Cold Spring Harbor Laboratory
Karel Svoboda, Ph.D.
Professor

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Dear committee members,

I am writing in support of **Dr. Karen Zito**, who is applying for a faculty position in your department. Karen has been working in my laboratory at Cold Spring Harbor Laboratory for ~4 years. In addition I have followed Karen's work in Corey Goodman's and Ehud Isacoff's labs at UC Berkeley, at least since the publication of her 1997 *Neuron* paper on the mechanisms of molecular clustering at synapses. Based on my many interactions with her it is clear that Karen is a highly creative and effective scientist. There is no doubt in my mind that she is on the way to become a leader in neuroscience research.

Karen's work first came to my attention when she published an influential paper on clustering of Shaker and Fasciclin II at synapses (1997 *Neuron*). She found that clustering is controlled by the Disc-Large protein, a PDZ scaffolding protein. To the best of my knowledge this was the first direct demonstration that PDZ containing proteins can act as 'synaptic organizers'. A second study published by Karen was even more beautiful and equally influential. Working in the developing fruit fly *in vivo* she imaged the growth of synapses over several days (1999 *Neuron*). She combined live cell imaging with retrospective immunofluorescence microscopy to begin to dissect the molecular steps leading to synapse formation. Her graduate work is a classic in the field of synaptogenesis and the approaches developed in her experiments are currently much imitated.

Naturally, I was delighted when Karen approached me about a postdoctoral position in my laboratory. My lab is interested in the formation and function of mammalian cortical synapses and their circuits in brain slices and *in vivo*. Based on two-photon microscopes that we custom-built in my laboratory we can observe the growth and function of *individual* synapses in intact neural tissues. Using intracellular electrodes we can measure the development of cortical maps, which reflect the ordered arrangement of synapses and which develop in an experience-dependent manner. Karen came to my laboratory with the idea to move her work on the molecular basis of synaptogenesis into mammalian cortex and to learn imaging and electrophysiological techniques to assay synaptic function.

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Karen decided to strike out in two directions. First, she developed a DNA array based screen for synaptogenic genes in neocortex *in vivo*. She recognized that, since most synapses in barrel cortex grow in a rapid burst, this system is an excellent source of tissue to discover genes associated with synapse formation. She invented methods to microdissect tissue from the mouse barrel cortex to extract mRNA for oligonucleotide array analysis. She has measured the expression of thousands of genes as a function of sensory experience and development. We are now (slowly) verifying interesting candidates using QPCR and *in situs*. Although not yet published, this data is of great importance to my lab and Karen's future work. In the meantime Karen has become a real microarray jock; for example she was asked by Professor Josh Dubnau to participate in writing a review on the application of microarrays to problems in neuroscience (*CONB*, 2003).

Second, Karen has helped to develop a directed screen for synaptogenic molecules. In this screen brain slice neurons are cotransfected with candidate genes (or activated and dominant negative variants) and a fluorescent protein or tagged synaptic protein. They are then imaged with 2-photon microscopy and analyzed automatically by computer. This provides a relatively high-throughput, objective assay to discover molecules that shape synaptic structure and density. Developing this assay made Karen into a master imager. Out of this screen came Neurabin I. Karen followed up with a structure-function analysis of the synaptogenic properties of Neurabin I. This caused her to learn synaptic physiology, sophisticated imaging assays including FRAP, and a variety of uncaging techniques. Using the interaction of Neurabin I and actin dynamics Karen discovered an important interaction domain of Neurabin 1. More interestingly, she demonstrated that postsynaptic actin polymerization can be sufficient to drive synapse formation (*Neuron*, 2004). I should state that these two projects (screen, structure-function analysis of Neurabin I) did not entirely overlap with my laboratory's mainstream; I would not have pursued them without Karen. This means that Karen has worked more independently than most other postdocs in my lab. As a result things went a bit more slowly than they might have, but Karen also learned an unusual amount.

Recently Karen has picked up a study with a focus more closely related to our other projets. She is now combining time-lapse imaging with glutamate uncaging and calcium imaging at the level of single synapses to probe the development of individual nascent synapses in brain slices. These physiological studies are turning into a very beautiful project.

Karen has a scholarly streak with interests ranging from molecules to systems. She asks important questions. Karen has benefited my lab by generously sharing her skill and experience in molecular biology and genetics. Karen's talents and energy extend well beyond science. For a couple of years she led a local postdoc working group.

She is also an accomplished musician, playing the flute and saxophone at a high level.

Karen would be an excellent addition to a Cell Biology or Neuroscience Department at a first rate research university. I recommend her without reservations.

If you should have further questions or concerns please feel free to contact me.

Sincerely,

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(Karel Svoboda)