

# Supporting Information

Contreras et al. 10.1073/pnas.1216953109

## SI Materials and Methods

The two-exponential kinetics of  $Q_p$  was reported by Horrigan and Aldrich (1) for BK channels in the absence of  $\text{Ca}^{2+}$ . They explained this behavior in terms of an allosteric model (Fig. S5A) in which the four voltage sensors have two states, resting,  $R$ , and active,  $A$ . The  $R$ - $A$  equilibrium constant,  $J$  is voltage dependent according to the relation  $J = J_0 \exp(-z_j eV/kT)$ , where  $J_0$  is the zero voltage equilibrium constant and every  $R$  to  $A$  transition results in the transfer of  $z_j$  elementary charges across the membrane. Membrane depolarization displaces the  $R$ - $A$  equilibrium toward  $A$ , and this process generates the fast gating current that when integrated in time gives  $Q_{\text{fast}}$ . Activation of the voltage sensors displaces the closed,  $C$ , to open,  $O$ , equilibrium toward the open state due to allosteric coupling, through the allosteric coupling constant  $D$ . The  $O$ - $C$  equilibrium constant,  $L$ , is also voltage dependent ( $L = L_0 \exp(-z_L eV/kT)$ ) and the closed-to-open transition displaces  $z_L$  elementary charges. Because the closed-to-open transition is slow, the charge transported contributes to the  $Q_{\text{pSlow}}$  component of  $Q_p$ . The allosteric coupling of the voltage sensors and the channel gate shifts toward  $A$  the voltage sensor equilibrium and the additional charge displaced by this rearrangement contributes to  $Q_{\text{pSlow}}$ . Therefore, the slow component of  $Q_p$  is dependent on the closed-to-open transition.

1. Horrigan FT, Aldrich RW (1999) Allosteric voltage gating of potassium channels II. Mslo channel gating charge movement in the absence of  $\text{Ca}^{2+}$ . *J Gen Physiol* 114(2): 305–336.

Our results on BK channels associated with the  $\beta 1$  auxiliary subunits show that  $Q_p$  also has a slow component but it appears at voltages where the closed-to-open transition is fully displaced toward the closed state. This result is incompatible with the H-C-A allosteric model, and, therefore we split the closed-to-open transition into two steps (Fig. S5B): The slow component of  $Q_p$  represents the charge transported due to the transition from the deeper closed state,  $C_0$ , to another closed state,  $C_1$ , and it is independent of the final transition from  $C_1$  to the open state. This latter transition is governed by the equilibrium constant  $M$ , which may be voltage-dependent and may displace charge, but the amplitude of the associated gating current is expected not to be measurable because the opening of the channel in the presence of  $\beta 1$  is very slow. We have augmented the two-tiered 10-state H-C-A allosteric model to a three-tiered 15-state model (Fig. S5B). Now a new allosteric coupling constant  $E$  must be added to complete the scheme.

Steady-state analysis of the gating charge, i.e., determination of  $Q_{\text{fast}}$  and  $Q_{\text{pSlow}}$  as a function of voltage is sufficient to make a first approximation of the equilibrium constants  $J$  and  $L$  as a function of voltage and the allosteric coupling constant  $D$ . We augmented the model to account for the kinetics of the processes, by splitting the equilibrium constant  $J$ ,  $L$ , and  $M$  into forward and backward rate constant (Fig. S5B).

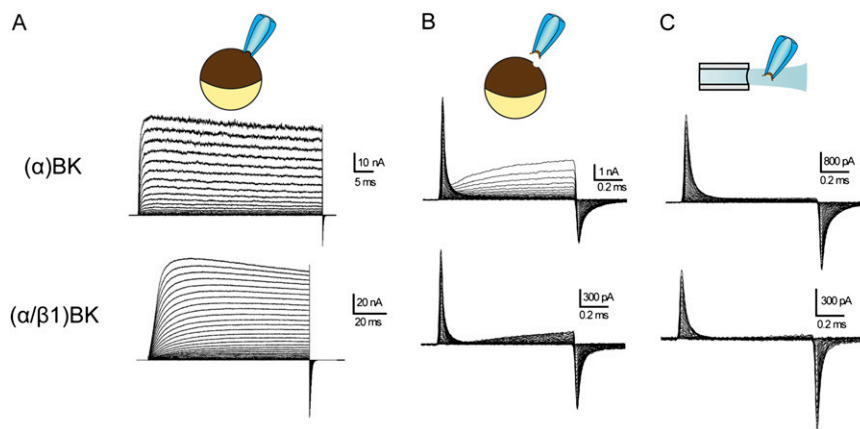


Fig. S1.  $I_K$  and  $I_G$  currents measured in the same macropatch.  $I_K$  currents were recorded in the cell-attached configuration (A) to ensure that BK channels were formed by the expected  $\alpha$  or  $\alpha/\beta$  complex. After patch excision (B),  $I_G$  currents were isolated in conditions of  $0 \text{ K}^+$  by repeated bath exchange of the internal side of the macropatch with a  $\text{K}^+$ -free solution (internal solution, see *Materials and Methods*).

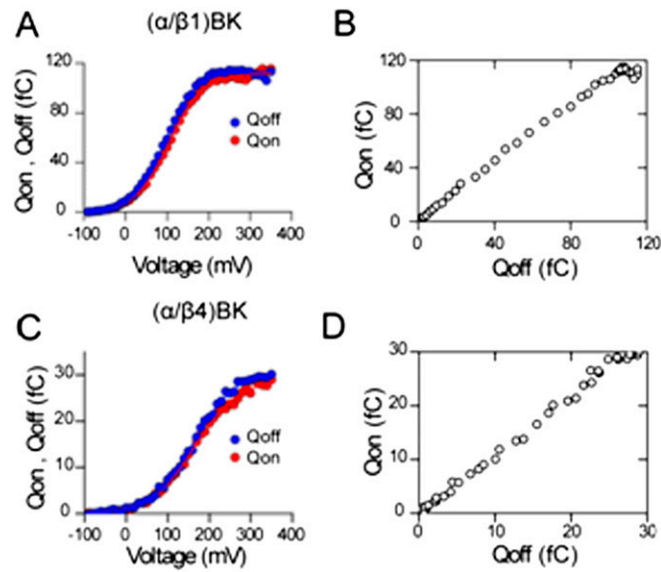


Fig. S2. ON and OFF gating currents. (A and C)  $Q_{ON}$  (red circles) and  $Q_{OFF}$  (blue circles) charge of  $(\alpha/\beta1)BK$  and  $(\alpha/\beta4)BK$  channel are equal. (B and D)  $Q_{ON}$  plotted against  $Q_{OFF}$  are linearly related.

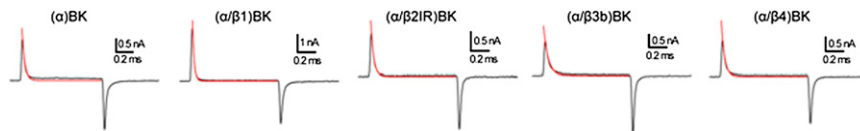
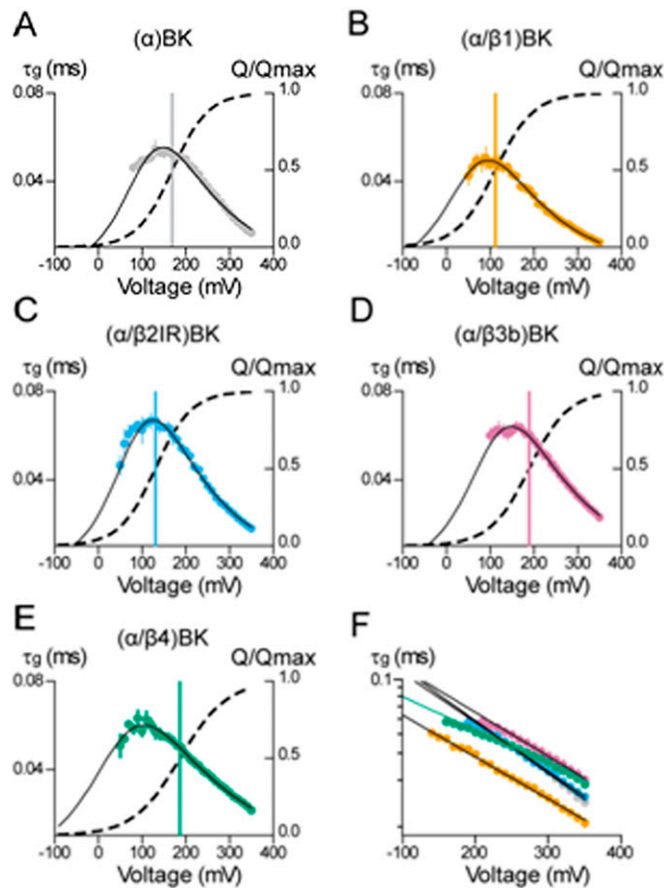
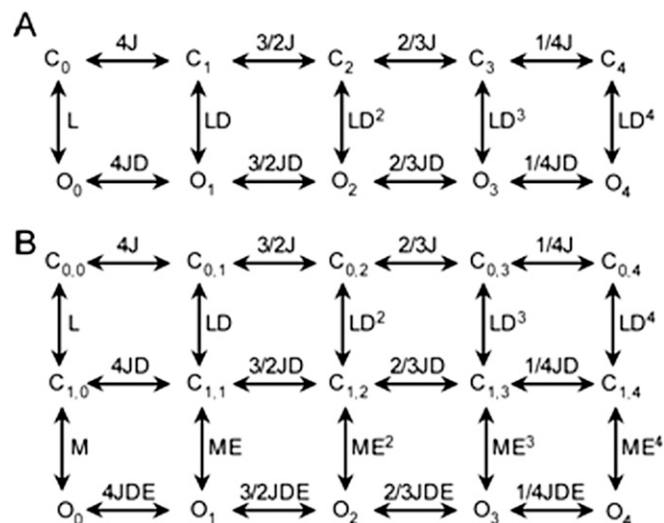


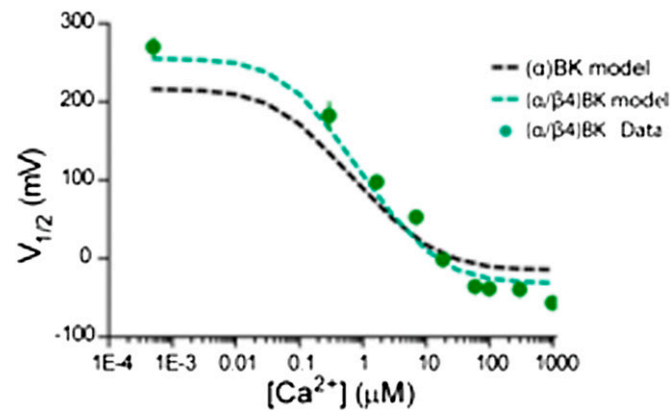
Fig. S3.  $I_{gON}$  is well fitted using a single exponential decay (red line).



**Fig. 54.** Kinetics of gating charge. (A–E) Exponential decays of  $I_{gON}$  were fitted with an exponential function and the time constants ( $\tau_g$ , mean  $\pm$  SEM) were plotted against the voltage. The solid black line is the best fit to a two-state model (Table S1). All graphs include the Boltzmann fit of the  $Q-V$  curve (dashed lines in A–E) and the corresponding  $V_h$  is indicated by a vertical line for the  $Q-V$  curve (solid colored line in A–E). (F) The linear range of each plot was fitted with an exponential decay function and is shown by black lines with the exception of  $(\alpha/\beta4)BK$ , which is shown by a green line.



**Fig. 55.** Allosteric models explain the complex kinetics of BK channels in the absence of calcium. (A) Horrigan, Cui, and Aldrich allosteric model (1). States within a row correspond to the possible combinations of the four independent voltage sensors. Subscripts indicate the number of voltage sensors in the active position. Resting/active equilibrium is governed by the voltage-dependent constant  $J$ . A single channel gates between two possible states, closed and open, with an equilibrium constant  $L$ . As voltage sensors reach the active state, channel opening is favored by an allosteric factor  $D$ . (B) Allosteric model in which the channel gate opens in two sequential steps. Equilibrium between the first and second row of closed states is governed by  $L$  and the allosteric factor  $D$ , whereas allosteric factors  $M$  and  $E$  describe equilibrium between  $C_{1,0}$  through  $C_{4,0}$  and  $O_1$  through  $O_4$ .



**Fig. S6.** Changes in  $z_j$  and  $V_{1/2}$  account for calcium sensitivity in  $(\alpha/\beta4)$ BK channels. Data from ref. 1 (bluish-green circles) are superimposed with simulation of  $(\alpha)$ BK (dashed black line) and  $(\alpha/\beta4)$ BK channels (dashed blue line) using the H–C–A model (Fig. 4A) with the following parameters:  $(\alpha)$ BK  $V_h = 140$  mV,  $z_j = 0.58$ ,  $z_L = 0.31$ ,  $D = 19$ ,  $C = 14$ ,  $E = 2.8$ ,  $L_0 = 1e-6$ , and  $K_d = 8.2$  (data from ref. 2). For  $(\alpha/\beta4)$ BK all parameters were the same except  $V_{1/2} = 186$  mV and  $z_j = 0.4$ .

1. Wang B, Rothberg BS, Brenner R (2006) Mechanism of beta4 subunit modulation of BK channels. *J Gen Physiol* 127(4):449–465.
2. Orio P, Latorre R (2005) Differential effects of beta 1 and beta 2 subunits on BK channel activity. *J Gen Physiol* 125(4):395–411.

**Table S1. Fit parameters of gating charge-voltage relationships for  $(\alpha)$ BK and  $(\alpha/\beta x)$ BK channels**

		$(\alpha)$ BK	$(\alpha/\beta1)$ BK	$(\alpha/\beta2IR)$ BK	$(\alpha/\beta3b)$ BK	$(\alpha/\beta4)$ BK
$V_h$ (mV)	Mean	169	112	130	189	186
	SD	12	12	17	9	6
$z$ ( $e_0$ )	Mean	0.63	0.58	0.61	0.56	0.48
	SD	0.06	0.06	0.04	0.02	0.02
	$N$	14	8	7	8	7

All of the corresponding  $Q(V)$  curves were displaced in the voltage axis by  $\Delta V = (V_h - \langle V_h \rangle)$ .  $V_h$  and  $z$  obtained from the fit of the  $Q(V)$  data to a Boltzmann function.

**Table S2. Fit parameters of the kinetics of gating charge**

Channel	$z$ ( $e_0$ )	$\delta$	$\alpha_0$ ( $s^{-1}$ )	$\beta_0$ ( $s^{-1}$ )
$(\alpha)$ BK	0.63	0.29	4.5	70
$(\alpha/\beta1)$ BK	0.58	0.30	7.5	28
$(\alpha/\beta2IR)$ BK	0.61	0.30	4.5	37
$(\alpha/\beta3b)$ BK	0.56	0.30	4.5	48
$(\alpha/\beta4)$ BK	0.48	0.28	7.0	19

Best fit to a two-states model with  $z$  constrained to the values found for the  $Q-V$  relation.