UNIVERSITY OF ILLINOIS
AT URBANA-CHAMPAIGN

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October 11, 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 Professor Brun:

This letter is in support of the application of John R. Kirby for a faculty position. I have known John for more than 15 years, first as a student at UIUC in the accelerated general chemistry sequence (chem. 107 – 108) for which I was the professor for many years, then as a teaching assistant in this same sequence and then as a friend. I know John very well, having had many in-depth discussions with him about scientific and non-scientific issues.

John is among the most talented students (top 1 – 2%) I saw in about 30 years at UIUC. He has a first rate intellect, an intense curiosity, both a broad and deep knowledge of biochemistry, a strong work ethic and very high standards. In addition, he has shown himself to be an outstanding teacher and mentor for undergraduates.

In terms of personality John has a strong, healthy and mature self-confidence, a broad-based and balanced outlook, a critical but constructive spirit of cooperation, very high ethical standards and a strong sense of personal priorities. He loves learning and sharing his knowledge with others.

John is already a scientist of considerable talents and accomplishments. I am confident that he will become a truly extraordinary scholar, researcher and teacher as his career unfolds. I recommend him to you in the highest possible terms.

Sincerely yours,

Steven S. Zumdahl
Professor Emeritus, Chemistry

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October 12, 2005

Dr. Yves Brun
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Dear Yves,

This letter is to enthusiastically support Dr. John Kirby's application for a faculty position in your Department. I have known John for about ten years. We met by discussing his poster at the Gordon Research Conference on Sensory Transduction In Microorganisms in January 1996. Following our initial meeting, John sought me out as someone to talk to about his thesis research, and we maintained an extensive correspondence by e-mail and telephone. We discussed experimental design, editing of manuscripts, career advice, etc. Once John moved on to his postdoctoral position and became more independent, our contact diminished, but we still correspond periodically and I see him at least once a year at professional meetings.

In order to fully appreciate the nature of our relationship and the magnitude of John's accomplishments it is necessary to candidly describe his situation in graduate school. A very large fraction of what is known about chemotaxis by *Bacillus subtilis* is due to the work of George Ordal's group. However, the fact that the Ordal group is to a first approximation the only laboratory in the world studying this topic means that the normal checks and balances of a competitive research field are largely absent. Many times there are no other labs to experimentally test or challenge models proposed by the Ordal group, with the consequence that the line between what is established fact and what is interpretation sometimes becomes blurred.

John established a fine record as a scientist in graduate school, but his ability to do so under these circumstances truly sets him apart. He conducted a series of careful experiments that overturned one of George's longstanding models to account for data from several publications. Although much of what is known about chemotaxis in *Escherichia coli* is applicable to *B. subtilis*, John's work concerned unique phenomenon where this is not the case. Thus, there were no precedents to guide him. John clearly proved his ability to operate as an independent scientist at an unusually early stage of his career.

It must be quite a challenge to directly contradict the work of one's thesis advisor. I think it is to John's credit that he persevered and sought advice from scientists outside his laboratory. Many graduate students would be too intimidated to do so. I have found him to be enthusiastic and thoughtful in our discussions. John is mature and willing to seriously consider the suggestions of others. He is unfailingly polite and gets along well with others. All of these characteristics suggest John has significant promise as a scientist. NIH recognized John's potential when it awarded him a Postdoctoral Fellowship and that confidence in John was further justified when he earned a RO1 grant.

John wisely chose a postdoctoral position (with David Zusman at UC Berkeley) that greatly broadened his training. He changed his focus to a substantially different organism, gained exposure to developmental biology, and learned techniques from molecular biology and genetics to supplement his largely biochemical background. Importantly, he once again proved his ability to make notable research discoveries.

In order to put John's postdoctoral work and his future research plans into a context where their significance can be fully appreciated, some background information is necessary. Although not all bacteria are motile, locomotion is widespread in prokaryotic species and is generally accompanied by an ability to guide movement in a beneficial direction. The information-processing network that controls chemotaxis in *E. coli* and *Salmonella typhimurium* is extraordinarily well understood. However, complete sequencing of many bacterial genomes in recent years has revealed that in several important respects, the *E. coli* chemotaxis signal transduction system is atypical. First, *E. coli* contains a signaling protein (CheZ) that most motile bacteria lack. We do not have a good understanding of how other species accomplish chemotaxis without CheZ. Second, *E. coli* lacks several chemotaxis proteins (CheC, CheD, CheV) that most motile bacteria possess. The function of these three proteins is incompletely understood. Third, many species of bacteria contain multiple sets of genes encoding apparent chemotaxis proteins. Although in some cases these multiple signaling pathways are used to control multiple motor types (e.g. flagella and pili) in the same organism, the reason for the existence of multiple sets of apparent chemotaxis proteins is generally unknown.

John's current and future research plans directly address the need, and opportunity, to investigate chemotactic signal transduction in carefully chosen species other than *E. coli*. John's postdoctoral discovery that at least one set of chemotaxis proteins in *Myxococcus xanthus* controls gene expression rather than motor behavior is both unexpected (in that it contradicts longstanding dogma based on an absence of information) and exciting (in that it provides a satisfying potential explanation for the existence and function of many "chemotaxis" genes in many species). His talk at the Bacterial Locomotion And Signal Transduction meeting in January 2002 and subsequent 2003 publication in the *Proceedings of the National Academy of Science of the U.S.A.* caused quite a stir as a result. John's future plans with *M. xanthus* are a logical extension of his current studies and involve working out the mechanism(s) by which apparent chemotaxis proteins regulate gene expression, as well as how multiple apparent chemotaxis pathways may function in the same organism.

In addition, John intends to resume investigation of *B. subtilis* chemotaxis. This is in many ways an ideal situation. First, there are excellent scientific reasons to study chemotaxis in *B. subtilis*. It has all the known chemotaxis proteins except CheZ and therefore is a good phylogenetically representative choice of model organism. This is the organism where we are most likely to figure out the roles of CheC, CheD, and CheV. Furthermore, *B. subtilis* has only one set of chemotaxis genes, which should simplify the analysis. Second, John already has the necessary experience for this work from his graduate student training. Third, with the impending retirement of Dr. Ordal, there will soon be no competition in *B. subtilis* chemotaxis research, and every reason to believe that big questions will still be awaiting answers.

I have no basis on which to personally evaluate John's teaching ability, other than to say that his seminar presentations are excellent. His teaching ability appears to be excellent, based on the teaching awards he has earned in the past, and the fact that he has been designated as a future instructor for the world-renowned summer course in Advanced Microbial Genetics at Cold Spring Harbor Laboratory.

In summary, I think John Kirby is an outstanding candidate for a faculty position, and I would be thrilled to have him a colleague in my own department. He has already proven himself during his three years as an Assistant Professor at Georgia Tech by establishing a new laboratory, attracting students and postdoctoral fellows to his research group, obtaining extramural funding in a highly competitive environment, teaching classes, and starting to publish independently. What more could a search committee want? I strongly encourage you to invite John for an interview and see for yourself. If I can provide any additional information, please do not hesitate to contact me.

Sincerely,

Robert B. Bourret, Ph.D.
Associate Professor

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October 10, 2005

Dr. Yves Brun
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Dear Yves, Re: John Kirby

I would like to give you my strongest recommendation for you to accept John Kirby's application as an assistant professor in the Systems Biology/Microbiology group in the Department of Biology.. He was a graduate student in my lab in the 90's (and previously an undergraduate research student). I have seldom seen someone who is as enthusiastic about science and about what he was working on than John Kirby. In my laboratory he really got people talking over ideas, very reminiscent of my own graduate student days in the Department of Biochemistry at Stanford in the late 60's.

He greatly advanced our understanding of receptor methylation in *Bacillus subtilis*. He focused on the effect of asparagine on McpB, the sole receptor for asparagine. McpB also recognizes other attractants, such as glutamate and histidine, but so does McpC. He made suitable receptor mutants so that methylation of McpB could be detected on SDS gels following fluorography, without interference of other, similarly sized receptors. With the resulting strain, he was the first to show that the methanol produced both on addition and also on removal of asparagine was accompanied by net receptor demethylation and that methylation levels spontaneously returned to the prestimulus value. In *Escherichia* coli attractant causes a net increase in receptor methylation and there is no return to prestimulus levels of methylation until attractant is removed; repellent causes a net demethylation. He also found that the great majority of methanol released in *B. subtilis* came from turnover, not net demethyation. In this connection, he was the first to produce evidence that methylation of different sites has different effects, in contrast to *E. coli*. Subsequent work, carried out after his graduation, has confirmed and extended this finding, experimental work that he designed before his departure, and we are finding that receptor methylation plays a somewhat different role in the two bacteria. He also produced evidence that the response regulator of the system, CheY-P, feeds back at the level of the receptor. He made many other findings as well. His

contributions, in terms of experiments performed, ideas generated, and quality of interactions fostered among the graduate students and undergraduates, were immense and put the work of the whole laboratory on a much firmer foundation than before.

He continued to break new ground in his work in the Zusman lab on *Myxococcus xanthus*. I think his discovery of new chemotaxis-type operons, encoding new receptors and other proteins, is very exciting. He is at the forefront of research into how chemotaxis-type operons are used to govern non-motility (e.g., developmental) processes. Kirby is very imaginative and at the same time very systematic and well-organized in his approach to new problems. His pioneering work on *B. subtilis* chemotaxis is helping in his new field. He is adept at making new observations and then carrying out the experiments needed to produce a satisfying story.

Kirby, as suggested above, is an excellent catalyst for excitement and enthusiasm in others. When he arrived at Berkeley, the Zusman lab always had their laboratory door shut and rarely had graduate students or undergraduate research students. He changed all that. He recruited both, kept the door open, and interacted with others in the lab and in other labs. He even frequently visited the Kaiser lab at Stanford (Most postdocs I know have trouble interacting with people in nearby buildings or even nearby floors in the same building). I am confident that, by his enthusiasm, he will also enrich scientific life in your Department.

Since moving to Georgia Tech as an Assistant Professor of Biology, Kirby has distinguished himself. He is making excellent progress in understanding the roles of the chemotaxis-type operons in the developmental program of *Myxococcus xanthus* and, since he was awarded a five-year NIH grant with a truly excellent score, he is, in my eyes and in the eyes of the PCMB study section, heading in the right direction, with the funding to make progress possible. He and his work are proving to be very visible in the microbiology community as evidenced by the invited talks he has given and by his role in the ASM (chair-elect of Division J (Ultrastructure and Function) and in the Cold Spring Harbor summer course structure (course on Advanced Microbial Genetics).

I think this is a marvelous opportunity to recruit a first-rate scientist to the Department. I give him my strongest endorsement in his candidacy to become a faculty member in Biology.

Yours sincerely,

George

George W. Ordal
Professor Emeritus of Biochemistry