

**Data-driven approach to decomposing complex enzyme kinetics with surrogate models**

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The temporal autocorrelation (AC) function associated with monitoring order parameters characterizing conformational fluctuations of an enzyme is analyzed using a collection of surrogate models. The surrogates considered are phenomenological stochastic differential equation (SDE) models. It is demonstrated how an ensemble of such surrogate models, each surrogate being calibrated from a single trajectory, indirectly contains information about unresolved conformational degrees of freedom. This ensemble can be used to construct complex temporal ACs associated with a “non-Markovian” process. The ensemble of surrogates approach allows researchers to consider models more flexible than a mixture of exponentials to describe relaxation times and at the same time gain physical information about the system. The relevance of this type of analysis to matching single-molecule experiments to computer simulations and how more complex stochastic processes can emerge from a mixture of simpler processes is also discussed. The ideas are illustrated on a toy SDE model and on molecular-dynamics simulations of the enzyme dihydrofolate reductase.

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**I. INTRODUCTION**

When enzymes and other proteins are probed at the single-molecule level, it has been observed in both experiments [1–3] and simulation studies [4–9] that conformational fluctuations at several disparate time scales have physically significant influence on both large-scale structure and biochemical function. In this paper, a method for using a collection of Markovian surrogate models [10–12] to predict kinetics that would often be considered non-Markovian is presented [13,14]. The ideas in Ref. [12] are extended to treat a system with more complex kinetics. The aim of the approach is to obtain a better quantitative understanding the factors contributing to complex time autocorrelations (ACs) associated with quantities modulated by slowly evolving conformational degrees of freedom. The focus is on systems where certain thermodynamically important conformational degrees of freedom evolve over an effective free-energy surface with relatively low barriers; this situation is often relevant to molecules undergoing a “population shift” or “selected-fit” mechanism [5,7] and the connection to “dynamic disorder” [1] is also discussed. The particular enzyme studied is dihydrofolate reductase (DHFR) because of its biological relevance to therapeutics and also due the complex kinetics associated with certain order parameters [7].

Surrogate models are used to describe the short-time dynamics. These surrogates are fairly simple phenomenological parametric stochastic differential equation (SDE) models. Specifically the Ornstein-Uhlenbeck (OU) process and an overdamped Langevin equation with a position-dependent diffusion function [10,12,15,16] are considered as the candidate surrogate models. Position-dependent diffusion is often observed when a few observables (or order parameters) are used to describe an underlying complex system such as a protein. Position-dependent noise models allow one to consider ACs having a different functional form than an expo-

ponential decay and it is demonstrated that this added flexibility can be of assistance in both understanding short and long time scale kinetics. Maximum-likelihood-type estimates utilizing transition densities, exact and approximate [17–19], are used to fit our surrogate SDE models. The fitting method does not require one to discretize [15] state space (the surrogates assume a continuum of states). The temporal AC is not used directly as a fitting criterion [14,20], but the surrogate models are able to accurately predict the AC after the model parameters are fit. Maximum-likelihood-based approaches employing accurate transition density approximations and a parametric structure poses several advantages in this type of application [21]. An accurate transition density of a parametric SDE facilitates goodness-of-fit tests appropriate for both stationary [22,23] and nonstationary time series [24]. The latter is particularly relevant to many systems (like the one considered here) where the diffusion coefficient is modulated by factors not directly monitored [12,13] and the hypothesis of a single one-dimensional surrogate model describing the modeled time series is questionable. Statistically testing the validity of various assumptions explicitly or implicitly behind a candidate surrogate model—such as Markovian dynamics, state-dependent noise, and/or regime switching—is helpful in both experimental and simulation data settings [25,26].

The type of modeling approach presented is attractive from a physical standpoint for a variety of reasons. The items that follow are discussed further in the Results and Discussion:

(i) In situations where the magnitude of the local fluctuations depend significantly on the instantaneous value of the order parameter monitored, simple exponential (or a finite mixture of exponentials [27]) can be inadequate to describe the relaxation and/or AC function [12]. The surrogates proposed can account for this situation when overdamped diffusion models can be used; additionally the estimated model parameters can be physically interpreted.

(ii) It has been observed that even in single-molecule trajectories that dynamic disorder can be observed due to ignoring certain conformational degrees of freedom [1,2,13]; the

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methods proposed here can be used to account for this type of variability and show promise for comparing frequently sampled single-molecule experimental time series to computer simulations where dynamic disorder is believed to be relevant.

(iii) Changes in conformational fluctuation magnitudes have been suggested to lead to physically interesting phenomena, so possessing a means for quantitatively describing an ensemble of dynamical responses can help one in better understanding the complex dynamics of enzymes, e.g., [4–6,9].

(iv) There is a general interest in showing how more complex stochastic processes arise from a collection of simpler parts [28–31]. It is discussed how, within a single trajectory, a continuous type of regime switching of Markovian surrogate models produces an AC that would often be considered non-Markovian.

The remainder of this paper is organized as follows. Section II reviews the background and presents the models considered. Section III introduces the modeling procedure used to approximate the AC function of a molecule experiencing multiple types of fluctuations. Section IV presents the Results and Discussion and Sec. V provides the Summary and Conclusions and this is followed by the Appendix.

## II. BACKGROUND AND METHODS

### A. Effective dynamics and statistical inference

The trajectory generated by a detailed molecular-dynamics (MD) simulation will be denoted by  $\{z_{ij}\}_{i=1}^N$ . The dynamics of the order parameter monitored is assumed to be complex (nonlinear, modulated by unobserved factors, etc.) even at the relatively short  $O(\text{ns})$  time intervals the order parameter time series is observed over. However, over short  $\approx 50\text{--}100$  ps time intervals a continuous SDE having the form

$$dz_t = \mu(z_t; \theta, \Gamma)dt + \sqrt{2}\sigma(z_t; \theta, \Gamma)dB_t \quad (1)$$

can often approximate the effective stochastic dynamics of the order parameter [10,12]. In the above  $\mu(\cdot)$  and  $\sigma^2(\cdot)$  are the nonlinear deterministic drift and diffusion functions (respectively) and  $B_t$  represents the standard Brownian motion [32]. The finite-dimensional parameter vector is denoted by  $\theta$  and  $\Gamma$  is used to represent unresolved lurking degrees of freedom that slowly modulate the dynamics [33].

The surrogate SDE models are formed by first dividing each trajectory into  $L$  temporal partitions. Each estimated parameter vector is denoted by  $\theta_\ell$  using the sequence  $\{z_{ij}\}_{i=T_{\ell-1}}^{T_\ell}$  and an assumed model, where  $\ell$  is an index of a partition,  $1 := T_0 < \dots < T_\ell < \dots < T_L := N$ , used to divide a time series into  $L$  disjoint local temporal windows. Within each of these windows, the data and the assumed model structure are used to compute  $\theta_\ell$  using maximum-likelihood-type methods (exact [19] and approximate [17] depending on the model). The parametric structures considered are presented in the next section. It is to be stressed that we estimate a collection of models,  $\{\theta_\ell\}_{\ell=1}^L$  for each trajectory. The differences in the estimated parameters are due in part to ran-

dom slowly evolving forces modulate the dynamics and also in part to unavoidable estimation uncertainty associated with a finite time series. It is demonstrated that a collection of surrogate model parameter vectors is needed to summarize conformational fluctuations inherent to many complex biomolecules. This procedure is repeated for each observed MD trajectory or time series.

The term ‘‘local diffusion coefficient’’  $\equiv \tilde{D}(z; \Gamma) := \sigma^2(z; \theta, \Gamma)$  is introduced in order to distinguish the coefficient in Eq. (1) from the diffusion coefficient usually implied in the physical sciences: the former is estimated from the observed data. The term ‘‘diffusion coefficient’’ used in statistical physics [34] is not necessarily the same as  $\tilde{D}(z; \Gamma)$ . If  $\Gamma$  does not modulate the dynamics, the two definitions are effectively identical. However, one theme of this paper is that some traditional dynamical summaries of statistical physics, such as diffusion coefficient and ensemble-based AC, can be modified or made less coarse by using a collection of surrogate models. Such a procedure may help in interpreting and understanding single-molecule time series.

### B. Candidate surrogate SDE models

Two local parametric SDE models are considered. MATLAB scripts illustrating how to obtain parameter estimates of both models from discretely observed data are available online [35]. The first is a linear constant additive noise process,

$$dz_t = B(A - z_t)dt + \sqrt{2}CdB_t. \quad (2)$$

The above SDE has a rich history in both the physical sciences [36] where it is usually referred to as the OU process and in econometrics where it is sometimes referred to as the Vasicek process [19]. The parameter vector to estimate in this model is  $\theta \equiv (A, B, C)$ . This model is appealing for a variety of reasons, one being that the exact transition density and maximum-likelihood parameter vector for a discretely sampled process [37] can be written in closed form, i.e., a numerical optimization is not needed to find the parameter vector because the parameter estimate can be written explicitly in terms of  $\theta$  and the observed data [19].

The second is a nonlinear position-dependent overdamped (PDOD) Langevin-type SDE [10,12,38],

$$dz_t = \beta[C + D(z_t - \psi_0)]^2[A + B(z_t - \psi_0)]dt + \sqrt{2}[C + D(z_t - \psi_0)]dB_t. \quad (3)$$

The variable  $\beta \equiv 1/(k_B T)$  is the inverse of the product of the Boltzmann constant and the system temperature.  $\psi_0$  represents a free parameter; in this paper it coincides with the umbrella sampling (US) point specified in the simulation. The parameter vector to estimate in this model is  $\theta \equiv (A, B, C, D)$ . Each parameter is estimated using the observed data and the transition density expansions [17] associated with Eq. (3) are used to construct a logarithmic likelihood cost function. A Nelder-Mead search is then used to find the  $\theta$  maximizing the associated cost function. The effective force in the above model is assumed to be linear in  $z$ , e.g.,  $F(z) := A + B(z - \psi_0)$  whereas the local diffusion coefficient function  $\equiv \tilde{D}(z; \Gamma) := [C + D(z - \psi_0)]^2$  is quadratic in  $z$ .

The overdamped appellation comes from multiplying the effective force by the effective friction (as determined by the Einstein relation [38]) corresponding to this diffusion function.

In this paper, all stochastic integrals used are Itô integrals. When a complex high-dimensional system with multiple time scales is approximated with a low-dimensional SDE possessing position-dependent noise the choice of the Itô or Stratonovich integral influences the interpretation of the drift function and the issue of which interpretation is “physically correct” is a nontrivial problem [39,40]. A related item is the so-called “noise-induced drift” [41,42]. Such a term is sometimes explicitly added to the drift [42]; one thermodynamic motivation for this is discussed further in Appendix Sec. 2.

An appealing feature of the data-driven modeling procedure presented here and elsewhere [10,12,25,33,43] is that various SDE models, of an explicitly specified form, can be considered, estimated, and tested using observed trajectories. Statistical hypothesis tests making use of the conditional distribution (not just moments) of the assumed surrogate model can then be used to test if the model assumptions are justified for the observed data. Tools from mathematical statistics [22,24] facilitate quantitatively and rigorously testing if certain features are required to adequately describe the stochastic dynamics. Many features, e.g., position-dependent noise, would be hard to statistically check using AC-based heuristic methods. Such heuristic checks are traditionally used in statistical physics, e.g., [40,44].

The data-driven models are used to approximate the stochastic evolution of black-box data and the estimated parameters do have a loose physical phenomenological interpretation. If one desires to compute unambiguous physical quantities from the estimated coefficients using a particular definition from statistical physics, the models can also be used to generate data for this purpose. For example, surrogate models can generate nonequilibrium (surrogate) work [10,11,33] and, under various assumptions, a well-defined thermodynamic potential of mean force (PMF) can be derived from such data [38,45]. This contrasts the case where one starts with a high-dimensional stochastic process (of known functional form) and then uses stochastic analysis to reduce the dimensionality of the system by first appealing to asymptotic arguments [39] and then possibly modifying the resulting equations to achieve a desired physical constraint [42]. In both analytical and data-driven cases, the goal is often to construct a single limiting low-dimensional evolution equation that can be used to predict statistical properties of the complex system valid over longer time scales [15,38–40]. It is not quantitatively clear at what time scale such an approximation (if any such a useful approximation exists at all) is valid over. Furthermore, it is usually difficult to determine if an equilibrium concept such as a PMF connects simply to trajectorywise kinetics in small complex systems experiencing fluctuations. Again, an appealing feature of the approach advocated here is that various statistical hypothesis tests [22–24] can be used to quantitatively assess the validity of proposed (reduced) evolution equations to see if physically convenient models are consistent with the observed data. For example, such tests can be used to determine the time one needs to wait before the inertia of the order parameter can be neglected [12].

Regarding the validity of using a *single* surrogate SDE to approximate “long-term”  $>O(\text{ns})$  trends, a main underlying theme of this paper and others [11,12,25,33] is that the presence of a lurking slowly evolving degree of freedom,  $\Gamma$ , can significantly complicate using a single equation and that methods for quantitatively accounting for this sort of variation are underdeveloped. Information in these types of models has proven useful in both theoretical chemistry computations [11,33] and in characterizing nanoscale experimental data [25,26,46]. Throughout this paper, it is shown how the *collection* of surrogate models can be linked with the ideas of dynamic disorder [1] to make quantitative statements about systems observed at the single-molecule level.

### III. METHOD FOR COMPUTING THE AC FUNCTION OF COMPLEX SYSTEMS

Before providing the algorithmic details of the method, the basic ideas and motivations behind the approach are sketched in words. It is assumed that a  $\Gamma$ -type coordinate slowly evolves (diffusively) over a relatively flat region of an effective free-energy surface. This evolution modulates the stochastic dynamics of the order parameter modeled, e.g., it changes the local diffusion coefficient function [12,13]. However, due to the almost continuous nature of the parameter change, a sudden or sharp change in the process dynamics is assumed difficult to detect in short segments of the time series (sudden regime changes or barrier crossings are not readily apparent in the data). Over longer time intervals, the changes become significant and the validity of a simple SDE model like the ones considered here to describe the global dynamics becomes suspect. However, if the evolution rules are updated as time progresses in the spirit of a dynamic disorder description [1], then there is hope for using a collection of these models to summarize the dynamics. Even if the data are truly stationary, some fluctuations due to a  $\Gamma$ -type coordinate may take a long time to be “forgotten” [2,14]. The idea proposed here is essentially to use the estimated model for a time commensurate with time interval length used for estimation and hypothesis testing and then suddenly switch model parameters. By doing this, one can take a collection of fairly simple stochastic models and construct another stochastic process possessing a more complex AC function.

One advantage of such a procedure is that an ensemble of elementary or phenomenological pieces can be constructed to gain a better understanding of how variation induced by slowly evolving fluctuations affects some system statistics and this information may help in better quantitatively understanding some recently proposed enzyme mechanisms [4–9]. This method is in line with the single-molecule philosophy that dynamical details should not be obscured by bulk averaging artifacts when possible. It is demonstrated how using a traditional AC summary of the data would obscure information of this sort on a toy example. Since the time scales at which simulations and single-molecule experiments span are rapidly converging, this type of dynamical summary can also be used to help in matching the kinetics of simulations and experiments and/or can be used to understand how more

complex dynamics emerge from simpler evolution rules [28–31].

Recall that for a single trajectory coming from a high-dimensional system, the time series data are divided into partitions and within each partition the parameters of both candidate models are estimated by methods discussed in the previous section. This results in a collection  $\{\theta_\ell\}_{\ell=1}^L$  for a each trajectory observed.

The algorithm goes as follows: The Euler-Maruyama [47] scheme is used here to simulate  $N_{MC}$  trajectories for each  $\theta \in \{\theta_\ell\}_{\ell=1}^L$ . The surrogate SDEs are recorded every  $\delta t$  and denote simulated order parameter time series by  $\{\{z_i^{s,(j)}\}_{i=T_{\ell-1}}^{T_\ell}\}_{\ell=1}^L$  with  $j=1, \dots, N_{MC}$ . To construct a new time series using the  $N_{MC}$  trajectories generated, set  $x^s = \{\}$ ,  $\ell = 1$  and for  $t=1$  to  $n \times L$  repeat the following:

- (1) Draw uniform integer  $u \in [1, N_{MC}]$ .
- (2) Set  $x^s = \{x^s, \{z_i^{s,(u)}\}_{i=T_{\ell-1}}^{T_\ell}\}$ .
- (3) Update counter  $\ell = \text{mod}(t, L) + 1$ .

The procedure described results in a new time series  $\{x_i^s\}_{i=1}^{N'}$ , where  $N' \equiv n \times N$ . Note that the time ordering of the original data is maintained and the last step forces the series  $\{x_i^s\}_{i=1}^{N'}$  to be periodic, so time lags  $> N\delta t$  cannot be resolved with this method. If the integer  $n > 1$ , the series  $\{x_i^s\}_{i=1}^{N'}$  contains more temporal samples than the original series. A larger sample size reduces the statistical uncertainty in an empirically determined AC. The issue of reducing uncertainty is subtle and is discussed in detail using the toy model presented in the next section. If the time ordering is believed to be irrelevant, the first step can be modified to drawing two random integers. The other random integer can be used to randomize the  $\ell$  index [48].

This procedure can then be repeated for each trajectory coming from a high-dimensional system. It is to be stressed that sudden and relatively infrequent regime switches (“barrier hopping”) cannot be described with this method. If the simulation or experiment is associated with a system possessing a jagged or rough free-energy surface with many small barriers and if a single trajectory can frequently sample the hops, then there is hope for using this method. However, note that the method is designed to treat relatively smooth regime changes (i.e., regime changes hard to identify by simple visual inspection). A discussion on how the surrogate models can be potentially used in more complex situations is briefly discussed later.

## IV. RESULTS AND DISCUSSION

### A. Toy model

In order to demonstrate the AC method on a simple example and illustrate some points in a controlled setting, we use the following SDE model:

$$dy_t^I = \kappa^0(\alpha^0 - y_t^I)dt + \eta^0 dB_t^1,$$

$$dy_t^{II} = \kappa^0(\alpha_t - y_t^{II})dt + \eta^0 dB_t^1,$$

$$dy_t^{III} = \kappa_t(\alpha_t - y_t^{III})dt + \eta_t dB_t^1,$$

$$d\alpha_t = 1/\tau_0(\alpha^0 - \alpha_t)dt + \sigma^\alpha dB_t^2,$$

$$d\kappa_t = 1/\tau_0(\kappa^0 - \kappa_t)dt + \sigma^\kappa dB_t^3,$$

$$d\eta_t = 1/\tau_0(\eta^0 - \eta_t)dt + \sigma^\eta dB_t^4, \quad (4)$$

where the constants  $\alpha^0, \kappa^0, \eta^0$  are meant to play the role of the surrogate parameters ( $A, B, C$ ) in the OU model. In the above expressions, superscripts are used simply to distinguish different constants or processes and do not represent exponentiation. Superscripts on the  $dB_t$  terms are used to distinguish separate independent standard Brownian motions. The roman numeral superscripts distinguish three cases: (I) the standard OU model, (II) an OU type model where the mean level  $\alpha$  evolves stochastically, and (III) an OU-type model where all parameters evolve stochastically. The parameter  $\tau_0$  dictates the time scale at which the OU parameters stochastically evolve. The evolution studied here is made to be slow relative to that dictated by  $\kappa^0$ . The (assumed unobserved) processes  $\alpha_t, \kappa_t, \eta_t$  are meant to mimic a dynamic-disorder-type [2] situation.

In addition, a fourth process referred to as “III (proxy)” will be evolved to demonstrate the AC method of Sec. III. This process is constructed by simply setting the parameters  $\alpha^0, \kappa^0, \eta^0$  equal to the corresponding parameters of process (III) at time  $t$  and then evolving this process like a standard OU model until the time index hits  $t+\mathcal{T}$  when the parameters are updated to those of process (III) at the same time. This procedure is then iterated. Randomizing  $\mathcal{T}$  had little influence on the accuracy here, but can be entertained. The processes above are simulated using the Euler-Maruyama scheme with time step size  $\delta s$  and the process is observed discretely every  $\delta t$  time unit. The remaining parameters are tuned to provide a parameter distribution consistent with those observed in some DHFR studies. These parameters are reported in the Appendix.

The toy model is used to investigate how variation induced by slowly evolving  $\Gamma$ -type factors influence the computed empirical AC on a controlled example where the assumptions behind the method introduced are satisfied. The features discussed are relevant to the DHFR system studied later and are also likely relevant to other single-molecules studies. The example is also used to highlight issues relevant to nonergodic sampling [13], i.e., when temporal averages are not equivalent to ensemble averages. In this type of situation, single-molecule data are particularly helpful. The use of the same Brownian motion term to drive three separate processes facilitates studying contributions to variance in these types of studies. In addition, the estimation is not carried out to keep the discussion simple and to remove an additional source of uncertainty.

The left panel of Fig. 1 plots the empirical AC computed by sampling four realizations from this process using 5000 observations uniformly spaced by  $\delta t=0.15$  ps. These time series lengths are commensurate with those used in typical MD applications [7,27,49]. One observes that the slowly evolving parameters do influence the AC measured. The fairly simple method of periodically updating the evolution parameters is able to mimic the AC associated with  $y^{III}$  for

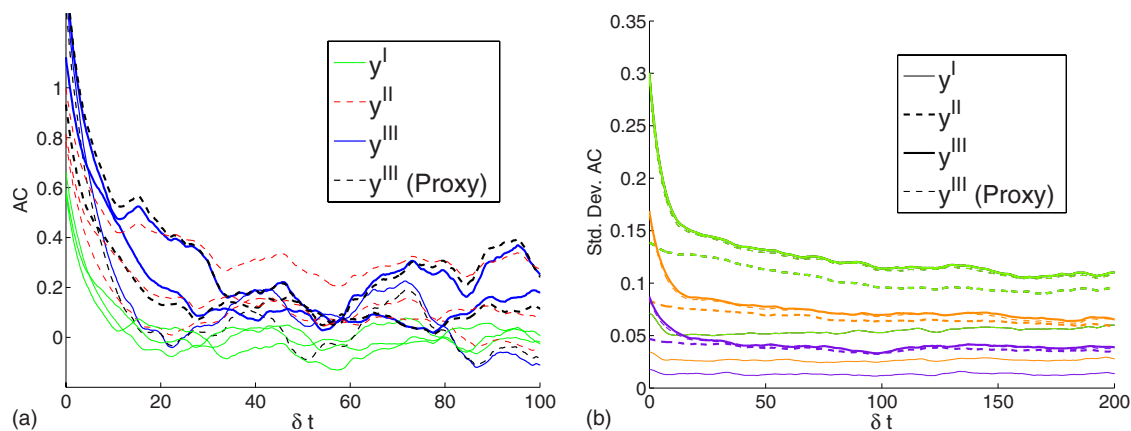


FIG. 1. (Color online) The (un-normalized) AC of four realizations, distinguished by different color shades in the plot, from four toy processes discussed in the text (left). The time lag  $\delta t$  reported is in ps. The standard deviation of the AC curves measured in a trajectorywise fashion from a population of 100 trajectories. The different line shades denote different temporal sample sizes (see text). The computed standard deviation reflects the variance in a population of 100 empirically determined ACs.

both short and long time scales. Furthermore, the variation induced by the relaxation and noise level (modulated by  $\kappa_i$  and  $\eta_i$ ) influences both the short- and longer-time responses. The stochastic response of a dynamic-disorder-type process is clearly richer than a single exponential. An advantage the surrogate approach offers over popular existing methods for treating this situation [27] is that other kinetic schemes, e.g., those associated with overdamped models with position-dependent diffusion, can be entertained. In enzymes associated with complex dynamics, other kinetic schemes may be needed to accurately reflect the stochastic dynamics of the order parameter monitored. For example, it is demonstrated in Fig. 3 that the PDOD surrogate is needed accurately captured relaxation kinetics even at short  $O(\text{ps})$  time scales. Over time scales relevant to experimentally accessible order parameters characterizing conformational fluctuations, one may need to account for dynamical responses more involved than a mixture of exponentials [12,14,50]. The procedure presented demonstrated how “elementary” pieces could be patched together to characterize relaxations and fluctuations occurring over longer time scales. This is attractive to both computer simulations and experimental data sets. In what follows the attention is shifted to focusing on limitations of using a single AC to describe single-molecule time series.

The right panel of Fig. 1 plots the standard deviation of the AC function associated with a trajectory population. For each observed trajectory, 100 different SDE trajectories were used to compute 100 empirical ACs from the time series associated with the trajectories. The pointwise standard deviation measured over the 100 ACs is plotted. The curve shadings distinguish different time series sample sizes. The three cases studied consisted of  $(0.5, 2, 8) \times 10^4$  discrete temporal observations; each time series was uniformly sampled with 0.15 ps between observations. Note that the influence of the evolving parameters on the measured AC is substantial. Recall that all  $y$  processes used common Brownian paths (so computer generated random numbers do not contribute to the differences observed). In addition, observe that the difference between  $y^{II}$  and  $y^{III}$  persists for a fairly long time and the length of time that this difference is measurably noticeable

depends on the temporal sample size used to compute the AC. In some applications, the variation induced by conformational fluctuations is important in computations [33] or to characterize a system [5,49]. The standard deviation in the measured AC here contains contributions coming from factors meant to mimic the influence unresolved conformational fluctuations whose influence persists for a fairly long time. In the AC computed with longer time series, i.e., spanning a larger time since the time between observations is fixed, the process has more time to “mix” and hence the difference between temporal and ensemble averages is reduced. Said differently, the influence of the initial conformation, or “memory,” diminishes. By using a single long-time series trajectory and only reporting one AC computed from this mixed series, these types of physically relevant fluctuations can get washed out by using a single AC function. This goes against the spirit of single-molecule experiments.

The example considered here is admittedly simple and was constructed to illustrate the types of assumptions behind the method introduced. If the dynamic disorder is induced by large kinetic barriers then one would need to construct more sophisticated processes for determining how and when the parameters regime switch. Combining the surrogate models with efforts along these lines, e.g., [51], may be able to help these more exotic situations. Exploring the various routes by which complex and/or heavy tailed ACs [2,14] can emerge from simpler dynamical rules can help in a fundamental understanding of the governing physics [28–31]. However, if the ensemble average decay rate is deemed as the only quantity of physical relevance then the collection of surrogate models can still potentially be used to help in roughly predicting the rate of decay of more complex ACs. This is particularly relevant to simulations where obtaining long enough trajectories to reliably calibrate models possessing complex AC exhibiting long-range dependence from observed data is problematic [12,14,52]. Even in cases where one only requires a coarse time decay of an ensemble of conformations for a physical computation [53] and can simulate for a long enough time to directly monitor kinetics, an understanding of the distribution of surrogate models esti-

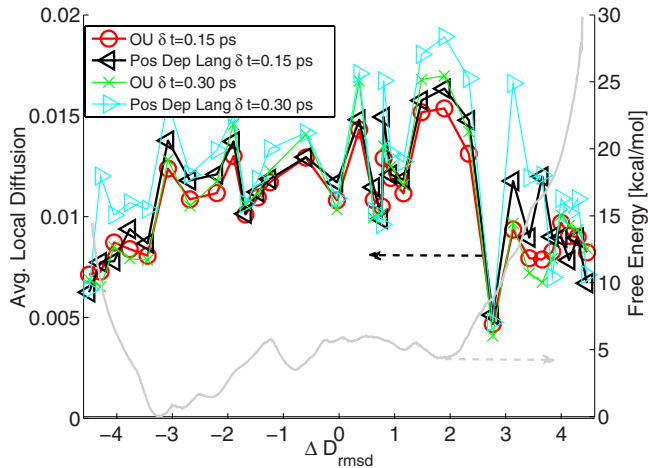


FIG. 2. (Color online) Average local diffusion function estimated (left axis) and free energy (right axis) as a function of order parameter. The population average of the estimated  $C^2$  ( $\text{\AA}^2/\text{ps}$ ) is plotted to give a feel for the position dependence of this quantity; it is stressed that the average alone is not adequate to describe the dynamics here due to a type of dynamic disorder phenomena [1]. The free energy (kcal/mol) was computed by Arora and Brooks III using methods described in Ref. [7].

ated will likely be of help in linking computer simulation force fields to single-molecule experimental time series. The remaining results use simulations of DHFR to illustrate some of these points.

## B. DHFR

### 1. DHFR simulation details

The detailed computational details are reported in Ref. [7]. Briefly, an order parameter denoted by  $\Delta D_{\text{rmsd}}$  was defined using the root-mean-square distance between two crystal structures [7]. This order parameter provides an indication of the proximity to the “closed” and “occluded” enzyme state and is reported in units of  $\text{\AA}$  throughout. The initial path between the closed and occluded conformations of DHFR was generated using the nudged elastic band (NEB) method [54]. Subsequently,  $\approx 50$  configurations obtained from NEB path optimization were subjected to US simulations. During these US simulations, production runs of 1.2 ps at 300 K were performed after equilibration using a weak harmonic restraint.

### 2. DHFR results

Figure 2 plots the average local diffusion coefficient of the surrogate SDE models using two different observation frequencies on the left axis, and on the right axis the free energy computed in Ref. [7] is plotted. Each surrogate model was estimated using 400 time series observations with either  $\delta=0.15$  or  $0.30$  ps separating adjacent observations corresponding to  $L=20$  or  $10$  (respectively). The average local diffusion coefficient demonstrates a relatively smooth increasing trend for a majority of the order parameter values explored, but then suddenly changes abruptly around  $\Delta D_{\text{rmsd}} \approx 3 \text{ \AA}$ . It has been observed that an interesting inter-

play between free energy, fluctuations, and stiffness exists in some enzyme systems [4–6,9] and this plot suggests that future works investigating some of the finer structural factors leading to this change may be worthwhile, although this direction is left to future work because it is outside the scope of this study.

It is to be stressed that the mean of each US window is not adequate to summarize the dynamics. That is, a single fixed parameter surrogate SDE like the ones considered here cannot mimic the longer-time statistics of the process. This is why the AC procedure introduced in Sec. III is needed. Figure 3 demonstrates that the individual PDOD models do capture simpler features that surrogates cannot. This is due partly to the position dependence of the local diffusion coefficient. The PDOD surrogate model combined with the procedure of Sec. III can accurately summarize the longer-time dynamics. These points are explained further in the discussion associated with Figs. 3–5.

The ability of the PDOD model to capture features that a single exponential (e.g., the AC associated with an OU process) cannot is demonstrated in Fig. 3. Results from four different US points, each possessing different degrees of position dependence on the noise, are shown. Here, the results obtained using both the OU and PDOD surrogates calibrated using  $\delta t=0.15$  ps with 400 temporal observations and the corresponding AC predictions are shown in the plot. The empirical ACs computed using the short segments of MD data used for surrogate model parameter estimation are also reported. Results with 400 blocks possessing observations spaced by  $\delta t=0.30$  ps were similar in their AC prediction, but hypothesis tests strongly rejected the assumption of a constant local diffusion function (see Fig. 5). The 400  $\delta t=0.15$  ps samples allowed the OU model structure to provide a better fit (as measured by the fraction rejected compared to the OU model calibrated using  $\delta t=0.30$  ps data) because the local diffusion function had less time to evolve or change value. For cases where the position dependence is moderate, the PDOD and OU surrogate models predict qualitatively similar AC functions. However, the PDOD model captures the short-time relaxation dynamics better than the OU for cases where the position dependence of the local diffusion is more substantial and hence for clarity we focus on the PDOD models in the remaining kinetic studies.

Figure 4 plots the empirically determined AC obtained from different MD production simulation data. The case labeled “in-sample” was the one used for the estimation of the local models reported in Fig. 3 and that labeled “out-of-sample” was computed by running a longer 3.6 ns simulation and computing the AC from the last 1.2 ns of this time series. The PDOD version of these models was used along with the procedure outlined in Sec. III using blocks of size 800 and randomizing the time index. The 400 block results were similar. Respecting the time ordering of the surrogate models only improved results marginally. Note also that the general trends of the long-time decay of the MD data are captured with the procedure and that there is a substantial difference between the in-sample and out-of-sample MD trajectories [55]. The physical relevance of such a variation was previously discussed and will be expanded on when results of stationary DHFR density prediction are shown. The primary

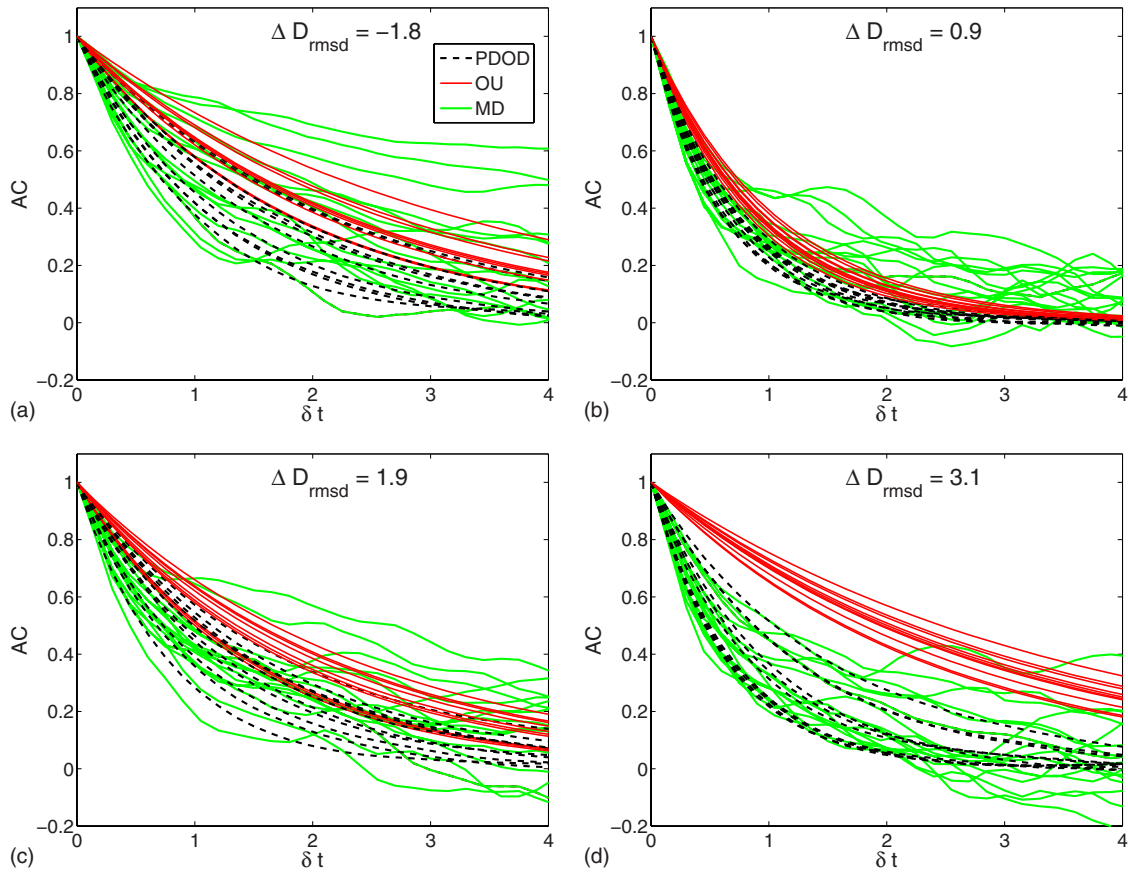


FIG. 3. (Color online) Normalized local AC function of MD data (more jagged curves) and that predicted by surrogates. The total time series of length 1.2 ns was divided into separate blocks, each containing 400 observations spaced by 0.15 ps (the time lag  $\delta t$  is reported in ps). These data were used to estimate a collection of surrogate models and a collection of MD ACs corresponding to US target points of  $\Delta D_{\text{rmsd}} \approx -1.8, 0.9, 1.9,$  and  $3.1$  Å. The AC corresponding to the estimate surrogate models is also plotted where the initial lag is normalized to unity to facilitate comparison (the raw units are shown in Fig. 4).

observation is that a collection of PDOD surrogate models was able to capture the basic relaxation trends of the enzyme that a single surrogate could not. Recall that even at short time scales a single exponential decay was inadequate to fit the data. Similar trends were observed for all 51 US windows explored. However, it is to be stressed that the procedure shown here is to *decompose* kinetics in the longest contiguous block of discrete time series observed. If complex dynamics occur over longer time scales and data are not available that directly sample these scales, then the method cannot be used to predict the long-time behavior that was not sampled.

The goodness-of-fit of the surrogate models using the two candidate SDEs is shown in Fig. 5 for various US windows. The median of the  $Q$ -test statistic introduced in [24] is reported. This test statistic under the null is asymptotically normally distributed with mean zero and unit variance, but has also been proven to be useful in small samples [12,24,33,43]. Recall that each MD time series (at each umbrella sampling window) was divided into small pieces. In the portions near the edges (larger  $|\Delta D_{\text{rmsd}}|$  values), where the position dependence of the noise is greatest, one observes that the OU model population has a median that would typically indicate a collection of poor dynamical models. If conformational fluctuations slowly modulate the dynamics, the

longer the time series one has, the likelihood of departing from any simple surrogate model increases [56]. Goodness-of-fit tests, like the ones presented here, can be used to quantitatively approximate when simple models begin departing from various assumptions.

The stationary density predicted by the surrogate OU models in a case where position dependence was shown to be marginal for the time interval data that were monitored is plotted in Fig. 6. Here, the mixture method discussed in Ref. [12] is reported due to its relevance to a collection of surrogates and dynamic disorder. The histogram of the 1.2 ns MD data is also plotted as well as the stationary density predicted by the average of the surrogate models taken at the US window near  $\Delta D_{\text{rmsd}} \approx 2$ . Using a single model obtained by aggregating all time series together in hope of reducing surrogate parameter uncertainty (i.e., the time series sample size increases) gives poor results relative to the MD data and mixture method. The mixture of OU models was calibrated using four sets of noncontiguously spaced 60 ps data (i.e., 400 entries spaced by 0.15 ps) were sampled every 300 ps from the MD process and this was used to compute four surrogate OU model parameters. The goodness-of-fit tests indicated that the local surrogates given the data were reasonable dynamical models. So portions where the “local equilibrium” density, i.e., the stationary density predicted by

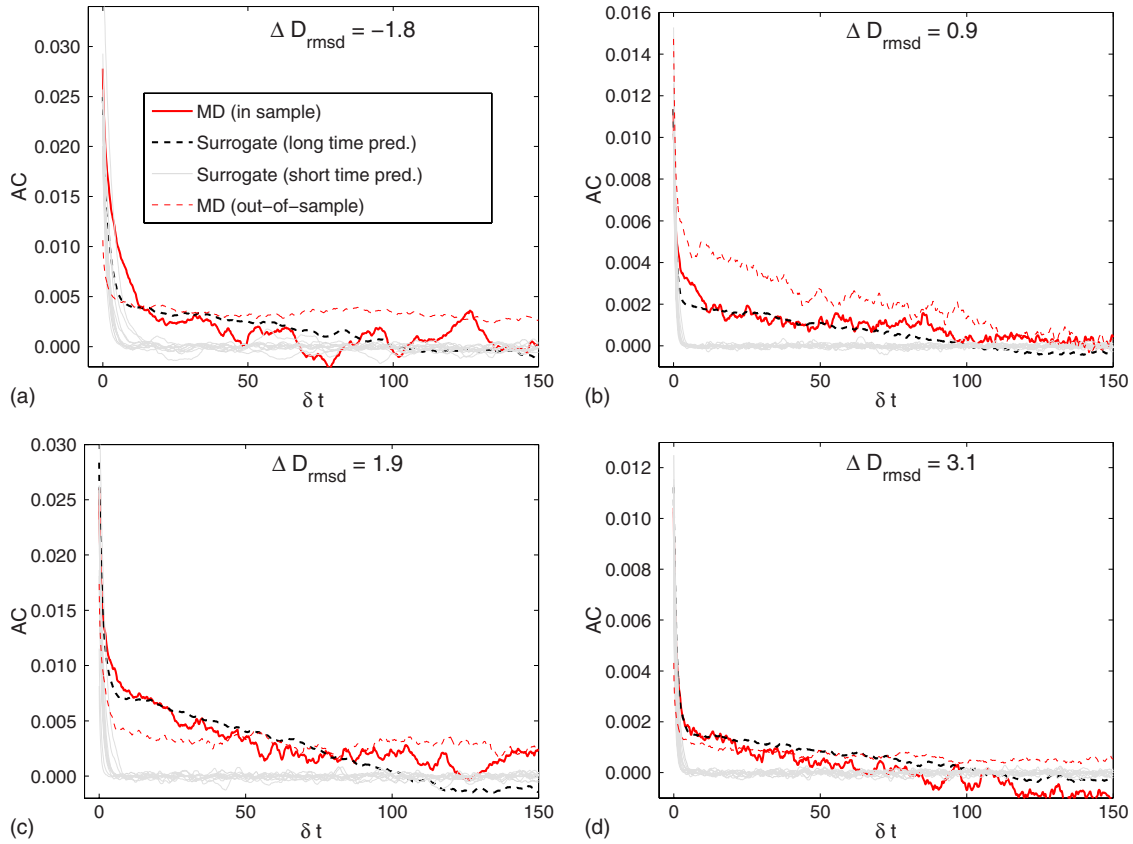


FIG. 4. (Color online) The global (un-normalized) AC prediction. The procedure introduced here is used along with PDOD data to predict the long-time AC (the time lag  $\delta t$  is reported in ps). The relaxation predicted by the individual surrogate (data shown in Fig. 3) are also shown to stress that the SDE parameters are not fixed, but evolving and any single surrogate cannot capture the richer long-time dynamics.

a surrogate with estimated parameters, possessing significant probability mass can be thought of as regions of phase space sampled due to fast-scale motion for a relatively fixed (and unobserved) value of  $\Gamma$  [12,25]. If variation in the conformational coordinate is important to thermodynamic averages, as the data here suggest to be the case in DHFR, then one needs to use a collection of local equilibrium densities [12]. The advantage of such an approach is that short bursts of simu-

lations started from different initial conditions can be run, then surrogate models can be calibrated and tested. If the surrogate is found suitable, it can then be used to make predictions on the local equilibrium density, and the variation in the local equilibrium densities can be used to partially quantify the degree to which a slow conformational degree of

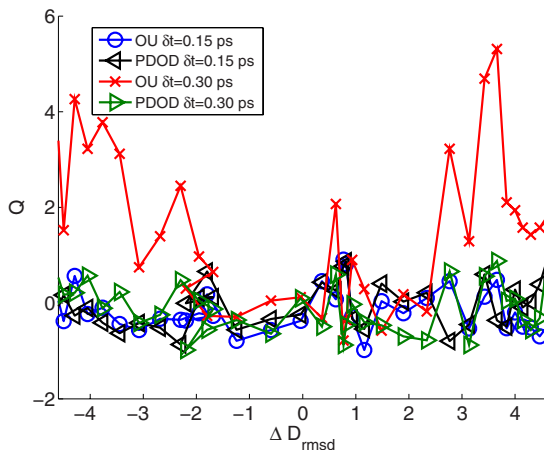


FIG. 5. (Color online) Goodness-of-fit tests. The test of Ref. [24] was computed given the parameter estimate and the observed data. The median of each umbrella sampling window is reported.

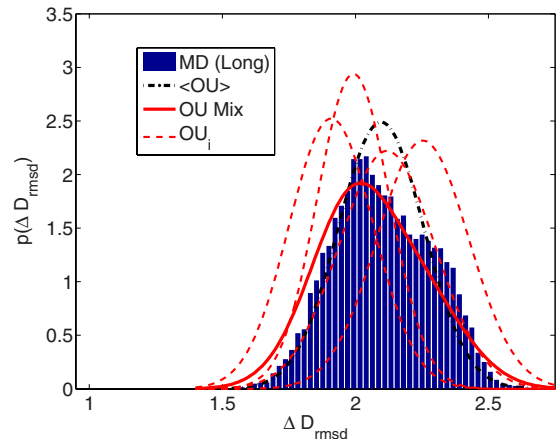


FIG. 6. (Color online) Stationary density/histogram prediction. The bars denote 1.2 ns MD data, the solid thick line denotes the mixture PDOD method (see text), the solid dotted line denotes the average PDOD model, and the thin lines denote the four local equilibrium densities used in constructing the mixture density.



freedom modulates the dynamics. This treatment is appealing when data on other physically relevant order parameters are either unknown or not easy to measure.

## V. SUMMARY AND CONCLUSIONS

Single-molecule experiments and simulations offer the potential for a detailed fundamental understanding of complex biomolecules without artifacts of bulk measurements obscuring the results. However, one must deal with complex multiscale fluctuations at this level of resolution and the factors contributing to the noise often contain physically relevant information such as quantitative information about conformational degrees of freedom [25]. The abundance of data available to researchers and recent advances in computational and statistical methods are allowing researchers to entertain new methods of summarizing information relevant to modeling systems at the nanoscale [25,33,46].

By applying surrogate models to the data coming from biased MD simulations of DHFR, it was demonstrated that a collection of stochastic dynamical models can be used to better understand the factors contributing to the shape of the autocorrelation function associated with fluctuations coming from multiple time scales. The surrogate models were estimated by appealing to maximum-likelihood-type methods [17–19] and were checked using goodness-of-fit tests, which utilized the transition density of the assumed surrogate. The tests used were appropriate for time correlated data. The time series data were not assumed to be stationary; the stationarity assumption is often suspect in simulation data. The tests used [24] indicated that taking the position dependence of the noise into account was required to provide a statistically acceptable model in many regions of phase space explored. For short time scales, the individual surrogate models (taking position dependence noise into account) were capable of predicting quantities outside the fitting criterion, e.g., a parametric likelihood function was fit but the models were able to predict short time scale autocorrelation functions and these physically based models were able to fairly accurately model relaxation kinetics that a simple exponential relaxation could not. Other enzyme systems have exhibited this type of behavior [12] and it is likely that future single-molecule experiments will yield data possessing this feature.

Perhaps more importantly, we demonstrated that a population of surrogate models was required to represent the complex dynamical system because an unobserved conformational degree freedom modulated the dynamical response and this feature had to be accounted for in order to predict autocorrelations valid for longer temporal trajectories. A method using parametric surrogate models calibrated over short time scales while at the same time respecting the variability induced by unresolved coordinates evolving over longer time scale was presented. The DHFR system was another instance where aggregating a collection of simpler dynamical models gave rise to a more complex stochastic process [12,28–31]. The basic idea is applicable to situations where a hidden slowly evolving degree of freedom modulates the dynamics and this coordinate evolves on an effective free-energy surface possessing relatively low barriers [12]. Issues associated with extensions were briefly discussed.

Even if a coarse system description, such as a single autocorrelation function, can be used to adequately approximate the physically relevant statistical properties of all experimentally accessible observables, the approach presented still has appeal. One circumstance where this is particularly relevant is when computer simulation trajectories are compared to frequently sampled experimental single-molecule time series [25]. In experimental time series, many conformational coordinates cannot typically be resolved [12,25], so constructing a simulation that matches all relevant degrees of freedom is highly problematic. Quantitative knowledge of how the variability induced by such hidden degrees of freedom is reflected in the surrogate model parameter distribution may help in refining force fields to match kinetic properties at multiple time scales. If the force fields are believed to be valid, then turning to the simulations for details of the structural dynamics can help us in understanding complex molecular machines [57]. This type of extra detail may also assist (or lead to new) methods for computing transition rates [58]. Furthermore, as nanotechnology demands higher resolutions at smaller length and time scales, one may want to avoid using a single autocorrelation function constructed by aggregating many mesoscopic or microscopic states, each possessing different dynamical features, because doing so may unnecessarily wash out physically relevant information. The phenomenologically motivated simple bottom-up strategy presented was one contribution in this direction.

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## APPENDIX

### 1. Toy model parameters

Here,  $\delta t = 0.15$ ,  $\delta s = \delta t / 50$ ,  $T = 200 \delta t$ ,  $\tau_0 = 120$ ,  $(\alpha^0, \kappa^0, \eta^0) = (4, 0.2, 0.5)$ ,  $(\sigma^\alpha, \sigma^\kappa, \sigma^\eta) = (6.5 \times 10^{-2}, 6.5 \times 10^{-3}, 1.9 \times 10^{-2})$ . The last set of parameters were selected to give the evolving OU parameters a stationary distribution characterized by three independent normals each having mean  $(\alpha^0, \kappa^0, \eta^0)$  and standard deviation  $(1/2, 1/20, 3/20)$ . The initial condition of each  $y$  process was set to  $\alpha^0$  and the OU parameters were all set to  $(\alpha^0, \kappa^0, \eta^0)$ . 100 batches of four independent Brownian motion processes were used to evolve the system.

### 2. Predicting quantities with surrogate models

The OU process is attractive for a variety of reasons. The conditional and stationary densities are both known analytically and it can be readily estimated from discrete data. Another appealing feature is that the AC function, denote this function by  $AC(t)$  [27], associated with a stationary process can readily be computed after parameter estimates are in hand, namely,  $AC(t) = \exp -Bt$ ; recall that the drift of the OU process is given by  $B(A - z)$ .

Unfortunately, these types of statistical summaries are more difficult to obtain with other SDEs. The position de-

pendence of the diffusion function and nonlinear models severely complicate obtaining analytical expressions for the autocorrelation function. Note that, once a single SDE model is estimated, a new large collection of sample paths can be simulated and quantities like the autocorrelation function associated with a given SDE model and  $\theta$  can be empirically determined (the computational cost of simulating a scalar SDE is typically marginal in relation to a MD simulation). This can be repeated for each surrogate SDE estimated from each MD path.

A stationary density, under mild regularity conditions, of a scalar SDE can often be expressed in closed form using only information contained in the estimated SDE coefficient functions via the relation [41,59]

$$p^{SD}(z;\Gamma) = \mathcal{Z}/\sigma(z)^2 \exp\left(\int_{z_{\text{REF}}}^z \mu(z')/\sigma(z')^2 dz'\right), \quad (\text{A1})$$

where in the above the SDE functions' dependence on  $\theta$  and  $\Gamma$  has been suppressed to streamline the notation.  $\mathcal{Z}$  represents a constant to ensure that the density integrates to unity and  $z_{\text{REF}}$  represents an arbitrary fixed reference point. When evaluating  $p^{SD}(\cdot)$ , one can encounter technical difficulties if the diffusion coefficient is allowed to take a zero or negative value (this is relevant to the PDOD model). Some heuristic computational approaches to dealing with this are discussed in Refs. [12,43].

Sometimes a thermodynamic motivation exists for expressing the stationary density of the high-dimensional molecular system in terms of some potential, denoted here by  $V(z)$ , which does not explicitly depend on the diffusion function [40,41]. In time-homogeneous scalar overdamped Brownian dynamics, where the forces of interest acting on  $z$  are believed to be related to the gradient of  $V(z)$ , a noise-induced drift term [41] can be added to the drift function and

this addition cancels out the contribution coming from the  $1/\sigma(z)^2$  term outside the exponential. The stationary density of the modified SDE can then be expressed as being proportional to  $\exp[-\beta V(z)]$  in such a situation. This type of modification has a thermodynamic appeal when  $z$  is the only important variable of the system and the fast-scale noise has been appropriately dealt with [39]. The utility of such an approach in describing the *pathwise* kinetics of trajectories is another issue and single-molecule studies are one area where the distinction may be important (one may not care as much about the stationary *ensemble* distribution).

However, when there are slowly evolving lurking variables like  $\Gamma$  modulating the dynamics (as is the case in many biomolecular systems), using simple expression like Eq. (A1) to approximate the stationary density of the high-dimensional system (with or without noise-induced drift corrections) is highly problematic. Note that the  $\Gamma$  variable has been retained in the left-hand side of Eq. (A1); the stationary density estimate is only meant to be valid for a fixed estimated SDE surrogate corresponding to one value  $\theta$ . In this paper and others, it is assumed that for a short time interval both  $\theta$  and  $\Gamma$  are effectively frozen. Given a model and short-time data, this can be tested using goodness-of-fit tests. However, over longer time scales,  $\Gamma$  evolves and modulates the dynamics so the estimated  $\theta$  evolves in time (this is why the situation can be thought of as a type of dynamic disorder [1]). For this long-time evolution, it is assumed that the form of a stochastic process depending only on  $z$  is completely unknown to the researcher. Furthermore, it was assumed that another order parameter (i.e., system observable) is unavailable or is unknown [12,25,50]. Hence, to approximate the stationary distribution of the high-dimensional molecular system, one would require a collection of  $p^{SD}$ 's (each with different  $\Gamma$ 's) to approximate this quantity. One procedure accomplishing this is presented in Ref. [12].

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