UNIVERSITY of VIRGINIA
DEPARTMENT of BIOLOGY

December 12, 2003

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington, IN 47405-7105

Dear Search Committee:

This letter is in support of Lance Davidson's application for a position in your department. Lance Davidson was a graduate student with Mimi Koehl and George Oster at UC Berkeley when I was on the faculty there. I was on his thesis committee. Toward the end of his time there I offered him use of my lab for his experiments and paid him from one of my grants as his other sources of support had dwindled and his research, which had turned from modeling to experimentation, was relevant to our goals. I kept in touch with his work at Berkeley as close as an advisor would have, and he spent a good bit of his time in our lab. He came to my lab as a postdoc after I moved to Virginia, and he began working on problems of common interest to our lab and Doug DeSimone's lab in the School of Medicine. He currently works in both the Keller and DeSimone labs. I support his application with great enthusiasm.

Lance is exceptionally well-qualified and has great potential for a research career. He was one of the best graduate students in my 15 year experience at U.C. Berkeley. He did an outstanding graduate thesis and published an excellent paper in **Development** (Davidson et al. 1995), and followed with another in **Developmental Biology** (Davidson et al., 1999), which he finished in my lab here at Virginia. In the first paper he did finite element computer simulations of five different mechanisms of primary invagination in sea urchin that have been proposed from various labs. He mapped out a parameter space of the mechanical properties of the extracellular matrix and the epithelial cells that would work for each of the five models. For each model he could make predictions about the mechanical properties of cells and matrix and cell shape changes that should occur with each model. It is an outstanding piece of work because unlike much of the modeling work, it makes predictions that can be experimentally tested in solid engineering and cell biological terms. Going beyond modeling, Lance set about testing these models, and in doing so, he has proved to be an exceptional experimentalist. He managed to make mechanical measurements of the elastic modulus of the sea urchin blastula, which is very small, and to make matters worse, it is ciliated and moves rapidly, making it difficult to constrain for measurements. Using this information he determined which models could work for a given species of sea urchin in the second paper. Overall, the work has

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is the best attempt thus far at a real biomechanical cellular analysis of mechanisms for a "simple" invagination and an exceptional interplay of modeling, biomechanics and experimentation.

Lance has the fairly rare combination of excellent mathematical and computer skills, biophysical skills, and experimental skills with cells and embryos; he shows no hesitation in getting his hands wet. He has the patience and the diligence to work out methods, to make up buffers, to deal with the idiosyncrasies of embryonic development, and to work with very small objects. He is very bright at doing math, physics, and computer work but yet does with ease the massive amount of pipeting of clear, minute quantities of liquids that is entailed by modern cell and molecular biology with ease.

He has learned a lot of cell biology, molecular biology, and development and has accomplished much in his work on frogs here in my lab and with Doug DeSimone. He developed a fluorescent whole mount in situ RNA hybridization procedure that allows us to look at expression of several genes in one cell, or to precisely correlate cell motility with gene expression, in 2 micrometer thick confocal slices (Davidson and Keller, 1999). This and careful imaging allowed him to resolve the cell behaviors involved in closing the neural tube of *Xenopus*. He discovered that it closes in a very different way than we had thought. The entire dorsal two thirds de-epithelializes and intercalates radially and mediolaterally to form an extended, single layer tube. In the process he also worked up methods of imaging neural tube closure at high resolution with methods adaptable to other species. He worked up a two-focal plane, two color confocal imaging system that has allowed us to resolve the protrusive activity of intercalating mesodermal and neural cells at the levels of resolution needed to determine how molecules in the planar cell polarity pathway, and downstream effectors polarize these cells (see his images and movies in Keller, R., Science 298, 1950-1954 (2002) www.sciencemag.org/cgi/content/full/298/5600/1950/DC1). He is currently exploiting these methods and the integrin and matrix reagents developed in Doug DeSimone's lab to probe the function of cell-fibronectin interactions in polarized cell behaviors underlying cell intercalation during convergent extension. He had previously used fibronectin substrates and these reagents to analyze the cell behavior underlying mesoderm migration (Davidson et al., 2001), and now has turned his attention to cell intercalation. Recently the components of the vertebrate version of the planar cell polarity pathway (PCP) (and also the wnt/calcium pathway), including Wnt, frizzled, dishevelled, several and downstream GTPases, have been implicated in controlling the polarized cell behavior thought to drive cell intercalation that we had described some years ago. The output of most experiments in this field are on the gross level, i.e., arrest of gastrulation, inhibition of convergent extension of activin treated animal caps, failure of "Keller explants" to extend, or failure of mediolateral elongation and alignment of cells, rather than direct assays of cell motility and polarized protrusive activity. As a result, these discoveries have implicated these genes and their encoded molecules in the process, which is a great advance, but they give very little insight into mechanism. Probing deeper will require 3 dimensional analysis of how the behavior and biomechanics of the intercalating cells changes on various manipulations, allowing us to relate molecule or gene to forces. Lance can accomplish this.

Lance has developed open-faced explants and fluorescent confocal imaging methods that allow him to probe the mechanism of how cell-cell and cell-matrix adhesion is involved in cell polarization, cell traction, and intercalation. These methods allow him to look directly at multiple cell functions in

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3 dimensional architecture. In a spectacular piece of work, he recently used Doug DeSimone's non-function blocking antibody against fibronectin to cy3-label the fibronectin fibrils in living explants. He then imaged the fibrils, the cells at the plane of fibronectin fibrils, and also 5 micrometers within the depth of the cell simultaneously in 3-level, 3-color (red-green-red on 3 channels) movies of the intercalating cells. My interpretation of his work is that he has discovered that the cells take the fibrils with them as they intercalate, rather than crawl on the fibrils. But he has also shown directly that interaction with the fibronectin fibrils is necessary but not sufficient for polarization, and intercalation, something Mungo Marsden in Doug's lab has previously shown indirectly. In short, the PCP pathway has multiple, sequential roles, some direct and others indirect and supportive in polarizing the cells that intercalate and produce convergent extension.

Lance is in a good position to sort out this type of problem. He is a master imager of cell behavior, as good or better than anyone else. He has been able to look at the cells in 3 dimensions and time with unparalleled resolution, and he understands the biomechanics that engages the local cell behavior to the macro tissue. Moreover, his skills and methods are broadly applicable in various developing systems, and will make a great contribution to enhancing this capability in his new institution, wherever that might be. Export of this technology to other labs will allow many more labs will be able to directly image these cell behaviors, enabling them to directly read out cell motility and cell polarity phenotypes of genetic and molecular manipulations of the polarity genes.

Lance adds another dimension to the problem and that is that he can analyze the most important output in morphogenic systems- the production and vectors of forces, as well as the tissue mechanical properties that transmit these forces. He designed a new model of the "Histowiggler", the biomechanical measuring machine that Steve Moore, Mimi Koehl and I used back at Berkeley to measure for the first time the increase in tissue stiffness that occurs with convergent extension. The new version is much more compact, more stable, more sensitive, allows a greater range of tests, and allows easier simultaneous imaging at high resolution of cell responses to mechanical manipulation of tissues. This machine can be built and used by anyone reasonably fluent in mechanics. It will open up new avenues of experimentation, since we can now more easily determine how genes affect mechanical properties of tissues.

Lance is a problem-oriented investigator who wants to learn how cells generate and integrate the collective forces that drive morphogenesis. He has worked with sea urchins and amphibians primarily but is not stuck on a system. He knows the cell biology and mechanics- what little is known- of the fly, the worm, and the fish. He is endlessly inventive and can be expected to attack the cellular and molecular biomechanics of morphogenesis in pretty much any system that offers opportunities. He stands apart from the usual candidate in that he is unique. He could work on the same gene in the frog, or work on the same zebrafish mutant affecting morphogenesis that many other labs are working on, and he would be able to answer different questions and to penetrate beyond their level of analysis with his approaches. In other words, he is unique and does things very few others will do. Compared to his competition, they are largely seeking to implicate a gene in a process. and then the going gets tough and they move on to another problem. Lance can actually find out how the machine works. In this regard, he would be a very valuable addition to any department that has investigators working on any of the systems in which morphogenesis can be

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genetically manipulated- he can take the whole enterprise into a new arena. He would be an outstanding complement to a strong genetics group, and he would be a very strong collaborator on joint work and program projects focused on a multilevel analysis. His microscopy and imaging skills coupled with his ability in biomechanics and cell/developmental biology makes him exceptionally valuable. He would make broad contributions to research and teaching, within and beyond his department.

Lance is a good teacher and mentor. He gets along with and works very well with others and shows no personality problems that would limit his career. He has mentored a number of undergraduates and graduate students in the lab, all of them successfully. He is competitive and assertive, and can hold his own in any interaction, but is fair, honest, trustworthy and generous. He will be a good colleague, a leader in his field, and a credit to the scientific community at large.

Lance stands at the interface of biomechanics and cell and developmental biology. As our manipulative skills and possibilities have expanded with molecular biology and molecular genetics, and our ability to image molecules and cells during morphogenesis with high resolution methods has increased, the last frontier is getting down, finally, to where the rubber meets the road in morphogenesis- molecular, cell and tissue biomechanics. Lance is one of the best of a very few of cross-trained individuals who can go there. Funding in this area and interest in it has increased dramatically in the last 5 years, and is, or about to be targeted by a number of NIH and NSF programs to develop the area between physics, biomechanics, nanotechnology, and biology. Lance will be able to set up an exceptional and unique program at the cutting edge.

Overall, Lance is one of the best people I have had. He is very similar in interest and range of ability to Jeff Hardin, one of my former students, and one of my best, now a successful Full Professor at Wisconsin. Both combine strong backgrounds in math and physics, keen imaginations, and excellent experimental expertise in embryology, imaging, cell biology, and biomechanics. He is one of the more versatile and broadly trained people out there. I recommend him very, very highly and without reservation.

Sincerely,

Ray Keller
Professor and Chair

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