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Supplemental Information

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Supporting Material

Determination of the stoichiometry between α and γ1 subunits of the BK channel using LRET

Willy Carrasquel-Ursulaez[1], Osvaldo Alvarez[1,2], Francisco Bezanilla[1,3,4] and Ramon Latorre[1]

[1]Centro Interdisciplinario de Neurociencia de Valparaíso, Universidad de Valparaíso, Valparaíso, Chile; [2]Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile; [3]Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, Illinois; [4]Institute for Biophysical Dynamics, University of Chicago, Chicago, Illinois

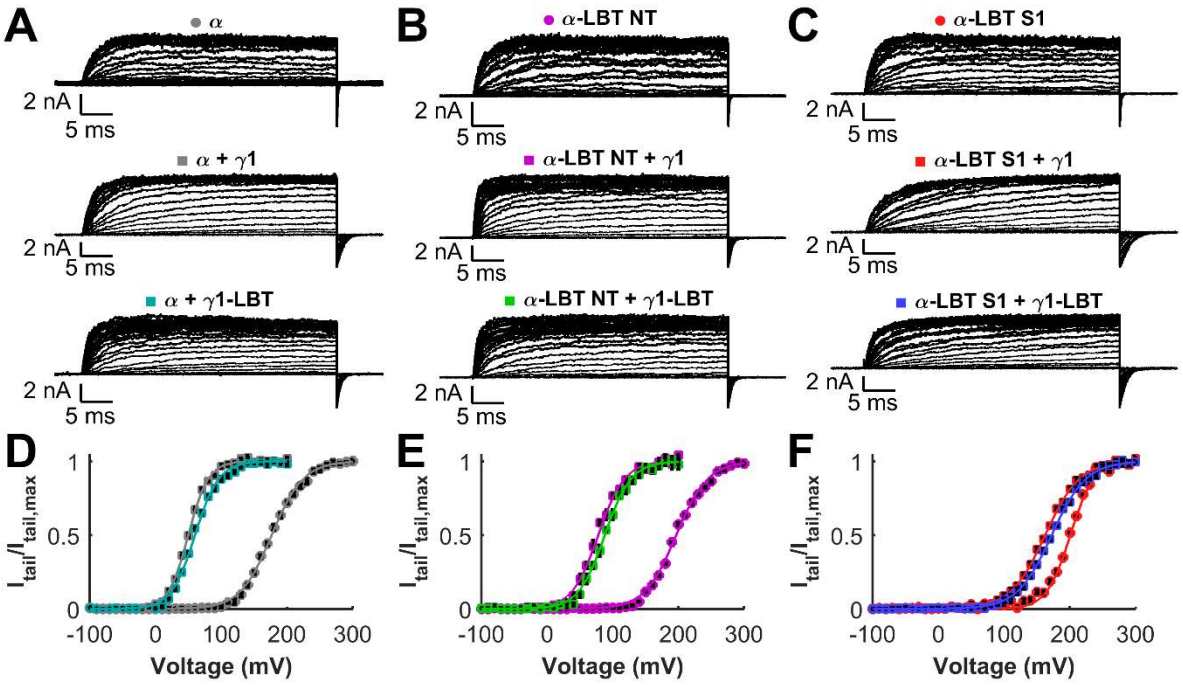

FIGURE S1. Biophysical characterization of LBT constructs.

(A-C) Representative records of BK channel macroscopic currents evoked by 40 ms voltage pulses (-100 to 200 or 300 mV; in 10 mV steps). BK pore was formed by the wild-type (wt) α subunit (A) and two different LBT-labeled α constructs, NT (B) and S1 (C). α (wt) and α-LBT constructs, were expressed in three different experimental conditions: α subunit alone (top); coexpressed with γ1 (wt) subunit (middle); and, coexpressed with γ1-LBT subunit (bottom). (D-F) Normalized tail currents versus voltage [($I_{tail}/I_{tail,max}$)-V] curves obtained from experiments like those in A-C. Data were fitted using Boltzmann function (solid lines). (D) For BK channels whose pore was formed by the α (wt) subunit, fitted parameters were (mean±S.E.M.): V_h=174±12 mV and zδ=1.06±0.17 e_0, for αBK channels (N=9); V_h=55±4 mV and zδ=1.72±0.04 e_0, for (α + γ1)BK channels (N=12); V_h=60±3 mV and zδ=1.46±0.07 e_0, for (α + γ1-LBT)BK channels (N=13). (E) For BK channels whose pore was formed by the α-LBT NT construct, fitted parameters were (mean±S.E.M.): V_h=195±7 mV and zδ=1.08±0.08 e_0, for (α-LBT NT)BK channels (N=4); V_h=76±5 mV and zδ=1.33±0.10 e_0, for (α-LBT NT + γ1)BK channels (N=6); V_h=82±7 mV and zδ=1.35±0.12 e_0, for (α-LBT NT + γ1-LBT)BK channels (N=4). (F) For BK channels whose pore was formed by the α-LBT S1 construct, fitted parameters were (mean±S.E.M.): V_h=200±3 mV and zδ=1.42±0.16 e_0, for (α-LBT S1)BK channels (N=4); V_h=159±10 mV and zδ=1.01±0.08 e_0, for (α-LBT S1 + γ1)BK channels (N=9); V_h=165±12 mV and zδ=1.01±0.09 e_0, for (α-LBT S1 + γ1-LBT)BK channels (N=5).

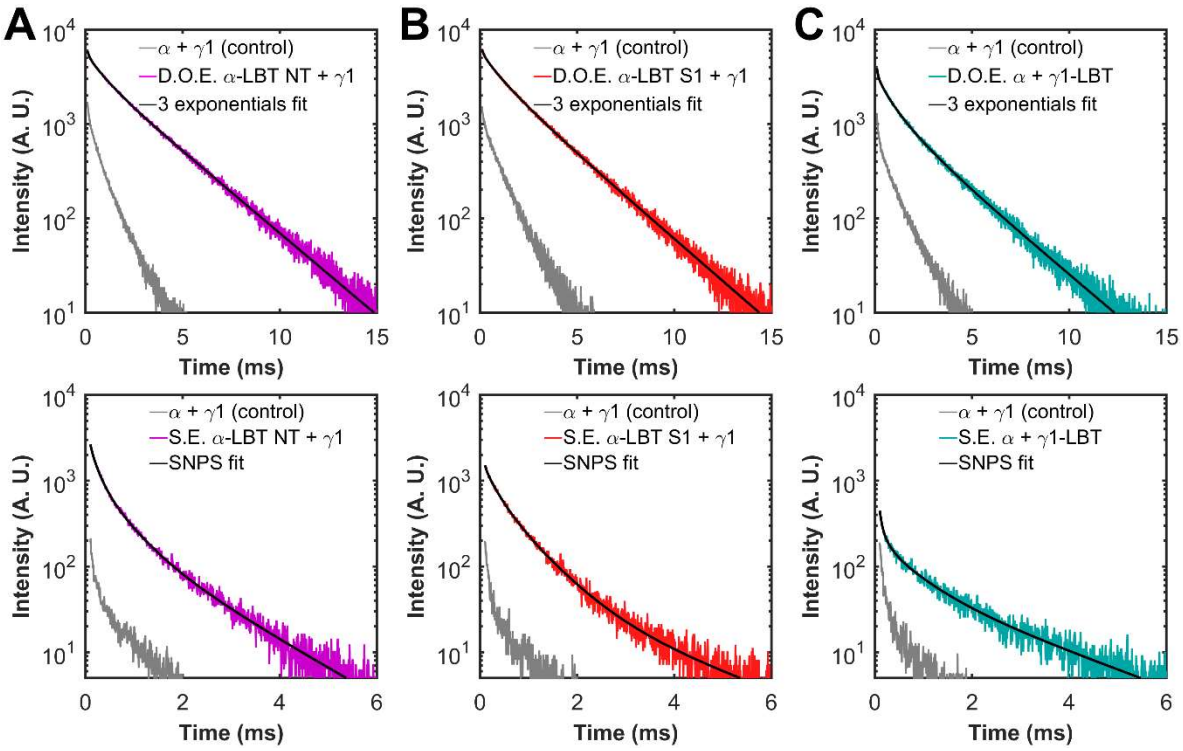

FIGURE S2. LRET measurements.

(A-C) Donor only emission (DOE; [top]) and sensitized emission (SE; [bottom]) lifetime measurements from the α-LBT NT (A) and S1 (B) constructs in the presence of γ1 (wt) (purple and red, respectively) and from the α (wt) in the presence of γ1-LBT (C; cyan). Three exponentials and SNPS fit (1) (black) are shown for DOE and SE traces, respectively. A control/background trace from (α + γ1)BK channels is also shown in each panel (gray). Experiments were performed while the voltage was clamped at -80 mV with the two-microelectrode voltage clamp technique. All traces were offset-subtracted. Note different time scales in DOE traces versus SE traces.

TABLE S1. Donor only time constants. Mean ± S.E.M.

BK channel composition	τ_{DOE} (ms)	N
α-LBT NT + γ1	2.46 ± 0.07	23
α-LBT S1 + γ1	2.34 ± 0.07	38
α + γ1-LBT	2.29 ± 0.07	19

TABLE S2. Sensitized emission time constants. Mean ± S.E.M.

BK channel composition	τ_1 (ms)	τ_2 (ms)	τ_3 (ms)	τ_4 (ms)	N
α-LBT NT + γ1	0.51 ± 0.03	0.63 ± 0.01	1.34 ± 0.04	1.45 ± 0.02	15
α-LBT S1 + γ1	0.71 ± 0.07	1.23 ± 0.12	1.42 ± 0.04	1.74 ± 0.06	5
α + γ1-LBT	1.12 ± 0.01	1.63 ± 0.01	1.69 ± 0.01	1.95 ± 0.01	12

METHODS

Mutagenesis. The cDNA coding for the human BK α-subunit (*KCNMA1*) from myometrium was provided by Dr. Ligia Toro (University of California, Los Angeles, CA). The cDNA coding for the human γ1 subunit (*LRRC26*) was kindly provided by Dr. Jiusheng Yan (University of Texas MD Anderson Cancer Center, Houston, TX) and Dr. Richard Aldrich (University of Texas, Austin, TX). The DNA sequence encoding LBT (YWDTNNDGWYEGDELLA (2)) was inserted in chosen positions of the α and γ1 subunits using standard molecular biology techniques. Insertions were confirmed by sequencing.

LRET acceptor. The LRET acceptor was the fluorophore BODIPY attached to the scorpion toxin iberiotoxin (IbTX). Labeled [D19C]IbTX was synthesized as previously described (3).

Heterologous protein expression. The wild-type and mutants cDNA were transcribed using T7 polymerase transcription kit (Ambion). The mRNA solution (50 nl) was injected into *Xenopus laevis* oocytes. For all experiments, α and α-LBT contructs RNA concentration were 250 ng/μl. In (α-LBT + γ1)BK experiments, γ1 mRNA concentration was 750 ng/μl (ratio $[RNA_{\gamma1}]/[RNA_{\alpha\text{-}LBT}]=3$). In (α-LBT + γ1-LBT)BK experiments, γ1-LBT mRNA concentration tested were 500, 375, 250, 187.5, 125, and 62.5 ng/μl (ratios $[RNA_{\gamma1\text{-}LBT}]/[RNA_{\alpha\text{-}LBT}]=2$, 1.5, 1, 0.75, 0.5, 0.25, respectively). In the last cases, the mix solutions were complemented with mRNA for γ1 wt at 0, 125, 250, 312.5, 375 and 437.5 ng/μl to assure that the total γ1 mRNA was constant and in excess along the experiments. An additional $[RNA_{\gamma1\text{-}LBT}]/[RNA_{\alpha\text{-}LBT}]$ ratio of 3 ($[RNA_{\gamma1\text{-}LBT}]$= 750 ng/μl; $[RNA_{\alpha\text{-}LBT}]$= 250 ng/μl) was tested. Oocytes were incubated in ND96 (96 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES, pH 7.6) solution at 18 °C for 3–5 days before recording.

Electrophysiology and LRET experiments. The electrophysiological characterization was made under patch clamp with an Axopatch 200B amplifier in the inside-out configuration. The bath and pipette solution both contained (in mM) K-MES 108, KCl 2, HEPES 10, and EGTA 5 (pH 7.4). The solution contained an estimated 0.8 nM Ca^{2+}, assuming the presence of 10 μM Ca^{2+} contaminant (4). During LRET experiments, the voltage was held to -80 mV using a CA-1B amplifier (Dagan Corp.) in the two-electrode voltage-clamp configuration. The internal resistance of the microelectrodes was in the 0.4–0.8 MΩ range and the pipette solution was 3 M KCl. During donor only emission (DOE) experiments, the bath solution contained NMDG-MES 110 mM HEPES 10 mM, $CaCl_2$ 2 mM, and $TbCl_3$ 10 μM (pH 7.4). For sensitized emission (SE) experiments, BODIPY-[D19C]IbTX stock solution was added to the bath solution until reaching 500 nM concentration and the oocytes were incubated for 5 min before recording.

Optical Setup. The optical setup used for simultaneous LRET and electrophysiology experiments was custom-designed as has been previously described (1,3). DOE and SE traces were obtained by averaging 16–25 pulses.

Donor only emission analysis. The analysis of the DOE decays was performed using the Decay Analysis software (1). The first 15 ms from each DOE trace was fitted to a three-exponential decay plus offset. The largest time constant (>2.25 ms) was considered as the decay time constant of Tb^{3+} bound to LBT (τ_{DOE}).

Sensitized emission analysis. The analysis of the SE decays was performed using the SNPS software (1). The position of the acceptor was the reported by (3). The first 6 ms from each SE trace was fitted to a Heyduk-constrained (5) tetra-exponential decay plus a two-exponential artifact plus offset. From

the output of the SNPS program the four time constants of the best fit to the (α-LBT + γ1)BK and (α + γ1-LBT)BK SE traces were obtained.

Stochiometry determination. The offset of (α-LBT + γ1-LBT)BK SE traces was calculated and substracted to the trace using Decay Analysis software (1). The offset-substracted trace was fitted with the Eq. 5 using a custom script using the Matlab function *lsqnonlin*, which solve nonlinear least-squares problems. The boundaries of the values of the six parameters were reasonably chosen and the six-element vectors of start values were randomly generated. For each experiment, 100 start values vectors were generated and 100 independent fits were made, to visually inspect that the solution was not a local minimum of the sum of the square of the weighted residuals.

SUPPORTING REFERENCES

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