**Text S2: Partitioned Monte Carlo sampling in a simplified model**

Monte Carlo sampling in the main text explores a 21-dimensional parameter space, making extensive coverage of the space impractical. To more extensively explore the parameter space and further test our conclusions that (i) overshoot occurs with negative open-loop gain or (ii) negative open-loop gain requires exogenous phosphorylation, we simplified the model. Any reactions not strictly necessary to demonstrate overshoot and negative open-loop gain were removed. The resulting simplified model has 13 parameters in the sample space (Figure S3A). We partitioned each parameter into 4 intervals, and sampled each once. This resulted in a sampling of 67,108,864 discrete regions of parameter space. We discarded cases that cause numerical computation errors. Figure S3B shows the distribution of exogenous phosphorylation ratios $J_E/(J_E + J_S)$ (fractions of RR phosphorylation not from cognate SHK) to have a lower bound of approximately $10^{-4}$ for effectively negative feedback\(^1\) (open-loop gain $\frac{\partial \ln [RRP]}{\partial [R_e]} |_{k_{phk}=0.1} < -0.01$) but not for positive ($\frac{\partial \ln [RRP]}{\partial [R_e]} |_{k_{phk}=0.1} > 0.01$). All cases found to exhibit feedback-dependent overshoot have effectively negative feedback (Figure S3C). Interestingly, the model frequently predicted significant overshoot for low negative open-loop gains. We conclude that extensive parameter sampling supports our conclusions.

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\(^1\) For clarity we only focused on the cases with absolute value of open loop-gains larger than 1% to discard the large number of cases with effectively zero open-loop gain.