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November 23, 2003

To the attention of

Biocomplexity Faculty Search Committee,
c/o **Prof. Rob de Ruyter van Steveninck,**
Biocomplexity Institute

Indiana University
Swain Hall West 117,
Bloomington IN, 47405-7105

Ref: (NW45910)R : Junior Faculty in Biocomplexity

Dear Prof. Rob de Ruyter van Steveninck,

I am writing to apply for the academic position available in your department and advertised on your web site.

I completed a post-doctorate at the Weizmann Institute of Sciences (Israel) with Professor Elisha Moses working on the development of a new technique of Scanning Electron Microscopy. I am currently involved in a second post-doctorate program at Princeton University working on the design of synthetic genetic networks with Professor Ron Weiss. I believe that my research background in physics and biology combined with my teaching experience make me a strong candidate for the position outlined in your notice.

After finishing my Ph.D. in the field of experimental non-linear physics, my wish was to turn to biology.

I began this transition using the knowledge I acquired in Instrumentation as a M.Sc. student. I joined the Weizmann Institute where I developed an advanced method of scanning electron microscopy allowing wet samples, such as live biological cells, to be examined. You can get a good idea of the technology looking at the web-site of a company we were working with and which bought the rights to this technology (www.quantomix.com).

I am currently working at Princeton University on the design of synthetic genetic networks. This discipline aims at getting a better understanding of protein networks through the building of synthetic ones. This new field has numerous industrial and medical applications.

I would like to set up a laboratory which will be studying natural and synthetic genetic networks. I am interested both in building artificial network for cell programming, and understanding more basic questions about the cell machinery connected with the functioning and evolution of simple genetic and biochemical networks.

As my curriculum vitae and my teaching statement show, I had excellent opportunities and several years experience teaching a variety of courses during my Ph.D. studies. On an undergraduate level, I taught Physics, both in classroom and laboratory settings. In a graduate program, I taught Instrumentation in Nuclear Physics, and Electronics in the laboratory. Among the most satisfying experiences for me as a teacher was instructing students on an individual basis in the laboratory. Finally, during my post-doctorate at the Weizmann Institute, I was privileged to mentor the work of some brilliant graduate students.

I included my curriculum vitae, statements of research interest and teaching philosophy, and would be happy to send you additional materials such as preprint, and proposed course syllabi. I will be available to meet with you for an interview at your convenience. I can be reached at my home phone number (609) 252 1669. Thank you for your consideration and I look forward to hearing from you.

Sincerely,
Stephan Thiberge, Ph.D.

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Research Interest

CONTENT

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1. Future research

1.1. Synthetic genetic networks

Today, we are still far from understanding even the simplest collective behavior of biomolecules, cells or organisms.

In recent years molecular biology has moved away from the study of individual components towards the study of many interacting components. For example, if the proteins involved in a particular pathway are known, the question is to know how the dynamic of those proteins is orchestrated: the order they are expressed, their rate of production, their regulation,... and finally why is this network the way it is? Could it be done differently, or has Nature found the ultimate way to do that?

The "systemic" approach seeks an appropriate, and if possible, quantitative description of cells and organisms. Both the theoretical and experimental methods necessary for such studies are at the beginning of their development.

One recent approach to this problem consists of designing artificial networks encoded in plasmids (circular DNA) which are introduced into the cells. Those small programs are executed using the cell machinery. Comparison of the actual observation with the predictions of their behavior improves tremendously our understanding of natural networks.

Beyond fundamental understanding of cell functioning, more practical aspects of this approach are of main interest. For a long time already, plants and cells are used and modified to produce desired chemicals (for example, the production of penicillin and other antibiotics by fungi and bacteria). Nowadays, we are on the way to improve considerably our ability to actually program organisms and cells to accomplish specific tasks. Drugs, sensors, actuators, nano-device productions are some of the applications envisioned. For example, it will be possible to rely on cells to detect the presence of toxic chemicals, stem cells will be ordered to produce specific tissues to repair a damaged organ.
I would like to set up a laboratory which research will be in this field. I am interested both in building artificial networks for cell programming, and understanding more basic questions about the cell machinery connected with the functioning and evolution of simple genetic and biochemical networks.

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Designing and building 'simple' networks with desired functionality

In the past years, simple networks have been build artificially and implemented in E.coli: logical gates, inverters, clocks... More recently in Prof. Ron Weiss laboratory, we have developed a pulse generator, a circuit that shows a transient response to a step-like input chemical signal. This has many similarities with the chemotactic response of bacteria. In the same laboratory, other circuits like filters, amplifier, triggered memory, toggle switch or tunable clocks are under development. Some circuits function independently in each cell. Some circuits include cell-cell communication components which couple the cell together.
The realization of those circuits is triggered by the necessity to build a library of functions in order to program cells, but the evidence for many of them at least, is that they are also part of the natural pathways.

I would like to pursue this effort to build "simple" artificial circuits.
All the circuits I mentioned are elementary parts for a future cell programing language. In this perspective, and beside the fact that many of them are not yet implemented, they will need to be improved. Because they were developed in just the last few years, they often lack some interesting characteristics. As an example, the only "clock" which has been constructed experimentally function asynchronously between cells. Another example is the "pulse generator" we did, lacks any adaptability, meaning that it cannot react to a further change in the input signal which is an important characteristic of the chemotactic system.
Generally speaking, actual designs are simple in concept but far from being as efficient as natural ones.

For most of the artificial circuits, the input signal, if any, is a particular chemical, either an inducer (meaning that it will promote the expression of a protein) or a repressor (meaning that it will block the transcription). The output of the circuit is the level of expression of a particular protein, generally a fluorescent protein because it can be measured easily.

Understanding pattern formation in organisms

In the development of a multicellular organism, the generation of spatial pattern is made with very high precision, both in time and space. How the scale of spatial pattern is established in an organism? What elements in the regulatory network gives the precision of such patterning?
With those questions in mind, I want to implement artificial circuits that generate spatial patterns. The cells will be grown on a gel substrate, which allows signaling molecules to diffuse and furnish the cells the nutrients they need. The circuits will incorporate cell-cell communication system including elements of the natural quorum sensing system.

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Dynamic and stochasticity of regulatory networks

Reactions in a cell involve a small number of molecules. The fluctuations in their number make cells a stochastic system. It appears that regulatory networks are either extremely robust to fluctuations or use them in the most intelligent way (like the lambda virus).

The effect of those fluctuations in a cascade of regulatory events remains unclear. Is the noise amplified in a cascade or is it decreased ? Does it have the same influence on long networks like those involved in cell differentiation, and for short ones, like those associated with changes in the environment?

With Prof. Ron Weiss, we are building an artificial circuit composed of a cascade of several components. Each element is added progressively one after the other. At each step, the behavior of the system is measured. We can measure in this way, how the noise is either amplified or decreased by adding new components to the network. A collaboration between Prof. Ron Weiss and I will continue on that subject.

I would like also to realize related studies on natural regulatory networks. I plan to study different natural regulatory networks of E.Coli. The Lac operon, the motility network responsible for the realization of propelling filaments of E.Coli and the SOS pathway are the systems I plan to study. The Lac system is a short network induced in the presence of lactose. The SOS response to cell damage is done by a long cascade of expression of different genes. It can be induced by UV irradiation, chemical shocks,...
I want to use simple green fluorescent protein reporter plasmids for different stages in those particular cascades, and measure the fluorescence on a cell-to-cell base. The protein expression levels will be measured revealing the dynamic of those pathways. The difference of expression between individual cells will be accessible revealing either noise amplification or control through the cascades.

The stochastic nature of protein expression can be measured experimentally looking at single cells, if necessary over a long period of time. Microscopy is the method I plane to use for this kind of measurements. I will use a set of tools I developed to follow cells over a long time period by microscopy. I plan also to develop simple microfluidic devices that will allow single cells to be easily isolated and observed. One advantage of this approach is that it can be automatized to get large statistics on single cell measurements.

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Theoretical approaches

Predicting the behavior of a given circuit or improving its behavior towards a certain direction is a need. I will continue as part of my activity to develop both deterministic and stochastic computer simulations of the genetic circuits designed, as well as mathematical models.

Instrumentation

As mentioned earlier, phase contrast and epifluorescence microscopy and microfluidics will be the techniques of choice. I don't plan to invest in Flow Cytometry equipments to begin with, but develop equivalent microfluidic devices (slower methods, but cheaper and allowing time-lapse measurements).

1.2. Cell imaging techniques:

Following my work as a post-doc at the Weizmann Institute, I keep some real interest in cell imaging techniques. I would like to realize few simple experiments using the tools I would already get for my main activity. This includes the microscope and the equipment to do molecular biology.

Studying molecular motor activity in cells:

For practical reasons, the dynamic of molecular motors have been essentially studied in vitro. I would like to study their behavior in their natural context: the cell. I would like to follow probes attached to motor proteins traveling on the cytoskeleton. In this way, we could also potentially observe how the cell organizes globally those transports. I would like to use Surface Enhanced Raman Scatering (SERS) particles (colloidal silver of 100 to 200nm diameter) or quantum dots as probes.

Developing a ferritin recombinant protein for wet SEM microscopy and soft X-ray microscopy:

Developing the wetSEM technique (see below), I used a protein called ferritin which contains a lot of Iron atoms to visualize the endocytic pathway of cells. The ferritin I used, extracted from

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bovine serum, was added to the cell growing medium, and took up into the cells via endocytosis. The intensity of the signal obtained by electron microscopy from regions containing ferritin was high enough to demonstrate the high interest of this molecule for cell labeling.
In collaboration with Prof. Elisha Moses from the Weizmann Institute, I plan to develop cell lines producing by themself the ferritin (recombinant protein). I believe this protein can be for the wet SEM and soft X-ray microscopy method what GFP is for fluorescence microscopy. Observations could be performed in the electron microscopy unit of the university if there is one available or by Prof.Moses team at the Weizmann Institute.

2. Present and past research

2.1. Electron Microscopy of wet samples. Research Associate at the Weizmann Institute of Sciences (November 1999 - February 2003)

I worked for the last three years at the Department of Complex Systems of the Weizmann Institute of Science (Israel) with Professor Elisha Moses, to develop a new method of Electron Microscopy which originality is to allow fully hydrated samples to be visualized. We studied mainly biological cells and investigated different questions using this new powerful tool.

Briefly, the method is the following. The sample is closed by a thin membrane able to isolate the wet sample from the vacuum of the microscope thus preventing any evaporation. But, the membrane is thin enough to let energetic electrons passing it, interacting with the sample and eventually being scattered back in direction of the detector.

The method allows cells to be observed with the resolution of electron microscopy without drying them, thus preventing any denaturation. It has also the advantage to give a resolution much higher than optical microscopy. Single molecule detection is possible when labeled with 10nm gold particles. The main limitation at this day is that no dynamic observation is possible since energetic electrons are killing the cells soon after being observed.

The images obtained are very similar to what Soft X-ray microscopy can give but at a cost much lower and a more accessibility to various potential users. Further, potential improvements are possible. I believe that some kind of dynamical experiments can be performed to a certain degree.

Two journal publications on this subject are in the process of publication:

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a. Scanning Electron Microscopy of Cells and Tissues under Fully Hydrated Conditions
S. Thiberge, A. Nechushtan, D. Sprinzak, O. Gileadi, V. Behar ,O. Zik, S. Michaeli, Y. Chowers, J. Schlessinger, E. Moses (submitted to Proceedings of the National Academy of Science of United States of America)
b. An Apparatus for Imaging Liquids, Cells and Other Wet Samples in the Scanning Electron Microscopy
S. Thiberge, O. Zik, E. Moses (submitted to Review of Scientific Instruments)

2.2. Synthetic genetic circuits. Research Associate at Princeton University (from March 2003)

In my present work at the department of Electrical Engineering at Princeton University, I am working in the laboratory directed by Professor Ron Weiss, to develop synthetic genetic network.
The laboratory research focuses on programming biological organisms by embedding synthetic biochemical logic circuits into cells and intercellular communication mechanisms. Biological organisms sense their environment, process information, and continuously react to both internal and external stimuli. We are extending their behavior by embedding biochemical logic circuitry that precisely controls intra- and inter-cellular processes. This engineering effort of constructing reliable in-vivo logic circuitry with predictable behavior enables a wide range of programmed applications. The application areas include drug and biomaterial manufacturing, programmed therapeutics, embedded intelligence in materials, environmental sensing and effecting, and nanoscale fabrication.
We are now pursuing a variety of interesting and challenging projects that include the prediction of the behavior of complex genetic circuits based on the "device physics" of their components, programming cells to genetically modify their embedded circuits in order to optimize performance and behavior, building signal processing circuits and two-way messaging capabilities into the intercellular communication systems.

Related Publication:

A synthetic genetic circuit exhibiting a pulse transient response. S. Basu, R.Mehreja, S.Thiberge, T. Ming-Cheng, R. Weiss (submitted to PNAS)

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2.3. Non-Linear Physics. PhD. Institut Non-Lineaire de Nice. CNRS. (October 1995 - October 1999)

I did my Ph.D. in the field of non-linear dynamics studying pattern formation, and the dynamic of structures under external forcing in liquid crystals samples.

Related Publications:

a. Is the electromechanical coupling the driving force of the perpendicular drift of first class cholesteric finger? L. Gil and S. Thiberge, J. Phys. II France, 7 (1997) 1499.
b. Inversion walls in homeotropic nematic and cholesteric layers, J.M. Gilli, S. Thiberge, A.. Vierheilig and F. Fried, Liq. Cryst., 23 (1997) 619.
c. Critical radius of loop defects in homeotropic liquid crystal, S. Thiberge, C. Chevallard, J.M. Gilli and A. Buka, Liq. Cryst., 26 (1999) 1225.

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Teaching Statement

My research focus is at the interface of Physics and Biology, and I am prepared to teach in both of these areas. In fact, I have already accumulated important experience in teaching others.

As a graduate student, I served as a teaching assistant for three years. I have been teaching different fields in Physics to undergraduates in the classroom and to graduates and undergraduates in the laboratory: general physics, electromagnetism, instrumentation and electronics. As a teaching assistant I was responsible for practicing sessions, exam reviews, grading problem sets and experimental reports, and examinations. Significant learning in my classroom arose from discussion in addition to traditional lecturing. My students were encouraged to expose their view and analysis and challenge each other's analysis.

My background includes applied experience in different fields: physics, soft-matter, non-linear dynamics, biophysics, molecular biology, cell biology, instrumentation, and microscopy techniques. I believe this experience will allow me to teach a variety of classes both in the core Physics curriculum and at the graduate level. In particular, I would enjoy teaching courses in biophysics, statistical physics, non-linear dynamics and instrumentation. I would also like to develop two graduate level courses. The first will investigate our actual understanding of genetic network functioning. We will describe some known natural regulatory networks and their dynamical behavior will be explained emphasizing how efficient and adapted those circuits can be. The second course will be a biophysics course. It will review what physics is bringing to biology based on the most recent research in the field. For example, we will talk about the new experimental techniques in single molecule manipulation and detection.

As a mentor for my Ph.D., Master and undergraduate students working in my laboratory, I will leave them the freedom and encourage initiative and ideas of their own. At the same time, I will be extremely attentive to their progress.

As a classroom teacher, I certainly want my students to learn the fundamental content of the course I teach. Beyond that, my wish is also to help them develop other useful skills, like good communication and multi-disciplinary skills.

I believe it is important for them to be able to communicate efficiently what they are doing to a non-scientist interlocutor as well as a colleague. I will stress oral communication as much as possible.
I will ask a student or a small group of students to explain a particular topic. It will require some bibliographical research which I believe is a good way to learn how to learn independently. I will ask the students to report on the progress of their work and guide them in order to make sure their presentation will include the elements I want to be taught in the class.
I will also ask them to explain shortly a research article. I believe it is an interesting exercise exposing the student to research methodologies, and developing critical thinking. Poster presentations are also among the exercises I would like to propose, to undergraduates at least.

I would like students to feel that what is taught prepares him or her to be an efficient participant in the socio-economic world. I am talking here especially about undergraduate students who are exposed to general courses. As a teacher, the thing I can do to help them feel this way is to build bridges between what is learned and the main challenges in research and industry. For example, teaching statistical physics, I would mention, one way or another, the 'fuel cells' which appear a very promising alternative source of energy, and the subject of interest for many new companies, especially in Canada. This kind of information can be given in different ways, through the course, exercises or even through exams.
This is actually a quiet easy thing to do, and I believe it is very helpful to build a broad knowledge and a better understanding of where science is going. Meanwhile, it helps the student to figure out where he or she is going.

In summary, I bring to the classroom a wide variety of experiences and a positive attitude toward teaching. I think that educating others is a high responsibility, a challenge and a privilege.
I hope to pass on this excitement to my Ph.D., Master and undergraduate students.