Drug delivery research is critical. Opportunities in areas such as siRNA-based therapies are immense, but delivery is a major obstacle since it is unclear how to effectively cross the cell’s plasma membrane [1]. Nature, however, already builds molecules that traverse this membrane. This special class of proteins is called protein transduction domains (PTDs), or cell-penetrating peptides [2]. I am inspired to learn the fundamental structural elements responsible for their ability to cross membranes by synthesizing and analyzing polymers designed to have similar biological properties to these PTDs.

The first aspect of my research project aims to understand what role aromatic elements have on promoting transduction of bifunctional protein transduction domain mimics (PTDMs) across both model membranes and mammalian cells. Previous studies have focused on poly-arginine’s unique ability to traverse the cell membrane. Results show that this molecule’s transduction efficiency is low by itself, but is significantly improved in the presence of the aromatic “activator” pyrenebutyrate [3]. Additional studies by my research group have indicated that incorporating hydrophobic moieties directly into the PTDMs they become self-activating. These experiments, coupled with the fact that many transmembrane proteins contain aromatic amino acids concentrated near the headgroups of the phospholipid bilayer [3], suggest that aromaticity, in addition to hydrophobicity, may play an important role in PTDM-membrane interactions. Therefore, I propose the incorporation of various aromatic functionalities, including electron-rich and electron-poor aromatic rings, into PTDMs synthesized by ring opening metathesis polymerization (ROMP). These systematic changes will enable me to understand the effects of aromaticity in transduction. It is expected that π-(cat)ion interactions can effectively modulate the activity of PTDMs[4].

The proposed bifunctional PTDMs are shown in Figure 1. To synthesize these molecules, a cyclic anhydride will be ring-opened using an alcohol and a DMAP catalyst. The protected guanidine functional group will then be added using EDC coupling. [5]. Lastly, PTDMs will be synthesized by ROMP using a 3rd generation Grubbs catalyst. In order to visualize the PTDMs within cells or vesicles, they will be end-labeled with a diazole dye as shown [6]. All polymers will be analyzed by 1H-NMR and 13C-NMR spectroscopy to verify chemical composition and by GPC to determine MW and PDI. Cellular uptake experiments using the HEK 293 cell line will be performed to determine which PTDMs are the most effective. Fluorescence activated cell sorting (FACS) will be used to measure the fluorescence of NBD-tagged polymers that successfully enter the cells using previously described methods [2].

From the activity of PTDMs determined in vitro, focused biophysical experiments will be performed to more deeply understand the fundamental interactions between PTDMs and biological membranes. Specifically, phosphatidylcholine (PC) vesicles will be prepared containing entrapped carboxyfluorescein using standard methods [6]. Additional vesicles will be made containing 10 mol% anionic (PS) or cationic (DOTAP) lipids. Based on the aromatic electron density schematics shown in Figure 2[7], it is expected that PTDMs containing electron withdrawing groups (NO₂, CN) will more effectively traverse vesicles containing negatively charged (PS) lipids. In contrast, PTDMs containing electron donating groups (OMe, CH₃) are expected to more effectively traverse vesicles containing cationic (DOTAP) lipids.