Exploring the enantioselective mechanism of halohydrin dehalogenase from *Agrobacterium radiobacter* AD1 by iterative saturation mutagenesis

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TABLE S1. Primers used in this study. All degenerate codons except for NNS were designed by using a free online software DC-Analyzer (http://cobi.uestc.edu.cn/resource/dc_analyzer/view).

Library	Primer	Primer Sequences	XXX representation
A	F86_*fw*	5'-GCACCAGAGXXXCAACCCATAGAT-3'	NDT, TGG, ATG, VMA
	L142_*rv*	5'-GTGTAGGTAGAXXXTTCCTTCCAAG-3'	AHN, CCA, CAT, TKB
B	T134_*fw*	5'-ACCTCTGCAXXXCCCTTCGGGCCTT-3'	NDT, TGG, ATG, VMA
	N176_*rv*	5'-CTGTGAAGATAXXXGGGTCCTATTG-3'	AHN, CCA, CAT, TKB
C	P84_*fw*	5'-TCGCAXXXGAGXXXCAACCCATAGA-3'	NNS
	F86_*rv*	5'-ATGGGTTGXXXCTCXXXTGCGAATA-3'	SNN
D	W139_*fw*	5'-TCGGGCCTXXXAAGGAACTTTCTAC-3'	NDT, TGG, ATG, VMA
	Y187_*rv*	5'-GGTTCTGTGGGXXXGAAGTAGGGAC-3'	AHN, CCA, CAT, TKB
E, F[a]	134_ism_*fw*	5'-ACCTCTGCAXXXCCCTTCGGGCCTT-3'	RYG, TGC
	176_ism_*rv*	5'-CTGTGAAGATAXXXGGGTCCTATTG-3'	KTK, GRM
G	139_ism_*fw*	5'-TCGGGCCTXXXAAGGAACTTTCTAC-3'	SHG, TGG, TAC

187_ism_*rv*	5’-GGTTCTGTGGGXXXGAAGTAGGGAC-3’	GNR, KTC

[a] Libraries E and F were constructed using same primers but with different templates (see TABLE S2)

TABLE S2. Construction of combinatorial libraries for evolving enantiocomplementary HheC mutants.

Libraries	Target positions	Template[a]
E (A + B)	F86/T134/L142/N176	L142F, F86L/L142K, L142M, L142S
F (C + B)	P84/F86/T134/N176	P84T/F86C, F86Y, P84A, P84V/F86P
G (F + D)	P84/F86/T134/W139/N176/Y187	P84V/F86P/T134C/N176A, P84V/F86P/T134A/N176A

[a] The plasmids of positive mutants obtained from first round mutagenesis were mixed with equal mole amount and used as a template for the construction of combinatorial libraries .

TABLE S3. Relative activity of selected positive mutants from CASTing libraries. All reactions were carried out using crude extracts of HheC mutants (0.06 mg) in 5 ml Tris-SO4 buffer (50 mM, pH 8.0) containing 5 mM 1, 3-dichloro-2-propanol.

Libraries	Mutants	Relative Activity [a] (%)	Mutants	Relative Activity [a] (%)
A (F86/L142)	L142Y	297	F86L/L142K	166
	L142M	168	L142S	134
	L142N	141	F86W	181
	L142F	425		
B (T134/N176)	T134C/N176Q	200	T134E/N176F	189
	T134V/N176Q	285	T134V	156
	T134A	100	N176A	103
	N176H	280		
C (P84/F86)	P84T/F86C	183	F86Y	156
	P84I/F86P	196	F86W	176

	P84A/F86P	305	P84V/F86P	270
	P84A	403		
D (W139/Y187)	W139Q/Y187E	126	W139Y	277
	W139V/Y187F	262	W139P	213
	W139G	23	Y187R	188
	W139M	258	W139V	291
	Y187P	133	Y187C	152
	Y187F	318	Y187S	118
	W139E	151	W139A/Y187D	167

[a] The activity of the wild-type HheC corresponding to 5.9 mol /min·mg was set as 100%.

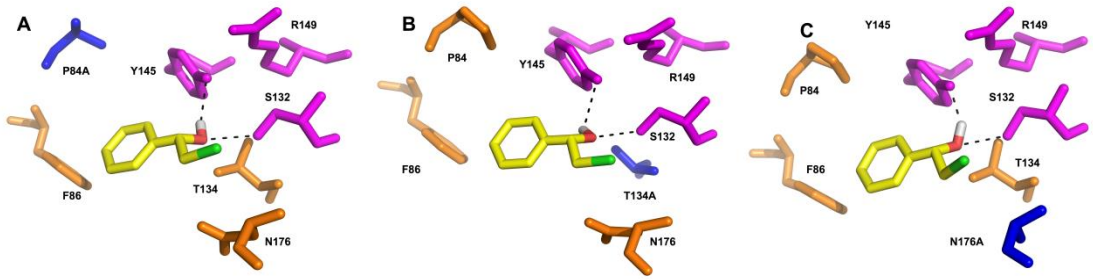

Figure S1. Representation of the structural models of mutants P84A (A), T134A (B) and N176A (C) with (*S*)-2-chloro-1-phenylethanol (2-CPE) docking at the active-site. The catalytic triad Ser132/Tyr145/Arg149 is shown in purple. The mutated residues are shown in blue. Hydrogen bonds are indicated as black dotted lines.

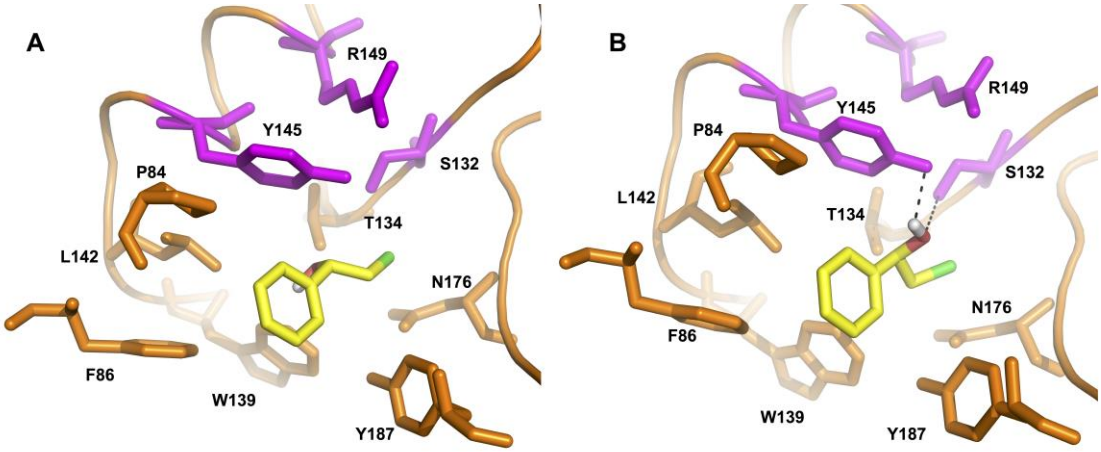

Figure S2. Representation of the binding modes of the enantiomers of 2-CPE in the active site of wild-type HheC. (A) (S)-2-CPE in the active site of HheC; (B) (R)-2-CPE in the active site of HheC. The catalytic triad Ser132/Tyr145/Arg149 is shown in purple. The other non-catalytic active-site residues are shown in orange. Hydrogen bonds are indicated as black dotted lines.

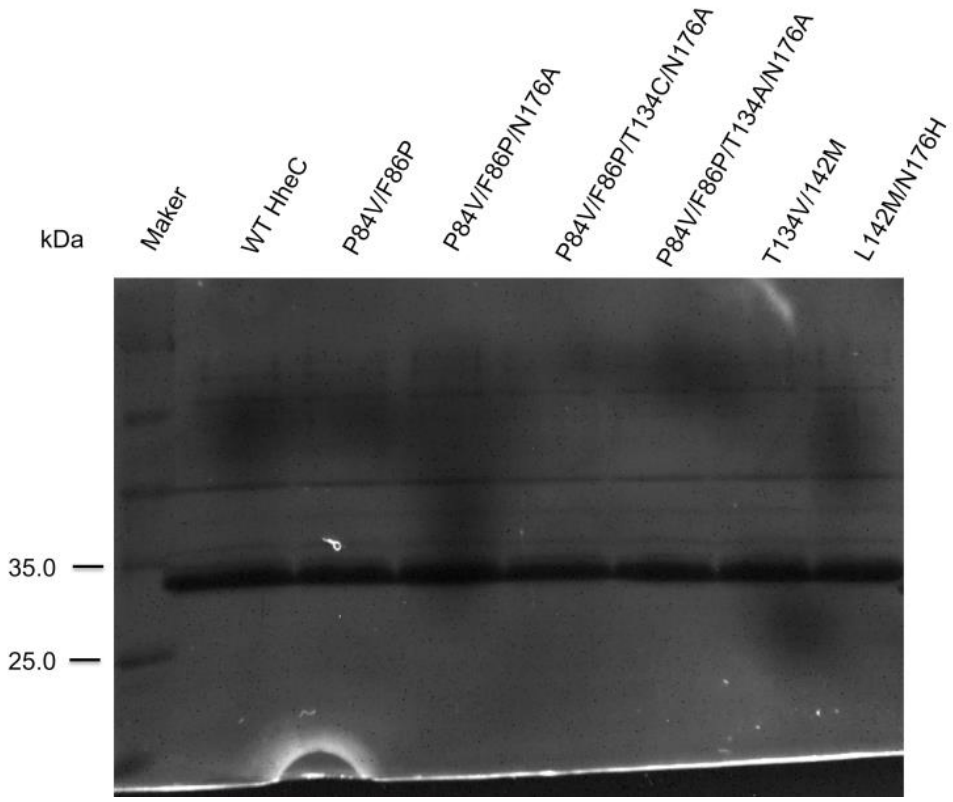

Figure S3. SDS-PAGE analysis of the purity of the wild-type HheC and its variants.

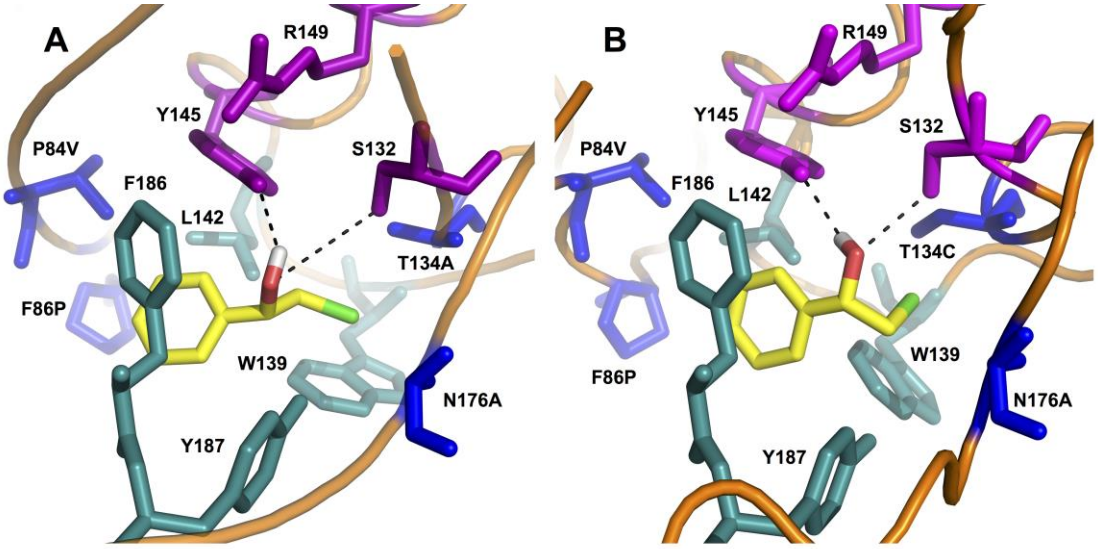

Figure S4. Representation of the structural models of two (*S*)-selective HheC mutants Pro84Val/Phe86Pro/Thr134Ala/Asn176Ala (A) and Pro84Val/Phe86Pro/Thr134Cys/Asn176Ala L142F/N176H (B) with (*S*)-2-CPE docked in the active site. The catalytic triad Ser132/Tyr145/Arg149 is shown in purple. The mutated residues are shown in blue. Hydrogen bonds are indicated as black dotted lines.