

SUPPLEMENTARY MATERIAL

Crystal structure of the plant symporter STP10 illuminates sugar uptake mechanism in Monosaccharide Transporter Superfamily

Peter Aasted Paulsen¹, Tânia F. Custódio¹, Bjørn Panyella Pedersen^{1,2}

1) Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10, DK-8000 Aarhus C, Denmark.

2) Aarhus Institute of Advanced Studies, Aarhus University, Høegh-Guldbergs Gade 6B, DK-8000 Aarhus C, Denmark.

Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

21 30 40 50 60 70

F39 D42 L43

M1a M1b I1 I2

MPA GGFVV GDGOKAYPGL V AFV IMTCIVVAAAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV SQMKKAKH D 73
 STP1 VVGSVMNV EEGTKAFPAKLL I LGVYFLFCVAAVAGGGLM I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKHRHH T 71
 STP2 NVA FEARKEAMAKSVSGGKLL I YFVVFVASTCVMVAAAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV LQEQEDRRRRRGNSEN 77
 STP3 MAG GGL ALDVSSAGNIDAKM I AVVVMSCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YLKKMSAH E 70
 STP4 MAG GGL ALDVSSAGNIDAKM I AVVVMSCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV LKQKASEAK T 71
 STP5 MAV VVS NANAPAFDAKMTM I YVYVFCVIMVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV WERKKKHHV E 68
 STP6 MAG GSF PTGVAKERAEQYQGGM I SYVYFICVIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKQAH E 74
 STP7 MAG VFS NGNSKSYFDAGM I YVYVIMTCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH E 73
 STP8 MAG GAFVSE GGGRSYEGGVT I AFVIMTCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH E 73
 STP9 MAG GAFVSE GGGRSYEGGVT I AFVIMTCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH E 73
 STP10 MAG GAFVSE GGGRSYEGGVT I AFVIMTCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH E 73
 STP11 MAG GAFVSE GGGRSYEGGVT I AFVIMTCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH E 73
 STP12 MPS VGV I GDGKKEYPGL I LYVTVTCIVVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH S 71
 STP13 MTG GGFAT SANGVEFE . . . AKI I TPIVYV I SCIMVAAVAGCGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH D 72
 STP14 MAG GALTDEG GLKRAHLYEHR I TSYVIFVAV I VSGM I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH E 74
 PKHUP1 MAG GGVV V SGRGLSTGDYRGG I VYVVMVAFMAACGGLL I FGDYD GISSGGVTSMDFFLTKR FFPFV YEKKKHAAH D 75

Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

80 90 100 110 120 130 140 150

Cys77 R142

TAY QYFDN QMLQ LFTSSLYLAAL I VASV FVMSV ITRKGR KRKVS MFI GGLA FCA G A L F N G A F V V S M L I I G R L L L G V G G F A N Q 154
 STP1 NOY C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 152
 STP2 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 151
 STP3 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 158
 STP4 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 151
 STP5 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 152
 STP6 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 149
 STP7 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 155
 STP8 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 150
 STP9 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 154
 STP10 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 154
 STP11 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 152
 STP12 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 153
 STP13 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 153
 STP14 NNH C Q F D S D L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 155
 PKHUP1 SP Y K Y D N Q L L Q L F T S S L Y L A A L I F A S L F L A S S Y V S R A F F G R R K V I F L G G V S F F F G A L A L L G S Q E L V M L I I G R L L L G F G G F A N Q 156

Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

160 170 180 190 200 210 220 230

Q177 I184 M6 SP motif

STP VYLSLSEMA HAK I R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 232
 STP1 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 231
 STP2 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 228
 STP3 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 235
 STP4 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 230
 STP5 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 227
 STP6 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 233
 STP7 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 228
 STP8 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 232
 STP9 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 232
 STP10 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 233
 STP11 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 229
 STP12 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 232
 STP13 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 233
 STP14 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 233
 PKHUP1 AV P L Y L S E M A P A K Y R G A L N I G F O M A I T I G I L I A N L I Y G T S K M A Q H . G W R V S L G L A A P A V V M V I G S F V L D I T N S M L E 233

Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

240 250 260 270 280 290 300 310

IC2 IC3 IC4 M7a Q295-6 N301 M7b

RGKNEEGKFA RKRTRGV DINDAEYESTVH A C S E L A S Q Y K D P Y R K L . L K P A S R P P F F I . G M L L Q L F Q O O F T G I N I A T I F Y A P V 309
 STP1 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 308
 STP2 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 306
 STP3 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 313
 STP4 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 307
 STP5 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 311
 STP6 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 304
 STP7 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 310
 STP8 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 305
 STP9 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 310
 STP10 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 309
 STP11 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 310
 STP12 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 306
 STP13 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 309
 STP14 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 311
 PKHUP1 RQHEEARTKTRRIRG DNDVDEEEDLVAEAKES K K S E T H P W K N I . M E R R K R P A L I F C S A I P P F F Q O O F T G I N I V M F Y A P V 312

Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

310 320 330 340 350 360 370 380

N332 M9 M10

LFKTLGFGDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 388
 STP1 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 388
 STP2 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 364
 STP3 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 390
 STP4 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 386
 STP5 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 390
 STP6 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 381
 STP7 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 387
 STP8 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 382
 STP9 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 389
 STP10 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 388
 STP11 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 388
 STP12 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 386
 STP13 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 387
 STP14 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 388
 PKHUP1 LFNTMIGFDDASLMSAVITGVV MLSTFVSIYGVDRYGRRLFLFEGG I O M F I C O A V V A A C I G A K F G V D G T P G E L P K W Y A I 391

Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

390 400 410 420 430 440 450 460 470

M10a M10b N433 T437 Cys449 M12

WILAFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 469
 STP1 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 469
 STP2 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 465
 STP3 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 467
 STP4 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 471
 STP5 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 471
 STP6 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 462
 STP7 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 468
 STP8 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 463
 STP9 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 470
 STP10 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 469
 STP11 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 470
 STP12 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 467
 STP13 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 468
 STP14 VVVFICLVYAGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 469
 PKHUP1 GILAVICTFTSGFAWSWGLPWGLV PSEICPLERSPAGQAINVSWMFFFTFLTAQGFLLTMLHLKFGFLVYFFASMVAVMTVVF 472

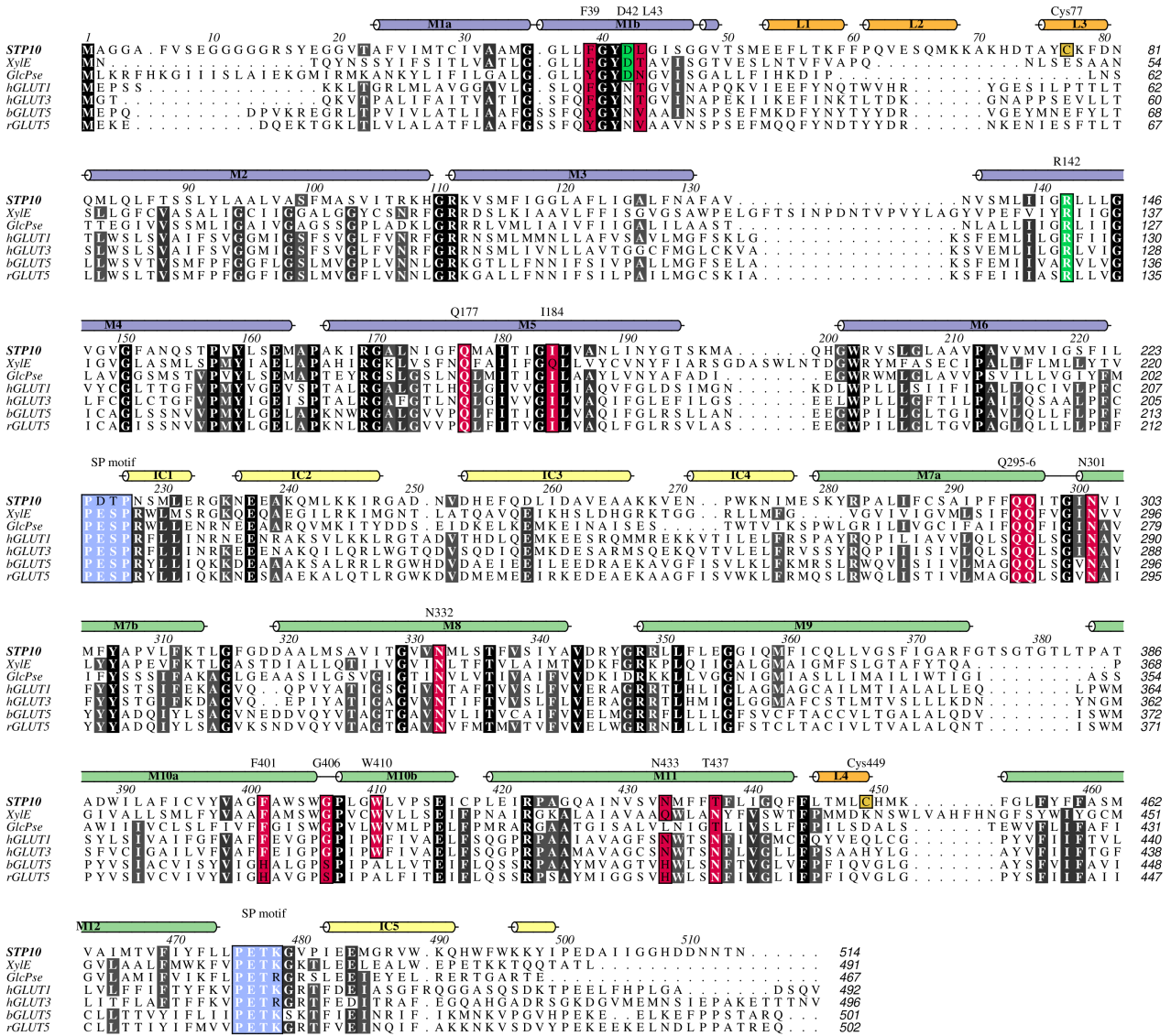
Crystal
STP1
STP2
STP3
STP4
STP5
STP6
STP7
STP8
STP9
STP10
STP11
STP12
STP13
STP14
PKHUP1

470 480 490 500

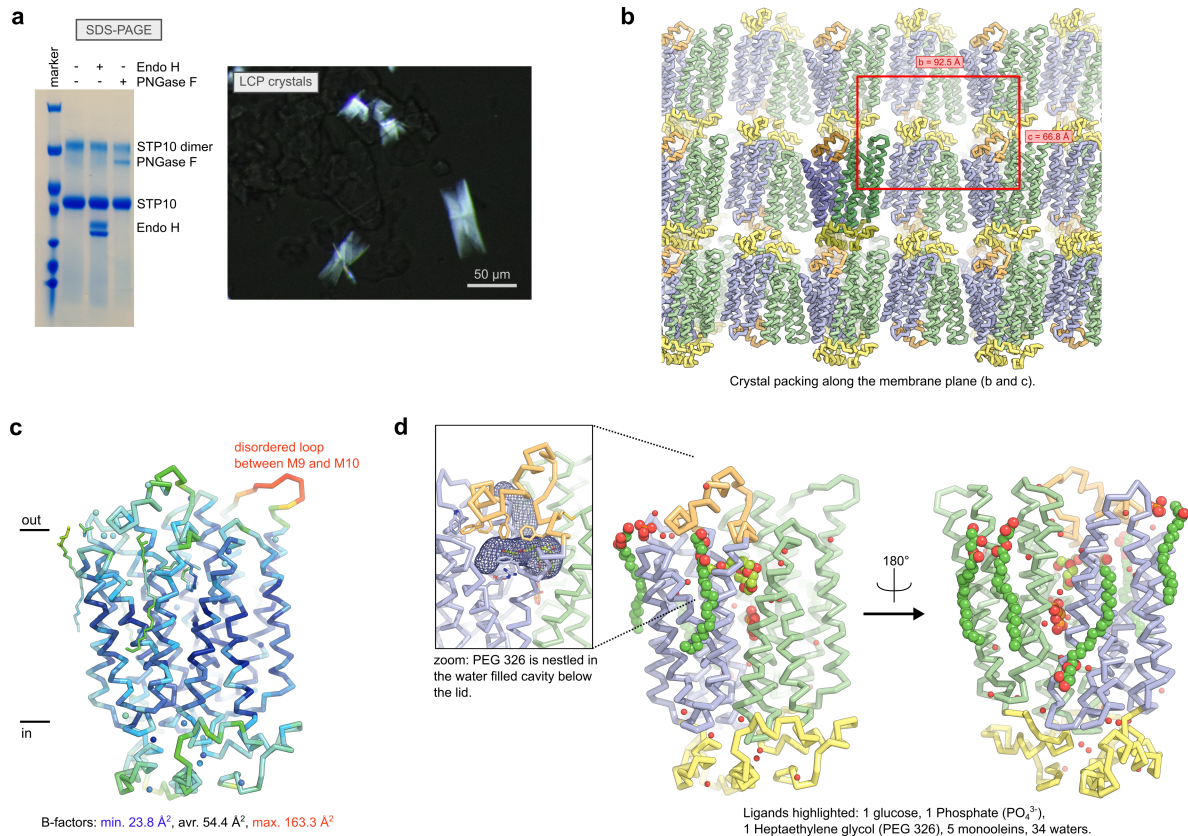
SP motif ICS ER export signal

VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 507
 STP1 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 522
 STP2 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 498
 STP3 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 514
 STP4 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 514
 STP5 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 506
 STP6 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 507
 STP7 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 513
 STP8 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 507
 STP9 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 517
 STP10 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 514
 STP11 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 514
 STP12 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 508
 STP13 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 526
 STP14 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 504
 PKHUP1 VYFLPETKGVPIIEEMGRVYIKQ RYKYPEDA IGG 534

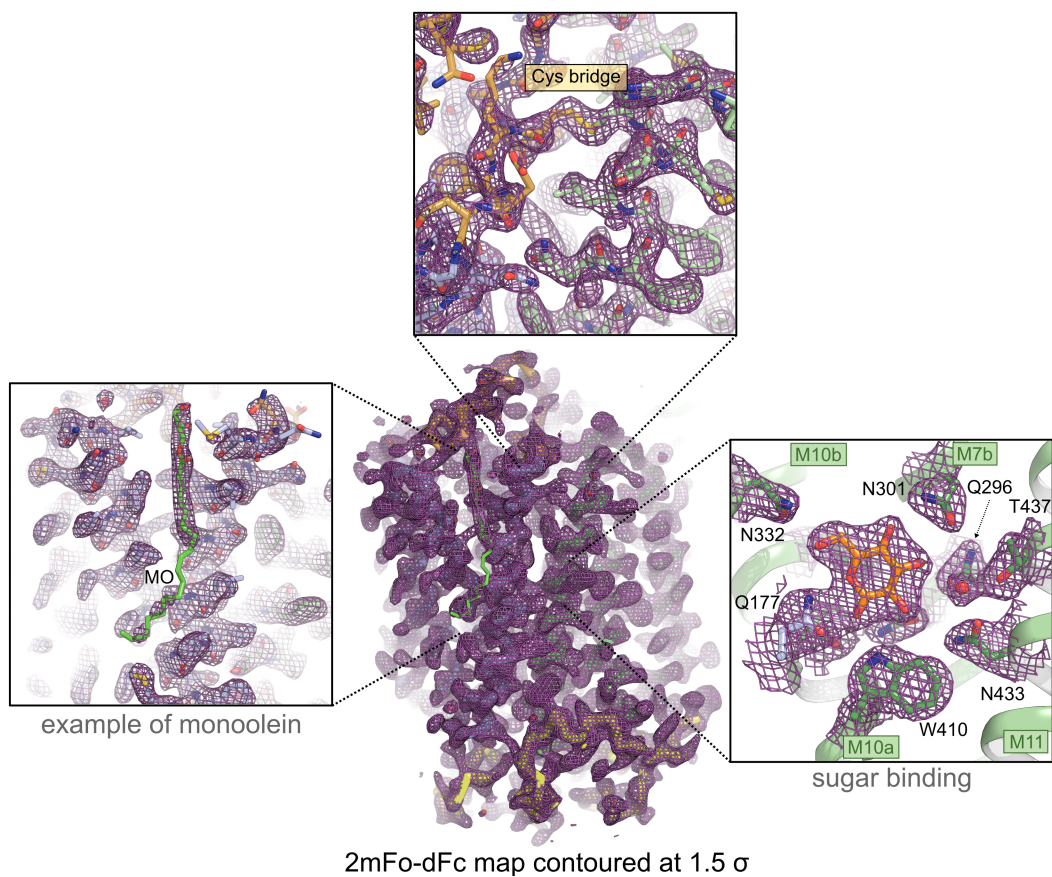
Supplementary Figure 1. Multiple sequence alignment of the *A. thaliana* Sugar Transport Family, with *Parachlorella kessleri* HUP1 included. Alignment between STP1 (accession number P23586), STP2 (accession number Q9LNV3), STP3 (accession number Q8L7R8), STP4 (accession number Q39228), STP5 (accession number Q93Y91), STP6 (accession number Q9SFG0), STP7 (accession number O04249), STP8 (accession number Q9SBA7), STP9 (accession number Q9SX48), STP10 (accession number Q9LT15), STP11 (accession number Q9FMX3), STP12 (accession number O65413), STP13 (accession number Q94AZ2), STP14 (accession number Q8GW61), *Parachlorella kessleri* HUP1 (accession number P15686). Conserved residues are highlighted with gray-scale, where black is perfectly conserved. Colored tubes represent α -helices found in the N domain (blue), Lid domain (orange), ICH domain (pale yellow) and C domain (green). Key residues are numbered above the α -helix markings. Residues highlighted in dark red participate in sugar binding. The proton donor/acceptor pair is highlighted in green. The cysteines forming the disulfide bridge between Lid domain and C domain are highlighted in dark yellow. Conserved motifs are highlighted in light blue.



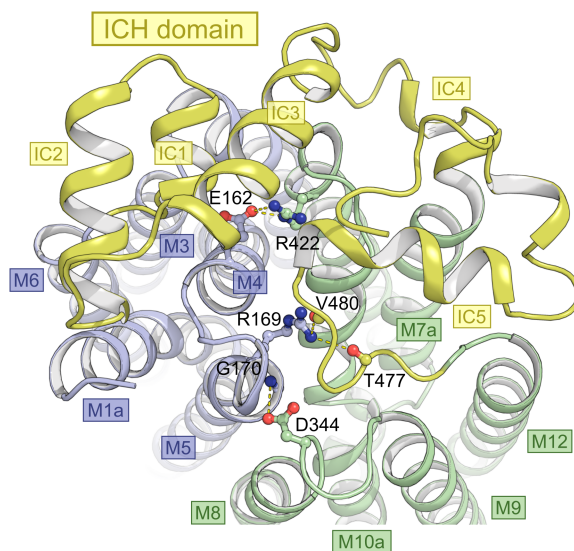
Supplementary Figure 2. Multiple sequence alignment of the *A. thaliana* STP10 with other Major Facilitator sugar transporters from the Sugar Porter group. Alignment between STP10 (accession number Q9LT15), XyleE (accession number P0AGF4), GlcPse (accession number A0A0H2VG78), hGLUT1 (accession number P11166), hGLUT3 (accession number P11169), bGLUT5 (accession number P58353), rGLUT5 (accession number P43427). Conserved residues are highlighted with gray-scale, where black is perfectly conserved. Colored tubes represent α -helices found in the N domain (blue), Lid domain (orange), ICH domain (pale yellow) and C domain (green). Key residues are numbered above the α -helix markings. Residues highlighted in dark red participate in sugar binding. The proton donor/acceptor pair is highlighted in green. The cysteines forming the disulfide bridge between Lid domain and C domain are highlighted in dark yellow. Conserved motifs are highlighted in light blue.



Supplementary Figure 3. Crystals and components of the asymmetric unit. **a**, SDS-PAGE gel of STP10 and polarized light photo of STP10 crystals. STP10 has a predicted glycosylation site at Asn134. While the crystal packing would allow the presence of glycosylation no density was observed, and incubating the STP10 sample over night with Endo H or PNGase F did not result in a mass-shift, indicating that STP10 is not glycosylated when overexpressed in yeast. **b**, Asymmetric unit and crystal packing of STP10. The unit cell is viewed perpendicular to the bc-plane, and the b and c axis highlighted in red. The asymmetric unit contains one molecule of STP10, as highlighted in darker colors. The packing is a nice example of type I packing normally obtained by LCP crystallography with the transmembrane regions packing in a lipid bilayer and a relatively low solvent content (54%). **c**, The backbone of STP10 colored by the atomic displacement factor (B-factor) with a rainbow gradient from low/blue (23.8 Å²) to high/red (163.3 Å²). There is a disordered loop between M9 and M10 with a significantly higher B-factor than the rest of the model. **d**, Backbone representation of STP10 with all the heterologous molecules found in the density highlighted. Besides STP10 we could confidently build 1 glucose, 1 phosphate, 1 PEG 326, 5 monoolein molecules and 34 waters.

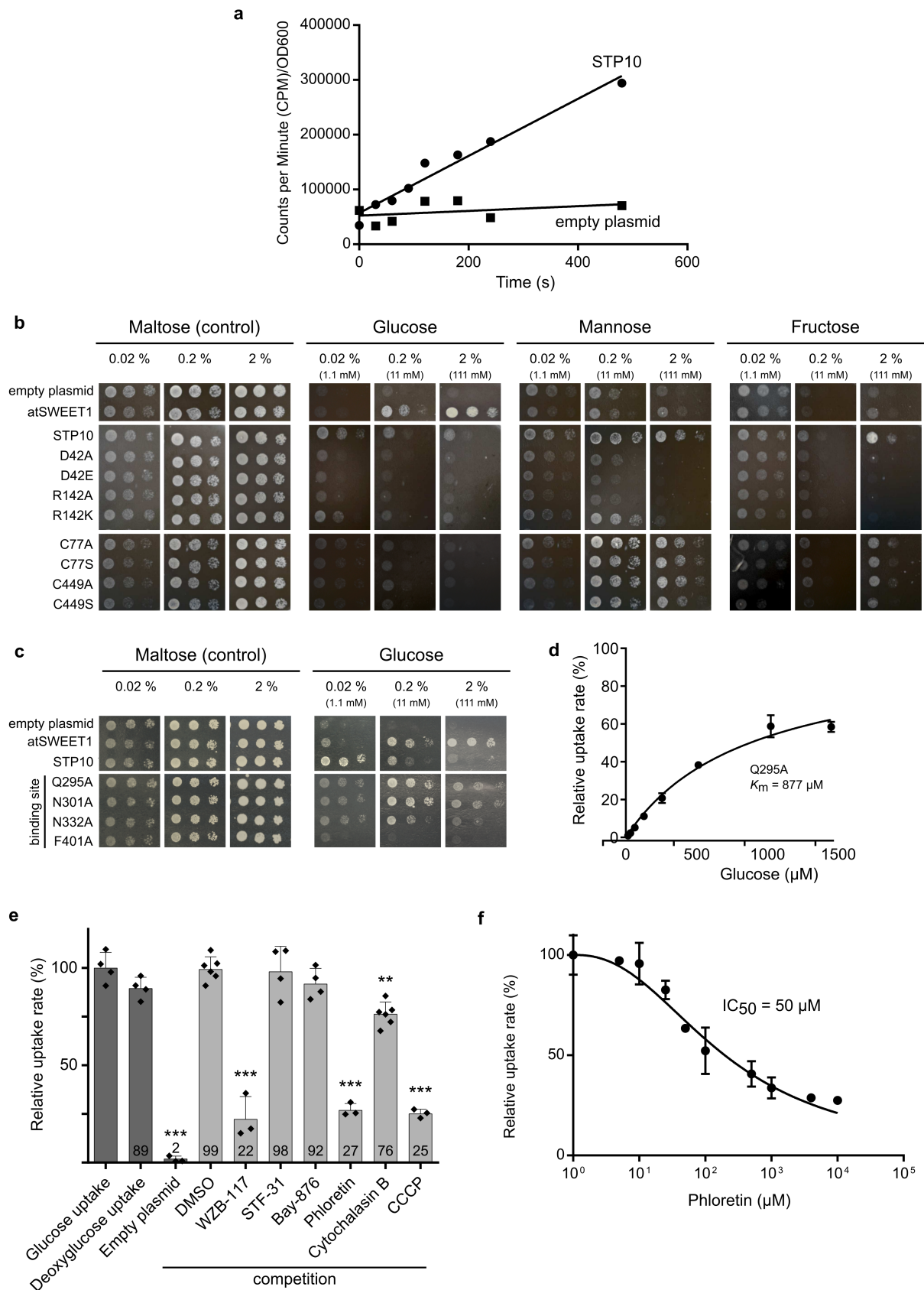


Supplementary Figure 4. Examples of electron density. 2mFo-DFc electron density calculated after the final refinement run (1.5 σ) in magenta with the final model overlaid. The three inserts highlight the high quality of the electron density for glucose binding and the disulfide bridge and an example of the density of the monoolein, which was weaker and is clearer at lower sigma levels than 1.5.



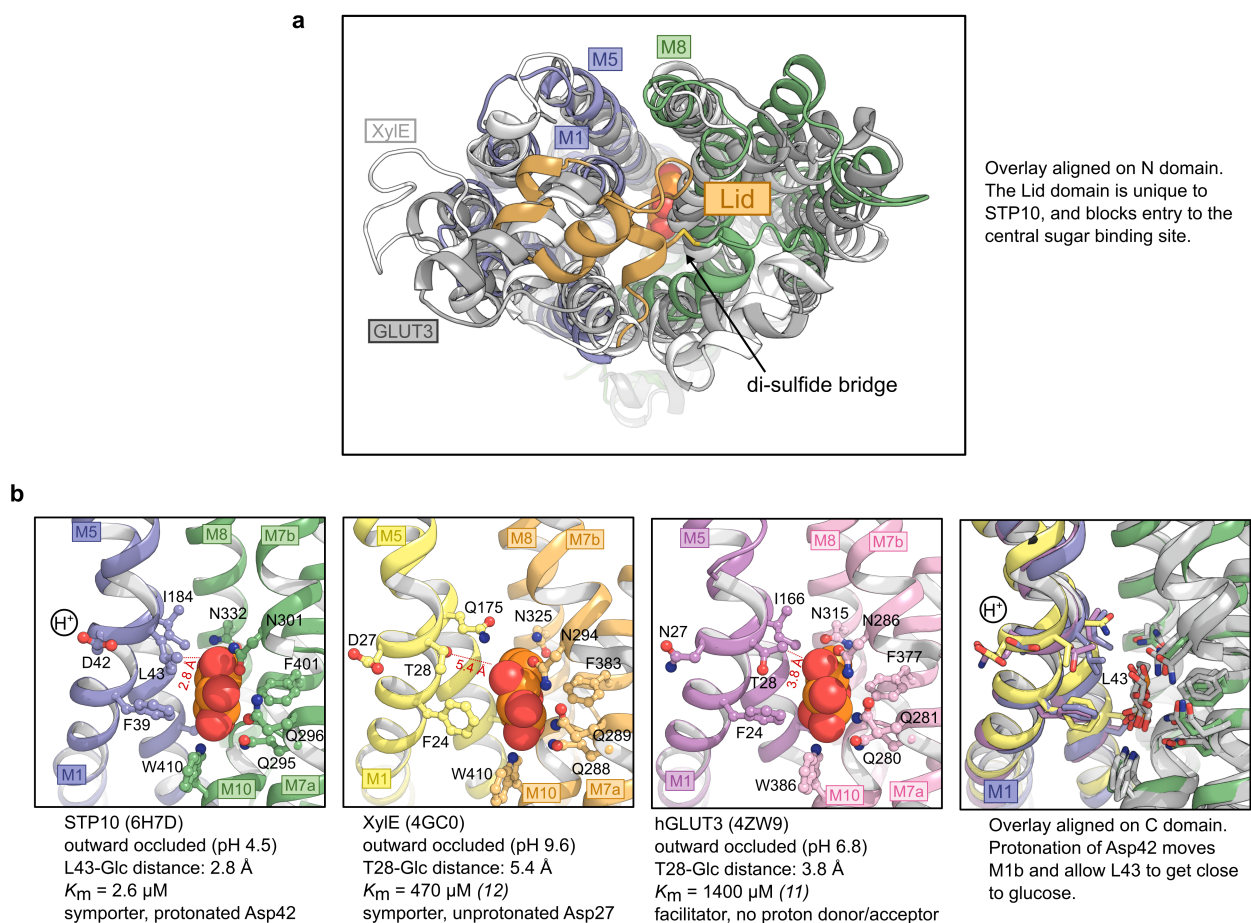
View from cytosolic side

Supplementary Figure 5. Closure towards the cytosolic side in STP10. View of STP10 from the cytosolic side. The opening between the N and C domain towards the cytosolic is closed and held in place by several highlighted interactions between the two domains. In particular by a double salt bridge from D344(M8) to the main chain nitrogen of Gly170(M5), and from Arg169(M5) to the main chain carbonyls of Thr477 and Val480, as well as a salt bridge from Glu162 (M4) to Arg422(M11). These regions are perfectly conserved in all STPs and several bacterial symporters (Supplementary Figure 2), and have also been observed in human sugar facilitators.



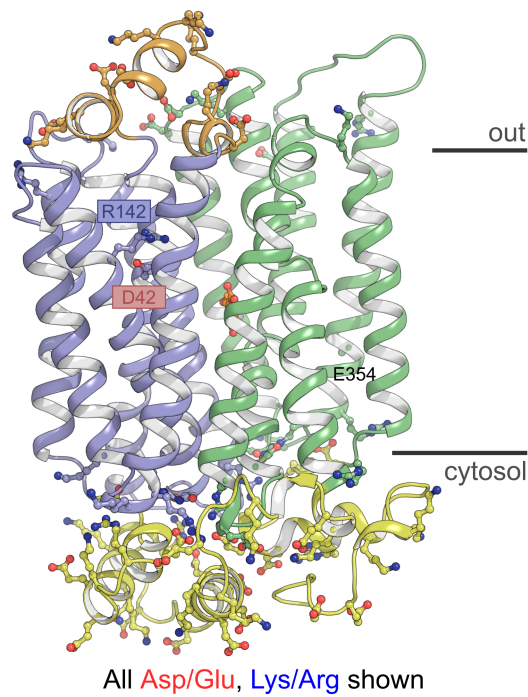
Supplementary Figure 6. Functional characterization of STP10. **a**, Uptake of glucose into EBY.VW4000 yeast strain expressing STP10 (circles) or empty plasmid (squares) per OD600 of cells at an initial outside concentration of 100 μM glucose at pH 5.0. **b**, Growth complementation of the *S. cerevisiae* hexose transport deficient strain, EBY.VW4000 by STP10, STP10 mutants and

Arabidopsis thaliana SWEET1 (positive control for glucose uptake) at different concentrations of sugars. **c**, Growth complementation of EBY.VW4000 by STP10 substrate binding site mutants. **d**, Determination of kinetic parameters for the transport of glucose measured using the EBY.VW4000 uptake assay for the mutant Q295A. Shown is a Michaelis-Menten fit to glucose titration. Data are mean \pm SD of three or more replicate experiments. **e**, STP10 inhibition determined by the EBY.VW4000 competition assay at pH 5.0. *, $P \leq 0.05$; **, $P \leq 0.01$; and ***, $P \leq 0.001$ by Student's *t* test. **f**, Dose response curve showing inhibition of STP10 activity by Phloretin. Data for all assays are mean \pm SD of three or more replicate experiments.

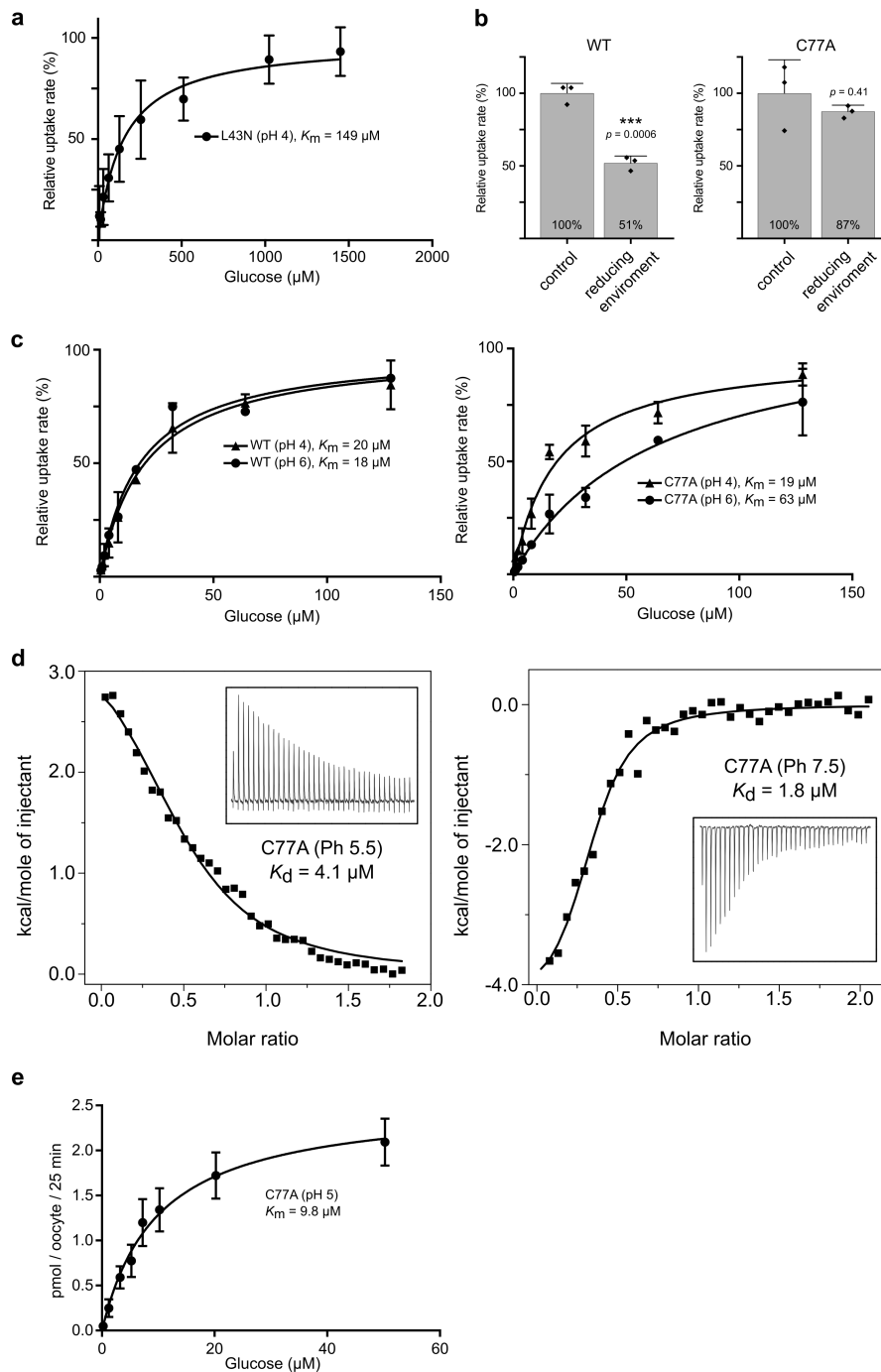


Supplementary Figure 7. Comparison of STP10 with XylE and hGLUT3. **a**, Overlay of the extracellular region in STP10, XylE and hGLUT3 (All in outward occluded conformations).

b, Comparison of the sugar binding sites in STP10, XylE and hGLUT3. All the polar interactions from the C domain are identical in the three cases, despite large differences in affinity. Differences are found in the N domain. In particular, the hydrophobic interaction between substrate and L43 in STP10 is replaced by a polar interaction from a Serine in XylE and Glut3. Only in STP10 is the proton donor/acceptor residue (Asp42) protonated, causing the M1 helix to bend away from Arg142 and move Leu43 towards the substrate.



Supplementary Figure 8. Identification of all charged residues in STP10. STP10 is shown in cartoon with all charged residues shown as sticks. Asp/Glu are red while Lys/Arg are blue. Only Asp42 and Arg142 are buried in the membrane plane and in position to form the proton donor/acceptor pair. There is a Glu354 that is also in the membrane and pointing towards the solvent. It is highly unlikely that this residues (that is not conserved in STPs), would have a functional role, but it might be implicated in determining the energy landscape of conformational change.



Supplementary Figure 9. Functional characterization of STP10 mutants L43N and C77A. a, Michaelis-Menten fit to glucose titration of STP10 mutant L43A at pH 4. **b**, Effect of reducing agent on glucose transport using the EB.Y.VW4000 uptake assay for WT and C77A. The uptake assay was done at pH 6.0 either under standard conditions or in a reducing environment (by addition of 2.5% (V/V) 2-Mercaptoethanol). ***, $P \leq 0.001$ by Student's t test. **c**, Determination of kinetic parameters for the transport of glucose measured using the EB.Y.VW4000 uptake assay. Shown is a Michaelis-Menten fit to glucose titration at pH 4 and pH 6 (left) and for the mutant C77A at pH 4 and pH 6 (right). Data are mean \pm SD of three or more replicate experiments. **d**, The

binding affinity between glucose and STP10-C77A was measured with Isothermal titration calorimetry at pH 5.5 and at pH 7.5. The apparent K_d does not change with pH. e, Determination of kinetic parameters for the transport of glucose by STP10 C77A measured using the *Xenopus* oocyte uptake assay at pH 5. Shown is a Michaelis-Menten fit to glucose titration. Data for all assays are mean \pm SD of three or more replicate experiments.

Supplementary Table 1. Sequence identity of STP10 to the *A. thaliana* Sugar Transport Family.

Name	Sequence %ID (Needle-Wunsch alignment)
STP1	59.4%
STP2	50.5%
STP3	46.5%
STP4	64.6%
STP5	48.2%
STP6	52.4%
STP7	53.7%
STP8	51.9%
STP9	84.3%
STP11	70.8%
STP12	60.5%
STP13	52.8%
STP14	50.7%

Supplementary Table 2. Sequence identity and Root Mean Square Deviation of STP10 to structures from the Sugar Porter group.

Name	PDB code	Sequence %ID (Needle-Wunsch alignment)	RMSD (Å) (all-atom fit to STP10)
XylE	4GC0	27.1%	1.86
XylE	4JA3	27.1%	2.71
XylE	4JA4	27.1%	3.04
XylE	4QIQ	27.1%	2.93
GlcPse	4LDS	27.8%	3.31
hGLUT1	4PYP	23.4%	3.50
hGLUT3	4ZWC	25.2%	1.89
hGLUT3	4ZW9	25.2%	1.83
bGLUT5	4YB9	24.0%	3.34
rGLUT5	4YBQ	26.0%	2.12

Supplementary Table 3. Data collection and refinement statistics

STP10:Glucose	
Data collection	
Space group	P 2 ₁ (#4)
No of crystals merged	2
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	51.7, 92.5, 66.8
α , β , γ (°)	90.0, 109.4, 90.0
Resolution (Å)	63 - 2.4 (2.5 - 2.4) *
<i>R</i> _{meas} (%)	30.6 (234.3)
<i>I</i> / σI	4.9 (0.9)
CC(1/2) (%)	99.1 (34.8)
Completeness (%)	99.8 (99.8)
Redundancy	6.6 (6.6)
Refinement	
Resolution (Å)	63 - 2.4 (2.51 - 2.4)
No. reflections (work/free)	22,078 / 1,162
<i>R</i> _{work}	20.3 (31.7)
<i>R</i> _{free}	26.8 (36.6)
No. atoms	
Protein	3,771
Ligand/ion	164
Water	34
<i>B</i> -factors	
Protein	53.9
Ligand/ion	68.7
Water	46.4
R.m.s. deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.01
Ramachandran plot	
Favored (%)	95.7
Outliers (%)	0.0
Rotamer outliers (%)	0.25
Clashscore	10.38
Molprobity score	1.84

*Values in parentheses are for highest-resolution shell.