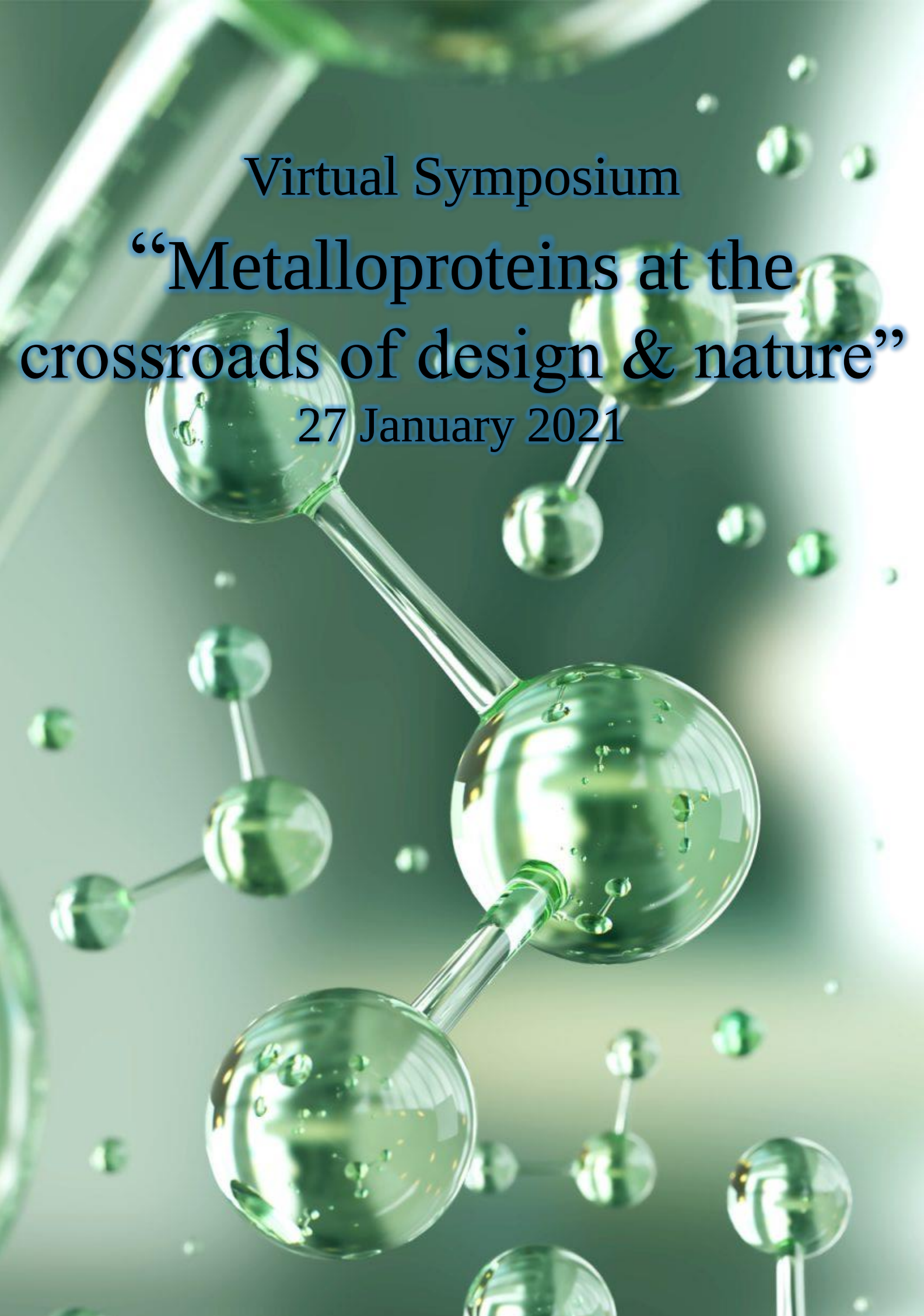

Virtual Symposium

“Metalloproteins at the crossroads of design & nature”

27 January 2021

RUTGERS

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Evolution of Nanomachines In Geospheres and Microbial Ancestors

Virtual Symposium

"Metalloproteins at the crossroads of design & nature"

27 January 2021, 12:30 PM EST

Timings (EST)	Topics/Sessions	Faculty/Speaker
12:30 – 12:40 PM	**Welcome note**	
	Keynote address	
12:40 – 1:40 PM	Emergent Complexity: Reconstructing primordial energy conversion processes in model metalloproteins	**Hannah Shafaat**, Ohio State University
	Session 1: Naturally Selected	
1:40 – 2:00 PM	Structural Changes of the Mn_4CaO_5 Cluster in the Oxygen-Evolving Complex of Photosystem II using X-ray diffraction and emission spectroscopy	**Ruchira Chatterjee** (Yano/Yachandra/Kern group) Lawrence Berkeley National Laboratory
2:00 – 2:20 PM	Understanding biosynthesis of copper-chelating natural products, methanobactins	**Yun Ji Park** (Amy Rosenzweig group) Northwestern University
2:20 – 2:40 PM	Hydrogen deuterium exchange within adenosine deaminase, a TIM barrel hydrolase, identifies networks for thermal activation of catalysis	**Shuaihua Gao** (Judith Klinman group) UC Berkeley
2:40 – 2:50 PM	Break	
	Session 2: Computationally defined	
2:50 – 3:10 PM	*De Novo* design and structural characterization of functional Mn-Porphyrin-Binding protein	**Samuel Mann** (Bill DeGrado group) UC San Francisco
3:10 – 3:30 PM	Redesign of a Copper Storage Protein into an Artificial Hydrogenase	**Pallavi Prasad** (Saumen Chakraborty group) University of Mississippi
3:30 – 3:50 PM	Cobalt-based Biomolecular Catalysts for Hydrogen Evolution from Water	**Jose Alvarez-Hernandez** (Kara Bren group) University of Rochester
3:50 – 4:10 PM	Synthetic models of enzymatic $[Fe_4S_4]$–alkyl intermediates and new insights into their electronic structures	**Mengshan Ye** (Daniel Suess group) Massachusetts Institute of Technology
4:10 – 4:30 PM	Deciphering the Dayhoff's hypothesis on the 'origins of ferredoxins'	**Bhanu P. Jagilinki** (Nanda/Falkowski group) Rutgers University
4:30 –4:45 PM	**Open Discussion and Closing Remarks**	

Keynote Lecture

Prof. Hannah Shafaat

Ohio State University

Emergent Complexity:
Reconstructing primordial energy conversion processes in model metalloproteins

Abstract:

Nature has evolved diverse systems to carry out energy conversion reactions. Metalloenzymes such as hydrogenase, carbon monoxide dehydrogenase, acetyl coenzyme A synthase, and methyl coenzyme M reductase use earth-abundant transition metals such as nickel and iron to generate and oxidize small-molecule fuels such as hydrogen, carbon monoxide, acetate, thioesters, and methane. These reactions are highly valuable to understand and harness in the context of the impending global energy and climate crisis. However, the native enzymes require complex multimetallic cofactors, are costly to isolate, highly sensitive to external conditions, and generally poorly suited for large-scale application. We are applying metalloprotein engineering approaches in order to better understand the mechanisms of the native enzymes and develop robust functional models with potential for implementation in anthropogenic energy conversion.

Robust metalloprotein scaffolds such as rubredoxin, ferredoxin, and azurin and have been converted into model systems that mimic the structure and function of complex nickel metalloenzymes. By introducing non-native metals and redesigning the primary and secondary coordination spheres, we have installed novel activity into these simple electron transfer proteins, including catalytic hydrogen evolution, carbon monoxide activation, and thioester generation. Adding key elements from the secondary and tertiary coordination spheres of hydrogenase and acetyl coenzyme A synthase enhances both activity and selectivity, pointing to functional roles of specific residues within the natural enzymes.

Steady-state and time-resolved optical, vibrational, and magnetic resonance spectroscopic techniques have been used in conjunction with bioanalytical methods and calculations to probe the active-site structures across different states and determine the catalytic mechanisms. These findings will be discussed in the context of identifying the

fundamental principles underlying highly active native enzymes and understanding how increasingly complex metalloenzymes for energy conversion may have evolved from minimal precursor scaffolds.

Ruchira Chatterjee

Yano/Yachandra/Kern group

Lawrence Berkeley National Laboratory

Structural Changes of the Mn_4CaO_5 Cluster in the Oxygen-Evolving Complex of Photosystem II using X-ray diffraction and emission spectroscopy

Abstract:

In oxygenic photosynthesis, light-driven oxidation of water to molecular oxygen is carried out by the oxygen-evolving complex (OEC) in Photosystem II (PS II), which is a multi-subunit protein complex. Recently, we reported the room temperature structures of PS II in the four (semi-)stable S-states (S_i, i = 0 to 3), that were advanced from the dark S_1 state to the S_2, S_3 and S_0 states by one, two or three laser flashes. These data show that a water molecule is inserted during the $S_2 \rightarrow S_3$ transition, as a new bridging O(H)-ligand between Mn1 and Ca[1]. To understand the sequence of events leading to the formation of this last stable intermediate state before O_2 formation, we conducted a simultaneous X-ray emission spectroscopy (XES)/X-ray diffraction (XRD) experiment. We recorded diffraction data of 2.01 to 2.23 Å resolution, and Mn X-ray emission spectroscopy data at several time points ranging between 50 to 400 µs during the $S_2 \rightarrow S_3$ transition. We observe several changes at the acceptor and donor sites. XES spectra from PS II solution samples demonstrate that the time constant for Mn oxidation during the $S_2 \rightarrow S_3$ transition and occurrence of O_X is observed over the same time range in the electron density[2] .

References:

1. Kern, **Chatterjee** *et al*. "Structures of the intermediates of Kok's photosynthetic water oxidation clock" Nature. (2018)

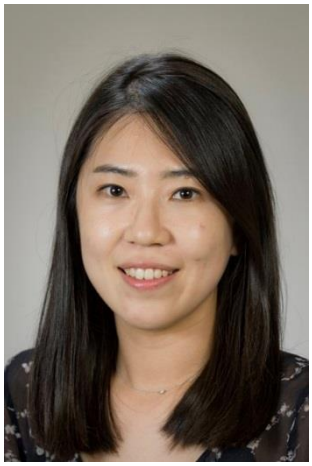
2. Ibrahim, Fransson, **Chatterjee** *et al*. "Untangling the sequence of events during the S2 → S3 transition in photosystem II and implications for the water oxidation mechanism" PNAS. (2020)

Yun Ji Park

Amy Rosenzweig group

Northwestern University

Understanding biosynthesis of copper-chelating natural products, methanobactins



Abstract:

Methanobactins (Mbns) are copper-chelating peptidic natural products, first discovered in methanotrophs. Copper is necessary for methanotrophs because their primary metabolic enzyme is copper-dependent particulate methane monooxygenase (pMMO). In order to meet their high needs for copper, some methanotrophs developed a specialized system where they produce Mbns to acquire copper from the environment under copper limited conditions.

Rosenzweig laboratory has been investigating how Mbns are synthesized, transported, and regulated in methanotrophs. My particular interest lies in understanding the biosynthesis of Mbns in methanotrophs. Mbn is ribosomally-synthesized and post-translationally modified peptide that is produced from a precursor peptide, MbnA. The MbnA comprises a leader peptide and a core peptide. The leader peptide is required to interact with Mbn biosynthesis proteins and eventually cleaved off by an unknown mechanism while the core peptide is post-translationally modified to form mature Mbn by a series of Mbn biosynthesis enzymes. The post-translational modifications happen at two specific cysteine residues in MbnA core peptide, resulting in the formation of the copper binding sites, oxazolone and thioamide groups. Previous studies from our laboratory has shown that two core Mbn biosynthesis proteins, MbnB and MbnC, form a heterodimeric iron-containing enzyme complex, MbnBC, and catalyze the formations of the oxazolone/thioamide pairs from the cysteine residues. The iron site in MbnB is believed to interact with MbnA in a presence of O2 to modify the cysteine residues. However, in vitro reaction stalled and generated an intermediate rather than mature Mbn. In this symposium, my current efforts to investigate how to complete the Mbn

biosynthesis in vitro and its reaction mechanisms will be discussed. This work will provide key insights into novel enzymatic chemistries performed by Mbn biosynthesis enzymes.

Shuaihua Gao

Judith Klinman group

UC Berkeley

Hydrogen deuterium exchange within adenosine deaminase, a TIM barrel hydrolase, identifies networks for thermal activation of catalysis

Abstract:

Proteins are intrinsically flexible macromolecules that undergo internal motions with time scales spanning femtoseconds to milliseconds. These fluctuations are implicated in the optimization of reaction barriers for enzyme catalyzed reactions. Time, temperature, and mutation dependent hydrogen–deuterium exchange coupled to mass spectrometry (HDX-MS) has been used to pursue the correlation of protein flexibility and chemical reactivity within the diverse and widespread TIM barrel proteins, targeting murine adenosine deaminase (mADA) that catalyzes the irreversible deamination of adenosine to inosine and ammonia. Using this technique, a map is constructed that illustrates the regions of protein that are proposed to be essential for the thermal optimization of active site configurations that dominate reaction barrier crossings in the native enzyme.

References:

Shuaihua Gao, *et al*. "Hydrogen–Deuterium Exchange within Adenosine Deaminase, a TIM Barrel Hydrolase, Identifies Networks for Thermal Activation of Catalysis" *J. Am. Chem. Soc*. (2020)

Samuel Mann

Bill DeGrado group

UC San Francisco

De Novo **design and structural characterization of functional Mn-Porphyrin-Binding protein**

Abstract:

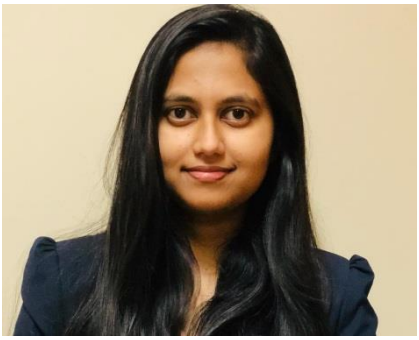
De novo protein design offers the opportunity to test our understanding of how metalloproteins perform difficult transformations. Attaining high-resolution structural information is critical to understanding how such designs function. There have been many successes in the design of porphyrin-binding proteins; however, crystallographic characterization has been elusive, limiting what can be learned from such studies as well as the extension to new functions. Moreover, formation of highly oxidizing high-valent intermediates poses design challenges that have not been previously implemented: (1) purposeful design of substrate/oxidant access to the binding site and (2) limiting deleterious oxidation of the protein scaffold. Here we report the first crystallographically characterized porphyrin-binding protein that was programmed to not only bind a synthetic Mn–porphyrin but also maintain binding site access to form high-valent oxidation states. We explicitly designed a binding site with accessibility to dioxygen units in the open coordination site of the Mn centre. In solution, the protein is capable of accessing a high-valent Mn(V)–oxo species which can transfer an O atom to a thioether substrate. The crystallographic structure is within 0.6 Å of the design and indeed contained an aquo ligand with a second water molecule stabilized by hydrogen bonding to a Gln side chain in the active site, offering a structural explanation for the observed reactivity.

Pallavi Prasad

Saumen Chakraborty group

University of Mississippi

Redesign of a Copper Storage Protein into an Artificial Hydrogenase



Abstract:

The increasing energy demand and the depleting fossil fuels call for the development of alternative clean energy sources from natural resources. Hydrogenases are a class of metalloenzymes that catalyze a (two electron) reversible interconversion of hydrogen (H_2) to protons, and protons back to hydrogen [H_2 ($\leftrightarrows$ $H^+ + H^-$) $\leftrightarrows$ $2H^+ + 2e^-$][1]. However, the innate complexity, oxygen sensitivity and low yield of these enzymes hinders their realization for being used as an alternative source of energy. The artificial hydrogenases can help overcome this hinderance by providing simpler functional models which can work under environmentally benign conditions. Protein engineering is a powerful tool to create these artificial metalloenzymes capable of *mimicking* the structure and function of naturally existing enzymes.

Herein, the construction of an artificial hydrogenase (ArH) by reengineering a Cu storage protein (Csp1) into a Ni-binding protein (NBP) employing rational metalloprotein design will be discussed[2]. Mutations were done to Csp1 to create the target tetrathiolate Ni binding site, followed by repacking of the hydrophobic core. Guided by the computational modeling, the NBP was expressed and purified in high purity. The NBP has a well-folded and stable construct, displaying native-like unfolding behavior. Spectroscopic and computational studies showed that NBP bound nickel in a distorted square planar geometry thus validating the design. Ni(II)-NBP is active for the photoinduced H_2 evolution and follows a reductive quenching mechanism. Ni(II)-NBP catalyzed H^+ reduction to H_2 electrochemically as well. Analysis of the catalytic voltammograms established a proton-coupled electron transfer mechanism. Electrolysis studies confirmed H_2 evolution with quantitative Faradaic yields. Therefore, these studies demonstrate an important scope of rational metalloprotein design in imparting functions into protein scaffolds that they have natively not evolved to possess the same function of the target metalloprotein constructs.

References:

1. Wolfgang Lubitz, *et al.* "Hydrogenases" Chem. Rev. (2014)

2. Dhanashree Selvan, **Pallavi Prasad**, *et al*. "Redesign of a Copper Storage Protein into an Artificial Hydrogenase" ACS Catal. (2019)

Jose Alvarez-Hernandez

Kara Bren group

University of Rochester

Cobalt-based Biomolecular Catalysts for Hydrogen Evolution from Water

Abstract:

The elucidation of structure-function relationships in metalloenzymes that catalyze redox reactions in nature has provided a foundation for the development of novel biomolecular catalysts for fuel production and small molecule activation[1]. In the Bren group, we use engineered enzymes and biomolecular complexes as catalysts for electrochemically and photochemically driven reactions, including the hydrogen evolution reaction (HER). Some remarkable features of our catalysts are their oxygen-tolerance and that they operate in 100% water as opposed to aprotic solvents or solvent mixtures. We have paid special attention to the roles that buffers play in proton transfer and electrocatalysis in water.

Investigations of the effects of buffer species on electrocatalysis have yielded new insights into catalytic mechanism and roles of buffer acids as proton shuttling agents. In electrocatalytic HER by CoMP11-Ac (a cobalt porphyrin-peptide) in water, two distinct catalytic regimes are identified as a function of the buffer p*Ka*. In the presence of buffers with p*K*a ≤ 7.4, a fast catalysis regime limited by diffusion of buffer is reached. The catalytic half-wave potential (Eh) shifts anodically as the buffer p*Ka* decreases from 7.4 to 5.6. With higher-p*Ka* buffers (p*Ka* > 7.7), an Eh = −1.42 V, proposed to reflect the Co(II/I) couple, is maintained independent of the buffer p*Ka*, consistent with rate-limiting protonation of the Co(I) species under these conditions[2]. We have also found that buffer-acid p*Ka* impacts the stoichiometry of the proton-coupled electron transfer events involved in the HER mechanism of a synthetic cobalt-porphyrin mini-enzyme (CoMC6*a), and increasing the steric hindrance of the

buffer acid species significantly decreases the catalytic current[3]. We conclude that buffer acids are the primary proton-donors for the catalytic HER under our experimental conditions and that buffer-acid p*Ka* directly impacts the rate of proton transfer steps of the catalytic cycle.

References:

1. Jennifer M. Le and Kara L. Bren. "Engineered Enzymes and Bioinspired Catalysts for Energy Conversion" ACS Energy Lett. (2019)
2. **Alvarez-Hernandez**, *et al*. "Buffer pKa Impacts the Mechanism of Hydrogen Evolution Catalyzed by a Cobalt Porphyrin-Peptide" Inorg. Chem. (2020)
3. Le, J. M, *et al*. "Tuning Mechanism through Buffer Acid Dependence of Hydrogen Evolution by a Cobalt Mini-enzyme" Biochemistry. (2020)

Mengshan Ye

Daniel Suess group

Massachusetts Institute of Technology

Synthetic models of enzymatic [Fe_4S_4]–alkyl intermediates and new insights into their electronic structures

Abstract:

The organometallic chemistry of Fe–S clusters has emerged as a new area in Fe–S enzyme biochemistry. In particular, alkylated [Fe_4S_4] clusters are now thought to be important intermediates in several classes of enzymes including radical SAM enzymes and enzymes involved in terpene biosynthesis. However, due to their transient nature, it has not been possible to fully characterize any enzymatic [Fe_4S_4]–alkyl intermediates and thus little is known about their geometric and electronic structures. To address this problem, our group has synthesized a series of alkylated/arylated $[Fe_4S_4]^{2+/+}$ clusters bearing a tridentate iminophosphorane ligand as synthetic models. Our Mössbauer spectroscopic and

computational studies suggest that the highly electron-releasing alkyl group at the apical site partially localizes the charge distribution within the $[Fe_4S_4]^{2+}$ cubane. Electron Paramagnetic Resonance (EPR) spectroscopic studies and single crystal X-ray diffraction analysis suggest the existence of complete charge polarization upon one-electron reduction. Ferric iron is localized at the alkylated site and antiferromagnetically couples with the remaining three ferrous iron sites, which leads to an unprecedented $S = 7/2$ ground state. By introducing various aryl groups with different electron-donating ability to the apical site of $[Fe_4S_4]^{+}$ cubane, we can modulate this localization effect and access high, intermediate and low spin states. These results point to the importance of (partially) localized electronic structures in Fe–S clusters bearing alkyl and potentially other strong-field ligands (e.g. hydrides in nitrogenase intermediates).

Bhanu Prakash Jagilinki

Nanda/Falkowski group

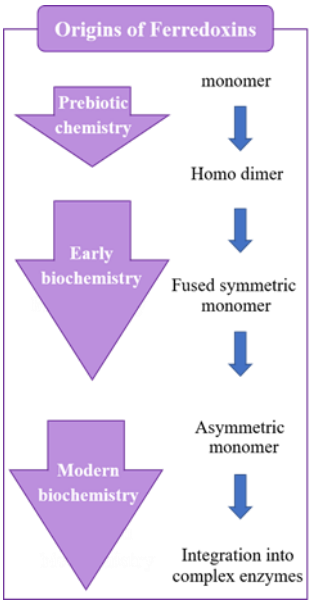
Rutgers University

Deciphering the Dayhoff's hypothesis on the 'origins of ferredoxins'

Abstract:

The modern day Ferredoxins are quite complex, however these proteins once have evolved from very small peptides several billion years ago, even before the existence of life on earth. The current evolutionary concepts on origins of ferredoxins was proposed by **Dayhoff**, more than five decades ago[1]. According to her hypothesis, the present-day ferredoxins could have evolved from smaller peptides binding a single [4Fe-4S] cluster, which later evolved into symmetric dimers. These symmetric dimers further transformed into larger proteins and fused to become symmetric monomers. The two domains of these symmetric ferredoxins then began to evolve independently as a result of divergent evolution leading to modern day asymmetric ferredoxins. The

ferredoxin motifs are also present in many complex metalloproteins such as hydrogenases and nitrogenases, where they essentially transport electrons to the active site of these enzymes. Although, the Dayhoff's model explains the utmost important events in the origins and evolution of ferredoxins, many of the intermediary states have not been studied yet. The abstract fig. illustrates the linear flowchart of 'origins of ferredoxins' depicting various stages of ferredoxins over an exceedingly long-time scale. Previously our lab had successfully demonstrated some key designs representing various stages in the evolution of ferredoxins such as minimal monomeric ferredoxins (Ambidoxin) and symmetric monomeric ferredoxins[2,3]. Currently, I am studying symmetric homo-dimers, an important intermediate event predicted to have happened in the early stage of biological events, that eventually triggered life on earth. Interestingly our symmetric homo-dimeric ferredoxins can mimic natural ferredoxins and can perform biological electron transfer *in vivo* as well. The success to our results so far, is a tribute to Prof. Dayhoff!



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1. Eck, R.V. and M.O. Dayhoff. "Evolution of the structure of ferredoxin based on living relics of primitive amino Acid sequences" Science. (1966)

2. Kim, J.D., *et al*. "Minimal Heterochiral de Novo Designed 4Fe-4S Binding Peptide Capable of Robust Electron Transfer" J Am Chem Soc. (2018)

3. Mutter, A.C., *et al*. "De novo design of symmetric ferredoxins that shuttle electrons in vivo" PNAS. (2019)