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Biocomplexity Faculty Search Committee,
c / o Prof. Rob de Ruyter van Steveninck,
Department of Physics,
Indiana University,
Swain Hall West 117,
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Dear Dr. de Ruyter van Steveninck,

I am writing to very highly recommend **Karen Zito** for a junior faculty position in your department. Simply put, Karen is outstanding. She has vision. She has a knack for choosing the right approach to important problems. She is a scholar and an excellent experimentalist. And she is energetic and enthusiastic. I think that Karen would make a great addition to your faculty. In the field of synaptogenesis and plasticity, Karen is one of the few who has both the deep training in molecular biology and genetics and a strong command of the leading optical techniques.

Karen is an exceptionally versatile and independent researcher. She single handedly brought cell biology and genetics into my lab as a graduate student in order to study synaptic targeting of membrane proteins and synapse development in Drosophila, and she has since extended herself into synaptogenesis in mouse barrel cortex in the lab of Karel Svoboda, where she branched into imaging and microarray analysis. Karen's approaches to synapse development are to my mind very promising, her intellectual ability is superior, and the breadth of her technical mastery is as impressive as her deep knowledge of the field.

Karen was one of the first two students to join my lab when I came to UC Berkeley in 1993. At the time she expected to study developmental genetics and had no background in neurophysiology or cell biology. Karen decided to join the lab because she became fascinated by the problem of how ion channels are targeted selectively to particular subcellular compartments. Even though I had little experience addressing problems of this sort it was hard to resist her enthusiasm or to deny the importance of the problem. Karen initiated the study on her own, introducing three systems to the lab

that I had never used: cultured CHO, HEK293 and COS cell lines for testing epitope tags that would be used to visualize channel localization, cultured MDCK cells to examine differential membrane targeting in a model polarized cell, and cultured brain slices of hippocampus, cortex and cerebellum to study targeting in neurons with intact circuitry. She worked out the best conditions for transfecting these, examining lipid, biolistic and viral delivery of genes, and learned confocal microscopy to follow channel localization. Aside from providing Karen with information about channel distribution and function and giving her training in electrophysiology, I was more her collaborator than teacher. Karen worked very hard, but ran into many obstacles with the preparations such as the difficulty of identifying pre versus postsynaptic expression in cultured brain slices, getting cells to transfect while maintaining their functional polarity, etcetera. After a stunning amount of hard work Karen had the strength of mind to admit to herself that alternative avenues to the question should be sought out. She settled on Drosophila as the choice preparation, in part because something was already known about the subcellular distribution of specific potassium channels in flies, and because of the power of fly genetics and of the UAS-GAL4 system to drive gene expression in specific cell types. We decided that this work would best be done in collaboration with Corey Goodman, and he became her co-supervisor.

With this more tractable preparation in hand, Karen made rapid progress and expanded her study to include the N-CAM-like homophilic adhesion molecule Fasciclin II (Fas II) in addition to the Shaker potassium channel. She succeeded in demonstrating that the PDZ interaction domains in the C-terminals of these two proteins are necessary and sufficient for their postsynaptic targeting, and that they depend for their localization on interaction with the PDZ protein Dlg. The demonstration that Fas II is associated via PDZ proteins with an ion channel (and likely with other membrane proteins and cytoplasmic enzymes) provided an intriguing new dimension to synaptic communication. It suggested that protein complexes in the presynaptic active zone and the postsynaptic membrane may communicate mechanically via adhesion molecules. These interactions could be mediated by homophilic Fas II interactions, or by heterophilic interactions, as shown concurrently by Sudhof's lab, and they could, in theory, mediate both anterograde and retrograde signaling--an exciting new concept.

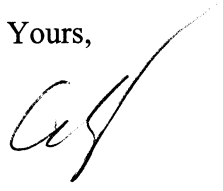
Karen's interest in the role of protein localization in synapse formation led her to design a method for watching synaptic development in time-lapse from the point of view of proteins that were concentrated by PDZ interactions in the synapse. To do this, she made a fusion gene with the transmembrane segment of CD8 at the N-terminal, GFP in the middle and the C-terminal of Shaker containing the PDZ interaction domain. As she had shown earlier, this protein was localized to postsynaptic membranes. The beauty of this was that the GFP was so bright that this PDZ-dependent synaptic clustering of protein could be followed non-invasively through the cuticle of developing larvae. By following synapse development in these intact animals, Karen obtained a first view of the process of synapse expansion in fly muscle. Both projects led to papers in *Neuron.*

Karen's work has spawned a major ongoing project in my lab and her synaptically targeted GFP constructs enabled one of the most beautiful series of papers from Corey's lab on synaptic homeostasis. Her work in Karel Svoboda's lab has been beautiful. I think that she'll do great things.

The key to evaluating Karen is to realize that much of what she has already accomplished she did with such independence that it can serve as an accurate predictor of the work she will do as an

assistant professor. I would rank Karen's intellect and research skills as among the very best that I have known among students and postdocs at Berkeley. She'll make a great colleague and teacher. I strongly recommend that you interview her and see for yourselves.

Yours,



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