Supplemental Figure 1

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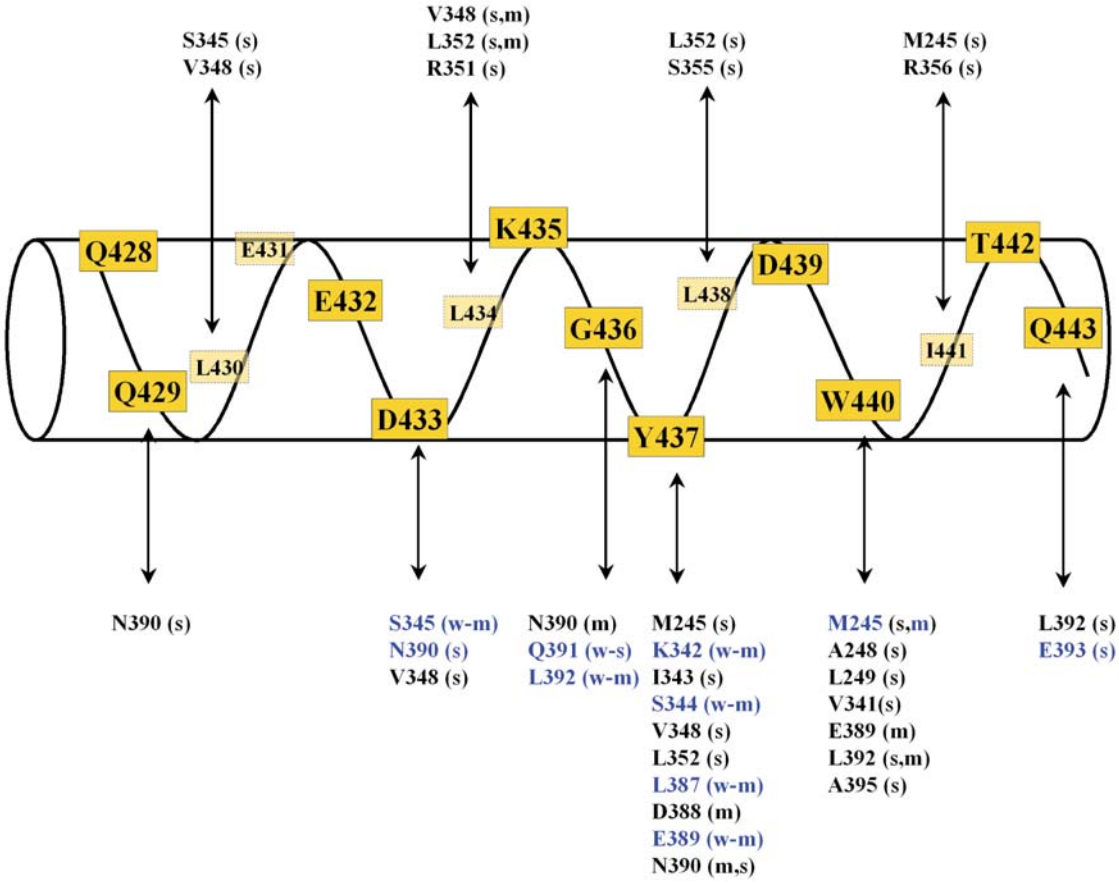
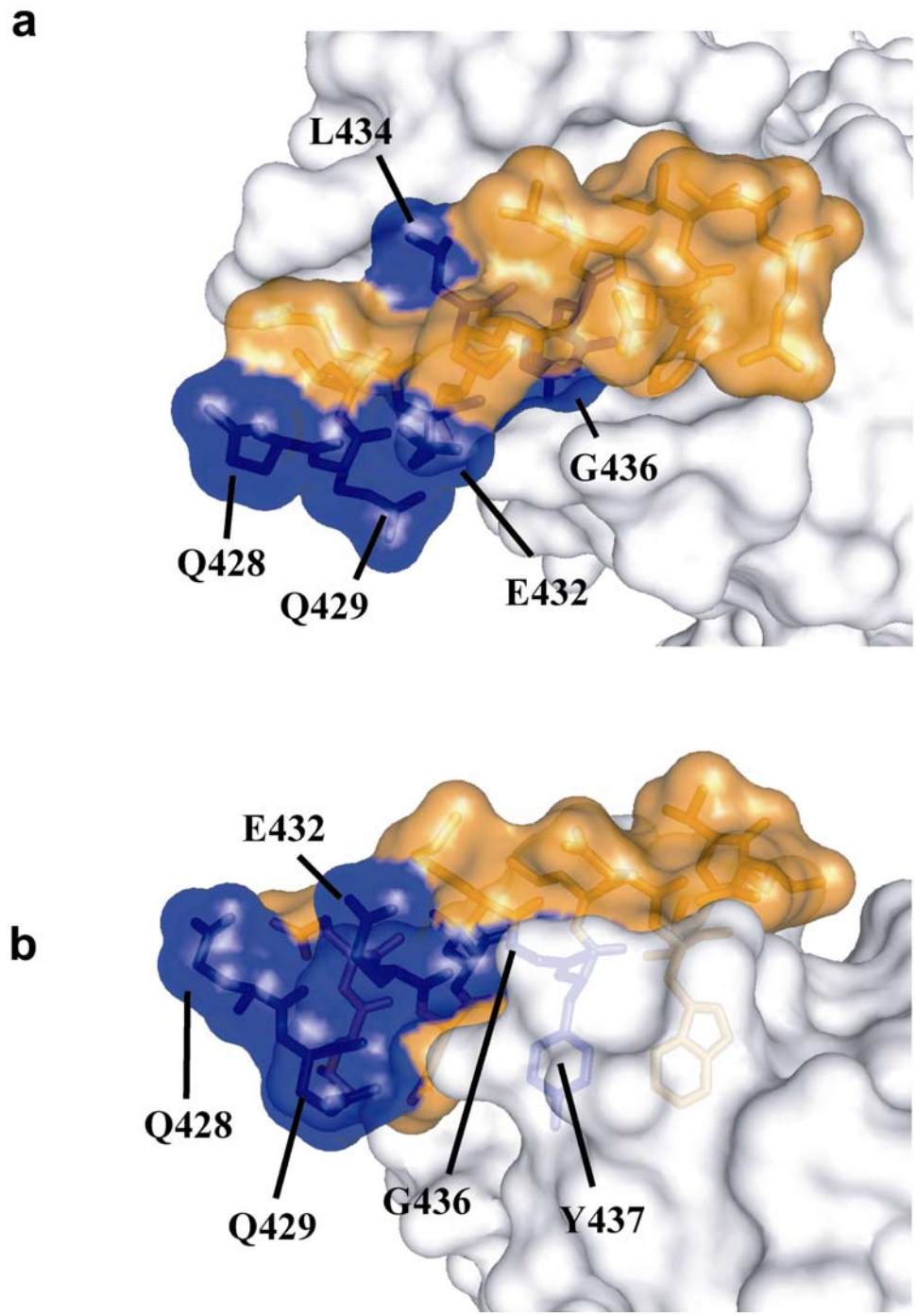


Supplemental Figure 1 Schematic representation of interactions between the AID peptide and $Ca_V\beta_{2a}$. All residues of $Ca_V\beta_{2a}$ with atoms closer than 4Å are indicated. 'm', indicates contacts with the main chain. 's', indicates contacts with the sidechain. Residues highlighted in blue form hydrogen bonds to the AID, either directly or via a water molecule ('w').

Supplemental Figure 2 **Van Petegem *et al.***



Supplemental Figure 2 a, b Location of AID residues implicated in Gβγ binding[1] (blue). Note that G_{436} and Y_{437} are buried in the complex.

Supplemental Table 1: Data collection, phasing and refinement.

	$Ca_V\beta_{2a}$		$Ca_V\beta_{2a}$-AID complex
	λ_1	λ_2	
Wavelength (Å)	1.0199	0.9796	1.1159
Resolution (Å)	30-1.97 (2.04-1.97)	30-2.20 (2.24-2.20)	30 – 2.00 (2.07-2.00)
Space group	P1		P1
Cell parameters (Å, °)	a=36.18, b=45.26, c=58.70 α=107.34, β=95.75, γ=97.00		a=36.76, b=45.33, c=60.65, α=96.813, β=102.459, γ=98.526
# unique reflections	24274 (2402)		24890 (2503)
R_{sym} * (%)	6.9 (30.5)	7.6 (29.2)	8.1 (38.5)
I/σ(I)	27.51 (2.45)	15.0 (4.2)	11.65 (2.06)
Completeness (%)	97.8 (96.5)	98.4 (97.5)	97.3 (96.1)
Mosaicity (°)	0.57		0.69
Phasing power **	0.36	1.24	n/a
Overall FOM□ (before/after density modification)	0.36 / 0.94		n/a
R_{cryst}/R_{free} (%)	18.55 / 21.32	n/a	19.97 / 24.15
# protein residues	283	n/a	299
# water molecules	147	n/a	138
Bond lengths RMSD (Å)	0.016	n/a	0.020
Bond Angles RMSD (°)	1.480	n/a	1.616
Mean overall B value ($Å^2$)	23.71	n/a	20.58
% Residues in core /disallowed regions of Ramachandran plot	93.5 / 0	n/a	93.1 / 0

- * $R_{sym} = \Sigma| I - \langle I \rangle | / \Sigma I$; I, intensity
- ** Phasing power = $[\Sigma_n|F_H|^2/\Sigma_n|E|^2]^{1/2}$; F_H, calculated heavy atom scattering factor; E, lack of closure error
- □ Figure of merit = $< |\Sigma_\alpha P(\alpha)e^{i\alpha}/\Sigma_\alpha P(\alpha)| >$; α, phase; $P(\alpha)$, phase probability distribution
- Values in parentheses refer to the highest resolution shell, where applicable

Supplemental Table 2: Accessibilities of AID residues in the AID-$Ca_V\beta_{2a}$ complex

Residue	Accessible surface area in AID-$Ca_V\beta_{2a}$ complex (Å^2)	Accessible surface area AID alone (Å^2)	% Accessible in AID-$Ca_V\beta_{2a}$ complex
Q 428	176	176	100.0
Q 429	125	135	92.5
L 430	49	123	39.8
E 431	88	109	80.7
E 432	101	112	90.1
D 433	**19**	**62**	**30.6**
L 434	**34**	**107**	**31.7**
K435	104	104	100.0
G 436	**1**	**39**	**2.5**
Y 437	**4**	**136**	**2.9**
L 438	62	97	63.9
D 439	77	92	83.7
W 440	**0**	**177**	**0.0**
I 441	**15**	**139**	**10.7**
T 442	124	124	100.0
Q 443	107	199	53.7

Comparison of the amount of accessible surface area of the AID. Values are shown for the conformation seen in the crystal structure in the presence and the absence of $Ca_V\beta_{2a}$. Residues that are in the complex (<35% accessible surface area are highlighted in bold.

Supplementary Methods:

Protein preparation Attempts to grow crystals of longer versions of rat $Ca_V\beta_{2a}$ failed due to solubility problems (full-length) or the formation of spherulites that could not be converted to crystals by extensive screening (constructs containing only the two conserved domains and V2). Therefore, we removed the V2 region and co-expressed the conserved domains as separate constructs corresponding to residues 17-145 (domain I) and 203-425 (domain II). Splitting the protein at the V2 loop has been shown to have minor effects on function[2]. Domain I was cloned into a modified pET28 vector (Novagen) containing in sequence, a His_6-tag, maltose binding protein (MBP), and a cleavage site for the Tobacco Etch Virus protease (TEV) (Gift of J.M. Berger). Domain II was cloned in pEGST [3] and expressed without affinity tags. The domains were co-expressed in *Escherichia coli* BL21(DE3) pLysS grown in 2xYT media at 37°C. Cells were lysed on ice by sonication in a buffer of 0.1M Tris-HCl, pH 8.0, containing 150 mM KCl, 10% sucrose, 1 mM EDTA, 5 mM β-mercaptoethanol, 1 mM PMSF. The complex was purified on a Poros20MC column (Perseptive Biosystems) in 250 mM KCl, 10 mM K-phosphate, pH 7.3, to retain His-tagged protein and eluted by a gradient to 300 mM imidazole in the same buffer. This step showed that domain II bound tightly to the His-tagged domain I. The protein was dialysed against 250 mM KCl, 10 mM K-phosphate, pH 7.3, and subjected to proteolytic cleavage with His-tagged TEV protease[4] at room temperature for ~12h. Uncleaved material, MBP, and protease were removed using a Poros20MC column and chromatographic conditions similar to those described above. Fractions that did not bind to the column were pooled and purified further with a Hiload SP column (Amersham) in 20mM MES, pH 6.3 and 1mM EDTA with a linear gradient from 250 mM to 600 mM KCl over eight column volumes. The resulting protein complex contained equal amounts of domains I & II and was dialysed against 20 mM KCl, 5 mM DTT, 10 mM Hepes, pH 7.0,

5 mM NaN_3 and concentrated to 5-15 mg ml^{-1} using Amicon Ultra concentrators (10K cutoff) (Millipore).

Selenomethionine-substituted domains I and II were co-expressed in BL21(DE3)pLysS cells in M9 minimal medium with 20% glucose as carbon source with the methionine biosynthesis pathway inhibited[5]. Purification was as described for native protein except that all buffers were supplemented with 5 mM methionine and 10 mM β-mercaptoethanol to prevent selenium oxidation.

The alpha interaction domain (AID) of human $Ca_v\alpha_1c$ (amino acid sequence QQLEEDLKGYLDWITQAE) was cloned into a modified pET27 vector (Novagen), pSV272, containing a His_6-tag, MBP, and a TEV cleavage site (gift of N. Pokala and T. Handel) and expressed in BL21(DE3)pLysS. Cells were lysed as described above. The purified complex of $Ca_V\beta_{2a}$ domains I+II was added to the lysate and the resulting complex with the AID was purified and concentrated using the protocol described for the I+II complex.

Crystallization and structure determination Complexes of native and selenomethionine-substituted $Ca_V\beta_{2a}$ domains I+II were crystallized by hanging-drop vapour diffusion[6] at 4°C by mixing 1μl of protein (5-15 mg ml^{-1}) with 1μl of well solution containing 0.1 M Tris-Cl , pH8.0, 0.2 M NaCl, & 10-20% PEG 4000. Crystals of the complex with AID were obtained in the same conditions by macroseeding with crystals of the $Ca_v\beta_{2a}$ domains I + II. X-ray diffraction data were collected at beamline 8.3.1 of the Advanced Light Source at Lawrence Berkeley National Laboratory. A two wavelength MAD-experiment was performed on crystals of SeMet-substituted domains I + II. The crystals belong to space group P1 and diffract to 1.97 Å. Location and refinement of seven selenium positions, phasing, and density modification were performed using CNS[7]. An

initial model was built using Arp/wArp[8] and manually completed using XtalView[9]. Two internal loops between η2 and α4, (residues 275-284) and α5 and α6 (residues 357-362) were not visible in the electron density. Two isolated stretches of residues at the N- and C-terminal ends of the NK domain (207-210 and 424-425) are ordered by crystal lattice contacts. One of these regions, the peptide RMPF (207-210), binds to a small groove on the surface of the SH3 domain of a neighbouring molecule in the lattice. We also measured a low-resolution dataset from a different crystal form (space group $P4_1$, data not shown), and solved this structure by molecular replacement. There were no significant changes in the main chains between the crystal forms. Crystals of the AID complex belong to the space group P1 and diffract to 2.00 Å. Due to significant differences in the unit cell dimensions, molecular replacement was performed with the uncomplexed structure using AMoRe[10]. All further refinement was performed using Refmac5[11], leading to final R/R_{free} factors of 18.55 % / 21.32% (uncomplexed) and 19.97 % and 24.15% (AID complex). Side chains and full residues with missing electron densities were not modelled.

References for Supplemental material:

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