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Supporting information for article:

Insight into the potential factors influencing catalytic directions in cellobiose 2-epimerase by crystallization and mutagenesis

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Table S1 . The enzymatic activities of the CEs.

CEs	*Cs*CE	*St*CE	*Bt*CE	*Rm*CE	*Ra*CE	*Fj*CE	*Ta*CE
Activity of Isomerization（U/mg）	3.55	2.37	0.43	0.078	0.001	0.0005	ND
Activity of Epimerization (U/mg)	43.18	51.12	46.75	311.4	38.82	48.54	13.5
Reference	This study	This study	This study	(Kuschel *et al.,* 2017)			(Chen *et al.*, 2015)

Table S2 The primers for construction of *Cs*CE mutants and cloning of different CEs.

Primers	5’-3’sequences
*Cs*CE_F	CAGCAAATGGGTCGCGGATCCATGGATATTACAAGGTTTAAG
*Cs*CE_R	TTGTCGACGGAGCTCGAATTCGTCAACCCTTTTTATTATC
*St*CE_F	CAGCAAATGGGTCGCGGATCCATGCCTCTTCCCACTAC
*St*CE_R	TTGTCGACGGAGCTCGAAT TCTCTTCGTTCCTCCTC
*Bt*CE_F	CAGCAAATGGGTCGCGGATCCATGAATACATTCGTAAATG
*Bt*CE_R	TTGTCGACGGAGCTCGAATTCCTTTCCAACCCTTTC
R170AF171A_F	GCCTTGAGCGAAAATGGAG
R170AF171A_R	GGCGTTTTCTTTTTCTTGCCAG
E174A_F	GCTAATGGAGTAATTGCCTCAAAAAC
E174H_F	CACAATGGAGTAATTGCCTCAAAAAC
E174D_F	GACAATGGAGTAATTGCCTCAAAAAC
E174_R	GCTCAAAAACCTGTTTTCTTTTTCTTG
N175K_F	AAGGGAGTAATTGCCTCAAAAACAATG
N175K_R	TTCGCTCAAAAACCTGTTTTCTTTTTC
G176D_F	GACTACACAGAACAGTTTGAGAGAG
G176D_R	ATTTTCCCTTTTGCATTTTGTCTC
G176K_F	AAGTACACAGAACAGTTTGAGAGAG
N175RG176D_F	GACTACACAGAACAGTTTGAGAGAG
N175RG176D_R	ACGTTCCCTTTTGCATTTTGTCTC
V177D_F	GATGCCTCAAAAACAATGAAC
V177D_R	ATCTCCATTTTCGCTCAAAAAC
I178D_F	GATGCCTCAAAAACAATGAAC
I178D_R	TACTCCATTTTCGCTCAAAAAC

Table S3 Data collection and refinement statistics

	*St*CE	*Bt*CE	*CsCE*
Data collection			
wavelength	0.9795	0.97946	1.0332
Space group	$P12_11$	C121	P222
Unit cell parameters			
a, b, c (Å)	67.53, 205.16, 67.72	176.98, 52.34, 129.60	55.69, 75.77, 91.17
α, β, γ (°)	90, 104.26, 90	90, 129.94, 90	90, 90, 90
Resolution (Å)	100-2.05（2.10-2.05）	50- 1.80(1.85-1.80)	29.62-1.54(1.57-1.54)
R_{merge}	0.042（0.381）	0.041(0.606)	0.10 (0.34)
Redundancy	1.86（1.77）	3.17(3.11)	4.0 (2.7)
Completement (%)	86.55（82.2）	97.6 (95.1)	96.3 (71.1)
Number of unique reflections	96356（6722）	83031(5958)	48931 (1690)
$\langle I/\sigma(I)\rangle$	12.94（2.11）	15.72 (1.64)	3.30 (1.54)
Refinement			
Resolution (Å)	2.05	1.80	1.54
R_{work}/R_{free}	0.1955/0.2669	0.1759/0.2100	0.159/0.188
Atoms			
Protein	13061	6068	3297
Water	599	400	204
EDO	8	48	24
ligand	0	0	0
RMSD			
Bond length (Å)	0.008	0.009	0.018
Bond angle (°)	0.898	0.885	1.74
Ramachandran (%)			
Favored	96.18	97.47	97.00
Allowed	3.82	2.53	3.00
Outliers	0	0	0
Mean B factor(Å^2)			
Protein	34.65	36.25	18.65
Ligand	42.09	39.23	-
Code in PDB	5ZIG	5ZHB	4Z4J

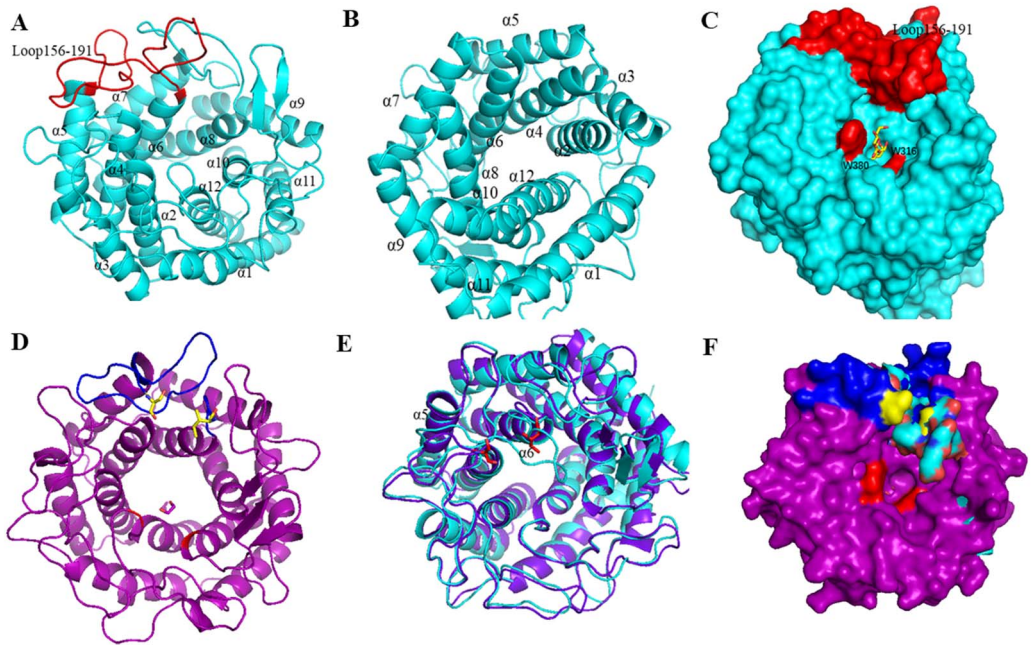

Figure S1 A and B, top view and upward view structures of *St*CE (cyan colored) and the distribution of α helices, β strands and the flexible loops in the structures. C, the surface shape of the *St*CE; the flexible loop and the two-tryptophan corresponding ligand binding (Trp380 and Trp316) were colored red. The ligand of glucosyl- β 1,4-mannose (yellow stick) from the *Rm*CE_ligand was docked here to depict the size of the active site. D, the top view structure of the *Bt*CE; residues 169-177 (blue colored) of the flexible loop in *Bt*CE were missing. E, the alignment of the structures of *St*CE (cyan) and *Bt*CE (purple). F, the overall structure of the *Bt*CE, and the missed loop 169-177 was corresponding to the loop 177-185 in *St*CE (cyan). These loops could partially cover the active site and also shrink the size of the entrance of the active site.

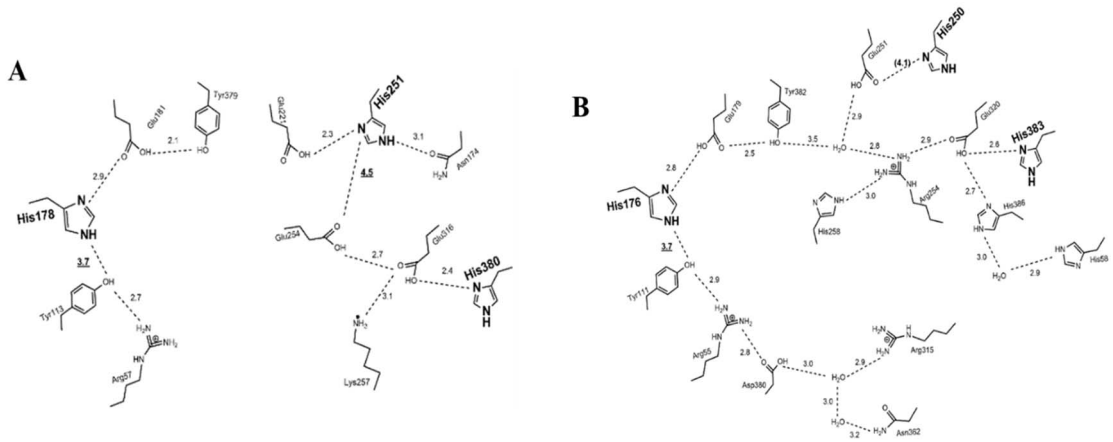

Figure S2 A and B, the distance of residues *Mm*MI(PDB: 5X32) and *Se*YihS (PDB: 2AFA). The molecular interactions in the H bond networks of the residues in the active site were shown in dashed lines and marked in Å, respectively.

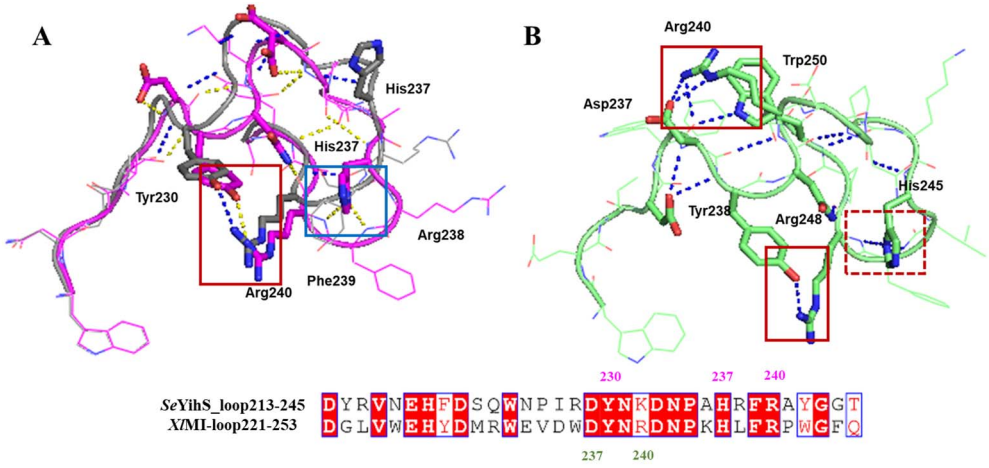

Figure S3 A) the changes in the hydrophilic interactions between residues in the flexible loops of apo_*Se*YihS (PDB: 2AFA) and *Se*YihS_Man (PDB: 2ZBL) during the ligand binding. The red and blue rectangles represented the interactions remained and newly formed in the *Se*YihS during the ligand binding. B, the molecular contacts of the apo-*Xl*MI (PDB: 3GT5). And the dashed rectangle in B indicated that the interactions may be kept once binding the ligands.