

DEPARTMENT OF MOLECULAR AND  
CELLULAR BIOLOGY  
HARVARD UNIVERSITY



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September 22, 2005

Dear Colleagues:

**Dr. Jie Xiao**, a postdoctoral fellow in the laboratory of my colleague in Chemistry & Chemical Biology, Professor Sunney Xie, has asked me to write a letter of recommendation on her behalf, and I am pleased to do so. I follow the work in the Xie laboratory with great interest, and I have interacted with Jie on several occasions both at Harvard and at various meetings where she has presented on her work. Jie and another fellow in the lab, Yu, have been spectacularly successful in developing a method for visualizing single protein molecules in a cell, one at a time. A key and clever idea in this work is tethering the green fluorescent protein tag to the membrane so that its diffusion is limited to two dimensions, thereby greatly improving the sensitivity of detection. Jie is co-first author on a paper that is under review for *Science* on this impressive story. Jie proposes to exploit this method to address to interesting and important problems in microbial biology: the interaction of sensor kinases with an important class of regulatory proteins called response regulators and the assembly of the cytokinetic machinery (divisome) in *E. coli*. My sense about Jie, who has expertise both in biophysics and molecular genetics, is that she has the intellectual tools to be successful in her proposed program of study. Also, she is coming from a truly outstanding laboratory. I hope that these brief comments are of assistance.

Sincerely,

A handwritten signature in black ink, appearing to be 'R. Losick', written in a cursive style.

Richard Losick



RICE

KATHLEEN S. MATTHEWS, DEAN  
AND STEWART MEMORIAL PROFESSOR

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September 21, 2005

Dear Colleagues,

This letter is written at the request of Dr. Jie Xiao, who is applying for a faculty position in your department. As a member of her Progress Review/Thesis Committees during her graduate studies, I observed her development and evaluated her research over a period of several years.

Dr. Xiao is a very bright and capable person, with a level of enthusiasm for her work that is rare. She appreciates feedback, discussion, and criticism and takes the information and applies it immediately to enhancing her research effort. She is thorough, thoughtful, reflective, and at the same time she is very proactive and capable in executing the experiments. As a graduate student, she undertook a very complex study of the RecA protein that is centrally involved in strand exchange during homologous DNA recombination. Her structural characterization of the key intermediate, the RecA triple-stranded DNA complex, using fluorescence resonance, was challenging and complex. The results that she obtained were exceptionally interesting and reflected a significant level of maturity in experimentation. She also characterized the kinetics of forming this RecA triple-stranded complex using rapid mixing fluorometry. Again, these experiments were very demanding, and she carefully and quantitatively analyzed the results.

Her work in Sunney Xie's laboratory at Harvard University is exciting. She is developing approaches to generate probes for real-time monitoring of gene expression within cells at the level of single molecules. Her systems range from bacterial to eukaryotic (specifically neural cells). This work has led to intriguing results that expand our understanding of gene expression in a range of biological systems.

In addition to her considerable experimental skills and intellectual capacity to design, understand, and interpret her experiments, Jie is a delightful person. She is friendly, open, interactive, and enthusiastic. A testimony to the overall positive perception of Dr. Xiao is that, following her presentations in progress reviews, all of her thesis committee members would note that we would welcome her into our laboratories at any time!

Based on my experience with her, I recommend Dr. Xiao to you with enthusiasm. She is an excellent researcher and would be a wonderful faculty colleague. If you should have any further questions, please do not hesitate to contact me.

Sincerely yours,

A handwritten signature in cursive script that reads "Kathleen Matthews".

Kathleen Matthews

September 26, 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 Dr. Brun:

I am writing to support Dr. Jie Xiao's application for junior position in your department. Jie's formal education is in biology, but her postdoctoral training is at the interface of biology and physical chemistry. Her current research interest is to improve the detection limit for green fluorescence proteins in live cells to the single molecule level. It is her unwavering determination and ability to work with physical chemists that has allowed her to make great progress towards this goal.

Jie is a postdoctoral scientist, working with Professor Sunney X. Xie in the Department of Chemistry and Chemical Biology at Harvard University. Last year I was on Sabbatical with them. After working with Jie for a whole year, I have become to know her very well. As a longtime collaborator with Sunney and biologist myself, I have observed the establishment of adequate biology capability in Sunney's lab. Jie played a critical role in building up the capability. Jie has managed to train several chemistry graduate students and postdoctoral associates in doing molecular biology, while learning the most advanced microscopic techniques from them. Jie's willingness to enter into physical chemistry may slow her down in terms of publication. However, her experience has put her at the forefront of discovery. She is combining biology and physical chemistry techniques to improve the detection limit in live cells.

Jie has worked on several projects involved in reporter development for low-level gene expression in live cells. Her first project was on the detection of single  $\beta$ -lactosidase in live *Escherichia coli* cells by single-molecule microscopy. Jie engineered an N-Terminal fusion of  $\beta$ -lactosidase with ubiquitin, an eukaryotic tag for rapid degradation by proteosomes in *E. coli*, so that the half life of the enzyme is shortened from hours to minutes. Using the native promoter of  $\beta$ -lactosidase gene under uninducing conditions, they observed the scholastic expression of the gene as *E. coli* cells occasionally produce a few copies of the enzyme. In the presence of the enzyme, the cell converts fluorogenic substrate DDAO-galactose into galactose and fluorescent DDAO. The rapid accumulation of DDAO makes the cell visible with a single-molecule microscope. Jie made a movie of the transient fluorescence of the single cells due to the scholastic production of the unstable  $\beta$ -lactosidase. The movie caused great excitement at the 2004 American Chemical Society Annual Fall Meeting in Philadelphia, as it was the first time that gene expression has been monitored in live cells in real time.

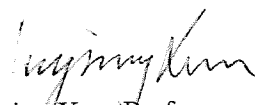
Jie's current project is to improve the detection limit of green fluorescence protein (GFP). I am impressed by what she has done. In literature, the detection limit in live *E. coli* cells is about 30 copies per cells. When she immobilized the GFP to non-diffusible cellular components, she detected single GFP molecules in live cells. The improvement is a simple concept that a diffusible GFP moves too fast to be captured by snapshots, whereas the immobilized GFP can be captured by snapshots with several ms exposures. This is a major contribution for the detection improvement and its application potential in biological research is anyone's imagination. She worked on this project closely with Dr. Ji Yu, who is a physical chemist. They have prepared a manuscript that is under review by *Science*. She has traced gene expression for many GFP tagged membrane proteins with time-lapse techniques, and she is analyzing the data and preparing manuscripts.

Jie's molecular techniques are impeccable. For example, she has constructed many gene expression reporters into *Escherichia coli* under the control of a *lac* operon in a short time frame. She used a Gateway cloning system to clone genes and then transfer the cloned gene with proper reporters into *E. coli* chromosome by using a  $\lambda$  recombinase system. I was most impressed with the use of the  $\lambda$  recombinase system because that is the system normally for gene inactivation not for reporter construction.

After working in Sunney's lab for several years, Jie has become familiar with setting up microscopes, lasers and cameras as well as their maintenance. She also can use and program computers for automated data collection. She understands and can use the statistical methods to analyze single-molecule data.

Jie is a highly devoted scientist. She works long hours and engages in enthusiastic discussions on research topics. Her accomplishment and training in both biology and single-molecule microscopy give her unmatched advantages in multidisciplinary research, where significant progress will be achieved in biology for the years to come. If you are hiring a molecular biologist using single-molecule microscopy to study gene expression in live cells, Dr. Jie Xiao is the best candidate. At a personal level, Jie is a wonderful person to work with as she is very considerate, helpful, and easy to get along.

Sincerely,



Luying Xun, Professor of Microbiology  
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**SCOTT F. SINGLETON, PHD**  
*Associate Professor, Director of  
Graduate Studies for New Students*

October 10, 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:

It is my great pleasure to write this letter on behalf of **Jie Xiao** who has applied for a **faculty position** in your department. Simply stated, Jie is among the very best students that I have known. Jie's intellectual skills are far above average and compare quite favorably to those of the best graduate students I have known. In addition, Jie's ability to set goals and her enthusiastic dedication to success are rare among students that I have known. In summary, Jie possesses many of the abilities – and the clearly demonstrated commitment – to become an outstanding independent scientist. Based on my experiences as Jie's graduate research advisor, allow me to elucidate my conclusions.

Jie conducted her Ph.D. thesis research in my laboratory from June 1998 until May 2002, during which time I served as her research and career mentor. In my lab, her projects involved the use of steady-state and time-dependent spectrofluorometry to elucidate key structural and kinetic parameters that define homologous DNA strand exchange mediated by the RecA protein of *Escherichia coli*. RecA plays essential roles in the bacterial processes of homologous recombination and recombinational DNA repair, during which DNA strand exchange between single-stranded (ssDNA) and homologous double-stranded DNA (dsDNA) molecules is facilitated. During this process, a RecA-triple-stranded DNA (RecA-tsDNA) complex is formed that serves as the key intermediate in strand exchange. When our work began, neither the structure nor the mechanistic mode of assembly and resolution of the RecA-tsDNA intermediate had been elucidated in molecular terms.

Jie developed a systematic and non-invasive method for isolating the helical structural information of the DNA bound inside a RecA filament using fluorescence resonance energy transfer (FRET) under physiologically relevant solution conditions. This work resulted in the publication of two papers: one invited for a special edition of *Nucleic Acid Sciences* (edited by Professor David Millar of Scripps Research Institute), and the second in the *Journal of Molecular Biology*. Our work was one of the first examples of the systematic use of FRET to extract helical geometry

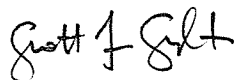
parameters for deformed DNA in a protein-DNA complex. Certainly, this work represents the first FRET-based structural characterization of a RecA-DNA complex. Importantly, Jie was able to propose a fundamentally new structure for the DNA in this complex. Moreover, the data allowed the construction of a model for the strand exchange intermediate. In Jie's second project area, she monitored RecA-mediated DNA strand pairing of short DNA substrates using stopped-flow spectrofluorometry. The use of intrinsic DNA fluorescence in the context of short DNA molecules enabled her to observe short-range interactions and local environmental change during the process. Jie was able to propose a molecular mechanism for the role played by RecA in DNA strand exchange. This work resulted in three manuscripts (two with another graduate student coauthor) that have been submitted for publication recently.

Jie's long-term interests include the application of biochemical and biophysical methods to the elucidation of molecular mechanisms in microbiology. I read her research prospectus and am thoroughly convinced of its substantial value to biology, pharmacology, and human medicine. This research will enhance our fundamental understanding of the complicated physiologies of pathogenic bacteria at the molecular level in nearly unprecedented ways. Moreover, her results have the potential to transform the way new drugs are discovered and evaluated.

Jie is very bright, self-motivated, creative, and diligent in her efforts. Indeed, she was an absolute joy to work with. During the projects described above, Jie has developed a broad base of experimental competencies. Moreover, Jie is remarkably independent and was instrumental in every stage of experimental design and interpretation. In addition to her research skills, Jie was also a responsible research group "citizen" and an effective communicator. She will make an exceptionally fine colleague.

In summary, Jie is one of the very best students that I have known in several academic settings: as an undergraduate with Ben Plummer and Mike Doyle at Trinity University (San Antonio, TX), as an NSF Graduate Fellow with Peter Dervan at the California Institute of Technology, as an NSF Postdoctoral Fellow with Steve Benkovic at the Pennsylvania State University (University Park, PA), at Rice University, and at UNC Chapel Hill. Indeed, Jie is the best graduate student I have seen at Rice or UNC, and she would compare favorably with the best at Caltech. Without any reservation, I give Jie my strongest recommendation. Without a doubt, Jie possesses the skills to become the kind of scientist who is a leader among her peers: she excelled as a graduate student and as a post-doc, and will stand out as an independent scientist. I urge you to grant Jie's application the full attention it merits: she will make an excellent addition to your department. If I may address any questions concerning Jie's candidacy, please feel free to contact me.

Sincerely yours,



Scott F. Singleton, Ph.D.



## HARVARD UNIVERSITY

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Professor of Chemistry

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November 29, 2005

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

### Confidential

Re: Dr. Jie Xiao's Candidacy for a Faculty Position

Dear Dr. Brun:

I am writing in most enthusiastic support of **Dr. Jie Xiao's** application for a faculty position in your department. Jie has been a postdoctoral fellow in my research group for three years. She is an outstanding young molecular biologist and I urge you to give her strongest consideration.

I first met Jie Xiao at the Biophysical Society meeting in 2002 where she won a poster award for her graduate work. I was delighted to attract her to my laboratory, where she joined my group's effort of taking single-molecule experiments into living cells. This was a new project, very difficult, but very exciting. We were trying to detect single copies of protein molecules, as they were generated one at a time in live *E. Coli* cells.

The central dogma of molecular biology states that DNA is transcribed to messenger RNA (mRNA) and mRNA is translated to protein. Gene expression, consisting of the transcription and the translation process, is an important biological process that is pertinent to every cell function, such as growth, division, signaling, and programmed death. Although the central dogma has been confirmed without doubt, the gene expression process has not been visualized in real time in a single living cell. For decades, biologists have been conducting *in vitro* scrutiny of m-RNAs and proteins by lysing the cell and measuring the property and reactivity of a large ensemble of molecules of a particular m-RNA or protein from many cells. Much information is lost or distorted in this reductionist approach.

Our approach is to develop new reporter proteins that allow *in vivo* observations, i.e. experiments on living cells. We were trying to detect single copies of protein molecules as they are generated in real time in live *E. Coli* cells. Jie adapted a fast maturing yellow fluorescence protein (YFP) in order to accomplish this. YFP is a popular reporter for monitoring gene expression in live cells. However, it has been impossible to image a single YFP molecule in cytoplasm with a fluorescence microscope because the molecule undergoes fast diffusion and its weak signal spreads to the entire cytoplasm during the image acquisition time and is overwhelmed by cellular autofluorescence. Our strategy was to immobilize the YFP molecule on the membrane in which the diffusion is significantly reduced. The fluorescence from a single YFP molecule is focused only on a few pixels of the CCD camera instead of the entire area of the cell, surpassing the autofluorescence background signal.

Like any first-of-its-kind experiment, the beginning is always tough. Although we began the effort before Dr. Xiao joined the group, we lacked the necessary biological expertise. It is fair to say, the turning point was when Jie joined our group. She quickly trained group members in molecular biology techniques, while at the same time she learned the ropes of sensitive detection with fluorescence microscopy. Jie came up with a plan for making a dozen fusion proteins and screening them, constantly adjusting the plan dependent on the experimental outcome. Working with Dr. Ji Yu, another postdoctoral fellow, she finally achieved our goal of developing such a reporter system.

With this reporter protein, Jie and Dr. Yu were able to see individual proteins being churned out one at a time. This is an unprecedented result! They made a major breakthrough in the demonstration of real-time observation of gene expression in living cells one protein molecule at a time. The experiment reveals that protein molecules are produced in randomly distributed bursts, and that the number of molecules in each burst follows a geometric distribution, which was never directly observed before. We were all on cloud nine! Jie's creative mind and her expertise in molecular biology guided her path to success.

Currently, this work is being reviewed for publication in *Science* magazine. This is the most biologically significant result my group has ever obtained. I believe it will open up exciting possibilities to probe gene expression at a quantitative level with single copy sensitivity.

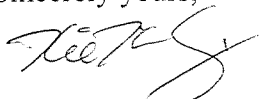
Jie has good communication skills. She speaks and writes English well and gives excellent presentations. I have observed her many presentations at our formal group meetings and at conferences. She is always very clear and extremely organized. She possesses the patience and positive attitude necessary to instruct students. I think she will become a wonderful teacher.

On a personal note, Jie is an intrinsically kind person, always ready to help people. She is an asset to the group and has demonstrated a good attitude towards failure, maintaining optimism despite setbacks. Jie is also a natural research collaborator for interdisciplinary research and will make a wonderful colleague. She is highly efficient in her work and multitasks well. Since recently becoming a mother, she has also shown that she can balance family life with her career.

I have had the good fortune to work with many outstanding postdoctoral fellows in my career. Many went on to academia: Bob Dunn at Kansas, Peng Chen at Cornell, Ji-xin Cheng at Perdue, Lukas Novotny at Rochester, Antoine van Ojien at Harvard Medical School, Eric Potma at UC Irvine, and Haw Yang at Berkeley. Jie is as brilliant and as determined as any of them, but she is unique in that she was the only one in my group who came from a biological background. I should mention that I made offers to six people with a biological background to start our effort on gene expression before Jie joined us, yet the lasers and microscopes in my lab intimidated each one. Jie, on the other hand, was unafraid to explore new terrain, and she saw only opportunity and embraced the challenge posed by this new research area.

In summary, Dr. Jie Xiao is an outstanding molecular biologist with biophysical training. She is a dedicated and talented young scientist who brings enormous energy and intellect to cutting edge research and she will continue to make important scientific contributions. Working with her has been a great pleasure for me. I give her my strong recommendation for the faculty position in your department and I urge you to give her an interview.

Sincerely yours,



X. Sunney Xie