```
ChbC (B. cereus)        FVAMIIAIISTEIYRFLVQKKMII-KMPETVPPAVTRSFAALIPGFIVVTVVWIIRLIFE     205
MalP (B. subtilis)      IGAIFISSIVV----FLHNRYFDK-KLPDFLGIFQGSTYIVMISFFIMIPIALAVSYIWP     194
MalT (B. cereus)        FVGIITGFLGA----TLYNKYYNYNKLPQALAFFNGKRFVPFVVIVWSTVTAIVLSLLWP     199
MalT (S. mutans)        FVGIISGFVGA----TAYNKYYNFRKLPDVLSFFNGKRFVPFVVIVRSTIVALILSVFWP     203
NagE (E. coli)          LAGIITGLVGG----AAYNRWSDI-KLPDFLSFFGGKRFVPIATGFFCLVLAAIFGYVWP     153
NagP (B. subtilis)      FGGIIAGLIAG----YTYNRFKDT-KLPEYLGFFSGRRLVPILTAIITIILAGIFGVVWP     151
PtsG (E. coli)          LGGIISGAIAA----YMFNRFYRI-KLPEYLGFFAGKRFVPIISGLAAIFTGVVLSFIWP     173
PtsG (B. subtilis)      FGGIIVGVLAA----LLFNRFYTI-ELPQYLGFFAGKRFVPIVTSISALILGLIMLVIWP     201

ChbC (B. cereus)        HTTFGSIHNVVGKLLQEP---LSILGASLWGAVIAVILVHVLWACGIHGATIVGGVMSPI     262
MalP (B. subtilis)      MV--QSGIGSLQSFLVAS---G-AVGVWIYTFLE-----RILIPTGLHHF-----IYTPF     238
MalT (B. cereus)        FI--QSGLNEFGRWIAASKDSAPIVAPFVYGTLE-----RLLLPFGLHHM-----LTIPM     247
MalT (S. mutans)        IV--QSGINGFGMWIASSQHTAPFLAPFLYGTLE-----RLLLPFGLHHM-----LTIPM     251
NagE (E. coli)          PV--QHAIHAGGEWIVSA---G-ALGSGIFGFIN-----RLLIPTGLHQV-----LNTIA     197
NagP (B. subtilis)      PI--QSCINSFGEWMLGL---G-GIGAGIFGLFN-----RLLIPLGLHHV-----LNNIF     195
PtsG (E. coli)          PI--GSAIQTFSQWAAYQ---NPVVAFGIYGFIE-----RCLVPFGLHHI-----WNVPF     218
PtsG (B. subtilis)      PI--QHGLNAFSTGLVEA---NPTLAAFIFGVIE-----RSLIPFGLHHI-----FYSPF     246
                                                                       **

ChbC (B. cereus)        WLSLMDQNRIA---FQAGQDVP------------------NTITAQFFDLW---------     292
MalP (B. subtilis)      IYGPAVA---------E-----GGIVTY--WAQHL-----GEYSQSAKPL-KELFPQGGF     276
MalT (B. cereus)        NYTELGGTYTMLTGSKVGQVVAGQDPLWLAWITDLNNLLANGDTKAYNDLLNNVVPARFK     307
MalT (S. mutans)        NYTQLGGTYVVLTGAQAGKHVLGQDPLWLAWVQDLIHLKGAGHMSQYHHLLTSVTPARFK     311
NagE (E. coli)          WFQIG--EFT----NAAGTVFHGDINRFYAGDGTA-----GMFMSGFFPI----------     236
NagP (B. subtilis)      WFQFG--EYN----GV-----TGDLARFFAKDPTA-----GTYMTGFFPI----------     229
PtsG (E. coli)          QMQIG--EYT----NAAGQVFHGDIPRYMAGDPTA------GKLSGGFLF----------     256
PtsG (B. subtilis)      WYEFF--SYK----SAAGEIIRGDQRIFMAQIKDGVQLTAGTFMTGKYPF----------     290
                                                                                   *

ChbC (B. cereus)        --IYMGGSGATLALVVGMLLFARSQQLKSLGRLSI----APGIFNINEMVTFGMPIVMNP     346
MalP (B. subtilis)      ALHGNSKIFGIPGIALAFYVTAKKEKKKLVAGLLIPVTLTAIVAGITEPIEFTFLFISPF     336
MalT (B. cereus)        AGQVIGSTAALMGIAFAMFRNVDKEKRAKYKPMFLSAALAVFLTGVTEPIEFMFMFIAPV     367
MalT (S. mutans)        VGQMIGSSGILMGLTLAMYRNVDPDKKEKYKGMFLSAAVAVFLTGVTEPLEYMFMFAALP     371
NagE (E. coli)          ------MMFGLPGAALAMYFAAPKERRPMVGGMLLSVAVTAFLTGVTEPLEFLFMFLAPL     290
NagP (B. subtilis)      ------MMFGLPAACLAMVVTAKPSKRKATAGMMIGFALTAFITGITEPIEFAFMFLSPL     283
PtsG (E. coli)          ------KMYGLPAAAIAIWHSAKPENRAKVGGIMISAALTSFLTGITEPIEFSFMFVAPI     310
PtsG (B. subtilis)      ------MMFGLPAAALAIYHEAKPQNKKLVAGIMGSAALTSFLTGITEPLEFSFLFVAPV     344
                                                                      **

ChbC (B. cereus)        LLLIPFIVVPVVLTIVSYFAMEWGLVARP---SGAAVTWTTPILFSGYLGSGGKISGVI-     402
MalP (B. subtilis)      LF-----AVHAVLAATMSTVMYMAGVVGN--MGGGLIEAVTLNWIPLFGSHGMTYVVQIL     389
MalT (B. cereus)        LY-----VVYAITTGLAFALADLINLRVH-AF--GFIELITRTPMMVNAGLTRDLINFVI     419
MalT (S. mutans)        LY-----LVYAVVQGLAFASADLIHLRVH-SF--GNIEFLTRTPMAIKAGLAMDIVNFIV     423
NagE (E. coli)          LY-----LLHALLTGISLFVATLLGIHAGFSFSAGAIDYAL---MYNLPAASQNVWMLLV     342
NagP (B. subtilis)      LY-----AVHAVLTGLSLFIVNWLGIRSGFSFSAGAIDYVL---SYGIAE---KPLLLLL     332
PtsG (E. coli)          LY-----IIHAILAGLAFPICILLGMRDGTSFSHGLIDFIV---LS---GNSSKLWLFPI     359
PtsG (B. subtilis)      LF-----AIHCLFAGLSFMVMQLLNVKIGMTFSGGLIDYFL---FGI-LPNRTAWWLVIP     395
                                                       *

ChbC (B. cereus)        LQLVNFALAFVIYLPFLKIWDKQKIAEEKGE     433
MalP (B. subtilis)      IGLSFTAIYFFVFRFLILKFNIATPGREKDE     420
MalT (B. cereus)        VSLVFFGLNFTLFNFLIKKFNLPTPGRAGNY     450
MalT (S. mutans)        VSVVFGVAMYFITNFMIKKFNLATSGRNGNY     454
NagE (E. coli)          MGVIFFAIYFVVFSLVIRMFNLKTPGREDKE     373
NagP (B. subtilis)      VGICYAAVYFIVFYVLIKALNLKTPGREDDD     363
PtsG (E. coli)          VGIGYAIVYYTIFRVLIKALDLKTPGREDAT     390
PtsG (B. subtilis)      VGLGLAVIYYFGFRFAIRKFNLKTPGREDAA     426
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Figure S1, related to Figure 1 and 4: Sequence alignment of representative functionally characterized EIIC domains. All sequences are members of the glucose (Glc) subfamily of the Glucose superfamily, except bcChbC, which is from the lactose (Lac) subfamily. The alignment contains residues from the start of TM4 in bcMalT to its C-terminus. The sequences are: ChbC from *B. cereus* (Uniprot Acc # Q72XQ0), MalP from *B. subtilis* (P54715), MalT from *B. cereus* (D8GZQ0), MalT from *Streptococcus mutans* (Q8DS05), NagE from *E. coli* (W8ZP98), NagP from *B. subtilis* (A0A085C1Y9), PtsG from *E. coli* (C3TDU2), and PtsG from *B. subtilis* (P20166). bcMalT shares only 23% identity with the maltose transporter MalP from *B. subtilis* and 30% identity with the glucose transporter PtsG from *E. coli*. Green residues are conserved in Glc family transporters; red residues are also conserved in bcChbC. Asterisks correspond to residues that bind substrate in the bcMalT crystal structure.

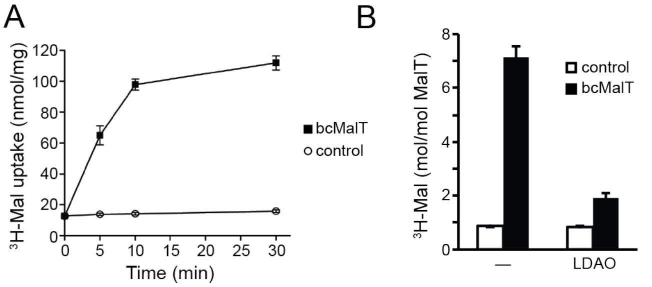

Figure S2, related to Figure 1: Transport activity of trypsinized bcMalT. A. Uptake of [^{3}H]maltose into proteoliposomes containing the trypsinized EIIC domain of bcMalT (black squares) or control liposomes without protein (white circles). Error bars are standard deviations of three technical replicates. **B**. Verification of [^{3}H]maltose uptake by comparing accumulation in proteoliposomes incorporating trypsinized bcMalT or control liposomes without protein in the presence or absence of 5 mM LDAO.

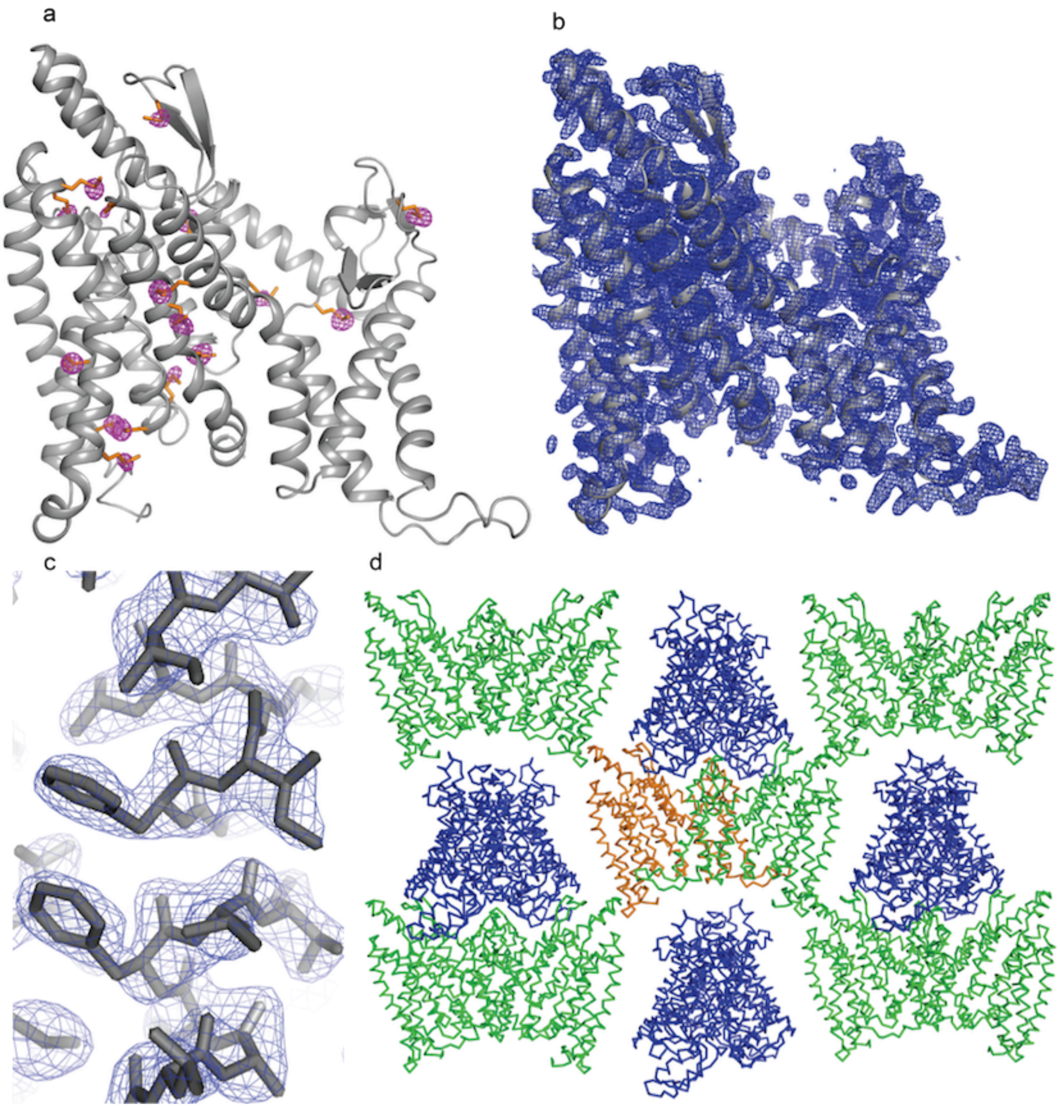

Figure S3, related to Figure 2. Structure determination of bcMalT. a. Anomalous difference map at 4.5 σ showing position of the selenium substructure. Selenomethionine sidechains are drawn as orange sticks. **b**. $2F_o$-F_c electron density map contoured at 1.5 σ of one bcMalT protomer. **c**. Closer view of the $2F_o$-F_c map at 1.5 σ illustrating sidechain densities. **d**. bcMalT crystallized as a homodimer with one protomer in the asymmetric unit (orange). There are extensive contacts at the periplasmic surface of the protein from another bcMalT homodimer (blue) and minor contacts between the helix following TM7 and HP1A.