Chapter 7: Summary and Outlook

The unchecked proliferation of DNA base pair mismatches in the human genome can have severe consequences, leading to mutagenesis. Proliferation of these single base lesions is generally the result of an absent or otherwise defective mismatch repair (MMR) machinery, which is responsible for the recognition and correction of these mutations. Unsurprisingly, deficiencies in the MMR pathway are associated with a variety of cancers, but the consequences of MMR-deficiency continue even further. The resistance that MMR-deficient cancers often exhibit to traditional chemotherapeutic agents renders them largely untreatable, particularly in the later stages of carcinogenesis. Worse, attempted treatment of these malignancies with therapies such as cisplatin or DNA alkylators simply enriches the population of cells exhibiting MMR-deficiency, often resulting in secondary cancers such as leukemia.

The necessity of treatment for MMR-related diseases, combined with the devastating side effects arising from traditional chemotherapeutics targeting healthy cells, has fueled our continued research of rhodium metalloinsertors. These complexes bind DNA mismatches both *in vitro* and in cells with exquisite precision, resulting in highly selective potency in MMR-deficicent cancer cells. A significant portion of my doctoral research has been devoted to uncovering *how* this unique biological activity occurs, and employing these discoveries in the development of increasingly complex structures.

In my early work in collaboration with Dr. Alexis Komor and Dr. Curtis Schneider, we uncovered an important structure activity relationship between the non-inserting ancillary ligands of a diverse group of metalloinsertors. These complexes displayed a broad range of biological activities that depended not on their DNA binding

affinities or cellular uptake, but rather their propensity to localize to the nucleus and avoid mitochondria, a characteristic that directly correlated to the lipophilicity of the ancillary ligands. Importantly, we discovered that metalloinsertors localize to the nucleus in concentrations sufficient for mismatch binding, whereas mitochondrial localization is detrimental to cell-selective cytotoxicity, thereby supporting the notion that our rhodium complexes target DNA mismatches in the genome.

Upon gaining a greater understanding of the biological activity of our rhodium complexes, my next goal for my thesis research was to synthesize more complicated structures for enhanced potency. Specifically, I sought to design bifunctional metalloinsertor conjugates, in hopes of conferring their cell-selective activity to another more potent therapeutic cargo, such as a platinum anticancer agent. I synthesized a bimetallic Rh-Pt complex, consisting of a rhodium metalloinsertor tethered to an oxaliplatin derivative. DNA binding studies showed that the complex interacts with DNA through both metalloinsertion at a mismatch and the formation of intrastrand Pt-DNA adducts. While the conjugate was not selective for MMR deficiency *in vitro*, it did exhibit enhanced cytotoxicity compared to cisplatin and oxaliplatin, as well as relative to its unconjugated Rh and Pt subunits.

Further development of additional new generations of metalloinsertor-platinum conjugates did not lead to the cell-selective targeting of platinum to mismatched DNA as we had initially intended. However, we constructed unique and complex structures that nevertheless revealed more information about how metalloinsertors function. I developed the first-generation conjugate derived from our newest family of metalloinsertor complexes – those containing an axial Rh—O bond – that is able to selectively target

platinum to mismatched DNA through the formation of non-classical adducts. I have also synthesized a new inserting ligand with two chelating environments, which has shown that metalloinsertion can place a second metal in the helix in place of the ejected mismatched bases. Most significantly, we discovered that the source of the nonselective toxicity of these conjugates is due to the initiation of apoptosis, rather than necrosis. This implies that the biological pathway leading to necrosis is a general hallmark of the cellular response to DNA mismatch recognition by metalloinsertors.

As we continue to unearth reasons why metalloinsertors do *not* exhibit cell selective cytotoxicity, we are just now beginning to discover why our most potent complexes do. The revelation that metalloinsertors are capable of inducing DNA damage in the genome, as well as selectively inhibiting transcription in MMR-deficient cells, marks an exciting new development in our study of these complexes. As we begin our *in vivo* studies of metalloinsertors, we enter a new frontier: the advancement of these complexes into the clinic as targeted chemotherapeutics.