BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ando, Nozomi

eRA COMMONS USER NAME (credential, e.g., agency login): nando1

POSITION TITLE: Associate Professor of Chemistry and Chemical Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology	B.S.	06/01	Physics
Cornell University	M.S.	05/04	Physics
Cornell University	Ph.D.	01/09	Physics
Massachusetts Institute of Technology	Postdoctoral	06/14	Chemistry

A. Personal Statement

The focus of my research program is to understand the molecular mechanisms of protein allostery. To do so, my lab uses X-ray scattering, crystallography, cryo-electron microscopy (cryo-EM), and bioinformatics. We are best known for our innovations in X-ray scattering, which allows us to interpret conformational heterogeneity in terms of protein motions and structural rearrangements (*Chem Rev* 2017). This approach has allowed us to map the conformational landscape of allosteric enzymes and identify evolutionary patterns (*JACS* 2017, *PNAS* 2018, *Nature Comm* 2019). Most recently, we were the first to solve a long-standing problem in X-ray crystallography by explaining the diffuse scattering signal from protein crystals that arise from correlated protein motions (*Nature Comm* 2020, *Nature Comm* 2023). Many of the systems we study are metalloenzymes as they perform reactions of evolutionary significance, and we are highly experienced in anaerobic methods (*PNAS* 2017, *JBC* 2021, *PNAS* 2023). As a recognized expert in the structural biology community, I have served as an elected member of the U.S. National Committee for Crystallography (USNC/Cr) and am currently serving on the leadership for MacCHESS at the Cornell Energy High Synchrotron Source (CHESS) as well as the Northeastern Collaborative Access Team (NE-CAT) at the Advanced Photon Source (APS).

As a faculty member, I have taken on various roles in service to the scientific community. Of these, my most important work has been focused on two areas. As a leader in the X-ray community, I have actively worked to promote the advancement and education of X-ray science and structural biology. As a program chair for the 2020 American Crystallographic Association (ACA) Meeting, I was able to develop a program with a strongly educational theme, geared towards students and postdocs. In 2023, I directed the Erice International School of Crystallography, which drew ~70 students internationally. At every stage of my career, I have also worked to promote the advancement of women and underrepresented groups in STEM. At Princeton, I served in various capacities to assess departmental climate, improve the experience of women faculty, and create an REU program to increase diversity in STEM. Currently, at Cornell Chemistry, I am the co-chair of the diversity, equity, and inclusion (DEI) committee, the faculty advisor for Cornell Chemists for Outreach and Graduate Inclusion (COrGI), and the Director of Graduate Studies.

Ongoing and recently completed projects that I would like to highlight include:

R35 GM124847
Ando (PI)
8/1/2017-7/31/2027
Protein Allostery and Catalysis beyond Bragg Diffraction

R00 GM100008
Ando (PI)
07/01/2014– 07/31/2017
Structural Characterizations of Transient and Heterogeneous Protein Complexes

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2001	Visiting Scholar, Center for Materials Science and Engineering, MIT, Cambridge, MA
2001-2008	Graduate Research Assistant, Department of Physics, Cornell University, Ithaca, NY with Sol M. Gruner (Dept. of Physics and Cornell High Energy Synchrotron Source)
2008-2010	HHMI Postdoctoral Associate, Department of Chemistry, MIT, Cambridge, MA with Catherine L. Drennan (HHMI, Depts. of Chemistry and Biology)
2010-2014	NIH Postdoctoral Fellow, Department of Chemistry, MIT, Cambridge, MA with Catherine L. Drennan (HHMI, Depts. of Chemistry and Biology)
2014-2018	Assistant Professor of Chemistry, Princeton University, Princeton, NJ
2018-2021	Assistant Professor of Chemistry & Chemical Biology, Cornell University, Ithaca, NY
2019-present	Graduate Faculty, Field of Biophysics, Cornell University, Ithaca, NY
2021-present	Associate Professor of Chemistry & Chemical Biology, Cornell University, Ithaca, NY
2022-present	Graduate Faculty, Field of Physics, Cornell University, Ithaca, NY
2024-present	Faculty Fellow, Atkinson Center for Sustainability, Cornell University, Ithaca, NY
2024-present	Director of Graduate Studies, Chemistry & Chemical Biology, Cornell University, Ithaca, NY

Other Experience and Professional Memberships

2006-	Member, Biophysical Society
2007	Mentor, Cornell University Expand Your Horizon Program
2008-	Reviewer for *Science, Nature, Nature Comm, JACS, Biochemistry, IUCr, Biophysical Journal, J Phys Chem, Langmuir, Nat Prod Rev, J Mol Biol, Chemical Science.*
2008	Training in the teaching of writing at the Cornell University Knight Institute
2009	HHMI MIT Mentoring Program in Chemical Biology
2010-2013	Member, American Physical Society
2010-	Member, American Chemical Society
2011-	Member, Protein Society
2011-2013	Elected Member, Cornell High Energy Synchrotron Source Executive User Committee
2014-2018	Proposal Reviewer, Cornell High Energy Synchrotron Source
2016	Organizer, *"Biomolecules in Motion"* Workshop, Cornell High Energy Synchrotron Source.
2016	Session chair, 2016 Diffraction Methods Gordon Research Conference
2016	Session chair, 21st Association for Crystallization Technology Larson Workshop
2017	Session chair, 2017 American Crystallographic Association Meeting
2017	Organizer, *"Measurement and Interpretation of Diffuse Scattering in X-Ray Diffraction for Macromolecular Crystallography"* Workshop, NSLS-II and CFN Meeting
2018	Session chair, 2018 Metallocofactors Gordon Conference
2019-present	Member, Structural Biology Oversight Committee, Cornell Cryo-EM Facility
2019-present	Faculty mentor, Chemical Biology Interface (CBI) Training Program, Cornell University
2019-2021	Elected member, U.S. National Committee for Crystallography (USNC/Cr)
2022-2023	Invited editor, *Methods in Enzymology*

Honors

2007	Best Instrumentation Poster Award, Cornell High Energy Synchrotron Source Users Meeting
2010	National Institutes of Health Ruth L. Kirschstein National Research Service Award (GM090486)
2012	Plenary speaker for 15th International Small Angle Scattering Conference, Sydney
2012	National Institutes of Health Pathway to Independence Award (GM100008)
2017	Invited Author, *Holy Grails in Chemistry* Special Issue of *Acc Chem Res*
2017	Invited Author, *Chemical Reviews*
2017	*Future of Biophysics* Burroughs Wellcome Fund Symposium Lecture, Biophysical Society.
2017	National Institutes of Health Maximizing Investigators' Research Award
2018	Invited Author, *Future of Biochemistry* Special Issue of *Biochemistry*
2020-2021	Program Chair, American Crystallographic Association Meeting
2020	Margaret C. Etter Early Career Award, American Crystallographic Association
2020	National Science Foundation CAREER Award
2020	*Future of Biophysics* Burroughs Wellcome Symposium Lecture, Biophysical Society
2022	Director, Erice International School of Crystallography
2022	Young Investigator Award, The Protein Society
2023	Editor, Methods in Enzymology
2024	Mildren Cohn Young Investigator Award, American Society for Biochem. & Molecular Biology

C. Contributions to Science

1) **Diffuse scattering from correlated motions in protein crystals**: Conventional crystallography involves analyzing sharp diffraction patterns, commonly called Bragg data. However, real crystals are not perfectly periodic and produce additional scattering between the Bragg peaks. This smooth background pattern, known as diffuse scattering, contains information about correlated displacements within the crystal but has been exceeding difficult to measure and interpret. My group is leading the world in the interpretation of macromolecular diffuse scattering, and we are making our software publicly available.
    a. Meisburger SP, Case DA, **Ando N**. (2023) "Robust total X-ray scattering workflow to study correlated motion of proteins in crystals." *Nature Communications* 14, 1228. PMCID: PMC9984388
    b. Meisburger SP, Case DA, **Ando N**. (2020) "Diffuse X-ray scattering from correlated motions in a protein crystal." *Nature Communications* 11, 1271. PMCID: PMC7062842
    c. Meisburger SP, Thomas WC, Watkins MB, **Ando N**. (2017) "X-ray scattering studies of protein structural dynamics." *Chem Rev* **117,** 7615–7672. PMCID: PMC5562295
    d. **Ando N**. Protein Folding & Dynamics Webinar (2021) – recording available online

2) **Evolution of allostery**: Ribonucleotide reductases (RNRs) are essential enzymes for all DNA-based life and have a fascinating evolutionary history that is thought to pre-date the oxygenation of the Earth. Among the RNR family the class Ib RNRs are unusual for two reasons: it lacks a regulatory domain that is prevalent in the rest of the RNR family, and it is found only in bacteria, including a number of well-known human pathogens. Using SAXS, crystallography, and cryo-EM, we discovered that a stunning form of convergent allostery had evolved in this class. Based on this discovery, we conducted a formal investigation of RNR evolution using phylogenetic inference, machine-learning methods, SAXS, and cryo-EM.
    a. Burnim AA, Xu D, Spence MA, Jackson CJ, **Ando N**. (2022) "Analysis of insertions and extensions in the functional evolution of the ribonucleotide reductase family." *Protein Sci*., 31:e4483.
    b. Burnim AA, Spence MA, Xu D, Jackson CJ*, **Ando N***. (2022) "Comprehensive phylogenetic analysis of the ribonucleotide reductase family reveals an ancestral clade and the role of insertions and extensions in diversification." *eLife*, 11: e79790. *co-corresponding.
    c. Thomas WC, Brooks PF, Burnim AA, Bacik J-P, Stubbe J, Kaelber JT, Chen JZ, **Ando N**. (2019) "Convergent allostery in ribonucleotide reductase." *bioRxiv* 504290 doi:10.1101/504290. *Nature Communications* 10, Article number: 2653. PMCID: PMC6572854
    d. **Ando N.** American Crystallographic Association (ACA) Etter Award Talk (2020) – recording available online

3) **Complex metalloenzymes**: My group is interested in understanding how life evolves and adapts to unusual environments. We have used various techniques to study metalloenzymes that perform challenging reactions with biomedical and evolutionary significance.

a. Watkins MB, Wang H, Burnim AA, **Ando N**. "Conformational switching and flexibility in methionine synthase studied by small-angle X-ray scattering and cryo-electron microscopy." (2023) *PNAS* 120, e2302531120. PMCID: PMC10293825
b. Illava G, Gillilan RE, **Ando N**. "Development of in-line anoxic small-angle X-ray scattering and structural characterization of an oxygen-sensing transcriptional regulator." (2023) J. Biol. Chem. 299: 105039. PMCID: PMC10425943
c. Parker MJ, Maggiolo AO, Thomas WC, Kim A, Meisburger SP, **Ando N***, Boal AK*, and Stubbe J*. (2018) "An endogenous dAMP ligand in *Bacillus subtilis* class Ib RNR promotes assembly of a noncanonical dimer for regulation by dATP." *PNAS* **55,** 201800356–10. *co-corresponding. PMCID: PMC5960316
d. Davis KM, Schramma K, Hansen W, Bacik J-P, Khare S, Seyedsayamdost M, **Ando N.** (2017) Structures of the peptide-modifying radical SAM enzyme SuiB elucidate the basis of substrate recognition. *PNAS* **114,** 10420–10425. PMCID: PMC5625900

4) **Service to the structural biology community:** I have a long track record of service to the field of structural biology. In addition to software contributions, a high-pressure small-angle X-ray scattering (SAXS) cell that I designed in my graduate studies has formed the basis for the recently established high-pressure biology (HP-Bio) beamline at the Cornell High Energy Synchrotron Source (CHESS). Notably, we have developed software for deconvolution of SAXS data, such as evolving factor analysis (EFA) and regularized alternating least-squares (REGALS). These and software packages for diffuse scattering data processing are available on our lab GitHub.
    a. Skou S, Gillilan RE, **Ando N.** (2014) "Synchrotron-based small-angle X-ray scattering (SAXS) of biomacromolecules in solution." *Nature Protocols* **9**, 1727–1739. PMCID: PMC4472361
    b. Meisburger SP, Taylor AB, Khan CA, Zhang S, Fitzpatrick PF, **Ando, N**. (2016) "Domain movements upon activation of phenylalanine hydroxylase characterized by crystallography and chromatography-coupled small-angle X-ray scattering." *JACS* **138,** 6506–6516. PMCID: PMC4896396
    c. Meisburger SP, Xu D, **Ando N**. (2021) "*REGALS*: a general method to deconvolve X-ray scattering data from evolving mixtures." *IUCrJ*, 8, 225-23.
    d. Ando Lab GitHub

5) **Allostery in a radical enzyme:** My postdoctoral work on class Ia ribonucleotide reductases (RNRs) set a new precedent for the use of SAXS to study transient and heterogeneous protein complexes. By combining SAXS with other biophysical techniques, we achieved a major milestone in understanding allosteric regulation of the class Ia RNR from *E. coli* and addressed a 50-year old mystery surrounding this complex system. This work has been included in the 6th edition of Lehninger, "Principles of Biochemistry."
    a. **Ando N**, Brignole EJ, Zimanyi CM, Funk MA, Yokoyama K, Asturias FJ, Stubbe J, Drennan CL. (2011) "Structural interconversions modulate activity of *Escherichia coli* ribonucleotide reductase." *PNAS* 108, 21046–21051. PMCID: PMC3248520
    b. Zimanyi CM, **Ando N**, Brignole EJ, Asturias FJ, Stubbe J, Drennan CL. (2012) "Tangled up in knots: Structures of inactivated forms of *E. coli* class Ia ribonucleotide reductase. "*Structure* 20, 1374–1383. PMCID: PMC3459064
    c. Minnihan EC, **Ando N**, Brignole EJ, Olshansky L, Chittuluru J, Asturias FJ, Drennan CL, Nocera D, Stubbe J. (2013) "Generation of a stable, aminotyrosyl radical-induced α2β2 complex of *Escherichia coli* class Ia ribonucleotide reductase." *PNAS* 110, 3835–3840. PMCID: PMC3593893
    d. **Ando N*,** Li H, Brignole EJ, Thompson S, McLaughlin MI, Page JE, Asturias FJ, Stubbe J, Drennan CL. "Allosteric inhibition of human ribonucleotide reductase by dATP entails the stabilization of a hexamer." 55, 373–381 (2016). *co-corresponding. PMCID: PMC4722859

Complete List of Published Work in Google Scholar:
https://scholar.google.com/citations?user=v-MyIFAAAAAJ&hl=en

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Ruma Banerjee, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): rbanerje

POSITION TITLE: Vincent Massey Collegiate Professor of Biological Chemistry

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Delhi University, Delhi, India	BS	1980	Botany
Delhi University, Delhi, India	MS	1982	Botany
Rensselaer Polytechnic Institute, N.Y.	Ph.D.	1987	Biochemistry
University of Michigan, Ann Arbor, MI	Postdoc	1991	Biophysics

A. Personal Statement

My research focuses on the enzymes, coenzymes and metabolic pathways that support and interact with the sulfur network in mammals. The enzymes that we study are involved in the biogenesis and oxidation of hydrogen sulfide (H_2S), and in the assimilation, trafficking and utilization of the essential B_{12} cofactor. The coenzymes that we study include vitamins B_2 (flavin), B_6 (pyridoxal phosphate), B_{12} (cobalamin) and heme, which support key junction enzymes that commit the flow of sulfur to metabolic tributaries and are major hubs of regulation. The metabolic pathways that we study are connected via H_2S signaling and include redox, oxygen, central carbon, nucleotide and lipid metabolism. Our contributions to science are characterized by their rigor and their breadth, as we integrate clinical, animal model, cellular and molecular data to tackle fundamental questions in the field. Collaborations with over three dozen investigators in four continents have contributed to broadening my research program. Throughout my career, I have been devoted to mentoring students (28 graduate and 51 undergraduate) and postdoctoral fellows (46) who have gone on to successful careers in various scientific spheres. The 2022 UROP outstanding mentor award, was both deeply meaningful and humbling. I have also been an active mentor to junior faculty, first at the NIH-funded Redox Biology Center (which I founded at the University of Nebraska) and later, through my participation in junior faculty mentoring workshops at the local (Michigan Medicine) and national (ASBMB) levels. I am committed to building and nurturing diversity in the biomedical sciences and have been active with ASBMB's NSF-funded IMAGE grant writing workshop, which provides mentorship to junior faculty and senior postdocs. I am the founding co-Director of the NIH-funded ASBMB MOSAIC program to provide culturally sensitive coaching and career development opportunities to diverse K99/R00 scholars and also am actively involved in the NIH-funded FIRST program at the University of Michigan to increase faculty diversity in the biomedical and health sciences. I am committed to providing an inclusive, and safe training environment that invites diverse perspectives. With an H-index of 94, the following sampling of recent reviews and articles (out of 295 total) captures areas in which we have made significant intellectual contributions while in Section C, our contributions to B_{12} biochemistry and sulfide homeostasis and signaling are described.

a. Banerjee, R., Gouda, H. and Pillay, S. (2021) Redox-linked coordination chemistry directs B_{12} trafficking. ***Acc Chem Rev*** 54(8), 2003-2013. *PMC8142554*

b. Filipovic, M.R., Zivanovic, J., Alvarez, B. and Banerjee, R. (2018) Chemical biology of H_2S signaling through persulfidation. ***Chem Rev*** 118:1253-1337*, PMC6029264*

c. Mishanina, T., Libiad, M. and Banerjee, R. (2015) Biogenesis of reactive sulfur species for signaling by sulfide oxidation pathways. ***Nature Chem Biol.*** 11(7):457-64*, PMC4818113*
d. Banerjee, R. and Ragsdale, S.W. (2003) The many faces of cobalamin: Catalysis by B_{12}-dependent enzymes, ***Ann. Rev. Biochem****.* 72:209-247

Active research support

RO1-DK45776 (NIH) 2/1/23-1/31/28
B_{12} Trafficking and Birth Defects
Overall Project Goals. To investigate the biochemistry of B_{12} trafficking and to characterize pathogenic mutations that lead to inborn errors of cobalamin metabolism.
Role: P.I.

R35-GM130183 (NIH) 1/1/24-12/31/28
Sulfide Metabolism and Signaling
Overall Project Goals: To investigate the role of H_2S homeostasis in cellular metabolism and redox signaling.
Role: P.I.

U54 CA280805-01 (NIH) 06/15/2023-06/14/2028
Michigan Program for Advancing Cultural Transformation (M-PACT) in Biomedical and Health Sciences
Overall Project Goals: This is an NIH FIRST grant to recruit 30 new faculty and support their success as independent investigators as well as to build a sustainable scientific community that is diverse, equitable, inclusive, and welcoming.
Role: Faculty Lead

B. Positions, Scientific Appointments, and Honors
Positions

1989-1991	Lecturer, Department of Biological Chemistry, University of Michigan
1991-1996	Assistant Professor, Biochemistry Department, University of Nebraska-Lincoln
1997-1999	Associate Professor, Biochemistry Department, University of Nebraska-Lincoln
2000-present	Professor, Biochemistry Department, University of Nebraska-Lincoln
2001	Acting Head, Department of Biochemistry, University of Nebraska-Lincoln
2002-2007	Director, Nebraska Redox Biology Center
2007-present	Vincent Massey Collegiate Professor of Biological Chemistry, Univ. of Michigan
2008-2019	Associate Chair, Department of Biological Chemistry, Univ. of Michigan
2020-present	Co-Director, ASBMB MOSAIC program
2022	Visiting Scientist, Massachusetts General Hospital, Harvard Medical School

Scientific Appointments

2020-present	Editorial Board, *Biochemistry*
2019-present	Member, Editorial Board, *Trends in Chemistry*
2019-present	Member, Minority Affairs Committee, ASBMB
2013-2024	Academic Council Member, Ashoka University, Delhi
2012-2022	Associate Editor, *Chemical Reviews*

2012-2022 Associate Editor, *Journal of Biological Chemistry*
2010-2020 Editorial Board, *Antioxidants and Redox Signaling*
2011-2015 Member, NIH MSFE Study Section
2009-2012 Council Member, ASBMB
2001-2012 Editorial Advisory Board, *Chemical Reviews*
2006-2009 Member, Award Selection Committee, American Chemical Society (Biol. Chem. Div.)
2006-2008 Member, ACS Committee on Professional Training
2005-2008 Editorial Board, *J. Inorg. Biochemistry*
2005-2008 Member, Publications Committee, ASBMB
2003-2005 Alternate Councilor, American Chemical Society (Biol. Chem. Div.)
1998-2003 Member, Biochemistry Study Section, NIH
2001 Member, Nominating Committee, American Chemical Society (Biol. Chem. Div.)

Honors

2023 Elected Member, American Academy of Arts and Sciences
2022 UROP Outstanding Mentor Award, University of Michigan
2022 David Green Lecturer, University of Wisconsin, Madison
2021 Elected Fellow, ASBMB
2019 Merck Award, ASBMB
2015 Endowment for Basic Sciences Recognition Award, University of Michigan
2013 Chair, NIH MSFA Study Section
2011 Elected Fellow, AAAS
2008 Chair, FASEB Conference of Folic Acid, B_{12} and One Carbon Metabolism
2008 Chair, Gordon Research Conference on Thiol-Based Redox Regulation and Signaling
2007 Vincent Massey Collegiate Professor of Biological Chemistry, University of Michigan
2006 Outstanding Research and Creative Activity Award, University of Nebraska
2006 Seth G.S. Medical College and K.E.M. Hospital Oration Award, Assoc. Clin. Biochemists, India
2006 Shorb Lecturer, University of Maryland
2003 George Holmes Distinguished University Professor of Biochemistry
2003 Chair, Gordon Research Conference on Cobalamins
2002-'07 Willa Cather Professor of Biochemistry
2001 Pfizer Award, American Chemical Society
2001 Co-Chair, Gordon Research Conference on Enzymes, Coenzymes & Metabolic Pathways
2001 Established Investigator, American Heart Association

C. Contributions to Science

1. *Reaction Mechanism and Regulation of B_{12} Enzymes*-B_{12} is a rare but essential vitamin utilized by only two mammalian enzymes that catalyze chemically disparate reactions, involving heterolytic versus homolytic cleavage of the cofactor's reactive cobalt-carbon bond. My early publications revealed how methylmalonyl-CoA mutase uses kinetic coupling as a strategy to control radical reactivity and quantum tunneling to traverse the H-atom transfer reaction coordinate. We have also studied two bacterial B_{12} enzymes with biotechnological potential that interconvert long-chain acyl-CoA substrates. These are IcmF, a fusion between isobutyryl-CoA mutase and it G-protein chaperone, and PCM, pivalyl-CoA mutase, which metabolizes tertiary carbons. We discovered that B_{12} translationally up-regulates human methionine synthase, which plays a key role in methylation homeostasis and in regulating cellular folate availability. We found that regulatory elements in the upstream open reading frame and an internal ribosome entry site are used for B_{12}-responsive regulation. We demonstrated that itaconyl-CoA, which is predicted to rise in individuals with CLYBL (citramalyl-CoA lyase) deficiency and is found in 2.7% of the population, rapidly inactivates methylmalonyl-CoA mutase. In elucidating the radical addition mechanism by which itaconyl-CoA inactivates the mutase, we captured crystallographically, the elusive 5'-deoxydenosyl radical, which is the workhorse of all coenzyme B_{12}-dependent enzymes.

a. Chowdhury, S. and Banerjee, R. (2000) Evidence for quantum mechanical tunneling in the coupled cobalt carbon bond homolysis-substrate radical generation step catalyzed by methylmalonyl-CoA mutase. ***J. Am. Chem.* Soc**. 122:5417-5418.

b. Li, Z., Kitanishi, K., Twahir, U.T., Cracan, V., Chapman, D., Warncke, K., Banerjee, R. (2017) Cofactor editing by the G-protein metallochapeone domain regulates the radical B_{12} enzyme IcmF. ***J Biol Chem*** 292:3977-

87. *PMC5354503* ***Editor's Pick***

c. Shen, H., Campanello, G., Flicker, D., Grabarek, Z., Hu, J., Luo, C., Banerjee, R. and Mootha, V.K. (2017) The human knockout gene CLYBL connects itaconate to vitamin B_{12} ***Cell*** *171: 771-782.e11*. *PMC5827971*

Highlighted in*:* Michael A. Reid, Jihye Paik, Jason W. Locasale, A Missing link to vitamin B_{12} metabolism, ***Cell***, *171, 736-737*

d. Ruetz, M., Campanello, G. C., Purchal, M., Shen, H., McDevitt, L., Gouda, H., Wakbayashi, S., Zhu, J., Rubin, E.J., Warncke, K., Mootha, V.K., Koutmos, M., and Banerjee, R. (2019) Itaconyl-CoA forms a stable biradical in methylmalonyl-CoA mutase and derails its activity and repair. ***Science*** 366:589-593 *PMC7070230*

Perspective in*:* Boal A. (2019) The immune system mimics a pathogen ***Science*** 366:574-575

2. *B_{12} Trafficking Pathway*-Although only two mammalian enzymes rely on B_{12} for activity, an elaborate network of chaperones shepherd the cofactor from its point of entry into cells to the two target enzymes that reside in the mitochondrion and in the cytoplasm, respectively. As the genes encoding the B_{12} chaperones have been described, our laboratory has been primarily responsible for ascribing functions, and helping explain clinical observations on patient responsiveness to different cofactor forms. Our functional studies are providing fascinating insights into novel strategies used to exert control over B_{12} reactivity via redox-linked coordination chemistry, and to facilitate transfer through direct protein-protein interactions. They are also revealing intricate inter-protein signaling mechanisms that operate to load and unload the cofactor to and from target enzymes. These studies are richly supported by crystallographic, spectroscopic and EM analyses.

a. Padovani, D., Labunska, T., Palfey, B.A., Ballou, D. P. and Banerjee, R. (2008) Adenosyltransferase tailors and delivers coenzyme B_{12}. ***Nature Chem. Biol****.,* 4(3):194-6

Highlighted in Bandarian V. Delivery of tailor-made cobalamin to methylmalonyl-CoA mutase. ***Nat Chem Biol***. 2008 Mar;4(3):158-9.

b. Lofgren, M., Padovani, D., Koutmos, M and Banerjee, R (2013) A switch III motif relays signaling between a B_{12} enzyme and its G-protein chaperone, ***Nat Chem. Biol.*** 9(9):535-9. *PMC3752380*

Commentary in: Toraya, T. (2013) G-protein signaling: A switch saves B_{12} radical status, ***Nat Chem. Biol.*** 9:530-1

c. Campanello, G.C., Ruetz, M., Dodge, G.J., Gouda, H., Gupta, A., Twahir, U.T., Killian, M. M., Watkins, D., Rosenblatt, D.S., Brunold, T. C., Warncke, K., Smith, J.L., Banerjee, R. (2018) Sacrificial cobalt-carbon bond homolysis in coenzyme B_{12} as a cofactor conservation strategy, ***J Am Chem Soc*** 140(41):13205-13208. *PMC6743335*. ***Communication highlighted as a spotlight***

d. Mascarenhas, R., Ruetz, M., Gouda, H., Heitman, N., Yaw, M., and Banerjee, R. (2023) Architecture of the human G-protein-methylmalonyl-CoA mutase nanoassembly for cofactor delivery and repair, ***Nature Comm*** Jul 19;14(1):4332 10.1038/s41467-023-40077-4. *PMC10356863*

3. *Sulfur-based Redox Homeostasis-*The mammalian sulfur network controls the flow of sulfur-containing amino acids into important cellular reagents: S-adenosylmethionine, glutathione, taurine and H_2S. My laboratory has been studying the reaction mechanisms and regulation of enzymes involved at key junction points in the sulfur metabolic network, using a combination of kinetic, structural, cellular and computational approaches. We have demonstrated that intracellular sulfur metabolism is key to controlling ambient redox poise inside and outside cells, which is used for signaling between cells in the immune and neuro-immune systems. We have developed a mathematical model for describing the regulatory switches that control low versus high flux of sulfur in response to availability and thus buffer circulating sulfur concentrations against large fluctuations. We complement theory with experiment to understand regulation of sulfur-based redox homeostasis at the macromolecular, cellular and organismal levels.

a. Mosharov, E. Cranford, M. and Banerjee, R. (2000) The quantitatively important relationship between homocysteine metabolism and glutathione synthesis by the transsulfuration pathway and its regulation by redox changes. ***Biochemistry*** 39*,* 13005-13011

b. Prudova, A., Bauman, Z., Braun, A., Vitvitsky, V., Lu, S. and Banerjee, R. (2006) AdoMet stabilizes Cystathionine β-synthase and modulates redox capacity: Relevance to Liver Disease, ***Proc. Nat. Acad. Sci****.* 103: 6489-94

c. Yan, Z., Garg, S., Kipnis, J. and Banerjee, R (2009) Modulation of extracellular redox remodeling by Regulatory T cells, ***Nature Chem. Biol****.* 5, 721 – 723, *PMC2760945*

Highlighted in News and Views by Rubartelli, A. and Sitia, *R.* ***Nature Chem. Biol****. (2009)* Chemo-metabolic regulation of immune responses by Tregs, 5: 709 – 710

d. Vitvitsky, V., Kumar, R., Libiad, M., Maebius, A., Landry, A.P. and Banerjee, R. (2021) The mitochondrial NADH pool is involved in hydrogen sulfide signaling and stimulation of aerobic glycolysis, ***J Biol Chem*** 296:100736, *PMC8165552*

4. *Enzymology of H_2S Signaling*-H_2S is a signaling molecule that affects physiological processes ranging from inflammation to cardiovascular function. My laboratory is investigating the enzymology of H_2S biogenesis and decay and the mechanism of sulfide-based signaling. We are elucidating mechanisms by which H_2S producing enzymes, which show substrate ambiguity and chemical promiscuity, control H_2S versus persulfide production. We discovered that the molecular mechanism of allosteric regulation in a key H_2S producing enzyme cystathionine beta-synthase, involves a regulatory heme and occurs via long-range signal transduction that controls the tautomeric state of the PLP cofactor in the active site. We also discovered that human sulfide quinone oxidoreductase utilizes a novel trisulfide redox motif to enhance sulfide oxidation. Finally, we have uncovered that the mammalian electron transport chain is plastic and opens up a new route for sulfide detoxification via reversal of complex II and utilization of fumarate as a terminal electron acceptor.

a. Landry, A.P., Moon, S., Kim, H., Yadav, P.K., Guha, A., Soo-Cho, U., Banerjee, R. (2019) A catalytic trisulfide in human sulfide quinone oxidoreductase catalyzes coenzyme A persulfide and synthesis and inhibits butyrate oxidation. ***Cell Chem Biol*** S2451-9456(19): 30315-0. *PMC6906606.* ***Cover article***

b. Kumar, R., Landry, A.P., Guha, A., Vitvitsky, V., Lee, H-J., Seike, K, Reddy, P., Lyssiotis, C.A. and Banerjee, R. (2022) A redox cycle with complex II promotes sulfide quinone oxidoreductase dependent H_2S oxidation, ***J Biol Chem*** 298(1):101435, *PMC8683732*
Selected as Editor's Pick and EP Highlight

c. Hanna, D.A., Diessl, J., Guha, A., Kumar, R., Andren, A., Lyssiotis, C. and Banerjee, R. (2024) H_2S preconditioning induces long-lived perturbations in O_2 metabolism. ***Proc Natl Acad Sci***, 121:e2319473121. *PMC10962982*

d. Kumar, R., Vitvitsky, V., Sethaudom, A., Singhal, R., Solanki, S., Alibeckhoff, S., Hiraki, H.L., Bell, H.N., Andren, A., Singhal, R., Baker, B.M., Lyssiotis, C.A., Shah, Y.M. and Banerjee, R. (2024) Sulfide oxidation promotes hypoxic angiogenesis and neovascularization. ***Nature Chem Biol*** 20(10):1294-1304. *PMC11584973*

BIOGRAPHICAL SKETCH

NAME: Ruetz, Markus

eRA COMMONS USER NAME (credential, e.g., agency login):mruetz

POSITION TITLE: Research assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Innsbruck, Innsbruck, Austria	Master	08/2008	Chemistry
University of Innsbruck, Innsbruck, Austria	PhD	03/2013	Chemistry
University of Innsbruck, Innsbruck, Austria	postdoctoral training	03/2014	Chemistry
University of Michigan, Ann Arbor, USA	postdoctoral training	03/2019	Biochemistry

A. Personal Statement

My research interests lie at the chemistry and biochemistry interface with a focus on vitamin B_{12}. Over the years, my research has encompassed many aspects of the chemical biology of this important cofactor, ranging from the synthesis of antivitamin B_{12} derivatives to the biochemical characterization of vitamin B_{12} dependent enzymes and chaperones. Vitamin B_{12} (cobalamin), described as the most beautiful cofactor in nature, presents a number of challenging chemical and biochemical facets for study, many of which remain to be realized. As an undergraduate and graduate student, I trained with Dr. Bernhard Kräutler at the University of Innsbruck on the synthesis of vitamin B_{12} derivatives with inert ligands (antivitamin B_{12}s) and characterized vitamin B_{12} degradation products. During my postdoctoral training under the supervision of Dr. Ruma Banerjee at the University of Michigan, I worked on intracellular B_{12} trafficking proteins. Specifically, I focused on the mitochondrial trafficking and target proteins: methylmalonyl CoA mutase (MMUT), the G-protein chaperone MMAA and the adenosyl transferase, MMAB. The GTPase activity of MMAA controls the movement of cobalamin cofactor to and from MUT via several “switch” elements. I showed that in addition to the canonical switch elements, the oligomeric state of human CblA regulates function, which can be compromised by clinical mutations. My recent study revealed that in the crystal structure of human MMUT in complex with MMAA, MMUT undergoes significant conformational changes, notably with the B_{12} binding domain rotating approximately 180 degrees. This rotation exposes the B_{12} domain to the solvent, which is proposed to facilitate the cofactor transfer from MMAB. I also characterized the *Mycobacterium tuberculosis* homologs of the human mitochondrial B_{12} proteins and chaperones and characterized the molecular mechanism by which the immunometabolite, itaconyl CoA, inhibits propionate metabolism. This study led to the capture of the 5’-deoxyadenosyl radical, >60 years after the discovery of coenzyme B_{12} and to its detailed biophysical and crystallographic characterization. A sampling of high impact publications that have resulted from my research on B_{12} chemical biology are listed below.

a. Mascarenhas, R*. **Ruetz, M***. Gouda, H. Heitman, N.;Yaw, M. Banerjee, R., Architecture of the human G-protein-methylmalonyl-CoA mutase nanoassembly for B_{12} delivery and repair. *Nat Commun* **2023,** *14* (1), 4332. PMC10356863 *co-first authors

b. **Ruetz M**, Campanello GC, Purchal M., Shen H., McDevitt L, Gouda H., Wakabayashi S, Zhu J, Rubin E.J., Warncke K., Mootha V.K., Koutmos M., Banerjee R; Itaconyl-CoA forms a stable biradical in methylmalonyl-CoA mutase and derails its activity and repair. ***Science***. 366(6465): 589 – 593, 2019, PMC7070230

Perspective in**:* Boal A. (2019) The immune system mimics a pathogen ***Science 366:574-575

c. Mascarenhas R*, **Ruetz M***, McDevitt L, Koutmos M, Banerjee R. Mobile loop dynamics in adenosyltransferase control binding and reactivity of coenzyme B_{12} **Proc Natl Acad Sci U S A**. 117(48):30412-30422, 2020 PMC: 7720225. *co-first authors

d. **Ruetz M**, Campanello GC, McDevitt L, Yokom AL, Yadav PK, Watkins D, Rosenblatt DS, Ohi MD, Southworth DR, Banerjee R; Allosteric Regulation of Oligomerization by a B_{12} Trafficking G-Protein is Corrupted in Methmalonic Acidurea. ***Cell Chem. Biol***. 26(7): 960-969, 2019.PMC6642015

e. **Ruetz M**, Gherasim C, Gruber K, Fedosov SN, Banerjee R, Kräutler B: Radical synthesis opens access to organometallic aryl-cobaltcorrins - 4-ethylphenyl-cobalamin, a potential 'antivitamin-B_{12}', ***Angew. Chem. Int. Ed***. ***Engl.*** 52(9): 2606-2610, 2013. PMC3843227
Selected as "hot paper" and on inside cover

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2007 - 2008	Teaching assistant, University of Innsbruck
2009 - 2013	University assistant, University of Innsbruck
2013 - 2014	Postdoctoral researcher, University of Innsbruck
2014 - 2019	Postdoctoral researcher, University of Michigan
2019 - 2020	Research Lab Specialist, University of Michigan
2020 - 2024	Research Investigator, University of Michigan
09/2024	Research Assistant Professor

Honors

07/2012	Invited Speaker at the 7th International Conference on Porphyrins and Phtalocyanines
08/2020	Invited Speaker at FASEB virtual The Folic Acid, Vitamin B12 and One-Carbon Metabolism Conference

Other Experience and Professional Memberships

2007-present	Member, Austrian Chemical Society
2017-present	Member, American Society of Biochemistry and Molecular Biology

C. Contributions to Science

1. **Early Career:** My early career contributions were focused on the synthesis of a vitamin B_{12} derivative with a modification in the linker region of vitamin B_{12} that connects the intramolecular coordinating dimethyl benzimidazole base with the corrin ring. It led to the following publication.
    a. Murtaza S, **Ruetz M**, Gruber K, Kräutler B, Isovitamin B_{12}: A vitamin B_{12} derivative that flips its tail. ***Chemistry***, 16(36): 10984-10988, 2010. PM20690123

2. **Graduate Career:** My graduate research contributions focused on the development of synthetic routes for cobalamins with an 'inert' organic ligand. The physiologically relevant forms of vitamin B_{12} are methylcobalamin and 5'-deoxyadenosylcobalamin with a cobalt-bound methyl- or 5'-deoxyadenosyl group, respectively. In mammals, these ligands are removed via an SN_2 reaction catalyzed by MMACHC (also known as CblC), a cytosolic enzyme that converts dietary cobalamins into a common intermediate for the downstream enzymes, methionine synthase and methylmalonyl-CoA mutase. I synthesized cobalamin derivatives with aryl ligands using radical chemistry. Cob(II)alamin with its unpaired electron on the cobalt center rapidly reacts with phenyl radicals (generated from aryl diazonium or iodonium salts). In collaboration with Dr. Ruma Banerjee, we showed that aryl cobalamins bind to human CblC, but are not further processed. The inhibition of CblC led to vitamin B_{12} deficiency, which was demonstrated in mice in collaboration with Dr. Ebba Nexo. Publications describing these results are listed below.
    a. **Ruetz M**, Gherasim C, Gruber K, Fedosov SN, Banerjee R, Kräutler B: Radical synthesis opens access to organometallic aryl-cobaltcorrins - 4-ethylphenyl-cobalamin, a potential 'antivitamin-B_{12}', ***Angew. Chem. Int. Ed. Engl***., 52(9): 2606-2610, 2013. PMC3843227

b. **Ruetz M**, Salchner R, Wurst K, Fedosov SN, Kräutler B: Phenylethynylcobalamin: A Light-Stable and Thermolysis-Resistant Organometallic Vitamin B12 Derivative Prepared by Radical Synthesis. ***Angew. Chem. Int. Ed. Engl***., 52(43): 11406-11409, 2013. PM24030966
c. Mutti E, **Ruetz M**, Birn H, Kräutler B, Nexo E: 4-Ethylphenyl-Cobalamin Impairs Tissue Uptake of Vitamin B_{12} and Causes Vitamin B_{12} Deficiency in Mice. ***PLoS ONE***, 8(9): e75312, 2013. PMC3779197

Another topic of my graduate research was the structural characterization of a vitamin B_{12} degradation product with an unusual blue color. These studies were performed in collaboration with Dr. Sergey Fedosov (Aarhus University). Using UV/visible, NMR spectroscopy, mass spectrometry as well as X-ray analysis, the structure of the blue corrinoid was solved and shown to contain a cleaved C-C bond in the periphery of the corrin ring with a newly formed double bond and a carbonyl function. The two moieties are within van der Waals contact and electrochemical reduction of the blue corrinoid allowed reconstitution of the corrin ring.

a. Fedosov SN, **Ruetz M**, Gruber K, Fedosova NU, Kräutler B: A blue corrinoid from partial degradation of vitamin B_{12} in aqueous bicarbonate - Spectra, structure and interaction with proteins of B_{12}-transport, ***Biochemistry***, 50(37): 8090-8101, 2011. PM21851077
b. **Ruetz M**, Fedosov SN, Kräutler B: Reconstitution of the B_{12} macrocycle by radical ring closure of a blue secocorrin, ***Angew. Chem. Int. Ed. Engl***. 51(27): 6780-6784, 2012. PM22700309
Selected as Inside cover

3. **Postdoctoral Career:** As a post-doctoral fellow, I joined the laboratory of Dr. Ruma Banerjee (University of Michigan) working on the intracellular trafficking of vitamin B_{12}. Mutations in the B_{12} trafficking proteins lead to severe pathological consequences. My studies focused on the mitochondrial branch of the B_{12} trafficking pathway. Key contributions from my research included the elucidation of the inactivation mechanism of human methylmalonyl CoA mutase (MMUT) as well as the homolog from *Mycobacterium tuberculosis* by itaconyl CoA, the coenzyme A form of the immunomodulatory metabolite, itaconate. Structural and spectroscopic studies revealed that the elusive 5'-deoxyadenosyl radical involved in catalysis was trapped by itaconyl-CoA. I characterized clinical mutations in human MMAA, a G-protein chaperone that regulates the cofactor transfer between MMUT and the adenosyl transferase MMAB, via three "switch" elements. Mutations in switch III in MMAA not only impair cofactor shuttling between MMAB and MMUT but also perturb the oligomeric distribution of the MMUT-MMAA complex. MMAA synthesizes adenosylcobalamin via a nucleophilic substitution reaction using ATP and cob(I)alamin. I helped elucidate the mechanism by which ATR catalyzes the homolytic cleavage of the cobalt-carbon in an unusual reversal of the nucleophilic chemistry that it uses to make the same bond, when the acceptor protein MCM is unavailable. The homolysis product cob(II)alamin, is more tightly bound than adenosylcobalamin, facilitating retention of the high-value cofactor. I was also involved in demystifying the thiol oxidase activity of *Caenorhabditis elegans* CblC, the B_{12} processing protein in the early steps of B_{12} trafficking. A sampling of publications from my postdoctoral work is listed below.

a. **Ruetz M**, Campanello GC, Purchal M., Shen H., McDevitt L, Gouda H., Wakabayashi S, Zhu J, Rubin E.J., Warncke K., Mootha V.K., Koutmos M., Banerjee R; Itaconyl-CoA forms a stable biradical in methylmalonyl-CoA mutase and derails its activity and repair. ***Science***. 366(6465): 589 – 593, 2019, PMC70702320
Perspective in*:* Boal A. (2019) The immune system mimics a pathogen ***Science*** 366:574-575
b. **Ruetz M**, Campanello GC, McDevitt L, Yokom AL, Yadav PK, Watkins D, Rosenblatt DS, Ohi MD, Southworth DR, Banerjee R; Allosteric Regulation of Oligomerization by a B_{12} Trafficking G-Protein is Corrupted in Methmalonic Acidurea. ***Cell Chem. Biol***. 26(7): 960-969, 2019. PMC6642015
c. Campanello, G.C., **Ruetz, M**., Dodge, G.J., Gouda, H., Gupta, A., Twahir, U.T., Killian,M. M., Watkins, D., Rosenblatt, D.S., Brunold, T. C., Warncke, K., Smith, J.L., Banerjee, R. (2018) Sacrificial cobalt-carbon bond homolysis in coenzyme B_{12} as a cofactor conservation strategy, ***J. Am. Chem. Soc.*** 140(41):13205-13208. ***Communication*** PMC6743335
Highlighted as a spotlight
d. **Ruetz M**, Kumutima J, Lewis BE, Filipovic MR, Lehnert N, Stemmler TL, Banerjee R. A distal ligand mutes the interaction of hydrogen sulfide with human neuroglobin. ***J. Biol. Chem***. 292(16): 6512-6528, 2017. PMC5399104

4. **Present Career.** I continued my research on human mitochondrial vitamin B12 trafficking proteins and enzymes as well as their *M. tuberculosis* homologues. The mechanism by which MMAB signals that its cofactor cargo is ready (AdoCbl) or not [cob(II)alamin] for transfer to MMUT, is not known. Biochemical and structural studies of the *M. tuberculosis* homolog of MMAB revealed ligand-induced ordering of the N-terminus, which organizes a dynamic cobalamin binding site and exerts exquisite control over coordination geometry, reactivity, and solvent accessibility. This study also highlights the importance of the lower axial ligand in vitamin B_{12} (dimethylbenzimidazole) in cofactor handover between proteins. In my previous studies, I made significant strides in understanding the MMUT and MMAA complex, which exists as an intricate oligomeric mixture ranging from linear 1:1 MMUT:MMAA (= M_1C_1) to annular (M_2C_2, M_3C_3) complexes. However, obtaining detailed structural insights proved challenging due to inherent heterogeneity within the complexes. Nonetheless, I successfully established specific conditions that allowed me to isolate and crystallize a homogenous M_2C_2 complex. The crystallographic analysis of this M_2C_2 complex yielded surprising results, revealing substantial conformational changes in both MMUT and MMAA. These findings shed light on the complex's dynamic nature and provided deeper insights into its functional mechanism. Moreover, the physiological significance of the $_{M2C2}$ complex was underscored by pathogenic patient mutations found in the MMUT-MMAA interface. These mutations hindered complex formation and consequently disrupted the efficient transfer of the cofactor, highlighting the importance of the complex for proper cellular functioning.
   Rhodibalamins, the rhodium analogues of vitamin B_{12}, are known as antimetabolites due to their antibacterial properties. My research demonstrated that these analogues bind effectively to human B_{12} chaperones MMACHC and MMAB, as well as to human and Mtb MMUT, without impairment. However, the rhodium-carbon bond in these complexes showed resistance to both homolytic and heterolytic cleavage. The structure of *Mtb* MMAB bound to adenosylrhodibalamin and inorganic triphosphate revealed a weakened yet intact Rh-C bond. This study highlights that while replacing cobalt with rhodium maintains structural mimicry, it compromises the chemical reactivity. This characteristic could be exploited to target human and bacterial B_{12} chaperones and enzymes effectively.

    a. Mascarenhas R*, **Ruetz M***, McDevitt L, Koutmos M, Banerjee R. Mobile loop dynamics in adenosyltransferase control binding and reactivity of coenzyme B12. **Proc Natl Acad Sci U S A**. 117(48):30412-30422, 2020 PMC: 7720225. *co-first authors
    b. Mascarenhas, R*. **Ruetz, M***. Gouda, H. Heitman, N.;Yaw, M. Banerjee, R., Architecture of the human G-protein-methylmalonyl-CoA mutase nanoassembly for B_{12} delivery and repair. **Nat Commun** *14* (1), 4332, 2023. PMC10356863 *co-first authors
    c. **Ruetz M***, Mascarenhas R, Widner F, Kieninger C, Koutmos M, Kräutler B*, Banerjee R. A Noble Metal Substitution Leads to B_{12} Cofactor Mimicry by a Rhodibalamin. **Biochemistry**, 63(15):1955-1962, 2024. PMC11540531 *corresponding author

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/markus.ruetz.2/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support
None

Completed Research Support
None