OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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NAME: Thompson, Samuel Mark

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

POSITION TITLE: Postdoctoral Research Fellow

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)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology	BS	09/2008	06/2013	Chemistry
University of California, San Francisco	PhD	09/2013	06/2020	Biophysics
Stanford University	Postdoctoral	09/2020	present	Bioengineering

A. Personal Statement

My long-term goal is to pursue an independent academic research program centered on engineering functional proteins to address issues in global public health, particularly the need for diverse antibiotics and chemotherapeutics that combat the rise of resistance both for infectious pathogens and for cancer. Furthermore, I aim to apply protein engineering to related projects for developing inexpensive biomedical sensors, carbon neutral fuel sources, and environmentally sustainable industrial materials that will all have an impact on global public health and address environmental risk factors for cancer patients. In particular, I aim to expand the usefulness of protein biotechnology by developing methods for using proteins as catalysts and nanomachines in chemical environments other than water (see **Proposal**).

My research training in material science, protein engineering, computational protein design, and structural and molecular biology began in November 2006, when I was synthesizing environmentally responsive microgels for drug delivery in the lab of Prof. Zhibing Hu (University of North Texas) during my junior year of high school. For that project, I finally succeeded in repeating a key method from the literature only by pursuing experiments months after the project had been abandoned in the lab. Although my mentor was impressed, I could not answer his question as to why I had maintained my efforts. I can only now put into words my long-held desire to achieve that which is perceived to be unreasonable. The challenges in generating functional nanomaterials in this project also convinced me of the extreme utility in protein engineering, where changing the primary amino acid sequence allows both for control over molecular structure at angstrom-level precision and for dramatic changes in molecular function.

During my undergraduate studies, I worked with Alice Ting (MIT, Stanford University) whose website highlighted research that “harnesses the natural power of enzymes.” Those words spoke the vision of the world that I held within me. In the Ting lab, I undertook the characterization of *E.coli* lipoate-protein ligase variants that would ligate coumarin fluorophores to a peptide tag for imaging proteins in living cells. In two and a half years of work, I completed experiments contributing to three publications, including my own independent project to engineer a ligase for a brighter and more photostable coumarin, Pacific Blue, another formerly abandoned project. To complement my experience engineering protein structure and function, I then worked on determining the structure of large enzymatic complexes by x-ray crystallography and 3D electron microscopy with Prof. Catherine Drennan (MIT) to expand my understanding of protein structure and familiarity with structural biology methods. Taking the skills I learned in the Drennan lab, I spent a year abroad developing methods for EM image processing at Osaka University, Japan, in the lab of Junichi Takagi. There, I once listened to my Japanese mentors describe how engineering enzymes for non-aqueous solvents was a grand challenge during their PhD training, but it had been largely abandoned in recent years. I decided in that moment, that this was the fundamental problem I wanted to address.

Convinced by the need for computational methods for tackling complex problems in protein engineering in general and for designing proteins for non-aqueous solvent environments, I then pursued my graduate studies in the lab of Prof. Tanja Kortemme (UCSF), a pioneer in computational protein design. There, I developed a high-throughput screen for a model enzyme to pair with computational models to predict the impact of mutations on function.

Currently, I am pursuing post-doctoral research with Polly Fordyce, an innovator in developing microfluidic approaches for dissecting protein function, and in collaboration with David Baker (University of Washington) a founder of the field of protein design. Here at Stanford, I fully intend to build off the momentum I have gained at MIT and UCSF because each research experience has been a deliberate step toward my present work. In fact, my long-term aim for engineering non-aqueous proteins was also my long-term aim for my successful applications to graduate school and the NSF Graduate Research Fellowship. My current project is a fantastic opportunity to pursue ideas that I have nurtured for years and that I aim to pursue in my own future lab. Support from the Alfred P. Giannini Fellowship will continue this trajectory of pursuing grand challenges in biotechnology research while grounding the research in health-related applications. This fellowship will be a bridge to a successful K99 application and independent research.

a. Photonic hydrogels with poly(ethylene glycol) derivative colloidal spheres as building blocks. Tong Cai, Guonan Wang, Samuel Thompson, Manual Marquez, and Zhibing Hu. Macromolecules 2008 41 (24), 9508-12.
b. Structure-guided engineering of a Pacific Blue coumarin ligase for specific protein imaging in living cells. Justin D. Cohen, Samuel Thompson, and Alice Y. Ting. Biochemistry 2011 50 (38), 8221-5.
c. Flex ddG: Rosetta Ensemble-Based Estimation of Changes in Protein-Protein Binding Affinity upon Mutation Kyle A. Barlow, Shane Ó Conchúir, Samuel Thompson, Pooja Suresh, James E. Lucas, Markus Heinonen, Tanja Kortemme. Journal of Physical Chemistry B 2018 122 (21) 5389-5399
d. Altered expression of a quality control protease in E. coli reshapes the in vivo mutational landscape of a model enzyme. Samuel Thompson,* Yang Zhang, Christine Ingle, Kimberly A. Reynolds, & Tanja Kortemme. eLife 2020 9 e53476. *As co-contributing author

B. Positions and Honors

Positions and Employment

2006-2008	High School Researcher, University of North Texas, Prof. Zhibing Hu
2008	High School Summer Researcher, University of Texas at Austin, Prof. Andrew Ellington
2008-2011	Undergraduate Researcher, MIT, Prof. Alice Ting
2011-2012	Undergraduate Exchange Researcher, Osaka University, Japan, Prof. Junichi Takagi
2011-2013	Undergraduate Researcher, MIT, Prof. Catherine Drennan
2013	Post-baccalaureate Researcher, National University of Singapore, Prof. Wen Shan Yew

Other Experience and Professional Memberships

2013-2017	Co-founder, UCSF Graduate Queer Alliance
2021	Designing for an Anti-Racist Stanford (Stanford d.School project course)
2021	Stanford-NIH Responsible Conduct of Research course

Honors

2007	Siemens Competition Regional Finalist
2008	Intel Talent Search Semifinalist
2008	Barry M. Goldwater Scholar
2008-2011, 2013	Eugene and Margaret McDermott Fund Recipient
2009	HHMI-MIT Summer Undergraduate Fellowship
2011	MIT Foreign Languages and Literature Distinguished Student
2012	Osaka University Frontier Lab Best Presentation
2013	MIT Department of Chemistry Research Award
2013	UCSF Chuan Lyu Chancellor Fellowship
2014	NSF Graduate Research Fellowship
2015	UCSF Quantitative Biosciences Consortium TA Award
2017	UCSF Mel Jones Memorial Research Award
2018	Protein Engineering Canada Best Presentation Award
2018	UCSF Dr. Herbert Landahl Mathematical Biophysics Student Excellence Award

C. Contributions to Science

Measuring the Impact of Cellular Factors on Protein Mutational Landscapes

The cellular environment acts as a transfer function modulating the relationship between the impact of mutations on individual protein function and broader phenotypic responses at the cellular level. During my PhD research with Prof. Kortemme (UCSF), I worked in collaboration with Prof. Kim Reynolds (UT Southwestern) and Prof. James Fraser (UCSF) to understand the cellular factors for *in vivo* deep mutational scans of all possible single point mutations to essential proteins in *S. cerevisiae* and *E. coli*. With Prof. Fraser we performed deep mutational scanning experiments in a classroom setting to elucidate how changes in selection pressure during stress response can explain why ubiquitin, a high conserved protein involved in protein quality control and signaling, often appears to be unusually tolerant to mutation in *in vivo* experiments. With Prof. Reynolds, I built a continuous culture set-up to screen libraries of mutants to the essential enzyme dihydrofolate reductase (DHFR). We discovered that 23% of single point mutations are advantageous and increase DHFR activity in our screening experiment despite the fact that the natural sequence is fixed in wild-type strains. We resolved these findings when we observed that the majority these mutations are destabilizing and are repressed by Lon protease, a component of the protein quality control network that is frequently knocked-out in lab strains of *E. coli*. These experiences prepared me to troubleshoot and perform high-throughput screens, including optimizing conditions and building custom experimental devices. Furthermore, they have given me practice in the far more difficult challenges of drawing scientific insight from large and convoluted biological datasets and developing targeted experiments to test hypotheses generated from screens. This work from 2014 to 2020 has resulted in 2 peer-reviewed publications, 2 manuscripts in progress, 4 presentations at international conferences, and 6 presentations at departmental and institute conferences.

a. Determination of Ubiquitin Fitness Landscapes Under Different Chemical Stresses in a Classroom Setting. David Mavor, Kyle A. Barlow, Samuel Thompson, et al. eLife 2016 5, 15802.
b. Altered expression of a quality control protease in *E. coli* reshapes the in vivo mutational landscape of a model enzyme. Samuel Thompson,* Yang Zhang, Christine Ingle, Kimberly A. Reynolds, & Tanja Kortemme. eLife 2020 9 e53476. *As co-contributing author

Modeling the Impact on Protein Function for Large Scale Mutational Datasets

Many protein engineering goals require mutations at a large number of positions (>10) on a protein scaffold or the generation of a completely new protein scaffold. Because the vast combinatorial size of amino acid combinations in protein sequence space is intractable for wetlab experiments, computational methods are often needed to identify protein sequences that have the desired structure and function. Furthermore, computational methods that can predict the relationship between sequence and function would be beneficial for understanding how mutations cause misfunction in cells and human disease, such as during oncogenesis. During my PhD research with Prof. Kortemme (UCSF), I developed computational models to address a fundamental problem in the representation of proteins *in silico*. While natural proteins undergo thermal fluctuations and functional proteins adopt multiple functional conformations, computational design models generally do not allow for fluctuations of the protein backbone structure. I combined flexible backbone protein models with models of proteins that adopt multiple functional conformations to rapidly sample sequences that are compatible with a more dynamic representation of the target proteins. We found that our model improved performance in experimental benchmarks for recovery of amino acid preferences from multiple sequence alignments of natural protein orthologues. An upcoming manuscript is currently in preparation to report our findings on this project. This research has given me valuable experience with state-of-the-art computation protein design software such as Rosetta. As both a user and a developer of Rosetta, I am well positioned to apply these techniques to my current research aims. This work from 2013 to 2020 has resulted in 1 peer-reviewed publication, 1 manuscript in progress, 2 presentations at international conferences, 1 presentation at a departmental/institute conference, and one module for a lab course at UCSF.

a. Flex ddG: Rosetta Ensemble-Based Estimation of Changes in Protein-Protein Binding Affinity upon Mutation. Kyle A. Barlow, Shane Ó Conchúir, Samuel Thompson, Pooja Suresh, James E. Lucas, Markus Heinonen, Tanja Kortemme. Journal of Physical Chemistry B 2018 122 (21) 5389-5399
b. tructurally distributed surface sites tune allosteric regulation. James W. McCormick, Marielle A.X. Russo, Samuel Thompson, Aubrie, Blevins, Kimberly A. Reynolds. eLife 2021 *10*, e68346.

c. Fundamentals to function: quantitative and scalable approaches for measuring protein stability. Beatriz Atsavapranee, Catherine D. Stark, Fanny Sunden, Samuel Thompson,* and Polly M. Fordyce. Cell Systems 2021 *12*(6), 547–560. *As co-contributing author

Engineering New Function into Enzymes to Image Proteins in Living Cells
To understand the molecular mechanisms behind how cells function and misfunction, it is important to understand how proteins behave in their natural cellular context. One major method for observing the behavior of proteins in living cells is florescence microscopy, but the large size of genetic tags such as GFP can alter the behavior of proteins of cells. In my undergraduate research with Prof. Alice Ting (MIT), I undertook the characterization of *E. coli* lipoate protein ligase variants that would ligate coumarin fluorophores to a small 13-amino acid peptide tag for imaging proteins in living cells. In two and a half years of work, I completed work contributing to three publications, including my own project to engineer a ligase for a brighter and more photostable coumarin, Pacific Blue, a project that had been initially abandoned by my postdoc mentor. In the course of this project, I established new screening protocols for activity using ultra performance liquid chromatography (UPLC) which increased the throughput of our experiments by two orders of magnitude and allowed me to identify a functional Pacific Blue Ligase from a combinatorial library. This research project would ultimately become a part of an educational video discussing how pKa can be used to understand pH dependent properties and how charge repulsion can impact binding. This research prepared me with fundamental tools for engineering proteins and for developing fluorescent reporter assays that will be invaluable in the experiments proposed in my aims. This work from 2008 to 2011 has resulted in 3 peer reviewed publications.

a. Yeast display evolution of a kinetically efficient 13-amino acid substrate for lipoic acid ligase. Sujiet Puthenveetil, Daniel S. Liu, Katharine A. White, Samuel Thompson, and Alice Y. Ting. Journal of the American Chemical Society 2009 131 (45), 16430-8.
b. A fluorophore ligase for site-specific labeling inside living cells. Chayasith Uttamanpinant, Katharine A. White, Hemanta Baruah, Samuel Thompson, Marta Fernádez-Suárez, Sujiet Puthenveetil, and Alice Y. Ting. Proceedings of the National Academy of Sciences 2010 107 (24), 10914-9.
c. Structure-guided engineering of a Pacific Blue coumarin ligase for specific protein imaging in living cells. Justin D. Cohen, Samuel Thompson, and Alice Y. Ting. Biochemistry 2011 50 (38), 8221-5.

Revealing Functionally Relevant Conformational Variation in Protein Complexes with Electron Microscopy
Characterizing proteins with unique and useful functions can hinge on the ability to set-up experimental environments to protect sensitive samples that cannot be studied with standard benchtop equipment. While working with Prof. Catherine Drennan (MIT), I analyzed the conformational changes in key enzymes of the Wood-Ljungdahl carbon fixation pathway from anaerobic microorganisms. Alongside my postdoctoral mentor, Dr. Ed Brignole, I developed what we understand to be the first method for anaerobic electron microscopy. This enabled me to reveal unexpected conformational flexibility in these enzymes that enables them to shield reactive intermediates from the solvent environment while shuttling them between three separate active sites. The insights we discovered may lead to engineering proteins that are capable of scrubbing CO_2 from industrial emissions. While researching abroad at the Institute for Protein Research at Osaka University, Japan. I worked with Profs. Junichi Takagi and Kenji Iwasaki to identify components of a mitochondrial pore complex (TIM) by cryo-EM. In this effort, I developed methods to perform digital size-exclusion on single-particle cryo-EM images to aid in the classification of TIM complexes comprised of varying subunit configurations. These two experiences prepared me to develop lab-scale experimental set-ups for working with proteins that require specialized environments and expanded my ability to apply structural biology techniques for large protein complexes. Furthermore, this work has allowed me to engage more deeply with the international scientific community and use my native English language skills for science communication. This work from 2011 to 2013 has resulted in 3 peer reviewed publications 2 presentations at international conferences, and 1 presentation at a departmental/institute conference.

a. Allosteric inhibition of human ribonucleotide reductase by dATP entails the stabilization of a hexamer. Nozomi Ando, Haoran Li, Edward J. Brignole, Samuel Thompson, Martin I. McLaughlin, Julia E. Page, Francisco J. Asturias, JoAnne Stubbe, Catherine L. Drennan. Biochemistry 2015 55 (2), 373-81
b. Conformational Freedom of the LRP6 Ectodomain Is Regulated by N-glycosylation and the Binding of

the Wnt Antagonist Dkk1. Kyoko Matoba, Emiko Mihara, Keiko Tamura-Kawakami, Naoyuki Miyazaki, Shintaro Maeda, Hidenori Hirai, Samuel Thompson, Kenji Iwasaki, Junichi Takagi. Cell Reports 2017 18 (1) 32-40.

c. Extensive conformational flexibility of carbon monoxide dehydrogenase/acetyl-CoA synthase revealed by electron microscopy. Steven E. Cohen, Edward J. Brignole, Elizabeth C. Wittenborn, Mehmet Can, Samuel Thompson, Stephen W. Ragsdale, & Catherine L. Drennan. Structure 2020 S0969-2126(20)30324-5.

Investing in Science Education, International Science Communication, and Research Mentorship

In addition to being a competent scientist, it is increasingly important to invest in the academic and scientific community. During my undergraduate work, I presented my research from the Ting lab as a real-life demonstration of the implications of pH and pKa as a part of Prof. Catherine Drennan's "Behind the Scenes at MIT" project to produce educational videos based on real-life research challenges. This video series is now freely available online for educators at all levels and is incorporated into the freshman chemistry courses at MIT. My experience demonstrating the capabilities of undergraduates was crucial when I worked at the Singapore University of Technology and Design (SUTD) as a TA under the Singapore-MIT Alliance. There, I convinced the department to start a peer-tutoring program and trained 11 freshman Chemistry students to run the program almost entirely autonomously, from drafting curriculum to mentoring other students. Today, I continue to facilitate the dissemination of Japanese research for English-language publication in collaboration with my colleagues Junichi Takagi (Osaka University) and Terukazu Nogi (Yokohama City University). In my PhD lab, I took leadership as lab manager and as mentor to three rotation students and two undergraduate students. Many of these students were from underrepresented minority populations and/or were first generation graduate students. Outside my lab, I co-founded the Graduate Queer Alliance (GQA) – the first organization for LGBTQ scientists on the Mission Bay campus – and served on leadership from 2013 to 2017. From 2014 to 2016, I moderated the GQA OUT in Science panel discussion for LGBTQ scientists in the Bay Area. In 2014, I served on the committee coordinating the DEI orientation for incoming UCSF graduate students. In 2015, I earned the QBI Teaching Assistant Award. In 2015 and 2017, I mentored first year students in writing proposals for the NSF GRFP. 30% (2 of 7) of my mentees earned a fellowship compared to a 15% award rate overall. In 2018, I co-authored a Career Column for *Nature* recommending concrete steps for improving communication between graduate students and their PIs. Currently, I am co-authoring an upcoming column recommending concrete anti-racist actions for academic labs in collaboration with a multi-racial group of former UCSF graduate students. This work is ongoing.

a. Structural basis for amyloidogenic peptide recognition by sorLA. Yu Kitago, Masamichi Nagae, Zenzaburo Nakata, Maho Yagi-Utsumi, Shizuka Takagi-Niidome, Emiko Mihara, Terukazu Nogi, Koichi Kato, Junichi Takagi. Nature Structure & Molecular Biology. 2015 22(3):199-206. *credited in acknowledgements
b. Application of the NZ-1 Fab as a crystallization chaperone for PA tag-inserted target proteins. Risako Tamura, Rika Oi, Satoko Akashi, Mika K Kaneko, Yukinari Kato, Terukazu Nogi. Protein Science. 2019 28(4):823-836. *credited in acknowledgements
c. Veuthey TL, Thompson S. Why you need an agenda for meetings with your principal investigator. Tess L. Veuthey & Samuel Thompson. Nature 2018 561(7722):277.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Stanford School of Medicine Dean's Postdoctoral Fellowship

(Role: Trainee, PI Polly Fordyce) 01/01/2021-12/31/2021

Mapping non-aqueous protein sequence space to expand the potential in protein engineering

Goal: To develop a platform for producing, screening, characterizing, designing, and engineering proteins that fold in water-immiscible organic solvents.