

Supplementary Material

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**Engineering of cellobiose phosphorylase for the defined synthesis of cellotriose**

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Table S1. Primers used for enzyme engineering; mutations are underlined

Construct	Template	Primer Sequences (5'-3')
A291R	CcCDP wild-type	CATGTTTGGTACCGCG <u>AGG</u> ACCCACGCGATCTTT
A291F	CcCDP wild-type	GGCATGTTTGGTACCGCG <u>TT</u> CACCCACGCGATCTTTGA
A294H	CcCDP wild-type	GGTACCGCGGGGACCCACT <u>AT</u> ATATCTTTGAAGATGTGACCT
A294F	CcCDP wild-type	GTACCGCGGGGACCCACT <u>TT</u> CATCTTTGAAGATGTGACC
Y330A	CcCDP wild-type	TACCCCGGATTACTATCGTAAGAA <u>AGC</u> CAACGAAGACACCC
Y330R	CcCDP wild-type	TACCCCGGATTACTATCGTAAGAA <u>ACG</u> CAACGAAGACACCC
K329R	CcCDP wild-type	CGGATTACTATCGTAAGA <u>G</u> ATACAACGAAGACACCCG
K329A	CcCDP wild-type	CCCGGATTACTATCGTAAG <u>G</u> CATACAACGAAGACACCCGT
K329W	CcCDP wild-type	ACCCCGGATTACTATCGTAAG <u>TGG</u> TACAACGAAGACACCCGTTTC
CcCDP_RV1	CcCDP wild-type	CGAAATAACGCAGCAGCGCTTCCAGCTCTTTTCG
CcCDP_RV2	CcCDP wild-type	CGTTGCTGGTCCACTCAAACAGCAGGTTCTTC
K51R	CuCBP_OCP2	TTTTACCGCGATGCAA <u>AG</u> GATGCGTCGGTTGACC
M52W	CuCBP_OCP2	TACCGCGATGCAAAG <u>TG</u> GCGTCGGTTGACCCG
M52R	CuCBP_OCP2	GCTTTTACCGCGATGCAAAG <u>AG</u> GCGTCGGTTGA
D156W	CuCBP_OCP2	TTCGTTGGAGTTTGTCTTATGG <u>TGG</u> GCCCAAGATGATCAGACTGAC
D156K	CuCBP_OCP2	CGTGGAGTTTGTCTTATGG <u>A</u> AGGCCCAAGATGATCAGACTG
D156R	CuCBP_OCP2	GTGGAGTTTGTCTTATGG <u>G</u> GCGCCCAAGATGATCAGAC
D156Y	CuCBP_OCP2	CGTGGAGTTTGTCTTATGG <u>T</u> ATGCCCAAGATGATCAGACTG
G502N	CuCBP_OCP2	AACTGCTTTTCCACCACGCCG <u>AA</u> TGAAAGCTTTCAAACCATCGAA
R166Y	CuCBP_OCP2	CAAGATGATCAGACTGACTACCA <u>ATA</u> AACCTGAGTATCGGCGAAGTG
CuCBP_OCP2_RV1	CuCBP_OCP2	TTGCGCTGCCATGAGGACTCTCCTGTTCTAC
CuCBP_OCP2_RV2	CuCBP_OCP2	AGACGCTGACCGACTCATGTAAACGTGAC
CuCBP_OCP2_RV3	CuCBP_OCP2	GGTACGTAGATACGCCGCCATGTGGAC

Table S2. Kinetic parameters towards cellobiose as acceptor<sup>a</sup>

Enzyme	$V_{\max}$ (U/mg)	$k_{\text{cat}}$ (s <sup>-1</sup> )	$K_m$ (mM)	$k_{\text{cat}}/K_m$ (M <sup>-1</sup> s <sup>-1</sup> )
CcCDP (wild-type)	10.4	19.3	16.8	1149
CcCDP_A294F	0.35	0.6	1.3	461
CcCDP_A294H	0.17	0.3	2.2	136
CcCDP_A291R	3.9	7.2	27.2	265
CcCDP_A291F	5.1	9.5	18.3	519
CcCDP_K329R	0.5	0.9	4.3	209
CcCDP_K329A	3.7	6.9	5.7	1210
CcCDP_K329W	0.5	0.9	4.2	214
CcCDP_Y330A	0.6	1.1	2.5	44
CcCDP_Y330R	0.7	1.3	10.5	124

<sup>a</sup> Using 50 mM  $\alpha$ G1-*P* as co-substrate in 100 mM MES buffer, at pH 7 and 55 °C