SUPPORTING INFORMATION

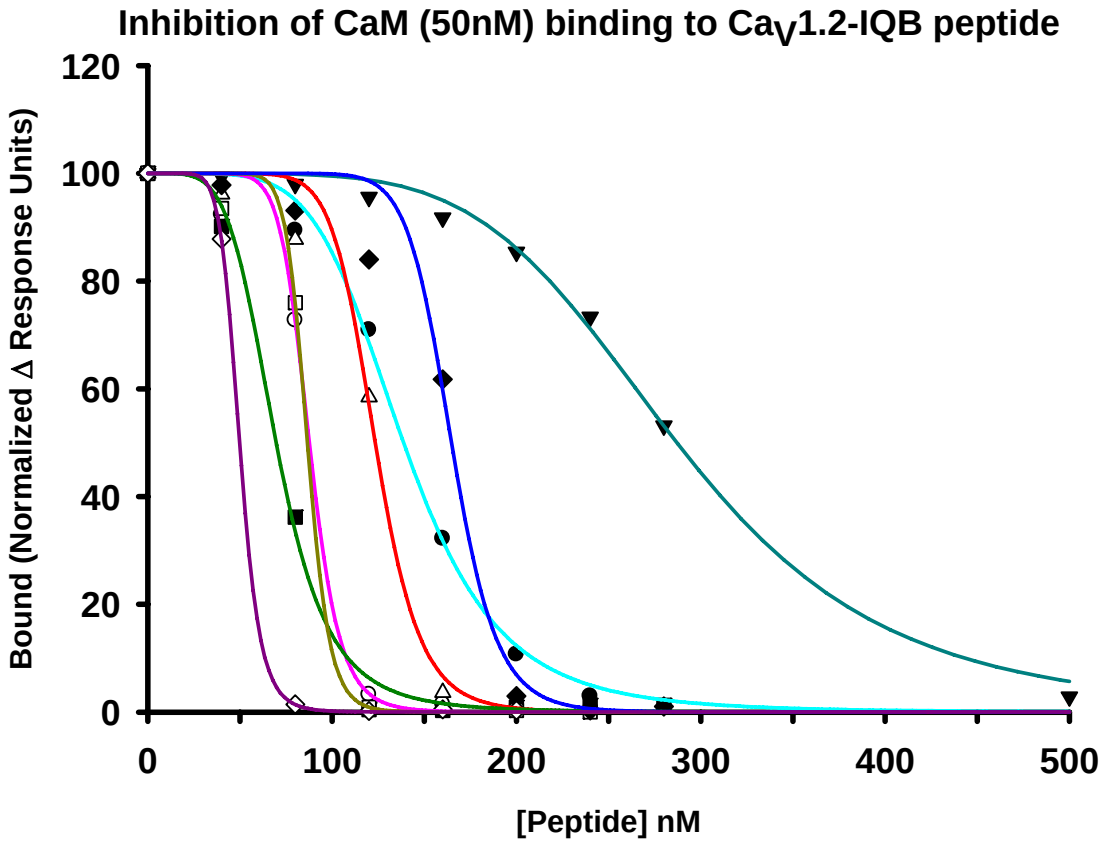
1. Determination of affinity of CaM for mutant IQ peptides: Surface Plasmon Resonance (SPR) with a Biacore3000 instrument (GE Biacore, Inc., Piscataway, NJ) was used to assess the CaM binding affinity to non-biotinylated peptides listed in **Table 2** through competition studies of CaM binding to the wild type IQ peptides. Sensor Chip SA was conditioned according to manufacturer's protocol and biotinylated wild type IQ peptides were immobilized to a Sensor Chip SA by loading 100 µL of 3 nM peptide in running buffer containing 3 mM $CaCl_2$, 30 mM MOPS, 100 mM KCl, 0.1 mg/ml BSA, 0.005% Tween-20, 0.02% NaN_3 (pH 7.5) at a flow rate of 30 µL/min. Biotin (5-10 µL of 300 nM) alone was immobilized to control flow cells on the chip and used to subtract bulk movement of CaM, to the chip during binding. Peptide immobilization was preceded and followed by a system desorb protocol to remove excess protein before another molecule was immobilized, desorb protocol was also performed at the end of each inhibition experiment. 50nM CaM with increasing concentration of peptides (0-280nM, and up to 1µM for the $Ca_V1.1$-H1532Y) were injected at a flow rate of 30 µL/min. All experiments were performed in triplicate.

IC50's were determined by fitting the normalized amplitudes of the plateau phase as a function of peptide concentration using the four parameter logistic model [$y = min + (max-min)/(1+(x/IC50)^{Hillslope})$], while the Ki's were derived from the Cheng-Prusoff equation $Ki=IC50/(1+[C]/K_d)$. (*Cheng Y, Prusoff WH (December 1973). "Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction". Biochem Pharmacol* ***22*** *(23): 3099–108.*)

In the figure normalized (0-100) differences in response units (NΔRU) for the inhibition of CaM binding to $Ca_V1.2$-IQB peptides are shown. The lines represent fits to a four parameter logistic model, with the constraints maximum NΔRU equal to 100, and minimum NΔRU positive or equal to zero. Similar response and Ki's in the same order of magnitude were derived in the case of inhibition of CaM binding to the $Ca_V1.1$-IQB.

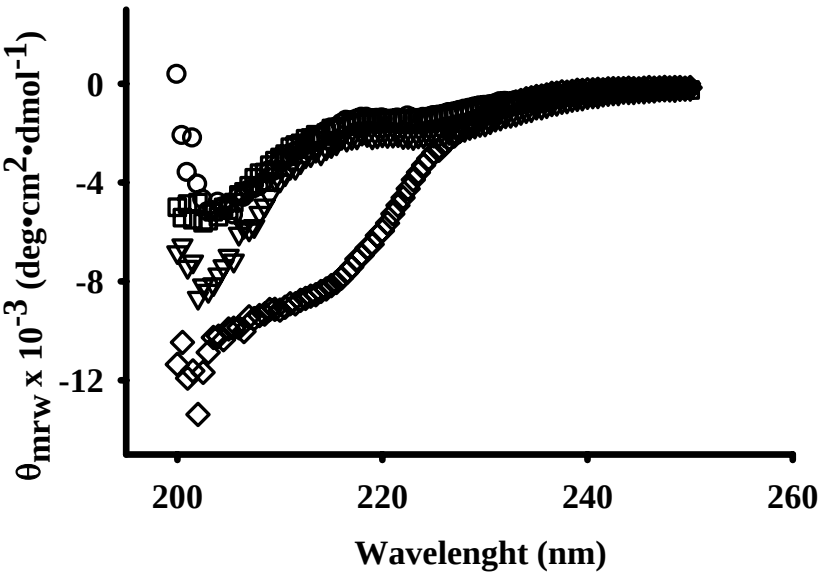
Peptide	Experimental points	Fit	Ki, (nM) $Ca_V1.2$-IQB
$Ca_V1.1$-IQ	◘	**cyan light**	6.60±0.95
$Ca_V1.2$-IQ	◘	**magenta**	4.17±0.14
$Ca_V1.1$-H1532Y-IQ	◘	**cyan**	13.67±0.24
$Ca_V1.2$-Y1675H-IQ	Δ	**red**	5.88±0.16
$Ca_V1.1$/H1532Y/M1537K	◘	**green**	3.33±0.16
$Ca_V1.1$/H1532Y/Q1540K	◘	**olive**	4.13±0.24
$Ca_V1.1$/H1532Y/E1542Q	◘	**blue**	7.85±0.26
$Ca_V1.1$/H1532Y/M1537K/Q1540K	◊	**purple**	2.37±0.03

Peptide $Ca_V1.1$-H1532Y-IQ show significant lower affinity that the wild type $Ca_V1.1$-IQ peptide, which raises the question of peptide conformation.


Inhibition of CaM (50nM) binding to Ca$_V$1.2-IQB peptide
Bound (Normalized Δ Response Units)
120
100
80
60
40
20
0
0
100
200
300
400
500
[Peptide] nM

2. Peptide conformation determination: Circular Dichroism (CD) spectra of Cav1.1-IQ, Cav1.2-IQ, Cav1.1-H1532Y, and Cav1.2-Y1675H peptides where acquired in a 62A DS CD spectrometer (AVIV Instruments, Lakewood, NJ), at 25˚C. Spectra were collected using a quartz cuvette with a 0.1 cm path length (Starna, Atascadero, CA) containing a solution with 0.5 mg/ml peptide in 5mM Tris buffer at pH 7.9.

In the figure molar ellipticity of mean residue weight [θ], is plotted as a function of wavelength. Represented data are the means of three trials that were collected with 0.5 mg/ml peptide in 5 mM Tris pH 7.9 buffer. Cav1.2-IQ, open circles; Cav1.1-IQ, open triangles; Cav1.2-Y1675H-IQ, open squares; Cav1.1-H1532Y-IQ, open diamonds.



Although we can not assign characteristics of secondary structure in the peptides tested here, since such interpretations require a model, our data suggest that the conformation of the Cav1.1-H1532Y peptide is different than the conformation the Cav1.1-IQ, Cav1.2-IQ and Cav1.2-Y1675H peptides assume in solution. The difference in conformation is made evident by the strong signal at 215 nm for the Cav1.1-H1532Y peptide, but the signal rapidly declines from 217 nm through 225 nm. In contrast, the circular dichroism signal from the other peptides remains steady through these wavelengths.

The above suggest that the low affinity of the Cav1.1-H1532Y peptide is likely to be due to the conformation it assumes that affects its interactions, rather than reflecting a weak interaction with CaM. On the other hand, the immobilization of the biotinylated Cav1.1-H1532Y peptide on the chip restores perhaps some stability for binding CaM that was lost by the mutation.