BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Lee, David John

eRA COMMONS USER NAME (credential, e.g., agency login): DJL002

POSITION TITLE: Postdoctoral Researcher

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Santa Cruz	B.S.	09/2005	06/2009	Biology
University of California, Santa Cruz	B.S.	09/2005	06/2009	Chemistry
University of California, San Diego	M.S.	09/2010	06/2012	Chemistry
University of California, San Diego	Ph.D.	06/2012	12/2016	Chemistry
University of California, San Diego	Postdoctoral	12/2016	10/2017	Structural Biology
University of California, San Francisco	Postdoctoral	11/2017	Current	Structural Biology

A. Personal Statement

My philosophical approach to science aligns with my greater philosophical approach to life—I like to know how things work. I like to take things apart, and learn how they function. I have found great satisfaction in my graduate work exploring how modular synthases produce a multitude of natural products. Using small molecules to study protein structure and protein function is analogous to using a wrench to work on a small engine, and philosophically draws me to chemical and structural biology.

During my undergraduate years, I was introduced to organic chemistry. I performed a substrate study, and enjoyed pushing a simple reaction to its limits to learn what guiding principles dictated its applicability. Similarly I was tasked with producing a family of glucosamine analogs, ultimately for incorporation into biological systems. I was especially interested in this project, and it was my first true introduction to chemical biology.

During graduate school and a brief postdoctoral position, I expanded on this interest in Professor Michael Burkart's laboratory at UC San Diego. The Burkart laboratory studies carrier protein dependent modular synthases and synthetases using structural and biophysical approaches. Collaborating with Professor Stan Opella, I learned solution state protein NMR techniques, solving multiple NMR structures of several different carrier proteins. I truly enjoy mentoring, and trained three undergraduate researchers and multiple graduate students in protein NMR. Together, we used NMR approaches to reveal specific information about proteins—specific pulse sequences to study dynamics, others to measure distance constraints for structural calculations, etc. We found that the carrier protein's cargo had significant ramifications on the carrier protein's structure, likely a structural handle to direct molecules through the pathway by tuning reaction partner protein affinity; small molecules with significant effects on protein structure. We focused on the mechanisms of substrate delivery from the carrier protein to the appropriate reaction partner, with minor conformations proving to be crucially important but difficult to study.

Ultimately, interest in minor conformational states and allostery guided my search for post-doctoral laboratories. I am engaged in my continuing training in Prof. James Fraser's laboratory, with additional technical training in Prof. Yifan Cheng's laboratory and collaboration with Prof. Ian Seiple's synthetic chemistry laboratory. This synergistic set of labs provides the ideal complement of skills to learn and study conformational heterogeneity, structural motions, and how function arises from structure in human-health

relevant systems. CryoEM studies on inhibitor-bound ribosomes, with the specific intent to understand and counteract antimicrobial resistance, perfectly tailor my continued training in chemical and structural biology. I have continued to learn and teach within the positive and collaborative environment at UCSF, rapidly advancing through CryoEM training and now guiding my labmates through CryoEM data collection and processing techniques. Additionally, UC San Francisco provides many career development opportunities, both academic and industrial interactions, and leadership and mentorship opportunities.

B. Positions and Honors

Positions and Employment

2007–2007 Intern, Medicinal Chemistry, University of Utah
2010–2010 Intern, Synthetic Chemistry, Intel Labs, Santa Clara
2010–2014 Teaching Assistant, UC San Diego
2016–2017 Professional Consultant, Biochemistry, Jones Day
2016–2017 Postdoctoral Researcher, UC San Diego
2017– Postdoctoral Researcher, UC San Francisco

Other Experience and Professional Memberships

2014–2017 Co-organizer, Natural Products Affinity Group, UC San Diego
2014– Member, Royal Society of Chemistry
2015– Member, American Chemical Society

Honors

2007 American Heart Association Summer Research Fellowship
2009 Dean's Award in Physical and Biological Sciences, UC Santa Cruz
2010 Harold Urey Award, UC San Diego
2014 Bruno Zimm Award, UC San Diego
2015 President's Dissertation Year Fellowship, UC San Diego

C. Contributions to Science

1. *Organic synthesis efforts towards chiral glucosamine analogs and asymmetric reduction of α,β-unsaturated ketones:* My early introduction to laboratory science and research, as an undergraduate, was predominantly through synthetic organic chemistry. As an American Heart Association Undergraduate fellow, I interned with Prof. Kuberan Balagurunathan at the University of Utah, synthesizing glucosamine analogs for future incorporation into heparan sulfate glycosaminoglycan chains. Heparan sulfate chains are crucial for intercellular communication and developmental processes, and we were attempting to modulate sulfation patterns of the chains through perturbation of the glucosamine monomers. After the end of the internship, I volunteered with Prof. Bakthan Singaram at the University of California, Santa Cruz, executing a substrate study to quantify and validate the efficacy of a chiral director, "Tar-B-NO_2". Tar-B-NO_2 facilitates asymmetric reduction of α,β-unsaturated ketones via directed hydride delivery. Prof. Singaram instilled in me the importance of applying science to benefit human health, ultimately culminating in my undergraduate thesis, "*Efforts towards an enantioselective synthesis of the HIV inhibitor abacavir".*
    a. Kim J, Bruning J, Park KE, **Lee DJ**, Singaram B. Highly enantioselective and regioselective carbonyl reduction of cyclic α,β-unsaturated ketones using TarB-NO2 and sodium borohydride. *Organic Letters.* 2009; 11(19):4358–61.

2. *Graduate work on Fatty Acid Synthases: Carrier protein structure* – During graduate school, I refocused my studies towards chemical biology and structural biology. Joining Prof. Michael Burkart's laboratory at the University of California, I began studying protein structure by NMR. I performed many studies of carrier proteins from carrier protein dependent modular synthases, including fatty acid synthases (FAS). Several studies of the bacterial FAS were carried out, with two primary focuses. Carrier proteins sequester and protect intermediates as they transport them from partner to partner, but the nature of sequestration is poorly understood. We characterized cargo-induced structural changes to the carrier protein. First, we turned to solution-state NMR methods, observing significant chemical shift

perturbations upon binding cargo corresponding to a global tightening of the carrier protein around the cargo. Additionally, we found that unnatural probes could be appropriately sequestered, encouraging us to explore covalent crosslinking by attaching modified cargo to carrier proteins.

a. Ishikawa F, Haushalter RW, **Lee DJ**, Finzel K, Burkart MD. Sulfonyl 3-alkynyl pantetheinamides as mechanism-based cross-linkers of acyl carrier protein dehydratase. *Journal of the American Chemical Society*. 2013; 135(24):8846–9. PMCID: PMC3713789
b. **Lee DJ***, Finzel K*, Burkart MD. Using modern tools to probe the structure-function relationship of fatty acid synthases. *ChemBioChem*. 2015; 16(4): 528–547. PMCID: PMC4545599

3. *Graduate work on Fatty Acid Synthases: Protein-protein interactions* – The protein-protein interactions required for fatty acid biosynthesis were observed by exploiting mechanism-based crosslinking probes. These probes allowed us to covalently trap the transient carrier protein-partner protein interaction for crystallographic and solution-state NMR evaluation. This approach was applied specifically to the *E. coli* acyl carrier protein and a partner dehydratase, yielding the first structural observations of an interaction between two partners within a fatty acid biosynthetic pathway. Additionally, NMR was used to observe the non-crosslinked *in vitro* interaction by titration, with regions of the protein perturbed in these titration experiments matching well with the interacting residues identified crystallographically. Molecular Dynamics simulations, supported by Residual Dipolar Coupling measurements, was used to quantify the flexibility of the carrier protein before and during interaction with the partner dehydratase. Together, these efforts allowed conclusive mapping of the acyl carrier protein and dehydratase interaction, with significant implications in future engineering and inhibition efforts.
    a. Sztain T, Patel A, **Lee DJ**, Davis T, McCammon JA, Burkart MD. One atom matters: modifying the thioester linkage affects structure of the acyl carrier protein. *Angewandte Chemie International Edition*. 2019, in press.
    b. **Lee DJ***, Nguyen C*, Haushalter RW*, Markwick PRL, Bruegger J, Caldara-Festin G, Finzel K, Jackson DR, Ishikawa F, O'Dowd B, McCammon JA, Opella SJ, Tsai S-C, Burkart MD. Trapping the dynamic acyl carrier protein in fatty acid biosynthesis. *Nature.* 2014; 505(7483): 427–31. PMCID: PMC4437705
    c. Beld J, **Lee DJ**, Burkart MD. Fatty acid biosynthesis revisited: structure eluctidation and metabolic engineering. *Molecular Biosystems.* 2015; 11(1): 38-59. PMCID: PMC4276719

4. *Graduate work on Hybrid polyketide synthase/non-ribosomal peptide synthetases* – Several pyrrole containing hybrid PKS/NRPS systems were studied and structurally characterized to engineer cross-pathway activity, between carrier proteins of closely related synthetases and non-cognate adenylation enzymes. Specifically, the pyoluteorin and prodigiosin carrier proteins were structurally characterized using traditional NOE solution-state NMR methods. The pyoluteorin carrier protein was also characterized bearing cargo, demonstrating structurally, for the first time, sequestration of cargo in a hybrid PKS/NRPS system. Computational and mutagenesis efforts were employed to produce a mutant prodigiosin carrier protein that could be acted upon by pyoluteorin biosynthetic partner proteins. Together, these efforts advance and highlight engineering opportunities in these modular synthases and synthetases, ideally to eventually allow development of custom synthases.
    a. Jaremko MJ, **Lee DJ**, Opella SJ, Burkart MD. Structure and substrate sequestration in the pyoluteorin type II peptidyl carrier protein PltL. *Journal of the American Chemical Society*. 2015; 137(36): 11546–9. PMCID: PMC4847951
    b. Jaremko MJ, **Lee DJ**, Patel A, Winslow V, Opella SJ, McCammon JA, Burkart MD. Manipulating protein–protein interactions in nonribosomal peptide synthetase type II peptidyl carrier proteins. *Biochemistry*. 2017; 56(40): 5269–73. PMCID: PMC5873958

5. *Graduate work on Polyketide Synthases* – Polyketide synthases, the secondary-metabolic relatives of fatty acid synthases, use similar proteins and pathways as fatty acid synthases but produce secondary metabolites by various alterations to tailoring and iterative chain extensions. Understanding the substrate recognition and sequestration of cargo is critical to the success of engineering efforts. Unfortunately, many polyketides are formed by repetitive elongation steps yielding an elongated, highly reactive polyketide that can spontaneously cyclize. Studying carrier protein sequestration in the biosynthesis of the antimicrobial polyketide actinorhodin required the preparation of atom-replaced geometrically and electronically similar substrate mimics. Both linear and cyclized atom-replaced

mimics were prepared. Subjecting these mimics to sequestration studies by solution-state protein NMR revealed that only the full-length and cyclized mimics were well sequestered, suggesting that the elongating polyketide remains within the partner ketosynthase until full elongation.

a. **Lee DJ***, Milligan JC*, Jackson DR*, Schaub AJ, Beld J, Barajas JF, Hale JJ, Luo R, Burkart MD, Tsai S-C. Molecular basis for interactions between an acyl carrier protein and a ketosynthase. *Nature Chemical Biology*. 2019; 15(7): 669-71. PMID: 31209348
b. Shakya G, Rivera H, **Lee DJ**, Jaremko MJ, La Clair JJ, Fox DT, Haushalter RW, Schaub AJ, Bruegger J, Barajas JF, White AR, Kaur P, Gwozdziowski ER, Wong F, Tsai S-C, Burkart MD. Modeling linear and cyclic PKS intermediates through atom replacement. *Journal of the American Chemical Society*. 2014; 136(48): 16792–9. PMCID: PMC4277753

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/david.lee.7/bibliography/57262207/public/?sort=date

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

YEAR	COURSE TITLE	GRADE
	University of California, San Diego	
2010	Enzyme Catalyzed Reactions	A
2010	Synthetic Methods/Organic Chemistry	A-
2010	Mechanisms/Organic Reactions	B-
2011	Synthesis of Complex Molecules	B+
2011	Structure and Properties of Organic Molecules	A
2011	Natural Products Chemistry	A
2011	Applied Spectroscopy	B+
2012	Protein NMR	A

BIOGRAPHICAL SKETCH

NAME: **Fujimori, Danica Galonić**

eRA COMMONS USER NAME: **DANICA_GALONIC**

POSITION TITLE: **Professor of Cellular and Molecular Pharmacology and Pharmaceutical Chemistry**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
University of Belgrade, Belgrade, Serbia	BSc	07/2000	Chemistry
University of Illinois, Urbana, IL	PhD	05/2005	Chemistry
Harvard Medical School, Boston, MA	Postdoc	06/2008	Biochemistry

A. Personal Statement

My research focuses on mechanisms, regulation, and biological function of post-transcriptional and post-translational modifications. In the area of protein methylation, we are investigating histone demethylases, a class of epigenetic eraser proteins that antagonize chromatin methylation. While it is well established that activities of these enzymes are regulated by chromatin environment and metabolic state of the cell, mechanisms by which this is achieved are poorly understood. Our work on defining the functional cross-talk between chromatin recognition and demethylation led to identification of new mechanisms by which chromatin reader domains modulate catalytic activity of histone demethylases. We uncovered a positive feedback regulation in demethylase KDM5A, enabled by allosteric communication between a chromatin reader domain and the catalytic domain in this enzyme. Furthermore, we are developing chemical probes for histone demethylases with a goal of utilizing these molecules for pharmacological target validation.

In area of infectious diseases, we investigate how modifications of the bacterial ribosome confer resistance to ribosome targeting antibiotics, using enzymology, microbiology and structural biology approaches. One such resistance mechanism is enabled by methylation of the ribosome by RNA methylating enzyme Cfr.

Our work combines biochemical reconstitution, mechanistic enzymology, chemical synthesis and cell biology, which provides an ideal multidisciplinary training ground for graduate students and postdoctoral scholars. My background combines chemical synthesis and mechanistic enzymology. I received a Ph.D. in organic chemistry from the University of Illinois at Urbana-Champaign in 2005, where I worked on chemical synthesis under the direction of David Gin and Wilfred van der Donk. As a Damon Runyon Cancer Research Foundation postdoctoral fellow in the lab of Chris Walsh lab at Harvard Medical School I gained expertize in mechanistic enzymology of complex systems.

Significant publications:

a. Ortiz Torres I, Kuchenbecker KM, Nnadi CI, Fletterick RJ, Kelly MJS, **Fujimori DG.** Histone Demethylase KDM5A is Regulated by its Reader Domain Through a Positive-Feedback Mechanism. *Nat Commun* 6: 6204 2015. PMID 25686748, PMCID: PMC5062987.

b. Longbotham JE, Chio CM, Dharmarajan V, Trnka MJ, Torres I, Goswami D, Ruiz K, Burlingame AL, Griffin PR, **Fujimori DG**. Histone H3 binding to the PHD1 domain of histone demethylase KDM5A enables active site remodeling, Nature Communications, 10: 94, 2019.

c. Korczynska M, Le DD, Younger N, Gregori-Puigjané E, Tumber A, Krojer T, Velupillai S, Gileadi C, Nowak RP, Iwasa E, Pollock SB, Ortiz Torres I, Oppermann U, Shoichet BK, **Fujimori DG**. Docking and Linking of Fragments to Discover Jumonji Histone Demethylase Inhibitors. *J Med Chem*, 59: 1580-98, 2016. PMCID: PMC5080985.

B. Positions and Honors

Positions and Employment:

2005-2008 Postdoctoral Fellow, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
2008-2014 Assistant Professor, Departments of Cellular and Molecular Pharmacology & Pharmaceutical Chemistry, University of California San Francisco
2013-present Member, Hellen Diller Family Comprehensive Cancer Center, University of California San Francisco
2014-2018 Associate Professor, Departments of Cellular and Molecular Pharmacology & Pharmaceutical Chemistry, University of California San Francisco
2018-present Professor, Departments of Cellular and Molecular Pharmacology & Pharmaceutical Chemistry, University of California San Francisco
2018-present Associate Director, Chemistry and Chemical Biology Graduate Program, University of California San Francisco

Other Experience and Professional Memberships:

2011 *Ad hoc* reviewer, NIH K99/R00
2011-Pres Grant review panelist and *ad hoc* reviewer, NSF
2012 *Ad hoc* reviewer, NIH MSFE study section
2015-2016 *Ad hoc* reviewer, NIH SBCB study section
2017-Pres Member, NIH SBCB study section

Honors and Awards:

2005 Damon Runyon Cancer Research Foundation Postdoctoral Fellowship
2007 NIH Pathway to Independence Award
2008 Sandler Program in Basic Sciences Opportunity Award
2009 Kimmel Scholar Award
2010 V Foundation Scholar Award
2011 Basil O'Connor Starter Scholar Research Award
2011 NSF Career Award
2011 Searle Scholar Award
2014 PBBR New Frontier Research Award
2014 UCSF Haile T. Debas Academy of Medical Educators Excellence in Teaching Award
2015 Chauncey D. Leake Lectureship in Cellular and Molecular Pharmacology
2015 Raymond and Beverly Sackler Sabbatical Exchange Program Award, UC Berkeley
2017 Byers Award, UCSF

C. Contribution to Science

1. **Histone demethylases**: Histone demethylases are a class of epigenetic eraser proteins. These enzymes antagonize lysine methylation in chromatin. Our work is centered on understanding the regulation of these enzymes, elucidating their roles in disease and developing pharmacological tools to probe the cellular functions of demethylases. We have uncovered previously unknown mechanistic links by which chromatin context impact demethylation catalysis. Our most significant contribution in this area is the discovery of allosteric regulation in KDM5A, an oncogenic demethylase. We determined that this protein is regulation by a positive feedback mechanism, where binding of the demethylation product to a reader domain within the demethylase stimulates demethylation activity. I served as a primary investigator on the following studies:
   a. Shiau C, Trnka MJ, Bozicevic A, Ortiz Torres I, Al-Sady B, Burlingame AL, Narlikar GJ, **Fujimori DG**. Reconstitution of Nucleosome Demethylation and Catalytic Properties of a Jumonji Histone Demethylase. *Chem Biol* 20: 494-9, 2013. PMCID: PMC3704229
   b. Ortiz Torres I, Kuchenbecker KM, Nnadi CI, Fletterick RJ, Kelly MJS and **Fujimori DG**. Histone Demethylase KDM5A is Regulated by its Reader Domain Through a Positive-Feedback Mechanism. *Nature Commun* 6: 6204. 2015. PMID 25686748, PMCID: PMC5080983.
   c. Pack LR, Yamamoto KR, **Fujimori DG**. Opposing chromatin signals direct and regulate the demethylase activity of KDM4C. *J Biol Chem*, 291: 6060-6070, 2016. PMCID: PMC4813556

d. Longbotham JE, Chio CM, Dharmarajan V, Trnka MJ, Torres I, Goswami D, Ruiz K, Burlingame AL, Griffin PR, **Fujimori DG**. Histone H3 binding to the PHD1 domain of histone demethylase KDM5A enables active site remodeling, Nature Communications, 10: 94, 2019.

2. **Tools for epigenetics and chromatin**: Our lab has developed and/or advanced several methods to study chromatin modifications by relying on chemoselective protein modification strategies. In addition, we have advanced the use of existing methods to elucidate the functions of unknown domains that interact with chromatin. Furthermore, we have developed highly potent small molecule inhibitors for demethylases. I served as a primary investigator or as co-investigator on the following studies:
   a. Le DD, Cortesi A, Myers SA, Burlingame AL, **Fujimori DG**. Site- and Regiospecific Installation of Methylarginine Analogs into Recombinant Histones and Insights into Effector Protein Binding. *J Am Chem Soc* 135: 2879-2882, 2013. PMCID: PMC4260808
   b. Dumesic PA, Homer CM, Moresco JJ, Pack LR, Coyle SM, Strahl BD, **Fujimori DG**, Yates III JR, Madhani HD. Product binding enforces the genomic specificity of a yeast polycomb repressive complex. *Cell* 160: 204-218, 2015. PMCID: PMC4303595
   c. Korczynska M, Le DD, Younger N, Gregori-Puigjané E, Tumber A, Krojer T, Velupillai S, Gileadi C, Nowak RP, Iwasa E, Pollock SB, Ortiz Torres I, Oppermann U, Shoichet BK, **Fujimori DG**. Docking and Linking of Fragments to Discover Jumonji Histone Demethylase Inhibitors. *J Med Chem*, 59: 1580-98, 2016. PMCID: PMC5080985.

3. **RNA methylation and its role in antibiotic resistance:** Modification of the peptidyltransferase center of the bacterial ribosome by Radical SAM enzymes RlmN and Cfr is mechanistically unique. In contrast to a majority of biological methylation substrates, which are electron rich, methylation substrates of these enzymes are electron poor. My lab has contributed to the discovery of a novel mechanism that these enzymes use to carry out methylation. Our work demonstrated that these enzymes, so-called Radical SAM methyl synthases, have several key mechanistic features, such as the formation of a unique covalent intermediate between the enzyme and the substrate as well as the ability to utilize *S*-adenosylmethionine both in homolyitc and in heterolytic fashion. Enzymatic methylations performed by these enzymes have important roles in the regulation of antibiotic susceptibility. I served as a primary investigator on the following studies:
   a. Yan, F, **Fujimori, DG**. RNA Methylation by Radical SAM Enzymes RlmN and Cfr Proceeds via Methylene Transfer and Hydride Shift. *Proc Natl Acad Sci USA* 108: 3930-34, 2011. PMCID: PMC3054002
   b. McCusker KP, Medzihradszky KF, Shiver AL, Nichols RJ, Yan F, Maltby DA, Gross CA, **Fujimori DG**. Covalent Intermediate in the Catalytic Mechanism of the Radical SAM Methyl Synthase RlmN Trapped by Mutagenesis. *J Am Chem Soc* 134: 18074-81, 2012. PMCID: PMC3499099
   c. Stojkovic V, Noda-Garcia L, Tawfik DS, **Fujimori DG**. Antibiotic Resistance Evolved via Inactivation of a Ribosomal RNA Methylating Enzyme*, Nucl Acids Res*, Aug 5. 2016. PMCID: PMC5062987.
   d. Stojkovic V, Chu T, Therizols G, Weinberg DE, **Fujimori DG**. miCLIP-MaPseq, a substrate identification approach for radical SAM RNA methylating enzymes. *J Am Chem Soc* 140: 7135-7143, 2018.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/danica.fujimori.1/bibliography/41758140/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

R01 GM114044 Fujimori (PI) 09/15/2015 - 08/31/2019
NIH/NIGMS
Allosteric Regulation in the KDM5 Family of Histone Demethylases
In this proposal are aiming to elucidate the mechanistic basis and the functional significance of allosteric regulation of histone demethylation catalyzed by the members of the KDM5 subfamily of jumonji histone demethylases.

New Frontiers Research Award Fujimori (PI) 06/15/2018 - 06/14/2019
UCSF Program for Breakthrough Biomedical Research
Disrupting Transcription through Targeting of Chromatin Methylation Readers
This award supports development of chemical probes that target epigenetic reader domains.

R01 AI137270 Fujimori (PI) 09/14/2018 - 08/31/2022
NIH/NIGMS
Radical SAM-dependent Methylation in Antibiotic Resistance
In this application we aim to investigate, using biochemical and structural studies, how aberrant methylation of the peptidyl transferase center of the ribosome impacts antibiotic susceptibility.

OVERLAP:
None.

Completed Research Support:

R01 AI095393 (Fujimori PI) 06/15/2011 - 05/31/2017
NIH/NIAID (NCE)
Radical SAM Methyltransferases
The proposed research aims to elucidate the mechanism of modification of ribosomal RNA by methyltransferases RlmN and Cfr. This modification renders bacteria resistant to several important classes of clinically used antibiotics, and its mechanistic understanding could lead to the development of new treatments for multi-drug resistant pathogens.

Catalyst Award Fujimori (PI) 07/01/2015 - 06/30/2016
UCSF Clinical & Translational Science Institute
Targeting Oncogenic KDM4 Demethylases
In this application we propose to expand our efforts to develop potent, selective and cell active inhibitors of histone demethylases through a combination of computational docking, chemical synthesis and activity assays.

Early Career Development Award Fujimori (PI) 01/15/2011 - 12/31/2015
National Science Foundation
Award ID: 1056143
A Chemical Approach to Elucidate the Mechanism of Radical SAM Methyltransferases
This early career development award offered general support of our research and teaching efforts, and provides funds for helping students from underrepresented groups spend a summer doing research.

New Frontier Research Award Fujimori (PI) 05/01/2014 - 04/30/2015
UCSF Program for Breakthrough Biomedical Research 07/31/2015 (NCE)
Regulation of Propagation in Chromatin Demethylation
The aim of this project was to define a molecular mechanism of spread of chemical signals that determine whether a gene is active or silent, determine the molecular mechanism of cross-talk between the reader and the catalytic domain in the histone demethylase KDM5A and elucidate the role of this cross-talk role in the propagation of chromatin demethylation.

Searle Scholar Award Fujimori (PI) 07/01/2011 - 06/30/2014
Kinship Foundation
Grant No. 11-SSP-157
Histone Demethylases in Cellular Regulation
The aim in this work was to identify substrates and develop chemical tools to help decipher the histone code as it related to dynamic changes in methylation caused by Jumonji C domain-containing histone demethylases.

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: Fraser, James Solomon

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

POSITION TITLE: Associate Professor of Bioengineering and Therapeutic Sciences

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
McGill University, Montreal, QC, Canada	B.Sc.	05/2005	Biology
University of California, Berkeley, CA	Ph.D.	12/2010	Molecular and Cell Biology

A. Personal Statement

The long-term goals of our research are to understand how protein conformational ensembles are reshaped by perturbations and to quantify how these perturbations impact protein function and organismal fitness. We are best known for creating multitemperature X-ray data collection approaches, which are especially powerful when paired with multiconformer computational modeling to reveal otherwise inaccessible features of conformational ensembles. My group has also pioneered methods to model and evaluate the data emerging from the “resolution revolution” in cryo-electron microscopy. I took advantage of a sabbatical to immerse myself in the practical aspects of electron microscopy data collection and processing. I have brought this new perspective back to UCSF, as my group integrates high resolution EM, X-ray, NMR, and computation to improve protein engineering and small molecule discovery. I care deeply about mentorship: my first three postdoctoral fellows are on their desired career trajectories (D. Keedy, Assistant Professor at CCNY; B. Hudson, Scientist at Relay Therapeutics; S. de Oliviera at Frontier Medicines); my first five graduate students have moved onto postdoctoral training (R. Woldeyes, with Wah Chiu/Stanford; D. Mavor, with Dan Bolon/UMass; B. Barad with Danielle Grotjahn/Scripps), transitioned into a staff scientist role (J. Biel, with the X-ray facility at UCSF), or launched successful careers in industry (A. van Benschoten, data scientist at Oracle). In addition, I have incorporated new technologies in teaching, establishing a deep sequencing-based project course that published multiple papers with student co-authors and a new “Methods in Structural Biology” class, with EM, X-ray, and NMR data collection at UCSF or the Advanced Light Source at LBNL.

Key Citations

1. Stojković V, Myasnikov AG, Young ID, Frost A, **Fraser JS,** Fujimori DG. High-resolution cryo-electron microscopy structure of the Escherichia coli 50S subunit and validation of nucleotide modifications. 2019. Preprint on BioRxiv: http://dx.doi.org/10.1101/695429
2. Li Q*, Pellegrino J*, Lee DJ, Tran AA, Wang R, Park JE, Ji K, Chow D, Zhang N, Brilot AF, Biel JT, van Zundert G, Borrelli K, Shinabarger D, Wolfe C, Murray B, Jacobson MP, **Fraser JS**, Seiple IB. Synthesis and Mechanism of Action of Group a Streptogramin Antibiotics That Overcome Resistance. 2019. Preprint on ChemRxiv: https://doi.org/10.26434/chemrxiv.8346107
3. Keedy DA*, Hill ZB*, Biel JT, Kang E, Rettenmaier TJ, Brandao-Neto J, Pearce NM, von Delft F, Wells JA, **Fraser JS**. An expanded allosteric network in PTP1B by multitemperature crystallography, fragment screening, and covalent tethering. *eLife.* 2018. PMCID: PMC6039181.
4. Otten R*, Liu L*, Kenner LR, Clarkson MW, Mavor D, Tawfik DS, Kern D, **Fraser JS.** Rescue of conformational dynamics in enzyme catalysis by directed evolution. *Nature Communications.* 2018. PMCID: PMC5883053.

B. Positions and Honors

Positions and Employment

2011-2012 QB3 at UCSF Faculty Fellow (Principal Investigator)
Department of Cellular and Molecular Pharmacology, UCSF
California Institute of Quantitative Biosciences (QB3)

2013-2016 Assistant Professor
Department of Bioengineering and Therapeutic Sciences, UCSF
California Institute of Quantitative Biosciences (QB3)

2016 - Consulting Professor
Department of Photon Science
SLAC National Accelerator Laboratory

2016 - Associate Professor
Department of Bioengineering and Therapeutic Sciences, UCSF
California Institute of Quantitative Biosciences (QB3)

2019 - Faculty Scientist
Molecular Biophysics and Integrated Bioimaging Division
Lawrence Berkeley National Lab

Other Experience

2007 - Author of problems/solutions manual for physical biochemistry textbook “The Molecules of Life” (Garland Science, Authors: John Kuriyan, Boyana Konforti, David Wemmer)
2008-2009 Assistant to Professor Howard Schachman for NIH Ethics Training (MCB 293C)
2013-2015 Advanced Light Source Proposal Review (Structural Biology), Panel Member
2015-2018 Linac Coherent Light Source (XFEL) Proposal Review Panel (BIO-C), Chair
2016- Beamline 8.3.1. at the Advanced Light Source, Head of Participating Research Team
2016- ASAPbio (Accelerating Science and Publication in biology) Board of Directors, Treasurer
2016- Relay Therapeutics, Consultant
2017- Quantitative Biosciences Institute of UCSF, Associate Director
2017- ALS-ENABLE P30 Resource, Deputy Director
2018 Protein Society Annual Symposium, Co-Chair
2018- PHENIX (Python-based Hierarchical ENvironment for Integrated Xtallography), Advisory Board
2019- UCSF Biophysics Graduate Program, Associate Director

Honors

2001-2005 Canadian Millennium Excellence Undergraduate Scholarship
2004 NSERC Undergraduate Summer Research Award (Mentor: Alan Davidson)
2006-2007 Natural Sciences and Engineering Research Council (Canada) Postgraduate Fellowship
2007-2010 Natural Sciences and Engineering Research Council (Canada) Doctoral Fellowship
2007-2010 National Science Foundation Graduate Research Fellowship
2010 EMBO Short Term Fellowship (Host: Dan Tawfik, Weizmann Institute, Israel)
2010 Warren DeLano Award for Structural Bioinformatics and Computational Biophysics
2011 Nicholas Cozzarelli Prize for Best Dissertation in Molecular and Cell Biology (UCB)
2011 Forbes 30 under 30 Science
2014 Searle Scholar, Kinship Foundation
2014 Pew Scholar, Pew Charitable Trusts
2014 Packard Fellow, The David and Lucile Packard Foundation
2017-2018 UCSF/Berkeley Sabbatical Exchange Fellowship (Host: Eva Nogales)

C. Contributions to Science

1. **Identifying hidden alternative conformations of proteins in biophysical data**. We study proteins as conformational ensembles. Although X-ray crystallography is an ensemble experiment, the results are typically summarized with a single static structure. As a graduate student, and now in my own lab, we have developed software to discover the structural ensembles present in the crystal. The ensemble nature of proteins highlighted by this work feeds into all of our mechanistic studies that interpret the functional effects of mutations, that characterize designed and artificially-evolved proteins, or that seek to modulate protein function with small molecules. We are expanding this direction to include modeling and validating protein

structural data generated by cryoelectron microscopy (using EMRinger and ensemble modeling) and through integrative approaches to discover cryptic sites.

a. Eshun-Wilson L, Zhang R, Portran D, Toso D, Lohr T, Vendruscolo M, Bonomi M, **Fraser JS**, Nogales E. Effects of α-tubulin acetylation on microtubule structure and stability. *PNAS*. 2019. PMCID: PMC6535015
b. van Zundert GCP*, Hudson BM*, Oliveira SHP, Keedy DA, Fonseca R, Heliou A, Suresh P, Borrelli K, Day T, **Fraser JS**, van den Bedem H. qFit-ligand reveals widespread conformational heterogeneity of drug-like molecules in X-ray electron density maps. *J Med Chem*. 2018. PMCID: PMC6820680.
c. Barad BA, Echols N, Wang RY, Cheng Y, DiMaio F, Adams PD, **Fraser JS**. EMRinger: Side-chain-directed model and map validation for 3D Electron Cryomicroscopy. *Nature Methods*. 2015. PMCID: PMC4589481.
d. **Fraser JS**, Clarkson MW, Degnan SC, Erion R, Kern D, Alber T. Hidden alternative structures of proline isomerase essential for catalysis. *Nature*. 2009. PMCID: PMC2805857.

2. **Creating multi-temperature X-ray data collection methods to inform mechanistic studies**. We recognized that the standard practice of cryocooling crystals could distort protein conformations. In both larger surveys and isolated mechanistic studies, we have demonstrated the value of room temperature data collection for revealing the structural basis of protein conformational dynamics, leading to new insights into the enzymes PTP1B, CypA, H-Ras, and DHFR, and increasing connections to dynamics studies from NMR and simulations. Additionally, we have identified how temperature can bias small molecule discovery, leading some fragment sites inaccessible at cryogenic temperatures, and the positioning of crucial water molecules in the flu ion channel M2.
    a. **Fraser JS**, van den Bedem H, Samelson AJ, Lang PT, Holton JM, Echols N, Alber T. Accessing protein conformational ensembles by room-temperature X-ray crystallography. *PNAS*. 2011. PMCID: PMC3182744.
    b. Fischer M, Coleman RG, **Fraser JS**, Shoichet BK. Incorporation of protein flexibility and conformational energy penalties in docking screens to improve ligand discovery. *Nature Chemistry*. 2014. PMCID: PMC4144196.
    c. Keedy DA*, Kenner LR*, Warkentin M*, Woldeyes RA*, Thompson MC, Brewster AS, Van Benschoten AH, Baxter EL, Hopkins JB, Uervirojnangkoorn M, McPhillips SE, Song J, Alonso-Mori R, Holton JM, Weis WI, Brunger AT, Soltis SM, Lemke H, Gonzalez A, Sauter NK, Cohen AE, van den Bedem H, Thorne RE**, Fraser JS**. Mapping the Conformational Landscape of a Dynamic Enzyme by XFEL and Multitemperature Crystallography. *eLife*. 2015. PMCID: PMC4721965.
    d. Biel JT, Thompson MC, Cunningham CN, Corn JE, **Fraser JS.** Flexibility and design: conformational heterogeneity along the evolutionary trajectory of a redesigned ubiquitin. *Structure*. 2017. PMCID: PMC5415430.
3. **Developing new X-ray diffuse and time-resolved scattering experiments to probe correlated motions in proteins**. A major limitation of most biophysical techniques is the inability to directly reveal correlations in motions between distinct regions of macromolecules. Diffuse scattering has the potential to reveal these motions; however, we currently lack the ability to collect, integrate, and refine diffuse scattering data. We are tackling each of these problems directly with collaborators: Michael Wall, Nicholas Sauter, Tom Terwilliger, and Paul Adams. Our long-term goal is to increase the information content of every X-ray diffraction experiment to reveal atomic level coupling at high resolution and improved models of grouped flexibility at low resolution. We are also taking advantage of the new capabilities of next-generation X-ray free electron laser (X-FEL) light sources to perform radiation damage-free imaging of proteins and to watch how protein ensembles respond when perturbed by rapid temperature jumps using the X-FEL.
    a. Thompson MC, Barad BA, Wolff AM, Cho HS, Schotte F, Schwarz DMC, Anfinrud P, **Fraser JS**. Temperature-Jump Solution X-ray Scattering Reveals Distinct Motions in a Dynamic Enzyme. *Nature Chemistry.* 2019. PMCID: PMC6815256.

b. Van Benschoten AH, Liu L, Gonzalez A, Brewster AS, Sauter NK, **Fraser JS**, Wall ME. Measuring and modeling diffuse scattering in protein X-ray crystallography. *PNAS*. 2016. PMCID: PMC4839442.
c. Wall ME, Van Benschoten AH, Sauter NK, Adams PD, **Fraser JS**, Terwilliger TC. Conformational dynamics of a crystalline protein from microsecond-scale molecular dynamics simulations and diffuse X-ray scattering. *PNAS*. 2014. PMCID: PMC4273327.
d. Thomaston JL, Woldeyes RA, Nakane T, Yamashita A, Tanaka T, Koiwai K, Brewster AS, Barad BA, Chen Y, Lemmin T, Uervirojnangkoorn M, Arima T, Kobayashi J, Masuda T, Suzuki M, Sugahara M, Sauter NK, Tanaka R, Nureki O, Tono K, Joti Y, Nango E, Iwata S, Yumoto F, **Fraser JS**, DeGrado WF. XFEL structures of the influenza M2 proton channel: Room temperature water networks and insights into proton conduction. *PNAS*. 2017. PMCID: PMC5754760

4. **Determining structures that influence microbial-host interactions**. I have a longstanding interest in microbiology, beginning from my undergraduate work with Alan Davidson (Toronto) on bacteriophage structure prediction that lead to the surprising discovery of a class of mobile immunoglobulin domains. I have collaborated with the Zusman lab (UC Berkeley) to determine the structure of FrzS, a key signaling regulator of Myxococcus xanthus, with the Fischbach lab (Stanford) to determine how the gut microbiome produces the neurotransmitter tryptamine, and with the Tawfik lab (Weizmann Institute, Israel) to determine the role of epistasis in restricting antibiotic resistance mutations. We are expanding this interest to include the interaction of human enzymes in degrading chitin molecules that can cause inflammation in the context of allergy and asthma, the hijacking of the proline isomerase CypA in lentiviral evolution, and structure-based antibiotic design using cryoEM.
    a. **Fraser JS**, Yu Z, Maxwell KL, Davidson AR. Ig-like domains on bacteriophages: a tale of promiscuity and deceit. *J Mol Biol.* 2006. PMID: 16631788.
    b. Williams BB, Van Benschoten AH, Cimermancic P, Donia MS, Zimmermann M, Taketani M, Ishihara A, Kashyap PC, **Fraser JS**, Fischbach MA. Discovery and characterization of gut microbiota decarboxylases that can produce the neurotransmitter tryptamine. *Cell Host Microbe*. 2014. PMCID: PMC4260654
    c. Dellus-Gur E, Elias M, Caselli E, Prati F, Salverda ML, de Visser JA, **Fraser JS**, Tawfik DS. Negative epistasis and evolvability in TEM-1 β-lactamase - The thin line between an enzyme's conformational freedom and disorder. *J Mol Biol*. 2015. PMCID: PMC4718737.
    d. Barad BA, Liu L, Diaz RE, Basillo R, Van Dyken SJ, Locksley RM, **Fraser JS**. Dissecting the chitinolytic activity of mammalian chitinases. Preprint on BioRxiv: http://dx.doi.org/10.1101/762336
5. **Identifying unifying concepts between systems and structural biology**. With Nevan Krogan, we have articulated the similarities in genetic epistasis and thermodynamic measurements and applied these insights to large-scale studies of point mutants and posttranslational modifications. This framework forms the basis for the UCSF graduate course that I direct, PUBS (Physical Underpinnings of Biological Systems), which uses deep sequencing to determine the context dependence of fitness effects of mutations. The class is taught through project-based learning where incoming students perform all library preparations, load samples directly on the MiSeq, and write all their own code to process sequencing data.
    a. Beltrao P, Albanèse V, Kenner LR, Swaney DL, Burlingame A, Villén J, Lim WA, **Fraser JS**, Frydman J, Krogan NJ. Systematic functional prioritization of protein posttranslational modifications. *Cell*. 2012. PMCID: PMC3404735
    b. Braberg H, Jin H, Moehle EA, Chan YA, Wang S, Shales M, Benschop JJ, Morris JH, Qiu C, Hu F, Tang LK, **Fraser JS**, Holstege FC, Hieter P, Guthrie C, Kaplan CD, Krogan NJ. From structure to systems: high-resolution, quantitative genetic analysis of RNA polymerase II. *Cell*. 2013. PMCID: PMC3932829
    c. **Fraser JS**, Gross JD, Krogan NJ. From systems to structure: bridging networks and mechanism. *Mol Cell*. 2013. PMCID: PMC3558917
    d. Mavor D, Barlow KA, Thompson S, Barad BA, Bonny AR, Cario CL, Gaskins G, Liu Z, Deming L, Axen SD, Caceres E, Chen W, Cuesta A, Gate R, Green EM, Hulce KR, Ji W, Kenner LR, Mensa B, Morinishi LS, Moss SM, Mravic M, Muir RK, Niekamp S, Nnadi CI, Palovcak E, Poss EM, Ross TD,

Salcedo E, See S, Subramaniam M, Wong AW, Li J, Thorn KS, Conchúir SÓ, Roscoe BP, Chow ED, DeRisi JL, Kortemme T, Bolon DN, **Fraser JS**. Determination of Ubiquitin Fitness Landscapes Under Different Chemical Stresses in a Classroom Setting. *eLife*. 2016. PMCID: PMC4862753

Complete List of 62 Publications in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/james.fraser.1/bibliography/public/

D. Research Support

Ongoing Research Support

Technologies, Methodologies & Cores Award Fraser (PI) 10/01/19 – 09/30/20
UCSF Program for Breakthrough Biomedical Research (PBBR)
Leveraging the Macromolecular Structure Group and Beamline Resources for High-throughput Liganding of Challenging Targets
The goal of this project is to set up an infrastructure for UCSF investigators to perform high-throughput soaking experiments.

R01 GM123159 Fraser (PI) 12/01/17 – 11/31/21
NIH/NIGMS
Resolving ensemble averaged conformations by multi-temperature x-ray crystallography
The objective of this research program is to experimentally access and computationally model multi-scale heterogeneity in allosteric protein-ligand complexes.

P30 GM0519206 Adams (PI) 07/01/17 – 06/30/22
NIH/NIGMS
ALS Efficiently Networking Advanced Beam Line Experiments (ALS-ENABLE)
Fraser administers the project as Deputy Director of Macromolecular Crystallography and performs outreach. Fraser is the deputy project director, overseeing the crystallography component of the project.

NSF 11-522 Snell (PI) 09/01/13 – 09/01/23
NSF - OIA - SCI & TECH CTRS
Biology with X-ray Lasers
The major goal of this center is to encourage the development of methods for biophysics using the newly developed x-ray free electron lasers (X-FEL). We participate by generating samples for X-FEL diffraction and comparing the resulting data to room temperature synchrotron datasets.

MCB 1714915 Herschlag (PI) 08/01/17 – 07/31/21
NSF
Collaborative Research: Systematic Investigation of the Structure, Dynamics, and Energetics of Hydrogen Bonds and the Protein Interior Using Ketosteroid Isomerase and Model Systems
The goal of this project is to determine the biophysical and mechanistic basis for enzyme catalysis.

R01 GM0517315 Holton (PI) 07/01/17 – 06/30/22
NIH/NIGMS
Eliminating Critical Systematic Errors In Structural Biology With Next-Generation Simulation
The goal of the project is to use simulations to explore systematic errors to enable improved modeling.

Completed Research Support

DP5 OD009180 Fraser (PI) 09/01/11 – 08/31/17
NIH/OSC
The Impact of Mutation on the Conformations and Recognition of Ubiquitin
This project used deep mutational scanning and biophysical characterization to study variants of Ubiquitin.