

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

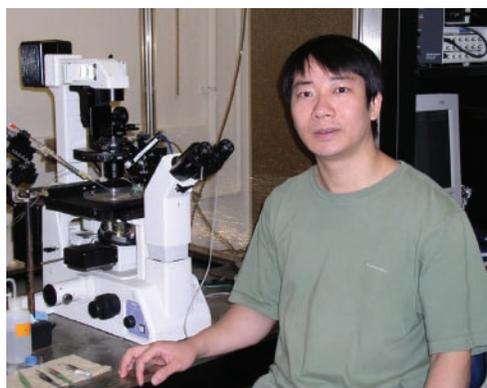
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*High voltage-activated (HVA)  $\text{Ca}^{2+}$  channels in cell membranes control diverse biological processes, such as muscle contraction and hormone release. They are composed of  $\alpha_1$ ,  $\alpha_2$ - $\delta$ ,  $\beta$ , and sometimes  $\gamma$ , subunits. The proper expression and function of HVA  $\text{Ca}^{2+}$  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 (an 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  $\text{Ca}^{2+}$  channel subunit or domain and suggest that  $\beta$  may be a multi-functional protein.*

There are four subfamilies of  $\text{Ca}^{2+}$  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  $\alpha$  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  $\beta$  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

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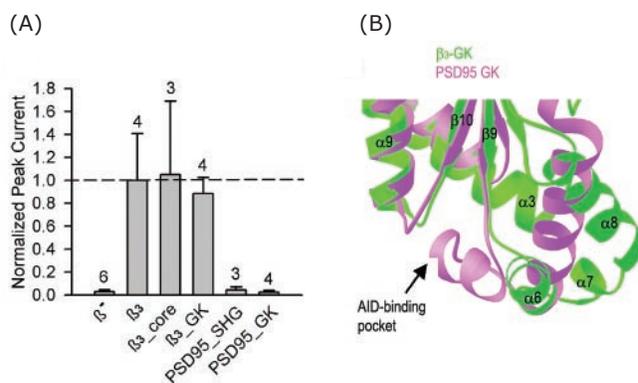
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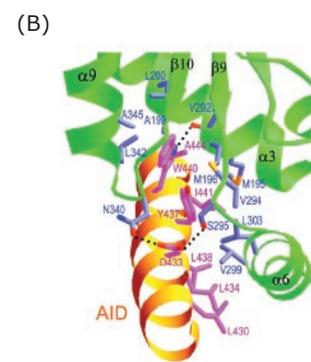
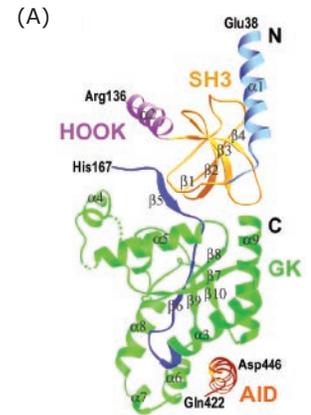
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possesses several amino acids critical for binding PXXP motifs (molecules that typically bind to SH3 domains), it is unlikely to interact with PXXP-containing proteins because the binding site is shielded by part of the HOOK. Likewise, although the overall structure of the  $\beta$  subunit GK domain ( $\beta$ -GK) is similar to that of the yeast GK domain, it is catalytically inactive. Thus, the function of the  $\beta$  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  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  channels (**Figure 2A**). A comparison of the structure of PSD95\_GK and  $\beta$ \_GK clearly reveals why: The AID-binding pocket is completely blocked in PSD95\_GK (**Figure 2B**). Our study thus provides a structural basis for the specificity of  $\text{Ca}^{2+}$  channel  $\beta$  subunits and MAGUK proteins.



**Figure 2.** Structural and functional comparisons of  $\beta$  with PSD95. (A) Whole-oocyte current measured in oocytes injected with Cav2.1 ( $\alpha_1$ ) and  $\alpha_2$ - $\delta$  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  $\beta_3$ .



**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.