Supplementary Material

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

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Table S1. Acquisition parameters for KcsA^{M} measurements.[a]

Backbone assignment:

Exp	^{1}H		^{15}N		^{13}CO/^{13}Cα		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

^{15}N backbone relaxation:

Exp	^{1}H		^{15}N		^{13}CO		recycle delay	relax delay[e]	No. scans	Exp time[f]
	Pts[b]	SW (Hz)	Pts	SW (Hz)	Pts	SW (Hz)	(sec)	(sec)		(hrs)
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
	Pts[b]	SW (Hz)	Pts[b]	SW (Hz)	Pts[b]	SW (Hz)		(hrs)
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

[a]All 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.

[b]Pts represent complex points acquired in each dimension.

[c]The third dimension is the ^{1}H (NOE-originator) acquisition parameters.

[d]The third dimension is the ^{15}N (first HMQC) acquisition parameters.

[e]The 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.

[f]Experiment 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

[h]Residual 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 $^1J_{C'C\alpha}$ coupling allowed to evolve during the ^{13}CO evolution as described in ref. [37].

Table S2. Chemical shift values for $KcsA^{M}$ in 25 mM sodium formate pH 4.2, 7% D_2O, as measured at B_0=14.1 T, Temp = 323 K

Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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% D_2O, as measured at B_0=14.1 T, Temp = 323 K

Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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% D_2O, as measured at B_0=14.1 T, Temp = 323 K

Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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 B_0=14.1 T, Temp = 323 K

Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$	Residue	$^{1}H^{N}$	^{15}N	^{13}CO	$^{13}C\alpha$
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% D_2O, as measured at B_0=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						

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

[b]RDC for the $^{1}H^{N}$ and ^{15}N 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.

[d]RDC for the ^{15}N nucleus of the appropriate residue and the $^{13}C'$ nucleus of the previous residue.

[e]RDC for the $^{13}C^{\alpha}$ and $^{13}C'$ nuclei of the appropriate residue

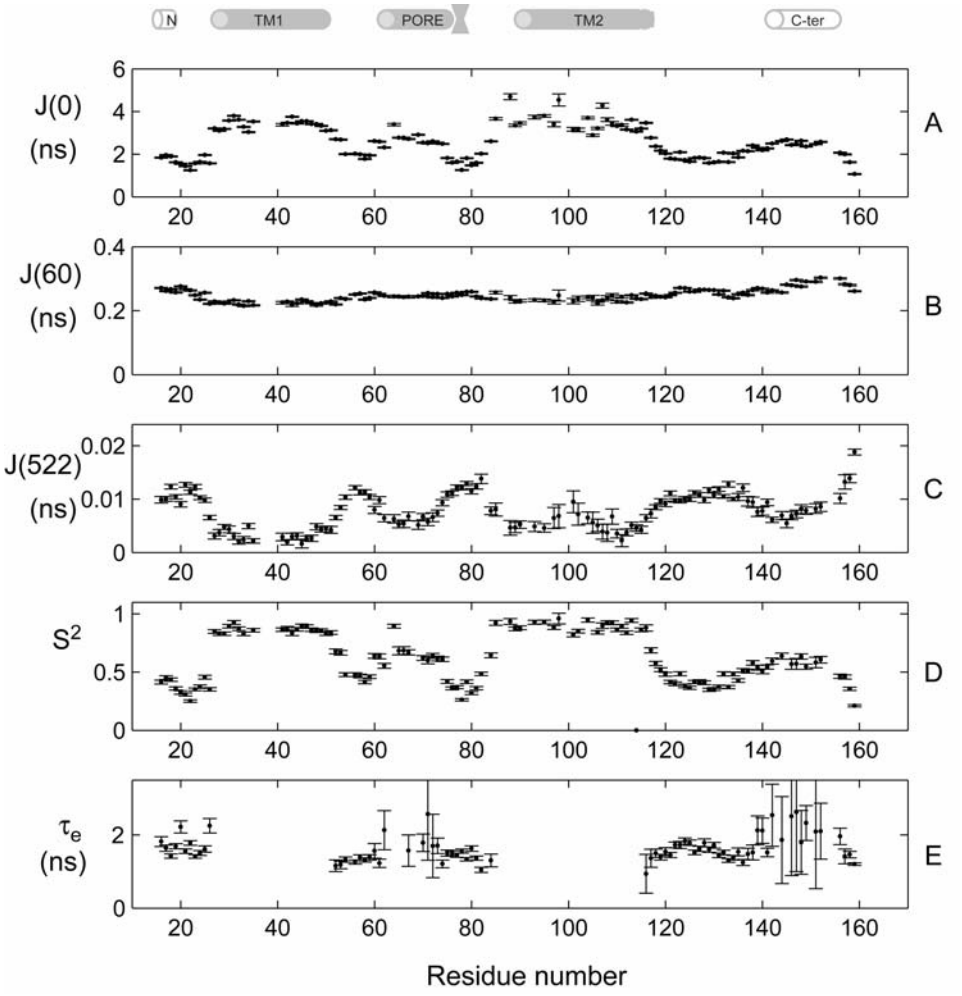

Figure S1. Analysis of ^{15}N relaxation data and ps–ns scale motions along the backbone of monomeric KcsA. Summarized are the results of spectral density mapping (panels A–C) and the model-free approach (panels D–E) for KcsA^{M}. Values of (A) J(0), (B) J(ω_N, 60.8 MHz), and (C) J(0.87ω_H, 522 MHz) 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 ~ 0.8-0.9) and fitted to model 1 of the Model-free analysis. Most other residues exhibit internal motion on the ns timescale with S^2 ~ 0.4-0.6. 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.