Supplementary Data

SOLUTION STRUCTURE OF THE $Na_V1.2$ C-TERMINAL EF-HAND DOMAIN

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Supplementary Table SI

Isoform	Mutation in CTD	I_{Na}	Voltage dependence of Inactivation	Kinetics of Slow Inactivation	Residue in $Na_V1.2$ CTD
$Na_V1.1$	F1808L	persistent	– shift	↔[1]	F1798
	D1866Y	persistent	+ shift	↔	D1856
	M1852T	decreased[2]		↔	M1842
$Na_V1.5$	V1777M	persistent	– shift		V1781
	E1784K	persistent	– shift	↔	E1788
	D1790G	↔	– shift with β subunit	↔	D1794
	Y1795insD	decreased, persistent	↔[3]	↔[4]	Y1799
	Y1795C	persistent	↔	↓	Y1799
	Y1795H	decreased, persistent	– shift	↑	Y1799
	W1798E[5]	persistent	– shift		W1802
	L1825P	decreased, persistent	– shift	↓	L1829
	R1826H	persistent		↔[6]	L1830
	I1853E[5]	persistent	– shift		I1857

Correspondence between isoforms was obtained through sequence alignment of Na_V1 C-terminal Domains, performed with CLUSTALW (1), with sequences of human Na_v1 channels (2) retrieved from NLM-NCBI. The references for the data above are as follows F1808L (3), D1866Y (4), M1852T (5), V1777M (6), E1784K (7,8), D1790G (9,10), Y1795insD (11,12), Y1795C/H (13,14), W1798E (13,14), L1825P (15), R1826H (16), I1853E (13,14). [1]F1808 was observed to have a larger proportion of channels exhibiting slow inactivation (5). [2]Lower relative expression is implicated as the cause of decreased current (5). [3]There is conflicting data on the voltage dependence of inactivation (11,12). [4]Kinetics of slow inactivation are only slightly increased over wild-type (11), with the predominant effect being on fast-inactivation (12). [5]W1798E and I1853E are synthetic mutants created to probe the helix I – IV interface (13,14). [6]The predominant effect for R1826H appears to be on fast inactivation (16).

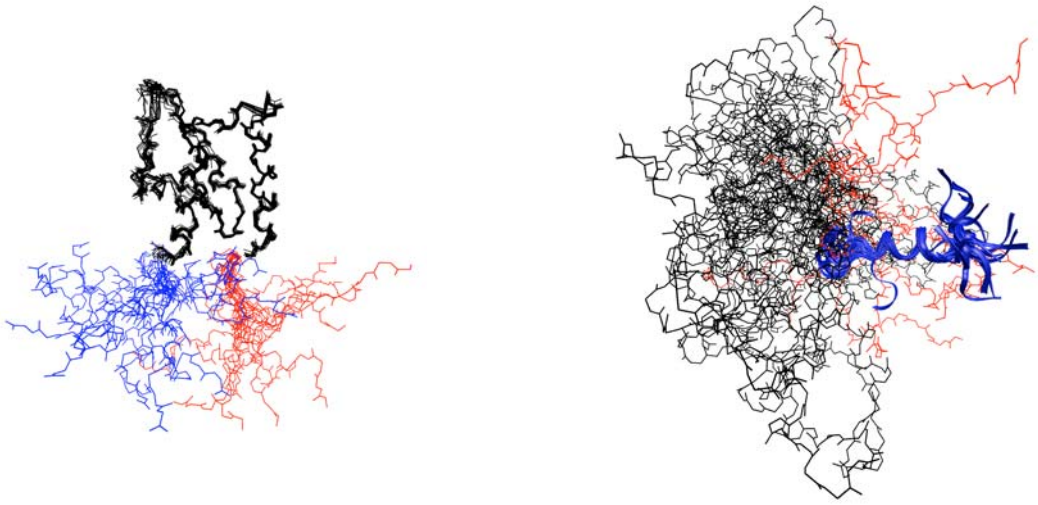

Figure S1. Superposition of $Na_V1.2$ (1777-1882) CTD structural ensemble. The N-terminal region (residues 1777-1789) is colored red, the core domain (residues 1790-1868) is colored black, and the C-terminal region (residues 1869-1882) is colored blue. (left) The ordered core residues 1790-1868 are superposed; the N- and C-terminal regions are disordered. (right) The residues 1870-1876 are superposed and are shown as a backbone ribbon to more clearly illustrate helix V. The N-terminal region is disordered and the core domain does not have a fixed orientation relative to helix V.

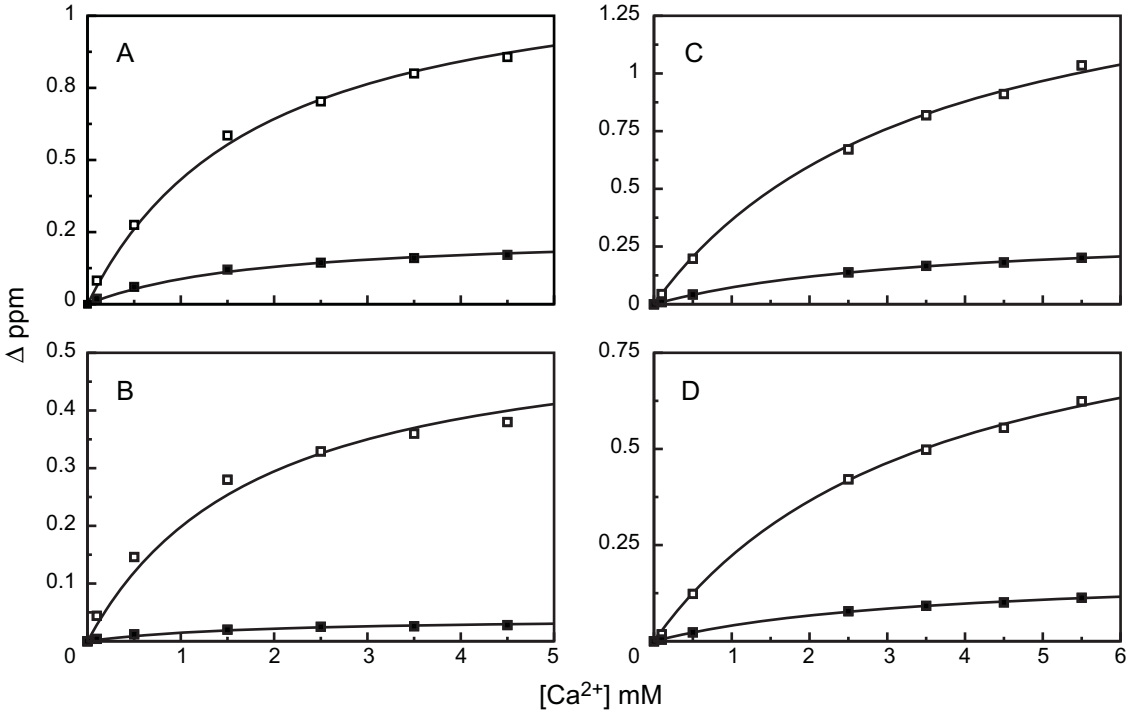

Figure S2. Calcium titration of $Na_V1.2$ and $Na_V1.5$. For $Na_V1.2$ representative residues L1790 (panel A) and L1830 (panel B) are shown. For $Na_V1.5$ residues L1786 (panel C) and R1826 (panel D) are shown. Plots show Ca^{2+} concentration versus change of the ^{1}H (closed symbols) and ^{15}N (open symbols) chemical shifts. Fitting of dissociation constants was performed globally with 103 and 83 residues, for $Na_V1.2$ and $Na_V1.5$ respectively, using Mathematica. Globally fitted dissociation rate constants are 1.65 ± 0.03 mM and 3.28 ± 0.13 mM for $Na_V1.2$ and $Na_V1.5$ respectively.

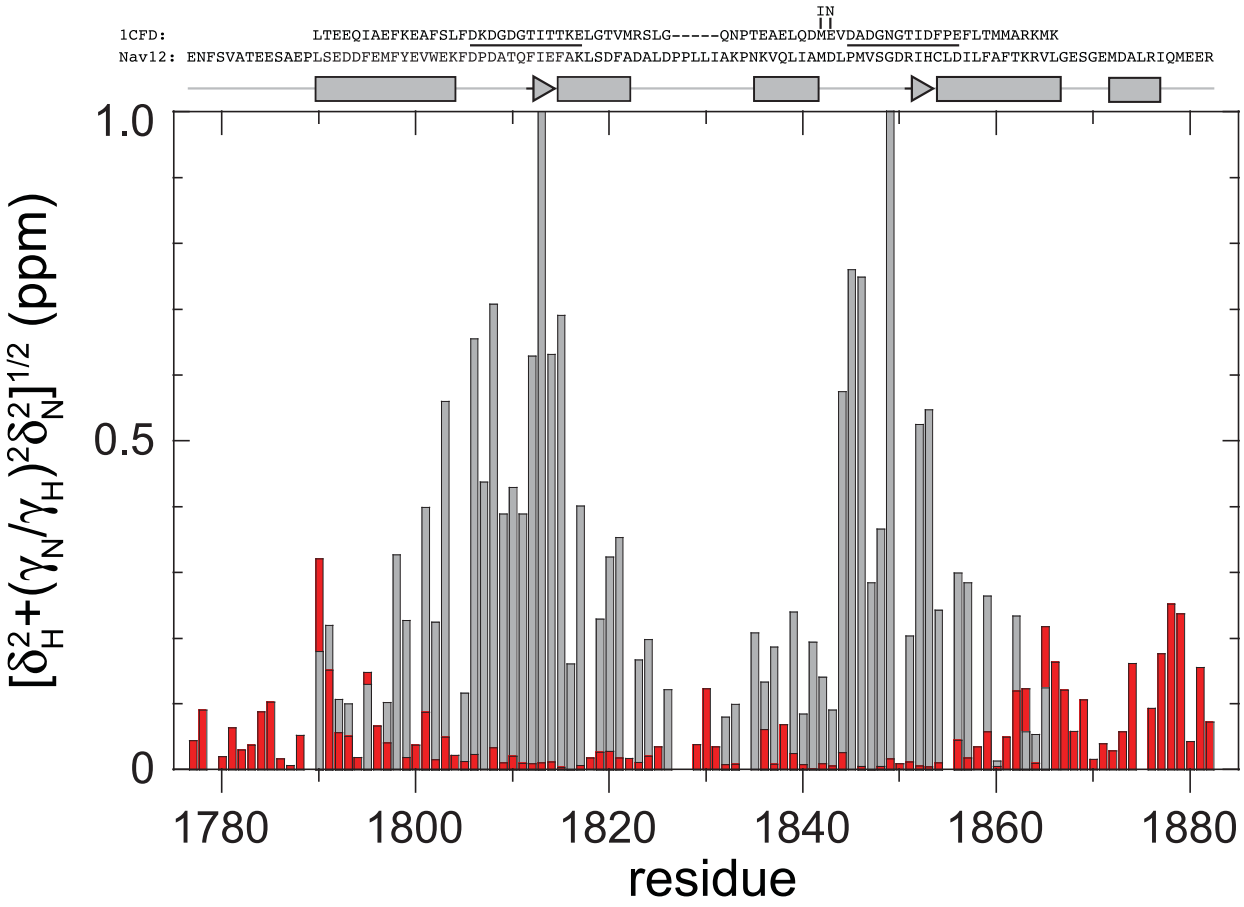

Figure S3. Calcium-induced shift perturbations for (red) $Na_V1.2$ and (grey) N-terminal EF hand pair of calmodulin. Residue T27 in calmodulin has a shift perturbation of 2.22 ppm and G60 has a shift perturbation of 1.23 ppm. Chemical shift differences between Ca^{2+}-bound and apo calmodulin were obtained from published chemical shift assignments (17,18). (top) Structure-based sequence alignment of $Na_V1.2$ (residues 1790-1866) and the N-terminal EF-hand pair of calmodulin (residues 4-77) (1CFD). The alignment was performed with CE (19). The helix II-III interhelical segment sequence (PPLLI) is not present in the calmodulin sequence and the indicated IN sequence in calmodulin is not present in $Na_V1.2$. The Ca^{2+} binding regions in calmodulin and the corresponding residues in $Na_V1.2$ are indicated by the two horizontal bars between the sequence designations. The secondary structure of $Na_V1.2$ is indicated schematically.

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