

Supporting Information

The Structure of Malto-Octaose bound *Eschericia coli* Branching Enzyme Suggests a Mechanism for Donor Chain Specificity

Table of contents

A. Figure S1.....	3-4
B. Figure S2.....	5
C. Figure S3.....	6
D. Figure S4.....	7
E. Crystallographic Table	8

<i>E.coli</i> /1-728	92	QNLIDDPYRF-GPLIQEMDA-----WLLSEGLHLRPYETLCAHADTMD---GVTGTRFSVWAPNARRVSVVGGFNYWDGRRHMPMR LRKESG	173
<i>Klebsiella</i> /1-728	92	QNLIDDPYRF-GPLIQDLVD-----WLLSEGLHLRPYETLCAHADTMD---GVVGTRFSVWAPNARRVSVVGGFNYWDGRRHMPMR LRKESG	173
<i>Y.Pestis</i> /1-727	92	PQIIIDDPYRF-GPLIQDIDS-----WLLAEGLHLRPYERLCAHLSLD---GVSGVSFAVWAPNAQRVSVVGGFNFWDGRRHMPMR LRRENG	173
<i>AgroTum</i> /1-735	101	EWAVTDPYSF-GPVLGPMDD-----YFVREGSHLRLLFDRMGCAHP LKLE---GVEGFHFAVWAPNARRVSVVGGFNNWDGRRHVMR FRKDTG	182
<i>MycroTub</i> /1-722	87	EPHTVADAYRF-LPTLGEVDL-----HLFAEGRHERLWEVLGAHPRSF TTDAGVVS GVSFAVWAPNAKGVSLIGFNGWNGHEAPMRVLGP SG	173
<i>RhodotermObam</i> /1-621	1	-----MSWLTEEDI-----RRWESGTFYDSYRKLGHPDD-----EGTWFCVWAPHADGVSVLGAFNDWNPEANPLERY-GGG	67
<i>Cyano</i> /1-793	115	EKVYDPYAFSSPYLTDEDI-----YLFSEGNHHRIFYEKLGAHVGEIN---GVKGVYFAVWAPNARNVSVIGDFNNWDGRRHMQMRKR-NYT	196
<i>Bac.Subtil</i> /1-627	1	-----MAAASPTAHDV-----YLFHEGSLFKSYQLFGSHYRELN---GKSGYFCVWAPHASEVRVAGDFNSWSGEEHVMHRVNDNG	74
<i>Bac.Caldo</i> /1-666	1	-----MIAANPTDLEV-----YLFHEGRLYQSYELFGAHVIRGG---GAVGTRFCVWAPHAREVRLVGSFNDWNGTNSPLTKVNDNG	74
<i>Bac.Stearothermoph</i> /1-652	1	-----MIAANPTDLEV-----YLFHEGSLYKSYELFGAHVINEG---GKVGTRFCVWAPHAREVRLVGSFNDWNGTDFRLEKVNDEG	74
<i>Yeast</i> /1-704	22	ADVLSERRYLADKWLYDITATPDGSSYQSLSKFARDSSKSYGLHANPET---KEITYKEWAPNAERAFLVGDFNNWDTTSHELKNKDEFG	108
<i>Rice</i> /1-755	24	KDHFNYRIKRYLDQKCLIEK-----HEGGLLEEFSGKYLKFGINTVD---GATIIYREWAPAAQEAQLIGDFNNWNGAKHKMEKD-KFG	101
<i>Potato</i> /1-861	109	LDHFRHRMKRYVDQKMLIEK-----YEGPLEEFAQGYLKFGRNED---GCIVYREWAPAAQEDVIGDFNCGNGSNHMMEKD-QFG	186
<i>Arab</i> /1-858	171	RNHLDYRYQYRKLRLEEIDK-----NEGGLEAFSGRYEFGFTRSA---TCITYREWAPAKAASLIGDFNNWNAKSDVMARN-DFG	248
<i>HomoSap</i> /1-702	42	AVDFQRRYKQFSQILKNIGE-----NEGGIDKFSRCYESFVHRCAD-----GGLYCKEWAPAGEVFLTGDFNGWNPFSYPYKKL-DYG	120
<i>E.coli</i> /1-728	174	IWELFIPGA-----HNCQLYKYEMIDANG-NLR LKSDPYAFEAQMRPETA---SLICGL-----PEKVV---QTEERKKANQFDAPIS IY	246
<i>Klebsiella</i> /1-728	174	IWELFVPGA-----LNCQLYKFELIDAHG-NLRVKSDPYAFEAQMRPESA---SLICGL-----PEKVA---QPPERKKANQFDAPIS IY	246
<i>Y.Pestis</i> /1-727	174	IWELFLPGI-----EAGEVYKFEIIDCHG-QVRLKADPYAFEAQMRPETA---SLISQL-----PDVVK---SSAARQKANDLCSPVSIY	246
<i>AgroTum</i> /1-735	183	IWEIFAPDV-----YAGCAYKFEVLGANGELLPLKADPYARRAELRPKNA---SVTAP E---LTQKWEDQAHREHWAQVDQRQRP I S IY	260
<i>MycroTub</i> /1-722	174	IWELFWPDP-----PCDGLYKFRVHGADG-VVTDRAADPYAFGETVPPQTA---SRVTS---SDYTGWDDWMAKRALRPVNEAMSTY	249
<i>RhodotermObam</i> /1-621	68	LWAGYVPGA-----RP GHTYKYRI RHGF---YQADKTDPAFYAMEPRTGSP IEGLAS I ITR---LDYTWHDDDEWRRKRGASLYEPVSIY	147
<i>Cyano</i> /1-793	197	IWELFVPEI-----GSGTVYKYEIKNSEG-HIYEKSDPYAGYERVPNTA---SIVVDI---DNIIQWHDDEWLKRRNSDPLKQPSVSY	274
<i>Bac.Subtil</i> /1-627	75	IWTLFIPGI-----GEKERYKYEIVTNNC-EIRLKADPYAIYSEVRPNTA---SLTYDL---EGYSWQDQKQWKKQKAKTLYEKPVIY	151
<i>Bac.Caldo</i> /1-666	75	VMTIVVPE-----LEGHLYKYEIITPDG-RVLLKADPYAFYSELRPHTA---SIVYDL---KGYEWNDSPWKRKKRRRIYDQPMVIY	151
<i>Bac.Stearothermoph</i> /1-652	75	VMTIVVPE-----LEGHLYKYEIITPDG-QVLFKADPYAFYSELRPHTA---SIAYDL---KGYEWNDSPWKRKKRRRIYDQPMVIY	151
<i>Yeast</i> /1-704	109	NFTITLHPLNGDFAIPHDSKIVMFI LPDG-SKIFRLPAWITRATOPSKET---SKQFGPAYEGRFVWPNKPYKFVHPRPKPSESVDSLRIY	197
<i>Rice</i> /1-755	102	ISIKISHVN-GKPAIPHNSKVKFRFRHGGG-AWVDRI PAWIRYATFDASKF---GAPYDG---VHWDPPACERYVFKHPRPKPDAPRIY	184
<i>Potato</i> /1-861	187	VMSIRIPDVG-SKPVIPHNSRYKFRFKHNG-VWVDRI PAWIKYATADATKF---AAPYDG---VYWDPPPSERYHFYKPRPKPRAPRIY	269
<i>Arab</i> /1-858	249	VWEIFLPPNADGSPAIPHGSRYKIRMDTPS-GIKDSIPAWIKYSVQPPGEI---PYNG---VYDPP EEDKYAFKHPRPKKPTSLRIY	329
<i>HomoSap</i> /1-702	121	KWELYIPPKQNKSVLPVHGSKLVVITSKSG-EILYRISPAWKYVVR EGDNV---NYDW-----IHWDP EHSYEFKHSRPPKPRSLRIY	200
<i>E.coli</i> /1-728	247	EVHLGSWRRHTD-----NNFWLSYRELADQLVPYAKWMGFTHLELLPINEHFDG SWGYQPTGLYAPTRRFGTTRDDFR	319
<i>Klebsiella</i> /1-728	247	EVHLGSWRRHTD-----NNFWLSYRELADQLVPYAKWMGFTHLELLPINEHFDG SWGYQPTGLYAPTRRFGTTRDDFR	319
<i>Y.Pestis</i> /1-727	247	EVHLGSWRRHTD-----NNFWLSYRELADQLVEYVKYMGFTHVELLPINEHFDG SWGYQPLGLYAPTRRRYGTTFEDFK	319
<i>AgroTum</i> /1-735	261	EVHAGSWQRR E-----DGTFLSWDELAALIPYCTDMGFTHIEFLPITEHPYDP SWGYQPTGLYAPSAFGDP EGF A	332
<i>MycroTub</i> /1-722	250	EVHLGSWRP-----GLSYRLARELTDYIVDQGFTHVELLPVAEHPFAGSWGYQVTSYAPTSRFGTTPDDFR	316
<i>RhodotermObam</i> /1-621	148	EVHLGSWRHRR-----PGESFYREIAEP LADYVQEMGFTHVELLPVEHPPYGSWGYQVVGYYAPTFRYGSQDLM	219
<i>Cyano</i> /1-793	275	EVHLGSWLHGSSAEKMP LLNGEADPVI VSEWNP GARFLSYELAEKLI PYVKDMGYTHIELLP IAEHPFDG SWGYQVTFGYSPTSRFRGPEDFM	368
<i>Bac.Subtil</i> /1-627	152	ELHLGSWKKHS-----DGRHYSYKELSQTLIPYIKEHGFTHIELLPVYEHPPYDR SWGYQGTGYSPTSRFGCPHDLM	223
<i>Bac.Caldo</i> /1-666	152	ELHFGSWKKKP-----DGRFYTYREMADELI SYVLDHGFTHIELLP LVEHPLDR SWGYQGTGYAVTSRYGTPHDFM	222
<i>Bac.Stearothermoph</i> /1-652	152	ELHFGSWKKK-----DGRFYTYREMADELI SYVLDHGFTHIELLP LVEHPLDR SWGYQGTGYAVTSRYGTPHDFM	222
<i>Yeast</i> /1-704	198	EAHVGISSPE-----PKITTYKEFTEKVLPRIKLYGDAIQLMAIMEHAYYASFGYQVTNFFAASSRFGTPEELK	267
<i>Rice</i> /1-755	185	EAHVGMSSGE-----PEVSTYREFADNVLPRI RANNYNTVOLMAIMEHSYYASFGYHVTNFFAVSSRSRGTPEDLK	254
<i>Potato</i> /1-861	270	EAHVGMSSSE-----PRVNSYREFADNVLPRI RANNYNTVOLMAIMEHSYYGSGFYHVTNFFAVSSRSRGNPDLK	339
<i>Arab</i> /1-858	330	ESHVGMSSSTE-----PKINTYANFRDDVLPRI KKLGYNAVQIMAIQEHAYYASFGYHVTNFFAVSSRSRGTPEDLK	399
<i>HomoSap</i> /1-702	201	ESHVGISSHE-----GKVASYKHFTCNVLPRI KGLGYNCIQLMAIMEHAYYASFGYQITSSFAASSRYGSP EELQ	270
<i>E.coli</i> /1-728	320	YFIDAHAAGLNVILDWVPGHFPPTDD-FALAE FDG---TNLYEHSDPREGYHQDWNTLIYNYGRREVS NFLVGNALYWI ERFGIDAL RVD AVAS	409
<i>Klebsiella</i> /1-728	320	YFINAAHAAGLNVILDWVPGHFPADD-FALAS FDG---TSLYEHSDPREGYHQDWNTLIYNYGRREVS NLYVGNALYWI ERFGIDAL RVD AVAS	409
<i>Y.Pestis</i> /1-727	320	AFVAKFHQAGINVLIDWVPGHFP SDE-HGLST FDG---TALY EYADPREGYHQDWNTLIYNYGRREVRNYLACNAFYWMERFGIDAL R I D AVAS	409
<i>AgroTum</i> /1-735	333	RFVNGAHKVGICGVLLDWVPAHFP TDE-HGLRW FDG---TALYEHADPRQGFHPDWNTAIYNFGR I EVMSYLLINNALYWAEFHLDGLRVD AVAS	422
<i>MycroTub</i> /1-722	317	ALVDA LHQAGIGVILDWVPAHFP KDA-WALGR FDG---TPLYEHSDPKRGEQLDWCTYVDFGRPEVRNFLVANALYWLQEFHIDGLRVD AVAS	406
<i>RhodotermObam</i> /1-621	220	YLIDYLHQRCIGVILDWVPSHFAADP-QGLVF FDG---TTLFEYDDPKMRYHPDWCTYVDFYKNPKGVRNFLI SNAFLWLEKYHVDGLRVD AVAS	309
<i>Cyano</i> /1-793	369	YFVDKCHENGIGVILDWVPGHFPKDS-HGLAY FDG---THLYEHADPR I GEHKWGT LVFNRYGRHEVRNFLVANVLFWFDKYHYVDGIRVD AVAS	458
<i>Bac.Subtil</i> /1-627	224	KFVDECHQQNIGVILDWVPGHFCCKDA-HGLYM FDG---EPLYEYKEERDRENWLGTANFDLGKPEVHSFLI SNAFLYWAFFYHIDGFRVD AVAN	313
<i>Bac.Caldo</i> /1-666	224	YFVDRCHQAELGVILDWVPGHFCCKDA-HGLYM FDG---APT YEYANEKDR ENYVWGTCANFDLGKPEVRSFLI SNAFLWLEYYHYVDGFRVD AVAN	313
<i>Bac.Stearothermoph</i> /1-652	223	YFVDRCHQAELGVIMDWVPGHFCCKDA-HGLYM FDG---APT YEYANEKDR ENYVWGTCANFDLGKPEVRSFLI SNAFLWLEYYHYIDGFRVD AVAN	312
<i>Yeast</i> /1-704	268	ELIDTAHSMGILVLLDVVHSHASKNVEDGLNMF DG-SDHOYFHSISSGRGEHPLWDSRLFN YGKFEVQRFLLANLAFYVDVYQFDGFRFDGVT S	360
<i>Rice</i> /1-755	255	YLVDKAHS LGLRVLDVHSHASNNVT DGLNGYDVGNQTHESYFHTGDRGYHKLWDSRLFN YANWEVLR FLLSNLRYWMOEFMFDGFRFDGVT S	348
<i>Potato</i> /1-861	340	YLIDKAHSLGLQVLVDVHSHASNNVT DGLNGYDVGGSQESYFHAGERGYHKLWDSRLFN YANWEVLR FLLSNLRWLLEENFDGFRFDGVT S	433
<i>Arab</i> /1-858	400	SLIDKAHELGLVLMDOI VSHASKNTLDGLDM FDG---TDGQYFHS GSRGYHMMWDSRLFN YGSWEVLR YLLSNARWLLEEKFDGFRFDGVT S	490
<i>HomoSap</i> /1-702	271	ELVDTAHSMTIIVLLDVVHSHASKNSADGLNMF DG-SDSCYFHS GPRGTHDLWDSRLFAYS SWEVLR FLLSNIRWLLEEYRFDGFRFDGVT S	361

- site I
- site II
- site III
- site IV
- site V
- site VI
- site VII
- site VIII
- site IX
- site X
- site XI
- site XII

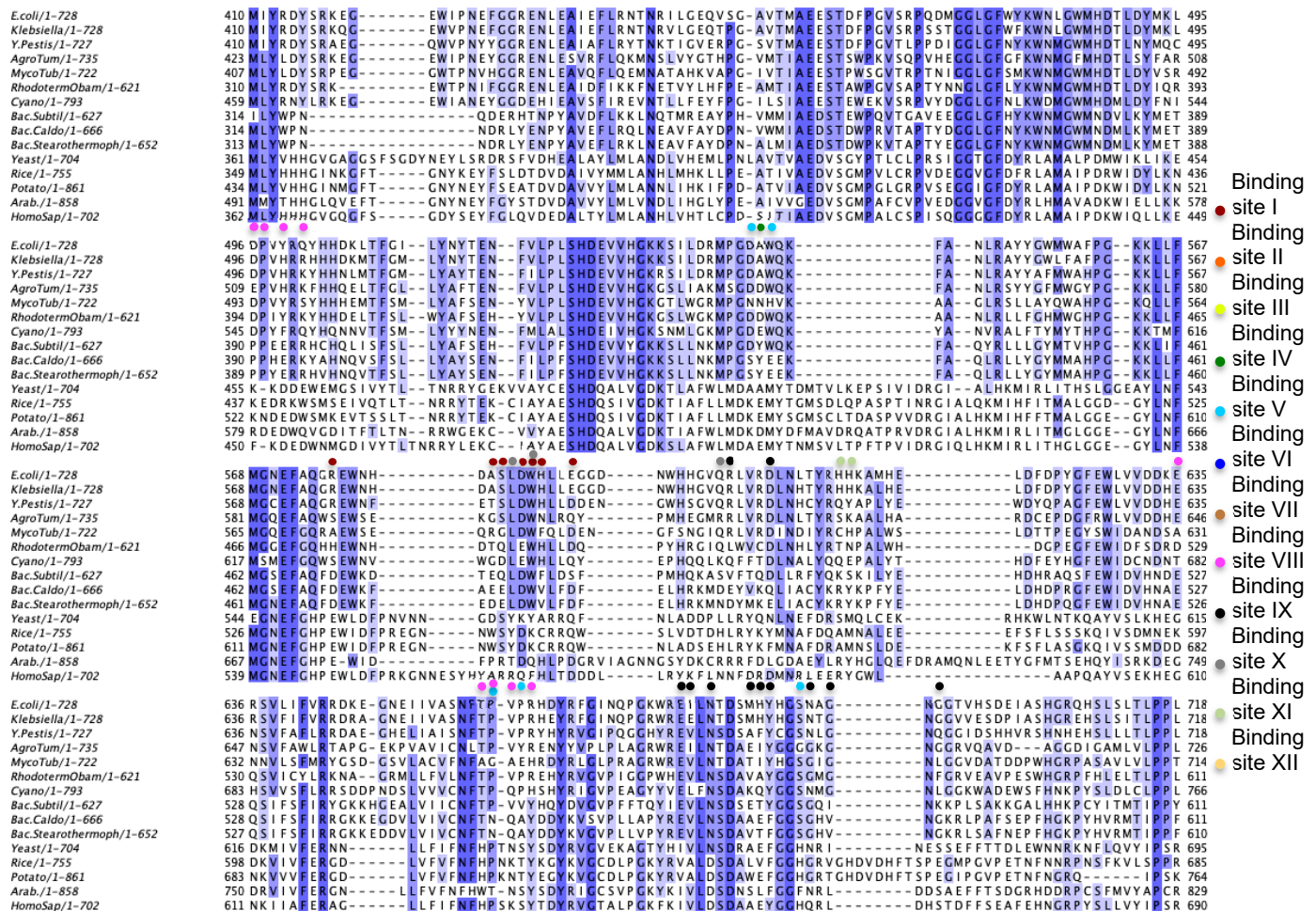


Figure S1. Sequence alignment of Branching Enzymes from various organisms. Identical residues are shown in blue. Amino acids in each binding site are shown in blue.

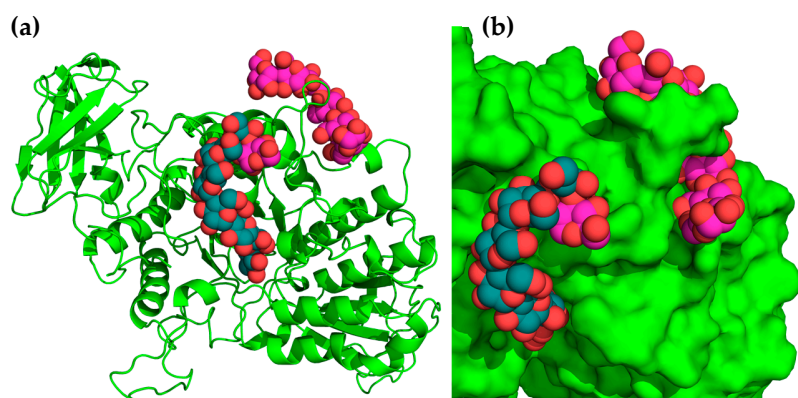


Figure S2. Site XII is close to the path of the donor chain. **(a)** The glucans bound in site I and XII (spheres, C, pink) of M8-bound EcBE (green ribbon) are shown with the M7 donor chain (spheres, C, blue-green) from the *Cyanospora* BE structure modeled into the structure. **(b)** EcBE is represented as a surface.

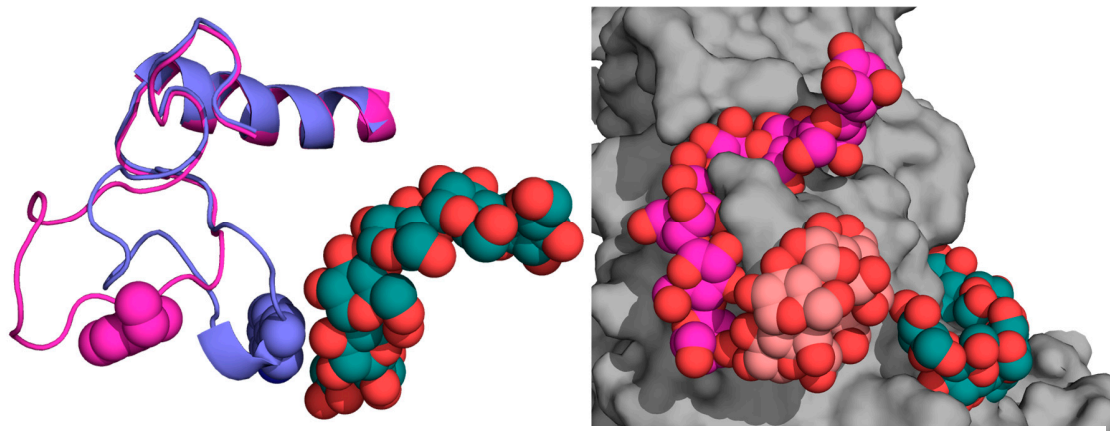


Figure S3. Overlay of the W399 loops from *Mycobacterium tuberculosis* (pink) and *Cyanothece* (blue) BE's. The active-site bound M7 donor chain from *Cyanothece* is shown as spheres, with C atoms colored blue-green. Right, M8-bound EcBE (grey surface) showing the site one bound glucan (Pink C atoms), with the cyclodextrin bound in site from the alpha-CD-bound EcBE structure (light red C atoms) and the *Cyanothece* M7 donor chain modeled onto the structure.

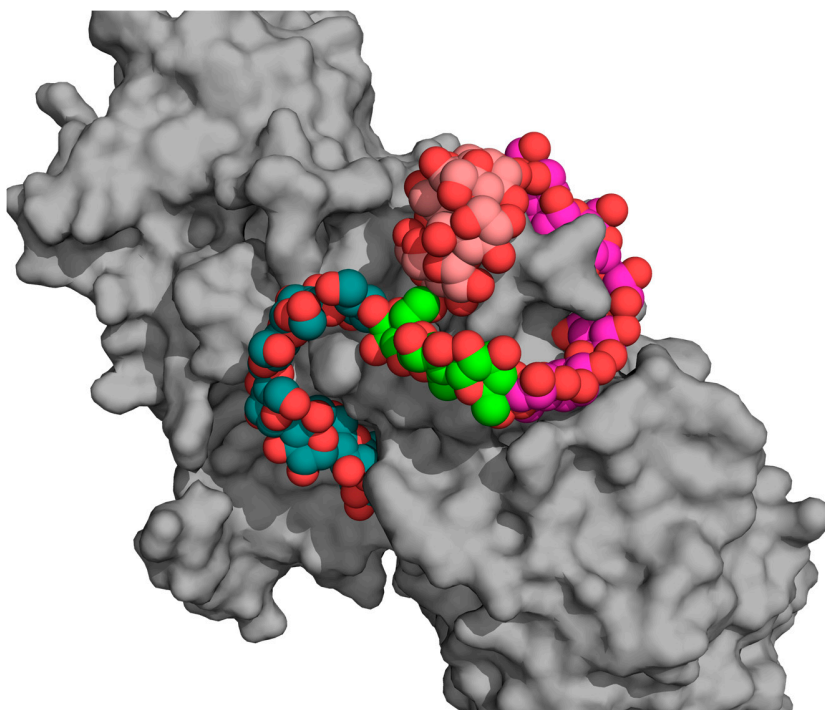


Figure S4. M8-bound EcBE structure showing site I-bound M8 (spheres, C, pink) with alpha-CD modeled into site VII (based on the alpha-CD bound EcBE structure, spheres, C, light red), active site bound M7 from *Cyanotheca* BE modeled into the active site (spheres, C, blue-green) representing the donor strand and three malto-oligosaccharides connecting M8 and the modeled donor strand showing how site I could represent the binding surface of the longer donor strand characteristic of *E. coli* BE.

Table SI. Data collection and refinement statistics M8-bound EcBE.

Wavelength (Å)	0.978
Resolution Range (Å)	38.85 – 3.00
Space group	P3221
a (Å)	146.489
b (Å)	146.489
c (Å)	294.92
a (°)	90
b (°)	90
γ (°)	120
Molecules per Asymmetric Unit	4
Total reflection	1065049
Unique Reflection	71397
Multiplicity	2.1
Completeness (%)	96.33
Average I/s	22.9
R _{merge} (%)	0.14
R _{work} (%)	0.2139
R _{free} (%)	0.3152
Reflections used in refinement	71269
Reflections used for R _{free}	3615
RMSD from ideal values	
Bond Angle	1.36
Bond Length (Å)	0.012
Average B factor	69.29
Number of water molecules	473
PDB ID	8SDB