



Enzymatic and Molecular Catalysts for Sustainable Fuels

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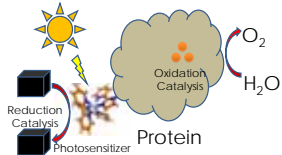
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Engineering Photoredox Enzymes: A Scaffold for Water Oxidation

One avenue toward sustainable fuel is to mimic photosynthesis, whereby organisms such as green plants harvest sunlight to effect rapid charge separation within a protein matrix. The separated charges subsequently drive redox processes, such as the splitting of water and the fixation of CO₂.

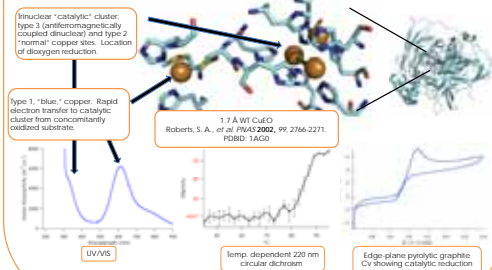


Our "photoredox enzyme" must possess the following features:

- Kinetically competent:** Should possess capacity to catalyze desired chemistry
- Robust:** Able to withstand oxidative stress and elevated temperatures to achieve high turnover
- Efficient:** Electron transfer and subsequent catalysis should proceed swiftly to produce useful amounts of product
- Photosensitive:** Able to make use of absorbed sunlight
- Cheap:** Make lots of it!

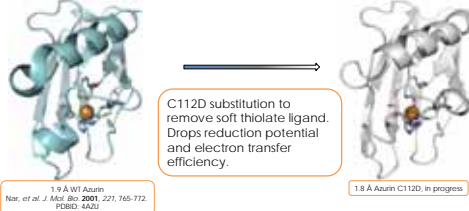
We have chosen the copper efflux oxidase (CuEO) from *E. coli* as a "fixer-upper."

- Competent:** CuEO catalyzes the 4 e⁻ reduction of dioxygen to water using three copper centers. **Microscopic reversibility!** We can drive the reaction in reverse if we tune the reduction potentials properly!
- Thermostable:** T_m ~ 85°C
- Easy to make/manipulate:** Prokaryotic, so we can easily modify with site-directed mutagenesis. Can overexpress wild-type in ~30 mg/L quantity.

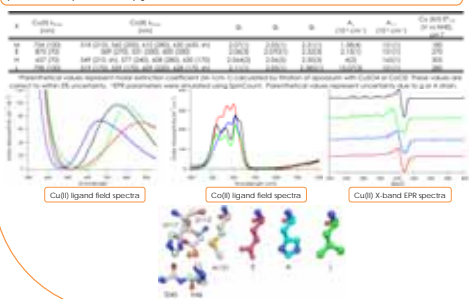


Engineering Photoredox Enzymes: Shoring Up The Electron Transfer Pathway

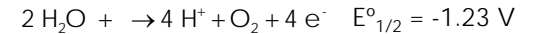
Oxidation chemistry limits our ligand set. We must not oxidize our protein in the process of splitting water! We used *P. aeruginosa* azurin's Cu site as a model for the CuEO T1 site in our search for a robust electron transfer center.



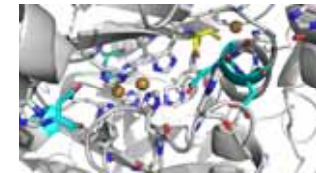
M121X substitution removes thioether: variable effects on reduction potential, spectroscopy...



Engineering Photoredox Enzymes: Tuning Cu(II/I)



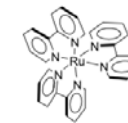
The oxidation of water to dioxygen requires us to significantly elevate CuEO's Cu(II/I) potentials.



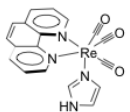
We have made several mutations near the trinuclear copper cluster in an attempt to raise its reduction potentials.

Of the mutants that we isolated, none have displayed electrocatalytic activity.

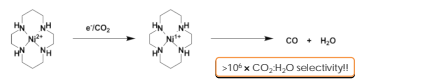
Engineering Photoredox Enzymes: Photosensitization



Removal of all but one strategically placed surface histidine will allow us to label CuEO with either a ruthenium bipyridine or rhenium tricarbonyl chromophore. Excited-state electron transfer chemistry will allow us to turn-over our CuEO!



Ni(cyclam) Electrocatalysts: CO₂ Reduction



Ni(cyclam)TON
Bely, Coll. Reppert, Sauvage *J. Am. Chem. Soc.* 1986, 108, 7641

CO dehydrogenase
Ni^{II}(η¹-CO)₂ interaction
H-bond to OCO
Fe^{II} as O-acceptor

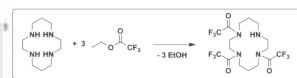


What makes Ni(cyclam) special?
Can the key points be replicated with tunable ligands?

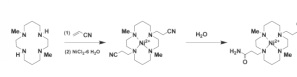
What role do hydrogen bonding interactions play?
Are N-H...OCO interactions required?

Spectroscopic signatures suggest Ni(CO) intermediate
Is this species mechanistically important?
Can bound CO be activated and reduced?

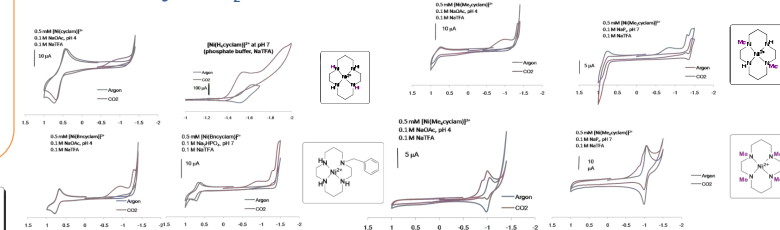
Will M-M cooperativity improve performance?
Do monometallic Ni^{2+/1+} offer maximum benefits?



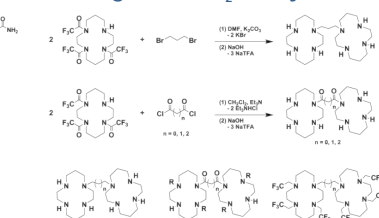
H-Bond Donors



Catalytic CO₂ Reduction



Ligands for Ni₂ Catalysts



CO₂ Summary

Availability of H-bonds important for CO₂ reduction
Do not appear necessary for CO₂ binding events

Trifluoroacetyl protecting groups permit multiple functionalizations
Mononuclear, binuclear, pendant arm donors, etc.
Acid chloride coupling looks promising

Several synthetic targets within reach - ready for Ni²⁺ metalation!
Electrochemical studies with and without CO₂ present
Probe role of CO binding

Photochemistry scheme for mechanistic studies to be developed
Choice of in situ reducing agents limited by Ni^{2+/1+} couple
Indole photoionization (ε₃₀₀)

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