Universal properties of mechanisms from two-state trajectories

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Finding the underlying mechanism from the statistical properties of an experimental two-state trajectory generated from dynamics in a complex on-off multisubstate kinetic scheme (KS) is the aim of many experiments. Since the data explicitly shows only transitions between substates of different states, information about the KS is lost, resulting in equivalence of KSs, i.e., the occurrence of different KSs that lead to the same data, in a statistical sense. In order to deal with this phenomenon, a canonical (unique) form of reduced dimensions (RD) is built from the data. RD forms are on-off networks with connections only between substates of different states, where the connections usually have nonexponential waiting time probability density functions. In this paper, we give a list of (about 50) relationships between properties of the data, the topology of reduced dimension forms, and features of KSs. Many of these relationships involve symmetries in RD forms, KSs, and the data and irreversible transitions in KSs. These relationships are useful both in theoretical analysis of on-off KSs and in the analysis of the data. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2825613]

I. INTRODUCTION

Finding a mechanism from a binary time series (Fig. 1) is a problem that appears in many fields in physical chemistry and biophysics,[1–33] ranging from studies on the photophysical properties of nanocrystals,[21–27] studies on the structural changes, and the activity of single biopolymers and small organic molecules[7–20,28–31] to numerical studies of complex systems, e.g., protein folding and reactions.[32,33] The mechanism of many of the processes that lead to binary time series can be described by a multisubstate on-off Markovian kinetic scheme[34–43] (KS). For example, KSs 2A-2D are on-off Markovian KSs that are frequently used for describing biophysical processes (Fig. 2). (In this paper, we call the KS by the figure it is shown in.) Thus, the mechanism is a network with a given time-independent wiring (i.e., connectivity). Each substate in the network has a unique observable value, on (rectangular substates in the figures) or off (circled substates in the figures). The observed two-state trajectory is generated by the random walk in the KS, in which explicitly observed are only transitions between substates of different states. (Here, we call the binary time series, also, a two-state trajectory or just a trajectory. The two states in the trajectory are called the on state and off state. The periods in the trajectory are also called events or waiting times.) The description of the experimental data within the framework of a random walk in a Markovian KS is fairly unrestrictive, because, in many cases, a model that couples different stochastic processes can be represented by a KS. (Adding substates and links to a given a KS is, in many cases, equivalent to coupling between stochastic processes.) Models for the processes mentioned above that are more specific and detailed than on-off KSs can be found in Refs. 44–62.

There are two fundamental questions in the analysis of two-state trajectories. One deals with the actual analysis of the data, e.g. Refs. 63–77, and will be addressed in a forthcoming publication. The second question is a conceptual question that asks whether the KS can be fully recovered from the statistical properties of the trajectory. In many cases, this task is not feasible, even when "analyzing" an infinitely long trajectory. The reason is that the spatial projection of the multidimensional KS into the two-dimensional data leads to a loss of information about the structure of the underlying KS, so two, or several, KSs can lead to identical trajectories in a statistical sense.[37–43] For example, the two KSs 2C and 2D are equivalent to each other, and cannot be resolved by a two-state trajectory. Here, the reason is that the single event waiting time probability density functions (WT-PDFs), $\phi_x(t)$, $x=\text{on}, \text{off}$, [see Fig. 1 and Eq. (A5)] from these KSs can be made the same, and the $\phi_x(t)$s contain all the information in the data due to the on-off connectivity in both KSs, both having a single gateway substate[39] (meaning that any on-off transition must involve substate 1_{on}). The best strategy to deal with the equivalence of on-off KSs in general is to use canonical forms.[37,42,43] A given KS is mapped into a single canonical form, but many KSs can be mapped to the same canonical form. (This is a signature of the loss of information in a single two-state trajectory that allows a unique construction only of a canonical form.) Recently, we found a map of KSs into new canonical forms, termed reduced dimensions (RD) forms.[43] A RD form is an on-off network, with connections only between substates of different states. The numbers of substates in this network are determined by the ranks, $R_{x,y}$, $x,y=\text{on},\text{off}$, of the two-dimensional WT-PDFs of successive events, $\phi_{x,y}(t_1,t_2)$ $x,y=\text{on},\text{off}$ [see Fig. 1 and Eq. (A7)]. Topologically, a RD form is the simplest on-off network that can generate the data. Each connection in a RD form has a unique WT-PDF, $\varphi_{x,ji}(t)$ for connecting substates $i_x \to j_y$, $x=\text{on},\text{off}$, which is not necessarily exponential.

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FIG. 1. A trajectory of an observable that fluctuates between two values, on and off, as a function of time. Such a trajectory is commonly obtained from single molecule experiments. In this paper, the data is described by a random walk in an on-off KS. Kinetic Monte-Carlo simulations are used to generate the data by a computer. WT-PDFs are easily constructed from this trajectory. For example, $\phi_{\text{on}}(t)$ is the histogram of the on durations, and $\phi_{\text{on,off}}(t_1,t_2)$ is the two-dimensional histogram of the intersection between successive on-off durations.

[The $\varphi_{x,ji}(t)$s are almost always multiexponentials.] For example, the RD form's topology of KSs 2C and 2D is shown in Fig. 3(B).

There are several important uses of RD forms. RD forms constitute a powerful platform for discriminating between KSs. A RD form is constructed more reliably from the data than a KS. The data-obtained RD form facilitates the search for the possible underlying KS in the space of KSs by indicating on special properties in the true underlying KS.

How can the RD forms be used to discriminate among KSs? Two KSs are equivalent if and only if they are mapped to the same RD form. Specifically, two KSs can lead to the same RD form, if and only if they share the same rank values, $R_{x,y}$s, of the $\phi_{x,y}(t_1,t_2)$s $x,y=\text{on},\text{off}$, the same number of exponentials in $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$, and the same functional form of the $\varphi_{x,ji}(t)$s. These properties are deduced in the mapping of a KS into a RD form. There are, however, several general topological properties of KSs that can be promptly translated into properties of RD forms, and thus can be used to distinguish KSs without the need for a full mapping. (Note that these general relationships are based on properties of the data, because RD forms are, by construction, objects that are equivalent to the data.) In particular, the on-off connectivity of the KS can be translated into the ranks of the $\phi_{x,y}(t_1,t_2)$ $x,y=\text{on},\text{off}$, the number of exponentials in

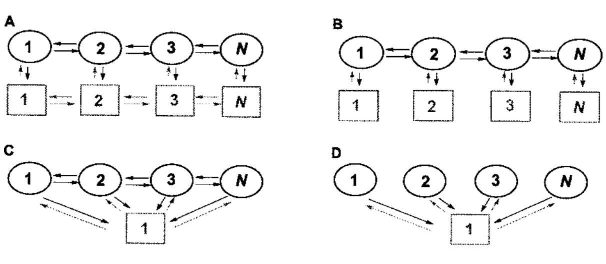

FIG. 2. (A)–(D) On-off KSs with only reversible transitions that are frequently used in describing biophysical processes. Here and along this paper, on substates are rectangles and off substates are circles. The KSs in (B)–(D) are particular realizations of the ladder on-off KS in (A), which has a linear coupling among substates of the same state, and a linear on-off coupling. (B) A special case of the ladder on-off KS in (A), obtained by taking to zero all the transition rates among the on substates. (C)–(D). The KS in (C) is obtained by taking to infinity all the transition rates among the on substates in KS (A), where the KS in (D) is obtained by taking to zero all the transition rates among the off substates in KS (C). Note that the KSs (A) and (B) can be resolved by a two-state trajectory, but not the KSs in (C) and (D).

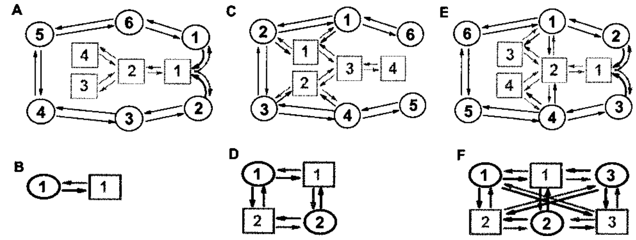

FIG. 3. A set of KSs with only reversible transitions, (A), (C), and (E), and the corresponding RD forms, (B), (D), and (F). The mapping between the KSs and the corresponding RD forms is discussed in the main text.

$\phi_x(t)$ is determined by the number of x substates in the KS, and the complexity of the $\varphi_{x,ji}(t)$s can also be deduced by the on-off connectivity of the KS. In this paper, we add many (about 50) new relationships between the data, properties of RD forms, and features of KSs to the above fundamental relationships. A convenient way to present these relationships is by the relative (to each other) rank values of the $\phi_{x,y}(t_1,t_2)$ $x,y=\text{on},\text{off}$ (Table I). For example, when all ranks have the same value, the KS has no detectable irreversible transitions or symmetry. Detectable irreversible transitions in the KS lead to $R_{\text{on,off}} \neq R_{\text{off,on}}$, and symmetry in the KS can lead to a scenario in which $R_{x,x}$ is the largest among all ranks. We have characterized three types of symmetry in KSs and five types of symmetry in RD forms. These emerge naturally when classifying all possible relative rank values. There are exotic combinations of relative rank values that can be associated with several different features in the KS. For example, the combination, $R_{\text{on,off}} > R_{\text{off,off}} > R_{\text{off,on}} = R_{\text{on,on}}$, indicates that the KS has irreversible transitions, and two different types of symmetry in the on state of the KS. We have also found that there are forbidden combinations of relative rank values. These findings constitute a complete characterization of on-off KSs and are translated into properties of RD forms. The network of relationships between KSs, RD forms, and two-state trajectories is useful in theoretical analysis of on-off KSs and in the actual analysis of the data.

This paper is laid out as follows: Sec. II introduces the RD forms and the map of KSs into RD forms. (This section presents a brief summary of the results of Ref. 43.) Section III gives the new relationships between RD forms, KSs, and two-state trajectories. Section IV summarizes and gives concluding remarks.

II. REDUCED DIMENSION FORMS

This section introduces the canonical forms of reduced dimensions and presents the mapping of on-off KSs into RD forms first given in Ref. 43. In this section, we make explicit mathematical discussion brief, where the full mathematical details are given in Appendices A and B. In what follows, x, $y=\text{on},\text{off}$.

A. Description of RD forms and some examples

RD forms are on-off networks with connections only between substates of different states (3B, 3D, and 3F). The topology of the RD form, i.e., the number of substates in the network, is the simplest topology that can reproduce the data. The trade off is that the $\varphi_{x,ji}(t)$s are (usually) sums of exponentials. The topology of a RD form is determined by the ranks, $R_{x,y}$s, of the corresponding $\phi_{x,y}(t_1,t_2)$s, e.g., for nonsymmetric KSs, $R_{x,y}$ for $x \neq y$ is the number of substates in state y in the RD form. [For discrete time, $\phi_{x,y}(t_1,t_2)$ is a matrix with a rank $R_{x,y}$. Thus, $R_{x,y}$ can be found from the data. $R_{x,y}$ is, in fact, the rank of the matrix $\sigma_{x,y}$ that appear in the double summation representation of $\phi_{x,y}(t_1,t_2)$,

$$\phi_{x,y}(t_1,t_2) = \sum_{i=1}^{L_x} \sum_{j=1}^{L_y} \sigma_{x,y,ij} e^{-\lambda_{x,i}t_1 - \lambda_{y,j}t_2}.$$

Matrix $\sigma_{x,y}$ is given in Eqs. (A8) and (A9) in terms of the underlying matrices in the master equation representation of the on-off process.] The $\varphi_{x,ji}(t)$s are determined by the mapping procedure of a KS into a RD form. The mapping is discussed in the next subsection, and the technical details to get the $\varphi_{x,ji}(t)$s given a mapping are spelled out in Appendix B. Here we note that $\varphi_{x,ji}(t)$ (for a KS as an underlying mechanism) is a weighted sum of exponentials with as many as L_x components,

$$\varphi_{x,ij}(t) = \sum_{H=1}^{L_x} \alpha_{x,iHj} e^{-\lambda_{x,H}t},$$

where, L_x is the number of exponentials in $\phi_x(t)$. It is straightforward to get the coefficients and the rates in the exponential expansion of $\varphi_{x,ji}(t)$ numerically, given the mapping of the KS into a RD form (Appendix B). Lastly, we note that RD forms are canonical forms in the sense that only one RD form can be constructed from an infinitely long two-state trajectory, and this RD form contains all the information in the two-state trajectory. RD forms are canonical forms of KSs because a given KS is mapped to a unique RD form.

The simplest topology for a RD form (3B) has one substate in each of the states, namely, $R_{x,y}=1$. Therefore, $\varphi_{x,11}(t)=\phi_x(t)$. For a 2×2 RD form (3D), e.g., when $R_{x,y}=2$, there are as many as four different $\varphi_{x,ji}(t)$s for each value of x. In general, for RD form with $L_{\mathrm{RD},x}$ substates in state x, there are as many as $2L_{\mathrm{RD,on}}L_{\mathrm{RD,off}}$ different WT-PDFs for the connections in the RD form. The number of amplitudes that describe these WT-PDFs is, $(L_{\mathrm{on}}+L_{\mathrm{off}})L_{\mathrm{RD,on}}L_{\mathrm{RD,off}}$.

B. Mapping a KS into a RD form

The mathematical basis for mapping a KS into a RD form is the path representation of the $\phi_{x,y}(t_1,t_2)$s (Appendices A and B). However, the mapping, to a large extent, can also be done based on topological analysis of the KS' on-off connectivity. This fairly simple topological analysis makes RD forms a powerful canonical representation of on-off KSs. In the topological analysis, we define initial substates and final substates in each of the KS' states. Initial substates in state x in the KS are those substates that get the flux from the final substates in state y in the KS. (In a reversible transition KS, each initial substate in state x is also a final substate; for example, substate 1_{on} in KS 3A is both an initial substate and a final substate of the on state. For an irreversible transition KS, an initial substate is not necessarily a final substate in that state; for example, in KS 6, all initial and final substates are distinct.) We denote by $\{N_x\}$ the group of initial x substates and by $\{M_x\}$ the group of final x substates. The number N_x (M_x) corresponds to the number of members in the group $\{N_x\}$ ($\{M_x\}$). (This convention is employed hereafter.) Now, in the mapping of a KS into a RD form, any initial substate in the KS is either clustered or mapped to itself. The fate of an initial substate in the KS is determined by a simple rule. For a nonsymmetric KS, initial-y-state substates in the KS that contribute to $R_{x,y}$ are mapped to themselves and those that do not contribute to $R_{x,y}$ are clustered, where initial-y-state substates in a cluster are all connected to the same final-x-state substate that contribute to $R_{x,y}$. (In this discussion, $x \neq y$ unless otherwise is explicitly indicated.) This simple rule reduces the KS dimensionality to that of the RD form. The mapping of a KS into a RD form uses only initial and final substates in the KS, but all the substates in the KS affect the form of the $\varphi_{x,ji}(t)$s.

To use the simple mapping rule in practice, we need to identify the initial substates that contribute to the ranks. There are only two different types of on-off connectivity in the KS associated with the different clustering procedures. For each clustering type, a different equation relates the ranks to the KS' on-off connectivity.

(a) The rank $R_{x,y}$ is determined by the equality

$$R_{x,y} = \tilde{M}_x + \tilde{N}_y. \tag{1}$$

In Eq. (1), $\tilde{M}_x < M_x$ with $\{\tilde{M}_x\} \in \{M_x\}$ and $\tilde{N}_y < N_y$ with $\{\tilde{N}_y\} < \{N_y\}$. Namely, when a rank $R_{x,y}$ is determined by Eq. (1), both final-x-state substates and initial-y-state substates contribute to this rank, but their numbers must be smaller than the size of their corresponding parent groups. To identify the specific substates that contribute to the rank, namely, to determine the substates in the subgroups $\{\tilde{M}_x\}$ and $\{\tilde{N}_y\}$, the minimum between M_x and N_y is first found. If $M_x = N_y$, $\{N_y\}$ is considered the smaller group for the following treatment. Once the smaller group is determined, we look for the subgroup of substates in it, denoted by $\{l\}$, that are connected *only* to a subgroup $\{s\}$ in the larger group, such that $l > s$. If subgroups $\{l\}$ and $\{s\}$ are found, the rank is determined by Eq. (1), where $\tilde{M}_x$ and $\tilde{N}_y$ are the numbers of members in the subgroups $\{\tilde{M}_x\}$ and $\{\tilde{N}_y\}$, respectively. The subgroups $\{\tilde{M}_x\}$ and $\{\tilde{N}_y\}$ are defined by $\{\tilde{N}_y\}=\{N_y\}-\{l\}$ and $\{\tilde{M}_x\}=\{s\}$ if $\{N_y\}$ is the smaller group, or $\{\tilde{M}_x\}=\{M_x\}-\{l\}$ and $\{\tilde{N}_y\}=\{s\}$ if $\{M_x\}$ is the smaller group. The generalization for cases with more pairs of special subgroups than one is straightforward: See the comment in Ref. 78 and the discussion in Appendix B.

The clustering procedure follows from the identification of the subgroups $\{\tilde{M}_x\}$ and $\{\tilde{N}_x\}$, $x=\mathrm{on},\mathrm{off}$, as explained in the first paragraph of this subsection. As an example for a full mapping, consider the KS 3E, which has $M_{\mathrm{off}}=N_{\mathrm{on}}$. For the calculations of the rank $R_{\mathrm{off,on}}$, $\{N_{\mathrm{on}}\}=\{1_{\mathrm{on}},2_{\mathrm{on}},3_{\mathrm{on}},4_{\mathrm{on}}\}$ is considered the smaller group. The subgroups among the on initial substates that are connected to smaller subgroups in the off final substates are $\{l\}_1=\{2_{\mathrm{on}},3_{\mathrm{on}}\}$ and $\{l\}_2=\{2_{\mathrm{on}},4_{\mathrm{on}}\}$, where the corresponding smaller subgroups $\{s\}_1$ and $\{s\}_2$ in the larger group $\{M_{\text{off}}\}=\{1_{\text{off}},2_{\text{off}},3_{\text{off}},4_{\text{off}}\}$ are $\{s\}_1=\{1_{\text{off}}\}$ and $\{s\}_2=\{4_{\text{off}}\}$. (Note that a given substate can appear in more than one subgroup, which means that the overall steady-state flux into this substate is divided into several contributions, but only once in $\{\tilde{N}_x\}$ and $\{\tilde{M}_y\}$). This identification of the special subgroups gives $\{\tilde{M}_{\text{off}}\}$ and $\{\tilde{N}_{\text{on}}\}$: $\{\tilde{M}_{\text{off}}\}=\{1_{\text{off}},4_{\text{off}}\}$ and $\{\tilde{N}_{\text{on}}\}=\{N_{\text{on}}\}-\{2_{\text{on}},3_{\text{on}},4_{\text{on}}\}=\{1_{\text{on}}\}$, and therefore enables finding the rank $R_{\text{off,on}}$, $R_{\text{off,on}}=2+1=3$. The mapping of the on-off KS into a RD form follows from the above identification of subgroups: The KS substate 1_{on} is mapped to itself, where the KS substates, $2_{\text{on}},3_{\text{on}}$ and $2_{\text{on}},4_{\text{on}}$, are clustered to give the RD form's substates 2_{on} and 3_{on}, respectively. By performing a similar analysis for the rank $R_{\text{on,off}}$, we find $R_{\text{on,off}}=3$ and that the off state mapping clusters the KS substates $1_{\text{off}},2_{\text{off}}$ into the RD form's substate 1_{off}, whereas the KS substates 3_{off} and 4_{off} are mapped to themselves into the RD form's substates 2_{off} and 3_{off}, respectively.

(b) When a rank $R_{x,y}$ is *not* determined by Eq. (1), it is determined by

$$R_{x,y}=\min(M_x,N_y). \tag{2}$$

In this case, only one type of substate contributes to the rank.

(1) When $M_x \geqslant N_y$, only initial-y-state substates contribute to $R_{x,y}$, so any initial-y-state substate is mapped to itself. Examples include:

- KSs 2A and 2B for $y=\text{on},\text{off}$. These KSs have $R_{\text{on,off}}=R_{\text{off,on}}=N$.
- KSs 2C, 2D, and 3A for $y=\text{on}$. These KSs have $R_{\text{on,off}}=R_{\text{off,on}}=1$.
- KS 3C for $y=\text{on}$. This KS has $R_{\text{on,off}}=R_{\text{off,on}}=2$.

(2) When $M_x<N_y$, only final-x-state substates contribute to $R_{x,y}$, and any initial-y-state substate is clustered. The number of such clusters is M_x, and all initial-y-state substates in a cluster are connected to the same final-x-state substate that contributes to the rank $R_{x,y}$. Examples include:

- KSs 2C, 2D, and 3A for $y=\text{off}$, for which, $R_{\text{on,off}}=R_{\text{off,on}}=1$. In all of these cases, the mapping into the RD form's single off substate clusters all the KS' off substates together.
- KS 3C for $y=\text{off}$, for which, $R_{\text{on,off}}=R_{\text{off,on}}=2$. The mapping in this example clusters the KS substates 1_{off}-2_{off} into one cluster, and the KS substates 3_{off}-4_{off} into another cluster. These clusters give rise to the RD form substates 1_{off} and 2_{off}, respectively.

III. SIGNATURES OF KS' PROPERTIES IN RD FORMS AND IN TWO-STATE TRAJECTORIES

This section presents about 50 new relationships between the RD forms, KSs, and two-state trajectories. These relationships are based on the ranks of the two-dimensional histograms and indicate on symmetry, irreversible transitions, and special connectivity in KSs. We start by listing important properties of RD forms.

- A RD form has the simplest topology that can reproduce the data.
- The topology of the RD form is obtained from the data without fitting.
- RD forms can represent KSs with symmetry and irreversible transitions because these canonical forms are built from all four $R_{x,y}$s.
- As shown in the preceding subsection, an important part in the mapping of a KS into a RD form is based on the on-off connectivity. As a result, RD forms constitute a convenient and powerful tool for discriminating between KSs. The basic rule is that two on-off KSs are equivalent if and only if they have the same RD form.

The above first two points are basic properties of RD forms, but the last two points connect KSs and RD forms. To use the third point in practice, we need to relate properties of KSs, such as symmetry and irreversible transitions, to the ranks. This is discussed later. We focus now on the fourth point above and elaborate on the ways in which RD forms can be used in discriminating between KSs. Basically, when different on-off KSs have different $R_{x,y}$ values or different number of exponentials in $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$, they are distinguishable by a two-state trajectory. Moreover, different on-off KSs are distinguishable by a two-state trajectory also when they have the same $R_{x,y}$ values and the same number of exponentials in $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$, but have different complexity in the WT-PDFs for the connections in the RD form. Because the complexity of the $\varphi_{x,ij}(t)$s can be deduced without actual calculations, RD forms are the most efficient canonical forms in discriminating between on-off KSs. To show how to use RD forms in discriminating between KSs, consider KSs 2A and 2B: The RD form of KS 2A has $2N^2$ connections whereas the RD form of KS 2B has $N(N+1)$ connections. In this example, the two on-off KSs lead to two RD forms with the same number of substates but with different connectivity. The more general case in which similar KSs lead to $\varphi_{x,ij}(t)$s with the same connectivity but different complexity happens when there are isolated clusters of x substates of different sizes in the KSs. For example, KS 4A has three isolated off clusters, two clusters with two substates and one substate with three substate. KS 4B also has three clusters of off substates, two clusters with three substates and one cluster with three substates (see Fig. 4). The different cluster sizes in the KSs lead to different complexity in their $\varphi_{x,ij}(t)$s. Thus, although both KSs have the same RD form's topology (3D) with the same number of exponentials in $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$, these KSs can be resolved by a two-state trajectory. The simplest scenario that enables discriminating KSs based on differences in $\varphi_{x,ij}(t)$s complexity involves different KSs with $R_{x,y}=1$ $(x,y=\text{on},\text{off})$ that have different $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$. This case, and also the opposite one—a

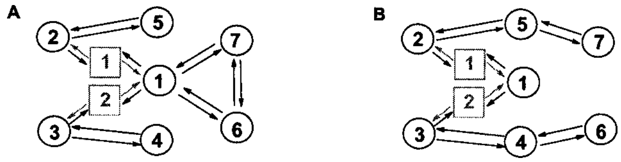

FIG. 4. KSs that can be resolved by a two-state trajectory. KSs (A) and (B) share the same rank values and the same number of components in the $\phi_x(t)$s, but still can be resolved by a two-state trajectory. The reason is that the corresponding RD forms of these KSs have different complexity for the WT-PDFs for the connections that are noticeable in the data.

case where different KSs with $R_{x,y}=1$ $(x,y=\text{on},\text{off})$ have the same $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$, and therefore the KSs cannot be resolved by the analysis of a trajectory, were extensively discussed in Refs. 35 and 37–42. For example, when the KSs 2C and 2D have the same $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$ they are equivalent. Equivalent KSs of rank three are shown in Figs. 5(A) and 5(B), whereas Fig. 5(C) shows a KS that can be discriminated from the other two, based on differences in RD forms' $\varphi_{x,ij}(t)$s.

The above discussion highlights the role for the rank values, the number of exponentials in $\phi_{\text{on}}(t)$ and $\phi_{\text{off}}(t)$, and the complexity of the $\varphi_{x,ji}(t)$s, in the characterization of KSs and RD forms. It turns out that these and other properties of KSs and RD forms can be unraveled by analyzing the relative (to each other) rank values. Table I gives these properties by considering all the combinations of relative rank values. In the following, important general properties are discussed in detail, such as symmetry in KSs, RD forms, and two-state trajectories. We start by considering a case where all the ranks have the same value, i.e., $R_{x,y}=R$ for $x,y=\text{on},\text{off}$. For this simple case, R is the number of substates in each of the states in the RD form, and the underlying on-off KS has no detectable symmetry or irreversible transitions. Additionally, for this case, the number of exponents in $\phi_x(t)$ is the number of substates in state x in the (simplest) underlying on-off KS that can generate the data. More generally, the $\phi_{x,y}(t_1,t_2)$s may have different rank values. For such cases, the underlying KS must have at least one of the following properties: Irreversible on-off connections, symmetry, and a special connectivity within the substates in a state. In particular,

- When $R_{\text{on,off}} \neq R_{\text{off,on}}$ and $R_{\text{on,off}}, R_{\text{off,on}} \geqslant R_{\text{on,on}}, R_{\text{off,off}}$, there are detectable irreversible on-off connections in the underlying KS.
- When $R_{x,y} \geqslant R_{\text{on,on}}, R_{\text{off,off}}$ $(x \neq y)$, $R_{x,y}$ is the number of substates in state y in the RD form.
- When $R_{x,x} > R_{y,y}, R_{\text{on,off}}, R_{\text{off,on}}$ $(x \neq y)$, $R_{x,x}$ is the number of substates of both states in the RD form, and there is symmetry in state y in the underlying KS.
- When $R_{x,z} > R_{z,z}$ $(x \neq z)$, there are irreversible on-off connections and a special connectivity in state x in the KS. In particular, $R_{z,z}$ is the *minimal* number of substates in state x of the KS among which the random walk must visit in each event in that state. This situation is exemplified in Fig. 6, and is called "a special wiring in the KS."

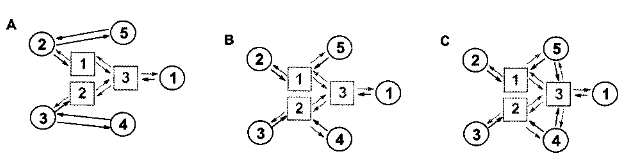

FIG. 5. KSs that cannot be resolved by a two-state trajectory (A)–(B) and a related distinct KS (C). KSs (A) and (B) can lead to the same RD form so they are equivalent on the level of the on-off data. KS (C) has different RD form's $\varphi_{x,ij}(t)$s than those of KSs (A) and (B).

In the second scenario above we used the term symmetry in the KS. Symmetry refers to a collection of properties in the KS, and consequently in the RD form. We define three types of symmetry in KS and five types of symmetry in RD forms, where two types out of the five are related to irreversible transitions in the KS. Type-one symmetry in state x in the KS leads to the reduction in the number of components in $\phi_x(t)$ but not to a reduction in the rank values. For example, this scenario is obtained in the linear on-off KS,

$$3_{\text{on}} - 2_{\text{on}} - 2_{\text{off}} - 3_{\text{off}} - 1_{\text{off}} - 1_{\text{on}},$$

when choosing,

$$k_{1_{\text{off}}1_{\text{on}}} = \lambda[1 + (1 - k_{2_{\text{off}}2_{\text{on}}} k_{2_{\text{off}}3_{\text{on}}}/\lambda)^{1/2}],$$

$$\lambda = \frac{k_{2_{\text{off}}3_{\text{on}}} + k_{3_{\text{off}}2_{\text{on}}} + k_{2_{\text{off}}2_{\text{on}}}}{2}.$$

(Here, the transition rate k_{ij} connects substates $j \to i$). This choice leads to a reduction of one component in $\phi_{\text{on}}(t)$, from a three component $\phi_{\text{on}}(t)$ into a two component $\phi_{\text{on}}(t)$, but all the ranks equal to 2. Importantly, the RD form of this KS reveals this symmetry, because $\varphi_{\text{on},11}(t)$ and $\varphi_{\text{on},22}(t)$ share some rates in their exponential expansions.

Such a tuning of transition rates can lead to a scenario where the number of components in $\phi_x(t)$ is smaller than $R_{y,y}$; take for example a KS similar to the above one with an additional on substate, substate 4_{on}, connected to substate 3_{off} by a rate, $k_{3_{\text{off}}4_{\text{on}}} = \lambda[1-(1-k_{2_{\text{off}}2_{\text{on}}} k_{2_{\text{on}}3_{\text{on}}}/\lambda)^{1/2}]$. For this case, $R_{\text{off,off}}=3$ with the other ranks equal 2, where the number of components in $\phi_{\text{on}}(t)$ is 2. This case represents type-three symmetry, which is characterized by a reduction in both the number of components in $\phi_z(t)$ and in rank value(s) that *can be detected*. (Note that both type-one and type-three symmetries (can) originate from tuning transition rate values. However, the degree of the tuning is different and leads to different signatures in the data.)

Type-two symmetry leads to a reduction in a rank value(s) but not in the number of components in the $\phi_x(t)$s. An example is a KS that has the same splitting probabilities, $p_{ji}=k_{ji}/k_i$ where $k_i=\Sigma_j k_{ji}$, for some substates i and i' in a state, but with $k_i \neq k_{i'}$. In particular, when choosing for the KS 7A,

$$k_{2_{\text{on}}2_{\text{off}}}/k_{1_{\text{on}}2_{\text{off}}} = k_{2_{\text{on}}1_{\text{off}}}/k_{1_{\text{on}}1_{\text{off}}} \equiv p_R/p_L,$$

with different values for $k_{2_{\text{off}}}$ and $k_{1_{\text{off}}}$, all the ranks, except of $R_{\text{on,off}}$, equal unity, but $R_{\text{on,off}}=2$. In the same time, both $\phi_x(t)$s can still have two components. See Fig. 7.

TABLE I. All possible combinations of relative rank values are examined, and their relationships to properties of RD forms and KSs are summarized in the table. In this table we denote by $T(i,j,k)$ the kth case in the column enumerated by j and the row enumerated by i.

	(1) $R_{\text{on,on}}=R_{\text{off,off}}\equiv\rho$	
(1) $R_{\text{on,off}}=R_{\text{off,on}}$ $R_{\text{off,on}}\equiv R$	(1) $R=\rho$. **Possible**. No symmetry and no irreversible transitions in the KS. R is the number of substates in each of the states in the RD form. (2) $R>\rho$. **Possible**. Irreversible transitions and a special wiring in both states in the KS (symmetries S1-S2). R is the number of substates in each of the states in the RD form. (3) $R<\rho$. **Impossible**. For this case, symmetries S3-S5 are needed in both states. However, this leads to case $T(1,1,1)$.	
(2) $R_{\text{on,off}}>R_{\text{off,on}}$ $R_{\text{off,on}}\equiv R$	(1) and (2) $R=\rho$ and $R>\rho$. **Possible**. Irreversible transitions and a special wiring in *both* states in the KS. $R_{x,y}$ for $x\neq y$ is the number of substates in state y in the RD form. (3)–(5) $R_{\text{on,off}}=\rho$, $R_{\text{on,off}}>\rho>R$, and $\rho>R_{\text{on,off}}$. **Impossible**. In all these cases, $R_{x,x}>R_{y,x}$, namely, symmetries S3-S5 in state y, but these also demand $R_{\text{on,off}}=R_{\text{off,on}}$.	
	(2) $R_{\text{on,on}}>R_{\text{off,off}}\equiv\rho$	
(1) $R_{\text{on,off}}=R_{\text{off,on}}$ $R_{\text{off,on}}\equiv R$	(1) $R=\rho$. **Possible**. Symmetries S3-S5 in state off. $R_{\text{on,on}}$ is the number of substates in each of the states in the RD form. (2) $\rho>R$. **Impossible**, as in $T(2,1,3–5)$. (3) $R_{\text{on,on}}>R>\rho$. **Possible**. Symmetries S3-S5 in the off state and symmetries S1-S2 in the on state. $R_{\text{on,on}}$ is the number of substates in each of the states in the RD form. (4) $R_{\text{on,on}}=R>\rho$. **Possible**. Symmetries S1-S2 in both states. Irreversible transitions and a special wiring in the KS. $R_{\text{on,on}}$ is the number of substates in each of the states in the RD form. (5) $R>R_{\text{on,on}}$. **Possible**, as above.	
(2) $R_{\text{on,off}}=R_{\text{off,on}}$ $R_{\text{off,on}}\equiv R$	(1) $R=\rho$	(1.1) $R_{\text{on,off}}>R_{\text{on,on}}$. **Impossible**. The different event ranks must be equal. (1.2) $R_{\text{on,off}}<R_{\text{on,on}}$. **Impossible**, as above. (1.3) $R_{\text{on,off}}=R_{\text{on,on}}$. **Impossible**, as above.
	(2) $R>\rho$	(2.1) $R>R_{\text{on,on}}$. **Possible**, irreversible transitions and symmetries S1-S2, in both states. $R_{x,y}$ for $x\neq y$ is the number of y substates in the RD form. (2.2) $R=R_{\text{on,on}}$. **Possible**, as above. (2.3) $R<R_{\text{on,on}}<R_{\text{on,off}}$. **Impossible**. Symmetry S3-S5 in the off state demands $R_{\text{off,on}}=R_{\text{on,off}}<R_{\text{on,on}}$, as in $T(2,2,1)$. (2.4) $R<R_{\text{on,on}}=R_{\text{on,off}}$. **Impossible**, as above. (2.5) $R<R_{\text{on,off}}<R_{\text{on,on}}$. **Impossible**, as above.
	(3) $\rho>R$	(3.1) $\rho>R_{\text{on,off}}$. **Impossible**, as in $T(2,2,2.3–2.5)$ and $T(2,2,1)$. (3.2) $\rho=R_{\text{on,off}}$. **Impossible**, as above. (3.3) $\rho<R_{\text{on,off}}<R_{\text{on,on}}$. **Impossible**, as above. (3.4) $\rho<R_{\text{on,off}}=R_{\text{on,on}}$. **Impossible**, as above. (3.5) $R_{\text{on,on}}<R_{\text{on,off}}$. **Impossible**, as above.
	(3) $R_{\text{off,off}}>R_{\text{on,on}}\equiv\rho$	
(1) $R_{\text{on,off}}=R_{\text{off,on}}$ $R_{\text{off,on}}\equiv R$	The same as $T(1,2)$.	
(2) $R_{\text{on,off}}>R_{\text{off,on}}$ $R_{\text{off,on}}\equiv R$	(1) $R=\rho$	(1.1) $R_{\text{on,off}}>R_{\text{off,off}}$. **Possible**. Symmetries S1-S2 in the on state and also symmetries S3-S5 in the on state. (The second condition reduces $R_{\text{on,on}}$ and $R_{\text{off,on}}$ from the value of $R_{\text{off,off}}$). $R_{\text{on,off}}$ is the number of off substates and $R_{\text{off,off}}$ is the number of on-substates in the RD form.

TABLE I. (*Continued.*)

	(1.2) $R_{\rm on,off}<R_{\rm off,off}$. **Impossible**. Symmetries S3-S5 in the on state demands the different event ranks to be the same.
	(1.3) $R_{\rm on,off}=R_{\rm off,off}$. **Possible**. Just the second part of $T(2,3,1.1)$. $R_{\rm on,off}$ is the number of off substates and $R_{\rm off,off}$ is the number of on substates in the RD form.
(2) $R>\rho$	(2.1) $R>R_{\rm off,off}$. **Possible**, symmetries S1-S2 in both states. $R_{x,y}$ for $x\neq y$ is the number of y substates in the RD form.
	(2.2) $R=R_{\rm off,off}$. **Possible**, as above.
	(2.3) $R<R_{\rm off,off}<R_{\rm on,off}$. **Possible**, as in $T(2,3,1.1)$.
	(2.4) $R<R_{\rm off,off}=R_{\rm on,off}$. **Possible**, as in $T(2,3,1.3)$.
	(2.5) $R<R_{\rm on,off}<R_{\rm off,off}$. **Impossible**. Symmetries S3-S5 in the on state demands that the different event ranks are the same.
(3) $\rho>R$	(3.1) $\rho>R_{\rm on,off}$. **Impossible**. Both same event ranks are larger than the "coupled" mixed event, as in $T(2,2,1)$ and $T(2,2,2)$.
	(3.2) $\rho=R_{\rm on,off}$. **Impossible**, as above.
	(3.3) $\rho<R_{\rm on,off}<R_{\rm off,off}$. **Impossible**, as above.
	(3.4) $\rho<R_{\rm on,off}=R_{\rm off,off}$. **Impossible**, as in $T(3,3,3.1)$.
	(3.5) $R_{\rm off,off}<R_{\rm on,off}$. **Impossible**, as above.

Finally, note that there is also a kind of symmetry that is undetectable in a single two-state trajectory. This symmetry can reduce, either or both, rank values and components in $\phi_x(t)$s, but in a way that cannot be inferred from the data. For example, take the linear four-substate KS,

$$2_{\rm on}-2_{\rm off}-1_{\rm off}-1_{\rm on},$$

and set $k_{1_{\rm off}1_{\rm on}}=k_{2_{\rm off}2_{\rm on}}\equiv\alpha$, $k_{2_{\rm off}1_{\rm off}}=k_{1_{\rm off}2_{\rm off}}$, $k_{1_{\rm on}1_{\rm off}}=k_{2_{\rm on}2_{\rm off}}\equiv\beta$. The trajectory generated by this KS is identical to a trajectory generated by a two-substate Markovian KS, with rates α and β for the on to off and off to on transitions, respectively. Such a scenario is not considered in the classification of symmetries used in Table I. (Note that it is possible to break this symmetry when collecting data while changing the external conditions, assuming that symmetry breaking is induced by these changes. Of course, when this symmetry breaking happens, the statistical properties of the various trajectories are different.)

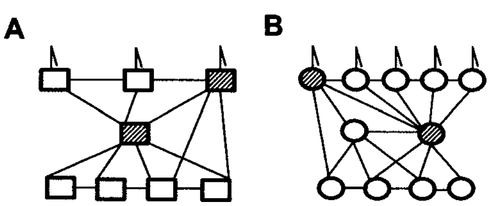

FIG. 6. An irreversible transition KS separated into two panels for a convenient illustration. The on substates are shown on the left and the off substates are shown on the right. The bottom substates in each of the states are initial ones and the top substates with the directional arrows are the final substates. An arrow represents a set of directional connections from the final substate to all the initial substates in the other state. This special KS has three different rank values: $R_{\rm on,off}=3$, $R_{\rm off,on}=4$, $R_{\rm on,on}=2$, and $R_{\rm off,off}=2$. The corresponding RD form has four on substates and three off substates. The stripped substates in state x are the substates that contribute to the rank $R_{y,y}$ ($x\neq y$), because these substates are the minimal number of substates among which the random walker must visit in each event in state x.

The above three symmetry types in KSs, together with the occurrence of irreversible transitions, are translated into five symmetry types in RD forms. These symmetries are characterized by different properties of the $\varphi_{x,ij}(t)$s. In particular,

- (S1) $\bar{\varphi}_{x,ij}(0)$ is the same for different values of j. Here, $\bar{\varphi}_{x,ij}(0)=(\int_0^\infty\varphi_{x,ij}(t)e^{-st}dt)_{s=0}$.
- (S2) $\bar{\varphi}_{x,ij}(0)$ is the same for different values of i.
- (S3) $\varphi_{x,ij}(t)$ is the same for different values of j.
- (S4) $\varphi_{x,ij}(t)$ is the same for different values of i.
- (S5) $F_{x,j}(t)=\Sigma_i\varphi_{x,ij}(t)$ is the same for different values of j, and $\varphi_{x,ij}(t)=\varphi_{x,i'j'}(t)$ for some indices, $i\neq i'$ and $j\neq j'$.

Note that symmetries (S1) and (S2) are automatically ful-

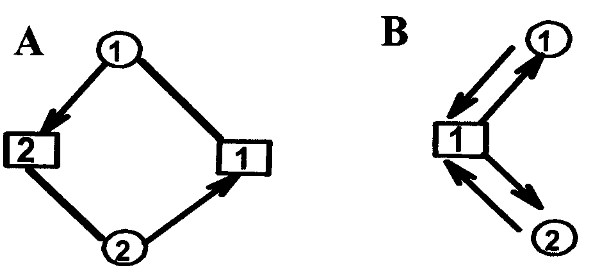

FIG. 7. A KS (A) and the RD form (B). The KS (B) has $L_{\rm on}=2$ (squared substates), $L_{\rm off}=2$ (circled substates), irreversible transitions, and $N_{\rm on}=M_{\rm on}=2$ and $N_{\rm off}=M_{\rm off}=2$. Imposing the equality on the ratios, $k_{2_{\rm on}2_{\rm off}}/k_{1_{\rm on}2_{\rm off}}=k_{2_{\rm on}1_{\rm off}}/k_{1_{\rm on}1_{\rm off}}\equiv p_R/p_L(p_L+p_R=1)$ with different values for $k_{2_{\rm off}}$ and $k_{1_{\rm off}}$, leads to symmetry in the KS in the sense that the ranks of the two-dimensional WT-PDFs of successive $x,y(=\text{on},\text{off})$ events are all equal to 1, except to $R_{\rm on,off}$ which equal to 2. However, both $\phi_x(t)$s can still have two components. The corresponding RD form (B) has one on substate and two off substates, and direction dependent WT-PDFs for the on to off connections, $\varphi_{\rm on,11}(t)=p_Lk_{1_{\rm off}1_{\rm on}}e^{-k_{1_{\rm off}1_{\rm on}}t}$, $\varphi_{\rm on,21}(t)=p_Rk_{2_{\rm off}1_{\rm on}}e^{-k_{2_{\rm off}1_{\rm on}}t}$, where $\varphi_{\rm off,11}=k_{1_{\rm off}}e^{-k_{1_{\rm off}}t}$, $\varphi_{\rm off,12}(t)=k_{2_{\rm off}}e^{-k_{2_{\rm off}}t}$.

filled when symmetries (S3)–(S4) are fulfilled, but when characterizing a RD form with symmetries (S1)–(S2), we mean that the more general type of symmetry is not observed. With this convention, symmetries (S1)–(S2) are associated with special wiring and irreversible transitions in KSs, whereas symmetries (S3)–(S5) are associated with symmetry in KSs. Using the above symmetries, we characterize properties in RD forms:

- A RD form with different number of substates, such that $R_{\text{on,off}} > R_{\text{off,on}}$, but *without* any kind of symmetry, must have $R_{\text{off,off}} = R_{\text{on,on}} = R_{\text{off,on}}$.
- Symmetries S1 and S2 in state x lead to a reduction of only $R_{y,y}$ $(x \neq y)$.
- Symmetry S3 in state x preserves only $R_{x,y}$ $(x \neq y)$.
- Symmetry S4 in state x preserves only $R_{y,x}$ $(x \neq y)$.
- Symmetry S5 in state x always leads to a reduction of $R_{y,y}$ and can also lead to a reduction of $R_{x,y}$ $(x \neq y)$.

In Table I we enumerate all the possibilities of relative rank values and relate each scenario to properties of KSs and RD forms. In Table I we use the above defined symmetries, S1–S5.

The last point in this section refers to symmetry on the level of the trajectory, also called time (microscopic) reversibility. A RD form can generate a two-state trajectory that preserves time reversibility even when it has irreversible connections. These can be balanced by the existence of direction dependent WT-PDFs for the connections. Microscopic reversibility in a RD form means that the $\phi_{x,y}(t_1,t_2)$s obtained when reading the two-state trajectory in the forward direction are the same as the corresponding $\phi_{x,y}(t_1,t_2)$s obtained when reading the two-state trajectory backwards.[75] Using matrix notation, microscopic reversibility means

$$\phi_{x,y}(t_1,t_2) = [\phi_{y,x}(t_1,t_2)]^T,$$

where T stands for the transpose of a matrix. We note that the above relation can be translated into a relation between the matrices $\sigma_{x,y}$ in the exponential expansion of $\phi_{x,y}(t_1,t_2)$s (Appendix A). For a mechanism that generates a microscopic reversible on-off trajectory, $\sigma_{x,y} = \sigma_{y,x}^T$, and, in particular, $\sigma_{x,x}$ is a symmetric matrix.

However, even when the condition of microscopic reversibility holds in the data, the underlying KS can still violate detailed balance. For on-off trajectories from on-off KSs, detailed balance violation in the KS can be deduced from peaked $\phi_x(t)$s even when the microscopic reversibility condition holds in the on-off data. Basically, detailed balance in a KS means that a transition from any given substate multiplied by the probability to occupy this substate in steady state, namely, the outgoing flux along the transition in infinite time, equals to the same quantity in the reverse direction.[76] This rule is translated to "no net flux" along closed loops in the KS and demands conditioning some transition rate values. It was shown that detailed balance in a KS is fulfilled when the numbers of conditioned transitions rates equals the difference between the number of double transitions and the number of substates plus one.[77,78] (Of course, irreversible transitions are not allowed in detailed-balanced KSs). For example, to fulfill detailed balance in KS 3A, one conditioned transition rate is required, whereas three conditioned transition rates are required for the KS 3C, and four conditioned transition rates are required for KS 3E. In terms of the terminology and definitions given in this paper, detailed-balanced KSs must lead to one of two scenarios in the data: All the ranks $R_{x,y}$ have the same value, and this value cannot exceed the number of components in the $\phi_x(t)$s, or one of the same event ranks, say, $R_{x,x}$, is larger than all other ranks, which are equal to each other and $L_x \geqslant R_{x,x}$ but $L_y < R_{x,x}$. A two-state trajectory that obeys the condition of microscopic reversibility with one of the above scenarios and nonpeaked $\phi_x(t)$s is most likely generated by a KS that fulfills detailed balance.

IV. SUMMARY AND CONCLUDING REMARKS

The problem of analyzing two-state trajectories in terms of complex on-off KSs emerges in many applications, e.g., single molecules studies.[1–31] Building the KS from the data is hard, and, in many cases, impossible, due to the loss of the information in the mapping of the multidimensional KS onto a two-state trajectory. The way to deal with this phenomenon uses canonical forms.[37,42,43] A single canonical form is associated with the data, but many KSs. Our analysis uses canonical forms of reduced dimensions (RD).[43] RD forms are on-off networks with connections only between substates of different states. The connections (usually) have nonexponential WT-PDFs. A RD form has the simplest topology that can reproduce the data, where the KSs complexity enters in the functional form of the WT-PDFs.

The mapping of the KS into a RD form is primarily determined by the ranks $R_{x,y}$, $x,y = \text{on,off}$, of the two-dimensional histograms of successive events, $\phi_{x,y}(t_1,t_2)$, x, $y = \text{on,off}$. [$R_{x,y}$ is also the rank of the matrix $\sigma_{x,y}$ whose elements appear in the exponential expansion of $\phi_{x,y}(t_1,t_2)$.] The topology of the RD form (i.e., the numbers of on substates and off substates) is determined by the four rank values. To translate the rank values into the topology of the RD form, one should use the results given in Table I. Because RD forms are built from all four ranks, they can represent any type of KS; namely, KSs with symmetry and/or irreversible transitions can also be mapped into RD forms. (For example, a symmetric KS can lead to a RD form with the same functional form for some WT-PDFs for the connections, and irreversible transitions in the KS can lead to a different number of substates in the two states of the RD form.)

The ranks $R_{x,y}$s can also be found from the structure of the on-off connectivity of the KS; see Eqs. (1) and (2). (The mathematical basis for the relationships between the ranks and the on-off connectivity of the KS is, in fact, the path representation of the $\phi_{x,y}(t_1,t_2)$s, see Appendix A.) Equations (1) and (2) classify all possible relationships between structures of the on-off connectivity of the KSs and the ranks of the $\phi_{x,y}(t_1,t_2)$s, and establish the fundamental relationships between reduced dimensions forms, kinetic schemes and two-state trajectories. The mapping of a KS into a RD form is uniquely determined by the identification of the substates in the on-off connectivity interface of the KS that contribute to the ranks, and then includes partial summations over Green's functions for irreversible on-off process (see Appendix B). In this paper, we unraveled hidden relationships between properties of on-off kinetic schemes, their canonical forms of reduced dimensions, and two-state trajectories. The relationships reported in this paper are based on the relative value of the ranks. Many of the new relationships are related to KSs with symmetries and irreversible transitions. A particular consideration was given to symmetries in KSs and in RD forms: Three types of symmetries were defined for KSs and five types for RD forms. Importantly, we have found forbidden combinations for the relative rank values. These findings, which are summarized in Table I, constitute a complete characterization of on-off KSs and are translated into properties of RD forms. The network of relationships between KSs, RD forms, and two-state trajectories is useful in theoretical analysis of on-off KSs, and also in the actual analysis of the data: They translate the rank values into the RD form's topology, and indicate on inconsistencies in the analysis when a forbidden combination of relative rank values is obtained.

Finally, note that along with the theoretical results presented in this paper, we developed a toolbox for constructing the RD form from finite data which will be presented in a future publication.

ACKNOWLEDGMENTS

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APPENDIX A: $\phi_x(t)$ AND $\phi_{x,y}(t_1,t_2)$

In this Appendix, we express the WT-PDFs for single periods, $\phi_x(t)$, $x=\text{on},\text{off}$, and for joint successive periods, $\phi_{x,y}(t_1,t_2)$, $x,y=\text{on},\text{off}$, in terms of both the master equation and the path representation. The relationships between the two representations is made. On-off KSs are commonly described in terms of the master equation, but our canonical forms are naturally related to the path representation.

1. Matrix formulation of the system

The master equation formalism is used to express $\phi_x(t)$ and $\phi_{x,y}(t_1,t_2)$. The treatment is fairly standard.[34–43] We start by introducing the equation of motion for the time-dependent occupancy probabilities of state x, $\mathbf{P}_x(t)$, $(\mathbf{P}_x(t))_i=P_{x,i}(t)$, $i=1,\ldots,L_x$, for the reversible (coupled) on-off process,

$$\frac{\partial}{\partial t}\begin{pmatrix}\mathbf{P}_{\text{on}}(t)\\ \mathbf{P}_{\text{off}}(t)\end{pmatrix}=\begin{pmatrix}\mathbf{K}_{\text{on}} & \mathbf{V}_{\text{off}}\\ \mathbf{V}_{\text{on}} & \mathbf{K}_{\text{off}}\end{pmatrix}\begin{pmatrix}\mathbf{P}_{\text{on}}(t)\\ \mathbf{P}_{\text{off}}(t)\end{pmatrix}. \tag{A1}$$

In Eq. (A1), matrix $\mathbf{K}_x$, with dimensions $[\mathbf{K}_x]=L_x,L_x$, contains transition rates among substates in state x and "irreversible" transition rates from substates in state x to substates in state y. (The irreversible transition rates appear, with negative signs, only on the diagonal of matrix $\mathbf{K}_x$). Matrix $\mathbf{V}_x$, with dimensions $[\mathbf{V}_x]=L_y,L_x$, contains transition rates between states $x\to y$, where $(\mathbf{V}_x)_{ji}$ is the transition rate between substates $i_x\to j_y$.

To obtain expressions for $\phi_x(t)$ and $\phi_{x,y}(t_1,t_2)$, we need to compute the occupancy probabilities of the coupled process at steady state, $\mathbf{P}_x(ss)$, $x=\text{on},\text{off}$, and the Green's function of the irreversible x process, $\mathbf{G}_x(t)$, $x=\text{on},\text{off}$. $\mathbf{P}_x(ss)$ is defined by

$$\mathbf{P}_x(ss)=\lim_{t\to\infty}\mathbf{P}_x(t)$$

and is found from Eq. (A1) for vanishing time derivative. The Green's function of state x for the irreversible process, $\mathbf{G}_x(t)$, obeys the equation

$$\partial\mathbf{G}_x(t)/\partial t=\mathbf{K}_x\mathbf{G}_x(t),$$

with the solution,

$$\mathbf{G}_x(t)=\exp[\mathbf{K}_x t]=\mathbf{X}\exp[\lambda_x t]\mathbf{X}^{-1}. \tag{A2}$$

The second equality in Eq. (A2) follows from a similarity transformation, $\lambda_x=\mathbf{X}^{-1}\mathbf{K}_x\mathbf{X}$, and all the matrices in Eq. (A2) have the dimensions L_x,L_x. $\phi_x(t)$ and $\phi_{x,y}(t_1,t_2)$ are given by

$$\phi_x(t)=\mathbf{1}_y^T\mathbf{V}_x\mathbf{G}_x(t)\mathbf{V}_y\mathbf{P}_y(ss)/N_x, \tag{A3}$$

and

$$\phi_{x,y}(t_1,t_2)=\mathbf{1}_x^T\mathbf{V}_y\mathbf{G}_y(t_2)\mathbf{V}_x\mathbf{G}_x(t_1)\mathbf{V}_y\mathbf{P}_y(ss)/N_x, \tag{A4}$$

where $N_x=\mathbf{1}_x^T\mathbf{V}_y\mathbf{P}_y(ss)$ and $\mathbf{1}_x^T$ is the summation row vector of $1,L_x$ dimensions. The expression for $\phi_{x,x}(t_1,t_2)$ is obtained from Eq. (A4) when plugging in the factor $\mathbf{V}_y\bar{\mathbf{G}}_y(0)$ (recall that the bar symbol stands for the Laplace transform of the function),

$$\phi_{x,x}(t_1,t_2)=\mathbf{1}_y^T\mathbf{V}_x\mathbf{G}_x(t_2)\mathbf{V}_y\bar{\mathbf{G}}_y(0)\mathbf{V}_x\mathbf{G}_x(t_1)\mathbf{V}_y\mathbf{P}_y(ss)/N_x.$$

Equation (A3) can be written as

$$\phi_x(t)=\sum_{i=1}^{L_x}c_{x,i}e^{-\lambda_{x,i}t},\quad \lambda_{x,i}>0,\ \forall\, i, \tag{A5}$$

where $c_{x,i}$, which is the ith element of vector $\mathbf{c}_x$, is given by

$$c_{x,i}=(\mathbf{c}_x)_i=(\mathbf{1}_y^T\mathbf{V}_x\mathbf{X})_i(\mathbf{X}^{-1}\mathbf{V}_y\mathbf{P}_y(ss)/N_x)_i, \tag{A6}$$

and $-\lambda_{x,i}$ is ith eigenvalue of matrix $\mathbf{K}_x$. Equation (A4) can be written in a double summation over weighted exponentials,

$$\phi_{x,y}(t_1,t_2)=\sum_{i=1}^{L_x}\sum_{j=1}^{L_y}\sigma_{x,y,ij}e^{-\lambda_{x,j}t_1-\lambda_{y,i}t_2}, \tag{A7}$$

where the matrix element $\sigma_{x,y,ij}$ is given by

$$\sigma_{x,y,ij}=(\mathbf{1}_x^T\mathbf{V}_y\mathbf{Y})_j(\mathbf{Y}^{-1}\mathbf{V}_x\mathbf{X})_{ji}(\mathbf{X}^{-1}\mathbf{V}_y\mathbf{P}_y(ss)/N_x)_j. \tag{A8}$$

For $x=y$, $\sigma_{x,x,ij}$ is given by

$$\sigma_{x,x,ij}=(\mathbf{1}_y^T\mathbf{V}_x\mathbf{X})_j(\mathbf{X}^{-1}\mathbf{V}_y\bar{\mathbf{G}}_y(0)\mathbf{V}_x\mathbf{X})_{ji}(\mathbf{X}^{-1}\mathbf{V}_y\mathbf{P}_y(ss)/N_x)_i. \tag{A9}$$

2. Path representation of the WT-PDFs

Our canonical forms are based on expressing the $\phi_{x,y}(t_1,t_2)$s in path representation that utilizes the on-off connectivity of the KS. As in the master equation's description, the on-off process is separated into two irreversible processes that occur sequentially, and we have for $\phi_{x,y}(t_1,t_2)$ $(x \neq y)$,

$$\phi_{x,y}(t_1,t_2) = \sum_{n_y=1}^{N_y}\left(\sum_{n_x=1}^{N_x} W_{n_x} f_{n_y n_x}(t_1)\right) F_{n_y}(t_2)$$
$$= \sum_{m_x \in \{M_x\}}\left(\sum_{n_x=1}^{N_x}\sum_{n_y=1}^{N_y} W_{n_x}\tilde{f}_{m_x n_x}(t_1)\omega_{n_y m_x}F_{n_y}(t_2)\right). \tag{A10}$$

(A sum $z_x \in \{Z_x\}$ is a sum over a particular group of Z_x substates.) Equation (A10) emphasizes the role of the KS's topology in expressing the $\phi_{x,y}(t_1,t_2)$s. N_x and M_x are the numbers of initial and final substates in state x in the KS, respectively. Namely, each event in state x starts at one of the N_x initial substates, labeled $n_x=1,\ldots,N_x$, and terminates through one of the M_x final substates, labeled $m_x=1,\ldots,M_x$, for a reversible on-off connection KS or $m_x=N_x+1-H_x,\ldots, N_x+M_x-H_x$, for an irreversible on-off connection KS, where $H_x(=0,1,\ldots,N_x)$ is the number of substates in state x that are both initial and final ones. (In each of the states the labeling of the substates starts from 1). An event in state x starts in substate n_x with probability W_{n_x}. The first passage time PDF for exiting to substate n_y, conditional on starting in substate n_x $(x\neq y)$, is $f_{n_y n_x}(t)$, and $F_{n_x}(t)=\Sigma_{n_y} f_{n_y n_x}(t)$. Writing $f_{n_y n_x}(t)$ as, $f_{n_y n_x}(t)=\Sigma_{m_x}\omega_{n_y m_x}\tilde{f}_{m_x n_x}(t)$, emphasizes the role of the on-off connectivity, where $\omega_{n_y m_x}$ is the transition probability from substate m_x to substate n_y, and $\tilde{f}_{m_x n_x}(t)\omega_{n_y m_x}$ is the first passage time PDF, conditional on starting in substate n_x, for exiting to substate n_y through substate m_x.

3. Relationships between the master equation and the path representation

All the factors in Eq. (A10) can be expressed in terms of the matrices of Eq. (A1). W_{n_x} and $f_{n_y n_x}(t)$ are related to the master equation by

$$W_{n_x} = (\mathbf{V}_y \mathbf{P}_y(ss))_{n_x}/N_x,$$

and

$$f_{n_y n_x}(t) = (\mathbf{V}_x \mathbf{G}_x(t))_{n_y n_x}.$$

$f_{n_y n_x}(t)$ can be further rewritten as

$$f_{n_y n_x}(t) = \sum_{m_x}\omega_{n_y m_x}\tilde{f}_{m_x n_x}(t),$$

and similarly for $(\mathbf{V}_x\mathbf{G}_x(t))_{n_y n_x}$ we have

$$(\mathbf{V}_x\mathbf{G}_x(t))_{n_y n_x} = \sum_k (\mathbf{V}_x)_{n_y k}(\mathbf{G}_x(t))_{k n_x}.$$

Note, however, that the factors in the right hand side in the above two sums are not equal but proportional,

$$\tilde{f}_{k n_x}(t) = \alpha_{x,k}(\mathbf{G}_x(t))_{k n_x}, \quad \alpha_{x,k} = -(\mathbf{K}_x)_{kk},$$

and

$$\omega_{n_y k} = (\mathbf{V}_x)_{n_y k}/\alpha_{x,k}.$$

APPENDIX B: THE $\varphi_{x,ij}(t)$s GIVEN A MAPPING

In this Appendix, we give expressions for the $\varphi_{x,ij}(t)$s, from any KS. We do not consider symmetric KSs separately, because symmetry does not change the functional form of the $\varphi_{x,ij}(t)$s. [Namely, even if symmetry forces reduction in the RD form topology, the functional form of the $\varphi_{x,ij}(t)$s will be unchanged.]

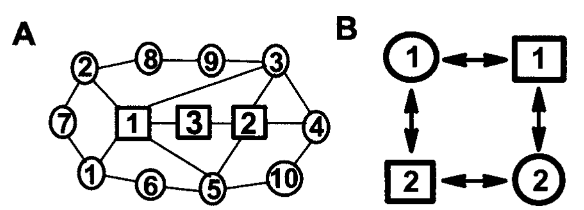
The $\varphi_{x,ij}(t)$s are uniquely determined by the clustering procedure in the mapping of a KS into a RD form. The clustering procedure is based upon the identification of substates in the on-off KS connectivity that contribute to the ranks $R_{x,y}$. The four ranks $R_{x,y}$s determine the RD form's topology, and the mapping determines the incoming flux and outgoing flux for each substate in the RD form. This makes RD forms legitimate canonical forms that preserve all the information contained in the two-state trajectory.

The technical details to obtain the $\varphi_{x,ij}(t)$s are spelled out below when considering separately the two types of clustering procedures (as discussed in II.B in the main text). (1) None of the terms in an external sum in Eq. (A10), after the first or the second equality, are proportional to each other, and (2) some of the terms in an external sum in Eq. (A10), after the first or second equality, are proportional to each other.

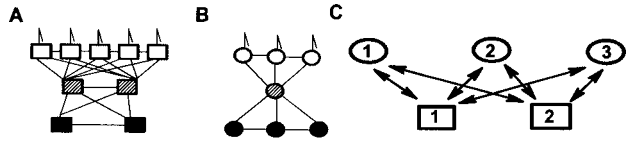
1. Clustering type 1

a. Reversible on-off connection KSs

Say, $M_x \geqslant N_y$, or equivalently $N_x \geqslant M_y$ (Scheme 1 with x=off). Based on the clustering procedure, there are N_y substates in each of the states in the RD form, and as many as $2N_y^2$ WT-PDFs for the connections in the RD form. Initial substates in state x are clustered, and the expression for $\varphi_{x,n_y i_x}(t)$ reads



SCHEME 1. A reversible connection KS, with $N_{\text{on}}=M_{\text{on}}=2$ and $N_{\text{off}}=M_{\text{off}}=5$. (B) The RD form of KS (A). The RD form's substate 1_{off} corresponds to the cluster of the KS's off substates 1_{off}-3_{off} and 5_{off} because these are connected to substate 1_{on} in the KS, which contributes to the rank $R_{\text{on,off}}$. The RD form's substate 2_{off} corresponds to the cluster of the KS's off substates 3_{off}-5_{off}, because these are connected to substate 2_{on} in the KS, which contributes to the rank $R_{\text{on,off}}$. Note that a particular initial substate can appear in more than a single cluster, which simply means that the overall steady-state flux into the substate is divided into several contributions. The initial on substates in the KS both contribute to $R_{\text{off,on}}$ so they are mapped to themselves in the RD form. The WT-PDFs for the connections can be obtained from Eqs. (B1) and (B2).



SCHEME 2. An example for a KS with irreversible on-off connections, and $N_{\mathrm{on}}=2$, $M_{\mathrm{on}}=5$, $N_{\mathrm{off}}=3$, and $M_{\mathrm{off}}=3$. The KS is divided into two panels shown on (A) (on state) and (B) (off state) for a convenient illustration. The RD form is shown on (C). The WT-PDFs for the connections can be obtained from Eqs. (B3) and (B4).

$$\varphi_{x,n_y i_x}(t) = \frac{1}{N_{x,m_y}} \sum_{n_x} P_{y,m_y}(ss)(\mathbf{V_y})_{n_x m_y} f_{n_y n_x}(t). \tag{B1}$$

In Eq. (B1), we use the normalization N_{x,m_y}, defined through the equations

$$N_x = \mathbf{1}_x^T \mathbf{V}_y \mathbf{P}_y(ss) = \sum_{m_y,n_x} P_{y,m_y}(ss)(\mathbf{V}_y)_{n_x m_y} = \sum_{m_y} N_{x,m_y} = \sum_{n_x} N_{x,n_x}.$$

As notation is concerned, we set in Eq. (B1) $j_y \to n_y$ because there are $n_y=1,\ldots,N_y$ substates in state y in the RD form, and we can also employ the meaning of n_y as the initial substates in state y in the underlying KS. Additionally, we associate m_y on the right hand side (RHS), which has the meaning of final substates in the underlying KS, with i_x on the left hand side (LHS), i.e., $m_y \to i_x$. Note that for a KS with only reversible on-off connections, $m_y=1,\ldots,M_y$, so the values of m_y and i_x can be the same.

The expression for $\varphi_{y,i_x n_y}(t)$ is different than that for $\varphi_{x,n_y i_x}(t)$ in both the normalization used and the factors that are summed, because of the mapping of the initial substates in state y to themselves. $\varphi_{y,i_x n_y}(t)$ is given by

$$\varphi_{y,i_x n_y}(t) = \frac{1}{N_{y,n_y}} \sum_{m_x} P_{x,m_x}(ss)(\mathbf{V_x})_{n_y m_x} \tilde{f}_{m_y n_y}(t) \tilde{\omega}_{m_y},$$

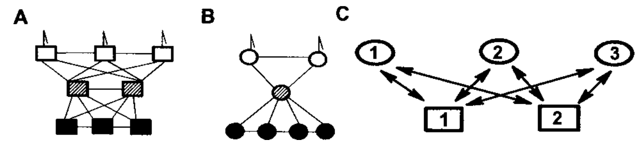
$$\tilde{\omega}_{m_y} = \sum_{n_x} \omega_{n_x m_y}. \tag{B2}$$

Note that here, $\varphi_{y,i_x n_y}(t) = \tilde{f}_{m_y n_y}(t)\tilde{\omega}_{m_y} = (\mathbf{G_y}(t))_{m_y n_y} \Sigma_{n_x} (\mathbf{V_y})_{n_x m_y}$. In Eq. (B2), we associate m_y on the RHS with i_x on the LHS, i.e., $m_y \to i_x$. Again, for a KS with only reversible transitions, the i_xs can have the same values as of the m_ys.

b. Irreversible on-off connection KSs

Obtaining the $\varphi_{x,ij}(t)$s for irreversible on-off connection KSs is similar to getting these WT-PDFs for reversible on-off connection KSs. The reason is that the clustering procedure is based on the *directional* connections between final substates in state x and initial substates in state y. However, some technical details may differ. We consider two cases.

a. Let $M_x \geq N_y$ and $M_y \geq N_x$ (Scheme 2). Then, the WT-PDFs for the connections are given by



SCHEME 3. An irreversible on-off connection KS with $N_{\mathrm{on}}=3$, $M_{\mathrm{on}}=3$, $N_{\mathrm{off}}=4$, and $M_{\mathrm{off}}=2$. The panels are divided as in Scheme 2. The WT-PDFs for the connections can be obtained from Eqs. (B5) and (B6).

$$\varphi_{x,n_y n_x}(t) = \frac{1}{N_{x,n_x}} \sum_{m_y} P_{y,m_y}(ss)(\mathbf{V_y})_{n_x m_y} f_{n_y n_x}(t) = f_{n_y n_x}(t), \tag{B3}$$

and

$$\varphi_{x,n_x n_y}(t) = \frac{1}{N_{y,n_y}} \sum_{m_x} P_{x,m_x}(ss)(\mathbf{V_x})_{n_y m_x} f_{n_x n_y}(t) = f_{n_x n_y}(t). \tag{B4}$$

Note that for this case any $\varphi_{z,ij}(t)$ equal to the corresponding $f_{ij}(t)$. This is an outcome of the KS' topology for which in both the on to off and the off to on connections, the number of initial substates in a given state is lower than the number of final substates in the other state.

b. Let $N_x > M_y$ and $N_y > M_x$ (Scheme 3). Then, the WT-PDFs for the connections are given by

$$\varphi_{x,j_y i_x}(t) = \frac{1}{N_{x,m_y}} \sum_{n_x} P_{y,m_y}(ss)(\mathbf{V_y})_{n_x m_y} \tilde{f}_{m_x n_x}(t) \tilde{\omega}_{m_x}, \tag{B5}$$

and

$$\varphi_{y,i_x j_y}(t) = \frac{1}{N_{y,m_x}} \sum_{n_y} P_{x,m_x}(ss)(\mathbf{V_x})_{n_y m_x} \tilde{f}_{m_y n_y}(t) \tilde{\omega}_{m_y}. \tag{B6}$$

In Eqs. (B5) and (B6), we use the mapping $m_y \to i_x$ and $m_x \to j_y$ between the RHS and the LHS indexes. (In particular, $m_y-(N_y-H_y)=i_x$ and $m_x-(N_x-H_x)=j_y$.)

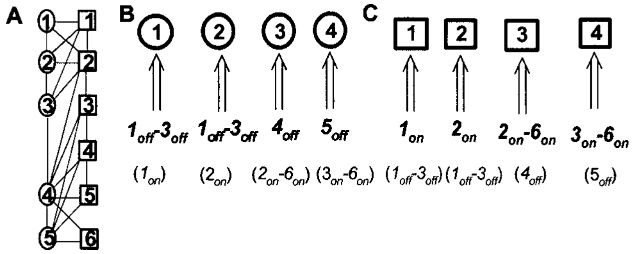
2. Clustering type 2: Reversible on-off connection KSs

We turn now to deal with cases in which some of the terms in Eq. (A10) are proportional, and therefore $\phi_{x,y}(t_1,t_2)$ is expressed by

$$\phi_{x,y}(t_1,t_2) = \sum_{n_y \in \{\tilde{N}_y\}} \left(\sum_{n_x=1}^{N_x} W_{n_x} f_{n_y n_x}(t_1) \right) F_{n_y}(t_2) + \sum_{m_x \notin \{\tilde{M}_x\}} \left(\sum_{n_x=1}^{N_x} W_{n_x} \tilde{f}_{m_x n_x}(t_1) \right) \times \left(\sum_{n_y \notin \{\tilde{N}_y\}} \omega_{n_y m_x} F_{n_y}(t_2) \right). \tag{B7}$$

We consider only KSs with reversible on-off connections, but the same analysis is relevant to KSs with irreversible on-off connections.

Let $M_x \leq N_y$, or equivalently $N_x \leq M_y$. (See Scheme 4 with x=off). So it follows that, $R_{x,y} < M_x$, which is a result of a special on-off connectivity. In particular, let $\{O_y\}$ and $\{O_x\}$



SCHEME 4. A reversible connection KS with $R_{x,y}=4$ $(x\neq y)$. The RD form's topology is shown on (B) and (C). The clustering procedure and the parent substates (in the parentheses) are indicated at the base of the double arrows. For example, substate 1_{off} in the RD form corresponds to the cluster of initial-off-substates 1_{off}-3_{off} in the KS. These are connected to substate 1_{on} in the KS. The WT-PDFs for the connections in the RD form can be obtained from Eqs. (B8)–(B13).

be the groups of substates in states y and x, respectively, such that the substates in $\{O_x\}$ are connected only to the substates in $\{O_y\}$, and $O_y<O_x$. (In Scheme 4, the group $\{O_{\mathrm{off}}\}$ contains the substates 1_{off}, 2_{off}, and 3_{off}, and the group $\{O_{\mathrm{on}}\}$ contains the substates 1_{on} and 2_{on}). Thus, both initial and final substates contribute to the rank $R_{z,z'}$, for $z\neq z'$, and the expressions for the $\varphi_{z,ij}(t)$s are distinct in each of the following three regimes:

(a) For $n_x\notin\{O_x\}$ and $n_y\notin\{O_y\}$,

$$\varphi_{x,j_y i_x}(t)=\frac{1}{N_{x,n_x}}\sum_{m_y}P_{y,m_y}(ss)(\mathbf{V}_y)_{n_x m_y}\tilde{f}_{m_x n_x}(t)\times\sum_{n_y\notin\{O_y\}}\omega_{n_y m_x},\tag{B8}$$

and

$$\varphi_{y,i_x j_y}(t)=\frac{1}{N_{y\in O_y,m_x}}\sum_{n_y}P_{x,m_x}(ss)(\mathbf{V}_x)_{n_y m_x}f_{n_x n_y}(t),\tag{B9}$$

where $N_{y\in O_y,m_x}=\Sigma_{n_y\in\{O_y\}}P_{x,m_x}(ss)(\mathbf{V}_x)_{n_y m_x}$, and we associate $n_x\to i_x$ and $m_x\to j_y$.

(b) For $n_x\notin\{O_x\}$ and $n_y\in\{O_y\}$,

$$\varphi_{x,j_y i_x}(t)=\frac{1}{N_{x,n_x}}\sum_{m_y}P_{y,m_y}(ss)(\mathbf{V}_y)_{n_x m_y}f_{n_y n_x}(t),\tag{B10}$$

and

$$\varphi_{y,i_x j_y}(t)=\frac{1}{N_{y,n_y}}\sum_{m_x}P_{x,m_x}(ss)(\mathbf{V}_x)_{n_y m_x}f_{n_x n_y}(t),\tag{B11}$$

where we associate $n_y\to j_y$ and $n_x\to i_x$.

(c) For $n_x\in\{O_x\}$ and $n_y\in\{O_y\}$

$$\varphi_{y,i_x j_y}(t)=\frac{1}{N_{y,n_y}}\sum_{m_x}P_{x,m_x}(ss)(\mathbf{V}_x)_{n_y m_x}\tilde{f}_{m_y n_y}(t)\times\sum_{n_x\in\{O_x\}}\omega_{n_x m_y},\tag{B12}$$

and

$$\varphi_{x,j_y i_x}(t)=\frac{1}{N_{x\in O_x,m_y}}\sum_{n_x\in\{O_x\}}P_{y,m_y}(ss)(\mathbf{V}_y)_{n_x m_y}f_{n_y n_x}(t),\tag{B13}$$

where we associate $n_y\to j_y$ and $m_y\to i_x$.

As a final note, we use O_y and O_x for expressing $R_{x,y}$. (This remark is complementary to the discussion in II.B). When $M_x<N_y$ and $\{O_x\}$ and $\{O_y\}$ are as defined above,

$$R_{x,y}=M_x-(O_x-O_y).\tag{B14}$$

This result can be generalized to the case of J groups in the underlying KS that are connected in the way defined above for the case of a single pair of groups. The generalized result reads

$$R_{x,y}=M_x-\sum_j(O_{x,j}-O_{y,j}).\tag{B15}$$

These expressions imply that $\tilde{M}_x$ and $\tilde{N}_y$ in Eq. (B7) are related to the KS's topology by

$$\tilde{M}_x=M_x-\sum_j O_{x,j},\tag{B16}$$

and

$$\tilde{N}_y=\sum_j O_{y,j}.\tag{B17}$$

When $M_x>N_y$ and there are groups $\{Z_x\}$ and $\{Z_y\}$, with $Z_x<Z_y$ such that substates in $\{Z_y\}$ are connected only to substates in $\{Z_x\}$, we define $O_x=M_x-Z_x$ and $O_y=N_y-Z_y$, and Eq. (B14) holds. For J such groups, we define $O_{x,j}=M_x/J-Z_{x,j}$ and $O_{y,j}=N_y/J-Z_{y,j}$, and Eqs. (B15)–(B17) hold.

For a KS with symmetry, $\tilde{M}_x$ and $\tilde{N}_y$ are chosen in a different way than the one that relies on the on-off connectivity; for such a case, the choice that makes the number of additives in the external sums of Eq. (B7) minimal simply groups the identical PDFs. The topology of the RD form is determined by the largest $R_{x,y}$.

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[78] For a case with I pairs of special subgroups, a summation over these subgroups is performed; for example, say that $\{N_y\}$ is the smaller group and there are I subgroups in it such that each has the same properties defined for the case of single pair of subgroups. Then, $\{\tilde{N}_y\}=\{N_y\}-\Sigma_{i=1}^{I}\{l\}_i$ and $\{\tilde{M}_x\}=\Sigma_{i=1}^{I}\{s\}_i$. Note that although the same substate can appear in different subgroups $\{l\}_i$ or $\{s\}_i$, a substate can appear only once in $\{\tilde{M}_x\}$ and $\{\tilde{N}_y\}$, and this is the meaning of the summation.