UNIVERSITY OF ILLINOIS
AT CHICAGO

Department of Physics (MC 273)
845 West Taylor Street, Room 2236
Chicago, Illinois 60607-7059

November 23, 2004

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington IN, 47405-7105

Dear Professor de Ruyter,

I write to you today to offer my highest recommendation that you hire Dr. Michael Poirier as a 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 assume that you will have separate letters focused on his postdoctoral work. Dr. Poirier, with a background combining basic physics and biology skills, is uniquely trained and positioned to take advantage of the research opportunities opening up for interdisciplinary biological physics research.

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, to directing my lab's experimental biophysical research on mitotic chromosome physical properties. He single-handedly established a new and successful experimental research program in my lab.

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 had 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 my Houchmandzadeh, Libchaber, Chatenay and myself 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 surprisingly large. 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). This implies that mitotic chromosomes undergo slow internal reorganizations similar to those found 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 chromosome bending elasticity is consistent with what we would expect based on chromosome stretching elasticity. What was surprising was that this was very different from bending behavior observed by Stefan Dimitrov's group in Grenoble for chromatids assembled in vitro using Xenopus egg extracts (J. Cell Biol., 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 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 re-condensation 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' model for mitotic chromosome structure, proposed by Laemmli and co-workers, has remained controversial 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). Subsequently, Dr. Poirier's work has attracted a good deal of attention. I have presented it in a few dozen invited talks, including a number at international biological meetings.

Very few graduate students make scientific contributions of the order of magnitude of Dr. Poirier's thesis work. I honestly rank him as one of the highest-achieving graduate students that I have known in my career, which includes students I have known at MIT, Cornell and the University of Chicago.

Dr. Poirier is extremely well qualified for the specific kinds of research he is proposing to do over the next few years. I note that he is planning to use micromanipulation, enzymatic and fluorescence techniques to study chromatin structure and dynamics. Dr. Poirier has learned these techniques since leaving my laboratory, in projects concerned with plasmid copy number, and on chromatin biophysics. In addition to having good background in chromatin biochemistry, Poirier has experience with low fluorophore-number fluorescence, and with the integration of computers, mechanics and optics that will be central to the type of research that Poirier is proposing.

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 chromatin biophysics. 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 a smashing success, and I expect him to excel at whatever he does in the future. I give my strongest recommendation that you hire Dr. Poirier as a faculty member in your department.

Yours truly,

John F. Marko
Associate Professor
University of Illinois at Chicago