Figures captions

Figure 1. Distribution of proton NOEs and correlation with heteronuclear NOEs for the complex of human CaM with the $Na_V1.5$ IQ motif. Histogram of ^{15}N NOE (top) and all protein NOEs restraints (bottom). Intraresidue NOEs are in white, sequential in light gray, medium-range in dark gray, and long-range in black. Calmodulin and IQ motif regions are labeled.

Figure 2. Three-dimensional solution structure of the complex of human CaM with the $Na_V1.5$ IQ motif. (a,b) Representation of the final ensemble of 20 conformers representing the solution structure depicted with all backbone atoms and superimposed on the N-lobe of CaM (a) and the C-lobe of CaM (b). α helices and the IQ motif are labeled. (c) Ribbon representation of the single representative conformer with α helices and β strands labeled. (d) Schematic drawing of the interactions between apo-CaM and the IQ motif. IQ motif residues exhibiting inter-molecular NOE with CaM residues (boxed) are indicated. Hydrophobic residues are colored yellow, acidic in red, basic in blue and polar in green. (e) Representation of the residues at the binding interface in the complex. CaM residues are labeled with transparent white fonts and IQ motif residues are labeled in black font. Hydrophobic residues are colored yellow, acidic in red, basic in blue and polar in green.

Figure 3. Comparison of the CaM-human $Na_V1.5$ IQ motif complex with structures of apo-CaM and other IQ motif complexes. (a) CaM/ IQ motif 1 of myosin V (PDB code **2IX7**), (b) CaM/ IQ motif 2 of myosin V (**2IX7**), (c) myosin A/myosin A tail interacting protein (**2QAC**), (d) CaM/myosin VI (**3GN4**), (e) apo-CAM (**1CFD**, NMR), and (f) apo-CaM (**1QX5**, x-ray). In each comparison the CaM/$Na_V1.5$ IQ complex is white. For clarity, only the α helices and IQ motif of the CaM/$Na_V1.5$ IQ motif complex in the first overlay are labeled. In panels a, b, c and d, only the C-lobe of CaM is shown. In panels e and f, only the N-lobe of CaM is shown.

Figure 4. Comparison of helix organization (a) and exposure of hydrophobic surface (b) in the CaM C-lobe structure in different conformational states. From left to right: apo-CaM (PDB code **1DMO**), Ca^{2+}-loaded CaM (**1CLL**) and the apo-CaM complex with the $Na_V1.5$ IQ motif. Coloring is based on a hydrophobicity scale ranging from white to red for hydrophilic to hydrophobic residues, respectively.

Figure 5. Conservation of residues in the IQ motif of Na_V and Ca_V channels. Na_V and Ca_V sequence alignments using the sequences (from top to bottom): $Na_V1.5$ (SWISSPROT **Q14524**), $Na_V1.1$ (**P35498**), $Na_V1.2$ (**Q99250**), $Na_V1.3$ (**Q9NY46**), $Na_V1.4$ (**P35499**), $Na_V1.6$ (**Q9UQD0**), $Na_V1.7$ (**Q15858**), $Na_V1.8$ (**Q9Y5Y9**), $Na_V1.9$ (**Q9UI33**), Cav1.1 (**Q13698**), Cav1.2 (**Q13936**), $Ca_V2.1$ (**O00555**), and Cav2.2 (**Q00975**). Residues are colored based on extent of sequence identity. The $Na_V1.5$ IQ motif structure is represented on the top of the alignment. The zigzag line represents α helix, straight lines non-helical regions, and dashed lines residues not present in the IQ motif construct. Stars highlight residues predicted to be important for CaM/$Na_V1.5$ IQ motif interaction. An open circle highlights Ser1904.

Figure 6. Potential steric clash in the interface between CaM and the $Na_V1.5$ IQ motif in the Ser1904Leu mutation associated with a Long QT syndrome. Packing of Ser1904 in the complex of $Na_V1.5$ IQ motif with CaM (left) and Ser1904Leu mutant (right). Ser1904 is packed in a CaM hydrophobic pocket formed by Ala88, Val91 and Phe92. Substitution of serine for leucine in the structure leads to a substantial steric clash with the leucine side-chain protruding into Val91 sidechain and the CaM backbone. Secondary structures and the key residues in the packing interaction are labeled.

Figure S1. Distribution of sequential and medium-range NOEs and chemical shift index. Strong, medium and weak NOE intensities are indicated by thick, medium, and thin lines, respectively. The chemical shift index is shown as vertical bars.