Supporting Information

Probing Allosteric Coupling of a Constitutively Open Mutant of the Ion Channel KcsA using Solid State NMR

Zhiyu Sun, Yunyao Xu, Dongyu Zhang, Ann E McDermott

Department of Chemistry, Columbia University, New York, NY, 10027

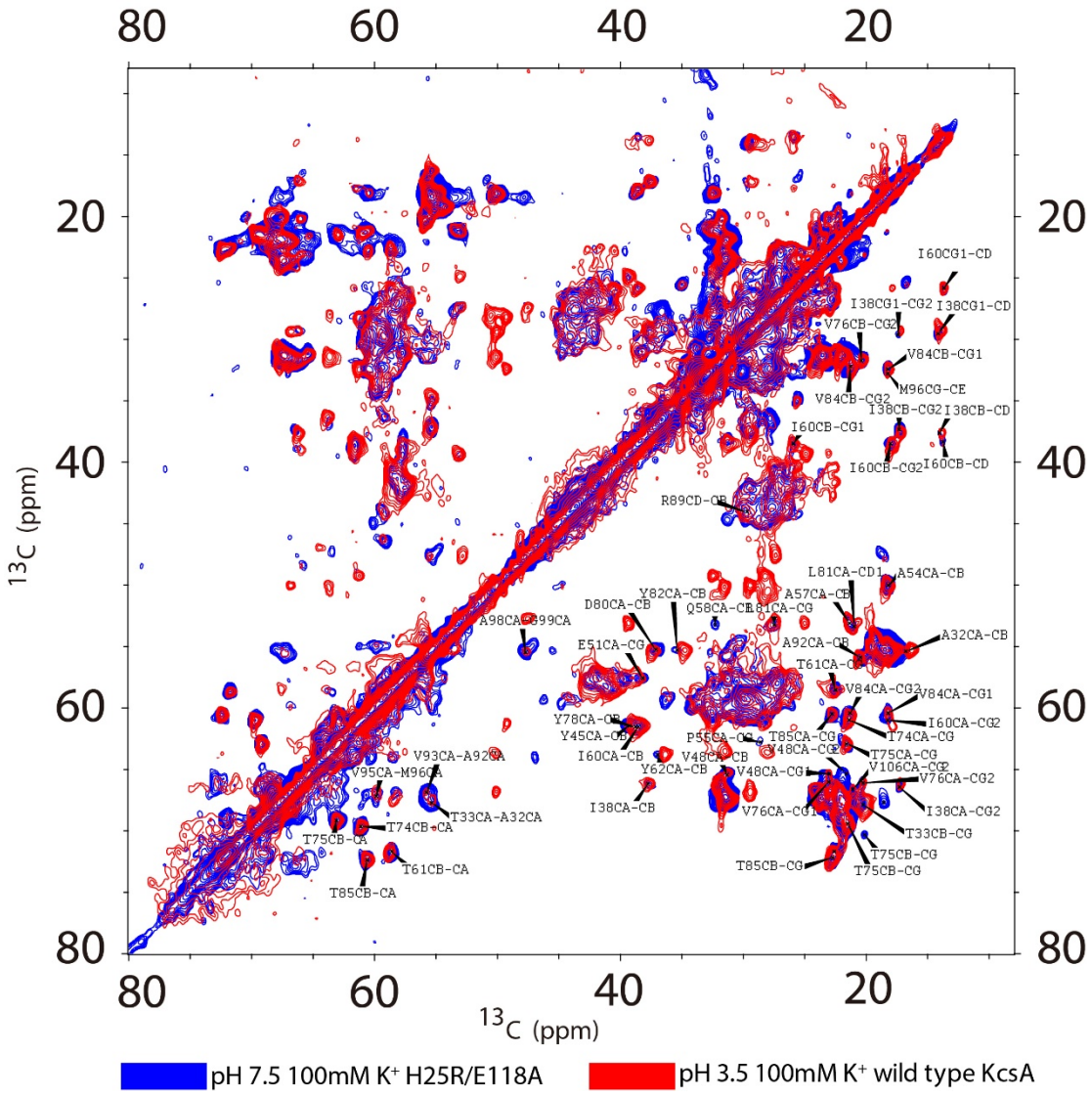

Fig. S1. 2D homonuclear ^{13}C spectra of two samples that represent the open state of KcsA: H25R/E118A at pH 7.5 and 100mM K^+ (blue) and wild type KcsA at pH 3.5 and 100 mM K^+(red). The proline peaks are present in the wild type KcsA but not in the H25R/E118A mutant, because wild type KcsA is expressed using the Bl21(de3) cell line, however, the H25R/E118A mutant is expressed in JM83, a proline-auxotrophic cell line. The spectra of these two models for the open state are largely identical.

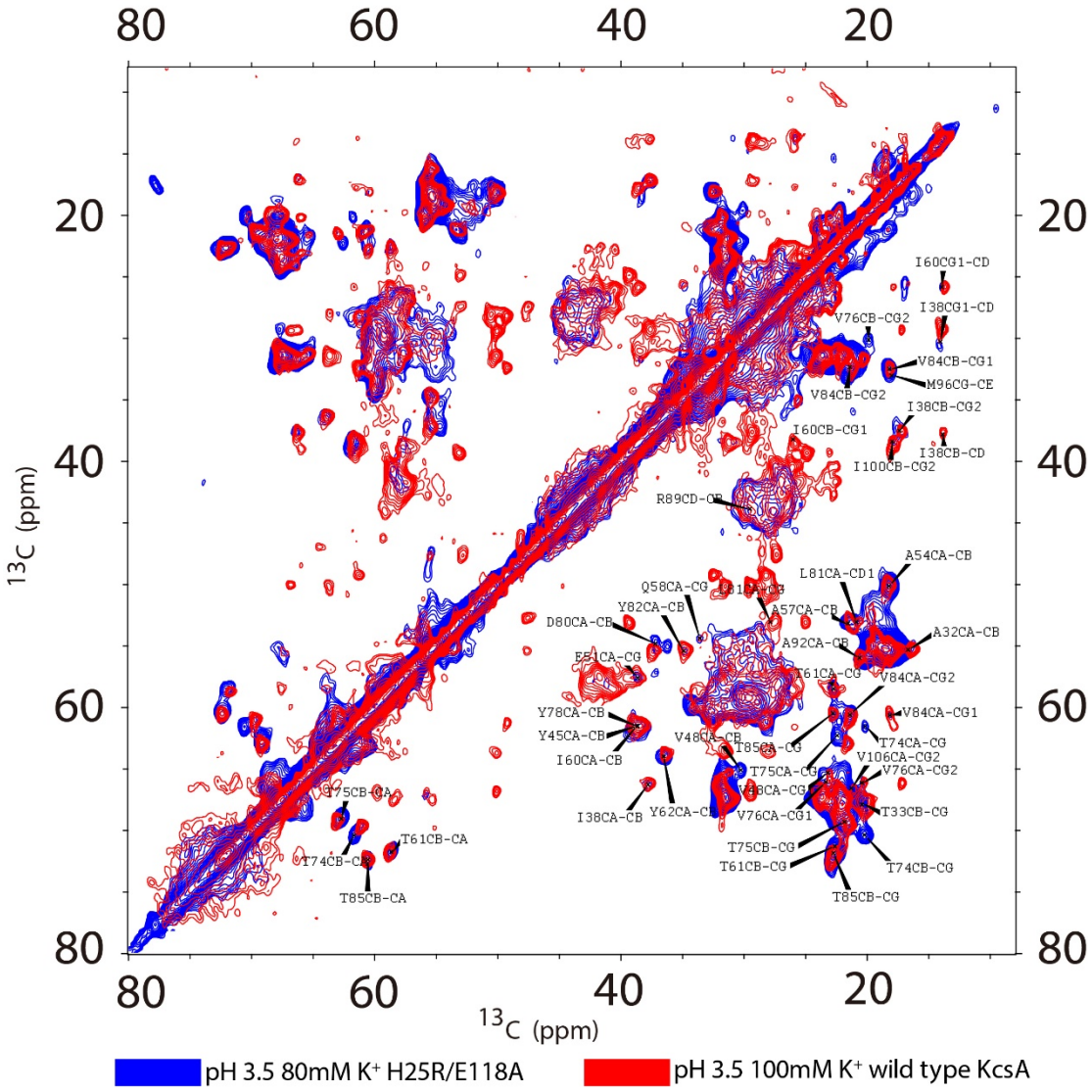

Fig. S2. 2D homonuclear ^{13}C spectra of H25R/E118A at pH 3.5 and 80mM K^+ and of wild type KcsA at pH 3.5 and 100 mM K^+ are overlaid. The majority of the peaks of the mutant at low pH are identical to those of the wild type at the same pH (with the notable exception of the marker peaks in the selectivity filter since the mutant in this case is a mixed state). This indicates that the mutant is robust with respect to pH as is the wild type. Also, the data suggests that the mutant has a somewhat weaker potassium binding affinity at pH 3.5 than at 7.5 (at this [K^+] concentration the mutant is 50% bound at pH 7.5, but the apo state is more favorable at pH 3.5).

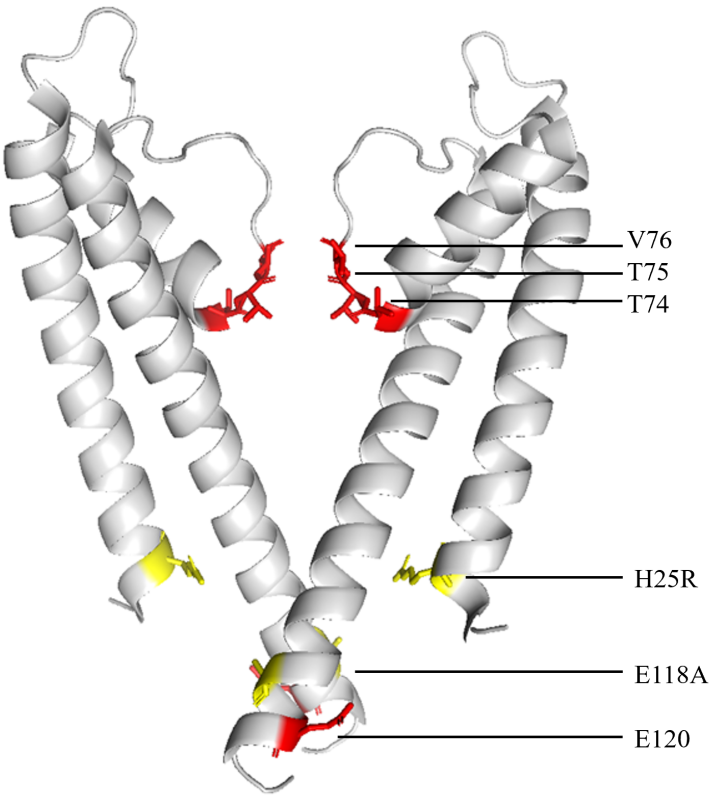

Fig. S3. Residues participating in the allosteric coupling are shown on a KcsA construct (PDB: 1k4C). Mutant residues, H25R and E118A, are colored yellow**.** Chemical shift markers used in this study are color red, T74, T75, V76, E120.

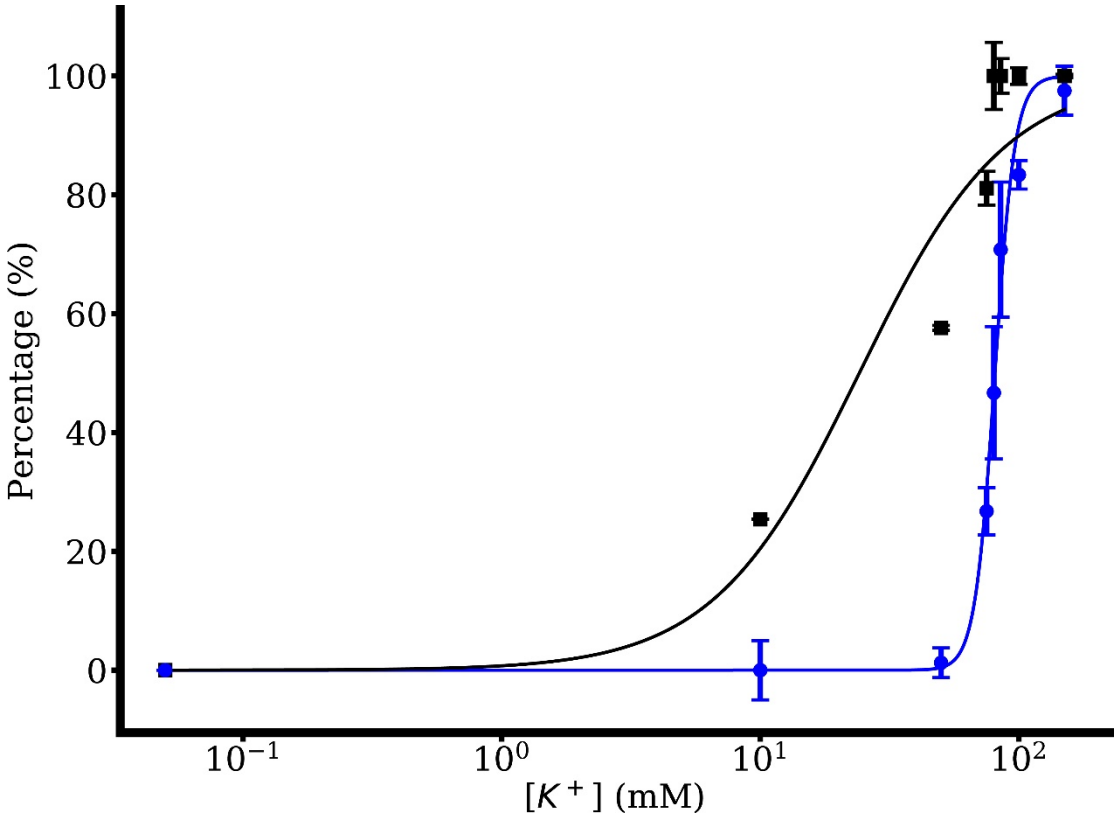

Fig. S4. The protonation state of E120 is monitored (percentage deprotonated state, black squares) in the same titration experiment where we probed binding at the selectivity filter (the percentage of the selectivity filter in the bound state, blue dots), both as a function of [K^+]. The populations were assessed based on peak intensities as described in the materials and methods. The black curve is the fit of the E120 data to the Hill equation with K_{app} =24 ± 7 mM (R-Square = 0.91) and a Hill coefficient of n = 1.5. The titration behavior indicates that there is allosteric coupling between the intracellular and extracellular gates even in this constitutively open mutant. Compared to the selectivity filter, the pH sensor titrates at a slightly lower concentration, suggesting the presence of a distinct potassium binding sites.

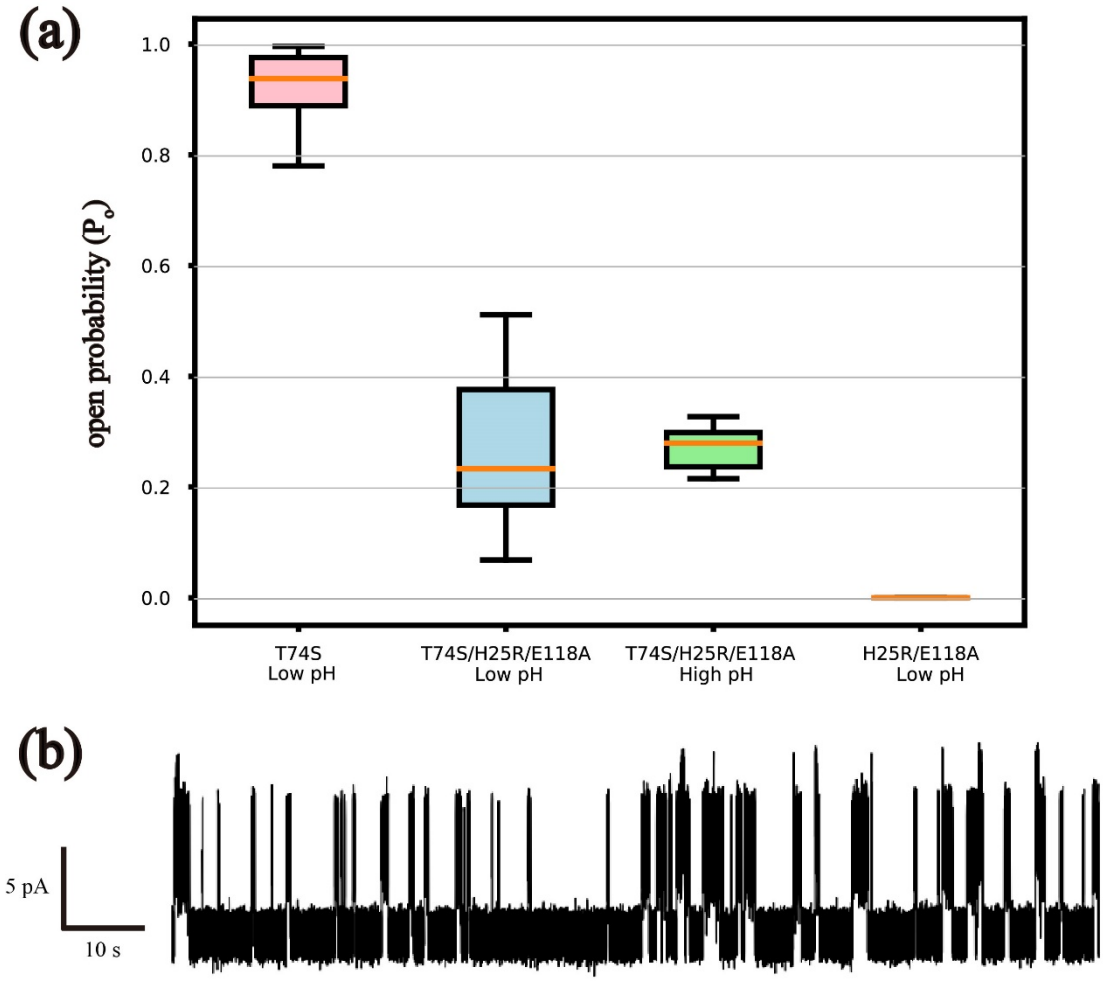

Fig. S5. Electrophysiology of the open pH gate mutant and T74S triple mutant. (a). box plot of channel open probability. Open probability of T74S low pH, H25RE118A_T74S low pH, H25RE118A_T74S high pH and H25RE118A low pH are 90 ± 10%, 30 ± 20%, 27 ± 4% and 0 ± 0%. The upper chamber buffer pH of T74S and H25R/E118A is 7 and the lower chamber buffer pH of the two samples is 4. The pH of both T74S/H25R/E118A buffer in the upper chamber are 7, but the pH of bath buffer in the chamber are 4 and 7 corresponding to low and high pH. (b). A trace of single channel T74S/H25R/E118A at +100 mV. The channel is 30% open compared to the 0% Po of inactivated H25R/E118A, which suggests that the allosteric coupling between the dual gates are attenuated by the T74S mutation.

model	Hill function			
equation	y= $Vmax\frac{x^n}{x^n+{k_d}^n}$ (Vmax set to 1)			
	k value (mM)	k std (mM)	Hill coefficient	adj r-square
WT pH7.5	0.0046	0.0009	1	0.967
WT pH3.5	15	1	1	0.991
H25RE118A pH7.5	100	60	1	0.533
H25RE118A pH7.5	81	1	11	0.986

Table. S1. Parameters involved in optimized simulations to the Hill equation.

Chemical shift markers of H25R/E118A at high [K+] and neutral pH							
	CA	CB	CG	CG1	CG2	CD	CO
T74	61.05	69.65	21.41	-	-	-	176.3
T75	63.03	69.63	21.08	-	-	-	172.4
V76	66.06	31.73	-	23.04	20.31	-	-
E120	59.40	36.31	34.11	-	-	177.30	-

Table. S2. Chemical shift markers of H25R/E118A at high [K+] and neutral pH

Chemical shift markers of H25R/E118A at low [K+] and neutral pH							
	CA	CB	CG	CG1	CG2	CD	CO
T74	61.18	69.94	20.92	-	-	-	177.2
T75	62.89	69.54	21.27	-	-	-	172.4
V76	66.06	31.33	-	23.11	20.27	-	-
E120	59.40	36.31	36.15	-	-	183.10	-

Table. S3. Chemical shift markers of H25R/E118A at low [K+] and neutral pH

	CA	CB	CG/CG1	CG2	CD/CD1	CE
A32	55.37	16.76	-	-	-	-
T33	67.72	67.87	20.24	-	-	-
I38	66.31	37.58	29.57	17.34	14.10	-
Y45	61.45	39.24	-	-	-	-
V48	65.38	31.31	23.01	21.62	-	-
E51	57.56	-	38.20	-	-	-
A54	50.02	18.23	-	-	-	-
P55	62.77	-	28.57	-	-	-
A57	53.09	21.36	-	-	-	-
Q58	53.21	32.29	-	-	-	-

I60	61.26	38.46	25.88	18.13	13.70	-
T61	58.57	71.68	22.52	-	-	-
Y62	63.81	36.97	-	-	-	-
T74	61.05	69.65	21.41	-	-	-
T75	63.03	69.63	21.08	-	-	-
V76	66.06	31.73	23.04	20.31	-	-
Y78	61.51	38.78	-	-	-	-
D80	55.25	37.13	-	-	-	-
L81	53.28	-	27.50	-	20.98	-
Y82	55.28	35.39	-	-	-	-
V84	60.42	32.31	18.22	21.31	-	-
T85	60.56	72.30	22.76	-	-	-
A92	55.81	20.46	-	-	-	-
M96	59.85	-	32.79	-	-	18.25

Table. S4. Assignment table of H25R/E118A at high [K+] and neutral pH

	CA	CB	CG/CG1	CG2	CD/CD1	CE
A32	55.36	16.74	-	-	-	-
T33	67.72	67.87	20.29	-	-	-
I38	66.31	37.61	29.67	17.34	14.07	-
Y45	61.47	39.10	-	-	-	-
V48	65.35	31.04	23.05	21.62	-	-
E51	57.57	-	38.31	-	-	-
A54	50.04	18.22	-	-	-	-
P55	62.77	-	28.57	-	-	-
A57	53.11	21.41	-	-	-	-
Q58	53.51	32.29	33.59	-	-	-
I60	61.29	38.46	25.89	18.13	13.73	-
T61	58.57	71.61	22.58	-	-	-
Y62	63.85	36.86	-	-	-	-
T74	61.18	69.94	20.92	-	-	-
T75	62.89	69.54	21.27	-	-	-
V76	66.06	31.33	23.11	20.27	-	-
Y78	61.51	38.75	-	-	-	-
D80	55.13	37.15	-	-	-	-
L81	53.22	-	27.58	-	20.95	-
Y82	55.32	35.27	-	-	-	-
V84	60.46	32.33	18.21	21.33	-	-
T85	60.54	72.25	22.76	-	-	-
A92	55.83	20.50	-	-	-	-
M96	59.85	-	32.85	-	-	18.19

Table. S5. Assignment table of H25R/E118A at low [K+] and neutral pH

	CA	CB	CG/CG1	CG2	CD/CD1	CE
A32	55.36	16.74	-	-	-	-
T33	67.72	67.87	20.29	-	-	-
I38	66.31	37.61	29.67	17.34	14.07	-
Y45	61.47	39.10	-	-	-	-
V48	65.35	31.04	23.05	21.62	-	-
E51	57.58	-	38.30	-	-	-
A54	50.04	18.22	-	-	-	-
P55	62.77	-	28.57	-	-	-
A57	53.11	21.41	-	-	-	-
Q58	53.51	32.29	33.59	-	-	-
I60	61.29	38.46	25.89	18.13	13.73	-
T61	58.57	71.61	22.58	-	-	-
Y62	63.85	36.86	-	-	-	-
T74	61.18	69.94	20.92	-	-	-
T75	62.89	69.54	21.27	-	-	-
V76	66.06	31.33	23.11	20.27	-	-
Y78	61.51	38.75	-	-	-	-
D80	55.13	37.15	-	-	-	-
L81	53.22	-	27.58	-	20.95	-
Y82	55.32	35.27	-	-	-	-
V84	60.46	32.33	18.21	21.33	-	-
T85	60.54	72.25	22.76	-	-	-
A92	55.83	20.50	-	-	-	-
M96	59.85	-	32.85	-	-	18.19

Table. S6. Assignment table of wild type KcsA at high [K+] and low pH