Electronic Supplementary Material (ESI) for Catalysis Science & Technology.

Engineering an alcohol dehydrogenase with enhanced activity and stereoselectivity toward diary ketones: reduction of steric hinderance and change of stereocontrol element

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Table S1. Van der Waals volume of twenty amino acids[1]. The small and uncharged amino acids used for shrinking mutagenesis were in bold.

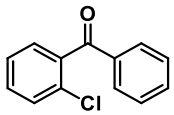
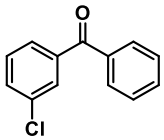
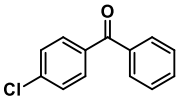
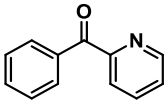
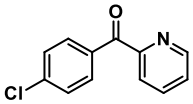
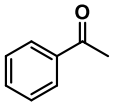
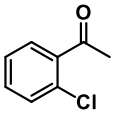
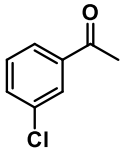
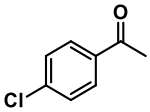
Amino acids	VDW volume ($Å^3$)
Glycine	**48**
Alanine	**67**
Serine	**73**
Cysteine	**86**
Proline	**90**
Aspartic	91
Threonine	93
Asparagine	96
Valine	**105**
Glutamic	109
Glutamine	114
Histidine	118
Isoleucine	124
Leucine	124
Methionine	124
Lysine	135
Phenylalanine	135
Tyrosine	141
Arginine	148
Tryptophan	163

Table S2. Comparison of gram-scale bioreduction with that of previously reported ADHs

Enzyme (best variant)	CPPK loading (mM)	S/C ($g \cdot g^{-1}$)	*ee* of (*R*)-CPPO	CPMK loading (mM)	*ee* of (*S*)-CPMO	S/C ($g \cdot g^{-1}$)	Reference
KpADH (Mu-S5)	not available	not available	91.7%	500[a]	97.4%	not available	[2]
TbSADH (T15)	not available	not available	98%	100	>99%	0.06	[3]
LkADH (seq5)	400	1.08	99.3%	400	98.1%	1.74	This study

[a]Accumulative concentration using fed-batch

Table S3. Analysis method of chiral HPLC and retention time of enantiomer.

ID	Substrate	Chiral column	Chromatographic conditions[a]	Retention time of (*S*)-enantiomer (min)	Retention time of (*R*)-enantiomer (min)
1a		OD-H	95:5	18.6	14.6
2a		OD-H	95:5	20.1	18.2
3a		OB-H	90:10	24	15
4a		AD-H	90:10	18.1	23.2
5a		OB-H	95:5	15.6	17.0
6a		OB-H	95:5	12.4	19
7a		OB-H	95:5	8.5	11.3
8a		OB-H	95:5	13.2	18.3
9a		OB-H	95:5	11.2	13.4

a The solvents used in HPLC are hexane and isopropyl alcohol

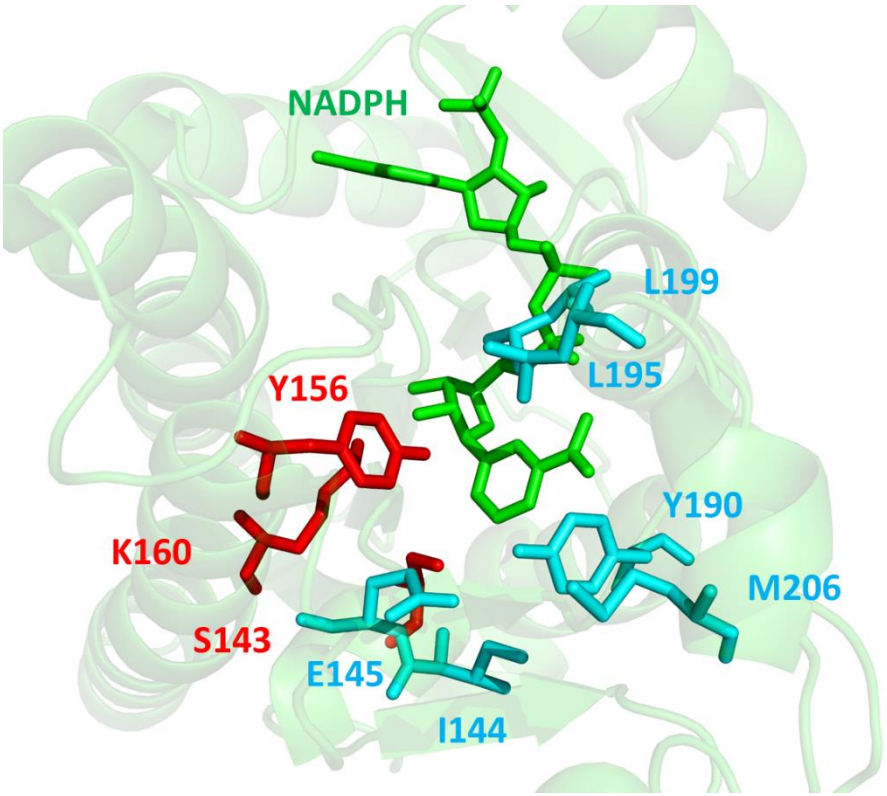


Figure S1. Residues selected for mutagenesis in this study. Catalytic residues were colored in red. Selected residues for mutagenesis were colored in cyan. NADPH was colored in green.

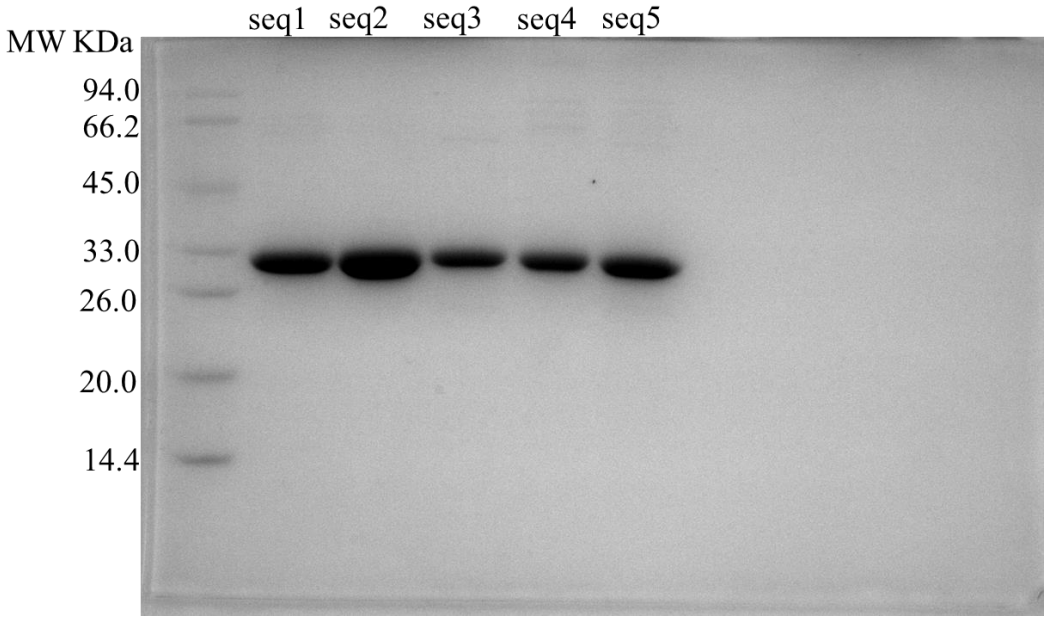


Figure S2. SDS-PAGE analysis of purified mutants seq1-5

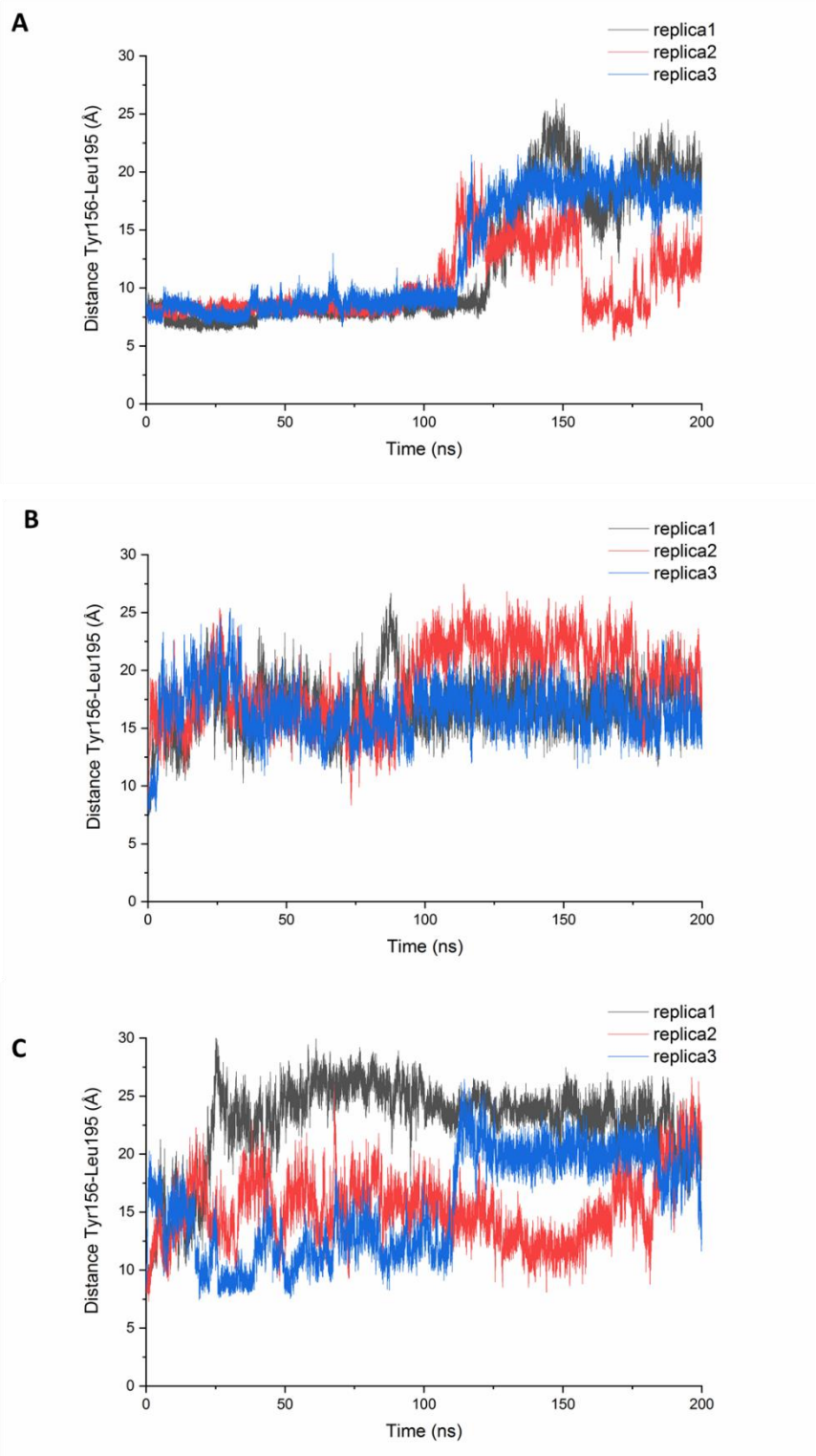


Figure S3. Distance between catalytic residue Tyr156 and binding loop residue Leu195. (A) WT (B) seq1 (Y190P) (C) seq5 (Y190P/I144V/L199V/E145C/M206F).

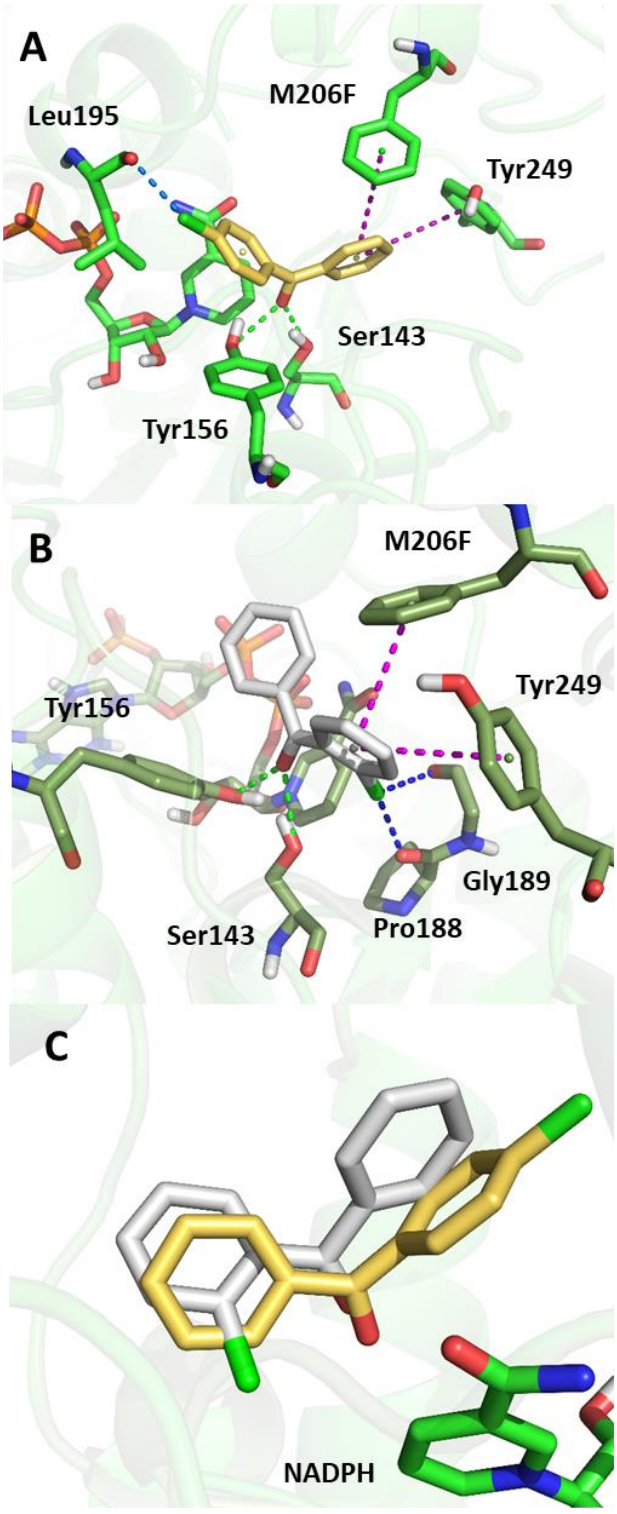


Figure S4. Opposite binding orientations of 2-chlorodiphenylketone and CPPK in seq5. 2-Chlorodiphenylketone and CPPK are represented in grey and yellow, respectively. Halogen bonds are represented in blue; π-π interactions in purple; hydrogen bonds in green. A. CPPK bound in pro-*R* pose. Halogen bonds formed between *para*-chlorine and Leu195. B. 2-chlorodiphenylketone bound in pro-*S* pose. Halogen bond formed between *ortho*-chlorine and Gly189 or Pro188. C. Overlay of the two substrate conformations, which bound in opposite orientations.

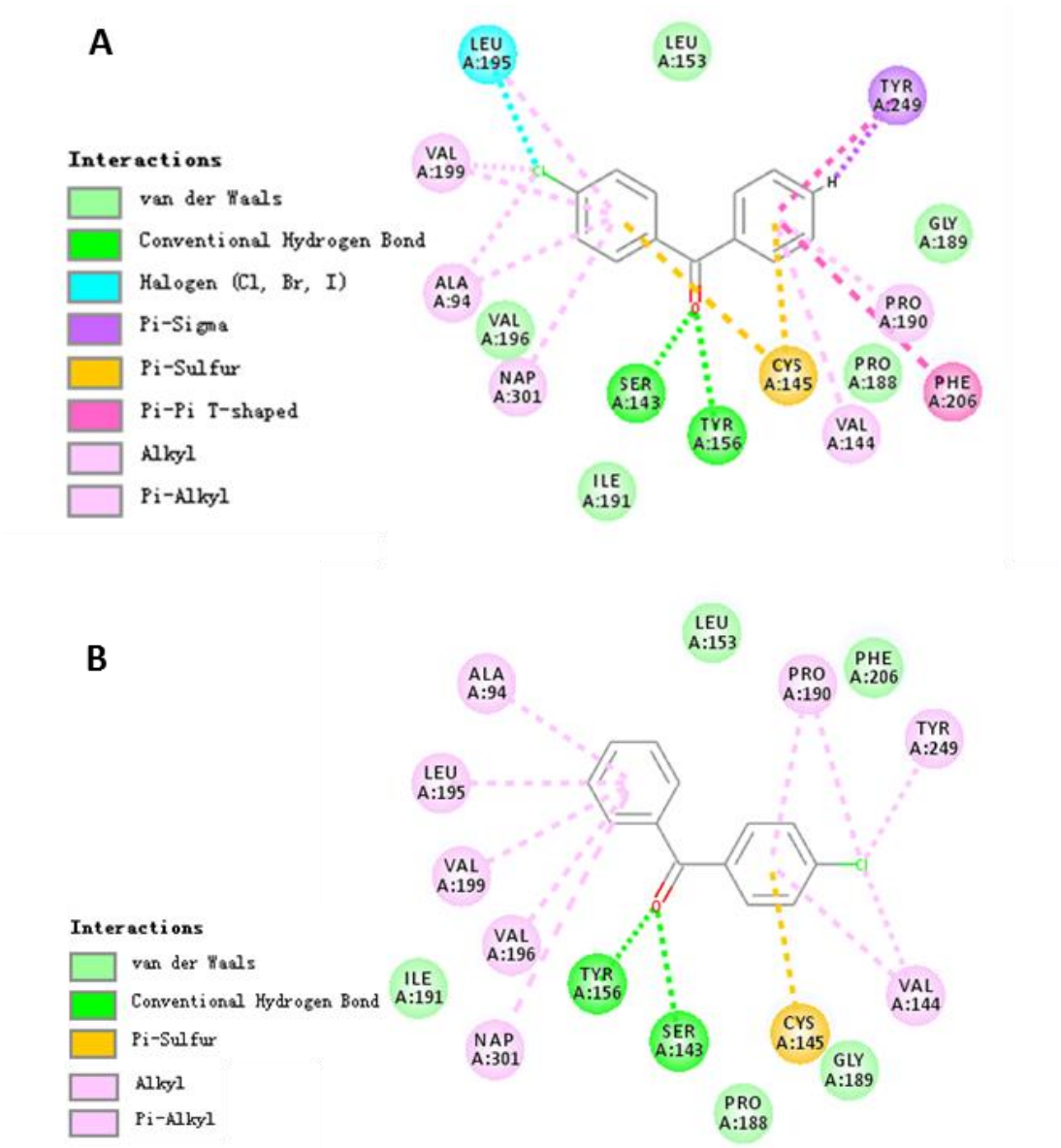


Figure S5. comparison of non-covalent interactions between pro-*R* and pro-*S* docking result through 2D interaction plot. No π-π stacking and halogen bond was observed in pro-*S* docking pose. (A) Interactions of pro-*R* docking conformation. (B) Interactions of pro-*S* docking conformation.

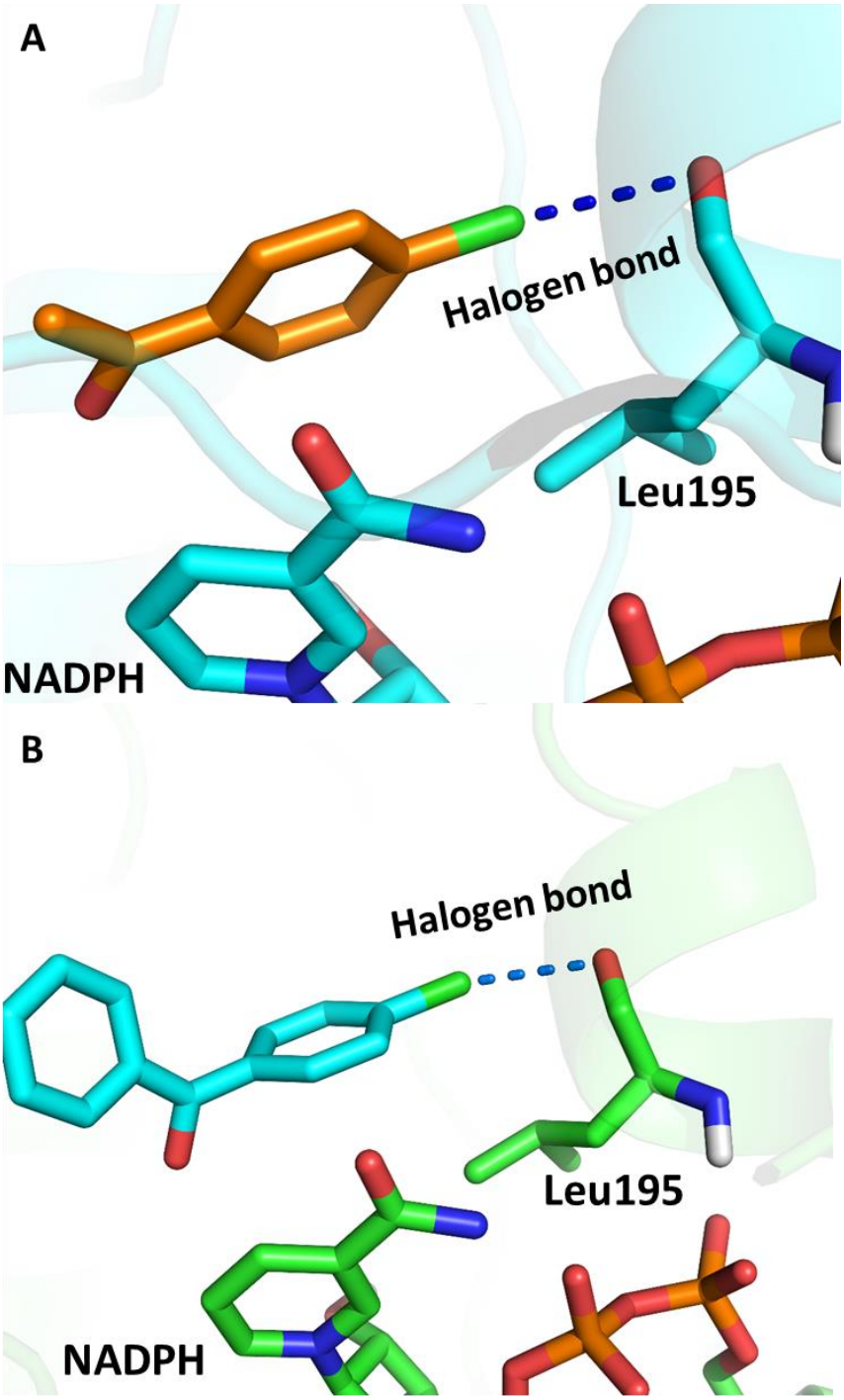


Figure S6. Similar halogen bond observed in 4-chloro-1-phenylethanone and CPPK. (A) pro-*R* docking pose of 4-chloro-1-phenylethanone (B) pro-*R* docking pose of CPPK.

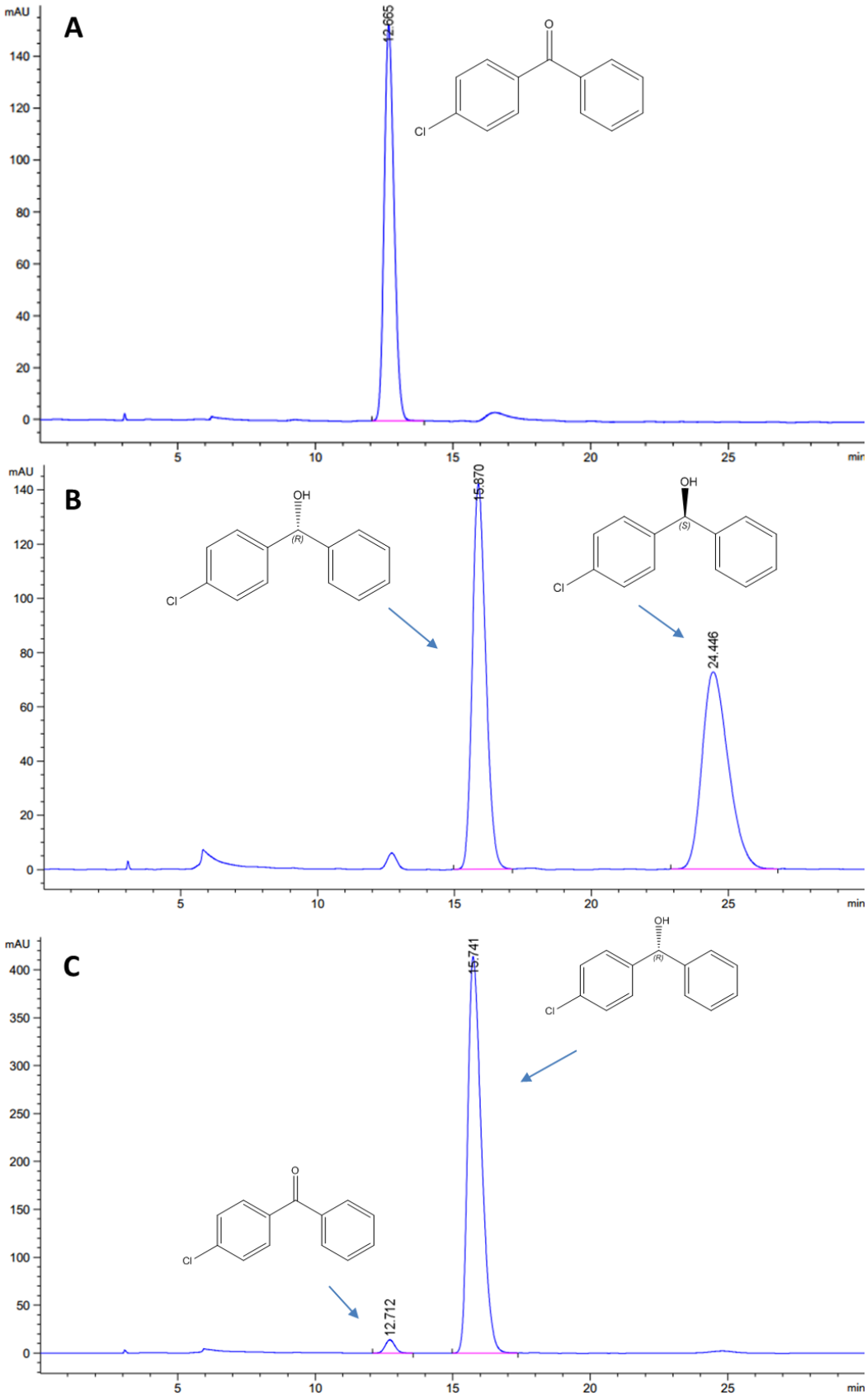


Figure S7. Chiral HPLC analysis of bioreduction of CPPK by seq5. (A)Substrate standard of CPPK (B) Racemic standard of CPPO (C) (*R*)-CPPO after 16h reaction.

1. Darby, N. J.; Creighton, T. E., *Protein structure*. IRL Press at Oxford University Press: 1993.
2. Zhou, J.; Wang, Y.; Xu, G.; Wu, L.; Han, R.; Schwaneberg, U.; Rao, Y.; Zhao, Y. L.; Zhou, J.; Ni, Y., Structural Insight into Enantioselective Inversion of an Alcohol Dehydrogenase Reveals a "Polar Gate" in Stereorecognition of Diaryl Ketones. *Journal of the American Chemical Society* **2018,** *140* (39), 12645-12654.
3. Liu, B.; Qu, G.; Li, J.-K.; Fan, W.; Ma, J.-A.; Xu, Y.; Nie, Y.; Sun, Z., Conformational Dynamics-Guided Loop Engineering of an Alcohol Dehydrogenase: Capture, Turnover and Enantioselective Transformation of Difficult-to-Reduce Ketones. *Advanced Synthesis & Catalysis* **2019,** *361* (13), 3182-3190.