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Biocomplexity Faculty Search
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December 22, 2004

Dear Search Committee,

I am writing on behalf of Dr. Vladimir Shinkarev who is applying to Indiana University, for a position in the Department of Physics. As you will know from his application, Dr. Shinkarev is a Research Associate Professor in the Department of Biochemistry, at the University of Illinois at Urbana-Champaign. Formerly, Vladimir was a Senior Scientist from the Moscow State University, a position of some seniority where he trained several graduate students. Like many in his position, however, life became untenable after the political turmoil of 1989 and he came to my lab in 1990, and he has now been associated with my group for more than 14 years. He is extremely creative and prolific, and his research experience and successes fit him extremely well for the position in biophysical chemistry, at Indiana University.

Vladimir is a very clever and energetic man, with a broad array of skills, both experimental and theoretical. He has diverse interests predicated on broad training in mathematics and biology, but his research work has been primarily in the molecular biophysics and biophysical chemistry of biological energy conversion, and on the functional, kinetic and energetic bases of membrane function in bioenergetics. However, he has shown himself to be extremely versatile in adopting new techniques, including structural methods quite distinct from the predominantly spectroscopic nature of his earlier work. His deep training and great facility with applied mathematics also make him especially adept at novel data analyses and at modeling processes, and he has been of great assistance to many people on this campus and elsewhere, in modeling their work. Vlad is a strong and original thinker and is extremely dedicated, working long hours with good productivity.

In my lab, Vlad has been tireless, working entirely independently but contributing greatly to all facets of our research program. From his earlier career, he had excellent facility with both optical (absorption and fluorescence) and electrical (membrane potential) measurements, and he is a master of kinetic and thermodynamic analyses relevant to membrane and protein function. When he arrived as a visiting scientist, Dr. Shinkarev brought with him a particular expertise in making electrical measurements on biological membrane systems. The technique, developed in Russia, is simple, extremely sensitive and has a very rapid response time. Vlad had been

involved with it since its inception and was responsible for some of its specific creative applications, especially in photosynthetic and respiratory electron transport systems. In my lab, he has made several significant contributions to the study of electron and proton transfer, using this electrometric method, as well as various forms of optical spectroscopy. His expertise has complemented the main thrust of my lab very well and he has contributed significantly and in many ways. He has also had many creative ideas – and good preliminary experimental results - for applying the electric potential method more generally to monitor diverse membrane phenomena, including membrane fusion and general transmembrane ionic activity.

From his publication record, it will be clear that his long-standing research interests have been, and remain, in mechanistic and physicochemical aspects of membrane transport activities, using spectroscopic and electrical techniques. However, a strong indication of his adaptability and technical capabilities is the work he did with scanning probe microscopy (SPM) methods. This arose from questions concerning the nanoscale structural character of the fused vesicle systems we use for direct measurements of membrane electric potential. These are formed by fusing membrane vesicles with a support material (usually collodion) impregnated with lipid. The method had been in use for over 25 years but there was absolutely no direct experimental evidence on the physical nature of the structures formed. In response to some rather improbable suggestions, Vladimir established a project at the Materials Science Research Laboratory, at UIUC, using near-field scanning optical microscopy (NSOM) and, over a period of a few weeks, demonstrated that the fusion structures were blister-like, formed from multiple fusions of vesicles, and with an intact internal aqueous phase. This knowledge allows an equivalent circuit analysis of the system and, therefore, unambiguous interpretation of the electric potential measurements. He then went further with this method and showed that a related technique, which had been proposed to result in membrane proteins incorporated into lipid *monolayers* on a solid Teflon support, also gave rise to blister-like bilayer structures and that these were almost certainly the source of the electrical signal.

From this it should be clear that when an important question arises, Vlad is quick to adopt and master whatever techniques may be available to provide an answer. In this case it was NSOM but, having got a taste for SPM, he then turned to atomic force microscopy (AFM), to investigate the supramolecular architecture of membrane complexes in native membranes. The goal here was to examine, for example, the association state of complexes like cytochrome bc_1, for which both monomers and dimers have been reported. (The X-ray structures may say "dimer" but there are persistent indications of monomer activity under certain conditions, including electron microscopic support for monomers in native membranes.) Typically, Vlad had intriguing data within a very short time of turning his hand to the problem. Recently, he visited colleagues at Baylor, to continue this work.

In the last 5 years or so, Vladimir's main activities have been directed towards the cytochrome (Cyt) bc_1 complex, under the primary NIH funding of my lab. He works essentially independently on this, as a peer collaborator with me and with Prof. Tony Crofts, in a nearby lab. Vlad initially invested a substantial amount of time in setting up new or refined experimental approaches, notably diode array and CCD absorption spectroscopy. For this, his exceptional mathematical skills were critical in establishing the analytical methods needed for handling the massive amounts of data that come from time-resolved spectroscopic array methods. Subsequently, his exceptional experimental and analytical skills have revealed highly significant properties from subtle observations, and his work has contributed greatly to our understanding of coupled charge transfer in this membrane, electron transport complex. Most notably, he was able to show that the effect of the membrane potential on the transmembrane electron transfer between the b-hemes of the bc_1 complex was much greater than had been previously suspected,

forcing a significant revision of the redox potentials of the b-cytochromes. He also showed that various inhibitors of the Cyt bc_1 complex affect the redox potential of the iron-sulfur protein, and in a manner that gave a clear mechanistic interpretation of the role of iron-sulfur protein in the active site turnover.

In Russia, Vlad had a long-standing interest in chlorophyll fluorescence as a probe of Photosystem II activity in oxygenic photosynthesis. Shortly after arriving in my lab, he conceived of a novel aspect of the well-known Kok cycle of S-states in the oxygen-evolving complex, that was amenable to subtle analysis by flash-probe fluorescence methods. After gaining some preliminary data, this work was funded by a grant from the USDA, which he conceived and largely wrote. I certainly had a significant input in the writing in an editorial sense, and for tactical reasons I was the PI with Vladimir as Co-PI, but the ideas were essentially his and the USDA panel knew exactly what was the real situation. The grant received the number one ranking in its year of review. We published several papers on this grant, but we did not pursue the project to a second round of funding because by that time my lab was funded by a new NIH grant on the cytochrome bc1 complex, also conceived with significant input from Vlad, and this has largely consumed his efforts at the bench since then. However, he has continued to be active in collaborations with others, where his data analysis and modeling skills have been greatly appreciated. He has also written two very substantial reviews on Photosystem I and II.

From the early success of this USDA grant on Photosystem II fluorescence and the oxygen-evolving apparatus, and his very substantial contributions to our on-going NIH funding for the Cyt bc1 work, it is evident that in the important area of federal funding Vladimir has proven the value of his ideas and conceptualization. His written language skills are good and, although I continue to be involved in shaping the final products of papers and grants so far, my input now is minimal and I am confident he will continue to gain federal grant support. His writing is easily as good as what one sees regularly in funded grant proposals and in published articles by native English speakers, and he has several single author reviews to his name, that are of considerable influence in the field. Most importantly, his ideas have contributed the basis of well-funded research.

In establishing his own experimental group, I would expect Vladimir to develop a strong independent research program using various membrane and bioenergetic systems to examine structure and function in membrane systems. Our funding from NIH on the cytochrome bc_1 complex stimulated his interest in the generation of reactive oxygen species (ROS) and their role in aging and apoptosis, and he has designed several prototype electrodes for assaying ROS production in optical cuvettes for simultaneous absorbance and electrical measurements. He has also made some very interesting and novel observations on membrane fusion, using the SPM methods outlined above, as well as the electrical potential measurements to follow the time course of the fusion process.

In fact, I am a bit hesitant to predict where Vlad might go as a fully independent scientist. I am aware that the research focus of my lab has constrained his creativity somewhat, and he would certainly have done many different things had my own agenda not cramped his style. Vlad is a brilliant experimentalist, and an outstanding "ideas man". He is also very congenial and an excellent collaborator, and he has been involved in several projects with other researchers on this campus and elsewhere, especially involving his exceptional modeling and analysis skills.

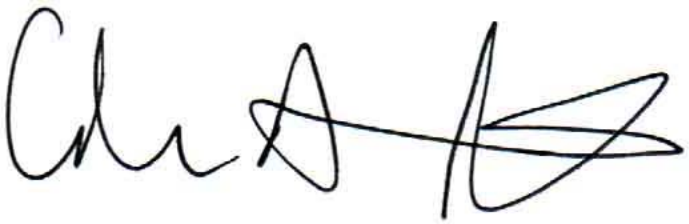
Vladimir's spoken English is very fluent and he speaks confidently. He has only a small amount of experience with teaching in English in a classroom setting, but, like his seminars, his class presentations are well organized and clear. Furthermore, he interacts very well with

students, one-on-one. In the lab he has been an effective and patient tutor, always looking for - and finding - good, pedagogically sound ways of explaining things. His primary research area might be termed physical biochemistry, but his broad training and his aptitude in mathematics have made it enviably easy for him to move into new areas, and to develop and assimilate novel data acquisition and analysis tools. He would certainly be able to teach in a variety of areas, including all areas of physical biochemistry, bioenergetics, and membrane biophysics, as well as many other topics at the graduate level. He would also be especially suited to teaching courses in biomathematics and "quantitative biology", an area of particular interested to NIH, these days.

The length of his appointment with me and his intention to seek a permanent position in this country are, of course, largely due to events in the Soviet Union, over a decade ago, although he may have been tempted to stay in the U.S., anyway. To this end he undertook all the immigration status changes necessary to facilitate his employment, and he is now a permanent resident. I might say, as further testimony to his resourcefulness, that he engaged the immigration process head-on and came out on top in record time!

The situation in Russia has been dire for persons of his ilk and we can be grateful for opportunities to obtain such expertise and feel we are helping them at the same time! His appointment as a Research Associate Professor is a rarity at the University of Illinois and reflects the appreciation of many people whom he has assisted in analysis and modeling of diverse and complex systems, but such a tentative situation does not reflect his proper standing in the academic community. I recommend him to you very strongly - he is a highly creative scientist and a very good colleague. If there is any possibility of an appointment at more advanced levels, Vladimir Shinkarev would be eminently suitable for such a position and it would accurately reflect his experience and his proven abilities.

Yours sincerely,



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