ALUMNUS PROFILE

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When I was asked to write this profile, I first identified a few key decisions I made over the last 20 years and the lessons I learned from those decisions. Those life lessons form the basis of what I'd like to share.

My life can be divided into 5 segments: 18 years in a small town in southern Illinois, 5 years as an undergraduate at University of Evansville, 6 years as a graduate student at Syracuse, 5 postdoctoral years at the University of California-San Diego, and 5 years as an assistant/associate professor at the University of Kentucky. I grew up in the small town of Sumner amid the cornfields of southern Illinois in a working-class family. I did well in school, but was never entirely devoted to my schoolwork. Instead, I preferred playing or watching baseball and basketball.

Sandy, Gabriella, and Matt Gentry

College was an expectation my parents instilled in me, but was mostly thought of as a means to getting a job that did not require physical labor and paid better than the majority of jobs in our area. So the assumption was I would go to college to become a pharmacist, engineer, physical therapist, or doctor. I was the first person in my family to go to college, and I was naive about the entire process. The thought of becoming a scientist was not part of the equation when I started my undergraduate studies.

LIFE LESSON #1

Explore your options and pay attention to what you do best.

I chose to attend the University of Evansville (UE), a small liberal arts school in southern Indiana, intending to become a physical therapist. Physical therapy (PT) school involved two years of prerequisite classes and then applying to a 3-year PT program. It was a competitive program and while I quickly learned that I did not know how to study, my grades were competitive enough to get into the program.

To meet one of the requirements for acceptance into the PT program, I volunteered in the physical therapy department of my local hospital. I soon discovered I did not enjoy working with patients and especially disliked the bodily fluids that were often a part of the job. I struggled to motivate myself to become a physical therapist. Acceptance into the PT program involved a series of interviews, and apparently the interviewers recognized I lacked any passion to become a PT, but instead saw it as a trade, a nonfactory job. I was devastated not getting into the PT program, but as that door closed, other opportunities led me on a fantastic journey much more exciting than I could have imagined.

I returned to UE after taking a year off and started taking upper level biology and biochemistry classes; suddenly everything seemed to click. I learned how to study–or more likely found something I truly wanted to learn–and studying was no longer a dreaded burden. I received an excellent education at UE in terms of knowing the fundamentals of biology, chemistry, and biochemistry, but I did not do any undergraduate research. I knew little about how science was actually done, including how one became a scientist, but I did realize I could not do science with just a bachelor's degree.

I applied to a range of graduate programs from Ivy League to the state schools, and I must confess I decided to apply to Syracuse University largely because I was an SU basketball fan. Watching Big East basketball games every Monday night, I had fallen in love with the Carrier Dome and the SU teams of the 1980s. I was so naive I didn't even realize it was common for a graduate student to receive a fellowship covering tuition and providing a stipend. When I spoke with graduate school contacts, the only program that mentioned this was SU. In the end, I declined other graduate schools to which I was accepted, because Syracuse was the only one that discussed finances; I headed for Upstate New York and the Carrier Dome.

I loved my first year of graduate school classes and flourished in them, receiving all A's. I did rotations with Saul Honigberg, Eleanor Maine, and Dick Hallberg. A project in the Hallberg lab determining the role of protein phosphatase 2A (PP2A) in cell division using the model organism *Saccharomyces cerevisiae* felt right to me and was the easiest for me to think about and conceptualize. However, the lab had a pretty tough reputation. Dick did not suffer fools and he had serious doubts about taking me as a graduate student, because I had never done research.

In the end, I was saved by Dick's long-time collaborator in both science and life, his wife, Liz Hallberg. Liz was an experimentalist

with fantastic bench-hands, and she trained everyone in the lab. However, a lack of research experience was not my only shortcoming; I also had little experience presenting science. The first lab meeting in which I came prepared to offer a one-hour presentation of my data lasted more than 2½ hours. Dick was conscientious about one's choice of words, and when I said anything inaccurate or wrong he made me repeat that slide. This was a humbling and embarrassing event, but I now appreciate Dick's strategy. He was very hard on us in-house, but he was our biggest advocate to others. His tough training greatly improved our ability to articulate our results and ideas, and impressed others when we went out into the world.

Read the literature and identify your rock.

When I joined Dick's lab in 1996, the focus was on deciphering cell division signals orchestrated by protein phosphatase 2A (PP2A) in yeast. Leland Hartwell, Tim Hunt, and Paul Nurse had discovered the master regulators of cell division in the 1980s. These included regulators of the cell cycle (Cdc28p in *S. cerevisiae* and Cdk1 in humans) along with cyclins–proteins responsible for driving cells through the different stages of the cell cycle. Their work in yeast defined the basis of how cells divide by describing a coordinated cell cycle and had direct correlations to cell division in all eukaryotic cells.

When Dick came to SU, he had just made a series of seminal discoveries on thermo-tolerance in *Tetrahymena* and yeast, including the discovery of heat shock protein 60 (Hsp60p). At SU, graduate student Yomin Shu performed a high copy suppressor screen using an hsp60 temperature-sensitive strain and discovered one of the subunits of PP2A to be a suppressor. Haifeng Yang, another of Dick's graduate students, elucidated how PP2A controlled the *S. cerevisiae* master cell cycle regulator Cdc28p and determined how loss of PP2A activity caused cell cycle defects. Graduate student Wei Jiang pioneering a new model organism, *Schizosaccharomyces pombe*, identified the PP2A genes in *S. pombe*, and defined downstream signaling events that PP2A controlled.

I initially took on several projects in the lab but did not make significant progress on any of them. I did well in classes, but my bench work went so poorly that after nearly two years of frustration, I seriously considered leaving with a master's degree. It was a trying time and I only survived because of my religious faith and prayer, as well as the support of my future wife and my family. Those rocks helped me through a difficult couple of years.

Now that I am a principal investigator, I am amazed at the patience Dick had in allowing me to find myself by giving me the space to develop as a scientist and learn from my mistakes. Dick taught me to think critically, to set up an experiment with the proper controls, and then how to analyze the data. My fortunes changed when I stopped constantly doing experiments and started to think about what questions to ask. Thinking also required reading and multiple trips trudging over to Carnegie Library.

The more I read and thought, the clearer it became that while multiple aspects of PP2A signaling were being untangled, little was understood about the regulation and dynamics of the PP2A subunits themselves. It was known that PP2A was a heterotrimeric complex comprised of a catalytic, a regulatory, and a structural subunit. Additionally, it was known that multiple genes encode for each subunit; there are 2 catalytic and 2 regulatory subunits, but only 1 structural subunit in yeast.

While these genes had been identified, there were multiple unanswered questions about the subunits, such as, What is the ratio of each subunit? Does the ratio change during the cell cycle? Where do the subunits localize during the cell cycle? Are the subunits modified after translation, and if so, how does this change their function? Due to the multidisciplinary nature of the department and attending so many ecology and evolution seminars, I began to think of PP2A as different species of enzymes rather than one enzyme and began to investigate the population dynamics of PP2A.

A way to answer these questions began to crystallize when I read a review article by Roger Tsien in *Annual Review of Biochemistry* (1998) on the green fluorescent protein (GFP). Tsien elegantly explained the natural history of GFP; its primary, secondary, and tertiary structure; and its use in generating GFP-tagged fusion proteins. I realized that generating GFP-fusions of PP2A genes might allow me to study the population dynamics of PP2A, and once these ideas coalesced, everything started going better.

I stopped juggling too many projects and focused on answering good questions. I generated fusions of the PP2A subunits to GFP, the influenza hemagglutinin (HA) epitope, and to the c-myc epitope. I subsequently integrated the gene fusions into the yeast genome, so the fused proteins would be produced at normal cellular levels. (Later, I did postdoctoral work at UC-San Diego when Roger Tsien won the 2008 Nobel Prize for his work with GFP.)

Work hard at the bench and stay afraid without letting your fear disable you.

With the appropriate tools in hand, Dick and I set out to define the population dynamics of the five PP2A proteins during the cell cycle. While our game plan was well devised, getting the results was not always so easy. Yeast cell cycle experiments require synchronizing cells in culture, such that the stages of cell growth and division are occurring in all cells at the same time. The experiments would last 48 to 72 hours and I had to take time-points every 1 to 2 hours. I recall living and sleeping in the conference room in Lyman Hall during the 2000 World Series as my Mets played the Yankees, and having to follow the games on the radio.

Another difficulty that Haifeng, Wei, and I constantly worried about was competition with far larger labs; we were often concerned we would be scooped. This fear drove us to work harder and in hindsight, it was a healthy fear. We also struggled with more than just our science, because most graduate students in biology served as a teaching assistant every semester. I remember being a TA for Marvin Druger in his famous Biology 121-123: general biology; for Melody Sweet in biology of organisms, and Ernie Hemphill in microbiology. At the time, it seemed like a nuisance, and it did slow down the progress of research. However, it gave me a lot more experience in teaching and lecturing than many of my colleagues have today and was definitely beneficial.

LIFE LESSON #4

Be scientifically savvy and social at meetings, but also have good data to discuss.

In the end, we were successful in defining the PP2A population dynamics by determining the relative concentration and localization of each of the PP2A subunits during the cell cycle. To our surprise, the PP2A subunit concentrations differed by more than 10-fold (Figure 1A). We then sought to define the localization of each PP2A subunit during the cell cycle. We elegantly demonstrated that the PP2A regulatory subunits take the heterotrimer to different locations in the cell, and these locations change during the cell cycle (Figure 1B). These data resulted in a

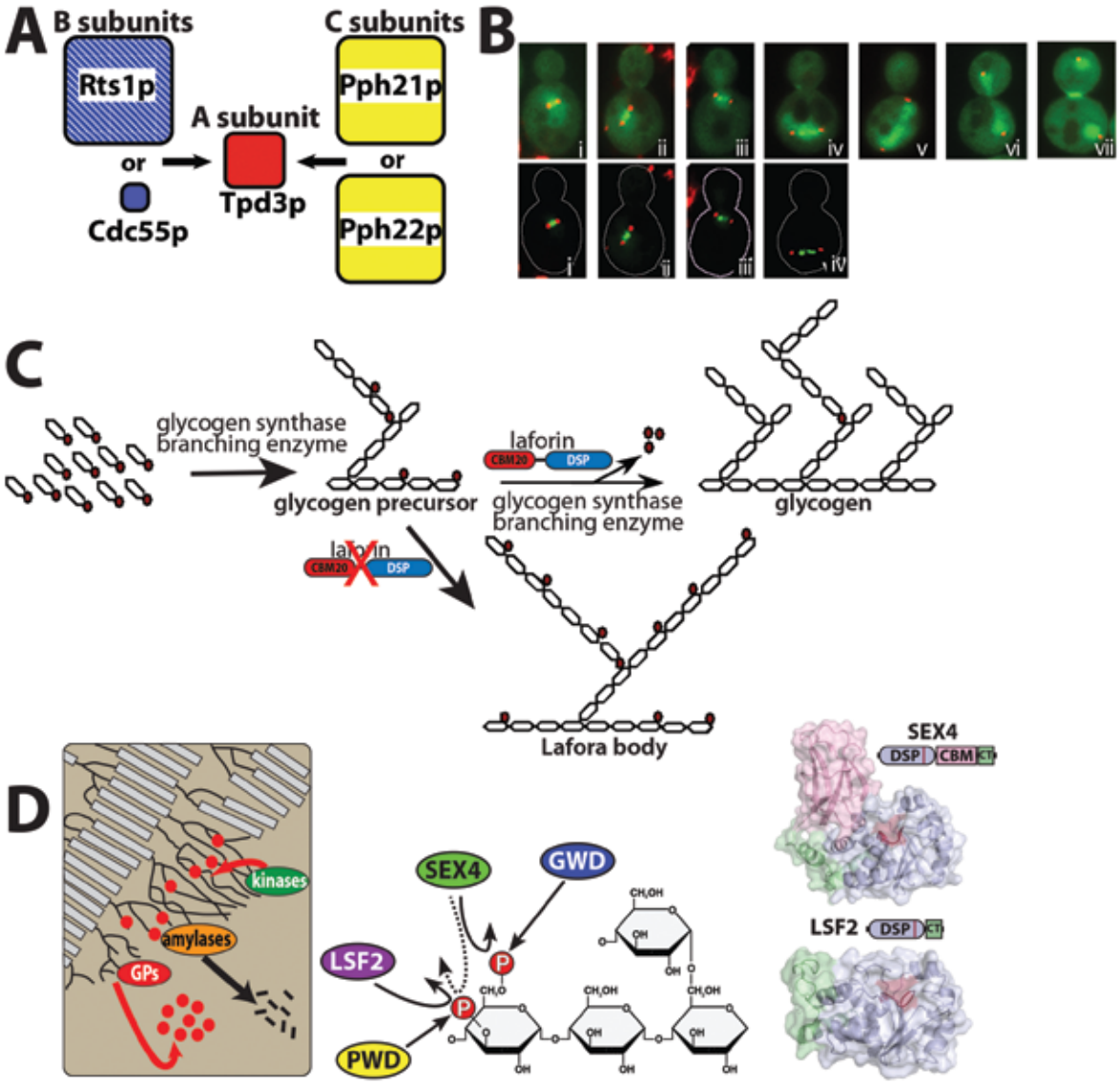

Figure 1: Summary of my scientific career. (A). Relative concentration of PP2A subunits. PP2A is a heterotrimeric protein complex comprised of one structural subunit (A), one regulatory subunit (B), and one catalytic subunit (C). The name of each protein is given inside or under the representative square. The size of each square is equal to the relative concentration of that subunit in the yeast *Saccharomyces cerevisiae.* **(B) Changes in PP2A localization throughout the cell division cycle.** We engineered a yeast strain to express GFP fused to the PP2A protein Rts1p and monitored its localization. Rts1-GFP is visualized in green and spindle pole bodies (SPBs) are visualized in red. Cells are displayed as they move through the cell cycle from S-phase (Panel i) through mitosis and the beginning of cytokinesis. Rts1-GFP is initially largely localized to the nucleus with intense staining between the SPBs (Panel i). As the SPBs move further apart, Rts1-GFP appears as two intense localization areas (Panel iv). Additional experiments demonstrated that Rts1-GFP was in a complex with other proteins at/near chromosomal centromeres. Panel v illustrates two SPBs within the mother cell and Rts1-GFP spanning the SPBs, while panel vi shows one SPB in the daughter cell and the other in the mother cell. Panel vii illustrates how Rts1-GFP localizes to the mother-bud neck before cytokineses while still maintaining localization within the nucleus. **(C) Model of Lafora body formation.** Glycogen synthesis involves the concerted efforts of glycogen synthase and branching enzyme covalently attaching glucose moieties. UDP-glucose is depicted on the far left as hexagons with phosphate (red circle). Glycogen contains a small amount of covalently linked phosphate that is present as both a phosphomonoester and phosphodiester (represented by red circles). Laforin dephosphorylates glycogen phosphomonoesters so that glycogen remains water-soluble. In the absence of laforin, glycogen phosphomonoesters accumulate and glycogen becomes both less branched and glucose chains become longer, eventually resulting in Lafora body formation. **(D) Starch degradation model.** Kinases phosphorylate (red circles) starch to facilitate starch solubility. Amylases bind and release glucose, but they cannot release glucose past the phosphate moieties. Glucan phosphatases (GPs) dephosphorylate starch so the process can repeat. Glucan Water Dikinase (GWD) phosphorylates the C6 position of glucose and this triggers Phosphoglucan Water Dikinase (PWD) to phosphorylate the C3 position. Once amylases act, Starch EXcess 4 (SEX4) preferentially dephosphorylates the C6 position and Like Sex Four 2 (LSF2) exclusively dephosphorylates the C3 position. We recently determined the crystal structures of SEX4 and LSF2, providing key insights into the molecular mechanism of this unique enzyme family.

publication in *Molecular Biology of the Cell* and opened numerous doors for the lab and myself.

After presenting my work at the annual American Society of Cell Biology meeting in Washington, D.C., I initiated conversations in person and via email with three other labs, and we began collaborations with investigators at Emory University, McGill University, and the Swiss Federal Institute of Technology (ETH) in Zurich. These collaborations were very fruitful, and I was able to visit each of these labs, including a 3-month stay in Yves Barral's lab in Zurich. These efforts resulted in papers published in *Developmental Cell, Eukaryotic Cell, Methods in Molecular Biology*, and *Journal of Virology*.

While science in Dick's lab was going great, all of our lives took a tragic hit when a tree limb fell on Liz and nearly killed her. It was a horrific accident and her body suffered enormous trauma. Dick rarely left Liz's bedside while she was in a coma, transported her to countless doctor visits, and helped her through many months of rehabilitation. Liz slowly rebounded but it was a difficult fight. (I returned to the biology department in 2012 to give a lecture and was delighted to learn Liz is still jogging, still loves her dog, and she and Dick are still inseparable.) While this was a terrible tragedy, it forced me to become independent and this independence was key for my postdoctoral years at UC-San Diego.

"While Dick (Hallberg) taught me how to do an experiment and to think critically, Jack (Dixon) taught me how to start a project and how to sell an idea."

I was at a phosphatase meeting in Snowmass, Colorado, during my next to last year in graduate school, when I first met and talked with Jack Dixon. I brought Jack over to my poster and went through it, knowing his lab was on a list of five I was interested in joining for postdoctoral work. I ended up interviewing in all the labs, and apparently my postdoctoral job talk was better than my first lab talk, because I was offered a position in each of the labs.

I chose Jack's lab just as he was in the process of moving from University of Michigan to UC-San Diego (UCSD). His lab at UCSD was almost a department by itself. He had senior scientists, postdocs, and graduate students working on 4 different human diseases, 3 different human pathogens, and 1 plant pathogen. The lab utilized multiple model organisms, with everything from mice to plants to algae to yeast growing in the lab. Whatever technique was needed we did, and we went where the science led us. Science moved a lot faster at UCSD than it did at SU, and it seemed what I could accomplish over a week at SU was done in 1 to 2 days at UCSD. A large part of this difference was that money was never a question, but also everyone in the lab was a really great scientist. Once again I felt overwhelmed; I considered leaving research to find a teaching position, but one great result changed my mind.

When I joined Jack's lab, I started working on dissecting the molecular mechanisms of Lafora disease (LD), an autosomal recessive neurodegenerative disorder. LD typically presents as a single epileptic seizure in the second decade of the patient's life and is then followed by progressive central nervous system degeneration, ending with the death of the patient within 10 years. LD is unique among epileptic disorders because of the neurodegeneration and also the accumulation of water-insoluble glucans (polymers of glucose) named Lafora bodies (LBs). Normal cells store glucose in the form of glycogen, but cells from LD patients make both glycogen and LBs. The LBs form in cells from nearly all tissue types, but cellular apoptosis is only observed in neurons.

Just before my joining Jack's lab, a team of gene hunters identified two genes that are mutated in patients with LD. One encodes a protein called laforin that was known to be a phosphatase, but the substrate was unknown. The second gene encodes the protein malin, but malin did not then have a known biochemical activity. We discovered malin is an E3 ubiquitin ligase that modulates the cellular levels of proteins involved in glycogen metabolism. Glycogen is constantly undergoing synthesis and degradation, and we demonstrated that malin controls the levels of enzymes involved in both processes. When malin is absent, cells aberrantly add glucose to glycogen, resulting in LB formation. Our results were published in *PNAS*, *The Journal of Biological Chemistry,* and *Genes & Development*.

Read, read, read the literature.... even the old stuff.

My second project involved determining the substrate of the phosphatase laforin, and this was more intractable. Laforin is a bi-modular protein with a carbohydrate binding module (CBM) at its amino terminus and a phosphatase domain at its carboxy terminus. After the discovery of its association with LD patients, Jack's lab demonstrated that the laforin CBM guided the enzyme to sites of glycogen synthesis, but it was still not known what laforin dephosphorylated. However, it was clear that LD revolves around glycogen metabolism: 1) LBs are aberrant glycogen bodies, 2) malin ubiquitinates are multiple proteins involved in glycogen metabolism, and 3) laforin contains a CBM that localizes it to glycogen. Additionally, Fischer and Krebs discovered in the 1950s that glycogen metabolism was regulated by reversible phosphorylation, making this one of the first pathways known to be regulated by phosphorylation. Subsequent findings in the 1960s and 1970s showed that multiple layers of glycogen metabolism are controlled by phosphorylation, and we hypothesized that laforin likely dephosphorylated one or more enzymes involved in glycogen metabolism. However, after nearly two years of a targeted search of glycogen metabolism enzymes as a laforin substrate, I had no good candidates.

I finally decided to step away from the bench and go back to what worked in the past: reading and thinking. I read everything I could find about glycogen and its sister macromolecule starch. Starch is the primary energy storage molecule in plants and algae, and like glycogen, is comprised of repeating units of glucose, i.e., glucose chains. However, glucose chains in starch are long enough that they form helices, which make starch water-insoluble, whereas glycogen is water-soluble.

This fact opened my eyes to the similarities between Lafora bodies (LBs) and starch; they are both water-insoluble glucose macromolecules. I eventually found a paper by a group that isolated and biochemically characterized LBs from human brains in the 1960s in which the writers reported LBs are, in fact, more like plant starch than human glycogen. Reading further, I learned plant physiologists more than five years previously had found two kinases that phosphorylate starch, and these kinases are essential for starch breakdown.

This was it. If there were starch kinases, then there had to be starch phosphatases; if there were starch phosphatases, then laforin was likely a glycogen phosphatase. After many years of experimentation in multiple model organisms, we have now demonstrated this is the case, and established a model of why loss of laforin leads to LD (Figure 1C). We published a paper in *The Journal of Biological Chemistry* describing laforin as the first glucan phosphatase and followed this up with a paper in *The Journal of Cell Biology* describing the first plant glucan phosphatase. We have subsequently discovered a second plant glucan phosphatase and published the first crystal structures of glucan phosphatases in *PNAS* and *The Plant Cell.* It has been a wild, crazy, sometimes scary, but fantastic ride linking a neurodegenerative disease with plant starch metabolism.

While Dick taught me how to do an experiment and to think critically, Jack taught me how to start a project and how to sell an idea. In Jack's lab, we had twice-monthly group meetings where a postdoc was expected to give organized polished talks, a skill I would need not only to present and write papers, but also to succeed in the art of grant writing. Jack taught his postdocs how to write grants by having us first organize the aims of a grant; then he edited our draft and returned it for further work. Jack would then put the final polish on the grant, in the process showing us how to crystallize and articulate key concepts. It also became clear to me that to be a successful scientist, I would need to learn the National Institute of Health's (NIH) granting system.

Learn to be a good salesman.

During my third postdoctorate year, NIH introduced a grant mechanism championed by Story Landis, director of the National Institute of Neurological Disorders and Stroke (NINDS), called the Pathway to Independence Grant, or K99/R00. This grant provides funding for 2 postdoctoral years, then transitions into 3 years of approximately $200,000 a year funding in an independent position. Another postdoc in Jack's lab, Fred Robinson, and I both applied for this grant. Together we learned the process of how to submit a grant, how to interact with NIH program officers, and how to get help from the university secretarial staff.

While at a Society for Neuroscience meeting, I had the chance to attend a seminar for postdocs by Landis. It was the end of a long day and I did not really want to hear another talk, but at the last minute I decided to go. To my surprise there were only a handful of postdocs there to hear this distinguished speaker. After she spoke, Landis asked if anyone had a question and no one raised a hand. She then scolded us for not asking questions, so I asked one about the K99/R00 mechanism.

After her talk, I nervously introduced myself and told her I had just applied for a K99. She looked at my nametag and said, "Oh, right, you had that interesting proposal about neurodegeneration and plants." She told me to email an NINDS program officer and talk with him about my proposal. I was stunned that she knew of my proposal and that she directed me to the person with whom I should discuss it. Surprisingly, Fred and I were both successful in obtaining a K99/R00, and while the science in my proposal was strong, I still believe meeting Landis helped.

Take advantage of opportunities and open as many doors as possible.

I have been at the University of Kentucky in the department of molecular and cellular biochemistry at the University of Kentucky's College of Medicine for 5 years and have made the transition from assistant professor to associate professor. I have been fortunate to get funding from NIH for the Lafora disease research, and I recently received an NSF CAREER award for our work on the plant glucan phosphatases (summarized in Figure 1D).

"My thinking today is still shaped by the holistic environment of the SU biology department, rather than just the narrow thinking of biochemistry or molecular biology, and I still draw on ideas and concepts from ecology and evolution."

My thinking today is still shaped by the holistic environment of the SU biology department, rather than just the narrow thinking of biochemistry or molecular biology, and I still draw on ideas and concepts from ecology and evolution. In my lab, we continue to work on elucidating the mechanisms of Lafora disease as well as deciphering the role of glucan phosphatases and kinases in starch metabolism. We utilize mouse models, cell culture models, algae, yeast, bacteria, and plants. Our basic premise is to generate hypotheses using bioinformatics and cell culture models, test these hypotheses *in vitro* using purified components, confirm these results using model organisms, and define the molecular mechanism of the enzymes using structural biology. While I rarely feel at ease or satisfied with what we have accomplished, I do feel that I am in a good position because of great mentors, my faith, good choices, and a great department.