Probing the Redox and Photophysical Properties of Ru(II)-Pt(II) Supramolecular Complexes as Efficient Photodynamic Therapy Agents

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

In Chemistry

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February 16, 2012
Blacksburg, VA

Keywords: supramolecular, ruthenium(II), platinum(II), photodynamic therapy, and DNA
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ABSTRACT

Mixed-metal Ru(II)-Pt(II) supramolecular complexes having the [(Ph_{2}phen)$_2$Ru(BL)PtCl$_2$]$^{2+}$ (Ph_{2}phen = 4,7-diphenyl-1,10-phenanthroline, and BL (bridging ligand) = dpp = 2,3-bis(2-pyridyl)pyrazine, or dpq = 2,3-bis(2-pyridyl)quinoxaline) structural motif were synthesized and their redox, photophysical, and photochemical properties studied. Subsequently the application of the Ru(II)-Pt(II) bimetallic complexes in light activated DNA modification and cytotoxicity were evaluated. The supramolecular design entails covalently coupling an efficient Ru(II) chromophore for photodynamic therapy (PDT) activity through a polyazine bridging ligand (dpp or dpq) to a cis-PtCl$_2$ bioactive site for covalent binding to biological substrates. The bioactive site is comparable to the first generation Pt-based chemotherapy agent cisplatin, cis-[PtCl$_2$(NH$_3$)$_2$]. The Ph$_2$phen ligand is known in [Ru(Ph$_2$phen)$_3$]$^{2+}$ to provide enhanced excited state lifetime and increase quantum efficiency for singlet oxygen generation in comparison to the phen analog ($\Phi_{1,02} = 0.97$, Ph$_2$phen and $\Phi_{1,02} = 0.54$, phen). The redox and photophysical properties were analyzed at each synthetic step providing systematic evaluation of the complex properties. The [(Ph$_2$phen)$_2$Ru(BL)PtCl$_2$](PF$_6$)$_2$ complexes display reversible Ru$^{III/II}$ oxidations at +1.61 (dpp) and +1.63 (dpq) V vs. Ag/AgCl with an irreversible Pt$^{IV/II}$ oxidation occurring prior at +1.51 V vs. Ag/AgCl. Four reversible ligand reductions occur at $-0.45$ (dpp$^{0/-}$), $-1.15$ (dpp$^{-2/-}$), $-1.33$ (Ph$_2$phen$^{0/-}$), and $-1.52$ (Ph$_2$phen$^{0/-}$) V vs. Ag/AgCl. For the [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$](PF$_6$)$_2$ complex, the first two reductions shift to more positive potentials at $-0.19$ and $-0.95$ V vs. Ag/AgCl, while the TL reductions remain generally
The electronic absorption spectroscopy for the \( [(\text{Ph}_2\text{phen})_2\text{Ru(BL)PtCl}_2](\text{PF}_6)_2 \), BL = dpp or dpq, complexes is dominated in the UV region by Ph\(_2\)phen (274 nm) and BL-based (310-320 nm) \( \pi \rightarrow \pi^* \) transitions and in the visible region by metal-to-ligand charge transfer (MLCT) transitions at 424 nm (Ru(\( d\pi \))\( \rightarrow \)Ph\(_2\)phen(\( \pi^* \)) \(^1\)CT) and 517 nm (Ru(\( d\pi \))\( \rightarrow \)dpp(\( \pi^* \)) \(^1\)CT) or 600 nm (Ru(\( d\pi \))\( \rightarrow \)dpq(\( \pi^* \)) \(^1\)CT). Steady-state and time-resolved emission spectroscopy shows that upon attaching Pt to the Ru monometallic precursor the \( \lambda_{\text{max}}^{\text{em}} \) shifts from 664 nm for \( [(\text{Ph}_2\text{phen})_2\text{Ru(dpp)}](\text{PF}_6)_2 \) to 740 nm for \( [(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2](\text{PF}_6)_2 \) and the excited state lifetime is reduced from 820 ns to 44 ns in accordance with the energy gap law. The \( \tau = 44 \) ns for the Ru(\( d\pi \))\( \rightarrow \)dpp(\( \pi^* \)) \(^3\)CT excited state was somewhat unexpected upon TL variation given the lack of formal involvement of Ph\(_2\)phen in the emissive state. This likely results from the Ph\(_2\)phen contribution to the formally Ru(\( d\pi \)) donor orbital. Although not typically done, given the complexity of the study the \( \Phi_{1O_2} \) was quantified for the \( [(\text{Ph}_2\text{phen})_2\text{Ru(BL)PtCl}_2]\text{Cl}_2 \) (BL = dpp, \( \Phi_{1O_2} = 0.07 \) or dpq, \( \Phi_{1O_2} = 0.03 \)) complexes supporting \(^1\)O\(_2\) generation via energy transfer from the \(^3\)MLCT excited state.

The thermal and photochemical interactions of the \( [(\text{Ph}_2\text{phen})_2\text{Ru(BL)PtCl}_2]\text{Cl}_2 \) (BL = dpp or dpq) supramolecular complexes were studied in the presence of DNA and U87MG cancer cells. Thermal binding at the cis-PtCl\(_2\) BAS in the Ru(II)-Pt(II) architecture was compared to cisplatin displaying similar reduced migration through the gel attributed to covalent binding to DNA. DNA photocleavage studies provided evidence of efficient strand cleavage when excited at 455 nm likely enhanced by producing \(^1\)O\(_2\) locally at the DNA target. DNA photobinding by the \( [(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2]\text{Cl}_2 \) complex was observed utilizing low energy light where typical Pt(II) agents do not absorb. This is the first example of MLCT excitation of a Ru(II)-Pt(II) complex to induce a photobinding event. MLCT excitation enhances electron density on the dpp
making the Pt(II) a weaker Lewis acid and promoting halide loss. In addition, this system is photoactivated with low energy red light in the therapeutic window. These studies validate the supramolecular design and show that coupling a Ru(II) chromophore for PDT activity and a cis-PtCl₂ binding moiety for covalent DNA targeting affords a complex applicable in photochemotherapies. Analysis of cytotoxicity in the dark for [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ and cisplatin afforded LC₅₀ values of 100 µM, which are confirmed by previous reports for cisplatin and the currently used chemotherapy, TMZ in U87MG cells. Photolysis of the [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ resulted in substantial reduction in the observed LC₅₀ values to approximately 5 µM. The enhanced cytotoxicity via excitation into the formally Ru(dπ)→BL(π*) CT excited state of [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ indicates that the bimetallic complex undergoes an efficient light activated mechanism of action. The Ru(II)-Pt(II) complex displays substantially lower LC₅₀ values through PDT action than currently used clinical treatments with LC₅₀ values of 100 µM.

The [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺ (BL = dpp or dpq) mixed-metal supramolecules utilizing the Ph₂phen TL have displayed surprising results. The direct coupling of the cis-PtCl₂ moiety to the (Ph₂phen)₂Ru(BL) chromophore display dramatically enhanced photophysical properties, relative to the bpy and phen systems with a longer excited state lifetime and improved light activated interactions with DNA, which was not previously observed for directly coupled Ru(II)-Pt(II) systems. The Ph₂phen TL positively influence the bioactivity compared to the typical deactivation observed in the bpy and phen systems. Probing the [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺ (BL = dpp or dpq) biological interactions confirms the importance of coupling an efficient light absorbing and ¹O₂ generating PDT-type unit with a cis-PtCl₂ DNA binding unit for applications in covalent DNA photomodification, DNA photocleavage, and photocytotoxicity. It is proposed
that excitation using visible light into the formally Ru(dπ)→BL(π*) CT excited state leads to enhanced electron density on the BL and weakened Lewis acidity at the Pt(II) center, which facilitates halide loss for efficient biological substrate modification. Upon coordination of the Ru(II)-Pt(II) complexes at the biological substrate, ^1^O_2 is localized providing effective targeting of the highly reactive oxygen species. The visible light induced activity of the [(Ph_2phen)_2Ru(BL)PtCl_2]^2+ (BL = dpp or dpq) supramolecules suggests a new mode of action in relation to cisplatin, which was further supported by the enhanced photocytotoxicity observed in the presence of U87MG cells. The results indicate that the Ru(II)-Pt(II) supramolecular structural motif hold great promise as a future photochemotherapy agent.
The goal of this research was to study the basic chemical properties and subsequent biological interactions of mixed-metal complexes composed of a Ru(II) chromophore for photodynamic therapy (PDT) activity coupled through a bis-bidentate polyazine ligand to a cis-PtCl₂ moiety for covalent DNA modification affording a supramolecule applicable in photochemotherapeutics. The [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺ (Ph₂phen = 4,7-diphenyl-1,10-phenanthroline and BL = dpp = 2,3-bis(2-pyridyl)pyrazine or dpq = 2,3-bis(2-pyridyl)quinoxaline) complexes were synthesized, and the redox, spectroscopic, photophysical, as well as the physicochemical properties characterized. Validation of the supramolecular architecture for thermal and photo bioactivity was evaluated in the presence of DNA and cultured U87MG cells.
Dedication

To my parents, Gains and Aileen Hopkins,

to my husband, Lt. Andrew L. Higgins, &

to all the cancer fighters in my life

“Cancer is so limited. It cannot cripple love. It cannot shatter hope. It cannot corrode faith. It cannot destroy peace. Cancer cannot kill friendships. It cannot suppress memories. It cannot silence courage. It cannot invade the soul. It cannot steal eternal life. And it cannot conquer the spirit.” — anonymous
Acknowledgements

I am profoundly grateful to my advisor, Prof. Karen J. Brewer, and collaborators Prof. Brenda S.J. Winkel and Dr. John L. Robertson for the opportunity to learn from their immeasurable experience. With their continued encouragement, support, and insight, I was able to push the inorganic photochemistry, molecular biology, and clinical application boundaries to reach the potential of this project. I would also like to thank the remainder of my advisory committee, Prof. Paul A. Deck and Prof. Gordon T. Yee, for their support and guidance in my research and during the milestones of my graduate career at Virginia Tech. I must mention Kristel Furchman and Vincent Dalfonzo from VMRCVM for their help with cell line maintenance. For help with $^{195}$Pt NMR I thank Hugo Azurmendi. Thanks to Prof. Webster Santos for use of his CD instrument.

From the Brewer group, I would like to thank all past and present members, especially Jessica D. Knoll, Travis A. White, Jing Wang, Dr. Shamindri M. Arachchige, Dr. David F. Zigler, and Dr. Avijita Jain for their support, direction, and useful discussions.

I was fortunate to have the opportunity to work with and mentor several exceptional undergraduate students including Norm Hurst, Lilit Stepanyan, Allison Tucker, Nima Vahidi, Abby Chase, Reece Prussin, and Shashwat Sinha. I would like to also acknowledge Rachel Hill, one of my most dedicated and motivated students whose life was tragically taken on April 16, 2007.

From Randolph-Macon College, I thank Prof. Serge Schreiner for developing my multidisciplinary research interests, honing my powerpoint skills, and providing encouragement through my PhD program. In addition, I thank Profs. Marchetti, Green, Foster, Martin, and Conway for their useful discussions and professional development. I was also fortunate enough to be mentored by Ms. Withers, Mr. Mahon, and Mr. Holmes at LaPlata High School. These teachers inspired greatness in learning and leadership.

To my many family members, friends, and mentors that have fought or been affected by cancer thank you for sharing your stories/battles and continuing to support cancer research. You make the results from this work meaningful and continue to provide the hope and motivation for finding a cure.

Finally, I would be amiss if I did not acknowledge my friends and family who have stuck through the roller coaster that is graduate school. My friends have always been there with the much-needed laugh and my family has always stood strong offering encouragement and perspective. Additionally, I must thank my parents for cultivating a desire to learn, teaching me the invaluable lesson of always getting back on the horse that threw you, and reminding me that anything is possible. To my husband, Andrew Higgins, who serves our country and has continued to support my endeavors from 6000 miles away without complaint, thank you a million times over.

All of these people have made me the person I am today and have helped me to this point in my education, for that I am forever grateful.
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<td>Singlet excited state</td>
</tr>
<tr>
<td>1GS</td>
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<td>$E^*_\text{ox}$</td>
<td>Excited state oxidation potential</td>
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<td>$E^*_\text{red}$</td>
<td>Excited state reduction potential</td>
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<tr>
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<td>$E_{1/2}$</td>
<td>Half-wave potential in voltammetry</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>$E_f$</td>
<td>Final potential</td>
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<td>$E_i$</td>
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<td>Eagle’s minimal essential medium</td>
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<td>Ethylenediamine</td>
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<td>$E_p^a$</td>
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<td>$E_p^c$</td>
<td>Cathodic peak potential</td>
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<td>$E_s$</td>
<td>Switching potential</td>
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<td>Electrospray ionization-mass spectrometry</td>
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<td>Food and Drug Administration</td>
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<td>Gram</td>
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<td>Highest occupied molecular orbital</td>
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<td>Cathodic peak current</td>
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<tr>
<td>isc</td>
<td>Intersystem crossing</td>
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<td>Rate constant for energy transfer</td>
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<td>Rate constant for internal conversion</td>
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<td>Concentration causing 50% mortality</td>
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<td>Light emitting diode</td>
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<td>Lumens</td>
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<td>Ligand to metal charge transfer</td>
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<td>Log$P$</td>
<td>Partition coefficient value</td>
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<td>Lowest unoccupied molecular orbital</td>
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m/z Mass-to-charge
mA Milliamps
MDR Multi-drug resistance
Me$_2$dpq 6,7-dimethyl-2,3-di(2-pyridyl)quinoxaline
Me$_2$phen 4,7-dimethyl-1,10-phenanthroline
Mebp-Mebpyp 4,4’-dimethyl-2,2’-bipyridine dimer
MePhtpy 4’-(4-methylphenyl)-2,2’:6’,2”-terpyridine
MHz Megahertz
min Minutes
mL Milliliter
mm Millimeter
mM Millimolar
mmol Millimol
n Number of electrons transferred per process
N Moles of complex
nm Nanometer
nM Nanomolar
NMR Nuclear magnetic resonance
ns Nanosecond
OAc Acetoxy group
ODBB o-dibenzoylbenzene
PDT Photodynamic Therapy
Ph$_2$phen 4,7-diphenyl-1,10-phenanthroline
phen 1,10-phenanthroline
ppm Parts per million
PS Photosensitizer
PTFE Polytetrafluoroethylene
Q Net charge
R# Resistor number
RH# Rheostat number
ROS Reactive oxygen species
rpm Rotations per minute
RT Room temperature
s Seconds
S# Switch number
SCE Saturated Calomel Electrode
TB Tris base
TBAH Tetrabutylammonium hexafluorophosphate
’Bu(tpy) 4,4’,4”-tri-tert-butyl-2,2’:6’,2”-terpyridine
TEAP Triethylammonium perchlorate
TIR Total internal reflection
TL Terminal ligand
TMZ Temozolomide
tpy 2,2’:6’,2”-terpyridine
U87MG Malignant glioblastoma cell line
UV-vis Ultra Violet-visible
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<td>W</td>
<td>Watts</td>
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<td>Zerner’s Intermediate Neglect of Differential Overlap</td>
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<td>Wavelength of irradiation</td>
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<tr>
<td>ìm</td>
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<tr>
<td>ìM</td>
<td>Micromolar</td>
</tr>
<tr>
<td>τ₀</td>
<td>Radiative lifetime</td>
</tr>
<tr>
<td>Φ₁O₂</td>
<td>Quantum yield of singlet oxygen generation</td>
</tr>
<tr>
<td>Φ_em</td>
<td>Quantum yield of emission</td>
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<td>Quantum yield of ³MLCT population</td>
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Preface

During my graduate student career at Virginia Tech, I have also been involved with several projects outside the scope of this dissertation, resulting in other publications. Following is a comprehensive list of publications pertaining to the research completed at Virginia Tech.


Chapter 1: Introduction

1.1. Cancer

Cancer, the primary cause of death in the US (under 85 years old), is a group of over 100 diseases broadly characterized by the uncontrollable growth of abnormal cells. The rapidly growing cells form masses or tumors, which obstruct the function of the surrounding tissues and/or organs. In a healthy patient, cells grow and divide in a highly regulated and controlled fashion. However, when damage occurs to the genetic material of the cell, the “checks and balances” system is disrupted allowing rapid cell division and growth.

Although each cancer type is the result of very specific genetic mutations, the general transformation from a healthy to a cancerous cell is shown in Figure 1.1. The origin of cancer is a result of DNA damage from environmental or internal factors including exposure to toxic chemicals, UV radiation, viruses, or predisposed genetic factors, ultimately resulting in gene mutations within a cell. Typically, several mutations, deletions, or amplifications must occur, providing for the up-regulation and secretion of tumor growth factor, or the down-regulation of tumor suppressor or DNA repair genes. The outcome of these mutations affords the conversion of a healthy cell to a cancerous cell. Cancerous cells, which proliferate rapidly and overtake healthy tissue vasculature as well as the immune system, typically result in the formation of a primary tumor. The primary tumor continues to grow interfering with the organ functionality. From the primary tumor, single cancerous cells can also start to invade the blood or lymph systems, relocating to new organs. This is called metastasis and the process that the cancer cells undergo is generally referred to as the “metastatic cascade.” Once the cancer cell unites with the target organ, the cancer cell can remain dormant or it can start replicating. If the cell is able
to replicate in the new organ, the disease is considered to have metastasized forming a malignant secondary tumor.

Figure 1.1: Flow chart displaying the general transformation process from a healthy cell to metastatic cancer. Healthy cells are exposed to factors that cause DNA damage, which results in mutations in various genes ultimately affording cancerous cells. Cancerous cells are then able to undergo the metastatic cascade to invade other organs forming a secondary malignant tumor.

1.1.1. Malignant Glioma

Brain cancer cases as a whole account for < 1.5% of the more than 1.5 million cancer cases diagnosed each year, however, survival upon diagnosis is low (approximately 50%) indicating improvement in clinical treatment is necessary.\textsuperscript{1,11-14} Of the types of brain cancers, glioblastoma multiforme (GBM) or malignant glioma is the most aggressive form with median survival being less than 2 years. GBM consists of a heterogeneous mixture of atypical astrocytes, which progress to the formation of a primary or secondary tumor.\textsuperscript{13,15} Primary tumors account for
approximately 60% of the cases and generally present in patients greater than 45 years of age. These tumors grow aggressively and manifest without previous lesions, resulting in rapid clinical symptom onset (headaches, personality changes, neurological and sensory losses, as well as seizures). Secondary tumors result from smaller astrocytic lesions (Grade I or II) and are more slowly developing, taking 5-10 years to present and generally occur in patients younger than 45 years of age. Ultimately, primary and secondary GBM are morphologically and clinically similar. The World Health Organization (WHO) designates these tumors as grade IV (Grade III and IV tumors are considered malignant gliomas and will grow more aggressively than Grade I or II), although the progression of genetic mutations is different for primary and secondary tumors. The genetic mutations result in invasion of adjacent brain matter, rapid relapse (< 7 months) due to inability to remove the entire tumor and drug resistance, as well as tumor heterogeneity. Approximately 30% of the GBM tumors occur in the temporal lobe, 25% in the parietal and frontal, and 20% in the occipital, Figure 1.2. Location in general makes these tumors difficult to treat.

Figure 1.2: Lateral (A) and axial (B) view of the brain, showing malignant glioma location percentage. MRI image of a malignant glioma tumor in the parietal lobe (C), used with permission from Mayfield Clinic.
1.1.2. Current Treatments and Treatment Limitations for Malignant Glioma

Currently there are several approaches to treat brain cancer including surgery, radiation, chemotherapeutics, and more typically a combination of the above therapies. The type of treatment used depends on the stage (I-IV), which is based on cell type, size, number, and grade of tumors (I-IV), and the involvement of the lymph nodes or the presence of metastasis. Although treatment has improved the median survival to 15 months, for the most part current cancer therapies have had a minimal impact on malignant glioma treatment and patient survival. Surgical resection is generally the first line of defense for newly diagnosed brain tumors, but is invasive, can result in neural damage or infection, and only a third of the patients with brain tumors are candidates. Whole brain radiation therapy (WBRT) can be used as a primary therapy or an adjuvant therapy, but can cause radiation sickness, patient fatigue, and may result in further genetic mutations producing secondary tumors. Chemotherapy when indicated, is clinically administered systemically to the whole body, which is complicated by the blood-brain barrier, lack of selectivity, and developed or intrinsic resistance. Due to the aggressiveness of malignant glioma, oncologists typically utilize a combination of therapies for patient treatment. A treatment regimen of surgery and WBRT, or chemotherapy (Temozolomide (TMZ, Figure 1.3), cisplatin, Figure 1.5, or carboplatin, Figure 1.8) and WBRT, or all three types of therapy are prescribed. The combination of therapies and aggressive treatment has resulted in the patient prognosis improving from certain death to median survival of 15 months. Survival past three years is less than 2%, indicating that new approaches must be developed.
The treatment limitations have resulted in further investigation of the malignant glioma genetics and pathology as well as the design and development of new chemotherapeutics. Although TMZ has shown improved efficacy compared to other drugs, it does not show activity against all forms of GBM due to the heterogeneity of the tumors. A therapy that is broadly active, yet targeted in regards to specific cellular receptors or via external stimulation may provide a new approach in the treatment of this consuming disease.

The chemotherapeutics commonly used for treatment of GBM, including TMZ, cisplatin, and carboplatin, all interact with DNA, which ultimately results in cell death. DNA is an ideal target for anticancer therapies, because it is the foundation for cellular replication. Inhibition of cellular replication or induction of cellular death in a single cancerous cell stops the exponential propagation of cancerous cells that would have occurred otherwise. In addition, induced cell death provides a mechanism for the body to remove dead cells. The DNA double helix provides two anionic phosphate backbones, nucleophilic bases (adenine, guanine, thymine, and cytosine), and the major or minor grooves as potential binding targets for drugs of the organic or inorganic nature. The binding motif or the mode in which the drug interacts with DNA is classified as ionic/electrostatic interaction, covalent modification, intercalation, or major/minor groove binding, Figure 1.4.\textsuperscript{3,27-36}
**Figure 1.4:** Representative drawing of the DNA double helix showing the phosphate backbone, nucleophilic bases, and major and minor grooves (A) and Watson-Crick base pairing (B). The drug represented as the red sphere is shown to interact with DNA via ionic, covalent, intercalative, as well as major/minor groove binding motifs (C).

### 1.2. Approved Metal-based Chemoserapeutic Treatments

The success of cisplatin as an anticancer drug has provided a basis for further metal-based drug development. Transition metal complexes offer useful properties that are promising for future chemotherapy agents. Typically, metal-based therapeutic treatments are positively charged affording enhanced interaction with negatively charged biomolecules. Furthermore, ligand
exchange studies of transition metal therapeutics suggest that hydrolysis is necessary before binding with biological ligands such as proteins, DNA, or other cellular substrates. The metal complex-bioactive site interactions typically interfere with cellular replication, which causes necrotic or apoptotic cell death. Cisplatin is one of the early metal-based therapeutics approved by the FDA for cancer therapy, however there is room for vast improvement and transition metal complexes hold promise for the future of chemotherapy.

1.2.1. Cisplatin: Mode of Cellular Interaction

In 1965, Rosenberg and co-workers were investigating the effect of electric fields on *Escherichia coli* bacteria, a study that would have a huge impact on the future of transition metal-based therapies. The group serendipitously discovered that upon electrolysis the Pt electrodes were releasing a compound that completely terminated bacterial replication, but not growth. The treated *E. coli*, which are normally 2-5 µm long and 1 µm in diameter, elongate to 300 times the normal size, Figure 1.5 A and B. After several trials and errors, it was finally identified that *cis*-[*Pt*II*Cl*2(NH3)2] (cisplatin), originally isolated in 1845 as Peyrone’s chloride, was the active agent in inhibiting bacteria and mammalian cell replication, Figure 1.5.

![Cisplatin structure](image)

Figure 1.5: Chemical structure of cisplatin, *cis*-[*Pt*II*Cl*2(NH3)2] and microscopy images of normal *E. coli* cells prior to cisplatin treatment (A), and elongated *E. coli* cells following cisplatin treatment (B). Micrographs were adapted from reference 39, © Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.
Further clinical studies showed that cisplatin inhibited the replication of rapidly dividing cells, caused apoptosis in cancer cells, and was approved by the FDA in 1978 as an anticancer agent. Currently cisplatin is used for the treatment of testicular and ovarian carcinoma, as well as bladder, head and neck tumors as a primary therapy or in combination with other therapies.

The proposed mechanism of cellular uptake by cisplatin is controversial. In the clinical setting, cisplatin’s physicochemical properties interfere with administration, requiring intravenous dispensation. Upon entering the blood stream, it is suggested that the complex stays intact due to increased chloride concentration. Interaction with cells results in drug uptake, however the uptake mechanism is unresolved. Some studies indicate passive diffusion via enhanced permeability and retention factor of tumor cells (leaky vasculature) while others suggest that an active transport mechanism using the copper transporter, Ctr1, facilitates cisplatin across the membrane. Either way, cisplatin enters the cell at which point the chloride concentration decreases affording sequential thermal ligand exchange to cis-[(NH₃)₂PtCl(OH₂)]⁺ and finally cis-[(NH₃)₂Pt(OH₂)₂]²⁺. The mono- and bis-aquated active products are thought to facilitate nuclear permeability and favorable DNA binding, Figure 1.6. In addition the hydrolyzed cisplatin products are available to interact with several intracellular substrates including proteins, membranes, and other nucleophilic sites. It is accepted that DNA is the lethal cellular target and is an ideal target for transition metal complexes.
Figure 1.6: Cisplatin is thought to stay intact within the bloodstream due to the high chloride concentration (100 mM), but will undergo hydrolysis after crossing the cell membrane as the concentration decreases (4 mM) inside the cell. Sequential chloride loss and hydrolysis, is important for drug partitioning across the nuclear membrane. Cisplatin can covalently bind to DNA in a variety of crosslinking motifs including 1,2-interstrand (A), 1,2-intrastrand (B), 1,3-intrastrand (C), and a mono-adduct with DNA and a protein (D), top figure. At guanine, cisplatin binds to the \( N^7 \) of guanine, bottom figure.

Cisplatin-DNA interactions have been well studied by NMR spectroscopy, X-ray crystallography, mass spectrometry, and gel electrophoresis. The predominant covalent
binding site for cisplatin is through the major groove of double-stranded DNA at the N\(^7\) of guanine (dG) or adenine (dA) due to the high nucleophilicity and easy accessibility of the bases, Figure 1.6. Cisplatin can form intrastrand adducts (with bases along a single strand), interstrand crosslinks (with bases on different strands), or mono-adducts (with a base on a single strand and/or a protein or other cellular substrate) as shown in Figure 1.6. The 1,2-d(GG) and 1,2-d(GA) intrastrand adducts account for ca. 60-70% and 20-25%, respectively of cisplatin binding followed by interstrand crosslinks and monoadducts. The study of cisplatin selectivity in intrastrand adducts has been further investigated using single-stranded DNA.\(^{54}\) Covalent adduct formation initiates the cell death process.

DNA covalent modification by cisplatin is regarded as the mode of action that induces cell death. X-ray crystallography studies show that covalently bound cisplatin causes localized distortion and unwinding of the DNA structure. Transcription and replication of the DNA is impeded, signaling DNA repair. The covalent adduct formation cannot be repaired, eliciting an apoptotic cellular response. An apoptotic cellular response (cell induced/controlled death mechanism) vs. a necrotic response (external damage or a bioenergetic catastrophe) is preferred in cancer treatment, Figure 1.7.\(^2,^{55},^{56}\) Necrotic cells release proinflammatory molecules that result in inflammation in the area of treatment, whereas apoptotic cells signal the body to dispose of the dead cells.\(^{57}\) Therefore, induction of apoptosis is a favorable mode of action because the body is able to dispose of the dead cells.
1.2.2. Cisplatin: Limitations

Though cisplatin effectively causes cell death, there are several drawbacks and limitations to cisplatin therapy. Cisplatin is not able to selectively target only rapidly replicating cancerous cells and therefore also enters rapidly replicating healthy cells, resulting in many side effects. Generally, patients are faced with fatigue, nausea, vomiting, nephrotoxicity, neurotoxicity, ototoxicity, alopecia, as well as suppressed white blood cell counts and bone marrow levels following treatments.\(^\text{41}\) Clinically cisplatin suffers from drug resistance, which limits the types of cancers that can be treated.\(^\text{58-62}\) Cellular resistance to cisplatin is classified as either intrinsic (cells are naturally resistant) or acquired (cells become resistant following multiple exposure to the drug). Intrinsic or acquired resistant cells are able to suppress Pt-DNA adduct formation (efflux mechanisms or glutathione (GSH) binding), promote DNA repair, tolerate a higher threshold of DNA damage and/or modify the regulation of the cell cycle.\(^\text{59, 61, 63-67}\)

Based on the successes and limitations of cisplatin a variety of transition metal complex analogs continue to be developed and studied.\(^\text{68-70}\)
1.2.3. Second- and Third-Generation Pt(II) Analogs

Following cisplatin, the second- and third-generation analogs, carboplatin and oxaliplatin, were developed and FDA approved in 1989 and 2002 respectively, Figure 1.8.\textsuperscript{69, 71} Comparable to cisplatin, the carboplatin and oxaliplatin complexes are square planar with \textit{cis} labile leaving groups and strongly bound amine groups.\textsuperscript{72} Though carboplatin is considered a second-generation drug, the mechanism of action and clinical activity is surprisingly similar to cisplatin.\textsuperscript{73} Carboplatin displays lower systemic toxicity while maintaining the efficacy of cisplatin, but does not show any additional activity against cisplatin resistant cell lines.\textsuperscript{74} Alternatively, oxaliplatin was the first successful chemotherapy agent that displayed no cisplatin cross-resistance.\textsuperscript{74, 75} The varied activity is attributed to improved water solubility, increased lipophilicity of the 1,2-diaminocyclohexane non-leaving group, and varied localized 1,2-d(GG) intrastrand DNA conformation.\textsuperscript{69, 76, 77} Oxaliplatin is approved for use worldwide, but is widely used as a secondary treatment for ovarian and metastatic colorectal cancers that displayed cisplatin and carboplatin drug resistance.\textsuperscript{78} Three platinum complexes have been approved for clinical treatment worldwide, three more have only been approved in Asia, and an additional ten complexes are in or have started clinical trials.\textsuperscript{79} However, the “magic bullet” cancer cure continues to elude researchers.
1.2.4. Future Directions for Pt(II) Therapies

Platinum therapies and other metal-based therapies targeted for DNA interactions were generally modeled from the parent drugs, cisplatin, carboplatin, or oxaliplatin. Therefore the inherent disadvantages of these parent complexes are typically also observed. To overcome the weaknesses of the parent drugs, several modifications for future therapies were suggested.\(^{45, 69, 80-83}\)

The first barrier experienced by cancer therapies is partitioning across the cellular membrane and cellular localization. This can be addressed through ligand choice or micelle/liposome-assisted delivery. A ligand with more lipophilic character facilitates partitioning of the metal complex across the cell membrane. Once the drug has entered the cytoplasm, a different mode of cellular death or a method to protect the drug from efflux mechanisms are necessary to overcome the developed drug resistance. Some methods to overcome cisplatin resistance include varying the structure around the metal center, incorporating photolabile ligands, and integrating sterically hindered non-leaving ligands to limit GSH deactivation at the metal-center. Though these modifications may improve the efficacy of the drug, the patient quality of life only improves marginally. Using a ligand that targets a specific receptor may provide a method to select for a specific cancer cell, however this method also limits activity in heterogeneous tumors. A drug that is selectively activated via a stimulus, e.g. external light source or internal pH or redox properties, provides a targeted therapy with a broad-based activity. It is also important to note

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**Figure 1.8**: Chemical structures of the second and third generation Pt(II) cancer therapies, carboplatin and oxaliplatin.

- **Carboplatin**
  - FDA approved
  - Lower systemic toxicity
  - Cross-resistance with cisplatin resistant cancer cells

- **Oxaliplatin**
  - FDA approved
  - Used as a secondary treatment for ovarian or metastatic colon cancer
  - No cisplatin cross-resistance
that although the parent drugs are often described as DNA targeting molecules, these are not the only interactions that occur in the cell. Therefore, in the pharmacological screening process, DNA-metal complex interactions may not directly correlate to the propensity of the drug as an anticancer therapy.

New approaches to cancer treatment with transition metal-based systems are now being explored. Anticancer drug development is moving towards more unconventional metal-based therapies with innovative mechanisms of action as well as evaluating previously overlooked systems (weed ed out by the prototypical structure-activity relationships developed nearly 40 years ago), which is exciting. Continued development in the area of inorganic pharmaceuticals as well as applying the drugs in a variety of therapies (radiation therapy, immunotherapy, or photodynamic therapy) opens the door to more selective treatment and improved patient outcomes.

1.3. Photodynamic Therapy: Mode of Action, General Considerations, Limitations, and Future Directions

Photodynamic therapy (PDT) is a relatively new and promising cancer treatment with the first FDA approved PDT agent in 1995 for the treatment of lung, esophageal, bladder, brain, and ovarian cancers. PDT utilizes a chromophore or photosensitizer (PS), light, and often molecular oxygen, which are independently non-toxic, however when combined initiate a toxic photochemical reaction only at the site of the cancer. The photochemical reaction generates the highly reactive singlet oxygen ($^1$O₂) species or other reactive oxygen species (ROS), which leads to direct apoptotic/necrotic cell death or indirect cell death via tumor vasculature damage. These photochemical reactions are successful due to their choice of PS and selective light targeting at the cancer site.
The photophysical properties of the PS afford interesting photochemical reactivity. In the presence of light, the PDT agent is typically excited from the singlet ground state (\(^1\text{GS}\)) to a short-lived singlet excited state (\(^1\text{ES}\)). From the \(^1\text{ES}\), radiative decay in the form of fluorescence or non-radiative decay in the forms of heat loss or intersystem crossing (isc) can occur. Non-radiative isc is the process of spin inversion to form a lower energy, longer-lived triplet excited state (\(^3\text{ES}\)). These excited state processes are shown in the Jablonski diagram, Figure 1.9. From the \(^3\text{ES}\), three photoreactions can occur: electron transfer to water or oxygen (Type I), energy transfer to oxygen (Type II), and direct interaction with the cellular target (Type III).\(^{90-92}\) The Type I reaction involves direct electron transfer from the \(^3\text{ES}\) of the PS to water, which forms radical reactive oxygen species (ROS). Alternatively, the Type II reaction involves energy transfer from the \(^3\text{ES}\) to molecular oxygen (\(^3\text{O}_2\)) forming the \(^1\text{GS}\) of the PS and the highly reactive \(^1\text{O}_2\). In both cases (Type I or Type II), reaction occurs from the \(^3\text{ES}\) to form ROS. Typical cellular targets include the plasma membrane, mitochondria, tumor vasculature, or DNA. Ultimately, the interaction results in necrotic or apoptotic cell death. Type III reactivity is considered the only mode that is an oxygen-independent PDT pathway. In Type III PDT activity, the PS interacts with cellular targets directly and causes cell death.
Figure 1.9: Jablonski diagram for a general PS with photodynamic therapy type I (electron transfer with H$_2$O or O$_2$) and type II (energy transfer with O$_2$) photoreactions labeled. $^1$GS = singlet ground state, $^1$ES = singlet excited state, $^3$ES = triplet excited state, $^3$Σ$_g$ = molecular oxygen ground state, $^1$Σ$_g$ = singlet oxygen highest energy excited state, $^1$Δ$_g$ = singlet oxygen lowest energy excited state, $k_{nr}$ = rate constant for non-radiative decay, $k_r$ = rate constant for radiative decay, $k_{isc}$ = rate constant for intersystem crossing non-radiative decay, $k_{en}$ = rate constant for energy transfer, $k_{et}$ = rate constant for electron transfer, $k_{ic}$ = rate constant for internal conversion.

The efficacy of singlet oxygen generation or other ROS is directly related to the localization at the target. The reported lifetime of the lowest lying $^1$O$_2$ state ($^1$Δ$_g$) in biological systems is generally 40 ns, which is short-lived with a small effective radius of approximately 10 nm.$^{93,94}$ It is suggested that singlet oxygen is only able to interact locally; therefore it is critical that the PS is localized near the desired substrate during ROS generation.

In certain cancer cases, the utilization of oxygen is not a viable option. Generally, aggressive tumors outgrow their vasculature, resulting in central pockets of hypoxia and necrosis. Applying a Type I or II PDT agent under hypoxic conditions further depletes the oxygen concentration until all drug activity is lost. Therefore, it is imperative to develop new
drug approaches that can utilize an oxygen environment, but can also function in an oxygen independent manner.

**Figure 1.10**: Cross-section of the epidermis, dermis, and subcutis, which overlays a graph representing light transmission depth (—, mm) for various wavelengths of light (nm) as a result of hemoglobin, melanin, and water absorptivity, as well as scattering and reflection. Adapted from references 95-97.

Ideally, PDT utilizes a non-toxic, water-soluble, and homogeneous PS, which strongly absorbs visible, non-thermal wavelengths of light and produces very toxic reactants upon exposure to light. The selectivity of PDT arises from the PS and the targeting provided by light delivery. The PS should be non-toxic and stable in dark conditions, so that healthy cells are not adversely affected during systemic treatment and display enhanced light-induced toxicity. PDT is made discriminatory by utilizing light within the therapeutic window (low energy, visible light) for activation of the drug only at the site of the tumor, Figure 1.10. The higher energy wavelength limit for the therapeutic window is generally 550 nm, which is past the intense absorptivity attributed to hemoglobin and melanin. The lower energy wavelength limit is 1200
nm, which is prior to water absorptivity. Development of a PS, which is easily tuned and selectively associates with cancer specific tissue, is desired. However, since PDT is a relatively young treatment option, ideal PS properties are still being developed.

An efficient PS, optimal light source, and carefully selected treatment parameters are necessary components for successful PDT cancer treatment. Several types of light sources including diode lasers, incandescent light, and LEDs, are available for PDT treatment. Light source modification (fiber optics, inflatable balloons, and implantable devices) as well as imaging technology has made it feasible to successfully treat areas otherwise inaccessible to light.

1.3.1. Traditional Porphyrin-based PDT Agents

The traditional PDT agents approved for cancer treatment are porphyrin-based and are categorized by generation. The first generation PDT agent, hematoporphyrin derivative (HpD or Photofrin®) was FDA approved for treatment of esophageal cancer in 1995. The drug is composed of a heterogeneous mixture of monomer or dimer porphyrin units, Figure 1.11, and does not efficiently populate its $^3\text{ES}$ limiting $^1\text{O}_2$ generation. However, the PS selectively associates in rapidly proliferating tissue and has been used for treatment in thousands of patients. Second generation PSs included chlorophyll and expanded porphyrin families, while third generation PDT agents incorporated specific biological targeting moieties, such as additional light absorbing metals or binding moieties. The clinically approved PDT agents display a different mechanism of action than other chemotherapies and do not negatively impact chemotherapy, radiation, or surgery allowing use in combination therapies. The use of light as an external stimulus affords targeted treatment at the site of the tumor. The unique PDT properties and mechanism of action have provided a large patient population with a better quality
of life and extended survival rates when other types of therapies failed, however, PDT remains underutilized in the field of clinical oncology due to the multi-disciplinary nature of the treatment.

![Chemical structure of Photofrin®](image)

**Figure 1.11**: Chemical structure of Photofrin® and pertinent chemical properties for application in PDT.

1.3.2. Ru(II)-based Chromophores for PDT Applications

Ru-based chromophores have shown promise as PDT agents due to their enhanced visible light absorption, effective intersystem crossing to form the $^3\text{MLCT}$ (triplet metal-to-ligand charge transfer) excited state, long-lived $^3\text{MLCT}$ excited state lifetimes, efficient $^1\text{O}_2$ generation, and their enhanced affinity for negatively charged biomolecules. One of the more efficient $^1\text{O}_2$ generating Ru(II)-based chromophores is $[\text{Ru(Ph}_2\text{phen)}_3]^{2+}$ (Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline), which displays superior properties compared to the $[\text{Ru(bpy)}_3]^{2+}$ (bpy = 2,2'-bipyridine) and $[\text{Ru(phen)}_3]^{2+}$ (phen = 1,10-phenanthroline) analogs. Figure 1.12 displays a Jablonski diagram for $[\text{Ru(Ph}_2\text{phen)}_3]^{2+}$ and its photogeneration of $^1\text{O}_2$. The $[\text{Ru(Ph}_2\text{phen)}_3]^{2+}$ is excited from the $^1\text{GS}$ to the singlet metal-to-ligand charge transfer ($^1\text{MLCT}$, $\lambda_{\text{max}}^{\text{abs}} = 470$ nm in CH$_3$CN at RT) excited state. The metal complex undergoes intersystem crossing with unit
efficiency to form the emissive $^3$MLCT excited state. The $^3$MLCT excited state radiatively ($k_r$) or non-radiatively ($k_{nr}$) decays to the ground state. In the presence of molecular oxygen, the emission from the $^3$MLCT excited state is quenched, a result of efficient energy transfer from the $^3$MLCT excited state to $^3$O$_2$ generating the $^1$GS of the metal complex and reactive $^1$O$_2$.

The determination of $^1$O$_2$ generation efficiency is important in characterizing effective PDT agents. The detection of $^1$O$_2$ can be probed indirectly by observing emission quenching of a $^1$O$_2$ quencher following photolysis of the PS or directly via emission from the lowest lying $^1$O$_2$ state ($\lambda_{\text{max}}^{\text{em}} = 1270\,\text{nm}$).\textsuperscript{112, 115-117} Studies on the generation of singlet oxygen ($\Phi_{\text{1O}_2}$) by $\text{[Ru(TL)$_3$]}^{2+}$, where TL = terminal ligand = Ph$_2$phen, phen, and bpy terminal ligands, using indirect methods are reported.\textsuperscript{110, 118, 119} The quantum yield of singlet oxygen formation ($\Phi_{\text{1O}_2}$) is the ratio of singlet oxygen formed per photons absorbed.\textsuperscript{120} For $\text{[Ru(Ph_2phen)$_3$]}^{2+}$ the $\Phi_{\text{1O}_2}$ in methanol was 0.97, which was significantly higher than the bpy ($\Phi_{\text{1O}_2} = 0.73$) or phen ($\Phi_{\text{1O}_2} = 0.54$).\textsuperscript{119, 121} In water the $\Phi_{\text{1O}_2}$ were 0.22, 0.24, and 0.42 for bpy, phen, and Ph$_2$phen, respectively. The authors indicate that in methanol the $^3$MLCT excited state is quenched only through energy transfer to $^3$O$_2$, whereas in water there is a competing pathway for these complexes. In both solvents, the enhanced activity by the $\text{[Ru(Ph_2phen)$_3$]}^{2+}$ complex was attributed to the long-lived $^3$MLCT lifetime, limited secondary dark reactions, increased photochemical stability, decreased rate constant for $^1$O$_2$ quenching, and enhanced variable wavelength excitation as compared to the bpy and phen analogs.
Figure 1.12: Chemical structure of [Ru(Ph₂phen)₃]²⁺ and the representative Jablonski diagram showing energy transfer with molecular oxygen. Ph₂phen = 4,7-diphenyl-1,10-phenanthroline, ¹GS = singlet ground state, ¹ES = singlet excited state, ³ES = triplet excited state, ³Σ₂g = molecular oxygen ground state, ¹Σ₂g = singlet oxygen highest energy excited state, ¹Δ₂g = singlet oxygen lowest energy excited state, ⁷nr = rate constant for non-radiative decay, ⁷r = rate constant for radiative decay, ⁷isc = rate constant for intersystem crossing non-radiative decay, ⁷en = rate constant for energy transfer, ⁷ic = rate constant for internal conversion.

The highly reactive ¹O₂ species is able to react with a variety of biological molecules. One that is of particular interest is the reaction of ¹O₂ with DNA. DNA-¹O₂ interactions generally result in strand cleavage or base oxidation, which precipitates cellular response mechanisms resulting in cell death. However, the ¹O₂ interactions with DNA require localization of the chromophore near DNA due to the small diffusion radius. The [Ru(Ph₂phen)₃]²⁺ complex showed the strongest interaction with DNA relative to the bpy and phen analogs indicating improved localization of ¹O₂ at the substrate.

One limiting factor for application of the Ru(II) complexes in PDT, also present for the porphyrin PSs, is direct targeting of a cellular substrate to localize reactivity. While the redox, spectroscopic, and photophysical properties of Ru-based chromophores can be easily tuned through ligand modification, cellular localization is not well understood. Additionally, hypoxic
tumor conditions limit the affectivity of PDT agents using Type I and II mechanisms, requiring probing of oxygen independent mechanisms.

It is proposed that a PS that performs multiple types of photoreactions in the presence and absence of oxygen, targets cellular substrates to localize reactivity, and strongly absorbs low energy visible light, may be able to overcome the fundamental limitations of current PDT agents. The future of PDT as a cancer treatment shows promise, but requires a multidisciplinary approach between biology, chemistry, and clinical oncology. This collaboration will provide the ability to design and prepare new, complex molecules; study the fundamental reactions with biomolecules; and evaluate the impact of complex living systems on the observed chemistry.

1.4. Ru(II)-Pt(II) Supramolecular Complexes for Biological Applications

For the purposes of this document, supramolecular complexes are defined as a system of individual components with specific roles, which are covalently coupled in a unique molecular architecture to perform complex functions. The supramolecular complexes of interest herein will focus on the covalent coupling of Ru-based light absorbers (LAs) to cis-PtCl₂ bioactive sites (BAS) through connectors or bridging ligands (BL) and their interactions with various biomolecules. The light absorbing unit consists of two terminal ligands (TL) and a bridging ligand (BL), examples shown in Figure 1.13, which covalently tethers additional LAs or a metal-based BAS. Modification of the LA components influences the redox, spectroscopic, and photophysical properties imparted onto the BAS. The cis-PtCl₂ BAS provides a metal known to covalently bind to DNA via ligand labilization as demonstrated by cisplatin. These systems couple PDT and chemotherapeutic agents into one molecule. The following is a brief review of the current Ru(II)-Pt(II) supramolecular complexes and their interactions with various biomolecules.
1.4.1. Redox and Photophysical Properties of Ru(II)-based Chromophores, 

\[(\text{TL})_2\text{Ru(BL)}\]^{2+}, where TL = bpy, phen, or Ph₂phen and BL = dpp, dpq, or dpb

Studies of Ru(II)-based LAs with the structural motif, \([\text{Ru(TL)}_3]^{2+}\), where the TL are polyazine ligands, provide the foundation for the design and study of covalently coupled light absorbing units. Replacing one TL with a polyazine BL affords the building block for supramolecular architecture expansion.\(^{124,125}\) The incorporation of a polyazine bridging ligand in the place of a terminal ligand affords complexes of the motif \([(\text{TL})_2\text{Ru(BL)}]^{2+}\) with unique properties.\(^{124,126}\) There are many bridging ligands that can be used as the covalent connector of subunits, but the focus for this review will be on bis-bidentate polyazine bridging ligands. These ligands offer strong metal-to-ligand binding, provide the site of localization of the lowest (\(\pi^*\)) acceptor orbital, and result in LA units with interesting spectroscopic and photophysical properties.\(^{127}\) Bridging ligands such as dpp, dpq, or dpb, (dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, and dpb = 2,3-bis(2-pyridyl)benzoquinoxaline) provide two bidentate binding sites (Figure 1.13). These ligands can connect two metals providing electronic coupling. The redox and photophysical properties of these Ru(II)-LAs are modulated upon incorporation of the BL and assembly into supramolecular architectures.
Electrochemistry analyzes redox active species. Cyclic voltammetry is a potential sweep method, which varies potential over time and measures the current, Figure 1.14 A, and is plotted as current response vs. applied potential, Figure 1.14 B. Analysis of the potential and reversibility (shape) of a redox couple provides information regarding the relative frontier orbital energetics and the possibility of a chemical reaction following the redox step. For example, in ferrocene (FeCp₂), the ratio of the anodic peak current ($i^a_p$) and the cathodic peak current ($i^c_p$) is one indicative of a reversible couple with no chemical reactions following the redox step, Figure 1.14 B.
1.14 B. In addition, the potential separation between the cathodic \(E_p^c\) and anodic \(E_p^a\) peaks should be independent of scan rate with

\[ \Delta E_p = E_p^c - E_p^a = \frac{59 \text{ mV}}{n} \text{ (at 25 °C)} \]

where \(n\) = the number of electrons transferred in the redox process. Generally, the half wave potential, \(E_{1/2}\), is reported for a reversible couple, defined using equation 1.2

\[ E_{1/2} = \frac{E_p^a + E_p^c}{2} \]

If \(\frac{i_p^a}{i_p^c} \neq 1\) or \(\Delta E_p > 59 \text{ mV}\) the couple is considered quasi-reversible. If there is no return wave then the system is considered irreversible and either the \(E_p^a\) or \(E_p^c\) is reported.

**Figure 1.14:** Potential waveform plotting potential (V) vs. time (s) (A) and cyclic voltammogram plotting current (µA) vs. potential (V) for ferrocene (B). \(E_i\) = Initial potential, \(E_s\) = switching potential, \(E_f\) = final potential, \(E_p^a\) = anodic peak potential, \(E_p^c\) = cathodic peak potential, \(i_p^a\) = anodic peak current, and \(i_p^c\) = cathodic peak current.

The \([(\text{TL})_2\text{Ru(BL)}]^2^+\) monometallic precursors are well studied.\textsuperscript{123,127,128} Electrochemical, spectroscopic, and photophysical data for a series of Ru(II) complexes with polyazine BLs are given in Table A-1, pages 124-125.\textsuperscript{129-137} The \([(\text{TL})_2\text{Ru(BL)}]^2^+\) complexes display Ru\textsuperscript{II/III}

25
oxidations that all occur at similar potential (+1.45-1.50 V vs. Ag/AgCl) indicative of a Ru(dπ)-based highest occupied molecular orbital (HOMO). Three reductions are commonly observed in the electrochemical window corresponding to three sequential one-electron ligand reductions. The first reduction is the BL$^{0/-}$ couple which indicates a BL(π*) lowest unoccupied molecular orbital (LUMO) in all the systems. However, the potential varies depending upon the BL. By extending the aromatic system of the BL, the π*-orbital is stabilized, shifting the potential more positive from approximately −1.0 (dpp) to −0.70 (dpq), and finally −0.60 (dpb) V vs. Ag/AgCl. The next two reductions in the monometallic complexes are TL$^{0/-}$ couples.

Typically, the Ru(II) monometallic complexes display a Ru(dπ)-based HOMO and ligand(π*)-based LUMO, specifically for the [(TL)$_2$Ru(BL)]$^{2+}$ motif a bridging ligand(π*)-based LUMO. Spectroscopic information may be correlated to electrochemical data as each provides a measure of the HOMO-LUMO gap. In spectroscopy, the lowest energy transition that occurs for the [(TL)$_2$Ru(BL)]$^{2+}$ complexes in electronic absorption spectroscopy is the Ru(dπ)→BL(π*) CT (charge transfer) transition. As predicted by the electrochemistry, the extended aromaticity from dpp to dpq to dpb affords stabilized BL(π*) orbitals and a smaller HOMO-LUMO gap, which is mirrored in the electronic absorption spectroscopy. For the dpp analogs the MLCT transitions occur in the 430-475 nm region, shifting to lower energy at 520-530 nm (dpq) or 550 nm (dpb). Characteristically, ligand-based π→π* transitions or intraligand (IL) transitions occur in the UV region, and several overlapping MLCT transitions occur in the visible. The Δ$E_{1/2}$ (V) between the $E_{1/2}$ for the Ru$^{IV/III}$ oxidation and the $E_{1/2}$ for the BL$^{0/-}$ reduction is an electrochemical measure of the HOMO-LUMO gap. The $E_{abs}$, converted to eV, using equation 1.3,

$$E_{abs} (eV) = \frac{hc}{\lambda} = \frac{1240 \ (eV \ nm)}{\lambda \ (nm)}$$  1.3


where $h$ is Planck’s constant ($4.14 \times 10^{-15}$ eV s), $c$ is the speed of light ($3.0 \times 10^{17}$ nm s$^{-1}$) and $\lambda$ is the measured wavelength (nm), provides a measure of the spectroscopic gap. There is a clear correlation between the $\Delta E_{1/2}$ (V) and $E_{\text{abs}}$ (eV),$^{138,139}$ supporting the Ru($d\pi$) and BL($\pi^*$) nature of the HOMO and LUMO in these systems, respectively ($E_{\text{abs}} = 1.14\Delta E_{1/2} + 0.15$, correlation = 0.98), Figure 1.15.$^{140,141}$

![Figure 1.15: Correlation of $\Delta E_{1/2}$ and $E_{\text{abs}}$ for the monometallic architecture, $[(\text{TL})_2\text{Ru(BL)}]^2^+$, supporting a Ru($d\pi$)-based HOMO and BL($\pi^*$)-based LUMO. TL = bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline, or Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline and BL = dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, or dpb = 2,3-bis(2-pyridyl)benzoquinoxaline.](image)

The photophyscis of Ru(II) complexes are described using Jablonski diagrams, Figure 1.16, and quantified using quantum yield and excited state lifetimes. Following excitation of the Ru-based LA in the visible region, the $^1\text{MLCT}$ excited state is populated. The $^1\text{MLCT}$ excited state undergoes intersystem crossing ($k_{\text{isc}}$) to populate the $^3\text{MLCT}$ excited state with unit efficiency and can radiatively decay (through the emission of a photon, $k_{r}$), non-radiatively decay (through a non-emissive pathway, $k_{\text{nr}}$), or react with another species ($k_{\text{rxn}}$).
**Figure 1.16**: Jablonski diagram for [(Ph2phen)2Ru(dpp)]2+ with energy levels for the 1MLCT and 3MLCT excited states determined experimentally. Ph2phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, 1GS = singlet ground state, 1MLCT = singlet metal-to-ligand charge transfer excited state, 3MLCT = triplet metal-to-ligand charge transfer excited state, knr = rate constant for non-radiative decay, kr = rate constant for radiative decay, kisc = rate constant for intersystem crossing non-radiative decay, krxn = rate constant for reaction decay.

Steady-state and time-resolved spectroscopy are used to quantify the quantum yield of emission and the lifetime from the 3MLCT excited state, respectively. Quantum yield is defined as the number of events occurring per photon absorbed in the system.\(^{120}\) For emission from the 3MLCT excited state, the quantum yield (\(\phi^{em}\)) is the product of the quantum yield of population of the emitting 3MLCT state, \(\phi_{pop}^{3MLCT}\), times the ratio of the radiative rate constant for emission divided by the sum of the deactivating pathways, equation 1.4

\[
\phi^{em} = (\phi_{pop}^{3MLCT}) \left( \frac{k_r}{k_{nr} + k_r + k_{rxn}} \right) = k_r \tau \quad \text{(1.4)}
\]

where, \(\phi_{pop}^{3MLCT}\) is 1 for the Ru(II) monometallic and bimetallic complexes, \(k_r\) and \(k_{nr}\) are radiative and non-radiative decay constants, respectively and \(k_{rxn}\) is the reaction decay constant from the 3MLCT excited state. The excited state lifetime of the 3MLCT excited state (\(\tau\)) is the inverse of the sum of the rate constants that deactivate the excited state, equation 1.5,
\[ \tau = \frac{1}{k_{nr} + k_r + k_{rxn}} \]

where, \( k_r \), \( k_{nr} \), and \( k_{rxn} \) are the same as equation 1.4.

The photophysical properties of Ru(II)-polyazine complexes directly influence the photochemistry occurring from the excited states. The excited state lifetime of a molecule impacts the probability of interaction, therefore, it is expected that a longer lifetime would afford a higher probability of substrate interaction. According to the energy gap law, as the energy gap (\( \Delta E \)) decreases the non-radiative decay rate of a metal complex increases exponentially as a result of increased vibronic coupling between the low energy excited state and ground state shown in Figure 1.17C.\(^{142}\) Increasing the non-radiative decay exponentially results in \( k_{nr} \) being the dominant factor in equation 1.5, and ultimately, a shorter-lived \( \tau \). In addition non-radiative decay may be influenced by an increase in the vibronic coupling due to an increase in average nuclear displacement (\( \Delta Q_e \)), Figure 1.17A.\(^{138, 143-145}\)

Emission for the \([\text{TL}]_2\text{Ru(BL)}\)_2\(^{2+}\) complexes, where TL = bpy, phen, or Ph\(_2\)phen and BL = dpp or dpq, is derived from the lowest lying Ru(\(d\pi\))\(\rightarrow\)BL(\(\pi^*\)) \(^3\)CT excited state. For the series, \([\text{TL}]_2\text{Ru(dpp)}\)_2\(^{2+}\), where TL = bpy, phen, and Ph\(_2\)phen, emissions at 680 nm (\( \Phi_{em} = 0.012 \), \( \tau = 380 \) ns), 660 nm (\( \Phi_{em} = 0.027 \), \( \tau = 460 \) ns), and 697 nm (\( \Phi_{em} = 0.036 \), \( \tau = 1000 \) ns) were reported, respectively.\(^{129, 131, 133}\) Despite the formally Ru(\(d\pi\))\(\rightarrow\)BL(\(\pi^*\)) \(^3\)CT nature of the excited state, the Ph\(_2\)phen analog displays significantly larger \( \Phi_{em} \) and \( \tau_0 \) values. Generally, the energy gap parameter, \( \Delta E \), largely influences the radiationless decay, however, in this case average nuclear displacement, \( \Delta Q_e \), may be of greater impact.\(^{146, 147}\) The \([\text{TL}]_2\text{Ru(dpq)}\)_2\(^{2+}\) series is incomplete, however emission was reported for bpy (\( \lambda_{max}^{em} = 760 \) nm and \( \tau_0 < 20 \) ns) and phen (\( \lambda_{max}^{em} = 760 \) nm and \( \tau_0 = 80 \) ns) systems. The decreased energy of emission from the \(^3\)MLCT
excited state for the dpq compared to the dpp analogs is consistent with the energy gap law. Emission data for the dpb analogs were not available.

Figure 1.17: Potential energy surfaces of the ground state (GS) and excited state (ES), showing the influence of average nuclear displacement ($\Delta Q_e$) and energy gap ($\Delta E$) on non-radiative decay rate ($k_{nr}$). The $k_{nr}$ with a normalized $\Delta Q_e$ and $\Delta E$ (B) increases as $\Delta Q_e$ increases (A), or $\Delta E$ decreases (C).

1.4.2. Redox and Photophysical Properties of Ru(II)-Pt(II) Complexes,

$[(TL)_2Ru(BL)PtCl_2]^{2+}$, where TL = bpy or phen and BL = dpp, dpq, or dpb

Coupling multiple subunits to produce supramolecular systems is made possible through the use of polyazine bridging ligands. In the late 1990s, Yam, et al. and Brewer, et al. separately reported Ru(II)-Pt(II) bimetallic complexes utilizing the bpy terminal ligand and different bridging ligands. The $[(bpy)_2Ru(dpdb)PtCl_2]^{2+}$ and $[(bpy)_2Ru(dpq)PtCl_2]^{2+}$ were reported by the Brewer group$^{33,148}$ and Yam, et al. synthesized and characterized $[(bpy)_2Ru(dpp)PtCl_2]^{2+}$. For comparison, the $[(phen)_2Ru(dpdb)PtCl_2]^{2+}$ and $[(phen)_2Ru(dpq)PtCl_2]^{2+}$ analogs were also
synthesized, Figure 1.18.\textsuperscript{130} Redox, spectroscopic, and photophysical properties of the previously reported Ru(II)-Pt(II) complexes are summarized in Table A-1, pages 125-126.

![Chemical Structures](image)

**Figure 1.18:** Chemical structures of reported Ru(II)-Pt(II) bimetallic complexes with the structural architecture, [(TL)$_2$Ru(BL)PtCl$_2$]$^{2+}$, where TL = bpy = 2,2'-bipyridine or phen = 1,10-phenanthroline and BL = dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, or dpb = 2,3-bis(2-pyridyl)benzoquinoxaline.

Cyclic voltammetry was used to characterize the redox properties of the bimetallic complexes. Upon scanning positively the electrochemistry for the bimetallic complexes confirms a reversible Ru$^{II/III}$ oxidation, with an irreversible Pt-based oxidation occurring as a shoulder on the anodic wave.\textsuperscript{130,150} In the series, [(TL)$_2$Ru(BL)PtCl$_2$]$^{2+}$, where TL = bpy or phen and BL = dpp, dpq, or dpb, the Ru$^{II/III}$ oxidation occurs at +1.61 (bpy, dpp), +1.67 (bpy, dpq), +1.65 (bpy, dpb), +1.67 (phen, dpq), and +1.65 (phen, dpb) V vs. Ag/AgCl. The data indicates that the
reversible component of the oxidation depends on the nature of the BL bound to Ru with minimal influence from the TL. TL or BL modification around the Ru(II) center did not affect the irreversible oxidation potential occurring at approximately +1.50 V vs. Ag/AgCl in all the complexes. An irreversible couple in this region is still observed upon switching the metal to Os, suggesting that it was related to a Pt-based oxidation.\textsuperscript{148} There is some controversy as to whether the oxidation is a Pt\textsuperscript{II/III}, Pt\textsuperscript{II/III/IV}, or Pt\textsuperscript{II/IV} couple.\textsuperscript{148, 150} Scanning reductively, two reversible BL-based reductions occur, corresponding to BL\textsuperscript{0/−} and BL\textsuperscript{−2/−}, which were shifted to a more positive potential upon platination. The positive potential shift is indicative of BL(π*) stabilization as a result of the decreased electron density on the ligand upon coordination of the Pt(II) center. Additionally, the BL(π*) orbital stabilization trend observed in the monometallics was the same for the bimetallic systems, with dpb(π*) orbitals being the most stabilized and occurring at the most positive potentials (−0.10, dpb\textsuperscript{0/−} and −0.77, dpb\textsuperscript{−2/−} V vs. Ag/AgCl) compared to (−0.50, dpp\textsuperscript{0/−} and −1.07, dpp\textsuperscript{−2/−} V vs. Ag/AgCl). TL\textsuperscript{0/−} reductions are only reported for [(bpy)\textsubscript{2}Ru(dpp)PtCl\textsubscript{2}]\textsuperscript{2+}, occurring more negative of the BL reductions at −1.45 V vs. Ag/AgCl. The electrochemical data provides insight into the orbital energetics of these Ru(II)-Pt(II) bimetallic systems, which display metal-based HOMOs and BL(π*)-based LUMOs.

The electronic absorption spectroscopy of the Ru(II)-Pt(II) complexes is characteristic of Ru(II) light absorbing chromophores with lower energy MLCT transitions occurring as a result of stabilized BL(π*) orbitals upon Pt(II) coordination. Although the Pt-based oxidation overlaps the reversible Ru\textsuperscript{II/III} couple, it is reported that the HOMO is localized on the Ru(dπ) orbital with the LUMO localized on the BL(π*) orbital. The electrochemistry also indicates that ligand variation from dpp to dpq to dpb results in stabilization of the BL(π*) or a reduced HOMO-LUMO gap, predicting a shift in the Ru(dπ)→BL(π*) CT transition to lower energy for this
series. As predicted in the [(bpy)$_2$Ru(BL)PtCl$_2$]$^{2+}$ analogs, the Ru(dπ)$\rightarrow$BL(π*) CT transitions shifts from 510 nm (dpp) to 580 nm (dpq) to 630 nm (dpb). In addition, compared to the respective [(bpy)$_2$Ru(BL)]$^{2+}$ monometallic precursors lowest energy MLCT transitions at 430 nm (dpp), 517 nm (dpq), and 550 nm (dpb), the bimetallic complexes lowest energy MLCT transition red shifts, consistent with BL(π*) orbital stabilization. The Ru(dπ)$\rightarrow$TL(π*) CT bands were reported at 410-420 nm and 420-430 nm for the bpy and phen complexes, respectively, however, due to the broad and overlapping nature of the transitions, assignment can be challenging. Characteristic of terminal and bridging ligand π→π* transitions, several strong absorptions occur in the UV region. The π→π* transitions occur at approximately 290 and 265 nm for bpy and phen, respectively, while the BL π→π* transitions are observed at lower energy between 340-400 nm. In addition, Yam, et al. proposed that a Pt(d)$\rightarrow$BL(π*) transition may contribute to the absorption between 330-400 nm, although this assignment uses data from Pt(II) monometallic complexes.

The photophysical properties reported for the [(TL)$_2$Ru(BL)PtCl$_2$]$^{2+}$ structural motif, where TL = bpy or phen and BL = dpp, dpq, or dpb, is limited. Similar to the monometallic precursors, it is expected that optical excitation populates a $^1$MLCT excited state, followed by population with unit efficiency of the emissive $^3$MLCT excited state via non-radiative intersystem crossing. The monometallic precursors are expected to be more strongly emissive with longer-lived $^3$MLCT excited state lifetimes compared to the related Ru(II)-Pt(II) bimetallic complexes. This observation is attributed to the lower energy $^3$MLCT states in the bimetallic complexes in accordance with the energy gap law. In most reports, bimetallic complex emission from the $^3$MLCT excited state transition, projected to occur at > 800 nm, is not reported due to the low PMT response in this region. Until the synthesis of the
[(Ph2phen)2Ru(dpp)PtCl2]2+ analog, only the [(bpy)2Ru(dpp)PtCl2]2+ complex was reported to display a measurable emission. Yam, et al. reported an emission from the 3MLCT excited state, which was shifted from 680 to 800 nm upon platination of the monometallic precursor.\textsuperscript{150} The lifetime was also reported as 450 ns, which was surprising considering the [(bpy)2Ru(dpp)]2+ lifetime (τ = 380 ns). It was expected that with a decrease in the energy gap between the excited state and ground state, the lifetime of the 3MLCT excited state would be shorter lived with an increased rate of non-radiative decay. However, the lengthened lifetime for the 3MLCT excited state was not discussed in detail.\textsuperscript{149,150} These directly coupled Ru(II)-Pt(II) bimetallic complexes with polyazine BLs often display short-lived 3MLCT states, a result of BL(π*) orbital stabilization upon platination, making their use in PDT unreported.

1.4.3. Discussion of Other Ru(II)-Pt(II) Architectures

Several Ru(II)-Pt(II) complexes with varied structural motifs from the series above have been reported, Figure 1.19. The electrochemical and spectroscopic data for the Ru(II)-Pt(II) complexes with varied architectural motifs are reported in Table A-1, pages 126-128.
Figure 1.19: Chemical structures of Ru(II)-Pt(II) complexes with varied molecular architectures. Mebpy-Mebpy = 4,4’-dimethyl-2,2’-bipyridine dimer, bpy = 2,2’-bipyridine, dpp = 2,3-bis(2-pyridyl)pyrazine, AB = 2,2’:3’,2’’:6”,2”-quaterpyridine, tpy = 2,2’:6’,2”-terpyridine, and MePhtpy = 4’-(4-methylphenyl)-2,2’:6’,2”-terpyridine.

The [(bpy)₂Ru(Mebp-Mebp)PtCl₂](ClO₄)₂ complex, where Mebpy-Mebpy = 4,4’-dimethyl-2,2’-bipyridine dimer, was published by Rillema et al. and studied for water reduction.
photocatalysis. The [(bpy)$_2$Ru(Mebpy-Mebpy)PtCl$_2$](ClO$_4$)$_2$ complex shows a reversible Ru$^{II/III}$ oxidation at +1.19 V vs. Ag/AgCl and two reversible ligand-based reductions. The first reduction was BL$^{0-}$ based (correlating to the Mebpy unit coordinated to the Pt-center) occurring at −1.27 V followed by a bpy$^{0-}$ reduction at −1.38 V vs. Ag/AgCl. The electronic absorption spectra revealed Ru(d$\pi$)→BL(π*) CT transitions occurring in the visible region at 457 nm, while the π→π* transitions were observed in the UV region. The sharp transition at 324 nm in the [(bpy)$_2$Ru(Mebpy-Mebpy)PtCl$_2$](ClO$_4$)$_2$ electronic absorption spectrum, which was also apparent in the [Pt(Me$_2$bpy)Cl$_2$] spectrum, was attributed to a Pt(d$\pi$)→BL(π*) transition. The photophysical properties for the Ru(II)-Pt(II) bimetallic complex were similar to [Ru(bpy)$_3$]$^{2+}$. Based on these studies, it was concluded that the Ru(II) and Pt(II) metal centers were largely electronically uncoupled. However, the Mebpy-Mebpy ligand provides free rotation about the ethyl bridge between the metal centers, which may provide flexible and interesting DNA binding capabilities, but no studies have been reported.

Two Ru(II)-Pt(II) bimetallic complexes using the bridging ligand, 2,2’:3’,2’’:6”,2”-quaterpyridine (AB), which has two distinct bpy binding sites, labeled A and B, were reported by Barigelletti and co-workers, Figure 1.19. The [(bpy)$_2$Ru(AB)PtCl$_2$]$^{2+}$ and [Cl$_2$Pt(AB)Ru(bpy)$_2$]$^{2+}$ systems displayed interesting electrochemical and spectroscopic properties due to the unequal coordination. In position A, the (bpy)$_2$Ru-unit is unhindered with properties that are similar to normal bpy coordination complexes while position B results in a steric distortion and perturbation of the properties. For both complexes, the Ru$^{II/III}$ oxidation was irreversible occurring at +1.38 or +1.47 V vs. Ag/AgCl for [(bpy)$_2$Ru(AB)PtCl$_2$]$^{2+}$ and [Cl$_2$Pt(AB)Ru(bpy)$_2$]$^{2+}$, respectively, becoming reversible with increased scan rate. Coordination of the cis-PtCl$_2$ moiety to the [(bpy)$_2$Ru(AB)]$^{2+}$, resulted in stabilization of the BL(π*) orbitals
from −1.30 V vs. Ag/AgCl to −0.98 (AB0/−) and −1.32 (AB−2/−) or −0.92 (AB0/−) and −1.27 (AB−2/−) V vs. Ag/AgCl for [(bpy)2Ru(AB)PtCl2]2+ or [Cl2Pt(AB)Ru(bpy)2]2+, respectively. The absorption spectroscopy for the [Cl2Pt(AB)]2+ monometallic complex displays a low intensity transition in the visible region (400 nm), while the [(bpy)2Ru(AB)]2+ monometallic complex and both Ru(II)-Pt(II) bimetallic complexes display strong Ru(dπ)→BL(π*) CT transitions at ca. 455 nm. The low energy 1MLCT transition for the bimetallic complexes is only slightly perturbed compared to the [(bpy)2Ru(AB)]2+ complex, indicating that coordination of the cis-PtCl2 unit has a small effect on the Ru(dπ)→BL(π*) transition. The emission for the Ru(II)-Pt(II) bimetallic complexes was labeled as a Ru-based 3MLCT since the Pt-based fragment was non-emissive.

The [Cl2Pt(AB)Ru(bpy)2]2+ complex displays emission at 624 nm (Φem = 0.007 and τ0 = 13 ns) compared to [(bpy)2Ru(AB)PtCl2]2+, which displays emission at 640 nm (Φem = 0.230 and τ0 = 280 ns). The weak emission and short-lived excited state lifetime for [Cl2Pt(AB)Ru(bpy)2]2+ is attributed to the thermal population of the 3LF (triplet ligand field) excited state. The emission spectra were recorded at room temperature in air-equilibrated CH3CN and there were no reports of the effects of molecular oxygen. Bioactivity of the [(bpy)2Ru(AB)PtCl2]2+ and

[Cl2Pt(AB)Ru(bpy)2]2+ systems with DNA has not been reported, although the isomeric differences would be an interesting study.

In 2006, Sakai, et al. reported the [(bpy)2Ru(bpy-(CONH-(CH2)3NH2)2)2PtCl2](PF6)2 bimetallic complex.154 The redox properties of the ruthenium precursors or the Ru(II)-Pt(II) complexes were not reported. The monometallic precursor, [(bpy)2Ru(bpy-(CONH-(CH2)3NH2)2)](CF3CO2)4 and Ru(II)-Pt(II) bimetallic complex, [(bpy)2Ru(bpy-(CONH-(CH2)3NH2)2)2PtCl2](PF6)2 displayed similar electronic absorption and emission spectral profiles. An increase in absorbance at 200 nm was reported for the bimetallic complex attributed to a Pt-
based transition. The lifetime for $^3$MLCT emission was collected under air-equilibrated room
temperature conditions and reported as 244 and 518 ns for the monometallic precursor and the
bimetallic complex, respectively. The increased lifetime is interesting because coordination of a
cis-PtCl$_2$ moiety generally quenches the $^3$MLCT excited state of the ruthenium chromophore.
However, coordination of the platinum unit affords a rigid metallocycle, resulting in decreased
vibronic motion yielding increased emission intensity. The biological interactions of the
[(bpy)$_2$Ru(bpy-(CONH-(CH$_2$)$_3$NH$_2$)$_2$)$_2$PtCl$_2$](PF$_6$)$_2$ bimetallic complex are summarized in
section 1.4.4.

The Brewer group reported an expanded Ru(II)-Pt(II) tetrametallic architecture in 2006
for multifunctional DNA interactions moving the site of localization of the lowest lying $^3$MLCT
state away from the Pt(II) center, which provided PDT activity.$^{155}$ More recently an expansive
series of tetrametallic complexes with the structural motif, [(TL)$_2$Ru(dpp)]$_2$Ru(BL')PtCl$_2$]$^{6+}$,
where TL = bpy or phen, BL' = bpm (2,2'-bipyrimidine), dpp or dpq were reported.$^{156}$ The
complexes display increased visible light absorption with high molar absorptivity values due to
the additional Ru(II) chromophore absorptions and a low energy tail that falls in the
phototherapeutic window. The redox chemistry for [(TL)$_2$Ru(dpp)]$_2$Ru(dpp')PtCl$_2$]$^{6+}$ shows the
first oxidation (+1.58 V vs. Ag/AgCl) is two overlapping 1e$^-$ oxidations attributed to terminal
Ru$^{II/III}$ oxidations. Reductively, the dpp$^{0/-}$ reduction is observed at −0.40 V vs. Ag/AgCl,
followed by the two consecutive terminal dpp$^{0/-}$ reductions at −0.60 V and −0.71 V vs. Ag/AgCl.
Stabilization of the dpp'(π*) orbital occurs upon platination, shifting the reduction potential more
positive of the terminal dpp$^{0/-}$ reductions. Based on the electrochemistry, the
[(TL)$_2$Ru(dpp)]$_2$Ru(BL')PtCl$_2$]$^{6+}$ complex displays terminal Ru(dπ)-based HOMOs and
dpp'(π*)-based LUMOs affording spatial separation between the frontier orbitals, indicating a
lower-lying non-emissive charge separated state ($^3$CS) is possible. The presence of a low-lying $^3$CS state was confirmed by comparing the photophysical properties of the

$$\left\{\text{(TL)}_2\text{Ru(dpp)}\right\}_2\text{Ru(dpp')}\}^{6+}$$ precursor and the Ru(II)-Pt(II) tetrametallic complexes, which both exhibit the same Ru(dπ)→dpp'(π*) $^3$MLCT excited state. The quantum yield of emission was reduced and the excited state lifetime decreased at RT for the tetrametallic complex ($\Phi_{em} = 0.0003$ and $\tau_0 = 100$ ns) indicative of intramolecular electron transfer to populate the $^3$CS state in comparison to the trimetallic precursor ($\Phi_{em} = 0.0006$ and $\tau_0 = 130$ ns). At 77 K, the excited state lifetime values for the trimetallic and tetrametallic were 1.7 $\mu$s, attributed to prohibited electron transfer to the low-lying $^3$CS state in rigid media. In comparison to the previously reported Ru(II)-Pt(II) bimetallic complexes, the expanded architecture in the tetrametallic complex provides spatial separation of the terminal Ru(dπ)→dpp'(π*) $^3$MLCT excited state and the Pt(II) BAS affording longer-lived $^3$MLCT states. The \{\{(TL)_2Ru(dpp)\}_2Ru(dpp')PtCl_2\}^{6+} tetrametallic complex provides increased visible light absorbance and long-lived $^3$MLCT excited state spatially separated, but coupled to a cis-PtCl_2 moiety, affording a complex which can undergo interesting photoreactivity with DNA, summarized in section 1.4.4.

Systematic modification in a structural motif provides further understanding of how the modifications influence the redox and spectroscopic properties. The first Ru(II)-Pt(II) structural motif with systematic modification and biological studies are a series of tpy based complexes. There have been several bimetallic complexes, which use the tridentate, 2,2':6',2"-terpyridine (tpy) ligand as well as extended tpy analogs, 4′-(4-methylphenyl)-2,2′:6′,2"-terpyridine (MePhtpy) and 4,4′,4"-tri-tet-butyl-2,2′:6′,2"-terpyridine (tBu_3tpy) with BL variation reported by the Brewer group. Incorporation of a tpy ligand into a bimetallic complex affords the structural motif, \{\{tpy)RuL(BL)PtCl_2\}^+$, where L is a monodentate ligand and the BL is dpp, dpq,
or dpb. The tpy ligand eliminates the Λ and Δ isomers of the tris-bidentate complexes and provides an open coordination site useful for tuning the Ru(II) chromophore. Similar to the bidentate analogs, the redox properties indicate a Ru(dπ) HOMO and BL(π*) LUMO. The stabilized BL(π*) orbital limits thermal population of the 3LF deactivation pathway present in the [Ru(tpy)₂]²⁺ complex providing interesting properties. In the structural motif,

([TL]RuCl(dpp)PtCl₂)⁺, where TL = tpy, MePhtpy, and 'Bu₃tpy, the RuⅡ/Ⅲ oxidation occurred at +1.12 V, +1.10 V, and +1.01 V vs. Ag/AgCl and the two dpp reductions were largely unperturbed in this series. The changes in the oxidation potentials display the effect of TL substituents on the Ru(II) metal center. The electron donating character of the 'Bu (tert-butyl) groups shifts the RuⅡ/Ⅲ oxidation more negative as a result of increased electron density at the Ru(II) center. In addition, the oxidation potentials occur more negative in comparison to the bpy analogs due to the σ-donating nature of the chloride ligand also providing increased electron density at the Ru(II) center. As a result, in the electronic absorption spectroscopy, the low-lying Ru(dπ)→BL(π*) CT transition occurs at lower energy 545 nm (tpy, dpp) vs. 510 nm (bpy, dpp). The [(tpy)RuCl(BL)PtCl₂]⁺ complexes, where BL = dpp, dpq, or dpb displayed similar trends in the reduction potentials as the tris-bidentate analogs, with the dpb(π*) orbitals being most stabilized and occurring at more positive potentials than dpq followed by dpp. The reductions for the BL⁰⁻ and BL⁻²⁻ occurring at −0.50 and −1.05 V (dpp), −0.33 V and −0.92 V (dpq), and −0.21 V and −0.82 V (dpb) vs. Ag/AgCl, were similar to the reductions observed in the bpy systems. The RuⅡ/Ⅲ oxidation was largely unperturbed in changing the BL. The effect of the monodentate ligand was studied by changing from a weak field chloride ligand to a strong field phosphine ligand, affording the [(tpy)Ru(PE₃Ph)(dpp)PtCl₂]²⁺ bimetallic complex. The monodentate phosphine ligand shifts the RuⅡ/Ⅲ oxidation potential more positive from +1.12 V
(Cl) to +1.55 V (PEt$_2$Ph) vs. Ag/AgCl, while the BL$^{0/-}$ and BL$^{-2/-}$ reductions remain generally unperturbed. In the electronic absorption spectroscopy of the [(tpy)Ru(L)(dpp)PtCl$_2$]$^{2+}$ complexes, the Ru(d$\pi$)\(\rightarrow\)BL(\(\pi^*\)) CT transition shifts from 544 nm (Cl) to higher energy at 506 nm (PEt$_2$Ph) attributed to a larger HOMO-LUMO gap. The emission spectroscopy for the Ru(II)-Pt(II) bimetallic complexes was not reported as it is expected to be short-lived and outside the detection limits of the instrumentation.

Varying the Ru(II)-Pt(II) architecture results in interesting redox and spectroscopic properties, with the opportunity for exciting photochemical reactivity. TL modification may provide isomer control and influence the steric and electronics in photochemical reactivity. The BL choice determines the electronic coupling between the two metal centers, which influences the redox and spectroscopic properties. Expanded systems provide for decoupling of the Ru(II)-based LA and Pt(II)-based BAS giving long-lived $^3$MLCT states. Few systematic studies exist to allow for a full understanding and exploitation of these Ru(II)-Pt(II) motifs. In the following section, the biological interactions available for the Ru(II)-Pt(II) complexes are reported.

1.4.4. DNA and Biological Interactions with Ru(II)-Pt(II) Bimetallic Complexes

Biological activity studies of the Ru(II)-Pt(II) bimetallic complexes have been limited to interactions with calf thymus DNA (CT-DNA) and plasmid DNA, as well as some bacterial studies.$^{130, 135, 148, 154, 155, 157, 159-164}$ Several methods have been used to study metal complex-DNA interactions including gel shift assay, DNA precipitation, and selective dialysis. Gel shift assay with plasmid DNA is by far the most common method used for studying DNA-metal complex interaction.

Gel shift assay monitors DNA migration through a gel with an applied voltage, which separates DNA molecules based on size, charge density, and shape, Figure 1.20. DNA fragments
with fewer base pairs, more compact structures, or more negatively charged move more rapidly through the gel leading to migration further toward the positive electrode. Plasmid DNA provides both supercoiled (Form I) and open circular (Form II) DNA in approximately 95% and 5% yield, respectively. The plasmid can undergo an unwinding event due to metal complex binding, a single strand cleavage event affording open circular DNA, or a double strand cleavage event resulting in linear DNA (Form III). Open circular DNA migrates the slowest through the gel appearing as a band towards the top of the gel, followed by bands attributed to linear and supercoiled DNA, Figure 1.20 C. For DNA binding studies, plasmid DNA may be linearized using a plasmid specific restriction enzyme and studied with metal complexes. Metal complex binding to DNA impacts size, shape, and charge of the DNA molecule adding complexity to gel shift assay interpretation. DNA is imaged in the gel via staining. A commonly used gel stain is ethidium bromide (EtBr), Figure 1.20 B. EtBr fluoresces approximately 20 times more intensely upon intercalation between the base pairs allowing for imaging of the gel on a transilluminator.
Figure 1.20: Schematics of gel shift assay process (A), staining by ethidium bromide (B), and a visualized gel presented as the negative black and white images with corresponding scanning electron microscopy images of supercoiled (form I), open circular (form II), and linear (form III) DNA (C). Microscopy images in figure C were adapted from reference 165 and reprinted with permission of W. H. Freeman and Company.

Cisplatin covalent binding to plasmid DNA was observed using gel shift assay.\textsuperscript{29, 168-171} Increasing cisplatin concentration in the presence of plasmid DNA resulted in slower migration of the supercoiled DNA, indicative of covalent modification. Cisplatin binding reached a saturation point between 4-10\%, resulting in the supercoiled DNA with bound metal complex coalescing with the open circular form as a result of metal binding and DNA unwinding. At concentrations greater than 10\%, the DNA started to condense or return to a more coiled form.
resulting in increased migration through the gel. However, the condensation of DNA is not observed for linearized forms. The covalent binding by cisplatin to DNA is generally used as a control for covalent DNA modification assayed using gel electrophoresis.

Brewer, et al. reported interactions of the monometallic precursors and Ru(II)-Pt(II) bimetallic complex with DNA. The monometallic analog precursors, \([\text{(bpy)}_2\text{Ru(dpq)}]^{2+}\), \([\text{(bpy)}_2\text{Ru(dpb)}]^{2+}\), \([\text{(phen)}_2\text{Ru(dpq)}]^{2+}\), and \([\text{(phen)}_2\text{Ru(dpb)}]^{2+}\) synthons showed no covalent binding to DNA, but should ionically bind or groove bind to DNA. The photoactivity of the monometallic complexes, \([\text{(bpy)}_2\text{Ru(dpp)}]^{2+}\), \([\text{(phen)}_2\text{Ru(dpp)}]^{2+}\), and \([\text{(Ph}_2\text{phen})_2\text{Ru(dpp)}]^{2+}\) in the presence of pUC18 plasmid DNA was also studied. The \([\text{(Ph}_2\text{phen})_2\text{Ru(dpp)}]^{2+}\) complex displays enhanced DNA association in comparison to the bpy and phen analogs consistent with the DNA interaction studies of the \([\text{Ru(TL)}_3]^{2+}\), where TL = bpy, phen, Ph\(_2\)phen. The longer-lived \(^3\text{MLCT}\) excited state and efficient quenching by oxygen afforded effective DNA photocleavage in the presence of oxygen by \([\text{(TL)}_2\text{Ru(dpp)}]^{2+}\) with TL bpy < phen < Ph\(_2\)phen.

The \([\text{(TL)}_2\text{Ru(BL)PtCl}_2]^{2+}\) (TL = bpy or phen, and BL = dpp, dpq, or dpb) molecular architectures have not been assayed for DNA photocleavage, but the covalent binding of the phen, dpq and phen, dpb systems were evaluated using gel shift assay. Coupling the Ru(II) LA unit to the \(\text{cis-PtCl}_2\) site afforded a positively charged molecule, which provided rapid association with the negatively charged DNA backbone. In addition, the Ru(II)-Pt(II) bimetallic complex displayed covalent modification of the DNA via thermal labilization of the chloride ligands causing more rapid retardation in migration of the metal bound DNA through the gel.

The \([\text{(bpy)}_2\text{Ru(bpy-(CONH-(CH}_2)_2\text{NH}_2)_2\text{PtCl}_2}]^{2+}\) complex was shown to covalently bind to DNA and then photocleave DNA upon irradiation with visible light in the presence of oxygen. Photoinduced electron transfer was suggested as a mechanism responsible for the
enhanced cleavage.\(^{154}\) Photoexcited reactants become more powerful oxidizing and reducing agents, which may provide useful information regarding energetically favorable biological redox activity with oxygen, DNA bases (specifically oxidation of guanine) or water.\(^{110,172-174}\) The excited state reduction (\(E_{\text{red}}^{*}\)) and oxidation (\(E_{\text{ox}}^{*}\)) potentials can be estimated using a revised Rehm-Weller equation 1.6 and 1.7, respectively,

\[
E_{\text{red}}^{*} = E_{\text{red}} + E^{0-0} \quad 1.6
\]

\[
E_{\text{ox}}^{*} = E_{\text{ox}} - E^{0-0} \quad 1.7
\]

where \(E_{\text{red}}\) and \(E_{\text{ox}}\) are the ground state reduction potentials and \(E^{0-0}\) is the energy of the transition from the thermally equilibrated excited state to the ground state, or the \(^3\)MLCT emission transition at 77 K in frozen glass.\(^{175}\) The mechanism of action for the \([(\text{bpy})_2\text{Ru(bpy-}(\text{CONH-}(\text{CH}_2)_2\text{NH}_2)_2\text{PtCl}_2)]^{2+}\) complex was thought to proceed through a direct electron transfer from the guanine to the photooxidized Ru(III) species which is bound through the platinum binding site affording improved activity.\(^{176}\) No DNA photocleavage experiments in the absence of oxygen and no redox properties were reported for the Ru(II)-Pt(II) complex to test this unusual hypothesis. It is more probable that the spatial separation of the Ru(II) chromophore from the Pt(II) binding moiety provides for efficient \(^1\)O\(_2\) generation. Therefore, the covalent binding to DNA in the presence of light affords localization of the \(^1\)O\(_2\) at the DNA resulting in DNA cleavage.

Multifunctional DNA interactions of the \([(\text{TL})_2\text{Ru(dpp})_2\text{Ru(dpp'})\text{PtCl}_2]\^{6+}\) complex were studied using gel shift assay. The Ru(II)-Pt(II) tetrametallic complex was reported to covalently bind DNA through the \(cis\)-PtCl\(_2\) binding unit and photocleave DNA in an oxygen-dependent manner through \(^1\)O\(_2\) generation.\(^{155}\) The \([(\text{TL})_2\text{Ru(dpp})_2\text{Ru(dpp'})\text{PtCl}_2]\^{6+}\) complex was the first example of a multifunctional DNA binding and photocleavage agent with polyazine
BLs. Several [(TL)RuCl(BL)PtCl$_2$]$^+$ analogs, where TL = tpy, MePhtpy, and $^1$Bu$_3$tpy and BL = dpp, dpq, or dpb displayed avid binding to DNA with enhanced retardation in DNA migration in comparison to cisplatin. In addition, the [(tpy)RuCl(dpp)PtCl$_2$]$^+$ complex was studied in the presence of $E. \text{coli}$ with promising results. Although the antibacterial photoactivity of the complexes was not reported, the Ru(II)-Pt(II) complex showed bacterial growth inhibition.

The Ru(II)-Pt(II) bimetallic complexes with reported biological interactions display covalent binding to DNA through the $\text{cis}$-PtCl$_2$ moiety, with only a handful of complexes displaying multifunctional DNA binding and photocleavage. Additional systematic studies of the Ru(II)-Pt(II) complexes are necessary to understand the photoactivity and produce highly active multifunctional molecules. Preliminary studies highlight the promise of supramolecular systems which couple Ru(II)-based LA units for PDT to Pt(II)-based BAS moiety for light activated treatment of cancer.

1.5. Conclusions and Future Directions for Ru(II)-Pt(II) Therapeutics

Cancer is a result of mutations at the molecular level, making DNA a possible target substrate for anticancer drug therapies. Transition metal-DNA association can occur via the anionic phosphate backbone (ionic binding), nucleosides (intercalation or covalent binding), and the major or minor grooves (major/minor groove binding). The most extensively studied transition metal-based complexes that covalently bind to DNA are cisplatin, carboplatin, and oxaliplatin. The Pt-based complexes display varied activity as a result of the leaving ligand lability, DNA site selectivity, as well as carrier ligand lipophilicity and steric hindrance. The second and third generation complexes have shown promise, with oxaliplatin being the first drug showing no cross-resistance with cisplatin. However, the Pt-based complexes still display
systemic toxicity and acquired cellular resistance limitations often resulting in poor quality of treatment for the patient.

PDT is a fundamentally different anticancer therapy, which utilizes a chromophore and light. The ideal PDT agent is non-toxic in the dark, has increased molar absorptivity values at low energy wavelengths, and interacts with biological substrates under aerobic and anaerobic conditions. Only recently were PDT agents FDA approved for cancer treatment. The development of a light targeted therapy is promising for future cancer treatments with limited systemic toxicity. The selectivity for cancerous cellular targets by PDT agents is primarily provided by the site of activation with light, which is challenging under hypoxic conditions due to oxygen dependence. Hypoxic tissues are prevalent in well-developed tumors. Therefore, incorporation of a cellular substrate targeting mechanism with a non-toxic chromophore may provide a new type of directed photoactivated therapy. Ru(II) polyazine complexes are well known to generate $\ce{O2^1}$ through energy transfer from the $\ce{3MLCT}$ state. One possibility for enhanced PDT action is coordination of a Ru(II)-based chromophore to a cis-PtCl$_2$ moiety, providing covalent binding to a biological substrate and targeted PDT activity.

Supramolecular chemistry as defined for this document combines individual subunits that have independent functions to form a compound that has multiple and more complicated roles. By incorporating a ruthenium-based chromophore through a polyazine bridging ligand, the photophysical features of the LA are imparted onto a cis-PtCl$_2$ covalent binding moiety affording an electronically coupled Ru(II)-Pt(II) bimetallic complex. One structural motif of the supramolecules of interest is $[(\text{TL})_2\text{Ru(BL)PtCl}_2]^{2+}$, which is the simplest construct coupling a Ru(II) LA for PDT activity and Pt(II) binding site for covalent modification of DNA. The building block method used to synthesize these molecular architectures offers many
opportunities to tune the photophysical and DNA binding characteristics by modifying the terminal and bridging ligands in the structural motif, Figure 1.21. Terminal ligand choice can influence the number of structural isomers of the complex, the redox and spectroscopic properties as well as excited state reactivity. Similarly by changing the bridging ligand the redox and spectroscopic properties are modified and the connectivity and electronic coupling between the Ru-chromophore and the Pt-binding site is altered. The complexes that have been synthesized offer a wide variety of redox, spectroscopic, and biological interactions; however none of the Ru(II)-Pt(II) complexes have been explored systematically with respect to interactions with DNA. Additionally, in vivo studies have not been widely reported; with only a few ex vivo analyses probing the interaction with bacterial cells reported. DNA and bacterial studies are helpful, but do not always translate to activity in mammalian cells.
Figure 1.21: The building block method displaying stepwise construction of supramolecular complexes. At each step the supramolecular architecture can be tuned or modified by altering the metal center, terminal ligand (TL), or bridging ligand (BL) and therefore the light absorber (LA) properties, and changing the bioactive site (BAS) metal center.
The aims of this work are to study in detail the basic chemical and biological activity of Ru(II)-Pt(II) systems aimed at coupling a Ru(II) chromophore PDT activity to a cis-PtCl₂ BAS for DNA covalent modification, affording metal complexes with promise as anticancer drugs. Exploration of the redox, spectroscopic, photophysical, and physicochemical properties of the [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺ bimetallic complexes, where BL = dpp or dpq, will be studied with an aim to correlate the therapeutic activity with DNA and for the treatment of cultured U87MG cells (malignant glioma). The structural architecture [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺, BL = dpp or dpq, affords several advantages in comparison to other Ru(II)-Pt(II) bimetallic complexes, known Ru(II) LAs, and the cisplatin model, Figure 1.22. Generally, directly coupling a Ru chromophore to Pt, results in deactivation of the ³MLCT excited state of the PDT agent and does not impact the reactivity at the Pt(II) BAS. It is proposed that by modifying the TLs, based on the enhanced excited state lifetime and Φ₁O₂ production, as well as increased lipophilicity observed for the [Ru(Ph₂phen)₂]²⁺ chromophore (compared to the bpy or phen analogs), the limitations of the previously reported directly coupled Ru(II)-Pt(II) complexes may be overcome. The BL variation will modulate the redox and spectroscopic properties allowing for modification of the lowest lying Ru(dπ)→BL(π*) orbital energetics, important in tuning absorption in the phototherapeutic window and ¹O₂ generation. The [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺ supramolecular complexes, where BL = dpp or dpq, were carefully designed to provide multifunctional interactions with biological substrates targeted at DNA. Ultimately, the results were better than expected with the [(Ph₂phen)₂Ru(BL)PtCl₂]²⁺ complexes impacting the reactivity at the Pt(II) BAS and dramatically enhancing the interactions with biomolecules.
Figure 1.22: Proposed Ru(II)-Pt(II) supramolecular design for multifunctional interactions with biological substrates.

Provides steric bulk around the cis-PtCl₂ bioactive site limiting possible glutathione deactivation.

Positively charged complex increases association with negatively charged biomolecules.

Non-inert chloride leaving ligands, which can be influenced by the excitation of the Ru(II)-chromophore.

Ru(ππ)-based HOMO and Ligand(π*)-based LUMOs affords large molar absorptivity values in the visible region.

The polyazine BL covalently and electronically couples the Ru(II)-chromophore and Pt(II)-bioactive site. Influences redox and spectroscopic properties.

The Ph₂phen TL reduces spectral gaps in the visible region, increases the lipophilicity for improved membrane permeability and ¹O₂ generation in comparison to the bpy and phen analogs.
Chapter 2: Materials and Methods

2. Materials

The starting materials RuCl$_3$•3H$_2$O, 4,7-diphenyl-1,10-phenanthroline (Ph$_2$phen), and LiCl were purchased from Alfa Aesar. K$_2$PtCl$_4$, 2,3-bis(2-pyridyl)pyrazine (dpp), 1,2-phenylenediamine, and KPF$_6$ were obtained from Sigma-Aldrich. All solvents were used as purchased from Fisher Scientific, unless otherwise noted. Doubly deionized water (ddH$_2$O) was filtered in lab unless otherwise noted. Whatman syringe filters (0.22 µm, PTFE) were purchased from Grace Davison Discovery Science. The chromatography materials, Sephadex LH-20, neutral alumina (80-200 mesh), and sand (20-30 mesh), were purchased from GE Healthcare Biosciences Corporation, Fisher Scientific, and Spectrum, respectively.

Spectral grade acetonitrile and DMF were obtained from Burdick and Jackson. Newly opened solvents were used without further purification, while solvents that were open already to atmospheric conditions were dried/purified using an alumina desiccant or distillation. Electrochemical grade tetrabutylammonium hexafluorophosphate (TBAH) supporting electrolyte was purchased from Fluka. 1,3-Diphenylisobenzofuran (DPBF) was purchased from Alfa Aesar.

Circular pUC18 plasmid DNA was purchased from Bayou Biolabs. Lambda DNA/HindIII molecular weight marker was obtained from Promega. Linearized DNA was prepared from circular pUC18, using the HindIII restriction enzyme and NE Buffer 2 from New England BioLabs. The calf thymus DNA (CT-DNA) was purchased from Rockland, Inc. and was dissolved in 0.1 M phosphate buffer. Electrophoresis grade boric acid, agarose, molecular biology grade glycerol and micropipette tips were purchased from Fisher Scientific. Sodium azide was purchased from Spectrum Chemicals.
U87MG cells were purchased from ATCC. Corning cell culture flasks with a canted neck and vented cap (75 and 25 cm², sterile packages of 5), and 500 mL disposable sterile individually wrapped bottle-top filters (0.22 µm pore, CA membrane) were purchased from Corning Biosciences. Cellstar 6 well plates (sterile packages of 5), individually wrapped sterile serological pipettes (5, 10, and 25 mL), heat inactivated (USDA approved) fetal bovine serum (FBS), and Eagle’s Minimal Essential Media (EMEM) with and without phenol red were purchased from Quality Biologicals, Inc. The RPMI 1640 media with L-glutamine and phenol red and RPMI 1640 media without L-glutamine and without phenol red were purchased from Cellgro, Inc. Gibco 1X Trypsin with 0.25% EDTA, L-glutamine, DMSO, and isopropanol were purchased from Sigma-Aldrich. Autoclavable micro-pipette man and micro-pipette tips for the cell culture hood were purchased from Thomas Scientific. Nuclease-free water was purchased from Ambion. Ethanol and Clorox bleach were used for sterilization and were purchased from Lab Safety Supply. Trypsin, L-glutamine, and FBS were stored in the freezer until used. Cells were cryo-preserved in a liquid nitrogen dewar.
3. Methods

3.1. Synthesis of cis-\([\text{PtCl}_2(\text{DMSO})_2]\)

The synthesis for cis-\([\text{PtCl}_2(\text{DMSO})_2]\) followed a previously reported method.\(^{177}\) The starting material, \(\text{K}_2\text{PtCl}_4\) (2.0 g, 5.0 mmol) was dissolved in 50 mL of room temperature ddH\(_2\)O. The solution was syringe-filtered into an Erlenmeyer flask and 2.0 mL DMSO (23.0 mmol) was added. The solution turned yellow and crystals formed overnight. The pale yellow crystals were isolated using vacuum filtration and were washed with ethanol, water, and diethyl ether.

3.2. Synthesis and Purification of \(\{(\text{Ph}_2\text{phen})_2\text{RuCl}_2\}\)

The monometallic starting material, \(\{(\text{Ph}_2\text{phen})_2\text{RuCl}_2\}\), was synthesized and purified using modification of a previously reported method.\(^{131, 178, 179}\) In a round bottom flask, 250 mL of DMF was heated at reflux with \(\text{RuCl}_3\cdot\text{H}_2\text{O}\) (2.0 g, 7.6 mmol), \(\text{Ph}_2\text{phen}\) (5.0 g, 15 mmol), and \(\text{LiCl}\) (2.5 g, 60 mmol) for 4 h. The solution was cooled to RT, 100 mL of acetone was added to the reaction mixture, and placed in the freezer for 12 h. The suspension was removed and added to a 5% by weight \(\text{LiCl}\) brine solution. The solid purple product was filtered, washed with water and ether and dried, while the orange filtrate containing \(\{\text{Ru(Ph}_2\text{phen})_3\}\text{Cl}_2\) was discarded. The \(\{(\text{Ph}_2\text{phen})_2\text{RuCl}_2\}\) product was further purified using alumina column chromatography with a 3:2 dichloromethane:acetone eluent. The initial purple band was collected leaving the orange band containing the \(\{\text{Ru(Ph}_2\text{phen})_3\}\text{Cl}_2\) complex adsorbed on the column. The solvent was removed and the solid was precipitated using 10 mL of dichloromethane into 400 mL diethyl ether, 3.8 g, 4.6 mmol with a 60% yield.
3.3. Synthesis and Purification of [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)]Cl<sub>2</sub> and [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)](PF<sub>6</sub>)<sub>2</sub>

The [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)]Cl<sub>2</sub> monometallic complex was synthesized following modification of a previously reported method. The [(Ph<sub>2</sub>phen)<sub>2</sub>RuCl<sub>2</sub>] (1.5 g, 1.8 mmol) starting material and dpp (0.70 g, 3.0 mmol) bridging ligand were added to 50 mL of 100% EtOH and heated at reflux for 2 h under atmospheric conditions. The reaction mixture was cooled to RT and the solvent was evaporated. The solid was purified by alumina chromatography using a 3:2 toluene:acetonitrile eluent, where the initial purple band containing [(Ph<sub>2</sub>phen)<sub>2</sub>RuCl<sub>2</sub>] was discarded, and the red band was collected. The solvent was removed under vacuum. For isolation of the chloride salt, the product was dissolved in a 10 mL of acetonitrile and flash precipitated into 300 mL diethyl ether, 1.4 g, 1.3 mmol, 70% yield. The [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)]Cl<sub>2</sub> was also metathesized to the PF<sub>6</sub><sup-></sup> salt by adding the product dissolved in 10 mL of water dropwise to a saturated aqueous KPF<sub>6</sub> solution. The solid was filtered and precipitated as described for the chloride salt 1.4 g, 1.2 mmol, 65%.

3.4. Synthesis and Purification of [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpq)]Cl<sub>2</sub> and [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpq)](PF<sub>6</sub>)<sub>2</sub>

The [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpq)]Cl<sub>2</sub> was synthesized by heating 50 mL of EtOH at reflux with [(Ph<sub>2</sub>phen)<sub>2</sub>RuCl<sub>2</sub>] (1.5 g, 1.8 mmol) and dpq (0.8 g, 2.8 mmol) for 3 h. The [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpq)]Cl<sub>2</sub> and [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpq)](PF<sub>6</sub>)<sub>2</sub> purification and isolation methods were the same as the dpp analog (section 3.3), producing a red-orange solid with 1.3 g, 1.2 mmol, 65% and 1.5 g, 1.1 mmol, 60% yields, respectively.
3.5. Synthesis and Purification of [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ and [(Ph₂phen)₂Ru(dpp)PtCl₂](PF₆)₂

The bimetallic complexes, [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ and [(Ph₂phen)₂Ru(dpp)PtCl₂](PF₆)₂, were prepared by reacting the respective monometallic precursor with a labile Pt(II) reagent. [(Ph₂phen)₂Ru(dpp)]Cl₂ (0.50 g, 0.47 mmol) and [PtCl₂(DMSO)₂] (1.0 g, 2.4 mmol) were heated at reflux in 25 mL of 100% EtOH for 2 h in the dark. The reaction mixture was cooled to RT and the solid was collected by vacuum filtration. The solid was dissolved in a minimal amount of 2:1 ethanol:acetonitrile, filtered through a fine frit, and the filtrate was purified by LH-20 size exclusion chromatography using 2:1 ethanol:acetonitrile eluent. The first red band was collected; solvent was removed under vacuum, and the complex was flash precipitated from 5 mL dry acetone into 200 mL diethyl ether, yield 0.47 g, 0.35 mmol, 75%. The Cl⁻ salt was used for all biological applications and was metathesized to the PF₆⁻ salt for characterization. Metathesis to the PF₆⁻ salt involved dissolving [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ in 10 mL water and adding drop wise to a saturated aqueous solution of KPF₆. The solid was filtered, dried, and precipitated from 5 mL acetonitrile into 200 mL diethyl ether, 0.51 g, 0.33 mmol, 70% yield.

3.6. Synthesis and Purification of [(Ph₂phen)₂Ru(dpq)PtCl₂]Cl₂ and [(Ph₂phen)₂Ru(dpq)PtCl₂](PF₆)₂

Synthesis of the [(Ph₂phen)₂Ru(dpq)PtCl₂]X₂, where X = Cl⁻ or PF₆⁻, was accomplished by substitution of [(Ph₂phen)₂Ru(dpq)]Cl₂ or [(Ph₂phen)₂Ru(dpq)](PF₆)₂ for the dpp analogs. The monometallic precursor, [(Ph₂phen)₂Ru(dpq)]Cl₂ (0.40 g, 0.36 mmol) and [PtCl₂(DMSO)₂] (0.80 g, 1.9 mmol) were heated at reflux in 25 mL of 100% ethanol for 3 h in the dark under atmospheric conditions. The purification and metathesis procedures were the same as the dpp
analog (section 3.5) producing a green colored product. The yields for the \( \text{Cl}^- \) or \( \text{PF}_6^- \) salts were 0.35 g, 0.25 mmol, 70% and 0.38 g, 0.23 mmol, 65% respectively.

### 3.7. ESI-Mass Spectrometry

The ESI-Mass spectrometry was performed by Virginia Tech Chemistry Department Analytical Services using an Agilent 6220 TOF instrument. The samples were dissolved in CH\(_3\)CN and directly injected into the instrument source.

### 3.8. NMR Spectroscopy

NMR spectroscopy was collected using a Bruker AVANCE 600 MHz NMR. The samples were dissolved in deuterated solvent and spectra were collected at room temperature. \(^{195}\text{Pt} \) NMR spectroscopy experiment was referenced to \( \text{K}_2\text{PtCl}_4 \) \( (\delta = -1614 \text{ ppm}) \).

### 3.9. Electrochemistry

The complexes were studied using cyclic voltammetry on an Epsilon potentiostat from Bioanalytical Systems. A three-electrode system was used; a platinum working electrode, a platinum wire auxiliary electrode, and a silver wire pseudo reference electrode, Figure 3.1. The scan rate for the studies was 100 mV/s. Following deoxygenation with argon, background scans of the supporting electrolyte/solvent system were completed prior to adding samples and showed no oxidative or reductive couples. Ferrocene was added as an internal standard after electrochemical analysis, \( (\text{FeCp}_2/\text{FeCp}_2^+ = 0.46 \text{ V vs. Ag/AgCl}) \), Figure 1.14.\(^{181,182}\) Analyses used a 0.1 M TBAH/CH\(_3\)CN supporting electrolyte/solvent system unless otherwise specified. This procedure provided \( E_{1/2} \) data ± 10 mV.
**Figure 3.1:** Electrochemistry setup for cyclic voltammograms using a Ag wire pseudo reference, Pt wire auxiliary, and Pt disc working, three electrode system. Samples were deoxygenated using Ar.

### 3.10. Coulometry

The oxidation of the Ru-Pt bimetallics was probed using coulometry to determine the number of electrons transferred. The coulometry experiment used a 10 mL beaker fitted with a separate auxiliary electrode compartment with a fine porous glass frit. A Pt wire auxiliary electrode, Ag wire pseudo reference electrode, and Pt disc working electrode were used for the cyclic voltammetry (CV) experiment to monitor progress, and the working electrode was switched to carbon cloth for the controlled potential electrolysis (CPE) experiment, shown in Figure 3.2. A 0.1 M TBAH/CH₃CN supporting electrolyte/solvent system was used and the concentration of the metal complexes was the same. The CV experiment was used to monitor the progress of the CPE experiment. The CPE potential and time was reset according to each CV and the net charge
was recorded for each segment until the oxidations were significantly shifted. Faraday’s Law was used to calculate the number of electrons, according to equation 3.1,

\[ Q = nFN \]  

where \( Q \) is the total charge passed through a substance or the net charge from CPE (C), \( n \) is the number of electrons transferred per molecule, \( F \) is Faraday’s constant (96,485 C/mol), and \( N \) is the mols of substance used in the experiment.

**Figure 3.2:** Coulometry setup for cyclic voltammetry and controlled potential electrolysis experiment.

### 3.11. Electronic Absorption Spectroscopy

Electronic absorption spectra were recorded using an Agilent 8453 diode array spectrophotometer with a 1 nm resolution. The spectra were collected in CH\(_3\)CN, DMF, or double deionized water (ddH\(_2\)O) at RT. Solutions for determining the extinction coefficient were
prepared gravimetrically and volumetrically diluted in accordance with Beer’s Law. The spectrophotometer was blanked using the appropriate solvent and the spectrum was collected using a 1 cm quartz cell. The values were reported as an average of three measurements. For DNA binding, photocleaving and cell culture studies, the metal complexes were measured in ddH$_2$O.

3.12. Emission Spectroscopy and Quantum Yield Determination

The room temperature emission spectra were recorded using a modified Photon Technology, Inc. QuantaMaster Model QM-200-45E. The system was modified to use a 150 W cooled xenon lamp excitation source, with emission collected at a right angle by a thermoelectrically cooled Hamamatsu 1527 photomultiplier tube operating in photon counting mode, Figure 3.3 A. Data were reported for both air-saturated and deoxygenated solutions. The air-saturated samples were used as prepared and the samples were then deoxygenated using ultra-high purity argon with sonication for 10 min prior to data collection. The emission grating was set to the 750 nm blaze, which was optimal for wavelengths in the visible region. The slit widths for the excitation and emission monochromators were adjusted to 6 nm, determining spectral resolution. Direct comparisons were made by collection of the data on the same day with the same instrumental/experimental settings. For 77 K emission, the samples were prepared as 4:1 ethanol:methanol absorbance-matched solutions. The solutions were sonicated in an NMR tube for 10 min and then immersed in a liquid nitrogen filled finger dewar. The samples were referenced to [Os(bpy)$_3$](PF$_6$)$_2$ standard, with $\Phi_{em} = 4.6 \times 10^{-3}$ in deoxygenated conditions, and the quantum yields calculated using equation 3.2,

$$\Phi_{sample} = \Phi_{standard} \times \frac{\text{Area}_{sample}}{\text{Area}_{standard}} \times \frac{\text{Absorbance}_{standard}}{\text{Absorbance}_{sample}}$$ 3.2
where $\Phi_{\text{sample}}$ is the quantum yield of the sample, $\Phi_{\text{standard}}$ is the quantum yield of the standard ($[\text{Os(bpy)}_3](\text{PF}_6)_2$, $\Phi^{\text{em}} = 4.6 \times 10^{-3}$), $\text{Area}_{\text{sample}}$ is the area under the sample emission profile, $\text{Area}_{\text{standard}}$ is the area under the standard emission profile, $\text{Absorbance}_{\text{standard}}$ is the measured absorbance for the standard, and $\text{Absorbance}_{\text{sample}}$ is the measured absorbance for the sample.

The spectra were corrected for detector response by multiplying the data by the manufacturer supplied correction file data, Figure 3.3 B.

Figure 3.3: Steady-state emission setup schematic (A) and detector response correction curve for 500-900 nm (B).

3.13. Excited State Lifetime Measurement

The excited state lifetime measurements were performed at room temperature and at 77 K. Measurements were collected using a Photon Technology, Inc. (PTI) PL2300 nitrogen laser equipped with a PL 201 continuously tunable dye laser as an excitation source (360-900 nm excitation source). For excitation wavelengths between 470-550 nm, a Coumarin 480 laser dye ($1 \times 10^{-3}$ M in ethanol), purchased from PTI, was used. A Hamamatsu R928 red-sensitive photomultiplier tube collected the time profile of the emission at right angles, Figure 3.4. The
signal was displayed on a LeCroy 9361 Dual 300 MHz oscilloscope. The data were fitted to a single exponential function using the equation 3.3,

$$ Y = A + Be^{-\frac{x}{c}} $$

where Y is the PMT response, A and B are scaling factors, x is time, and c is τ in seconds. The initial time prior to the laser pulse is removed before analysis. This system provides lifetime data ± 5 ns.

**Figure 3.4**: Time-resolved emission spectroscopy setup.

**3.14. Singlet Oxygen Quantum Yield Determination**

Singlet oxygen quantum yields were determined by a previously established method measuring the emission quenching of DPBF (1,3-diphenylisobenzofuran). In the presence of singlet oxygen produced from the metal complexes, DPBF is converted to the non-emissive o-dibenzoylbenzene (ODBB). The [Ru(Ph\text{2phen})\text{3}]Cl\text{2}, [(Ph\text{2phen})\text{2Ru(dpp)PtCl})\text{2}]Cl\text{2}, and [(Ph\text{2phen})\text{2Ru(dpq)PtCl})\text{2}]Cl\text{2} samples were absorbance matched, $A_{500\ nm} = 0.08$ AU in air-saturated methanol, with a final DPBF concentration of 20 µM in a 2 mL sample. The emission spectroscopy instrumentation was the same for this experiment however the slit width for the excitation and emission monochromators set at 2 nm. The samples were photolyzed using an
Oriel 1000 W Xe arc lamp fitted with a narrow bandpass filter ($\lambda_{irr} = 460$-$540$ nm), Figure 3.5, for varied time periods from 0 to 15 min.

**Figure 3.5:** The emission quenching scheme for the DPBF experiment and the absorption spectrum of the narrow bandpass that was fitted on the Xe arc lamp for the experiment. DPBF = 1,3-diphenylisobenzofuran, $^1$PS = singlet ground state for a photosensitizer, $^3$PS = triplet excited state for a photosensitizer.

Following photolysis the emission was analyzed, exciting the sample at 408 nm and detecting the emission of the DPBF at 480 nm. The $\Phi_{1O_2}$ were determined by fitting the data to a linear trend line, equation 3.4,

$$\frac{I_o - I_t}{I_o} = m(t) + 0$$  \hspace{1cm} (3.4)

where t is time, $I_o$ is the emission intensity at photolysis time = 0 min, and $I_t$ is the emission intensity at photolysis time = t min. The slope was referenced to the standard $[Ru(Ph_2 phen)_3]Cl_2$, with $\Phi_{1O_2} = 0.97$ \textsuperscript{119} to calculate the $\Phi_{1O_2}$ for both Ru-Pt bimetallic complexes, equation 3.5,

$$\frac{m_{\text{sample}}}{m_{\text{standard}}} = \frac{\Phi_{\text{sample}}}{\Phi_{\text{standard}}}$$  \hspace{1cm} (3.5)

where $m_{\text{sample}}$ is the slope of the Ru-Pt bimetallic sample, $m_{\text{standard}}$ is the slope of the $[Ru(Ph_2 phen)_3]Cl_2$ standard, $\Phi_{\text{standard}}$ is the singlet oxygen quantum yield for the standard (0.97), and $\Phi_{\text{sample}}$ is the singlet oxygen quantum yield for the Ru-Pt bimetallic sample.
Covalent Binding of Metal Complexes to Linear pUC18 DNA

Agarose gel electrophoresis was used to analyze covalent binding of the bimetallic complexes to linear pUC18 DNA and was compared to cisplatin, which is known to covalently bind to DNA. Master solutions of the metal complexes were prepared in ddH$_2$O. The concentrations were varied by preparing solutions of 5:1, 10:1, and 20:1 BP (base pairs) of pUC18 DNA to MC (metal complex) in 100 mM NaH$_2$PO$_4$ buffer (pH = 7.0). The samples were incubated for 1 hour at 37 °C. A Tris-Boric Acid (TB) buffer solution was prepared with 45 mM Tris base and 45 mM boric acid (pH = 7.4). To assay covalent DNA binding, 10 μL of the sample solutions (0.1 μg of linear pUC18 DNA) were mixed with 2 μL glycerine-based loading dye and loaded into the wells of a 30 ml gel prepared with 0.8% w/w agarose and 20% w/w TB buffer. Electrophoresis was performed using Owl Separation Systems, Inc., Model B1A electrophoresis state set at 105 V for 1.5 h. The gels were stained for 30 min in 0.5 µg/mL ethidium bromide (EtBr), destained for 15 min in ddH$_2$O, and visualized on a Fisher Scientific FBTI-88 transilluminator. Photographs were taken using an Olympus E-320 digital camera equipped with a Peca Products Inc. EtBr filter. The reported images were color inverted to the negative and converted to black and white.

DNA Photocleaving Studies

Agarose gel electrophoresis was also used to assay photocleavage of circular plasmid pUC18 DNA by the complexes. Metal complex/DNA solutions were prepared with ddH$_2$O and 100 mM NaH$_2$PO$_4$, forming a 20:1 BP:MC ratio. A LED array constructed by our group, consisting of eight thermostated cells using 5 W Luxeon Royal Blue Star LEDs (λ$_{max}$ = 455 nm, total average flux = 2.0 ± 0.1 x 10$^{19}$ photons/min) was used for photolysis. The 1 mL samples were photolyzed for 1 hour under air-saturated conditions or in the presence of a singlet oxygen...
quencher, sodium azide (0.1 M). The sodium azide samples were prepared by adding in the following order: sodium azide, buffer, DNA, water, and then MC. Identical samples were incubated at either RT or 37 °C in the dark for 1 hour. Electrophoresis and imaging were completed using section 3.15.

3.17. Photobinding of [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)PtCl<sub>2</sub>]<Cl<sub>2</sub> to pUC18 Plasmid DNA and Gel Shift Assay

Gel electrophoresis was used to assay photobinding of circular plasmid pUC18 DNA by [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)PtCl<sub>2</sub>]<sup>2+</sup>. The metal complex/DNA solution was prepared with ddH<sub>2</sub>O and 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, forming a 5:1 BP:MC ratio. A LED array, consisting of eight temperature-controlled cells using 5 W Luxeon Royal Blue Star LEDs (λ<sub>max</sub> = 455 nm, total average flux = 2.0 x 10<sup>19</sup> photons/min) or a Xe arc lamp, fitted with 455 and 590 nm cutoff filters (λ<sub>max</sub> ≥ 590 nm, total average flux = 6.0 x 10<sup>19</sup> photons/min), and a temperature-controlled cell holder, were used for photolysis, Figure 3.6. The 3 mL samples were photolyzed for 0, 2.5, 5, 10, 20, 30, 45, and 60 min under air saturated conditions. The control samples were incubated at RT and 37 °C in the dark. Electrophoresis and imaging were completed using section 3.15.
3.18. Photobinding of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ to CT-DNA and Selective Precipitation Assay

Calf thymus DNA (CT-DNA) that was exposed to [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]$^{2+}$ was selectively precipitated from solution using an adapted version of a previously reported method, Figure 3.7. The solid CT-DNA was hydrated in ddH$_2$O. The DNA container was placed on ice and the DNA solution was sonicated for three 30 s pulses. The concentration of the sonicated DNA was measured using the base pair molar absorptivity value $\varepsilon_{260} = 13,200$ M$^{-1}$ cm$^{-1}$. The [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]$^{2+}$ metal complex was dissolved in water. A stock metal complex-DNA
solution was prepared with ddH$_2$O and 0.1 M NaH$_2$PO$_4$, forming a 5:1 BP:MC ratio forming a total volume of 18 mL. The stock solution was split into six 3 mL quartz cells and was photolyzed using a LED array or Xe arc lamp. Both the dark controls (RT and 37 °C) and photolyzed samples (455 nm or ≥ 590 nm) were deoxygenated with Ar for 20 min prior to exposure to the experimental conditions through a septum. At each time point, 0, 2.5, 5, 10, 20, 30, 45, and 60 min, a 500 µL aliquot was removed. The DNA was precipitated using 20 µL 5 M NaCl and 2000 µL of 95% ethanol. The sample was centrifuged for 3 min at 13000 rpm.

The amount of metal complex that was unbound to DNA remained in the supernatant and the absorption at 525 nm was measured by an Agilent 8453 spectrophotometer. A blank sample was prepared using the same components without metal complex. The supernatants of each of the samples were measured and the absorbance at 525 nm was recorded. These experiments were repeated in triplicate. The change in absorptivity ($A_t/A_0$) vs. time was graphed.

**Figure 3.7**: Schematic of the selective precipitation method.

3.19. **Photobinding of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ to CT-DNA and DNA Melting Point Assay**

Similar to the above preparation, stock metal complex-DNA solution was prepared with ddH$_2$O and 0.1 M NaH$_2$PO$_4$, forming a 5:1 BP:MC ratio and a total volume of 6 mL. The stock
solution was split into two 3 mL cuvettes. The metal complex-DNA sample and DNA control sample were photolyzed using the reported LED array and conditions from the selective precipitation experiment. Separately a metal complex-DNA solution was purged with Ar and placed in the dark at 37 °C for 60 min. The melting point was studied using the change in $A_{260}$ vs. temperature using a JASCO-815 CD instrument with a thermally controlled cell compartment. The temperature was equilibrated for 20 s prior to taking the absorption reading at 260 nm and readings were taken every 3 °C from 50-101 °C. The melting point was determined from the $A_{260}$ vs. temperature graph. Melting point temperatures for the control sample were the same as the manufacturer’s report.

3.20. Partition Coefficient

The partition coefficient was measured as an approximation of the complex’s ability to partition across a cellular membrane, Figure 3.8. The buffer and octanol solvents were equilibrated and separated. The equilibrated solvents were then used for the experiments. The complexes were dissolved in 30 mL of buffer and 30 mL of octanol was added. The sample was stirred at 1000 rpm for 30 min and added to a separatory funnel for 12 h. The layers were separated and the concentration was determined using an electronic absorption spectrophotometer and the molar absorptivity values of the complex. The $\log P$ or partition coefficient was determined using equation 3.6,

$$
\log P = \log \left( \frac{[\text{octanol}]_f}{[\text{buffer}]_f} \right)
$$

3.6

where $[\text{octanol}]_f$ and $[\text{buffer}]_f$ are the concentrations of the octanol and buffer after separation.
3.2.1. Construction of LED Array

Multipurpose aluminum (Alloy 6061) U-channel pieces (4” base x 2” legs x 12” length) were purchased from McMaster-Carr Supply. The Luxeon Star cyan (510 nm, 130 lm at 700 mA) and red-orange (615 nm, 130 lm at 700 mA) Rebel pre-mounted (10 mm square base) LEDs, pre-cut 10 mm x 10 mm adhesive squares, and Luxeon Star Carclo TIR (total internal reflection) 29.8 degree frosted lens (10 x 10 mm base, FWHM (full width at half maximum) = 29.8 mm) with integrated legs were purchased from Quadica, Inc. The 19 mm x 19 mm x 13 mm heat sink (APF19-19-13CB/A01), Arctic Silver thermal adhesive, waterproof wiring nuts and Scotch-Weld epoxy adhesive were purchased from Mouser Electronics. Kester silver solder, soldering iron, 16 gauge multi-strand insulated wire, and heat-shrink tubing were purchased from RadioShack. The two Xitanium LED indoor dimmable drivers (30 W, 700 mA, 42 V) with constant current output and trailing dimmer compatibility were purchased from Philips Lighting. The trailing Levitron dimmers (TB103) were purchased from Lowe’s. The aluminum body was machined using the Virginia Tech Chemistry Department machine shop.

**Figure 3.8:** Schematic of cell membrane and representative examples of the partitioning experiment over time.
The LED placement was based on the dimensions of the six well plates used for cell culture experiments, shown in Figure 3.9. The VT Chemistry Department machine shop was used to bevel six 20 mm x 20 mm squares that were located directly above each well for the heat sinks in the U-shaped aluminum piece. Centered in the 20 mm x 20 mm square, a 10 mm x 10 mm square was stenciled and removed, leaving a 1 mm thick outer edge for mounting the heat sinks. At the opposite end, two 2 mm holes were bored through the aluminum for the wiring. The aluminum surfaces were cleaned well using isopropyl alcohol. The heat sink base was covered with Arctic Silver thermal adhesive and mounted according to the adhesive directions into the beveled squares. The 10 mm x 10 mm pre-mounted LED was then adhered onto the 10 mm x 10 mm exposed heat sink base using the Artic Silver thermal adhesive. The LEDs were wired in series using 16-gauge multi-strand insulated copper wire. Wire connections from the anode of the first LED base to the cathode of the next LED base were soldered using silver with the wire connectors on the first and last LEDs being longer to go through the end of the array. Each wire connector was protected from the external environment using heat-shrink tubing. The anode and cathode wires were connected to the driver using waterproof wire nuts. The driver was connected to a 2-pole straight blade plug. A trailing dimmer was used to control the brightness of the LEDs. A Carclo lens with legs was mounted on each LED base using Scotch-Weld epoxy adhesive being careful not to interfere with the wiring. Both the 510 and 615 nm LED arrays were used for sample photolysis outside the cell culture hood, as the sterility could not be controlled.
Figure 3.9: Schematic showing the dimensions of the 6-well plate (A), U-shaped piece (gray, end viewpoint) and beveling of the 20 x 20 mm square (maroon) and the 10 x 10 mm (orange) cut out for the heat sinks and LED bases (B), wiring diagram for the LED arrays (C), and the stepwise LED array construction (D).

3.22. Routine Cell Culturing

The glioblastoma cell line (U87MG) was continuously cultured from passage 1. The cells were cultured in the ATCC® suggested media, Eagle’s minimal essential medium (EMEM), supplemented with 10% fetal bovine serum (FBS). The cells were incubated at 37 °C with a 5% CO₂ atmosphere and the media was refreshed every other day. Upon reaching 70-80% confluency the cells were harvested using 1X Trypsin (0.25% EDTA), washed further with
media, and centrifuged at 1000 RPM for 10 min. The old media was aspirated, cells were suspended in fresh media, and split to continue subculturing or for experimental treatment. Experiments were carried out from passage 1 through 45.

To cryo-preserve early cell passages, cells were harvested and suspended in freezing media (EMEM with 10% FBS and 5% DMSO). The media with cells was added to cryovials, the vials were placed in the “Mr. Frosty” Nalgene® cryo freezing container filled with isopropanol alcohol, and this apparatus was placed in a −60 °C freezer overnight. The vials were then stored in a liquid nitrogen dewar until needed.

3.23. Dark U87MG Metal Complex Treatment using Cisplatin and [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂

The U87MG cells were harvested from 75 cm² flasks and split into 6 well plates. The cells were seeded at 2.0 x 10⁶ cells/well and were incubated for 18 h. Stock solutions (3.75 x 10⁻⁴ M) of cisplatin and [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ were made by dissolving metal complexes in water. The control samples were cells treated with media only and cells treated with volumes of water correlating to the amount of metal complex added. The media used for the treatment of the cells was 1640 RPMI media without L-glutamine and phenol red that was supplemented with 10% FBS, 1% antibiotic/antimitotic, and 1% L-glutamine. The media was added to the cell cultures followed by the addition of the varied water and metal complex volumes. The cells were treated for 1 h, then the media was replaced with culturing media, and the cells were incubated for another 72 h. Media was removed, but reserved (to include any cells that were dead but suspended) and then cells were harvested using trypsin. The cells were stained with Trypan blue for 2 min and counted. Cells that were golden in color were considered live cells, while cells
darkly stained blue were considered dead. Growth inhibition and cell viability were calculated based on the cells treated with media only.

3.24. **Photocytotoxicity U87MG Studies Using Cisplatin and**

\[(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2\text{Cl}_2\]

The U87MG cells were harvested from 75 cm$^2$ flasks and split into 6 well plates. The cells were seeded at 2.0 x 10$^6$ cells/well and were incubated for 18 h. Stock solutions (3.75 x 10$^{-4}$ M) of cisplatin and \[(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2\text{Cl}_2\] were used. Both dark control and photolysis experiments were completed. Similar to above the control samples were cells treated with media only and cells treated with volumes of water correlating to the amount of metal complex added. The media used for the treatment of the cells was 1640 RPMI media without L-glutamine and phenol red that was supplemented with 10% FBS, 1% antibiotic/antimitotic, and 1% L-glutamine. The media was added to the cell cultures followed by the addition of the varied water and metal complex volumes. The cells were treated in the dark for 15 min, light for 30 min, and then in the dark for another 15 min. The media was then replaced with culturing media and the cells were incubated for another 72 h. Media was removed but reserved (to account for cells in suspension) and the remaining cells were harvested using trypsin. The cells were stained with Trypan blue for 2 min and counted. Cells that were golden in color were considered live cells, while cells darkly stained blue were considered dead, Figure 3.10. Growth inhibition and cell viability were calculated based on the cells treated with media only.
Figure 3.10: Schematic of 6-well plate for treatment and expanded view of a representative hemocytometer counting area with live cells (golden) and dead cells (stained blue).
Chapter 3: Results and Discussion

Mixed-metal supramolecular complexes consisting of Ru(II)-light absorbers coupled through polyazine BLs to a Pt(II) BAS are developed for targeted PDT therapy. The molecular architecture [((Ph₂phen)₂Ru(BL)PtCl₂)]²⁺, where BL = dpp or dpq, couples an efficient UV and visible light absorbing chromophore imparting PDT activity onto a cis-PtCl₂ moiety affording covalent binding to nucleophilic cellular substrates and affording localized activity. The application of the Ph₂phen TL required overcoming synthetic difficulties, however the persistence proved worthwhile resulting in a complex where the Ph₂phen TL provided a dramatic positive influence on the bioactivity and photophysical properties of the Ru(II)-Pt(II) supramolecular systems, not observed in bpy or phen analogs and not necessarily predicted given the formally Ru(dπ)→BL(π*) nature of the photoactive states. The research described herein provides insight into the redox and photophysical properties of these Ru(II)-Pt(II) complexes and how those properties impact the photochemistry in the presence of DNA and the U87MG cancer cell line. In-depth studies of the Ru(II)-Pt(II) bimetallic complexes with systematic variation provides new understanding of the effect of each part of the molecular architecture on the redox and photophysical properties. Validation of the supramolecular architecture design for multifunctional photoactivity and covalent localization with biomolecules was investigated through interaction of the bimetallics with native DNA. Further studies probing the effect of the Ru(II)-Pt(II) complexes in more complicated systems were analyzed using U87MG cancer cells.
4. **Metal Complex Synthesis and Purification**

The mixed-metal complexes were synthesized using a building block approach which allows for ease of construction, purification, and characterization, Figure 4.1. \(^{184}\) Each building block is added stepwise at each synthetic step with purification and analysis of each intermediate product. The synthesis begins at the Ru(II) site allowing Pt(II) complexation to occur in the final step because the cis-PtCl\(_2\) site is not amenable to adsorption chromatography. The Ph\(_2\)phen terminal ligand was coordinated to the Ru-metal center to produce the [(Ph\(_2\)phen)\(_2\)RuCl\(_2\)] LA unit. The TL complexation step was similar, however purification was modified quite dramatically from previously published methods. \(^{131, 178, 179}\) Heating the reaction vessel initially produces an orange product, which turns to a dark purple over 4 h. The solution was cooled to RT and acetone was added. The acetone/DMF solution was added to a 5% by weight LiCl brine wash to remove the DMF. This step was added from the original Meyer, et al. preparation affording a dark purple product which was collected and an orange filtrate which was discarded. Additionally, column chromatography was necessary for the Ph\(_2\)phen complex because the [Ru(Ph\(_2\)phen)\(_3\)]\(^{2+}\) byproduct is quite emissive (\(\Phi^{em} = 0.36\))\(^{185, 186}\) and difficult to remove following coordination of the BL. The initial procedure for adsorption chromatography, reported by Brewer, et al. employed a 3:2 dichloromethane:acetonitrile eluent was modified to a 3:2 dichloromethane:acetone system to limit ligand exchange observed using CH\(_3\)CN producing [(Ph\(_2\)phen)\(_2\)Ru(CH\(_3\)CN)\(_2\)]\(^{2+}\) during the process. Column chromatography provides quick separation of excess ligand, [Ru(Ph\(_2\)phen)\(_3\)]\(^{2+}\), and the product [(Ph\(_2\)phen)\(_2\)RuCl\(_2\)]. Overall the modifications to the original synthetic procedure have resulted in reproducible products with 60% yield improved from 40%. In addition, the [(Ph\(_2\)phen)\(_2\)RuCl\(_2\)] product is free of detectable [Ru(Ph\(_2\)phen)\(_3\)]\(^{2+}\) analyzed using emission
analysis and found to be > 98% [(Ph₂phen)₂RuCl₂]. Attachment of the BL, where BL = dpp or dpq, was straightforward and produced high yields of the monometallic, [(Ph₂phen)₂Ru(BL)]²⁺ complex. Column chromatography was effective at separating the desired monometallic from the [(Ph₂phen)₂Ru(BL)Ru(Ph₂phen)₂]⁴⁺ bimetallic byproduct, unreacted ligand, and unreacted starting material. The yield was generally 65% for both BLs. Coordination of a cis-PtCl₂ moiety to the BL produces the title bimetallic complexes [(Ph₂phen)₂Ru(BL)PtCl₂](PF₆)₂ (BL = dpp or dpq). The labile cis-[PtCl₂(DMSO)₂] platination agent resulted in the highest purity and yield of the desired bimetallic, 65%. Purification of the bimetallic complex using size exclusion chromatography yielded separation of the title bimetallic complexes from the starting materials. The [(Ph₂phen)₂Ru(BL)PtCl₂](PF₆)₂ (BL = dpp or dpq) complexes prepared here are free of detectable [(Ph₂phen)₂Ru(BL)]²⁺ using emission analysis and detectable cis-[PtCl₂(DMSO)₂] using NMR spectroscopy resulting in > 95% [(Ph₂phen)₂Ru(BL)PtCl₂](PF₆)₂ product. The [(Ph₂phen)₂Ru(BL)]Cl₂ and [(Ph₂phen)₂Ru(BL)PtCl₂]Cl₂ complexes (BL = dpp or dpq) were synthesized as chloride salts and metathesized to PF₆⁻ salts for characterization.
Figure 4.1. Building block method used in the synthesis of [(Ph₂phen)₂(dpq)PtCl₄]²⁺. Adapted from reference 184. Ph₂phen = 4,7-diphenyl-1,10-phenanthroline and dpq = 2,3-bis(2-pyridyl)quinoxaline, modified from references 123, 167, and 168.
5. Metal Complex Characterization

5.1. ESI-MS

The ESI mass spectral data for monometallic precursors and title bimetallic complexes were consistent with their formulation, Table 5.1. The isotopic distribution patterns for the molecular ion fragments were simulated using UMIST MS isotope pattern calculator.\textsuperscript{187} The theoretical patterns were graphed and are compared to the experimental spectral data showing agreement, Figure 5.1 A-E.

**Table 5.1**: ESI-mass spectral data for [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(BL)](PF\textsubscript{6})\textsubscript{2} and [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(BL)PtCl\textsubscript{2}](PF\textsubscript{6})\textsubscript{2}, where BL = dpp or dpq.

<table>
<thead>
<tr>
<th>Complex Fragment ( ^{a} )</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>[(Ph\textsubscript{2}phen)\textsubscript{2}RuCl\textsubscript{2}]</td>
<td>836</td>
</tr>
<tr>
<td>[(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)]\textsuperscript{2+}</td>
<td>500</td>
</tr>
<tr>
<td>[(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)PtCl\textsubscript{2}]\textsuperscript{2+}</td>
<td>633</td>
</tr>
<tr>
<td>[(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpq)]\textsuperscript{2+}</td>
<td>525</td>
</tr>
<tr>
<td>[(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpq)PtCl\textsubscript{2}]\textsuperscript{2+}</td>
<td>658</td>
</tr>
</tbody>
</table>

\( ^{a} \) Ph\textsubscript{2}phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, and dpq = 2,3-bis(2-pyridyl)quinoxaline.
Figure 5.1 A: ESI-mass spectral data for \([\text{Ph}_{2}\text{phen}]_{2}\text{RuCl}_{2}\) in CH$_3$CN experimental (top) and theoretical (bottom).
Figure 5.1 B: ESI-mass spectral data for [(Ph<sub>2</sub>phen)<sub>2</sub>Ru(dpp)](PF<sub>6</sub>)<sub>2</sub> in CH<sub>3</sub>CN experimental (top) and theoretical (bottom).
Figure 5.1 C: ESI-mass spectral data for $[(\text{Ph}_2\text{phen})_2\text{Ru(dpq)}](\text{PF}_6)_2$ in CH$_3$CN experimental (top) and theoretical (bottom).
Figure 5.1 D: ESI-mass spectral data for \([\text{Ph}_2\text{phen}]_2\text{Ru(dpp)PtCl}_2](\text{PF}_6)_2\) in CH$_3$CN experimental (top) and theoretical (bottom).
Figure 5.1 E: ESI-mass spectral data for $[(\text{Ph}_2\text{phen})_2\text{Ru(dpq)}\text{PtCl}_2]^{2+}$ in CH$_3$CN experimental (top) and theoretical (bottom).
5.2. NMR Spectroscopy

$^{195}$Pt NMR spectroscopy was used to analyze complexation of platinum to the monometallic precursor, determine purity relative to the cis-[PtCl$_2$(DMSO)$_2$] starting material, and compare to previously reported Ru(II)-Pt(II) bimetallic complexes. It is important to assay the presence of other Pt containing species in the samples, but it typically not reported by other labs. The chemical shifts are reported in Table 5.2. The reaction of [PtCl$_2$(DMSO)$_2$] and [(Ph$_2$phen)$_2$Ru(BL)](PF$_6$)$_2$, where BL = dpp or dpq, was monitored by $^{195}$Pt NMR over time. The $^{195}$Pt resonance intensity at $-2980$ ppm corresponding to [PtCl$_2$(DMSO)$_2$] decreased as the starting material was consumed and the growth of a resonance at either $-2170$ or $-1970$ ppm increased corresponding to the formation of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$ or [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$](PF$_6$)$_2$, respectively. The bimetallic complexes were also analyzed by $^{195}$Pt NMR spectroscopy following purification and showed only the broad single resonances. The chemical shift of the [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$ at $-2170$ ppm was consistent with a set of previously reported [(TL)ClRu(dpp)PtCl$_2$](PF$_6$) analogs, where TL = tpy ($-2200$ ppm), MePhtpy ($-2200$ ppm), or tBu$_3$tpy ($-2220$ ppm) analogs.$^{161}$ A shift of 200 ppm downfield was observed for the [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$](PF$_6$)$_2$ compared to [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$ as a result of deshielding by the dpq ligand. The bimetallic purity is $>98\%$ with respect to the platinum starting material as no signal at $-2980$ ppm was observed in the $^{195}$Pt NMR of the isolated bimetallic systems.
Table 5.2: \(^{195}\)Pt NMR spectral shifts for Pt(II) precursors and Ru(II)-Pt(II) complexes.

<table>
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<tr>
<th>Metal Complex (^a)</th>
<th>(\delta) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{K}_2\text{PtCl}_4)</td>
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</tr>
<tr>
<td>(\text{cis-}[\text{PtCl}_3(\text{DMSO})_2])</td>
<td>(-2980)</td>
</tr>
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<td>(<a href="%5Ctext%7BPF%7D_6">\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2</a>_2)</td>
<td>(-2170)</td>
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<tr>
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</tr>
<tr>
<td>(<a href="%5Ctext%7BPF%7D_6">\text{MePhtpy)}\text{ClRu(dpp)PtCl}_2</a>)</td>
<td>(-2200)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BPF%7D_6">\text{MePtptpy})\text{ClRu(dpp)PtCl}_2</a>)</td>
<td>(-2220)</td>
</tr>
<tr>
<td>(<a href="%5Ctext%7BPF%7D_6">\text{tBu}_3\text{tpy})\text{ClRu(dpp)PtCl}_2</a>)</td>
<td>(-1970)</td>
</tr>
</tbody>
</table>

\(^a\) Ph\(_2\)phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, tpy = 2,2':6',2''-terpyridine, MePtptpy = 4'-(4-methylphenyl)-2,2':6',2''-terpyridine, and tBu\(_3\)tpy = 4,4',4''-tri-tert-butyl-2,2':6',2''-terpyridine.

5.3. **Electrochemistry**

Several electrochemical techniques were used to probe the redox properties of the electroactive species produced in the synthetic chemistry. The potentials of the redox processes, the relative frontier orbital energetics, and the number of electrons per molecule for certain processes were studied. The oxidative and reductive couple’s reversibility and the relative orbital energetics were studied using cyclic voltammetry (CV). The electrochemical data are summarized in Table A-1, pages 128-129, and shown in Figure 5.2.\(^{184}\) Cyclic voltammetry in a 0.1 M TBAH/CH\(_3\)CN supporting electrolyte/solvent system was completed at each step in the building block synthesis. Reversible Ru\(^\text{II/III}\) oxidations and reversible ligand\(^{0/-}\) couples were observed with \(\Delta E_p = E_p^c - E_p^a \approx \frac{59 \text{ mV}}{n}\) and \(i_P \approx 1\).

The electrochemical properties of the \([\text{Ph}_2\text{phen})_2\text{RuCl}_2\] starting material were investigated as a means of characterization and a test for redox active impurities. The \([\text{Ph}_2\text{phen})_2\text{RuCl}_2\] displayed a reversible Ru\(^\text{II/III}\) oxidation at +0.39 V and two overlapping reversible TL\(^{0/-}\) reductions at −1.51 and −1.60 V vs. Ag/AgCl. The strongly σ-donating character of the chloride ligands results in increased electron density at the Ru(II) center shifting the oxidation potential to
less positive potentials. The electrochemistry was consistent with the oxidation and reduction potentials reported for [(bpy)_2RuCl_2]. The bpy analog was reported to have a reversible Ru^{II/III} oxidation at +0.35 V and the ligand based reductions occurred between −1.50 V to −2.0 V vs. Ag/AgCl. In addition, a Ph_2phen^{0/-} reduction at −1.27 V vs. Ag/AgCl resulting from the [Ru(Ph_2phen)_3]^{2+} byproduct was not observed, indicative of > 90% purity.

Coordination of a π-acceptor BL in place of the strongly σ-donating chloride ligands is expected to affect the redox properties of the complex. Stabilization of the Ru(dπ) orbitals upon coordination of the BL shifts the Ru^{II/III} couple to positive potentials. The [(Ph_2phen)_2Ru(BL)](PF_6)_2 complexes displayed the typical reversible Ru-based oxidation, reversible one electron BL^{0/-} reduction, and two TL^{0/-} couples. The monometallic synthon, [(Ph_2phen)_2Ru(dp)](PF_6)_2, displays a reversible Ru^{II/III} oxidation at +1.45 V vs. Ag/AgCl, and reversible dp^{0/-}, Ph_2phen^{0/-}, Ph_2phen^{0/-} reductions at −0.98 V, −1.33 V, and −1.52 V vs. Ag/AgCl, respectively, consistent with previous reports. The Ru^{II/III} reversible oxidation and two TL^{0/-} reversible reductions were largely unperturbed upon changing the BL from dpp to dpq, while the BL^{0/-} reversible reduction shifted to −0.71 V vs. Ag/AgCl for the dpq analog. The CVs of the [(Ph_2phen)_2Ru(BL)](PF_6)_2 complexes as a function of BL establishes the BL as the LUMO in these complexes, which was still in question in previous reports. The electrochemistry of the [(Ph_2phen)_2Ru(BL)](PF_6)_2 monometallic complexes (BL = dpp or dpq) correlates well with previously reported [(TL)_2Ru(dp)](PF_6)_2 (TL = bpy (−1.16 V vs. Ag/AgCl) or phen (−1.02 V vs. Ag/AgCl) and for both the (bpy and phen) dpq analogs the couple occurred at −0.73 V vs. Ag/AgCl. Platination of the monometallic precursors stabilizes the BL(π*) orbital resulting in BL^{0/-} and BL^{−/2}− couples occurring prior to the two TL^{0/-} couples. The dp^{0/-} and dp^{−/2}− reductions occur at −0.45 and −1.15 V vs. Ag/AgCl, respectively,
compared to −0.19 and −0.95 V vs. Ag/AgCl for the sequential dpq reductions. Additionally following platination, the communicative nature of the BLs affords a less electron rich Ru(II) metal center and perturbation of the Ru$^{II/III}$ oxidation. A small shift to positive potential was observed for the Ru$^{II/III}$ couple from +1.45 V (dpp or dpq monometallic synthon) to +1.61 V and +1.63 V vs. Ag/AgCl for dpp and dpq, respectively, following coordination of the electron deficient cis-PtCl$_2$ moiety. Prior to the Ru$^{II/III}$ oxidation an irreversible Pt-based oxidation occurs as a shoulder at approximately +1.51 V vs. Ag/AgCl for both dpp and dpq bimetallic analogs.
Figure 5.2: Cyclic voltammograms of [(Ph₂phen)₂RuCl₂] (A), [(Ph₂phen)₂Ru(dpp)](PF₆)₂ (B), [(Ph₂phen)₂Ru(dpp)PtCl₂](PF₆)₂ (C), [(Ph₂phen)₂Ru(dpq)](PF₆)₂ (D), and [(Ph₂phen)₂Ru(dpq)PtCl₂](PF₆)₂ (E) in 0.1 M TBAH/CH₃CN supporting electrolyte/solvent system, using a Pt working electrode, Pt auxiliary electrode, Ag pseudo reference electrode and a ferrocene internal standard (FeCp₂/FeCp²⁺ = 0.46 V vs. Ag/AgCl), recorded at 100 mV/s. Adapted from reference 184. Ph₂phen = 4,7-diphenyl-1,10-phenanthroline, BL = dpp = 2,3-bis(2-pyridylyl)pyrazine, and dpq = 2,3-bis(2-pyridylyl)quinoxaline.
Systematic evaluation of the redox properties as each building block is added allows a direct measure of interconnections within these supramolecules. The metals and ligands are all electroactive in the solvent/electrolyte system, which provides an assay of subunit addition and complex purity at each step of the synthesis. The assignment of the nature of the redox couples and orbital energetics are possible through such monitoring and by systematic variation of the BL in these systems. For the \([\text{[(Ph}_2\text{phen)}_2\text{Ru(BL)}](\text{PF}_6)_2\) and \([\text{[(Ph}_2\text{phen)}_2\text{Ru(BL)PtCl}_2](\text{PF}_6)_2\) complexes the electrochemistry predicts a Ru(d\(\pi\))-based HOMO and a BL(\(\pi^*\))-based LUMO indicating a Ru(d\(\pi\))\(\rightarrow\)BL(\(\pi^*\)) MLCT lowest lying excited state, important in understanding the spectroscopy, photophysics, and photochemistry of these supramolecules. The less negative reduction potential showing a lower energy dpq(\(\pi^*\)) orbital vs. dpp(\(\pi^*\)) results in a reduced HOMO-LUMO energy gap in the dpq complexes, suggesting a lower energy MLCT excited state compared to the dpp analogs.

5.4. Coulometry

The overlapping quasi-reversible oxidations of the \([(\text{Ph}_2\text{phen)}_2\text{Ru(BL)PtCl}_2](\text{PF}_6)_2\) (BL = dpp or dpq) are generally assumed to be overlapping Ru and Pt oxidations but was subjected to further investigation. This couple has been assigned in the literature for related Ru(II)-Pt(II) complexes as overlapping Ru and Pt oxidation, but not thoroughly investigated. Coulometry was used to determine the number of electrons transferred in the oxidative couple for assignment of an overlapping Ru and Pt oxidation.\textsuperscript{130, 148-150, 164} For each trial the samples were concentration matched, and the amount of net charge transferred, \(Q\), was determined. The experiments were conducted in triplicate. The number of electrons, \(n\), passed with electrolysis at +1.50 V vs. Ag/AgCl was calculated using equation 5.1,

\[
Q = nFN
\]  

5.1
The number of electrons transferred were $2.75 \pm 0.23$ and $2.89 \pm 0.27$ for
$[(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2](\text{PF}_6)_2$ and $[(\text{Ph}_2\text{phen})_2\text{Ru(dpq)PtCl}_2](\text{PF}_6)_2$, respectively. The three electrons transferred establish overlapping Ru and Pt oxidations that are attributed to a one electron Ru$^{II/III}$ couple expected to be reversible and an irreversible two electron Pt$^{II/IV}$ couple at these potentials.

5.5. Electronic Absorption Spectroscopy

The electronic absorption spectroscopy of the complexes has been studied. The spectroscopy of these complexes are shown in Figure 5.3, displaying efficient UV and visible light absorptivity. The spectroscopic transitions in these complexes are broad and overlapping, making it difficult to assign individual transitions. Generally, Ru(II)-polyazine complexes and the Ru(II)-Pt(II) bimetallic complexes display strong TL and BL intraligand ($\pi \rightarrow \pi^*$) transitions in the UV region as well as Ru(d$\pi$)$\rightarrow$TL($\pi^*$) and Ru(d$\pi$)$\rightarrow$BL($\pi^*$) CT bands in the visible. Based on the electrochemistry, the HOMO and LUMO for the monometallic and bimetallic complexes are Ru(d$\pi$) and BL($\pi^*$) based. Therefore, the Ru(d$\pi$)$\rightarrow$BL($\pi^*$) CT transition is expected to be the lowest lying electronic transition, red shifting from BL = dpp to dpq upon platination of the monometallic precursors.

The transitions in the UV and visible region of the electronic absorption spectroscopy were assigned and the molar absorptivity values reported in RT CH$_3$CN for the mono- and bimetallic complexes. The $[(\text{Ph}_2\text{phen})_2\text{RuCl}_2]$ complex displays typical Ph$_2$phen $\pi \rightarrow \pi^*$ transitions at 274 nm ($\varepsilon = \text{ca. } 65,000 \text{ M}^{-1}\text{cm}^{-1}$). In the visible region, several overlapping transitions occur at 470 ($\varepsilon = \text{ca. } 7,000 \text{ M}^{-1}\text{cm}^{-1}$), 555 ($\varepsilon = \text{ca. } 14,000 \text{ M}^{-1}\text{cm}^{-1}$), and 670 nm ($\varepsilon = \text{ca. } 4,000 \text{ M}^{-1}\text{cm}^{-1}$), which are attributed to Ru(d$\pi$)$\rightarrow$Ph$_2$phen($\pi^*$) $^1$CT. In the UV region of the electronic absorption spectroscopy for $[(\text{Ph}_2\text{phen})_2\text{Ru(BL)}](\text{PF}_6)_2$, where BL = dpp or dpq, IL (IL = intraligand)
transitions occur and are assigned as Ph$_2$phen $\pi \rightarrow \pi^*$ transitions at 274 nm ($\varepsilon = \text{ca. 90,000}$ M$^{-1}$cm$^{-1}$), dpp $\pi \rightarrow \pi^*$ transitions at 310 nm ($\varepsilon = \text{ca. 35,000 M}^{-1}\text{cm}^{-1}$) or dpq $\pi \rightarrow \pi^*$ transition at 320 nm ($\varepsilon = \text{ca. 34,000 M}^{-1}\text{cm}^{-1}$). The larger molar absorptivity values for the Ph$_2$phen $\pi \rightarrow \pi^*$ transitions from [(Ph$_2$phen)$_2$RuCl$_2$] to [(Ph$_2$phen)$_2$Ru(BL)](PF$_6$)$_2$ are expected, considering the BL $\pi \rightarrow \pi^*$ transitions overlap with and contribute to the Ph$_2$phen. $^1$MLCT transitions occur in the visible region for each $\pi$-acceptor TL and BL. The Ru(d$\pi$)\text{Ph$_2$phen($\pi^*$)} CT transitions are relatively unperturbed for the series of monometallic and bimetallic complexes, occurring at 424 nm ($\varepsilon = \text{approximately 18,000 M}^{-1}\text{cm}^{-1}$). Platination of the [(Ph$_2$phen)$_2$Ru(dpp)](PF$_6$)$_2$ complex results in a red shift for the Ru(d$\pi$)\text{dpp($\pi^*$)} CT transition from 474 nm ($\varepsilon = 15,000$ M$^{-1}$cm$^{-1}$) for the monometallic to 520 nm ($\varepsilon = 11,400$ M$^{-1}$cm$^{-1}$) for the bimetallic due to stabilized BL($\pi^*$) acceptor orbitals upon platination. Similar to the dpp analog, the Ru(d$\pi$)\text{dpq($\pi^*$)} CT transition at 522 nm ($\varepsilon = 11,200$ M$^{-1}$cm$^{-1}$) shifts to lower energy at 600 nm ($\varepsilon = 9,800$ M$^{-1}$cm$^{-1}$) upon platination of the monometallic precursor. In addition, each of the complexes displays a broad and weak low energy transition tail attributed to the $^3$MLCT transition.

The electronic absorption spectroscopy of [(TL)$_2$RuCl$_2$] complexes, where TL = bpy, phen, or Ph$_2$phen displays the importance of the TL in the visible region. The $\pi \rightarrow \pi^*$ transitions for bpy, phen and Ph$_2$phen occur at 288 nm ($\varepsilon = \text{ca. 45,700 M}^{-1}\text{cm}^{-1}$),$^{178,189}$ 267 nm ($\varepsilon = \text{ca. 72,500 M}^{-1}\text{cm}^{-1}$),$^{191}$ and 274 nm ($\varepsilon = \text{ca. 65,000 M}^{-1}\text{cm}^{-1}$), respectively. In the visible region the spectral profile differs between the three analogs, however the transitions are attributed to higher and lower energy Ru(d$\pi$)\text{TL($\pi^*$)} CT transitions.$^{179,189}$ In the analogous systems, $\lambda_{\text{max}}^{\text{abs}}$ were reported at 376 nm ($\varepsilon = \text{ca. 15,850 M}^{-1}\text{cm}^{-1}$) and 550 nm ($\varepsilon = \text{ca. 15,500 M}^{-1}\text{cm}^{-1}$) with a low energy shoulder at ca. 650 nm for bpy, whereas a single transition was reported at 496 nm ($\varepsilon = \text{ca. 10800 M}^{-1}\text{cm}^{-1}$) for phen (spectral profile was not shown in patent literature).$^{189,191,192}$
Compared to the bpy system, the Ph₂phen complex displays fewer spectral gaps and an enhanced low energy MLCT tail in the visible region. The TL variation in these systems displays the value of Ph₂phen for enhanced absorptivity.
Figure 5.3: Electronic absorption recorded in RT CH$_3$CN (solid line), room temperature steady-state emission recorded in deoxygenated RT CH$_3$CN (dashed line), and 77 K steady-state emission recorded in 4:1 EtOH:MeOH glass (dotted line) normalized profiles for the Ru(II)-monometallic and Ru(II)-Pt(II) bimetallic complexes. Adapted from reference 184.
Analysis of the electronic absorption spectroscopy of \([(\text{Ph}_2\text{phen})_2\text{Ru(BL)}](\text{PF}_6)_2\) and \([(\text{Ph}_2\text{phen})_2\text{Ru(BL)PtCl}_2](\text{PF}_6)_2\) complexes, where BL = dpp or dpq, also display similarities to and differences from the bpy or phen analogs. In both the monometallic and bimetallic complexes, the \(\text{TL}(\pi \rightarrow \pi^*) \lambda_{\text{max}}^\text{abs}\) occur in the UV region of the electronic absorption spectroscopy at 280 nm, 260 nm, and 270 nm and the \(\text{Ru}(d\pi) \rightarrow \text{TL}(\pi^*)\) CT transitions occur at 410 nm, 410 nm, and 424 nm for bpy, phen, and Ph\(_2\)phen, respectively. The \(\text{Ru}(d\pi) \rightarrow \text{TL}(\pi^*)\) CT transitions are more easily assigned in the bimetallic complexes, as platination stabilizes the BL(\(\pi^*\)) orbital and shifts the \(\text{Ru}(d\pi) \rightarrow \text{BL}(\pi^*)\) CT transitions to lower energy, affording separation and improved resolution. In the \([(\text{TL})_2\text{Ru(dpp)PtCl}_2](\text{PF}_6)_2\), TL = bpy, phen, or Ph\(_2\)phen, there is approximately a two-fold increase in the \(\text{Ru}(d\pi) \rightarrow \text{TL}(\pi^*)\) CT molar absorptivity for Ph\(_2\)phen compared to the bpy and phen analogs providing enhanced spectral coverage throughout the UV and visible compared to the reduced absorptivity in the 400-500 nm region observed for bpy and phen analogs, Figure 5.4.\textsuperscript{130, 148-150, 164} Generally the dpp-based complexes display dpp(\(\pi \rightarrow \pi^*\)) transitions at 280-300 nm, while the dpq(\(\pi \rightarrow \pi^*\)) transitions are shifted to lower energy at 320-350 nm, due to stabilization of the dpq(\(\pi^*\)) orbital. The \(\text{Ru}(d\pi) \rightarrow \text{BL}(\pi^*)\) CT transitions for the \([(\text{TL})_2\text{Ru(dpp)}](\text{PF}_6)_2\) complexes occur at 460 nm (bpy), 465 nm (phen), and 474 nm (Ph\(_2\)phen) nm and for the dpq analogs at 517 nm (bpy), 520 nm (phen), and 520 nm (Ph\(_2\)phen). Upon platination the \(\text{Ru}(d\pi) \rightarrow \text{BL}(\pi^*)\) CT transition shifts to lower energy due to stabilization of the BL(\(\pi^*\)) orbital. The \(\text{Ru}(d\pi) \rightarrow \text{BL}(\pi^*)\) CT transitions in \([(\text{Ph}_2\text{phen})_2\text{Ru(BL)PtCl}_2](\text{PF}_6)_2\) occur at 520 nm (dpp) and 600 nm (dpq). The shift to low energy of the \(\text{Ru}(d\pi) \rightarrow \text{BL}(\pi^*)\) CT transitions upon substitution of dpq for dpp are also reported for the previously studied bpy and phen analogs.\textsuperscript{130, 164}
Figure 5.4: Electronic absorption and steady-state room temperature emission spectra for the [(TL)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$ analogs, where TL = bpy (blue), phen (red), and Ph$_2$phen (green). Recorded in RT CH$_3$CN. bpy = 2,2’-bipyridine, phen = 1,10-phenanthroline, Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline, and dpp = 2,3-bis(2-pyridyl)pyrazine.

5.6. Emission and Photophysics of [(Ph$_2$phen)$_2$Ru(dpp)](PF$_6$)$_2$, [(Ph$_2$phen)$_2$Ru(dpq)](PF$_6$)$_2$, and [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$

The [(Ph$_2$phen)$_2$Ru(BL)](PF$_6$)$_2$ (BL = dpp and dpq) and [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$ complexes display emission from their lowest lying Ru($d\pi$)$\rightarrow$BL($\pi^*$) $^3$MLCT excited state in both fluid solution and frozen glass media. Emission spectra are shown in Figure 5.3 and 5.4 and summarized in Table A-1, pages 128-129. The photophysics of the monometallic and bimetallic complexes is described using a Jablonski diagram, Figure 5.5 with the calculated rate constants reported in Table 5.3. The Ru(II)-polyazine and the Ru(II)-Pt(II) bimetallic complexes undergo excitation throughout the UV and visible to populate singlet excited states that undergo non-radiative internal conversion and intersystem crossing with unit efficiency to populate the lower energy $^3$MLCT excited state. Emission is observed from the lowest lying Ru($d\pi$)$\rightarrow$BL($\pi^*$) $^3$CT excited state with interesting photophysical properties and a probe into photochemical reactivity. Ru(II) polyazine monometallic complexes are more emissive than the related bimetallic complexes in accordance with the energy gap law due to the lower-lying $^3$MLCT
states of the bimetallic complexes. In fluid media the observed emission bands are broad. The emission bands in glass media are blue shifted with more narrowed bands and vibronic structure.\textsuperscript{195-197}

\textbf{Figure 5.5}: Representative Jablonski diagram for [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)](PF\textsubscript{6})\textsubscript{2} (A) and [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)PtCl\textsubscript{2}](PF\textsubscript{6})\textsubscript{2} (B) with energy levels for the \(^1\)MLCT and \(^3\)MLCT excited states determined experimentally. Ph\textsubscript{2}phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, \(^1\)GS = singlet ground state, \(^1\)MLCT = singlet metal-to-ligand charge transfer excited state, \(^3\)MLCT = triplet metal-to-ligand charge transfer excited state, \(k_{nr}\) = rate constant for non-radiative decay, \(k_{r}\) = rate constant for radiative decay, \(k_{isc}\) = rate constant for intersystem crossing non-radiative decay.

Steady-state and time-resolved emission spectroscopies were used to quantify the emission quantum yields and excited state lifetimes of the \(^3\)MLCT excited state complexes as well as the related rate constants. The [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)](PF\textsubscript{6})\textsubscript{2} monometallic complex displays an intense emission from the Ru(d\pi)\rightarrow dpp(\pi*) \(^3\)CT state at RT in deoxygenated CH\textsubscript{3}CN at \(\lambda_{\text{max}}^{\text{em}} = 664\) nm (\(\Phi^{\text{em}} = 3.5 \times 10^{-2}, \tau = 820\) ns, \(k_{r} = 4.3 \times 10^{4}\) s\(^{-1}\), \(k_{nr} = 1.2 \times 10^{6}\) s\(^{-1}\)). The \(\Phi^{\text{em}}\) and \(\tau\) for [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)](PF\textsubscript{6})\textsubscript{2} analog are higher than the bpy (\(\Phi^{\text{em}} = 1.2 \times 10^{-2}, \tau = 380\) ns, \(k_{r} = 3.2 \times 10^{4}\) s\(^{-1}\), \(k_{nr} = 2.6 \times 10^{6}\) s\(^{-1}\)) or phen (\(\Phi^{\text{em}} = 2.7 \times 10^{-2}, \tau = 460\) ns, \(k_{r} = 5.9 \times 10^{4}\) s\(^{-1}\), \(k_{nr} = 2.1 \times 10^{6}\) s\(^{-1}\)) analogs. This is surprising considering the formally Ru(d\pi)\rightarrow BL(\pi*) \(^3\)CT nature of the emissive excited state. The TL generally has minor impact on the emissive properties of
Ru(dπ)→BL(π*) 3CT states. In the case of Ph2phen, the TL likely contributes to the formally Ru(dπ) donor orbital in the Ru(dπ)→BL(π*) 3CT state as a means to perturb the properties of 3MLCT excited states. At 77 K in 4:1 EtOH:MeOH glass the emission band narrows and shifts to 607 nm with an extended lifetime, \( \tau = 5.4 \, \mu s \) with introduction of vibronic structure characteristic of 3MLCT excited state.\(^{195,197} \) The [(Ph2phen)2Ru(dpq)](PF6)2 has a red shifted emission relative to the dpp system in RT deoxygenated CH3CN centered at 758 nm (\( \Phi_{\text{em}} = 1.1 \times 10^{-4} \), \( \tau = 20 \, \text{ns} \), \( k_r = 5.5 \times 10^3 \, \text{s}^{-1} \), \( k_{\text{nr}} = 5.0 \times 10^7 \, \text{s}^{-1} \)). At 77 K the dpq system displays an emission at 715 nm (\( \tau = 1.7 \, \mu s \)) in 4:1 EtOH:MeOH glass. The red shift for the emission from the dpq monometallic complex vs. the dpp analog is consistent with the lower-lying dpq(π*) acceptor orbital and a stabilized 3MLCT state predicted by the redox properties and electronic absorption spectroscopy. The shorter-lived, weaker emission for the dpq vs. dpp analog is consistent with the lower energy 3MLCT excited state in accordance with the energy gap law.\(^{138,143} \)

**Table 5.3:** Photophysical data at room temperature for [(TL)2Ru(dpp)](PF6)2 and [(TL)2Ru(dpp)PtCl2](PF6)2, where TL = bpy, phen, or Ph2phen, recorded in RT CH3CN.

<table>
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<th>Metal Complex (^a)</th>
<th>( \lambda_{\text{max}}^{\text{em}} ) (nm)</th>
<th>( \Phi_{\text{em}} )</th>
<th>( \tau ) (s)</th>
<th>( k_r ) (s(^{-1}))</th>
<th>( k_{\text{nr}} ) (s(^{-1}))</th>
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<tr>
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<tr>
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<td>4.3 \times 10^{4}</td>
<td>1.2 \times 10^{6}</td>
</tr>
<tr>
<td><a href="PF6">(bpy)2Ru(dp)PtCl2</a>2</td>
<td>760</td>
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<td>4.6 \times 10^{-8}</td>
<td>8.7 \times 10^{3}</td>
<td>2.2 \times 10^{7}</td>
</tr>
<tr>
<td><a href="PF6">(phen)2Ru(dp)PtCl2</a>2</td>
<td>750</td>
<td>5.0 \times 10^{-4}</td>
<td>5.6 \times 10^{-8}</td>
<td>8.9 \times 10^{3}</td>
<td>1.8 \times 10^{7}</td>
</tr>
<tr>
<td><a href="PF6">(Ph2phen)2Ru(dp)PtCl2</a>2</td>
<td>740</td>
<td>4.1 \times 10^{-4}</td>
<td>4.4 \times 10^{-8}</td>
<td>9.3 \times 10^{3}</td>
<td>2.3 \times 10^{7}</td>
</tr>
</tbody>
</table>

\(^a\) bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline, Ph2phen = 4,7-diphenyl-1,10-phenanthroline, and dpp = 2,3-bis(2-pyridyl)pyrazine.

The photophysical properties of the [(Ph2phen)2Ru(BL)PtCl2](PF6)2 (BL = dpp or dpq) were studied. The [(Ph2phen)2Ru(dpp)PtCl2](PF6)2 complex emits at 740 nm (\( \Phi_{\text{em}} = 4.1 \times 10^{-4} \), \( \tau = 44 \) \mu s).
ns, \( k_r = 9.3 \times 10^3 \text{ s}^{-1}, k_{nr} = 2.3 \times 10^7 \text{ s}^{-1} \) in RT deoxygenated CH\(_3\)CN and 694 nm (\( \tau = 2.3 \mu \text{s} \)) at 77 K. The RT emission and excited state lifetime for the [(TL)\(_2\)Ru(dpp)PtCl\(_2\)](PF\(_6\))\(_2\) analogs were (\( \lambda_{\text{max}}^{\text{em}} = 760 \text{ nm}, \Phi^{\text{em}} = 4.0 \times 10^{-4}, \tau = 46 \text{ ns}, k_r = 8.7 \times 10^3 \text{ s}^{-1}, k_{nr} = 2.3 \times 10^7 \text{ s}^{-1}, \text{bpy} \)) and (\( \lambda_{\text{max}}^{\text{em}} = 750 \text{ nm}, \Phi^{\text{em}} = 5.0 \times 10^{-4}, \tau = 56 \text{ ns}, k_r = 8.9 \times 10^3 \text{ s}^{-1}, k_{nr} = 1.8 \times 10^7 \text{ s}^{-1}, \text{phen} \)). The results for the Ph\(_2\)phen\(^{184}\) and phen Ru(II)-Pt(II) bimetallic complexes were comparable to the bpy\(^{149,150}\) analog, supporting the same lowest lying Ru(d\(\pi\)) \( \rightarrow \) dpp(\(\pi^*\)) excited state in these complexes. Emission was not detected for [(Ph\(_2\)phen)\(_2\)Ru(dpq)PtCl\(_2\)](PF\(_6\))\(_2\), which is expected given the predicted lower energy, weaker emission in this system.

In the presence of molecular oxygen, using air-saturated CH\(_3\)CN solutions, the emissions display reduced quantum yields and shortened excited state lifetimes. The monometallic complex, [(Ph\(_2\)phen)\(_2\)Ru(dpp)](PF\(_6\))\(_2\) displayed an approximately 50% reduction in quantum yield and lifetime in aerated solution (\( \Phi^{\text{em}} = 1.5 \times 10^{-2} \) and \( \tau = 386 \text{ ns} \)). The [(Ph\(_2\)phen)\(_2\)Ru(dpp)PtCl\(_2\)](PF\(_6\))\(_2\) and [(Ph\(_2\)phen)\(_2\)Ru(dpq)](PF\(_6\))\(_2\) complexes showed quenching of their RT emissions, but to a lesser extent with \( \Phi^{\text{em}} = 3.6 \times 10^{-4} \) and \( \tau = 40 \text{ ns} \) and \( \Phi^{\text{em}} = 9.6 \times 10^{-5} \) and \( \tau = 18 \text{ ns} \) in aerated solution, respectively. The quenching of Ru(II) polyazine \(^3\)MLCT excited states by \(^3\)O\(_2\) is well established, occurring through energy transfer to generate \(^1\)O\(_2\).\(^{116}\)

Emission from the lowest energy \(^3\)MLCT state for [(Ph\(_2\)phen)\(_2\)Ru(BL)](PF\(_6\))\(_2\) and [(Ph\(_2\)phen)\(_2\)Ru(dpp)PtCl\(_2\)](PF\(_6\))\(_2\) provides a probe into the energy and reactivity of this state. In going from BL = dpp to dpq in the [(Ph\(_2\)phen)\(_2\)Ru(BL)](PF\(_6\))\(_2\) systems, the emission maxima shifted to lower energy and the lifetime of emission was significantly reduced as a result of the stabilized acceptor orbital in dpq vs. dpp. Coordination of the Pt(II) metal center resulted in a shift of the emission maximum by approximately 100 nm toward lower energy for the dpp bimetallic analog consistent with the redox properties showing a stabilized dpp(\(\pi^*\)) acceptor.
orbital upon platination (−0.98 V, monometallic and −0.45 V, bimetallic vs. Ag/AgCl). The 
$^3$MLCT energy shifts to lower energy upon platination in all [(TL)$_2$Ru(dpp)PtCl$_2$](PF$_6$)$_2$ (TL = bpy, phen, or Ph$_2$phen) analogs, Table 5.3. The quantum yields of emission and the
excited state lifetimes are significantly reduced in the bimetallic complexes, attributed to
stabilization of the BL($\pi^*$) orbitals affording a lower energy $^3$MLCT state with more rapid non-
radiative decay to the ground state ($k_{nr} \approx 2.0 \times 10^6$ s$^{-1}$, monometallic analogs vs. $k_{nr} \approx 2.0 \times 10^7$
s$^{-1}$, bimetallic analogs). All the Ph$_2$phen complexes reported herein show emission quenching in
the presence of molecular oxygen indicating reactivity of $^3$O$_2$ with the $^3$MLCT excited state to
generate reactive oxygen species known to be $^1$O$_2$ in related Ru(II) polyazine systems, but was not clearly demonstrated or quantified in the Ru(II)-Pt(II) systems prior to these
studies.

5.7. Singlet Oxygen Quantum Yield Determination for [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$
and [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$

The quantum yield for singlet oxygen production was indirectly determined via reactivity of
$^1$O$_2$ produced following photolysis of the metal complexes with 1,3-diphenylisobenzofuran
(DPBF) leading to formation of the non-emissive o-dibenzoylbenzene (ODBB), Figure 5.6. In
section 5.6, quenching of the $^3$MLCT emission by $^3$O$_2$ suggests $^1$O$_2$ production, but does not
establish or quantitate $^1$O$_2$ generation leading to these studies to establish the mechanism of
oxygen quenching of the $^3$MLCT emissions. The [Ru(TL)$_3$]Cl$_2$ complexes, where TL = bpy,
phen, and Ph$_2$phen all display emission quenching in the presence of oxygen with, $\Phi_{102} = 0.73,$
0.54 and 0.97 in methanol, respectively. Using [Ru(Ph$_2$phen)$_3$]Cl$_2$ as a standard
reference ($\Phi_{102} = 0.97$) the $\Phi_{102}$ for the Ru(II)-Pt(II) complexes were determined. For the
bimetallic complexes [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ and [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$, a $\Phi_{102}$ of
0.07 and 0.03 was determined, respectively. The [(Ph₂phen)₂Ru(dpq)PtCl₂]Cl₂ complex generates \(^1\)O₂ even though measurement of emission from the \(^3\)MLCT excited state was not detectable by our system. This establishes for the first time that a Ru(II)-Pt(II) complex produces \(^1\)O₂, important in the application as PDT agents. The lower yield of \(^1\)O₂ from dpq vs. dpp is attributed to the expected lower energy and shorter-lived \(^3\)MLCT excited state. The [(Ph₂phen)₂Ru(BL)PtCl₂]Cl₂ complexes, where BL = dpp or dpq, afford reasonable \(^1\)O₂ generation quantum efficiencies suggesting they can function as PDT agents. The Ru(II)-Pt(II) complexes also have the distinct advantage of photogenerating \(^1\)O₂ at the DNA target via covalent localization of the supramolecule via covalent binding through the cis-PtCl₂ unit.

Figure 5.6: Plot of DPBF emission profile from \(t_i\) (black) to \(t_f\) (blue) (A), and plot of DPBF emission quenching values vs. photolysis time as a result of \(^1\)O₂ generation by [Ru(Ph₂phen)₃]Cl₂ (■), [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ (♦) and [(Ph₂phen)₂Ru(dpq)PtCl₂]Cl₂ (♦) with linear trend lines and correlation values. Recorded in RT MeOH. DPBF = 1,3-diphenylisobenzofuran, \(t_i\) = time initial, \(t_f\) = time final, Ph₂phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, and dpq = 2,3-bis(2-pyridyl)quinoxaline.
6. Metal Complex-Biological Interactions

Incorporation of a Ru(II)-chromophore capable of generating $^1$O$_2$ through $^3$MLCT excited state energy transfer with a cis-PtCl$_2$ BAS able to covalently bind to DNA, via a polyazine BL, affords supramolecular complexes able to perform targeted PDT. The [((Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ and [((Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ supramolecular complexes were designed to thermally bind to the nucleophilic DNA target providing localized $^1$O$_2$ generation at DNA resulting in photocleavage. The photochemical studies of Ru(II)-Pt(II) complexes in the presence of biomolecules provides insight into the multifunctional binding and PDT activity for these supramolecular complexes.

6.1. Covalent Binding of [((Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ and [((Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ to DNA

The covalent binding of cisplatin and the new [((Ph$_2$phen)$_2$Ru(BL)PtCl$_2$]Cl$_2$ complexes, where BL = dpp or dpq, to linear double-stranded pUC18 plasmid DNA was compared and assayed using gel electrophoresis to test the functioning of the cis-PtCl$_2$ BAS. The cis-PtCl$_2$ moiety of the title supramolecules is designed to provide covalent binding to DNA similar to cisplatin, cis-[PtCl$_2$(NH$_3$)$_2$]. Linear DNA is a well-behaved substrate to assay covalent binding to DNA. Linear plasmid DNA presents as a single DNA shape with migration changes due to enhanced size and reduced anionic charge upon cationic metal binding. The covalent binding of the cis-PtCl$_2$ site of cisplatin and the Ru(II)-Pt(II) complexes to DNA is expected to cause a retardation in migration of the covalently modified DNA through the agarose gel demonstrating covalent modification of the DNA.$^{168-171}$

Gel electrophoresis shift assays of the metal complex binding to DNA are presented in Figure 6.1. Each gel shows a lambda molecular weight marker ($\lambda$), a DNA control (C), and DNA
incubated with a 5:1, 10:1, and 20:1 ratios of DNA base pairs (BP) to each metal complex (MC) for 1 hour at 37 °C in the dark. The DNA in the gels is stained with ethidium bromide (EtBr), imaged via the red EtBr emission, and presented as negative black and white images. Retardation of the DNA migration through the gel is observed for cisplatin and the title bimetallic complexes at all BP:MC ratios. The slowing of migration in the presence of metal complex is attributed to covalent modification of the DNA, which causes a change in the mass, charge, and/or tertiary structure of the DNA molecule following metal binding. DNA migration for both the Ru(II)-Pt(II) bimetallic complexes and cisplatin changed upon incubation with the complexes in a concentration dependent manner. As the ratio of BP:MC decreases with increased metal loading, the DNA migration through the gel increases, consistent with concentration dependent covalent modification of DNA. The Ru(II)-Pt(II) gels, Figure 6.1 B and 6.1 C, also display reduced EtBr dye emission intensity in lanes 5:1, 10:1, and 20:1 BP:MC relative to the DNA control, C. The reduced emission by EtBr is characteristic of the Ru(II)-Pt(II) bimetallic bound to DNA, but is not observed for cisplatin and is either a result of energy transfer quenching of the EtBr excited state by bound Ru(II)-Pt(II) complexes or inhibition of EtBr intercalation, upon metal binding. Both quenching of EtBr emission and reduced staining are expected for these systems upon metal complex binding to the DNA.

The Ru(II)-Pt(II) complexes show thermal covalent binding to DNA comparable to cisplatin, with increased changes in DNA migration with increased metal loading. Additional evidence of covalent DNA modification by the Ru(II)-Pt(II) complexes is provided by the EtBr emission quenching. Reduced EtBr emission would not be observed in our metal complex treated DNA lanes in the absence of covalently attached [(Ph2phen)2Ru(BL)PtCl2]Cl2 (BL = dpp or dpq).
These studies display the *cis*-PtCl$_2$ BAS retains its thermal DNA binding function when incorporated into the Ru(II)-Pt(II) supramolecular architecture.

![Figure 6.1: Gel electrophoresis photos showing covalent binding of *cis*- [PtCl$_2$(NH$_3$)$_2$] (A), [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ (B), and [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ (C) with linear pUC18 DNA. The lanes for each gel correspond to: λ) lambda molecular weight marker, C) linear pUC18 DNA control, 5) 5:1 BP:MC, 10) 10:1 BP:MC, and 20) 20:1 BP:MC. Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline and BP:MC = base pair to metal complex ratio. Adapted from reference 184.

6.2. DNA Photocleaving Studies with [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ and [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$

The [(Ph$_2$phen)$_2$Ru(BL)PtCl$_2$]Cl$_2$ complexes, where BL = dpp or dpq, were assayed as DNA photocleavage agents to test the PDT activity of the Ru(II) LA site. The directly coupled Ru(II)-Pt(II) systems in the literature generally showed covalent binding to DNA via the Pt(II) BAS, but no PDT activity from the Ru(II) chromophore due to deactivation upon Pt(II) coordination.$^{130,148}$ The photophysical studies showed, in the presence of visible light, the bimetallic complexes were able to generate singlet oxygen via the Ru(II)-chromophore. DNA photocleavage experiment was used to assay the Ru(II)-Pt(II) supramolecular complex multifunctional interactions with DNA to establish the validity of the supramolecular design. Theoretically, the mixed-metal complexes should covalently bind to DNA through the *cis*-PtCl$_2$ moiety and in the presence of light and oxygen locally generate $^1$O$_2$ at the DNA resulting in strand cleavage, while in the absence of oxygen only covalent modification would be observed. The experiments were
completed using supercoiled pUC18 plasmid DNA at a 20:1 BP:MC ratio and the solutions were photolyzed for 1 h under both air saturated and $^1\text{O}_2$ quenching conditions.

The gel electrophoretic studies assaying DNA photocleavage are shown in Figure 6.2. The pUC18 plasmid was used as received with approximately 95% of the sample as supercoiled (no cleavage) and 5% open circular (single strand cleavage), which migrate at different rates due to their varied size and shape.\textsuperscript{165} Binding of the metal complexes to supercoiled DNA results in unwinding of the supercoil and slowed migration, while cleavage converts supercoiled DNA to open circular DNA.\textsuperscript{122} The complexes bind to DNA at RT and 37 °C in the dark, lanes \textbf{RT} and \textbf{37}, with similar changes in migration as reported in the covalent binding studies. The 20:1 BP:MC ratio was chosen so that the difference between the dark/thermal reactions and the photolyzed samples was clear.

The photochemical studies of the [\((\text{Ph}_2\text{phen})_2\text{Ru(dpp)}\text{PtCl}_2\)\text{Cl}_2 and \((\text{Ph}_2\text{phen})_2\text{Ru(dpq)}\text{PtCl}_2\)\text{Cl}_2 complexes in the presence of DNA, light, and oxygen, afforded DNA strand cleavage. The light activated DNA strand cleavage via an O$_2$ dependent pathway is shown in lane *\textbf{Atm} in Figure 6.2. For the \((\text{Ph}_2\text{phen})_2\text{Ru(dpp)}\text{PtCl}_2\)\text{Cl}_2 complex, single strand cleavage to form open circular DNA is observed and for the \((\text{Ph}_2\text{phen})_2\text{Ru(dpq)}\text{PtCl}_2\)\text{Cl}_2 system linearization of the DNA occurs, consistent with cleavage of both DNA strands near the same location. The cleavage of DNA by these systems is unusual given most directly coupled Ru(II)-Pt(II) bimetallic complexes are not photoactive due to the shortened $^3\text{MLCT}$ lifetime as a result of Pt(II) coordination.
Figure 6.2: Electrophoretic mobility studies of the interaction of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$](A) and [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ (B) with circular pUC18 plasmid DNA. The lanes for each gel correspond to: λ) lambda molecular weight marker (24, 9.4, 6.6, 4.4, 2.2, 2.0, and 0.56 kb), C) linear pUC18 DNA control, RT) 20:1 BP:MC ratio incubated at RT for 1 hour, 37) 20:1 BP:MC incubated at 37 °C for 1 hour, *Atm) 20:1 BP:MC ratio photolyzed for 1 hour under air saturated conditions, and *NaN$_3$) 20:1 BP:MC ratio photolyzed for 1 hour in the presence of the singlet oxygen quencher, sodium azide (NaN$_3$).

The DNA photocleavage via $^1$O$_2$ generation was tested using a $^1$O$_2$ quencher, sodium azide (lanes *NaN$_3$).$^{117}$ In the presence of sodium azide, there is no DNA photocleavage observed for the bimetallic complexes. Covalent binding was also inhibited by azide. Sodium azide showed efficient $^1$O$_2$ quenching with no increase of open circular band intensity in the gels. Covalent binding inhibition provides evidence that azide may interfere with the ligand exchange at the cis-PtCl$_2$ moiety. It is suggested in other cis-PtCl$_2$ systems that azide acts as an excellent ligand with a higher nucleophilicity constant than H$_2$O, which may inhibit DNA binding for the bimetallic systems.$^{198,199}$ It must also be noted that the typical deoxygenation technique using a 30 min Ar purge and blanket during the photolysis still displayed some level of conversion from supercoiled to open circular DNA, attributed to the enhanced oxygen sensitivity of the (Ph$_2$phen)$_2$Ru$^{II}$(BL) chromophore unit observed for the [Ru(Ph$_2$phen)$_3$]Cl$_2$ complex.$^{112}$

The bimetallic complex-induced photocleavage of DNA using visible light is enhanced compared to previously reported Ru(II)-Pt(II) analogs. The [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ and
[(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ systems show enhanced photocleavage under lower light flux (LED vs. arc lamp excitation) and with lower metal loading (20:1 vs. 5:1 BP:MC ratios) than previously observed.$^{154,155}$ This variation is a result of TL modification to Ph$_2$phen. Though the $^3$MLCT is formally Ru(d$\pi$)$\rightarrow$BL($\pi^*$) CT, contribution from the TL to the formally Ru(d$\pi$) orbital enhances the activity of the title bimetallic complexes. The Ph$_2$phen TL in the Ru-chromophore provides efficient absorptivity throughout the visible region and population of the $^3$MLCT excited state. Enhanced $^3$MLCT excited state lifetimes are observed leading to measurable $^1$O$_2$ generation.$^{122}$ Ru(II) chromophore localization at the DNA via cis-PtCl$_2$ binding provides for targeted delivery of singlet oxygen allowing high efficiency of DNA cleavage by the photo-generated $^1$O$_2$. The studies validate the supramolecular design as light activated agents capable of targeting nucleophilic DNA for applications in PDT therapy.

6.3. Photobinding of [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ to pUC18 Plasmid DNA and Gel Shift Assay

UV and near UV-light excitation in several Ru(II), Rh(III), and Pt(IV) systems affords photobinding to DNA as a result of light-induced ligand labilization through ligand field (LF) and less commonly ligand-to-metal charge transfer (LMCT) states.$^{200-203}$ MLCT excited states are preferred states for DNA modification as they have increased molar absorptivity compared to LF states, are tunable, and occur in the visible region of the spectrum. Photolysis of the [(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl$_2$ bimetallic complex in the visible region, where Pt(II) complexes do not typically absorb light (Figure 6.3), results in charge transfer from the Ru(II) center to the dpp BL affording increased electron density at the Pt(II) site. It is predicted that the decreased Lewis acidity at the cis-PtCl$_2$ site may facilitate halide loss and provide for enhanced DNA binding via MLCT excitation. This would provide a new paradigm for DNA photomodification.
Figure 6.3: Electronic absorption spectroscopy of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ (orange) and cis-[PtCl$_3$(NH$_3$)$_2$] (blue) recorded in RT water. Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine.

Photobinding of a metal complex to DNA assayed by gel electrophoresis is expected to slow the migration of the supercoiled band as a result of relaxation (unwinding) of the DNA, decreased charge of the DNA, and the size of the DNA-metal complex structure, until the DNA is saturated with metal complex or other photoreactions occur.$^{170}$ The Ru(II)-Pt(II) bimetallic complex thermal binding and photobinding properties to pUC18 plasmid DNA were assayed using gel electrophoresis.

Gel electrophoresis was used to assay thermal and photobinding of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ to DNA, which is shown Figure 6.4. The [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ complex was incubated at RT (dark), 37 °C (dark) and photolyzed ($\lambda_{irr} = 455$ nm or $\geq 590$ nm) in the presence of pUC18 plasmid DNA for 0, 2.5, 5, 10, 20, 30, 45, and 60 min at a 5:1 BP:MC ratio. At RT minimal binding occurred supported by the similarity between the control and incubated samples. Under photolysis conditions at $\lambda_{irr} = 455$ nm or $\geq 590$ nm migration retardation enhancement of the Form I DNA band was observed with eventual coalescence and formation of a more intense Form II band. The slowing of Form I DNA
Figure 6.4: Gel electrophoresis mobility shift assay showing the binding of [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ to pUC18 plasmid DNA following incubation at room temperature (A) and 37 °C (B) and photobinding after 455 nm (C) or ≥ 590 nm (D) irradiation. The lanes correspond to: λ) lambda molecular weight marker, C) pUC18 DNA plasmid control, and 0, 2.5, 5, 10, 20, 30, 45, and 60) 5:1 BP:MC solutions photolyzed for 0, 2.5, 5, 10, 20, 30, 45, and 60 min, respectively. Ph₂phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, and BP:MC = base pair to metal complex ratio. Adapted from reference 204.

migration in the presence of visible light is indicative of Ru(II)-Pt(II) bimetallic photobinding, a mechanism of action previously unobserved for MLCT excited states.²⁰⁴ This provides a new paradigm for DNA modification, DNA photobinding via MLCT excitation. The increased intensity of the Form II DNA band provides evidence for photocleavage of the pUC18 DNA facilitated by the generation of O₂ from the ³MLCT state. Visible light excitation promoting DNA photobinding, where typical Pt(II) complexes do not absorb, displays the importance of the Ru(II)-Pt(II) supramolecular architecture. The spectroscopic properties of the Ru(II) chromophore are imparted onto the coupled cis-PtCl₂ bioactive site affording multifunctional
interactions with DNA. Red light excitation (within the therapeutic window) inducing a DNA photobinding event has not been reported in the literature making the therapeutic light photobinding by the Ru(II)-Pt(II) complex a very significant observation.

6.4. Photobinding of [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ to CT-DNA and Selective Precipitation Assay

In addition to gel electrophoresis, binding and photobinding of [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ to calf thymus DNA (CT-DNA) was probed using a selective DNA precipitation technique. DNA is exposed to the metal complex followed by DNA precipitation such that metal complex bound to the DNA is removed from solution. The amount of metal complex not bound to the CT-DNA, was determined using electronic absorption spectroscopy of the supernatant. The ratio in the 1MLCT (λₘₐₓabs = 525 nm) absorbance of the supernatant over time (average of three experiments) was plotted as Aₜ/A₀ vs. time, Figure 6.5.

The [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ complex thermal and photobinding to DNA were compared using selective DNA precipitation. Metal complex binding in the dark at RT and 37 °C were used as control samples. The dark control at RT shows minimal change in the metal complex absorbance over time, similar to the results by gel electrophoresis, with ca. 15% of the metal complex bound to the DNA at 60 min. The control samples correlated well with the gel electrophoresis studies, showing that in the dark binding was not efficient. Under photolysis conditions, photobinding of the Ru(II)-Pt(II) complex to CT-DNA was observed with enhanced efficiency. Irradiation of the complex and CT-DNA at 455 nm resulted in a rapid change in absorbance up to 10 min. A steady state was reached by 30 min and was used as the estimated metal complex saturation point. The [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂-treated samples using lower energy wavelength irradiation, λ_irr ≥ 590 nm, reached a similar level of saturation at 60 min. Red
light induced photobinding is slower initially consistent with the previous pUC18 photobinding studies.

Figure 6.5: Selective precipitation of [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ bound CT-DNA at RT in the dark (•), 37 °C in the dark (■), irradiated at ≥ 590 nm (♦) and irradiated at 455 nm (♦). Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine. Adapted from reference 204.

The well-established dimensions of DNA provides a method to experimentally measure the size of the [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ binding site. For comparison, the semi-empirical method, ZINDO (Zerner’s Intermediate Neglect of Differential Overlap), was used to calculate the optimized geometry in Scigress to determine complex molecular size.$^{206-209}$ Based on the observed BP:MC ratio or the point at which the DNA is fully saturated by bound metal complex and the known distance between each base pair (3.4 Å)$^{210}$, the binding site may be determined. For $\lambda_{irr} = 455$ nm or ≥ 590 nm, the DNA is fully saturated with approximately 0.55 metal
complex per 5 DNA BP, indicating a binding site size of 32 Å, which correlates to the theoretical optimized value of 20 Å, represented in Figure 6.6.

![Diagram](image)

**Figure 6.6:** ZINDO optimized geometry of \([\text{Ph}_2\text{phen}]_2\text{Ru(dpp)}\text{PtCl}_2\)Cl\(_2\) where \(\bullet = \text{Ru}, \cdot = \text{N}, \circ = \text{H}, \circledast = \text{C}, \odot = \text{Pt}, \circledcirc = \text{Cl}\) (A) and representation of the experimentally determined binding site for \([\text{Ph}_2\text{phen}]_2\text{Ru(dpp)}\text{PtCl}_2\)Cl\(_2\) (B). Ph\(_2\)phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine.

The gel shift assay and selective precipitation experiments demonstrate \([\text{Ph}_2\text{phen}]_2\text{Ru(dpp)}\text{PtCl}_2\)Cl\(_2\) photobinding to DNA via low-energy MLCT excitation, the first example of MLCT photobinding and the first photobinding event promoted by red light in the therapeutic window using any type of excited state. Previously reported DNA photobinding agents require UV or rarely high energy visible excitation functioning through LF or LMCT states. The cis-[Rh(phen)\(_2\)Cl\(_2\)]\(^+\) monometallic complex, where phen = 1,10-phenanthroline displayed ionic binding to DNA in the dark, dependent on ion concentration.\(^{200}\) Photolysis (\(\lambda_{\text{irr}} > 330\) nm) of the Rh(III) complex with DNA results in population of the lowest lying \(^3\)LF excited state initiating ligand exchange to form covalent bonds to DNA. The Pt(IV)-based metal complexes using iodide or azide as the reducing ligands have been reported to form reactive Pt(II) species, via LMCT excitation. Irradiation (\(\lambda_{\text{irr}} > 375\) nm) of the *cis, trans-*
[Pt(en)(I)2(OAc)2] (en = ethylenediamine and OAc = acetoxy group) complex affords 25% binding in one hour. The cis-[Ru(bpy)2(NH3)2]2+ complex displayed efficient DNA photobinding (60-80%, 15 min) with irradiation > 345 nm. Population of the 3LF excited state resulted in photo-ligand exchange of the ammines to form covalent bonds to DNA, however excitation of the lower energy MLCT state did not provide photoactivity. The cis-[Rh2(µ-O2CCH3)2(CH3CN)6]2+ bimetallic complex displays facile thermal and photo hydrolysis resulting in efficient interaction with biomolecules. In the presence of light (λirr > 455 nm) and linear pUC18 DNA, the Rh-Rh bimetallic photobinds to DNA via weak metal centered excitation. Comparatively, the [(Ph2phen)2Ru(dpp)PtCl2]Cl2 complex shows efficient DNA photobinding fully saturating the DNA in one hour utilizing low energy MLCT excitations, even red light in the therapeutic window. Binding is more rapid under photolysis vs. thermal conditions, displaying the light-induced selectivity and targeted nucleophilic binding to DNA as well as further supporting the necessity of the supramolecular architecture.

6.5. Photobinding of [(Ph2phen)2Ru(dpp)PtCl2]Cl2 to CT-DNA and DNA Melting Point Assay

The effects of [(Ph2phen)2Ru(dpp)PtCl2]2+ photobinding to CT-DNA was examined using melting point (Tm), determining the temperature at which the DNA denatures. The solutions of CT-DNA, 5:1 BP:MC incubated at 37 °C in the dark, and photolyzed at 455 nm for 60 min were heated from 60 to 100 °C in 2.5 °C increments. The absorbance at 260 nm was recorded and plotted vs. temperature, Figure 6.7. CT-DNA controls showed a melting point (reported and confirmed by experiment) of 87 °C. The solutions incubated at 37 °C, showed minimal change in melting point occurring at 86 °C. The samples that were photolyzed at 455 nm showed a
decreased Tm value of 7 °C, indicative of DNA destabilization. This provides further evidence of photomodification by the Ru(II)-Pt(II) complex via photobinding.

**Figure 6.7:** DNA melting curves for native CT-DNA control (◆), CT-DNA incubated at 37 °C with [(Ph2phen)2Ru(dpp)PtCl2]Cl2 (◇), and CT-DNA photolyzed with [(Ph2phen)2Ru(dpp)PtCl2]Cl2 at 455 nm (◆) with the Tm marked using a dashed line for clarity. Ph2phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine.

### 6.6. Partition Coefficient

The partition coefficient is a physicochemical property that estimates a drug’s hydrophilicity or lipophilicity. The partition coefficient value is correlated with distribution in the body, ability to cross the blood-brain barrier (BBB, interface of the central nervous system and blood21) or cell membrane.213-215 The partition coefficient measures the relative concentration of a molecule in equilibrated immiscible solvents, in this case equilibrated phosphate buffer (pH 7.4) and n-octanol. Partition coefficients using octanol and water are reported as LogP<sub>O/W</sub>, where a LogP<sub>O/W</sub> value of 0 represents equal concentrations of the molecule in the octanol and water phases. A LogP<sub>O/W</sub> value greater than 0 indicates a larger concentration of the molecule in the octanol phase and therefore a more lipophilic molecule. In drug design a balance of hydrophilicity and lipophilicity is desired. For optimal oral absorption or central nervous system penetration a LogP<sub>O/W</sub> ≅ +1.5 ± 1.0 is desired.216-219 For Pt(II) complexes and more recently Ru(II) β-
carboline complexes, increased lipophilicity has favored cellular and tissue uptake.\textsuperscript{220-222} It is important to note that favorable lipophilicity modifications can be counterbalanced with decreased reactivity.

The Log\textsubscript{O/W} values for the Ru(II)-Pt(II) bimetallic complexes were measured using a modified shake-flask method and compared to the previously reported complexes, data summarized in Table 6.1. Cisplatin, carboplatin, and TMZ, which have or are currently used in the treatment of malignant glioma, are reported to have Log\textsubscript{O/W} values of −2.50, −2.30, and −0.24, respectively.\textsuperscript{220,223} It is important to note that currently the accepted clinical treatment for malignant glioma is TMZ, ascribed to the improved permeability of the BBB and the varied mechanism of action compared to cisplatin and carboplatin. The related [(TL)RuCl(dpp)PtCl\textsubscript{2}]Cl (TL = tpy, MePhtpy, and \textsuperscript{1}Bu\textsubscript{3}tpy) bimetallic complexes displayed dramatically varied Log\textsubscript{O/W} values of −2.00 (tpy), −0.39 (MePhtpy), and +4.00 (\textsuperscript{1}Bu\textsubscript{3}tpy), attributed to the varying degrees of TL lipophilicity. The [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)PtCl\textsubscript{2}](PF\textsubscript{6})\textsubscript{2}, [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpp)PtCl\textsubscript{2}]Cl\textsubscript{2}, and [(Ph\textsubscript{2}phen)\textsubscript{2}Ru(dpq)PtCl\textsubscript{2}]Cl\textsubscript{2} complexes displayed Log\textsubscript{O/W} values of +1.26, +0.55, and +0.99, respectively. The values suggest that passive diffusion into tissue or cells would be favorable and can be tuned via ligand and counterion choice. Although Log\textsubscript{O/W} values are often used as a predicative measure, they do not necessarily correlate well to \textit{in vivo} efficacy due to the complexity of disease treatment. Additionally, it is thought that the “normal” cell membrane and BBB is disrupted by tumor growth providing a “leaky membrane” and resulting in weak correlations between lipophilicity and toxicity.
Table 6.1: LogP$_{O/W}$ values for known anticancer agents and reported Ru(II)-Pt(II) complexes.

<table>
<thead>
<tr>
<th>Metal Complex $^a$</th>
<th>LogP$_{O/W}$</th>
</tr>
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<tbody>
<tr>
<td>Cisplatin</td>
<td>-2.50</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>-2.30</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>-0.24</td>
</tr>
<tr>
<td><a href="PF$_6$">(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$</a>$_2$</td>
<td>+1.26</td>
</tr>
<tr>
<td>[(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl</td>
<td>+0.55</td>
</tr>
<tr>
<td>[(Ph$_2$phen)$_2$Ru(dpq)PtCl$_2$]Cl</td>
<td>+0.99</td>
</tr>
<tr>
<td>[(tpy)ClRu(dpp)PtCl$_2$]Cl</td>
<td>-2.00</td>
</tr>
<tr>
<td>[(MePhtpy)ClRu(dpp)PtCl$_2$]Cl</td>
<td>-0.39</td>
</tr>
<tr>
<td>[(tBu$_3$tpy)ClRu(dpp)PtCl$_2$]Cl</td>
<td>+4.00</td>
</tr>
</tbody>
</table>

$^a$ Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline, dpp = 2,3-bis(2-pyridyl)pyrazine, dpq = 2,3-bis(2-pyridyl)quinoxaline, tpy = 2,2':6',2''-terpyridine, MePhtpy = 4'-(4-methylphenyl)-2,2':6',2''-terpyridine, and tBu$_3$tpy = 4,4',4''-tri-tert-butyl-2,2':6',2''-terpyridine.

6.7. Routine Cell Culturing

The U87MG cancer cell line is classified as grade IV with an infinite life span in vitro.$^{224}$ The U87MG cells were cultured from frozen cells purchased from ATCC®. The cells are slow growing, with varied sizes, shapes, and cell densities in early growth, Figure 6.8 A. From frozen cultures, the cells take approximately 10 days to reach 60% confluency. Cells were grown to 65-80% confluency prior to splitting, Figure 6.8 B. Cells were generally seeded at 4.0 × 10$^6$ cells in a 75 cm$^2$ flask and reached splitting confluency (3.0 × 10$^7$ cells) in a week. The U87MG line showed consistent growth and morphology to passage 45, however for passages 45-50 the cells started displaying irregularities at which time the line was discarded. In addition, the cells could be frozen, but generally did not survive multiple freeze-thaw cycles. For each treatment, a total of 2.0-3.0 × 10$^8$ cells were necessary requiring several passages to reach the necessary concentration.
Figure 6.8: Microscopy images (100X) of U87MG cells showing early growth morphology (A) and at 65% confluency prior to splitting (B).

6.8. Dark U87MG Metal Complex Treatment using Cisplatin and 

\[(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2\text{Cl}_2\]

The cytotoxicity of U87MG cells upon treatment by cisplatin and 

\[(\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2\text{Cl}_2\] was studied under dark conditions. The cells were seeded in 6-well plates at $2.0 \times 10^6$ cells/well and were incubated for 18 h prior to treatment. Stock solutions of the metal complex were made at the same concentration in water. The cells were prepared for treatment by aspirating the old media and adding new media with adjusted volumes to account for treatment. Cells with media only and cells treated with corresponding water volumes were used as controls. Water, cisplatin and Ru(II)-Pt(II) concentrations were varied from 1, 10, 25, 50, 75, 100, 125, 150, and 250 µM. Following treatment for 1 h in the dark, the treatment media was removed and replaced with fresh media. The samples were grown for 72 h following treatment, harvested, and counted. Cells that were darkly stained blue were considered dead and cells that remained golden were considered live. The % viability (relative to media only) vs. concentration
was graphed, Figure 6.9. The dark cytotoxicity studies were replicated in duplicate for the water control and cisplatin and triplicate for the Ru(II)-Pt(II) complex.

Figure 6.9: Dark cytotoxicity study graphed as % Viability (U87MG cells treated/U87MG cells media only) vs. Concentration (µM) for the water control (♦), cisplatin (■), and [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ (●). Ph₂phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-bis(2-pyridyl)pyrazine.

Generally cell viability following treatment is reported as LC₅₀, the concentration value at which 50% cell death occurred or IC₅₀, the concentration value at which 50% cell inhibition occurred. For the dark cytotoxicity studies, the water control had minimal effect on the cells as expected. The LC₅₀ for cisplatin and [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ following 1 hour treatment and 72 hour harvesting is ca. 100 µM. There is a large deviation in the samples, which is attributed to error in counting an average of 1.5-2.0 × 10³ cells per sample and staining, both the Ru(II)-Pt(II) bimetallic and cisplatin show concentration dependent activity. Compared to
previous reports, the U87MG LC<sub>50</sub> for cisplatin under similar conditions was near 100 µM and IC<sub>50</sub> for TMZ following 6 or 72 hour treatment was near 100 µM. The large LC<sub>50</sub> and IC<sub>50</sub> values for cisplatin and TMZ are concerning because 100 µM is on the higher end of a clinically achievable dosage and for cisplatin the large values are compounded by poor BBB permeability clinically. To lower the dose of cisplatin and temozolomide, while maintaining activity, new delivery systems, concomitant chemotherapies, and combination therapies have been employed.

6.9. Photocytotoxicity U87MG Studies using Cisplatin and 
\(((\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2)\text{Cl}_2\)

The photocytotoxicity of cisplatin and \(((\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2)\text{Cl}_2\) in the presence of U87MG cells was studied. The light source was the lab-built LED array, which was fitted for 6-well plate treatment. The samples (0, 10, and 20 µM) were prepared and harvested using the same methods as the dark samples. The cells were incubated in the dark for 15 min, photolyzed for 30 min (λ<sub>irr</sub> = 510 nm), and left in the dark for a final 15 min. The experiments were repeated in duplicate and graphed as % viability (cells treated/media only) vs. concentration, Figure 6.10.

Photolysis of \(((\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2)\text{Cl}_2\) affords enhanced U87MG cytotoxicity. The dark control for the \(((\text{Ph}_2\text{phen})_2\text{Ru(dpp)PtCl}_2)\text{Cl}_2\) is shown for comparison. In the dark at 25 µM ca. 85% viability was observed for the Ru(II)-Pt(II) complex, which was similar to the photolyzed cisplatin sample. Upon photolysis, the % viability dramatically decreases to 10% for the Ru(II)-Pt(II) complex displaying enhanced light-induced toxicity as expected based on the design of this supramolecule. The selectivity induced by light activation is impressive as the LC<sub>50</sub> value is 100 µM under dark thermal conditions, which is reduced to ca. 5 µM under photolysis conditions. Furthermore, the morphology of the dead cells was mostly apoptotic (not necrotic), an important
distinction in clinical applications. It is expected that the Ru(II)-Pt(II) complex would also be
effective using light excitation in the therapeutic window, but further studies are necessary to
optimize treatment conditions. Though these are preliminary studies, the

\[(\text{Ph}_2\text{phen})_2\text{Ru(dpp)}\text{PtCl}_2\text{Cl}_2\] complex photocytotoxicity provides the first example of a mixed-
metal complex with enhanced light induced activity against U87MG cells and shows promise in
targeted PDT applications. This complex also displays the lowest known LC$_{50}$ for U87MG cells
to date.

**Figure 6.10**: Photocytotoxicity study ($\lambda_{\text{irr}} = 510$ nm unless otherwise noted), graphed as %
Viability (U87MG cells treated/U87MG cells media only) vs. Concentration ($\mu$M) for the water
control (●), \([(\text{Ph}_2\text{phen})_2\text{Ru(dpp)}\text{PtCl}_2]\text{Cl}_2\) dark (●), cisplatin (■), and
\([(\text{Ph}_2\text{phen})_2\text{Ru(dpp)}\text{PtCl}_2]\text{Cl}_2\) (●). Ph$_2$phen = 4,7-diphenyl-1,10-phenanthroline and dpp = 2,3-
bis(2-pyridyl)pyrazine.
Chapter 4: Concluding Remarks

Mixed-metal Ru(II)-Pt(II) supramolecular complexes were designed, synthesized, characterized, and studied using electrochemistry, electronic absorption spectroscopy, and emission spectroscopy (steady-state and time-resolved) for applications in light targeted anticancer therapy. The structural motif employed for this work was \([(\text{Ph}_2\text{phen})_2\text{Ru}B\text{L})\text{PtCl}_2]^2+\), which covalently coupled a Ru(II) chromophore for PDT activity through a bis-bidentate polyazine bridging ligand (dpp or dpq) to a Pt(II) BAS for covalent binding to nucleophilic biological substrates. The redox and photophysical properties were analyzed at each building block step providing systematic evaluation of the complex properties and demonstrating perturbation of subunit properties upon incorporation into the assembly.

Basic chemical properties of the Ru(II)-Pt(II) complexes were studied. The electrochemistry of the \([(\text{Ph}_2\text{phen})_2\text{Ru}B\text{L})]^2+ \text{and } [(\text{Ph}_2\text{phen})_2\text{Ru}B\text{L})\text{PtCl}_2]^2+ \text{(BL = dpp or dpq)}\) systems displayed reversible one electron oxidations and reductions. The electrochemistry suggested that the HOMO was localized on the Ru(dπ) orbitals and the LUMO on the BL(π*) orbitals, which would indicate a lowest lying Ru(dπ)→BL(π*) CT excited state, supported by the electronic absorption spectroscopy. In the bimetallic systems, the enhanced absorptivity provided by the Ru(dπ)→\text{Ph}_2\text{phen}(π*) CT transitions was noted as the spectral gaps were reduced in the visible region of the spectrum compared to the bpy and phen analogs. Tuning of the HOMO-LUMO gap was achieved by systematic variation of the BL allowing for increased absorption in the phototherapeutic window. Emission derived from the 3MLCT excited state was observed, with the monometallic precursors having higher energy excited states with longer excited state lifetimes compared to the bimetallic complexes, in accordance with the energy gap law. The emission from the 3MLCT excited state was quenched and the excited state lifetime reduced in
the presence of molecular oxygen, indicative of energy transfer to generate $^{1}\text{O}_2$. The $\Phi_{1O2}$ was quantified for the $[(\text{Ph}_2\text{phen})_2\text{Ru(BL)}\text{PtCl}_2]^2+$ (BL = dpp, $\Phi_{1O2} = 0.07$ or dpq, $\Phi_{1O2} = 0.03$) complexes, which provided evidence that these complexes could generate $^{1}\text{O}_2$ and possibly function as PDT type agents.

The thermal and photochemical interactions of the $[(\text{Ph}_2\text{phen})_2\text{Ru(BL)}\text{PtCl}_2]\text{Cl}_2$ (BL = dpp or dpq) supramolecular complexes were studied in the presence of DNA and a more complicated cellular system, the U87MG cancer cell line. The DNA interactions of the molecules were analyzed using gel shift assay or selective precipitation. Thermal binding at the $\text{cis}$-$\text{PtCl}_2$ BAS in the Ru(II)-Pt(II) architecture was observed and compared to cisplatin, $\text{cis}$-$[\text{PtCl}_2(\text{NH}_3)]$. Both $[(\text{Ph}_2\text{phen})_2\text{Ru(BL)}\text{PtCl}_2]\text{Cl}_2$ (BL = dpp or dpq) treated DNA samples displayed similar reduced migration through the gel as cisplatin treated samples demonstrating covalent modification of the DNA. Upon confirmation of thermal covalent binding to DNA via the $\text{cis}$-$\text{PtCl}_2$ unit, the activity of the (Ph$_2$phen)$_2$Ru(BL)-LA unit in the Ru(II)-Pt(II) complex was examined by photolyzing the metal complex-DNA solutions under air equilibrated conditions and in the presence of a $^{1}\text{O}_2$ quencher. It was expected that metal binding to DNA would occur and, in the absence of a $^{1}\text{O}_2$ quencher, localize $^{1}\text{O}_2$ at the DNA substrate, affording strand cleavage or in the presence of a $^{1}\text{O}_2$ quencher prevent photochemical activity. Both bimetallic complexes photocleave DNA through a $^{1}\text{O}_2$ mediated pathway. DNA binding and photocleavage was inhibited in the presence of the $^{1}\text{O}_2$ quencher, sodium azide, attributed to the azide interfering with ligand exchange at the $\text{cis}$-$\text{PtCl}_2$ BAS. Light induced cleavage was observed under air-equilibrated conditions providing evidence of targeted $^{1}\text{O}_2$ as a result of covalent localization of the Ru(II)-Pt(II) complex. This is unique for directly coupled Ru(II)-Pt(II) systems and is a result of the properties imparted by subunit identity.
The complexes display unprecedented reactivity with DNA when exposed to visible light. DNA photobinding by the [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ complex was evaluated. The CT-DNA/metal complex solutions were photolyzed at λ_{irr} = 455 ± 10 nm and ≥ 590 nm, where typical Pt(II) complexes do not absorb. Photoinduced binding to DNA was demonstrated via MLCT excitation. This is the first example of MLCT excitation affording a photobinding event and in addition the first complex that utilizes low energy red light excitation in the therapeutic window to induce photobinding. The Ru(dπ)→BL(π*) CT excited state leads to enhanced electron density on the BL making Pt a weaker Lewis acid in the excited state and facilitating halide loss. This provides for enhanced covalent attachment of the supramolecule to DNA.

These studies validate the supramolecular design and show that coupling a Ru(II) chromophore for PDT activity and a cis-PtCl₂ binding moiety for covalent localization affords a complex applicable as a targeted PDT drug. The cytotoxicity and photocytotoxicity of [(Ph₂phen)₂Ru(BL)PtCl₂]Cl₂ toward U87MG cells was evaluated and compared to cisplatin. The U87MG cancer cell line is considered a grade IV astrocytoma and provides an ex vivo model for metal complex treatment. In the dark, [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ and cisplatin afforded LC₅₀ values of 100 µM consistent with previous reports for cisplatin and similar to the currently used chemotherapy, TMZ.²³,²²⁵,²²⁶ This finding confirms the anticancer properties of these supramolecules. Using a LED array for visible light excitation, the metal complex/cell solutions were photolyzed (λ_{irr} = 510 nm) for 30 minutes and displayed significantly enhanced toxicity compared to the cells that were not photolyzed. The LC₅₀ values for the dark [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ and cisplatin treated samples were similar to the photolyzed cisplatin samples, however, the photolyzed Ru(II)-Pt(II) complex the LC₅₀ was ca. 5 µM. Drugs that function at µM concentration against U87MG cancer cells are unknown. The enhanced
photocytotoxicity via MLCT excitation of the [(Ph₂phen)₂Ru(dpp)PtCl₂]Cl₂ complex displays the selective targeting for applications in photochemotherapy. In addition the photocytotoxicity studies provides further evidence that the supramolecular design is necessary and imparts photoactivity at the cis-PtCl₂ BAS affording a targeted therapeutic. The studies also imply that the Ru(II)-Pt(II) complex acts in a fundamentally different method than cisplatin, which would be beneficial in typical MDR cancers.
Chapter 5: Future Directions

The supramolecular [(Ph$_2$phen)$_2$Ru(BL)PtCl$_2$]$^{2+}$ (BL = dpp or dpq) design was validated through detailed and systematic studies of the redox, photophysical, and photochemical properties, providing the basis for development of simplified Ru(II)-Pt(II) bimetallic complexes in the field of targeted PDT agents. However, several questions remain regarding the Ru(II)-Pt(II) bimetallic systems and how they function. The future directions for this project are aimed at probing the photochemical reactions leading to the active complex and the photoproducts of the Ru(II)-Pt(II) complexes. In addition, structural modifications to the Ru(II)-Pt(II) bimetallic systems providing enhanced visualization and targeted activity in cells are proposed.

Studying the photochemistry of the Ru(II)-Pt(II) complexes prior to interaction with a biological substrate provides insight into the mechanism of complex activation. In the photobinding experiments, it is suggested that visible light irradiation of the [(Ph$_2$phen)$_2$Ru(dpp)PtCl$_2$]Cl$_2$ bimetallic complex induces charge transfer from the Ru(II) center to the dpp BL affording increased electron density at the Pt(II) site, facilitating halide loss and DNA binding. This mechanism is more targeted and efficient than the prototypical thermal binding observed in platinum based anticancer agents and is expected to be oxygen independent, which is promising for activity in hypoxic environments. The visible light induced activity occurring at the cis-PtCl$_2$ site is exciting, however confirmation of photoinduced ligand exchange processes at the Pt bioactive site, the light absorbing unit influence on Pt ligand exchange, and the environment impact (ion concentration and pH) need in-depth investigation. These studies could provide promising results with regards to treatment optimization and provide evidence for a new mechanism of targeted oxygen independent activity, which is important in the application of these complexes as photochemotherapeutics.
More detailed studies of the metal complex-biological target adducts are necessary. Studies in the presence of DNA display that the Ru(II)-Pt(II) complexes bind to DNA, but in cell cultures there is no confirmation that the metal complexes reach DNA and if they do is the binding strong enough in vivo to inhibit replication or undergo photochemistry events. Evaluation of binding by electrophoresis provides a change in the rate of migration as a result of covalent modification, but does not provide any evidence that the complex inhibits DNA replication. Preliminary studies in the Brewer lab are underway, utilizing polymerase chain reaction (PCR), a laboratory technique used to rapidly replicate DNA to investigate whether metal binding to DNA can inhibit replication and if it does what extent of bound metal complex prevents replication. Further studies include investigations of the Ru(II)-Pt(II) treated bacterial or cell culture systems, including DNA extraction, cell fractionation, or microscopy. Confirmation of the metal complex attached to DNA and the concentration breakdown of the complex within the cell system would help to understand the fundamental pharmacological properties. Furthermore, it would be interesting to determine other substrates the metal complexes may competitively bind to in the place of DNA or provide insight into new binding targets. These studies are pivotal in understanding the fundamentals of the Ru(II)-Pt(II) bimetallic complexes.

Finally, structural modification within the architecture to improve localization of the Ru(II)-Pt(II) complexes or to observe localization in real time provide the next steps in the development of these supramolecules. Therapeutic nanoparticles are one of the directions scientists are focusing on to overcome typical drug delivery limitations and to introduce active targeting within the tumor. Recently, Ru(II)-polypyridyl functionalized Au nanoparticles were reported as cellular imaging agents. The Ru(II)-functionalized nanoparticles were visualized in
HeLa (ovarian) cancer cells using confocal fluorescence microscopy and showed aggregation in the cytosol and nucleus within 4 hours. Cells did not display significant apoptosis or inhibitory cell growth effects, however localization of the complexes was clearly observed. It is proposed that the TL in the $[(\text{TL})_2\text{Ru(BL)PtCl}_2]^{2+}$ (TL = polypyridyl terminal ligand and BL = dpp or dpq) architecture could be functionalized and attached to the nanoparticle surface. In addition, attachment of the complex to a nanoparticle provides concentrated delivery of the metal complexes. Alternatively, fluorescently tagging the TL on the Ru(II) LA may provide a method to observe migration of the complex over time in the cell via confocal microscopy without greatly perturbing the PDT activity. Generally fluorescent tags are increasingly lipophilic and therefore must be balanced with hydrophilicity in drug design. It is also necessary that the fluorescent tag is spatially separated, which limits emission quenching of the fluorescent tag by the Ru(II)-Pt(II) complex.\textsuperscript{235-237} The structural modifications allow non-invasive monitoring of the Ru(II)-Pt(II) bimetallic complexes for understanding transport and ultimate localization of these complexes.