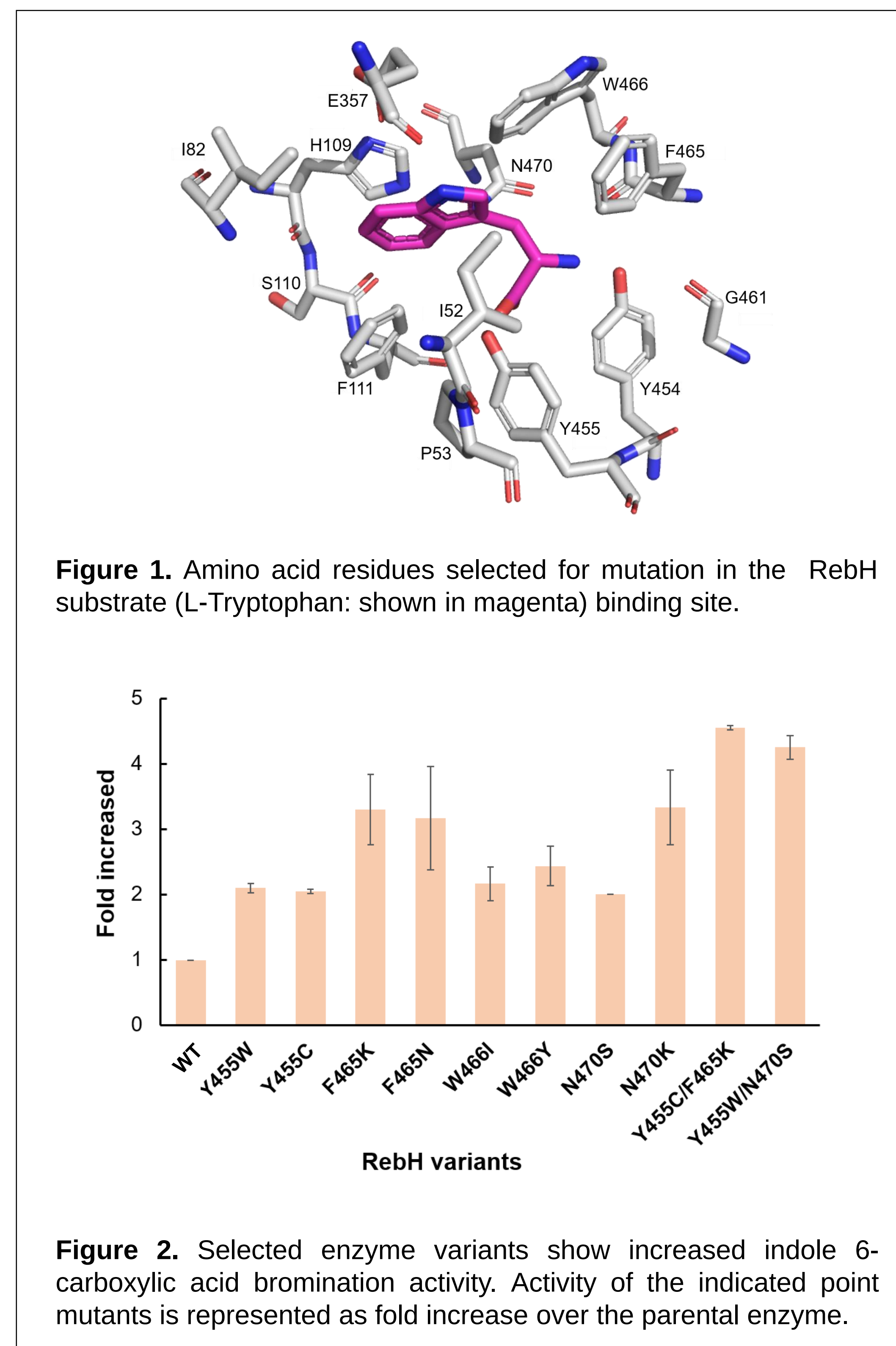


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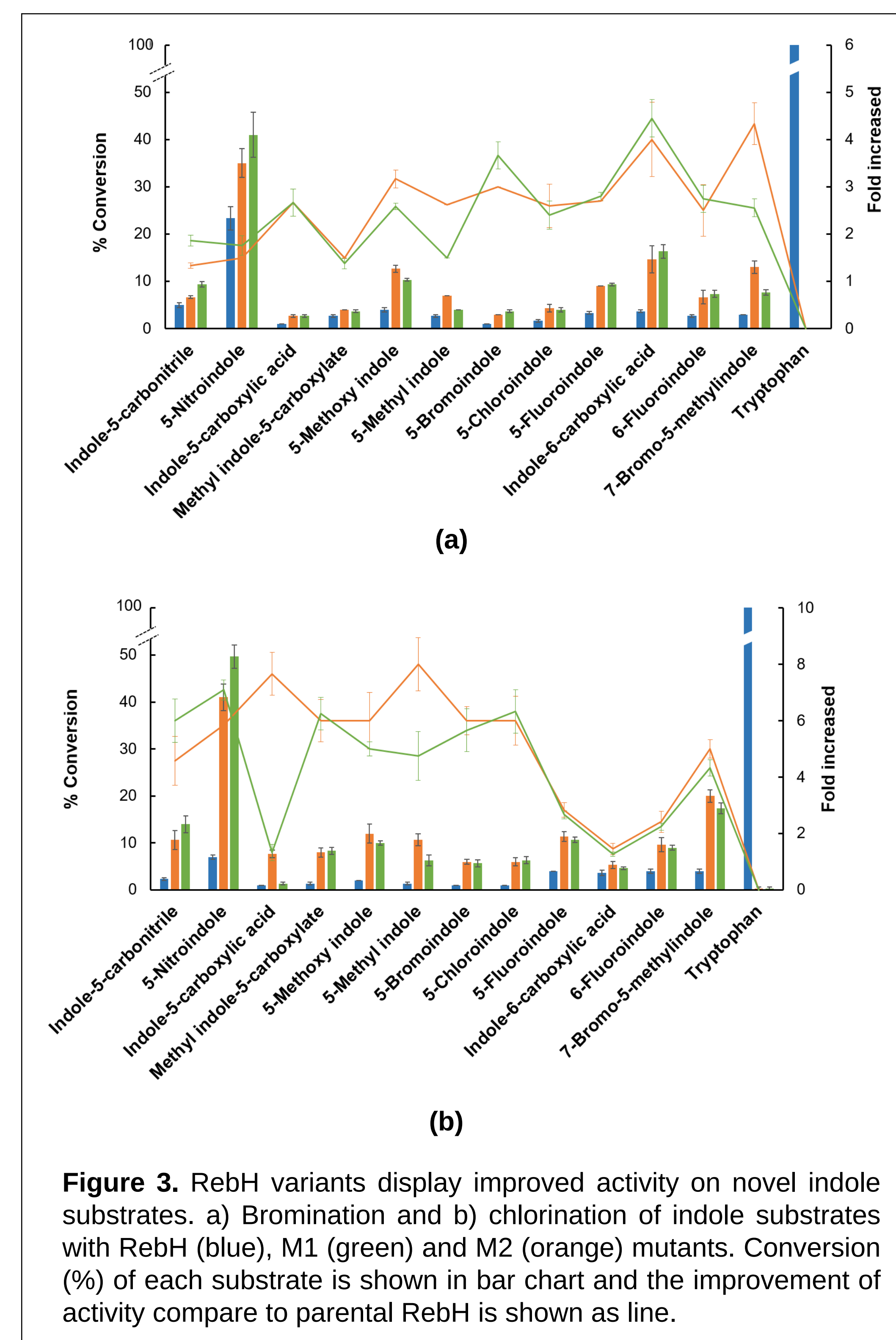
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Abstract: Regioselective halogenation of aromatic hydrocarbons is extremely valuable due to the importance of halogenated arenes as intermediates in organic synthesis and as active molecules in pharmaceuticals, agrochemicals and other materials. Halogenation can abruptly alter therapeutic activity, metabolism and half-life of a diverse range of drug molecules including antibiotics, anticancer agents, and psychoactive compounds. Furthermore, halogenated arenes are frequently used in transition metal-catalyzed cross-coupling reactions, which is extremely useful in synthetic chemistry. Conventional chemical halogenation generally requires activated starting materials, harsh reaction conditions and multistep synthesis, and often suffers from poor regioselectivity. On the other hand, enzymatic reactions can display high selectivity and the reactions take place under mild conditions. Although natural enzymes evolved to act on specific substrates, their substrate scopes can be improved by using protein engineering approach. Halogenase enzymes (like RebH) can play an important role in selective halogenation of industrially important aromatic hydrocarbons, including various indole derivatives. We developed two RebH variants M1 (Y455C/F465K) and M2 (Y455W/N470S) with expanded substrate scope, by a structure-guided protein engineering approach. Both variants catalyzed halogenation of various indole derivatives more efficiently than the parent enzyme to produce important pharmaceutical intermediates. Interestingly, both mutants showed complete switch of substrate specificity from tryptophan towards other indole derivatives; they were completely inactive on their natural substrate tryptophan. Computational modelling and MD simulations highlight key roles for the active site Y455 and F465 residues in mediating the dramatic switch in substrate specificity away from tryptophan towards the non-natural substrates.



Conclusion: Catalytic scope of a halogenase enzyme was expanded using semi-rational enzyme engineering approach. Two mutants have been identified that show complete switch of substrate specificity; they are highly efficient in halogenating various indole compounds but are completely inactive towards their natural substrate tryptophan. The mutants also demonstrated synthesis of pharmaceutical precursors by site-selective halogenation of several indole compounds that has not been reported for enzymatic halogenation. In addition to their use in halogenating novel substrates for synthetic chemistry, these enzymes with altered substrate selectivity can potentially be exploited as a metabolic engineering tool for biosynthesis of novel natural products.



<i>Lentzea aerocolonigenes</i> (RebH)	KIDMYRAGMAINAPASDDAQLIYGNFEEFRNFWNNSNYICVL
<i>Actinopolyspora mortivallis</i>	KIRCYDAGLAINAPATDDAQVYYGNFEEFRNFWNNSNYISVL
<i>Nonomuraea jiangxiensis</i>	KMDMYRAGLPINNPPSSDASHYYANFEEFRNFWNNGNYICVL
<i>Nocardiopsis halotolerans</i>	KVEMYSAGLAINQPSSDDAGVYIGDFEEFRNFWNNSNYICVL
<i>Pseudonocardia</i> sp. EC080610-09	KIASYRAGLPVNPASDDARHYIYGNFEEELRNFWNNSNYICIL
<i>Salinispora pacifica</i>	KISAYKAGLPINAPTDE-STYYGNFEAEFRNFWTNGSYICVF
<i>Streptomyces venezuelae</i>	KISAYKAGLPINSPITDE-STYYGNFEAEFRNFWTNGSYICIF
<i>Actinomadura</i> sp. RB68	KMAMYKAGLAINQPITGE-SDYYGNFETEFRNFWTNGSYICIF
<i>Frankia Canadensis</i>	KISAYRAGLPINSPITDE-STYYGNFEAEFRNFWTNGSYICIF
<i>Lentzea fradiae</i>	KIAQYRAGLAVNMPVTDE-ATYYENFEAEFRNFWTNSYYCIF
<i>Embleya scabrispora</i>	KISLYRAGFPVNTSQAGE-RVYYGNSEVDNKNFWNNTNYWCIF
<i>Mycobacterium marinum</i>	KVAMYKAGVPVNPPIATE-SSYYSNFDAEFENFWTNGSYICIF
<i>Embleya hyaline</i>	KISLYRAGFPVNTSQAGE-RVYYGNSEVDNKNFWNNTNYWCIF
<i>Lentzea albidocapillata</i>	KVAMYKAGLAVNMPVTDE-GNYYGNFEAEFRNFWSNANYICVF
<i>Saccharothrix espanaensis</i>	KVRMYKAGLAVNMPITDE-STYYSSFDAEFNFWNNSNYICIF

Figure 4. Sequence alignment of RebH with other bacterial tryptophan 7-halogenase enzymes showing the conservation at Y455, F465 and N470 residues.

