BIOGRAPHICAL SKETCH

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NAME: Strecker, Jonathan

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

POSITION TITLE: 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
University of Calgary, Calgary, Canada	B.HSc	05/2010	Biomedical Science
University of Toronto, Toronto, Canada	Ph.D.	11/2016	Molecular Genetics
Broad Institute, Cambridge MA	Postdoctoral Fellow	08/2023	Molecular Biology

A. Personal Statement

The sequencing of the human genome revealed the genetic blueprint for life and allows us to start to understand the genetic causes of disease and how differences in our DNA can impact human health. This motivated me to become a scientist and my graduate work focused on how cells protect their genome in response to DNA double strand breaks. Advances in genome editing inspired me to pursue ways to repair the genetic code during my postdoctoral work and I began working on CRISPR-Cas proteins. In addition to being programmable nucleases, I discovered that CRISPR-Cas systems can cooperate with diverse proteins like DNA transposases and proteases resulting in new programmable functions and enabling new biotechnologies. Finally, my investigation into bacterial antiviral systems has uncovered new parallels to eukaryotic immunity and I am motivated to continue to explore the microbial world to uncover new immune proteins and systems useful for biotechnology and research.

With this background I have now started my own research group and my vision is to discover and characterize novel enzymes that are controlled via nucleic acid recognition and use these discoveries to develop molecular tools for cellular control. I am motivated by the following questions: How do cells respond to nucleic acids? What other programmable functions exist in nature? And can we use these natural systems to build tools to better understand the human genome and disease? Discovering new proteins linked to nucleic-acid recognition is not only a fascinating research topic, but I believe will provide powerful new ways to manipulate and control cells. While advances in sequencing technologies have generated tremendous insight into gene function, mutations that cause disease, and unique cell types based on gene-expression signatures, we lack the ability to use genomic and transcriptomic information to control cells and to test biological hypotheses. For example, to activate gene expression in specific cell types, or

to trigger cell death in response to deleterious mutations, and the ability precise targeting, edit, and control of specific cells is a long-term goal of my work.

a. **Strecker J,** Gupta GD, Zhang W, Bashkurov M, Landry MC, Pelletier LP, Durocher D. (2016). DNA damage signalling targets the kinetochore to promote chromatin mobility. *Nature Cell Biology* 18, 281-90 (2016)

b. **Strecker, J**, Ladha A, Gardner Z, Schmid-Burgk J, Makarova KS, Koonin EV, Zhang F. RNA-guided DNA insertion with CRISPR-associated transposases. *Science* 365:48-53. (2019)

c. Gao L*, Wilkinson, ME*, **Strecker J***, Makarova KS, Macrae RK, Koonin EV, Zhang F. Prokaryotic innate immunity via pattern recognition of conserved viral proteins. *Science* 377:eabm4096 (2022)

d. **Strecker J*,** Demircioglu FE*, Li D, Faure G, Wilkinson ME, Gootenberg JS, Abudayyeh OO, Nishimasu H, Macrae RK, Zhang F. RNA-activated protein cleavage with a CRISPR-associated endopeptidase. *Science* 378:add7450 (2022)

B. Positions and Honors

Positions and Employment

2023–	Assistant Professor, Department of Genetics, Harvard Medical School Assistant Investigator, Department of Molecular Biology, Massachusetts General Hospital
2016–2023	Postdoctoral fellow with Dr. Feng Zhang, Broad Institute of MIT & Harvard
2010–2016	Graduate student with Dr. Daniel Durocher, Samuel Lunenfeld Research Institute, University of Toronto
2007–2010	Research assistant with Dr. Glen Armstrong, University of Calgary

Fellowships/Scholarships

Postdoctoral Fellowships

2021–2023	Charles A King Postdoctoral Fellowship
2017–2020	Human Frontiers Science Program Fellowship

Graduate Scholarships

2013–2016	CIHR Frederick Banting and Charles Best CGS D
2012	Ontario Graduate Scholarship
2011	NSERC Alexander Graham Bell CGS M Scholarship

C. Contribution to Science: Publications

A list of all publications can be found at the link below:
Pubmed (https://pubmed.ncbi.nlm.nih.gov)

1. Graduate Career

My graduate work focused on how cells maintain genome stability in response to DNA double-strand breaks using budding yeast as a model. First, I interrogated the distinction

between external ends of chromosomes, or telomeres, and internal ends that are formed by a DNA-double strand break and identified a genome stability mechanism that helps control the fate of DNA ends by allowing short telomeres to be extended while preventing the inappropriate addition of new telomeres to DNA double-strand breaks (Strecker et al. 2017). It was previously shown that a DNA double-strand break increases mobility of chromosomes and that the Mec1 kinase is required for this phenomenon, but the phosphorylation target was unknown. I established live-cell microscopy methods in the lab to study the mobility of broken chromosomes and identified a key DNA damage-induced phosphorylation site at the kinetochore that is required for increased mobility. Surprisingly, kinetochore mutants that are unable to be phosphorylated and therefore prevent DNA damage-induced chromatin mobility are not defective in DNA repair. This work challenged the prevailing notion that chromatin mobilities promotes the homology search and DNA repair and proposed a new function for this phenomenon involving activation of the spindle checkpoint and the arrest of the cell cycle. (Strecker et al. 2016).

a. **Strecker J**, Gupta GD, Zhang W, Bashkurov M, Landry MC, Pelletier LP, Durocher D. (2016). DNA damage signalling targets the kinetochore to promote chromatin mobility. *Nature Cell Biology* 18, 281-90 (2016)

b. **Strecker J***, Stinus S*, Caballero MP, Szilard RK, Chang M, Durocher D. A sharp Pif1-dependent threshold separates DNA double-strand breaks from critically short telomeres. *Elife* 6:e23783 (2017)

c. Orthwein A, Fradet-Turcotte A, Noordermeer SM, Canny MD, Brun CM, **Strecker J**, Escribano-Diaz C, Durocher D. Mitosis inhibits DNA double-strand break repair to guard against telomere fusions. *Science* 111:89-93 (2014)

d. Chung DK, Chan JN, **Strecker J**, Zhang W, Ebrahimi-Ardebili S, Lu T, Abraham KJ, Durocher D, Mekhail K. Perinuclear tethers license telomeric DSBs for a broad kinesin- and NPC-dependent DNA repair process. *Nature Communications* 6:7742 (2015)

2. Genome editing technologies

The initial goal of my postdoctoral work was to develop new genome editing technologies. A major focus in the field has been to explore the diversity of CRISPR-Cas nucleases and I led the effort investigating the Cas12b family for use in human cells. A key advance in this work was the observation that Cas12b preferentially cuts the non-target DNA strand, thus reducing DNA double-strand break potential, and the protein engineering strategies I developed to overcome this limitation through the identification of improved variants. My work established Cas12b as a third RNA-guided platform for effective human cell editing (Strecker et al. 2019) and similar mutagenesis strategies have been successfully used to improve other CRISPR-Cas nucleases.

a. **Strecker J**, Jones S, Koopal B, Schmid-Burgk J, Zetsche B, Gao L, Makarova KS, Koonin EV, Zhang F. Engineering of CRISPR-Cas12b for human genome editing. *Nature Communications* 10(1):212. (2019)

b. Zetsche B, **Strecker J**, Abudayyeh O, Gootenberg J, Scott D, Zhang F. A survey of genome editing activity for 16 Cas12a orthologs. *The Keio Journal of Medicine* https://doi.org/10.2302/kjm.2019-0009-OA (2019)

c. Schmid-Burgk J, Gao L, Li D, Gardner Z, **Strecker J**, Lash B, Zhang F. Highly parallel profiling of Cas9 variant specificity. *Molecular Cell* 78(4):794-800.e8 (2020)

3. New functions of CRISPR-Cas systems and bacterial immune proteins

I became interested in new functions of CRISPR and was inspired by an association with Tn7-like transposons. Starting from the native cyanobacterial hosts, I designed assays to study these mobile elements and elucidated the mechanism of type V-K CRISPR-associated transposase (CAST) systems (Strecker et al. 2019). This work was a significant advance in the field as it identified a new function for CRISPR beyond adaptive immunity and pointed to a new strategy for targeted DNA insertion without host cell machinery. Subsequent work revealed how these mobile elements return to conserved chromosome attachment sites (Saito*, Ladha*, Strecker*, Faure* et al. 2021), a key step in their natural life cycle.

Inspired by my work on CAST systems, I turned to another gene of unknown function, the CRISPR-associated protease (CASP) Csx29 and identified the protein substrate and function. Although originally hypothesized to promote programmed cell death, my work established that Csx29 cleaves a sigma factor inhibitor to regulate a transcriptional response in cells. (Strecker et al. 2022). My work also engineered CASP reporters for RNA sensing applications in vitro and in human cells paving the way for future bioengineering efforts.

Bacteria possess numerous antiviral proteins of unknown mechanisms and one recently identified system resembles NOD-like receptors (NLRs) involved in innate immunity in plants and animals. In collaboration with two colleagues, we discovered that these defense proteins are in fact ancestral pattern recognition receptors that bind to conserved viral proteins leading to activation of endonuclease effector domains and cell suicide (Gao*, Wilkinson*, Strecker* et al. 2022). This work revealed a surprising evolutionary connection to eukaryotic immunity and establishes the paradigm of NLR-based pattern recognition across all domains of life.

a. **Strecker, J**, Ladha A, Gardner Z, Schmid-Burgk J, Makarova KS, Koonin EV, Zhang F. RNA-guided DNA insertion with CRISPR-associated transposases. *Science* 365(6448):48-53. (2019)

b. **Strecker J***#, Demircioglu FE*, Li D, Faure G, Wilkinson ME, Gootenberg JS, Abudayyeh OO, Nishimasu H, Macrae RK, Zhang F. RNA-activated protein cleavage with a CRISPR-associated endopeptidase. *Science* 378:add7450 (2022)

c. Gao L*, Wilkinson, ME*, **Strecker J***, Makarova KS, Macrae RK, Koonin EV, Zhang F. Prokaryotic innate immunity via pattern recognition of conserved viral proteins. *Science* 377:eabm4096 (2022)

d. Saito M*, Ladha A*, **Strecker J***, Faure G*, Neumann E, Altae-Tran G, Macrae R, Zhang F. Dual modes of CRISPR-associated transposon homing. *Cell* 184(9):2441–2453.e18 (2021)

D. Additional Information: Research Support

Startup Funds 09/01/2023–

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NAME: Liu, Yang

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

POSITION TITLE:

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)	END DATE MM/YYYY	FIELD OF STUDY
Hong Kong University of Science and Technology	BS	06/2018	Biochemistry and Cell Biology
University of Texas Southwestern Medical Center	PHD	05/2023	Molecular Biophysics
Massachusetts Institute of Technology	Postdoctoral Fellow	04/2024	Bioengineering
Massachusetts General Hospital	Research Fellow	Current	Molecular Biology

A. Personal Statement

My research focuses on elucidating the mechanisms of novel CRISPR-associated systems, with a particular emphasis on the CRISPR-associated protein kinase (CASK) system. As a research fellow in Dr. Jonathan Strecker's lab, I am leveraging my expertise in biochemistry and structural biology to investigate the function and molecular basis of this newly discovered RNA-guided protein kinase system.

During my Ph.D. studies under Dr. Xiaochun Li, I gained extensive experience in protein purification, X-ray crystallography, and cryo-electron microscopy. I successfully determined the structures of several key proteins involved in lipid metabolism and morphogen signaling, including Porcupine (PORCN) and Dispatched-1 (DISP1). These studies provided crucial insights into the mechanisms of Wnt acylation and Hedgehog ligand secretion, contributing to our understanding of these essential developmental pathways and informing potential therapeutic strategies for cancers driven by aberrant morphogen signaling.

In my current role, I am applying my structural biology skills to the characterization of the CASK system. This research aims to expand our understanding of CRISPR systems beyond their well-known nuclease activities and potentially uncover new RNA-guided functions with applications in genome engineering and molecular diagnostics. My background in biochemistry and structural biology, combined with the cutting-edge resources and collaborative environment in Dr. Strecker's lab, positions me well to make significant contributions to this exciting field.

I am committed to pushing the boundaries of our understanding of biological systems and driven by the potential impact of our discoveries on both basic science and future biotechnological applications. This research not only aligns with my long-standing interest in complex biological mechanisms but also offers the opportunity to contribute to the development of transformative technologies.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

Honors

2023	Dean’s Discretionary Award, UT Southwestern Medical Center
2022	Best Poster Presentation, FASEB Science Research Conference
2022	Ze Zhang Graduate Student Award, UT Southwestern Medical Center
2018	First Honor, Hong Kong University of Science and Technology

2017	Dean List, Hong Kong University of Science and Technology
2014 - 2016	University Scholarship, Hong Kong University of Science and Technology

C. Contribution to Science

1. Elucidating the dimerization-dependent mechanism of cholesterol transporter NPC1L1: During my graduate work, I determined novel cryo-EM structures of the cholesterol transporter NPC1L1. The structures revealed that mutation of a key tryptophan residue to arginine (W347R) converts NPC1L1 from a dimer to a monomer. Functional studies showed the monomeric mutant has attenuated cholesterol uptake compared to wildtype NPC1L1, likely due to faster endocytosis rate rather than impaired cholesterol binding. These findings advance our mechanistic understanding of cholesterol uptake mediated by this important transporter.

    a. Long T*, **Liu Y***, Qin Y, DeBose-Boyd R, Li X. Structures of dimeric human NPC1L1 provide insight into mechanisms for cholesterol absorption. Science Advances. 2021 August 20; 7(34).

2. Defining the catalytic mechanism of the oncogenic enzyme porcupine: I determined multiple cryo-EM structures of porcupine (PORCN), including complexes with its palmitoleoyl-CoA substrate, Wnt3A peptide substrate, palmitoylated Wnt3A product, and LGK974 inhibitor. By comparing the substrate- and product-bound structures, I elucidated PORCN's direct, one-step catalytic mechanism for transferring palmitoleoyl groups to Wnt proteins. The inhibitor complex revealed LGK974 occupies the palmitoyl-CoA binding pocket, explaining its competitive inhibitory mechanism. Molecular dynamics simulations provided further evidence that PORCN's catalytic pocket specifically recognizes the palmitoleoyl chain length. Overall, these structural insights advance our understanding of PORCN's pivotal role in oncogenic Wnt signaling activation and will facilitate development of improved PORCN inhibitors to treat Wnt-driven cancers.

    In summary, I have determined cryo-EM structures elucidating mechanisms of key proteins involved in cholesterol homeostasis and Wnt signaling regulation. My contributions enhance our fundamental knowledge of these biological processes and aid design of new therapeutics.

    a. **Liu Y***, Qi X*, Donnelly L, Elghobashi-Meinhardt N, Long T, Zhou R, Sun Y, Wang B, Li X. Mechanisms and inhibition of Porcupine-mediated Wnt acylation. Nature. 2022 July 13; 607(7920):816-822.