The primary objectives of this work are the development and characterization of model nickel catalysts for CO activation and carbon-carbon bond forming reactions. These catalysts will serve as functional models of acetyl coenzyme A synthase (ACS), a nickel-containing enzyme that combines carbon monoxide and a cationic methyl group to generate an acetyl moiety followed by acyl transfer to coenzyme A, an alkyl thiol. This reaction is a biological analog of the industrially prevalent Monsanto process, which relies on Rh or Ir compounds, and replacing these precious elements with earth-abundant metals would be highly valuable. A model protein system was proposed as a scaffold for design of an active catalytic center with carefully constructed secondary and tertiary environments, allowing precise tailoring of activity and identification of critical molecular components of an effective catalyst (Figure 1).

The specific aims of this research project are:

1) To design and generate nickel-substituted macromolecules capable of supporting two-electron chemical transformations, as evidenced by the ability to access three distinct redox states within a narrow potential range. A complete description of the electronic and geometric structures of each oxidation state will be obtained using advanced spectroscopic and computational techniques.

2) To incorporate substrate binding pockets and design a coordinatively unsaturated nickel-containing active site within a macromolecular scaffold in order to enable reactivity towards carbon-carbon bond formation and acyl transfer.

We have made significant progress towards these goals over the last year.

1) Nickel-containing macromolecules capable of two-electron transformations

Using nickel-substituted azurin (NiAz) as a model protein scaffold, we have demonstrated that two reversible, one-electron couples are accessible. Using protein electrochemistry and potentiometric titrations, the Ni\(^{II}\), Ni\(^{III}\), and Ni\(^{IV}\) azurin species within wild-type azurin have been generated. Equivalent oxidation states have been accessed within a mutant of azurin (M121A) that features a substrate binding pocket. These species have been characterized using an array of spectroscopic techniques in conjunction with computational analyses to obtain structural information (Figure 2), and important similarities between NiAz and native ACS have been noted. These results were published in J. Am. Chem. Soc. in 2017.

While this recent work indicated that the (His)\(_{2}\)(Cys) ligation environment is sufficient to support two one-electron couples, the redox potentials remain energetically separated by approximately 1.5 V. In order to reduce the potential drop between the two transitions, increased covalency may be needed. This adaptation is typically seen in sulfur-rich binding sites that support multielectron transformations, such as iron-sulfur clusters and heme centers. Towards this end, we have generated and begun to characterize mutants with increased sulfur-containing ligands in place of one of the histidine residues. Hypsochromic shifts of the primary absorption features suggests perturbation of the electronic structure, which are likely to accompany a shifted reduction potential. Work aimed at characterizing the independent reduction potentials of each of the mutants as a function of ligand covalency is ongoing using spectroscopic and computational techniques.

2) Substrate binding and reactivity in nickel-containing macromolecules

By introducing the M121A mutation at the top of the metal-binding site, we have installed a substrate binding pocket. Our recent work has shown that M121A Ni\(^{III}\)Az is capable of binding CO with moderate binding affinity (K\(_D\) \(\sim\) 30 \(\mu\)M), comparable to that of native ACS, and substantial CO bond activation indicative of a high degree of orbital overlap between the nickel and carbon centers (Figure 3). In addition to binding CO, we have also shown interaction between the nickel center and a methyl group, and this work was published in JACS in 2017. We have also noted that,
like native ACS, the Ni(I)-CO Az species is photolabile at cryogenic temperatures, suggesting a low activation barrier for rebinding (Figure 4). The photochemical properties of this reaction and the changes in CO binding affinity as a function of primary and secondary sphere changes are currently being explored.

Moving beyond carbon monoxide binding, current work is aimed at characterization of the methyl-bound species. Our prior work demonstrated that addition of methyl iodide to M121A NiAz in the presence of excess reducing agent results in an EPR-silent species with visible optical absorption bands. This interaction occurs with a binding affinity of ~100 µM, weaker than that reported for binding to CO (Figure 5). Through optimization of experimental conditions, we are now able to isolate the M121A NiAz species in the absence of excess reducing agent. Upon mixing with methyl iodide, a new transient species has been identified with a broad optical absorption feature in the visible region of the spectrum and unique EPR properties. Isotopic substitution and kinetic analysis have been performed, implicating nucleophilic attack from the reduced metal center. Additional evidence supporting this claim is found using methylco(III)binamide as a methyl donor. This putative Ni-CH₃ Az species is only the second such organometallic species to be identified within a biological system and a rare example of a low-coordinate, high-valent Ni-alkyl species with “normal” ligands (i.e., ligands that are not highly fluorinated or redox-active). Decay of this species proceeds exclusively through generation of methane, consistent with reactivity observed in ACS. This work is in preparation for submission.

The identification and characterization of well-defined Ni(I)-CO and Ni-CH₃ species lay an important foundation for exploring reactivity with multiple substrates, which form the basis for present and future work on this project.

This project has had a significant impact on my career. The data that we were able to obtain from this grant contributed to a successful application to the DOE Early Career Research Program and a publication in JACS. This has raised the profile of my research program, leading to an invitation to speak at a Gordon Research Conference.

Additionally, this grant has provided substantial training opportunities for graduate and undergraduate students in my lab. The primary student on the project over the last year has been Anastasia Manesis. Through this grant support, she has been able to focus on research and advance the project, as well as travel to a Gordon Research Conference to disseminate her results. Anastasia is graduating in December but will stay for a couple months as a postdoc on this project to wrap up things. This grant has also provided summer support for an undergraduate researcher (Timothy O’Connor) and a younger graduate student (Camille Schneider), who is collaborating with Anastasia to measure the electron transfer kinetics for NiAz.