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Supporting Material

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Supplementary data

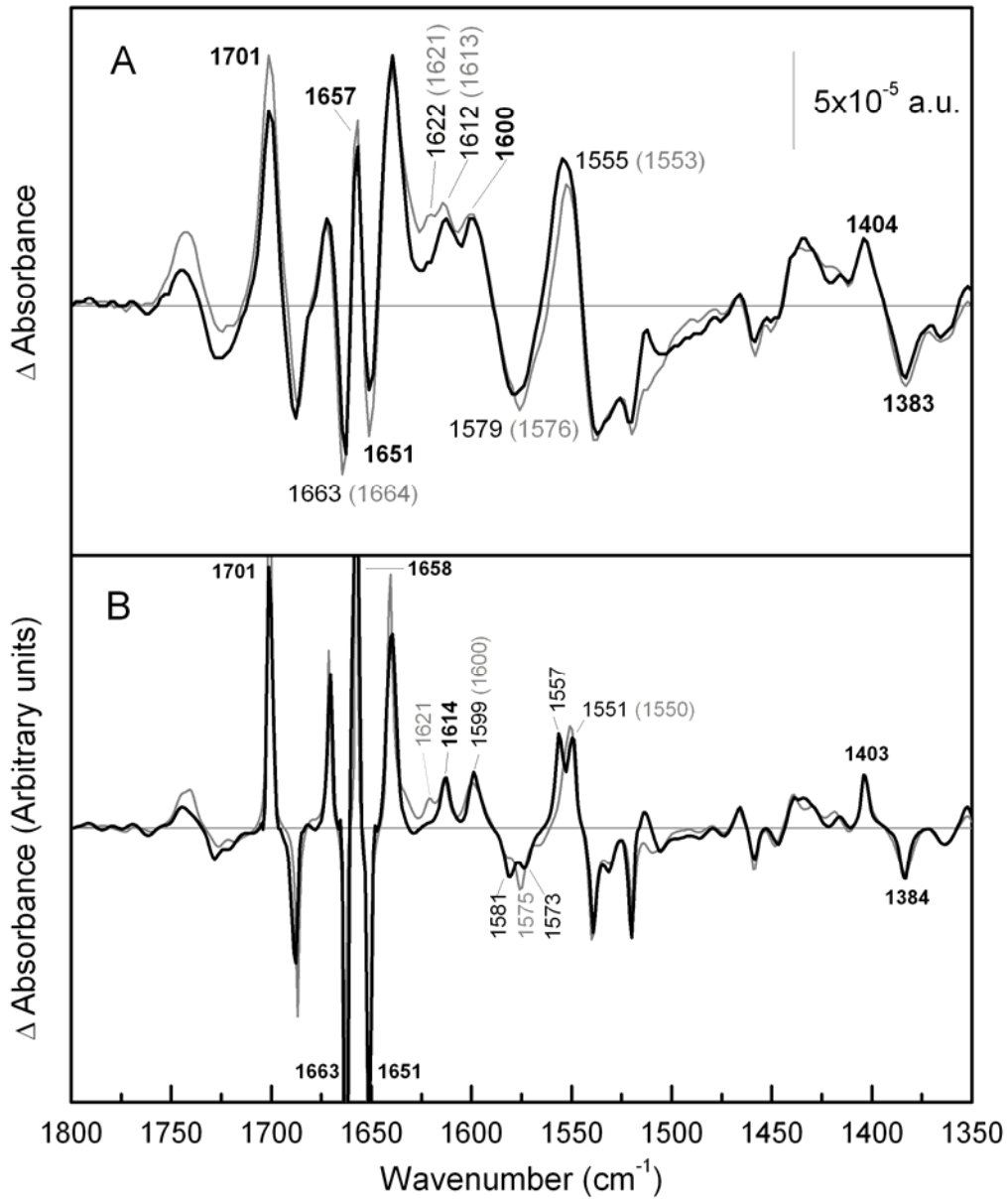

Fig. S1. Comparison of Na^+-induced difference spectra of Cys-less and WT permeases. (A) *(solid line)* difference spectrum of Cys-less in 20 mM MES, 100 mM KCl, 10 mM NaCl, pH 6,6 minus Cys-less in 20 mM MES, 110 mM KCl, pH 6,6; *(shaded line)* difference spectrum of WT under the same conditions. (B) deconvoluted difference spectra of top panel.

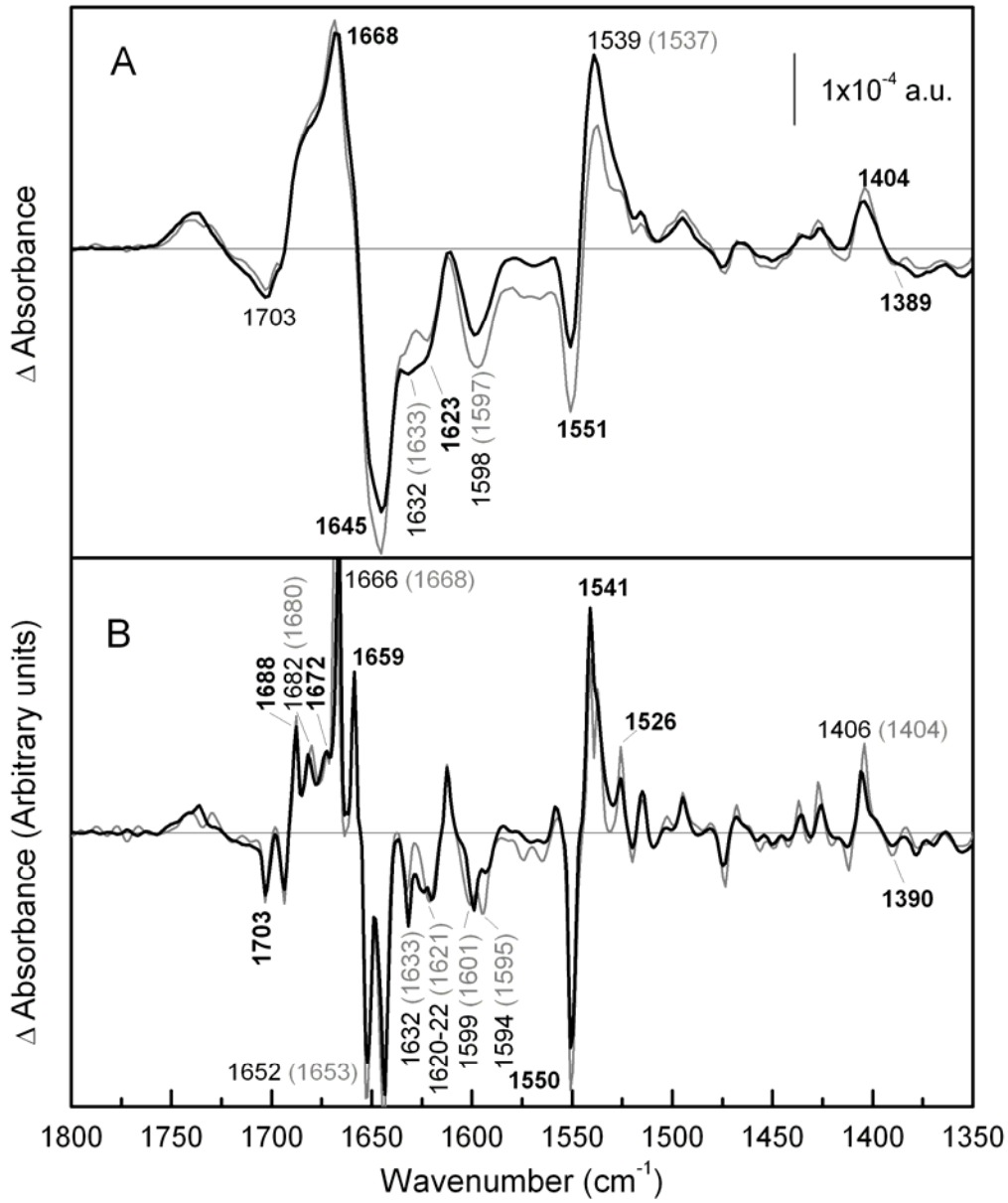

Fig. S2. Comparison of melibiose-induced difference spectra of Cys-less and WT permeases in the presence of Na^+. (A) *(solid line)* difference spectrum of Cys-less in 20 mM MES, 100 mM KCl, 10 mM NaCl, 10 mM melibiose, pH 6.6 minus Cys-less in 20 mM MES, 100 mM KCl, 10 mM NaCl, pH 6.6; *(shaded line)* difference spectrum of WT under the same conditions. (B), deconvoluted difference spectra of (A).

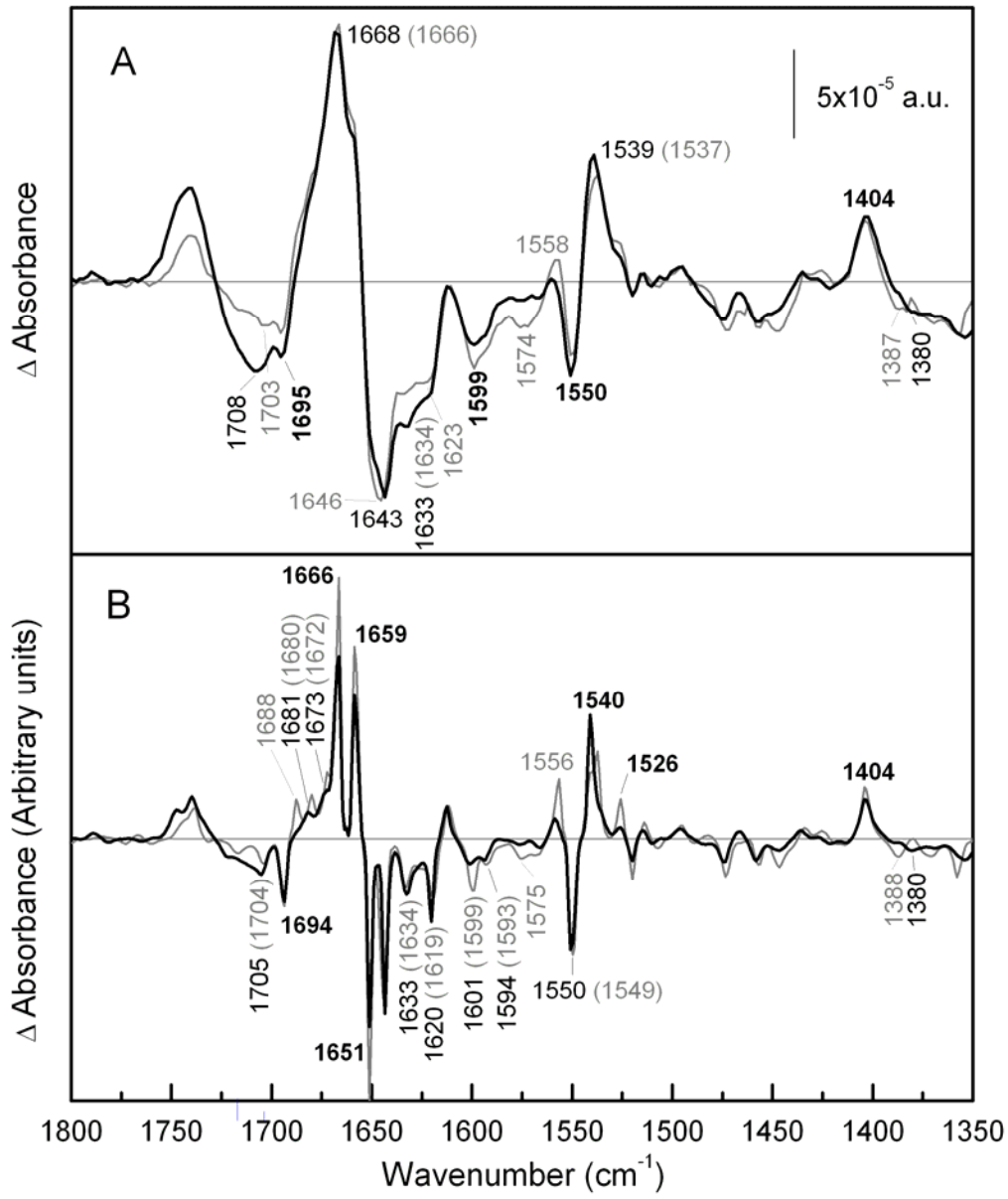

Fig. S3. Comparison of melibiose-induced difference spectra of Cys-less and WT permeases, in the presence of H^+. (A) *(solid line)* difference spectrum of Cys-less in 20 mM MES, 100 mM KCl, 50 mM melibiose, pH 6.6 minus Cys-less in 20 mM MES, 100 mM KCl, pH 6.6; *(shaded line)* difference spectrum of WT under the same conditions. (B), deconvoluted difference spectra of (A).

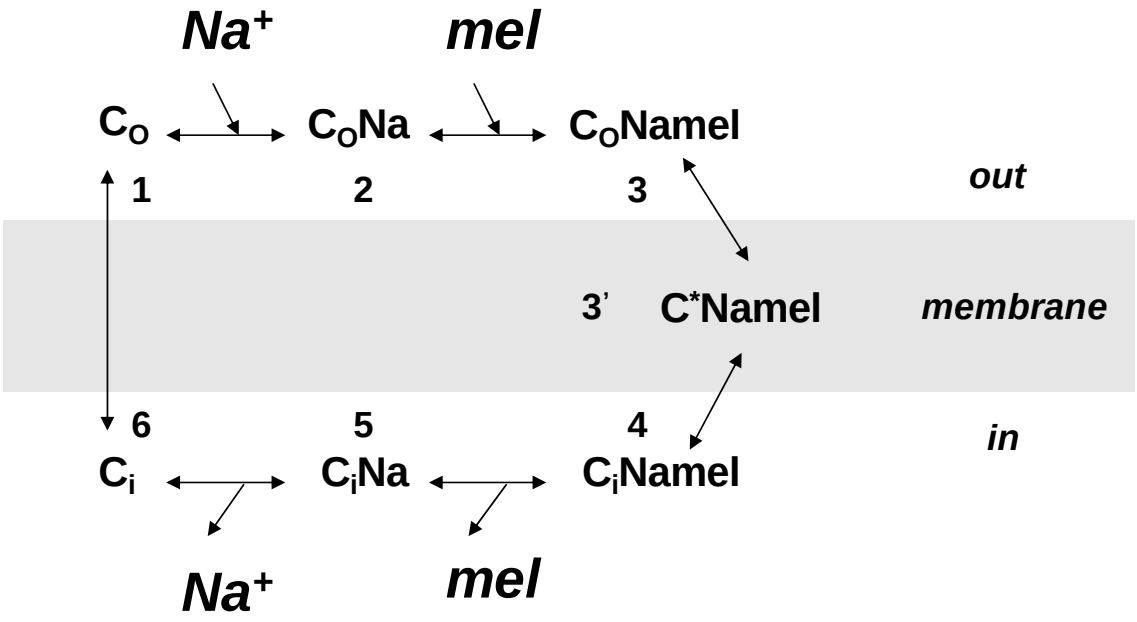

Fig. S4**.** Kinetic model of Na^+-sugar cotransport by MelB in physiological orientation. This kinetic model is adapted from an extended 6-state kinetic model initially suggested for MelB running in a backward direction by Meyer-Lipp et al (39). The $\mathbf{C_O}$, **C*** and $\mathbf{C_i}$ indicate MelB states with an outward-facing, occluded or inward-facing conformation, respectively. Starting from C_O (empty carrier), binding of Na^+ and then melibiose successively drives the outward-facing transporter into the $\mathbf{C_ONa}$ and $\mathbf{C_ONamel}$ states (5). The outward-facing to inward-facing carrier reorientation towards the cytoplasm ($\mathbf{C_ONamel \leftrightarrow C_iNamel}$) is proposed to include an intermediate occluded state (**C*Namel**). After sequential release of melibiose ($\mathbf{C_iNamel \leftrightarrow C_iNa}$) and Na^+ ($\mathbf{C_iNa \leftrightarrow C_i}$) into the inner compartment (3), MelB cycling ends by a return of the empty carrier towards the outer membrane surface ($\mathbf{C_i \leftrightarrow Co}$).