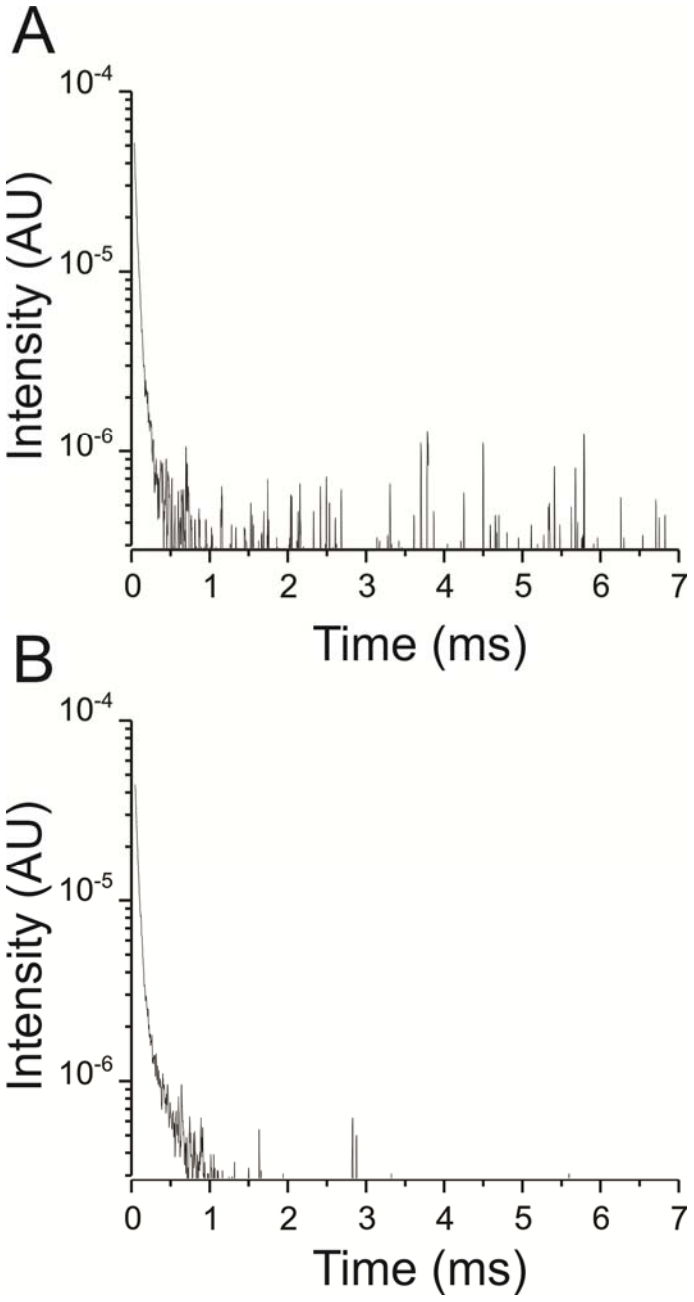
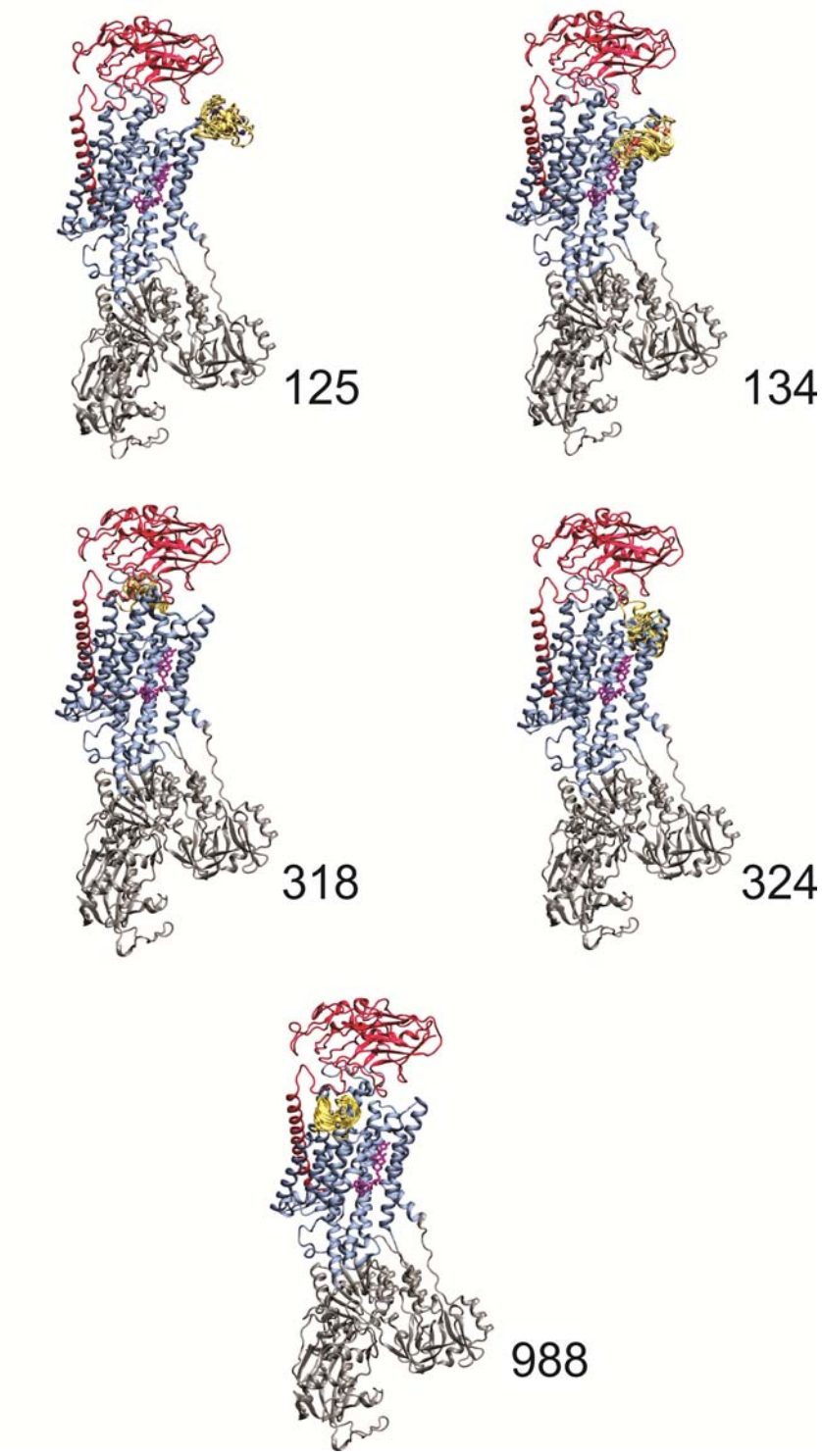
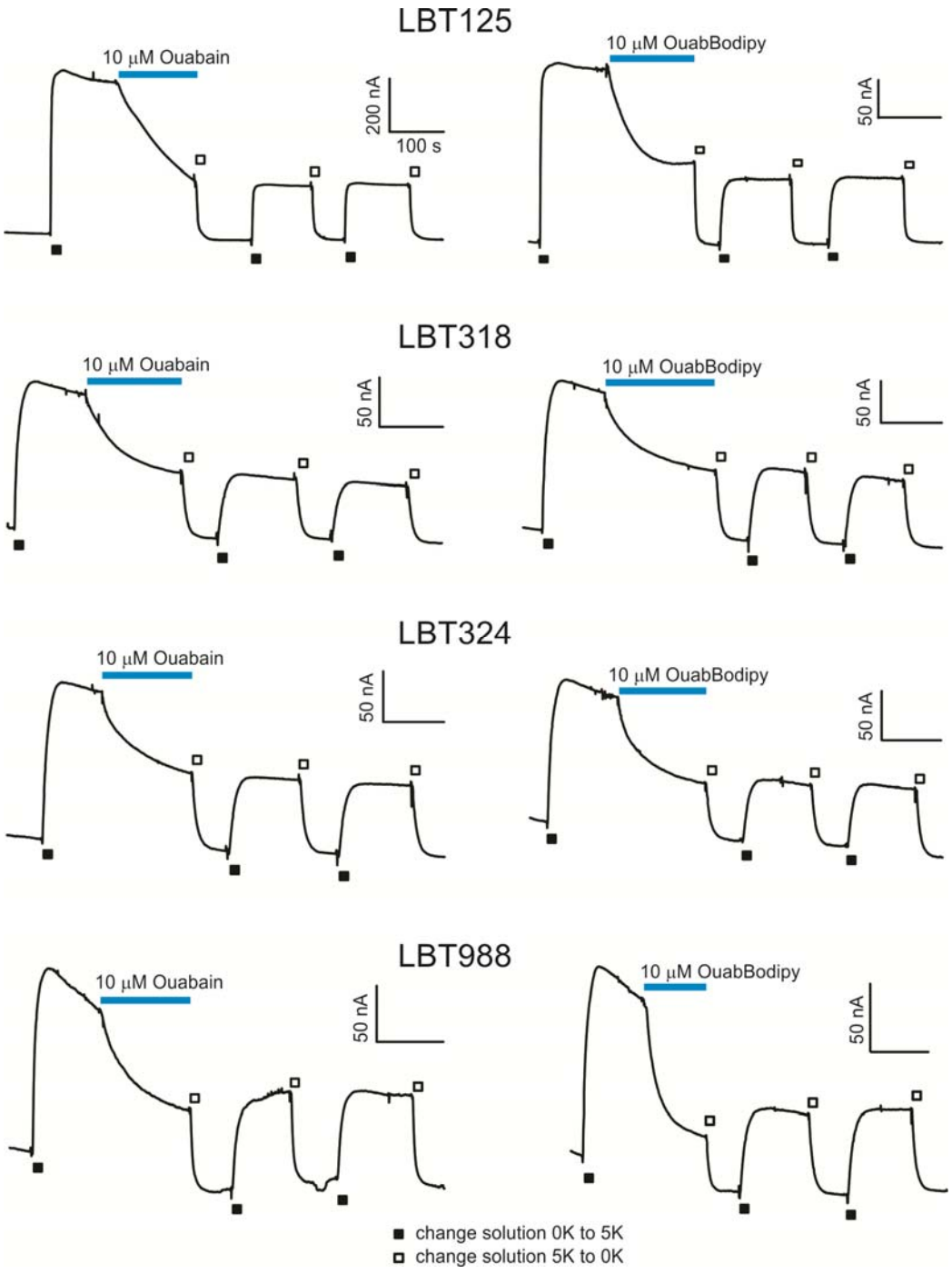


Supplemental Fig. S1. Fluorescent signal from uninjected oocytes. *A*. Tb^{3+} bound luminescence in the absence of acceptor. The characteristic fluorescent signal (τ_D ~2.5 ms) emanating from the excitation of for Tb^{3+} bound to synthetic LBTs in solution or LBTs inserted within a coding region is absent in uninjected oocytes. *B.* Sensitized emission. Similarly, uninjected oocytes lack any fluorescence decay with time constants > 0.5 ms upon light excitation in the presence of Bodipy-Fl Ouabain. The fast fluorescence decays observed results from a combination of gate switching, delayed fluorescence and Tb^{3+} bound to unspecific sites within the oocyte's membrane which is subject to water collisional quenching.



Supplemental Fig. S2. Homology models of all squid LBT- Na^+/K^+ ATPase constructs that produced functional pumps. The β subunit is shown in red, modeled LBT positions in yellow, transmembrane segments of the α subunit in blue and intracellular domains in gray. The Bodipy-Fl Ouabain is shown in stick representation (violet). All 10 models of each LBT are shown superimposed.



Supplemental Fig. S3. Cardiotonic inhibition of LBT construct Na^+/K^+ ATPases. *Left colum*, Ouabain. *Right column*, Bodipy-Fl Ouabain. In the absence of external K^+ (initial conditions of all experiment), pumps are electrically quiescent. Upon addition of 5 mM K^+ (filled squares), pumps begin to transport $3Na^+$ by $2K^+$ producing a large outward current. Exposure with 10 μM ouabain or Bodipy-Fl Ouabain resulted in an irreversible pump current reduction. Electrophysiological experiments were performed with standard two-microelectrode voltage clamp technique. Pump current from LBT 134 was not detected, even though LRET signal could be occasionally measured. Likely, the turnover rate from these pumps is too slow to be detected by electrophysiological techniques.