Supplementary data 6. Fast buffering by ATP

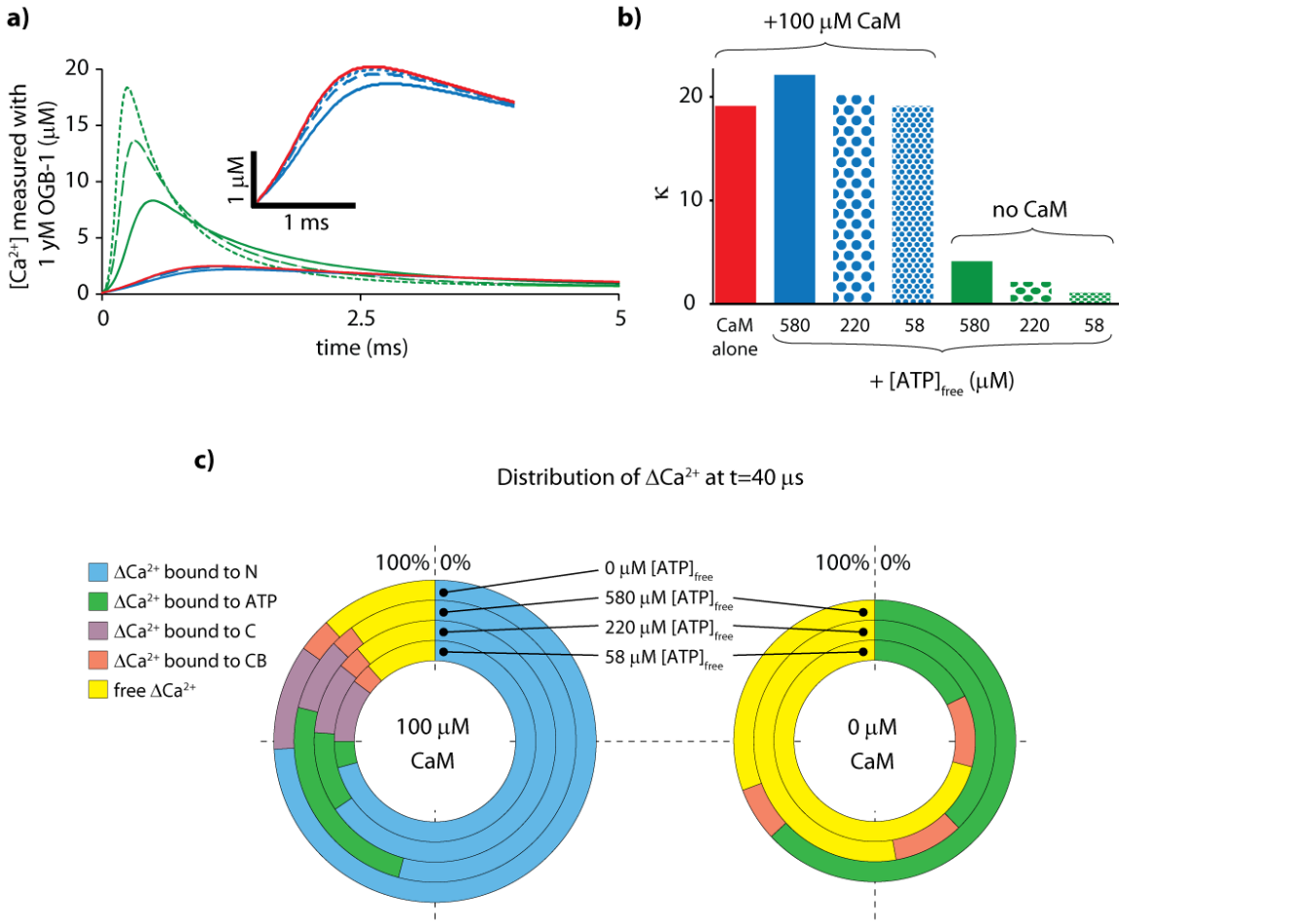
It has been suggested that ATP can contribute significantly to the fast buffering capacity of a cell[28]. To investigate this possibility, we repeated the 50 μM Ca^{2+} influx in the model (with CaM and CB) with ATP added to the system. For the properties of ATP we used $K_{dCa(^{2+})}$=200 μM, $K_{dMg(^{2+})}$=100 μM and $k_{onCa(^{2+})}$=2×10^8 $M^{-1}s^{-1}$ [28]. The binding and unbinding rates of Mg^{2+} to ATP were considered too slow to play a significant role in the studied timespan[28]. With 4 mM MgATP [4,28], and 100 nM free Ca^{2+}, 580 μM ATP will be free and available to buffer a rise in Ca^{2+}. Under these conditions, the Ca^{2+} signal observed with OGB-1 is only slightly smaller compared to the situation without ATP (Supplementary Fig. 7a, solid blue vs. red line). The κ determined from the system with 580 μM free ATP only increases from 19 to 22 (Supplementary Fig. 7b). In comparison, when the simulation was repeated with no CaM and only ATP as a fast buffer, the observed [Ca^{2+}]peak was >8 μM (Supplementary Fig. 7a solid green line) indicating this system had a buffering capacity of κ=4 (Supplementary Fig. 7b), which is substantially smaller compared to values found for fast buffering in spines of CA1 pyramidal cells measured with OGB-1 (κ=~20) [5,25]. Clearly ATP under these conditions (4 mM $[MgATP]_{total}$) will only minimally contribute to the buffering capacity. We attempted to actually measure the kinetic properties of Ca^{2+} binding to ATP and found it to be technically extremely difficult because CaATP and MgATP can form complexes with zwitterions such as pH buffers like HEPES[29] that are generally used in biological research:

$$\left[Ca^{2+}\right]+\left[ATP\right]\underset{K_d=200\,\mu M}{\rightleftharpoons}\left[CaATP\right]+\left[HEPES\right]\underset{K_d=1mM}{\rightleftharpoons}\left[CaATPHEPES\right] \quad \text{(eq. S8)}$$

$$\left[Mg^{2+}\right]+\left[ATP\right]\underset{K_d=100\,\mu M}{\rightleftharpoons}\left[MgATP\right]+\left[HEPES\right]\underset{K_d=1mM}{\rightleftharpoons}\left[MgATPHEPES\right] \quad \text{(eq. S9)}$$

It is important for our measurements that the pH is strongly buffered since the kinetic properties on CBPs strongly depend on pH, and pH changes upon uncaging of DMn. Normally, we use a high concentration of HEPES which would further complicate dynamic measurements of Ca^{2+} binding to ATP making it impossible to measure it in this way. To ensure a stable acidity, HEPES is very often used in experiments as a pH buffer [4,5,30]. , In the experiments[30] from which it was suggested that ATP can significantly contribute to the fast Ca^{2+} buffering capacity of a cell 10 mM HEPES was used. Unfortunately, this amount of HEPES was not accounted for when estimating the free [ATP][28]. Using an estimated a K_d of 1 mM for HEPES to either CaATP and MgATP[29], we calculated that 220 μM ATP is free to buffer the Ca^{2+} rise in the presence of 10 mM HEPES total, 4 mM MgATP total and 100 nM free Ca^{2+}. This means that with 4 mM MgATP and 10 mM HEPES added[4,5,30] for the system modeled here (with 100 μM CaM and 30 μM CB) the Ca^{2+} signal measured with OGB-1 to a $\Delta[Ca^{2+}]_{total}$ of 50 μM would be almost identical to a system without the ATP (Supplementary Fig. 7a, long dash blue). The κ only increases from 19 to 20 in the system without ATP (Supplementary Fig. 7b, large dot blue). Moreover, in unperturbed cells the $[ATP]_{total}$ is around 0.9 mM [31] and the $[Mg^{2+}]_{free}$ around 1 mM [32,33] which would leave only 58 μM ATP to buffer incoming Ca^{2+}, leading to a negligible increase in buffering capacity (Supplementary Fig. 7a and b, short dash/small dot blue). By itself ATP has some buffering capacity (Supplementary Fig. 7b, green).

Therefore, it will account for some of the fast buffering of CaM (Supplementary Fig. 7c) and will slightly increase the capacity of a whole system with CaM and CB present. However, the contribution is minimal under most experimental conditions (with HEPES) and negligible in unperturbed cells.



Supplementary Figure 7. ATP as a fast Ca^{2+} buffer. Single-compartment simulations of Ca^{2+} dynamics in a dendritic spine of a hippocampal CA1 pyramidal cell containing 100 μM CaM, 30 μM CB and varying amounts of free ATP. At t=0, $[Ca^{2+}]_{total}$ was rapidly (τ=10 μs) increased by 50 μM. a) $[Ca^{2+}]_{free}$ as it would be measured with 1 yM OGB-1 ($[Ca^{2+}]_{OGB-1}$ with no ATP (red), 580 μM (solid blue), 220 μM (long dash blue) and 58 μM (short dash blue) free ATP present. These are the expected values of free ATP under the recording conditions of 4 mM $[MgATP]_{total}$ and 4 mM $[MgATP]_{total}$ with 10 mM $[HEPES]_{total}$ and the physiological condition of 0.9 mM $[ATP]_{total}$ with 1 mM $[Mg^{2+}]_{free}$ (respectively). For comparison, simulations are also shown of the $[Ca^{2+}]_{OGB-1}$ when no CaM is present (green lines). b) From the $[Ca^{2+}]_{OGB-1}$ curves the buffer capacity (κ) only slightly increases due to the addition of ATP to the system (compare red to blue bars) as the buffering capacity of the ATP by itself is minimal (green bars). ATP does take over some of the fast buffering by CaM as shown in the distribution of the added Ca^{2+} after 40 μs (c left figure). However, when CaM was taken out of the system there is a substantial increase in unbuffered Ca^{2+} at t=40 μs (c right figure).