RAPID COMMUNICATIONS

PHYSICAL REVIEW E **74**, 010902(R) (2006)

Generalized Haldane equation and fluctuation theorem in the steady-state cycle kinetics of single enzymes

Hong Qian*
Department of Applied Mathematics, University of Washington, Seattle, Washington 98195, USA

X. Sunney Xie†
Department of Chemistry and Chemical Biology, Harvard University, Cambridge, Massachusetts 02138, USA
(Received 1 August 2005; revised manuscript received 13 February 2006; published 12 July 2006)

Enyzme kinetics are cyclic. We study a Markov renewal process model of single-enzyme turnover in nonequilibrium steady state (NESS) with sustained concentrations for substrates and products. We show that the forward and backward cycle times have identical nonexponential distributions: $\Theta_+(t)=\Theta_-(t)$. This equation generalizes the Haldane relation in reversible enzyme kinetics. In terms of the probabilities for the forward (p_+) and backward (p_-) cycles, $k_BT\ln(p_+/p_-)$ is shown to be the chemical driving force of the NESS, $\Delta\mu$. More interestingly, the moment generating function of the stochastic number of substrate cycle $\nu(t)$, $\langle e^{-\lambda\nu(t)}\rangle$, follows the fluctuation theorem in the form of Kurchan-Lebowitz-Spohn-type symmetry. When $\lambda=\Delta\mu/k_BT$, we obtain the Jarzynski-Hatano-Sasa-type equality $\langle e^{-\nu(t)\Delta\mu/k_BT}\rangle\equiv 1$ for all t, where $\nu\Delta\mu$ is the fluctuating chemical work done for sustaining the NESS. This theory suggests possible methods to experimentally determine the nonequilibrium driving force *in situ* from turnover data via single-molecule enzymology.

DOI: 10.1103/PhysRevE.74.010902 PACS number(s): 87.10.+e, 05.70.Ln, 02.50.−r, 05.40.−a

Most biochemical reactions in a living cell have nonzero flux J and nonzero chemical driving force $\Delta\mu$. The nonequilibrium state of such a reaction is sustained by continuous material and energy exchange with and heat dissipation into its environment [1]. Hence, to understand the state of a biochemical network in an open environment, it is necessary to be able to experimentally measure both J and $\Delta\mu$ *in situ*. A large literature exists on measuring J, but none exists on directly measuring $\Delta\mu$. One could in principle compute $\Delta\mu$ from *in situ* measurements of the concentrations of the substrate and product of a reaction if its equilibrium constant is known [2]. Alternatively, one should be able to obtain $\Delta\mu$ from fluctuating cycle kinetics of a single enzyme directly. This possibility has been recently investigated in term of stochastic simulations [3]. Here we exam this idea through an analytical model.

Enzyme kinetics are complex mainly due to the many possible intermediates in the form of enzyme-substrate complexes. Recent laboratory measurements with high resolution at the single-molecule level give the waiting time distributions for enzyme cycles [4]. This motived the present Markov renewal process (MRP) model, also known as the extended kinetics model in the theory of motor proteins [5]. In terms of the MRP, the kinetics of a single enzyme becomes a stochastic sequence of forward and backward cycles as a function of time. We shall denote the number of forward and backward cycles by $\nu_+(t)$ and $\nu_-(t)$, as shown in Fig. 1.

It is obvious that the cycle time distributions give information on the kinetics. In this Rapid Communication we show that the key nonequilibrium thermodynamic quantity, $\Delta\mu$, can be obtained from stochastic data on single-enzyme cycle $\nu(t)\equiv\nu_+(t)-\nu_-(t)$ via two equalities

$$\Delta\mu = k_BT\ln[\langle\nu_+(t)\rangle/\langle\nu_-(t)\rangle], \tag{1}$$

$$\langle e^{-\nu(t)\Delta\mu/k_BT}\rangle = 1 \qquad \forall\, t, \tag{2}$$

where $\langle\cdots\rangle$ is the ensemble average for repeated measurements of $\nu(t)$ in a steady state. Equation (1) generalizes a result well known for one-step chemical reactions [1,6]. Equation (2) is a version of the fluctuation theorem (FT) in nonequilibrium statistical mechanics. The FT for the probability distribution of entropy production of a nonequilibrium steady-state (NESS) was first discovered in deterministic dynamical systems [7]. Kurchan, Lebowitz, and Spohn (KLS) introduced a parallel theory in terms of stochastic dynamics [8] which is more appropriate for single-enzyme experiments [4,9,10]. It was shown that the generating function, i.e., an exponential average, of a work functional $W(t)$ possesses a certain symmetry in the limit of $t\to\infty$. Crooks introduced a heat functional $Q(t)$ and showed that similar symmetry is valid for all finite t [11]: $c_\lambda(t)=c_{1-\lambda}(t)$ where $c_\lambda(t)=\langle e^{-\lambda Q(t)/k_BT}\rangle$. Since $Q(t)$ and $W(t)$ differ by a stationary term while both increase without boundy, Crooks' result im-

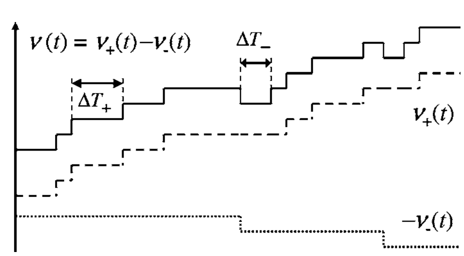

FIG. 1. The solid line illustrates ideal data on single-enzyme cycling as a function of time, $\nu(t)$, which can be decomposed into $\nu_+(t)$ and $\nu_-(t)$, shown as dashed and dotted lines. The starting positions are arbitrary. ΔT_+ and ΔT_- are forward and backward cycle times.

*Electronic address: qian@amath.washington.edu
†Electronic address: xie@chemistry.harvard.edu

mediately yields that of KLS. The symmetry in the generating function implies the FT for Q [12].

The symmetry implies that $\ln\langle e^{-Q(t)/k_BT}\rangle=0$. This is analogous to the Jarzynski equality [13], which is surprising since $\langle Q(t)\rangle=-k_BT\ln e^{-\langle Q(t)\rangle/k_BT}$ is the mean heat dissipated from the NESS, which certainly is not equal to 0; it should always be greater than 0. The Jarzynski equality provides the possibility obtaining a function of state such as the free energy from a nonstationary heat functional $Q(t)$ with finite t. This was proposed and experimentally tested for the mechanical work functional on single biological macromolecules such as RNA [14,10].

The difference between the FTs for $W(t)$ in the limit of infinite t and for $Q(t)$ with any finite t is crucial to real experiments. In heuristic thermodynamic terms, the work functional $W(t)$ [8] is related to the $\Delta\mu^0$ of a reaction and the heat functional $Q(t)$ [11] to $\Delta\mu$. While the former is determined by the transition rate constants, and hence is experimentally accessible in short time, the latter depends on the stationary probability. For cyclic enzymatic turnovers, however, $W=Q$. Hence, the FT associated with enzyme cycle kinetics is particularly simple, and experimentally accessible [3]. Generalizing the Jarzynski equality to open systems, Hatano and Sasa's equality for the NESS [13] also suggested the possibility of the computing chemical driving force for single-molecule chemical reactions in NESS (see [3,15]).

To show Eqs. (1) and (2), there are two strategies. One is based on traditional Markov models, i.e., master equations, for single-enzyme kinetics. Then both equations can be show as conseqeunces of the existing FTs [8,11]. An alternative, the more insightful approach is to model the kinetics in terms of a MRP with cycle kinetics. In our model, we shall show a surprising equality between the forward and backward cycle time distributions: $\Theta_+(\tau)=\Theta_-(\tau)$. With this equality, Eq. (1) becomes obvious, and Eq. (2) can be shown in elementary terms, in Eqs. (7)–(11) below.

The equality $\Theta_+(\tau)=\Theta_-(\tau)$ turns out to be a very important relation in enzyme kinetics. This is a key result of this work. It has to do with microscopic reversibility. There is experimental evidence for it, as well as theoretical models proving equal mean time $\langle\Delta T_+\rangle=\langle\Delta T_-\rangle$ [16,17]. We shall give a proof for the equal distribution with sequential enzyme kinetics. The proof for more general systems will be published elsewhere [18].

The detailed kinetic scheme of an enzyme-catalyzed biochemical reaction $A\rightleftharpoons B$ is usually very complex [19]. But if one considers only the net number of steady-state turnovers from A to B, $\nu(t)$, it can be represented by a continuous-time, discrete-state one-dimensional random walk with cumulative cycle time distribution functions $\Theta_\pm(t)$ for the forward and the backward stochastic transition times ΔT_+ and ΔT_-: $\Theta_\pm(0)=0$, $\Theta_\pm(\infty)=1$, and $\Theta_\pm(t)$ are nondecreasing. This is a class of stochastic models known as MRPs [20] which has wide applications in single-enzyme kinetics and motor protein stepping [21,5]. See Fig. 2 in which $w_\pm(t)=p_\pm\Theta_\pm(t)$ and $p_++p_-=1$. p_+ (p_-) is the eventual probability of the enzyme binding A (B) and converting it to B (A). We shall also denote $w(t)=w_+(t)+w_-(t)$.

We discover that a necessary condition for Eqs. (1) and (2) is that the cycle time distributions for the forward and backward steps are equal: $\Theta_+(t)=\Theta_-(t)$. We call this the equality generalized Haldane equation [22].

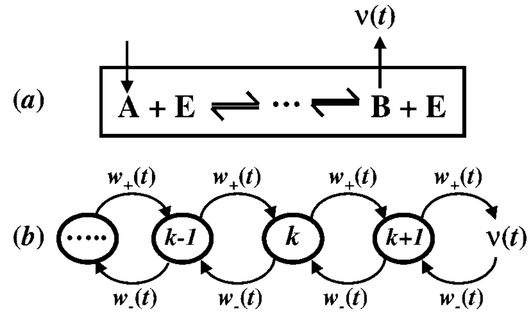

FIG. 2. (a) Schematics for an enzyme reaction converting substrate A to product B. In a NESS, the concentrations for A and B, c_A and c_B, are controlled through feedback by an experimenter. The cumulative number of B taken out by the time t is denoted by $\nu(t)$, $-\infty<\nu(t)<\infty$. (b) The integer-valued $\nu(t)$ is most naturally modeled by a random walk with forward and backward time distributions $w_+(t)$ and $w_-(t)$ [5].

The position of the random walker in Fig. 2(b), $\nu(t)$, models the net number of enzyme turnovers. Let $\nu_0=0$, $\Delta\nu_1$, $\Delta\nu_2,\ldots,\Delta\nu_\ell,\ldots$ ($\Delta\nu=\pm1$) be successive increments of the turnover number, and $T_0=0$, $\Delta T_1,\Delta T_2,\ldots,\Delta T_\ell,\ldots$ ($\Delta T\geq0$) be the corresponding increments in time. Then the probabilistic meaning of $w_\pm(t)$ is the joint probability for continuous ΔT and binary $\Delta\nu$:

$$w_\pm(t)=\Pr\{\Delta\nu_\ell=\pm1,\Delta T_\ell\leq t\}\quad(\ell\geq1). \tag{3}$$

The equation $\Theta_+(t)=\Theta_-(t)$ leads to $w_\pm(t)=p_\pm w(t)$. That is, the random variables $\Delta\nu_\ell$ and ΔT_ℓ are statistically independent.

To show the equality $\Theta_+(t)=\Theta_-(t)$ for forward and backward cycles, we consider a sequential enzyme reaction as shown in Fig. 3(a) and a corresponding exit problem [23] shown in Fig. 3(b). Starting at the central position E, $w_+(t)$ and $w_-(t)$ are the cumulative probabilities of reaching $B+E$ and $A+E$. Since only the first and last steps are irreversible,

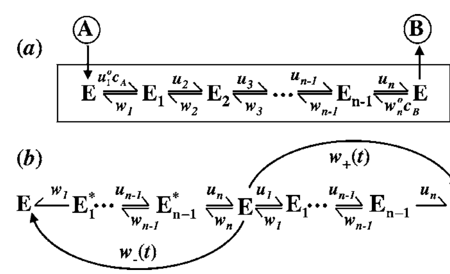

FIG. 3. (a) A schematic for an enzyme reaction converting A to B. The transition time distribution of a single enzyme converting A to B, $w_+(t)$, and converting B to A, $w_-(t)$, is intimately related to the exit problem shown in (b) in which u_1 and w_n are pseudo-first-order rate constants that depend on the concentrations of A and B, respectively: $u_1=u_1^oc_A$, $w_n=w_n^oc_B$. The scheme in (b) has been used to compute steady-state one-way flux in Hill's theory on biochemical cycle kinetics [25,6].

$w_+(t)$ and $w_-(t)$ both have $2n+1$ exponential terms with the same eigenvalues, one of which is 0. Thus both can be written as $a_0+a_1e^{-\lambda_1 t}+a_2e^{-\lambda_2 t}+\cdots+a_{2n}e^{-\lambda_{2n}t}$. With some straightforward algebra, it can be shown that for all $0\le m\le 2n$ [24]

$$\frac{1}{w_1w_2\cdots w_n}\frac{d^m w_-(0)}{dt^m}=\frac{1}{u_1u_2\cdots u_n}\frac{d^m w_+(0)}{dt^m}. \tag{4}$$

Since the functions $w_+(t)$ and $w_-(t)$ are completely determined by these initial conditions, which satisfy the linear algebraic system, we have

$$\frac{w_+(t)}{w_-(t)}\equiv\prod_{\ell=1}^{n}\left(\frac{w_\ell}{u_\ell}\right)=e^{-\Delta\mu/k_BT}, \tag{5}$$

independent of t. That is, $\Theta_-(t)=\Theta_+(t)$.

The meaning of the equality now becomes clear: We recall that u_1 and w_n are pseudo-first-order rate constants: $u_1=u_1^o c_A$ and $w_n=w_n^o c_B$. In a chemical equilibrium,

$$\frac{c_B}{c_A}=\frac{u_1^o u_2\cdots u_{n-1}u_n}{w_1w_2\cdots w_{n-1}w_n^o}, \tag{6}$$

that is, $w_+(t)=w_-(t)$. Therefore, in a chemical equilibrium not only does the average $w_+(\infty)=w_-(\infty)$, i.e., the forward flux equals the backward flux, but the detailed kinetics for the transition time distributions has to be equivalent: There is absolutely no statistical difference between the forward and backward reactions. In a NESS when Eq. (6) does not hold true, $w_+(t)\neq w_-(t)$. But the difference is only in the total probability $p_+=w_+(\infty)$ and $p_-=w_-(\infty)$, the distribution functions $\Theta_+(t)=\Theta_-(t)$ still hold true. This equality is essential to the KLS symmetry below. It is known that microscopic reversibility has to be satisfied even when a mesoscopic system is in a nonequilibrium steady state [8].

For the number k of successive renewal events (forward plus backward turnovers) within time $[0,t]$, let us denote $(\nu_k,T_k)=\Sigma_{\ell=1}^{k}(\Delta\nu_\ell,\Delta T_\ell)$. The moment-generating function for $\nu(t)$ is

$$g_\lambda(t)\equiv\langle e^{-\lambda\nu(t)}\rangle=\sum_{n=-\infty}^{\infty}e^{-\lambda n}\sum_{k=0}^{\infty}\Pr\{\nu_k=n,T_k\le t,T_{k+1}>t\} \tag{7}$$

$$=\sum_{k=0}^{\infty}\left(\sum_{n=-k}^{k}e^{-\lambda n}\Pr\{\nu_k=n\}\right)\times\Pr\{T_k\le t,T_{k+1}>t\} \tag{8}$$

$$=\sum_{k=0}^{\infty}(p_+e^{-\lambda}+p_-e^{\lambda})^k\Pr\{T_k\le t,T_{k+1}>t\}. \tag{9}$$

Equation (8) is obtained because of the independence between ν_k and T_k. Then from Eq. (9) we have the KLS symmetry

$$g_\lambda(t)=g_{\lambda^*-\lambda}(t)\quad\forall\ t, \tag{10}$$

where $\lambda^*=\ln(p_+/p_-)$. Furthermore,

$$g_{\lambda^*}(t)\equiv\langle e^{-\nu(t)\Delta\mu/k_BT}\rangle=g_0(t)=1, \tag{11}$$

if $\ln(p_+/p_-)=\Delta\mu/k_BT$ holds true. We recognize that $\nu(t)\Delta\mu$ is the external chemical work done to the system in a NESS. Hence Eq. (11) is analogous to the Jarzynski equality for a cycle.

If we let $t\to\infty$ in Eq. (5), we have $\ln(p_+/p_-)\equiv\lambda^*=\Delta\mu/k_BT$, which is needed in deriving Eq. (11). This generalizes the well-known result for single-step chemical reactions [25,6] to any complex enzyme reaction cycle.

We are now also in a position to show Eq. (1). The mean number of net turnovers can be computed from the $g_\lambda(t)$ given in Eq. (9):

$$\langle\nu(t)\rangle=\langle\nu_+(t)\rangle-\langle\nu_-(t)\rangle=-\left[\frac{dg_\lambda(t)}{d\lambda}\right]_{\lambda=0} \tag{12}$$

$$=(p_+-p_-)\sum_{k=0}^{\infty}k\Pr\{T_k\le t,T_{k+1}>t\} \tag{13}$$

$$=(p_+-p_-)\times(\text{mean no. of cycles in time } t). \tag{14}$$

Therefore, $\frac{\langle\nu_+\rangle}{\langle\nu_-\rangle}=\frac{p_+}{p_-}$. Furthermore, in the limit of large t [23], $\langle\nu(t)\rangle\approx(p_+-p_-)t/\langle T_1\rangle$, where $\langle T_1\rangle=\int_0^\infty t\,dw(t)$ is the mean time for one cycle, forward or backward. When $p_+=p_-$, the steady-state flux $J=\lim_{t\to\infty}\langle\nu(t)\rangle/t=0$ as expected. When $p_+>p_-$, $J>0$.

Studying enzyme-catalyzed biochemical reactions *in situ* requires methods for measuring $\Delta\mu$, the NESS chemical driving force. Currently none exists. We propose obtaining $\Delta\mu$ from stochastic cycle data of a single-enzyme molecule, $\nu(t)$, via (i) an equality similar to that of Jarzynski and Hatano-Sasa, $\langle e^{-\nu(t)\Delta\mu/k_BT}\rangle=1$; or simply (ii) $k_BT\ln[\langle\nu_+(t)\rangle/\langle\nu_-(t)\rangle]$. We developed a MRP model for enzyme cycles with arbitrary complex mechanism, and found an equality between the forward and backward cycle time distributions based on microscopic reversibility. This equality is a generalization of what is known as the Haldane relation for reversible enzyme kinetics and recent results in [17]. The model enables us to establish a FT and above equalities (i) and (ii) for any t. Noting that $(1/t)\langle\nu(t)\rangle=J$, one thus obtains both the flux J and the driving force $\Delta\mu$ for a reaction in a NESS from the fluctuating $\nu(t)$. The statistical accuracies associated with these measurements were discussed in [3].

We thank C. Asbury, M. Fisher, C. Jarzynski, A. Kolomeisky, W. Min, U. Seifert, Y.-Y. Shi, A. Szabo, H.-Y. Wang, and J. Yu for discussions.

[1] For recent reviews, see H. Qian, J. Phys.: Condens. Matter **17**, S3783 (2005); H. Qian and D. A. Beard, Biophys. Chem. **114**, 213 (2005).

[2] H. Qian and E. L. Elson, Proc. Natl. Acad. Sci. U.S.A. **101**, 2828 (2004); W. J. Heuett and H. Qian, J. Chem. Phys. **124**, 044110 (2006).

[3] W. Min, L. Jiang, J. Yu, S. C. Kou, H. Qian, and X. S. Xie, Nano Lett. **5**, 2373 (2005).

[4] B. P. English *et al.*, Nat. Chem. Biol. **2**, 87 (2006); S. C. Kou, B. J. Cherayil, W. Min, B. P. English, and X. S. Xie, J. Phys. Chem. B **109**, 19068 (2005); X. S. Xie and H. P. Lu, J. Biol. Chem. **274**, 15967 (1999).

[5] A. B. Kolomeisky and M. E. Fisher, J. Chem. Phys. **113**, 10867 (2000).

[6] H. Qian, Phys. Rev. E **64**, 022101 (2001).

[7] G. Gallavotti, Phys. Rev. Lett. **77**, 4334 (1996); G. Gallavotti and E. G. D. Cohen, *ibid.* **74**, 2694 (1995); J. R. Dorfman, *An Introduction to Chaos in Nonequilibrium Statistical Mechanics* (Cambridge University Press, New York, 1999); D.-Q. Jiang, M. Qian, and M.-P. Qian, *Mathematical Theory of Nonequilibrium Steady-State, Lecture Notes in Mathematics*, vol. 1833 (Springer, New York, 2004).

[8] J. Kurchan, J. Phys. A **31**, 3719 (1998); J. L. Lebowitz and H. Spohn, J. Stat. Phys. **95**, 333 (1999); P. Gaspard, J. Chem. Phys. **102**, 8898 (2004).

[9] H. Qian, Phys. Rev. E **65**, 016102 (2002); J. Phys. Chem. B **106**, 2065 (2002).

[10] J. Liphardt, S. Dumont, S. B. Smith, I. Tinoco, and C. Bustamante, Science **296**, 1832 (2002); E. H. Trepagnier, C. Jarzynski, F. Ritort, G. E. Crooks, C. J. Bustamante, and J. Liphardt, Proc. Natl. Acad. Sci. U.S.A. **101**, 15038 (2004).

[11] G. E. Crooks, Phys. Rev. E **60**, 2721 (1999); D.-Q. Jiang, M. Qian, and F.-X. Zhang, J. Math. Phys. **44**, 4174 (2003); U. Seifert, Phys. Rev. Lett. **95**, 040602 (2005); H. Qian, J. Phys. Chem. B **109**, 23624 (2005).

[12] Symmetry $c_\lambda = c_{1-\lambda}$ means $\Sigma_a e^{-\lambda a}\,\Pr\{Q/k_BT=a\} = \Sigma_a e^{-\lambda a} e^{-a}\,\Pr\{Q/k_BT=-a\}$ $\forall$ λ. Hence $\Pr\{Q/k_BT=a\}/\Pr\{Q/k_BT=-a\}=e^a$. The symmetry is a mathematical version of the FT.

[13] C. Jarzynski, Phys. Rev. Lett. **78**, 2690 (1997). While Jarzynski's equality was first established for time-dependent conservative systems, it was latter shown that it is also valid for the NESS of open systems; see T. Hatano and S. I. Sasa, *ibid.* **86**, 3463 (2001).

[14] G. Hummer and A. Szabo, Proc. Natl. Acad. Sci. U.S.A. **98**, 3658 (2001).

[15] U. Seifert, Europhys. Lett. **70**, 36 (2005).

[16] M. Nishiyama, H. Higuchi, and T. Yanagida, Nat. Cell Biol. **4**, 790 (2002); N. J. Carter and R. A. Cross, Nature (London) **435**, 308 (2005).

[17] A. B. Kolomeisky, E. B. Stukalin, and A. A. Popov, Phys. Rev. E **71**, 031902 (2005).

[18] H. Wang and H. Qian (unpublished).

[19] I. H. Segel, *Enzyme Kinetics* (John Wiley Interscience, New York, 1975).

[20] E. Cinlar, *Introduction to Stochastic Processes* (Prentice-Hall, Englewood Cliffs, NJ, 1975). A MRP is a combination of Markov processes with renewal theory. It has nonexponential sojourn time for each transition. It becomes a Markov process if the distributions of the sojourn times are all exponential and independent of the next state; it becomes a Markov chain if the sojourn times are all equal to 1, and it becomes a renewal process if there is only one state.

[21] H. Qian, Biophys. Chem. **67**, 263 (1997); H. Qian and E. L. Elson, *ibid.* **101**, 565 (2002).

[22] The Haldane relation in reversible enzyme kinetics [19] states that the steady-state velocities for forward and backward enzyme reactions, V_f and V_r, have the same saturation dependence on the substrate and product concentrations: $V_f=\alpha_f[S]/(1+[S]/K_S+[P]/K_P)$, $V_r=\alpha_r[P]/(1+[S]/K_S+[P]/K_P)$, where $\alpha_f/\alpha_r=K_{eq}$, the equilibrium constant between S and P. Also see [17].

[23] H. M. Taylor and S. Karlin, *An Introduction to Stochastic Modeling*, 3rd ed. (Academic Press, New York, 1998).

[24] The initial condition leads to $\Sigma_{\ell=0}^{2n} a_\ell = \Sigma_{\ell=1}^{2n} a_\ell \lambda_\ell = \cdots = \Sigma_{\ell=1}^{2n} a_\ell \lambda_\ell^{n-1} = 0$, but $d^n w_-(0)/dt^n = \Sigma_{\ell=1}^{2n} a_\ell \lambda_\ell^n = w_1 w_2 \cdots w_n$ and $d^n w_+(0)/dt^n = \Sigma_{\ell=1}^{2n} a_\ell \lambda_\ell^n = u_1 u_2 \cdots u_n$. Furthermore, $d^{n+1} w_-(0)/dt^{n+1} = \Sigma_{\ell=1}^{2n} a_\ell \lambda_\ell^{n+1} = -w_1 \cdots w_n \Sigma_{k=1}^{n} (w_k+u_k)$, $d^{n+1} w_+(0)/dt^{n+1} = \Sigma_{\ell=1}^{2n} a_\ell \lambda_\ell^{n+1} = -u_1 \cdots u_n \Sigma_{k=1}^{n} (w_k+u_k)$.

[25] T. L. Hill, *Free Energy Transduction and Biochemical Cycle Kinetics* (Springer-Verlag, New York, 1989); *Free Energy Transduction in Biology* (Academic Press, New York, 1977).