UNIVERSITY of GUELPH

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ANNOUNCEMENT: Interested Members of the University Community are Invited to attend the Final Oral Examination for the Degree Master of Science of

Corey Kelly

of the Department of Physics, on Monday, November 7th, 2011 at 10:00 a.m. Science Complex, Room 1504, University of Guelph.

Thesis Title: **Effect of antimicrobial agents on MinD protein oscillations in Escherichia Coli**

Examination Committee: Dr. Paul Garrett Chair,
Dr. John Dutcher, Advisor
Dr. Cezar Khursigara
Dr. Leonid Brown

ABSTRACT

The Min protein system regulates cell division in the bacterium Escherichia coli. The protein MinD undergoes a pole-to-pole oscillation, antagonizing formation of the division septum at the cell poles, thereby confining the septum formation to the mid-cell. The MinD oscillation period is 40 s at room temperature in healthy cells, but has been shown to be sensitive to stress on the cell. By fluorescently labeling MinD with green fluorescent protein (GFP), we are able to measure the MinD oscillation period as an in situ metric of cell viability using high resolution total internal reflection fluorescence (TIRF) microscopy.

We have made several improvements to the method by which we measure and analyse the MinD oscillation period. A microscopy flow cell was designed and constructed and it provides temperature control and stability to a precision of 0.05 °C in addition to allowing controlled addition of bacterial cells and reagents of interest to the imaging region of the flow cell. This flow cell enabled us to make a precise measurement of the temperature dependence of the MinD oscillation period, for which we observed an Arrhenius dependence with an activation energy of 11.8 kcal/mol. We developed a centroid-tracking method, performed in a custom MATLAB program, to extract the values of the MinD oscillation periods from our time series of TIRF microscopy images.

We measured the effect on the MinD oscillation period of exposure to the cationic antimicrobial peptide polymyxin B (PMB) and the related compound polymyxin B nonapeptide (PMBN), which does not have antimicrobial activity. Exposure to PMB resulted in a 60% increase in the average MinD oscillation period tau, whereas exposure to PMBN resulted in an 20% decrease in tau. After exposure to PMB and PMBN, we measured the Arrhenius temperature dependence of the MinD temperature dependence and calculated the associated activation energy Ea. We found that exposure to PMB resulted in a 40% increase in Ea, whereas exposure to PMBN did not significantly change the value of Ea. These results indicate that careful measurements of the MinD oscillation can yield information that can be helpful in evaluating the mechanism of action of antimicrobial compounds.

By: Corey Kelly
Advisor: Dr. John Dutcher

CURRICULUM VITAE

EDUCATIONAL BACKGROUND:

BSc (Honours), Memorial University of Newfoundland, 2009.

ACADEMIC AWARDS:

2008 NSERC USRA
2009 NSERC USRA
2009 NSERC CGSM
2010 OGS
2011 CAP Congress Best Student Talk (DMBP Division)
2011 CAP Congress Best Student Talk (Overall)

PUBLICATIONS:

Kelly, C.J., Giuliani, M., Dutcher, J. (2011) Precise Measurements of Min Protein Oscillations in Bacterial Cells Using TIRF Microscopy. Physics in Canada. July-September 2011. (In press.)