

TOWARDS AN ADVANCED STATISTICAL THEORY
OF BIOCHEMICAL PROCESSES*

M. KURZYŃSKI

Institute of Physics, A. Mickiewicz University
Umultowska 85, 61-614 Poznań, Poland
e-mail: kurzphys@main.amu.edu.pl*(Received February 27, 1998)*

The slow character of internal dynamics of native proteins, recently becoming more and more apparent, causes the hitherto used theories of chemical reactions to be inadequate for description of most biochemical reactions. The consequence is a challenge to physicists theoreticians to construct a contemporary, truly advanced statistical theory of biochemical processes based on simple but realistic models of microscopic dynamics of biomolecules involved. A few points which seem to be important in that future theory are presented in this paper. Perhaps the most important one is the possibility of predominance of the short initial-condition dependent stage of protein involved reactions over the main stage described by the standard kinetics. This initial stage, and not that described by the standard kinetics, is expected as responsible for the coupling of component reactions in the complete catalytic cycles and more complex processes of biological free energy transduction.

PACS numbers: 87.15. He, 87.15. Rn, 05.40. +j

1. Introduction

The great complexity of biological matter can account for the fact that for a long time construction of any reasonable statistical theory of biological processes was considered a hopeless task. Biochemical reactions were interpreted in terms of a simple transition state theory [1] neglecting any intramolecular dynamics of enzymatic proteins involved. However, the situation has rapidly changed. Essential progress in studies of protein dynamics accomplished in the 1980-th and the 1990-th [2–7] has made it possible to approach a truly advanced statistical theory of biochemical processes based

* Presented at the Marian Smoluchowski Symposium on Statistical Physics, Zakopane, Poland, September 1–10, 1997.

on simple but realistic models of phenomena underlying microscopic dynamics of biomolecules. The slow character of intramolecular dynamics implies the need for radical changes in the hitherto assumed description of most biochemical processes involving proteins [7,8]. In this short lecture we present a few points which seem to be important in the construction of a future statistical theory of these processes.

2. Stochastic intramolecular dynamics of native proteins

The universal, statistically independent units of biochemical processes are supramolecular multienzyme protein complexes [9] of the size of an order of 25 nm (Fig. 1).¹ From the dynamical point of view it is essential to distinguish within their body between solid-like fragments of protein secondary structures (α -helices or β -pleated sheets) and liquid-like surrounding regions, either nonpolar (domain interiors, lipid membrane environment), or polar ones ('channels' between domains, water environment). Many experiments performed with the help of various techniques [2-7] indicate the existence of a reach, purely stochastic dynamics of conformational transitions taking place in these liquid-like regions of the complexes. The conformational transition dynamics is much slower than the usual vibrations of periods varying from 10^{-14} s (localized N-H or C-H stretching vibrational modes) to 10^{-11} s (collective vibrational modes involving the whole domains). The spectrum of relaxation times characterizing conformational transitions spreads over many orders of magnitude from 10^{-11} s (local side chain rotations or hydrogen bond rearrangements on the protein surface) to hours or even years (the mean waiting-time for protein spontaneous unfolding in physiological conditions).

At least in the range from 10^{-11} to 10^{-7} s the relaxation time spectrum of conformational transition dynamics looks practically like a quasi-continuous one [7]. There are two classes of models provided hitherto by literature, which display this property [7,10]. In the first, 'protein machine' class of models [7,11], the dynamics of conformational transitions is represented by a quasi-continuous diffusion in a certain effective potential along a few 'mechanical' coordinates, *e.g.* angles or distances describing mutual orientation of approximately rigid fragments of secondary structure or larger structural elements. The spectrum of reciprocal relaxation times for dynamics of such a type is more or less homogeneous. Otherwise, in the second class of models the dynamics is assumed to look alike in every time scale, *i.e.*, the spectrum

¹ An important exception, out of scope of the present discussion, are protein microfilaments or microtubules forming highly organized systems which take part in large-scale movements of the biological cell.



Fig.1. Schematic cross-section of the universal statistically independent unit of biochemical processes, a supramolecular multienzyme protein complex. Heavily shaded are solid-like fragments of protein secondary structures, medium shaded are nonpolar liquid-like regions, and weakly shaded are polar liquid-like regions. Black are individual catalytic centers usually localized at two neighbouring solid-like elements.

of reciprocal relaxation times has approximately a self-similarity symmetry. The latter is considered to be a generic property of glassy materials, thus we refer to this second, more extensive class of models as 'protein glass'.

Time scaling can originate either from a hierarchy of barrier heights in the conformational potential energy landscape (the 'fractal time'), or from a hierarchy of bottlenecks (the entropy barrier heights) in the network joining conformations between which direct transitions take place (the 'fractal space') [13]. A hierarchy of energy barrier heights was proposed originally

by Frauenfelder and coworkers in order to give a unitary interpretation of results of the pioneer studies of small ligand rebinding to heme proteins after a laser flash photolysis in various conditions [4–6] and a particular mathematical realization of such a hierarchy, especially predisposed for the application to proteins, seem to offer certain spin-glass models [14]. Most experimental observations supporting the protein glass picture of dynamics can be, however, equally well interpreted in terms of the hierarchy of both the energy and the entropy barrier heights. Mathematical realization of hierarchical networks are fractal lattices [7, 12, 15]. The process of diffusion on a lattice can (but does not have to) be interpreted as directly representing the motion of structural defects in the liquid-like regions between solid-like fragments of secondary structure.

3. Two special features of protein involving reactions: control and gating by intramolecular dynamics

It is the slow purely stochastic dynamics of conformational transitions that can effect the essential majority of reactions involving proteins, thus any adequate statistical theory of these reactions has to be a development of the stochastic theory of reaction rates [7, 12].

When considering a unimolecular reaction

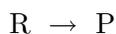


one assumes that the set of internal states (in our case, conformational substates) of the molecule involved is divided into two subsets corresponding to chemical species R and P (Fig. 2(a)). In both subsets regions R^\ddagger and P^\ddagger are to be distinguished, referred to as the transition states of the reaction, composed of those conformational substates between which direct transitions take place. Any reversible reaction can be formally divided into two irreversible reactions



after introducing the imagined conformational substate, referred to as the limbo state, the transition probability from which to any other conformational substate vanishes (Fig. 2(b)).

Usually the reaction is assumed to be an *activated process* which means that, as a result of a bottleneck in the transition states of either the energetic or entropic origin, the events of the molecule leaving the state R or P are very rare when compared to the time of interconformational equilibration within R or P. In such cases, for an actual or formally imagined irreversible reaction



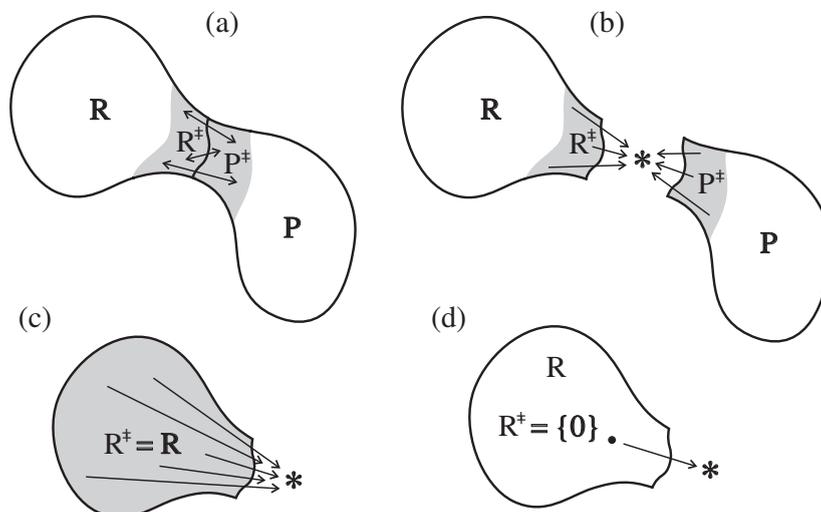


Fig. 2. Stochastic theory of single unimolecular reaction. (a) A schematic partition of the set of molecule microstates (here, conformational substates) into two subsets corresponding to different chemical species. In both subsets regions are to be distinguished, referred to as the transition states, between which direct transitions take place. A bottleneck in the transition states, of either the energetic or entropic origin, causes reaction to be an activated process. (b) Any reversible reaction can be formally divided into two irreversible reactions after introducing the imagined limbo state *. (c) If the transition state R^\ddagger is identical with the whole subset R, one can describe the entire time course of reaction, including the initial-condition dependent stage, in terms of a fluctuating rate parameter or a *fluctuating barrier*. (d) If the transition state is reduced to a single conformational substate 0 (the gate) one speaks about the *gated* reaction.

the mole fraction $C(t)$ of molecules being at time t in the chemical state R (equal to the sum of occupation probabilities at time t of all substates composing R) obeys the usual kinetic equation

$$\frac{d}{dt}C(t) = -\kappa C(t) \quad (1)$$

of the solution tending exponentially to zero with the relaxation time equal to the reciprocal reaction rate constant κ^{-1} . In general κ^{-1} is to be decomposed into two time components:

$$\kappa^{-1} = \kappa_{\text{tst}}^{-1} + \kappa_{\text{ctr}}^{-1}. \quad (2)$$

The first component in Eq. (2) determines the time needed to cross the boundary under the assumption (made in the transition state theory) that

the transition state R^\ddagger is in a local equilibrium with the rest of microstates composing the chemical state R . As a result of transition this equilibrium is, however, disturbed. It is the second component in Eq. (2) that determines the time needed for restoring this equilibrium. If the second component is much smaller than the first component the reaction is well described by the transition state theory, possibly with a certain transmission coefficient smaller than unity. If, on the contrary, the second term prevails, the reaction is referred to as *controlled* by processes of intramolecular dynamics.

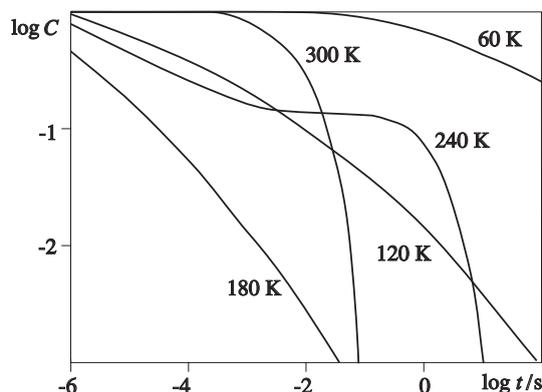


Fig. 3. A draft of time dependence of the experimentally observed rebinding of CO molecules after the photodissociation of CO-bound sperm wale myoglobin in 3:1 v/v glycerol-water at CO pressure 1 bar and various temperatures. $C(t)$ represents the fraction (monitored with the help of the absorption IR spectra) of the myoglobin molecules that have not rebound CO at time t after the laser flash. The log-log plot. In low temperatures only the unimolecular reaction of CO rebinding from the protein interior is observed. Its time course is evidently non-exponential. The exponential stage observed at 240 K and in higher temperatures is attributed to the bimolecular reaction of CO rebinding from the solution. The latter process masks the exponential stage of the unimolecular CO rebinding reaction. After Refs. [16] and [17]. Almost identical result has been reported more recently in Ref. [18] for the horse myoglobin with the only difference that the exponential stage of unimolecular CO rebinding is not completely masked.

It should be stressed that in general Eq. (1) is valid only after a short initial period dependent on the initial distribution of conformational substates. During the initial period a more general equation

$$\frac{d}{dt}C(t) = -f(t) \quad (3)$$

is appropriate, with $f(t)$ having a meaning of the first-passage time distribution density [7, 12]. The initial-condition dependent stage of the reaction

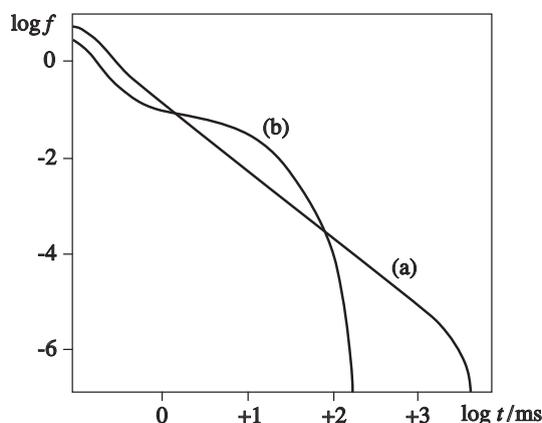


Fig. 4. A draft of time dependence of the closed time (a) and open time (b) distribution density $f(t)$, *cf.* Eq. (3), observed with the help of the patch clamp technique for the protein K^+ channel of NG 108–15 cells. The log-log plot. Both curves show a short-time non-exponential behaviour. In the case (a) the pre-exponential stage includes the changes of $f(t)$, *i.e.* $C(t)$, by five orders of magnitude and fits very well to the power law of the form $t^{-\alpha}$. After Ref. [20].

can, however, occur only for reactions controlled by internal dynamics of the molecules involved. Two kinds of experiments show clearly the occurrence of such a stage in the case of protein involving processes: studies of small ligand rebinding to heme proteins in various conditions after a laser flash photolysis [4–6, 16–18] (Fig. 3) and direct observations, with the help of the patch clamp technique, of fluctuations of the ionic current flowing through single protein channels [19, 20] (Fig. 4). The important conclusion arising from these experiments is that some (and presumably almost all) biochemical reactions are controlled by the intramolecular stochastic dynamics of proteins involved.

The observability, in both kinds of experiments, of the initial-condition dependent stage of reaction and its predominance over the main exponential stage result from the confinement of the initial distribution of conformational substates only to the transition state R^\ddagger or P^\ddagger [7, 12]. Statistical theories of reactions involving molecules in the initial state confined to the reaction's transition state develop towards two opposite limits. In the one extreme the entire microscopic dynamics of the molecule is assumed to take place within the transition state ($R^\ddagger = R$, *cf.* Fig. 2(c)). This enables one to apply the kinetic equation (1) in the whole time domain provided that the rate parameter κ a random function of time needed only to be appropriately averaged (the picture of 'fluctuating barriers' [4–6] or 'dynamical disorder' [21]). The opposite extreme is based on the assumption that the transition state R^\ddagger is

reduced to a single conformational substate 0 being a ‘gate’ for the reaction which is thus referred to as the *gated reaction* [2, 7, 11, 12] (Fig. 2(d)). Neither of both approaches can be considered as corresponding to the actual situation, but the assumption of gating seems to be better physically justified. The important indirect argument for this approximation is stationary Michaelis–Menten kinetics observed for the majority of enzymatic reactions, which could not be realized for the reactions being simultaneously controlled and *not* gated by the intramolecular dynamics [11, 22].

4. Importance of the initial-condition dependent stage of reactions

In Fig. 5 some results of computer simulations performed in our laboratory are shown of the time course of reaction gated by intramolecular dynamics modelled by random walk on the finite Sierpiński gasket [15]. The initial microstate was assumed as strictly confined to the gate. The varying parameter q represents the ratio of the probability of leaving the lattice to the probability of transition between the neighbouring sites. In the lin-log plots quoted in Fig. 5(a) the exactly exponential long-time decay is apparent with the relaxation time of the value close to κ_{tst}^{-1} for $q = 0.01$ and to κ_{ctr}^{-1} for $q = 100$ (*cf.* Eq. (2)). It is clearly seen that more strongly is the reaction controlled by the intramolecular dynamics more dominant is the initial, nonexponential stage of the reaction. The latter is better exposed in the log-log plots quoted in Fig. 5(b).

The pre-exponential stages of the simulated reactions are well described by the analytical formula [15]

$$C_{\text{ini}}(t) = \exp(\eta t)^{2\alpha} \operatorname{erfc}(\eta t)^\alpha, \quad (4)$$

where the symbol erfc denotes the complementary error function, η^{-1} is a certain unit of time and the value of the exponent α is determined by the value of the spectral dimension \tilde{d} of the lattice:

$$\alpha = 1 - \frac{\tilde{d}}{2} \quad (5)$$

(= 0.317 for the Sierpiński gasket). In the limit of short times Eq. (4) represents the stretched-exponential law and in the limit of long times, the algebraic power law:

$$C_{\text{ini}}(t) \approx \begin{cases} \exp[-2(\eta t)^\alpha/\sqrt{\pi}] & \text{for } t \ll \eta^{-1} \\ (\eta t)^{-\alpha}/\sqrt{\pi} & \text{for } t \gg \eta^{-1} \end{cases}. \quad (6)$$

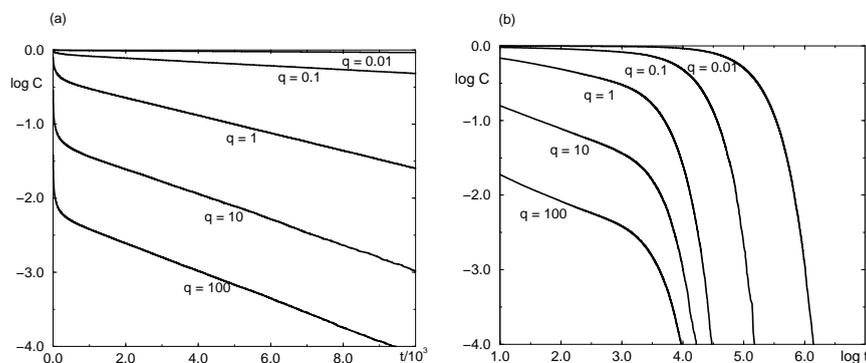


Fig. 5. Time course of reaction gated by an intramolecular dynamics with the initial microstate confined to the transition state. Results of computer simulations for the model of conformational transition dynamics representing random walk on the Sierpiński gasket. The lattice has been limited to $(3^6 - 3)/2$ sites (cluster of the 6-th order). Some 10^5 walkers started at the same site which simultaneously is the only gate to exit the lattice. The ratio q of the probability of leaving the lattice to the probability of transition between the neighbouring sites was assumed to vary in the wide range of values between 0.01 and 100. Time is measured by the number of steps in which transitions were randomly generated. Survival probabilities vs time are plotted in the lin-log (a) and the log-log (b) scales. After Ref. [15].

The moment of crossing over to the exponential stage of the reaction depends on the size of the lattice and the probability to leaving it relative to the probability of transition between the neighbouring sites. The smaller the lattice and the lower the probability of leaving it (or, equivalently, the higher the probability of jumping between the lattice sites), the earlier the exponential stage of the reaction begins. The crossover from the non-exponential decay, Eqs (4) and (6), to the exponential decay with the ‘chemical’ relaxation time κ^{-1} can be described with the help of a simple formula

$$C(t) = [(1 - a) C_{\text{ini}}(t) + a] e^{-\kappa t}. \quad (7)$$

with a denoting the level (concentration) from which the exponential decay begins. The combined formulae (7) and (4) comprise three dimensionless parameters α , a and the ratio $b \equiv \kappa/\eta$. Two of those parameters: a and b depend on temperature in the Arrhenius manner so that it should be no problem to describe in these terms, possibly with minor modifications, a time course of any experimentally observed reactions including its variation with temperature (*cf.* Figs 3 and 4).

The necessary condition for the presence of a certain initial-condition dependent stage of protein involving reaction is not only the slow character

the intramolecular conformational transition dynamics but also the special preparation of the initial conformational substates of the protein to be confined to the transition state of the reaction. The latter condition is realized, however, only in the special experiments mentioned above. Usually, the initial distribution of conformational substates is not very different from the local equilibrium. It is thus not a surprise that no initial-condition stages are observed in standard biochemical kinetic experiments.

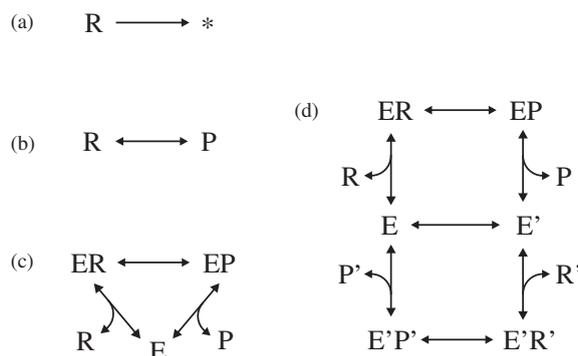


Fig. 6. From single irreversible (a) and reversible (b) reaction through the complete catalytic cycle (c) to the system of coupled reactions in which biological free energy transduction is carried out (d).

Nevertheless, the initial-condition dependent stages of reactions appear important also in standard conditions, provided that a steady-state is realized. These stages and not the following ones described by the standard kinetics, are expected to be responsible for the coupling of component reactions in the complete catalytic cycles shown in Fig. 6(c) (this was proven specifically for the particular protein machine model of intramolecular dynamics [11]) and more complex processes of biological free energy transduction (Fig. 6(d)). Importance of the latter statement, if it is actually true, can hardly be overestimated.

5. Summary

In the construction of the future, truly advanced statistical theory of biochemical processes the following points have to be taken into account:

- (a) The native proteins involved in these processes reveal a reach purely stochastic intramolecular dynamics of conformational transitions, much slower than the usual vibrational dynamics. At least in the range from 10^{-11} to 10^{-7} s the relaxation time spectrum of conformational transition dynamics is practically quasi-continuous. Two classes of math-

emathical models of these dynamics seem reasonable, we referred symbolically to as protein-machine and protein-glass.

- (b) The essential majority of reactions involving proteins are controlled and, presumably, also gated by this stochastic intramolecular dynamics. This means that the rate of biochemical processes is determined by the mean first-passage time through the gate composed of a small number of conformational substates of the protein involved.
- (c) Of special importance is the short initial-condition dependent stage of biochemical reactions, neglected in the description of the reaction in terms of the standard kinetics. This stage is directly observed in experiments in which the especially prepared initial conformational substates of the protein are confined to the transition state of the reaction.
- (d) The initial-condition dependent stage, and not that described by the standard kinetics, is expected as responsible for the coupling of component reactions in the complete catalytic cycles and more complex processes of biological free energy transduction.

The study has been supported in part by the Polish State Committee for Scientific Research (project 2 P03B 161 12) and in part by the Alexander von Humboldt Foundation.

REFERENCES

- [1] A. Fersht, *Enzyme Structure and Mechanisms*, 2nd edn., Freeman, New York 1985.
- [2] J.A. McCammon, S.C. Harvey, *Dynamics of Proteins and Nucleic Acids*, Cambridge University, Cambridge 1987.
- [3] C.L. Brooks III, M. Karplus, B.M. Pettitt, *Proteins: A Theoretical Perspective of Dynamics, Structure and Thermodynamics, Advances in Chemical Physics* vol. 71, Wiley, New York 1988.
- [4] H. Frauenfelder, F. Parak, R.D. Young, *Annu. Rev. Biophys. Biophys. Chem.* **17**, 451 (1988).
- [5] H. Frauenfelder, P.J. Steinbach, R.D. Young, *Chem. Scr.* **29A**, 145 (1989).
- [6] H. Frauenfelder, S.G. Sligar, P.G. Wolynes, *Science* **254**, 1598 (1991).
- [7] M. Kurzyński, *Prog. Biophys. Molec. Biol.*, review article in print.
- [8] M. Kurzyński, *FEBS Lett.* **328**, 221 (1993).
- [9] G.R. Welch, ed. *Organized Multienzyme Systems: Catalytic Properties*, Academic Press, Orlando 1985.
- [10] M. Kurzyński, in: *Diffusion Processes — Experiment, Theory, Simulations*, ed. A. Pękalski, *Lecture Notes in Physics*, vol. **438**, Springer, Berlin 1994, p295.

- [11] M. Kurzyński, *Biophys. Chem.* **65**, 1 (1997).
- [12] M. Kurzyński, *Acta Phys. Pol.* **B28**, 1853 (1997).
- [13] A. Blumen, H. Schnörrer, *Angew. Chem. Int. Ed.* **29**, 113 (1990).
- [14] D.L. Stein, ed., *Spin Glasses in Biology*, World Scientific, Singapore 1992.
- [15] M. Kurzyński, K. Palacz, P. Chełminiak, *Proc. Natl. Acad. Sci. USA*, submitted for publication.
- [16] R.H. Austin, K.W. Beeson, L. Eisenstein, H. Frauenfelder, I.C. Gunsalus, *Biochemistry* **14**, 5355 (1975).
- [17] P.J. Steinbach, A. Ansari, J. Berendzen, D. Braunstein, K. Chu, B.R. Cowen, D. Ehrenstein, H. Frauenfelder, J.B. Johnson, D.C. Lamb, S. Luck, J.R. Mourant, G.U. Nienhaus, P. Ormos, R. Philipp, A. Xie, R.D. Young, *Biochemistry* **30**, 3988 (1991).
- [18] F. Post, W. Doster, G. Karvounis, M. Settles, *Biophys. J.* **64**, 1833 (1993).
- [19] L. S. Liebovitch, J.M. Sullivan, *Biophys. J.* **52**, 979 (1987).
- [20] M.S.P. Sansom, F.G. Ball, C.L. Kerry, R. McGee, R.L. Ramsey, P.N.R. Usherwood, *Biophys. J.* **56**, 1229 (1989).
- [21] R. Zwanzig, *Acc. Chem. Res.* **23**, 148 (1990).
- [22] J. Ninio, *Proc. Natl. Acad. Sci. USA* **84**, 663 (1987).
- [23] T.L. Hill, *Free Energy Transduction and Biochemical Cycle Kinetics*, Springer, New York 1989.