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Dear Search Committee,

Enclosed please find my application materials for an Assistant Professor position in the Department of Biology and the Biocomplexity Institute at Indiana University. As you can see from my research plan, I have initiated a highly creative and topical research project in the laboratory of James Broach at Princeton, based on the engineering of G protein-coupled receptors (GPCRs). My project is directed at investigating the fundamental mechanisms of membrane receptor structure/function, using genetic tools that extend to the fields of biomolecular engineering, 'synthetic biology' and computational biology. This is an innovative project with abundant opportunities for collaboration across the many areas of biology relating to GPCR signaling.

My work in the Broach lab grew out of an interest in signal transduction developed during my Ph.D. work with Robert Deschenes at the University of Iowa. At Iowa I developed biochemical assays for characterization of histidine kinase signaling in yeast. Among my accomplishments were biochemically characterizing the phosphoryl transfer pathway from the yeast histidine kinase to its downstream targets, biochemical analysis of an activated histidine kinase mutant, and characterization of chimeric histidine kinase modules to test a model for the regulatory mechanism of histidine kinase signaling. My assays were also utilized to test lead compounds for antimicrobials that were isolated in screens targeting histidine kinase activity.

In the Deschenes lab I developed an interest in receptor engineering, reasoning that a system for rapidly engineering cellular receptors could be useful, and that developing such a system would teach us much about how receptors function. My work in the Broach lab has allowed me to pursue these interests and to lay the groundwork for a successful research program.

Thank you for your consideration,

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Engineering chemical switches based on G protein-coupled receptors.

G protein-coupled receptors (GPCRs) occupy an esteemed place in the life sciences. They are encoded by the largest family of genes in the genome, comprising ~3% of all genes, and they are disproportionately well represented as drug targets, with nearly half of all drugs on the market targeting GPCRs [1]. The goal of my research is to engineer chemical switches based on GPCRs, applying tools created in the pharmaceutical industry for high-throughput screening of receptors. My research entails exploring structure-activity relationships (SARs) between ligands and GPCRs, which will generate valuable data to aid drug development. It also involves developing computational and experimental methodologies for rapidly engineering receptors with desirable properties, which will lead to new cell-based chemical sensing technologies.

My proposed research involves three specific aims:
1. Create new chemical sensors based on GPCR arrays.
2. Analyze ligand/receptor interfaces.
3. Test computational tools for transmembrane protein design.

Background and current studies

Numerous tools have been developed for functional analysis of GPCRs, due to the ongoing interest in drugs that target this class of receptors. A yeast-based system for heterologous expression of GPCRs has been developed that offers a convenient, robust system for screening receptor activity [2]. This system is optimized for 'real world' high throughput screening, with receptor signaling coupled to growth and colorimetric outputs. Over sixty human GPCRs have been functionally expressed in this system to date. The yeast system offers critical advantages over cell culture-based methods with respect to screening receptor mutants. It is possible to construct and express libraries of mutant receptors directly in yeast. Moreover, yeast cells grow faster, are less susceptible to contamination, and are more genetically stable than cell cultures.

Olfaction and Receptor Engineering

My research in receptor engineering has grown out of a broader effort in the Broach lab to study olfaction by characterizing olfactory receptors (ORs) in yeast. It is generally accepted that olfactory perception functions via a combinatorial mechanism in which individual chemical ligands stimulate different sets of ORs [3]. We had anticipated that ORs expressed in yeast could be used as chemical sensors, but I hypothesized that it might also be possible to modify ordinary chemosensitive GPCRs to function in sensory arrays. In parallel with efforts to functionally express ORs, I initiated a 'proof of principle' project in which I generated mutants of a non-olfactory chemical receptor that can be utilized together as a rudimentary chemical sensor [5]. Engineering non-olfactory chemical receptors to have novel signaling properties serves as a means to learn about fundamental mechanisms of chemical sensing by GPCRs and offers an opportunity to test models of olfaction.

To engineer GPCRs I utilized a directed evolution (DE) approach. DE simply refers to the practice of using successive rounds of mutagenesis and selection to isolate mutant proteins with desirable properties. My experimental strategy is predicated on a model for protein engineering in which there is a synergistic relationship between structure-based design and screening techniques. Even in cases in which it is not feasible to design receptors using structural/computational models alone, efficient screening tools make it possible to screen libraries that have enough diversity to overcome weaknesses in available design tools.

Supportive of this assumption, I have been able to isolate mutants using only simple structural models to design mutant libraries for screening. The synergy of this approach arises from the fact that improvements in receptor modeling and increases in screening throughput each enhance the power of the experimental system, which will make it possible to accomplish progressively more sophisticated design goals.

I have demonstrated that DE can be utilized to introduce new signaling properties into the human UDP-glucose receptor (P2Y14), and I showed that a pair of mutants can serve as an effective chemical sensor (Fig. 1). Alone, the UDP-glucose receptor cannot be used to distinguish one stereioisomer of UDP-glucose from another. With two receptors, one with significant changes in ligand specificity, it becomes possible to utilize the ratio of responses from the different receptors to differentiate one UDP-sugar stereoisomer from another. As in the olfactory system, it is possible to discriminate chemical ligands over a range of concentrations with a single measurement from each receptor.

My specific aims build on this successful application of mutagenesis and screening to GPCR engineering. The three aims are highly interdependent but have distinct experimental goals. Aim 1 continues the 'synthetic biology' trajectory of my initial experiments, exploring the limits of DE. Aim 2 focuses on application of DE techniques to building and refining structural models for drug development. Aim 3 describes one of several possible strategies for incorporating new computational techniques into receptor design.

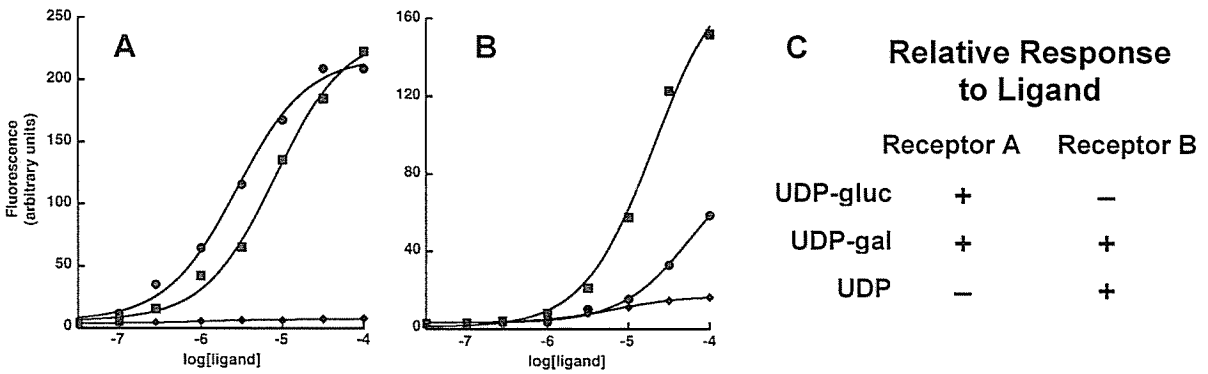

Figure 1. Dose response curves for two UDP-glucose receptor mutants (A and B) for three ligands each: UDP-glucose (●), UDP-galactose (■), and UDP (◆). For each ligand β-galactosidase reporter gene activity is plotted with increasing concentrations of ligand. Over a range of concentrations the ratio of responses from a single measurement uniquely identifies the ligand (C). Represented qualitatively for simplicity, UDP-glucose, UDP-galactose and UDP can be differentiated. Note that UDP-glucose and UDP-galactose differ by a single chiral center. UDP is an antagonist/weak partial agonist of 'A'.

Aim 1. Create new chemical sensors (Synthetic biology)

The overarching goal of this aim is to develop chemosensory arrays capable of discriminating among large numbers of chemically and stereochemically related compounds. Olfactory detection is still the state of the art for detection of many chemicals, as demonstrated by the continuing use of drug- and bomb-sniffing canines. To explore the combinatorial potential of 'pseudo-olfactory' sensing, I propose to identify receptor mutants with greater breadth and diversity in ligand binding. GPCRs bind a set of scientifically and economically relevant chemical ligands, but the ligand specificities of naturally occurring receptors are comparatively narrow relative to the diversity of chemical analytes of analytical interest. Thus, an important challenge in creating sensory arrays is to generate receptors that bind ligands or families of ligands (pharmacophores) that are not bound by naturally occurring receptors.

Using improved screening and computational techniques I plan to explore the practical limits of DE-assisted receptor design. In the near term I intend to work with two chemical receptors, the UDP-glucose receptor and the human melatonin receptor, to broaden and diversify ligand binding specificity. Longer term it may be possible to focus on engineering other aspects of receptor functionality, like ligand efficacy, allosteric regulation, receptor trafficking or G-protein coupling. Each functional property will present its own unique challenges in design of receptor mutants and implementation of screening strategies.

Aim 2. Analyze ligand/receptor interfaces. (Drug Design)

The goal of this aim is to test and refine structural models of how ligands bind to GPCRs. GPCR structure/function relationships are of paramount importance to the pharmaceutical industry. To explore these relationships, chemical libraries based on known ligands are created to map structure-activity relationships (SARs) between a receptor and its ligands. SARs are essentially careful descriptions of how chemical modifications to the ligand affect receptor activity [6]. To test models based on SAR data, chemically complementary mutations have been constructed for some receptors, most notably for receptors of biogenic amines [7], but this mode of analyzing receptor/ligand interactions has generally been limited to directed mutagenesis of specific amino acids. Now that I have accumulated critical experience mutagenizing and selecting GPCRs in yeast, I propose to systematically characterize how changes in amino acid sequence alter responses to chemically related ligands for the UDP-glucose receptor. Screening libraries of mutants is a more information-rich approach than directed mutagenesis, as the results of a typical experiment will reveal which residues are subject to genetic selection for a particular compound. This aim involves essentially the same techniques as in Aim 1, while focusing on generating data to help understand how ligands interact with the wild type receptor. This aim depends critically on obtaining relatively large libraries of compounds that interact with the receptor. To that end, I intend to collaborate with the Jacobson group at NIH, which has conducted SAR analysis for several receptors in the nucleotide receptor (P2Yx) subfamily and is currently generating ligands and computational models for analysis of the UDP-glucose receptor.

Aim 3. Test computational tools for design of transmembrane receptors. (Bioinformatics)

I am eager to apply computational tools to experimental design, with an eye toward testing and validating computational methodologies. Clearly, ligand docking algorithms could be tested in support of the first two aims, but it I am also interested in characterizing 'inverse folding' algorithms that could facilitate design of receptor chimeras or mutant libraries. For instance, creation of chimeric receptors could be a means of generating ligand binding diversity in receptors, and it has been reported that some chimeric ORs with N-terminal helices from another receptor have been functional [8]. A major obstacle to constructing chimeric GPCRs is the potential for incompatibility of the interhelical contacts between TM domains. Inverse folding algorithms involve the computational prediction of amino acids that are compatible with a predefined protein backbone [9, 10]. I am collaborating with the Floudas group at Princeton to test an inverse-folding algorithm that has been designed to account for potential flexibility/abiguity in the peptide template [11]. The algorithm highlights residues that would be expected in interfere with interhelical packing in a chimera, and proposes sets of potentially compatible alternatives. We hope to test a number of chimeras between receptors that are functional in the yeast system, as well as chimeras designed to help ORs localize and function in yeast.

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Experience

I have accumulated substantial classroom experience as a teaching assistant for undergraduate chemistry and molecular biology classes. I have worked as a teaching assistant at four institutions: the University of Wisconsin, Madison; Cornell College in Mt. Vernon, Iowa; the University of Iowa; and Princeton University. I have supervised laboratory classes for general chemistry, organic chemistry and molecular biology, and I have led discussion sections for general, organic and biochemistry. Some of the laboratory courses required preparing a brief introductory lecture, as well. Prior to attending graduate school I edited and 'published' lab manuals customized for different institutions. These were some of the first examples of electronically published books. At Princeton I served as a teaching assistant for a course for non-majors entitled 'Genes, Health and Society', an introduction to human genetics. I also taught a 1 semester-hour seminar course introducing Princeton Molecular Biology majors to reading scientific literature.

Interests

My teaching interests include undergraduate and graduate level biochemistry, molecular biology and molecular genetics. I have teaching experience in organic chemistry, especially laboratory courses, although I realize organic is generally left to practicing organic chemists at the university level. Some schools, including Princeton, are moving to an introductory bio/organic chemistry course, which I would be qualified to teach. I would enjoy teaching nonscience majors, too, and I could teach courses for nonmajors in anything from physics to molecular biology. I have sufficient experience and training in enzymology and pharmacology (the underlying math being the same) to teach these at the undergraduate level, and I would be comfortable teaching these at the graduate level with some guidance. Finally, I would be able to teach undergraduate or graduate level classes on principles of protein engineering.

Teaching Philosophy

Pedagogical or philosophical considerations are of secondary importance to developing a clear vision of what one is trying to teach. I have never taught a course often enough to get beyond this point, and I did not develop this philosophy myself. I have been fortunate enough to work with a number of professors who have asserted that thoughts about pedagogy often mask uncertain thinking about educational goals and priorities, and I have come to believe that this is true. In the sciences, I see many conversations about science literacy hamstrung by the assumption that pedagogical issues lie at the root of science illiteracy. If classes were made more accessible, more appealing, the logic goes, then students would be drawn to study science. This thinking obscures the fact that there is little discussion or agreement about what scientific facts and concepts should be an essential part an average person's education.

To this end, I believe there is a critical need for leadership by research universities in the area of science education for nonscientists. Essentially all experts agree that priorities in science education need to be reevaluated in the US. In the postgenomic era over half of the population is destined to have cancer and 80-90% of families will undergo prenatal genetic testing. With development of pharmacogenetic profiling and additional genetic tests, there will be an unprecedented need for members of the public to achieve rudimentary scientific literacy just to understand their own healthcare decisions.

Educational leadership in our decentralized educational system rests squarely in the hands of the most prestigious, wealthiest educational institutions—the 'brand name' institutions of the Ivy League and other large research universities. I have seen research scientists, and even university presidents excoriate political and educational institutions (usually high schools) for perpetuating scientific illiteracy. However, the research universities that employ leading scientists do not have academic standards requiring their graduates to understand what a gene is, nor do they have any standard whatsoever for their incoming students with regard to science literacy. In the absence of leadership or standards at the university level, it is very difficult for AAAS, NSF, NAS or high school science educators to persuade local school districts that updated science curricula are a priority.

Research scientists are not indifferent to these issues but they generally lack the time and resources to invest extra effort into teaching or developing new curricula. There are at least two ways that research scientists could collectively address their institutions' unmet teaching responsibilities. One would be to 'partner', using business parlance, with smaller colleges and universities to develop standards and curricula, lending big university prestige and any needed advisory capacity to a pilot program that would be executed first by educational specialists on a smaller scale. The alternative is quite simply to ask university trustees for the institutional support needed to address new educational responsibilities. Neither option necessarily implies adding to the teaching responsibilities of the research faculty, a step that would probably only create confusion and make the institution less competitive with respect to research. On the contrary, it means finding the appropriate people assume responsibility for devising and executing a plan for educating nonscientists.

There has been a regrettable reluctance on the part of research scientists to step forward and assert that there may be a basic set of scientific facts that any educated person should know. Courses that cover the basic elements of science literacy have been developed at numerous institutions. These courses typically cover rudimentary concepts of physics, chemistry and biology in such a manner that students are familiarized with scientific phenomena and are challenged to perform some calculations, but are not burdened with unmanageable theory and abstraction. The physical world is the 'operating system' of life, and it is not too much to ask that universities require their graduates to master a descriptive understanding of physical reality.