

IUSB Faculty Research Grant Final Report

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What Makes A Cell Crawl

The Faculty Research Grant was a wonderful experience for my summer students and myself. From this grant I have learned much about IUSB and the process of conducting full-time research at an undergraduate institution, both the benefits and the limitations. The FRG was for the summer 2003, and I spent 12 weeks working constantly on the project. During the summer 2003 I had five undergraduate students conducting research in my laboratory, including Victoria Pysh, a recipient of a SMART summer fellowship.

1)-Description of grant supported activity.

In the project, there were four main specific aims. I proposed to accomplish aims 1 and 2, and to get a good start on aims 3 and 4.

Specific Aim 1:

By the end of the 12-week period, I identified a technique to purify actin from *Dictyostelium*, rather than from rabbit muscle, thus accomplishing specific aim number one, but through slightly different means. In the future I will use both rabbit muscle actin, and *Dictyostelium* non-muscle actin in my studies. It was much more challenging preparing actin without an ultracentrifuge, but through the use of column chromatography and differential polymerization, coupled with long spins in the high-speed centrifuge in my laboratory, it was possible.

Specific Aim 2:

This was the bulk of the summer project. It took one full week to make up all the buffers for the process of membrane purification, but after the cells started growing to large enough populations for membrane production, the process went smoothly. *Dictyostelium* cells were successfully grown in 12-liter batches, processed and their membranes purified. These membranes were pooled and kept in the cold room in dialysis tubing with numerous protease inhibitors.

Specific Aim 3 & 4:

While I proposed to get a good start on aims 3 & 4, technical hurdles inhibited me from completing these aims.

2)-Were you able to complete the project? Describe any difficulty you had.

The entire project was not completed during the summer. This is because it is the beginning of a long-term project that currently involves two undergraduates. The main problems encountered were the loss of membranes due to the use of a fixed angled rotor (instead of a swinging-bucket rotor), and the loss of membranes after the summer was over due to a person bumping the stir plate in the cold-room. This caused the stir

plate to speed up, and the magnet ripped the dialysis tubing apart, releasing the purified membranes into the dialysis buffer.

From the membrane preparations I learned to expect only 50% as much membranes from the fixed-angled rotor, so I do more preps and pool the membranes. As for keeping the membranes away from other people, I now keep the finished products in a much safer place. I am trying to talk the department into purchasing the swinging-bucket rotor that would make membrane preparation much more efficient. A new setback to finishing the project is the malfunction of a Perkin-Elmer LS-3 Fluorimeter that I purchased to conduct the actin polymerization assays. I have to ship the piece of equipment to an independent repair person in Tennessee to have it repaired. After this is accomplished, things should move forward at a steady pace.

The last hurdle to the research was a simple matter of space. With numerous students working in the same lab with me, it became very crowded and painful. The department has allocated more space to me, but having two different labs in different places does not seem to be working well. I am currently trying to locate a single laboratory where all of my equipment and students will be more functional.

3)-Did, or will the project result in a specific product – a manuscript, composition, syllabus, etc? If so, please describe and indicate state of development.

This project is ongoing, with multiple approaches. I am currently making more actin, membranes, and have all of the lipid probes to finish the third aim. I have a summer student (Steve Duleh) working to identify Rac-binding proteins in *Dictyostelium*. These proteins could very well be the effectors activated through the diacylglycerol pathway. This addresses specific aim number four, and will lead to a good presentation at the American Society for Cell Biology.

A manuscript will be submitted from the research conducted under this grant, and the research being conducted by Steve Duleh and Aimee Marino. The data will be used as preliminary data in my NSF grant that I will be resubmitting this year. The manuscript currently contains an introduction and materials and methods. I will have to get some more results to finish the manuscript for publication.

Again, the FRG was a wonderful experience, letting me determine what can be accomplished at IUSB during specific time intervals.