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October 1, 2005

Dear Dr. Yves Brun and Search Committee,

**Evaluation of Dr. Kurt Thorn for an Assistant Professorship Position.**

I know Dr. Kurt Thorn through informal interactions while he has been at the Bauer Center for Genomics Research (CGR). He worked on septin proteins, an interest of my group, and we discussed his project and future directions every few months. I also heard him present his work formally at a few departmental retreats. I am struck by how careful and quantitative a scientist he is, and I think he has a good set of skills and ideas to make an impact in modern basic biological research.

Dr. Thorn came to the CGR with very ambitious plans to use FRET technology to annotate the whole yeast genome with protein interactions. In the end he spent far more time that he expected developing and characterizing the FRET methodology on one interesting protein complex and then using it to gain some interesting structural and biological insight. FRET is appealing, simple in concept and sounds easy. The reality is that to do FRET well inside a living cell is difficult, requires careful calibration and correction of data to produce reliable data. To his credit, once Dr. Thorn realized the magnitude of the challenge he set out to optimize his microscope and solve all the problems in a systematic way, and ended up with probably the highest quality FRET data for a protein complex in cells anywhere. He did this essentially by himself, attesting to his ability to work independently. Dr. Thorn became quite interested in the biology of the bud neck and proposes to take a kind of in vivo structure approach to dissecting this biology. I think this is quite a creative idea, and will reveal both detailed mechanism in yeast, and probably some generally relevant ideas and/or mechanisms.

When comparing Dr. Thorn to his peers, it's important to realize he chose an independent fellowship over a conventional post doc, and therefore lacked the benefit of walking in to

an established experimental system with close personal mentoring. He has benefited from exposure to cutting edge systems biology at the Bauer Center, and from the experience of having to set up his own mini-program. That experience means he is more independent that many of his peers and may have an easier transition to running his own group.

Dr. Thorn is one of the most methodical and serious young scientists I have interacted with, and I rate his skills in quantitative experimental work as outstanding. I'm less clear as to his creativity, since the progress he shared with me was mostly a problem solving exercise in a particular methodology. I am confident he can take on tough experimental challenges and solve them, and that he is well versed in modern cell and systems biology, as well as quantitative microscopy.

Sincerely,

Timothy Mitchison, PhD

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October 4, 2005

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Dear Selection Committee:

I am writing to support Kurt Thorn's application for a faculty position. He did his graduate work with Ron Vale at UCSF, making fundamental contributions to our understanding of the molecular mechanism of microtubule motors, and since October 2001, he has been fellow of the Bauer Center at Harvard.

The Bauer Center is a unique interdisciplinary initiative, which provides an unusually supportive environment for research. The intellectual core of the Bauer Center is formed by ten fellows; outstanding young researchers who have been appointed to five year positions to develop their own, completely independent research program. The fellows are given funding to support a research group of three and are also eligible to apply for grants. They come from a diverse set of backgrounds, including genomics, molecular and cellular biology, organismal and evolutionary biology, computer science, mathematics, and physics. Their enthusiasm for interdisciplinary collaboration with other fellows and the faculty in surrounding departments is a key criterion in their selection, and all of the fellows are have been involved in active collaborations with each other and the surrounding faculty. Their research areas are diverse but interwoven with each other, ranging from computational methods of defining genetic circuits and mathematical modeling to studying the reciprocal interactions between gene expression and organismal behavior in animals and plants. They are described in detail on our web site (http://www.cgr.harvard.edu)

Kurt came to the Bauer Center with an ambitious and ingenious proposal to use fluorescence resonance energy transfer (FRET) to study intracellular signaling in real time. In brief, the idea was to use yeast molecular genetics to couple cyan fluorescent protein to a phosphopeptide binding protein and green fluorescent protein to a protein that contained a phosphorylation site of interest, and then follow the energy transfer that resulted from binding of the phosphopeptide binding protein to the phosphorylated substrate to monitor protein phosphorylation inside living cells. In the end, this project failed for a number of technical reasons, of which the most serious was that previous claims to have measured FRET in yeast turned out to be substantially exaggerated.

Undaunted, Kurt went on to do a very thorough job of identifying and then solving the problems with FRET in yeast. His innovations included developing novel media that minimized

autofluorescence, building a microscope that minimized fluctuations in illumination, and developing much better algorithms for reliably calculating the extent of FRET. He then applied these methods to a biologically interesting problem, the structure of septin polymers in budding yeast. The septins are a set of proteins, first identified through cell division mutants, that are required for cytokinesis in many eukaryotic cells. Work from Tim Mitchison's lab showed that they could polymerize in vitro and that the repeating unit of this polymer was made up of fiver different septin subunits, but the structure of this repeating unit was completely unknown.

Kurt set out to lift this veil by making detailed measurements of FRET between the different septin subunits in living cells at two different points in the cell cycle, G1 and mitosis. The essence of the problem is the same as that of solving protein structures by NMR, finding the set of related structures that produces intersubunit distances that are consistent with the measurements, albeit at a substantially lower level of resolution. The product of this work is a new hypothesis about the structure of the septin complex, the interactions between complexes that allow it to form a linear polymer, and the side-to-side associations between adjacent polymers that likely play an important role in cytokinesis. A revised version of the paper describing this work is currently being considered by Nature Cell Biology. In addition Kurt has published a useful methods paper on fluorescently tagging proteins in yeast and is preparing another on methods for making accurate FRET measurements in yeast and comparing the pros and cons of different donor-acceptor pairs.

One could view the septin paper and the two technical papers as a modest return on Kurt's time at the Bauer Center, but I don't take this position. Kurt arrived with no knowledge of yeast genetics or cell biology and he and a single technician learnt these fields, did an enormous amount of careful troubleshooting and then made an extraordinary number of painstaking measurements to determine the structure of the septin complex. In doing so, Kurt set the bar for future work on FRET in yeast, produced an important and testable model for the structure of the septin complex, and amassed reagents and techniques that he can use for a full scale assault on the problem of cytokinesis.

Although we know that actomyosin rings, septins, and positioning information from spindles are essential for cytokinesis, our fundamental understanding of the molecular mechanisms that tell furrows when and where to form and then cause their contraction remains abysmal. Kurt proposes to tackle this problem using genetics, microscopy, and cell biology. He has identified a large number of proteins that localize near the septin ring and will use FRET to measure their physical interaction with septins and a variety of genetic techniques to detect functional interactions. His goal is to identify all the proteins that play important roles in cytokinesis, and then explore the interactions between them to answer the key questions about the regulation and mechanism of cytokinesis. In addition, he is setting out to purify the septin complex from yeast cells so he can use the combination of chemical cross-linking and mass spectrometry to produce a higher resolution structure that can inform both genetic and cell biological dissection of the functions of this complex.

Since cytokinesis has been something of a graveyard for ambitious graduate students and post-docs, it is worth asking what Kurt's chances of success are. I think they are very good. First, he has learnt all the different fields he needs for a comprehensive attack on this problem. Second, he has shown that he can develop and improve techniques, something that will be essential for tackling this difficult problem. Third, he has the dedication and determination to take the long view of an important problem and not be distracted by short term tangents. Finally, he is incredibly realistic about what he has achieved and is good at setting a series of successive,

attainable goals that will lead to the ultimate prize. The majority of post-docs and fellows I have interacted with have a tendency to overestimate the importance of their contributions, whereas I have found that my and Kurt's assessments of where his work stands have almost always been perfectly matched.

In addition to his strengths as a scientist, Kurt will make an excellent colleague. He is deeply interested in a wide range of scientific problems and has a quick and critical mind. He likes working with others and has formed a very effective collaboration with Mike Laub, one of the other Bauer Center fellows, to study the localization and interaction of two component signaling proteins in bacteria. Kurt is a clear and compelling speaker and questioner and he will make an excellent teacher. Finally, he is unusually responsible and has played an important role in organizing seminars and other activities at the Bauer Center.

The best way of summarizing my view of Kurt would be to compare him to John Kilmartin at the MRC in Cambridge. More than fifteen years ago, Kilmartin quietly announced that he was going to purify the yeast spindle pole body to homogeneity, identify its components, and use the knowledge to really get to grips with how microtubule organizing centers were assembled and how they nucleated microtubules. For the next few years, nothing was heard, but then he produced a series of crucial papers demonstrating that he had fulfilled his promise and the proteins that he and his small number of associates discovered have revolutionized studies of the spindle pole body, the proteins that attach to the yeast centromere DNA, and our view of microtubule organizing centers. Although none of John's papers appeared in Nature, Science, or Cell, everyone in the field acknowledges that it was his unstinting efforts that identified the vast majority of the components that have made the advances of the last few years possible.

Like Kilmartin, I suspect that Kurt will run a smallish lab, that it will not produce annual papers that grace the Holy Trinity of journals, and that for some time he will be under-appreciated in the larger world of biology. But like Kilmartin, I expect his low-key combination of determination, interest and ingenuity in developing techniques, rigor, and commitment to an important unsolved biological problem will lead to major breakthroughs in the future.

Based on the bare bones of his track record as both a graduate student and a Bauer Center fellow, Kurt will undoubtedly look worse than some other candidates. But in both places, he has done much more than the published record shows, worked with an admirable level of independence, and been an important, well liked, and universally respected member of a high-powered intellectual community. My strong feeling is that the solid training he has received, his experience with tackling hard problems, his focus on a single goal, and his ability to set manageable goals will lead him to make very important contributions to the central question of understanding how cells divide. I recommend Kurt to you very strongly.

Yours sincerely,

A W Murray

Andrew W. Murray
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Co-Director, Bauer Center for Genomics Research
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