iScience, Volume *26*

Supplemental information

Cardiolipin binding enhances KcsA channel gating via both its specific and dianion-monoanion interchangeable sites

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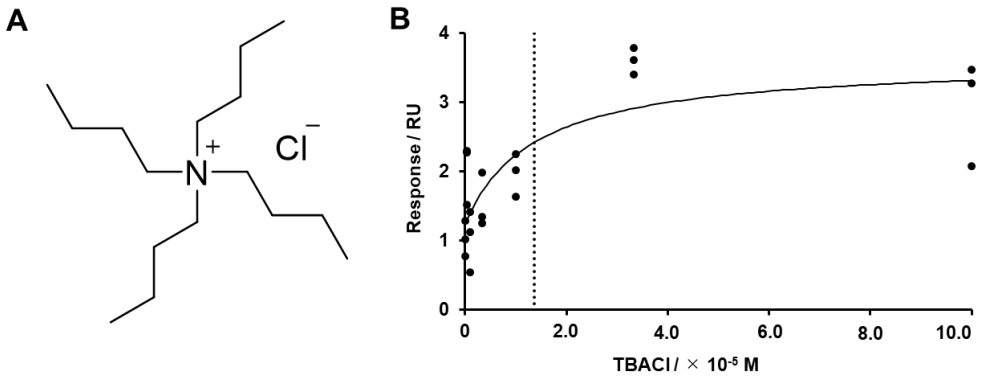

Figure S1. SPR analysis of the interactions between injected tetrabutylammonium chloride (TBACl) and wt-KcsA immobilized on the C_6-SAM modified sensor chip, related to Figure 1. (A) Structure of TBACl. **(B)** The affinity of TBA towards wt-KcsA was assessed from the concentration dependence of the response at 180 s by a fit of Langmuir isotherm binding model. 7 concentrations of TBA were injected in the range of 100 nM to 100 μM dissolved in acidic buffer [10 mM succinic acid, pH 4.0, 200 mM KCl, 3 mM EDTA, 0.5 %(v/v) Tween 20] at a flow rate of 30 μL min^{-1}, to obtain a set of sensorgrams. The affinity was calculated as 13 ± 12 μM, which is coincident with the previous channel recording [S1].

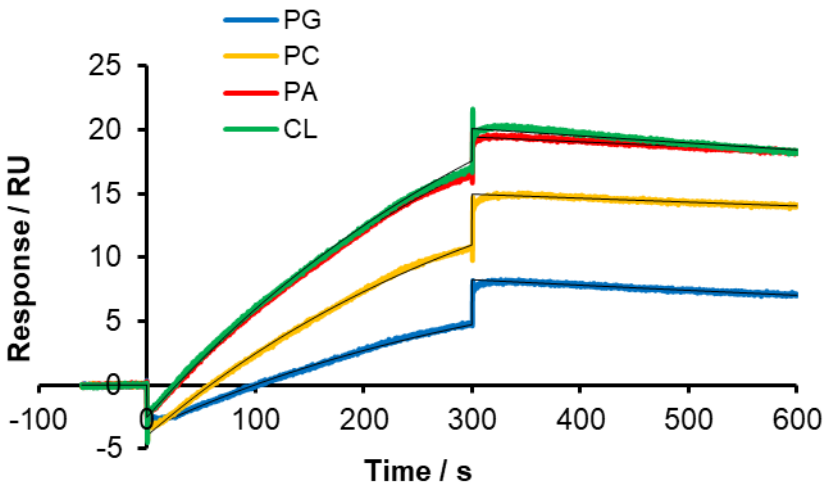

Figure S2. SPR sensorgrams showing the interactions of PG, PC, PA and CL with fl-KcsA immobilized on the C_6-SAM modified sensor chip, at pH 7.5, related to Figure 3. 100 μM of the lipids dissolved in neutral buffer [10 mM HEPES, pH 7.5, 200 mM KCl, 3 mM EDTA, 0.5%(v/v) Tween20] were injected at a flow rate of 30 μL min^{-1}. Colored lines indicate experimentally obtained sensorgrams, while black lines indicate theoretical curves (1:2 heterogeneous ligand binding model).

Table S1. Particle sizes of lipids in Tween 20-containing buffer, as detected by dynamic light scattering measurement.[a] Related to Figure 1AB.

Lipids	Acidic buffer [b] / nm	Neutral buffer [c] / nm
PC	10.1 ± 1.8	10.0 ± 2.5
PG	6.2 ± 2.1	10.3 ± 2.2
CL	7.9 ± 1.6	11.2 ± 5.1
PA	8.4 ± 2.8	11.0 ± 3.1

[a] Data are presented as mean ± SD (***n*** = 3).

[b] 10 mM succinic acid [pH 4.0], 200 mM KCl, 3 mM EDTA, 0.05% (v/v) Tween 20.

[c] 10 mM HEPES [pH 7.5], 200 mM KCl, 3 mM EDTA, 0.05% (v/v) Tween 20.

Table S2. Affinities K_A and theoretical maximum response R_{max} of lipids toward fl-KcsA, as determined using SPR method.[a] Related to Figure 3.

Lipid	pH 4.0 (active state)		pH 7.5 (resting state)	
	K_A	R_{max}	K_A	R_{max}
PG	$(1.40 \pm 0.15) \times 10^5$	78.9 ± 0.5	$(4.29 \pm 0.33) \times 10^4$	15.5 ± 2.1
	$(6.06 \pm 0.35) \times 10^4$	11.0 ± 0.3	$(8.83 \pm 5.89) \times 10^3$	1.13 ± 0.66
PC	$(1.18 \pm 0.06) \times 10^5$	58.7 ± 1.2	$(9.55 \pm 2.29) \times 10^4$	31.6 ± 2.8
	$(7.26 \pm 1.54) \times 10^3$	14.8 ± 2.0	$(4.03 \pm 3.63) \times 10^4$	$(1.38 \pm 0.98) \times 10^{-3}$
PA	$(2.45 \pm 0.33) \times 10^5$	96.1 ± 0.6	$(9.33 \pm 3.36) \times 10^4$	37.9 ± 1.8
	$(1.28 \pm 0.04) \times 10^5$	24.1 ± 0.4	$(3.97 \pm 1.74) \times 10^4$	1.48 ± 0.98
CL	$(9.86 \pm 4.56) \times 10^8$	93.7 ± 10.1	$(5.49 \pm 2.11) \times 10^4$	44.3 ± 3.6
	$(4.60 \pm 0.30) \times 10^4$	167 ± 3	$(3.68 \pm 2.07) \times 10^4$	2.86 ± 1.70

[a] Data are presented as mean ± SD ($\boldsymbol{n}$ = 3). The affinity is shown as the binding constants K_A (M^{-1}). Each value was calculated from the sensorgram by fitting to a 1:2 heterogeneous ligand model [S2,S3]. R_{max} was corrected for number of immobilized proteins as 1000 RU.

The heterogeneous ligand binding model assumes the following two competitive equilibriums.

$$A + \begin{cases} B \underset{k_{off1}}{\overset{k_{on1}}{\rightleftharpoons}} AB & K_{A1} = k_{on1} / k_{off1},\ R_{max1}\text{: maximum capacity 1} \\ C \underset{k_{off2}}{\overset{k_{on2}}{\rightleftharpoons}} AC & K_{A2} = k_{on2} / k_{off2},\ R_{max2}\text{: maximum capacity 2} \end{cases}$$

In this study A is lipid, and B and C are different binding sites in KcsA, one of which we assumed specific and the other non-specific.

Table S3. Kinetic data of the interaction between fl-KcsA and lipids at pH 4.0*. Related to Figure 3.

	k_{on}	S.D.	k_{off}	S.D.
CL	2087	160	2.556×10^{-6}	1.442×10^{-6}
PA	950.5	55.0	7.443×10^{-3}	2.30×10^{-4}
PC	63.63	1.16	5.391×10^{-4}	3.65×10^{-5}
PG	939.1	28.9	1.553×10^{-2}	7.1×10^{-4}

*Kinetic data were obtained from the larger K_A values in Table S2.

Table S4. Affinities K_A and theoretical maximum response R_{max} of lipids toward ΔM0-KcsA at pH 4.0, as determined using SPR method.[a] Related to Figure 4.

Lipid	K_A	R_{max}
PG	$(5.58 \pm 0.74) \times 10^4$	112 ± 8
	$(1.20 \pm 0.58) \times 10^4$	48.2 ± 4.7
PC	$(1.03 \pm 0.24) \times 10^5$	65.6 ± 14.8
	$(1.43 \pm 0.60) \times 10^3$	92.9 ± 32.7
PA	$(5.89 \pm 0.74) \times 10^4$	174 ± 3
	$(4.83 \pm 0.48) \times 10^4$	32.7 ± 1.4
CL	$(1.58 \pm 0.25) \times 10^9$	94.1 ± 33.4
	$(3.95 \pm 1.11) \times 10^4$	169 ± 12

[a] Data are presented as mean ± SD (***n*** = 3). The affinity is shown as the binding constants K_A (M^{-1}). Each value was calculated from the sensorgram by fitting to a 1:2 heterogeneous ligand model. R_{max} was corrected for amount of immobilized proteins as 1000 RU. For heterogeneous ligand model, refer to Table S2 caption.

Table S5. Open probability (P_{open}) of wt- and ΔM0-KcsA channels in lipid bilayers of various compositions.[a] Related to Figure 6.

Channel	Lipid [b]	P_{open}	SD	*N*
Full-length	PG	0.170	0.065	5
	PA	0.174	0.098	5
	CL	0.304	0.059	8
	PC	0.013	0.012	3
ΔM0	PG	0.020	0.007	9
	PA	0.023	0.017	10
	CL	0.058	0.046	10
	PC	0.018	0.018	10

[a] The lipid bilayers were formed by the contact bubble bilayer (CBB) method (see Methods).

[b] Anionic lipids (PG, PA, and CL) are mixed with PC at an anionic lipid/PC weight ratio of 1/3.

Supplemental note

As described in the text, the observed sensorgrams were fitted to a 1:2 heterogeneous ligand binding model that assumed that the lipid binds to KcsA at two different sites (Tables S2 and S4). The smaller association constants (K_A) were considered to be due to non-specific or less relevant binding, and larger K_A values were regarded as specific binding. Figure S3 shows sensorgrams obtained from three consecutive SPR measurements between CL solution and immobilized ΔM0-KcsA. Evidently, the amount of the CL binding in the second and third analysis was reduced compared with that in the first analysis. The 1:2 heterogeneous ligand binding fitting (Table S6) clearly shows that the R_{max1} related to K_{A1} (larger association constant) is significantly reduced in the second and third analysis, while R_{max2} related to K_{A2} (smaller association constant) is not vary affected among the analysis. This suggests that CL bound to the specific site in the first analysis was not completely removed by the washing, and thus the amount of CL that can bind to the specific site was reduced in the second and third analysis, resulting in smaller R_{max1} values. On the other hand, CL bound to non-specific site was mostly removed by washing, and thus the amount of CL bound to non-specific site, corresponding to R_{max2}, was not changed among the consecutive three analysis. This data strongly supports the notion that larger K_{A1} and smaller K_{A2} values are associated with specific and non-specific bindings, respectively.

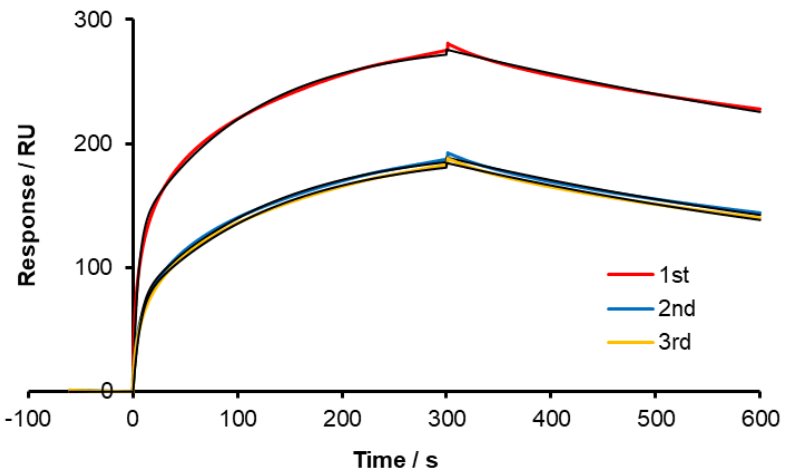

Figure S3. SPR sensorgrams showing the interactions of CL with ΔM0-KcsA immobilized on the C_6-SAM modified sensor chip, at pH 4.0, related to Figures 3 and 4. 100 μM of the lipids dissolved in acidic buffer [10 mM succinic acid, pH 4.0, 200 mM KCl, 3 mM EDTA, 0.5%(v/v) Tween20] were injected at a flow rate of 30 μL min^{-1} for 180 s, and then washed with the same acidic buffer for 180 s, and further washed with regeneration solution (10 mM NaOH, 0.5% (v/v) Tween 20) for 3×30 s. This sequential analysis was repeated three times, and each sensorgram was recorded. Colored lines indicate experimentally obtained sensorgrams for the 1st (red), 2nd (blue), and 3rd (yellow) analysis, and while black lines indicate theoretical curves (1:2 heterogeneous ligand binding model).

Table S6 Affinities K_A and theoretical maximum response R_{max} of lipids toward ΔM0-KcsA.[a] Related to Figures 3 and 4.

	CL specific		***nonspecific***	
	K_{A1} / M^{-1}	R_{max1} / RU	K_{A2} / M^{-1}	R_{max2} / RU
1st	1.86×10^9	133	5.23×10^4	183
2nd	1.46×10^9	76	3.35×10^4	162
3rd	1.42×10^9	73	3.28×10^4	162

[a] Each value was calculated from the sensorgram by fitting to a 1:2 heterogeneous ligand model. R_{max} was corrected for amount of immobilized proteins as 1000 RU. For heterogeneous ligand model, refer to Table S2 caption.

Supplemental references

S1 Iwamoto, M., Shimizu, H., Inoue, F., Konnno, T., Sasaki, Y. C., Oiki, S. (2006) Surface Structure and Its Dynamic Rearrangements of the KcsA Potassium Channel upon Gating and Tetrabutylammonium Blocking. *J. Biol. Chem*. **81**, 28379–28386.

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S3 Khalifa, M. B., Choulier, L., Lortat-Jacob, H., Altschuh, D., Vernet, T. (2001). BIACORE data processing: an evaluation of the global fitting procedure. *Anal. Biochem.* **293**, 194–203.