

Supporting Material - A Gate-Free Pathway for
Substrate Release from the Inward-Facing State of the
 Na^+ -Galactose Transporter

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Table S1: Averaged interaction energies (kcal/mol) between the substrate and various subsets of the environment (protein, water, or individual residues) calculated for the unbound state obtained after Unbinding Event I ($t = 81-96$ ns) and the Silent Phase ($t = 130-200$ ns).

Environment	Unbinding Event I	Silent Phase
protein	-31.90 ± 3.51	-59.61 ± 1.30
water	-32.96 ± 5.26	-16.58 ± 0.99
N64	-0.48 ± 1.03	-1.62 ± 1.20
E68	-15.48 ± 4.41	-5.65 ± 2.84
Q69	-0.16 ± 0.47	-4.22 ± 0.85
E88	1.28 ± 0.32	-35.38 ± 1.23
S91	-0.16 ± 0.62	1.36 ± 0.35
N142	-2.18 ± 0.03	-0.75 ± 0.01
N260	0.22 ± 0.39	-1.67 ± 1.14
Y263	-2.55 ± 0.92	-1.81 ± 0.65
K294	-1.18 ± 0.31	2.27 ± 1.56
Q428	-1.24 ± 3.23	-1.38 ± 0.06

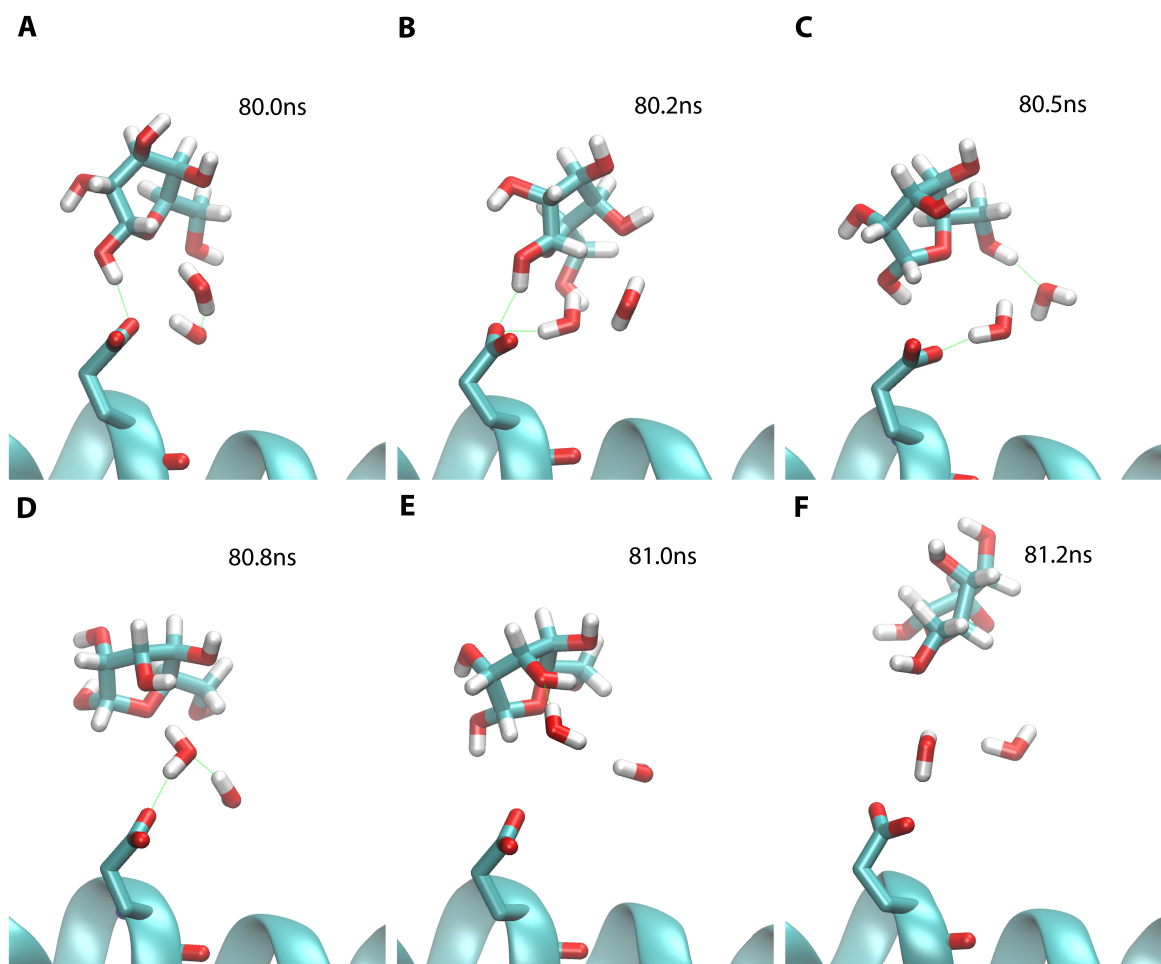


Figure S1: Lubricating effect of water in unbinding of the substrate from E88. Several snapshots taken from the equilibrium simulation showing the facilitating effect of water in detachment of the substrate from E88 through competing for H-bonds. Substrate, E88, and two water molecules in the region are shown in stick representations. Snapshots are taken at $t = 80.0$ ns (A), 80.2 ns (B), 80.5 ns (C), 80.8 ns (D), 81.0 ns (E), and 81.2 ns (F) of the equilibrium simulation.

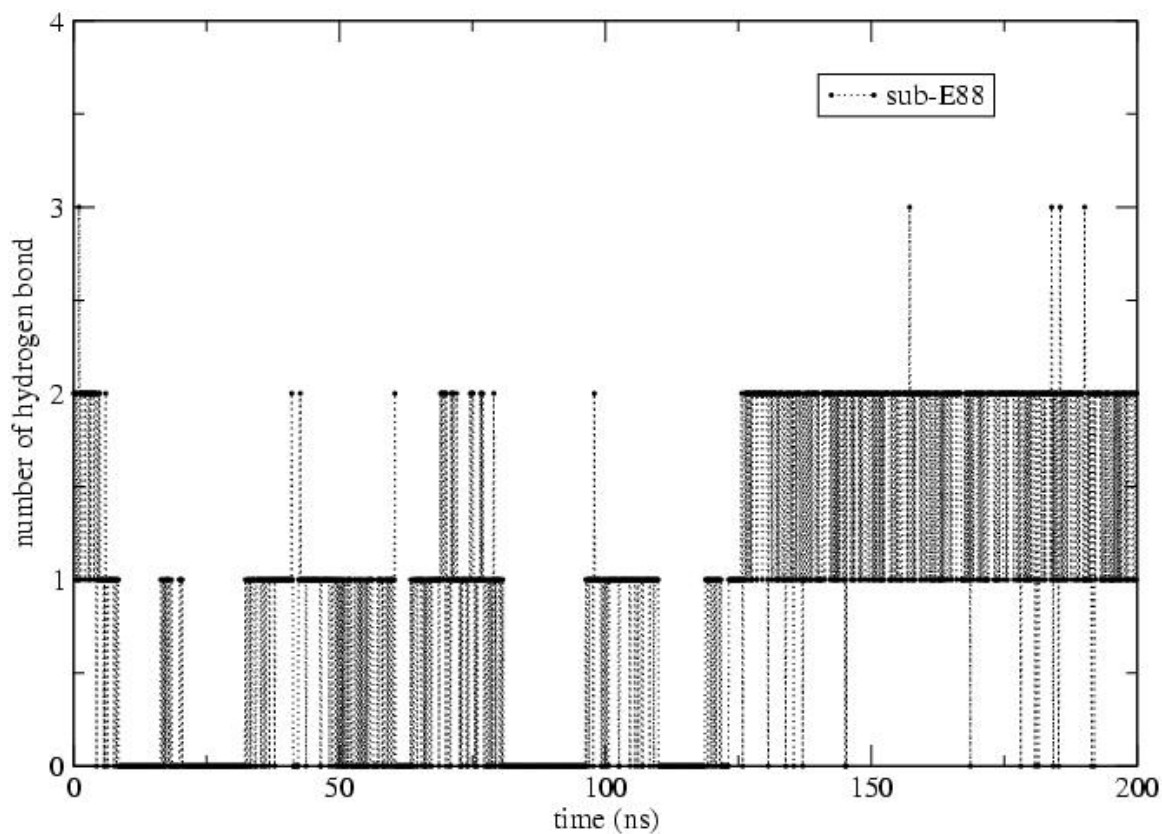


Figure S2: The number of H-bonds between the substrate and E88 during the 200 ns equilibrium simulation. A heavy atom distance cutoff of 3.0 Å and an angle cutoff of 30° were used to define H-bonds

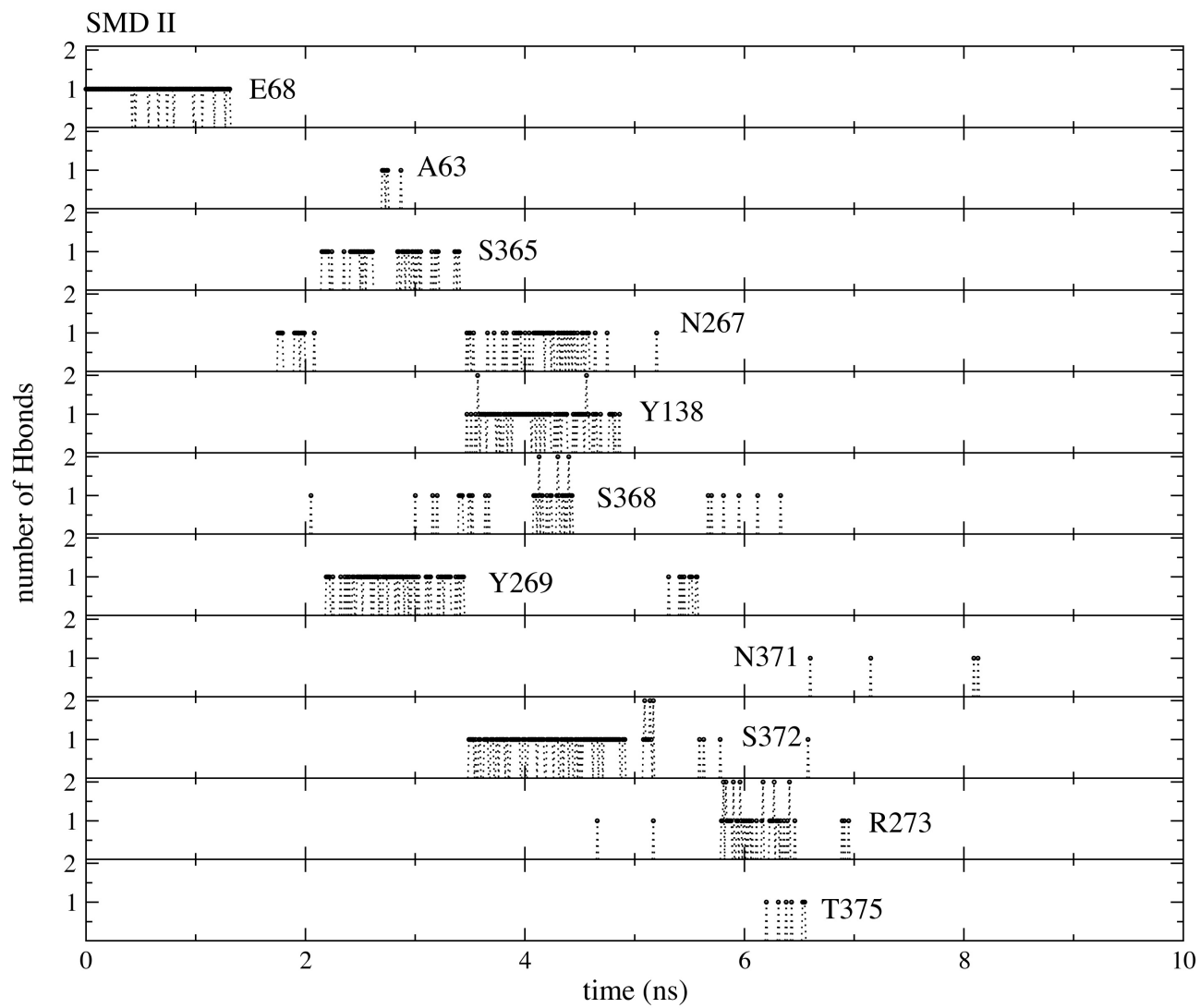


Figure S3: The number of H-bonds between the substrate and the residues lining the substrate exit pathway as the function of time in the simulation SMD-II.

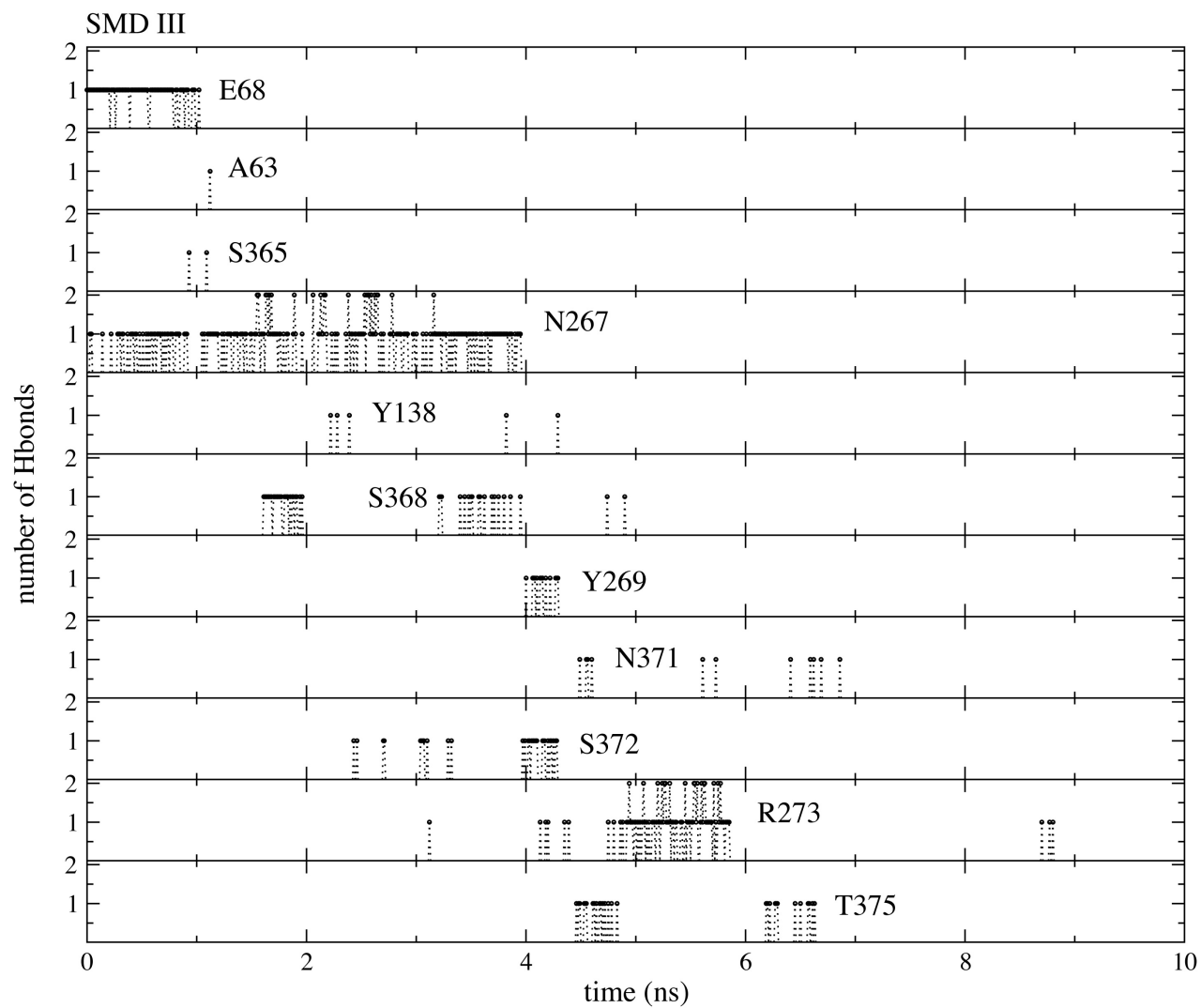


Figure S4: The number of H-bonds between the substrate and the residues lining the substrate exit pathway as the function of time in the simulation SMD-III.

10 20 30 40 50

SGLT_VIBPA 1 - - - - - MSNIEHGLSFI DIMVFAIYVAIIIGVGLWVSRDKKGTQKST E42

SC5A1_HUMAN 1 MDSSTWSPKTTAVTRPVEHELIRNAADISIIVYFVVVMAVGLWAMFSTN - - RGTVG56

SC5A2_RABIT 1 MEEHMEAGSRLGLG - - - DHGALIDNPADI AVIAAYFLLVIGVGLWVSMCRTN - - RGTVG53

SC5A3_CANFA 1 - - - - - MRAVLETADIAIVALYFILVMCIGFFAMWKS N - - RSTVS37

SC5A4_PIG 1 MASTLSPSTVTKTPGPPEISERIQNAADISVIVYFVVVMAVGLWAMLRTN - - RGTVG56

SC5AB_HUMAN 1 MESGTS SPQPPQLDPLDAFPQKGLEP GDIAVLVLYFLFVLT VGLWSTVTKK - - RDTVK56

PUTP_ECOLI 1 - - - - - MAISTPMLVTFVCVYIFGMILIGFIAWRSTK - - - - NFD33

60 70 80 90 100 110

SGLT_VIBPA 43 DYFLAGKSLPWWAVGASLIAANI SAEQFIGMSGSGYSIGLAIASYEWM SAI - - - - - TL95

SC5A1_HUMAN 57 GFFLAGRSMVWVPI GASLFASNIGSGHFVGLAGTGAASGIAIGGF EWNALV - - - - - LV109

SC5A2_RABIT 54 GYFLAGRSMVWVPGASLFASNIGSGHFVGLAGTGAANGLAVAGFEWNA LF - - - - - VV106

SC5A3_CANFA 38 GYFLAGRSMTWVAIGASLFVSNIGSEHFIFLAGSGAASGFAVGAWEFNALL - - - - - LL90

SC5A4_PIG 57 GFFLAGRDVTWWPMGASLFASNIGSGHFVGLAGTGAASGIAIAAFEWNA LL - - - - - LL109

SC5AB_HUMAN 57 GYFLAGGDMVWVPGASLFASNVGSGHFIFLAGSGAATGISVSAYELNGLF - - - - - SV109

PUTP_ECOLI 34 DYILGGRSLGPFVFTAL SAGASDMSGWLLMGLPGAVFLSGISESWIAIGLTLGAWINWK91

120 130 140 150 160 170

SGLT_VIBPA 96 IIVGKYFLPIFIEKGIYTIPEFVEKRFN - - KKLKTI LAVFWISLYIFVNLT SVLYLGG151

SC5A1_HUMAN 110 VVLGWLFPVIYIKAGVVTMP EYLRKRFGG-QRIQVYLSLSL SLYIFTKISADIFSGA166

SC5A2_RABIT 107 LLLGWLFPVYLTAGVITMPQYLRKRFGG-HRIRLYLSVLSL SLYIFTKISVDMFSGA163

SC5A3_CANFA 91 QLLGWVFIPIYIRSGVYTMP EYLSKRFGG-HRIQVYFAALS LLYIFTKLSVDLYSGA147

SC5A4_PIG 110 LVLGWFFVPIYIKAGVMTMP EYLRKRFGG-KRLQIYLSLSL SLYICVALRISSDIFSGA166

SC5AB_HUMAN 110 LMLAWIFLPIYIAGQVTMP EYLRKRFGG-IRIPILAVLYL SLYIFTKISVDMYAGA166

PUTP_ECOLI 92 LVAGR LRVHT EYNNNA LTPDYFTGR FEDKSRILR IISALV ILLFFTIYCASGIVAGA149

180 190 200 210 220 230

SGLT_VIBPA 152 LALETILGIPLMYSILGLALFALVYSIYGGLSAVYVTDV IQVFFLV LGGFMTTYMAV S209

SC5A1_HUMAN 167 IFINLALGLNLYLAIFLLLAIT ALYTI TGGLAAVIYTDTLQTVIMLV GSLILTGF AFH224

SC5A2_RABIT 164 VFIQQA LGWNIYASVIALLGITMVYTVTGG LAALMYTDVTQTFV I IAGAFILTGY AFH221

SC5A3_CANFA 148 LFIQES LGWNLYVSVILLIGMTALLTVTGG LVAVIYTDTLQALLMIV GALT LMI SMM205

SC5A4_PIG 167 IFIKLALGLDLYLAIFSLLAITAIYTI TGGLASVIYTDTLQTIIMLIGSFI LMGFAFV224

SC5AB_HUMAN 167 IFIQQS LHLDLYLA IAGLLAITAVYTVAGGLAAV IYTDALQTLIMLIGALT LMGYSFA224

PUTP_ECOLI 150 RLFESTFGMSYETALWAGAAAT ILYTFI GGFLAVSWTDTVQASLMIFALILT PVIV I I207

240 250 260 270 280

SGLT_VIBPA 210 FIGGTDGWFAVSKMVDAA P - - - - - GHFEMILDQSNPQYMNLP G I A250

SC5A1_HUMAN 225 EVGGYDAFMEKYMKA IPTIVSDGN - - - TT FEKCYT - PRADSFHIFRDPLTGDLPWP G278

SC5A2_RABIT 222 EVGGY SGLFDKYMGA MTSLTVSEDP AVGNISS SCYR - PRPDSYHL LRDPTVTDLPWP A278

SC5A3_CANFA 206 EIGGFEEVKRRYMLASPNVTS ILLTYNLSNTNS CNVHPKKDALKMLRNPTDEDV PWP G263

SC5A4_PIG 225 EVGGY ESFTEKYMNA IPTIVEGDN - - - LTISP KCYT - PQGDSFHIFRDAVTDLPWP G278

SC5AB_HUMAN 225 AVGGMEGLKEKYFLA LASNRSNS - - - - - SCGL - PREDAFHIFRDPLTSDLPWP G273

PUTP_ECOLI 208 SVGGFGDSL EVIKQKS - - - - - IENV DMLKG - - - - - 232

300 310 320 330 340

SGLT_VIBPA 251 VLI GGLWVANLYYWG FNQYIIQRT LAAKSVSEAKGIVFAAF LKLVFPFLV LPGAIAA308

SC5A1_HUMAN 279 FIFG-MSI LTLWYWCTDQVIVQRCLSAKNM SHVKGGCILCGY LKLMMPFIMVMPGMI S335

SC5A2_RABIT 279 LLLG- LTI VSGWYWCSDQVIVQRCLAGRN LTHIKAGC ILCGY LKLTMPFLMVP GMI S335

SC5A3_CANFA 264 FVLG- QTPASVWYWCADQVIVQRCLSAKNIAHAKGSTLMAGFLKLLP MFIIVVPGMI S320

SC5A4_PIG 279 MIFG- M TVVAAWYWCTDQVIVQRCLSGKDM SHVKAACIMCGY LKLLPFLMVP GMI S335

SC5AB_HUMAN 274 VLF G-MSI PSLWYWCTDQVIVQRCLSAKNL SHAKGGA LMAAYLKVLP LFIIMVFP GMI S330

PUTP_ECOLI 233 - - LNFVA IISLMGWGLG- YFGQPHILARFMAADSHHSIVHARRISMTWMI LCLAGAV A287

350 360 370 380 390 400

SGLT_VIBPA 309 YVITSDPQLMASLGDIAATNLP SAANADKAYP- WLTQFLPVGVKGVVFAALAAAIVS S365

SC5A1_HUMAN 336 RILYTEK IACVVPSECEKYCGTKVGCTNIAYPTLVV E LMPNGLRGLMLSVMLASLMS S393

SC5A2_RABIT 336 RILYPDEVACVAP EVC KRVC GT EVGCSNIAYPRLVV K LMPNGLRGLMLAVMLAALMS S393

SC5A3_CANFA 321 RILFADDIACINPEHCMQVCGSRAGCSNIAYPRLV MKLV PVGLRGLMMAVMI IAA LMS D378

SC5A4_PIG 336 RILYTEK VACVVPSECVKHC GT EVGCSNYAYP LLMELMP SGLRGLMLSVMLASLMS S393

SC5AB_HUMAN 331 RILFPDQVACADPEICQKICSNP SGCSDIAYPKLVLEL LPTGLRGLMMAVMVAALMS S388

PUTP_ECOLI 288 VGGFG - - - IAYFNDHPALAGAVNQNA ERV FIE LAQILFNPW IAGILLSA I LAAVMST 341

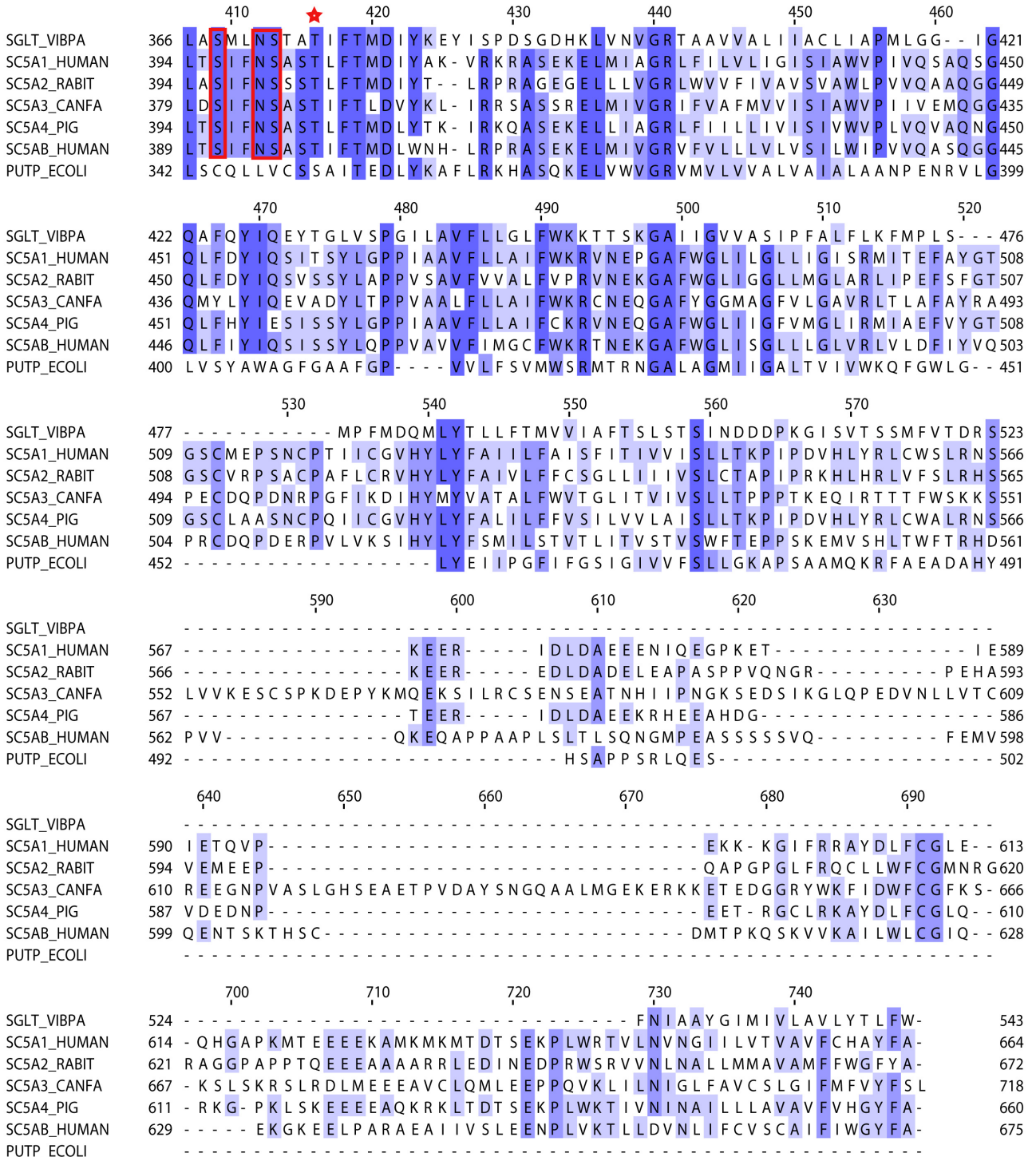


Figure S5: Sequence alignment of several members of the solute:sodium symporter (SSS) family (2.A.21) obtained from the Transporter Classification Database (www.tcd.org). SGLT_VIBPA: glucose or galactose:Na⁺ symporter from *V. parahaemolyticus*, vSGLT; SC5A1_HUMAN: human glucose or galactose:Na⁺ symporter; SC5A2_RABIT: rabbit nucleoside or glucose:Na⁺ symporter; SC5A3_CANFA: myoinositol:Na⁺ symporter from *Canis familiaris*, SMIT1; SC5A4_PIG: porcine glucose:Na⁺ symporter 3 (low affinity); SC5AB_HUMAN: human myoinositol:Na⁺ symporter, SMIT2; PUTP_ECOLI, proline:Na⁺ symporter from *E. coli*. The red boxes indicate the residues lining the substrate's release pathway characterized in our simulations of vSGLT, which are conserved in sugar:Na⁺ transporters but changed in the proline:Na⁺ symporter. The red stars indicate other residues lining the substrate's release pathway.