Department of
Chemical and Environmental Engineering

2013—2014 Seminar Series

Friday December 6, 2013

9:30—10:30 AM

WCH 205/206

Anne Skaja Robinson

Catherine and Henry Boh Professor of Engineering

Chair of Chemical and Biomolecular Engineering

Department of Chemical and Biomolecular Engineering

Tulane University

Adenosine receptor expression and biophysical characterization: Role of the protein and lipid characteristics in trafficking and activity

Proteins that reside in the cell membrane represent the most difficult challenges for expression and isolation, because they are partially hydrophobic, flexible, and unstable in isolation. However, they are among the most important of all proteins, as they play key roles in almost every cellular process, and represent over a third of all proteins. Here, I will highlight our studies of expression G-protein coupled receptors (GPCRs), in which we try to understand the importance of the membrane protein sequence or composition in the proper trafficking within our heterologous host, *S. cerevisiae*. Over the last eight years, we have had great success expressing the human adenosine A_2a (A_2aR) in yeast. Here, typical yields were ~ 7 mg active $A_{2A}R$ per liter of culture; activity was verified by ligand-binding assays. To understand why some receptors are trafficked correctly to the cell surface, and others are not, we utilize protein chimeras of similar receptor families to determine critical receptor regions.

Because of the ability to purify mg quantities of active $A_{2A}R$ protein, we have been able to carry out biophysical studies to understand the role of detergent on the protein stability. Unlike soluble proteins, for membrane proteins the protein-lipid interactions are as important as protein-protein interactions. Here, I describe our studies of the effects of different membrane-mimetic environments on activity and stability. Through this combination of cellular and *in vitro* approaches, we can better understand the limitations to large-scale production and biophysical studies of the G-protein-coupled receptors – the largest known protein superfamily, and the targets of 30-50% of drug discovery efforts.