1 Kramers theory of diffusive reactions with multiple kinetic barriers

In this section, we present a detailed derivation of some key equations of Kramers theory of diffusive reactions with multiple kinetic barriers, which are discussed in Section II of the main text.

Let us consider a Brownian particle moving in one-dimensional potential landscape \( G(x) \), such as the one sketched in Fig. 1 of the main text. It is assumed that the mean thermal energy of the Brownian particle is much smaller than the free energy barrier height. The Brownian particle starts diffusing from an initial location \( x_0 \) lying somewhere in some region of space \( \Omega \) with boundary \( \partial \Omega \). In the following we will consider the case when \( \Omega = (-\infty, x_e] \). The key question that we want to address is: how long does it take on average for the Brownian particle to diffuse from \( x_0 \) to the boundary \( \partial \Omega \) and hence leave the set \( \Omega \)? The starting point for answering this question is the Fokker-Planck equation, which describes the time evolution of the probability \( p(x, t|x_0) \) that the Brownian particle will be at position \( x \) at time \( t \) given that
it started at position \( x_0 \) at \( t = 0 \). The Fokker-Planck equation reads (Eq. (1) of the main text):

\[
\frac{\partial}{\partial t} p(x, t| x_0) = \frac{\partial}{\partial x} \left[ \frac{1}{\gamma} \frac{\partial G(x)}{\partial x} p(x, t| x_0) \right] + D \frac{\partial^2}{\partial x^2} p(x, t| x_0)
\]

(S1)

where \( \gamma \) is the frictional coefficient (note: we assume to be in the large friction limit so that the inertia term can be neglected), \( D \) is the diffusion coefficient and \( \gamma D = \frac{1}{\kappa} k_B T \).

The key quantity of interest is the first passage time \( \tau(x_0) \), defined as the first time at which a Brownian particle starting at \( x_0 \) hits the point \( x_e \) defining the boundary of \( \Omega \). Since \( \tau(x_0) \) is a random variable, we consider the probability distribution \( T(t| x_0) \) that \( \tau(x_0) \) equals \( t \). According to the theory of first passage times [1], \( T(t| x_0) \) is computed as follows

\[
T(t| x_0) = -\frac{dQ(t| x_0)}{dt}, \quad \text{(S2)}
\]

where \( Q(t| x_0) \) is the probability that at time \( t \) a Brownian particle starting at \( x_0 \) has not yet reached the boundary \( \partial \Omega \), i.e.

\[
Q(t| x_0) = \int_{\Omega} p(x, t| x_0) dx. \quad \text{(S3)}
\]

To prove the equation \( T(t| x_0) = -\frac{dQ(t| x_0)}{dt} \), we note that \( Q(t| x_0) \) can be also interpreted as the probability that the first passage time is larger than \( t \), that is \( Q(t| x_0) = \text{Prob}[\tau > t] \). Hence:

\[
Q(t| x_0) = \int_t^\infty T(s| x_0) ds. \quad \text{(S4)}
\]

Taking the derivative with respect to \( t \) on both sides, yields

\[
\frac{dQ(t| x_0)}{dt} = -T(t| x_0), \quad \text{(S5)}
\]

which is what we wanted to prove. The average first passage time is therefore given by (integration by parts)

\[
\langle \tau(x_0) \rangle = \int_0^\infty t \ T(t| x_0) dt = -\int_0^\infty t \ \frac{dQ(t| x_0)}{dt} dt \quad \text{(S6)}
\]

\[
= \int_0^\infty Q(t| x_0) dt = \int_0^\infty dt \int_{\Omega} dx \ p(x, t| x_0).
\]
This is Eq. (3) of the main text. We now combine Eq. (S6) with Eq. (S1) to obtain a differential equation for $\langle \tau(x_0) \rangle$, which is given by:

$$-rac{1}{\gamma} \frac{dG(x_0)}{dx_0} \frac{d\langle \tau(x_0) \rangle}{dx_0} + D \frac{d^2 \langle \tau(x_0) \rangle}{dx_0^2} = -1$$  \hspace{1cm} (S7)

subject to the following boundary condition

$$\langle \tau(x_0) \rangle = 0 \text{ for } x_0 \in \partial\Omega, \text{ i.e. } \langle \tau(x_e) \rangle = 0.$$  \hspace{1cm} (S8)

To prove Eq. (S7), we first notice that if $p(x, t|x_0, t_0)$ satisfies the forward Fokker-Planck equation

$$\frac{\partial}{\partial t} p(x, t|x_0, t_0) = L_{FP}(x)p(x, t|x_0, t_0)$$

with respect to the variables $(x, t)$, with the Fokker-Planck operator

$$L_{FP}(x) = \frac{\partial}{\partial x} \left[ \frac{1}{\gamma} \frac{\partial G(x)}{\partial x} \right] + D \frac{\partial^2}{\partial x^2},$$  \hspace{1cm} (S9)

then $p(x, t|x_0, t_0)$ satisfies the backward Fokker-Planck equation with respect to the variables $(x_0, t_0)$, i.e.

$$\frac{\partial}{\partial t_0} p(x, t|x_0, t_0) = -L_{FP}^\dagger(x_0)p(x, t|x_0, t_0)$$  \hspace{1cm} (S10)

where the adjoint Fokker-Planck operator is

$$L_{FP}^\dagger(x_0) = -\frac{\partial}{\partial x_0} \left[ \frac{1}{\gamma} \frac{\partial G(x_0)}{\partial x_0} \right] + D \frac{\partial^2}{\partial x_0^2}.\hspace{1cm} (S11)$$

Thus, applying the adjoint Fokker-Planck operator to $\langle \tau(x_0) \rangle$, we find

$$L_{FP}^\dagger(x_0)\langle \tau(x_0) \rangle = \int_0^\infty dt \int_\Omega dx \ L_{FP}^\dagger(x_0)p(x, t|x_0, t_0)$$  \hspace{1cm} (S12)

$$= -\int_0^\infty dt \int_\Omega dx \ \frac{\partial}{\partial t_0} p(x, t|x_0, t_0),$$

where in the last step we used the backward Fokker-Planck equation (S10). Using the stationarity condition $p(x, t|x_0, t_0) = p(x, t-t_0|x_0, 0)$, we can rewrite the above equation as

$$L_{FP}^\dagger(x_0)\langle \tau(x_0) \rangle = -\int_0^\infty dt \int_\Omega dx \ \frac{\partial}{\partial t_0} p(x, t|x_0, t_0)$$  \hspace{1cm} (S13)

$$= \int_0^\infty dt \int_\Omega dx \ \frac{\partial}{\partial t} p(x, t|x_0, 0) = -1,$$

S3
which is Eq. (S7).

To solve Eq. (S7), we multiply on both sides by $e^{-\beta G(x_0)}$, yielding

$$\left( \frac{d}{dx_0} e^{-\beta G(x_0)} \right) \frac{d\langle \tau(x_0) \rangle}{dx_0} + e^{-\beta G(x_0)} \frac{d^2 \langle \tau(x_0) \rangle}{dx_0^2} = -\frac{1}{D} e^{-\beta G(x_0)} \quad (S14)$$

This equation can be rewritten as

$$\frac{d}{dx_0} \left( e^{-\beta G(x_0)} \frac{d\langle \tau(x_0) \rangle}{dx_0} \right) = -\frac{1}{D} e^{-\beta G(x_0)} \quad (S15)$$

Integrating once, we find

$$e^{-\beta G(x_0)} \frac{d\langle \tau(x_0) \rangle}{dx_0} = -\frac{1}{D} \int_{-\infty}^{x_0} e^{-\beta G(z)} dz. \quad (S16)$$

Then we integrate another time using the boundary condition $\langle \tau(x_e) \rangle = 0$ and

$$\langle \tau(x_0) \rangle = -\frac{1}{D} \int_{x_e}^{x_0} e^{\beta G(y)} \int_{-\infty}^{y} e^{-\beta G(z)} dz. \quad (S17)$$

The solution to Eq. (S7) with the boundary condition (S8) is therefore:

$$\langle \tau(x_0) \rangle = \frac{1}{D} \int_{x_e}^{x_0} dy \int_{-\infty}^{y} dz \ e^{\beta [G(y) - G(z)]}. \quad (S18)$$

This is Eq. (6) in the main text. The integrals in Eq. (S18) can be evaluated using the saddle point approximation or Laplace’s method [3]. To this end, we need to maximize the integrand over the integration range of Eq. (S18), i.e. we need to find $\max_{z \leq y} [G(y) - G(z)]$. Let us denote the points in the range of integration of Eq. (S18) where the integrand in Eq. (S18) is maximal as $y = x^*$ and $z = x_*$, and so using the following expansions (note that $G''(x^*) < 0$ since $x^*$ is a maximum point):

$$G(y) = G(x^*) - \frac{|G''(x^*)|}{2} (y - x^*)^2 + \text{higher order terms} \quad (S19)$$

and

$$G(z) = G(x_*) + \frac{G''(x_*)}{2} (z - x_*)^2 + \text{higher order terms}, \quad (S20)$$
we find:

$$\langle \tau(x_0) \rangle = \frac{2\pi}{\beta D \sqrt{|G''(x^*)| G''(x_*)}} e^{\beta[G(x^*) - G(x_*)]},$$  \hspace{1cm} (S21)  

where we have used the result for Gaussian integrals \( \int e^{-\alpha x^2} dx^2 = \sqrt{\pi/\alpha} \) for \( \alpha > 0 \). The transition rate from \( x_0 \) to \( x_e \) is the inverse of the average first hitting time, \( k(x_0 \rightarrow x_e) = 1/\langle \tau(x_0) \rangle \), and so, using \( \beta D = 1/\gamma \), we find:

$$k(x_0 \rightarrow x_e) = \frac{\sqrt{|G''(x^*)| G''(x_*)}}{2\pi\gamma} e^{-\beta \Delta G^\dagger} = \frac{\omega_1 \omega_2}{2\pi\gamma} e^{-\beta \Delta G^\dagger},$$  \hspace{1cm} (S22)  

where

$$\Delta G^\dagger = G(x^*) - G(x_*) = \max_{x_0 \leq z \leq x_e} [G(y) - G(z)],$$  \hspace{1cm} (S23)  

and \( \omega_1 \) and \( \omega_2 \) are the curvatures (i.e. second derivatives) of the potential at \( x_* \) and \( x^* \), respectively.

### 2 Details on MC and MD simulations

#### 2.1 Diffusion of a single particle on a free energy landscape

To test the validity of key results Eqs. (S22) and (S23) (which are Eqs. (8) and (9) in the main text), numerical evidence is collected from Monte Carlo simulations of a particle moving in a
Figure S2: Eq. (S23) is used to determine the relevant barrier for three examples (a, b and c) of free energy (left) and enthalpy (middle) landscapes and comparison to MC computer simulations of the escape times, measured as the average number of MC steps needed to reach the global potential minimum (right).
one-dimensional box. The probability of acceptance of moves obey the Metropolis-Hastings algorithm, reflecting the Brownian particle’s sensitivity to the surrounding energy landscape $\Delta G(x)$.

The particle is initially placed at the centre of the leftmost well and displaced randomly every step. To simulate diffusive processes under various temperatures, the width of the uniform step size distribution is set to be proportional to temperature, as described by Einstein’s relation $D = k_B T / \gamma$. The average number of steps taken to reach the rightmost well for the first time is equivalent to $\langle \tau(x_0) \rangle$. As Equation (S22) states that $\langle \tau(x_0) \rangle$ depends exponentially on the relevant barrier height, an Arrhenius plot of $\log(\langle \tau(x_0) \rangle)$ against $\beta = 1/k_B T$ is expected to yield a straight line with the barrier height as the slope.

The first set of landscapes tested consist of three square wells separated by two square barriers defined on the interval $x \in [0, 1]$, with box boundaries $G(x) \to \infty$ for $x < 0$ and $x > 1$. Although unrealistic, simulations of square landscapes are particularly useful because on these landscapes, exact solutions to Eq.(S1) is obtainable through analytical methods, and can be easily compared to reflect accuracy of the results from simulation and test soundness of the simulation setup. It should be stressed that the theoretical results only become exact in the high barrier limit $G \gg k_B T$, but practically this condition is difficult to satisfy due to an exponential increase in CPU time with barrier height. This introduces a ”systematic error” to the $\Delta G^\ddagger$ extracted from the Arrhenius plot, though it is in fact a property intrinsic to Eq.(S1)’s exact solution that reflects thermal effects of finite temperature. It is also expected that the limited number of crossings sampled results in a random spread in the plot.

Another subtlety arises from the fact that the landscape explicitly imposed in the algorithm is an enthalpy landscape, whereas entropy changes are implicitly imposed by the widths of potential wells accessible to the particle. To connect these simulations with theory, we can simply rewrite Eq. (S22) using $G = H - TS$ and absorbing the entropy contribution into the
prefactor, we obtain:

$$k(x_0 \rightarrow x_e) \sim e^{-\beta \Delta H^\ddagger}, \quad (S24)$$

where the activation enthalpy $\Delta H^\ddagger$ is the quantity measured from simulations. However, the analogous statement:

$$\Delta H^\ddagger = H(x^*) - H(x_e) = \max_{x_0 \leq z \leq y \leq x_e} [H(y) - H(z)] \quad (S25)$$

is not true because the criterion for determining the activation energy still follows Eq. (S23). Although reactions on the landscapes tested in this work do not involve any significant entropic change, it is not uncommon to find condensed phase reactions with enthalpy and free energy landscapes so drastically different that the height ordering of minima and maxima becomes different. Any potential confusion may be resolved by realizing that the relevant activation energy barrier is determined by the free energy landscape, while the measured temperature dependence of rate constants only reflect the enthalpic contribution to this barrier. In other words, the relevant barrier is found by using Eq. (S23) on the free energy landscape; the measured $\Delta H^\ddagger$ is then the enthalpy change associated with the relevant free energy barrier; $\Delta H^\ddagger$ need not correspond to the highest enthalpy change. The same applies to experimental measurements of real chemical reactions (see e.g. Figure 4 of the main text).

Since physical energy landscapes of reactions are usually smooth rather than square, we moved on to test the reaction rates on a set of smooth landscapes, in which case the external force on the diffusing particles are continuous and we may find the numerical solution of Eq. (S1) from Molecular Dynamics simulations. Additionally, the friction coefficient $\gamma$ is set explicitly in MD, which allows the transition between ballistic and diffusive regimes to be controlled. This is in contrast to MC which could only probe diffusive reaction due to the random walk nature of the MC moves.

The results of MD are shown in Fig. S3. We also find that the results in Eq. (S22) and
Figure S3: **Relevant barriers found from MD simulations on smooth landscapes**: red arrows mark the enthalpy barrier that enters the exponent of Arrehenius equation. Reaction starts from $x = 0.15$ and is recorded as finished when the diffusing particle crosses $x = 0.80$.

Eq. (S23) were indeed obeyed both in MC and MD when testing on the same smooth landscapes.

### 2.2 Coarse-grained model for amyloid nucleation

We used coarse-grained Monte Carlo model for primary and secondary amyloid nucleation developed in [6] and [7]. The model, potentials, and the notation are kept as in the original papers. The parameters of the model are summarised in Fig. S4. The exact parameters used in this work are as follows. For both primary and secondary nucleation: $\epsilon_{\beta\beta} = 60k_B T$, $\epsilon_{s\beta} - \epsilon_{ss} = k_B T$, and $\mu_{s \rightarrow \beta} = 20k_B T$. For primary nucleation: $\epsilon_{ss} = 4.5k_B T$, and concentration of monomers $c = 1.8 \text{mM}$. For secondary nucleation: $\epsilon_{ss} = 4k_B T$, $\epsilon_{sf} = 6k_B T$, $\epsilon_{i\beta} - \epsilon_{ii} = k_B T$, $\epsilon_{if} = 1k_B T$, $\epsilon_{si} = 8k_B T$, $\epsilon_{ii} = 16k_B T$, $\mu_{s \rightarrow i} = 10k_B T$, $\mu_{i \rightarrow \beta} = 10k_B T$, and the concentration of monomers $c \approx 0.15 \text{mM}$.

### 2.3 Results of Coarse-grained simulations of amyloid aggregation
Coarse-grained model: possible interactions in the system.

- Interactions between monomers: $\epsilon_{SS}$, $\epsilon_{ii} = 16kT$, $\epsilon_{i\beta} = 60kT$
- Interactions with the fibril: $\epsilon_{sf} = 6kT$, $\epsilon_{if} = 1kT$

$\epsilon_{Si} = 8kT$, $\epsilon_{S\beta} = \epsilon_{SS} + 1kT$, $\epsilon_{i\beta} = \epsilon_{ii} + 1kT$

Figure S4: Coarse-grained model: possible interactions in the system.

Coarse-grained simulations of temperature dependence of primary nucleation.

(a) Two regimes are observed: a low-temperature regime, where the nucleation proceeds via stable pre-nucleation oligomers ("two-step nucleation"), and a high-temperature regime, where the nucleation proceeds by protein conversion either in solution or within small unstable dimers ("one-step nucleation"). (b) The size of the nucleating oligomer increases as the nucleating mechanism changes from high to low temperatures.

3 Analysis of three state kinetics using the master equation

3.1 Spectral analysis of the rate matrix

This section contains an explicit spectral analysis of the rate matrix for the kinetics in a three state system using a chemical master equation approach. The first part is mostly textbook
The (chemical) master equation for three subsequent states 1,2,3 depicted in Fig. S6 reads

\[
\begin{pmatrix}
\dot{p}_1 \\
\dot{p}_2 \\
\dot{p}_3
\end{pmatrix}
= \begin{pmatrix}
k_{21}p_2 - k_{12}p_1 \\
k_{12}p_1 + p_2(-k_{21} - k_{23}) + k_{32}p_3 \\
k_{32}p_2 - k_{32}p_3
\end{pmatrix},
\]

(S26)

where \(p_1, p_2, p_3\) are respectively, the time dependent populations, and \(k_{ij}\) denotes the kinetic rate constant between state \(i\) and \(j\). Note that \(p_1 + p_2 + p_3 = 1\). This master equation can be conveniently re-written in matrix form as

\[
\dot{p} = Kp,
\]

(S27)

with

\[
K = \begin{pmatrix}
-k_{12} & k_{21} & 0 \\
k_{12} & -k_{21} - k_{23} & k_{32} \\
0 & k_{23} & -k_{32}
\end{pmatrix}
\]

(S28)
and \( p = \{p_1, p_2, p_3\} \). The solution of this equation is in general

\[
p(t) = p(0) \exp(-Kt),
\]

where \( p(0) \) is the vector of initial conditions. Alternatively the solution can be expanded in eigenfunctions \( \Phi_\lambda \) of \( K \), which are given by

\[
K \Phi_\lambda = \lambda \Phi_\lambda,
\]

so that the solution can be written as

\[
p(t) = \sum_\lambda c_\lambda \Phi_\lambda e^{\lambda t}.
\]

The largest eigenvalue is \( \lambda_0 = 0 \) and its corresponding eigenvector is \( \Phi_0 = p_{eq} \). For the three state system the other two eigenvalues can be expressed analytically as

\[
\begin{align*}
\lambda_1 &= -\frac{1}{2} \left( k_{sum} - \sqrt{(k_{sum})^2 - 4(k_{12}k_{23} + k_{12}k_{32} + k_{21}k_{32})} \right) \\
\lambda_2 &= -\frac{1}{2} \left( k_{sum} + \sqrt{(k_{sum})^2 - 4(k_{12}k_{23} + k_{12}k_{32} + k_{21}k_{32})} \right),
\end{align*}
\]

where \( k_{sum} = k_{12} + k_{21} + k_{23} + k_{32} \).

The eigenvalues appear in the exponents of the solution, and can be identified with exponential relaxation processes. The relaxation time of those processes are given by \( \tau = -1/\lambda \). The largest eigenvalue corresponds to infinite relaxation time, i.e. the equilibrium distribution. The other two relaxation times \( \tau_1 \) and \( \tau_2 \) depend on the specifics of the problem. When the times are similar to each other, the system exhibits typically three state kinetics, in which the processes interfere. In contrast, when the times are very disparate, the time scale separation make one relaxation process the dominate one, and the entire process can be described essentially by two-state kinetics with a single exponential relaxation time. For that to be the case we require that the fraction \( \tau_2/\tau_1 \) becomes smaller than a threshold \( \alpha \), e.g. \( \alpha \approx 0.1 \). This threshold value
is a bit arbitrary, and should be in principle small, but in practice an order of magnitude in time scale can be considered sufficiently different to obtain effective two state kinetics.

We can thus write

$$\frac{\tau_2}{\tau_1} = \frac{\lambda_1}{\lambda_2} = \frac{1 - \sqrt{1 - 4D}}{1 + \sqrt{1 - 4D}} < \alpha,$$  \hspace{1cm} (S33)

with

$$D = \frac{k_{12}k_{23} + k_{12}k_{32} + k_{21}k_{32}}{(k_{12} + k_{21} + k_{23} + k_{32})^2}. \hspace{1cm} (S34)$$

Up to now we made no assumption. To make progress we assume that $\alpha$ is sufficiently small to guarantee that $D$ is also small. Then we can expand the square root in Eq. S33 in terms of D, and can replace the condition $\tau_2/\tau_1 < \alpha$ by

$$\frac{k_{12}k_{23} + k_{12}k_{32} + k_{21}k_{32}}{(k_{12} + k_{21} + k_{23} + k_{32})^2} < \alpha. \hspace{1cm} (S35)$$

Note that we would arrive at the same condition by first solving Eq. S33 for $D$ and then expand the r.h.s. in $\alpha$, and take the leading order.

This condition again depends on the specifics of the three state problem, e.g which rates are dominant in the system. The number of distinct possibilities is sufficiently large to refrain from doing an exhaustive analysis of this condition. However, to connect with the problem in the main text we can make the assumption, also made in the main text, that once the final state is reached, the reverse process is unlikely, i.e. that the process is almost irreversible. This means the $k_{32}$ is much smaller than any of the other rates. Hence, the above condition simplifies to

$$\frac{k_{12}k_{23}}{(k_{12} + k_{21} + k_{23})^2} < \alpha. \hspace{1cm} (S36)$$

Now we still have a number of possible cases. Suppose first that one single rate is dominant, i.e. much higher than the other two. Then we can distinguish three cases:

1) $k_{12}$ is dominant, i.e. $k_{12} \gg k_{23}, k_{21}$ This would reduce the condition to

$$\frac{k_{12}k_{23}}{(k_{12})^2} = \frac{k_{23}}{k_{12}} < \alpha. \hspace{1cm} (S37)$$
That is, the system would be effectively described by two-state kinetics if the rate over the second barrier is much slower than the rate over the first.

2) $k_{23}$ is dominant, i.e., $k_{23} \gg k_{12}, k_{21}$. The condition is then

$$\frac{k_{12}k_{23}}{(k_{23})^2} = \frac{k_{12}}{k_{23}} < \alpha,$$

(S38)

the reverse of case 1). This would basically mean that the kinetics is completely determined by the rate over the first barrier. Thus, the system would be 2 state if the rate over the first barrier is much slower than the one over the second.

3). $k_{21}$ is dominant, i.e., $k_{21} \gg k_{23}, k_{12}$, leading to

$$\frac{k_{12}k_{23}}{(k_{21})^2} < \alpha.$$  

(S39)

Here there is not much direct insight to be gained.

The other extreme, namely that a single rate is much smaller than the other two, yields 3 additional cases: 4) First, we consider the case that $k_{12} \ll k_{23}, k_{21}$. The condition then becomes

$$\frac{k_{12}k_{23}}{(k_{21} + k_{23})^2} < \alpha.$$  

(S40)

When the rates out of intermediate state 2 are about equal, i.e. $k_{23} \approx k_{21} \gg k_{12}$, this reduces to

$$\frac{k_{12}k_{23}}{(k_{21} + k_{23})^2} \approx \frac{k_{12}}{4k_{23}} < \alpha,$$

(S41)

very similar to case 2, but with an extra factor 4.

5) Of course we can also consider $k_{21} \ll k_{23}, k_{12}$, leading to

$$\frac{k_{12}k_{23}}{(k_{12} + k_{23})^2} < \alpha.$$  

(S42)

When rates $k_{12} \approx k_{23}$, we obtain

$$\frac{k_{12}k_{23}}{(k_{12} + k_{23})^2} \approx \frac{1}{4} < \alpha,$$

(S43)
which is in fact, never fulfilled. Hence, for this case we cannot expect effective two state
kinetics under any circumstances.

6) Finally we can consider \( k_{23} \ll k_{21}, k_{12} \), yielding,

\[
\frac{k_{12}k_{23}}{(k_{12} + k_{21})^2} < \alpha
\]  
(S44)

which again, when assuming that \( k_{12} \approx k_{21} \), simplifies to

\[
\frac{k_{12}k_{23}}{(k_{12} + k_{21})^2} \approx \frac{k_{23}}{4k_{12}} < \alpha,
\]  
(S45)

very similar to case 1, but with an extra factor 4.

The above analysis is in terms of the individual rate constants of the 3 state process. How-
ever, as in the main text, one would also like expressions in terms of the free energies of the
stable states and the barriers between them. For simplicity we express the rates in terms of free
energies energy using the Arrhenius-Eyring expression

\[
k_{ij} = \nu \exp(-\beta \Delta G_{ij}),
\]  
(S46)

where \( \Delta G_{ij} \) denotes the barrier height for the transition from \( i \) to \( j \), and \( \beta = 1/k_B T \) is the
reciprocal temperature.

Denoting the (absolute) free energies of the stable states as \( G_1, G_2, G_3 \) and the absolute free
energies of the barriers as \( G_{12}^*, G_{23}^* \), we have for the rate constants’

\[
k_{12} = \nu \exp(-\beta(G_{12}^* - G_1))
\]  
(S47)

\[
k_{21} = \nu \exp(-\beta(G_{12}^* - G_2))
\]  
(S48)

\[
k_{23} = \nu \exp(-\beta(G_{23}^* - G_2))
\]  
(S49)

\[
k_{32} = \nu \exp(-\beta(G_{23}^* - G_3)),
\]  
(S50)

where we assume that all prefactors are identical. The condition for case 1) is now

\[
\frac{k_{23}}{k_{12}} = \exp(-\beta(G_{23}^* - G_2)) / \exp(-\beta(G_{12}^* - G_1)) < \alpha
\]  
(S51)
which, taking the logarithm, turns into

\[-(G_{23}^* - G_2) + G_{12}^* - G_1 < k_B T \ln \alpha\] (S52)

or

\[(G_{23}^* - G_2) - (G_{12}^* - G_1) > -k_B T \ln \alpha.\] (S53)

Thus, the condition for 2-state kinetics is fulfilled when the second barrier is higher than the first by at least a few \(k_B T\). For case 2) the situation is reversed:

\[\frac{k_{13}}{k_{23}} = \exp(-\beta(G_{12}^* - G_1))/\exp(-\beta(G_{23}^* - G_2)) < \alpha,\] (S54)

which, taking the logarithm, turns into

\[(G_{12}^* - G_1) - (G_{23}^* - G_2) > -k_B T \ln \alpha,\] (S55)

or the condition for 2-state kinetics is fulfilled when the first barrier is higher than the second by at least a few \(k_B T\).

More complicated is the third case

\[\frac{k_{12}k_{23}}{(k_{21})^2} = \exp(-\beta(G_{12}^* - G_1)) \exp(-\beta(G_{23}^* - G_2))/\exp(-2\beta(G_{12}^* - G_2)) < \alpha,\] (S56)

which, taking the logarithm, turns into

\[-(G_{12}^* - G_1) - (G_{23}^* - G_2) + 2(G_{12}^* - G_2) < k_B T \ln \alpha.\] (S57)

This condition can be simplified to

\[-G_{12}^* - G_1 + G_{23}^* + G_2 = (G_{23}^* - G_1) - (G_{12}^* - G_2) > -k_B T \ln \alpha,\] (S58)

which states that 2 state kinetics is recovered when the second barrier height, measured with respect to state 1, is larger than the first barrier with respect to state 2, by at least a few \(k_B T\).
Table 1: Summary of three state kinetic analysis, detailing the six cases’ condition for effective two states kinetics. For the barrier conditions we have taken \( \kappa_0 \).

<table>
<thead>
<tr>
<th>case</th>
<th>rate condition</th>
<th>2-state condition</th>
<th>2-state barrier condition</th>
<th>effective rate ( k_f )</th>
<th>landscape</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( k_{12} \gg k_{23}, k_{21} )</td>
<td>( \frac{k_{23}}{k_{12}} &lt; \alpha )</td>
<td>( (G^<em>_2 - G_2) - (G^</em>_1 - G_1) \gtrsim 2.3k_BT )</td>
<td>( k_{23} )</td>
<td><img src="image1" alt="Diagram 1" /></td>
</tr>
<tr>
<td>2</td>
<td>( k_{23} \gg k_{12}, k_{21} )</td>
<td>( \frac{k_{12}}{k_{23}} &lt; \alpha )</td>
<td>( (G^<em>_1 - G_1) - (G^</em>_2 - G_2) \gtrsim 2.3k_BT )</td>
<td>( k_{12} )</td>
<td><img src="image2" alt="Diagram 2" /></td>
</tr>
<tr>
<td>3</td>
<td>( k_{21} \gg k_{23}, k_{12} )</td>
<td>( \frac{k_{12}k_{23}}{(k_{21})^2} &lt; \alpha )</td>
<td>( (G^<em>_2 - G_1) - (G^</em>_1 - G_2) \gtrsim 2.3k_BT )</td>
<td>( \frac{k_{12}k_{21}}{k_{23}} )</td>
<td><img src="image3" alt="Diagram 3" /></td>
</tr>
<tr>
<td>4</td>
<td>( k_{12} \ll k_{23} \approx k_{21} )</td>
<td>( \frac{k_{12}}{k_{23}} \ll \alpha )</td>
<td>( (G^<em>_2 - G_1) - (G^</em>_1 - G_2) \gtrsim 9.2k_BT )</td>
<td>( \frac{k_{12}k_{23}}{k_{21}k_{23}} \approx \frac{1}{2}k_{12} )</td>
<td><img src="image4" alt="Diagram 4" /></td>
</tr>
<tr>
<td>5</td>
<td>( k_{21} \ll k_{23} \approx k_{12}, )</td>
<td>( \frac{1}{4} \ll \alpha )</td>
<td>never</td>
<td>N/A</td>
<td><img src="image5" alt="Diagram 5" /></td>
</tr>
<tr>
<td>6</td>
<td>( k_{23} \ll k_{21} \approx k_{12} )</td>
<td>( \frac{k_{23}}{k_{12}} \ll \alpha )</td>
<td>( (G^<em>_2 - G_1) - (G^</em>_2 - G_1) \gtrsim 9.2k_BT )</td>
<td>( \frac{k_{12}k_{23}}{k_{21}k_{23}} \approx \frac{1}{2}k_{23} )</td>
<td><img src="image6" alt="Diagram 6" /></td>
</tr>
</tbody>
</table>
The cases 4 and 6 can be analysed similarly. Examples of each of these conditions are given in Table 3.1.

When the conditions are fulfilled we can express the two state kinetics by effective rate constants $k_f$ and $k_b$. The sum of these rate constants is equal to the inverse of the longest timescale, $k_f + k_b = 1/\tau_1 = -\lambda_1$, which in turn corresponds to the smallest eigenvalue. Inserting Eq S32 and approximating for $D \ll 1$ we obtain

$$k_f + k_b = \frac{1}{2}k_{\text{sum}} \left( 1 - \sqrt{1 - 4D} \right) \approx k_{\text{sum}} D = \frac{k_{12}k_{23} + k_{12}k_{32} + k_{21}k_{32}}{(k_{12} + k_{21} + k_{23} + k_{32})}. \quad (S59)$$

When we, as above, assume that the reverse $k_{32}$ is much smaller than any of the other rates, and by construction also $k_b \ll k_f$, if follows that

$$k_f = \frac{k_{12}k_{23}}{k_{12} + k_{21} + k_{23}}. \quad (S60)$$

This expression, when $k_{12}$ is also small, reduces to the well known steady state expression

$$k_f^\text{ss} = \frac{k_{12}k_{23}}{k_{21} + k_{23}}. \quad (S61)$$

When $k_{12} \ll k_{23} \approx k_{23}$ we recover case 4, and the effective rate constant is about $k_f = \frac{1}{2}k_{12}$ (see also Table 3.1).

The other case $k_{21} \ll k_{12}, k_{23}$ (case 5) gives

$$k_f = \frac{k_{12}k_{23}}{k_{12} + k_{23}}, \quad (S62)$$

but is in fact never achieved since the 2 state condition is never fulfilled.

The third possibility $k_{23} \ll k_{12}, k_{21}$ leads to

$$k_f = \frac{k_{12}k_{23}}{k_{12} + k_{21}}. \quad (S63)$$

When $k_{23} \ll k_{21} \approx k_{12}$ we recover case 6, and the effective rate constant is about $k_f = \frac{1}{2}k_{23}$ (see also Table 3.1).
Finally, the opposite cases lead to case 1-3. For instance, $k_{12} \gg k_{21}, k_{23}$ yields case 1

$$k_f = \frac{k_{12}k_{23}}{k_{12} + k_{21} + k_{23}} \approx k_{23},$$  \hspace{1cm} (S64)

whereas $k_{23} \gg k_{12}, k_{21}$ yields case 2

$$k_f = \frac{k_{12}k_{23}}{k_{12} + k_{21} + k_{23}} \approx k_{12},$$  \hspace{1cm} (S65)

and finally for $k_{21} \gg k_{12}, k_{23}$ this gives case 3

$$k_f = \frac{k_{12}k_{23}}{k_{12} + k_{21} + k_{23}} \approx \frac{k_{12}k_{23}}{k_{21}}$$  \hspace{1cm} (S66)

These results are also summarised in Table 3.1. We note that the above analysis is indeed in agreement with the results from Kramers’ theory.

### 3.2 Time dependent relaxation

Instead of making approximations as was done in the previous section we can also solve the time dependent population numerically, using Eq. S29. We illustrate this for two cases. First, we make the reverse process $k_{32} = 0.000001$ and $k_{21} = 0.000001$, very unlikely (where we take the unit of time dimensionless and arbitrary). The rate across the second barrier is set to $k_{23} = 0.00001$. The $k_{12}$ varies between 0.000001 and 1. We show in figure S7 solutions to Eq. S29, starting from population $p_1 = 1$, and $p_2, p_3 = 0$. Also shown are the corresponding free energy landscapes. The rates are chosen such that for these energy landscapes $G_{12}^* > G_{23}^*$. For each of the settings the intermediate 2 first slowly fills up, followed by a steady (initially linear, later exponential) increase of state 3. In the equilibrium most of the population is in state 3. Note that the population in intermediate 2 reaches steady state fast for $k_{12} < k_{21}$ but overshoots dramatically for the opposite case $k_{12} > k_{21}$, before relaxing back to the steady state value.

Next, we make the reverse process $k_{21} = 0.0001$, less unlikely, while keeping $k_{23} = 0.00001$. The $k_{12}$ varies between 0.000001 and 1. We show in figure S8 solutions to Eq. S29,
Figure S7: In the right column, the time dependent populations for state 2 (blue) and state 3 (red) are plotted for, from top to bottom, $k_{12} = 0.000001, 0.0001, 0.01, 1$. The unit of time is arbitrary and dimensionless. The left column shows the corresponding free energy landscape. For these free energy landscapes $G_{12}^* > G_{23}^*$. 
starting from population $p_1 = 1$, and $p_2, p_3 = 0$. Also shown are the corresponding free energy landscapes. The rates are chosen such that for these energy landscapes $G_{12}^* < G_{23}^*$. For each of the settings the intermediate 2 first quickly fills up, followed by a steady (initially linear, later exponential) increase of state 3. In the equilibrium most of the population is in state 3. Note that the population in intermediate 2 reaches steady state fast for $k_{12} < k_{21}$ but overshoots dramatically for the opposite case $k_{12} > k_{21}$, before relaxing back to the steady state value.
Figure S8: In the right column, the time dependent populations for state 2 (blue) and state 3 (red) are plotted for, from top to bottom, \( k_{12} = 0.000001, 0.0001, 0.01, 1 \). Unit of time is arbitrary and dimensionless. The left column shows the corresponding free energy landscape. For these free energy landscapes \( G_{12}^* < G_{23}^* \).
References and Notes


