Fig. 6. A snapshot of the lattice Monte Carlo simulation. A snapshot from the computer simulation with cartoons of the distinct types of events considered in our kinetic Monte Carlo simulation. The stimulus, kinases, phosphatases, and scaffold proteins are represented as discrete objects on a lattice. The proteins can hop randomly to adjacent sites unless a target site is occupied by another protein. Each kinase can potentially be activated by an upstream kinase if the appropriate upstream kinase is activated and located at a proximal site. The rate of phosphorylation, determined by the activation barrier for the reaction, is varied. Phosphatases, upon encountering active kinases, can deactivate the kinase. Kinases can bind to sites on the scaffold protein with a binding energy, E_2, and disassociate from its scaffold binding site with energy, E.

Fig. 7. Effects of kinase numbers and catalysis rates. Plots of $\phi \equiv \left\langle \frac{C^*}{A^*} - 1 \right\rangle$ for different values of kinases and kinetic parameters. Black lines represent the constrained case, red lines indicates the unconstrained case. (*a* and *b*): The ratio of A/B/C/scaffold is 1:1:10:1. (*c* and *d*) This concentration ratio is 1:10:10:1. *a* and *c* show cases of high phosphatase activity. *b* and *d* show cases of low phosphatase activity. $\sigma = 0.25$ and 1,200 generic phosphatases are present in *a*-*d*. For concentration ratios of 1:1:10:1, the results are qualitatively the same as in Fig. 2. For concentration ratios of 1:10:10:1, results do not change qualitatively for high phosphatase activity. However, for low phosphatase activity (*d*), scaffolding the cascade has little effect on signaling, because kinases in free solution do almost all of the signaling in both the scaffolded and nonscaffolded scenarios. (*e* and *f*) Qualitative results do not change upon variation of catalytic rates. (*e*) $E_3 = 4$ and $E_4 = 4$. (*f*) $E_3 = 4$ and $E_4 = 10$.

Fig. 8. How simulation results can depend on protein mobility. On the abscissa, λ ($\lambda \equiv \frac{P_{Diffusion}}{P_{\mathrm{Re}action}}$), which sets a relative protein mobility is varied. The ordinate represents the difference in phosphatase deactivation energy and kinase phosphorylation energy barrier E_4-E_3, and $E_3 = 0$. We calculate the ratio of active kinases in the presence of equimolar

amounts of scaffold protein with a strong binding affinity ($E_2 = 20$) to the number of active kinases in an unscaffolded system ($E_2 = 0$). For this particular calculation, a 1:1:1:1 ratio of kinases and scaffolds is used along with 800 generic phosphatases, and a strong stimulus is present ($\sigma = 0.25$). In this case, scaffolding the signaling system becomes more (less) important as phosphatase activity increases (decreases) and protein diffusion decreases (increases).

Fig. 9. Differential phosphatase kinase-phosphatase interactions can influence the signaling function of scaffold proteins. Three situations are considered where the first [MAP3K (A), diamonds], second [MAP2K (B), circles], and third [MAPK (C) crosses] members of the cascade are protected from any phophatase mediated inactivation. (*a* and *b*) The specified kinase, A (MAP3K), B (MAP2K), or C (MAPK) is protected from phosphatase-mediated deactivation only when bound to the scaffold. (*c* and *d*) The specified kinase is protected from phosphatases regardless of whether or not it is attached to the scaffold. Kinases bound to the scaffold cannot interact with their downstream substrates that are present in solution. Again high (*a* and *c*) and low (*b* and *d*) constitutive phosphatase levels are studied. Signal output, θ ($\theta \equiv \left\langle \frac{C^*}{[A]_0} \right\rangle$), is plotted against varying kinase-scaffold disassociation energies, *E*.

Fig. 10. Variations in scaffold concentration. Shown below are representative results for how the difference in the amplification factor, $\Delta\varphi$ ($\phi \equiv \left\langle \frac{C^*}{A^*} - 1 \right\rangle$), for scaffolded (and tightly bound $E = 20$) and nonscaffolded signaling depends upon relative scaffold concentration, ξ ($\xi \equiv \frac{[Scaffold]}{[A]_0}$). The ratio of kinase concentrations is 1:1:10, and σ = 0.25 ($\sigma \equiv \frac{[S^*]}{[A]_0}$). High (*a*) and low (*b*) constitutive phosphatase levels are considered. (*a*) As expected, amplification effects become prominent when a significant number of scaffolds are present and are most pronounced at $\zeta = 1$, the optimal value of scaffold concentration; however, amplification effects persist for scaffold concentrations ~75%

greater than the optimal value. Beyond this threshold value of scaffold expression, the prozone effect is dominant and amplification is abrogated. (*b*) In addition to the inhibitory prozone effect at high scaffold concentrations, under these conditions, scaffolds are inhibitory at all concentrations, and thus, increases in the scaffold concentration results in a monotonic decrease in signal output.