

Table S1. Statistics for crystallography data collection and refinement, Related to Figure 1 and 3.

	D57A	Q20A
Data Collection ^a		
Space group	<i>P</i> 2 ₁	<i>C</i> 222 ₁
Cell dimensions (Å)		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	47.02, 36.9, 47.51	63.71, 189, 90.86
<i>α</i> , <i>β</i> , <i>γ</i> (°)	90, 104.27, 90	90, 90, 90
Wavelength (Å)	1.03327	1.03327
Resolution (Å)	50.00-2.80 (2.85-2.80)	29.76-2.78 (2.93-2.78)
R _{merge} (%)	25.9 (>1)	15.8 (>1)
<i>I</i> / <i>σ</i> (<i>I</i>)	13.73 (1.33)	11.39 (0.92)
Completeness (%)	96.9 (73.7)	98 (87.3)
Redundancy	9.3 (2.3)	6.3 (3.9)
Refinement		
Resolution	29.01-2.79 (2.89-2.79)	29.76-2.78 (2.88- 2.78)
Unique reflections	3928 (289)	13933 (1130)
<i>R</i> _{work} / <i>R</i> _{free}	0.242/0.269	0.227/0.267
Number of atoms		
Protein	1326	2729
Ligands/PEG	41	
B-factors (Å ²)		
Protein	56.38	86.4
Ligands/PEG	59.95	
R.m.s deviations		
Bond lengths (Å)	0.005	0.005
Bond angles (°)	0.88	1.20

^a High-resolution shell is shown in parentheses.

Table S2. Table of simulations performed, Related to Figure 2, 3, 4, 6 and 7.

Initial structure	Substrate	Simulation size	Simulation No.	Duration (μ s)	Final conformational state
Outward open (WT) LbSemiSWEET	Glucose	64 Å × 64 Å × 75 Å 113 lipids 9 Na ⁺ ; 13 Cl ⁻ ~4,900 waters ~32,800 atoms	1	2.18	Outward open; glucose dissociates (1.78 μ s)
			2	1.84	Outward open
			3	0.82	Outward open; glucose dissociates (0.34 μ s)
			4	5.39	Inward open
			5	0.29	Outward open; glucose dissociates (0.28 μ s)
			6	2.69	Outward open
			7	2.39	Outward open
			8	2.95	Outward open
			9	5.88	Inward open; glucose unbinds (1.80 μ s)
			10	1.95	Outward open
Outward open (WT) LbSemiSWEET (Round 2)	Glucose	65 Å × 67 Å × 77 Å 127 lipids 11 Na ⁺ ; 15 Cl ⁻ ~5,400 waters ~35,900 atoms	11	3.02	Occluded
			12	3.02	Outward open; glucose dissociates (1.50 μ s)
			13	3.04	Outward open
			14	3.05	Outward open
			15	6.02	Inward open
			16	5.02	Inward open
			17	5.04	Occluded
			18	5.02	Outward open; glucose dissociates, rebinds (1.60 μ s, 4.30 μ s)
			19	5.06	Outward open; glucose dissociates (3.00 μ s)
			20	5.03	Occluded
Outward open (WT) LbSemiSWEET	None	64 Å × 64 Å × 75 Å 113 lipids 9 Na ⁺ ; 13 Cl ⁻ ~4,900 waters ~32,800 atoms	21	2.23	Outward open
			22	2.18	Inward open
			23	1.89	Outward open
			24	2.57	Outward open
			25	2.35	Outward open
			26	2.56	Outward open
			27	2.51	Outward open
			28	2.86	Outward open
			29	2.86	Outward open
			30	2.37	Inward open
Outward open (WT) LbSemiSWEET (Round 2)	None	65 Å × 67 Å × 77 Å 127 lipids 11 Na ⁺ ; 15 Cl ⁻ ~5,400 waters ~35,900 atoms	31	3.02	Outward open
			32	3.03	Outward open
			33	3.03	Inward open
			34	3.04	Outward open
			35	5.02	Inward open
			36	3.05	Outward open
			37	3.04	Outward open

			38	3.04	Outward open
			39	3.03	Outward open
			40	4.17	Outward open
Occluded	Glucose	$63 \text{ \AA} \times 62 \text{ \AA} \times 79 \text{ \AA}$	41	1.49	Occluded
LbSemiSWEET		113 lipids	42	1.48	Occluded
PDBID: 4QNC		9 Na ⁺ ; 13 Cl ⁻	43	1.46	Occluded
		~4,800 waters	44	1.28	Inward open
		~32,400 atoms	45	1.14	Inward open
			46	0.63	Occluded
			47	0.69	Inward open
Occluded	None	$63 \text{ \AA} \times 62 \text{ \AA} \times 79 \text{ \AA}$	48	1.32	Inward open
LbSemiSWEET		113 lipids	49	1.12	Occluded
PDBID: 4QNC		9 Na ⁺ ; 13 Cl ⁻	50	1.15	Inward open
		~4,800 waters	51	0.95	Inward open
		~32,400 atoms	52	0.95	Occluded
			53	0.65	Inward open
			54	0.62	Inward open
Inward open	None	$65 \text{ \AA} \times 60 \text{ \AA} \times 95 \text{ \AA}$	55	2.43	Inward open
EcSemiSWEET		111 lipids	56	2.22	Inward open
PDBID: 4X5M		12 Na ⁺ ; 20 Cl ⁻	57	2.22	Inward open
		~7,400 waters			
		~40,000 atoms			
Outward open (D57A)	Glucose	$65 \text{ \AA} \times 67 \text{ \AA} \times 77 \text{ \AA}$	58	3.04	Inward open
LbSemiSWEET (Round 2)		127 lipids	59	3.03	Outward open
		11 Na ⁺ ; 15 Cl ⁻	60	3.03	Occluded
		~5,400 waters	61	3.02	Outward open
		~35,900 atoms	62	3.04	Outward open
Outward open (I60A)	Glucose	$65 \text{ \AA} \times 67 \text{ \AA} \times 77 \text{ \AA}$	63	3.01	Outward open
LbSemiSWEET (Round 2)		127 lipids	64	3.05	Outward open
		11 Na ⁺ ; 15 Cl ⁻	65	3.04	Outward open
		~5,400 waters	66	3.02	Outward open
		~35,900 atoms	67	3.02	Outward open
Outward open TM1 alone (Round 2)	None	$55 \text{ \AA} \times 55 \text{ \AA} \times 75 \text{ \AA}$	68	1.02	N.A.
		99 lipids	69	1.03	N.A.
		8 Na ⁺ ; 9 Cl ⁻	70	1.03	N.A.
		~3,400 waters			
		~24,000 atoms			