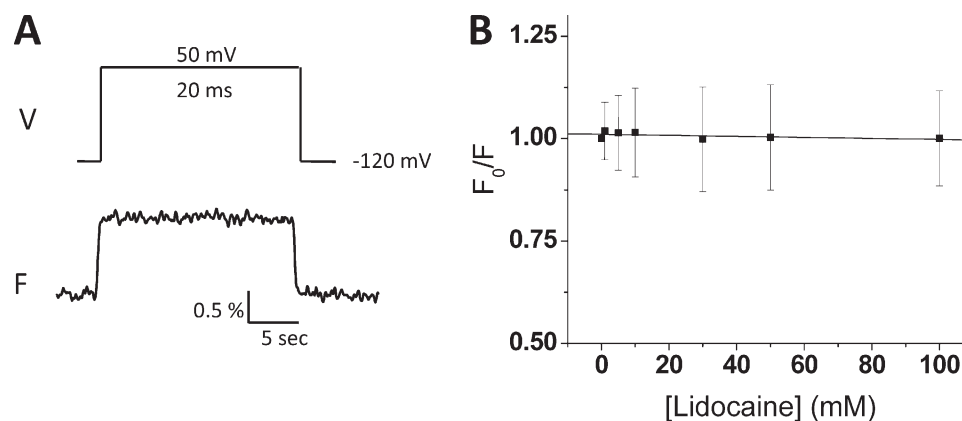


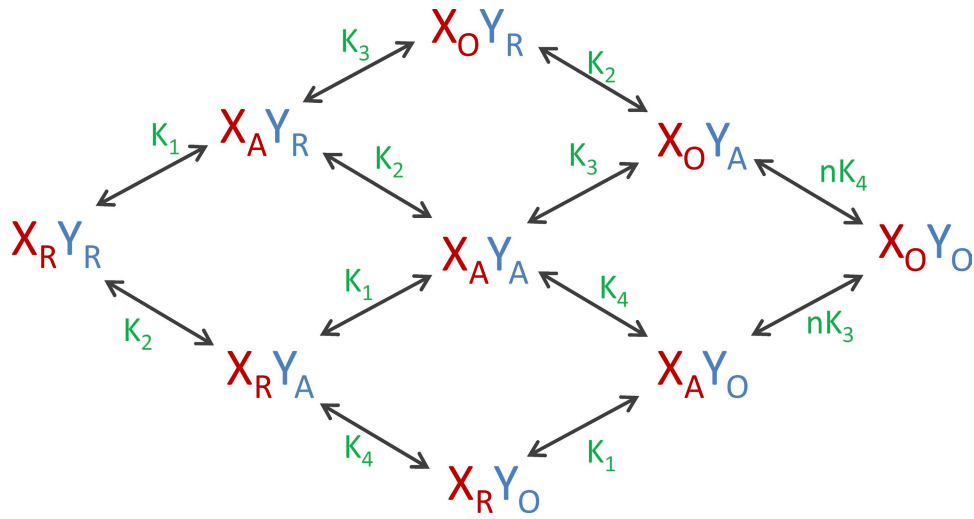
Muroi et al., <http://www.jgp.org/cgi/content/full/jgp.200810103/DC1>



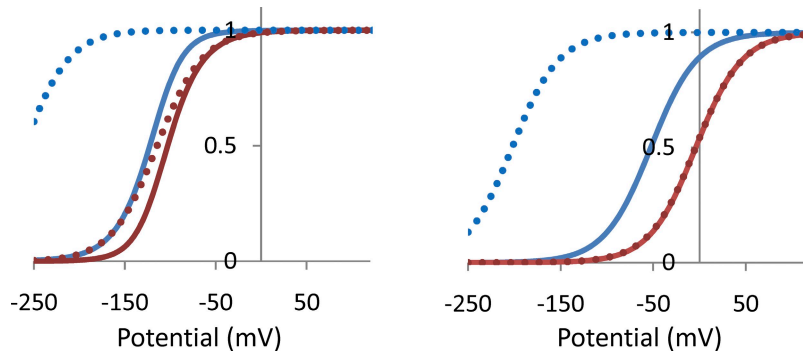
**Figure S1.** The effect of lidocaine on rhodamine fluorescence in the lipid membrane. (A) Membrane localization of this fluorophore was first confirmed by measuring a fast voltage-dependent fluorescence response in labeled un-injected oocytes. This fluorescence response is likely to be an electrochromic signal that results from an interaction of the fluorophore dipole moment with the electric field. Membrane potential-dependent fluorescence traces from oocytes incubated in octadecyl rhodamine B were elicited by a pulse to +50 mV from -120 mV. An average of 10 individual traces is shown. (B) Fluorescence intensities from labeled un-injected oocytes were measured in different concentrations of lidocaine. A Stern-Volmer plot of octadecyl rhodamine quenching by lidocaine is shown. The data were fitted to the following equation:

$$\frac{F_0}{F} = 1 + K_D [Q],$$

where  $F_0$  is the maximum fluorescence intensity without lidocaine,  $F$  is fluorescence intensity in different concentrations of lidocaine,  $[Q]$  is lidocaine concentration, and  $K_D$  is the Stern-Volmer quenching constant. Thus, lidocaine is unlikely to quench rhodamine fluorescence either in an aqueous solution (up to 100 mM lidocaine; not depicted) or in a membrane environment. The time-dependent changes in octadecyl rhodamine B fluorescence intensity was corrected by recording in the absence of lidocaine. The data represent the mean  $\pm$  propagation of errors derived from standard deviations for each data set.



**Figure S2.** A state diagram depicting a simple model system. This hypothetical channel consists of two subunits, where each subunit undergoes two transitions: R to A and A to O. These subunits were coupled via the last transition. If one of the subunits was in state A, the forward transition to O was favored by a factor  $n$  when the second subunit was also in the state O. The gating scheme is similar to the one used to describe the Shaker gating (Zagotta, W.N., T. Hoshi, and R.W. Aldrich. 1994. *J. Gen. Physiol.* 103:321–362).



**Figure S3.** Simulated fluorescence–voltage curves of the simple model system (Fig. S2) in response to voltage sensor perturbations. The left panel shows the fluorescence–voltage curves when the second A to O transition is made favorable ( $K_3$  and  $K_4 = 10$ ), and the right panel shows the fluorescence–voltage curve when the A to O transition is not favored ( $K_3$  and  $K_4 = 0.1$ ). The parameters to generate the fluorescence–voltage plots are shown in Table S1. Solid lines depict the fluorescence–voltage curves when both the domains were coupled to each other ( $n = 10$ ) before perturbation. Dashed lines represent the fluorescence–voltage curves when the input  $V_m$  corresponding to the R→A transition for subunit Y was left-shifted by  $-150$  mV.

TABLE S1  
*Parameters to Simulate the Fluorescence–Voltage Relationships in the Model System in Response to Voltage Sensor Perturbations*

		Subunit X	Subunit Y
Coupling term, $n$		$10$	
Input $V_m$ values		0	$-50$ $\downarrow$ $-200$
	Large $K_3$ and $K_4$	$-101.80$ $\downarrow$	$-123.49$ $\downarrow$
Output $V_m$ values		$-114.49$	$-260.48$
	Small $K_3$ and $K_4$	$-3.92$ $\downarrow$	$-52.72$ $\downarrow$
		$-4.23$	$-202.41$

The input  $V_m$  values define  $K_1$  and  $K_2$  as described in Materials and methods. The output  $V_m$  values were obtained by fitting the simulated fluorescence–voltage curves of subunits X and Y with a Boltzmann function. When input  $V_m$  value of subunit Y is changed from −50 to −200 mV in this model system, the output  $V_m$  values of both subunits shift depending on their  $K_3$  and  $K_4$ .