Rutgers-NASA Annual ENIGMA Astrobiology Symposium

ENIGMA: Evolution of Nanomachines In Geospheres and Microbial Ancestors

How did proteins evolve to become the predominant catalysts of life on Earth?

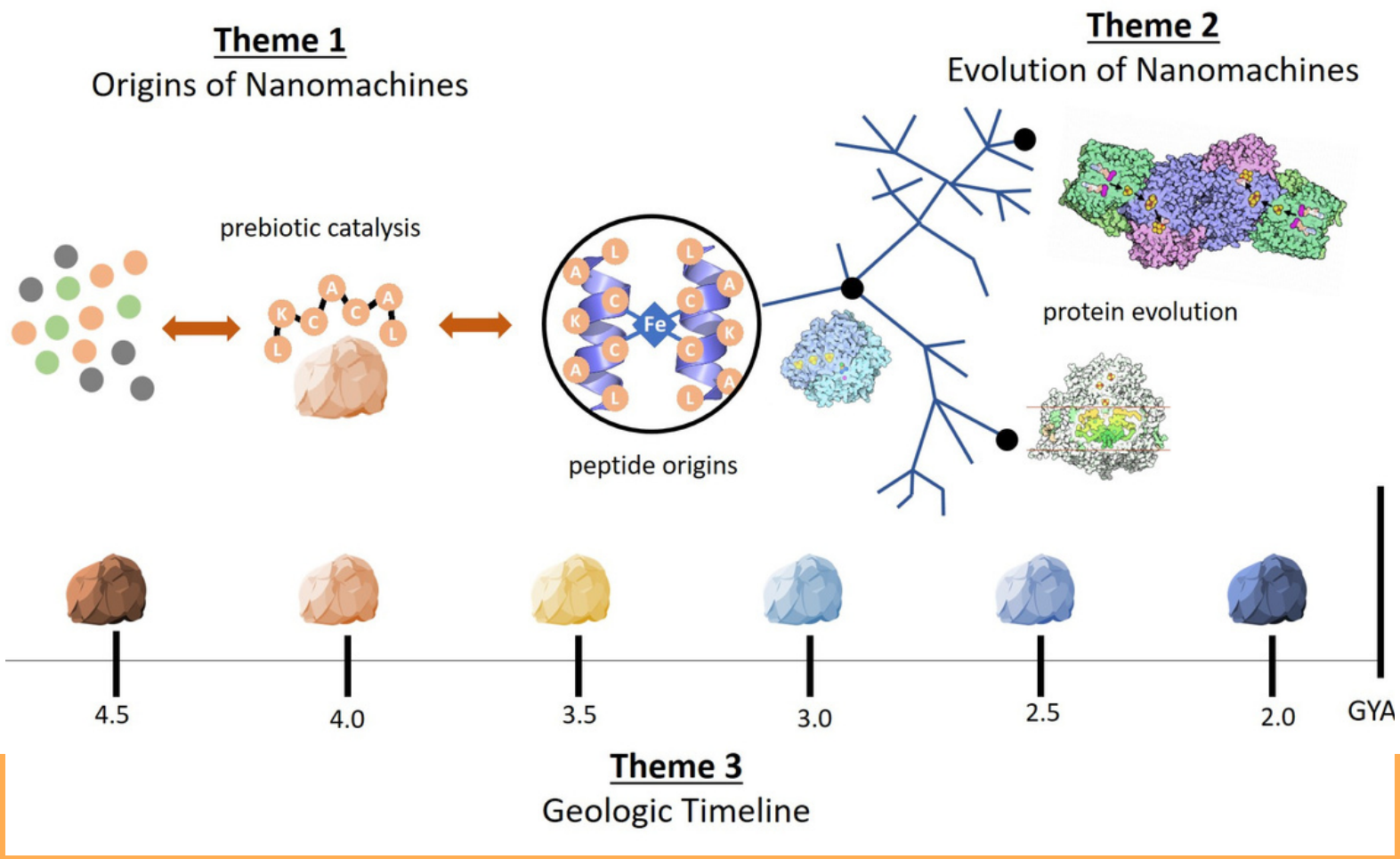
June 8-9, 2021

Tuesday 10:00am-3:00pm EDT

Wednesday 11:00am-3:00pm EDT

Virtual symposium to inform astrobiological research and help us to better understand the origins and evolution of life.

enigma.rutgers.edu



ENIGMA: Evolution of **N**anomachines **I**n **G**eospheres and **M**icrobial **A**ncestors. The NAI team explores catalysis of electron transfer reactions by prebiotic peptides to microbial ancestral enzymes to modern nanomachines, integrated over four and a half billion years of Earth's changing geosphere. Theme 1 focuses on the synthesis and function of the earliest peptides capable of moving electrons on Earth and other planetary bodies. Theme 2 focuses on the evolutionary history of "motifs" in extant protein structures. Theme 3 focuses on how proteins and the geosphere co-evolved through geologic time.

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Evolution of Nanomachines In Geospheres and Microbial Ancestors

Day 1 - June 8th

Time - EDT	Speaker	Title
10:00am-10:15am	Paul G. Falkowski, PI ENIGMA, Rutgers University	Welcome and Introduction
10:15am-10:30am	Dror Noy, Migal Galilee Research Institute	Dan Tawfik Memorial
10:30am-11:15am	Jeremy England, Georgia Institute of Technology	Self-Organization of Lifelike Behaviors
11:15am-12:00pm	Sara Walker, Arizona State University	Planetary Systems Biochemistry: Inferring the Laws of Life at a Planetary Scale
12:00pm-12:15pm	Aaron Martinez, Rutgers University	Biomarkers in the Geologic Record
12:15pm-12:30pm	Break	
12:30pm-1:15pm	Gonen Ashkenasy, Ben-Gurion University of the Negev	The Systems Chemistry of Peptide Networks
1:15pm-1:30pm	Douglas Pike, Rutgers University	A Protein Structure Sequence: Decoding the Deeper Structural Evolution of Proteins Upon Which Amino Acids Vary
1:30pm-2:15pm	Betul Kacar, University of Arizona	Between a Rock and a Living Place: A Molecular Paleobiology Approach to Explore Life's Origins and Early Evolution
2:15pm-2:30pm	Saroj Poudel, Rutgers University	Expansion of Positively Charged Cavities Enabled the Evolution of Substrate Specificity in Rubisco.
2:30pm-3:00pm	Open Discussion	

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Evolution of Nanomachines In Geospheres and Microbial Ancestors

Team Leaders

Paul G. Falkowski
PI ENIGMA Program
Bennet L. Smith Endowed Chair

Vikas Nanda
Theme 1
Professor
Center for Advanced Biotechnology and Medicine

Yana Bromberg
Theme 2
Associate Professor
Dept. of Biochememistry and Microbiology

Nathan Yee
Theme 3
Professor
Dept. of Environmental Sciences

Speakers

Jeremy England

Jeremy England is a Principal Research Scientist in the Department of Physics at the Georgia Institute of Technology. He serves as a Senior Director in Artificial Intelligence and Machine Learning at GlaxoSmithKline. From 2011 to 2019, he was Assistant and then Associate Professor in the Department of Physics at MIT, where he led a research group in studying the nonequilibrium statistical mechanics of life-like self-organization.

Abstract: Self-Organization of Lifelike Behaviors. Life is a multifarious bundle of distinct physical phenomena that are distinctive, but not unique to, living things. Self-replication, energy harvesting, and predictive sensing are three such phenomena, and each can be given a clear physical definition. In this talk, we will report recent progress in understanding what physical conditions are required for the spontaneous emergence of these various lifelike behaviors from assemblages of simple, interacting components.

Sara Imari Walker

Sara Walker is an astrobiologist and theoretical physicist interested in the origin of life and how to find life on other worlds. While there are many things to be solved, she is most interested in whether or not there are 'laws of life' - related to how information structures the physical world - that could universally describe life here on Earth and on other planets.

At Arizona State University she is Deputy Director of the Beyond Center for Fundamental Concepts in Science, Associate Director of the ASU-Santa Fe Institute Center for Biosocial Complex Systems and Associate Professor in the School of Earth and Space Exploration. She is also a member of the External Faculty at the Santa Fe Institute. She is active in public engagement in science, with appearances on "Through the Wormhole", the World Science Festival and NPR's Science Friday.

Abstract: Planetary Systems Biochemistry: Inferring the Laws of Life at a Planetary Scale. Currently, no general theory exists that explains what life is. While many definitions for life do exist, these are primarily descriptive, not predictive, and they have so far proved insufficient to explain the origins of life, or to provide rigorous constraints on what properties we might expect all examples of life to share (e.g., in our search for life in alien environments). In this talk I discuss new approaches to understanding what universal principles might explain the nature of life and elucidate the mechanisms of its origins, focusing on recent work in our group elucidating regularities and law-like behavior of biochemical networks on Earth from the scale of individual organisms to the planetary scale.

Aaron Martinez

Aaron Martinez
Post-doctoral Researcher
Rutgers University

Abstract: Biomarkers in the Geologic Record. Rocks record life. The evolutionary history of life on Earth is intimately tied to geologic processes through intricate and diverse biogeochemical cycles. Records of past geobiological processes are preserved within ancient rocks – sometimes faithfully, but often with artistic license. Rates of evolutionary change and leaps in organismal and ecological complexity vary enormously across geologic time, but profound restructuring of marine ecology has often been associated with episodes of major climatic and/or environmental change that have punctuated and reshaped the course of life on Earth. We use organic geochemical proxies such as lipid biomarkers and light stable isotopes to probe the dynamics of microbial communities during environmental perturbations. We aim to distinguish the primary drivers of both biological and environmental change, and their downstream manifestations, during transitional periods in Earth's history to better understand the past trajectory of life as well as inform the potential consequences of changing climates and environments on our planet today.

Gonen Ashkenasy

Gonen Ashkenasy conducted a PhD research in organic chemistry at the Weizmann Institute of Science (Israel), and postdoctoral research in peptide chemistry at the Scripps Research Institute (CA, USA).

Since 2006 he runs the *Systems Chemistry* laboratory at the Ben-Gurion University (Israel), focusing on the study of molecular replication networks as tools to develop and investigate complex chemical systems.. In 2011, he was promoted to tenured Associate Professor, and in 2015 to Professor of Chemistry. Ashkenasy has recently served as the chair of the Israel Society for Astrobiology and the Study of the Origin of Life (ILASOL; 2013-2016), vice-chair of the European COST action CM1304 focusing on the 'Emergence and Evolution of Complex Chemical Systems' (2013-2017), and co-chair of the first Gordon Research Conference on Systems Chemistry (2018). He is currently the Head of the Chemistry Department at Ben-Gurion University.

Abstract: The Systems Chemistry of Peptide Networks. Systems Chemistry aims to develop complex molecular networks showing emergent 'life-like' properties; i.e., properties that go beyond the sum of the characteristics of the individual constituents of the system. For more than a decade, our group has focused on the development of such systems using peptide-based replication networks. Three of the current challenges related to the design of complex chemical systems will be discussed in my talk: (i) evolving 'self-synthesizing materials' through utility of the replicating networks for various applications, such as catalysisand electron transfer,(ii) a search for synergism between molecules from different molecular families (Nucleic acid, Peptides, Sugars etc.) and (iii) design and analysis of chemical systems operating far-from-equilibrium by the incorporation of nonlinear processes and feedback loops. Future directions, involving the combination of these studies towards the formation of synthetic cells will be discussed.

Douglas Pike

Douglas Pike
Laboratory Researcher II
Rutgers University

Abstract: A Protein Structure Sequence: Decoding the Deeper Structural Evolution of Proteins Upon Which Amino Acids Vary. There exists no formal quantitative method of defining the phylogenetic relationships of proteins based solely on their structural similarity. Similarity which could predate much of amino acid based phylogenetics by hundreds of millions of years, where a relatively small set of stable fold motifs are diversified functionally by subsequent amino acid mutation. To address this, we propose a new method of quantitatively defining identical and similar protein structure by extracting and statistically comparing one of the most fundamental geometric scalar parameters of three-dimensional protein structure, the number of residues-per-turn. A particular number of residues-per-turn is a geometric constraint for all hydrogen bonding of protein structure and is proportional to all major geometric components of structure, rise, pitch and radius. By binning this parameter, we can reduce the dimensionality of protein structure into a sequence comprised of a new structural alphabet. Using this structural alphabet one can employ traditional phylogenetic sequence methods to trace protein structural relationships that existed prior to significant diversification of amino acid sequences. Additionally, using traditional sequence tools, consensus and ancestral structural motifs can be identified, then templated and modeled in three-dimensions for reconstruction of complete putative ancestral folds.

Betul Kacar

Betül Kaçar is an Assistant Professor of Astrobiology at the University of Arizona at the Departments of Astronomy, Lunar and Planetary Laboratory and Molecular and Cell Biology. She is also an associate professor at the Earth-Life Science Institute at the Tokyo Institute of Technology. She directs a NASA Astrobiology Research Center exploring the essential attributes of life, its origins and how they should shape our notions of habitability and the search for life on other worlds

Kaçar was named NASA Early Career Faculty Fellow in 2019. In 2020, she received the Scialog fellowship for her studies on life in the universe by the Research Corporation and Science Advancement. Kacar partnered with the 2020 UN Women Generation Equality Campaign to support education of girls and women globally and delivered a talk during the UN Women meeting for the Commission on the Status of the Women in 2021.

Abstract: Between a Rock and a Living Place: A Molecular Paleobiology Approach to Explore Life's Origins and Early Evolution. The geologic record and the genetic content of extant organism are two complementary datasets, providing insights into the history of how key biomolecules have shaped global environmental and macroevolutionary trends. Organismal survival depends on how well critical biological components can adapt to their environments, reflecting an ability to optimize efficiently to changing conditions. While the geologic record provides an array of biologically independent indicators of macroscale atmospheric and oceanic composition, it provides little in the way of the behavior of the biomolecules that influenced the compositions of such reservoirs. By reconstructing sequences of proteins that might have been present in ancient organisms, we can downselect and experimentally study a subset of possible variants that may have been optimized to these ancient conditions. In this talk, I will survey our group's efforts towards establishing a molecular paleobiology approach. I will further discuss how exploring the early evolution of ancient biomolecules, protein-cofactor interaction interfaces and metallosystems contribute to our understanding of origins and first life on Earth.

Saroj Poudel

Saroj Poudel
NASA Post-doctoral Researcher
Rutgers University

Abstract: Expansion of Positively Charged Cavities Enabled the Evolution of Substrate Specificity in Rubisco. Ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the most abundant enzyme on Earth that incorporates CO2 into photosynthetic metabolism. The enzyme predominantly catalyzes the addition of the primary substrate carbon dioxide (CO2) from the environment to ribulose 1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate (i.e., carboxylation), each of which subsequently is reduced to an aldehyde in the Calvin–Benson–Bassham cycle. However, its catalytic rate per molecule of protein is extremely slow and the binding of the primary substrate, CO2, is competitively displaced by O2. While the reaction with the former leads to the productive formation of organic carbon, reaction with the latter leads to a metabolically futile, but energetically costly pathway. Hence, carbon fixation by RuBisCO is highly inefficient; indeed, in higher C3 plants, about 30% of the time the enzyme mistakes CO2 for O2. Using genomic and structural analysis, we identify regions around the catalytic site that play key roles in discriminating between CO2 and O2. Our analysis identified positively charged cavities directly around the active site, which are expanded as the enzyme evolved with higher substrate specificity. The residues that extend these cavities have recently been under selective pressure, indicating that larger charged pockets are a feature of modern RuBisCOs, enabling greater specificity for CO2. This work will highlight key structural features that enabled the enzyme to evolve improved CO2 sequestration in an oxygen-rich atmosphere and discuss future work that may guide the engineering of more efficient RuBisCOs.

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Evolution of Nanomachines In Geospheres and Microbial Ancestors

Day 2 - June 9th

Time - EDT	Speaker	Title
11:00am-11:15am	Paul G. Falkowski, PI ENIGMA, Rutgers University	Welcome and Introduction
11:15am-12:00pm	Birte Hoecker, Bayreuth University	Evolution of Proteins from Subdomain-sized Fragments
12:00pm-12:15pm	Ariel Aptekmann, Rutgers University	Mebipred: Protein Sequence Metal-binding Prediction Web Server and Standalone Tool
12:15pm-12:30pm	Break	
12:30pm-1:15pm	Dennis Dean, Virginia Tech	Aspects of Nitrogenase Catalysis and Assembly
1:15pm-1:30pm	Corday Selden, Rutgers University	Tracing N_2 Fixation in the (Marine) Environment
1:30pm-2:15pm	Markus Ribbe, University of California, Irvine	Reactivities of Isolated Nitrogenase Cofactors
2:15pm-2:30pm	Jennifer Timm, Rutgers University	Reconstructing a Primordial Hydrogenase
2:30pm-3:00pm	Open Discussion	

Birte Hoecker

Birte Höcker is Professor of Biochemistry at Bayreuth University (Germany). She studied biology in Göttingen and at Carleton University in Ottawa (Canada). She earned her Ph.D. in biochemistry from the University of Cologne (Germany). 2003 - 2005 she worked as a postdoctoral fellow at Duke University in Durham, NC (USA) on computational protein design. In 2006 she started her own research group at the Max Planck Institute for Developmental Biology in Tübingen (Germany) investigating protein evolution and design. Her lab relocated in 2016 to the University of Bayreuth where she became a full professor.

Her main research interests are the evolution and design of protein folds and functions. For her work on the evolution of enzymes she was awarded the Biology price of the Akademie der Wissenschaften zu Göttingen In 2010. In 2015 she received the Otto-Meyerhof-Award of the German Society for Biochemistry and Molecular Biology (GBM). The same year she was awarded a prestigious ERC Consolidator grant to work on the evolution of proteins from subdomain-sized fragments. In 2020 now she and her colleagues Heiner Linke and Paul Curmi received an ERC Synergy grant to design autonomous motor proteins from non-motor parts thereby exploring how complex protein functions can develop from simpler building blocks.

Abstract: Evolution of Proteins from Subdomain-sized Fragments. Nature has generated an impressive diversity of proteins using mechanisms such as recombination of smaller, sub-domain sized protein fragments that serve as building blocks in a Lego-like manner. We want to understand how these diverse structures and functions evolved and how we can apply observed mechanism to problems of protein design. Using bioinformatics tools we identify possible evolutionary links between protein folds and use this knowledge to construct hybrid proteins from subdomain sized fragments, thereby establishing a new design approach based on fragment recruitment. At the same time, our approach offers a rigorous test for the identification of minimal determinants of protein structure and function and allows us to test our understanding of protein evolution.

Ariel Aptekmann

Ariel Aptekmann
Post-doctoral Researcher
Rutgers University

Abstract: Mebipred: Protein Sequence Metal-binding Prediction Web Server and Stand-alone Tool. Metal-binding proteins have a central role in maintaining life processes. Nearly one-third of known protein structures contain metal ions that are used for a variety of needs, such as catalysis, DNA/RNA binding, protein structure maintenance, etc. Identifying metal-binding proteins is thus crucial for our understanding of mechanisms of cellular activity. However, experimental annotation of protein metal-binding activity is severely lacking, while computational techniques are imprecise and of limited applicability.

We developed a novel machine learning-based method, "mebipred", for identifying metal-binding proteins from sequence-derived features. Our method is nearly 90% accurate in identifying proteins that bind ten ubiquitously present, metal-containing ligands. Mebipred is reference-free, i.e. no alignments are involved, and outperforms other prediction methods, both in speed and accuracy. It can also identify protein metal-binding capabilities from short peptide stretches. Thus, it may be useful in annotation of microbiome metal requirements inferred from translated sequencing reads. In our analysis of available microbiome data, for example, we found that ocean and soil microbiomes use a more diverse set of metals than human host-related ones. For human microbiomes, physiological conditions explain the observed metal preferences, while subtle changes in ocean sample ion concentration affect the abundance of metal-binding proteins. In a similar manner, the gradients in Iron and Magnesium concentration associated with changes in depth on Black Sea metagenomic samples, correlate with changes in the number of proteins predicted to bind those ions. These results, in line with our expectation, show mebipred can give meaningful information on the metal requirements of microbiomes.

Dennis Dean

Dennis R. Dean is a professor of Biochemistry in the College of Agricultural and Life Sciences and has been a member of the Virginia Tech faculty for 36 years.

Dean attended Wabash College (B.A. 1973) and is a Purdue University College of Science Distinguished alumnus (Ph.D. 1979). He was an NIH Post-Doctoral Fellow at the University of Wisconsin and a Staff Scientist at the Kettering Research Laboratory before joining the Virginia Tech faculty in 1985. He is the founding director of the Virginia Tech Fralin Life Science Institute and holds the titles of University Distinguished Professor and Stroobants Professor of Biotechnology.

Abstract: Aspects of Nitrogenase Catalysis and Assembly. Nitrogenase is the only catalytic component of biological nitrogen fixation and there are three genetically distinct but mechanistically similar nitrogenase types. The various nitrogenases are designated as Mo-dependent, V-dependent and Fe-only, a nomenclature that reflects the metal compositions of their active sites. In this presentation, mechanistic features that are common to all three nitrogenase systems, including the role of the central carbide contained within all three nitrogenase active site types, will be summarized. Genetic studies that have explored the role of molecular scaffolds and a recently discovered "gatekeeper" protein involved in controlling active site cofactor distribution will also be discussed.

Corday Selden

Corday Selden
Post-doctoral Researcher
Rutgers University

Abstract: Tracing N2 Fixation in the (Marine) Environment. Biological dinitrogen (N2) fixation represents the primary supply of bioavailable nitrogen for primary production and, consequently, plays an important role in global climate. I will discuss how our expanding understanding of diazotroph (N2 fixer) diversity affects our view of the factors that regulate this important process, and thus nitrogen homeostasis, on a global scale in the modern ocean. I will close with a note regarding how isotope metallomics may help use trace nitrogen metabolism through deep time.

Markus Ribbe

Markus Ribbe is the Chancellor's Professor at the Departments of Molecular Biology & Biochemistry, and Chemistry at the University of California, Irvine. He is an elected fellow of the American Academy of Microbiology and American Association for the Advancement of Science.

The focus of Ribbe's research is the assembly and mechanism of nitrogenase, one of the most complex metalloenzymes known to date. Nitrogenase can be appreciated from the perspective of the useful agricultural and industrial products it generates, namely, ammonia, hydrogen and hydrocarbons. Since the beginning of his independent career, Ribbe has focused his efforts on investigating the biosynthesis of the Mo-nitrogenase from *Azotobacter vinelandii* and, in particular, the unique metal centers of its MoFe protein component: FeMoco and P-cluster. Results of these studies have firmly established nitrogenase MoFe protein as a model system that could be used to deduce the general mechanism of metal cluster assembly and develop successful strategies for synthesizing bio-inspired catalysts for industrial usage. More recently, Ribbe expanded his research to the investigation of the structure and function of the "alternative" nitrogenases from *A. vinelandii* and nitrogenases from other organisms. His group discovered that V-nitrogenase can convert CO and CO2 to hydrocarbons under ambient conditions, and that this reactivity is extended to the isolated nitrogenase cofactors, cofactor variants and synthetic cofactor mimics in an ATP-independent manner. These findings provide a potential blueprint for developing cost-efficient processes for industrial production of biofuels in the future.

Abstract: Reactivities of Isolated Nitrogenase Cofactors. Isolated nitrogenase cofactors can reduce CO, CN-, and CO2 to short-chain hydrocarbons in the presence of a strong reductant. Isotope-labeling experiments demonstrate reductive condensation of formaldehyde and acetaldehyde into alkanes and alkenes, as well as condensation of aldehydes with CO, by isolated nitrogenase cofactors, pointing to aldehyde-derived species as possible intermediates of nitrogenase-catalyzed CO reduction. Deuterium-labeling experiments further suggest formation of a cofactor-bound hydroxymethyl intermediate upon activation of formaldehyde, as well as release of C2H4 upon β-hydride elimination of an acetaldehyde-derived hydroxyethyl intermediate. These findings establish reductive condensation of aldehydes as a previously unobserved reactivity of a biogenic catalyst while shedding light on the mechanism of the enzymatic CO reduction and C-C bond formation.

Jennifer Timm

Jennifer Timm
Post-doctoral Researcher
Rutgers University

Abstract: Reconstructing a Primordial Hydrogenase. Today, metabolism and catalysis in every organism is carried out by a complex set of proteins. However, at the beginning when life emerged and abiotic processes transition to biotic ones, the proteins and molecules involved must have been a lot simpler than what we find today. While presumably simpler than today, the fundamental chemical and physical processes are most likely highly similar if not identical. One of the most ancient processes for energy production and transfer in living organisms is the reversible oxidation of molecular hydrogen, a molecule highly abundant in early earth's atmosphere. The protein catalyzing this reaction is hydrogenase. Simple, primordial versions of hydrogenase are likely to have occurred very early on in the emergence of life and our work is aimed to reconstruct a simple peptide molecule that could have been at the very beginning of hydrogenase evolution. We designed a peptide based on the chemical requirements necessary to emulate a hydrogenase active site, binding Nickel ions. We report the successful design of a nickel-binding peptide capable of robustly producing molecular hydrogen from protons under a wide variety of conditions. Biophysical and structural investigation strongly indicate the peptide to form a di-nickel cluster analogous to Acetyl-CoA synthase, an ancient protein central to metabolism in all life forms. This could indicate the ability to catalyze multiple different reactions and supports the hypothesis that early life forms might have started off using simpler peptide-based molecule generalists.