1. PRF #57403-DNI6
2. Project Title: A macromolecular nickel catalyst for carbon-carbon bond formation
3. PI: Hannah S. Shafaat, The Ohio State University

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**).

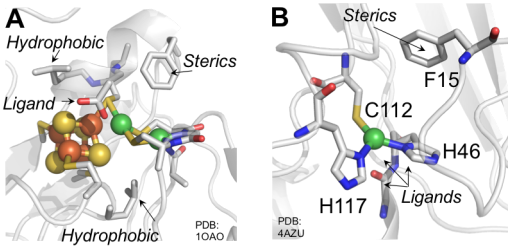

Figure 1. Structures of the active sites of (A) acetyl coenzyme A synthase and (B) nickel-substituted azurin (NiAz). Active sites shown with conserved secondary sphere amino acids and their proposed function.

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^{I}, Ni^{II}, and Ni^{III} 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.

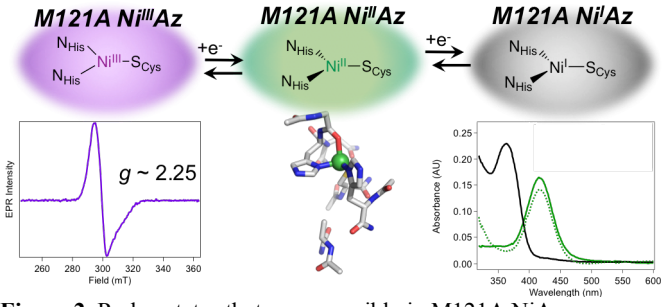

Figure 2. Redox states that are accessible in M121A NiAz.

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 $Ni^{I}Az$ is capable of binding CO with moderate binding affinity (K_D ~ 30 μM), 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.

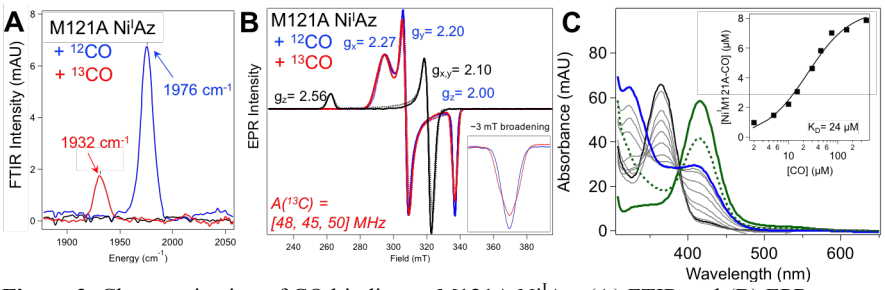

Figure 3. Characterization of CO binding to M121A Ni^{I}Az. (A) FTIR and (B) EPR spectra of M121A Ni^{I}-CO prepared with natural abundance (blue) or ^{13}CO (red). Pertinent spectroscopic metrics labeled on figure. (C) Optical titration to measure CO binding affinity. Ni^{II}Az shown in green. Spectra of M121A Ni^{I}Az shown in all panels in black.

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 Ni^{I}Az 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 Ni^{I}Az 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-CH_3 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.

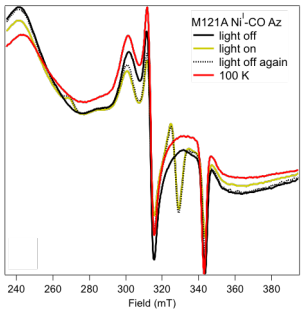

Figure 4. Photolability of Ni^{I}-CO species. CW X-band EPR investigation of CO photolability in 150 μM M121A Ni^{I}-CO Az in 50 mM HEPES, pH 8.0, prepared with 6 mM Eu^{II}DTPA. Spectra were collected at 5 K with 20 mW power. Spin quantitation reveals ~20% of the M121A Ni^{I}-CO Az sample is photolabilized to generate M121A Ni^{I}Az.

The identification and characterization of well-defined Ni^{I}-CO and Ni-CH_3 species lay an important foundation for exploring reactivity with multiple substrates, which form the basis for present and future work on this project.

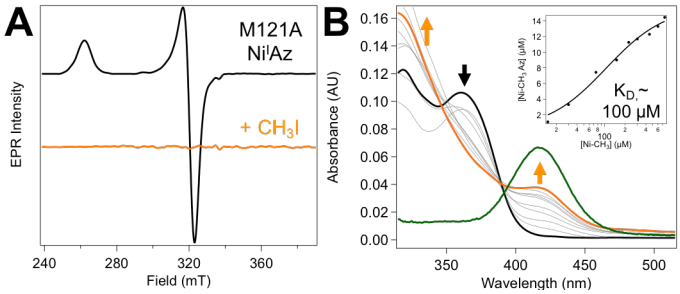

Figure 5. M121A NiAz titration with CH_3I. (A) CW X-band EPR of reduced M121A Ni^{I}Az (black trace) and following addition of CH_3I (orange trace). (B) 15 μM M121A Ni^{II}Az (green line) prepared in 50 mM HEPES buffer, pH 8.0. Addition of CH_3I to M121A Ni^{I}Az (black line) shows conversion to a new, EPR-silent species (orange). (Inset) Binding curve for $-CH_3$ binding to Ni^{I}Az.

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.