

Supplementary materials

Rational Design and Modification of NphB for Cannabinoids Biosynthesis

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Supplementary Tables

Table S1: Streamlined nomenclature for the 16 selected prenyltransferase sequences.

Simple name	Locus	Source
N1	WP_057602682.1	<i>Streptomyces_sp._Root1310</i>
N2	WP_059197833.1	<i>Streptomyces_antibioticus</i>
N3	1ZB6_A	<i>Streptomyces_sp.</i>
N4	WP_122184786.1	<i>Streptomyces_triticirhizae</i>
N5	WP_047018069.1	<i>unclassified_Streptomyces</i>
N6	WP_125936269.1	<i>Streptomyces_sp._WAC_06738</i>
N7	AQU65790.1	<i>Streptomyces_niveus</i>
N8	WP_150163952.1	<i>Streptomyces</i>
N9	WP_181139734.1	<i>Streptomyces_sp._Ru71</i>
N10	WP_173165263.1	<i>Phytohabitans_suffuscus</i>
N11	WP_103786661.1	<i>Streptomyces_sp._Ru71</i>
N12	WP_164253484.1	<i>Streptomyces_sp._S4.7</i>
N13	OQR64855.1	<i>Streptomyces_sp._B9173</i>
N14	WP_150213418.1	<i>Streptomyces_venezuelae</i>
N15	WP_027750390.1	<i>Streptomyces_sp._CNH287</i>
N16	REG00998.1	<i>Asanoa_ferruginea</i>

Supplementary Figures

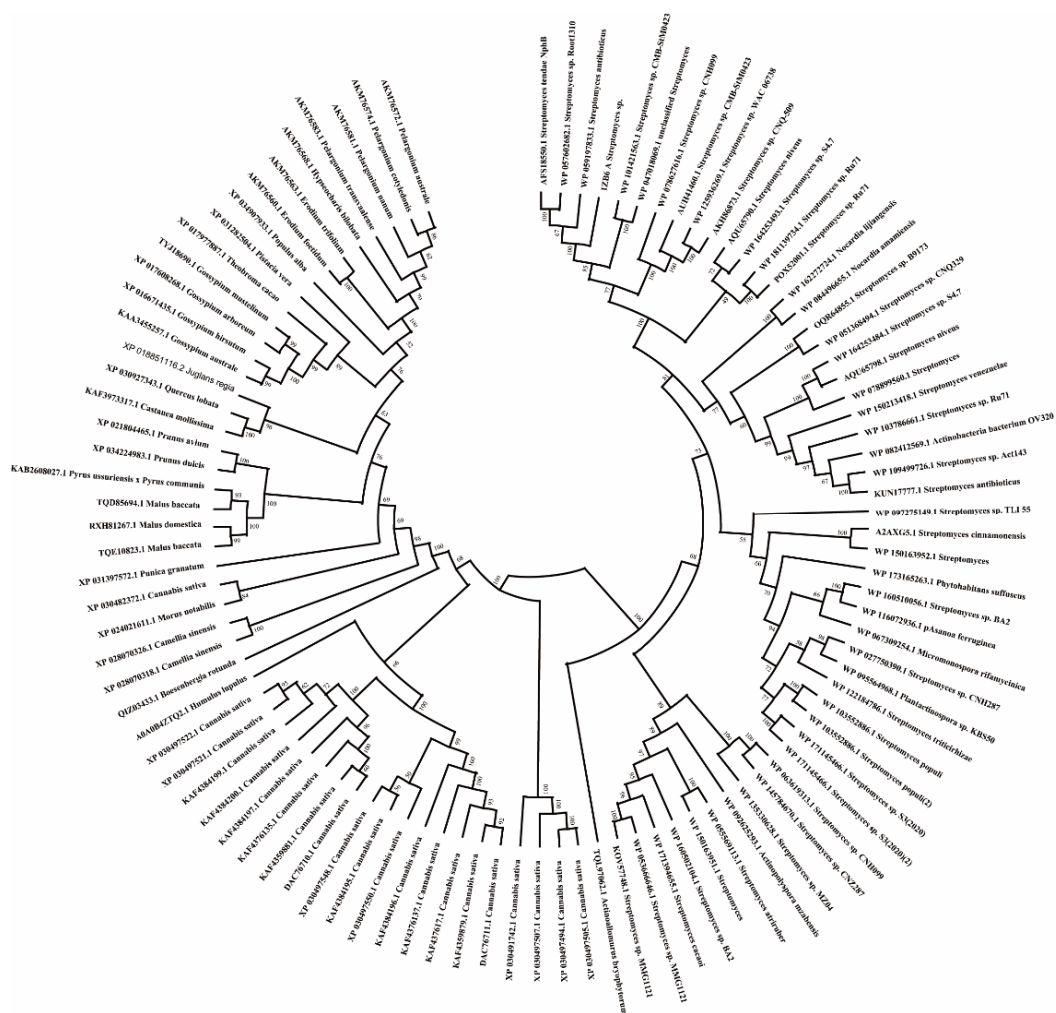


Figure S1: The construction of a gene tree based on the homologous of NphB.

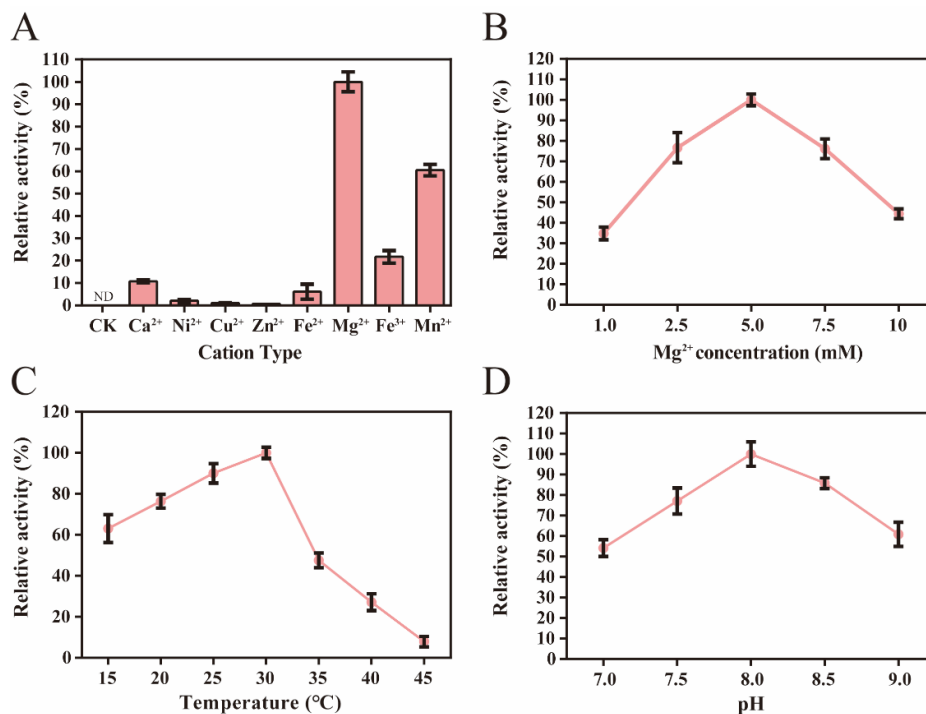


Figure S2: Investigating the reaction conditions influencing NphB's efficiency in CBG synthesis. **(A)** The effect of diverse cation types on enzymatic activity, using the activity in the control without added metal ions (CK) as a baseline, with magnesium ion catalytic activity normalized to 100%, and ND signifies 'not detected'. **(B)** Evaluation of the optimal magnesium ion concentration for the reaction. **(C)** Determination of the most favorable temperature for the reaction. **(D)** Assessment of the ideal pH for the reaction. Error bars indicate standard deviation; experiments performed in triplicate.

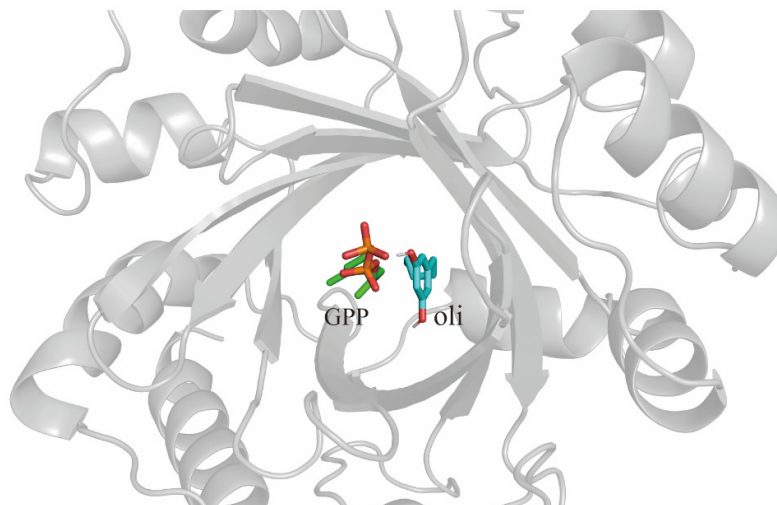


Figure S3: Protein NphB model construction and substrate docking

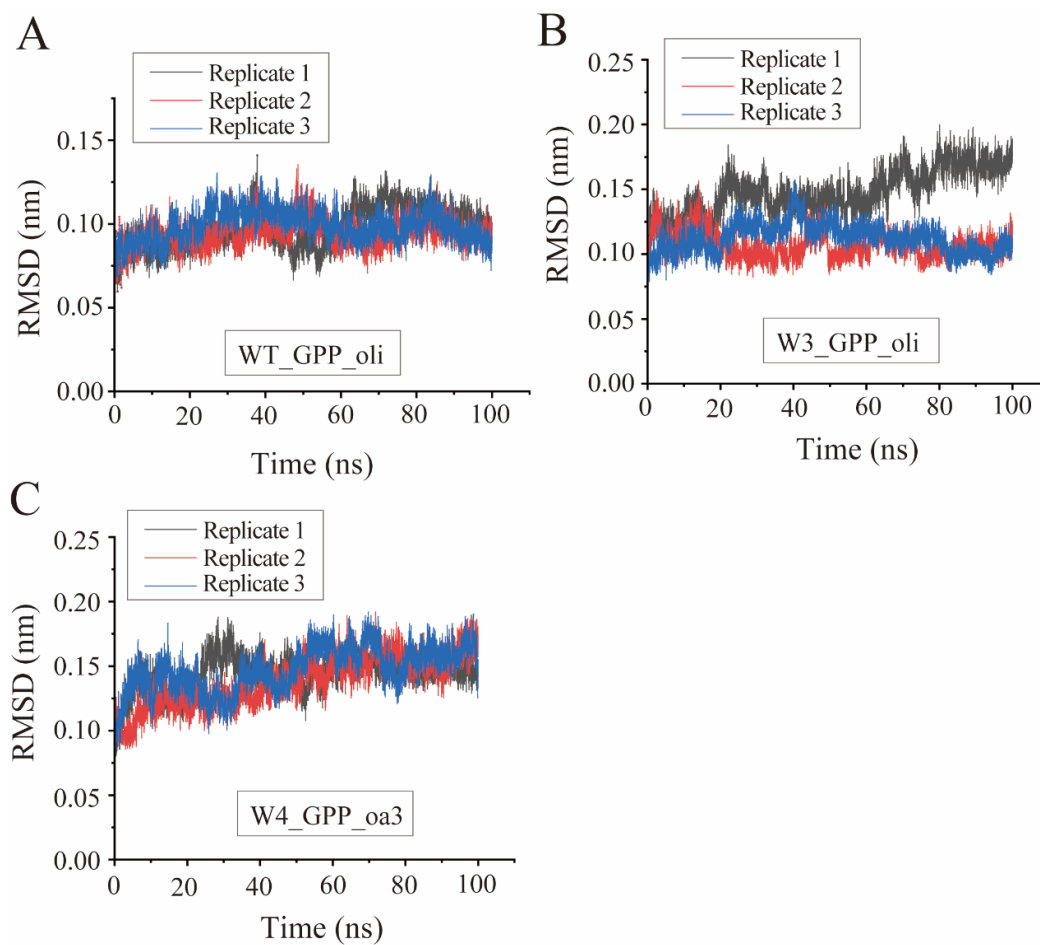


Figure S4: The RMSD values of the wild-type (A), mutant W3 (B) and mutant W4 (C).

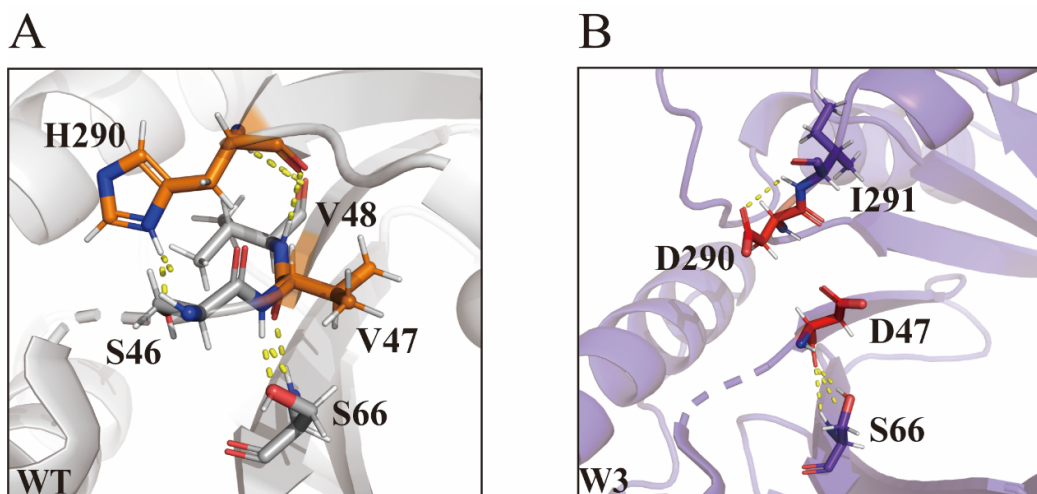


Figure S5: Analysis of the hydrogen bond network at positions 47 and 290 in both the wild-type and W3 mutant strains. (A) In the wild-type enzyme, H290 engages in hydrogen bonding with S46 and V48, while V47 is hydrogen-bonded to S66, resulting in a total of eight hydrogen bond pairs. (B) In the W3 mutant, D290 forms a single hydrogen bond with I291, and V47 retains its hydrogen bond with S66, resulting in a total of four hydrogen bond pairs. The H290D mutation in the mutant diminishes the interactions of D290 with neighboring residues, easing the restrictions on the alpha-helix.

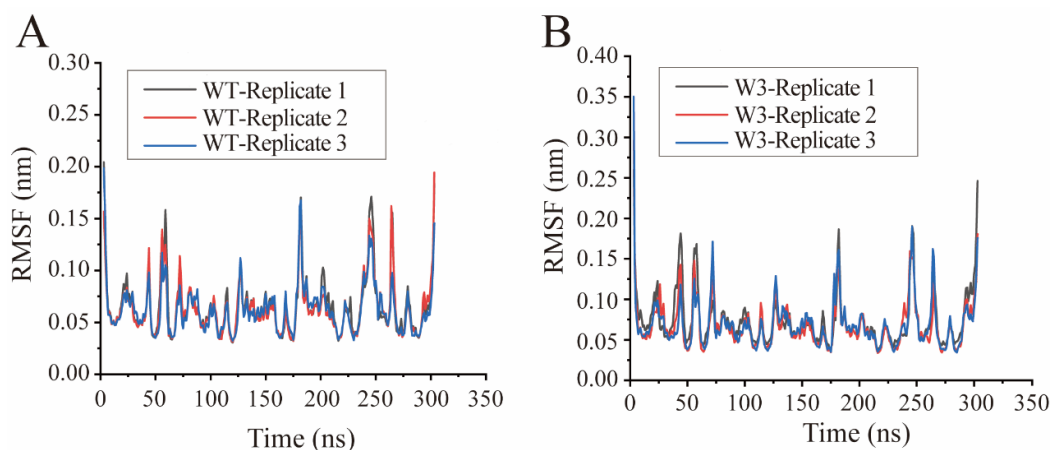


Figure S6: The RMSF values of the wild-type (A) and mutant W3 (B). Residue F176 is located on the beta-sheet within the TIM barrel. Analysis of the protein RMSF values shows that the RMSF value for F176 in the wild-type protein is 0.0376 nm, and in the mutant W3, it is 0.044 nm, indicating minimal fluctuation of the residue and a relatively stable side chain spatial position.

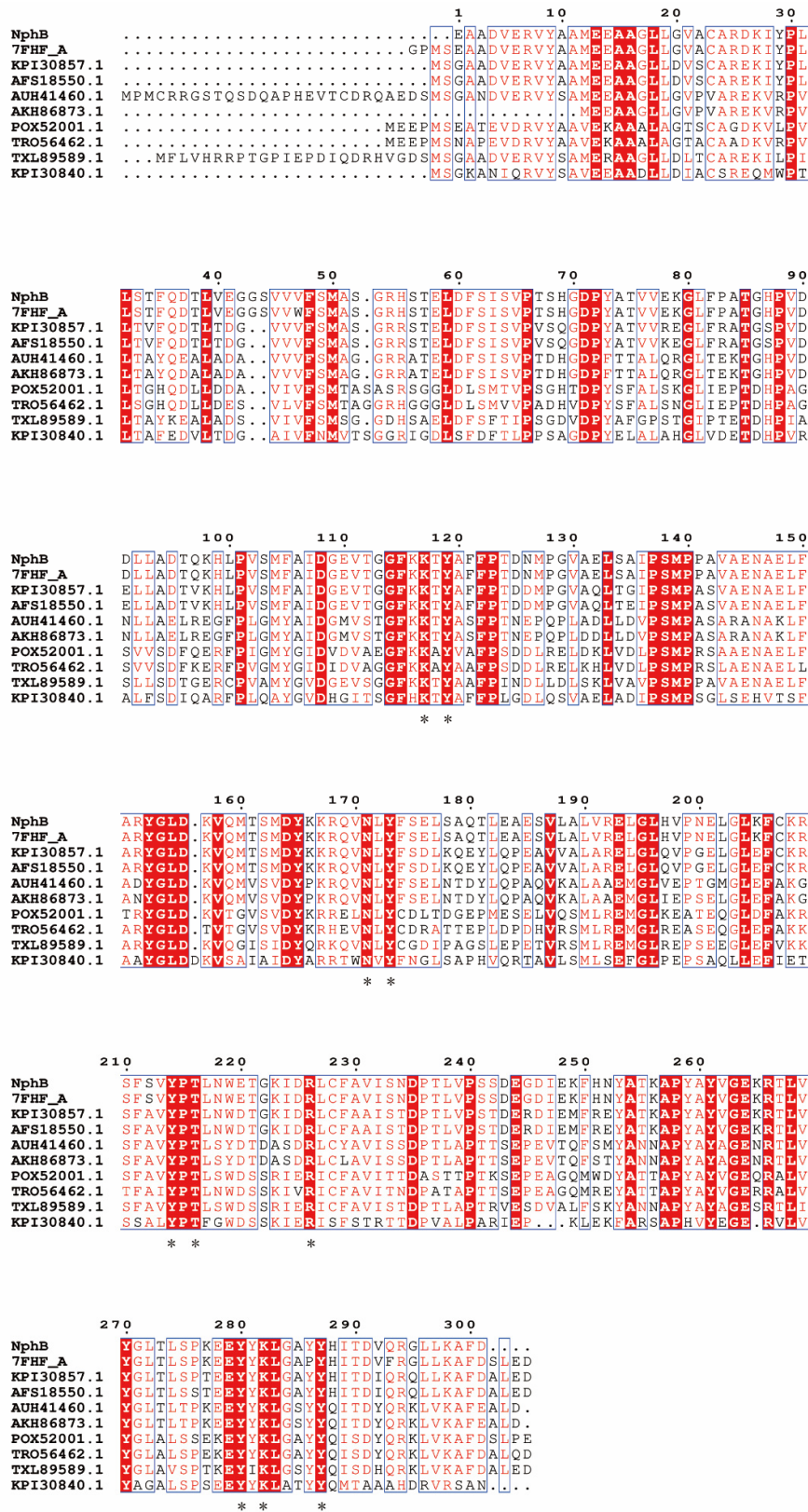


Figure S7: Alignment of amino acid sequences of NphB with its homologous sequences.

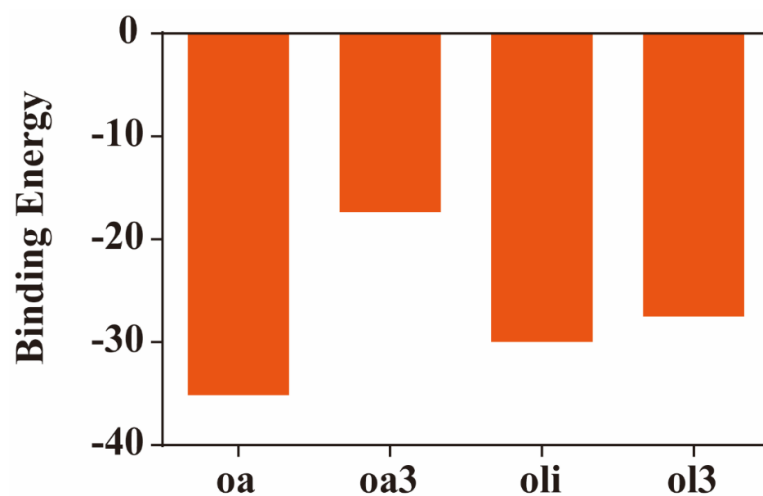


Figure S8. The predicted binding free energies by molecular docking oa/oa3/oli/ol3 into WT-NphB.

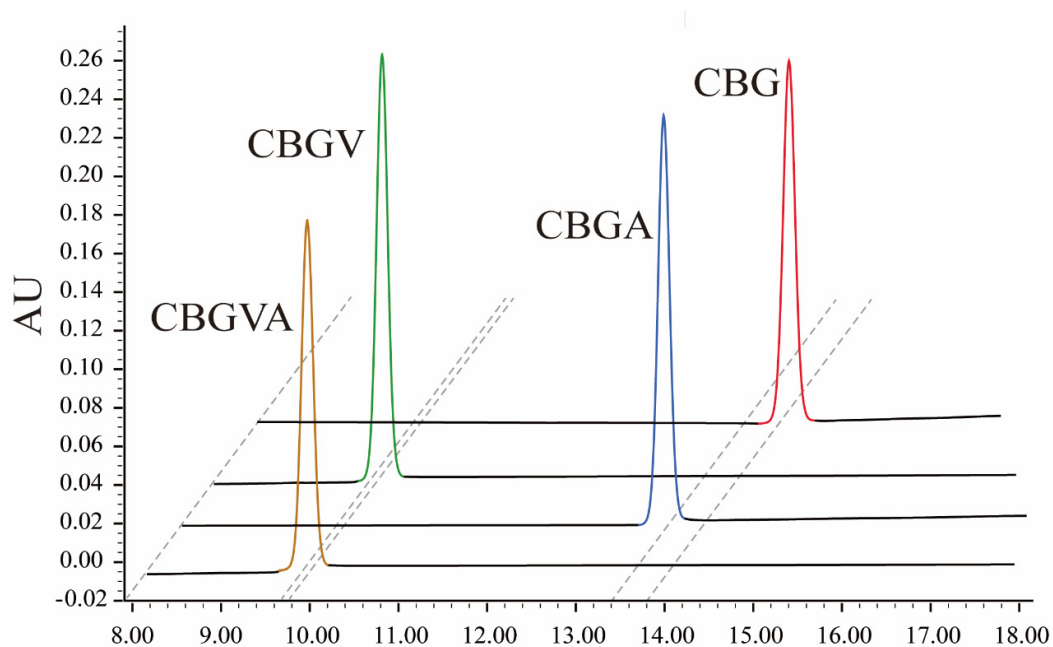


Figure S9. High-performance liquid phase detection of CBG, CBGA, CBGV and CBGVA.