Structural analyses of Ca$^{2+}$/CaM interaction with Na$_V$ channel C-termini reveal mechanisms of calcium-dependent regulation

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Ca$^{2+}$ regulates voltage-gated Na$^+$ (Na$_V$) channels, and perturbed Ca$^{2+}$ regulation of Na$_V$ function is associated with epilepsy syndromes, autism and cardiac arrhythmias. Understanding the disease mechanisms, however, has been hindered by a lack of structural information and competing models for how Ca$^{2+}$ affects Na$_V$ channel function. Here we report the crystal structures of two ternary complexes of a human Na$_V$ cytosolic C-terminal domain (CTD), a fibroblast growth factor homologous factor and Ca$^{2+}$/calmodulin (Ca$^{2+}$/CaM). These structures rule out direct binding of Ca$^{2+}$ to the Na$_V$ CTD and uncover new contacts between CaM and the Na$_V$ CTD. Probing these new contacts with biochemical and functional experiments allows us to propose a mechanism by which Ca$^{2+}$ could regulate Na$_V$ channels. Further, our model provides hints towards understanding the molecular basis of the neurologic disorders and cardiac arrhythmias caused by Na$_V$ channel mutations.
Voltage-gated Na\(^+\) (Na\(_V\)) channels underlie the rapid upstroke of action potentials. Mammalian Na\(_V\) channels are pseudotetramers with six-transmembrane segment repeats joined by intracellular linkers and flanked by intracellular N- and C-termini. The four repeats, each of which contains a voltage sensor, assemble to form a central pore. Although recent crystal structures of the tetrameric bacterial Na\(_V\) channel from *Arcobacter butzleri* (Na\(_V\)Ab) provided detail about the pore and voltage sensors\(^{1,2}\), Na\(_V\)Ab tetramers lack the intracellular linkers and termini of mammalian Na\(_V\) channels. Those components are of particular interest because they confer isoform-specific regulatory effects, serve as sites of interaction for critical modulatory proteins and are loci for many disease-causing mutations.

The C-terminal domain (CTD) is of special interest because it exerts powerful effects upon channel inactivation\(^3\) and is the interaction site for several auxiliary proteins that modulate channel function, such as calmodulin (CaM) and fibroblast growth factor homologous factors (FFHs), both of which regulate excitability through their CTD interactions\(^4,5\). Moreover, many disease-causing mutations localize to Na\(_V\) CTDs or their associated proteins. Key examples include mutations in SCN1A and SCN2A (which encode the neuronal channels Na\(_V\)1.1 and Na\(_V\)1.2, respectively) that lead to various epilepsy syndromes, ataxia and autism\(^6–8\) or in the CTD of Na\(_V\)1.5, the cardiac Na\(_V\) channel encoded by SCN5A, which is a hotspot for mutations causing arrhythmias, cardiomyopathy and sudden infant death syndrome\(^9–14\). Likewise, mutations in FFHs or CaM have been associated with neurodegenerative disorders, cognitive deficits and arrhythmias\(^14–16\). Structural information about Na\(_V\) CTDs, however, has been limited. How the associated regulatory proteins influence channel function and how mutations in the CTDs or associated auxiliary proteins perturb channel function and at the molecular level are not well understood.

Among the proteins associated with Na\(_V\) CTDs, CaM is of particular interest because it acts as a sensor for Ca\(^2+\), which serves as a critical signal of electrical activity, providing powerful feedback regulation upon Na\(_V\) channel function\(^7\). Still, how Ca\(^2+\) and CaM affect Na\(_V\) channels have been controversial since sequence analysis of Na\(_V\) channels first revealed the presence of potential CaM-binding sites, including an ‘IQ’ motif\(^8\) and a potential Ca\(^2+\)-binding site within the CTD\(^9\). Obtaining an understanding for CaM regulation of Na\(_V\) channels has been further complicated by apparent isoform-specific regulation. For example, CaM affects inactivation properties of the neuronal Na\(_V\)1.6 but not the skeletal muscle Na\(_V\)1.4 (ref. 19). For the cardiac Na\(_V\)1.5, CaM affects several different properties, including channel inactivation and persistent current\(^20,21\). Nevertheless, the identification of disease-causing mutations within or near the Na\(_V\) IQ motifs of several Na\(_V\) isoforms\(^6,20,22–24\) highlights important roles for CaM. The potential significance of CaM-binding to Na\(_V\) channels has been further spotlighted by recent exome-sequencing studies in which searches for repeated rare variants or *de novo* mutations associated with autism identified SCN1A and SCN2A among the small list of loci\(^25–27\); several of these catalogued Na\(_V\) mutations cluster in and around the IQ motif.

A major barrier to understanding how Ca\(^2+\) and CaM act on Na\(_V\) channels has been that structural information is limited to Ca\(^2+\)-free CaM (apoCaM) interacting with the CTD. While such...
studies defined an interaction between the decalcified C-lobe of CaM and the IQ motif that alludes to NaV function and did not provide insight into mechanisms for IQ motif disease mutations, including a familial autism mutation in the neuronal NaV1.2 channel and a cardiac arrhythmia mutation in the cardiac NaV1.5 (ref. 20) that fall outside of the apoCaM contact sites.

Here we present crystal structures of NaV1.2 and NaV1.5 CTDs bound to Ca2+/CaM. Comparison with our previous structure obtained with apoCaM reveals novel and unexpected Ca2+/CaM contacts and stark differences in the overall conformation of the ternary complex, including a Ca2+-dependent interaction between the CaM N-lobe and an extended helix that contains the IQ motif. Together, these findings provide a basis for understanding the effects of specific disease-causing mutations within NaV CTD domains.

**Results**

**Ternary complex structures of a NaV CTD and FHF and Ca2+/CaM.** To define how Ca2+ regulates NaV channels, we solved crystal structures of complexes containing a NaV CTD, Ca2+/CaM and a FHF. FHFs are constitutive NaV subunits in the brain and heart and their inclusion allows us to compare Ca2+/CaM structures with our previous complex containing apoCaM. We tested several different combinations of FHFs and NaV CTDs with Ca2+/CaM and eventually succeeded in crystallizing two ternary complexes: a 6xHis-tagged human NaV1.5 CTD (amino acids 1,773–1,940), human FGF12B and CaM; and a 6xHis-tagged human NaV1.2 CTD (amino acids 1,777–1,937), human FGF13U and CaM. The sequences of the NaV CTDs are highly conserved among the subtypes (76% identities between NaV1.5 and NaV1.2, and 91% of the amino acids are conserved; Fig. 1a) and the solution structures of the proximal NaV1.2 CTD and NaV1.5 CTD are nearly identical and the solution structures of the proximal NaV1.2 CTD and NaV1.5 CTD are nearly identical.

Likewise, FGF12B and FGF13U are highly conserved (69% identities) and their crystal structures in the absence of any binding partners are similar. Thus, we anticipated significant similarities between the NaV1.2- and NaV1.5-containing complexes.

Both complexes were expressed in *Escherichia coli* and purified in the presence of 2 mM Ca2+ by Co2+-affinity chromatography followed by size exclusion chromatography. The two ternary complexes (combined *Mw* ~ 60 kDa) were stable and eluted in a single peak on a size exclusion column (Supplementary Fig. 1). Their individual profiles were highly similar to each other and to what we observed for the ternary complex containing the NaV1.5 CTD, FGF13U and CaM purified in EGTA, for which we had demonstrated a stoichiometry of 1:1:1 (ref. 30).

The complex containing NaV1.2 CTD, FGF13U and CaM (hereafter referred to as NaV1.2/Ca2+2) was crystallized in the C2 space group with two copies of the ternary complex in each asymmetric unit. The crystals were grown in the presence of 2 mM Ca2+ and diffracted to 3.02 Å Bragg spacings. The experimental phases were derived by single anomalous dispersion from selenomethionine (SeMet)-substituted crystals and improved by twofold non-crystallographic averaging, which yielded a good-quality electron density map (Supplementary Fig. 2A). The final model contains the NaV1.2 amino acids 1,773–1,929, FGF13U amino acids 11–158 and the CaM amino acids 7–149. The final model was refined to a resolution of 2.5/24.6% (Table 1). The ternary complex containing NaV1.5 CTD, FGF12B and CaM (hereafter referred to as NaV1.5/Ca2+2) was crystallized in the P3121 space group with one copy of the ternary complex in each asymmetric unit. The crystals were grown in the presence of 2 mM Ca2+ and diffracted anisotropically to 3.8/5.4/6.0 Å Bragg spacings. Molecular replacement was performed to obtain the phase (see Methods for the details). The final model contains the NaV1.5 amino acids 1,786–1,927, FGF12B amino acids 12–152, and the CaM amino acids 7–148. Despite the resolution limit, the model refined to good statistics (Rwork/Rfree of 26.2/31.8%) and good geometry (Table 1 and Methods for the refinement). 2Fo–Fc OMIT map shows a good-quality electron density, supporting the accuracy of the model given the resolution (Supplementary Fig. 2B). There is no significant difference in the refinement statistics when the data were truncated to 6.0 Å (Table 1).

Figure 1b,c shows the overall architecture of the NaV1.2/Ca2+2 and NaV1.5/Ca2+2 ternary complexes, respectively. In both complexes, the NaV CTD comprises one globular domain that contains an EF-hand fold followed by an extended helix that contains the IQ motif. We refer to this helix as the IQ domain. The FHF binds to the CTD globular domain and CaM binds to the IQ domain. When the NaV1.5/Ca2+2 and the NaV1.2/Ca2+2 structures were superimposed with respect to their IQ domain, structural conservation was observed within the region containing the IQ domain and CaM (r.m.s.d. of 1.7 Å; Fig. 1d).

When the two structures (stripped of their respective CaM molecules) are superimposed relative to their respective CTD’s globular domains together with FHFs, both NaV CTD globular domains and FHFs are very similar to the r.m.s.d. of 0.89 Å, with their IQ domains out of register (Fig. 1e) because of different angles between their CTD’s globular domain (along with FHFs) and their IQ domain. The different angle may reflect an isoform-specific structural difference or inherent flexibility between the two domains of NaV CTD.

To focus on the Ca2+-dependent conformational changes, we also superimposed our previously solved structure of a ternary complex of NaV1.5 CTD, FGF13 and CaM crystallized in the absence of Ca2+ (ref. 30), hereafter referred to as NaV1.5/-Ca2+. With the CaM molecules stripped for clarity, Fig. 1e shows that the difference in angle between the respective NaV CTD global domains and IQ domains is even more pronounced between NaV1.5/Ca2+2 and NaV1.5/-Ca2+ than between NaV1.2/Ca2+2 and NaV1.5/Ca2+2 (Fig. 1e). Although it is possible that these rigid-body motions between the CTD global domains and their respective IQ domains are associated with Ca2+ binding, these motions can also be due to inherent flexibility between these two domains. As the NaV1.2/Ca2+2 structure provides higher resolution than the NaV1.5/Ca2+2 structure and the differences are otherwise minimal, subsequent analyses focus only on the NaV1.2/Ca2+2 structure.

**A Ca2+-dependent CaM N-lobe interaction with the IQ domain.** The most significant effects of Ca2+ are the changes in the interactions between CaM and the respective IQ domains as shown in Fig. 2a,b, in which the NaV1.2/Ca2+2 and NaV1.5/-Ca2+2 structures are aligned by their IQ domains. The CaM in the NaV1.5/-Ca2+2 structure adopts an extended conformation with the α-helical interlobular linker between the CaM/N- and C-lobes holding the N-lobe away from the C-lobe that binds to the proximal portion (IQ motif) of the IQ domain. NMR structures of Ca2+-free CaM and an isolated IQ domain peptide from either NaV1.2 or NaV1.5 also showed an interaction between the CaM C-lobe and the IQ motif, but not the CaM N-lobe and the IQ domain. In our new NaV1.2/Ca2+2 structure, in contrast, the α-helical interlobular linker is unwound, thereby allowing the Ca2+-bound CaM N-lobe to envelope the distal portion of the IQ domain and provide additional contacts between the linker and the IQ domain and between the CaM N-lobe and the IQ domain that are not present in the absence of Ca2+. These new contacts include residues mutated in channelopathies, as discussed below.

Superposition of NaV1.5/-Ca2+2 and NaV1.2/Ca2+2 structures shows that there are only minor conformational changes with
Fourier peaks in the Ca$^{2+}$ only weak peaks were observed in the Ca$^{2+}$ 'closed' conformation in the NaV1.2/Ca$^{2+}$ structure. However, occurred within the N-lobe of CaM, which assumes a (NaV1.2/Ca$^{2+}$) and the distal C-terminal portion of the IQ domain is driven mainly through van der Waals forces (Fig. 2e), burying Na$\nu_1$.2 hydrophobic side chains (Leu1,920, Leu1,921, Val1,925 and Val1,928) and has functional and disease-related implications. First, this additional interaction between the distal IQ domain and the CaM N-lobe offers an explanation for a previous report that an Ala1,924Thr mutation in the cardiac NaV1.5 channel (equivalent to Val1,928 in NaV1.2) causes the life-threatening arrhythmia Brugada Syndrome and eliminates the Ca$^{2+}$/CaM-dependent slow inactivation observed for the wild-type NaV1.5 channel$^{20}$. We hypothesized that the Ala1,924Thr mutation in the NaV1.5 CTD affected the affinity for Ca$^{2+}$/CaM and tested the hypothesis by isothermal calorimetry (ITC). Indeed, in the presence of saturating 5 mM Ca$^{2+}$, the mutation reduced the affinity for Ca$^{2+}$/CaM by approximately threefold compared with the wild-type NaV1.5 CTD (Supplementary Fig. 4A and Table 2). In contrast, the affinity of the mutant NaV1.5 CTD for CaM in the absence of Ca$^{2+}$ was mildly increased (Table 2). Second, we found that the CaM N-lobe interaction with the distal IQ domain provides a significant boost to the affinity of Ca$^{2+}$/CaM for the Na$\nu_1$.5 CTD. A previous report using ITC$^{58}$ had found the affinity of Ca$^{2+}$/CaM for a Na$\nu_1$.5 peptide containing the IQ motif to be ~2.1 mM. Those thermodynamic parameters, however, were obtained in experiments employing an IQ domain in which the newly discovered CaM N-lobe contact site is truncated. We therefore performed ITC with a longer Na$\nu_1$.5 CTD (through amino acid 1,940) and observed a significantly higher affinity for both Ca$^{2+}$/CaM and apoCaM (~100 nM, as shown in Supplementary Fig. 4A,B and Table 2). To assure that the lower $K_d$ values we obtained were indeed

| Table 1 | Data collection, phasing and refinement statistics. |
|---|---|---|
| SeMet Na$\nu_1$.2/CaM/FGF13U/Ca$^{2+}$ | Na$\nu_1$.5/CaM/FGF12B/Ca$^{2+}$ | Na$\nu_1$.5/CaM/FGF12B/Ca$^{2+}$ with 6 Å cutoff |
| **Data collection** | | |
| Space group | C2 | P3,21 | P3,21 |
| Wavelength (Å) | 0.9792 | 1.0 | 1.0 |
| **Cell dimensions** | | | |
| $a$, $b$, $c$ (Å) | 153.13, 86.11, 109.40 | 115.199, 115.199, 120.107 | 115.199, 115.199, 120.107 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 | 90.0, 90.0, 90.0 |
| Resolution (Å) | 50.00–3.02 (3.07–3.02) | 50.00–3.80 (3.87–3.80)* | 50.00–6.00 (6.10–6.00) |
| $R_{\text{work}}$ (%) | 16.4 (48.3) | 6.9 (39.2) | 5.7 (24.1) |
| $I/\sigma I$ | 16.8 (1.5) | 34.7 (1.8) | 53.0 (7.6) |
| Completeness (%) | 94.3 (66.6) | 46.1 (13.2) | 92.6 (61.5) |
| Redundancy | 12.5 (5.6) | 7.2 (2.2) | 8.5 (6.5) |
| **SAD phasing** | | | |
| Figure of merit | 0.30 (50.0–3.02 Å) | | |
| **Refinement** | | | |
| Resolution (Å) | 48.23–3.02 (3.09–3.02) | 41.75–3.84 (5.00–3.54)* | 41.75–6.00 (7.00–6.00) |
| Completeness (%) | 91.8 (53.0) | 40.53 (12.91) | 92.89 (81.72) |
| No. of reflections | 26,179 | 3,700 | 2,301 |
| $R_{\text{work}}/\text{free}$ | 21.48/24.64 | 26.01/31.75 | 23.61/31.82 |
| Ramachandran (%) | | | |
| Favour | 95.7 | 95.5 | 95.5 |
| Outliers | 0.4 | 0.5 | 0.5 |
| $R_{\text{m.s.d}}$ Bond lengths (Å) | 0.011 | 0.011 | 0.012 |
| Bond angles (°) | 1.04 | 0.996 | 1.093 |

*SAD, single anomalous dispersion; SeMet, selenomethionine.

The crystal diffracts anisotropically to 3.8/5.4/6.0 Å.

The data treated with ellipsoidal truncation and anisotropic scaling were used for refinement (1).

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because of the longer CTD and not because of technical differences, we measured the affinity of CaM for the NaV1.5 CTD truncated at amino acid 1,924, for which we obtained a value of 2.0 ± 0.4 μM in 5 mM Ca2+ (Table 2 and Supplementary Fig. 4B). This result is in excellent agreement with the value previously obtained38, thereby allowing us to benchmark our thermodynamic data against that report. Thus, the ~20-fold higher affinity for CaM obtained with the longer CTD highlights critical contributions of the more distal IQ domain residues. Comparison of the N values of interactions for Ca2+/CaM with the two CTDs by ITC (Table 2) further demonstrates the importance of the more distal residues in the IQ domain. For the
shorter CTD, the N value of the Ca$^{2+}$/CaM–CTD interaction is close to ~0.5 (0.38 in our measurement, and 0.56 previously reported$^{38}$), suggesting that one Ca$^{2+}$/CaM can bind two CTDs. The N value we obtained for the longer CTD was doubled (0.84), suggesting that one Ca$^{2+}$/CaM binds one CTD, and fits well with our crystallographic observation that both N- and C-lobes simultaneously bind to different sites within the IQ domain of NaV1 CTD. Consistent with our observation, N value of the interaction between Ca$^{2+}$/CaM and Ala1924Thr of the longer CTD is reduced to half of the wild type (0.42; Table 2). Thus, these data underlined the importance of the interaction between CaM and the more distal region of the IQ domain and supported a model in which there are simultaneous interactions of the two CaM lobes (at different sites on the IQ domain).

**Ca$^{2+}$-dependent changes and disease mechanisms.** While the new CaM N-lobe contact with the NaV1.2 CTD is the most obvious Ca$^{2+}$-dependent structural change, we also identified additional new Ca$^{2+}$/CaM contact sites within the NaV1.2 IQ domain, which may provide insight for several other NaV channelopathies. For example, Arg1918 (Fig. 3a,b), which contacts Asp79 and Ser82 in the CaM interlobular linker in the Ca$^{2+}$-loaded complex (but not in the apoCaM structure), was reported in a patient with febrile seizures and childhood absence epilepsy$^{23}$, and mutation of the equivalent Arg1928 in the homologous NaV1.1 was found in a patient with severe myoclonic epilepsy of infancy$^{24}$.

In addition, of particular interest was a familial autism mutation Arg1902Cys in NaV1.2 (ref. 7) especially since recent analyses of rare de novo mutations in subjects with autism have identified SCN2A as one of a handful of high-confidence autism spectrum disorder genes$^{25,39}$. Arg1902 sits at the hinge between the CTD globular domain and the IQ domain helix (Fig. 3c). We had previously found that the Arg1902Cys mutation conferred a Ca$^{2+}$/CaM-dependent conformational change indicated by a significant Ca$^{2+}$-dependent shift in migration on a size exclusion column of a NaV1.2 CTD/CaM binary complex that was not observed with the wild-type complex$^{31}$. Although Arg1902 (or the Arg1898 equivalent in NaV1.5) does not make any direct contacts with CaM either in the presence or in the absence of Ca$^{2+}$, it interacts with the side chain of Glu1905 (Glu1901 in NaV1.5) one turn below along the IQ domain helix and also forms cation–π interaction with the side chain of Tyr98 in FGF13U in the NaV1.5/Ca$^{2+}$ structure. Tyr98 in FGF13 and Glu1905 in NaV1.2 (Glu1901 in NaV1.5) also interact with Lys95 within the third CaM EF-hand in the CaM C-lobe. Interestingly, Glu1905 in NaV1.2, Tyr98 in FGF13U and Lys95 in CaM are the only residues within the ternary complex that make interactions with each of the other partners. Thus, we suspected that the relayed interactions from Lys95 (CaM) through Glu1905 (NaV IQ domain) to Arg1902 (Nav globular domain), stabilized by the cation–π interaction with Tyr98 in FGF13, suppress the Ca$^{2+}$-dependence of NaV1.2 channels and that disruption of these interactions would affect Ca$^{2+}$-dependent regulation of NaV1.2 channels (Fig. 3c). We tested this hypothesis in two ways.

First, we expressed either wild-type or Arg1902Cys-mutant NaV1.2 along with FGF14, the best characterized neuronal FHF$^{4,31,40}$ in HEK293 cells, in which endogenous CaM is abundant, and recorded Na$^{+}$ currents in the presence of saturating internal Ca$^{2+}$ or nominally zero internal Ca$^{2+}$ (Supplementary Fig. 5). For wild-type NaV1.2, inclusion of 10 μM Ca$^{2+}$ in the pipette did not affect peak current density, the V1/2 of activation or the V1/2 of steady-state inactivation. In contrast, for the Arg1902Cys mutant the addition of Ca$^{2+}$ induced a large approximately −10 mV shift in the V1/2 of steady-state activation and inactivation (Fig. 3d, Supplementary Fig. 6A and Supplementary Table 1). To test further our hypothesis that disruption of the NaV CTD to CaM relay affected Ca$^{2+}$-dependent regulation of NaV1.2, we ablated the key intermediary by mutating NaV1.2 Glu1905 to Gln. Recordings from the Glu1905Gln-mutant channels phenocopied those from the Arg1902Cys-mutant channels (Supplementary Fig. 6A and Supplementary Table 1). Second, to test the proposed role of Arg1902 in the relayed interactions in NaV1.2 CTD/apoCaM, we measured the affinity of apoCaM for the wild-type and Arg1902Cys-mutant NaV1.2 CTDs by ITC. Consistent with our hypothesis, the Arg1902Cys mutation reduced affinity of apoCaM for the NaV1.2 CTD significantly (Table 3 and Supplementary Fig. 4C). We did not observe a difference in affinity for Ca$^{2+}$/CaM between the wild-type and the Arg1902Cys-mutant CTD (Table 3 and Supplementary Fig. 4D). As our ITC measurement in the presence of Ca$^{2+}$ could be complicated by an additional binding process because of Ca$^{2+}$-loading of the CaM C-lobe, which is essentially unoccupied in the NaV1.2/Ca$^{2+}$ structure (Supplementary Fig. 3), we therefore prepared CaM in which the third and fourth EF hands were mutated to ablate Ca$^{2+}$ binding to the CaM C-lobe$^{41}$ and repeated the ITC measurements with this crippled CaM$^{44}$ mutant. The ITC experiment with the CaM$^{44}$ mutant showed a reduced affinity for the Arg1902Cys mutant compared with the wild CTD in the presence of Ca$^{2+}$ (Table 3 and Supplementary Fig. 4E). Thus, these data are consistent with the previously observed shift in mobility on gel filtration of the Arg1902Cys/CaM binary complex$^{33}$ and suggest that by disrupting the relayed interactions to CaM the familial autism NaV1.2 Arg1902Cys mutation revealed Ca$^{2+}$-dependent effects upon channel function that were suppressed in the wild-type channel.

We next investigated whether the Ca$^{2+}$-dependent interaction of the CaM N-lobe was required for the Ca$^{2+}$-dependent regulation of NaV1.2 exposed by the Arg1902Cys mutation. We mutated Val1925, one of the NaV1.2 hydrophobic side chains buried by the calcified CaM N-lobe (see Fig. 2e), to Lys and examined how this mutant affected the Ca$^{2+}$-dependent shift in activation and steady-state inactivation in the context of the Arg1902Cys mutant. With this additional Val1925Lys mutation, the Arg1902Cys mutant no longer displayed any Ca$^{2+}$-dependent effects on either activation or steady-state inactivation.

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**Table 2 | Thermodynamic parameters for Ca interaction with the NaV1.5 CTD.**

| Titrant       | N   | Cell        | $K_a$ (nM) | Δ$H$ (kcal mol$^{-1}$) | Δ$S$ (cal mol$^{-1}$ deg$^{-1}$) | N value |
|---------------|-----|-------------|------------|------------------------|-------------------------------|---------|
| Ca$^{2+}$/CaM | 3   | 1,773-1924  | 1,990 ± 358$^*$ | −3.77 ± 0.27           | 12.4 ± 0.5                    | 0.38 ± 0.02 |
| apoCaM       | 4   | 1,773-1940  | 88 ± 6      | −10.69 ± 0.06          | −4.2 ± 0.3                     | 0.94 ± 0.03 |
| Ca$^{2+}$/CaM | 3   | 1,773-1940  | 132 ± 9     | −9.94 ± 0.33           | −2.4 ± 0.1                     | 0.83 ± 0.05 |
| Ca$^{2+}$/CaM | 3   | 1,773-1940 (A1924T) | 497 ± 32$^*$ | −7.81 ± 0.09           | 2.1 ± 0.2                      | 0.42 ± 0.02 |
| apoCaM       | 3   | 1,773-1940 (A1924T) | 61 ± 4$^*$  | −11.16 ± 0.14          | −5.1 ± 0.3                     | 0.70 ± 0.01 |

CaM, calmodulin; CTD, C-terminal domain. $^*$P<0.05 compared with NaV1.5 CTD (amino acids 1,773-1,940) plus Ca$^{2+}$/CaM.
Ca\(^{2+}\) binding is restricted to CaM. Our analyses suggested that Ca\(^{2+}\)-dependent regulation of Na\(_V\) channel function derives from Ca\(^{2+}\)-dependent changes in the interaction between CaM and the Na\(_V\) CTD, yet it has previously been suggested that Ca\(^{2+}\) also affects Na\(_V\) channels by binding directly to an EF-hand motif within the Na\(_V\) CTD influences Na\(_V\) channel function\(^{18,34,42}\). To query whether Ca\(^{2+}\) can bind directly to the Na\(_V\) CTD EF-hand motif, we used our anomalous scattering studies. Even though we detected anomalous difference signals from many sulphur atoms (in methionines) whose signal is approximately twofold weaker than that for Ca\(^{2+}\), we did not detect anomalous difference signal for Ca\(^{2+}\) within the proposed Ca\(^{2+}\)-binding loops (Fig. 4a) and we observed a strong signal for Ca\(^{2+}\) bound to the CaM N-lobe (Supplementary Fig. 3). Comparing the acidic and polar residues proposed to coordinate Ca\(^{2+}\) in the putative Na\(_V\) CTD EF-hand\(^{18}\) with those in the Ca\(^{2+}\)-binding EF hands in the CaM N-lobe of the associated CaM provides an explanation. While both EF hands of the Ca\(^{2+}\)-loaded CaM N-lobe contain a sufficient number of acidic and polar residues...
Table 3 | Thermodynamic parameters for CaM interaction with the NaV1.2 CTD.

| Titrant       | N  | Cell | $K_d$ (nM) | $\Delta H$ (kcal mol$^{-1}$) | $\Delta S$ (cal mol$^{-1}$ deg$^{-1}$) | $N$ value |
|---------------|----|------|------------|-----------------------------|----------------------------------|-----------|
| apoCaM        | 3  | WT   | 36 ± 6     | -4.54 ± 0.1                 | 18.6 ± 0.2                        | 1.02 ± 0.02 |
| Ca$^{2+}$/CaM | 3  | WT   | 1,713 ± 333| 6.01 ± 0.5                  | 47.0 ± 1.3                        | 0.76 ± 0.02 |
| Ca$^{2+}$/CaM$_{34}$ | 3  | WT   | 388 ± 22   | -13.85 ± 0.4                | -17.9 ± 1.4                       | 0.86 ± 0.05 |
| apoCaM        | 3  | R1902C | 958 ± 80*  | -3.28 ± 0.3                 | -2.1 ± 0.2                        | 0.58 ± 0.13 |
| Ca$^{2+}$/CaM | 3  | R1902C | 1,510 ± 187| 6.19 ± 0.9                  | 48.0 ± 2.8                        | 0.74 ± 0.03 |
| Ca$^{2+}$/CaM$_{34}$ | 3  | R1902C | 2,931 ± 123* | -12.53 ± 0.9 | 17.4 ± 2.8 | 0.49 ± 0.02 |

CaM, calmodulin; CTD, C-terminal domain; WT, wild type.
*P<0.05 compared with WT.

Discussion

Whether and how Ca$^{2+}$ contributes to the regulation of voltage-gated Na$_V$ currents has been a focus of significant controversy since potential CaM-binding sites were first identified within Na$_V$ CTDs. Our new structural model with Ca$^{2+}$/CaM bound to the NaV1.2 CTD, in context with previous structures demonstrating the interaction of apoCaM with various Na$_V$ CTDs, reveals novel and unexpected interactions between the Ca$^{2+}$/apoCaM and the Na$_V$ CTD. The apoCaM structures showed that the CaM C-lobe is anchored to the signature IQ motif within the extended IQ domain. On the basis of our new structural, biochemical and functional data, we propose that a major action of Ca$^{2+}$ is to induce a conformational switch in the anchored CaM so that the CaM N-lobe swings into contact with the distal IQ domain, while the Ca$^{2+}$-free CaM C-lobe remains anchored to the IQ motif (Fig. 2b). Interestingly, the new contact site for the calcified CaM N-lobe sits within a previously identified peptide that, when isolated from the adjacent IQ motif peptide, could only bind Ca$^{2+}$/apoCaM, in contrast to the IQ motif that supported apoCaM binding over Ca$^{2+}$/CaM binding.

In addition, we observed that Ca$^{2+}$ induces rearrangements between the Na$_V$ CTD and the CaM intralobular linker, and between the Na$_V$ CTD and the CaM C-lobe (Fig. 3b). We hypothesize that, together, these conformational changes may be propagated to the adjacent domain IV (DIV) transmembrane region of the channel to thereby affect Na$_V$ function in an isoform-specific manner. Since our structures do not contain the transmembrane region of the channel, our model cannot explain how the conformational changes propagate to DIV. However, it is known that the conformational change of DIV voltage sensor (S4) is the rate-limiting step for channel inactivation. Thus, the Na$_V$ CTD is in an advantageous position to affect channel gating.

Interestingly, the specific Ca$^{2+}$/CaM-dependent effects appear to vary among different Na$_V$ channels. Our data add to that concept in which we found that the wild-type NaV1.2 channel, not previously studied, was insensitive to Ca$^{2+}$/CaM for the parameters we studied at either nominally zero or saturating (~10 μM) intracellular Ca$^{2+}$. While the specific concentrations of internal Ca$^{2+}$ studied here are outside the range of physiologic Ca$^{2+}$ in neurons, these two levels allowed us to explore the bounds of Ca$^{2+}$, and correlate to our structures, obtained in the absence or presence of Ca$^{2+}$. These functional studies were also performed in the presence of a FFH, which was a component of the crystallized ternary complexes. Whether FFHs influence the Ca$^{2+}$ dependence of Na$_V$ currents has not yet been analysed. However, their inclusion in the functional studies is appropriate not only because of their presence in the crystal structures but also because of growing evidence that FFHs are important regulators of Na$_V$ currents in the neurons and not likely bind Ca$^{2+}$; Ca$^{2+}$-dependent effects on Na$_V$ channel function are more likely mediated via CaM.

Table 3 | Thermodynamic parameters for CaM interaction with the NaV1.2 CTD.

| Titrant       | N  | Cell | $K_d$ (nM) | $\Delta H$ (kcal mol$^{-1}$) | $\Delta S$ (cal mol$^{-1}$ deg$^{-1}$) | $N$ value |
|---------------|----|------|------------|-----------------------------|----------------------------------|-----------|
| apoCaM        | 3  | WT   | 36 ± 6     | -4.54 ± 0.1                 | 18.6 ± 0.2                        | 1.02 ± 0.02 |
| Ca$^{2+}$/CaM | 3  | WT   | 1,713 ± 333| 6.01 ± 0.5                  | 47.0 ± 1.3                        | 0.76 ± 0.02 |
| Ca$^{2+}$/CaM$_{34}$ | 3  | WT   | 388 ± 22   | -13.85 ± 0.4                | -17.9 ± 1.4                       | 0.86 ± 0.05 |
| apoCaM        | 3  | R1902C | 958 ± 80*  | -3.28 ± 0.3                 | -2.1 ± 0.2                        | 0.58 ± 0.13 |
| Ca$^{2+}$/CaM | 3  | R1902C | 1,510 ± 187| 6.19 ± 0.9                  | 48.0 ± 2.8                        | 0.74 ± 0.03 |
| Ca$^{2+}$/CaM$_{34}$ | 3  | R1902C | 2,931 ± 123* | -12.53 ± 0.9 | 17.4 ± 2.8 | 0.49 ± 0.02 |

CaM, calmodulin; CTD, C-terminal domain; WT, wild type.
*P<0.05 compared with WT.
cardiomyocytes in which Na\textsubscript{V}1.2 and Na\textsubscript{V}1.5 are expressed.

Nevertheless, the familial autism mutation Arg1902Cys introduced a large Ca\textsuperscript{2+}-dependent shift in both channel activation and steady-state inactivation (Fig. 3 and discussed below). A gain-of-function effect of a channelopathic mutation is reminiscent to the mechanism by which mutations in Na\textsubscript{V}1.5 lead to Long QT Syndrome\textsuperscript{9,10} and in some Na\textsubscript{V}1.2 mutations associated with epilepsy\textsuperscript{46}. Combined with analysis of the Na\textsubscript{V}1.2/Ca\textsuperscript{2+} structure, our functional data suggest that the relayed interactions from Lys95 (CaM) through Glu1905 (Nav IQ domain) to Arg1902 (Nav globular domain) mask a Ca\textsuperscript{2+} interaction from Lys95 (CaM) through Glu1905 (Nav IQ domain) to Arg1902Cys familial autism mutant when the relay is disrupted. It is noteworthy that an Asp96Val mutation adjacent to Lys95 in CaM, recently reported in a patient with an arrhythmia syndrome, was also associated with moderate cognitive impairment\textsuperscript{14}.

Taken together, we suggest that similar Ca\textsuperscript{2+}-induced conformational changes of CaM in both Na\textsubscript{V}1.2 and Na\textsubscript{V}1.5 (interactions of the CaM N-lobe to the distal IQ domain of Na\textsubscript{V} CTD) might be responsible for Ca\textsuperscript{2+}-dependent regulation, and that their functional effects are isoform-specific. The concept that Ca\textsuperscript{2+}-dependent regulation may be isoform-specific is consistent with a recent report showing that a rapid increase in intracellular Ca\textsuperscript{2+} diminished transient Na\textsubscript{V} currents through Na\textsubscript{V}1.4, but not through the Na\textsubscript{V}1.5 isoform\textsuperscript{47}. With regard to Na\textsubscript{V}1.2, only Arg1902Cys or Glu1905Gln unveiled a Ca\textsuperscript{2+}-dependent functional effect. We reasoned that there are possible reasons for these isoform-specific differences of Ca\textsuperscript{2+} dependence. First, in the context of the full-length channels, it is possible that the conformational changes at the CTD could propagate to the DIV transmembrane region of the channel differently depending on the isoforms. The apparent isoform-specific difference in the angle between the Na\textsubscript{V} globular domain and the extended IQ domain reported herein (Fig. 1e) provide one possibility. For example, perhaps the difference in the angle within Na\textsubscript{V}1.2 masks the Ca\textsuperscript{2+}-dependent changes in Na\textsubscript{V}1.2 wild-type channel function initiated by the Ca\textsuperscript{2+}-dependent CaM N-lobe interaction. Second, it is possible that there are unexamined functional parameters that are more relevant to Ca\textsuperscript{2+}-dependent regulations that are less-isoform-specific. Third, it is possible that Ca\textsuperscript{2+}/CaM does not mediate the observed Ca\textsuperscript{2+}-dependent changes in functions of Na\textsubscript{V}1.2 Arg1902Cys or Glu1905Gln. However, the fact that Val1925Lys mutation (in the background of Arg1902Cys) abolishes the Ca\textsuperscript{2+}-dependent functional effect eliminates this possibility, as Val1925Lys would disrupt the binding of the CaM N-lobe to the distal IQ motif of Na\textsubscript{V}1.2.

While mutagenesis studies have yielded suggestions about how Ca\textsuperscript{2+}/CaM regulates Na\textsubscript{V} channel function\textsuperscript{18,19,21,38,42,48,49}, human disease mutations can be particularly revealing. By identifying new Ca\textsuperscript{2+}/CaM-dependent contacts with the Na\textsubscript{V} CTD and demonstrating Ca\textsuperscript{2+}-dependent conformation changes within the complex, our data provide a context in which to consider the effects of disease mutations that affect Na\textsubscript{V} CTD–CaM interaction. Several epilepsy mutations in Na\textsubscript{V}1.1 or Na\textsubscript{V}1.2 are in residues that make different contacts with Ca\textsuperscript{2+}/CaM compared with apoCaM (Fig. 3) as are additional Na\textsubscript{V}1.1 and Na\textsubscript{V}1.2 mutations associated with sporadic and familial cases of autism\textsuperscript{25,26}. Further, our analysis of the effects of the Na\textsubscript{V}1.2 Arg1902Cys familial autism mutation demonstrates that disruption of the wild-type interaction between the CaM C-lobe and the CTD induces a Ca\textsuperscript{2+}-dependent change for the mutant Na\textsubscript{V}1.2 channel function. The marked hyperpolarizing shift in both Na\textsubscript{V}1.2 activation and inactivation induced by the Arg1902Cys autism mutation would affect neuronal excitability in Na\textsubscript{V}1.2-expressing neurons, thus leading to an imbalance between excitation and inhibition known to drive neuropsychiatric phenotypes\textsuperscript{50}. In addition, our identification of the Ca\textsuperscript{2+}-dependent CaM N-lobe interaction with the distal IQ domain, not predicted by previous structural studies, provides a molecular mechanism for the Ca\textsuperscript{2+}-dependent dysfunction of the Na\textsubscript{V}1.5 Ala1924Thr Brugada Syndrome mutation\textsuperscript{51}. Finally, our data provide a potential mechanism for the recently described mutations in CaM associated with arrhythmias and cognitive deficits\textsuperscript{14}.

The Ca\textsuperscript{2+}-loaded ternary complexes present several unusual and novel features for a CaM-containing complex. Among these are the dissimilar conformations of the CaM N-lobe and CaM C-lobe when calcified CaM is bound to the IQ domain (Fig. 2c). While the different conformations of the individual CaM lobes in our structures mirror the CaM lobe conformations seen in the SK K\textsuperscript{+} channel structure, the interactions between CaM and its target peptide(s) are markedly different. Within the SK K\textsuperscript{+} channel homotrimer, the calcified CaM N-lobe wraps around one helix from the C terminus of a protomer but the apoCaM C-lobe interacts with two helices from a different protomer within the tetramer\textsuperscript{51}. Split roles for CaM lobes have also been suggested for Ca\textsuperscript{2+}-dependent regulation of CaV channels\textsuperscript{52–54}, with one lobe responsible for mediating changes to global Ca\textsuperscript{2+}, while the other responds to changes in local Ca\textsuperscript{2+}. Comparison of our structures with Ca\textsubscript{V} Ca\textsuperscript{2+} channel-derived structure provides an interesting contrast in structure and mechanisms by which Ca\textsuperscript{2+} regulates ion channel function, particularly since voltage-gated Na\textsuperscript{+} channels and Ca\textsuperscript{2+} channels are similar in sequence within their proximal CTDS. The similarities include not only the IQ domain to which CaM binds but also an EF hand motif in the Ca\textsubscript{V} L-type Ca\textsuperscript{2+} channel that was hypothesized to serve as a site for Ca\textsuperscript{2+}-dependent regulation\textsuperscript{55} but (similar to the EF hand in the Na\textsubscript{V} CTD) has also never been shown to bind Ca\textsuperscript{2+} with a physiologically meaningful affinity. In spite of these similarities in sequence, structures of the Ca\textsubscript{V} CTDS bound to CaM are surprisingly different from what we observe for the Na\textsubscript{V} CTDS. Foremost among these differences is that the Ca\textsubscript{V} CTD/CaM complexes crystallized as a dimer of CTDS, and each CTD interacted with two CaM molecules for an overall stoichiometry of four CaM and two CTDS\textsuperscript{56,57}. Whether the dimerization of channels observed in the structure is functionally relevant has been debated. Nevertheless, the 2:1 stoichiometry between CaM and the Ca\textsubscript{V}1.2 CTD contrasts markedly with the 1:1 stoichiometry between CaM and a Na\textsubscript{V} CTD. With these differences, it is not surprising that the overall fold of the Ca\textsubscript{V}1.2 CTD does not resemble the Na\textsubscript{V} CTDs.

Even focusing specifically on the interactions between Ca\textsuperscript{2+}/CaM and the respective IQ domains, the structures reveal stark differences (Fig. 5a–c). First, the arrangement of CaM with respect to the Na\textsubscript{V} IQ domain is very different than Ca\textsubscript{V} IQ domains. In both Ca\textsubscript{V}1.2 and Ca\textsubscript{V}2.1 IQ domains, CaM wraps around the IQ motif of Na\textsubscript{V}1.2/Ca\textsubscript{2+}/CaM footprint on the Na\textsubscript{V} IQ domain is longer. The signature IQ motif in Ca\textsubscript{V} channels forms a central anchor for Ca\textsuperscript{2+}/CaM interactions with both CaM lobes, while the IQ motif residues in the Na\textsubscript{V} structures form contacts mostly with the CaM’s Ca\textsuperscript{2+}-free C-lobe. Thus, the Ca\textsuperscript{2+}/CaM N-lobe is located further towards the C terminus on the Na\textsubscript{V} IQ domains compared with the Ca\textsubscript{V} IQ domains. A search of the Dali database\textsuperscript{58} suggesting that the CaM conformation in the Na\textsubscript{V}1.2/Ca\textsuperscript{2+} and Na\textsubscript{V}1.5/Ca\textsuperscript{2+} structures is novel.
CaM wraps around the NaV IQ domains in the left-handed manner in contrast to the right-handed wrapping seen in CaV structures and in CaMKII. The positions of IQ(M) amino-acid residues on the IQ domains or the 302AI303 amino-acid residues in the CaMKII autoinhibitory peptide are demarcated with two black lines. The cartoon figures are shown below each structure to illustrate the difference of the CaM–NaV1.2 interactions relative to the IQ domains in the NaV1.5/-Ca2+ structure (a), the NaV1.2/Ca2+ structure (b), a CaV1.2 structure (c), a CaM binding peptide (d, PDB ID: 3DVM) and autoinhibitory (CaM binding) peptide in CaMKII (e, PDB ID: 1CDM). CaM N-lobe and C-lobe are coloured yellow and green, respectively, and the orientation of IQ motifs or autoinhibitory peptide is indicated with a colour gradient (N terminus blue and C terminus red). The positions of IQ(M) amino-acid residues on the IQ domains or the 302AI303 amino-acid residues in the CaMKII autoinhibitory peptide are demarcated with two black lines. The cartoon figures are shown below each structure to illustrate the difference of the CaM–NaV1.2 interactions.

CaM wraps around the NaV IQ domains in the left-handed manner in contrast to the right-handed wrapping seen in CaV structures and in CaMKII.

Together with this novel interaction mode between CaM and the NaV1.2 CTDs, our structural, biochemical and functional data provide a new framework for understanding how CaM affects NaV channels in physiology and disease.

**Methods**

**Molecular biology.** The following plasmids, for protein expression and purification, have been previously described: for crystallization the human NaV1.5 CTD (amino acids 1,773–1,940) and NaV1.2 (amino acids 1,777–1,937) were cloned into pET28 (Novagen)22; the human FGF13U (accession no. NM_033642) and FGF12B were cloned into the second multiple cloning site of pETDuet-1 (Novagen)23; and CaM was cloned into pSGC02 (ref. 24). For Isothermal titration calorimetry, human NaV1.5 CTD amino acids 1,773–1,924, NaV1.5 CTD amino acids 1,773–1,940 (and the Ala1924Thr mutant) were cloned into pET28. For Site-directed mutagenesis was performed with QuikChange (Strategene).

**Recombinant protein expression and co-purification.** The three plasmids for His6–Nav1.5 CTD, FGF12B and CaM or His6–Nav1.2 CTD, FGF13U and CaM were co-electroporated into BL-21 (DE3) cells. Proteins were grown in LB medium or M9 medium as described30. Cells were harvested and resuspended in 300 mM NaCl, 20 mM Tris–HCl, 5 mM imidazole, 2 mM CaCl2, pH 7.5, supplemented with EDTA-free protease inhibitor mixture (Roche). The initial purification protocol has been previously described30. Additional purification was performed using gel filtration on a Superdex 200 10/300 l column on an AKTA FPLC (GE Healthcare) in 300 mM NaCl, 20 mM Tris–HCl, 5 mM imidazole, 2 mM CaCl2, pH 7.5. Protein concentrations were determined using UV absorbance with Thermo NanoDrop and were concentrated to 20 mM in 12 in above buffer for crystallization. For ITC experiments, the single plasmid was electroporated into BL-21 (DE3) cells and the proteins were expressed after induction with 1 mM isopropyl-1-thio-β-D-galactopyranoside for 16 h at 20 °C. CaM protein was purified as previously described31.

**Crystallization and structure determination.** Crystals were grown by vapour diffusion with the sitting-drop method. His6–Nav1.5 CTD, FGF12B and Ca2+/CaM crystals were obtained from the SeMet-substituted complex using single anomalous dispersion. The serious anisotropy of amplitudes were corrected using the UCLA Diffraction Anisotropy Server (http://services.mbi.ucla.edu/anisoscope)32 and was used for the refinement. The final model contains one complex in the asymmetric unit and is of good quality with R/Refree of 26.0/31.8% (Table 1).

**Isothermal titration calorimetry.** Experiments were performed with an ITC-200 (MicroCal) at 20 °C. The solutions containing the wild-type NaV1.5 CTD, Nav1.2 CTD, NaV1.2 CTD R1902C mutation, NaV1.5 CTD amino acids 1,773–1,924, truncation mutant or A1924T mutant (25–35 μM) were titrated with one injection of 5 μl and 27 injections of 10 μl of solutions containing CaM or CaM2+ (240–310 μM). ITC experiments were repeated with different preparations and different concentration at least three times to confirm thermodynamic parameters and stoichiometry values. The binding isotherms were analysed with a single site-binding model using the Microcal Origin version 7.0 software package (Originlab Corporation), yielding binding enthalpy (ΔH), stoichiometry (n), entropy (ΔS) and association constant (Ks). Results are presented as mean ± s.e.; statistical significance was assessed using a two-tailed Student’s t-test and was set at P < 0.05.

**Electrophysiology.** Human embryonic kidney (HEK) 293 T cells (ATCC) were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum. The cells were plated on 60-mm tissue culture dishes and grown to 65–75% confluence, and then transfected using Lipofectamine 2000 (Invitrogen) with a total 6 μg of cDNAs encoding NaV1.2, FGF14b, and β2 at a ratio of 2:1:1. One day after transfection, the cells were re-plated on coverslips coated with 50 μg/ml poly-D-lysine (Sigma) for electrophysiological recordings. Transfected cells were identified by green fluorescent protein fluorescence. Na+ currents were recorded using the whole-cell patch-clamp technique at room temperature (20–22 °C) 48–72 h after transfection. Electrode resistance ranged from 2.5 to 2.5 MΩ. Currents were filtered at 2.9 kHz and digitized at 20 Hz using an EPC 10 USB patch amplifier (HEKA Elektronik). Cells were allowed to stabilize for 7–10 min after the whole-cell configuration was established. Cells expressing peak current amplitude > 6,000 pA were excluded from kinetic analyses because of suboptimal voltage control, as were cells exhibiting peak current amplitudes < 600 pA to avoid contamination by endogenous currents. All cells were included in analyses of current density. The liquid junction potential, series resistance and leak current for these recordings were not corrected, and cells were discarded if series resistance was > 8 MΩ. The bath solution contained (in mM): NaCl 124, TEA-Cl 20, CaCl2 2.0, MgCl2 1, HEPES 5, glucose 10, pH 7.3 (adjusted with NaOH). The intracellular ‘0 Ca2+’ solution contained (in mM): CaCl2 60, -aspartic acid 80, 1,2-bis(o-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA) 10, HEPES 10, pH 7.35 (adjusted with CsOH). The intracellular ‘10 μM Ca2+’ solution contained (in mM): CaCl2 60, L-aspartic acid 80, BAPTA 1, CaCl2, 1, HEPES 10, pH 7.30

Figure 5 | CaM conformations when bound to Na+ channels compared with Ca2+ channels and CaMKII. (a–e) Unique arrangement of CaM relative to the IQ domains in the NaV1.5/Ca2+ structure (a), the NaV1.2/Ca2+ structure (b), a CaV1.2 structure (c), a CaM binding peptide (d, PDB ID: 3DVM) and autoinhibitory (CaM binding) peptide in CaMKII (e, PDB ID: 1CDM). CaM N-lobe and C-lobe are coloured yellow and green, respectively, and the orientation of IQ motifs or autoinhibitory peptide is indicated with a colour gradient (N terminus blue and C terminus red). The positions of IQ(M) amino-acid residues on the IQ domains or the 302AI303 amino-acid residues in the CaMKII autoinhibitory peptide are demarcated with two black lines. The cartoon figures are shown below each structure to illustrate the difference of the CaM–NaV1.2 interactions.
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Author contributions

C.W., S.-Y.L. and G.S.P. designed the study. C.W., B.C.C., H.Y. and H.-G.G. performed experiments. All authors analysed the data. S.-Y.L. and G.S.P. wrote the manuscript.

Disclaimer

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Additional information

Accession Numbers: Atomic coordinates and structure factors for the reported crystal structures have been deposited in the Protein Data Bank under accession codes 4JPZ and 4JQ0 for the Na\(_v\)1.2/Ca\(^{2+}\) and Na\(_v\)1.5/Ca\(^{2+}\) structures, respectively.

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