

University of Maryland Baltimore Graduate School

Announcement of Doctoral Dissertation Defense*

Candidate: Rebecca A. Bozym

Date, Time, and Place: August 24, 2007 11:00 AM, RM102 N Greene Street

Dissertation Title: Measuring Intracellular Free Zinc using Carbonic Anhydrase as a Fluorescent Biosensor

Dissertation Abstract**:

Zinc is a metal ion of great importance in the biological world. It is a cofactor in over 300 enzymes and is necessary for proper function, growth, and development of the human body. Zinc is also a vital component in the immune and reproductive systems, and in zinc fingers of transcription factors. Zinc has also been implicated in apoptosis where there is still debate over whether it is protective or pro-apoptotic. Recent papers have also focused on zinc and its relation to oxidative stress as well as mitochondrial function and membrane potential disruption. It has been proposed that oxidative stress could result in the release of zinc from binding sites such as metallothionein; where it would go on to induce apoptosis by inhibiting electron transport chain enzymes and/or other enzymes present in the mitochondria. However it is also thought that an increase in the amount of intracellular zinc could also initiate these events. A necessary starting point in understanding the many roles of zinc is a quantitative measurement of biologically active zinc, i.e. readily exchangeable zinc. Due to the modest amounts of zinc and the presence of other divalent cations zinc measurements require high selectivity and sensitivity. In order to achieve the necessary level of selectivity and sensitivity we have employed carbonic anhydrase as the sensor transducer for zinc binding due to its picomolar affinity for zinc. This system is based on resonance energy transfer from a zinc-bound aryl sulfonamide to the fluorescent label on carbonic anhydrase. Zinc levels are then measured by taking the ratio of fluorescence intensities at two different excitation wavelengths. Using this system we have successfully measured the level of labile zinc in resting PC-12 cells. Zinc levels and occurrence of apoptosis were also measured with varying amounts of available zinc in the media. In order to monitor zinc levels in mitochondria, an expressible system was developed with DsRed2 fused to carbonic anhydrase. In order to specifically monitor the mitochondrion, a mitochondrial target sequence was fused to the 5' end of carbonic anhydrase thus creating an expressible biosensor to measure free zinc in cells.

Dissertation Committee Chair (name and title): Richard B. Thompson, Associate Professor

Dissertation Committee Members (names and titles):

Dr. David Weber, Professor

Dr. Chris Geddes, Professor

Dr. Veronika Szalai, Assistant Professor

Dr. Michael Johnson, Professor

The Open Presentation is open to the university community and invitees of the candidate. Any member of the Graduate Faculty may observe the Final Examination. Only committee members may vote. For more information, see **Procedures for Examination of the Doctoral Dissertation.*

***You must type your abstract on this form in the space provided.*

Updated: February 24, 2006