Quality control of overexpressed membrane proteins

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Supporting Information

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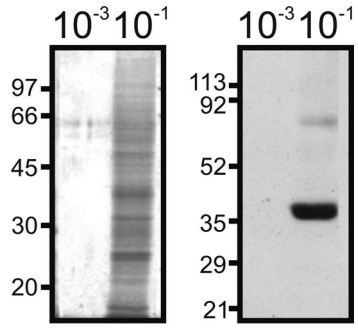

Fig. S1. Insoluble inclusion bodies isolated from cells expressing LacS to different levels. Expression of LacS by *Escherichia coli* MC1061 cells was induced with the percentages of L-arabinose indicated above the panels. Molecular masses (in kDa) and positions of the marker proteins are indicated on the left of each panel. (*Left*) Coomassie-stained gel. (*Right*) Immunoblot decorated with anti-His tag antibody.

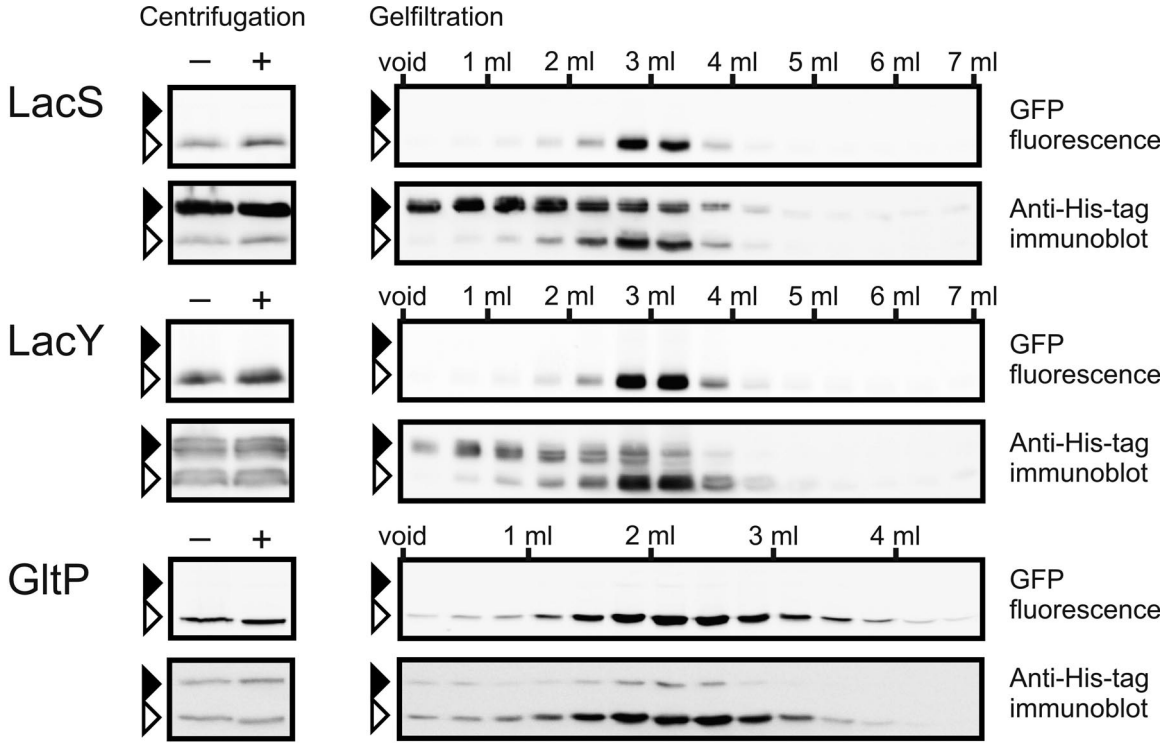

Fig. S2. Size-exclusion chromatography (SEC) on FC-12-solubilized cells. *E. coli* MC1061 cells were induced with 1×10^{-1}% (wt/vol) L-arabinose for 4 h at 25°C (LacS and LacY), and *E. coli* C43 cells were induced with 0.4 mM IPTG for 16 h at 17°C (GltP). Disrupted cells were solubilized with 1% (wt/vol) FC-12, and after ultracentrifugation the supernatant was analyzed by SEC. Samples taken before (−) and after (+) ultracentrifugation, and relevant fractions from the SEC were analyzed by *in gel* fluorescence and immunodetection using an anti-His tag antibody. Black and white arrows indicate the positions of nonfluorescent and fluorescent species of the GFP fusion proteins, respectively.

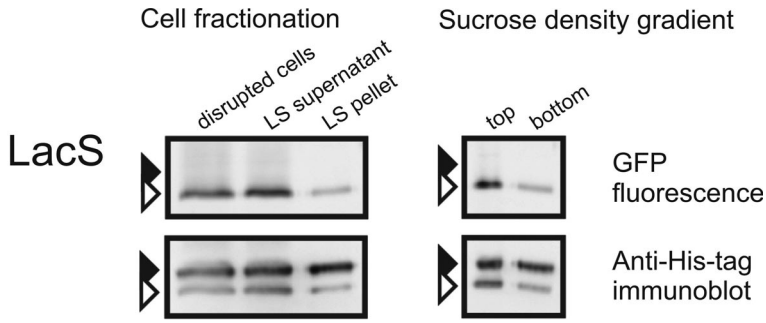

Fig. S3. Isolation of membrane vesicles. *E. coli* MC1061 cells were induced with 1×10^{-1}% (wt/vol) L-arabinose for 4 h at 25°C to allow expression of LacS. Cells were disrupted by two passes through a French pressure cell. (*Left*) The disrupted cells before centrifugation and the supernatant (LS supernatant) and pellet (LS pellet) after fractionation by low-speed centrifugation were analyzed. (*Right*) The supernatant of the low-speed centrifugation was further fractionated by sucrose density centrifugation. The top (low density; 5 ml) and bottom (high density; 1 ml) fractions of the sucrose gradient were analyzed.

Table S1. Proteins analyzed in this study

Protein	Function	Source organism	Transporter family	Family nr.	Size, kDa
LacY	Lactose/H^+ symporter	*E. coli*	MFS	2.A.1	46.5
GltP	Glutamate/H^+ symporter	*E. coli*	DAACS	2.A.23	47.2
EcClC	Cl^-/H^+ antiporter	*E. coli*	ClC	2.A.49	50.3
NhaA	Na^+/H^+ antiporter	*E. coli*	NhaA	2.A.33	41.4
DctA	Dicarboxylate/H^+ symporter	*E. coli*	DAACS	2.A.23	45.4
LacS(ΔIIA)	Lactose/H^+ symporter	*S. thermophilus*	GPH (MFS)	2.A.2	52.3
YdjN	Unknown	*E. coli*	DAACS	2.A.23	48.7
SstT	Serine/Na^+ symporter	*E. coli*	DAACS	2.A.23	43.5
GlpF	Glycerol facilitator	*E. coli*	MIP	1.A.8	29.8

MFS, Major Facilitator Superfamily; DAACS, Dicarboxylate/Amino Acid:Cation (Na^+ or H^+) Symporter Family; ClC, Chloride Carrier/Channel Family; NhaA, Na^+:H^+ Antiporter (NhaA) Family; GPH, Glycoside-Pentoside-Hexuronide:Cation Symporter Family; MIP, Major Intrinsic Protein Family. Family names and numbers are derived from the Transport Classification Database [Saier MH, Jr (1988) Molecular phylogeny as a basis for the classification of transport proteins from bacteria, archaea, and eukarya. *Adv Micro Physiol* 40:81–136].