



University at Buffalo
The State University of New York

Department of Microbiology and Immunology
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October 15, 2005

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

Subject: Recommendation for Dr. Laurence Brewer

Dear Dr. Brun:

I have known Dr. Brewer for several years. We first worked as colleagues during my postdoctoral tenure at UC Davis, where we collaborated on a project to study DNA helicases at the single molecule level. This work culminated with a publication in Nature. Dr. Brewer was instrumental in the success of this project bringing to it his knowledge of physics, in particular his knowledge of lasers and fluid dynamics, two critical components of the experimental setup.

Following the conclusion of this work, Dr. Brewer has continued on to study additional DNA binding proteins and extended his collaboration with Dr. Baskin of UC Davis. My expertise is in the field of DNA motor proteins and Dr. Baskin's expertise lies in the field of contractile motor proteins. Thus the collaboration with more biologically oriented colleagues is not novel for Dr. Brewer. In fact, he does this extremely well. To further enhance his collaborative efforts and to aid in his own projects, he has further developed his knowledge and understanding of biological systems by taking courses at U.C. Berkeley in biochemistry and structural biology. This knowledge of biological systems added to his pre-existing extensive physics background make Dr. Brewer an outstanding and knowledgeable colleague.

Dr. Brewer brings novel, exciting and constantly evolving approaches to the study of DNA condensation/compaction which as I am sure you are aware, is critical to the development active sperm. His extensive knowledge of physics and understanding of the behaviour of protein-nucleic acid interactions will make him an excellent colleague at Indiana University.

During the years I have known Dr. Brewer, I have found him to be an outstanding biophysicist and I highly recommend him for the position.

Sincerely yours,

A handwritten signature in cursive script, appearing to read 'P. R. Bianco'.

Piero R. Bianco, Ph.D.



October 11, 2005

Yves Brun
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Chair and Search Committee:

I am writing this letter to provide my enthusiastic support for Dr. Laurence Brewer's application for the position at Indiana University. I have worked and collaborated closely with Dr. Brewer for nearly a decade, and thus feel qualified to assess Dr. Brewer's capabilities as an independent research investigator.

Dr. Brewer and I began working 9 years ago out of a common interest in developing methods for isolating and manipulating single DNA molecules and using single molecule techniques to answer questions about protein-DNA interactions that are difficult to obtain from ensemble measurements. Together we obtained an LLNL Laboratory Directed Research and Development award that provided the funding we needed to set up a confocal based fluorescence imaging system that was configured for optical trapping and for performing single DNA molecule manipulations and studying DNA-protein interactions. Larry built the microscope and optical trapping system from scratch, and together we designed a two-channel flow cell for use in monitoring the effect of protein binding on the condensation of individual DNA molecules. Our first studies focused on examining kinetics and dynamics of the process of DNA condensation into toroids when protamine, the basic nuclear protein that packages DNA in sperm cells, binds to DNA. This work progressed exceptionally well, and resulted in the publication of three excellent publications. The first appeared in *SCIENCE*. Three additional papers have been completed on this work and published. These efforts examined the on and off-rates of protamine and subsets of the protein's DNA binding domain, the effect of protamine binding on the rate of toroid coiling, the dynamics of the condensation process, and the factors that lead to toroid formation. Experiments focused on protamine and the binding of other sperm basic nuclear proteins (TP1 and TP2) to DNA have provided considerable new insight into the process of DNA condensation and inactivation in sperm by protamines and the transition proteins. It also provided the preliminary data needed for Dr. Brewer's application to NIH for a Career Development award to continue the single molecule work, focused on a single molecule study of the process of chromatin reorganization in the developing mammalian spermatid. Larry succeeded and was awarded a five year Career Development grant from NIH to continue the research.

Working in collaboration with Drs. Ron Baskin and Steve Kowalczykowski at UC Davis, Dr. Brewer helped set up a similar system at UC Davis and initiate a series of single molecule experiments using RecBCD, a helicase and nuclease that plays a critical role in DNA recombination. These studies focused on working out methods to monitor the function and movement of the enzyme down the length of a lambda phage DNA molecule,



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and allowed us to directly measure the rate of protein movement and processivity for single RecBCD molecules. This work also progressed extremely well, and resulted in a paper that was published in NATURE.

More recently, Dr. Brewer also carried out a study to characterize the binding of another DNA packaging protein, ABF2p, that condenses circular mtDNA molecules in yeast. In collaboration with myself, Drs. Alex Noy (LLNL) and Enoch Baldwin (UC Davis), Dr. Brewer was able to show that ABF2p binding condenses DNA into nucleoid-like structures and that only very weak forces (~ 0.6 pN) hold the DNA-protein complex in a condensed state. A paper describing this work has also been published. After spending time to redesign the optical traps, microscope and data collection system, Larry has just initiated experiments to measure the forces generated when ABF2 and protamine bind to DNA and initiate its condensation.

During the period of our collaboration, both Dr. Brewer's knowledge of biophysics and his single molecule expertise have increased dramatically. Eight years ago, his research was confined primarily to instrument design using his engineering and physics background. Several years ago he took a series of courses at UC Berkeley to strengthen his knowledge of protein structure and function, biochemistry and biomolecular processes. His recent extensive, avid reading of the single molecule literature has helped him progress rapidly to the point that he now understands the work in the single molecule field as well as anyone and better than most. Dr. Brewer has advanced rapidly in the field of single molecule biophysics via his own initiative and through successful collaborations with other experts in molecular and structural biology.

While I cannot evaluate Dr. Brewer's teaching capabilities, I am convinced his research expertise in single molecule biophysics would make an excellent addition to your program. Larry gets excited about the biophysical aspects of biomolecular interactions, and he is constantly learning about new systems that might benefit from single molecule studies. Working at LLNL and in close proximity to UC Davis and UC Berkeley, he learned very quickly about the advantages of highly collaborative research efforts. As an independent investigator, he works very carefully. And yet he critically evaluates every aspect of his results and interpretations.

Dr. Brewer's expertise and ongoing research involving single molecule studies and his highly collaborative nature would make him an excellent acquisition by any university.

Clearly the prospect of Larry accepting a position in Indiana is not one I would like to consider, as it would most certainly make our active and highly successful collaboration more difficult. But...Larry would be an excellent choice for this position, and I couldn't recommend anyone for this position more highly.

Respectfully,

Rod Balhorn
Biomedical Division Leader