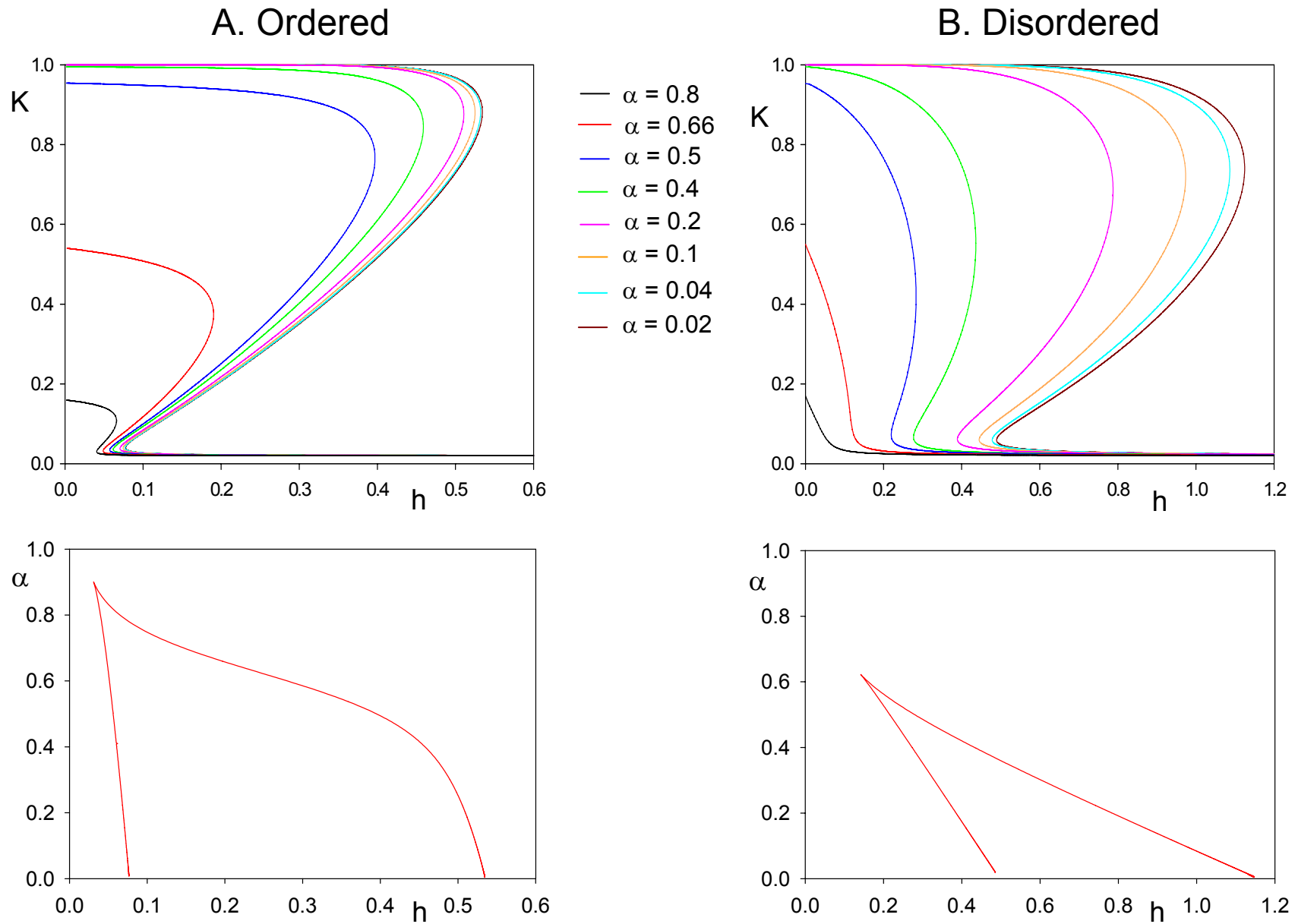
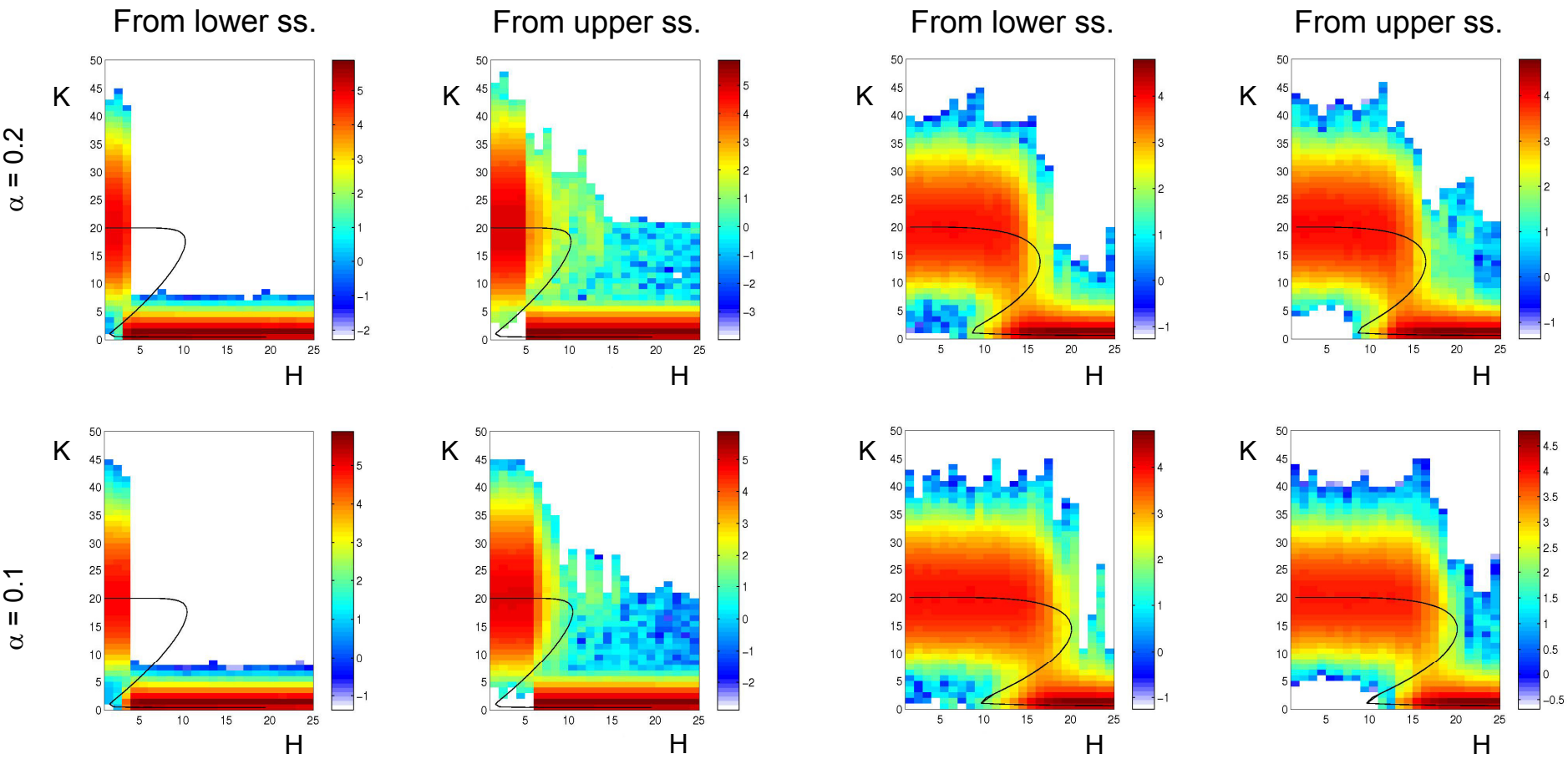
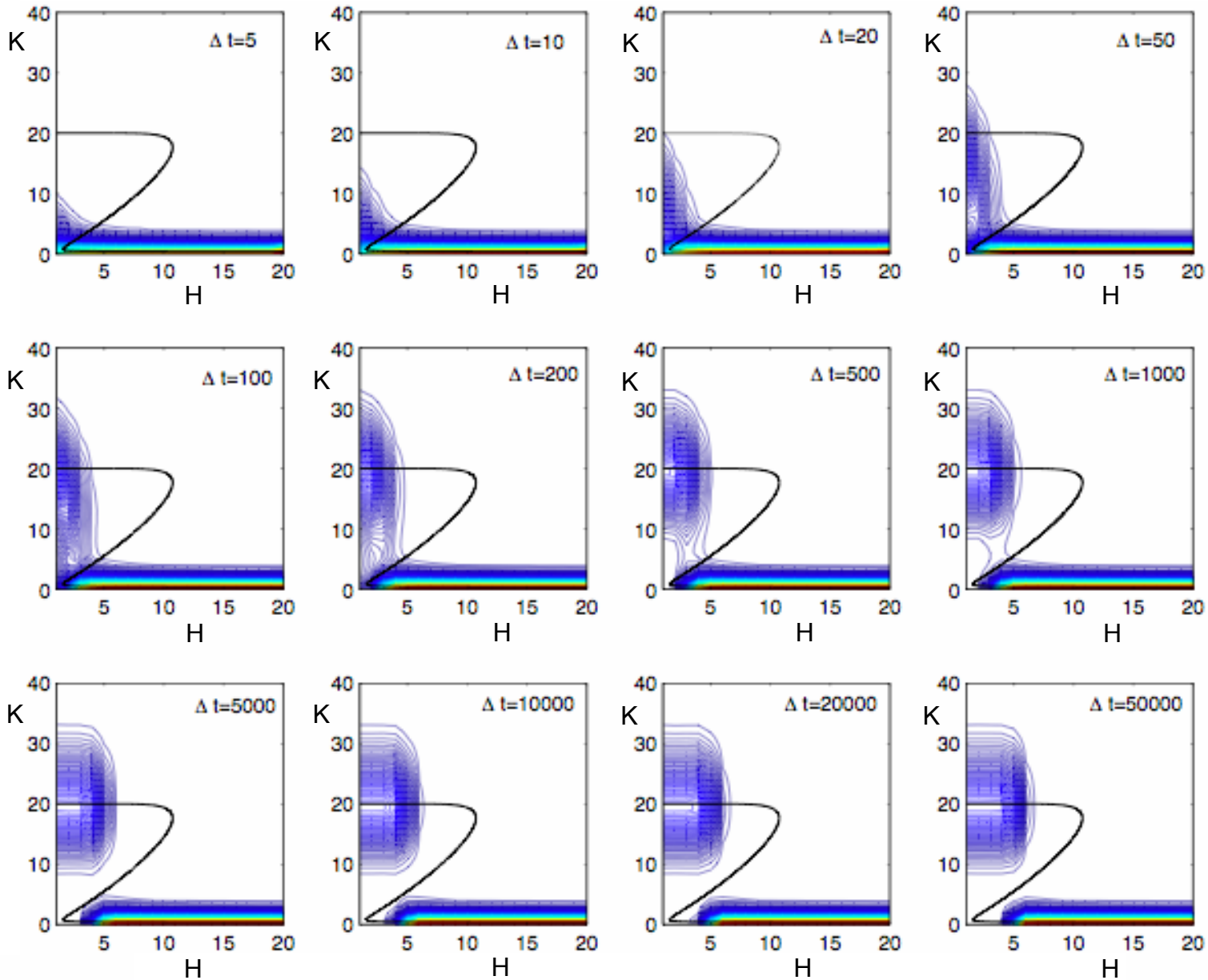


Suppl. Figure A1: One- and two-parameter bifurcation diagrams for the double-negative feedback loop with multiplicative decay of enzymatic activity for each successive phosphorylation step. (A) Ordered and (B) disordered distributive mechanism. First row: steady state level of kinase (K) is plotted against the phosphatase activity (h) for different values of the multiplicative constant (α). Second row: location of the SN bifurcation points (the turning points of the curves in row 1) as functions of αi. Parameter values: $\kappa_s = 0.02$, $\kappa_d = 0.02$, $\kappa_a = 0.98$, $X_T = 1$, $N = 10$.



Suppl. Figure A2: Effect of the number of molecules on the behavior of the switch. Stochastic simulations are carried out for the double-negative feedback loop, with distributive phosphorylation (Panel A: Ordered, and Panel B: Disordered), with multiplicative decay of enzyme activity (Row 1: $\alpha = 0.2$; Row 2: $\alpha = 0.1$). Parameter values: $V = 20$, $N = 10$, $\kappa_s = 0.02$, $\kappa_d = 0.02$, $\kappa_a = 0.98$. In each panel, the left column corresponds to initializing the stochastic simulations in the lower steady state predicted by the deterministic equations, and the right column to initializing in the upper steady state. In each simulation H is held fixed at the number of molecules indicated on the abscissa. In panel A, the total time of each simulation is 106 arbitrary time units; in panel B it is 105 au. During each of these simulations, we record the total time, T_i, spent with a particular number, i, of kinase molecules, and we plot these times using a color code to indicate log10(T_i).



Suppl. Figure A3. Response of the stochastic double-negative feedback loop to decreasing levels of phosphatase. Stochastic simulations as in Fig. 9, but in this case *H* starts at 20 and decreases by 1 each Δt time units until $H = 0$.

Supplementary Material B: Stochastic Formulation

In order to apply Gillespie's algorithm {Gillespie, 2007 #14} to the double-negative feedback loop, we must rewrite the differential equations in terms of numbers of molecules rather than concentrations. In this section, let K and H = the numbers of molecules of kinase and phosphatase, respectively, and $X_0, X_1, \ldots, X_N$ = the numbers of molecules of the different phosphorylated forms of X, in a cell of volume V. Then,

$$\frac{dK}{dt} = \kappa_s - (\kappa_d + \frac{\kappa_a}{V} \cdot X_a) \cdot K$$

and for an ordered, distributive phosphorylation mechanism:

$$\frac{dX_0}{dt} = \frac{1}{V}\left(H \cdot X_1 - K \cdot X_0\right)$$
$$\frac{dX_i}{dt} = \frac{1}{V}\left(K \cdot X_{i-1} - \left(K + H\right) X_i + H \cdot X_{i+1}\right), \text{ for } i = 1,...,(N\text{-}1)$$
$$\frac{dX_N}{dt} = \frac{1}{V}\left(K \cdot X_{N-1} - H \cdot X_N\right)$$

For a disordered, distributive phosphorylation mechanism:

$$\frac{dX_0}{dt} = \frac{1}{V}\left(H \cdot X_1 - N \cdot K \cdot X_0\right)$$
$$\frac{dX_i}{dt} = \frac{1}{V}\left((N-i+1)K \cdot X_{i-1} - \left((N-i)K + iH\right) X_i + (i+1)H \cdot X_{i+1}\right), \text{ for } i = 1,...,(N\text{-}1)$$
$$\frac{dX_N}{dt} = \frac{1}{V}\left(K \cdot X_{N-1} - N \cdot H \cdot X_N\right)$$