Supplementary Information

Noncanonical catalytic-relay geometry and PET-oligomer accommodation in a CheB-like α/β-hydrolase from *Pseudomonas marincola*

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Online Resource 1: Supplementary tables and figures supporting model provenance, validation, catalytic-relay measurements, pocket mapping, and docking analyses

Structural Chemistry — Research

S1. Model provenance and confidence

The ColabFold run used version 1.6.2, AlphaFold2-ptm, five model parameter sets, three recycles, seed 0, MMseqs2 UniRef/environmental MSAs, no templates, and one Amber relaxation. The rank-1 prediction was model 5, seed 000, with mean pLDDT 87.99 and pTM 0.84 [13–15]. SWISS-MODEL produced six models; model 2 was selected as the experimental-template cross-check because it used PDB 6YMZ rather than an obsolete database model [12,16].

Table S1. Complete SWISS-MODEL model screen.

Model	Template	Coverage	Identity	Similarity	GMQE	Pred. lDDT	QMEAN4 Z	QMEAN6 Z
1	A0A423NFA6.1.A	100.00%	83.78%	0.552	0.847	0.847	n/a	n/a
2	6ymz.1.A	89.63%	36.20%	0.383	0.638	0.638	-3.257	-2.803
3	7esg.1.A	94.41%	38.87%	0.387	0.513	0.513	-11.229	-9.932
4	2v0n.1.A	59.31%	20.18%	0.290	0.298	0.298	-3.284	-3.841
5	6od1.1.A	56.65%	20.66%	0.294	0.301	0.301	-5.670	-5.175
6	3cwo.1.A	47.34%	22.47%	0.305	0.263	0.263	-9.417	-9.107

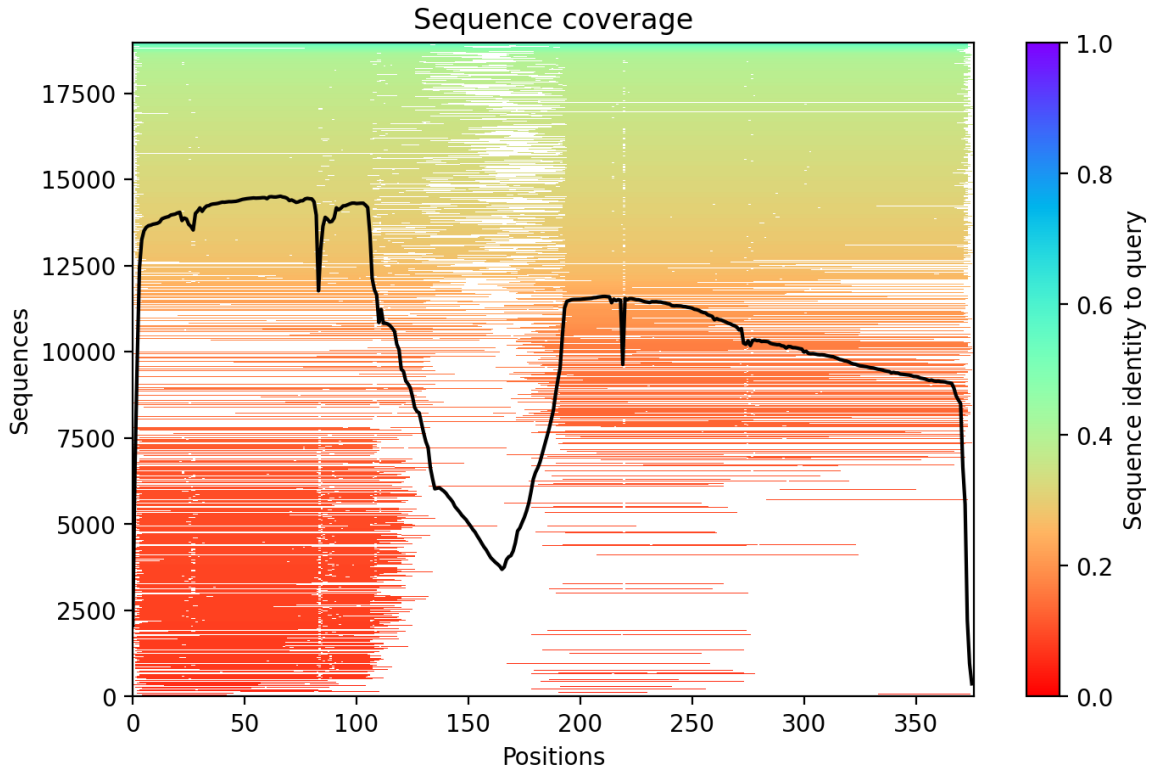

Fig. S1 ColabFold multiple-sequence-alignment depth and sequence coverage used for the five ranked predictions. The low-complexity central insertion has reduced effective support

S2. MolProbity validation

MolProbity values were taken from the supplied all-atom geometry reports [18,19]. The relaxed ColabFold structure is the appropriate source for reporting model quality; the separate hydrogen-added receptor run was used for docking preparation and had a higher clashscore because hydrogen placement changes the all-atom contact calculation.

Table S2. Detailed MolProbity statistics.

Metric	ColabFold relaxed	ColabFold H-added	SWISS-MODEL 2 H-added
Clashscore	1.05	5.23	9.29
MolProbity score	1.46	1.93	2.13
Poor rotamers	4/302 (1.32%)	4/302 (1.32%)	5 (1.67%)
Favoured rotamers	289/302 (95.70%)	289/302 (95.70%)	288 (96.00%)
Ramachandran outliers	14/374 (3.74%)	14/374 (3.74%)	9 (2.42%)
Ramachandran favoured	335/374 (89.57%)	335/374 (89.57%)	344 (92.47%)
Cβ deviations	3/352 (0.85%)	3/352 (0.85%)	5 (1.43%)
Bad bonds	0/2868	0/2868	0/2854

Metric	ColabFold relaxed	ColabFold H-added	SWISS-MODEL 2 H-added
Bad angles	16/3897 (0.41%)	16/3897 (0.41%)	27/3878 (0.70%)
CaBLAM outliers	6 (1.6%)	6 (1.6%)	12 (3.2%)

S3. Catalytic-relay distance matrix

Table S3. Complete selected heavy-atom distances (Å).

Structure	Ser Oγ–His ND1	Ser Oγ–His NE2	His ND1–Asp OD1	His ND1–Asp OD2	His NE2–Asp OD1	His NE2–Asp OD2
ColabFold	2.850	4.917	6.576	4.646	4.795	2.690
SWISS-MODEL 2	2.930	4.941	6.682	4.728	4.702	2.635
6SBN	4.583	2.630	3.129	2.823	4.984	4.936

Note: 6SBN alternative atom-pair distances were calculated from the supplied PDB coordinates. The main text reports only the closest residue-pair contacts.

S4. CASTpFold pocket mapping

Table S4. CASTpFold topography and catalytic-atom membership.

Structure	Probe	Relevant pocket	SA area (Å²)	SA volume (Å³)	Catalytic membership	Interpretive caution
ColabFold	1.4 Å	Pocket 2	589.268	476.045	Ser200 and His227; Asp320 not in same pocket	Substantial depression; relay edge is fragmented
SWISS-MODEL 2	2.0 Å	Pocket 1	502.494	612.840	Ser200 and His227; Asp320 not in same pocket	Probe differs; volume not directly comparable
6SBN	1.4 Å	Pocket 8	11.784	2.961	Ser171 Oγ; Asp217 and His249 assigned elsewhere	Open groove fragmented into small pocket IDs

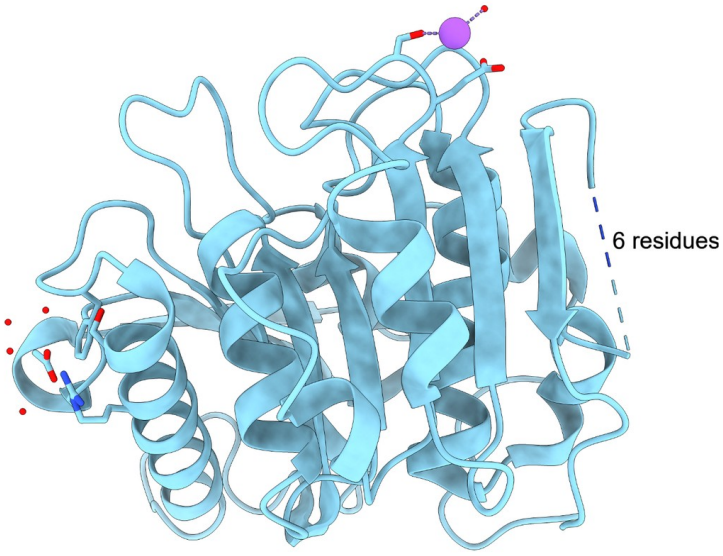

Fig. S2 CASTpFold visualization of 6SBN pocket 8. The small surface feature includes Ser171 Oγ but should not be interpreted as the full catalytic groove; Asp217 and His249 are assigned to other topographic pockets

S5. Docking run manifest and geometry screen

Table S5. Complete 12-run AutoDock Vina production manifest.

Receptor	Ligand	Seed	Exhaust.	Requested	Returned	Best score	Runtime (s)
ColabFold	BHET	101	32	20	20	-5.788	159.59
ColabFold	BHET	202	32	20	20	-6.006	159.02
ColabFold	BHET	303	32	20	20	-6.026	160.64
ColabFold	MHET	101	32	20	20	-6.036	83.13
ColabFold	MHET	202	32	20	20	-6.095	80.7
ColabFold	MHET	303	32	20	20	-6.027	81.18
6SBN	BHET	101	32	20	20	-5.252	179.18
6SBN	BHET	202	32	20	20	-5.29	178.6
6SBN	BHET	303	32	20	19	-5.094	179.23
6SBN	MHET	101	32	20	20	-5.268	82.65
6SBN	MHET	202	32	20	20	-5.187	83.08

Receptor	Ligand	Seed	Exhaust.	Requested	Returned	Best score	Runtime (s)
6SBN	MHET	303	32	20	20	-5.148	83.47

Table S6. All poses passing the permissive catalytic-geometry screen.

Receptor	Ligand	Seed	Pose	Score	Distance (Å)	Angle (°)	Min. contact (Å)
ColabFold	BHET	101	10	-5.576	3.676	89.7	2.795
ColabFold	BHET	101	18	-5.453	4.039	87.9	2.705
ColabFold	MHET	101	13	-5.475	4.464	97.4	2.773
ColabFold	MHET	202	8	-5.576	4.277	103.2	2.746
ColabFold	MHET	202	10	-5.480	3.530	97.3	2.850

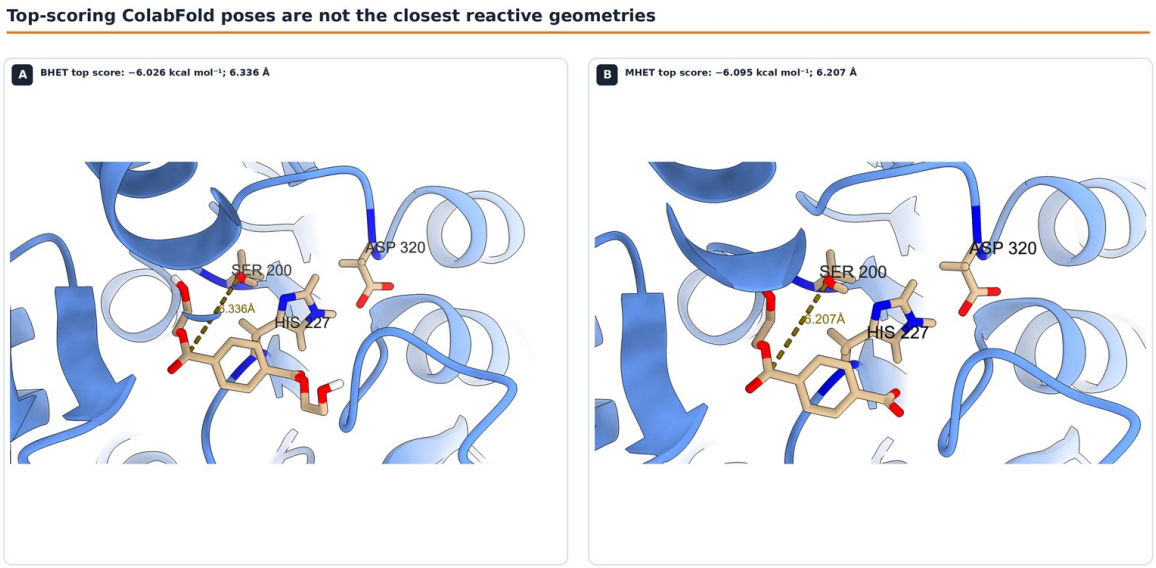

Fig. S3 Highest-scoring ColabFold poses are catalytically non-ideal. (A) BHET seed 303 pose 1. (B) MHET seed 202 pose 1. Their reactive carbonyls remain 6.336 and 6.207 Å from Ser200 despite more favourable Vina scores than the retained near-attack-like candidates

S6. Supplementary data file map

Online Resource 2 (ESM_2.zip) contains the target FASTA sequence; ColabFold rank-1 coordinates and confidence/configuration files; SWISS-MODEL model 2 coordinates and reports; a consolidated MolProbity table; curated CASTpFold pocket files; docking inputs, all production pose/score outputs, the complete 239-pose geometry screen, selected poses, and a plain-text parameter record. The archive does not include the pilot docking results or duplicate screenshots.