



The Department of
Pharmacology

Proudly Presents the
Seminar Series:

Signaling

Neuroscience

Genomics

Frontiers in Pharmacology

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“Using FRET to measure the binding affinity, binding kinetics, and structure of membrane protein complexes: Application to phospholamban and the cardiac calcium pump.”

Membrane protein complexes present a special challenge to traditional methods of biochemical characterization. Proteins in lipid bilayers cannot be rapidly mixed or diluted for binding experiments, and detergent solubilization disrupts many important interactions. They are also notoriously resistant to structure determination; of the 36,000 protein structures published in the Protein Data Bank, only ~100 are membrane proteins. Thus, there is a great need to complement classical methods with new approaches to the binding affinity, kinetics, and structure of membrane protein complexes.

We have used fluorescence resonance energy transfer (FRET) to investigate the interactions of the membrane protein phospholamban (PLB) and its regulatory target, the cardiac calcium pump (SERCA). These proteins play a central role in cardiac function and pathophysiology, and are considered high-value therapeutic targets for the treatment of heart failure. Our experiments provide insight into the effects of mutation and phosphorylation on the affinity of PLB-SERCA and PLB-PLB binding interactions. Distance constraints obtained by FRET discriminate among models of the PLB pentamer quaternary structure. In addition, we will discuss the Förster Transfer Recovery (FTR) technique, a new approach to membrane protein complex subunit exchange kinetics.

Friday, October 26th

10:00 am

**Auditorium (Room 1005)
in GBSF**