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Fluctuations and mass action law breakdown in statistical thermodynamics of small systems

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The statistical equilibrium of chemical reaction of the type $\mathbf{PQ} \rightleftarrows \mathbf{P} + \mathbf{Q}$, taking place within small but macroscopic closed vesicle, is considered using statistical physics approach. It is shown, that mass action law, being the result of mean-field-type approximation, breaks down for sufficiently small vesicle volume and/or equilibrium constant of reaction, when mean number of free "**P**" particles, $\langle p \rangle$, within the vesicle becomes of order one or less. At the same time, the Nernst equation is shown to be applicable for systems of arbitrary volume and it gives the relation $\Delta F \sim -\ln\langle p \rangle$ for free energy "payment" for one "**P**" particle liberation from the vesicle. Due to fluctuations the true $\langle p \rangle$ and $(-\Delta F)$ values are essentially lower than corresponding mass action law predictions. The same effect of fluctuations leads to essential random inhomogeneity in the ensemble of vesicles, prepared under equivalent macroscopic conditions, and to non-Gaussian distributions of these vesicles over total number of particles within them or over ΔF value. Estimations show the effects of fluctuations to be essential for vesicles with sizes of order 10^2–10^3 Å, which is just the typical order of magnitude for many biological vesicles. Corresponding possible explanations of some experimental results in bioenergetics are discussed briefly.

INTRODUCTION

The problems of thermodynamics and statistical physics of small systems are of special importance for biophysics and biochemistry (for review see Refs. 1–9). The fundamental equations of equilibrium and linear irreversible thermodynamics have been applied to a theoretical study of different kinds of chemical and biochemical systems.[1-15] For a quantitative description of energy transduction in biological membranes, one needs to calculate the free energy changes in the processes of transmembrane particle transfer from small closed vesicles to surrounding medium or backward. First, it is very important to estimate the free energy change after proton transfer through the energy-transducing membranes of mitochondria, chloroplasts and chromatophores.[3-6,16-18] However, due to small dimensions of these vesicles the number of certain particles inside a single vesicle can be very low: The average number of certain free particles $\langle p \rangle$, may be even less then one. For example, for hydrogen ions inside a single thylakoid of higher plant chloroplasts $\langle p \rangle \cong 0.3$ at *p*H 7.[19] In accordance with the above mentioned there is the problem of adequate calculation of particle concentrations inside the vesicles and free energy change ΔF, accompanying transmembrane particle transfer.

For macroscopic vesicles this problem is trivial and can be easily solved using the well-known thermodynamic formulas of Nernst type, $\Delta F = k_B T \ln(c_{in}/c_{out})$, where c_{in} and c_{out} are corresponding concentrations, which can be determined under a chemical equilibrium state by mass action law.

For small vesicles, however, the fluctuations are of special importance. The problem with calculation of the particles mean concentrations and free energy changes are, therefore, rather complicated. For other chemical and biochemical systems, the noise theory was used to analyze the fluctuations in state probabilities, transition and cyclic fluxes in different kinds of equilibrium or steady state processes.[6-15] The treatment of our problem based on the classical thermodynamic approach carried out by two of us[20] has led to a paradoxical result: For sufficiently small vesicles, the transfer of even one particle along the concentration gradient can be thermodynamically unfavorable. With a similar problem with apparent violation of the second law of thermodynamics had faced Westerhoff and his colleagues[7,9] who used kinetic equations and nonequilibrium thermodynamics for analysis of the problem of energy coupling in small vesicles and output reaction in channeled systems.

This apparent violation of the second law of thermodynamics might be the consequence of the breakdown of mass action law due to fluctuations and some "quantum" peculiarities of small systems. The explanation of this sort of paradoxes is obviously connected with discrete nature of the process of particle transfer. The number of transferred particles is the integer, whereas the average number of free particles within the vesicle $\langle p \rangle$ might be even much less than one. For the same reason, the traditional thermodynamic approach cannot account for discrete nature of distribution of particle number within one vesicle over the ensemble of vesicles prepared under identical macroscopical conditions.

It leads to the necessity to reanalyze the sense, the form

and applicability of mass action law and Nernst equation for the special case of a sufficiently small system. This is the subject of the present paper.

THE MODEL

Let us consider the following simplified model. We start our treatment with one closed vesicle of inside volume V. The vesicle and surroundings contain neutral particles "**P**" and "**Q**" which participate in reversible chemical association reaction $\mathbf{PQ} \rightleftarrows \mathbf{P} + \mathbf{Q}$. Except for this reaction, in all other respects the system is supposed to be ideal. The vesicle wall is impenetrable for "**P**," "**Q**" and "**PQ**" particles.

The system thermodynamical state is defined by the total numbers, P and Q, of "**P**" and "**Q**" particles (free as well as bound in "**PQ**" form) inside the vesicle; vesicle volume V and equilibrium constant K [In traditional chemical thermodynamics the equilibrium constant K is determined macroscopically by the mass action law, i.e., by the expression of the type $[\mathbf{P}][\mathbf{Q}]/[\mathbf{PQ}]$. In our case we must use the microscopic approach and interpret K as a characteristic of single "**PQ**" molecule: $K = v_0^{-1} \exp(-\Delta E/k_B T)$, where ΔE is energy difference between "**P**" + "**Q**" and "**PQ**" states, v_0 is a well-known entropic factor with dimension of volume. In other words, K is the ratio of corresponding direct and inverse rate constants (see the Appendix).]. K value determines the average distribution of "**P**" and "**Q**" particles inside the vesicle between free and bound forms.

We are interested in the case, when the K value is so low, that the thermodynamically averaged number of free particles inside the vesicle is extremely small.

We shall calculate the free energy difference, ΔF, between two identical vesicles one of which contains P and Q and other $(P-1)$ and Q corresponding particles. This value ΔF corresponds to the free energy change after the unidirectional transfer of one "**P**" particle from a vesicle to outside.

In real biological systems containing energy transducing membranes, two mechanisms of particle transfer across the membrane exist, which in the cases of interest for us differ essentially by characteristic time scales. The time scale of the first mechanism (passive transport) is by three or four orders of magnitude larger than the time scale of the second mechanism (unidirectional selective active transfer) which is realized by special device.[16] The functioning of this device is realized before the passive transmembrane transfer influence the state of the system. This is why the vesicle wall in our model can be considered to be impenetrable.

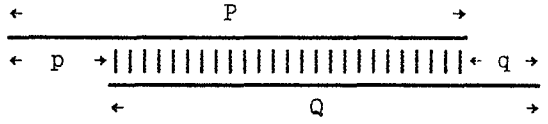
In real systems there are, of course, a great number of vesicles. However, every single vesicle is functioning independently of the others. Therefore, for our problem the physical meaning has ΔF value just for a single vesicle. Later in this paper we will return to the question regarding averaging over the ensemble of vesicles.

STATISTICAL TREATMENT 1: THE VESICLE WITH FIXED NUMBER OF PARTICLES

Although the number of free "**P**" particles in the vesicle is very low, the vesicle as a whole is sufficiently large to be considered as a statistical system.

At first we shall use an approach of equilibrium statistical mechanics. We shall describe briefly in the Appendix an alternative kinetic approach, which is also of principal interest.

Let us denote numbers of free "**P**" and "**Q**" particles, respectively, as p and q, according to the following scheme:



It is clear from this scheme, that $m = P - p = Q - q$ is the number of "**PQ**" molecules.

Let us consider an integer p as the dynamic variable of our system. If p is fixed, we have the mixture of three ideal gases with numbers of particles p, q, and m, and the partition function of our system is, therefore, equal to $[K^m$ factor corresponds to energies of m bonds **P**–**Q**. For the sake of simplicity we have written down the configurational part of the partition function only. The whole expression contains, of course, an additional factor arising from integration over all generalized momentums and including a Planck constant. This factor corresponds to the so-called "quantum volume." Trivial calculations show this factor to cancel out in the final expression]

$$Z(V,K,Q,P) = \sum_{p_{\min}}^{P} z_p, \quad z_p = \left[\frac{V^p}{p!}\right] * \left[\frac{V^q}{q!}\right] * \left[\frac{(VK)^m}{m!}\right], \tag{1}$$

where (see the scheme) $q = (Q - P) + p$, $m = P - p$, and

$$p_{\min} = \begin{cases} 0 & \text{if } Q \geqslant P \\ P - Q & \text{if } Q < P \end{cases}.$$

For the observables $\langle p \rangle$ and ΔF we have the following obvious expressions [factor c_{out}^{-1} in formula (3) corresponds to chemical potential $k_B T \ln(c_{\text{out}})$ of "**P**" particles in macroscopic surrounding medium. It must be introduced, because ΔF includes not only free energy change of the vesicle inner part, but surroundings as well]:

$$\langle p \rangle = \sum_{p_{\min}}^{P} p z_p \Big/ \sum_{p_{\min}}^{P} z_p, \tag{2}$$

$$\exp(-\Delta F/k_B T) = Z(V,K,Q,P-1)/[Z(V,K,Q,P) c_{\text{out}}]. \tag{3}$$

First of all, it is easy to see from Eqs. (2) and (3), that the following formula is valid and exact for our system:

$$\Delta F = -k_B T \ln(\langle p \rangle / V c_{\text{out}}). \tag{4}$$

Of course, it is the equation of the Nernst type. One would bear in mind, however, that $\langle p \rangle$ in this formula is to be calculated in the framework of statistical approach. In conventional chemical thermodynamics equilibrium concentration $\langle p \rangle / V$ in the Nernst equation can be calculated using mass action law. However, this law for a small system can, as we shall see later, break down due to fluctuations.

Let us discuss now calculation of average $\langle p \rangle$ value according to Eq. (2). In the routine macroscopic case, when V is sufficiently large, one may use the saddle point approximation, i.e., replace both sums in Eq. (2) by their highest terms.

This leads to replacing $\langle p \rangle$ by p^*, where p^* is the number of the highest term, or, physically, the most probable p value. Using conventional Stirling formula, it is easy to find

$$KV(P-p^*) = p^*(Q-P+p^*) \text{ (if } V \text{ is sufficiently large).} \quad (5)$$

Thus, we obtain the conventional expression of mass action law. We can conclude this law to be just the result of saddle point approximation. In other words, this law is valid only if fluctuations can be neglected. Consequently, this law is the typical example of the mean field type approximation.

It must be born in mind that in all cases of interest for us the number of free particles p is negligible in comparison with total numbers P and Q, but not with the difference

$$n = P - Q = p - q. \quad (6)$$

In these cases p^* is really determined by two parameters only, n and

$$\kappa = KVQ \cong KVP, \quad (7)$$

because one can rewrite Eq. (5) as follows:

$$\kappa = p^*(p^* - n). \quad (8)$$

Moreover, the exact Eq. (2) in these cases really gives an expression for $\langle p \rangle$ just in terms of the two parameters, n and κ, because upper limits in Eq. (2) do not play any role and for comparatively small p values $1/m! = 1/(P-p)! \cong P^p/P! \cong Q^p/P!$: Therefore,

$$\langle p \rangle = \sum_{p_{\min}} p y_p \Big/ \sum_{p_{\min}} y_p, \quad y_p(n,\kappa) = \kappa^p/p!(p-n)!. \quad (9)$$

Using Eq. (9) in the next section, we can discuss the nontrivial case, when volume of the system V is comparatively small and fluctuations of p value become essential, because p is an integer. In this case, the main contribution to both sums in formula (9) give few terms with small (in comparison with P) p values, and it leads to some essential deviation from the results of saddle point approximation.

DISCUSSION 1

To illustrate the difference between solutions of the correct Eq. (9) and mass action law predictions, let us consider the simplest limiting case: $n = 0$ (i.e., $p_{\min} = 0$), $\kappa \ll 1$. In this case we can replace both sums in Eq. (9) by its first nonzero terms; this leads to expression $\langle p \rangle \cong \kappa$, whereas mass action law (8) gives $p^* \cong \kappa^{1/2}$. We see, that in our limiting case $p^* \gg \langle p \rangle$; we shall see later, that p^* always exceeds $\langle p \rangle$.

The results of very simple numerical calculations using Eq. (9) are presented in Fig. 1, where average $\langle p \rangle$ value vs n is shown for a few values of κ in the logarithmic scale. For comparison the corresponding predictions of mass action law is drawn in the figure as a firm line.

As we have seen above, mass action law is formally exact for nondiscreet continuous medium, when sums in Eqs. (2) or (9) can be replaced by integrals. (It is usual situation for mean-field-type approximation. For example, Debay–Hukkel approximation is formally exact for continuous electrolytes.) One may say, that discrete nature of matter manifests itself in the most evident form in the deviations of mass action law predictions from the correct mean $\langle p \rangle$ value. Let us discuss briefly the physical meaning of these deviations.

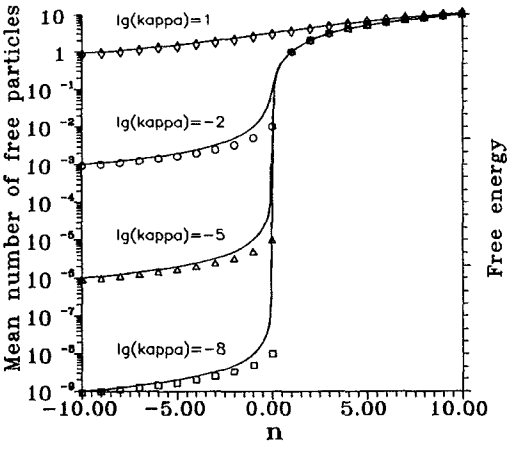


FIG. 1. Mean number of free "**P**" particles within the vesicle, $\langle p \rangle$, in logarithmic scale (left axis) or free energy "payment" of one "**P**" particle liberation in linear scale (right axis) vs n—the difference between total numbers of "**P**" and "**Q**" particles for the few values of κ parameter [see Eq. (7) or (12) for its definition; for the example of pure water, where $c = 1$ mol/l and $pK = 14$, the values of $\kappa = 10^{-8}, 10^{-5}, 10^{-2}, 10$, which are used in the figures of the present paper, correspond to cubic vesicles with sizes $a \cong 175$, 550, 1750, 5500 Å, respectively]. Symbols are the results of statistical theory, whereas solid lines, which are drawn for comparison, correspond to mass action law predictions.

As shown in Fig. 1 the real mean number of free "**P**" particles $\langle p \rangle$ for the system in the sufficiently small volume V is essentially lower, than corresponding mean value predicted by mass action law. While it is the property of thermodynamically equilibrated system, its most clear physical explanation we can give using the following kinetic arguments.

Let us consider the mixture of "**P**" and "**Q**" particles equilibrated with respect to the reaction $\mathbf{P} + \mathbf{Q} \rightleftarrows \mathbf{PQ}$. If the equilibrium constant K has a sufficiently low value, then the concentrations of free "**P**" and "**Q**" particles are rather small and the acts of the decay of the "**PQ**" molecules are the rare events. However, as soon as the "**PQ**" molecule has been decomposed, the liberated fragments, i.e., free "**P**" and "**Q**" particles, begin random walking independently of each other. For each individual particle, its random walking would continue until it meets corresponding partner. Therefore, the time interval of this walking is rather large due to small concentration of free partners. On the other hand, in a sufficiently small closed volume V the fragments "**P**" and "**Q**", appeared after "**PQ**" decay act, cannot go far away from each other. Consequently, the probability of their collision and association is considerably higher, than in macroscopic system. This is why, the equilibrium concentration of free particles within a small volume V, $\langle c_p \rangle_V = \langle p \rangle / V$, is essentially smaller than in macroscopic mixture, $\langle c_p \rangle_{\text{macro}}$.

For a macroscopic system mass action law is practically exact and gives the true value of equilibrium concentration. Therefore, $\langle c_p \rangle_{\text{macro}} = p^*/V$ and, consequently, $p^* > \langle p \rangle$: most probable p value (p^*) is always greater, than the aver-

age one ($\langle p \rangle$), or, in other words, mass action law always gives some exaggeration for mean p value.

Of course, this result can be explained not only kinetically, as we have done, but also in terms of equilibrium theory.

Let us discuss now the free energy "payment," ΔF, for the transfer of one particle. In accordance with Eq. (4) we have $-\Delta F/k_B T = \ln\langle p \rangle - \ln(Vc_{\text{out}})$ and, therefore, one can estimate ΔF value using linear scale axis on the right-hand side of Fig. 1.

The main question is whether particle transfer is thermodynamically favorable ($\Delta F < 0$) or not ($\Delta F > 0$). In accordance with Eq. (3) $\Delta F < 0$ inequality takes place under usual condition $\langle p \rangle / V = \langle c_p \rangle_V = c_{\text{in}} > c_{\text{out}}$. It is easy to interpret this condition graphically using Fig. 1: $\Delta F < 0$ if and only if mean $\langle p \rangle$ value in Fig. 1 for given n and κ lies lower than the level of $\ln(Vc_{\text{out}})$. However, if one tries to formulate this condition in terms of vesicle parameters n and κ, as an inequality of the type $n > n_{\min}(\kappa, c_{\text{out}})$, then it is necessary to take into account great deviations of true dependence $n_{\min}(\kappa, c_{\text{out}})$ from the corresponding predictions of mass action law; these deviations are, as it is clear from Fig. 1, of special significance in the region of comparatively small n ($|n| \lesssim 10$, $\kappa \lesssim 1$), as it is shown in Fig. 2.

Thus, a lot of situations exist when the behavior of the vesicle depends strongly on the total number of particles within it, P. In a real system, this number is determined during the process of vesicle preparation. However, in any real preparation process the number of particles can be controlled with some finite accuracy only. Accordingly, in the next section we shall discuss the role of inhomogeneity of the ensemble of vesicles with respect to number of "P" particles within a vesicle.

STATISTICAL TREATMENT 2: ENSEMBLE OF VESICLES WITH FIXED CHEMICAL POTENTIAL OF "P" PARTICLES

In this section we shall suppose a vesicle to be prepared in the special surrounding medium during a sufficiently long time. In such a situation the system is thermodynamically equilibrated with respect to the number of "P" particles within the vesicle. The chemical potential of "P" particles in the preparation surroundings let be μ. Our first question is the following: What is the probability $w_{\text{P}}(V,K,Q,\mu)$ to find a vesicle in which the number of "P" particles equals P?

The answer is clear:

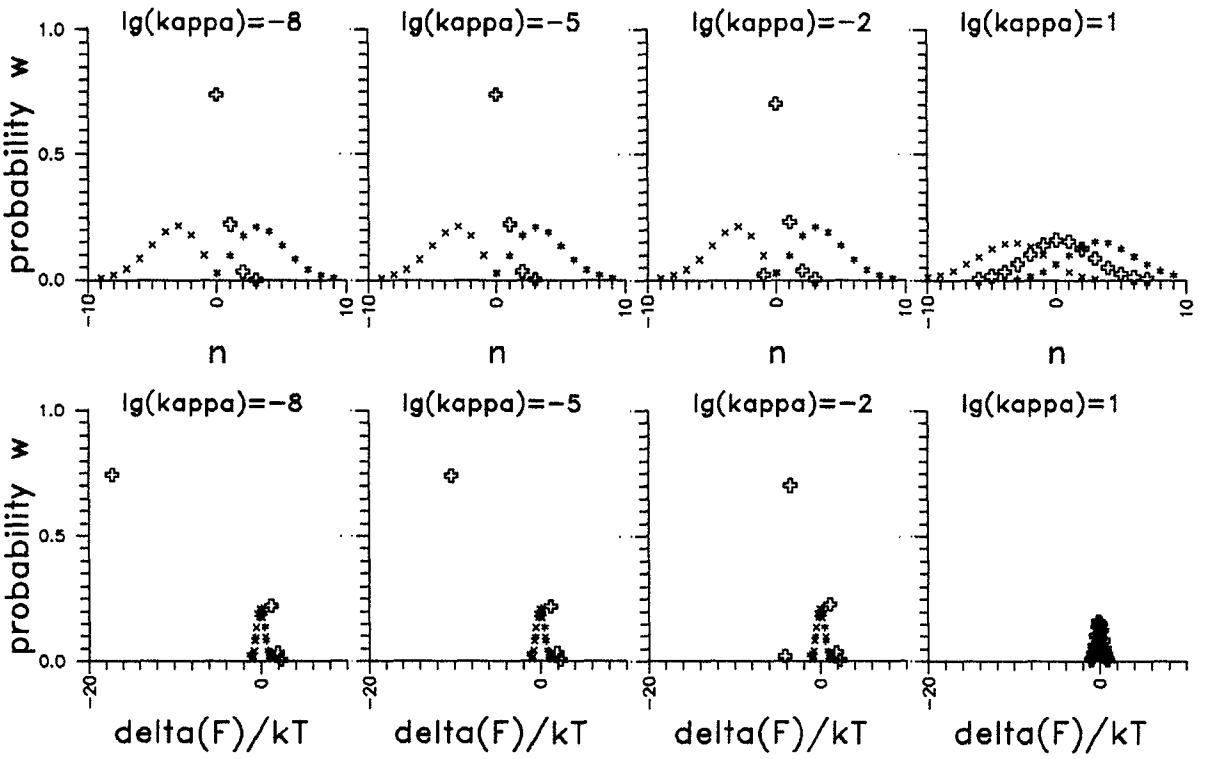


FIG. 2. Probability distributions for vesicles over different numbers of "P" particles, i.e., over n values (upper series of graphics) and over different possible ΔF values (lower series) for the few values of κ parameter (the same as in Fig. 1) and for three different values of chemical potential of "P" particles in the surrounding preparation medium. As the characteristic of this chemical potential one can use averaged difference between numbers of "P" and "Q" particles within volume $V = a^3$ (equals to vesicle volume) in macroscopic preparation surroundings: let's denote this as $\langle n \rangle_\mu$. Small crosses, large crosses and small stars correspond to $\langle n \rangle_\mu \cong -3.6, 0.3, 3.6$, respectively. The distributions are practically Gaussian if $\kappa > 1$, fluctuations lead to great effects when $\kappa \ll 1$ and $\langle n \rangle_\mu < 1$.

$$W_P(V,K,Q,\mu) = \exp(-P\mu/k_BT)Z(V,K,Q,P)/\zeta(V,K,Q,\mu),$$

$$\zeta(V,K,Q,\mu) = \sum_{P=0}^{\infty} \exp(-P\mu/k_BT)Z(V,K,Q,P). \quad (10)$$

In the macroscopic case W_P distribution is practically Gaussian.

When, however, $\kappa \lesssim 1$ and $|P-Q| \ll P,Q$ the probability $W_P(=W_n)$ depends only on κ Eq. (7) [as $\langle p\rangle$ in Eq. (9)]:

$$W_P(V,K,Q,\mu) \cong W_n(\kappa,\mu) \cong \frac{\exp(-n\mu/k_BT)\sum_{p=p_{\min}} y_p(n,\kappa)}{\sum_n \exp(-n\mu/k_BT)\sum_{p=p_{\min}} y_p(n,\kappa)}.$$

As shown in Fig. 2(a), there exist an essential deviation of the $W_P(=W_n)$ distribution from the Gaussian one. [The results, presented in Fig. 2, have been obtained numerically using the following recurrence relation, which can be proved using Eq. (10):

$$W_{n+1}(V,K,Q,\mu)/W_n(V,K,Q,\mu) = \exp(\mu/k_BT)\langle p\rangle_{\kappa n}.]$$

In particular, mean value $\overline{P}$ differs essentially from most probable one.

DISCUSSION 2

The physical reason of the distribution W_P deviation from the trivial Gaussian one is, of course, the same effect of fluctuations, which we have discussed above.

Now we can return to the problem concerning averaging over ensemble of vesicles. If we have a macroscopic system with a multitude of independently functioning vesicles, then in experiments of the different types one actually measures different characteristics of the system, such as, for example, probability to find a vesicle with positive ΔF, W, and average free energy

$$\overline{\Delta F} = \sum_{P=0}^{\infty} W_P(V,K,Q,\mu)\Delta F(V,K,Q,P) \cong \sum_n W_n(\kappa,\mu)\Delta F(\kappa,n), \quad (11)$$

where ΔF is the corresponding value for one vesicle, which was calculated above. An interesting feature of Eq. (11) is the following one: The second factor, ΔF, characterizes the single vesicle under the "working" conditions, but the first factor, $W_P(=W_n)$, is the characteristic of our system under another condition, realized during system preparation. Averaging procedures of such a type are well known in the theory of disordered systems.[21]

It is interesting to reformulate Eq. (11) in the following way

$$\overline{\Delta F} = \sum_{\Delta F} W_{\Delta F}(\kappa,\mu)\Delta F, \quad (12)$$

where $W_{\Delta F}(\kappa,\mu)$ is the probability to find the vesicle with given ΔF value in the system with fixed κ and μ. It is easy to find $W_{\Delta F}$ distribution using Figs. 1 and 2a, and the result shown in Fig. 2b. To explain this result, let us notice that, in accordance with Fig. 1, ΔF for any given κ can have only discrete set of values. It is clear from Fig. 2b, that measured averaged $\overline{\Delta F}$ value in fluctuational regime can differs dramatically from most probable one. Using $W_{\Delta F}$ distribution or Fig. 2b it is easy to find also another observable value, defined above W: $W = \Sigma_{\Delta F>0} W_{\Delta F}$

CONCLUSION

Using conventional notations, i.e., dimensions (mol/l) for concentrations and expression of the type $K = 10^{-pK} \times N_A$ (mol/l) for equilibrium constant K, we can rewrite κ definition (7) as follows:

$$\kappa = ca^6 \times 10^{-pK} \times 3.6 \times 10^{-8}, \quad (12)$$

where $a = V^{1/3}$ (Å) is linear size of the cubic volume V and $c = [\mathbf{PQ}]/V$ (mol/l) is concentration of "**PQ**" molecules. Let us discuss briefly some typical concrete situations.

The simplest application of above theory is the case when "**PQ**" is pure water, so $\mathbf{P} = \kappa^+$, and $\mathbf{Q} = \mathbf{OH}^-$. (Simple estimations, carried out by two of us,[22] show the role of electrical charges to be qualitatively insignificant.) In this case, as it is well known, $c \times 10^{-pK} \cong 10^{-14}$ and, therefore, the crossover value of a, corresponding to $\kappa \sim 1$, is of order $a \simeq 4000$ Å. It is just a typical order of magnitude for real biological vesicles. Consequently, the fluctuational regime with $\kappa < 1$ can occur in reality.

Our model is restricted to the systems containing only one kind of "**PQ**" particles. As to biological systems, there always exist, in addition to $\mathbf{OH}^-$, many other chemical groups (for example, acid and basic groups in proteins), which can change the number of free protons. Of course, its concentration is incomparably smaller, than water's one, but this can be compensated by their pK value. It seems to be quite natural, however, to suppose, that certain fluctuational effects can be essential in systems with small ($\lesssim 1$) mean number of free particles.

For the sake of clarity we can formulate the following paradoxical statement based on the afore-described conclusions. Two small but macroscopic (with linear size of order of hundreds of angstroms), vesicles identical in all respects except the difference between total numbers of protons, which can be as small as one, can differ dramatically from each other: The liberation of single proton from one vesicle to outside medium is thermodynamically favorable, whereas from another one-unfavorable.

As a conclusion we shall try to apply our results for qualitative explanation of one experimental result, which, to our knowledge, have not been explained up to now.

ATP synthesis in the energy-transducing vesicles of higher plants, thylakoids, require proton ejection from the acidic vesicle interior to the alkaline outside medium through the channel of the ATP synthase across the thylakoidal membrane. This process is initiated by electron transport along the electron transfer chain (ETC) in the energy transducing thilakoidal membrane from water to ultimate ETC electron acceptor, NAD Electron transport, in its turn, is initiated by light absorption. At physiological intrathyla-

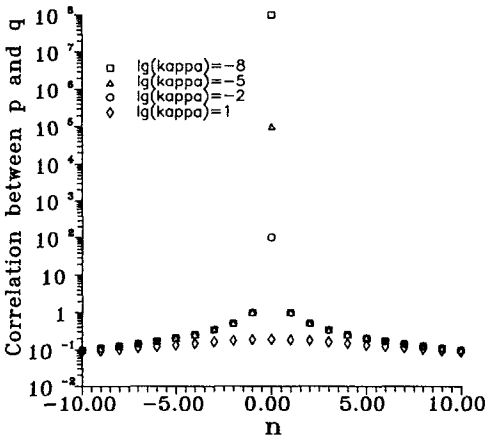


FIG. 3. Correlator $\langle pq\rangle/\langle p\rangle\langle q\rangle - 1$ vs $n = P - Q$ for the few values of κ (the same as in Fig. 1).

koidal average pH value under stationary conditions (continuous illumination) the synthesis of one ATP molecule from ADP and P_i requires the passage of ~ 2 electrons through ETC (see, e.g., Refs. 16–18).

Experiments with single short light flashes, using dark adapted chloroplasts from higher plants or chromatophores from photosynthetic bacteria,[23–25] when during one light flash only one electron passes through every ETC (The duration of light flashes in these experiments were $10\,\mu$s and 30 ns, whereas the "recovery time" of light absorbing chlorophyll reaction center exceeds $600\,\mu$s), have shown, however, that ATP is formed under this conditions, but its yield per one ETC, in this case, corresponds approximately to the half of the maximal possible one. Just this can be explained on the basis of present paper results, Eq. (11) and Fig. 3: at average pH value about one-half of all thylakoids in the sample have inside pH value (or $c_{\text{in}} = \langle[\mathbf{H}^+]\rangle/V$ above) too high to make proton ejection thermodynamically favorable.

APPENDIX: KINETIC APPROACH

We shall suppose for the sake of simplicity, that the diffusion rate is infinite and, as a result, all the space gradients are zero. In this case the kinetic equation deals with the function $W_p(t)$ only, where $W_p(t)$ is the probability to find a system at the time moment t in the state, where the number of free "**P**"-particles equals p. The kinetic equation has a form

$$\begin{aligned}\frac{d}{dt}W_p(t) = {} & k_+(P-p+1)W_{p-1}(t) \\ & + (k_-/V)(p+1)(p-n+1)W_{p+1}(t) \\ & - k_+(P-p)W_p(t) \\ & - (k_-/V)p(p-n)W_p(t), \qquad (13)\end{aligned}$$

where k_+ and k_- are corresponding rate constants and for the boundary cases $p = 0$ and $p = P$ we mean obvious special additional conventions of the type $W_{-1} = W_{P+1} = 0$. First and second terms in Eq. (13) describe an increase of W_p due to "**PQ**"-molecule decay in the $(p-1)$ state and their creation in the $(p+1)$ state, respectively; third and forth terms describe decrease of W_p due to decay and creation of "**PQ**" particles in the p state, respectively.

Kinetic equation (13) satisfies the obvious requirement

$$\frac{d}{dt}\sum_{p=0}^{P} W_p(t) = 0.$$

It is easy to verify, that at $t\to\infty$ the solution of kinetic equation tends to the time-independent stationary limit, which is proportional just to the partition function z_p [see Eq. (1)], i.e., $W_p(t\to\infty)\cong z_p$, as it must to be from the physical point of view (of course, $K = k_+/k_-$).

Using Eq. (13) one can derive the so-called master equation for mean value $\langle p(t)\rangle$ (it is, of course, the first "link" of the "chain" of equations for correlators of the type $\langle pq\rangle$, etc.); it has the form

$$\frac{d}{dt}\langle p(t)\rangle = k_+\langle P - p(t)\rangle - (k_-/V)\langle p(t)q(t)\rangle$$

instead of the conventional dynamic mass action law

$$\frac{d}{dt}p(t) = k_+[P - p(t)] - (k_-/V)p(t)q(t).$$

The comparison of last two equations gives us a new point of view for the qualitative explanation of the effects of fluctuations described above: it is clear, that discreteness of p value leads to the statistical interdependency between p and q fluctuations, which, in its turn, becomes essential when $p\sim 1$. This leads to correlations between p and q, and to essential difference between $\langle pq\rangle$ and $\langle p\rangle\langle q\rangle$ values (Fig. 3).

[1] T. L. Hill, *Thermodynamics of Small Systems, Part 1* (Benjamin, New York, 1963).
[2] T. L. Hill, *Free Energy Transduction in Biology* (Academic, New York, 1977).
[3] T. L. Hill, Proc. Natl. Acad. Sci. USA **76**, 232 (1979).
[4] S. R. Caplan and A. Essig, *Bioenergetics and Linear Nonequilibrium Thermodynamics. The Steady State* (Harvard University, Cambridge, Massachusetts and London, England, 1983).
[5] H. V. Westerhoff and K. Van Dam, *Thermodynamics and Control of Biological Free-Energy Transduction* (Elsevier, New York, 1987).
[6] G. R. Welch and D. B. Kell, in *The Fluctuating Enzyme*, edited by G. R. Welch (Wiley–Interscience, New York, 1986), pp. 451–492.
[7] H. V. Westerhoff and F. Kamp, in *Organization of Cell Metabolism*, edited by G. R. Welch and J. S. Clegg (Plenum, New York, 1987), pp. 339–356.
[8] T. L. Hill and Y-D. Chen, Proc. Natl. Acad. Sci. USA **76**, 3654 (1979).
[9] H. V. Westerhoff and Y-D. Chen, Proc. Natl. Acad. Sci. USA **82**, 3222 (1985).
[10] J. Stucki, Eur. J. Biochem. **109**, 257 (1980).
[11] J. L. Lebowitz and J. K. Perkus, Phys. Rev. **124**, 1673 (1961).
[12] D. A. McQuarrie, J. Chem. Phys. **38**, 433 (1963).
[13] D. A. McQuarrie, Appl. Probability **8**, 1 (1967).
[14] P. Glansdorf and I. Prigogine, *Structure, Stability and Fluctuations* (Wiley–Interscience, London, 1971).
[15] Y-D. Chen, J. Chem. Phys. **59**, 5810 (1973).
[16] L. A. Blumenfeld, *Physics of Bioenergetic Processes* (Springer, Berlin, 1983).
[17] D. C. Nichols, *Bioenergetics. An Introduction to the Chemiosmotic Theory* (Academic, London, 1982).

[18] V. P. Skulachev, *Bioenergetics* (Springer, New York, 1988).
[19] L. A. Blumenfeld, R. M. Davydov, and A. N. Tikhonov J. Molecular Liquids **42**, 231 (1989).
[20] A. N. Tikhonov and L. A. Blumenfeld, J. Phys. Chem. (USSR) **64**, 1729 (1990).
[21] J. M. Ziman, *Models of Disorder* (Cambridge University Press, Cambridge, MA, 1979).
[22] A. N. Tikhonov and L. A. Blumenfeld, Biophysics (USSR) (in press).
[23] P. Graber, E. Schlodder, and H. T. Witt, Biochim. Biophys. Acta **461**, 426 (1977).
[24] D. A. Harris and M. Baltshefsky, Biochim. Biophys. Res. Comm. **86**, 1248 (1979).
[25] L. A. Blumenfeld, M. G. Goldfield, and L. G. Dmitrovsky, Studia Biophysica **65**, 69 (1977).