Jessie Garcia ♦ Research Statement

Engineering of a General Methyltransferase Biosensor

Nature and evolution have worked together to create pathways for the synthesis of complex bioactive natural products. Large multimodular biocatalysts within these pathways direct regio- and chemospecific reactions, leading to diverse classes of natural products. The complexity and versatility of these molecules are likely the reason they continue to be important leads for new medicines. However, advances in engineering these biosynthetic pathways to generate libraries of natural product variants are limited by technical barriers, specifically, the screening of hits with biologically active properties. The research described herein proposes to alleviate this constraint, by developing a general methyltransferase biosensor using a SAH-recognizing transcription factor to screen methyltransfer reactions. Methyltransferases (MTases) are ubiquitous post-modifying enzymes involved in several biosynthetic pathways, and SAM-dependent methyltransferase activity has been identified as a target for psychiatric, anti-viral, anticancer and anti-inflammatory drug design.[1]

Natural products are a logical source of drug discovery because biological pathways conveniently catalyze their assembly. Consequently, researchers aim to exploit and engineer these pathways in order to uncover novel antimicrobial or anticancer properties with increased potency and target specificity.[2] Polyketides, for example, are a class of natural products that compromise a large portion of commercially available pharmaceuticals, including the widely studied antibiotic erythromycin A and the immunosuppressant rapamycin. Similarly, terpenes, which characterize the largest class of natural products, contain important medicines such as the anticancer agent, taxol. Additionally, alkaloids, non-ribosomal peptides and ribosomally-synthesized and post-translationally modified peptides (RiPPs) are diverse classes of biologically active natural compounds. Fascinatingly, all of the above-mentioned classes utilize panels of post-modifying MTases. MTases catalyze methylation reactions of carbon, nitrogen and oxygen atoms common across all three domains of life, but most natural products are substrates of O-MTases (**Figure 1**).[3] For this reason, we hypothesize that a fluorescent, genetically encoded MTase biosensor would enable the high-throughput engineering of methyltransfer reactions implicated in natural product biosynthesis—all while more effective, cheaper and faster than traditional methods.

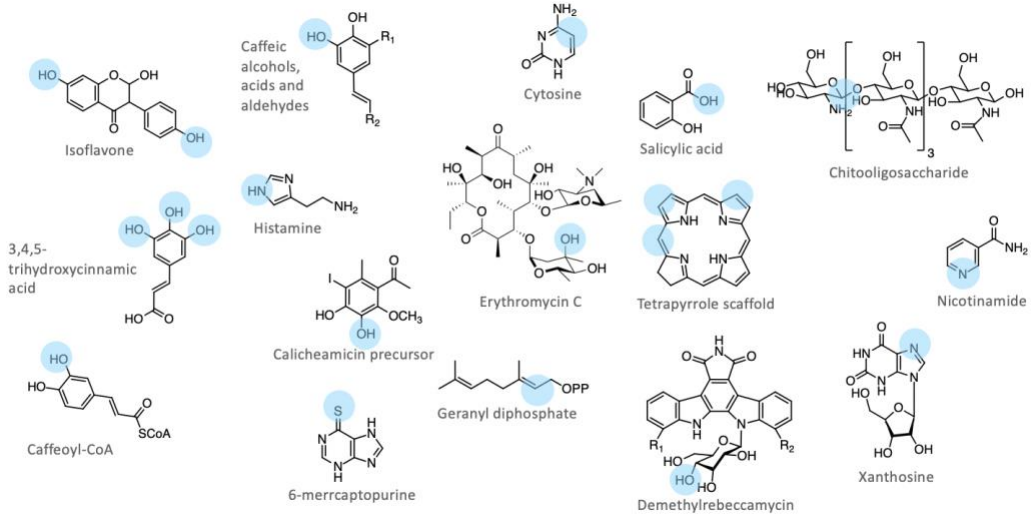

Figure 1. Examples of natural product O-MTase substrates. Methyl acceptors are highlighted with a blue circle.

Aim 1: Development of the fluorescent, genetically encoded methyltransferase biosensor. S-adenosyl methionine (SAM) is the electron-deficient methyl-donating cofactor of MTase enzymes, produced via ATP-dependent adenosylation of methionine. The transfer of a methyl from SAM to the methyltransferase produces S-adenosyl homocysteine (SAH) as a byproduct (**Figure 2**).[3]

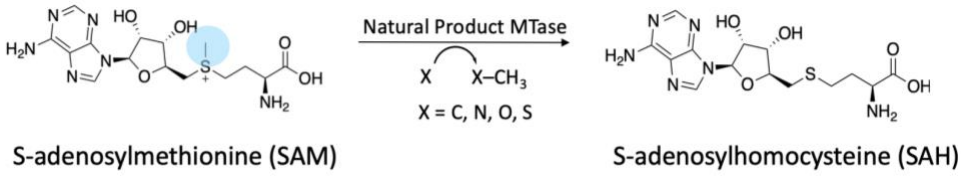

Figure 2. Natural product MTases (NPMTs) use co-substrate SAM, producing SAH as a byproduct.

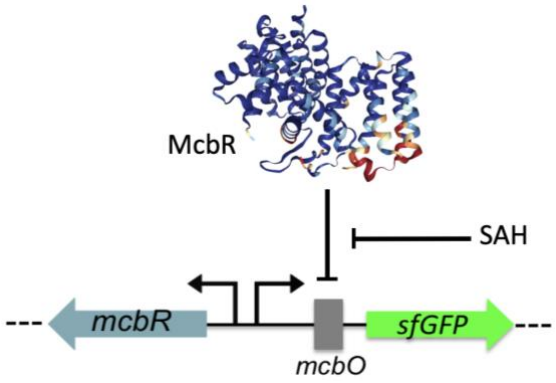

Figure 3. MTase biosensor where transcriptional repressor, McbR, binds to its cognate operator sequence, mcbO. McbR regulates the expression of a reporter gene, GFP, in the presence of SAH.

A SAH-sensing transcription factor, McbR, can be used within a biosensor to detect SAH as a measure of cofactor turnover. We hypothesize this biosensor to detect SAH concentrations and induce fluorescent output with GFP, where fluorescent output would indicate late-stage natural product methylation by a MTase. **Figure 3** shows the layout of the biosensor, which will be constructed within a plasmid system. GFP fluorescence will be induced by the presence of SAH, and fluorescence-activated cell sorting (FACS) can be used to quickly screen for hits with the highest fluorescent output.

Aim 2: Determine difference in SAH levels in cells that expressed or did not express active MTases. For a biosensor of this nature to function properly, it must be sensitive to SAH levels in cells that express or do not express MTases, and not towards basal levels of SAH *in vivo*. This can be experiment can be performed with SAH in *E. coli* BL21 cells. SAH levels can be quantified using mass spectrometry. **Aim 3: Use of biosensor for directed evolution of MTases.** A MTase biosensor would indispensably aid in engineering efforts of natural product variants by accelerating the rate of high throughput screening and selections, in addition to the identification of functionally important sequences. MTase biosensor-guided screening of engineered natural products can be applied using liquid cultures of microbe supernatants, with the identification of hits by measuring GFP fluorescence in a high-throughput format.

Intellectual Merit/Broader Impacts: The tailoring modifications of SAM-dependent MTases are necessary to achieve full bioactivity of natural products and are therefore integral to their biosynthesis. For this reason, they are the perfect target for a high-throughput screening biosensor. The ability to screen millions of mutant enzymes with a MTase biosensor would greatly increase the chances of discovering beneficial mutations for the production of non-natural natural products. This work, at the interface of molecular biotechnology, chemical biology, and medicinal chemistry, could provide a blueprint for how enzyme engineering projects are designed and decoded in the near future. My mentorship, under the guidance of Dr. Gavin Williams at NC State University, will aid in my growth as a competent, interdisciplinary chemist and future educator.

1. Yuan, C. S. Methods of Determining SAM-Dependent Methyltransferase activity using a mutant SAH hydrolase 6,610,504 B1, Aug. 26, 2003.
2. Quinn, R. The re-emergence of natural products for drug discovery in the genomics era. Nature Rev. Drug Discovery 2015, 14, 111-129.
3. Noel, J. Architectures, mechanisms and molecular evolution of natural product methyltransferases. Nat. Prod. Rep. 2012, 29, 1238.