Plants such as the opium poppy have been extensively studied for their production of benzylisoquinoline alkaloids (BIAs), a class of specialized metabolites with many useful and potent pharmacological properties. Naturally occurring BIAs such as morphine and noscapine have been widely used since ancient times, but recent advances in synthetic biology and protein engineering provide exciting new opportunities for creating novel compounds with novel or improved pharmacological properties. N-methyltransferases (NMTs) play key roles in several different branches of BIA-metabolism. Hundreds of BIA NMT gene sequences from a wide variety of plants can be separated into three general groups based on function and sequence identity. Recent work from our group led to the determination of the first molecular structure of an NMT involved with BIA biosynthesis (pavine-NMT from *Thalictrum flavum*, M.A. Torres *et al.*, *J. Biol. Chem.* 291:23403, 2016). More recently, we have determined the structures of enzymes from the two other groups of NMT’s involved with BIA biosynthesis. Using pavine-NMT from *T. flavum* as a search model, we used molecular replacement to solve the structures of tetrahydroprotoberberine-NMT (52% sequence identity, $d_{\text{min}} = 1.8$ Å) and coclaurine-NMT (63% sequence identity, $d_{\text{min}} = 2.2$ Å) from *Glaucium flavum*. These structures reveal a high level of structural conservation in the overall protein fold, arrangement of catalytic residues at the active site and substrate-binding site for $S$-adenosylmethionine. A subset of residues within the binding site for the methyl-acceptor alkaloid substrate appear to define the unique substrate recognition specificities of each group of NMT’s. To further explore these structure-function relationships, we have undertaken mutagenesis studies in combination with differential scanning fluorimetry, enzyme activity measurements and structure determination of enzymes with alkaloid substrates or substrate-analogs. Our presentation will discuss some of our recent progress in defining structure-function relationships in NMT enzymes and initial steps towards engineering altered substrate preferences for synthetic biology applications.