enzyme, so the native enzyme is not affected. If the cofactor binding pocket of the targeted PKMT, in this case a SMYD protein, is mutated in the correct way, it can be specifically engineered to take up a synthetic SAM analogue when the native enzyme would only accept SAM. The Luo Laboratory has synthesized several SAM analogues, notably those containing a terminal alkyne group such as 4-propar-gyloxy-but-2-enyl (Pob)-SAM \[9\] and (E)-hey-2-en-5-ynyl (Hey)-SAM \[2\]. These SAM analogues have been used recently to profile the substrates of methyltransferases PRMT1 and G9a, respectively, using targeted mutations to their cofactor binding pockets \[2\], \[9\]. The terminal alkyne group present on both synthetic cofactors is able to undergo high-efficiency click chemistry with azide dyes, allowing for analysis of labeled substrate proteins.

Given this method’s success in the PKMT family, the goal of our research is to extend it to the SMYDs. Beginning with SMYD2 and SMYD3, the cofactor-binding pockets of the SMYDs will be engineered to accept synthetic SAM analogues, facilitating labeling of their protein substrates and allowing them to be profiled.

**Materials and Methods**

**Molecular Cloning:** DNA sequences were originally contained in pET28-MHL bacterial plasmid vector. The protein inserts were amplified using the Qiagen® HotStar® Hi-Fidelity Polymerase Chain Reaction Kit. To prepare for the SMYD insert, the mammalian plasmid vector pcDNA3, containing an unwanted insert, was obtained and digested with appropriate restriction enzymes (Hi-Fidelity EcoR1, Nhe1 and/or Not1). The PCR product of each SMYD sequence was digested with the same restriction enzymes, and then ligation was done using the T4 DNA Ligase enzyme. All enzymes were obtained from New England Biolabs, Inc®. A 1:6 ratio of insert:vector was used for ligation, and successful reactions were transformed into TOP10 *E. coli* cells. After sequencing, successful clones were subsequently identified and transformed into DH5α *E. coli* cells, where DNA was harvested using the Qiagen® Hi-Speed® Maxiprep kit®.