

UNIVERSITY OF OXFORD  
DEPARTMENT OF CHEMISTRY

Hagan Bayley  
Professor of Chemical Biology  
tel: +44 1865 275948  
fax: +44 1865 275905  
bayley@tamu.edu

October 22, 2003

**CONFIDENTIAL**

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

Dear Professor Glazier:

It has been said that ability, energy and character should be the main criteria when considering a person for any job. While **Liviu Movileanu** would not be a very top candidate with respect to any one of these three ingredients, he has a rare blend of qualifications that place him overall in the top 10% of candidates for junior faculty positions.

I have known Liviu since he joined my laboratory at Texas A&M in November, 1998, as a postdoctoral associate. He has truly prospered and remains on a strong upward trajectory. Recently, I moved to Oxford University and Liviu has continued his work in Texas, rejoining my laboratory there after a brief and difficult experience as a junior faculty member in the Netherlands. I intend to phase out the laboratory in Texas by the end of 2004.

Liviu has an excellent balance of intellect and practical ability. He can both get difficult experiments done and, when necessary, reason about their implications with a high level of analysis. By now, he has a broad knowledge of biophysics, as you will see from his other letters. Over the last 3 years, he has

developed the creativity and independence of thought necessary to become a funded, tenure-track faculty member at a first-rate institution.

Liviu's primary project has been to study the interaction of the polymer polyethylene glycol (PEG) with a protein pore, staphylococcal  $\alpha$ -hemolysin. At first sight, this must appear rather esoteric. In fact, the work has yielded fundamental insight into questions of basic science: for example, into how polymers behave in confined spaces. It also has practical implications for the development of a class of sensors that work by single molecule detection, known as stochastic sensors.

Liviu has worked on this project both independently and, where required, as a member of a team. In 2000 (*J. Am. Chem. Soc.* **122**, 2411-2416 (2000)), with Stefan Howorka, he showed that single polymer chains could be tethered inside the protein pore, an interesting feat of protein engineering. Single channel current recordings on these constructs gave the first hint that polymer movement might be monitored by this means. The paper was striking enough to be selected by Phil Szuromi as an Editor's Choice in Science.

Liviu and Stefan followed this work up very rapidly with what I consider to be one of the most remarkable papers to come from my laboratory (*Nature Biotechnology* **18**, 1091-1095 (2000)). They attached biotin to the free end of the tethered polymer. In a single-channel experiment they were able to show that the biotin moves rapidly back-and-forth through the protein from one side of the lipid bilayer to the other. This was achieved by capturing the biotin for a short time with a mutant streptavidin of weakened affinity. While this experiment provided interesting information about the movement of a confined polymer, it also provided the basis for sensing macromolecules that cannot partition into the lumen of the pore. In effect, the polymer provides a link between the interior of the pore, which controls ion flux, and the external solution where the macromolecular analyte binds. This innovation was recognized in a News & Views article in the same issue of *Nature Biotechnology*.

Not all of Liviu's work has been as glitzy. In parallel, he has carried out highly detailed, fundamental investigations of polymer partitioning into pores. By studying the rate at which sulfhydryl-directed PEG reagents of various molecular masses react with engineered cysteine residues inside the lumen of the  $\alpha$ -hemolysin pore, he was able to map the interior geometry of the protein (*J. General Physiology* **117**, 239-251 (2001)). The work was recognized as an advance by Arthur Karlin, who wrote an editorial about it for the journal. While exploring the theoretical implications of this work, entirely on his own initiative, Liviu was able to show (*Proc. Natl. Acad. Sci. USA* **98**, 10137-10141 (2001)) that the PEG chains partition into the nanoscale lumen of the pore according to a

scaling law devised some years earlier by De Gennes. Direct tests of scaling laws at nanometer dimensions are rare in the literature. Recently, Liviu managed to reinforce this finding by studying the non-covalent interaction of PEG molecules with single  $\alpha$ -hemolysin pores (**Biophys. J.** 85, 897-910 (2003)). This work required the execution of current recordings on an unusually rapid timescale, for which Liviu had to grapple with several tricky experimental and interpretative issues.

Liviu is a superb team player as exemplified by his work with Stefan Howorka, also a postdoctoral associate, and George Miles, an MD-PhD student. With Stefan, Liviu studied duplex formation by short oligonucleotides covalently attached to the  $\alpha$ -hemolysin pore. They were able to show that the thermodynamics of duplex formation is much the same as that found in solution (**Proc. Natl. Acad. Sci. USA** 98, 12996-13001 (2001)). However, the single channel technique is far better able to catch nuances of the kinetics of hybridization. One of Liviu's major contributions to this work was to devise a practical way to carry out single-channel recordings over a range of temperatures. With this strong start, his colleague Stefan has been able to go on and use the technology for a variety of applications.

With George Miles, Liviu has solved a long-standing problem in the toxin literature. With Eric Gouaux, we showed some years ago that  $\alpha$ -hemolysin forms a heptameric pore. The question arose of whether the pore formed from the related binary toxin, leukocidin, which contains two different subunits, both related to  $\alpha$ -hemolysin, is also a heptamer. By using a subunit counting technique based on chemical modification during single channel recording, Liviu and George showed that leukocidin contains four subunits of each type and is therefore an octamer (**Protein Science** 11, 894-902 (2002)).

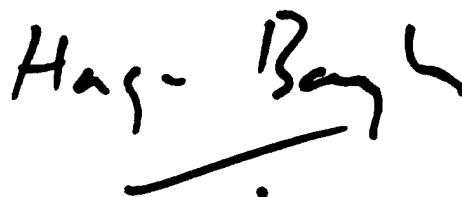
When I look back over his time in the laboratory, I feel amazed that Liviu has accomplished so much. He has truly taken advantage of a favorable environment to carry out first rate research and build up a fine publication record in high quality journals. I have read Liviu's Research Plan and I believe it describes an excellent course of action, which is befitting of federal funding. Protein translocation across membranes is a very important topic that is hardly explored at the molecular level. Liviu proposes means by which detailed information about translocation can be obtained at the single molecule level by using a cutting edge combination of biochemistry and biophysics. During the last year, he has obtained what were originally interesting preliminary data, which have now reached the stage where they can be written up for publication. He has also expanded his practical abilities by carrying out his own mutagenesis and protein expression, including the difficult task of making heteromeric pores.

Liviu's speaking skills have improved greatly during his time in College Station. He is by now a very good speaker, both on his own work and in journal clubs. His talks are rich in content and brightened by humor. For example, his analogy between experiments with ligands on the polymer and fishing is both apt and amusing. His approach bodes well for his future as a teacher. Liviu needs to become more efficient at writing, but that is not unusual for a person at his stage of development.

Liviu is highly motivated and dependable. He can work extremely hard when the situation warrants it. He is mature and well-organized. You will find him immediately likeable and extremely good natured. He gets on very well with others and can be an excellent team player when this is needed. He is very patient in explaining experimental details to others, and this again indicates his strong potential as an educator. Liviu is well respected by his juniors and peers, and I expect him to perform well in leadership roles.

In summary, Liviu is an excellent prospect. He has a proven record as a researcher and he is a fine prospect as a teacher. He is unusually well rounded. Not only is he bright and knowledgeable, but also persistent and untiring in pursuit of his goals. I do hope you will interview Liviu; you will enjoy meeting this talented young scientist. Please call me at +44-1865-275948, if you think I can tell you more.

Yours sincerely

A handwritten signature in black ink that reads "Hagan Bayley". The signature is written in a cursive style with a long horizontal stroke underneath the name.

Hagan Bayley, PhD