Published in final edited form as:

Biomol NMR Assign. 2018 October ; 12(2): 283–289. doi:10.1007/s12104-018-9824-5.

Backbone Resonance Assignments of Complexes of Apo Human Calmodulin Bound to IQ Motif Peptides of Voltage-Dependent Sodium Channels Na_v1.1, Na_v1.4 and Na_v1.7

Holly M. Isbell¹, Adina M. Kilpatrick², Zesen Lin¹, Ryan Mahling¹, and Madeline A. Shea^{1,*}

¹Department of Biochemistry, Roy J. and Lucille A. Carver College of Medicine University of Iowa, Iowa City, Iowa 52242-1109, USA

²Department of Physics and Astronomy, Drake University Des Moines, IA, 50311-4516, USA

Abstract

Human voltage-gated sodium (Na_v) channels are critical for initiating and propagating action potentials in excitable cells. Nine isoforms have different roles but similar topologies, with a pore-forming α -subunit and auxiliary transmembrane β -subunits. Na_v pathologies lead to debilitating conditions including epilepsy, chronic pain, cardiac arrhythmias, and skeletal muscle paralysis. The ubiquitous calcium sensor calmodulin (CaM) binds to an IQ motif in the C-terminal tail of the α -subunit of all Na_v isoforms, and contributes to calcium-dependent pore-gating in some channels. Previous structural studies of calcium-free (apo) CaM bound to the IQ motifs of Na_v1.2, Na_v1.5, and Na_v1.6 showed that CaM binding was mediated by the C-domain of CaM (CaM_C), while the N-domain (CaM_N) made no detectable contacts. To determine whether this domain-specific recognition mechanism is conserved in other Na_v isoforms, we used solution NMR spectroscopy to assign the backbone resonances of complexes of apo CaM bound to peptides of IQ motifs of Na_v1.1, Na_v1.4, and Na_v1.7. Analysis of chemical shift differences showed that peptide binding only perturbed resonances in CaM_C; resonances of CaM_N were identical to free CaM. Thus, CaM_C residues contribute to the interface with the IQ motif, while CaM_N is available to interact elsewhere on the channel.

Keywords

Voltage-Gated Sodium Channel; Molecular Recognition; Domain Interactions; Allosteric; EF-hand protein; Calcium signaling

Biological Context

Voltage-gated sodium (Na_v) channels are essential for the initiation and propagation of action potentials in excitable cells, including neurons, and skeletal and cardiac myocytes. Na_v channels are large transmembrane proteins consisting of a major pore-forming α -subunit and one or more auxiliary β -subunits (Mantegazza and Catterall 2012; Messner and Catterall 1985). A membrane depolarization event leads to the opening of the pore and the

*Corresponding author: Telephone: (319) 335-7885, FAX: (319) 335-9570, madeline-shea@uiowa.edu.

rapid influx of sodium ions from the extracellular space into the cytosol, resulting in the rising phase of an action potential. Within milliseconds, the pore is closed in a process called fast inactivation (Hodgkin and Huxley 1952).

Nine mammalian Na_V isoforms (Na_V1.1 – Na_V1.9) have been identified and functionally characterized. Although the nine channels share similar overall topologies, they are differentially expressed in tissues and have specialized functional roles (Mantegazza and Catterall 2012). Na_V1.1, Na_V1.2, Na_V1.3, and Na_V1.6 are crucial for neuronal excitability in the central nervous system (CNS). Na_V1.4 and Na_V1.5 are the primary sodium channels in skeletal and cardiac muscle, respectively. Na_V1.7 is broadly expressed in the peripheral nervous system (PNS), where it initiates and conducts action potentials in axons and was recently recognized to act in the CNS, playing a role in weight regulation (Branco et al. 2016). Na_V1.8 and Na_V1.9 are predominantly distributed in the PNS.

Na_V channelopathies lead to serious diseases affecting nervous system function, heart rhythm, and skeletal muscle contraction (see review in (Huang et al. 2017)). Pathologies of isoforms expressed in the CNS include Na_V1.1 defects that are associated with Dravet syndrome, severe epilepsy, sleep disturbance, and cognitive impairment. Identified Na_V1.2 mutations underlie seizure and autism spectrum disorders. Mutations in Na_V1.4 and Na_V1.5 are associated primarily with neuromuscular and cardiac diseases, including skeletal muscle paralysis and myotonia, and cardiac arrhythmia syndromes. Defects in Na_V1.7 are known to cause altered pain perception and response to anesthetics, leading to disorders in which subjects feel no pain (congenital insensitivity to pain), or have chronic pain.

The activity of sodium channels is modulated through interactions with multiple protein partners, including the essential eukaryotic calcium sensor calmodulin (CaM) (Mori et al. 2000). CaM is a small, acidic protein (148-residues) composed of two globular domains (CaM_N and CaM_C) connected by a flexible linker. Each domain binds two calcium ions *via* a pair of EF-hand calcium-binding motifs. Although the two domains have a high degree of sequence and structural similarity, they act as distinct calcium sensors and often play separable functional roles in regulating the activity of target proteins, as originally discovered in a genetic screen of *Paramecium*. (Kink et al. 1990)

CaM binds to a well-conserved IQ motif [IQxxx[R,K]Gxxx[R,K] found in the intracellular C-terminal domain of the α -subunit of all Na_V isoforms (Mori et al. 2000; Theoharis et al. 2008; Sarhan et al. 2012; Gabelli et al. 2016; Hovey et al. 2017; Kim et al. 2004) and regulates the function of some isoforms in a calcium-dependent manner (Young and Caldwell 2005; Ben-Johny, Yue, and Yue 2016; Yan et al. 2017; Pitt and Lee 2016). However, the mechanisms of calcium- and CaM-dependent regulation of channel function are complex and remain controversial.

Our previous thermodynamic and solution NMR studies showed that both calcium-depleted (apo) CaM and calcium-saturated CaM bind the IQ motif of Na_V1.2 with high affinity, and that CaM_C was necessary and sufficient for binding in the absence or the presence of calcium (Theoharis et al. 2008; Feldkamp, Yu, and Shea 2011; Hovey et al. 2017; Mahling,

Kilpatrick, and Shea 2017). CaM_N did not influence CaM binding to the Nav1.2 IQ motif, and it retained structural and energetic properties of the free domain.

Similarly, in high-resolution structures of apo CaM bound to Nav1.5 (2L53, 4OVN, 4DCK) or Nav1.6 (3WFN) IQ motif peptides, CaM_N did not contribute to the interface (Wang et al. 2012; Reddy Chichili et al. 2013; Gabelli et al. 2014). The solution NMR structure (2L53) showed that CaM_N could adopt multiple orientations relative to CaM_C and the IQ region. No high-resolution structures are available for CaM bound to the IQ motifs of the six other Nav isoforms: 1.1, 1.3, 1.4, 1.7, 1.8, and 1.9.

To provide insight into the structural determinants of CaM recognition of the IQ motifs of these Nav channels, we have used solution NMR spectroscopy to assign the backbone resonances of ¹³C, ¹⁵N-labeled complexes of apo CaM bound to peptides corresponding to the IQ motifs of Nav1.1, Nav1.4, and Nav1.7. These channels have similar IQ motif sequences, but different functions and expression patterns. The three complexes are hereafter referred to as apo CaM+Nav1.1_{IQp}, apo CaM+Nav1.4_{IQp}, and apo CaM+Nav1.7_{IQp}.

In all three complexes, residues in CaM_C underwent large chemical shift changes upon peptide binding, while residues in CaM_N matched those in free CaM. These data indicate that residues in the C-domain of apo CaM are necessary and sufficient to mediate binding to the IQ motifs of these three channels. Moreover, the distribution of chemical shift perturbations induced in CaM_C upon peptide binding is consistent with atomic resolution structures of apo CaM bound to the IQ motifs of Nav1.2, Nav1.5 and Nav1.6 where the Q residue of the IQ motif is cradled by the loop between helices F and G of CaM_C suggesting that the structural features of this interaction are conserved in these three Nav isoforms.

Materials and Experiments

Protein Expression and Purification

Complexes of CaM with Nav1.1_{IQp}, Nav1.4_{IQp}, or Nav1.7_{IQp} were obtained by bacterial co-expression of CaM with GST-tagged sequences corresponding to the IQ motifs of the three channels. Full-length mammalian CaM was expressed using a pT7-7 vector (Ampicillin^R) (Sorensen and Shea 1998). IQ peptides were expressed using a modified form of a pBG101 vector (Kanamycin^R) containing an N-terminal Glutathione-S-Transferase (GST) tag and a 3C protease cleavage site (Damo et al. 2013). The original vector was a generous gift from Walter Chazin, Director of the Center for Structural Biology at Vanderbilt University.

The plasmid was modified to code for residues 1911–1937 of human Nav1.1 [KRKQEEVSAVIIQRAYRRHLLKRTVKQ], residues 1723–1749 of human Nav1.4 [KRKHEEVCAIKIQRAYRRHLLQRSMKQ], or residues 1885–1911 of human Nav1.7 [KRKQEDVSATVIQRAYRRYRLRQNVKN]. Because the 3C protease site is between the sequences for GST and the IQ motif, the three peptides used in this study contain a 4-residue N-terminal tag having the sequence Gly-Pro-Gly-Ser (GPGS); these residues were identified with negative integers (−4 to −1) in the BMRB deposits.

Escherichia coli BL21(DE3) cells co-transformed with plasmids for both CaM and GST-Na_v IQ motif peptides were grown in 4 baffled flasks containing 1 L cultures of LB at 37°C to an OD₆₀₀ of 0.7. Cells were pelleted by centrifugation at 4,000 rpm, 4°C for 20 min, combined and exchanged into 1 L of minimal media containing 1 g/L ¹⁵NH₄Cl (CIL) and 4 g/L ¹³C₆-glucose (CIL) (Marley, Lu, and Bracken 2001). After a recovery period (1 h), expression was induced with 1 mL of 0.8 M IPTG. Four hours after addition of IPTG, cells were pelleted by centrifugation at 4,000 rpm, 4°C, for 20 min, and stored at -20°C. Thawed cell pellet was resuspended in 30 mL of lysis buffer (50 mM HEPES, 100 mM KCl, 1 mM MgCl₂, 5 mM NTA, 50 μM EGTA, 0.01% NaN₃ (w/v), pH 7.4), and sonicated. Cell lysate was centrifuged at 20,000 rpm, 4°C for 50 min; supernatant was passed through a 0.22 μm sterile PVDF filter, mixed with 10 mL Glutathione Sepharose resin (Amersham Biosciences), gently rocked at 4°C, and washed with lysis buffer (5 column volumes (CV)), a salt wash buffer (lysis buffer with 500 mM KCl, 5 CV), and lysis buffer (5 CV) at 4°C.

To release the apo CaM-IQp complex, resin was incubated with 3C protease for 4 h at 22 to 25 °C with gentle rocking. CaM-IQp complexes were collected, concentrated and exchanged into 10 mM imidazole, 100 mM KCl, 100 μM EDTA, and 0.01% (w/v) NaN₃, at pH 6.5. The apo CaM+Na_v1.4_{IQp} and apo CaM+Na_v1.7_{IQp} complexes were further purified by size exclusion chromatography on a Superdex75 10 × 300 mm column (ÅKTA FPLC, GE Healthcare) in matching buffer. Protein purity (> 95%) was assessed by SDS-PAGE, UV/Vis spectroscopy, and rpHPLC. Protein concentrations were determined with the BCA assay (Pierce Biotechnology; Rockford IL).

NMR Experiments

NMR spectra were collected at 25°C (298 K) using 600 MHz Varian Unity Innova or 500 MHz Bruker AVANCE II NMR spectrometers equipped with triple resonance gradient probes. NMR samples were ¹³C,¹⁵N-labeled complexes of apo CaM bound to Na_v1.1_{IQp} (0.85 mM), Na_v1.4_{IQp} (1.1 mM), or Na_v1.7_{IQp} (1.0 mM), in 10 mM imidazole, 100 mM KCl, 100 μM EDTA, 0.01% (w/v) NaN₃, at pH 6.5. All spectra were processed using NMRPipe (Delaglio et al. 1995) and analyzed using CCPN Analysis (Vranken et al. 2005). Backbone resonance assignments were made using 2D ¹H-¹⁵N HSQC and 3D HNCACB, HNCO, HN(CA)CO, and HN(CO)CACB (for apo CaM+Na_v1.4_{IQp} and apo CaM +Na_v1.7_{IQp}) or CBCA(CO)NH (for apo CaM+Na_v1.1_{IQp}) experiments in 90% H₂O/10% D₂O.

Order parameters were predicted using TALOS+ (Shen et al. 2009). Chemical shift perturbations δ (ppm) were calculated with Eq. 1

$$\Delta \delta = \sqrt{(\Delta \delta_{HN})^2 + (\Delta \delta_N \cdot 0.10134)^2} \quad [1]$$

where δ_{HN} and δ_N are the observed changes in chemical shifts for ¹H_N and ¹⁵N respectively.

Extent of Assignment and Data Deposition

Assigned ^1H - ^{15}N HSQC spectra are shown for apo CaM+Nav1.1IQp (Fig. 1), apo CaM+Nav1.4IQp (Fig. 2), and apo CaM+Nav1.7IQp (Fig. 3). Given the predominantly α -helical topology of CaM and CaM-bound IQ peptides, the spectra are reasonably well dispersed. For each spectrum, a central crowded region is expanded to the right to provide greater clarity. For apo CaM, 93%, 96%, and 97% of the backbone $^1\text{H}_\text{N}$ and ^{15}N resonances of non-proline residues were assigned in apo CaM+Nav1.1IQp, apo CaM+Nav1.4IQp, and apo CaM+Nav1.7IQp, respectively. Crosspeaks were not observed for the first two residues (A1 and D2) in all complexes. The majority of the other missing resonances correspond to residues in calcium-binding site IV and flanking regions, consistent with previous findings from solution NMR studies of apo CaM complexes with Nav1.2 and Nav1.5 IQ motifs (Feldkamp, Yu, and Shea 2011; Chagot and Chazin 2011; Mahling, Kilpatrick, and Shea 2017). The backbone $^1\text{H}_\text{N}$ and ^{15}N assignments of non-proline residues in the IQ motif peptides are 97% complete in the apo CaM+Nav1.4IQp, and apo CaM+Nav1.1IQp complexes, missing only the first residue [G(-4)] in the GPGS tag, and 94% complete in the apo CaM+Nav1.1IQp complex, missing G(-4) and G(-2) in the GPGS tag.

For apo CaM+Nav1.1IQp, 98% of the $^{13}\text{C}\alpha$, 93% of the $^{13}\text{C}\beta$, and 97% of the $^{13}\text{C}'$ resonances were assigned for CaM, and 94% of the $^{13}\text{C}\alpha$ and $^{13}\text{C}'$, and 90% of the $^{13}\text{C}\beta$ resonances were assigned for Nav1.1IQp. For apo CaM+Nav1.4IQp, 99% of the $^{13}\text{C}\alpha$, 98% of the $^{13}\text{C}\beta$, and 97% of the $^{13}\text{C}'$ resonances were assigned for CaM, and 97% of the $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and $^{13}\text{C}'$ resonances were assigned for Nav1.4IQp. For apo CaM+Nav1.7IQp, 99% of the $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and $^{13}\text{C}'$ resonances were assigned for CaM, and 97% of the $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and $^{13}\text{C}'$ resonances were assigned for Nav1.7IQp. With the exception of the $\text{C}\beta$ resonance of S1918 in Nav1.1IQp, all missing assignments in the IQ peptides correspond to residues in the N-terminal GPGS tag.

TALOS+ was used to predict order parameters for apo CaM and the IQ motif peptides in the three complexes (Fig. 4A, 4B, 4C) using $^1\text{H}_\text{N}$, ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and $^{13}\text{C}'$ chemical shifts (Shen et al. 2009). The values predicted for apo CaM were similar to those observed in the apo CaM+Nav1.2IQp complex (Mahling, Kilpatrick, and Shea 2017), and agree well with previously determined high-resolution structures of free apo CaM such as 1CFD (Kuboniwa et al. 1995) and apo CaM in complex with the Nav1.5 IQ motif (Chagot and Chazin 2011). A colored bar above Fig. 4A shows the typical secondary structure of CaM. Residues predicted to have lower order parameters map to the calcium-binding loops (yellow), the loops between the EF-hands within each domain (gray), and the linker connecting CaM_N and CaM_C (gray).

The Nav1.1, Nav1.4, and Nav1.7 IQ motif peptides are predicted to have reduced order parameters only at the N- and C-termini (Fig. 4D, 4E, 4F). This is consistent with the peptides forming single amphipathic α -helices when bound to apo CaM, as was observed in high-resolution structures of apo CaM bound to the Nav1.2, Nav1.5, and Nav1.6 IQ motifs (Chagot and Chazin 2011; Feldkamp, Yu, and Shea 2011; Wang et al. 2012; Reddy Chichili et al. 2013; Gabelli et al. 2014).

To investigate the perturbations of apo CaM caused by binding to the Nav IQ peptides and determine the roles of the two CaM domains in the interaction with the Nav1.1, Nav1.4, and Nav1.7 IQ motifs, we calculated backbone amide chemical shift perturbations (CSPs) of CaM residues in apo CaM+Nav1.1_{IQp}, apo CaM+Nav1.4_{IQp}, and apo CaM+Nav1.7_{IQp} relative to free apo CaM (1CFD) (Kuboniwa et al. 1995). The histograms depicted in Fig. 4G, 4H, and 4I, show that, in all three complexes, resonances for CaM_C residues underwent large chemical shift perturbations upon peptide binding, with the largest perturbations observed in the loop connecting the two EF-hands of CaM_C. The large and numerous CSPs in CaM_C, and the very small CSPs in CaM_N (judged to be at the level of noise), demonstrate that apo CaM binds to the Nav1.1, Nav1.4, and Nav1.7 IQ motifs exclusively through CaM_C, as was observed previously in NMR studies of apo CaM bound to an IQ motif of Nav1.2 IQ motif (Mahling, Kilpatrick, and Shea 2017), 2KXW (Feldkamp, Yu, and Shea 2011) and Nav1.5 (Chagot and Chazin 2011).

These data show that a conserved interaction interface between apo CaM and the IQ motifs of Nav1.1, Nav1.2, Nav1.4, Nav1.5, and Nav1.7 is independent of CaM_N, and that apo CaM_C residues experience similar chemical environments when bound to IQ motif peptides. Additional structural studies are needed in order to understand the molecular basis of isoform-specific differences in CaM regulation of channel function, and additional electrophysiology studies are needed to clarify why investigators disagree over the functional roles of CaM in regulating these channels.

The backbone NMR assignments of the three complexes described here were deposited with the Biological Magnetic Resonance Data Bank and have BMRB accession numbers **27190** (apo CaM+Nav1.1_{IQp}), **27332** (apo CaM+Nav1.4_{IQp}), and **27260** (apo CaM+Nav1.7_{IQp}).

Acknowledgments

We thank Liping Yu and C. Andrew Fowler of the University of Iowa Carver College of Medicine (UI CCOM) NMR facility for technical assistance. These studies were supported by University of Iowa ICRU Fellowships (HMI, ZL), UI Carver College of Medicine FUTURE in Biomedicine Fellowship (AMK), NIH T32 NS045549 (RM), NIH R01 GM57001 (MAS), and UI OVPRED Major Research Grant (MAS). The content is solely the responsibility of the authors and does not necessarily represent the official views of any supporting organization or agency.

References

- Ben-Johny M, Yue DN, and Yue DT. 2016 'Detecting stoichiometry of macromolecular complexes in live cells using FRET', *Nature Communications*, 7: 13709.
- Branco T, Tozer A, Magnus CJ, Sugino K, Tanaka S, Lee AK, Wood JN, and Sternson SM. 2016 'Near-Perfect Synaptic Integration by Nav1.7 in Hypothalamic Neurons Regulates Body Weight', *Cell*, 165: 1749–61. [PubMed: 27315482]
- Chagot B, and Chazin WJ. 2011 'Solution NMR Structure of Apo-Calmodulin in Complex with the IQ Motif of Human Cardiac Sodium Channel Nav1.5', *J Mol Biol*, 406: 106–19. [PubMed: 21167176]
- Damo SM, Feldkamp MD, Chagot B, and Chazin WJ. 2013 'NMR studies of the interaction of calmodulin with IQ motif peptides', *Methods Mol Biol*, 963: 173–86. [PubMed: 23296611]
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, and Bax A. 1995 'NMRPipe: a multidimensional spectral processing system based on UNIX pipes', *Journal of Biomolecular NMR*, 6: 277–93. [PubMed: 8520220]

- Feldkamp Michael D., Yu Liping, and Shea Madeline A.. 2011 'Structural and Energetic Determinants of Apo Calmodulin Binding to the IQ Motif of the Na_v1.2 Voltage-Dependent Sodium Channel', *Structure*, 19: 733–47. [PubMed: 21439835]
- Gabelli SB, Boto A, Kuhns VH, Bianchet MA, Farinelli F, Aripirala S, Yoder J, Jakoncic J, Tomaselli GF, and Amzel LM. 2014 'Regulation of the Na_v1.5 cytoplasmic domain by calmodulin', *Nature Communications*, 5: 5126.
- Gabelli SB, Yoder JB, Tomaselli GF, and Amzel LM. 2016 'Calmodulin and Ca²⁺ control of voltage gated Na⁺ channels', *Channels (Austin)*, 10: 45–54. [PubMed: 26218606]
- Hovey L, Fowler CA, Mahling R, Lin Z, Miller MS, Marx DC, Yoder JB, Kim EH, Tefft KM, Waite BC, Feldkamp MD, Yu L, and Shea MA. 2017 'Calcium triggers reversal of calmodulin on nested anti-parallel sites in the IQ motif of the neuronal voltage-dependent sodium channel Na_v1.2', *Biophysical Chemistry*, 224: 1–19. [PubMed: 28343066]
- Huang W, Liu M, Yan SF, and Yan N. 2017 'Structure-based assessment of disease-related mutations in human voltage-gated sodium channels', *Protein Cell*, 8: 401–38. [PubMed: 28150151]
- Kim J, Ghosh S, Liu H, Tateyama M, Kass RS, and Pitt GS. 2004 'Calmodulin mediates Ca²⁺ sensitivity of sodium channels', *Journal of Biological Chemistry*, 279: 45004–12. [PubMed: 15316014]
- Kink JA, Maley ME, Preston RR, Ling K-Y, Wallen-Friedman MA, Saimi Y, and Kung C. 1990 'Mutations in Paramecium Calmodulin Indicate Functional Differences between the C-Terminal and N-Terminal Lobes in Vivo', *Cell*, 62: 165–74. [PubMed: 2163766]
- Kuboniwa H, Tjandra N, Grzesiek S, Ren H, Klee CB, and Bax A. 1995 'Solution structure of calcium-free calmodulin', *Nature Struct. Biol.*, 2: 768–76. [PubMed: 7552748]
- Mahling R, Kilpatrick AM, and Shea MA. 2017 'Backbone resonance assignments of complexes of human voltage-dependent sodium channel Na_v1.2 IQ motif peptide bound to apo calmodulin and to the C-domain fragment of apo calmodulin', *Biomol NMR Assign.*
- Mantegazza M, and Catterall WA. 2012 'Voltage-Gated Na⁺ Channels: Structure, Function, and Pathophysiology.' in Noebels JL, Avoli M, Rogawski MA, Olsen RW and Delgado-Escueta AV (eds.), *Jasper's Basic Mechanisms of the Epilepsies* (Bethesda (MD)).
- Marley J, Lu M, and Bracken C. 2001 'A method for efficient isotopic labeling of recombinant proteins', *Journal of Biomolecular NMR*, 20: 71–5. [PubMed: 11430757]
- Messner DJ, and Catterall WA. 1985 'The sodium channel from rat brain. Separation and characterization of subunits', *Journal of Biological Chemistry*, 260: 10597–604. [PubMed: 2411726]
- Mori M, Konno T, Ozawa T, Murata M, Imoto K, and Nagayama K. 2000 'Novel Interaction of the Voltage-Dependent Sodium Channel (VDSC) with Calmodulin: Does VDSC Acquire Calmodulin-Mediated Ca²⁺-Sensitivity?', *Biochemistry*, 39: 1316–23. [PubMed: 10684611]
- Pitt GS, and Lee SY. 2016 'Current view on regulation of voltage-gated sodium channels by calcium and auxiliary proteins', *Protein Science*, 25: 1573–84. [PubMed: 27262167]
- Reddy Chichili VP, Xiao Y, Seetharaman J, Cummins TR, and Sivaraman J. 2013 'Structural basis for the modulation of the neuronal voltage-gated sodium channel Na_v1.6 by calmodulin', *Sci Rep*, 3: 2435. [PubMed: 23942337]
- Sarhan MF, Tung CC, Van Petegem F, and Ahern CA. 2012 'Crystallographic basis for calcium regulation of sodium channels', *Proc Natl Acad Sci U S A*, 109: 3558–63. [PubMed: 22331908]
- Shen Y, Delaglio F, Cornilescu G, and Bax A. 2009 'TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts', *Journal of Biomolecular NMR*, 44: 213–23. [PubMed: 19548092]
- Sorensen BR, and Shea MA. 1998 'Interactions between domains of apo calmodulin alter calcium binding and stability', *Biochemistry*, 37: 4244–53. [PubMed: 9521747]
- Theoharis NT, Sorensen BR, Theisen-Toupal J, and Shea MA. 2008 'The Neuronal Voltage-Dependent Sodium Channel Type II IQ Motif Lowers the Calcium Affinity of the C-Domain of Calmodulin', *Biochemistry*, 47: 112–23. [PubMed: 18067319]
- Vranken WF, Boucher W, Stevens TJ, Fogh RH, Pajon A, Llinas M, Ulrich EL, Markley JL, Ionides J, and Laue ED. 2005 'The CCPN data model for NMR spectroscopy: development of a software pipeline', *Proteins*, 59: 687–96. [PubMed: 15815974]

- Wang C, Chung BC, Yan H, Lee SY, and Pitt GS. 2012 'Crystal Structure of the Ternary Complex of a Nav C-Terminal Domain, a Fibroblast Growth Factor Homologous Factor, and Calmodulin', Structure.
- Yan H, Wang C, Marx SO, and Pitt GS. 2017 'Calmodulin limits pathogenic Na⁺ channel persistent current', J Gen Physiol, 149: 277–93. [PubMed: 28087622]
- Young KA, and Caldwell JH. 2005 'Modulation of skeletal and cardiac voltage-gated sodium channels by calmodulin', Journal of Physiology, 565: 349–70. [PubMed: 15746172]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. ^1H , ^{15}N HSQC Spectrum of apo CaM+Nav1.1IQP.

Backbone resonance assignments are labeled in black for CaM and green for Nav1.1IQP. Numbers correspond to the full-length sequence of each protein. Residues in the N-terminal tag (GPS) of Nav1.1IQP are labeled with negative numbers. Peaks contoured in red were aliased. The crowded central region (denoted by a dashed box) was expanded to the right for clarity of labeling.

Figure 2. ^1H , ^{15}N HSQC Spectrum of apo CaM+Nav1.4-IQp.

Backbone resonance assignments are labeled in black for CaM and green for Nav1.4-IQp.

Numbers correspond to the full-length sequence of each protein. Residues in the N-terminal

tag (GPS) of Nav1.4-IQp are labeled with negative numbers. Peaks contoured in red were

aliased. The crowded central region (denoted by a dashed box) was expanded to the right for clarity of labeling.

Figure 3. ^1H , ^{15}N HSQC Spectrum of apo CaM+Nav1.7IQP.

Backbone resonance assignments are labeled in black for CaM and green for Nav1.7IQP.

Numbers correspond to the full-length sequence of each protein. Residues in the N-terminal tag (GPGS) of Nav1.7IQP are labeled with negative numbers. Peaks contoured in red were aliased. The crowded central region (denoted by a dashed box) was expanded to the right for clarity of labeling.

Figure 4. Predicted Order Parameters and Analysis of Chemical Shift Perturbations

Order parameters predicted by TALOS+ (Shen et al. 2009) for apo CaM (Fig. 4A, 4B, 4C) and Nav IQ peptides (Fig. 4D, 4E, 4F) using the assigned chemical shifts of the apo CaM + Nav1.1IQ_p, apo CaM + Nav1.4IQ_p, and apo CaM + Nav1.7IQ_p complexes. Colored bars above figures depict CaM and the IQp. The CaM_N helices A, B, C and D are blue, the CaM_C helices E, F, G, and H are red. The four calcium-binding sites (I, II, III, IV) are yellow, and the loops and interdomain linker are gray. The peptides are green, highlighting the initial IQ and terminal basic residue in the IQ motif [IQxxx[R,K]Gxxx[R,K]]. Histograms (Fig. 4G, 4H, 4I) show chemical shift perturbations ($\Delta\delta$, ppm, Eq. 1) of apo CaM residues upon binding Nav1.1IQ_p, Nav1.4IQ_p, or Nav1.7IQ_p. Proline residues (P43, P66) and residues that were unassigned in either free apo CaM or the complexes are marked by small negative bars.