**Supplementary data 2. Fit results of CB compared to earlier experiments**

Berggard et al.[1] reported some cooperativity in the binding of $Ca^{2+}$ to CB while we found that the simplest model without any cooperativity fit the data quite well. However, the cooperativity found by Berggard et al. [1] is actually quite small. We calculated from their described Adair binding curves an $n_H$ of 1.2–1.3. We incorporated this small cooperativity but found that the fit results were indistinguishable from using no cooperativity. This makes it impossible to confirm or reject the existence of such small cooperativity with our system. We chose to use the model without cooperativity as it is the least complex (least number of parameters to be fitted), making the fit results more reliable (less degrees of freedom). Furthermore, the cooperativity described by Berggard et al.[1] could be different from ours because of the difference of temperature ath which the experiments were performed (theirs was conducted at 20–25°C, ours at 35°C).

In other earlier measurements[2] we found similar kinetics and steady state binding affinity for CB as reported here, but we also found that CB has at least one different binding site with different kinetics. Besides the difference in temperature at which these experiments were preformed (20°C), it is also likely that an improvement of the fitting model underlies this difference. In our earlier measurements on CB, we did not consider the detailed kinetic uncaging properties of DMn[3] and assumed that DMn uncaged with a single time constant.