

M.D.-Ph.D. Statement of Intent

When I took organic chemistry, I remember how much fun it was pushing arrows from nucleophiles to electrophiles and working through the logic of chemical syntheses. In my mind, there was a mechanistic beauty behind chemical reactions that I found both satisfying and intriguing. Chemistry was in a class by itself, and its charm lay in the congruity of its interlocking pieces.

But my focused appreciation of pure chemistry was to expand dramatically when I began working in research labs. Each lab I worked in exposed me to new and different ways to thread chemistry into unsolved problems in that particular field. I found that chemistry, though valuable in itself, had extrinsic worth in its many beneficial applications. Suddenly, I discovered that this subject which I enjoyed so much was in fact the universal language spoken by molecular biologists, microchip specialists, pharmaceutical designers, and antibiotics seekers.

In the summer of 1998, while working in Dr. Peter Schultz's bioorganic chemistry lab, I saw how creative organic chemistry could be used to modify the protein-manufacturing machinery of molecular biology. Schultz and co-workers had developed a method that was capable of expanding the traditional number of amino acids accessible by the genetic code. By designing a tRNA complementary to the UAG stop-codon, they had found a way to site-specifically incorporate unnatural amino acids into a growing peptide at the ribosome. Schultz's innovation opened the door for a host of *in vivo* protein structure/function studies not possible before. The project that I joined was one such structure/function study that sought to investigate the locomotion of the Shaker channel, a voltage-gated membrane ion channel whose mechanism of opening and closure was poorly understood. My role was the synthesis of an unnatural amino acid ultimately to be used in real-time fluorescence spectroscopy studies. I synthesized a keto-derivative of tyrosine and then acylated the dinucleotide pdCpA with this amino acid, a sequence that involved five steps in total. The added ketone moiety on tyrosine allowed for fluorophore-binding specificity in later spectroscopy experiments with the Shaker ion channel. These experiments were done later in the lab of our collaborator in biology, but the underlying tools that made them possible were crafted by the bioorganic chemists.

The applications of chemistry are far and wide. The San Francisco Bay Area, where I live, is flooded with biotechnology companies that use chemical tools regularly. But just south of the Bay Area is Silicon Valley, home of the computer/information technology revolution. I was eager to see how other fields such as the semiconductor industry utilized chemistry to solve problems. I had a chance to see this during my next research experience at IBM Almaden Research Labs in the summer of 1999. There, I discovered the crucial roles that polymer and organic chemistry play in the race towards computer chip miniaturization. As an NSF-funded summer intern with Dr. Richard DiPietro, my project centered on synthesizing and evaluating a family of novel cyclic olefin ester monomers and their corresponding polymers as potential photoresist materials for 193nm lithography (which means "writing with light"). The most exciting feature of this research project was the broad range of experimental techniques it involved. After preparing photoresist formulations of the materials synthesized, I had the opportunity to test their photolithographic performance in a cleanroom environment (with required bunny suit attire). To do this, the newly-synthesized photoresists were spin-coated onto silicon wafers which were then exposed to an incremental light exposure program using a 193nm Microstepper. One photoresist gave very encouraging results and was further studied in a follow-up imaging experiment to determine resolution capability. A beautiful 18,600X SEM photograph of the silicon wafer cross-section taken after development showed clearly resolved lines spaced 130nm apart. Indeed, writing with light at such high resolutions relies on the finely-tuned molecular properties at the level of the photoresist¹.

At each turn, my research experiences brought me in touch with areas that were using a "molecular mindset" to solve problems in their respective fields. In my next two research endeavors, I decided to shift gears and apply this mindset to research potentially beneficial to human health. From May to Sept. 2001, I was an intern with the medicinal chemistry group at Tularik, a biopharmaceutical company in South San Francisco. This company's objective is to create small molecule drugs that regulate gene expression. There, I worked with Dr. Sharon McKendry on a project focused on developing pharmaceuticals to treat hypercholesterolemia. Previously, the company had identified an important nuclear orphan receptor that plays a key role in down-regulating endogenous cholesterol breakdown. With

¹ Aggarwal, S., DiPietro, R., Allen, R. Synthesis and evaluation of novel monomers and polymers for 193nm lithography. Poster presentation given at IBM Almaden Research Labs. August 11, 1999. A technical paper was submitted as part of the NSF GOALI grant CHE9625628. See included abstract.

high-throughput screening of their vast chemical library, a promising antagonist to this receptor had been identified. My role in advancing the project was to assist in the preliminary structure/activity relationship studies by designing and synthesizing chemical analogues to the lead compound. This work entailed devising synthetic routes to compounds of interest based on literature and Beilstein searches. It also gave me the opportunity to think like medicinal chemist when considering what features of molecular structure would yield drugs with increased activity. During the internship, I attended weekly joint meetings with the medicinal chemistry and molecular biology groups in which we discussed new directions in light of potency assay results. At one of these meetings, a compound that I had synthesized was chosen to move from *in vitro* and whole cell assays to animal model experiments. Although the drug candidate turned out to give poor pharmacokinetics, the fact that it had been taken to that next level made me realize just how close and potentially impacting the chemistry we were doing was to human health².

I continued down this path of clinically-relevant research with my most recent research experience at the University of Hawai'i. The research that I conducted there under the guidance of Dr. Thomas Hemscheidt was my most independent and extensive work. As an NSF-funded intern under MarBEC³, I began a project dealing with antibiotics discovery. Given the widespread misuse and over-prescription of antibiotics by the medical community, new antibiotics that target novel features of cellular machinery are desperately needed to combat the rise of multi-drug resistant pathogens of clinical concern. The goal of the project was to develop a new strategy to screen for antibiotics from marine bacteria sources. It has long been assumed that marine organisms produce antibiotic substances in response to limited nutrients. Most conventional screening programs utilize cultures of a single organism and assess antibiotic production in stationary phase, i.e. when the number of cells is no longer increasing. In this project, we pursued the idea that antibiotic production might be induced if at least two bacteria are cultured together in a single vessel. Using these conditions, we intended to mimic the competition for space and nutrients that occurs in bacterial communities residing in biofilms on the surfaces of marine seaweed and algae. Of particular interest were those marine bacteria that showed no activity when cultured singly and induced antibiotic activity when cultured in the presence of a competitor. To search for such pairings, a novel co-cultured matrix screening technique was employed with sixteen unidentified bacterial species isolated from seaweed and algae samples collected from the shores of O'ahu. Using this technique coupled with a well-diffusion antibiotic screening assay, three pairings of interest were identified. Various follow-up experiments were conducted with these bacterial pairings to learn more about this phenomenon of induced antibiotic production. At the end of my work there, what emerged was a proof-of-principle—demonstration that this co-culturing methodology could be expanded and fine-tuned to aid in the search for new antibiotics.^{4,5}

With this diverse research experience to draw from, I feel better equipped to work towards finding solutions to long-standing problems in the medical sciences. The interface of chemistry and neuroscience presents a wide variety of such problems. In particular, my studies in philosophy have led me to marvel at the human capacity for performing higher-order brain functions such as learning and memory. What is the exact nature of cell-cell signaling that underlies these remarkable phenomena? Because scientists can only probe as far deep as the tools at their disposal allow, what sorts of chemical tools can be developed to probe the brain and nervous system to help find answers to these kinds of questions? I believe that research oriented along these lines will turn out to be greatly beneficial for the study of neurological diseases, as well as for the design and development of pharmaceuticals to treat these diseases. Recently, I have begun working in Dr. Pam England's chemical neurobiology lab at UC San Francisco to learn more about how to confront research problems in the area of learning and memory.

To really begin to unravel the vastly complex workings of the human brain, I feel that the problem must be approached from two sides: both macroscopically and microscopically. With M.D. training comes the macroscopic view: an understanding of where the brain fits in with the rest of the human body and the

² Medicinal chemistry internship final presentation. September 8, 2000. Tularik, Inc.

³ Marine Bioproducts Engineering Research Center, an NSF-funded collaboration between academia and industry

⁴ Aggarwal, S. and Hemscheidt, T. Elicitation of antibioticly active secondary metabolites from co-cultured marine bacteria. Presentation given at MarBEC Summer Undergraduate Research Fellowship Symposium. August 2, 2001. Paper submitted for publication in upcoming *Proceedings from the MSURF 2001 Final Symposium*. Future publications are anticipated. See included abstract.

⁵ Aggarwal, S. and Hemscheidt, T. Elicitation of antibiotics. Presentation given at the Marine Bioproduct Engineering Research Center Industrial Advisory Board Meeting. August 6-7, 2001.

opportunity to work with patients to better grasp disease phenotypes. Likewise, with Ph.D. training comes the microscopic view: a comprehension of disease mechanism and “hard-wiring” on cellular and molecular levels. The combined M.D./Ph.D. course of study would give me the ability to simultaneously approach the problem from these two complementary angles and make the connections hopefully leading to the betterment of human health. For, in the end, behind the “Abstract” and “Materials and Methods” sections is hidden the human being—someone that I do not want to lose sight of.

Thank you for considering my application,
Sunil Aggarwal