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Jeremy Bennett, Faculty Search Coordinator
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Dear Dr. Bennett:

I am writing this letter in support of Dr. Jiaxin Li's application for a position in the Department of Biology at Indiana University. Jiaxin received his Ph.D. in my laboratory in 1998. During his time here he concurrently pursued degree in our department and in computer science and bioinformatics, receiving an M.S. in Computer Engineering in 1999. He used his abilities in bioinformatics to deduce the evolutionary history of the arsenic resistance proteins, which was a valuable addition to his wet bench research. He continued as a postdoctoral associate with Tom Gelehrter at the University of Michigan, was the Director of the Bioinformatics Unit at Mount Desert Island Biological Laboratory and is currently in a similar position at the Virginia Bioinformatics Institute.

Jiaxin did outstanding work as a graduate student, and I would have no hesitation in giving him my strongest recommendation. His research in my laboratory focused on the structure and function of the As(III)/Sb(III)-dependent ArsA enzyme, the catalytic subunit of the arsenite-translocating ATPase. This is a membrane-bound complex encoded on bacterial resistance plasmids which functions to provide resistance to the toxic oxyanions arsenate, arsenite, and antimonite. The ATPase pumps the anions out of the cell as fast as they enter, so that the intracellular concentration never reaches a toxic level. The Ars ATPase is one of the best model systems for multidrug resistance and is frequently on the schedule at meetings about P-glycoprotein and related MDR systems. It is also the first enzyme shown to have a biological requirement for arsenic.

In addition, arsenicals and antimonials have been and are still used as antimicrobial agents, for example Salvarsan, the first antimicrobial agent, and Pentostam, the treatment of choice for leishmaniasis. They are widely used as herbicides, pesticides, growth supplements and crop ripening agents. Pressure-treated wood is soaked in arsenic, which is why decks don't rot (and why nothing

grows within a foot radius of the posts). Ortho Crabgrass Killer II has methane arsenate as its sole active ingredient. This selects for arsenic resistant bacteria in lawns and fields, and those bacteria eventually find their way into the water supply. Similarly, the use of arsenicals as feed supplements for pigs and turkeys sometimes results in the spread of resistant bacteria through undercooked food. Since the plasmids which bear these organisms carry multiple drug resistances, they spread resistances, which is a most serious clinical problem in hospital settings.

Jiaxin first came to my laboratory as a rotational student - each student does three rotations before choosing a lab. He worked with two postdocs on the mechanism of allosteric activation of ArsA. The enzyme is activated by binding of arsenite or antimonite. However, it was not known whether activation required binding of the metalloid oxyanions or whether soft metal chemistry was involved. To provide a mechanistic explanation for allosteric activation, Jiaxin mutated arsA by site-directed mutagenesis, demonstrating through alteration of the codons for cysteine residues that activation required metal-thiol chemistry. Jiaxin's work led to a 1995 publication - he is one of the few students in our department to become a JBC author for a 10-week rotation. The crystal structure has since verified his predictions, showing that As(III) or Sb(III) forms a complex with three cysteines in ArsA, an absolutely novel metal-thiol structure in biology!

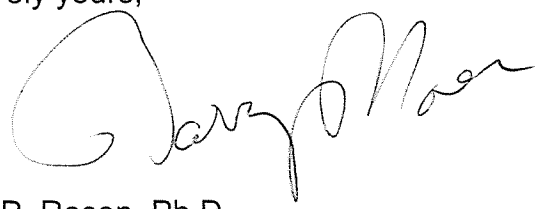
His project upon joining the lab was analysis of the nucleotide binding sites of the ArsA ATPase. He has used a combination of classical genetics - second site suppressor analysis - and biochemistry to demonstrate that the two nucleotide binding sites of ArsA interact to form the catalytically competent species. His work led to a new model for the mechanism of allosteric activation, where formation of the novel metal center pulls together the two halves of the protein, forming an interface of the two ATP sites. This supplanted the previous model, where I hypothesized that the protein dimerized, and the two sites interacted between monomers of the dimer. Jiaxin not only disproved that model, but he wasn't afraid to tell me that I was wrong. It takes a lot of courage to contradict the pet hypotheses of the head of the lab! Again, his predictions have been borne out by the crystal structure.

Jiaxin's research resulted in six publications, including three first-authored papers in the Journal of Biological Chemistry and Molecular Microbiology, plus several book chapters. Our papers are a collaborative effort, but Jiaxin always writes the first drafts. He has a very good idea of what constitutes good (and bad) scientific writing - at my request as an editorial board member, Jiaxin frequently reviewed papers for the Journal of Biological Chemistry. In fact, his command of written English is better than mine - I usually ask him to critique our manuscripts because he's the best at it in the lab.

Jiaxin's seminars are well organized and professionally presented. On a personal level he is very congenial and interacts well with his colleagues. He has used tools of molecular biological and biochemistry and is absolutely unafraid to try any technique. He works both hard and well. Since I am frequently away, he used his own initiative to design experiments. He learns easily from others and was always willing to teach new members of the lab techniques such as DNA sequencing and site directed mutagenesis. He is a genuinely nice person who gets along well with others.

In summary, I would recommend Jiaxin Li with highest enthusiasm. Please call me if you require additional information.

Sincerely yours,



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