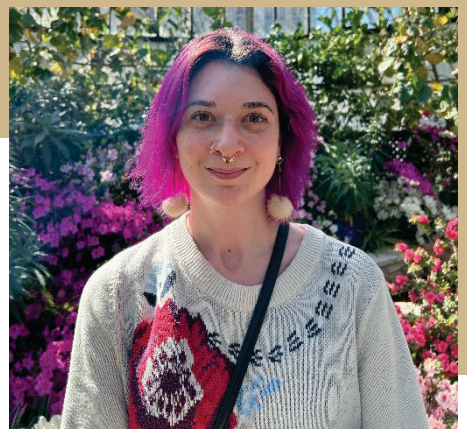
BIOCHEMISTRY SEMINAR



Cara Trench

Graduate student | Lyon Group | Purdue University

"Identification of Isoform-Specific Differences in Phospholipase Cb Regulation"

Cardiovascular disease (CVD) is the leading cause of death worldwide. The most common types of CVD are coronary artery disease and coronary heart disease, accounting for a combined nearly 50% of CVD cases. Implicated in both of these diseases is phospholipase C (PLC) activity. These enzymes cleave phosphatidylinositol-4,5-biphosphate (PIP2) at the inner leaflet of the plasma membrane, leading to increased formation of secondary messengers diacylglycerol (DAG) and Inositol 1,4,5-trisphosphate (IP3), which in turn increase intracellular Ca2+ and activate protein kinase C (PKC). Of the six subfamilies of PLC, the PLCβ subfamily is of special interest as they vary structurally from other subfamilies and they are activated downstream of G protein-coupled receptors through direct binding of the Gαq and Gβγ subunits. The four PLCβ isoforms vary in their basal activities and in their sensitivity to activation by heterotrimeric G proteins. However, the molecular basis of these differences has not been investigated. The proteins vary most in the sequence of their proximal and distal C-terminal regulatory domains (CTDs). Based on studies of PLCβ3, the proximal CTD inhibits basal activity, but is displaced by Gαq-GTP binding. The distal CTD also binds to activated Gαq, as well as the membrane. Whether the CTDs fulfill similar roles in other PLCb enzymes has not been experimentally determined. We recently determined the cryo-electron microscopy reconstruction of human PLCβ2, revealing striking differences in the organization of its proximal CTD. We are using cell-based studies to determine how the proximal and/or distal CTD contributes to basal and G protein-stimulated activities across all four PLCβ isoforms. These studies will reveal, at the residue level, isoform-specific differences that dictate their regulation. Such insights can be used to develop new therapeutic strategies for modulating the activity of PLCβ isoforms in cardiovascular disease.

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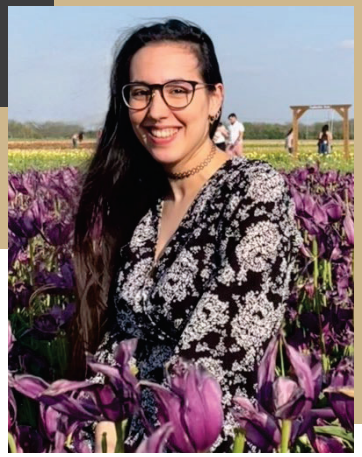

Stephanie Barrios

Graduate student | Lyon Group | Purdue University

"Investigating the Guanine Nucleotide Exchange Factor Activity of Phospholipase Cε"

Heart failure is the leading cause of death worldwide, with current treatments only managing symptoms rather than stopping disease progression. Phospholipase Cε (PLCε) plays a vital role in heart health by regulating cardiac function. In response to the activation of different cell surface receptors, PLCε can be recruited to either the plasma membrane or the perinuclear membrane through the direct binding of small GTPases. PLCε recruitment to the plasma membrane is driven by the RhoA GTPase, where its activation protects cardiomyocytes from ischemicalreperfusion injury. Translocation of PLCε to the perinuclear membrane is driven by the Rap1A GTPase and results in maximum cardiac contractility. However, dysregulated or sustained signaling through this pathway results in cardiovascular disease. This process is driven by the CDC25 guanine nucleotide exchange factor (GEF) domain within PLCε. This domain binds to inactive Rap1A (Rap1A·GDP) and catalyzes nucleotide exchange to activate the G protein. The newly activated Rap1A·GTP can then binds to the C-terminus of PLCε, sustaining activation. This proposed feed-forward activity increases pro-hypertrophic gene expression, leading to cardiac hypertrophy and heart failure. Very little is known about the CDC25 domain or its specificity for Rap1A. We have now expressed and purified the region of PLCε that houses its GEF activity and found that the CDC25 and PH domains for a functional module. We have also found that this CDC25/PH module can only catalyze nucleotide exchange when Rap1A is localized to a model membrane. We are using site-directed mutagenesis and functional assays to identify the molecular basis for its specificity for Rap1A over other closely repeated Rap GTPases. This research will provide new insights into cardiac signaling and open new avenues in developing small molecules that block PLCε-dependent activation of Rap1A. Such inhibitors could disrupt the pathological feedback loop that leads to cardiac hypertrophy, offering a potential therapeutic approach for heart failure.