Appendix: A kinetic model to explain the switch between activating and inhibitory autophosphorylation tendencies between CaMKII-α and CaMKII-β

A simple kinetic model is built to explain the observed differences in the balance between activating and inhibitory autophosphorylation. The model is based on a CaMKII dimer, which avoids the extremely large number of intermediate states that need to be considered for a CaMKII dodecamer. Phosphorylation at the inhibitory site (Thr 305/306) can occur both in *cis* and in *trans*, whereas Thr 286 can only be phosphorylated in *trans*.

We wrote a series of chemical reactions that can lead to autophosphorylation at Thr 286 and Thr 305/306. A representative set of values for the forward and reverse rate constants are shown below. The reaction kinetics corresponding to these equations were simulated using Berkeley Madonna, a general-purpose differential equation solver (http://www.berkeleymadonna.com/). We monitored the accumulation of all species that are phosphorylated at either Thr 286 or Thr 305/306 under different rates of *trans*-phosphorylation, keeping the rates of *cis*-phosphorylation constant (Figure 5-figure supplement 1a-b).

The on and off rates for Ca^{2+}/CaM are set to 10^5 M^{-1} sec^{-1} (denoted onrate) and 10 sec^{-1} (denoted offrate) respectively, corresponding to a dissociation constant of 10^{-4} M^{-1} prior to CaM trapping. We mimicked the effect of CaM trapping by ignoring the dissociation of Ca^{2+}/CaM from species that are phosphorylated at Thr 286. We ignore the effects of phosphatases in this simulation, and set the rate of dephosphorylation to be negligible (10^{-9} sec^{-1}, denoted reverserate). The initial concentrations of CaM and CaMKII are set to 10^{-4} M and 10^{-9} M, respectively. The equations considered are summarized in Appendix-figure 1, where K denotes the kinase (CaMKII) and C denotes CaM. Phosphorylation on Thr 286 or Thr 305/306 is denoted by 286 and 306, respectively.

We ran the kinetic simulations for two sets of parameters. For the first set, the value of k_{cat} for the kinase reaction is set to 1 sec^{-1} and 0.1 sec^{-1} for the *trans*-phosphorylation of Thr 286 (denoted rate286) and Thr 305/306 (denoted rate306), respectively. The rate of *cis*-phosphorylation of Thr 305/306 (denoted ratecis306) is set to 0.1 sec^{-1}. Under these conditions, the simulation shows a dominant accumulation of species that are phosphorylated on Thr 286 (Figure 5-figure supplement 1a). Next, to mimic the effect of increasing the kinase-hub linker length, the rate of *trans*-phosphorylation was reduced by 10-fold for both Thr 286 and Thr 305/306 (i.e., 0.1 sec^{-1} and 0.01 sec^{-1}, respectively), while keeping the rate of *cis*-phosphorylation unaltered. Under these conditions, the simulations show a higher accumulation of the inhibitory phosphorylation (Figure 5-figure supplement 1b).

Appendix-figure 1: A summary of the reactions considered in the kinetic model

Reaction 1: $\mathrm{KK + C} \underset{\text{offrate}}{\overset{\text{onrate}}{\rightleftharpoons}} \mathrm{KKC}$

Reaction 2: $\mathrm{KKC + C} \underset{\text{offrate}}{\overset{\text{onrate}}{\rightleftharpoons}} \mathrm{KCKC}$

Reaction 3: $\mathrm{KCKC} \underset{\text{reverserate}}{\overset{\text{rate286}}{\rightleftharpoons}} \mathrm{KCKC286}$

Reaction 4: $\mathrm{KCKC286} \underset{\text{reverserate}}{\overset{\text{rate286}}{\rightleftharpoons}} \mathrm{KC286KC286}$

Reaction 5: $\mathrm{KCKC286} \underset{\text{onrate}}{\overset{\text{offrate}}{\rightleftharpoons}} \mathrm{KKC286 + C}$

Reaction 6: $\mathrm{KKC} \underset{\text{reverserate}}{\overset{\text{rate306}}{\rightleftharpoons}} \mathrm{K306KC}$

Reaction 7: $\mathrm{K306KC} \underset{\text{onrate}}{\overset{\text{offrate}}{\rightleftharpoons}} \mathrm{K306K + C}$

Reaction 8: $\mathrm{KKC286} \underset{\text{reverserate}}{\overset{\text{rate306}}{\rightleftharpoons}} \mathrm{K306KC286}$

Reaction 9: $\mathrm{KK} \underset{\text{reverserate}}{\overset{\text{ratecis306}}{\rightleftharpoons}} \mathrm{K306K}$

Reaction 10: $\mathrm{K306K} \underset{\text{reverserate}}{\overset{\text{ratecis306}}{\rightleftharpoons}} \mathrm{K306K306}$

Reaction 11: $\mathrm{KKC} \underset{\text{reverserate}}{\overset{\text{ratecis306}}{\rightleftharpoons}} \mathrm{K306KC}$

Reaction 12: $\mathrm{KKC286} \underset{\text{reverserate}}{\overset{\text{ratecis306}}{\rightleftharpoons}} \mathrm{K306KC286}$

$$\frac{d[\mathrm{C}]}{dt} = -\mathrm{onrate}_1[\mathrm{KK}][\mathrm{C}] + \mathrm{offrate}_1[\mathrm{KKC}] - \mathrm{onrate}_2[\mathrm{KKC}][\mathrm{C}] + \mathrm{offrate}_2[\mathrm{KCKC}] - \mathrm{onrate}_5[\mathrm{KKC286}][\mathrm{C}] + \mathrm{offrate}_5[\mathrm{KCKC286}] - \mathrm{onrate}_7[\mathrm{K306K}][\mathrm{C}] + \mathrm{offrate}_7[\mathrm{K306KC}]$$

$$\frac{d[\mathrm{KK}]}{dt} = -\mathrm{onrate}_1[\mathrm{KK}][\mathrm{C}] + \mathrm{offrate}_1[\mathrm{KKC}] - \mathrm{ratecis306}_9[\mathrm{KK}] + \mathrm{reverserate}_9[\mathrm{K306K}]$$

$$\frac{d[\mathrm{KKC}]}{dt} = +\mathrm{onrate}_1[\mathrm{KK}][\mathrm{C}] - \mathrm{offrate}_1[\mathrm{KKC}] - \mathrm{onrate}_2[\mathrm{KKC}][\mathrm{C}] + \mathrm{offrate}_2[\mathrm{KCKC}] - \mathrm{rate306}_6[\mathrm{KKC}] + \mathrm{reverserate}_6[\mathrm{K306KC}] - \mathrm{ratecis306}_{11}[\mathrm{KKC}] + \mathrm{reverserate}_{11}[\mathrm{K306KC}]$$

$$\frac{d[\mathrm{KCKC}]}{dt} = +\mathrm{onrate}_2[\mathrm{KKC}][\mathrm{C}] - \mathrm{offrate}_2[\mathrm{KCKC}] - \mathrm{rate286}_3[\mathrm{KCKC}] + \mathrm{reverserate}_3[\mathrm{KCKC286}]$$

$$\frac{d[\mathrm{KCKC286}]}{dt} = +\mathrm{rate286}_3[\mathrm{KCKC}] - \mathrm{reverserate}_3[\mathrm{KCKC286}] - \mathrm{rate286}_4[\mathrm{KCKC286}] + \mathrm{reverserate}_4[\mathrm{KC286KC286}] - \mathrm{offrate}_5[\mathrm{KCKC286}] + \mathrm{onrate}_5[\mathrm{KKC286}][\mathrm{C}]$$

$$\frac{d[\mathrm{KC286KC286}]}{dt} = +\mathrm{rate286}_4[\mathrm{KCKC286}] - \mathrm{reverserate}_4[\mathrm{KC286KC286}]$$

$$\frac{d[\mathrm{KKC286}]}{dt} = +\mathrm{offrate}_5[\mathrm{KCKC286}] - \mathrm{onrate}_5[\mathrm{KKC286}][\mathrm{C}] - \mathrm{rate306}_8[\mathrm{KKC286}] + \mathrm{reverserate}_8[\mathrm{K306KC286}] - \mathrm{ratecis306}_{12}[\mathrm{KKC286}] + \mathrm{reverserate}_{12}[\mathrm{K306KC286}]$$

$$\frac{d[K306KC]}{dt} = +\text{rate306}_{6}[KKC] - \text{reverserate}_{6}[K306KC] - \text{offrate}_{7}[K306KC] + \text{onrate}_{7}[K306K][C] + \text{ratecis306}_{11}[KKC] - \text{reverserate}_{11}[K306KC]$$

$$\frac{d[K306K]}{dt} = +\text{offrate}_{7}[K306KC] - \text{onrate}_{7}[K306K][C] + \text{rate306cis}_{9}[KK] - \text{reverserate}_{9}[K306K] - \text{ratecis306}_{10}[K306K] + \text{reverserate}_{10}[K306K306]$$

$$\frac{d[K306KC286]}{dt} = +\text{rate306}_{8}[KKC286] - \text{reverserate}_{8}[K306KC286] + \text{ratecis306}_{12}[KKC286] - \text{reverserate}_{12}[K306KC286]$$

$$\frac{d[K306K306]}{dt} = +\text{ratecis306}_{10}[K306K] - \text{reverserate}_{10}[K306K306]$$