Mini review

STATISTICAL PROPERTIES OF THE DICHOTOMOUS NOISE GENERATED IN BIOCHEMICAL PROCESSES [#]

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Abstract: Dichotomous noise detected with the help of various single-molecule techniques convincingly reveals the actual occurrence of a multitude of conformational substates composing the native state of proteins. The nature of the stochastic dynamics of transitions between these substates is determined by the particular statistical properties of the noise observed. These involve non-exponential and possibly oscillatory time decay of the second order autocorrelation function, its relation to the third order autocorrelation function, and a relationship to dwell-time distribution densities and their correlations. Processes gated by specific conformational substates are distinguished from those with fluctuating barriers. This study throws light on the intriguing matter of the possibility of multiple stepping of the myosin motor along the actin filament per ATP molecule hydrolyzed.

Key words: Single-molecule techniques, Time autocorrelation functions, Dwell-time distribution densities, Molecular gear

INTRODUCTION

As recently as ten years ago, the picture of a protein molecule in its native state displaying a rich stochastic dynamics of conformational transitions [1] was unacceptable to the majority of molecular biophysicists, who identified the protein tertiary structure simply with a single conformational substate. This

situation has dramatically changed, and now the view that, under the appropriate environmental conditions, we have to do with a native state ensemble rather than a single native state has become commonly accepted [2]. Single-molecule techniques played a considerable role in changing this point of view, in particular the patch-clamp technique [3, 4], single fluorophore detection using confocal or total internal reflection microscopy [5-7], and motility assays of molecular motors using optical tweezers [8]. The goal of this paper is to consider in some detail the statistical properties of the dichotomous noise observed with the help of these techniques.

THE STOCHASTIC THEORY OF RATE PROCESSES

The time behaviour of molecular systems with purely stochastic internal dynamics is described adequately by the stochastic theory of rate processes [9]. To introduce its main concepts, let us start with the simple picture shown in Fig. 1. A protein macromolecule fluctuates between many conformational substates divided between two experimentally distinguishable states, R and P. These can be the 'closed' and 'open' states of a protein channel that conducts an ionic current through a membrane; the 'off' and 'on' states of a fluorophore bound to the protein, the transitions between which are observed as blinks in the fluorescence microscopy; or the states of a molecular motor attached to and detached from its track. The change of state occurs through transitions between the distinguished substates in R and the distinguished substates in P, jointly forming what is called the transition state of the process.
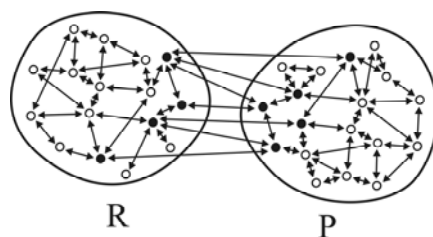

Fig. 1. A schematic partition of the set of conformational substates (white and black circles) of the protein molecule into two subsets corresponding to different experimentally distinguishable species, R and P. The arrows denote purely stochastic transitions between the substates. The substates represented by the black circles form the transition state of the process. In fact, a much larger number of substates is expected.

The stochastic dynamics is determined by a system of master equations

$$\dot{p}_l(t) = \sum_{l'} \left[w_{ll'} p_{l'}(t) - w_{l'l} p_l(t) \right] \tag{1}$$

where $p_l(t)$ denotes the probability of the molecule being in the substate l at time t, $w_{ll'}$ is the transition probability per unit time from the substate l' to l,

and the dot is the time derivative. In the appropriate linear combination of probabilities

$$X_k(t) = \sum_l X_{kl} p_l(t),$$ (2)

the system of linear equations (1) is decoupled into the system of independent linear equations

$$\dot{X}_k(t) = -\tau_k^{-1} X_k(t).$$ (3)

The normal modes of relaxation X_k can be interpreted as the mean values of some dynamic variables X_k determined for substates labelled with the index l. If the transition probabilities satisfy the detailed balance condition, the coefficients τ_k^{-1} are real and positive, and have the meaning of reciprocal relaxation times. Hence, the system of equations (1) determines a specific spectrum of reciprocal relaxation times. If the process represents a single reaction, there is a gap in the spectrum between the reciprocals of the longest and the next shorter relaxation times, τ_1 and τ_2 (Fig. 2). This corresponds to a time-scale separation: the partial equilibrium between the substates within states R and P is reached much faster than the complete equilibrium between the distinguished states. The ground value 0 of the spectrum (the infinite relaxation time) is related to the sum of all probabilities, which remains constant during the evolution.

If the detailed balance condition is broken, i.e. if our system trends towards the steady state rather than equilibrium, some coefficients τ_k^{-1} can be complex, which corresponds to the presence of damped oscillations forerunning one or more cycles of probability fluxes.
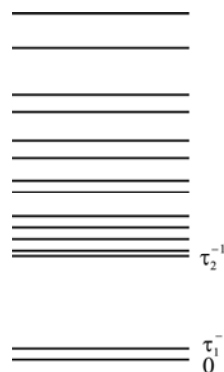

Fig. 2. A schematic spectrum of reciprocal relaxation times characterizing the stochastic dynamics of the molecular system under consideration.

The dynamic variable that describes the transitions between states R and P is a characteristic function of, e.g. the state P:

$$\mathcal{P}_l = \begin{cases} 0 & \text{if } l \in R \\ 1 & \text{if } l \in P. \end{cases} \tag{4}$$

The variable $\mathcal{P}$ changes its value during the stochastic evolution of the system in time. A schematic realization (a 'trajectory') of the stochastic process $\mathcal{P}(t)$ is shown in Fig. 3.
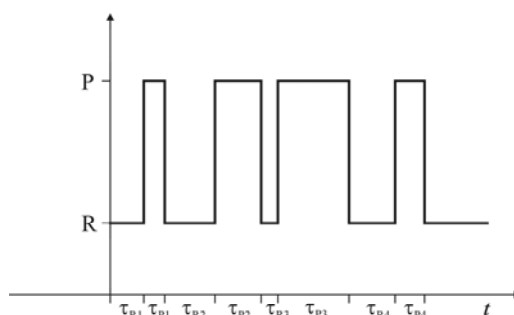

Fig. 3. Schematic 'telegraphic noise' recorded for a single molecule experiencing transitions between the two states R and P. Successive dwell times in R and P are denoted.

Statistically, the process $\mathcal{P}(t)$ can be characterized in two ways. The first is the non-equilibrium ensemble average:

$$P(t) = \sum_l p_l(t)\mathcal{P}_l . \tag{5}$$

The quantity P has the meaning of the fraction of the molecules in state P. The time course $P(t)$ depends on the preparation of the initial state of the ensemble, determined by the probabilities $p_l(0)$. In the case of time-scale separation (Fig. 2), the asymptotic long-time behaviour of $P(t)$ is exponential and determined by the standard kinetic equation

$$\dot{P} = -\tau_1^{-1}(P - P^{\text{eq}}), \tag{6}$$

where τ_1 is the longest relaxation time and P^{eq} is the equilibrium (or stationary) molar fraction of the molecules in state P.

The short-time behaviour of $P(t)$ depends on shorter relaxation times. Even if the initial state is the partial equilibrium state (separately in R and P), some non-exponential initial stage of the reaction should occur, because the very reaction initially disturbs the partial equilibrium. However, the more the initial state differs from the partial equilibrium state, the stronger the effect. In the historic

experiment performed in 1975 by Frauenfelder *et al.* [10], an ensemble of CO-bound myoglobin molecules in the transition state were prepared by breaking the heme-CO bond in a non-thermal way using a laser flash, and the process of CO rebinding to the heme in various conditions after photolysis was observed (Fig. 4). At 300 K, only the bimolecular reaction of binding from the solution was observed, with its usual exponential time course. The essential novelty of the experiment was to study the process at low non-physiological temperatures. In such conditions, what is observed reveals the clearly non-exponential time course that can be ascribed to the initial stage of the unimolecular reaction of CO binding from the protein matrix.
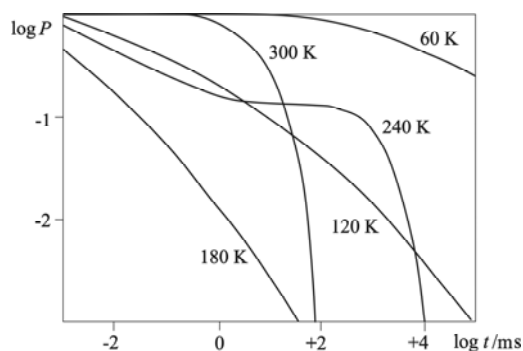

Fig. 4. A sketch of the time dependence of the rebinding of CO molecules after the photodissociation of CO-bound sperm whale myoglobin at various temperatures. After Frauenfelder *et al.* [10].

We already mentioned that for enzymatic reactions proceeding in an open system, some of the parameters τ_k are complex, which corresponds to the presence of oscillations. Such oscillations can actually be observed in the transient stage of the reaction before $P(t)$ reaches its steady-state value, provided that the behaviour of all the molecules forming the ensemble is synchronized. This is possible when the reaction takes place in a small volume enabling the substrate diffusion to occur rapidly enough to start the cycles of all the enzymes from the same substate [11].

TIME AUTOCORRELATION FUNCTIONS AND DWELL-TIME DISTRIBUTION DENSITIES

The second way to statistically characterize the process under discussion is to consider the equilibrium or the steady-state averages, the latter in the case of an open system. This approach applies well to single-molecule experiments because, following ergodicity, the equilibrium or stationary averages can be replaced by the time average. Besides the equilibrium or stationary average of

the process $\mathcal{P}(t)$, which is constant in time and equals the equilibrium or the stationary value of P,

$$C_1 = \langle \mathcal{P} \rangle \equiv P^{\text{eq}}, \tag{7}$$

one also considers the equilibrium or stationary autocorrelation functions of the second order:

$$C_2(t) = \langle \mathcal{P}(t)\mathcal{P} \rangle; \tag{8}$$

or the third order:

$$C_3(t,t') = \langle \mathcal{P}(t+t')\mathcal{P}(t)\mathcal{P} \rangle. \tag{9}$$

Following Onsager's fluctuation regression theorem, the dynamics of the non-equilibrium ensemble average $P(t)$ is identical to that of the equilibrium ensemble average $C_2(t)$, but only if the initial state of the former is the partial equilibrium state [12]. For Markovian two-state processes, the second order autocorrelation function $C_2(t)$ is a single exponential. A non-exponential time dependence indicates that the process is non-Markovian and indicates the presence of substates and the corresponding transition dynamics (Fig. 5).
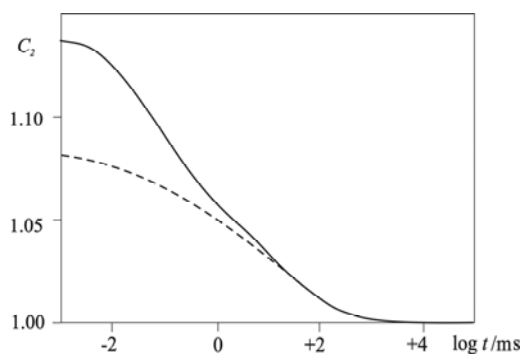

Fig. 5. The sketched time dependence of the correlation function $C_2(t)$ of the fluorescence signal emitted by a single horseradish peroxidase molecule immobilized in the light cavity of a confocal microscope. The short-time course does not follow the curve (dashed line) that fits the long-time exponential behaviour. After Edman *et al.* [6].

A measure of the non-Markovianity is a deviation from the Smoluchowski-Chapman-Kolmogorov equation for transition probabilities in the process $\mathcal{P}(t)$ [4], which in terms of the time autocorrelation functions (7-9) can be characterized by a non-Markovian or 'memory landscape' function [11, 13, 14]:

$$\phi(t,t') = C_3(t,t')/C_2(t) - C_2(t')/C_1. \tag{10}$$

For transient processes in open systems, the function $\phi(t,t')$ is periodic, and the function $C_2(t)$ can but does not have to be periodic. Determining both functions from the experiment enables the establishment of some general features of the substate dynamics [13]. However, for a more detailed characterization of this dynamics, more information is needed.

The process $\mathcal{P}(t)$ defines the three stochastic chains:

- $\mathcal{T}_R(n)$, the dwell time in R during an event n;
- $\mathcal{T}_P(n)$, the dwell time in P during an event n;
- $\mathcal{T}_c(n)$, the total cycle time during an event n.

The values of these chains are continuous and positive. The corresponding distribution density functions are denoted $f_R(t)$, $f_P(t)$ and $f_c(t)$, respectively. They can be determined directly from the single-molecule experiments on the formation of the statistics of successive dwell times recorded in a particular realization of the process $\mathcal{P}(t)$ (Fig. 3). Fig. 6 presents an example of the functions $f_R(t)$ and $f_P(t)$ determined in a patch-clamp experiment. Both curves show short-time non-exponential behaviour. This indicates the non-Markovianity of the process. For a Markovian process, the dwell time distribution density function would be Poissonian, i.e. exponential.
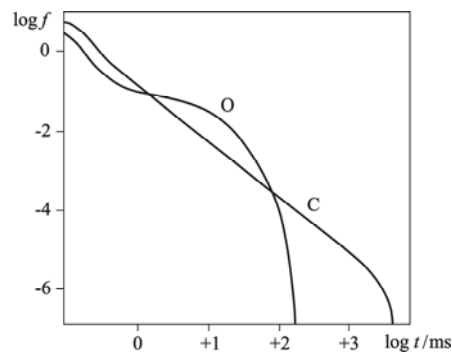

Fig. 6. A sketch of the time dependence of the closed time (C) and open time (O) distribution density functions observed with the help of the patch-clamp technique for a certain protein K^+ channel. After Samson *et al.* [3].

The functions $f_R(t)$, $f_P(t)$ and $f_c(t)$ can be relatively easily related to the second order autocorrelation function $C_2(t)$ [15], provided that the two chains $\mathcal{T}_R(n)$ and $\mathcal{T}_P(n)$ are non-correlated. A correlation means that a molecule in state P keeps the memory of its recent residence in state R. A measure of the correlation is the two-time function $f_{\alpha,\beta}(t,t',n)$, $\alpha,\beta = R, P$, determining the

distribution density of the dwell time in β, provided a distribution in α at n events ago is given [5, 16]. For uncorrelated processes, it holds that

$$f_{\alpha,\beta}(t,t',n) = f_\alpha(t)f_\beta(t')$$ (11)

A lack of correlation takes place when the dynamics in one of the distinguishable states is assumed to always start from the same substate (a 'gate'). In previous publications, I have referred to such two-state processes without memory as gated processes [1, 9]. Flomenbom *et al.* [7, 16] use the notion of reducible semi-Markov processes, whereas Bruno *et al.* [17] refer to processes of the manifest interconductance rank 1 form. Two-state processes with a transition memory are usually referred to as processes with fluctuating barriers.

THE PROBLEM OF MOLECULAR GEAR

The gating mechanism of enzymatic reactions is a sufficient condition for the Michaelis-Menten steady-state kinetics observed in many experiments [18, 19]. However, this mechanism cannot explain, for example, the intriguing phenomenon of the multiple stepping of molecular motors per nucleoside triphosphate molecule hydrolyzed, which has recently been the object of heated discussions.

Fundamental to the physical theory of biological molecular motors is the assumption that to make a single step along its track, the motor molecule has to hydrolyze at least one molecule of nucleoside triphosphate, in particular ATP [20]. This assumption was recently questioned by a group of Japanese biophysicists from the Yanagida laboratory. Joining a specific nanometry technique with fluorescence microscopy, they showed that the myosin head can make several steps along the actin filament per ATP molecule hydrolyzed [21, 22]. This observation was confirmed in some other laboratories for myosin [23], dynein [24, 25] and kinesin [26].

In Fig. 7A, the classical Lymn-Taylor-Eisenberg kinetic model of the mechanochemical cycle of the actomyosin motor is shown. The notation used is explained in the caption. The concentration of ADP is assumed to be fixed, so that the ATP hydrolysis can effectively be treated as a unimolecular reaction $T \leftrightarrow P_i$. A denotes the actin filament before and A_{tr} after the translation of the myosin head by a unit step. An external load attached to the statistical ensemble of myosin heads (organized, in the case of myofibrils, into a system of thick filaments [20]) influences the free energy involved in binding the myosin heads to thin actin filaments. The associated changes in the binding free energy can be expressed as changes in the effective rather than the actual concentrations of the actin filament before and after translation. As a consequence, the actomyosin motor can be effectively treated as a chemochemical machine that couples the free energy donating reaction $T \leftrightarrow P_i$ with the free energy accepting reaction

$A \leftrightarrow A_{tr}$ [18, 19]. The ratio of the fluxes of the second and the first reaction is referred to as the degree of coupling of the two processes.
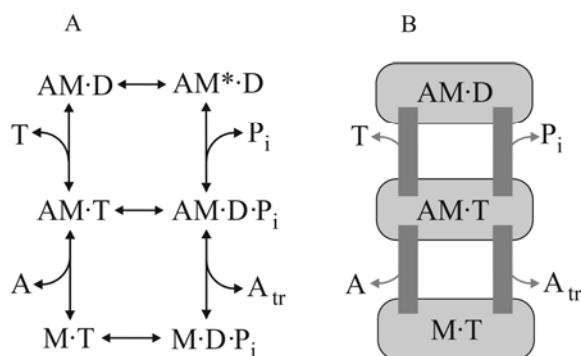

Fig. 7. The mechanochemical cycle of the actomyosin motor. A – The classical Lymn-Taylor-Eisenberg kinetic model. M denotes the myosin head, A and A_{tr} denote the actin filament before and after translation by a unit step; and T, D and Pi respectively stand for ATP, ADP and inorganic phosphate. The asterisk distinguishes the high free energy conformation of the myosin head. B – The proposed generalization of the model with a quasi-continuum of conformational substates. See the text for discussion. After [18], modified.

In terms of the conventional chemical kinetics shown in Fig. 7A, it is impossible to explain a multiple use of the free energy donated by the first reaction because in this kinetics, partial equilibrium within intramolecular degrees of freedom has to be attained before each successive kinetic step. The memory of the preceding reaction can be kept only when the intramolecular dynamics is slow enough for a transition to the next step to take place before the partial equilibration. I proposed a model in which the lack of partial equilibrium is secured by the presence of a multitude of conformational transitions which have to be treated on an equal footing with the chemical transformations [18, 19]. Such a case is schematically illustrated in Fig. 7B, where the 'black boxes' represent more or less complex networks of conformational substates, and the thickened vertical lines denote transitions, possibly between many conformational substates.

Jointly with Przemysław Chełminiak, I developed a technique [18] that enables calculation of the steady-state fluxes in kinetic schemes like the one shown in Fig. 7B, assuming that binding-rebinding reactions with substrates are gated by specific conformational substates of the protein macromolecule. However, transition through a gate consisting of a single conformational substate results in a loss of memory, so the calculated degree of coupling between the reaction $A \leftrightarrow A_{tr}$ and $T \leftrightarrow P_i$ is always smaller than unity [19]. The transition states have to consist of more substates, i.e. we have to consider a case with fluctuating barriers. In Fig. 8, the results of numerical simulations for a particular model

with gates consisting of two substates are shown. The search for a reasonable model of the conformational dynamics for the myosin head is in progress.
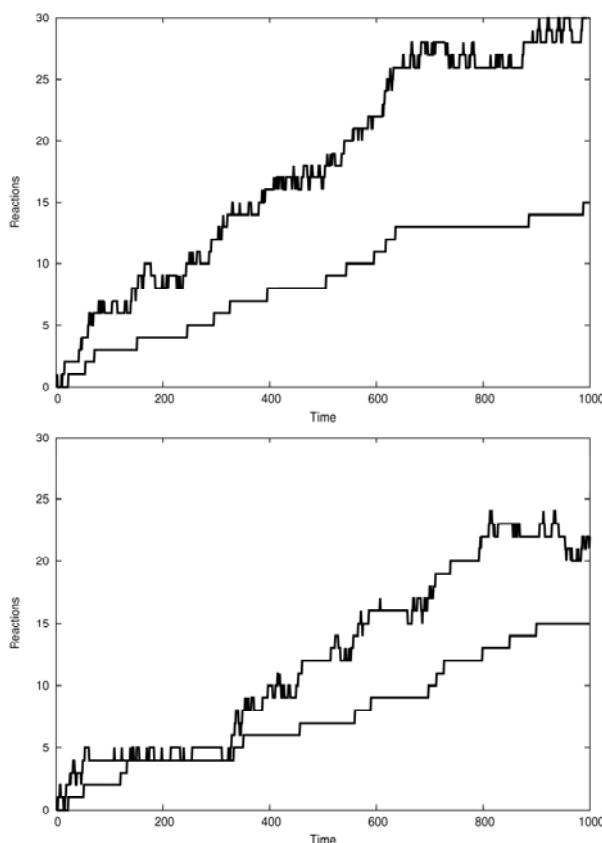

Fig. 8. Exemplary simulations of the time course of reaction 1 (ATP hydrolysis, the lower curve) coupled to reaction 2 (filament detachment, translation, and reattachment, the upper curve). The stochastic dynamics is determined by a particular realization of the scheme presented in Fig. 7B with the transition states of the reactions from AM.T to M.T consisting of two substates (M. Torchała and M. Kurzyński, unpublished results). Above, the case with no slippage. Below, the case with a slippage.

CONCLUDING REMARKS

Protein macromolecules do not resemble small molecules of conventional physical chemistry with rapidly equilibrating intramolecular dynamics. Rather, they resemble highly organized assemblies of mechanical elements: levers, hinges, springs and triggers. Sometimes, some electrical elements such as conductors, semi-conductors and insulators can be distinguished. It seems possible to describe them in the same terms as the common macroscopic machines. However, they are not macroscopic but mesoscopic systems, and they

function due to thermal fluctuations. Energy is borrowed from and returned to the surroundings. In molecular machines, ATP hydrolysis only favours one direction of these processes. Therefore, biomolecular processes have to be described in the same way as common chemical reactions, but with a multitude of highly organized conformational substates taken into account [19].

It is often sufficient to assume that biomolecular reactions are gated by certain conformational substates of the enzymatic proteins involved [18], but there are cases where such an assumption is an oversimplification. An interesting example considered here is the possibility of a multiple stepping of biomolecular motors along their tracks per ATP molecule hydrolyzed. A sophisticated statistical analysis would enable one to assess whether a given reaction is gated or not, and this paper constitutes an introduction to such a method of analysis.

REFERENCES

1. Kurzyński, M. A synthetic picture of intramolecular dynamics of proteins. Towards a contemporary statistical theory of biochemical processes. **Progr. Biophys. Molec. Biol.** <u>69</u> (1998) 23-82.
2. Caflish, A. Network and graph analyses of folding free energy surfaces. **Curr. Opin. Struct. Biol.** <u>16</u> (2006) 71-79.
3. Sansom, M.P.S., Ball, F.G., Kerry, C.J., McGee, R., Ramsey, R.J. and Usherwood, P.N.R. Markov, fractal, diffusion, and related models of ion channel gating. A comparison with experimental data from two ion channels. **Biophys. J.** <u>56</u> (1989) 1229-1243.
4. Fuliński, A., Grzywna, Z., Mellor, I., Siwy, Z. and Usherwood, P.N.R. Non-Markovian character of ionic current fluctuations in membrane channels. **Phys. Rev. E** <u>58</u> (1998) 919-924.
5. Lu, H.P., Xun, L. and Xie, S. Single-molecule enzymatic dynamics. **Science** <u>282</u> (1998) 1877-1882.
6. Edman, L., Foeldes-Papp, Z., Wennmalm, S. and Rigler, R. The fluctuating enzyme: a single molecule approach. **Chem. Phys.** <u>247</u> (1999) 11-22.
7. Flomenbom, O., Velonia, K., Loos, D., Masuo, S., Cotlet, M., Engelborghs, Y., Hofkens, J., Rowan, A.E., Nolte, R.J.M., Van der Auweraer, M., de Schryver, F.C. and Klafter, J. Stretched exponential decay and correlations in the catalytic activity of fluctuating single lipase molecules. **Proc. Natl. Acad. Sci. USA** <u>102</u> (2005) 2368-2372.
8. Knight, A.E., Veigel, C., Chambers, C. and Molloy, J.E. Analysis of single-molecule mechanical recordings: application to acto-myosin interactions. **Progr. Biophys. Molec. Biol.** <u>77</u> (2001) 45-72.
9. Kurzyński, M. **The Thermodynamic Machinery of Life**, Springer, Berlin, 2006, Appendixes B and D.
10. Frauenfelder, H., Sligar, S.G. and Wolynes, P.G. The energy landscapes and motions in proteins. **Science** <u>254</u> (1991) 1598-1602.

11. Lerch, H.P., Mikhailov, A.S. and Hess, B. Conformational-relaxation models of single-enzyme kinetics. **Proc. Natl. Acad. Sci. USA** <u>99</u> (2002) 15410-15415.
12. Keyes, T. Principles of mode-mode coupling theory, in: **Statistical Mechanics, Part B: Time-Dependent Processes** (Berne, B.J., Ed.), Plenum, New York, 1977, 259-309.
13. Edman, L. and Riegler, R. Memory landscapes of single-enzyme molecules. **Proc. Natl. Acad. Sci. USA** <u>97</u> (2000) 8266-8271.
14. Quian, H. and Elson, E.L. Single-molecule enzymology: stochastic Michaelis-Menten kinetics. **Biophys. Chem.** <u>101</u> (2002) 565-576.
15. Boguna, M., Berezhkovskii, A.M. and Weiss, G.H. Residence time densities for non-Markovian systems. The two-state system. **Physica A** <u>282</u> (2000) 474-485.
16. Flomenbom, O. and Silbey, R.J. Utilizing the information content in two-state trajectories. **Proc. Natl. Acad. Sci. USA** <u>103</u> (2006) 10907-10910.
17. Bruno, W.J., Yang, J. and Pearson, J.E. Using independent open-to- closed transitions to simplify aggregated Markov models of ion gating kinetics. **Proc. Natl. Acad. Sci. USA** <u>102</u> (2006) 6326-6331.
18. Kurzyński, M. and Chełminiak, P. Mean first-passage time in the stochastic theory of biochemical processes. Application to actomyosin molecular motor. **J. Statist. Phys.** <u>110</u> (2003) 137-181.
19. Kurzyński, M. **The Thermodynamic Machinery of Life**, Springer, Berlin, 2006, Chapter 9, see also errata.
20. Howard, J. **Mechanics of Motor Proteins and the Cytoskeleton**, Sinauer, Sunderland, 2001.
21. Kitamura, K., Tokunaga, M., Iwane, A.H. and Yanagida, T. A single Mosin head moves along an actin filament with regular steps of 5.3 nanometers. **Nature** <u>397</u> (1999) 129-134.
22. Kitamura, K., Tokunaga, M., Esaki, S., Iwane, A.H. and Yanagida, T. Mechanism of muscle contraction based on stochastic properties of single actomyosin motors observed *in vitro*. **Biophysics** <u>1</u> (2005) 1-19.
23. Liu, X. and Pollack, G.H. Stepwise sliding of single actin and myosin filaments. **Biophys. J.** <u>86</u> (2004) 353-358.
24. Kojima, H., Kikumoto, M., Sakakibara, H. and Oiwa, K. Mechanical properties of a single-headed processive motor, inner-arm dynein subspecies-c of *Chlamydomonas* studied at the single molecule level. **J. Biol. Phys.** <u>28</u> (2002) 335-345.
25. Mallik, R., Carter, B.C., Lex, S.A., King, S.J. and Gross, S.P. Cytoplasmic dynein functions as a gear in response to load. **Nature** <u>427</u> (2004) 649-652.
26. Shao, Q. and Gao, Y.Q. On the hand-over-hand mechanism of kinesin. **Proc. Natl. Acad. Sci. USA** <u>103</u> (2006) 8072-8077.