

Dr. John Wheeler- Wheeler Group Research Projects

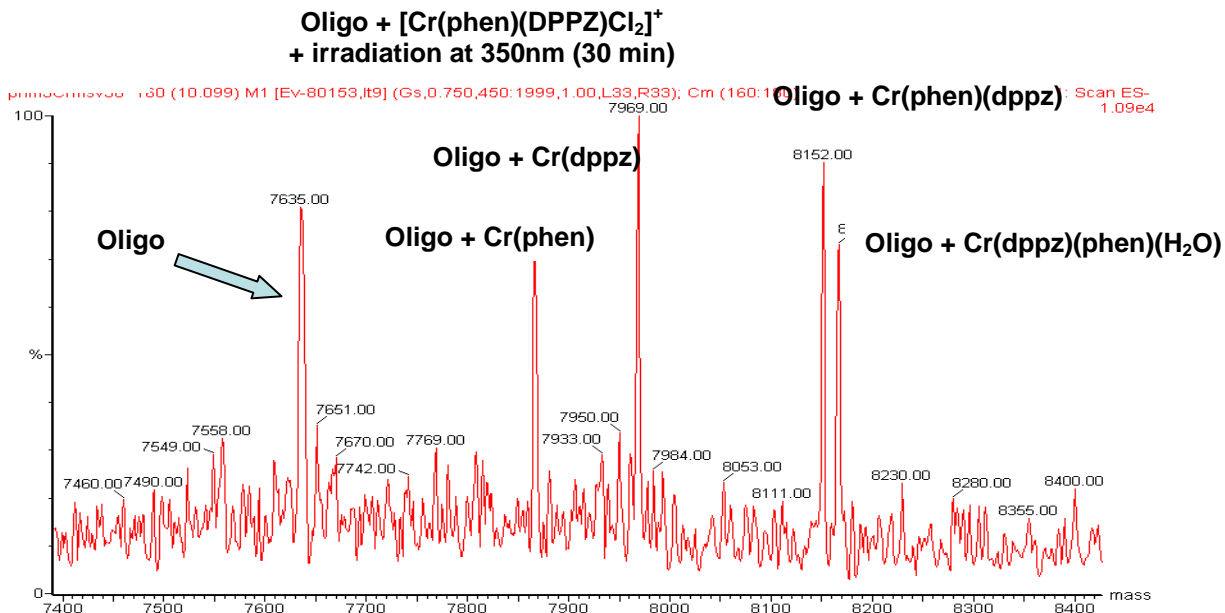
Research in our labs involves applying analytical chemistry to a diverse group of chemical problems. Students intending to pursue advanced degrees in analytical, physical, environmental or biochemistry, the health sciences, or entering directly into the workplace will benefit from the research experiences encountered in these applications.

Chiral Separations/ TM-DNA Binding Investigations

Over the past several years our group (in collaboration with Professor Kane-Maguire's group) has become very interested in the interaction of certain transition metal (TM) complexes that bind strongly to DNA and exhibit the potential to behave as anti-cancer agents when excited by light (i.e., *photocleavage agents*). In some of our earliest studies, we noted that the chirality inherent in the DNA double helix was sufficiently unique to permit enantiomeric analysis of TM complexes (i.e., Λ and Δ isomers) using a separations technique known as capillary electrophoresis (CE). As a result, in the recent past a significant number of presentations and publications have resulted from our efforts to develop new methods for separating chiral TM systems using chiral resolving agents such as antimonyl-*d*-tartrate and sulfonated β - and γ -cyclodextrins (*Susan Bailey, Brad Herbert, 2005; Stephanie Bass, 2006; Floyd Stanley 2007-2008; Sara McCord 2008*). In addition to demonstrating stereospecificity in DNA binding, work in our labs has provided considerable evidence and detail for the site and mode of DNA interaction for $\text{Cr}(\text{diimine})_3^{3+}$ compounds in particular. (*Page Bridges, Sandy Wheeler, 2006-2008; Angela Zeigler, 2008*). Numerous bioanalytical techniques are routinely used to study the binding behavior of these complexes with DNA, including CE, HPLC with mass spectrometry (LC-MS), emission and UV-Vis absorption spectroscopy, circular dichroism (CD), equilibrium dialysis, cyclic voltammetry (CV), viscometric titration and most recently gel electrophoresis and isothermal titration calorimetry (ITC).

In the summer of 2009, we will continue our collaborative efforts with a focus on characterizing and improving the molecular design of $\text{Cr}(\text{diimine})_3^{3+}$ systems that interact with DNA. Using electrospray ionization mass spectrometry (ESI-MS) (a technique that permits analysis of intact duplex DNA oligomers with molecular weights on the order of 10,000 daltons), we are able to consider not only general aspects of DNA binding such as groove preference (e.g., major vs. minor groove) and sequence selectivity (e.g., A-T vs. G-C), but also important changes to the DNA structure and the Cr(III) "pro-drug" targets following photoactivation. Recent studies in our lab have suggested possible covalent adduct formation between the Cr(III) complexes and DNA following exposure to light, most likely due to coordination with the purine bases (i.e., guanine and adenine) (*Chris Priedemann, 2006-2008; Thomas Powers, 2007-2008*, see Figure below). Demonstrating the presence, site and nature of these interactions is of paramount interest, one of the most successful anti-cancer drugs in use today (*cisplatin*) relies on covalent bond formation with nucleophilic sites on guanine during the cell division stage for its chemotherapeutic activity. As one might expect, the chemistry of the ligands associated with the parent Pt(II) complex (or Cr(III) complex in our case) is critical to the binding mechanism associated with the drug's efficacy. *Thus, an important long term goal is to*

understand the nature of the covalent interaction so as to permit the synthetic development of compounds that maximize selective and controlled binding while retaining the unique advantages of the Cr(III) complexes afforded by the photoactivation mechanism. Methodologies have now been developed to permit the separation and analysis of custom-designed sequences of up to 25 base pairs. We have also developed the capacity to examine the formation of covalent bonds between the Cr(III) complexes and smaller molecules (Melissa Allen, 2007; Marian Helsel, 2008; Chris Priedemann, 2008) that contain the basic guanine structural motifs, such as 9-ethylguanine and 9-methylguanine (see Kane-Maguire Research Projects).



A second focus this summer will involve a continuing investigation of the thermodynamics of binding between our Cr(III) complexes and DNA. Using isothermal titration calorimetry (ITC), we have recently demonstrated that the formation of an intercalation complex between $[\text{Cr}(\text{diimine})_2(\text{diimine}')^{3+}]$ (where diimine' is a planar intercalator such as dipyridophenazine, DPPZ) and DNA is *entropically* (rather than *enthalpically*) driven (see Figure below), and likewise have corroborated binding constants (K_{DNA}) obtained through independent spectroscopic means (Jay Forsythe, 2007-2008). This observation is intriguing and rather unique among DNA binders, which more typically exhibit $-\Delta H_{\text{binding}}$. We anticipate extending these studies to include a broader range of complexes with varied ligand chemistries over extended temperature ranges in order to accurately report values for the thermodynamic quantities of interest. One very active area of investigation involves an additional binding site on the DNA strand, possibly in the major groove, which is accessible by smaller $\text{Cr}(\text{diimine})_3^{3+}$ complexes and at elevated temperatures by the larger complexes such as $[\text{Cr}(\text{dmp})_2\text{DPPZ}]^{3+}$ (below). This is particularly intriguing, since reports of other metal systems (e.g., $[\text{Ru}(\text{phen})_2\text{DPPZ}]^{2+}$) have conflicted in literature reports in the past regarding the site of binding with DNA (i.e., *major* versus *minor* groove), and the ITC data we are observing displays opposite signs (endothermic vs exothermic) for the binding enthalpy, suggesting the possibility of interaction in *both* grooves. We will

continue to further investigate these phenomena using ITC, ΔT_m studies and dialysis coupled with UV/Vis and circular dichroism in the summer of 2007.

