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Dr. Yves Brun
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22 October 2005

Dear Dr. Brun:

Dr. Di Jiang has asked that I write you concerning his application for your position in. development and evolution. I have had the pleasure of knowing Di for the past 4 or 5 years and have been extremely impressed by his intellectual curiosity, drive and ability to assimilate a whole field of information very rapidly.

Di is a very energetic young man who tackles big questions in biology. Just how broad these questions are is developed in his research interest statement. His background is in immunology and he has worked with ascidians (I have worked on ascidian biology for over 40 years) for only a few years yet he already has made major discoveries into their fertilization, genetics, morphogenesis and population structure. His achievements in such diverse areas speak well for what we can expect from him in the future. Di's success in such diverse areas is the result of very high intelligence coupled with a real drive to understand basic questions in biology. In my opinion Di is a rising star in the field of invertebrate biology. I am most gratified that Di has recognized the potential for research on ascidians and chosen to pursue questions into their development and evolution.

Di is a very articulate young man with a perfect command of English. I have discussed biology and worked with him frequently, especially last summer when I spent several weeks working with him at UC Santa Barbara. He speaks clearly and confidently about his research. He presented his findings to several people highly respected in ascidian research a couple of years ago at the University of Washington where it was very well received. During the summer I had the opportunity of collecting with Di, observing him interacting with other post docs and graduate students and presenting his findings at a meeting. In all these interchanges I was impressed by Di's ability to impart his enthusiasm to the group including his mentor Dr. Smith. You can be assured that Di will interact well with other members of your department.

If you have any other questions about Di or just want to discuss him or his work further, do not hesitate to email or phone me. I am very enthusiastic about Di's past and future research and would be glad to discuss it further.

Sincerely,

Charles C. Lambert
Professor Emeritus

UNIVERSITY OF CALIFORNIA, SANTA BARBARA

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO



SANTA BARBARA • SANTA CRUZ

KATHY FOLTZ
ASSOCIATE PROFESSOR
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October 13, 2005

Dr. Yves Brun
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RE: Application of **Dr. Di Jiang** for Assistant Professor position

Dear search committee members:

It is my pleasure to recommend Dr. Di Jiang for your advertised position for an Assistant Professor. Dr. Jiang has been a postdoctoral fellow here at UCSB in the Department of Molecular, Cellular and Developmental Biology and the Marine Science Institute since 2001, and I have come to know him fairly well during that time. I am sure that his advisor, Dr. Bill Smith, will comment specifically on Di's research, so I will limit my comments in that area to more general terms and focus on Di's excellent potential as a researcher and teacher.

Di has been working on the development of ascidians, particularly the development of the notochord, with a broader question of how the vertebrate nervous system might have evolved. He has framed his research questions carefully and is likely to make excellent progress in this area. He uses a nice combination of observational and comparative approaches with molecular techniques that can provide quite a bit of insight into a problem.

As you can see from his CV, Di has a very broad background in terms of his formal training in the life sciences. His command of the literature and salient problems in a wide variety of areas is nothing short of astounding. Di can be counted on to ask insightful, pertinent questions in journal clubs, seminars and during informal discussions on Friday afternoons over beer. Many students and faculty seek him out to discuss new ideas or to chat about a recent paper they have read or are trying to write. Di always seems to know the background literature, and in the rare case where he does not, he quickly picks up details and zeroes in on the key issues. One of the reasons that Di is so impressive in this regard is that his breadth and wide interests are complemented by his ability to focus on a problem at hand and to make progress on that problem. He is a hard worker and his deep thinking and scholarly perspective is a perfect partner for his experimental prowess. He is not afraid to tackle new techniques or new

questions and it is very likely that in his career he will pursue directions of research based on where the data lead him.

Di's scholarly approach to science – really, a Renaissance approach – will come through in his teaching. While he has not had a lot of experience in this regard, his natural passion for science, his curiosity, and his commitment to “being a scientist” will lend themselves well to teaching. Students ought to respond to this approach – Di's excitement will be contagious. I have observed this in informal settings here, where Di has been interacting with and mentoring younger graduate students. His written and spoken command of the English language are excellent. I say this based on having reviewed drafts of his manuscripts and hearing him speak in formal and informal settings.

Di is “always in the lab” and I have lost track of how many times (too numerous to count!) that he has popped into my office or lab to show me some new result or to discuss a paper or new observation. Actually, I am really going to miss him when he leaves – it is a real pleasure to have a colleague who just loves biology for the sake of biology and who is constantly learning and exploring. His enthusiasm is infectious and his energy limitless. As you are probably aware, UCSB is a wonderful place to work on marine invertebrates (our campus sits right on the Pacific ocean) and one of the first things Di did when he arrived as postdoc was to sit in on our undergraduate classes in invertebrate zoology. These courses are taught by Dr. Armand Kuris and Di figured out immediately that this would be the quickest way to come up to speed on the local invertebrates. He has become an expert on local ascidian species since arriving here and has played an important role in establishing the methodologies for genetic approaches in this organism. As a specific example of this, Di has made some important and interesting observations regarding self-fertilization in a local ascidian species and had a recent paper in the journal *Biological Bulletin* describing these results.

In summary, I think that Di Jiang will make a wonderful faculty member and colleague, and I give him my highest recommendation. In fact, I'd be happy to have him as a colleague here at UCSB. If you have any questions, please feel free to contact me.

Sincerely,

Kathy Foltz

A handwritten signature in cursive script, appearing to read "Kathy Foltz". The signature is written in black ink and is positioned to the right of the typed name "Kathy Foltz".



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Oct. 12, 2005

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Dear Dr. Brun,

Di Jiang has asked me to write a letter of recommendation. I am very happy to do so. Di has been a postdoc in my laboratory since 2001. Before entering my lab, Di had done a short postdoc Ajay Chitnis at NIH working on zebrafish neural development. Di heard me speaking at a conference in the summer of 2000 in which I presented results on our efforts on establishing the ascidian *Ciona* as a genetic model system. Di quickly realized the great potential of *Ciona* for studying chordate development and evolution. Ascidiates, such as *Ciona*, are among the simplest extant chordates. The simplicity of ascidiates can be seen at several levels: *Ciona* embryos contain many fewer cells and cell types than embryos of vertebrates; the *Ciona* genome is only about 5% the size of the typical vertebrate genome; and the number of genes is estimated to be about half that of the typical vertebrate. Di's recently published work on ascidian notochord development (which I will describe below) exemplifies the degree of sophisticated analysis that is possible with ascidiates, and that is either not feasible, or much more difficult, in other chordate model systems, such as zebrafish or mice. At the time that Di joined my laboratory many of the techniques for *Ciona* genetics were in early development, and once coming to my lab Di has been able to push the field forward at an incredible pace. For example, he recently published the first manuscript describing the positional cloning of an ascidian mutation.

Di's first project when entering to my laboratory came from his interest in vertebrate CNS development. In a small project that was aimed primarily with getting his feet wet with a new experimental system, Di isolated the ascidian ortholog of the *engrailed* gene and examined its expression by in situ hybridization. In vertebrates, *engrailed* is expressed at the junction between the mid-brain and hind-brain (MHB). To our amazement, Di found that the *Ciona* *engrailed* was expressed in two bilaterally opposed cells at what is called the "neck region" of the CNS. Thus it would appear that

the MHB junction of the *Ciona* nervous system consists of only two cells. In vertebrates the MHB is an important source of inductive factors that play an important role in patterning the CNS. To add further evidence for his hypothesis concerning the correspondence of the engrailed-expressing cells to the vertebrate MHB, Di showed that the same cells express the ortholog of the vertebrate Pax-2, 5 and 8 genes. Di's results highlight both the simplicity of the ascidian embryo, as well as the high degree of conservation among chordate embryos.

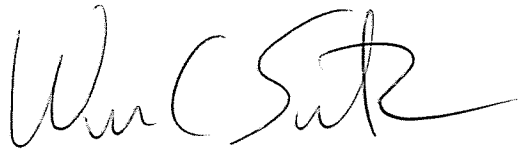
After this first study Di made a very important observation. Researchers in my laboratory had just begun to screen for developmental mutants of *Ciona* using a chemical mutagenesis protocol. Di observed that the wild population of *Ciona* carried many interesting spontaneous mutations. Wild *Ciona* are available in the Santa Barbara area by the thousands. *Ciona* are hermaphrodites with the ability to self-fertilize. This means that one can screen for recessive mutations simply by collecting wild animals and inducing them to spawn eggs and sperm. The resulting embryos are then screened for mutations that occur at the expected Mendelian ratio. Di found that one could rapidly screen through hundreds of animals this way. To my knowledge, no other animal has been screened for pre-existing mutations to the degree that *Ciona* has been by Di. Using this route Di identified a number of interesting mutations, two of which he has studied in detail. Di identified the first of these mutants, *aimless*, because exactly 1/4 of the progeny from a wild animal had short tails. Di was able to outcross and propagate this line in the laboratory. Di quickly discovered that embryos homozygous for the *aimless* mutation had a defect in notochord development. In all chordates the notochord forms by a process called convergent extension (C/E) in which the developing notochord cells move to the midline to form a column. In *aimless* embryos C/E is disrupted. Di's efforts were key to developing techniques for positionally cloning mutant loci in *Ciona*, and with amazing speed was able to identify the *aimless* genetic lesion as being in the gene *prickle*, which is a component of the planar cell polarity (PCP) pathway. In further characterization of the *aimless* mutant Di made several observations that are of great significance for understanding chordate morphogenesis. First he observed that intracellular components of the PCP pathway are polarized in the axis of C/E, but not in *aimless* mutants. This type of polarization had not been observed before in chordates, and now Di had genetic tools to understand this better. A second and unexpected observation of Di's was that following C/E the notochord cells become polarized in second axis (the anterior/posterior axis), and that this polarization is also dependent on the PCP pathway. I think that this second observation of Di's exemplifies his enormous ability. Researchers have been looking at notochords for over a hundred years, but nobody had noticed this anterior/posterior polarization of the cells (which can be seen in unstained embryos). However, once it is pointed out it's obvious to anyone. This is an entirely new area that has great potential for understanding the development and evolution of chordates, and Di is the only one working on it (my lab will not continue in this area).

Di's other major recent project concerned an "albino" mutation that he discovered. Ascidiarians contain two prominent pigmented (melanized) cells in the CNS. The role of one of these cells is obvious. It serves to shield the photoreceptors from stray light. The other is found within the gravity sensing organ of the ascidian. It was not clear what role melanin was serving within this organ, and Di's mutants were ideal for testing the hypothesis that they play a role in gravity sensation. On his own, Di devised

ingenious behavioral assays to examine the swimming behavior of the wild type and mutant larvae. Di's published results are now the definitive treatment on this subject. He showed that the gravity sensing organ in the albinos was intact, albeit without melanin. However, the mutants did not show the normal negative gravitropism. No other metazoan appears to use melanin in quite this way. These findings have important implications for the evolution of sensory structures.

I should close by giving my overall assessment of Di. He is one of the anchors and stars of my group, and he will be impossible to replace. Among Di's strongest traits (and there are many) are his native curiosity and creativity. He is a researcher in the truest sense. Equally important is how Di combines his curiosity and creativity with an ability to focus on a problem and produce top-notch results. In my nine years at UC Santa Barbara I have had nine postdocs (including Di) work with me. If I had to rank them, I would have to place Di at the top. While I have worked with many talented people, none of them has had the combination of talents found in Di. I should also add that Di would make a wonderful colleague and mentor. His curiosity and enthusiasm are infectious.

Sincerely,

A handwritten signature in black ink, appearing to read "W. Smith". The signature is fluid and cursive, with a large initial "W" and a long, sweeping tail.

William Smith, Professor of Biology

**Kewalo Marine Lab
Pacific Biomedical Research Center
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41 Ahui Street
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October 21, 2005

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Dear Search Committee,

I have been asked to write a letter for Dr. **Di Jiang** for the faculty opening in your department. I first met Di when he was a student in the Woods Hole embryology course in the summer of 2000. At that time he was a postdoc at the NIH working on zebrafish neurogenesis. Di has an interesting history completing a medical degree in China and earning his Ph.D in immunology at the NIH. He is now working on a marine invertebrate model, and the students and faculty at Woods Hole are partly responsible for this interesting career change.

I cannot speak to the quality of his earlier, more medically related work, but Di is now interested in understanding the molecular basis of morphogenesis. He has chosen the ascidian model system due to the fact that it is highly amenable to optical visualization, the genome has been completely sequenced, and Di's lab (Bill Smith's lab) has pioneered the use of genetics. Di has focused on understanding the origin and function of the notochord. Much of his work has been first-class work on the role of the non-canonical wnt pathway on convergent extension and axial cell polarity, but his natural sense of curiosity and technical confidence is revealed by the range of questions that he has addressed. He has published on problems ranging from genetics and molecular biology, to behavioral physiology and biomechanics. Each of these has been a solid contribution and has been driven by important empirical observations. He has also played a large role in developing techniques to artificially culture ascidians and be able to perform genetic screens in labs around the country. Previously, these animals were difficult to study at locations distant to a steady supply of fresh seawater.

Di has extremely bright, talented and hard working. He has excellent attitude, gets along with others and is fun to talk to about science and how things work. He has an excellent publication record and has clearly thought through his future research program. His desire to use both genetic, molecular, behavioral, and comparative approaches is surely a testament to his broad range of future research questions. I think that Di will make an excellent member of an active, interdisciplinary research department and will be valued by both his colleagues and the students around him. I urge you to take a serious look at this talented young scientist as he has an outstanding future ahead of him.

Sincerely,



Mark Q. Martindale, Ph.D. (808) 539-7330 (office) mqmartin@hawaii.edu



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October 17, 2005

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RE: Recommendation for Dr. Di Jiang

Dear Mr. Brun,

It is my pleasure to recommend Dr. Di Jiang for a faculty position in the Department of Biology at Indiana University. I have known Dr. Jiang approximately 13 years during which time he was first employed as a Research Assistant in my laboratory, then advanced to a graduate student in Genetics when the joint George Washington University/National Institutes of Health program was established, and finally carried out postdoctoral work on zebrafish at the NIH in the laboratory of A.J. Chitnis. Prior to coming to the NIH, he was a student at St. Mary's College in Maryland after emigrating to the U.S. following his participation in the student demonstrations that were crushed by the Tiananmen Square Massacre. During the time that I have known Di, I have found him to be a thoughtful and dedicated scientist and a pleasure with which to work. He was one of the best graduate students that I have encountered at the NIH and I think he has a very promising future in the academic field.

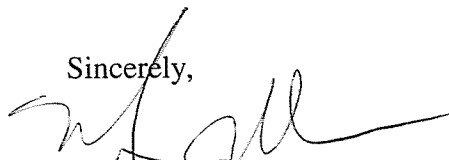
When Di first undertook his thesis work, he was most interested in how progenitor cells differentiate in the thymus. We had established several systems in the lab to study early steps in T cell development and he immersed himself in studies designed to reveal the key participating molecules. He was successful in using P53 knockout mice to establish a role for this protein in an early checkpoint in thymic development. This was a surprising result, although it is now generally accepted because previously P53 had been thought to primarily regulate the apoptotic response to DNA damage. He next attempted an ambitious strategy to retrovirally tag early thymic progenitors to carry out fate mapping during mouse thymic development. This idea was nicely thought out, but we encountered a variety of technical difficulties that in the end became insurmountable. Although we have not yet succeeded in this endeavor, we learned a great deal about stages at which early thymic progenitors are transfectable by murine retroviruses. Given the challenges of the tagging project, at my behest Di turned his attention to T cell receptor-

induced apoptosis that occurs during negative selection in thymocyte differentiation. He carried out a number of studies that implicate specific caspase proteases. During these studies he hypothesized that there must be a member of the death-inducing tumor necrosis factor gene family that mediated thymocyte death and set out to scan the EST (expressed sequence tag) database at Genebank for such a molecule. His efforts were successful and we are presently characterizing a novel TNF-like molecule. Though it is too early to tell if this is responsible for thymocyte deletion, its expression pattern strongly suggests that it will have a role in lymphocyte regulation and possibly a role in the central nervous system. Therefore I expect this work will generate another important contribution to the literature. So it should be clear that Di is creative, he gets excited by new ideas and pursues them with great vigor. This made him a very successful member of our group.

When leaving my laboratory, Di expressed interest in pursuing work on fundamental aspects of development in simpler systems. He joined the lab of A. J. Chitnis and has been productive during that experience. Dr. Chitnis would be a more appropriate person to comment on his contributions during his postdoc there. About a year or so ago, Di became interested in the nascent field of ascidian research. He was attracted to his new area because of the simplicity of this organism and its usefulness for genetic research. His visit to Dr. Smith's lab at UCSB convinced him that this was an important system to explore as the basis for his career and he decided to pursue a second postdoctoral fellowship there. I think this is a brilliant move for Di since he has a deep interest in basic aspects of development. It also will be terrific for Dr. Smith to have a highly trained investigator join his team that has experience in development in mammals (the immune system) and zebrafish. I think that Di's proposal may also be potentially valuable for developing new lines of investigation for cancer research using a simple system. The utility of simple organisms for significant insights into cancer and mammalian biology is well demonstrated. For example, there is no doubt that the study of programmed cell death in *C. elegans* has shed light on the mechanisms of apoptosis in mammalian cells and how they can become deranged and lead to malignancy. One could reasonably hope that penetrating analyses of ascidian development might yield insights into the basic mechanisms of human malignancy.

I think you will find Di a welcome addition to Indiana University. He is a first-rate investigator, with a formidable molecular biology background and a strong interest in developmental biology. He was one of the hardest working individuals in my lab and, when he left the lab, I missed his company in the late evening hours when I do my experiments. He is dedicated to science and I think he has the ability to be a highly successful independent investigator. I think his career has been greatly advanced by the training in Dr. Smith's laboratory and he could play an important role in this new field of investigation. I strongly recommend him with the greatest enthusiasm as an ideal candidate for a faculty position in your department

Sincerely,

A handwritten signature in black ink, appearing to read "M. Lenardo", written in a cursive style.

Michael J. Lenardo, M.D.
Senior Investigator