BIOGRAPHICAL SKETCH

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NAME: Rousseau, Denis Lawrence

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

POSITION TITLE: Professor and University Chair

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
Bowdoin College, Brunswick, ME	BA	06/1962	Chemistry
Princeton University, Princeton, NJ	MS	06/1964	Physical Chemistry
Princeton University, Princeton, NJ	PhD	06/1967	Physical Chemistry
University of Southern California, Los Angeles, CA	Post-doc	09/1969	Physics

A. Personal Statement

I am a Professor of Biochemistry and was formerly the University Chair of the Department of Physiology and Biophysics. I have extensive knowledge of the structure, function and spectroscopy of many different heme proteins, especially cytochrome *c* oxidase (C*c*O), which I have been studying for several years. My group has been a leader in using innovative approaches to address important biochemical questions including structure/function studies of hemoglobin, nitric oxide synthase, heme oxygenase and protein folding. All of the studies were supported by NIH grants on which I was the PI. I developed rapid mixing Raman difference spectroscopy to determine the ligand structures during the catalytic reaction of bovine C*c*O (bC*c*O) which has resulted in the identification of all of the intermediates in the oxygen reduction reaction. I recently initiated the use of Serial Femtosecond X-ray Crystallography (SFX), with an X-ray Free Electron Laser (XFEL), to solve several crystal structures of bovine C*c*O (bC*c*O), including equilibrium states, transient intermediates and photoproducts. These studies have allowed us to formulate a new mechanism of how the redox chemistry is coupled to proton translocation. Recognizing the power of single particle cryo-EM to complement the crystallographic studies, over the past year we initiated a new approach for the study of bC*c*O under near-native conditions. We have embedded bC*c*O in a nanodisc and solved its structure by cryo-EM to a resolution of 2.1 Å, opening up a totally new method for studies of bC*c*O, especially for its interaction with cellular effectors.

Recent Citations

a. I Ishigami,I.; Zatsepin, N.; Hikita, M.; Conrad, C.; Nelson,G.; Coe, J.; Basu, S.; Grant, T.; Seaberg, M.H.; Sierra, R. G.; Hunter, M. S.; Fromme, P.; Fromme, R.; Yeh, S.-R.; Rousseau, D. L. Crystal structure of CO-bound cytochrome *c* oxidase determined by serial femtosecond X-ray crystallography at room temperature. ***Proc. Nat. Acad. Sci (USA)*** 114, 8011-8016 (2017). (PMCID: PMC5544322)

b. Ishigami, I.; Lewis-Ballester, A.; Echelmeier, A.; Brehm, G.; Zatsepin, N.; Grant, T.; Jesse Coe, J.; Lisova, S.; Nelson, G.; Zhang, S.; Dobson, Z.; Boutet, S.; Sierra, R.; Batyuk, A.; Fromme, P.; Fromme, R.; Spence, J.; Ros, A.; Yeh, S.-R.; Rousseau, D. L., Snapshot of an Oxygen Intermediate in the Catalytic Reaction of Cytochrome C Oxidase. ***Proc Nat Acad Sci (USA)*** 116, 3572-3577 (2019). (PMCID: PMC6397517)

c. Ishigami, I.; Russi, S.; Cohen, A.; Yeh, S. R.; Rousseau, D. L.; Temperature-dependent structural transition following X-ray-induced metal center reduction in oxidized cytochrome c oxidase. ***Journal of Biological Chemistry*** 298, 101799 (2022) doi:10.1016/j.jbc.2022.101799 . (PMCID: PMC8971940)

d. Ishigami, I, Carbajo S, Zatsepin N, Hikita M, Conrad C E., Nelson G, Coe J, Basu S, Grant T, Seaberg M H., Sierra R G., Hunter M S., Fromme P, Fromme R, Rousseau D L and Yeh S-R. Detection of a Geminate Photoproduct of Bovine Cytochrome c Oxidase by Time-Resolved Serial Femtosecond Crystallography. ***J. Am. Chem. Soc. (Communication)*** 145, 22305-22309, (2023). doi: 10.1021/jacs.3c07803. (PMID: 37695261)

e. Ishigami, I., Sierra R. G., Su, Z., Peck A, Poitevin F, Lisova S, Hayes B, Moss F, Boutet S, Sublett R E., Yoon C. H, Yeh S-R , and Rousseau D L. Structural insights into functional properties of the oxidized form of cytochrome c oxidase. ***Nature Communications*** 14, 5752 (2023). doi: 10.1038/s41467-023-41533-x (PMID: 37717031).

B. Positions and Honors

Professional Experience:

2022-Present	Professor, Dept. of Biochemistry, Albert Einstein College of Medicine, Bronx, NY
1998-2021	Chairman, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY
1996-2021	Professor, Dept. of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY
1985-1995	Adjunct Professor, Dept. of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA.
1969-1995	Member of Technical Staff, AT&T Bell Laboratories, Murray Hill, NJ

Honors:
Advisory Council on Research (Bell Laboratories) (1975-1977).
Fellow of American Physical Society (1979).
Division of Biological Physics, American Physical Society: Member of Executive Committee (1982-1985); Vice Chairperson (1995-1996); Chairperson (1997-1998; Chairperson (2004-2005).
Distinguished Technical Staff Award (Bell Laboratories) (1983).
Member of technical Review Committee, NJ Commission on Science and Technology (1985-1989).
Fellow of the Optical Society of America (1990).
Canvassing Committee for Alfred Bader Award in Bioinorganic and Bioorganic Chemistry (1992-1994).
Ad hoc member of NIH and NSF Review Panels multiple times.

C. Contributions to Science

I. Early studies at Bell Labs: Polywater and Resonance Raman Theory. My independent research career began when joined Bell Telephone Laboratories in the Physics Research Division. I continued the work I had started a few months earlier on determining the properties of "Polywater", a newly discovered viscous, petroleum-like substance, claimed to consist of pure H_2O. This material had been proposed as a new form of water with properties so unique that is was thought that it would be a life changing material. Initially in my spectroscopic studies, I used infrared spectroscopy to assure that the Polywater I had formed was the same as that reported by others. I then carried out a variety of chemical analyses from which I determined that Polywater consisted of multiple contaminants. Although, these initial studies would seem to put to rest the idea that there was a more stable form of water that had not been previously discover, it was claimed by its proponents that their polwater was "pure" and mine was contaminated and that I could not account for the features in the infrared spectrum. However, considerable effort I was able to show by infrared spectroscopy that biological contaminants could account for the entire phenomenon. The polywater episode is an example of how scientist can become self-deluded in the search for truth.
After my studies on Polywater were completed, I focused on the determination of the mechanism of resonance Raman scattering, using molecular iodine as an example. It was selected because we could detect both fluorescence and Raman scattering from it. Our studies led to a series of papers published in *Physical Review*

Letters culminating in a paper in *The Journal of Chemical Physics* in which I was able to clearly define the differences between fluorescence and Raman scattering, a controversial issue at that time.

a. Rousseau, D. L.; Porto, S. P., Polywater: polymer or artifact? ***Science*** 167, 1715-19 (1970). (PMID: 17729617)

b. Rousseau, D. L., "Polywater" and sweat:similarities between the infrared spectra. ***Science*** 171, 170-172 (1971). (PMID: 5538826)

c. Rousseau, D. L., Case Studies in Pathological Science. ***American Scientist*** 80, 54-63 (1992).

d. Rousseau, D. L.; Williams, P. F., Resonance Raman scattering of light from a diatomic molecule. ***Journal of Chemical Physics*** 64 (9), 3519-3537 (1976).

II. Hemoglobin and Myoglobin: Dynamics and Cooperativity. Having clarified our understanding of resonance Raman scattering, I focused on applying the technique to the study of biological systems, starting with determining the biophysical properties of hemoglobins and myoglobins to the mechanisms of cooperativity and the role of dynamics in their functions. At the time, the functional role of protein dynamics was just being recognized and whether the energetics of hemoglobin cooperativity was localized over a few bonds or was distributed over many bonds was very controversial. We carried out many studies on both hemoglobin and myoglobin identifying the dynamics that was occurring at the heme in these proteins and the types of coupling that was occurring. These studies demonstrated how localized changes at the heme could propagate as a protein quake. Our studies of cooperativity in hemoglobin showed that localized changes at the hemes were too small to account for the cooperativity, but the changes due to ligand binding at the heme would propagate through the iron-histidine linkage to the rest of the protein.

a. Ondrias, M. R.; Rousseau, D. L.; Simon, S. R., Resonance Raman detection of structural dynamics at the active site in hemoglobin. ***Proceedings of the National Academy of Sciences of the United States of America*, 79** (5), 1511-14 (1982). (PMID: 6951193)

b. Sassaroli, M.; Dasgupta, S.; Rousseau, D. L., Cryogenic stabilization of myoglobin photoproducts. ***Journal of biological chemistry*** 261 (29), 13704-13 (1986). (PMID: 3759989)

c. Rousseau, D. L.; Ondrias, M. R., Resonance Raman scattering studies of the quaternary structure transition in hemoglobin. ***Annual review of biophysics and bioengineering*** 12, 357-80 (1983). (PMID: 6347041)

d. Ondrias, M. R.; Rousseau, D. L.; Simon, S. R., Structural changes at the heme induced by freezing hemoglobin. ***Science*** 213 (4508), 657-59 (1981). (PMID: 7256263)

III. Heme Oxygenase and Nitric Oxide Synthase: Intermediates and Mechanisms. In the 1990s my group carried out many studies on heme oxygenase (HO) and nitric oxide synthase (NOS). We were able to characterize the equilibrium species of HO and identify the two intermediates in the heme breakdown: the alpha-hydroxy heme and verdoheme species. In addition, from our resonance Raman studies we showed that the oxygen atom was highly bent it the initial Oxy-complex, facilitating the subsequent chemistry. Our spectroscopic conclusion was later verified by our crystallographic study. Studies of NOS were done to mechanism of the enzyme. By determining the ligand interactions and by characterizing a mutant of the enzyme that stabilized a transient intermediate, we were able to formulate a reaction scheme that accounts for both of the two steps of the catalytic reaction.

a. Takahashi, S.; Matera, K. M.; Fujii, H.; Zhou, H.; Ishikawa, K.; Yoshida, T.; Ikeda-Saito, M.; Rousseau, D. L., Resonance Raman spectroscopic characterization of alpha-hydroxyheme and verdoheme complexes of heme oxygenase. ***Biochemistry*** 36 (6), 1402-10 (1997). (PMID: 9063888)

b. Takahashi, S.; Ishikawa, K.; Takeuchi, N.; Ikeda-Saito, M.; Yoshida, T.; Rousseau, D. L., Oxygen-Bound Heme-Heme Oxygenase Complex: Evidence for a Highly Bent Structure of the Coordinated Oxygen. ***Journal of the American Chemical Society*** 117 (22), 6002-6006 1995).

c. Li, D.; Kabir, M.; Stuehr, D. J.; Rousseau, D. L.; Yeh, S. R., Substrate- and isoform-specific dioxygen complexes of nitric oxide synthase. ***Journal of the American Chemical Society*** *129* (21), 6943-51 (2007). (PMID: 17488012)

d. Sabat, J.; Egawa, T.; Lu, C.; Stuehr, D. J.; Gerfen, G. J.; Rousseau, D. L.; Yeh, S. R., Catalytic intermediates of inducible nitric-oxide synthase stabilized by the W188H mutation. ***Journal of Biological Chemistry*** 288 (9), 6095-106 (2013). (PMID: 23269673)

IV. Protein Folding: Biphasic Mechanisms. In the late 1990s we started to study protein folding because we had two new approaches: submillisecond mixers we had developed to enable identification of early intermediates and the use of resonance Raman scattering to study the role of hemes during the folding of cytochrome *c*. We discovered that the folding proceeded by a two-step mechanism: a hydrophobic collapse followed by a conformational search for a global minimum. This new paradigm was published in back-to-back papers in *Nature Structural Biology*, and was summarized in a subsequent paper. By capitalizing on our rapid mixing technology we were able to study other proteins by using Trp fluorescence in addition to Raman scattering to determine the time dependence of early intermediates and thereby unravel the folding mechanisms.

a. Takahashi, S.; Yeh, S. R.; Das, T. K.; Chan, C. K.; Gottfried, D. S.; Rousseau, D. L., Folding of cytochrome c initiated by submillisecond mixing. ***Nature Structural Biology*** 4 (1), 44-50 (1997). (PMID: 8989323)

b. Yeh, S. R.; Takahashi, S.; Fan, B.; Rousseau, D. L., Ligand exchange during cytochrome c folding. ***Nature Structural Biology*** 4 (1), 51-56 (1997). (PMID: 8989324)

c. Yeh, S.-R.; Han, S.; Rousseau, D. L., Cytochrome c Folding and Unfolding: A Biphasic Mechanism. ***Accounts of Chemical Research*** 31 (11), 727-736 (1998). (PMID: 10881185)

d. Yeh, S. R.; Ropson, I. J.; Rousseau, D. L., Hierarchical folding of intestinal fatty acid binding protein. ***Biochemistry*** 40 (14), 4205-10 (2001). (PMID: 11284675)

V. Cytochrome Oxidase: Intermediates and Functional Mechanisms. My group first started studying equilibrium forms of cytochrome oxidase in the 1980s. Also during that period, we developed rapid mixing devices to be able to determine the catalytic intermediates formed in the reaction of the enzyme with oxygen. All of this came to fruition at about 1990 when we were able to identify the major intermediates in the catalytic reaction including the initial oxygen bound species, the ferryl intermediate and the hydroxyl final intermediate. By monitoring the intensity of the progressive changes in the intermediates we were able to follow their time dependence and thereby define the catalytic mechanism of the reduction of oxygen to water. The major issue that remains in the understanding of the cytochrome oxidase function is the mechanism by which the oxygen reduction is coupled to the proton translocation. We recently summarized the current models for the important process and are currently carrying out experiments to test the models.

a. Han, S. W.; Ching, Y. C.; Rousseau, D. L., Primary intermediate in the reaction of oxygen with fully reduced cytochrome c oxidase. ***Proceedings of the National Academy of Sciences of the United States of America*** 87 (7), 2491-95 (1990). (PMID: 2157201)

b. Han, S.; Ching, Y. C.; Rousseau, D. L., Ferryl and hydroxy intermediates in the reaction of oxygen with reduced cytochrome c oxidase. ***Nature*** 348 (6296), 89-90 (1990). (PMID: 2172834)

c. Han, S.; Takahashi, S.; Rousseau, D. L., Time dependence of the catalytic intermediates in cytochrome c oxidase. ***Journal of Biological Chemistry*** 275 (3), 1910-19 (2000). (PMID: 10636892)

d. Ishigami, I.; Hikita, M.; Egawa, T.; Yeh, S. R.; Rousseau, D. L., Proton translocation in cytochrome c oxidase: insights from proton exchange kinetics and vibrational spectroscopy. ***Biochimica et Biophysica Acta*** 1847 (1), 98-108 (2015). (PMCID: PMC4254173)

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/denis.rousseau.1/bibliography/44199113/public/?sort=date&direction=ascending

BIOGRAPHICAL SKETCH

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NAME: Yeh, Syun-Ru

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

POSITION TITLE: Professor of Physiology and Biophysics

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
National Cheng Kung University, Taiwan	B.S.	06/1985	Chemistry
National Tsing Hua University, Taiwan	M.S.	06/1988	Physical Chemistry
University of Maryland, College Park, MD	Ph. D.	06/1993	Biophysical Chemistry
Harvard Medical School, Boston, MA	Postdoc	07/1994	Photobiology
Princeton University, Princeton, NJ	Postdoc	10/1996	Biophysics
AT&T Bell laboratories, Murray Hill, NJ	Postdoc	10/1996	Biophysics
Albert Einstein College of Medicine, Bronx, NY	Postdoc	09/1997	Biophysics

A. Personal Statement

My research interest lies at the interface between chemistry and biology. I am passionate about the mechanisms by which biological functions of proteins, in particular hemeproteins, are encoded in their structures. In addition, I am active in developing new biotechnologies for attaining new knowledge inaccessible by conventional techniques. The current focus of my lab is centered on heme-based dioxygenases and oxidases. The long-term goal of my lab is to delineate molecular mechanisms underlying the biological functions of these important heme-containing enzymes and to comprehend how these mechanisms contribute to human health and disease. Apart from my research work, I am committed to supporting researchers in the community, by sharing the biochemical protocols and biophysical tools that I developed in the lab. In addition, I am active in reviewing manuscripts and grant applications, and in recruiting students, postdocs and faculty members, in particular women and minorities, to our college.

B. Positions and Honors

Positions and Employment

2010-Present Professor, Albert Einstein College of Medicine, Bronx, NY
2004-2010 Associate Professor, Albert Einstein College of Medicine, Bronx, NY
1998-2004 Assistant Professor, Albert Einstein College of Medicine, Bronx, NY
1997-1998 Instructor, Albert Einstein College of Medicine, Bronx, NY

Service and Honors

Fellow, American Physical Society — Elected 2012
DOE Review Panel Member, BSSD Beamline Review Panel — 2024
NIH Review Panel Member, Special Emphasis Panel, ZRG1 MBBC-L — 2024

NIH Ad hoc Review Panel Member, MSFC study section	2024
NIH Review Panel Member, Special Emphasis Panel ZTR1 RD 8 (01)	2024
NIH Ad hoc Review Panel Member, MSFA study sections	2022
NSF Review Panel Member / Ad hoc Reviewer	2010-Present
Session Leader, Gordon Research Conference (Chemistry & Biology of Tetrapyrroles)	2022
Symposium organizer ("Heme enzymes: Structure and function"), International Conference on Porphyrins and Phthalocyanines (ICPP)	2012-Present
Chang-An Yu Eminent Scholar Lectureship, Oklahoma State University	2011
Visiting Lecturer of the Chemistry Research Promotion Center, National Science Council, Taiwan	2012
International Advisory Committee, National Key Lab of Biochemical Engineering Chinese Academy of Sciences, Beijing, China	2012-15
External Scientific Advisory Committee Member, NIGMS MBRS-SCORE Program at the University of Puerto Rico	2004-07
Fellow, National Synchrotron Radiation Research Center, Taiwan	1988
Excellent Youth Award, Taiwan	1985

Institutional Service at Einstein

Committee on Appointments & Promotions	2015-18, 23
Information Technology Faculty Advisory Committee (IT FACE)	2011-15
Steering Committee for NIH biophysics Training Grant	2009-14
Sue Golding Academic Affair Committee	2002-07
Sue Golding Qualifying Examination Committee	2006-07

C. Recent Contributions to Science

The major goal of my lab is to elucidate molecular mechanisms of hemeproteins that underly human physiology and pathophysiology, and to develop new biotechnologies that enable the exploration of important biochemical events. We have made important contributions to the field by promoting the understanding of the structural, functional, and folding mechanisms of a wide variety of hemeproteins, including cytochrome *c* oxidases (C*c*O), tryptophan dioxygenases, indoleamine 2,3-dioxygenases, nitric oxide synthases, cytochrome P450s, cytochrome bc1, cytochrome c, HutZ, PGRMC1 and globins, as documented in the literature. I have been collaborating with the Rousseau group on mechanistic studies of C*c*O for almost two decades. In addition, C*c*O, current research is focused on three human heme-based dioxygenases, including hIDO1, hIDO2 and hTDO. The important achievements that my lab made in recent years are highlighted below.

Cytochrome *c* oxidases (C*c*O). C*c*O offers an interesting contrast to the dioxygenases, as it catalyzes the reduction of O_2 to water, instead of inserting O_2 into an organic substrate. My lab has a long-standing collaboration with Dr. Denis Rousseau's lab on the delineation of the mechanism by which the oxygen reduction reaction is coupled to proton translocation. With spectroscopic methods, in particular optical absorption, resonance Raman and EPR spectroscopies and x-ray crystallography, we unraveled unique structural characteristics of C*c*O underlying its proton pumping mechanism. Recently, we have made the following new achievements: **(i)** we determined the first room-temperature crystal structure of C*c*O in complex with CO, a surrogate of O_2, with the state-of-the art serial femtosecond crystallography (SFX) technique, **(ii)** we determined the room-temperature structure of an oxygen intermediate of C*c*O for the first time with a novel mix-and-inject coupled SFX technique, **(iii)** we detected a 100 ns geminate photoproduct of C*c*O by combining optical laser photolysis with SFX and **(iv)** we settled a long-standing controversy by using resonance Raman spectroscopy and SFX to determine the structure of the oxidized state of C*c*O.

a. "Structural insights into functional properties of the oxidized form of cytochrome c oxidase." Ishigami, I, Sierra RG, Su, Z, Peck A, Poitevin F, Lisova S, Hayes B, Moss F, Boutet S, Sublett R E, Yoon CH, Yeh S-R , and. Rousseau D L. **Nature Communications.** 14, 5752, 2023. PMID: 37717031.

b. "Detection of a Geminate Photoproduct of Bovine Cytochrome c Oxidase by Time-Resolved Serial Femtosecond Crystallography." Ishigami, I, Carbajo S, Zatsepin N, Hikita M, Conrad CE., Nelson G, Coe J, Basu S, Grant T, Seaberg MH, Sierra RG, Hunter MS, Fromme P, Fromme R, Rousseau DL and Yeh S-R. **J. Am. Chem. Soc**.145, 22305-22309, 2023. PMID: 37695261

c. "Temperature-dependent structural transition following X-ray-induced metal center reduction in oxidized cytochrome c oxidase." Ishigami I, Russi S, Cohen A, Yeh SR, Rousseau DL. **J Biol Chem.** 298, 101799, 2022. PMID: 35257742

d. "Snapshot of an oxygen intermediate in the catalytic reaction of cytochrome c oxidase." Ishigami I, Lewis-Ballester A, Echelmeier A, Brehm G, Zatsepin NA, Grant TD, Coe JD, Lisova S, Nelson G, Zhang S, Dobson ZF, Boutet S, Sierra RG, Batyuk A, Fromme P, Fromme R, Spence JCH, Ros A, Yeh SR, Rousseau DL. **Proc Natl Acad Sci U S A.** 116, 3572, 2019. PMID: 30808749

Human Indoleamine 2,3-dioxygenases (hIDOs). The original isoform of IDO (named IDO1) was first discovered in 1963; however, nothing was known about the human isoform until early 21th century, when we and others pioneered the molecular studies of the enzyme (hIDO1). With a combination of biochemical and biophysical techniques, we revealed the unique structural and functional properties of hIDO1 and defined its ferryl-based dioxygenase mechanism for the first time. More recently we made the following important breakthroughs: **(i)** we solved the long-awaited structure of the Trp bound hIDO1, which uncovered a variety of unique structural features of the enzyme, including the 2nd Trp binding site, which accounts for its substrate-inhibition behavior, **(ii)** we solved the structures of hIDO1 in complex with a group of inhibitors with diverse pharmacophores, which provides important blueprints for structure-based drug design, and **(iii)** we solved three structures of hIDO1 in complex with a frontline hIDO1 inhibitor in clinical trials, BMS-986205, which demonstrate for the first time that a full binding trajectory of a drug to its protein target can be experimentally mapped out with atomic resolution. In addition to hIDO1, we made important advances in the comprehension of the mostly unknown 2nd isoform of hIDO (named hIDO2, which was first identified in 2007): **(i)** we developed a robust expression and purification protocol for this previously unisolatable protein, **(ii)** we solved the first structure of hIDO2 in complex with Trp, which offers the much-needed structural information for rational drug design, and **(iii)** we designed and tested a group of mono, dual and pan inhibitors targeting hIDO1, hIDO2 and/or hTDO, demonstrating for the first time that all three types of inhibitors can be derived from a single pharmacophore.

a. "Indoleamine 2,3-dioxygenase (IDO)-1 and IDO-2 activity and severe course of COVID-19." Guo L, Schurink B, Roos E, Nossent EJ, Duitman JW, Vlaar AP, van der Valk P, Vaz FM, Yeh SR, Geeraerts Z, Dijkhuis A, van Vught L, Bugiani M, Lutter R; also on behalf of the Amsterdam UMC COVID-19 Biobank Study Group. **J Pathol.** 256, 256-261, 2022. PMID: 34859884

b. "Conformational Plasticity in Human Heme-Based Dioxygenases." Pham KN, Lewis-Ballester A, Yeh SR. **J Am Chem Soc.** 143,1836-18452021, 2021. PMID: 33373218

c. "Structural Basis of Inhibitor Selectivity in Human Indoleamine 2,3-Dioxygenase 1 and Tryptophan Dioxygenase." Pham KN, Lewis-Ballester A, Yeh SR. **J Am Chem Soc.** 141, 18771-18779, 2019. PMID: 31682426

d. "Structural Insights into Substrate and Inhibitor Binding Sites in Human Indoleamine 2,3-Dioxygenase 1" Lewis-Ballester A, Pham KN, Batabyal D, Karkashon S, Bonanno JB, Poulos TL, Yeh SR, **Nat Commun.** 8, 1693, 2017. PMID: 29167421

Human tryptophan dioxygenase (hTDO). TDO was first discovered in rat liver in 1936; however, the first study of the human isoform was not reported until 2007 by my lab. We pioneered a robust preparation protocol for hTDO, which allowed us to define its unique structural and functional characteristics that distinguish it from hIDOs for the first time. In addition, we demonstrated that, like hIDO1, hTDO follows the ferryl-based dioxygenase mechanism. In the past few years, we made several important new advances: **(i)** we solved the first structure of hTDO, which offers the much-needed information for structure-based drug design, **(ii)** we solved the first structure of a transient oxygen intermediate of hTDO, which provides new insights into the ferryl-based dioxygenase mechanism, **(iii)** we identified a previously unknown 2nd Trp binding site in hTDO and defined its

role in regulating ubiquitination-linked proteasome degradation of the enzyme in liver cells, **(iv)** we identified several posttranslational modification sites and a bipartite degron in hTDO that underly the unusually short cellular lifetime of the enzyme, and **(v)** we solved the structure of hTDO in complex with an inhibitor in clinical trials, which offers new insights into inhibitor-selectivity in hTDO *versus* hIDO1.

a. "Characterization of the structural determinants of the ubiquitin-dependent proteasomal degradation of human hepatic tryptophan 2,3-dioxygenase." Liu Y, Kim SM, Wang Y, Karkashon S, Lewis-Ballester A, Yeh SR, Correia MA. **Biochem J**. 478, 1999, 2021. PMID: 27762317

b. "Structural Basis of Inhibitor Selectivity in Human Indoleamine 2,3-Dioxygenase 1 and Tryptophan Dioxygenase." Pham KN, Lewis-Ballester A, Yeh SR, **J Am Chem Soc.**141, 18771, 2019. PMID: 31682426

c. "Structure, function and regulation of human heme-based dioxygenases" Lewis-Ballester A, Pham KP, Liao M, Correia MA, Yeh SR. (2019). CHAPTER 9: Structure, Function and Regulation of Human Heme-based Dioxygenases. In M. Ikeda-Saito, & E. Raven (Eds.), Dioxygen-dependent Heme Enzymes (13 ed., pp. 181-221). (RSC Metallobiology; Vol. 2019-January, No. 13). Royal Society of Chemistry. doi: 10.1039/9781788012911-00181

d. "Molecular basis for catalysis and substrate-mediated cellular stabilization of human tryptophan 2,3-dioxygenase." Lewis-Ballester A, Forouhar F, Kim SM, Lew S, Wang Y, Karkashon S, Seetharaman J, Batabyal D, Chiang BY, Hussain M, Correia MA, Yeh SR*, Tong L.* **Sci Rep.** 6, 35169, 2016. PMID: 27762317

Development of novel biotechnologies. I have a keen interest in developing novel biotechnologies to address important biochemical questions. In Bell labs, I discovered a novel electrokinetic phenomenon, which later formed the core technology of the first-ever FDA-approved diagnostic for molecular typing of donor and recipient red blood cells for blood transfusions by BioArray Solutions Ltd. Later, I used a similar technology to design the first microfluidic solution mixer, which allows visualization of fast biological reactions in a μs time window that was previously unachievable. More recently, I am collaborating with the SSRL, LCLS and ASU teams in developing time-resolved serial femtosecond x-ray crystallography (SFX) with an x-ray free electron laser as the light source, for which I made important contributions in designing/testing a new mix-and-inject device (see Ref 3a above) and a novel sample injector. I am also actively involved in the development of concentric-flow microfluidic electrokinetic sample holder (coMESH)-based injector/mixer for its application in SFX, as well as in serial millisecond x-ray crystallography (SMX), a new technique that uses the more accessible synchrotron, instead of the free electron laser, as the light source. These novel x-ray crystallographic techniques enable macromolecular structural determination at room-temperature without radiation damage under either equilibrium or kinetic conditions.

a. "Three-dimensional-printed gas dynamic virtual nozzles for x-ray laser sample delivery." Nelson G, Kirian RA, Weierstall U, Zatsepin NA, Faragó T, Baumbach T, Wilde F, Niesler FB, Zimmer B, Ishigami I, Hikita M, Bajt S, Yeh SR, Rousseau DL, Chapman HN, Spence JC, Heymann M. **Opt Express.** 24, 11515, 2016. PMID: 27410079

b. "Design and evaluation of a passive alcove-based microfluidic mixer." Egawa T, Durand JL, Hayden EY, Rousseau DL, Yeh SR. **Anal Chem.** 81, 1622, 2009. PMID: 19140669

c. "Ultrafast microfluidic mixer and freeze-quenching device." Lin Y, Gerfen GJ, Rousseau DL, Yeh SR. **Anal Chem.** 75, 5381, 2003. PMID: 14710815

d. "Assembly of ordered colloidal aggregates by electric-field-induced fluid flow." Yeh SR, Seul M, Shraiman BI. **Nature.** 386, 57, 1997. PMID: 28943661

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BIOGRAPHICAL SKETCH

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NAME: Ishigami, Izumi

eRA COMMONS USER NAME (credential, e.g., agency login): IZUMI0912

POSITION TITLE: Research Assistant Professor

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
Himeji Institute of Technology, Hyogo	BS	03/2006	Life Science
University of Hyogo	MS	03/2009	Molecular Biology
University of Hyogo	PHD	03/2012	Molecular Biology
Albert Einstein College of Medicine	Postdoctoral Fellow	03/2017	Biophysics

A. Personal Statement

I have the expertise, experience, and motivation necessary to successfully carry out the research work described in this proposal by Dr. Rousseau. I am an experienced vibrational spectroscopist and X-ray crystallographer. Most of my career has focused on solving biological problems related to hemeproteins, in particular mammalian cytochrome *c* oxidase (C*c*O), guanylate cyclase, HutZ, myoglobins and hemoglobins. From a technical viewpoint, I have ample experience in using optical absorption, FTIR and resonance Raman spectroscopy, as well as microspectroscopy-guided X-ray crystallography, Xe-pressurized X-ray crystallography and serial femtosecond X-ray crystallography (SFX), to study biological molecules. My previous successes in developing and testing continuous-flow resonance Raman spectroscopy and mix-and-inject serial X-ray crystallography demonstrate my technical skills and knowledge. I have isolated C*c*O from beef hearts and purified the dimeric form of the protein for all of our studies. In addition, I developed all of the procedures needed to make diffraction quality microcrystals used in our SFX studies. More recently I have developed the techniques to isolate monomeric bovine C*c*O and embed it in nanodiscs. I am proficient at solving crystal structures and I have recently developed expertise to make cryoEM sample grids and process cryo-EM data. I have been working closely with Dr. Rousseau since I joined Einstein. I believe I am qualified and well-prepared to make significant contributions to the research proposed ln this project.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2020 – present Research Assistant Professor, Albert Einstein College of Medicine, Bronx, NY
2017 – 2020 Associate, Albert Einstein College of Medicine, Bronx, NY

Honors

2017 Joe Wong Poster Award, 2017 SSRL/LCLS User's Meeting
2017 Poster prize, BioXFEL 4th International Conference

C. Contributions to Science

1. The major theme of my research work has been focused on mammalian C*c*O. Since I joined Einstein, I have developed a procedure to purify C*c*O from bovine hearts (bC*c*O). I have established reproducible protocols for the generation of large crystals of bC*c*O for structural determination at synchrotron centers and small nanocrystals for serial femtosecond X-ray crystallography (SFX). In addition, I am involved in the development and testing of time-resolved SFX (TRSFX). With these techniques I have solved several important structures of bCcO, including the first structure of a transient oxygen intermediate of bC*c*O, the structure of the oxidized enzyme and the structure of a geminate 100 ns photoproduct. These structural data offer new insights into the mechanism by which C*c*O harvests the energy derived from the oxygen reduction chemistry to drive proton translocation.

   a. Ishigami, I., Sierra R. G., Su, Z., Peck A, Poitevin F, Lisova S, Hayes B, Moss F, Boutet S, Sublett R E., Yoon C. H, Yeh S-R , and. Rousseau D L. Structural insights into functional properties of the oxidized form of cytochrome c oxidase. *Nature Communications* Volume: 14, P: 5752, 2023. doi: 10.1038/s41467-023-41533-x PMID: 37717031.

   b. Ishigami, I, Carbajo S, Zatsepin N, Hikita M, Conrad C E., Nelson G, Coe J, Basu S, Grant T, Seaberg M H., Sierra R G., Hunter M S., Fromme P, Fromme R, Rousseau D L, and Yeh S-R. Detection of a geminate photoproduct of bovine cytochrome c oxidase by time-resolved serial femtosecond crystallography. *J. Am. Chem. Soc. (Communication)* 145, 22305-22309, 2023. doi: 10.1021/jacs.3c07803. PMID: 37695261

   c. Ishigami I, Russi S, Cohen A, Yeh SR, Rousseau DL. "Temperature-dependent structural transition following X-ray-induced metal center reduction in oxidized cytochrome c oxidase."J Biol Chem. 2022 Apr;298(4):101799. doi: 10.1016/j.jbc.2022.101799. PMID: 35257742

   d. Ishigami I, Lewis-Ballester A, Echelmeier A, Brehm G, Zatsepin NA, Grant TD, Coe JD, Lisova S, Nelson G, Zhang S, Dobson ZF, Boutet S, Sierra RG, Batyuk A, Fromme P, Fromme R, Spence JCH, Ros A, Yeh SR, Rousseau DL. "Snapshot of an oxygen intermediate in the catalytic reaction of cytochrome c oxidase." Proc Natl Acad Sci U S A. 116, 3572-77, 2019. PMID: 30808749 PMCID: PMC6397517.

   e. Ishigami I, Zatsepin NA, Hikita M, Conrad CE, Nelson G, Coe JD, Basu S, Grant TD, Seaberg MH, Sierra RG, Hunter MS, Fromme P, Fromme R, Yeh SR, Rousseau DL. "Crystal structure of CO-bound cytochrome *c* oxidase determined by serial femtosecond X-ray crystallography at room temperature." Proc Natl Acad Sci U S A. 2017;114, 8011-8016. PubMed PMID: 28698372; PubMed Central PMCID: PMC5544322.

   f. Nelson G, Kirian RA, Weierstall U, Zatsepin NA, Faragó T, Baumbach T, Wilde F, Niesler FB, Zimmer B, Ishigami I, Hikita M, Bajt S, Yeh SR, Rousseau DL, Chapman HN, Spence JC, Heymann M. "Three-dimensional-printed gas dynamic virtual nozzles for x-ray laser sample delivery." Opt Express. 2016 May 30;24(11):11515-30. PubMed PMID: 27410079; PubMed Central PMCID: PMC5025224.

2. To understand the full mechanism of C*c*O, the structural properties of transient intermediates must be determined. With resonance Raman spectroscopic studies, I was able to reveal important structural characteristics of bCcO underlying its proton translocation mechanism.

   a. Ishigami I, Hikita M, Egawa T, Yeh SR, Rousseau DL. "Proton translocation in cytochrome c oxidase: insights from proton exchange kinetics and vibrational spectroscopy." Biochim Biophys Acta. 2015 Jan;1847(1):98-108. PubMed PMID: 25268561; PubMed Central PMCID: PMC4254173.

   b. Ishigami I, Nishigaki T, Shinzawa-Itoh K, Yoshikawa S, Nakashima S, Ogura T. "An intermediate conformational state during ligand binding to cytochrome c oxidase detected by time-resolved resonance Raman analyses of heme peripheral groups." Chem. Lett. 2012; 41:178-180.

3. In addition to CcO, I have investigated ligand binding reactions and allosteric regulations in several different hemeproteins, including guanylate cyclase, HutZ, myoglobins and hemoglobins.

   a. Uchida T, Sekine Y, Dojun N, Lewis-Ballester A, Ishigami I, Matsui T, Yeh SR, Ishimori K. "Reaction intermediates in the heme degradation reaction by HutZ from Vibrio cholerae." Dalton Trans. 2017 Jun 27;46(25):8104-8109. doi: 10.1039/c7dt01562c. PubMed PMID: 28607990; PubMed Central PMCID: PMC5876723.

   b. Nishimura R, Shibata T, Ishigami I, Ogura T, Tai H, Nagao S, Matsuo T, Hirota S, Shoji O, Watanabe Y, Imai K, Neya S, Suzuki A, Yamamoto Y. "Electronic control of discrimination between O2 and CO in myoglobin lacking the distal histidine residue." Inorg Chem. 2014 Jan 21;53(2):1091-9. PubMed PMID: 24377722.

   c. Nishimura R, Shibata T, Tai H, Ishigami I, Ogura T, Nagao S, Matsuo T, Hirota S, Imai K, Neya S, Suzuki A, Yamamoto Y. "Relationship between the electron density of the heme Fe atom and the vibrational frequencies of the Fe-bound carbon monoxide in myoglobin." Inorg Chem. 2013 Mar 18;52(6):3349-55. PubMed PMID: 23445324.

   d. Kanaori K, Tajiri Y, Tsuneshige A, Ishigami I, Ogura T, Tajima K, Neya S, Yonetani T. "T-quaternary structure of oxy human adult hemoglobin in the presence of two allosteric effectors, L35 and IHP." Biochim Biophys Acta. 2011 Oct;1807(10):1253-61. PubMed PMID: 21703224

   e. Kitanishi K, Kobayashi K, Kawamura Y, Ishigami I, Ogura T, Nakajima K, Igarashi J, Tanaka A, Shimizu T. "Important roles of Tyr43 at the putative heme distal side in the oxygen recognition and stability of the Fe(II)-O2 complex of YddV, a globin-coupled heme-based oxygen sensor diguanylate cyclase." Biochemistry. 2010 Dec 14;49(49):10381-93. PubMed PMID: 21067162.

4. Recently I have expanded my research into heme model complexes. I have studied the intriguing new chemistry catalyzed by a group of heme model complexes, where the electronic properties are systematically tuned to enhance their catalytic activities. Based on these studies, I have reported two high impact papers in top line journals.

   a. Mondal P, Ishigami I, Yeh SR, Wijeratne GB. "The Role of Heme Peroxo Oxidants in the Rational Mechanistic Modeling of Nitric Oxide Synthase: Characterization of Key Intermediates and Elucidation of the Mechanism" Angew Chem Int Ed Engl. 2022 Sep 28. doi: 10.1002/anie.202211521. Online ahead of print. PMID: 36169890

   b. Mondal P, Ishigami I, Gérard EF, Lim C, Yeh SR, de Visser SP, Wijeratne GB "Proton-coupled electron transfer reactivities of electronically divergent heme superoxide intermediates: a kinetic, thermodynamic, and theoretical study." Chem Sci. 2021 May 27;12(25):8872-8883. doi: 10.1039/d1sc01952j. eCollection 2021 Jul 1, PMID: 34257888.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/izumi.ishigami.1/bibliography/55286606/public/