Supporting Information

Crystal Structure of GH49 Dextranase from *Arthrobacter oxidans* KQ11: Identification of Catalytic Base and Improvement of Thermostability Using Semirational Design Based on B-Factors

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The sequence gene of Aodex (the red sequence numbered the signal peptide, and the rest sequence numbered the Aodex):

ATGCCCGGAACAGGGCTGGGCCGATTGGCCAAACGCATGACAGCGGCCGCCGCCGTCTTCCTTATCAGCAGTGGCGCCGT
CCTCCCGGCGCAGGCAGCCACGACAGCGGGGCACACCCCCTCTACGGCGCCTGCTGCGCCTACCGACAAACACGCCATTA
CCACTGCGGACAACGGCAACCTTCACACCTGGTGGCATGACAACGCCGTTTTCAACACCACCGGCCCAACTGGCAACGAC
GAAGTGCGGCGGTCCTCCTTCTACGACCTGCAGGTGGCCCAGGAGAACCAGCCGGACAAGGCTTATGACGCCTTCACTTA
CATGAGCATCCCCCGCAGCGGCAAGGACAAGATCGGCTACACCAAGGAAGACGGCGCCGAATTCTCCTCCCAGGCCGGCC
TTACCATGAGCTGGTCCAGCTTCGAATATGCCAAAGACGTCTGGGTGGACGTAAGCCTCCGCACCGGCCAGACCATCACCT
CAGCGGACCAGGTGCAGATCCGCCCCAGCAGCTACAACTTCGAAAAGCAGCTCGTTGACGCGGACACCGTCAAAATCAAA
GTGCCCTACTCCGATGCCGGCTACCGGTTCTCAGTGGAATTCGAGCCACAGTTGTATACGGCCTACAACGACATGTCCGGG
GACAGCGGCAAGCTGACCACCGAAGCCGAGGGCAACCGCGCCATCCACACCGAACCCCGCAATTCAATGATGATCTTCGC
GGAACCTAAACTCCGCGGAGAGCAAAAGGAACGATTGGTTCCCACAGAGGAGTCGGGCAGCATCCATTACCCTGCCGAAG
GTGAGGTGACCAACCTCAATGCCGTTACCGAGGAAATCATCTACTTCAAGCCCGGCACCTACAGCATGGGATCGGACTAC
CACGCGGTCCTGCCGCCCAACGTCAAGTGGGTCTACCTGGCACCGGGCGCTTACGTAAAGGGAGCCTTCCGGTTCCTCCAT
GACAACCAGAGCCAGTACAAGGTCACCGGCTACGGTGTCCTCTCCGGCGAGCAGTACGTGTACGAGGCAGACACCAATAA
CAACTACAACCACCTCAGCGGAGCGTCCAACTGCCACTCGTCGTGCGTGAAGATGCTGCAGTTCGCTTCCGCCGATGCCGA
GCAGAAGCTGGACCTTCAGGGCGTTACTGTCGCCGAGCCCCCGTACCACTCCTTTGTGGTCTACGGAAACGAGCAGACCTT
CCACATGAACGTTGAGAACTACAAGCAGGTGGGCAGCTGGTACTGGCAGACCGACGGCATCGAACTGTACAAGGGCAGC

ACCATGAAGAACACCTTCTTCAACGCGAACGACGACGTGCTGAAGATGTACCACAGCGACGTCACCATCGATAACACCGT
GATTTGGAAGAACGAGAACGGACCCGTGATCCAGTGGGGCTGGACGCCCCGGAACATCGACAACGTGAACGTCACCAAC
ACCACGGTCATCCACAACCGGATGTACTGGAAGGACGTCAAGTACAACACCTGCATCCTGAACTCCTCGTCGCACTGGGA
AGACATGGGTTCCACCACCAAGGCGGATCCCAACACCACCGTGAAGAACATGCGCTTCGAGAACATCACGGTGGAGGGCA
TGACCAACTGTGCCATCCGCGTCTATGCCCTCTCCGATACCGAGAACATCCACGTGAAAAACCTCAACATCGACGCTTGGA
ACGGGCTGGACTGGACCTCACAGGTCAGCCACCTCAAGCGCTACACCAACCCTGCCGGCGAAAAAGTCACAATCGGAAAC
GAGATTCCTGACGGCAACGGACTAGCCCTGGAGAACTACTCCGTGGGCGGCGAAGTGATCGAAAAAACCGCCGACAACTG
GGCCGACCACCAGCTGGGCCGCATCGGCTTCGACGGCGAAAACTGGAACAGCTGGAACGCCTGGCGGACCACCCCGTAG

The surface of the crystal structure of Aodex included three large cavities, three large protrusions, and one large flat region:

The largest cavity (surface area = 799.153 Å^2; volume = 1794.341 Å^3) was composed of 15 aa (positions: 123, 125, 126, 127, 128, 129, 218, 219, 415, 417, 438, 459, 460, 488, and 490). The second largest cavity (surface area = 744.459 Å^2; volume = 1673.825 Å^3) was composed of 14 aa (positions: 77, 82, 83, 107, 108, 109, 113, 138, 312, 314, 336, 338, 386, and 387). The third largest cavity (surface area = 897.343 Å^2; volume = 2028.901 Å^3) was composed of 14 aa (positions: 295, 318, 346, 360, 363, 364, 365, 368, 390, 391, 394, 396, 418, and 420) (Figure 2A). The largest protrusion (surface area = 3067.323 Å^2; volume = 12616.679 Å^3) was composed of 24 aa (positions: 57, 58, 59, 79, 192, 246, 247, 248, 249, 250, 251, 256, 257, 258, 266, 267, 269, 271, 272, 275, 277, 282, 284, and 431). The second largest protrusion (surface area = 2863.407 Å^2; volume = 9825.691 Å^3) was composed of 22 aa (positions: 213, 214, 215, 218, 222, 223, 224, 225, 227, 492, 495, 565, 566, 567, 590, 591, 592, 611, 613, 617, 618, 630, and 631). The third largest protrusion region (surface area = 897.343 Å^2; volume = 2028.901 Å^3) was composed of 17 aa (positions: 323, 325, 373, 374, 375, 377, 398, 400, 426, 473, 475, 476, 519, 523, 579, 580, 582, and 585) (Figure 2B). The large flat region (surface area = 638.080 Å^2; volume = 1061.563 Å^3) was composed of 12 aa (positions: 261, 262, 263, 264, 265, 266, 270, 272, 275, 276, 277, and 278) (Figure 2C).

Table S1. Sequences of the oligonucleotides used for site-directed mutagenesis.

Target sites	Oligonucleotide sequences (the nucleotide changes are underlined)
S354F	5’-CAACTACAACCACCTCTTCGGAGCGTCCAACT-3’
	5’-AAGAGGTGGTTGTAGTTGTTATTGGTGTCTG-3’
G355F	5’-ACTACAACCACCTCAGCTTTGCGTCCAACT-3’
	5’-AAAGCTGAGGTGGTTGTAGTTGTTATTGGT-3’
A356F	5’-AACCACCTCAGCGGATTTTCCAACTGCCACT-3’
	5’-AAATCCGCTGAGGTGAAAGTAGTTGTTATTG-3’
S357F	5’-AACCACCTCA GCGGAGCGTT CAACTGCCACT-3’
	5’-AACGCTCCGC TGAGGTGGTT GTAGTTGTTA-3’
S357F/G355F	5’-ACCACCTCAGCTTCGCGTTCAACTGCCACTC-3’
	5’-GAGTGGCAGTTGAACGCGAAGCTGAGGTGGT-3’
Q418G	5’-TACAGTTCGATGCCGTCGGTGCCCCAGTACCAGCTGCCCACCT-3’
	5’-AGGTGGGCAGCTGGTACTGGGGCACCGACGGCATCGAACTGTA-3’
D420G	5’-CCCTTGTACAGTTCGATGCCGGCCGTCTGCCAGTACCAGCTGC-3’
	5’-GCAGCTGGTACTGGCAGACCGGCGGCATCGAACTGTACAAGGG-3’
E423G	5’-AGACCGACGGCATCGGCCTGTACAAGGGC-3’
	5’-GCCGATGCCGTCGGTCTGCCAGTACCAG-3’
D439G	5’-TGGTACATCTTCAGCACGTCGGCGTTCGCG TTGAAGAAGGTGT-3’
	5’-ACACCTTCTTCAACGCGAACGCCGACGTGCTGAAGATGTACCA-3’
D440G	5’-TCAACGCGAACGACGGCGTGCTGAAGAT-3’
	5’-CCGTCGTTCGCGTTGAAGAAGGTGTTC-3’
W507G	5’- CCTGAACTCCTCGTCGCACGGGGAAGACAT -3’
	5’- CGTGCGACGAGGAGTTCAGGATGCAGGTGT -3’

Table S2. Amino acid compositions of Aodex and Dex49A.

	Amino acid composition	
	Aodex	Dex49A
Ala	6.50%	5.80%
Arg	3.10%	2.30%
Asn	8.70%	7.50%
Asp	6.30%	5.80%
Cys	0.70%	1.00%
Gln	3.70%	3.80%
Glu	6.50%	2.60%
Gly	7.20%	9.60%
His	2.70%	2.30%
Ile	4.40%	7.70%
Leu	4.80%	4.20%
Lys	5.10%	3.00%
Met	2.40%	2.10%
Phe	3.20%	4.20%
Pro	3.60%	5.80%
Ser	7.20%	11.00%
Thr	8.00%	6.80%
Trp	3.10%	2.60%
Tyr	5.50%	4.50%
Val	7.50%	7.30%

Figure S1. Plate-type crystals, with maximum dimensions of 0.2 mm × 0.1 mm × 0.1 mm, appeared within a month in the reservoir solution, where one drop contained 1 μL of Aodex (10 mg/mL) and 1 μL of reservoir solution.

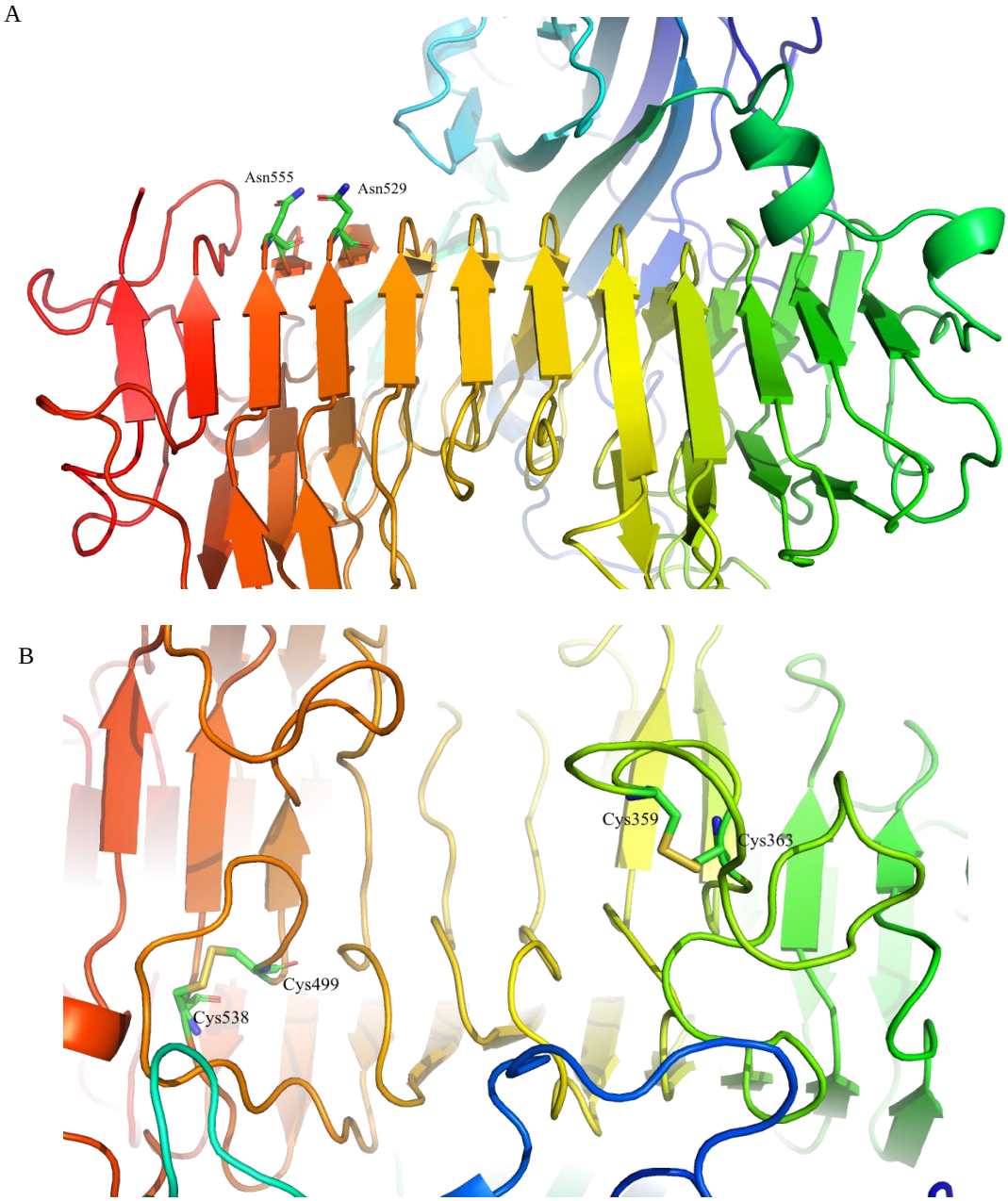

Figure S2. A: The asparagine ladder formed by Asn529 and Asn555. B: The two Aodex disulfide bridges (Cys359-Cys363 and Cys499-Cys538).