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## Structural Basis of the $\alpha_1$–$\beta$ Subunit Interaction of Voltage-Gated $Ca^{2+}$ Channels

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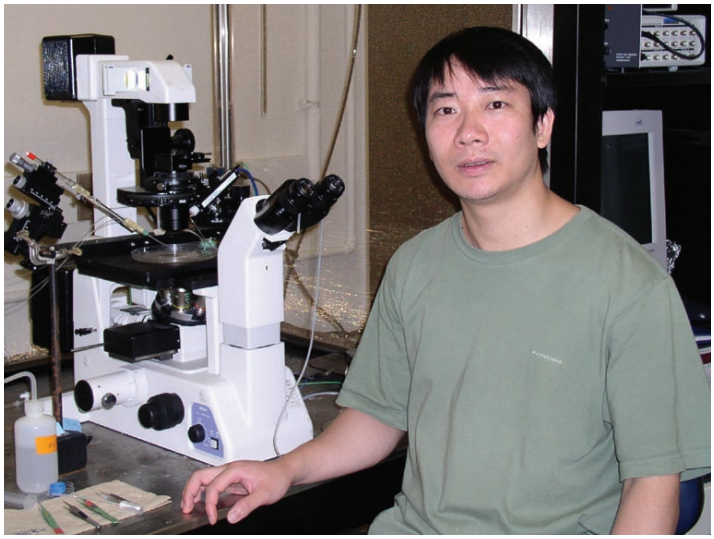
*High voltage-activated (HVA) $Ca^{2+}$ channels in cell membranes control diverse biological processes, such as muscle contraction and hormone release. They are composed of $\alpha_1$, $\alpha_2$-$\delta$, $\beta$, and sometimes $\gamma$, subunits. The proper expression and function of HVA $Ca^{2+}$ channels are critically dependent on the $\beta$ subunit, which binds directly to the $\alpha$ interaction domain (AID) in $\alpha_1$, presumably through the $\beta$ interaction domain (BID). We have solved the crystal structure of the conserved core region of $\beta_3$, alone and in complex with AID, and of $\beta_4$ alone. The structures show that the $\beta$ core contains two common protein-interacting domains (a Src homology 3 (SH3) domain and a guanylate kinase (GK) domain) and that AID does not bind to BID. These represent the first crystal structures of a $Ca^{2+}$ channel subunit or domain and suggest that $\beta$ may be a multi-functional protein.*

There are four subfamilies of $Ca^{2+}$ channel $\beta$ subunits ($\beta_1$-$\beta_4$). Each $\beta$ subunit contains five regions, with the three middle regions forming a core that can perform most functions of full-length $\beta$. The crystal structure of the $\beta_3$ core in complex with a 49-amino acid segment of $\alpha_1$, which contains the 18-amino acid α interaction domain (AID) (at 2.6 Å-resolution), shows that the three regions comprising the $\beta$ core are: an Src homology 3 (SH3) domain, a guanylate kinase (GK) domain, and a "HOOK" region that connects them (**Figure 1a**). The SH3 and GK domains interact intramolecularly. The AID forms an $\alpha$ helix and binds to a hydrophobic (water-fearing) groove in the GK domain. The interactions between AID and $\beta$ are extensive, involving hydrophobic interactions, hydrogen bonds, and an ion pair (**Figure 1b**). These interactions form the basis for the high affinity binding between $\alpha_1$ and $\beta$. Most of the amino acids involved in the interactions are conserved in both subunits. The structure also reveals that the β interaction domain (BID) spans all three regions of the SH3-HOOK-GK motif and is thus crucial for the intramolecular interaction between the SH3 and GK domains, as well as their structural integrity. But, it is not directly involved in binding AID (**Figure 1a**).

The overall structure of the unliganded (unbound) $\beta_3$ core is very similar to that of the $\beta_3$ core bound to AID, indicating that AID binding does not change the $\beta$ subunit structure. Also, as predicted by the high amino acid similarity, the structure of the $\beta_4$ core is nearly identical to that of the $\beta_3$ core.

The $\beta$ subunit SH3 domain ($\beta$-SH3) is structurally similar to canonical SH3 domains, but, unlike the continuous configuration of the latter, $\beta$-SH3 is split, with a separate, flexible HOOK region inserted between the first four $\beta$ strands and the last one. Furthermore, although $\beta$-SH3 possesses several amino acids critical for binding PXXP motifs (molecules that typically bind to SH3 domains), it is unlikely to interact with PXXP-containing proteins because the binding site is shielded by part of the HOOK. Likewise, although the overall structure of the $\beta$ subunit GK domain ($\beta$-GK) is similar to



*Yu-hang Chen*

that of the yeast GK domain, it is catalytically inactive. Thus, the function of the β subunit SH and GK domains remains to be determined.

Interestingly, the SH3-HOOK-GK motif is also a common structural signature of a family of proteins called membrane-associated guanylate kinase homologs (MAGUKs). Many members of this family, including PSD95, are concentrated at the boundaries between neurons, or "synapses," as are HVA $Ca^{2+}$ channels, and play a central role in clustering and organizing both pre- and post-synaptic ion channels and neurotransmitter receptors. Electrophysiological studies indicate that the PSD95 SH3-HOOK-GK and PSD95_GK do not interact with HVA $Ca^{2+}$ channels (**Figure 2a**). A comparison of the structure of PSD95_GK and β_GK clearly reveals why: The AID-binding pocket is completely blocked in PDS95_GK (**Figure 2b**). Our study thus provides a structural basis for the specificity of $Ca^{2+}$ channel β subunits and MAGUK proteins.

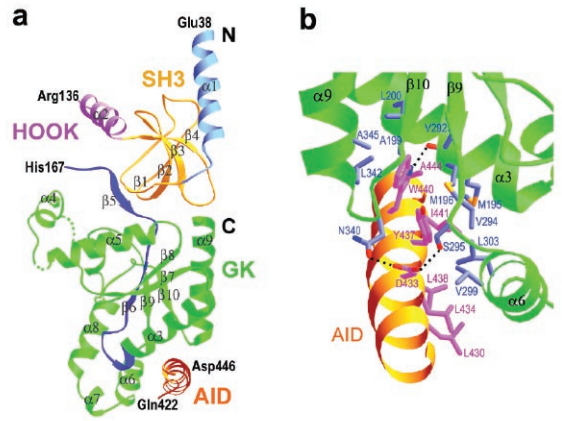



**Figure 1.** *Crystal structure of the $\beta_3$ core in complex with its $\alpha_1$ binding partner (AID). (**a**) The $\beta_3$ core can be divided into three regions: an SH3-domain (gold), a HOOK region (purple) and a GK domain (green). The AID and BID are colored in orange and blue, respectively. (**b**) Close-up of the interface between $\beta_3$ and AID. Most residues involved in the interactions are shown.*

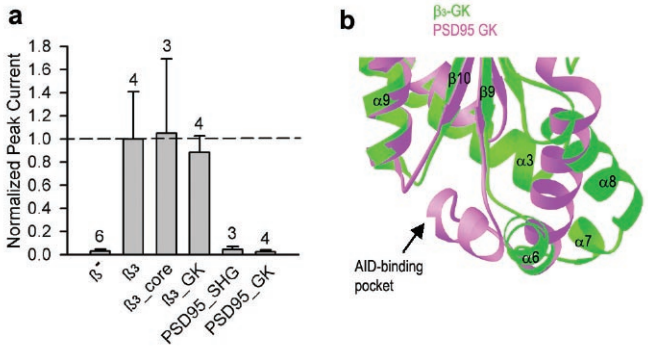



**Figure 2.** *Structural and functional comparisons of β with PSD95. (**a**) Whole-oocyte current measured in oocytes injected with Cav2.1 ($\alpha_1$) and $\alpha_2$-δ only ($\beta^-$) or together with the indicated construct. $\beta_3$_GK represents amino acids M177-G366 of $\beta_3$; PSD95_SHG represents the SH3-HOOK-GK motif (amino acids G473-L767) of PSD95; and PSD95_GK represents the GK domain (amino acids H575-S755) of PSD95. The current was normalized using the mean current obtained with wild-type $\beta_3$. (**b**) Superposition of the GK domain of unliganded $\beta_3$ (green) and PSD95 (1KJW, pink). Only the region around the AID binding site is shown. Secondary structures are labeled only for $\beta_3$.*