Supplementary data 5. Simulating 1 yM dye to measure [Ca^{2+}]

In a simulation the [Ca^{2+}] can be directly determined and does not have to be measured as such. However, to make a comparison with what would be measured in real measurements in cells, we calculated the Ca^{2+} signal that would be measured by adding to our simulation 1 yM (10^{-24} M) of OGB-1 (Fig. 2a, K_d=170 nM, k_{on}=10^9 $M^{-1}s^{-1}$), a dye often used for Ca^{2+} measurements in neurons. Such small concentration of OGB-1 is impossible to use in a real experiment. However, in a simulation it will not influence the actual [Ca^{2+}] but can still be used to reflect the limit of [Ca^{2+}] that would be imposed by the kinetic properties of OGB-1 in a real experiment ([Ca^{2+}]$_{OGB-1}$)[5,25]. In our simulation, the [Ca^{2+}]$_{OGB-1}$ has a peak amplitude of 2.5 μM. The limit of [Ca^{2+}] that could be measured with a dye in a real experiment is basically what is determined with the 'added buffer' approach used to determine κ [22,23,24]. By measuring the [Ca^{2+}] under different Ca^{2+} indicator concentrations one interpolates the [Ca^{2+}] that would be measured with no indicator present at all. Here we simply do the same by using an infinitesimally small amount of dye in the simulation. With a known Δ[Ca^{2+}]$_{total}$ of 50 μM this means that fast buffer capacity (κ) of the whole system (including fast buffering by CB and the C-terminus) is 19, which is comparable to values found for fast buffering in spines of CA1 pyramidal cells [5,25].