Supporting Information

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Exactly Solvable Model

In this section we provide the details of the derivation of the exact rate equations (Eqs. **6** and **7** in the main text) for the many-particle model described in the *Theory* section. In this model, an immobile substrate with N sites is surrounded by M enzymes in volume V diffusing with the diffusion coefficient D. The enzymes are considered as point particles that do not interact with each other. The substrate can be in states $S_0, S_1, \ldots, S_N$ corresponding to the number of modified sites. Instantaneous irreversible transitions between the substrate states (site modification), $S_i \to S_{i+1}$, occur with the intrinsic rate constant κ_i when an enzyme and the substrate are separated by the contact radius R. The enzymes do not change in this model, so that their concentration $[E]$ is a constant. We are looking for the concentration of the substrate with i modified sites, $[S_i(t)]$.

When diffusion is fast and the system is well mixed, kinetics of S_i is described by conventional rate equations. For a substrate with two sites,

$$S_0 + E \xrightarrow{\kappa_0} S_1 + E \xrightarrow{\kappa_1} S_2 + E,$$

the conventional rate equations are

$$\begin{aligned}\frac{d[S_0]}{dt} &= -\kappa_0[E][S_0]\\ \frac{d[S_1]}{dt} &= \kappa_0[E][S_0] - \kappa_1[E][S_1]\\ \frac{d[S_2]}{dt} &= \kappa_1[E][S_1].\end{aligned} \qquad \textbf{[S1]}$$

These equations can be written in matrix form as

$$\frac{d[\boldsymbol{S}(t)]}{dt} = -\boldsymbol{K}_{CH}[E][\boldsymbol{S}(t)], \qquad \textbf{[S2]}$$

where $[\boldsymbol{S}]$ is the vector of concentrations with components $[S_0(t)]$, $[S_1(t)]$ and $[S_2(t)]$ and $\boldsymbol{K}_{CH}$ is the rate matrix

$$\boldsymbol{K}_{CH} = \begin{pmatrix} \kappa_0 & 0 & 0\\ -\kappa_0 & \kappa_1 & 0\\ 0 & -\kappa_1 & 0\end{pmatrix}. \qquad \textbf{[S3]}$$

The eigenvalues of this rate matrix are κ_0, κ_1, and 0.

For a substrate with N sites,

$$S_0 + E \xrightarrow{\kappa_0} S_1 + E \xrightarrow{\kappa_1} \ldots \xrightarrow{\kappa_N} S_N + E.$$

$[\boldsymbol{S}]$ in Eq. **S2** is a vector with components $[S_i(t)]$, $i = 0, \ldots, N$ and $\boldsymbol{K}_{CH}$ is the rate matrix whose only nonzero elements are $[\boldsymbol{K}_{CH}]_{ii} = -[\boldsymbol{K}_{CH}]_{i+1,i} = \kappa_i$, $i = 0, 1, \ldots, N-1$, and $[\boldsymbol{K}_{CH}]_{NN} = 0$. This matrix has eigenvalues κ_i, $i = 0, \ldots, N-1$ and a zero eigenvalue, which is related to conservation of the concentration, $\sum_{i=0}^{N}[S_i(t)] = [S_{tot}]$.

To take the rate of finite diffusion into account, we consider the probability distribution $P_i(r_1, \ldots, r_M, t)$ that the substrate has i sites modified and the enzymes are located at $r_1, r_2, \ldots, r_M$ at time t. The substrate concentration is obtained by integrating the distribution with respect to the positions of all of the enzymes ($d\boldsymbol{r}_m = 4\pi r_m^2 dr_m$) and taking the thermodynamic limit (i.e., M and V increase in such a way that their ratio is a constant, $M/V = [E]$):

$$[S_i(t)]/[S_{tot}] = \lim_{\substack{M,V\to\infty\\ M/V=[E]}} \int_V P_i \, d\boldsymbol{r}_1 \ldots d\boldsymbol{r}_M. \qquad \textbf{[S4]}$$

P_i satisfies the diffusion equation, which depends on all enzyme coordinates:

$$\frac{\partial P_i}{\partial t} = D\sum_{m=1}^{M} \nabla_{r_m}^2 P_i. \qquad \textbf{[S5]}$$

The transitions between the substrate states are described by boundary conditions obtained by equating the diffusive (left-hand side) and reactive (right-hand side) fluxes at contact:

$$4\pi R^2 D \frac{\partial}{\partial r_m} P_i = \kappa_i P_i - \kappa_{i-1} P_{i-1}, \quad r_m = R, \qquad \textbf{[S6]}$$

where κ_{-1} is set to zero in the boundary condition for P_0. Here the term with κ_{i-1} in the right-hand side describes the increase in P_i due to transitions $i-1 \to i$ and the term with κ_i corresponds to the decrease due to transitions $i \to i+1$. Because we assume that all enzymes are randomly distributed, the initial condition is $P_i(t=0) = V^{-M}[S_i(0)]/[S_{tot}]$, which is consistent with Eq. **S4**.

It is convenient to write the above equations in matrix form for the vector $\boldsymbol{P}$ with components $P_0, P_1, \ldots, P_N$:

$$\begin{aligned}\frac{\partial \boldsymbol{P}}{\partial t} &= D\sum_{m=1}^{M} \nabla_{r_m}^2 \boldsymbol{P}\\ 4\pi R^2 D \frac{\partial}{\partial r_m}\boldsymbol{P} &= \boldsymbol{K}_{CH}\boldsymbol{P}, \quad r_m = R.\end{aligned} \qquad \textbf{[S7]}$$

Initially, $\boldsymbol{P}(t=0) = V^{-M}[\boldsymbol{S}(0)]/[S_{tot}]$. Note that the same matrix, $\boldsymbol{K}_{CH}$, enters the conventional kinetics rate equation, Eq. **S2**, and the boundary condition in Eq. **S7**.

To solve the above equations, we first transform the vector $\boldsymbol{P}$ with the matrix that diagonalizes the rate matrix $\boldsymbol{K}_{CH} = \boldsymbol{T}\boldsymbol{\Lambda}_{CH}\boldsymbol{T}^{-1}$. Specifically, we let $\boldsymbol{P} = \boldsymbol{T}\boldsymbol{P}'$. As the result we get a set of uncoupled many-particle equations for P_i':

$$\begin{aligned}\frac{\partial \boldsymbol{P}'}{\partial t} &= D\sum_{m=1}^{M} \nabla_{r_m}^2 \boldsymbol{P}'\\ 4\pi R^2 D \frac{\partial}{\partial r_m}\boldsymbol{P}' &= \boldsymbol{\Lambda}_{CH}\boldsymbol{P}', \quad r_m = R.\end{aligned} \qquad \textbf{[S8]}$$

The initial condition is $\boldsymbol{P}'(t=0) = V^{-M}\boldsymbol{p}^0$, where we introduce for convenience $\boldsymbol{p}^0 \equiv \boldsymbol{T}^{-1}\boldsymbol{S}(0)/S_{tot}$.

Eq. **S8** is solved exactly the same way as in the Smoluchowski approach. That is, because all enzymes are independent, it can be shown by direct substitution into Eq. **S8** that the solution for each component P_i' can be presented as a product of pair distributions that describe the reaction of the substrate with one enzyme:

$$P_i'(r_1, \ldots, r_M, t)/p_i^0 = V^{-M}\prod_{m=1}^{M} g_i(r_m, t), \qquad \textbf{[S9]}$$

where $g_i(r, t)$ satisfies the diffusion equation

$$\frac{\partial}{\partial t}g_i(r,t) = D\nabla_r^2 g_i. \qquad \textbf{[S10]}$$

The boundary condition is the Collins–Kimball radiation boundary condition with the eigenvalue of $\boldsymbol{K}_{CH}$, $\lambda_i = \kappa_i$, as the intrinsic rate:

$$4\pi R^2 D\frac{\partial}{\partial r}g_i(r,t)|_{r=R} = \lambda_i g_i(R,t). \qquad \textbf{[S11]}$$

Initially, $g_i(r, 0) = 1$. For the zero eigenvalue, $g_i(r, t) = 1$ for all times.

Now we substitute $\boldsymbol{P} = \boldsymbol{TP}'$ with $\boldsymbol{P}'$ in Eq. **S9** into Eq. **S4** and integrate it with respect to all r_m. Using the equivalence of all enzymes, we find that the vector of the substrate concentrations is given by

$$[\boldsymbol{S}(t)] = \boldsymbol{TF}(t)\boldsymbol{T}^{-1}[\boldsymbol{S}(0)], \qquad \textbf{[S12]}$$

where

$$F_{ij}(t) = \lim_{\substack{M,V\to\infty \\ M/V=[E]}} \left(\int_V g_i(r,t)\frac{d\boldsymbol{r}}{V}\right)^M \delta_{ij}. \qquad \textbf{[S13]}$$

In the next step we get the rate equations by differentiating Eq. **S12**. Differentiating $F_{ij}(t)$ with respect to time and taking the thermodynamic limit, we have

$$\begin{aligned}\frac{dF_{ij}(t)}{dt} &= \lim_{\substack{M,V\to\infty \\ M/V=[E]}} \frac{M}{V}\left(\int_V \frac{\partial g_i}{\partial t}d\boldsymbol{r}\right)\left(\int_V g_i\frac{d\boldsymbol{r}}{V}\right)^{M-1}\delta_{ij} \\ &= [E]\left(\int_V \frac{\partial g_i}{\partial t}d\boldsymbol{r}\right)F_{ij}(t).\end{aligned} \qquad \textbf{[S14]}$$

Using the above equation in the time derivative of Eq. **S12**, we have

$$\begin{aligned}\frac{d[\boldsymbol{S}(t)]}{dt} &= -\boldsymbol{K}(t)[E][\boldsymbol{S}(t)] \\ \boldsymbol{K}(t) &= \boldsymbol{T}\boldsymbol{\Lambda}(t)\boldsymbol{T}^{-1},\end{aligned} \qquad \textbf{[S15]}$$

where $\boldsymbol{\Lambda}(t)$ is a diagonal matrix with elements

$$k_i(t) = -\int \frac{\partial g_i(r,t)}{\partial t}4\pi r^2 dr. \qquad \textbf{[S16]}$$

Another expression for $k_i(t)$ can be obtained by integrating Eq. **S10** with respect to r and using the boundary condition in Eq. **S11**. In this way we find that

$$k_i(t) = \kappa_i g_i(R,t). \qquad \textbf{[S17]}$$

The diffusion-influenced rate coefficient $k_i(t)$ can be found analytically by solving Eqs. **S10** and **S11**. As a result, we get the well-known expression for the time-dependent rate coefficient (1):

$$k_i(t) = \kappa_i g_i(R,t) = \kappa_i(\epsilon_i + (1-\epsilon_i)e^{\tau_i}\mathrm{erfc}\sqrt{\tau_i}), \qquad \textbf{[S18]}$$

where $i = 0, \ldots, N-1$, $k_N(t) = 0$. Here $\tau_i = Dt/(\epsilon_i R)^2$ and ϵ_i is the escape probability:

$$\epsilon_i = \frac{k_D}{\kappa_i + k_D}, \qquad \textbf{[S19]}$$

where $k_D = 4\pi DR$ is the Smoluchowski's diffusion-limited rate constant.

The matrix Eq. **S15** is exact for the model (Eq. **6** in the main text). Solving this equation, we get the vector of the substrate concentrations:

$$[\boldsymbol{S}(t)] = \boldsymbol{T}e^{-[E]\int_0^t \boldsymbol{\Lambda}(t')dt'}\boldsymbol{T}^{-1}[\boldsymbol{S}(0)], \qquad \textbf{[S20]}$$

where $\exp(-[E]\int_0^t \boldsymbol{\Lambda}(t')dt')$ is the diagonal matrix with the elements $\exp(-[E]\int_0^t k_i(t')dt')$ on the diagonal.

For a substrate with two sites, the substrate concentrations (assuming that initially the substrate is unmodified) are

$$\begin{aligned}[S_0(t)]/[S_{tot}] &= e^{-[E]\int_0^t k_0(t')dt'} \\ [S_1(t)]/[S_{tot}] &= \frac{\kappa_0}{\kappa_0-\kappa_1}\left(e^{-[E]\int_0^t k_1(t')dt'} - e^{-[E]\int_0^t k_0(t')dt'}\right).\end{aligned} \qquad \textbf{[S21]}$$

The concentration of S_2 is related to that of S_0 and S_1 by the conservation condition:

$$[S_2(t)] = [S_{tot}] - [S_0(t)] - [S_1(t)]. \qquad \textbf{[S22]}$$

When diffusion is fast, $k_i(t) \to \kappa_i$, and this solution reduces to the conventional chemical kinetics result obtained by solving Eq. **S1**:

$$\begin{aligned}[S_0(t)]/[S_{tot}] &= e^{-[E]\kappa_0 t} \\ [S_1(t)]/[S_{tot}] &= \frac{\kappa_0}{\kappa_0-\kappa_1}\left(e^{-[E]\kappa_1 t} - e^{-[E]\kappa_0 t}\right).\end{aligned} \qquad \textbf{[S23]}$$

At times longer than the diffusion time, $t \gg R^2/D$, the rate coefficients approach their steady-state values, $k_i(t) \to \kappa_i\epsilon_i$, and the solution in Eq. **S21** becomes

$$\begin{aligned}[S_0(t)]/[S_{tot}] &= e^{-[E]\kappa_0\epsilon_0 t} \\ [S_1(t)]/[S_{tot}] &= \frac{\kappa_0}{\kappa_0-\kappa_1}\left(e^{-[E]\kappa_1\epsilon_1 t} - e^{-[E]\kappa_0\epsilon_0 t}\right).\end{aligned} \qquad \textbf{[S24]}$$

Note that Eq. **S24** cannot be obtained from Eq. **S23** by simply replacing $\kappa_0 \to \kappa_0\epsilon_0$ and $\kappa_1 \to \kappa_1\epsilon_1$. However, Eq. **S24** can be obtained by solving the rate equations that correspond to the kinetic scheme with the additional connection between S_0 and S_2 (Fig. 2*B* in the main text):

$$\begin{aligned}\frac{d[S_0]}{dt} &= -\kappa_0\epsilon_0[E][S_0] \\ \frac{d[S_1]}{dt} &= \kappa_0\epsilon_0\epsilon_1[E][S_0] - \kappa_1\epsilon_1[E][S_1] \\ \frac{d[S_2]}{dt} &= \kappa_1\epsilon_1[E][S_1] + \kappa_0\epsilon_0(1-\epsilon_1)[E][S_0].\end{aligned} \qquad \textbf{[S25]}$$

Eqs. **S21** and **S23** (with κ_i replaced by $\kappa_i\epsilon_i$, $i = 0, 1$) and Eq. **S24** were used in Fig. 2 (main text) to calculate the exact, scheme A, and scheme B kinetics, respectively.

The above analysis can be extended to the reversible reaction described by the scheme

$$S_i + E \underset{\kappa_i^r}{\overset{\kappa_i^f}{\rightleftharpoons}} S_{i+1} + E. \qquad \textbf{[S26]}$$

Conventional rate equations of chemical kinetics for this reaction is given by Eq. **S2** with $\boldsymbol{K}_{CH}$ containing forward and reverse rate constants. For example, for $n = 2$ $\boldsymbol{K}_{CH}$ is

$$\boldsymbol{K}_{CH} = \begin{pmatrix} \kappa_0^f & -\kappa_0^r & 0 \\ -\kappa_0^f & \kappa_0^r + \kappa_1^f & -\kappa_1^r \\ 0 & -\kappa_1^f & \kappa_1^r \end{pmatrix}. \qquad \textbf{[S27]}$$

The vector of probability distributions $\boldsymbol{P}(t)$ satisfies Eq. **S7**, with the rate matrix $\boldsymbol{K}_{CH}$ corresponding to the reaction in Eq. **S26**. The modified rate equations are still given by Eq. **S15** (and the kinetics by Eq. **S20**) in which $\boldsymbol{T}$ is the matrix of eigenvectors of $\boldsymbol{K}_{CH}$ and $\boldsymbol{\Lambda}(t)$ is a diagonal matrix with elements

$$k_i(t) = \lambda_i\left(\epsilon_i + (1-\epsilon_i)e^{\tau_i}\mathrm{erfc}\sqrt{\tau_i}\right)$$
$$\epsilon_i = \frac{k_D}{\lambda_i + k_D}, \qquad \tau_i = \frac{Dt}{R^2\epsilon_i^2}, \qquad \textbf{[S28]}$$

where λ_i are the eigenvalues of $\boldsymbol{K}_{CH}$.

Multisite Modification via Michaelis–Menten Mechanism

In this section we derive the rate equations for diffusion-influenced multisite modification with the Michaelis–Menten mechanism. For the modification of the substrate with two sites, our results will correspond to the scheme in Fig. 3*B* (main text).

Let us start with the simplest binding reaction $S + E \rightleftharpoons SE$, where a substrate S and an enzyme E bind reversibly and form a complex SE. The association occurs when the diffusing reactants come in contact and is described by a partially reactive boundary condition involving the intrinsic rate constant κ^a. The complex can dissociate with the dissociation rate constant κ^d to form a contact pair of S and E. The ordinary chemical kinetics rate equations for this reaction (i.e., in the limit of fast diffusion) are

$$\frac{d[S]}{dt} = -\kappa^a[E][S] + \kappa^d[SE] = \frac{d[E]}{dt} = -\frac{d[SE]}{dt}. \qquad \textbf{[S29]}$$

The above equations assume that the enzymes and substrates are well mixed and uncorrelated. The rate equations that do not use that assumption involve the pair distribution function:

$$\frac{d[S]}{dt} = -\kappa^a\rho(R,t) + \kappa^d[SE] = \frac{d[E]}{dt} = -\frac{d[SE]}{dt}, \qquad \textbf{[S30]}$$

where $\rho(r,t)$ is the distribution function of pairs S and E separated by r. When $r \to \infty$, the enzyme and substrate are uncorrelated, so that $\rho(r,t) \to [E][S]$. The boundary condition for ρ relates the diffusion flux at $r = R$ to the flux owing to association and dissociation. Because the flux at contact must be equal to the total rate of the reaction, we have

$$4\pi R^2 D\frac{\partial}{\partial r}\rho|_{r=R} = \kappa^a\rho(R,t) - \kappa^d[SE], \qquad \textbf{[S31]}$$

where $D = D_E + D_S$ is the relative diffusion coefficient. The first term in the right-hand side describes the enzyme–substrate pairs that disappear owing to association and the second term corresponds to the pairs formed when the complexes dissociate.

Eq. **S30** and the boundary condition, Eq. **S31**, are formally exact for the microscopic model described above. To close the theory we need the equation for $\rho(r,t)$. The determination of the exact pair distribution function is a complicated many-body problem. When the enzyme and substrate are not in contact ($r > R$), this function changes due to relative diffusion of the enzyme and substrate and due to reaction with other molecules. There are various techniques of approximating the pair distribution function. We use the following arguments to find arguably the simplest approximation valid at small concentrations. The pair distribution function changes on various time scales. There are fast microscopic changes on times comparable to the diffusion time, that is, the time during which the molecule diffuses through the distance comparable to the reaction radius. The distribution function also changes on a slow macroscopic time scale, which corresponds to the reaction rate. When these times are well separated (e.g., owing to small reactant concentration), we can assume that on times longer that the diffusion time the distribution function changes in time only implicitly through the time-dependent concentrations. However, the distribution function does change in space, reflecting the spatial correlations that appear owing to diffusion. Thus, we assume that

$$D\nabla_r^2\rho = 0. \qquad \textbf{[S32]}$$

The above Eqs. **S30**–**S32**, with the requirement that $\rho \to [E][S]$ as $r \to \infty$ constitute the approximate theory that leads to the rate equations with the steady-state rate constants. To show this, we solve Eq. **S32** subject to the boundary condition in Eq. **S31**. In this way we find

$$\rho(r) = [E][S] - \frac{R}{r}\frac{q}{\kappa^a}\left(\kappa^a[E][S] - \kappa^d[SE]\right), \qquad \textbf{[S33]}$$

where $q = \kappa^a/(\kappa^a + k_D)$ is the capture probability and $k_D = 4\pi DR$ is the diffusion-limited rate constant. Note that this pair distribution function depends on time only through the macroscopic concentrations $[E(t)]$, $[S(t)]$ and $[SE(t)]$. Substituting the above equation into Eq. **S30**, we get

$$\frac{d[S]}{dt} = -\kappa^a\epsilon[E][S] + \kappa^d\epsilon[SE] = \frac{d[E]}{dt} = -\frac{d[SE]}{dt}, \qquad \textbf{[S34]}$$

where $\epsilon = 1 - q = k_D/(\kappa^a + k_D)$ is the escape probability.

The resulting equation is the same as the conventional chemical rate equation, Eq. **S29**, but with diffusion-influenced rate constants, which are the original rate constants scaled by the escape probability ϵ.

This is the simplest theory that takes diffusion into account. The theory is valid when the concentrations of S and E are small (the volume fraction is much less than 1). The resulting rate equations involve time-independent rate constants. This description is appropriate for times longer than the diffusion time, R^2/D, and before the power-law asymptotics sets in. When E's are in excess and do not change in time, the concentration of S obtained using Eq. **S34** approaches equilibrium exponentially with the exponent $([E]\kappa^a + \kappa^d)\epsilon$, where $\epsilon = k_D/(\kappa^a + k_D)$. At short times the theory may not be applicable; however, these times are not significant if the relaxation time is longer than the diffusion time, $([E]\kappa^a + \kappa^d)\epsilon R^2/D \ll 1$. The first term in this inequality, which is less than $[E]k_DR^2/D = [E]4\pi R^3$, is small when the volume fraction is small, $[E]4\pi R^3/3 \ll 1$. The second term puts the following constrains on the dissociation rate constant: $\kappa^d\epsilon R^2/D \ll 1$. When κ^a is comparable to k_D, the dissociation time $1/\kappa^d$ must be longer than the diffusion time R^2/D.

The above theory can be systematically improved (2–6). If Eq. **S32** is replaced by the time-dependent diffusion equation for the deviations $\delta\rho(r,t) = \rho(r,t) - [E][S]$, $\partial\delta\rho/\partial t = D\nabla_r^2\delta\rho$, then the theory becomes valid at short times (4). However, the resulting rate equations involve memory kernels instead of rate constants. Even more complex equations that account for three-particle correlations give a better description at larger concentrations and the exact (power-law) long-time asymptotics (4). These theories as well as accurate many-particle stochastic simulations (7, 8) can be used to establish the range of validity of Eq. **S34** more accurately.

Now we generalize the above theory to multisite modification. A substrate with N sites is modified via the Michaelis–Menten mechanism, converting S_0 to S_N ($i = 0, 1, \ldots N-1$):

$$S_i + E \underset{\kappa_i^d}{\overset{\kappa_i^a}{\rightleftharpoons}} S_iE \xrightarrow{\kappa_i^c} S_{i+1} + E. \qquad \textbf{[S35]}$$

As before, we assume that association (with the rate constant κ_i^a), dissociation (κ_i^d), and catalytic modification (κ_i^c) are isotropic.

The rate equations of the conventional chemical kinetics are (κ^c_{-1}, κ^a_N, and κ^d_N are set to zero)

$$\frac{d[S_i]}{dt} = -\kappa^a_i[E][S_i] + \kappa^d_i[S_iE] + \kappa^c_{i-1}[S_{i-1}E]$$
$$\frac{d[S_iE]}{dt} = \kappa^a_i[E][S_i] - \kappa^d_i[S_iE] - \kappa^c_i[S_iE]$$
$$\frac{d[E]}{dt} = \sum_{i=0}^{N-1}\frac{d[S_i]}{dt}. \qquad \textbf{[S36]}$$

The theory that accounts for diffusion is obtained from the conventional chemical rate equations by replacing the bimolecular rate $\kappa^a_i[E][S_i] \to \kappa^a_i\rho_i(R,t)$ similar to Eq. **S30**:

$$\frac{d}{dt}[S_i] = -\kappa^a_i\rho_i(R,t) + \kappa^d_i[S_iE] + \kappa^c_{i-1}[S_{i-1}E]$$
$$\frac{d}{dt}[S_iE] = \kappa^a_i\rho_i(R,t) - \kappa^d_i[S_iE] - \kappa^c_i[S_iE] \qquad \textbf{[S37]}$$

and the equation for E is the same as in Eq. **S36**. Here $\rho_i(r,t)$ is the pair distribution function for E and S_i. As in the binding reaction, we assume that $\rho_i(r,t)$ depends on time only through the macroscopic concentrations. Therefore, the distribution function satisfies the Laplace equation

$$D\nabla^2_r\rho_i = 0 \qquad \textbf{[S38]}$$

with the boundary conditions

$$4\pi R^2 D\frac{\partial}{\partial r}\rho_i|_{r=R} = \kappa^a_i\rho_i(R) - \kappa^d_i[S_iE] - \kappa^c_{i-1}[S_{i-1}E] \qquad \textbf{[S39a}$$

$$\rho_i(r\to\infty) = [E][S_i]. \qquad \textbf{[S39b]}$$

Here $D = D_E + D_{S_i}$ is the relative diffusion coefficient assumed to be independent of i. The first term in the right side of Eq. **S39a** describes the depletion of the distribution function owing to association of E and S_i, the second term corresponds to the pairs formed due to dissociation of the complex S_iE, and the third term describes pairs formed from $S_{i-1}E$ upon catalysis.

Eqs. **S37**–**S39** specify the approximate theory for the diffusion-influenced substrate modification via the Michaelis–Menten mechanism. The main assumptions (i.e., small concentrations so that ρ_i is not affected by three-particle correlations and separation of time scales so that ρ_i depends on time only implicitly via the concentrations) are made in Eq. **S38**.

To find the rate equations for the macroscopic concentrations, we note that the solution of the Laplace equation for ρ_i is $\rho_i(r) = [E][S_i] + c/r$. The constant c is then found from the boundary condition, Eq. **S39a**:

$$\rho_i(r) = [E][S_i] - \frac{R}{r}\frac{q_i}{\kappa^a_i}\left(\kappa^a_i[E][S_i] - \kappa^d[S_iE] - \kappa^c_{i-1}[S_{i-1}E]\right), \qquad \textbf{[S40]}$$

where $q_i = \kappa^a_i/(\kappa^a_i + k_D)$ is the capture probability for E and S_i. This capture probability is related to the escape probability by $\epsilon_i = 1 - q_i$. Then we substitute $\rho_i(R)$ into Eq. **S37** and rewrite the term $\kappa^c_i[S_iE]$ in the equation for $[S_iE]$ as $\kappa^c_i(\epsilon_{i+1} + q_{i+1})[S_iE]$. The resulting rate equations are

$$\frac{d[S_i]}{dt} = -\kappa^a_i\epsilon_i[E][S_i] + \kappa^d_i\epsilon_i[S_iE] + \kappa^c_{i-1}\epsilon_i[S_{i-1}E] \qquad \textbf{[S41a]}$$

$$\frac{d[S_iE]}{dt} = \kappa^a_i\epsilon_i[E][S_i] - \kappa^d_i\epsilon_i[S_iE] - \kappa^c_i\epsilon_{i+1}[S_iE] + \kappa^c_{i-1}q_i[S_{i-1}E] - \kappa^c_i q_{i+1}[S_iE]. \qquad \textbf{[S41b]}$$

In these equations, the terms corresponding to the conventional rate equations in Eq. **S36** are multiplied by the escape probabilities. Two additional terms with the capture probabilities q_i in the right-hand side of Eq. **S41b** correspond to the new reaction channels in the reaction scheme between complexes, $S_{i-1}E \to S_iE$ and $S_iE \to S_{i+1}E$.

The above equations correspond to the kinetic scheme where the adjacent bound complexes are connected. The rate constant of the new reaction channel is equal to the catalytic rate constant multiplied by the corresponding probability that an enzyme and a substrate bind starting from contact (the capture probability). For the two-site modification, the above equations are (κ^a_2, κ^d_2, κ^c_{-1}, q_2 are zero and $\epsilon_2 = 1$)

$$\frac{d[S_0]}{dt} = -\kappa^a_0\epsilon_0[S_0][E] + \kappa^d_0\epsilon_0[S_0E]$$
$$\frac{d[S_1]}{dt} = -\kappa^a_1\epsilon_1[S_1][E] + \kappa^d_1\epsilon_1[S_1E] + \kappa^c_0\epsilon_1[S_0E]$$
$$\frac{d[S_2]}{dt} = \kappa^c_1[S_1E]$$
$$\frac{d[S_0E]}{dt} = \kappa^a_0\epsilon_0[S_0][E] - \left(\kappa^d_0\epsilon_0 + \kappa^c_0\right)[S_0E]$$
$$\frac{d[S_1E]}{dt} = \kappa^a_1\epsilon_1[S_1][E] - \left(\kappa^d_1\epsilon_1 + \kappa^c_1\right)[S_1E] + \kappa^c_0(1-\epsilon_1)[S_0E]$$
$$\frac{d[E]}{dt} = -\kappa^a_0\epsilon_0[S_0][E] + \left(\kappa^d_0\epsilon_0 + \kappa^c_0\epsilon_1\right)[S_0E] - \kappa^a_1\epsilon_1[S_1][E] + \left(\kappa^d_1\epsilon_1 + \kappa^c_1\right)[S_1E]. \qquad \textbf{[S42]}$$

The total concentrations of S and E do not change, so that

$$[S_0] + [S_1] + [S_2] + [S_0E] + [S_1E] = [S_{tot}]$$
$$[E] + [S_0E] + [S_1E] = [E_{tot}], \qquad \textbf{[S43]}$$

where $[S_{tot}]$ and $[E_{tot}]$ are the total concentrations of the substrates and enzymes. These equations correspond to the kinetic scheme in Fig. 3*B* in the main text. When $\epsilon_0 = \epsilon_1 = 1$, they correspond to the ordinary kinetic scheme in Fig. 3*A*.

Diffusion-Modified Rate Equations for the Dual Phosphorylation–Dephosphorylation Cycle. The diffusion-modified kinetic scheme in Fig. 3 *B* and *C* for dual cycle with distributive mechanism is equivalent to the following set of reactions:

$$S_0 + E \underset{\kappa^d_0\epsilon_0}{\overset{\kappa^a_0\epsilon_0}{\rightleftharpoons}} S_0E \xrightarrow{\kappa^c_0\epsilon_1} S_1 + E$$
$$S_1 + E \underset{\kappa^d_1\epsilon_1}{\overset{\kappa^a_1\epsilon_1}{\rightleftharpoons}} S_1E \xrightarrow{\kappa^c_1} S_2 + E$$
$$S_2 + F \underset{\kappa^d_2\epsilon_2}{\overset{\kappa^a_2\epsilon_2}{\rightleftharpoons}} S_2F \xrightarrow{\kappa^c_2\epsilon_3} S_1 + F$$
$$S_1 + F \underset{\kappa^d_3\epsilon_3}{\overset{\kappa^a_3\epsilon_3}{\rightleftharpoons}} S_1F \xrightarrow{\kappa^c_3} S_0 + F$$
$$S_0E \xrightarrow{\kappa^c_0(1-\epsilon_1)} S_1E$$
$$S_2F \xrightarrow{\kappa^c_2(1-\epsilon_3)} S_1F. \qquad \textbf{[S44]}$$

Here the escape probabilities are $\epsilon_i = k_D/(\kappa_i^a + k_D)$, $i = 0, 1, 2, 3$. When all escape probabilities are equal to 1, we recover the ordinary kinetic scheme with intrinsic rate constants, which do not take diffusion into account. The last two reaction channels, $S_0E \to S_1E$ and $S_2F \to S_1F$, disappear.

These reactions correspond to the following rate equations:

$$\begin{aligned}
\frac{d[S_0]}{dt} &= -\kappa_0^a\epsilon_0[S_0][E] + \kappa_0^d\epsilon_0[S_0E] + \kappa_3^c[S_1F] \\
\frac{d[S_1]}{dt} &= -\kappa_1^a\epsilon_1[S_1][E] + \kappa_1^d\epsilon_1[S_1E] + \kappa_0^c\epsilon_1[S_0E] \\
&\quad -\kappa_3^a\epsilon_3[S_1][F] + \kappa_3^d\epsilon_3[S_1F] + \kappa_2^c\epsilon_3[S_2F] \\
\frac{d[S_2]}{dt} &= -\kappa_2^a\epsilon_2[S_2][F] + \kappa_2^d\epsilon_2[S_2F] + \kappa_1^c[S_1E] \\
\frac{d[S_0E]}{dt} &= \kappa_0^a\epsilon_0[S_0][E] - \left(\kappa_0^d\epsilon_0 + \kappa_0^c\right)[S_0E] \\
\frac{d[S_1E]}{dt} &= \kappa_1^a\epsilon_1[S_1][E] - \left(\kappa_1^d\epsilon_1 + \kappa_1^c\right)[S_1E] + \kappa_0^c(1-\epsilon_1)[S_0E] \\
\frac{d[S_2F]}{dt} &= \kappa_2^a\epsilon_2[S_2][F] - \left(\kappa_2^d\epsilon_2 + \kappa_2^c\right)[S_2F] \\
\frac{d[S_1F]}{dt} &= \kappa_3^a\epsilon_3[S_1][F] - \left(\kappa_3^d\epsilon_3 + \kappa_3^c\right)[S_1F] + \kappa_2^c(1-\epsilon_3)[S_2F] \\
\frac{d[E]}{dt} &= -\kappa_0^a\epsilon_0[S_0][E] + \left(\kappa_0^d\epsilon_0 + \kappa_0^c\epsilon_1\right)[S_0E] \\
&\quad -\kappa_1^a\epsilon_1[S_1][E] + \left(\kappa_1^d\epsilon_1 + \kappa_1^c\right)[S_1E] \\
\frac{d[F]}{dt} &= -\kappa_2^a\epsilon_2[S_2][F] + \left(\kappa_2^d\epsilon_2 + \kappa_2^c\epsilon_3\right)[S_2F] \\
&\quad -\kappa_3^a\epsilon_3[S_1][F] + \left(\kappa_3^d\epsilon_3 + \kappa_3^c\right)[S_1F].
\end{aligned} \tag{S45}$$

The total concentrations of the substrate S, kinase E and phosphotase F do not change, so that

$$\begin{aligned}
&[S_0] + [S_1] + [S_2] + [S_0E] + [S_1E] + [S_1F] + [S_2F] = [S_{tot}] \\
&[E] + [S_0E] + [S_1E] = [E_{tot}] \\
&[F] + [S_1F] + [S_2F] = [S_{tot}].
\end{aligned} \tag{S46}$$

These equations are solved numerically to obtain the data shown in Fig. 4 in the main text.

Finite Reactivation Time. In this section we consider the case in which the enzyme becomes inactive right after modifying the substrate. The inactive enzyme ($E^\star$) reactivates with the rate constant $k^\star$:

$$\begin{aligned}
&S_i + E \underset{\kappa_i^d}{\overset{\kappa_i^a}{\rightleftharpoons}} S_iE \xrightarrow{\kappa_i^c} S_{i+1} + E^\star \\
&E^\star \xrightarrow{k^\star} E.
\end{aligned} \tag{S47}$$

The theory that accounts for diffusion in this model involves time-dependent memory kernels instead of rate constants because pair association depends both on diffusion and enzyme reactivation. However, at times longer than both the diffusion time, R^2/D, and the reactivation time, $1/k^\star$, it is possible to get the rate equations with the time-independent rate constants. The reactivation time is assumed to be much shorter than the concentration relaxation, and the concentration of inactive enzymes, $E^\star$, is assumed to be much less than the total enzyme concentration.

At times longer than the reactivation time, both the ordinary chemical kinetics rate equations and the diffusion-modified rate equations are the same as those with instantaneous reactivation (Eqs. **S36** and **S37**, respectively). However, the pair distribution function of S_i and E, ρ_i, is now coupled with the distribution function $\rho_i^\star$ of S_i and the inactive enzyme $E^\star$. The pairs with the inactive enzyme convert to those with the active enzyme, so that the equations for ρ_i and $\rho_i^\star$ are

$$\begin{aligned}
D\nabla_r^2\rho_i + k^\star\rho_i^\star &= 0 \\
D\nabla_r^2\rho_i^\star - k^\star\rho_i^\star &= 0.
\end{aligned} \tag{S48}$$

The boundary conditions describe the pairs that disappear (positive terms) and are formed (negative terms) at contact:

$$\begin{aligned}
4\pi R^2 D\frac{\partial}{\partial r}\rho_i|_{r=R} &= \kappa_i^a\rho_i(R,t) - \kappa_i^d[S_iE] \\
4\pi R^2 D\frac{\partial}{\partial r}\rho_i^\star|_{r=R} &= -\kappa_{i-1}^c[S_{i-1}E]
\end{aligned} \tag{S49}$$

when $r \to \infty$, $\rho_i \to [E][S_i]$ and $\rho_i^\star \to 0$.

Eqs. **S37**, **S48**, and **S49** include the effect of diffusion on the substrate modification when the enzyme reactivation time is finite. We now further rearrange these coupled equations to get the rate equations that involve only macroscopic concentrations. As can be verified by substitution, the solution of Eqs. **S48** and **S49** for the pair distributions can be written as

$$\begin{pmatrix} \rho_i(r) \\ \rho_i^\star(r) \end{pmatrix} = \begin{pmatrix} [E][S_i] \\ 0 \end{pmatrix} + \begin{pmatrix} f_{11}(r) & f_{12}(r) \\ f_{21}(r) & f_{22}(r) \end{pmatrix} \begin{pmatrix} \kappa_i^d[S_iE] - \kappa_i^a[E][S_i] \\ \kappa_{i-1}^c[S_{i-1}E] \end{pmatrix} \tag{S50}$$

if we require $f_{\beta\alpha}(r)$ to satisfy

$$\begin{aligned}
D\nabla_r^2 f_{1\alpha} + k^\star f_{2\alpha} &= 0 \\
D\nabla_r^2 f_{2\alpha} - k^\star f_{2\alpha} &= 0
\end{aligned} \tag{S51}$$

with the boundary condition

$$\begin{aligned}
4\pi R^2 D\frac{\partial}{\partial r}f_{1\alpha}|_{r=R} &= \kappa_i^a f_{1\alpha} - \delta_{1\alpha} \\
4\pi R^2 D\frac{\partial}{\partial r}f_{2\alpha}|_{r=R} &= -\delta_{2\alpha},
\end{aligned} \tag{S52}$$

where $\delta_{\beta\alpha}$ is 1 when $\alpha = \beta$ and 0 otherwise. When $r \to \infty$, $f_{\beta\alpha} \to 0$. Unlike Eqs. **S48** and **S49**, the equations for f's do not involve concentrations and depend only on the parameters of an isolated pair. From Eq. **S50**, the pair distribution that enters Eq. **S37** is

$$\kappa_i^a\rho_i(R) = \kappa_i^a\epsilon_i[E][S_i] + \kappa_i^d q_i[S_iE] + \kappa_{i-1}^c q_i^\star[S_{i-1}E], \tag{S53}$$

where q_i, ϵ_i, and $q_i^\star$ are defined as

$$\begin{aligned}
q_i &= 1 - \epsilon_i = \kappa_i^a f_{11}(R) \\
q_i^\star &= \kappa_i^a f_{12}(R).
\end{aligned} \tag{S54}$$

Substituting $\kappa_i^a\rho_i(R)$ in Eq. **S53** into Eq. **S37**, we get the diffusion-modified rate equations (the equation for $[E]$ is the same as that in Eq. **S37**):

$$\frac{d[S_i]}{dt} = -\kappa_i^a\epsilon_i[E][S_i] + \kappa_i^d\epsilon_i[S_iE] + \kappa_{i-1}^c\epsilon_i^\star[S_{i-1}E] \tag{S55a}$$

$$\begin{aligned}
\frac{d[S_iE]}{dt} &= \kappa_i^a\epsilon_i[E][S_i] - \kappa_i^d\epsilon_i[S_iE] - \kappa_i^c\epsilon_{i+1}^\star[S_iE] + \kappa_{i-1}^c q_i^\star[S_{i-1}E] \\
&\quad - \kappa_i^c q_{i+1}^\star[S_iE].
\end{aligned} \tag{S55b}$$

These equations are the sought-for rate equations that involve only macroscopic concentrations.

To understand the meaning of q_i, ϵ_i, and $q_i^\star$, consider the irreversible binding of a single enzyme and a single substrate, which are initially separated by the contact radius R. If the enzyme is initially in the inactive state, it first converts to the active state and then binds to the substrate. Let $B(t|1)$ (or $B(t|2)$) be the probability that the enzyme and substrate are bound at time t, given that initially the enzyme is in the active (or inactive) state in contact with the substrate. At long times, $B(t|1)$ and $B(t|2)$ tend to the capture probabilities for the enzyme that is initially in the active and inactive states, respectively.

The probabilities $B(t|\alpha)$ increase owing to binding. The rate of the increase is

$$\frac{dB(t|\alpha)}{dt}=\kappa_i^a G_{1\alpha}(R,t|R). \qquad \textbf{[S56]}$$

Here $G_{\beta\alpha}(r,t|r')$ is the time-dependent probability density for an enzyme to be in r in state β at time t provided it was initially in r' in state α. The states α and β refer to the active (α, $\beta=1$) and inactive (α, $\beta=2$) states of the enzyme. When the enzyme is in state 1, it can bind to the substrate with the intrinsic rate constant κ_i^a. This probability density satisfies the following equation:

$$\begin{aligned}\frac{\partial}{\partial t}G_{1\alpha}&=D\nabla_r^2 G_{1\alpha}+k^\star G_{2\alpha}\\ \frac{\partial}{\partial t}G_{2\alpha}&=D\nabla_r^2 G_{2\alpha}-k^\star G_{2\alpha}\end{aligned} \qquad \textbf{[S57]}$$

with the partially absorbing boundary condition for the enzyme in the active state and reflecting for the enzyme in the inactive state:

$$\begin{aligned}4\pi R^2 D\frac{\partial}{\partial r}G_{1\alpha}|_{r=R}&=\kappa_i^a G_{1\alpha}\\ 4\pi R^2 D\frac{\partial}{\partial r}G_{2\alpha}|_{r=R}&=0.\end{aligned} \qquad \textbf{[S58]}$$

Initially, $G_{\beta\alpha}(t=0)=\delta(r-r')/4\pi r^2$ when $\alpha=\beta$ and 0 otherwise.

Solving Eq. **S56**, we have the probability to be bound at time t:

$$B(t|\alpha)=\kappa_i^a\int_0^t G_{1\alpha}(R,t'|R)dt'. \qquad \textbf{[S59]}$$

In the next step we are going to relate the probabilities $B(t|\alpha)$ to the quantities in Eq. **S54**. First, let us note that the probability density $G_{\beta\alpha}$ is related to $f_{\beta\alpha}$ by

$$f_{\beta\alpha}(r)=\int_0^\infty G_{\beta\alpha}(r,t|R)dt. \qquad \textbf{[S60]}$$

This relation can be verified by integrating both sides of Eq. **S57** for $G_{\beta\alpha}(r,t|R)$ with respect to t from 0 to ∞, using $G_{\beta\alpha}(t\to\infty)=0$. The resulting equation is the same as Eq. **S51**, except for the term with the delta function, $\delta(r-r')/4\pi r^2=\delta(r-R)/4\pi R^2$, which comes from the initial condition. This term is 0 when $r>R$ and contributes to the boundary condition, which becomes exactly the same as in Eq. **S52**.

Using the above relation, Eq. **S60**, in Eq. **S54** and comparing the result with Eq. **S59**, we find that

$$\begin{aligned}q_i&=\kappa_i^a\int_0^\infty G_{11}(R,t|R)dt=\lim_{t\to\infty}B(t|1)\\ q_i^\star&=\kappa_i^a\int_0^\infty G_{12}(R,t|R)dt=\lim_{t\to\infty}B(t|2).\end{aligned} \qquad \textbf{[S61]}$$

Thus, q_i and $q_i^\star$ are the long-time limit of the probabilities that the enzyme and substrate are bound (i.e., the capture probabilities) given that they are initially in contact. q_i is the capture probability for the enzyme that is initially in the active state, and $q_i^\star$ is the capture probability for the enzyme that is initially in the inactive state.

The explicit expressions for the capture probabilities in Eq. **S54** are found by solving Eq. **S51** with the boundary condition in Eq. **S52**. The solution is $f_{11}(r)=c_{11}/r$, $f_{22}(r)=c_{22}\exp(-\sqrt{k^\star/D}(r-R))/r$, $f_{12}(r)=c_{12}/r-f_{22}(r)$, where $c_{\beta\alpha}$ are constants. Because the enzyme cannot convert from the active to inactive state in the course of the irreversible reaction, $f_{21}(r)=0$. The constants $c_{\beta\alpha}$ are found from the boundary condition. The expressions for f_{11} and f_{12} are

$$\begin{aligned}f_{11}(r)&=\frac{1}{4\pi Dr}\frac{k_D}{\kappa_i^a+k_D}\\ f_{12}(r)&=\frac{1}{4\pi Dr}\frac{1}{1+\sqrt{k^\star\tau_d}}\left(1+\sqrt{k^\star\tau_d}\,\frac{k_D}{\kappa_i^a+k_D}-e^{-\sqrt{k^\star/D}(r-R)}\right),\end{aligned} \qquad \textbf{[S62]}$$

where $\tau_d=R^2/D$ is the diffusion time. Substituting these into Eq. **S54**, we find the capture probabilities

$$\begin{aligned}q_i&=\frac{\kappa_i^a}{k_D+\kappa_i^a}=1-\epsilon_i\\ q_i^\star&=\frac{\sqrt{k^\star\tau_d}}{1+\sqrt{k^\star\tau_d}}\cdot\frac{\kappa_i^a}{k_D+\kappa_i^a}=1-\epsilon_i^\star.\end{aligned} \qquad \textbf{[S63]}$$

To understand the capture probability for the enzyme that is initially in the inactive state, $q_i^\star$, note that the enzyme is first reactivated, being still around the substrate, and then binds with the substrate. Therefore, the corresponding capture probability is a product of two factors: (*i*) the probability that the enzyme is reactivated and comes to contact with the substrate and (*ii*) the probability that the enzyme in the active state in contact with the substrate is "captured." The second factor is the same as q_i. To find the first factor (with the square roots), one has to solve the same problem as for $q_i^\star$, but in the limit of perfectly absorbing sphere ($\kappa_i^a\to\infty$). The capture probabilities are related to the escape probabilities $\epsilon_i=1-q_i$ and $\epsilon_i^\star=1-q_i^\star$. The latter is given in Eq. **10** in the main text.

The rate equations in Eq. **S55** are applicable for the modification of substrates with many sites. They are the same as Eq. **S41** for the modification with instantaneous reactivation, except the escape and capture probabilities multiplying the catalytic rate are replaced by $\epsilon^\star$ and $q^\star$, corresponding to the enzyme that is initially in the inactive state. The rate equations correspond to the kinetic scheme where adjacent bound states are connected. The rate constants of the new connections $S_{i-1}E\to S_iE$ are proportional to the capture probabilities $q_i^\star$ for the enzyme that is in the inactive state initially. When the reactivation time is much longer than the diffusion time, $q_i^\star\to 0$. In this case the only effect of diffusion is to rescale the association and dissociation rate constants with corresponding escape probabilities, ϵ_i, whereas the catalytic rate constants are unmodified and no new reaction channels appear in the kinetic scheme.

For the substrate with two sites, the rate equations, Eq. **S55**, are (since κ_2^a, κ_2^d, κ_{-1}^c, $q_2^\star$ are zero and $\epsilon_2^\star = 1$)

$$\frac{d[S_0]}{dt} = -\kappa_0^a\epsilon_0[S_0][E] + \kappa_0^d\epsilon_0[S_0E]$$

$$\frac{d[S_1]}{dt} = -\kappa_1^a\epsilon_1[S_1][E] + \kappa_1^d\epsilon_1[S_1E] + \kappa_0^c\epsilon_1^\star[S_0E]$$

$$\frac{d[S_2]}{dt} = \kappa_1^c[S_1E]$$

$$\frac{d[S_0E]}{dt} = \kappa_0^a\epsilon_0[S_0][E] - \left(\kappa_0^d\epsilon_0 + \kappa_0^c\right)[S_0E]$$

$$\frac{d[S_1E]}{dt} = \kappa_1^a\epsilon_1[S_1][E] - \left(\kappa_1^d\epsilon_1 + \kappa_1^c\right)[S_1E] + \kappa_0^c q_1^\star[S_0E]$$

$$\frac{d[E]}{dt} = -\kappa_0^a\epsilon_0[S_0][E] + \left(\kappa_0^d\epsilon_0 + \kappa_0^c\epsilon_1^\star\right)[S_0E]$$
$$-\kappa_1^a\epsilon_1[S_1][E] + \left(\kappa_1^d\epsilon_1 + \kappa_1^c\right)[S_1E]. \qquad \textbf{[S64]}$$

The total concentrations of S and E conserve, so that

$$[S_0] + [S_1] + [S_2] + [S_0E] + [S_1E] = [S_{tot}]$$
$$[E] + [S_0E] + [S_1E] = [E_{tot}]. \qquad \textbf{[S65]}$$

These equations correspond to the following set of reactions:

$$S_0 + E \underset{\kappa_0^d\epsilon_0}{\overset{\kappa_0^a\epsilon_0}{\rightleftharpoons}} S_0E \xrightarrow{\kappa_0^c\epsilon_1^\star} S_1 + E$$

$$S_1 + E \underset{\kappa_1^d\epsilon_1}{\overset{\kappa_1^a\epsilon_1}{\rightleftharpoons}} S_1E \xrightarrow{\kappa_1^c} S_2 + E$$

$$S_0E \xrightarrow{\kappa_0^c\left(1-\epsilon_1^\star\right)} S_1E. \qquad \textbf{[S66]}$$

These reactions are the same as those in the kinetic scheme in Fig. 3*B* (main text), except the escape and capture probabilities that multiply κ_0^c are replaced by $\epsilon_1^\star$ and $q_1^\star = 1 - \epsilon_1^\star$.

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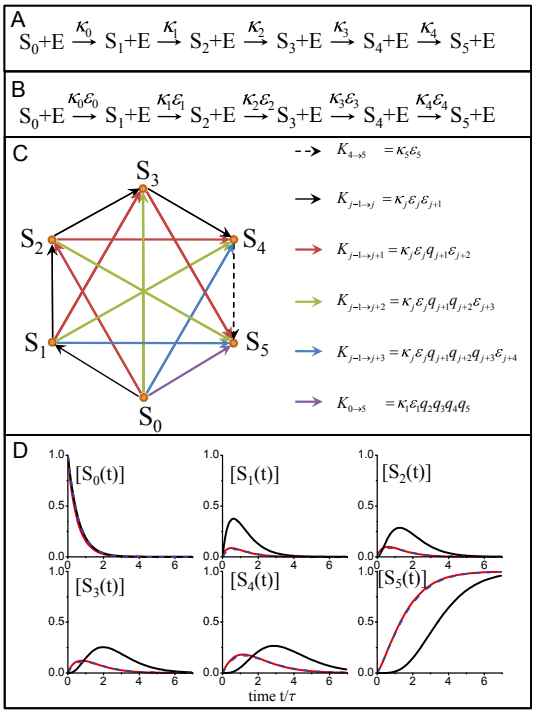

Fig. S1. Five-site modification using exactly solvable model. (*A*) Kinetic scheme corresponding to the conventional chemical kinetics. (*B*) Kinetic scheme in *A* with the rate constants replaced by the diffusion-influenced ones with the escape probabilities $\epsilon_i = k_D/(\kappa_i + k_D)$. (*C*) Diffusion-modified kinetic scheme with new reaction channels between the species. The rate constants are scaled with the escape, ϵ_i, and capture, $q_i = 1 - \epsilon_i$, probabilities. (*D*) Kinetics of six states. The kinetics corresponding to schemes in *B* (black lines) and *C* (dashed blue lines) are compared with the exact solution (red lines) from Eq. **S20**. Initially, the substrate is unmodified. $\kappa_i = (5 - i)k_D$, $i = 0, \ldots, 5$, $R = 1$, $D = 1$, $[S_{tot}] = 1$, $\tau^{-1} = [E]k_D/2$, $[E]4\pi R^3/3 = 0.01$.

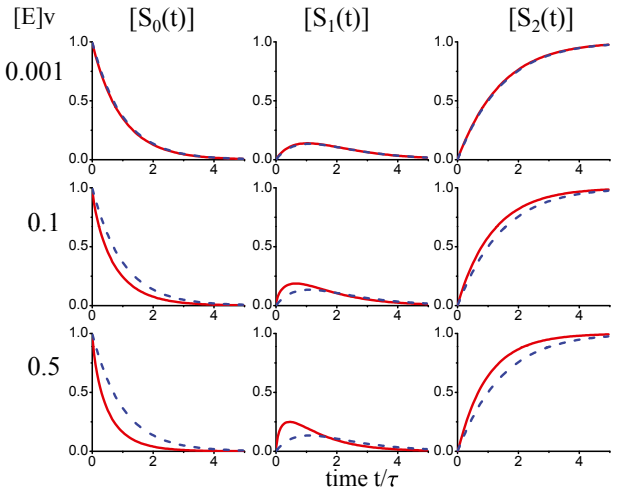

Fig. S2. Effect of enzyme concentration on the kinetics of a simple model of dual modification $S_0 + E \rightarrow S_1 + E \rightarrow S_2 + E$. Kinetics predicted by the scheme in Fig. 2*B* (main text) (dashed blue lines, Eq. **S24**) is compared with the exact kinetics (red lines, Eq. **S21**) at various enzyme concentrations. Time is normalized to $\tau = (\kappa_0 \epsilon_0 [E])^{-1}$. Parameters are the same as in Fig. 2 (main text), except the apparent volume fraction $v[E] = 0.001$, 0.1, and 0.5; $v = 4\pi R^3/3$.

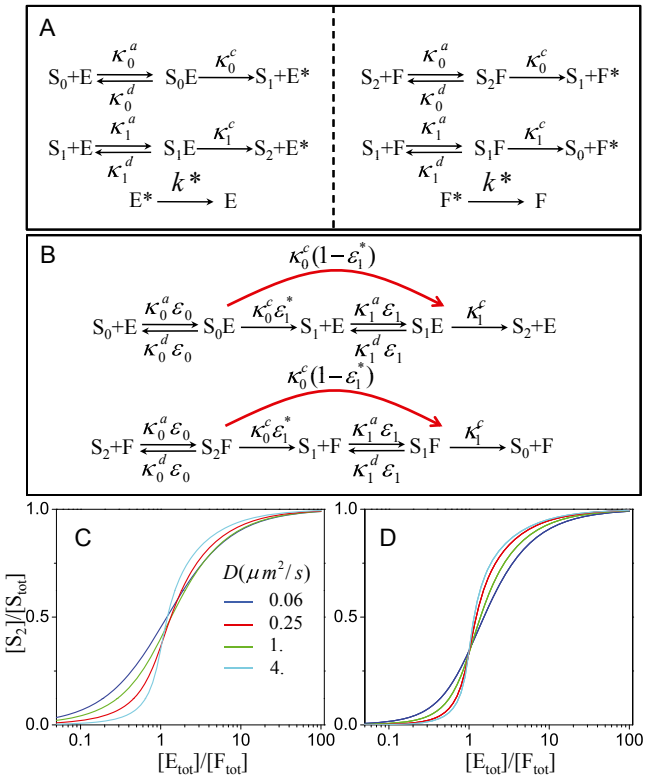

Fig. S3. Steady-state input–output relations in a double phosphorylation cycle. (*A*) Kinetic scheme describing double phosphorylation by a kinase E and dephosphorylation by a phosphotase F. After modification, *E* and *F* are in the inactive state (E* and F*) and reactivate with the relaxation rate k^*. For simplicity, the phosphorylation rate constants are the same as those for dephosphorylation. (*B*) Diffusion-modified kinetic scheme, valid when $[E] \gg [E^\star]$, with the rate constants scaled by the escape probabilities, $\epsilon_i = k_D/(\kappa_i^a + k_D)$, $i = 0$, 1 and $\epsilon_1^* = 1 - \beta\kappa_1^a/((\kappa_1^a + k_D)$, $\beta = \sqrt{k^*\tau_d}/(1 + \sqrt{k^*\tau_d})$, $k_D = 4\pi RD$, $\tau_d = R^2/D$. Additional reaction channels (red) connect the bound complexes and appear owing to diffusion when the enzyme reactivation is fast. (*C*) Steady-state population of doubly phosphorylated substrate as a function of the ratio of kinase and phosphotase concentrations for fast enzyme reactivation for various diffusion coefficients. The reactivation time is $\ln 2/k^* = 1\ \mu s$. The steady-state concentrations are calculated according to the scheme (*B*). The parameters are the same as in ref. 1. $\kappa_0^a = 0.027\ nM^{-1}s^{-1}$, $\kappa_1^a = 0.056\ nM^{-1}s^{-1}$, $\kappa_0^d = 1.35\ s^{-1}$, $k_1^d = 1.73\ s^{-1}$, $k_0^c = 1.5s^{-1}$, $k_1^c = 15s^{-1}$, $R = 5nm$, $[E_{tot}] + [F_{tot}] = 100\ nM$, $[S_{tot}] = 200\ nM$. (*D*) The same as *C* for slow enzyme reactivation (the reactivation time $\ln 2/k^* = 10\ ms$).

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