## ESSAY #3: "Nitrogen"

### Summary of Current Research

My current work with Dr. Raganasik is on the activation of nitrogen regulated promoters. Earlier work in this laboratory has shown that transcription of the *glnALG* operon of *E. coli,* coding for glutamine synthetase and two nitrogen regulatory proteins, is initiated at *glnAp2* by $\sigma^{54}$-RNA polymerase. Under nitrogen-limiting conditions the regulatory protein $NR_I$ is phosphorylated by $NR_{II}$. $NR_I$-phosphate ($NR_I$-P) binds to two sites, located 100 and 130 bp upstream from the transcription start site, and catalyzes the isomerization of the closed $\sigma^{54}$-RNA polymerase-*glnAp2* complex to the open complex. We have now shown that $NR_I$-P cannot activate a $\sigma^{70}$ promoter, like the *lac* promoter, containing $NIR_I$-binding sites but can activate other $\sigma^{54}$ promoters like *nifHp.* These results suggested that the activation by $NR_I$-P is not solely dependent on the presence of the $NR_I$-binding sites but also on the nucleotide sequence of the downstream promoter region. In collaboration with Dr. Vona White, we studied the effect of phosphorylation of $NR_I$ on its DNA binding properties. Our results showed that phosphorylation greatly increases cooperative binding of the activator to two adjacent binding sites, and that the interaction between two $NR_I$-P dimers is required for activation.

Furthermore, I am studying the activation of *glnHp2,* also a nitrogen-regulated promoter, of the glutamine permease operon of *E. coli.* I have shown that open complex formation at *glnHp2* is activated by $NR_I$-P, and that the activation is stimulated by integration host factor (IHF), a DNA-bending protein. IHF binds to a site between the $NR_I$-binding sites and the promoter. A single nucleotide mutation in *glnHp2* (T to G at position -14) increases the affinity of $\sigma^{54}$-RNA polymerase for the promoter. Using an *in vitro* transcription assay, I showed that the stimulation by IHF takes place only in promoters that form weak closed complexes with RNA polymerase. Activation of the mutant promoter is not stimulated by IHF. On the other hand, when the binding sites for $NR_I$ or IHF were placed on the opposite side of the DNA helix, IHF inhibited the open complex formation. We conclude from these results that the stiffening produced by the IHF-induced bend can facilitate or prevent interactions between the upstream bound $NR_I$-P and the closed RNA polymerase-promoter complex, depending on the relative location of the binding sites for these proteins. In this manner a regulatory protein, IHF, can stimulate or block transcription without itself contacting either the RNA polymerase or the activator.

Recently, we have found that $\sigma^{54}$ binds specifically to the *glnHp2* promoter in the absence of the core RNA polymerase subunits. In collaboration with Weslia Carson and Malcolm Blick (AFRC Nitrogen Fixation Laboratory, University of Sussex, England), we have demonstrated a new role for core RNA polymerase in transcription: that it assists the binding of $\sigma^{54}$ to promoter DNA. An altered form of $\sigma^{54}$ with a deletion within the N terminus showed increased DNA binding properties. Our results suggest $\sigma^{54}$ has a latent DNA binding activity which is revealed by core RNA polymerase.

Graduate applications may require a statement of purpose, an autobiography, an essay or two or six on assigned topics, a summary of research, or all of the above. All these writing assignments are slightly different, and you should be sensitive to the instructions. Remember, **RTGDQ.** This two-part essay is in response to the assignment: "Describe your research experience to date."

Research summaries are a common part of laboratory science applications. The focus should be on the science, not the scientist. Presenting your research in a clean, impersonal format—as this candidate did—is the best approach.

From: Asher, D. Graduate Admissions Essays 3rd edition, 2008, Crown Publishing, New York.

## #3 "Nitrogen" *(continued)*

Be sure to name your advisers, as it is bad form to imply that you did high-level work all by yourself, without any advice or counsel from a senior scientist.

### Summary of Previous Research

As a postdoctoral associate in the laboratory of Dr. Kirschner, I was involved in two research projects. One project was to characterize the *ams* gene, which is involved in decay of *Escherichia coli* mRNA. I cloned the *ams* gene by complementation, mapped the transcription start site, and determined the size and the N terminus sequence of the *ams* protein. We used a T7 RNA polymerase-promoter system to overexpress the *ams* locus. We determined the nucleotide sequence of the *ams* gene, and these data showed that the C terminus of the protein has homology to a mitochondrial ribosomal protein of *Neurospora crassa*. More recently, it has been shown that *ams* encodes RNase E, an endoribonuclease that plays a general role in the chemical decay of *E. coli* mRNA. The other project was the characterization and secretion of protease III (*ptr*) of *E. coli*. I mapped and sequenced the *ptr* promoter and determined the N terminus sequence of protease III. This N terminus contains a signal sequence which is needed for secretion of protease III to the periplasm. Using the cloned *ptr* gene and protease III-alkaline phosphatase fusions, we found evidence that protease III is also secreted to the growth medium.

In my Ph.D. work, I studied the chemistry and ultrastructure of the spore wall of *Aspergillus nidulans* conidia, and the effect of cell wall components in the uptake of chemicals. I used electron microscopy to study the ultrastructure of the conidial wall and found that it is composed of several layers. The outer layer is covered by regularly arranged fibers (rodlets) which can be removed by sonication. Chemical analysis of purified conidial walls from a wild-type strain showed the presence of neutral sugars (glucose, galactose and mannose), protein, chitin, melanin and small amounts of lipid. Chemical fractionation experiments showed the presence of alpha-1,3-glucan in the electron-dense outer layer. Conidial walls of a white mutant strain lacked melanin and alpha-1,3-glucan. I also purified the rodlets, which contain protein and melanin in equal amounts and some carbohydrate. Histidine, aspartic acid, glutamic acid, glycine, and alanine are the most prominent amino acids of the rodlet protein. The presence of melanin and possible cross-linkages between it and amino acids like aspartic acid, glutamic acid and glycine makes the rodlets resistant to proteases. I also studied the effect of conidial wall components on the uptake of sugar analogs and amino acids by conidia. Conidia were first treated with sonication to remove the rodlet layer, and then with proteases or glucanases. The removal of glucan and protein from the outer layer of the conidial wall allowed an increased uptake of 2-deoxy-D-glucose, 3-O-methyl-glucose and L-alanine. These results indicate that certain components of the conidial wall act as a barrier to penetration of chemicals.

## ESSAY #17: "Tobacco Mosaic Virus and the Eiffel Tower"

"What is the most important difference between tobacco mosaic virus and the Eiffel Tower?" my professor asked on the last day of my introductory biochemistry class, as he put two slides of the structures up on the screen. "Both are made of precise building blocks which elegantly come together to form the whole unit," he explained, "but only the virus knows how to put itself together." At this point, I had a *Eureka!* response. I truly recognized the beauty and complexity of life at the molecular level. That's when I first knew that I wanted to undertake biomedical research.

Since then, my decision to pursue graduate study has been confirmed by both my undergraduate course work and my research experience. While studying immunology in my sophomore year, I learned for the first time not only the facts about the workings of the immune system, but also the ideas and experiments that led to their discovery. As I became exposed to the experimental side of the information found in the textbook, I began to appreciate the sophisticated thought processes and energy required by scientific research.

The most influential experience in persuading me to attend graduate school, however, has been my current independent research project, which will culminate in an honors thesis. I am examining the antigenicity of a protein in a novel drug delivery system. (Please see the accompanying research summary.) I am eager to bring the concepts I have learned in my project to the level of a graduate program of study. First, I discovered how the power of perseverance can overcome obstacles. When my experimental system, the ELISA, suddenly stopped working, careful troubleshooting led to the discovery of a minor technical problem. Through this experience, I learned how to critically dissect an experiment to find the root of error. In addition, the graduate student with whom I have been working for almost two years has taught me the ability to take an idea and follow it while at the same time demonstrating to me the balancing act involved in allocating time, money, and energy to a project when the direction your results will take you is unknown. My research sponsor, with her contagious energy, has also influenced me with her enthusiastic approach to attacking new research areas, and has motivated me to work harder to reach my goals and the goals of the lab.

The pathobiology graduate program at the College of Physicians and Surgeons of Columbia University is of interest to me for several reasons. First, the affiliation of the University with Presbyterian Hospital, Milstein Hospital, the Institute for Cancer Research and the Institute for Human Nutrition provides students with the opportunity to combine basic scientific studies with clinical applications. The resources available at the hospitals and centers aid students in immediately applying what they learn in the classroom and laboratory to situations where disease demands immediate attention. In addition, the location of the college in Manhattan is attractive because of its proximity to other research institutions and medical schools. Such a dynamic group of scientists provides many opportunities for the exchange of fresh ideas and collaborative efforts. Finally, the range of research conducted by the faculty is appealing. The studies of Nicole Sucio-Foca are of particular interest to me

Here is a superb example of using an experiential opening for a science-oriented essay.

There are many career paths and research opportunities related to medicine, pharmaceuticals, and biotechnology that do not involve an M.D. degree. This Ph.D. candidate in medical pathobiology will have many exciting opportunities to contribute to medical science, without suffering through a few years of 80- and 100-hour sleepless clinical internships.

Trace the history of your interest in the topic.

This is a model answer to the question "Why here?" Every program wants an answer to this question, whether they explicitly ask it or not.

## #17: "Tobacco Mosaic Virus and the Eiffel Tower" *(continued)*

because they involve the creation of peptide vaccines, an area of immunological research which has much potential for the treatment or prevention of many diseases.

Once in graduate school, I hope to pursue studies related to the development of vaccines. My interest in this topic stems not only from my course work specific to immunology but also from an additional academic experience in the course, "The Burden of Disease in Developing Nations." In this class I learned that although vaccines are currently available to treat a myriad of diseases, some of these vaccines are useless to people in the developing world because they degrade under the conditions of high temperature or humidity which are often found in these countries. Multiple lines of research can thus address both the development of new vaccines and the improvement of currently existing vaccines so that they may be useful to the greatest number of people.

In trying to create new vaccines for diseases for which they are currently not available, several approaches from immunology, biochemistry, molecular genetics, and organic chemistry can be considered. For example, an understanding of whether a humoral or cell-mediated immune response is best suited to fight a particular disease is needed. Immunologic techniques involving animal models and cell culture studies can be used to determine how B and T cells interact to fight disease. Furthermore, specific pathogenic macromolecules can be used as the antigen in a vaccine rather than an entire protein. This method requires the isolation and purification of protein subunits using biochemical assays such as gel electrophoresis, column chromatography, and protein sequencing. In addition, the gene encoding an antigen can also be used to develop a vaccine. Recombinant DNA techniques such as screening of genomic cDNA libraries, gene sequencing, and the polymerase chain reaction can be use to isolate, characterize, and amplify a specific gene. Finally, specific protein antigens can be chemically synthesized. This method requires not only a rigorous use of synthesis design from organic chemistry, but also principles from biochemistry to determine protein sequence and folding, as the conformation of a protein domain and not just its amino acid sequence is often recognized by antigen presenting cells. Thus, x-ray crystallography and FTIR must be employed. All of these lines of research can lead to the development of new vaccines.

After graduate school, I will consider a career in the pharmaceutical industry. The ability to see an idea about a molecular process evolve into a product which will help make people's lives healthier is my motivation for this choice. However, I am also considering a career in academia because I am interested in the possibility of combining research with teaching and interacting with undergraduates. I am currently tutoring genetics students and have previously tutored organic chemistry students, and the one-on-one interaction has enabled me to teach and learn at the same time. Through my involvement in Women in Science and Engineering as a biochemistry affinity group leader, I have been able to advise students about the selection of courses, summer jobs, and potential professors with whom to do an independent study. The teaching experience which has proven to be the most challenging is serving as a ninth-grade religious education instructor for the past three years. I have

## #17: "Tobacco Mosaic Virus and the Eiffel Tower" *(continued)*

prepared my own lessons and led discussions with a group of twenty, sometimes less than enthusiastic, fourteen-year-olds. Trying to capture their attention has forced me to be creative in my style and presentation of material. Thus, my involvement with students may persuade me to enter the academic research world.

My past experiences have well prepared me to pursue graduate education at the College of Physicians and Surgeons. My undergraduate education in the competitive atmosphere at Brown has enabled me to not merely reiterate ideas stated by my professors but to apply the concepts I have learned to unfamiliar situations. During my four years here both my study skills and my ability to process information have sharpened, as evidenced by an improvement in my grades within my major from a 3.0 grade point average freshman year to a 3.6 junior year. The lack of self-confidence which plagued me during my first two years here was induced by both insufficient study skills and an unusually rigorous course load, wherein I completed my inorganic and organic chemistry courses in three semesters rather than four and took physical chemistry, a course usually reserved for upperclassmen, my sophomore year. In addition, my interactions with people within the Brown community outside the classroom have prepared me for the intellectual atmosphere at Columbia. The need to write and speak effectively on issues of importance, whether it involves a change in the housing policy or creating a new concentration, are requisite to enact positive change. One initiative which I undertook was the creation of a Web site for Women in Science and Engineering, to help create better communication among women scientists both at Brown and at other universities. Therefore, by combining my diverse undergraduate experiences, I will be able to grow as a researcher in your pathobiology graduate program while contributing my ideas about both the research interests of my colleagues and issues facing the Columbia community.

When your grades have markedly improved, always point that out. Readers may look at your GPA without noting your GPA *trend*. Help them see what you want them to see.

Service to your academic community, and the other "communities" to which you belong, is almost always regarded as a positive sign of character and maturity.

## ESSAY #19: "Iditarod Dogs and Molecular Biology"

### Statement of Purpose

They call it the last frontier. Last summer I set out for Alaska, to see the true wildness left in this world. This spirit of adventure took me to Homer on the Kenai peninsula, where I became the "dog handler" for Iditarod musher Jack Berry and his sixty huskies. Although I came to Alaska to live among the wild, I found myself spending all my free time teaching English pronunciation to a Brazilian doctor and arguing plant physiology with the old women of the Homer Garden Club, when I wasn't hitchhiking the fifteen miles to the Homer Public Library. For better or for worse I'm obsessed with learning, and I want to take my pursuit of knowledge to a far greater scale.

Theoretical physicists are in pursuit of the grand unifying theory, the set of equations that will make compatible all of this world's macro- and microcosms. As I see it, there is a similar grand objective in the world of biology. I feel a drive to elucidate the mechanisms of life through molecular studies. There are ways, paths, lines of thinking that converge the realm of the biological with the domain of chemical logic. I know that a solid understanding of the physical function of proteins can be that unifying link.

Now it is the rainy winter of my fourth and final year at Reed College. I have been an enthusiastic biochemistry and molecular biology major enrolled in what is possibly the best program of its kind. This past spring I worked independently on a project to determine the preferred conformation of dehydrated isosorbide. While this was interesting in its own right, I think that the knowledge obtained through studies of organic chemistry is most relevant when applied toward macromolecules. Aside from being fascinating structures, they have a significance reaching far beyond the laboratory. I've chosen the topic of my undergraduate thesis with these greater interests in mind. For this thesis I am pipetting toward a crystal structure of xylose isomerase that contains a single active site mutation. I find it absolutely amazing that proteins can catalyze reactions and am obsessed with the relationship between their function and structure. Enzymes catalyze reactions, but an amino acid polymer is also capable of much more. Motor proteins, G-proteins, the amalgamations in the SNARE hypothesis—cells have created proteins for an intense diversity of uses. I am lucky to be a structural biologist at a time when the techniques necessary to decipher the form of these proteins are uncovered. I am intrigued by the functional structure of proteins, and value any laboratory method that can provide molecular insight. I chose to apply to Scripps because I have been uncommonly impressed by the structural research I've seen published by Scripps researchers. Orton Gilula's and Nigel Unwin's investigations of the structure and functional mechanisms of gap-junction and ion channels are especially intriguing. I find ion channels to be wondrous edifices. Ion channels are contraptions straight out of a Dr. Seuss story book: one massive protein that chooses to allow specific ions through it, if and only if it is satisfied with the chemical and electrical environment surrounding it. This, truly, is a level of chemistry where biological decisions are made.

Notice how this graduate-school candidate uses personal stories and a genuine enthusiasm to package his approach to science. This applicant also told me that he wore a Hawaiian shirt to his interviews, to stand out from the crowd in the minds of interviewers who may meet with a dozen candidates in a day. These techniques worked for him, but only because they matched his effusive personality.

Notice the answer to "Why here?" and the mention of specific researchers of interest to this candidate. Customize your essays, or lose out to the candidates like this one, who do.

## #19: "Iditarod Dogs and Molecular Biology" *(continued)*

I could drone on for pages about the research that I find fascinating, as Scripps has a collection of amazing resources. I would love the opportunity to work in a laboratory with this talent. I am enrolling in graduate school to learn more and to understand greater biological systems so that I will be able to apply my molecular knowledge to my own research. I'm fascinated by the biology of the cell; with a thorough understanding of the techniques available to the protein scientist, I will finally possess the ability to address the basic hows and whys of cell function.

Two years ago I spent a semester abroad with the School for Field Studies in the Pacific island nation of Palau. With every mangrove and coral reef transect we took, I wanted to know: "Why do these angel fish live here? How can these trees grow out of the salt sea?" The only answers my professors could give: "Because the fish do best in this biotic environment . . . because they've evolved and adapted for longer than you can imagine." The answers available are just not satisfactory, but I know that with more training I could find those answers for myself. I want answers with a mechanism: answers that resemble not statistical spreadsheets, rather blueprints of ingenious design; answers that might detail how membrane proteins balance harsh extra-cellular conditions with a cytoplasm that is conducive to life. These are answers I need to find, and I'm too stubborn to quit now. I need to go to graduate school, for I've only just learned the principles of protein structure and function. I want to be an expert. The MCSC Program holds the resources that can enable me to continue my quest.

## ESSAY #25: "Where Drive and Talent Can Take You—Biomedical Engineering"

### Molecular Biophysics Essay

This essay was written by the same person as the last one. Although there is a modicum of overlap, notice how different this presentation is from the prior example.

Ever since I was young, I have wanted to understand not just how the body solved complex engineering problems, but also how these mechanisms worked at a fundamental level. In particular, I am interested in applying computer and experimental techniques to biological problems (and vice versa). Of particular interest is the use of computational tools in analyzing issues relating to sequence analysis/protein engineering, protein folding (and the reverse problem).

I think this is why I find the pursuit of a Ph.D. almost a necessity, given my need to satiate my mind's curiosity for seeking deeper knowledge in this area. In fact, I think this methodology has been reflected throughout my life, as I have always strived to challenge myself to find answers and overcome various obstacles. When I arrived in the United States, I knew only one word in English (the word "no"). Yet, I was determined to work hard in order to catch up. Graduating valedictorian of my class in high school and serving as co-president of Science Olympiad proved that it could be done.

I went on to Carnegie Mellon University, not just because of the merit-based scholarships, but also because I wanted to gain deeper understanding of computer techniques and their applications in biology/medicine. While I majored in electrical and computer engineering with a minor in biomedical engineering, I took as many relevant biology and chemistry courses as I could within the curriculum, including advanced courses like 03-510 (Computational Biology) and 42-680 (Bioinstrumentation). This spring, I am scheduled to take a biochemistry and second organic chemistry class. I expect to have the necessary prerequisites for advanced biochemistry study by graduation.

I am confident that I will succeed in advanced courses given my background. I also feel that I have some rather unique advantages as a non-biology major. Having a heterogeneous entering class composed not only of biology/biochemistry majors allows for more diversity in thought processes. Having undergone a similar curriculum with similar experiences, biology majors will likely have similar perspectives when encountering a novel problem. In contrast, an individual like myself may have a unique, novel contribution. Many of the breakthroughs in the past have come from interdisciplinary study resulting in a link between two previously unknown areas which went on to serve as the basis for an invention (and a new area for research). Two recent examples include the invention of DNA-based computers and biochips. In each case, a biologist or an electrical/computer engineer working alone could not have carried the project to fruition.

I have always strived to complement my education/experiences in computational approaches with fundamental biology. I have gained experience with biology both at the molecular level and at the systems level which allows me to see the big picture. When chosen by the Ohio governor to represent the state, I went to the Lawrence Berkeley Laboratory for a program on "Life Sciences and Biotechnology." Here, I attended both lectures and

## #25: "Biomedical Engineering" *(continued)*

Name your advisers.

did lab work involving biotechnology. Professors included Dr. Marian Diamond, Nobel Prize winner Dr. Glenn Seaborg, Dr. Sylvia Spenger (on the Human Genome Project), Dr. Mina Bissell, and Dr. Jeff O'Neil (Calgene). At a summer course at Case Western Reserve University entitled "Biotechnology and Genetic Engineering," I gained additional knowledge in experimental techniques.

Make a laundry list of laboratory skills. Don't assume your reader will know what you can do.

From the aforementioned programs, I have gained experience with such biological techniques as electrophoresis, Southern blots, transformations, recombinant DNA techniques, PCR, chromatography, and sequence analysis. Through class/lab work and various programs, I have become knowledgeable in computer techniques used in biology such as coding region identification (via base composition, codon bias/preference, etc.), BLAST/FASTA algorithms for sequence match scoring methods, multiple sequence alignment, general similarity and homology methods (e.g., dynamic programming, dot-matrix methods, usage of hashing), secondary structure prediction (methods like Chou-Fasman, Garnier-Osguthorpe-Robson, SOPMA, etc.), and hydophobicity analysis.

In terms of bioinstrumentation, I have learned about NMR, IR, and techniques for novel instrumentation design. This past summer, I worked on a signal processing project in the Tachycardia Research group of St. Jude Medical's Pacesetter (a company formerly owned by Siemens). I designed and built a stimulus waveform generator. Then, I initiated a research study with the group's manager involving pain thresholds (in over 30 individuals) and co-authored "Sural Nerve Sensory Thresholds of Defibrillation Waveforms" which has been submitted and accepted. It will be presented at the next American College of Cardiology Scientific Session and published in the organization's peer-reviewed *Journal of the American College of Cardiology.*

Trace the history of your interest. This applicant is able to trace this particular idea back to freshman year.

One example where I applied computational/engineering techniques to biology (as I hope to do in my career) goes back to the first semester of my freshman year. I independently wrote my own grant proposal based on an idea I thought of while at the Lawrence Berkeley Lab which involved application of neural networks to the classification of DNA fingerprints. When my proposal won a SURG grant, I carried out the project independently under mentor Prof. Jose Moura, editor-in-chief of *IEEE Transactions on Signal Processing.* This endeavor, combined with academic achievements, resulted in my recognition via honorable mention as part of the all-USA College Academic Team competition (published in *USA Today.*) Only one Carnegie Mellon student is known to have ever been recognized in this competition. Since it involved novel coding techniques deployed on a PC and supercomputing environment, the work also led to an article (which I wrote) in the "Cross Platform Strategies" section in five magazines in 82 countries.

For the past several semesters, I have been working on a research project on the "system level" at the University of Pittsburgh Medical Center. It involves analysis of EEG signals from brain waves and design of a barbiturate drug infusion system with Dr. Marc Bloom, the director of neuroanesthesia at the University of Pittsburgh's medical center. The results

## #25: "Biomedical Engineering" *(continued)*

of my project are rather exciting. The control system was tested on a live rhesus monkey and later revised. We are currently discovering and classifying relations between various patient variables and the sedative state and already have some interesting correlations. As we approach the next phase, this information will be combined "to establish an entirely new approach to patient modeling and the use of control in bioengineering systems" (as originally stated in the NSF grant from a group of three professors which I helped to form last semester).

I believe I can make a significant contribution to the current literature during graduate school and eventually lead efforts in innovation upon graduating from the University of Pennsylvania. I think the result from my experience at Motorola's Speech Technology Laboratory is an example of such an endeavor. Not only did I put forth a new idea, I also implemented it in a prototype and wrote the first draft of the patent (which I presented to the other co-inventors for input). The patent was submitted to the patent office in July after approval by the Motorola review committee.

I also have experience in writing, a skill which is obviously imperative for researchers who wish to communicate their findings with others. As a writer for several newspapers, I have had the chance to interview people such as Ohio's governor, a U.S. Congressman, a CEO (FORE Systems), three current and past presidents of Carnegie Mellon, and a managing editor from *U.S. News & World Report.*

I have found several faculty that share my interests at the University of Pennsylvania. In Prof. Lewis's lab, I am interested in the work going on related to computational methods used for studying protein-nucleic acid complexes. Papers from Prof. Sharp's research in structure of protein at the molecular level (using computational techniques as an option) also piqued my interest. I see a possible match with Prof. Wroblewski's work involving image processing and protein structure. I am open to working in other labs involving a combination of computational and experimental techniques in biochemistry as well.

Address the question, "Why here?" This candidate wrote different, and successful, applications to Caltech, Stanford, MIT, Berkeley, and others. Customize, customize, customize! Of course, it helps to be as accomplished as this scholar.