Ca²⁺/CaM interaction with voltage-gated Na⁺ channels

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Gardill et al. (1) describe a crystal structure for a complex of Ca²⁺-loaded calmodulin (CaM) and the cardiac Na_v1.5 Na⁺ channel intracellular C terminus (C-terminal domain [CTD]) (Protein Data Bank [PDB]: 6MUD). They found, as we reported (2) with ternary complexes (PDB: 4JQ0 and 4JPZ) of Na_vCTD, Ca²⁺-CaM, and the fibroblast growth factor homologous factor (FHF) FGF13B (3, 4), that Ca²⁺ occupies only the CaM N-lobe while the CaM C-lobe adopts a semiopen conformation found when apoCaM binds a target peptide (5). Gardill et al., however, state that we had interpreted our structures as having Ca²⁺ bind to the C-lobe, thus creating confusion in the context of agreement.

In our ternary complex structures, we observed 2Fo-Fc electron density peaks in all 4 sites in CaM (both N- and C-lobes). Nevertheless, having noted that the C-lobe adopts a semiopen conformation, we hypothesized either low Ca²⁺ occupancy in the C-lobe or the C-lobe bound unknown divalent metals (e.g., Mg²⁺). Thus, we performed a long-wavelength anomalous diffraction experiment showing robust anomalous Fourier peaks in the N-lobe yet very weak or no signals in the C-lobe (supplementary figure 3 in ref. 2), supporting our hypothesis. Because the 2 mM Ca²⁺ we used for crystallization might have forced the binding of Ca²⁺ in the C-lobe and we had not included Mg²⁺, the most reasonable explanation is low C-lobe Ca²⁺ occupancy. At this resolution (~3 Å), reliable occupancy refinement of Ca²⁺ is not possible, so—consistent with standard crystallographic practices—

we deposited the coordinates with Ca²⁺ in the C-lobe while clearly stating in the manuscript that the “the affinity for Ca²⁺ in the CaM C-lobe of Na_v1.2/Ca²⁺ is low (compared with the CaM N-lobe) and...the CaM C lobe is only partially occupied with Ca²⁺” (2). In a subsequent review (6), we emphasized: “Ca²⁺ binding is limited to the N-lobe of CaM.” Thus, the statement from Gardill et al., “Although a previous structure [4JQ0] was previously reported as a Ca²⁺/CaM complex, we show here that this is a misinterpretation and that the C-lobe was in the apo form,” creates a false narrative of our analyses. Additionally, their comments, “It is clear that a structure [our PDB: 4JQ0], previously interpreted as a fully Ca²⁺-occupied CaM...” and “Previously, Wang et al. published a crystal structure that was proposed to represent a complex between Ca²⁺/CaM and the CT of Na_v1.2 and Na_v1.5” distort our analyses.

Furthermore, Gardill et al. claim that the FHF induces a ~90° twist between the CTD globular domain and the long α-helix to which the CaM C-lobe binds, but do not acknowledge that others and we had previously reported this FHF-dependent twist (6–8). Additionally, Gardill et al. report that the affinity for CaM increases with a longer CTD, mirroring what we had previously reported (2), but without noting our results. Thus, we aim to emphasize the general agreement between our previously published results/interpretations and this new report, which are instead presented as contrasting results in Gardill et al. (1).

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The authors declare no competing interest.

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