

422 **Appendix: A kinetic model to explain the switch between activating and inhibitory**  
 423 **autophosphorylation tendencies between CaMKII- $\alpha$  and CaMKII- $\beta$**

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

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

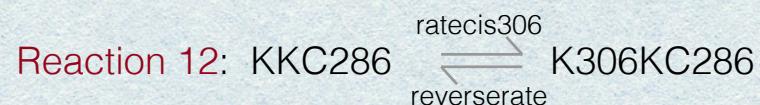
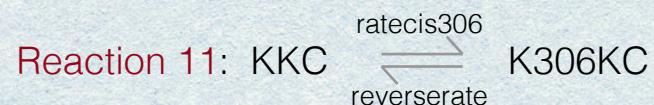
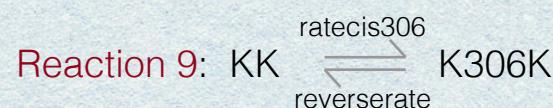
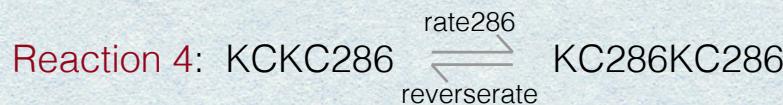
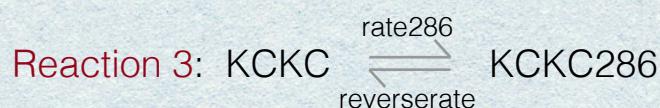
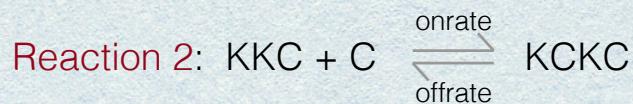
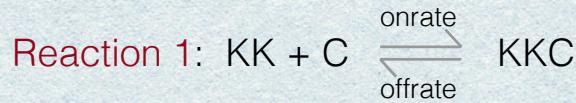
436       The on and off rates for  $\text{Ca}^{2+}/\text{CaM}$  are set to  $10^5 \text{ M}^{-1} \text{ sec}^{-1}$  (denoted onrate) and  $10 \text{ sec}^{-1}$   
 437 (denoted offrate) respectively, corresponding to a dissociation constant of  $10^{-4} \text{ M}^{-1}$  prior to CaM  
 438 trapping.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

443 the kinase (CaMKII) and C denotes CaM. Phosphorylation on Thr 286 or Thr 305/306 is denoted  
444 by 286 and 306, respectively.

445 We ran the kinetic simulations for two sets of parameters. For the first set, the value of  
446  $k_{cat}$  for the kinase reaction is set to 1 sec<sup>-1</sup> and 0.1 sec<sup>-1</sup> for the *trans*-phosphorylation of Thr 286  
447 (denoted rate286) and Thr 305/306 (denoted rate306), respectively. The rate of *cis*-  
448 phosphorylation of Thr 305/306 (denoted ratecis306) is set to 0.1 sec<sup>-1</sup>. Under these conditions,  
449 the simulation shows a dominant accumulation of species that are phosphorylated on Thr 286  
450 (Figure 5-figure supplement 1a). Next, to mimic the effect of increasing the kinase-hub linker  
451 length, the rate of trans-phosphorylation was reduced by 10-fold for both Thr 286 and Thr  
452 305/306 (i.e., 0.1 sec<sup>-1</sup> and 0.01 sec<sup>-1</sup>, respectively), while keeping the rate of *cis*-phosphorylation  
453 unaltered. Under these conditions, the simulations show a higher accumulation of the inhibitory  
454 phosphorylation (Figure 5-figure supplement 1b).

455

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



$$\frac{d[C]}{dt} = \underset{1}{-\text{onrate}[KK][C]} + \underset{1}{\text{offrate}[KKC]} - \underset{2}{\text{onrate}[KKC][C]} + \underset{2}{\text{offrate}[KCKC]} - \underset{5}{\text{onrate}[KKC286][C]} \\ + \underset{5}{\text{offrate}[KCKC286]} - \underset{7}{\text{onrate}[K306K][C]} + \underset{7}{\text{offrate}[K306KC]}$$

$$\frac{d[KK]}{dt} = \underset{1}{-\text{onrate}[KK][C]} + \underset{1}{\text{offrate}[KKC]} - \underset{9}{\text{ratecis306}[KK]} + \underset{9}{\text{reverserate}[K306K]}$$

$$\frac{d[KKC]}{dt} = \underset{1}{+\text{onrate}[KK][C]} - \underset{1}{\text{offrate}[KKC]} - \underset{2}{\text{onrate}[KKC][C]} + \underset{2}{\text{offrate}[KCKC]} - \underset{6}{\text{rate306}[KKC]} + \underset{6}{\text{reverserate}[K306KC]} \\ - \underset{11}{\text{ratecis306}[KKC]} + \underset{11}{\text{reverserate}[K306KC]}$$

$$\frac{d[KCKC]}{dt} = \underset{2}{+\text{onrate}[KKC][C]} - \underset{2}{\text{offrate}[KCKC]} - \underset{3}{\text{rate286}[KCKC]} + \underset{3}{\text{reverserate}[KCKC286]}$$

$$\frac{d[KCKC286]}{dt} = \underset{3}{+\text{rate286}[KCKC]} - \underset{3}{\text{reverse rate}[KCKC286]} - \underset{4}{\text{rate286}[KCKC286]} + \underset{4}{\text{reverserate}[KC286KC286]} \\ - \underset{5}{\text{offrate}[KCKC286]} + \underset{5}{\text{onrate}[KKC286][C]}$$

$$\frac{d[KC286KC286]}{dt} = \underset{4}{+\text{rate286}[KCKC286]} - \underset{4}{\text{reverserate}[KC286KC286]}$$

$$\frac{d[KKC286]}{dt} = \underset{5}{+\text{offrate}[KCKC286]} - \underset{5}{\text{onrate}[KKC286][C]} - \underset{8}{\text{rate306}[KKC286]} + \underset{8}{\text{reverserate}[K306KC286]} \\ - \underset{12}{\text{ratecis306}[KKC286]} + \underset{12}{\text{reverserate}[K306KC286]}$$

$$\frac{d[K_{306}KC]}{dt} = +\text{rate306}[KKC]_6 - \text{reverserate}[K_{306}KC]_6 - \text{offrate}[K_{306}KC]_7 + \text{onrate}[K_{306}K][C]_7$$

$$+\text{ratecis306}[KKC]_{11} - \text{reverserate}[K_{306}KC]_{11}$$

$$\frac{d[K_{306}K]}{dt} = +\text{offrate}[K_{306}KC]_7 - \text{onrate}[K_{306}K][C]_7 + \text{rate306cis}[KK]_9 - \text{reverserate}[K_{306}K]_9$$

$$-\text{ratecis306}[K_{306}K]_{10} + \text{reverserate}[K_{306}K_{306}]_{10}$$

$$\frac{d[K_{306}KC286]}{dt} = +\text{rate306}[KKC286]_8 - \text{reverserate}[K_{306}KC286]_8 + \text{ratecis306}[KKC286]_{12} - \text{reverserate}[K_{306}KC286]_{12}$$

$$\frac{d[K_{306}K_{306}]}{dt} = +\text{ratecis306}[K_{306}K]_{10} - \text{reverserate}[K_{306}K_{306}]_{10}$$