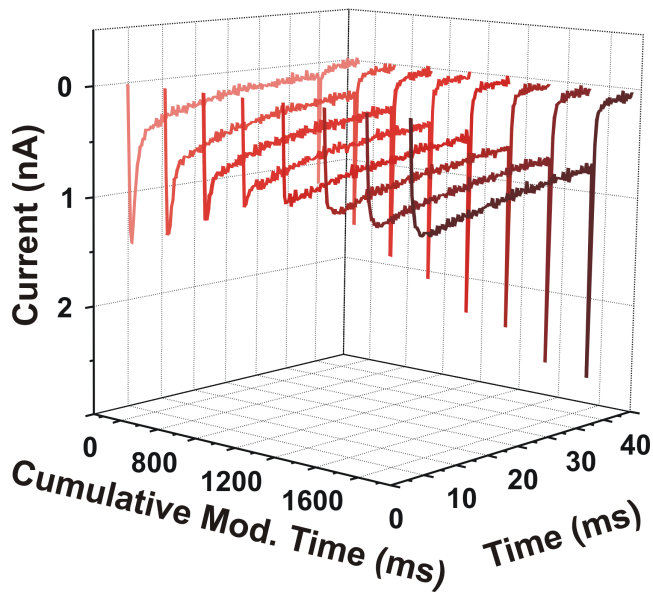


**Supplementary Figure 1.** MTSET application onto closed and open WSW channels does not reduce current amplitude. (a) Closed state modification experiment for WSW channels using 10 mM MTSET. After a total of 48 seconds exposure to MTSET, the initial and final current amplitude remain the same. (b) Open state experiment for WSW channels using 20 mM MTSET. After a cumulative MTSET exposure time of two seconds onto open WSW channels, no change in current amplitude is observed.

## A/W438C Open State



**Supplementary Figure 2.** Reaction time course of A/W438C with MTSET while channels are open. Initial currents (pink) recorded from inside-out patches containing A/W438C channels reveals that replacement of the tryptophan at this position with cysteine results in a near complete return of fast-inactivation. Repeated application of MTSET onto these channels while they are open (pink to dark red) progressively removes fast-inactivation and renders channels much like the WSW background. Of the three sites in the WSW mutant, this particular position is closest to the cytoplasm.