

Supplementary information

Exploring the enzymatic and antibacterial activities of novel Mycobacteriophage Lysin B enzymes

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Table S1: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of the antibiotics used in the antibacterial activity assay against *M. smegmatis*.

Antibiotic	MIC	MBC
Rifampicin	0.6 µg/ml	1.2 µg/ml
Isoniazid	58.59–234.375 µg /ml	500 µg /ml
Ciprofloxacin	0.005–0.01 µg/ml	0.01–0.02 µg/ml
Pyrazinamide	7.5 mg/ml	13.5 – >13.5 mg/ml
Colistin	2 µg/ml	2 µg/ml
Protamine sulfate	20 µg/ml	20 µg/ml

Figure S1

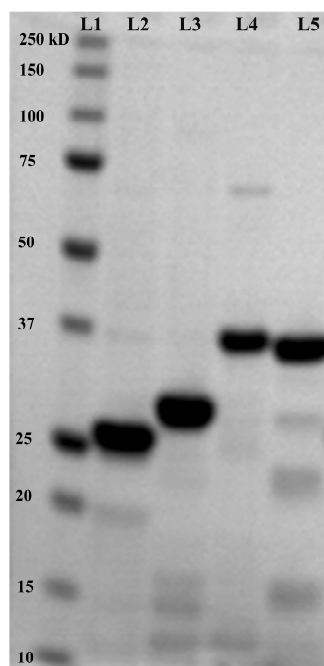


Figure S1. SDS-PAGE of purified LysB-His₆ enzymes. L1 Precision Plus Protein™ All Blue Prestained Standards (Bio-Rad), L2: LysB-D29 (MW: 29.3 kDa), L3: LysB-Omega (MW: 31.5 kDa), L4: LysB-Saal (MW: 37.4 kDa), L5: LysB-Obama12 (MW: 36.7 kDa).

Figure S2. Nucleotide sequences of the codon optimized genes used in the current study

- **LysB–D29 (Uniport ID: O64205)**

ATGAGCAAGCCCTGGCTGTTACCGTTACGGCACGGGCCAGCCCGATCCCCTCGGGCCTGGCCTGCC
TGCCGATACGGCACGCGACGTACTTGACATCTACCGGTGGCAGCCCATCGGCAACTACCCCGCTGCGG
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TCTTCTGGGGTAATCCCATGCGGCAGAAGGGCTTTGCCCACTCTGACGAGTGGATCCACCCGGTCGCT
GCCCCTGACACCCTCGGAATCCTCGAGGACCGGCTCGAAAACCTGGAGCAGTACGGCTTCGAGGTCCG
CGACTACGCCCACGACGGTGACATGTACGCCTCCATCAAAGAGGACGACCTGCACGAATACGAGGTG
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ATCGAGCTTGGCCAGCGTCCGATCACCGAGGGAATTGCGTTGGCGGGAGCCATCATCGACGCCCTCAC
GTTCTTCGCCCCTCTCGTATGGGCGACAAGTGGCCGCACCTCTACAACCGCTACCCGGCGGTGAGT
TCCTACGACAGATCCACCACCACCACCACCTGA

- **LysB–DS6A (Uniprot ID: G8I4E1)**

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CCCCGCATCTGACTTATGACGTAGGTCTGCCATCGATTACCTTCGCCAAGTTGCGCGCGACCGCCG
TTGGCTTTAGCCGCGCATCACCATCATCACTAA

- **LysB–Omega (Uniprot ID: Q854L1)**

ATGTTGAACGGTGAATTTTACGTTCCGGCAGGGACAATCATTACTGCCAACGGGACAGGGTTCCAGA
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- **LysB–Saal (Uniprot ID:W6AV51)**

ATGCGCATCGACGGTCAATATGTGGGACTGGGGCTTGGGGATTCTTCAGACGAGATCCGTAAAAATCAA
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GCCCCAAGCTATCGCTATCACAAAGGCGCTTTTGGATGCCGGTATGTTCTTCGCTAAACGTACAGGCC
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- **LysB–Echild (Uniprot ID: W0LJP4)**

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- **LysB–Enkosi (Uniprot ID: A0A0M4R3S7)**

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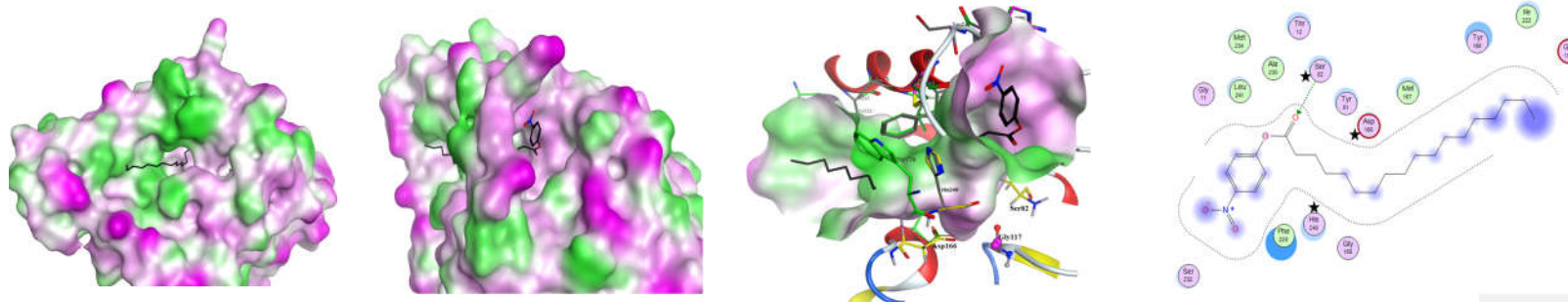
• **LysB–Obama 12 (Uniprot ID:W0LJG8)**

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CCCAGGGACGGGAATGACATATTACGAATACGCGGTCAAGCATTTGCGCGACACCGCAGATGCGCGTT
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• **LysB–Pumpkin (Uniprot ID: C9DCK3)**

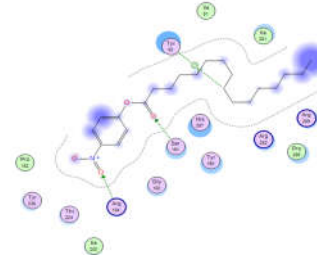
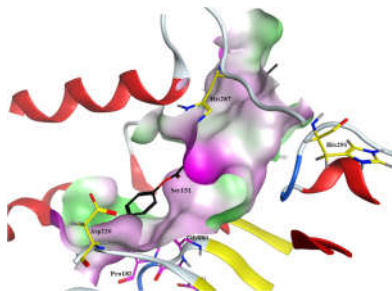
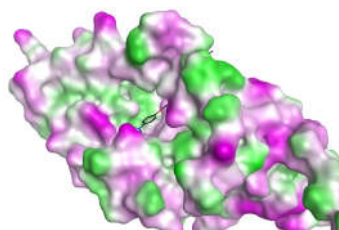
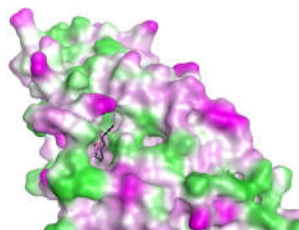
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CTGTCCAAGGGACCCGCCGACATTACCTTAGCTGAAACCGGACGTGTAGCCCGTGCTGTGTGGCAT
TTAAATCGCATTGGTGCCAAGACATTAGCGCGCGCCTCACATCACCATCATCATCACTAA

Aa. LysB-D29 like orientation (Tunnel conformation)

LysB-D29: Orientation pose 163, *p*NPS, S = -8.07

B. Inverted LysB-D29 orientation

LysB-Obama12: Orientation pose 160, $pNPP$, $S = -6.5$

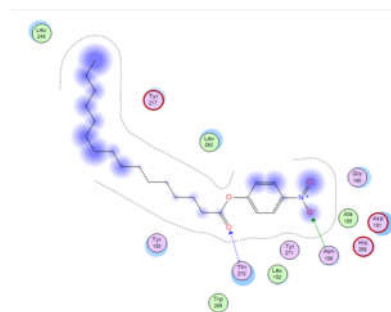
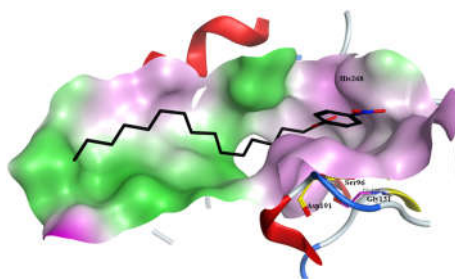
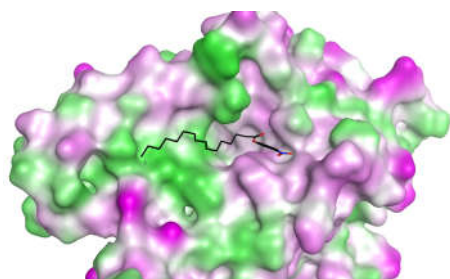


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⚡. Long shallow bowl

LysB-Omega, no detectable orientation pose, *p*NPP



LysB–Saal: Orientation pose 165, p NPP, $S = -7.5$

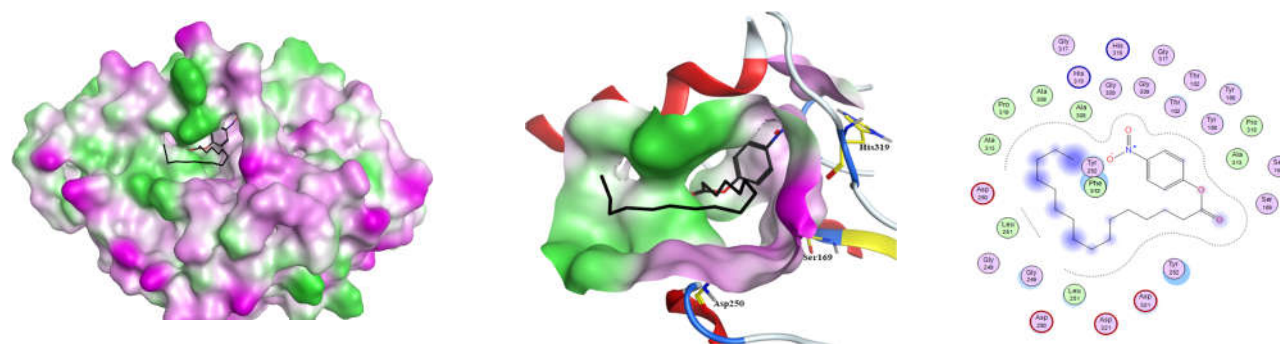


Figure S3: 3D conformations of poses of *p*NP ligands upon docking to LysB proteins. Each diagram illustrates (in order) the overall surface of protein with its ligand, the shape of active site with the docked ligand, the interactions of ligand atoms with different residues of the corresponding protein. Hydrophilic residues (pink color), hydrophobic residues (green color), *p*NP ligands (Black color). Orientation pose: ligand–protein conformation where the catalytic Ser faces ligand’s ester bond (C=O) with no H-bond formation. NDP: (No Detected Pose) neither binding nor orientation pose were detected. Stars indicate catalytic triad residues of LysB–D29.