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September 20, 2005

Yves Brun
Systems Biology/Microbiology Faculty Search
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To Whom It May Concern:

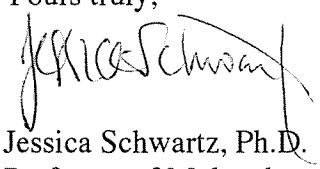
I am pleased to write in support of the application of Maria Diakanova, Ph.D. for a tenure-track faculty position in the Department of Biology at Indiana University. I have known Dr. Diakonova since 1999, when she joined the laboratory of my collaborator Dr. Christin Carter-Su. Since our labs are next to each other and we have lab meetings together, I know Dr. Diakanova and her work quite well. She has brought sophisticated expertise in imaging, and a mature and professional approach to research to the lab. I believe she is motivated for an independent position as a faculty member.

Dr. Diakonova is a highly trained and productive cell biologist who is making her mark in understanding mechanisms for regulation and movement of biologically important molecules in cells. Her early training focused on cytoskeletal structure and on internalization of growth factor receptors and signaling molecules such as phospholipase C gamma. She extended her skills to analysis of phagocytosis prior to joining the Carter-Su lab, where she then began investigation of growth hormone signaling, with a focus on using imaging techniques to study functions of the adapter molecule SH2B beta. It was immediately evident that Dr. Diakonova was more than a postdoc. She functioned as a matureinvestigator poised to bring her insights and set of skills to a new and important biological question. Although GH signaling was a new area for her, she clearly had a foundation in understanding cell growth, and dived in energetically. It was she who brought to light the unexplored function of growth hormone on cell motility. I was impressed at the variety and array of assays that she used to identify and validate this consistent but subtle response. When she first assembled a movie of ruffling of GH-treated cells we were enthralled. Most exciting was the role that she identified for SH2B beta as an adapter mediating this

response. It should be emphasized that Dr. Diakonova worked quite independently, drawing on her cell biology and imaging skills, to accomplish these important observations. This provided a launching point for her to develop an independent line of investigation. She is systematically analyzing signaling components regulating actin-dependent motility, where SH2B beta appears to serve as a critical link between receptor tyrosine kinases and the actin cytoskeleton. She has combined biochemical approaches with her cell biological skills, and has optimized use of Listeria monocytogenes to understand how SH2B beta regulates the actin cytoskeleton. It was with great diligence that she made this creative idea come to fruition. She is now also performing a related set of studies addressing the role of the serine-threonine kinase PAK1 in cytokine-regulated JAK2 dependent signaling. She has successfully competed for funding for these studies from the Human Growth Foundation and the Michigan Diabetes Research and Training Center, and currently holds an NIH R21 grant. The work is novel, cutting edge and has potential clinical implications.

In addition to her scientific accomplishments, I am sure that Dr. Diakonova will be successful in running her own research program. Her organizational skills were evident from the outset, when she pitched in for our research team by supervising the cell culture facility used by all of the investigators in our two labs. Not only did she introduce efficient innovations to the cell culture facility, but she also voluntarily undertook systematic classification of the cell lines which had accumulated in our research groups over the years. This was a daunting project which has since proved to be an invaluable resource for all of us. It was clear when she was ready to launch out on her own, and she successfully obtained independent research grants. She has assembled an energetic lab group which has included research assistants, postdoctoral fellows and a visiting faculty member on sabbatical. She has supervised undergraduates and, I believe, has the patience to be an effective teacher. Thus, I recommend Dr. Diakonova to you as a creative, productive investigator who has established an exciting research program on a novel and important problem. I am sure that she will be a professional and interactive colleague who can make valuable contributions to your department.

Yours truly,



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To Whom It May Concern:

I am delighted to recommend Dr. Maria Diakonova for a tenure-track faculty position in the Department of Biology and Biocomplexity Institute, Indiana University. Dr. Diakonova has been working with me for the past 6 and a half years, first as a senior postdoctoral fellow and for the past 3 years, as an Assistant Research Scientist. As a postdoctoral fellow, she became an indispensable member of the laboratory, known and sought out for her knowledge of cell biology, all types of microscopy, and tissue culture, and recognized and valued for her work ethic, straightforward communication style, and fearlessness in tackling and skill in mastering new techniques.

When she joined the laboratory, Dr. Diakonova took over a project looking at the role of the ubiquitously expressed adapter protein SH2-Bβ in the regulation of the actin cytoskeleton. SH2-Bβ was originally identified in our laboratory as a binding protein of the tyrosine kinase JAK2. In response to growth hormone (GH) binding to its receptor, JAK2 is activated and the resulting phosphorylated tyrosine 813 in JAK2 binds SH2-Bβ, which is in turn phosphorylated on tyrosines by JAK2. SH2-Bβ has also been shown to be recruited to the activated forms of a number of receptor tyrosine kinases, including the receptors for insulin, insulin-like growth factor-1, platelet-derived growth factor, nerve growth factor and fibroblast growth factor, making SH2-Bβ a widely utilized adapter protein. She and Dr. Herrington documented that overexpression of SH2-Bβ enhances GH-stimulated membrane ruffling and overexpression of a dominant negative form of SH2-Bβ blocks it. She added a new level of sophistication to these studies by rigorously quantifying her measurements using Metamorph software analysis. She also introduced videomicroscopy to the laboratory, doing extremely labor-intensive recordings and quantification. Having decided that she needed to look at the physiological consequences of membrane ruffling, Dr. Diakonova independently introduced three separate assays to quantify

the effects of SH2-Bβ on cellular motility. Her tour de force was using a large series of mutants to identify a region of SH2-Bβ that was necessary for the stimulatory effects of SH2-Bβ on cellular motility. She then went on to show that the small GTP binding protein, Rac, bound to that same region of SH2-Bβ, suggesting that the function of SH2-Bβ may be to recruit actin regulating proteins such as Rac to activated tyrosine kinases and the cell membrane.

Dr. Diakonova has continued to work on these studies in an independent manner, introducing on a routine basis new techniques to the laboratory. For example, she independently arranged to spend time in Dr. Gary Borisy's laboratory at the University of Chicago where she used platinum replica(s) and colloid gold to localize endogenous SH2-Bβ to the actin cytoskeleton in membrane ruffles and filopodia. She also initiated a collaboration with Dr. Joel Swanson to use Listeria to investigate further the role of SH2-Bβ in the regulation of the actin cytoskeleton. Her Listeria experiments provide the most compelling evidence to date that SH2-Bβ directly regulates the actin cytoskeleton. When they infect cells, Listeria use the cell's machinery to produce long actin tails, which are required for Listeria to move in the cell and infect other cells. Dr. Diakonova showed that when Listeria infect cells overexpressing SH2-Bβ, they move at a significantly faster rate than when they infect untransfected cells. Conversely, they produce very short tails and move at a much slower rate when they infect cells overexpressing a dominant negative form of SH2-Bβ. Masha then spent considerable effort establishing an *in vitro* assay using Xenopus oocyte extracts – another tour-de-force as she is one of only a few people in the world who has gotten this assay to work – and showed that direct addition of substantially purified SH2-Bβ enhances the motility of Listeria. She then developed an international collaboration with Dr. Marie-France Carlier to study the effect of SH2-Bβ on a totally reconstituted system using ActA coated beads and a limited number of defined actin regulating proteins. Together they established that SH2-Bβ can increase the motility of these coated beads, but only in the presence of the ActA binding protein VASP. Masha then showed that of these defined proteins, SH2-B binds, either directly or indirectly, to VASP and actin. Furthermore, she showed that SH2-Bβ causes bundling of actin (another new assay to the laboratory) and that SH2-Bβ enhancement of Listeria motility require VASP. Thus, Masha's work not only identifies SH2-Bβ as an important, novel regulator of the actin cytoskeleton, but also provides strong evidence that it may serve as a direct link between receptor tyrosine kinases (to which it is recruited) and the actin cytoskeleton (to which it also binds). This is a very important finding, since it is well known that ligands that bind to receptor tyrosine kinases regulate the actin cytoskeleton but the signaling pathways and proteins by which they do so are only beginning to be understood. This area of identifying signaling pathways linking membrane receptors to the actin cytoskeleton represents one of the most interesting, timely and important areas of cell biology research with relevance to many different human diseases. This Listeria work is currently under review.

During this time, Masha also initiated and has nearly completed a study characterizing the phosphorylation of the Rac effector protein Pak by JAK2. Pak has been implicated in a number of important cellular functions, including regulation of the actin cytoskeleton and cell survival. Regulation of Pak activity by tyrosyl phosphorylation is another new and interesting area of study relevant to cell biology as again it relates the interface between growth factors and regulation of the actin cytoskeleton.

As alluded to above, Masha works relatively independently in the laboratory and has for some time. She is our expert on the actin cytoskeleton and cell motility. She seeks out and attends appropriate meetings on her own. She seeks out collaborators. She is tenacious in going after novel techniques needed for her research and in every case has been successful in figuring out the necessary methodology even though many others in the field have been unsuccessful in doing so. She has written three grants and gotten them funded. In every case, she was quick to hire people and make significant headway on her proposed projects. Most impressive is her work using Listeria as a model system, which resulted in her obtaining an NIH grant.

Overall, Masha has overcome many obstacles in her life, yet has never been deterred from striving to carry out novel and important research. She is smart, works extremely hard, and is always willing to master a new technique, no matter how difficult or tedious. She is always ready to take suggestions, and is aggressive about getting the advice that she needs to accomplish her goals. She gets along well with the wide range of students, fellows, and technicians in my laboratory, who value the advice that she willingly and readily provides regarding cell biology, microscopy and cell motility. In addition to training her most recent technicians and postdoctoral fellows, she has trained a number of undergraduate students who enjoyed working with her a great deal. Her English is quite good and she would have no trouble communicating with colleagues and students in the department. Although I have not seen her give a formal classroom lecture, her ability to present her work at laboratory meetings and at internal seminars would suggest that she would do a conscientious, thorough job of teaching. I would also predict that she would make an excellent colleague, serving as a valuable resource for others in the department. For these reasons, I recommend her to you most highly and without qualification for a tenure-track faculty position at Indiana University.

Sincerely,

Christin Carter-Su

Christin Carter-Su, Ph.D.
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MEDICAL COLLEGE OF WISCONSIN

October 3, 2005

Cardiovascular Center

Dear Dr Brun

The purpose of this letter to write a strong document of support for Maria Diakonova, who is considered for the Assistant Professor position in your Department. I have known Maria Diakonova for 18 years since she started to work at the Institute of Cytology Russian Academy of Science in St.-Petersburg, Russia. We have continued our interactions later when she pursued her scientific career in Germany and USA. Dr. Diakonova is a creative and gifted scientist. While working in Russia she has proposed an exciting research program to study the role of cytoskeleton in signal transduction. While working in west laboratories her works were always at the cutting edge of science. Her cell biology skills are superb. Dr. Diakonova has all necessary knowledge, energy and creativity to become one of the most valuable addition to any scientific department. Dr. Diakonova is one of the most capable scientists I have ever met and I believe she will have an outstanding career in academic science. She is well organized and highly motivated.

In summary, Dr. Diakonova is the perfect candidate for the position of Assistant Professor and I recommend her without any reservation.

Sincerely

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