ROBERT R. PUCCINELLI

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EDUCATION:

2020 – *present* **Ph.D. Bioengineering**, Biosensor Design and Characterization

UC Berkeley - UCSF Joint Graduate Program in Bioengineering

2010 – 2014 **B.S. Physics,** Biophysics

B.S. Biological Sciences, Microbiology and Immunology

University of California, Merced

Cumulative GPA: 3.675, STEM GPA: 3.754

RESEARCH EXPERIENCE:

Ph.D. Candidate, UCB-UCSF Joint Graduate Program in BioengineeringJoseph DeRisi, PhD; *Biosensors*Aug. 2020 – present

Autoimmune diseases are notoriously challenging to diagnose. Many symptoms are not disease specific and most diseases are not characterized by a small, well-defined set of autoantigens. Producing diagnostics for all subtypes of a disease is not commercially viable with traditional methods due the rarity of some subtypes and the diversity of the targets. Phage immunoprecipitation sequencing (PhIP-seq) is an emerging research method that allows for massively multiplexed screening of autoantigens against a patient sera and does not share the same limitations of current methods. My work was focused on translating the labor intensive benchtop process into an automated process using multilayer microfluidics. By translating the format of the protocol, the method could be extended to include previously inaccessible forms of characterization such as linking antibody-antigen binding strength to disease severity or directly quantifying the number of autoantibodies in patient sera.

Research Associate II, Chan Zuckerberg Biohub

Rafael Gómez-Sjöberg, PhD; Scientific Instrumentation Development

Jun. 2017 – Aug. 2020

Chan Zuckerberg Biohub was launched by Mark Zuckerberg and Priscilla Chan with the intention to cure, prevent, or manage every disease within 100 years. The Bioengineering Platform sat in a unique position as it sought to support collaborative efforts between Stanford, UCSF, UC Berkeley, internal scientists and international affiliates by building instrumentation on-demand to address new scientific questions or enhance current scientific capabilities. I joined as the first member of the Bioengineering Platform.

Independent Projects

- Collaborated with protein scientists to develop a protein purification system to improve throughput while maintaining flow rate control at cost-sensitive prices. The final product was a fully programmable, parallelized system that was 1/10th the cost of commercial single-channel purifiers. The feature set included: up to 4 purification columns, an 8-to-1 buffer selector, solenoid valves for flow path control, peristaltic pumps for flow rate control, and a fraction collector supporting 10 fractions per column
- Collaborated with internal scientists to develop a high-density tube capping-decapping system to enable the use of cryotubes with unique IDs for storing and tracking sensitive samples. The semi-automated unit reduced cap transfer time from 10 minutes to 2 minutes for a set of 96 tubes
- Collaborated with members of the CZ Biohub Microbiome Initiative to organize instrumentation in a large anaerobic chamber and developed motorized axes to transport samples across the chamber

Contributions to Team Projects

- Developed Arduino code and a LCD module for an ultra-low cost turbidity meter that was built and deployed to Uganda within 3 days to replace faulty equipment in a UC Berkeley CEND protein purification workshop at Makerere University
- Developed an electronic demultiplexer that selectively activates one of several LED light sources on a custom UV microscope using a single LED driver and PC-controlled IO
- Developed a capillary holder mechanism for imaging in a tightly confined dual light sheet microscope
- Developed a digital, parallelized reagent degassing module for a custom in-situ transcriptomics microscope
- Developed an auto-sampler that aliquots the output of a microfluidic device into PCR tubes
- Developed a cantilevered arm and plate chiller that collects and preserves rare cell populations acquired from a custom single cell picker system

COVID-19 Responsibilities

- Addressed supply chain failure for sample collection tubes in March by designing and building a parallelized media dispensing system for empty collection tubes. This system could fill tubes at an average rate of 0.75s per tube. Multiple copies of this system were produced to support UCSF's, Stanford's and CLIAHUB's independent testing efforts. CLIAHUB is a COVID-19 testing lab funded by CZ Biohub
- Trained and led CLIAHUB essential workers to produce 55,000 tubes over 2 months. Tube production consisted of racking tubes onto the machine, dispensing medium, unracking/capping tubes, and labeling. An external company was eventually contracted to produce tubes for our testing efforts
- Packaged and distributed 150,000 testing kits to our partner institutions, studies and departments of public health all across the state of California
- Trained CLIAHUB essential workers on chemical and biohazard waste disposal and managed communication between our testing team and UCSF EH&S. At peak, CLIAHUB produced 1000 gallons of solid waste per week before more efficient testing protocols were implemented
- Produced a variety of small tools to facilitate internal testing efforts such as laser cutting cardboard racks to organize incoming samples while also maintaining label visibility

Life Sciences Research Professional, Stanford University

Sep. 2014 – May 2017

Polly Fordyce, PhD; Biophysical Interactions in Microfluidics

My research in the Fordyce Lab was centered around employing the MITOMI microfluidic device, a multiplexer with >1500 sample chambers that mechanically traps molecular interactions, for high throughput investigations of transcription factor – DNA oligo binding equilibrium and kinetics to access fine resolution data. As the first member of the Fordyce Lab, this required me to assemble optical, fluidic, and microarray systems, independently conduct each experiment, write software to analyze image datasets and train other members.

Instrumentation Assembly

- Assembled and maintained several PC-controlled epifluorescent microscopes
- Constructed a DeRisi-style microarryer to enable local microarray printing
- Constructed several PC-controlled microfluidic pneumatic control setups

Data Collection and Processing

- Automated microfluidic device control and time series imaging protocol
- Isolated fluorescent images from TIFF stacks, flat-field corrected images, and stitched channels
- Processed complete image datasets with a custom image analysis tool to evaluate TF-DNA binding events for >1500 isolated chambers. Reduced analysis from 120 minutes to 15 minutes per dataset

Sample Preparation

- PCR extended basic helix-loop-helix TF DNA such as Max and c-Myc to include transcription/translation sites before cloning into a plasmid and transforming competent E. coli for expansion and sequencing
- Prepared DNA oligo libraries with a fluorescent tag before printing onto glass slides with DeRisi-style microarrayers with printing solutions that were optimized for our setups
- Fabricated two-layer MITOMI devices with PDMS and aligned to >1500 DNA spots on glass slide

Undergraduate Research Assistant, University of California, Merced

Oct. 2011 – May 2013

Kara McCloskey, PhD; Cardiac Tissue Engineering

Stem cells that have not attained an adult-like state are unsuitable for clinical applications due to their propensity to revert to a multipotent state and induce tumorigenic growth. My research focused on differentiating mouse and human embryonic stem cells into immature cardiomyocytes and investigating the effects of surface topography and electrical pacing on advancing their maturation to an adult-like state. Consistently differentiating mature cardiomyocytes would be milestone in the path to restoring function to regions of the heart that has been irreversibly damaged by hypoxia.

Tissue Culture

- Maintained multiple lines of mouse and human stem cells including thawing, feeding, passing and freezing of cultures so that excess cells were always ready for experiments in the lab
- Directed differentiation of multiple lines of stem cells towards the myocardial lineage with physical techniques, such as embryoid body formation, chemical techniques, such as scheduled growth factor exposure and feeding, and environmental techniques, such as electrical stimulation
- Cultures were tagged with fluorescently labeled with antibodies and imaged or FACS sorted

Additional Responsibilities

- Trained incoming students on cell culture, stem cell culture, and fluorescent microscopy to provide the with a basic skill set for their own projects
- Drafted experimental design for the project, directed and oversaw progress of the project, and discussed troubleshooting measures with my post-doctoral mentor
- Prepared and maintained reagent and media inventory

Undergraduate Research Assistant, University of California, Merced

Jan. 2013 – Oct. 2014

Ajay Gopinathan, PhD; Computational Cellular Biophysics

f-Actin is a cytoskeletal element that plays significant roles in cell motility, endocytosis and defining cell shape by polymerizing against regions of the cell membrane with positive curvature. For my capstone in physics, I modeled actin polymerization events at the cellular scale with a 2D, coarse-grained, nearest-neighbor Monte Carlo simulation whereby parameters for membrane-surface binding events, membrane curvature and elasticity, in addition to actin polymerization were used to evaluate cell shape modulation over time. This has predictive power for applications in tissue engineering where nano- and microscale topography is utilized to influence stem cell differentiation with cell shape.

Computational Modeling

- Constructed model for actin dynamics at the cellular level de novo by considering resistive and propulsive forces in the system including membrane bending, membrane adhesion and actin polymerization
- Generated different modes of actin dynamics such as cell spreading and cyclical actin wave generation by tuning parameters in the model

- Model was applied to different surface topographies to evaluate their influence on the localization of actin polymerization and cell shape
- Examine the literature for relevant energetic and rate constants

PREPRINTS:

- 1. **Portable low-cost optical density meter.** <u>Puccinelli RR</u>, et al. *bioRxiv*. 2021 May. doi: 10.1101/2021.05.14.444207
- Supervised self-collected SARS-CoV-2 testing in indoor summer camps to inform school reopening. Cooch P, <u>CLIAHUB Consortium</u>, et al. medRxiv. 2020 Oct. doi:10.1101/2020.10.21.20214338

PUBLICATIONS:

- 1. **Remoscope: a label-free imaging cytometer for malaria diagnostics.** Lebel P, et al. *Trans. R. Soc. Trop. Med. Hyg.* 2025 Jul. doi:10.1093/trstmh/traf070
- 2. **Open-source milligram-scale, four channel, automated protein purification system.** <u>Puccinelli RR</u> et al. *PLOS One*. 2024 Feb. doi:10.1371/journal.pone.0297879
- 3. A handheld luminometer with sub-attomole limit of detection for distributed applications in global health. Lebel P, et al. *PLOS Global Public Health* . 2024 Feb. doi: 10.1371/journal.pgph.0002766
- 4. Comparison of SARS-CoV-2 reverse transcriptase polymerase chain reaction and BinaxNOW rapid antigen tests at a community site during an Omicron surge: a cross-sectional study. Schrom J, et al. *Annals of internal medicine*. 2022 May. doi: 10.1186/s13690-021-00647-8
- 5. Community transmission of severe acute respiratory syndrome coronavirus 2 disproportionately affects the Latinx population during shelter-in-place in San Francisco. Chamie G, <u>CLIAHUB Consortium</u>, et al. *Clinical Infectious Diseases*. 2021 Aug. doi: 10.1093/cid/ciaa1234
- 6. **SARS-CoV-2 PCR and antibody testing for an entire rural community: methods and feasibility of high-throughput testing procedures**. Appa A, <u>CLIAHUB Consortium</u>, et al. *Archives of Public Health*. 2021 Jul. doi:10.1101/2020.05.29.20116426
- 7. Performance Characteristics of a Rapid Severe Acute Respiratory Syndrome Coronavirus 2 Antigen Detection Assay at a Public Plaza Testing Site in San Francisco. Pilarowski G, CLIAHUB Consortium, et al. The Journal of Infectious Diseases. 2021 Jan. doi: 10.1093/infdis/jiaa802
- 8. Universal PCR and antibody testing demonstrate little to no transmission of SARS-CoV-2 in a rural community. Appa A, <u>CLIAHUB Consortium</u>, et al. *Open Forum Infect. Dis.* 2020 Oct. doi:10.1093/ofid/ofaa531

- 9. **Rapid deployment of SARS-CoV-2 testing: The CLIAHUB.** Crawford ED, et al. *PLOS Pathogens*. 2020 Oct. doi:10.1371/journal.ppat.1008966
- 10. Identification of a polymorphism in the N gene of SARS-CoV-2 that adversely impacts detection by RT-PCR. Vanaerschot M, <u>CLIAHUB Consortium</u>, et al. *J Clinical Microbiology*. 2020 Oct. doi:10.1128/JCM.02369-20
- 11. Community Transmission disproportionately affects Latinx population during Shelter-in-Place in San Francisco. <u>CLIAHUB Consortium</u>, et al. *Clinical Infectious Diseases*. 2020 Aug. doi:10.1093/cid/ciaa1234
- 12. Clinical features, diagnostics, and outcomes of patients presenting with acute respiratory illness: A retrospective cohort study of patients with and without COVID-19. CLIAHUB Consortium, et al. EClinicalMedicine. 2020 Aug. doi:10.1016/j.eclinm.2020.100518
- 13. Diversification of DNA binding specificities enabled SREBP transcription regulators to expand the repertoire of cellular functions that they govern in fungi. Del Olmo Toledo V, Puccinelli R, Fordyce PM, Pérez JC. *PLoS Genet.* 2018 Dec. doi:10.1371/journal.pgen.1007884
- 14. **Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris.** <u>Logistical coordination</u>; et al. *Nature*. 2018 Oct. doi:10.1038/s41586-018-0590-4
- An Open-Source, Programmable Pneumatic Setup for Operation and Automated Control of Single- and Multi-Layer Microfluidic Devices. Brower K, <u>Puccinelli R</u>, Markin CJ, Shimko TC, Longwell SA, Cruz B, Gómez-Sjöberg R, Fordyce PM. *HardwareX*. 2018 Apr; doi:10.1016/j.ohx.2017.10.001
- 16. **Optimized Sequence Library Design for Efficient In Vitro Interaction Mapping.** Orenstein Y, Puccinelli R, Kim R, Fordyce P, Berger B. *Cell Systems*. 2017 Sep. doi:10.1016/j.cels.2017.07.006

GRADUATE TEACHING EXPERIENCE:

Fab in Lab, UCSF Summer 2019

Instructors: Joseph DeRisi and Wallace Marshall

Teaching Staff: Joanna Cabrera, Rafael Gómez-Sjöberg, Eric Lam, Paul Lebel, <u>Robert Puccinelli</u>, Kevin Yamuchi BP 219; Course evaluation: 4.8/5.0 (university and department averages of 4.3)

Microfluidic Device Design Laboratory, Stanford

Winter 2017

Instructors: Polly Fordyce and Stephen Quake

Teaching Staff: Kara Brower, Diego Oyarzun, Robert Puccinelli, Adam White

BIOE 301D/GENE 207

POSTER PRESENTATIONS:

September 2016 **2016 Stanford Department of Genetics Annual Retreat**

Poster Presentation: Influence of Flanking Sequences in Transcription Factor -

DNA Binding

Robert R. Puccinelli and Polly M. Fordyce

September 2015	2015 Stanford Department of Genetics Annual Retreat
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Poster Presentation: Resolving functional networks in a highly proficient enzyme C. Markin, R. Puccinelli, C. Guegler, F. Sunden, I. AlSadhan, P. Fordyce, D. Herschlag

March 2014 **2014 Undergraduate Research Poster Competition, UC Merced**

Poster Presentation: Modeling Actin Dynamics on Nanoscale Topography

Robert R. Puccinelli and Ajay Gopinathan

*Certificate of Achievement awarded for best poster presentation

March 2013 Student Research Poster Competition, UC Merced

Poster Presentation: Roles for Electrical and Topographical Cues in the Maturation

of Derived Cardiomyocytes.

Robert R. Puccinelli, William S. Turner, Kara E. McCloskey

August 2012 Sixth Annual Summer Research Symposium, UC Merced

Poster Presentation: Combinatorial Effects of Nanoscale Topography and Electrical

Stimulation in Stem Cell Fate

Robert R. Puccinelli, William S. Turner, Kara E. McCloskey

AWARDS/HONORS:

2014	Graduation with Honors, University of California, Merced
2014	Outstanding Student Award, University of California, Merced
2014	Best Poster Presentation, UC Merced Research Week 2014
2007	Eagle Scout, Boy Scouts of America