Systematic Design and Validation of Ion Channel Stabilization of Amphipathic α -Helical Peptides incorporating Tryptophan Residues

Keita Shigedomi¹, Satoshi Osada^{1*}, Masoud Jelokhani–Niaraki², Hiroaki Kodama¹

¹Department of Chemistry and Applied Chemistry, Faculty of Science and Engineering, Saga

University, Saga 840-8502, Japan

²Department of Chemistry, Faculty of Science, Wilfrid Laurier University, Waterloo, Ontario N2L

3C5, Canada

Supporting Information Content Two Supporting Figures -Figure S1: Near-UV CD spectra of BKBA20 and Trp-containing analogs. Figure S2: Lipid solubilization assay.

Lipid solubilization assay.

DPPC (4.4 mmol) was dissolved in 100 mL of chloroform. The chloroform stock solutions were then evaporated into thin films using a nitrogen stream and further dried in vacuo overnight. The thin lipid film was then suspended in 1 mL of 50 mM phosphate buffer (pH 7.4) and vortexed for 30 min at 60°C. LUVs were prepared using a LiposoFast extruder (Avestin, Canada) through a 100 nm filter41. Lipid dispersions were passed through a filter for a total of 21 times. The solutions were mixed at 200 μ M peptide concentration and 1 mM DPPC concentration to give P/L ratios of 1/5 and incubated at 25°CThe kinetics of DPPC solubilization were measured by UV absorbance at 595 nm every 2 mins for 30 minutes on Model 680XR microplate reader (Bio-Rad).

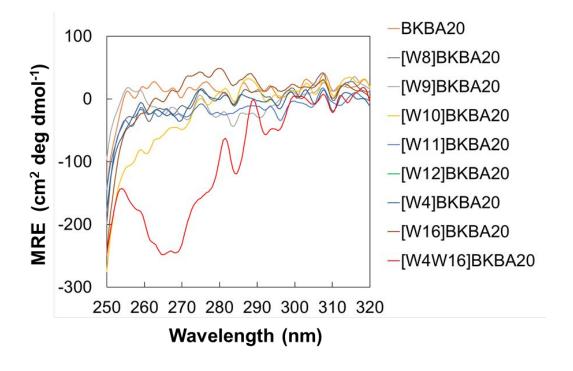


Figure S1. Near-UV CD spectra of BKBA20 and Trp-containing analogs in 50 mM phosphate buffer (pH 7.4). [W4W16]BKBA20 indicated the characteristic CD band in the 255-290 nm range, corresponding to the L_a and L_b bands of the Trp, which is sensitive to the environment.

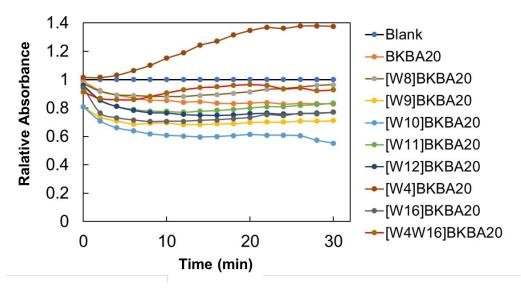


Figure S2. Lipid solubilization assay. BKBA20 and Trp-containing analogs dissolved in 50 mM phosphate buffer and mixed with DPPC LUVs. Turbidity was monitored by measuring the absorption at 595 nm every 2 minutes for 30 min. The peptide and DPPC concentrations were 200 μ M and 1 mM, respectively, to give the P/L ratios of 1/5.