***Development of a High-Throughput FRET-Based Assay***

***to Enable Rapid Amine-Acid Reaction Discovery***

**Andrew Outlaw**

Advisor: Tim Cernak

3rd Year Seminar

Medicinal Chemistry Program

February 28, 2023

The physicochemical properties of a molecule have a profound and direct link to its medicinal function. However, the traditional path to developing new life-saving medicines is largely facilitated by a select few chemical transformations. By far, the most popular of these transformations is the uniting of a carboxylic acid and an amine to form the amide bond. The highly robust nature of this reaction and vast availability of amine and acid feedstocks largely contribute to the popularity of the amide coupling in medicinal chemistry. However, it is also possible to envision an array of currently undeveloped theoretical transformations enabled by the coupling of amines and acids that impart distinct and interesting physicochemical properties beyond that of the amide motif.

The Cernak lab is interested in surveying this expanse of unexplored amine-acid coupling space using High-Throughput Experimentation (HTE) techniques and liquid handling automation to expedite reaction discovery and optimization. While modern robotics platforms make it trivial to setup high-throughput experiments in 24, 96, 384, and 1536-well plate formats, the process of extracting key information from large HTE data sets is still highly time-consuming. Conventional chromatographic analysis of 1,536 reaction datapoints can take upwards of 3 days of instrument runtime, compared to the single day it takes to setup the 1536-well plate experiment. Even after instrument runtime, reaction deconvolution can require several days alone to determine what, if any, products were made in each reaction well amongst the host of other chemical species present. These factors often lead to long turnaround times on largescale experiments where a large portion of the time spent is in data collection and analysis to distill down what worked and what didn’t, before proceeding forward.

To combat this analytical bottleneck in the HTE workflow and enable faster exploration of amine-acid coupling space, we are developing a Fluorescence Resonance Energy Transfer (FRET) based screening assay capable of giving near instantaneous feedback on coupling of dye-tagged reaction substrates. We have synthesized a Perylene-tagged amine and BODIPY-tagged carboxylic acid that are capable of functioning as a FRET pair to give indication of amine-acid coupling through fluorescence analysis in a fraction the time as conventional chromatographic methods. Ultimately, through introducing FRET-based reaction analysis into our HTE workflow, we will run a large-scale screening campaign of amine-acid coupling space to showcase the use of FRET for general purpose reaction discovery/optimization and in the identification of novel amine-acid coupling reactions.