Discreteness-Induced Transitions in Autocatalytic Systems

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(Dated: July 29, 2004)

Abstract

To study the dynamics of chemical processes, we often adopt rate equations to observe the change in chemical concentrations. However, when the number of the molecules is small, the fluctuations cannot be neglected. We often study the effects of fluctuations with the help of stochastic differential equations.

Chemicals are composed of molecules on a microscopic level. In principle, the number of molecules must be an integer, which must only change discretely. However, in analysis using stochastic differential equations, the fluctuations are regarded as continuous changes. This approximation can only be valid if applied to fluctuations that involve a sufficiently large number of molecules. In the case of extremely rare chemical species, the actual discreteness of the molecules may critically affect the dynamics of the system.

To elucidate the effects of the discreteness, we study an autocatalytic system consisting of several interacting chemical species with a small number of molecules through stochastic particle simulations. We found novel states, which were characterized as an extinction of molecule species, due to the discrete nature of the molecules. We also observed a strong dependence of the chemical concentrations on the size of the system, which was caused by transitions to the novel states.

I. INTRODUCTION: DISCRETE REACTION SYSTEMS

In nature, there exists a variety of systems that involve chemical reactions. Some are on a geographical-scale, while others on a nano-scale. Chemical reactions are an integral part of life, including all living forms of life.

To study the dynamics of reaction systems, we often adopt rate equations in order to observe the change in chemical concentrations. In rate equations, we regard the concentrations as continuous variables; the rate of the reaction as a function of the concentrations. In macroscopic systems, there are a vast number of molecules; thus, continuous representations are usually applicable.

When the concentration of a certain chemical is small, fluctuations in the reactions or flow can be significant. We often handle such systems with the help of stochastic differential equations, in which we regard noise as a continuum description of the fluctuations [1, 2]. Such

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an approximation is useful when the number of molecules is intermediate. The employment of stochastic differential equations led to some important discoveries such as noise-induced order \[3\], noise-induced phase transitions \[4\], and stochastic resonance \[5\].

In stochastic differential equations, still quantities of chemicals are regarded as continuous variables. Essentially, on a microscopic level, chemicals are composed of molecules. The number of molecules should be an integer \((0, 1, 2, \cdots)\), which changes discretely. Fluctuations are derivatives of discrete stochastic changes; thus, continuum descriptions of fluctuations are not always appropriate and can be doubted. For chemicals with a small number of molecules of the order of 1, a single molecule is extremely significant; therefore, the discreteness in the number is significant.

Biological cells appear to be a good example. The size of the cells is of the order of microns, in which nano-scale “quantum” effects can be ignored. However, in cells, some chemicals act at extremely low concentrations of the order of pM or nM. Assuming that the typical volume of a cell ranges from 1 to \(10^3\) \(\mu m^3\), the concentration of one molecule in the cell volume corresponds to \(1.7\) pM–1.7 nM. It is probable that the molecular numbers of some chemicals in a cell are of the order of 1, or sometimes reach 0.

If such chemicals play only a minor role, we can safely ignore these chemicals. However, this is not always the case. In biological systems, chemical species with a small number of molecules may critically affect the behavior of the entire system. For example, there exist only one or a few copies of genetic molecules such as DNA, which are important to characterize the behavior, in each cell. Further, some experiments show that doses of particular chemicals at concentrations of the order of pM or fM may alter the behavior of the cells (e.g., \[6, 7\]). Biological systems also include positive-feedback mechanisms such as autocatalytic reactions, which may amplify single molecular changes to a macroscopic level. The effects due to small molecular numbers in cells have been noticed only recently, both theoretically \[8, 9\] and experimentally \[10\].

At present, we focus on the possible effects of molecular discreteness. To study such effects, we should adopt an appropriate method to handle molecular discreteness. Some numerical methods to investigate reaction systems that take into account discreteness and stochasticity already exist (we briefly review these methods; see Appendix). Among the methods, we adopted Gillespie’s direct method, which is popular and frequently used.

Furthermore, some works related to molecular discreteness also exist. For example, Blumenfeld et al. showed that the mass action law may breakdown in a small system \[11, 12\]. Stange et al. studied the synchronization of the turnover cycle of enzymes \[13, 14\].

We regard it important to identify the phenomena for which molecular discreteness is essential.

Through stochastic simulations, we show that discreteness can induce transitions to novel states in autocatalytic systems \[15\], which may affect macroscopic chemical concentrations \[16\].
II. DISCRETENESS-INDUCED TRANSITIONS

A. Model

We consider a simple autocatalytic network (loop) with $k$ chemicals. We consider $X_i$ chemicals and assume

$$X_i + X_{i+1} \rightarrow 2X_{i+1}$$

reactions between these chemicals ($i = 1, 2, \cdots, k$; $X_{k+1} \equiv X_1$). All the reactions are irreversible.

For the reactor, we assume a well-stirred container with volume $V$. The set of $N_i$, the number of $X_i$ molecules, determines the state of the system. The container is in contact with a chemical reservoir, in which the concentration of $X_i$ is fixed at $s_i$. The flow rate of $X_i$ between the container and the reservoir is $D_i$, which corresponds to the probability of the flowing out of a molecule per time unit\(^1\).

We can consider the continuum limit as $V \rightarrow \infty$. In the continuum limit, the change of $x_i$, the chemical concentration $X_i$ in the container, follows the rate equation

$$\frac{dx_i}{dt} = r_{i-1}x_{i-1} - r_ix_ix_{i+1} + D_i(s_i - x_i),$$

(1)

where $r_i$ is the rate constant of the reaction $X_i + X_{i+1} \rightarrow 2X_{i+1}$, and $X_0 \equiv X_k$.

For simplicity, we consider the case with equivalent chemical species, given as $r_i = r$, $D_i = D$, and $s_i = s$ for all $i$ ($r$, $D$, $s > 0$). By this assumption, the rate equation has only one attractor: a stable fixed point $x_i = s$ for all $i$. For any initial condition, each $x_i$ converges to $s$, the fixed point value. Around the fixed point, $x_i$ vibrates with the frequency $\omega_p \equiv rs/\pi$.

If the number of molecules is finite but fairly large, we can estimate the dynamical behavior of the system using a Langevin equation, obtained by adding a noise term to the rate equation. Each concentration $x_i$ fluctuates and vibrates around the fixed point. An increase in the noise (corresponding to a decrease in the number of molecules) merely boosts the fluctuation.

However, when the number of molecules is small, the behavior of the system is completely different. First, we investigate the case when $k = 4$, which is the smallest number of species to show the novel states described below.

B. Novel States Induced by the Discreteness

Subsequently, we investigate the dynamical behavior of the system with a small number of molecules. In order to detect the phenomena for which the discreteness of the number of molecules is crucial, we employ stochastic simulations.

\(^1\) $D_i$ is the diffusion rate across the surface of the container. Here, we choose the flow proportional to $V$, to have a well-defined continuum limit. One might assume the flow proportional to $V^{2/3}$, considering the area of the surface. By rescaling $D_i$, the model can be rewritten into the case with $V^{2/3}$ for finite $V$. 


Here, we adopt Gillespie’s direct method\(^2\). The frequency (expected number per unit time)

- of the reaction \(X_i + X_{i+1} \rightarrow 2X_{i+1}\) is \(P_{R_i} \equiv rx_i x_{i+1} V = rN_i N_{i+1} / V\);
- of the outflow of \(X_i\) is \(P_{O_i} \equiv Dx_i V = DN_i\);
- of the inflow of \(X_i\) is \(P_{I_i} \equiv DsV\).

In the continuum limit (\(V \rightarrow \infty\)), these frequencies agree with the rate equation. We calculate these frequencies with the current \(N_i\), and stochastically decide when and which event will occur next.

In this case, by an appropriate conversion of \(D, V,\) and \(t\), we can set \(r\) and \(s\) to be 1 without loss of the generality (\(rs/D\) and \(sV\) are the only independent parameters). We assume that \(r = 1\) and \(s = 1\) for the purpose of further discussion. The total number of molecules in the container, \(N_{\text{tot}} = \sum N_i\), is approximately \(4sV\) on an average. By varying \(V\), we can control the average number of molecules without changing the continuum limit.

First, we consider the case of a large \(V\), i.e., both the number of molecules in the container and flow of molecules between the container and the reservoir are large. As expected, the behavior of the system is similar to that of the rate equation with noise. As shown in Fig. 2, each \(N_i\) fluctuates and vibrates around the fixed point value \(N_i = V\) (i.e. \(x_i = 1\)). This is still in the realm of stochastic differential equations.

However, when \(V\) is small, we observe novel states that do not exist in the continuum limit. As shown in Fig. 3, continuous vibrations disappear. Furthermore, two chemicals are dominant and the other two are mostly extinct (\(N_i = 0\)). In Fig. 3, at \(t < 520\), \(N_1\) and \(N_3\) dominate the system and \(N_2 = N_4 = 0\) for the most part. We call such a state the 1-3 rich state. Reversely, at \(t > 520\), \(N_2\) and \(N_4\) are large and usually \(N_1 = N_3 = 0\). We call this state the 2-4 rich state.

These states appear because of the following reason. In this system, the production of \(X_i\) molecules requires at least one \(X_i\) molecule as a catalyst. If \(X_i\) becomes extinct, the production of \(X_i\) halts. \(N_i\) never regains before an \(X_i\) molecule flows in.

In the rate equation (eq. (1)), the concentration \(x_i\) is a continuous variable, which can be an infinitesimal but positive value. The consumption rate of \(x_i\) is proportional to \(x_i\) itself; thus, \(x_i\) cannot reach 0 exactly within finite time, even if it can go to 0 asymptotically as \(t \rightarrow \infty\).

In fact, the number of molecules must be an integer. Transitions from \(N_i = 1\) to \(N_i = 0\) are probabilistic and may happen in finite time. For transitions to occur, it is important that the consumption rate of \(x_i\) does not converge to 0 at \(N_i = 1\). The average interval of a molecule flowing in is \(1/DV\) for each chemical. If \(D\) and \(V\) are small enough to ensure that the inflow interval is longer than the time scale of the reactions, it is likely that the state of the system \(N_i = 1\) drops to \(N_i = 0\) before an \(X_i\) molecule enters.

When \(N_i\) reaches 0, \(N_{i+2}\) is also likely to become 0. For example, if we assume that \(N_2 = 0\), then \(N_1\) is likely to increase because the consumption of \(X_1\) halts; \(N_3\) is likely to decrease because the production of \(X_3\) halts. Thus, this results in \(N_1 > N_3\). When

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\(^2\) For systems with a large number of chemical species, the next reaction method would be more suitable.
two variables, \( N \) is conserved at \( N \). More molecules flow in or out throughout the switching, the total number of molecules (\( N \) state with vibration around the fixed point with large \( V \) switching states. We clearly observe a symmetry-breaking transition between a continuous in concentrations: \( x \) fluctuations. When \( V \) large imbalances, such as (\( N \) the 1-3 rich state (as well as the 2-4 rich state).

\[ x \equiv (x_1 + x_3) - (x_2 + x_4) \]

Around the fixed point, \( z \approx 0 \); in the 1-3 rich state, \( z \approx 4 \); in the 2-4 rich state, \( z \approx -4 \).

The distribution of \( z \) is shown in Fig. 4. When \( V \geq 128 \), a single peak appears around \( z = 0 \), which corresponds to the fixed point. By decreasing \( V \), the peak broadens with fluctuations. When \( V \leq 32 \), double peaks appear at \( z \approx \pm 4 \), which correspond to the switching states. We clearly observe a symmetry-breaking transition between a continuous vibration around the fixed point with large \( V \) and the switching states with small \( V \). This is a discreteness-induced transition (DIT) that occurs with decrease of \( V \), which is not seen in continuum descriptions.

We introduce another index \( y \equiv (x_1 + x_2) - (x_3 + x_4) \), which represents the difference in concentrations: \( x_1 - x_3 \) in the 1-3 rich state and \( x_2 - x_4 \) in the 2-4 rich state. The distribution of \( y \) is shown in Fig. 5. There are double peaks around \( y = \pm 3 \), which imply large imbalances, such as (\( N_1, N_3 \) = (3.5\( V \), 0.5\( V \)) and (0.5\( V \), 3.5\( V \)), between \( N_1 \) and \( N_3 \) in the 1-3 rich state (as well as the 2-4 rich state).

C. Single Molecular Switch

We investigate some properties of the switching states.

First, we examine how each \( N_i \) changes in a switching event. We assume the 1-3 rich state with \( N_1 = N_{1\text{ini}}, \) \( N_3 = N_{3\text{ini}}, \) and \( N_2 = N_4 = 0 \). Here, one \( X_2 \) molecule flows in (\( N_2 = 1 \)) at \( t = 0 \), which starts up the reactions. Assuming that \( DV \) is so small that no more molecules flow in or out throughout the switching, the total number of molecules \( N \) is conserved at \( N = N_{1\text{ini}} + N_{3\text{ini}} + 1, \) and \( N_4 \) is always 0. We can represent the state with two variables, \( N_1 \) and \( N_2 \).

In this system, only the following types of reactions

- \( X_1 + X_2 \rightarrow 2X_2 \) and
- \( X_2 + X_3 \rightarrow 2X_3 \)
may change \( N_i \); the others never take place. \( N_1 \) monotonously decreases. When \( N_2 \) reaches 0, these reactions halt completely, and the switching is completed. Evidently, \( N_{1\text{fin}} + N_{3\text{fin}} = N_{1\text{ini}} + N_{3\text{ini}} + 1 \), where \( N_{1\text{fin}} \) and \( N_{3\text{fin}} \) are the final values of \( N_1 \) and \( N_3 \), respectively.

The frequency of the reaction \( X_1 + X_2 \rightarrow 2X_2 \) is

\[
f_1(N_1, N_2) = N_1 N_2 / V,
\]

and that of \( X_2 + X_3 \rightarrow 2X_4 \) is

\[
f_2(N_1, N_2) = N_2 N_3 / V = N_2 (N - N_1 - N_2) / V.
\]

We obtain the Master equation

\[
\frac{dP(N_1, N_2, t)}{dt} = f_1(N_1 + 1, N_2 - 1)P(N_1 + 1, N_2 - 1, t) + f_2(N_1, N_2 + 1)P(N_1, N_2 + 1, t) \\
- \{f_1(N_1, N_2) + f_2(N_1, N_2)\} P(N_1, N_2, t) \\
= \frac{1}{V} \left\{ (N_1 + 1)(N_2 - 1)P(N_1 + 1, N_2 - 1, t) \\
+ (N_2 + 1)(N - N_1 - N_2 - 1)P(N_1, N_2 + 1, t) \\
- N_2 (N - N_2) P(N_1, N_2, t) \right\}
\]

for \( P(N_1, N_2, t) \), the probability of residence in the state \( (N_1, N_2) \) at time \( t \). The initial condition is \( P(N_{1\text{ini}}, 1, 0) = 1 \); otherwise \( P(N_1, N_2, 0) = 0 \).

We can easily follow the Master equation numerically. We investigate the relationship between the initial state \( (N_{1\text{ini}}, N_{3\text{ini}}) \) and the final state \( (N_{1\text{fin}}, N_{3\text{fin}}) \).

If the first reaction is \( X_2 + X_3 \rightarrow 2X_3 \), \( N_2 \) instantaneously reaches 0, and as a result \( N_{3\text{fin}} = N_{3\text{ini}} + 1 \). The system fails to switch.

If \( N_{1\text{ini}} > N_{3\text{ini}} \), it is more probable that the first reaction is \( X_1 + X_2 \rightarrow 2X_2 \). Subsequently, the system carries out further reactions. In this case, it is probable that \( N_{1\text{ini}} \approx N_{3\text{fin}} \), i.e., the system swaps \( N_1 \) and \( N_3 \), as shown in Fig. 6. Consequently, we observe successive switching (as seen in Fig. 3).

When \( N_{1\text{ini}} \gg N_{3\text{ini}} \), the system is likely to reach \( N_{1\text{fin}} = 0 \) and break the 1-3 rich state. A large imbalance between \( N_1 \) and \( N_3 \) results in an unstable 1-3 rich state.

\[ \text{D. Conditions for Switching States} \]

Now, we investigate the requirements for the transitions to the switching states. The rate of residence of the switching states for several \( D \) and \( V \) is shown in Fig. 8. For approximately \( DV < 1 \), we observe the switching states. If \( DV < 0.1 \), the system mostly stays in the switching states.

Subsequently, we expect that switching states appear even for large \( V \) if \( D \) is very small. In fact, we observe switching states for \( V = 10^4 \) as shown in Fig. 9. Furthermore, in this case also, a single molecule can induce switching.

Strictly speaking, if \( DV \) is the same, the rate of residence is a little smaller for larger \( V \). If \( V \) is large, the system takes longer to reach \( N_i = 0 \) even if the rate of reaction and the
initial concentrations are the same. Thus, it is less likely to exhibit switching states for the same interval of the inflow, $1/DV$.

In general, some reactions are much faster than the inflow. If the number of molecules which enter within the time scale of the reactions is of the order of 1 for a certain chemical, the reactions may consume all the molecules of the chemical, and the molecular discreteness of the chemical becomes significant. In other words, for the effect of the discreteness to appear in a system with several processes, it is important that the number of events of a process within the time scale of another process is of the order of 1.

Once the system is in the switching state, it is fairly stable and difficult to escape, especially if $DV$ is small. To escape the 1-3 rich state and regain continuous vibration, at least one $X_2$ molecule and one $X_4$ molecule should flow in and coexist. It is required that after an $X_2$ molecule flows in, an $X_4$ molecule should flow in before $N_2$ returns to 0, or vice versa.

We put one $X_2$ molecule into the system in the 1-3 rich state at $t = 0$, and one $X_4$ molecule in at $t = \tau$. We assume that $DV$ is so small that no more molecules flow in or out. Then, we judge whether the system escapes from the 1-3 rich state. In due course, the system returns to the 1-3 rich (or sometimes 2-4 rich) state because there is no further flow; thus, we should judge at the right moment. Here, if $N_i > 0$ for all $i$ at $t = 8$ (i.e., waiting about 2.5 times longer than the period of the oscillation around the fixed point), we consider that the 1-3 rich state has been interrupted. We measure the probability of interruption for various initial conditions and the delay $\tau$ as shown in Fig. 10.

The system requires adequate timing of inflow to escape the switching states, which may amplify the imbalance between the stability of each state. For example, to escape the 1-3 rich, $N_1 > N_3$ state, it is required that an $X_2$ molecule flows in, and then an $X_4$ molecule flows in with a certain delay $\tau$, as shown in Fig. 10. Thus, the frequency of escape from the 1-3 rich state is approximately proportional to the product of the inflow frequencies of $X_2$ and $X_4$. If each $D_i$ or $s_i$ is species-dependent, the stability of the 1-3 and 2-4 rich states may strongly depend on $D_i s_i V$, the inflow frequency of $X_i$.

Furthermore, if at the outset $N_1 \approx N_3$, it is difficult for the system to escape from the 1-3 rich state. In some cases where the parameters are species-dependent, the flows or the switching may lead to $N_1 \approx N_3$, which stabilizes the 1-3 rich state.

These conditions are important to stabilize particular states and affect the macroscopic behavior of the system (see Section III).

### E. Stability and Shape of the Network: $k \neq 4$ case

To close the section, we briefly discuss the cases where $k \neq 4$. Figure 11 shows the time series of each $N_i$ for $k = 3, 5, 6$. When $k = 6$, 1-3-5 rich and 2-4-6 rich states appear. However, these states are less stable than the 1-3 and 2-4 rich states for $k = 4$. These states collapse when any of the rich chemicals vanish; thus, they are unstable for large $k$. When $k$ is odd (3, 5, ⋅⋅⋅), there are no stable states where particular chemicals are extinct. However, additional reactions, or a variety of $r_i$ or $s_i$, may stabilize or destabilize the states such that it is not always true that loops with odd-$k$ are unstable.

The discreteness-induced transitions are not limited in the autocatalytic loop. We apply the abovementioned discussions to certain segments of a complicated reaction network with a slight modification.
III. ALTERATION OF CONCENTRATIONS BY THE DIT

In the preceding section, we show that the discreteness of the molecules can induce transitions to novel “switching” states in autocatalytic systems.

For the case where \( k = 4 \) with uniform parameters, the 1-3 rich state and the 2-4 rich state are equivalent. In due course, the system alternates between the 1-3 rich and 2-4 rich states. The long-term averaged concentrations are still the same as the continuum limit value, \( \bar{x}_i = 1 \).

It will be important if macroscopic properties, such as the average concentrations, can be altered. We show that the discreteness-induced transitions may alter the long-term averaged concentrations.

A. Model

Once again, here, we adopt the autocatalytic reaction loop

\[ \text{X}_i + \text{X}_{i+1} \rightarrow 2\text{X}_{i+1} \]

for the \( k = 4 \) species. Now we consider the case where the parameters \( D_i, s_i, \) or \( r_i \) are species-dependent. In the continuum limit, the concentration \( x_i \) is governed by the rate equation

\[
\frac{dx_i}{dt} = r_{i-1}x_{i-1}x_i - r_i x_i x_{i+1} + D_i (s_i - x_i).
\]  

(3)

The rate equation does not contain the volume \( V \); hence, the average concentrations should be independent of \( V \).

As discussed in the preceding section, for the transitions to the switching states to occur, it is necessary that the interval of the inflow is longer than the time scale of the reactions. In this model, the inflow interval of \( X_i \) is \( \sim 1/D_i s_i V \), and the time scale of the reaction \( X_i + X_{i+1} \rightarrow 2X_{i+1} \) in order to use \( X_i \) up is \( \sim 1/r_i x_{i+1} \). If all the chemicals are equivalent, the discreteness of all the chemicals equally take effect, and the 1-3 and 2-4 rich states coordinately appear at \( DV \approx r \).

Now, since the parameters are species-dependent, the effect of discreteness may be different for each species. For example, assuming that \( D_1 s_1 < D_2 s_2 \), the inflow interval of \( X_1 \) is longer than that of \( X_2 \). Thus, the discreteness of the inflow of \( X_1 \) may be significant for larger \( V \).

To demonstrate a possible effect of the discreteness on the macroscopic properties of the system, we measure each average concentration \( \bar{x}_i \), sampled over a long enough time to allow transitions between the 1-3 and 2-4 rich states, by Gillespie’s direct method. Note that every \( \bar{x}_i \) does not depend on \( V \) in the continuum limit. Generally, in discrete simulations, the effect of the discreteness varies with \( V \) and alters every \( \bar{x}_i \). When \( V \) is very large, the discreteness does not matter and \( \bar{x}_i \) is almost equal to the continuum limit value. In contrast, when \( V \) is small, the discreteness causes \( \bar{x}_i \) to be very different from the continuum limit.

We first investigate the case where each \( s_i \) is species-dependent (i.e., each inflow rate is species-dependent), and each \( D_i \) and \( r_i \) are uniform (\( D_i = D, r_i = 1 \)). Later, we briefly discuss the case where \( r_i \) is inhomogeneous.
As mentioned in Section II D, for the effect of the discreteness to appear, it is important that the interval of events of a process is of the order of or longer than the time scale of another process. As regards the inflow, there are two indices to determine how the discreteness appears:

- The inflow interval, \( \frac{1}{D_s V} \),
- The number of molecules at equilibrium, \( s_i V \).

If the inflow interval, \( \frac{1}{D_s V} \), is longer than the time scale of the reactions, the reactions may exhaust the chemical before the chemical enters, and the inflow discreteness becomes significant.

Furthermore, if \( s_i V \) is smaller than 1, \( N_i \) can reach 0 because of the outflow. In such cases, the relation between the inflow interval and the outflow time scale is also important.

The approach time from \( N_i = n \gg 1 \) to \( N_i = 0 \) is of the order of \( \frac{1}{D_s V} \log n \). If the inflow interval of the chemical that causes the switching to raise \( N_i \) is long enough to allow all \( X_i \) molecules to flow out, the inflow discreteness may alter the stability of the states drastically.

From this point of view, we classify the mechanism in cases I, I', and II as follows.

**B. Case I: inflow discreteness and reaction rate**

We start with the simplest case, \( s_1 = s_3 > s_2 = s_4 \). In this case, the rate equation has a stable fixed point with \( \forall i : x_i = s_i \). When \( V \) is large, each \( x_i \) fluctuates around the fixed point, and each average concentration \( \bar{x}_i \) is in accordance with the fixed point value. When \( V \) is small, \( \bar{x}_i \) depends on \( V \). Fig. 12 shows each \( \bar{x}_i \) as a function of \( V \). The difference between \( \bar{x}_1 \) and \( \bar{x}_2 \) increases for small \( V \).

By decreasing \( V \), first \( N_2 \) and \( N_4 \) reach 0 and the 1-3 rich state appears. To reach \( N_2 = 0 \) or \( N_4 = 0 \), the inflow interval of \( X_2 \) or \( X_4 \) should be longer than the time scale of the reactions. We set \( x_1 = O(1) \); thus, the condition to achieve the 1-3 rich state is approximately \( \frac{1}{D_1 V}, \frac{1}{D_3 V} > \frac{1}{r} \); that for the 2-4 rich state is \( \frac{1}{D_1 V}, \frac{1}{D_3 V} < \frac{1}{r} \).

If \( V \) satisfies \( \frac{1}{D_1 V}, \frac{1}{D_3 V} < \frac{1}{r} < \frac{1}{D_2 V}, \frac{1}{D_4 V} \), the 1-3 rich state appears but the 2-4 rich state does not. Thus, \( \bar{x}_1 \) and \( \bar{x}_3 \) increase. We actually observed this at \( V \approx \frac{1}{D_2 V} \), as shown in Fig. 12.

For smaller \( V \) that fulfills both the 1-3 and 2-4 rich states, the imbalance between the 1-3 and 2-4 rich states does not disappear. Once the system is in the 1-3 rich state, adequate timing for the \( X_2 \) and \( X_4 \) inflow is required to escape the state. Thus, the frequency of escape from the 1-3 rich state is approximately proportional to \( D^2 s_2 s_4 V^2 \), the product of the inflow frequencies of \( X_2 \) and \( X_4 \). The average residence time in the 1-3 rich state as well as the 2-4 rich state is the reciprocal of the escape frequency. The ratio of the average residence time in the 1-3 rich state to that in the 2-4 rich state is \( \approx \frac{s_1}{s_2 s_4} \). In addition, after escaping the switching states, the system tends to reach the 1-3 rich state rather than the 2-4 rich state because of the biased inflow. Thus, the ratio of the total residence time in the 1-3 rich state to that of the 2-4 rich state is larger than \( \frac{s_1}{s_2 s_4} \); hence, \( \bar{x}_1, \bar{x}_3 \gg \bar{x}_2, \bar{x}_4 \), even for a small difference in \( s_i \).
C. Case I': imbalance of inflow discreteness

In Case I, we consider the imbalance between the $X_1$, $X_3$ pair and the $X_2$, $X_4$ pair. If another imbalance exists between the $X_2$ and $X_4$ inflows, the switching induced by these chemicals in the 1-3 rich state may be unbalanced. We consider the case where $s_1 = s_3 > s_2 > s_4$. In this case, the 1-3 rich state is more stable than the 2-4 rich state, which is identical to Case I. In the 1-3 rich state, the system can be switched from $N_1 > N_3$ to $N_1 < N_3$ by an $X_2$ molecule; and from $N_1 < N_3$ to $N_1 > N_3$ by an $X_4$ molecule. Now, the inflow rate of $X_2$ is larger than $X_4$; thus, switching from $N_1 > N_3$ to $N_1 < N_3$ is more probable than vice versa, and the system tends to stay in the $N_1 < N_3$ state. Consequently, $\bar{x}_1 < \bar{x}_3$, as shown in Fig. 12. This effect requires switching states. When $V$ is large, $\bar{x}_1$ and $\bar{x}_3$ are almost the same.

D. Case II: inflow and outflow

Here, we consider the case where $s_1 = s_2 > s_3 = s_4$. In this case also, the rate equation has a stable fixed point $^3$. When $V$ is small, both the 1-3 and the 2-4 rich states appear. Here, we consider $s_4 V$, the number of $X_4$ molecules when the concentration of $X_4$ in the container and in the reservoir are at equilibrium. If $s_4 V < 1$, $N_4$ reaches 0 without undergoing any reaction. The system takes $\frac{1}{D} \log n$ time units to reach from $N_4 = n \gg 1$ to $N_4 = 0$. The reaction $X_4 + X_1 \rightarrow 2 X_1$ also uses $X_4$ such that $N_4$ decreases faster if $s_1$ is large.

If $N_4 > 0$, the inflow of $X_3$ may switch from $N_2 > N_4$ to $N_2 < N_4$ and raise $N_4$ again. The inflow interval of $X_3$ is $\sim \frac{1}{D s_3 V}$. If the interval is much shorter than the approach time to $N_4 = 0$, the switching maintains $N_4 > 0$. If the interval is longer, $N_4$ reaches 0 before switching, and the 2-4 rich state is easily destroyed.

In the 1-3 rich state, the system tends to maintain $N_1 < N_3$ because the $X_2$ inflow is frequent. However, the $X_1$ inflow is also large enough to maintain $N_1 > 0$. The 1-3 rich state retains its stability.

In conclusion, the 1-3 rich state is more stable than the 2-4 rich state. In the 1-3 rich state, $N_1 < N_3$ is preferred, and $\bar{x}_3$ increases. At this stage, it is possible that $\bar{x}_2 \ll \bar{x}_3$ despite the fact that $s_2 \gg s_3$, as shown in Fig. 13.

E. Amplification by Discreteness

In summary, the difference in the “extent of discreteness” between chemical species induces novel transitions. The “extent of discreteness” depends on $V$; thus, we observe transitions by changing $V$. The transition reported in Section II is regarded as a second order transition involving symmetry breaking (see Figs. 4 and 5), while the transition in this section

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$^3$ Generally, if $D \ll rs_1$, $x_1, x_3 \approx (s_1 + s_3)/2$ and $x_2, x_4 \approx (s_2 + s_4)/2$. 
corresponds to the first order transition without symmetry breaking (see Fig. 13) in terms of thermodynamics.

We classified the mechanism in cases I, I', and II. These mechanisms can be combined. For example, we demonstrate the case where \( s_1 = 0.09, s_2 = 3.89, \) and \( s_3 = s_4 = 0.01. \) In this case, each \( \bar{x}_i \) shows a three-step change with \( V, \) as shown in Fig. 15.

When \( V \) is large, \( \bar{x}_1, \bar{x}_3 \ll \bar{x}_2, \bar{x}_4, \) since \( s_1 + s_3 \ll s_2 + s_4. \) At \( V \approx 10^3, \) the discreteness of the \( X_3 \) inflow becomes significant, and the 2-4 rich state appears. In the 2-4 rich state, the system tends to remain at \( N_2 > N_4 \) because of the inflow imbalance between \( X_1 \) and \( X_3, \) as observed in Case I'. Figure 16 shows the distribution of \( x_2. \) The major peak corresponds to the 2-4 rich, \( x_2 > x_4 \) state for the cases when \( 128 \leq V \leq 512. \)

On the other hand, in the 2-4 rich state, the outflow of \( X_4 \) depresses \( N_4 \) toward \( s_4 V, \) as observed in Fig. 16. By decreasing \( V, \) the imbalance between \( N_2 \) and \( N_4 \) increases because the rate of switching, which again raises \( N_4, \) decreases in proportion to \( V. \) Finally, at \( V \approx 10^2, \) the 2-4 rich state loses stability, as seen in Case II. Now, the 1-3 rich state is preferred despite the fact that \( s_1 + s_3 \ll s_2 + s_4. \) \( \bar{x}_3 \) increases to \( \approx 2, \) which is more than 30 times as large as that in the continuum limit.

For extremely small \( V, \) the 1-3 rich state is also unstable because \( N_1 \) and \( N_3 \) easily reach 0. In such a situation, typically only one chemical species is in the container. The system is dominated by diffusion, and \( \bar{x}_2 \) increases again due to the large \( s_2. \)

Note that the chemical that becomes extinct depends not only on the flows but also on the reactions. In some cases, we observe smaller \( \bar{x}_3 \) for larger \( s_3. \)

**F. Asymmetric Reaction Case**

It is possible that each \( r_i \) varies with the species. In such cases, we can discuss the effect of discreteness in a similar way. However, the change of \( \bar{x}_i \) with \( V \) is different from the case with asymmetric flows.

For example, we assume that \( r_1 = r_3 > r_2 = r_4 \) and \( \forall i : s_i = 1. \) In the continuum limit or in the case of large \( V, \) \( \bar{x}_2 = \bar{x}_4 > \bar{x}_1 = \bar{x}_3, \) as shown in Fig. 15. In contrast, when \( V \) is small, \( \bar{x}_i \approx 1. \) If \( V \) is very small, such that the total number of molecules is mostly 0 or 1, reactions rarely take place. The flow of chemicals dominate the system; thus, \( \bar{x}_i \approx s_i. \)

If both the reactions and the flows are species-dependent, we simply expect the behavior to be a combination of the abovementioned cases. Even this simple system can exhibit a multi-step change in concentrations along with a change in \( V. \) It is not limited to the simple reaction loop. In fact, we observe this kind of change in concentrations with a change in the system size in randomly connected reaction networks. For a large reaction network with multiple time scales of reactions and flows, the discreteness effect may exhibit behavior that is more complicated. Our discussion is largely applicable to such cases if we can define the time scales appropriately.

As seen in this paper, the discreteness of molecules can alter the average concentrations. When the rates of inflow and/or the reaction are species-dependent, transitions between the discreteness-induced states are imbalanced. This may alter the average concentrations drastically from those of the continuum limit case.
IV. DISCUSSION

We demonstrated that molecular discreteness may induce transitions to novel states in autocatalytic systems, and that may result in an alteration of the macroscopic properties such as the average chemical concentrations.

In biochemical pathways, it is not anomalous that the number of molecules of a chemical is of the order of $10^2$ or less in a cell. There are thousands of protein species, and the total number of protein molecules in a cell is not very large. For example, in signal transduction pathways, some chemicals work at less than 100 molecules per cell. There exist only one or a few copies of genetic molecules such as DNA; furthermore, miRNAs and tRNAs are not present in large numbers. Thus, regulation mechanisms involving genes are quite stochastic. Molecular discreteness naturally concerns such rare chemicals.

One of the authors, Kaneko, and Yomo recently provided the “Minority Control conjecture,” which propounds that chemical species with a small number of molecules governs the behavior of a replicating system, which is related to the origin of heredity \[17, 18, 19\]. Matsuura et al. experimentally demonstrated that a small number of genetic molecules is essential for evolution \[20\]. Molecular discreteness should be significant for such chemicals, and may be relevant to characters of genetic molecules.

Until now, we have modeled reactions in a well-stirred medium, where only the number of molecules is taken into account while determining the behavior. However, if the system is not mixed well, we should take into account the diffusion in space. Both the total number of molecules and the spatial distributions of the molecules may be significant. From a biological point of view, the diffusion in space is also important because the diffusion in cells is not always fast as compared with the time scales of the reactions. If the reactions are faster than the mixing, we should consider the system as a reaction-diffusion one, with discrete molecules diffusing in space. The relation between these time scales will be important, as indicated by Mikhailov and Hess \[14, 21, 22\]. As regards these time scales, we recently found that the spatial discreteness of molecules within the so-called Kuramoto length \[2, 23, 24\], over which a molecule diffuses in its lifetime (lapses before it undergoes reaction), may yield novel steady states that are not observed in the reaction-diffusion equations \[25\]. There is still room for exploration in this field, e.g., pattern formation.

Our result does not depend on the details of the reaction and may be applicable to systems beyond reactions, such as ecosystems or economic systems. The inflow of chemicals in a reaction system can be seen as a model of intrusion or evolution in an ecosystem; both systems with discrete agents (molecules or individuals), which may become extinct. In this regard, our result is relevant to studies of ecosystems, e.g., extinction dynamics with a replicator model by Tokita and Yasutomi \[24, 27\]. The discreteness of agents or operations might also be relevant to some economic models, e.g., artificial markets.

Most mathematical methods that are applied to reaction systems cannot account for the discreteness. Although the utility of simulations have become convenient with the progress of computer technology, it might be useful if we could construct a theoretical formulation applicable to discrete reaction systems. On the other hand, in recent years, major advances have been made in the detection of a small number of molecules and fabrication of small reactors, which raises our hopes to demonstrate the effect of discreteness experimentally.

We believe that molecular discreteness is of hidden but real importance with respect to biological mechanisms, such as pattern formation, regulation of biochemical pathways, or evolution, to be pursued in the future.
Acknowledgments

This research is supported by grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (11CE2006, 15-11161). Y.T. is supported by a research fellowship from the Japan Society for the Promotion of Science.

Appendix: Methods for Simulating Discrete Reaction Systems

Since the 1970s, several methods have been suggested for simulating discrete reaction systems. We briefly review some of these methods: StochSim method, Gillespie’s methods, and their improved versions.

These methods are based on chemical master equations. In chemical master equations, we define the state of the system as the number of molecules in each chemical; the reaction process as a series of transitions between the states. We consider each event, i.e., a reaction event between molecules, inflow or outflow of a molecule, as a transition. They take place stochastically with a certain frequency (probability per time unit) determined by the current state.

For the simulations used in this study, we adopted Gillespie’s direct method for simplicity. We also attempted a direct simulation and the next reaction method, and confirmed that our result does not depend on the simulation method.

A. Direct Simulation with Fixed Time Step

First, let us consider a very simple approximation, which looks similar to the Euler method for differential equations.

In this method, we fix the time step as $\Delta t$. Assuming that the frequency of the event-i is $a_i$, the average number of the event-i for each step is $a_i \Delta t$. If $\sum a_i \Delta t \ll 1$, we approximately assume that at most one event occurs at each step, and the probability of the event-i is $a_i \Delta t$ (it is possible that no event occurs in the step). We select an event with a random number, change the state according to the event, and recalculate each $a_i$.

B. StochSim Method

From this simple concept, we can derive some variations. The so-called StochSim method is one such variation.

The StochSim method adopts random sampling of molecules. For bimolecular reactions, we randomly choose two molecules from the system, and decide whether they react or not.

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4 Gillespie demonstrated that the chemical master equations are exact for gas-phase, well-stirred systems in thermal-equilibrium.

5 The next reaction method would be more suitable if the system contains more chemical species and reactions.
with certain probability. The method requires three random numbers in total for each step. In case there are some single molecular (first-order) reactions, we choose the second molecule from the molecules in the system and some pseudo-molecules (dummies). If the second molecule is a pseudo-molecule, then we select the single molecular reaction of the first molecule, and determine whether it occurs.

In the StochSim method, $\Delta t$ is restricted by the fastest reaction (with the largest reaction rate per pair of molecules). If most of the bimolecular pairs do not react with each other, or the reaction probability varies with the species, this method may be impractical because no reaction occurs in most steps.

C. Gillespie’s Direct Method

Incidentally, if the system consists of discrete molecules, it is typical that each frequency $a_i$ changes only when an event actually occurs. Taking this into account, Gillespie suggested two exact simulation methods: the Direct Method [30] and the First Reaction Method [31]. In these methods, we do not fix the time step. Instead, we calculate the time lapse until the next event.

In the direct method, first, we consider the total frequency of the events, $a = \sum a_i$. If $a_i$ does not change until the next event, the time lapse until the next event, $\tau$, is exponentially distributed as $P(\tau) = ae^{-a\tau} (0 < \tau)$. We determine the time lapse $\tau$ with an exponentially distributed random number.

Subsequently, the probability that the next event is $i$ is $a_i/a$. We determine which event occurs with a random number. We then set the time $t$ forward by $\tau$, update the state according to the event, and recalculate each frequency $a_i$. Iterate the above steps until the designated time elapses.

D. Gillespie’s First Reaction Method

The first reaction method is similar to the direct method. It is based on the fact that $\tau_i$, the time lapse until the next event-$i$, is exponentially distributed as $P(\tau_i) = a_i e^{-a_i \tau_i} (0 < \tau_i)$. Only the event with minimum $\tau_i$ actually occurs. We update the state, recalculate each $a_i$, and generate all $\tau_i$ again with the new corresponding $a_i$.

In the first reaction method, we need as many random numbers each step as the types of events. We calculate all $\tau_i$, choose the earliest, and discard the others. Generally, the processor time to generate random numbers is very large; hence, a large amount of time is wasted for several types of events.

E. Next Reaction Method

To solve this performance problem, Gibson and Bruck proposed a refinement of the first reaction method: Next Reaction Method [32]. Although, in general, Monte Carlo simulations require independency of random numbers, they proved a safe way of recycling random numbers, which drastically promotes efficiency.
In the next reaction method, we store $t_i$, the absolute time when the next event-$i$ occurs, instead of $\tau_i$. In the first step, we have to calculate $t_i = \tau_i$ for every $i$, according to the exponential distribution $P(\tau_i) = a_i e^{-a_i \tau_i}$ ($0 < \tau_i$). We choose the event with the smallest $t_i$. According to the event, we set the time $t$ forward to $t_i$, change the state, and recalculate each $a_i$.

In steps that follow, we recalculate each $t_i$ as follows.

1. As regards the event just executed, we should recalculate $t_i = t + \tau_i$ with the exponential distribution of $\tau_i$, identical to the first step.

2. As regards other events whose frequency $a_i$ has changed, we should recalculate the corresponding $t_i$. For such events, we convert $t_i$ as

$$t_{\text{new}} = t_{\text{old}} + \frac{a_{\text{old}}}{a_{\text{new}}}(t_{\text{old}} - t).$$

$t_{\text{old}}$ is the $t_i$ before the event and $t_{\text{new}}$ is that after the event.

With this conversion, the actual frequency is adjusted from $a_{\text{old}}$ to $a_{\text{new}}$, without using random numbers$^6$.

3. As for other events whose frequency $a_i$ has not changed, we do not need to recalculate the corresponding $t_i$.

Subsequently, we choose the smallest $t_i$, and proceed further.

If the event executed does not influence $a_i$, we do not need to recalculate the concerned $a_i$ and $t_i$ (except for those of the event just executed). Thus, it is useful to manage the dependency of $a_i$ on each event. With this intention, we prepare a dependency graph that shows which $a_i$ should be updated after event-$j$ (event-$i$ depends on event-$j$). In a large reaction network, such as biochemical pathways, one chemical species can react with only a small part of the chemicals in the entire system. In such cases, recalculation is not required for irrelevant chemical species; hence, we can accelerate simulations with help of a dependency check.

It is also important to find the smallest $t_i$ quickly. For this purpose, we use a heap, a binary tree in which each node is larger than or equal to its parent. The root is the smallest at any instance$^7$.

The next reaction method requires only one random number per event, and executes much

$^6$ Note that Gibson and Bruck mathematically proved the validity of the method and did not mention numerical errors in their paper. In some cases, repetition of the conversion might be numerically dangerous. In case there are various time scales of reactions, incautious coding may result in numerical errors (e.g., rare events would not occur forever).

$^7$ The heap sort is an $O(N \log N)$ sorting algorithm. If only one $t_i$ has changed in the step, the cost of resorting is $O(\log N)$. 
faster than first reaction method, especially in case of many chemicals and reactions\textsuperscript{8}.

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\textsuperscript{8} While the next reaction method is an exact algorithm, there are also some approximate methods derived from Gillespie’s algorithms, e.g., refs. \textsuperscript{34, 35}. 

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FIG. 1: Time series of the concentration $x_i$ at the continuum limit for $r = 1$, $s = 1$, and $D = 1/32$. With $D > 0$, the rate equation has a stable fixed point $\forall i : x_i = 1$. Each $x_i$ converges to the fixed point.

FIG. 2: Time series of the number of molecules $N_i$ for $V = 512$ and $D = 1/256$. Each $N_i$ fluctuates around the fixed point (as seen in Fig. 1) but does not reach 0.
FIG. 3: Time series of $N_i$ for $V = 32$ and $D = 1/256$. In this stage, $N_i$ can reach 0, and the switching states appear. In the 1-3 rich state, the system successively switches between the $N_1 > N_3$ and $N_1 < N_3$ states. The interval of switching is much longer than the period of continuous vibration ($\approx \pi$) observed in Figs. I and II. Around $t = 520$, a transition occurs from the 1-3 rich to the 2-4 rich state.

FIG. 4: The probability distribution of the index $z = (x_1 + x_3) - (x_2 + x_4)$, sampled over $5 \times 10^6$ time units. ($z$ is actually a discrete value. Here, we show the distribution as a line graph for visibility.) $D = 1/128$. When $V$ is large ($V \geq 256$), there appears a single peak around $z = 0$, corresponding to the fixed point state $\forall i : x_i = 1$. For $V \leq 64$, the distribution has double peaks around $z = \pm 4$. The peak $z \approx 4$ corresponds to the 1-3 rich state, with $N_1 + N_3 \approx 4V$, $N_2 = N_4 = 0$. $z \approx -4$ corresponds to the 2-4 rich state as well.
FIG. 5: The probability distribution of the index \( y = (x_1 + x_2) - (x_3 + x_4) \), sampled over \( 5 \times 10^6 \) time units, \( D = 1/128 \). When \( V \) is large, there appears a single peak around \( y = 0 \), corresponding to the fixed point. When \( V \) is small and the system is in the switching states, the index \( y \) shows an imbalance between the rich (non-zero) chemicals. For example, in the 1-3 rich state, \( y \) corresponds to \( x_1 - x_3 \) since \( N_2 = N_4 = 0 \) for the most part. The distribution shows double peaks around \( y = \pm 3 \). Assuming that \( x_1 + x_3 = 4 \) and \( x_2 = x_4 = 0 \) in the 1-3 rich state, \( y = \pm 3 \) correspond to \((x_1, x_3) = (3.5, 0.5), (0.5, 3.5)\). Both the chemicals are likely to have a large imbalance.

FIG. 6: Probability density for the switch from \((N_1, N_3) = (N_{1ini}, N_{3ini})\) to \((N_{1fin}, N_{3fin})\). We assume a switching event triggered by a single \( X_2 \) molecule in the 1-3 rich state, and we set the initial conditions as \( N_2 = 1 \) and \( N_3 = 0 \). Assuming that there is no further flow \( (D = 0) \), we numerically follow the master equation (eq. 2) until \( t = 50 \) (sufficiently large to ensure \( N_2 = 0 \) for most cases). For each initial condition, we show the transition probabilities of the final states \((N_{1fin}, N_{3fin})\). \( V = 32, N_{1ini} + N_{3ini} = 128 \). The system shows high probabilities around \( N_{3fin} = N_{3ini} + 1 \) (immediately terminated) and \( N_{3fin} = N_{1ini} \) (switching; \( N_{1ini} > N_{3ini} \)).
FIG. 7: Probability for $N_2 = 0$, i.e., reactions have already stalled, as a function of $t$ (in other words, the cumulative distribution of the time when $N_2$ reaches 0). $V = 32$, $N_{1\text{ini}} + N_{3\text{ini}} = 128$. In the case where $N_{1\text{ini}} < N_{3\text{ini}}$ or $N_{1\text{ini}} \approx N_{3\text{ini}}$, the probability increases just after the reactions start. For such initial conditions, the reactions terminate near the initial state, as shown in Fig. 6. If $N_{1\text{ini}} \gg N_{3\text{ini}}$, the probability steeply increases at $t \approx 4$, which corresponds to the switching. The system takes $\approx 4$ time units to complete the switching.

FIG. 8: The rate of residence of the switching states plotted against $DV$, the inflow frequency, sampled over $10^6$ ($V > 1024$), $10^7$ ($32 < V \leq 1024$), and $10^8$ ($V \leq 32$) time units. Here, we define the 1-3 rich state as continuation of the state in which at least one of $N_2$ or $N_4$ is 0 for 8 time units or longer. Thus, the system may contain states with only one or no chemical, especially for small $V$. We observe the switching states for $DV < 1$. 
FIG. 9: Time series of $N_i$ for $V = 10^4$ and $D = 10^{-6}$. For such a small $D$, we observe the switching states for relatively large $V$. In this case also, a single molecule can induce switching. One $X_1$ molecule flows in ($N_1 = 1$), propagates to more than $10^4$, and then returns to 0.

An initial large imbalance, such as $N_1^{ini} : N_3^{ini} = 120 : 8$ (15 : 1), makes it easier to escape the 1-3 rich state. The system is unlikely to escape from the state when $N_1^{ini} \approx N_3^{ini}$ or $N_1^{ini} < N_3^{ini}$.

The probability is maximum at $\tau \approx 2$, which approximately corresponds to a half of the period of the vibration around the fixed point.

FIG. 10: Probability for interruption of the 1-3 rich state as a function of the delay $\tau$, sampled over $10^5$ times for each condition. We assume the system where $N_2 = N_4 = 0$. We inject an $X_2$ molecule at $t = 0$, then an $X_4$ molecule at $t = \tau$ (no further flow). If $\forall i : N_i > 0$ at $t = 8$, we ascertain that the system has escaped from the 1-3 rich state. $V = 32$, $N_1^{ini} + N_3^{ini} = 4V (= 128)$. An initial large imbalance, such as $N_1^{ini} : N_3^{ini} = 120 : 8$ (15 : 1), makes it easier to escape the 1-3 rich state. The system is unlikely to escape from the state when $N_1^{ini} \approx N_3^{ini}$ or $N_1^{ini} < N_3^{ini}$. The probability is maximum at $\tau \approx 2$, which approximately corresponds to a half of the period of the vibration around the fixed point.
FIG. 11: Time series of $N_i$ for $k = 3, 5, 6$, with $V = 64$ and $D = 1/256$. For the case where $k = 6$, the transition occurs from the 1-3-5 rich state to the 2-4-6 rich state at $t \approx 10080$. For the cases where $k = 3$ and $k = 5$, there are no stable states such as the 1-3 rich state when $k = 4$ or 1-3-5 rich state when $k = 6$. 
FIG. 12: The average concentration $\bar{x}_i$ as a function of $V$, sampled over $10^6$ ($V > 1024$), $10^7$ ($32 < V \leq 1024$), and $10^8$ ($V \leq 32$) time units (same for Fig. 14). $r = 1$ and $D = 1/128$. (a) Case I: $s_1 = s_3 = 1.7$, $s_2 = s_4 = 0.3$. When $V$ is small, the 1-3 rich state appears, and the difference between $\bar{x}_1$ and $\bar{x}_2$ increases. (b) Case I': $s_1 = s_3 = 1.9$, $s_2 = 0.19$, $s_4 = 0.01$. When $V$ is small, $\bar{x}_2$ and $\bar{x}_4$ decrease, identical to Case I. In this case, the imbalance between $\bar{x}_1$ and $\bar{x}_3$ appears at the same time. (Figures 12, 14, 15, and 17 are reproduced from ref. [16] by permission of the publisher.)

FIG. 13: Probability distribution of $y \equiv (x_1 + x_2) - (x_3 + x_4)$, for Case I': $s_1 = s_3 = 1.9$, $s_2 = 0.19$, $s_4 = 0.01$, $r = 1$, and $D = 1/128$. There is a peak around $y = 0$ for large $V$. The difference in $s_i$ has a slight effect on $\bar{x}_i$. For the case where $V \leq 1024$, there appears another peak at $y \approx -0.8$, which corresponds to the 1-3 rich, $N_1 < N_3$ state.
FIG. 14: The average concentration $\bar{x}_i$, for Case II: $s_1 = s_2 = 1.99$, $s_3 = s_4 = 0.01$, $r = 1$, and $D = 1/128$. For small $V$, the 1-3 rich state is preferred, and $\bar{x}_3$ increases.

FIG. 15: The average concentration $\bar{x}_i$ for $s_1 = 0.09$, $s_2 = 3.89$, $s_3 = s_4 = 0.01$, $r = 1$, and $D = 1/64$, sampled over $5 \times 10^6$ ($V > 32$) or $5 \times 10^8$ ($V \leq 32$) time units. By decreasing $V$, first, the 2-4 rich state appears, as seen in Case I. Then, the 2-4 rich state becomes unstable and gives way to the 1-3 rich state, as seen in Case II. When $V$ is extremely small ($V < 0.5$), the flow of molecules governs the system, and $\bar{x}_2$ increases again.
FIG. 16: Time series of $N_i$ for $s_1 = 0.09, s_2 = 3.89, s_3 = s_4 = 0.01, r = 1$, and $D = 1/64$. (a) $V = 256$. The 2-4 rich state is dominant (Case I'). Inflow of $X_3$ molecules induces switching from $N_2 > N_4$ to $N_2 < N_4$, which prevents $N_4$ from decreasing to 0. (b) $V = 32$. Now, the $X_3$ inflow is rare, which allows $N_4$ to reach 0 before the switching. Thus, the 2-4 rich state is unstable (Case II).

FIG. 17: Distribution of $x_2$ for $s_1 = 0.09, s_2 = 3.89, s_3 = s_4 = 0.01, r = 1$, and $D = 1/64$, sampled over $5 \times 10^6$. For large $V$, a single peak around $x_2 = 2$ appears, which corresponds to the fixed point in the continuum limit. At $V \approx 10^3$, double peaks appear around $x_2 = 1$ and $x_2 = 3$, which correspond to the 2-4 rich state. By decreasing $V$, the two peaks spread apart. At $V \approx 10^2$, the skirt of the low-density (left) peak touches $x_2 = 0$, implying that $N_2$ (and $N_4$) is likely to reach 0, and thus, the 2-4 rich state loses stability. A peak at $x_2 = 0$ steeply grows by decreasing $V$ further.
FIG. 18: The average concentration $\bar{x}_i$ for $\forall i : s_i = 1$ and $D = 1/128$ with inequivalent reaction constants. For small $V$, the flows of molecules dominate the system. Thus, $\bar{x}_i \approx 1$, which simply reflects $s_i = 1$; this does not depend on how the continuum limit is imbalanced by the reactions. (a) $r_1 = r_3 = 1$ and $r_2 = r_4 = 0.9$. (b) $r_1 = r_2 = 2$ and $r_3 = r_4 = 1$. 