Supporting Information to Accompany:

Identification of an Atypical Calcium-Dependent Calmodulin Binding Site on the C-terminal domain of GluN2A

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Derivation of Equation 1

The total concentration of Alexa-CaM equals $[R_0]$ and free Alexa-CaM $[R]$ has a fluorescent enhancement equal to ε_1. The stoichiometrically quenched complex $[RL]$ has an enhancement equal ε_2. Thus,

$$[R_0] = [R] + [RL]$$

The total ligand added $[L_0]$ in the titration of Alexa-CaM equals free $[L]$ plus bound $[RL]$:

$$[L_0] = [L] + [RL]$$

and the dissociation constant for complex formation (K) is:

$$K = \frac{[R] + [L]}{[RL]}$$

Substituting for $[R] = [R_0] - [RL]$ and $[L] = [L_0] - [RL]$ and solving for $[RL]$ gives:

$$[RL] = \frac{2\,[R_0][L_0]}{[R_0] + [L_0] + K + \sqrt{([R_0] + [L_0] + K)^2 - 4\,[R_0][L_0]}}$$

The fluorescence measurement (F), at any concentration of $[L_0]$ is the sum of that from $[RL]$ and $[R]$:

$$F = \varepsilon_1[R] + \varepsilon_2\,[RL]$$

Substituting for $[R] = [R_0] - [RL]$ gives:

$$F = \varepsilon_1[R_0] - (\varepsilon_1 - \varepsilon_2)[RL]$$

Substituting for $[RL]$ then gives the expression for fluorescence in terms of $[R_0]$, $[L_0]$, K and, the enhancements, ε_1 and ε_2.

$$F = \varepsilon_1[R_0] - (\varepsilon_1 - \varepsilon_2)\left[\frac{2\,[R_0][L_0]}{[R_0] + [L_0] + K + \sqrt{([R_0] + [L_0] + K)^2 - 4\,[R_0][L_0]}}\right]$$

in equation (1), $a = \varepsilon_1\,[R_0]$ and $b = \varepsilon_1 - \varepsilon_2$ where $\varepsilon_2 < \varepsilon_1$.

In the absence of quenching agent $[L_0] = 0$, $F = \varepsilon_1[R_0]$, an estimate of the *x* – intercept was used as an initial fitting estimate for *a* and the value of F at saturating quenching agent gave an estimate of $\varepsilon_2[R_0]$.

The initial estimate for *b* used for fitting the data was taken from:

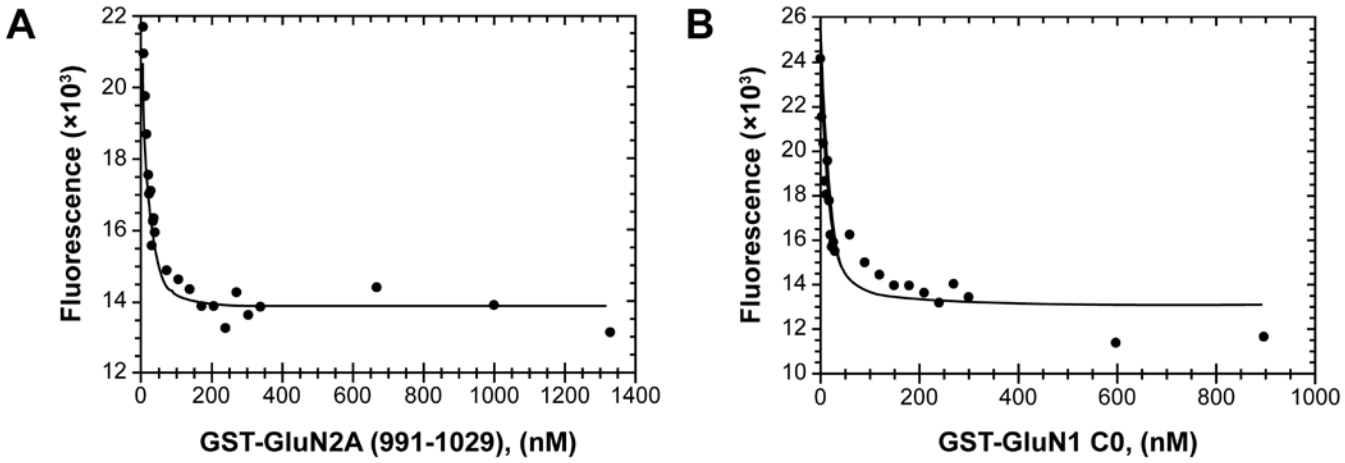
$$b = \frac{F\text{ at }X\text{–intercept} - F\text{ at saturating quenching agent}}{R_0}$$

where:

$$b = \frac{\varepsilon_1 R_0 - \varepsilon_2 R_0}{R_0} = (\varepsilon_1 - \varepsilon_2)$$

Supplementary Figure 1.

(A) GluN2A and (B) GluN1-C0 quench the fluorescence emission of Alexa Fluor-labeled calmodulin.



Titration of (A) GluN2A (991-1029) or (B) GluN1-C0 (834-864) quenched Alexa-488-labeled calmodulin. Data were fit to a single hyperbolic curve using equation 1 to derive binding affinities of 5.2 $\pm$ 2.4 nM (a = 2.17 $\pm$ 0.04 x 10^4, b = 3.86 $\pm$ 1.42 x 10^2, $[R_0]$ = 20 $\pm$ 7 nM, r^2 = 0.999) for GluN2A, and 5.9 ± 5.5 nM (a = 2.29 $\pm$ 0.09 x 10^4, b = 5.03 $\pm$ 3.75 x 10^2, $[R_0]$ = 20 $\pm$ 13 nM, r^2 = 0.996) for GluN1-C0. Points represent fluorescence determined in quadruplicate; standard errors lie within the data points. Fluorescence of GluN2A (991-1029) or GluN1-C0 (834-864) proteins in the absence of Alexa-CaM was not above background and was fixed at 0.