# Structural insights into the mechanisms of substrate recognition and catalysis for the N-methyltransferases involved in benzylisoquinoline alkaloid metabolism

**Dean Lang[1], Jeremy Morris[2], Peter Facchini[3], Kenneth Ng[4]**

*[1]No affiliation given, [2]University of Calgary, [3]University of Calgary, [4]University of Windsor*

*delang@ucalgary.ca*

Methyltransferases (MTs) are a diverse family of enzymes responsible for the addition of a methyl group from a donor molecule (typically S-adenosyl methionine) to target substrates. Methyltransferases are found widely across nature and are critical for many cellular activities, including central metabolism, DNA and RNA processing, signal transduction, and protein localization. The ubiquity of MTs in multiple contexts highlights the importance of methyl transfer for many biological processes. In addition to their intrinsic importance to biology, the diversity of sequences and 3-dimensional structures of MTs, particularly in the domain responsible for recognizing methyl acceptor substrates, underlines the importance of characterizing the broad range of molecular mechanisms for substrate recognition and catalysis used within this enzyme family.

MTs play a key role in the production of benzylisoquinoline alkaloids (BIAs), a class of specialized metabolites produced in plants such as the opium poppy. High value natural (morphine, codeine) and non-natural (oxycodone, naloxone) BIAs are considered essential medicines. Several novel or rare BIAs are being actively studied for their potent and diverse pharmacological properties, and to explore their potential as lead compounds in drug discovery. However, the promiscuity of many of the natural enzymes involved with BIA biosynthesis, including MTs, is widely believed to limit the yields of targeted, high-value products from engineered metabolic pathways. As a result, there is great interest and urgency to develop a rational molecular basis for controlling the specificity and activity of BIA biosynthetic enzymes, including MTs. A diversity of BIA MTs have been shown to methylate the multiple oxygen atoms (OMT) or central nitrogen atom (NMT) within the BIA substrate. While several functionally distinct BIA OMTs are known, all characterized BIA NMTs can be grouped into 3 functional classes.

Previously, the structures of two functionally distinct BIA NMTs have been reported, showing surprisingly distinct modes of acceptor substrate recognition [1, 2]. Recently, we reported structure-function studies of the third distinct class of NMTs involved with BIA biosynthesis, the tetrahydroprotoberberine NMT from Glaucium flavum (dmin = 1.6-1.8 Å) [3]. Our newest structures highlight the importance of complementarity between the uniquely L-shaped substrate recognition pocket and the (S)-cis configuration of the BIA substrate for stereospecific substrate selection.. This presentation will focus on how our most recent results provide novel insights into the functional roles of conserved residues within and near the enzyme active site, and the roles of the size and shape of the substrate recognition pocket in the selection of the appropriate BIA substrate.

*[1] Torres, M. A. Hoffarth, E. Eugenio, L. Savtchouk, J. Chen, X. Morris, J. S. Facchini, P. J. & Ng, K. K. S. (2016) J. Biol. Chem. 291, 23403-23415.*

*[2] Bennett, M. R. Thompson, M. L. Shepherd, S. A. Dunstan, M. S. Herbert, A. J. Smith, D. R. M. Cronin, V. A. Menon, B. R. K. Levy, C. Micklefield, I. (2018). Angew Chem 57, 10600-10604.*

*[3] Lang, D. E. Morris, J. S. Rowley, M. Torres, M. A. Maksimovich, V. A. Facchini, P. K. Ng, K. K. S. (2019) J. Biol. Chem. 294, 14482-14498.*