BIOGRAPHICAL SKETCH

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NAME: Jared E. Toettcher

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POSITION TITLE: Assistant Professor, Department of Molecular Biology, Princeton University

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 California Berkeley, CA	BS	05/2004	Bioengineering
Mass. Institute of Technology, Cambridge, MA	PhD	08/2009	Biological Engineering
University of California, San Francisco	Postdoctoral	11/2014	Cell Biology / Biochem

A. Personal Statement

My laboratory is interested in developing a quantitative understanding of how signaling pathways control cell decisions, and in re-engineering this logic to drive desired cell behaviors for therapeutic benefit. The proposed work is at the heart of this research program, focusing on how time-varying optogenetic inputs can be used to probe the dynamic decoding of the Ras/Erk signaling pathway. It builds on my 10+ years of experience as a researcher studying the dynamics of cancer signaling pathways, including those controlled by p53, Ras and PI3K. As a graduate student, I performed quantitative studies on how the pulses of p53 activity observed in vitro and in vivo initiate cell cycle arrest after DNA damage. As a postdoc, I developed the first optogenetic inputs to precisely control growth factor signaling through Ras and PI 3-kinase, and identified downstream signaling programs that are dynamically regulated by Ras/Erk activity, including the delayed release of STAT3 activating ligands and induction of EMT genes.

Currently, the major goal of the Toettcher lab is to study how extracellular and intracellular signals drive fundamental cellular processes – growth, differentiation, and death. In every multicellular organism, cell decisions are controlled by inputs that are presented at particular times and at particular locations in a tissue. We therefore reason that to understand these processes, we must be able to control when and where a particular signaling process is active. My laboratory has two main thrusts. First, we aim to develop new tools and approaches to control protein activity in live cells, focusing primarily on optogenetics. With these tools comes a growing capability to deliver precise inputs to specific cells in a tissue, and turn inputs on and off at desired times. Second, we apply these tools to dissect cell fate choices in canonical systems in cell and developmental biology. Our model organisms of choice are the early fly embryo, where major questions still remain about the interpretation of morphogen gradients, and mammalian cells, where signaling pathway dynamics are thought to control patterns of gene expression. We focus primarily on the core, conserved molecular pathways downstream of receptor tyrosine kinases (RTKs), especially Ras/Erk and PI 3-kinase/Akt signaling.

B. Positions and Honors

Positions and Employment

2002-2004	Undergraduate Research, David Schaffer and Adam Arkin, UC Berkeley
2004-2009	Graduate Research, MIT and Harvard Medical School labs of Bruce Tidor and Galit Lahav
2009-2014	Postdoctoral Fellow, UC San Francisco, labs of Wendell Lim and Orion Weiner
2015-present	Assistant Professor, Department of Molecular Biology, Princeton University Associated Faculty, Department of Chemical & Biological Engineering, Princeton University Associated Faculty, Lewis Sigler Institute for Integrative Genomics, Princeton University Member, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ

Academic and Professional Honors

2000	National Merit Scholarship
2000	UC Berkeley Regents' & Chancellor's Scholarship
2004	Phi Beta Kappa
2004	MIT Presidential Fellowship
2009	Ruth Kirschstein Postdoctoral Fellowship (declined)
2009	Cancer Research Institute-Irvington Institute Fellowship
2016	NIH New Innovator Award

Invited speaker of more than 30 lectures, including: American Society for Cell Biology Annual Meeting (2016), Keystone Symposium on Optogenetics (2015), FASEB meeting on Protein Phosphorylation and Signaling (2014), iCEMS International Symposium on Light Control in Cell Biology (2014), Cold Spring Harbor (2009, 2013), INSERM Optogenetics Conference (2012), NASA AMES Research Center (2012), Society of Toxicology (2011).

C. Contribution to Science

One of my major contributions to science is the development of optogenetic tools for probing and controlling the major mammalian cell signaling pathways downstream of receptor tyrosine kinases (RTKs). I was an early developer of this class of approaches, focusing specifically on the fast, reversible Phy/PIF optogenetic system in mammalian cells. A large part of my research program now involves the development and application of these tools to questions in how RTKs drive cell decisions in both normal and diseased cells. I developed the first light-gated inputs to control both Ras and PI 3-kinase. By coupling each to live-cell reporters, we were able to measure how spatial and temporal information is transmitted in each pathway, identifying fundamental properties of both pathways (*e.g.* both pathways are low pass filters for transmitting dynamic information, and even genetically identical cells have high cell-to-cell variability in their Ras-to-Erk dose responses). This direct control also made it possible for us to identify signaling responses that are activated by specific Ras dynamics, such as a novel signaling circuit whereby Ras drives STAT3 activation in neighboring cells. Finally, we were also able to "close the loop" on PI 3-kinase signaling, using real-time measurements of pathway output to automatically update the inputs we deliver to control signaling with unprecedented, seconds-timescale resolution.

a. **Toettcher JE**, Gong D, Lim, WA, and Weiner OD. "Light-based feedback for controlling intracellular signaling dynamics." *Nature Methods* **8**, 837-839 (2011). PMID: 21909100.

b. **Toettcher JE**, Weiner OD, and Lim, WA. "Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module." *Cell* **155**:1422-1434 (2013). PMID: 24315106.

c. Johnson HE, Goyal Y, Pannucci N, Schupbach G, Shvartsman SY, **Toettcher JE**. "The spatiotemporal limits of developmental Erk signaling." *Developmental Cell* 40:185-192 (2017). PMID: 27135534.

d. Wilson MZ, Ravindran PT, Lim WA, **Toettcher JE**. "Tracing information flow from Erk to target gene induction reveals mechanisms of dynamic and combinatorial control." *Molecular Cell* **67**:1-13 (2017). PMID: 28826673.

A second thrust of our research has been to develop entirely new optogenetic tools, with a particular focus on protein phase separation. Recently it has become clear that in many physiologically relevant contexts, proteins and nucleic acids can condense and separate out from the cytosol. This process is familiar from nature and drives processes like dew condensation on blades of grass, but its role in driving intracellular processes is only beginning to be understood. In collaboration with the Brangwynne laboratory, we developed the *OptoDroplet* system, the first optogenetic tool to precisely control protein phase separation in living cells. Efforts in the lab are ongoing to study how protein coalescence into liquid-like droplets can be used to enhance signaling and metabolic flux or inhibit cellular processes by sequestering proteins from their substrates.

a. Shin Y, Berry J, Pannucci N, Haataja M, **Toettcher JE****, Brangwynne CP**. Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. *Cell* **168**: 159-171 (2017). PMID: 28041848. (** Co-corresponding authors)

Another major contribution of my research was to elucidate the systems-level properties of the mammalian DNA damage response and cell cycle arrest. Both the DNA damage response and cell cycle are complex, feedback-connected signaling pathways, with multiple connections bridging the two processes. I therefore developed the first mathematical model spanning both processes in order to interrogate what role each connection might play to ensure an appropriate cell cycle arrest in response to damage. This model allowed us to interrogate each arrest mechanism separately and in combination, revealing a novel role for p53-dependent repression of cyclin B to prevent improper re-entry into the wrong phase of the cell cycle and undergoing a second round of DNA replication without an intervening mitosis. It also allowed us to “zoom in” on the network regulating p53, which exhibits pulses of activity even in response to a constant DNA damage stimulus. Studying such activity pulses has been a longstanding challenge across many systems because standard tools (e.g. gene knockouts or pharmacological perturbations) typically destroy the dynamics altogether, rather than altering features such as the period or amplitude of each pulse. Our model was able to predict specific combinations of interventions that could be used to tune the amplitude, frequency, and stability of p53 pulses to separate the effects of these dynamic properties.

a. Apgar JF, **Toettcher JE**, Endy D, White FM, Tidor B. Stimulus design for model selection and validation in cell signaling. *PLoS Computational Biology* **4**(2): e30 (2008). PMID: 18282085.
b. **Toettcher JE**, Loewer A, Ostheimer GJ, Yaffe MB, Tidor B, Lahav G. Distinct mechanisms act in concert to mediate cell cycle arrest. *Proceedings of the National Academy of Sciences* **106**(3):785-790 (2009). PMID: 19139404.
c. **Toettcher JE**, Mock C, Batchelor E, Loewer A, and Lahav G. "A synthetic-natural hybrid oscillator in human cells." *Proceedings of the National Academy of Sciences* **107**, 17047-52 (2010). PMID: 20837528.

Featured as “Editor’s Choice” in Ray LB. Oscillator fine-tuning. Science Signal. ***3****(143):ec315.*

* denotes corresponding authorship.

Complete List of Published Work in myBibliography (18 Publications):
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/48425251/?sort=date&direction=descending

D. Research Support

Current Research Support

Schmidt Transformative Technology Fund Award (PI Avalos, Toettcher, Kevrekidis) 2/1/2017 – 1/31/2019
Closed-loop metabolic engineering: development of a photobioreactor to optimize biofuel production
Bioreactors typically contain organisms such as yeast that have been genetically modified to produce fuels, chemicals or medicines. To improve bioreactor efficiency, we will create an integrated approach to monitoring and controlling the organisms' metabolic activity. The approach will involve developing yeast strains where metabolic genes can be switched on or off when light is shone on the yeast, allowing researchers to turn on or

off the production of the chemicals. Using live-cell biosensors of chemical production, chemical production can then be optimized in a closed-loop system.
Role: PI

DP2 EB024247 (PI Toettcher) 9/30/2016 – 8/31/2021
Harnessing optogenetics to diagnose and therapeutically rewire cancer cell signaling
This project would enable us to develop optogenetics as a diagnostic and therapeutic tool for cancer. We will develop light-gated protein inputs to both diagnose tumors with unknown mutational profiles, and to therapeutically rewire cancer signaling in order to amplify apoptotic signaling or rewire growth inputs to apoptotic outputs.
Role: PI

U01 DA040601 (PI Brangwynne) 10/1/2015 – 9/31/2018
Optogenetic Droplets: Using Light to Control Nucleoplasmic Phase Separation
The main goal of this study is to use light to control biophysical phase transitions of nuclear proteins, harnessing the Cry2 and Phy/PIF systems.
Role: Sub-contractor

Completed Research Support

Rutgers/CINJ/ACS Early Investigator Pilot Project (ID 8292) (PI Toettcher) 11/1/2016 – 10/31/2017
Developing an Optogenetic Approach to Detect Signaling Alterations in Cancer
I will develop an approach termed “optogenetic profiling”, which aims to directly measure how growth signaling is altered in tumor cells by measuring cellular responses to a rich set of input stimuli. Rooted in engineering, this approach is akin to probing an electronic circuit with a different signals to characterize its function, and can be highly informative even when the exact wiring diagram is unknown. The main hypothesis of our work is that mutations in the Ras/PI3K pathways will alter how input stimuli are transmitted to downstream responses. To test this hypothesis, our primary objective is to measure Ras/Erk and PI3K/Akt signaling in tumor cell lines and compare signaling with drug sensitivity and mutational status.

Molecular Biology Innovation Grant, Princeton University (PI Toettcher) 2/1/2016 – 1/31/2017
All-optical morphogen patterning to test models of cell fate control
This grant for high-risk, high-reward research provides seed funding for setting up Drosophila research in our lab and developing optogenetic control over the Bicoid transcription factor to interrogate the interpretation of morphogen gradients with light.
Role: PI

New Ideas in the Natural Sciences Innovation Grant (PI Toettcher, Ploss) 2/1/2015 – 1/31/2017
Dissecting signaling complexity using cellular optogenetics *in vivo*
The main goal of this study is to generate the first knock-in mice expressing optogenetic activators of Ras and PI3K to enable multiple future projects in cancer biology and immunology.
Role: PI