C · H · O · R · I

Children's Hospital Oakland Research Institute

Elizabeth C. Theil, Ph.D.
Senior Scientist

October 24, 2005

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

Dear Dr. Brun,

Re: Xiaofeng Liu, Ph.D.

It is with great pleasure and enthusiasm that I recommend Dr. Xiaofeng Liu to you for a faculty position in Systems Biology/Microbiology in the Department of Biology and Biocomplexity Institute at Indiana University. Xiaofeng came to my Group four years ago as a new Ph.D. and I have watched him mature into an independent scientist of enormous energy, creativity, commitment and talent with a broad approach to investigating protein structure and function in bacteria and eukaryotes related to medicine and the environment. He is the best postdoctoral associate I have ever had in my Group and in the top 0.4% of the over 75 chemists/biologists I have mentored (undergraduate, pre- and post-doctoral) at North Carolina State University (NCSU), and now at CHORI, a private research institute, and at UC-Berkeley. Collaborative interactions with colleagues and their trainees at institutions such as Stanford, Duke, Emory, Michigan, Minnesota, and UNC-Chapel Hill, leading faculty searches at NCSU during a period of rapid departmental turnover where we evaluated ~ 2000 applications over time, as well as knowledge gained by participation in both national and international scientific communities, increase further the basis for my comparison.

Effective communication is one of Xiaofeng's strengths. He had direct teaching experience for seven years in both biochemistry and chemistry as a post- baccalaureate lecturer (3 years in China), and as a graduate student (4 years in the US). While I have not personally observed him in the classroom, I have observed him giving talks at National meetings and fielding tough questions with aplomb. His Group Meeting presentations are always clear and well–prepared. He is a leader in the lab and has made solid friendships at CHORI. Xiaofeng has an easy and productive manner when instructing undergraduates in our lab and has recruited several undergraduate premeds to careers in research science.

When Xiaofeng joined my Group, he had just received his Ph.D. in Biochemistry from Iowa State University, studying the structure and functions of brain hexokinase and was the first author or co-author of three unusually strong, solid papers in outstanding journals (*Biochemistry, J. Biol. Chem. and J. Mol. Biol.*). His doctoral research laid the foundation for studies of hexokinase-mitochondrial interactions and the role of hexokinase in apoptosis. Papers based on his seminal work as a graduate student are continuously appearing. Xiaofeng is also first author on a paper about the mechanisms of catalyzing esterification of solid acids and holds a patent for the synthesis of epoxy resins, based on post-baccalaureate activities in China. What impressed me particularly about Xiaofeng at the time he interviewed for my group was his intellectual independence and strength, and his outstanding experimental ability, coupled with a depth of character that was rare to see in someone of his age.

Ferritin structure/function studies, the subject of his research in my Group, coupled his previous experience in catalysis, spectroscopy, and protein engineering with new expertise in gated pores, iron biochemistry and protein chimeras. During the time he has been here, his intellectual development

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burgeoned and his productivity increased. Xiaofeng is the first author on two Proc. Nat'l. Acad. Sci. (USA) papers (2 or 3 authors), one a cover article on how ferritin pores unfold, the other on a direct demonstration of the Fe ligand sets in the di-iron ferritin ferroxidase sites; he is also coauthor on two other papers and a review with me in *Accounts of Chemical Research,* also a cover article. Before Xiaofeng leaves, he will be first author on 2-3 more papers from our Group (one is currently in revision) and 1-2 papers with our colleague Ed Solomon (Stanford) on Fe (II) interactions with the ferritin catalytic site, using MCD.

The work Xiaofeng has completed in my Group on vertebrate "maxi-ferritins," the 24 subunit protein nanocages that form iron minerals and are characteristic of all life forms, has been truly revolutionary. When he first arrived, we had only a rudimentary knowledge of the properties of the pore helices and gates from mutagenesis, coupled with assays of chelation rates. Xiaofeng discovered the pore regions of the ferritin protein nanocages were very sensitive to heat, melting (CD spectroscopy) at least 30 degrees below the cage itself, and to chaotropes. With low concentration of unfolding reagents such as urea, at physiological (millimolar) concentrations, the pores melt close to body temperatures. In search of a combinatorial library (10-12 peptides), he found two that bind to the ferritin pore with high affinity, using both closed (wild type) and open (mutant) proteins, in the selection and finding two with high affinity, that change pore function and have the potential for novel, rapid iron chelators for medicine. A second project has been the direct identification of the functional iron ligands at the ferritin catalytic site, using protein chimeras with a host that was naturally inactive catalytically. For the last several years he has received part support from a Fellowship provided by the Cooley's Anemia Foundation; his proposal received the top score.

Developing a new, independent research program has taken some of Xiaofeng's time over the last year. The project explores the behavior of Bacillus-specific paired mini-ferritins (12 subunit polypeptide nanocages), or Dps proteins, which he characterized from *B. anthracis*, finding complementary activities of hydrogen peroxide reduction by Fe (II) and DNA protection in each Dps protein. His kinetic characterization studies and genomic comparison with other bacillus Dps proteins, as well as plans to examine physiological function of the two proteins in cells with Dps deletions, form the basis for a manuscript in revision, 1-2 more manuscripts in development, and an NIH R03 proposal he submitted October 1, 2005. The proposal is well-developed and constructed, in my opinion, and lays the foundation for a research program rich in biochemistry and microbiology, and, because Dps proteins scavenge iron and resist host oxidants during infection, a project with fundamental medical and environmental impact.

In my opinion, Xiaofeng will be an outstanding appointee to a tenure- track position, an enormous asset to any department he joins, and will become as valued a colleague to you as he has become to me. His teaching experiences and his training as a biochemist at Iowa State were outstanding, his accomplishments in my group are without peer, his ideas and strategies are well developed, and his enthusiasm and dedication are boundless. Beyond his scientific talents, he has always conveyed a personal depth and understanding that is extraordinary. If I have not managed to convey effectively that Xiaofeng personifies the best and brightest of young academic scientists, please call or write to me for more information.

Sincerely,

Elizabeth C. Theil, Ph.D.
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Professor, Nutrition Science & Toxicology, University of California-Berkeley (adj.)
University Professor of Biochemistry and Physics, North Carolina State University (former)