

Supporting Information

Enhancing ω -transaminase catalysis of bulky substrates

guided by binding mechanism

**Haidong Teng¹, Haoran Yu^{1, 2}, Zhehang Xu³, Lanxin Xiao^{1, 2}, Xinghao Yuan¹,
Lirong Yang^{1, 2}, Jianping Wu^{1, 2}, Gang Xu^{*1}**

¹ Institute of Bioengineering, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou, Zhejiang 310058, China.

² ZJU-Hangzhou Global Scientific and Technological Innovation Centre, Hangzhou, Zhejiang 311200, China.

³ University College London, Gower Street, London WC1E 6BT, United Kingdom.

Corresponding author: Gang Xu (email: xugang_1030@zju.edu.cn)

Table of Contents

1 Supplementary Figures

Supplementary Fig. 1 Reaction mechanism for the transamination of KBP to ABP with isopropylamine as an amino donor.

Supplementary Fig. 2 Conformational analysis of KBP and PMP in MD simulations.

Supplementary Fig. 3 Identification of key residues governing substrate tunnel and active pocket.

Supplementary Fig. 4 Time evolution of the geometric parameters distance $d(C_{\text{LIG}}-N_{\text{PMP}})$ and angle $\angle(N_{\text{PMP}}-C_{\text{LIG}}-O_{\text{LIG}})$ over the last 200 ns of 300 ns MD simulations for each variant.

Supplementary Fig. 5 Time evolution of the geometric parameters distance $d(O_{\text{LIG}}-N_{\text{PMP}})$ over the last 200 ns of 300 ns MD simulations for WT.

Supplementary Fig. 6 Analysis of WT GaMD simulations.

Supplementary Fig. 7 Free energy profiles along the minimum free energy path (MFEP) for WT, 6M, and 13M variants.

Supplementary Fig. 8 Funnel metadynamics (FM) analysis of the 6M variant.

Supplementary Fig. 9 Funnel metadynamics (FM) analysis of the 13M variant.

Supplementary Fig. 10 Ligand position on the binding path as a function of path metadynamics simulation time.

Supplementary Fig. 11 Block analysis carried out on the path metadynamics trajectory data.

Supplementary Fig. 12 RMSD of molecular dynamics simulations.

Supplementary Fig. 13 Metadynamics simulation of the 6M variant using $d(C_{\text{LIG}}-N_{\text{PMP}})$ and $\angle(N_{\text{PMP}}-C_{\text{LIG}}-O_{\text{LIG}})$ as collective variables.

Supplementary Fig. 14 Metadynamics simulation of the 13M variant using $d(C_{\text{LIG}}-N_{\text{PMP}})$ and $\angle(N_{\text{PMP}}-C_{\text{LIG}}-O_{\text{LIG}})$ as collective variables.

Supplementary Fig. 15. Comparison of sampled configurations with $d(C_{\text{LIG}}-N_{\text{PMP}}) \leq 0.28$ nm extracted from metadynamics simulations of 6M and 13M.

2 Supplementary HPLC spectrums

Supplementary Fig. 16 HPLC analysis of 3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol and 3-([1,1'-biphenyl]-4-yl)-2-ketopropan-1-ol.

Supplementary Fig. 17 HPLC analysis of chiral amine.

3 Supplementary NMR spectrums

Supplementary Fig. 18 The ^1H NMR spectrum of (*R*)-3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol.

Supplementary Fig. 19 The ^{13}C NMR spectrum of (*R*)-3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol.

4 Supplementary Tables

Supplementary Table 1 Binding free energies of the WT, 6M, and 13M variants calculated by

MM/GBSA.

Supplementary Table 2 Comparison of the catalytic binding conformation of BmATA WT with those reported in the literature.

Supplementary Table 3 Key geometric parameters of the dominant conformation A identified from 2D density map for each variant.

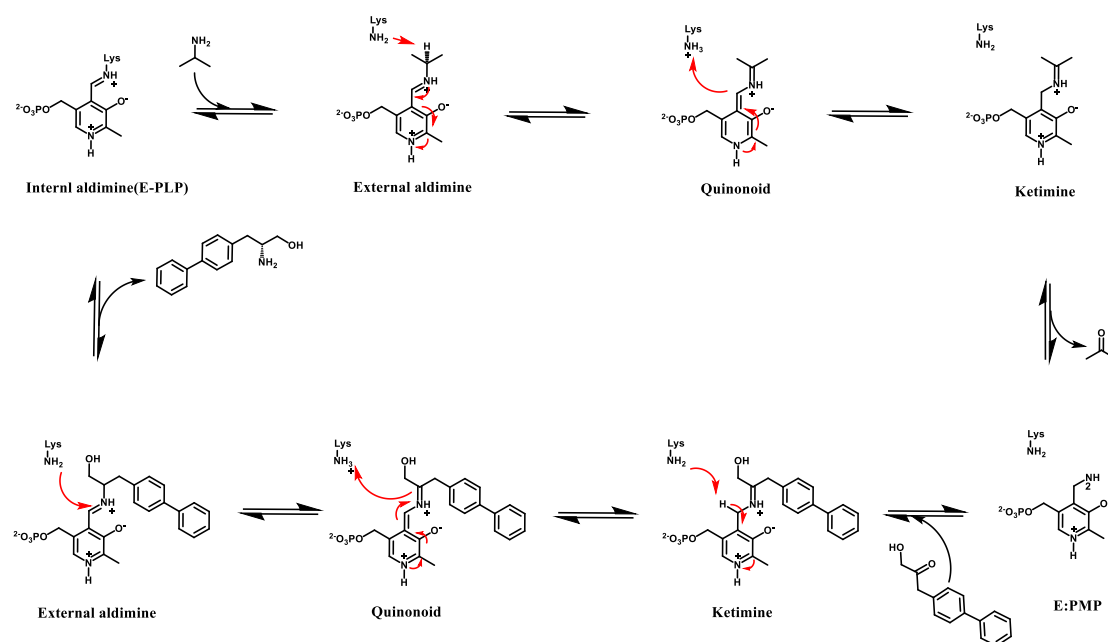
Supplementary Table 4 Primers used in this study.

5 Gel

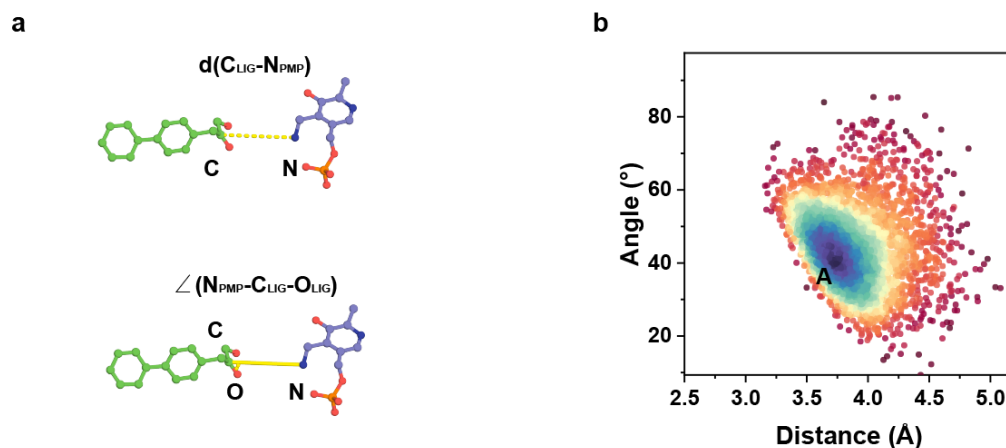
Supplementary Fig. 20 SDS-PAGE gel characterization of purified enzyme.

6 Supplementary References

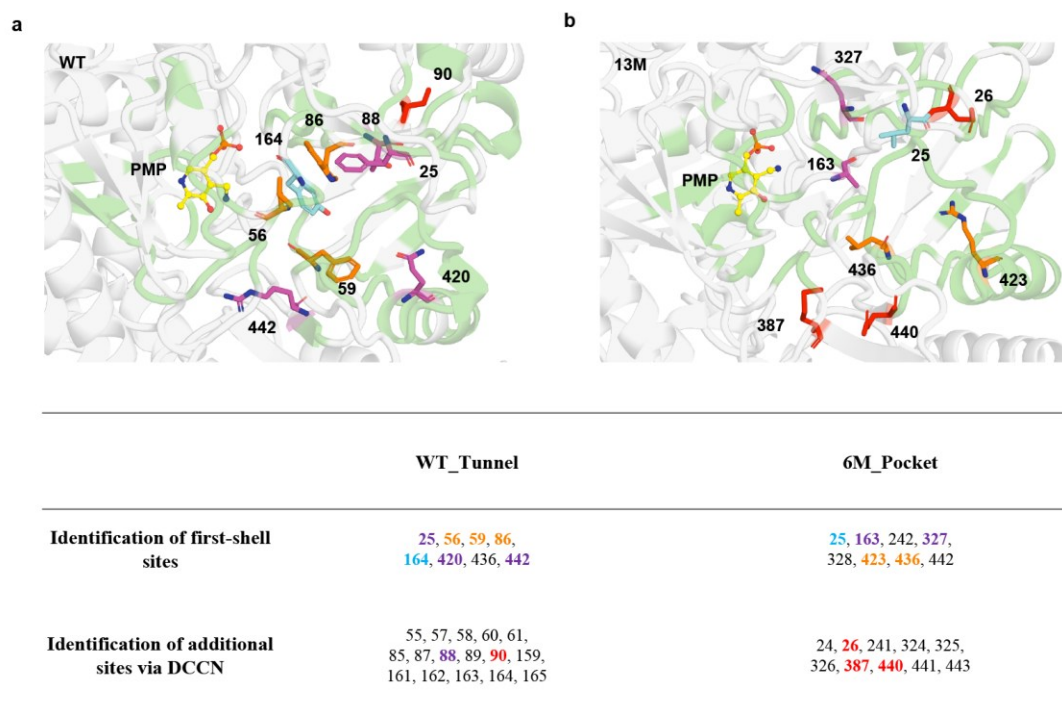
1 Supplementary Figures



Supplementary Fig. 1 Reaction mechanism for the transamination of KBP to ABP with isopropylamine as an amino donor. Reductive amination pathway of ω -transaminase: the amino group of catalytic residue Lys reacts with the aldehyde group of PLP to form an internal aldimine. This is followed by substitution with the amino group from the amino donor, leading to the formation of external aldimine 1. Proton transfer, facilitated by Lys, generates quinonoid intermediate 1, which undergoes an internal hydrogen rearrangement to form ketimine 1. Ketimine 1 then hydrolyzes to produce E-PMP and releases the ketone product, completing the first half of the transamination reaction. The second half proceeds analogously through ketimine 2, quinonoid intermediate 2, and external aldimine 2, ultimately releasing the amine product.

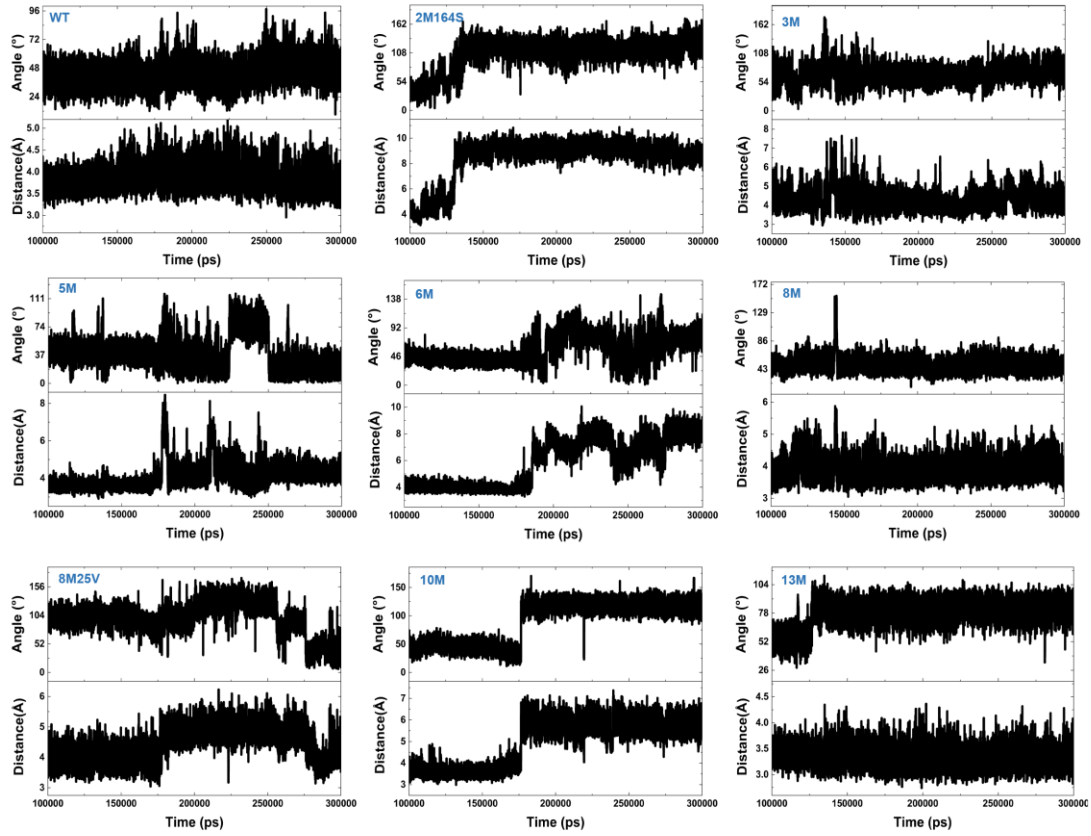


Supplementary Fig. 2 Conformational analysis of KBP and PMP in MD simulations. **a** The yellow dashed line represents the distance $d(C_{\text{LIG}}-N_{\text{PMP}})$. The angle between the yellow lines represents the angle $\angle(N_{\text{PMP}}-C_{\text{LIG}}-O_{\text{LIG}})$. The nitrogen atom of the amino group ($-\text{NH}_2$) on PMP is denoted as N_{PMP} . C_{LIG} denotes the carbonyl carbon of substrate KBP, O_{LIG} denotes the carbonyl oxygen of substrate KBP. **b** Molecular dynamics analysis of the WT. Statistical data of $d(C_{\text{LIG}}-N_{\text{PMP}})$ and $\angle(N_{\text{PMP}}-C_{\text{LIG}}-O_{\text{LIG}})$ were used to generate the 2D density maps. Point colour ranges from red (low density) to blue (high density). Data were derived from the last 200 ns of 300 ns MD trajectories.

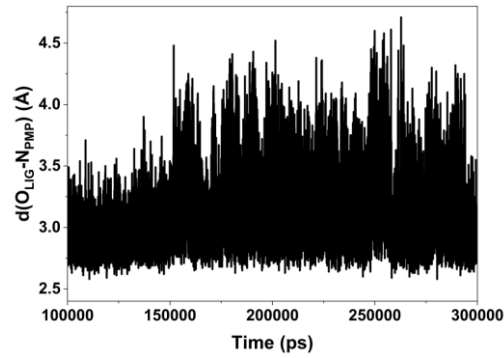


Supplementary Fig. 3 Identification of key residues governing substrate tunnel and active pocket.

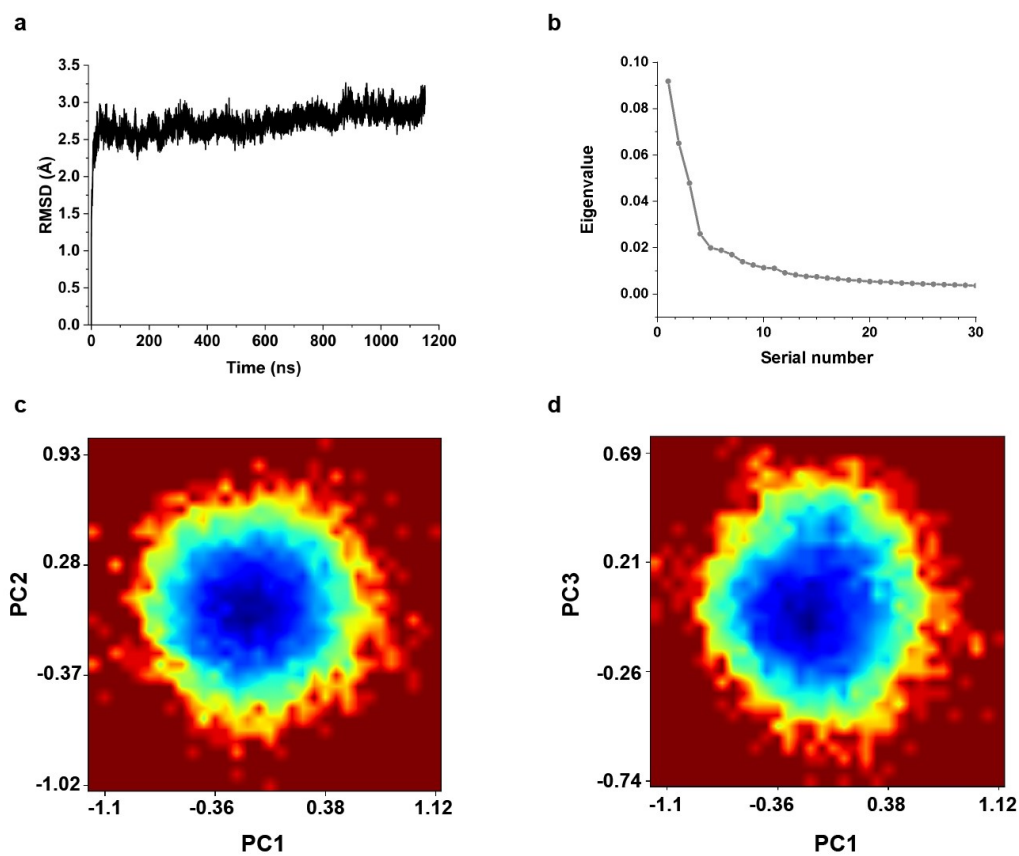
a&b PMP (yellow) is shown in ball-and-stick representation. Residues are color-coded by functional role: magenta, sites that widen the substrate tunnel without disrupting binding stability (25, 88, 420, 442) or that optimize binding stability without severely compromising tunnel accessibility (163, 327); orange, trade-off sites between tunnel widening and active-pocket binding stability (56, 59, 86, 423, 436); blue, sites influencing stereoselectivity (25, 164); red, distal sites that modulate tunnel and pocket dynamics indirectly (26, 90, 387, 440). The table summarizes first-shell contact residues and additional sites identified via DCCN for iterative saturation mutagenesis.



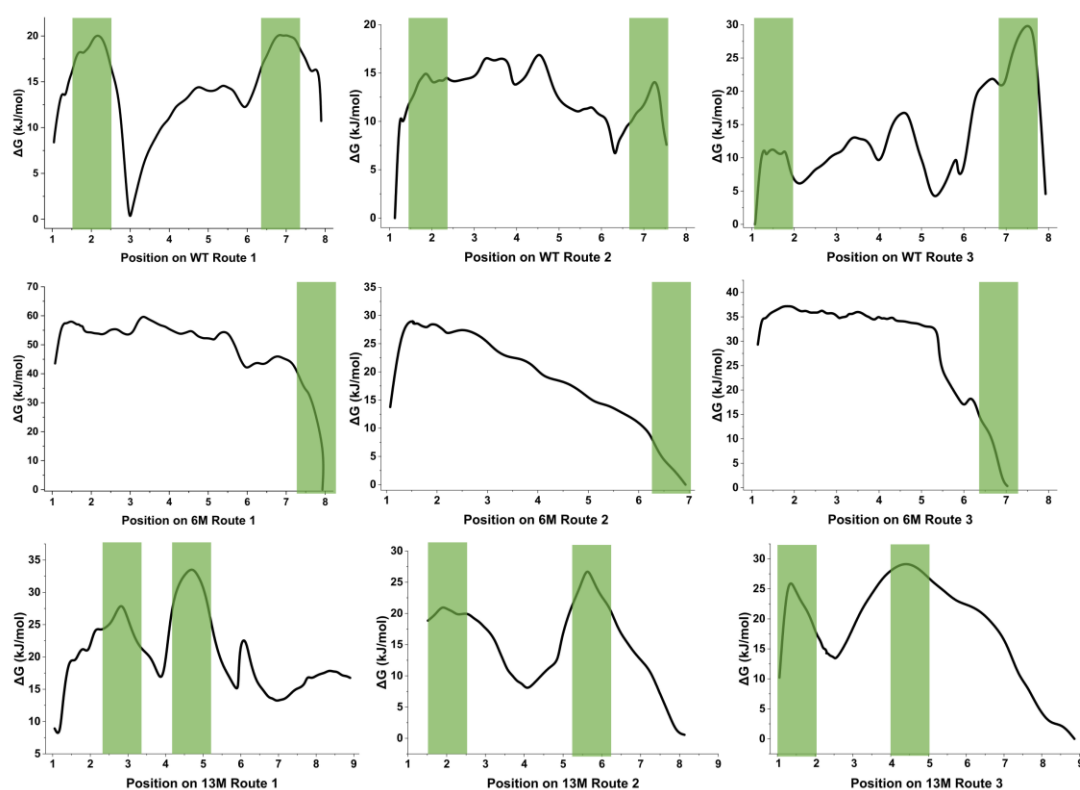
Supplementary Fig. 4 Time evolution of the geometric parameters distance $d(\text{C}_{\text{LIG}}-\text{N}_{\text{PMP}})$ (lower sub-panels) and angle $\angle(\text{N}_{\text{PMP}}-\text{C}_{\text{LIG}}-\text{O}_{\text{LIG}})$ (upper sub-panels) over the last 200 ns of 300 ns MD simulations for each variant.



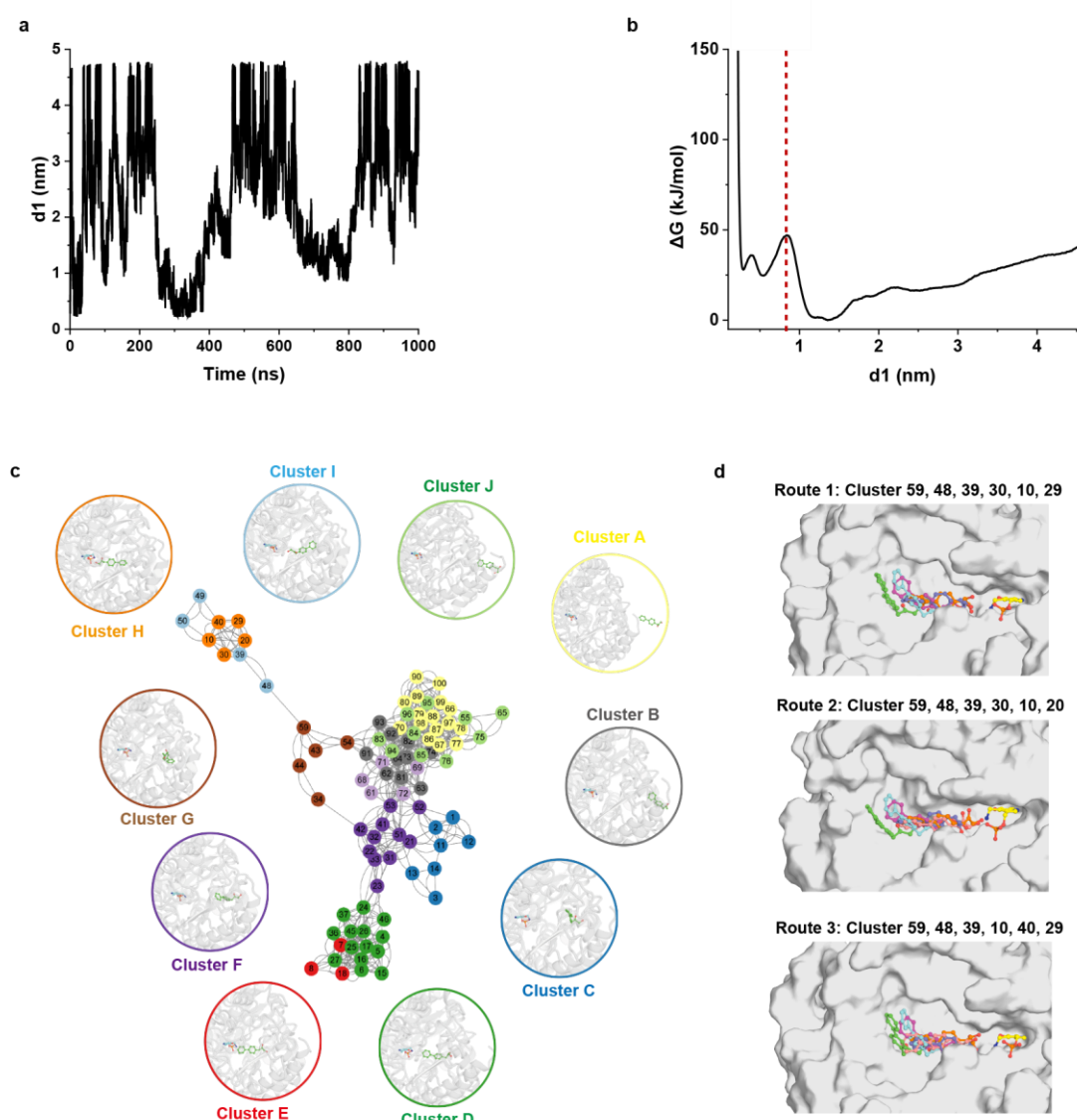
Supplementary Fig. 5 Time evolution of the geometric parameters distance $d(\text{O}_{\text{LIG}}-\text{N}_{\text{PMP}})$ over the last 200 ns of 300 ns MD simulations for WT.



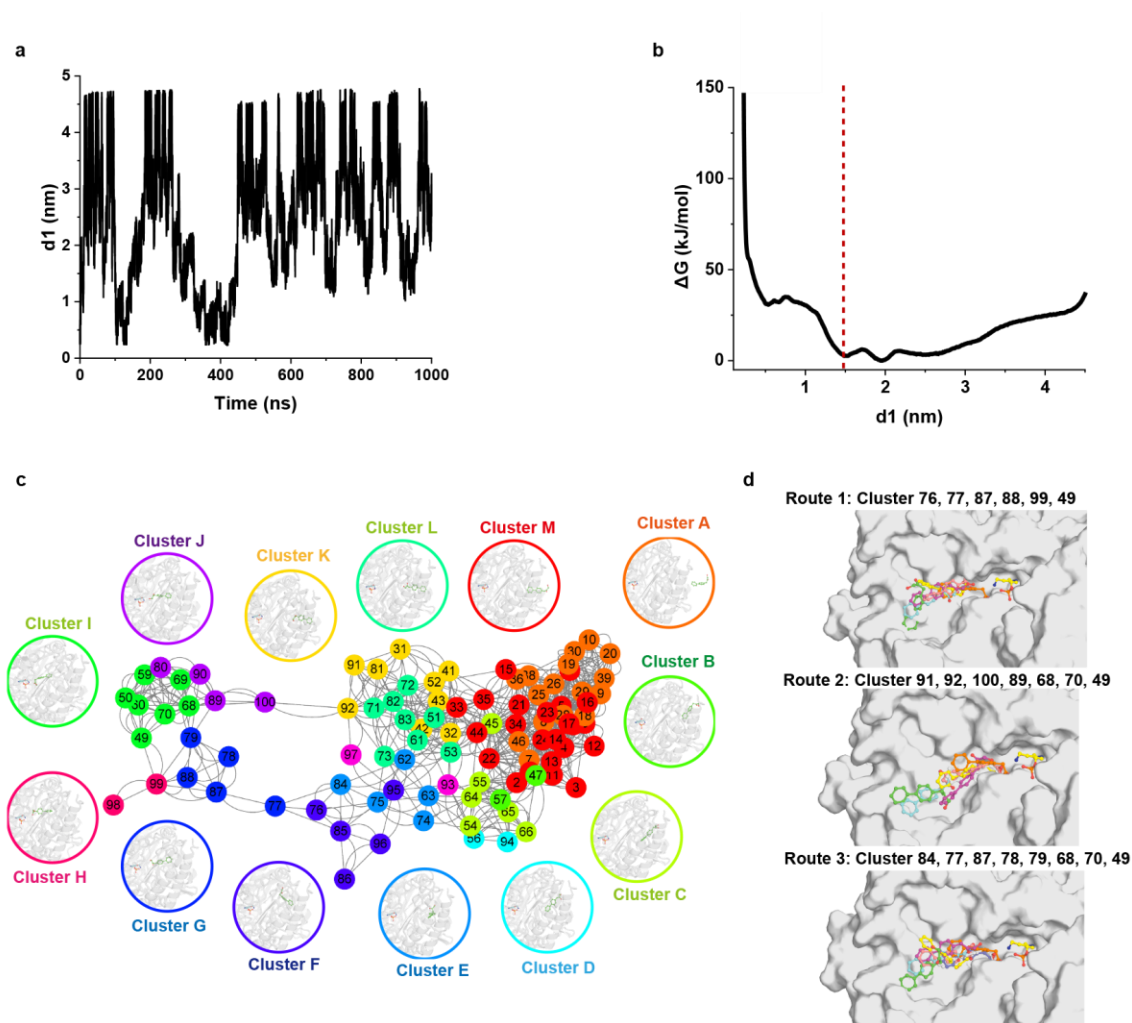
Supplementary Fig. 6 Analysis of WT GaMD simulations. **a** RMSD of WT. **b** Principal component analysis of the CA atoms of WT tunnel residues. **c&d** Free energy landscape of the CA atoms of WT tunnel residues.



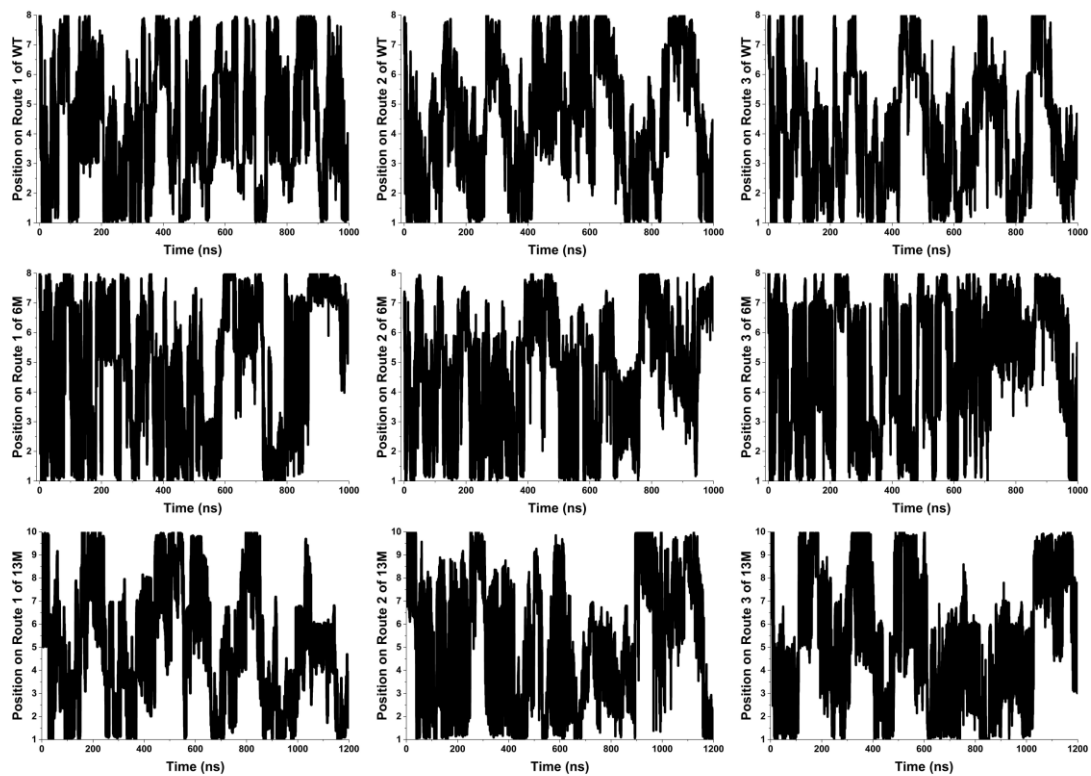
Supplementary Fig. 7 Free energy profiles along the minimum free energy path (MFEP) for WT, 6M, and 13M variants, each derived from three independent path metadynamics simulations (Routes 1-3). The green-shaded regions correspond to the key states marked by yellow star on the free energy surfaces (Fig. 3, 5&7a).



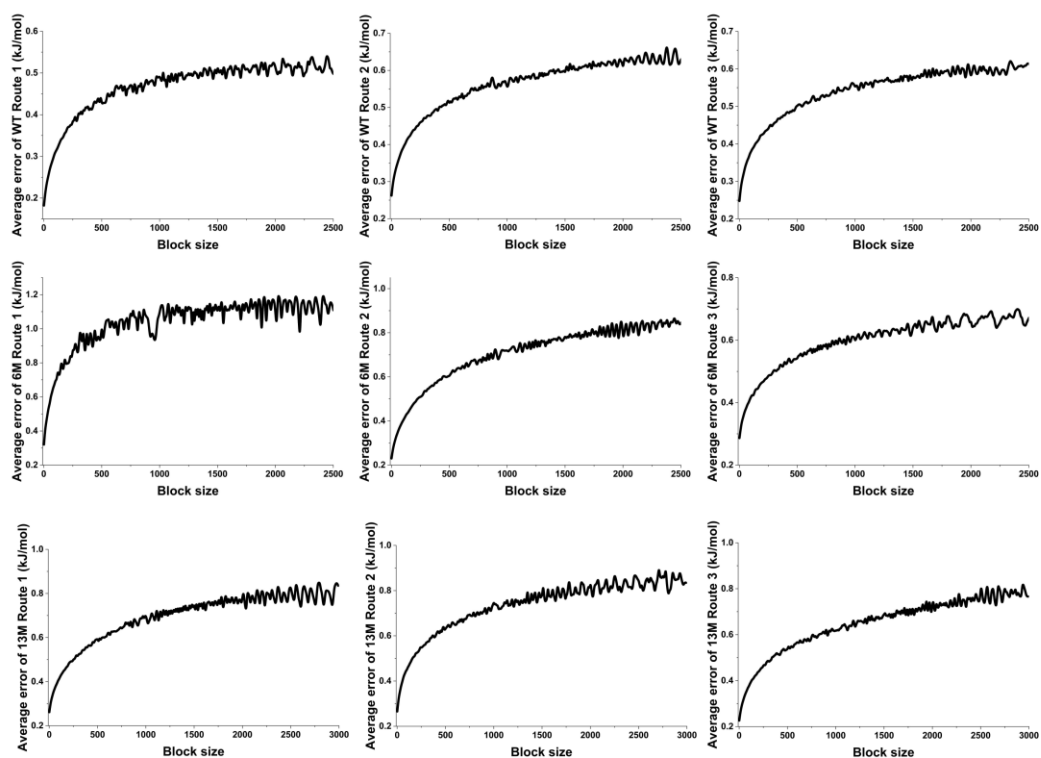
Supplementary Fig. 8 Funnel metadynamics (FM) analysis of the 6M variant. **a** Ligand-protein distance $d1$ as a function of the FM simulation time. **b** Free energy landscape of substrate binding to 6M obtained using FM simulations. The red dashed line marks the $d1$ value corresponding to the high-energy barrier region. **c** Projection of FM simulation data onto an interpretable 2D self-organizing map grid (2D-SOM). Communities are represented on the network, with nodes colored according to community assignment. The representative conformations of the communities are shown in cartoon representation with the PMP and ligand in ball-and-stick model. **d** The three guess paths selected based on the SOM are displayed separately. PMP and ligand are displayed in a ball-and-stick model, while the protein is shown in surface style.



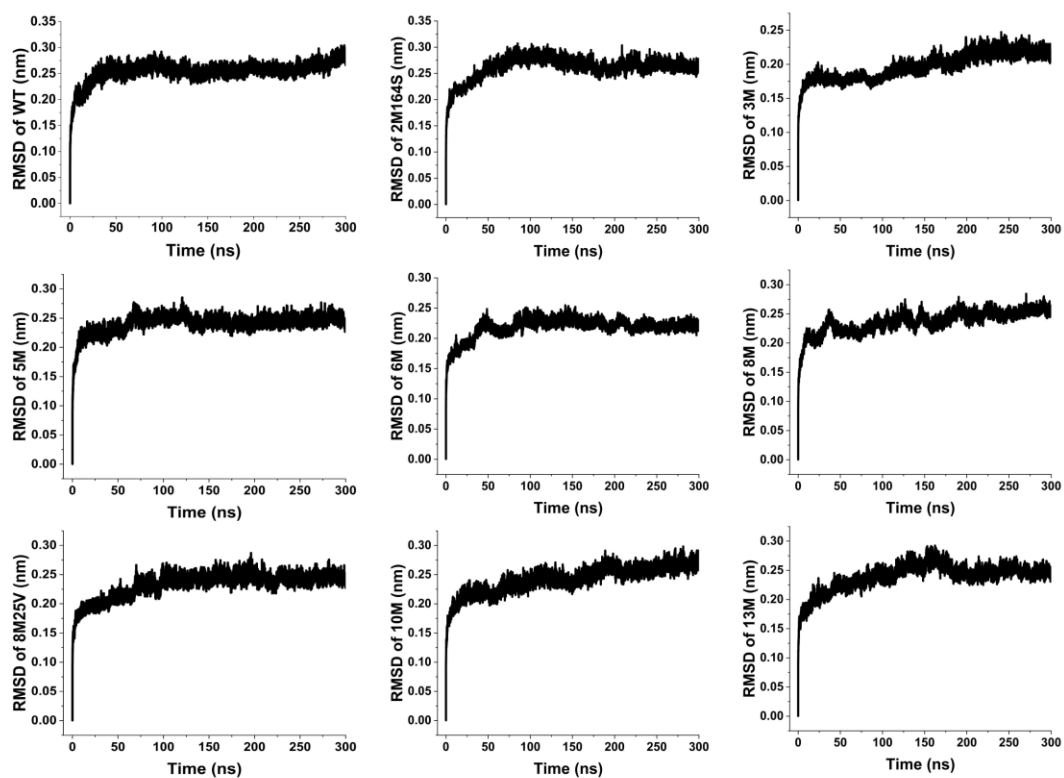
Supplementary Fig. 9 Funnel metadynamics (FM) analysis of the 13M variant. **a** Ligand-protein distance $d1$ as a function of the FM simulation time. **b** Free energy landscape of substrate binding to 13M obtained using FM simulations. The red dashed line marks the $d1$ value corresponding to the high-energy barrier region. **c** Projection of FM simulation data onto an interpretable 2D self-organizing map grid (2D-SOM). Communities are represented on the network, with nodes colored according to community assignment. The representative conformations of the communities are shown in cartoon representation with the PMP and ligand in ball-and-stick model. **d** The three guess paths selected based on the SOM are displayed separately. PMP and ligand are displayed in a ball-and-stick model, while the protein is shown in surface style.



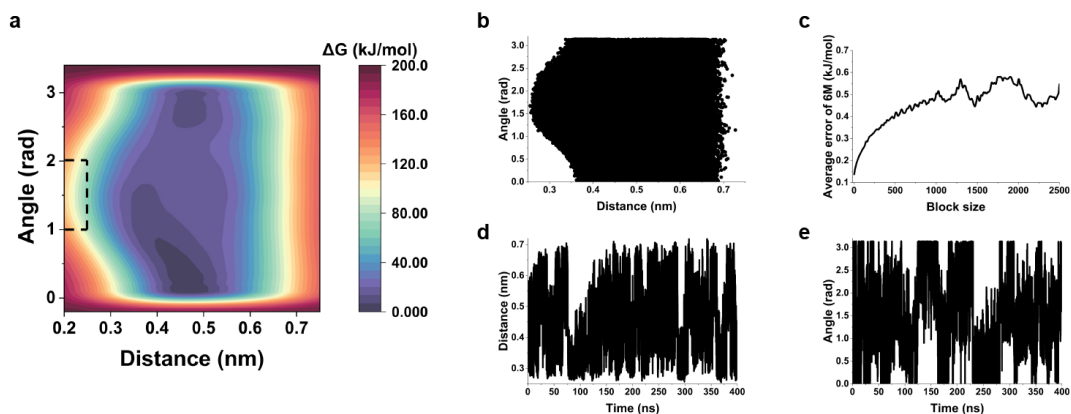
Supplementary Fig. 10 Ligand position on the binding path as a function of path metadynamics simulation time.



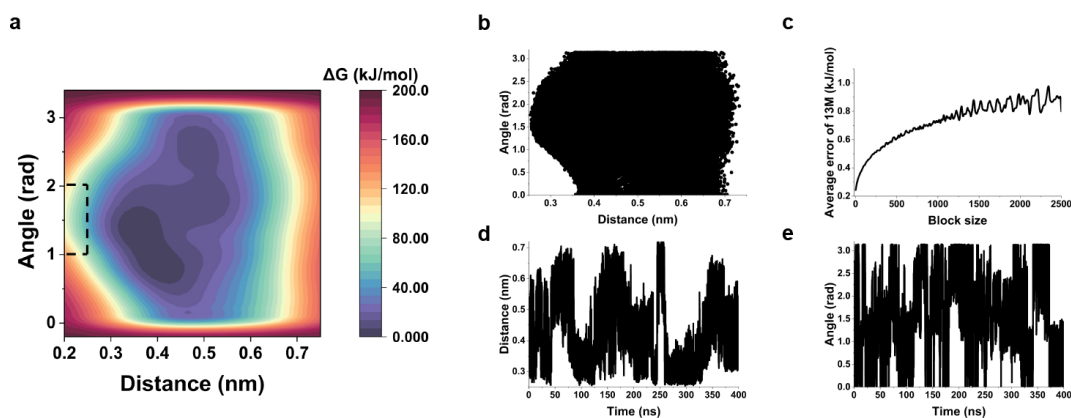
Supplementary Fig. 11 Block analysis carried out on the path metadynamics trajectory data. The average error associated with the free energy is shown as a function of increasing block size. All nine simulations (three independent routes per variant) reached a plateau or near-plateau, supporting reasonable convergence.



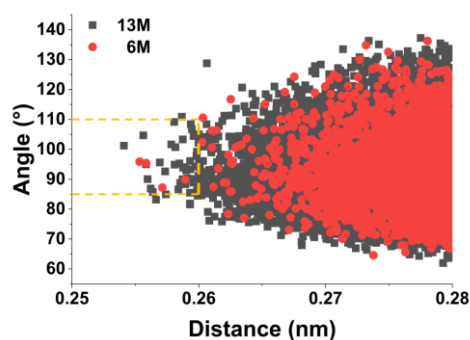
Supplementary Fig. 12 RMSD of molecular dynamics simulations.



Supplementary Fig. 13 Metadynamics simulation of the 6M variant using $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$ as collective variables. **a** Free energy surface of the 6M reconstructed from metadynamics simulation as a function of the distance $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and the angle $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$. The dashed box highlights the region where $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}}) \leq 0.28$ nm, with the associated angular range of $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$. **b** Distribution of sampled configurations projected onto the two collective variables throughout the simulation. **c** Block analysis carried out on the 6M metadynamics trajectory data. **d&e** Time evolution of the distance $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and the angle $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$ over the 400 ns metadynamics trajectory.

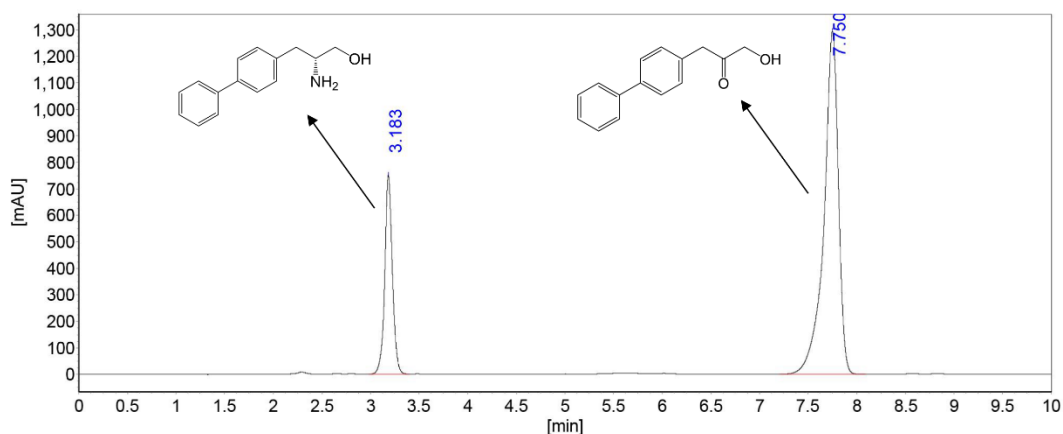


Supplementary Fig. 14 Metadynamics simulation of the 13M variant using $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$ as collective variables. **a** Free energy surface of the 13M reconstructed from metadynamics simulation as a function of the distance $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and the angle $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$. The dashed box highlights the region where $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}}) \leq 0.28$ nm, with the associated angular range of $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$. **b** Distribution of sampled configurations projected onto the two collective variables throughout the simulation. **c** Block analysis carried out on the 13M metadynamics trajectory data. **d&e** Time evolution of the distance $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and the angle $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$ over the 400 ns metadynamics trajectory.

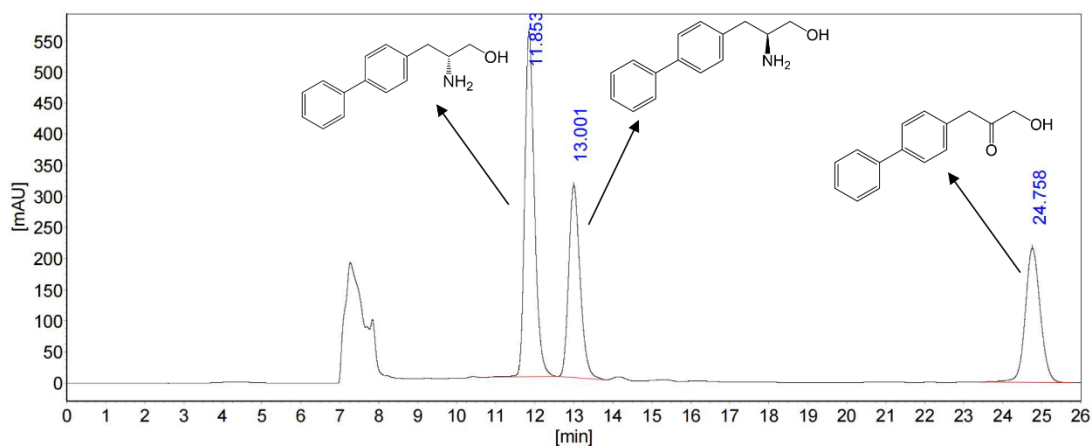


Supplementary Fig. 15. Comparison of sampled configurations with $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}}) \leq 0.28$ nm extracted from metadynamics simulations of 6M and 13M. Configurations are projected onto the distance $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}})$ and the angle $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$. The dashed box highlights the region where $d(\text{C}_{\text{LIG}}\text{-N}_{\text{PMP}}) \leq 0.26$ nm, indicating the angular range of $\angle(\text{N}_{\text{PMP}}\text{-C}_{\text{LIG}}\text{-O}_{\text{LIG}})$ (approximately $85^\circ\text{-}110^\circ$) adopted upon close approach to covalent bond formation.

2 Supplementary HPLC spectrums

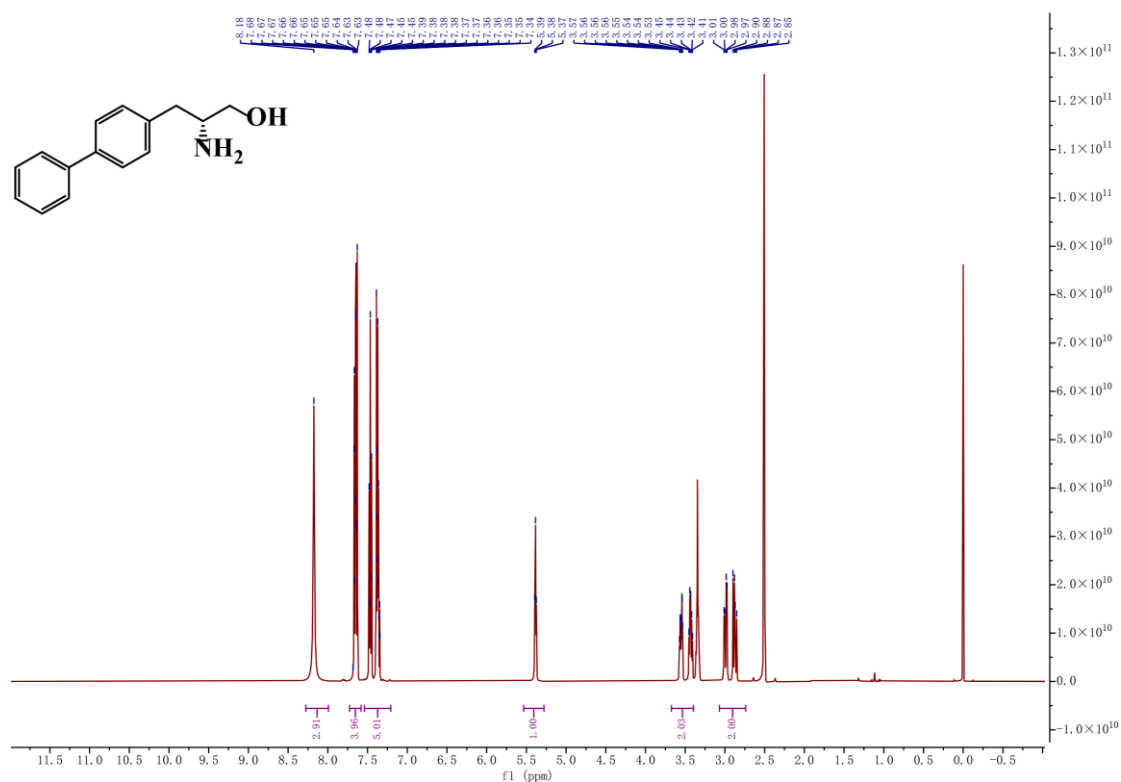


Supplementary Fig. 16 HPLC analysis of 3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol and 3-([1,1'-biphenyl]-4-yl)-2-ketopropan-1-ol. The retention times of 3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol and 3-([1,1'-biphenyl]-4-yl)-2-ketopropan-1-ol are 3.183 min and 7.750 min, respectively. Product concentration was determined with a C18 column (Pentulips QS-C18). Mobile phase: 0.1% trifluoroacetic acid aqueous solution: acetonitrile = 45:55; flow rate: 1 mL/min; column temperature: 30 °C; detection wavelength: 256 nm (UV absorbance).

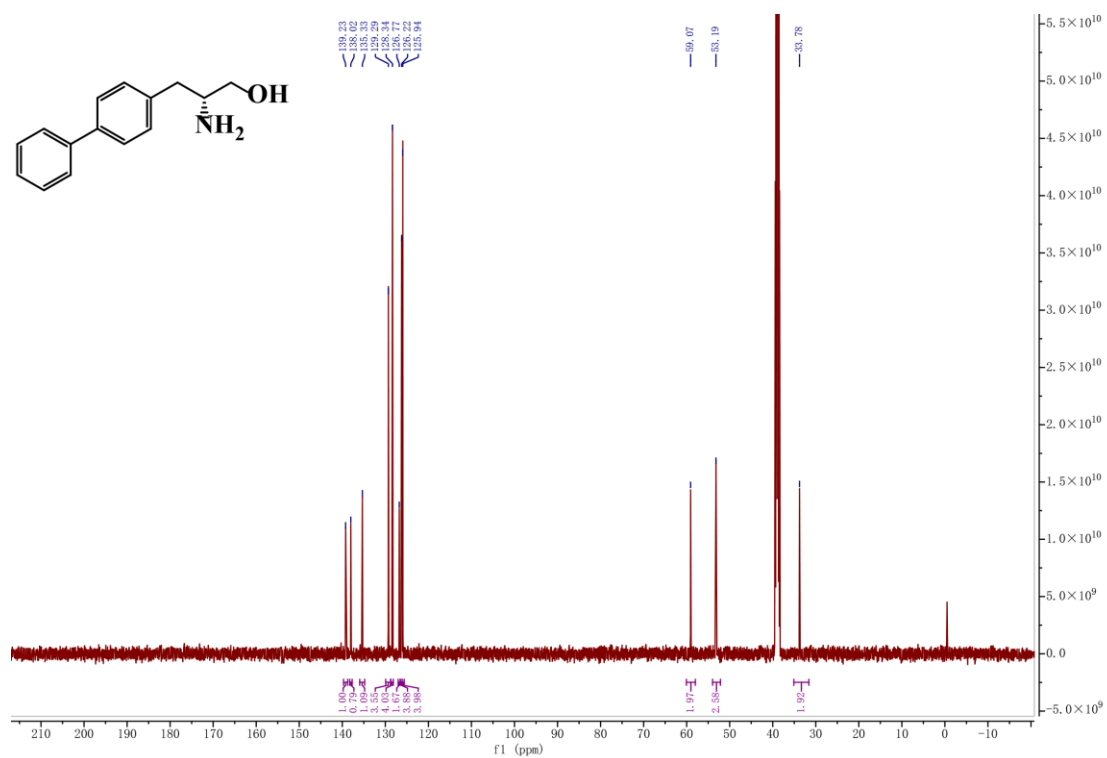


Supplementary Fig. 17 HPLC analysis of chiral amine. The retention times of (R)-3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol, (S)-3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol and 3-([1,1'-biphenyl]-4-yl)-2-ketopropan-1-ol are 11.85 min, 13.00 min and 24.75 min, respectively. Product enantiomeric excess (e.e. value) was determined with a chiral column (CHIRALPAK AY-3R). Mobile phase: 20 mM ammonium bicarbonate aqueous solution: acetonitrile = 35:65; flow rate: 0.4 mL/min; column temperature: 25 °C; detection wavelength: 256 nm (UV absorbance).

3 Supplementary NMR spectrums



Supplementary Fig. 18 The ^1H NMR spectrum of (R)-3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol. ^1H NMR (500 MHz, DMSO) δ 8.18 (s, 3H), 7.80–7.55 (m, 4H), 7.56–7.24 (m, 5H), 5.38 (t, $J = 5.0$ Hz, 1H), 3.70–3.39 (m, 2H), 3.11–2.76 (m, 2H).



Supplementary Fig. 19 The ¹³C NMR spectrum of (R)-3-([1,1'-biphenyl]-4-yl)-2-aminopropan-1-ol. ¹³C NMR (126 MHz, DMSO) δ 139.23, 138.02, 135.33, 129.29, 128.34, 126.77, 126.22, 125.94, 59.07, 53.19, 33.78.

4 Supplementary Tables

Supplementary Table 1 Binding free energies of the WT, 6M, and 13M variants calculated by MM/GBSA

Variant	MM/GBSA (kcal/mol)
WT	-50.69±4.30
6M	-29.25±6.31
13M	-32.03±2.56

Supplementary Table 2 Comparison of the catalytic binding conformation of BmATA WT with those reported in the literature

Variant	(<i>S</i>)-selective transaminase from <i>Paraburkholderia phymatum</i> (BPTA) ¹ M78F/W82A/I284F/T440Q	(<i>R</i>)-selective transaminase from <i>Rhodobacter</i> sp. 140A (RbTA) ² Y125A	BmATA WT
Substrate	(<i>S</i>)-1-amino-1-phenylpropane	<i>N</i> -heterocyclic amines	KBP
Activity (U/mg)	215.46-fold higher than WT	1.23±0.02	0.0065±0.0012
d(C _{LIG} -N _{PMP}) ^[a] (Å)	4.86	/	3.84±0.27
d(O _{LIG} -N _{PMP}) ^[b] (Å)	/	< 3.5	3.10±0.29
MM/PBSA (kcal/mol)	-36.33	/	/
MM/GBSA (kcal/mol)	/	-33.64	-50.69±4.30

[a] The distance between the carbonyl carbon atom of substrate and the amino group of PMP. Data are presented as mean values ± SD.

[b] The distance between the carbonyl group of the substrate and the amino group of PMP. Data are presented as mean values ± SD.

Supplementary Table 3 Key geometric parameters of the dominant conformation A identified from 2D density map for each variant

Variant	d(C _{LIG} -N _{PMP}) (Å) ^[a]	∠(N _{PMP} -C _{LIG} -O _{LIG}) (°) ^[a]	Proportion (%) ^[b]
WT	3.74±0.10	42.24±3.94	33.10
2M164S	9.21±0.26	119.66±5.64	36.38
3M	4.21±0.23	68.70±7.10	37.56
5M	3.65±0.12	42.51±4.86	23.58
6M	3.90±0.16	41.80±3.48	25.77
8M	3.76±0.11	46.73±4.14	37.38
8M25V	3.96±0.16	99.59±6.39	17.66
10M	3.6±0.10	45.52±4.10	18.52
13M	3.27±0.09	81.98±3.79	36.30

[a] Data are presented as mean values ± SD.

[b] The proportion was defined as the fraction of MD frames residing within the dominant density region, i.e., the connected region encompassing the KDE peak at ≥60% of maximum density.

Supplementary Table 4 Primers used in this study

Variants	Name	Primers
WT	25-F	CTGATGCGCACGNNKAGCACGCAGAATG
	25-R	MNNCGTGCGCATCAGATATTTGCGATC
	56-F	CTGCTGGATTTTNNKAATCAGCTGTATTG
	56-R	MNNAATCCAGCAGACGGGTACCA
	59-F	TTTTTAATCAGNNKTATTGTGTTAAC
	59-R	MNNCTGATTAATAAATCCAGCAGAC
	86-F	TATGGTTTTGTGNNKGATACATATAG
	86-R	MNNCAGAAAACCATAACGATCCAGTG
	164-F	CGTCTGCGTAGTNNKCGTAGCGGTC
	164-R	MNNACTACGCAGACGGGTACGGTG
	420-F	CAGATTCCGACCNNKATTATTATGAAAAAAGC
	420-R	MNNGGTCGGAATCTGATTCGGATTG
	436-F	CTGATTGGTGGTNNKATGCCGAATAC
	436-R	MNNACCACCAATCAGAACACCTTTTTC
	442-F	CCGAATACCATGNNKATTGGTGCAAG
	442-R	MNNCATGGTATTCGGCATAACACCAC
3M	55-F	CCGTCTGCTGGATNNKTTTAATCAGCTGTATTGTGTTAACCTGG
	55-R	AMNNATCCAGCAGACGGGTACCATCCGGCATA
	57-F	TNNKCAGCTGTATTGTGTTAACCTGGGTCAGA
	57-R	CACAATACAGCTGMNNAATAAATCCAGCAGACGGGT
	58-F	TAATNNKCTGTATTGTGTTAACCTGGGTCAGA
	58-R	CACAATACAGMNNATTAATAAATCCAGCAGACGGG
	60-F	GCTGNNKTGTGTTAACCTGGGTCAGAAAAATC
	60-R	GGTTAACACAMNNCAGCTGATTAATAAATCCAGCAG
	61-F	TNNKGTTAACCTGGGTCAGAAAAATCCGAAAG
	61-R	GACCCAGGTTAACMNNATACAGCTGATTAATAAATCCAGCA
	85-F	GGTTTTNNKTGGGATACATATAGCACCGATTATAAA
	85-R	GTATCCCAMNNAATCCATAACGATCCAGTGCTT
	87-F	GGTTTTGTGTGGNNKACATATAGCAC
	87-R	MNNCCACACAAAACCATAACGATCC
	88-F	TTTGTGTGGGATNNKTATAGCACCG
	88-R	MNNATCCACACAAAACCATAACGATC
	89-F	GGATACANNKAGCACCGATTATAAAGCAAAAGC
	89-R	CGGTGCTMNNTGTATCCACACAAAACCATAACG
	90-F	GTGGGATACATATNNKACCGATTATAAAG
	90-R	MNNATATGTATCCACACAAAACCATAAC
	159-F	CACCGTANNKCGTCTGCGTAGTTATCGTAGCG
	159-R	GCAGACGMNNTACGGTGGCTGCACCACCTGTC
	161-F	GTAACCCGTNNKCGTAGTTATCGTAGCGGTCTGGT

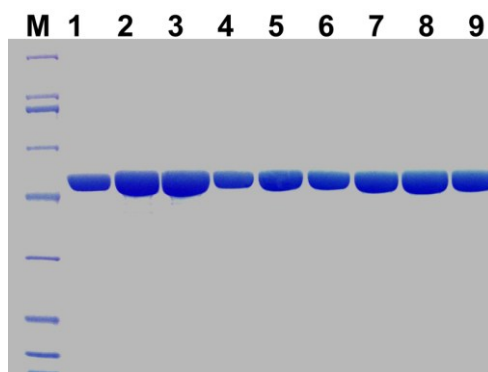
Supplementary Table 4 (continued)

Variants	Name	Primers
3M	161-R	CTACGMNNACGGGTTACGGTGGCTGCACCACC
	162-F	GTAACCCGTCTGNNKAGTTATCGTAGCGGTCTGGTTGG
	162-R	CTMNNCAGACGGGTTACGGTGGCTGCACCACC
	163-F	CGTCTGCGTNNKTATCGTAGCGGTCTGGTTGGTG
	163-R	CGATAMNNACGCAGACGGGTTACGGTGGCTGC
	165-F	TNNKAGCGGTCTGGTTGGTGAAAAATTCTGAAA
	165-R	CAACCAGACCGCTMNNATAACTACGCAGACGGGTTACGG
6M	163-F	GTCTGCGTNNKATGCGTAGCGGTCTGGTTGGT
	163-R	ACGCATMNNACGCAGACGGGTTACGGTGGCTG
	242-F	TCAGGGTNNKGGTAGCGCAATGCCGCCATATG
	242-R	CGCTACCMNNACCCTGACTTACTTCCGTGATTACT
	327-F	CTGGGAANNKGTGAGCACATATGCAGGTCATCC
	327-R	TGCTCACMNNTTCCCAGCGATGACGATCCATA
	328-F	GCTGGGAAAGTNNKAGCACATATGCAGGTCATCCG
	328-R	GCTMNNACTTTCCCAGCGATGACGATCCATAA
	423-F	CGACCTCGATTATTNNKAAAAAAGCACTGGAAAAAGGTGTT
	423-R	MNNAATAATCGAGGTCGGAATCTGATTTCGGAT
	436-F	TGGTGGTNNKATGCCGAATACCATGCATATTG
	436-R	TCGGCATMNNACCACCAATCAGAACACCTTTTTTC
	442-F	GNNKATTGGTGCAAGCCTGAATGTTAGCCGTG
	442-R	GGCTTGACCAATMNNCATGGTATTTCGGCATAACACCA
10M	24-F	GATGCGCNNKTTGAGCACGCAGAATGAATATCA
	24-R	TGCTCAAMNNGCGCATCAGATATTTGCGATCC
	26-F	CACGTTGNNKACGCAGAATGAATATACGCCGG
	26-R	TCTGCGTMNNCAACGTGCGCATCAGATATTTG
	241-F	AGTCAGNNKGCAGGTAGCGCAATGCCGCCATA
	241-R	CTACCTGCMNNCTGACTTACTTCCGTGATTACTGCTG
	324-F	GGATCGTCATNNKTGGGAACAGGTGAGCACATATG
	324-R	CCCAMNNATGACGATCCATAAATTCTGCAATT
	325-F	TCATCGCNNKGAACAGGTGAGCACATATGCAGG
	325-R	CCTGTTCMNNNGCGATGACGATCCATAAATTCTG
	326-F	TCGCTGGNNKCAGGTGAGCACATATGCAGGTC
	326-R	TCACCTGMNNCCAGCGATGACGATCCATAAAT
	387-F	TGGTCTGNNKTGGATTGTTGATATTGTTAATGCAAA
	387-R	CAATCCAMNNCAGACCATAACCATCAAAATTACCA
	440-F	GCCGAATNNKATGCATATTGGTGCAAGCCTGA
	440-R	TATGCATMNNATTTCGGCATAAGACCACCAATC
	441-F	GCCGAATACNNKCATATTGGTGCAAGCCTGAATG
	441-R	TATGMNNGGTATTTCGGCATAAGACCACCAATC

Supplementary Table 4 (continued)

Variants	Name	Primers
10M	443-F	CCATGCATNNKGGTGCAAGCCTGAATGTTAGC
	443-R	TGCACCMNNATGCATGGTATTCGGCATAAGAC

5 Gel



Supplementary Fig. 20 SDS-PAGE gel characterization of purified enzyme. M: Marker, 1: WT, 2: 2M164S, 3: 3M, 4: 5M, 5: 6M, 6: 8M, 7: 8M25V, 8: 10M, 9: 13M.

6 Supplementary References

1. Xie, Y. et al. Engineering the large pocket of an (*S*)-selective transaminase for asymmetric synthesis of (*S*)-1- amino-1-phenylpropane. *Catal. Sci. Technol.* **11**, 2461-2470 (2021).
2. Li, F. et al. Redesigning an (*R*)-selective transaminase for the efficient synthesis of pharmaceutical N-heterocyclic amines. *ACS Catal.* **13**, 422-432 (2023).