

Supplementary Information for:

Maltodextrin Transport in the Extremely Thermophilic, Lignocellulose Degrading Bacterium *Anaerocellum bescii* (f. *Caldicellulosiruptor bescii*)

Hansen Tjo ¹, Virginia Jiang ¹, Jerelle A. Joseph ^{1,2}, Jonathan M. Conway ^{1,2,3,4,5,#}

Author Affiliations:

¹ Department of Chemical and Biological Engineering, Princeton University, Princeton, NJ 08544, USA

² Omenn-Darling Bioengineering Institute, Princeton University, Princeton, NJ 08544, USA

³ Department of Molecular Biology, Princeton University, Princeton NJ 08544, USA

⁴ Andlinger Center for Energy and the Environment, Princeton University, Princeton, NJ 08544, USA

⁵ High Meadows Environmental Institute, Princeton University, Princeton, NJ 08544, USA

#Corresponding Author: Jonathan M. Conway jmconway@princeton.edu

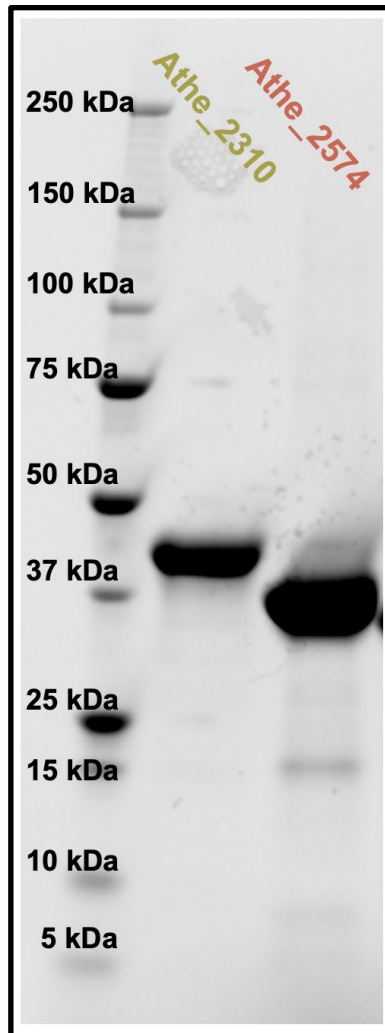


Figure S1: SDS-PAGE gel of purified Athe_2310 and Athe_2574 used in this study.

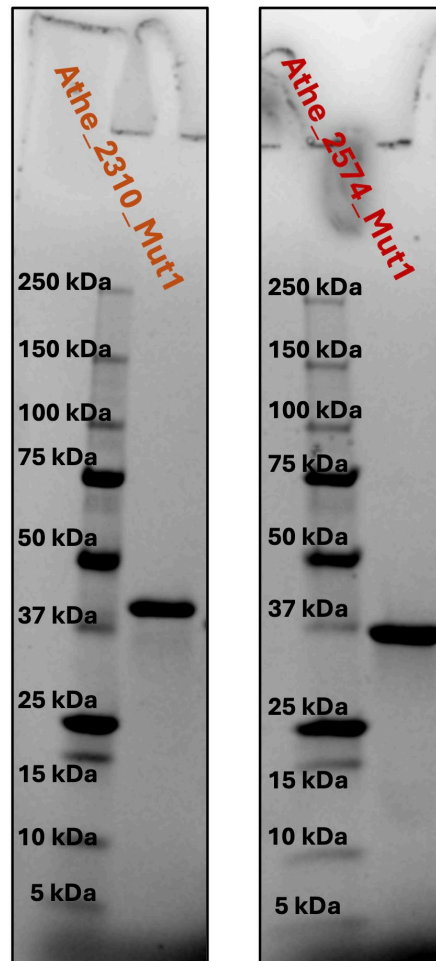


Figure S2: SDS-PAGE gel of purified MBP mutants *Athe_2310_Mut1* and *Athe_2574_Mut1* used in this study.

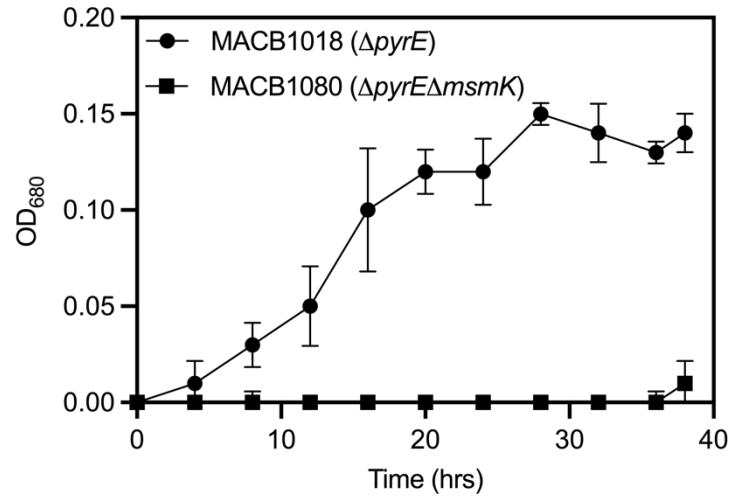


Figure S3: Growth curves of *msmK* deletion *A. besicii* strain MACB1080 ($\Delta pyrE \Delta msmK$) and parent strain MACB1018 ($\Delta pyrE$) on maltose substrate. MACB1080 is unable to grow on maltose, indicating that the Athe_2308 – 2310 and Athe_2574 – 2578 transporters rely on MsmK for ATP-powered maltodextrin uptake. All growth curves were performed in triplicate; error bars indicate the standard deviation about the mean.

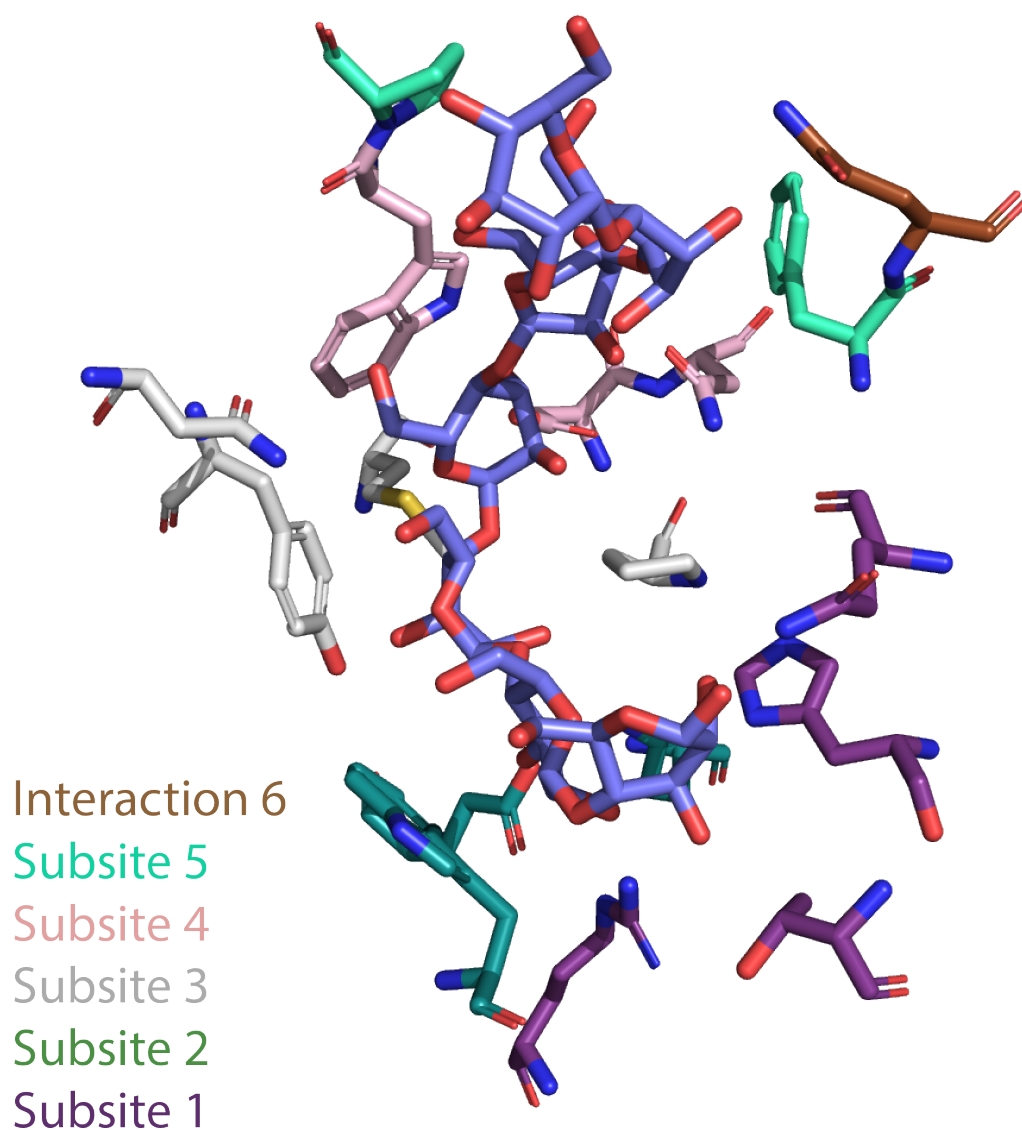


Figure S4: Predicted orientation of maltoheptaose (G7) binding in an AlphaFold2 predicted structure of Athe_2574. Additional glucosyl residues that cannot occupy one of the existing five subsites within the binding pocket are stabilized by hydrogen bonding interactions with residues on the protein surface, such as shown by Interaction 6.

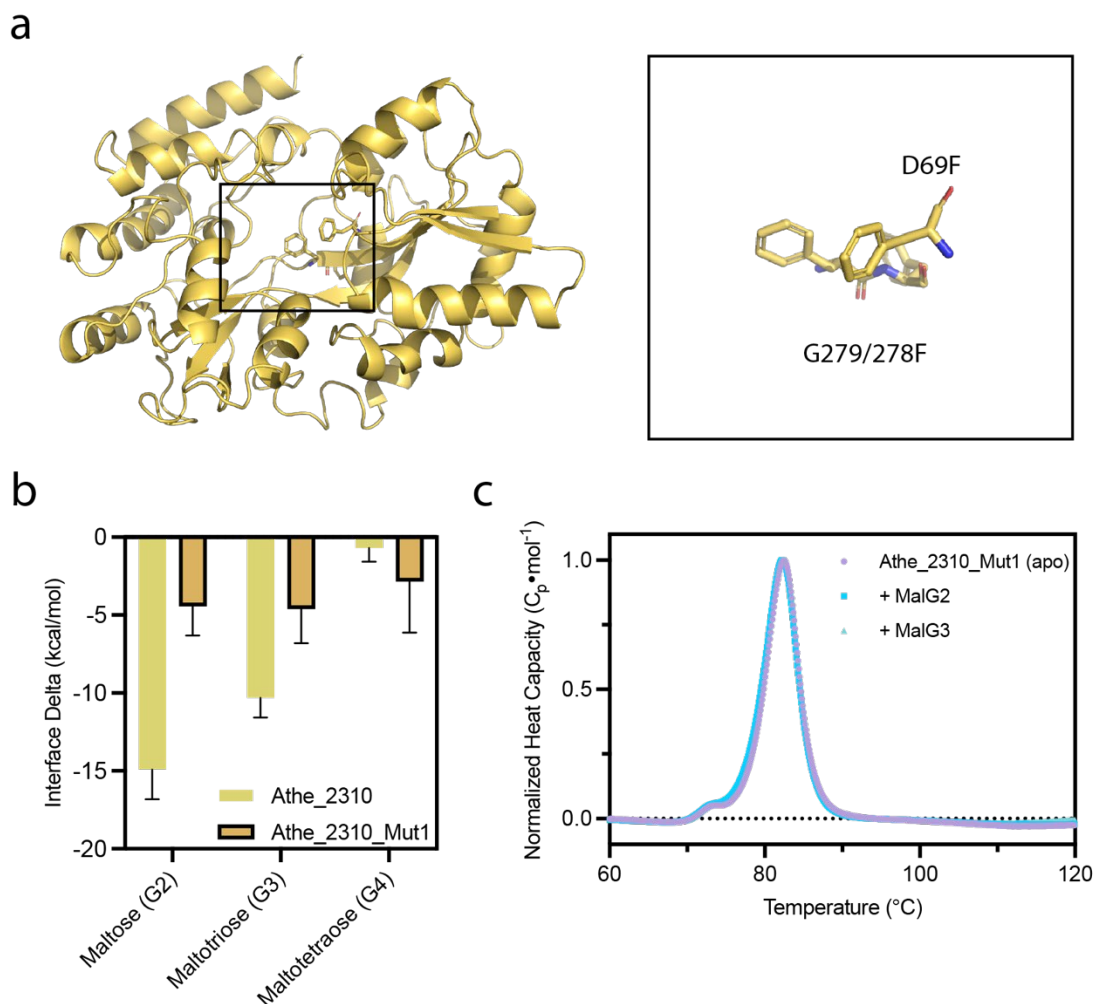


Figure S5: a) AlphaFold2 predicted structure of Athe_2310_Mut1. A close-up view of the binding pocket illustrates all mutated residues to perturb ligand coordination. b) Interface energy deltas, corresponding to free energies of binding, show that simulated Athe_2310_Mut1 binds maltodextrin substrates (G2 – G4) worse than wild-type Athe_2310 ($n = 200$). c) Normalized DSC screens of Athe_2310_Mut1 mixed with its cognate maltodextrins show minimal changes to its melting temperature, indicating an absence of ligand-induced conformational change as shown in Figure 4.

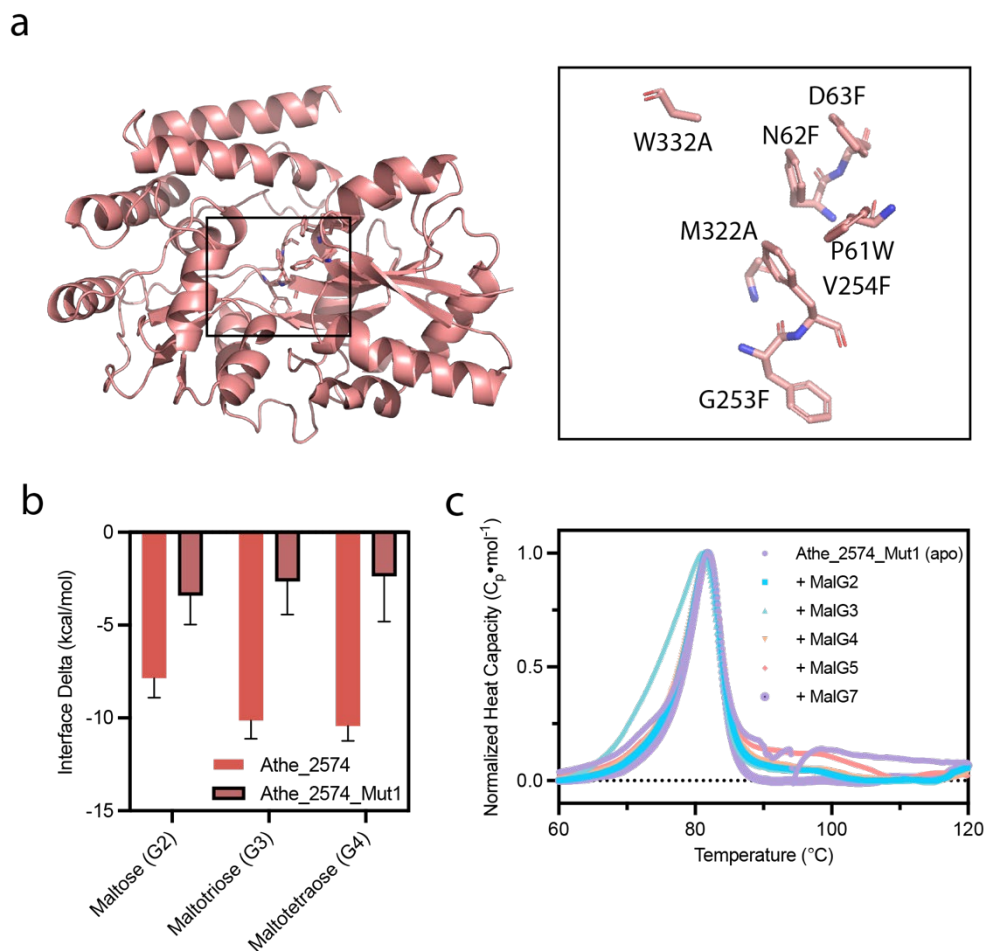


Figure S6: a) AlphaFold2 predicted structure of Athe_2574_Mut1. A close-up view of the binding pocket illustrates all mutated residues to perturb ligand coordination. b) Interface energy deltas, corresponding to free energies of binding, show that simulated Athe_2574_Mut1 binds its maltodextrin substrates (G2 – G4) worse than wild-type Athe_2574 ($n = 200$). c) Normalized DSC screens of Athe_2574_Mut1 mixed with various maltodextrins show minimal changes to its melting temperature, indicating an absence of ligand-induced conformational change as shown in Figure 4.

Table S1: Table of primers used in this study.

Primer	DNA Sequence (5' – 3')	Application
HT001_F	GCTGCCACCGCTGAGCAATAACTAG	pRSF1-b Vector.FOR (backbone linearization)
HT002_F	cttgctgctgcatccacgtgatg	pRSF1-b Vector.REV (backbone linearization)
HT005	CACGTGGATGACGACGACAAGGCTT CTAAAAAACAGGTCACAATTACTTAT GTTCG	pHT002 Fragment FOR (Insert amplification from <i>A. bescii</i> genomic DNA)
HT006	TTGCTCAGCGGTGGCAGCTTACTTT GAAGTTTTTACAACCTTCTTTTAATTC TTGTCC	pHT002 Fragment REV (Insert amplification from <i>A. bescii</i> genomic DNA)
HT134	tgagatccggctgctaacaag	pCri8a Vector.FOR (backbone linearization)
HT135	catggcgccctggaagtaaag	pCri8a Vector.REV (backbone linearization)
HT136	tttacttcagggcgccatggttACATCCAAAA ACAGCTTGTTGTC	pHT003a Fragment.FOR Athe_2574 (Insert amplification from <i>A. bescii</i> genomic DNA)
HT137	ctttgtagcagccgatctcaTTATTGCATCT GAGCAATACCTTGCTTG	pHT003a Fragment.REV Athe_2574 (Insert amplification from <i>A. bescii</i> genomic DNA)

Table S2: Amino acid sequences of all proteins analyzed in this study. Residues comprising the signal peptide sequence are marked in red.

Protein	Amino Acid Sequence
Athe_2310 (native with signal peptide) (JGI Accession: YP_002574153)	MKRFI AVMVLI AFSVGLFLAFGPANSNA ASKKQVTITYVRGKDETHATE KIIKEFMKKNPDI NVIYKENPSDTGQNHDQLVTVLSAGGSDIDVFDMDV IWPAEFAQAGYTLPLDRFIKRDKTNLNDYIKGTIDAARFKGQMWAFFPR FIDAGLLYYRKDIVPQNELPKTWDDLIKVAKKYKGKNGTKYGFLMQAK QYEGLVCD AIEYIASYGGKVVD ESGNIVVNNQGTIDGLNMMRKVITSGI VPPNINTFTEVETHTAFINGLSVFARNWPYMWAMINSPQSKVRGKVGIL LPLPKGSKGSAACLGGMVGINKFSKNPEASWRLLKFLVQKEGQKL MAIYNGNVPVYKPLFNDKDV IKANPLIGDKKFIEAILAAVPRPVSP IYPKI SDVMQIELSNIVNGKKDVKTAVADM DKKLKEVVKTSK
Athe_2574 (native with signal peptide) (JGI Accession: YP_002574412)	MKNLKRIL TVALIIT FAVVALIPLSGVFAT SKKQLVWWSHLTQDEVKALQ PIADKWGKENG YTVKVITDQGSFQSFQTAAMSGKGPDIMFGIPHDNL GAFWKAKLLEAVPANLIDKKNFVSTALDACSFEGLYALPIAMETYALF YNTSKVKEAPKTMSQLITLAKKYGFMYDVNNFYFSFAFIAQNGGYVFK NKGSLDPNDIGLATNGAIKGLSLIRDFVQTYKFMPKDIKGDIAGNFKQ NQKIAFYISGPWDVQDFIKAKVPFAVAPLPKTDDGKPTPSFVGVAFAF VSAKSKNKDAAFKLMKYL VENSALT LFKVGHRI PVLNKVLTSSEVKAD KIMSAFAEQAKVGIPMPNIP EMSAVWPVANNALSLITTGKATPKQAAD AMVKQIKQGIAQMQ
Athe_2310 (cloned in <i>E. coli</i>)	MAHHHHHHVDDDDKASKKQVTITYVRGKDETHATEKIIKEFMKKNPDI NVIYKENPSDTGQNHDQLVTVLSAGGSDIDVFDMDVIWPAEFAQAGY TLPLDRFIKRDKTNLNDYIKGTIDAARFKGQMWAFFPRFIDAGLLYYRKD IVPQNELPKTWDDLIKVAKKYKGKNGTKYGFLMQAKQYEGLVCD AIEY IASYGGKVVD ESGNIVVNNQGTIDGLNMMRKVITSGIVPPNINTFTEVE THTAFINGLSVFARNWPYMWAMINSPQSKVRGKVGILPLPKGSKGSA ACLGGMVGINKFSKNPEASWRLLKFLVQKEGQKLMAIYNGNVPVY KPLFNDKDV IKANPLIGDKKFIEAILAAVPRPVSP IYPKISDVMQIELSNIV NGKKDVKTAVADM DKKLKEVVKTSK
Athe_2574 (cloned in <i>E. coli</i>)	MGSSHHHHHSSGENLYFQGAMVTSKKQLVWWSHLTQDEVKALQPI ADKWGKENG YTVKVITDQGSFQSFQTAAMSGKGPDIMFGIPHDNLGA FWKAKLLEAVPANLIDKKNFVSTALDACSFEGLYALPIAMETYALFY N TSKVKEAPKTMSQLITLAKKYGFMYDVNNFYFSFAFIAQNGGYVFKNK GGSLDPNDIGLATNGAIKGLSLIRDFVQTYKFMPKDIKGDIAGNFKQ KIAFYISGPWDVQDFIKAKVPFAVAPLPKTDDGKPTPSFVGVAFAFVS AKSKNKDAAFKLMKYL VENSALT LFKVGHRI PVLNKVLTSSEVKADKIM SAFAEQAKVGIPMPNIP EMSAVWPVANNALSLITTGKATPKQAADAMV KQIKQGIAQMQ
Athe_2310_ pointmut #D69F G279F G278F (Athe_2310_Mut1)	MAHHHHHHVDDDDKASKKQVTITYVRGKDETHATEKIIKEFMKKNPDI NVIYKENPSDTGQNHDQLVTVLSAGGSDIDVFDMFVIWPAEFAQAGY TLPLDRFIKRDKTNLNDYIKGTIDAARFKGQMWAFFPRFIDAGLLYYRKD IVPQNELPKTWDDLIKVAKKYKGKNGTKYGFLMQAKQYEGLVCD AIEY IASYGGKVVD ESGNIVVNNQGTIDGLNMMRKVITSGIVPPNINTFTEVE THTAFINGLSVFARNWPYMWAMINSPQSKVRGKVGILPLPKGSKGSA ACLFFWMVGINKFSKNPEASWRLLKFLVQKEGQKLMAIYNGNVPVYK

	PLFNDKDVIKANPLIGDKKFIEAILAAVPRPVSPYIPKISDVMQIELSNIV NGKKDVKTAVADMCKLKEVVKTSK
Athe_2574_pointmut #G253F V254F P61W N63F D64F M322A W332A (Athe_2574_Mut1)	MGSSHHHHHHSSGENLYFQGAMKQLVWWSHLTQDEVKALQPIADK WGKENG YTVKVITDQGSFQSFQTAAMSGKGPDIMFGIFHFFLGAFWK AKLLEAVPANLIDKKNFVSTALDACSFEGKLYALPIAMETYALFYNTSK VKEAPKTMSQLITLAKKYGFMYDVNNFYFSFAFIAQNGGYVFKNKGG SLDPNDIGLATNGAIKGLSLIRDFVQTYKFMPKDIKGDIAKGNFQNQKI AFYISGPWDVQDFIKAKVPFAVAPLPKTDDGKPTPSFFVQAAAFVSAK SKNKDAAFKLMKYL VENSALT LFKVGH RIPVLNKVLTSSSEVKADKIMSA FAEQAKVGIPAPNIPEMSAVAPVANNALSLITTGKATPKQAADAMVKQI KQGIAQM
TmMBP3 (PDB: 6DTQ)	MAVKITMTSGGVGKELEV LKKQLEM FHQQYPDIEVEIIPMPDSSTERH DLYVTYFAAGETDPDVLMLDVIWPAEFAPFLEDLTADKDYFELGEFLP GTVMSVTVNGRIVAVPWFTDAGLLYYRKDLLEKYGYDHAPRTWDELV EMAKKISQAEGIHGFVWQGARYEGLVCD FLEYLWSFGGDVLDES GK VVIDSPEAVAALQFMVDLIYKHKVTPEGVTTYMEEDARRIFQNGEAVF MRNWPYAWSLVNSDESPIKGVGVAPLPMGPGGRRRAATLGGWVLGI NKFSSPEEKEAAKKLIKFLT SYDQQLYKAINAGQNPTRKAVYKDPK LK EAAPFMVELLGVFINALPRPRVANYTEVSDVIQRYVHAALTRQTTSED AIKNI AKELKFLLGQHHHHH
TMBP (PDB: 1EU8)	IEEGKIVFAVG GAPNEIEYWKGVIAEF EKYPGVTVELKRQATDTEQR RLDLVNALRGKSSDPDVLMDVAWL GQFIASGWLEPLDDYVQKDNY DLSVFFQSVINLADKQGGKLYALPVYIDAGLLYYRKDLLEKYGYSKPP ETWQELVEMAQKIQSGERETNPFWGFVWQGKQYEGLVCD FVEYV YSNGGSLGEFKDGKWVPTLNK PENVEALQFMVDLIHKYKISPPNTYT EMTEEPVRLMFQQGNAAFERNWPYAWGLHNADDSPVKGVGVAPL PHFPGHKSAATLGGWHIGISKYSDNKALAWEFVKFVESYSVQKGFAM NLGWNPGRVDVYDDPAVVSKSPHLKELRAVFENAVPRPIVPPYQLS EIIQKYVNSALAGKISPQEALDKAQKEAEELVKQYS
TtMBP (PDB: 6J9W)	QSGPVIRVAGDSTAVGEGGRWMKEMVEAWGKKTGTRVEYIDSPADT NDR LALYQQYWAARSPD VDVY MIDVIWPGIVAPHALDKPYL TEAE LK EFFPRIVQNN TIRGKLTSLPFFT DAGILYYRKDLLEKYGYTSPRTWNE LEQMAERVM EGERRAGNRDFWGFVFQGKPYEGLTCDALEWIYSHG GGRIVEPDGTISVNNGRAALALNRAHGWWGRIAPQGVTSYAE E EARN VWQQGNSLFMRNWPYAYALGQAEGSPIRGKFGVTLPKASADAPNA ATLGGWQLMVSAYSRYPK EAVDLVKYLASYEVQKDN AVRLSRLPTRP ALYTDRDVLARNPWFRDLLPVFQNAVSRPSDVAGARYNQVSEAIWTE VHSVLTGRKKGEQAVRDLEARIRRLRHHHHH
PfuMBP (PDB: 1ELJ)	MKIEEGKVVIWHAMQPNELEV FQSLAE EYMALCPEVEIVFEQKPNLED ALKAAIPTGQGPD LFIWAHDWIGKFAEAGLLEPID EYVTEDLLNEFAPM AQDAMQYKGHYALPFAAETVAIIYNKEMVSEPPKTFDEMKAIMEKYY DPANEKYGIAPINAYFISAIAQAFGGYYFDDKTEQPGLDK PETIEGFK FFFTEIW PYMAPTGDYNTQQSIFLEGRAPMMVNGPWSINDVKKAGIN FGVVLPIIKD GKEYWPRPYGGVKLIYFAAGIKNKDAAWKFAKWLTT SEESI KTLALELGYIPVLTKVLDDPEIKNDPVIYGFQQAVQHAYLMPKS PKMSAVWGGVDGAINEILQDPQNADIEGILKKYQQEILNNMQG

TmMBP1 (PDB: 6DTU)	MQPKLTIWCSEKQVDILQKLGEFEKAKYGVVEVQYVNFQDIKSKFLT AAPEGQGADIIVGAHDWVGELAVNGLIEPIPNFSDLKNFYETALNAFSY GGKLYGIPYAMEAIALIYNKDYVPEPPKTMDELIEIAKQIDEEFGGEVR GFITSAAEFYIAPFIFGYGGYVFKQTEKGLDVNDIGLANEGAIGVKLL KRLVDEGILDPSDNYQIMDSMFREGQAAMIINGPWAIKAYKDAGIDYG VAIPDLEPGVPARPFVGVQGFVMVNAKSPNKLLAIEFLTSFIAKKETMY RIYLGDPRLPSRKDVLELVKDNPDVVGFTLSAANGIPMPNVPQMAAV WAAMNDALNLVVNGKATVEEALKNAVERIKAQIQS
TmMBP2 (PDB: 6DTS)	MQTKLTIWCSEKQVDILQKLGEFEKAKYGIPVEVQYVDFGSIKSKFLTA APQGQGADIIVGAHDWVGELAVNGLIEPIPNFSDLKNFYDTALKAFSY GGKLYGVYPYAMEAVALIYNKDYVDSVPKTMDELIEKAKQIDEEYGGEV RGFIYDVANFYFSAPFILGYGGYVFKETPQGLDVTDIGLANEGAVKGA KLIKRMIDEGVLT PGDNYGTMDSMFKEGLAAMIINGLWAIKSYKDAGIN YGVAPIPELEPGVPAKPFVGVQGFMINAKSPNKVIAMEFLT NFIARKET MYKIYLADPRLPARKDVLELVKDNPDVVAFTQSASMGTPMPNVP PMA PVWSAMGDALSIINGQASVEDALKEAVEKIKAQIEKGSHHHHHH

Table S3: DNA sequences of gene synthesis for maltodextrin-binding protein mutants.

Protein	DNA Sequence
Athe_2310_ pointmut #D69F G279F G278F (Athe_2310_Mut1)	GCGAGCAAAAAACAGGTAACCATTACCTATGTGCGTGGCAAAGAT GAAACGCATGCGACCGAAAAAATTATTAAGAGTTTATGAAAAAA ACCCGGATATTAACGTGATTTACAAAGAAAACCCGAGCGATACGG GCCAGAACCATGATCAACTGGTTACCGTGCTGAGCGCAGGTGGCA GCGATATTGATGTGTTTCGATATGTTTCGTGATCTGGCCGGCGGAATT CGCGCAGGCGGGCTATACCCTGCCGCTGGATCGCTTCATTAAACG CGATAAAACCAATCTGAACGATTACATTAAAGGCACCATTGATGCG GCCCCGCTTTAAAGGCCAGATGTGGGCGTTTCCGCGCTTCATTGAT GCGGGCCTGCTGTATTATCGTAAAGATATTGTGCCGCAGAACGAA CTGCCGAAAACCTGGGACGATCTGATTAAAGTGGCCAAAAAATATA AAGGCAAAAATGGCACCAAATACGGCTTTCTGATGCAGGCGAAAC AGTATGAAGGCCTGGTGTGCGATGCGATTGAATACATTGCGTCTTA TGGCGGCAAAGTGGTGGATGAAAGCGGAAATATTGTGGTGAATAA TCAGGGCACCATTGATGGCTTAAATATGATGCGCAAAGTGATTACC AGCGGCATTGTGCCGCCGAACATTAAACAGTTCACCGAAGTGGA ACGCATACCGCGTTTATTAATGGCCTGAGCGTATTTGCGCGCAACT GGCCGTATATGTGGGCGATGATTAAACAGCCCGCAGAGCAAAGTGC GCGGCAAAGTAGGCATTCTGCCGCTGCCGAAAGGCAGCAAAGGC AGCGCCGCGTGTCTGTTTTTCTGGATGGTTGGCATTAAACAATTTA GCAAAAACCCGGAAGCGTCATGGCGCCTGCTGAAATTTCTGGTGC AGAAAGAAGGCCAAAAACTGATGGCGATCTATAACGGTAACGTGC CGGTGTATAAACCGCTGTTTAACGATAAAGATGTTATTAAGCGAA CCCGCTGATCGGCGATAAAAAATTCATTGAAGCGATTCTGGCGGC AGTGCCGCGCCCGGTGAGCCCGATCTATCCGAAAATTAGCGATGT GATGCAGATCGAACTGAGCAACATTGTAAATGGCAAAAAAGATGTG AAAACCGCAGTGGCGGATATGGATAAAAAACTGAAAGAAGTGGTG AAAACCAGCAAATAA
Athe_2574_pointmut #G253F V254F P61W N63F D64F M322A W332A (Athe_2574_Mut1)	AAACAGCTGGTGGTGTGGAGCCATCTGACCCAGGATGAAGTGAAA GCGCTGCAGCCGATTGCGGATAAATGGGGTAAAGAAAACGGTTAT ACCGTGAAAGTGATTACCGATCAGGGCAGCTTTCAGAGCTTCCAG ACCGCCGCGATGAGTGGCAAAGGCCCGGATATTATGTTTGGCATT TTTCATTTTTTTCTGGGCGCGTTCTGGAAAGCGAAACTGCTGGAAG CGGTGCCGGCGAACCTGATTGATAAAAAAACTTTGTTAGCACCG CGCTGGATGCGTGTAGCTTTGAGGGCAAACCTGTATGCGCTGCCGA TCGCGATGGAAACCTACGCGCTGTTTTACAACACCAGCAAAGTGA AAGAAGCGCCTAAAACCATGAGCCAGCTGATTACCCTGGCCAAAA AATATGGCTTTATGTATGATGTGAACAATTTTTATTTTAGCTTTGCG TTTATTGCGCAGAATGGCGGCTATGTGTTCAAAAACAAAGGCGGTT CCCTGGATCCGAACGATATTGGCCTGGCGACCAACGGCGCCATTA AAGGTCTGAGCCTGATTCGCGATTTTGTGCAGACCTATAAATTTAT GCCGAAAGATATTAAGGTGATATTGCCAAAGGCAATTTTCAGAAC CAGAAAATTGCGTTCTATATTAGCGGCCCGTGGGATGTGCAGGAC TTTATCAAAGCGAAAGTGCCGTTTCGCGGTGGCCCCGCTGCCGAAA ACCGATGATGGCAAACCGACCCCGAGCTTTTTTTTTGTGCAGGCG GCGTTTGTGAGCGCGAAAAAGCAAAAATAAAGATGCCGCGTTTAAA

	CTGATGAAATACCTGGTGGAAAACAGCGCCCTGACCCTGTTCAA GTTGGCCATCGTATTCCGGTCCTGAATAAAGTCCTGACCTCAAGC GAAGTGAAAGCCGATAAAATTATGAGCGCGTTTGCCGAACAGGCG AAAGTTGGGATCCCGGCGCCGAATATTCCGGAATGTCGGCCGTG GCCCCGGTGGCCAACAACGCGCTGAGCCTGATTACCACCGGCAA AGCAACCCCGAAACAAGCAGCCGATGCGATGGTTAAACAGATTAA ACAGGGCATCGCGCAGATGTAA
--	---