

## Control of hydroxylation regioselectivity by hyoscyamine 6 $\beta$ -hydroxylase as revealed by crystallographic and QM/MM studies

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Hyoscyamine 6 $\beta$ -hydroxylase (H6H) is a bifunctional 2-oxoglutarate/Fe(II)-dependent dioxygenase that catalyzes the two final steps in the biosynthesis of scopolamine [1], that is a regioselective hydroxylation of hyoscyamine at the C6 position, followed by a formation of the epoxide ring utilising the installed hydroxy group [2]. The combination of crystallographic and computational studies on H6H:hyoscyamine complex provided insight into the substrate binding and the selectivity of the enzymatic reaction [3].

The QM/MM studies reveal that the regioselectivity of the hydroxylation reaction is dictated by only a few residues (i.e. Lys-129, Tyr-326, Lys-330), which promote the reaction occurring at the C6 site and at the same time hinder the alternative channel proceeding at the neighbouring (C7) position. Notably, the electronic properties of the reactants, that is hyoscyamine and the active site, do not favour any of the reaction channels, which suggests that switching regioselectivity of the oxygen rebound and thus obtaining other potentially useful alkaloids, may be achieved by targeting the residues in vicinity of the reactants.

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