High-Pressure Biophysics and Biotechnology at the Department of Physics: 2008 Update

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For Students

If you are thinking about getting involved in professional school, or would like hands-on experience doing exciting science, read on...

An important goal of my laboratory is to ACTIVELY involve students in the research process. You are encouraged to initiate and develop projects, and are given the freedom and guidance to do so. The earlier you get involved and STAY involved, the more substantial and meaningful the experience will be. See me if interested.

Lab Members

It (l to r) Michael Eldridge (graduate student), Erica Raber (graduate student), Joshua Jasensky (physics)

High pressure, fluorescence-based sensing of calcium ions

Sara Savage (’09), Eric W. Frey (’09)

To be presented at the 5th International Conference on High Pressure Biophysics and Biotechnology, La Jolla, CA Sept 15-20, 2008.

Conference Abstract

Fluorescence-based sensors offer a number of advantages over the use of ion-sensitive microelectrodes or optical fibre sensors. These include lower cost, ease of use, sensitivity and selectivity, and the ability to observe a range of calcium concentrations in vivo. In this study we report the development of a new pressure fluorimeter that allows the measurement of fluorescence intensity under a range of hydrostatic pressures. The system is based on a standard optical microscope, with imaging and data analysis performed with a computer program.

Methanol-denatured dynamics of NADH probed using fluorescence spectroscopy

Joshua Jasensky (’09, US), Jumaid Farooqi (GS ’09), Daniel Horne (’08)

To be presented at OSAPS, Oct 10-11, 2008.

NADH is an intermediate in several redox reactions and a metabolic co-factor useful for non-invasively probing cellular metabolic activity. NADH folding-unfolding transition and intermolecular NADH interactions in solution can be probed using fluorescence spectroscopy. Future studies involve solution and in vivo investigations under pressure.

A calibration approach for rapid fluorescence lifetime determination for applications using time-gated detection and finite pulse width excitation

Scott B. Keller (’08), Jonathan A. Dudley (’07), Katherine Binzel (’07), Joshua Jasensky (’09), Hector Michael DePedro (GS ’08), Eric W. Frey (’09)


Paper Abstract

Time-gated techniques are useful for the rapid sampling of excitation-state (fluorescence) emission decay in the time domain. Gated detectors coupled with bright, economical, nanosecond-pulsed laser light sources like flashlamps and nitrogen lasers are an attractive combination for biological and biomedical applications. Here we present a calibration approach for lifetime determination that is non-destructive and that does not assume a negligible instrument response function (i.e., a negligible excitation pulse width). Such time-gated fluorescence decay-time measurements are also used as a calibration reference. A fast avalanche photodiode and GHz-bandwidth digital oscilloscope is used to record fluorescent emission decay-time from different calcium sensitive analogs. The measured decay-time is a function of calcium concentration and is proportional to the lifetime and can be determined with good reproducibility (typically <100 ps error for data with poor signal-to-noise (~2:1)). Results are presented for different analogs using flow-through systems. A fluorescence lifetime imaging application is proposed. In conclusion, a calibration-based approach is a valuable analysis tool for rapid determination of lifetime in applications using time-gated detection and finite pulse width excitation.

High-pressure, fluorescence-based sensing of calcium ions

Sara Savage (’09), Eric W. Frey (’09)

To be presented at the 5th International Conference on High Pressure Biophysics and Biotechnology, La Jolla, CA Sept 15-20, 2008.

Conference Abstract

Recent calcium ions are nearly ubiquitous in cellular signaling and control. Methods for calcium-ion sensing at high pressures are valuable in investigating pressure effects on cellular function. Optically-active, calcium-sensitive dyes provide the means for quantitative ion sensing at physiological concentrations. Here we present high-pressure techniques for calcium-ion sensing using the Fluo4 calcium-sensitive dye. We find that calcium concentration and ion binding properties of the dye can be determined using an Arrhenius expression. The effective pK under pressure was determined using a two-state bound/unbound model for fluorophore/calcium ion dissociation. Assuming an Arrhenius relation, the effective pK was determined using a two-state bound/unbound model for fluorophore/calcium ion dissociation. The percent deviation is a useful parameter on the nitrogen laser is also shown that the area is a useful parameter on the nitrogen laser is also shown that the area is a useful parameter on the nitrogen laser is also shown that the area is a useful parameter.