Supplementary Information to

Calmodulin as a Direct Detector of Ca^{2+} Signals

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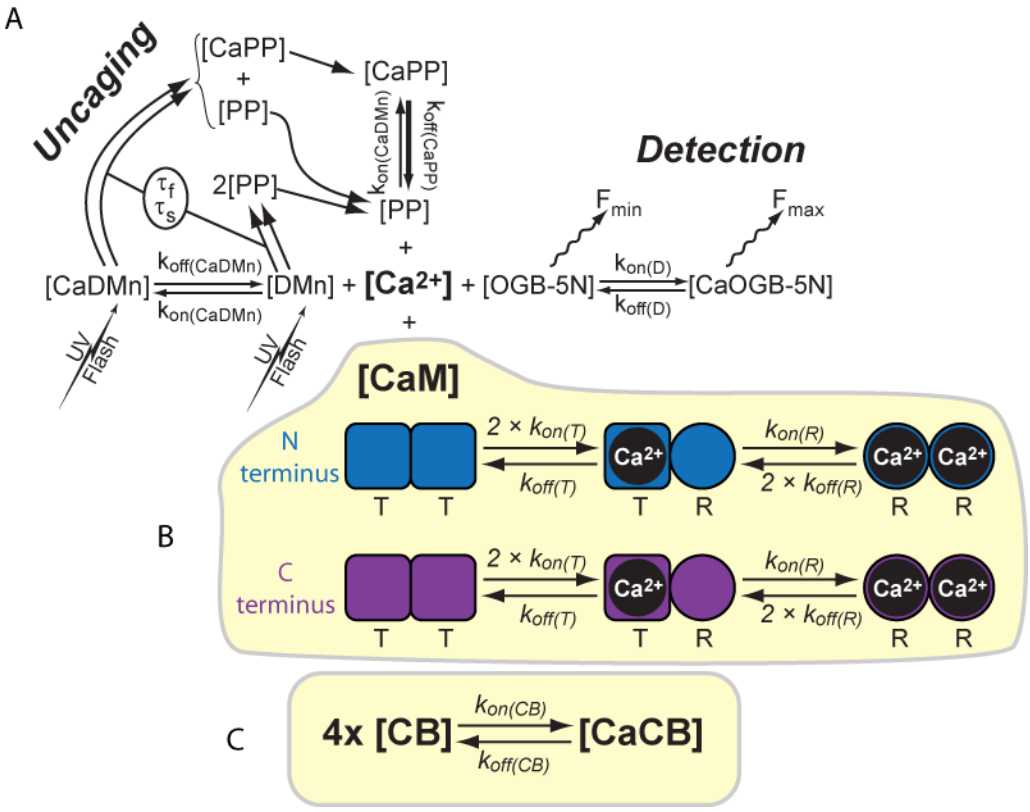

Supplementary Figure 1. Schematics of the simulated reactions. A) To determine the kinetics of the binding of Ca^{2+} to CaM we used a mathematical model that simulated all processes occurring in the reaction chamber. The properties of DMn, its photoproducts (PP) and OGB-5N were determined in independent experiments. The properties of CaM and CB were determined by fitting the uncaging data with the mathematical model. B) CaM contains two high affinity Ca^{2+}-binding sites on its C terminus and two lower affinity sites on its N terminus. The steady-state affinity for Ca^{2+} of these sites has been measured in several ways. These (and other) measurements indicate that each pair has positive cooperativity. We incorporated cooperativity in the model by including an allosteric effect, as shown. In our model, the cooperativity is based on the ability of the binding sites to occur in two states, one with a low affinity for Ca^{2+} (T) and one with a high affinity (R). A binding site is in the T state, when the other binding site in the cooperative pair has no Ca^{2+} bound. A binding site is in the R state, when the other site has Ca^{2+} bound. In this model, the transitions from T to R and vice versa are considered to occur instantaneously. This type of model has been used successfully to simulate kinetics of cooperative binding sites in calretinin[35] and gives an intuitive insight into the kinetics of cooperative binding. **C)** For CB there are no indications that there are cooperative interactions between its four binding sites. Therefore, CB was modeled as having four independent binding sites. A model with all four sites being identical resulted in acceptable fits.