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Dear Faculty Search Committee,

I am extremely pleased to recommend Dr. Zefeng Wang for a faculty position in the Department of Biology at Indiana University. Zefeng came highly recommended from a very productive and successful Ph.D. at Johns Hopkins and I was delighted that he chose to join my lab over others where he had offers. He is a highly intelligent and hard working scientist who simply 'gets things done' in the lab no matter whether this involves molecular biology or computational work or a combination of both. His scientific ability and bold ideas led to his being awarded a prestigious Damon Runyon Fellowship which supports his work in my lab.

Since joining my lab in 2002, Zefeng has done superb work, culminating in a wonderful project on the role of exonic splicing silencers (ESSs) in human pre-mRNA splicing, the results of which were published in the Dec. 17, 2004 issue of *Cell* (with Zefeng as first author), accompanied by a commentary written by Dr. Xian-Dong Fu of UC San Diego. In this study, Zefeng designed and synthesized a GFP-based splicing reporter system, constructed a clone library containing random decanucleotides inserted into the test exon of this reporter, created a library of stably transfected human 293 cells using the Flp-In (site-specific recombination) system, and screened this library using FACS for 10-mers with ESS activity. In all, he identified 141 ESS 10-mers (133 unique) sequences, representing the first large-scale experimental screen for ESSs in any species, and nicely complementing my lab's previous work on characterizing exonic splicing enhancers (ESEs). Zefeng went on to do extensive additional experimental and computational characterization of these sequences, for example, showing that all tested ESS 10-mers function in a heterologous exon context and in a second cell type (HeLa). Furthermore, he identified a set of over-represented hexamers in these sequences, called the FAS-hex3 set, and showed that these hexamers have many distinctive properties, including: over-representation in alternatively spliced (AS) exons, suggesting a possible role in control of AS; over-

representation in pseudoexons (pairs of decoy splice sites that are not recognized as exons), suggesting a role in pseudoexon suppression; and under-representation in constitutive exons with weak splice sites. All of these observations are consistent with important biological roles for these sequences in control of splicing. He also studied a database of known exonic mutations in the HPRT gene that are known to cause exon skipping and found that there was a significant tendency for these mutations to create FAS-hex3 hexamers, suggesting a possible application to the prediction of exon skipping mutations in human disease genes. Some of this computational work was done in collaboration with a graduate student in the lab, Gene Yeo.

In a related project (a portion of which is described in the Cell paper), Zefeng contributed to a splicing simulation algorithm, ExonScan, which was developed by Michael Rolish, an EECS (computer science) masters student in the lab. Incorporation of the FAS-hex3 hexamers into this splicing simulator gave substantial improvements in its accuracy, suggesting that the ESSs identified play important roles in pre-mRNA splicing specificity. Two technicians, Matt Mawson and Vivian Tung, worked under Zefeng's supervision on the experimental aspects of the ESS project. Zefeng generously suggested that they be included as coauthors on his paper (which they were), evidence of his gracious spirit.

I am also deeply indebted to Zefeng for almost single-handedly bringing "wet" biology to my formerly "dry" computational biology lab. In my mind, I think about my lab's history in two phases: BZ (Before Zefeng), when we focused almost exclusively on computational analyses, often collaborating with experimental labs to test predictions; and AZ (After Zefeng), where now we conduct many of our own experiments, allowing the lab to go in many interesting directions that would not otherwise have been possible. In addition to five or six lab members who do primarily computational work, there are currently three postdocs in the lab who are primarily experimental in their focus, as well as one or two graduate students. Zefeng's efforts in setting up the wet side of my lab, and quickly obtaining positive results, really paved the way for the experimental work that is now being carried out.

Over the past year, Zefeng has led two other projects in the lab, both of which are nearing completion and will be written up and submitted (with Zefeng as first author on both papers) to high profile journals by the end of 2005. In the first, he adapted his splicing reporter system to screen for intronic splicing silencers (ISSs). To date, he and another postdoc in the lab have identified over 100 ISS decamers, which cluster into several interesting new motifs, including one which resembles the binding site for the splicing repressor PTB. In the other, he has tested the hypothesis that ESSs play a role in splice site definition and has shown very convincingly that most/all of the classes of ESSs identified in his earlier work can inhibit usage of upstream 3' splice sites and of downstream 5' splice sites. He has also showed that tethering of a

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specific SR protein (ASF/SF2) and of a specific hnRNP protein (A1) to an exon can mimic the effects of ESEs and ESSs, respectively, on 5' splice site selection. He has conducted other experiments that illuminate the mechanism by which ESSs have their effects on splice site choice and show that ESSs play a role in regulation of natural alternative splice site exons. He has also worked on a couple of computational projects in order to develop his bioinformatics skills. In the course of this work, he has shown a strong facility for programming and computational work and has rapidly learned bioinformatics techniques.

During his time in my lab, Zefeng has designed and executed his research in a very independent manner, with little supervision from me, leading me to believe that he has very strong potential for a successful independent research career, and I look forward to collaborating with him on various projects when he is running is own lab. He has also taken advantage of opportunities to teach, organizing with another postdoc in the lab a seminar course on RNA splicing and disease for advanced MIT undergraduates. He is also an active participant in MIT's RNA journal club, and frequently volunteers to teach the theory and practice of molecular biology to others in the lab. He is also an excellent lab citizen, contributing to the organization of the lab as well as to lab social events. He is well liked by other lab members, including those whom he supervises, and those whom he collaborates with. He is that rare individual who is motivated not by a desire for personal advancement, but for the pure joy of doing science and contributing to human knowledge. In summary, Zefeng is an outstanding scientist with outstanding promise for an independent academic career and I recommend him enthusiastically. Please don't hesitate to call if you have any questions about Zefeng.

Sincerely,

Christopher B. Burge

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