26th Enzyme Mechanisms Conference

January 6 - 9, 2019
Loews Hotel
New Orleans, LA

Back in the Big Easy after 50 years!

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About

The Enzyme Mechanisms Conferences have brought together academic and industrial scientists to discuss new ideas at the forefront of mechanistic enzymology. The goal is to foster collegial interactions among chemists and biochemists who seek to understand the chemical basis for enzymatic catalysis and regulation of enzyme action, and those who apply that knowledge for practical applications.

The EMC has been held biennially since it was founded in 1969 by Tom Bruice, Bill Jencks, and Myron Bender. Over the past 48 years, the conference has provided an outstanding forum for the presentation and discussion of the most exciting advances in our understanding of the mechanisms of enzyme action and their application to pharmaceutical design and action and to plant health.

To celebrate the 50 year anniversary of the conference, the 26th EMC is being held in New Orleans, which was the site of the first EMC in 1969.

50 years of EMC

1969 Bill Jencks, New Orleans
1971 Tom Bruice, Santa Barbara
1973 Paul Boyer, Los Angeles
1975 Al Mildvan, San Juan
1977 Joe Coleman, Tucson
1979 George Kenyon, La Jolla
1981 Perry Frey, Sanibel Island
1983 Judith Klinman, Asilomar
1985 Gene Cordes, Tarpon Springs
1987 Tony Fink, Asilomar
1989 Paul Bartlett, St. Pete
1991 Joe Villafranca, San Diego
1993 John Gerlt, Key Largo
1995 Dale Poulter, Scottsdale
1997 John Kozarich, Naples
1999 Richard Armstrong, Napa
2001 Vern Schramm, Marco Island
2003 Frank Raushel, Galveston
2005 JoAnne Stubbe, Asilomar
2007 Chris Whitman, St. Pete
2009 Karen Allen, Tucson
2011 John Richard and Tina Amyes, St. Pete
2013 Tom Meek, San Diego
2015 Ken and JoAnn Johnson, Galveston
2017 Richard Silverman, St. Pete
2019 Vahe Bandarian, New Orleans

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General information

Registration and Conference Check-in: The registration desk in Parish Hall will be open Sunday January 6th from 1:00 pm to 7:30 pm, and on Monday January 7th from 7:00 am to 10:30 am. If you are unable to obtain your registration material during these times, please see Vahe Bandarian.

Badges: Conferees and registered guests are kindly asked to wear their badge at all times while attending the scientific sessions and social functions.

Opening Reception: The conference Welcome and Opening Reception will be held on Sunday January 6th from 7:00 pm to 9:00 pm in the *Parish Hall*. Conferees and registered guests are welcome to attend.

Lecture Sessions: The nine scientific sessions will be held in the *Louisiana Ballroom*.

Poster sessions: Poster sessions will be held in the *Pointe Coupee* and *La Fourche* rooms 3:00 – 5:00 pm on Monday and Tuesday. Posters will be on display throughout the conference. Free munchies and a cash bar will be available. Presenters of ODD numbered posters should be available to discuss their posters on Monday. Presenters of EVEN numbered posters should be available to discuss their posters on Tuesday. Posters may be mounted on Monday morning and should be removed by Wednesday evening.

Breakfast: A full breakfast will be available for conferees and registered guests outside the *Louisiana Ballroom* beginning at 7:30 am on Monday, Tuesday, and Wednesday.

Lunch: A lunch buffet will be provided to conferees and guests on Wednesday between the morning and afternoon sessions.

Closing Banquet: The closing banquet will be held on Wednesday from 6:00 – 9:00 pm in *Parish Hall* and *Louisiana Ballroom.* Cocktails and light hors d'oeuvres will be served from 6:00 pm, followed by dinner at 7:00 pm. Conferees and registered guests are welcome to attend.

Registered guests: Registered guests are invited to the opening reception, breakfasts, coffee breaks, lunch on Wednesday, and the closing banquet.

26th Enzyme Mechanisms Conference

Schedule

Sunday, January 6

7:00 – 9:00 pm Reception: *Laissez les bon temps rouler!*

Monday, January 7

7:30-8:30 Breakfast

8:25 Welcome

Session 1 – From the active site to the bedside
Chair: Richard Silverman, Northwestern

8:30 **Chaitan Khosla** – Stanford
"An allosteric disulfide bond and its role in celiac disease pathogenesis"

9:05 **Tom Meek** – Texas A & M
"2 Infectious diseases, 2 enzymes, and maybe 2 lead compounds"

9:40 **Vern Schramm** – Albert Einstein College of Medicine
"Transition state analogues for ribosyl transferases"

10:15-10:45 Coffee break

Session 2 – Promiscuity and enzymes – what happens in the active site stays there?
Chair: John Richard, SUNY Buffalo

10:45 **Shelley Copley** – University of Colorado Boulder
"Bumps in the road toward evolution of a new enzyme"

11:20 **Chris Whitman** – UT Austin
"Analysis of the fused 4-oxocrotonate tautomerases: Implications for divergence in the tautomerase superfamily"

11:55 **John Gerlt** – UIUC
"Genomic enzymology: Discovery of novel enzymes and metabolic pathways"

12:30 Lunch (not provided)

3:00 – 5:00 pm **Poster Session I (Odd numbered posters, cash bar)**

Session 3 – The holy trinity – Enzymes, Coenzymes, and Metabolic Pathways
Chair: Squire Booker, Penn State University

7:00 **Elizabeth Sattely** – Stanford
"Discovery and engineering of plant chemistry for plant and human health"

7:35 **Mo Seyedsayamdost** – Princeton
"New metalloenzyme-catalyzed transformations in natural product biosynthesis"

8:10 **Tadhg Begley** – Texas A&M University
"Mechanistic studies on the futalosine-dependent menaquinone biosynthetic pathway"

8:45 **Wulf Blankenfeldt** – Helmholtz Centre for Infection Research
Poster talk: "The alkylquinolone repertoire of *Pseudomonas aeruginosa* is linked to structural flexibility of the FabH-like Pseudomonas quinolone signal biosynthesis enzyme PqsBC"

Tuesday, January 8

7:30-8:30 Breakfast

Session 4 – A biosynthetic menagerie
Chair: Karen Allen, Boston University

8:30 **Janet Smith** – University of Michigan
"Deciphering the activities of natural product biosynthetic enzymes: Not all domains are what they appear to be"

9:05 **Yi Tang** – UCLA
"Discovery and characterization of a SAM-dependent pericyclase"

9:40 **Amy Rosenzweig** – Northwestern
"Biosynthesis of methanobactin"

10:15-10:45 Coffee Break

Session 5 – Picking on the bass
Chair: Ken Johnson, UT Austin

10:45 **Craig Cameron** – Penn State
"Targeting a viral polymerase for antiviral therapy: Insight from studies of single molecules and single, infected cells"

11:20 **Peter Weigele** – New England BioLabs
"Pathways of thymidine hypermodification"

11:55 **Jane Jackman** – Ohio State
"A new direction for RNA polymerases: 3'-5' polymerase mechanism and function"

12:30 Lunch (not provided)

3:00 – 5:00 pm **Poster Session II (Even numbered posters, cash bar)**

Session 6 – A good drug is hard to find
Chair: Elham Behshad, Incyte

7:00 **Alan Rendina** – GSK
"Uncovering novel Inhibitor sites and mechanisms takes both screening and sleuthing – Tales from the big pharma trenches"

7:35 **John Quinn** – Genentech
"Real-time, label-free measurement of inhibitor affinity and kinetics"

8:10 **Karen Maegley** – Pfizer
"Targeting protein arginine methyltransferase 5 (PRMT5) for cancer therapy; development of a SAM competitive inhibitor with potent antitumor activity and a decrease liability for developing resistance"

8:45 **Norbert Reich** – UC Santa Barbara
Poster talk: "DNMT3A mutations in AML patients cause loss and gain of function and differential regulation by protein partners"

Wednesday, January 9

7:30-8:30 Breakfast

Session 7 – Sugar Bowl!
Chair: Frank Raushel, Texas A&M

8:30 **Hazel Holden** – UW Madison
"N-Formylated sugars – are they the kiss of death?"

9:05 **Stephen Withers** – UBC
"Discovery of CAZymes for cell surface glycan removal through metagenomics: Towards universal blood"

9:40 **Jianyong Jiang** – UW Madison
Poster talk: "Specificity, function and regulation of protein O-GlcNAc modification"

9:55 **Liz Hedstrom** – Brandeis
Poster talk: "Dynamic characteristics of GMP reductase complexes revealed by high resolution ^{31}P field cycling NMR relaxometry"

10:15-10:45 Coffee Break

Session 8 – When metal ions and electrons go marching in!
Chair: Aimin Liu, UTSA

10:45 **Stephen Ragsdale** – University of Michigan
"Coupling of electron transfer and chemical reactions to drive organometallic and radical-based enzymatic catalysis by nickel enzymes"

11:20 **Marty Bollinger** – Penn State University
"How iron- and 2-(oxo)glutarate-dependent oxygenases direct their common ferryl intermediate to different outcomes"

11:55 **Sean Elliott** – Boston University
"Radical SAM enzymes, viewed through an electrochemical lens"

12:30-1:30 Lunch buffet

2:00 **Founders Award lecture: Melanie Higgins** – UBC
"Characterization of a heme-dependent N-N bond forming enzyme"

Session 9 – Farewell to the Big Easy with a biosynthetic tryptich
Chair: Gene Cordes

2:30 **Wilfred van der Donk** – UIUC and HHMI
"Posttranslational modifications during cyclic peptide biosynthesis"

3:05 **Judith Klinman** – UC Berkeley
"PQQ biosynthesis: Getting close to the finish line"

3:40 **Dale Poulter** – University of Utah
"Isopentenyl diphosphate isomerase. Breaking up is hard to do"

6:00 Closing banquet: *Second line*
Cocktails and hors d'oeuvres, dinner at 7 pm

POSTERS

P1. Specificity, function and regulation of protein O-GlcNAc modification
Jiaoyang Jiang

P2. Intradiol ring-cleavage dioxygenase from the polyphagous spider mite herbivore *Tetranychus urticae Koch*
Leily Daneshian, Caleb R. Schlachter, Jose Amaya, Vincent Klapper, Nicky Wybouw, Tomasz Borowski, Thomas Van Leeuwen, Vojislava Grbic, Miodrag Grbic, Thomas M. Makris, Maksymilian Chruszcz

P3. From kinetic studies of enzyme catalysis and inhibition to discovery of novel "mechanism-based" inhibitors of *Mycobacterium tuberculosis* isocitrate lyase
Megan Moynihan, Hsiao-Ling Huang, Inna Krieger, Andrew Murkin, James Sacchettini, Thomas D. Meek, Truc V. Pham

P4. Role of the KDO glycosyltransferase KpsS in the Biosynthesis of the polysialyltransferase acceptor for *Escherichia coli* K1
Nicholas D. Lanz, Vireak Thon, and Willie F. Vann

P5. Novel radical SAM enzymology in futalosine-dependent menaquinone biosynthesis
Sumedh Joshi, Dmytro Fedoseyenko, Hannah Manion, Derek Gagnon, Rodrigo Ducati, Vern Schramm, R. David Britt, Tadhg P. Begley

P6. An Alternative catabolic pathway for ascorbate includes a benzilic acid rearrangement and a unique NAD^+-dependent decarboxylase
Tyler M. M. Stack, Brian San Francisco, Michael S. Carter and John A. Gerlt

P7. Investigation of the reaction mechanisms of the Fe(II)- and 2- oxoglutarate-Dependent ethylene-forming enzyme, EFE
Rachelle A. Copeland, Katherine M. Davis, Tokufu Kent C. Shoda, Elizabeth J. Blaesi, Amie K. Boal, Carsten Krebs, J. Martin Bollinger, Jr.

P8. Development of enzyme-coupled reactions for monitoring the ATP-ase activity of Hsp-70/Hsp-40 chaperone system
Cristina C. Clement[1], Janet Gonzalez[2], Kateryna Morozova[1]

P9. Prediction of golden gate assembly using a comprehensive analysis of T4 DNA ligase end-joining fidelity and bias
Vladimir Potapov, Jennifer L. Ong, Rebecca Kucera, Bradley W. Langhorst, Katharina Bilotti, Eric Cantor, Barry Canton, Thomas F. Knight, Thomas C. Evans, Jr., and Gregory J. S. Lohman, John Pryor

P10. Identifying the activities of hypothetical proteins in a putative biosynthetic cluster from *Streptomyces griseofuscus*
Trevor R. Melkonian, Madeline A. Rettmann, Nicholas R. Silvaggi

P11. Detection of dynamic protein networks for ligand-induced allostery and thermal activation/adaptation using multi-temperature hydrogen deuterium exchange
Adam R. Offenbacher

P12. Template-assisted synthesis of adenine-mutagenized cDNA by a diversity-generating retroelement protein complex
Sumit Handa, Partho Ghosh

P13. Regulation of PTEN by C-terminal phosphorylation
Daniel R. Dempsey, Hanjie Jiang, Jay H. Kalin, Stephanie Henriquez, Zan Chen, Sandra B. Gabelli, Philip A. Cole

P14a. DNMT3A mutations in AML patients cause loss and gain of function and differential regulation by protein partners
Jonathan Sandoval, Abigale Muise and Norbert Reich

P14b. The highly specific, cell cycle–regulated methyltransferase from *Caulobacter crescentus* relies on a novel DNA recognition mechanism
Norbert O. Reich, Eric Dang, Martin Kurnik, Sarath Pathuri, Clayton B. Woodcock

P15.Structural and Functional Characterization of FosM from *Mycobacterium abscessus*
Madeline R. Shay, Skye Travis, Matthew K. Thompson

P16. Toward deciphering the biosynthesis of DNA thymidine hypermodifications in bacteriophages
Yan-Jiun Lee, Nan Dai, Stephanie Muller, Shannon Walsh, Chudi Guan, Ivan Correa & Peter Weigele

P17. Mechanism of 6-hydroxynicotinate 3-monooxygenase (NicC), a flavin-dependent decarboxylative hydroxylase involved in aerobic nicotinic acid catabolism
Scott W. Perkins and Mark J. Snider

P18. Steric enforcement of cis rather than trans epoxide formation by fosfomycin-producing (S)-2-hydroxypropylphosphonate epoxidase
Shengbin Zhou, Juan Pan, Katherine M. Davis, Irene Schaperdoth, Amie K. Boal, Carsten Krebs, J. Martin Bollinger, Jr.,

P19. Mechanism of dioxygen activation by peroxide-dependent P450 decarboxylase OleT
Courtney E. Wise, Nathan L. Poplin, Thomas M. Makris

P20. Elucidating the Mechanism of C-S Bond Cleavage in Isethionate Sulfite-Lyase
C. D. Dawson, S. C. Peck, S. M. Irwin, E. P. Balskus, C. L. Drennan

P21. CYP epoxygenases metabolize omega-3 endocannabinoids to form a novel class of anti-inflammatory lipid mediators
Josephine E. Watson and Aditi Das

P22. Using ketoreductases for the chemoenzymatic synthesis of a stereotriad
Kaan Kumru

P23. Structural and functional studies of modular polyketide synthase domains for venemycin synthesis
Adrian Keatinge-Clay, Melissa Hirsch

P24. Characterization of a heme-dependent N-N bond forming enzyme
Melanie A Higgins, Yi-Ling Du, Katherine Ryan

P25. Nicotinamide riboside (NR), a multifaceted molecule
Ai Tran, Song Zheng, Ryota Yokose, Stacia Rymarchyk, Yana Cen

P26. Filling in the gaps: Complete biosynthetic pathwayto fosfomycin in Pseudomonads
Max Simon and Wilfred A. van der Donk

P27. Cytochrome P450-catalyzed hydroxylation initiating ether formation in Platensimycin biosynthesis
Jeffrey D. Rudolf, Liao-Bin Dong, Xiao Zhang, Hans Renata, and Ben Shen

P28. Coming Back from the Dead: Insight into the Regeneration of the Auxiliary Cluster of *E. coli* Lipoyl Synthase in the Biosynthesis of the Lipoyl Cofactor
Erin L. McCarthy and Squire J. Booker

P29. Biocatalytic carbon-hydrogen and carbon-fluorine bond hydroxylation promoted by a histidyl-ligated heme enzyme, LmbB2
Yifan Wang, Ian Davis, Inchul Shin, Daniel J. Wherritt, Wendell P. Griffith, Kednerlin Dornevil, Keri L. Colabroy, and Aimin Liu

P30. Structural and functional studies of a *gem*-dimethylating methyltransferase from a trans-acyltransferase assembly line.
Jessica L. Meinke, M. Rachel Mehaffey, Drew T. Wagne, Zhicheng Zhang2, Ningze Sun, Jennifer S. Brodbelt, Adrian T. Keatinge-Clay

P31. Kinetic Basis for improved specificity of CRISPR/Cas9 high fidelity variants
Mu-Sen Liu, Shanzhong Gong, Helen-Hong Yu, David W. Taylor, Kenneth A. Johnson

P32. Studies of the four enzymes catalyzing methyllincosamide backbone transformation and their mechanistic implications
Shao-An Wang, Chia-I Lin, Richiro Ushimaru, Jiawei Zhang, Eita Sasaki and Hung- wen Liu

P33. Post-translational control and dynamics of the human iron-sulfur cluster assembly complex
Seth Van Andel, Steven Havens, Dr. David Barondeau

P34. *In vitro* reconstitution, analysis, and engineering of the

venemycin modular polyketide synthase
Takeshi Miyazawa and Adrian T. Keatinge-Clay

P35. Ketoreductases as biocatalysts in the synthesis of chiral diketides
Mireya Luna-Robles, Zhicheng Zhang, & Adrian Keatinge-Clay, Mireya Luna-Robles

P36. From enzyme mechanisms to rational inhibitor design: Potential inhibitors of essential enzymes from human pathogens.
Ardala Katzfuss, Peter Tyler, Vern Schramm, Thomas D. Meek, and Demetrios Kostomiris

P37. General chemoenzymatic route to two-stereocenter triketides
Zhicheng Zhang, Alexis J. Cepeda, Mireya L. Robles, Kaan Kumru, Jina Zhou, Zhicheng Zhang

P38. Using a fluorescent unnatural amino acid to characterize the role of conformational dynamics in high fidelity DNA replication
Kenneth Johnson, Tyler Dangerfield

P39. Effects of vibrational and structural perturbations in formate dehydrogenase
Chethya Ranasinghe, Phil Pagano, Qi Guo, Amnon Kohen, Christopher M. Cheatum

P40. Exploring the mechanism of enzyme Evolution
Priyanka Singh, Donald Hilvert and Amnon Kohen

P41. Dibenzothiophene catabolism: mechanistic studies and first enzymatic synthesis of a chiral biphenyl
Avick Kumar Ghosh, Sanjoy Adak and Tadhg P. Begley

P42. Phytoene desaturase: biochemical characterization and enzymatic activity
Brian K. Barr, Cassandra Cairns, Ruben Ferreira de Carvalho, Najuma Babirye, Faith Osinaga, Loc Le, Lindsey Staszewski and Abigail McLaughlin

P43. High-field EPR and DFT studies of tryptophan-based radicals
Teruaki Koto, Ian Davis, James R. Terrell, Alexander Kozhanov, J. Krzystek and Aimin Liu

P44. Mechanism of a Class C radical SAM Thiazole methyl transferase
Zhengan Zhang, Nilkamal Mahanta, Graham A. Hudson, Douglas A. Mitchell and Wilfred A. van der Donk

P45. Insights into AMS/PCAT transporters from biochemical and structural characterization of a double glycine motif protease
Silvia C. Bobeica, Shi-Hui Dong, Liujie Huo, Nuria Mazo, Martin I. McLaughlin, Gonzalo Jiménez-Osés, Satish K. Nair and Wilfred A. van der Donk

P46. Active site structure of full-length and split-LanBs revealed through the use of non-reactive substrate mimics

Ian R. Bothwell, Dillon P. Cogan, Satish K. Nair, and Wilfred A. van der Donk

P47. On the mechanism of the L-Arginine Oxidase MppP from *Streptomyces Wadayamensis*
Nemanja Vuksanovic[1], Lanlan Han[1], Nicholas R. Silvaggi

P48. Polyubiquitin-based DUB probes reveal mechanisms of ubiquitin chain recognition and processing by ubiquitin-specific protease
Prajwal Paudel, Qi Zhang, Charles Leung, Harrison Greenberg, Yusong Guo, Yi- Hsuan Chern, Aiping Dong, Yanjun Li, Masoud Vedadi, Yufeng Tong and Zhihao Zhuang

P49. Towards novel chemotherapy for Chagas disease: Design, synthesis and kinetic studies of Cruzain inhibitors
Linfeng Li, Bala C. Chenna, Thomas D. Meek

P50. Characterization of hypoxanthine-guanine phosphoribosyltransferase
Ryan Bringenberg, Dr. Ardala Katzfuss, Dr. Thomas Meek

P51. New cofactors for bacterial class 1 ribonucleotide reduction
Amie K. Boal

P52. Reassignment of the human aldehyde dehydrogenase ALDH8A1 (ALDH12) to the kynurenine pathway in tryptophan catabolism
Ian Davis, Yu Yang, Daniel Wherritt, and Aimin Liu

P53. Protein Quaternary Structure as a Mean to Regulate Enzyme Activity in Kynurenine Pathway
Yu Yang, Ian Davis, Tsutomu Matsui, and Aimin Liu

P54. Probing cofactor biogenesis in cysteine dioxygenase: C-F bond cleavage with unnatural tyrosine
Jiasong Li, Ian Davis, Teruaki Koto, Wendell Griffith, Inchul Shin, Yifan Wang, Daniel J. Wherritt, and Aimin Liu

P55. New insights into mycofactocin biosynthesis
Richard Ayikpoe, Cameron Robertson, John. A. Latham

P56. Probing the catalytic mechanism of tryptophan 2,3-dioxygenase and molecular basis for hypertryptophanemia due to enzyme deficiency linked to noncatalytic tryptophan binding site.
Inchul Shin, Daniel Wherrit,Wendell P. Griffith, Ryan Altman, Patrick Ferreira, Aimin Liu

P57. Elucidating the role of lysine succinylation on the function of isocitrate lyase 1 from *Mycobacterium tuberculosis*
Drake Mellott, Zhipeng Wang, Wenshe Liu, Thomas Meek

P58. Transient histone H4 phospho-serine and acetyl-Lysine distinctly govern the kinetics of arginine R3 methylation catalyzed by PRMT1 and PRMT5

Emmanuel S. Burgos, Adam Haimowitz, Bogos Agianian, Evripidis Gavathiotis and David Shechter

P59. Structural characterization of a hydroxyproline dehydratase from *C.*
Lindsey R. F. Backman, Yolanda Y. Huang, Michael A. Funk, Brian Gold, Ronald T. Raines, Emily P. Balskus, and Catherine L. Drennan

P60. Structure and mechanism of a nicotine-degrading enzyme, NicA2: Towards design of tools and therapeutics
Margarita A. Tararina, Song Xue, Kim D. Janda, Bruce A. Palfey and Karen N. Allen

P61. Structure and inhibition kinetics of trehalose 6-phosphate phosphatase
Katherine H. O'Toole, Christine M. Harvey, Lucy Lin, Kim D. Janda, Karen N. Allen

P62. SufS, a Type II cysteine desulfurase, is regulated through changes in the dimer interface.
Jack A. Dunkle, Michael Bruno, F. Wayne Outten, and Patrick A. Frantom

P63. Rieske non-heme iron enzymes in natural product biosynthetic pathways
Jianxin Liu, April L. Lukowski, Alison R. H. Narayan, Jennifer Bridwell-Rabb

P64. Substrate binding in 2,4'-dihydroxyacetophenone dioxygenase
Catherine Haley Cave, Gabrielle C. Connor, Jason A. Weeks, Gerard T. Rowe, and Kenneth M. Roberts

P65. Structural and biochemical characterization of the *N*-prenyltransferase from domoic acid biosynthesis reveals basis for unusual activity
Jonathan R. Chekan and Bradley S. Moore

P66. A low-lying dark state controls the deactivation of excited anionic and neutral flavin semiquinones in nitronate monooxygenase
Dan Su, Mohammad Pabel Kabir, Yoelvis Orozco-Gonzalez, Samer Gozem, and Giovanni Gadda

P67. Kinetic viscosity effects reveal a protein isomerization in the reductive half-reaction of nitronate monooxygenase
Maria Vodovoz, Dan Su, Giovanni Gadda

P68. Probing carrier protein and substrate recognition of the second cyclization domain of yersiniabactin synthetase by docking and molecular dynamics
Andrew Gnann, Yuan Xia, Jessica Soule, Daniel P. Dowling

P69. Characterization of an $FMNH_2$-dependent monooxygenase involved in methanesulfinate utilization in *Pseudomonas fluorescens*
Jessica Soule, Daniel P. Dowling, Denyce K. Wicht

P70. Kinetic and mechanistic characterization of a potent inhibitor of human arginase
Yu Chen, Sterling C. Eckard, Elaine Ginn, Tezcan Guney, Jarek Kalisiak, Manmohan R. Leleti, Lixia Jin, Stephen W. Young, Matthew J. Walters, Ulrike Schindler, Jay P. Powers

P71. Kinetic Characterization of L-arginine dehydrogenase from *Pseudomonas aeruginosa*
Giovanni Gadda, Archana Iyer, Madeline Weaver, Joanna Quaye

P72. Heavy enzyme kinetic isotope effects on the reaction of tyrosine phenol-lyase
Robert S. Phillips, Andreea I. Iorgu, Derren J. Heyes and Sam Hay

P73. Getting a grip on protein dynamics: Ligand induced conformational changes in GT-B enzymes
Erika A. Taylor, Patrick A. Frantom, Jozafina Milicaj, Cody Hecht, Wen Chen, Bakar Hassan, Frank Tucci, Courtney Petersen and Jacquelyn S. Turri

P74. Conserved residues have different roles in homologous OSBS enzymes
Margaret E. Glasner, Thomas Dunham and Denis Odokonyero

P75. Directed evolution of NSAR activity
Benjamin C. Morse and Margaret E. Glasner

P76. The important role of a second-shell amino acid in determining *N*-succinylamino acid racemase reaction specificity
Dat P Truong, Simon Rousseau, Jamison Huddleston, Frank M. Raushel, James Sacchettini, & Margaret E. Glasner

P77. A New Flavoenzyme Catalyzed Baeyer-Villiger Type Rearrangement in the Bacterial Folate Catabolic Pathway
Tadhg P. Begley, Sanjoy Adak

P78. Expanding and applying the chemical potential of stereospecific vanadium-dependent haloperoxidases from Streptomyces bacteria
Shaun M. K. McKinnie, Zachary D. Miles and Bradley S. Moore

P79. Discovery of an NRPS-like choline synthetase in fungi
Yang Hai, Arthur Huang, and Yi Tang

P80. Structural, biochemical and genetic characterization of a unique NRPS protein AB826 from *A. baumannii*
Ketan D. Patel, Andrew Gulick

P81. The structural basis of β-lactone formation catalyzed by a nonribosomal peptide synthetase
Dale Kreitler, Jason Schaffer, Erin Gemmell, Timothy Wencewicz, Andrew Gulick

P82. Resolving the mechanism of an NRPS-independent siderophore synthetase from hypervirulent *Klebsiella pneumoniae*
Lisa S. Mydy, Andrew M. Gulick

P83. Click chemistry approach for the optimal synthesis of antibody-drug conjugates
Wenshe Liu, Alfred Tuley, Erol Vatansever

P84. LanCLs: Non-canonical glutathionylation enzymes in mammalian systems
Kuan-Yu Nick Lai, Jitka Dadova, Benjamin Davis, Wilfred van der Donk

P85. Dre1 and Nar2 are the partner proteins of the Nbp35-Cfd1 cytosolic iron sulfur cluster assembly scaffold
Christa N. Mole

P86. Probing the equilibrium conformational ensemble of transglutaminase 2 (TG2)
Anita DeSantis[1], Alex D Hondros[1], Kimberly A Kew, and Tonya N Zeczycki[1]

P87. Design, synthesis, and mechanistic evaluation of aminotransferase inhibitors for the treatment of epilepsy, addiction, and liver cancer.
Matthew J. Moschitto, Jose I. Juncosa, Kenji Takaya, Hoang V. Le, Pathum M. Weerawarna, Romila Mascarenhas, Dan Catlin, Peter Doubleday, Dali Liu, Stephen L. Dewey, Richard B. Silverman

P88. New Insights on nickel-pincer nucleotide cofactor biosynthesis and function
Joel A. Rankin, Matthias Fellner, Jian Hu, Robert P. Hausinger

P89. Disulfide formation kinetics of two-cysteine peroxiredoxins and a model for peroxide sensing
Derek Parsonage, Stephanie Portillo-Ledesma, Lía M. Randall, Joaquín Dalla Rizza, P. Andrew Karplus, Ana Denicola, Gerardo Ferrer-Sueta, Leslie B. Poole

P90. Transition State Interrogation: β-glucosidase from *Thermotoga maritima*
Ross A. Zaenglein, Larry D. Byers

P91. Substitution of methyl group with amino: Novel flavoenzyme converts vitamin into antibiotic
Isita Jhulki and Tadhg P.Begley, Isita Jhulki

P92. Structural properties that promote catalysis for enzymes involved in sulfur metabolism
Richard A. Hagen, Jeff S. McFarlane, Annemarie S. Chilton, Audrey L. Lamb, and Holly R. Ellis

P93. An opine on opines: The biosynthesis of opine metallophores in bacterial pathogens
Jeffrey S. McFarlane, Cara L. Davis, Graham R. Moran and Audrey L. Lamb

P94. 3,4-dihydroxy-2-butanone 4-phosphate synthase (RibB) of riboflavin biosynthesis has a mononuclear magnesium active site
Nikola Kenjic, Graham R. Moran, Audrey L. Lamb

P95. Biosynthesis of the capsular polysaccharide modifications found in *Campylobacter jejuni* NCTC 11168
Zane W. Taylor, Alexander S. Riegert, Haley A. Brown, Hazel M. Holden, and Frank M. Raushel

P96. Deciphering the enzymatic function of the bovine enteric infection related protein YfeJ from *Salmonella enterica* serotype Typhimurium
Yuan Zhi, Tamari Narindoshvili , Helene Andrews-Polymenis, and Frank M. Raushel

P97. Elucidating the mechanism of enzymes utilized during the bacterial degradation of lignin in the protocatechuate 4,5-cleavage pathway
Tessily N. Hogancamp, Mark F. Mabanglo, Seth A. Cory, Dongju Lee, and Frank M. Raushel

P98. Mechanistic Characterization of *Sb*-PTE a phosphotreisterase from *Sphingobium* sp. TCM1 capable of hydrolyzing flame retardants
Andrew N. Bigley, Dao Feng Xiang, Tamari, Narindoshivili, Frank M. Raushel

P99. Investigation of blunt end joining by DNA ligases
Robert J. Bauer, Alexander Zhelkovsky, Evan Burke, Vladimir Potapov, Jennifer Ong, Pierre Davy, Thomas C. Evans Jr., and Gregory J.S, Lohman, Katharina Bilotti

P100. Kinetic characterization of an ultra-high fidelity DNA polymerase
Kinjal Dave, Andrew Gray and Nicole Nichols

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Witzgall F, Depke T, Hoffmann M, Empting M, Brönstrup M, Müller R & Blankenfeldt W

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ABSTRACTS

Oral Sessions

An Allosteric Disulfide Bond and its Role in Celiac Disease Pathogenesis

Chaitan Khosla

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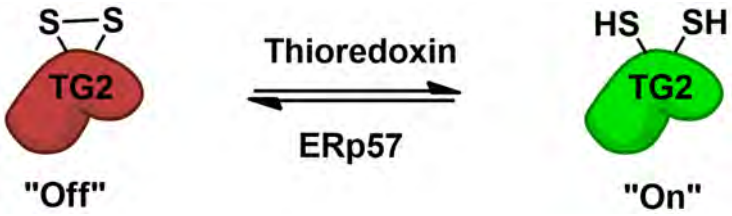
Human transglutaminase 2 (TG2) is a ubiquitous but non-essential enzyme that plays a critical role in celiac disease pathogenesis. This lecture will focus on the chemistry and biology of an unusual disulfide bond that comprises a redox-active switch for reversibly activating the enzyme in the extracellular matrix.



2 Infectious Diseases, 2 Enzymes, and Maybe 2 Lead Compounds

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Marketed drugs which are either enzyme inhibitors or covalent inactivators almost universally display time-dependent kinetics, and many of these have been rationally designed from the structures of both enzyme and their substrates. Essential enzymes that contain active-site cysteines are particularly interesting as drug targets, since the nucleophilicity of this rarely-occurring residue may be exploited by the formation of both reversible and irreversible covalent adducts from electrophilic substrate analogues, often via thia-Michael reactions. The isocitrate lyases (ICLs) of *Mycobacterium tuberculosis* and the trypanosomal cysteine proteases, cruzain and rhodesain, of respectively, *Trypansoma cruzi*, and *Trypanosomal brucei* contain active-site cysteines, and are essential enzymes to these respective causative agents of tuberculosis, Chagas's disease, and African sleeping sickness. Supporting the design of reversible covalent inactivators of *Mtb* ICL1 and cruzain has been detailed kinetic analysis, including (pre-)steady-state analysis, pH studies, solvent kinetic isotope effects, and site-directed mutagenesis, which has unveiled mechanistic details we have attempted to exploit for inactivator design. 2-vinyl-isocitrate (2-VIC) is a mechanism-based inactivator of ICL1/2 in which catalysis generated a Michael substrate, 2-vinyl-glyoxylate, which reacts with active-site Cys_{191} at very high efficiency[1]. We have used this as a starting point to develop other covalent inactivators. For cruzain, our kinetic characterization of its mechanism reveals that in the free enzyme both active-site Cys_{25} and His_{162} are both neutral, and for its most optimal substrates, the acylation half-reaction of catalysis is much faster than de-acylation.[2] These findings have encouraged the synthesis and characterization of a novel family of potent, time-dependent inhibitors, the most potent of which exhibit anti-trypanocidal activities in cell cultures of *T. brucei* and *T. cruzi*, and for the latter, in a cellular model of infection.

1. Truc V. Pham, Andrew S. Murkin , Margaret M. Moynihan,1, Lawrence Harris , Peter C. Tyler, Nishant Shetty, James C. Sacchettini, Hsiao-ling Huang, and Thomas D. Meek (2017) "Mechanism-based inactivator of isocitrate lyases 1 and 2 from Mycobacterium tuberculosis" *Proc. Natl. Acad. Sci. USA 114,*7617-7622.

2. X. Zhai, and T. D. Meek (2018) "Catalytic Mechanism of Cruzain from *Trypanosoma cruzi* as Determined from Solvent Kinetic Isotope Effects of Steady-State and Pre-Steady-State Kinetics" *Biochemistry* 57, 3276-3190.

Transition State Analogues for Ribosyl Transferases

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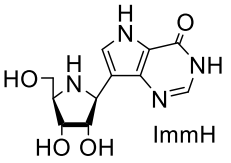
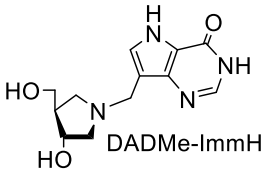
Transition state analogues can occasionally be developed by understanding enzymatic transition states from kinetic isotope effect experiments. Five transition state analogues exemplify roles in understanding the catalytic function of their cognate enzymes and some potential uses toward human medicine.



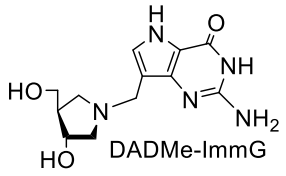
23 pM bovine PNP

ImmH mimics the transition state of bovine purine nucleoside phosphorylase (PNP) and binds tightly to bovine and human PNPs. It has been approved in Japan for use against resistant or relapsed peripheral T-cell lymphoma under the name Mundesine®.



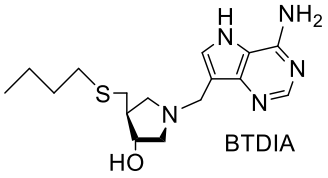
9 pM human PNP

DADMe-ImmH mimics the human PNP transition state, providing an improved fit to the catalytic site. Solid-state NMR of ImmH and DADMe-ImmH bound to human PNP reveals strong distortional forces for ImmH. DADMe-ImmH has completed phase 2 clinical trials for gout. A single oral dose completely inhibits erythrocyte PNP for the 120 day lifetime of the cells. Inhibitor exchange studies reveal dynamic rebinding as the mechanism for its long lifetime on the target enzyme.



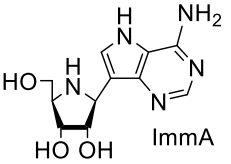
0.3 nM malaria PNP

DADMe-ImmG is a pM inhibitor of human PNP and a 0.3 nM inhibitor of the *P. falciparum* PNP. This purine auxotroph requires hypoxanthine formed by PNP in erythrocytes or the parasite. PNP inhibition is lethal to *P. falciparum* under in vivo hypoxanthine conditions. Parasites cultured for several years under increasing drug pressure undergo PNP gene amplification and catalytic site mutations that decrease inhibitor binding and catalytic function.



36 pM *H. pylori* MTAN

BTDIA is a transition state analogue of bacterial 5′-methylthioadenosine nucleosidases (MTANs). *H. pylori* and *C. jejuni* use MTAN in the futalosine pathway of menaquinone synthesis, but most bacteria of the gut microbiome do not. BTDIA and related transition state analogues prevent the growth of *H. pylori* and *C. jejuni* but do not block the growth of the gut microbiome.



87 pM *T. vaginalis* PNP

ImmA is a transition state analogue of the PNP from *T. vaginalis*, a common STD. It is also a prodrug for chain termination of viral RNA polymerases. It has entered clinical trials as an antiviral.

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Bumps in the road toward evolution of a new enzyme

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New enzymes often evolve from promiscuous activities of existing enzymes by a process of gene duplication and divergence. When a new enzyme is required for fitness or even survival, some microbes in a community may be more successful than others in meeting that evolutionary challenge. Some microbes may not have a promiscuous enzyme that can be recruited to carry out a new function. A promiscuous activity in others may be too weak to affect fitness, and it may take several mutations – an unlikely scenario – to raise the efficiency of the promiscuous activity to an effective level. Mutations that enhance an inefficient promiscuous activity may have unacceptable consequences for the native activity. Finally, some microbes may not be able to duplicate a gene encoding a weak-link enzyme.

We are studying the evolution of a new enzyme from a promiscuous activity of ProA (γ-glutamyl phosphate reductase). ProAs from several microbes have an inefficient promiscuous ability to catalyze reduction of *N*-acetyl glutamyl phosphate, a reaction normally carried out by ArgC in the arginine synthesis pathway. In *E. coli,* this promiscuous activity is too inefficient to support growth if *argC* is deleted. However, a mutation that changes Glu383 to Ala enables ProAs from 9 different microbes to substitute for ArgC in Δ*argC E. coli*. The effects of the same mutation on the native and promiscuous activities are strikingly different in the various orthologs, supporting the idea that some microbes may be more successful than others in the initial recruitment of a promiscuous enzyme to serve a new function.

In Δ*argC E. coli* carrying a gene E383A ProA, massive amplification of the region surrounding the *proBA* operon occurs. This is the pre-requisite for divergence of a new enzyme. In the same system in *S. enterica*, gene amplification, surprisingly, does not occur, although growth rate improves substantially in 250 generations. These findings suggest that evolution of a new enzyme may be possible in some organisms but not in others, and that adaptation may take circuitous routes toward improved fitness that do not improve the performance of the inefficient enzyme.

Analysis of the Fused 4-Oxalocrotonate Tautomerases: Implications for Divergence in the Tautomerase Superfamily

Bert-Jan Baas, Shoshana Brown, Rebecca Davidson, Jake LeVieux, Eyal Akiva, Brenda Medellin, Yan Jessie Zhang, Patricia C. Babbitt, and Christian P. Whitman

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The tautomerase superfamily (TSF) is a group of structurally homologous proteins characterized by two notable features. First, they are constructed from a single β–α–β structural unit (58-80 amino acids) or two fused β–α–β units (110-150 amino acids). Second, all of the experimentally characterized members have a catalytic amino-terminal proline (Pro-1) that functions as a general acid or general base depending on the pK_a of the prolyl nitrogen. Analysis of the sequence similarity network for the TSF provides many new insights. One is the observation that a significant fraction (~3%) of TSF members lack Pro-1, where serine, isoleucine, and alanine are the residues most frequently found in place of Pro-1. Second, sequence links between the 4-oxalocrotonate tautomerase (4-OT) and *cis*-3-chloroacrylic acid dehalogenase (*cis*-CaaD) subgroups combined with other results might suggest steps in the evolution of proteins in the *cis*-CaaD subgroup from an ancestral one in the 4-OT subgroup. Finally, a closer look at one of these sequence links, the "fused 4-OT", uncovered a subset of similar sequences with some intriguing properties. The combined results are consistent with the hypothesis that the *cis*-CaaD-like enzymes evolved from a 4-OT-like ancestor and might reflect a combinatorial mechanism used by nature to diversify function.

GENOMIC ENZYMOLOGY: DISCOVERY OF NOVEL ENZYMES AND METABOLIC PATHWAYS

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The number of proteins in the UniProt database (>134M sequences in Release 2018_10) is increasing with a doubling time of ~2.5 years; however, ≥50% of the proteins have uncertain, unknown, or incorrect functions. This lecture will describe the use of publicly accessible ("democratized") web tools for generating 1) protein sequence similarity networks (SSNs; EFI-EST; efi.igb.illlinois.edu/efi-est/) to explore sequence-function space in protein families and 2) genome neighborhood networks (GNNs; EFI-GNT; efi.igb.illlinois.edu/efi-gnt/) for identifying functionally linked genes/proteins in metabolic pathways. We also have developed a web tool (EFI-CGFP, not yet "democratized") for chemically guided functional profiling (CGFP) that maps metagenome abundance to clusters in SSNs, thereby allowing prioritization of "unknowns" for functional assignment. Examples will be given of novel metabolic pathways that have been discovered using SSNs and GNNs. The lecture will also describe the need for authors to include UniProt accession IDs in manuscripts so that the reliability of functional annotations in the databases can be improved. Supported by NIH P01GM118303.

Discovery and Engineering of Plant Chemistry for Plant and Human Health

Elizabeth Sattely

Stanford University

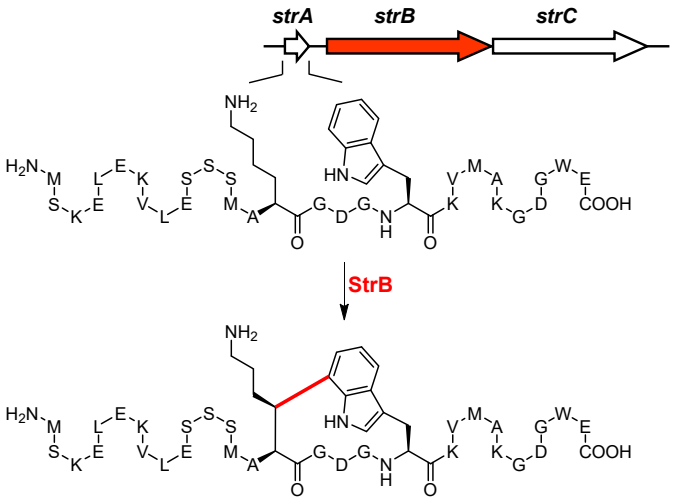
Plants produce an impressive array of molecules important for both plant and human health. The discovery of biosynthetic pathways for plant natural products has classically been a slow process; as a consequence, few complete pathways are known and even fewer have been engineered. New plant genome sequences offer an opportunity to increase the rate of pathway discovery, but many medicinal plants have not yet been sequenced and genomic data alone is often not sufficient to elucidate pathway genes. This seminar will describe our efforts to merge transcriptomics, untargeted metabolomics, bioinformatics, and engineering to rapidly uncover complete plant pathways and novel enzyme chemistry for known and new molecules, not only in the model plant *Arabidopsis* but also non-model plants.

New Metalloenzyme-Catalyzed Transformations in Natural Product Biosynthesis

Kelsey R. Schramma, Leah B. Bushin, Alessio Caruso, Kenzie A. Clark, Ryan J. Martinie, Clarissa C. Forneris, and Mohammad R. Seyedsayamdost

Department of Chemistry, Princeton University

Microbial natural products continue to inspire chemists and biologists alike with their fascinating structures and exquisite biological activities. Investigations into their biosynthetic pathways not only provides possible means of creating these molecules in the laboratory but can also reveal unusual enzymatic reactions. In this talk, I will present new transformations that my group has discovered during the biosynthesis of complex natural products, with a focus on carbon-carbon bonds installed by metalloenzymes. Results from detailed analyses are pointing to new mechanistic paradigms that are likely wide-spread in biological systems. Our efforts are expanding the scope of reactions that microorganisms have at their disposal in concocting bioactive natural products.



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Mechanistic Studies on the Futalosine-Dependent Menaquinone Biosynthetic Pathway

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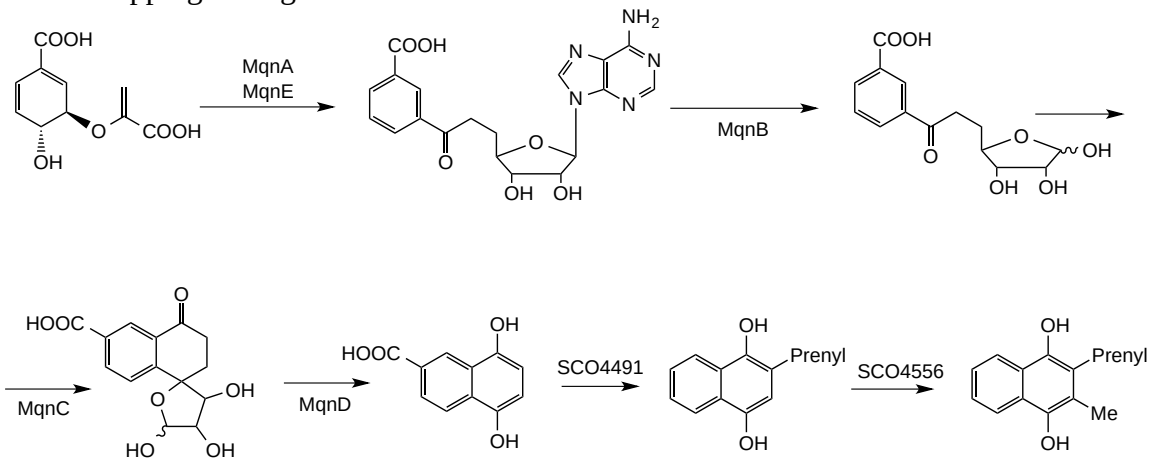
The futalosine-dependent menaquinone biosynthesis discovered by Tohru Dairi in 2008[1] is shown below. MqnE[2-5] and MqnC[6] are both radical SAM enzymes. My lecture will describe recent mechanistic studies on these two enzymes and the development of new radical trapping strategies.



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The Alkylquinolone Repertoire of *Pseudomonas aeruginosa* is Linked to Structural Flexibility of the FabH-like Pseudomonas Quinolone Signal Biosynthesis Enzyme PqsBC

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Pseudomonas aeruginosa is a bacterial pathogen that causes life-threatening infections in immunocompromised patients. It produces a large armory of saturated and mono-unsaturated 2-alkyl-4(1H)-quinolones (AQs) and AQ N-oxides (AQNOs) that serve as signaling molecules to control the production of virulence factors. In addition, AQs are involved in membrane vesicle formation and iron chelation, and they also have antibiotic properties. It has been shown that the β-ketoacyl-acyl-carrier protein synthase III (FabH)-like heterodimeric enzyme PqsBC catalyzes the last step in the biosynthesis of the most abundant AQ congener, 2-heptyl-4(1H)-quinolone (HHQ), by condensing octanoyl-coenzyme A (CoA) with 2-aminobenzoylacetate (2-ABA), but the basis for the large number of other AQs/AQNOs produced by *P. aeruginosa* is not known. Here, we demonstrate that PqsBC uses different medium-chain acyl-CoAs to produce various saturated AQs/AQNOs and that it also biosynthesizes mono-unsaturated congeners. Further, we determined the structures of PqsBC in four different crystal forms at 1.5 to 2.7 Å resolution. Together with a structure from a previous report, these data reveal that PqsBC adopts open, intermediate, and closed conformations that alter the shape of the acyl-binding cavity and explain the promiscuity of PqsBC. The different conformations also allow us to propose a model for structural transitions that accompany the catalytic cycle of PqsBC that might have broader implications for other FabH-enzymes, for which such structural transitions have been postulated but have never been observed.

Reference

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DECIPHERING THE ACTIVITIES OF NATURAL PRODUCT BIOSYNTHETIC ENZYMES: NOT ALL DOMAINS ARE WHAT THEY APPEAR TO BE

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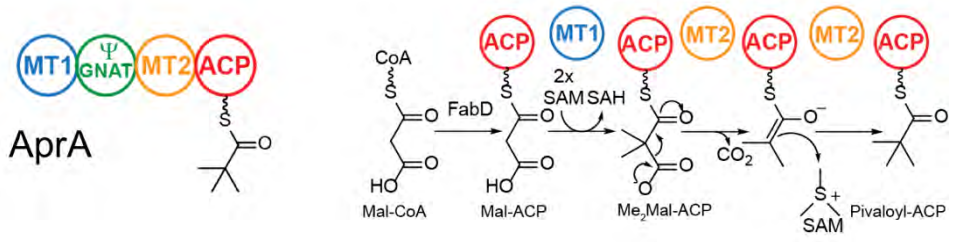
The unusual *t*-butyl group in two natural products, Sec61 inhibitor apratoxin A and PKC inhibitor bryostatin, inspired an investigation into the biosynthetic origins of the *t*-butyl group. The apratoxin A *t*-butyl group is formed as pivaloyl acyl carrier protein (ACP) by the polyketide synthase (PKS) loading module of the apratoxin A biosynthetic pathway (Figure) and by a module within BryX in the bryostatin pathway. These modules contain a GCN5-related N-acetyltransferase-like (GNAT) domain flanked by two methyltransferase domains (MT1 and MT2) of distinctly different sequence and an ACP domain. Pivaloyl-ACP synthesis is primed by the fatty acid synthase malonyl acyltransferase (FabD), which provides the initial acyl-transfer step to form malonyl-ACP[1]. The rare iron-dependent MT1 then catalyzes two methyl transfer reactions to form dimethylmalonyl-ACP[2]. MT2 is closely related to the *C*-methyltransferases of polyketide extension modules[3], but catalyzes an unusual coupled decarboxylation and methylation to form pivaloyl-ACP[1]. The inactive GNAT domain is truncated relative to other PKS GNAT domains and hence is designated as a "pseudo" GNAT (ΨGNAT). In other pathways with active GNAT domains, we determined that the primary/only activity is decarboxylation. The PKS GNATs and malonyl-CoA decarboxylase from primary metabolism have a common ancestor whereas the eukaryotic *N*-acetyltransferases are a different branch of the superfamily tree.



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Discovery and Structural Characterization of a SAM-Dependent Pericyclase

Yi Tang

UCLA

Pericyclic reactions are among the most powerful synthetic reactions for making multiple carbon carbon and carbon-heteroatom bonds in a regio- and stereoselective manner. Despite their prevalence in organic synthesis, only a handful of naturally-occurring enzymes have been characterized to catalyze pericyclic reactions and related [4+2] cycloadditions. We recently discovered a novel pericyclase from the biosynthetic pathway of leporin B from Aspergillus flavus. LepI is an S-adenosylmethionine (SAM)-dependent pericyclase that shows sequence homology to classic O-methyltransferases. Biochemical characterization showed LepI can catalyze the stereoselective dehydration to yield a reactive (E)-quinone methide that can undergo bifurcating intramolecular Diels-Alder (IMDA) and hetero-Diels-Alder (HDA) cyclizations from an ambimodal transition state, as well as a [3,3]-retro-Claisen rearrangement to recycle the IMDA product into leporin C (Ohashi et al, Nature, 2007). We solved the X-ray crystal structures of SAM-bound LepI and in complex with a substrate analog, the product leporin C, and a retro-Claisen reaction transition-state analog to understand the structural basis for the multitude of reactions. Structural and mutational analysis revealed how Nature evolves a classic methyltransferase active site into one that can serve as a dehydratase and a multifunctional pericyclase. Catalysis of both sets of reactions employs His133 and Arg295, two active site residues that are not found in canonical methyltransferases. An alternative role of SAM, which is not found to be in direct contact with the substrate, is also proposed.

Images

References

BIOSYNTHESIS OF METHANOBACTIN

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Methanobactins (Mbns) are ribosomally-produced, post-translationally modified natural products that bind copper with high affinity. Some methanotrophic bacteria secrete Mbns as a means to acquire copper for their primary metabolic enzyme, particulate methane monooxygenase (pMMO). Genome mining has led to the identification and classification of operons encoding the Mbn precursor peptide (MbnA) as well as potential biosynthesis, transport, and regulatory proteins. These operons are found in a range of bacteria, including non-methanotrophs, consistent with a broader role in and perhaps beyond copper homeostasis.[1] The known and predicted Mbn structures are diverse, but all Mbns characterized thus far bind copper with two nitrogen-containing heterocycles and two neighboring thioamide groups. Two proteins encoded by all Mbn operons, MbnB and MbnC, form a heterodimeric, iron-containing enzyme complex that installs these ligands.[2] For some Mbns, biosynthesis is completed by a transamination reaction catalyzed by the aminotransferase MbnN.[3] Other aspects of Mbn transport and biosynthesis remain unclear as does its broader role in microbial metal homeostasis.

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TARGETING A VIRAL POLYMERASE FOR ANTIVIRAL THERAPY: INSIGHT FROM STUDIES OF SINGLE MOLECULES AND SINGLE, INFECTED CELLS.

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With each viral outbreak, it becomes clearer that we have yet to master the ability to predict accurately newly emerging or reemerging viruses. One mission of our laboratory has been the creation of fundamental knowledge on the chemistry and biology of the viral RNA-dependent RNA polymerase (RdRp) to facilitate development of broad-spectrum antiviral therapeutics and RdRp-mechanism based strategies for viral attenuation and vaccine development. Our goal is to enable a rapid response to a viral outbreak independent of etiology. This lecture will describe our recent studies of antiviral ribonucleos(t)ide analogues that exploit a high-throughput, magnetic-tweezers assay for polymerase elongation and a high-throughput, microfluidics-based assay to monitor viral infection dynamics in single cells. The major conclusions of this lecture are: (1) the least-fit members of a viral population survive when challenged with a non-obligate chain terminator; (2) nucleotide-induced polymerase backtracking represents a third mechanistic class of antiviral ribonucleotide; (3) backtracking may represent an intermediate on path for RdRp-catalyzed template switching; and (4) RdRp-catalyzed recombination represents a target for development of both antiviral therapeutics and vaccine candidates.

Biosynthesis of thymidine hypermodifications

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Recently, the thymidine hypermodifications 5-(2-aminoethoxy)methyluridine (5-*Ne**O*mdU) and 5-(2-aminoethyl)uridine (5-*N*edU) were discovered in the genomic DNAs of the *Salmonella* phage ViI and the *Pseudomonas* phage M6, respectively. These complex DNA modifications protect the DNA from restriction endonucleases and likely stabilize viral DNA packed at high density in the phage capsid. We have reconstituted the complete biosynthesis of 5-*Ne**O*mdU and 5-*N*edU on double stranded DNA *in vitro* from purified enzymes and substrates. Biosynthesis of these thymidine hypermodifications requires the sequential activity of at least three enzymes on the DNA: 1) a 5-hydroxymethylpyrimidine kinase, 2) an enzyme catalyzing group transfer and predicted to have a glycosylase like helix-hairpin-helix fold, and 3) a pyridoxal phosphate-dependent decarboxylase. The synthesis of 5-*N*edU in phage M6 requires an additional isomerization step carried out by a radical S-adenosyl methionine-dependent enzyme breaking an N-C bond between the modifying group and the methyl carbon of 5-hmdU and resulting in the formation of a C-C bond. Insights into the mechanisms of each of these enzymes will be discussed.

A new direction for RNA polymerases: 3'-5' polymerase mechanism and function

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Department of Chemistry and Biochemistry and Center for RNA Biology, The Ohio State University, Columbus, OH 43210

Canonical DNA and RNA polymerases synthesize nucleic acids in the 5'-3' direction, utilizing a metal ion-dependent active site to incorporate nucleotide triphosphates into the growing polynucleotide chain. Surprisingly, a family of 3'-5' RNA polymerases has been identified that utilizes the 3'-hydroxyl of the incoming nucleotide to attack an activated 5'-phosphate, and thus elongate the 5'-ends of RNA substrates. The founding member of this enzyme family, *Saccharomyces cerevisiae* $tRNA^{His}$ guanylyltransferase (Thg1), uses this chemistry to incorporate a single essential guanosine nucleotide into $tRNA^{His}$ molecules[1]. However, the unexpected discovery that members of this enzyme family are capable of incorporating multiple Watson-Crick base paired nucleotides using the 3'-end of the substrate RNA as a template suggested that additional biological functions that take advantage of this non-canonical polymerase type activity might remain to be discovered[2]. Indeed, we have demonstrated that members of the Thg1 family known as Thg1-like proteins (TLPs) play diverse roles in RNA repair in species from bacteria to eukaryotes[3]. As the first known class of biological 3'-5' polymerases, the mechanism of these enzymes is of significant interest. Our structural and kinetic approaches have revealed surprising similarities between Thg1/TLP family 3'-5' polymerase active site and canonical 5'-3' polymerases, raising interesting questions about the evolution of nucleic acid biosynthetic machinery[4, 5]. These shared features include the use of similar two metal ion active site architecture and fidelity of base pair selection driven by kinetic preferences for correct base pairing interactions with an incoming nucleotide triphosphate. Additional mechanistic features that explain substrate selectivity of diverse family members are currently being explored. The 3'-5' polymerases also present an attractive alternative to the canonical 5'-3' polymerases that are ubiquitously utilized in biotechnology, and progress toward engineering these enzymes to perform generalized 3'-5' addition to be exploited for other applications will also be discussed.

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Uncovering Novel Inhibitor Sites and Mechanisms Takes Both Screening and Sleuthing – Tales from the Big Pharma Trenches

Alan Rendina

GlaxoSmithKline

Knowledge of enzyme mechanisms and the methods used to elucidate them have been employed in small molecule drug discovery to uncover novel inhibitor binding sites and mechanisms starting from screening approaches. During discovery campaigns, we try to rapidly identify novel mechanisms of modulation/inhibition, and these often rely on combining activity-based assays with binding assays. Providing a team with mechanistic and structural information about binding, X-ray structure or binding region, facilitates rapid rational design of inhibitors and is often the goal of our MOA studies. In this talk we will discuss 3 examples of the biochemical and biophysical studies that facilitated the progression of screening hits and increased our understanding of the structures and mechanisms of the targeted enzymes. A series of indoleamine-2,3-dioxygenase (IDO1; trp catabolism and immunosuppression) inhibitors for cancer or HIV were optimized using cell-based assays initially, and later shown to have a unique mechanism via binding to the apo (non heme) form of the enzyme. Inhibitors of the ecto-5'-nucleotidase, CD73, an enzyme that catalyzes the conversion of extracellular AMP to adenosine with important implications for adenosine-mediated immunosuppression in the tumor microenvironment, were discovered by high throughput screening and found to bind to a novel site that traps the enzyme in an inactive open conformation. High throughput screening of a GPCR protein kinase, GRK2, implicated in heart failure, in conjunction with a key binding assay led to the discovery of inhibitors that were more traditional ATP competitive hinge binders, but also a series that compete with protein substrates, a far less common mechanism.

Real-time, label-free measurement of inhibitor affinity and kinetics

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The kinetic properties of affinity complex formation are increasingly being recognized as important in many cases [1] in drug development. Real-time, label-free biophysical techniques have become the gold standard [2] in kinetic analyses because they are quantitative, apply to almost all target classes, and can measure binding/unbinding in real-time and without reporter labels. While the measurable kinetic range spans several orders, very slow and very fast kinetic interactions remain outside the kinetic detection limit. For example, transient enzyme reaction kinetics is not generally accessible by current label-free methods, and like early "hits" obtained from primary screening, form unstable affinity complexes that can only be observed as steady-state affinity isotherms in label-free biosensing. Here we show extension of the accessible kinetic range for both tight binders and transient binders. Modeling of hydrodynamic dispersion was shown to extend the kinetic range into the sub second regime and a multiphysical numerical model provided pseudo-experimental data for validation. We outline the current and future potential of these developments with reference to our work supporting pipeline projects at Genentech.

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Targeting Protein Arginine Methyltransferase 5 (PRMT5) for Cancer Therapy; Development of a SAM Competitive Inhibitor with Potent Antitumor Activity and a Decreased Liability for Developing Resistance.

Karen Maegley

Pfizer

Protein arginine methyltransferase 5 (PRMT5) acts as a splicing regulator and transcriptional coactivator via symmetric dimethylation of arginine residues within a variety of cellular proteins. Known PRMT5 substrates include Sm proteins which serve as accessory proteins of the spliceosome. Reduced methylation of the spliceosome through inhibition or loss of PRMT5 protein results in aberrant splicing and the generation of alternative transcripts for a variety of different genes including those involved in regulation of the cell cycle. As such, PRMT5 is a compelling target for the treatment of cancers which have become dependent on alternative splicing pathways. The discovery of adenosine as an efficient inhibitor of PRMT5 sparked a medicinal chemistry effort that lead to the nucleoside-like inhibitor PF-06855800. PF-06855800 is an 11 pM, S-adenosyl-L-methionine (SAM) competitive PRMT5 inhibitor with >100,000 fold selectivity over other methyltransferases. Treatment with PF-06855800 results in loss of methylation of Sm proteins and antitumor activity against both solid tumors and lymphomas. Resistant cell lines generated against PF-06855800 and the substrate competitive inhibitor EPZ015666 revealed point mutations in the PRMT5 enzyme localized to the binding site for each inhibitor. Characterization of the mutant enzymes showed a loss of inhibitor binding affinity consistent with loss of efficacy in cellular assays, suggesting that these mutations are largely responsible for the generated resistance. Only partial resistance to PF-06855800 was observed compared to EPZ015666. Crystallographic comparison of protein-inhibitor interactions between the SAM analog S-adenosylhomocysteine and PF-06855800 suggests that generation of PRMT5 mutant enzymes which block inhibitor binding, while simultaneously minimizing loss of SAM binding affinity, is unlikely, which may impede the development of resistance toward PF-06855800.

DNMT3A mutations in AML patients cause loss and gain of function and differential regulation by protein partners

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Eukaryotic DNA methylation, an integral epigenetic process, prevents genomic instability by regulating the expression of oncogenes and tumor suppressor genes. The importance of dysregulated DNA methylation in diverse blood cancers including Acute Myeloid Leukemia (AML) pathogenesis is highlighted by the strong correlation between mutations in the *de novo* DNA methyltransferase gene, DNMT3A, and adult patients with poor prognoses. We show that clinically observed DNMT3A mutations result in dramatic changes in enzyme activity, including mutations that lead to 6-fold hypermethylation and 3-fold hypomethylation of the *p15* human promoter. Our results provide insights into the clinically observed heterogeneity of *p15* methylation in AML. Cytogenetically normal AML (CN-AML) constitutes 40-50% of all AML cases, is the most epigenetically diverse AML subtype and has pronounced changes in DNA methylation in non-CpG regions. We identified a subset of mutations in DNMT3A that lead to 2-8 fold enhancements in the enzyme's ability to perform non-CpG methylation. Many of these mutations map to regions on the protein that are well known to interact with partner proteins, which themselves contribute to AML, such as Thymine DNA glycosylase (TDG). Using a functional mapping of TDG-DNMT3A interactions, we provide evidence that TDG and DNMT3L bind distinct regions of the DNMT3A surface. Furthermore, DNMT3A mutations cause significant and diverse changes in the ability of regulatory partner proteins to affect DNMT3A function. Our results present a link between DNMT3A mutations and the disruption of the epigenetic landscape in AML.

N-FORMYLATED SUGARS – ARE THEY THE KISS OF DEATH?

Hazel M. Holden and James B. Thoden

Department of Biochemistry, University of Wisconsin-Madison

Bacteria produce an astonishingly diverse array of carbohydrate-based macromolecules that serve important physiological roles. Approximately 11 years ago, my laboratory turned its research attention to those enzymes that are involved in the biosynthesis of novel sugars found attached to antibiotics, antifungals, anthelmintics, and antitumor agents. In addition to being found on natural products, however, unusual sugars have also been observed on the lipopolysaccharides or LPS of Gram-negative bacteria. There is growing evidence that the O-antigens of the LPS play important physiological roles including effective colonization of host tissues, protection from phagocytosis and serum-mediated killing, and resistance to antimicrobial peptides.

The occurrence of deoxysugars on the bacterial LPS has been known for more than 30 years. Due to the increased sensitivities of such techniques as NMR, however, it is becoming apparent that the O-antigens are far more complicated than originally thought. Recent research has demonstrated that some pathogenic Gram-negative bacteria contain quite remarkable *N*-formylated dideoxysugars. Indeed, my laboratory has shown that *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has all the necessary enzymes to produce *N*-formylated sugars. Our recent investigations on the enzymes involved in the biosynthesis of these sugars will be presented.

Discovery of CAZYmes for Cell Surface Glycan Removal Through Metagenomics: Towards Universal Blood

Stephen G. Withers[1], Peter Rahfeld[1], Drew Huff[1], Kevin Mehr[1], Zach Armstrong[1], Jacob Wardman[1], Connor Morgan-Lang[2] and Steven Hallam[2].

[1] Depts. of Chemistry and Biochemistry and Michael Smith Laboratories, Univ of B.C.

[2] Dept of Microbiology and Immunology, Univ of B.C.

Gaining access to enzymes that are able to optimally degrade glycosides of interest under specific conditions can be very challenging. Traditionally this has been achieved by screening of isolated bacterial strains for that activity, then either directly purifying or cloning and expressing that candidate….but this only works well if you have access to such libraries and ways of growing them all.

I shall describe our approach in which we screen the diverse gene repertoire present within the “silent majority” of microorganisms that have not been cultured through metagenomic approaches. Total DNA from an environment of interest is extracted, fragmented into chunks containing either ~ 1 gene (3-5 kB) or ~30-40 genes (40-50 kB) and transformed into E. coli. After picking colonies into 384 well plates we screen them for the activities of interest using custom-synthesized substrates. After hits are validated we sequence interesting fosmids and clone and express any obvious CAZymes: if not obvious we identify the responsible gene through small insert libraries. Using this approach we have identified useful glycosidases of novel specificity from a variety of environments ranging from forest soils and bioreactors through to human and beaver gut contents. In particular we have screened human gut metagenomic libraries for enzymes that can be used to remove the Gal or GalNAc residues that function as the antigenic determinants from A and B type red blood cells, thereby generating “universal” O type blood. A set of efficient enzymes of a new class has been identified and characterised and used to convert units of A blood to O. Enzymes have also been identified that remove O-glycans from glycoproteins. High-throughput screening of libraries for glycoside phosphorylases will also be discussed if time permits.

Specificity, Function and Regulation of Protein O-GlcNAc Modification

Jiaoyang Jiang

School of Pharmacy, University of Wisconsin-Madison

The *N*-acetylglucosamine (O-GlcNAc) modification is an essential glycosylation that has been identified on over 1,000 proteins. It dynamically modulates protein functions and regulates numerous biological processes in physiology and disease. O-GlcNAc modification is added by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA). Despite recent progress, challenges remain to decipher the biological roles of O-GlcNAc modification and its regulation by OGT and OGA on a broad range of substrates that lack an apparent sequence motif. In this poster, I will present our recently developed structural biology and chemical biology strategies to start revealing the specificity, function and regulation of O-GlcNAc modification.

DYNAMIC CHARACTERISTICS OF GMP REDUCTASE COMPLEXES REVEALED BY HIGH RESOLUTION ^{31}P FIELD CYCLING NMR RELAXOMETRY

Masha M. Rosenberg[‡], Tianjiong Yao[‡], Alfred G. Redfield[§], Mary F. Roberts[¶] and Lizbeth Hedstrom[‡£*]

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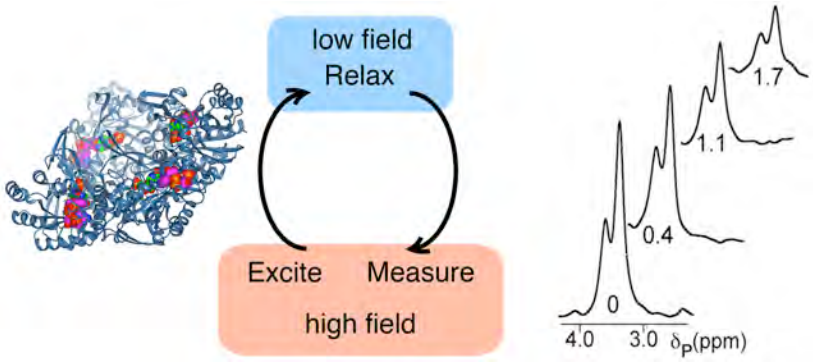
We used an underappreciated NMR technique, subtesla high resolution field cycling ^{31}P NMR relaxometry, to interrogate the dynamics of enzyme bound substrates and cofactors in guanosine-5'-monophosphate reductase (GMPR). These experiments reveal distinct binding modes and dynamic profiles associated with the ^{31}P nuclei in the Michaelis complexes for the deamination and hydride transfer steps of the catalytic cycle. Importantly, the substrate is constrained and the cofactor is more dynamic in the deamination complex E•GMP•NADP$^+$, while the substrate is more dynamic and the cofactor is constrained in the hydride transfer complex E•IMP•NADP$^+$. dIMP and dGMP are poor substrates, and the dynamics of the cofactor complexes of dGMP/dIMP are disregulated relative to GMP/IMP. The substrate 2'-OH interacts with Asp219. Counterintuitively, loss of Asp219 makes both substrates and cofactors less dynamic. These observations suggest that the interactions between the substrate 2'-OH and Asp219 coordinate the dynamic properties of the Michaelis complexes, and these dynamics are important for progression through the catalytic cycle.



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2. Rosenberg, Masha M.; Redfield, Alfred G.; Roberts, Mary F. and Hedstrom, Lizbeth. *Substrate and Cofactor Dynamics on Guanosine Monophosphate Reductase Probed by High Resolution Field Cycling ^{31}P NMR Relaxometry*. J. Biol. Chem. 291, 22988-22998 (2016).

COUPLING OF ELECTRON TRANSFER AND CHEMICAL REACTIONS TO DRIVE ORGANOMETALLIC AND RADICAL-BASED ENZYMATIC CATALYSIS BY NICKEL ENZYMES

Mehmet Can, Seth Wiley, Thanyaporn Wongnate, Anjali Patwardhan, Logan Giles, Ritimukta Sarangi, Stephen W. Ragsdale

University of Michigan and Stanford Synchrotron Radiation Lightsource

This lecture focuses on studies understanding the chemical basis for enzymatic catalysis by nickel enzymes involved in the microbial one-carbon cycle. I will describe several fundamental principles uncovered in our recent studies of methyl-SCoM reductase (MCR), CO dehydrogenase (CODH) and acetyl-CoA synthase (ACS). MCR is the key enzyme in the radical-based mechanism of methane activation and synthesis. CODH and ACS catalyze the key steps in the organometallic Wood-Ljungdahl pathway (WLP) of anaerobic CO and CO_2 fixation. In both systems, electron transfer and chemical reactions are coupled to drive the key catalytic steps.

A series of unstable low-valent intermediates react with substrates to form the organometallic bonds of the WLP, starting with Co(I)-B_{12} and proceeding through a series of Ni-carbon bonds [1] on ACS involving unstable Ni(I) and Ni(III) intermediates (Eq. 1 & 2). This effect can be understood as an EC reaction, in which the metal ion undergoes an electron transfer (E) immediately followed by a chemical reaction (C). The Ni-based low-valent and organometallic intermediates, proposed over 30 years ago, have now been trapped and characterized using photolysis, electron paramagnetic resonance, X-ray absorption spectroscopy, transient kinetics and infrared spectroscopy. Tight coupling of the redox and ligation states of metallocofactors in these EC reactions is similar in principle to proton-coupled electron transfer reactions. In methanogenesis, the low-valent Ni(I) intermediate of MCR reacts with a methyl-thiolate (methyl-SCoM) to form a methyl radical intermediate (Eq. 3).[2] The work has spurred activity among chemists who are modeling these Ni-based catalysts with metal complexes to rapidly and specifically activate CO_2 and methane.

Wood Ljungdahl Pathway:

ACS: $Ni^{2+} \rightleftharpoons Ni^{1+} \xrightarrow{CO} Ni^{1+}-CO$ (Eq. 1)

B_{12} $Co^{2+} \rightleftharpoons Co^{1+} \xrightarrow{CH_3^+} Co^{3+}-CH_3$ (Eq. 2)

Methanogenesis (MCR):

$Ni^{2+} \rightleftharpoons Ni^{1+} \xrightarrow[RSH]{CH_3SR} Ni^{2+}-SR + \{CH_3^{\bullet}\}$ (Eq. 3)

References

[1] Can, M., et al. (2017) X-ray Absorption Spectroscopy Reveals an Organometallic Ni-C Bond in the CO-Treated Form of Acetyl-CoA Synthase, Biochemistry **56**, 1248-1260.

[2] Wongnate, T., et al. (2016) The radical mechanism of biological methane synthesis by methyl-coenzyme M reductase, Science **352**, 953-958.

Mechanistic and Structural Analysis of How Nonheme-Iron Enzymes Direct Different Oxidation Reactions

Rachelle A. Copeland[1], Shengbin Zhou[1], Juan Pan[1], Noah P. Dunham[2], Ryan J. Martinie[1], Eliott S. Wenger[1], Wei-cheng Chang[1], Alexey Silakov[1], Carsten Krebs[1,2], Amie Boal[1,2], J. Martin Bollinger, Jr.[1,2]

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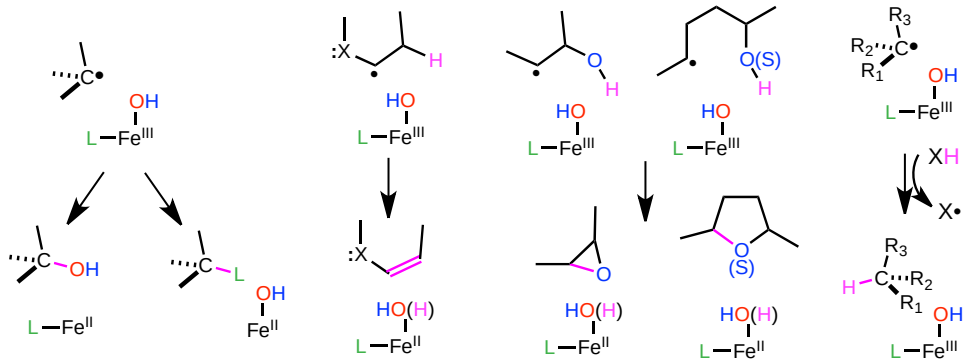
Mononuclear nonheme iron(II) enzymes abstract hydrogen (H•) from unactivated aliphatic carbon centers to initiate hydroxylation, halogenation, desaturation, cyclization, endoperoxidation, epimerization, ring-expansion, and C–C-coupling/fragmentation reactions (some examples are shown in the ***Scheme***). Available evidence suggests that a largely conserved intermediate, containing a variably coordinated Fe(III) cofactor and substrate carbon radical (C•), decays by multiple divergent pathways, involving: radical coupling of the C• with an Fe(III) ligand (OH or L) to form a new C–O/L bond; transfer of a second H• from an adjacent carbon to form an olefin; anchimerically assisted electron transfer to produce a formal carbocation that reacts as an acid or electrophile, leading to desaturation or heterocyclization; transfer of H• to the C•, but on the opposite face of the original C–H bond; addition of O_2 to the radical to form a reactive peroxyl radical; or addition of the C• to an olefin to produce a new C–C bond and migrate the radical (which then reacts via one of the other possible steps). By (i) mapping these reaction pathways, (ii) solving and comparing structures of "resting" states, stable mimics of intermediate states, and the reactive intermediates themselves, and (iii) altering sequences/structures (both rationally and randomly) to alter outcomes, we aim to define the structural and dynamical bases for selection of a particular pathway by individual members of this large enzyme family. I will present our most recent analysis of one or more of the less well-studied reactions catalyzed by these versatile enzymes.

Scheme



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RADICAL SAM ENZYMES, VIEWED THROUGH AN ELECTROCHEMICAL LENS

Lindsey M. Walker,[1] Sheila Bonitatibus,[1] Stephanie J. Maiocco,[1] Squire J. Booker,[2,3,4] Vahe Bandarian,[5] and Sean J. Elliott[1]

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The Radical S-Adenosylmethionine (RS) enzyme superfamily makes use of $[Fe_4S_4]$ clusters to engage in spectacular transformations that span a wide-range of chemical space, and many members of the superfamily have one or more additional redox active cofactors, including other FeS clusters of variable compositions (e.g., $[Fe_4S_4]$ and $[Fe_2S_2]$). However, the electrochemical properties of the RS superfamily have been poorly described, historically. And, as all canonical family members require reduction to the $[Fe_4S_4]^{1+}$ redox state in order to function, understanding the redox potentials of the diverse family members has long proven a tantalizing challenge. Here, efforts using protein film electrochemistry to reveal the redox chemistry of RS metallocofactors will be described. Studies of several SPASM/Twitch domain proteins that span different kinds of catalytic chemistry will be examined in a comparative fashion, demonstrating the unique insights that can come from direct electrochemistry: resolution of individual $[Fe_4S_4]$ cluster redox potentials;[1,2] transformations of the clusters themselves;[3] and questions of proton-coupled electron transfer,[1] will all be addressed, particularly with emphasis upon the possibility of closely spaced redox couples[4]. Collectively, these data allow us to view the bioinformatic diversity of the ARE superfamily in new light, depicting how sequence-based families correlate to function and to the redox chemistry of the metallocofactors.

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Characterization of a heme-dependent N-N bond forming enzyme

Melanie A Higgins[1], Yi-Ling Du[2], Katherine Ryan[1]

[1]*Department of Chemistry, The University of British Columbia, Vancouver Canada.*
[2]*Institute of Pharmaceutical Biotechnology, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, China*

There is a long-standing mystery of how nitrogen-nitrogen (N-N) bonds are formed in natural products. Piperazic acid is one of the most common N-N bond containing non-proteinogenic amino acids, found in a variety of natural product peptides, such as kutznerides and polyoxypeptins. Our group recently discovered an enzyme that cyclizes N-hydroxylated L-ornithine, forming an N-N bond, to produce L-piperazic acid using a previously undescribed heme-dependent mechanism. Here I will present structural and biochemical analysis on this enzyme to gain further insight into this novel mechanism of enzymatic N-N bond formation.

Posttranslational Modifications during Cyclic Peptide Biosynthesis

Wilfred van der Donk

Department of Chemistry and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign

The genome sequencing efforts of the first decade of the 21st century have revealed that ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a very large class of peptide natural products. These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast.[1] The defining post-translational modification of most RiPPs is a macrocyclization step, but additional tailoring reactions also take place such as oxidation, halogenation, and reduction.
This presentation will discuss investigations of the mechanisms of these remarkable catalysts as well as discovery of unanticipated new biosynthetic reactions.

References

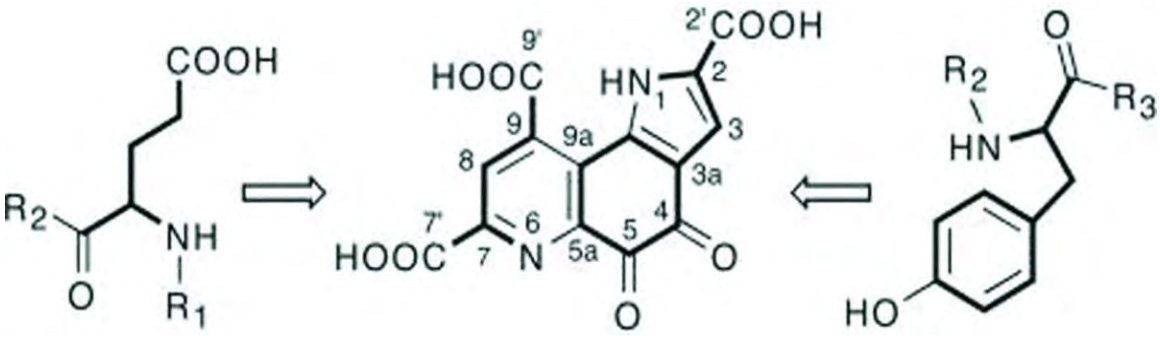
(1) Arnison, P. G., *et al.* (2013) Ribosomally synthesized and post-translationally modified peptide natural products: overview and recommendations for a universal nomenclature, *Nat. Prod. Rep. 30*, 108-160.

PQQ Biosynthesis: Getting Close to the Finish Line

Judith P. Klinman

Departments of Chemistry and of Molecular and Cell Biology, California Institute for Quantitative Biology, University of California, Berkeley, CA 94720

Pyrroloquinoline quinone (PQQ) is a bacterial redox cofactor that is critical for C1 metabolism in many microorganisms, a disproportionate number of which are opportunistic human pathogens[1]. Bioinformatic analyses of sequenced prokaryotic genomes indicate a conserved operon that is comprised, at a minimum, of 5 open reading frames encoding PqqA, B, C, D and E. To date, the following identifications have been made: PqqA is the peptide substrate, PqqD is a peptide chaperone required for the action of the Radical Sam enzyme PqqE on PqqA, and PqqC is an oxidase. This presentation will focus on evidence that the remaining essential biosynthetic enzyme PqqB is an iron-dependent hydroxylase, catalyzing oxygen insertion reactions that are the source of the quinone moiety of the mature PQQ cofactor. The demonstrated reactions of PqqB are unprecedented within the metallo β-lactamase protein family and expand the catalytic repertoire of non-heme iron hydroxylases. This resolution of PqqB activity allows a nearly complete description of PQQ biosynthesis and the essential open reading frames encoding its production.



Scheme Legend: The structure of PQQ (center) contains the carbon skeletons of two amino acids, Glu and Tyr derived from the C-terminus of PqqA.

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Isopentenyl Diphosphate Isomerase. Breaking Up is Hard to Do

C. Dale Poulter

Department of Chemistry, University of Utah

Type 1 and type 2 isopentenyl diphosphate:dimethylallyl diphosphate isomerase (IDI-1 and IDI-2) catalyze the interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), the two fundamental building blocks for biosynthesis of isoprenoid compounds. Several studies indicate that the mechanism for the isomerization by the two independently evolved isoforms of IDI involves a protonation-deprotonation sequence. These include linear free energy correlations, observation of tight binding behavior of transition state/reactive intermediate analogues, and alkylation of active site residues by proton-activated substrate analogues. Protonation-deprotonation by IDI-1 is catalyzed by active-site glutamate and cysteine residues and by fully reduced FMN by IDI-2. Patterns of deuterium incorporation into IPP and DMAPP during isomerizations catalyzed by IDI-1 and IDI-2 were determined by NMR spectroscopy and, in the case of IDI-1 indicate that at least two rounds of isomerization occur within a single binding event. We used mass spectrometry to further investigate this phenomenon for both isoforms of IDI and discovered that a single binding event produces d_1-d_3 isotopologues of both IPP and DMAPP as the result of up to five rounds of isomerization before dissociation. Analysis of the distributions of IPP and DMAPP isotopologues during the time course of the reactions shows that isomerization proceeds by a concerted reaction or through a very short-lived carbocationic intermediate.

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P1

Specificity, Function and Regulation of Protein O-GlcNAc Modification

Jiaoyang Jiang

School of Pharmacy, University of Wisconsin-Madison

The *N*-acetylglucosamine (O-GlcNAc) modification is an essential glycosylation that has been identified on over 1,000 proteins. It dynamically modulates protein functions and regulates numerous biological processes in physiology and disease. O-GlcNAc modification is added by O-GlcNAc transferase (OGT) and removed by O-GlcNAcase (OGA). Despite recent progress, challenges remain to decipher the biological roles of O-GlcNAc modification and its regulation by OGT and OGA on a broad range of substrates that lack an apparent sequence motif. In this poster, I will present our recently developed structural biology and chemical biology strategies to start revealing the specificity, function and regulation of O-GlcNAc modification.

P2

Intradiol ring-cleavage dioxygenase from the polyphagous spider mite herbivore *Tetranychus urticae Koch*

Leily Daneshian, Caleb R. Schlachter, Jose Amaya, Vincent Klapper, Nicky Wybouw, Tomasz Borowski, Thomas Van Leeuwen, Vojislava Grbic, Miodrag Grbic, Thomas M. Makris, Maksymilian Chruszcz
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Aromatic compounds exist in the environment as either man-made, or natural products. Oftentimes, they pose as environmental contaminants [1, 2]. Due to their propensity of having high resonance energy and sometimes electron withdrawing groups, this renders them very stable and resistant to biodegradation [1, 2]. Their resistance to degradation is problematic as they can accumulate in animal and plant tissues to toxic levels [1]. To combat these aromatic environmental contaminants, there are metalloproteins such as intradiol ring cleavage dioxygenases (ID-RCDs) that catabolize these compounds into non-toxic metabolites by utilizing a non-heme iron in the active site [3, 4]. An aromatic contaminant, catechol and catechol derivatives (i.e. substituted with halides, or other functional groups) are common substrates for ID-RCDs. These dioxygenases detoxify catechol and its derivatives by cleaving the aromatic ring between C1 and C2, resulting in the formation of cis, cis-muconic acid that can be further metabolized into succinate and acetyl-CoA [2, 3].17 genes that code for "intradiol dioxygenase-like" subgroup are present in *Tetranychus urticae* (also known as two-spotted spider mite), which is an important agricultural pests [4]. As a defense mechanism to pests, plants produce aromatic compounds [5] and we hypothesize that some of these compounds are detoxified by enzymes form the "intradiol dioxygenase-like" subgroup. To understand the function of these proteins, we have structurally and functionally characterized one of them (*tetur07g02040*). The active site of the *T. urticae* enzyme contains an Fe^{3+} cofactor that is coordinated by two histidine and two tyrosine residues. We demonstrated that this protein is indeed an intradiol ring-cleavage dioxygenase, as the enzyme is able to cleave catechol and 4-methylcatechol. *Tetur07g02040* is not only the first spider mite dioxygenase that has been characterized at the molecular level but is also the first structurally characterized intradiol ring-cleavage dioxygenase originating from a eukaryote [6].

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P3

From Kinetic Studies of Enzyme Catalysis and Inhibition to Discovery of Novel "Mechanism-Based" Inhibitors of *Mycobacterium tuberculosis* Isocitrate Lyase

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Isocitrate lyase (ICL) represents a potential drug target for tuberculosis treatment for its essentiality in the persistent stage of *Mycobacterium tuberculosis* (*Mtb*) infection. Since high-throughput screening campaigns were unsuccessful in identifying suitable inhibitors, these enzymes present a promising target for a rational approach. Understanding the catalytic mechanism would assist in new strategies for inhibiting the isocitrate lyases (ICLs). The retro-aldol catalytic cleavage and the nucleophilic active-site cysteine inspired an adaptation of isocitrate analogs as ICL mechanism-based inactivators. Finally, a mechanistic comprehension of ICL inhibition by known inactivators and substrate analogs imparts essential structural-activity features of potential inhibitors. Recently we reported 2-VIC as a mechanism-based inactivator of both ICL1 and ICL2 whose action relies on the retro-aldol cleavage of the enzyme. However, the inactivation by 2-VIC is rather slow, and impeded by DTT. Therefore improvements are needed for more effective inactivation of ICL. A survey of different analog substrates emphasized the significance of *cis* conformation of succinate fragment in ICL1 active-site. Additionally, the active-site loop movement during catalysis can be advantageous to design inhibitors that only bind to E-glyoxylate complex, providing more selectivity against other acting on the same metabolic pathway (isocitrate dehydrogenase and aconitase). Here we discovered that *cis*-2,3-epoxysuccinate acts as a mechanism-based inactivator of *Mtb* ICL1 which is 750-fold more potent than 2-VIC and evades thiol protection. Co-crystallization of ICL1 and *cis*-epoxysuccinate showed that ICL1 inactivation by this compound occurs via a covalent linkage with active-site cysteine 191. Differential gel electrophoresis of *E.coli* lysate, pre-treated with and without *cis*-epoxy-succinate, asserted that *cis*-epoxy-succinate has a notable selectivity for ICL. We are working toward *cis*-2,3-epoxysuccinate analog pro-drugs that have better cellular permeability and cell growth inhibition effects. On the other hand, we assembled kinetic data and mutagenesis studies to explain the catalytic mechanism of ICL in depth and Lys189 was identified as a potential catalytic base involving in the first deprotonation step during isocitrate cleavage. Hence, Lys189, along with Cys191, comprises another susceptible nucleophilic active-site residue for development of novel inactivators.

P4

Role of the KDO Glycosyltransferase KpsS in the Biosynthesis of the Polysialyltransferase Acceptor for *Escherichia coli* K1

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Many pathogenic bacteria produce polysaccharide capsules to evade immune recognition and prevent lysis by the host immune response. *Neisseria meningitidis* groups B and C as well as *Escherichia coli* serogroups K1 and K92 are coated with a polysialic acid (PSA) capsule. The PSA chain is synthesized by polysialyltransferases that makes an α-2,8 linkage for *E. coli* K1 and *N. meningitidis* serotype B, an α-2,9 linkage for *N. meningitidis* serotype C, or an alternating α-2,8, α-2,9 linkage for *E. coli* K92. These transferases cannot initiate synthesis of PSA *de novo*, but rather, a more complex set of machinery is required to generate an appropriate acceptor. This acceptor has been proposed to be a phosphatidylglycerol lipid anchor with a short keto-deoxyoctulosonate (KDO) linker to one or more sialic acid residues. Synthesis of this acceptor requires at least three enzymes in *E. coli* K1: KpsS, KpsC, and NeuE. In this report, we have characterized KpsS, the first enzyme in the pathway for acceptor synthesis and a membrane associated beta KDO glycosyltransferase. Much of the machinery required to produced bacterial capsules consists of transmembrane or membrane-associated enzymes. Isolation and characterization of these enzymes in an active state has proven challenging. Here, we have purified KpsS in a soluble and active form and have begun to investigate its function. We show that KpsS can transfer a KDO residue to a fluorescent labeled phosphatidylglycerol lipid substrate. The enzyme tolerates various lengths of lipid tails on the phosphatidylglycerol substrate, including fluorescent tags that facilitate the characterization of the substrate and product; however, the length of the tails significantly impacts the rate of the reaction. The length of the tail also impacts the binding affinity of the substrate for the enzyme. We have also shown that the enzyme can transfer modified KDO residues. Furthermore, we have isolated the product of the KpsS reaction and analyzed its structure by NMR. That KpsS is the first KDO transferase in the pathway is confirmed by the further modification of this purified product by KpsC with additional KDO units.

P5

Novel Radical SAM Enzymology in Futalosine-Dependent Menaquinone Biosynthesis

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Menaquinone (Vitamin K) is a membrane-bound electron transfer cofactor that is an essential component for all the domains of life. In humans, it plays a critical role in the blood clotting cascade and bone morphogenesis. Recently, a new menaquinone biosynthetic pathway was discovered in *S. coelicolor* that uses radical chemistry to assemble the aromatic core of menaquinone. Aminofutalosine synthase (MqnE) and dehypoxanthine futalosine cyclase (MqnC), radical SAM enzymes, catalyze the key C-C bond formation reactions in this pathway. MqnE has a unique ability to add the 5'-deoxyadenosyl radical to the substrate - rather than abstract a hydrogen atom, which constitutes a new catalytic motif in radical SAM enzymology. Both MqnE and MqnC reaction sequences involve radical additions to a benzene ring followed by formation of an aryl radical anion intermediate. These type of arene addition reactions even though widely used in natural product biosynthesis, are poorly understood. We have developed new radical trapping strategies based on fast, radical triggered, carbon-halogen bond fragmentation reactions leading to successful trapping of captodative and aryl radical intermediates in the MqnE mechanism. Lower catalytic efficiency of MqnC and challenges in substrate analog synthesis had precluded similar studies on MqnC catalyzed reaction. Here we report an improved *in vitro* reconstitution of MqnC reaction and the development of a robust chemo-enzymatic route for the synthesis of halogenated substrate analogs of MqnC. In summary, our biochemical studies on MqnE have elucidated the radical rearrangement catalyzed by MqnE and have set the stage for the detailed mechanistic studies on MqnC catalyzed reaction.

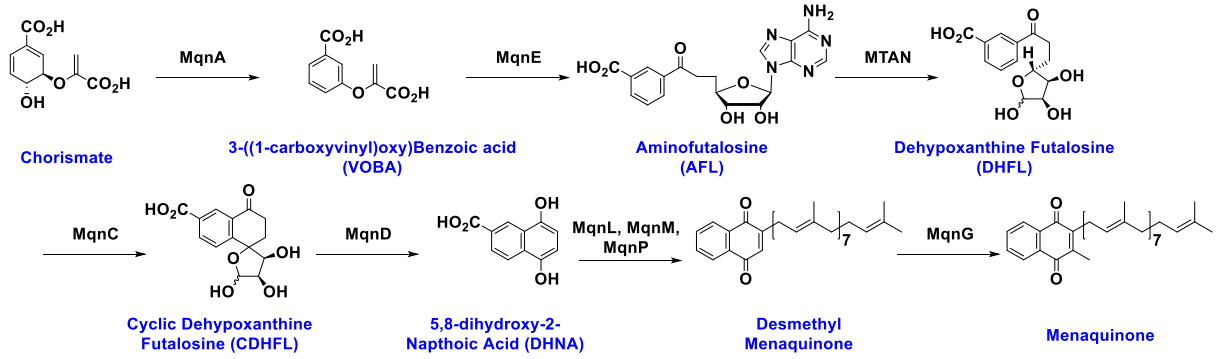

Figure 1: Futalosine dependent menaquinone biosynthesis pathway

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P6

An Alternative Catabolic Pathway for Ascorbate Includes a Benzilic Acid Rearrangement and a Unique NAD^+-dependent Decarboxylase

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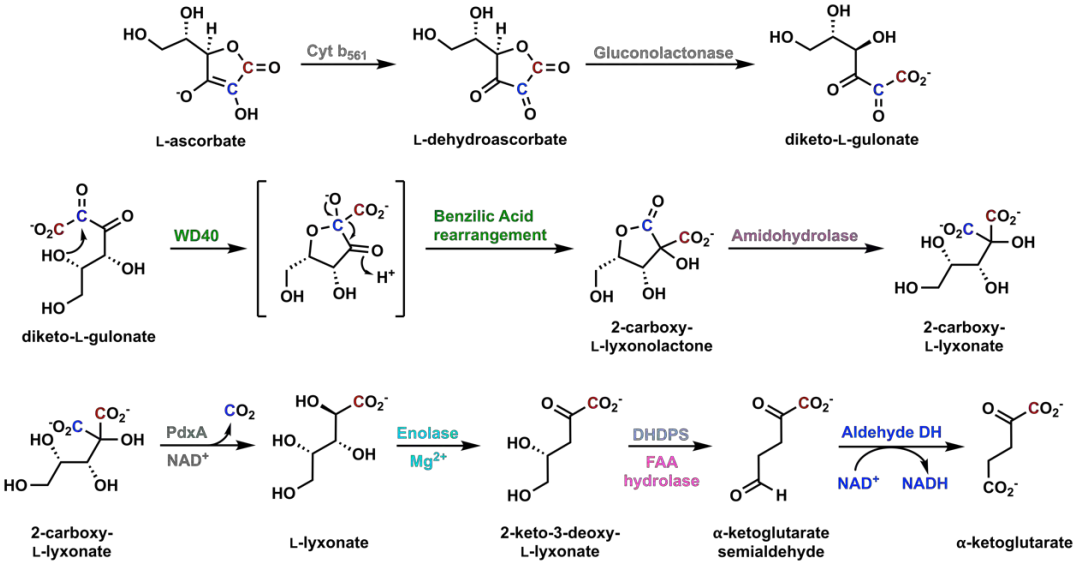
As part of our efforts to annotate enzymes of unknown function and characterize bacterial metabolic pathways, we discovered that *Cupriavidus necator* ATCC 17699 (formerly *Ralstonia eutropha* H16) can catabolize L-ascorbate. pProkaryotes are able to catabolize L-ascorbate, requiring an operon which encodes the utilization of L-ascorbate (*ula*).[1] As the *C. necator* genome does not encode the *ula* operon, the pathway responsible for this growth was unclear. RNA-Seq transcriptomic experiments identified the genes that are upregulated when *C. necator* is cultured with L-ascorbate as the sole carbon source, and a combination of sequence similarity networks and genome neighborhood networks guided the selection of target proteins. We found eight enzymes with highly upregulated transcripts and co-occurrent genes which informed the construction of an alternative catabolic pathway for ascorbate. In this pathway, L-ascorbate is first oxidized by a cytochrome b_{561} and then ring opened by a gluconolactonase to form L-2,3-diketogulonate. A protein predicted to have a WD40-like fold then catalyzes an unprecedented benzilic acid rearrangement where the C5 alcohol attacks the C2 ketone, followed by a migration of the C1 carboxylate and simultaneous lactone formation, yielding 2-carboxy-L-lyxonolactone. This lactone is hydrolyzed by an amidohydrolase to yield 2-carboxy-L-lyxonate. An enzyme from the PdxA family of alcohol dehydrogenases utilizes a prosthetic NAD^+ to catalyze a stereospecific decarboxylation and form the five-carbon acid sugar L-lyxonate. The pathway then follows a previously reported pathway for the degradation of L-lyxonate.[2] Bioinformatic analysis shows this alternative catabolic pathway can be found in at least 300 species of Actinobacteria and Proteobacteria, including the gut-dwelling *Enterobacter cloacae*, and the pathogens *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. These bioinformatic analyses also suggest there are deviations to this reported pathway, and these are currently being investigated.



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P7

Investigation of the Reaction Mechanisms of the Fe(II)- and 2-Oxoglutarate-Dependent Ethylene-Forming Enzyme, EFE

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The ethylene-forming enzyme (EFE) from *Pseudomonas syringae* is a bifunctional iron(II)- and 2-oxoglutarate-dependent (Fe/2OG) oxygenase. In the primary reaction, L-arginine activates EFE for catalysis of 2-oxoglutarate fragmentation to ethylene and three equivalents of CO_2. In the secondary reaction, EFE instead hydroxylates C5 of L-arginine, coupling this process to the oxidative decarboxylation of 2OG to succinate. While the secondary reaction conforms to the usual *modus operandi* of Fe/2OG oxygenases, the primary, ethylene-producing reaction deviates significantly. To gain insight into the mechanisms of these fundamentally distinct reactions, we conducted transient-state spectroscopic experiments using C5-deuterated L-arginine as substrate. Our results demonstrate that, consistent with precedent, L-arginine hydroxylation is initiated by an iron(IV)-oxo (ferryl) intermediate. The decay of this intermediate is slowed when C5 of L-arginine is deuterated, owing to a large kinetic isotope effect (^{2}H-KIE) on hydrogen atom transfer. The product partition ratio is unperturbed by C5 deuteration, suggesting that the reaction pathway bifurcates – and the reaction outcome is determined – upstream of the observed ferryl complex. Substitution of the iron ligand Asp191 by Glu produces modest changes in the X-ray crystal structure of the reactant complex yet alters the reaction outcome markedly: ethylene production is abolished, the partition ratio shifts in favor of L-arginine hydroxylation, and the ferryl complex accumulates almost four-fold higher than in the wild-type reaction. The results imply that the unusual ethylene-forming reaction has more rigid stereoelectronic requirements than the canonical oxidative decarboxylation leading to ferryl formation and two-electron oxidation of the prime substrate, L-arginine.

P8

Development of enzyme-coupled reactions for monitoring the ATP-ase activity of Hsp-70/Hsp-40 chaperone system

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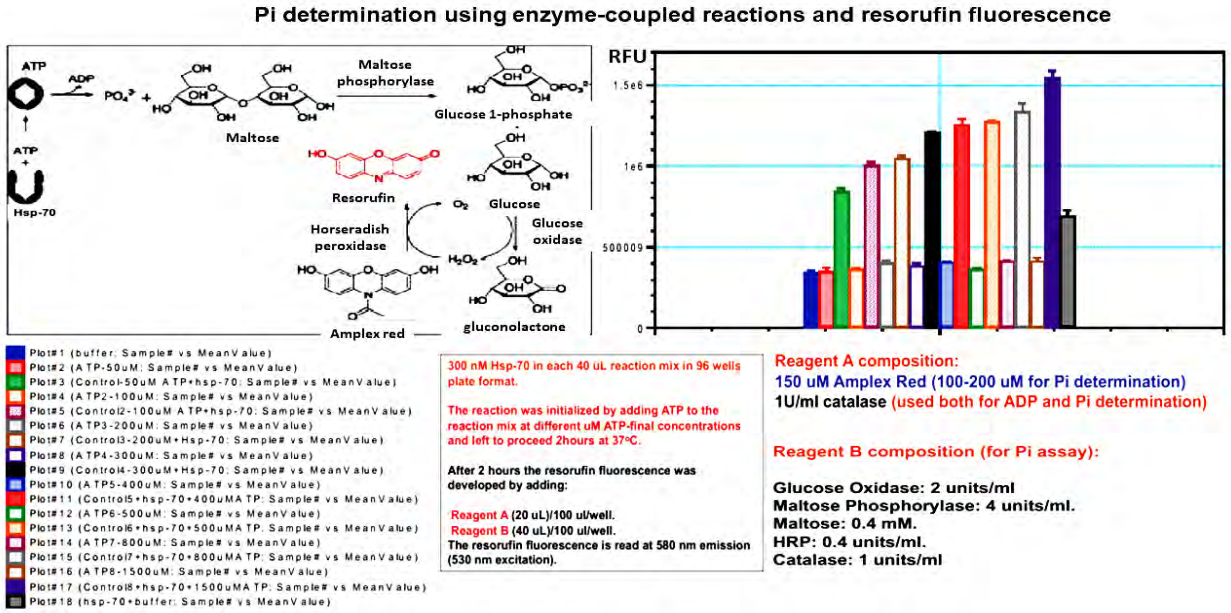
In response to environmental stress, there is an increase in the expression of heat shock proteins. Heat shock proteins are molecular chaperones that maintain cell homeostasis through various cellular mechanisms. Heat shock protein 70 is a specific family of heat shock proteins that uses an ATP-driven mechanism to bind its client proteins. The overexpression of Hsp70 has been shown to prevent apoptosis and lead to tumorigenesis, but the inhibition of Hsp70 has been linked to an increase in apoptosis and tumor cell death. These anti-apoptotic functions of Hsp-70 prompted many groups of researchers toward development of sensitive assays for monitoring the ATP-ase activity of this chaperone which can further be used for discovery of new potential competitive inhibitors of its ATP-ase activity. This research presents the development of resorufin fluorescence-based assays for monitoring the ATP-ase activity of Hsp-70 by measuring the inorganic phosphate (Pi) or the adenosine diphosphate (ADP) production during the multi turn-over enzymatic reactions. Enzyme-coupled reactions were developed to convert the Pi or ADP in hydrogen peroxide which in turn was used to oxidize the reagent amplex red into resorufin which emits fluorescence at 590 nm upon excitation at 530 nm. The non-hydrolysable ATP analog AMP-PNP showed inhibition of human Hsp-70 ATP-ase activity in the low mM range.



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P9

Prediction of Golden Gate Assembly Using a Comprehensive Analysis of T4 DNA Ligase End-Joining Fidelity and Bias

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1

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T4 DNA ligase is a key enzyme in molecular and synthetic biology that catalyzes the efficient end-joining of DNA fragments. Despite this widespread use, no comprehensive studies of ligation fidelity (discrimination against the ligation of substrates containing mismatched base pairs) and bias (preferential ligation of particular sequences over others) have been carried out in end-joining ligation contexts. Ligase promiscuity and bias can be problematic for methodologies that rely on high fidelity and high efficiency ligation. For example, Golden Gate assembly enables the simultaneous joining of multiple DNA fragments in one step, but ligation of mismatched overhangs can result in large insertions or deletions and low efficiency joining can lead to truncated constructs. In this study, we applied Pacific Biosciences Single-Molecule Real-Time sequencing technology to directly sequence the products of a highly multiplexed ligation reaction. This method has been used to profile the ligation of all three-and four-base 5′-overhangs by T4 DNA ligase under typical ligation conditions. We report the relative frequency of all ligation products with or without mismatches, and show that these data accurately predict the frequency of ligation errors in practical DNA assembly applications. Use of the comprehensive fidelity and bias data has enabled the design of highly complex one-pot DNA assemblies of up to 25 fragments with high efficiency and fidelity.

P10

Identifying the Activities of Hypothetical Proteins in a Putative Biosynthetic Cluster from *Streptomyces griseofuscus*

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The enzyme MppP from *Streptomyces wadayamensis* is the prototype of a family of pyridoxal 5'-phosphate (PLP)-dependent enzymes that use dioxygen to perform a 4-electron oxidation of L-Arg to 4-hydroxy-2-ketoarginine in the biosynthesis of L-enduracididine (L-End). L-End is a non-proteinogenic amino acid found in a number of nonribosomal peptide antibiotics. Sequence analysis of MppP homologs suggests that MppP-like enzymes—and the PLP-dependent L-Arg oxidase activity—are present in a larger variety of genomic contexts than was initially thought. Herein we describe structural and biochemical studies of enzymes from an MppP-containing gene cluster from *Streptomyces griseofuscus*. This putative biosynthetic cluster contains open reading frames predicted to code for a protein with no homology to any characterized protein (UnkA), one with weak sequence identity to an L-amino acid ligase (SgrAAL), a flavin-dependent oxygenase (SgrFDO), a ketoarginine-3-methyltransferase (SgrMT), a proclavaminate amidinohydrolase (SgrAH), an MppP homolog (SgrMppP), and a GNAT family N-acetyltransferase (SgrNAT). Thus far, we have expressed and purified three of these proteins: SgrMT, SgrAH, and SgrMppP. The crystal structure of SgrMppP shows that the *S. griseofuscus* homolog is nearly identical in structure to the prototypical *S. wadayamensis* MppP. As might be expected from the high degree of structural similarity, SgrMppP possesses the same catalytic activity as *S. wadayamensis* MppP. It produces the same mixture of 4-hydroxy-2-ketoarginine, the fully oxidized product, and 2-ketoarginine, an abortive, partially oxidized product. The steady-state kinetic parameters were measured using an oxygen electrode, and closely match those of *S. wadayamensis* MppP. Although SgrMppP catalyzes exactly the same reaction as *S. wadayamensis* MppP with very similar kinetics, it has a distinct biochemical role, since there are no homologs of the other L-End-producing enzymes (*e.g.* MppQ and MppR) in *S. griseofuscus*. We were interested to find out in what other biosynthetic contexts the L-Arg oxidase activity of MppP is used. To this end, we set about determining the structures and identifying the catalytic activities of the other proteins in this putative biosynthetic cluster. To date, we have determined the structure of SgrAH and found that the active site of this protein is very similar to the proclavaminate amidinohydrolase (PAH) from *Streptomyces clavuligerus*. Preliminary experiments indicate that L-Arg, 2-ketoarginine, and 4-hydroxy-2-ketoarginine are *not* substrates for SgrAH. Preliminary experiments with SgrMT suggest that it is an Fe(II)-dependent enzyme like the Fe(II) and SAM-dependent methytransferase MppJ from *Streptomyces hygroscopicus*. These preliminary experiments have allowed us to formulate a hypothetical biosynthetic scheme that we are beginning to test experimentally.

P11

Detection of Dynamic Protein Networks for Ligand-Induced Allostery and Thermal Activation/Adaptation using Multi-Temperature Hydrogen Deuterium Exchange

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Hydrogen deuterium exchange (HDX) has emerged as a powerful tool to assess how a protein's conformation changes upon ligand, inhibitor, and/or allosteric effector binding as well as from protein-protein or protein-membrane interactions. Nearly all reports are centered on analysis from a single temperature, though it is clear from Nature that proteins, and especially enzymes, require thermal activation for function. The large size of enzymes, together with the frequent sequestration of their active sites from bulk solvent, raises the important question of how thermal activation is transmitted from solvent to the reaction center. Assignments of specific pathways for energy transmission and even dynamic allosteric regulation within (or throughout) a protein remains a daunting challenge. The recent applications of multi-temperature HDX have begun to demonstrate its unique capabilities to structurally resolve such dynamic networks relevant to both thermal activation/adaptation and dynamic allostery. Because of the sensitivity of HDX to detect to dynamic changes in the protein matrix from point mutations, this approach also has the potential to resolve alterations to the dynamic framework of a protein arising from molecular diseases. Applications to various systems will be presented.

P12

Template-assisted synthesis of adenine-mutagenized cDNA by a diversity-generating retroelement protein complex

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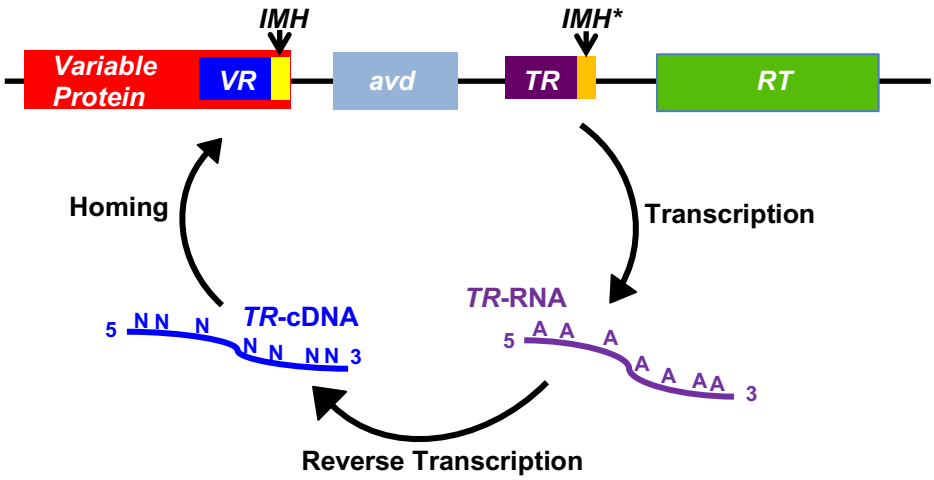
Diversity-generating retroelements (DGRs) create unmatched levels of protein sequence variation through mutagenic retrohoming. Transfer of information is carried out from an invariant template region (TR), through an RNA intermediate, to a variable region (VR) of the protein. During this transfer adenine specific mutagenesis is introduced by the DGR reverse transciptase (RT) and pentameric accessory variability determinant (Avd) protein complex. This selective infidelity at adenines during transfer is a hallmark of DGRs from disparate bacteria, archaea, and microbial viruses. We recapitulated selective infidelity *in-vitro* for the prototypical Bordetella bacteriophage DGR. A complex of the DGR RT and Avd protein along with DGR RNA were sufficient for synthesis of template-primed covalently linked RNA–cDNA molecules. The RNA–cDNA molecules appear to be branched and most plausibly linked through 2′-5′ phosphodiester bonds. This bRT-Avd complex displayed unprecedented promiscuity while reverse transcribing adenines of either DGR or non-DGR RNA templates. The bRT-Avd processivity was strictly dependent on the template, and this restriction was due to a noncoding segment downstream of TR referred as spacer, which specifically bound Avd and created a privileged site for processive polymerization. These results have defined the early steps in a novel pathway for massive sequence diversification.



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P13

Regulation of PTEN by C-terminal Phosphorylation

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PTEN is a tumor suppressor that is linked to different cancers, Cowden syndrome, and autism spectrum disorders. As the major negative regulator of the PI3K/AKT pathway, it catalyzes the hydrolysis of the 3' phosphate of phosphatidylinositol 3,4,5-trisphosphate (PIP3), a lipid second messenger, to PIP2 which reduces cell growth and proliferation by limiting AKT's activation. PTEN's cellular function is regulated by phosphorylation of its C-terminal tail at positions Ser380, Thr382, Thr383, and Ser385, which drives a conformational change from an open to a closed state resulting in inhibited enzymatic activity, reduced plasma membrane binding, and paradoxically, increased cellular stability. Herein, we present our recent findings that provide greater insight into the structural and mechanistic basis for the regulatory role of phosphorylation of PTEN's C-terminal tail. We also present a simple method that harnesses the vast availability of commerical N-hydroxysuccinimide esters for site-specific labeling of protein N-termini. This new "one pot" labeling strategy was instrumental in our efforts to gain a more detailed understanding of the mechaistic basis for how C-tail phosphorylation enhances PTEN's cellular stability.

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P14a

DNMT3A mutations in AML patients cause loss and gain of function and differential regulation by protein partners

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Eukaryotic DNA methylation, an integral epigenetic process, prevents genomic instability by regulating the expression of oncogenes and tumor suppressor genes. The importance of dysregulated DNA methylation in diverse blood cancers including Acute Myeloid Leukemia (AML) pathogenesis is highlighted by the strong correlation between mutations in the *de novo* DNA methyltransferase gene, DNMT3A, and adult patients with poor prognoses. We show that clinically observed DNMT3A mutations result in dramatic changes in enzyme activity, including mutations that lead to 6-fold hypermethylation and 3-fold hypomethylation of the *p15* human promoter. Our results provide insights into the clinically observed heterogeneity of *p15* methylation in AML. Cytogenetically normal AML (CN-AML) constitutes 40-50% of all AML cases, is the most epigenetically diverse AML subtype and has pronounced changes in DNA methylation in non-CpG regions. We identified a subset of mutations in DNMT3A that lead to 2-8 fold enhancements in the enzyme's ability to perform non-CpG methylation. Many of these mutations map to regions on the protein that are well known to interact with partner proteins, which themselves contribute to AML, such as Thymine DNA glycosylase (TDG). Using a functional mapping of TDG-DNMT3A interactions, we provide evidence that TDG and DNMT3L bind distinct regions of the DNMT3A surface. Furthermore, DNMT3A mutations cause significant and diverse changes in the ability of regulatory partner proteins to affect DNMT3A function. Our results present a link between DNMT3A mutations and the disruption of the epigenetic landscape in AML.

P14b

The highly specific, cell cycle–regulated methyltransferase from *Caulobacter crescentus* relies on a novel DNA recognition mechanism

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Two DNA methyltransferases, Dam and β-class cell cycle–regulated DNA methyltransferase (CcrM), are key mediators of bacterial epigenetics. CcrM from the bacterium *Caulobacter crescentus* (CcrM *C. crescentus*, methylates adenine at 5'GANTC3') displays 10^5–10^7 fold sequence discrimination against non-cognate sequences. However, the underlying recognition mechanism is unclear. Here, CcrM *C. crescentus* activity was either improved or mildly attenuated with substrates having one to three mismatched base pairs within or adjacent to the recognition site, but only if the strand undergoing methylation is left unchanged. By comparison, single-mismatched substrates resulted in up to 10^6-fold losses of activity with α- (Dam) and ɣ-class (M.HhaI) DNA methyltransferases. We found that CcrM *C. crescentus* has a greatly expanded DNA-interaction surface, covering six nucleotides on the 5' side and eight nucleotides on the 3' side of its recognition site. Such a large interface may contribute to the enzyme's high sequence fidelity. CcrM *C. crescentus* displayed the same sequence discrimination with single-stranded substrates, and a surprisingly large ($> 10^7$ fold) discrimination against ssRNA was largely due to the presence of two or more riboses within the cognate (DNA) site, but not outside the site. Results from C-terminal truncations and point mutants supported our hypothesis that the recently identified C-terminal, 80-residue segment is essential for dsDNA recognition, but is not required for single-stranded substrates. CcrM orthologs from *Agrobacterium tumefaciens and Brucella abortus* share some of these newly discovered features of the *C. crescentus* enzyme, suggesting that the recognition mechanism is conserved. In summary, CcrM *C. crescentus* uses a previously unknown DNA recognition mechanism.

P15

Structural and Functional Characterization of FosM from *Mycobacterium abscessus*

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Mycobactrium abscessus subspecies *Bolletii* was first isolated in 2006 from patients with chronic pneumonia and cystic fibrosis.[1] It is highly pathogenic and is an increasing cause of human pulmonary disease and infections of the skin and soft tissue.[2,3] FosM is a putative fosfomycin resistance enzyme from *M. abscessus* subsp. *bolletii*.[4] It is a 134 amino acid protein that shares approximately 30% sequence identity with other Vicinal Oxygen Chelate (VOC) fosfomycin resistance enzymes and maintains nearly every amino acid found in the active site of the FosA and FosB fosfomycin resistance enzymes from *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively. This protein represents the first of its type found in any *Mycobacterium* species and likely represents a new class of fosfomycin resistance enzyme. We have expressed, purified, and initially characterized FosM. Preliminary time trace kinetic data using ^{31}P NMR suggest that FosM is an M^{2+}-dependent thiol transferase. Kinetic activity has been observed with both GSH and L-cysteine as thiol substrates, with metal activation similar to other VOC fosfomycin resistance enzymes. However, *Mycobacteria* do not produce GSH, and we believe FosM utilizes the natural thiol found in *Mycobacteria*, mycothiol (MSH), as its substrate. Furthermore, the VOC superfamily of metalloenzymes is characterized by a 3D domain-swapped arrangement of tandem βαβββ motifs in which both subunits of the homodimeric enzyme participate in coordination of two metal ions and formation of U-shaped active sites in the enzyme. We have obtained an initial crystal structure of apo FosM that appears to maintain this general homodimeric, domain-swapped orientation. We are currently working to obtain MSH in order to demonstrate that FosM is a new class of fosfomycin resistance enzyme from *Mycobacteria*.

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P16

Toward deciphering the biosynthesis of DNA thymidine hypermodifications in bacteriophages

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Bacterial viruses (bacteriophages) contain the greatest diversity of modified nucleotides in their DNA where a variety of molecules, including sugars, amino acids, or polyamines, are found appended on the nucleobases of their genomic DNA to circumvent the endonuclease-mediated host defenses. These bacteriophages' unique DNA modifications, specifically called DNA hypermodifications, are formed through bacteriophage-encoded biosynthetic pathways, with steps occurring before and after replication of bacteriophage DNA.

Historically, it has been known that virion DNAs from the *Delftia* phage ΦW-14 and the *Bacillus* phage SP10 contain the hypermodified pyrimidines α-putrescinylthymidine and α-glutamylthymidine, respectively. These thymidine modifications are derived from 5-hydroxymethyl-2'-deoxyuridine (5-hmdU), where the 5hmdU is incorporated into DNA polymer by the phage-encoded polymerase during DNA replication. Then a fraction of DNA 5-hmdU is further modified by phage-encoded enzymes resulting in a "hypermodified" base via a pyrophosphorylated intermediate presumably catalyzed by 5hmdU specific kinases. Moreover, like ΦW-14 and SP10, the *Pseudomonas* phage M6 and the *Salmonella* phage ViI encode kinase homologs predicted to phosphorylate 5-hmdU DNA but have uncharacterized nucleotide content.[1] We report the discovery and characterization of two bases, 5-(2-aminoethoxy)methyluridine (5-*N*e*O*mdU) and 5-(2-aminoethyl)uridine (5-*N*edU), in the virion DNA of ViI and M6 phages, respectively. Furthermore, we have developed a LC/MS based activity assay to search for the enzymes responsible for the biochemical transformation and we show that recombinant expression of four gene products encoded by phage ViI and M6 is sufficient to reconstitute the formation of 5-*N*e*O*mdU and 5-*N*edU *in vitro*. These findings reveal an expanded diversity in the types of naturally occurring DNA modifications and point to an unexplored diversity of DNA modification enzymes and the underlying biochemistry of their formation.

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P17

Mechanism of 6-hydroxynicotinate 3-monooxygenase (NicC), a flavin-dependent decarboxylative hydroxylase involved in aerobic nicotinic acid catabolism

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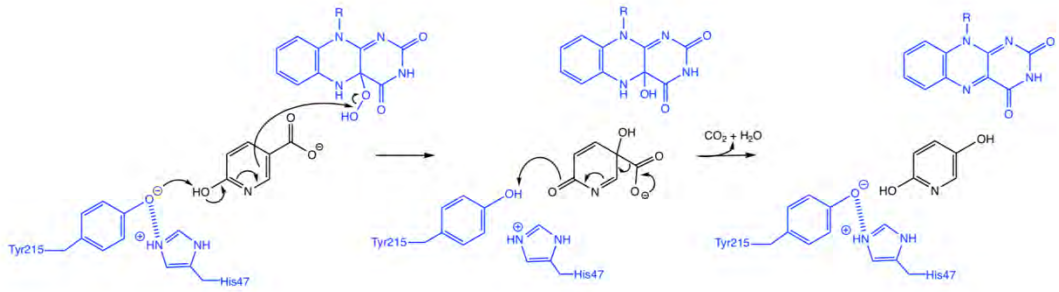
N-Heterocyclic aromatic compounds (*N*HACs), often found in agrochemicals, pharmaceuticals, and personal care products, are pervasive environmental contaminants. Bacterial nicotinic acid catabolism is a useful model system for investigating structural and mechanistic details of *N*HAC degradation to improve bioremediation methods. 6-Hydroxynicotinate 3-monooxygenase (NicC) catalyzes the FAD-dependent decarboxylative hydroxylation of 6-hydroxynicotinic acid (6-HNA) to 2,5-dihydroxypyridine (2,5-DHP) with concomitant oxidation of NADH in the aerobic degradation pathway of nicotinic acid by several genera of soil bacteria.[1] Two possible mechanisms for NicC were previously proposed.[2] We present results from mass spectrometric analyses as well as steady-state and transient-state kinetic studies with substrate analogues and enzyme variants that support an electrophilic aromatic substitution mechanism. Active site residues Tyr215 and His47, observed to be critical for substrate binding and in catalyzing the rate of hydroxylation, are postulated to function as a catalytic dyad to form an H-bonding network that deprotonates the 6-OH group and promotes hydroxylation of 6-HNA by FADHOOH with subsequent decarboxylation. These results help refine the structural model for understanding substrate binding and specificity.



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P18

Steric enforcement of *cis* rather than *trans* epoxide formation by fosfomycin-producing (*S*)-2-hydroxypropylphosphonate epoxidase

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ABSTRACT:

(*S*)-2-hydroxypropylphosphonate [(*S*)-2-HPP] epoxidase (HppE) is a nonheme-iron peroxidase that cyclizes its substrate to the (1*R*,2*S*) *cis*-epoxide, fosfomycin. HppE reportedly removes the *pro-R* hydrogen from C1 and forms the new C1–O bond of the epoxide with inversion. Here we show that the ring-closure stereochemistry is not absolute but is strongly biased (~ 95:5) toward inversion – yielding the *cis* product – by steric control. Published structures of the HppE•Fe^{II}•(*S*)-2-HPP and HppE•Zn^{II}•fosfomycin complexes reveal distinct pockets for the C3 methyl group of the substrate and product and identify three hydrophobic residues, Leu120, Phe182, and Leu193, that are close to C3 in one complex or the other. Replacement of Leu193, proximal to C3 of the substrate, with the bulkier Phe enhances selectivity (*cis*:*trans* ~ 100:0), whereas the Leu120Phe substitution in the product-C3 pocket diminishes it (~ 80:20). Remarkably, the ring-closure stereoselectivity is partially or completely *reversed* by a bulk-reducing substitution in the substrate-C3 pocket (Phe182Ala; *cis*:*trans* ~10:90), trifluoro substitution of C3 (~ 20:80), or both (~ 0:100). The fluorine effect, most likely arising from the unfavorable eclipsing of the CF_3 and PO_3^{2-} groups in the trajectory for inversion, is partly counteracted by the more constrained substrate-C3 pocket of the Leu193Phe variant (*cis*:*trans* ~ 57:43). In light of the hypothesis advanced in a recent computational study that the initial C1 radical intermediate undergoes facile inversion, the results pinpoint specific enzyme-substrate contacts that may drive this step to ensure formation of the more potently antibacterial *cis*-epoxide.

P19

Mechanism of Dioxygen Activation by Peroxide-Dependent P450 Decarboxylase OleT

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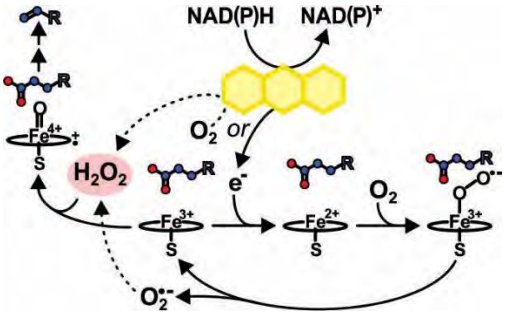
OleT, a recently discovered member of the CYP152 family of cytochrome P450s, catalyzes a unique decarboxylation reaction, converting free fatty acids into 1-olefins and carbon dioxide in a peroxide-dependent manner.[1, 2] The C-C cleavage reaction proceeds through hydrogen atom abstraction by an iron(IV)-oxo intermediate known as compound I.[2, 3] The capacity of this enzyme for generating important commodity chemicals and liquid biofuels has inspired a flurry of investigations seeking to maximize its biosynthetic potential. One common approach has sought to address the limitations imposed by the H_2O_2 co-substrate, particularly for *in vivo* applications. Numerous reports have shown relatively efficient decarboxylation activity with various combinations of the enzyme with pyridine nucleotides, biological redox donors, and dioxygen, implicating a mechanism in which OleT can generate Compound I via a canonical P450 O_2-dependent reaction scheme. We have applied transient kinetics, cryoradiolysis, and steady state turnover studies to probe the precise origins of OleT turnover with surrogate redox systems.[4] Results show electron transfer from several redox partner proteins to be prohibitively sluggish.[4] Further, the enzyme is unable to form the hydroperoxo-ferric intermediate that serves as a critical precursor to Compound I via O_2-driven catalysis.[4] Though ferrous OleT readily binds O_2, autoxidation of both the enzyme and redox system results in generation of H_2O_2, which is ultimately responsible for turnover.[4] These conclusions illuminate several strategies for improving OleT for downstream biocatalytic applications.



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P20

Elucidating the Mechanism of C-S Bond Cleavage in Isethionate Sulfite-Lyase

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Among the many bacteria associated with the human gut microbiome (HGM) the sulfate reducing bacteria (SBR) respire anaerobically using sulfite as a terminal electron acceptor, releasing sulfide. SRB have garnered recent interest for their implication in Irritable Bowel Syndrome (IBS), as the sulfide they release can damage the mucus lining of the colon. To better understand the etiology of IBS we must better understand how SRB harness sulfite from the HGM. Sources of sulfite comprise several organosulfonates, including isethionate that is believed to be produced by the HGM through deamination of the abundant metabolite taurine. Although isethionate-sulfite lyase activity was anticipated based on in vivo studies, the corresponding enzymes had been unknown, hampering efforts to inhibit pathological overgrowth of SRBs. Transcriptomic analysis and biochemical characterization performed in the Schleheck and Balskus labs, respectively, have identified isethionate-sulfite lyase enzymes of the glycyl radical enzyme (GRE) superfamily. To better understand the molecular mechanism of this enzyme we solved a crystal structure of the Bilophila wadsworthia enzyme. Surprisingly the active site differs markedly from what is predicted by sequence alignments to other structurally characterized GREs, but maintains essential catalytic features expected of GREs including a cysteine residue, a glycine residue, and a glutamate residue. This structure also reveals two unanticipated arginine residues in the active site that appear to be in a suitable conformation to coordinate the sulfate group of the substrate isethionate. Structural characterization of the enzyme substrate complex is being pursued to elucidate how SRB harness sulfite from a metabolite in the HGM.

P21

CYP Epoxygenases Metabolize Omega-3 Endocannabinoids to form a Novel Class of Anti-inflammatory Lipid Mediators

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In the endocannabinoid (eCB) system, eCBs are endogenously synthesized cannabinoid-like molecules that activate cannabinoid receptors and possess physiological relevance in inflammation, cancer, pain and other disease conditions in humans. Omega-3 eCBs, docosahexaenoic ethanolamide (DHEA) and eicosapentaenoic ethanolamide (EPEA), in particular have been implicated in brain development and reduction of cancer cell migration. One possible mechanism of how eCBs play a role in disease is through the downstream metabolism by cytochrome P450 epoxygenases (CYPs). Of the 57 CYP isozymes, we have demonstrated two brain CYPs, CYP2J2 and CYP2D6, are responsible for the conversion of omega-3 eCBs into eCB epoxides. The omega-3 eCB epoxides formed are DHEA-epoxides and EPEA-epoxides (EDP-EAs and EEQ-EAs) that are anti-neuroinflammatory, vasodilatory and activate cannabinoid receptors. Herein, we investigate the role of CYPs mediated metabolism of eCBs to form dual functional molecules, eCB epoxides, that may target multiple receptors. We have developed a novel lipidomics LC/MS-MS method for endogenous quantification of eCB epoxides and performed cell based-assays to identify their biological and pharmacological significance.

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P22

Using Ketoreductases for the Chemoenzymatic Synthesis of a Stereotriad

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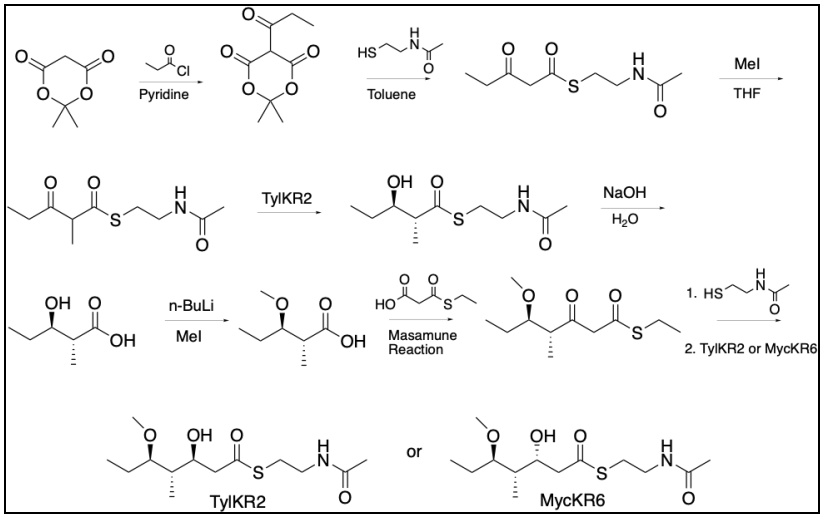
Polyketides are diverse natural products of high biological activity produced from complex molecular machines called polyketide synthases (PKSs). Of particular focus here is the ketoreductase (KR), one of the domains within PKSs which reduces carbonyl groups stereoselectively and controls stereocenters in the polyketide. KRs have been used here to produce 2 stereotriads that would traditionally be difficult to synthesize. A diketide was first synthesized chemically and reduced with a tylosin KR from *Streptomyces fradiae* (TylKR2). This reduced diketide was then extended into a triketide through the Masamune C-acylation reaction. Finally, TylKR2 and a mycolactone KR from a bacterial artificial chromosome (MycKR6) were found to reduce S-(2-acetamidoethyl) (4*R*,5*R*)-5-methoxy-4-methyl-3-oxoheptanethioate to S-(2-acetamidoethyl) (3*S*,4*S*,5*R*)-3-hydroxy-5-methoxy-4-methylheptanethioate in the case of TylKR2 and to S-(2-acetamidoethyl) (3*R*,4*S*,5*R*)-3-hydroxy-5-methoxy-4-methylheptanethioate in the case of MycKR6. The reductions were performed *in vitro* using the glucose dehydrogenase regeneration system with $NADP^+$. In this way, each of the 3 stereocenters was set chemoenzymatically using KRs to form a stereotriad. With similar KRs, it is likely that all 8 possible stereoisomers could be synthesized. Ultimately, polyketide building blocks could be extremely useful functional groups to experiment with in drug synthesis and may result in more potent or effective medicines. Additionally, this work could be extended into a methodology for setting as many stereocenters as desired - possibly one day allowing for customizable polyketide synthesis in the same way oligonucleotides and polypeptides can be made today.



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P23

Structural and functional studies of modular polyketide synthase domains for venemycin synthesis

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Polyketides are bacteria-derived secondary metabolites which often exhibit high biological activity. These natural products have a wide variety of structural characteristics, but are equivalently assembled from acyl starter units and malonyl/methylmalonyl extender units by polyketide synthases (PKSs) through a pathway analogous to fatty acid biosynthesis. The domains of these modular PKSs, which typically contain acyltransferases, ketosynthases, ketoreductases, dehydratases and enoylreductases can be selectively manipulated to control both the regio- and stereochemistry of substituents. For example, venemycin, a natural product derivative of *Streptomyces venezuelae*, can be biosynthesized *in vitro*, using PKS VemG and VemH enzymes to catalyze the elongation of 3,5-dihydroxybenzoic acid. Derivatives of venemycin can be obtained through the construction of a unique expression vector and large scale synthesis can be performed for spectroscopic analysis of the metabolite.

P24

Characterization of a heme-dependent N-N bond forming enzyme

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There is a long-standing mystery of how nitrogen-nitrogen (N-N) bonds are formed in natural products. Piperazic acid is one of the most common N-N bond containing non-proteinogenic amino acids, found in a variety of natural product peptides, such as kutznerides and polyoxypeptins. Our group recently discovered an enzyme that cyclizes N-hydroxylated L-ornithine, forming an N-N bond, to produce L-piperazic acid using a previously undescribed heme-dependent mechanism. Here I will present structural and biochemical analysis on this enzyme to gain further insight into this novel mechanism of enzymatic N-N bond formation.

P25

Nicotinamide Riboside (NR), A Multifaceted Molecule

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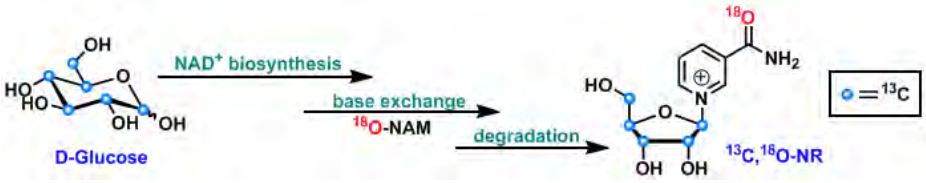
Nicotinamide riboside (NR), a trace nutrient found in milk, is a potent NAD^+ elevating agent. Increasing cellular NAD^+ concentration was thought to provide beneficial effects on healthspan and lifespan extension.[1,2,3] Repletion of the intracellular NAD^+ pool using NR, therefore, has been suggested as a novel therapeutic for the treatment of metabolic and age-related diseases.[4,5,6] We developed a chemo-enzymatic synthesis of NR with the specific incorporation of isotope labels into the ribose ring and nicotinamide. We took advantage of a pre-existing enzymatic synthesis of NAD^+[7] and coupled it with enzyme catalyzed "base exchange" and degradation for the production of isotopically labeled NRs. Using these isotopically labeled NRs, we demonstrate that in mammalian cells NR can be converted to NAD^+ independent of decomposition. We also establish the importance of NRK-dependent pathway in maintaining NAD^+ biosynthetic capacity when salvage pathway is compromised, further highlighting the homeostatic mechanism in NAD^+ biosynthesis.



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P26

FILLING IN THE GAPS: COMPLETE BIOSYNTHETIC PATHWAY TO FOSFOMYCIN IN PSEUDOMONADS

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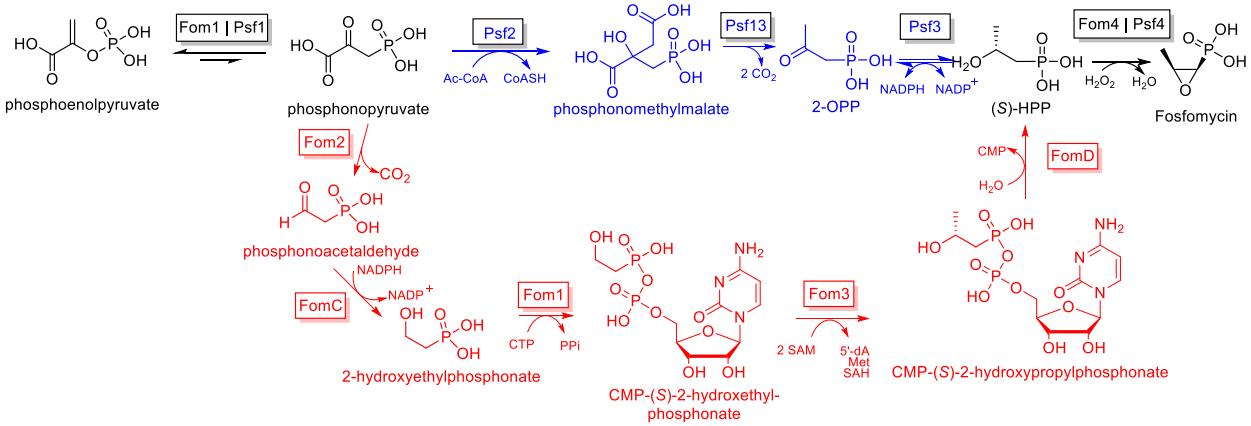
Fosfomycin is a clinically utilized, highly effective broad-spectrum antibiotic. Its mode of action involves covalent inhibition of MurA, the first committed step of bacterial peptidoglycan biosynthesis. Although the biosynthesis of fosfomycin has been observed in multiple streptomycetes (Fom) and pseudomonads (Psf), the complete biosynthetic pathway has only been fully elucidated recently in Gram-positive streptomycetes. Surprisingly, the biosynthetic gene clusters from these two organisms are drastically different, sharing only the enzymes catalyzing the first and last steps: the conversion of the primary metabolite PEP to phosphonopyruvate (PnPy), and the transformation of (*S*)-2-hydroxypropylphosphonate ((*S*)-2-HPP) to fosfomycin, respectively. Whereas canonical phosphonate biosynthetic pathways overcome the highly thermodynamically unfavorable conversion of PEP to PnPy via decarboxylation to phosphonoacetaldehyde (PnAA), no such decarboxylase is encoded in the pseudomonad gene cluster. Instead, a homo-citrate synthase homolog condenses acetate from acetyl-coenzyme A onto this PnPy intermediate to form phosphonomethylmalate (Pmm). Pmm then requires two subsequent decarboxylations to afford the presumed next intermediate, 2-oxopropylphosphonate (2-OPP). Here, we show the conversion of Pmm to 2-OPP via a single biosynthetic enzyme, Psf13, in a heterologous producer. Through *in vivo* co-expression of the gene cluster from *Pseudomonas syringae* PB-5123 in *E. coli* BL21(DE3), we show accumulation of 2-OPP after induction of Psf1, Psf2, and Psf13, the first three genes in the pathway. The unusual Psf13 enzyme is likely metal-dependent and shares homology with histidinol-phosphate phosphatase enzymes. The resulting 2-OPP is then reduced by Psf3 to form (*S*)-2-HPP, followed by the Psf4-catalyed epoxidation to fosfomycin. We have also characterized the thymidylate-like kinase, Psf8, that serves as part of the self-resistance mechanism, akin to FomB from streptomycetes. This catalyzes the formation of fosfomycin pyrophosphate from fosfomycin monophosphate. These two newly characterized enzymes expand the diversity of phosphonate chemistry and shed new light on a classic antibiotic.



P27

Cytochrome P450-Catalyzed Hydroxylation Initiating Ether Formation in Platensimycin Biosynthesis

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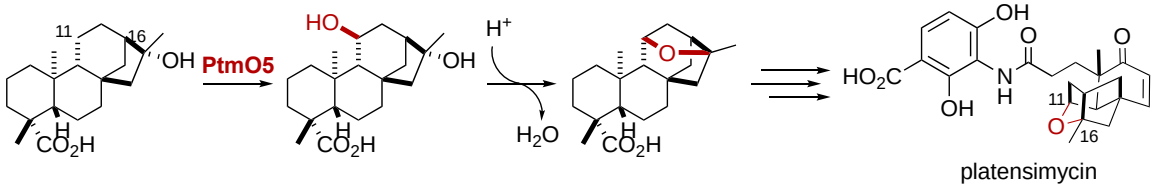
Platensimycin (PTM) and platencin (PTN) are highly functionalized bacterial diterpenoid natural products that target bacterial and mammalian fatty acid synthases.[1,2] The biosynthetic pathways of PTM and PTN diverge at the cyclization of *ent*-copalyl diphosphate. Two distinct type I diterpene synthases form the skeletons of PTM and PTN, (16*R*)-*ent*-kauran-16-ol and *ent*-atiserene, respectively. Before *ent*-kauranol resumes its parallel biosynthesis with *ent*-atiserene, it is processed by a cytochrome P450 to form the characteristic 11*S*,16*S*-ether linkage of PTM. The isolation of 16*R*-hydroxyl intermediates and congeners from the PTM producing strain suggested that ether formation goes through an 11*S*,16*R*-diol intermediate, due to the necessary inversion of stereochemistry at C-16.[3] Here, we report that (i) the in vitro characterization of PtmO5, revealing that PtmO5 stereoselectively hydroxylates C-11, (ii) the ether oxygen in PTM originates from molecular oxygen, and (iii) ether formation, through the 11*S*,16*R*-diol intermediate, is non-enzymatically facilitated by the presence of acid.[4] P450s of Streptomycete origin are diverse in sequences and functions, and possess enormous potential in natural products biosynthesis and enzymology.[5]



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P28

Coming Back from the Dead: Insight into the Regeneration of the Auxiliary Cluster of *E. coli* Lipoyl Synthase in the Biosynthesis of the Lipoyl Cofactor

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Lipoyl synthase (LipA in bacteria, LIAS in humans, Lip5 in yeast) is a radical SAM (RS) enzyme that catalyzes the second step of the *de novo* biosynthesis of lipoic acid, an essential cofactor known for its prominent roles in energy metabolism and the degradation of certain amino acids, among others. All RS enzymes contain one [4Fe–4S] cluster typically coordinated by cysteine residues lying in a highly conserved Cx_3Cx_2C motif. This iron-sulfur (Fe–S) cluster supplies the electron during the reductive cleavage of SAM to generate a 5′-deoxyadenosyl 5′-radical (5′-dA•) capable of hydrogen abstraction from an unactivated carbon center. Lipoyl synthase coordinates a second 'auxiliary' cluster that has been shown to be sacrificed as a sulfur source during catalysis, leaving LipA in an inactive state in the absence of a system to regenerate it (1-5). As such, LipA typically catalyzes no more than one turnover *in vitro.* Recently, we have provided evidence that *E. coli* NfuA, an Fe–S cluster-containing protein suggested to serve as an intermediate in Fe–S cluster delivery, restores *E. coli* LipA for subsequent turnover in a non-rate limiting step (6). In an additional study, we have investigated the role of the domain architecture of *E. coli* NfuA and provided insight into the mechanism by which *E. coli* NfuA recognizes *E. coli* LipA (in review). Efforts to elucidate the mechanistic details of the regeneration of *E. coli* LipA's auxiliary cluster are ongoing.

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P29

Biocatalytic Carbon-Hydrogen and Carbon-Fluorine Bond Hydroxylation Promoted by a Histidyl-Ligated Heme Enzyme, LmbB2

Yifan Wang[†], Ian Davis[†], Inchul Shin[†], Daniel J. Wherritt[†], Wendell P. Griffith[†], Kednerlin Dornevil[†], Keri L. Colabroy[‡], and Aimin Liu[†]

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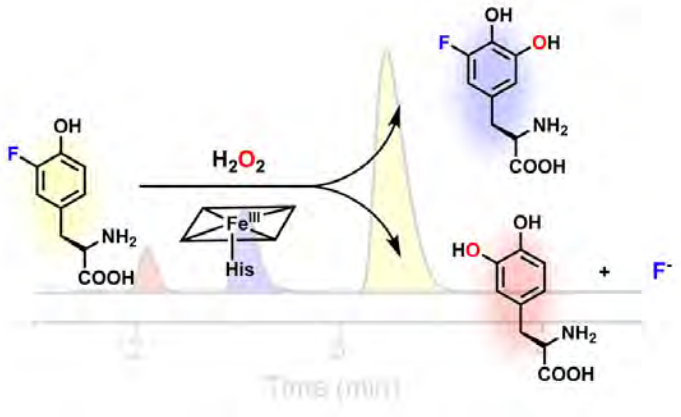
Heme enzymes are well known for performing a broad spectrum of chemistries. Herein, we report a mechanistic study on a new heme-dependent L-tyrosine hydroxylase LmbB2 with selected tyrosine analogs. LmbB2 is a peroxygenase-like enzyme hydroxylating L-tyrosine to L-3,4-dihydroxyphenylalanine (DOPA) in the presence of hydrogen peroxide. However, the cofactor of this enzyme is ligated by a proximal histidine but not cysteine. By testing tyrosine analogs without a phenol group: L-phenylalanine and *O*-methyl-L-tyrosine, we demonstrate that the phenol is essential for reacting with a high-valent heme intermediate and thus, activating the aromatic C-H bond. Spectroscopic and reaction data also indicate LmbB2 can oxidize L-tyrosine analogs with ring-deactivated substituents such as 3-nitro-, fluoro-, chloro-, iodo-L-tyrosine. The most interesting observation was obtained with 3-fluoro-L-tyrosine as the substrate. The LmbB2-mediated catalytic reaction yielded two hydroxylated products with nearly equal population: oxidative C-H bond cleavage at C5 to generate 3-fluoro-5-hydroxyl-L-tyrosine and a C-F bond oxygenation at C3 to generate L-DOPA. A histidyl-ligated heme enzyme-mediated hydroxylation on both C-H and C-F bond with multiple turnovers is unprecedented in literature. Thus, this finding reveals a significant potential of biocatalysis in C-H and C-F bond cleavage. Based on the experimental results, two catalytic pathways are proposed to explain how the enzyme hydroxylates the C-H and C-F bond resulting in a proton and fluoride leaving group, respectively. This study broadens the understanding of heme enzyme catalysis and sheds light on enzymatic applications in medicinal and environmental fields.



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P30

Structural and Functional Studies of a *gem*-Dimethylating Methyltransferase from a *trans*-Acyltransferase Assembly Line.

Jessica L. Meinke[1], M. Rachel Mehaffey[2], Drew T. Wagner[1], Zhicheng Zhang[2], Ningze Sun[1], Jennifer S. Brodbelt[2], Adrian T. Keatinge-Clay[1,2]

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Polyketides are complex bioactive secondary metabolites produced in a variety of bacteria and are synthesized via enzymatic assembly lines called modular polyketide synthases (PKSs). The intricate chemistry of each polyketide is set through the modular composition within these megadalton polypeptides. Many polyketides from the *trans*-acyltransferase assembly lines must incorporate α-methyl groups using *S*-adenosyl-methionine (SAM)-dependent methyltransferase domains, as most pathways do not utilize α-substituted extender units. The *gem*-dimethyl moiety within the polyketide disorazol is installed by a singular methyltransferase domain iteratively operating on an α-unsubstituted substrate. The 1.75 Å- resolution crystal structure of the *gem*-dimethylating methyltransferase from the third module of the disorazol assembly line yields insight into the intricate organization required to attach these two methyl groups. Activity assays of point mutants on β-ketoacyl chains linked to an acyl carrier protein and *N*-acetylcysteamine provide additional insights into the roles of active site residues.

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P31

Kinetic Basis for Improved Specificity of CRISPR/Cas9 High Fidelity Variants

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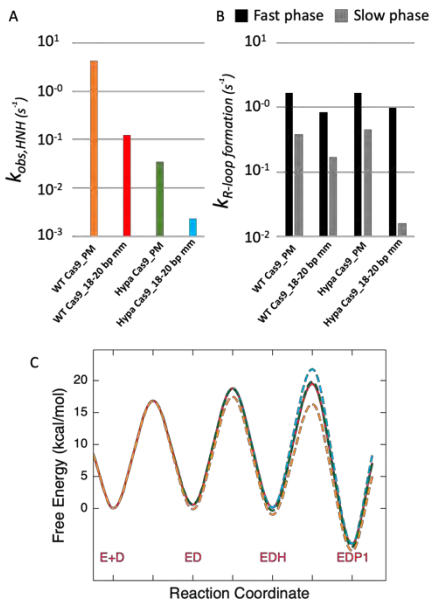
CRISPR/Cas9-mediated is a powerful, programmable genome editing tool that has been widely used for many applications. While engineered Cas9s have been reported to minimize the off-target cleavage efficiency compared with wild type Cas9 *in vivo*, a rational mechanism for enhanced specificity has not been put forward. Here, we provide kinetic characterization of the Hyper-accurate Cas9 variant (HypaCas9) to provide a basis for improved discrimination against off-target cleavage. We show that chemistry is the rate-limiting step for off-target cleavage in HypaCas9 and is much slower than on-target cleavage in WTCas9, where DNA unwinding is the rate-limiting step. In addition, the intrinsic cleavage rate and non-productive DNA binding state has also greatly been affected in engineered Cas9s. We find that HypaCas9 gains discrimination mainly through slowing down chemistry and rebalancing kinetic partitioning to favor release rather than cleavage of the bound substrate.



Free energy profile for WTCas9 and HypaCas9 for on-target and off-target cleavage. (A) $k_{obs,HNH}$ cleavage rates for on-target and off-target cleavage by WTCas9 and HypaCas9. The WTCas9 on-target cleavage was cited from Gong et al.[2] WTCas9 on-target cleavage is colored orange; WTCas9 off-target cleavage is colored red; HypaCas9 on-target cleavage is colored green; and HypaCas9 off-target cleavage is colored blue. (B) The two-steps for R-loop formation rates for on-target and off-target cleavage of WTCas9 and HypaCas9 were estimated by stopped-flow fluorescence measurements. The fast phase of R-loop formation is colored black; the slow phase of R-loop formation is colored gray. (C) The free energy profile was calculated using transition state theory: $\Delta G^{\ddagger} = RT[\ln(kT/h) - \ln(k_{obs})]$ kcal/mol using the rate and equilibrium constants derived from globally fitting all experiments. WTCas9 on-target cleavage is from Gong et. al.[2] WTCas9 on-target cleavage is colored orange (dashed line); WTCas9 off-target cleavage is colored red; HypaCas9 on-target cleavage is colored green (dashed line); and HypaCas9 off-target cleavage is colored blue (dashed line).

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STUDIES OF THE FOUR ENZYMES CATALYZING METHYLLINCOSAMIDE BACKBONE TRANSFORMATION AND THEIR MECHANISTIC IMPLICATION

Shao-An Wang[1], Chia-I Lin[1], Richiro Ushimaru[1], Jiawei Zhang[1], Eita Sasaki[1] and Hung-wen Liu[1]

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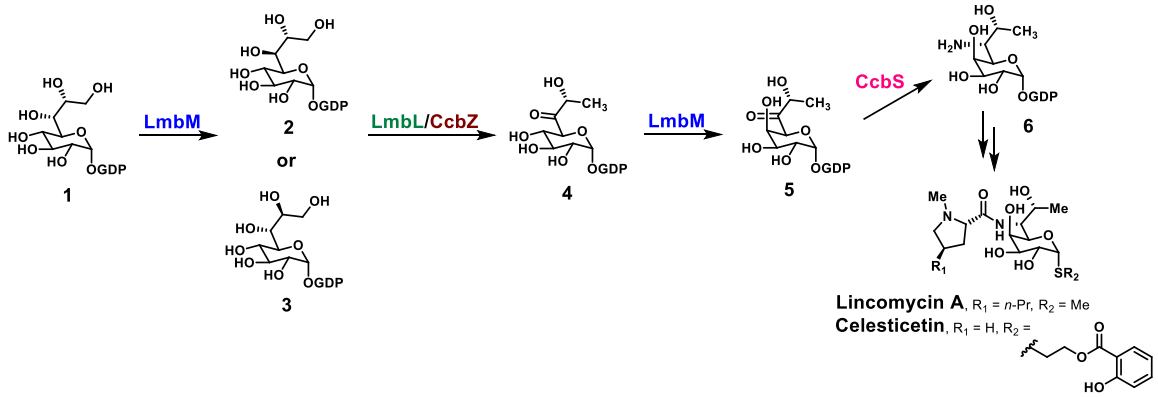
The structure of lincomycin A and celesticetin consists of an unusual eight-carbon methylthiolincosamide (MTL) core decorated with the pendent amino acid derivative *N*-methyl-4-propyl-l-proline (*N*-methyl PPL). Our group previously identified GDP-D-*erythro*-α-D-*gluco*-octose (**1**) as a key precursor[1,2] leading toward the immediate substrate for thiol incorporation, GDP-aminooctose (**6**).[3] We have found four enzymes encoded in the lincomycin (*lmb*)/celesticetin (*ccb*) biosynthetic gene clusters responsible for the transformation from **1** to **6**, including NAD-dependent epimerase LmbM, the two NAD(H)-dependent oxidoreductases LmbL and CcbZ, and the PLP-dependent transaminase CcbS. Further *in vitro* enzymatic assays revealed the unexpected dual function of LmbM, and the LmbMLZ catalysis essentially involves three enzymatic reactions: an initial LmbM-catalyzed C6- or C7-epimerization of **1**, the following LmbL/CcbZ-catalyzed 6,8-dehydration, and LmbM-catalyzed C4-epimerization of **4**. Our isotope mechanistic probes also indicate that LmbL/CcbZ catalyzes 6,8-dehydration initiated via an unexpected C7-oxidation. In all, the unusual dual function of LmbM and the interplay between the Lmb and Ccb enzymes exemplifies the complication in high carbon sugar biosynthesis and is adding new insights to the carbohydrate biochemistry.



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P33

Post-Translational Control and Dynamics of the Human Tron-Sulfur Cluster Assembly Complex

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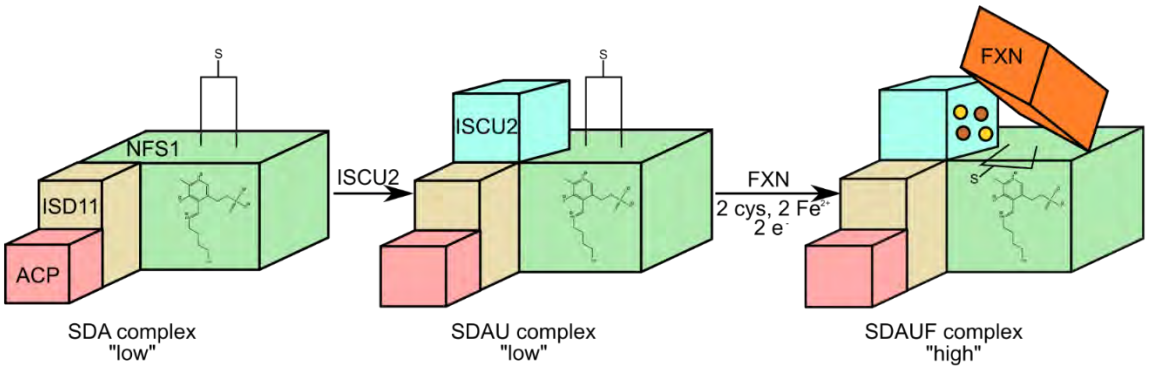
Texas A&M University Department of Chemistry

The mitochondrial iron-sulfur (Fe-S) cluster assembly complex is responsible for the biosynthesis and distribution of essential Fe-S cluster cofactors[1]. The resulting Fe-S proteins function in processes such as DNA replication and repair, RNA modification, substrate activation, respiration, and the biosynthesis of other protein cofactors[1]. The central enzyme in the mitochondrial Fe-S cluster assembly complex is the cysteine desulfurase NFS1, which is unstable in the absence of its binding partners ISD11 and the acyl-carrier protein (ACP)[2]. Interestingly, this complex has been crystallized in two distinct dimeric forms that have the same NFS1-ISD11-ACP (named SDA) core, but these SDA units arrange across two different interfaces to form fundamentally distinct quaternary structures[2-3]. In addition, the Dancis and Pain groups recently identified a modifying kinase that appears to regulate the yeast SDA complex through phosphorylation[4]. Together, these and more recent studies suggest a model in which the activity of the cysteine desulfurase complex is controlled by protein binding and post-translational modification events that shift the equilibrium between the two architectural forms. Here, we have combined structure-function studies of phosphomimics and directly phosphorylated human complex with chemical biology approaches to incorporate non-canonical amino acid to spin-label the complex for EPR distance measurements. We anticipate these studies will provide new insights into the regulation of the human Fe-S cluster assembly complex with implications for human disease.



Adapted scheme for Fe-S biosynthesis[5]

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P34

In vitro reconstitution, analysis, and engineering of the venemycin modular polyketide synthase

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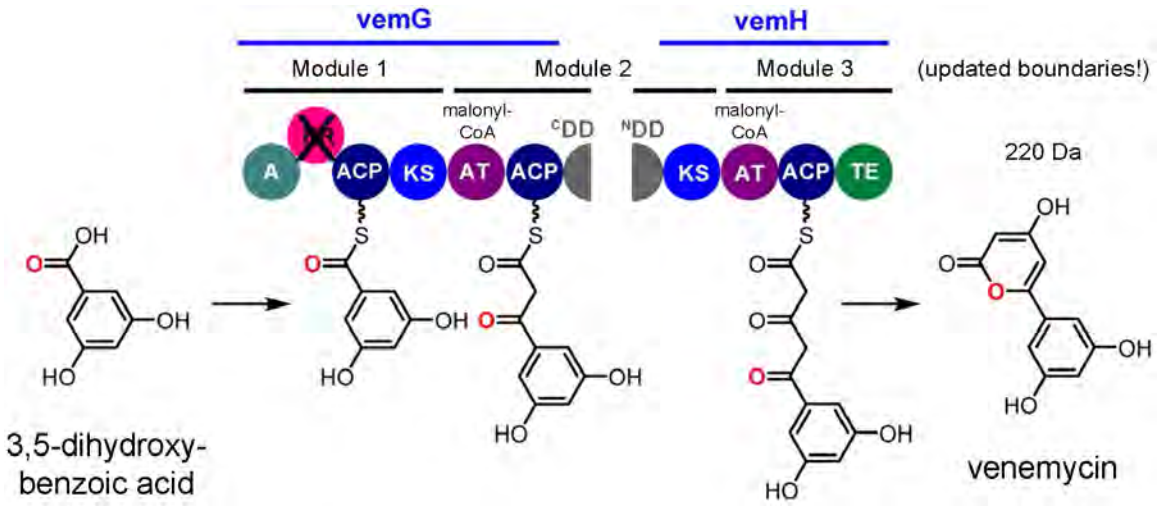
Modular polyketide synthases (PKSs) are enzymatic assembly line responsible for the biosynthesis of structurally and pharmacologically diverse polyketide compounds. Despite extensive studies, there have been a few reports of *in vitro* reconstitution and characterization of a naturally occurring PKS, and the enzymatic products have only been detected by mass spectrometry. Here we report the *in vitro* reconstitution of the venemycin PKS from *Streptomyces venezuelae*. The PKS is composed of two subunits, VemG and VemH, both of which express well in *Escherichia coli*. Scaled-up *in vitro* synthesis generates sufficient venemycin for NMR analysis. Taking advantage of the highly active enzymatic assembly line as a new platform for PKSs studies, we further demonstrated PKSs engineering based on evolutionary co-migratory units (updated module definition)[1]. We swapped Module 3 of VemH with the termination module of the pikromycin PKS. The resulting chimeric PKS expressed well in *E. coli* and incubation with VemG yielded a novel venemycin derivative. This indicates that the linker between between KS and AT is well suited as a fusion site to generate chimeric PKSs.



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P35

Ketoreductases as Biocatalysts in the Synthesis of Chiral Diketides

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Ketoreductases (KRs) control the majority of stereocenters in polyketides. These KRs are capable of setting different combinations of stereocenters at the α and β position, resulting in complex polyketides with multiple chiral centers.[1] To better understand the mechanisms of stereocontrol in KRs, a uv-active thiophene moiety, NAC and pantetheine handle were used in diketide substrates. The thiophene moiety allows for easier chiral HPLC analysis and is used to test the ability of KRs to set stereocenters with bulky diketides at the γ position. Typically NAC handles are used in the biosynthesis of diketide chiral building blocks, however, a pantetheine handle might allow for a greater level of stereocontrol than the NAC-linked diketides because of the greater homology with the native phosphopantetheinyl arm-linked moieties observed in nature.[2] The goal is to explore the relationship between the handle, and γ region of the substrate and the stereochemical outcome of ketoreductases.

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P36

From enzyme mechanisms to rational inhibitor design: Potential inhibitors of essential enzymes from human pathogens.

Ardala Katzfuss, Peter Tyler, Vern Schramm, Thomas D. Meek, and Demetrios Kostomiris

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The use of small molecules to inhibit the catalytic function of enzymes has been an important tool for treating human diseases. One approach to discovering novel enzyme inhibitors is through rational design. In the rational design approach information regarding the interactions of an enzyme with both discrete and transient chemical species during the catalytic cycle is utilized to inform the chemical synthesis of enzyme inhibitors. Of particular interest are small molecules that can approximate the characteristics of the transition state, converting the features of the transition state into binding energy. Currently, the chemical mechanisms of D-alanyl-D-alanine ligase from *Mycobacterium tuberculosis* and hypoxanthine-guanine phosphoribosyltransferase from *Trypanosoma cruzi* are being investigated.

D-alanyl-D-alanine ligase (Ddl) is an enzyme which functions in forming the terminal D-alanyl-D-alanine dipeptide of the pentapeptide of nascent peptidoglycan. During catalysis, the donor D-alanine undergoes a phosphoryl transfer from the γ-phosphate of ATP[1], forming the activated acyl-phosphate intermediate. This reactive intermediate is then captured by the amino group of the acceptor D-alanine forming the dipeptide product. Phosphinate and phosphonate analogs, which are presumably akin to the transition state for the formation of the amide bond, have previously been reported as time-dependent inhibitors[2]. Similarly to the antibiotic D-cycloserine[3], both of these inhibitors are phosphorylated in the active site of the enzyme, suggesting that chemical groups that can elicit a non-productive phosphoryl transfer in the enzyme active site may serve as tight-binding inhibitors.

Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes the formation of IMP and GMP from the reaction of phosphoribosyl pyrophosphate and the corresponding purine. In *T. cruzi*, the function of this enzyme is essential for purine incorporation from the host into the parasites' nucleotide pool. HGPRT is a member of the *N*-ribosyltransferase family, of which several potent transition-state analog inhibitors have previously been reported[4]. Accordingly, the transition state structure of the enzyme from *T. cruzi* should be able to be solved using the well-developed methods for the transistion-state analysis of this family of enzymes.

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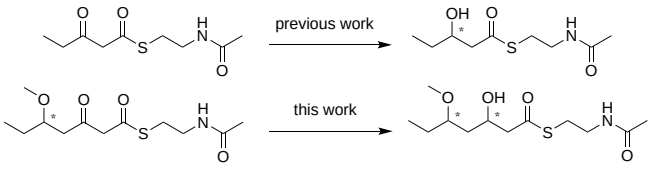
P37

General Chemoenzymatic Route to Two-Stereocenter Triketides

Zhicheng Zhang, Alexis J. Cepeda, Mireya L. Robles, Kaan Kumru, Jina Zhou, Zhicheng Zhang

UT Austin

Modular polyketide synthases (PKSs) are enzymatic assembly lines that fuse small carbon fragments into complex chiral products. Re-engineering these factories to synthesize designer polyketides is not yet practical; however, our group has collaborated with PKS enzymes excised from their assembly lines to produce libraries of diketide chiral building blocks. Here, the synthetic logic of PKSs is further employed in the chemoenzymatic generation of triketide fragments. Each of the four stereoisomers of a two-stereocenter triketide were constructed in an environmentally-friendly manner using a combination of C-acylation and two PKS ketoreductases (KRs) possessing opposite stereoselectivities. The routine described is general and can be developed to access longer and more stereocomplex compounds.



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P38

Using a Fluorescent Unnatural Amino Acid to Characterize the Role of Conformational Dynamics in High Fidelity DNA Replication

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The DNA polymerase of bacteriophage T7 is a model enzyme for understanding high fidelity DNA replication. Crystal structures of the open, binary (E-DNA) complex and the closed, nucleotide bound ternary (E-DNA-dNTP) complex reveal large structural changes as nucleotide binds and the fingers domain closes around the correct base pair. However, the contribution of this structural transition to DNA replication fidelity has been greatly disputed, and its measurement has been further complicated by the difficulty in site specific fluorescent labeling of the T7 DNA polymerase using traditional cysteine – maleimide coupling chemistry. The work presented here determines the contribution of the motions of the fingers domain of the T7 DNA polymerase to its high DNA replication fidelity, through direct measurement using a fluorescent unnatural amino acid incorporated site-specifically in the fingers domain of the enzyme. Each step in the pathway of correct nucleotide incorporation was measured using stopped flow and quench flow techniques, and data from all experiments were globally fit in Kintek Explorer to derive rate constants for each step in the pathway. Our data support a model where the conformational change preceding chemistry is not rate-limiting but is still the major determinant of fidelity, and provides new information on post-chemistry conformational dynamics of the enzyme and their contribution towards DNA replication fidelity.

P39

Effects of Vibrational and Structural Perturbations in Formate Dehydrogenase

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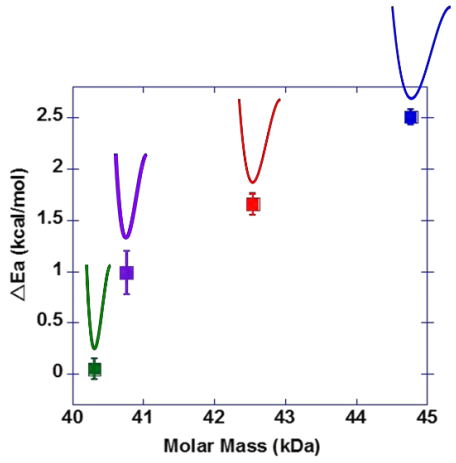
Understanding the role of enzyme dynamics is instrumental in designing inhibitors, biomimetic catalysts and novel enzymes. Enzymes show a broad array of motions ranging from seconds and milliseconds to picoseconds (ps) and femtoseconds (fs). Unlike dynamics at the slow, seconds-nanoseconds timescale, investigation of fast fs-ps timescale motions is challenging, due to lack of suitable probes and techniques. Isotopic labeling of enzymes, by growing the cells in minimal media enriched with C-13, N-15 and H-2, is a technique recently employed to perturb enzyme motions.[1] This method diminishes vibrational motions in the fs-ps time scale quite significantly, as demonstrated in the present study by using the model enzyme, formate dehydrogenase (FDH).[2] Examination of the hydride transfer step catalyzed by differently labeled FDHs, namely, N-15 (singly labeled), C-13; N-15 (doubly labeled), C-13; N-15; H-2 (triply labeled) and native enzyme (unlabeled), is realized by analyzing the temperature dependence of intrinsic kinetic isotope effects (KIEs). The results delineate a mass effect on hydride transfer reaction. Further, two-dimensional infrared spectroscopic studies, which directly probe active-site dynamics are performed on the labeled and unlabeled FDHs. Interestingly, pseudo-static components (Δ_0) which reflect motions that are extended further out from the active site, and thermal denaturation measurements correspondingly suggest altered heterogeneity in FDH upon N-15 labeling. This effect is compensated for, when H-2 is introduced in the triply labeled FDH. Preliminary results on temperature dependence of KIEs for several active-site mutations (structural perturbations) reveal the coupling of active site motions to the hydride transfer coordinate in FDH.[3]



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P40

Exploring the Mechanism of Enzyme Evolution

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Enzyme motions on a broad range of time scales can play an important role in various intra- and intermolecular events, including substrate binding, catalysis of the chemical conversion, and product release. The relationship between protein motions and catalytic activity is of contemporary interest and heated controversy. Enzyme catalysis has been studied extensively, but the role of enzyme dynamics in the catalyzed chemical conversion is still an enigma. Our objectives are to gain a better understanding of how covalent bond activation in enzymes evolved. We trace the evolving nature of the chemical step in dihydrofolate reductase (DHFR), specifically a C-H→C hydride transfer in a primitive form of the enzyme evolving to a mature catalyst. Our hypothesis is that as a primitive enzyme evolves: the active site of the enzyme will more effectively be able to reorganize to achieve an ideal transition state. To investigate this hypothesis, one has to start from a primitive enzyme, in which the chemical step is far from ideal, and follow that step as the enzyme evolves. The model system for our study is a circularly permuted DHFR (cpDHFR) as a primitive starting point for directed evolution and the chemical step is followed through measurements of temperature dependence of intrinsic kinetic isotope effects (KIE). KIEs were measured to follow the evolution of the chemical step from a poor transition-state ensemble in the primitive enzyme to an accurate transition-state ensemble for mature variants. The cpDHFR appears to be quite primitive, not only because its catalytic efficiency is slower by several orders of magnitude, but also because of the steep temperature dependence of its intrinsic KIEs. Since very little is known about the changing physical nature of the chemistry along the evolutionary path of an enzyme, our study sheds light on this interesting biological question and broaden our understanding of the evolution of drug resistance in pathogens and cancer.

P41

Dibenzothiophene catabolism: mechanistic studies and first enzymatic synthesis of a chiral biphenyl

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Dibenzothiophene is one of the sulfur containing molecules present in crude petroleum that produces sulfur dioxide on fossil fuel combustion[1]. Traditional metal catalyzed hydrodesulfurization is not efficient enough to remove such a sulfur atom embedded in the carbon skeleton. The search of an alternative method lead to the discovery of the dibenzothiophene catabolic pathway in *Rhodococcus erythropolis*[2]. Our study is focused on DszA, the third enzyme of this pathway, which carries out a flavin (FMN) dependent C-S bond cleavage to convert dibenzothiophene sulfone (DBT sulfone) to 2-(hydroxybiphenyl) sulfinate (HBPS). Detailed mechanistic studies[3] revealed that the reaction proceeds via production of an FMN-N5-oxide, a recently discovered flavin oxidation state[4]. Investigation of the product stereochemistry lead us to design a substrate analogue, which unveiled that the enzyme DszA specifically produces "S"-enantiomer of the product. To the best of our knowledge, this is the first enzymatic synthesis of a chiral biphenyl- an important moiety present in various natural products and drug molecules.

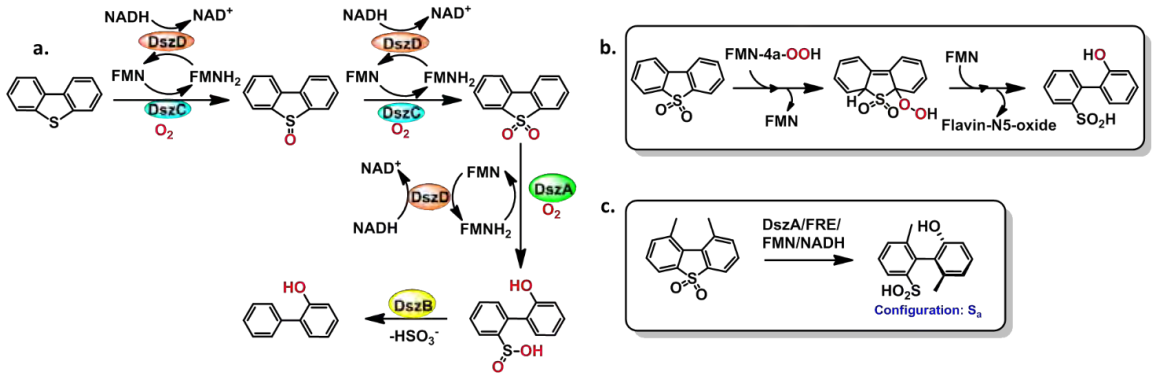

Image1a. Dibenzothiophene catabolism pathway; b. Proposed mechanistic scheme of DszA reaction. c. Studies on product stereochemistry of DszA.

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P42

Phytoene Desaturase: Biochemical Characterization and Enzymatic Activity

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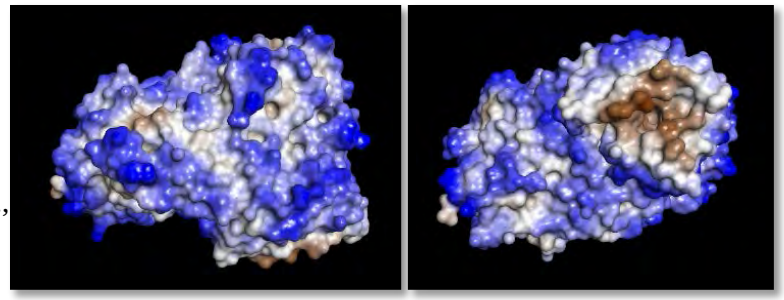
Phytoene Desaturase (PDS) is a key enzyme in the early stages of the carotenoid biosynthetic pathway in cyanobacteria, green algae and plants.[1] PDS uses a ping-pong, bi-bi mechanism to catalyze two consecutive $2e^-$ oxidations and isomerizations, converting phytoene (PT) to phytofluene (PF) and then to ζ-carotene (zCar). After each carotenoid oxidation the reduced FAD is reoxidized using plastoquinone. The published structure[1] (PDB code 5MOG) is tetrameric, with FAD adjacent to a long hydrophobic tunnel apparently devoid of reactive functional groups. PDS has both gene sequence and mechanistic similarities to enzymes such as monoamine oxidase and protoporphyrinogen oxidoreductase; yet the reactions catalyzed by this flavoenzyme are distinct enough to make the enzyme worthy of detailed study. Further, there is interest in bioengineering green microalgae to produce carotenoid co-products (nutraceuticals) such as lutein. Thus mechanistic studies of PDS have the potential to improve the economic prospects of commercializing algae for biofuels production.

We have begun characterization of PDS from *Chlorella vulgaris* UTEX 395 and rice (*Oryza sativa*). We have purified the substrates PT and PF from red tomatoes and developed HPLC protocols for their analysis. We have prepared His-tagged PDS constructs from both organisms in pET-26b(+), optimized *E. coli* strain and growth conditions for protein production and purified active rice PDS.

Currently we are characterizing steady-state rice PDS activity in phosphatidyl choline-liposomes containing PT and decylplastoquinone, with the addition of the inhibitor norflurazon, followed by HPLC analysis. We have found that maximal PDS activity requires both PT and the quinone to be added prior to liposome formation. As both PF and zCar products accumulate, we have developed an appropriate method to calculate Specific Activity; and developed a way to consider concentrations of nonpolar carotenoids buried in the hydrophobic core of liposome bilayers. We also have plans to develop new assay methods that will be useful to facilitate mechanistic analysis of the enzyme.



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P43

High-Field EPR and DFT studies of Tryptophan-Based Radicals

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Tryptophan–based radicals play crucial roles in many biochemical reactions, and hence attracted attention for molecular characterization with use of various spectroscopical techniques including HF-EPR spectroscopy. The *g*-anisotropy (Δg) of tryptophan radicals has been explained mainly by spin density as well as the presence of protonation on the nitrogen atom of the indole ring. However, tryptophan and its derivatives-based radicals have relatively a smaller Δg, making it harder to discriminate the difference in Δg between protonated cation radical forms and deprotonated neutral radical forms. Likewise, the substitution effects on tryptophan also remain unclear.

We attempted to interpret the origin of the difference in Δg observed in HF-EPR spectra in terms of the difference in orbital energies, principal axes of *g*-tensors, spin densities under oxygen substitution, by means of DFT calculations. The difference in Δg of EPR spectra are consistent with Δg predicted by DFT calculations and can be explained by larger extent of the contribution of NBMO of oxygen as well as larger extent of spin delocalization in protonated cation radical forms. Meanwhile, in neutral radical forms, Δg of EPR spectra can be explained mainly by large spin density mainly on pyrrole ring, regardless of oxygen substitution, making the difference in Δg smaller.

Our Δg analysis in combination of HF-EPR with DFT computation will help us to discriminate different types of radicals, even with relative small Δg, paving the way to comprehend bioorganic molecular mechanisms more clearly.

This work was supported by the National Institutes of Health (NIH) Grant R01GM108988.

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P44

Mechanism of a Class C Radical SAM Thiazole Methyl Transferase

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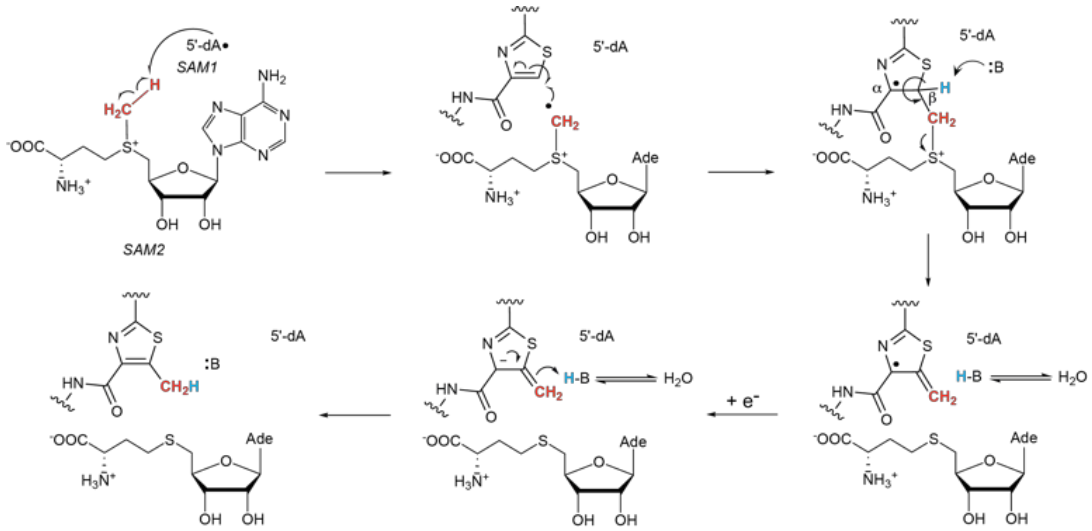
The past decade has seen the discovery of four different classes of radical *S*-adenosylmethionine (rSAM) methyltransferases that methylate unactivated carbon centers. Whereas the mechanism of class A is well understood, the molecular details of methylation by class B-D are not. Herein, we present detailed mechanistic investigations of the class C rSAM methyltransferase TbtI involved in the biosynthesis of the potent thiopeptide antibiotic thiomuracin. TbtI *C*-methylates a Cys-derived thiazole during posttranslational maturation. Product analysis demonstrates that two SAM molecules are required for methylation and that one SAM (SAM1) is converted to 5'-deoxyadenosine and the second SAM (SAM2) is converted to *S*-adenosyl-L-homocysteine (SAH). Isotope labeling studies show that a hydrogen is transferred from the methyl group of SAM2 to the 5'-deoxyadenosine of SAM1 and the other two hydrogens of the methyl group of SAM2 appear in the methylated product. In addition, a hydrogen appears to be transferred from the β-position of the thiazole to the methyl group in the product. We also show that the methyl protons in the product can exchange with solvent. A mechanism consistent with these observations is presented that differs from other characterized radical SAM methyltransferases.



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P45

Insights into AMS/PCAT Transporters from Biochemical and Structural Characterization of a Double Glycine Motif Protease

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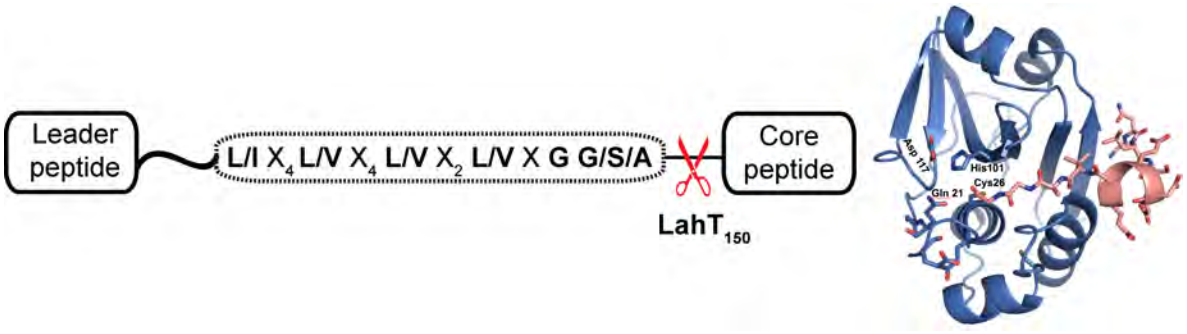
Leader peptide removal at a double glycine motif by a protease is the final step in the biosynthesis of ribosomally synthesized and post translationally modified natural products.[1] Dual-functional ATP-binding cassette transporters export antimicrobial[2] or quorum signaling peptides in Gram-positive bacteria.[3] Their substrates contain a leader sequence that is excised by an N-terminal peptidase C39 domain at a double Gly motif.[4] We characterized the protease domain (LahT150) of a transporter from a lanthipeptide biosynthetic operon in Lachnospiraceae and demonstrate that this protease can tracelessly remove leader peptides by recognizing a short amino acid stretch at the C-terminus of the leader region. This promiscuous enzyme is able to cleave leader peptides from unrelated ribosomally synthesized and post translationally modified peptides (RiPPs) from Gram negative or Gram positive bacteria, stemming from different leader peptide families such as Nif11, nitrile hydratase or even glycocins. The 2.0 Å resolution crystal structure of the protease domain in complex with a covalently bound leader peptide demonstrates the basis for substrate recognition across the entire class of such transporters. The structural data also provide a model for understanding the role of leader region recognition in the translocation cycle,[5] and the function of degenerate, non-functional C39-like domains (CLD) in substrate recruitment in toxin exporters in Gram-negative bacteria.[6]



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P46

Active Site Structure of Full-Length and Split-LanBs Revealed Through the Use of Non-Reactive Substrate Mimics

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The class I lantibiotic nisin, from *Lactococcus lactis*, has been employed extensively for agricultural and food preservative applications for decades. Despite this, the biosynthesis of class I lantibiotics is still not full understood on a molecular basis. Herein, we describe our efforts to examine the structure and mechanism of class I lantibiotic and thiopeptide dehydratases using inert substrate mimics. We report the development of a glutamylated-NisA analogue as a structural probe for the elimination domain of NisB, NisA-Dap_3^{Glu}. In addition, we also report the development of a puromycin analogue, 5′-phosphoryl-*N6*-desmethylglutamycin, to mimic glutamyl-$tRNA^{Glu}$ and probe the structure of the glutamylation domain of the split-LanB, TbtB, involved in thiomuracin biosynthesis (from *Thermobispora bispora*). Our results provide structural rational for the mode of substrate recognition and yield further insight into how these enzymes carry out their enzymatic transformations.

P47

On the mechanism of the L-Arginine Oxidase MppP from *Streptomyces Wadayamensis*

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The non-proteinogenic amino acid L-enduracididine (L-End) is found in a number of cyclic peptide antibiotics, such as teixobactin, mannopeptimycin and enduracidin.
The biosynthesis of L-End begins with L-arginine and involves the action of three enzymes, MppP, MppQ, and MppR, in the case of mannopeptimycin. Our recent work showed that MppP is an unprecedented PLP-dependent L-Arg oxidase that catalyzes a 4-electron oxidation to give 4-hydroxy-2-ketoarginine. It also releases the partially-oxidized abortive product 2-ketoarginine. Several outstanding questions remained about the mechanistic details of the MppP-catalyzed oxidation of L-Arg. First, is the 4-electron-oxidized, β-γ unsaturated intermediate hydroxylated on the enzyme in a stereospecific manner? If so, what is the configuration of the 4-hydroxy-2-ketoarginine produced? The reduction of dioxygen is thermodynamically unfavorable. How, then, is the presumed superoxide anion stabilized in the active site to promote its formation? Through detailed pre-steady state kinetics studies, X-ray crystallography, and NMR spectroscopy, we have provided answers to these questions and raised others. Crystallographic structures of the enzyme with L-Arg and each of the products bound suggest that MppP catalyzes a stereo-specific hydroxylation at Cγ to produce 4(S)-hydroxy-2-ketoarginine. The stereospecificity of the hydroxylation reaction was confirmed by NMR experiments in H_2O and D_2O, the results of which are consistent with the presence of a single enantiomer. The structure of the enzyme with 4(S)-hydroxy-2-ketoarginine bound shows that His29, which is absolutely conserved among MppP-like proteins, forms a hydrogen bonding interaction with the hydroxyl group of the product. This observation suggests that His29 may play a role in activating dioxygen for catalysis. This work represents a significant advance in our understanding of this unusual class of PLP dependent enzymes.

P48

Polyubiquitin-Based DUB Probes Reveal Mechanisms of Ubiquitin Chain Recognition and Processing by Ubiquitin-Specific Protease

Prajwal Paudel[1], Qi Zhang[2], Charles Leung[2], Harrison Greenberg[1], Yusong Guo[3], Yi-Hsuan Chern[4], Aiping Dong[2], Yanjun Li[2], Masoud Vedadi[2,4], Yufeng Tong[2,4,5] and Zhihao Zhuang[1]

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Ubiquitin-specific proteases (USPs) comprise the largest family of deubiquitinases (DUBs) and regulate diverse cellular processes in humans. In order to investigate the ubiquitin chain linkage specificities of USP and how it recognizes and processes polyubiquitin chains, we developed novel triubiquitin probes with orthogonal sets of linkages allowing either directed cleavage or covalent trapping of DUBs. Specifically, the probes contain either a non-cleavable linkage or a Michael acceptor-containing linkage between distal and middle ubiquitin, and a native isopeptide linkage between middle and proximal ubiquitin. Using this set of novel activity-based triubiquitin probes (ABP), we demonstrated that the USP9X catalytic domain has an exo-cleavage preference for K48-linkage and an endo-cleavage preference for K11-linkage. Together with a crystal structure of the USP9X catalytic domain at 2.5 Å resolution, the role of a novel β-hairpin insertion in USP9X was investigated with the triubiquitin probes. Our results revealed an interesting role of the β hairpin in binding of K63-linked triubiquitin to USP9X. Overall, unique structural features of USP9X unravelled in the high-resolution crystal structure together with novel triubiquitin probes are critical for understanding polyubiquitin chain recognition and processing by USPs.

P49

Towards Novel Chemotherapy for Chagas Disease: Design, Synthesis and Kinetic Studies of Cruzain Inhibitors

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Chagas disease, caused by the parasitic protozoa *Trypanosoma cruzi* (*T. cruzi*), affects approximately 8 million people in Mexico, Central and South America. Cruzain is the major protease expressed by *T. cruzi* throughout the parasitic life cycle, thus it becomes a promising antichagas target. The most effective inactivator of this enzyme—K777—employs a vinyl-sulfone moiety that acts as a Michael acceptor and irreversibly modifies Cys_{25} in the catalytic site. To avoid potential toxicity caused by such irreversible mode of action, our lab focuses on compounds with two types of rationally-designed warheads that inhibit cruzain in a reversible, time-dependent fashion. The vinyl heterocycle consists of peptidomimetic scaffold that orients the molecules into binding pockets and a conjugated vinyl heterocyclic warhead allows fine-tuning of its electrophilicity. The masked aldehyde is essentially a δ-lactol that may mitigate the hyperreactivity of aldehyde. It also offers a chance to control the aldehyde exposure via substitution on the phenol ring. The potency of these two classes of compounds is not only demonstrated on cruzain level, but the inhibition has also been observed in cell culture of *T. cruzi* and *T. brucei*.

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P50

Characterization of hypoxanthine-guanine phosphoribosyltransferase

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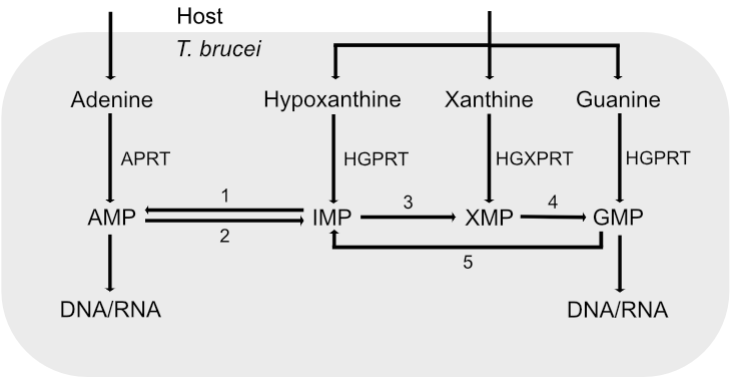
Human African Trypanosomiasis (HAT) is a neglected tropical disease caused the parasitic protozoan *Trypanosoma brucei*. Currently there are few approved treatments all of which lack oral availability and have severe side effects. In addition, a lack of an available vaccine, emerging drug resistance, and the inability of vector control to completely eliminate the disease, create a necessity for new chemotherapies (1). One approach for the development of new drugs is to target essential enzymes for the development of mechanism-based inhibitors. Like all parasitic protozoa, *Trypanosoma brucei gambiense* and *Trypanosoma brucei Rhodesiense* are auxotrophic for purines and must salvage them from mammalian hosts (3). *T. Brucei* salvages purines via the phosphoribosyl transferases (PRTases) which have been shown to be essential to the parasite (4, 5). The hypoxanthine-guanine-xanthine phosphoribosyl transferase (HG(X)PRT) enzymes are responsible for catalytic conversion of the dominate blood purines, hypoxanthine, guanine, and xanthine, into inosine monophosphate, guanosine monophosphate, and xanthosine monophosphate, respectively (4). The essentiality of the PRTases for trypanosomes, but not humans, make them an ideal drug target.



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P51

New cofactors for bacterial class I ribonucleotide reduction

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Ribonucleotide reductase (RNR) enzymes are required in all organisms for DNA replication and repair. All RNRs additionally share a common nucleotide reduction mechanism, initiated in the catalytic α subunit by a Cys thiyl radical. Class I enzymes use remarkably varied inorganic chemistry in a separate β subunit to generate the thiyl radical transiently on each turnover. Prior to 2007, the only known subclass (Ia) of class I RNR was defined by its use of a diiron cluster and O_2 to generate a tyrosyl radical (Tyr•) initiator. Recently, we reported two new bacterial class I RNR subclasses, Id and Ie. Class Id enzymes use a stable high-valent Mn_2 cluster as a direct metal-centered Cys oxidant. The Id RNR requires superoxide for activation, scavenging the oxidant from solution, similarly to superoxide dismutase. X-ray crystal structures of Id β reveal an open cavity and a neighboring positively-charged lysine residue to enable efficient O_2^- capture. Class Ie enzymes completely lack transition metals, at least in their activated form. They require a NrdI activase to convert a conserved Tyr (the one that normally harbors the Y• in class Ia enzymes) to a post-translationally modified semiquinone radical (DOPA•). Crystallographic analysis identified this novel cofactor, verified the absence of metal, and suggested how the oxidant might be tuned to ensure sufficient potency for initiating nucleotide reduction. Our work highlights the predominance of Mn-based RNR activity in microbes, thought to be important in escape of iron-dependent nutritional immunity. Class Ie enzymes – found in streptococcal and mycoplasmal pathogens linked to scarlet fever, pneumonia, and flesh-eating bacterial infections – may represent the most extreme RNR-based adaptation to metal-targeting innate immunity of the host.

P52

Reassignment of the Human Aldehyde Dehydrogenase ALDH8A1 (ALDH12) to the Kynurenine Pathway in Tryptophan Catabolism

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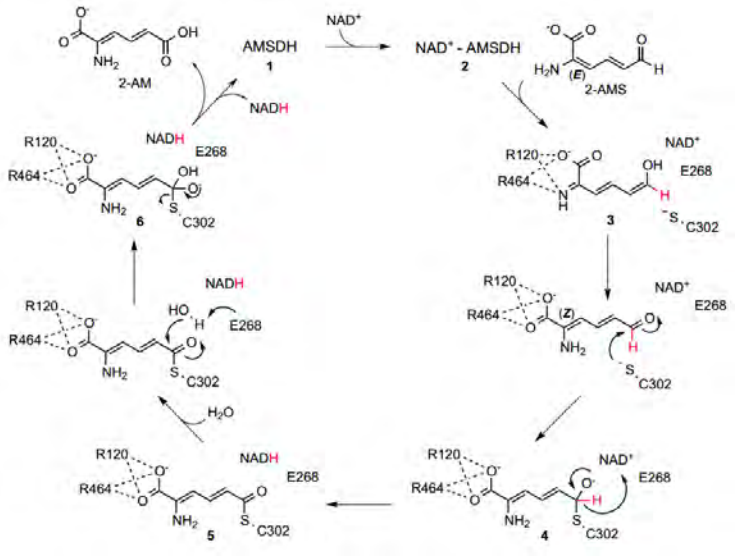
The kynurenine pathway is the primary route for L-tryptophan degradation in mammals. Intermediates and side products of this pathway are involved in immune response and neurodegenerative diseases. This makes the study of enzymes, especially those from mammalian sources, of the kynurenine pathway worthwhile. Recent studies on a bacterial version of an enzyme of this pathway, 2-aminomuconate semialdehyde (2-AMS) dehydrogenase (AMSDH), have provided a detailed understanding of the catalytic mechanism and identified residues conserved for muconate semialdehyde recognition and activation.[1,2] Findings from the bacterial enzyme have prompted the reconsideration of the function of a previously identified human aldehyde dehydrogenase, ALDH8A1 (or ALDH12), which was annotated as a retinal dehydrogenase based on its ability to preferentially oxidize 9-cis-retinal over trans-retinal. This molecular-level study establishes an additional enzymatic step in an important human pathway for tryptophan catabolism.[3]



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P53

Protein Quaternary Structure as a Mean to Regulate Enzyme Activity in Kynurenine Pathway

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Kynurenine pathway is the primary metabolic pathway to degrade tryptophan by providing a pool of metabolites including quinolinic acid (QA), the universal biosynthetic precursor to NAD^+ and related to protection against ageing and numerous diseases[1]. QA is a derivative from non-enzymatically decay of α-amino-β-carboxymuconate-ε-semialdehyde (ACMS), the substrate of ACMS decarboxylase (ACMSD). Because of neurotoxic characters, QA is critically regulated in mammalian cells. The specific activity of ACMSD was demonstrated to exhibit concentration-dependent and to be associated with its oligomeric states[2]. In this study, a tetramer model of ACMSD with the head-on dimer of dimers was demonstrated by performing size-exclusive chromatography coupled small angle X-ray scattering (SEC-SAXS) experiments. Dissociation profile of ACMSD from high to low concentration monitored by measuring specific activity is required three exponential functions to describe. The data indicates that ACMSD present in three distinct phases, with only dimer or high-ordered oligomeric states being catalytically active. We found that the three decay phases included ACMSD dissociation from higher oligomer to dimer, from dimer to the inactive monomeric form, and finally, protein degradation. Mutating His110, a residue located at the dimer-dimer interface in crystal structures, changed the dissociation profile by increasing the higher-ordered oligomer portion. Moreover, the effects of pH and ionic strength on the oligomeric states and specific activity of ACMSD were also studied and indicated that the tetramer formation of ACMSD is associated with multiple interaction mechanisms including His110-involved network and hydrophobic interaction under low pH and other interaction under high pH environment. Collectively, these results suggest that the dynamic protein oligomerization status is an enzyme activity regulatory factor and hence affecting the pathway profiling at a critical crossroad of the pathway junction.

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P54

Probing Cofactor Biogenesis in Cysteine Dioxygenase: C-F Bond Cleavage with Unnatural Tyrosine

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Cysteine dioxygenase (CDO; EC 1.13.11.20) is a mononuclear non-heme iron protein that catalyzes the conversion of L-cysteine to cysteine sulfinic acid, a crucial enzymatic activity for sulfur metabolism in mammals. A posttranslationally generated Cys93–Tyr157 cofactor, adjacent to the iron ion, is reported to increase mammalian CDO's catalytic efficiency by around ten-fold. It appears that the crosslink enhances activity by positioning the Tyr157 hydroxyl group to enable multiple H-bonding interactions with the organic substrate and dioxygen in the ternary complex and presumably later stage of the oxidation chemistry. Here, we performed the genetic incorporation of 3,5-flurotyrosine (F_2-Tyr) into human cysteine dioxygenase (CDO), resulting in a selective alteration of Tyr157 to its fluorine substituted form, F_2-Tyr157 CDO[1]. After a substantial effort, we were able to isolate both uncrosslinked and crosslinked forms of the native enzyme and CDO variant with F_2-Tyr. We have determined the crystal structures of the immature, cofactor-free CDO structure. Surprisingly, we observed a self-processing carbon-fluorine bond scission chemistry performed by the non-heme iron center of the dioxygenase, as shown by the crystallographic, high-resolution mass spectrometry, and ^{19}F NMR spectroscopy. This observation is very intriguing because the carbon-fluorine bond is stronger than the corresponding carbon-hydrogen bond. We also obtained the crystal structure of a ternary complex of F_2-Tyr157 CDO with bound L-cysteine and nitric oxide (NO), which is a structural analog to the ternary complex in the catalytic pathway for the cofactor formation. Our studies have revealed new insights into the power of the non-heme iron in the dioxygenase and shed lights into the biogenesis of the Cys–Tyr cofactor in human CDO.

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P55

New Insights into Mycofactocin Biosynthesis

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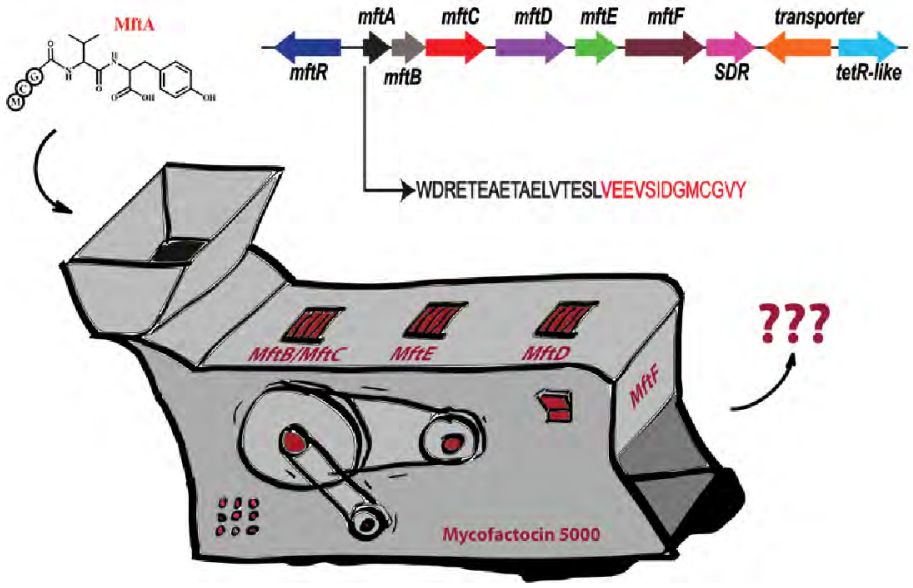
Abstract: Mycofactocin is a ribosomally synthesized and post-translationally modified peptide (RiPP) natural product. Despite being one of the most abundant RiPP biosynthetic systems across a number of bacterial genera, its biosynthesis and function still remain enigmatic. Current efforts to understand the biosynthesis and function of mycofactocin have aimed at *in vitro* reconstitution of each enzyme in the pathway to gain insights into their role and function in the maturation of mycofactocin. Hitherto, the first step in the biosynthesis of mycofactocin is the SAM-dependent oxidative decarboxylation of the C-terminal tyrosine by the radical SAM enzyme MftC in the presence of MftB followed by a subsequent carbon-carbon bond formation between the penultimate valine and decarboxylated tyrosine on the precursor peptide MftA to form MftA*. The peptidase MftE hydrolyzes the last two residues of MftA* to liberate the carbon skeleton (AHDP) of mycofactocin. Here, we use HPLC analysis, LC/MS and NMR techniques to demonstrate the chemistry of another step in mycofactocin biosynthesis.



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P56

PROBING THE CATALYTIC MECHANISM OF TRYPTOPHAN 2,3-DIOXYGENASE AND MOLECULAR BASIS FOR HYPERTRYPTOPHANEMIA DUE TO ENZYME DEFICIENCY LINKED TO NONCATALYTIC TRYPTOPHAN BINDING SITE

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Tryptophan 2,3-dioxygenase (TDO) is a heme enzyme that catalyzes the first and committed step of the kynurenine pathway by inserting molecular dioxygen into L-tryptophan. Along with its sister enzyme, indoleamine 2,3-dioxygenase (IDO), TDO has gained significant biomedical interest as an immune checkpoint that cancer cells hijack and exploit by elevating the expression level to evade immune surveillance. In a mechanistic point of view, there has been a long debate between concerted and stepwise oxygen insertion. We trapped and identified the monooxygenated reaction product by employing synthetic mechanistic probes which were designed for investigating the role of substrate α-amino group.[1] The product analysis results corroborate the presence of a substrate-based epoxyindole as a result of a stepwise O-atom transfer during catalysis and provide the first substantial experimental evidence for the involvement of the substrate α-amino group in the epoxide ring-opening. In the collaboration with a clinician, we reported the first human case of hypertryptophanemia in which two rare variants, c.491Adup and c.324G>C were revealed by sequencing *TDO2* gene.[2] We provided a molecular level of understanding for its pathogenicity by showing that c.491Adup does not produce soluble protein and c.324G>C results in a catalytically less efficient M108I enzyme. The mutation site is over 30 Å apart from the catalytic site but close to the noncatalytic tryptophan binding site. Our study revealed that the M108I mutation disrupts the noncatalytic binding site thereby making enzyme more susceptible to proteolytic degradation. Based on the molecular understanding, these new findings will guide us to design new concept inhibitors targeting TDO and IDO.

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P57

Elucidating the Role of Lysine Succinylation on the Function of Isocitrate Lyase 1 from *Mycobacterium tuberculosis*

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The causative agent of tuberculosis, *Mycobacterium tuberculosis* (*Mtb*), is able to persist in humans undetected for decades through remodeling its metabolism and manipulating the body's immunological defenses. One enzyme essential for *Mtb*'s metabolic shift, and subsequent survival in the host, is isocitrate lyase 1 (ICL), the gatekeeper of the glyoxylate shunt. Recent proteomic studies uncovered that ICL undergoes lysine succinylation at three sites, K189, K322, and K334. Post-translational modification can serve as a dynamic modifier of protein activity and because $K189_{succ}$ is positioned in a highly conserved active site loop, it has the potential to modulate ICL activity and thereby regulate entry of metabolites into the glyoxylate shunt. Preliminary results display that amino acid substitutions in ICL that mimic succinylation effect ICL activity. Further, structural and biophysical studies highlight how these modifications alter protein structure and function. Ongoing studies utilize chemical and synthetic biology approaches to prepare succinylated ICL for kinetic and biophysical studies. Additionally, using quantitative proteomic analysis, we will test how changes in carbon source and growth condition, mimicking those that occur in the host, affect the level of ICL succinylation in the virulent H37Rv strain of *Mtb*. Together, these studies will provide direct insight into the effects of post-translational modification on ICL's essentiality during infection and aid in the development of therapeutics designed to target the glyoxylate shunt pathway.

P58

Transient histone H4 phospho-Serine and acetyl-Lysine distinctly govern the kinetics of Arginine R3 methylation catalyzed by PRMT1 and PRMT5

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The protein arginine methyltransferases PRMT1 and PRMT5 deposit the majority of the asymmetric and symmetric dimethylarginine marks, respectively. Associated with gene regulation, these epigenetic '*writers*' are often dysregulated in cancer cells, altering their tumorigenic phenotypes.[1] *In vitro*, these enzymes prefer unmethylated substrates, suggesting discrete biological roles for mono- and dimethylarginine-modified proteins.[2] Previous reports highlighted the importance of lysine acetylation in modulating substrate recognition by type I and type II PRMTs.[3-4]

To elucidate a potential biologically-relevant cross-talk between various post-translational modifications (PTMs) and arginine methytlation at H2A/H4R3, we modulated the *in vivo* levels of histones acetylation through inhibition of histone deacetylases (HDAC). Concomitant with lysine hyper-acetylation, we observed an overall hypo-phosphorylation at serine S1 on both H2A and H4; furthermore, inhibition of casein kinase-2 (CK-2) recapitulated such an inverse correlation. These observations revealed a previously unknown biological crosstalk between H4S1ph and H4 acetylation.

We designed a state of the art analytical platform to interrogate methyltransferases: the 1-Step EZ-MTase provides a simple, yet robust solution to the continuous monitoring of enzymatic rates.[5] Using H4 peptides, we established that the short-lived phospho-Serine and acetyl-Lysines distinctly govern the kinetics of Arginine R3 methylation. This encryption of histone tail is decoded differently by PRMTs with S1ph being detrimental to PRMT5 catalysis.

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P59

STRUCTURAL CHARACTERIZATION OF A HYDROXYPROLINE DEHYDRATASE FROM *C. DIFFICILE*

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The glycyl radical enzyme (GRE) family utilizes a glycyl radical cofactor to catalyze difficult chemical reactions in a variety of microbial metabolic pathways. Although glycyl radical enzymes are widely encoded and expressed by bacteria found in the gut microbiome, these enzymes remain largely uncharacterized. Recently, a new glycyl radical enzyme was discovered to catalyze the dehydration of *trans*-4-hydroxy-L-proline (4-Hyp) to 1-pyrroline-5-carboxylic acid. Bioinformatics studies by the Balskus lab show that this hydroxyproline dehydratase (HypD) is the second most prominent GRE in the human gut microbiome and is encoded by 360 bacterial genomes, including the human pathogen *C. difficile*[1]. HypD presents a pathway for bacteria to reverse 4-Hyp post-translational modifications, the most common post-translational modification in animals which was previously thought to be irreversible. Furthermore, the bacteria that encode HypD are known to use 4-Hyp as an electron acceptor during amino acid fermentation, their primary method of generating adenosine triphosphate (ATP). However, the enzyme responsible for assimilating 4-Hyp into this pathway has remained unknown until now. HypD could be the missing puzzle piece to understanding how these bacteria use the abundant metabolite 4-Hyp in energy production, while also symbiotically providing humans with a method for recycling this common amino acid. In order to elucidate the mechanism for how HypD performs the dehydration of hydroxyproline, we have engaged in structural studies of HypD from *C. difficile*. Progress with the structural analyses will be presented.

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P60

Structure and mechanism of a nicotine-degrading enzyme, NicA2: Towards design of tools and therapeutics

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Several tobacco soil bacteria have adapted to using nicotine as a growth substrate and evolved biochemical strategies for using it as a primary source of carbon and nitrogen. Among these nicotine-catabolizing bacteria is *Pseudomonas putida*, which uses the enzyme nicotine oxidoreductase (NicA2) to catalyze the first committed step of nicotine degradation via the pyrrolidine pathway. NicA2 is a flavoenzyme, part of the monoamine oxidase (MAO) family that oxidizes *S*-nicotine to *N*-methyl-myosmine, followed by non-enzymatic hydrolysis to form pseudooxynicotine. Taking advantage of its unique evolutionary adaptation, we aim to refine the inherent catalytic function and structural features of NicA2 to develop a biotherapeutic for nicotine addiction and nicotine poisoning and tools for nicotine biosensor development. Recently, we have published the first crystal structure of NicA2 at 2.2 Å resolution[1]. Structural analysis of NicA2 depicted an overall conserved amine oxidase fold along with a conserved FAD binding domain. However, the substrate-binding domain contained unusual differences from monoamine oxidases with a unique composition of the canonical aromatic cage (W427 and N462), which flanks the flavin isoalloxazine ring. Additionally, the X-ray crystallographic structure of NicA2 in complex with the substrate, *S*-nicotine, has been resolved to 2.6 Å resolution[2]. The crystal structure reveals a predominantly hydrophobic binding site with a solvent-exclusive cavity. The hydrophobic character of the active site is consistent with binding of a deprotonated substrate and supports a hydride-transfer mechanism.

Preliminary mechanistic studies report that NicA2 is specific for *S*-nicotine with a K_m of 44 nM, but with a very slow catalytic rate (k_{cat} of 6.64×10^{-3} s^{-1})[3], yielding an "apparently efficient" enzyme with $k_{cat}/K_m = 1.5 \times 10^5$ $M^{-1}s^{-1}$. Our goal is to identify the factors contributing to the mechanistic and substrate binding properties of NicA2 in efforts to improve its biotherapeutic potential. To address the low k_{cat}, the apparent tight binding and slow rate of NicA2 was further explored using stopped-flow and single-turnover kinetics. Additionally, site-directed mutagenesis and mechanistic studies probe the functional role of the aromatic cage and its role in catalysis. Site-directed mutagenesis of the residues W427 and N462 to resemble those of MAO family members is used to investigate the role of N462 in substrate binding and specificity. Overall, these studies reveal the rate-limiting reaction step to be in the half-reaction with oxygen, and together with structural information, yield insight into the specificity of NicA2 as well as highlighting the possibilities for protein engineering to enhance catalytic activity.

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P61

Structure and inhibition kinetics of trehalose 6-phosphate phosphatase

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Enzymes in the trehalose biosynthetic pathway have recently been explored as potential targets for a variety of bacterial and parasitic diseases including tuberculosis (*Mycobacterium tuberculosis*) and elephantiasis (*Brugia malayi* and *Wuchereria bancrofti*). Among these enzymes, trehalose 6-phosphate phosphatase (T6PP) catalyzes the dephosphorylation of trehalose 6-phosphate (T6P), producing trehalose as a product[1]. T6PP knockdown models of *Caenorhabditis elegans* and *M. tuberculosis* have confirmed that lack of T6PP leads to organism death[2,3]; however, it is the buildup of trehalose 6-phosphate (T6P), rather than lack of trehalose, that is lethal[2]. Furthermore, this biosynthetic pathway is not present in higher-ordered species including mammals. Therefore, this is an optimal target for designing therapeutics for tuberculosis and helminth-borne diseases. X-ray crystal structures were determined previously in our laboratories of *Salmonella typhimurium* (*St*) T6PP in complex with trehalose and an inhibitory probe, OGS[4]. Herein we present the X-ray structure of *St*T6PP in complex with trehalose 6-sulfate, a substrate analog, to 2.5 Å (R_{work}/R_{free}= 0.24/0.26). Sequence conservation and structural analyses identified three highly conserved residues (Glu123, Lys125, Glu167) as important for substrate binding.

Additionally, through screening the Johns Hopkins Clinical Compound Library, Closantel was identified as a lead candidate for inhibitor design of T6PP. Using kinetic analyses, it was determined that Closantel is a slow-binding inhibitor of T6PP with a K_I^{*app} of 4.5 μM for *Bm*T6PP. Furthermore, Closantel analogs were synthesized with various substitutions on the center ring, replacing the cyano moiety with an ether linkage, and replacing the iodo groups with chloro substituents (Figure 1). The analogs were screened (at 10 μM) against *Bm*T6PP at $2K_m$ T6P, and compounds that decreased *Bm*T6PP activity to a greater extent than Closantel were further characterized. The preliminary inhibition constants, K_I^{*app} for SB-7 & SB-16 and IC_{50} of SB-21 are 10.8, 5.5, 11.6 μM, respectively. Future work will focus on elucidating X-ray crystal structures of T6PP (*St* or *Ce*) in complex with Closantel and lead analogs to identify the binding modes. Structural analyses are critical in development of more selective and potent inhibitors of T6PP. Future studies will also decipher the mechanism of T6P toxicity.

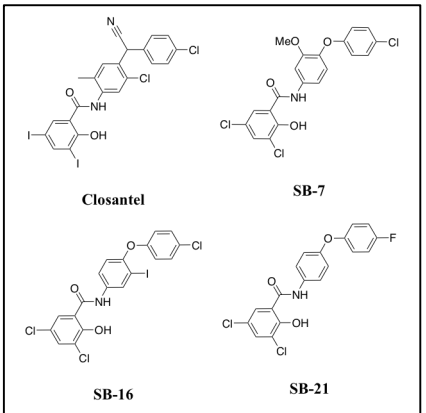

Figure 1. Structures of Closantel and lead analogs

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P62

SufS, a Type II Cysteine Desulfurase, is Regulated Through Changes in the Dimer Interface.

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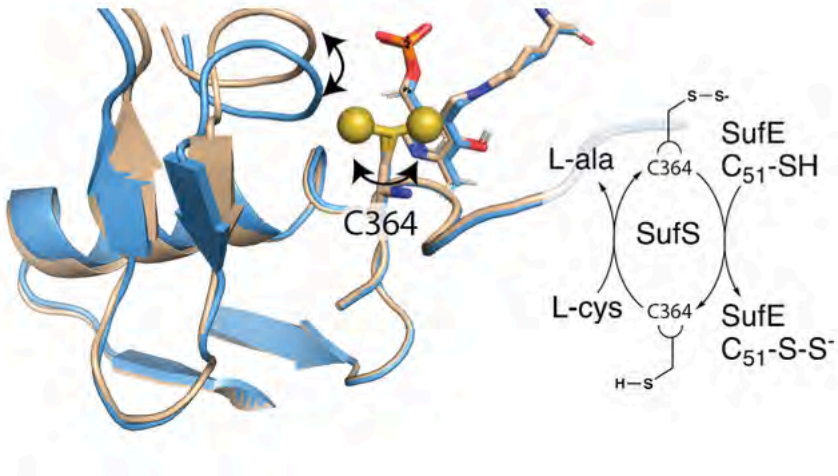
SufS is a type II cysteine desulfurase and acts as the initial step in the Suf Fe-S cluster assembly pathway. In *Escherichia coli* this pathway is utilized under conditions of oxidative stress and is resistant to reactive oxygen species. Mechanistically this means SufS must shift between protecting a covalent persulfide intermediate and making it available for transfer to the next protein partner in the pathway, SufE. Previous HDX-MS results suggest that the conserved SufS dimer interface is sensitive to the catalytic state of the enzyme (*Biochemistry*, **2018**, *57*, 5210). Site-directed mutagenesis of residues at the interface resulted in decreased k_{cat}/K_{SufE} values, consistent with long-range structural changes mediated by the architecture of the dimer interface. Here, we report five x-ray crystal structures of SufS (wildtype and four variants) including a new structure of SufS containing an inward facing persulfide intermediate on C364. Additional structures of SufS variants with substitutions at the dimer interface show changes in dimer geometry and suggest a conserved β-hairpin structure plays a role in mediating interactions with SufE. These new structures, along with previous data, identify an interaction network capable of communication between active-sites of the SufS dimer coordinating the shift between desulfurase and transpersulfurase activities in a potential half-sites mechanism.



P63

Rieske non-heme iron enzymes in natural product biosynthetic pathways

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The biosynthesis of the neurotoxin saxitoxin (and structurally related paralytic shellfish toxins) by freshwater cyanobacteria has been traced to a gene cluster that encodes enzymes involved in building, exporting, and functionalizing the tricyclic saxitoxin scaffold[1-2]. Recently we demonstrated that three of these enzymes are Rieske oxygenases that catalyze site- and stereo-selective hydroxylation reactions[3]. SxtH performs a hydroxylation reaction on a linear saxitoxin intermediate; whereas SxtT and GxtA hydroxylate the polycyclic saxitoxin core, a more advanced biosynthetic intermediate. Here, we describe three X-ray crystal structures that reveal how these enzymes, which share more than eighty-percent sequence identity with one another, selectively hydroxylate different biosynthetic intermediates. We have identified structural motifs that govern the site-selectivity and substrate specificity of these enzymes. Finally, using the information gathered from these studies, we have successfully interconverted the activity of SxtT and GxtA by mutation of only two protein residues.

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P64

Substrate Binding in 2,4'-Dihydroxyacetophenone Dioxygenase

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The enzyme, 2,4'-dihydroxyacetophenone dioxygenase (DAD), catalyzes the oxidative cleavage of the α-hydroxyketone side-chain of 2,4'-dihydroxyacetophenone (DHAP). While this unique reaction bears some similarity to that of the intradiol dioxygenases (IDs), the facts that the cleavage occurs off-ring and that there is no sequence or structural homology between DAD and the IDs strongly support a unique reaction system. Further, an important aspect of the ID reaction is the bidentate binding of the catechol substrate to the active site Fe^{3+}. In the study presented here, we screened various substrate analogs and common Fe^{3+} ligands as inhibitors of DAD. We found that substrate analogs that included a phenol group were much more successful inhibitors than both substrate analogs that mimicked the α-hydroxyketone cleavage site and general bidentate ligands of Fe^{3+}. Our preliminary conclusions are that DHAP likely binds to DAD with a monodentate geometry and that this ligand-metal interaction is less important to binding than the presence of the phenolic hydroxyl moiety at the distal end of DHAP. Computational modeling demonstrates a strong pair of hydrogen bonds between the phenol of DHAP and Trp-61 and Asp-63 which hold DHAP such that the α-hydroxyketone cleavage site is oriented in proximity to the active-site metal.

P65

Structural and Biochemical Characterization of the *N*-Prenyltransferase from Domoic Acid Biosynthesis Reveals Basis for Unusual Activity

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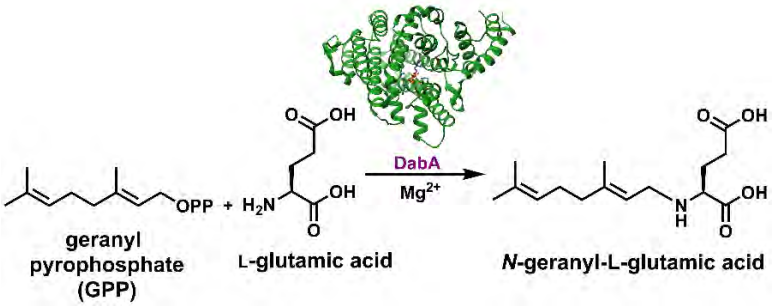
Domoic acid is a natural product produced by marine diatoms that can bioaccumulate in the food web and produce seizures, memory loss, and death in humans[1]. A particularly large bloom of these algae in 2015 on the west coast of the United States led to beach closures and damage to the fishing industry. Using a transcriptomics approach, we recently identified and characterized the domoic acid biosynthetic enzymes. The first committed step of the biosynthesis is the *N*-geranylation of L-glutamic acid by DabA[2]. *N*-prenylation is uncommon in natural products and often employs an ABBA prenyltransferase for formation. However, DabA is highly divergent from any known enzyme by primary amino acid sequence and is only identifiable as a terpene cyclase by secondary structure analysis. Notably, terpene cyclases typically catalyze intramolecular cyclization reactions, not intermolecular prenylations[3]. To confirm DabA's structural assignment as a terpene cyclase and identify the basis for catalysis, we crystallized DabA and solved co-crystal structures with both the *N*-geranyl-L-glutamate enzymatic product and a non-hydrolyzable GPP substrate mimic. A combination of this structural data and mutagenesis has allowed us to suggest a model for how the terpene cyclase fold of DabA has adapted to enable prenyltransferase chemistry. Furthermore, analysis of orthologs that are selective for a shorter prenyl substrate instead of GPP enabled us to propose the basis for substrate selectivity. Together, these results give insights into the mechanistic details of an important but divergent member of the well-studied terpene cyclase family.



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P66

A Low-Lying Dark State Controls the Deactivation of Excited Anionic and Neutral Flavin Semiquinones in Nitronate Monooxygenase

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ABSTRACT

Fluorescent cofactors like flavins can be exploited to probe their local environment with spatial and temporal resolution (1-4). While the fluorescence properties of oxidized flavins have been studied extensively (5-8), this is not the case for the one- and two-electron reduced states of flavins. Both neutral and anionic semiquinones have proven particularly challenging to study, as they are unstable in solution and are transient, short-lived species in many catalytic cycles. Here, we report that nitronate monooxygenase from *Pseudomonas aeruginosa* PAO1 (*Pa*NMO) is capable of stabilizing both semiquinone forms anaerobically for hours, enabling us to study their spectroscopy in a constant protein environment. We find that in the active site of *Pa*NMO the anionic semiquinone exhibits no fluorescence and the neutral semiquinone shows a relatively strong fluorescence, with a behavior that violates Kasha-Vavilov's rule. The fluorescence properties are discussed in the context of TD-DFT calculations, which reveal low-lying dark states in both systems.

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P67

Kinetic Viscosity Effects Reveal a Protein Isomerization in the Reductive Half-Reaction of Nitronate Monooxygenase

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3-Nitropropionate (3-NPA) is a toxin that is produced in various plants, insects, and fungi as a protection mechanism against predators. This toxin naturally occurs as an ester glycoside and can be hydrolyzed by the intestinal flora. Once hydrolyzed, the toxin can be fatal by irreversibly inhibiting succinate dehydrogenase and fumarase, halting the Krebs cycle and the electron transport chain.[2] Nitronate monooxygenase from *Neurospora crassa* (*Nc*NMO), an FMN-dependent enzyme, catalyzes the oxidation of 3-NPA via a single electron transfer.[1] In this study, we investigated the kinetic mechanism of *Nc*NMO using steady-state and rapid kinetics with 3-NPA as substrate in the presence of various concentrations of viscosigen. The rate of the reductive half-reaction, which is not diffusion-controlled, increased with added viscosigen suggesting that the electron transfer is gated by a slower conformational change

This study was supported in part by grant CHE-1506518 from the NSF (G.G.)

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P68

Probing Carrier Protein and Substrate Recognition of the Second Cyclization Domain of Yersiniabactin Synthetase by Docking and Molecular Dynamics

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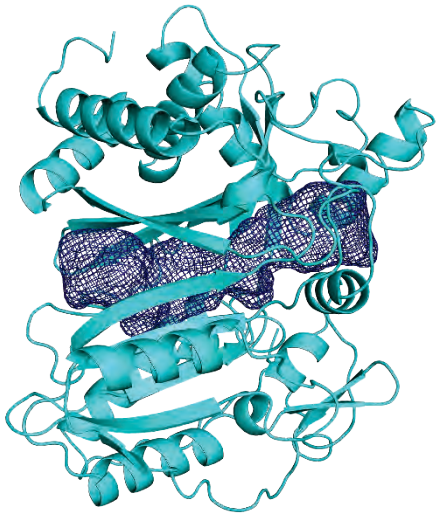

Yersiniabactin synthetase is a mixed nonribosomal peptide synthetase/polyketide synthase (NRPS/PKS) that catalyzes condensation/heterocyclization reactions yielding thiazoline rings. These moieties hold interest as key features of important natural products with functions including chelation, as in yersiniabactin[1], and antitumor therapy, as in epothilones[2]. NRPS/PKS systems often carry out their transformations with high specificity in complex chemical contexts. This behavior relies on recognition events between non-catalytic domains (i.e. carrier proteins that covalently tether intermediates to deliver them to active sites) and catalytic domains as well as recognition events between catalytic domains and their substrates.[3] Here we report a high-resolution crystal structure of the second cyclization domain of Yersiniabactin synthetase, which takes covalently tethered cysteine and hydroxyphenyl-thiazolinyl groups as substrates. This apo structure is the first structure of one of the three cyclization domains in Yersiniabactin synthetase. Additionally, the active site tunnel in this structure is more open than in previous condensation/cyclization domain structures from other systems, a feature that may be beneficial for docking and molecular dynamics simulations investigating the molecular interactions that underlie recognition events important for catalysis. So far, such simulations have led to the identification of several potential interactions between the upstream carrier protein and the second cyclization domain as well as between active site tunnel residues and the phosphopantetheine arm that tethers the hydroxyphenyl-thiazolinyl intermediate to the carrier protein. Docking studies of potential interactions between the cyclization domain and homology models of the downstream carrier protein are ongoing. Molecular dynamics and induced fit docking are being used to generate potential catalytic poses for the initial condensation reaction between tethered cysteine and the tethered hydroxyphenyl-thiazolinyl intermediate.

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P69

Characterization of an $FMNH_2$-dependent monooxygenase involved in methanesulfinate utilization in *Pseudomonas fluorescens*

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In the soil dwelling bacterium *Pseudomonas fluorescens, sfnG* (for sulfone utilization) and the *msuEDC* operon (for methanesulfinate utilization) code for enzymes involved in sulfur assimilation. SfnG converts the organosulfur compound dimethylsulfone ($DMSO_2$), a product of atmospheric dimethylsulfide oxidation, to methanesulfinate. Subsequently, products of the *msuEDC* operon oxidize methanesulfinate to sulfite, which can be used in downstream pathways to synthesize biomolecules such as cysteine or the gasotransmitter H_2S. Although the activities of MsuE and MsuD have been reported for sulfite production from methylsulfonate, the biochemical role of MsuC has only recently been reported. This enzyme requires reduced flavin, supplied by the flavin reductase MsuE, to catalyze the oxidation of methanesulfinate to methanesulfonate with concomitant reduction of oxygen. In order to characterize the $FMNH_2$-dependent monooxygenase, MsuC and MsuE were recombinantly expressed and purified. MsuC has been analyzed by circular dichroism to confirm its overall secondary structure, and an assay to determine the apparent steady state kinetics of MsuC is under development. This multi-enzyme assay is complicated by the requirement of molecular oxygen for substrate turnover. Free reduced flavin and oxygen can react to form hydrogen peroxide, and H_2O_2 can non-enzymatically oxidize methanesulfinate to methanesulfonate. To combat this side reaction, catalase is added to neutralize hydrogen peroxide, and the generation of reduced flavin must be optimized for the assay conditions. We present the optimization of flavin reduction conditions for MsuE and kinetic characterization of MsuC.

P70

Kinetic and Mechanistic Characterization of a Potent Inhibitor of Human Arginase

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Arginase is a metalloenzyme that catalyzes the hydrolysis of L-arginine to produce L-ornithine and urea. Two isozymes of arginase exist in mammals. Arginase 1 (ARG1) is expressed in liver and myeloid cells and plays a key role in the urea cycle whereas arginase 2 (ARG2) is expressed in the mitochondria of non-hepatic tissues. Adequate levels of L-arginine are crucial for T cell proliferation and activity.

In cancer patients, arginase released by myeloid-derived suppressor cells (MDSC) and neutrophils in the tumor microenvironment increases the rate of L-arginine breakdown, reducing L-arginine levels and suppressing T cell function. It is believed that inhibition of arginase may facilitate the rescue of exhausted T cells from suppression enabling them to mount a more effective anti-tumor immune-response.

We have developed a potent, reversible and competitive small molecule arginase inhibitor. This inhibitor (Compound 1) inhibits both recombinant human ARG1 and endogenous arginase prepared from human granulocytes with a K_i of 2-4 nM. Compound 1 inhibits human ARG1 activity with an IC_{50} = 16.5 nM in the presence of 3.5% human serum albumin (HSA) and 0.1% alpha-1-acid glycoprotein (AAG) using an L-arginine substrate concentration of 0.8 mM. Comparable potency was observed in human plasma spiked with recombinant ARG1. Elevated levels of arginase were observed in plasma derived from cancer patients relative to that of normal healthy subjects. High concentrations of ARG1 (5-15 nM) results in suppression of T cell proliferation *in vitro*. Restoration of T cell proliferation was observed by addition of Compound 1. We have also shown Compound 1 inhibits arginase in species utilized for preclinical safety and pharmacology studies.

P71

Kinetic Characterization of L-Arginine Dehydrogenase from *Pseudomonas aeruginosa*

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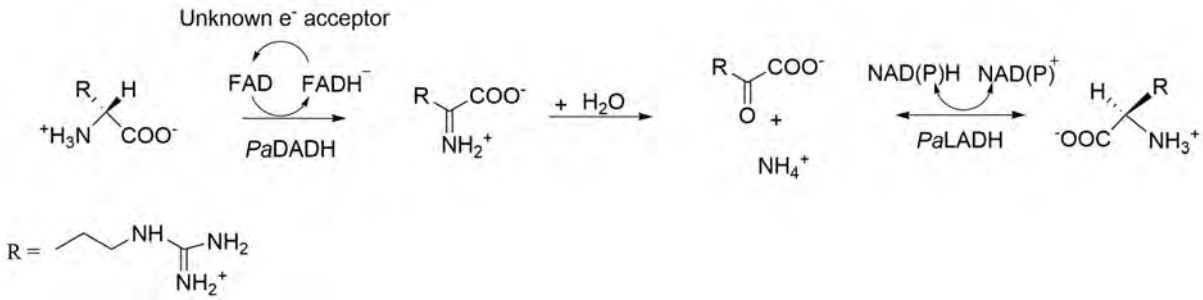
L-arginine dehydrogenase from *Pseudomonas aeruginosa* (*Pa*LADH) is an enzyme that converts 2-ketoarginine and ammonia to L-arginine, oxidizing NAD(P)H in the process. *Pa*LADH is part of a two-enzyme system along with *P. aeruginosa* D-arginine dehydrogenase (*Pa*DADH) that catalyzes the conversion of D-arginine to L-arginine in pseudomonads. *Pa*DADH catalyzes the oxidation of D-arginine into 2-ketoarginine and ammonia[1]. This two-enzyme racemic system has enabled *P. aeruginosa* to gain an edge over other bacteria, enabling it to survive solely on D-arginine as a nitrogen and carbon source[2].

We hypothesize that the two enzymes *Pa*LADH and *Pa*DADH, interact with each other to form a protein complex to catalyze the conversion of D-arginine to L-arginine. Here, we report the Steady-state kinetic parameters for *Pa*LADH with L-arginine, 2-ketoarginine, $NAD(P)^+$ and NAD(P)H, and sedimentation velocity analytical ultracentrifugation data to analyze the *Pa*DADH- *Pa*DADH complex formation.



Scheme 1. The two-enzyme racemic system for D-arginine catabolism in P. aeruginosa. PaDADH is D-arginine dehydrogenase and PaLADH is L-arginine dehydrogenase.

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P72

HEAVY ENZYME KINETIC ISOTOPE EFFECTS ON THE REACTION OF TYROSINE PHENOL-LYASE

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Tyrosine phenol-lyase (TPL) catalyzes the pyridoxal-5'-phosphate (PLP)-dependent β-elimination of L-tyrosine to give phenol and ammonium pyruvate. The key intermediate in the mechanism is the 2-aminoacrylate complex of PLP formed by cleavage of the C_β-C_γ bond of L-tyrosine. Aminoacrylates of TPL can be stabilized by binding 4-hydroxypyridine, an isoelectronic analogue of phenol. We have obtained X-ray crystal structures of aminoacrylate intermediates of TPL formed from L-tyrosine, 3-fluoro-L-tyrosine, *S*-ethyl-L-cysteine and L-serine, and measured the effect of hydrostatic pressure on the formation of aminoacrylate intermediates for "light" (unlabeled) and "heavy" (^{2}H isotopically labeled) TPL using stopped-flow spectroscopy. The kinetics of aminoacrylate formation are pressure-dependent (Figure 1). The rate of formation of the aminoacrylate intermediate from *S*-ethyl-L-cysteine decreases with increasing pressure, and the reaction rate for "heavy" TPL is slower than for "light" TPL, with $^{H}k/^{D}k = 1.30 \pm 0.05$ at 1 bar. In contrast, for the reaction of L-tyrosine, a significant positive curvature can be observed for the pressure dependence of the reaction rate, indicating high compressibility, and the reaction of "heavy" TPL is faster than for "light" TPL, with $^{H}k/^{D}k = 0.85 \pm 0.04$. These results suggest that the rate-limiting step in aminoacrylate formation is different for the two substrates, and that the reaction of L-tyrosine may be coupled with enzyme vibrations.

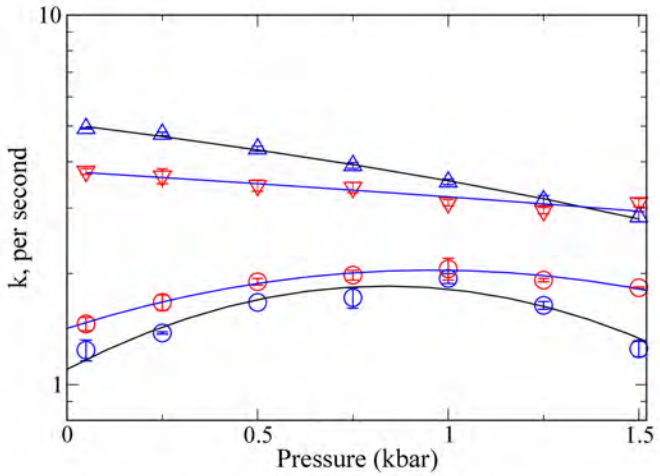

Figure 1. Effect of hydrostatic pressure on the formation of aminoacrylate intermediates of TPL. Blue circles, "light" TPL with L-tyrosine; red circles, "Heavy" TPL with L-tyrosine; blue triangles, "light" TPL with *S*-ethyl-L-cysteine; red triangles, "heavy" TPL with *S*-ethyl-L-cysteine.

P73

Getting a Grip on Protein Dynamics: Ligand Induced Conformational Changes in GT-B Enzymes

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Glycosyltransferases (GTs) are a highly diverse group of enzymes, with over 140 different EC activities reported in the Carbohydrate-Active enZYme (CaZY) database (www.cazy.org). GTs are responsible for the transfer of a sugar moiety onto another substrate to allow the biosynthesis or functionalization of glycans, proteins, lipids and natural products. The GT-B class of glycosyltransferases is of particular interest due to the divergence of the chemical reactions catalyzed (including enzymes that catalyze the addition of sugars with either inversion or retention mechanisms - based upon the stereochemical outcome of the anomeric carbon of the donor sugar) while having a conserved overall structure (of two Rossman-like domains connected by a linker region and no co-enzyme). Enzymes of the GT-B structural family have been observed to adopt both "open" and "closed" conformations, and while it is generally accepted that these two states interconvert during catalysis, the mechanisms controlling interconversion between these two states, as well as the kinetic nature of these transitions, is poorly understood. A collaboration was born from the shared interest in investigating these problems using evolutionarily distinct enzymes – the Heptosyltransferases of the GT-9 family and the GlcNAc transferases of the GT-4 family which includes MshA. These enzymes are collectively being characterized by circular dichroism, fluorescence, UV-vis spectrophotometric steady state kinetics, stop flow pre-steady state kinetics, isothermal titration calorimetry, and H/D exchange studies to allow for a full understanding of the nature of these enzymes and the trigger(s) for undergoing the open-to-closed transition. By studying MshA and the Heps, we hope to gain a better understanding of the importance of conserved dynamics for GT-Bs, and by extension, develop a strategy for developing potent GT inhibitors that disrupt protein dynamics.

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CONSERVED RESIDUES HAVE DIFFERENT ROLES IN HOMOLOGOUS OSBS ENZYMES

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Conserved amino acids are commonly presumed to have the same function in homologous proteins. However, the role of any amino acid is also tied to its structural context. To investigate how the roles of conserved amino acids depend on their structural context, we examined *o*-succinylbenzoate synthase enzymes from *Thermobifida fusca* (TfOSBS) and *Escherichia coli* (EcOSBS) that share only 22% sequence identity. Rapid divergence of these enzymes due to sizable insertions and deletions led to differences in active site size and electrostatic interactions. Analysis of the structural interactions between the two proteins, however, suggests that two conserved active site residues (R128/G254 in TfOSBS and R159/G288 in EcOSBS) play similar roles in substrate binding. Surprisingly, mutating these amino acids severely decreased the catalytic efficiency of EcOSBS, but was only mildly deleterious in TfOSBS. Instead, the G254A mutation in TfOSBS compromises thermostability, while G288A in EcOSBS does not affect thermostability. To determine whether this difference arises from the mesophilic versus thermophilic natures of the two species, we determined the effects of the same mutations in *Amycolatopsis mediterranei* OSBS (AmOSBS), a mesophilic Actinobacteria in the same subfamily as TfOSBS. The R124M and G252A mutations in AmOSBS had the same effects as the mutations in TfOSBS, indicating that the divergent roles of these amino acids stem from structural divergence of the two subfamilies, rather than adaptation to thermophilicity. Extending this analysis to other active site positions demonstrates that many other positions play different roles in thermostability and activity in the two enzymes. These differences are due not only to structural divergence between the enzymes, but also changes in conformational dynamics.

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Directed Evolution of NSAR Activity

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The fitness landscape of proteins that evolve new activities and the propensity to evolve along multiple or different mutational paths is not well understood. Promiscuous proteins, which have a second chemical activity that is biologically irrelevant, provide a suitable system to study protein evolution. Promiscuous and in cases biologically relevant NSAR (*N*-succinyl amino acid racemase) activity evolved in an ancestral OSBS (*o*-succinyl benzoate synthase) in the NSAR/OSBS enzyme subfamily and later evolved into a biologically relevant function. However, the amino acid substitutions and structural changes that were required to gain NSAR activity are not known. Given the relatively unknown nature of the fitness landscape and the large sequence diversity among NSAR/OSBS proteins, are there multiple mutational trajectories to gain NSAR activity? Furthermore, do these trajectories differ based on which protein sequence is used as the template? To answer these questions, a directed evolution experiment has been devised in which survival of an L-methionine auxotrophic strain requires the conversion of D-methionine to L-methionine using an NSAR-dependent metabolic pathway from *Geobacillus kaustophilus*. The mutations in the survivors will identify which residues are required for the gain of NSAR activity. Starting from different non-promiscuous protein templates, the mutational trajectory leading to increases in NSAR activity will be tracked by determining effects of mutations that affect NSAR activity in successive rounds of evolution. Collectively, these results will show how evolvable NSAR activity is in the NSAR/OSBS family and if it is possible have different mutational trajectories to evolve the same activity.

P76

THE IMPORTANT ROLE OF A SECOND-SHELL AMINO ACID IN DETERMINING *N*-SUCCINYLAMINO ACID RACEMASE REACTION SPECIFICITY

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Several lines of evidence show that catalytic promiscuity, which refers to the ability of an enzyme to catalyze more than one reaction in the same active site, plays a role in the evolution of new enzyme functions. Studying catalytic promiscuity can help identify structural features that predispose an enzyme to evolve new functions. Our research will address this problem using the catalytically promiscuous *N*-succinylamino acid racemase/*o*-succinylbenzoate synthase (NSAR/OSBS) subfamily, which is a branch of the OSBS family. We found that the residue R266 is conserved in the NSAR/OSBS subfamily, in which most members catalyze both NSAR and OSBS reactions. However, the homologous position is usually hydrophobic in other nonpromiscuous OSBS subfamilies, which lack NSAR activity. R266 is a second-shell amino acid that is close to the catalytic K263, but it does not contact the substrates, suggesting that R266 affects the catalytic mechanism, rather than substrate binding. Mutating R266 to glutamine in *Amycolatopsis* NSAR/OSBS reduces both NSAR and OSBS activities, but it is more deleterious for NSAR activity. Specifically, the R266Q mutant decreases the rate of proton exchange between the alpha proton of the NSAR substrate and the general acid/base K263 without affecting that of the other general acid/base K163. Preliminary analysis of the crystal structure of *Amycolatopsis* NSAR/OSBS R266Q shows that K263 forms a salt bridge with the conserved metal ligand D239, which normally forms a salt bridge with R266 in the wild type protein. This interaction reduces the acid/base reactivity of K263 in the NSAR reaction. However, this interaction is less deleterious for the OSBS reaction because K263 forms a cation-π interaction with the OSBS substrate and/or the intermediate, rather than acting as a general acid/base. Together, the data explain the switch in the reaction specificity by the R266Q mutant and show that R266 is important for determining NSAR reaction specificity.

P77

A New Flavoenzyme Catalyzed Baeyer-Villiger Type Rearrangement in the Bacterial Folate Catabolic Pathway

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Folic acid is an important cofactor which is present in cells as a family of structurally related derivatives made of 2-amino-4-hydroxypteridine linked through a methylene carbon to p-amino-benzoylpolyglutamate.[1] Folate mediated one-carbon metabolism occurs both in mitochondria and cytoplasm. Cytoplasmic folate mediated one-carbon metabolism is essential for the de novo synthesis of purines and thymidylate, and for methylation of homocysteine to methionine. Unlike most bacteria and yeast, mammals cannot synthesize folates *de novo*, therefore, folic acid has to be supplied through the diet to meet their daily requirements. Folate deficiency can result in many health problems. Pregnant women with folate deficiency are more likely to give birth to premature infants, and infants with neural tube defects.[2] Therefore, elucidating the biochemical mechanism of folate catabolism is critical for understanding the relationship between folate metabolism and folate deficiency diseases. I have identified a gene cluster in *Mesorhizobium loti* that catabolizes folic acid (Figure 1). Detailed biochemical and structural studies suggest a unique flavoenzyme mediated *Baeyer-Villiger* type rearrangement of an imine. Since the gene cluster responsible for the folate catabolism is widespread amongst bacteria, it is important to examine its presence in the human gut microbiome and its connection to diseases like neural tube defects. My future studies will be in that direction.

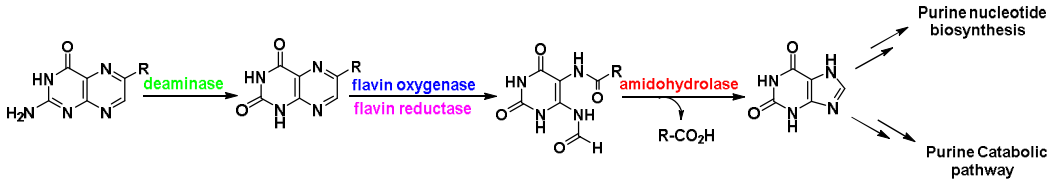

Figure 1: Folate catabolic pathway in *Mesorhizobium loti*

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P78

Expanding and applying the chemical potential of stereospecific vanadium-dependent haloperoxidases from *Streptomyces* bacteria

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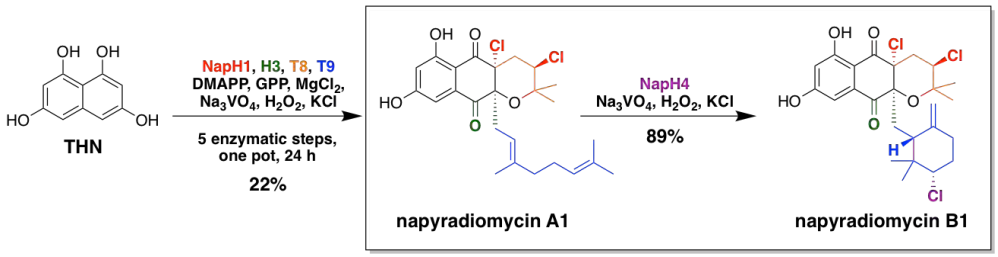
One of the most unique classes of enzymatic halogenases are the vanadium-dependent haloperoxidases (VHPOs), which catalyze a two-electron oxidation of aqueous halide ions in the presence of co-substrate hydrogen peroxide and vanadate (VO_4^{3-}). While algal and fungal VHPOs release diffusable hypohalous acid,[1] oxidized halenium ions are proposed to be enzymatically directed in *Streptomyces* homologs. This rationalizes the regio- and enantioselective halogenation reactions observed in actinomycete-derived meroterpenoid natural products. Recent efforts within our lab have uncovered fascinating and chemically diverse enzymology from this class of enzymes, including chloronium-induced etherifications,[2] α-hydroxyketone rearrangements,[3] and aromatic halogenation[4,5] reactions with high selectivities.We recently interrogated NapH4, a novel VHPO within the napyradiomycin biosynthetic gene cluster, that catalyzes a remarkably high yielding, diastereoselective, and stereospecific chloronium-induced terpenoid cyclization.[4] By establishing two stereocenters and a new carbon-carbon bond, NapH4 represents a novel biological activity for this enzyme family. Furthermore, we have successfully employed meroterpenoid VHPO and ABBA aromatic prenyltransferase enzymology in the total enzyme synthesis of chemically complex meroterpenoids, such as napyradiomycin B1, that have yet to be chemically synthesized.[4]



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P79

Discovery of an NRPS-like choline synthetase in fungi

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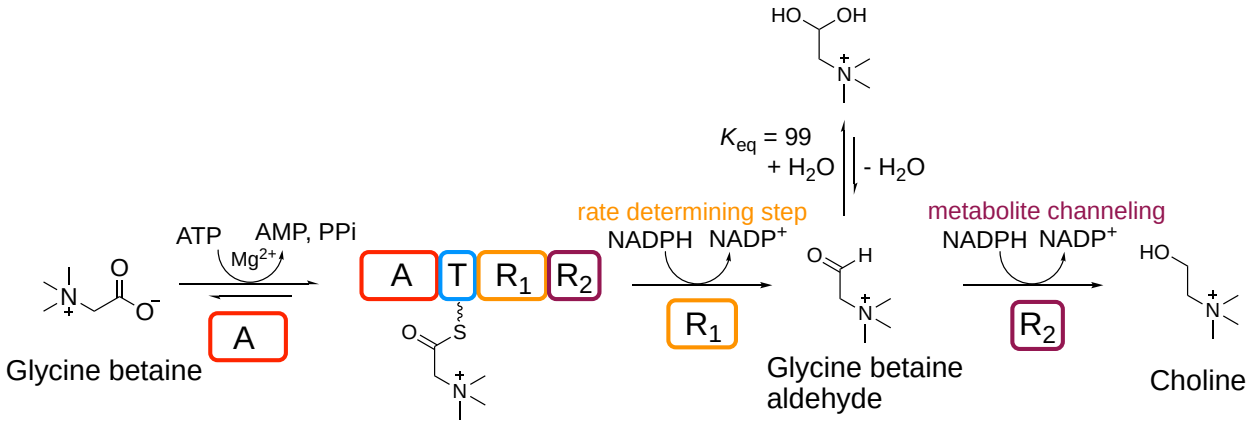
An orphan nonribosomal peptide synthetase (NRPS)-like protein (ATRR), with a unique domain architecture A-T-R_1-R_2 (A, adenylation domain; T, thiolation domain; R_1, thioester reductase domain; R_2, short chain dehydrogenase/reductase (SDR) domain) resembling that of carboxylic acid reductase A-T-R, is widely conserved among filamentous ascomycetes (*Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, etc). Using a structure-guided (homology model) approach integrating bioinformatics and mechanistic enzymology, we elucidated the function of ATRR as choline synthetase. The substrate glycine betaine, a compatible osmolyte, is activated A domain by ATP-dependent adenylation, followed by transthioesterification. The T-domain tethered intermediate is reduced at R_1 domain yielding glycine betaine aldehyde, which is channeled to the R_2 domain and further reduced into choline. Biochemical characterization suggests the substrate specificity is achieved by cation - π interaction. Discovery of this new enzyme reveals a hidden reductive pathway from glycine betaine to choline, shedding new light onto choline biogenesis in filamentous fungi.



P80

Structural, biochemical and genetic characterization of a unique NRPS protein AB826 from *A. baumannii*

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Acinetobacter baumannii is an emerging opportunistic bacterial pathogen with increasing incidences of highly drug resistant strains. *A. baumannii* along with five other pathogens form a group of "ESKAPE bugs" which have the potential to escape from commonly used antimicrobials. Exploration of unknown gene clusters from these bacteria is important to understand the physiology and pathogenesis as well as finding new drug targets. Herein, we investigated the genetic organization, structure and biochemical characterization of an unprecedented protein AB826 from *A. baumannii* strain 307. AB826 protein consists of a unique architecture with an N-terminal adenylation domain, a carrier domain and a C-terminal domain of unknown function (DUF). Crystal structure of the isolated adenylation domain was solved at 2.0 Å to investigate the substrate binding pocket. Adenylation domain did not adenylate any of the twenty proteogenic amino acid in biochemical assay which indicated AB826 may activate a novel precursor. Analysis of the AB826 gene cluster revealed its presence in many bacterial and fungal families but not in higher eukaryotes. Present investigation explores the function of AB826 protein in *A. baumannii* towards our goal of identifying its potential product.

P81

The structural basis of β-lactone formation catalyzed by a nonribosomal peptide synthetase

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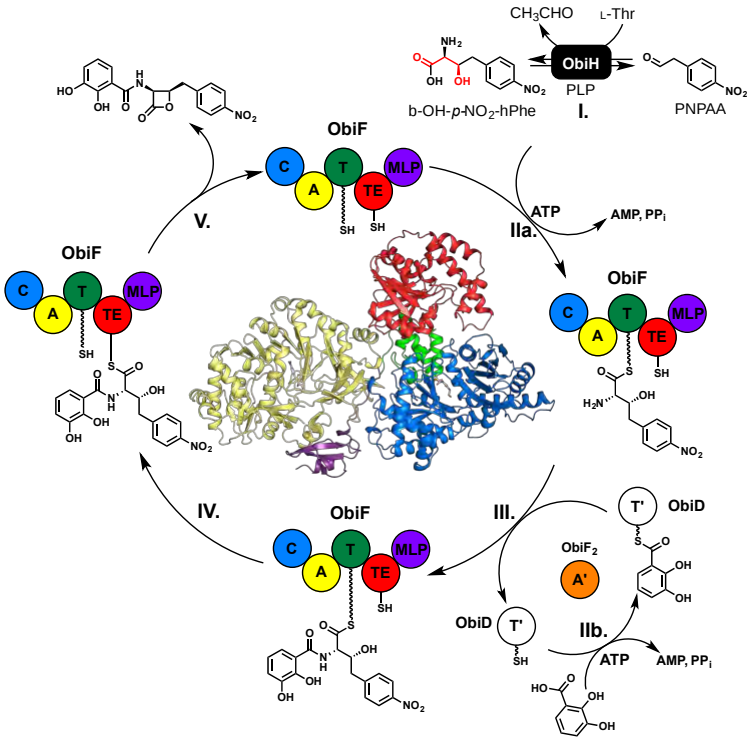
Non-ribosomal peptide synthetases (NRPSs) are modular, multidomain enzymes that produce many important natural products in an 'assembly line' fashion. Terminal NRPS modules often contain thioesterase (TE) domains that catalyze hydrolysis or cyclization reactions, which release the tethered natural product. β-Lactone natural products contain highly strained four-membered ring systems that make these compounds well-suited for the inhibition of hydrolases, transferases, ligases, and oxidoreductases. Recent biochemical and genetic studies have demonstrated that β-lactone formation in obafluorin, an *N*-acyl-α-amino-β-lactone with antimicrobial properties, is catalyzed by the TE domain of the *Pseudomonas fluoroscens* NRPS module ObiF. To establish the structural basis for this important ring-forming reaction and to identify key residues involved in this process we obtained an X-ray crystal structure of a homologous ObiF module from *Burkholderia diffusa*. The *B. diffusa* ObiF structure displays a novel domain architecture in addition to a TE domain that possesses a Cys-His-Asp catalytic triad configuration similar to that of a standalone, proof-reading TE domains. These newly identified, unexpected structural features should facilitate the identification of biosynthetic gene clusters which produce novel β-lactones.



P82

Resolving the Mechanism of an NRPS-Independent Siderophore Synthetase from Hypervirulent *Klebsiella pneumoniae*

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Klebsiella pneumoniae is an opportunistic human pathogen, affecting those with compromised immune systems, especially in hospitals or long-term care facilities. Alarmingly, this pathogen has gained new awareness due to its extreme drug resistance. In addition, a strain of hypervirulent *K. pneumoniae* (hvKP) emerged in the Asian Pacific Rim in the 1980s, infecting previously healthy and younger individuals. Hallmarks of hvKP include severe pneumonia, pyogenic liver abscess, and necrotizing fasciitis. These strains have additional virulence factors, including increased production of siderophores, which are small-molecules that chelate iron. Aerobactin is the NRPS-independent siderophore largely responsible for virulence in hvKP. Its biosynthetic components, regulated by the *iucABCD* genes, are thus attractive drug targets. Aerobactin contains two N^6-acetyl-N^6-hydroxylysine molecules, one ligated to each of the primary carboxyl groups of citrate. One of the synthetases of aerobactin, IucA, is known to catalyze the first ligation of an N^6-acetyl-N^6-hydroxylysine to citrate via an adenylated intermediate, but the rest of the mechanism remains unclear. Steady state kinetics have revealed the order of addition of substrates for IucA, and site-directed mutagenesis experiments have allowed us to probe the relevance of specific residues in the active site. Testing the 20 proteinogenic amino acids and derivatives of N^6-acetyl-N^6-hydroxylysine as nucleophiles with citrate and ATP resulted in distinct substrate specificity for IucA with its physiological substrate. In addition, a homolog of IucA from *Yersinia pseudotuberculosis* maintains nearly the same steady state kinetic values, illustrating the conservation of activity across bacterial species. Overall, this information guides the functional characterization of IucA and contributes insight to other NRPS-independent siderophore synthetases.

P83

Click Chemistry Approach for the Optimal Synthesis of Antibody-Drug Conjugates

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Since its conceptualization in 2001,[1] click chemistry has been utilized for widespread applications. However, initially the copper-catalyzed azide alkyne cycloaddtion (CuAAC) struggled to find many uses in bioconjugation due to the toxicity of Cu(I) and relatively low reaction rates. A variety of improvements have been introduced to adapt this reaction to biological systems including the use of strained alkynes,[2] copper-chelating azides,[3] and/or other copper-chelating ligands[4]. These efforts were aimed at speeding up the reaction while decreasing the Cu(I) toxicity (or totally removing the copper in the case of activated alkyne). In this study, we investigated kinetics of ligand-accelerated CuAAC reaction by reacting a copper-chelating azide and a non-chelating azide with excess amounts of alkyne in pseudo-first order reaction conditions. The results demonstrated the copper-azide binding process to be the rate-limiting step in the case of non-chelating azides. Furthermore, it was observed that alkynes with different electron donating and withdrawing properties did not alter the reaction rate significantly. These results indicated that reaction rate of CuAAC with non-chelating azides is not affected by alkyne concentration. Following this study, the reaction was applied to the synthesis of antibody drug conjugates (ADCs). A two-step conjugation strategy was devised (Figure 1). In the first step the azide moiety was installed onto antibody through reduced cysteines. In the second step, linker-drug molecules with alkyne moiety were used in ligand-accelerated CuAAC to form stable triazole linkages. In a cell-based study, the ADCs generated by this approach was shown to have similar efficacy and less side-toxicity with respect to the widely used maleimide conjugation technique. The next aim in the project is to use PEGylated drug-linker molecules for conjugation to improve the pharmokinetics of ADCs.

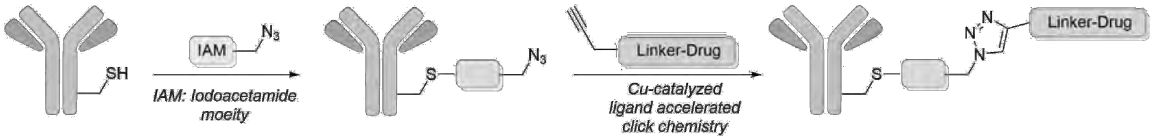

Figure 1 Two-step ADC Conjugation Strategy

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P84

LanCLs: Non-canonical glutathionylation enzymes in mammalian systems

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Lanthionine-containing peptides or lanthipeptides are ribosomally synthesized and post-translationally modified peptides (RiPPs) found in bacteria. The lanthionine rings are installed by consecutive dehydration and cyclization post-translational modifications (PTMs). These PTMs involves first dehydration of Ser/Thr residues on precursor peptides. Then, LanC enzymes (or LanC-like domains) mediate the cyclization of cysteine onto the dehydroamino acid residues. It is intriguing that mammalian genomes encodes for LanC-like proteins (LanCLs) despite the absence of an encoded dehydratase homolog. The protein structures of human LanCL1 and LanCL2 have been solved and resemble NisC (a bacterial LanC enzyme); both structures contain conserved two-layered alpha-helix barrels that bind Zn^{2+} and other putative active site residues. LanCL1 and 2 are ubiquitously expressed in various tissues but the expression pattern of LanCL3 still remains to be examined due to the lack of a specific antibody. In addition, human LanCL1 and LanCL2 have been shown to bind to glutathione (GSH) and LanCL1 possesses glutathione transferase activity by standard glutathione-S-transferase assays. Thus, we hypothesized that LanCLs have different functions or exploit different substrates in eukaryotic cells..

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P85

Dre2 and Nar1 are the partner proteins of the Nbp35-Cfd1 cytosolic iron sulfur cluster assembly scaffold

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The **c**ytosolic **i**ron sulfur cluster **a**ssembly (CIA) scaffold builds iron sulfur cluster cofactors for cytosolic and nuclear enzymes. In fungi and animals, the scaffold comprises two homologous ATPases, called Nbp35 and Cfd1 in yeast. We previously proposed that the role of ATP hydrolysis is to regulate interaction with at least one partner protein. Herein, we identify the Dre2 and Nar1 as Nbp35-Cfd1's partner proteins. Dre2 and Nar1 each independently bind to the Nbp35-Cfd1 complex with low micromolar affinities and stimulate its ATP hydrolysis activity more than 10-fold. Neither affects ATP hydrolysis activity of the Nbp35 or the Cfd1 homodimers. Our results establish that the CIA scaffold uses its ATPase domain to regulate dynamic interactions with its Dre2 and Nar1 partner proteins during assembly and transfer of its nascent FeS clusters.

P86

Probing the equilibrium conformational ensemble of transglutaminase 2 (TG2)

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A multifunctional protein's inherent flexibility, capacity to adopt multiple, stable conformations, and its ability to perform numerous, often disparately regulated, functions are inextricably linked[1-4]. As is the case for transglutaminase 2 (TG2), the "open" state conformation is associated with TG2's Ca^{2+}-dependent, transamidase activity[5], while the "closed" state conformation is associated with the mutually exclusive GTPase activity[6]. Purportedly under allosteric control, this functional conformational switch between "open" and "closed" states is proposed to be accompanied by both large-scale conformational changes and significant secondary structural rearrangements[5, 6]. The TG2 apoenzyme populates similar "open" and "closed" conformations at equilibrium; however, the structural architecture and dynamic properties of these highly populated apoenzyme conformations remains unknown. To begin understanding the dynamic nature of the TG2 apoenzyme, we have undertaken an extensive biophysical study of the denaturant-induced unfolding properties of TG2 WT and mutant forms. As previously shown to be important to protein dynamics[7-9], the interplay between structural rearrangements and protein folding/unfolding may be key to both realizing accessible TG2 apoenzyme conformations and defining TG2's inherent dynamic nature. Using a combination of site-directed mutagenesis, limited-proteolysis mass spectrometry, and denaturant unfolding studies, we have identified gross structural differences in the observed TG2 conformations populated at equilibrium; examined the differences in population distributions arising from destabilizing mutation incorporation; and finally, determined how GTP binding alters the population distribution and unfolding properties. By establishing the interplay among protein structural rearrangements, protein unfolding, and the equilibrium conformational ensemble of TG2, the studies presented here contribute to advancing our understanding of both the allosteric mechanisms regulating TG2 activities and the role protein unfolding/folding has in conformational switching and protein dynamics.

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Design, synthesis, and mechanistic evaluation of aminotransferase inhibitors for the treatment of epilepsy, addiction, and liver cancer.

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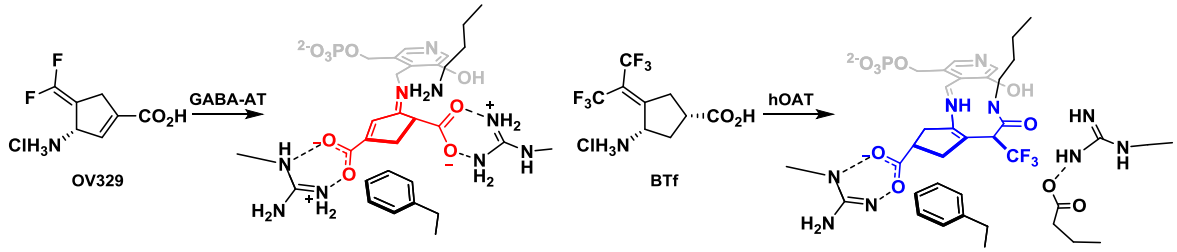
Epilepsy and addiction are diseases of the central nervous system. One cause for epilepsy is a drop in the concentration of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Raising GABA levels has been shown to terminate seizures. At the same time, raising GABA levels antagonizes the release of dopamine from the nucleus accumbens. To increase GABA concentrations, we look to inhibit the enzyme responsible for GABA degradation, GABA aminotransferase (GABA-AT) through the use of mechanism-based enzyme inactivators (MBEIs). MBEIs are inactive compounds, which are activated by an enzyme's normal mechanistic machinery to form reactive intermediates. Using knowledge of the mechanism of action of previous GABA-AT inhibitors, we developed **OV329**, which was determined to be 10 times more potent than known inhibitors of GABA-AT. Through crystallography, we determined that **OV329** inactivates GABA-AT through hydrolysis of the difluoromethylene group. **OV329** was shown to block the release of dopamine in a mouse model of addiction and reduce seizures.

Over the course of the previous studies, inhibitors were assayed against a second aminotransferase enzyme, ornithine aminotransferase (hOAT). Inhibition of hOAT has been shown as a possible treatment of hepatocellular carcinoma, the most common form of liver cancer. One particular inhibitor, (1S,3S)-3-amino-4-(perfluoropropan-2-ylidene)cyclopentane-1-carboxylic acid hydrochloride (**BTf**), was selective for hOAT over GABA-AT. Through crystallography, proteomics, molecular modeling, and targeted synthesis, we show that **BTf** inactivates hOAT after fluoride elimination and attack by an active site lysine.



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P88

New Insights on Nickel-Pincer Nucleotide Cofactor Biosynthesis and Function

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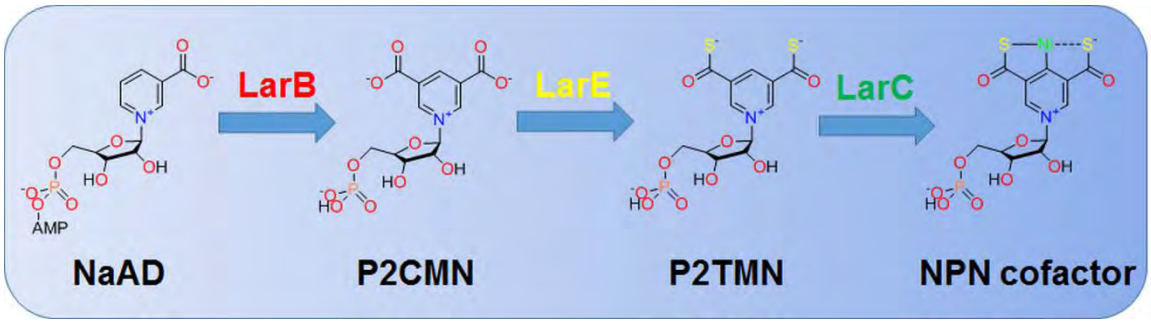
Lactate racemase from *Lactobacillus plantarum* contains a nickel-pincer nucleotide (NPN) cofactor covalently bound to Lys184 of the LarA protein.[1] This NPN cofactor is synthesized by a nicotinic acid adenine dinucleotide (NaAD) carboxylase/hydrolase (LarB),[2] an ATP-dependent sacrificial sulfur transferase (LarE),[3,4] and a CTP-dependent cyclometallase (LarC).[5] Here, we report new insights related to the biosynthesis and function of this coenzyme. We solved the structure of a LarA–like, non-lactate racemase protein of *Thermoanaerobacterium thermosaccharolyticum*, a thermophilic bacterium, for which the substrate is unknown. We obtained high quality datasets (3.3 Å resolution) of crystals for *L. plantarum* LarB, and its structure determination is in progress. We purified a multi-Cys LarE from a second thermophilic bacterium, *Thermotoga maritima*, and found it possesses a brown color and may contain an [4Fe4S] cluster. The mono-Cys $LarE_{Lp}$ acts stoichiometrically during NPN biosynthesis, and we propose $LarE_{Tm}$ functions catalytically. Additional new findings on NPN synthesis and function will be detailed.



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P89

DISULFIDE FORMATION KINETICS OF TWO-CYSTEINE PEROXIREDOXINS AND A MODEL FOR PEROXIDE SENSING

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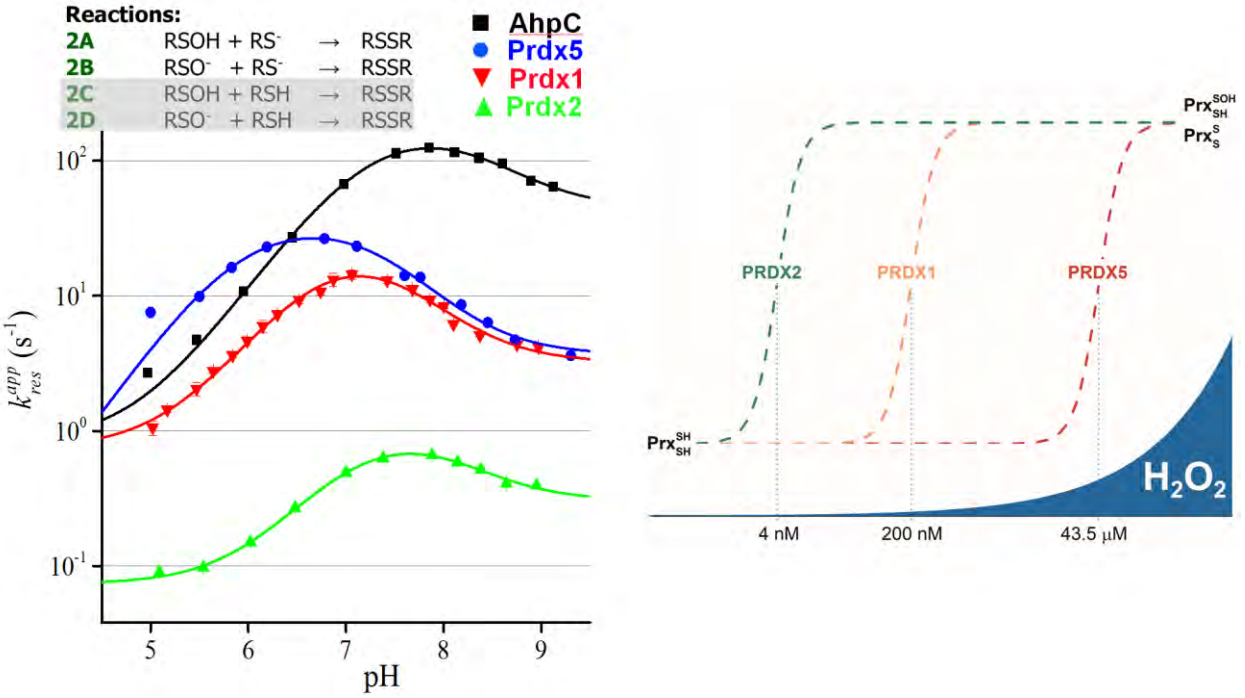
Two-cysteine peroxiredoxins (Prx) have a three-step catalytic cycle consisting of (1) reduction of peroxide and formation of sulfenic acid on the enzyme, (2) condensation of the sulfenic acid with a thiol to form disulfide, also known as resolution, and (3) reduction of the disulfide by a reductant protein. By following changes in protein fluorescence, we have studied the pH dependence of reaction 2 in human peroxiredoxins 1, 2 and 5, and in *Salmonella typhimurium* AhpC and obtained rate constants for the reaction and pK_a values of the thiol and sulfenic acid involved for each system. The observed reaction 2 rate constants span two orders of magnitude, but in all cases reaction 2 appears to be slow compared to the same reaction in small-molecule systems, making clear that the rates are limited by conformational features of the proteins. For each Prx, reaction 2 will become rate limiting at some critical steady-state concentration of H_2O_2 producing the accumulation of Prx as sulfenic acid. When this happens, an alternative and faster-resolving Prx (or other peroxidase) may take over the antioxidant role. The accumulation of sulfenic acid Prx at distinct concentrations of H_2O_2 is embedded in the kinetic limitations of the catalytic cycle, and may constitute the basis of a H_2O_2–mediated redox signal transduction pathway requiring neither inactivation nor posttranslational modification. The differences in the rate constants of resolution among Prx coexisting in the same compartment may partially explain their complementation in antioxidant function and stepwise sensing of H_2O_2 concentration.



Biochemistry 57, 3416-3424 (2018). PMID: 29553725

P90

Transition State Interrogation: *β*-glucosidase from *Thermotoga maritima*

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Tulane University

Glucosidases catalyze the breakdown of glycosides and oligosaccharides. Retaining glucosidases work through a double displacement mechanism, with formation of a glycosyl enzyme intermediate and subsequent hydrolysis. A number of studies have been aimed at more fully elucidating the mechanisms of these enzymes. In this work *β*-glucosidase from *Thermotoga maritima*, a hyper thermophilic bacterium, is investigated. A series of artificial phenolic substrates were synthesized covering a large range of leaving group pK_a values. Kinetic constants were determined for these substrates and a Brønsted Plot constructed. The plot for k_{cat} is biphasic demonstrating a clear change in the rate limiting step. Rates for substrates with a pK_a less than 8.4 show no dependence on the leaving group ability. While poorer substrates exhibit a negative slope (-0.40), indicative of negative charge buildup on the leaving phenol in the transition state. Plots of k_{cat}/K_M show no break, suggesting that chemical steps are limiting over the entire range observed. A change in the rate limiting step is also reflected in the temperature effects on the steady-state kinetics for individual substrates[1].

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P91

SUBSTITUTION OF METHYL GROUP WITH AMINO: NOVEL FLAVOENZYME CONVERTS VITAMIN INTO ANTIBIOTIC

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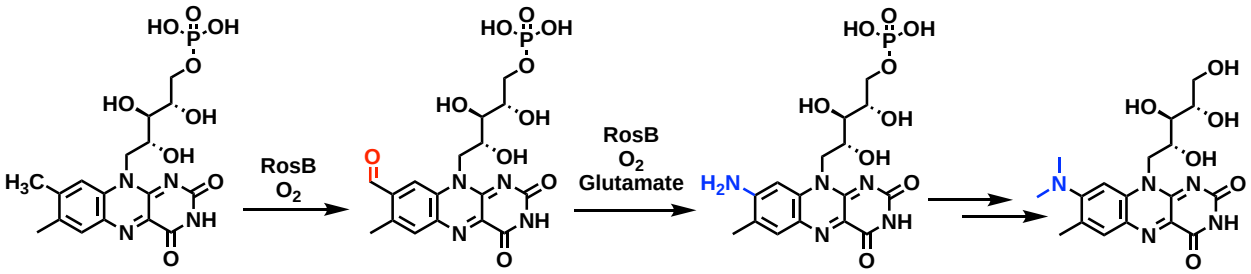
Roseoflavin is a bright red-colored anti-vitamin biosynthesized by *Streptomyces davawensis* and *Streptomyces cinnabarinus*. It is the only naturally occurring riboflavin analog that exhibits antibiotic properties. Earlier studies show that roseoflavin is biosynthesized from riboflavin itself by substitution of the C8 methyl with a dimethylamino group[1]. Recently, the methyltransferase catalyzing the final step has been characterized and a sub-genomic fragment containing genes necessary for the biosynthesis has also been cloned[2-3]. However, the biosynthesis of the key intermediate 8-amino-flavin remains largely unexplored. Herein, we report the identification and characterization of a single flavin-dependent enzyme, RosB that replaces the C8 methyl group of Flavin mononucleotide (FMN) and uses the amino group of glutamate to convert FMN to 8-amino-FMN via the intermediacy of 8-formyl-FMN[4]. The enzyme was cloned, overexpressed and purified, and subsequently tested for the predicted activity. A mechanism for the RosB-catalyzed reaction is proposed based on the identification of reaction products and intermediates. In the first step of this reaction, the enzyme oxidizes the C8 methyl group of FMN to a formyl group using molecular oxygen. Next, the intermediate, 8-formyl-FMN catalyzes the transfer of the amino group from the glutamate to itself in a fashion, similar to that observed for a PLP-dependent aminotransferase. The resulting reactive FMN intermediate then rearranges to yield 8-amino-FMN and the C8 methyl group is lost as formate. To gain further insights into this unprecedented amine transfer reaction various substrate analogs were designed and studied. Additionally, the crystal structure of the enzyme containing a bound inhibitor complemented the results of the biochemical analysis. Overall, this work reveals the existence of yet another incredible new flavin transformation involved in natural product biosynthesis.



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P92

STRUCTURAL PROPERTIES THAT PROMOTE CATALYSIS FOR ENZYMES INVOLVED IN SULFUR METABOLISM

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Sulfur metabolism is an essential metabolic process for bacterial survival. When sulfur is limiting in the environment, multiple enzymes are expressed that allow the organism to utilize alternative sulfur sources. Some of these enzymes are two-component systems that consist of a FMN reductase and monooxygenase that together catalyze the desulfonation of organosulfonate compounds. The FMN reductases and monooxygenases in these two-component systems have similar properties, and likely utilize similar mechanisms for desulfonation. The SsuE FMN reductase in the alkanesulfonate monooxygenase system contains a π-helix located at the tetramer interface that has been proposed to be generated by the insertion of a Tyr residue in a conserved α4 helix found in canonical flavin reductases.[1] An FMN reductase (MsuE) involved in methanesulfonate desulfonation contains a His residue (His126) at a comparable region as the Tyr residue in sequence alignments with SsuE. Therefore, FMN reductases involved in sulfur acquisition may have evolved with alternative insertional residues to generate the π-helix.

Initial studies were focused on evaluating the role of the Tyr118 π-helix insertional residue. Variants of Tyr118 were generated, to evaluate how these alterations affect the catalytic properties and structural integrity of the π-helix.[2,3] The structure of the Y118A SsuE π-helix was converted to an α-helix, similar to the FMN-bound members of the NADPH:FMN reductase family. Conversely, deletion of Try118 disrupted the secondary structural properties of the π-helix, generating a random coil region in the middle of the α4 helix. Structure-based sequence analysis identified a similar Tyr residue in a stable α4 helix of a FMN-bound reductase in the NADPH:FMN reductase family, suggesting that the insertional residue alone is not solely responsible for generating the π-helix. In addition to containing the π-helix, the MsuE FMN reductase involved in the desulfonation of methanesulfonates is structurally similar to SsuE in modeling studies. Functional analyses of MsuE and SsuE demonstrated that both enzymes were able to provide reduced flavin to the alternate monooxygenase though protein-protein interactions.[3] However, exchanging the π-helix insertional residue of each enzyme did not result in equivalent kinetic properties. Results from structural and functional studies of the FMN-dependent reductases suggest that additional structural adaptions occur to provide the altered gain of function associated with the π-helix.

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P93

An Opine on Opines: The Biosynthesis of Opine Metallophores in Bacterial Pathogens

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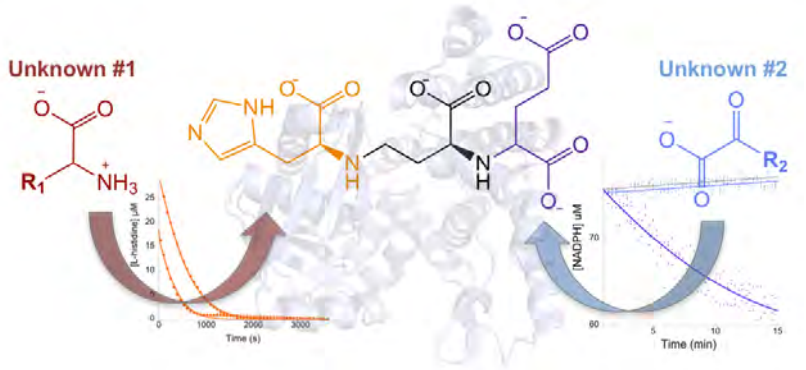
An uncharacterized biosynthetic operon found in pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Yersinia pestis* was proposed as the source of a novel metallophore in 2015[1]. Growing evidence demonstrates the important role this operon plays in metal acquisition and virulence, including in lung and burn wound infections (*P. aeruginosa*) and in blood and heart infections (*S. aureus*). In 2016, the *S. aureus* metallophore product was elucidated and named staphylopine[2]. In 2017, we identified the metallophore product in *P. aeruginosa*, naming it pseudopaline[3].

Staphylopine and pseudopaline are produced in two biosynthetic steps by a nicotianamine synthase and an opine dehydrogenase. We have examined substrate specificity and determined steady-state kinetic parameters for both nicotianamine synthase and opine dehydrogenase enzymes from multiple species, and have generated X-ray crystal structures and collected transient state kinetic data for the opine dehydrogenases. Our work provides the first kinetic analysis of a nicotianamine synthase and reveals a non-processive mechanism that is dependent on S-adenosyl-L-methionine and either L- or D-histidine. We currently have promising nicotianamine synthase crystals for upcoming diffraction studies. Importantly, only one nicotianamine synthase X-ray crystal structure, from an archeal species, has been solved to date. Our studies of opine dehydrogenases suggest varied substrate specificity. For example, *S. aureus* opine dehydrogenase incorporates pyruvate as an α-keto acid substrate and uses NADPH as a co-substrate, while *P. aeruginosa* opine dehydrogenase uses α-ketoglutarate and either NADH or NADPH. We have solved X-ray crystal structures for the opine dehydrogenases ranging from 1.9 - 2.5 Å revealing a structural basis for the NAD(P)H specificity[4]. We are currently completing a single turnover analysis of hydride transfer by the *S. aureus* opine dehydrogenase to complement our full steady-state analysis of both the forward and reverse reactions. This work contributes to our understanding of two enzymes involved in virulence that form a unique pathway generating a new metallophore class, the opine metallophores.



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P94

3,4-dihydroxy-2-butanone 4-phosphate synthase (RibB) of riboflavin biosynthesis has a mononuclear magnesium active site

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Riboflavin (vitamin B2) is a precursor for the synthesis of the cofactors FAD and FMN. Like other vitamins, riboflavin cannot be synthesized by mammals and must be obtained from the diet. Bacteria and plants synthesize riboflavin in five steps using the enzymes RibA-E. The focus of this study is the 3,4-dihydroxy-2-butanone 4-phosphate synthase, otherwise known as RibB. RibB is a magnesium dependent enzyme that catalyzes the conversion of the sugar ribulose-5-phosphate (Ru5P), a product of the pentose phosphate pathway, into 3,4-dihydroxy-2-butanone-4-phosphate (DHBP). The reaction catalyzed by RibB is an unusual deformylation reaction in which the fourth carbon of the five carbon sugar is removed as formate. Bacher and colleagues[1] have proposed that the mechanism is dependent on a skeletal rearrangement linking C3 and C5. We propose a fragmentation mechanism, in which the bond between carbons C3 and C4 is broken allowing formation of the new link between carbons C3 and C5 followed by release of formate. To test these competing hypotheses, we are using NMR and X-ray crystallography to observe the intermediates formed during catalysis. We have produced ^{13}C labeled Ru5P for use in NMR experiments and shown enzymatic formation of DHBP and formate. We also show the 1.1 Å structure of apo RibB. Previously reported product bound structures of RibB have been modeled with two metal ions in the active site[2]. However, we report here intrinsic tyrosine fluorescence and activity assay profiles that indicate that RibB requires a single magnesium for full activity.

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P95

Biosynthesis of the Capsular Polysaccharide Modifications found in *Campylobacter jejuni* NCTC 11168

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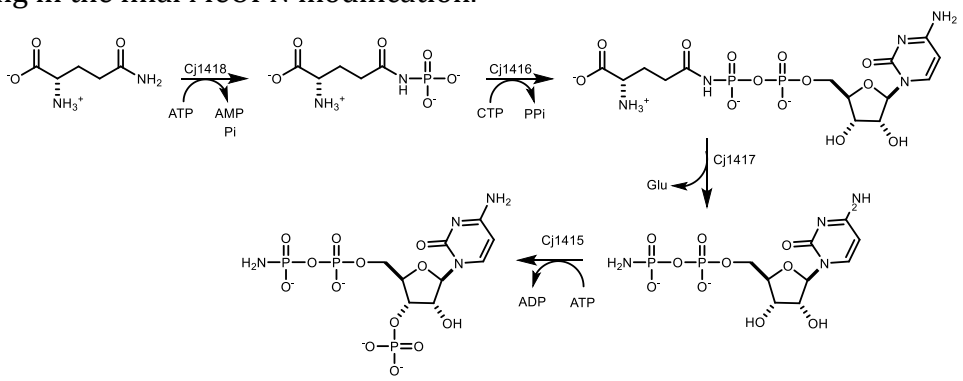
Campylobacter jejuni is a Gram-negative pathogenic bacterium that is the leading cause of gastroenteritis worldwide. Like many other Gram-negative bacteria, *C. jejuni* produces a capsular polysaccharide (CPS) that helps improve the overall fitness of the organism and contributes to its pathogenicity. Previously, the structure of the CPS from the NCTC 11168 strain was determined, and has revealed the presence of two unique modifications. The first modification is an aminoglycerol modification appended to glucoronate residues on the capsule at C-6. The other modification is an *O*-methyl phosphoramidate (MeOPN), that can be found on C-4 of a heptose and C-3 of an N-acetyl galactofuranose residue. Investigations into the role of MeOPN on the CPS suggest that it is involved in the evasion of immune responses, and serum resistance. Currently the biosynthesis of the aminoglycerol and MeOPN modifications found on the CPS are unknown.

We have characterized the first four enzymes involved in the biosynthesis of the phosphoramidate moiety of the MeOPN modification. The first enzyme, Cj1418, is a novel glutamine kinase that catalyzes the ATP dependent phosphorylation of the amide nitrogen of L-glutamine, resulting in L-glutamine phosphate. Next, the nucleotidyl transferase, Cj1416 uses L-glutamine phosphate to displace pyrophosphate from CTP, forming CDP-L-glutamine. CDP-L-glutamine is then hydrolyzed by Cj1417, releasing glutamate and cytidine diphosphoramidate. Cj1415 catalyzes the phosphorylation of the 3'-hydroxyl group of cytidine diphosphoramidate. The resulting cofactor is very similar to the 3'-phosphoadenylyl sulfate (PAPS) that is used by many biological systems for the transfer of sulfate. Presumably 3'-phophosphocytidine diphosphoramidate is used to transfer the phosphoramidate moiety to the capsule, and then methylated resulting in the final MeOPN modification.



P96

Deciphering the Enzymatic Function of the Bovine Enteric Infection Related Protein YfeJ from *Salmonella enterica* serotype Typhimurium

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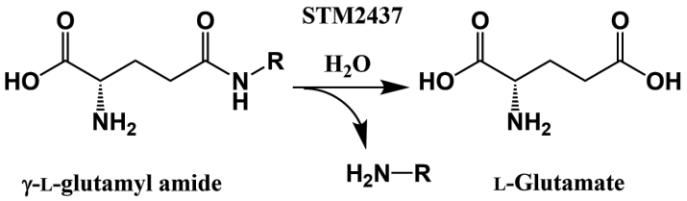
Non-typhoidal *Salmonella*, which can colonize the gastrointestinal system of cattle, cause significant food-borne bacterial diseases in humans. The use of a library of single-gene deletions (SGD) in *Salmonella enterica* serotype Typhimurium allowed identification of several proteins that are under selection in the intestine of cattle. *STM2437* (*yfeJ*) encodes one of these proteins and it is currently annotated as a Type I glutamine amidotransferase. STM2437 was purified to homogeneity and its catalytic properties with a wide range of γ-glutamyl derivatives were determined. Catalytic efficiency toward the hydrolysis of L-glutamine was extremely weak with a value of k_{cat}/K_m of 20 M^{-1} s^{-1}. γ-L-Glutamyl hydroxamate was identified as the best substrate for STM2437, with a value of k_{cat}/K_m of 9.6 x 10^4 M^{-1} s^{-1}. A homology model of STM2437 was built based on the known crystal structure of a protein of unknown function (PDB id: 3L7N) and γ-L-glutamyl hydroxamate was docked into the active site based on the binding of L-glutamine in the active site of carbamoyl phosphate synthetase. Acivicin was shown to inactivate the enzyme by reaction with the active site cysteine residue and the subsequent loss of HCl. Mutation of Cys91 to serine completely abolished catalytic activity.



P97

Elucidating the Mechanism of Enzymes Utilized During the Bacterial Degradation of Lignin in the Protocatechuate 4,5-Cleavage Pathway

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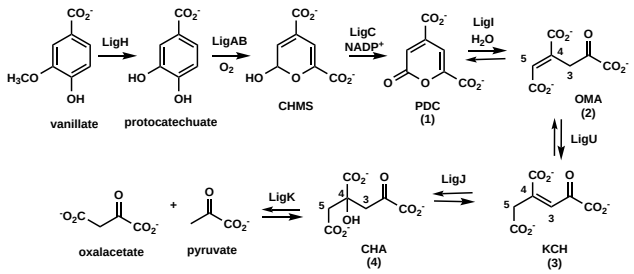
Lignin is the most abundant aromatic bio-polymer in the world. The enzymes LigU and LigJ from the soil bacterium *Sphingobium* sp. SYK-6 play an important role in the degradation of the lignin monomer unit guaiacyl in the protocatechuate 4,5-cleavage pathway, but their structures and mechanistic details have not been elucidated. Previous studies have shown that guaiacyl is converted into vanillate, which is demethylated to protocatechuate and then further metabolized via the 4,5-cleavage pathway to pyruvate and oxaloacetate by the combined actions of enzymes LigAB, LigC, LigI, LigU, LigJ, and LigK. Using NMR spectroscopy LigU was shown to function as a 1,3-allylic isomerase where the double bond between carbons 5 and 4 of (4E)-oxalomesaconate (**2**) migrates to carbons 4 and 3 forming (3*Z*)-2-keto-4-carboxy-3-hexenedioate (**3**). We determined the crystal structure of LigU in the absence of bound ligands in the active site to a resolution of 2.5 Å. LigU is structurally related to enzymes with the diaminopimelate epimerase fold and is composed of a central α-helix that is wrapped by antiparallel β-strands to make a β-barrel. The kinetic constants for the isomerization of OMA (**2**) to KCH (**3**) by LigU at pH 8.0 are 1300 s^{-1}, 170 μM, and 7.7 x 10^6 M^{-1} s^{-1} for k_{cat}, K_M, and k_{cat}/K_M, respectively. We have determined the three-dimensional structures of the apo-form, substrate, and product bound structures of the LigJ hydratase at 2.20 Å, 1.65 Å, and 2.02 Å resolution, respectively, by x-ray crystallography. The structure of the product bound complex shows that CHA, 4-carboxy-4-hydroxy-2-oxoadipate (**4**) is formed in the S-configuration. The kinetic constants for the hydration of KCH (**3**) to CHA (**4**) by LigJ at pH 8.0 are 25 s^{-1}, 10 μM, and 2.6 x 10^6 M^{-1} s^{-1} for k_{cat}, K_M, and k_{cat}/K_M, respectively. The mutant E284Q has no observable catalytic activity and this mutant was used to co-crystallize the substrate, KCH (**3**) with LigJ. Based on the kinetic parameters and the structure of the bound substrate in the active site, we propose that Glu-284 initiates the hydration reaction by abstraction of a proton from a bound water molecule that attacks C4 of KCH (**3**). Glu-284 then functions as a general acid to protonate C3 with an overall syn-addition of water to the C4/C3 double bond.



P98

Mechanistic Characterization of *Sb*-PTE a Phosphotreisterase from *Sphingobium* sp. TCM1 Capable of Hydrolyzing Flame Retardants

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The phosphotriesterase from *Sphingobium* sp.-TCM1 (*Sb*-PTE) is unique among phosphotriesterase enzymes in that it is able to hydrolyze organophosphorus flame retardants (OPFR). While not neurotoxins, OPFR are toxic compounds commonly used as additives in plastics and durable foam products with wide spread environmental contamination. OPFR are not substrates for typical phosphotriesterase enzymes due to a lack of an activated leaving group. The reaction catalyzed by Sb-PTE shows little dependence on the leaving group with only about a 2-fold difference in catalytic rates between a phenyl and *p*-nitrophenyl leaving group. Additionally, Sb-PTE is able to cleave any one of the three ester bonds present in a substrate including cleavage of simple alkyl groups. In some substrates the cleavage of a methyl group can proceed at the same rate as *p*-nitrophenyl cleavage. To probe the mechanistic aspects of *Sb*-PTE which gives rise to these abilities, a Bronsted analysis was carried out using a series of diethyl phosphotriesters with substituted phenols and a series dibutyl phosphotriesters with phenyl or activated butyl groups. It was found that there is very little dependence on the leaving group p*Ka* ($\beta = -0.3$) and that there is no upper plateau at low pK_a values suggesting that the chemical step is rate limiting for all substrates. Heavy atom isotope effects with ^{18}O in both bridging and non-bridging positions are in agreement with this finding. Solvent viscosity effects demonstrate that the low dependence on pK_a is not the result of forward commitment and confirms that the chemical step is rate limiting. The solvent kinetic isotope effects in D_2O were found to be unity, demonstrating that proton transfer to the leaving group is not rate limiting even with simple alkyl leaving groups. These findings suggest that Sb-PTE catalyzed reaction may have a much earlier transition state from that seen in the reaction catalyzed by the phosphotriesterase from Pseudomonas diminuta, despite very similar metal centers.

P99

Investigation of blunt end joining by DNA ligases

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While T4 DNA ligase is frequently used to join blunt-ended DNA fragments in modern molecular biology protocols, few mechanistic details of blunt-end ligation have been reported. To further elucidate the mechanism of blunt end joining, we investigated the substrate specificity of DNA ligases in this challenging reaction using several approaches. First, wild-type DNA ligases (phage T3, T4, and T7 DNA ligases, *Paramecium bursaria* chlorella virus 1 (PBCV1) DNA ligase, human DNA ligase 3, and *Escherichia coli* DNA ligase) were tested for their ability to ligate DNA fragments with several difficult to ligate end structures (blunt-ended termini, 3′- and 5′-single base overhangs, and 5′-two base overhangs). We found significant differences in end structure preference between ligases and observed no correlation of ligase activity on blunt DNA ends with their activity on single base overhangs. In addition, DNA binding domains were fused to PBCV1 DNA ligase to explore whether modified binding to DNA would lead to greater activity on these difficult to ligate substrates. These engineered ligases indeed showed both an increased binding affinity for DNA and increased activity, but did not alter the relative substrate preferences of the ligase, indicating active site structure plays a role in determining substrate preference. In addition to end structure preferences, we investigated the tolerance for nonstandard nucleobases in blunt end joining. We tested sugar, backbone, and nucleobase modifications in a variety of sequence contexts and found varying degrees of inhibition on blunt end ligation. Finally, we used a Pacific Biosciences single molecule sequencing assay to examine the sequence specificity of blunt-end joining. We observed a pairwise bias across the ligation junction, with purines preferentially ligating across from purines and pyrimidines preferentially ligating across from pyrimidines. This result suggests a role for base-stacking in stabilization of the ternary complex necessary for blunt ligation.

P100

Kinetic Characterization of an Ultra-high Fidelity DNA Polymerase

Kinjal Dave, Andrew Gray and Nicole Nichols

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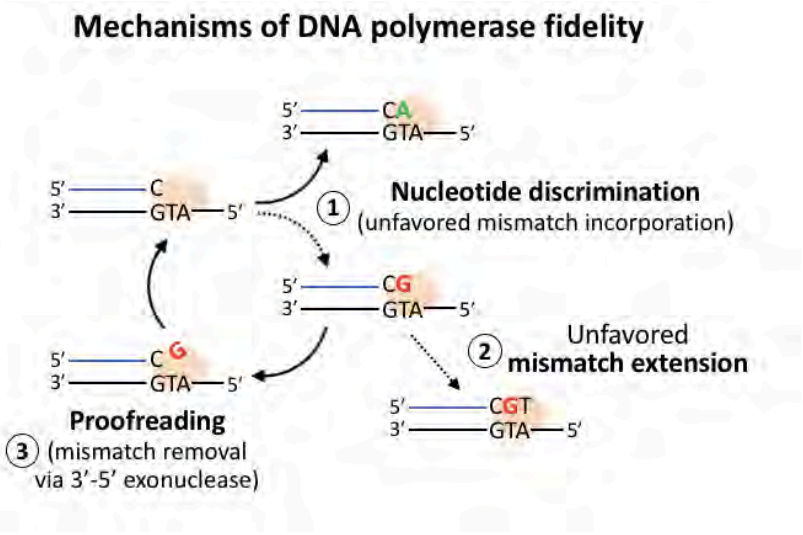
Accuracy during DNA replication is essential for maintaining genetic integrity and viability in all living organisms. DNA polymerases achieve this via several mechanisms, including selective incorporation of correct nucleotides (nucleotide discrimination), delayed synthesis following incorporation of incorrect nucleotides (low rate of mismatch extension), and removal of misincorporated nucleotides via 3'-5' exonuclease activity (proofreading). These activities act synergistically to ensure faithful replication, both *in vivo* and for purified DNA polymerases in *in vitro* methods such as PCR, cloning, library amplification for Next Generation Sequencing (NGS), and molecular diagnostics for cancer and other diseases. Understanding polymerase fidelity mechanisms thus carries significance for these and other applications in biotechnology and medicine. Q5 DNA Polymerase is a commercially available, engineered polymerase belonging to the B family of DNA polymerases. It has robust 3'-5' exonuclease (proofreading) activity and single-molecule sequencing studies indicate that it has ~280-fold higher fidelity than *Taq* DNA polymerase. While widely used as an ultra-high fidelity DNA polymerase, however, how Q5 achieves this high fidelity has not been previously described. In this work, we report both steady state and pre-steady state kinetic studies of Q5 including rates of correct and incorrect nucleotide incorporation, 3'-5' exonuclease activity, and mismatch extension. Parallel kinetic studies using Vent DNA polymerase, a Family B DNA polymerase with lower fidelity, are shown to highlight the unique properties of Q5 that likely contribute to its high fidelity.



P101

Discovery and Characterization of Isethionate Sulfite-Lyase, a C–S Bond-Cleaving Glycyl Radical Enzyme

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Glycyl radical enzymes (GREs) are highly abundant in anaerobic microbial communities and possess great functional diversity[1]. GREs contain a conserved glycine radical that is post-translationally installed with a partner activating enzyme, or activase. The activase belongs to the radical *S*-adenosylmethionine (SAM) enzyme family. The activase contains at least one [4Fe-4S] cluster and uses SAM to generate a co-factor-based radical species that is then capable of generating a radical on the active site glycine of the GRE. GREs have been linked to production of disease-associated microbial metabolites, DNA synthesis pathways, and carbohydrate metabolism[2]. However, the majority of GREs remain uncharacterized, making further study compelling. Here we present efforts to elucidate the function and study the biochemical properties of the GRE isethionate sulfite-lyase (IslA) from *Bilophila wadsworthia*. While it was known that *B. wadsworthia* is able to use taurine as a sole terminal electron acceptor, the metabolic pathway was incomplete. Proteomics experiments identified IslA as a candidate enzyme involved in taurine and isethionate metabolism in *B. wadsworthia*. Biochemical and microbiological tools were used to demonstrate that IslA can cleave isethionate into acetaldehyde and sulfite. Sulfite can then be used in respiration as a terminal electron acceptor[3]. This is the first biochemically confirmed C–S bond cleavage by a GRE, adding novel functional diversity to this enzyme family[4]. Additionally, we have used structural information to better understand this new chemical functionality. We hope to both elucidate how IslA allows *B. wadsworthia* to access terminal electron acceptors in the anaerobic gut environment, and also how to predict what other uncharacterized GREs may cleave C–S bonds.

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P102

Synthetic Cannabinoid Biology

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Cannabinoids are compounds originally derived from the plant, *Cannabis sativa* that binds to cannabinoid receptors such as CB_1 and CB_2 in the human body. They have been used as a therapeutic herb for centuries due to their bioactivity. However, research into their therapeutic potential has been largely implicated by social and legal issues. Recent developments in cannabinoids legalization has seen a rapid expansion in their production for therapeutic and research use. Currently, cultivation of the plant, *C. sativa* supplies majority of the demand for cannabinoids in the market. This "green rush" presents a new set of challenges such as environmental and cost issues, variable and unspecific products generated leading to problems such as substance abuse due to the psychoactive components produced as a mixture in plant extracts. We propose an alternative method of heterologous production using micro-organisms that promises to be more environmentally sustainable, less costly and a high selectivity for specific products. Through this, many minor cannabinoids previously produced in low yields in *C.* sativa may be engineered to be produced in high yields. Preliminary results conducted in this study suggest the feasibility of engineering micro-organisms such as the bacteria, *Escherichia coli* and the yeast strain, *Saccharomyces cerevisiae* in expressing the heterologous biosynthetic pathway and producing specific cannabinoids. Additionally, novel and unnatural cannabinoids can also be generated by exploiting specific "branch-points" in the biosynthetic pathway that can be subsequently tested for bioactivity and therapeutic potential. Using precursor-directed combinatorial biosynthesis, novel and unnatural cannabinoid precursors were produced in this study. Other methods of diversifying the cannabinoid library will include protein engineering and using orthologues identified using computational and bioinformatics tools.

P103

Structural and biochemical insights into the catalytic mechanism of class I fumarate hydratases

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Here we describe a novel [4Fe-4S] cluster-containing metalloprotein: cytosolic fumarate hydratase from *Leishmania major* (LmFH-2). Like aconitase, it uses a [4Fe-4S] cluster to coordinate substrate, but is not structurally similar to aconitase. In fact, LmFH-2 has a unique protein fold. Importantly, metal-dependent FHs are found in parasites that cause Neglected Tropical Diseases (NTD), such as leishmaniases, Chagas disease and sleeping sickness, whereas humans employ a metal-independent FH. NTDs affect approximately one-sixth of the world's population, and due to the appearance of resistance to current medicines, there is an increased interest in the development of innovative strategies to combat NTD. To explore the potential of FHs as anti-NTD targets, we have determined a series of structures of *Leishmania major* FH, including a structure with the substrate malate bound to the unique iron of the cluster. This structure, site-directed mutagenesis and kinetics provide insights into the catalytic mechanism of the class I (metal-dependent) FHs, a mechanism that is likely to be conserved among FHs from NTD-causing organisms.

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P104

Active-Site Brønsted Acid-base Catalysts Destabilize Mandelate Racemase and Related Subgroup Enzymes*

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Enzymes of the enolase superfamily (ENS) are mechanistically diverse, yet share a common partial reaction, i.e., the metal-assisted, Brønsted base-catalyzed abstraction of the α-proton from a carboxylate substrate to form an enol(ate) intermediate. The catalytic machinery responsible for the deprotonation reaction has been conserved, while divergent evolution has led to numerous ENS members that catalyze different overall reactions.[1] The ENS is divided into seven subgroups based on the identities of the Brønsted acid-base catalysts and the ligands of the metal ion.[2] The active site of the mandelate racemase (MR) subgroup contains an H-bonded Asp-His dyad with the Asp and His located at the ends of the sixth and seventh β-strands, respectively, and a Brønsted acid-base group (e.g., Lys, Arg, His, Tyr) that is often present at the end of the second β-strand.[3] Using differential scanning calorimetry, we have examined the thermostability (T_m) of four members of the MR subgroup: MR, D-tartrate dehydratase, L-talarate/galactarate dehydratase, and L-fuconate dehydratase. Substitution of the active-site Lys by Met in all four enzymes increased the kinetic stability of the folded state with the effect being most prominent in MR (ΔT_m = 8.6 °C). Substitution of the active-site His by Asn also had a stabilizing effect on MR, but not on the other enzymes. The thermostability of wild-type MR was increased in the presence of the substrate analogue (*S*)-atrolactate and the transition state analogue inhibitor benzohydroxamate. K166M and K166A MRs both exhibited enhanced thermostability (T_m = 72.5 °C), relative to wild-type MR (T_m = 63.9 °C), while restoration of the positive charge at position 166 by introduction of an Arg reduced the observed T_m value to a value (64.2 °C) similar to that of wild-type MR. These findings suggest that the active sites of MR subgroup enzymes are destabilized by the Lys Brønsted acid-base catalyst.

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P105

The Dynamic Conformational Landscapes of the Protein Methyltransferase SETD8

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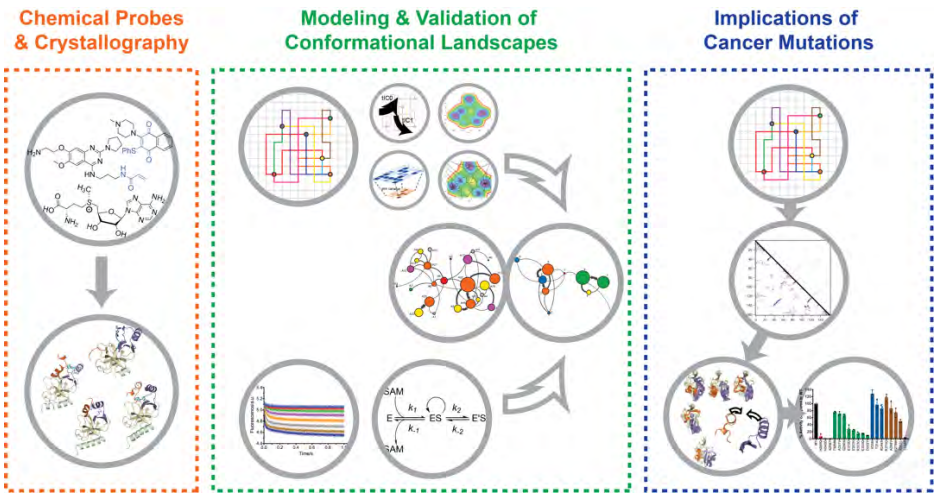
Elucidating conformational heterogeneity of proteins is essential for understanding protein functions and developing exogenous ligands for chemical perturbation. While structural biology methods can provide atomic details of static protein structures, these approaches cannot in general resolve less populated, functionally relevant conformations and uncover conformational kinetics. Here we demonstrate a new paradigm for illuminating dynamic conformational landscapes of target proteins. SETD8 (Pr-SET7/SET8/KMT5A) is a biologically relevant protein lysine methyltransferase for *in vivo* monomethylation of histone H4 lysine 20 and nonhistone targets. Utilizing covalent chemical inhibitors and depleting native ligands to trap hidden high-energy conformational states, we obtained diverse novel X-ray structures of SETD8. These structures were used to seed massively distributed molecular simulations that generated six milliseconds of trajectory data of SETD8 in the presence or absence of its cofactor. We used an automated machine learning approach to reveal slow conformational motions and thus distinct conformational states of SETD8, and validated the resulting dynamic conformational landscapes with multiple biophysical methods. The resulting models provide unprecedented mechanistic insight into how protein dynamics plays a role in SAM binding and thus catalysis, and how this function can be modulated by diverse cancer-associated mutants. These findings set up the foundation for revealing enzymatic mechanisms and developing inhibitors in the context of conformational landscapes of target proteins.



Reference: S. Chen et al., bioRxiv (2018), doi:10.1101/438994.

P106

ASSEMBLY OF SYNTHETIC BACTERIAL MICROCOMPARTMENTS IN YEAST

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Abstract

Bacterial microcompartments (MCPs) are prokaryotic organelles constructed from self-assembling protein units and are found in some bacteria species to aid in niche metabolic processes. Bacterial MCPs permit entry of small, polar molecules while sequestering hydrophobic and potentially toxic metabolites, making them orthogonal to lipid-enclosed eukaryotic organelles in terms of chemical selectivity. We wish to investigate if bacterial MCPs can be assembled in a eukaryotic cell as a synthetic bio-nanoreactor. We report that the carboxysome, a type of MCP found in cyanobacteria to improve carbon fixation, can be expressed in the yeast Saccharomyces cerevisiae, which is a popular eukaryotic cell factory. A hierarchical DNA assembly system was adopted and expanded to facilitate rapid building and testing of MCP expression cassettes. Biophysical characterization involving both fluorescence and electron microscopy indicate that the various shell proteins have assembled in yeast to form electron-dense structures resembling β-carboxysomes. Our work demonstrates that synthetic MCPs may serve as novel platforms for exerting spatial control of biochemical processes.

P107

Glutamine 451 Confers Sensitivity to Oxidative Inhibition and Heme-Thiolate Sulfenylation of Rabbit Cytochrome P450 4B1

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Human cytochrome P450 (P450) Family 4 enzymes and other drug metabolizing P450s are redox sensitive, showing a loss of activity resulting from preincubation with H_2O_2 and recovery with mild reducing agents (1,2). This inhibition is due to sulfenylation (Cys-SOH) of the heme-thiolate ligand, as determined by chemopreoteomic and spectral techniques (1). This phenomenon may have implications in observed disease-drug interactions where increases in drug half-lives of P450 substrates occurred in patients with inflammatory diseases (e.g., influenza and autoimmunity). Interestingly, human P450 1A2 was determined to be redox insensitive. To determine the mechanism behind this redox sensitivity, molecular dynamics (MD) simulations were employed using the crystal structure of rabbit P450 4B1 (3,4). Mouse P450 4b1 was found to be sulfenylated in kidney microsomes and shares 85% identity with its human and rabbit homologs. This includes an identical amino acid sequence surrounding the heme-thiolate cysteine. In simulating either the thiolate ($Cys\text{-}S^-$) or the sulfenic acid at the heme-ligation site, MD revealed Glu-451 in either an "open" or "closed" conformation, respectively, between the cytosol and heme-thiolate cysteine. Mutation to either a leucine (Q451L) or glutamate (Q451E) abrogated the redox sensitivity, suggesting that this "open" conformation allows for reduction of the sulfenic acid and thiolate religation to the heme iron.

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P108

Synthetic Alkaloid Enzymology: Characterization of Clavine Oxidases (CloA)

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Abstract

Ergot alkaloids are amino acid-derived nitrogen-containing compounds that are synthesized primarily by filamentous fungi in the phylum *Ascomycota*. These compounds share similar chemical structures to neurotransmitters and can act on the same receptors with varying effects (1). The ability of ergot alkaloids to interact with these receptors underlines their importance as pharmacologically important drugs for the treatment of neurological disorders such as Parkinson's, Alzheimer's and migraines. Most ergot alkaloid drugs are semi-synthetically derived from the compounds lysergic acid and lysergol, which makes them valuable precursors for the development of novel ergot alkaloid drugs (2). Clavine Oxidase (CloA) is a putative cytochrome P450 identified in the ergot alkaloid biosynthesis pathway and a key enzyme that catalyses the formation of lysergic acid from the precursor alkaloid agroclavine (3).

We show for the first time that CloA from *Claviceps purpurea* and homologs can be functionally expressed in *Saccharomyces cerevisiae* with varying activities and discuss the strategies for the characterisation of CloA substrate binding and activity. The knowledge gained from this study would be useful for the engineering of CloA for improved catalytic efficiency as well as potentially diversifying the ergot alkaloid pathway for the synthesis of novel ergot alkaloid compounds.

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P109

DYNAMIC CHARACTERISTICS OF GMP REDUCTASE COMPLEXES REVEALED BY HIGH RESOLUTION ^{31}P FIELD CYCLING NMR RELAXOMETRY

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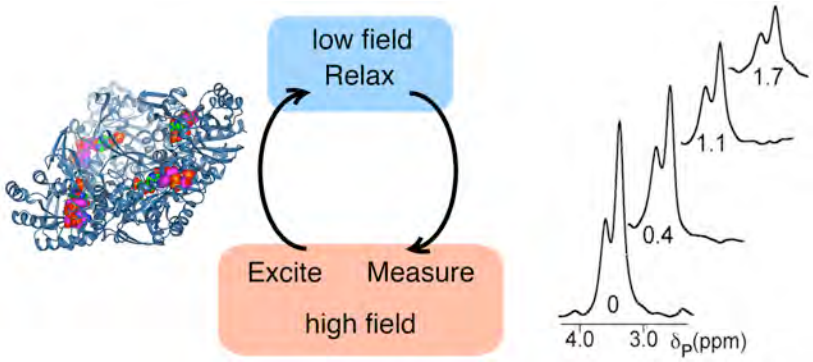
We used an underappreciated NMR technique, subtesla high resolution field cycling ^{31}P NMR relaxometry, to interrogate the dynamics of enzyme bound substrates and cofactors in guanosine-5'-monophosphate reductase (GMPR). These experiments reveal distinct binding modes and dynamic profiles associated with the ^{31}P nuclei in the Michaelis complexes for the deamination and hydride transfer steps of the catalytic cycle. Importantly, the substrate is constrained and the cofactor is more dynamic in the deamination complex E•GMP•NADP$^+$, while the substrate is more dynamic and the cofactor is constrained in the hydride transfer complex E•IMP•NADP$^+$. dIMP and dGMP are poor substrates, and the dynamics of the cofactor complexes of dGMP/dIMP are disregulated relative to GMP/IMP. The substrate 2'-OH interacts with Asp219. Counterintuitively, loss of Asp219 makes both substrates and cofactors less dynamic. These observations suggest that the interactions between the substrate 2'-OH and Asp219 coordinate the dynamic properties of the Michaelis complexes, and these dynamics are important for progression through the catalytic cycle.



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P110

SYNTHETIC ENZYMOLOGY FOR NEXT-GENERATION-OMICS APPLICATIONS

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At present, the preferred method to synthesize DNA is via a chemical process called phosphoramidite-based oligonucleotide synthesis (Matteucci & Caruthers, 1980; LeProust, 2015). Due to the requirement of multiple steps to incorporate one nucleotide, coupling efficiency is limited, which caps the feasible length of synthesized oligonucleotides (Kosuri & Church, 2014). This limitation poses challenges in applications such as the creation of whole synthetic organism which could be used as model organism in various scientific studies (Venter *et al.*, 2010; Richardson *et al.*, 2017). Hence, there is a demand to improve on the process of *de novo* synthesis of DNA. Enzymatic synthesis of oligonucleotides has the potential to achieve better efficiency and accuracy as compared to the chemical synthesis due to its relative simplicity. Terminal deoxynucleotidyl transferase (TdT), a template-independent DNA polymerase from Family X (Bollum, 1974), is a prime candidate for this. Briefly, one could use TdT with blocked nucleotides to direct the incorporation of nucleotides one at a time in the desired order. Moreover, this process is also more environmentally friendly compared to chemical synthesis as it is carried out in aqueous media. The challenge is in modifying TdT to efficiently incorporate modified nucleotides. We began by evolving a thermostable and more active variant of TdT to serve as a chassis for further modification to enable modified nucleotide incorporation. To this end, assays were established to screen for active and potentially thermostable TdT, resulting in a significantly improved TdT enzyme. Assays will be now established to screen for TdT mutants which are able to incorporate modified nucleotides. This study highlights our efforts at TdT engineering and transforming it into a potential tool to write DNA.

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P111

Non-active Site Residues Inactivate the N-terminal Domain of the Human Hexokinase 2

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Cancer utilizes glucose at elevated levels to support its growth and proliferation, historically known as "Warburg effect". Targeting glucose metabolism in cancer cells to limit its growth will enhance patients' survival rate. Hexokinase catalyzes glucose phosphorylation, and is a major step in regulation of glycolysis. One of four isozymes found in humans, hexokinase 2 (HK2), the first enzyme of the glycolytic pathway, catalyzes the phosphorylation of glucose for its activation and further metabolism. Moreover, gene expression profiling experiments of different types of cancer showed high expression levels of HK2. Binding of HK2 to the outer-mitochondrial membrane (OMM) plays an important role in inhibiting apoptosis in cancer, where cancer cells tolerate extreme physiological conditions with low pH due to their high lactate production, and hypoxic conditions at which cancer cells survive in low oxygen environment. Various biological studies also highlighted the importance of HK2 in tumor metastasis making it an ideal target for the characterization of cancer metabolism and development of new class of cancer therapeutics.

We determined the crystal structure of human HK2 in complex with glucose and glucose-6-phosphate (PDB code: 2NZT). HK2 is a homodimer with catalytically active N- and C-terminal domains linked by a seven-turn α-helix. The biochemical and biophysical characterization of HK2 revealed the role of the N-terminal domain in catalysis, and more importantly its role in the thermodynamic stabilization of the full-length enzyme. Binding of HK2 to the OMM is facilitated by the mitochondrial-binding peptide (MBP), an α-helix at the beginning of the N-terminus of HK2. The deletion of the MBP, the first 16 amino acids of HK2 (Δ16-HK2), altered the stability and catalytic activity of the full-length (FL) enzyme. The Δ16-HK2 variant was conformationally more stable than the FL-HK2. Mutating the catalytic residue (D209A) of the N-terminal but not C-terminal (D657A) altered the thermodynamic stabilization effect of glucose on the enzyme.

Further analysis revealed the importance of the linker helix joining the N- and C-terminal domains on the catalytic function of the N-terminal domain of HK2. Decreasing the size of the linker inactivated the N-terminal domain. The human hexokinase isozymes 1 and 3 contain inactive N-terminal domain. Altering the catalytic activity of the N-terminal domain of HK2 at the linker helix can reduce its tumorigenic effect. The linker helix can be used as unique site for the inhibition of the N-terminal domain of HK2. Further structural and biochemical analysis of the molecular mechanisms of human HK2 in cancer metabolism and apoptosis will accelerate the design and development of safe and specific anticancer therapeutics.

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P112

SYNTHETIC ENZYMOLOGY AND THE FOUNTAIN OF YOUTH

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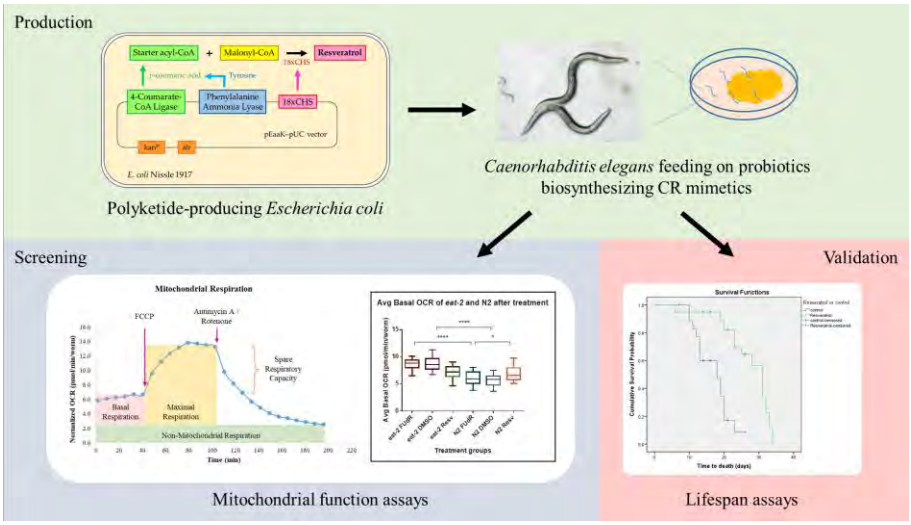
Caloric restriction (CR) is an intervention that can increase maximal lifespan in organisms, but its application to humans remains challenging. A more feasible approach to achieve lifespan extension is to develop CR mimetics that target biochemical pathways affected by CR. Recent studies in the engineering and structural characterization of polyketide synthases (PKSs) have facilitated their use as biocatalysts to produce novel polyketides. Here we show that by establishing a combinatorial biosynthetic route in Escherichia coli and exploring the substrate promiscuity of a mutant PKS from alfalfa, 413 potential anti-ageing polyketides were biosynthesized. In this approach, novel acyl-CoA precursors generated by promiscuous acid-CoA ligases were utilized by PKS to generate polyketides which were then fed to Caenorhabditis elegans to study their potential efficacy in lifespan extension. It was found that CR mimetics like resveratrol can counter the age-associated decline in mitochondrial function and increase the lifespan of C. elegans. Using the mitochondrial respiration profile of C. elegans supplemented for 8 days with 50 µM resveratrol as a blueprint, we can screen our novel polyketides for potential CR mimetics with improved potency. The same platform can also be applied to the screening of alkaloids and cannabinoids for anti-ageing properties. This study highlights the utility of synthetic enzymology in the development of novel anti-ageing therapeutics.



P113

MUTAGENESIS OF ACTIVE-SITE LOOPS IN TRYPTOPHAN HYDROXYLASE: CONTRIBUTIONS IN SUBSTRATES BINDING AND FORMATION OF CATALYTIC INTERMEDIATE

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Tryptophan hydroxylase (TrpH) is an aromatic amino acid hydroxylase (AAH) that catalyzes the pterin-dependent hydroxylation of tryptophan to 5-hydroxytryptophan in the rate-limiting step in the biosynthesis of serotonin. Structural studies of AAHs have shown that the positions of active-site loops differ significantly between the free enzyme and the substrate-bound enzyme complex.[1-3] To understand the role of loop movements in TrpH, Tyr125 in loop 124-134 and Ile366 in loop 365-371 were mutated. The mutant enzymes were characterized by steady-state and rapid kinetic methods. K_{cat} for I366N TrpH is 1-2 orders of magnitude slower compared to wild-type and Y125A TrpH. The effect of increasing the viscosity of the solvent on the kinetic parameters suggests that a conformational change associated with substrate binding has become rate-limiting in I366N TrpH. Rapid-kinetic parameters of single turnover reactions were used to determine the individual rate constants in the enzyme catalysis. The rate constant for formation of the first catalytic intermediate decreased by 3 orders of magnitude in I366N TrpH compared to wild-type TrpH. This confirms that the I366N mutation disrupts an early step in the overall reaction.[4] However, the kinetic parameters for Y125A TrpH are within a factor of two of those for the wild-type enzyme. Overall, the mechanism is consistent with binding of the amino acid and pterin substrate to form the ternary complex being directly coupled to closure of the 365-371 loop over the active site and formation of the reactive $Fe^{IV}O$.

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P114

Development of a cell-free and *in vivo* platform for the screening of microbial lipases

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Lipases (E.C.3.1.1.3) are a group of enzymes with great commercial value due to their ability to catalyze a wide range of reactions ranging from hydrolysis to esterification and alcoholysis.[1] These enzymes are ubiquitous in nature but microbial lipases are more useful for industrial applications due to their increased thermostability and ease of expression in other microbial hosts.[2, 3] Although numerous microbial lipases have recently been characterized, the function of most of these enzymes are currently unknown. With recent advancements in synthetic biology, we describe a high-throughput, cell-free approach for the characterization of over 100 microbial lipases.[4] Coupled with a colorimetric assay, lipase activity was detected against triacylglycerides of various chain length. The high correlation between the cell-free and *in vivo*-based screening of lipases suggests that cell-free synthesis can be used reliably for the functional assignment of unknown lipases.

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P115

Mechanism of inhibition of *Mycobacterium tuberculosis* isocitrate lyase by 3-nitropropionate

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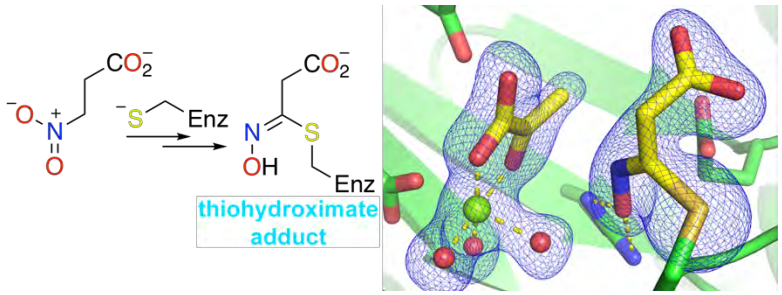
Mycobacterium tuberculosis, the causative organism of tuberculosis, is responsible for the highest mortality rate among bacterial pathogens. Persistent *M. tuberculosis* infections depend on the glyoxylate shunt, a carbon-conserving bypass in the tricarboxylate cycle consisting of isocitrate lyase (ICL) and malate synthase. Since these enzymes are absent in humans but essential to mycobacteria, they are potential drug targets. ICL catalyzes the reversible retro-aldol cleavage of isocitrate into succinate and glyoxylate. 3-Nitropropionate (3-NP) is an analog of succinate that demonstrates slow-onset inhibition of the enzyme, which we show is attributable to its conjugate base, propionate-3-nitronate (P3N). P3N, prepared from 3-NP at pH 13, was found to inhibit the enzyme 70 times faster than 3-NP at pH 7.5. Through jump-dilution kinetics, P3N was found to be an irreversible inhibitor of ICL from *M. tuberculosis*, unlike the enzyme from *Pseudomonas indigofera* (Schloss & Cleland, 1982). Electrospray ionization mass spectrometry showed a time-dependent increase of 101 Da in the mass of ICL following incubation with 3-NP or P3N (119 Da for neutral form), which was accelerated in the presence of glyoxylate. An inverse solvent kinetic isotope effect (KIE) of 0.56 was measured on k_{inact}/K_I, indicating that a cysteine residue, assumed to be Cys191, must lose its proton during formation of the inhibited complex. A normal deuterium KIE of 3.53 on k_{inact}/K_I was measured using [3,3-$^2H_2$]-3-NP, indicating that formation of P3N is rate limiting. An X-ray crystal structure of the inactivated complex reveals a thiohydroximate adduct between the inhibitor and Cys191. A mechanism is proposed for this unprecedented modification. It is hoped that nitro groups can be used more generally as masked electrophiles for the design of selective covalent inhibitors.



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P116

Spectroscopic and Kinetic Studies of Catalytically Versatile Non-Heme Iron Enzymes

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Iron(II) and 2-Oxoglutarate (2OG) dependent non-heme iron (Fe(II)/2OG) enzymes catalyze a large array of chemical transformations. Many of these transformations are key steps in the biosynthesis of natural products having antibiotic and antimicrobial activities. In recent years, studies on hydroxylation and halogenation reactions have revealed key details of Fe(II)/2OG enzyme catalysis, namely the discovery of the ferryl species as the key intermediate for C-H bond activation, and the utilization of the rebound mechanism (OH-rebound for hydroxylation and halide-rebound for halogenation) to complete functional group installation. However, mechanistic details are lacking on the reactions that the rebound mechanism is not operative, such as desaturation and epoxidation, which prevent the emergence of a unified picture for Fe(II)/2OG enzyme catalysis. To this end, we are using spectroscopic and kinetic tools to elucidate reaction mechanisms of several newly discovered Fe(II)/2OG enzymes that utilize non-rebound pathways to catalyze reactions. Here, we present our recent results from the studies on a novel bi-functional Fe(II)/2OG enzymes, AsqJ from *Aspergillus nidulans*, which catalyzes a stepwise oxidation (desaturation and epoxidation) in the biosynthesis of a quinolone-type fungal alkaloid, 4'-methoxy-viridicatin. Recent results on other novel Fe(II)/2OG enzymes will also be presented**.** The implications of these results to the overall understanding of Fe(II)/2OG enzyme catalysis will be briefly discussed.

P117

Lysine-2,3-aminomutase's role in salt tolerance in Methanogenic Archaea

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Fluctuating salinity/osmolarity is a major environmental stressor that cells must cope with using various strategies. To prevent water loss and maintain cell turgor under high salinity conditions, many microorganisms accumulate osmolytes. The most common osmolytes are known as compatible solutes, which are charged and/or polar small organic molecules, such as polyols, betaines, and certain amino acid derivatives, that do not interfere with normal cellular operations when they are present at high concentrations.[1] Methanogenic archaea uniquely employ β-amino acids and their derivatives as compatible solutes, most commonly β-glutamate and N^{ε}-acetyl-β-lysine.[2] N^{ε}-acetyl-β-lysine biosynthesis from α-lysine requires two enzymes, lysine-2,3-aminomutase (LAM) and an acetyltransferase, and the genes encoding these two enzymes, *ablA* and *ablB*, are found in tandem in the genomes of several methanogens. Both genes were previously shown to be essential for growth on high salt in *Methanococcus maripaludis* JJ.[3] Here, we generated a deletion of both *ablA* and *ablB* in *M. maripaludis* S2 in order to further understand the importance of these genes in our model methanogen, as well as to use as a tool for studying other potential compatible solute biosynthesis enzymes. The *M. maripaludis* S2 Δ*ablA*Δ*ablB* strain exhibits slow growth on high salt, but surprisingly is not completely inhibited. This suggests that without N^{ε}-acetyl-β-lysine, *M. maripaludis* S2 synthesizes and/or transports another molecule to use as a compatible solute. Another strain, *M. maripaludis* C7, contains two genes that are annotated as LAM, MmarC7_0106 and MmarC7_1783. We assumed MmarC7_0106 was the actual LAM since it is located on the genome next to an acetyltransferase, and we hypothesized that MmarC7_1783 could be making a different compatible solute. When MmarC7_0106 was expressed in the Δ*ablA*Δ*ablB* strain, the slow growth phenotype was nearly completely rescued, indicating that β-lysine is sufficient for growth on high salt. This may explain why the genomes of some methanogens only encode LAM without the associated acetyltransferase. When MmarC7_1783 was expressed in the Δ*ablA*Δ*ablB* strain, the slow growth phenotype was not rescued, indicating that MmarC7_1783 is not a LAM and that it likely does not synthesize another compatible solute. We additionally expressed the methanogenic LAM (MmarC7_0106) in *E. coli* for *in vitro* characterization. We show that purified MmarC7_0106 contains a [4Fe-4S] cluster and catalyzes the conversion of lysine to β-lysine in the presence of *S*-adenosyl-L-methionine (SAM) and dithionite, consistent with the expected radical SAM reaction.

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P118

Synthetic Alkaloid Enzymology: Next-generation production of active pharmaceutical ingredients and nutrichemicals

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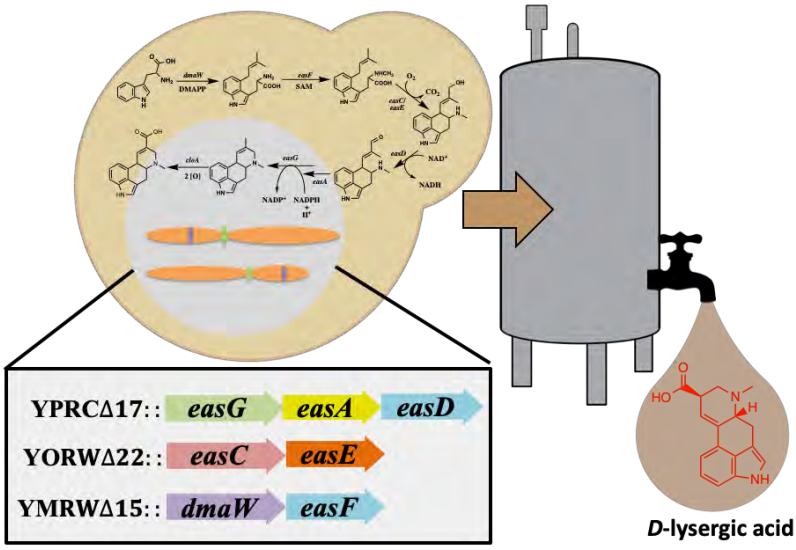
Abstract

Ergot alkaloids and their semi-synthetic derivatives are known for their potent biological effects on the nervous system. These properties have led to their use in the treatment of several neurological disorders such as Parkinson's and Alzheimer's disease. Their production however, is limited by the difficulty in cultivation of the ergot fungi. Our goal is to reconstitute the ergot pathway in *Saccharomyces cerevisiae*, to produce a strain producing lysergic acid that can be easily scaled for industrial fermentation. To this end, we have adapted a golden gate-based cloning methodology (YeastFab[1]) to easily construct customized expression cassettes for integration into the yeast genome.

A prototype strain containing the seven genes required for the production of agroclavine (the direct precursor to lysergic acid) integrated into the yeast genome, has been demonstrated to be capable of producing a yield of up to 55 μg/L. Further optimization and addition of the enzyme *cloA* would see improvements made to the yield and completing the pathway to lysergic acid. The development and improvement to this strain would be essential for laying the groundwork towards a more efficient commercial production method, as well as the discovery of novel ergot alkaloid therapeutics.



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P119

THE RADICAL SAM ENZYME BIOTIN SYNTHASE: CHARACTERIZATION OF RATE-LIMITING HYDROGEN ATOM ABSTRACTION USING PRIMARY KINETIC ISOTOPE EFFECTS

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Biotin synthase is a radical SAM enzyme that catalyzes the terminal step in the biotin biosynthetic pathway: the insertion of a sulfur atom between the C6 methylene and C9 methyl groups of dethiobiotin. Biotin synthase carries out this insertion in two sequential half-reaction cycles. In the first half reaction, reductive cleavage of SAM generates a 5'-deoxyadenosyl radical that abstracts a hydrogen atom from the C9 methyl, generating a putative dethiobiotinyl radical. This radical is then quenched by formation of a new C-S bond with the μ-sulfide of a $[2Fe\text{-}2S]^{2+}$ cluster, generating an intermediate in which 9-mercaptodethiobiotin is tightly bound as a ligand to the remnant cluster.[1] Following dissociation of 5'-deoxyadenosine and methionine and binding of a second equivalent of SAM, a second half-reaction targeted at the C6 position closes the thiophane ring. The formation of 9-mercaptodethiobiotin ($k_1 \approx 0.15$ min^{-1}) is significantly slower than ring closure to biotin ($k_2 \approx 0.5$ min^{-1}),[2] and on this basis we proposed that the rate-limiting step was likely to be hydrogen atom abstraction from the C9 position of dethiobiotin. We probed this possibility using (9-$^2H_3$-*methyl*)-dethiobiotin in varying mixtures with unlabeled dethiobiotin and observed primary ^{2}H KIEs ranging from 6 – 50, depending on various factors including substrate concentration, reaction time, and reaction temperature. In an effort to understand this wide range for observed KIE values, we discovered an unusual kinetic anomaly. Biotin synthase is a homodimeric enzyme that exhibits half-of-sites activity, and we discovered that the enzyme is ~3-4-fold more active when only one active site is occupied ($k_{cat} \geq 0.5$ min^{-1}), while enzyme with both active sites occupied is significantly slower ($k_{cat} = 0.1$ min^{-1}). There is no observable cooperative effect on substrate binding, but rather this effect is expressed entirely on catalysis. With this kinetic model in hand, we were able to fully model the range of KIE values and can report $k_H/k_D = 35$ for the slower saturated enzyme and $k_H/k_D \approx$ 50-60 for the faster subsaturated enzyme. These large KIE values suggest that hydrogen atom quantum mechanical tunneling is occurring in the transition state, which we further probed using an Arrhenius analysis. The results suggest significant contributions from tunneling to the reaction trajectory, but also suggest that rate-limiting hydrogen atom transfer is at least partially gated by thermal motions within the active site.

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P120

Enzyme Covalent Inactivation Kinetics and Selectivity for 3rd-Generation EGFR Tyrosine Kinase Inhibitor Osimertinib

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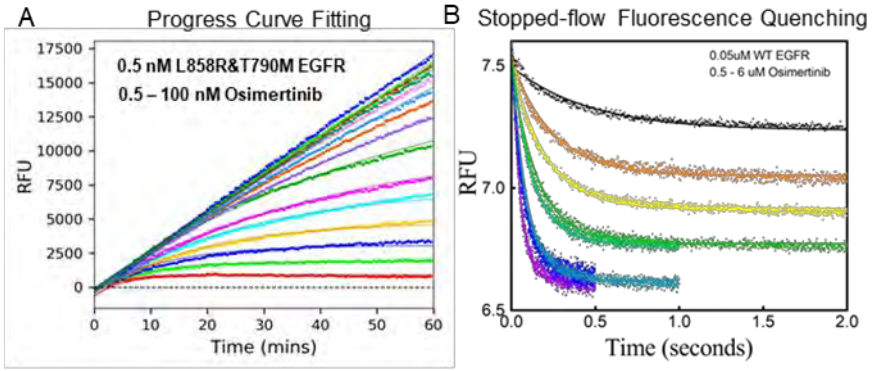
Osimertinib[1] (Tagrisso®) is a 3rd-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) approved for treating patients with EGFRm advanced non-small cell lung cancer (NSCLC), and for patients with T790M positive NSCLC, following disease progression on first or second-generation EGFR-TKIs. It bears the acrylamide warhead which covalently modifies the Cys797 in the active site of EGFR kinase domain. Osimertinib differentiates from early generations of EGFR TKIs in that it potently targets the sensitizing (Ex19del or L858R) and resistance mutations (T790M) but is less active against the wildtype[1]. Here we developed methodologies to measure the enzyme inactivation kinetics for Osimertinib and 2nd-generation inhibitors to gain additional insight into their mechanisms of action. k_{inact}/K_i values were determined from the global fit of progress curves using numerical integration by Kintek Explorer[2] (Fig. A). K_i values were measured by following the EGFR intrinsic fluorescence quenching kinetics using the stopped-flow instrument (Fig. B). Using an Osimertinib analog which lacks the acrylamide warhead, we confirmed that the fluorescence quenching is only due to the initial formation of the enzyme-inhibitor complex. The data recapitulate the data in pre-clinical tumor models and the clinical observations and demonstrates that Osimertinib is very potent inhibitor of EGFR bearing the activating mutations or T790M mutation compared to the wild type, unlike 2nd-gen inhibitors such as Afatinib and Dacomitinb which have a smaller margin to wildtype and are less potent towards T790M EGFR.



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P121

HUMAN GLYCEROL-3-PHOSPHATE DEHYDROGENASE: X-RAY CRYSTAL STRUCTURES THAT GUIDE THE INTERPRETATION OF MUTAGENESIS STUDIES

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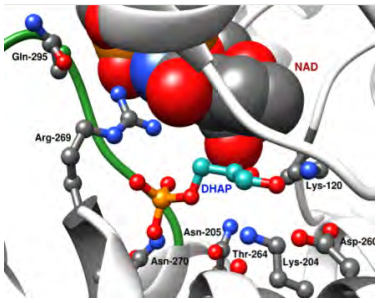

Glycerol-3-phosphate dehydrogenase (*hl*GPDH) serves as a catalyst for the reversible redox conversion of dihydroxyacetone phosphate (DHAP) and reduced nicotinamide adenine dinucleotide (NADH) to *L*-glycerol 3-phosphate (*L*-G3P) and NAD^+, respectively. X-ray crystal structures are reported for unliganded, binary E•NAD, and ternary E•NAD•DHAP complexes of wildtype *hl*GPDH at 1.9 Å, 2.1 Å, and 2.1 Å resolutions, respectively. The ternary complex shows the carbonyl oxygen of enzyme-bound DHAP positioned to accept a proton from the side chain cation of K120 during *hl*GPDH hydride transfer from NADH to form the product *L*-G3P. Kinetic parameters are reported for the reduction of DHAP catalyzed by the K120A, K120A/R269A and K120A/Q295A mutants of *hl*GPDH. The K120A mutation results in similar increases in the activation barrier to wildtype *hl*GPDH-catalyzed reduction of the whole substrate DHAP and to phosphite–dianion activated reduction of glycolaldehyde, so that that these transition states show similar interactions with the cationic K120 side chain. The K120A mutation results in a 5.3 kcal/mol transition state destabilization, and 3.0 kcal/mol of the lost transition state stabilization is rescued by 1.0 M ethylammonium cation. The effect of K120A and Q295A mutations on transition state stability for the wildtype *hl*GPDH catalyzed reaction is independent of the presence of the second side chain. The K120A mutation results in a 2.4 kcal/mol reduction in the 9.1 kcal/mol transition state destabilization from the R269A mutation of wildtype *hl*GPDH, so that optimal transition state stabilization by the individual side chains is only observed when both are present at the enzyme active site. These results provide strong evidence that the enzyme rate acceleration is due mainly or exclusively to transition state stabilization by electrostatic interactions with polar amino acid side chains.

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P122

IS GUANIDINE A SUBSTRATE FOR UREA CARBOXYLASE?

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Urea amidolyase is a multifunctional biotin-dependent enzyme that catalyzes the HCO_3^- and ATP-dependent cleavage of urea into ammonia and CO_2. UAL is comprised of two enzymatic components: urea carboxylase (UC) and allophanate hydrolase (AH). These enzyme activities are encoded on separate but proximally related genes in prokaryotes. It has recently been reported that prokaryotic UC can be regulated by a guanidine-sensitive riboswitch and that UC from *Oleomonas sagarenesis* catalyzes the cleavage of ATP with a catalytic efficiency that is ~40-fold higher in the presence of guanidine compared to urea (1). These results suggested that UC preferentially catalyzes the carboxylation of guanidine to carboxyguanidine *en route* to its eventual decomposition by AH to ammonia and CO_2. Here, we test this hypothesis by examining the catalytic activity of *Pseudomonas syringae* UC and AH in the presence of urea and guanidine. We confirm that, compared with urea, ATP is cleaved with a much higher catalytic efficiency in the presence of guanidine. However, we did not detect any production of ammonia from guanidine in the presence of AH. Furthermore, no product peak corresponding to ^{13}C-carboxyguanidine was observed by ^{13}C-NMR in the presence of guanidine and *Ps*UC. These results suggest that either carboxyguanidine is not a product of *Ps*UC or that the carboxyguanidine product is too unstable to be detected or channeled in our assays. Two proteins encoded by genes that are closely associated with UC and AH do not serve as catalysts, nor do they facilitate channeling of carboxyguanidine from UC to AH. In summary, while guanidine serves to accelerate the rate of ATP cleavage in UC, we have not yet found evidence that carboxyguanidine is a product of the reaction catalyzed by urea carboxylase.

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P123

Mechanisms of Transcriptional Downregulation of Gene Expression by U/A Base Pairs in DNA

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dUTP is a close structural congener of dTTP and can be readily incorporated into DNA opposite to adenine during DNA replication leading to non-mutagenic U/A base pairs (“uracilation”). We now report that U/A pairs located within DNA transcription templates optimized for either T7 RNA polymerase (T7 RNAP) or human RNA polymerase II (pol II) have strong inhibitory effects on transcription. The data for T7 RNAP establishes that even a single U/A pair can inhibit promoter binding and transcription initiation up to 30-fold, and that inhibitory effects on transcription elongation are also possible. Sequencing of the mRNA products transcribed from uniformly uracilated DNA templates by T7 RNAP indicated an increased frequency of transversion and insertion mutations compared to all T/A templates. Strong inhibitory effects of U/A pairs were also observed with human RNA poll II transcription using an engineered human cell line that lacked any uracil excision activity. At the highest levels of template uracilation, transcription by RNA pol II was completely blocked. These findings establish that U/A pairs in DNA are inhibitory to transcription and in some cases, mutagenic. In human cells, uracilation of a gene promoter region could silence transcription by mechanisms involving inhibition of RNA polymerase activity or decreased binding of multiple transcription factors.

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BIOREMEDIATION OF GOLD USING SYNTHETIC ENZYMOLOGY

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With the rise in consumerism, electronic waste is a large and growing problem in society today. Due to the high concentration of gold present in this waste, there is interest in the treatment and recovery of these precious metals. Current treatment methods such as chemical cyanidation and leaching have proven to be environmentally unsustainable. Thus, environmentally friendly, sustainable alternatives of recovering gold are sought after. One potential solution lies in the use of metal reductases like mercuric reductase (MerA).

Investigation into MerA's substrate promiscuity revealed that the enzyme not only reduces divalent mercury ions but can also reduce metal ions with other valencies. Of particular interest is the gold trivalent metal ion, Au^{3+}. Reduction of gold by MerA was enhanced over 15-fold using a combination of enzyme engineering techniques showing that enzymes can be engineered to improve reduction of trivalent metal ions in a typically divalent metal ion reduction system. Recovery of gold from electronic waste leachate using the mutants generated was also demonstrated and hence, further serves to establish the benefits of synthetic enzymology, particularly in gold bioremediation.

P125

Mechanistic Enzymology of Tryptophan Kynurenine Pathway

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Tryptophan, an essential amino acid in humans, is used as the precursor for serotonin and melatonin in the brain. However, the vast majority is catabolized through the kynurenine pathway, producing numerous neuroactive compounds, the biosynthetic precursor for NAD/NADH, and ultimately energy via the tricarboxylic acid cycle. Dysregulation of this pathway directly correlates to neurological disorders, infectious diseases, and cancer. This poster presents a summary of our recent discoveries on the mechanistic enzymology of the pathway, especially on the key regulatory enzyme indolamine 2,3-dioxygenase/tryptophan 2,3-dioxygenase (IDO/TDO) and the enzymes sitting at the crossroads where the pathway branches due to intrinsic instability of the metabolites. These enzymes include 3-hydroxyanthranilate-3,4-dioxygenase (HAO), 2-amino-3-carboxy-muconate-6-semialdehyde decarboxylase (ACMSD), and a new enzyme 2-aminomuconate semialdehyde dehydrogenase (AMSDH). This poster will provide an update that highlights our recent advances in the mechanistic enzymology studies of these enzymes of the tryptophan kynurenine pathway.

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P126

In Silico Docking-Guided Evolution of Geobacillus Kaustophilus Lactonase towards Enhanced Quorum Quenching

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A thermostable lactonase from *Geobacillus kaustophilus* (GKL) was used as the template for the *in vitro* evolution of enhanced quorum quenching activity. We developed a generalizable computational protocol including exhaustive generation of point mutations lining the GKL active site and mutant evaluation based on ligand docking. This facilitated the identification of mutants that have better or broader range of lactonase activity compared to the wild type. We have also created a robust and tunable directed evolution platform to screen for enhanced quorom quenching activity against a broad range of AHLs. We have also provided structural evidence of a productive active site architecture in GKL.

P127

Functionally Important Conformational Sub-States in Human Ribonuclease Family

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Eight members of the human pancreatic ribonuclease (RNase) family have diverse biological functions including angiogenesis and host defense (cytotoxic and anti-pathogen activities), but share common structural scaffold and catalyze the hydrolysis of ribonucleic acid (RNA). However, the catalytic efficiency and rate of dynamics among these enzymes differ by more than a million (10^6) folds. Emerging evidence from other enzyme systems propose that internal motions drive the sampling of short-lived minor population of conformations (called as *sub-states*) that can contain features to promote various steps during the function of an enzyme. Therefore, quantitatively characterizing the conformational sub-states in the catalytic cycle of enzymes, including substrate binding, structural rearrangements leading to the transition state, product formation and product release will enable us to obtain detailed insights in the role of enzyme dynamics in catalysis by RNases. We have used a combination of theoretical modeling, computer simulations, steady state kinetics and NMR experiments. Our studies indicate that RNases superfamily can be classified into sub-families with designated biological functions and conserved dynamical behavior. Further, computer simulations show diverse binding preferences across the human RNase family and the conformational sub-states in various steps of the catalytic cycle differ among the members belonging to each of the sub-family. Moreover, we observe a ~10^6 fold difference in the catalytic rates of the human RNases. Additionally, we are using hybrid QM/MM method to model the catalysis of the single stranded RNA that would enable us to identify the distinct conformational sub-states sampled by each of the members of human RNase family in their respective minimum energy reaction pathway.

P128

Using secondary ^{18}O kinetic isotope effects to determine the transition state structure for phosphite dehydrogenase

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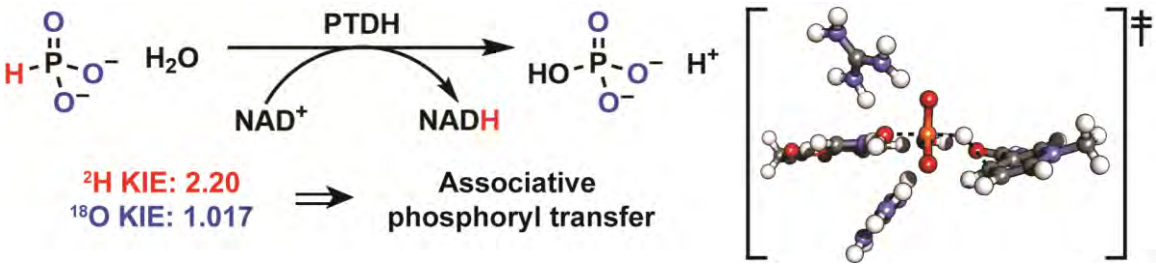
Phosphite dehydrogenase (PTDH) catalyzes the oxidation of phosphite to phosphate with the concomitant reduction of NAD^+. This process represents a formal phosphoryl transfer reaction with an unusual hydride donor and a hydroxide acceptor. Despite decades of study, the relative timing of bond-making and bond-breaking in this reaction remains unclear. In order to differentiate between a tight, phosphorane-like transition state and a loose, metaphosphate-like transition state, primary H/D and secondary ^{18}O kinetic isotope effects (KIEs) were determined for the PTDH-catalyzed reaction. The modest H/D KIE and the large, normal ^{18}O KIE suggested that the reaction proceeds through a particularly tight transition state. To aid in the interpretation of these KIEs, quantum mechanical calculations were used to generate a series of transition state structures within a model of the active site of PTDH. ^{18}O and H/D KIEs were calculated for each of the optimized structures and the experimentally observed KIEs were used as boundary constraints to arrive at a transition state structure that correctly predicts the experimental KIEs. This analysis resulted in a geometric model of the transition state for the PTDH-catalyzed phosphoryl transfer that involves significant nucleophilic participation and very little displacement of the hydride leaving group. This tight transition state is likely a consequence of the extremely poor leaving group requiring significant P-O bond formation to expel the hydride. The identified transition state for the phosphoryl transfer catalyzed by PTDH differs dramatically from the dissociative processes that occur in most phosphoryl transfer reactions.



P129

Stereospecific Radical-mediated B_{12}-dependent Methyl Transfer by the Fosfomycin Biosynthesis Enzyme Fom3

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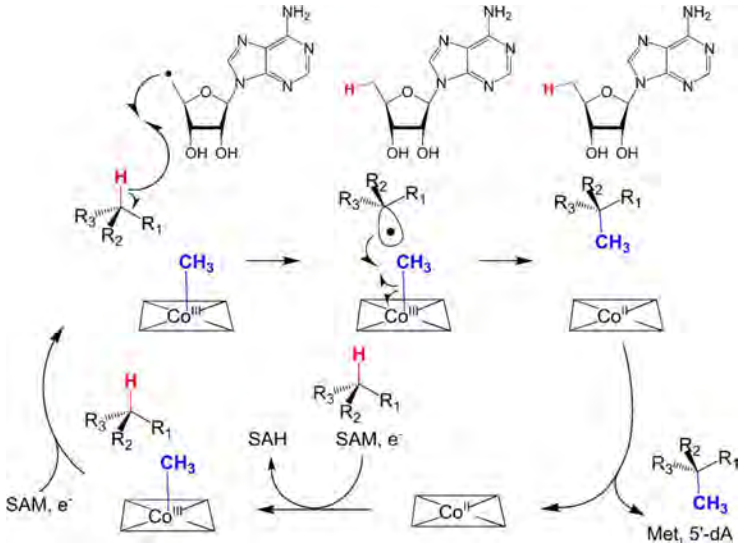
[a]Department of Chemistry, [b]Carl R. Woese Institute for Genomic Biology, and [c]Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Fom3, the antepenultimate enzyme in *Streptomyces* fosfomycin biosynthesis, is a class B cobalamin-dependent radical SAM methyltransferase that catalyzes methylation of (5′-cytidylyl)-2-hydroxyethylphosphonate (2-HEP-CMP) to form (5′-cytidylyl)-2-hydroxypropylphosphonate (2-HPP-CMP).[1, 2] The insolubility of the enzyme without its B_{12} cofactor has made characterization challenging. Recently, *in vivo* incorporation of B_{12} into Fom3 was achieved by co-expression in *E. coli* with a B_{12} uptake system.[3] Reactions of *S. wedmorensis* Fom3 prepared by this method show that the initiator 5′-deoxyadenosyl radical stereospecifically abstracts the *pro-R* hydrogen atom from the C2 position of 2-HEP-CMP. Conversion of the product to fosfomycin by the downstream enzymes FomD and Fom4[4] demonstrates that the methyl transfer is stereospecific, producing only (2*S*)-2-HPP-CMP. The transferred methyl group originates from the as-isolated enzyme during a single turnover, but from SAM thereafter; B_{12} isolated from Fom3 reaction mixtures contains methyl groups derived from SAM. These results support a model in which Fom3 catalyzes methyl transfer from SAM to B_{12} and the resulting methylcobalamin is the proximal methyl source for the reaction.[5]



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P130

Tackling Insecticide Resistance: Structural and Kinetic characterization of Arylalkylamine *N*-acyltransferase from *Tribolium castaneum*

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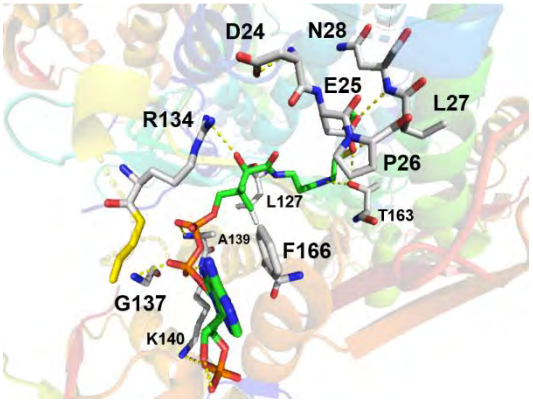
The issue of insecticide resistance, combined with strict regulations on usage, has meant that the identification of novel insecticide targets has never been more important. Resistance to current insecticides has seen the need for higher dosing, and a growing number of trusted insecticides becoming obsolete. It is, therefore, vital to approach this issue by not just implementing new insecticides, or by more stringent regulation and mixing, but by carefully selecting new chemical targets.[1] By increasing the number of possible targets, we have a better chance to resist natural evolution through genetic adaption.

Arylalkylamine *N*-acyltransferases (AANATs) have been suggested as potential new targets.[2] These promiscuous enzymes are involved in the *N*-acylation of biogenic amines to form *N*-acylarylalkylamines. In insects, this process is a key step in melanism, as well as hardening of the cuticle. AANAT-catalyzed acetylation also facilitates the removal of biogenic amines in insects (a role performed by monoamine oxidase in mammals). AANATs have also been implicated in the biosynthesis of fatty acid amides – important cell signalling molecules, the origin of which largely remains unknown.[3] Importantly, the unique nature of each AANAT isoform characterized indicates that while catalyzing similar reactions, each organism accommodates an assembly of AANATs relatively exclusive to that organism. This implies a high potential for selectivity in insecticide design, while also maintaining polypharmacology.

The red flour beetle, *Tribolium castaneum*, offers an ideal model organism, being regarded as one of the most common secondary pests of all plant commodities in the world. Presented here is a thorough kinetic and structural analysis of two AANATs found in *T. castaneum*, which were determined to catalyze the formation of short chain *N*-acylarylalkylamines, with short-chain acyl-CoAs (C2-C10) functioning in the role of acyl-donor. A combination of kinetic analysis, crystallography, mutagenesis, ITC, and sequence analysis shines light on some approaches possible to utilize these enzymes in insecticide design.



Crystal structure of *Tc*-AANAT0 active site

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P131

Tackling Insecticide Resistance: Structural and kinetic characterization of arylalkylamine N-acyltransferase from *Tribolium castaneum*

Brian G. O'Flynn, Karin Claire Prins, Nasha M. Rois-Guzman, Gabriela Suarez, Britney A. Shepherd, and David J. Merkler

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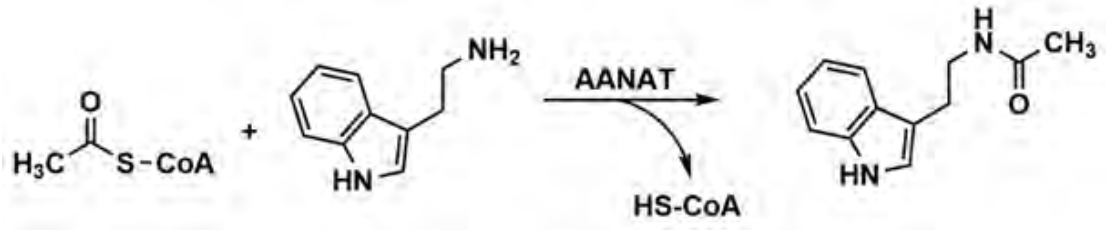
Growing resistance to current insecticides has, in recent years, seen the need for higher dosing, and a growing number of trusted insecticides becoming obsolete. It is vital to approach this issue by not just implementing new insecticides, or by more stringent regulation and mixing, but by carefully selecting new chemical targets.

Arylalkylamine N-acyltransferases (AANATs), involved in the N-acylation of biogenic amines to form N-acylamides, (Fig. 1) have been suggested as potential new targets. While catalyzing similar reactions, each organism accommodates an assembly of AANATs relatively exclusive to that organism. This implies a high potential for selectivity in insecticide design, while also maintaining polypharmacology.



Tribolium castaneum, offers an ideal model organism, being regarded as one of the most common secondary pests of all plant commodities in the world. Presented here is a kinetic summary of two AANATs found in T. castaneum, which were determined to catalyze the formation of short chain N-acylamines, with short-chain acyl-CoAs (C2-C10) functioning in the role of acyl-donor. Crystallography and mutagenesis also offer us insight into the chemical mechanism of the putative dopamine N-acyltransferase of T. castaneum, TcAANAT0.

P132

Remote Binding Energy in 3α-Hydroxysteroid Dehydrogenase/Carbonyl Reductase Catalysis

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The binding energy of enzyme and substrate is utilized for lowering the activation energy to facilitate the reaction[1]. We found that 3α-hydroxysteroid dehydrogenase/ carbonyl reductase (3α-HSD/CR) utilizes the remote binding energy of the active site with the non-reacted portion of androsterone to accelerate the reaction[2]. The uniform binding energy from the B-ring of steroids with the active site of 3α-HSD/CR equally contributes 2.1 kcal/mol to interact with both the transition state and ground state of the ternary complex, leading to the similarity in k_{cat} for 2-decalol and cyclohexanol. Meanwhile, the differential binding interactions of the remote BCD-ring and CD-ring of androsterone with the active site of 3α-HSD/CR contribute 8.5 and 6.4 kcal/mol to the stabilization of the transition state, respectively. Here, we analyze the enthalpic and entropic contributions of the remote binding energy in 3α-HSD/CR catalyzed reaction of NAD^+ with androsterone versus the truncated substrate analogs by analyzing the temperature-dependent kinetic parameters through steady-state kinetics. The effects of temperature on k_{cat}/K_M for 3α-HSD/CR acting on androsterone, 2-decalol, and cyclohexanol indicate the reactions are entropically favorable. It is endothermic and entropy-driven for the binding of E-NAD^+ complex with either 2-decalol or cyclohexanol to form the ternary complex, and both enthalpically and entropically unfavorable for the subsequent conversion to the transition state, based on the thermodynamic analysis from the temperature-dependent values of K_M and k_{cat}, respectively. These results suggest solvation entropy play an important role in the binding process through both the desolvation from the solute molecules and the releases of bound water molecules from the active site into bulk solvent. As compared to thermodynamic parameters of 3α-HSD/CR acting on cyclohexanol, the hydrophobic interaction of the B-ring of steroids with the active site of 3α-HSD/CR contributes to catalysis by increasing exclusively the entropy of activation ($T\Delta\Delta S^{\ddagger}$=1.8 kcal/mol), while the BCD-ring of androsterone significantly lower $\Delta\Delta H^{\ddagger}$ by 10.4 kcal/mol with slight entropic penalty of -1.9 kcal/mol. Therefore, the remote non-reacting sites of androsterone may induce the conformational change of the substrate binding loop with entropic cost for better interactions with transition state to decrease the activation of enthalpy, resulting in significant increases in catalytic efficiency k_{cat}/K_m by 1.8×10^6-fold.

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P133

Kinetics of A Radical Mediated Ring Contraction

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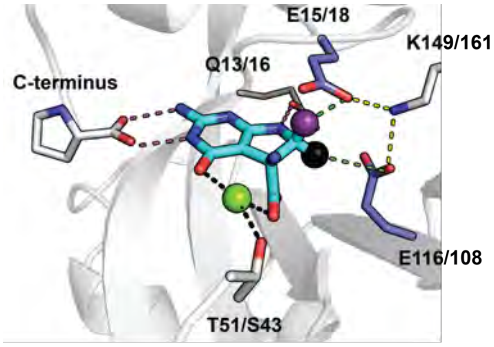
The radical S-adenosyl-L-methionine (SAM) superfamily are a large group of enzymes that are identified on the basis of a CX_3CX_2C motif, known as the radical SAM signature sequence[1]. All members characterized to-date contain at least one [4Fe-4S] coordinated by the CX_3CX_2C, which is used to reductively cleave SAM often forming a 5'-deoxyadenosyl radical[2,3]. 7-Carboxy-7-deazaguanine (CDG) synthase (QueE) is a member of the radical SAM enzyme that catalyzes the radical-mediated ring contraction of 6-carboxy-5,6,7,8-tetrahydropterin (CPH_4) to form (CDG), a pyrrolopyrimidine core, which is elaborated further to form the hypermodified RNA bases queuosine and archaeosine, as well as a variety of deazapurine-containing natural products[4,5]. This poster will describe our recent studies on the stereoselective proton elimination that leads to retention of C7-proS hydrogen of the substrate in the CDG product.



*Bm*QueE structure; *Bm/Bs* numbering

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P134

Thiamin Biosynthesis in Yeast: THI5 - a Remarkable 'Suicide' Enzyme

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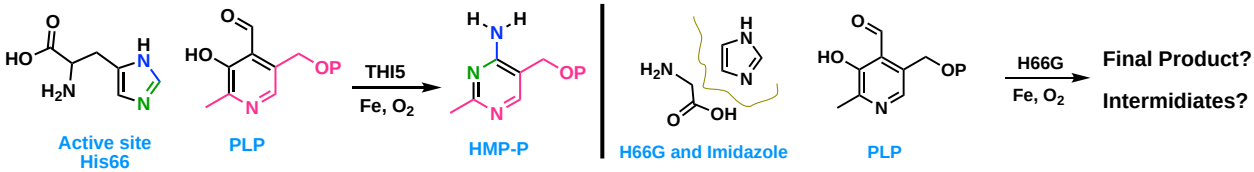
Thiamin pyrophosphate is an essential cofactor in all forms of life. It is a derivative of Vitamin B_1. Grain products are its primary source and the recommended daily allowance for humans is 1.2 mg. Thiamin is also an important commercial chemical and it is widely used as a food additive and flavoring agent. Thiamin dependent enzymes play an important role in carbohydrate and branched chain amino acid metabolism that include transketolase, acetolactate synthase, α-ketoacid decarboxylases and α-ketoacid oxidases. In all cases, thiamin stabilizes an acyl carbanion biosynthon. The biosynthesis of Thiamin pyrophosphate in *S.* cerevisiae involves some enzymes that catalyze complex and intriguing chemical transformations. The pyrimidine synthase (THI5) in this thiamin biosynthesis pathway utilizes Pyridoxal phosphate (PLP) and its active site Histidne residue (His66) in presence of molecular oxygen and Fe(II) to form 4-amino-2-methyl-5-hydroxymethyl pyrimidine phosphate(HMP-P). ThI5 is a remarkable 'suicide' enzyme and the mechanistic details of this complicated rearrangement is still to be addressed. We did mutagenesis on THI5 to explore the mechanism by using the H66G mutant and providing imidazole externally as the active site histidine mimic. The purpose of this experiment was to obtain any intermediate involved in the pathway or check whether H66G can complete the reaction to form HMP-P. We found a proposed intermediate is being formed with these reaction conditions by analyzing the reaction through LC-MS. Further to probe the origin of atoms in the intermediate we used labeled imidazole, labeled PLP and 18-O labeled oxygen. These experiments provide us insight into of the initial step of THI5 catalyzed reaction. We will characterize this proposed intermediate by NMR spectroscopy and also try other proposed intermediates to complete our understanding of this highly complex biochemical reaction.



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P135

Mechanistic Characterization of the Radical SAM Enzyme TYW1

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There are over 100 hundred documented modifications of the four canonical RNA bases found throughout the three kingdoms of life. The majority of modified RNA bases are found in tRNA. Wyosine bases are amongst some of the most complex RNA modifications and are characterized by a tricyclic ring. They are derived from a genetically encoded guanosine at position 37 of $tRNA^{Phe}$ in archaea and eukarya. The characteristic tricyclic ring is formed during the second step of the biosynthesis of these modified bases by the radical *S*-adenosyl-L-methionine (SAM) enzyme TYW1. TYW1 catalyzes the condensation of *N*-methylguanosine containing tRNA with two carbons, which we have shown to be derived from pyruvate. This poster will discuss our most recent biochemical and structural studies of TYW1 to understand how this enzyme catalyzes a complex radical mediated ring formation.

P136

A Click Chemistry Approach to Reveal the Chromatin-Dependent Histone H3 K36 Deacylase Nature of SIRT7

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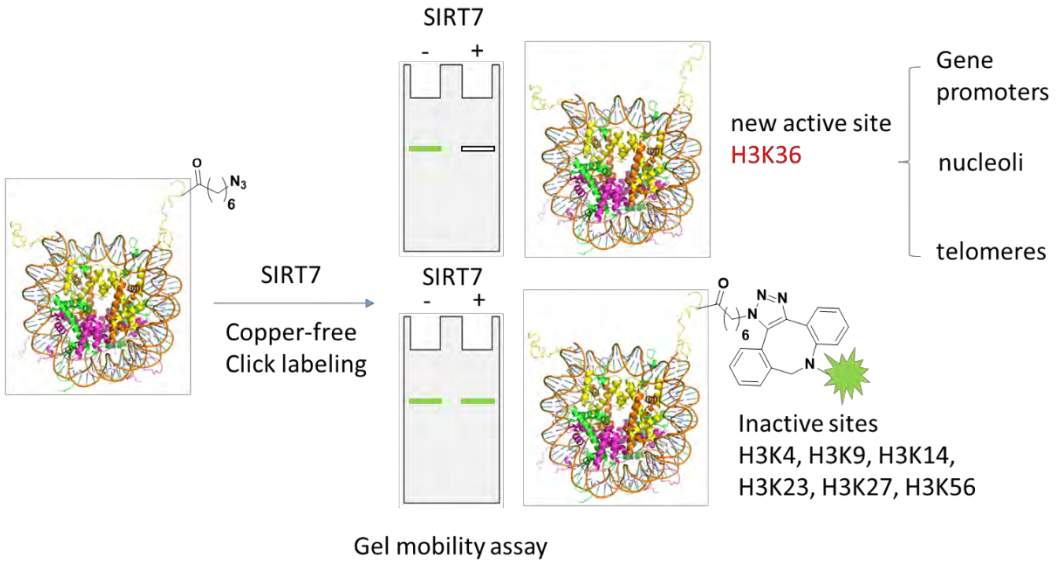
[⊥] *Geriatric Research, Education, and Clinical Center, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304*

As a member of the NAD^+ dependent Sirtuin deacylase family, SIRT7 plays an important role in numerous cellular biological processes *in vivo*, majorly through its deacylation activity toward a wide range of targets, including histone H3.[1] Using acetyl-peptides as substrates, SIRT7 was shown to selectively remove histone H3 K18 acetylation.[2] But recent discoveries call for SIRT7 deacylation target profiling on intact nucleosome substrates, which shows higher activity than peptides or histone complexes.[3]

In this research, we used an engineered pyrrolysyl-tRNA synthetase mutant together with the amber suppressor $tRNA_{CUA}^{Pyl}$, and genetically encoded N^{ε}-(7-azidoheptanoyl)-L-lysine (AzHeK) by amber codon in *Escherichia coli* for recombinant expression of a number of AzHeK-containing histone H3 proteins. Nucleosomes containing AzHeK at different sites were assembled *in vitro* and subjected to a straightforward SIRT7 deacylation assay, in which the reaction products were labelled by a dibenzocyclooctyne (DBCO) conjugated fluorescent dye, electrophoresized and analyzed directly in a native gel. Besides confirming the previously reported H3 K18, we discovered a new and much more active Histone H3 target K36 for SIRT7. The newly discovered K36 was confirmed *in vitro* with acetyl-nucleosomes and *in vivo* with SIRT7 overexpressed cell model. Moreover, SIRT7-deficient cells exhibited H3 K36 hyperacetylation globally at rDNA sequences in nucleoli and at select SIRT7 target loci, demonstrating the physiologic importance of SIRT7 in determining endogenous H3 K36 acetylation levels.



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Mechanistic, Structural, and Spectroscopic Studies of SkfB, a sactipeptide maturase from *Bacillus subtilis*

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Sactipeptides are a subclass of ribosomally synthesized and post-translationally modified peptides (RiPPs). They contain a unique thioether bond, referred to as a sactionine linkage, between the sulfur atom of a cysteine residue and the alpha-carbon of an acceptor residue. These linkages are formed via radical chemistry and are essential for the spermicidal, antifungal, and antibacterial properties of sactipeptides. Enzymes that form these linkages, called sactisynthases, are AdoMet radical enzymes in the SPASM/Twitch subgroup whose structures are incompletely characterized. Here we present the X-ray crystal structure to 1.29-Å resolution,Mössbauer analysis, and a mechanistic study of SkfB, a sactisynthase from *Bacillus subtilis* involved in making sporulation killing factor (SKF). Notably, both crystallography and Mössbauer analyses suggest that SkfB can bind a [2Fe-2S] cluster at the auxiliary cluster site, which has been observed only once before in a SPASM/Twitch auxiliary cluster site. It is well known that Radical SAM enzymes employ a 4Fe–4S cluster to bind and reductively cleave SAM to generate a 5′-deoxyadenosyl radical. SkfB utilizes this radical intermediate to abstract the α-H atom at Met40 to initiate cross-linking. However, the roles of auxiliary cluster sites of SPASM/Twitch domain containing RiPP maturases are not known. We have shown that auxiliary clusters are required for thioether formation in SkfB and other sactipeptide maturases. We made use of a substrate analogue with a cyclopropylglycine (CPG) moiety replacing the wild-type acceptor residue side chain forgoes thioether cross-linking for an alternative radical ring opening reaction. This alternative reactivity also takes place with an SkfB variant in which the auxiliary Fe–S cluster is absent. Therefore, the CPG-containing peptide uncouples H atom abstraction from thioether bond formation, limiting the role of the auxiliary cluster to promoting thioether cross-link formation. CPG proves to be a valuable tool for uncoupling H atom abstraction from peptide modification in RiPP maturases and demonstrates potential to leverage RS enzyme reactivity to create noncanonical amino acids.

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Synthetic Enzymology: Directed Computational Evolution of Quorum-Quenching Lactonases.

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Synthetic Enzymology has been increasingly used to engineer enzymes within biological systems for purposeful function. In this presentation, we will highlight the use of directed computational evolution, a synergy of the use of a generalizable computational protocol and a tunable directed evolution platform, to develop quorum-quenching lactonases for use as anti-virulence therapeutic biomolecules. Although traditional directed evolution platforms have been successfully used in the past to engineer enzymes, the unwieldily large enzyme sequence space limits current efforts in the purposeful design of enzymes. With our current computational strategy, coupled with a robust directed evolution platform, we have been successful in identifying mutagenesis hotspots within our enzyme progenitors, accelerating our efforts to produce enzymes with better substrate binding profiles and enhanced catalytic efficiencies. This will eventually translate into various biomedical and bioremediation efforts currently underway in our laboratory.

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The Alkylquinolone Repertoire of *Pseudomonas aeruginosa* is Linked to Structural Flexibility of the FabH-like Pseudomonas Quinolone Signal Biosynthesis Enzyme PqsBC

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Pseudomonas aeruginosa is a bacterial pathogen that causes life-threatening infections in immunocompromised patients. It produces a large armory of saturated and mono-unsaturated 2-alkyl-4(1H)-quinolones (AQs) and AQ N-oxides (AQNOs) that serve as signaling molecules to control the production of virulence factors. In addition, AQs are involved in membrane vesicle formation and iron chelation, and they also have antibiotic properties. It has been shown that the β-ketoacyl-acyl-carrier protein synthase III (FabH)-like heterodimeric enzyme PqsBC catalyzes the last step in the biosynthesis of the most abundant AQ congener, 2-heptyl-4(1H)-quinolone (HHQ), by condensing octanoyl-coenzyme A (CoA) with 2-aminobenzoylacetate (2-ABA), but the basis for the large number of other AQs/AQNOs produced by *P. aeruginosa* is not known. Here, we demonstrate that PqsBC uses different medium-chain acyl-CoAs to produce various saturated AQs/AQNOs and that it also biosynthesizes mono-unsaturated congeners. Further, we determined the structures of PqsBC in four different crystal forms at 1.5 to 2.7 Å resolution. Together with a structure from a previous report, these data reveal that PqsBC adopts open, intermediate, and closed conformations that alter the shape of the acyl-binding cavity and explain the promiscuity of PqsBC. The different conformations also allow us to propose a model for structural transitions that accompany the catalytic cycle of PqsBC that might have broader implications for other FabH-enzymes, for which such structural transitions have been postulated but have never been observed.

Reference

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