Supplementary Information

Prokaryotic solute/sodium symporters: versatile functions and mechanisms of a transporter family

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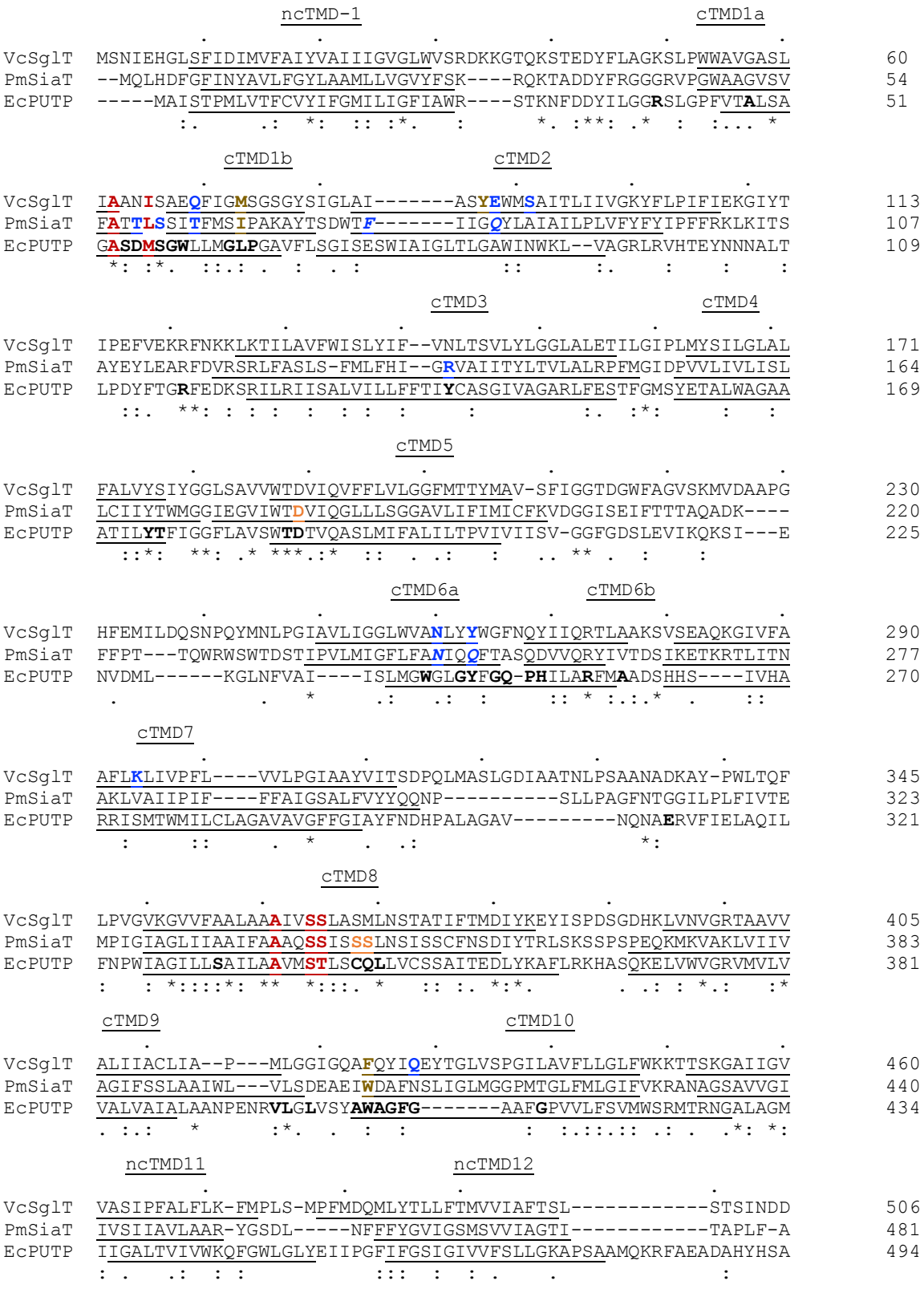
ncTMD-1
cTMD1a
VcSglT MSNIEHGLSFIDIMVFAIYVAIIIGVGLWVSRDKKGTQKSTEDYFLAGKSLPWWAVGASL 60
PmSiaT --MQLHDFGFINYAVLFGYLAAMLLVGVYFSK----RQKTADDYFRGGGRVPGWAAGVSV 54
EcPUTP -----MAISTPMLVTFCVYIFGMILIGFIAWR----STKNFDDYILGGRSLGPFVTALSA 51
cTMD1b
cTMD2
VcSglT IAANISAEQFIGMSGSGYSIGLAI-------ASYEWMSAITLIIVGKYFLPIFIEKGIYT 113
PmSiaT FATTLSSITFMSIPAKAYTSDWTF-------IIGQYLAIAILPLVFYFYIPFFRKLKITS 107
EcPUTP GASDMSGWLLMGLPGAVFLSGISESWIAIGLTLGAWINWKL--VAGRLRVHTEYNNNALT 109
cTMD3
cTMD4
VcSglT IPEFVEKRFNKKLKTILAVFWISLYIF--VNLTSVLYLGGLALETILGIPLMYSILGLAL 171
PmSiaT AYEYLEARFDVRSRLFASLS-FMLFHI--GRVAIITYLTVLALRPFMGIDPVVLIVLISL 164
EcPUTP LPDYFTGRFEDKSRILRIISALVILLFFTIYCASGIVAGARLFESTFGMSYETALWAGAA 169
cTMD5
VcSglT FALVYSIYGGLSAVVWTDVIQVFFLVLGGFMTTYMAV-SFIGGTDGWFAGVSKMVDAAPG 230
PmSiaT LCIIYTWMGGIEGVIWTDVIQGLLLSGGAVLIFIMICFKVDGGISEIFTTTAQADK---- 220
EcPUTP ATILYTFIGGFLAVSWTDTVQASLMIFALILTPVIVIISV-GGFGDSLEVIKQKSI---E 225
cTMD6a
cTMD6b
VcSglT HFEMILDQSNPQYMNLPGIAVLIGGLWVANLYYWGFNQYIIQRTLAAKSVSEAQKGIVFA 290
PmSiaT FFPT---TQWRWSWTDSTIPVLMIGFLFANIQQFTASQDVVQRYIVTDSIKETKRTLITN 277
EcPUTP NVDML------KGLNFVAI----ISLMGWGLGYFGQ-PHILARFMAADSHHS----IVHA 270
cTMD7
VcSglT AFLKLIVPFL----VVLPGIAAYVITSDPQLMASLGDIAATNLPSAANADKAY-PWLTQF 345
PmSiaT AKLVAIIPIF----FFAIGSALFVYYQQNP----------SLLPAGFNTGGILPLFIVTE 323
EcPUTP RRISMTWMILCLAGAVAVGFFGIAYFNDHPALAGAV---------NQNAERVFIELAQIL 321
cTMD8
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PmSiaT MPIGIAGLIIAAIFAAAQSSISSSLNSISSCFNSDIYTRLSKSSPSPEQKMKVAKLVIIV 383
EcPUTP FNPWIAGILLSAILAAVMSTLSCQLLVCSSAITEDLYKAFLRKHASQKELVWVGRVMVLV 381
cTMD9
cTMD10
VcSglT ALIIACLIA--P---MLGGIGQAFQYIQEYTGLVSPGILAVFLLGLFWKKTTSKGAIIGV 460
PmSiaT AGIFSSLAAIWL---VLSDEAEIWDAFNSLIGLMGGPMTGLFMLGIFVKRANAGSAVVGI 440
EcPUTP VALVAIALAANPENRVLGLVSYAWAGFG-------AAFGPVVLFSVMWSRMTRNGALAGM 434
ncTMD11
ncTMD12
VcSglT VASIPFALFLK-FMPLS-MPFMDQMLYTLLFTMVVIAFTSL----------STSINDD 506
PmSiaT IVSIIAVLAAR-YGSDL-----NFFFYGVIGSMSVVIAGTI----------TAPLF-A 481
EcPUTP IIGALTVIVWKQFGWLGLYEIIPGFIFGSIGIVVFSLLGKAPSAAMQKRFAEADAHYHSA 494
VcSglT DPKGISVTSSMFVTDRSFNIAAYGIMIVLAVLYTLFW 543
PmSiaT PAKQLSLDDSET----SEN---------- 496
EcPUTP PPSRLQES---------- 502

Supplementary Figure S1. Amino acid sequence alignment of the SSS family transporters SGLT of *V. parahaemolyticus* (vSGLT), SiaT of *P. mirabilis* (PmSiaT) and PutP of *E. coli* (EcPutP). The alignment was performed with Clustal Omega [1]. Amino acid sequences forming TMDs are underlined. TMDs are numbered as described in Figure 2a. Amino acids highlighted in red coordinate sodium at the Na2 site, while amino acids in orange belong to the recently described Na3 site of PmSiaT. Amino acids labeled in blue constitute the central substrate binding site. Amino acids highlighted in blue and *italic* interact with the substrate via water molecules. Amino acids highlighted in brown form the outer "thin" hydrophobic gate. For PutP, amino acids of particular functional significance are highlighted in **bold** (compare also Table 2). Information was taken from the following references: vSGLT [2,3], PmSiaT [4], EcPutP [5-9].

Suplementary Table S1. Amino acids involved in sodium and substrate binding in PutP, vSGLT and SiaT[a]

Function	PutP	vSGLT	SiaT
Na2 site	cTMD1: A53, M56 cTMD8: A337, S340, T341	cTMD1: A62, I65 cTMD8: A361, S364, S365	cTMD1: A56, L59 cTMD8: A339, S342, S343
Na3 site	cTMD5: D187 (?) cTMD8: S340 (?), C344 (?), Q345 (?)	no experimental evidence	cTMD5: D182 cTMD8: S342, S345 S346
central substrate binding site	cTMD1: S54, S57 (?) cTMD6: Y248, P252 (?) cTMD8: C344, M369 (?)	cTMD1: Q69 cTMD2: E88, S91 cTMD6: N260 cTMD7: K294 cTMD10: Q428	cTMD1: T58, S60, T63 cTMD2: F78, Q82 cTMD3: R135 cTMD6: N247, Q250
2nd substrate binding site[b]	cTMD1: S57, W59 (?) cTMD6: W244, Y248 (?) cTMD10: L398, S402 (?)	cTMD1: S66 (?) cTMD6: Y269, R273 (?) cTMD8: S365, S368 (?)	no experimental evidence

[a]The data of the sodium and the central substrate binding sites of vSGLT and PmSiaT were taken from the analyses of respective crystal structures [4,10]. For PutP, the amino acids proposed to be involved in sodium and substrate binding were identified by amino acid replacements in combination with comprehensive analyses of transport kinetics, ligand affinities and site directed labeling approaches [5-8]. The location of a 2nd substrate binding site in PutP and vSGLT was predicted based on computational analyses in combination with amino acid replacements, substrate binding and transport studies [11].

[b]Of note: Structural alignments between LeuT and vSGLT revealed that the crystallographically identified galactose-binding site in vSGLT [10] is located in a more extracellular location relative to the central substrate-binding site in LeuT. Therefore, the existence of an additional galactose-binding site in vSGLT was suggested and experimentally tested that aligns to the central binding site of LeuT [11]. Following this logic, the amino acids of vSGLT listed in the table under "2nd substrate binding site" constitute the central binding site while the amino acids listed under "central binding site" form a more external binding site.

Supplementary Table S2. Information of the isolates from the SMART database used for Figure 5.

Number in Figure 5	Isolates (Organisms/SMART ID)
1	***Candidatus* Nitrosocaldus cavascurensis/ A0A2K5ARA0_9ARCH (A0A2K5ARA0)**
2	***Pseudomonas stutzeri*/ A0A0H3Z2P6_PSEST (A0A0H3Z2P6)**
3	***Pseudomonas fluorescens Q2-87*/J2EGW4_PSEFL (J2EGW4)**
4	***Pseudomonas corrugata*/ A0A1B3C9J6_9PSED (A0A1B3C9J6)**
5	***Actinospica acidiphila*/ UPI00052494D7**
6	***Streptomyces griseorubens*/ UPI00056BF26D**
7	***Streptomyces albus*/ UPI000689E04F**
8	***Streptomyces gilvosporeus*/ A0A1V0TJU0_9ACTN (A0A1V0TJU0)**

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