Frontiers in Metallobiochemistry

June 7-10, 2006
Penn State’s University Park Campus
State College, Pennsylvania

An outreach program of the Eberly College of Science
25th Summer Symposium
In Molecular Biology
June 7-10, 2006

"Frontiers in Metallobiochemistry"

Program and Abstracts

Berg Auditorium
Life Sciences Building
The Pennsylvania State University
University Park Campus
NOTES

About the Symposium

The Summer Symposium in Molecular Biology is an autonomous faculty program administered by the Department of Biochemistry and Molecular Biology at Penn State University. This internationally recognized program follows these symposium objectives:

- Broaden the body of knowledge relating to the symposium theme
- Provide a vehicle for information exchange and technology transfer between Penn State and other academic and industrial communities
- Provide an internationally recognized forum for molecular biology and biotechnology research and education, financially accessible to undergraduate and graduate students, and post-doctoral scholars

The roster of distinguished keynote speakers who have addressed past symposia includes: Alexander Rich, David Baltimore, Paul Berg, Charles Yanofsky, Joseph Gall, Seymour Benzer, Robert Gallo, Luc Montagnier, Arthur Kornberg, J Michael Bishop, Jeff Schell, Phillip Sharp, Gregory Petsko, Peter Dervan, Robert Horvitz, Christopher Walsh, David Feltz, George Rose, Thomas Shenk, Ron Evans, C. David Allis, Stuart Orkin, and Harald von Boemer. Each year a program is developed relative to current research directions in the medical and molecular biological sciences. Since 1982 the symposium has addressed a broad range of research topics, including: oncogenes, DNA protein interactions, AIDS, neurobiology, nuclear structure, microbial differentiation, plant/bacteria symbiosis, regulation of gene expression, cell cycle control, growth factors and receptors, transgenic expression, molecular interactions in plant development, cell growth and regulation; structure/function in proteins and enzymes, molecular mechanisms of toxicity, chromosomal controls of gene expression, apoptosis, microbial structural biology, immune-neuro endocrine interactions, chromatin structure and DNA function, protein and RNA folding problems, emerging viral diseases, xenobiotic receptors in toxicology and carcinogenesis, hematopoiesis and immune cell function, and comparative and functional genomics.

The wealth of genome sequence data presents unprecedented opportunities and formidable challenges. Biologists must deduce function, but they need bioinformatic tools to separate the important sequences from the less critical ones. Comparative methods are a front-rank approach to this problem. We invite you to attend our annual symposium, which this year will explore the evolutionary methods driving comparative genomics and the functional elements revealed by them, including protein families. Integration of this information by systems-based approaches to better understand physiology will also be featured.
NOTES

Program Sponsors
The Penn State Faculty of the Summer Symposium in Molecular Biology wishes to acknowledge and thank the University and industry sponsors of the 2005 symposium:

Penn State University Park Sponsors:
Department of Veterinary and Biomedical Sciences
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Dr. James L. McDonel (Biochemistry and Molecular Biology)

Symposium Coordinators:
Betsy R. King (Biochemistry and Molecular Biology)
Carolyn Andersen (Conferences and Institutes)

Symposium Conveners:
Dr. Squire Booker (Associate Professor of Biochemistry and Molecular Biology)
Dr. J. Martin Bollinger, Jr. (Associate Professor of Biochemistry and Molecular Biology, Associate Professor of Chemistry)

Industry Exhibits:
Tom Rodgers (VWR International, Inc.)
General Information:

Breaks will be held in the Life Sciences building unless otherwise noted.

Preparation for speakers will be held:

In the Life Sciences Building, Berg Auditorium. Powerpoint presentations can be given to the session chair for loading into the Mac or PC computer on the stage.

Invited speaker presentations will be strictly limited to 35 minutes total, with a recommendation of 30 minutes for the presentation and 5 minutes for questions and discussion.

Thursday, June 8, 2006

Industrial Exhibits – Life Sciences Building, Verne M. Willaman Gateway

NOTE - Set-up for industrial exhibits will be on Wednesday, June 7, from 7:00 p.m. - 9:00 p.m.

Over forty industrial exhibitors will be presenting their latest equipment and supplies.

The exhibits will be open from 10:00 a.m. until 3:00 p.m. on Thursday, June 8.

A boxed lunch will be provided between Noon and 1:30 p.m. Thursday in the in the Verne M. Willaman Gateway of the Life Sciences Building to all participants. It is advised that you try to pick up your boxed lunch promptly at noon since the number of lunches is limited.

Special Thanks to VWR for co-organizing the industrial exhibits. VWR can be found on the Internet at http://www.vwr.com.

Friday, June 9, 2006

Poster Presentations – Life Sciences Building, Verne M. Willaman Gateway

7:30 p.m. – 10:00 p.m.

Posters are to be set up for the entire poster session on Friday evening. Each Poster has been assigned a number. Check for your poster presentation number in this proceedings book by looking up your name in the author index in the back of the book and then going to the page where your abstract is found. The poster number is given by your abstract. Set up of posters in
the Life Sciences Building, Verne M. Willaman Gateway can be done Friday evening, between 6:30 p.m. and 7:30 p.m.

Removal of posters is to be immediately following the evening poster session. Posters must be removed from the Life Sciences Building, Verne M. Willaman Gateway promptly at or before 10:00 p.m. Friday, June 9, 2006.

Program Schedule

Penn State's Twenty-fifth Summer Symposium in Molecular Biology

"Frontiers in Metallobiochemistry"

Berg Auditorium
Life Sciences Building
The Pennsylvania State University
Wednesday, June 7, 2006

5:30 p.m. - 7:00 p.m. Registration (Berg Auditorium Foyer)

6:15 p.m. - 7:45 p.m. Reception (Berg Auditorium Foyer)

8:00 p.m. - 8:15 p.m. Opening Remarks (Drs. Booker and Bollinger)

8:15 p.m. - 9:00 p.m. Evening Lecture (Berg Auditorium)
   Structure/function correlations over non-heme iron enzymes
   Edward Solomon, Ph.D. (Stanford)

Thursday, June 8, 2006

7:30 - 9:30 a.m. Registration for those not yet registered (Berg Auditorium Foyer)

8:30 a.m. - Noon Session I (Berg Auditorium)
   Microbial Systems
   Chair: Juliette Lecomte (Penn State)

8:30 a.m. - 9:15 a.m. Regulation of iron-sulfur cluster biosynthesis in Cyanobacteria
   John H. Golbeck, Ph.D. (Penn State)

9:15 a.m. - 10:00 a.m. The microbe electric: Nanowires, capacitors, and other proteins involved in extracellular electron transfer to metals and electrodes in Geobacter
   Derek Lovley, Ph.D. (University of Massachusetts)

10:00 a.m. - 10:30 a.m. Morning Break

10:00 a.m. - 3:00 p.m. Industrial Exhibits (Life Sciences Building, Verne M. Willaman gateway)

10:30 a.m. - 11:15 a.m. Enzymatic halogenation catalysts
   Christopher T. Walsh, Ph.D. (Harvard Medical School)

11:15 a.m. - 12:00 noon The active site of hydrogenases
   Rudolf Thauer, Ph.D. (Max Planck institute)

12:00 noon - 2:00 p.m. Lunch Break

Thursday, June 8, 2006 (continued)

2:00 p.m. - 5:30 p.m. Session II (Berg Auditorium)
   Complex Cofactors and Novel Reactivity
   Chair: Carsten Krebs (Penn State)

2:00 p.m. - 2:45 p.m. The unusual heme of cystathionine beta-synthase
   Judith Burstyn, Ph.D. (University of Wisconsin)

2:45 p.m. - 3:30 p.m. Beyond the ligands: the role of the second coordination shell in modulating the electronic structure and reactivity of copper sites in proteins
   David Dooley, Ph.D. (Montana State University)

3:30 p.m. - 4:00 p.m. Afternoon Break

4:00 p.m. - 4:45 p.m. Acetyl-CoA synthase/carbon monoxide dehydrogenase: A bifunctional Ni-Fe-S-containing enzyme with an organometallic reaction mechanism
   Paul Lindahl, Ph.D. (Texas A&M University)

4:45 p.m. - 5:30 p.m. The mechanism of reductive cleavage of S-adenosylmethionine to initiate a radical reaction: the case of lysine 2,3-aminomutase
   Perry A. Frey, Ph.D. (University of Wisconsin)

5:30 p.m. - 7:30 p.m. Program Break

7:30 p.m. - 9:00 p.m. Evening Lecture (Berg Auditorium)
   Intermediates along the nitrogenase reaction pathway
   Lance Seefeldt, Ph.D. (Utah State University)

Friday, June 9, 2006

8:30 a.m. - Noon Session III (Berg Auditorium)
   Metalloprotein Synthesis
   Chair: Ann Valentine (Yale University)

8:30 a.m. - 9:15 a.m. The active-site metal centre of [NiFe]-hydrogenases: Synthesis and assembly
   August Böck, Ph.D. (University of Munich)
9:15 a.m. – 10:00 a.m.  Role of Sco1/Sco2 in the assembly of the CuA site in cytochrome oxidase
Dennis Wings, Ph.D. (University of Utah)

10:00 a.m. – 10:30 a.m.  Morning Break

10:30 a.m. – 11:15 a.m.  Copper trafficking in a hyperthermophile
Amy Rosenzweig, Ph.D. (Northwestern University)

11:15 a.m. – 12:00 noon  Transporting copper across membranes in yeast and mammals
Dennis Thiele, Ph.D. (Duke University Medical Center)

12:00 noon – 2:00 p.m.  Lunch Break

2:00 p.m. – 5:30 p.m.  Session III (Berg Auditorium)
Oxygen Activation
Chair: Michael Green (Penn State)

2:00 p.m. – 2:45 p.m.  Risky business of Rieske Oxygenases
S. Ramaswamy, Ph.D. (University of Iowa)

2:45 p.m. – 3:30 p.m.  How proteins control electrons: Protons
Anne-Frances Miller, Ph.D. (University of Kentucky)

3:30 p.m. – 4:00 p.m.  Afternoon Break

4:00 p.m. – 4:45 p.m.  Oxygen activation by a mixed-valent diiron cluster and C-H cleavage by an iron-superoxide intermediate in the glycol cleavage reaction catalyzed by myo-inositol oxygenase
J. Martin Bollinger, Jr., Ph.D. (Penn State)

4:45 p.m. – 5:30 p.m.  Substrate binding, electron transfer, and O2 activation in Rieske dioxygenases: Three sides of the same coin
John Lipscomb, Ph.D. (University of Minnesota)

5:30 p.m. – 7:30 p.m.  Program Break

7:30 p.m. – 10:00 p.m.  Poster Presentations (Verne M. Willaman Gateway)

7:30 p.m. – 10:00 p.m.  Concurrent Poster Reception (Verne M. Willaman Gateway)

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Saturday, June 10, 2006

8:30 a.m. – Noon  Session V (Berg Auditorium)
Methods in Metalloenzymology
Chair: Carsten Krebs (Penn State)

8:30 a.m. – 9:15 a.m.  Ribonucleotide reductases: something for everyone
JoAnne Stubbe, Ph.D. (MIT)

9:15 a.m. – 10:00 a.m.  Spectroscopic characterization of radical intermediates in enzymic reactions
George H. Reed, Ph.D. (University of Wisconsin)

10:00 a.m. – 10:30 a.m.  Morning Break

10:30 a.m. – 11:15 a.m.  Site-specific chemistry at a unique Fe site of the FeS1 cluster in ferredoxin: thioredoxin reductase
Vincent Huynh, Ph.D. (Emory University)

11:15 a.m. – 12:00 noon  Electrochemical enzymology: Probing dynamics and mechanism at an electrode
Sean J. Elliot, Ph. D. (Boston University)

12:00 noon  Symposium Closing Remarks
NOTES

**Invited Speakers**

**Evening Lecture**  
Wednesday, June 7, 2006

Edward Solomon  
Monroe E. Spaght Professor of Chemistry  
Department of Chemistry  
Stanford University  
S G MUDD, Rm. 141  
Stanford, California, 94305-5080

**Session I**  
**Microbial Systems**

John Golbeck  
Professor  
Department of Biochemistry and Molecular Biology, and  
of Chemistry  
The Pennsylvania State University  
310 South Frear Laboratory  
University Park, PA. 16802

Derek Lovley  
Distinguished University Professor  
Department of Microbiology  
University of Massachusetts  
203 Morrill Science Center IVN  
639 North Pleasant Street  
Amherst, MA 01003

Christopher T. Walsh  
Hamilton Kuhn Professor  
Department of Biological Chemistry and Molecular  
Pharmacology  
Harvard Medical School  
240 Longwood Ave.  
Boston, MA. 02115

Rudolf Thauer  
Professor  
Max Planck Institute für terrestrische Mikrobiologie  
Philips-Universität Marburg  
Karl-von-Frisch-Straße  
D-35043 Marburg / Germany
Session II

Complex Cofactors and Novel Reactivity

Judith Burstyn
Professor
University of Wisconsin-Madison
5327a Chemistry (Farrington Daniels Wing)
1101 University Ave.
Madison, WI 53706

David M. Dooley
Department of Chemistry and Biochemistry
Montana State University
108 Gaines Hall
Bozeman, MT 59717

Paul Lindahl
Professor
Department of Chemistry
Texas A&M University
College Station, TX 77842

Perry A. Frey
Robert H. Abeles Professor of Biochemistry
University of Wisconsin–Madison
546B Enzyme Institute
1710 University Ave.
Madison, WI 53726-4087

Evening Lecture
Thursday, June 8, 2006

Lance Seefeldt
Professor
Department of Chemistry and Biochemistry
Utah State University
0300 Old Main Hill
Logan, UT 84322-0300

Session III

Metalloprotein Synthesis

August Böck
Professor
University of Munich
Department Biologie I
Maria-Ward-Strasse 1a
D-80638 Munich, Germany

Dennis Winge
Professor
Department of Biochemistry
University of Utah
4C416 SOM
30 North, 1900 East
Salt Lake City UT 84132-2408

Amy Rosenzweig
Irving M. Klotz Research Professor of Chemistry
Department of Chemistry and of Biochemistry, Molecular Biology and Cell Biology
Northwestern University
Cook Hall 4137
2220 Campus Dr.
Evanston, IL 60208

Dennis Thiele
Professor
Department of Pharmacology and Cancer Biology
Duke University Medical Center
C351 LSRC
Box 3813
Durham, NC 27710

Session IV

Oxygen Activation

S. Ramaswamy
Associate Professor
Department of Biochemistry
University of Iowa
51 Newton Road
4-611 BSB
Iowa City, Iowa 52242

Anne-Frances Miller
Associate Professor
Department of Chemistry–Physics Building
113 Chemistry Building
University of Kentucky
Lexington, KY 40506-0055

J. Martin Bollinger, Jr.
Associate Professor
Department of Biochemistry and Molecular Biology and of Chemistry
The Pennsylvania State University
208 Althouse Laboratory
University Park, PA 16802
Session V

Methods in Metalloenzymology

JoAnne Stubbe
Novartis Professor of Chemistry
Department of Chemistry and of Biology
Massachusetts Institute of Technology
Room 18-598
77 Massachusetts Ave.
Cambridge, MA. 02139-4307

George H. Reed
Professor
Department of Biochemistry
University of Wisconsin–Madison
Room 464 Enzyme Institute
1710 University Ave.
Madison, WI. 53726-4087

Vincent Huynh
Samuel Candler Dobbs Professor
Department of Physics
Emory University
N226 Mathematics and Science Center Building
400 Dowman Drive
Atlanta, GA. 30322-2430

Sean J. Elliot
Assistant Professor
Department of Chemistry
Boston University
590 Commonwealth Ave.
Boston, MA 02215

Invited Speaker Abstracts
Structure/function correlations over non-heme iron enzymes

Edward I. Solomon; Department of Chemistry, Stanford University, Stanford, CA 94305, USA
E-mail: edward.solomon@stanford.edu

Non-heme iron active sites are found in a wide range of enzymes which perform different biological functions requiring dioxygen. These reactions often involve dioxygen activation by a ferrous active site which is generally difficult to study with most spectroscopic methods. A new spectroscopic methodology has been developed utilizing variable temperature, variable field magnetic circular dichroism (VTVH MCD) which enables one to obtain detailed insight into the geometric and electronic structure of the non-heme ferrous active site and probe its reaction mechanism on a molecular level. This spectroscopic methodology will be presented and applied to a number of key mononuclear non-heme iron enzymes leading to a general mechanistic strategy for O₂ activation. These studies will then be extended to consider the new features present in the binuclear non-heme iron enzymes. The nature of the oxygen intermediates will be considered relative to those of heme enzymes and general electronic structure/reactivity correlations for O₂ activation by non-heme iron enzymes will be discussed.
Session I

Microbial Systems
Regulation of iron-sulfur cluster biosynthesis in Cyanobacteria

John H. Golbeck; Department of Biochemistry and Molecular Biology, Department of Chemistry, The Pennsylvania State University, University Park, PA 16802
Tel: 1 814 865 1163; Fax: 1 814 863 7024; e-mail: jhg5@psu.edu

Research Objectives: As oxygenic phototrophs, cyanobacteria are voracious consumers of iron due to the large number of membrane-bound photosynthetic reaction centers, Photosystems I and II. Photosystem I alone incorporates three [4Fe-4S] clusters (F_{x}, F_{y} and F_{z}), and services at least four molecules of soluble plant-type ferredoxin (which contain [2Fe-2S] clusters) during electron transfer, for a total contingent of 20 iron and sulfur atoms per reaction center. The need to assemble and maintain a large number of iron-sulfur clusters in an oxygen-saturated environment during growth, metabolism, and reproduction leads to the following questions: Is Suf or Isc the indispensable iron-sulfur cluster assembly system in cyanobacteria? Are the Suf and Isc regulons in cyanobacteria governed by the same genetic control elements as in Escherichia coli? Is SufA, IscA or Nfh (which is similar to the C-terminal domain of NifU) the essential iron-sulfur cluster scaffold in cyanobacteria? Given its special status, are additional factors required to assemble the interpolypeptide F_{y} cluster in Photosystem I?

Research Results: The experimental approach involves a mechanistic analysis of the suf and isc regulons, and a search for transcriptional regulators based on the isolation of suppressor mutants. Deletion mutagenesis shows that unlike in E. coli, iscS1, iscS2, iscA and fdx are dispensable in Synechococcus sp. PCC 7002, whereas sufB, sufC, sufD, sufS and sufE are required. This fits well with the finding that in eukaryotes, the isc system is located in the relatively anoxic environment of mitochondria, whereas the Suf system is located in the oxic environment of chloroplasts. An open reading frame named sh0088 was identified in Synechocystis sp. PCC 6803 that rescues photosynthetic competence in a psaC variant in which a serine replaced a cysteine as a ligand to the F_{y} iron-sulfur cluster. This gene, which has been seen since named sufR, is located directly upstream of the conserved sufBCDS operon in all cyanobacterial genomes sequenced to-date. The SufR protein has a predicted N-terminal domain that contains a helix-loop-helix motif characteristic of DNA-binding proteins, and a C-terminal domain that contains four conserved cysteine residues that provides ligands to a [4Fe-4S] cluster. SufR has been shown to function as a transcriptional repressor to regulate the expression of the suf regulon in a manner similar to the function of IscR in the isc regulon in E. coli. Interestingly, sufR is absent in E. coli, and iscR is absent in cyanobacteria. SufA, IscA have been proposed to function as alternative scaffolds in the assembly of FeS clusters in bacteria. Demonstrating their non-essential nature in cyanobacteria, the sufA, iscA, and sufA/iscA variants grew on light with doubling times that were similar to the wild type. In contrast, attempts to inactivate the nfu gene only resulted in stable merodiploids. These results imply that Nfu, and not SufA or IscA, is likely the essential FeS scaffold protein. Under iron-limiting conditions, the iscA and sufA deletions grew better than the wild-type, consistent with a function in signalling the status of iron-sulfur clusters rather than assembling iron-sulfur clusters. A membrane-bound rubredoxin, RubA, was found to be indispensable for the assembly of the F_{x} cluster in Photosystem I. Its function is obscure, although one hint is provided by the fact that F_{x} must be assembled in growing cells undergoing vigorous oxygenic photosynthesis. One intriguing possibility is that rubredoxin guarantees a proper redox environment for the assembly of the F_{x} cluster by shunting electrons from the preceding low potential (-730 mV vs H_{2}) A_{1} quinone to an alternative electron acceptor.
The microbe electric: Nanowires, capacitors, and other proteins involved in extracellular electron transfer to metals and electrodes in *Geobacter*

Derek Lovley; Department of Microbiology, UMASS-Amherst

The hallmark physiological characteristic of *Geobacter* species is their ability to transfer electrons onto extracellular electron acceptors such as Fe(III) oxides, humic substances, and electrodes. Unlike other well-studied Fe(III)-reducing microorganisms, *Geobacter* species do not release compounds that can serve as electron shuttles between the cell surface and the surface of insoluble electron acceptors. This suggests that they must directly transfer electrons onto the insoluble electron acceptors. This unique ability is thought to be account for the fact that *Geobacteraeae* are the predominant Fe(III)-reducing microorganisms in a variety of sedimentary environments and in aquifers undergoing anaerobic bioremediation as well as on the surface of electrodes harvesting electricity from waste organic matter. Initial studies on the mechanisms for extracellular electron transfer in *Geobacter sulfurreducens* focused on the role of c-type cytochromes. These cytochromes are very abundant in the cells as is visually apparent from the red color of cultures. Furthermore, there are more than 100 c-type cytochromes in the genomes of the *Geobacter* species that have been sequenced. Most of the cytochromes are multiheme with 2-27 predicted heme groups and are located in the periplasm or outer membrane. Genetic studies have identified inner-membrane, periplasmic, and outer-membrane cytochromes that are essential for optimal Fe(III) reduction in *G. sulfurreducens*. Although a number of these cytochromes are considered to function primarily in electron transfer to Fe(III), several outer-membrane cytochromes appear to impact on transcription of other cytochrome genes whereas other impact on translation of the gene transcripts. Proteomic analysis of a mutant deficient in a general secretory pathway identified a putative multi-copper protein that accumulates in the periplasm of the secretary mutant. A mutant deficient in the multi-copper protein lacked the ability to reduce insoluble Fe(III) and Mn(IV) oxides, but readily reduced soluble electron acceptors, including chelated Fe(III). Biochemical and genomic analysis of *Pelobacter* species, which are phylogenetically intertwined with cytochrome-rich members of the *Geobacteraeae*, demonstrated that they lacked the abundant c-type cytochromes found to be essential for Fe(III) oxide reduction in *G. sulfurreducens*, yet *Pelobacter carbinolicus* was capable of Fe(III) oxide reduction. These results led to a search for other proteins exposed on the outer surface of the cell that might be serve as the final conduit between cells and Fe(III) oxide. Comparison of *Geobacter* grown on soluble, chelated Fe(III) versus cells grown on Fe(III) oxide revealed that the cells specifically expressed pili and flagella during growth on Fe(III) oxide. The flagella are necessary for motility related to a novel chemotaxis to iron. Deletion of the gene for PilA, the structural protein for the pili, inhibited Fe(III) oxide reduction, reintroduction of the gene restored pilin formation and Fe(III) oxide reduction. Fe(III) oxides appeared to specifically associate with the pili. Atomic force microscopy of the pili with a conducting tip indicated that the pili were electrically conductive. These results suggest that pilus are the final conduit for electron transfer from the cell to Fe(III) oxides. The pili are also required for maximal power production in microbial fuel cells under conditions in which thick (>50 μm) biofilms form on the anode surface. These results indicate that the pili are also involved in long-range electron transfer to anode surfaces in microbial fuel cells. In summary, the study of electron transfer to extracellular electron acceptors in *Geobacter* species is clearly in its infancy, but it is clear that a relatively sophisticated complex of proteins is involved in this process.
Enzymatic halogenation catalysts

Christopher Walsh; Harvard Medical School

More than 4500 natural products contain carbon-halogen bonds, biased towards organochlorine and organobromine linkages. Two categories of oxidative enzymes are known to activate and install halogens on cosubstrate scaffolds. The first comprises haloperoxidases which use hydrogen peroxide to generate -OCI or -OBr equivalents by way of heme iron cofactors or vanadate cofactors in the haloperoxidase active sites. The second category are \( \text{O}_2 \)-dependent halogenases that reduce \( \text{O}_2 \) during halide oxidation. A large set of such halogenases use the dihydroflavin coenzyme FADH\(_2\). These halogenate electron-rich substrates at aromatic and heteroaromatic rings. A second set of biosynthetic halogenases work at unactivated methyl groups of cosubstrates and are nonheme iron enzymes that use \( \text{O}_2 \) and \( \alpha \)-ketoglutarate as cosubstrates. Mechanistic studies of these two sets of \( \text{O}_2 \)-consuming halogenases will be presented.
The active site of hydrogenases

Rudolf K. Thauer; Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Straße, D-35043 Marburg, Germany

Hydrogenases are enzymes that catalyze the production or utilization of H₂. There are three types known to date, the [NiFe]-hydrogenases, the [FeFe]-hydrogenases and the [Fe]-hydrogenases. The three enzyme types are phylogenetically not related but have an unusual structural feature in common. Their active site iron contains CO as natural ligand, which is not found in any other metalloenzymes. This common feature is a sign for convergent evolution indicating that the catalytic mechanism of hydrogen activation in the different hydrogenases is probably similar and that therefore one can learn from one of the enzyme for the others.

The crystal structures of only two of the three hydrogenase types have been reported. The [NiFe]-hydrogenases, which are found in bacteria and archaea, are composed of at least two different subunits, a larger one harboring a binuclear [NiFe] active site center with two cyanide and one CO molecules bound to the iron, and a smaller one harboring iron-sulfur clusters. The [FeFe]-hydrogenases, which are found in bacteria and eucarya, have a catalytic subunit with a binuclear [FeFe]-center with three CO and two cyanide molecules bound to the two iron. The [FeFe]-center is directly linked via a bridging cysteine sulfur to an [4Fe-4S] cluster. The [Fe]-hydrogenase (Hmd), which is found only in some methanogenic archaea, is composed of two identical subunits and contains two iron atoms per homodimer. The iron is complexed within a cofactor, which can be reversibly extracted and which is essential for enzyme activity. Until now only a crystal structure of the Hmd apoenzyme is available. There is, however, spectroscopic information on where and how the iron is bound.

Mössbauer spectroscopic analysis indicates that Hmd contains a mononuclear iron center and that the electronic state of the iron site is low spin Fe(0) or low spin Fe(II). Infrared spectroscopy revealed that 2 CO ligands are bound to the iron site in an angle of 90°. An additional CO or cyanide ligand is bound to the iron site, when external CO or KCN are added to Hmd samples. Binding of external CO, cyanide and H₂ was found to be mutually exclusive as evidenced by competitive inhibition of enzyme activity. X-ray absorption spectroscopic analysis revealed that two CO molecules, one sulfur and one or two nitrogen/oxygen atoms are in binding distance to iron. The sulfur ligand is most probably provided by Cys176, which is conserved and essential for hydrogenase activity as revealed by mutational analysis. All available evidence indicates that the active site iron in Hmd has the function of a Lewis base facilitating - in conjunction with a Lewis acid A⁺ - the heterolytic cleavage of H₂:

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[\text{Fe}^+] + \text{H}_2 + \text{A}^+ \rightleftharpoons [\text{Fe}\text{H}^+] + \text{HA}
\]

NOTES

Session II
Complex Cofactors and Novel Reactivity
The unusual heme of cystathionine beta-synthase

Judith N. Burstyn; Departments of Chemistry and Pharmacology, University of Wisconsin, Madison, WI

Human cystathionine beta-synthase (CBS) is a PLP-dependent enzyme that unexpectedly also contains heme. CBS catalyzes the condensation of serine and homocysteine to form cystathionine, chemistry that is solely performed by the PLP co-factor. Although not required for catalysis, heme influences the catalytic reactivity of the enzyme. The CBS heme is one of relatively few in which a histidine imidazole and a cysteine thiolate serve as ligands. This low-spin, electron rich coordination environment confers unusual reactivity upon the CBS heme. Ferrous CBS is unstable, reacting at physiological pH via a pH-gated electron transfer process to generate ferric CBS, or at modest temperatures to generate a product in which the thiolate ligand is replaced. These processes influence the catalytic reactivity of the CBS heme; under normal assay conditions ferrous CBS loses activity with time and gradually forms the thiolate-free, ligand-switched product. Substrate binding influences the rate of the ligand switch process in ferrous CBS and alters the reactivity of the CBS heme toward exogenous ligands, such as CO. These observations will be discussed in light of a proposed redox-sensing role for the CBS heme.
Beyond the ligands: the role of the second coordination shell in modulating the electronic structure and reactivity of copper sites in proteins

David M. Dooley; Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717

The roles of metal-ion ligands in determining the electronic structures and reactivities of metalloprotein active sites has been extensively explored for decades. Sophisticated physical methods, synthetic modeling, and, increasingly, modern theoretical and computational approaches have provided a wealth of information. Investigators have gained a broad and deep understanding of structure – reactivity relationships in metalloproteins governed by metal-ion ligand bonding. However, the roles of active-site structural elements beyond the ligands, and weak interactions the second coordination shell are much more poorly understood, although recognized to be important. Using examples from a plastocyanin, a blue-copper electron-transfer protein, and galactose oxidase, a Type 2 copper oxidase, the roles of second coordination shell residues will be examined. In both cases the results indicate that second coordination shell effects can modulate the reactivity of the metal ion in functionally significant respects.
Acetyl-CoA Synthase/Carbon Monoxide Dehydrogenase: A bifunctional Ni-Fe-S-containing enzyme with an organometallic reaction mechanism

Paul A. Lindahl; Departments of Chemistry and Biochemistry/Biophysics, Texas A&M University, College Station TX 77843-3255; Email: Lindahl@mail.chem.tamu.edu

The title enzyme, abbreviated ACS/CODH, is contained within the most ancient living organisms – namely anaerobic, thermophilic archaebacteria and bacteria that grow chemolithotrophically on CO₂ as their sole carbon source. These organisms play a significant role in the global carbon cycle and some of them are human pathogens. ACS/CODH’s are 300 kDa αβ₂ tetramers, the β₂ portion of which catalyzes the reversible reduction of CO₂ to CO at a Ni-Fe-S active site called the C-cluster. The α subunits contain another Ni-Fe-S active site called the A-cluster which catalyzes the synthesis of acetyl-CoA from CO, Coenzyme A, and a methyl group transferred onto the enzyme from an autonomous 90 kDa corrinoid protein. The A- and C-clusters are separated by a distance of ~ 70 Å but they interact via an extensive molecular tunnel through which CO migrates. Migration is regulated by the conformational status of the α subunits and by the CO concentration. Besides connecting A- and C-clusters, the tunnel also connects the C-cluster of one β subunit with the C-cluster of the other β subunit, but the reason for this has not been established.

Besides providing further details regarding the structure and general properties of these enzymes, the presentation will highlight a major controversy which is currently the focus of research activity. At issue is the number of electrons required for reductively activating the A-cluster and the site(s) at which it(they) localize(s) on the A-cluster. The A-cluster contains an [4Fe4S] cluster bridged to 2 Ni ions. Proposals involve 1 or 2 electron reduction of the cubane and/or Ni ions. In the end, we will be left with the intriguing but controversial possibility that a Ni(0) atom is generated during reductive activation. If true, this would be the first example of a zero-valent transition metal in biology. Previously, such an oxidation state has been the exclusive domain of Organometallic chemistry. Other aspects of the catalytic mechanism also appear to be borrowed from the standard repertoire of organometallic reactions. The Ni(0) species attacks the methyl group of the corrinoid protein, forming a Ni-methyl intermediate. CO inserts into this species, forming a Ni-acetyl intermediate. Attack by CoA promotes a reductive elimination reaction in which the product acetyl-CoA forms and Ni(0) is regenerated. Using stopped-flow, we have recently obtained kinetic information for each step of the catalytic mechanism, and our attempts to generate the outlines of a kinetic model will be presented. We have also recently probed the function of the tunnel by combining site-directed mutagenesis with activity and biophysical measurements. Our results suggest that CO/CO₂ may enter/exit the tunnel through dynamic connections with a second, newly discovered hydrophilic channel.
The mechanism of reductive cleavage of S-adenosylmethionine to initiate a radical reaction: the case of lysine 2,3-aminomutase

Perry A Frey; University of Wisconsin-Madison

The radical SAM enzyme lysine 2,3-aminomutase (LAM) catalyzes the interconversion of L-lysine and L-\(\tilde{\text{U}}\)-lysine by a radical mechanism that has been characterized by rapid mix-freeze quench EPR spectroscopy. The mechanism requires the actions of pyridoxal phosphate (PLP), S-adenosylmethionine (SAM), and a [4Fe-4S] cluster. The radical isomerization mechanism will be presented in an overview of the available evidence. The radical isomerization mechanism focuses attention on the mechanism of radical initiation. LAM and the other radical SAM enzymes facilitate the reductive cleavage of SAM by the [4Fe-4S]\(^{1+}\) cluster to form the 5'-deoxyadenosyl radical as a transient intermediate. The 5'-deoxyadenosyl radical initiates radical isomerization by abstraction of the 3-pro-\(R\) hydrogen atom from the lysyl-side chain. The recent focus of research has been the mechanism of reductive cleavage of SAM by the [4Fe-4S] cluster. The main barrier to cleavage is electron transfer to the sulfonium center of SAM, a process characterized by a mid-point potential of about \(-1.5\) \(V\) for generic trialkylsulfonium ions in aqueous solutions. The reduction potential of the [4Fe-4S] cluster in LAM in the presence of SAM is \(-0.43\) \(V\) for the (2+/1+) transition, more than one volt higher than for generic sulfonium ions in solution. Spectroscopic and x-ray crystallographic evidence implicate a mechanism facilitated by inner sphere electron transfer and an increase in the ligation of a unique iron in the iron-sulfur complex upon electron transfer. Evidence indicates that this mechanism significantly elevates the mid-point potential for electron transfer to SAM. Complementary evidence indicates that the binding of lysine lowers the mid-point potential of the [4Fe-4S] cluster, so that it approaches the value for SAM at the active site. The current model suggests that the binding of SAM to LAM elevates the reduction potential of the [4Fe-4S] cluster to the physiological range (\(-0.43\) \(V\)) and allows the cluster to be reduced to [4Fe-4S]\(^{1+}\) in the complex of LAM and SAM. The binding of lysine then lowers the reduction potential of the cluster to near that of SAM, which is bound to the [4Fe-4S] cluster in the active site. The near parity of reduction potentials allows electron transfer and radical formation to take place. Supported by Grant No. DK28607 from the National Institute of Diabetes and Digestive and Kidney Diseases.
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Intermediates along the nitrogenase reaction pathway

Lance C. Seefeldt, Dennis R. Dean, and Brian M. Hoffman 1, 4Department of Chemistry and Biochemistry, Utah State University, Logan, UT; 3Department of Biochemistry, Virginia Tech University, Blacksburg, VA; 4Northwestern University, Evanston, IL

The biological fixation of N₂ to ammonia is a central reaction in the global nitrogen cycle and is essential to all living organisms. This reaction occurs exclusively in microbes that contain the enzyme nitrogenase. Nitrogenase catalyzes the fixation of N₂ according to the reaction shown in equation 1.

\[ \text{N}_2 + 8e^- + 16MgATP + 8H^+ \rightarrow 2\text{NH}_3 + 3H_2 + 16MgADP + 16P_i \]

The most widely distributed and investigated nitrogenase is comprised of two component proteins called the MoFe protein and the Fe protein. The Fe protein functions to deliver electrons one at a time to the MoFe protein in a reaction requiring the hydrolysis of MgATP. The MoFe protein contains the site of N₂ binding and reduction, a novel metallocluster called the FeMo-cofactor. Our work over the last few years has focused on addressing some of the outstanding questions about the N₂ reduction mechanism of nitrogenase. Where does N₂ bind, how is it reduced, and what are the intermediates along the reaction pathway?

Very little is known about the nature of the reaction intermediates along the N₂ reduction pathway on nitrogenase, but is clear that the enzyme catalyzes this reaction by the sequential addition of protons and electrons to a metal bound N₂ molecule. Based on N₂ reduction mechanisms deduced for organometallic compounds done by Chatt and Schrock and coworkers, and theoretical calculations, two different reaction pathways for the nitrogenase catalyzed reaction seem possible (Figure 1).

These two mechanisms share end-on binding of N₂ to a single metal, but differ in the site of proton addition and the reaction intermediates. These competing mechanisms are referred to as the distal and alternating mechanisms to reflect the different site of proton addition.

Recently, we have succeeded in trapping nitrogenase during reduction of three different nitrogenous substrates: N₂, diazene, and hydrazine. Characterization of these bound states by EPR and ENDOR spectroscopy is revealing the nature of the bound intermediates. Some key features that are already evident include: each intermediate is bound to FeMo-cofactor, each appears to be bound through a single N atom, and each appears to represent a unique state along the reaction pathway reflecting early, middle and late steps in the reduction mechanism. These results will be presented coupled with our current thinking on the mechanism of N₂ reduction by nitrogenase.
The active-site metal centre of [NiFe]-hydrogenases: Synthesis and assembly

August Böck; Department of Biology I, Microbiology, University of Munich, Munich, Germany

[NiFe]-hydrogenases are heterodimeric enzymes consisting of a large subunit which carries the active-site metal centre and a small subunit with three or less iron sulphur clusters which lead the electrons to or from the active site. The nickel of the centre is coordinated via the thiolates of four cysteine residues from the protein backbone of the large subunit, two of which also serve as ligands bridging the nickel with the iron. The Fe of the centre in addition carries three non-protein ligands, which are in the classical case one CO and two CN moieties. The core machinery for the synthesis and integration of the centre requires the activities of at least seven gene products, which are designated as accessory proteins (for rev. see ref.1, 2). Six of them are designated as hyp genes. The following functional entities have been differentiated, mainly during the genetic and biochemical analysis of the maturation of the E. coli hydrogenase 3, which is a cyttoplasmically located enzyme. (i) HypF transfers the carbamoyl moiety from carbamoylphosphate to the carboxyterminal cysteine residue of the HypE protein resulting in HypE-thiocarboxamide. HypE then dehydrates the thiocarboxamide to the thiocyanate in an ATP-dependent mechanism. (ii) In a subsequent reaction, HypE-SCN delivers the cyanide to some acceptor group in a complex formed by the maturation proteins HypC and HypD. During this process HypF interacts with HypE and HypE-CN undergoes transient complex formation with the HypCxHypD heterodimer. Based on the fact that HypC is also found in a complex with the precursor of the large subunit a putative role in the transfer of the cyanide group has been postulated. The source of the CO ligand is still unknown. (iii) Nickel insertion succeeds iron incorporation and requires the activities of the HypA and HypB proteins and, for optimal efficiency, the function of the SlyD protein. The requirement for HypA (a zinc protein with nickel binding capacity) and HypB (a GTPase, also able to bind nickel) is not absolute because their activity can be replaced by high nickel concentrations both in vivo and in vitro. (iv) The seventh essential protein is an endopeptidase which removes an oligopeptide from the C-terminal end of the precursor of the large subunit once nickel has been inserted. The metal appears to be a decisive recognition motif for the endopeptidase in the cleaving process. The C-terminal cleavage of the precursor results in a conformational change and in the closing and internalisation of the centre.

In addition to this core maturation machinery there are additional accessory proteins which are involved in maturation of the small subunit and the coordination of maturation of the two subunits and their joint export in microorganisms which export the enzyme into the periplasmic space.

Role of Sco1/Sco2 in the assembly of the CuA site in cytochrome oxidase

D.R. Winge, P. Cobine, Y-C. Hong, and F. Pierrel; University of Utah Health Sciences Center, Salt Lake City UT 84132, USA

Sco1 and Sco2 are metallochaperones required for copper delivery to the CuA site in the Cox2 subunit of mammalian cytochrome c oxidase (CcO). The function of human Sco1 and Sco2 is dependent on copper ion binding. Expression of soluble domains of human Sco1 but not Sco2 in the yeast cytoplasm is dependent on the co-expression of human Cox17. Two conserved cysteines and a histidyl residue, known to be important for both copper-binding and in vitro function in yeast Sco1, are also critical for in vivo function of human Sco1 and Sco2. Human and yeast Sco proteins can bind either a single Cu(I) or Cu(II) ion. The Cu(II) site yields S-Cu(II) charge transfer transitions that are not bleached by weak reductants or chelators. The Cu(I) site exhibits trigonal geometry, while the Cu(II) site resembles a type II Cu(II) site with a higher coordination number. Replacement of Asp238 in human or yeast Sco1 abrogates the Cu(II) visible transitions, and in yeast Sco1 attenuates Cu(II), but not Cu(I), binding. Both the mutant yeast and human proteins are nonfunctional suggesting the importance of both Cu(I) and Cu(II) coordination for normal Sco function. The only known missense mutation in human Sco1, a P174L substitution in the copper-binding domain, is associated with a fatal neonatal hepatopathy. Immortalized fibroblasts from a SCO1 patient show a severe deficiency in CcO activity that is partially rescued by overexpression of P174L Sco1. The mutant protein retains the ability to bind Cu(I) and Cu(II) normally when expressed in bacteria, but Cox17-mediated copper transfer is severely compromised both in vitro and in a yeast cytoplasmic assay. Defective Cox17-mediated copper maturation of Sco1, and subsequent failure of CuA site maturation, appears to be the basis for the inefficient assembly of the cytochrome c oxidase complex in SCO1 patients.
Copper trafficking in a hyperthermophile

Amy C. Rosenzweig: Departments of Biochemistry, Molecular Biology, and Cell Biology and of Chemistry, Northwestern University, Evanston, IL 60208, USA

The P-type ATPases translocate cations across membranes using the energy provided by ATP hydrolysis. CopA from Archaeoglobus fulgidus is a hyperthermophilic ATPase responsible for the cellular export of Cu" and is a member of the heavy metal P1β-type ATPase subfamily, which includes the related Wilson and Menkes disease proteins. The P1β-type ATPases comprise eight transmembrane helices, a large ATP binding domain (ATPBD), an actuator domain (A-domain), and N- or C-terminal soluble metal binding domains (MBDs). Binding sites responsible for metal coordination during transport are located within the membrane. With the exception of solution structures of several MBDs, structural information for P1β-type ATPases is lacking. Crystal structures of the sarcoplasmic reticulum (SERCA1) Cu²⁺-ATPase have been determined and provide insight into the conformational changes that P2-type ATPases undergo during catalysis. Due to significant architectural differences between P1β-type and P2-type ATPases, homology modeling based on the SERCA1 structures is of limited utility in understanding P1β-type ATPase function and transport mechanisms on the molecular level. We have determined the crystal structures of the ATPBD and the A-domain from A. fulgidus CopA to 2.3 Å and 1.65 Å resolution, respectively. These structures provide insight into catalysis as well as into the likely structural and functional effects of various mutations that lead to Wilson and Menkes diseases. In addition, we have characterized a novel copper chaperone that delivers Cu" to CopA in A. fulgidus. Unlike previously characterized Atx1-like copper chaperones, A. fulgidus CopZ contains a unique cysteine-rich N-terminal domain in addition to a C-terminal copper binding domain with the typical conserved CXXC motif. Surprisingly, spectroscopic data indicate the presence of a [2Fe-2S] cluster in the N-terminal domain. The crystal structure of the CopZ N-terminal domain has been determined to 1.8 Å resolution, revealing an additional metal center and a novel fold.
Transporting copper across membranes in yeast and mammals

Dennis J. Thiele; Department of Pharmacology and Cancer Biology and the Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, North Carolina 27710 (email: dennis.thiele@duke.edu)

Copper serves as a critical co-factor for a wide variety of enzymatic reactions as well as in key roles in signal transduction and gene expression. While copper is essential for virtually all life forms around the globe, interactions with reactive oxygen species generate hydroxyl radical, which causes damage to lipids, proteins and nucleic acids. Our laboratory is interested in understanding how organisms accumulate sufficient copper levels to drive key biological processes and respond to times of copper deprivation or overload. A key component for copper uptake from yeast to human cells is the Ctr1 protein, a homotrimeric integral membrane protein that functions to deliver copper across the plasma membrane in a specific, high affinity and energy-independent fashion. Here we describe the results of studies aimed at the characterization of Ctr1 subcellular location, regulation, mechanism of action and physiological function in copper transport. A membrane protein with primary structural homology similar to that of Ctr1, denoted Ctr2, is also conserved from yeast to humans. Yeast Ctr2 is an integral membrane of the vacuole, a compartment involved in the storage of amino acids, phosphate, metals and other nutrients and which is functionally analogous to the mammalian lysosome. Under conditions of copper deprivation Ctr2 functions to mobilize copper from the lumen of the vacuole to the cytosol, where it can be distributed to copper-dependent enzymes. While Ctr1 and Ctr2 mobilize copper across distinct membrane compartments, biochemical, cell biology and genetic studies suggest that these structurally related proteins move copper across membranes via similar mechanisms.
Session IV
Oxygen Activation
Risky business of Rieske Oxygenases

S. Ramaswamy; Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA 52242

Rieske non-heme iron oxygenases (RO) catalyze the first step in the activation of inert carbon, nitroaromatic and several other compounds that are degraded by bacteria. They add molecular oxygen to these compounds to activate them. Bacteria use these activated compounds as a source of carbon. ROs hence play a key role in the global carbon cycle. ROs are multi-component systems with a reductase component, often a ferredoxin component and a terminal oxygenase component. In collaboration with the group of David Gibson we have determined the structures of a number of oxygenase components by X-ray crystallography. The structures include, wild type proteins, mutant forms of the protein as well as several complexes. We also used intermediate trapping as a tool to elucidate possible mechanisms of dioxygenation.

Our results demonstrate that oxygen activation and attack of the carbon-carbon bond occurs after a side-on binding of oxygen to the mono-nuclear iron. This results in the formation of a cis-dihydrodiol. This lead to the hypothesis ‘the orientation of the substrate in its ground state governs the site of attack by activated dioxygen’. We investigated this by determining structures of mutant forms of the enzyme that had varied regio-selectivity of product formation. Nature provides a ready set of mutant enzymes that have different substrate specificity and regio-specificity of product formation. We have also determined the structures of number of different ROs in complex with substrates. Our results suggest that the hypothesis is generally true.

Our investigations have also led us to the identification of the possible base (proton donor) in the reaction mechanism. ROs catalyze a variety of reactions other than dioxygenation – monoxygenation, sulfoxidation, demethylation etc. The identification of the catalytic base led to the hypothesis that ‘the structural basis for the type of reaction is controlled by the presence of a water ligated to the catalytic base’. The talk will present the results of these studies.

How proteins control electrons: protons

Enine Yikilmaz, David W. Rodgers, and Anne-Frances Miller. 1Department of Chemistry, University of Kentucky, Lexington KY 40506, U.S.A.; 2Department of Chemistry, The Johns Hopkins University, Baltimore MD 21208 U.S.A.; 3Department of Molecular and Cellular Biochemistry, and Center for Structural Biology, University of Kentucky Medical Centre, Lexington KY 40536, U.S.A. *Correspondence to A.-F. M. afm@uky.edu, tel. (859) 257-9349, fax (859) 323-1069.

In Fe-containing superoxide dismutase, FeSOD, reduction of the Fe$^{3+}$ is accompanied by protonation of a coordinated OH$. However, the coordinated solvent appears to hydrogen bond with a conserved Glutamine, Glu69. We have proposed that this H-bond can strongly influence the midpoint potential of the proton-coupled reduction of Fe (E_m). [1,2] We now show that mutation of this Glu to His subtly alters the active site structure, but preserves 30% activity. In contrast, mutation to Glu otherwise preserves the active site structure, but inactivates the enzyme. Redox titrations indicate that mutation of Glu69 to His increases the reduction potential by 240 mV, whereas mutation to Glu appears to increase it by more than 660 mV. We find that this suffices to explain the mutant's loss of activity, although additional factors likely contribute. Simple electrostatic interaction between Fe$^{3+}$ and anionic Glu' would tend to lower rather than raise the E_m, and both mutants have WT-like Fe electronics. However, since His69 and Glu69 appear to act as less effective hydrogen bond donors, or even hydrogen bond acceptors from coordinated solvent, we conclude that hydrogen bonding with a residue with a greater tendency to accept a hydrogen bond favors coordinated H$_2$O (relative to OH$^-$) and thereby stabilizes the associated Fe$^{2+}$ state. Thus, strong coupling between electron transfer and proton transfer, plus H-bond-mediated control over protonation of the redox-coupled proton acceptor, can explain the strongly increased potentials we have produced, in a conserved structural context. This mechanism is likely at work in many other enzymes, and may be useful in designing novel redox active sites, or in modifying the activity of existing ones.

† AFM is pleased to thank the N.S.F. for financial support; MCB 0125939.

Proposed model for alterations in active site H-bonding that would be consistent with the observed increases in E_m as well as the X-ray crystal structures determined. The left panel depicts the H-bonds entraining the WT-FeSOD Glu69 to donate an H-bond to coordinated solvent, the centre panel depicts the structure of Q69H-FeSOD in magenta, overlain on the structure of the WT in grey, the right panel depicts the structure of Q69E-FeSOD in red, overlain on the structure of the WT in grey. H., Helt, G., Struhl, K. and Gingeras, T. R. (2004) Mapping of transcription factor binding sites points to wide-spread antisense transcription along human chromosomes 21 and 22. Cell 116: 499-510.
Oxygen activation by a mixed-valent diiron cluster and C-H cleavage by an iron-superoxide intermediate in the glycol cleavage reaction catalyzed by myo-inositol oxygenase

J. Martin Bollinger, Jr., 1, 2 Carsten Krebs, 1, 3 Gang Xing, 1 Eric Barr, 1 Lee Hoffart, 1 Yinghui Dao, 1 K. Sandeep Prabhu, 1 Ryan J. Arner, 1 C. Channa Reddy, 3 Departments of Biochemistry and Molecular Biology, 1 Chemistry, 1 and Veterinary and Biomedical Sciences, 4 Penn State University, University Park, PA 16802

myo-Inositol oxygenase (MIOX) catalyzes the ring-opening, glycol-cleaving, four-electron oxidation of cyclohexan-1,2,3,5,6,7-ol (myo-inositol, MI) to D-glucorurate by a single equivalent of dioxygen (Eq. 1). The reaction initiates the only pathway in humans for catabolism of MI, the sugar backbone of cell-signaling phosphoinositides. Recent spectroscopic and kinetic studies on the mouse kidney enzyme produced in Escherichia coli have established that MIOX can contain a dinuclear non-heme iron cluster. 2, 3 Unlike all non-heme diiron enzymes and oxidases characterized prior to MIOX, which activate dioxygen with fully-reduced (II/II) diiron cofactors, 1 MIOX activates O2 with the mixed-valent (II/III) form of its cofactor. 4 The diiron(II/III) cluster directly coordinates the substrate via an alkoxide bridge, which is most likely contributed by C1. 1 The MIOX 3+MI complex reacts with O2 to form a (formally) (superoxo)diiron(II/III) complex, G, which is characterized by a rhombic g = (2.05, 1.98, 1.90) EPR signal and accumulates only when the substrate contains deuterium. 5 G converts to a second intermediate, a diiron(II/III)-containing complex, H, which has a g = (1.92, 1.76, 1.54) EPR signal and accumulates in the reaction with either protium- or deuterium-containing substrate. 2, 4 The G-to-H conversion is associated with a substrate deuterium kinetic isotope effect of at least 5, indicating that G abstracts hydrogen from the substrate. 4 The kinetic and spectroscopic data on MIOX provide the first example of O2 activation by a mixed-valent diiron cluster and the most direct evidence yet reported for C-H cleavage by a superoxide-level metalloenzyme intermediate.

\[
\begin{align*}
\text{HO-} & \quad \text{OH} \\
\text{HO-} & \quad \text{OH} \\
\text{HO-} & \quad \text{OH}
\end{align*}
\]

\[
\text{G} \quad \text{O2} \quad \text{MIOX} \quad \text{H2O} + \text{H+}
\]

Substrate binding, electron transfer, and O₂ activation in Rieske dioxygenases: Three sides of the same coin

Matthew B. Nehergall, Sarmistha Chakrabarty, Matt D. Wolfe, Michael M. Mboghuni, and John D. Lipscomb; Department of Biochemistry, Molecular Biology, and Biophysics and the Center for Metals in Biocatalysis, University of Minnesota, Minneapolis, MN 55455

Rieske dioxygenases catalyze insertion of both atoms of oxygen from O₂ into unactivated aromatic substrates to yield cis-dihydrodiole products. This is initiated by degradation of many aromatics that enter the biosphere. Crystallographic studies have shown that most Rieske di- and mono-oxygenases have two types of metal centers in the same subunit of their hydroxylase component, a 2Fe-2S Rieske cluster and a mononuclear iron site. Typically, 3 subunits are aligned head-to-tail so that the Rieske cluster of one subunit is located adjacent to the mononuclear iron site of another subunit, thus forming the active unit. The complete system also includes an NADH-linked reductase and often one or two electron carrier components to supply the 2 e⁻ required by the stoichiometry. We have developed single turnover systems for both benzoate (BZDO) and naphthalene (NDO) dioxygenases which show that the fully reduced hydroxylase component alone is sufficient to carry out the complete reaction. During this process, both of the metal sites are oxidized by one equivalent, showing that the O₂ activation process requires 2 (and only 2) e⁻. This suggests that the reactive form of oxygen is either an Fe(III)peroxo adduct or the equivalent generated by cleaving the O-O bond. Accordingly, the cis-diol products are formed when H₂O₂ is used to supply both electrons and oxygen. BZDO can be prepared with both metal centers fully oxidized. The peroxide shunt remains functional, clearly demonstrating that the 2 reducing equivalents from peroxide are sufficient for the reaction to proceed. Probe experiments using NO as an O₂ surrogate to yield an EPR active complex of the mononuclear Fe(II) suggest that the entry of O₂ is tightly regulated. When the Rieske cluster is reduced, substrate must be bound before NO (or O₂) can bind, presumably to prevent release of reactive species. ENDOR studies of the NO adducts show that reduction of the Rieske cluster shifts the relative orientations of the active site substrate and Fe(II), perhaps allowing the small molecule to access the iron. There are other indications of tight coupling between the metal centers across the subunit boundary. Significantly, the rate of e⁻ transfer between the metal centers is controlled by both the residues in the e⁻ transfer pathway and the nature of the substrate in the active site. Optimization of substrate type, oxygen source, and metal redox state has allowed the detection of reaction cycle intermediates for the first time in this enzyme class.
Session V
Methods in Metalloenzymology
Ribonucleotide reductases: Something for everyone

JoAnne Stubbe; Department of Chemistry and of Biology; MIT
Spectroscopic characterization of radical intermediates in enzymic reactions

George H. Reed; Department of Biochemistry, University of Wisconsin—Madison, Madison, WI

Organic free radicals play fundamental roles in several aspects of biological processes. Metallocofactors are frequently involved in the generation of these reactive species. Adenosylcobalamin (AdoCbl) is one such metallocofactor that initiates free radical chemistry through homolysis of its cobalt-carbon bond in the active sites of AdoCbl-dependent enzymes. A general scheme for the catalytic cycles of AdoCbl-dependent enzymes is shown below.

The net result of the reaction is a switch in the positions of a group X and a hydrogen atom on adjacent carbon atoms. The AdoCbl-dependent enzymes lower the barrier to cobalt-carbon bond cleavage, shepherd the reactive intermediates to prevent radical side reactions, facilitate the rearrangement, and foster re-formation of the cobalt-carbon bond following product formation. The 5′-deoxyadenosyl radical (Ado−) is a transient intermediate that hasn’t been observed spectroscopically. Depending on the enzyme and the substrate, substrate or product based radicals may be trapped in the steady-state of the reaction. Our recent experiments have focused on two AdoCbl-dependent enzymes, diol dehydrase (DDH), and ethanolamine ammonia-lyase (EAL), that catalyze eventual elimination of the group −X, −OH and −NH₂, respectively, to form aldehyde products.

With diol dehydrase we have used an analog of AdoCbl, 3′,4′-anhydro-AdoCbl, that forms an allylically stabilized radical upon cobalt-carbon bond cleavage in the active site. We have analyzed the complicated, triplet EPR spectra of the intermediates to determine the position of the anhydroadenosyl radical in relation to the g-axis system of the low-spin cobalt of cob(II)alamin in the presence and absence of substrate.

Experiments with EAL have focused on understanding the barrier to and mechanism of the rearrangement step. We have used 15N-isotope effects (IE’s) to examine the contribution of C-N bond breaking/making to the kinetic parameter, V/K. We have used 1H primary and secondary IE’s to allow access to different steady-state intermediates. In the latter studies, we have used 13C-labeling of the 2H-labeled substrates to identify the position of the unpaired electron in the free radical intermediates. Rapid-mix, freeze-quench sampling for EPR measurements has been coordinated with the time course of cobalt-carbon bond cleavage measured by stopped-flow spectrophotometry.

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Site-specific chemistry at a unique Fe site of the Fe₄S₄ cluster in ferredoxin: thioredoxin reductase

Boi Hanh Huynh,1 Elizabeth M. Walters,2 Ricardo Garcia-Serres,1 Guy N. L. Jameson,1 Dominique A. Glauser,3 Florence Bourquin,3 Wanda Manieri,2 Peter Schürmann,3 and Michael K. Johnson2; 1Department of Physics, Emory University, 2Department of Chemistry and Center for Metalloenzyme Studies, University of Georgia, and 3Laboratoire de Biochimie Végétale, Université de Neuchâtel

Ferredoxin-thioredoxin reductase (FTR) catalyzes the reduction of the disulfide in thioredoxin (Trx) in two one-electron steps using a unique active site comprising a Fe₄S₄ cluster and an adjacent redox-active disulfide. It plays a key role in the light-regulated activation of chloroplast enzymes by transforming the light signal received by photosystem I in the thylakoid membrane into a disulfide/dithiol interchange redox-signal between Trx and the target enzymes. We have used a combination of spectroscopic methods (Mössbauer, EPR, and resonance Raman) to characterize the active site of FTR in various forms of the enzyme, including wild-type FTR, point-mutation variants at each of the active-site cysteine residues, stable analogs of the one-electron-reduced FTR-Trx heterodisulfide intermediate, and methyl viologen-reduced FTR. The results reveal novel site-specific Fe₄S₄-cluster chemistry in oxidized, one-electron reduced and two-electron reduced forms of FTR. In the resting enzyme, a weak interaction between the Fe₄S₄ cluster and the active-site disulfide promotes charge buildup at a unique Fe site and primes the active site to accept one electron from ferredoxin with concomitant cleavage of the active-site disulfide. In one electron-reduced analogs, cleavage of the active-site disulfide is accompanied by coordination of one of the cysteine residues that form the disulfide to yield an unusual [Fe₄S₄]⁷⁺ cluster with two cystinate ligands at a unique Fe site. The most intriguing result is that the methyl viologen-reduced FTR, in which the disulfide is reduced to a dithiol, contains an unprecedented [Fe₄S₄]⁷⁺ cluster comprising both a valence-delocalized Fe⁶⁺Fe⁴⁺ pair, with the unique Fe site being the valence-localized high-spin Fe⁶⁺ site. These results provide molecular level insights into the catalytic mechanism of FTR. On the basis of these results, two viable mechanisms are proposed.
Electrochemical Enzymology: Probing dynamics and mechanism at an electrode

Sean J. Elliot; Department of Chemistry, Boston University
1. Characterizing the catalytic mechanism of the prototypic \( \gamma \)-class carboxylic anhydrase, Cam
Sabrina A. Zimmerman and James G. Ferry; Pennsylvania State University, University Park, PA

2. Probing subunit interactions in human ribonucleotide reductase by fluorescence probe and photo cross-linking agents
A Quamru H. Hassan, JoAnne Stubbe; Department of Chemistry, Massachusetts Institute of Technology

3. Purification of periplasmic nitrate reductases: \textit{Sulfurospirillum barnesii} SES-3
Courtney Sparacino, Ganesh Naik, John Stolz and Partha Basu; Department of Chemistry and Biochemistry, and Biological Sciences, Duquesne University Pittsburgh, PA 15282

4. Characterization of the outer-membrane heme-receptor ShuA from the heme uptake system of \textit{Shigella dysenteriae}: Substrate specificity and identification of the heme-protein ligands
Kimberly A. Burkhard and Angela Wilks; Department of Pharmaceutical Sciences, School of Pharmacy, 20 Penn Street, University of Maryland, Baltimore, MD 21201

5. Syntheses and characterization of a new class of molybdenum monooxo complexes
Eranda I. Perera and Partha Basu; Duquesne University, Department of Chemistry and Biochemistry, Pittsburgh, PA 15282

6. ADP-BeFx and ADP-AlFx stabilize a complex formed by sigma54 transcription factor and AAA+ ATPases
Baoyu Chen, Michael Leon Dosunlu, Sacha De Carlo, David E. Wemmer, Eva Nogales, Timothy R. Hoover, Elena Kondrashkina, and B. Tracy Nixon

7. Functional studies of the transcription factor NikR: A global regulator of nickel homeostasis in \textit{H. pylori}
N.S. Dosanjh and S.L.J. Michel; Department of Pharmaceutical Sciences, University of Maryland, Baltimore MD 21201

8. Characterization and mechanistic studies of \textit{Escherichia coli} lipoyl synthase: A member of the "radical SAM family of enzymes"
Robert M. Cicchillo, David F. Ivig, Natasha M. Nesbitt, Camelia Gogonea, Loretta Tu, Matthew G. Souder, Carsten Krebs, and Squire J. Booker; Department of Biochemistry, Microbiology, and Molecular Biology, Penn State University, 101 Ailhouse Laboratory, University Park, PA 16802
9. Ascorbic acid scaffold as a mechanism-based inhibitor of heme oxygenase from *Pseudomonas aeruginosa*

Mehul N. Bhakta, Ayodele Olabisi, Kandatege Winalasema and Angela Wilks; Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD 21201, and Department of Chemistry, Wichita State University, Wichita, KS 67260

10. Crystal structure of α-ketoglutarate non-heme iron halogenase CytC3

Cintyu Wong, Danica Galonic, Christopher T. Walsh and Catherine L. Drennan; 1Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA; 2Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

11. Phylogenetic metal variations as a predictor of species-specific porphobilinogen synthase druggability

Sarah H. Lawrence, Ursula Ramirez, G. Doug Markham, Eileen K. Jaffe; Fox Chase Cancer Center, Philadelphia, PA

12. Reaction between peroxynitrite and cytochrome P450BM3 yields nitrosyl

Rachel Behan, Lee M. Hoffart, Kari L. Stone, Carsten Krebs and Michael T. Green; 1Department of Chemistry and 2Department of Biochemistry and Molecular Biology, The Pennsylvania State University

13. Kinetics of dissimilatory iron reduction in *Shewanella oneidensis* MR-1: scaling from purified proteins to whole cell cultures

Dan Rossi, Susan Brantley, Ming Tiong; 1Department of Biochemistry and Molecular Biology; 2Department of Geosciences; 3Department of Biochemistry and Molecular Biology

14. Characterization of PscB, an iron-sulfur protein from the photosynthetic reaction center of *Chlorobium vibrioforme*

Bharat Jagannathan and John H. Golbeck; 1Department of Biochemistry and Molecular Biology; 2Department of Chemistry, Pennsylvania State University, University Park, PA 16802

15. Implications of metal coordination on NikR specificity: An update on the Zn(II)-bound NikR structure

Christine M. Phillips, Eric Schreiter, Yayi (Stephanie) Guo, Catherine L. Drennan; Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

16. A new generation of dithiolene ligands

Barbara Seril Mitasev and Partha Basu; Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, PA

17. Halogenation of unactivated carbon centers in natural product biosynthesis: Trichlorination of leucine during barbamide Biosynthesis and mechanistic Insight into halogenation process

Danica P. Galonic, Frédéric H. Vaillancourt, Eric W. Barr, Carsten Krebs, J. Martin Bollinger, Jr., and Christopher T. Walsh*; 1Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115 and 2Department of Biochemistry and Molecular Biology and Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

18. Synthetic ribonucleosides as mutagenic antiviral agents

Jocelyn P. Edathil, Jason D. Graci, Craig E. Cameron, and Blake R. Peterson; Department of Chemistry and Biochemistry and Molecular Biology, The Pennsylvania State University

19. Spectroscopic studies of high-valent Intermediates of chloroperoxidase

Kari L. Stone, Rachel K. Behan, Lee M. Hoffart, Carsten Krebs, and Michael T. Green*; The Pennsylvania State University, Department of Chemistry, University Park Pennsylvania, 16802, USA, email: kll174@psu.edu

20. Altering substrate specificity of Phosphatidylcholine-Preferring Phospholipase C of *Bacillus cereus* by random mutagenesis of the headgroup binding pocket

Nina M. Gooday, Paul J. Hergetrother, Micheleen M. Harris, William Corbett, and Stephen F. Martin; 1Presentor (formerly Nina M. Antikainen) Department of Chemistry, The Pennsylvania State University, 414 Warrik Laboratory, University Park, PA 16802; 2Department of Chemistry, University of Illinois, Urbana, IL 61801; 3Department of Chemistry and Biochemistry and The Institute of Cellular and Molecular Biology, The University of Texas at Austin, Austin, Texas 78712

21. A novel difteric-peroxo intermediate in the O2 reaction of Toluene/o-Xylene Monoxygenase Hydroxylase I100W variant, that decays to difteric state via a FeIV/FecIII transient

Ricardo García-Serres, Leslie J. Murray, Viviana Izzo, Sunil Naik, Boi Hanh Huynh, and Stephen J. Lippard; Department of Physics, Emory University, Atlanta, Georgia and 1Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts.

22. The effect of iron binding on the function of NUP475, a non-classical zinc finger protein involved in inflammatory response

Seung Jae Lee, Robert C. diTargiani, Sarah L. J. Michel; Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore
23. WrbA from *Escherichia coli* and a homologue from *Archaeoglobus fulgidus* are NAD(P)H:Quinone reductases
Eric V. Patridge*, Susana L. Andrade*, Oliver Eisnle*, and James G.erry*
*Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802; Institut für Mikrobiologie und Genetik, Georg-August-Universität, Justus-von-Liebig-Weg 11, 37077 Gottingen, Germany

24. MA3736 from *Methanosarcina acetivorans* is an iron-sulfur protein with disulfide reductase activity
Daniel J. Lesser and James G.erry; Pennsylvania State University, Department of Biochemistry and Molecular Biology, University Park, PA

25. Characterization of a lipoyl synthase from *Thermus thermophilus*
Kyung-Hoon Lee and Squire J. Booker; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

26. Determination of the cysteine residues that coordinate the [4Fe-4S] cluster in quinolinate synthases
Amy Griffiths, Allison Saunders, Jeffrey Stromberg, Loretta Tu, and Squire J. Booker; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

27. The function of bicarbonate in the reaction catalyzed by *Escherichia coli* cyclopropane fatty acid synthase
David F. Twig and Squire J. Booker; Department of Biochemistry, Microbiology, and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

28. Electrochemical characterization of cytochrome *C*54 from *Nitrosomonas europaea*
Pulcu, G. S.*, Hooper, A. B.*, and Sean J. Elliott*; *Department of Chemistry, Boston University, Boston, MA 02215; †Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108

29. Biochemical and structural characterization of thiorledoxins from *Archaeoglobus fulgidus*
Hector H. Hernandez†, Sean J. Elliott*, and Catherine L. Drennan†; (1)Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, (2)Department of Chemistry, Boston University, Boston, MA

30. Electrochemical investigation of CuO, a multicopper oxidase involved in copper homeostasis in *E. coli*
Tao Ye, William R. Monfort, and Sean J. Elliott; *Chemistry Department, Boston University, Boston, MA 02215; †Department of Biochemistry and Molecular Biophysics, University of Arizona, 1306 E. University Blvd, Tucson, Arizona, 85721-0041

31. Structural investigation of RebP and RebC.
Katherine S. Ryan, Annaliese R. Howard-Jones, Christopher T. Walsh, and Catherine L. Drennan; †Department of Biology, ‡Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, 02139; §Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

32. Role of protein environment in redox regulation of phylloquinone and its effects on electron transfer rates in Photosystem I
Nithya Srinivasan and John H. Golbeck; Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802

33. The role of molybdenu and ModE in gene regulation in the green sulfur bacterium *Chlorobium tepidum*
Christina Cress, Julia Maresca and Donald Bryant; The Pennsylvania State University

34. The role of the C-8 and C-12 of methylations of bacteriochlorophyll c in the green sulfur bacterium *Chlorobium tepidum*
Aline Gomez Maqneo Chew, Niels-Ulrik Frigaard, and Donald A. Bryant; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA

35. Mutagenesis and characterization of the highly conserved cysteine residues in the C-terminal domain of the transcriptional repressor SurR of *Synechocystis* sp. PCC 6803
Yingxian Wu, Ramakrishnan Balasubramanian, Tao Wang, Lee M. Hoffart, Carsten Krebs, Guozhong Shen, Donald A. Bryant, and John H. Golbeck; †Department of Biochemistry and Molecular Biology, ‡Department of Chemistry, The Pennsylvania State University, University Park, PA 16802 USA

36. Nitric oxide mediates the deactivation of diphtheria toxin repressor by iron oxidation and S-nitrosation
Michelle M. Splier, Douglas A. Mitchell, and Michael A. Marletta; †Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109; ‡Department of Chemistry and §Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720
37. Binding of artificial multimetallic peptides with double stranded DNA
Lauren A. Levine and Mary Elizabeth Williams; Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, Pennsylvania 16802

38. Structural insight into antibiotic fosfomycin biosynthesis by a mononuclear iron enzyme
Danny Yan1, Luke J. Higgins1, Feng Yan2, Pinghua Liu2,3, Hung-wei Liu1 and Catherine L. Drennan1; 1Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139; 2Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712; 3Department of Chemistry, Boston University, Boston, MA 02215

39. Iron sulfur clusters in Type I homodimeric photosynthetic reaction centers
Mark Heinicke1, Gaozhong Shen1, Rutfat Agalarov1, Carsten Krebs1,2, and John H. Golbeck1,2; 1Department of Biochemistry and Molecular Biology, 2Department of Chemistry, The Pennsylvania State University, University Park, PA 16802 USA

40. Spectroscopic studies on de novo designed binuclear iron active sites
Caleb B. Bell III1, Pin-pin Wei1, Jennifer Callhoun3, William F. DeGrado2 and Edward I. Solomon1; 1Department of Chemistry, Stanford University, Stanford, California 94305, 2Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104

41. Identification of an anaerobic bacteriochlorophyll c C-7 hydroxylase in brown-colored green sulfur bacteria
Julia A. Maresca, Tao Li, and Donald A. Bryant; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA

42. Biomimetic formation of titanium phosphate and protein encapsulation
Kathryn E. Cole, Andrea N. Ortiz, Martin A. Schoenfliess and Ann M. Valentine1; 1Department of Chemistry, Yale University, New Haven, Connecticut, 06511; 2Department of Chemistry, California State Polytechnic University, Pomona, California 91768; 3Department of Geosciences and Center for Environmental Molecular Science, Stony Brook University, Stony Brook, New York, 11794-2100

43. A Mg2+-dependent ATPase that generates mechano-chemical force for clamp loading: ensemble and single-molecule studies
Zhihao Zhuang1, R. Derike Smiley2, Anthony J. Berdi1, Gordon G. Hammes1 and Steve J. Benkovic3; 1Department of Chemistry, The Pennsylvania State University, 414 Wartik Lab, University Park, PA, 16802; 2Department of Biochemistry, Duke University Medical Center, Box 3711, Durham, NC 27710; 3Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106

44. A unified mechanism for the α-ketoglutarate-dependent dioxygenases: Evidence for a C-H-cleaving Fe(IV) complex in prolyl-4-hydroxylation
Lee M. Hoffart1, Eric W. Barr1, Robert B. Guyer1, J. Martin Bollinger, Jr.,2 and Carsten Krebs1,2; 1Departments of 1Biochemistry and Molecular Biology, and 2Chemistry, The Pennsylvania State University

45. The role of the heme group in the structure of b hemoproteins: The sensory PAS domain of Bradyrhizobium japonicum FixL
Daniel A. Landfried and Juliette T. J. Lecomte; Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

46. Evidence for non-native structure in the isolated heme-binding loop of cytochrome b5
Ronald Davis, Jr.; Department of Chemistry, The Pennsylvania State University

47. Substrate triggering of O2 reactivity in the Fe(II)- and α-ketoglutarate-dependent monoxygenase/chlorinase enzymes, SyrB2 and Cytc3
Gretchen Koch1, Megan Matthews2, Danica Galonici2, Eric Barr3, Chris Walsh3, Carsten Krebs1, J. Martin Bollinger Jr., 4; 4Department of Biochemistry and Molecular Biology and Chemistry, Penn State University and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

48. Binding of substrate, product, and analogs to the mixed-valent non-heme diriron(II/III) cofactor in myo-inositol oxygenase
Yinghui Diao1, Sun Hee Kim2, Gang Xing3, Eric W. Barr1, Mads-Jacob K. Kilgaard2, Lee Hoffart1, Brian M. Hoffman2, Carsten Krebs3,4, and J. Martin Bollinger Jr.1; 1Department of Biochemistry and Molecular Biology and 2Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802, 3Department of Chemistry, Northwestern University, Evanston, Illinois 60208-3113, 4Department of Chemistry, Technical University of Denmark, Kgs. Lyngby, Denmark, 2800
49. Simultaneous activation of the substrate and O₂ by a mixed-valent, non-heme, diiron(II/III) cluster and cleavage of a C-H bond by a diiron(III/III)-superoxide complex in myo-inositol oxygenase
Gang Xing¹, Yinghuai Diao¹, Eric W. Barr², Lee Hoffart¹, K. Sandeep Prabhu³,
Ryan Arner², C. Channa Reddy², Carsten Krebs¹,³, and J. Martin Bollinger Jr.¹,³,
¹Department of Biochemistry and Molecular Biology, ²Department of Veterinary
and Biomedical Sciences, and ³Department of Chemistry, The Pennsylvania State
University, University Park, Pennsylvania 16802

50. Biochemical characterization of T4 UvsW helicase: DNA unwinding and
strand annealing activities
Scott W. Nelson and Stephen J. Benkovic; Department of Chemistry, The
Pennsylvania State University, University Park, Pennsylvania, 16802

51. Caught in the act: Functional evolution in GTP cyclohydrolase II
homologs of S. coelicolor
James E. Spoonamore¹, Annie L. Dahlgran¹, Jesus Hernandez, Joon S. Kim¹, Neil
E. Jacobsen¹, Vahé Bandarian¹,³; ¹Department of Biochemistry and Molecular
Biophysics, University of Arizona, 1041 E. Lowell St., Tucson, AZ 85721;
³Department of Chemistry, University of Arizona, 1306 E. University Ave.,
Tucson, AZ 85721

52. Investigating the interaction between ribonucleoside triphosphatase reductase and thioredoxin in Lactobacillus leichmannii
C. Ainsley Davis, Nicole J. DeNisco, and Catherine L. Drennan; Massachusetts
Institute of Technology, Department of Chemistry, Cambridge, MA 02139
1. Characterizing the catalytic mechanism of the prototypic γ-class carbonic anhydrase, Cam

Sabrina A. Zimmerman and James G. Ferry; Pennsylvania State University, University Park, PA

The crystal structure of Cam reveals active site residues Gln75, Asn73, and Asn202 that potentially may participate in catalysis. Site-specific amino acid replacement variants of these residues in zinc and cobalt forms of Cam were kinetically investigated. Residues Gln75 and Asn73 were previously proposed to comprise a hydrogen bond network, similar to the characterized Thr199/Glu106 hydrogen bond network in α-class carbonic anhydrases that orients the catalytic metal-bound hydroxide as well as lowers pKₐ of the hydroxide to make it a better nucleophile. Gln75 variants showed large decreases in k-cat/K_m relative to wild-type and a loss of the pKₐ in pH vs. k-cat/K_m profiles previously attributed to ionization of the metal-bound hydroxide, supporting the proposed catalytic role of Gln75 acting analogously to α-class residue Thr199. Kinetic analyses of Asn73 variants were consistent with a role in hydrogen bonding with Gln75 to position it for optimal interaction with the catalytic hydroxide. Cam residue Asn202 was proposed to act analogous to the backbone amide of Thr199 in α-class carbonic anhydrases and the conserved glutamine in "plant-type" β-class carbonic anhydrases to stabilize the transition states of HCO₃⁻ and aid in product removal. Asn202 variants showed substantial decreases in k-cat/K_m relative to wild-type consistent with this proposed role. Based on structural and kinetic data, we present a catalytic mechanism of Cam involving Gln75, Asn73, and Asn202 that also suggests a role for Glu62 not previously recognized. Further, the results suggest that the γ-, β-, and α-class carbonic anhydrases each independently evolved residues to carry out conserved catalytic roles for carbonic anhydrase activity.
2. Probing subunit interactions in human ribonucleotide reductase by fluorescence probe and photo cross-linking agents

A Quamruil Hassan, JoAnne Stubbe*, Department of Chemistry, Massachusetts Institute of Technology

The overall goal of this research project is to better understand the subunit interactions in human ribonucleotide reductase (hRNR), and the effect of allosteric regulators (i.e. dATP, ATP) on these interactions. Human ribonucleotide reductase, which consists of two subunits (hR1 and hR2) supplies the dNTP pools needed for cell survival. The subunit interactions of hR1 and hR2 (hR2+hR1) have long been known to play a key role for hRNR activity during DNA replication and repair. Recently, another subunit (p53R2) regulated by tumor suppressor p53 was discovered, and has been shown to interact with hR1 and play a distinct role in p53-dependent DNA repair pathways. Very little is known about the interactions between p53R2 and hR1 (p53R2+hR1). Herein we propose to investigate the subunit interactions in hRNR by labeling the residues at the putative interface of hR2+hR1 and p53R2+hR1 with fluorescence probe and photo cross-linking agent. A successful outcome of this project could provide a new way to inhibit hRNRs, and compliment hRNR inhibitors, Gemzar® and Hydrea®, presently used clinically for the treatment of cancer.

3. Purification of periplasmic nitrate reductases: *Sulfospirillum barnesi* SES-3

Courtney Sparacino, Ganesh Naik, John Stolz and Partha Basu; Department of Chemistry and Biochemistry, and Biological Sciences, Duquesne University Pittsburgh, PA 15282

Molybdenum containing enzymes catalyze a variety of redox reactions in prokaryotes and eukaryotes. It has been shown that defects in genes encoding these proteins can cause disease in humans. Native bacterial flora can transform oxygen to reactive species which then can cause human disease. For example, the reduction of nitrate to nitrite may be involved in the generation of reactive NO which leads to disease like cancer. In depth investigations on the molybdenum containing enzymes exhibit substantial structural diversity. We are interested in the investigating the structure function relationship of nitrate reductases (NR) was studied. Periplasmic nitrate reductase (Nap) that reduces the nitrate to nitrite from a soil bacterium, *Sulfospirillum barnesi* SES-3, was chosen as a model for this study. Genomic investigation revealed the presence of the large NapA subunit necessary for nitrate reduction. It is important to note that SES-3 is a close relative to other *e*-proteobacteria e.g., *Campylobacter jejuni* and *Helicobacter pylori* that are pathogenic.

SES-3 was grown in a nitrate enriched media. Cells were lysed in a sucrose buffer and the membrane fraction was solubilized with a detergent (CHAPS). The CHAPS soluble membrane proteins were fractionated with salt and dialyzed and purified by ion exchange chromatography (DEAE resin). At each step of purification the samples were analyzed for the presence of the NapA by denaturing gel electrophoresis, nitrite and ammonium assays using reduced methyl viologen as the electron donor. Post chromatographic samples were analyzed with non-denaturing gel electrophoresis, western blot and atomic absorption. In this poster we will discuss the details of the purification and characterization of the periplasmic nitrate reductase from SES-3.
4. Characterization of the outer-membrane heme-receptor ShuA from the heme uptake system of *Shigella dysenteriae*: Substrate specificity and identification of the heme-protein ligands

Kimberly A. Burkhart and Angela Wilks; Department of Pharmaceutical Sciences, School of Pharmacy, 20 Penn Street, University of Maryland, Baltimore, MD 21201

*Shigella dysenteriae* like many bacterial pathogens has evolved outer-membrane receptor mediated pathways for the uptake and utilization of heme as an iron-source. While many heme-uptake systems have been identified little is known of the molecular mechanisms by which heme is ligated or transferred in such proteins. As a first step toward understanding the mechanism of heme uptake we have undertaken a spectroscopic and mutational analysis of the outer-membrane receptor ShuA of *S. dysenteriae*. Expression and purification of the outer-membrane receptor gave a single band of molecular mass 73 kDa on SDS-PAGE. Initial spectroscopic analysis of the protein in either detergent micelles or lipid bicontinuous revealed residual heme remained bound to the protein at a Soret maxima at 413 nm. However, iotration of the protein with exogenous heme gave a Soret at 437 nm in detergent micelles or 402 nm lipid bicontinuous. In contrast to the binding of free heme, transfer from hemoglobin yielded a Soret maximum identical to that of the isolated protein, suggesting hemoglobin is most likely the physiological substrate of the ShuA receptor. Further analysis revealed that the substrate specificity of the receptor was specific to heme and not the haptoglobin:hemoglobin complex or myoglobin. The rate of heme transfer from hemoglobin was also dependent on both the oxidation state and quaternary structure. On the basis of sequence alignment of all known bacterial outer-membrane heme receptors, two conserved histidine residues were identified as potential heme-coordinating ligands. Site directed mutagenesis of His-86 and/or His-420 resulted in a loss of stable complex formation and a distribution of heme between the receptor and substrate based on their relative heme-binding affinities. These findings taken together strongly suggest that His-86 and His-420 are essential for substrate recognition, heme coordination and heme transfer.

5. Syntheses and characterization of a new class of molybdenum mono/oxo complexes

Eranda I. Perera and Partha Basu; Duquesne University, Department of Chemistry and Biochemistry, Pittsburgh, PA 15282

Mononuclear molybdenum enzymes catalyze variety of reactions, which have significant impact on human health. The molybdenum centers in molybdoenzymes are coordinated by at least one pyranopterin cofactor. The pyran ring of the pterin co-factor was found to deviate from planarity by 40° and the pyrazine ring of the pterin co-factor also confirms to be distinctly puckered. Least square superposition reveals that the deviation of the bicyclic pterin system is not fixed, indicating the flexibility in these systems. Additionally the fold angle of the dithiolene chelate varies form structure to structure. The role of the cofactor may be to modulate the reduction potential of the metal center and mediate electron transfer processes to other redox active centers by modulating the interaction between the metal and the sulfur orbitals. Such interaction can be controlled by the conformation of the pyrazine ring.

In order to probe the effect of structure on the redox property of the metal center, we have designed and synthesized a new series of dithiolene ligands. Reaction of N,N diammino ethene (where R= methyl, isopropyl and phenyl) with diethyl oxalate produce N,N- 2R piperazene 2,3 diones. These diones were reacted with Lawesson’s reagent to convert into respective thiocarboxamides which were subsequently reacted with several molybdenum containing starting materials e.g., Mo(CO)2(MeCN)5, Mo(CO)2Cl2, Mo2O3Cl4(OPPB)2 and MoO(THF)3Cl to make molybdenum model complexes.

Reaction between N,N, piperazene 2,3 dithione and Mo(CO)2(MeCN), resulted in a Mo(CO)4 complex coordinated by a single dithione ligand which has been characterized crystallographically. We will also discuss the reactions of the thioamides with Mo(CO)2Cl2, Mo2O3Cl4(OPPB)2 and MoO(THF)3Cl resulting in bis N,N- R-piperazene mono(oxo) molybdenum chloride. This poster will discuss the syntheses and spectroscopic characterization of these complexes.

6. ADP-BeFx and ADP-AlF₄ stabilize a complex formed by sigma54 transcription factor and AAA+ ATPases

Baoyu Chen,¹ Michaeleneen Doucelleff,² Sacha De Carlo,¹ David E. Wemmer,²,³ Eva Nogales,⁴ Timothy R. Hoover,⁵ Elena Kondrashkina,⁶ and B. Tracy Nixon.⁷

¹Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA; ²Department of Chemistry, University of California, Berkeley, CA 94720, USA; ³Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; ⁴Howard Hughes Medical Institute, Department of Molecular and Cellular Biology, University of California at Berkeley, and Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA; ⁵Department of Microbiology, University of Georgia, Athens, GA 30602, USA; ⁶BioCAT at APS/Argonne National Lab, Illinois Institute of Technology, 9700 S. Cass Ave, Argonne, IL 60439, USA.

Abstract: The AAA+ ATPases of bacterial enhancer binding proteins (EBPs) stimulate transcription of a special set of adaptive genes in bacteria by utilizing energy from ATP hydrolysis to remodel sigma54-form of RNA polymerase. In order to understand the structural basis for regulated assembly and function of these ATPases, small- and wide-angle X-ray scattering experiments (SAXS/WAXS) were done to obtain low-resolution solution structures of the AAA+ ATPases in its apo state and when bound to various nucleotide or nucleotide analogs. Two-component signal transduction receptor domain is frequently used by EBPs to regulate the assembly of the AAA+ ATPase ring. The use of BeFx as a phosphorylation mimic to stably activate the receiver domain facilitated the structural characterization of a fully activated full-length EBp, NucC, based on which we observed a novel positive mechanism of the AAA+ ATPases ring assembly, as opposed to the negative mechanism for Nuc1 and DcdD. Upon assembly, the ATPase rings undergo conformational changes as they hydrolyze ATP. The use of ADP-BeFx-Mg as an ATP ground state analogue, ADP-AlF₄-Mg as an ATP hydrolysis transition state analogue, and ADP-Mg as product state enabled us to dissect the dynamic ATP hydrolysis process into several distinct static snapshots, which disclosed that transitions between these conformations are harnessed to perform mechanical work on the polymerase. The ATP-ground state (ADPBeF₄) and the transition state (ADPAIF₄) share similar structural characteristics, both extending the conserved GAFTGA loops of the central pore region to make contact with the sigma54 component of the polymerase. After hydrolysis of ATP, transition to the ADP-product state results in a large conformational change that is coupled with modification and release of the polymerase, which is now “powered-on” to start transcription. Based on these observations, we propose a “hug, kiss and run” model to describe the activation of sigma54-bound RNA polymerase by AAA+ ATPases.

7. Functional studies of the transcription factor NikR: A global regulator of nickel homeostasis in H. pylori

N.S. Dosanjh and S.L.J. Michel; Department of Pharmaceutical Sciences, University of Maryland, Baltimore MD 21201

NikR, a prokaryotic nickel binding transcription factor, is essential for the global regulation of nickel homeostasis in H. pylori. Specifically, NikR regulates the expression of the nickel dependent enzyme urease which is critical for the organisms’ survival in the acidic environment of the stomach. Further genes directly regulated by NikR include NixA, a nickel permease, hpn, a cytoplasmic nickel storage protein, and Fur, a global regulator of iron acquisition and utilization. It is of interest to note that the target operator sequences of these aforementioned genes do not contain any identifiable palindromes for NikR binding. Furthermore the exact mechanism of nickel-induced DNA binding remains unknown. The aims of the work described here include both investigating the metal ion requirements for NikR’s DNA binding function and determining the structural basis for the recognition of such varied target operator sequences.

H. pylori NikR was readily expressed and purified as a soluble protein with considerable α-helical secondary structure with evidence of tertiary fold. UV/visible spectroscopy revealed the protein binds stoichiometric quantities of nickel with picomolar affinities determined. Fluorescence anisotropy studies using the puReA operator revealed that the apo- and nickel bound forms of H. pylori NikR are unable to evoke DNA binding. Unexpectedly, a secondary low-affinity binding metal (Magnesium) was found to be essential for inducing NikR binding to puReA with nanomolar affinity. Interestingly, substitution of nickel with copper at the high affinity site also induced a DNA binding response suggesting that this site is functionally selective for metal ions that favor a square planar coordination geometry.

The results attained suggest that H. pylori NikR function is mechanistically distinct to that of its homologue E. coli NikR with respect to its metal ion requirements and DNA binding activity.

E-mail: ndosanjh@rx.umd.edu
8. Characterization and mechanistic studies of Escherichia coli lipoyl synthase: A member of the “radical SAM family of enzymes”

Robert M. Cicchillo*, David F. Iwig, Natasha M. Nesbitt, Camelia Gogonea, Loretta Tu, Matthew G. Souder, Carsten Krebs, and Squire J. Booker; Department of Biochemistry, Microbiology, and Molecular Biology, Penn State University, 101 Althouse Laboratory, University Park, PA 16802

Lipoic acid (6,8-thioctic acid) is an essential cofactor in several multienzyme complexes that are involved in energy metabolism, such as the pyruvate dehydrogenase complex, the 2-oxoketoglutarate dehydrogenase complex, the branched-chain oxo-acid dehydrogenase complex, and the glycine cleavage system. The biosynthesis of this cofactor involves the insertion of two sulfur atoms into two completely unactivated carbon atoms of protein bound octanoyl groups. Lipoyl synthase, the protein thought to catalyze sulfur insertion, has been cloned and purified by immobilized metal affinity chromatography. Indeed, both lipoyl synthase and biotin synthase belong to a superfamily of enzymes that use S-adenosyl-L-methionine (AdoMet) and iron sulfur clusters to generate high-energy carbon-centered radicals that are intermediates in catalysis. Proteins that are members of this superfamily have a conserved iron sulfur cluster-binding motif consisting of cysteine residues in a CXXC type pattern. Lipoyl synthase deviates from other members of this class in that it contains an additional conserved set of cysteines lying in the motif CXCXCHC. We have shown, through site-directed mutagenesis, UV-visible spectroscopy, EPR Spectroscopy, and Mössbauer spectroscopy, that the active enzyme contains two [4Fe-4S] clusters that lie within the conserved cysteine motifs. We have also demonstrated that the synthesis of one molecule of lipoic acid requires the consumption of two equivalents of AdoMet. Recently we have obtained experimental evidence through isotope labeling studies that lipoyl synthase itself donates the sulfur atoms that are inserted into positions C6 and C8 of the octanoyl group.

9. Ascorbic acid scaffold as a mechanism-based inhibitor of heme oxygenase from Pseudomonas aeruginosa

Mehul N. Bhakta1, Ayodele Olabisi1, Kandatege Wimalasena2 and Angela Wilks1; Department of Pharmaceutical Sciences, University of Maryland, Baltimore, MD 21201, and Department of Chemistry, Wichita State University, Wichita, KS 67260

Iron is an essential element needed for the survival of all bacteria. In pathogenic bacteria, the primary source of iron available to the pathogen upon infection is in the form of heme, which is complexed to various heme proteins of the host. Therefore, pathogenic bacteria, such as Pseudomonas aeruginosa (pa), have developed sophisticated mechanisms to acquire and transport heme across the outer membrane and periplasm, and deliver to a group of monoxygenases known as heme oxygenases (pa-HO). HO catalytically oxidizes heme to biliverdin, CO, and free iron, in the presence of molecular oxygen and NADPH. Although, the enzymology of HO has been studied extensively using oxygen surrogates, DFT calculations, various spectroscopic techniques, and site-directed mutagenesis over the past decades, the mechanism in which HO degrades heme remains controversial.

Ascorbic acid (AA) has been used extensively as an exogenous reductant to characterize the mechanism of oxygen activation in many heme-proteins, including HOs. AA and NADPH-cytochrome P450-reductase (CPR) support HO reactions, and are mechanistically similar in that both use molecular oxygen to initiate the catalytic cycle. Therefore, we have designed a mechanism-base probe based on AA scaffold, 5,6-O-isopropylidine-2-O-allyl-ascorbic acid (1), in order to gain insight into the mechanism of HO. Preliminary results show that 1 is able to support HO-cycle effectively, and the final product of the reaction is biliverdin. These and other observations will be presented in terms of the HO mechanism, and possible inactivation of HO by 1.
10. Crystal structure of α-ketoglutarate non-heme iron halogenase CytC3

Cintyu Wong¹, Danica Galonic¹, Christopher T. Walsh² and Catherine L. Drennan¹; ¹Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA; ²Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115, USA

Halogenated natural products, such as antibiotics and metabolites, are important for the organisms' survival. Different classes of halogenase enzymes involved in the biosynthesis of these halogenated natural products have been discovered. One class of the halogenase enzymes belong to the α-ketoglutarate (αKG) non-heme Fe(II) dependent dioxygenase family. These enzymes utilize the decarboxylation of αKG to catalyze oxidative reactions, usually hydroxylation, through hydrogen abstraction of unactivated carbons. CytC3, which belongs to this family, is an αKG-Fe(II) dependent halogenase that catalyzes the chlorination of L-aminobutyric acid (ABA) to L-amino-1-carboxycyclopropane (ACC) in cytothriienin A biosynthesis. The crystal structure of CytC3 with αKG and iron is described here to ~2.2 Å resolution. Structural comparison of CytC3 with other halogenase SyrB2 will be presented.

11. Phylogenetic metal variations as a predictor of species-specific porphobilinogen synthase druggability

Sarah H. Lawrence, Ursula Ramirez, G. Doug Markham, Eileen K. Jaffe; Fox Chase Cancer Center, Philadelphia, PA

Porphobilinogen synthase (PBGS, 5-aminolevulinate dehydratase, EC 4.2.1.24) catalyzes a fundamental step in the biosynthesis of the tetrapyrrole pigments and its activity is essential to nearly all free-living organisms. PBGS exists in an equilibrium of "morpheins", alternate quaternary structures whose interconversion is governed by a ligand-induced shift in the monomer structure, and has served as the prototype to examine this novel mechanism of activity regulation. The human and pea PBGS have been shown to exist as an equilibrium of high-activity octamers and low-activity hexamers. Many PBGS's contain two metal binding sites per monomer: one at the enzyme active-site (usually zinc) and one at an allosteric site (usually magnesium). The human PBGS has zinc at the active site but lacks the allosteric metal binding site, while the pea PBGS (and PBGS's from a number of pathogenic bacteria) has magnesium at the allosteric site, and a metal other than zinc at the active site. This variation in metal usage is one factor that imparts species-specific differences in the morphein equilibrium, and creates an attractive target for drug development. A compound that could trap a bacterial PBGS as an inactive hexamer may retain the human PBGS as an active octamer. In silico docking was performed to identify small molecules that bind at the subunit interfaces of a hexameric pea PBGS structural model, shifting the equilibrium towards the hexamer and inhibiting the enzyme's activity. Starting from a sub-library of about 100,000 molecules available from Life Chemicals, Inc., compounds with the best scores were manually sorted according to binding orientation and solubility estimates. 76 promising compounds were purchased; thirteen were insoluble and five were incompatible with the assay methodology. Four of the remaining compounds inhibited the activity of pea PBGS in a time- and dose-dependent manner at micromolar concentrations. Of these, three did not inhibit the activity of the human PBGS, supporting the potential of this approach for drug discovery.
12. Reaction between peroxynitrite and cytochrome P450BM3 yields nitrosyl

Rachel Behan\(^1\), Lee M. Hoffart\(^6\), Kari L. Stone\(^6\), Carsten Krebs\(^{ab}\), and Michael T. Green\(^1\); \(^a\)Department of Chemistry and \(^b\)Department of Biochemistry and Molecular Biology, The Pennsylvania State University

Peroxynitrite has come into the limelight in recent years. Its effects on biomolecules have been implicated in several disease states such as neurodegenerative, chronic inflammatory, gastrointestinal tract, and cardiovascular disorders, as well as viral and bacterial infections. Peroxynitrite has been shown to inactivate PG\(_L\) synthase, a thiolate-ligated heme protein involved in cardiovascular function. This discovery prompted the investigation of the mechanism of PG\(_L\) synthase inactivation using bacterial P450s as models. It has previously been shown that the reaction between cytochrome P450BM3 and peroxynitrite results in the formation of compound II. In an effort to characterize this intermediate we discovered that the intermediate was not an Fe(IV)oxo complex, but rather a ferric nitrosyl. We have used Mössbauer, resonance Raman, and stopped-flow spectroscopies to characterize the peroxynitrite-generated intermediate, as well as the P450BM3 nitrosyl complex.

13. Kinetics of dissimilatory iron reduction in Shewanella oneidensis MR-1: scaling from purified proteins to whole cell cultures

Dan Ross\(^1\), Susan Brantley\(^2\), Ming Tien\(^3\); \(^1\)Department of Biochemistry and Molecular Biology; \(^2\)Department of Geosciences; \(^3\)Department of Biochemistry and Molecular Biology

Shewanella oneidensis MR-1 is a gram-negative facultative anaerobe that has a versatile respiration system capable of utilizing a large number of terminal electron acceptors. Genetic studies have identified most if not all of the proteins involved in iron reduction; however, the mechanism of iron reduction is yet to be determined. Our research is focused on how this bacterium is able to utilize solid iron forms as its terminal electron acceptor. Solid iron oxides are the predominant form which iron is found on the earth’s crust. In order to fully understand how this process works, we have taken a biochemical/kinetics approach. We have isolated purified proteins to obtain kinetic constants from transient-state kinetic studies using a stop flow. We have also obtained steady-state kinetic constants as well as rate constants from whole cell cultures. Furthermore, we can obtain molecular rate constants if the concentration of the catalyst is known. Proteins can be quantitated through Western-blot analysis using polyclonal antibodies. Taken together these studies allow for a determination of rates across scales that yield insight to reaction mechanisms involved in dissimilatory iron reduction.

\(^1\)der180@psu.edu
\(^2\)brantley@escs.psu.edu
\(^3\)mtt3@psu.edu
14. Characterization of PscB, an iron-sulfur protein from the photosynthetic reaction center of Chlorobium vibrioforme

Bharat Jagannathan and John H. Golbeck. Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802

Chlorobium vibrioforme is a green-sulfur bacterium that performs anaerobic photosynthesis using Type I reaction centers, i.e. iron-sulfur clusters serve as the terminal electron acceptors. The iron-sulfur clusters are harbored in a protein denoted PscB, which is bound to the reaction center on the cytoplasmic side of the membrane. This study involves the initial characterization of recombinant PscB, with the ultimate aim of elucidating the NMR solution structure of the unbound protein. Based on the amino acid sequence and the presence of two [4Fe-4S] clusters (S = 1/2) in the dithionite-reduced protein. The identity of the second iron-sulfur cluster is under active investigation. The absence of any spectral features around g = 4 to 6 tend to rule out the presence of a high spin (S = 3/2) [4Fe-4S] cluster. Instead, a [3Fe-4S] cluster was found when the protein was oxidized with potassium ferricyanide. This is in contrast to PsaC, its counterpart in Photosystem I, and PslB in Helitobacterium modesticaldum wherein a complex EPR spectrum is obtained due to dipolar interaction upon the reduction of the two terminal [4Fe-4S] clusters. It is interesting that while one iron-sulfur cluster-binding motif is the traditional sequence of CXXCXXCXXXXCP, the other is an unusual sequence of CXXCXXCXXXXCP. We are investigating whether electron transfer in Chlorobium reaction centers is regulated through a [4Fe-4S] cluster interconversion. A detailed structural and functional characterization will enable the complete understanding of the unusual properties of the Fe-S clusters in PscB.

15. Implications of metal coordination on NikR specificity: An update on the Zn(II)-bound NikR structure

Christine M. Phillips, Eric Schreiter, Yayi (Stephanie) Guo, Catherine L. Drennan. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

The concentration of transition metals within the cell must be tightly regulated. If the concentration of a given transition metal is too low the cell may not be able to perform life-sustaining processes, while high levels of metals are poisonous to the cell and can initiate apoptosis. Nickel concentration within E. coli is regulated by a transcription factor NikR that blocks the transcription of an ABC-type transporter specific for nickel. When the concentration of nickel in the cell is high, nickel binds to NikR, which in turn binds to the nik operon, preventing transcription of NikABCDE, the importer complex.

The NikR protein has been shown to bind not only Ni(II), but a number of transition metals including Cd(II), Co(II), Cu(II), and Zn(II) [1]. The affinities of these metals to NikR vary according to the Irving-Williams series: Co(II) < Ni(II) < Cu(II) ≥ Zn(II) [2]. With other metals bound at 1:1 stoichiometry, the affinity of NikR for the nik operon is as follows: (1.1 ± 0.2) x10⁸ for Co(II); (5 ± 1) x10⁷ for Ni(II); (3 ± 1) x10⁶ for Cu(II); and (2.7 ± 0.4) x10⁵ for Zn(II) [1]. To understand how metal binding geometries influence the affinity of NikR for DNA, we are determining a series of X-ray structures of the Metal Binding Domain (MBD) of NikR with different metals bound. These structures can then be compared to the Ni(II)-MBD structure [3] to examine the coordination geometries and parameters that report on structural flexibility (B-factors, occupancies). Here we will discuss the progress on the Zn(II)-MBD X-ray structure.

16. A new generation of dithiolene ligands

Barbara Serli Mitasev and Partha Basu; Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, PA

Molybdenum is present in the enzyme nitrogense as part of a multinuclear cluster with seven iron atoms, and in a mononuclear center in other molybdoenzymes. Mononuclear molybdenum enzymes catalyze a variety of reactions that are important in the metabolism of nitrogen and sulfur-containing compounds. In the active site of mononuclear molybdenum enzymes, the metal is coordinated to one or two equivalents of the pyranopterin (or molybdopterin) cofactor. With the exception of membrane bound respiratory nitrate reductase (Nar), in all cases the tricyclic ring remains intact. In Nar, however, a ring opened form has been observed. The key features of pyranopterin cofactor are: a) the pyran ring can undergo ring scission which may influence the reactivity; b) the dithiolene moiety as well as the pyridine unit are redox noninnocent and can influence the redox chemistry; c) high degree of metal-sulfur covalency present in the Mo-dithiolene can be modulated by distortion at the pyran ring as well as the pyrazine ring of the cofactor.

In recent years, many molybdenum complexes have been synthesized and investigated as models for molybdenum enzymes. In the majority of the systems, the metal is coordinated to simple unfuctionalized dithiolene. Very recently two reports outlined the synthesis of molybdenum complexes with dithiolene ligands that contain the pyran feature of the cofactor: [MoO(II)I] (II= flavanyl-1,3-dithiolate) and [MoO(II)I] (II= 2,3-dihydro-2H-pyran-4,5-dithiolate). We have developed a strategy for synthesizing a new generation of ligands based on the structure of the pyranopterin.

The synthesis of these new compounds will be discussed.

References:

17. Halogenation of unactivated carbon centers in natural product biosynthesis: Trichlorination of leucine during barbamide Biosynthesis and mechanistic insight into halogenation process

Danica P. Galonie, Frédéric H. Vaillancourt, Eric W. Barn, Carsten Krebs, J. Martin Bollinger, Jr., and Christopher T. Walsh; Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115 and Department of Biochemistry and Molecular Biology and Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

Halogenation is a common structural feature of more than 4500 natural products of both marine and terrestrial origin. This biosynthetic modification has a profound influence on the biological activity of the resulting compounds. Recently, our laboratory described a novel class of halogenating enzymes capable of carrying out halogenations at aliphatic carbon centers of pepitidyl carrier protein-linked amino acid residues. These enzymes are nonheme FeIII halogenases that require oxygen, α-ketoglutarate and chloride for their activity. The in vitro reconstitution of leucine halogenation during barbamide biosynthesis has been accomplished. It has been demonstrated that the triple chlorination of the unactivated pro-B methyl group of the pepitidyl carrier protein-tethered L-Leu substrate is carried out by the tandem action of two nonheme iron(III)-dependent halogenases, BarB1 and BarB2. Investigation of the substrate specificities of each of the halogenating enzymes revealed their complementary roles in the generation of trichloroleucine.

Mechanism of aliphatic halogenation process was studied in the context of chlorination of L-2-amino-butyric acid tethered to pepitidyl carrier protein CysC2 with aliphatic halogenase CysC3 from cytorigenin system. Stopped-flow absorption experiments and Mössbauer spectroscopy have provided evidence for the intermediacy of a kinetically competent high-spin FeIII oxo species.
18. Synthetic ribonucleosides as mutagenic antiviral agents

Jocelyn P. Edathil, Jason D. Graci, Craig E. Cameron, and Blake R. Peterson; Department of Chemistry and Biochemistry and Molecular Biology, The Pennsylvania State University

Lethal mutagenesis promoted by small molecules is a novel strategy to combat infection by RNA viruses. This mechanism is exploited by the broad-spectrum antiviral drug Ribavirin. This drug comprises a ribonucleoside that is converted to the triphosphate in vivo, is incorporated into the viral RNA genome, and promotes viral mutagenesis by templating incoming nucleosides. The resulting viral mutagenesis dramatically decreases viral fitness and infectivity. Based on this mechanism of action, we are designing antiviral agents based on ribonucleosides with potentially greater mutagenic potential than Ribavirin. Hydrophobic universal bases that template all four nucleosides are currently under investigation as lethal mutagens. Ribonucleosides utilizing the isocarboxystyryl scaffold have been explored in this endeavor. The design, synthesis and evaluation of hydrophobic ribonucleosides as antiviral agents will be presented.

19. Spectroscopic studies of high-valent Intermediates of chloroperoxidase

Kari L. Stone, Rachel K. Behan, Lee M. Hoffart, Carsten Krebs, and Michael T. Green*; The Pennsylvania State University, Department of Chemistry, University Park Pennsylvania, 16802, USA, email: kl1174@psu.edu

Using a combination of density functional theory calculations and Mössbauer spectroscopy, we have examined chloroperoxidase compound II (CPO-II). The Mössbauer spectrum of CPO-II shows for the first time the presence of two distinct ferryl species.[1] Together with Mössbauer experiments coupled with density functional calculations, cryogenic γ-irradiation experiments, and a previous EXAFS investigation, we assign the major species as the protonated ferryl. We find that CPO-II is ~70% protonated. The Mössbauer parameters of the minority component are indicative of an Fe(IV)-oxo unit. Surprisingly, the ratio of the two species is pH invariant, but this is in agreement with the unchanging absorption spectrum of CPO-II over its pH stability. The results of our spectroscopic investigations of CPO-II will be presented.

20. Altering substrate specificity of Phosphatidylcholine-Preferring Phospholipase C of Bacillus cereus by random mutagenesis of the headgroup binding pocket

Nina M. Goodey, Paul J. Hergenrother, Micheleen M. Harris, William Corbett, and Stephen F. Martin; *Presenter (formerly Nina M. Antikainen)
Department of Chemistry, The Pennsylvania State University, 414 Wartik Laboratory, University Park, PA 16802; †Department of Chemistry, University of Illinois, Urbana, IL 61801; ‡Department of Chemistry and Biochemistry and The Institute of Cellular and Molecular Biology, The University of Texas at Austin, Austin, Texas 78712

Phosphatidylcholine-Preferring Phospholipase C of Bacillus cereus (PLC<sub>BC</sub>) is a monomeric 28.5 kDa phosphodiesterase that contains three zinc ions in its active site. PLC<sub>BC</sub> catalyzes the hydrolysis of the phosphodiester bond of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidyl-L-serine (PS) with the ratios of the corresponding specificity constants, k<sub>cat</sub>/K<sub>M</sub>, being approximately 10:7:1 to provide a diacylglycerol (DAG) and the corresponding phosphatidylated head group (Figure 1). Three residues in the substrate binding pocket of PLC<sub>BC</sub> were randomly varied via region-specific random mutagenesis and variants with altered substrate specificities were identified and characterized. This study led to a better understanding of the determinants for substrate specificity in the PLC<sub>BC</sub> binding pocket. (Antikainen et al. 2003 Biochemistry 42, 1603)

![Figure 1](image)

Figure 1. General structures of three families of phospholipids, PC, PE, and PS. The dotted line indicates the phosphodiester bond that is hydrolyzed by PLC<sub>BC</sub>.

21. A novel diferric-peroxo intermediate in the O<sub>2</sub> reaction of Toluene/o-Xylene Monoxygenase Hydroxylase II100W variant, that decays to diferric state via a Fe<sup>III</sup>Fe<sup>IV</sup> transient

Ricardo García-Serres, †Leslie J. Murray, ‡Viviana Izzo, ‡Sunil Naik, †Boi Hanh Huynh, and Stephen J. Lippard; †Department of Physics, Emory University, Atlanta, Georgia and ‡Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts.

The reactions of different Toluene Monoxygenase Hydroxylase (wild type as well as 1100W variant) or diferric Phenol Hydroxylase II104Fe/F198W variant from Pseudomonas stutzeri OX1 with dioxygen have been studied by stopped-flow and rapid freeze-quench techniques in conjunction with EPR and Mössbauer spectroscopies. For the three systems, the first intermediate observed is an EPR-silent species with no apparent UV-vis absorption, which shows up in the Mössbauer spectra as a quadrupole doublet with parameters ($\beta$ = 0.55 ± 0.04 mm/s and $\Delta E_Q$ = 0.64 ± 0.06 mm/s) characteristic of high-spin Fe<sup>III</sup>. High-field Mössbauer shows diamagnetism, indicating antiferromagnetical coupling between the two Fe<sup>III</sup> ions, presumably bridged by a peroxo moiety. The Mössbauer parameters differ, however, from those of peroxodicirrifer intermediates reported for other diiron proteins (MMOH, R2, Ferritin, 89D), which all have $\beta$ > 0.60 mm/s and $\Delta E_Q$ > 1.00 mm/s, suggesting an unusual core structure for the peroxo complex in TomOH and PH. Moreover, no diferryl complex is detected subsequent to the first transient in any of the three time courses studied, as opposed to MMOH, where H<sub>peroxo</sub> decays to diferryl Q, which hydroxylates methanol. Interestingly, for the TomOH II100W variant, the peroxodiciron(III) transient decays via a mixed-valent Fe<sup>II</sup>Fe<sup>IV</sup> complex spin-coupled to a W<sup>+</sup> radical.

![Complex Diagram](image)

This Fe<sup>II</sup>/Fe<sup>IV</sup>-W<sup>+</sup> complex reaches a maximum at ~3s, and then decays to yield a diferric product. Results of MALDI-TOF experiments indicate hydroxylation of the W100 position in that product. In the light of these results, the catalytic mechanism of diiron aromatic hydroxylases will be discussed.
22. The effect of iron binding on the function of NUP475, a non-classical zinc finger protein involved in inflammatory response

Seung Jae Lee, Robert C. d’Targiani, Sarah L. J. Michel; Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore

The protein NUP475 (also known as tristetraprolin and tis11) regulates the inflammatory response by binding to AU-rich sequence elements located at the 3’ untranslated region of cytokine mRNAs forming a complex that is degraded by the exosome. The nuclear acid binding region of NUP475 is comprised of two CysX6CysX5CysX1His domains that are activated in the presence of zinc. A two-domain construct of NUP475 (NUP475-2D) has been cloned and over-expressed in E. coli. NUP475-2D picks up visible red coloration from the expression media, unless it is expressed under iron-restricted conditions. The iron binding properties of NUP475-2D and the effect of iron substitution on RNA recognition have been investigated. Both Fe(II) and Fe(III) bind to NUP475-2D and a full titration of Fe(III) with NUP475-2D revealed that this metal ion binds with micromolar affinity. Upon reconstitution of NUP475-2D with either Fe(II) or Fe(III), the protein recognizes a canonical RNA binding sequence, UUUUUUUUUUU with nanomolar affinity. Substitution of a single or both adenines results in a decreased affinity of NUP475-2D for the RNA molecule, demonstarting that both Fe(II)-NUP475-2D and Fe(III)-NUP475-2D selectively recognize a physiologically relevant RNA sequence. The relative affinities of Fe(II)-NUP475-2D and Fe(III)-NUP475-2D for the series of RNA sequences mirror those observed for Zn(II)-NUP475-2D and suggest that iron is a viable substitute for zinc in this protein.

23. WrbA from *Escherichia coli* and a homologue from *Archaeoglobus fulgidus* are NAD(P)H: Quinone reductases

Eric V. Patridge*, Susana L. Andrade*, Olivier Einsle*, and James G. Ferry*;
*Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802; " Institut fur Mikrobiologie und Genetik, Georg-August-Universitat, Justus-von-Liebig-Weg 11, 37077 Gottingen, Germany

The so-called tryptophan repressor binding protein (WrbA) from *Escherichia coli* is the only one characterized of the 200 estimated homologues, which span all domains of life. The 21 kDa flavoprotein exhibits a dimer-tetramer equilibrium, with one FMN cofactor bound per monomer. Only a few articles have been published addressing the function of WrbA: one suggests that EcWrbA enhances binding of the tryptophan repressor to its DNA target and another suggests it does not. Literature concerning the global expression of stress-response genes shows that wrbA is upregulated in a variety of environmental conditions (pH, salt, and diaxie), under transcriptional control of RpoS (the stress-response sigma factor). The wrbA gene has also been shown to be upregulated in the early stages of the stationary phase, indicating that WrbA could play a role in preparing the cell for long-term maintenance under stress conditions. Through sequence alignment, WrbA appears to be a member of the flavodoxin family and has significant similarity to several flavodoxins, iron-sulfur flavoproteins, and quinone reductases. Within the genome of *Archaeoglobus fulgidus*, wrbA is clustered with rubredoxin, nigerythrin, and neelaredoxin, which could be consistent with an operon that addresses oxidative stress. We have cloned wrbA from *E. coli* and from *A. fulgidus* and have used *E. coli* to express and purify both encoded proteins (EcWrbA and AfWrbA). Like EcWrbA, the 21 kDa monomer of AfWrbA forms a homodimer, as estimated by size-exclusion chromatography, and HPLC analysis shows the presence of a FMN cofactor. We have shown that both EcWrbA and AfWrbA transfer electrons directly from NAD(P)H to several quinones, DCIP, and potassium ferricyanide. We have also qualitatively demonstrated that Rd and Nir are both reduced in the presence of NADH and AfWrbA, under anaerobic conditions. We hypothesize that WrbA functions in response to environmental stress to alleviate oxidative stress, potentially having multiple roles.
24. **MA3736** from *Methanosarcina acetivorans* is an iron-sulfur protein with disulfide reductase activity

Daniel J. Lessner and James G. Ferry; Pennsylvania State University, Department of Biochemistry and Molecular Biology, University Park, PA

We are using *Methanosarcina acetivorans* as a model organism to elucidate the oxidative stress response in the methanogenic archaea. The genome of *M. acetivorans* contains a conserved gene cluster (MA4664-MA3743) that encodes homologs of enzymes known to function in response to oxidative stress in other anaerobes and proteins of unknown function. MA3736 encodes a protein of unknown function, but contains a Cys-X-X-Cys (CXXC) motif similar to the active site of alkylperoxide reductase (AhpD) from *Mycobacterium tuberculosis*. AhpD is the disulfide reductase partner to AhpC, a peroxiredoxin. Heterologously-expressed MA3736 was purified and demonstrated in vitro protein disulfide reductase activity under anaerobic conditions. In addition to the CXXC cestines (Cys67 and Cys70), MA3736 contains two additional cysteine residues (Cys39 and Cys107). Analysis of cysteine to serine variants of MA3736 demonstrated that Cys67 and Cys70 are necessary for protein disulfide reductase activity. Additional analysis of MA3736 and variants revealed the presence of a putative chromophore, consistent with an iron-sulfur cluster. Cys67 and Cys70 were necessary for binding of the putative iron-sulfur cluster. In addition, protein disulfide reductase activity was dependent on the presence of EDTA. Size-exclusion chromatography revealed that the C67S/C70S variant is a trimer, whereas wildtype MA3736 and the non-active site cysteine variant (C39S/C107S) are in a larger oligomeric state. However, upon incubation with EDTA, wildtype MA3736 and the C39S/C107S variant were converted to a smaller oligomeric state. These results suggest MA3736 contains an intermolecular iron-sulfur cluster that is coordinated by the active-site cysteines, maintaining MA3736 in an inactive state. Loss of the cluster results in conversion to a smaller active-form. Therefore, we propose MA3736 contains an iron-sulfur cluster that controls oligomerization as a mechanism to regulate protein disulfide reductase activity.

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25. **Characterization of a lipoil synthase from Thermus thermophilus**

Kyung-Hoon Lee and Squire J. Booker; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

Lipoic acid is a sulfur-containing cofactor found in most prokaryotic and eukaryotic organisms. In *Escherichia coli* and other organisms, it is essential for the function of several important enzymes involved in oxidative and single carbon metabolism including pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (2-OGDH), branched-chain 2-oxo-acid dehydrogenase, acetoacetyl-CoA thiolase, and the glycine cleavage system (1). In its functional form it is attached to the epsilon amino group of a specific lysine amino acid located on one of the subunits of each complex. In *E. coli*, free lipoic acid can be attached via the action of lipoate protein ligase A (LpLA), which constitutes the exogenous pathway for formation of the lipoic acid. The endogenous pathway is a derivative of fatty acid biosynthesis, and involves two enzymatic activities. Octanoyltransferase (LpB) catalyzes the transfer of the eight-carbon fatty acyl chain from octanoyl--acyl carrier protein to the appropriate lipoil carrier protein. Lipoil synthase (LpA) then catalyzes insertion of sulfur atoms into C--H bonds at positions six and eight.

Our laboratory has been interested in characterizing the detailed reaction mechanisms of both enzymes involved in the endogenous biosynthesis of the lipoic acid with a particular focus on LpA. LpA is a member of a recently established class of metalloenzymes that use S-adenosyl-L-methionine (SAM) as the precursor to a high-energy 5′-deoxyadenosyl 5′-radical (5′-dA). These enzymes all contain [4Fe-4S] clusters, which participate intimately in the cleavage reaction. In efforts to obtain an X-ray crystal structure of the enzyme, we are interested in obtaining and characterizing a more thermally stable form of LS. Herein we present the cloning and purification of a lipoil synthase from *Thermus thermophilus* HB8, which displays about 53% identity with the protein from *E. coli*. We employ a combination of spectroscopic and (Mössbauer, EPR, and UV-visible) analytical methods (iron, sulfide, and protein quantification) to characterize the iron-sulfur clusters of the protein, and also characterize the efficiency by which the protein catalyzes sulfur insertion.
26. Determination of the cysteine residues that coordinate the [4Fe-4S] cluster in quinolinate synthases

Amy Griffths, Allison Saunders, Jeffrey Stromberg, Loretta Tu, and Squire J. Booker; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

Quinolinate acid is a key intermediate in the biosynthetic pathway for nicotinamide adenine dinucleotide (NAD), an essential cofactor in a variety of biological redox reactions. In prokaryotic organisms, quinolinate acid is formed via the action of two enzymes, L-aspartate oxidase (NadB), which generates iminoaspartate from L-aspartate, and quinolinate synthase (NadA), which catalyzes a condensation reaction between dihydroxyacetone phosphate and iminoaspartate. Characterization of the protein by a number of physical methods, including electron paramagnetic resonance, Mössbauer, and UV-visible spectroscopies, as well as quantification of bound iron and sulfide, indicate the presence of one [4Fe-4S] cluster per polypeptide in *Escherichia coli* NadA [Ciocchillo, R. M., Tu, L., Stromberg, J. A., Hoffart, L. M., Krebs, C., and Booker, S. J. (2005) *J. Am. Chem. Soc.*, 127, 7310-7311], which is believed to be essential for catalysis. In addition, the protein contains nine cysteine residues, three of which lie in a CX3CX2C motif characteristic of those in which the cysteines therein act as ligands to [4Fe-4S] proteins. Analysis of variants containing cysteine to serine substitutions in NadA from *E. coli* indicate that only one of the cysteine residues in this motif is an actual ligand to the cluster.

27. The function of bicarbonate in the reaction catalyzed by *Escherichia coli* cyclopropane fatty acid synthase

David F. Iwig and Squire J. Booker; Department of Biochemistry, Microbiology, and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

Cyclopropane fatty acid (CFA) synthase catalyzes the formation of cyclopropane rings in the acyl chains of phospholipid unsaturated fatty acids. The CFA synthase reaction involves the formal transfer of a methylene group from the methyl moiety of S-adenosyl-L-methionine (AdoMet) to an unactivated cis-olefin of an unsaturated fatty acid, producing one equiv of S-adenosyl-L-homocysteine and one proton per cis-cyclopropane ring formed. Our lab has previously determined using isotope and elemental effects that the reaction proceeds through a rate-limiting methyl transfer followed by fast ring closure and deprotonation steps.

Cyclopropane mycolic acid (CMA) synthases are present in slow-growing, pathogenic species of mycobacteria such as *Mycobacterium tuberculosis* and *M. leprae*, and share ~35% similarity to *E. coli* CFA synthase. Recently, the crystal structures of three CMA synthases have been reported which revealed the presence of bicarbonate in the respective active sites of the CMA synthases [Huang, C., Smith, C. V., Glickman, M. S., Jacobs, W. R., Sacchettini, J. C. (2002) *The Journal of Biological Chemistry* 277, 11559-11569]. Subsequently, our lab demonstrated the dependence of CFA synthase activity on the presence of bicarbonate. Herein, through use of kinetic isotope effects and substrate analogues, we describe the function that bicarbonate plays in the CFA synthase reaction mechanism.
28. Electrochemical characterization of cytochrome C₅₅₄ from *Nitrosomonas europaea*

Pulcu, G. S.; Hooper, A. B.; and Sean J. Elliott; "Department of Chemistry, Boston University, Boston, MA 02215; "Department of Biochemistry, University of Minnesota, St. Paul, Minnesota 55108

In Elliott Lab we work on different redox active proteins and enzymes from different organisms. The small tetraheme cytochrome C₅₅₄ from *Nitrosomonas europaea* has been characterized electrochemically using the technique of protein film voltammetry (PFV). Cyt C₅₅₄ from the chemosynthetic soil bacterium *Nitrosomonas europaea* plays a key role in the conversion of ammonia to nitrite, the basis of *N. europaea* bioenergetics. Cyt C₅₅₄ is a 25 kDa electron transfer protein with four c-type hemes, which serves as a mediator between Hydroxylamine oxidoreductase (HAO) and electron acceptors in the periplasm, the identities of which are unknown. While HAO catalyzes the four-electron oxidation of NH₂OH to NO₂⁺, the four electrons generated are transferred to cyt C₅₅₄. Our goal is to clarify the ET mechanism of cyt C₅₅₄, using PFV, which allows for the rapid interrogation of all of the redox cofactors of the cytochrome. We have started to study the electrochemical behavior of cyt C₅₅₄ on different gold electrode surfaces. Reversible protein films display fast electron transfer, and redox potentials of -195, -518, -398 and -195mV vs SCE are resolved in the PFV experiment. Results from the variation of the electrode surface indicates a dynamic behavior of one of the heme centers, that is dependent upon the use of either mercaptoundecanoic acid or mercaptobenzoic acid as an electrode-modifying reagent. Scan-rate variation has indicated that there must be an EC mechanism taking place at the Heme 2 center and pH-dependent PFV is currently being used to investigate the proton-coupled nature of the observed redox reactions.

suakks@chem.bu.edu

29. Biochemical and structural characterization of thioredoxins from *Archaeoglobus fulgidus*

Hector H. Hernandez¹, Sean J. Elliott², and Catherine L. Drennan¹; (1)Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, (2) Department of Chemistry, Boston University, Boston, MA

Thioredoxins (TRXs) are small proteins responsible for maintaining the reduction potential in cells. They have been implicated in response to inflammation, oxidative stress, and apoptosis. TRXs also mediate the re-reduction of ribonucleotide reductases (RNRs). TRXs are characterized by a CxxC motif in the active site. The reduction potential of TRX is thought to be a function of the specific sequence of amino acids between the conserved cysteines in the active site. To investigate the relationship of the CxxC motif to activity, we have identified the *A. fulgidus* genes encoding four TRX (aTRX-1, aTRX-2, aTRX-3, aTRX-4) proteins by sequence homology, and have cloned these genes. The genes were sequenced and the recombinant (His)_6-tagged TRX proteins have been expressed and purified. Our progress with the electrochemical and structural characterization of these proteins will be presented.
30. Electrochemical investigation of CueO, a multicopper oxidase involved in copper homeostasis in E. coli

Tao Ye,1 William R. Montfort2 and Sean J. Elliott3; 1Chemistry Department, Boston University, Boston, MA 02215; 2Department of Biochemistry and Molecular Biophysics, University of Arizona, 1306 E. University Blvd, Tucson, Arizona, 85721-0041

A direct electrochemical study using protein film voltammetry (PFV) of the multicopper oxidase CueO will be presented. Copper proteins perform many critical biological functions, the many of which are coupled the chemistry of dioxygen. Here, we have investigated how Nature couples one electron transfer events through a blue copper center (CuII/CuI) to either the 2 or 4 electron reduction of O2 in E. coli the potential toxicity of copper along with dioxygen reactivity, require complex systems of copper homeostasis, which include the enzyme CueO. CueO contains three classical copper-binding sites, namely, Type I (T1), T2, T3, and a putative regulatory site (rCu) that binds a fifth copper. In initial studies, we have investigated the redox properties of the multiple redox-active copper ions of CueO using direct cyclic voltammetry (CV). By examining both the non-turnover and turnover signals, we have elucidating the reduction potential associated with the resting enzyme, as well as that of catalysis. In the meantime, any coupled chemical reactions are of equal interest. Two approaches have been utilized to measure the formal reduction potential of CueO T1 center in vitro, which is approximately 300mV vs. standard hydrogen electrode (S.H.E.). Moreover, electrochemical experiments have been further carried out to evaluate the influence of inhibitors upon the redox characteristics of CueO.

email: yetao@bu.edu

31. Structural Investigation of RebP and RebC

Katherine S. Ryan,1 Annailese R. Howard-Jones,1 Christopher T. Walsh,1 and Catherine L. Drennan2; 1Department of Biology, 2Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, 02139, 
1Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Rebcamycin is an indolocarbazole alkaloid with anti-tumor properties produced by the bacterium Lechevaliera aerocolonigenes. Key to the biosynthesis of rebcamycin is the multi-electron oxidation of the compound chromopyrrole acid. This remarkable transformation – involving an aryl-aryl coupling and two oxidative decarboxylations – is carried out by the enzyme pair RebP and RebC.

RebP, a cytochrome P450 protein, is responsible for catalysis while RebC appears to modulate its activity affecting product distribution [Sánchez, et. al (2005) PNAS 102: 461-6]. We are interested in determining the structures of both RebP and RebC in order to facilitate an understanding of this enzymatic reaction.
32. Role of protein environment in redox regulation of phyloquinone and its effects on electron transfer rates in Photosystem I

Nithya Srinivasan and John H. Golbeck; Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802

Photosystem I is a multisubunit pigment-protein complex that converts photons to chemical bond energy. The Photosystem I reaction center is comprised of a Psaa/PsbA heterodimer and two bifurcating branches of electron transfer cofactors consisting of six chlorophyll molecules (including P700, the primary electron donor, and A680, the primary electron acceptor), two phyloquinones, and one [4Fe-4S] cluster, F5. Phyloquinone acts as an intermediate, oxidizing the primary electron acceptor A680 and reducing the F5 iron sulfur cluster. In prior work, two kinetic phases of phyloquinone oxidation have been measured, ca. 10 ns and 200 ns, by time resolved optical spectroscopy, which are attributed to electron transfer through the PsbbA-branch and PsaaA-branch, respectively. Curiously, only the slow kinetic phase has been measured by transient EPR spectroscopy. The 2.5 Å X-ray crystal structure indicates that the phyloquinones in the electron transfer chain are hydrogen bonded to Leu718Psaa and Leu697PsbA and are π-stacked with Trp589Psaa and Trp648PsbA. The H-bond and π-stacking are thought to be the primary determinants of the low redox potential (ca. -730 mV) of the phyloquinones. The premise of the experiment is that changing the environment of the quinones should alter their redox potential, which according to Marcus theory, should result in a change in the rate of electron transfer. The experimental program is to substitute a bulky amino acid in place of Leu718Psaa and Leu697PsbA in the expectation of precluding quinone binding on the PsaaA-side and PsbbB-side respectively. If the two kinetic phases are due to electron transfer up the two branches of cofactors, then when the PsaaA-branch phyloquinone is missing, only the fast kinetic phase will be seen; conversely, when the PsbbB-branch phyloquinone is missing, then only the slow kinetic phase will be seen. The rate of electron transfer will be determined by both time-resolved optical spectroscopy in the near-UV and by transient EPR spectroscopy. Similar studies are planned for variants in which the π-stacking is altered by substitution of Trp589Psaa and Trp648PsbA with isoleucine. This study is therefore designed to elucidate the influence of the protein environment on the midpoint potential of a bound quinone cofactor.

33. The role of molybdenum and ModE in gene regulation in the green sulfur bacterium Chlorobium tepidum

Christina Cress1, Julia Maresca2 and Donald Bryant3; The Pennsylvania State University

Molybdenum is a transition metal found in trace amounts in the environment, and molybdenum-containing cofactors are frequently required for nitrogen and sulfur metabolism. The protein ModE is an important regulator of molybdenum uptake and utilization in many bacterial species. A modE homolog was identified in the genome of the green sulfur bacterium Chlorobium tepidum, and several putative ModE-binding sites have been predicted. The putative modE regulon comprises six operons in the Chl. tepidum genome, including the modABC molybdate transporter, two nir operons that encode for molybdenum nitrogenase genes and several molybdopterin biosynthesis genes. This study investigates the effects of limiting nitrogen and molybdenum concentrations on growth and gene expression in wild-type Chl. tepidum and a modE mutant strain. ModE is only the second regulatory protein to be characterized in green sulfur bacteria, and these experiments describe how it enables green sulfur bacteria react to different environmental conditions.

1 cmc365@psu.edu
2 jam636@psu.edu
3 dab14@psu.edu
34. The role of the C-8\(^2\) and C-12\(^1\) methylations of bacteriochlorophyll \(c\) in the green sulfur bacterium \textit{Chlorobium tepidum} 

Aline Gomez Maqueo Chew, Niels-Ulrik Frigaard, and Donald A. Bryant; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA

Bacteriochlorophyll \(c\) (BChl \(c\)) accounts for more than 90% of the chlorophyll in the green sulfur bacterium \textit{Chlorobium tepidum}. Chlorosomes, the light harvesting antennae of green bacteria, can contain more than 200,000 BChl \(c\) or \(e\) molecules that form self-aggregates. The pigments are methylated at the C-8\(^2\) and C-12\(^1\) carbons, but the roles of these methylations have not been previously described. The \(bchQ\) and \(bchR\) genes, which encode the C-8\(^2\) and C-12\(^1\) methyltransferases of \textit{C. tepidum}, respectively, were identified by targeted inactivation. Strains harboring segregated mutations in both genes and a double mutant strain were obtained; complete segregation of alleles was confirmed by PCR. The proteins encoded by the \(bchQ\) and \(bchR\) genes belong to the radical-SAM superfamily of proteins, and thus these enzymes are related to the oxygen-independent isocyclic ring cyclase, BchL. The pigmentation of the mutants was analyzed by reverse-phase HPLC, HPLC-mass spectrophotometry, and absorption spectroscopy. The HPLC elution profiles and mass spectroscopic analysis of the chlorophylls in the mutants confirmed the anticipated changes in methylation patterns. The in vivo absorption spectra show that the mutants have a narrower Qy peak and are red-shifted to varying degrees. The mutants show slower growth rates than wild type especially at low light intensities. Interestingly, the mutants have a lower BChl \(c\) content in relation to protein and other pigments. Our results demonstrate that these methylations have several important roles in the adaptation of \textit{C. tepidum} to low light.

35. Mutagenesis and characterization of the highly conserved cysteine residues in the C-terminal domain of the transcriptional repressor SuFR of \textit{Synechocystis} sp. PCC 6803

Yinxian Wu\(^1\), Ramakrishnan Balasubramanian\(^1\), Tao Wang\(^1\), Lee M. Hoffart\(^1\), Carsten Krebs\(^1,2\), Gaozhong Shen\(^1\), Donald A. Bryant\(^1\), and John H. Golbeck\(^1,2\); \(^1\)Department of Biochemistry and Molecular Biology, \(^2\)Department of Chemistry, The Pennsylvania State University, University Park, PA 16802 USA

SuFR functions as a negative transcriptional regulator of the \textit{sunf} regulon in cyanobacteria. The SuFR protein from cyanobacteria contain two important features 1) an N-terminal domain that contains a helix-loop-helix, DNA-binding motif that is characteristic of DNA-binding proteins; and 2) a C-terminal domain that contains four conserved cysteine residues, which may provide ligands for coordinating an Fe/S cluster. The four cysteine residues at the C-terminus of SuFR are separated by 12, 13, and 14 amino acid residues respectively. This highly conserved \(CX_2\)\(CX_2\)\(CX_2\)\(CX_4\)C arrangement constitutes a unique Fe/S binding motif. Site-directed mutagenesis was used to generate mutated \(sufr\) genes with specific cysteine codons changed to either serine or alanine. The wild-type and variant SuFR proteins were expressed and purified. The Fe/S clusters in SuFR variants were reconstituted for initial analysis by EPR spectroscopy. Additionally, \(^57\)Fe was used in Fe/S cluster reconstitution to allow Mössbauer spectroscopic measurements. Alkylation experiments using iodoacetamide, followed by protease digestion and mass spectrometry, have been used to identify those cysteine ligands that do not provide ligands to the \([4Fe-4S]\) cluster. The results show that SuFR harbors a single \([4Fe-4S]\) cluster in its native state and that cysteines 164 and 206 coordinate the \([4Fe-4S]\) cluster. Currently, we are further investigating the cysteine ligands in SuFR using a site-specific cleavage strategy with NTGB and mass spectrometry. The data will provide further information to clarify the roles of the cysteine residues that ligate the \([4Fe-4S]\) cluster and that could play a role as a redox switch in regulation.
36. Nitric oxide mediates the deactivation of diphtheria toxin repressor by iron oxidation and S-nitrosation

Michelle M. Spiering1, Douglas A. Mitchell2, and Michael A. Marletta3,4;
1Department of Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109; 2Department of Chemistry and 3Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Diphtheria toxin repressor (DtxR) belongs to a family of iron-activated repressors that control gene expression in Corynebacterium diphtheriae and other prokaryotes. In vivo and in vitro reporter gene systems and a direct DNA-binding assay were used to investigate the deactivation of DtxR by nitric oxide (NO) and its aerobic decomposition products. Escherichia coli grown aerobically in the presence of NO expressed beta-galactosidase activity from a reporter gene, while no beta-galactosidase activity was expressed in the absence of NO. In a direct DNA-binding assay, DtxR was deactivated in a NO-concentration dependent manner. DtxR deactivation was associated with both iron oxidation and cysteine modification. NO binds to the ferrous iron in DtxR to form a ferrous-nitrosyl complex that decays to ferric iron. In reactions of DtxR with NO, evidence of a ferrous-nitrosyl complex of DtxR was obtained by electron absorbance and EPR spectroscopy and a specific ferric iron chelator was used to monitor the formation of ferric iron. NO is also capable of modifying C102 of apo-DtxR to form nitrosothiols and inactive disulfide-linked dimeric DtxR under aerobic conditions. Bound metal protected C102 from NO-induced disulfide formation. NO had no effect on apo-DtxR(C102D); however, ferrous iron-bound DtxR(C102D) was susceptible to deactivation by NO. DtxR is sensitive to changes in the intracellular iron concentration as well as oxidative conditions experienced by pathogens during an immune response. Since iron-activated prokaryotic repressors regulate the expression of many virulence determinants, deactivation by NO may be important in pathogenesis.

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1current address: Department of Chemistry, Pennsylvania State University, University Park, PA 16802

37. Binding of artificial multimetallic peptides with double stranded DNA

Lauren A. Levine and Mary Elizabeth Williams*; Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, Pennsylvania 16802

Metal containing intercalation complexes are a common synthetic starting point for therapeutics and imaging in biological systems. The optimization of these systems to provide higher selectivity and specificity is a continuing challenge since most of these complexes bind non-specifically. To try to increase the binding affinity and selectivity, we tether inorganic intercalation complexes to a polyamide backbone. The artificial oligopeptides are prepared using solid phase peptide synthesis methods and subsequently titrated with the metal complex. Thermodynamic studies are performed using metal-bound tetrapeptides with an oligomeric double stranded DNA. Isothermal titration microcalorimetry (ITC), circular dichroism (CD), and UV thermal melts were used to determine the binding constants, free energies, entropies, enthalpies and melting temperatures for the DNA-oligomer complexes. Polyvalent, cooperative binding of the oligopeptides results in higher binding affinities that depend on the number of metal complex intercalators. These results suggest that tethered multimetallic systems may find use as new chemotherapeutic agents with greater efficacy.
38. Structural insight into antibiotic fosfomycin biosynthesis by a mononuclear iron enzyme

Danny Yun1, Luke J. Higgins1, Feng Yan2, Pinghua Liu3,4, Hung-wen Liu5 and Catherine L. Drennan1; 1Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139; 2Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712; 3Department of Chemistry, Boston University, Boston, MA 02215

The enzyme S-(2)-hydroxypropylphosphonic acid epoxidase (HppE) from Streptomyces wedmorensis utilizes a mononuclear iron center and molecular oxygen to catalyze a radical–initiated dehydrogenation of its substrate, S-2-hydroxypropylphosphonic acid, with the retention of its hydroxyl oxygen in the product, fosfomycin. This mechanism constitutes a new chemical strategy for epoxide formation and puts HppE in a class of its own within the mononuclear non-heme iron enzyme superfamily. The unusual carbon-phosphorus-bond-containing antibiotic fosfomycin inhibits the enzyme UDP-GlcNAc enolpyruvyl transferase (MurA in Escherichia coli), which catalyzes the first committed step in bacterial cell wall peptidoglycan biosynthesis.

The reaction is a two-electron oxidation and is mechanistically atypical because it is independent of α-ketoglutarate or any other known cofactor and results in incorporation of the hydroxyl oxygen of the substrate, rather than an atom of O₂, into the epoxide ring. Several HppE crystal structures have been determined by our laboratory in collaboration with Hung-wen Liu’s laboratory in order to provide information about the mechanism of this unique enzyme. Crystallographic studies are ongoing to determine how HppE activates molecular oxygen for this reaction.


39. Iron sulfur clusters in Type I homodimeric photosynthetic reaction centers

Mark Heinickel1, Gaohong Shen1, Rufat Agalarov1, Carsten Krebs1,2 and John H. Golbeck1,2; 1Department of Biochemistry and Molecular Biology, 2Department of Chemistry, The Pennsylvania State University, University Park, PA 16802 USA

Only two types of photosynthetic reaction centers (RCs) exist in nature. Type I RCs have iron sulfur clusters as terminal electron acceptors, and Type II RCs have quinones as terminal electron acceptors. There exist two subclasses of Type I RCs, heterodimeric as found in Photosystem I, and homodimeric as found in heliobacteria and green sulfur bacteria, and, similarly, there exist two subclasses of Type II RCs, the purple bacterial RC and Photosystem II. The Heliobacteriaceae, which are obligately anaerobic phototrophs that contain the unique pigment bacteriochlorophyll g, contain homodimeric Type I photosynthetic reaction centers (RCs). Similar to anaerobic green sulfur bacteria, the reaction centers from Heliobacteria are homodimeric. At this point, it is unclear how many polypeptides are present in the heliobacterial reaction center. It is also unclear if the same number and type of electron transfer cofactors exist as in other Type I RCs. Here, we report the low temperature EPR spectra of the Fe/S clusters in the RC from Heliobacterium modesticaldum (HbRC). A Fe/S cluster is a Fe/S cluster with a ground spin state of S = 3/2, similar to the [4Fe-4S] cluster found in the Fe-protein of the nitrogenase enzyme from Azotobacter vinelandii. The final two Fe/S clusters, F₇ and F₈, are harbored in a loosely bound ferredoxin-like protein termed PshB. We have cloned and expressed the PshB gene and the purified PshB protein was rebound to HbRC cores after reconstitution of the F₇ and F₈ clusters.
40. Spectroscopic studies on de novo designed binuclear iron active sites

Caleb B. Bell III, Pin-pin Wei, Jennifer Calhoun, William F. DeGrado and Edward I. Solomon. Department of Chemistry, Stanford University, Stanford, California 94305, Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA 19104

The DF family of de novo designed proteins have a four helix bundle that coordinates divalent metals in ligand environments similar to many binuclear non-heme iron enzymes, including ribonucleotide reductase (RR), Δ^2-desaturase (Δ^2D) bacterioferritin (BF) and methane monoxygenase (MMO). Despite highly similar active site structures, these enzymes perform diverse biological functions involving oxygen activation, and react through structurally and electronically different intermediates. The ferrous reconstituted DF2t and DF3c members of the DF family react with dioxygen and generate spectroscopically observable species. These systems offer ideal models to systematically study the factors that tune diiron sites for differential oxygen chemistry. A variety of spectroscopic methods are being used to study the effects of variations in the primary and secondary shell amino acids on the structures and reactivity of these de novo designed binuclear iron active sites.

41. Identification of an anaerobic bacteriochlorophyll c C-7 hydroxylase in brown-colored green sulfur bacteria

Julia A. Maresca, Tao Li, and Donald A. Bryant; Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA

Two pigment phenotypes exist among green sulfur bacteria: brown species produce bacteriochlorophyll (BChl) e as their antenna pigment and isorenieratene as the primary carotenoid, and green species synthesize chlorobactene as the main carotenoid and BChl c or d for the antenna. BChl e has a formyl group at the C-7 position of the chlorin ring, which changes the absorption properties of the molecule dramatically and enables the brown species to live at much lower light intensities than green species and in environments more enriched in green wavelengths. In some organisms that carry out oxygenic photosynthesis, chlorophyll (Chl) b, like BChl e, has a C-7 formyl group. The enzyme that formylates Chl a to Chl b is an oxygenase which uses molecular oxygen as the substrate. Because GSB are strict anaerobes, the equivalent reaction in these organisms must have a different mechanism. Comparative analysis of the recently sequenced genomes of 6 green- and 3 brown-colored GSB has identified a 6.5-kb gene cluster responsible for isorenieratene biosynthesis and tentatively for BChl e biosynthesis. The putative BChl e-specific hydroxylase in this cluster is a member of the BioB family of enzymes, and as such may employ a radical mechanism to perform the anaerobic oxidation of BChl c.
42. Biomimetic formation of titanium phosphate and protein encapsulation

Kathryn E. Cole,† Andrea N. Ortiz,‡ Martin A. Schoonen§ and Ann M. Valentine¶; †Department of Chemistry, Yale University, New Haven, Connecticut, 06511; ‡Department of Chemistry, California State Polytechnic University, Pomona, California 91768; §Department of Geosciences and Center for Environmental Molecular Science, Stony Brook University, Stony Brook, New York, 11794-2100

Biomineralization describes the use of inorganic structures by organisms. Diatoms are one of the most well studied examples of biomineralization. Their intricate siliceous frustules reflect a controlled deposition of silica in vivo. The RS peptide, a repeat unit of a cationic polypeptide isolated from the diatom *Cylindrotheca fusiformis*, and poly(allylamine), a synthetic mimic of the long-chain species-specific polymamines isolated from diatoms, are active in promoting the formation of nanostructured silica. In addition to silica, diatoms are also known to incorporate titanium into their frustules. We have shown that the (bio)molecules active in biosilification also induce the formation of nano- and micro-structured titanium phosphate spheres. The morphology of the spheres can be controlled by the (bio)molecular template, the temperature, and the pH. Finally, green fluorescent protein can be encapsulated into the titanium phosphate spheres, yielding a green-glowing solid. The controlled deposition of nano- and micro-sized titanium phosphate spheres and the encapsulation of protein may provide useful for technological applications.

43. A Mg²⁺-dependent ATPase that generates mechano-chemical force for clamp loading: ensemble and single-molecule studies

Zhihao Zhuang†, R. Derike Smiley, Anthony J. Berdis*, Gordon G. Hammes and Steve J. Benkovic‡; †Department of Chemistry, The Pennsylvania State University, 414 Wartik Lab, University Park, PA, 16802; ‡Department of Biochemistry, Duke University Medical Center, Box 3711, Durham, NC 27710; *Department of Pharmacology, School of Medicine, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44106

Processive DNA replication is crucial for successful duplication of genomic DNA. Since the DNA polymerase alone is not processive, a toroidal clamp protein is required to topologically link the polymerase to DNA. The clamp loader protein, as a matchmaker, converts chemical energy derived from ATP hydrolysis to the mechano-chemical force for clamp loading onto DNA. The bacteriophage T4 clamp loader (gp44/62), as a member of AAA+ protein family, is critical for clamp (gp45) loading and polymerase holozyme assembly. In this study we demonstrated the ATP dependent binding of clamp loader to primer-template DNA through surface plasmon resonance (SPR) studies. The stoichiometry of ATP hydrolysis catalyzed by gp44/62 in clamp-loading was investigated using rapid-quench experiment through controlled mixing. We also demonstrated the inter-domain conformational change of clamp loader as the result of nucleotide analogue MANT-ATP binding using the stopped-flow FRET (fluorescence resonance energy transfer) spectroscopy. In order to probe the dynamic clamp interface closing during clamp loading, a FRET pair was introduced across the clamp protein interface and the distance change was measured as the clamp encounters a clamp loader-DNA complex and is loaded onto DNA. Furthermore, single-molecule fluorescence microscopy was exploited to probe the possible pathways for clamp loading and polymerase holozyme assembly. We uncovered three novel pathways of holozyme formation catalyzed by clamp loader, which is in turn powered by ATP hydrolysis.
44. A unified mechanism for the \(\alpha\)-ketoglutarate-dependent dioxygenases: Evidence for a C-H-cleaving Fe(IV) complex in prolyl-4-hydroxylase

Lee M. Hoffart,1 Eric W. Barr,1 Robert B. Guyer,1 J. Martin Bollinger, Jr.,1,2 and Carsten Krebs1,2; Departments of 1Biochemistry and Molecular Biology, and 2Chemistry, The Pennsylvania State University

The Fe(II)- and \(\alpha\)-ketoglutarate-dependent dioxygenase catalyze activation of molecular oxygen at a mononuclear, non-heme Fe(II) site to oxidative decarboxylation of \(\alpha\)-ketoglutarate and oxidation of their substrates (usually hydroxylation). It has been proposed that members of this family employ a conserved catalytic mechanism. Recent dissection of the mechanism of TauD (taurine-\(\alpha\)-ketoglutarate dioxygenase, a member of the family) included detection and characterization of two reaction intermediates, the hydrogen atom abstracting Fe(IV)=O complex, J, and an enzyme-product(s) complex, setting the stage for direct evaluation of the hypothesis for a common mechanism.

Here accumulation of two intermediates is established in the reaction of a prolyl-4-hydroxylase (P4H) from Paramecium bursaria Chlorella virus I, a model system for human prolyl-4-hydroxylases that have essential roles in collagen biosynthesis and oxygen sensing. The similarity of the spectroscopic properties of the P4H intermediates to those in the TauD reaction lends credence to the hypothesis of a conserved mechanism for the entire family. The first intermediate observed in the mechanism is a high-spin Fe(IV) complex that appears to be the P4H homologue of J in TauD. Additionally a large substrate deuterium kinetic isotope effect on its decay shows that the P4H Fe(IV) complex is responsible for cleaving the C-H bond. The second intermediate appears to correspond to the enzyme-product(s) complex observed in TauD.

45. The role of the heme group in the structure of \(b\) hemoproteins: The sensory PAS domain of Bradyrhizobium japonicum FixL

Daniel A. Landfried and Juliette T. J. Lecomte; Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

FixL is an oxygen-binding sensor responsible for the regulation of nitrogen fixation in rhizobia. The sensor domain (FixLH) is a 106 residue member of the PAS domain family, and utilizes a heme cofactor for ligand binding. This domain does not exhibit the traditional \(\alpha\)-helix rich structure typically associated with heme-binding domains; rather, there is a conserved complex \(\alpha/\beta\) fold topology with a higher degree of \(\beta\)-strand structure. Therefore FixL provides an interesting system with which to study the role of a heme cofactor in determining the structure of the functional holoprotein. Denaturation experiments of FixLH were monitored by UV-vis and far-UV CD spectrophotometry, and it was found that the heme group contributes significantly to establishing the secondary and tertiary structure of the PAS domain. Comparison of the solvent accessibility of the heme in FixLH to other heme-binding proteins was made to further understand the role of the heme as a stabilizing factor in the domain and in \(b\) hemoproteins in general.

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46. Evidence for non-native structure in the isolated heme-binding loop of cytochrome b₅

Ronald Davis, Jr.; Department of Chemistry, The Pennsylvania State University

The water-soluble domain of cytochrome b₅ contains a heme-binding region that, in contrast to the rest of the protein, retains very little of its structure on heme removal. Studies of holo- and apocytochrome b₅ and chimeric constructs consisting of the heme binding region of b₅ grafted to an alternate support protein indicate that a specific conformational restriction of the binding region termini may be essential to its function. To further investigate this, three peptide constructs were synthesized and characterized to investigate the behavior of the heme-binding region independently of the remaining protein. The first peptide corresponds to the entire heme-binding loop sequence (Leu32 - Thr73). The second and third peptides correspond to each half of the original binding loop sequence (Leu32 - Gly52 and Gly52 - Thr73). Circular dichroism in solvents of varying 2,2,2-trifluoroethanol concentration demonstrates that the C-terminal half of the binding loop sequence has a greater global helical propensity than the N-terminal half. Furthermore, 2D NMR data suggest that the region of greatest helical propensity in the peptides corresponds to a region that takes on a β-turn in the holoprotein. Such non-native structural propensities may play a role in the kinetics or thermodynamics of heme binding in cytochrome b₅.

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47. Substrate triggering of O₂ reactivity in the Fe(II)- and α-ketoglutarate-dependent monoxygenase/chlorinase enzymes, SyrB2 and CytC3

Gretchen Koch1, Megan Matthews2, Danica Galonicic3, Eric Barr3, Chris Walsh3, Carsten Krebs1, J. Martin Bollinger Jr.1; Department of Biochemistry and Molecular Biology1 and Chemistry2, Penn State University and Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School3

SyrB2 and CytC3 are "tailoring enzymes" of nonribosomal peptide synthetase megacomplexes. They install chlorine on alkyl groups in the biosyntheses of the natural products, syringomycin and cytotoxicin A, respectively. Recent studies have revealed that they are structurally and functionally similar to the Fe(II)- and α-ketoglutarate dioxygenases, which use His2Asp/Glu-coordinated mononuclear iron(II) centers to couple the oxidative decarboxylation of αKG with the hydroxylation of alkyl groups on a variety of substrates [Vaillancourt, F.H., Yin, J., and Walsh, C.T. (2005). Proc. Natl. Acad. Sci. USA 102, 10111-10116]. In both hydroxylation and chlorination, a high-spin Fe(IV)-oxo intermediate initiates transformation of the substrate by abstracting a hydrogen atom. Both types of enzymes have evolved to avoid forming the potentially oxidizing high-valent-iron intermediate until the substrate is bound. In both cases, the reactivity of the Fe(II) cofactor toward O₂ is dramatically enhanced upon binding of the substrate (substrate triggering). It is believed that this triggering arises, at least in part, from substrate-induced dissociation of a water ligand from the site to which O₂ binds, but the nature of the conformational coupling of substrate binding to water dissociation is not understood. In the case of the chlorinases, the substrate is expansive and complex. It consists of a thiolation protein domain linked to a phosphopantetheine (PPant) arm that is charged with an amino acid (linked as thioester). Recent experiments have suggested that all three parts of the substrate are required to trigger optimal O₂ reactivity and initiate chlorination. The nature of this remarkable simultaneous sensing of the amino acid substrate, the cofactor/linker, and the protein interaction partner to trigger the oxygenases is being probed by use of chemically modified substrate constituents. In particular, (1) the amino acid specificity of both the triggering phenomenon and the chlorination outcome and (2) whether mechanical coupling through the PPant arm or merely the simultaneous presence of all three constituents is required for substrate triggering are being investigated.
48. Binding of substrate, product, and analogs to the mixed-valent non-heme diiron(II/III) cofactor in myo-inositol oxygenase

Yinghui Diao¹, Sun Hye Kim², Gang Xing³, Eric W. Barr¹, Mads-Jacob K. Klitgaard⁴, Lee Hoffart¹, Brian M. Hoffman⁵, Carsten Krebs⁶, and J. Martin Bollinger, Jr.¹,⁴
¹Department of Biochemistry and Molecular Biology and
²Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802
³Department of Chemistry, Northwestern University, Evanston, Illinois 60208-3113
⁴Department of Chemistry, Technical University of Denmark, Kgs. Lyngby, Denmark, 2900

The mixed-valent diiron(II/III) cofactor in myo-inositol oxygenase (MIOX) activates oxygen for the unique glycol-cleaving, four-electron oxidation of myo-inositol (MI) to D-glucorurate.¹,² H-ENDOR experiments have established that at least one non-exchangeable hydron of MI and at least one exchangeable hydron are strongly coupled to the electron spins of the diiron(II/III) cluster (S = 1/2) and that the substrate binds via at least one bridging oxygen. Direct coordination rationalizes the dramatic perturbations to the UV-visible absorption, EPR, and Mössbauer spectra of the diiron(II/III) cluster upon binding of either the substrate or the product.³ These perturbations have been used to determine that binding of MI to MIOX at 5 °C is characterized by kₐ = 0.26 ± 0.03 mM⁻¹ s⁻¹, k₋¹ = 0.15 ± 0.05 s⁻¹ and K₈ = 0.6 ± 0.3 mM. EPR spectra of mixed-valent MIOX in the presence of 50 mM of each of five different inositols that are epimeric with respect to MI at a single carbon have shown that scylo-inositol (the 2-epimer), D-chiro-inositol (the 3-epimer) and neo-inositol (the 5-epimer) bind reasonably well and give the same EPR signal as the MI complex. By contrast, L-chiro-inositol and epi-inositol (the 4- or 6-epimer) bind weakly, if at all. The 6-dehydro (ketone) analogue binds but elicits an EPR spectrum distinct from that of the MI complex. The use of specifically-deuterium-labeled MI and deoxy analogues will reveal which oxygen (or oxygens) of MI bridges or (bridge) the Fe ions of the cofactor. The flexibility of the active site to accommodate MI analogues with an epimeric, oxidized, or reduced carbon center will permit such analogues to be used as sensitive probes of the enzyme's unique mechanism.


49. Simultaneous activation of the substrate and O₂ by a mixed-valent, non-heme, diiron(II/III) cluster and cleavage of a C-H bond by a diiron(III/III)-superoxide complex in myo-inositol oxygenase

Gang Xing¹, Yinghui Diao¹, Eric W. Barr³, Lee Hoffart¹, K. Sandeep Prabhu¹, Ryan Amer, C. Channa Reddy³, Carsten Krebs⁶, and J. Martin Bollinger, Jr.¹,³
¹Department of Biochemistry and Molecular Biology, ²Department of Veterinary and Biomedical Sciences, and ³Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

myo-Inositol oxygenase (MIOX) activates O₂ for the unique glycol-cleaving, four-electron oxidation of myo-inositol (MI) to D-glucorurate (DG). EPR and Mössbauer spectra of the recombinant Mus musculus enzyme produced in E. coli have shown that MIOX contains a dinuclear non-heme-iron cluster that is perturbed by binding of MI in a manner consistent with direct bridging coordination.¹ Stopped-flow absorption and freeze-quench EPR spectroscopies in combination with product analysis have established that, unlike any previously characterized non-heme diiron oxygenase, MIOX uses the mixed-valent, diiron(II/III) form of its cofactor to activate oxygen.² When the complex of the mixed-valent enzyme and deuterium-labeled MI is mixed with O₂, a formally (superoxido)diiron(II/III) intermediate, G, in which the superoxide is probably coordinated to a single iron, accumulates. It decays to a second intermediate state, H, which has a diiron(II/III) cluster. Conversion of G to H has a deuterium kinetic isotope of at least 5, indicating that the superoxide complex abstracts hydrogen from MI.³ The slowest step in the reaction is decay of H and regeneration of the reactant MIOX-MI complex.² H could be a product complex, but it is more likely to be a mechanistically informative intermediate, because addition of DG or its aldehyde reduced product, L-gulonate, to the enzyme does not elicit the EPR signal characteristic of H. Thus, determination of the nature of H will likely distinguish which among several possible, equally unprecedented mechanisms is operating in MIOX.


50. Biochemical characterization of T4 UvsW helicase: DNA unwinding and strand annealing activities

Scott W. Nelson and Stephen J. Benkovic; Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania, 16802

UvsW protein belongs to the SFII helicase family and is one of three helicases found in T4 phage. UvsW governs the transition from origin-dependent to origin-independent replication through the dissociation of R-loops located at the T4 origins of replication. Additionally, in vivo evidence suggests that UvsW plays a role in recombination-dependent replication and/or DNA repair. Here, the biochemical properties of UvsW helicase are described. UvsW is a double-stranded DNA dependent ATPase with a turnover number of 55 sec\(^{-1}\) and a \(K_M\) of 180 \(\mu\)M. UvsW unwinds a wide variety of substrates, including those resembling stalled replication forks and recombination intermediates. UvsW also contains a potent single-strand DNA annealing activity, both in the presence and absence of nucleotide cofactor. This annealing activity can be inhibited by T4 single-stranded DNA-binding protein (gp32) or a small 8.8 kD polypeptide (UvsW.1). Fluorescence resonance energy transfer experiments indicate that UvsW and UvsW.1 form a tight complex, suggesting that the UvsW helicase exists primarily as a heterodimer in vivo. Fusion of UvsW and UvsW.1 results in a 68 kD protein having identical properties as the UvsW-UvsW.1 complex, indicating that the binding locus of UvsW.1 is near the C-terminus of UvsW. The biochemical properties of UvsW show a strong similarity to the RecQ protein family and suggest that the annealing activity of these helicases may also be modulated by protein-protein interactions.

51. Caught in the act: Functional evolution in GTP cyclohydrolase II homologs of S. coelicolor

James E. Spoonamore\(^1\), Annie L. Dahlgran\(^1\), Jesus Hernandez, Joon S. Kim\(^2\), Neil E. Jacobsen\(^3\), Vahe Bandarian\(^4\); \(^1\)Department of Biochemistry and Molecular Biophysics, University of Arizona, 1041 E. Lowell St., Tucson, AZ 85721; \(^2\)Department of Chemistry, University of Arizona, 1306 E. University Ave., Tucson, AZ 85721

The genome sequence of \(S. coelicolor\) contains three open reading frames (SCO 1441, SCO 2687 and SCO 6655) with significant (> 40 %) amino acid identity to GTP cyclohydrolase II (GCH II), which catalyzes the committed step in the biosynthesis of riboflavin. The physiological significance of the redundancy of these proteins in \(S. coelicolor\) is not known. However, the gene contexts of the three proteins are different, suggesting that they may serve alternate biological niches. Each of the three proteins was overexpressed in \(E. coli\) and characterized to determine if their functions are biologically overlapping. As purified, each protein contains one molar equivalent of zinc per mole of protein and utilizes guanosine 5'-triphosphate (GTP) as substrate. Two of these proteins (SCO 1441 and SCO 2687) produce the canonical product of GCH II, 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate (APy). Remarkably, however, one of the three proteins (SCO 6655) converts GTP to 2-amino-5-formylamino-6-ribosylamino-4(3H)-pyrimidinone 5'-phosphate (FAPy), as shown by UV-visible spectrophotometry, mass spectrometry and NMR. Comparison of the sequences of these proteins and mapping onto the structure of the \(E. coli\) GCH II protein [Ren, J., Kotaka, M., Lockyer, M., Lamb, H. K., Hawkins, A. R., and Stammens, D. K. (2005) J. Biol. Chem. 280, 36912-36919] allowed identification of a switch residue, Met120, which appears to be responsible for the altered fate of GTP observed with SCO 6655; a Tyr is found in the analogous position of all proteins that have been shown to catalyze the conversion of GTP to APy. The Met120Tyr variant of SCO 6655 acquires the ability to catalyze the conversion of GTP to APy, suggesting a role for Tyr120 in the late phase of the reaction. Our data are consistent with duplication of GCH II in \(S. coelicolor\) promoting evolution of a new function and suggest a strategy for functional evolution in this class of proteins.
52. Investigating the interaction between ribonucleoside triphosphosphate reductase and thioredoxin in Lactobacillus leichmannii

C. Ainsley Davis, Nicole J. DeNisco, and Catherine L. Drennan; Massachusetts Institute of Technology, Department of Chemistry, Cambridge, MA 02139

Ribonucleotide reductases (RNRs) catalyze the conversion of nucleotides to deoxynucleotides (dNTPs) in all organisms and thus play an essential role in DNA biosynthesis and repair. This function makes RNRs attractive targets for anticancer and antiparasitic chemotherapies since inhibition of these enzymes disrupts the dNTP balance within the cell, leading to cell death. Previous work has shown that thioredoxin (Trx) / thioredoxin reductase systems are responsible for the re-reduction of class I and class II RNRs through disulfide exchange reactions involving conserved cysteine residues. To study this re-reduction reaction, we have selected proteins from Lactobacillus leichmannii, an ideal model system due to the relative simplicity of its ribonucleoside triphosphate reductase (RTPR) and the presence of only one Trx in its genome. We have cloned Trx from Lactobacillus leichmannii and purified the protein to homogeneity. To investigate which of the cysteine residues at the C-terminus of RTPR are more reactive toward Trx, we have prepared RTPR mutant proteins C731S and C736S. Further, to investigate which of the cysteines in the conserved Trx ‘CGPC’ motif are more reactive toward RTPR, we have prepared C285S and C315S Trx mutant proteins. Preliminary results of the disulfide-mediated cross-linking of RTPR with Trx will be presented.

Author Index

Agaliarov, Rafat 80, 123
Andrade, Susana L. 78, 107
Arner, Ryan 82, 133
Arner, Ryan J. 59
Balabanraman, S 79, 119
Bandarian, Valve 82, 135
Barr, Eric W 59, 77, 81, 82, 101, 128, 131, 132, 133
Basu, Partha 75, 76, 87, 89, 100
Behan, Rachel K. 76, 77, 96, 103
Bell III, Caleb B. 80, 124
Benkovic, Stephen J. 81, 127
Berdís, Anthony J. 81, 127
Bhakta, Mehal N. 76, 93
Böck, August 44
Bollinger, Jr., J. Martin 59, 77, 81, 82, 101, 128, 131, 132, 133
Booker, Squire J. 75, 78, 92, 109, 110, 111
Bourquin, Florence 69
Brainley, Susan 76, 97
Brown, Eric N. 55
Bryant, Donald A. 79, 80, 117, 118, 119, 125
Burkhard, Kimberly A. 75, 88
Bursyn, Judith N. 33
Calhoun, Jennifer 80, 124
Cameron, Craig E. 77, 102
Carredano, Enrique 55
Chakrabarty, Sarmistha 61
Chen, Baoyu 75, 90
Chew, Aline Gomez 79, 118
Maquero 75, 92
Cicchillo, Robert M. 75, 92
Cobine, P. 47
Cole, Kathryn E. 80, 126
Corbett, William 77, 104
Cress, Christina 79, 117
Dahigran, Ann L. 82, 135
Davis, C. Ainsley 82, 136
Davis, Jr., Ronald 81, 130
De Carlo, Sacha 75, 90
Dean, Dennis R. 41
DeGrado, William F. 80, 124
DeNisco, Nicole J. 82, 136
Diao, Yinghui 59, 81, 82, 123, 133
d'Targiani, Robert C. 77, 106
Dooley, David M. 35
Dosanjh, N.S. 75, 91
Douxchel, Michaelene 75, 90
Douxchel, Michaelene 90
Drennan, Catherine L. 76, 78, 79, 80, 82, 94, 99, 113, 115, 122, 136
Edelhoch, Jocelyn P. 77, 102
Einsele, Oliver 78, 107
Eklund, Hans 55
Elliot, Sean J. 78, 79, 112, 113, 114
Ferraro, Daniel J. 55
Ferry, James G. 75, 78, 85, 107, 108
Frey, Perry A. 39
Friemann, Rosemarie 55
Frigsörd, Niels-Ulrik 79, 118
Gakhar, Lokesh 55
Galosid, Danica P. 76, 77, 81, 94, 101, 131
Garcia-Serrés, Ricardo 69, 77, 105
Gibson, David T. 55
Glauser, Dominique A. 69
Gogonea, Camelia 75, 92
Gogonea, Camelia 92
Golbeck, John H. 23, 76, 78, 90, 98, 116, 119, 123
Goodey, Nina M. 77, 104
Graci, Jason D. 77, 102
Green, Michael T. 76, 77, 96, 103
Griffiths, Amy 78, 110
Guo, Yai (Stephanie) 76, 99
<table>
<thead>
<tr>
<th>Name</th>
<th>Page</th>
<th>Name</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guyer, Robert B.</td>
<td>81, 128</td>
<td>Lecomte, Juliette T. J.</td>
<td>81, 129</td>
</tr>
<tr>
<td>Hammes, Gordon G.</td>
<td>81, 127</td>
<td>Lee, Kyung-Hoon</td>
<td>55, 78, 109</td>
</tr>
<tr>
<td>Harris, Micheleen M.</td>
<td>77, 104</td>
<td>Lee, Seung Jae</td>
<td>77, 106</td>
</tr>
<tr>
<td>Hassan, A. Quanmu</td>
<td>75, 86</td>
<td>Lessner, Daniel J.</td>
<td>55, 78, 108</td>
</tr>
<tr>
<td>Heinmickel, Mark</td>
<td>80, 123</td>
<td>Levine, Lauren A.</td>
<td>80, 121</td>
</tr>
<tr>
<td>Hergenrother, Paul J.</td>
<td>77, 104</td>
<td>Li, Tao</td>
<td>80, 125</td>
</tr>
<tr>
<td>Hernandez, Hector H.</td>
<td>78, 113</td>
<td>Lindahl, Paul A.</td>
<td>37</td>
</tr>
<tr>
<td>Hernandez, Jesus</td>
<td>82, 135</td>
<td>Lippard, Stephen J.</td>
<td>77, 105</td>
</tr>
<tr>
<td>Hoffart, Lee M.</td>
<td>59, 76, 77, 79, 81, 82, 96, 103, 119, 128, 132, 133</td>
<td>Liu, Hung-wen</td>
<td>80, 122</td>
</tr>
<tr>
<td>Hoffman, Brian M.</td>
<td>41, 81, 132</td>
<td>Liu, Pinghua</td>
<td>80, 122</td>
</tr>
<tr>
<td>Hooper, A. B.</td>
<td>78, 112</td>
<td>Lovley, Derek</td>
<td>25</td>
</tr>
<tr>
<td>Hoover, Timothy R.</td>
<td>75, 90</td>
<td>Malik, Zulfiqar Ali</td>
<td>55</td>
</tr>
<tr>
<td>Horng, Y-C.</td>
<td>47</td>
<td>Manieri, Wanda</td>
<td>69</td>
</tr>
<tr>
<td>Howard-Jones, Annaleise R.</td>
<td>79, 115</td>
<td>Maresca, Julia A.</td>
<td>79, 80, 117, 125</td>
</tr>
<tr>
<td>Huphah, Boi Hamb</td>
<td>67, 77, 105</td>
<td>Markham, G. Doug</td>
<td>76, 95</td>
</tr>
<tr>
<td>Iwig, David F.</td>
<td>75, 78, 92, 111</td>
<td>Marletta, Michael A.</td>
<td>79, 120</td>
</tr>
<tr>
<td>Izzio, Viviana</td>
<td>77, 105</td>
<td>Martin, Stephen F.</td>
<td>77, 104</td>
</tr>
<tr>
<td>Jacobsen, Neil E.</td>
<td>82, 135</td>
<td>Matthews, Megan</td>
<td>81, 131</td>
</tr>
<tr>
<td>Jaffe, Eileen K.</td>
<td>76, 95</td>
<td>Mbughuni, Michael M.</td>
<td>61</td>
</tr>
<tr>
<td>Jagannathan, Bharat</td>
<td>76, 98</td>
<td>Michel, Sarah L. J.</td>
<td>75, 77, 91, 106</td>
</tr>
<tr>
<td>Jameson, Guy N. L.</td>
<td>69</td>
<td>Miller, Anne-Frances</td>
<td>57</td>
</tr>
<tr>
<td>Jensen, Maya Ivacovic</td>
<td>55</td>
<td>Mitsaev, Barbara Serli</td>
<td>76, 100</td>
</tr>
<tr>
<td>Johnson, Michael K.</td>
<td>69</td>
<td>Mitchell, Douglas A.</td>
<td>79, 120</td>
</tr>
<tr>
<td>Karlsson, Andreas</td>
<td>55</td>
<td>Montfort, William R.</td>
<td>79, 114</td>
</tr>
<tr>
<td>Kauppi, Bjorn</td>
<td>55</td>
<td>Montfort, William R.</td>
<td>79, 114</td>
</tr>
<tr>
<td>Kim, Joon S.</td>
<td>82, 135</td>
<td>Murray, Leslie J.</td>
<td>77, 105</td>
</tr>
<tr>
<td>Kim, Sun Hee</td>
<td>81, 132</td>
<td>Naik, Ganesh</td>
<td>75, 87</td>
</tr>
<tr>
<td>Kilgaard, Mads-Jacob K.</td>
<td>81, 132</td>
<td>Naik, Sunil</td>
<td>77, 105</td>
</tr>
<tr>
<td>Koch, Gretchen</td>
<td>81, 131</td>
<td>Neibergall, Matthew B.</td>
<td>61</td>
</tr>
<tr>
<td>Kondrashkina, Elena</td>
<td>75, 90</td>
<td>Nelson, Scott W.</td>
<td>82, 134</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>59, 75, 76, 77, 79, 80, 81, 82, 92, 96, 101, 103, 119, 123, 128, 131, 132, 133</td>
<td>Neishitt, Natasha M.</td>
<td>75, 92</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>75, 90</td>
<td>Nixon, B. Tracy</td>
<td>75, 90</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>75, 90</td>
<td>Nogales, Eva</td>
<td>75, 90</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>76, 95</td>
<td>Okerlund, Adam</td>
<td>55</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>76, 95</td>
<td>Olabisi, Ayodele</td>
<td>76, 93</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>76, 95</td>
<td>Ortiz, Andrea N.</td>
<td>80, 126</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>76, 95</td>
<td>Parales, Juanito V.</td>
<td>55</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>76, 95</td>
<td>Parales, Rebecca E.</td>
<td>55</td>
</tr>
<tr>
<td>Krebs, Carsten</td>
<td>76, 95</td>
<td>Patridge, Eric V.</td>
<td>78, 107</td>
</tr>
<tr>
<td>Landfried, Daniel A.</td>
<td>81, 129</td>
<td>Peera, Eranda L.</td>
<td>75, 89</td>
</tr>
<tr>
<td>Lawrence, Sarah H.</td>
<td>76, 95</td>
<td>Peterson, Blake R.</td>
<td>77, 102</td>
</tr>
<tr>
<td>Phillips, Christine M.</td>
<td>76, 99</td>
<td>Pierrul, F.</td>
<td>47</td>
</tr>
<tr>
<td>Prodhoo, K. Sandeep</td>
<td>59, 82, 133</td>
<td>Pulcu, G. S.</td>
<td>78, 122</td>
</tr>
<tr>
<td>Ramaswamy, S.</td>
<td>55</td>
<td>Ramirez, Ursula</td>
<td>76, 95</td>
</tr>
<tr>
<td>Reddy, C. Channa</td>
<td>59, 82, 133</td>
<td>Reed, George H.</td>
<td>67</td>
</tr>
<tr>
<td>Rodgers, David W.</td>
<td>57</td>
<td>Rosenzweig, Amy C.</td>
<td>49</td>
</tr>
<tr>
<td>Rossi, Dan</td>
<td>76, 97</td>
<td>Ryan, Katherine S.</td>
<td>79, 115</td>
</tr>
<tr>
<td>Saunders, Allison</td>
<td>78, 110</td>
<td>Schoonen, Martin A.</td>
<td>80, 126</td>
</tr>
<tr>
<td>Schreiter, Eric</td>
<td>76, 99</td>
<td>Schwinn, Peter</td>
<td>69</td>
</tr>
<tr>
<td>Schmehl, Peter</td>
<td>71</td>
<td>Seefeldt, Lance C.</td>
<td>41</td>
</tr>
<tr>
<td>Shen, Gaozhong</td>
<td>79, 80, 119, 123</td>
<td>Smiley, R. Derike</td>
<td>81, 127</td>
</tr>
<tr>
<td>Solomon, Edward I.</td>
<td>19, 80, 124</td>
<td>Souder, Matthew G.</td>
<td>75, 92</td>
</tr>
<tr>
<td>Spacino, Courtney</td>
<td>75, 87</td>
<td>Sponnemos, James E.</td>
<td>82, 135</td>
</tr>
<tr>
<td>Spiering, Michelle M.</td>
<td>79, 120</td>
<td>Sriniwasan, Nidnya</td>
<td>79, 116</td>
</tr>
<tr>
<td>Strole, John</td>
<td>75, 87</td>
<td>Stone, Karl L.</td>
<td>76, 77, 96, 103</td>
</tr>
<tr>
<td>Stromberg, Jeffrey</td>
<td>78, 110</td>
<td>Stubbe, JoAnne</td>
<td>65, 75, 86</td>
</tr>
<tr>
<td>Thauer, Rudolf K.</td>
<td>29</td>
<td>Tien, Ming</td>
<td>76, 97</td>
</tr>
<tr>
<td>Thiele, Dennis J.</td>
<td>50</td>
<td>Tu, Loretta</td>
<td>75, 78, 92, 110</td>
</tr>
<tr>
<td>Wilkins, Angela</td>
<td>75, 76, 88, 93</td>
<td>William, Mary A.</td>
<td>80, 121</td>
</tr>
<tr>
<td>Wilmilasena, Kandetege</td>
<td>76, 93</td>
<td>Winters, Elizabeth</td>
<td>69</td>
</tr>
<tr>
<td>Winge, D.R.</td>
<td>47</td>
<td>Wang, Tao</td>
<td>79, 119</td>
</tr>
<tr>
<td>Wolff, Matt D.</td>
<td>61</td>
<td>Wei, Pin-pin</td>
<td>80, 124</td>
</tr>
<tr>
<td>Wong, Cindy</td>
<td>76, 94</td>
<td>Wenmner, David E.</td>
<td>75, 90</td>
</tr>
<tr>
<td>Wu, Yangxian</td>
<td>79, 119</td>
<td>Wilks, Angela</td>
<td>80, 121</td>
</tr>
<tr>
<td>Xing, Gang</td>
<td>59, 81, 122, 133</td>
<td>Williams, Mary A.</td>
<td>80, 121</td>
</tr>
<tr>
<td>Yan, Feng</td>
<td>80, 122</td>
<td>Ye, Tao</td>
<td>79, 114</td>
</tr>
<tr>
<td>Yiklilmaz, Ennie</td>
<td>57</td>
<td>Yu, Chi Li</td>
<td>55</td>
</tr>
<tr>
<td>Yun, Danny</td>
<td>80, 122</td>
<td>Zhuang, Zhihao</td>
<td>81, 127</td>
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<td>Zimmerman, Sabrina A.</td>
<td>75, 85</td>
<td>Zimmermann, Sabrina A.</td>
<td>75, 85</td>
</tr>
</tbody>
</table>
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