Transfer of Kv3.1 Voltage Sensor Features to the Isolated Ci-VSP Voltage-Sensing Domain

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Supporting Material

Construct Name	AA of Ci-VSP replaced[a]	AA of Kv3.1 transferred[b]	Δ R/R (%)[c]
VSFP2.3 (Ci-VSP R217Q)			12.1
C1	227-240	321-334	9.6
C2	227-239	321-333	11.3
C3	227-238	321-332	8.3
C4	227-237	321-331	9.3
C 5	227-236	321-330	10.7
C 6	224-236	319-330	12.9
C 7	222-236	317-330	2.2
C 8	217-236	312-330	2.1
C 9	215-236	310-330	0.2
C 10	206-236	300-330	1.3
C 11	194-236	290-330	0.4
C 12	189-236	285-330	0.6
C 13	189-226	285-320	0.5
C 14	188-226	284-320	0.5
C 15	188-236	284-330	3.3
C 16	188-239	284-333	-
C 17	178-236	274-330	1.3
C 18	173-236	269-330	-
C 19	145-236	241-330	-
C 20	144-236	212-330	5.2
C 21	143-236	210-330	5.6
C 22	142-240	209-334	3.0
C 23	142-239	209-333	6.5
C 24	142-238	209-332	3.5
C 25	142-237	209-331	6.4
C 26	142-236	209-330	5.8
C 27	142-235	209-329	6.4
C 28	142-234	209-328	5.5
C 29	141-236	208-330	-
C 30	140-236	207-330	3.1
C 31	139-236	206-330	2.4
C 32	138-236	205-330	1.7
C 33	136-236	203-330	1.0
C 34	130-236	197-330	-
C 35	125-236	192-330	-
C 36	121-236	188-330	-
C 37	117-236	191-330	-
C 38	110-236	184-330	-
C 39	1-236	1-330	-
C 40	1-226	1-320	-

Table S1 Summary of chimeric constructs with regions of Ci-VSP VSD replaced by portions of Kv3.1.
[a]Amino acids removed from Ci-VSP to be replaced by the homologous amino acids of Kv3.1 [b]Amino acids of Kv3.1 that replaced the corresponding amino acids of Ci-VSP. [c]Ratiometric FRET responses from a two-step voltage protocol of -100 mV to 60 mV from a holding potential of -70 mV at room temperature.

	τ_1 on	τ_2 on	% τ_1	τ_1 off	$V_{1/2}$ fast	$V_{1/2}$ slow	$V_{1/2}$ off	ΔR/R (%)
VSFP2.3	3.0 ± 0.4	69.2 ± 4.3	26.6	91.6 ± 4.2	-28.3 ± 1.3	-48.6 ± 1.2	-28.2 ± 0.9	15.2 ± 0.2
VSFP2.3 RT	6.0 ± 1.2	120. ± 4 7.1	24.3	145.4 ± 5.4	-17.8 ± 2.7	-48.1 ± 1.0	-26.0 ± 0.3	15.6 ± 0.2
C1	2.6 ± 0.6	34.9 ± 1.8	62.8	12.8 ± 0.6	11.0 ± 2.1	≥20	≥20	17.9 ± 0.03
C1 RT	5.1 ± 0.5	62.1 ± 6.2	59.8	33.2 ± 4.1	n/a[a]	≥20	≥20	17.5 ± 1.4
C2	2.2 ± 0.4	39.2 ± 1.7	59.7	17.0 ± 0.6	-20.0 ± 2.2	≥20	≥20	19.7 ± 0.06
C2 RT	6.5 ± 0.3	72.7 ± 2.5	56.4	36.0 ± 3.3	-29.8 ± 2.0	≥20	≥20	13.9 ± 0.04
C5	2.1 ± 0.5	36.8 ± 2.7	60.1	13.4 ± 1.5	-17.9 ± 1.2	≥20	≥20	14.8 ± 0.2
C5 RT	2.8 ± 0.4	43.2 ± 3.8	58.1	15.8 ± 1.9	-8.6 ± 1.0	≥20	≥20	14.6 ± 0.2
C25	1.8 ± 0.1	n/a	100	1.8 ± 0.2	-27.6 ± 1.8	n/a	-25.6 ± 0.5	6.4 ± 0.3
C25 RT	2.3 ± 0.4	n/a	100	3.9 ± 0.2	-34.3 ± 2.2	n/a	-36.4 ± 0.8	6.1 ± 0.8
K229R	2.0 ± 0.4	34.1 ± 2.4	49.1	16.8 ± 2.1	-63.6 ± 3.0	n/a	-69.2 ± 2.0	12.7 ± 0.4

Table S2 Temperature dependency of the chimeric Ci-VSP-Kv3.1 VSD and properties of chimera C5 with K229R mutation.

	τ_1 on	τ_2 on	% τ_1	τ_1 off	ΔR/R (%)
C7	37.7 ± 6.3	-		29.5 ± 1.4	2.2
C15	15.5 ± 6.0	60.7 ± 42.6	43.1	33 ± 4	3.3
C22	2.1 ± 0.3	-		3.8 ± 0.4	3.0
C24	2.9 ± 0.3	-		3.6 ± 0.3	3.5
C30	4.3 ± 0.6	-		5.5 ± 1.1	3.1
C31	5.5 ± 0.6	-		31 ± 6	2.4

Table S3 Kinetic properties of the chimeric Ci-VSP-Kv3.1 VSD that display FRET signals between 2-5% at room temperature. C8 was not analyzed as the fluorescence signal was biphasic.