Structure *18*

Supplemental Information

Conformational Changes During the Gating of a Potassium Channel Revealed by Structural Mass Spectrometry

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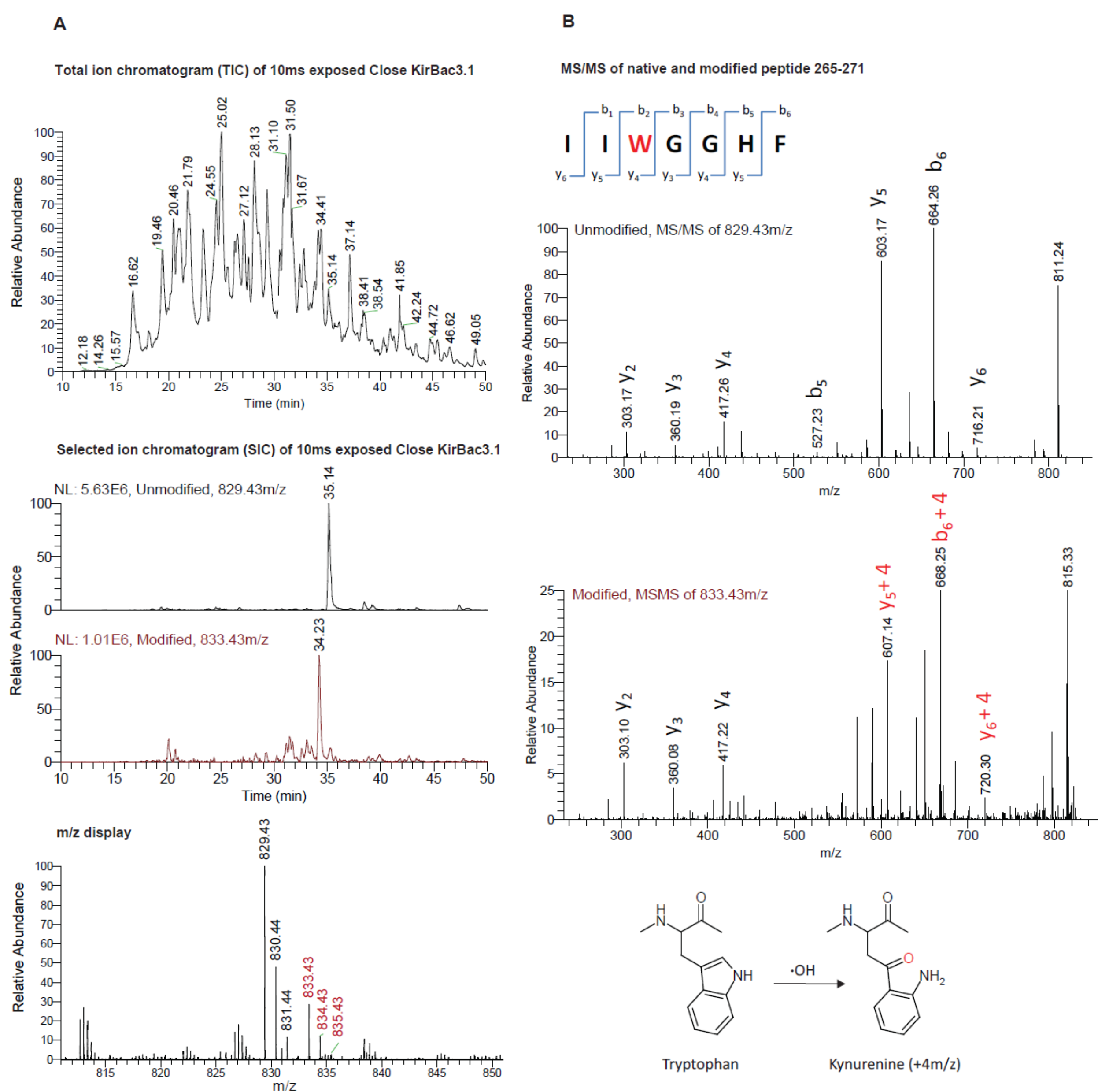

Fig. S1. Example of LC-LTQ-Orbitrap-MS data used to determine modification rate constant and identify site of modification. (A) The top panel shows a typical total ion current chromatogram of a peptic digest of X-ray irradiated (10ms) Closed KirBac3.1 represented by software XCalibur 2.01 (Thermo Electron) with scan filter for the range 400-1800m/z. The middle panel shows the selected ion chromatogram for the native (unmodified) and modified (+4 m/z shift, single peak) peptide 265-271 and their relative abundance after 10ms of X-ray irradiation. The bottom panel shows the m/z display of $(M+H)^+$ charge state and corresponding isotopic distributions. (B) MS/MS fragmentation of native and modified peptide 265-271, which shows +4 modification at W267 (kynurenine formation). The fraction of unmodified peptide at any exposure is calculated by the following equation:

$$\text{Fraction Unmodified} = \frac{\text{Unmodified peak area}}{(\text{Unmodified peak area} + \text{Modified peak area})}$$

The peak areas are calculated from the selected ion chromatogram. A time evolution of SICs are used to calculated fraction unmodified vs. exposure time. Hydroxyl radical modification rate constants are determined from the single exponential curve fitting as described in Figure 1*B* and Methods.

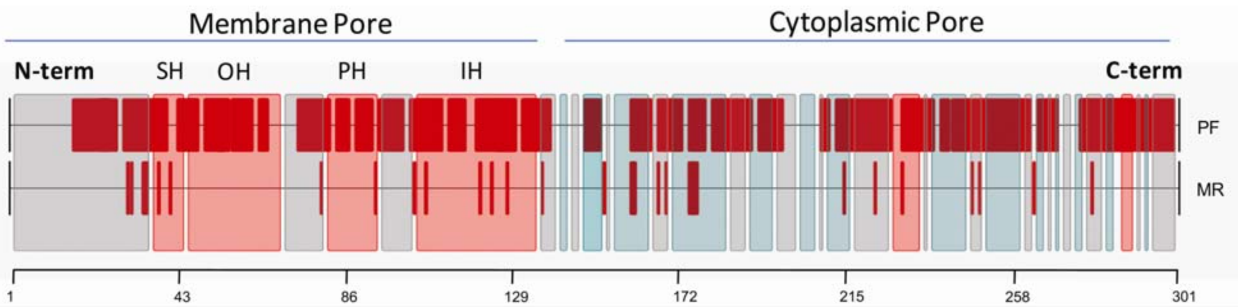

Fig. S2. Sequence Coverage: 92% sequence coverage was obtained from the LC-MS analysis of pepsin fragments (PF)(Figure S1 and Table 1). Primary sequence of KirBac3.1 covered by the PFs and location of modified residues (MR) identified by MSMS are marked in red blocks. The secondary structure consisting of loops, helix and sheets are represented by translucent grey, red and green background respectively. SH,OH,PH,IH represents Slide Helix, Outer Helix (TM1), Pore Helix and Inner Helix (TM2) respectively. Position of the modified residues in the three dimensional structure of KirBac3.1 are represented in Figure 1*C*.

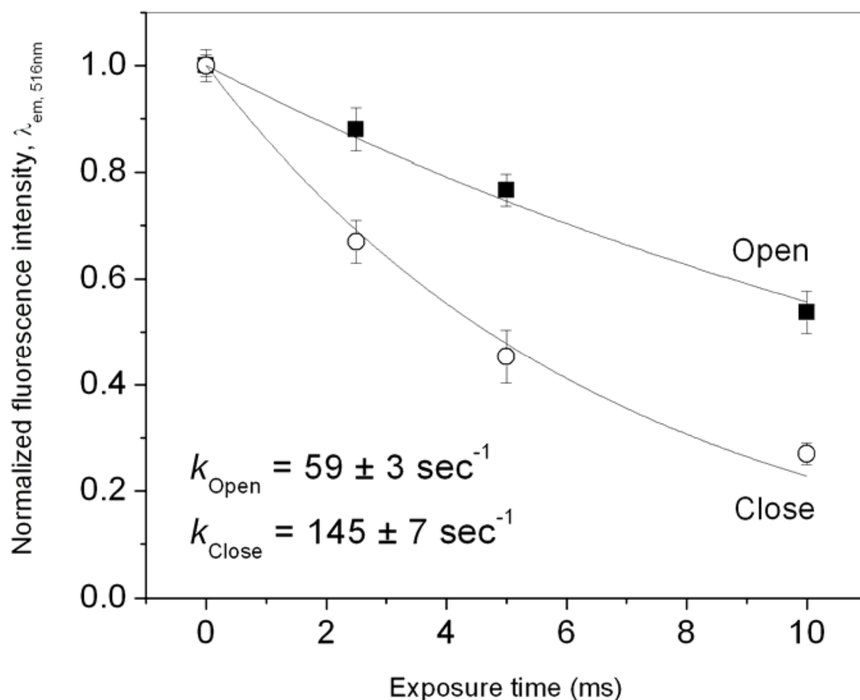

Fig. S3. Alexa 288 radiolysis and determination of X-ray dose: Dose response plots of 2µM Alexa in presence of 5µM closed and open KirBac3.1 solution in 50mM $MgCl_2$, 150mM KCl, 0.02% Tri-DM, 10mM Na-Cacodylate pH 7 and 1mM EDTA, 150mM KCl, 0.02% Tri-DM, 10mM Na-Cacodylate pH 7 respectively. Alexa solution in 5µl volume inside a PCR tube were irradiated with focused X-ray of energy 5-12keV (mirror angle=5.5, bender value = 8.0, ~ 10 fold more intense than the unfocused bending magnet source) for 0-10ms. After irradiation the solution is diluted (1:500) prior to fluorescence analysis. A Turner Biosystems TBS-380 fluorometer is used to determine the emission intensity at 516nm with an excitation wavelength of 496 nm. The solid line represents the fitting of data to a first-order reaction kinetics [Y=A*exp(-k.t) + y_0, A=1 and y_0=0]. The rate constant k, is a measure of effective hydroxyl radical dose on the sample. The EDTA in the open state sample is a •OH radical scavenger and reduces the X-ray dose by a factor of ~ 2.45. The rate constant k_{open} is corrected by multiplying the factor 2.45 ($k_{open\text{-}corrected}$ = k_{open} X 2.45) and ratio of $k_{open\text{-}corrected}$ and k_{closed} was determined (Figure 1B and Table 1).