Supporting Information

Smirnova *et al*. 10.1073/pnas.0803577105

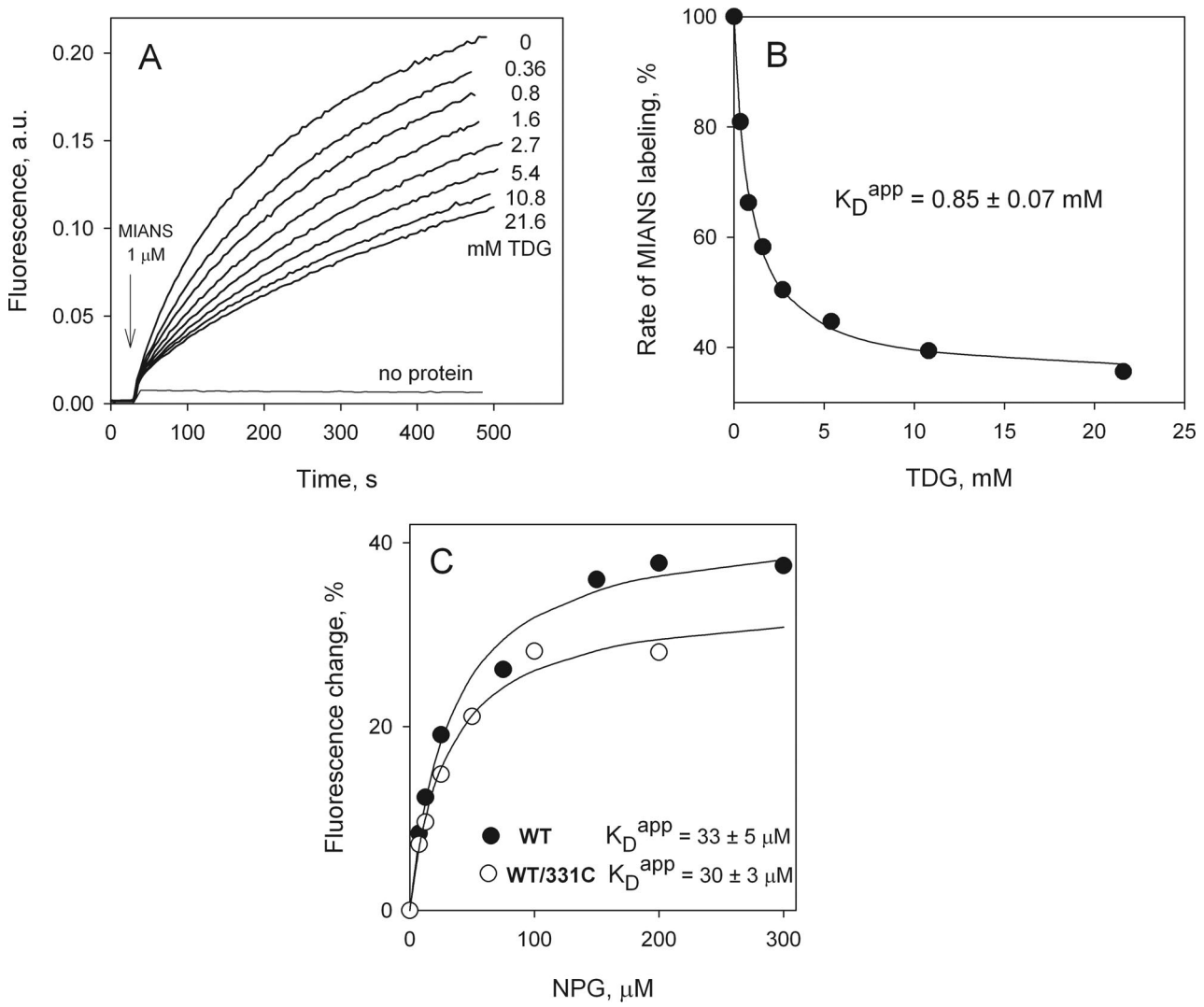

Fig. S1. Affinity of purified unlabeled LacY for sugar. (*A*) TDG affinity measured by TDG protection of Cys-148 against alkylation by MIANS. Rates of wild-type LacY (0.5 μM, final concentration) labeling by MIANS at given TDG concentrations were recorded at excitation and emission wavelengths of 330 and 415 nm, respectively, in the 50 mM NaP_i buffer (pH 7.5), 0.02% DDM. (*B*) Dependence of initial rates of MIANS labeling (from *A*) on TDG concentrations. (*C*) NPG affinity measured for wild-type LacY (●) and for unlabeled V331C/LacY (○) by using the direct sugar binding assay based on Trp→NPG FRET (31). Displacement of NPG at given concentrations with an excess of TDG was detected as change in Trp emission of LacY (0.4 μM) in 50 mM NaP_i (pH 7.5), 0.02% DDM excited at 295 nm. (*B* and *C*) Solid lines are hyperbolic fits to the data with estimated K_d^{app} shown.

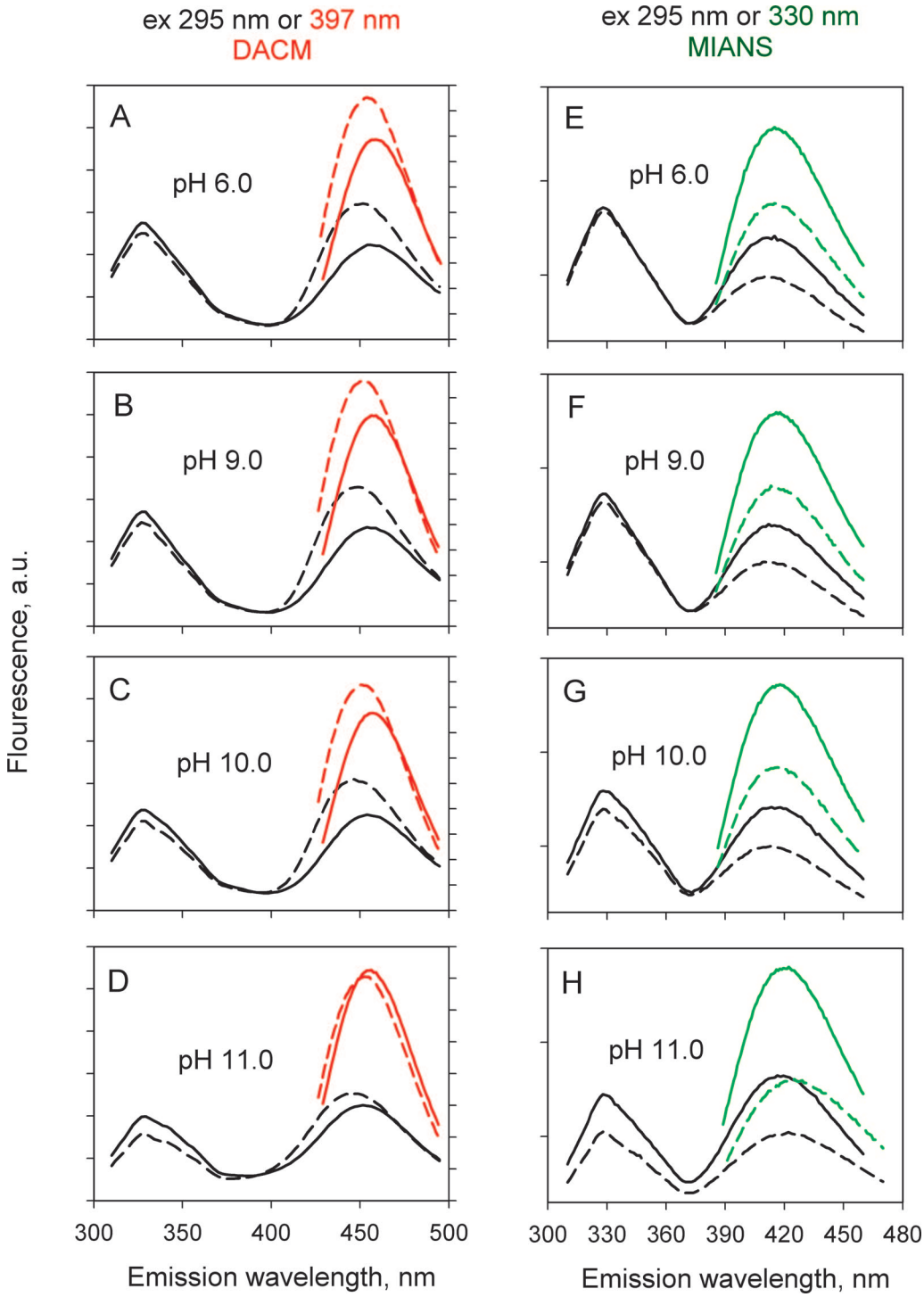

Fig. S2. TDG effect on emission spectra of DACM-labeled (*A–D*) or MIANS-labeled V331C LacY (*E–H*) at different pH values. Emission spectra were recorded at 0.4 μM protein (final concentration) before TDG addition (solid lines) and after adding a saturating TDG concentration (broken lines). Excitation wavelengths are 295 nm (black lines), 397 nm (red lines), and 330 (green lines). Emission maxima are shown for Trp at 330 nm, for DACM at 450 nm, and for MIANS at 415 nm. Vertical scales corresponding to fluorescence intensity are shown with ticks separated by 0.05 arbitrary units. (*A–D*) The right vertical scales are for DACM fluorescence (excitation 397 nm).

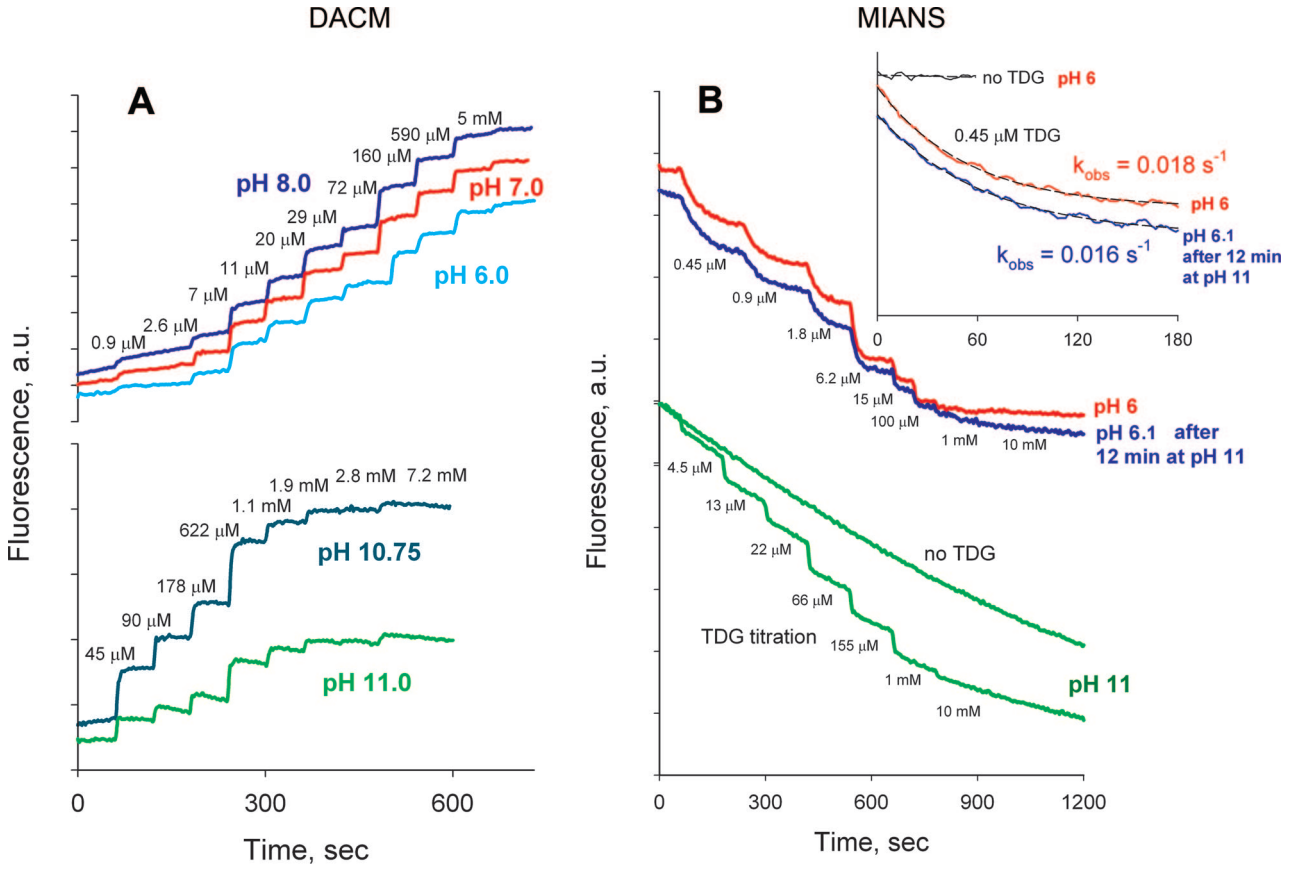

Fig. S3. TDG titration time traces at different pH values. (*A*) DACM-labeled V331C LacY titrations at a given pH are shown with final TDG concentrations indicated. Excitation and emission wavelengths are 397 and 440 nm, respectively. (*B*) MIANS-labeled V331C LacY titrations with given TDG concentrations at pH 6 and 11. Traces at pH 11 and pH 6.1 are shifted vertically for clarity. A control experiment demonstrating LacY stability at pH 11 is presented as blue lines for TDG titration of the protein preincubated at pH 11.0 for 12 min and then diluted 20-fold into acidic buffer to a final pH of 6.1. (*Inset*) Sugar-binding rates at 0.45 μM TDG before and after incubation at pH 11.0 with k_{obs} values estimated from single exponential fits to the data (0.018 and 0.016 s^{-1}, respectively).

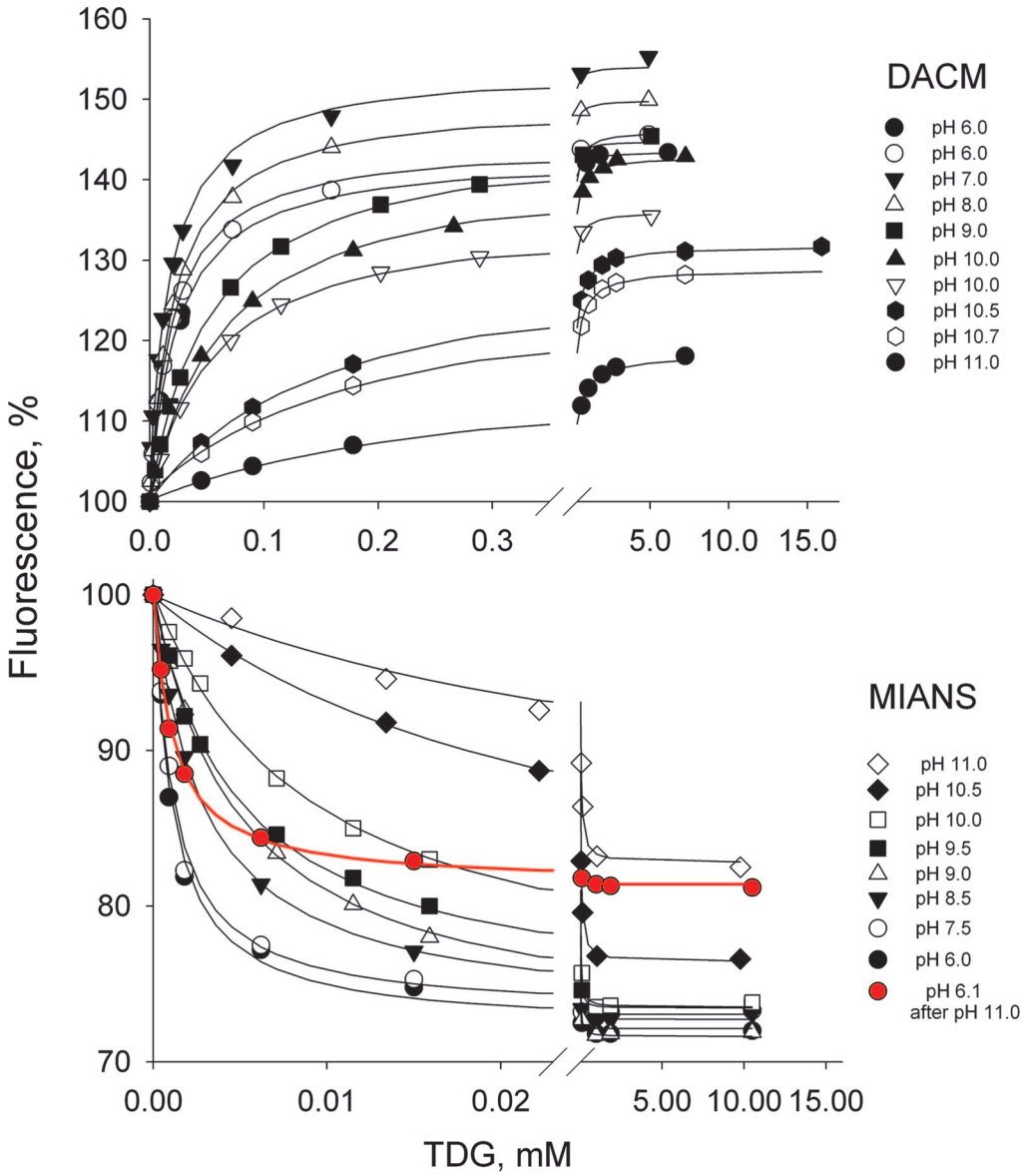

Fig. S4. Affinity for TDG at different pH values measured with DACM- and MIANS-labeled V331C LacY. Titration data at the indicated pH are plotted versus TDG concentration and fitted with hyperbolic equations (solid lines). Excitation and emission wavelengths were 397 and 440 nm for DACM-labeled or 330 and 415 nm for MIANS-labeled LacY. A control experiment for MIANS-labeled LacY preincubated at pH 11.0 for 12 min and then titrated at pH 6.1 is shown in red.

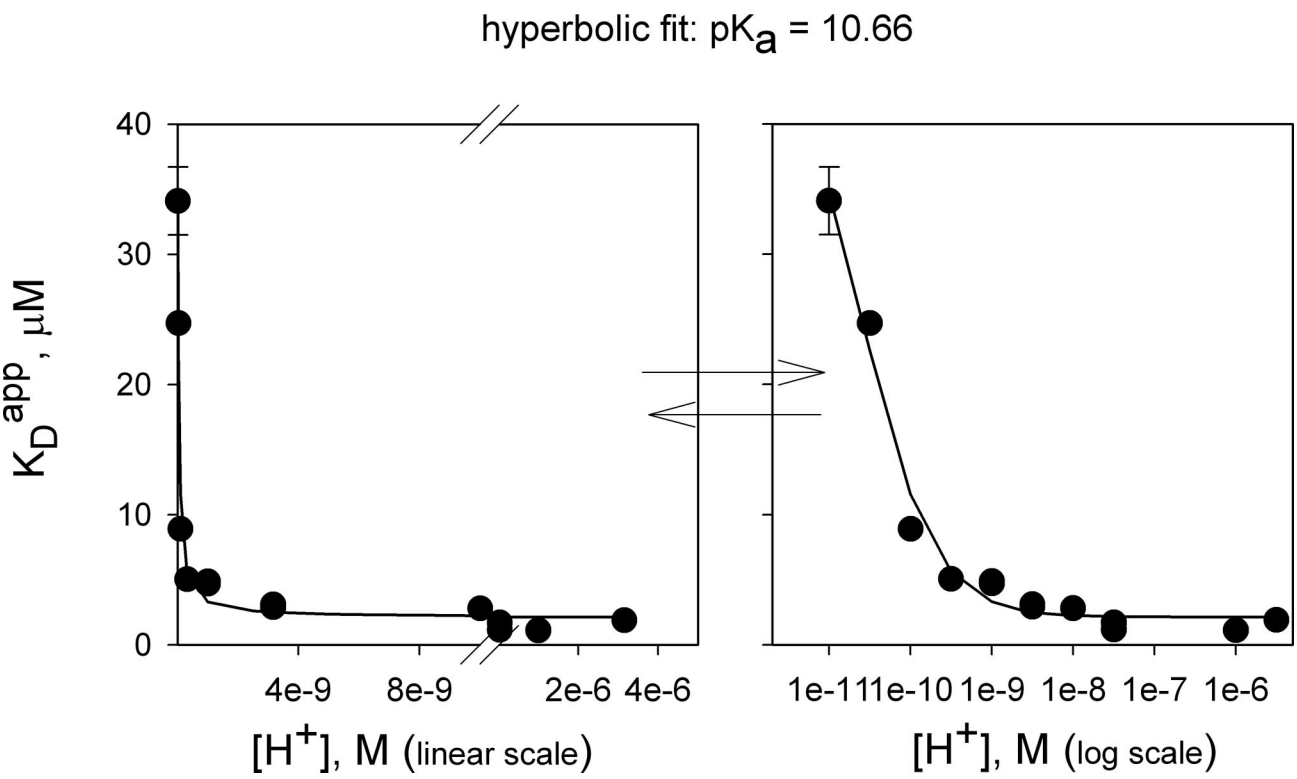

Fig. S5. Affinity of MIANS-labeled V331C LacY for TDG as a function of H^+ concentration. Data from Fig. S4 (MIANS) are plotted as K_d^{app} versus H^+ concentration are shown on linear and log scale. Solid line is a hyperbolic decay fit to the data. The pK_a value (10.66) is estimated as logarithm of the H^+ concentration required for half-maximum effect (2.188×10^{-11} M).

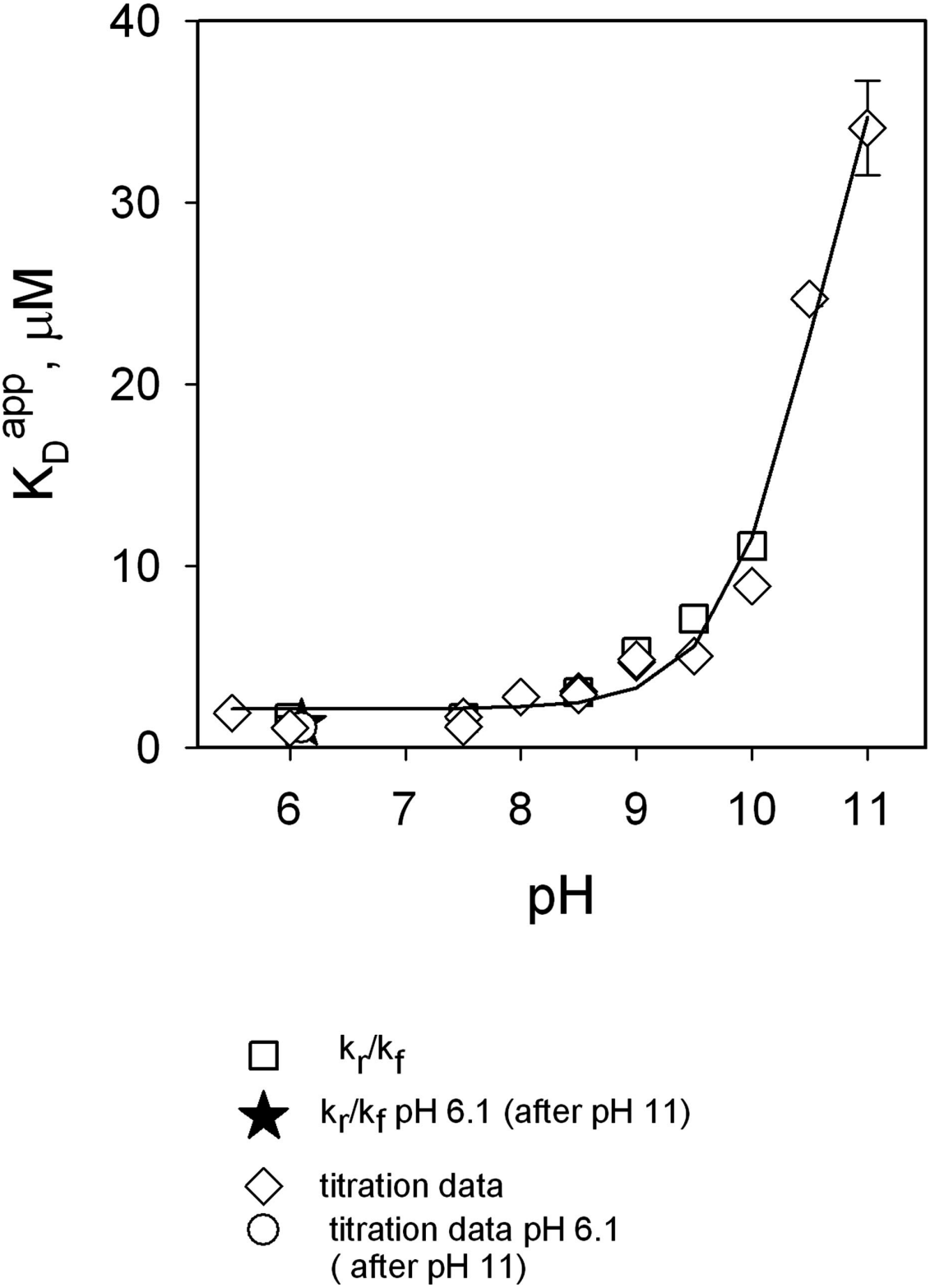

Fig. S6. K_d^{app} values calculated from kinetic parameters and from TDG titrations of MIANS-labeled V331C LacY at different pH values. Data plotted are K_d^{app} calculated as k_r/k_f from kinetic experiments (□) or titration data (◇). ⋆, ○, control experiments for LacY preincubated at pH 11.0 for 12 min and assayed at pH 6.1 in kinetic experiments and TDG titrations, respectively.

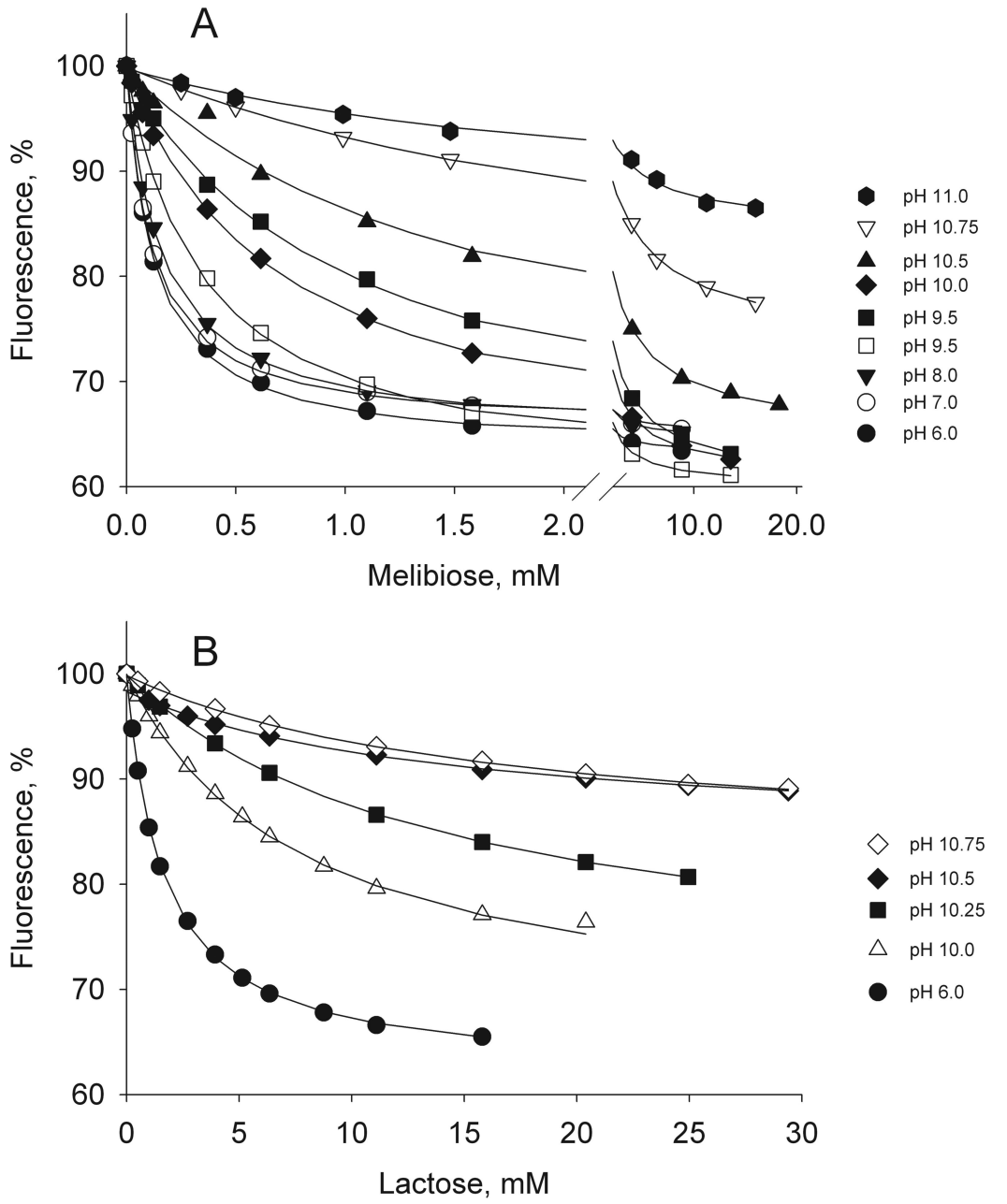

Fig. S7. Affinity for melibiose (*A*) or lactose (*B*) at different pH values measured with MIANS-labeled V331C LacY. Titration data at the indicated pH are plotted versus sugar concentrations and fitted with hyperbolic equation (solid lines). Excitation and emission wavelengths were 330 and 415 nm, respectively.