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December 7, 2005

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

Dear Yves Brun,

It is my pleasure to recommend Dr. Ke Hu for a Faculty position in the Department of Biology at Indiana University. I have known Ke since she joined my lab as a post-doctoral Associate in September 2003. During this time she was honored as a prestigious Leukemia and Lymphoma Society Special Fellow, an extremely competitive fellowship awarded to outstanding post-docs who are close to starting their independent careers. To start off, I must first say that Ke is clearly the independent, intellectual, and technical star of my current lab of 9 members (6 post docs, each supported by their own fellowships, 2 grad students (one supported by HHMI), and a technician). I rank Ke in the top 5% of the very outstanding post docs and students I have had the pleasure of interacting with over my short 6 years as a faculty member (2 former post docs with faculty positions at UCSF and Netherlands Cancer Institute). She is extremely hard-working, unstoppably determined, deeply thoughtful, thorough, independent and capable. Ke has a longstanding association as a TA in Cold Spring Harbor, one of the best microscopy courses in the country, through her association with John Murray from Penn. She is one of the most knowledgeable people I know in terms of thorough understanding of state of the art light microscopy techniques, and an absolute expert in molecular and cell biology. In the following letter, I will comment on Ke's performance and achievements since I have known her in my lab. Clearly, Ke's prior achievements during her PhD at University of Pennsylvania were also exceptional.

In my lab we are interested in the dynamics of the cytoskeletal polymers, microtubules and actin, and how they generate forces on the extracellular environment to drive directed cell motility in normal and invasive cells. In general terms, Ke's completely unique and very challenging project in my lab was to measure the molecular interactions between the actin cytoskeleton and focal adhesion proteins to determine the molecular mechanism by which cells transmit biochemical and mechanical information from the actomyosin cytoskeleton through integrin-mediated focal adhesions to the extracellular matrix (ECM) in cell migration. In order to achieve this, she had to develop a completely novel quantitative microscopic method and computational analysis scheme for measuring protein-protein interactions at the cell-ECM interface, and to apply it to migrating epithelial cells to get some very novel and important mechanistic insight into the fundamental basis of cell migration. She has in fact, in two years, achieved this remarkable goal, and is currently in the throes of submitting a manuscript to *Science*. In order to achieve this, Ke, who came to me as a parasitologist with some background on the cytoskeleton of parasites and an outstanding training in light microscopy, had to learn the field of cell biology of the vertebrate actin cytoskeleton, cell-adhesion, and tissue cell migration. She also had to work in close collaboration with a mathematician, Dr. Jin Li in the Computer Vision lab of Gaudenz Danuser at Scripps to guide the biological thinking behind the development of the very sophisticated image analysis algorithms.

My lab developed a novel quantitative light microscopic assays called fluorescent speckle microscopy (FSM), which allows quantitative analysis of the assembly/disassembly and movement of molecules within macromolecular assemblies in living cells. FSM had only been performed with conventional epi-fluorescence microscope and used to study only cytoskeletal biopolymers. To initiate her project, Ke had to prove that the principle of FSM could be combined with total internal reflection fluorescence (TIRF) microscopy and applied to study the dynamics of focal adhesion structures where forces in the cytoskeleton are transferred to the extracellular matrix. Ke collected a host of focal adhesion protein cDNAs, cloned them into a GFP vector with a truncated the CMV promoter to drive low level expression for FSM, expressed the constructs in epithelial cells, and produced TIRF images showing for the first time that TIRF-FSM could be used to visualize the dynamics of non-polymeric macromolecular assemblies; i.e. focal adhesions. This was one specific aim of my NIH GM67230 technology development grant achieved in a couple months by a single post-doc!!

Ke's next goal was to develop a new technology called Correlational TIRF-FSM for measuring interactions between actin filaments and focal adhesion proteins during cell migration. She worked intensely with Lin Ji to extend current particle tracking based analysis of molecular dynamics in FSM image in one spectral channel to correlative particle tracking in two spectrally distinct channels in TIRF-FSM. The goal of this development was to quantitatively analyze the degree of correlation between the motion of fluorescent molecules labeled with spectrally distinct fluorophores, such that a high degree of correlated molecular motion indicates an interaction or "kinematic coupling" between the molecules. To quantitatively describe the kinematic coupling of actin and focal adhesion molecules, Ke and Lin had to conceptualize and implement two new metrics. A "direction correlation score" describes the coherence in direction between the different molecules, and a "speed correlation score" represents how the similar the speed of movement of focal adhesion molecules along the actin axis compares with the speed of actin itself.

Ke then applied this exciting technology to do some fascinating cell biology. Ke has used her correlational analysis of two-channel TIR-FSM data of fluorescent actin along with GFP-conjugates of a variety of focal adhesion proteins (integrin αv, paxillin, α-actinin, zyxin, talin, vinculin, and FAK) to screen for molecules that may mediate the dynamic linkage between actin and the ECM to mediate cell migration. Her study represents "first times" for a number of important acheivements. First, previous studies of focal adhesion dynamics in migrating cells have treated individual focal adhesions as single entities, and features of intra-adhesion molecular dynamics have never been explored. Second, a comparison of the molecular dynamics of multiple focal adhesion molecules has never been performed in a single cell type, even by standard methods such as FRAP. And finally, no quantitative analytical comparison of actin and adhesion molecule dynamics measured simultaneously in living migrating cells has ever been performed. It should be pointed out that these are VERY technically demanding, time consuming experiments that require exceptional skill in live-cell high resolution imaging, and Ke cranked out more high quality data than I thought was possible.

Examination of only the GFP-focal adhesion channel of her two-channel datasets surprisingly revealed two classes of protein dynamics exist within individual focal adhesions, with some molecules coupling to the stationary ECM that is bound to the substrate, and some molecules kinematically coupling to the dynamic actin cytoskeleton. She next used her correlational FSM software to verify and quantitatively analyze the degree of kinematic coupling between GFP-focal adhesion molecules and actin. This revealed a low degree of kinematic coupling between actin and integrin, FAK, zyxin and paxillin, as indicated by low direction and speed correlation scores. In contrast and quite remarkably, her analysis revealed a high degree of kinematic coupling between actin motion in the cell and GFP-conjugates of α-actinin, talin, and vinculin within focal adhesions. By mapping individual correlation scores onto their local

positions in the cell she found these molecules exhibited a varying degree of kinematic coupling with local actin motion between and within individual focal adhesions. She has now shown that strong kinematic coupling of actin and vinculin immediately precedes release of a focal adhesions from the ECM, and that this coupling can be engaged by activation of the small GTPase Rac1. Thus, Rac1 may activate the link between actin and vinculin to loosen the grip of focal adhesions on the environment when adhesions turn over during cell migration. Thus, her work is an example of her ability to do creative and thorough technology development and apply it do excellent, thoughtful mechanistic cell biology.

As an example of her independence, while she was carrying out these experiments, at night, and unbeknownst to me, she developed a purification protocol for a crude preparation of focal adhesions from cultured cells, set up a collaboration with the Yates lab (one of the best Mass Spec labs in the world), and performed a proteomic analysis of focal adhesions. She got peptide sequence on the components of the crude focal adhesion prep (with proper controls), cloned four of the proteins she identified, subcloned them into GFP vectors, and began simple functional analysis by expressing them in cells and performing FRAP experiments.

Ke's plan is to apply her knowledge and expertise in cytoskeletal cell biology, proteomics, parasitology, and state of the art microscopy to study the cytoskeleton of the malaria parasite. I realize that it is non-traditional for a post doc to initiate a research program that is so clearly independent of her post-doctoral studies. However, I have 100% confidence that Ke has the personal drive, focus, and uncommon level of intellectual capability to implement a productive research program on this very unique and medically relevant problem. In summary, I feel that Ke would be an excellent addition to your program. She is armed with state-of-the-art techniques in biochemistry, molecular biology, and cutting edge light microscopy that will allow her to answer important questions about the role of the cytoskeleton in parisitology, for an exciting and productive career. Please do not hesitate to contact me if you have further questions regarding her application.

Sincerely,

Clare Waterman-Storer
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December 06, 2005

Dear Dr Brun,

It is my pleasure to recommend Dr Ke Hu most enthusiastically for a faculty position in the Department of Biology at Indiana University, Bloomington. Dr Hu is a dedicated and thoughtful scientist who has had great training and is tackling the problem of understanding the pathogenic model organism Toxoplasma with world-class cell biology. Ke has to be one of the best and most dedicated scientists of her generation. I urge you to interview her and get a sense of her ability and scientific talents.

I first met Ke in October 2000 while running a course at Cold Spring Harbor Laboratory (see below). Since then, I have spent two weeks each year working with Ke, so I have known and worked with her through the latter part of her PhD and postdoctoral studies. Ke's thesis focused on *Toxoplasma gondii*, an important intracellular pathogen that is the leading cause of congenital birth defect and a devastating opportunistic parasite in immuno-compromised patients. Its pathogenesis is directly related to its uncontrolled, fast replication and invasion into a host cell, the understanding of which requires a thorough study of its basic cell biology. Because of its small size, it has been extremely difficult to study the internal structure of the parasite. Dr. Hu is one of the first people to apply advanced light microscopy to study the proliferation and invasion of living parasite. She developed numerous fluorescent markers to follow various aspects of cell replication in living cell. In addition, she discovered a new tubulin polymer in the major invasion organelle involved in the cell motility of the parasite. In her first postdoctoral position with Dr. John Murray at University of Pennsylvania, she purified this organelle and identified more than 200 new components and at the same time revealed novel cytoskeletal structure features in the parasite never suspected before. This work was a critical insight, and essentially opened a whole research direction for the Toxoplasma field. The apical conoid is also critical for infectivity, so Ke's work opens up new targets for therapy. Her work is of tremendous importance, not only because she contributed greatly to molecular and cellular parasitology of *Toxoplasma gondii*, but also for developing *Toxoplasma gondii* into an extremely attractive model system for studying cell division and cytoskeleton biogenesis in general, a notion that was oblivious to most researchers in main-stream cell biology before her work.

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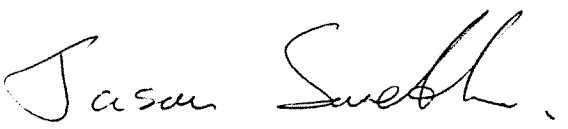
Since Oct 2000, I have had the opportunity to work with Ke once a year in the course I run (together with John Murray and Abby Dernburg) at Cold Spring Harbor Laboratory entitled Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging. Officially, John, Abby, and I are "Instructors", while Ke is listed as an "Assistant Instructor". These titles reflect CSHL administration's requirements. We run our course so that we are all instructors—we all lecture, design and run labs, and do the grunt work. Ke gives two lectures in the course and these talks are always clear and well-presented. This annual course has also given me a chance to see Ke develop through her PhD and postdoctoral studies. Ke has always done beautiful work and always had incisive questions, but has really matured in the last two years as a confident scientific colleague. She has a real breadth of knowledge and often leads the questions during our course seminars (given by invited external speakers). In short, there is a very strong intellect and a tireless dedication to experimental design and work. She is a great colleague, commits herself to great lectures and student mentoring, will certainly drive a very active and world-competitive research program. Moreover, Ke has achieved a competitive advantage—in her PhD!—which is now so necessary: she has crossed many disciplines, and mastered them all, and is now prepared to become a great cell biologist.

Before I close, a word about me—I am an American, working and living in the UK. After almost 8 years, I have come to appreciate the European style of reference letters. There is no use in claiming that every job candidate is the next Messiah, thus my letters are taking on the more reserved tones of my local colleagues. Without bombast or exaggeration, I will say that Ke is one of the best young scientists I have come across—if I could I would hire in my own department.

In short, her Ph.D thesis was extremely productive, and Ke has demonstrated her ability to perform world-class research, organize, teach, and perform as a great colleague. The quality and quantity of her output and dedication has always impressed me and I believe her to be one of the top young scientists in the world today. It has provided a great foundation for the next step in her career. She has the systems, tools, and reagents to make a great start. She has also succeeded in her first efforts at securing funding, having been awarded multiple post-doctoral fellowships. I strongly suggest you interview her and get to know her more.

Please do not hesitate to contact me if you require any further information.

Sincerely,



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