Biophysical Journal, Volume *112*

Supplemental Information

Probing Conformational Changes during the Gating Cycle of a Potassium Channel in Lipid Bilayers

Elwin A.W. van der Cruijsen, Alexander V. Prokofyev, Olaf Pongs, and Marc Baldus

SUPPLEMENT INFORMATION

Probing conformational changes during the gating cycle of a Potassium channel in lipid bilayers

E.A.W. van der Cruijsen[1,+], A.V. Prokofyev[2,+], O. Pongs[2,*] , and M. Baldus[1,*]

[1]NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, the Netherlands.

and

[2] Institute of Cellular Neurophysiology, Department of Physiology, University of the Saarland, 66421 Homburg, Germany

[+] contributed equally to this work

[*] To whom correspondence should be addressed:

oupon@t-online.de (OP) or m.baldus@uu.nl (MB)

SUPPLEMENT INFORMATION

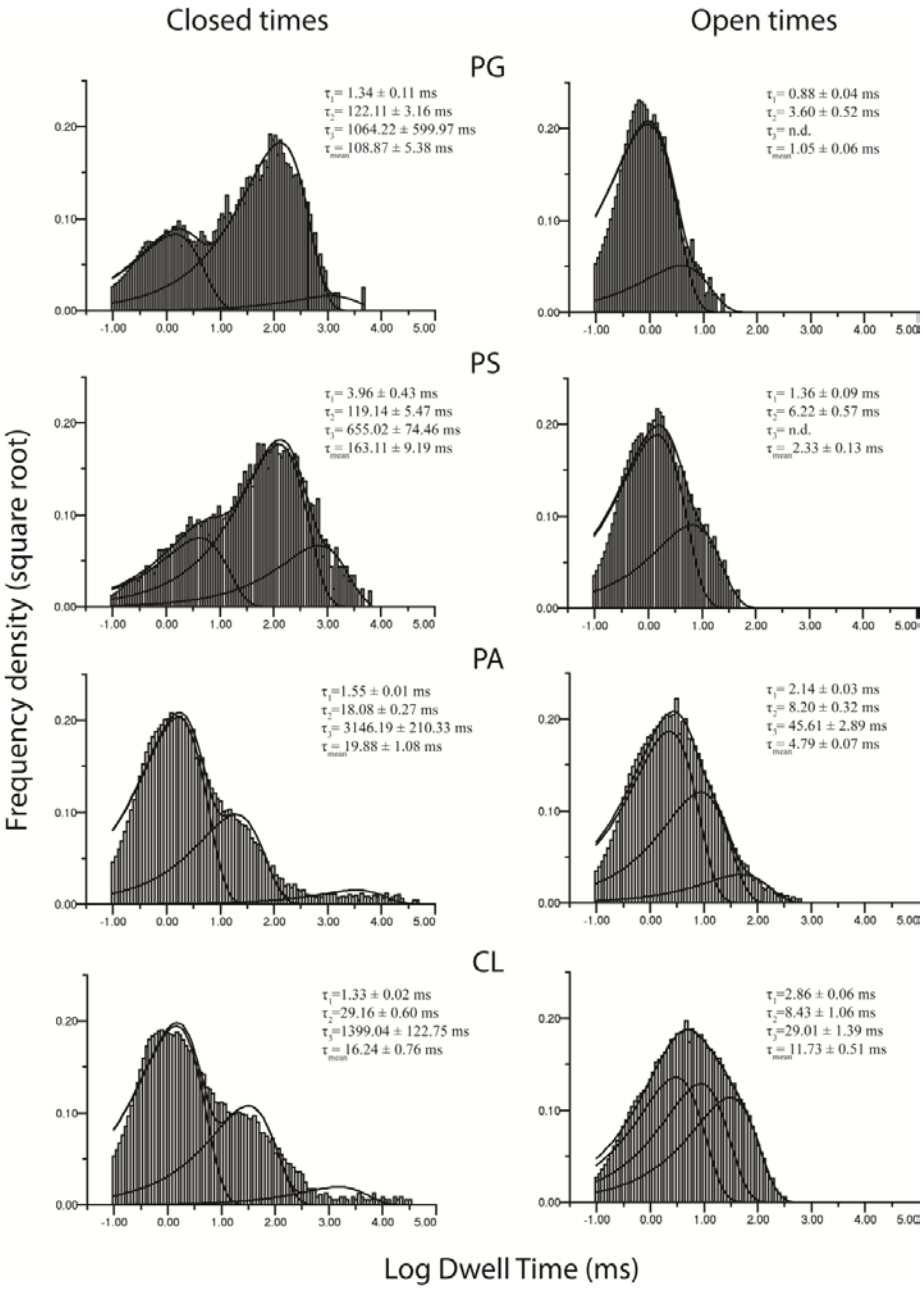

Figure 1. Representative dwell time histograms of KcsA recorded in different anionic phospholipids. All experiments were performed in symmetrical 150 mM KCl solution at pH = 7.0 on the *cis* side and pH 4.0 on the *trans* side. Lipid bilayers were composed of 70 % anionic lipid and 30 % neutral DOPC. Time constants (τ) were calculated from a three exponential fits (see equations on the page 48) to log dwell time distributions. Time constants (τ) are given in ms as mean ± std (see Table1).

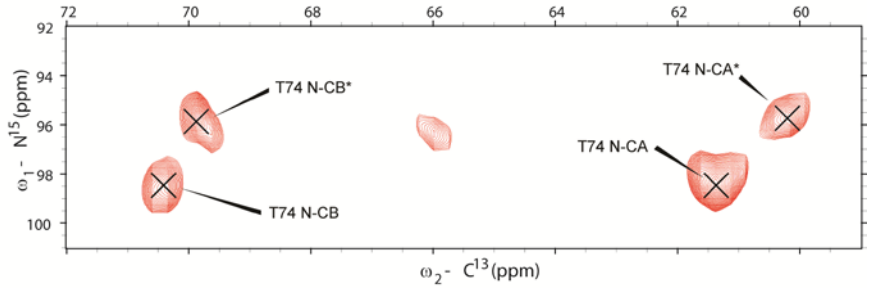

Figure 2. NCACX spectra of the key residues of KcsA selectivity filter recorded in CL proteoliposomes. Experiments were conducted in the presence of 150 mM KCl at pH 4.0 at 500MHz ^{1}H-frequency. Effective sample temperature -273 K. KcsA was reconstituted into proteoliposomes composed of DOPC and CL at 3/7 molar ratio. Correlations denoted with "*" relate to chemical shifts corresponding to the conductive conformation of the selectivity filter.

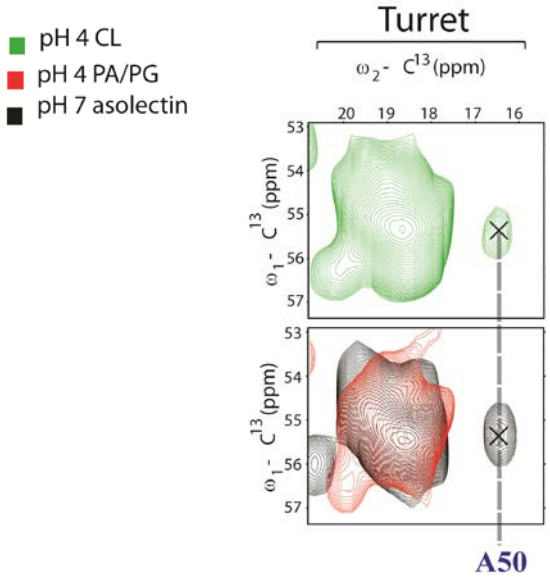

Figure 3. ^{13}C-^{13}C intra-residue correlation spectra reveal an extended TM1 α-helix in CL-rich liposomes. Cutouts of ^{13}C-^{13}C intra-residue correlation spectra reveal in both asolectin (pH 7) and CL-rich (pH 4) bilayers a characteristic correlation for Ala50 that is diagnostic for an extended TM1 α-helix.

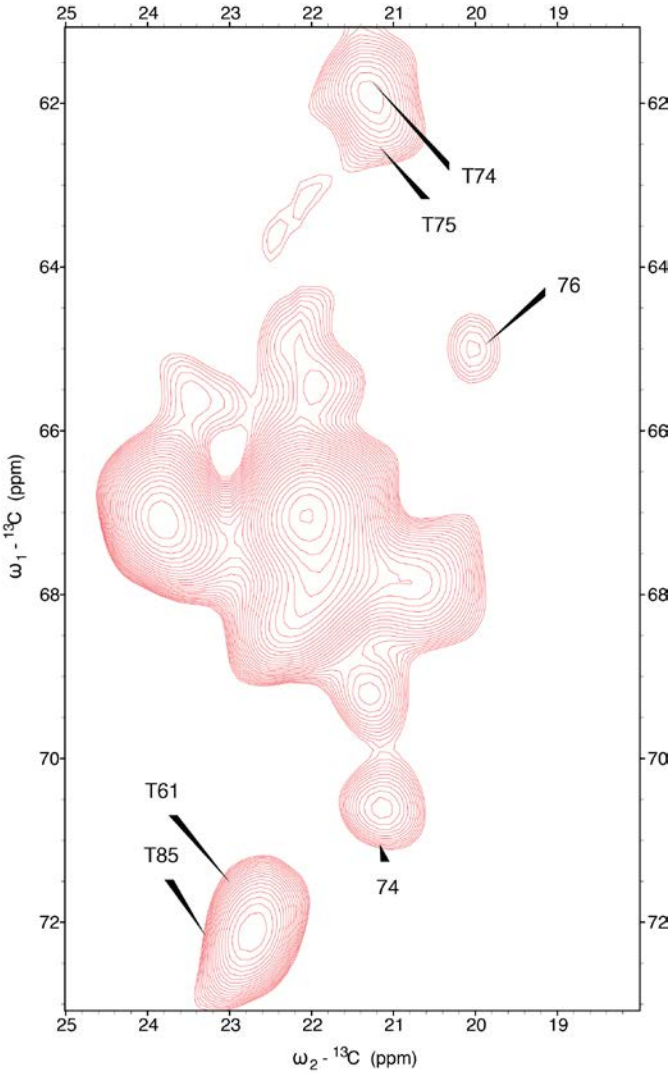

Figure 4. Identification of a Val76 correlation in the closed-collapsed state. Cut-out of a $^{13}C,^{13}C$ correlation experiment using a 30 ms mixing time and 12 kHz MAS (273 K) at a 500 MHz ^{1}H wide-bore NMR spectrometer (Bruker Biospin).

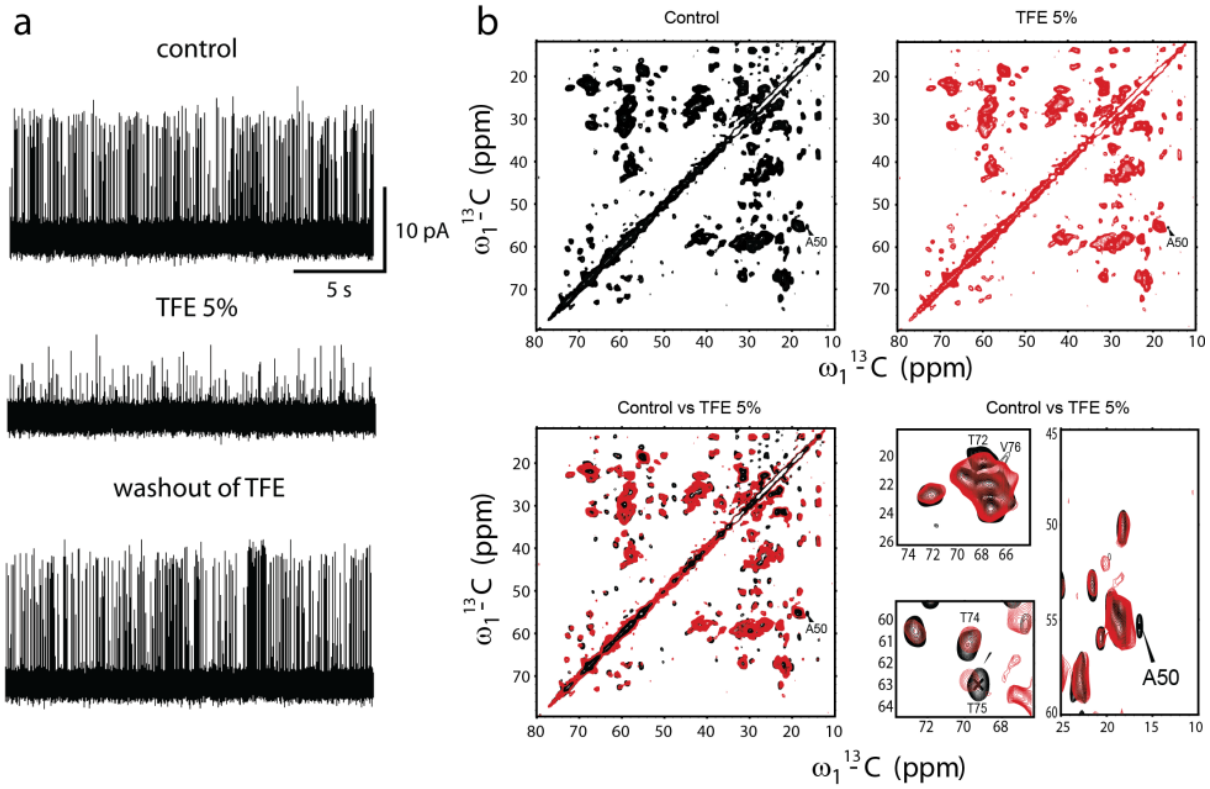

Figure 5. Influence of TFE on KcsA function and structure. (a) - Influence of TFE on KcsA single channel properties. Experiments were performed at +100 mV in symmetrical 150 mM KCl solution at pH = 7.0 on the cis side and pH 4.0 on the trans side. Lipid bilayers were composed of DOPC and DOPG at 7/3 molar ratio. TFE was applied from cis side. (b) ^{13}C-^{13}C ssNMR correlation spectra using (top, left) a control sample of ^{13}C,^{15}N labeled KcsA at 50 mM K^+ in asolectin bilayers at pH 7. (top, right) same experiment after addition of 5% TFE 5% using 150 mM K^+ and lipid bilayers with DOPC/DOPG=3/7 (molar) and pH 7. Overlays and zoom-ins are presented at the bottom. 2D PDSD data sets were recorded using a 30 ms mixing time and 12 kHz MAS (273 K) at a 500 MHz ^1H wide-bore NMR spectrometer (Bruker Biospin).

SUPPLEMENT INFORMATION

Table 1

		PG		PS		PA		CL	
		τ, ms	a*i*	τ, ms	a*i*	τ, ms	a*i*	τ, ms	a*i*
open times	τ_1	0.88 ± 0.04	0.94 ± 0.02	1.36 ± 0.09	0.80 ± 0.02	2.14 ± 0.03	0.69 ± 0.07	2.86 ± 0.06	0.38 ± 0.07
	τ_2	3.60 ± 0.52	0.06 ± 0.02	6.22 ± 0.57	0.20 ± 0.02	8.20 ± 0.32	0.30 ± 0.06	8.43 ± 1.06	0.35 ± 0.03
	τ_3	n.d.	< 0.001	n.d.	< 0.001	45.61 ± 2.89	0.02 ± 0.01	29.01 ± 1.39	0.28 ± 0.04
	τ_{mean}	1.05 ± 0.06		2.33 ± 0.13		4.79 ± 0.07		11.73 ± 0.51	
closed times (inactivated)	τ_1	1.34 ± 0.11	0.16 ± 0.01	3.96 ± 0.43	0.13 ± 0.04	1.55 ± 0.01	0.80 ± 0.01	1.33 ± 0.02	0.75 ± 0.02
	τ_2	122.11 ± 3.16	0.83 ± 0.01	119.14 ± 5.47	0.76 ± 0.05	18.08 ± 0.27	0.20 ± 0.01	29.16 ± 0.60	0.25 ± 0.02
	τ_3	1064.22 ± 599.97	0.01 ± 0.002	655.02 ± 74.46	0.11 ± 0.05	3146.19 ± 210.33	0.01 ± 0.001	1399.04 ± 122.75	0.01 ± 0.001
	τ_{mean}	108.87 ± 5.38		163.11 ± 9.19		19.88 ± 1.08		16.24 ± 0.76	

Table 1. Dwell times for open and closed (inactivated) states of KcsA recorded in different anionic phospholipid environments. Mean open and closed (inactivated) times were calculated from a three exponential fit to log dwell time distributions. Open and closed times (τ) are given in ms as mean ± std. Area values (a) are given as mean ± std. These values were calculated from 6 to 10 experiments. *n.d.* - not detectable.

Residue	CS Ca (50 mM K+)	Δ CS 0 mM K+	Δ CS DOPA pH 4 50 mM K+	Δ CS DOPG	Δ CS CL (1) At pH4 and (pH 3.5)	Δ CS CL (2) At pH4 and (pH 3.5)
50	55.4	-0.1	-	-	-	
53	45.1	0.4	0	0	0	
54	49.8	0.4	0.1	0.4	0.3	
56	45.1	0.4	0	0	0	
57	52.8	0.6	0.5	0.5	0.4	
60	61.7	-0.3	-0.3	-0.3	-0.2	
61	58.9	-0.2	-0.4	-0.3	-0.2	
65	56.0	-0.4	0.9	0.3	0.3	
74	61.2	0.7	0.4	0.4	0.4 / -0.2	0.7 / -0.2
75	63.1	-0.8	-0.7	-0.7	-0.8 / 0	0.4 / 1.0
76	66.1	-1.1	-1.1	-1.1	-1.2 / -0.2	-1.0
77	48.7	-1.2	-1.7	-1.7	-1.7 / 0	-1.8 / -0.5
79	45.5	2.0	1.4	1.4	1.4 / -0.1	1.2 / 0.5
80	55.4	-0.5	-0.8	-0.8	-0.2	-1.0
83	61.3	0	-0.2	-0.2	-0.2	0
84	60.4	0.1	0.3	0.2	0.3	0.6

Table 2. Summary of Ca chemical shifts and their changes

Chemical shift (CS) of Ca of KcsA in the closed conductive state. The following columns are the delta chemical shifts with respect to the closed conductive state. In case of CL lipids where peak doubling occurs numbers values for conformation 1 and 2 are separated by a "/".

State	PDB ID	Mutation	RMSD [Angstrom]
C	1K4C	P2A, L90C	2.0
O	3FB8	H25Q, L90C, R117Q, E120E, R121Q, R122Q, H124Q	2.8
I	3F5W	H25Q, L90C, R117Q, E120E, R121Q, R122Q, H124Q	3.3
I*	1K4D	P2A, L90C	2.3

Table 3. Summary of X-ray structures used in Figure 6
Channel states, PDB IDs, RMSD and reported mutations compared to wild-type KcsA are given.