^{200, 209, 210}$ can be largely ascribed to differences in the relative concentration of electrochemiluminophores. For example, under the conditions shown in Figure 3.2, the ECL obtained when applying potentials 100 mV beyond the oxidation of [Ru(bpy)$_3$]$^{2+}$ (0.89 V vs. Fe$^{0+/+}$) and the reduction of [Ir(ppy)$_3$] $^{-2.67}$ V vs.Fe$^{0+/+}$ aris
ed predominantly (but not entirely) from the [Ir(ppy)$_3$]$_2*$ emitter. However, as the concentration of [Ru(bpy)$_3$]$^{2+}$ in the mixture was increased, we observed an increase in the characteristic emission from [Ru(bpy)$_3$]$^{2+}$* and decrease from [Ir(ppy)$_3$]$_2*$ (Figure 3.3).
Figure 3.3: Annihilation ECL spectra (0.99 V to −2.77 V vs. Fe⁰/⁺) for a mixture of [Ir(ppy)₃] (0.25 mM) and [Ru(bpy)₃]²⁺ (0.015–0.060 mM) in acetonitrile with 0.1 M TBAPF₆. For each spectrum, a two-step potential pulse was applied at 0.5 Hz for 12 s.

3.4.2 Energy Transfer in Mixed Annihilation ECL Systems

Prior photoluminescence studies of the [Ru(dtb-bpy)₃][Ir(ppy)₂(CN)₂]₂ soft salt indicated Förster (resonance) energy transfer between the donor (Ir) and acceptor (Ru) complexes, with considerable overlap between their MLCT emission and absorption bands. However, electron transfer between ground and excited states of the complexes could not be ruled out in this system, and this process has been ascribed as the major pathway for energy transfer in photoluminescence studies of related soft salts (such as [Ir(Me-ppy)₂(dtb-bpy)][Ir(df-ppy)₂(CN)₂]) that exhibit very little overlap between emission and absorption bands. In ECL experiments, significant quantities of the ground state oxidised and reduced species are generated, which must also be considered. The absence of iridium-based ECL from the [Ru(dtb-bpy)₃][Ir(ppy)₂(CN)₂]₂ soft salt, for example, was ascribed to electron transfer from the electrochemically reduced [Ir(ppy)₂(CN)₂]⁻ species to [Ru(dtb-bpy)₃]²⁺ (reaction 11).
(11) \([\text{Ir}(ppy)_2(CN)_2]^{2-} + [\text{Ru} \text{dtb-bpy)}_3]^{2+} \rightarrow [\text{Ir}(ppy)_2(CN)_2]^+ + [\text{Ru} \text{dtb-bpy)}_3]^{2+}\)

In contrast, the absence of ECL from the iridium(III) luminophore in mixtures of \([\text{Ru}(bpy)_3][\text{Cl}_2]\) and \([\text{Ir}(\text{df-ppy})_2(bpy)][\text{PF}_6]\) was tentatively postulated\(^{200}\) to involve oxidative quenching of the electronically excited \([\text{Ir}(\text{df-ppy})_2(bpy)]^+\) resulting in the direct formation of \([\text{Ru}(bpy)_3]^{2+}\) (reaction 12).

(12) \([\text{Ir}(\text{df-ppy})_2(bpy)]^+ + [\text{Ru}(bpy)_3]^{3+} \rightarrow [\text{Ir}(\text{df-ppy})_2(bpy)]^{2+} + [\text{Ru}(bpy)_3]^{2+}\)

In the \([\text{Ru}(bpy)_3][\text{PF}_6]_2 – [\text{Ir}(ppy)_3] \) system, as with the system discussed above, the ECL of the iridium complex was efficiently quenched by the ruthenium-complex. The emission of \([\text{Ir}(ppy)_3]\) overlaps with the MLCT absorption band of \([\text{Ru}(bpy)_3]^{2+}\) (Figure 3.4), but to a much lesser extent than that of \([\text{Ir}(ppy)_2(CN)_2]^{2-}\) and \([\text{Ru} \text{dtb-bpy)}_3]^{2+}\),\(^{212}\) and therefore Förster resonance energy transfer could be anticipated to make only a minor contribution to the \([\text{Ru}(bpy)_3][\text{PF}_6]_2 – [\text{Ir}(ppy)_3] \) mixed annihilation ECL system.

**Figure 3.4:** Absorption spectrum of 10 µM \([\text{Ru}(bpy)_3]^{2+}\) (red line) and photoluminescence emission spectrum \((\lambda_{\text{ex}} = 380 \text{ nm})\) of 10 µM \([\text{Ir}(ppy)_3]\) (green line), in acetonitrile.

To examine the feasible electron transfer pathways, we plotted the electrochemical potentials (from Figure 3.1) of both electrochemiluminophores and superimposed the
corresponding potentials for their electronically excited states (Figure 3.5 and AI.4a), which have been estimated based on the low temperature (77 K) photoluminescence emission spectra.\textsuperscript{208, 216} In this depiction, the species shown above the arrows at the top of the graph are the strongest oxidants and the species below the arrows at the bottom of the graph are the strongest reductants.

Figure 3.5: Redox potentials for ground states (blue dots) and electronically excited states (red dots) within the [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}–[Ir(ppy)\textsubscript{3}] mixed annihilation ECL system.

Considering first the electron transfer between the ground and excited states of the most stable oxidation state: the [Ir(ppy)\textsubscript{3}] complex in its \textsuperscript{3}MLCT excited state is a strong reductant that can donate an electron to [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} (reaction 13 and Figure AI.4b; $\Delta G \approx -0.41$ eV). It can be estimated that the back electron transfer to generate the excited ruthenium complex (reaction 14) is marginally energy insufficient ($\Delta G \approx +0.04$ eV), but it should be noted that (a) this is small compared to the combined estimation error of the excited state potentials and $\Delta G$, and (b) the electron exchange
may be a concerted process in which the overall energetics are favourable (Figure AI.4c).

\[
(13) \quad \text{[Ir(ppy)]}^* + \text{[Ru(bpy)]}^{2+} \rightarrow \text{[Ir(ppy)]}^+ + \text{[Ru(bpy)]}^2+
\]

\[
(14) \quad \text{[Ir(ppy)]}^- + \text{[Ru(bpy)]}^2+ \rightarrow \text{[Ir(ppy)]}^+ + \text{[Ru(bpy)]}^2^*+
\]

The excitation process of annihilation ECL (unlike that of photoluminescence) generates significant quantities of the oxidised and reduced complexes (near the electrode surface) and therefore the contribution of these species to energy transfer must also be considered. Figure 3.5 suggests a series of additional energetically feasible electron-transfers that may contribute to the observed quenching of the [Ir(ppy)], ECL and enhancement of [Ru(bpy)], ECL within the mixed system (reactions 15-17).

\[
(15) \quad \text{[Ir(ppy)]}^* + \text{[Ru(bpy)]}^{3+} \rightarrow \text{[Ir(ppy)]}^+ + \text{[Ru(bpy)]}^{2^*}
\]

\[
(16) \quad \text{[Ir(ppy)]}^- + \text{[Ru(bpy)]}^{2+} \rightarrow \text{[Ir(ppy)]}^+ + \text{[Ru(bpy)]}^+
\]

Reaction (15) is analogous to that postulated by Moon, et al.\textsuperscript{200} (reaction (12)) to explain the absence of ECL from [Ir(df-ppy)(bpy)]\textsuperscript{+} when combined with [Ru(bpy)]\textsuperscript{3+}. Interestingly, whilst this pathway is certainly feasible within our system (Figure 3.5), an examination of the reduction potentials within the mixed annihilation ECL system for which it was originally proposed suggests that it is unlikely to explain the energy transfer observed in that case (Figure AI.5). Reaction (16) is analogous to reaction (11), proposed by Swanick, et al.\textsuperscript{210} to explain the lack of ECL from the iridium component of the [Ru(dtb-bpy)]\textsuperscript{2–}–[Ir(ppy)\textsubscript{2}(CN)\textsubscript{2}]\textsuperscript{–} system and an unexpectedly large electrochemical current for the [Ir(ppy)\textsubscript{2}(CN)\textsubscript{2}]\textsuperscript{–} reduction. These electron transfers (reactions (16) and (12)) are energetically feasible in both systems (Figure 3.5 and AI.6). Based on this process, Swanick, et al.\textsuperscript{210} concluded that the electronically excited [Ir(ppy)\textsubscript{2}(CN)\textsubscript{2}]\textsuperscript{–} was not formed in their system. However, it is
also possible that some of this excited state species is formed but then effectively quenched via electron exchange and resonance energy transfer, analogous to those discussed above. Moreover, the significant ion pairing interactions$^{212}$ of the soft salt facilitate efficient quenching within that mixed annihilation ECL system.

3.4.3 An Additional Route for Enhancement in Mixed Annihilation ECL Systems

The above discussion focuses on energy transfer between concomitant ECL systems under conditions that would be suitable to attain the excited state of either metal complex in isolation (i.e., the applied potentials are generally beyond the first reduction and oxidation of both complexes). Under these conditions, considerable enhancement of the ECL of one complex in the mixture has been observed (compared to the annihilation ECL of that complex in isolation).$^{200, 210}$

Figure 3.6 shows that under conditions in which the generation of only one excited state is energetically feasible, we observed an additional mechanism of enhancement that does not involve the energy transfer pathways discussed above. In the $[\text{Ru(bpy)}_3]^2$––$[\text{Ir(ppy)}_3]$ mixed annihilation ECL system for example, applying potentials of 0.99 V and −1.82 V (vs. Fe$^{0\,+/+}$) results in the formation of $[\text{Ru(bpy)}_3]^+$, $[\text{Ir(ppy)}_3]^+$ and $[\text{Ru(bpy)}_3]^+$, but not $[\text{Ir(ppy)}_3]^-$ (Figure 3.1). In this case, the $[\text{Ir(ppy)}_3]^*$ emitter is not formed (Figure 3.2), because there is insufficient free energy in the mixed annihilation ECL reaction of $[\text{Ir(ppy)}_3]^+$ and $[\text{Ru(bpy)}_3]^+$ ($\Delta G_{es} \sim +0.41$ eV, based on the data shown in Figure 3.5). In contrast, the electronically excited $[\text{Ru(bpy)}_3]^2$* species is generated by the reaction of $[\text{Ru(bpy)}_3]^+$ with not only $[\text{Ru(bpy)}_3]^+$ ($\Delta G_{es} \sim −0.48$ eV), but also $[\text{Ir(ppy)}_3]^+$ (reaction (8) above; $\Delta G_{es} \sim −0.04$ eV). As the concentration of $[\text{Ir(ppy)}_3]$ in the mixture is increased, so too is the concentration of $[\text{Ir(ppy)}_3]^+$ when 0.99 V is applied, which increases the probability that $[\text{Ru(bpy)}_3]^+$ species (generated at the applied potential of −1.82 V) is oxidised to
form [Ru(bpy)$_3$]$^{2+}$, thus increasing the observed ECL from the ruthenium complex emitter (Figure 3.6). An approximately linear increase ($R^2 = 0.996$) in the annihilation ECL (integrated peak area) of 6 μM [Ru(bpy)$_3$]$^{2+}$ was observed with [Ir(ppy)$_3$] concentration up to 30 μM. Beyond this point, the ECL intensity was approximately 25-fold greater than that of the annihilation ECL of [Ru(bpy)$_3$]$^{2+}$ in the absence of the iridium complex.

**Figure 3.6:** ECL intensity of [Ru(bpy)$_3$]$^{2+}$ (6 μM) in the presence of different concentrations of [Ir(ppy)$_3$]. In each case, a two-step potential pulse was applied at 0.5 Hz for 12 s. Applied potentials: 0.99 V and −1.82 V vs. Fe$^{0}$ (i.e., 0.1 V beyond the oxidation and first reduction potential of [Ru(bpy)$_3$]$^{2+}$, respectively). All complexes were prepared in acetonitrile with 0.1 M TBAPF$_6$. Average RSD: 7.5%. In all cases, only the emission from the ruthenium complex was observed. The ECL spectrum for a mixture of 6 μM [Ru(bpy)$_3$]$^{2+}$ and 100 μM [Ir(ppy)$_3$]$_2$, under these applied potentials, is shown in Figure AI.7.

It should be noted that the linear range and relative intensities were found to vary depending on the timespan of the applied potential, the presence of trace amounts of oxygen prior to analysis and relative concentrations of [Ru(bpy)$_3$]$^{2+}$ and [Ir(ppy)$_3$]. Moreover, emission was sometimes also observed at the counter electrode.
therefore we employed a collimating lens (focussed on the working electrode) to eliminate interference. ECL spectra were obtained to confirm that only the characteristic orange emission from $[\text{Ru(bpy)}_3]^{2+*}$ was generated in this system using these applied potentials (Figure AI.7).

We also examined the enhancing effect of a non-emissive iridium complex, $[\text{Ir(ppz)}_3]$, on the annihilation ECL of $[\text{Ru(bpy)}_3]^{2+}$. The oxidation potential of $[\text{Ir(ppz)}_3]$ (0.38 V vs. Fc$^{0+/+}$; Figure AI.8) is slightly higher than that of $[\text{Ir(ppy)}_3]$, but unlike $[\text{Ir(ppy)}_3]$, $[\text{Ir(ppz)}_3]$ has a luminescence quantum yield below 0.01 at room temperature due to efficient population of a non-emissive metal-centred excited state.\cite{39} Applying potentials of $-1.82$ V and $0.99$ V to a mixture of 6 μM $[\text{Ru(bpy)}_3]^{2+}$ and 60 μM $[\text{Ir(ppz)}_3]$ to generate $[\text{Ru(bpy)}_3]^+$, $[\text{Ru(bpy)}_3]^{3+}$ and $[\text{Ir(ppz)}_3]^+$, gave 59-fold (±2) ECL (integrated peak area) from $[\text{Ru(bpy)}_3]^{2+*}$, compared to that generated from $[\text{Ru(bpy)}_3]^{2+}$ in the absence of $[\text{Ir(ppz)}_3]$.

3.4.4 A Comparison of Mixed Metal Complex Annihilation ECL Systems

For a quantitative comparison of the three previously reported mixed metal-complex annihilation ECL systems, we examined the emission spectra of the Ru complex at a series of different concentrations (0.005 mM to 0.12 mM) in the presence and absence of the respective Ir complex (at 0.12 mM). We also tested the Ir complex (0.12 mM) in the absence of the Ru complex. For these experiments, a higher frequency electrochemical pulse (10 Hz) was used over the same acquisition time to enhance the ECL intensities.

For each system, potentials were selected to include both the first oxidation of each complex and the first reduction of each complex. In the $[\text{Ru(bpy)}_3]^{2+}$–$[\text{Ir(ppy)}_3]$ and $[\text{Ru(dtb-bpy)}_3]^{2+}$–$[\text{Ir(ppy)}_3](\text{CN})_2^-$ systems (Figure 3.5, AI.6a, AI.9 and AI.10), pulsing 0.1 V beyond the first reduction of the Ir complex will also reach the 2nd, 3rd and to
some extent the 4th reduction of the Ru complex. Whereas, in the [Ru(bpy)₃]²⁺–[Ir(df-ppy)₃(bpy)]⁺ system (Figure AI.5a), the first reduction potentials of the Ru and Ir complexes are similar.

The measured ECL spectra (Figure AI.11) were deconvoluted into the two characteristic emission bands (defined by the ECL spectrum of each individual complex at 0.12 mM) using the Solver function of Excel (for examples, see Figure 3.7 and AI.12). This enabled not only the comparison of the absolute ECL intensities of the Ru and Ir complexes within each system without interference (Figure AI.13), but also their ECL intensities relative to that of a standard solution for each complex (Figure 3.8).

Figure 3.7: The deconvolution of the annihilation ECL spectrum (black plot) from 0.03 mM [Ru(bpy)₃][(PF₆)₃] and 0.12 mM [Ir(ppy)₃] into the characteristic spectra of the two metal complexes (green and red plots, for which the spectral distributions were derived from the ECL of the individual complexes at 0.12 mM), using the Solver function of Microsoft Excel software. The ECL was generated using a two-step potential pulse (0.99 V and −2.77 V vs. Fe⁰/⁺) applied at 10 Hz for 12 s. Complexes were prepared in acetonitrile containing 0.1 M TBAPF₆. Additional examples are shown in Figure AI.12.
Figure 3.8: Annihilation ECL intensities from: (a) [Ru(bpy)$_3$][(PF$_6$)$_2$] and [Ir(ppy)$_3$]; (b) [Ru(dtbbpy)$_3$][(PF$_6$)$_2$] and [TBA][Ir(ppy)$_2$(CN)$_2$]; or (c)
[Ru(bpy)₃][PF₆]₂ and [Ir(df-ppy)₂(bpy)][PF₆], in acetonitrile containing 0.1 M TBAPF₆. The green plot is the ECL intensity of the Ir complex in the mixed solutions, relative to that of an individual standard of the Ir complex (0.12 mM) in the absence of the Ru complex. The red and grey plots are the ECL intensities of the Ru complex (from 0 mM to 0.12 mM) with and without the presence of 0.12 mM Ir complex, respectively, relative to that of an individual standard of the Ru complex (0.12 mM). The absolute ECL intensities are shown in Figure AI.13. In each case, a two-step potential pulse was applied at 10 Hz for 12 s. The applied potentials were: (a) 0.99 V, −2.77 V vs. Fe⁰/⁺, (b) 0.83 V, −2.81 V vs. Fe⁰/⁺, (c) 1.20 V, −1.82 V vs. Fe⁰/⁺. ECL spectra from each mixed system were deconvoluted into their two characteristic components (Figure AI.12).

Under these experimental conditions, we observed an efficient quenching of the annihilation ECL of [Ir(ppy)₃] in the presence of increasing concentrations of [Ru(bpy)₃]²⁺ (Figure 3.8a). However, this did not translate to an enhancement of the ECL of the ruthenium complex at all concentrations. The grey and red plots in Figure 3.8a show the ECL intensity of the [Ru(bpy)₃]³⁺ complex at various concentrations in the absence and presence of [Ir(ppy)₃], using the same applied potentials. At [Ru(bpy)₃]²⁺ concentrations of 0.06 mM and 0.12 mM, the ECL intensities of both [Ir(ppy)₃] and [Ru(bpy)₃]²⁺ were below that of the corresponding individual complex. It is possible that this quenching involves electron transfer from [Ir(ppy)₃] or from the reduced [Ir(ppy)₃]⁻ to [Ru(bpy)₃]²⁺*. Alternatively, the excitation pathway to the [Ru(bpy)₃]²⁺* occurring via the concomitant iridium system may be less efficient than that of the direct annihilation of [Ru(bpy)₃]³⁺ and [Ru(bpy)₃]⁺.

We observed similar trends for the [Ru(dtb-bpy)₃]²⁻-[Ir(ppy)₂(CN)₂]⁻ system (Figure 3.8b), which is not surprising, considering the similarity of their redox potentials (Figure AI.6). The quenching of the iridium complex in this system was far less efficient ($K_{SV} = 25$; Figure AI.14) than that of [Ir(ppy)₃] with [Ru(bpy)₃]²⁺ ($K_{SV} = 9.7 \times 10³$).
However, the ECL quantum yield of \([\text{Ir(ppy)}_2(\text{CN})_2]^-\) is far lower than that of \([\text{Ir(ppy)}_3]\), and the relative ECL intensities of the individual complexes at the same concentration (0.12 mM) under these conditions were found to increase in the order: 
\([\text{Ir(ppy)}_2(\text{CN})_2]^- \ll [\text{Ru(bpy)}_3]^{2+} < [\text{Ru(dtb-bpy)}_3]^{2+} \ll [\text{Ir(ppy)}_3]\) (Figure AI.13). Therefore, although the iridium complex is quenched less efficiently in the \([\text{Ru(dtb-bpy)}_3]^{2+} - [\text{Ir(ppy)}_2(\text{CN})_2]^-\) system, its contribution to the overall ECL emission is lower than that of the iridium complex of the \([\text{Ru(bpy)}_3]^{2+} - [\text{Ir(ppy)}_3]\) system (compare, for example, Figure AI.12b and AI.12d). A very minor contribution to the overall ECL emission was observed from the iridium luminophore in the \([\text{Ru(dtb-bpy)}_3]^{2+} - [\text{Ir(ppy)}_2(\text{CN})_2]^-\) system at a 1:2 concentration ratio (Figure AI.12h), and to an ever lesser extent in the same stoichiometric ratio in the \([\text{Ru(dtb-bpy)}_3][\text{Ir(ppy)}_2(\text{CN})_2]_2\) soft salt at 0.06 mM. In general agreement with the result of Swanick, et al., the ECL from these complexes at that concentration ratio arose almost entirely from the ruthenium component, but some \([\text{Ir(ppy)}_2(\text{CN})_2]^-*\) was formed at all concentration ratios examined in our study. It is possible that a minor emission from \([\text{Ir(ppy)}_2(\text{CN})_2]^-*\) was hidden in the noise of the ECL spectra obtained in that study, but it is also feasible that use of cyclic voltammetry to obtain ECL (rather than chronoamperometry) resulted in greater quenching of the iridium complex, and favoured the observed enhancement in ECL from the ruthenium complex.

In the case of the \([\text{Ru(bpy)}_3]^{2+} - [\text{Ir(df-ppy)}_2(\text{bpy})]^+\) system (Figure 3.8c), the quenching of the ECL from the iridium complex (\(K_{sv} = 125\)) was more efficient that observed in \([\text{Ru(dtb-bpy)}_3]^{2+} - [\text{Ir(ppy)}_2(\text{CN})_2]^-\) system, but still far less efficient than that of the \([\text{Ru(bpy)}_3]^{2+} - [\text{Ir(ppy)}_3]\) system. Moon, et al. observed emission from only the ruthenium complex of the \([\text{Ru(bpy)}_3]^{2+} - [\text{Ir(df-ppy)}_2(\text{bpy})]^+\) system, even at a stoichiometric ratio of 1:4. Under our conditions, we observed an emission from both
complexes at that ratio (Figure AI.12f) although as the concentration of the ruthenium complex was increased to a stoichiometric ratio of 1 : 1, the contribution from the iridium complex decreased to less than 5% of the integrated ECL spectrum. The mixed system produced considerably greater ECL from [Ru(bpy)$_3$]$^{2+}$ than that of the individual complex. At the same [Ir(ppy)$_2$(CN)$_2$]$^-$ concentration (0.12 mM), the degree of enhancement decreased with increasing concentration of [Ru(bpy)$_3$]$^{2+}$ (Figure 3.8c and AI.15). At stoichiometric ratios of 1 : 2 and 1 : 1, the enhancement was 2.0-fold and 1.7-fold, respectively, which was in reasonable agreement with the approximately 2-fold enhancement for a 3 : 2 mixture reported by Moon, et al.\textsuperscript{200}. 
3.5 Conclusions

The apparent differences in emission properties (i.e., luminescence observed from a single luminophore or a combination of two luminophores) in the preliminary explorations of annihilation ECL of mixed metal complexes can largely be ascribed to the relative concentrations of the two complexes used in the respective studies, a variable that until now has been largely overlooked. Other important factors include the relative ECL quantum yields (or relative intensities) of the individual and mixed annihilation ECL reactions, and the efficiency of various energy transfer pathways. In two previous publications, the observed energy transfer was tentatively attributed to a specific electron-transfer reaction, but it is likely to arise from a combination of several concomitant pathways that may include resonance energy transfer or electron transfer/exchange between the numerous oxidised, reduced, ground and/or excited states of the complexes within the mixed annihilation ECL system. The feasibility and relative efficiency of these pathways are dependent on the inherent electrochemical and photophysical characteristics of the metal complexes and the applied electrode potentials, which will need to be carefully considered to create annihilation ECL systems containing near-equimolar mixtures of metal complexes that are capable of simultaneous emissions from two distinct luminophores.

3.6 Acknowledgements

This research was funded by the Australian Research Council (DP160103046).
Chapter Four: Blue Electrogenerated Chemiluminescence from Water-Soluble Iridium Complexes Containing Sulfonated Phenylpyridine or Tetraethylene Glycol Derivatised Triazolylpyridine Ligands

Emily Kerr, a Egan H. Doeven, *a Gregory J. Barbante, a,b Timothy U. Connell, c Paul S. Donnelly, c David J. D. Wilson, d Trent D. Ashton, a Frederick M. Pfeffer, a and Paul S. Francis *a

*a Ms Emily Kerr, Dr Egan H. Doeven, Dr Gregory J. Barbante, Dr Trent D. Ashton, Dr Frederick M. Pfeffer, and Dr Paul S. Francis, Centre for Chemistry and Biotechnology, Deakin University, Geelong, Victoria 3220, Australia. E-mail: egan.doeven@deakin.edu.au; paul.francis@deakin.edu.au

b Current affiliation: University of Tasmania, Hobart, Tasmania 7001, Australia.

c Dr Timothy U. Connell, and Dr Paul S. Donnelly, School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Victoria 3010, Australia.

d Dr David J. D. Wilson, Department of Chemistry and La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria 3086, Australia.

**Authorship Statement**

1. **Details of publication and executive author**

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Electrogenated Chemiluminescence from Water-Soluble Iridium Complexes Containing Sulfonated Phenylpyridine or Tetraethylene Glycol Derivatized Triazolylpyridine Ligands</td>
<td><em>Chem. Eur. J.</em>, 2013, 19, 1487-1495</td>
</tr>
<tr>
<td></td>
<td>DOI: 10.1002/chem.201502037</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organisation and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul Francis</td>
<td>Centre for Chemistry and Biotechnology; School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td><a href="mailto:paul.francis@deakin.edu.au">paul.francis@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

2. **Inclusion of publication in a thesis**

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>Yes/No</th>
<th>If Yes, please complete Section 3 if No, go straight to Section 4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes/No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. **HDR thesis author’s declaration**

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above. (If the same, write “as above”)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emily Kerr</td>
<td>Centre for Chemistry and Biotechnology; School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td>Electrochemical and luminescence Studies of Ruthenium and Iridium Metal-Chelates</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication. (For example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically, etc.).

Designed and planned experiments with the assistance of supervisors. Conducted all electrochemical, spectroscopic and ECL experiments independently (with the exception of photographs and quantum yields which were collected with Egan Deoversen). Prepared and revised the appropriate sections of the manuscript (e.g. ECL, spectroscopy and electrochemistry sections).

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below. [Signature and date: Emily Kerr 14/12/16]

4. **Description of all author contributions**

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan J. Deoversen</td>
<td>Assisted with taking photographs of ECL and collecting quantum yield data. Assisted with planning experiments. Involved in conceptualisation of project and identification of target complexes. Assisted with revision of manuscript.</td>
</tr>
<tr>
<td>Gregory J. Barbante</td>
<td>Involved in conceptualisation of project and identification of target complexes. Assisted with planning experiments. Assisted with revision of manuscript.</td>
</tr>
<tr>
<td>Timothy U. Connolly</td>
<td>Undertook a portion of the chemical synthesis experiments and prepared relevant sections of the manuscript. Assisted with revision of manuscript, oversee aspects of chemical synthesis.</td>
</tr>
<tr>
<td>Paul S. Donnelly</td>
<td>Completed all DFT calculations and prepared the relevant section for publication. Contributed to intellectual content of the manuscript.</td>
</tr>
<tr>
<td>Trent D. Ashton</td>
<td>Design and execution of a portion of the chemical synthesis experiments and prepared relevant sections of the manuscript.</td>
</tr>
<tr>
<td>Frederick N. Pfeffer</td>
<td>Assisted with revision of manuscript, oversee aspects of chemical synthesis.</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td>Involved in conceptualisation of project. After receiving initial draft, completed the majority of revisions, contributed to intellectual content of manuscript and finalised submission to journal.</td>
</tr>
</tbody>
</table>
5. **Author Declarations**

I agree to be named as one of the authors of this work and confirm:

*1. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
2. that there are no other authors according to these criteria,
3. that the description in Section 4 of my contribution(s) to this publication is accurate,
4. that the date on which these findings are based are stated as set out in Section 7 below.*

If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further:

*5. consent to the incorporation of the publication into the candidate's HDR thesis submitted to Deakin University and, if the higher degree is awarded, the subsequent publication of the thesis by the university (subject to relevant copyright provisions).*

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan H. Dooven</td>
<td></td>
<td>15/12/20</td>
</tr>
<tr>
<td>Gregory J. Barbante</td>
<td></td>
<td>15/12/16</td>
</tr>
<tr>
<td>Timothy U. Connell</td>
<td></td>
<td>15/12/2016</td>
</tr>
<tr>
<td>Paul S. Donnelly</td>
<td></td>
<td>16/12/16</td>
</tr>
<tr>
<td>David J. Willson</td>
<td></td>
<td>15 Dec 2016</td>
</tr>
<tr>
<td>Trent D. Aspin</td>
<td></td>
<td>05/01/2017</td>
</tr>
<tr>
<td>Frederick N. Pfeffer</td>
<td></td>
<td>15th Dec 2016</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td></td>
<td>16/12/2016</td>
</tr>
</tbody>
</table>

6. **Other contributor declarations**

I agree to be named as a non-author contributor to this work.

<table>
<thead>
<tr>
<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
</table>

*If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author.*

7. **Data storage**

The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting. If the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB</td>
<td>WP Kc 5.127</td>
<td>19/02/2017</td>
<td>Paul Francis</td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based.

If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
4.1 Abstract

Incorporating phenylpyridine- and triazolopyridine-based ligands decorated with methylsulfonate or tetraethylene glycol (TEG) groups, we have created a series of iridium(III) complexes for green and blue electrogenerated chemiluminescence under analytically useful aqueous conditions, with tri-n-propylamine as a co-reactant. The relative ECL intensities of the complexes were dependent on the sensitivity of the photodetector over the wavelength range and the pulse-time of the applied electrochemical potential. In terms of the integrated area of corrected ECL spectra, using a pulse-time of 0.5 s, the intensities of the iridium complexes were between 18% and 102% that of [Ru(bpy)₃]²⁺. However, when the intensities were measured with a typical bialkali photomultiplier tube, the signal of the most effective blue emitter, [Ir(df-ppy)₂(pt-TEG)]⁺, was over 1200% that of the orange-red emitter [Ru(bpy)₃]²⁺. A combined experimental and theoretical investigation of the electrochemical and spectroscopic properties of the iridium(III) complexes indicated that the greater intensity from [Ir(df-ppy)₂(pt-TEG)]⁺ compared to the other iridium(III) complexes resulted from a combination of many factors, rather than being significantly favored in one area.
4.2 Introduction

There is great interest in cyclometalated iridium(III) complexes as alternatives to ruthenium(II) diimine chelates for luminescence-based analysis.\textsuperscript{13, 14, 145, 208} Preliminary explorations of their photoluminescence and electrochemiluminescence (ECL) properties, predominantly in organic solvents, have shown an impressive range of emission colors and/or superior luminescence efficiencies,\textsuperscript{36, 44, 46, 148, 149, 218-220} but the translation of these properties to real-world analytical applications has been hampered by the poor aqueous solubility of many available iridium(III) complexes.

Common strategies to increase the water solubility of cyclometalated iridium(III) complexes include the replacement of one ligand with either a neutral diimine (N\(^{\text{N}}\)) compound to impart an overall positive charge to the complex\textsuperscript{70, 221} or a derivative containing highly polar sulfonate\textsuperscript{52, 70, 72, 222} or saccharide groups\textsuperscript{48} (\textit{e.g.}, Figure 4.1: I-III). In these complexes, however, the LUMO energy is often largely determined by the ancillary N\(^{\text{N}}\) ligand,\textsuperscript{52, 72, 222} resulting in a significantly red-shifted emission. This effect can be counteracted by introducing electron withdrawing fluorine groups on the cyclometalating rings (to stabilize the HOMO level),\textsuperscript{208} but this also increases the hydrophobicity of the complex.\textsuperscript{223} Alternatively, the use of a 1,2,3-triazol-4-ylpyridine species as the ancillary ligand can impart an even greater emission energy than the corresponding homoleptic cyclometalated complexes ([Ir(ppy)]\(_3\)) or [Ir(df-ppy)]\(_3\)], which has been used to create cationic iridium(III) complexes that exhibit green or blue ECL (Figure 4.1: IV, V).\textsuperscript{36, 47, 209} We recently examined the blue chemiluminescence from an overall-neutral iridium(III) complex with an ancillary 1,2,3-triazol-4-ylpyridine ligand with a sulfonate substituent (Figure 4.1: VI), under aqueous conditions.\textsuperscript{71} In an alternative approach, Zanarini, \textit{et al.}\textsuperscript{77} doped hydrophobic
neutral iridium(III) complexes into silica nanoparticles with a hydrophilic polyethylene glycol outer shell for ECL in aqueous media.\textsuperscript{77}

\textbf{Figure 4.1:} Various iridium(III) complexes previously examined for photoluminescence, chemiluminescence and/or ECL detection: (I) (2,2′-bipyridine-κN\textsuperscript{1},κN\textsuperscript{1′})bis[2-(2-pyridinyl-κN)phenyl-κC]iridium(1+)\textsuperscript{70} (IIa, R = H) [[3,3′-(1,10-phenanthroline-4,7-diyl-κN,κN10)bis[benzenesulfonato]](2-)bis[2-(2-pyridinyl-κN)phenyl-κC]iridate(1-);\textsuperscript{70-72} (IIb, R = F) bis[3,5-difluoro-2-(2-pyridinyl-κN)phenyl-κC][[(1,10-phenanthroline-4,7-diyl-κN\textsuperscript{1},κN\textsuperscript{10})bis[benzenesulfonato]](2-)iridate(1-);\textsuperscript{72, 223} (III) [[[2,2′-bipyridine]-4,4′-diyl-κN\textsuperscript{1},κN\textsuperscript{1′}]bis(methylene) bis[1-thio-β-D-glucopyranoside]]bis[2-(2-pyridinyl-κN)phenyl-κC]iridium(1+);\textsuperscript{78} (IV) [2-(1-methyl-1H-1,2,3-triazol-4-yl-κN\textsuperscript{3})pyridine-κN]bis[2-(2-pyridinyl-κN)phenyl-κC]iridium(1+);\textsuperscript{47} (V) bis[3,5-difluoro-2-(2-pyridinyl-κN)phenyl-κC][2-[1-(phenylmethyl)-1H-1,2,3-triazol-4-yl-κN\textsuperscript{3}]pyridine-κN]iridium(1+);\textsuperscript{36, 209} and (VI) bis[3,5-difluoro-2-(2-pyridinyl-κN)phenyl-κC][2-[1-(phenyl-4-sulfonate)-1H-1,2,3-triazol-4-yl-κN\textsuperscript{3}]pyridine-κN]iridium.\textsuperscript{71}
Herein we examine the ECL of various iridium(III) complexes that combine strategies to impart water solubility with the manipulation of emission wavelength towards the blue end of the visible spectrum. To avoid the bathochromic influence of the ancillary diimine ligand (e.g. in II and III), we have utilized triazolylpyridines, in some cases in conjunction with electron-withdrawing fluorine groups on the phenyl ring of the ppy ligands to maximize the HOMO-LUMO gap. To create water-soluble complexes incorporating this functionality, we have utilized sulfonated phenylpyridines (in Figure 4.2: VII-IX) and tetraethylene glycol (TEG) derivatised triazolylpyridines (in VIII, X). The electrochemical, photoluminescence and ECL properties of these complexes are compared with those of the widely used [Ru(bpy)$_3$]$^{2+}$ species, in addition to two previously reported complexes containing a bathophenanthroline-disulfonate (BPS) ligand (Figure 4.1: IIa (R = H) and IIb (R = F)).

Figure 4.2: Water soluble iridium(III) complexes containing sulfonate groups on the two phenylpyridine-based ligands and/or a TEG group on the triazolylpyridine ligand.
4.3 Experimental Section

4.3.1 Absorption and Photoluminescence Emission Spectra.

UV-visible absorption and photoluminescence spectra were collected at a complex concentration of 10 µM in Milli-Q water. UV-Visible absorption spectra were collected using 1 cm path length quartz cells with a Cary 300 Bio UV/Vis spectrophotometer (Varian Australia, Mulgrave, Vic., Australia). Photoluminescence spectra were collected using a Cary Eclipse Spectrofluorimeter (Varian Australia), with a 1 cm quartz cuvette (excitation set to 380 nm, 5 nm bandpass, 1 nm data interval, PMT voltage: 800 V). Correction factors for emission spectra were established using a spectral irradiance standard (Optronic Laboratories, model OL 245m) with constant current source (model OL 65A).

Absolute quantum yields and CIE data were obtained using a Horiba JY Nanolog 3 fluorescence spectrophotometer equipped with TBX Picosecond photon detection module/Symphony II (1LS-256-OE) LN2 cooled CCD detectors, iHR-320 emission monocromator (100 g mm\(^{-1}\) grating), 450 W xeon arc (1200 g mm\(^{-1}\) grating) and NanoLED excitation sources and Fluorohub single-photon counting controller. Samples were prepared at 10 µM and absolute quantum yields (average of three replicates) and CIE data were collected using the xeon arc lamp as the excitation source at 350 or 400 nm, 2.5 nm bandpass, a 150 mm QuantaPhi integrating sphere and the Symphony II LN2 cooled CCD detector and calculated using the supplied Fluorescence (Horiba JY) software.

4.3.2 Electrochemistry and ECL.

Complex stock solutions (1 mM or 0.25 mM) were prepared in either 0.1 M phosphate buffer solution adjusted to pH 7.5 using KOH or 50:50 ACN:(0.1 M phosphate buffer solution, adjusted to pH 7.5 using KOH) depending on the solubility
of the complex, and diluted to appropriate concentrations with phosphate buffer prior to analysis. Experiments were performed using an Autolab PGSTAT 101 or PGSTAT 128N potentiostat (Metrohm Autolab B.V., Netherlands). A custom built light-tight Faraday cage enclosed the cylindrical glass cell with quartz window base and Teflon cover with spill tray. A conventional three electrode configuration consisting of glassy carbon working electrode (3 mm diameter) shrouded in Teflon (CH Instruments, Austin, TX, USA), Ag/AgCl reference electrode and platinum wire counter electrode was used throughout.

Oxidation potentials of the complexes were determined using square wave voltammetry (0.005 V step, 0.02 V amplitude, 25 Hz), at a complex concentration of 0.5 mM in 0.1 M phosphate buffer, adjusted to pH 7.5 using 1 M KOH. All potentials quoted are in reference to Ag/AgCl. ECL spectra were collected using an Ocean Optics CCD, model QE65pro.

A collimating lens, custom cell holder and optic fiber (1.0 m, 1.0 mm core diameter) were used to interface the electrochemical cell with the CCD detector and each acquisition was triggered using the potentiostat in conjunction with a HR 4000 Break-Out box. Complex concentrations were 10 µM for relative ECL intensities, and 10 or 50 µM for representative ECL spectra, in 0.1 M phosphate buffer, adjusted to pH 7.5 using 1 M KOH, with a co-reactant concentration of 10 mM TPrA. Prior to collection of each ECL spectra, the platinum counter electrode was flamed and the glassy carbon working electrode was polished on 0.3 µm and then 0.05 µm alumina powder with water on a felt pad. The electrode was rinsed in acetone and sonicated in water (1 min), and then positioned 2 mm from the bottom of the quartz windowed cell. ECL spectra were collected using a 0.5 s or 5 s pulse to 100 mV past the oxidation potential of the
complex, for relative ECL and representative ECL spectra respectively. Relative ECL intensities were calculated from the integrated peak area of two replicates.

4.3.3 Synthesis and Characterisation.

$^1$H NMR spectra were recorded at 500 MHz and $^{13}$C$[^1]$H NMR spectra were collected at 125.7 MHz on a Varian FT-NMR 500 spectrometer (Varian, California, USA). All chemical shifts are referenced to residual solvent peaks and are quoted in ppm relative to TMS. ESI-MS was recorded on an Agilent 6510 ESI-TOF LC/MS mass spectrometer (Agilent, California, USA). HPLC traces were acquired using an Agilent 1200 Series HPLC system with an SGE Analytical Science ProteCol C18 HPH125 120 Å column (4.6 × 150 mm, 5 μm) (Trajan, Ringwood, Australia), a gradient elution of H$_2$O-CH$_3$CN/0.1% trifluoroacetic acid, 0-40% CH$_3$CN, a 1 mL min$^{-1}$ flow rate over 28 min, and were monitored at $\lambda = 220, 254, 280$ and 320 nm. Full details of the synthesis and characterization are included in Appendix II.

4.3.4 Computational Methods.

Density functional theory (DFT) calculations were carried out within the Gaussian 09 suite of programs.$^{225}$ Ground state geometries were optimized in the absence of solvent with the mPW1PW91$^{226, 227}$ functional in conjunction with the def2-SVP basis set and associated core potential.$^{228}$ The mPW1PW91 functional has previously been demonstrated to yield reliable results for such systems.$^{203, 229}$ All structures are characterized as minima with no imaginary frequencies. Single-point energy calculations were carried with the def2-TZVP basis set and core potential.$^{228}$

The polarizable continuum model (PCM)$^{230}$ self-consistent reaction field (SCRF) was used to model solvent effects at the gas-phase optimized geometries with a solvent of water, consistent with the experimental system. Equivalent calculations with
acetonitrile solvent produced very similar results and are subsequently not discussed in the manuscript. Frontier MO energies were calculated using DFT MOs with mPW1PW91, PBE, B3LYP, BP86, and wB97XD. An SCF convergence criteria of $10^{-8}$ a.u. was employed throughout.
4.4 Results and Discussion

4.4.1 Design and Preparation of Iridium(III) Complexes

Previous approaches to introduce sulfonate groups into iridium(III) complexes for chemiluminescence or ECL detection to improve their aqueous solubility have predominantly focused on modification of the ‘ancillary’ ligand,\textsuperscript{71, 72, 223} introduced into the heteroleptic iridium(III) complex through reaction with a dichloro-bridged cyclometalated iridium dimer (e.g. [Ir(ppy)\textsubscript{2}(\mu-Cl)]\textsubscript{2}). The commercially available BPS ligand is a convenient option as a sulfonated ancillary ligand,\textsuperscript{72, 223} but the resulting iridium(III) complexes exhibit considerably lower ECL intensities than [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}.\textsuperscript{72} For example, \textit{IIa} and \textit{IIb} gave 14\% and 2\% the intensity of [Ru(bpy)\textsubscript{3}]\textsuperscript{2+}, respectively, when using tri-n-propylamine (TPrA) as co-reactant in buffered aqueous solution, with a 10 s integration time. Moreover, the inclusion of this ancillary ligand exerts a significant bathochromic shift compared to the homoleptic cyclometalated complex,\textsuperscript{71, 223} limiting the scope for short-wavelength ECL emitters based on this approach.

We previously prepared an iridium(III) complex with an ancillary sulfonated triazolopyridine-based ligand (\textit{VI}).\textsuperscript{71} Initial attempts to synthesize the sulfonated ligand and then form the complex were ineffective, due to the difficulty in purifying the products. Nevertheless, the target was obtained by first preparing the analogous thiol ligand, then forming the iridium(III) complex, before oxidizing the thiol to the sulfonate (\textit{VI}).\textsuperscript{71} Although the monosulfonate complex exhibited the desired blue luminescence, it was less soluble than \textit{IIb}; indeed, the maximum concentration of \textit{VI} in aqueous solution was \textasciitilde 10 \textmu M.
We have devised new strategies to improve the aqueous solubility of iridium(III) complexes that exhibit short-wavelength (green and blue) emissions for ECL detection, including the introduction of sulfonate groups on the phenylpyridine-based cyclometalating ligands and/or the addition of a TEG group on the ancillary triazolylpyridine ligand. We used the commercially available sulfonated phenylpyridine (1, Figure 4.3) to first form the dichloro-bridged iridium(III) dimer (2), and then the heteroleptic complexes VII and VIII by introducing a triazolylpyridine ligand with benzyl (3a) or TEG (3b) substituent. The peripheral functional groups were electronically isolated from the central complex by methylene spacers to minimize their influence on the electronic and spectroscopic properties of the complex. Based on previous studies with non-sulfonated analogues and our computational evidence (discussed below), we anticipated these complexes to exhibit green luminescence, which could be blue-shifted by incorporating fluorine groups on the phenyl rings of the phenylpyridine ligands. However, the corresponding sulfonated difluoro-phenylpyridine ligand was not commercially available, so we pursued two pathways to prepare water-soluble complexes for blue ECL. Firstly, ligand 3b was added to the conventional [Ir(df-ppy)$_2$(μ-Cl)]$_2$ dimer to form complex IX, which contained a TEG group, but no sulfonate functionality. Secondly, a sulfonate was introduced onto the pyridine group of the difluoro-phenylpyridine ligands. To avoid difficulties in separating sulfonated products and precursors, we prepared dichloro-bridged iridium(III) dimer 5 from the hydroxyl analogue 4 (Figure 4.4). After forming the heteroleptic complex XI with ligand 3a, the hydroxyl groups were converted to sulfonates via the chloro species to form the target complex X.
The addition of the TEG group was the most efficient strategy for improving the solubility in water. Complexes VIII and IX containing the TEG-functionalised ligand dissolved readily at 1 mM in the aqueous phosphate buffer solution (PBS). Complexes VII and X containing sulfonated-phenylpyridine ligands were prepared at 1 mM in a
1:1 mixture of PBS:acetonitrile, whereas complexes IIa and IIb, containing the sulfonated diphenylphenanthroline ligand, were prepared at 0.25 mM in 1:1 PBS:acetonitrile. Each of these stock solutions was then diluted to the desired concentration in the aqueous PBS.

4.4.2 Electronic Spectroscopy

The absorption spectrum of each iridium(III) complex exhibited characteristic intense $\pi$-$\pi^*$ ligand centered transitions (240-300 nm), and weak $d$-$\pi^*$ metal-to-ligand transitions above 300 nm (Table 1; and Figure AII.1).\textsuperscript{239, 240} In the photoluminescence spectra (Figure AII.2), a significant, consistent hypsochromic shift was observed from the non-fluorinated complexes to their fluorinated analogues due to HOMO stabilization (where the electron withdrawing nature of the fluorine substituent causes a decrease in the Coulombic repulsion charges of the phenyl substituent, increasing the energy required to remove an electron from the HOMO).\textsuperscript{241} Complexes IIb, X and IX exhibited maximum photoluminescence intensities at wavelengths 77, 27 and 29 nm shorter than their similar non-fluorinated counterparts, IIa, VII and VIII, respectively (Table 1).
Table 1. Electrochemical and spectroscopic properties of [Ru(bpy)_3]^{2+} (Ru), [Ir(ppy)_2(BPS)]^- (IIa), [Ir(df-ppy)_2(BPS)]^- (IIb), [Ir(ppy-SO_3)_2(ptb)]^- (VII), [Ir(ppy-SO_3)_2(pt-TEG)]^- (VIII), [Ir(df-ppy)_2(pt-TEG)]^+ (IX), and [Ir(df-ppy-SO_3)_2(ptb)]^- (X).

<table>
<thead>
<tr>
<th></th>
<th>E^0 vs Ag/AgCl</th>
<th>Absorbance</th>
<th>Photoluminescence</th>
<th>ECL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E^0 /V</td>
<td>λ_{max}/nm</td>
<td>λ_{max}/nm</td>
<td>Φ_p^{[a]}</td>
</tr>
<tr>
<td>Ru</td>
<td>1.07</td>
<td>244, 255, 287, 420^{[b]}, 460^{[b]}</td>
<td>622</td>
<td>3.9 (4.0^{[c]})</td>
</tr>
<tr>
<td>IIa</td>
<td>1.16</td>
<td>256, 268, 283, 380^{[b]}</td>
<td>634</td>
<td>1.9</td>
</tr>
<tr>
<td>IIb</td>
<td>1.52</td>
<td>291, 374^{[b]}</td>
<td>557</td>
<td>12.8</td>
</tr>
<tr>
<td>VII</td>
<td>1.09</td>
<td>253, 268, 390^{[b]}</td>
<td>482, 512, 557^{[s]}</td>
<td>6.7</td>
</tr>
<tr>
<td>VIII</td>
<td>1.09</td>
<td>254, 267, 390^{[b]}</td>
<td>482, 512, 557^{[s]}</td>
<td>9.9</td>
</tr>
<tr>
<td>IX</td>
<td>1.43</td>
<td>246, 362^{[b]}</td>
<td>456, 483, 521^{[s]}</td>
<td>8.4</td>
</tr>
<tr>
<td>X</td>
<td>1.47</td>
<td>252, 368^{[b]}</td>
<td>462, 489, 528^{[s]}</td>
<td>12.8</td>
</tr>
</tbody>
</table>

^{[a]} Room temperature aqueous solution (not degassed). ^{[b]} Broad. ^{[c]} Reported by Suzuki and co-workers.^{242} ^{[s]} Shoulder.

The replacement of the benzyl substituent on the triazolylpyridine ligand of complex VII with a TEG group (in complex VIII) caused only a very minor (4 nm) change in the emission wavelength, because the first oxygen of the TEG group is electronically separated from the complex by two methylene spacers. Similarly, the addition of methylsulfonate groups on the difluorophenylpyridine ligands did not have a significant effect. The ability to manipulate the solubility of iridium(III) complexes without significant alteration of the HOMO or LUMO and hence, emission properties, is useful for any future development of water-soluble metal chelates with emissions spanning the visible spectrum. Complexes containing a triazolylpyridine ligand...
exhibited structured photoluminescence spectra, in agreement with previous observations.\textsuperscript{36, 47}

### 4.4.3 Electrochemistry

The oxidation potential of each complex (Table 1) was determined using square wave voltammetry. Complexes containing cyclometalated df-ppy ligands (\textit{IIb}, \textit{X} and \textit{IX}) exhibited higher oxidation potentials (by 340-380 mV) than the related complexes with two ppy ligands (\textit{IIa}, \textit{VII} and \textit{VIII}, respectively). We observed reversible electrochemistry for complexes \textit{IIa}, \textit{VII} and \textit{VIII}, in the aqueous phosphate buffer. However, the oxidation potentials of complexes \textit{IIb}, \textit{IX} and \textit{X} were high in relation to electrolysis of the solvent and electrochemical properties were only clearly discernible through square wave voltammetry. In accordance with photoluminescence results, the addition of TEG or sulfonate groups with methylene spacers did not strongly influence the electrochemical behavior of the complex; complexes \textit{VII} and \textit{VIII} both had near identical oxidation potentials, and those of complexes \textit{IX} and \textit{X} were 40 mV apart.

### 4.4.4 Theoretical Calculations

Density functional theory (DFT) calculations were employed to examine the electronic structure of each complex. The frontier MOs of [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} are already well-characterized,\textsuperscript{71, 189} with a HOMO that is predominantly metal-centered and a LUMO that is distributed equally amongst the three bipyridine ligands. The triplet-state spin density shares the same spatial extent as the singlet HOMO and LUMO. Both results support the description of the lowest excited state being labelled as metal-to-ligand charge transfer (MLCT).
Analysis of the HOMO and LUMO of \([\text{Ir(df-ppy)}_2\text{(BPS)}]^+\) (IIb) and \([\text{Ir(ppy-SO}_3)_2\text{(pt-TEG)}]\) (VIII) (Figure 4.5) is illustrative of the results for the iridium(III) complexes considered here (frontier MO and triplet spin density surfaces for all complexes are included in Table S1). The singlet HOMO of the IIb is principally composed of the iridium d and the phenyl \(\pi\) orbitals, distributed equally across the ppy ligands (i.e., metal and ligand based). The LUMO is localized on the phenanthroline component of the ancillary BPS ligand (i.e., ligand based). The triplet-state spin density shares the same spatial extent as the singlet-state HOMO and LUMO. Taken together, this suggests that the lowest excited state may be attributed to a mixture of MLCT and LLCT (i.e., metal-ligand-to-ligand CT, MLLCT). The frontier MOs of IIa are almost identical to that of IIb. For \([\text{Ir(ppy-SO}_3)_2\text{(pt-TEG)}]\) (VIII), the HOMO is similarly composed of a mixture of the iridium d and phenyl \(\pi\) orbitals of the phenylpyridine ligands, and the LUMO is localized on the TEG derivatised ptb-based ligand. The triplet state spin density surface shares the same spatial extent as the singlet LUMO and HOMO, for which the lowest excited state is also characterized as MLLCT.
Figure 4.5: Contour plots for the HOMO and LUMO of (a) [Ir(dfppy)$_2$(BPS)]$^+$ (IIb) and (b) [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^-$ (VIII). BP86/def2-TZVP//mPW1PW91/ def2-SVP with water solvent (SCRF, IEFPCM).

Mulliken population analysis of fragment contributions (metal, ppy-based ligands and ancillary BPS/ptb-based ligands) to the HOMO and LUMO (Figure 4.6) enables a comparison of the electronic structure of these iridium(III) complexes. In each case, the metal contributes 39-46% of the HOMO while the phenyl (π) ring of the phenylpyridine ligand contributes 50-58%. The LUMO is almost exclusively composed of the ancillary ligand for IIa, IIb and IX (~95%), while for VII, VIII and X the LUMO has a slightly smaller ancillary ligand contribution (74-82%). Fragment populations are consistent with a MLLCT description of the lowest excited state for the iridium(III) complexes. In comparison, the HOMO of [Ru(bpy)$_3$]$^{2+}$ has a metal d orbital contribution of 82% while the LUMO has a 99% contribution from the bpy ligand, which supports a MLCT description of the excited state.
Figure 4.6: Contribution to (a) LUMO and (b) HOMO of metal centre and ligands in [Ru(bpy)$_3$]$_2^{2+}$ (Ru), [Ir(ppy)$_2$(BPS)]$^+$ (IIa), [Ir(df-ppy)$_2$(BPS)]$^+$ (IIb), [Ir(ppy-SO$_3$)$_2$(ptb)]$^+$ (VII), [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^+$ (VIII), [Ir(df-ppy)$_2$(pt-TEG)]$^+$ (IX) and [Ir(df-ppy-SO$_3$)$_2$(ptb)]$^+$ (X) (BP86/def2-TZVP calculations).

For the iridium(III) complexes under investigation, there is very little overlap between the singlet-state HOMO and LUMO (i.e., they are largely orthogonal) and the triplet spin density surface shares the same spatial extent as the singlet LUMO and HOMO, which indicates that the HOMO and LUMO energies can be independently ‘tuned’ by appropriate substitution of donor/acceptor groups. The exception is X, for
which there is some overlap of the HOMO and LUMO, and the triplet-state spin density has negligible contribution on the ancillary ptb-based ligand, which is where the LUMO is centered.

Calculated MO energies are illustrated in Figure 4.7, and while the absolute MO energies (and HOMO-LUMO gaps) are quite dependent on DFT method, the trend across the series of complexes is independent of method. The BP86/def2-TZVP calculated HOMO-LUMO gaps (1.8-2.5 eV) are significantly smaller than equivalent mPW1PW91 (3.5-4.3 eV), B3LYP (3.1-3.9 eV) and PBE (3.5-4.3 eV) results. The BP86 results align closest with experimental electrochemistry results (see above) and also yield the smallest degree of spin-contamination in the oxidized and reduced forms of the complexes (see below), which matches previous results.36

Figure 4.7: Comparison of MO energies (left axis) and electrochemical properties (right axis) for: [Ru(bpy)₃]²⁺ (Ru), [Ir(ppy-SO₃)(ptb)]⁻ (VII), [Ir(ppy-SO₃)(pt-TEG)]⁻ (VIII), [Ir(ppy)(BPS)]⁻ (IIa), [Ir(df-ppy)(pt-TEG)]⁺ (IX) and [Ir(df-ppy-SO₃)(ptb)]⁺ (X), and [Ir(df-ppy)(BPS)]⁺ (IIb) (BP86/def2-TZVP calculations).

The hypsochromic shift observed in the photoluminescence spectra from the non-fluorinated complexes to their fluorinated analogues (IIa to IIb; VII to X; and VIII to IX) due to HOMO stabilization is consistent with calculated MO energies. The
fluorinated analogues have HOMO energies 0.2-0.5 eV lower in energy than the respective non-fluorinated complex, whereas the LUMO energies are almost identical for the IIa-IIb and X-VII pairs with the LUMO of fluorinated IX being stabilized relative to non-fluorinated VIII by 0.35 eV. The result is that the HOMO-LUMO gap is consistently larger for the fluorinated analogues by 0.2-0.5 eV (with shorter wavelength photoluminescence).

4.4.5 Electrogenerated Chemiluminescence

Each metal complex was investigated under oxidative potentials with TPrA as co-reactant in an aqueous phosphate buffer (pH 7.5). We visually observed the ECL from each complex at the electrode surface as moderate to intense emissions, including green and blue ECL (Figure 4.8). The bluest and brightest emission was observed from complex IX. In each case, the spectral distribution of the ECL (Figure 4.9) was found to match that of the photoluminescence (Table 1).

**Figure 4.8:** Photographs of ECL of [Ru(bpy)]$_2^{2+}$ and the Ir$^{III}$ complexes at the working electrode surface (left to right): [Ru(bpy)]$_2^{2+}$, [Ir(ppy)$_2$(BPS)]$^+$ (IIa), [Ir(df-ppy)$_2$(BPS)]$^+$ (IIb), [Ir(ppy-SO$_3$)$_2$(ptb)]$^-$ (VII), [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^-$ (VIII), [Ir(df-ppy)$_2$(pt-TEG)]$^+$ (IX) and [Ir(df-ppy-SO$_3$)$_2$(ptb)]$^+$ (X). The complexes were prepared at 10 $\mu$M in 0.1 M phosphate buffer solution with 10 mM TPrA as co-reactant. ECL was generated by pulsing 100 mV over the oxidation potentials of each complex for 0.5 s. Images were captured with a Canon 6D camera (Canon Inc., Japan) and 100 mm Tamron F2.8 macro lens (Tamron Inc., Saitama City, Japan). Exposure details: F2.8, ISO 3200, 4 s.
Figure 4.9: ECL spectra of \([\text{Ir(ppy)}_2(\text{BPS})]^+ (\text{IIa}), [\text{Ir(df-ppy)}_2(\text{BPS})]^+ (\text{IIb}), [\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+ (\text{IX}), [\text{Ir(ppy-SO}_3\text{)}_2(\text{pt-TEG})]^+ (\text{VIII}), [\text{Ir(df-ppy-SO}_3\text{)}_2(\text{ptb})]^+ (\text{X}), \) and \([\text{Ir(ppy-SO}_3\text{)}_2(\text{ptb})]^+ (\text{VII}).\)

Relative ECL intensities, in terms of the integrated area of the corrected ECL spectra, were comparable to \([\text{Ru(bpy)}_3]^{2+}\), decreasing in the order: \text{IX} (102%) > \text{IIb} (82%) > \text{VIII} (47%) > \text{VII} (35%) > \text{IIa} (30%) > \text{X} (18%). However, the relative intensities of some complexes were dependent on the applied potential pulse-time. For example, at a pulse-time of 5 s, the intensity of complex \text{IX} was only 48% that of \([\text{Ru(bpy)}_3]^{2+}\), but as the pulse-time was decreased to 0.1 s, the relative ECL intensity of \text{IX} increased to 221% that of the ruthenium complex (Figure AII.3). A similar trend was observed for the other blue ECL complex \text{X}, but the intensities of the green emitters \text{VII} and \text{VIII} remained at approximately the same proportion of that of
[Ru(bpy)₃]²⁺ irrespective of the timespan of the applied pulse (0.1 to 5.0 s). These effects are most likely due to increased passivation of the electrode surface and electrolysis of the solvent (leading to quenching by molecular oxygen) at the high electrochemical potentials required to oxidize IX and X, compared to VII, VIII and [Ru(bpy)₃]²⁺.

To rationalize the relative ECL intensities of the iridium(III) complexes, several fundamental parameters should be considered. The reaction pathway can be summarized as follows, in which M is the metal complex electrochemiluminophore, TPrA⁺⁺ is the aminium radical cation of the co-reactant, and TPrA* is the corresponding a-aminoalkyl carbon-centered radical (Pr₂NC’CHCH₂CH₃).²⁴¹ The product of reactions (5 and 6) has been identified as Pr₂N’C=HCH₂CH₃, which hydrolysates to form Pr₂NH and CH₃CH₂CH₂CHO.²⁴³

(1) \( M - e^- \rightarrow M^+ \) (oxidation)
(2) \( TPrA - e^- \rightarrow TPrA^{*+} \) (oxidation)
(3) \( M^+ + TPrA \rightarrow M + TPrA^{*+} \) (electron transfer)
(4) \( TPrA^{*+} \rightarrow TPrA^* + H^+ \) (deprotonation)
(5) \( M^+ + TPrA^* \rightarrow M^* + \text{other products} \) (excited state)
(6) \( M^+ + TPrA^* \rightarrow M + \text{other products} \) (ground state)
(7) \( M^* \rightarrow M + h\nu \) (emission)

The strong reductant TPrA* (\( E^o \approx -1.7 \text{ V vs Ag/AgCl} \))¹⁷ may also generate the corresponding M⁻ species:

(8) \( M + TPrA^* \rightarrow M^- + \text{other products} \) (electron transfer)
(9) \( M^- + M^+ \rightarrow M^* + M \) (excited state)
(10) \( M^- + TPrA^{*+} \rightarrow M^* + TPrA \) (excited state)
Intense co-reactant ECL with TPrA could be expected from complexes that have: (i) suitable redox potentials to generate the excited state emitting species; (ii) sufficient stability in their relevant oxidation states; and (iii) high luminescence quantum yields.

Firstly, with respect to factor (i), the ECL intensity of each complex was obtained at an applied potential sufficient to oxidize the metal complex (reaction 1), which was well beyond that required to oxidize the co-reactant (reaction 2; $E^o \approx 0.83$ to 0.95 V vs Ag/AgCl).\textsuperscript{17} Reaction 3 is therefore energetically feasible, and can enable more efficient generation of TPrA$^+\cdot$\textsuperscript{44} but this reaction plays only a minor role when the concentration of the metal complex is low.\textsuperscript{244} The reaction of the oxidized metal complex with TPrA$^-$ requires sufficient excess energy to not only attain the excited state (reaction 5), but also kinetically inhibit the formation of ground state (reaction 6). This relationship, which can be expressed as Eqn I (where $E^o(TPrA^-)$ is the reduction potential of the radical),\textsuperscript{50} means that shorter wavelength (i.e., blue-shifted) ECL places a greater demand on the oxidation potential of the metal complex.

\begin{align*}
\text{I.} \quad \Delta G &= E^o(TPrA^-) - E^o_{ox} + E_{MLCT}
\end{align*}

The position of each metal complex with respect to the energy required for reactions 3 and 5 is depicted in Figure 4.10. This shows that in each case under investigation, there is sufficient energy for both of these reactions. Moreover, it illustrates one of the difficulties in devising metal complexes capable of efficient deep-blue ECL: the increasingly limited window between the ‘wall of energy sufficiency’ for reaction 5 and the oxidation boundary of the aqueous solvent. We could not directly reduce the complexes at the electrode under aqueous conditions, but the potential for [Ru(bpy)$_3$]$^{1+/2+}$ is well known\textsuperscript{17} and the reduction potential for complexes VII, VIII, IX and X can be reasonably estimated from those of their analogues without
methylsulfonate or TEG groups in acetonitrile. Based on this data and reactions 2 and 3, reaction 9 would be favorable for each of these iridium(III) complexes, and reaction 10 might be feasible for complexes VII and VIII. However, these reactions depend on the formation of the reduced M’ species through reaction 7. Unlike the [Ru(bpy)₃]²⁺ system, this is not energetically feasible for VII and VIII. Based on the quasi-reversible reduction potential of [Ir(df-ppy)₂(ptb)]⁺ of -2.14 V vs Fe⁰/⁺ in acetonitrile (where TPrA⁺ is approximately -2.1 V vs Fe⁰/⁺), complexes IX and X could be anticipated to be borderline cases in aqueous solution. The co-reactant ECL of the green and blue emitting iridium(III) complexes with TPrA in aqueous solution can therefore be considered to predominantly comprise reactions 1-6.

II. \[ \Delta G = E^0(M^-) - E^0(M^{+}) + E_{MLCT} \]

III. \[ \Delta G = E^0(M^-) - E^0(TPrA^{++}) + E_{MLCT} \]

Figure 4.10: Oxidation potentials vs the wavelengths of maximum ECL intensity (at room temperature), indicating that there is sufficient energy in each system under investigation for both reaction 5 and reaction 3.
From a molecular orbital perspective, ECL is favored under these circumstances by stabilization of the H(S)OMO of the M⁺, which accepts an electron from the HOMO level of TPrA in reaction 3, and stabilization of the LUMO level of M⁺, which accepts an electron from TPrA* in reaction 5. We previously observed, for a series of related iridium(III) complexes in acetonitrile solvent, a correlation between M⁺ LUMO stabilization and relative ECL intensity. Moreover, the iridium(III) complex (M) LUMO levels were a good predictor of the M⁺ LUMO energies (although the absolute scale of the energies varied significantly with the DFT functional). The relative ECL intensities of the complexes under investigation, however, showed a much poorer correlation with LUMO stabilization (Figure 4.11). Only BP86 results are presented, as this functional was least affected by spin contamination in the unrestricted calculations.

**Figure 4.11**: Comparison of the calculated LUMO energies (BP86/def2-TZVP) for the complexes (M) and oxidised complexes (M⁺) compared to the relative ECL intensity using TPrA as co-reactant, for: [Ir(ppy-SO₃)₂(ptb)]⁺ (VII), [Ir(ppy-SO₃)₂(pt-TEG)]⁺ (VIII), [Ir(df-ppy-SO₃)₂(ptb)]⁺ (X), [Ir(df-ppy₂)(pt-TEG)]⁺ (IX), [Ir(ppy₂)(BPS)]⁺ (IIa), [Ir(df-ppy₂)(BPS)]⁺ (IIb) and [Ru(bpy)₃]²⁺ (Ru).
With respect to factor (ii), the stability of the oxidized (M⁺) species can to some extent be ascertained by the reversibility of the M/M⁺ redox couple in the corresponding cyclic voltammogram. As noted earlier, the oxidation of IIa, VII and VIII (and [Ru(bpy)₃]²⁺), in the aqueous phosphate buffer was reversible, but the potentials of the other complexes, IIb, IX and X, were only clearly discernible through square wave voltammetry as they bordered the potential limit of the aqueous solvent (Figure 4.10). Whilst reducing the stability of the complex, oxidation of the solvent may also lead to greater quenching from O₂. The influence these parameters could both be expected to increase with the electrode potential required to oxidize the metal complex: IX < X < IIb.

With respect to factor (iii), a poor correlation between ECL intensities and the photoluminescence quantum yields has previously been reported (due to the much greater influence of other factors), but in cases where other parameters are similar, the quantum yield can certainly be a determining factor. In this study, the lower quantum yields of IIa and VII can in part explain their relatively low ECL intensities (Table 1).

The calculation of relative ECL intensities (Table 1) from the integrated corrected emission spectra is most appropriate for comparisons of the fundamental properties of the complexes relating to the process of ECL. However, in an analytical setting, the ECL signal is generally measured using a photodetector with considerably different sensitivity across the visible region, and therefore the particular wavelengths of maximum emission will have significant influence on the relative intensities. To illustrate this point, we examined the relative ECL intensity of the orange-red emitter [Ru(bpy)₃]²⁺ and the most effective blue emitter IX, at a concentration of 0.1 µM (with a 0.5 s pulse time) using three different photomultiplier tubes (PMTs). When an
extended-range trialkali S20 PMT (ET Enterprises model 9828B) was used, the signal for **IX** was only 42% that of *[Ru(bpy)₃]^{2+}*. In contrast, when we utilized cheaper bialkali green- and blue-sensitive PMTs (9125B and 9124B), the ECL signal for **IX** was 498% and 1213% that of *[Ru(bpy)₃]^{2+}* respectively.
4.5 Conclusions

Highly water soluble iridium(III) complexes capable of moderate to high ECL intensities (relative to $[\text{Ru(bpy)}_3]^{2+}$) were successfully prepared by introducing methylsulfonate or TEG groups onto the ligands. The use of TEG groups was found to be the best option in terms of both ECL intensity and solubility in aqueous solution. When comparing the integrated area of the corrected ECL spectra and using a 0.5 s pulse time, IX was the only iridium(III) complex to give an ECL intensity exceeding that of $[\text{Ru(bpy)}_3]^{2+}$ (Table 1). The greater intensity from IX compared to the other iridium(III) complexes under investigation appears to be the result of a combination of factors, rather than it being considerably favored in one area. Other complexes had higher quantum yields, were less inhibited by the potential window of the solvent and electrode passivation, and/or had more exergonic reaction steps, but they were disfavored to a greater extent in at least one of the other parameters. An examination of relative ECL intensities using three different photomultiplier tubes highlighted the importance of photodetector sensitivity over the wavelength range when comparing emitters with distinctly different spectral distributions.

4.6 Acknowledgements

The Australian Research Council (ARC) provided funding for this research, and PSD and PSF are ARC Future Fellows. We also thank La Trobe University, NCI-NF and VPAC for generous grants of computing resources.
Chapter Five: Analytically Useful Blue Chemiluminescence from a Water-Soluble Iridium(III) Complex Containing a Tetraethylene Glycol Functionalised Triazolylpyridine Ligand

Zoe M. Smith $^a$, Emily Kerr $^a$, Egan H. Doeven $^b$, Timothy U. Connell $^{ac}$, Neil W. Barnett $^a$, Paul S. Donnelly $^c$, Stephen J. Haswell $^b$ and Paul S. Francis $^{*a}$

$^a$Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Deakin University, Locked Bag 20000, Geelong, Victoria 3220, Australia. E-mail: paul.francis@deakin.edu.au

$^b$Centre for Regional and Rural Futures. Deakin University, Locked Bag 20000, Geelong, Victoria 3220, Australia

$^c$School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Victoria 3010, Australia

Published in Analyst, Royal Society of Chemistry, 2016, volume 141, issue 7, pages 2140-2144. Reproduced with permission from the Royal Society of Chemistry.
**Authorship Statement**

1. **Details of publication and executive author**

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
</table>
| Analytically useful blue chelate luminescence from a water-soluble indium(III) complex containing a tetraalkylphosphonium functionalised triazolopyridine ligand | Analyser, 2016, 141, 2146-2144  
DOI: 10.1039/C5AN00144F |

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin, Organisation and address</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paul Francis</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td><a href="mailto:paul.francis@deakin.edu.au">paul.francis@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

2. **Inclusion of publication in a thesis**

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>Yes/No</th>
</tr>
</thead>
</table>
| If Yes, please complete Section 3  
If No, go straight to Section 4. |

3. **HDR thesis author’s declaration**

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above (if the same, write &quot;as above&quot;)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emily Kerr</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td>Electrochemical and Luminescence Studies of Ruthenium and Indium Metal-Chelates</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication. For example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

Designed and planned experiments with assistance from co-author. With Zoe M. Smith, collected all published data (with the exception of synthesis), developed and optimised procedures for analysis. Assisted with manuscript revisions.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: [Signature]  
14/12/16

4. **Description of all author contributions**

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contributor(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoe M. Smith</td>
<td>Collected all published data together with Emily Kerr. Prepared first draft of manuscript. Assisted with revisions.</td>
</tr>
<tr>
<td>Egan H. Donovan</td>
<td>Assisted with taking photographs of CL. Assisted with revision of manuscript.</td>
</tr>
<tr>
<td>Timothy L. Connell</td>
<td>Synthesised all complexes used in this study, prepared relevant sections for publication and assisted with manuscript revision.</td>
</tr>
<tr>
<td>Neil W. Bennett</td>
<td>Assisted with revision of manuscript and guided experimental design.</td>
</tr>
<tr>
<td>Paul S. Donnelly</td>
<td>Assisted with revision of manuscript and oversaw aspects of chemical synthesis.</td>
</tr>
<tr>
<td>Stephen J. Haswell</td>
<td>Assisted with revision of manuscript and guided experimental design.</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td>Involved in conceptualisation of project and planning of experiments. After receiving initial draft, involved in the revision process, contributed to intellectual content of manuscript and finalised submission to journal.</td>
</tr>
</tbody>
</table>
5. Author Declarations
I agree to be named as one of the authors of this work and confirm:
1. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
2. that there are no other authors according to these criteria,
3. that the description in Section 4 of my contribution(s) to this publication is accurate,
4. that the date on which these findings are stored are set out in Section 7 below.
If this work is to form part of an HDR thesis as described in Sections 2 and 3, I further
consent to the incorporation of the publication into the candidate's HDR thesis submitted to Deakin
University and, if the higher degree is awarded, the subsequent publication of the thesis by the
university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoe M. Smith</td>
<td></td>
<td>15/11/15</td>
</tr>
<tr>
<td>Egan H. Dooven</td>
<td></td>
<td>15/11/15</td>
</tr>
<tr>
<td>Timothy L. Connell</td>
<td></td>
<td>19/11/15</td>
</tr>
<tr>
<td>Neil W. Barnett</td>
<td></td>
<td>19/11/15</td>
</tr>
<tr>
<td>Paul E. Donnelly</td>
<td></td>
<td>16/12/15</td>
</tr>
<tr>
<td>Stephen J. Haswell</td>
<td></td>
<td>15th December 2015</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td></td>
<td>16/11/2016</td>
</tr>
</tbody>
</table>

6. Other contributor declarations
I agree to be named as a non-author contributor to this work.

<table>
<thead>
<tr>
<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
</table>

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of
  Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to
  suggest that the person would object to being named as author.

7. Data storage
The original data for this project are stored in the following locations. (The locations must be within an appropriate
institutional setting, if the executive author is a Deakin staff member and data are stored outside Deakin
University, permission for this must be given by the Head of Academic Unit within which the executive author is
based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date Lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB</td>
<td>WPK's 5.127</td>
<td>19/02/2017</td>
<td>Paul Francis</td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based.
If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis
with the publication.
5.1 Abstract

We examine [Ir(df-ppy)$_2$(pt-TEG)]$^+$ as the first highly water soluble, blue-luminescent iridium(III) complex for chemiluminescence detection. Marked differences in selectivity were observed between the new complex and the conventional [Ru(bpy)$_3$]$^{2+}$ reagent, which will enable this mode of detection to be extended to new areas of application.
5.2 Introduction

Cyclometalated iridium(III) complexes exhibiting high luminescence efficiencies and a wide range of emission colours\textsuperscript{208, 218, 249} are seen as promising alternatives to the ruthenium(II) complexes traditionally employed for photoluminescence, chemiluminescence and electrochemiluminescence (ECL). The use of iridium(III) complexes offers opportunities to manipulate detection selectivity, shift the emission into the most sensitive wavelength range of conventional photomultiplier tubes, and develop multi-colour detection systems.\textsuperscript{28, 44, 46, 47, 149, 250} However, the poor aqueous solubility of many available iridium(III) complexes has limited their application in these areas. In cases where only low concentrations of the luminophore are required, such as photoluminescence protein staining, cellular imaging and molecular probes,\textsuperscript{221, 222, 251} or ECL labelling for immunoassay,\textsuperscript{110, 181} sufficient solubility has been derived by including a neutral ligand to impart an overall positive charge to the complex, or a derivative containing sulfonate or saccharide groups.\textsuperscript{47, 70, 71, 78, 221, 222, 252-254} For chemiluminescence detection systems in which the metal complexes are used at relatively high concentrations to detect reducing agents (such as tertiary amines),\textsuperscript{7} these approaches have not resulted in adequate solubility and/or have restricted emission wavelengths to regions of green to red light. Nevertheless, explorations of iridium(III) complexes as chemiluminescence reagents\textsuperscript{68-71, 223, 255, 256} have shown differences in the selectivity of their light producing reactions, and for some analytes, more intense emissions than those from analogous reactions with conventional ruthenium(II)-based reagents.\textsuperscript{70, 71, 223}

We previously examined a series of chemiluminescence reactions of $\text{[Ir(C}^\text{N})_2(BPS)]^-$ complexes\textsuperscript{71, 223} (where $C^\text{N}$ represents the cyclometalating phenylisoquinoline (piq), phenylpyridine (ppy), phenylbenzothiazole (bt) or
difluorophenylpyridine (df-ppy) ligands, and BPS is bathophenanthroline disulfonate), which exhibited red, orange, yellow and green emissions, respectively. The BPS ligand imparted significant solubility in aqueous solution, but a 1 : 1 mixture of water : acetonitrile was required to prepare the reagents at 1 mM.\textsuperscript{71,223} Using a flow-injection analysis manifold, the reagents were oxidised with cerium(IV) sulfate before reacting with a variety of analytes. Greater chemiluminescence intensities were generally obtained using [Ir(df-ppy)\textsubscript{2}(BPS)]\textsuperscript{−} or [Ir(bt)\textsubscript{2}(BPS)]\textsuperscript{−} than with [Ru(bpy)]\textsuperscript{2+}, but the blank responses from the reaction between oxidised iridium complexes and solvent were also greater, which reduced the anticipated improvements in the signal-to-blank ratios.\textsuperscript{71,223} The inclusion of the BPS ligand in these complexes also induced a bathochromic shift in the emission when compared to their homoleptic counterparts, restricting the highest energy emission, exhibited by [Ir(df-ppy)\textsubscript{3}]\textsuperscript{−} (Figure 5.1b), to the green region of the spectrum ($\lambda_{\text{max}} = 549$ nm; Figure AIII.1),\textsuperscript{71} whereas the corresponding neutral [Ir(df-ppy)]\textsubscript{3} complex (Figure 5.1a), which is not soluble in water, emits blue light ($\lambda_{\text{max}} = 495$ nm).\textsuperscript{36}

Figure 5.1: Comparison of [Ir(df-ppy)\textsubscript{2}(L)]\textsuperscript{n} complexes, where L is: 2-(2,4-difluorophenyl)pyridinato (df-ppy);\textsuperscript{36,148} bathophenanthroline-disulfonate (BPS);\textsuperscript{40} bathophenanthroline-1,2,3-triazol-4-ylpyridine (STP);\textsuperscript{71} or 2-triethoxyethanol[4-(2-pyridinyl)-1H-1,2,3-triazole-1-yl] (pt-TEG).\textsuperscript{40,238}
In a previous attempt to create a water-soluble iridium(III) complex exhibiting blue chemiluminescence,\textsuperscript{71} we synthesised a sulfonated derivative of 1-phenyl-1,2,3-triazol-4-ylpyridine (STP) as an alternative ancillary ligand to BPS. Although blue chemiluminescence was observed from [Ir(df-ppy)\textsubscript{2}(STP)] (Figure 5.1c and AIII.1) upon reaction with cerium(IV) sulfate and pharmaceuticals such as codeine, furosemide and ofloxacin, the aqueous solubility of the complex was poor (limited to \( \sim 10^{-5} \) M) and its chemiluminescence intensities (and signal/blank ratios) were generally very low compared to those of [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} and the [Ir(C\textsuperscript{3}N)\textsubscript{2}(BPS)]\textsuperscript{-} complexes. However, a close analogue of [Ir(df-ppy)\textsubscript{2}(STP)] bearing a polyethylene glycol substituent ([Ir(df-ppy)\textsubscript{2}(pt-TEG)]\textsuperscript{+}; Figure 5.1d) was recently identified as a promising candidate for ECL detection,\textsuperscript{40} with high aqueous solubility and co-reactant ECL signals with tri-\textit{n}-propylamine that were over twelve times greater than those of [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} when measured with a typical bialkali photomultiplier tube. Herein, we report our investigation of [Ir(df-ppy)\textsubscript{2}(pt-TEG)]\textsuperscript{+} as the first highly water-soluble iridium(III) complex exhibiting blue chemiluminescence.
5.3 Experimental Section

5.3.1 Chemicals

[ Ir(df-ppy)₂(pt-TEG)] [Cl] and [Na][Ir(ppy-SO₃)₂(pt-TEG)] were prepared as previously described.⁴⁰ [Ru(bpy)₃]Cl₂·6H₂O was purchased from Strem (MA, USA).

5.3.2 Flow Injection Analysis Setup

Flow injection analysis was used to reproducibly combine the reactants. The manifold was assembled as described previously,²⁵ which included a GloCel chemiluminescence detector (Global FIA, MA, USA) with dual-inlet serpentine flow-cell²⁶ and an Electron Tubes model 9125B photomultiplier tube (ETP, NSW, Australia). The aqueous metal-complex reagent solutions were injected (70 μL) into a carrier line containing the 1 mM cerium(IV) sulfate (in 0.05 M H₂SO₄), which merged with the analyte solution within the detection flow-cell. A flow rate of 3.5 mL min⁻¹ per line was used in all experiments.

5.3.3 Absorption and Emission Spectroscopy Experiments

Absorption spectra were obtained using Cary 300 Spectrophotometer. Photoluminescence spectra were obtained with a Cary Eclipse fluorescence spectrophotometer (5 nm excitation and emission band pass) and corrected for the wavelength dependence of the detector response and monochromator transmission.²²⁴ Chemiluminescence spectra were obtained by replacing the photomultiplier tube in the flow injection analysis manifold with an Ocean Optics QE65Pro spectrometer with CCD detector (10 s spectra integration time, each acquisition manually triggered in concert with reagent injection), which was interfaced with the chemiluminescence flow-cell via fibre optic cable (1 mm core diameter, 1.0 m length) and collimating lens
(30 mm diameter, 350–2000 nm). This enabled measurement of the chemiluminescence spectrum after each injection, as the light-producing reaction mixture passed through the flow-cell. The presented chemiluminescence spectra are each an average of those obtained from three injections of the reagent solution into the flow injection analysis manifold.
5.4 Results and Discussion

We compared the chemiluminescence responses of \([\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+\) with that of \([\text{Ru(bpy)}_3]^2+\) and a related iridium(III) complex that exhibits green luminescence \((\text{[Ir(ppy-SO}_3)_2(\text{pt-TEG})]^-, \text{Figure 5.2a, AIII.2 and AIII.3}), using flow injection analysis methodology, and reagent concentrations of 1 mM, 0.1 mM and 0.01 mM (Figure 5.3), representing the wide range adopted in previous analytical applications.\(^7\)

![Figure 5.2: (a) Normalised absorption spectrum of cerium(IV) sulfate (black line), and normalised photoluminescence emission spectra of \([\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+\) (blue line; \(\lambda_{\text{max}} = 456, 483 \text{ nm}\)), \([\text{Ir(ppy-SO}_3)_2(\text{pt-TEG})]^-\) (green line; \(\lambda_{\text{max}} = 482, 512 \text{ nm}\)), and \([\text{Ru(bpy)}_3]^2+\) (red line; \(\lambda_{\text{max}} = 618 \text{ nm}\)), at 10 \(\mu\text{M}\) in aqueous solution. (b) Normalised chemiluminescence spectra for 1 mM \([\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+\) (blue line) and \([\text{Ru(bpy)}_3]^2+\) (red line), with 1 mM cerium(IV) sulfate and 50 \(\mu\text{M}\) furosemide. The vibrational structure in the photoluminescence emission spectrum of \([\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+\) was not observed in the corresponding chemiluminescence spectrum due to the considerably lower resolution of the CCD spectrometer configuration.\(^7\)
The compounds selected for the comparison: codeine, furosemide, 1-(4-trifluoromethylphenyl)piperazine, 1-(4-trifluoromethylphenyl)piperidin-4-ol and ofloxacin (Figure A4.4), have previously been shown to elicit light upon reaction with various ruthenium(II) and iridium(III) complexes under acidic conditions using cerium(IV) sulfate as an oxidant. Similar trends in the relative chemiluminescence intensities (and signal-to-blank ratios) were observed across the different reagent concentrations (Figure 5.3 and S5†), but the three reagents exhibited markedly different selectivity. Using [Ru(bpy)₃]²⁺, the greatest intensities were elicited by ofloxacin and the piperidinol derivative, whereas using [Ir(ppy-SO₃)(pt-TEG)]⁻, the piperazine derivative elicited a much greater response than the other four compounds. Lower signals (and S/B ratios) were observed using [Ir(df-ppy)(pt-TEG)]⁺ than [Ru(bpy)₃]²⁺, with the exception of the reaction with furosemide, which exhibited a four-fold greater S/B ratio with the blue-light emitting iridium(III) reagent (at 1 mM and 0.1 mM metal complex concentration).
Figure 5.3: Relative chemiluminescence (signal-to-blank) response for [Ru(bpy)$_3$]$^{2+}$, [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^{-}$, and [Ir(df-ppy)$_2$(pt-TEG)]$^{+}$ at (a) 1 mM, (b) 0.1 mM, and (c) 0.01 mM reagent concentration, with cerium(IV) sulfate (1 mM) and various pharmaceuticals and related compounds (1 μM), using flow injection analysis methodology.
The mechanism for the light-producing reactions of ruthenium- and iridium-complexes with various amine-containing compounds upon chemical or electrochemical oxidation involves numerous competing reaction pathways, the most dominant of which depends on the reaction conditions, and the properties of not only the metal complex, but also the amine and its radical oxidation products. The differences in the selectivity of the \([\text{Ru(bpy)}_3]^{2+}, \text{Ir(ppy-SO}_3)_2(\text{pt-TEG})\] and \([\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+\) complexes towards these analytes can be attributed to factors such as their oxidising strength \((E_{\text{ox}} = 1.06\ \text{V}, 1.09\ \text{V}\) and \(1.43\ \text{V vs. Ag/AgCl, respectively}\), ligand structure and overall charge (2+, 1− and 1+, respectively), which influence the rate of reaction leading to the emitting species. Similar reasoning can be made for the deleterious light-producing reaction with the solvent that produces the ‘blank’ response. The chemiluminescence intensity of the three complexes with any particular analyte (or the solvent) will also be limited by the luminescence quantum yield of each complex.

The optimum reagent concentration, in terms of chemiluminescence S/B ratios, was found to be both analyte and reagent dependent, in agreement with our previous investigations. In that prior work, stopped-flow experiments indicated that the changes in S/B ratio arose from the influence of concentration on the rates of the competing reactions of analyte and solvent with the reagent, coupled with the dependence of the chemiluminescence signal measured in a flow-injection analysis system on the reaction kinetics.

The sensitivity of the \([\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+\) reagent towards furosemide compared to the other analytes under investigation is similar under certain conditions to that observed for \([\text{Ir(df-ppy)}_2(\text{BPS})]^+\) (Figure 5.1b) which also has a much higher \(E_{\text{ox}} (1.52\ \text{V vs. Ag/AgCl})\) than \([\text{Ru(bpy)}_3]^{2+}\). Comparison of the
chemiluminescence intensity of $[\text{Ru(bpy)}_3]^{2+}$ and $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$ with compounds similar in structure to furosemide (Figure 5.4 and 5.5) highlighted the remarkable difference in selectivity between the two reagents. Removing the aniline group from furosemide, or replacing its furan-2-ylmethyl substituent with a benzyl group on the anilinic nitrogen reduced the chemiluminescence intensity with $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$ by an order of magnitude (Figure 5.6). Piretanide, which possesses a tertiary aniline group, gave significantly greater chemiluminescence intensity upon reaction with $[\text{Ru(bpy)}_3]^{2+}$ (and cerium(IV)) than the other compounds, but this was not observed for $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$.

**Figure 5.4:** Photographs of the chemiluminescence reactions of (a) $[\text{Ru(bpy)}_3]^{2+}$ and 1-(4-trifluoromethylphenyl)piperidin-4-ol, (b) $[\text{Ir(ppy-SO}_3\text{)}_2(\text{pt-TEG})]^-$ and 1-(4-trifluoromethylphenyl)piperazine, and (c) $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$ and furosemide, in aqueous solution. The metal complex reagents were continuously merged with an oxidant solution (cerium(IV) sulfate in 0.05 M $\text{H}_2\text{SO}_4$) at a T-piece shortly prior to mixing with the other reactant solution within a transparent serpentine flow-cell. A Canon 6D camera with 50 mm f1.8 lens were used (Canon, Japan). The exposure time and reactant concentrations were adjusted to produce similar emission intensities.
Figure 5.5: Loop diuretics and related compounds.

Figure 5.6: Chemiluminescence responses (signal/blank ratios) of various loop diuretics and related compounds: (1) furosemide, (2) 4-chloro-3-sulfamoylbenzoic acid, (3) N-benzyl-4-chloro-5-sulfamoylanthranilic acid, (4) piretanide, (5) bumetanide at 1 μM, with [Ru(bpy)$_3$]$^{2+}$ (red columns) and [Ir(dfppy)$_2$(pt-TEG)]$^+$ (blue columns), using flow injection analysis methodology. Reagent concentration: 0.1 mM. Oxidant: 1 mM cerium(IV) sulfate in 0.05 M H$_2$SO$_4$. A comparison of signal/blank ratios for the two complexes with the same y-axis scale is shown in Figure AIII.6.
Previously examined iridium(III) complexes often exhibited higher chemiluminescence intensities than [Ru(bpy)$_3$]$^{2+}$, but also showed greater blank responses from the corresponding reaction with the solvent. However, this was not the case for [Ir(df-ppy)$_2$(pt-TEG)]$^+$, which gave lower blank responses than [Ru(bpy)$_3$]$^{2+}$. We suspected that this might be due to partial absorption of the blue emission of [Ir(df-ppy)$_2$(pt-TEG)]$^+$ by the cerium(IV) oxidant, but the overlap of their emission and absorption spectra is minimal (Figure 5.2a). Consequently, there was no red-shift in the chemiluminescence emission compared to the photoluminescence in the absence of cerium(IV) (Figure 5.2b): visually, the light emitted from the reaction of [Ir(df-ppy)$_2$(pt-TEG)]$^+$ with cerium(IV) sulfate and furosemide (Figure 5.4) was the same blue colour as the corresponding photoluminescence (Figure AIII.3).

Calibrations for furosemide (Figure AIII.7) prepared using flow injection analysis methodology with 0.1 mM [Ir(df-ppy)$_2$(pt-TEG)]$^+$ (and 1 mM cerium(IV) sulfate in 0.05 mM H$_2$SO$_4$) showed a superior limit of detection ($1 \times 10^{-8}$ M; 3$\sigma$) than that obtained with [Ru(bpy)$_3$]$^{2+}$ ($7 \times 10^{-8}$ M; 3$\sigma$) under the same conditions. These detection limits are comparable to those reported in previous studies based on chemiluminescence reactions with [Ru(bpy)$_3$]$^{2+}$ ($8 \times 10^{-9}$ M to $2 \times 10^{-7}$ M), [Ru(BPS)$_3$]$^{4-}$ ($1 \times 10^{-9}$ M to $3 \times 10^{-8}$ M), and [Ir(df-ppy)$_2$(BPS)]$^-$ ($1 \times 10^{-8}$ M), where cerium(IV) was used as the oxidant.
5.5 Conclusions

These preliminary investigations of the [Ir(df-ppy)(pt-TEG)]⁺ complex as a chemiluminescence reagent reveal a viable approach to develop new detection systems based on chemically induced blue luminescence from metal complexes under analytically useful aqueous conditions. The use of polyethylene glycol groups is a more effective option to enhance solubility in water than previous attempts involving ligands with sulfonate groups. The ability to shift the emission bands into the blue region of the visible spectrum is advantageous for the development of miniaturised analytical devices with low-cost photodetectors. Moreover, the striking differences in the selectivity of these novel chemiluminescence reagents compared to traditional ruthenium(II) polypyridine complexes will expand the scope of chemiluminescence detection into new areas of application.

5.6 Acknowledgements

We thank the Australian Research Council (DP140100439, FT100100646) for funding this research.
Chapter 6: A Comparison of Commercially Available Screen Printed Electrodes for Electrogeminated Chemiluminescence Applications

Emily Kerr\textsuperscript{a,b}, Richard Alexander\textsuperscript{c}, Paul S. Francis\textsuperscript{a}, George M. Whitesides\textsuperscript{b}, Gregory J. Barbante\textsuperscript{d}, Egan H. Doeven\textsuperscript{c,*}

\textsuperscript{a}Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment, Deakin University, Geelong, Victoria 3220, Australia.

\textsuperscript{b}Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA.

\textsuperscript{c}Centre for Regional and Rural Futures. Deakin University, Geelong, Victoria 3220, Australia.

\textsuperscript{d}ARC Training Centre for Portable Analytical Separation Technologies (ASTech), University of South Australia, Future Industries Institute, Mawson Lakes, 5095, Australia

Authorship Statement

1. Details of publication and executive author

<table>
<thead>
<tr>
<th>Title of Publication</th>
<th>Publication details</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Name of executive author</th>
<th>School/Institute/Division if based at Deakin; Organisation and address if non-Deakin</th>
<th>Email or phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egan Doeven</td>
<td>Centre for Rural and Regional Futures, Deakin University</td>
<td><a href="mailto:Egan.doeven@deakin.edu.au">Egan.doeven@deakin.edu.au</a></td>
</tr>
</tbody>
</table>

2. Inclusion of publication in a thesis

<table>
<thead>
<tr>
<th>Is it intended to include this publication in a higher degree by research (HDR) thesis?</th>
<th>If Yes, please complete Section 3. If No, go straight to Section 4.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes/</td>
<td></td>
</tr>
</tbody>
</table>

3. HDR thesis author's declaration

<table>
<thead>
<tr>
<th>Name of HDR thesis author if different from above (if the same, write &quot;as above&quot;)</th>
<th>School/Institute/Division if based at Deakin</th>
<th>Thesis title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emily Kerr</td>
<td>Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment</td>
<td>Electrochemical and Luminescence Studies of Ruthenium and Iridium Metal-Chelates</td>
</tr>
</tbody>
</table>

If there are multiple authors, give a full description of HDR thesis author's contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)

Designed and planned experiments with assistance from co-author. Collected all electrochemical and ECL data with the exception of ECL bead-based assay experiments. Collected SEM images together with Richard Alexander and Kara Splittas. Developed and optimised procedures for analysis. Completed manuscript revisions with assistance from co-author.

I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.

Signature and date: [Signature] 14/1/12

4. Description of all author contributions

<table>
<thead>
<tr>
<th>Name and affiliation of author</th>
<th>Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richard Alexander</td>
<td>Machine flow cells and optimised design of flow cell, collected SEM images with Emily Kerr and Kara Splittas, Prepared relevant sections of manuscript for publication.</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td>Assisted with manuscript revisions and general project supervision. Discussion of experimental design and manuscript structure.</td>
</tr>
<tr>
<td>George M. Whitesides</td>
<td>Assisted with manuscript revisions and general project supervision. Discussion of experimental design and manuscript structure.</td>
</tr>
<tr>
<td>Gregory J. Bamberge</td>
<td>Synthesised one sample used in analysis. Assisted with manuscript revisions and planning of experiments.</td>
</tr>
<tr>
<td>Egan H. Doeven</td>
<td>Completed ECL bead-based assay experiments, assisted with manuscript revisions and planning of experiments. Contributed to intellectual content of the manuscript.</td>
</tr>
</tbody>
</table>
5. Author Declarations
   I agree to be named as one of the authors of this work, and confirm:
   i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,
   ii. that there are no other authors according to these criteria.
   iii. that the description in Section 4 of my contribution(s) to this publication is accurate,
   iv. that the data on which these findings are based are stored as set out in Section 7 below.

If this work is to form part of an HDR thesis as described in Sections 3 and 4, I further
v. consent to the incorporation of the publication into the candidate’s HDR thesis submitted to Deakin
   University and, if the higher degree is awarded, the subsequent publication of the thesis by the
   university (subject to relevant Copyright provisions).

<table>
<thead>
<tr>
<th>Name of author</th>
<th>Signature*</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Richard Alexander</td>
<td></td>
<td>16/02/2017</td>
</tr>
<tr>
<td>Paul S. Francis</td>
<td></td>
<td>14/1/2017</td>
</tr>
<tr>
<td>George M. Whitesides</td>
<td></td>
<td>14/1/2017</td>
</tr>
<tr>
<td>Gregory J. Burkharte</td>
<td></td>
<td>16/3/2017</td>
</tr>
<tr>
<td>Egan H. Donevan</td>
<td></td>
<td>15/1/2017</td>
</tr>
</tbody>
</table>

6. Other contributor declarations
   I agree to be named as a non-author contributor to this work.

<table>
<thead>
<tr>
<th>Name and affiliation of contributor</th>
<th>Contribution</th>
<th>Signature* and date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yi Heng Bai</td>
<td>Assisted with bead-based ECL assay</td>
<td>16/02/2017</td>
</tr>
<tr>
<td>Kara Spletha</td>
<td>Collected SEM images</td>
<td>16/02/2017</td>
</tr>
</tbody>
</table>

* If an author or contributor is unavailable or otherwise unable to sign the statement of authorship, the Head of Academic Unit may sign on their behalf, noting the reason for their unavailability, provided there is no evidence to suggest that the person would object to being named as author.

7. Data storage
   The original data for this project are stored in the following locations. (The locations must be within an appropriate institutional setting if the executive author is a Deakin staff member and data are stored outside Deakin University, permission for this must be given by the Head of Academic Unit within which the executive author is based.)

<table>
<thead>
<tr>
<th>Data format</th>
<th>Storage Location</th>
<th>Date lodged</th>
<th>Name of custodian if other than the executive author</th>
</tr>
</thead>
<tbody>
<tr>
<td>USB</td>
<td>WP Ks 5.127</td>
<td>16/02/2017</td>
<td>Paul Francis</td>
</tr>
</tbody>
</table>

This form must be retained by the executive author, within the school or institute in which they are based.

If the publication is to be included as part of an HDR thesis, a copy of this form must be included in the thesis with the publication.
6.1 Abstract

We examine a series of commercially available screen printed electrodes (SPEs) for their suitability for application in electrochemical and electrogenerated chemiluminescence (ECL) detection systems. We have compared the SPEs in ECL experiments using cyclic voltammetry with both a free-complex and bead-based ECL assay. We observed the most intense ECL signals from unmodified carbon-based SPEs; three varieties were tested Zensor (100%), DropSens (62%) and Kanichi (45%). In general, we observed lower ECL intensities from SPEs comprising combinations of nanomaterials and carbon (21-48%), despite their high electro-active surface area. The ECL intensity from platinum (9%) and gold (16%) SPEs was much lower than carbon-based alternatives. The intensity from platinum electrodes could be enhanced by ~3-fold with the addition of a surfactant.
6.2 Introduction

Since the development of the personal glucometer, a device that employs screen printed electrodes to provide diabetic patients with accurate blood glucose measurements, many recent advances in electrochemical detection techniques have used commercially available or in-house produced screen printed electrodes (SPEs) for the development of point-of-care diagnostic systems; example targets include heavy metals, pesticides, ethanol, dopamine, pathogenic DNA or specific antigens.\textsuperscript{266-276} Electрогenerated chemiluminescence (ECL) is a technique routinely employed in a range of bioanalytical assays, in an ECL experiment, an applied electrochemical potential results in the formation of excited states and light emission at an electrode surface.\textsuperscript{1, 277} This light emission can be exploited and measured in a range of ECL assays to provide a highly sensitive and selective method for the detection of various biological molecules of interest.\textsuperscript{14, 17, 125, 130} Research groups have recently begun to couple ECL detection strategies with disposable SPEs; the potential of this combination to provide enhanced sensitivity along with simplified and low-cost devices presents a convenient solution to many draw-backs of initial proof-of-concept point-of-care devices.\textsuperscript{139, 177, 178, 238, 278, 279} ECL offers many advantages when compared to conventional electrochemical, fluorescent or chemiluminescent detection techniques as it does not require precise current monitoring or an external light source; ECL also offers accurate spatial and temporal control over the reaction procedure.\textsuperscript{14}

A number of electrode characteristics are important for developing highly sensitive analytical ECL and electrochemical applications, including: (i) fast electron transfer rates; (ii) highly reproducible electrode surfaces to improve assay precision; (iii) high electroactive surface area to maximise signal; (iv) high electrode surface stability, to improve the reproducibility between potential scans and prevent electrode
passivation; (v) wide electrochemical potential window; (vi) low background current; and (vii) for ECL, a significantly hydrophobic electrode surface, to permit efficient oxidation of the frequently employed co-reactant tripropylamine (TPrA).\textsuperscript{280-282} Previously, Kadara, \textit{et al.}\textsuperscript{283} and Fanjul-Bolado, \textit{et al.}\textsuperscript{284} conducted thorough electrochemical characterisations of a range of in-house produced and commercially available SPEs.\textsuperscript{283, 284} Following these studies, a wide variety of SPEs that exploit advances in electro-active materials, such as modified electrodes incorporating carbon nanofibers (CNF), carbon nanotubes (CNT), gold nanoparticles (GNP), graphene (GPH), ordered mesoporous carbon (OMC) and combinations of these materials, have become available from commercial suppliers, alongside traditional alternatives such as carbon, platinum and gold electrodes.\textsuperscript{104, 118, 285-289} Herein, we interrogate 13 commercially available varieties of electrodes for their electrochemical and ECL properties.
6.3 Experimental

6.3.1 Chemicals

Potassium ferrocyanide (AJAX, Australia >98%), potassium chloride (LabServ, Australia >99%), sodium chloride (Sigma-Aldrich, Australia >99.5%), potassium phosphate monobasic (Sigma-Aldrich, Australia >99%), potassium phosphate dibasic (Sigma-Aldrich, Australia >98%), tris (Sigma-Aldrich, Australia >99%), borate (Sigma-Aldrich, Australia >99%) potassium hydroxide (Sigma-Aldrich, Australia >85%), hydrochloric acid (Chem-Supply, Australia 32%), tripropylamine (Sigma-Aldrich, Australia >98%), tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate ([Ru(bpy)₃]Cl₂·6H₂O, Sigma-Aldrich, Australia, >99.5%), Tween 20 (polyethylene glycol sorbitan monolaurate, T-20, Sigma Aldrich, Australia), and Triton X-100 (polyethylene glycol tert-octylphenyl ether, TX-100, Ajax Chemicals, Australia) were used as supplied. All samples were prepared in milli-Q water (18.2 MΩ cm⁻¹). Biotinylated 89mer ssDNA used as a nucleic acid proxy assay was purchased from Integrated DNA Technologies, USA (details in SI), Bis(2,2'-bipyridine)-(4-methyl-4′-carboxypropyl-2,2′-bipyridine)ruthenium(II)-bis(hexafluorophosphate) (Ru-COOH) was used as supplied by Prof. Paul Francis’s lab, (Deakin University, Waurn Ponds, Australia). Dynabeads d280 streptavidin coated 2.8 µm paramagnetic beads (Invitrogen) were purchased from Life Technologies (Australia), and were washed in binding buffer (0.5 M NaCl, 50 mM Tris.HCl, pH 8.0) three times prior to use. Beads were used at a concentration of 2 mg/mL and stored in binding buffer unless otherwise specified.
6.3.2 Procedure

We machined custom SPE holders from 10 mm thick cast poly(methyl methacrylate) (PMMA) sheets using a Datron M7HP CNC mill (Datron AG, Germany). SPE holders were designed using SolidWorks 2015 CAD package (Dassault Systems, France), while G-code CNC toolpaths were created using Siemens NX 10 CAD/CAM package (Siemens, Germany). 3D drawings of these holders are shown in Figure AIV.1. These holders were designed to house the SPEs for analysis and to reproducibly interface the cells with the detector. For the paramagnetic particle based experiments, the holders were designed to hold a 3 × 4 mm diameter rod shaped N42 rare earth magnet (Aussie Magnets, Australia) beneath the working electrode position, to facilitate particle capture at the electrode surface. Two detectors were used: solution phase ECL was detected using a standard photomultiplier tube (extended-range trialkali S20 PMT, ET Enterprises model 9828B), while ECL from the magnetic bead based assay was detected using an AdvanSiD (Italy) 3×3 mm silicon photon multiplier (ASD-RGB3S-P), to remove any effect of the magnetic field on the PMT. The SiPM was biased at 33 V and interfaced with an AdvanSiD ASD-EP-EB-N amplifier board. Data from the SiPM was recorded using an eDAQ401 (eDAQ, Australia) data recording unit using the supplied eDAQ Chart software.

For electrochemical and ECL (PMT) experiments, we used a custom-built, light-tight, faraday cage and an Autolab PGSTAT 101 or PGSTAT 128 N (Metrohm Autolab B.V., Netherlands) potentiostat with accompanying NOVA software. We purchased the following varieties of electrodes from DropSens (http://www.dropsens.com/): unmodified carbon (DS-C), ordered mesoporous carbon (DS-OMC), carbon nanotubes (DS-CNT), carbon nanofiber (DS-CNF), graphene (DS-GPH), gold (BT-250 model, DS-Au) platinum (DS-Pt), carbon with gold
nanoparticles (DS-GNP), carbon nanofiber with gold nanoparticles (DS-CNFGNP), carbon nanotubes with gold nanoparticles (DS-CNT-GNP), and graphene with gold nanoparticles (DS-GPH-GNP). We also purchased carbon electrodes from eDAQ (http://www.edaq.com/, Zensor and Kanichi varieties). Each SPE contained a three-electrode configuration with varying working electrode surfaces (DropSens varieties 4 mm working electrode diameter, Kanichi and Zensor, 3 mm working electrode diameter), carbon auxiliary electrode and Ag/AgCl reference electrode. For electrochemical experiments, we prepared analytes in either 1 M KCl or 0.1 M phosphate buffer solution (PBS), pH 7.5. For ECL experiments, we prepared solutions at the appropriate concentration in ECL buffer; 0.1 M PBS, pH 7.5 containing 100 mM TPrA. We calculated relative ECL intensities from the integrated area of the PMT response from three cyclic voltammetry (CV) cycles between 1.6 and -1.2 V at 0.1 V/s and adjusted each result proportionally to the geometric working electrode surface area.

For DNA assay comparison experiments, we prepared an Ru-DNA-biotin construct that could be immobilised on paramagnetic particles, following the procedure detailed by Zhou, et al.\textsuperscript{181} we first prepared the N-hydroxysuccinimide (NHS) intermediate in DMF from the Ru-COOH complex, followed by bioconjugation with the NH\textsubscript{2} terminated DNA sequence. The DNA was isolated and the Ru-DNA-biotin conjugate concentration quantified by UV-visible spectrometry. This purified Ru-DNA-biotin conjugate was then bound to the paramagnetic beads by streptavidin-biotin interaction. The beads (with bound Ru-DNA-biotin construct) were then washed and re-suspended in binding buffer at 2 mg/mL for later use.

To perform ECL experiments on the Ru-DNA-bead constructs, the paramagnetic beads were re-suspended in ECL buffer at 2 mg/mL. The SPE to be
tested was mounted in the holder, then 80 µL of ECL buffer was pipetted into the well in the holder located over the electrode area. 5 µL of the Ru-DNA-bead solution (10 µG paramagnetic particles) was then carefully pipetted over the working electrode area, where the beads were rapidly captured at the working electrode surface by the magnet located directly underneath. The detector was then mounted to the top of the cell (the SiPM fits in the machined recess) and ECL performed in an identical fashion to the solution phase experiments.

We employed a handheld digital multi-meter to measure the conductive path resistance (Dick Smith Electronics, Q-1559). For Scanning Electron Microscopy (SEM) images (Zeiss Supra 55VP Scanning Electron Microscope; Zeiss, Germany), we employed an acceleration voltage of 12 kV and either an in-lens or secondary electron detector. We collected contact angles for each electrode using a contact angle goniometer (Ramè-Hart, USA).
6.4 Results and Discussion

6.4.1 Electrochemical Properties

We tested each electrode for its electrochemical properties (Table 1) using potassium ferro/ferricyanide, a thoroughly studied outer-sphere redox couple. The peak-to-peak separation of the redox couple ($\Delta E_p$) for each electrode was greater than anticipated for a one-electron transfer process (59 mV). Although not ideal, these results are consistent with those of both Fanjul-Bolado et al. and Banks et al., who proposed that the irreversibility of the ferro/ferricyanide couple at these electrode surfaces results from a combination of electrode characteristics including the nature of the ink used to produce the electrode, the amount of organic binder incorporated into the electrode, the temperature employed in the curing process, the degree of formation of oxygenated species at the electrode surface, the hydrophilicity of the electrode surface and the electrode material itself.

The electro-active area ($A_{r-s}$) of the electrode was calculated from scan rate studies of 1 mM potassium ferrocyanide in 1 M KCl, using the Randles-Sevcik equation (I).  

\[
I. \quad i_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2}
\]

Where $i_p$ is the peak current, $n$ is the number of electrons participating in the electron transfer reaction, $A$ working electrode area, $D$ is the diffusion coefficient and $v$ is the scan rate. We have included representative scan rate studies and graphs of the variation of $i_p$ with scan rate in Figures AIV.2 and AIV.3.
Table 1. Electrochemical properties of various commercially available SPEs.

<table>
<thead>
<tr>
<th></th>
<th>$A_{rs}$ (cm²) (a, b)</th>
<th>$A_{real}$ (c)</th>
<th>Conductive path resistance (Ω) (d)</th>
<th>ΔE (mV)</th>
<th>$I_c/I_a$</th>
<th>TPrA oxidation (µmol/cm²) (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zensor</td>
<td>0.058</td>
<td>0.82</td>
<td>115</td>
<td>131</td>
<td>1.05</td>
<td>14.8</td>
</tr>
<tr>
<td>DS-C</td>
<td>0.136</td>
<td>1.08</td>
<td>477</td>
<td>156</td>
<td>1.00</td>
<td>17.0</td>
</tr>
<tr>
<td>Kanichi</td>
<td>0.098</td>
<td>1.39</td>
<td>196</td>
<td>126</td>
<td>1.01</td>
<td>12.9</td>
</tr>
<tr>
<td>DS-OMC</td>
<td>0.137</td>
<td>1.09</td>
<td>262</td>
<td>76</td>
<td>0.96</td>
<td>2.5</td>
</tr>
<tr>
<td>DS-CNT</td>
<td>0.192</td>
<td>1.53</td>
<td>279</td>
<td>81</td>
<td>0.99</td>
<td>15.1</td>
</tr>
<tr>
<td>DS-CNF</td>
<td>0.242</td>
<td>1.93</td>
<td>376</td>
<td>81</td>
<td>1.01</td>
<td>14.6</td>
</tr>
<tr>
<td>DS-GPH</td>
<td>0.179</td>
<td>1.43</td>
<td>353</td>
<td>91</td>
<td>0.97</td>
<td>4.8</td>
</tr>
<tr>
<td>DS-Pt</td>
<td>0.244</td>
<td>1.94</td>
<td>1</td>
<td>65</td>
<td>1.01</td>
<td>2.6</td>
</tr>
<tr>
<td>DS-Au</td>
<td>0.205</td>
<td>1.63</td>
<td>1</td>
<td>70</td>
<td>0.99</td>
<td>2.4</td>
</tr>
<tr>
<td>DS-GNP</td>
<td>0.169</td>
<td>1.35</td>
<td>254</td>
<td>111</td>
<td>1.06</td>
<td>9.9</td>
</tr>
<tr>
<td>DS-CNF-GNP</td>
<td>0.254 (f)</td>
<td>2.02</td>
<td>202</td>
<td>70</td>
<td>0.99</td>
<td>10.8</td>
</tr>
<tr>
<td>DS-GPH-GNP</td>
<td>0.282 (f)</td>
<td>2.24</td>
<td>259</td>
<td>70</td>
<td>1.00</td>
<td>13.2</td>
</tr>
<tr>
<td>DS-CNT-GNP</td>
<td>0.198 (f)</td>
<td>1.57</td>
<td>291</td>
<td>91</td>
<td>1.05</td>
<td>10.2</td>
</tr>
<tr>
<td>GC</td>
<td>0.108</td>
<td>1.54</td>
<td>4</td>
<td>70</td>
<td>1.02</td>
<td>1</td>
</tr>
</tbody>
</table>

(a) Calculated using equation 1, (average RSD = 5%, n = 3). (b) Also commonly referred to as ‘roughness factor’. Calculated using equation 2. (d) Average RSD = 2%, n = 3. (e) calculated using $mmol = \left(\frac{Q}{nF_A}\right) \times 1000$, where $Q$ is the charge at the electrode surface (the area under the TPrA oxidation CV peak, in coulombs), $n$ is the number of electrons transferred in the reaction, $F$ is the faraday constant (96485 C mol⁻¹) and $A$ is the geometric electrode area (cm²). (f) $n = 1$. The roughness factor ($A_{real}$) can also be calculated using the following equation;

$$II. \quad A_{real} = \frac{A_{rs}}{A_{geo}}$$

where $A_{geo}$ is the geometric area of the electrode. As expected, the modified electrodes generally showed an increase in $A_{real}$ when compared to standard carbon electrodes (except DS-OMC), and electrodes modified with two different nano-
materials (e.g. nanotubes and gold nano-particles) exhibited a further increase in $A_{\text{real}}$. High resistance can adversely affect the reversibility of electron transfer reactions at the electrode surface.\textsuperscript{290} We observed minimal resistance in both DS-Au and DS-Pt electrodes, due to the high conductivity of gold and platinum metals. Carbon electrodes showed much higher resistance. The Zensor displayed the lowest resistivity (115 $\Omega$) of the carbon electrodes. The resistivity of the DropSens carbon-based varieties varied between 202 and 477 $\Omega$. The Kanichi electrodes, unlike DropSens and Zensor varieties, do not have an underlying silver track between the working electrodes and the connectors and displayed the highest resistivity at 1966 $\Omega$.

6.4.2 SEM Characterisation

SEM images showed distinct variations between the different modified electrodes (Figures 6.1 and AIV.2). Unmodified Kanichi, DropSens and Zensor carbon electrodes displayed a similar surface profile, with visible graphitic particles surrounded by a binding polymer, as previously observed by Banks and co-workers.\textsuperscript{283} Carbon electrodes modified with GNPs showed a similar surface structure to DS-C electrodes with the addition of GNPs present on the surface ranging between ~30-100 nm in diameter. Graphene modified electrodes exhibited distinct graphene ‘shards’ and electrodes modified with both graphene and GNPs displayed spherical GNPs distributed across the graphene shards. Electrodes modified with carbon nanotubes and carbon nanofibers both exhibited ‘web-like’ appearances and the respective GNP derivatives showed spherical GNPs embedded in the web-like surface. Platinum and gold electrodes both displayed distinct metallic crystalline ‘bead’ structures. It is possible to visualise the electroactive surface area on each electrode variety using the SEM images, as the exposed edge or plane-like surfaces are the predominant source of electron transfer in carbon-based electrodes.\textsuperscript{283, 291}
Figure 6.1. SEM characterisation of commercially available SPE varieties. A) Kanichi. B) DS-GNP. C) DS-GPH. D) DS-GPH-GNP. E) DS-CNT. F) DS-CNFM. G) DS-CNT-GNP. H) DS-CNFM-GNP. I) DS-Pt. J) DS-Au. Additional SEM images are included in Figure AIV.2.

6.4.3 Electрогенерated Chemiluminescence

CVs of [Ru(bpy)₃]²⁺ in PBS showed a reversible oxidation peak at 0.92 V vs Ag/AgCl (representative CVs are shown in Figure 6.2. For all CVs, see Figure AIV.5). CVs of gold or gold nanoparticle modified electrodes also exhibited additional waves corresponding to the formation of gold-oxides at the electrode surface. The formation of surface oxides began at ~0.7 V vs Ag/AgCl (appearing as a shoulder on the [Ru(bpy)₃]²⁺ oxidation wave) and in the reverse sweep, we observed the corresponding reduction of the surface oxide layer at ~0.3 V vs Ag/AgCl.²⁵,²⁴⁴,²⁹² We also collected CVs of TPrA at 100 mM (Figure AIV.6) to monitor the extent of TPrA oxidation, an important, rate-limiting step in ECL.⁶,¹⁷,²⁴⁴
Figure 6.2. Representative CVs of 1 mM [Ru(bpy)$_3$]$_{2+}$ in 0.1 M PBS (scan rate 0.1 V/s). A) Kanichi. B) DS-CNT. C) DS-Au. D) DS-GNP. E) DS-GPH. F) DS-GPH-GNP.

The generalised mechanism for [Ru(bpy)$_3$]$_{2+}$ ECL with TPrA is outlined in reaction steps 1–9 below:

(1) [Ru(bpy)$_3$]$_{2+}$ – e$^-$ → [Ru(bpy)$_3$]$_{3+}$

(2) TPrA – e$^-$ → TPrA$^{+*}$

(3) [Ru(bpy)$_3$]$_{3+}$ + TPrA → [Ru(bpy)$_3$]$_{2+}$ + TPrA$^{+*}$

(4) TPrA$^{+*}$ → TPrA$^+$ + H$^+$

(5) [Ru(bpy)$_3$]$_{3+}$ + TPrA$^+$ → [Ru(bpy)$_3$]$_{2+}$ + Pr$_2$N=C=H$_2$CH$_3$

(6) [Ru(bpy)$_3$]$_{2+}$ + TPrA$^+$ → [Ru(bpy)$_3$]$^+$ + Pr$_2$N=C=H$_2$CH$_3$

(7) [Ru(bpy)$_3$]$_{3+}$ + [Ru(bpy)$_3$]$^+$ → [Ru(bpy)$_3$]$_{2+}$ + [Ru(bpy)$_3$]$_{2+}$

(8) [Ru(bpy)$_3$]$^+$ + TPrA$^{+*}$ → [Ru(bpy)$_3$]$_{2+}$ + TPrA
(9) \([\text{Ru(bpy)}_3]^{2+*} \rightarrow [\text{Ru(bpy)}_3]^{2+} + h\nu (\lambda_{\text{max}} = 620 \text{ nm})\)

For our ECL comparison experiments, we selected relatively low \([\text{Ru(bpy)}_3]^{2+}\) concentrations (1 × 10\(^{-7}\) M and 1 × 10\(^{-8}\) M) to reflect the low concentrations of metal complex present in bioanalytical ECL applications.\(^3,17\) In magnetic bead based assays, where \([\text{Ru(bpy)}_3]^{2+}\) is localised too far away from the electrode to undergo direct oxidation, and at low concentrations of \([\text{Ru(bpy)}_3]^{2+}\), the amount of formation of \([\text{Ru(bpy)}_3]^{3+}\) via reaction 1 is small and ECL is predominantly produced by reactions 2, 4, 6, 8, and 9. We calculated ECL intensities relative to the Zensor carbon electrodes and (corrected for differences in geometric working electrode area), as shown in Figure 6.3. DS-C and Zensor electrodes displayed the highest ECL intensities, followed in decreasing order by DS-CNT > DS-GPH > Kanichi > DS-GNP > DS-CNF > DS-OMC > DS-GPH-GNP > DS-CNT-GNP > DS-Pt > DS-Au and DS-CNF-GNP. This trend was consistent within experimental error across the two concentrations we examined (1 × 10\(^{-7}\) M and 1 × 10\(^{-8}\) M).
Figure 6.3. ECL intensities from various SPEs relative to Zensor, conducted in pH 7.5 PBS, 100 mM TPrA. A) $1 \times 10^{-7}$ M $[\text{Ru(bpy)}_3]^{2+}$. B) $1 \times 10^{-8}$ M $[\text{Ru(bpy)}_3]^{2+}$ (n = 3). The intensities are corrected for the difference in geometric working electrode area (Zensor and Kanichi SPEs have 3 mm diameter, whereas DS varieties have 4 mm diameter).
We selected the five electrodes exhibiting the highest ECL intensities (Zensor, DS-C, DS-CNT, DS-GPH and Kanichi) to carry out a magnetic bead-based DNA assay. We chose a bead-based assay using DNA bound to a magnetic bead to mimic a nucleic bead-based assay testing for a short length polymerase chain reaction (PCR) product. Using pre-bound DNA eliminates the experimental variability associated with primer recognition and binding, meaning any variation in signal will result only from differences in the working electrode material, relative ECL intensities are summarised in Figure 6.4. Zensor electrodes displayed the highest relative ECL intensity followed by DS-C and Kanichi. We observed a significant decrease in the relative ECL from both DS-CNT and DS-GPH electrodes in the bead-based assay experiments, when compared to experiments with free complex.

![Figure 6.4. Relative ECL intensity (corrected for working electrode area) for bead-based DNA assay (100 mM TPrA, 0.2 M PBS, pH 7.5, n = 3).](image-url)
Carbon-based electrodes are ideal for analytical ECL applications because they: have fast and efficient TPrA oxidation;\textsuperscript{15, 17, 244} are relatively hydrophobic (contact angle measurements for each electrode are included in Table S1),\textsuperscript{280, 281, 293} allowing high concentrations of TPrA to be present at the electrode surface; and have low rates of surface oxide formation compared to noble metal electrodes.\textsuperscript{244} These three factors result in the high ECL response we observed from carbon-based electrodes compared to DS-Pt, DS-Au, and electrodes with GNPs. Despite many publications employing nanostructured carbon materials for diagnostic ECL applications,\textsuperscript{182, 294} including GNPs,\textsuperscript{295} OMC,\textsuperscript{296} GPH,\textsuperscript{297} and CNTs,\textsuperscript{129, 140} we observed no enhancement of the ECL signal from electrodes composed of these materials in our experiments when compared to unmodified carbon SPEs. Generally, nanomaterial electrodes exhibit superior ECL signals in assays where the functionality of the nanomaterial is physically integrated into the assay procedure.\textsuperscript{13, 74, 104, 119, 129, 131-133, 136, 140, 182, 271, 288, 289, 294, 295, 298-301} For example, Guo, \textit{et al.}\textsuperscript{297} developed an ‘in-electrode’ biosensor by functionalising two separate graphene sheets with a capture antibody (this sheet was then immobilised on the electrode surface) and a detection antibody (this secondary sheet was then functionalised with an electrochemiluminophore). When the bioconjugate was assembled on the electrode surface, the two conductive graphene sheets served to extend the effective working electrode area, meaning all of the electrochemiluminophores were within the distance required for direct oxidation to produce ECL, unlike a conventional bead type assay, where the electrochemiluminophores may be located far outside of the electrode double layer.\textsuperscript{297} Many nanomaterials also find applications with alternative co-reactants and luminophores which are beyond the scope of this evaluation.\textsuperscript{131, 289, 294, 300} Although commercially available SPEs composed of carbon and nanomaterials showed no
increase in ECL intensity when compared to unmodified carbon electrodes in our cyclic voltammetry experiments; nanomaterials do present opportunities for innovative exploitation and modification when compared to classic carbon, platinum or gold electrodes; such as the aforementioned example. In the case of platinum and gold SPEs, and to a lesser extent in gold nanoparticle modified electrodes, a significant cathodic ECL signal was also observed, triggered by the reaction of $[\text{Ru(bpy)}_3]^{2+}$ with reactive oxygen species (ROS). These ROS are formed upon reduction of the oxide layers at noble metal electrodes and the reduction of dissolved oxygen species. Negligible cathodic ECL was observed at carbon-based electrodes.

6.4.4 Effect of the Addition of Surfactants on ECL Intensity

Surfactants such as Tween20 or Triton X-100 are regularly employed to enhance ECL at the electrode surface; the structures of these surfactants are displayed in Figure AIV.7. Surfactants increase the hydrophobicity of noble metal electrode surfaces, thereby increasing the concentration of TPrA at the electrode surface and facilitating higher rates of TPrA oxidation. We investigated the effect of two surfactants, Tween20 (T20) and Triton X-100 (TX), at concentrations of 0.1% and 1% at both DS-Pt and DS-Au electrodes on ECL intensity (Figure 6.5). We observed minimal enhancement at DS-Au electrodes with the addition of surfactant, but DS-Pt electrodes exhibited up to 3-fold enhancement with the addition of 0.1% TX. We observed lower ECL intensities at both DS-Pt and DS-Au electrodes with the addition of either 1% T20 or TX. As expected, we found no significant ECL enhancement with the addition of surfactant at DS-C electrodes (Figure AIV.8).
6.4.5 Stability of ECL Over Multiple Potential Cycles

Another important characteristic of electrodes for ECL applications is the stability of the ECL response over multiple scans or potential cycles. Traditional electrodes are polished between ECL measurements to refresh the electrode surface and ensure reproducible results, but SPEs cannot be polished. To investigate this property, we conducted three potential scans at 0.1 V/s between 1.6 V to -1.2 V (vs Ag/AgCl) and measured the ECL response for each scan (Figures AIV.5 and AIV.9). Zensor, Kanichi and DropSens carbon electrodes generally displayed a consistent
decrease in ECL with scan number. This results from several factors including deprotonation of the TPrA radical cation by oxygen containing surface species, increased oxidative consumption of the TPrA radical species, and passivation of the electrode surface caused by the attachment of dipropylamine (a side-product of TPrA’ oxidation) to the electrode surface after oxidation. A generalised reaction mechanism for this process has been included in the supplementary information. The net result of these three factors is a steady passivation of the electrode surface, causing a decrease in ECL signal with scan number. In contrast, we observed an increase in ECL response with scan number when using electrodes composed of platinum and certain carbon nanomaterials. Presumably, in the case of platinum electrodes this increase results from the intense cathodic ECL signal. In the case of SPEs modified with nanomaterials, it is possible that the nanomaterials may also be inherently stable and ‘resistant’ to passivation when compared to unmodified carbon (graphite) electrodes. When compared to a classic 3 mm GC electrode, we observed poor relative ECL intensities from commercially available SPEs; ranging from 4% for DS-Pt electrodes to 47% for Zensor electrodes (GC 100%). However, we observed a consistent decrease with scan number similar to that observed for unmodified carbon SPEs. Commercially available SPEs are considerably cheaper and easier to use (they do not require polishing), and therefore, SPEs are suitable for single-use experiments and applications where disposability is preferred; for example, dealing with bio-hazards, biological samples or in systems that are frequently contaminated (e.g. RNA assays).
6.4.6 Considerations and Future Work

We selected cyclic voltammetry, in stead of chronoamperometry, for ECL generation in our experiments because it provides information about the potential dependence of ECL processes; this is of particular interest to our research group.\textsuperscript{142, 148} Furthermore, we observed higher variability in chronoamperometry experiments (average RSD 9%, n = 3) when compared to cyclic voltammetry experiments (average RSD 3%, n = 3) and it was not possible to detect ECL using chronoamperometry from all electrodes at the same concentration of [Ru(bpy)$_3$]$^{2+}$ (see Figure AIV.10). However, we observed different relative ECL intensities from chronoamperometry experiments (we used 0.5 s pulse to 1.4 V \textit{vs} Ag/AgCl) when compared to cyclic voltammetry experiments; presumably, the reduced timespan of the applied potential, means that electrode passivation is minimised and effects such as the electro-active surface area become more important for ECL intensity. A follow-up study should investigate the difference in relative ECL intensities generated by cyclic voltammetry \textit{vs} chronoamperometry. This comparison would be a useful tool for scientists to develop and improve their assays by carefully evaluating the optimal ECL generation mechanism for their electrode variety and application.
6.5 Conclusions

We have compared a variety of commercially available SPEs for their application in ECL sensing. In our cyclic voltammetry experiments, unmodified carbon-based SPEs displayed the highest relative ECL intensities (Zensor 100%, DS-C 61% and Kanichi 45%), the incorporation of nanomaterials did not significantly enhance the ECL intensity in our experiments (such as GNPs 19%, CNTs 45%, CNFs 21%, GPH 48% and OMC 21%); despite their high electro-active surface areas. Platinum and gold electrodes exhibited poor relative ECL intensities (16% and 10%), due to their high rates of surface oxide formation and inefficient oxidation of TPrA. However, the ECL signal at platinum electrodes can be enhanced ~3-fold with the addition of a surfactant. Our results also demonstrate that these SPEs should only be used once—especially for long cyclic voltammetry experiments—as we observed a significant change in ECL intensity over repeated scans and SPEs cannot be polished to refresh the electrode surface.

6.6 Acknowledgements

The authors would like to acknowledge Dr Yi Heng Nai, for his assistance with the bead-based DNA assay and Miss Kara Spilstead, for collection of SEM images. The present work was carried out with the support of the Deakin Advanced Characterisation Facility. E.K. thanks Endeavour Scholarships and Fellowships, DFAT, Australian Government. M.T.F.A.
Conclusions and Future Work

This work investigates the electrochemical and luminescent properties of a variety of ruthenium and iridium metal chelates. Mixtures of iridium and ruthenium metal chelates for annihilation ECL has been thoroughly investigated and multiple previously conflicting observations have been consolidated. Multi-colour ECL has been observed from multiple combinations of emitters and the observed colour has been found to depend on a number of factors including the quantum efficiency and concentration of the luminophores as well as the applied potential and energy transfer pathways available in each system. This section of work has particularly interesting potential applications in the area of photovoltaics and for this research to be translated to a real-world application significant effort should be invested to further evaluate the energy transfer processes available in each system prior to incorporating an optimised system into a thin film display.

A variety of water soluble iridium complexes have been evaluated for their suitability for ECL in aqueous solution with co-reactant TPrA. One emitter, [Ir(dfppy)2(pt-TEG)]+, was found to exhibit intense ECL with co-reactant TPrA and is particularly promising for incorporation into bio-assays as a NA or antibody label. However, for any of these water soluble iridium complexes to be successfully incorporated into a bioanalytical ECL assay, they must first be tested with a wide variety of co-reactants to establish a system with optimal energetics; as both direct and indirect ECL pathways with TPrA are precluded in label-based bio-assays with most blue-emissive iridium complexes due to electrode proximity and energetics.

Chemiluminescence studies of water soluble iridium complexes revealed striking differences in selectivity when compared to [Ru(bpy)]32+ and provide interesting
opportunities for expanding the current variety of analytes that can be detected using chemiluminescence. Future studies in this work should involve the strategic structural modification of these iridium complexes and testing a wider variety of analytes to fully evaluate their selectivity.

Finally, a range of commercially available SPEs have been compared for their suitability for bioanalytical ECL assays. Graphitic carbon SPEs showed the highest ECL intensities when using cyclic voltammetry for ECL generation. In order to fully evaluate each SPE for use in ECL experiments the electrodes should also be compared using chronoamperometric ECL generation. The comparatively short time-frame of a chronoamperometry ECL experiment would minimise variables such as electrode passivation that may disproportionately disadvantage modified electrodes.
References

26. G. J. Barbante, Doctor of Philosophy, La Trobe University, 2011.
127. E. Doeven, Doctor of Philosophy, La Trobe University, 2012.
139. S. Wang, L. Ge, Y. Zhang, X. Song, N. Li, S. Ge and J. Yu, Lab Chip, 2012, 12, 4489-4498.


Appendices

Appendix I

Chapter 3: New Perspectives on the Annihilation Electrogenerated Chemiluminescence of Mixed Metal Complexes in Solution

A 1.1 Energetics of Annihilation ECL

A detailed account of this topic can be found within the excellent explanation of “Marcus theory in the qualitative and quantitative description of electrochemiluminescence phenomena” by Andrzej Kapturkiewicz (Adv. Electrochem. Sci. Eng., 1997, 5, 1-60). A brief summary adapted to our description of the annihilation ECL of mixed metal complex systems is presented below:

The Gibbs free energy of the annihilation reaction to form ground electronic state products (reaction 3c in our paper) can be calculated as:

$$\Delta G_0 = E^\circ_{D+/D} - E^\circ_{A/A^-} - w_r + w_p$$

where $w_r$ is the energy required to bring the reactants together to the most probable separation distance at which the electron transfer takes place, and $w_p$ is the energy required to bring the product into the precursor complex.

Similarly the Gibbs free energy for the formation of products with one in an electronically excited state (reactions 3a and 3b in our paper) can be calculated as:

$$\Delta G_0 = E^\circ_{D+/D} - E^\circ_{A/A^-} - w_r + w_p + E_{es}(D^*)$$

$$\Delta G_0 = E^\circ_{D+/D} - E^\circ_{A/A^-} - w_r + w_p + E_{es}(A^*)$$
These equations are not strictly correct because of the combination of Gibbs energy and energy terms. To overcome this inconsistency, the entropic contribution $\Delta S$ can be calculated as follows:

$$
\Delta S = -\frac{\partial \Delta G_0}{\partial T} = -\frac{\partial}{\partial T} \left[ (E_{D+/D^0}^\circ - E_{A/A^0}^\circ) - w_r + w_p \right] \partial T
$$

However, only small values for $\Delta S$ (compared than those for the isolated ions) are generally obtained, due to the correction for the Coulombic interaction energy terms, and at least in the first approximation, the entropic contribution can be neglected.

Figure A1.1. (a) Sectioned view and (b) 3D depiction of the custom built cell holder for ECL.
Figure A1.2. (a) A 3D representation of the ECL of the \([\text{Ru}(bpy)_3]^{2+}\)-[Ir(ppy)_3] mixed annihilation system spectra showing ECL intensity versus emission wavelength and applied reductive potential (V vs Fe^{0/+/+}). Data were obtained using an automated chronoamperometry procedure with an oxidative potential of 0.43 V and a series of reductive potentials spaced 50 mV apart, using 0.01 mM \([\text{Ru}(bpy)_3]^{2+}\) and 0.24 mM \([\text{Ir}(ppy)_3]\) in acetonitrile with 0.1 M TBAPF_6.

(b) The corresponding portion of a cyclic voltammogram of 0.25 mM \([\text{Ru}(bpy)_3]^{2+}\) and 0.25 mM \([\text{Ir}(ppy)_3]\) (0.1 M TBAPF_6, acetonitrile), showing: (1) \([\text{Ru}(bpy)_3]^{1+/2+}\), (2) \([\text{Ru}(bpy)_3]^{0/1+}\); (3) \([\text{Ru}(bpy)_3]^{1-/0}\), and (4) a combination of \([\text{Ir}(ppy)_3]^{1-/0}\) and \([\text{Ru}(bpy)_3]^{2+/1-}\).
Figure A1.3 (a) 3D representations (normalised ECL intensity versus emission wavelength and applied reductive potential) for the [Ru(bpy)$_3$]$_{2+}$-[Ir(ppy)$_3$] mixed annihilation system using three different concentrations of [Ru(bpy)$_3$]$_{2+}$: (a) 0.005 mM, (b) 0.010 mM, and (c) 0.015 mM, where the X axis is potential (V vs Fc$^{0+/+}$) and Y axis is wavelength (nm). Each data set was obtained using 0.24 mM [Ir(ppy)$_3$], with an oxidative potential of 0.99 V and a series of reductive potentials spaced 50 mV apart. Complexes were prepared in acetonitrile with 0.1 M TBAPF$_6$. 
Figure AI.4. (a) Electron transfer between the ground and excited states of the most stable oxidation state complexes in the \([\text{Ru(bpy)}_3]^{2+}-[\text{Ir(ppy)}_3]\) mixed annihilation ECL system; (b) Generalised depiction of stepwise process; and (c) Concerted electron exchange.
Figure A1.5. (a) Redox potentials for ground and excited states within the $[\text{Ru}(bpy)_3]^{2+}$-[Ir(df-ppy)$_2$(bpy)]$^+$ system examined by Moon and co-workers (J. Am. Chem. Soc., 2014, 136, 3705-3712), showing the reaction proposed by the authors to account for the absence of ECL from the iridium-complex component. (b) The analogous electron transfer in the $[\text{Ru}(bpy)_3]^{2+}$-[Ir(ppy)$_3$] system.

Data for Figure A1.5(a): Moon, et al.\textsuperscript{200} reported the electrochemical potentials of the $[\text{Ru}(bpy)_3]^{2+}$ complex in acetonitrile as 0.91 V and -1.24 V vs Fc$^{0+/+}$. The oxidative potential is in reasonable agreement with previous data, but the reductive potential is significantly different (c.f. Figure 1 in main text),\textsuperscript{216} which appears to be due to an uncharacteristic artifact within their cyclic voltammogram\textsuperscript{200} for that complex. For Figure A1.5(a), we used the potentials for the ground and $^3$MLCT excited state of...
[Ru(bpy)_3]^{2+} reported by Juris, et al.\textsuperscript{216} (ground state: 1.26 V and -1.35 V; excited state: -0.87 V and 0.78 V vs SCE), referenced to the ferrocene/ferrocenium couple using the conversion constant of -380 mV in acetonitrile at 25°C,\textsuperscript{308} to give: -1.25 V and 0.40 V vs Fe^{0/+} (excited state); 0.88 V, -1.73 V vs Fe^{0/+} (ground state), which are in good agreement with our data. The subsequent reduction potentials were from our data (Figure 1).

The potentials reported by Moon, et al.\textsuperscript{200} for the [Ir(df-ppy)_2(bpy)]\textsuperscript{+} complex (1.23 V and -1.34 V vs Fe^{0/+}) in acetonitrile are also questionable, because the difference between these potentials of 2.57 V is unexpectedly low for an iridium complex exhibiting green luminescence. Unfortunately, the previously reported potentials for [Ir(df-ppy)_2(bpy)]\textsuperscript{+} are inconsistent.\textsuperscript{200, 309, 310} For Figure AI.5(a), we used the data reported by Singh, et al.\textsuperscript{310} which included an estimation of the potentials of the excited state complex. Although Singh, et al.\textsuperscript{310} stated that their potentials (obtained in acetonitrile) were reported relative to the ferrocene/ferrocenium redox couple, this does not appear to be the case, considering the values presented for well-known complexes such as [Ir(ppy)_3]. After correction (by -0.40 V) to account for this, we obtain 1.26 V and -1.72 V vs Fe^{0/+} (ground); -1.20 V and 0.74 V vs Fe^{0/+} (excited). The ground state oxidative potential 1.26 V vs Fe^{0/+} is similar to that reported by Moon, et al.\textsuperscript{200} (1.23 V vs Fe^{0/+}) and the reductive potential (-1.72 V vs Fe^{0/+}) is visually coincident with the second reduction peak on the cyclic voltammogram presented by Moon, et al.\textsuperscript{200} for that complex.

To confirm our selection of this data, we synthesised and characterised the [Ir(df-ppy)_2(bpy)][PF_6] complex (details in A 1.2). Our cyclic voltammetry (E\textsubscript{ox} = 1.20 V vs Fe^{0/+}, E\textsubscript{red} = -1.72 V vs Fe^{0/+}; Figure AI.9a) was in reasonable agreement with that of Singh, et al.\textsuperscript{310} after the correction noted above.
181

Figure AI.6. (a) Redox potentials for ground and excited states within the [Ru(dtb-bpy)₃]²⁺-[Ir(ppy)₂(CN)₂]⁻ system reported by Swanick and co-workers (Chem. Eur. J., 2015, 21, 7435-7440), showing the pathway for the electrocatalytic reduction of the ruthenium complex proposed by the authors to account for the absence of emission from the iridium component of the mixed system. (b) The analogous electron transfer in the [Ru(bpy)₃]²⁺-[Ir(ppy)₃]⁻ system.

Data for Figure AI.6(a): The electrochemical potentials for the ground state [Ir(ppy)₂(CN)₂]⁻ complex were obtained from Swanick, et al.²¹⁰ (0.98 V (irreversible) and -2.32 V vs SCE) and referenced to the ferrocene/ferrocenium couple using the

---

**Table**

<table>
<thead>
<tr>
<th>Potential/V (vs Fc⁰⁺)</th>
<th>[Ru(dtb-bpy)₃]²⁺</th>
<th>[Ir(ppy)₂(CN)₂]⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 8**

(a) [Graph of potential vs Fc⁰⁺ for [Ru(dtb-bpy)₃]²⁺ and [Ir(ppy)₂(CN)₂]⁻]

---

**Figure 13**

(b) [Graph of potential vs Fc⁰⁺ for [Ru(bpy)₃]²⁺ and [Ir(ppy)₃]⁻]
conversion constant\(^{308}\) of -380 mV in acetonitrile at 25°C (to give 0.60 V and -2.70 V vs Fe\(^{0+/+}\)). The potentials for the excited-state [Ir(ppy)\(_2\)(CN)\(_2\)]\(^*\) complex (-2.11 V and 0.01 V vs Fe\(^{0+/+}\)) were estimated as the difference between the potentials of the ground state complex and the \(E^{00}\) energy of the low-temperature \(^3\)MLCT emission, which was reported by Li, et al.\(^{59}\) as 458 nm (2.71 eV) in 2-methyltetrahydrofuran at 77 K, and by Chan, et al.\(^{311}\) as 457 nm (2.71 eV) in 4:1 (v/v) ethanol:methanol at 77 K. We note that the potentials obtained in acetonitrile by Swanick, et al.\(^{210}\) for the ground state [Ir(ppy)\(_2\)(CN)\(_2\)]\(^-\) are similar to those obtained in dimethylformamide by Li, et al.\(^{59}\) (0.50 V (irreversible) and -2.78 V vs Fe\(^{0+/+}\)) and in dichloromethane by Di Censo, et al.\(^{312}\) (0.55 V (quasi-reversible) and -2.69 V vs Fe\(^{0+/+}\)).

The electrochemical potentials for the ground state \([\text{Ru}(\text{dtb-bpy})_3]^{2+}\) complex were obtained from Swanick, et al.\(^{210}\) (1.11 V and -1.45 V vs SCE) and referenced to the ferrocene/ferrocenium couple using the conversion constant of -380 mV in acetonitrile at 25°C,\(^{308}\) to give 0.73 V and -1.83 V vs Fe\(^{0+/+}\), which was in good agreement with previously published data.\(^{216}\) The subsequent reduction potentials (-1.99 V, -2.24 V and -2.98 V vs Fe\(^{0+/+}\)) were derived from the electrochemical data of the \([\text{Ru}(\text{dtb-bpy})_3][\text{Ir}(\text{ppy})_2(\text{CN})_2]_2\) soft salt.\(^{210}\) The excited-state potentials (-1.43 V and 0.34 V vs Fe\(^{0+/+}\)) were estimated based on the low temperature \(E^{00}\) emission energy, which was reported by Juris, et al.\(^{216}\) to be 575 nm (2.16 eV) in 4:1 (v/v) methanol:ethanol at 77 K.

To confirm the oxidation and reduction potentials, we synthesised the \([\text{Ru}(\text{dtb-bpy})_3][(\text{PF}_6)_2]\) and \([\text{TBA}][\text{Ir}(\text{ppy})_2(\text{CN})_2]\) complexes (details on pages S16-S17). Our cyclic voltammetry \((E_{\text{ox}} = 0.73 \text{ V}, E_{\text{red}} = -1.83 \text{ V}, -2.01 \text{ V}, -2.30 \text{ V vs Fe}^{0+/+}\) for \([\text{Ru}(\text{dtb-bpy})_3][(\text{PF}_6)_2]\), and \(E_{\text{ox}} = 0.58 \text{ V}, E_{\text{red}} = -2.71 \text{ V vs Fe}^{0+/+}\) for \([\text{TBA}][\text{Ir}(\text{ppy})_2(\text{CN})_2]\);
Figure A.10a was in good agreement with that reported by Swanick and co-workers.\textsuperscript{210}

**Figure A1.7.** Normalised annihilation ECL spectra (applied potentials: 0.98 V and -1.82 V vs Fc\textsuperscript{0/+}) for a mixture of 0.006 mM [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} and 0.100 mM [Ir(ppy)\textsubscript{3}] (red plot), and [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} only (blue plot), in acetonitrile with 0.1 M TBAPF\textsubscript{6}. In this experiment the applied electrode potentials are 100 mV beyond the oxidation and first reduction of the [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} complex. At 0.98 V, the [Ir(ppy)\textsubscript{3}] complex is oxidised, but at -1.83 V it is not reduced. As described in our previous work (Kerr et al., *Chemical Science*, 2015, 6, 472-479), the annihilation ECL reaction between [Ru(bpy)\textsubscript{3}]\textsuperscript{2+} and [Ir(ppy)\textsubscript{3}] is sufficiently energetic to form [Ru(bpy)\textsubscript{3}]\textsuperscript{2+*}, but not [Ir(ppy)\textsubscript{3}]\textsuperscript{*}.\textsuperscript{210}
Figure A1.8. Cyclic voltamogram of (a) \([\text{Ir(ppz)}_3]\), (b) a mixture of \([\text{Ir(ppz)}_3]\) and \([\text{Ru(bpy)}_3]^{2+}\), and (c) \([\text{Ru(bpy)}_3]^{2+}\), showing \(E^0\) values. Scan rate: 0.1 V s\(^{-1}\). Complexes at 0.25 mM with 0.1 M TBAPF\(_6\) supporting electrolyte in acetonitrile.
Figure A1.9. (a) Cyclic voltammograms of $[\text{Ir(df-ppy)$_2$(bpy)}][\text{PF}_6]$ (green line) and $[\text{Ru(bpy)$_3$}][\text{(PF}_6)_2$] (red line) at 0.5 mM with 0.1 M TBAPF$_6$ supporting electrolyte in acetonitrile. Scan rate: 0.1 V s$^{-1}$. Potentials for $[\text{Ir(df-ppy)$_2$(bpy)}][\text{PF}_6$]: $E_{\text{ox}} = 1.20$ V vs Fc$^{0/+}$ and $E_{\text{red}} = -1.72$ V vs Fc$^{0/+}$. (b) Absorption spectrum (dashed black line) and corrected photoluminescence emission spectrum (green line) of $[\text{Ir(df-ppy)$_2$(bpy)}][\text{PF}_6$] at 10 μM. These metal complexes were used by Moon et al. (J. Am. Chem. Soc., 2014, 136, 3705-3712) in their examination of the ECL of mixtures of metal complexes for flexible emissive displays.
Figure AI.10. (a) Cyclic voltammograms of [TBA][Ir(ppy)$_2$(CN)$_2$] (green line) and [Ru(dbtb-bpy)$_3$][(PF$_6$)$_2$] (red line) at 0.2 mM with 0.1 M TBAPF$_6$ in acetonitrile, scanning to the oxidation ($E_{ox} = 0.58$ V and 0.73 V vs Fe$^{0+/+}$) and first reduction ($E_{red} = -2.71$ V and -1.83 V vs Fe$^{0+/+}$) potentials. The 2$^{nd}$ and 3$^{rd}$ reductions of [Ru(dbtb-bpy)$_3$][(PF$_6$)$_2$] were at -2.01 V and -2.30 V vs Fe$^{0+/+}$. When scanning beyond the first reduction of [Ru(dbtb-bpy)$_3$][(PF$_6$)$_2$]
(e.g., dashed line), we observed a large peak at -1.76 V vs Fe^{III/+} during the return scan. Scan rate: 0.1 V s^{-1}. (b) Absorption spectrum (dashed line) and corrected emission spectrum (green line) of [TBA][Ir(ppy)_2(CN)_2] at 10 μM. (c) Absorption spectrum and corrected emission spectrum of [Ru(dtb-bpy)_3][(PF_6)_2] at 10 μM. These complexes were previously examined by Swanick et al. (Chem. Eur. J., 2015, 21, 7435-7440) in their study of the ECL of the [Ru(dtb-bpy)_3][Ir(ppy)_2(CN)_2_2] soft salt.
Figure A1.11. Annihilation ECL spectra from (a) 0 and 0.005-0.120 mM [Ru(bpy)$_3$][(PF$_6$)$_2$] with 0.12 mM [Ir(ppy)$_3$], using 0.99 V and -2.77 V vs Fe$^{0+/+}$; (b) 0.005-0.120 mM [Ru(bpy)$_3$][(PF$_6$)$_2$], using 0.99 V and -2.77 V vs Fe$^{0+/+}$; (c) 0 and 0.005-0.120 mM [Ru(dtb-bpy)$_3$][(PF$_6$)$_2$] with 0.12 mM [TBA][Ir(ppy)$_2$(CN)$_2$], using 0.83 V and -2.81 V vs Fe$^{0+/+}$; (d) 0 and 0.005-0.120 mM [Ru(dtb-bpy)$_3$][(PF$_6$)$_2$], using 0.83 V and -2.81 V vs Fe$^{0+/+}$; (e) 0.005-0.120 mM [Ru(bpy)$_3$][(PF$_6$)$_2$] with 0.12 [Ir(df-ppy)$_2$(bpy)][(PF$_6$)$_2$], using 1.20 V and -1.82 V vs Fe$^{0+/+}$; (f) 0 and 0.005-0.120 mM [Ru(bpy)$_3$][(PF$_6$)$_2$], using 1.20 V and -1.82 V vs Fe$^{0+/+}$. In each case, complexes were prepared in acetonitrile containing 0.1 M TBAPF$_6$. A two-step potential pulse was applied at 10 Hz for 12 s. The arrows show the change in emission intensity with increase in ruthenium complex concentration (after deconvolution procedure show in Figure A1.12 was applied).
Figure AI.12. Examples of the deconvolution of the annihilation ECL spectra from mixed system into the characteristic spectra of the two individual metal complexes, using the Solver function of Microsoft Excel software. Complexes: (a) 0.01 mM [Ru(bpy)₃][(PF₆)₂] and 0.12 mM [Ir(ppy)₃]; (b) 0.03 mM [Ru(bpy)₃][(PF₆)₂] and 0.12 mM [Ir(ppy)₃]; (c) 0.01 mM [Ru(dtb-bpy)₃][(PF₆)₂] and 0.12 mM [TBA][Ir(ppy)₂(CN)$_2$]; (d) 0.03 mM [Ru(dtb-bpy)₃][(PF₆)₂] and 0.12 mM [TBA][Ir(ppy)₂(CN)$_2$]; (e) 0.01 mM [Ru(bpy)₃][(PF₆)₂] and 0.12 [Ir(df-ppy)$_2$(bpy)][PF₆]; (f) 0.03 mM [Ru(bpy)₃][(PF₆)₂] and 0.12 [Ir(df-ppy)$_2$(bpy)][PF₆]; in acetonitrile containing 0.1 M TBAPF₆. In each case, the ECL was generated using a two-step potential pulse was applied at 10 Hz for 12 s. The applied potentials were: (a,b) 0.99 V and -2.77 V vs Fe$^{0+/+}$; (c,d) (0.83 V and -2.81 V vs Fe$^{0+/+}$; (e,f) 1.20 V and -1.82 V vs Fe$^{0+/+}$. 
Figure A1.12 (continued). (g) A repeat of Figure A.12e showing the sum of the two individual components (orange plot) overlaid onto the original ECL spectrum (black plot). (h) Annihilation ECL spectrum and deconvoluted emissions for a mixture of 0.06 mM [Ru(dtb-bpy)₃][(PF₆)₂] and 0.12 mM [TBA][Ir(ppy)₂(CN)₂]. Inset: zoomed in section of the graph showing the emission from the Ir complex.
Figure AI.13. Annihilation ECL intensities from: (a) [Ru(bpy)$_3$][(PF$_6$)$_2$] and [Ir(ppy)$_3$]; (b) [Ru(dtb-bpy)$_3$][(PF$_6$)$_2$] and [TBA][Ir(ppy)$_2$(CN)$_2$]; or (c) [Ru(bpy)$_3$][(PF$_6$)$_2$] and [Ir(df-ppy)$_2$(bpy)][PF$_6$], in acetonitrile containing 0.1 M TBAPF$_6$. The green plots are the ECL intensities of the Ir complex in the mixed solutions. The red and grey plots are the
ECL intensities of the Ru complex (from 0 to 0.12 mM) with and without the presence of 0.12 mM Ir complex, respectively. In each case, a two-step potential pulse was applied at 10 Hz for 12 s. The applied potentials were: (a) 0.99 V and -2.77 V vs Fc⁰⁺⁺, (b) (0.83 V and -2.81 V vs Fc⁰⁺⁺, (c) 1.20 V and -1.82 V vs Fc⁰⁺⁺. The ECL spectrum from each mixed solution was deconvoluted into its two characteristic components as shown in Figure A.12. The ECL intensities for the Ru and Ir complexes relative to standards of each individual complex are shown in Figure 3.8.
Figure AI.14. Quenching of the ECL of the Ir complex in solutions containing: (a) [Ir(ppy)$_3$] and [Ru(bpy)$_3$][(PF$_6$)$_2$]; (b) [Ir(df-ppy)$_2$(bpy)][PF$_6$] and [Ru(bpy)$_3$][(PF$_6$)$_2$] (white dots), or [TBA][Ir(ppy)$_2$(CN)$_2$] and [Ru(dtb-bpy)$_3$][(PF$_6$)$_2$] (black dots). The ECL was generated by applying potentials of sufficient magnitude to alternately oxidise and reduce both complexes in solution, as described in Figure AI.13. The deconvolution process (shown in Figure AI.12) was applied to all ECL spectra. $I_0$ is the integrated ECL peak area of Ir complex at a concentration of 0.12 mM in the absence of the Ru complex, whereas $I$ is the integrated ECL peak area of the Ir complex at a concentration of 0.12 mM in the presence of different concentrations of the Ru complex. The slope of the first three points of graph (a) is 957. Although this is lower than when including all points on this graph, is still much greater than the slopes calculated for the plots in graph (b).
Figure AI.15. Green plot and left axis: the ECL intensity from 0.12 mM \([\text{Ir(df-ppy)}_2(bpy)]\text{[PF}_6\text{]} \) in the presence of various concentrations of \([\text{Ru(bpy)}_3]\text{[(PF}_6\text{)}_2] \) (0.005 mM to 0.12 mM). Red plot and right axis: the ratio of the ECL from \([\text{Ru(bpy)}_3]\text{[(PF}_6\text{)}_2] \) in the presence and absence of 0.12 mM \([\text{Ir(df-ppy)}_2(bpy)]\text{[PF}_6\text{]} \). Conditions as described in Figure AI.13.

A 1.2 Synthesis and Characterisation of Metal Complexes

NMR data was collected using a Varian FT-NMR 400 spectrometer or Varian FT-NMR 500 spectrometer (Varian, CA, USA). \(^1\text{H}\) NMR spectra recorded at 400 or 500 MHz and \(^{13}\text{C}\{^1\text{H}\}\) NMR spectra recorded at 101 MHz and 126 MHz respectively. Chemical shifts were referenced to residual solvent signals and quoted in ppm relative to tetramethylsilane (TMS). \(^{19}\text{F}\) NMR spectra were collected at 470 MHz and chemical shifts were quoted relative to an internal standard of hexafluorobenzene (δ -164.9 ppm). ESI-MS spectra were recorded on an Agilent 6510 ESI-TOF LC/MS mass spectrometer (Agilent, CA, USA). Microwave reactions were carried out using a Biotage Initiator microwave reactor (Biotage, Uppsala, Sweden).

\([\text{Ir(df-ppy)}_2(bpy)]\text{[PF}_6\text{]} \)

Prepared by previously reported procedure\(^{313}\) with some modifications. A mixture of \([\text{Ir(ppy)}_2(\text{Cl})]_2 \) (0.30 g, 0.25 mmol) and 2,2′-bipyridine (0.076 g, 0.49 mmol) in
CH₂Cl₂/MeOH (30 mL, 2:1, v/v) was shielded from light and heated at reflux for 5 h. The mixture was cooled to ambient temperature and potassium hexafluorophosphate (0.10 g, 0.54 mmol) was added, then the mixture was shielded from light and stirred at ambient temperature for 2 days. The mixture was filtered to remove a colourless precipitate before removing the solvent by evaporation under reduced pressure. The crude product was loaded onto a silica gel column and eluted with CH₂Cl₂/acetone (15:1 v/v). The relevant fractions were collated and the solvent removed by evaporation under reduced pressure to afford a bright yellow solid (0.30 g, 0.34 mmol, 71%). ¹H-NMR (500 MHz; DMSO-d₆): δ  5.62 (dd, 2H, J = 8.4, 2.3 Hz), 6.97 (m, 2H), 7.24 (m, 2H), 7.71 (m, 4H), 7.93 (m, 2H), 8.04 (m, 2H), 8.31 (m, 4H), 8.90 (d, 2H, J = 8.2 Hz). ¹⁹F NMR (470 MHz; DMSO-d₆): δ -106.52, -104.37, -67.97. ESI-MS (+ve ion) m/z [M]+ 729.126 (experimental), 729.13 (calculated for [C₃₂H₂₀F₄IrN₄]+).

[Ru(dtb-bpy)₃]Cl₂

A microwave vial (10-20 mL) charged with [Ru(cod)Cl₂]ₙ (0.21 g, 0.75 mmol), 4,4′-di-tert-butyl-2,2′-bipyridine (0.61 g, 2.3 mmol) and dimethylformamide (10 mL) was sealed then heated to 150°C (30 min) then at 160 °C (30 min). The reaction was cooled to ambient temperature and an orange precipitate was observed upon addition of diethyl ether. The precipitate was isolated by filtration, washed with diethyl ether then air dried to afford an orange powder (0.32 g, 0.33 mmol, 44%). ¹H NMR (400 MHz; CD₃CN): δ  8.55 (s, 6H, pyrH), 7.55 (m, 6H, pyrH), 7.39 (m, 6H, pyrH), 1.41 (s, 54H, tBuH ). ¹³C NMR (101 MHz; CDCl₃): δ  163.4, 157.9, 151.7, 125.4, 122.8, 36.3, 30.5. ESI-MS (+ve ion) m/z [M]+²⁺ 453.308 (experimental), 453.24 (calculated for [C₅₄H₇₂N₆Ru]²⁺).
[TBA][Ir(ppy)$_2$(CN)$_2$]

[TBA][Ir(ppy)$_2$(CN)$_2$] was prepared by modification to a previously reported procedure. To a mixture of the dimeric iridium(III) complex [Ir(ppy)$_2$(Cl)$_2$] (0.24 g, 0.22 mmol) in dichloromethane (30 mL), was added tetrabutylammonium cyanide (0.62 g, 2.3 mmol). The reaction mixture was heated at reflux for 19 h then the volume of the solvent was reduced under a stream of N$_2$. A yellow precipitate was observed after addition of petroleum spirits (boiling range: 40-60°C, 60 mL). The precipitate was collected by filtration, washed with petroleum spirits and air-dried to afford a yellow powder (0.27 g, 0.34 mmol, 78%). $^1$H NMR (400 MHz; DMSO-d$_6$): δ 9.54 (m, 2H, pyrH), 8.07 (d, $^3$J$_{HH}$ = 8.4 Hz, 2H, ArH), 7.89 (m, 2H, pyrH), 7.66 (d, $^3$J$_{HH}$ = 7.6 Hz, 2H, pyrH), 7.30 (m, 2H, ArH), 6.73 (m, 2H, pyrH), 6.61 (m, 2H, ArH), 6.09 (m, 2H, ArH), 3.16 (m, 8H, CH$_2$), 1.56 (m, 8H, CH$_2$), 1.30 (m, 8H, CH$_2$), 0.93 (t, $^3$J$_{HH}$ = 7.3 Hz, 12H, CH$_3$). $^{13}$C{($^1$H) NMR (101 MHz; DMSO-d$_6$): δ 167.9, 164.0, 153.3, 144.4, 136.1, 130.78, 130.73, 128.2, 123.6, 122.5, 119.9, 118.9, 57.5, 23.1, 19.2, 13.5. ESI-MS (-ve ion) m/z [M]$^-$ 553.157 (experimental), 553.10 (calculated for [C$_{24}$H$_{16}$IrN$_4$]-).

[Ru(dtb-ppy)$_3$][Ir(ppy)$_2$(CN)$_2$]$_2$

The [Ru(dtb-ppy)$_3$][Ir(ppy)$_2$(CN)$_2$]$_2$ salt was prepared by modification to a previously reported procedure. A mixture of DCM (40 mL), [Ru(dtb-bpy)$_3$][Cl]$_2$ (0.099 g, 0.10 mmol) and [TBA][Ir(ppy)$_2$(CN)$_2$] (0.16 g, 0.20 mmol) was washed with water (7 × 20 mL) followed with brine (40 mL). The organic phase was dried (MgSO$_4$) and the solvent was removed. The product was recrystalised from a minimum of dichloromethane layered with diethyl ether to afford a red crystalline solid (0.065 g, 32%). $^1$H NMR (500 MHz; CD$_3$CN): δ 9.66 (m, 4H, pyrH), 8.51 (m, 6H, pyrH), 7.97
(m, 4H, ArH), 7.85 (m, 4H, pyrH), 7.64 (m, 4H, pyrH), 7.58 (m, 6H, pyrH), 7.40 (m, 6H, pyrH), 7.21 (m, 4H, ArH), 6.80 (m, 4H, pyrH), 6.70 (m, 4H, ArH), 6.22 (m, 4H, ArH), 1.42 (s, 54H, CH3). 13C{1H} NMR (126 MHz; CD3CN): δ 169.3, 165.1, 163.3, 157.9, 154.7, 151.7, 145.7, 137.0, 132.19, 132.09, 129.6, 125.5, 124.6, 123.6, 122.5, 121.3, 119.9, 36.3, 30.5. ESI-MS (+ve ion) m/z [M]2+ 453.248 (experimental), 453.24 (calculated for [C54H72N6Ru]2+). ESI-MS (-ve ion) m/z [M]- 553.114 (experimental), 553.10 (calculated for [C24H16IrN4]-).
Appendix II

Chapter 4: Blue Electrogenerated Chemiluminescence from Water-Soluble Iridium Complexes Containing Sulfonated Phenylpyridine or Tetraethylene Glycol Derivatised Triazolylpyridine Ligands

Details of Synthesis and Characterization

4-(Pyridin-2-yl)phenyl)methane sodium sulfonate (1) was purchased from SunaTech (P.R. China).

1-Benzyl-4-(2-pyridyl)-1,2,3-triazole (3a) was prepared over two steps on small scale following the procedure of Hiroki and co-workers,\textsuperscript{314} and on a larger scale following De Cola and co-workers,\textsuperscript{199} which gave a 54% yield (750 mg).

1-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl)-4-(2-pyridyl)-1,2,3-triazole (3b) was prepared as previously described.\textsuperscript{238,315}

(2-(2,4-Difluorophenyl)pyridin-4-yl)methanol (4): A mixture containing 2-bromopyridine-4-methanol (569 mg, 3.03 mmol) and K\textsubscript{2}CO\textsubscript{3} (839 mg, 6.07 mmol, 2.0 equiv) in PhCH\textsubscript{3} (18 mL), EtOH (1.5 mL) and H\textsubscript{2}O (3 mL) was purged with N\textsubscript{2} for 45 mins. Pd(PPh\textsubscript{3})\textsubscript{4} (177 mg, 0.15 mmol, 0.05 equiv) and 2,4-difluorobenzeneboronic acid (666 mg, 4.22 mmol, 1.4 equiv) were added and the solution was stirred at 80°C for 80 min. The reaction mixture was diluted with EtOAc (50 mL), washed using H\textsubscript{2}O (50 mL) and brine (50 mL), then dried (MgSO\textsubscript{4}), filtered and concentrated under reduced pressure. The resultant brown powder was suspended in CH\textsubscript{2}Cl\textsubscript{2} (5 mL), cooled to 0°C and the solid was collected by vacuum filtration, washing with cold CH\textsubscript{2}Cl\textsubscript{2} to give 4 (621 mg, 93%) as a beige powder. \textsuperscript{1}H NMR (270 MHz, DMSO-\textit{d}\textsubscript{6})
δ 8.63 (d, J = 4.9 Hz, 1H), 7.99 (app. td, J_{app} = 9.2, 6.7 Hz, 1H), 7.71 (s, 1H), 7.39 (ddd, J = 11.4, 9.4, 2.7 Hz, 1H), 7.35-7.32 (m, 1H), 7.23 (ddd, J = 8.5, 2.7, 1.0 Hz, 1H), 5.51 (t, J = 5.9 Hz, 1H), 4.61 (d, J = 5.9 Hz, 2H). Data is consistent with the literature.

Dimer [Ir(ppy-CH$_2$SO$_3$)$_2$(μ-Cl)]$_2$ (2): IrCl$_3$·xH$_2$O (150 mg, 411 μmol) and 1 (264 mg, 972 μmol) were suspended in a 1:1 mixture of 2-ethoxyethanol and H$_2$O (15 mL). The mixture was sparged with nitrogen gas for 30 min and then heated at reflux in the dark for 16 h under an inert atmosphere. During this time, full dissolution occurred and the solution turned yellow in color. The solution was diluted with H$_2$O (50 mL) and concentrated under reduced pressure and the solid dried in vacuo. The crude reaction mix was redissolved in H$_2$O (20 mL) and loaded in 3-4 mL aliquots onto a Maxi-Clean™ SPE reversed-phase cartridge (C18, 900 mg) (Grace, Rowville, Australia). The product was eluted using H$_2$O and the remaining colored impurities were removed from the cartridge using methanol. The solvent was removed to give a mixture of the product and excess 1 as a dark yellow powder (340 mg). A yield was estimated by relative integration of peaks in the $^1$H NMR (72%), this product was used without further purification. $^1$H NMR (500 MHz; D$_2$O): δ 3.59 (d, J = 13.5 Hz, 1H), 3.71 (d, J = 13.5 Hz, 1H), 6.10 (d, J = 1.6 Hz, 1H), 6.75 (dd, J = 8.0, 1.6 Hz, 1H), 7.41 (ddd, J = 7.3, 5.7, 1.6 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.95-7.89 (m, 2H), 8.81 (d, J = 5.7 Hz, 1H). MS (ESI) m/z 687.02 ([C$_{24}$H$_{18}$N$_2$O$_6$S$_2$Ir] requires 687.02). HPLC $R_T$ = 12.6 min.

Dimer [Ir(df-ppy-CH$_2$OH)$_2$(μ-Cl)]$_2$ (5) was prepared according to literature procedure with slight modification. Iridium trichloride hydrate (0.25 g, 0.7 mmol) was combined with compound 4 (0.64 g, 2.87 mmol), dissolved in a mixture of 2-ethoxyethanol (20 mL) and water (10 mL), and heated to reflux for 24 h under an inert
atmosphere. The solution was cooled to room temperature, and water (15 mL) was added to aid precipitation and the yellow precipitate was collected on a glass frit. The precipitate was washed with ethanol (10 mL) and ether (10 mL) and then recrystallised in hot methanol, and cooled to give crystals of \([\text{Ir(dfppy-CH}_2\text{OH})_2\text{Cl}]_2\) (Yield 0.45 g, 49%). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 4.83 (d, \(J = 5.56\) Hz, 2H), 4.86 (d, \(J = 5.35\) Hz, 2H), 5.12 (dd, \(J = 2.3, 8.7\) Hz, 1H), 5.73-5.82 (m, 3H), 6.77-6.87 (m, 2H), 7.46 (d, \(J = 5.2\) Hz, 1H), 7.58 (d, \(J = 5.7\) Hz, 1H), 8.24 (s, 1H), 8.28 (s, 1H), 9.42 (d, \(J = 6.1\) Hz, 1H), 9.65 (d, \(J = 6.1\) Hz 1H). \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 98.9, 99.1, 99.3, 99.6, 112.2, 112.3, 114.0, 114.1, 120.0, 120.2, 120.5, 120.7, 121.2, 121.7, 127.4, 128.1, 150.2, 150.3, 150.8, 152.2, 155.9, 156.6, 156.7, 157.1, 159.0, 159.1, 159.7, 159.8, 160.9, 161.0, 161.1, 161.2, 161.7, 161.9, 162.0, 162.9. \(^{19}\)F NMR (470 MHz, DMSO-\(d_6\)) \(\delta\) -108.00 (s, 1F), -109.18 (s, 1F), -110.07 (s, 1F), -111.14 (s, 1F). MS (ESI\(^+\)) \(m/z\) 633.08 ([M-2L-2Cl]\(^+\) requires 633.06). 

**Ir complexes IIa, IIb and IX** were prepared as previously described.\(^{52, 70, 223, 238}\) The commercially available bathophenanthroline-sulfonate (BPS) ligand used in complexes IIa and IIb was found to predominantly contain the \(m\)-\(m'\) isomer.\(^{265}\)

**Ir complex VII**: The crude dimer 2 (69 mg, 32 \(\mu\)mol) and 3a (31 mg, 131 \(\mu\)mol) were dissolved in a 1:1 mixture of acetonitrile and \(\text{H}_2\text{O}\) (4 mL) and stirred in the dark at ambient temperature for 16 h under an inert atmosphere. The solution was diluted with \(\text{H}_2\text{O}\) (10 mL) and the acetonitrile removed under reduced pressure. The solution was extracted with dichloromethane (3 \(\times\) 50 mL) to remove excess 3a. The remaining solution was loaded in 3-4 mL aliquots onto a Maxi-Clean\(^{\text{TM}}\) SPE reversed-phase cartridge (C18, 900 mg). Excess ligand 1 was eluted using water and then the product was eluted in a 1:1 mixture of acetonitrile and \(\text{H}_2\text{O}\). The solution was concentrated under reduced pressure and dried \textit{in vacuo} to give the product as a bright yellow
powder (38 mg, 62%). $^1$H NMR (500 MHz; $D_2$O, 20% CD$_3$CN): δ 3.65 (d, $J$ = 13.6 Hz, 1H), 3.66 (d, $J$ = 13.6 Hz, 1H), 3.73 (d, $J$ = 13.6 Hz, 1H), 3.79 (d, $J$ = 13.5 Hz, 1H), 5.51 (m, 2H), 6.19 (d, $J$ = 1.5 Hz, 1H), 6.27 (d, $J$ = 1.5 Hz, 1H), 6.98-6.95 (m, 2H), 7.04-7.01 (m, 2H), 7.10 (dd, $J$ = 7.7, 1.4 Hz, 1H), 7.31-7.25 (m, 4H), 7.55 (dd, $J$ = 5.8, 0.7 Hz, 1H), 7.62 (d, $J$ = 8.1 Hz, 1H), 7.67 (dd, $J$ = 5.8, 0.7 Hz, 1H), 7.70 (d, $J$ = 8.1 Hz, 1H), 7.83-7.77 (m, 3H), 7.95-7.92 (m, 2H), 8.01 (m, 2H), 8.74 (s, 1H). $^{13}$C NMR (126 MHz; $D_2$O, 20% CD$_3$CN): δ 56.2, 57.8, 120.7, 121.0, 123.7, 124.4, 124.6, 125.2, 125.5, 125.6, 125.8, 127.1, 127.8, 128.9, 130.0, 130.1, 134.5, 134.5, 134.6, 134.7, 135.2, 139.5, 139.6, 140.6, 144.6, 144.9, 146.6, 149.6, 149.7, 150.0, 150.3, 151.7, 167.5, 167.8. HRMS (ESI) $m/z$ 923.12 ([C$_{38}$H$_{30}$N$_6$O$_6$S$_2$Ir]$^+$ requires 923.13). HPLC $R_T$ = 19.8 min.

**Ir complex VIII**: The crude dimer 2 (65 mg, 30 μmol) and 3b (35 mg, 108 μmol) were dissolved in a 1:1 mixture of acetonitrile and H$_2$O (4 mL) and stirred in the dark at ambient temperature for 16 h under an inert atmosphere. The solution was diluted with H$_2$O (10 mL) and the acetonitrile removed under reduced pressure. The solution was extracted with dichloromethane (3 × 50 mL) to remove excess 3b. The remaining solution was loaded in 3-4 mL aliquots onto a Maxi-Clean™ SPE reversed-phase cartridge (C18, 900 mg). Excess ligand 1 was eluted using water and then the product was eluted in a 1:1 mixture of acetonitrile and H$_2$O. The solution was concentrated under reduced pressure and dried in vacuo to give the product as a bright yellow powder (37 mg, 59%). $^1$H NMR (500 MHz; $D_2$O): δ 3.36-3.30 (m, 2H), 3.44-3.39 (m, 2H), 3.55-3.48 (m, 6H), 3.66-3.63 (m, 2H), 3.88-3.72 (m, 7H), 4.58-4.55 (m, 2H), 6.35 (d, $J$ = 1.3 Hz, 1H), 6.39 (d, $J$ = 1.2 Hz, 1H), 6.92 (dd, $J$ = 7.7, 0.6 Hz, 1H), 6.98 (d, $J$ = 7.5 Hz, 1H), 7.11-7.05 (m, 2H), 7.33-7.30 (m, 1H), 7.65 (d, $J$ = 8.1 Hz, 1H), 7.70 (d, $J$ = 8.7 Hz, 1H), 7.71 (d, $J$ = 6.9 Hz, 1H), 7.77 (d, $J$ = 5.7 Hz, 1H), 7.85-7.81 (m,
2H), 7.91 (d, J = 5.5 Hz, 1H), 7.99 (t, J = 8.0 Hz, 3H), 8.15 (d, J = 8.0 Hz, 1H), 8.87 (s, 1H).\textsuperscript{13}C NMR (126 MHz; 80\% D_{2}O, 10\% CD_{3}CN): δ 52.6, 57.8, 61.2, 69.0, 70.3, 70.4, 70.5 (2C), 72.6, 120.8, 121.0, 123.6, 124.5, 124.6, 125.3, 125.6 (2C), 125.8, 127.7, 129.0, 132.6, 134.2, 134.5 (2C), 134.9, 139.6 (2C), 140.6, 144.8, 145.0, 146.6, 149.6, 149.8, 150.0, 150.3, 151.8, 167.4, 167.7. HRMS (ESI) \textit{m/z} 1009.17 ([C_{39}H_{40}N_{6}O_{10}S_{2}\text{Ir}]^{+} \text{ requires} 1009.19). HPLC \textit{RT} = 15.4 min.

**Ir complex XI:** The ligand (3a) (0.037 g, 0.156 mmol) and the dichloro-bridged dimer (5) were dissolved in a solution of 2-ethoxyethanol (10 mL) and dichloromethane (10 mL). The mixture was heated at reflux for 24 h under an inert atmosphere. The solvent was removed by rotary evaporation and the residue was suspended in 2 M HCl (10 mL), sonicated for 2 min and the yellow precipitate was collected. The yellow solid was washed with water (15 mL) followed by ether (10 mL) to give crystals (0.12 g, 88\%). \textsuperscript{1}H NMR (500 MHz, DMSO-\textit{d}_{6}) δ 4.75 (s, 4H), 5.60 (dd, J = 2.4, 8.5 Hz, 1H, ), 5.68 (dd, J = 2.4, 8.4 Hz, 1H), 5.78 (s, 2H), 6.88 (m, 1H), 6.98 (m, 1H), 7.15-7.23 (4H, m), 7.38 (m, 3H), 7.55-7.58 (m, 1H), 7.61 (d, J = 6 Hz, 1H), 7.70 (d, J = 6 Hz, 1H), 7.78 (m, 1H), 8.20-8.26 (m, 3H), 8.44 (d, J = 7.95 Hz,1H) 9.34 (s, 1H)\textsuperscript{19}F NMR (470 MHz, DMSO-\textit{d}_{6}) δ -107.73 (s, 1F), -108.82 (s, 1F), -109.87 (s, 1F), -110.83 (s, 1F). MS (ESI\textsuperscript{+}) \textit{m/z} 869.19 ([M-Cl]^{+} \text{ requires} 869.18).

**Ir complex XII:** The precursor XI (0.05 g, 0.055 mmol) and SOCl\textsubscript{2} (10 \textmu L, 0.12 mmol) were dissolved in dichloromethane (10 mL) and was heated at reflux for 24 h. The solvent was removed by rotary evaporation to give a yellow solid. The yellow solid was dissolved in chloroform (10 mL), washed with saturated bicarbonate (2 × 15 mL) and then water (15 mL). The organic layer was dried with MgSO\textsubscript{4}, filtered and then the solvent removed under reduced pressure to give a light yellow powder [Ir(df-
ppy-CH₂Cl₂(ptb)][Cl] (0.043 g, 83%). 1H NMR (500 MHz, DMSO-d₆) δ 5.02 (s, 4H), 5.60 (dd, J = 2.4, 8.4 Hz, 1H), 5.68 (dd, J = 2.4, 8.4 Hz, 1H), 5.78 (s, 2H), 6.92 (m, 1H), 7.03 (m, 1H), 7.22 (3H, m), 7.31 (m, 3H), 7.39 (m, 4H), 7.57 (m, 1H), 7.72 (d, J = 6 Hz, 1H), 7.78 (d, J = 6 Hz, 1H), 7.81 (d, J = 6 Hz, 1H), 8.23 (td, J = 1.4, 7.8, 9.3 Hz, 1H), 8.32 (m 3H), 8.44 (d, J = 8 Hz, 1H) 9.34 (s, 1H). MS (ESI⁺) m/z 905.12 ([M-Cl⁺] requires 905.12).

**Ir complex X**: A mixture containing XII (147 mg, 0.16 mmol) and Na₂SO₃ (200 mg, 1.58 mmol, 10.1 equiv) in acetone/H₂O (1:1, 5 mL) was heated at 50°C for 48 h. After this time the reaction mixture was cooled at 4°C to give a precipitate which was collected by vacuum filtration and washed using ethanol/H₂O (3:1) and Et₂O. The yellow powder was then dissolved in hot ethanol and any remaining solid removed by vacuum filtration (2 cycles). The filtrate was reduced in vacuo (ca. 2-3 mL) and precipitation occurred. The solid was collected by vacuum filtration to give a yellow powder, which was then dissolved in acetonitrile/ethanol (1:1, ca. 10 mL). Upon standing a yellow precipitate formed and was collected by vacuum filtration to give X (23 mg 14%) as a yellow solid. ¹H NMR (500 MHz, DMSO-d₆) δ 9.26 (s, 1H), 8.45 (d, J = 8.0 Hz, 1H), 8.22 (br s, 2H), 8.19 (dd, J = 7.9, 1.3 Hz, 1H), 7.81 (d, J = 5.6 Hz, 1H), 7.59-7.54 (m, 3H), 7.46-7.39 (m, 3H), 7.33 (d, J = 7.0 Hz, 2H), 7.21 (ddd, J = 12.9, 6.1, 1.5 Hz, 2H), 6.96 (ddd, J = 11.1, 9.4, 2.4 Hz, 1H), 6.87 (ddd, J = 11.0, 9.4, 2.2 Hz, 1H), 5.80 (m, 2H), 5.63 (dd, J = 8.3, 2.3 Hz, 1H), 5.57 (dd, J = 8.5, 2.3 Hz, 1H), 3.99-3.92 (m, 4H). ¹⁹F NMR (470 MHz, DMSO-d₆) δ -107.69 (d, J = 10.3 Hz, 1F), -108.83 (d, J = 9.5 Hz, 1F), -109.48 (d, J = 10.3 Hz, 1F), -110.43 (d, J = 9.5 Hz, 1F). HRMS (ESI⁺) m/z 995.8 ([M-Na⁺] requires 995.1).
Figure AII.1. UV-Vis absorbance spectra of [Ru(bpy)$_3$]$^{2+}$ (Ru), [Ir(ppy)$_2$(BPS)]$^+$ (IIa), [Ir(df-ppy)$_2$(BPS)]$^+$ (IIb), [Ir(ppy-SO$_3$)$_2$(ptb)]$^+$ (VII), [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^+$ (VIII), [Ir(df-ppy)$_2$(pt-TEG)]$^+$ (IX), and [Ir(df-ppy-SO$_3$)$_2$(ptb)]$^+$ (X).
Figure AII.2 Photoluminescence spectra of $[\text{Ir}(\text{ppy})_2(\text{BPS})]^-$ (IIa), $[\text{Ir}(\text{ppy})_2(\text{BPS})]^-$ (IIb), $[\text{Ir}(\text{df-ppy})_2(\text{pt-TEG})]^-$ (IX), $[\text{Ir}(\text{ppy-SO}_3)_2(\text{pt-TEG})]^-$ (VIII), $[\text{Ir}(\text{df-ppy-SO}_3)_2(\text{ptb})]^-$ (X), and $[\text{Ir}(\text{ppy-SO}_3)_2(\text{ptb})]^-$ (VII).
Figure AII.3. Influence of electrochemical pulse time on ECL intensity. Columns: red = [Ru(bpy)$_3$]$^{2+}$; green = [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^+$ (VIII); blue = [Ir(df-ppy)$_2$(pt-TEG)]$^+$ (IX). (a) Absolute ECL intensity. (b) ECL intensity relative to that of [Ru(bpy)$_3$]$^{2+}$ at the same pulse time.
Figure AII.4. A) NMR spectra. [Ir(ppy-SO$_3$)$_2$(ptb)][Na] (VII). 90% D$_2$O, 10% CD$_3$CN. Residual solvent marked with an asterisk.
Figure AII.4. B) [Ir(ppy-SO\textsubscript{3})\textsubscript{2}(pt-TEG)][Na] (VIII). 90% D\textsubscript{2}O, 10% CD\textsubscript{3}CN. Residual solvent marked with an asterisk.
Figure AII.4. C) \([\text{Ir(df-ppy-SO}_3\text{)}_2\text{(ptb)}][\text{Na}]\) (X).
Table AII.1. Contour plots for the HOMO and LUMO energy levels and triplet spin densities. BP86/def2-TZVP//mPW1PW91/def2-SVP (with SCRF-IEFPCM water solvent).

<table>
<thead>
<tr>
<th>Compound</th>
<th>HOMO</th>
<th>LUMO</th>
<th>Triplet spin density</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ru(bpy)$_3^{2+}$] (Ru)</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>[Ir(ppy)$_2$(BPS)]$^-$</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>(IIa)</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" alt="Image" /></td>
</tr>
<tr>
<td>[Ir(df-ppy)$_2$(BPS)]$^-$</td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td>(IIb)</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
</tr>
<tr>
<td>[Ir(ppy-SO$_3$)$_2$(ptb)]$^-$</td>
<td><img src="image16" alt="Image" /></td>
<td><img src="image17" alt="Image" /></td>
<td><img src="image18" alt="Image" /></td>
</tr>
<tr>
<td>(VII)</td>
<td><img src="image19" alt="Image" /></td>
<td><img src="image20" alt="Image" /></td>
<td><img src="image21" alt="Image" /></td>
</tr>
<tr>
<td>[Ir(ppy-SO$_3$)$_2$(pt-T EG)]$^-$</td>
<td><img src="image22" alt="Image" /></td>
<td><img src="image23" alt="Image" /></td>
<td><img src="image24" alt="Image" /></td>
</tr>
<tr>
<td>(VIII)</td>
<td><img src="image25" alt="Image" /></td>
<td><img src="image26" alt="Image" /></td>
<td><img src="image27" alt="Image" /></td>
</tr>
<tr>
<td>HOMO</td>
<td>LUMO</td>
<td>Triplet spin density</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>([\text{Ir}(df\text{-ppy})_2(pt\text{-TEG})]^+) (IX)</td>
<td>![Image](176x672 to 290x733)</td>
<td>![Image](296x667 to 409x733)</td>
<td>![Image](431x669 to 544x732)</td>
</tr>
</tbody>
</table>

| [\text{Ir}(df\text{-ppy-SO}_3)_2(ptb)] (X) | ![Image](176x558 to 289x645) | ![Image](296x557 to 410x645) | ![Image](431x548 to 531x645) |
Appendix III

Chapter 5: Analytically Useful Blue Chemiluminescence from a Water-Soluble Iridium(III) Complex Containing a Tetraethylene Glycol Functionalised Triazolylpyridine Ligand

Figure AIII.1. Chemical structure and selected properties of [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^-$ green emitter ($\lambda_{\text{max}} = 482, 512$ nm) soluble in water $E_{\text{ox}} = 1.09$ V vs Ag/AgCl.

Figure AIII.2. Photoluminescence of [Ir(df-ppy)$_2$(pt-TEG)]$^+$ (left), [Ir(ppy-SO$_3$)$_2$(pt-TEG)]$^-$ (middle), and [Ru(bpy)$_3$]$^{2+}$ (right) at 1 mM in aqueous solution under ultraviolet light (LED: $\lambda_{\text{max}} = 370$ nm).
**Figure AIII.3.** Compounds selected for the comparison of the chemiluminescence intensities.

**Figure AIII.4.** Relative chemiluminescence (signal-to-blank) response for [Ru(bpy)$_3$]$^{2+}$ and [Ir(df-ppy)$_2$(pt-TEG)]$^+$ at 1 mM reagent concentration, with cerium(IV) sulfate (1 mM) and various pharmaceuticals and related compounds (10 µM), using flow injection analysis methodology.
Figure AIII.5. (a) Normalised absorption spectrum of cerium(IV) sulfate (black line), and normalised photoluminescence emission spectra of $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$ (blue line) and $[\text{Ru(bpy)}_3]^{2+}$ (red line). (b) Normalised chemiluminescence spectra for 1mM $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$ (blue line) and $[\text{Ru(bpy)}_3]^{2+}$ (red line), with 1 mM cerium(IV) sulfate and 50 µM furosemide. Photoluminescence and chemiluminescence spectra were corrected for the relative wavelength sensitivity of the spectrometers. The chemiluminescence spectra were obtained under analytically relevant conditions by replacing the photomultiplier tube in the flow injection analysis manifold with a spectrometer with CCD detector. This enabled measurement of the chemiluminescence spectrum for each injection, during the time that the light-producing reaction mixture passed through the detection flow-cell. The vibrational structure in the photoluminescence emission spectrum of $[\text{Ir(df-ppy)}_2(\text{pt-TEG})]^+$ was not observed in the corresponding chemiluminescence spectrum due to the considerably lower resolution of the CCD spectrometer configuration.
Figure AIII.6. Calibrations for furosemide prepared using flow injection analysis methodology with 0.1 mM [Ir(dfppy)$_2$(pt-TEG)]$^+$ (blue plot) or [Ru(bpy)$_3$]$^{2+}$ (red plot) and 1 mM cerium(IV) sulfate in 0.05 mM H$_2$SO$_4$. Each point is an average of three replicate injections. The precision was evaluated using a $3 \times 10^{-7}$ M furosemide solution ($n = 5$), which showed a relative standard deviation of less than 2% with both reagents. The limits of detection ($1 \times 10^{-8}$ M and $7 \times 10^{-8}$ M, respectively) were established using a smaller range calibration at low concentrations.
Appendix IV

Chapter 6: A Comparison of Commercially Available Screen Printed Electrodes for Electrogenerated Chemiluminescence Applications

Biotinylated 89mer ssDNA sequence: 5’-/5AmMC6/GAT GCA AGG TCG CAT ATG AGA TTT CTG TGG CAT CCT GGC GCT CCC CAC CAG TCT CCA TTT GTT CAT ATG ATC GTT TGG TGC CTT GAG AC/3Bio/-3’.

General reaction mechanism for electrode fouling by dipropylamine.

1. TPrA' - e' → Pr₂N+C=H₂CH₃
2. TPrA'' + TPrA' → TPrA + Pr₂N+C=H₂CH₃
3. Pr₂N=C=H₂CH₃ + H₂O → Pr₂NH + CH₃CH₂CHO
4. Pr₂NH - e' → Pr₂NH''
5. Pr₂NH'' → Pr₂N' + H'
6. Pr₂N' + GC
   Au
   GC
   Au
   N(CH₂CH₂CH₃)₂

We used the following equation to account for differences in geometric working electrode area in order to calculate relative ECL intensities;

\[
\left( \frac{ECL(SPE)}{A_{geo(SPE)}} \div \frac{ECL(Zensor)}{A_{geo(Zensor)}} \right) \times 100\%
\]
<table>
<thead>
<tr>
<th></th>
<th>Contact angle of water on different electrode surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zensor</td>
<td>132° ± 1</td>
</tr>
<tr>
<td>DS-C</td>
<td>122° ± 1</td>
</tr>
<tr>
<td>Kanichi</td>
<td>132° ± 1</td>
</tr>
<tr>
<td>DS-OMC</td>
<td>125° ± 1</td>
</tr>
<tr>
<td>DS-CNT</td>
<td>107° ± 1</td>
</tr>
<tr>
<td>DS-CNF</td>
<td>150° ± 1</td>
</tr>
<tr>
<td>DS-GPH</td>
<td>117° ± 1</td>
</tr>
<tr>
<td>DS-Pt</td>
<td>99° ± 1</td>
</tr>
<tr>
<td>DS-Au</td>
<td>106.5 ± 1</td>
</tr>
<tr>
<td>DS-GNP</td>
<td>108° ± 1</td>
</tr>
<tr>
<td>DS-CNT-GNP</td>
<td>117° ± 1</td>
</tr>
<tr>
<td>DS-CNF-GNP</td>
<td>140° ± 1</td>
</tr>
<tr>
<td>DS-GPH-GNP</td>
<td>97° ± 1</td>
</tr>
</tbody>
</table>
Figure AIV.1. CAD drawings of electrochemical cells used to house SPEs. (1,2) Base with hole below working electrode for magnet. (3) Flow cell for free-complex assays. (4) Cell with open top for bead-based assays. (5) Adaptor used to interface cells with PMT.
Figure AIV.2. Representative scan rate study of 1 mM potassium ferrocyanide in 1 M KCl at a Zensor electrode.
B

Scan Rate$^{1/2}$ (V s$^{-1}$)

C

Scan Rate$^{1/2}$ (V s$^{-1}$)
Figure AIV.3. Variation of peak current with scan rate, used to calculate the electroactive area of each electrode. Representative from 1 mM potassium ferrocyanide, 1 M KCl. A) Zensor. B) DS-C. C) Kanichi. D) DS-OMC. E) DS-CNT. F) DS-CN. G) DS-GPH. H) DS-Pt. I) DS-Au. J) DS-GNP. K) DS-CNT-GNP. L) DS-CN-GNP. M) DS-GPH-GNP.
Figure AIV.4. SEM images of commercially available SPEs. A) DS-C. B) Zensor. C) DS-GNP, with measurements of GNP diameter. D) DS-GPH-GNP. E) DS-CNT. F) DS-OMC.
Figure AIV.5. CVs of 1 mM \([\text{Ru(bpy)}_3]^{2+}\) in 0.1 M PBS (scan rate 0.1 V/s). A) Zensor. B) DS-C. C) DS-OMC. D) DS-CNf. E) DS-Pt. F) DS-CNf-GNP. G) DS-CNf-GNP. H) GC.
Graphs E and F show the PMT response (V) as a function of potential (V vs Ag/AgCl) for different scans and concentrations. The graphs illustrate the electrochemical behavior at various potentials and concentrations.
I

PMT Response (V)

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
2.0
Potential (V vs Ag/AgCl)

Scan 1
Scan 2
Scan 3
100 mM TPrA

100 mM TPrA (Current (μA))

J

PMT Response (V)

6
4
2
0
-0.5
0.0
0.5
1.0
1.5
2.0
Potential (V vs Ag/AgCl)

Scan 1
Scan 2
Scan 3
100 mM TPrA

100 mM TPrA (Current (μA))
Figure AIV.6. Representative ECL response from $1 \times 10^{-7}$ M $[\text{Ru(bpy)}_3]^{2+}$, 100 mM TPrA, 0.1 M PBS, pH 7.5 at different electrodes (solid lines, primary axis) and a representative CV of 100 mM TPrA in 0.1 M PBS (dotted line, secondary axis). A) Zensor. B) DS-C. C) Kanichi. D) DS-OMC. E) DS-CNT. F) DS-CN. G) DS-GPH. H) DS-Pt. I) DS-Au. J) DS-GNP. K) DS-CNT-GNP. L) DS-CN-GNP. M) DS-GPH-GNP.
Figure AIV.7. Structures of (A) Triton X-100 (n = 9-10) and (B) T-20 (w + x + y + z = ~20).

Figure AIV.8. Effect of 0.1% TX on ECL intensity, relative to ECL response from each electrode variety with no surfactant (0.1 M PBS pH 7.5, 100 mM TPrA, n = 3).
**Figure AIV.9.** Variation of ECL response with scan number. Scans 2 and 3 are calculated relative to scan 1 for each electrode variety. Each with 100 mM TPrA, 0.1 M PBS, pH 7.5, $1 \times 10^{-7}$ M $[\text{Ru(bpy)}_3]^{2+}$. 
Figure AIV.10. ECL response from chronoamperometry experiments. The signal is the integrated area from a 0.5 s pulse to 1.4 V vs Ag/AgCl. Each with 100 mM TPrA, 0.1 M PBS, pH 7.5, $2 \times 10^{-7}$ M $[\text{Ru(bpy)}_3]^{2+}$. No ECL response from DS-Pt electrodes was observed at $2 \times 10^{-7}$ M. The ECL response from Kanichi was insignificant compared to the background response at $2 \times 10^{-7}$ M $[\text{Ru(bpy)}_3]^{2+}$, therefore, we compared Kanichi electrodes relative to the response of Zensor at $1 \times 10^{-6}$ M. All ECL responses are corrected for differences in geometric working electrode area.