THE UNIVERSITY OF ILLINOIS AT CHICAGO

DEPARTMENT OF PHYSICS

M/C 273 · 845 WEST TAYLOR STREET · ROOM 2236 · CHICAGO · ILLINOIS 60607-7059

TELEPHONE: (312) 996-6105
EMAIL: jmarko@uic.edu

FAX (312) 996-9016
WEB: : http://www.uic.edu/ jmarko

May 20, 2003

Faculty Search Committee
c/o Prof. James Glazier
Dept. of Physics, Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

Confidential Recommendation for **Michael Guy Poirier**

Dear Search Committee Members,

I write to you today to offer my highest recommendation that you hire Dr. Michael Poirier as a junior faculty member in your department. I make this recommendation on the basis of the strength of the research that Dr. Poirier did as a graduate student in my laboratory at the University of Illinois at Chicago; I devote most of this letter to discussion of that work. I assume you will solicit separate letters from other people who will be able to comment on his postdoctoral work.

Dr. Poirier was a graduate student in my research group from fall 1997 through the end of 2001. In four years Dr. Poirier went from being a beginning physics graduate student with essentially zero knowledge of biology, to directing my lab's experimental research on mitotic chromosome physical properties. He more-or-less singlehandedly established a new and successful experimental research program.

While in my lab, Dr. Poirier worked on biophysical study of chromosome structure, using a unique combination of force-extension and biochemical techniques. His approach was to study chromosome structure by unfolding the chromatin while observing changes in its elastic and morphological properties. Dr. Poirier's thesis work answered several fundamental biophysical questions about chromosome physical properties and folding.

First, he had to build our lab – we started in October 1997 with an empty space. Next, he had to learn a number of techniques completely foreign to physics students: biochemical handling and purification, cell culture, cell microsurgery, to name a few. In this training period Dr. Poirier showed an amazing level of enthusiasm for studying outside of the traditional physics curriculum, and he either took or audited the biology and biochemistry courses needed to bring him up to the basic technical level necessary to do our cell-biological work. This was being done at the same time that he was completing rigorous Physics Ph.D. course and exam requirements.

By late 1998 Dr. Poirier collected his first publishable data characterizing the elastic response of mitotic chromosomes (Poirier et al, Mol. Biol. Cell 11, 269-276, 2000). This marked the first time that mitotic chromosomes from a cell were isolated and precisely studied micromechanically, and was a huge improvement over work done by my collaborators and I at Rockefeller University a few years earlier. The results presented in Dr. Poirier's 2000 paper provide a precise baseline for further combined biochemical-mechanical studies of chromosomes.

Dr. Poirier came to the realization that mitotic chromosomes had a number of interesting basic biophysical properties which warranted further study. First, he realized that mitotic chromosomes showed a slow dynamics when put under stress, which implied that their 'internal' viscosity was far greater than that of water. Systematic experiments showed that the viscosity of mitotic chromatin is about 100,000 times that of water (Poirier et al, Phys. Rev. Lett. 86, 360-363, 2001). Another way to express this is that the time associated with the internal dynamics of chromatin in a mitotic chromosome is in the 1 second range. This implies that mitotic chromosomes undergo slow internal reorganizations similar to those occuring in entangled polymer systems.

Dr. Poirier also noticed that mitotic chromosomes both in cells and then removed from cells displayed appreciable bending stiffness. His subsequent experiments showed that their bending elasticity are consistent with what we would expect based on their stretching elasticity. What was surprising here was that this was very different from bending behavior observed by Stefan Dimitrov's group in Grenoble for 'artificial' chromatids assembled using Xenopus egg extracts (JCB 1999). The implication of Dr. Poirier's measurements is that the in-vitro assembled mitotic chromatids and mitotic chromosomes assembled inside cells have profoundly different internal structures. He has published two articles (Poirier et al, Phys. Rev. Lett. 88, 228103, 2002; Poirier et al, Mol. Biol. Cell. 13, 2170, 2002) on the bending properties of mitotic chromosomes.

Dr. Poirier then went on to study the effect of short-duration shifts in ionic strength on mitotic chromosomes. I will mention two key results. First, he found that high ionic strengths could trigger an almost instantaneous unfolding of mitotic chromosomes to about 15 times their native volume, followed by a similarly rapid recondensation to the native form when ionic strength was returned to physiological. This behavior provided another clear hint that there is tremendous internal flexibility of mitotic chromatin. Second, he found that divalent and higher valence ions at low concentration could hypercondense chromosomes to as little as 1/3 of their native volume. This indicates that at least 2/3 of a native mitotic chromosome is actually aqueous solution which can be rapidly squeezed out (Poirier et al, J. Cellular Biochem. 85, 422, 2002).

The technique of spraying small quantities of reactants into the vicinity of a micromanipulated chromosome, introduced in Dr. Poirier's 2000 MBC paper and developed further in his work on chromatin conformational change triggered by ions, has opened the possibility of carrying out biochemical modifications of chromosome structure, while assaying kinetics of those modifications through force response. This technique played an important role in the final part of Dr. Poirier's thesis

research, where he used a variety of nucleases to study DNA connectivity in mitotic chromosomes.

The 'protein scaffold' proposed by Laemmli and co-workers has remained a controversial idea since the late 1970s. The reason for this is the biochemically violent nature of the histone depletion and subsequent EM preparation used in the classical scaffold studies. Dr. Poirier, using his combined biochemical-micromechanical approach, discovered that cutting DNA alone was sufficient to completely disconnect mitotic chromosomes. He then used blunt-cutting restriction enzymes to estimate how often cuts needed to be made to significantly reduce chromosome elasticity; cutting sufficiently infrequently (roughly less often than one cut per 15 kb) results in no detectable change in chromosome elastic response (Poirier and Marko, PNAS 99, 15393, 2002).

This final study indicates that the traditional 'contiguous protein scaffold' model of mitotic chromosome structure will have to be revised. Dr. Poirier has shown that mitotic chromosomes have a non-DNA internal structure which is mechanically disconnected in the native state. This, coupled with traditional protein-discovery approaches being applied to non-histone proteins, is going to lead to a major revision of the way we think about mitotic chromosome structure. Our PNAS paper was advertised on the cover of PNAS and was discussed in a separate PNAS Commentary (A. Belmont, PNAS 99, 15855, 2002). Thus Dr. Poirier's work has already attracted a good deal of attention. I have presented it in a few dozen invited talks, including a number at international biological meetings.

I think that very few graduate students make scientific contributions of the order of magnitude of Dr. Poirier's work. I honestly can rank him as the highest-achieving person at his career stage (new Ph.D.) that I have known over the past five years. How many graduate students start from an empty room and obtain truly fundamental new results in four years?

In my opinion, at the time that he left my lab Dr. Poirier's main weakness was his lack of training in advanced techniques in molecular biology. His choice of a postdoc in Prof. Chatenay's lab (from a number of other opportunities in top biology labs) was perfect to address this shortcoming, since Chatenay's lab is focused on combining molecular genetics and biophysical live-celll micromanipulation techniques. Dr. Poirier has focused his postdoc on, among other things, learning E. coli microbiology and molecular genetic techniques. He is at present developing methods for studying phenotypic variation within monoclonal colonies of E. coli, using an elegant combination of few-molecule fluorescence, microfluidic cell handling, and molecular-genetic techniques.

For biophysics junior faculty positions, the questions of whether the candidate will fit into the educational mission of a physics department is sometimes contentious. The case of Dr. Poirier should not be difficult in this regard. His Ph.D. is in physics, with an advisor whose main work is in theoretical condensed matter physics. He has published papers in physics journals. He did teaching assistant work and took his Ph.D. prelim exam in a physics department. In short, his preparation for teaching in a physics department is absolutely typical of assistant professor hires in physics departments.

Dr. Poirier has what it takes to be a successful academic scientist of the first rank. His Ph.D. training in biophysics, when combined with the advanced molecular biological training that he is at present receiving, will allow him to become a leader in biological physics. Dr. Poirier is a natural pioneer, ready to figure out ways to do things that when first proposed, sound impossible. His research in my lab was an unqualified success, and I expect him to excel at whatever he does in the future. I can't think of another young person at his stage of development with the same potential for scientific achievement. I give Dr. Poirier my strongest recommendation for a junior faculty position.

Yours truly,

John F. Marko
Associate Professor
Department of Physics
University of Illinois at Chicago
(312)996 6064/6105
jmarko@uic.edu