BIOGRAPHICAL SKETCH

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NAME: Gitai, Zemer

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

POSITION TITLE: 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
Massachusetts Institute of Technology, Cambridge, MA	B.S.	05/1996	Biology
University of California, San Francisco, San Francisco, CA	Ph.D.	07/2003	Cell Biology
Stanford University, Stanford, CA	Postdoctoral	07/2005	Biology

A. Personal Statement

My research group focuses on bacterial cell biology, host-pathogen interactions, and antibiotic development. To understand how bacterial cells establish and maintain subcellular organization we develop new methods for imaging bacteria to study bacterial growth, with an emphasis on quantitative approaches to studying bacterial cell shape, cytoskeletal dynamics, and the way in which bacteria sense and respond to their mechanical environments. Most recently we have developed a deep interest in using our expertise in bacterial physiology to develop new antibiotics, and in particular those with new mechanisms of action that target Gram-negatives. We developed a promising candidate that kills Gram-positive and Gram-negative bacteria with no detectable resistance.

Citations:

1. Martin JK, Sheehan JP, Bratton BP, Moore GM, Mateus A, Li SH, Kim H, Rabinowitz JD, Typas A, Savitski MM, Wilson MZ, **Gitai Z.** A Dual-Mechanism Antibiotic Kills Gram-Negative Bacteria and Avoids Drug Resistance. *Cell*. 2020 May 22:S0092-8674(20)30567-5. doi: PMID: 32497502
2. Martin NR, Blackman E, Bratton BP, Bartlett TM, and **Gitai Z.** The evolution of bacterial shape complexity by a curvature-inducing module. *Nature Microbiology.* 2021 Jul;6(7):910-920. doi: 10.1038/s41564-021-00924-w. Epub 2021 Jun 28. PMID: 34183815
3. Kaletsky R, Moore RS, Vrla GD, Parsons LL, **Gitai Z**, Murphy CT. *C. elegans* "reads" bacterial non-coding RNAs to learn pathogenic avoidance. *Nature.* 2020 Sep 9. doi: 10.1038/s41586-020-2699-5
4. Bartlett TM, Bratton BP, Duvshani A, Miguel A, Sheng Y, Martin NR, Nguyen JP, Persat A, Desmarais SM, VanNieuwenhze MS, Huang KC, Zhu J, Shaevitz JW, **Gitai Z.** A Periplasmic Polymer Curves *Vibrio cholerae* and Promotes Pathogenesis. *Cell*. 2017 Jan 12;168(1-2):172-185.e15. PubMed PMID: 28086090; PubMed Central PMCID: PMC5287421.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2022 Founder, Board Member, Scientific Advisory Board Chair, Consultant – ArrePath, Inc.
2019 Member, American Association for the Advancement of Science
2015 Professor, Department of Molecular Biology, Princeton University, Princeton, NJ

2012-2019 Director of Graduate Studies, Department of Molecular Biology, Princeton University
2012-2015 Associate Professor, Princeton University, Department of Molecular Biology, Princeton, NJ
2005-2012 Assistant Professor, Princeton University, Department of Molecular Biology, Princeton, NJ
2003-2005 Postdoctoral Fellow, Stanford University, Developmental Biology Department, Stanford, CA
2003 Member, American Society of Microbiology
2000 Member, American Society of Cell Biology

Honors

2021 Organizer, EMBO-EMBL Mechanobiology at the Cell Surface Symposium, Heidelberg
2017 Organizer, Transitions in Biology Symposium, Princeton Center for Theoretical Science
2016 Edwin Grant Conklin Endowed Professorship, Princeton University
2016 Chair, Bacterial Cell Surfaces Gordon Conference
2016 Organizer and Chair, “Bacterial Cell Biology” symposium, ASCB General meeting
2015 NIH Director's Pioneer Award, NIH
2014 Helen Shipley Hunt Innovation Award, Princeton University
2013 Human Frontiers Science Program Award, Human Frontiers
2012 Organizer and Chair, “Mechano-Microbiology” symposium, American Society for Microbiology (ASM) General meeting, ASM
2011 Chair and Organizer of "Cellular Organization: Location, Location, Location" Session at the American Society for Microbiology (ASM) General Meeting
2011 Chair of American Society of Microbiology Division J (Cell and Structural Biology), ASM
2010 Organizer of ASCB Special Interest Subgroup on the Cell Biology of Metabolic Pathways, ASCB General Meeting, 2010
2008 Profiled as “Scientist to Watch” by The Scientist, The Scientist Magazine
2008 Human Frontiers Science Program Young Investigator Award, Human Frontiers Science Program
2008 NIH Director’s New Innovator Award recipient, NIH
2007 Chair and organizer of “Bacterial Cell Biology” minisymposium at the American Society for Cell Biology (ASCB) General meeting, 2007
2007 Beckman Young Investigator Award, 2007, Beckman
2004 Ruth Kirschstein NIH Postdoctoral Fellowship, NIH
2003 Stanford Program in Genomics Postdoctoral Fellowship, Stanford
1996 Howard Hughes Pre-Doctoral Fellowship

C. Contributions to Science

1. ***How to build a rod: Shape determination by the MreB actin-like bacterial cytoskeleton***

My group has played a key role in discovering the functions and regulation of the bacterial actin-like MreB cytoskeleton, establishing it as a central coordinator of bacterial cell elongation. Early on we focused on the functions of MreB in C. crescentus, but for the past decade we have primarily focused on how it mediates the elongation of E. coli. There we were among the first to show that MreB forms a highly dynamic discontinuous structure and that proper cell shape formation requires a feedback circuit: MreB directs the sites of new cell wall assembly, while the process of cell wall synthesis in turn rotates MreB around the cell circumference to promote the uniform redistribution of MreB filaments. We also discovered important functions for the mechanical properties of cytoskeletal polymers: MreB polymers localize to regions of the cell with negative curvature, and its geometric enrichment can explain large-scale patterns of growth. Specifically, MreB is excluded from the cell poles and enriched at invaginated areas where the cell needs more growth, thereby using local curvature measurements to achieve a globally smooth rod-like growth pattern. More recently we have focused on the regulation of MreB by interacting factors like RodZ, which regulates both MreB assembly and curvature enrichment, and AimB, which inhibits its assembly.

a. Bratton BP, Shaevitz JW, Gitai Z*, Morgenstein RM. MreB polymers and curvature localization are enhanced by RodZ and predict E. coli's cylindrical uniformity. Nature Commun. 2018 Jul 18;9(1):2797. PubMed PMID: 30022070; PubMed Central PMCID: PMC6052060.
*ZG is a corresponding author (Morgenstein and Bratton completed most of the work as postdocs in my lab, and the paper was published after Morgenstein started his own group at OSU).

b. Shi H, Bratton BP, Gitai Z*, Huang KC. How to Build a Bacterial Cell: MreB as the Foreman of E. coli Construction. Cell. 2018 Mar 8;172(6):1294-1305. doi: 10.1016/j.cell.2018.02.050. PubMed PMID: 29522748; PubMed Central PMCID: PMC5846203.
*ZG is a corresponding author (equal contribution with KCH).
c. Morgenstein RM, Bratton BP, Nguyen JP, Ouzounov N, Shaevitz JW, Gitai Z. RodZ links MreB to cell wall synthesis to mediate MreB rotation and robust morphogenesis. PNAS. 2015 Oct 6;112(40):12510-5. PubMed PMID: 26396257; PubMed Central PMCID: PMC4603514.
d. van Teeffelen S, Wang S, Furchtgott L, Huang KC, Wingreen NS, Shaevitz JW, Gitai Z. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. Proc Natl Acad Sci U S A. 2011 Sep 20;108(38):15822-7. PubMed PMID: 21903929; PubMed Central PMCID: PMC3179079.

2. ***The how and why of cell curvature in pathogens and free-living bacteria: formation, function, and regulation of bacterial curvature***

After rods and spheres, curved shapes represent the most common morphology of bacteria, but far less is known about these systems. My lab has addressed this gap by discovering new mechanisms by which bacteria become curved, how they regulate their curvature, and how being curved provides selective benefits to bacteria. These efforts have focused on two curved bacterial species, Caulobacter crescentus and Vibrio cholerae. In the curved pathogen, V. cholerae, we used a high-throughput imaging approach to discover a novel periskeletal polymer, CrvA, that assembles along the inner face of these bacteria and causes them to become curved by locally reducing the rate of cell wall insertion. The presence of curved mutants also enabled us to determine a functional benefit for V. cholerae curvature. Curved cells can better penetrate dense matrices, which promotes their colonization of the gut mucus and thereby enhances their virulence. Meanwhile in the freshwater bacterium, C. crescentus, we discovered a metabolic enzyme, CTP Synthase, that assembles into cytoskeletal polymers that in turn regulate cell curvature. The assembly of this enzyme depends on its metabolic state, thereby coupling cell shape and metabolism. While cell curvature has no detectable benefit in standard lab growth conditions, we found that the curved shape promotes C. crescentus surface colonization and biofilm formation by causing cells to arc in flow, thereby bringing polar adhesins closer to the surface and enhancing surface attachment.

a. Martin NR, Blackman E, Bratton BP, Bartlett TM, and Gitai Z. The evolution of bacterial shape complexity by a curvature-inducing module. Nature Microbiology. 2021 Jul;6(7):910-920. doi: 10.1038/s41564-021-00924-w. Epub 2021 Jun 28. PMID: 34183815
b. Bartlett TM, Bratton BP, Duvshani A, Miguel A, Sheng Y, Martin NR, Nguyen JP, Persat A, Desmarais SM, VanNieuwenhze MS, Huang KC, Zhu J, Shaevitz JW, Gitai Z. A Periplasmic Polymer Curves Vibrio cholerae and Promotes Pathogenesis. Cell. 2017 Jan 12;168(1-2):172-185.e15. PubMed PMID: 28086090; PubMed Central PMCID: PMC5287421.
c. Persat A, Stone HA, Gitai Z. The curved shape of Caulobacter crescentus enhances surface colonization in flow. Nature Commun. 2014 May 8;5:3824. PubMed PMID: 24806788; PubMed Central PMCID: PMC4104588.
d. Barry RM, Bitbol AF, Lorestani A, Charles EJ, Habrian CH, Hansen JM, Li HJ, Baldwin EP, Wingreen NS, Kollman JM, Gitai Z. Large-scale filament formation inhibits the activity of CTP synthetase. Elife. 2014 Jul 16;3:e03638. PubMed PMID: 25030911; PubMed Central PMCID: PMC4126345.

3. ***Mechano-microbiology: how bacteria use forces to sense and respond to their hosts***

Our studies on the functions of curved shapes (described above) indicated that bacteria have adapted to their mechanical environments, as most of the benefits of specific shapes are not detectable in mechanically uniform environments. The ability of bacteria to sense and respond to chemical features of their environment such as nutrients and signaling molecules has long been appreciated. But our discoveries pioneered a new appreciation that bacteria also sense and respond to mechanical features of their environment such as fluid flow and surface association. These mechanical cues are ubiquitous in nature and particularly prevalent in animal hosts, such that we then focused much of our efforts in this area on understanding how bacterial pathogens such as Pseudomonas aeruginosa overcome the mechanics of the host for colonization or utilize host mechanics as cues to regulate virulence factor induction. Specifically, we found that P. aeruginosa utilize their polarized pili to twitch upstream in the presence of flow. This behavior enables them to colonize environments such as the branched flow networks of the cardiovascular system that sweep away most bacteria. Meanwhile we

also discovered that P. aeruginosa uses two different sensory systems, long-range pili and the short-range PilY1 factor to sense the presence of stiff surfaces as a cue to induce virulence factor expression. We proposed that broad-host-range pathogens like P. aeruginosa might use surface stiffness as a host-independent signal, explaining how they induce virulence factors in response to such chemically-distinct hosts. Finally, we recently described a flow-sensing pathway in P. aeruginosa, defining a new sensory modality of bacterial rheosensing.

a. Koch MD, Fei C, Wingreen NS, Shaevitz JW, **Gitai Z**. Competitive binding of independent extension and retraction motors explains the quantitative dynamics of type IV pili. *PNAS* 2021 Feb 23;118(8):e2014926118. doi: 10.1073/pnas.2014926118. PMID: 33593905. PMCID: PMC7923367
b. Vrla GD, Esposito M, Zhang C, Kang Y, Seyedsayamdost MR, **Gitai Z.** Cytotoxic alkyl-quinolones mediate surface-induced virulence in *Pseudomonas aeruginosa*. *PLoS Pathogens* 2020 Sep 14;16(9):e1008867. doi: 10.1371/journal.ppat.1008867.
c. Sanfilippo JE, Lorestani A, Koch MD, Bratton BP, Siryaporn A, Stone HA, **Gitai Z**. Microfluidic-based transcriptomics reveal force-independent bacterial rheosensing. *Nature Microbiology*. 2019 May 13. Pubmed PMID: 31086313.
d. Persat A, Inclan YF, Engel JN, Stone HA, **Gitai Z**. Type IV pili mechanochemically regulate virulence factors in *Pseudomonas aeruginosa*. *PNAS*. 2015 Jun 16;112(24):7563-8. PubMed PMID: 26041805; PubMed Central PMCID: PMC4475988.

4. ***The cell biology of growth: integrating translation, metabolism, and spatial organization***

Most of my lab's work has focused on how bacteria grow from the perspective of cell shape formation. But bacterial growth also requires regulation and coordination of the key elements of biomass production: protein synthesis and metabolism. Interestingly, we discovered that there are surprising layers of cell biological regulation in these putatively "core" processes. As discussed above, we found that metabolic enzymes like CTP Synthase can assemble into large-scale structures in a manner that depends on metabolic state. We also showed that co-assembling multiple enzymes into a single polymer can regulate metabolic flux to alter which branch of a pathway intermediates diffusively interact with. In a follow-up study we recently showed that synthetic formation of phase-separated enzyme clusters can similarly be used to direct metabolic flux. Most recently, we have used ribosome profiling to study how protein translation is regulated in different growth states. Specifically we discovered that at the same growth rate, *E. coli* cells use 3 very different strategies to achieve the same protein production rate depending whether their growth is limited by carbon, nitrogen, or phosphorous. This study revealed that in carbon and nitrogen limitation *E. coli* actually grow sub-optimally: in carbon limitation they accumulate excess inactive ribosomes while in nitrogen they translate slowly. In addition to revising one of the fundamental "growth laws of bacteria" (i.e. it is not true that to grow faster bacteria need to make more ribosomes), in the course of this work we developed a new ribosome profiling method that proved successful in profiling mitochondrial translation, which had previously proved intractable. In collaboration with the Rabinowitz lab at Princeton we thus applied our new mitochondrial ribosome profiling approach to discover a new method by which folate inhibitors kill cancer cells. Here we showed that folate is required for tRNA methylation, which is essential for mitochondrial translation and whose inhibition prevents fast growth of mammalian cells.

a. Li SH, Li Z, Park JO, King CG, Rabinowitz JD, Wingreen NS, **Gitai Z**. *Escherichia coli* translation strategies differ across carbon, nitrogen and phosphorus limitation conditions. *Nature Microbiol*ogy. 2018 Aug;3(8):939-947. Pubmed PMID: 30038306.
b. Li SH, Nofal M, Parsons LR, Rabinowitz JD, **Gitai Z**. Monitoring mammalian mitochondrial translation with MitoRiboSeq. *Nature Protocols*. 2021 Jun;16(6):2802-2825. doi: 10.1038/s41596-021-00517-1. PMID: 33953394.
c. Morscher RJ, Ducker GS, Li SH, Mayer JA, **Gitai Z**, Sperl W, Rabinowitz JD. Mitochondrial translation requires folate-dependent tRNA methylation. *Nature*. 2018 Feb 1;554(7690):128-132. PubMed PMID: 29364879; PubMed Central PMCID: PMC6020024.
d. Castellana M, Wilson MZ, Xu Y, Joshi P, Cristea IM, Rabinowitz JD, **Gitai Z***, Wingreen NS. Enzyme clustering accelerates processing of intermediates through metabolic channeling. Nature Biotechnol. 2014 Oct;32(10):1011-8. PubMed PMID: 25262299; PubMed Central PMCID: PMC4666537. *ZG is a corresponding author (equal collaboration with the NSW lab).

5. ***Developing new strategies to understand and modulate host-microbe interactions:***
In the past several years my lab has become increasingly focused on using our expertise in bacterial cell biology and pathogenesis to understand and combat pathogenesis. In our first endeavor, we combined quantitative imaging, machine learning, proteomics, and CRISPRi genetics to screen for new antibiotics with novel mechanisms of action. This led to the identification of a promising compound, Irresistin-16, that is effective against a broad spectrum of Gram-negative and Gram-positive bacteria with no detectable resistance. Irresistin-16 simultaneously disrupts both folate metabolism, by directly inhibiting dihydrofolate reductase, and membrane integrity, by selectively permeabilizing bacterial membranes. Irresistin-16 also has favorable pharmacological properties and effectively treated a *Neisseria gonorrhoeae* vaginal infection *in vivo*. In parallel, we are using imaging-based approaches to identify additional compounds. For example, we recently collaborated with the Donia lab to discover a small molecule, MHQ, produced by *P. aeruginosa,* that causes *P. aeruginosa* itself to detach from surfaces. Since surface attachment is necessary for bacterial colonization and virulence but not for growth, we are currently developing this compound as an inhibitor of *P. aeruginosa* pathogenesis. Such anti-infectives that disrupt pathogenesis but not growth have the potential to limit the emergence of resistance by reducing the selective pressures that drive the evolution of resistance. Finally, we have worked with the Murphy lab to show that hosts sense and respond to bacterial sRNAs, and we are currently working to develop sRNA-based antibacterial interventions.
    a. Martin JK, Sheehan JP, Bratton BP, Moore GM, Mateus A, Li SH, Kim H, Rabinowitz JD, Typas A, Savitski MM, Wilson MZ, **Gitai Z.** A Dual-Mechanism Antibiotic Kills Gram-Negative Bacteria and Avoids Drug Resistance. *Cell*. 2020 May 22:S0092-8674(20)30567-5. doi: PMID: 32497502
    b. Kaletsky R, Moore RS, Vrla GD, Parsons LL, **Gitai Z**, Murphy CT. *C. elegans* “reads” bacterial non-coding RNAs to learn pathogenic avoidance. *Nature.* 2020 Sep 9. doi: 10.1038/s41586-020-2699-5
    c. Scheffler RJ, Sugimoto, Bratton BP, Ellison CK, Koch MD, Donia MS, **Gitai Z**. *Pseudomonas aeruginosa* detachment from surfaces via a self-made small molecule. *J Biol Chem.* 2021 Jan-Jun;296:100279. doi: 10.1016/j.jbc.2021.100279. Epub 2021 Jan 12. PMID: 33450229
    d. Moore RS, Kaletsky R, Lesnik C, Cota V, Blackman E, Parsons LR, **Gitai Z**, Murphy CT. The role of the Cer1 transposon in horizontal transfer of transgenerational memory. *Cell*. 2021 Aug 3:S0092-8674(21)00881-3. doi: 10.1016/j.cell.2021.07.022. PMID: 34363756

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