BIOGRAPHICAL SKETCH

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POSITION TITLE: Postdoctoral researcher

HOMETOWN: Virginia Beach, Virginia

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Berkeley	BS	12/2014	Bioengineering
University of California, San Diego	PhD	10/2020	Bioengineering
University of Washington	Postdoc	Current	Biochemistry

A. Personal Statement

Thousands of biochemical reactions occur simultaneously in each cell. These events are more than just a random collection of molecular interactions and reactions but need to be organized into segregated compartments to ensure specificity and efficiency. This is particularly true in signal transduction where the same central signaling molecule directs numerous cellular processes, a phenomenon called pleiotropy. My central hypothesis is that spatiotemporal compartmentation of intracellular signaling molecules enables functional specificity, and this homeostasis is hijacked in diseases. In investigating this hypothesis, my approach is to expand the molecular toolkit using *de novo* and modular protein design to measure, track, and perturb complex signaling pathways.

My academic training and research experience have provided me with an excellent background in multiple biological disciplines including molecular biology, biochemistry, microscopy, and computational modeling. As an undergraduate, I conducted research with Dr. Song Li at UC Berkeley to understand the biophysical determinants of cell stemness, with Dr. Dilworth Parkinson at LBNL to use x-ray microtomography to visualize complex biological materials in 3-D, and with Dr. Edward Guo at Columbia to understand the biomechanical forces shaping bone development and regulation. As a doctoral student at UCSD with Dr. Jin Zhang, my research focused on understanding how signaling specificity is encoded by developing and deploying fluorescence-based biosensors. Using protein and gene engineering techniques, I developed a new class of biosensors that can measure the native signaling dynamics around protein hubs and biochemical activity indicators with double-digit dynamic ranges. With these technologies, I probed how pleiotropic signaling molecules encode specificity through spatiotemporal organization. For instance, I discovered the liquid-liquid phase separation of the PKA regulatory subunit RIα which acts as a principle organizer for cAMP/PKA signaling and this system is disrupted in liver cancer. During this time, I received several awards to fund my research such as the NSF GRFP.

For my postdoctoral training, I am working at University of Washington with Dr. David Baker and Dr. Dustin Maly in continuing to engineer proteins for understanding the spatiotemporal regulation of cell signaling. Using computational protein design, I developed an assay to measure neutralizing antibodies against several SARS-CoV-2 variants (now part of Monod, a company Jason co-founded). I developed and utilized deep-learning algorithms to design *de novo* protein sensors to measure the activity and environment of the central signaling molecule Ras, which is mutated in a third of cancers. These Ras tools identified upstream factors necessary to activate cytosolic Ras in oncogenic condensates and revealed a new signaling scaffold protein that enables compensatory Ras signaling at the golgi and mitochondria during RasG12C inhibitor resistance. I also designed *de novo* proteins that bind and activate Hsp70 chaperones and applied this to dissolve condensates related to signaling (e.g. RIα condensates, EML4-Alk granules) to understand the cellular function of these systems. With these protein engineering tools, Jason is excited to further expand the molecular toolkit for studying pleiotropic signaling networks by using protein engineering/design to generate new sensors and perturbators. Jason is

particularly convinced and interested in exploring the role of condensates as critical organizers of various signaling networks, as seen throughout his and other's studies. Broadly, these protein engineering methodologies/tools can be applied to various biomolecules to answer fundamental questions in the cellular logic of organizing complex biochemical networks.

B. Positions and Honors

Positions and Employment

2015 - 2015 Researcher, Xip Diagnostics
2020 - current Postdoctoral Researcher, University of Washington

Other Experience and Professional Memberships

2012 - 2014 Member, Biomedical Engineering Society
2015 - 2020 Board Member, Queer and Trans in STEM
2015 - 2020 Coordinator, Jacobs Undergraduate Mentorship Program
2015 - 2020 Teaching Assistant, Miramar Community College
2016 - 2020 Member, American Society for Pharmacology and Experimental Therapeutics
2021 - current Member, Out in STEM
2021 - current Co-founder and scientific advisor, Monod
2021 - current Member, American Society for Biochemistry and Molecular Biology
2021 - current Member, American Association for Cancer Research
2023 - current Member, American Society for Cell Biology
2023 - current Member, Biomedical Engineering Society

Honors

2017 - 2020 Predoctoral Fellowship, National Science Foundation
2022 - 2024 Postdoctoral Fellowship, Helen Hay Whitney Foundation
2024 - 2029 NCI K99/R00, National Institutes of Health

C. Contributions to Science (*co-first author, # co-corresponding author)

1. **Graduate Career:** My doctoral work centered on understanding how signaling cascades encode specificity through spatiotemporal regulation, knowledge of which will enable the design of more effective and targeted therapeutics. To this end, I designed genetically-encoded, fluorescence-based biosensors such as called fluorescent sensors targeted at endogenous proteins (FluoSTEP) and excitation ratiometric activity indicators (ExRai). With these engineered sensors, I discovered a novel principle organizer of cAMP/PKA signaling through liquid-liquid phase separation of the PKA regulatory subunit RIα. This signaling compartment acts as a dynamic cAMP buffer necessary for homeostatic compartmentation of cAMP, disruption of which by a fusion oncoprotein associated with fibrolamellar carcinoma leads to uncontrolled cell growth. With fluorescence-based biosensors and reaction-kinetics based computational modeling, I also uncovered the mechanism and revealed the impact of biphasic RhoA activation as I teased apart the two pathways that are responsible for this biphasic RhoA activation and showcased that these pathways enable both rapid activation and longer-term "memory" in RhoA signaling and RhoA-mediated transcription.
    a. **Zhang J.Z., Lu T.W., Stolerman L.M., Tenner B., Yang J., Zhang J.F., Falcke, M., Rangamani P., Taylor S.S., Mehta S., Zhang J. (2020) Phase separation of a PKA regulatory subunit controls cAMP compartmentation and oncogenic signaling. Cell *182*, 1531-1544.e15.**
    b. Zhang J.Z., Nguyen A., Miyamoto S., McCulloch A.D., Brown J.H., Zhang J. (2020) Histamine-induced biphasic activation of RhoA allows for persistent RhoA signaling. PLoS Biology *18*, e3000866.
    c. **Tenner B.*, Zhang J.Z.*, Huang B., Mehta S., Zhang J. (2020) Fluorescent biosensors for monitoring compartmentalized signaling within endogenous microdomains. Science Advances *7*, eabe4091.**
    d. Zhang J.F., Liu B., Hong I., Mo A., Roth R.H., Tenner B., Lin W., Zhang J.Z., Johnson R.C., Molina R.S., Drobizhev M., Taylor S.S., Hughes T.E., Tian L., Huganir R.L., Mehta S., Zhang J. (2021)

An ultrasensitive biosensor for high-resolution kinase activity imaging in awake mice. Nature Chemical Biology *17*, 39-46.

e. Ahn S.H., Qin S., Zhang J.Z., McCammon J.A., Zhang J., Zhou H.X. (2021) Characterizing Protein Kinase A (PKA) subunits as macromolecular regulators of PKA RIα liquid-liquid phase separation. Journal of Chemical Physics*. 154*, 221101.

2. **Postdoctoral Career:** As a postdoctoral fellow, I focused on decoding the complexities in Ras signaling by developing and utilizing *de novo* protein tools. Melding deep-learning, computational modeling, structural biology, and protein biochemistry, protein design is achievable and has led to the construction of a generalizable biosensor for rapid and sensitive detection of various protein targets such as antibodies and disease biomarkers. I further designed these sensors for intracellular tracking of cellular processes and as a synthetic biology tool to understand cellular responses and dynamics. I also used deep-learning based computational design to develop *de novo* binders for the highly charged C-termini of Ras isoforms to differentiate the signaling from different Ras isoforms and *de novo* activators of Hsp70 chaperones. With these Hsp70 activators, I created a first-in-class perturbator to dissolve specific condensates to uncover their biochemical and cellular roles.

   a. Zhang J.Z.*#, Yeh H.W.*, Walls A.C., Wicky B.I.M, Sprouse K., VanBlargan L.A., Treger R., Quijano-Rubio A., Pham M.N., Kraft J.C., Haydon I.C., Yang W., DeWitt M., Chow C., Carter L., Wener M.H., Stewart L., Veesler D., Diamond M.S., Baker D.# (2022) Thermodynamically coupled biosensors for detecting neutralizing antibodies against SARS-CoV-2 variants. Nature Biotechnology *40*, 1336–1340.

   b. Zhang J.Z.#, Nguyen W.H., Greenwood N., Ong S.E., Maly D.J.#, Baker D.# Computationally designed sensors detect endogenous Ras activity and signaling effectors at subcellular resolution. (2024) Nature Biotechnology https://doi.org/10.1038/s41587-023-02107-w

   c. Zhang J.Z.#, Ong S.E., Baker D., Maly D.J.# Single-cell signaling analysis reveals that Major Vault Protein facilitates RasG12C inhibitor resistance. (Accepted at Nature Chemical Biology) Biorxiv DOI: 10.1101/2023.10.02.560617v1

   d. Zhang J.Z.#, Greenwood N., Hernandez J., Cuperus J.T., Huang B., Queitsch C., Gestwicki J.E., Baker D.# De novo designed Hsp70 activator dissolves intracellular condensates. (Under review at Nature Communications) Biorxiv DOI: 10.1101/2023.09.18.558356v1

   e. Zhang J.Z.#, Wu K., Liu C.X., Li X., Baker D#. De novo design of Ras isoform selective tools by targeting their highly charged C-terminus. In preparation.

   f. Yeh H.W.*, Norn C.*, Kipnis Y., Tischer D., Pellock S.J., Evans D., Ma P., Lee G.Y., Zhang J.Z., Anishchenko I., Coventry B., Cao L., Halabiya S., DeWitt M., Carter L., Houk K.N., Baker D. (2023) De novo design of luciferases using deep learning. Nature. *614*, 774–780.

   g. Yang E.C., Divine R., Miranda M., Borst A., Sheffler W., Khmelinskaia A., Zhang J.Z., Goldbach N., Choi H.J., Abedi M., Lubner J., Hendel S., Fallas J., Ueda G., Norris A., Wysocki V., King N.P., Baker D. Computational design of non-porous pH-responsive antibody nanoparticles. (2024) Nature Structural and Molecular Biology https://www.nature.com/articles/s41594-024-01288-5

   h. Huang B.*, Abedi M.*, Ahn G.*, Coventry B.*, Sappington I., Tang C., Wang R., Schlichthaerle T., Zhang J.Z., Wang Y., Goreshnik I., Chiu C.W., Chazin-Gray, A., Chan S., Gerben S., Murray A., Wang S., O'Neill J., Yeh R., Misquith A., Wolf A., Tomasovic L.M., Piraner D.I., Gonzalez M.J.D., Bennett N.R., Venkatesh P., Ahlrichs M., Dobbins C., Yang W., Wang X., Sahtoe D., Vafeados D., Mout R., Shivaei S., Cao L., Carter L., Stewart L., Sapngler J.D., Roybal K.T., Greisen P.J., Li X., Bernardes G.J.L., Bertozzi C.R., Baker D. Designed Endocytosis-Triggering Proteins mediate Targeted Degradation and amplify signaling. Nature (accepted) Biorxiv: 10.1101/2023.08.19.553321

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