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Structures of SemiSWEET transporters in two distinct conformations

Yan Xu^{#1}, Yuyong Tao^{#1}, Lily S. Cheung^{#2}, Chao Fan¹, Li-Qing Chen², Sophia Xu³, Kay Perry⁴, Wolf B. Frommer², and Liang Feng^{1,@}

¹ Department of Molecular and Cellular Physiology, 279 Campus Drive, Stanford University School of Medicine, Stanford CA 94305

² Department of Plant Biology, Carnegie Institution for Science, 260 Panama St., Stanford, CA 94305

³Department of Biology, Stanford University, Stanford, CA 94305

⁴NE-CAT and Dep. of Chemistry and Chemical Biology, Cornell University, Building 436E, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne, IL 60439

# These authors contributed equally to this work.

Abstract

SemiSWEETs and SWEETs are mono- and disaccharide transporters present from Archaea to higher plants and humans¹⁻³. SWEETs play crucial roles in cellular sugar efflux processes, i.e. phloem loading⁴, pollen nutrition⁵ and nectar secretion⁶. Their bacterial homologs, SemiSWEETs, are among the smallest known transporters^{1,3}. Here we show SemiSWEET, consisting of a triple-helix-bundle (THB), forms a symmetric parallel dimer to create the translocation pathway. Two SemiSWEET isoforms were crystallized in apparent open and occluded states, indicating that SemiSWEETs/SWEETs are transporters that undergo rocking-type movements during the transport cycle. The topology of THB is similar to the basic building block in MFS transporters (GLUTs, SUTs), indicating that they may have evolved from an ancestral THB into a parallel configuration to produce 6/6+1 transmembrane-helix pores for SemiSWEETs/SWEETs, and an antiparallel configuration of 2×2 THBs to generate 12 transmembrane-helix pores for MFS transporters. Given the similarity of SemiSWEETs/SWEETs to PQ-loop amino acid transporters and mitochondrial MPC organic acid transporters, the structures characterized here may also be relevant for other *Mtn3* clan transporters⁷⁻⁹.

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@author for correspondence: Liang Feng Tel: 609-933-2897 liangf@stanford.edu.

Author Contributions.

Y.X., W.B.F. and L.F. conceived and designed experiments. Y.X., Y.T. performed expression, purification, crystallization, data collection and crystallography, C.F. performed functional experiments, S. X. performed biochemical characterization, L.C., L.-Q.C., performed alignments and functional experiments, K.P. performed data collection and assisted crystallography, L.F. contributed to crystallization, data collection and crystallography. Y.X., Y.T., L.C., C.F., L.-Q.C., W.B.F. and L.F. analyzed the data. L.F. and W.B.F. wrote the manuscript.

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Sugars produced by photosynthesis serve as key energy sources for humans. In both plants and animals, sugars are transported across cellular membranes as a means of distribution throughout the body^{10,11}. While sugar transporters are essential for translocation in plants, human transporters play critical roles in glucose homeostasis; mutations lead to diseases such as diabetes, glucose malabsorption, or epilepsy^{10,11}. Striking similarities exist among the sugar transporter proteins used by plants and animals. Animal and human genomes encode three major classes of sugar transporters: Major Facilitator Superfamily (MFS)-type transporters of the GLUT family (SLC2)¹¹, sodium-dependent glucose transporters of the SGLT family (SLC5)¹⁰ and the recently identified SWEET/SemiSWEET sugar transporters (SLC50)¹⁻³. Plant genomes contain homologs of GLUT transporters, specifically STP glucose/H⁺ symporters and SUT sucrose/H⁺ symporters¹², as well as SWEET transporters¹. Major breakthroughs in understanding how these transporters function resulted from solving atomic structures for the prototype of the MFS transporter family, lactose permease¹³, as well as GLUT¹⁴ and SGLT homologs¹⁵. Both MFS and SGLT transporters are built from 12 transmembrane helices (TM), but form fundamentally different structures: MFS transporters are composed of four structurally related triple helix bundles (THBs) arranged in antiparallel format; whereas SGLTs consist of two 5-TM bundles in antiparallel arrangement.

Until now, there was limited information on the structure of SWEETs and their bacterial SemiSWEET homologs^{2,3}. Plant SWEETs play crucial roles in intercellular transport and cellular secretion. Specific isoforms are key for cellular efflux as a first step for phloem loading⁴, pollen nutrition⁵ and nectar secretion⁶, and they also play key roles in pathogen susceptibility^{2,4,16}. The human genome contains a single SWEET homolog, which functions as a glucose transporter². The finding that the *Ciona intestinalis* SWEET is essential indicates that animal and human SWEETs play important roles in physiology¹⁷. SWEETs are unique in that eukaryotic isoforms are predicted to be heptahelical with an internal THB repeat²; while prokaryotic SemiSWEET contain only 3 TMs³.

To determine the structure and function of SemiSWEETs/SWEETs, two SemiSWEETs were crystallized in different states. The basic unit in both structures is a THB arranged as a 1-3-2 bundle, and two THBs are arranged in parallel to form the actual conduit. Six TMs are thus sufficient to form the pore. Moreover, the detection of two distinct states indicates that SemiSWEETs/SWEETs do not function as sugar channels, but rather as transporters that undergo rocking movements. We suggest that the eukaryotic heptahelical SWEETs form a similar structure in which a SemiSWEET-like dimer is fused via an inversion linker helix (TM4). We also show that pairs of Trp and Asn residues in the pore are essential for SemiSWEET and SWEET function.

The structure of a SemiSWEET from *Vibrio sp.* N418 (VsSemiSWEET; Extended Data Fig. 1) was determined at 1.7Å resolution from crystals grown in the lipid cubic phase (LCP) (Extended Data Table 1). The protomer of VsSemiSWEET contains 3 TMs and a nonconserved extra N-terminal amphipathic α -helix (AH). Within the protomer, TM3 is sandwiched between TM1 and TM2 and there is little direct contact between TM1 and TM2 (Fig. 1). This arrangement of TM helices shares similarities with the triple-helix repeat of MFS transporters¹⁸ although SemiSWEET and MFS belong to unrelated folds (Supplementary Discussion). The orientation of the protomer relative to the membrane was

inferred using the ‘inside positive rule’¹⁹, which is in good agreement with the observation that the cytosolic C-terminus of *Arabidopsis* SWEET11 was phosphorylated *in vivo*²⁰.

In the VsSemiSWEET crystal, two molecules that are related by a two-fold axis perpendicular to the membrane tightly interact to form a dimer (Fig. 1b & Extended Data Fig. 2). At the dimer interface, TM1 of one protomer packs against TM2 of the other protomer, and vice versa. When viewed from the extracellular side, the backbone forms a basket-like structure, with an opening to the extracellular side, while the intracellular side was sealed by loops L1-2 (Fig. 1b). Several lines of evidence support the physiological relevance of dimer formation. First, the interface between the subunits is extensive, encompassing $\sim 1,970\text{\AA}^2$. Second, the dimer is formed in the lipid bilayer environment of the crystal (Extended Data Fig. 2). The majority of non-packing hydrophilic residues within the membrane points to the center of the dimer interface, compatible with a putative translocation route at the interface of subunits. Third, consistent with the structure, VsSemiSWEET dimerizes in solution and remains as a dimer during SDS-PAGE, indicating stable dimer assembly (Extended Data Fig. 3a). Cross-linked BjSemiSWEET³ products also migrate as dimers (Extended Data Fig. 3b). Together, our structural and biochemical observations strongly suggest formation of the transport pore of VsSemiSWEET by a dimer.

From bioinformatic analyses, e.g. as in PFAM, SemiSWEETs belong to the ‘*MtN3 clan*’ and are distantly related to the ‘*PQ-loop*’ family in this clan, defined by a conserved PQ-dipeptide motif^{7,21,22}. Contradictory to the assumption that the PQ-motif is positioned in a loop region, the PQ-motif is embedded in the membrane and part of TM1 in VsSemiSWEET (Fig. 1d). The role of the conserved glutamine in SemiSWEETs is revealed by analyzing the dimer interface: the CO and NH group of the side chain amide group of Gln29 hydrogen bonds with NH and CO from two consecutive backbone amides right before TM2 of the other protomer, not only bringing the L1-2 loop to the dimer interface, but stabilizing the L1-2 loop conformation (Fig. 1d). The proline preceding the glutamine induces a kink in the helix, probably increases the flexibility of the transmembrane helix to allow formation of a glutamine backbone interaction, or potentially facilitates its disruption during the transport cycle.

The crystal structure indicates that the 3-TM protomer cannot form an enclosed compartment for substrate transport. Instead, there is a solvent-filled cavity between the two protomers at the central two-fold axis (Fig. 1e). The cavity transverses approximately halfway across the membrane and is completely separated from the lipid bilayer by the surrounding 6 TMs, but remains accessible from the extracellular side (Fig. 1c). This open cavity measures $9.2\text{\AA}$ at the narrowest point and is sufficient to allow small molecules to freely diffuse in or out. Among amino acids lining the cavity, Trp59 and Asn75 (Fig. 1e) are most conserved across species, constituting the only two invariant residues in 66 SemiSWEET sequences analyzed. Both residues strategically sit at a similar level above the bottom of the open cavity. Their side chains surround the center of the cavity, form a putative binding pocket, and are most likely within range to interact with substrates given the size and geometry of the cavity. It is noteworthy that both residues are also highly conserved in 3-TM repeats of the SWEETs^{2,4} and MPC2^{8,9}, supporting their functional importance.

To further investigate the transport mechanism, we focused on a SemiSWEET from *Leptospira biflexa serovar Patoc* (named LbSemiSWEET) that shares significant homology (44% identity and 63% similarity) with the known sugar transporter BjSemiSWEET³ (Extended Data Fig. 1). The structure of LbSemiSWEET was determined at 2.4Å resolution from crystals grown in LCP (Extended Data Table 1). In one asymmetric unit, two molecules tightly interact with each other, forming a dimer in the lipid bilayer (Fig. 2 & Extended Data Fig. 4). The dimer interface of LbSemiSWEET is highly similar to that of VsSemiSWEET, despite only modest sequence similarity (15% identity), strongly supporting the notion that the dimeric architecture is a common feature of SemiSWEETs.

At the interface of two protomers, LbSemiSWEET contains a large cavity right above its center (Fig. 2b). In contrast to VsSemiSWEET, the cavity of LbSemiSWEET is completely sealed from solvent. Near the extracellular side, Asp57 from one protomer forms hydrogen bonds with Tyr51 from the other protomer (Fig. 2c), shielding the cavity from the extracellular solution. This may explain the high conservation of Asp57 and Tyr51 across SemiSWEET, SWEET and MPC families^{2,4,8,9}: they form the cap on top of the cavity in the “occluded” state; cross-protomer interactions may facilitate the formation of this conformation. At the center of the cavity, there is a strong non-protein electron density, the identity of which cannot be unambiguously determined at this resolution (Fig. 2d). The flatshaped density is surrounded by Trp48 and Asn64 (equivalent to Trp59 and Asn75 in VsSemiSWEET) from both protomers. The antiparallel aromatic ring of Trp from each protomer is within 4Å from the putative substrate, and may interact with and stabilize the putative substrate in the pocket. The precise mode of the interaction is unclear, possibly involving hydrogen bonds and stacking interactions. The Asn side chains point to the putative substrate and are in close proximity, likely contributing to substrate binding. These structural observations implicate critical roles for Trp48 on TM2 and Asn64 on TM3 in substrate binding and translocation.

To assess the roles of Trp48 and Asn64 in sugar transport, we generated alanine substitution mutants in LbSemiSWEET and tested transport activity. In cell-based radiotracer uptake assays, the glucose uptake deficient *E. coli* strain²³ expressing wild-type LbSemiSWEET showed significantly higher glucose uptake compared to controls, consistent with the homology-based prediction that LbSemiSWEET transports sugar. When Trp48 or Asn64 were mutated to alanine, glucose uptake was markedly reduced to a level similar as in controls (Fig. 3a). We did not detect significant glucose uptake activity for VsSemiSWEET (data not shown). Furthermore, alanine substitutions of corresponding Trp and Asn in both THB repeats in *Arabidopsis* SWEET1 (Extended Data Fig.1), a glucose transporter, failed to complement the growth phenotype of a hexose uptake-deficient yeast strain (Fig. 3b). The mutations had no significant effect on plasma membrane localization of SWEET1 in yeast (Extended Fig. 5). These results demonstrate that Trp and Asn play critical roles in sugar transport in both SemiSWEETs and plant SWEETs and further support the notion that SemiSWEETs and SWEETs share the same basic architecture.

Alternating access is the prevailing model to explain substrate transport by transporters^{24,25}. Our structural observations support an alternating access mechanism in SemiSWEETs. In the crystal, VsSemiSWEET adopts an outward open conformation, while LbSemiSWEET is

captured in an occluded state (Fig 1c&2b). Although LbSemiSWEET and VsSemiSWEET share modest sequence identity, the monomer of LbSemiSWEET superimposes well onto that of VsSemiSWEET, with a main-chain Root-mean-square deviation (RMSD) of 1.1 Å over 66 aligned residues (Fig. 4a). By contrast, pronounced differences were observed between dimers of LbSemiSWEET and VsSemiSWEET (Fig. 1b, 2a, 4b). The dimer interface of VsSemiSWEET opens more towards the extracellular side compared with LbSemiSWEET and is ~10 Å wider at the extracellular surface (Fig. 4b). This is achieved mainly through a ~10-degree rotation of the protomer around an axis near the intracellular membrane surface (Fig. 4a). To a lesser extent, slight bending of the transmembrane helices of LbSemiSWEET towards the center contributes to its more closed conformation. The conformational differences between VsSemiSWEET and LbSemiSWEET indicate a “rocker-switch” mechanism and bear interesting parallels to structurally unrelated transporter families, such as MFS proteins and ATP-binding cassette (ABC) transporters, in which rigid body rocking between transmembrane subdomains provides alternating access to the substrate^{18,19,26,27}. We propose that a similar rocking-type movement of two SemiSWEET subunits will result in two additional states: an “inward open” conformation and an “occluded empty” state to complete a transport cycle (Extended Data Fig 7a). It still remains to be determined whether the transport cycle is coupled to proton transfer or operates by facilitated diffusion. SWEETs show properties that are consistent with a facilitated diffusion or uniport, including pH-independence and low affinity^{2,4}. More detailed functional analysis informed by the structure may help to determine the exact transport mechanism.

Eukaryotic SWEETs consist of two SemiSWEET-like units fused via an inversion linker transmembrane helix. It was not clear whether SWEETs could be large enough to form a pore from a single heptahelical subunit or have to form a higher oligomer³. In light of the SemiSWEET structures with putative substrate binding sites at the center of the dimer interface, we propose that the two THBs in a single SWEET are able to form the transport route. Mutagenesis analysis of SWEET is compatible with this hypothesis by showing the functional conservation of key residues between SemiSWEET and SWEET proteins. SWEETs might form multibarrelled oligomers as part of a regulatory mechanism. Such regulation has been observed in GLUT glucose transporters²⁸, AMT1 ammonium transporters²⁹ and NRT1 nitrate transporters³⁰. Finally, since mitochondrial pyruvate carriers (MPC)^{8,9} contain a related THB, our data is consistent with the observation that two copies of MPC are required to produce the functional transporter by likely forming a hetero-dimer.

Methods

Protein expression and purification

Full-length *Leptospira biflexa* serovar *Patoc* SemiSWEET (LbSemiSWEET, GI: 183221000) and *Vibrio* *sp.* N418 SemiSWEET (VsSemiSWEET, GI:497419526) genes were codon optimized and cloned into the pJexpress411 vector containing sequences for a 3C protease cleavage site and a His-tag (DNA2.0 Inc.). *E. coli* BL21(DE3) was transformed and cells were first grown at 37°C until the OD₆₀₀ reached 0.8, then the temperature was

lowered to 22°C, and overexpression was induced by 0.2 mM isopropyl β -D-thiogalactoside (IPTG). Cells were harvested 16 hr post induction and lysed in a buffer containing 50 mM Tris-HCl, pH8.0, 150mM NaCl by sonication. Membrane proteins were solubilized by incubating the lysate with 2% of (w/v) dodecyl- β -D-maltopyranoside (DDM; Anatrace) at 4°C for 2 hr. Cell debris was removed by centrifugation at 16,000rpm for 45 min. Supernatant was collected and incubated with HisPur Cobalt resin (ThermoScientific) at 4°C for 2 hr. Resin was washed with a buffer containing 20mM Tris-HCl pH8.0, 150mM NaCl, 0.1% DDM. His-tag was cleaved by PreScission and protein was purified by gel filtration (Superdex-200, GE Healthcare) in a buffer containing 10mM Tris-HCl pH8.0, 150mM NaCl, 0.03% DDM. Peak fractions were collected, concentrated, flash-frozen in liquid nitrogen and stored at -80°C. All SemiSWEET mutants were generated by inverse PCR, and were overexpressed and purified in the same way as the wild-type protein. Selenomethionine (Se-Met) labeled protein was purified similarly. After the cells were lysed by sonication, cell debris was removed by centrifugation and the supernatant was collected and applied to ultra-centrifugation at 50,000rpm for 1 hr. Membrane fraction was solubilized with 2% of (w/v) DDM at 4°C for 2 hr. Membrane debris was removed by ultra-centrifugation and the supernatant was incubated with Co-resin and the following steps were the same as above for native protein.

Crystallization

Purified VsSemiSWEET and LbSemiSWEET were reconstituted into monoolein (protein/lipid ratio = 1/1.5) by mixing in a coupled glass syringe system. Crystals were grown in glass sandwich plates at 20°C. VsSemiSWEET crystals appeared in conditions containing 30% PEG 400, 100mM MgCl₂ and 50 mM MES pH6.5 in two days and grew to full size in about one week. The precipitant solution to obtain LbSemiSWEET crystals consisted of 10% PEG 400, 150 mM NH₄SO₄ and 100 mM HEPES pH7.5. The crystals were harvested by mesh grid loops (MiTeGen) in about one week and flash frozen in liquid nitrogen.

Data Collection

Both the native and anomalous data of VsSemiSWEET were collected on the same crystal at beamline 24ID-C of Advanced Photon Source. After collection of a complete native data set at 0.97920 Å, the same crystal was used for native sulfur SAD data collection at 1.7712 Å. 540 degrees of data were collected in three separate wedges to minimize radiation damage and increase redundancy. The wedges were merged using XDS and SCALA as implemented in RAPD (<https://github.com/RAPD/RAPD>)^{31,32}. The diffraction data of LbSemiSWEET were collected at beamline 23ID-B and 23ID-D of Advanced Photon Source. Typically, the crystals were initially rastered with a 80 by 50 μ m beam with fourfold attenuation and 1 s exposure and subsequently sub-rastered with a 20 or 10 μ m beam. Data collection was performed with a 10 μ m beam. In most cases, wedges of 20-40° could be collected from each crystal except few cases where more than 100° wedges data were collected. The diffraction data were processed and merged with HKL2000³³.

Structure determination

For VsSemiSWEET, sulfur sites were determined using ShelxC/D³⁵. Phasing was improved with AutoSol and an initial model was built using AutoBuild from PHENIX as implemented

in RAPD. This model was used as the search model to perform molecular replacement against the native data using Phaser³⁶. The model was completed through manual rebuilding in Coot and iterative refinement with phenix.refine. In the final model, 97.9% residues fell in the favored region and 2.1% residues were in the allowed region in the Ramachandran plot. The initial phase of LbSemiSWEET was obtained by molecular replacement using a search model created by Phenix.Sculptor based on sequence alignment with linker loops removed³⁵. The phase was further improved by MR-SAD using Phaser and extended to 2.4 Å through 4-fold averaging with the program DM^{36,37}. The model was manually rebuilt in Coot and iteratively refined in Phenix^{35,38}. In the final model, 100% residues fell in the favored region in the Ramachandran plot. All structures were checked by MolProbity and figures were prepared using PyMOL^{39,40}.

Crosslinking of SemiSWEET

The protein was purified as described above except the buffer containing 10mM HEPES, pH7.5, 150mM NaCl and 0.05% DDM was used for gel filtration. Around 15 µg of protein was used in each reaction containing no disuccinimidyl suberate (DSS), 0.02 mM, 0.2 mM, and 2 mM DSS, respectively. The reaction was carried out at room temperature for 30 min and stopped by 50 mM Tris-HCl, pH 8.5. Aliquots of the reaction mixture were run on SDS-PAGE gel (Any kD™ Mini-Protean® TGX™ gel, Bio-Rad) and stained with Coomassie dye.

Cell-based uptake assay

The glucose transporter-deficient *E. coli* strain JM1100 was obtained from Yale *E. coli* Genetic Stock Center. Uptake assays were performed similarly as a published protocol⁴¹. SemiSWEET wild-type (WT) and variants containing indicated point mutations were subcloned into the pQE80 vector. The *E. coli* cells containing plasmids were grown in Luria-Bertani medium at 37°C and were induced with 50 µM IPTG for 30 min when OD₆₀₀ reached 1.5. Cells harvested by centrifugation were washed twice with MK buffer (150mM KCl, 5mM MES pH 6.5) before being resuspended in the MK buffer to OD₆₀₀ of 2.0. 20mM glycerol was added to energize cells 5 min before a mixture of 2 µM D- [¹⁴C]glucose and 1mM D-glucose was added to start the uptake reaction. After 1 min incubation with glucose, cells were filtered onto 0.22-µm cellulose acetate filter membranes (Millipore) under low gentle vacuum. The membranes were immediately washed with 1 ml of ice-cold MK buffer three times and then taken for liquid scintillation counting. Control experiments were carried out on cells containing an empty vector of pQE80. All experiments were performed at room temperature and repeated at least three times. SemiSWEET variants showed similar membrane expression level as the WT protein by preparing the membrane fraction and following the same protocol as for purification of WT.

Plasmid constructs

SWEET1 mutants were generated by site direct mutagenesis on the pDONR221-SWEET1 vector using the following primers:

Construct	Forward primer	Reverse primer
SWEET1-W ⁵⁶ A	GCgtatggacttcctttgtgtc	agcagagaggagacagttgag
SWEET1-N ⁷³ A	GCtggcacaggagcagtgat	gattgtgctcacaagtgtgtt
SWEET1-W ¹⁷⁶ A	GCgttcgtctatggctaatcgg	cgaagtaccacaggaagac
SWEET1-N ¹⁹² A	GCtgggtttggatgtgcatta	tgggattgcaacaaaagg

The sequences of *SWEET1* and *SWEET1* mutants were cloned into the yeast expression vectors pDRf1-GW and pDRf1-GW-eGFP³ by LR reaction (Life Technologies). The empty vector negative control was generated by replacing the Gateway cassette in the destination vectors by the sequence CAGCTG.

Complementation of the yeast mutant EBY4000

The yeast strain EBY4000 [*hxt1-17 ::loxP gal2 ::loxP stl1 ::loxP agt1 ::loxP ydl247w ::loxP yjr160c ::loxP*]⁴² was transformed with *SWEET1*, *SWEET1* mutants and the empty vector using the LiAc/SS carrier DNA/PEG method⁴³. Transformants were selected on SD medium supplemented with 2% maltose and auxotrophic requirements. For complementation growth assays, cells were grown in liquid medium containing 1% yeast extract, 2% peptone and 2% maltose (YPM) to an OD₆₀₀ of ~0.6, then diluted to OD₆₀₀ ~0.2 in water. Ten-fold serial dilutions were plated on SD media containing either 2% maltose (as control) or 2% glucose in addition to the respective auxotrophic requirements. Growth was documented by scanning (CanoScan, Canon) after 4 days at 30 °C. A representative sample from three experiments is shown in Fig.3.

Fluorescence microscopy

Fluorescence imaging of yeast was performed on a Leica TCS SP5 microscope. Cells were grown in YPM medium to an OD₆₀₀ of ~0.6 and then transferred to water for imaging. Fluorescence was detected with a glycerol-corrected 63x objective at 488 nm excitation and 492-600 nm emission. Image analysis was performed using Fiji software (<http://fiji.sc>).

Extended Data

Extended Data Figure 1. Sequence alignment

Secondary structure is shown above the alignment. Protein sequences were aligned using ClustalW and manually adjusted. N-terminal part (first THB) and C-terminal part (second THB) of AtSWEET1 were separately used for alignment and labeled as “AtSWEET1-N” and “AtSWEET1-C” respectively.

Extended Data Figure 2. VsSemiSWEET dimer in the crystal

Crystal packing in the lipid bilayer environment shows the formation of the dimer. Two protomers are colored in green and purple.

Extended Data Figure 3. Subunit stoichiometry of SemiSWEET

a, Purified VsSemiSWEET (predicted M.W. = 11.1 kDa) remains as dimer on SDS-PAGE.

b, Crosslinking of purified BjSemiSWEET (theoretical M.W.=10.1 kDa) in detergent solution. Increasing amounts of disuccinimidyl suberate (DSS), 0.02 mM, 0.2 mM, and 2 mM DSS were used to crosslink BjSemiSWEET. The protein was analyzed by SDS-PAGE and stained by Coomassie.

Extended Data Figure 4. Crystal packing of LbSemiSWEET

Crystal lattice structure of LbSemiSWEET shows dimer formation in the membrane. Two protomers are colored in red and blue respectively.

Extended Data Figure 5. Conserved Trp and Asn residues in AtSWEET1 are required for transport activity

a, Functional analysis of AtSWEET1-GFP fusion transport activity in the EBY4000 yeast strain. The point mutations W56A, N73A, W176A and N192A failed to complement the growth defect of EBY4000 in SD medium supplemented with 2% glucose as the only carbon source. Growth was unaffected in control medium containing 2% maltose. An empty vector and AtSWEET1-GFP are used as the negative and positive controls, respectively. **(b-g)** Membrane localization of the wild type and mutant AtSWEET1 was confirmed by imaging of GFP. The empty vector negative control **(b)** shows cytoplasmic localization of the GFP; while the wild type and W56A, N73A, W176A and N192A mutants **(c-g)** show membrane localization suggesting that the phenotype of the mutated transporters is not due to mislocalization.

Extended Data Figure 6. TM arrangements in SWEET, MFS and GPCR

The ribbon representations of three transmembrane helices unit in SWEET (left panel), GPCR (middle panel) and MFS (right panel) are shown on the top level and their respective topology is shown below. The same color scheme is used in all three proteins.

Extended Data Figure 7. Proposed transport model and pathway to build SWEET and MFS

a, Alternating access model for SemiSWEET transport. Two protomers are colored in purple and green. The putative substrate is shown as orange hexagon. **b**, A proposed pathway to

construct SemiSWEET/SWEET or MFS from the primitive THB. The same color scheme is used for all THB. The position of TM3 relative to TM1 and TM2 of the THB in SWEET or MFS is illustrated by colored circles viewing down from the N-terminus. Note that the THB in SWEET and MFS are roughly mirror-imaged.

Extended Data Table 1

Data collection and refinement statistics

	LbSemiSWEET (Native)	LbSemiSWEET (SeMet)	VsSemiSWEET (Native)	VsSemiSWEET (Sulfur-SAD)
Data Collection				
Space group	$P2_1$	$P2_1$	$I4_122$	$I4_122$
Cell dimensions (Å)				
a, b, c (Å)	36.91,51.83,46.96	36.85,51.70,47.05	58.24,58.24,172.92	58.28,58.28,173.24
α, β, γ (°)	90.00,94.85,90.00	90.00,94.85,90.00	90.00,90.00,90.00	90.00,90.00,90.00
		<i>Peak</i>		
Wavelength (Å)	0.9794	0.9793	0.9792	1.7712
Resolution (Å)	50.00-2.40 (2.44-2.40)	50.00-2.85 (2.95-2.85)	55.19-1.70 (1.73-1.70)	86.62-2.10 (2.21-2.10)
R_{merge} (%)	20.3 (55.6)	14.3 (62.4)	6.7 (88.3)	8.0 (73.1)
$I/\sigma I$	11.1 (2.0)	8.5 (1.5)	15.0 (0.8)	45.8 (6.4)
Completeness (%)	95.5 (79.4)	96.2 (99.0)	97.5 (76.9)	99.7 (98.4)
Redundancy	8.0 (2.3)	2.8 (2.7)	5.2 (2.0)	31.0 (30.1)
Refinement				
Resolution	36.78-2.40 (3.00-2.40)		55.22-1.70 (1.75-1.70)	
Unique reflections	6683 (3191)		15505 (936)	
$R_{\text{work}}/R_{\text{free}}$	26.76/27.85		19.82/20.60	
Number of atoms				
Protein	1332		760	
Lipids/PEG	146/-		104/13	
Water	14		65	
B-factors (Å ²)				
Protein	41.42		23.46	
Lipids/PEG	44.24/-		52.95/48.51	
Water	40.98		43.11	
R.m.s deviations				
Bond lengths (Å)	0.005		0.003	
Bond angles (°)	0.976		0.811	

Native LbSemiSWEET data were collected from 11 crystals and others were from one crystal.

*Highest resolution shell is shown in parenthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structure of VsSemiSWEET

a, Ribbon representation of a VsSemiSWEET protomer viewed from the side of the membrane. Helices in THB were colored in blue, yellow and red. **b**, Ribbon representation of the VsSemiSWEET dimer. Two protomers are colored in purple and green. **c**, A slab view of the VsSemiSWEET dimer to show the central cavity, colored by electrostatic potential. **d**, Close-up view of the PQ motif near the dimer interface. Inter-protomer hydrogen bonds between Q29 on TM1 and backbone amides on L1-2 are shown as dashed lines. **e**, Solvent accessible surface area in the cavity. Solvent accessible surface is shown as the cyan mesh. Protein is shown as grey ribbon with invariant residues W59 and N75 as sticks.

Figure 2. Structure of LbSemiSWEET

a, Two views of LbSemiSWEET dimer in ribbon representation. Two protomers are colored in red and blue respectively. **b**, A slab view of LbSemiSWEET dimer in electrostatic potential showing the central cavity. **c**, Residues capping the cavity. Extracellular view of LbSemiSWEET is shown in ribbon with two protomers colored in red and blue. Hydrogen bonds between Y51 and D57 are shown as dashed lines. **d**, Two views of electron density map of a putative substrate in the cavity. The $f_o - f_c$ map contoured at 3.0σ is displayed as a red mesh. W48 and N64 are shown as sticks. TM1 is removed for clarity on the left.

Figure 3. Glucose transport by LbSemiSWEET or AtSWEET1

a, Glucose uptake activity of LbSemiSWEET in *E. coli* cells. W48A or N64A mutations abolished glucose uptake (control: empty vector). Transport activities were normalized to wild-type (WT) LbSemiSWEET (mean $\pm$ s.e.m., $n=3$). Uptake in WT was significantly different from control and mutants (two-tailed T-test, $p<0.01$). **b**, Functional analysis of AtSWEET1 transport activity in the hexose transport defective yeast strain EBY4000. W56A and N73A (first THB), and W176A and N192A (second THB) mutants failed to complement the growth defect of EBY4000 in SD medium supplemented with 2% glucose as sole carbon source. Growth was unaffected in control medium containing 2% maltose. Empty vector and AtSWEET1 were used as the negative and positive controls, respectively.

Figure 4. Alternating access by rigid-body rocking of two protomers

a, Structural comparison of LbSemiSWEET and VsSemiSWEET dimer. Two structures are superimposed only by protomer A. Left panel shows protomer A of LbSemiSWEET (in red) and VsSemiSWEET (in purple), which structurally align well. Right panel shows protomer B of LbSemiSWEET (in blue) and VsSemiSWEET (in green) with ~10 degree rotation between them. **b**, Ribbon representation showing VsSemiSWEET opens up more to the extracellular side compared with LbSemiSWEET. TM1 is removed for clarity and selected residues are shown as sticks. The cross-protomer distance between L2-3 is shown as dashed lines.