The proton electrochemical gradient induces a kinetic asymmetry in the symport cycle of LacY

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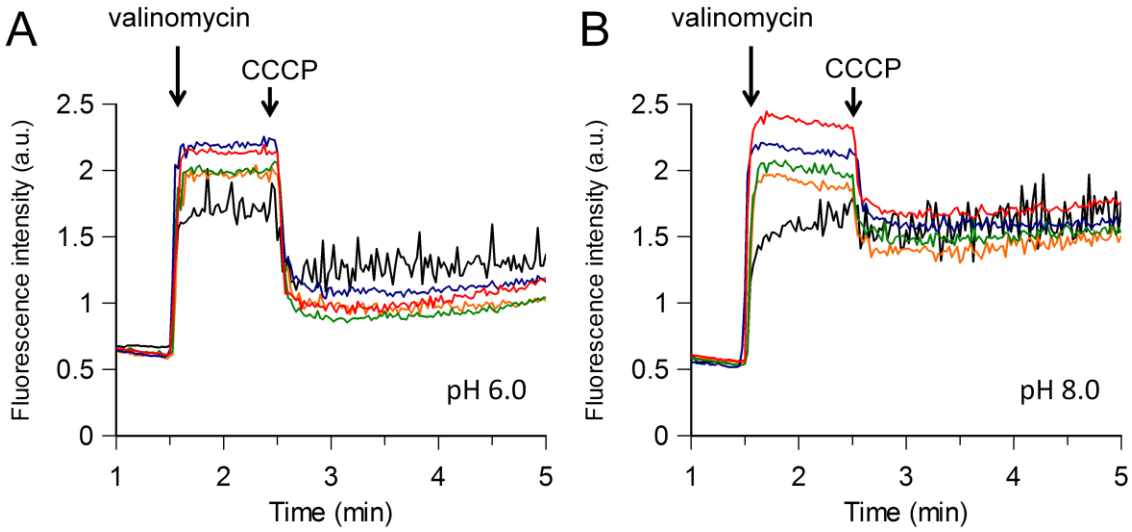

Fig. S1. Assessment of imposed membrane potential (*interior positive*) by potential-sensitive oxonal dye DiBAC4(3). RSO membrane vesicles at a protein concentration of 2 mg/mL in 100 mM NaPi at pH 6.0 (A) or pH 8.0 (B) were incubated with 4 μM DiBAC4(3) at 25 ^{o}C for 30 min, then 50 μL of RSO membrane vesicles were diluted into 2 mL of 100 mM phosphate buffer at a given ratio of Na^+/K^+ at the same pH (black: no potassium, orange: 25% potassium, green: 50% potassium, blue: 75% potassium and red: 100% potassium). Fluorescence emission of DiBAC4(3) was monitored at 516 nm with excitation at 490 nm. Where indicated by the arrows, valinomycin or CCCP was added to a final concentration of 25 μM, respectively. Based on the percentage of fluorescence change at each tested pH, a membrane potential of at least +75 mV was estimated upon dilution into pure KP_i.

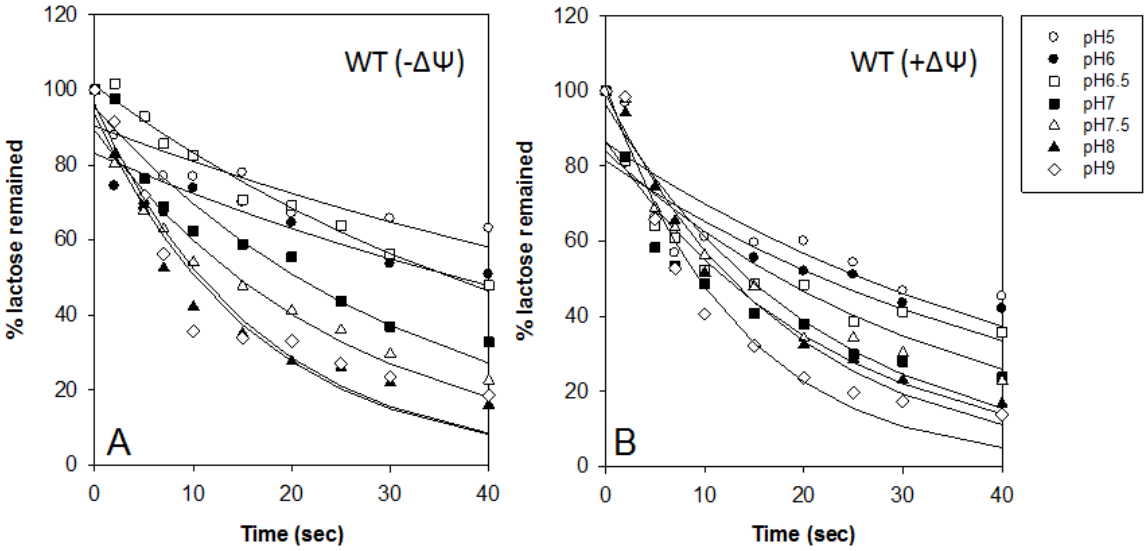

Fig. S2. Efflux by RSO vesicles with WT LacY in the absence (A) or presence (B) of ΔΨ (*interior positive*) at different pHs. Efflux experiments were performed as described in *Materials and Methods* and data are plotted as an exponential fit.

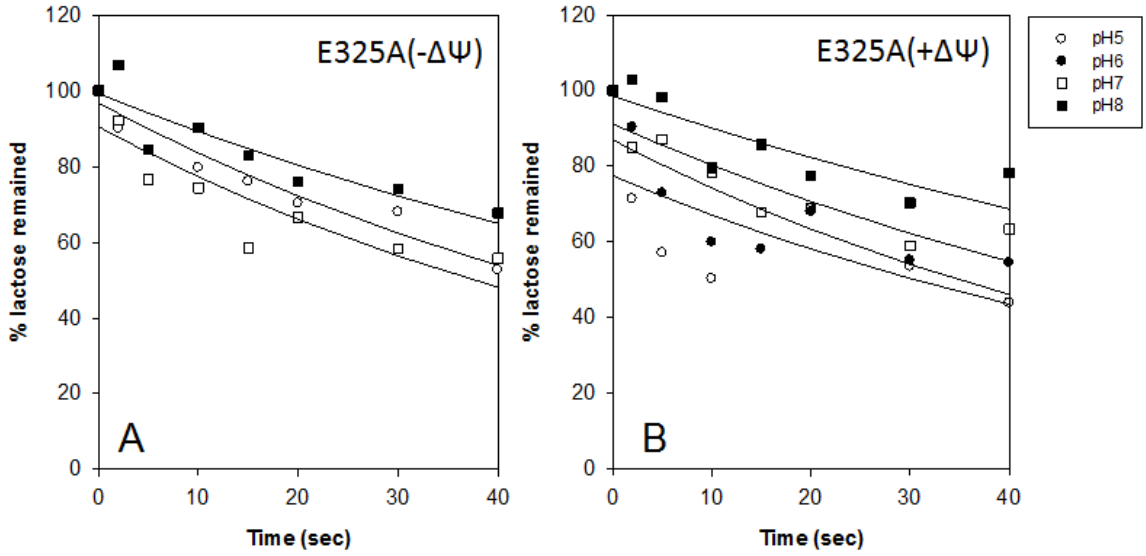

Fig. S3. Efflux by RSO vesicles with mutant E325A LacY in the absence (A) or presence (B) of ΔΨ (*interior positive*) at different pHs. Efflux experiments were performed as described in *Materials and Methods* and data are plotted as an exponential fit.

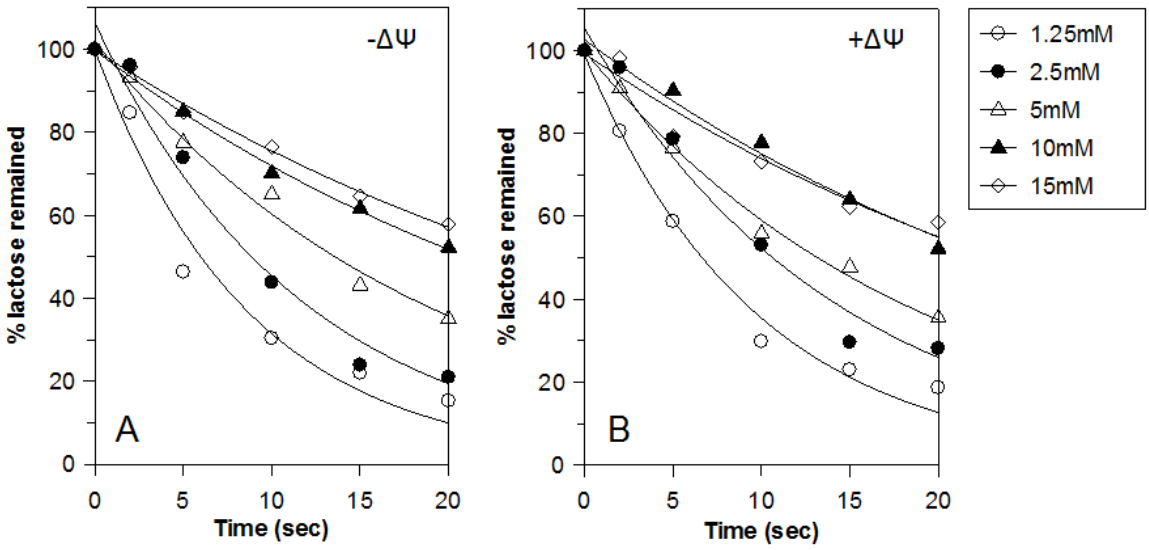

Fig. S4. Efflux by RSO vesicles with WT LacY equilibrated with different concentrations of [^{14}C]lactose in the absence (A) or presence (B) of ΔΨ (*interior positive*) at pH7.5. Efflux experiments were performed as described in *Materials and Methods* and data are plotted as an exponential fit.

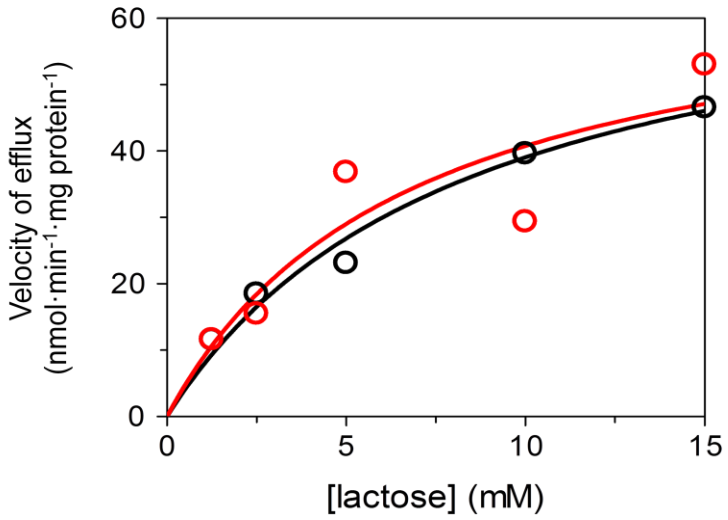

Fig. S5. Effect of imposed ΔΨ (*interior positive*) on the kinetics of lactose efflux. RSO membrane vesicles were equilibrated with [^{14}C]lactose at given concentrations overnight at 4°C, then efflux experiments in the absence (black) and presence of ΔΨ (*interior positive*) (red) were performed at pH 7.5 as described in *Materials and Methods*. K_m and V_{max} were obtained by plotting the amount of lactose effluxed in the first 10s as a function of lactose concentration with Michaelis–Menten equation.