Samuel E. Holtzen, Ph.D.

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Education and Training

Postdoctoral Fellow, Biology and Biological Engineering 2019-2024
California Institute of Technology
Doctor of Philosophy, Molecular Cell and Developmental Biology 2019-2024
University of Colorado Boulder
Higher Education Research Experiences (HERE) Intern 2018-2019
Oak Ridge National Laboratory
Bachelor of Science in Biochemistry, Pre-Health Concentration 2013-2017
Georgia Institute of Technology, Cum Laude

Teaching and Mentorship Experience

Analytical and Quantitative Light Microscopy, Teaching Assistant Spring 2023 and 2024
Principal Instructor: Dr. Amy Palmer

Phage Discovery Laboratory, Teaching Assistant Spring 2020
Principal Instructor: Dr. Nancy Guild and Megan Greening

Principles of Genetics, Teaching Assistant Spring 2020
Principal Instructor: Dr. Jennifer Knight

Developmental Biology, Teaching Assistant Fall 2019
Principal Instructor: Dr. Jennifer Knight

Research Experience

Repetitive element-based spatial barcoding for rapid high-throughput screening 2024-Present

Advisor: David Van Valen, Assistant Professor, California Institute of Technology

Cellular barcoding can allow researchers to identify single-cell perturbations in mixed populations. Spatial optical barcoding can accelerate these so-called pooled optical screens by accelerating the speed at which these barcodes can be read out. I am developing a method of spatial barcoding that uses dCas9 and gRNAs to target repetitive elements (REs) in the genome. The patterns of these gRNA-RE complexes can be read out with multicolor FISH. A deep learning model trained on these patterns can determine the identity of these gRNAs with 90-99% accuracy. Since the encoding space is only limited by the number of discernible patterns and FISH colors, this technique has a very large encoding space and requires only one multicolor FISH step for readout.

Computational design and *in vitro* validation of novel metabolite biosensors 2024-Present

Advisor: David Van Valen, Assistant Professor, California Institute of Technology

There is a distinct lack of well-characterized binding proteins for use in fluorescent biosensors designs that are specific to less common metabolites. The advent of computational protein design tools such as RoseTTAFold Diffusion and LigandMPNN, and heuristic-driven protein folding tools like AlphaFold has enabled our ability to build bespoke proteins on demand. I am currently designing and testing novel sensing domains for use in biosensors for less-studied

metabolites involved in immune metabolism. Through this, I hope to illuminate understudied aspects of metabolism in an array of cells as they grow, divide, and differentiate.

Mapping the zinc requirements of the mammalian cell cycle 2020-2024

Advisor: Amy Palmer, Professor of Biochemistry, University of Colorado Boulder

Mild to moderate zinc deficiency causes a bifurcation in cell fate and increased DNA damage response in a cell culture model. This phenotype has been demonstrated by several studies, but to date, no mechanism has been defined for these hallmark phenotypes. My thesis research aims to decouple the causes, consequences, and sequence of events that cause zinc deficient cells to enter a quiescent state.

Cellular dynamics of free zinc throughout the mammalian cell cycle 2020-2023

Advisor: Amy Palmer, Professor of Biochemistry, University of Colorado Boulder

The Palmer group has engineered a live-cell genetically encoded zinc sensor with high dynamic range within the physiological zinc range in mammalian cells. We have developed a method of monitoring cytosolic zinc using long-term live-cell imaging of single cells. Using this system, we have identified zinc dynamics in the mammalian cell cycle. Additionally, we monitor these dynamics after depletion of a transcription factor involved in the response to high zinc. I was responsible for data stewardship and analysis of terabytes of high-content live-cell imaging experiments.

Engineering *Pseudomonas putida* KT2440 for the valorization of lignin 2018-2019

Advisor: Adam Guss, Biosciences Division, Oak Ridge National Lab

I worked with a graduate student to engineer the metabolism of *Pseudomonas putida* to catabolize lignin monomers into value-added chemicals. Specifically, I developed and optimized extraction techniques of fatty alcohols and polyhydroxyalkanoate monomers for analysis on our group's GC-MS. In addition, I was the caretaker of the GC-MS and performed routine maintenance and troubleshooting.

Biomarker analysis for validating Earth analogue sampling techniques 2016-2018

Advisor: Amanda Stockton, Assistant Professor of Chemistry & Biochemistry, Georgia Institute of Technology

As an undergraduate research assistant, my task was the cataloging and curation of precious Icelandic tephra samples from field expeditions. In addition, I developed a pipeline for extraction of DNA from low-biomass samples and library preparation for 16S rRNA community analysis. Additionally, I aided in the development of an *in situ* co-culturing chip for unculturable microbes. I was afforded the opportunity to embark on a field expedition in June and July 2018 to the Icelandic highlands to sample volcanic tephra from the active Holuhraun lava field.

Skills and Expertise

Laboratory Skills: Mammalian cell culture, high-content live-cell imaging, molecular cloning, Western blot analysis, next-generation sequencing library preparation and analysis, fluorescence microscopy, single- and multi-color flow cytometry, ATAC-seq, RNA-seq, quantitative PCR, high-content microscopy, microscope maintenance

Data Skills: image analysis, data pipeline development, machine learning, statistics, high performance computing

Programming Languages: Python, MATLAB, Bash, R, SQL

Languages: English (native speaker), French (limited working proficiency)

Publications and Other Works

In Press:

Ocampo, D., Damon, L., Sanford, L., **Holtzen, S.**, Jones, T., Allen, M., Dowell, R., Palmer, A. (2024). Cellular zinc status alters chromatin accessibility and binding of TP53 to DNA. *Life Science Alliance*, e202402638

Holtzen, S., Rakshit, A., Palmer, A. (2024). Measuring cell cycle Zn^{2+} dynamics using a FRET-based biosensor. STAR Protocols.

Holtzen, S., Rakshit, A., Palmer, A. (2024). Measuring labile cytosolic Zn^{2+} using an in-situ calibration of a genetically encoded FRET sensor. STAR Protocols.

Holtzen, S., Navid, E., Kainov, J., Palmer, A. (2024). Transient Zn^{2+} deficiency induces replication stress and compromises daughter cell proliferation. *Proceedings of the National Academy of Sciences*, *121(19),* e2321216121.

Rakshit, A.*, **Holtzen, S. E.***, Lo, M. N., Conway, K. A., & Palmer, A. E. (2023). Human cells experience a Zn^{2+} pulse in early G1. *Cell Reports*, *42*(6).

Tan, G. K., Simpson, A., **Holtzen, S.**, Amador, E., Cable, M. L., Cantrell, T., ... & Stockton, A. M. (2022). Spatial Variation in Results of Biosignature Analyses of Apparently Homogeneous Samples from Mars Analogue Environments in Iceland. *ACS Earth and Space Chemistry, 6*(6), 1472-1481.

Rader, E., Simpson, A., Amador, E., Fraser, J. M., **Holtzen, S.**, Hanna, A., ... & Stockton, A. (2020). Preferably Plinian and Pumaceous: implications of microbial activity in modern volcanic deposits at Askja Volcano, Iceland, and relevancy for Mars exploration. *ACS Earth and Space Chemistry*, *4*(9), 1500-1514.

*These authors contributed equally

Conference Abstracts:

Untangling the Role of Zinc in the Mammalian Cell Cycle and DNA Damage Response, **Samuel Holtzen**, Joseph Kainov, Elnaz Navid, Lottie Steward, Amy Palmer, Chemical Biology and Physiology, 2022, Conference Abstract

Production of medium chain length alcohols from lignin-derived aromatic compounds in Pseudomonas putida, Jay Huenemann, Joshua Elmore, **Samuel Holtzen**, Adam Guss, SIMB Annual Meeting and Exhibition, 2019, Conference Abstract

Biomarker and Geochemical Assay Validation in Mars Analog Sites: Lessons from the FELDSPAR (Field Exploration and Life Detection Sampling for Planetary Analog Research), Diana Gentry, Elena Sophia Amador, Morgan L Cable, Thomas Cantrell, Nosheen Chaudry, Thomas Cullen, Zachary A Duca, **Samuel Holtzen**, Malene B Jacobsen, David King, Jessica Kirby, Heather Catherine McCaig, Gayathri Murukesan, Erika Rader, Adrianne Reeder, Vincent Rennie, Edward Schwieterman, Alexander Michael Sessa, Adam Stevens, Scot M Sutton, George Kenneth Tan, Chang Yin, David Cullen, Wolf Geppert, Amanda M Stockton, Project, 2019, Conference Abstract

Evaluation of spatial variation in life-detection assay results in apparently homogeneous samples from Mars analog environments in Iceland, George Kenneth Tan, Amanda M Stockton, Morgan L Cable, Wolf Geppert, David Cullen, Diana Gentry, Elena Sophia Amador, Adam Stevens, Thomas Cantrell, Zachary A Duca, **Samuel Holtzen**, Heather Catherine McCaig, Jarah Whitehead, Gayathri Murukesan, Vincent Rennie, Jessica Kirby, Thomas Cullen, Astrobiology Science Conference, 2019, Conference Abstract

A Recent Volcanic Eruption, Holuhraun, in the Central Highlands of Iceland as a Mars analog: The 2018 Field Campaign of FELDSPAR, Amanda M Stockton, Elena Amador, Morgan L Cable, David Cullen, Julia Fraser, Diana Gentry, Wolf Geppert, **Samuel Holtzen**, Gayathri Murukesan, Erica Raider, Adrianne Reeder, Alexander Michael Sessa, Astrobiology Science Conference, 2019, Conference. Abstract