

Supplementary Material

Local and global structure of the monomeric subunit of the potassium channel KcsA probed by NMR

Jordan H. Chill, John M. Louis, Frank Delaglio and Ad Bax

Table S1. Acquisition parameters for KcsA^M measurements.^a**Backbone assignment:**

Exp	¹ H		¹⁵ N		¹³ CO/ ¹³ C α		Other		No. scans	Exp time
	Pts ^b	SW (Hz)	Pts	SW (Hz)	Pts	SW (Hz)	Pts	SW (Hz)		
tHNCO	512	8090.6	32	1459.8	40	1509.2	---	---	4	16
tHN(CA)CO	512	8090.6	32	1581.3	44	1509.2	---	---	8	22
tHNCA (ct)	512	8090.6	36	1581.3	36	1509.2	---	---	8	64
NOE- ¹⁵ N-HMQC ^c	512	11160.7	56	1864.7	---	---	116	7201.2	4	40
3D- ¹⁵ N-HMQC- NOE- ¹⁵ N-HMQC ^d	512	11160.7	50	1864.7	---	---	72	1864.7	4	26

¹⁵N backbone relaxation:

Exp	¹ H		¹⁵ N		¹³ CO		recycle delay (sec)	relax delay ^e (sec)	No. scans	Exp time ^f (hrs)
	Pts ^b	SW (Hz)	Pts	SW (Hz)	Pts	SW (Hz)				
R ₁	512	8090.6	32	1459.8	32	1509.2	2.7	0.64	4	22
R ₂ ^g	512	8090.6	32	1459.8	32	1509.2	2.2	0.080	4	23
NOE	512	8090.6	36	1581.3	40	1509.2	3.0	---	4	42
η_{xy}	512	8389.3	46	1459.8	42	---	2.0	0.0162	4	40

Residual dipolar couplings:^h

Exp	¹ H		¹⁵ N		¹³ CO		No. scans	Exp time (hrs)
	Pts ^b	SW (Hz)	Pts ^b	SW (Hz)	Pts ^b	SW (Hz)		
D _{NH} ⁱ	512	8090.6	40	1581.3	34	1509.2	8	45
D _{NC} ^j	512	8090.6	36	1459.8	36	1509.2	8	44
D _{CC} ^k	512	8090.6	40	1581.3	64	1509.2	8	40

^aAll measurements were conducted at a field of 14.1 T (with the exception of the two NOE-HMQC experiments conducted at 18.8 T) and at 323 °K. Typical sample concentrations were 0.6-0.8 mM of KcsA^M in 200-250 mM SDS and an appropriate buffer.

^bPts represent complex points acquired in each dimension.

^cThe third dimension is the ¹H (NOE-originator) acquisition parameters.

^dThe third dimension is the ¹⁵N (first HMQC) acquisition parameters.

^eThe relax-delay parameter represents the value of Δ in the experiments of ref. [26], e.g. the full delay in R_1 and R_2 measurements and the half-delay in the η_{xy} measurement.

^fExperiment times represent the total time necessary for acquiring back-to-back reference and attenuated experiments.

^g R_2 was measured as an $R_{1\rho}$ measurement against a spin-lock field of 1.8 kHz with an appropriate correction for offset effects

^hResidual dipolar couplings were measured in anisotropic environment by soaking the KcsA^M sample in the desired buffer into a polyacrylamide gel polymerized from a solution containing 4.9% (w/v) acrylamide (AA), 0.1% (w/v) 2-(acrylamido)2-methyl-1-propanesulfonic acid (AMPS), 0.17% (w/v) bis(acrylamide) (BIS), 0.1% (w/v) ammonium persulfate, 0.1% (v/v) tetramethylethylenediamine (TEMED).

ⁱ D_{NH} couplings were extracted from an tr-HNCO-based experiment as described in ref. [35].

ⁱ D_{NC} couplings were extracted from an tr-HNCO-based experiment as described in ref. [36].

ⁱ D_{CC} couplings were extracted from an tr-HNCO-based experiment with the $^1J_{C^{\alpha}C^{\beta}}$ coupling allowed to evolve during the ¹³CO evolution as described in ref. [37].

Table S2. Chemical shift values for KcsA^M in 25 mM sodium formate pH 4.2, 7% D₂O, as measured at B₀=14.1 T, Temp = 323 K

Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca	Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca
L 16	8.24	122.7	177.1	56.4	T 61	7.5	114.9	174.9	62.6
L 17	7.71	117.7	177.6	54.9	Y 62	7.78	121.4		58.8
G 18	7.95	107.9	174.9	45.3	P 63			177.2	64.9
R 19	7.82	119.6	176.7	56.3	R 64	7.61	117.5	177	58.6
H 20	8.22	117.5	174.9	55.2	A 65	7.69	121.2	180.2	54.1
G 21	8.06	109.3	174.3	45.4	L 66	7.86	119.8		57.2
S 22	7.92	115.7	174.3	58.2	W 67			178.4	
A 23	7.99	125.1	177.2	52.2	W 68	7.92	120.1	179	59.2
L 24	7.65	119.7	177.3	55	S 69	7.97	116.7	175.9	61.7
H 25	8.04	119	175.1	54.8	V 70	7.83	122	177.9	65.2
W 26	7.63	120.7	177.5	59.1	E 71	8.12	119.9	178.1	57.9
R 27	7.75	120.4	177.3	59	T 72	7.73	113.7	175.5	64
A 28	7.57	121.3	178.4	54.2	A 73	7.77	124.6	178.5	53.8
A 29	7.87	118.8	180.1	54.3	T 74	7.71	109.4	175.3	62.8
G 30	8.49	107.7	174.4	47	T 75	7.69	115	175.2	62.8
A 31	8.16	123.9	178.5	55	V 76	7.69	120.8	176.4	62.8
A 32	8.19	118	178.3	54.9	G 77	7.84	110.1	174	45
T 33	7.67	113.8	175.8	67.5	Y 78	7.76	119.9	176.5	58.2
V 34	7.74	120.2	177.2	66.8	G 79	8.09	109.4	174.3	45.7
L 35	7.86	118.7	177.9	57.5	D 80	7.87	118.3	174.8	53.1
L 36	8.03	118.4	178.2	57.4	L 81	7.76	120.9		55
V 37	7.99	118.2	177.5	67	Y 82				
I 38	8.07	119.4	177.6	65.8	P 83			176.8	63.4
V 39	8.25	119.5	177.9	66.8	V 84	7.75	118.3	177	63.4
L 40	8.32	119.9	180.2	57.8	T 85	7.65	114.4	175.7	62.9
L 41	8.56	121.3	178.5	57.9	L 86	7.88	123.2	177.4	57.3
A 42	8.75	121.5	179.7	55	W 87	7.7	118.3	177.6	59.3
G 43	8.84	105	175.1	47	G 88	7.94	106	176	46.8
S 44	8.07	117.9	175.6	61	R 89	7.69	120.9		58.3
Y 45	8.08	122.6	177.3	61	L 90			178.5	
L 46	8.18	118.3	178.7	57.5	V 91	8.04	118.1	177.1	66.4
A 47	8.15	121.1	179.5	54.9	A 92	7.56	119.9	179.9	54.9
V 48	7.84	118.3	178.5	66	V 93	7.73	117.3	177.4	66.2
L 49	7.97	120.7	179.7	57.5	V 94	7.97	120.2	177.6	66.9
A 50	8.18	121.2	179.9	54.1	V 95	8.24	118.4	178.4	66.2
E 51	7.98	117	177.7	57.1	M 96	7.86	119.7	178.8	58.6
R 52	7.98	118	177.2	56.5	V 97	8.37	118.9	178.2	65.8
G 53	7.83	108.6	173.5	45	A 98	8.51	123.2		54.1
A 54	7.83	124.2		50.2	G 99				
P 55			177.8	63.5	I 100			177	
G 56	8.17	108.8	174.3	45.5	T 101	7.87	114.6	175.4	63.6
A 57	7.76	122.8	177.9	52.8	S 102	7.93	116.7	175	59.5
Q 58	8.01	117.7	176.6	56.2	F 103	8.2	121.9	177.8	60
L 59	7.93	121.9	177.3	56.5	G 104	8.39	109.2	175.2	46.6
I 60	7.49	115.7	175.4	61.2	L 105	7.86	121.7	178.9	57.1

Table S2 (cont.)

Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	$^{13}\text{C}\alpha$	Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	$^{13}\text{C}\alpha$
V 106	7.83	118.4	177.6	66	A 133	7.92	120.6	178.7	53.9
T 107	7.93	113.6	176.5	66.1	E 134	7.94	117.2	177.6	57.8
A 108	7.82	123.4	180.3	54.8	E 135	7.85	119.4	177.4	57.1
A 109	7.93	122.3	179.9	55.1	A 136	8.09	122.8	179	54
L 110	8.23	118.8	178.8	57.7	Y 137	8.15	119.3	177.1	59.8
A 111	8.39	121	179.5	55.2	T 138	7.98	114.9	175.6	63.9
T 112	7.93	113.4	176.8	66.1	R 139	7.93	121.8	177.6	57.2
W 113	8.16	123.9	178	60.9	T 140	7.86	114.7	175.5	64.1
F 114	8.6	118.7	177.7	61.1	T 141	7.9	114.6	176.1	63.4
V 115	8.19	118.2	178.2	65.1	R 142	7.82	122.6	177.2	57.2
G 116	7.77	108.5	175.6	46	A 143	7.85	123.2	179	53.2
R 117	7.47	120.7		56.5	L 144	7.92	119	178.2	56.8
E 118					H 145	8.11	116.8	175.9	57.2
Q 119			176.3		E 146	8	118.7	177.9	57.3
E 120	7.81	120.3	176.6	57	R 147	7.79	119	177.3	57.5
R 121	7.92	120.3	176.9	57	F 148	8.01	118.5	176.7	59.2
R 122	7.89	120.4	176.9	56.5	D 149	8.09	118.5	177.5	55.7
G 123	7.99	108.7	174.1	45.2	R 150	7.87	119.8	178.2	58.6
H 124	7.95	117.3	174.5	55.5	L 151	7.66	120.2	178.3	57.2
F 125	8.06	120.5	176.2	58.8	E 152	8.08	117.7	177.8	58.7
V 126	7.73	120.1	176.3	63	R 153	7.62	118.3	178.1	58.1
R 127	7.85	121.5	177	56.8	M 154	7.69	118.8	177.5	57.8
H 128	8.11	117.7	175.1	55.9	L 155	7.8	119.3	178.1	56.2
S 129	8.01	115.9	175	59.1	D 156	8.03	117.4	176.3	54.1
E 130	8.13	122		57.2	D 157	8.02	118.1	177.1	53.9
K 131			177.8	57.6	N 158	7.92	118.4	174.8	53.8
A 132	7.91	122.7	179	53.9	R 159	7.77	119.7		56.2
					R 160				

Table S3. Chemical shift values for KcsA^M in 25 mM MES pH 6.0, 7% D₂O, as measured at B₀=14.1 T, Temp = 323 K

Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca	Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca
L 16	8.22	122.8	177.1	56.6	T 61	7.49	114.7	174.9	62.7
L 17	7.7	117.9	177.6	54.9	Y 62	7.74	121.6	174.6	58.8
G 18	7.95	108	174.9	45.4	P 63			177.1	64.9
R 19	7.8	119.6	176.7	56.5	R 64	7.61	117.6	176.9	58.6
H 20	8.21	117.5	174.9	55.3	A 65	7.66	121.2	180	54.1
G 21	8.05	109.4	174.3	45.5	L 66	7.81	119.7	178.4	57.2
S 22	7.91	115.7	174.2	58.4	W 67	7.84	120.1	178.3	59.4
A 23	7.95	125	177.2	52.3	W 68			178.7	59.2
L 24	7.66	120	177.4	55.1	S 69	7.91	116.6	175.8	61.4
H 25	8.04	119.1	175.2	54.7	V 70	7.8	122	177.8	65.1
W 26	7.62	120.7	175.9	59.3	E 71	8.1	120.3	178	58
R 27	7.74	120.2	177.4	59.1	T 72	7.7	113.4	175.4	64
A 28	7.55	121.1	178.5	54.4	A 73	7.76	124.7	178.4	53.7
A 29	7.88	118.7	180.3	54.5	T 74	7.71	109.8	175.3	62.6
G 30	8.54	107.8	174.4	47.1	T 75	7.71	115	175.1	62.6
A 31	8.15	124	178.5	55.1	V 76	7.68	120.8	176.4	62.6
A 32	8.18	117.9	178.3	55	G 77	7.89	110.4	174.1	45
T 33	7.68	113.8	177.6	67.8	Y 78	7.75	119.9	176.5	58.3
V 34	7.73	120.2	177.2	66.9	G 79	8.08	109.5	174.2	45.6
L 35	7.85	118.7	177.8	58	D 80	7.88	119.4	175.5	53.8
L 36	8.05	118.5	178.5	57.8	L 81	7.71	120.8	176.2	55.1
V 37	8	118.3	177.3	67.3	Y 82	7.69	120.1	174.4	59.1
I 38	8.06	119.2	177.5	65.3	P 83			176.8	63.7
V 39	8.24	119.5	177.8	66.9	V 84	7.73	118	177.2	63.5
L 40	8.31	119.8	180.2	57.9	T 85	7.68	115.2	177.4	62.8
L 41	8.55	121.3	178.5	57.8	L 86	7.84	123.1	178.2	57.3
A 42	8.75	121.5	179.8	55	W 87	7.7	118.4	177.5	59.3
G 43	8.83	105	175.1	47.1	G 88	7.93	105.5	176.4	46.8
S 44	8.06	118	175.6	62.9	R 89	7.68	121.2	177.6	58.5
Y 45	8.07	122.6	177.3	61.1	L 90	7.84	119.9	178.5	57.8
L 46	8.17	118.4	178.7	57.6	V 91	8.01	118.1	177.1	66.6
A 47	8.14	121.1	179.6	54.9	A 92	7.45	119.8	180.2	55
V 48	7.84	118.3	178.5	66	V 93	7.68	117.5	177.4	66.1
L 49	7.96	120.7	179.7	57.5	V 94	7.93	120.4	177.6	67
A 50	8.15	121.3	179.9	54.2	V 95	8.19	118.1	178.3	66.3
E 51	7.96	117.2	177.7	57.2	M 96	7.66	120.1	175.4	55.7
R 52	7.98	118	177.2	56.6	V 97	8.22	119	175.7	62.1
G 53	7.82	108.7	173.5	45	A 98	8.28	122.1		52.8
A 54	7.82	124.2	175.6	50.4	G 99			173.9	
P 55			177.9	63.5	I 100	7.62	119.6	175.6	61
G 56	8.17	108.8	174.3	45.5	T 101	7.81	115.4	174.7	61.6
A 57	7.75	122.8	177.9	52.8	S 102	7.75	115.8	175	58.3
Q 58	8.01	117.6	176.7	56.2	F 103	8.25	122.6	177.7	60.1
L 59	7.92	121.8	177.4	56.6	G 104	8.39	108.8	175.4	46.6
I 60	7.47	115.6	175.5	61.2	L 105	7.78	121.7	179.1	57.4

Table S3 (cont.)

Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	$^{13}\text{C}\alpha$	Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	$^{13}\text{C}\alpha$
V 106	7.8	118.8	177.7	65.9	A 133	7.91	121.6	178.5	53.6
T 107	7.93	113	176.7	65.9	E 134	7.96	118	177.7	57.6
A 108	7.71	123.5	180.3	54.8	E 135	8.02	120.2	177.1	57.1
A 109	7.83	122.3	180.1	54.8	A 136	8.02	123.2	178.7	53.7
L 110	8.19	119	178.8	57.7	Y 137	8.07	119.5	176.9	59.5
A 111	8.31	121	179.6	55.4	T 138	7.94	115.7	175.4	63.5
T 112	7.89	113.3	176.7	66.1	R 139	8	122.2	177.5	57.5
W 113	8.11	123.9	178	60.7	T 140	7.9	115.2	175.5	64.1
F 114	8.52	118.5	177.8	61.1	T 141	7.92	114.8	175.9	63.6
V 115	8.08	117.9	178	64.9	R 142	7.87	123.1	177.4	57.4
G 116	7.72	108.8	175.3	45.8	A 143	7.83	123	179.2	53.5
R 117	7.35	120.6	177.2	56.5	L 144	7.94	119.1	178.3	57
E 118	7.93	119.9	177	56.9	H 145	8.1	116.6	176.2	57.8
Q 119	7.91	119	176.6	56.8	E 146	7.98	118.7	178.1	59.2
E 120	7.87	119.5	176.4	56.3	R 147	7.76	118.9	177.4	57.6
R 121	7.85	120.5	176.6	56.2	F 148	7.97	118.9	176.6	59.2
R 122	7.91	120.5	176.9	56.5	D 149	8.07	119.8	178.1	56.6
G 123	8	108.8	174.1	45.3	R 150	7.8	119.2	178.4	58.8
H 124	7.93	117.3	174.4	55.6	L 151	7.64	120.4	178.2	57.3
F 125	8.01	120.3	175.8	58.6	E 152	8.11	118.1	178.4	58.9
V 126	7.63	120	176	62.7	R 153	7.66	118.3	178.2	58.3
R 127	7.84	121.9	176.8	56.6	M 154	7.69	119	178.1	58
H 128	8.12	118.2	174.8	55.9	L 155	7.76	118.9	178.3	57.7
S 129	7.99	116	175	58.9	D 156	7.97	118.7	177.2	55
E 130	8.23	122.6	176.9	57.1	D 157	8.04	118.9	176.5	54.7
K 131	7.92	120.9	177.2	57.1	N 158	7.89	117.7	175	53.8
A 132	7.9	123.5	178.5	53.5	R 159	7.72	120	175.8	56.2
					R 160	7.64	125.8	180.5	56.9

Table S4. Chemical shift values for KcsA^M in 20 mM Tris pH 8.0, 7% D₂O, as measured at B₀=14.1 T, Temp = 323 K

Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca	Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca
L 16	8.28	122.2	177.1	56.3	T 61	7.54	114.7	174.4	62
L 17	7.73	118	177.6	54.9	Y 62	7.7	122.2	174.4	57.7
G 18	7.98	108	174.8	45.4	P 63			176.9	64.2
R 19	7.8	119.7		56.2	R 64	7.66	118.2	176.5	57.5
H 20					A 65	7.71	121.2	178.9	53.2
G 21					L 66	7.7	118.9	178	56.2
S 22			174.1	58.2	W 67	7.59	119.4	176.7	58.6
A 23	8	125	177.1	52.2	W 68	7.61	119.3	177.1	58
L 24	7.69	119.9	177.2	55.1	S 69	7.75	116.3	175	59.3
H 25	8.03	119.2	175.4	55.1	V 70	7.73	121.9	177	63.7
W 26	7.63	120.6	176.2	59.1	E 71	8.15	122.2	177.7	57.7
R 27	7.8	120.5	177.4	59.1	T 72	7.75	114.2	175	63
A 28	7.64	121	178.5	54.4	A 73	7.93	124.9	178.1	53.3
A 29	7.91	118.6	180.3	54.5	T 74	7.77	110.5	175.1	62.3
G 30	8.59	107.7	174.3	47	T 75	7.78	115.4	174.8	62.2
A 31	8.18	123.9	178.4	55	V 76	7.73	120.8	176.2	62.3
A 32	8.21	117.9	178.3	54.8	G 77	7.97	110.6	174	44.9
T 33	7.71	113.8	175.8	67.7	Y 78	7.78	119.9	176.4	58.1
V 34	7.78	120.2	177.1	66.7	G 79	8.11	109.5	174.1	45.5
L 35	7.89	118.8	178.2	57.9	D 80	7.92	120.1	175.8	54.3
L 36	8.09	118.7	178.2	57.9	L 81	7.72	120.8	177.6	54.9
V 37	8.03	118.2	177.5	67	Y 82	7.82	120.3	177.4	56
I 38	8.11	119.2	177.5	65.5	P 83			176.7	63.7
V 39	8.29	119.5	177.9	66.8	V 84	7.77	117.9	177.1	63.5
L 40	8.34	119.9	180.1	57.8	T 85	7.7	115.2	175.9	62.7
L 41	8.58	121	178.6	57.7	L 86	7.93	123.3	177.2	57.4
A 42	8.82	121.6	179.7	54.8	W 87	7.75	118.2	177.4	59.2
G 43	8.79	105	175.2	47	G 88	7.94	105.2	176.5	46.7
S 44	8.07	117.6	175.7	62.9	R 89	7.71	121.3	177.6	58.2
Y 45	8.13	122.5	177.3	60.9	L 90	7.87	119.9	178.5	57.7
L 46	8.21	118.7	178.5	57.3	V 91	8.03	118.1	177	66.4
A 47	8.05	120.7	179.7	54.5	A 92	7.44	119.7	180.4	54.7
V 48	7.7	117.4	178.2	64.9	V 93	7.71	117.6	177.3	65.8
L 49	7.88	120.9	178.9	56.8	V 94	7.94	120.4	177.6	66.8
A 50	7.97	121.2	179.1	53.7	V 95	8.2	117.9	178.6	66
E 51	7.81	117.8	177.4	57.2	M 96	7.75	120	178.9	58.4
R 52	7.92	118.3	177	56.2	V 97	8.29	119	177.8	65.3
G 53	7.88	108.4	173.2	44.8	A 98	8.35	122.4		53.9
A 54	7.84	124	175.5	50.3	G 99			175.1	45.6
P 55			177.6	63.4	I 100	7.72	119.7	176.8	62.2
G 56	8.21	108.5	174.3	45.4	T 101	7.86	115.1	174.9	62.6
A 57	7.76	122.7	177.9	52.8	S 102	7.8	116.2	174.9	58.4
Q 58	8.04	117.3	176.6	56.2	F 103	8.25	122.1	177.5	59.8
L 59	7.91	121.6	177.2	56.3	G 104	8.35	108.5	175.6	46.4
I 60	7.46	115.6	175.4	61	L 105	7.75	121.6	179.1	57.2

Table S4 (cont.)

Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	^{13}Ca	Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	^{13}Ca
V 106	7.77	118.8	177.6	65.7	A 133	7.99	122.6	178.2	53
T 107	7.95	112.4	176.7	65.7	E 134	8.09	118.5	177.4	57.4
A 108	7.66	123.5	180.2	54.5	E 135	8.06	120.4	177	56.9
A 109	7.79	122.2	180.1	54.6	A 136	7.94	123.3	178.3	53.1
L 110	8.16	118.8	178.6	57.5	Y 137	8.06	119.6	176.6	58.7
A 111	8.22	120.8	179.6	55.1	T 138	7.93	116.3	175	62.8
T 112	7.86	113.2	176.4	65.6	R 139	8.1	122.6	177.3	57.2
W 113	8.06	123.4	177.7	60.3	T 140	7.96	115.4	175.3	63.6
F 114	8.41	117.8	177.7	60.6	T 141	7.94	114.9	175.6	63.3
V 115	7.95	117.3	177.5	63.9	R 142	7.95	122.9	177.1	57.4
G 116	7.75	109.5	174.7	45.2	A 143	8	123.1	179.2	53.3
R 117	7.24	120.4	176.5	55.9	L 144	7.98	119.2	178.2	57.1
E 118	8.11	121.5	176.7	56.6	H 145	8.04	116.4	176.3	58
Q 119	7.99	120.1	176.2	56	E 146	7.89	118.6	178	58
E 120	8.17	121.1	176.4	56.4	R 147	7.69	118.4	177.1	57.1
R 121	7.99	121.1	176.6	55.9	F 148	7.89	119	176.4	58.1
R 122	7.96	120.9	176.8	56.4	D 149	8.08	121.1	178	56.7
G 123	8.04	108.6	174	45.2	R 150	7.86	118.4	178	58
H 124	7.9	117.7	174.6	55.9	L 151	7.6	120.1	177.9	56.8
F 125	8.01	119.7	175.5	58.3	E 152	8.06	119.2	178.1	58.7
V 126	7.59	119.4	175.9	62.2	R 153	7.73	118.2	177.8	57.7
R 127	7.85	122		56.1	M 154	7.77	119	177.6	57.4
H 128					L 155	7.8	119.7	177.9	56
S 129					D 156	7.87	120	177.3	55.4
E 130					D 157	8.11	120	176.8	55.2
K 131			176.6	56.2	N 158	8.01	117.6	175.1	53.6
A 132	7.99	124.5	177.9	52.5	R 159	7.77	120.2	175.6	56.2
					R 160	7.61	126.4		57.2

Table S5. Chemical shift values for KcsA^{TET} in 25 mM MES pH 6.0, as measured at B₀=14.1 T, Temp = 323 K

Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca	Residue	¹ H ^N	¹⁵ N	¹³ CO	¹³ Ca
L 16	8.22	122.6	177.4	56.5	T 61	7.15	109.9	173.9	58.2
L 17	7.68	117.6	177.8	54.9	Y 62	8.98	122.4		63.3
G 18	7.95	107.9	175.2	45.5	P 63			177.9	66.4
R 19	7.78	119.5	177	56.4	R 64	6.74	113.8	178.5	58.9
H 20	8.2	117.4	175.2	55.4	A 65	7.79	120.6	178.9	54.8
G 21	8.05	109.1	174.6	45.6	L 66	8.58	122.8	180	57.2
S 22	7.83	115.6	174.6	58.4	W 67	7.86	121.4	176.8	59.5
A 23	7.94	124.9	177.4	52.5	W 68	8.33	120.3	180.2	59.8
L 24	7.54	118.7	176.8	54.9	S 69	9.41	124.5	176.2	63.2
H 25	7.77	117.9	174.4	54.6	V 70	7.66	124.8	178.1	66
W 26	7.68	121.6	176.8	57.9	E 71	7.98	120.1	180.2	58.5
R 27	7.73	120.1	176.5	56.2	T 72	7.89	119.6	173.6	67.2
A 28	7.81	123.4	178.5	53.6	A 73	7.57	120.8	177.1	54.8
A 29	7.83	119.2	179	53.3	T 74	6.98	100.1	178	61.5
G 30	7.76	107.8	174.7	46.2	T 75	7.3	116	173.6	62.2
A 31	8.08	123.6	179.2	55	V 76	7.09	119.7	176.4	65.2
A 32	8.07	118.1	178.6	54.8	G 77	8.16	107.1	175.7	47.8
T 33	7.48	114.6	176.2	67.1	Y 78	6.09	117.4	178	56.9
V 34	7.73	121.1	177.1	67	G 79	8.96	102.2	174.4	47.1
L 35	7.75	119.3	176.9	58	D 80	9.61	115.6		54.4
L 36	7.85	117.6	178.2	57.9	L 81				
V 37	8.26	117.5	177.9	67.4	Y 82				
I 38	8.26	120	177.8	66	P 83			176.1	61.1
V 39	8.21	119.8	178.7	67	V 84	10.75	116.4	176.7	61
L 40	8.46	118.3	180.8	57.8	T 85	8.7	116.8	174.7	60
L 41	8.77	120.8	180.5	57.8	L 86	8.05	123.6	178.7	58.4
A 42	8.82	121.5	180	54.7	W 87	7.17	116	178.7	59
G 43	8.9	106.6	175.4	47	G 88	8.42	107.6	176.6	46
S 44	7.7	115.6	175.2	62.8	R 89	7.77	123.3	178	59.7
Y 45	7.47	121.4	177.1	61.2	L 90	7.87	120	179.8	58.2
L 46	7.95	116.4	178.2	57.1	V 91	8.23	119.1	178.8	66.5
A 47	8.09	120	178.8	55.4	A 92	8.44	121	179.2	55.7
V 48	7.22	116.3	178.6	65	V 93	8.21	119	177.8	67.5
L 49	7.41	119.5	179.4	57.4	V 94	7.55	119.9	178	67
A 50	7.76	118.1	179.3	54.1	V 95	8.43	120.1	179.4	67.2
E 51	7.82	114.9	177.9	57.4	M 96	9.16	120.6	177.6	59.7
R 52	7.93	116.9	177.9	59.3	V 97	8.33	117	178.2	67.5
G 53	8.3	110.6	174.6	44.5	A 98	8.61	119.8	180.1	54.2
A 54	7.67	126.4	176.4	49.7	G 99	8.85	112.1	174.6	46.7
P 55			178.3	63.2	I 100	8.5	124	181	65.6
G 56	8.5	111.7	174.4	44.9	T 101	6.85	114.9	177.6	67.1
A 57	7.29	121.9	177.5	52.9	S 102	7.69	116.3	176.5	62.8
Q 58	8.63	116.4	176.9	54.7	F 103	9.09	124.4	177.8	62
L 59	7.85	125.1	174.6	53	G 104	8.14	106.9	177.1	46.6
I 60	6.88	107.1	175.9	61.3	L 105	7.31	122.5	179.9	57.4

Table S5 (cont.)

Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	$^{13}\text{C}\alpha$	Residue	$^1\text{H}^{\text{N}}$	^{15}N	^{13}CO	$^{13}\text{C}\alpha$
V 106	7.65	121.8	178.2	66.7	A 133	7.86	121.7	178.6	53.5
T 107	8.11	110.8	177.8	65	E 134	7.94	118.2	178.1	57.7
A 108	7.6	124.7	181	54.8	E 135	8	120.2	177.4	57.3
A 109	7.68	122.7	181.2	54.7	A 136	7.99	123.1	179	53.7
L 110	8.27	120.7	178.7	57.7	Y 137	8.07	119.5	177.2	59.4
A 111	8.53	122.1	179.8	55.6	T 138	7.94	115.7	175.7	63.6
T 112	7.92	114.1	177.3	66.3	R 139	7.98	122	177.9	57.6
W 113	8.15	125.1	178.5	61.3	T 140	7.84	115	175.8	64.3
F 114	8.63	119.7	178.2	61.3	T 141	7.88	114.5	176.3	63.9
V 115	8.19	117.7	178.8	65.5	R 142	7.82	122.9	177.6	57.7
G 116	7.63	107.5	176	46.1	A 143	7.81	123.1	179.7	53.7
R 117	7.24	121.2	177.2	56.5	L 144	7.92	118.8	178.5	57.1
E 118	7.87	120.9	177	57	H 145	8.03	116.6	176.6	58.1
Q 119	0	0	177.6	57.1	E 146	7.97	118.7	178.4	58.2
E 120	7.9	119	177.6	56.9	R 147	7.64	119.1	177.1	56.5
R 121	7.9	119.2	177	56.8	F 148	7.9	119.2	176.8	58.7
R 122	7.82	120.4	177.3	56.8	D 149	8.02	120	178.3	57
G 123	7.97	108.9	174.5	45.4	R 150	7.74	119.3	178.6	56.5
H 124	7.86	117.3	174.7	55.7	L 151	7.57	120.3	178.4	57.2
F 125	7.96	120.2	176	58.7	E 152	8.07	118.1	178.4	58.9
V 126	7.54	119.7	176.2	62.5	R 153	7.66	118.4	178.4	58.3
R 127	7.77	121.8	176.9	56.5	M 154	7.68	118.8	177.9	57.7
H 128	8.05	118.2	175	55.8	L 155	7.73	119.4	178.5	57.8
S 129	7.93	116	175.1	58.7	D 156	7.89	119	177.5	55.4
E 130	8.21	122.9	177.1	57.2	D 157	8	119.2	176.9	55.2
K 131	7.86	120.8	177.4	56.9	N 158	7.85	117.6	175.3	53.8
A 132	7.86	123.5	178.6	53.3	R 159	7.67	120	175.9	56.3
					R 160	7.57	126.1	181.1	57.1

Table S6. Residual dipolar couplings for KcsA^M in 25 mM sodium formate pH 4.2, 7% D₂O, as measured at B₀=14.1 T, Temp = 323 K^a

Residue	D_{NH}^b	Δ_{NH}^c	D_{NC}^d	Δ_{NC}	D_{CC}^e	Δ_{CC}
L 16	-4.0	1.0	-2.7	0.5		
L 17	0.4	1.0	2.4	0.5	4.2	1.0
G 18	-4.6	1.0	0.4	0.5	0.9	1.0
R 19	-19.1	1.0	0.8	0.5	0.0	1.0
H 20	-10.5	1.0	0.8	0.5	-1.6	1.0
G 21	-5.8	1.0	-0.6	0.5	-1.9	1.0
S 22	-0.8	1.0	-0.6	0.5	-1.7	1.0
A 23	1.9	1.0	-0.3	0.5	0.1	1.0
L 24	6.0	1.0	-0.4	0.5	-1.3	1.0
H 25	-0.9	1.0	0.2	0.5	1.7	1.0
W 26	-12.3	1.0	0.8	0.5	-1.1	1.0
R 27	-22.8	1.0	0.1	0.5	0.3	1.0
A 28	-20.6	1.2	0.6	0.5	3.7	1.0
A 29	-21.8	1.2	1.8	0.5	1.2	1.0
G 30	-25.4	1.6	0.5	0.6	-0.4	1.0
A 31	-29.4	1.4	2.2	0.5	2.2	1.0
A 32	-29.3	1.0	0.4	0.5	1.1	1.0
T 33	-28.3	1.5	1.4	0.6	-6.2	1.0
V 34	-27.2	1.0				
L 35			2.4	0.5		
L 36			1.2	0.5		
V 37	-27.4	1.3	1.3	0.5		
I 38					0.4	1.0
V 39	-27.5	2.6	1.1	0.7	0.0	1.0
L 40	-28.1	1.5	2.4	0.6	0.9	1.0
L 41	-28.6	1.6	0.3	0.6	-0.8	1.0
A 42	-30.4	1.6	2.1	0.5	0.9	1.0
G 43	-24.7	1.4	-0.3	0.5	-0.7	1.0
S 44	-21.0	1.5	2.1	0.6	1.8	1.0
Y 45	-25.4	1.5	0.5	0.5	2.6	1.0
L 46	-25.2	1.2	0.9	0.5	1.0	1.0
A 47	-18.6	1.1	0.4	0.5	1.2	1.0
V 48	-22.3	1.5	1.2	0.5	0.0	1.0
L 49	-21.7	1.2	0.6	0.5	0.0	1.0
A 50	-20.0	1.2	-0.2	0.5	0.9	1.0
E 51	-14.1	1.3	2.2	0.6	0.7	1.0
R 52	-9.8	3.2	-0.3	0.5	-1.1	1.0
G 53	-11.2	1.0	1.4	0.5	-0.1	1.0
A 54	-2.5	1.0	-0.9	0.5		
P 55					0.0	1.0
G 56	1.7	1.0	0.2	0.5	-0.1	1.0
A 57	1.5	1.0	-0.9	0.5	-1.6	1.0
Q 58	4.5	1.0	0.4	0.5	2.0	1.0
L 59	9.1	1.4	-0.9	0.5	-0.7	1.0
I 60	-0.2	1.0	1.0	0.5	-0.8	1.0
T 61	-2.6	1.0	0.1	0.5	0.0	1.0
Y 62			-0.3	0.5		
P 63					0.2	1.0
R 64	-3.5	1.0	-0.1	0.5	-3.3	1.0
A 65	-1.5	1.0	1.6	0.5	1.7	1.0

Table S6 (cont.)

Residue	D_{NH}	Δ_{NH}	D_{NC}	Δ_{NC}	D_{CC}	Δ_{CC}
L 66	4.2	1.0	-1.0	0.5		
W 67						
W 68	0.4	1.9	1.3	0.6	-0.2	1.0
S 69	-0.3	1.0	-0.3	0.5	3.9	1.0
V 70	3.1	1.0	0.6	0.5	0.0	1.0
E 71	-1.7	1.0	-0.2	0.5	-1.6	1.0
T 72	-1.5	1.0	-0.1	0.5	0.0	1.0
A 73	0.6	1.0	-1.0	0.5	0.3	1.0
T 74	-1.2	1.0	1.1	0.5	0.0	1.0
T 75	-5.6	1.0	-0.2	0.5	-1.4	1.0
V 76	-2.3	1.0	0.0	0.5	-0.1	1.0
G 77	0.6	1.0	0.3	0.5	-0.4	1.0
Y 78	-0.8	1.0	0.1	0.5	-1.0	1.0
G 79	-0.9	1.0	-0.3	0.5	-0.6	1.0
D 80	4.3	1.0	0.2	0.5		
L 81			-1.2	0.5		
Y 82						
P 83					-2.2	1.0
V 84	-2.1	1.0	0.0	0.5	-1.5	1.0
T 85	-3.7	2.2			-0.2	1.0
L 86	0.6	4.0	0.4	0.7	-1.2	1.0
W 87			-1.6	0.7	-3.6	1.9
G 88	-12.3	3.1			-3.1	1.0
R 89	-2.1	3.2	-0.1	0.8		
L 90						
V 91	-6.9	2.5	1.3	0.5	-2.5	1.3
A 92	-6.7	2.3	-2.6	0.8	-1.6	1.4
V 93	-1.4	2.8			3.0	1.0
V 94	-3.7	1.8	0.6	0.6		
V 95	-18.6	3.2	-1.3	0.8		
M 96					1.1	1.0
V 97	1.9	2.2	1.4	0.9		
A 98						
G 99						
I 100					4.0	1.5
T 101					-2.3	1.0
S 102	-16.2	3.4			-3.1	1.0
F 103	-8.8	1.8	-0.2	0.5	0.0	1.0
G 104	-3.1	1.9	0.0	0.8		
L 105	-6.0	4.0			2.1	3.5
V 106	-14.8	2.5			-4.2	2.3
T 107						
A 108	7.1	3.0			1.7	1.2
A 109	-6.7	3.1	1.2	0.8	-2.7	1.0
L 110	-13.4	1.5	-1.3	0.6	-1.6	1.0
A 111	-1.9	1.9	-0.5	0.7	3.3	1.0
T 112	2.6	1.3	2.0	0.6	0.3	1.0
W 113	-11.3	1.4	-1.0	0.6	-4.7	1.0
F 114	-8.9	1.4	-0.1	0.6		
V 115	4.7	1.1	-0.4	0.5	2.8	1.0

Table S6 (cont.)

Residue		D_{NH}	Δ_{NH}	D_{NC}	Δ_{NC}	D_{CC}	Δ_{CC}
G	116	0.7	1.4	2.8	0.7	-0.4	1.0
R	117	-10.9	1.2	-1.2	0.7		
E	118						
Q	119						
E	120	-5.1	1.4	-1.0	0.5		
R	121	7.3	2.2	-0.3	0.5		
R	122	-4.9	2.2			-0.8	1.0
G	123	-3.0	1.0	0.2	0.5	-0.2	1.0
H	124	0.1	1.0	0.1	0.5	-0.7	1.0
F	125	1.6	1.0	-1.0	0.5	-1.2	1.0
V	126	2.3	1.0	0.5	0.5	0.6	1.0
R	127	1.6	1.0	-0.1	0.5	-1.1	1.0
H	128	-4.3	1.0	1.1	0.5	-0.7	1.0
S	129	0.6	1.0	-1.3	0.5	-1.4	1.0
E	130	3.5	1.0	-0.3	0.5		
K	131					-0.1	1.0
A	132	9.5	1.0	-0.3	0.5	-2.1	1.0
A	133	9.8	1.0	-0.1	0.5	0.0	1.0
E	134	10.7	1.0	-0.8	0.5	-2.9	1.0
E	135	4.2	1.0	-0.1	0.5	0.1	1.0
A	136	8.7	1.0	-1.6	0.5	-2.6	1.0
Y	137	9.7	1.1	-0.3	0.5	0.5	1.0
T	138	13.2	1.0	-0.9	0.5		1.0
R	139	11.0	1.0	0.3	0.5	-0.7	1.0
T	140	9.9	1.0	-1.0	0.5	-1.9	1.0
T	141	9.8	1.0	-0.3	0.5		
R	142						
A	143	11.2	1.0	-0.3	0.5	-0.5	1.0
L	144	13.7	1.0	-1.3	0.5	-0.8	1.0
H	145	12.6	1.0	-1.0	0.5		
E	146	13.7	1.0	-0.2	0.5	1.0	1.0
R	147	9.4	1.0	0.9	0.5	4.0	1.0
F	148			0.1	0.5		
D	149	8.1	1.0	-1.6	0.5	-2.0	1.5
R	150	8.7	3.2			0.9	1.0
L	151	14.4	1.0	-0.2	0.5	-0.6	1.0
E	152	8.2	1.2	0.2	0.5		
R	153	5.8	1.6	-0.8	0.5	-0.7	1.0
M	154	12.1	1.0	-0.9	0.5		
L	155						
D	156			-0.4	0.5		
D	157	2.3	1.5	-0.1	0.5	-0.1	1.0
N	158	6.1	1.0	0.0	0.5	-0.4	1.0
R	159	-2.2	1.0	0.3	0.5		
R	160						

^aRDCs were measured as described in [35-37]. Missing couplings are due to spectral overlap or insufficient signal-to-noise in the spectrum.

^bRDC for the ¹H^N and ¹⁵N nuclei of the appropriate residue.

^c Δ_{ij} represents the experimental error for residual coupling D_{ij} . Errors in peak position were estimated using the empirical relationship $\Delta = LW/(2*SN)$, where LW is the linewidth at half-height (in Hz) and SN is the signal-to-noise ratio. As this represents a lower limit for measurement accuracy, the minimum errors in D_{NH} , D_{NC} and D_{CC} used for purposes of structural fitting were set to 1.0, 0.5 and 1.0 Hz, respectively.

^dRDC for the ¹⁵N nucleus of the appropriate residue and the ¹³C' nucleus of the previous residue.

^eRDC for the ¹³C ^{α} and ¹³C' nuclei of the appropriate residue

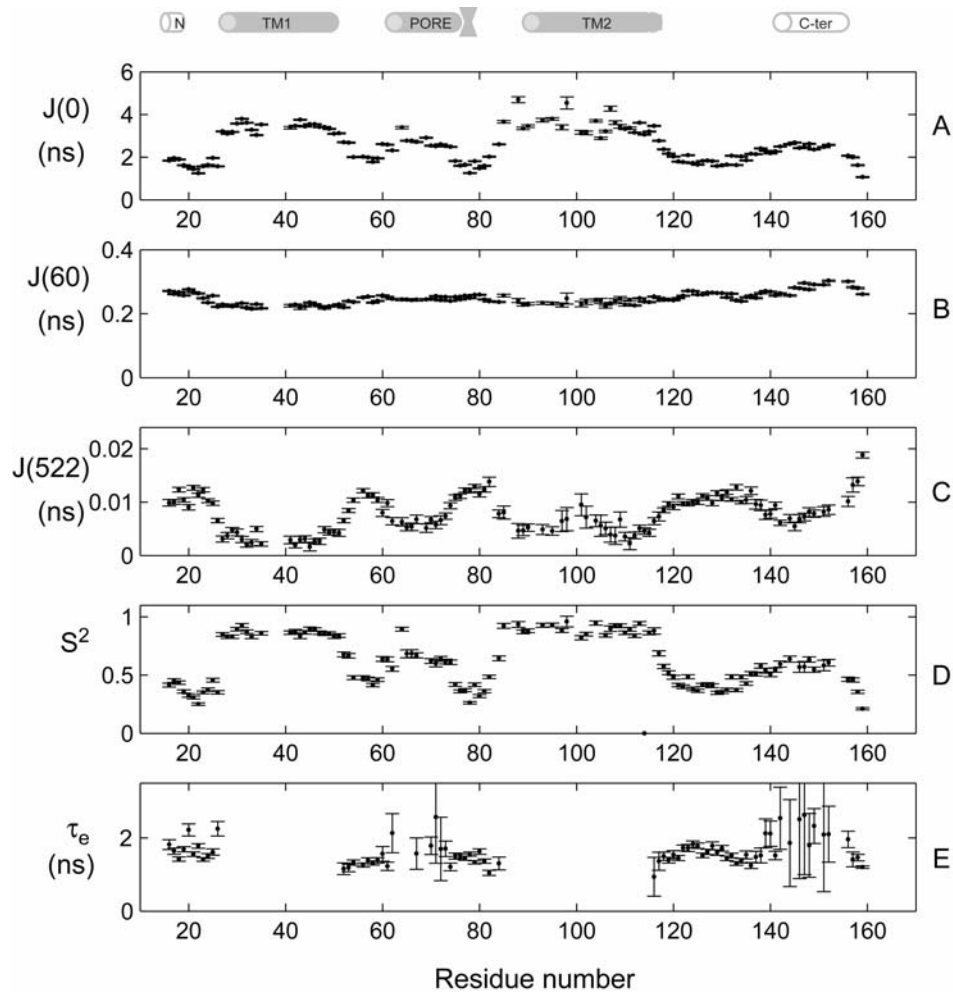


Figure S1. Analysis of ^{15}N relaxation data and ps–ns scale motions along the backbone of monomeric KcsA. Summarized are the results of spectral density mapping (panels A–C) and the model-free approach (panels D–E) for KcsA^M. Values of (A) $J(0)$, (B) $J(\omega_{\text{N}}, 60.8 \text{ MHz})$, and (C) $J(0.87\omega_{\text{H}}, 522 \text{ MHz})$ are shown and calculated using standard formulae (Farrow et al., 1995, *J. Biomol. NMR*, 6, 153–162.). (D) Generalized squared order parameter S^2 , and (E) timescale of internal motions, τ_e . The global tumbling time was estimated as 9.8 ns under the assumption of isotropic rotational diffusion, which is necessary in the absence of a high-resolution structure. Residues in the transmembrane domains (including the kinked TM2^K segment) are well structured ($S^2 \sim 0.8\text{--}0.9$) and fitted to model 1 of the Model-free analysis. Most other residues exhibit internal motion on the ns timescale with $S^2 \sim 0.4\text{--}0.6$. The two helices in the extra-membranal domain (PORE, residues 62–74, and the C-TER helix, residues 141–154) exhibit an intermediate S^2 value of 0.6–0.7. Structural elements of KcsA (ref. 2) appear above the data; helices are represented by cylinders and the selectivity filter is designated by the hourglass.