

Supplementary Information

A Non-Canonical Nucleophile Unlocks a New Mechanistic Pathway in a Designed Enzyme

Amy E. Crossley¹, Rebecca Crawshaw¹, Florence J. Hardy¹, Linus Johannissen¹, Thomas M. Lister¹, Emilie F. Gérard¹, Zachary Birch-Price¹, Richard Obexer¹, Sam Hay¹, Anthony P. Green^{1*}

*Corresponding author

¹Manchester Institute of Biotechnology, School of Chemistry, University of Manchester, Manchester, UK.

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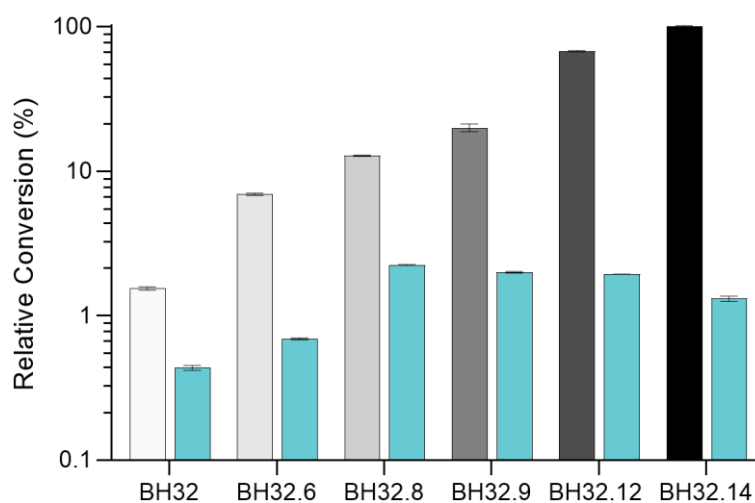


Figure 1: Identification of a suitable starting point for evolution. Relative conversions of BH32 and selected evolved descendants with either His (grey scale) or MeHis (blue) as the catalytic nucleophile at position 23. Biotransformations were performed using **1** (15 mM), **2** (1.5 mM) and enzyme (60 μ M) in PBS (pH 7.4) with 3% (v/v) MeCN as cosolvent and analysed following 5 h incubation at 30 °C. Error bars represent the standard deviation of measurements made in triplicate.

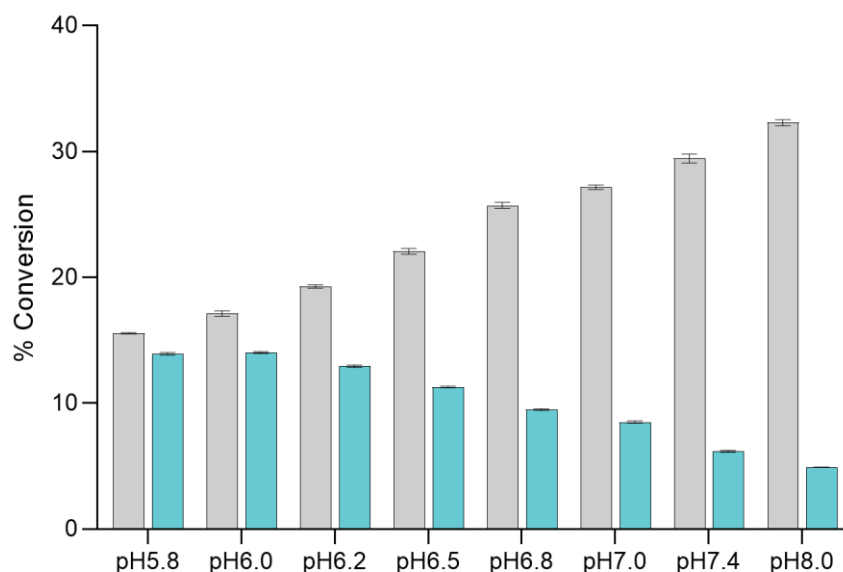


Figure 2: Comparison of pH profiles of BH32.8 and BH_{MeHis}1.0. Reaction conversions of BH32.8 (grey) and BH_{MeHis}1.0 (blue) across a range of pHs ranging from pH 5.8 to 8.0. Reactions performed using **1** (15 mM), **2** (1.5 mM), 60 μ M enzyme in PBS at the stated pH using 3% (v/v) MeCN as cosolvent and analysed following 21 h incubation at 30 °C. Error bars represent standard deviation of measurement made in triplicate. In addition to MBH product **3** an aldol side product (**S1**) was formed in low abundance (data not shown).

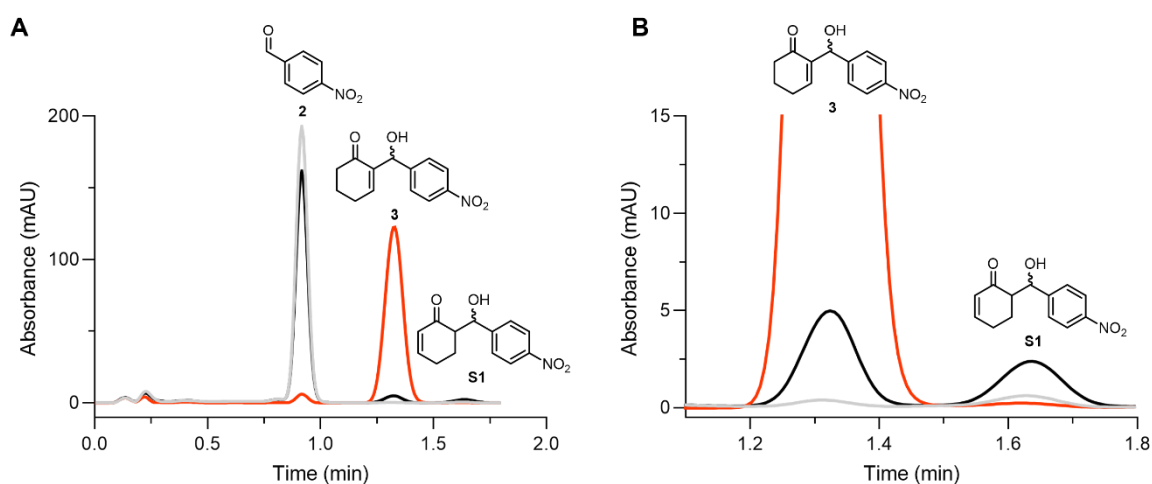


Figure 3: UPLC analysis of the MBH reaction catalysed by either BH_{MeHis}1.0 or BH_{MeHis}1.8. **A)** Ultra-high performance liquid chromatography (UPLC) trace for the MBH reaction between 2-cyclohexen-1-one (**1**) and 4-nitrobenzaldehyde (**2**) forming MBH product (**3**) and an aldol side product (**S1**). **B)** zoom of UPLC trace in **A** between 1.1 and 1.8 min. After evolution, BH_{MeHis}1.8 (red, 10 μ M) forms MBH product **3** as the exclusive product as opposed to the starting variant BH_{MeHis}1.0 (grey, 10 μ M or black, 60 μ M). Reactions performed using **1** (15 mM), **2** (1.5 mM), PBS pH 6.0 with 20% (v/v) DMSO as cosolvent and analysed following 23 h incubation at 30 °C.

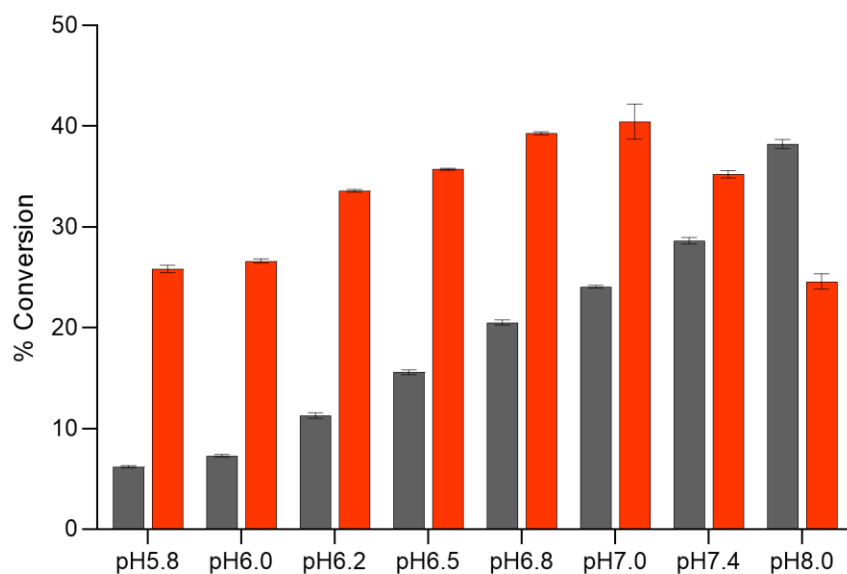


Figure 4: pH profiles of BH_{MeHis}1.8 and BH32.14. Reaction conversions of either BH_{MeHis}1.8 (red, 3 μ M) or BH32.14 (grey, 30 μ M) achieved across a range of pHs between 5.8 to 8.0. Reactions were performed using **1** (15 mM), **2** (1.5 mM) in PBS at the stated pH using 3% (v/v) MeCN as cosolvent and analysed by UPLC following 2 h incubation at 30 °C. Error bars represent standard deviation of measurement made in triplicate.

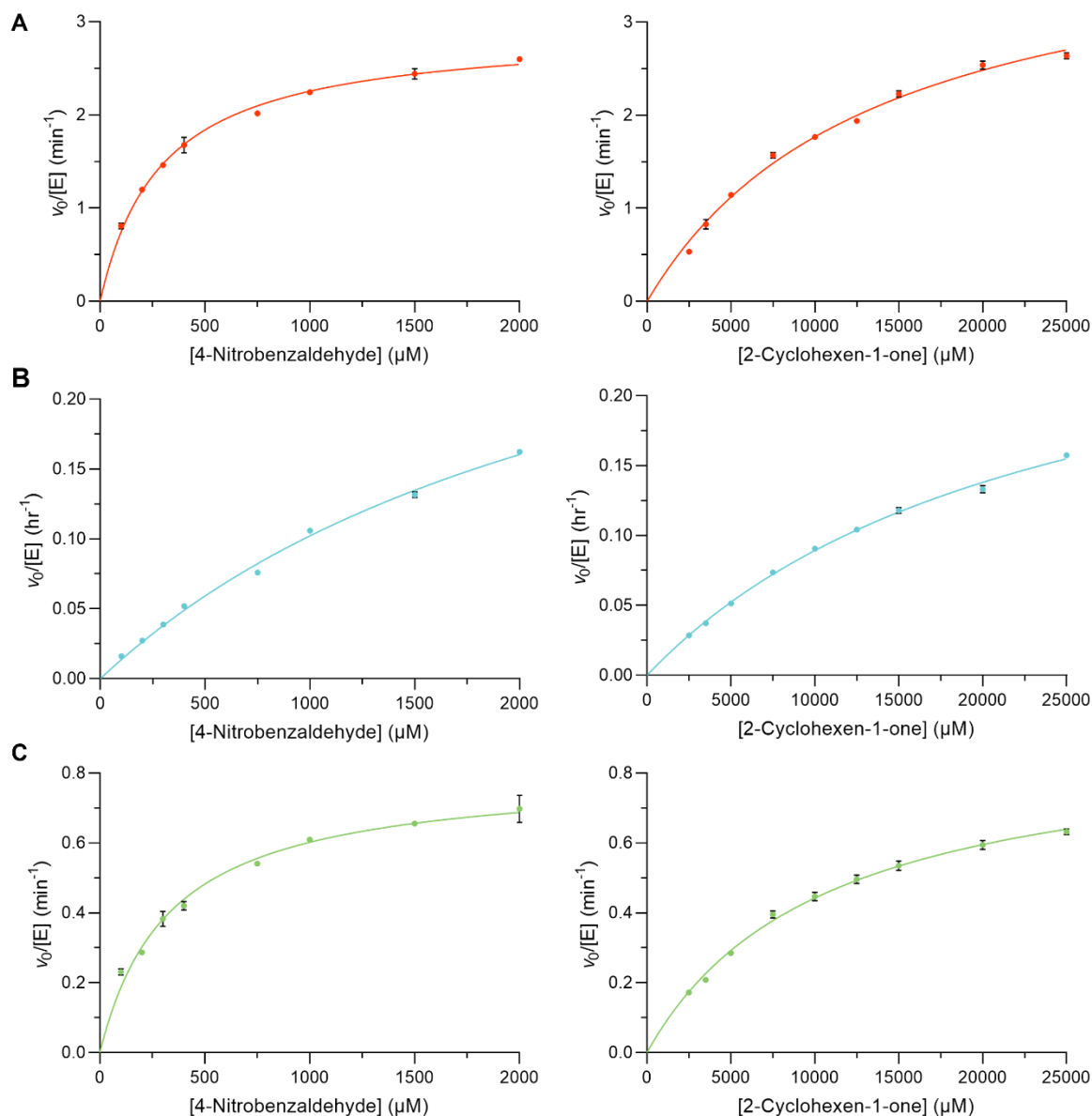


Figure 5: Kinetic characterization of BH_{MeHis}1.0, BH_{MeHis}1.8 and BH_{MeHis}1.8 MeHis23His. Michaelis-Menten plots for the MBH reaction between **1** and **2** catalysed by either: **A)** BH_{MeHis}1.8, **B)** BH_{MeHis}1.0 and **C)** BH_{MeHis}1.8 MeHis23His. Assays were performed at either a fixed concentration of **1** (25 mM) and varying concentrations of **2**, or a fixed concentration of **2** (2 mM) and varying concentrations of **1**. The plots show the averaged initial rates of triplicate data which were fitted to the Michaelis-Menten equation using Origin software.

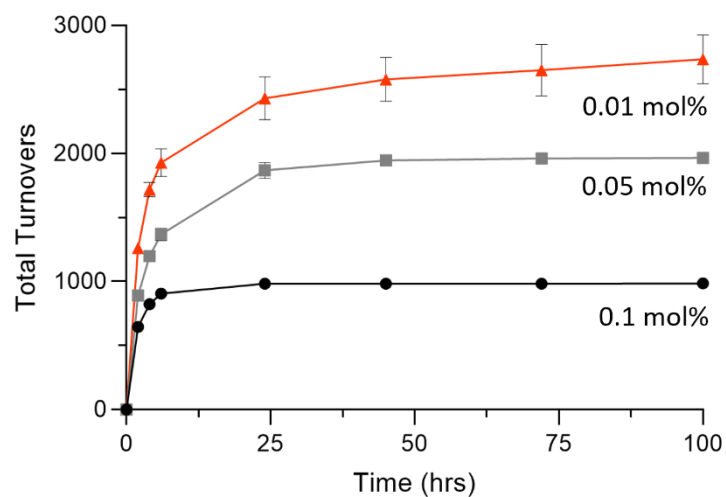


Figure 6: Total turnover numbers achieved by BH_{MeHis}1.8. Time-course to determine the total turnover number of BH_{MeHis}1.8. Reactions were performed using **1** (50 mM) and **2** (10 mM) in PBS pH 7.0 with 20% (v/v) DMSO as cosolvent, using either 0.1 mol% (black), 0.05 mol% (grey) or 0.01 mol% (red) of BH_{MeHis}1.8. Error bars represent standard deviation of measurement made in triplicate.

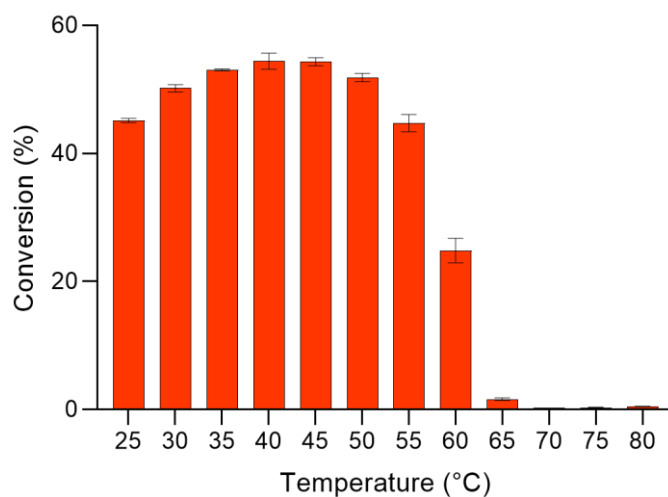


Figure 7: Temperature profile of BH_{MeHis}1.8. Reaction conversions of BH_{MeHis}1.8 across a range of temperatures from 25 to 80 °C after 2 h incubation. Reactions were performed using **1** (15 mM), **2** (1.5 mM) in PBS pH 7.0 using 3% (v/v) MeCN as cosolvent and 3 μM BH_{MeHis}1.8. Error bars represent standard deviation of measurement made in triplicate. Aldol by-product (**S1**) only observed at temperatures above 60 °C (data not shown).

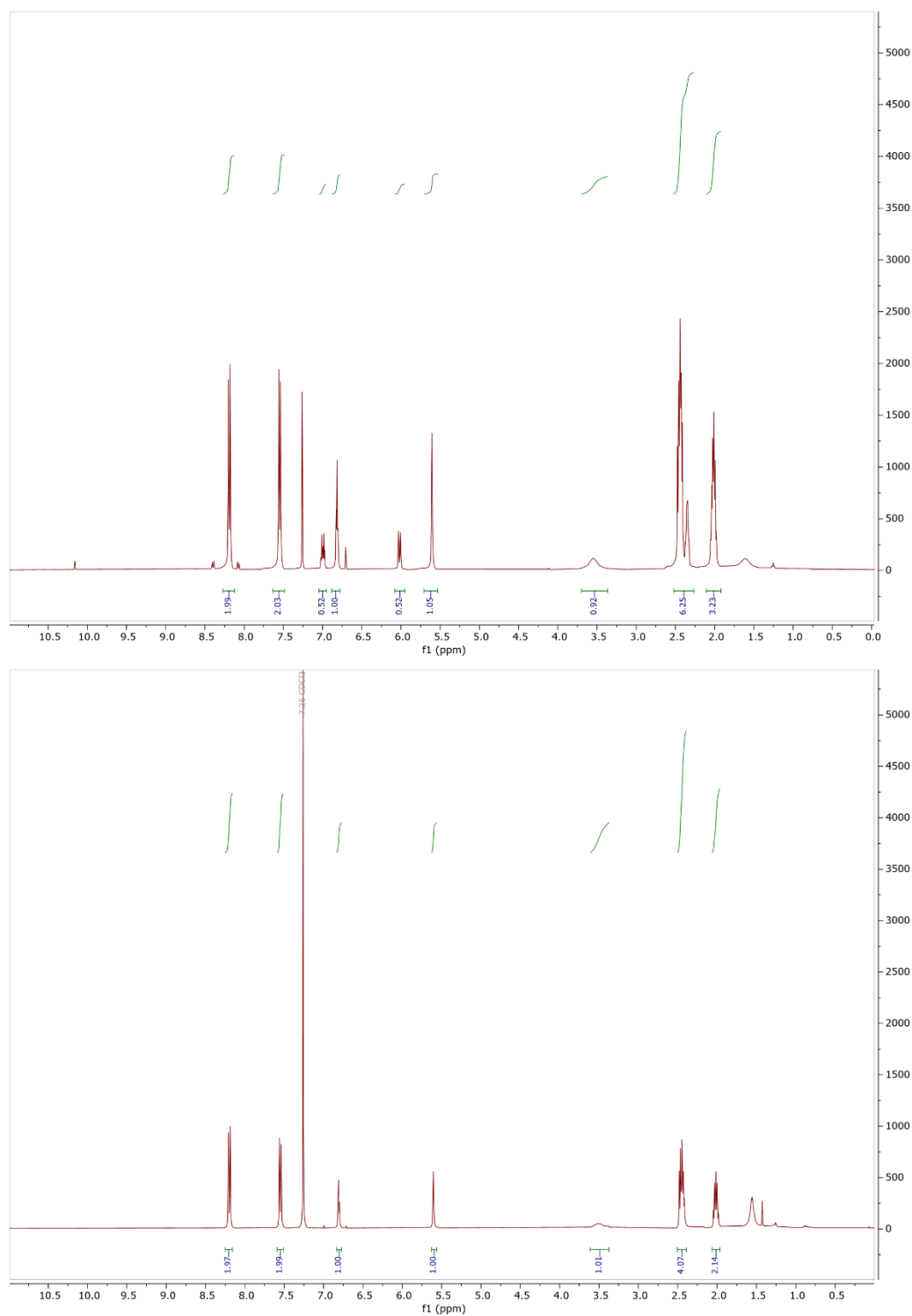


Figure 8: NMR traces of crude reaction and purified **3 produced from a preparative-scale biotransformation.** ¹H NMR traces (400 MHz; CDCl₃) showing; Top: crude product extracted from the preparative-scale biotransformation of BH_{MeHis} 1.8 (10 μM) using **1** (50 mM) and **2** (10 mM) in PBS pH 7.0 with 20% (v/v) DMSO as cosolvent for 13 h at 30 °C. Bottom: Isolated MBH product **3** following purification by flash chromatography.

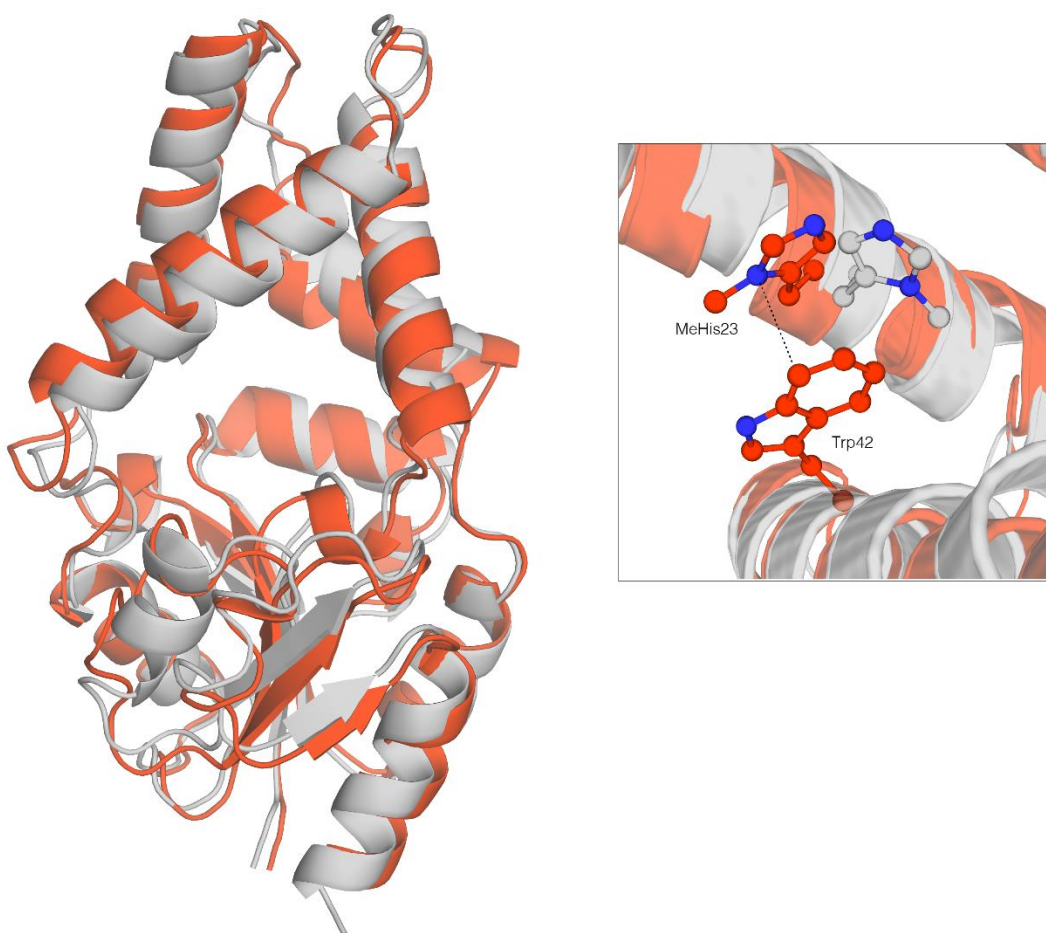


Figure 9: Crystal structures of BH_{MeHis}1.0 and BH_{MeHis}1.8. A cartoon presentation (left) of the superimposed coordinates of BH_{MeHis}1.0 (grey) and BH_{MeHis}1.8 (red). Mutations installed during evolution cause minimal changes to the overall protein fold, with a secondary structure r.m.s.d of 0.47 Å. The MeHis23 catalytic nucleophile is shown as sticks in both structures. A zoom of the active site (right) shows a ~120° rotation in the imidazole ring has occurred during evolution.

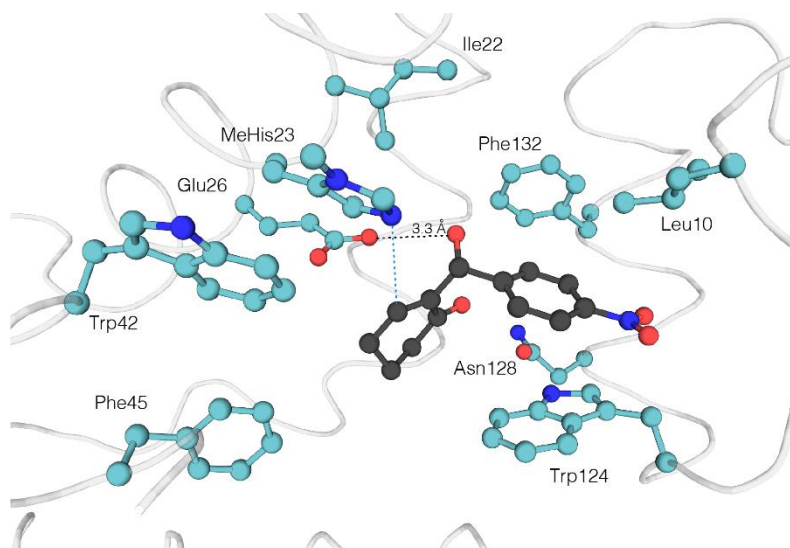


Figure 10: Docking of MBH product **3 into the crystal structure of BH_{MeHis}**1.8**.** The product **3** (shown as atom-coloured sticks, carbons black) was docked into the crystal structure of BH_{MeHis}**1.8** using MolsoftICM64-Pro (version 3.9-2d). To ensure a productive pose for catalysis, a distance restraint of 4 Å between the MeHis and the position of nucleophilic attack was imposed on the calculation (shown as a blue dashed line). Glu26 is within hydrogen-bonding distance of O1 of **3** (black dashed line).

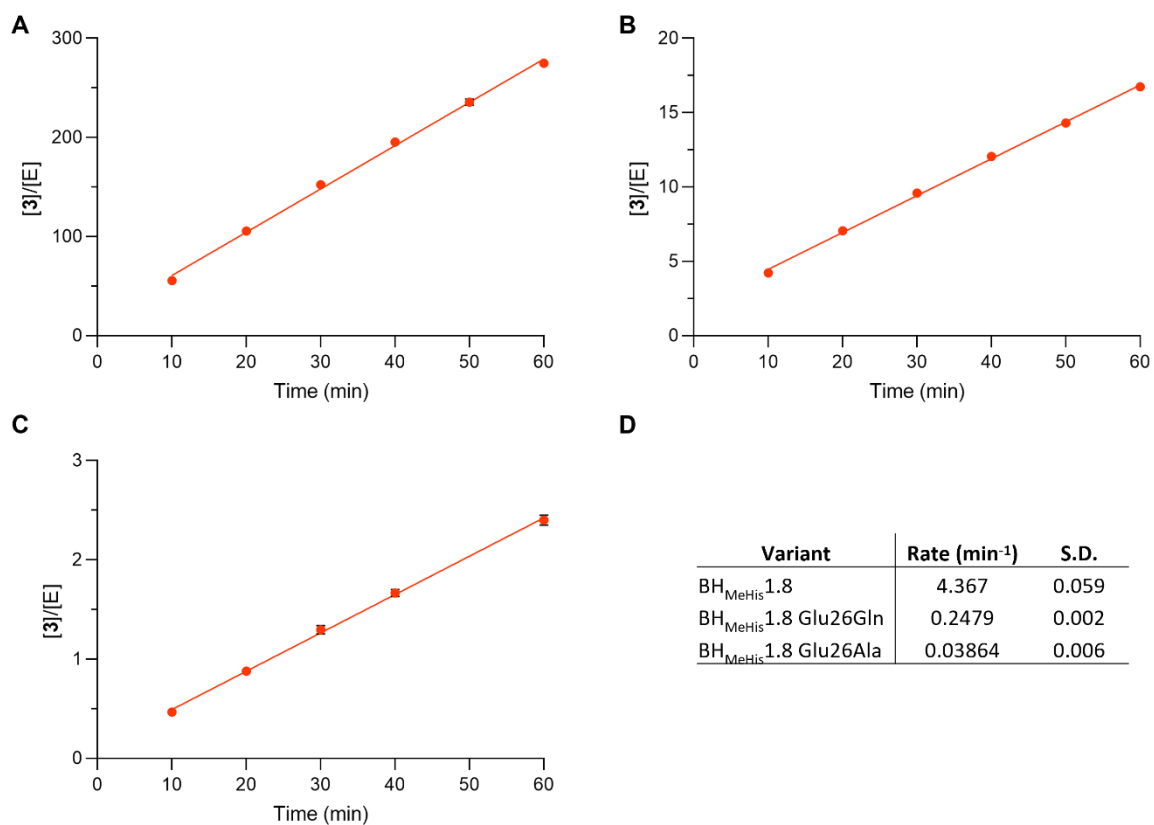


Figure 11: Changes in reaction rate upon mutation of Glu26 in BH_{MeHis}1.8 to either Gln or Ala. Reactions were performed using **1** (25 mM), **2** (2 mM) in PBS pH 7.0 with 3% (v/v) MeCN as cosolvent. **A)** BH_{MeHis}1.8, **B)** BH_{MeHis}1.8 Glu26Gln and **C)** BH_{MeHis}1.8 Glu26Ala. **D)** Table of rates determined from linear plots with standard deviation of triplicate measurements stated.

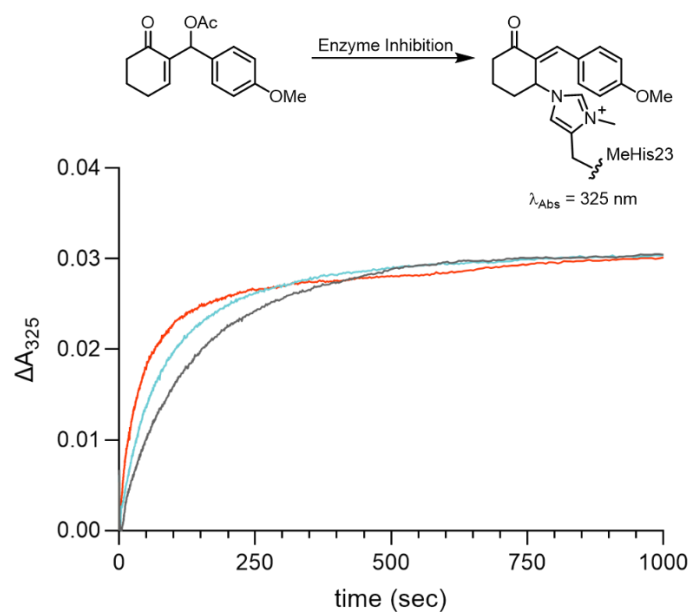


Figure 12. Time course of the inhibition of MBH1.8 and its variants. Stopped flow analysis of mechanistic inhibitor binding, through absorbance measurements at 325 nm to BH_{MeHis}1.8 (red), BH_{MeHis}1.8 Glu26Ala (blue) and BH_{MeHis}1.8 MeHis23His (grey). Reactions were performed using (4-methoxyphenyl)(6-oxocyclohex-1-en-1-yl)methyl acetate inhibitor (25 μ M) in PBS pH 7.0 with 3% (v/v) acetonitrile as cosolvent and 10 μ M enzyme at room temperature.

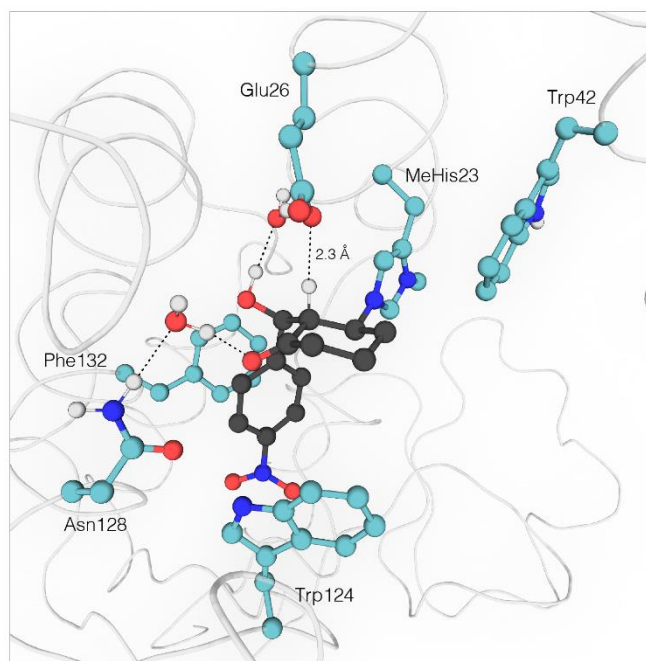


Figure 13: Representative MD snapshot of BH_{MeHis}1.8:Int2H complex where the proton has been transferred from Glu(H)26 to Int2 (model B) from a 500 ns simulation. Int2 (black) and key amino acid residues (blue) are shown in ball and stick representation with hydrogen bonds shown as black dashed lines.

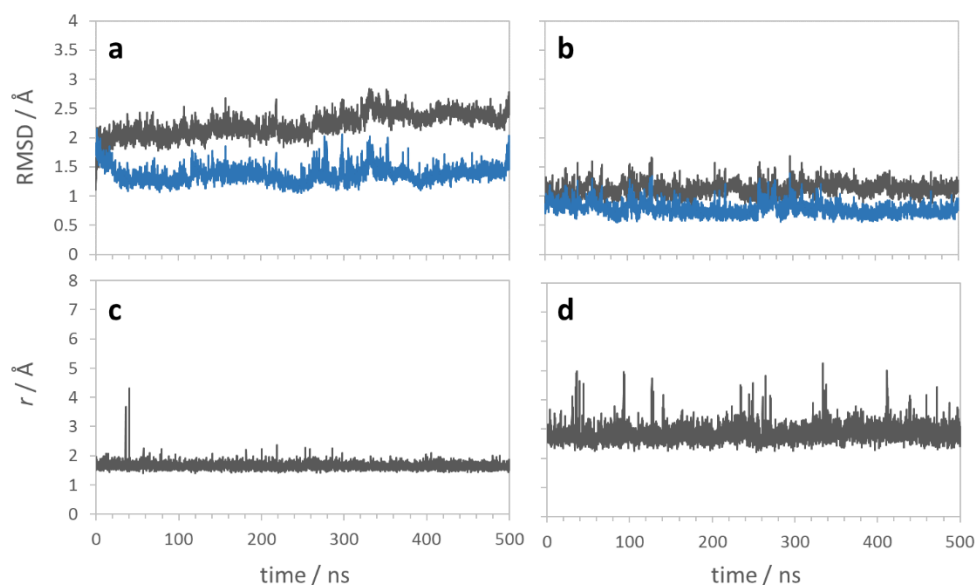


Figure 14. Structural parameters for MD simulation of BH_{MeHis}1.8:Int2 complex with a protonated glutamic acid (Glu(H)26) (model A). (a) protein heavy-atom RMSD relative to the first (grey) and average (blue) structure, (b) active site RMSD relative to the first (grey) and average (blue) structure. The active site is defined as residues with at least one atom within 5 Å of the MeHis23_Int2 adduct in the starting structure (residues number 10, 11, 14, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 42, 45, 49, 68, 91, 92, 94, 122, 124, 128, 132). (c) O-H distance between the putative proton donor Glu(H)26 and the C3-alkoxide of Int2. (d) O-H distance between the C2 proton and the GluH26 carbonyl oxygen.

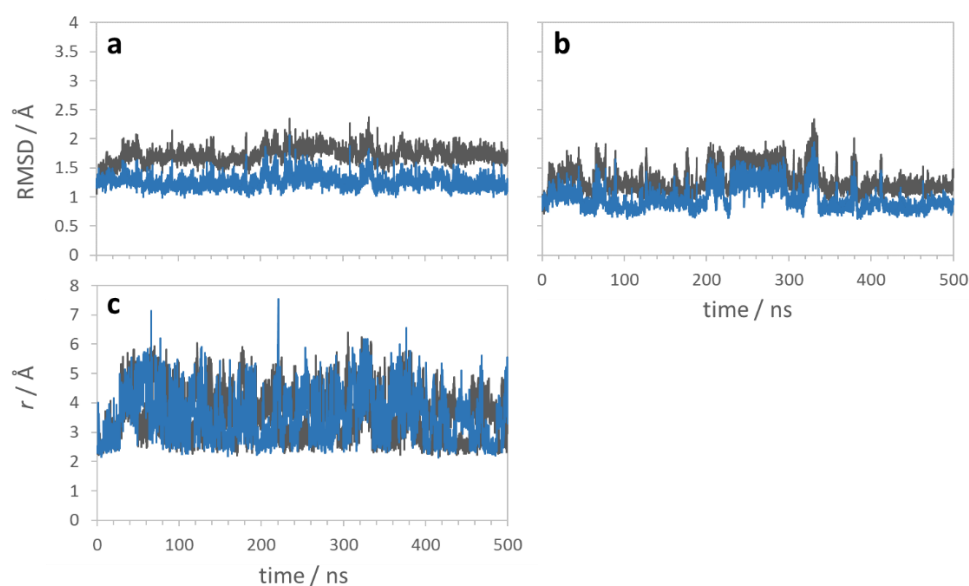


Figure 15. Structural parameters for MD simulation of BH_{MeHis}1.8:Int2H complex where the proton has been transferred from Glu(H)26 to Int2 (model B). (a) protein heavy-atom RMSD relative to the first (grey) and average (blue) structure, (b) active site RMSD relative to the first (grey) and average (blue) structure. The active site is defined by the same residues as for model A (Supplementary Figure 14). (c) O-H distances between the C2-proton and each of the two oxygen atoms of Glu26 (one atom in blue and one in grey).

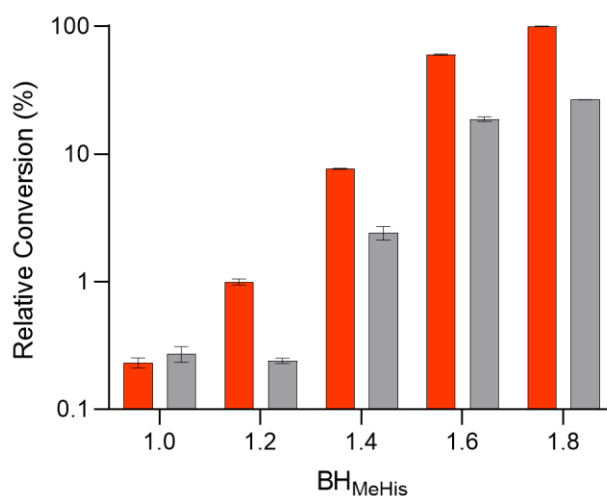


Figure 16: Changes in activity along the evolutionary trajectory upon mutation of MeHis23 nucleophile to histidine. Relative conversions of variants along the evolutionary trajectory of BH_{MeHis}1.8 with either MeHis (red) or His (grey) as the catalytic nucleophile at position 23. Biotransformations were performed using **1** (15 mM), **2** (1.5 mM) and enzyme (1.5 μ M) in PBS pH 6.0 with 3% (v/v) MeCN as cosolvent and analysed following 3 h incubation at 30 °C. Error bars represent the standard deviation of measurements made in triplicate. To eliminate errors arising from determination of low conversions, variants BH_{MeHis}1.0, BH_{MeHis}1.0 MeHis23His and BH_{MeHis}1.2 MeHis23His were monitored over a longer timeframe and conversions were extrapolated using linear regression.

1. Supplementary Tables 1-9

Table 1: Directed evolution of BH_{MeHis}1.8. Strategy employed for each round of evolution using either random mutagenesis or site-saturation mutagenesis, stating mutations introduced in each round. Starting template (BH_{MeHis}1.0) originated from our previous MBH evolution¹ where His23 has been mutated to MeHis.

Round	Description	Clones screened	Beneficial mutations	Best variant ^[1]
1	Saturation mutagenesis of positions mutated during BH32.14 evolution – L10, A19, Y20, V22, L24, I26, M27, L42, Y45, E46, Y56, L64, E70, D125, P128, A129, F132, F154, Y177, D180	1760	I26E, I26T, M27S, L42S, Y45R, L64A, P128F	BH_{MeHis}1.1 = <i>BH_{MeHis}1.0</i> ^[2] + I26E_L64A
2	Combinatorial active site saturation testing (CASTing) of two 'hotspots' L42 and Y45 simultaneously randomised	1408	L42E/Y45R, L42V/Y45V, L42W/Y45F, L42Y/Y45F	BH_{MeHis}1.2 = <i>BH_{MeHis}1.1</i> + L42W_Y45F
3	Saturation mutagenesis of active site positions and flexible loop regions – S9, I14, A19, Y20, V22, L24, M27, A49, F53, L87, W88, S91, L92, A95, N123, D125, P128, A131, F132, K174	1760	L24V, W88K, W88Q, S91G	BH_{MeHis}1.3 = <i>BH_{MeHis}1.2</i> + L24V_W88Q_S91G
4	Random mutagenesis of the entire gene	1936	R124W/E173G/G32A, T146S/E51D, V71I/Y177F, V71A/I215N/D66G, K74N/F161L, Q96R/K139R/D185G, E46D/K202N/K184E, E46D/L47Q, F161I/K202I/S217T	
5	Saturation mutagenesis of positions identified from random mutagenesis – G32, E46, L48, E51, D66, V71, K74, E85, Q96, R124, K139, T146, F161, E173, Y177, V178, K184, D185, K202, I215, S217, I223	1936	Q96R, R124W, F161L, Y177V	BH_{MeHis}1.4 = <i>BH_{MeHis}1.3</i> + Q96R_R124W_F161L
6	Saturation mutagenesis of 'hotspots' – V22, M27, E46, F53, Y56, D66, E85, L92, A95, D125 P128, F132, F154, E173, K174, Y177, I215 and active site positions – Y34, K39, L41, K110, Y116, L122, K155, A175, V178, E207, A212	1936	E46D, Y56G, D66R, E85G, L92V, P128N, P128W, F154A	BH_{MeHis}1.5 = <i>BH_{MeHis}1.4</i> + E46D_E85G_L92V_P128N_F154A
7	Saturation mutagenesis of active site positions and flexible loops – S9, L10, V22, Y34, K39, L41, W42, A49, F53, Y56, D66, E85, G91, A95, L122, D125, E127, N128, F154, K155, Y177, I215	1936	V22I, V22L, A49F, I215R	BH_{MeHis}1.6 = <i>BH_{MeHis}1.5</i> + V22I_A49F_I215R
8	Random mutagenesis of entire gene	1936	R26H/L165M, E100G, Y34H/S189T, L122M, S15T/K39N/E100G, D43N/E100G/S189P, R62H/D227A/N191I/ G193D, E100G/R220H, D66Y, S15T/E173V, K115E, V176A	
9	Saturation mutagenesis of positions identified from random mutagenesis – S15, E26, M27, Y34, K39, D43, R62, D66, E100, L112, K115, L122, N123, D125, L165, E173, V176, S189, N191, G193, R220, D227	1936	Y34H, Y34S, K39R, D66F, D66Y, E100G	BH_{MeHis}1.7 = <i>BH_{MeHis}1.6</i> + K39R_D66F_E100G
10	Saturation mutagenesis of active site positions and flexible loop regions – A20, R50, E51, Y56, L68, L87, M94, A95, R97, Y98, G99, L101, Y102, T126, A129, F132, T146, K155, A175, Y177, E207, A212	1936	R50K, G99P, T146V, K155M, K155Y	BH_{MeHis}1.8 = <i>BH_{MeHis}1.7</i> + R50K_K155Y

^[1] The gene sequences used as the template each round of evolution shown in italics

^[2] BH_{MeHis}1.0 = BH32.8 with His23MeHis mutation

Table 2: Enantiomeric excess of BH_{MeHis}1.0, BH_{MeHis}1.8 and selected variants. Reactions were performed using **1** (15 mM), **2** (1.5 mM), PBS pH 6.0 with 20% (v/v) DMSO as cosolvent and analysed by UPLC following 23 h incubation at 30 °C. All reactions ran with 10 µM enzyme apart from BH_{MeHis}1.0 (60 µM).

^a Preparative-scale biotransformation. Performed using enzyme (10 µM), **1** (50 mM), **2** (10 mM), PBS pH 7.0 with 20% (v/v) DMSO as cosolvent.

Variant	e.e.(%)
BH _{MeHis} 1.0	55
BH _{MeHis} 1.8	90
BH _{MeHis} 1.8 MeHis23His	89
BH _{MeHis} 1.8 Glu26Gln	97
BH _{MeHis} 1.8 ^a	91

Table 3: Kinetic characterization of BH_{MeHis}1.0, BH_{MeHis}1.8 and BH_{MeHis}1.8 MeHis23His. Kinetic constants derived from global fitting of the combined V_0 vs [1] and V_0 vs [2] steady state kinetic data (Supplementary Figure 5) using a random order binding model. Saturating conditions for either **1** or **2** were not achieved for BH_{MeHis}1.0. K_M values quoted are the *apparent* Michaelis constants. N.D. = not determined.

Variant	k_{cat} (min ⁻¹)	$K_M(1)$ (μM)	$K_M(2)$ (μM)
BH _{MeHis} 1.0	N.D.	N.D.	N.D.
BH _{MeHis} 1.8	4.50 ± 0.19	12020 ± 1034	323 ± 24
BH _{MeHis} 1.8 MeHis23His	1.13 ± 0.05	12190 ± 1079	293 ± 22

Table 4: Effect of cosolvent on BH_{MeHis}1.8 activity. Reaction conversions of BH_{MeHis}1.8 with varying cosolvent loadings from 3% to 50% (v/v) of either MeCN or DMSO. Reactions performed using **1** (15 mM), **2** (1.5 mM) in PBS pH 7.0 with 3 μ M BH_{MeHis}1.8 and 2 h incubation at 30 °C. Standard deviation of measurements made in triplicate. N.D. = not detectable.

Cosolvent	Conversion (%)	S.D.
3% MeCN	40.19	0.36
5% MeCN	38.15	0.75
10% MeCN	29.94	0.78
15% MeCN	15.81	0.23
20% MeCN	5.74	0.34
30% MeCN	0.27	0.04
40% MeCN	N.D.	N.D.
50% MeCN	N.D.	N.D.
3% DMSO	45.41	0.06
5% DMSO	46.52	0.16
10% DMSO	50.04	0.13
15% DMSO	50.24	0.96
20% DMSO	50.04	0.62
30% DMSO	41.08	1.37
40% DMSO	12.90	0.44
50% DMSO	N.D.	N.D.

Table 5: Conversions of MBH reactions catalysed by BH_{MeHis}1.0, BH_{MeHis}1.8 and selected variants. MBH reaction conversions of BH_{MeHis}1.0, BH_{MeHis}1.8 and selected variants after 2 h using **1** (15 mM), **2** (2 mM) in PBS pH 7.0 with 3% MeCN as cosolvent.

^a Conversion for preparative-scale biotransformation performed using **1** (50 mM), **2** (10 mM) in PBS pH 7.0 with 20% (v/v) DMSO as cosolvent.

Variant	Catalyst loading (mol%)	Time (h)	Conversion (%)
BH _{MeHis} 1.0	0.1	2	<0.5
BH _{MeHis} 1.8	0.1	2	26
BH _{MeHis} 1.8 MeHis23Ala	0.1	2	<0.5
BH _{MeHis} 1.8 MeHis23His	0.1	2	6.6
BH _{MeHis} 1.8 Glu26Gln	0.1	2	1.3
BH _{MeHis} 1.8 Glu26Ala	0.1	2	<0.5
BH _{MeHis} 1.8 ^a	0.1	13	96

Table 6: Data collection and refinement statistics.

^aValues in parentheses are for highest resolution shell. ^bR-free was calculated using ~5% of the data separate from the rest

	BH_{MeHis} 1.0	BH_{MeHis} 1.8
PDB ascension number	8BP1	8BP0
Wavelength (Å)	0.9762	0.9763
Resolution range	61.5 - 1.72 (1.782 - 1.72) ^a	38.15 - 2.621 (2.714 - 2.621) ^a
Space group	P 31 2 1	P 21 21 21
Unit cell dimensions a, b, c, (Å) α, β, γ (°)	71.01, 71.01, 120.3 90, 90, 120	35.46, 42.43, 152.5 90, 90, 90
Total reflections	765674 (70921)	193638 (18122)
Unique reflections	37997 (3733)	7422 (715)
Multiplicity	20.2 (19.0)	26.1 (25.3)
Completeness (%)	99.94 (99.71)	99.81 (99.58)
Mean I/sigma(I)	20.45 (1.00)	9.87 (2.25)
Wilson B-factor	30.52	33.11
R-merge	0.08128 (1.691)	0.2535 (0.8861)
R-meas	0.08336 (1.736)	0.2585 (0.9041)
R-pim	0.01838 (0.3912)	0.04998 (0.1782)
CC _{1/2}	1 (0.709)	0.996 (0.964)
CC*	1 (0.911)	0.999 (0.991)
Reflections used in refinement	37987 (3733)	7411 (713)
Reflections used for R-free	1865 (170)	400 (38)
R-work	0.1811 (0.2491)	0.1902 (0.2495)
R-free ^b	0.2021 (0.2687)	0.2419 (0.3119)
CC (work)	0.960 (0.826)	0.958 (0.942)
CC (free)	0.959 (0.805)	0.912 (0.706)
Number of non-hydrogen atoms	2170	1962
macromolecules	1948	1867
ligands	38	44
solvent	200	77
Protein residues	231	230
RMS(bonds)	0.004	0.002
RMS (angles)	0.74	0.45
Ramachandran favoured (%)	98.67	95.11
Ramachandran allowed (%)	1.33	4.89
Ramachandran outliers (%)	0	0
Rotamer outliers (%)	0	3.08
Clashscore	3.24	2.1
Average B-factor	38.59	36.87

Table 7: Kinetic isotope effect (KIE) and solvent kinetic isotope (SKIE) effects for BH_{MeHis}1.8 and selected variants. Reactions were performed with the relevant enzyme (1 μ M BH_{MeHis}1.8, 3 μ M BH_{MeHis}1.8 MeHis23His and 10 μ M BH_{MeHis}1.8 Glu26Gln) using **1** or **S2** (25 mM), **2** (2 mM) in both deuterated and non-deuterated PBS buffer at pH 7.0 with 3% (v/v) MeCN as cosolvent. KIE and SKIE values calculated from reactions performed in triplicate.

Variant	KIE (H ₂ O)	KIE (D ₂ O)	SKIE (1)	SKIE (S2)
BH _{MeHis} 1.8	1.7	1.7	0.9	0.9
BH _{MeHis} 1.8 Glu26Gln	4.0	4.2	0.6	0.6
BH _{MeHis} 1.8 MeHis23His	1.9	1.5	0.6	0.5

Table 8: Experimental and calculated masses of apo enzymes.

Variant	Expected Mass	Observed Mass
BH _{MeHis} 1.0 (BH32.8 His23MeHis)	27593.73	27593.4
BH _{MeHis} 1.1	27567.60	27567.4
BH _{MeHis} 1.2	27624.66	27624.4
BH _{MeHis} 1.3	27522.52	27522.2
BH _{MeHis} 1.4	27546.59	27546.2
BH _{MeHis} 1.5	27387.36	27387.0
BH _{MeHis} 1.6	27520.51	27520.2
BH _{MeHis} 1.7	27508.55	27508.4
BH _{MeHis} 1.8	27515.54	27515.5
BH _{MeHis} 1.8 MeHis23His	27501.51	27501.2
BH _{MeHis} 1.8 MeHis23Ala	27435.44	27435.3
BH _{MeHis} 1.8 Glu26Ala	27457.50	27457.3
BH _{MeHis} 1.8 Glu26Gln	27514.52	27514.2
BH _{MeHis} 1.8 (strep tag)	27732.84	27732.4
BH _{MeHis} 1.8 MeHis23His (strep tag)	27718.81	27718.3
BH32.8 (BH _{MeHis} 1.0 MeHis23His)	27579.70	27579.2
BH _{MeHis} 1.1 MeHis23His	27553.57	27553.4
BH _{MeHis} 1.2 MeHis23His	27610.63	27610.4
BH _{MeHis} 1.3 MeHis23His	27508.49	27508.2
BH _{MeHis} 1.4 MeHis23His	27532.56	27532.2
BH _{MeHis} 1.5 MeHis23His	27373.33	27373.2
BH _{MeHis} 1.6 MeHis23His	27506.48	27506.4
BH _{MeHis} 1.7 MeHis23His	27494.52	27494.4
BH32 His23MeHis	27648.58	27648.6
BH32.6 His23MeHis	27525.55	27545.4
BH32.9 His23MeHis	27716.77	27716.6
BH32.12 His23MeHis	27529.59	27529.4

Table 9: Cloning primer sequencing

Name	Primer Sequence
BH32-BH32.6_His23MeHis_F	GGCGCTGCTAAATCC TAG CTGAAAATTATGGAGGAAGTG
BH32-BH32.6_His23_R	GGATTTAGCAGCGCC
BH32.8_His23MeHis_F	GGCGCTTATAAAGTG TAG CTGAAAATTATGGAGGAAGTG
BH32.8_His23_R	CACTTTATAAGCGCCTTC
BH32.9-BH32.14_His23MeHis_F	GGCGCTTATAAAGTG TAG TTTAAAATTATGGAGG
BH32.9-BH32.14_His23_R	CACTTTATAAGTGCCTTCAA
BH _{MeHis} 1.8_MeHis23Ala_F	GGCGCTTATAAAATT GCG GTGAAAGAGATGGAGGAAG
BH _{MeHis} 1.8_MeHis23His_F	GGCGCTTATAAAATT CAC GTGAAAGAGATGGAGGAAG
BH _{MeHis} 1.8_MeHis23_R	AATTTTATAAGCGCCTTCAACG
BH _{MeHus} 1.8_Glu26Ala_F	AAAATTTAGGTGAAA GCG ATGGAGGAAGTGCTGG
BH _{MeHus} 1.8_Glu26Gln_F	AAAATTTAGGTGAAA CAG ATGGAGGAAGTGCTGG
BH _{MeHus} 1.8_Glu26_R	TTTCACCTAAATTTTATAAGCGCCTTC
BH _{MeHis} 1.1-1.2_MeHis23His_F	GGCGCTTATAAAGTG CAC CTGAAAGAGATGGAGGAAG
BH _{MeHis} 1.1-1.2_MeHis23_R	CACTTTATAAGCGCCTTCA
BH _{MeHis} 1.3-1.5_MeHis23His_F	GGCGCTTATAAAGTG CAC GTGAAAGAGATGGAGGAAG
BH _{MeHis} 1.3-1.5_MeHis_R	CACTTTATAAGCGCCTTCA
BH _{MeHis} 1.6-1.7_MeHis23His_F	GGCGCTTATAAAATT CAC GTGAAAGAGATGGAGGAAG
BH _{MeHis} 1.6-1.7_MeHis23_R	AATTTTATAAGCGCCTTCAACG

Table 10: Primer sequences used to generate DNA libraries.

Flanking Primers	
NDE_F	catgcatCATATGATTCGTGCGGTA
XHO_R	atgcatgcCTCGAGAGAGCCCTG
Round 1	
L10NNK_F	GTATTCTTTGATAGC NNK GGTACTCTGATTAGCGT
L10_R	GCTATCAAAGAATACCGC
A19NNK_F	ATTAGCGTTGAAGGC NNK TATAAAGTGTAGCTGAAAATTATGG
A19_R	GCCTTCAACGCTAATC
Y20NNK_F	AGCGTTGAAGGCGCT NNK AAAAGTGTAGCTGAAAATTATGG
Y20_R	AGCGCCTTCAACG
V22NNK_F	GAAGGCGCTTATAAA NNK TAGCTGAAAATTATGGAGGA
V22_R	TTTATAAGCGCCTTCAAC
L24NNK_F	GCTTATAAAGTGTAG NNK AAAATTATGGAGGAAGTGC
L24_R	CTACACTTTATAAGCGCC
I26NNK_F	AAAGTGTAGCTGAAA NNK ATGGAGGAAGTGCTG
I26_R	TTTCAGCTACACTTTATAAGC
M27NNK_F	GTGTAGCTGAAAATT NNK GAGGAAGTGCTGGGT
M27_R	AATTTTCAGCTACACTTTATAAG
L42NNK_F	AACCCGAAAACCCTG NNK GACGAATACGAGAACTG
L42_R	CAGGGTTTTTCGGGTT
Y45NNK_F	ACCCTGCTGGACGAA NNK GAGAACTGGCTCGC
Y45_R	TTCGTCCAGCAGG
E46NNK_F	CTGCTGGACGAATAC NNK AAACTGGCTCGCG
E46_R	GTATTCGTCCAGCAGG
Y56NNK_F	GAAGCGTTCTTAAC NNK GCGGGCAAACCG
Y56_R	GTTAGAGAACGCTTCGC
L64NNK_F	AAACCGTATCGTCCG NNK CGTGATATCCTGGAAGA
L64_R	CGGACGATACGGTTT
E70NNK_F	CGTGATATCCTGGAA NNK GTAATGCGTAACTGGC
E70_R	TTCCAGGATATCACGC

D125NNK_F	GTGATCCTGAATAGG NNK ACCGAGCCGGC
D125_R	CCTATTCAGGATCACGC
P128NNK_F	AATAGGGATACCGAG NNK GCCACGGCATTCC
P128_R	CTCGGTATCCCTATTAG
A129NNK_F	AGGGATACCGAGCCG NNK ACGGCATTCTGGA
A129_R	CGGCTCGGTATCC
F132NNK_F	GAGCCGGCCACGGCA NNK CTGGACGCACTGG
F132_R	TGCCGTGGCCG
F154NNK_F	GAAGAAGCTGGTTTC NNK AAACCGCACCCAC
F154_R	GAAACCAGCTTCTTCAG
Y177NNK_F	GGCGAGAAAGCAGTG NNK GTTGGTGACAACCCG
Y177_R	CACTGCTTTCTCGCC
D180NNK_F	GCAGTGACGTTGGT NNK AACCCGGTCAAAGAC
D180_R	ACCAACGTACACTGC
Round 2	
L42x_Y45x_1	AACCCGAAAACCCTG NDT GACGAA NDT GAGAAACTGGCTCGC
L42x_Y45x_2	AACCCGAAAACCCTG VHG GACGAA VHG GAGAAACTGGCTCGC
L42x_Y45x_3	AACCCGAAAACCCTG NDT GACGAA VHG GAGAAACTGGCTCGC
L42x_Y45x_4	AACCCGAAAACCCTG VHG GACGAA NDT GAGAAACTGGCTCGC
L42x_Y45x_5	AACCCGAAAACCCTG NDT GACGAA TGG GAGAAACTGGCTCGC
L42x_Y45x_6	AACCCGAAAACCCTG TGG GACGAA NDT GAGAAACTGGCTCGC
L42x_Y45x_7	AACCCGAAAACCCTG VHG GACGAA TGG GAGAAACTGGCTCGC
L42x_Y45x_8	AACCCGAAAACCCTG TGG GACGAA VHG GAGAAACTGGCTCGC
L42x_Y45x_9	AACCCGAAAACCCTG TGG GACGAA TGG GAGAAACTGGCTCGC
L42x_Y45x_R	CAGGGTTTTTCGGTT
Round 3	
NDE_S9NNK_F	gagatatacatATGATTCGTGCGGTATTCTTTGAT NNK CTGGGTACTCTGATTAGC
I14NNK_F	AGCCTGGGTACTCTG NNK AGCGTTGAAGGCGCT
I14_R	CAGAGTACCCAGGCT
A19NNK_F	ATTAGCGTTGAAGGC NNK TATAAAGTGTAGCTGAAAGAG
A19_R	GCCTTCAACGCTAATC
Y20NNK_F	AGCGTTGAAGGCGCT NNK AAAAGTGTAGCTGAAAGAGA

Y20_R	AGCGCCTTCAACG
V22NNK_F	GAAGGCGCTTATAAA NNK TAGCTGAAAGAGATGGAG
V22_R	TTTATAAGCGCCTTCAAC
L24NNK_F	GCTTATAAAGTGTAG NNK AAAGAGATGGAGGAAGTG
L24_R	CTACACTTTATAAGCGCC
M27NNK_F	GTGTAGCTGAAAGAG NNK GAGGAAGTGCTGGG
M27_R	CTCTTTCAGCTACACTTT
A49NNK_F	GAATACGAGAAACTG NNK CGCGAAGCGTTCT
A49_R	CAGTTTCTCGTATTCGTC
F53NNK_F	CTGGCTCGCGAAGCG NNK TCTAACTATGCGGGC
F53_R	CGCTTCGCGAGCC
L87NNK_F	AAATACCCTGAAAAC NNK TGGGAAATCTCCCTG
L87_R	GTTTTCAGGGTATTTGAAAC
W88NNK_F	TACCCTGAAAAC NNK TGGGAAATCTCCCTGCGT
W88_R	CAAGTTTTTCAGGGTATTTGA
S91NNK_F	AACTTGTGGGAAATC NNK CTGCGTATGGCGC
S91_R	GATTTCCACAAGTTTTCA
L92NNK_F	TTGTGGGAAATCTCC NNK CGTATGGCGCAACGC
L92_R	GGAGATTTCCACAAGT
A95NNK_F	ATCTCCCTGCGTATG NNK CAACGCTACGGCGAG
A95_R	CATACGCAGGGAGAT
N123NNK_F	GTTGGCGTGATCCTG NNK AGGGATACCGAGCC
N123_R	CAGGATCACGCCAAC
D125NNK_F	GTGATCCTGAATAGG NNK ACCGAGCCGGC
D125_R	CCTATTCAGGATCACGC
A131NNK_F	ACCGAGCCGGCCACG NNK TTCTGGACGCAC
A131_R	CGTGGCCGGCTC
F132NNK_F	GAGCCGGCCACGGCA NNK CTGGACGCACTGG
F132_R	TGCCGTGGCCG
K174NNK_F	GGCGTTAAAGGCGAG NNK GCAGTGACGTTGGT
K174_R	CTCGCCTTTAACGCC
Round 5	

G32NNK_F	ATGGAGGAAGTGCTG NNK GACTATCCGCTGAACC
G32_R	CAGCACTTCCTCCAT
E46NNK_F	CTGTGGGACGAATTT NNK AAACTGGCTCGCGAA
E46_R	AAATTCGTCCACAGG
L48NNK_F	GACGAATTTGAGAAA NNK GCTCGCGAAGCG
L48_R	TTTCTCAAATTCGTCCCA
E51NNK_F	GAGAAACTGGCTCGC NNK GCGTTCTCTAACTATGCG
E51_R	GCGAGCCAGTTTCTC
D66NNK_F	TATCGTCCGGCGCGT NNK ATCCTGGAAGAAGTAATGC
D66_R	ACGCGCCGGAC
V71NNK_F	GATATCCTGGAAGAA NNK ATGCGTAAACTGGCG
V71_R	TTCTCCAGGATATCACG
K74NNK_F	GAAGAAGTAATGCGT NNK CTGGCGGAAAAGTACG
K74_R	ACGCATTACTTCTTCCA
E85NNK_F	GGTTTCAAATACCCT NNK AACTGCAGGAAATCGG
E85_R	AGGGTATTTGAAACCGT
Q96NNK_F	GGCCTGCGTATGGCG NNK CGCTACGGCGAGC
Q96_R	CGCCATACGCAGG
R124NNK_F	GGCGTGATCCTGAAT NNK GATACCGAGCCGGC
R124_R	ATTCAGGATCACGCC
K139NNK_F	GACGCACTGGGCATC NNK GACCTGTTCGATTCCA
K139_R	GATGCCCAGTGCGT
T146NNK_F	CTGTTTCGATTCCATC NNK ACGTCTGAAGAAGCT
T146_R	GATGGAATCGAACAGGT
F161NNK_F	CCGCACCCACGCATC NNK GAACTGGCTCTGAAGA
F161_R	GATGCGTGGGTGC
E173NNK_F	GCCGGCGTTAAAGGC NNK AAAGCAGTGTACGTTGG
E173_R	GCCTTTAACGCCGG
Y177NNK_F	GGCGAGAAAGCAGTG NNK GTTGGTGACAACCCG
Y177_R	CACTGCTTTCTCGCC
V178NNK_F	GAGAAAGCAGTGAC NNK GGTGACAACCCGGTC
V178_R	GTACACTGCTTTCTCGC

K184NNK_F	GGTGACAACCCGGTC NNK GACGCGGGTGGTT
K185_R	GACCGGGTTGTCACC
D185NNK_F	GACAACCCGGTCAAA NNK GCGGGTGGTTCTAAG
D185_R	TTTGACCGGGTTGTC
K202NNK_F	ATCCTGCTGGATCGT NNK GGTGAGAAACGTGAATTC
K202_R	ACGATCCAGCAGGAT
I215NNK_F	GATAAGGCGGACTTT NNK GTCTCCGACCTGC
I215_R	AAAGTCCGCCTTATCC
S217NNK_F	GCGGACTTTATCGTC NNK GACCTGCGCGAAGTT
S217_R	GACGATAAAGTCCGC
I223NNK_F	GACCTGCGCGAAGTT NNK AAGATTGTTGACGAACTGA
I223_R	AACTTCGCGCAGGTC
Round 6	
V22NNK_F	GAAGGCGCTTATAAA NNK TAGGTGAAAGAGATGGAGG
V22_R	TTTATAAGCGCCTTCAAC
M27NNK_F	GTGTAGGTGAAAGAG NNK GAGGAAGTGCTGGG
M27_R	CTCTTTCACCTACACTTTAT
Y34NNK_F	GAAGTGCTGGGTGAC NNK CCGCTGAACCCG
Y34_R	GTCACCCAGCACTTC
K39NNK_F	TATCCGCTGAACCCG NNK ACCCTGTGGGACG
K39_R	CGGGTTCAGCGGATA
L41NNK_F	CTGAACCCGAAAACC NNK TGGGACGAATTTGAGAA
L41_R	GGTTTTCGGGTTCAGC
E46NNK_F	CTGTGGGACGAATTT NNK AAACTGGCTCGCGAA
E46_R	AAATTCGTCCACAGG
F53NNK_F	CTGGCTCGCGAAGCG NNK TCTAACTATGCGGGCA
F53_R	CGCTTCGCGAGC
Y56NNK_F	GAAGCGTTCTCTAAC NNK GCGGGCAAACCGTAT
Y56_R	GTTAGAGAACGCTTCGC
D66NNK_F	TATCGTCCGGCGCGT NNK ATCCTGGAAGAAGTAATGC
D66_R	ACGCGCCGGAC
E85NNK_F	GGTTTCAAATACCCT NNK AACTTGCAGGAAATCGG

E85_R	AGGGTATTTGAAACCGT
L92NNK_F	TTGCAGGAAATCGGC NNK CGTATGGCGCGAC
L92_R	GCCGATTTCTGCAA
A95NNK_F	ATCGGCCTGCGTATG NNK CGACGCTACGGC
A95_R	CATACGCAGGCCGAT
K110NNK_F	GTGGTGGAAGTACTG NNK TCTCTGAAAGGTAAATATCACG
K110_R	CAGTACTTCCACCACTT
Y116NNK_F	TCTCTGAAAGGTAAA NNK CACGTTGGCGTGATC
Y116_R	TTTACCTTTCAGAGATTTCAGT
L122NNK_F	CACGTTGGCGTGATC NNK AATTGGGATACCGAGC
L122_R	GATCACGCCAACGTG
D125NNK_F	GTGATCCTGAATTGG NNK ACCGAGCCGGC
D125_R	CCAATTCAGGATCACGC
P128NNK_F	AATTGGGATACCGAG NNK GCCACGGCATTCTCG
P128_R	CTCGGTATCCCAATTCA
F132NNK_F	GAGCCGGCCACGGCA NNK CTGGACGCACTGGG
F132_R	TGCCGTGGCCG
F154NNK_F	GAAGAAGCTGGTTTC NNK AAACCGCACCCACG
F154_R	GAAACCAGCTTCTTCAG
K155NNK_F	GAAGCTGGTTTCTTT NNK CCGCACCCACGC
K155_R	AAAGAAACCAGCTTCTTCA
E173NNK_F	GCCGGCGTTAAAGGC NNK AAAGCAGTGTACGTTGG
E173_R	GCCTTTAACGCCGG
K174NNK_F	GGCGTTAAAGGCGAG NNK GCAGTGTACGTTGGT
K174_R	CTGCCTTTAACGCC
A175NNK_F	GTAAAGGCGAGAAA NNK GTGTACGTTGGTGACA
A175_R	TTTCTGCCTTTAACGC
Y177NNK_F	GGCGAGAAAGCAGTG NNK GTTGGTGACAACCCG
Y177_R	CACTGCTTCTCGCC
V178NNK_F	GAGAAAGCAGTGTAC NNK GGTGACAACCCGGTC
V178_R	GTACACTGCTTCTCGC
E207NNK_F	AAAGGTGAGAAACGT NNK TTCTGGGATAAGGCGG

E207_R	ACGTTTCTCACCTTTACG
I215NNK_F	GATAAGGCGGACTTTNNKGTCTCCGACCTGCG
I215_R	AAAGTCCGCCTTATCC
A212NNK_F	GAATTCTGGGATAAGNNKGACTTTATCGTCTCCGAC
A212_R	CTTATCCCAGAATTCACGT
Round 7	
NDE_S9NNK_F	gagatatacatATGATTCGTGCGGTATTCTTTGATNNKCTGGGTACTCTGATTAGC
NDE_L10NNK_F	gagatatacatATGATTCGTGCGGTATTCTTTGATAGCNNKGGTACTCTGATTAGCGT
V22NNK_F	GAAGGCGCTTATAAANNKTAGGTGAAAGAGATGGAG
V22_R	TTTATAAGCGCCTTCAAC
Y34NNK_F	GAAGTGCTGGGTGACNNKCCGCTGAACCCG
Y34_R	GTCACCCAGCACTTC
K39NNK_F	TATCCGCTGAACCCGNNKACCCTGTGGGACG
K39_R	CGGGTTCAGCGGATA
L41NNK_F	CTGAACCCGAAAACCNNKTGGGACGAATTTGATAAACT
L41_R	GGTTTTCGGGTTCAGC
W42NNK_F	AACCCGAAAACCCTGNNKGACGAATTTGATAAACTGGC
W42_R	CAGGGTTTTCGGGTT
A49NNK_F	GAATTTGATAAACTGNNKCGCGAAGCGTTCTCT
A49_R	CAGTTTATCAAATTCGTCCC
F53NNK_F	CTGGCTCGCGAAGCGNNKTCTAACTATGCGGGC
F53_R	CGCTTCGCGAGCC
Y56NNK_F	GAAGCGTTCTCTAACNNKGCGGGCAAACCGTAT
Y56_R	GTTAGAGAACGCTTCGC
D66NNK_F	TATCGTCCGGCGCGTNNKATCCTGGAAGAAGTAATGC
D66_R	ACGCGCCGGAC
E85NNK_F	GGTTTCAAATACCCTNNKAACTTGCAGGAAATCGG
E85_R	AGGGTATTTGAAACCGT
G91NNK_F	AACTTGCAGGAAATCNNKGTGCGTATGGCGC
G91_R	GATTTCTGCAAGTTTTAG
A95NNK_F	ATCGGCGTGCGTATGNNKCGACGCTACGGCGAG
A95_R	CATACGCACGCCGAT

L122NNK_F	CACGTTGGCGTGATC NNK AATTGGGATACCGAGAAT
L122_R	GATCACGCCAACGTG
D125NNK_F	GTGATCCTGAATAGG NNK ACCGAGAATGCCAC
D125_R	CCAATTCAGGATCACGC
E127NNK_F	CTGAATTGGGATAC NNK AATGCCACGGCATT
E127_R	GGTATCCCAATTCAGGAT
N128NNK_F	AATTGGGATACCGAG NNK GCCACGGCATTCTG
N128_R	CTCGGTATCCCAATTCA
F154NNK_F	GAAGAAGCTGGTTTC NNK AAACCGCACCCACG
F154_R	GAAACCAGCTTCTTCAG
K155NNK_F	GAAGCTGGTTTCTTT NNK CCGCACCCACGC
K155_R	AAAGAAACCAGCTTCTTCA
Y177NNK_F	GGCGAGAAAGCAGTG NNK GTTGGTGACAACCCG
Y177_R	CACTGCTTTCTCGCC
I215NNK_F	GATAAGGCGGACTTT NNK GTCTCCGACCTGCG
I215_R	AAAGTCCGCCTTATCC
Round 9	
NDE_S15NNK_F	gagatatatacatATGATTCGTGCGGTATTCTTTGATAGCCTGGGTACTCTGATT NNK GTTGAAGGCGC TTATAAAA
E26NNK_F	AAAATTTAGGTGAAA NNK ATGGAGGAAGTGCTG
E26_R	TTTACCTAAATTTTATAAGCGCC
M27NNK_F	ATTTAGGTGAAAGAG NNK GAGGAAGTGCTGGGT
M27_R	CTCTTTCACCTAAATTTTATAAGC
Y34NNK_F	GAAGTGCTGGGTGAC NNK CCGCTGAACCCGAAA
Y34_R	GTCACCCAGCACTTC
K39NNK_F	TATCCGCTGAACCCG NNK ACCCTGTGGGACGAA
K39_R	CGGGTTCAGCGGATA
D43NNK_F	CCGAAAACCCTGTGG NNK GAATTTGATAAACTGTTTCGCG
D43_R	CCACAGGGTTTTTCGG
R62NNK_F	GCGGGCAAACCGTAT NNK CCGGCGCGTGATATC
R62_R	ATACGGTTTGCCCGC
D66NNK_F	TATCGTCCGGCGCGT NNK ATCCTGGAAGAAGTAATGC

D66_R	ACGCGCCGGAC
E100NNK_F	GCGCGACGCTACGGC NNK CTGTACCCGGAAGTG
E100_R	GCCGTAGCGTCG
L112NNK_F	GAAGTACTGAAATCT NNK AAAAGGTAAATATCACGTTGGC
L112_R	AGATTTCACTACTTCCACC
K115NNK_F	AAATCTCTGAAAGGT NNK TATCACGTTGGCGTG
K115_R	ACCTTTCAGAGATTTTCAGT
L122NNK_F	CACGTTGGCGTGATC NNK AATTGGGATACCGAGAAT
L122_R	GATCACGCCAACGTG
N123NNK_F	GTTGGCGTGATCCTG NNK TGGGATACCGAGAATG
N123_R	CAGGATCACGCCAAC
D125NNK_F	GTGATCCTGAATTGG NNK ACCGAGAATGCCACG
D125_R	CCAATTCAGGATCACGC
L165NNK_F	ATCCTCGAACTGGCT NNK AAGAAAGCCGGCGTT
L165_R	AGCCAGTTCGAGGAT
E173NNK_F	GCCGGCGTTAAAGGC NNK AAAGCAGTGTACGTTGG
E173_R	GCCTTTAACGCCGG
V176NNK_F	AAAGGCGAGAAAGCA NNK TACGTTGGTGACAACC
V176_R	TGCTTTCTCGCCTTT
S189NNK_F	AAAGACGCGGGTGGT NNK AAGAACCTGGGTATGAC
S189_R	ACCACCCGCGTCT
N191NNK_F	GCGGGTGGTTCTAAG NNK CTGGGTATGACTAGCAT
N191_R	CTTAGAACCACCCGC
G193NNK_F	GGTCTAAGAACCTG NNK ATGACTAGCATCCTGCT
G193_R	CAGGTTCTTAGAACCACC
R220NNK_F	CGTGTCTCCGACCTG NNK GAAGTTATTAAGATTGTTGACGAA
R220_R	CAGGTCGGAGACACG
XHO_D227NNM_R	TGGTGGTGCTCGAGAGAGCCCTGACCGTTCAGTTC MNNA ACAATCTTAATAACTTCGCG
Round 10	
A20NNK_F	AGCGTTGAAGGCGCT NNK AAAAATTTAGGTGAAAGAGATGG
A20_R	AGCGCCTTCAACG

R50NNK_F	TTTGATAAACTGTTT NNK GAAGCGTTCTCTAACTATG
R50_R	AAACAGTTTATCAAATTCGTCC
E51NNK_F	GATAAACTGTTTCGC NNK GCGTTCTCTAACTATGCG
E51_R	GCGAAACAGTTTATCAAATTCG
Y56NNK_F	GAAGCGTTCTCTAAC NNK GCGGGCAAACCG
Y56_R	GTTAGAGAACGCTTCGC
L68NNK_F	CCGGCGCGTTTTATC NNK GAAGAAGTAATGCGTAACT
L68_R	GATAAAACGCGCCGG
L87NNK_F	AAATACCCTGGAAAC NNK CAGGAAATCGGCGTG
L87_R	GTTTCCAGGGTATTTGAAAC
M94NNK_F	GAAATCGGCGTGC NNK GCGCGACGCTAC
M94_R	ACGCACGCCGATTTC
A95NNK_F	ATCGGCGTGC NNK CGACGCTACGGC
A95_R	CATACGCACGCCGAT
R97NNK_F	GTGCGTATGGCGCGA NNK TACGGCGGGCTGTA
R97_R	TCGCGCCATACGC
Y98NNK_F	CGTATGGCGCGACGC NNK GGCGGGCTGTAC
Y98_R	GCGTCGCGCCATA
G99NNK_F	ATGGCGCGACGCTAC NNK GGGCTGTACCCGG
G99_R	GTAGCGTCGCGC
L101NNK_F	CGACGCTACGGCGGG NNK TACCCGGAAGTGGTG
L101_R	CCCGCCGTAGCG
Y102NNK_F	CGCTACGGCGGGCTG NNK CCGGAAGTGGTGGAA
Y102_R	CAGCCCGCCGTAG
T126NNK_F	ATCCTGAATTGGGAT NNK GAGAATGCCACGGCA
T126_R	ATCCCAATTCAGGATCAC
A129NNK_F	TGGGATACCGAGAAT NNK ACGGCATTCTGGAC
A129_R	ATTCTCGGTATCCCAATT
F132NNK_F	GAGAATGCCACGGCA NNK CTGGACGCACTGG
F132_R	TGCCGTGGCATTCTC
T146NNK_F	CTGTTTCGATTCCATC NNK ACGTCTGAAGAAGCTG
T146_R	GATGGAATCGAACAGGT

K155NNK_F	GAAGCTGGTTTCGCT NNK CCGCACCCACGC
K155_R	AGCGAAACCAGCTTC
A175NNK_F	GTAAAGGCGAGAAA NNK GTGTACGTTGGTGACAA
A175_R	TTTCTCGCCTTTAACGC
Y177NNK_F	GGCGAGAAAGCAGTG NNK GTTGGTGACAACCCG
Y177_R	CACTGCTTTCTCGCC
E207NNK_F	AAAGGTGAGAAACGT NNK TTCTGGGATAAGGCG
E207_R	ACGTTTCTCACCTTTACG
A212NNK_F	GAATTCTGGGATAAG NNK GACTTTCGTGTCTCCG
A212_R	CTTATCCCAGAATTCACGT

2. DNA and protein sequence for the most active variant BH_{MeHis}1.8

ATGATTTCGTGCGGTATTCTTTGATAGCCTGGGTACTCTGATTAGCGTTGAAGGCGCTTATAAAATTTA
GGTGAAAGAGATGGAGGAAGTGCTGGGTGACTATCCGCTGAACCCGCGAACCCTGTGGGACGAATTTG
ATAAACTGTTTAAAGGAAGCGTTCTCTAACTATGCGGGCAAACCGTATCGTCCGGCGCGTTTTATCCTG
GAAGAAGTAATGCGTAAACTGGCGGAAAAGTACGGTTTCAAATACCCTGGAAACTTGCAGGAAATCGG
CGTGCGTATGGCGCGACGCTACGGCGGGCTGTACCCGGAAGTGGTGGAAGTACTGAAATCTCTGAAAG
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CGGTCAAAGACGCGGGTGGTTCTAAGAACCTGGGTATGACTAGCATCCTGCTGGATCGTAAAGGTGAG
AAACGTGAATTCTGGGATAAGGCGGACTTTCGTGTCTCCGACCTGCGCGAAGTTATTAAGATTGTTGA
CGAACTGAACGGTCAGGGCTCTCTCGAGCACCACCACCACCACCAC

MIRAVFFDSLGLT LISVEGAYKI (**MHS**) VKEMEEVLGDYPLNPRTLWDEFDKLFKEAFS NYAGKPYRPA
RFILEEVMRKLA EKYGFKYPGNLQEIGVRMARRYGGLYPEVVEVLKSLKGKYHVGVI LNWDTENATAF
LDALGIKDLFDSITTSEEAGFAYPHPRILELALKKAGVKGEKAVYVGDNPVKDAGGSKNLGMTSILLD
RKGEKREFWDKADFRVSDLREVIKIVDELNGQGSLEHHHHHH

MHS = MeHis