Supporting information

Rational Design of Dehydrogenase/reductases Based on Comparative Structural Analysis of Prereaction-State and Free-State Simulations for Efficient Asymmetric Reduction of Bulky Aryl Ketones

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Supporting tables

Table S1. Primers used for site-directed mutagenesis

Primer	Sequence (5'-3')
Mu0-H145A forward[a]	GCCTCAATAGCCGGTACAGTAGCGGC
Mu0-H145A reverse	GTACCGGCTATTGAGGCCATATTCACAATAG
Mu0-H145C forward	GCCTCAATATGCGGTACAGTAGCGGC
Mu0-H145C reverse	GTACCGCATATTGAGGCCATATTCACAATAG
Mu0-H145G forward	GCCTCAATAGGCGGTACAGTAGCGGC
Mu0-H145G reverse	GTACCGCCTATTGAGGCCATATTCACAATAG
Mu0-H145F forward	GCCTCAATATTCGGTACAGTAGCGGC
Mu0-H145F reverse	GTACCGAATATTGAGGCCATATTCACAATAG
Mu0-Y188A forward	GACCTGGTGCTATCATGACACCATTGTTGTC
Mu0-Y188A reverse	GTCATGATAGCACCAGGTCCCACAGCATTGC
Mu0-Y188C forward	CCTGGTTGTATCATGACACCATTGTTGTC
Mu0-Y188C reverse	GGTGTCATGATACAACCAGGTCCCACAGC
Mu0-Y188G forward	CCTGGTGGTATCATGACACCATTGTTGTC
Mu0-Y188G reverse	GGTGTCATGATACCACCAGGTCCCACAGC
Mu0-Y188F forward	CCTGGTTTCATCATGACACCATTGTTGTC
Mu0-Y188F reverse	GGTGTCATGATGAAACCAGGTCCCACAGC
Mu0-A94Q forward	CCGGAATACAAGGTCCGGCTGAATTGAC
Mu0-A94Q reverse	GGACCTTGTATTCCGGCATTATTACATGC
Mu0-A94R forward	CCGGAATACGCGGTCCGGCTGAATTGAC
Mu0-A94R reverse	GGACCGCGTATTCCGGCATTATTACATGC
P-M85A forward[b]	CGTCGCTGGCCCCCTGCC
P-M85A reverse	GGGGGCCAGCGACGCCCGC
P-M85G forward	CGTCGGTGGCCCCCTGCC
P-M85G reverse	GGGGGCCACCGACGCCCGC
P-M85S forward	CGTCAGTGGCCCCCTGCC
P-M85S reverse	GGGGGCCACTGACGCCCGC

[a]Of which, Mu0 represents the parent EbSDR8-G94A/S153L in mutagenesis. [b]Of which, P represents the parent PpYSDR in mutagenesis.

Table S2. HPLC and GC methods for various prochiral ketones and their corresponding chiral alcohols products

Sub.[a]	Column	Method	Retention time of substrate (min)	Retention time of *R*-product (min)	Retention time of *S*-product (min)
1a	HYDRODEX β-TBDAc Column (25 m×0.25 mm, Diacel, Japan)	GC was performed with a temperature program of 1 min at 120℃ followed by heating to 180℃ at a rate of 10℃/min.	7.13	7.37	7.25
2a	HYDRODEX β-TBDAc Column (25 m×0.25 mm, Diacel, Japan)	GC was performed with a temperature program of 15 min at 125℃.	10.44	14.27	13.81
3a	HYDRODEX β-TBDAc Column (25 m×0.25 mm, Diacel, Japan)	GC was performed with a temperature program of 12 min at 115℃.	8.09	10.31	10.63
4a	Chiralcel OD-H (4.6 mm×250 mm, 5 μm, Diacel, Japan)	HPLC was performed at 210 nm and 30℃ using hexane : isopropanol (98:2, v/v) as mobile phase at a flow rate of 1.0 mL/min.	10.30	20.94	10.73
5a	Chiralcel OD-H (4.6 mm×250 mm, 5 μm, Diacel, Japan)	HPLC was performed at 210 nm and 30℃ using hexane : isopropanol (95:5, v/v) as mobile phase at a flow rate of 1.0 mL/min.	6.78	12.01	10.16
6a	Chiralpak AD-H column (4.6 mm×250 mm, 5 μm, Diacel, Japan)	HPLC was performed at 254 nm and 30℃ using hexane : isopropanol (90:10, v/v) as mobile phase at a flow rate of 1.0 mL/min.	6.91	10.54	13.45

[a]Sub. = substrate.

Supporting figures

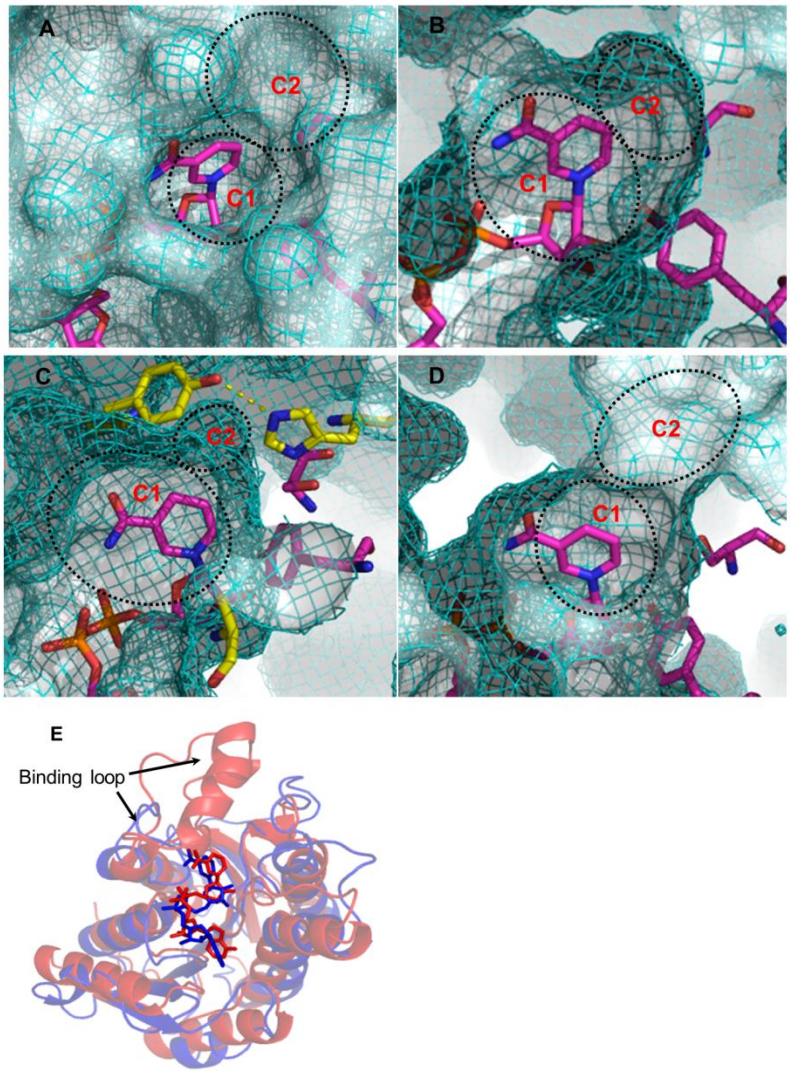

Figure S1. Substrate binding pockets of different dehydrogenase/reductases: (A) Dehydrogenase/reductase PED[1]. (B) Dehydrogenase/reductase Hped[2]. (C) Dehydrogenase/reductase EbSDR8-Mu03. (D) Dehydrogenase/reductase PpYSDR4. (E) Alignment of PpYSDR (blue) to EbSDR8-Mu0 (red). The substrate binding pocket is shownd as surface and mesh. The C1 and C2 are marked with dashed circles. The hydrogen bond is shown as yellow dash. A94, H145 and Y188 of Mu0 are shown as yellow sticks. The cofactor and catalytic sites (Ser and Tyr) are shown as pink sticks.

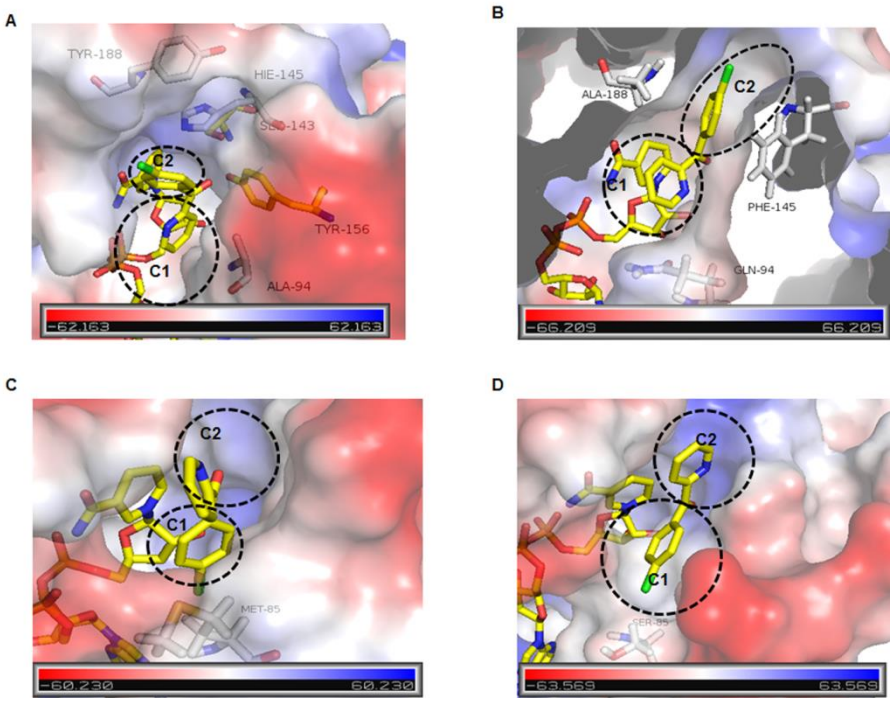

Figure S2. The contact potential analysis of substrate binding pockets of Mu0 (A), Mu14 (B), PpYSDR (C) and Mu17 (D). The cofactors and substrate 6a are shown as yellow sticks. The mutant sites are shown as white sticks. The substrate binding pocket are shown as surfaces colored by contact potential and the cavities C1 and C2 are marked with dash circles.

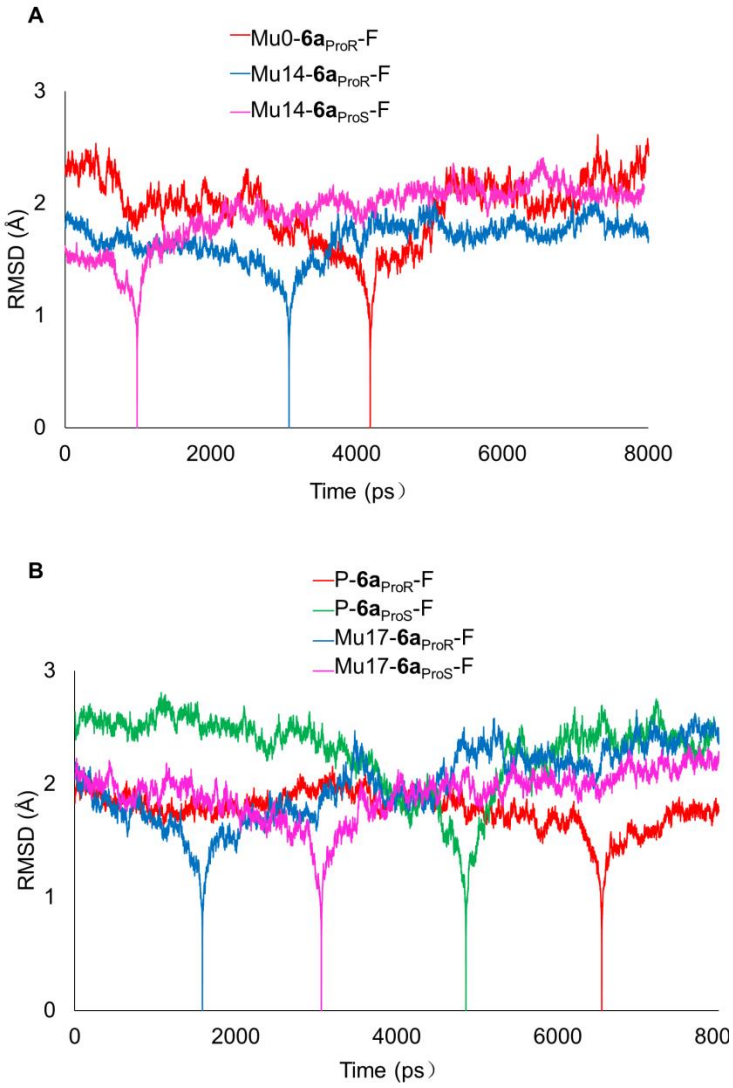

Figure S3. RMSD analyses of enzymes assembled with **6a** to their lowest energy structure in free-state simulation: (A) Mu0-**6a**$_{ProR}$ (red), Mu14-**6a**$_{ProR}$ (blue) and Mu14-**6a**$_{ProS}$ (pink); (B)P-**6a**ProR (red), P-**6a**ProS (green), P-**6a**ProR (blue) and P-**6a**ProS (pink). The value decreased to 0 at the time when the lowest energy structure occurs.

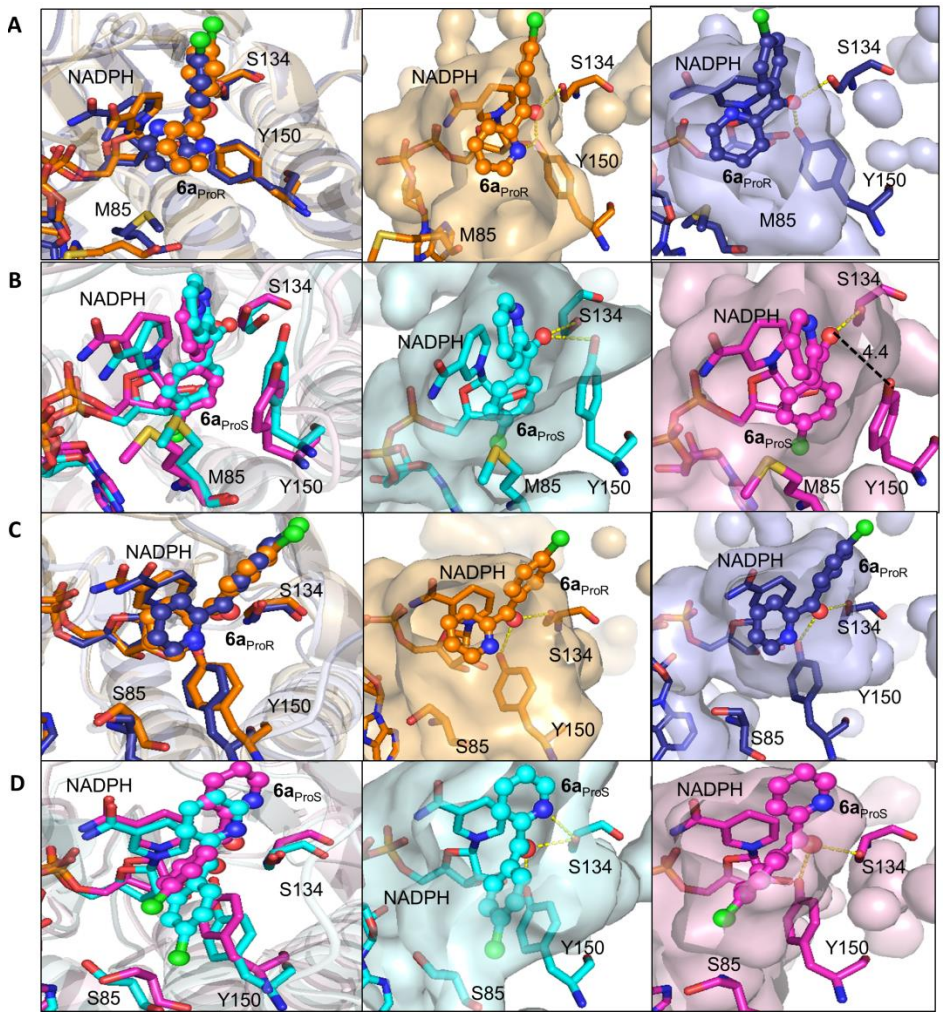

Figure S4. Binding modes of **6a** to P and Mu17 in prereaction-state simulation and free-state simulation: (A) Binding modes of **6a**$_{proR}$ to P in prereaction-state simulation (orange) and free-state simulation (blue); (B) Binding modes of **6a**$_{proS}$ to P in prereaction-state simulation (cyan) and free-state simulation (pink); (C) Binding modes of **6a**$_{proR}$ to Mu17 in prereaction-state simulation (orange) and free-state simulation (blue); (D) Binding modes of **6a**$_{proS}$ to Mu17 in prereaction-state simulation (cyan) and free-state simulation (pnk). Key residues are shown as sticks and substrates are shown as balls and sticks. The hydrogen bonds are shown as yellow dashes and the distances are shown with black dashes and values (Å).

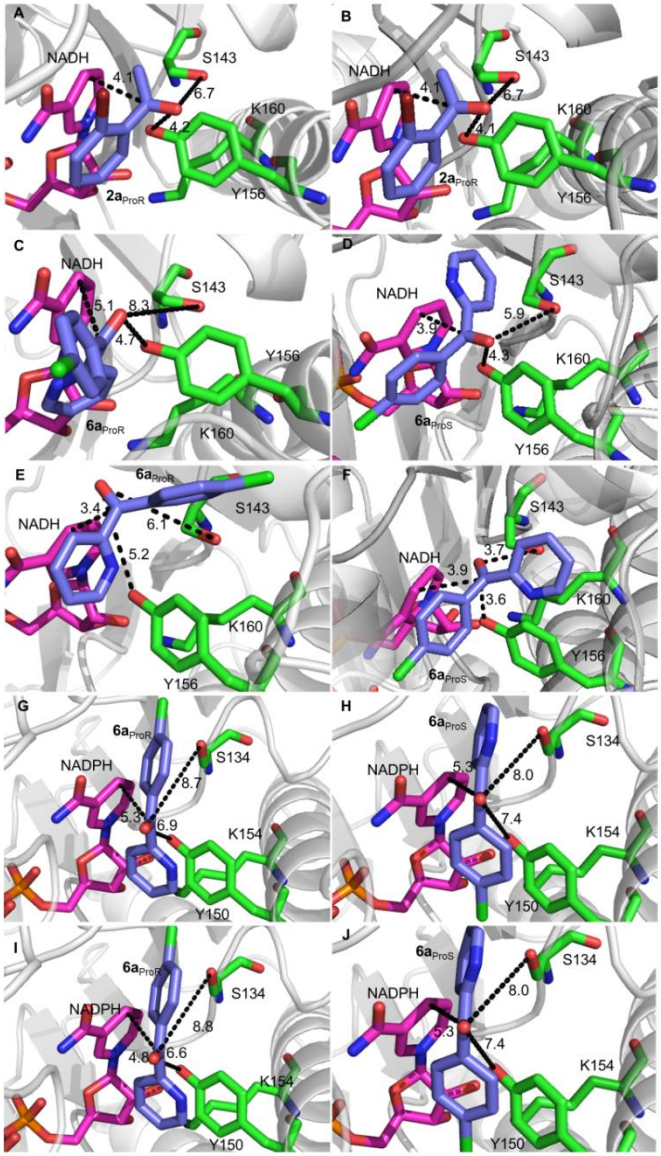

Figure S5. Starting conformation of enzyme-substrate complexes: (A) Mu0-**1a**$_{ProR}$; (B) Mu1-**1a**$_{ProR}$; (C) Mu0-**6a**$_{ProR}$; (D) Mu0-**6a**$_{ProS}$; (E) Mu14-**6a**$_{ProR}$; (F) Mu14-**6a**$_{ProS}$; (G) P-**6a**$_{ProR}$; (H) P-**6a**$_{ProS}$; (I) Mu17-**6a**$_{ProR}$; (J) Mu17-**6a**$_{ProS}$. The enzymes are shown as cartoons, the cofactors are shown as pink sticks, the active sites are shown as green sticks and the substrates are shown as blue sticks. The distances are shown with black dashes and values (Å). The docking binding energies (kcal/mol) for each starting conformations are -4.99, -4.90, -5.51, -5.96, -6.70, -6.92, -6.10, -5.92, -6.24 and -6.15 respectively.

References

1. Hoffken, H. W.; Duong, M.; Friedrich, T.; Breue, M.; Hauer, B.; Reinhardt, R.; Rabus, R.; Heider, J., Crystal Structure and Enzyme Kinetics of the (S)-Specific 1-Phenylethanol Dehydrogenase of the Denitrifying Bacterium Strain EbN1. *Biochemistry* **2006,** *45*, 82-93.
2. Busing, I.; Hoffken, H. W.; Breuer, M.; Wohlbrand, L.; Hauer, B.; Rabus, R., Molecular Genetic and Crystal Structural Analysis of 1-(4-Hydroxyphenyl)-Ethanol Dehydrogenase from '*Aromatoleum aromaticum*' EbN1. *J. Mol. Microbiol. Biotechnol.* **2015,** *25*, 327-339.