# Discovery and characterization of oxygen-directed methyltransferases acting on pharmaceutically relevant natural product scaffolds

Kristina Haslinger

Department of Chemical and Pharmaceutical Biology, University of Groningen, Groningen, Netherlands

Oxygen-directed methylation is a ubiquitous reaction in natural product pathways catalyzed by O-methyltransferases (OMTs). Methyl groups influence the bioactivity of the resulting products by altering their water solubility, membrane permeability, and stability, and by providing crucial structural features for cellular targeting. Therefore, methylation is frequently used by medicinal chemists in the design of bioactive molecules. Compared to enzymatic methylation, however, achieving regioselective methylation can be challenging with chemical methods. Thus, promiscuous OMTs are valuable biocatalytic tools for sustainable synthesis and optimization of known bioactive scaffolds in drug development. This work focuses on identifying and applying novel OMTs for diversifying various privileged natural product scaffolds. With the help of our recently developed rapid in vitro screening platform for OMTs, we identified two bacterial OMTs with intriguing properties: an OMT from Streptomyces avermitilis with robust and high catalytic activity both in vitro and in vivo, and an OMT from Desulforomonas acetoxidans with medium catalytic activity despite various sequence deviations from other known bacterial OMTs including absence of the canonical catalytic triad. I will show that these sequence features are conserved among homologues of the latter enzyme and that its crystal structure does not reveal how the absence of the catalytic triad is compensated for. Both OMTs methylated a wide range of catechol-like substrates, including flavonoids, coumarins, hydroxybenzoic acids and their respective aldehydes, an anthraquinone and an indole. One enzyme also accepted a steroid. Interestingly, certain non-catechol flavonoids and hydroxybenzoic acids were also methylated. This study expands the knowledge on substrate preference and structural diversity of bacterial catechol OMTs and paves the way for their use in biocatalytic synthesis routes.