BIOGRAPHICAL SKETCH

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NAME: Zhang, Feng

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

POSITION TITLE:Core Member

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
Harvard University, Cambridge, MA	AB	06/2004	Chemistry
Stanford University, Stanford, CA	PHD	06/2009	Chemistry

A. Personal Statement

The recent advent of precise genome editing technologies promises to revolutionize the way thousands of human diseases are treated: instead of a drug that can ameliorate symptoms, tools exist that could potentially correct the underlying genetic changes causing the disease. The goal of my research program is to realize this potential by developing novel modular therapeutic platforms for treating human diseases. Broadly, this work can be divided in two: continued development of molecular and cellular tools for correcting and treating disease (cargo) and novel modes of delivery of these therapeutics. Although these can be viewed as separate lines of work, the overarching goal is to create a complete suite of tools for cellular and genetic manipulation that can be used interchangeably with an array of delivery vehicles.

a. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, **Zhang F**. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013 Feb 15;339(6121):819-23. PMCID: PMC3795411.
b. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DBT, Shmakov S, Makarova KS, Semenova E, Minakhin L, Severinov K, Regev A, Lander ES, Koonin EV, **Zhang F.** C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*. 2016 Aug 5;353(6299):aaf5573. PMCID: PMC5127784
c. Strecker J, Ladha A, Gardner Z, Schmid-Burgk JL, Makarova KS, Koonin EV, **Zhang F**. RNA-guided DNA insertion with CRISPR-associated transposases. *Science.* 2019 Jul 5;365(6448):48-53. PMCID: PMC6659118
d. Segel M, Lash B, Song J, Ladha A, Liu CC, Jin X, Mekhedov SL, Macrae R, Koonin EV, **Zhang F.** Mammalian retrovirus-like protein PEG10 packages its own mRNA and can be pseudotyped for mRNA delivery. *Science.* 2021 Aug 20;373(6557):882-889. NIHMSID1736872

B. Positions and Honors

Positions and Employment

1997 – 1999 Research Assistant with John Levy, Ph.D., Human Gene Therapy Research Institute, Des Moines, IA
2000 – 2001 Research Assistant with Don Wiley, Ph.D., Harvard University, Cambridge, MA
2002 – 2004 Research Assistant with Xiaowei Zhuang, Ph.D., Harvard University, Cambridge, MA
2004 – 2009 Graduate Student with Karl Deisseroth, M.D., Ph.D., Stanford University, Stanford, CA
2009 – 2010 Junior Fellow, Society of Fellows, Harvard University, Cambridge, MA
2011 – Core Member, Broad Institute of MIT and Harvard, Cambridge, MA

2011 – 2016	Assistant Professor of Neuroscience and Biological Engineering, MIT, Cambridge, MA
2011 –	Investigator, McGovern Institute for Brain Research at MIT, Cambridge, MA
2015 – 2019	Robertson Investigator, New York Stem Cell Foundation
2016 – 2019	Associate Professor (with tenure) of Neuroscience and Biological Engineering, MIT
2017 –	James and Patricia Poitras Professor in Neuroscience
2018 –	Investigator, Howard Hughes Medical Institute
2019 –	Professor of Neuroscience and Biological Engineering, MIT

Select Honors

2014	Alan T. Waterman Award (for optogenetics and CRISPR-Cas9), National Science Foundation
2015	Tsuneko and Reiji Okazaki Award (for CRISPR-Cas9), Nagoya University
2016	Canada Gairdner International Award (for CRISPR-Cas9, shared with Charpentier & Doudna)
2016	Tang Prize (for CRISPR-Cas9, shared with Charpentier & Doudna)
2016	New York Stem Cell Foundation – Robertson Stem Cell Prize
2017	Blavatnik Award for Young Scientists – National Award Winner
2017	Lemelson-MIT prize
2017	Albany Medical Center Price in Medicine and Biomedical Research (for CRISPR-Cas9, shared with Mojica, Marraffini, Charpentier & Doudna)
2018	Vilcek Prize for Creative Promise in Biomedical Science
2018	Elected Member, National Academy of Sciences
2018	Keio Medical Science Prize
2018	Harvey Prize
2019	Mallinckrodt Foundation Scholar
2020	Elected Fellow, National Academy of Inventors
2021	Richard Lounsbery Award
2021	Edward Novitski Prize, Genetics Society of America

C. Contributions to Science

A list of all publications can be found at the link below:
http://www.ncbi.nlm.nih.gov/sites/myncbi/feng.zhang.4/bibliography/44154648/public

1. **Development of Cas9 for genome editing:** I developed the nuclease from CRISPR-Cas9, a microbial defense system, for mammalian genome editing, demonstrating for the first time that these bacterial proteins could be reprogrammed to achieve precise editing in a eukaryotic genomic context. At the time we began working on Cas9, it was unknown if it could function in eukaryotic cells, and we devised a strategy to systematically test and optimize Cas9 for in vivo mammalian genome editing. When we reported these results (Cong et al., 2013), it sparked a wave of interest, and thousands of laboratories are now using the CRISPR-Cas9 system for a variety of applications. My group continued to pioneer the development of CRISPR-Cas9 technology, including improving its specificity (e.g., Slaymaker et al., 2015); applying it for mouse models of disease (e.g., Wang et al., 2013); optimizing a Cas9 ortholog to achieve more efficient in vivo delivery (Ran et al., 2015); and developing methods to assess and improve the specificity of Cas9. Cas9 has now been used in clinical trials to successfully treat multiple genetic diseases.

    a. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA, **Zhang F**. Multiplex genome engineering using CRISPR/Cas systems. *Science*. 2013 Feb 15;339(6121):819-23. PMCID: PMC3795411.
    b. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, **Zhang F**. In vivo genome editing using Staphylococcus aureus Cas9. *Nature*. 2015 Apr 9;520(7546):186-91. PMCID: PMC4393360.
    c. Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, **Zhang F**, Jaenisch R. One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. *Cell.* 2013 May 9;153(4):910-8. PMCID: PMC3969854
    d. Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, **Zhang F**. Rationally engineered Cas9 nucleases with improved specificity. *Science.* 2016 Jan 1;351(6268):84-8. PMCID: PMC4714946

2. **Discovery and development of RNA-guided enzymes**: Our work with CRISPR-Cas9 inspired us to explore the natural diversity of CRISPR-Cas systems to discover novel systems with useful properties for molecular technologies. We first identified and characterized Cas12a (previously known as Cpf1) and adapted it for use in mammalian genomes (Zetsche et al., 2015); clinical trials using Cas12a to treat genetic diseases are now underway. Using a bioinformatics approach, we discovered the Cas13 family of RNA-guided RNA-targeting enzymes (Abudayyeh et al., 2016), which we engineered for RNA knock-down and precision RNA base editing. We also discovered and developed additional DNA- and RNA-targeting CRISPR systems for use in mammalian cells, including Cas12b and Cas13a. We continued to expand our efforts, leading to the discovery and characterization of a CRISPR-associated transposon (CAST) system, which we showed could be engineered to achieve precise RNA-guided gene insertion in *E. coli* (Strecker et al., 2019b). Recently, we uncovered a new family of RNA-guided enzymes, termed OMEGAs, that are among some of the most abundant bacterial proteins (Altae-Tran et al., 2021). We showed that some of these OMEGAs can be harnessed for gene editing in human cells. Together, characterizing and adapting these programmable enzymes will provide many new tools for molecular biologists and serve as the foundations for new technologies. This work shed new light on the biology of RNA-guided systems and their evolutionary origins.

    a. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz S, Joung J, van der Oost J, Regev A, Koonin EV, **Zhang F**. Cpf1 is a single RNA-guided endonuclease of a Class 2 CRISPR-Cas system. *Cell*. 2015 Oct 22; 162(3):759-771. PMCID: PMC4638220
    b. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DBT, Shmakov S, Makarova KS, Semenova E, Minakhin L, Severinov K, Regev A, Lander ES, Koonin EV*, **Zhang F*.** C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*. 2016 Aug 5;353(6299):aaf5573. PMCID: PMC5127784
    c. Strecker J, Ladha A, Gardner Z, Schmid-Burgk JL, Makarova KS, Koonin EV, **Zhang F**. RNA-guided DNA insertion with CRISPR-associated transposases. *Science.* 2019 Jul 5;365(6448):48-53. PMCID: PMC6659118
    d. Altae-Tran H, Kannan S, Demircioglu FE, Oshiro R, Nety SP, Mckay LJ, Dlakic M, Inskeep WP, Makarova KS, Macrae RK, Koonin EV, **Zhang F.** The widespread IS200/605 transposon family encodes diverse programmable RNA-guided endonucleases. *Science.* 2021

3. **Development of new molecular technologies:** I have developed a number of new molecular technologies enabling a deeper understanding of biology and improving human health. As a graduate student, I played a central role in the development of the optogenetics technology, which allows the use of light to control neural activity in specific populations of cells in the brain. As a PI, I have continued to innovate and develop powerful technologies, such as large-scale loss- and gain-of-function screening methodologies (e.g., Shalem et al., 2013), which we have applied to a number of questions in human health. To advance our understanding of the brain, we developed complementary approaches to profile the transcriptomes of single nuclei, which is particularly helpful for studying neurons and other difficult to isolate cells (Habib et al., 2016). We have also developed nucleic acid detection technologies based on CRISPR systems that are being used to advance human health. Our original detection platform, SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing), uses Cas13 to detect a target nucleic acid sequence and a quenched fluorophore reporter. In early 2020, we began adapting SHERLOCK for detection of the SARS-Cov-2 genome, first reporting a lateral-flow strip-based readout and then working to develop a version of the diagnostic, STOPCovid, that is suitable for at-home use (Joung et al., 2020). To complement our genome editing technologies, we developed a novel RNA delivery modality, SEND, which can accommodate large RNA cargoes, such as Cas9 and a guide RNA, and uses endogenous components, which may make it less immunogenic than other delivery modalities.

    a. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG, **Zhang F**. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*. 2014 Jan 3;343(6166):84-7. PMCID: PMC4089965.
    b. Habib N, Li Y, Heidenreich M, Swiech L, Avraham-Davidi I, Trombetta JJ, Hession C, **Zhang F**, Regev A. Div-Seq: Single-nucleus RNA-Seq reveals dynamics of rare adult newborn neurons. *Science* 2016 Aug 26;353(6302):925-8. PMCID: PMC5480621

c. Joung J, Ladha A, Saito M, Kim N-G, Woolley A, Segel M, Barretto R, Ranu A, Macrae R, Faure G, Ioannidi E, Krajeski R, Bruneau R, Huang M-L, Yu X, Li J, Walker B, Hung D, Greninger A, Jerome K, Gootenberg JS, Abudayyeh OO, **Zhang F**. Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. *New England Journal of Medicine* 2020 Oct 8;383(15):1492-1494. PMCID: PMC7510942

d. Segel M, Lash B, Song J, Ladha A, Liu CC, Jin X, Mekhedov SL, Macrae R, Koonin EV, **Zhang F.** Mammalian retrovirus-like protein PEG10 packages its own mRNA and can be pseudotyped for mRNA delivery. *Science.* 2021 Aug 20;373(6557):882-889. PMID: 34413232

4. **Dissemination of Technologies:** In addition to developing novel technologies, I have driven the dissemination of molecular technologies we develop. I have established web-based resources to share protocols and reagent information, established and maintained online discussion forums to facilitate the exchange of expertise (e.g., crispr@googlegroups.com), and provided detailed protocols for each of the technologies developed. We have also hosted multiple workshops aimed at teaching users how to use CRISPR-Cas technology in their own work. Finally, via the non-profit Addgene, we have fulfilled numerous reagent requests (>62,000) to help researchers adopt these new technologies as quickly and effortlessly as possible. These reagents are being used for a broad range of research efforts.

   a. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, **Zhang F**. Genome engineering using the CRISPR-Cas9 system. *Nat Protoc*. 2013 Nov;8(11):2281-308. PMCID: PMC3969860.
   b. Sanjana NE, Shalem O, **Zhang F**. Improved vectors and genome-wide libraries for CRISPR screening. *Nat Methods*. 2014 Aug;11(8):783-4. PMCID: PMC4486245.
   c. Joung J, Konermann S, Gootenberg JS, Abudayyeh OO, Platt RJ, Brigham MD, Sanjana NE, **Zhang F**. Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. *Nat Protoc*. 2017 Apri; 12(4):828–863. PMCID: PMC5526071
   d. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO & **Zhang F**. SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocols* (2019). Oct;14(10):2986-3012. PMCID: PMC6956564

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

Active support

NIH 1 DP1 HL141201-01 Zhang, Feng (PI) 09/30/2017 – 07/31/2022
Exploration of diverse mobile genetic elements for precision genome manipulation
The goal of this project is to develop a suite of tools designed to achieve precise genome surgery for repairing disease-causing changes based on microbial genetic elements, which have evolved to perform a range of DNA manipulations.
Role: PI

HHMI Zhang, Feng (PI) 01/21/2020 – 01/20/2027
Investigator
Role: PI

NIH 2R01HG009761-05 Zhang, Feng (PI) 08/25/2021 – 07/31/2025
Advancing programmable RNA-targeting tools for research and therapeutics
This project aims to mine natural diversity for enzymes that can programmably recognize RNA sequences, characterize these enzymes, and develop them into molecular tools.
Role: PI

The Milky Way Research Foundation Zhang, Feng (PI) 09/01/2021 – 08/31/2024
Reverse engineering epigenetic programming of aging and development of therapeutic interventions
The goal of this project is to combine well-established screening technologies, such as CRISPR-mediated gain- and loss-of-function screens, with novel technologies we develop, such as combinatorial epigenetic modulators and engineered aging reporters.
Role: PI

BIOGRAPHICAL SKETCH

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NAME: Demircioglu, F. Esra

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

POSITION TITLE: Staff Scientist

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
Middle East Technical University, Turkey	B.S.	06/2006	Molecular Biology and Genetics
University of Goettingen, Germany	M.S.	04/2008	Molecular Biology
University of Goettingen, Germany	Ph.D.	11/2011	Molecular Biology, Biochemistry
Massachusetts Institute of Technology, USA	Postdoctoral	10/2019	Biochemistry, Structural Biology
Broad Institute of MIT and Harvard, USA	Staff Scientist	Present	Biochemistry, Structural Biology

A. Personal Statement

As a staff scientist specializing in biochemistry and structural biology, my goal in the Zhang lab is to contribute to a number of projects aimed at characterizing and engineering molecular systems. Preparing me for this role, I have 10+ years of experience in working with challenging protein complexes, a solid background in protein crystallography, an increasing amount of experience in cryo-EM, strong motivation, and collaborative and leadership skills. During my postdoctoral training, my research mainly focused on protein crystallography, but I also got experience in cryo-EM specimen preparation while characterizing a self-assembling protein in its filamentous form. Since MIT did not have an internal cryo-EM center at the time, I used the UMass Medical School Cryo-EM Core Facility for this project for both sample preparation and cryo-EM imaging, and I collaborated with Prof. Edward Egelman and his colleagues at the University of Virginia to determine the structure of our filamentous target. During my time in the Zhang lab up till now, I contributed to discovery and biochemical characterization of OMEGA systems, and recently shifted my focus to CRISPR-associated signaling machineries, which I would like to study using cryo-EM. Until last year, my cryo-EM experience was fairly limited, and largely self-taught in terms of image processing, structure determination, and validation. However, the learning process is much accelerated for me since then, as we are a larger crew of structural biologists in the lab right now, and I have a chance to work with colleagues who have extensive cryo-EM expertise. Also, I will soon be participating in NCCAT's TP1 program for further training. In the long run, I plan to become a highly skilled cryo-EM scientist, integrate my expertise to the biodiscovery pipelines, and help establish a structural biology platform in the Zhang lab. My background and motivation is of highest relevance for the current application.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2018	Participant, MIT I-Corps TFP: Essentials program certification, Cambridge, MA
2015, 2018	Postdoctoral member, American Society for Cell Biology (ASCB)
2014	Participant, SBGrid/NE-CAT - Quo Vadis Structural Biology? Data Processing in Crystallography, Harvard Medical School, Cambridge, MA
2008	Participant, abc6 Applications of Biocalorimetry, Heidelberg, Germany

Honors

2011	Invited Speaker, 8th Molecular Mechanisms of Exocytosis & Endocytosis meeting, Edinburgh, Scotland
2008	GGNB (Goettingen Graduate Schools for Neurosciences and Molecular Biosciences) Excellence Fellowship, Goettingen, Germany

C. Contributions to Science

1. During my graduate studies, I worked with SNAREs, a group of proteins involved in vesicle fusion. I initiated a project on poorly characterized members of the SNARE family, which function during ER-to-Golgi trafficking. Through intricate ITC, fluorescence anisotropy and FRET experiments, I characterized the interaction mode between the SNARE protein Sed5 and its regulating SM protein Sly1. I found that Sly1 loosens a closed, inaccessible conformation of Sed5 and facilitates SNARE complex formation. My findings revealed how the original binding mode between SM proteins and SNAREs might have evolved.

    a. **Demircioglu, F.E.**, Burkhardt, P., and Fasshauer, D. (2014) The SM protein Sly1 accelerates assembly of the ER-Golgi SNARE complex. Proc. Natl. Acad. Sci. U.S.A. 111, 13828–13833.

2. During my postdoctoral training, I developed tools to biochemically characterize a number of nuclear envelope proteins associated with neuromuscular diseases. My research particularly focused on TorsinA, a neuronal, nuclear membrane-associated AAA+ ATPase with an unknown function. TorsinA is medically relevant, since it becomes a disease-causing molecule when its single glutamate residue at position 302 or 303 (deltaE) is deleted, leading to a prevalent neuromuscular disorder, primary dystonia. Furthermore, TorsinA, unlike its homologs, needs external activators for its ATPase activity, and TorsinAdeltaE cannot bind to these activators. I set out to understand the molecular basis of these observations using a structural biology approach. I first obtained the crystal structure of a nanobody-bound TorsinA-activator complex and characterized the activator interaction. Through structural analysis I discovered the nanobody's "connector" role between TorsinA and the activator. Implementing this knowledge also enabled me to assemble and crystallize a nanobody-bound TorsinAdeltaE-activator complex, providing a structural platform for drug development for treatment of primary dystonia. Using cryo-electron microscopy, I next characterized TorsinA in its self-assembled filamentous form. Bioinformatics and electrostatic potential analysis of these filaments led to the hypothesis that TorsinA may engage lipids. Supporting this, I observed that TorsinA tubulates membranes in vitro. Together, these findings not only help us understand the membrane defects that occur upon TorsinA dysfunction, but they also suggest entirely new characteristics of AAA+ ATPases.

    a. Sosa, B. A., **Demircioglu, F. E.,** Chen, J. Z., Ingram, J., Ploegh, H. L., and Schwartz, T. U. (2014) How lamina-associated polypeptide 1 (LAP1) activates Torsin. Elife 3:03239

    b. **Demircioglu, F. E.,** Sosa, B.A., Ingram, J., Ploegh, H.L., and Schwartz, T.U. (2016) Structures of TorsinA and its disease-mutant complexed with an activator reveal the molecular basis for primary dystonia. Elife 5:213.

c. Schwartz, T.U., **Demircioglu, F.E.,** Sosa, B.A. Crystal structures of human Torsin-A and methods of determining and using the same. U.S. patent 9,823,250 issued November 21, 2017.

d. **Demircioglu, F.E.,** Zheng, W., McQuown, A.J., Maier, N.K., Watson, N., Cheeseman, I.M., Denic, V., Egelman, E.H., and Schwartz, T.U. (2019) The AAA+ ATPase TorsinA polymerizes into hollow helical tubes with 8.5 subunits per turn. Nat Commun 10:3262.

3. During the beginning of my time in the Zhang lab as a staff scientist, I assisted protein purification and biochemistry workflow of multiple projects, focusing on CRISPR systems as well as therapeutic delivery tools. This period also helped me establish a system in the lab for protein work and build the lab space with necessary equipment and tools. Later, I started working with a team of PhD students to help investigate the evolutionary origin of CRISPR proteins. Using phylogenetic analysis, RNA sequencing, and biochemical experiments, we reconstructed the evolution of CRISPR systems from IS200/IS605 transposons. We discovered that the transposon-encoded IscB and TnpB proteins are the likely ancestors of the widely-used CRISPR effectors Cas9, and Cas12, respectively, and that the noncoding RNA (ncRNA) part of the transposon is an ancient form of guide RNA, functioning similarly to the tracrRNA and crRNA components of canonical CRISPR systems. These systems, which we call OMEGA (obligate mobile element–guided activity), are highly abundant, functionally diverse, and expand to all domains of life, including eukaryotic genomes, making them a trove of potential new biotechnologies. In addition to leading the efforts to biochemically characterize OMEGA molecules, I have also performed brief cryo-EM experimentation with a bacterial TnpB, but with limited success, mainly arising from preferred particle orientation distribution of the sample on cryo-EM grids. During the past few months, I switched my focus to some other important structural targets that our lab absolutely is interested in pursuing. I started helping another team of colleagues, working with CRISPR-associated signaling machineries, and I already collected promising cryo-EM data from these new molecules. Our hope is to structurally characterize these unique machineries, understand their mechanism of action, and engineer them toward novel molecular technologies.

a. Altae-Tran, H.*, Kannan, S.*, **Demircioglu,F.E**., Oshiro, R., Nety, S.P., McKay, L.J., Dlakić, M., Inskeep, W.P., Makarova, K.S., Macrae, R.K., Koonin, E.V., Zhang, F. (2021) The widespread IS200/IS605 transposon family encodes diverse programmable RNA-guided endonucleases. Science 374, 57–65

Complete List of Published Work in Google Scholar:
https://scholar.google.com/citations?hl=en&user=c405LNwAAAAJ&view_op=list_works&sortby=pubdate

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Wilkinson, Max Edward

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

POSITION TITLE: Postdoctoral Fellow

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
University of Otago	BSc (hons)	05/2015	Biochemistry
University of Cambridge	PhD	05/2019	Molecular biology
Massachusetts Institute of Technology	Postdoctoral		Molecular biology

A. Personal Statement

I am a postdoc in Feng Zhang's lab at the Broad Institute of Harvard and MIT, focusing on using cryo-EM to characterize new biological systems, including transposons and prokaryotic phage defense systems. I have extensive experience in cryo-EM, from my doctoral training at the MRC Laboratory of Molecular Biology in Cambridge, UK, where I worked in Kiyoshi Nagai's lab to unveil the structural basis for pre-mRNA splicing. This entailed many years of sample preparation and optimization, and many hours at cryo-electron microscopes of various sizes around the world. I am therefore well placed for carrying structural biology projects from conception to completion.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2021 – Present Postdoctoral fellow, Broad Institute of Harvard and MIT, Cambridge, MA, USA
2019 – 2021 Career development fellow, MRC Laboratory of Molecular Biology, Cambridge, UK

Honors

2019 Scaringe Award of the RNA Society, for outstanding graduate student work
2018 MRC LMB Perutz Student Prize for outstanding PhD work
2015 Trinity College Krishnan-Ang Studentship
2014 Cambridge-Rutherford Memorial Scholarship (full PhD funding)
2014 University of Otago Prince of Wales Prize (top graduating bachelors student)

C. Contributions to Science

1. In my previous research, I have been studying how nucleic acids function within molecular machines. As an undergraduate I studied CRISPR-Cas systems. I used bacterial genetics, biochemistry, and X-ray crystallography to examine how bacteria can recognize foreign DNA from mobile genetic elements and integrate it into CRISPR arrays. (Wilkinson *et al*., 2016; Fagerlund *et al*., 2017).

a. **Wilkinson, M.E.,** Nakatani, Y., Staals, R.H., Kieper, S.N., Opel-Reading, H.K., McKenzie, R.E., Fineran, P.C., Krause, K.L. (2016) Structural plasticity and in vivo activity of Cas1 from the type I-F CRISPR-Cas system. *Biochem. J.* 473, 1063–1072.
b. Fagerlund, R.D.*, **Wilkinson, M.E***, Klykov, O.*, Barendregt, A., Pearce, F.G., Kieper, S.N., Maxwell, H.W.R, Capolupo, A., Heck, A.J.R., Krause, K.L., Bostina, M., Scheltema, R.A., Staals, R.H.J., Fineran, P.C. (2017) Spacer capture and integration by a type I-F Cas1-Cas2-3 CRISPR adaptation complex. *Proc. Natl. Acad. Sci. U. S. A.* 114, E5122–E5128.

2. For my PhD I decided to apply the growing power of cryo-electron microscopy (cryo-EM) to a molecular machine fundamental to eukaryotic gene expression: the spliceosome. The spliceosome removes introns from pre-mRNA. Decades of biochemical studies indicated that the spliceosome uses an RNA-based active site to perform two reactions: branching, where a conserved adenosine within the branchpoint sequence (BPS) attacks the 5'-splice site (5'SS), and exon ligation, where the 5'SS attacks the 3'-splice site (3'SS). My PhD work aimed to understand the mechanism of these two reactions by solving structures of the yeast spliceosome at key catalytic stages by cryo-EM. I purified and solved the structure of a step one spliceosome (C complex) which revealed the active site of the spliceosome and showed how the catalytic snRNA core brings together the BPS and 5'SS for nucleophilic attack within an intricate shell of proteins. The structure accounted for many genetic and biochemical studies of the spliceosome and showed the role of numerous splicing factors.
    a. Galej, W.P., **Wilkinson, M.E.,** Fica, S.M., Oubridge, C., Newman, A.J., Nagai, K. (2016) Cryo-EM structure of the spliceosome immediately after branching. *Nature* 537, 197–201.

3. Next, I wanted to understand how the spliceosome recognizes the 3'SS to perform the second step of splicing. Although the other two hallmarks of an intron, the 5'SS and BPS, are recognized by base-pairing to snRNAs, the recognition mechanism of the 3'SS, defined only by an AG dinucleotide and a preceding pyrimidine, was not understood. I purified the post-catalytic spliceosome stalled after exon-ligation and solved its structure by cryo-EM. The structure showed a large conformational rearrangement where the BPS:U2 snRNA helix undocks from the active site, creating a cavity in the active site for docking of the 3'SS. The 3'SS AG is not recognized by snRNAs but by other nucleotides within the intron itself: the 3'SS(-2) adenosine pairs with the branchpoint adenosine, and the 3'SS(-1) guanosine pairs with the 5'SS(+1) guanosine. Exon ligation is therefore the only state during splicing where all the main sequences of an intron – the starting GU, the ending AG, and the branchpoint adenosine – come together, explaining their conservation across eukaryotes. Since the branchpoint adenosine and 5'SS(+1) guanosine are linked due to step one chemistry, this mechanism suggests coupling between steps one and two: the spliceosome, through step one chemistry, makes a structure (the branch) that becomes an integral component of the active site for step two chemistry. This mechanism explains the use of the unusual 2'-5' linked RNA in spliceosomal introns, and could explain why pre-mRNA splicing uses a branching mechanism, justifying the existence of the lariat intron.
    a. **Wilkinson, M.E.**, Fica, S.M., Galej, W.P., Norman, C.M., Newman, A.J., Nagai, K. (2017) Postcatalytic spliceosome structure reveals mechanism of 3'-splice site selection. *Science* 358, 1283–1288.

4. I also solved, and helped solve, numerous other spliceosome structures throughout PhD, providing unprecedented mechanistic insight into pre-mRNA splicing and suggesting avenues for further experimental investigation. I summarized this work in a now highly cited review article.
    a. **Wilkinson, M.E.*,** Charenton, C.*, Nagai, K.* (2020) RNA Splicing by the Spliceosome. *Annu. Rev. Biochem.* 89.
    b. **Wilkinson, M.E.*,** Fica, S.M.*, Galej, W.P.*, Nagai, K. (2021) Structural basis for conformational equilibrium of the catalytic spliceosome. *Mol. Cell* 81(7), 1358-1362.
    c. Charenton, C.*, **Wilkinson, M.E.*,** Nagai, K. (2019) Mechanism of 5' splice site transfer for human spliceosome activation. *Science* 364, 362–367.
    d. Fica, S.M. , Oubridge, C., **Wilkinson, M.E.,** Newman, A.J., Nagai, K. (2019) A human postcatalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation. *Science* 363, 710–714.

e. Fica, S.M., Oubridge, C., Galej, W.P., **Wilkinson, M.E.,** Bai, X.C., Newman, A.J., Nagai, K. (2017) Structure of a spliceosome remodeled for exon ligation. *Nature* 542, 377–380.

BIOGRAPHICAL SKETCH

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NAME: Seiichi Hirano

eRA COMMONS USER NAME (credential, e.g., agency login): N/A

POSITION TITLE: Postdoctoral Fellow

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
The University of Tokyo; Tokyo, Japan	B.S.	03/2015	Biochemistry
The University of Tokyo; Tokyo, Japan	M.S.	03/2017	Biology
The University of Tokyo; Tokyo, Japan	Ph.D	03/2020	Biology

A. Personal Statement

My research goal is to elucidate how DNA-binding proteins (e.g., Cas effectors, transcription factors) search for their target sites within a vast and complex genomic landscape. Some proteins use DNA sequence-specificity to recognize target sites, while other proteins combine sequence-specificity with an RNA guide. For instance, the RNA-guided CRISPR effector Cas9 requires a short nucleotide motif, called a PAM, to rapidly scan the genome for target sites that are compatible with the RNA guide. In my graduate work, I studied the DNA sequence-specificity of Cas9 (i.e., the PAM-based recognition) from the viewpoint of biochemistry and structural biology. During my postdoctoral research, I have extended this work to characterize novel RNA-guided DNA endonucleases. For this work, I have combined biochemical studies with structural biology, using both Cryo-EM and AlphaFold. In the future, I would like to study genomic specificity from the micro (e.g., structural biology) to the macro (e.g., bioinformatics) perspectives.

B. Positions, Scientific Appointments, and Honors

Positions:
2020 – current Postdoctoral fellow, Broad Institute of MIT and Harvard

Honors:
2020 – 2022 Overseas Research Fellowships (Japan Society for the Promotion of Science)
2017 – 2020 Research Fellowships for Young Scientists (Japan Society for the Promotion of Science)
2016 Poster prize (The 39th Annual Meeting of the Molecular Biology Society of Japan)

C. Contributions to Science

1. Structural studies of the Cas9 protein

The microbial CRISPR-Cas9 system helps bacteria and archaea defend themselves against invading phage. The Cas9 protein is an RNA-guided endonuclease that uses RNA/DNA complementarity to recognize specific DNA sequences, which are then cleaved. In 2013, it was reported that Cas9 can be engineered for

programmable genome editing in human cells, a tool that revolutionized molecular biology and holds great potential therapeutically for the correction of disease-causing mutations. To further engineering efforts to improve and extend the Cas9 technology as well as provide a deeper understanding of its natural mechanism, I examined the biochemical, biophysical, and structural aspects of Cas9. Together, these works revealed new information about how Cas9 finds its target regions in the complex landscape of the human genome.

a. Shibata, M.*, Nishimasu, H.*, Kodera, N., **Hirano, S.**, Ando, T., Uchihashi, T., and Nureki, O. (2017). Real-space and real-Time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy. Nat. Commun. *8*, 1–9.

b. **Hirano, S.**, Abudayyeh, O.O., Gootenberg, J.S., Horii, T., Ishitani, R., Hatada, I., Zhang, F., Nishimasu, H., and Nureki, O. (2019) Structural basis for the promiscuous PAM recognition by *Corynebacterium diphtheriae* Cas9. Nat. Commun. 10, 1–10.

2. Engineered Cas9 with expanded PAM range

Cas9 requires a short nucleotide motif, called a PAM, to rapidly scan the genome for target sites that are compatible with the RNA guide. Although the PAM requirement is typically only 3 to 4 bases, depending on the Cas9 ortholog, this substantially reduces the availability of target sites in the human genome. This may be particularly problematic for therapeutic applications of Cas9, where it is crucial that gene editing occur at the site of the disease-causing mutation. To address this challenge, I worked to engineer Cas9 to change its PAM preference and unlock the full space of targetable sites. In addition, I conducted structural studies of Cas9 variants to better understand the mechanism of PAM preference, information that can further future engineering efforts.

a. Nishimasu, H., Shi, X., Ishiguro, S., Gao, L., Hirano, S., Okazaki, S., Noda, T., Abudayyeh, O.O., Gootenberg, J.S., Mori, H., et al. (2018). Engineered CRISPR-Cas9 nuclease with expanded targeting space. Science 361, 1259–1262.

b. Hirano, S.*, Nishimasu, H.*, Ishitani, R., and Nureki, O. (2016). Structural Basis for the Altered PAM Specificities of Engineered CRISPR-Cas9. Mol. Cell 61, 886–894.

3. Structural studies of other RNA-guided enzymes

Cas9 is just one example of an enzyme that uses a small RNA to direct its enzymatic activity. I have also studied the structures other RNA-guided enzymes, including the Piwi protein, an Argonatue protein that uses piRNAs to silence transposons. Recently, a new class of RNA-guided enzymes, called OMEGAs, were discovered. This diverse class of proteins includes the likely ancestors of the Cas9 protein, as well as other Cas effectors. OMEGA proteins tend to be much smaller than other Cas effectors, but they require a larger RNA. To understand this trade-off at the structural level, I solved the structure of an OMEGA protein in complex with its guide RNA and target DNA. Together, these studies enable comparisons between RNA-guided proteins and provide clues into the evolution of this powerful mode of regulation.

a. Crystal structure of Drosophila Piwi.
Yamaguchi S, Oe A, Nishida KM, Yamashita K, Kajiya A, **Hirano S**, Matsumoto N, Dohmae N, Ishitani R, Saito K, Siomi H, Nishimasu H, Siomi MC, Nureki O. Nat Commun. 2020 Feb 12;11(1):858. doi: 10.1038/s41467-020-14687-1.PMID: 32051406

b. **Hirano S**, Kappel K, Altae-Tran H, Faure G, Wilkinson ME, Kannan S, Demircioglu FE, Makarova KS, Koonin EV, Macrae RK, Zhang F. Structure of RNA-guided nickase IsrB in complex with guide RNA and target DNA. (*Under review*)