College of Arts and Sciences

Department of Chemistry

THE UNIVERSITY OF
ALABAMA
ARTS & SCIENCES

October 25, 2005

Yves Brun, Systems Biology/Microbiology Faculty Search
Department of Biology, Indiana University
Jordan Hall 142, 1001 E 3rd Street
Bloomington, IN 47405-7005

Dear Dr Brun:

Please find enclosed my application for the position in Systems Biology/Microbiology, consisting of my curriculum vita and a statement of research and teaching interests, along with copies of some representative publications.

My research over the past several years has focused on structure/function analyses of Photosystem I, combining site-directed mutagenesis with advanced spectroscopic analysis (optical, infrared, and electron spin resonance). We have also launched a related project on disassembly and degradation of multi-subunit membrane protein complexes, using Photosystem I as a model system.

I should let you know that I am also applying to an open position in the Dept. of Chemistry at IU. My research interests fall somewhere between microbiology/cell biology and chemistry. I was trained in a biochemistry department (although I was doing mainly cell biology and genetics) and I am currently in a chemistry department, so I am at home now in both environments.

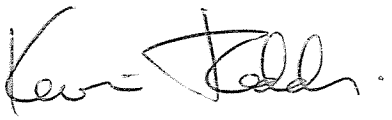
I ask that you keep this application confidential. I would prefer that the University of Alabama not know at this point in time that I am looking elsewhere. Your understanding is appreciated. Please do not hesitate to contact me if you have any questions about this application.

Shelby Hall
Box 870336
Tuscaloosa, Alabama 35487-0336
(205) 348-5954
FAX (205) 348-9104

You should expect receive letters of recommendation soon from my referees:

- Robert Blankenship (Professor and Chair, Dept. of Arizona State Univ.)
- Arthur Grossman (Carnegie Institute and Dept. Plant Biol., Stanford Univ.)
- Sabeeha Merchant (Professor, Dept. of Chemistry and Biochem, UCLA)
- A. William Rutherford (chef de service, Bioenergetics section, CEA-Saclay)
- Francis-André Wollman (section director, Institute of Physico-Chemical Biology)

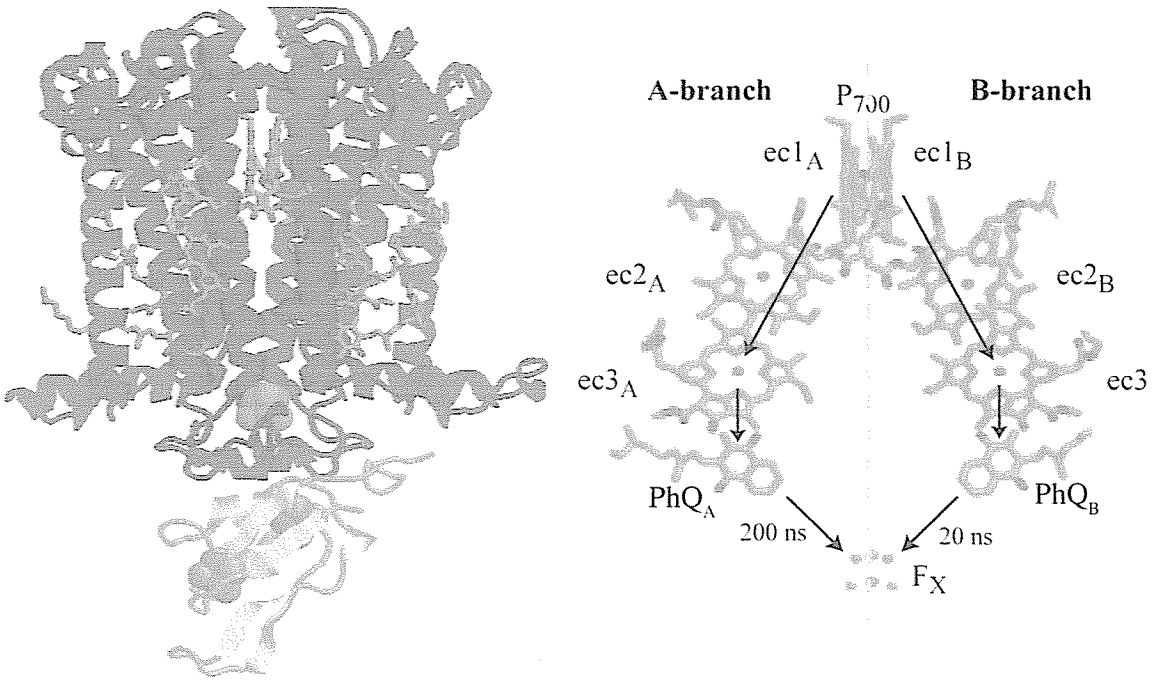
Best regards,



Kevin Redding
Associate professor of Chemistry
Adjunct associate professor of Biological Sciences
The University of Alabama

Overview of current research projects and plans for future

All of our current research revolves around Photosystem I (PS1). This is a key component of the biochemical machinery used by plants to convert light energy into a chemical form usable by organisms. It is found in all photosynthetic organisms and is almost identical in composition from cyanobacteria ("blue-green algae") to higher plants, species separated by more than 1.5 billion years of evolution. Based upon recent studies (including our own), it has become generally accepted that all photosystems are related. In other words, nature invented the basic photosystem design once, and then specialized it for different purposes. This invention is one of the most important ones (if not *the* most important) in our biosphere, having enabled biological organisms to harvest orders of magnitude more energy than before. About half of our research has to do with understanding at a physical/chemical level how this energy conversion nanodevice works. The other half is concerned with using PS1 as a model system to understand in general how proteins embedded in biological membranes are degraded.



On the right is a view of the arrangement of the core of PS1 (core portions of PsaA and PsaB shown in red and blue, respectively, PsaC in yellow), derived from the *Thermosynechococcus* crystal structure (Jordan et al., 2001), with electron donor side at the top. On the right is an expanded view of the core cofactors; the pseudo-C_2 symmetry axis is shown as a dotted gray line dividing the two branches. Reduction of the ec3 Chl(s) occurs in <100 ps, with subsequent electron transfer to the phylloquinone (PhQ) on a similar timescale. In the nanosecond timescale, the electron is transferred to F_X and then to F_A/F_B (not shown).

Currently funded projects

Genetic/biophysical analysis of the phylloquinone-binding site of Photosystem I: We have initiated a genetic study of Photosystem 1 (PS1) in order to understand how the polypeptide environment tunes the properties of the phylloquinone electron transfer cofactors in PS1 (PhQ; see figure). This study involves creating mutations near the

quinones and using advanced spectroscopic techniques, including time-resolved optical and EPR (electron paramagnetic resonance) spectroscopy, to assess the effects of such changes. The questions we are asking include:

- What role does the protein environment play in tuning the properties of the phylloquinone (especially its very low reduction potential)?
- Which environmental differences are responsible for the 10-fold difference in electron transfer rate between the A-side and B-side quinones?

The project is supported by an Energy Biosciences grant (DE-FG02-00ER15097) from the Department of Energy. We are currently in the third year of a 3-year grant, which represents a renewal of a grant that originally started in August 2000. (A renewal application for 3 more years is currently under consideration.) This project has resulted so far in publication of 6 papers (*J. Biol. Chem.*, *Proc. Natl. Acad. Sci.*, *J. Photochem. Photobiol., Chem. Phys. Lett.*, and *Biochemistry*), 2 proceedings articles, 2 book chapters, with 1 paper in press (*PNAS*). It has involved 2 graduate students: Mr. Brent Boudreaux, an MS student who started as an undergraduate researcher in my lab, and Ms. Feifei Gu, a PhD student who just finished in August. At various times, 5 different undergraduate researchers have made contributions. For ~3 years, we were joined by Dr. Alexander Petrenko, an expert in computational chemistry, who just moved to Montana State U., and has been replaced by Dr. Narasimhulu Kuppala Venkata (previously of the Weizmann Inst.), an expert in high-field and pulsed EPR. The DOE grant has also partially supported Dr. Tatyana Konovalova, our EPR facility manager.

Examining directionality of electron transfer within Photosystem I: All photosynthetic reaction centers contain two symmetric branches of cofactors down which electrons could potentially be transferred (see figure above). An unexpected discovery of the project above is that both symmetry-related phylloquinones seem to be involved in electron transfer, which implies that electrons can travel down either of the potential pathways within PS1 (Guergova-Kuras et al., 2001). We have initiated a new project to try to uncover the factors that govern the relative use of the two branches of electron transfer cofactors. The questions we are asking include:

- What role does the protein environment play in directing electron transfer down the 2 symmetric branches of cofactors?
- What mechanisms are used to direct electron transfer when there more than one potential pathway?
- How is asymmetry introduced into initially symmetric proteins?

Like the PhQ project, this one also makes use of site-directed mutagenesis of the PS1 genes (*psaA* and *psaB*) in two model photosynthetic organisms: a green alga (*Chlamydomonas reinhardtii*) and a cyanobacterium (*Synechocystis* PCC6803). It relies heavily upon kinetic techniques, including pump-probe spectroscopy (in the picosecond and nanosecond timescales) and transient EPR, in order to asses the functional consequences of the mutations we introduce. Ms. Yajing Li, an MS student who graduated 2 years ago, made the first series of mutants, and Mr. Rajiv Luthra, a new

student, is now working on this project. Three undergraduates and a high school teacher have also made contributions. Since June of last year, this project has been funded by a 5-year NSF CAREER award (MCB-0347935). It supports 2 PhD students and a postdoctoral fellow, who will split his/her time between my lab and the Rappaport group at the *Institut de Biologie Physico-Chimique* in Paris, where we have a strong collaboration to do the ns pump-probe with a device allowing measurements *in vivo* (a unique capability). I have recently hired Dr. Audrius Jasaitis, an expert in ultra-fast spectroscopy, as the postdoc on this project.

Disassembly and degradation of Photosystem I: A fundamental question in biochemistry is how integral membrane proteins are degraded. We have an excellent opportunity to answer this question with PS1. The questions we are asking include:

- What is the sequence of events when a multimeric membrane protein is degraded?
- Which proteins take part in the processes of recognizing aberrant membrane proteins, disassembling and degrading them?
- What mechanisms do they employ?

We have made use of the techniques used to study PS1 structure and function to set up an *in vitro* biochemical assay for disassembly and degradation of PS1 (Henderson et al., 2003). We have also isolated mutants in the degradation pathway. The combination of genetics and biochemistry should prove to be a powerful approach. This project was started by 4 undergraduates, one of whom, (Nathan Henderson) stayed in my lab to obtain his MS (2002); he set up the *in vitro* assay and was the first author on the *JBC* paper describing it. Ms. Jianying Zhang, a Ph.D. candidate in her fourth year, leads the genetics sub-project and works with Walter Evans, an undergraduate researcher who has worked one year on the project. Ms. Galina Gulis, a new graduate student, is now trying to combine the genetics and biochemistry to create a biochemical complementation system. This project is supported by an NIH AREA grant (GM66345-01), which was recently renewed in April for 2 more years.

New projects

Re-engineering Photosystem I as a bioremediator: This is a risky project with the goal of redesigning the PhQ-binding site of Photosystem I, so that it can accommodate other organic molecules as targets of reduction. The chlorophyll radical anion that serves as donor to the PhQ is one of the most reducing components in the biosphere ($E_m \approx -1$ V). We have focused upon chlorinated aromatic molecules that are also long-lived environmental pollutants, such as polychlorinated biphenyls (PCBs) and pentachlorophenol, as these might be reductively dehalogenated. The ultimate goal is to produce an oxygen-tolerant organism capable of dechlorinating such molecules. We have made some progress, and all the experiments have been carried out by myself and four different undergraduates over the past 2.5 years.

Recapitulating evolution – introducing asymmetry into heliobacterial reaction centers: This is a spin-off of my NSF project on directionality of electron transfer in PS1. Heliobacteria are primitive anaerobic photoheterotrophs in the GC-rich Gram-positive group of eubacteria. They possess a very simple photosynthetic apparatus with no external antenna system and a homodimeric reaction center, thus purely symmetric. Therefore, no gene duplication event, allowing subsequent diversification of the two sides of the reaction center, ever occurred in this lineage. This gives us a powerful system to ask the question: how might nature take an initially symmetric system and modify it into a very asymmetric system? We will engineer a duplication of the gene encoding the reaction center protein into the genome of *Heliobacterium modesticaldum*, which is currently being sequenced (http://genomes.tgen.org/). Then we will be able to introduce asymmetry in a completely controlled fashion. This will allow us to see if we can use the rules Nature seems to have used to make asymmetric reaction centers, to drive electron transfer down only one branch, and perhaps to explore ways that Nature might not have used. Furthermore, starting with a symmetric reaction center is a powerful system for examining the effects of asymmetric environments upon cofactors; it can be difficult to tease apart the effects of single changes in dimeric proteins that have many differences between the two sides. Bradford Bullock, a new grad student, just started on this sub-project, and has gotten a glovebox fitted for growth of heliobacteria and has started developing transformation protocols for *H. modesticaldum*.

Teaching Interests

The primary reason for my choosing an academic position was the opportunity to teach. This is a vocation that I enjoy and for which I think I have a natural ability. During my time at U.A., I have tried to expand my repertoire of teaching skills. I have constructed a website for each course that I have taught. On these, I have all of the essential material for each course, including syllabi, lecture schedules, class notes, assignments, and exams. I find my students appreciate this convenient access to the material. I have also used multi-media components during my courses, as well as more traditional use of overheads and the chalkboard. I put a lot of myself into my courses, and I expect a lot from my students. I have continuously attempted to improve my performance as a teacher. I have employed feedback from my students and from successful educators in my institute and my field.

Biochemistry I and II

My main focus has been the Biochemistry course, which I have taught for six years. This is a two-semester series for graduate students and advanced undergraduates, and I have had sole responsibility for it. It covers everything from the basic biomolecules (amino acids & polypeptides, carbohydrates, nucleic acids, and lipids) to proteins structure and function, enzymology (kinetic and mechanistic analysis), metabolism, signal transduction and regulation, and finally to molecular biology. One of the important features of this course is a take-home exam in the first semester that gives students the opportunity to solve complex kinetic and structural problems. I teach them how to use protein structure visualization programs, such as RasMol, which are critical for success..

The Relationship between Evolution and Religion, Philosophy, and Ethics

I developed this discussion-based seminar 3 years ago as part of the Blount Undergraduate Initiative (http://www.as.ua.edu/blount/), after hearing about poll of senior U.A. biology majors in which a majority claimed that they "did not believe in evolution." I understood that the cause of this "disbelief" was not the lack of convincing science, but the perceived conflict with religious beliefs and values, and that there was a real need for a course that dealt with the implications of biological evolution. One of our responsibilities as scientists is to educate young people not just in the "facts" of science and how science works, but also in the implications of scientific knowledge. The intention of this course was to create a safe place to give students the opportunity to explore important questions as far as they chose to go, such as: What is evolution and the evidence for it? What are the strengths and weaknesses of other hypotheses (e.g. "intelligent design")? Is evolutionary theory inconsistent with religious interpretations? What do our origins say about us? How did consciousness arise? What are the societal implications of evolution? The heart of the seminar was a series of forums led by teams of students that had taken on one of the special-topic books, and was assisted by a paper-less system of assignments and a set of electronic bulletin boards divided into topics.

Diversity of Bioenergetic Mechanisms (in process of designing now)

As part of my NSF CAREER award, I am designing a course on the diversity of bioenergetics, available to graduate and senior undergraduate students. It will be a survey of how organisms on this planet make a living, in terms of obtaining energy, fixing carbon, etc. An almost exclusive focus on eukaryotic systems (especially "higher" animals) has severely narrowed the diversity of biochemical systems that students are exposed to. Most biochemistry courses deal with fermentation and aerobic organotrophic metabolism, so this course focuses on the more "exotic" bacteria, including the following:

- Oxygenic photosynthetic bacteria (*Synechocystis*, *Anabaena*)
- Anoxygenic phototrophs: purple sulfur bacteria (*e.g. Rhodobacter capsulatus*), green sulfur bacteria (*Chlorobium tepidum*), green nonsulfur bacteria (*Chloroflexus aurantiacus*), and heliobacteria (*Heliobacillus mobilis*)
- Fe^{2+}-oxidizing bacteria (*Thiobacillus ferrooxidans*)
- Sulfur bacteria (*Thiobacillus ferrooxidans, Thiobacillus denitrificans*)
- H_2-oxidizing bacteria (*Ralstonia eutropha*)
- Nitrifying bacteria (*Nitrosomonas, Nitrobacter, Brocadia anammoxidans*)
- Methanotrophs (*Methylomonas*, *Methylosinus*)
- Methanogenic archaebacteria (*Mathanocaldococcus jannaschi*)
- Bacteriorhodopsin-based photosynthetic archaebacteria (*Halobacterium*)

The students will meet with the instructor twice a week for lectures and discussion on what is known about how these organisms extract energy from their environment. In the last part of the course, discussion will focus on the evolutionary origins of life and bioenergetic pathways. The students will be expected to perform a significant amount of literature research and will present a specific topic to the class. Assuming that there are enough students, they will be divided into teams, each of which will focus on a specific enzyme or pathway; each participant would be in 2-3 different teams. There will be a lab associated with the course; about 1/3 of the contact hours will be lab-related. The aim is not to have an artificial separation between "lecture" and "lab". As different species are discussed, we will be growing them in the laboratory and performing various physiological and biochemical experiments with them. These will include use of various sensors to measure O_2 uptake (of aerobic bacteria), O_2 evolution from oxygenic phototrophs, enzymatic assays, CO_2 uptake, H_2 evolution and uptake, *etc.*