SUPPORTING MATERIAL 1

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I. Generalization to non-zero background Ca^{2+} concentration

In the case of non-zero [Ca^{2+}] infinitely far from channel, equilibrium relationships given by Eq. 9 are transformed to the form (1,2):

$$\begin{cases} b_\infty^* = 2\varepsilon\, c_\infty\,, \\ b_\infty^{**} = c_\infty b_\infty^* / 2 = \varepsilon c_\infty^2 . \end{cases} \tag{S1}$$

Recall that we set the buffer concentration scale to the concentration of free buffer at inifinity, therefore b_∞=1. Thus, the total concentration parameters given by Eqs. 13 are uniquely determined by the non-dimensional background Ca^{2+} concentration, c_∞:

$$\begin{cases} b_T = 1 + b_\infty^* + b_\infty^{**} = 1 + \varepsilon c_\infty \left(2 + c_\infty\right)\,, \\ c_T = c_\infty + \dfrac{\nu_2}{2}\left(b_\infty^* + 2b_\infty^{**}\right) = c_\infty \left[1 + \nu_1 \left(1 + c_\infty\right)\right]. \end{cases} \tag{S2}$$

This transforms Eq. 12 to the form

$$\begin{cases} \lambda_1 \nabla^2 b = 2\varepsilon b \left[\dfrac{1}{r} - \dfrac{\nu_2}{2}\left(b^{**} - b + b_T\right) + c_T\right] + b + b^{**} - b_T\,, \\ \lambda_2 \nabla^2 b^{**} = \left[\dfrac{1}{r} - \dfrac{\nu_2}{2}\left(b^{**} - b + b_T\right) + c_T\right]\left(b + b^{**} - b_T\right) + 2b^{**}\ . \end{cases} \tag{S3}$$

Note that the Taylor series of the solution up to leading order O(r) is the same as in the c_∞=0 case, and is given by Eq. 17:

$$\begin{cases} b = b_0 + \dfrac{\varepsilon b_0}{\lambda_1} r + O(r^2)\,, \\ b^{**} = b_0^{**} + \dfrac{b_0^{**} + b_0 - b_T}{2\lambda_2} r + O(r^2). \end{cases} \tag{S4}$$

Here b_0=b(0) and b_0^{**}=$b^{**}(0)$ are the nondimensionalized concentrations of free and fully bound buffer at channel location, r=0; both are unknown *a priori*, as in the case c_∞=0.

The transformation x=1/r, converts Eq. S3 to the form (cf. Eq. 18)

$$\begin{cases} \lambda_1 x^4 b_{xx} = 2\varepsilon b\left[x - \frac{\nu_2}{2}\left(b^{**} - b + b_T\right) + c_T\right] + b + b^{**} - b_T, \\ \lambda_2 x^4 b^{**}_{xx} = \left[x - \frac{\nu_2}{2}\left(b^{**} - b + b_T\right) + c_T\right]\left(b + b^{**} - b_T\right) + 2b^{**}, \end{cases} \tag{S5}$$

whose solution has the following long-range asymptotic expansion in powers of x (cf. Eq. 19):

$$\begin{cases} b = 1 - b_1 x + O\left(x^2\right), \\ b^{**} = b^{**}_\infty + b^{**}_1 x + O\left(x^2\right), \end{cases} \tag{S6}$$

with expansion coefficients given by

$$\begin{cases} b^{**}_\infty = \varepsilon c_\infty^2, \\ b_1 = 2q\varepsilon\left(c_\infty + 1\right), \\ b^{**}_1 = 2q\varepsilon c_\infty\left(1 + \varepsilon c_\infty\right). \end{cases} \tag{S7}$$

In these expressions the generalized parameter q has a more complex form than in the case $c_\infty=0$, where it reduces to $q = \left(1+\nu_1\right)^{-1}$:

$$q = \left[b_T\left(1+\nu_1\right) + 2\nu_1 c_\infty\left(1-\varepsilon\right)\right]^{-1} \tag{S8}$$

It is important to note that the long-range b^{**} series shown above starts with terms of order O(1) and O(x), in contrast to the case $c_\infty=0$, whereby b^{**} series starts with terms of order O(x^2) (see Eq. 20). The reason is intuitively clear: if $c_\infty \neq 0$, then a non-zero fraction of buffer will be fully bound even infinitely far from the channel. In contrast, in the case $c_\infty=0$, both bound buffer states approach zero as $r \to +\infty$ and $c_\infty \to 0$, with fully bound buffer decaying faster than b^* and [Ca^{2+}], which explains the quadratic dominant term in $b^{**}(x)$ in Eq. 20.

We find that the best approximants achieving sufficient accuracy in large portions of parameter space are the simpler analogues of the **ExpExp** and **ExpPáde** approximants that we considered in the case $c_\infty=0$. Namely, we choose the exponential *ansatz* for the free buffer variable b, analogous to Eq. 21:

$$\begin{aligned} b(r) &= 1 - b_1 \frac{1-\exp\left(-\alpha_1 r\right)}{r} \\ &= 1 - \alpha_1 b_1 + \frac{b_1}{2}\alpha_1^2 r + O(r^2), \\ b(x) &\sim 1 - b_1 x + O\left(x^2\right). \end{aligned} \tag{S9}$$

Note that it automatically matches Eq. S6 to order O(x). Using terms up to order O(r) in Eq. S4, and noting that Eq. S9 gives $b_0 = 1 - \alpha_1 b_1$, we obtain

$$\frac{b_1}{2}\alpha_1^2\lambda_1 = \varepsilon\left[1-\alpha_1 b_1\right]. \qquad \text{(S10)}$$

This is a quadratic equation for the unknown parameter α_1. Recalling the definition of b_1 in Eq. S7, we find that this equation always has one real positive root:

$$\alpha_1 = \frac{\varepsilon}{\lambda_1}\left[\sqrt{1+\frac{2\lambda_1}{\varepsilon b_1}}-1\right] = \frac{\varepsilon}{\lambda_1}\left[\sqrt{1+\frac{\lambda_1}{q\varepsilon^2\left(1+c_\infty\right)}}-1\right]. \qquad \text{(S11)}$$

As in the c_∞=0 case, the best b^{**} approximant accepts two possible simple forms that define the approximant type, and match Eq. S6 to the same order O(x):

ExpExp Approximant: *ansatz* for b^{**} is also an exponential, with one free parameter α_2:

$$\begin{aligned} b^{**}\left(r\right) &= b_\infty^{**} + b_1^{**}\frac{1-\exp\left(-\alpha_2 r\right)}{r} \\ &= b_\infty^{**} + \alpha_2 b_1^{**} - \frac{b_1^{**}}{2}\alpha_2^2\, r + O(r^2). \end{aligned} \qquad \text{(S12)}$$

Matching this expansion to the corresponding expansion in Eq. S4, and recalling that $b_0 = 1 - \alpha_1 b_1$ according to Eq. S9, we obtain the following equation for α_2:

$$\begin{aligned} -\lambda_2\alpha_2^2 b_1^{**} &= b_0^{**} + b_0 - b_T \\ &= b_\infty^{**} + \alpha_2 b_1^{**} + 1 - \alpha_1 b_1 - b_T \end{aligned} \qquad \text{(S13)}$$

Since $b_T = 1 + b_\infty^{*} + b_\infty^{**}$ according to Eq. S2, we obtain a quadratic equation

$$-\lambda_2\alpha_2^2 b_1^{**} = \alpha_2 b_1^{**} - \alpha_1 b_1 - b_\infty^{*}, \qquad \text{(S14)}$$

with real positive root

$$\alpha_2 = \frac{1}{2\lambda_2}\left[\sqrt{1+4\lambda_2 A}-1\right], \qquad \text{(S15)}$$

where

$$A = \frac{\alpha_1 b_1 + b_\infty^{*}}{b_1^{**}} = \frac{q\alpha_1\left(1+c_\infty\right)+c_\infty}{qc_\infty\left(1+\varepsilon c_\infty\right)}. \qquad \text{(S16)}$$

ExpPadé Approximant: *ansatz* for b^{**} is a bilinear function, which depends on one free parameter, β:

$$\begin{aligned} b^{**}(r) &= b_\infty^{**} + \frac{b_1^{**}}{\beta + r} \\ &= b_\infty^{**} + \frac{b_1^{**}}{\beta}\left(1 - \frac{r}{\beta}\right) + O\left(r^2\right). \end{aligned} \tag{S17}$$

Matching this expansion to the corresponding expansion in Eq. S4, recalling that $b_T = 1 + b_\infty^{*} + b_\infty^{**}$ and $b_0 = 1 - \alpha_1 b_1$, we obtain

$$\begin{aligned} -2\lambda_2 \frac{b_1^{**}}{\beta^2} &= b_0^{**} + b_0 - b_T \\ &= b_\infty^{**} + \frac{b_1^{**}}{\beta} + 1 - \alpha_1 b_1 - b_T = \frac{b_1^{**}}{\beta} - \alpha_1 b_1 - b_\infty^{*}. \end{aligned} \tag{S18}$$

Multiplying by β^2 and dividing by b_1^{**}, we obtain the quadratic equation

$$A\beta^2 - \beta - 2\lambda_2 = 0. \tag{S19}$$

This equation has a real positive root

$$\beta = \frac{1 + \sqrt{1 + 8\lambda_2 A}}{2A}, \tag{S20}$$

where the value of A is given in Eq. S16, and α_1 is given by Eq. S11. Note once again that the ansatz parameters are all real and positive, regardless of model parameter values.

We note that the limit $c_\infty \rightarrow 0$ in any of the two b^{**} *ansätze* above is singular and does not yield any of the approximants in Table 1: this is clear from the noted difference in the order of the dominant term in the long-range asymptotic expansion of b^{**} in the case c_∞=0 (Eq. 20) vs. $c_\infty \neq 0$ (Eq. S6).

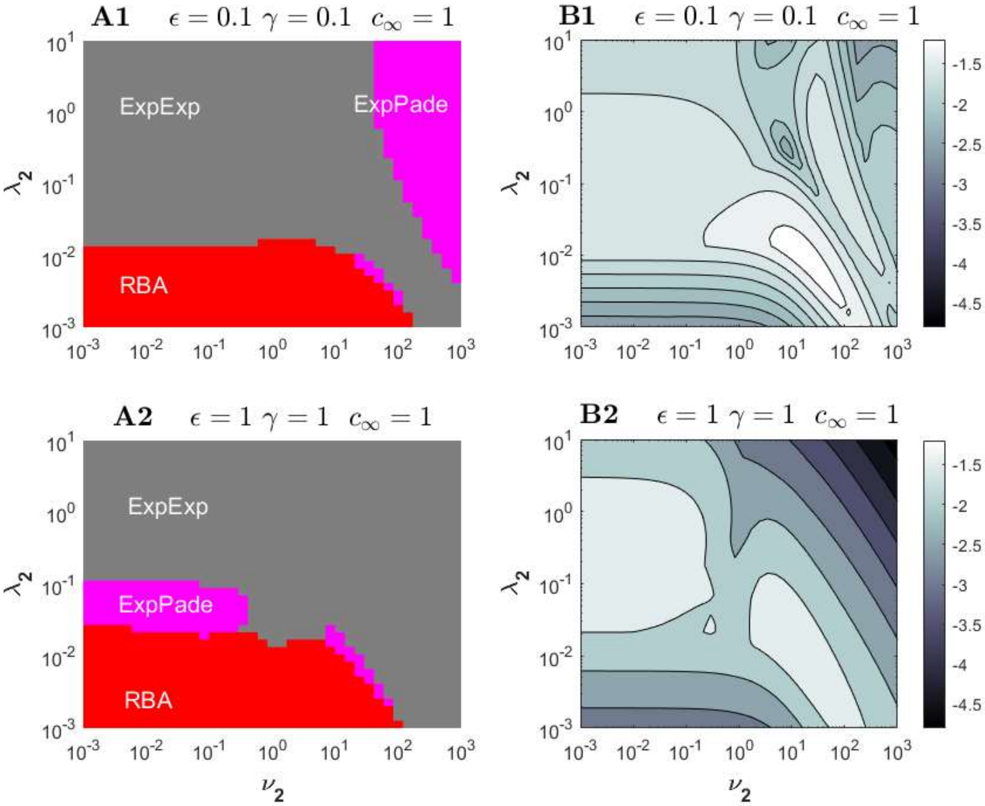

Figure S1. Best approximants (A1 and A2) and combined accuracy of free and fully bound buffer state approximations (B1 and B2) in the (ν_2, λ_2) parameter plane, as given by the error measure in Eq. 27, for the case c_∞=1, with parameters ε and γ fixed to two combinations: in A1 and B1, $\varepsilon = \gamma = 0.1$; in A2 and B2, $\varepsilon = \gamma = 1$. Each color in A1 and B1 marks the parameter region of best performance for the following approximants: RBA (*red*), ExpPadé (*magenta*), and ExpExp (*gray*). The gray-scales in B1 and B2 indicate the $\log_{10}$ error values. Darker shades represent better accuracy, according to the error bars on the right of each panel.

Although the approximant considered here are simpler than any of the approximants for the case c_∞=0 summarized in Tables 1-3, they nevertheless achieve qualitative accuracy in large portions of parameter space, as demonstrated by the combined buffer concentration error measure results shown in Figure S1. Finding even more accurate approximants for the $c_\infty \neq 0$ is a topic of further investigation.

II. General Rapid Buffering Approximation for 2:1 Buffers

Here we present the most general form of the Rapid Buffering approximation (RBA) for a buffer with two Ca^{2+} binding sites. The derivation below follows the one in (1), but we adapt it to the new and simpler non-dimensionalization adopted in this work, whereby the buffer concentration variables are re-scaled by the background free buffer concentration, B_∞, rather than the total buffer concentration. This allows us to consider binding-dependent buffer mobility and $c_\infty \neq 0$, whereas these two generalizations were treated separately in (1). We start by generalizing Eq. 12 of the main manuscript to the case of binding-dependent buffer mobility,

$$\begin{cases} \lambda_1 \nabla_\rho^2 b \;=\; 2\varepsilon c\, b - b^*, \\ \lambda_2 \nabla_\rho^2 b^{**} = -c\, b^* + 2 b^{**}, \\ b + \delta_B^* b^* + \delta_B^{**} b^{**} = b_T, \\ c + \frac{\nu_2}{2}\left(\delta_B^* b^* + 2\delta_B^{**} b^{**}\right) = \frac{1}{r} + c_T, \end{cases} \tag{S21}$$

where the extra parameters characterizing the change of buffer mobility upon Ca^{2+} binding are

$$\delta_B^* = \frac{D_B^*}{D_B}, \quad \delta_B^{**} = \frac{D_B^{**}}{D_B}, \tag{S22}$$

while the non-dimensional buffer mobility of the fully bound buffer state is redefined according to (cf. Eq. 14):

$$\lambda_2 = \frac{D_B^{**}}{L^2\, k_{1,2}^-}. \tag{S23}$$

The integration constants in the conservation laws in Eq. S21 are related to the total (fee plus bound) buffer and Ca^{2+} concentrations, and obey a more generalized version of Eqs. 13, 36:

$$\begin{cases} b_T = 1 + \delta_B^* b_\infty^* + \delta_B^{**} b_\infty^{**}, \\ c_T = c_\infty + \frac{\nu_2}{2}\left(\delta_B^* b_\infty^* + 2\delta_B^{**} b_\infty^{**}\right). \end{cases} \tag{S24}$$

Since RBA is defined by reaction equilibrium, we equate the reaction terms on the right-hand side of Eq. S21 to zero, which yields (recall that in our non-dimensionalization b_∞=1) (1,2):

$$\begin{aligned} &b^* = 2\varepsilon c\, b, \;\; b^{**} = \varepsilon c^2 b, \\ &b_\infty^* = 2\varepsilon c_\infty, \quad b_\infty^{**} = \varepsilon c_\infty^2. \end{aligned} \tag{S25}$$

Thus, the buffer conservation laws in Eq. S24 becomes

$$\begin{aligned} b_T &= 1+\varepsilon c_\infty\left(2\delta_B^{*}+\delta_B^{**}c_\infty\right) \\ &= b\left(1+\varepsilon c\left(2\delta_B^{*}+\delta_B^{**}c\right)\right). \end{aligned} \tag{S26}$$

Along with Eq. S25, this gives

$$\begin{cases} b = b_T \Big/ \left[1+\varepsilon\, c\left(2\delta_B^{*}+\delta_B^{**}c\right)\right], \\ b^{*} = 2\varepsilon cb = 2\varepsilon c b_T \Big/ \left[1+\varepsilon\, c\left(2\delta_B^{*}+\delta_B^{**}c\right)\right], \\ b^{**} = \varepsilon\, c^2 b = \varepsilon\, c^2 b_T \Big/ \left[1+\varepsilon\, c\left(2\delta_B^{*}+\delta_B^{**}c\right)\right]. \end{cases} \tag{S27}$$

Therefore, the Ca^{2+} conservation law in Eq. S21 becomes (recalling that $\varepsilon\nu_2 = \nu_1$)

$$c+\nu_1 c\left(\delta_B^{*}+\delta_B^{**}c\right)\frac{1+\varepsilon\, c_\infty\left(2\delta_B^{*}+\delta_B^{**}c_\infty\right)}{1+\varepsilon\, c\left(2\delta_B^{*}+\delta_B^{**}c\right)} = \frac{1}{r}+c_T \ , \tag{S28}$$

where the total [Ca^{2+}] at infinity defined in Eq. S24 becomes

$$c_T = c_\infty\left[1+\nu_1\left(\delta_B^{*}+\delta_B^{**}c_\infty\right)\right]. \tag{S29}$$

Eq. S28 is readily converged to a cubic equation for *c*, which has a unique real and positive root,

$$c(r) = \frac{S(r)}{A(r)}+A(r)+F(r), \tag{S30}$$

where the auxiliary functions *A*, *F*, *R*, *S*, *G* depend on model parameters according to

$$\begin{cases} A = \left[\sqrt{R^2-S^3}+R\right]^{1/3}, \\ R = F\left(F^2-\dfrac{3G}{2}\right)+\dfrac{1+c_T r}{2\delta_B^{**}\varepsilon r}, \\ S = F^2-G, \\ F = \dfrac{1}{3}\left(\dfrac{1}{r}+c_T-b_T\nu_2-2\dfrac{\delta_B^{*}}{\delta_B^{**}}\right), \\ G = \dfrac{1}{3\delta_B^{**}}\left[\delta_B^{*}\left(\nu_2 b_T-2\left(\dfrac{1}{r}+c_T\right)\right)+\dfrac{1}{\varepsilon}\right]. \end{cases} \tag{S31}$$

Here the fractional power should be understood as a principle root. This expression produces the real positive root when implemented verbatim in MATLAB (Mathworks, Inc.), and generalizes Eq. 19 in (1).

Buffer concentrations are uniquely determined from this expression using the equilibrium conditions, Eq. S27.

Figure S1 summarized the accuracy of this approximation for the case c_∞=1, comparing RBA to the closed-form approximants derived above. In that figure, buffer mobility is assumed to be binding independent, i.e. $\delta_B^* = \delta_B^{**} = 1$. Figures 1-6 in the main text of the manuscript compare this RBA approximation with other approximants in the simpler case of zero background Ca^{2+} concentration, c_∞=c_T=0, b_T=1, under the same constraint $\delta_B^* = \delta_B^{**} = 1$; in this case the expressions for auxiliary functions *R*, *F* and *G* in Eq. S31 are significantly simplified.

SUPPORTING CITATIONS

1. Matveev, V. 2018. Extension of Rapid Buffering Approximation to Ca2+ Buffers with Two Binding Sites. Biophysical Journal. 114: 1204–1215.

2. Saftenku, E.E. 2012. Effects of Calretinin on Ca(2+) Signals in Cerebellar Granule Cells: Implications of Cooperative Ca(2+) Binding. *Cerebellum.* 11: 102–120.